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AGING

From Evolution to Modern Biology to Anti-Aging

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Aging

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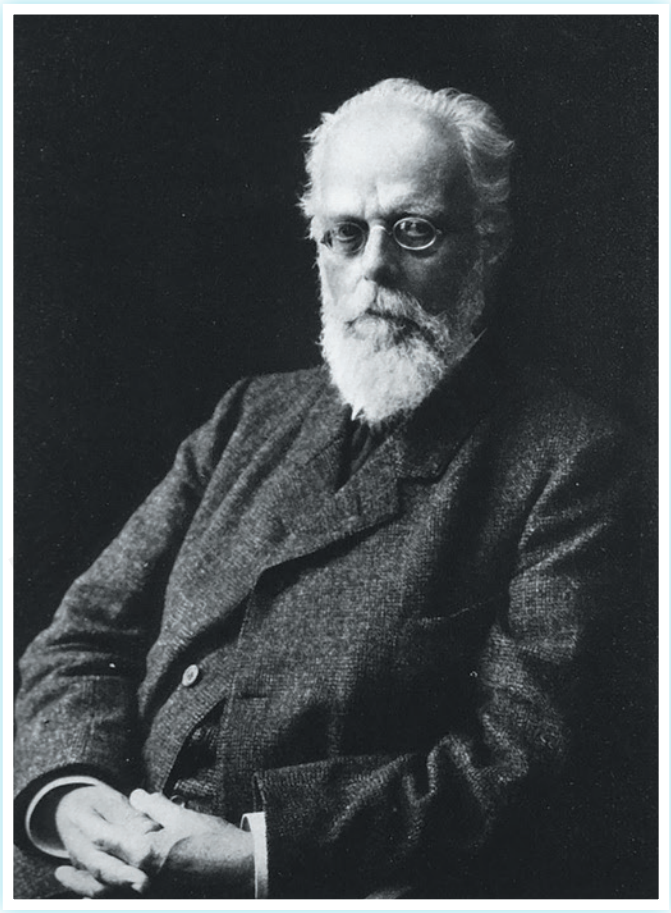
To my wife, Liliane, who defies aging … by not aging!
and to:

August Weismann FRS, HonFRSE, LLD (1834 – 1914)
German evolutionary biologist and
Father of the Evolutionary Theory of Aging



Unknown author - Edwin G. Conklin, "August Weismann"
Proceedings of the American Philosophical Society,
Vol. 54, No. 220. (Oct. - Dec., 1915), pp. iii-xii.

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Preface

Around 300,000 generations ago, the human species split from an ancient ancestor that we share with chimpanzees. Since then, human life expectancy at birth has doubled and doubled again over the last 200 years. Further, according to the World Health Organization (WHO), between the years 2000 and 2050, the proportion of the world's population over 60 years of age is anticipated to double from about 11% to 22%. This trend begs the question of why aging occurs? That question goes back much further than the theory proposed by Weismann in the 1800s. For example, Shakespeare addressed it in his "seven ages of man" and religious texts have done so for millennia. In ancient times, people believed that just as a machine will begin to deteriorate after a certain number of uses, the human body likewise deteriorates in direct proportion to its use. But, the body is not a machine! The modern version of this theory recognizes that the number of heartbeats does not predict lifespan. Instead, researchers have focused on the speed at which an organism processes oxygen. There is indeed some evidence, when comparing species, that creatures with faster oxygen metabolisms die younger and those with lower metabolisms have longer lifespans.

But, what is aging? Simply stated, it is the slow decline of function over time considered to be an inevitable fact of the human condition. As cells divide and reproduce in our bodies, they gradually deteriorate and, as a result, our mental and physical health decline. Recent scientific data show that longevity is associated with the successful management of chronic diseases, not the absence of any disease! The process of aging and its rate are still not fully understood and continue to baffle the medical and scientific communities. Various theories (more than 30 of them) have therefore been posited in, thus far, unsuccessful attempts to uncover the primary cause(s) of aging. They

involve many interdependent and interconnecting genetic, biochemical, and physiological processes. However, they all relate to signs, symptoms, and risk factors that do not reach to the root cause(s) of aging. Let us pause and remember that signs, symptoms, and risk factors are not causation … merely indications of a process. Nonetheless, as their understanding grows, the resulting findings could lead to better health, less disability, greater independence in later life and, potentially, longer lifespans. But, no matter which theory(ies) of aging will turn out to be correct or if, instead, aging may be the partial or total sum of a number of them, the true bottom line is that aging is universal. Fortunately, certain lifestyle factors and predispositions may defer one's demise to a degree and, at the very least, offer a better quality to the quantity of a lifespan.

In this volume, I analyze what is currently known about the aging process including most of the theories of aging advanced so far. I also address the issue of what can be done about aging, covering such aspects as anti-aging behaviors, discussing therapies for retarding or reversing aging, and offering guidelines for healthy aging. In addition, I answer some frequently asked questions, outline some latest developments and research in aging, and summarize resources available.

A good understanding of aging and the associated human diseases cannot be had without a serious preliminary grounding in the basic sciences. For this reason, I provide primers on Biology; Evolution; Genetics; Epigenetics; Ecogenetics; and Stem cells. Other useful primers consisting of Toxicology and Toxicogenomics; Pharmacogenetics and Pharmacogenomics; Epidemiology; and Paleopathology would also be useful but may go far beyond the scope of this volume. (I cover these other topics in a separate antology titled: "The Odyssey of Humanity's Diseases: Epigenetic and Ecogenetic Modulations from Ancestry Through Inheritance, Environment, Culture, and Behavior", Volumes 1, 2, and 3), In addition, for those interested readers who are eager to go beyond the main text of the several chapters herein, I also provide numerous sidebars and all-embracing selected references. Mindful of the

very extensive available literature on the subject of aging, I have classified such references by topic for convenient use.

Notwithstanding the gloomy and concerning aspect of aging for many, I nonetheless hope that the reader may derive some of the great satisfaction I myself gained in writing about it.

PART A

ONCE UPON A TIME

Introduction to Part A

This Part A includes two chapters. **Chapter 1** explores the essence of aging. While consciousness and complex thinking have gradually emerged more than 100,000 years ago in Africa, our captivation with aging dates essentially to the last 200 years. This occurred especially as we entered an era where most individuals have to face with great concern the prospects of watching their bodies, their minds, and their cognition slowly decline with time - robbing them of their very humanity by chronic and neurodegenerative diseases.

August Weismann, the German evolutionary biologist, is considered the father of the Evolutionary Theory of Aging**.** He advanced the idea that aging is a beneficial trait that evolved to cleanse the population of old worn-out individuals. However, being keenly aware that Charles Darwin's natural selection can only work when a phenotype is relevant to fitness, he also realized that aging by itself is unlikely to have an advantage, which contradicts his earlier idea of a beneficial cleansing mechanism. On the other hand, Peter Medawar argued that aging, at least in sexually reproducing organisms with a difference between the soma and the germ line, is a result of the declining force of natural selection with age. He also proposed that aging was the necessary result of constitutional mutations, accumulated in the germ line over evolutionary time, reducing fitness late in life. However, these concepts by themselves are not sufficient to explain aging.

Several views of aging developed and will be briefly summarized at first, pending more elaborated discussions in subsequent chapters. In the "teleological view" (which, actually, never went away), aging was seen as something that befell humans but spared the gods. Modern scientists, of course, require something more than a divine whim! In the "evolutionary

view", aging is a natural outcome of evolution. However, some isolated cases seemingly contradict this view and can almost always be logically explained without the need of creation or intelligent design. In the "genetically-programmed view", aging is a process genetically programmed in our DNA in which each of us has a biological clock set to go off at a particular time to signal our bodies first to age and then to die. Still, it is very difficult to accept that the elegant series of developmental switches and checkpoints that create an organism is so crudely interrupted by a seemingly random, aimless process that leads to our demise.

However, experimental evidence rests firmly on the side of a non-programmed view, with the caveat that it still may be feasible and even simpler than we would have guessed to forestall aging and the chronic diseases that aging enables. It is relatively easy to think of aging as a genetically-modulated process that can support both programmed and non-programmed aspects. Proponents of programmed aging often argue that the process of organismal degeneration and death has all the hallmarks of evolved adaptation.

Continuing research is likely to change the way we think about aging, perhaps showing us that it may still be feasible, and perhaps even easier than we would have guessed, to forestall aging and the accompanying chronic and neurodegenerative diseases that aging enables. There are several ways to intervene in the aging process and extend the lifespan of the organism, including "dietary restriction" or/and many genetic and pharmacologic interventions that delay aging, whether it is programmed or not.

Human beings and members of other species, especially animals, age and die. In contrast, many species can be considered potentially immortal. Even within humans and other mortal species, having lost the ability to die, cancer cells have the potential for immortality.

Chapter 2 is dedicated to the life and works of August Weismann. His main contribution involved the "germ plasm theory", according to which inheritance (in a multicellular animal) only takes place by means of the germ cells—the gametes such as egg cells and sperm cells. Other cells of the body—somatic cells—do not function as agents of heredity. The hereditary material, the germ plasm, is transmitted only by the gonads. Somatic cells (of the body)

develop afresh in each generation from the germ plasm. The effect is that germ cells produce somatic cells and are not affected by anything the somatic cells learn or, therefore, any ability an individual acquires during its life. Genetic information cannot pass from soma to germ plasm and on to the next generation. Biologists refer to this concept as the *Weismann's barrier*. If true, this idea, rules out the inheritance of acquired characteristics as proposed by Jean-Baptiste Lamarck. However, Weismann's later views became more nuanced, insisting, like Darwin, that a variable environment was necessary to cause variation in the hereditary material.

The idea of the Weismann's barrier is central to the modern synthesis of the early 20th century. It preceded the rediscovery of Gregor Mendel's work. Weismann is much admired today. Fellow German Ernst Mayr ranked him as the second most notable evolutionary theorist of the 19th century after Charles Darwin, the most important evolutionary thinker between Darwin and the evolutionary synthesis around 1930–1940, and "one of the great biologists of all time."

1

The essence of aging

Contents

1

The essence of aging

"Only the Gods can never age and can never die.
All else in the world, almighty Time obliterates, crushes all to nothing."
(Oedipus to Theseus. Sophocles, Oedipus at Colonus)

Consciousness and complex thinking have gradually emerged more than 100,000 years ago in Africa, most likely gripping our distant ancestors. Closer to us and, more particularly, for the last 200 years, humanity has been enthralled with aging, especially as we entered an era where most individuals have to face with great concern the prospects of watching their bodies, their minds, and their cognition slowly decline with time - robbing them of their very humanity by chronic and neurodegenerative diseases. Since the majority of all deaths in developed countries are caused by highly age-related diseases (such as cancer, stroke, and cardiovascular diseases), it is essential to understand aging and senescence to devise ways to prevent or treat these diseases. This is exacerbated by the fact that in most countries of the world, elders are the most rapidly growing segment of the population so that, in the not too distant future, up to 20% of the global population will be over age sixty, defining the 21st century demographics. It is also important therefore to prevent and treat those age-related diseases through a better understanding of the aging and senescence process.

As an introduction, let us ponder why aging exists and initially peruse through some of the advanced views on aging.

Why aging exists?

August Weismann is best known for his germ plasm theory in which, for the first time, he distinguished a germ line from the soma. He also originated the idea that aging is a beneficial trait, evolved to cleanse the population of old worn-out individuals. These early ideas set the stage for the later realization that the chance of individuals to contribute to the future ancestry of their population declines with age. However, Weismann later rejected some of his own earlier positions, likely including his adaptive theory of aging, as he was keenly aware that natural selection can only work when a phenotype is relevant to fitness. Further, he also realized that aging by itself is unlikely to have an advantage, which contradicts his earlier idea of a beneficial cleansing mechanism.

Nearly forty years after Weismann's death, Medawar argued that aging, at least in sexually reproducing organisms with a difference between the soma and the germ line, is a result of the declining force of natural selection with age. He also proposed that aging was the necessary result of constitutional mutations, accumulated in the germ line over evolutionary time, that reduce fitness late in life. However, Medawar's concepts by themselves are not sufficient to explain aging. Why do genetic variants with adverse effects late in life emerge, leading to symptoms of senescence at ages frequently reached (an illustration of "antagonistic pleiotropy", i.e., when the same gene variant controls a phenotypic trait with beneficial effects at an early age and adverse effects later.)

The teleological view

Aging must have fascinated humans almost immediately thereafter. Naturally, the first recorded attempts at explanation were entirely in terms of religion. For the ancients, the teleological component of aging was always clear: To keep humans in their place, aging was seen as something that befell humans but spared the gods. Possibly because of this religious trigger to

immortality, aging came to be seen as an active program of decay that could be prevented provided one could discover the correct way to do it. This point of view never went away! Modern scientists, of course, require something more than a divine whim!

The evolutionary view

Experimental evidence supporting the "evolutionary theory of aging" has been obtained for several animal species, including animals in the wild. It is often pointed out, especially by proponents of programmed aging (see below), that some isolated cases seemingly contradict the evolutionary theory of aging. But, even these exceptional cases can almost always be explained within the boundaries of evolutionary logic and are always logically explained without the need of creation or intelligent design - two alternatives that are neither logical nor supported by a similar mountain of scientific evidence as evolution theory.

The genetically-programmed view

In this perspective, aging is viewed as a process genetically programmed in our DNA. That is, genetic information in the organism does not only specify its development but also its demise. The process is supposed to have emerged during evolution as a series of germ line mutations that were selected on the basis of a particular gain in fitness. Accordingly, each of us has a biological clock set to go off at a particular time to signal our bodies first to age and then to die. However, it is very difficult to accept that the elegant series of developmental switches and checkpoints that create an organism is so crudely interrupted by a seemingly random, aimless process that leads to our demise. Yet, a majority of experts in the science of aging believe that this is exactly what is happening - a genetically programmed series of events that increases fitness of the individual.

The logic of programmed aging appeals especially to molecular biologists whose science is imbued with signaling pathways and genetically-controlled functional networks and for whom aging could only become a topic of study when explained as a series of signaling steps that bring life to a close.

Moreover, recent results with model organisms have provided evidence that aging can be affected by manipulating single genes or through the administration of single drugs - greatly strengthening deterministic positions.

Programmed aging has also been considered in individual-based models with competition between parents and progeny. Yang presented a model according to which aging is selected to benefit the group in viscous populations, i.e., populations in which offsprings stay around rather than dispersing. The benefit of aging in this model is to promote survival of genetically fitter young progenies who would suffer competition from their parents who had already acquired improved abilities with age. Hence, this basically goes back to the original Weismann's hypothesis, but this time based on the benefit of capturing inherited, superior abilities in the progeny rather than the elimination of individuals already damaged by wear-and-tear to reduce the burden to the group. Yet another model, also based on competition between parents and progeny, is from Martins, who proposed that aging serves as a pruning mechanism to get rid of older individuals harboring less well optimized genotypes who managed to survive by chance.

In summary, programmed aging theories provide dubious theoretical arguments as to how a process of organismal deterioration and death could have emerged during evolution. None can boast of some serious experimental support. In addition, they all suffer from the fact that aging is a gradual process, without a critical age or threshold when the hypothetical mechanism would kick in to abruptly increase death rate. There is in fact experimental evidence that contradicts programmed aging. Indeed, it has now been established that in multiple species, possibly including humans, death rate at extreme old age starts to slow down rather than exponentially increase further as one would expect if aging was programmed. Hence, the conclusion must be that the case for programmed aging is a weak one at best.

However, aging is not programmed based on adaptive evolutionary change because evolution optimizes for fitness, not for longevity. Indeed, as it emerged, diversified and perpetuated over almost four billion years, life has no vested interest in healthy aging or immortality, but merely in reproduction.

The genetically-modulated view

It is relatively easy to think of aging as a genetically-modulated process that can support both programmed and non-programmed aspects. In this context, we can find species having different reproductive strategies and corresponding lifespans that undergo programmed aging (see Sidebar 1.1 on semelparous and iteroparous life cycles). Death following reproduction has been documented in various species from a wide diversity of taxa, across bacteria, plants, and almost all animal classes.

There are multiple explanations for a semelparous life style, all based on logical evolutionary reasoning. Natural selection should favor physiological processes that enable individuals to maximize offspring. In species with a low probability of reproducing more than once (because of a brief life span or due to long migration such as for certain eels or in different species of salmon), selection should favor an extreme mobilization of the resources for reproduction in order to maximize offspring. The rapid decline and death of such individuals would be merely a consequence of their intense reproductive effort. Whether or not this is truly "accelerated aging" is debatable, but there is no reason to assume that the process is itself a selectable trait offering a competitive advantage to the individual and/or its offspring.

In iteroparity, adult members of a species reproduce repeatedly during life, often with great variation in breeding schedule. Intermittent reproduction is associated with organisms, such as humans, with fairly long life spans living in seasonal environments that can vary drastically over time. Still, not maximally using the first breeding opportunity and spreading out reproduction over time, is evolutionarily intriguing because of the risk of not surviving.

While there can be no doubt that in semelparous organisms, specific, genetically-controlled processes bring life to a close shortly after reproduction, the existence of a similar mechanism is much less clear for iteroparous organisms. There are two key problems here. First, the process takes a long time and it remains unclear why evolution could not come up with a cleaner, more rapid process to end life (as it evidently did with semelparous organisms). To drag it on like this seems to serve no purpose. Second, aging

as a selectable trait is difficult to act on by natural selection because the force of natural selection significantly weakens with age.

The adaptive trait view

Proponents of programmed aging often argue that the process of organismal degeneration and death has all the hallmarks of evolved adaptation. It is controlled by genes that have often been conserved across extensive phylogenies and shows pathophysiological changes that are often very similar from species to species. While this is not in conflict with non-adaptive explanations for aging, it is true that, at first glance, it seems more compatible with programmed aging. The first and easiest way to explain aging as an adaptive trait is to invoke group selection, in this case meaning that aging of the individual occurs for the benefit of the group, which shares genetic alleles.

As we have already seen, Weismann was the first to propose that aging evolved to get rid of weak, worn-out individuals to preserve resources for the healthy young who still need to reproduce. There are two problems with this: (a) As noticed almost immediately by Weismann himself, this hypothesis seeks to explain the problem of aging by aging itself, an obvious example of circular reasoning; and (b) the controversy about group selection since the object of natural selection is first and foremost the individual. However, as recognized by Ernst Mayr, this does not rule out group selection, i.e., when there is a relationship between the fitness of an individual and the properties of the group. Indeed, one could imagine that certain characteristics, such as the emergence of sentinels to warn for predators, could be subject to group selection because the fitness of individuals belonging to such a group may be higher than that of individuals from non-sentinel groups. Nonetheless, it is difficult to find such an advantage for altruistic aging.

Mitteldorf rather proposed that a major target of natural selection at the group level is demographic homeostasis. As he argued, aging could have evolved based on its contribution to stabilizing population dynamics, helping prevent population growth overshoot. Later, he also proposed a group benefit of senescence in limiting the spread of infectious epidemics through the regulation of population dynamics. This makes sense because overpopulation

often results in famine or epidemic disease, which could wipe out the entire population. Aging, then, could have evolved as a means for the group or even species to control its death rate. While the problem remains that the process simply takes too long to be of any use, especially in wild populations where most members of a species die of age-extrinsic causes, it is difficult to see why young adults are not at least equally well-suited as targets in this model.

Genetic determinants of aging

A number of genetic components of aging have been identified using model organisms, ranging from the simple budding yeast *Saccharomyces cerevisiae* to worms such as *Caenorhabditis elegans* and fruit flies (*Drosophila melanogaster*). Study of these organisms has revealed the presence of at least two conserved aging pathways.

Gene expression is imperfectly controlled, and it is possible that random fluctuations in the expression levels of many genes contribute to the aging process as suggested by a study of such genes in yeast. Individual cells, which are genetically identical, nonetheless can have substantially different responses to outside stimuli, and markedly different lifespans, indicating that epigenetic factors play an important role in gene expression and aging as well as genetic factors.

The ability to repair DNA double-strand breaks declines with aging in mice and humans. A set of rare hereditary genetic disorders, each called progeria, has been known for some time. Progeroid syndromes are a group of diseases that cause individuals to age faster than usual, leading to them appearing older than they actually are. Patients born with progeria typically live to an age of mid-teens to early twenties. Progeria is a specific type of progeroid syndrome, also known as Hutchinson–Gilford syndrome (or Hutchinson–Gilford progeroid syndrome, HGPS). There, a single gene mutation is responsible for causing progeria. The gene, known as lamin A (LMNA), makes a protein necessary for holding the nucleus of the cell together. When this gene gets mutated, an abnormal form of lamin A protein called progerin is produced. Sufferers exhibit symptoms resembling accelerated aging, including wrinkled skin. The cause of HGPS was reported in the journal *Nature* in May 2003,

suggesting that DNA damage, not oxidative stress, is the cause of this form of accelerated aging.

Another study also indicated that aging may shift activity toward short genes or shorter transcript length and that this can be countered by interventions.

Extending the organism's lifespan

Let us now revisit the peculiar finding that it is relatively easy, at least in animal models, to intervene in the aging process and extend the lifespan of the organism. This seems paradoxical if aging really is the effect of a decline in natural selection and, by extension, likely a highly variable and multi-factorial process. Put another way, if aging is caused by the decline in function of many different processes, it would seem difficult to alter the process by one genetic mutation. Yet, it is irrefutable that this is possible. In fact, reduced or ablated expression of hundreds of individual genes (up to 5% of the respective gene sets) lead to lifespan extension in worms and yeast, and similar observations have been made in flies and mice based on more limited studies to date.

One way to resolve this apparent conflict is to propose that while aging is not adaptive, species come pre-equipped with programs that can be turned on to delay aging. More accurately, they can be turned on for other naturally selected reasons but, when activated, delay the aging process. The best example would be "dietary restriction", a reduced calorie intake without malnutrition that has been demonstrated in many laboratories to significantly increase life span. A reduction in available nutrients converts species from an unabated focus on reproduction, to allocation of resources toward long-term survival, presumably until resources become once again abundant. This re-allocation of resources, which leads to activation of stress resistance and turnover of damaged molecules in cells, may be just the ticket to forestall many features of aging and extend lifespan. Indeed, many genetic and pharmacologic interventions that delay aging are proposed to phenocopy dietary restriction.

From a more philosophical perspective, slowing aging as a means of extending the healthy period of life seems feasible whether aging is programmed or

not. If one takes the programmed view, interventions should be sought that disrupt the program, thus avoiding aging. But, extending lifespan may be just as easy from the non-programmed perspective. In this case, the most likely strategy would be to find interventions that enhance programs selected to promote health during early adulthood; in other words, improving the function of pro-health pathways rather than disrupting pro-aging ones. This may be less difficult than it seems. Evolution has had billions of years to optimize fitness in species, but at older ages, when the force of natural selection has greatly declined, it may be relatively easy to tweak existing pathways to prolong their normative function and delay aging. This is perhaps consistent with findings that a surprisingly large number of genetic mutations enhance organismal lifespan.

Of course, many of these lifespan-extending interventions may have deleterious age-extrinsic consequences on important aspects of fitness, making them undesirable, particularly outside the laboratory. Nevertheless, it seems from the current perspective that while aging is not likely programmed, it will still be possible to target aging as a means of extending human lifespan and, more importantly, prevent the onset of a wide spectrum of chronic diseases that are increasingly plaguing humanity.

Aging versus immortality

Human beings and members of other species, especially animals, age and die. Fungi, too, can age. In contrast, many species can be considered potentially immortal: For example, bacteria fission to produce daughter cells, strawberry plants grow runners to produce clones of themselves, and animals in the genus Hydra have a regenerative ability by which they avoid dying of old age.

Early life forms on Earth, starting at least 3.7 billion years ago, were single-celled organisms. Such organisms (prokaryotes, protozoans, algae) multiply by fission into daughter cells, thus, do not age and are potentially immortal under favorable conditions.

Aging and mortality of the individual organism became possible with the evolution of sexual reproduction, which occurred with the emergence of the

fungal/animal kingdoms approximately a billion years ago, and the evolution of seed-producing plants 320 million years ago. The sexual organism could henceforth pass on some of its genetic material to produce new individuals and could itself become disposable with respect to the survival of its species. This classic biological idea has, however, been perturbed recently by the discovery that the bacterium *E. coli* may split into distinguishable daughter cells, which opens the theoretical possibility of "age classes" among bacteria.

In artificial cloning, adult cells can be rejuvenated to embryonic status and then used to grow a new tissue or animal without aging. Normal human cells however die after about 50 cell divisions in laboratory culture (the so-called Hayflick's limit).

On stem cells and cancer

The subject of stem cells is treated in a primer to this subject in Chapter 11. Here, regarding stem cells and cancer, the following preliminary observations will suffice. Even within humans and other mortal species, there are cells with the potential for immortality. Examples include cancer cells which have lost the ability to die when maintained in a cell culture (such as the HeLa cell line) and specific stem cells such as germ cells (which produce ova and spermatozoa).

Stem cells are cells in the beginning stages of growth. They are found mostly in the bone marrow and, to a lesser extent, in the blood. All stem cells begin life in the same way. Then, they mature into different types of blood cells. Young, immature stem cells are also called 'hematopoietic' (or blood-forming) stem cells. In the bone marrow, they divide, forming new cells for the body. During the process of blood cell maturity, the cells eventually form into white or red blood cells. The mature cells travel into the blood to perform the function they are meant to do in the body, but a small number of the immature stem cells (called 'peripheral' stem cells) are also released into the blood.

Stem cell transplants are used to treat some types of cancer, particularly those of the blood or immune system such as leukemia, multiple myeloma,

or lymphoma. The procedure involves harvesting (taking) healthy stem cells from bone marrow, blood, or cord blood (from a newborn).

Types of stem cell transplants

There are primarily two different types of stem cell transplant procedures.

- 'Autologous' stem cell transplants involve using a patient's own stem cells, taken from their blood, that are then given back after cancer treatment.
- 'Allogenic' stem cell transplants involve harvesting stem cells from a donor, then giving the cells to the recipient via an IV transfusion. The donor can be a family member or a non-related person from donor organizations such as the National Marrow Donor Program (NMDP).

Sub-types

The specific sub-type of stem cell therapy depends on where the cells are harvested:

- The bone marrow: A bone marrow transplant.
- The blood: Peripheral blood stem cell transplant.
- Cord blood: Cord blood transplant.

During cancer treatment, bone marrow is damaged, either by cancer itself or by the chemotherapy or radiation treatment. Stem cell therapy is a way to replenish the bone marrow with healthy stem cells.

Conclusions and take-aways

- Consciousness and complex thinking have gradually emerged more than 100,000 years ago in Africa, most likely fascinating our distant ancestors.
- For the last 200 years, humanity has been captivated with aging, especially as we entered an era where most individuals have to face with great concern the prospects of watching their bodies, their minds,

and their cognition slowly decline with time - robbing them of their very humanity by chronic and neurodegenerative diseases.

- Weismann originated the germ plasm theory in which he distinguished a germ line from the soma. He also advanced the idea that aging is a beneficial trait, evolved to cleanse the population of old worn-out individuals. Nonetheless, he may have rejected these ideas as he was keenly aware that natural selection can only work when a phenotype is relevant to fitness. Further, he also realized that aging by itself is unlikely to have an advantage, which contradicts his earlier idea of a beneficial cleansing mechanism.
- Medawar argued that aging, at least in sexually reproducing organisms with a difference between the soma and the germ line, is a result of the declining force of natural selection with age. He also proposed that aging was the necessary result of constitutional mutations, accumulated in the germ line over evolutionary time, that reduce fitness late in life. However, these concepts by themselves are not sufficient to explain aging.
- In the teleological view (that never went away), aging was seen as something that befell humans but spared the gods. Modern scientists, of course, require something more than a divine whim!
- In the evolutionary view, aging is a natural outcome of evolution. However, some isolated cases seemingly contradict this view and can almost always be logically explained without the need of creation or intelligent design.
- In the genetically-programmed view, aging is a process genetically programmed in our DNA - a genetically programmed series of events that increases fitness of the individual. Accordingly, each of us has a biological clock set to go off at a particular time to signal our bodies first to age and then to die. However, it is very difficult to accept that the elegant series of developmental switches and checkpoints that creates an organism is so crudely interrupted by a seemingly random, aimless process that leads to our demise.
- Recent results with model organisms have provided evidence that aging can be affected by manipulating single genes or through the administration of single drugs - greatly strengthening deterministic positions.

- Programmed aging has also been considered in individual-based models with competition between parents and progeny in which aging is selected to benefit the group in viscous populations, i.e., populations in which offspring stay around rather than dispersing or in which aging serves as a pruning mechanism to get rid of older individuals harboring less well optimized genotypes who managed to survive by chance.
- Experimental evidence rests firmly on the side of a non-programmed view, with the caveat that it still may be feasible and even easier than we would have guessed to forestall aging and the chronic diseases that aging enables.
- It is relatively easy to think of aging as a genetically-modulated process that can support both programmed and non-programmed aspects. While there can be no doubt that in semelparous organisms, specific, genetically-controlled processes bring life to a close shortly after reproduction, the existence of a similar mechanism is much less clear for iteroparous organisms.
- Proponents of programmed aging often argue that the process of organismal degeneration and death has all the hallmarks of evolved adaptation. The first and easiest way to explain aging as an adaptive trait is to invoke group selection.
- Continuing research is likely to change the way we think about aging, perhaps showing us that it may still be feasible, and perhaps even easier than we would have guessed, to forestall aging and the accompanying chronic and neurodegenerative diseases that aging enables.
- It is relatively easy, at least in animal models, to intervene in the aging process and extend the lifespan of the organism. While aging is not adaptive, species come pre-equipped with programs that can be turned on to delay aging. More accurately, they can be turned on for other naturally selected reasons but, when activated, delay the aging process. The best example would be "dietary restriction" that has been demonstrated in many laboratories to significantly increase life span. Many genetic and pharmacologic interventions that delay aging are proposed to phenocopy dietary restriction.
- Slowing aging as a means of extending the healthy period of life seems feasible whether aging is programmed or not.

- Many of the lifespan-extending interventions may have deleterious age-extrinsic consequences on important aspects of fitness, making them undesirable particularly outside the laboratory. While aging is not likely programmed, it will still be possible to target aging as a means of extending human lifespan and, more importantly, prevent the onset of a wide spectrum of chronic diseases that are increasingly plaguing humanity.
- Human beings and members of other species, especially animals, age and die. In contrast, many species can be considered potentially immortal, for example, bacterial fission produces daughter cells, strawberry plants grow runners to produce clones of themselves, and animals in the genus Hydra have a regenerative ability by which they avoid dying of old age.
- Early life forms on Earth, starting at least 3.7 billion years ago, were single-celled organisms, which multiply by fission into daughter cells and, thus, do not age and are potentially immortal under favorable conditions.
- Aging and mortality of the individual organism became possible with the evolution of sexual reproduction wherein the sexual organism could henceforth pass on some of its genetic material to produce new individuals and could itself become disposable with respect to the survival of its species.
- Even within humans and other mortal species, there are cells with the potential for immortality: cancer cells which have lost the ability to die when maintained in a cell culture (such as the HeLa cell line), and specific stem cells such as germ cells (which produce ova and spermatozoa). In artificial cloning, adult cells can be rejuvenated to embryonic status and, then, used to grow a new tissue or animal without aging.
- Normal human cells die after about 50 cell divisions in laboratory culture (the so-called Hayflick's limit).
- Stem cells are cells in the beginning stages of growth. They are found mostly in the bone marrow and, to a lesser extent, in the blood. All stem cells begin life in the same way; then, they mature into different types of blood cells. Young, immature stem cells (also called

'hematopoietic' or blood-forming stem cells) form in the bone marrow, divide, and form new cells for the body.

- Stem cell transplants are used to treat some types of cancer, particularly those of the blood or immune system such as leukemia, multiple myeloma, or lymphoma. The procedure involves harvesting (taking) healthy stem cells from bone marrow, blood, or cord blood (from a newborn).
- Sidebar 1.1 treats the subjects of semelparity and iteroparity, which are are two contrasting reproductive strategies available to living organisms. Sidebar 1.2 outlines AnAge, a curated database of animal aging and longevity.

Sidebar 1.1 – Semelparity and iteroparity

Semelparity (or monocarpy or 'big bang' reproduction) and iteroparity (or polycarpy) are two contrasting reproductive strategies available to living organisms. A species is considered semelparous if it is characterized by a single reproductive episode before death, and iteroparous if it is characterized by multiple reproductive cycles over the course of its lifetime. Iteroparity can be further divided into 'continuous iteroparity' (primates including humans and chimpanzees) and 'seasonal iteroparity' (birds, dogs, etc.). In semelparous species, death after reproduction is part of an overall strategy that includes putting all available resources into maximizing reproduction, at the expense of future life. In any iteroparous population, there will be some individuals who die between their first and second reproductive episodes. Semelparity and iteroparity are not alternative strategies, but extremes along a continuum of possible modes of reproduction. The above distinction is also related to the difference between annual and perennial plants. An annual is a plant that completes its life cycle in a single season, and is usually semelparous. Perennials live for more than one season and are usually (but not always) iteroparous.

Semelparity

A classic example of a semelparous organism is the Pacific salmon (*Oncorhynchus spp.*), which lives for many years in the ocean before

swimming to the freshwater stream of its birth, spawning, and dying. Other semelparous animals include many insects, including some species of butterflies, cicadas, and mayflies, many arachnoids, and some mollusks such as some species of squid and octopus. Semelparity also occurs in smelt and capelin, but is very rare in vertebrates other than bony fish. In amphibians, it is known only among some Hyla frogs including the gladiator frog. In reptiles, it is known in only a few lizards such as the Labord's chameleon of southwestern Madagascar, Sceloporus bicanthalis of the high mountains of Mexico, and some species of Ichnotropis from dry savanna areas of Africa. Among mammals, it exists only in a few didelphid and dasyurid marsupials. Annual plants, including all grain crops and most domestic vegetables, are semelparous. Long-lived semelparous plants include century plant (agave), Lobelia telekii, and some species of bamboo.

This form of lifestyle is consistent with r-selected strategies as many offspring are produced and there is low parental input, as one or both parents die after mating. All of the male's energy is diverted into mating with repression of the immune system. High levels of corticosteroids are sustained over long periods of time. This triggers immune and inflammatory system failure and gastrointestinal hemorrhage, which eventually leads to death.

Iteroparity

An example of an iteroparous organism is a human—humans are biologically capable of having offsprings many times over the course of their lives. Iteroparous vertebrates include all birds, most reptiles, virtually all mammals, and most fish. Among invertebrates, most mollusks and many insects (for example, mosquitoes and cockroaches) are iteroparous. Most perennial plants are iteroparous.

Sidebar 1.2 – Database of animal aging and longevity

AnAge is a curated database of aging and life history in animals, including extensive longevity records. It was primarily developed for comparative biology studies, particularly studies of longevity and aging but it can also be useful for ecological and conservation studies and as a reference for zoos

and field biologists. It is made freely available to everyone under the terms and conditions described in HAGR's license.

Database entries

The database can be searched using keywords or phrases, or else terms at a taxonomic level, relating to the species or common name of the organism of interest. The search can be conducted for multiple species. Other tools and datasets are also available, including statistics, literature search, etc. Of special interest to research on aging are the few animals that appear not to age. The raw data in the database can be downloaded.

Component databases

The following component databases are available:

- Gene Database (GenAge)
- Species Database (AnAge)
- Dietary Restriction Database (GenDR)
- Longevity Variants Database (LongevityMap)
- Cell Senescence Database (CellAge)
- Drugs Database (DrugAge)
- External Databases
- Digital Aging Atlas
- LibAge (beta version)
- Gene Expression
- Cancer
- Genome Sequencing
- Evolution of Aging
- Senescence.info
- Genomics of Aging and Rejuvenation.

2

August Weismann ... the father of the evolutionary theory of aging

Contents

2

August Weismann . . . the father of the evolutionary theory of aging

Early Years

August Friedrich Leopold Weismann FRS, HonFRSE, LLD was born on 17 January 1834 in Frankfurt am Main (Figure 2.1), the son of high school teacher Johann (Jean) Konrad Weismann (1804–1880) and his wife Elise (1803–1850), née Lübbren. His father was a graduate of ancient languages and theology and his mother was the daughter of the county councillor and mayor of Stade. He had a typical 19th century bourgeois education, receiving music lessons from the age of four, and drafting and painting lessons from Jakob Becker (1810–1872) at the Frankfurter Städelsche Institut from the age of 14. His piano teacher was a devoted butterfly collector who introduced him to the collecting of imagos and caterpillars. But, studying the natural sciences was out of the question due to the cost involved and the limited job prospects. A friend of the family, chemist Friedrich Wöhler (1800–1882), recommended studying medicine. A foundation from the inheritance of

Weismann's mother allowed him to take up studies in Göttingen. Following his graduation in 1856 (at the early age of 22), he wrote his dissertation on the synthesis of hippuric acid in the human body. In 1867, he married Mary Dorothea Gruber from whom he got a son, Julius Weismann (1879–1950), a composer.

Early scientific career

Immediately after university, Weismann took on a post as assistant at the Städtische Klinik (city clinic) in Rostock. He successfully submitted two manuscripts, one about hippuric acid in herbivores and one about the salt content of the Baltic Sea, which won him two prizes. The paper about the salt content dissuaded him from becoming a chemist, since he felt himself lacking in apothecarial accuracy.

After a study visit to see Vienna's museums and clinics, he visited Italy (in 1859) and Paris (in 1860). He returned to Frankfurt as personal physician to the banished Archduke Stephen of Austria at Schaumburg Castle from 1861 to 1863. During the war between Austria, France, and Italy in 1859, he became Chief Medical Officer in the military and, on a leave from duty, he walked through Northern Italy and the County of Tyrol. After a sabbatical in Paris, he worked with Rudolf Leuckart (1822-1898) at the University of Gießen. He graduated as a physician and settled in Frankfurt with a medical practice in 1868.

From 1863, he was privatdozent in comparative anatomy and zoology, extraordinary professor from 1866, and full professor from 1873 to 1912. He became the first holder of the Chair in Zoology and Director of the Zoological Institute at Albert Ludwig University of Freiburg in Breisgau (Figure 2.2). He retired in 1912.

Figure 2.1 - City Hall of Frankfurt, Germany

Early works

Weismann's earlier work was largely concerned with purely zoological investigations, one of his earliest works dealing with the development of the Diptera. Microscopical work, however, became impossible for him owing to his impaired eyesight and he, therefore, turned his attention to wider problems of biological inquiry.

Figure 2.2 – The University of Freiburg, Germany

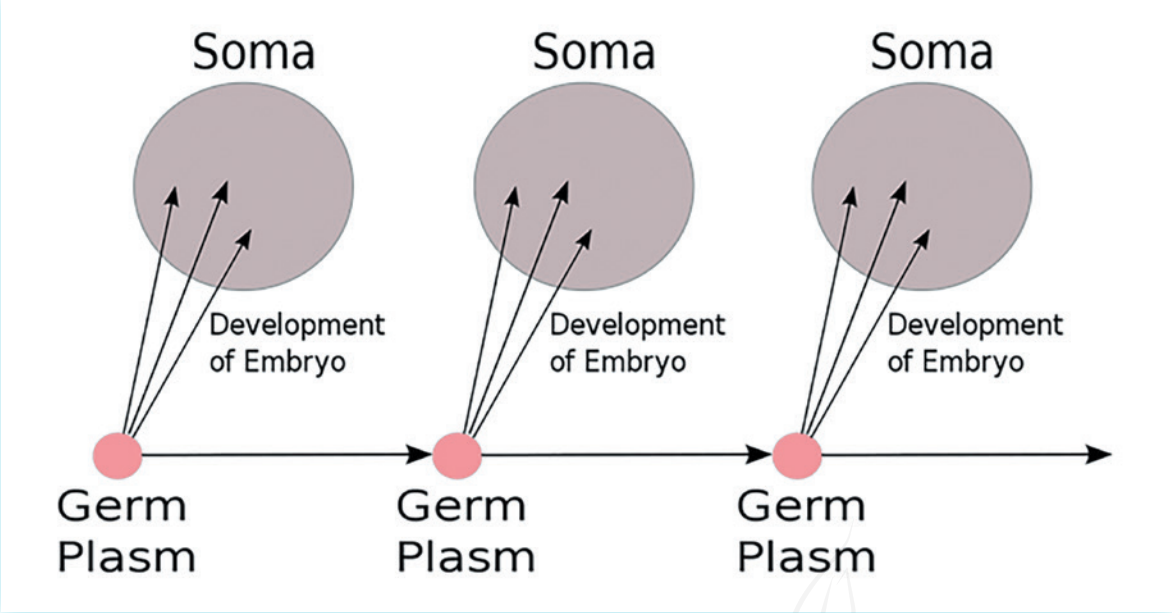
Deutschland, Baden-Württemberg, Freiburg im Breisgau, Institut für Biologie II + III, Schänzlestr.

Source: Kamahele

Main scientific contributions

Weismann was a German evolutionary biologist. His main contribution involved the "germ plasm theory" (se Figure 2.3), at one time also known as "Weismannism", according to which inheritance (in a multicellular animal) only takes place by means of the germ cells—the gametes such as egg cells and sperm cells. Other cells of the body—somatic cells—do not function as agents of heredity. The hereditary material, the germ plasm, is transmitted only by the gonads. Somatic cells (of the body) develop afresh in each generation from the germ plasm.

Figure 2.3 - Weismann's germ plasm theory



Reference: Ian Alexander

The effect is that germ cells produce somatic cells and are not affected by anything the somatic cells learn or, therefore, any ability an individual acquires during its life. Genetic information cannot pass from soma to germ plasm and on to the next generation. Biologists refer to this concept as the "Weismann's barrier". If true, this idea, rules out the inheritance of acquired characteristics as proposed by Jean-Baptiste Lamarck (1744-1829). Weismann became one of the first biologists to deny Lamarckism entirely. However, a careful reading of Weismann's work over the span of his entire career shows that he had more nuanced views, insisting, like Darwin, that a variable environment was necessary to cause variation in the hereditary material.

The idea of the Weismann's barrier is central to the modern synthesis of the early 20th century, though scholars do not express it today in the same terms. In Weismann's opinion the largely random process of mutation, which must occur in the gametes (or stem cells that make them) is the only source of change for natural selection to work on. Weismann's ideas preceded the rediscovery of Gregor Mendel (1822-1884)'s work, and though Weismann

was cagey about accepting Mendelism, younger workers soon made the connection.

Weismann is much admired today. Fellow German Ernst Mayr (1904-2005) ranked him as the second most notable evolutionary theorist of the 19th century after Charles Darwin (1809-1882), the most important evolutionary thinker between Darwin and the evolutionary synthesis around 1930–1940, and "one of the great biologists of all time".

Contributions to evolutionary biology

At the beginning of Weismann's preoccupation with evolutionary theory was his grappling with Christian creationism as a possible alternative. In his work *Über die Berechtigung der Darwin'schen Theorie* (On the justification of the Darwinian theory), he compared creationism and evolutionary theory. He concluded that many biological facts can be seamlessly accommodated within evolutionary theory, but remain puzzling if considered the result of acts of creation.

After this work, Weismann accepted evolution as a fact on a par with the fundamental assumptions of astronomy (e.g. heliocentrism). His position towards the mechanism of inheritance and its role for evolution changed during his life. Three periods can be distinguished.

Work on cells

Weismann's work on the demarcation between germ-line and soma can scarcely be appreciated without considering the work of (mostly) German biologists during the second half of the 19th century. This was the time that the mechanisms of cell division began to be understood. Eduard Bogumił Strasburger (1803–1874), Walther Flemming (1843-1905), Heinrich Wilhelm Gottfried von Waldeyer-Hartz (1836 – 1921), and the Belgian Édouard Joseph Louis Marie Van Beneden (1846 – 1910) laid the basis for the cytology and cytogenetics of the 20th century. Strasburger, the outstanding botanical physiologist of that century, coined the terms "nucleoplasm" and "cytoplasm". He said "*new cell nuclei can only arise from the division of other cell nuclei*". Van Beneden discovered how chromosomes combined at meiosis, during

the production of gametes, and discovered and named "chromatin". Walther Flemming, the founder of cytogenetics, named "mitosis", and pronounced *omnis nucleus e nucleo* (which means the same as Strasburger's dictum). The discovery of mitosis, meiosis, and chromosomes is regarded as one of the 100 most important scientific discoveries of all times, and one of the 10 most important discoveries in cell biology.

The significance of meiosis for reproduction and inheritance was first described in 1890 by Weismann, who noted that two cell divisions were necessary to transform one diploid cell into four haploid cells if the number of chromosomes had to be maintained. Thus, the work of the earlier cytologists laid the ground for Weismann, who turned his mind to the consequences for evolution, which was an aspect the cytologists had not addressed. All this took place before the work of Mendel had been rediscovered.

- **During 1868–1881/82:** Like many other 19th century scientists, among them Charles Darwin, Weismann started out believing that the observed variability of individuals of one species is due to the "*inheritance of sports*" (Darwin's term). He believed, as written in 1876, that "... *transmutation of species is directly due to the influence of the environment*". He also wrote, "… *if every variation is regarded as a reaction of the organism to external conditions, as a deviation of the inherited line of development, it follows that no evolution can occur without a change of the environment*". This is close to the modern use of the concept that changes in the environment can mediate selective pressures on a population, so leading to evolutionary change. Weismann also used the classic Lamarckian metaphor of use and disuse of an organ.
- **During 1882–1895:** Weismann's first rejection of the inheritance of acquired traits came in a lecture in 1883, titled "On inheritance" (*Über die Vererbung*). Again, as in his treatise on creation vs. evolution, he attempted to explain individual examples with either theory. For instance, the existence of non-reproductive castes of ants, such as workers and soldiers, cannot be explained by inheritance of acquired characters. Germ plasm theory, on the other hand, does so effortlessly. Weismann used this theory to explain Lamarck's original examples for

"use and disuse", such as the tendency to have degenerate wings and stronger feet in domesticated waterfowl.

- **During 1896–1910:** Weismann worked on the embryology of sea urchin eggs and, in the course of this, observed different kinds of cell division, namely "equatorial division" and "reductional division", terms he coined (*Äquatorialteilung* and *Reduktionsteilung,* respectively). His germ plasm theory states that multicellular organisms consist of germ cells containing heritable information, and somatic cells that carry out ordinary bodily functions. The germ cells are influenced neither by environmental influences nor by learning or morphological changes that happen during the lifetime of an organism, which information is lost after each generation. As he proposed it, the concept was referred to as "Weismannism" in his day. [See, for example, in the book "An examination of Weismannism" by George Romanes.] This idea was illuminated and explained by the rediscovery of Gregor Mendel's work in the early years of the 20th century (see Mendelian inheritance).

Experiments on the inheritance of mutilation

The idea that germ line cells contain information that passes to each generation unaffected by experience and independent of the somatic (body) cells, came to be referred to as the "Weismann's barrier". It is frequently quoted as putting a final end to the theory of Lamarck and the inheritance of acquired characteristics (what Lamarck claimed was the inheritance of characteristics acquired through effort, or will).

Weismann conducted the experiment of removing the tails of 68 white mice, repeatedly over 5 generations. He reported that no mice were born in consequence without a tail or even with a shorter tail. He stated that "901 young were produced by five generations of artificially-mutilated parents and, yet, there was not a single example of a rudimentary tail or of any other abnormality in this organ". Weismann was aware of the limitations of his experiment, and made it clear that he embarked on the experiment precisely because, at the time, there were many claims of animals inheriting mutilations (he refers to a claim regarding a cat that had lost its tail having numerous tail-less offspring). There were also claims of Jews born without foreskins. None of these claims, he said, were backed up by reliable evidence

that the parent had in fact been mutilated, leaving the perfectly plausible possibility that the modified offspring were the result of a mutated gene. The purpose of his experiment was to lay the claims of inherited mutilation to rest. The results were consistent with Weismann's germ plasm theory.

Evolution of Weismann's views on the evolution of aging

Weismann's scientific views evolved significantly over the course of his life. When becoming older, he stopped writing about the "injuriousness" of the old, changed his evolutionary views, and considered old organisms not as harmful but simply neutral for the biological species. Thus, he wrote:

> *"…in regulating duration of life, the advantage to the species, and not to the individual, is alone of any importance ….. It is of no importance to the species whether the individual lives longer or shorter, but it is of importance that the individual should be enabled to do its work towards the maintenance of the species…. The unlimited existence of individuals would be a luxury without any corresponding advantage".*

He further corrected his own earlier theory of programmed death, stating that " *….. aging is not an adaptive trait but rather simply a neutral trait".*

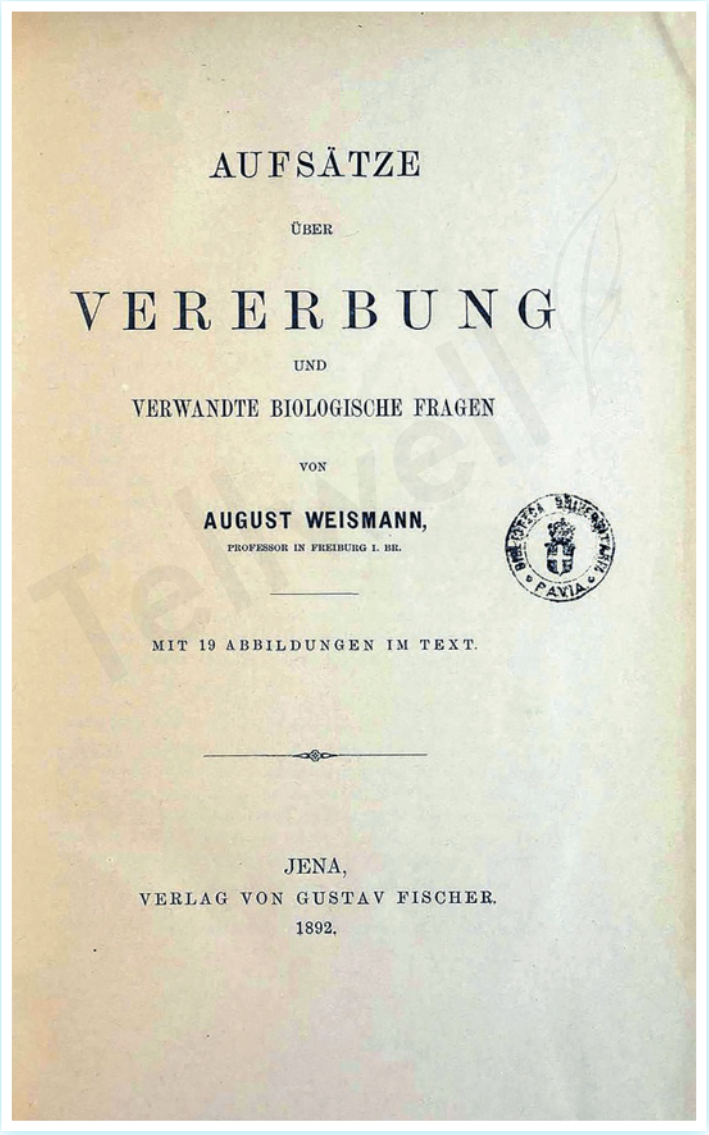
Weismann should be credited with at least four contributions to aging studies:

- Suggesting the first evolutionary theory of aging.
- Abandoning his own theory when he understood that it was incorrect.
- On a theoretical basis alone, correctly predicting the existence of a cell division limit without having any data at all.
- Formulating the germ plasm theory, i.e., that the body is strictly divided into two types of cells: the "germ cells" (sperm or ova cells), which are the only cells transmitting hereditary information to the offspring, as opposed to all other "somatic cells", with a prophetic claim of "*the perishable and vulnerable nature of the soma*".

Awards and Honors

Weismann was elected an International Member of the American Philosophical Society (APS) in 1906. He was awarded the Linnean Society of London's Darwin-Wallace Medal in 1908. He was elected an International Member of the United States National Academy of Sciences (NAS) in 1913.

Figure 2.4 - *Aufsätze über Vererbung und verwandte biologische Fragen*


AUFSÄTZE

ÜBER

VERERBUNG

UND

VERWANDTE BIOLOGISCHE FRAGEN

VON

AUGUST WEISMANN,

PROFESSOR IN FREIBURG I. BR.

MIT 19 ABBILDUNGEN IM TEXT.

JENA,
VERLAG VON GUSTAV FISCHER.
1892.

Source: Weismann, August – Aufsätze über Vererbung und verwandte biologische Fragen

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And so, after this brief journey into the life and times of Weismann, we bid him farewell, paying him a grateful homage and tribute at his resting place in Frieburg, Germany. It is now time to turn our attention to the scientific basis of the aging process.

PART B

WHY DO WE AGE?

Introduction to Part B

Part B includes three chapters. **Chapter 3** discusses senescence and the biology of aging. The aging process is a complex interplay of various factors, the major contributors being: Genetics (family risk factors), environment (geographical area and corresponding climatology, and air quality, exposure to ionizing and harmful electromagnetic radiations), and lifestyle (sedentary lifestyle and lack of physical activity, stress, smoking, drinking, and diet). Aging itself is the collection of the early stages of the various age-related diseases. It proceeds in a downward spiral such that the more we age, the more our self-repair functions decline and the less able our body is to stop aging. Thus, we age faster and faster! The *Gompertz–Makeham law of mortality* states that the human death rate is the sum of an age-dependent component (the *Gompertz function*) which increases exponentially with age and an age-independent component (the *Makeham term*). A distinction will be made between "proximal aging" (i.e., age-based effects that come about because of factors in the recent past) and "distal aging" (i.e., age-based differences that can be traced to a cause in a person's early life. Now, "chronological age" is a measurement of how long you have lived whereas "biological age" is a prediction of how long you have left and how likely you are to become chronically ill. Determining biological age requires medical tests for telomere length and biomarkers of DNA methylation, which is the process by which DNA is changed throughout lifetime. Unlike chronological age, biological age can be changed. In this context, a longevity calculator will also be presented and discussed.

The 13 hallmarks of aging will be set forth along with their characterization. Regarding the determination of the biological age of different tissues or systems or overall, research and development is ongoing for further biomarkers, detection systems, and software systems.

Life span, like other phenotypes, is selected for in evolution. Traits that benefit early survival and reproduction will be selected for, even if they contribute to an earlier death (so-called "antagonistic pleiotropy effect" - see later). The "disposable soma effect" refers to an entire genetic program in which the organism diverts limited resources from maintenance to reproduction. The biological mechanisms which regulate lifespan probably evolved with the first multicellular organisms more than a billion years ago.

Rare human mutations can cause accelerated aging diseases. Further, different parts of the body may age distinctly and at different rates. Similarly, functions may distinctly decline with aging. Normal human cells die after about 50 cell divisions in laboratory culture (the so named "Hayflick's limit"). On the other hand, senescence (or biological aging) is the gradual deterioration over time of the functional characteristics in living organisms, the resulting effects of which could be delayed. We can distinguish between "cellular senescence", "organismal senescence", and "actuarial senescence".

Chapter 4 sets forth our understanding of the aging process. A brief overview of six major categories of aging theories and their variations will be provided, pending further more detailed discussions in subsequent chapters. These include: Programmed theories, hormonal theories, error theories, genetic theory, biochemical theory, and environmental damage theories.

In **Chapter 5,** medical myths about aging are outlined. Overall, most of the myths surrounding age seem to center on inevitability – the inevitable gradual crumbling into dust as lives become increasingly unbearable, boring, passionless, and painful. However, although certain aspects of health might decline with age, none of the above is inevitable for everyone. A positive psychological outlook on aging can benefit the physical aspects of aging.

3

Senescence and the biology of aging

Contents

3

Senescence and the biology of aging

We age faster and faster!
Evolutionarily, natural selection has wrought careful compromises in senescence.

This Chapter will discuss human aging and senescence, relegating to Chapters 16 and 17 a review of the associated modern science research and technologies that may enable to prolong life. From an individual's viewpoint, the latter proposition may sound appealing, providing this longer existence is also a healthy life. From an evolutionary standpoint (see Chapter 11), our bodies have evolved to be disposable vessels that carry genes over succeeding generations (the so-called "disposable soma"). This explains not only general senescence, but also why, in youth, neurodegenerative diseases (including dementia), cancer, cardiovascular problems, arthritis and many other diseases are guarded against but crammed into old age once the reproduction function is no longer the evolutive goal or even possible. Such diseases would also have to be treated if a long and healthy life was to become routine. Moreover, even a healthy brain having evolved to accommodate 70-80 years or more of memories and functions may age badly and become unable to cope when asked to perform similarly

for 150 years. From a societal point of view, a longer lived population may pose additional new problems such as, for example, exacerbation of the existing social and economic structures, equitable access to anti-senescence treatment, perhaps discrimination against workers (either older or younger), wedding and retirement concepts, altered lifestyles, etc.

In the 21st century, researchers are only beginning to investigate the biological basis of aging, even in relatively simple and short-lived organisms, such as yeast, let alone mammals of which little is known. The classic biological idea of how life appeared, evolved, and multiplied unfolds something like the following. About 3.7 billion years ago, early life forms appeared on Earth and multiplied by fission into *identical* daughter cells. Many species (e.g., bacteria, strawberry plants, animals of the genus Hydra, etc.) could somehow regenerate themselves, thus avoiding dying of old age – they can justifiably be considered potentially immortal! Later, with the emergence of the fungal/animal kingdoms approximately a billion years ago, and the evolution of seed-producing plants about 320 million years ago, aging and mortality of the individual organisms only became possible because of the evolution of sexual reproduction. Henceforth, the sexual organism could pass on some of its genetic material to produce new individuals and could itself become disposable with respect to the survival of its species. Recently, however, the above idea has been perturbed by the discovery that the bacterium *E. coli* may split into *distinguishable* daughter cells, opening the theoretical possibility of "age classes" among bacteria.

Even within mortal species including humans, there are potentially immortal cells – witness, cancer cells (which do not die when maintained in a cell culture such as the HeLa cell line) and specific stem cells such as germ cells. Further, in artificial cloning, adult cells can be rejuvenated into embryonic status to grow a new tissue or animal without aging. Otherwise, normal human cells die after about 50 cell divisions in laboratory culture (the *Hayflick's limit* - see Sidebar 3.1).

A distinction can be made between "proximal aging" (age-based effects that come about because of factors in the recent past) and "distal aging" (age-based differences that can be traced to a cause in a person's early life, such as childhood poliomyelitis).

Aging is among the greatest known risk factors for most human diseases. Of the roughly 150,000 people who die each day across the globe, about two-thirds—100,000 per day—die from age-related causes. In industrialized nations, the proportion is higher, reaching 90%.

Contrasting aging and senescence

"Aging" is the process of growing older from birth onward. It is the collection of the early stages of the various age-related diseases. It proceeds in a downward spiral such that the more we age, the more our self-repair functions decline and the less able our body is to stop aging. Thus, we age faster and faster! On the other hand, "senescence", the process of bodily deterioration or general dwindling of prowess experienced by all as time takes its toll that occurs in older ages, is manifested in an increased susceptibility to many diseases and a decreasing ability to repair damage.

In a world in which all causes of premature death would have been eliminated, so that all deaths result from the effects of aging, we would live hearty, healthy lives until approximately age 85 when we would nearly all die. By contrast, eliminating the effects of senescence, so that death rates do not increase with age but remain throughout life at the level of 18-year olds, say about 10 per 1000 a year for young adults in India in 1990, some people would still die at all ages but half the population would still live to age 300!

Research on senescence seems to be discovering the value of an evolutionary point of view (see discussion in later chapters of this book). Gerontologists are realizing that the mechanisms that cause senescence may not be mistakes but compromises carefully wrought by natural selection. An evolutionary view suggests that more than a few genes are involved in senescence and that some of them have functions crucial to life. These genes express their various effects in a seemingly coordinated cluster of escalating genes, because any gene whose deleterious effects occur earlier than those of other genes will be selected against the most strongly. Selection will act on it and other genes to delay its effects until they are in synchrony with those of other genes that cause senescence.

Since aging in laboratory animals has been successfully postponed, an extension of a maximum lifespan of ~ 20%, we posit a similar or greater result is likewise potentially achievable in humans. Our purpose would then be the minimization of senescence and possible extension of maximum lifespan.

Contributing factors to the aging process

The aging process is a complex interplay of various factors involving genetics, environment, and lifestyle (an eminent example of epi/eco-genetics discussed at length in Chapters 7-9 of this book) that can be partially influenced. The major contributors to why we age are further identified below (acronym: GEL for Genetics, Environment, and Lifestyle):

Genetics (family history)

Family risk factors (for example, heart disease, cancer, obesity, diabetes or dementia) elevate the risk a progeny will contract such diseases. Understanding such factors and the role played by the environment and lifestyle can help control the risk through lifestyle dynamics.

Environment

The geographical location and the corresponding climate and air quality cannot be altered by any single individual unless that individual moves to a different location. Exposures to ionizing and other harmful electromagnetic radiations (whether occupational or elective for medical diagnostic, therapeutic and other procedures) can be controlled only in part but may not be practical choices for many.

Lifestyle

We can distinguish here the four factors that follow (acronym: DESS for Diet, Exercise, Stress, Sleep)

- ***Diet****:* Diet may be the most important modifiable contributor to the aging process. In North America, unfortunately, the standard diet is primarily composed of refined carbohydrates, sugar, trans fat, saturated fat, sodium, animal protein, and high calories. That type of diet provides a poor supply of vital nutrients to sustain, maintain, or repair aging cells. As a consequence, the rates of heart disease, cancer, diabetes, obesity, stroke, arthritis, and dementia are high.
- ***Exercise*** *- Sedentary lifestyle and lack of physical activity:* Studies of different populations and evaluation of specific variables such as mortality rates, longevity, and rates of chronic disease, have evidenced a very interesting pattern in that the more physically active the population the longer its lifespan and the lower its incidence of chronic disease. Sedentary lifestyle, including prolonged sitting and unhealthy sleep patterns, leads to the advancement of disease and hastens the aging process: arteries become stiff, thicken, and fill with plaque. When this occurs, blood pressure rises and so will the risk of heart disease and stroke. Without continual mechanical stimulation and external loading, muscle and bone mass are lost with a net gain of body fat. The tissues in the joints will not remodel or repair themselves without continual, external stimulation from loading forces. In addition, the immune and hematological systems will weaken if not challenged by continual physical effort. Lastly, the brain ages unimpeded without the improved blood supply afforded from regular exercise. Thus, regular exercise is vitally important, especially in sedentary situations.
- ***Stress:*** **Stress** can affect the aging process and cause disease. Emotional stress, especially chronic stress, causes the body to adapt in such a way as to place a lot more 'wear-and-tear' on the organs. Under stress, the adrenal gland secretes hormones which increase blood pressure, influence fat oxidation, brain neurochemistry, stomach acid secretion, inflammation, and digestive function. If the stress is not managed properly or alleviated, heart problems develop, digestion is impaired, memory or concentration is impaired, insulin metabolism is affected, and chronic fatigue sets in.
- ***Sleep:*** Optimizing sleep improves brain function. Impediments to good sleep should be addressed and corrected including: Treating sleep apnea (if diagnosed), getting restful sleep (~ 8 hours/day) without

sleeping pills that can compromise cognitive function, and generally practicing good sleep hygiene

Other important lifestyle factors include:

- ***Smoking:*** **Smoking** is the most preventable cause of premature aging and death. It damages arteries by increasing blood stickiness, oxidizing low density lipoprotein (LDL) cholesterol molecules, enhancing the inflammatory response inside the artery, and directly influencing endothelial function. Smoking also negatively influences enzymes which keep arteries relaxed, resulting in higher blood pressure. Further, it increases the concentration of carbon monoxide in red blood cells (RBC), which limits the oxygen carrying capacity of the circulatory system. All of the above effects greatly increase the aging of the circulatory system leading to myocardial infarction (MI) or heart attack, stroke, and peripheral arterial disease (PAD). Smoking can also cause cancers of the lung, esophagus, mouth, tongue, stomach, and pharynx by producing abnormal pre-cancerous cellular growth. It can take a great toll upon the appearance of the skin (more wrinkles, discoloration, and tightness of the facial skin).
- ***Drinking:*** Excessive **d**rinking (binge drinking, excessive alcohol intake, alcoholism) is directly responsible for accelerated aging, disease, and premature death. Too much alcohol can damage brain cells, liver cells, and affect nutrient absorption. It has also been associated with higher rates of cancers of the breast, esophagus, stomach, and liver.

Aging symptoms

The long observed symptoms of aging are summarized in Table 3.1 by age range and associated effects. As seen, aging is among the greatest known risk factor for most human diseases. Thus, as indicated earlier, of the roughly 150,000 people who die each day across the globe, about two-thirds die from age-related causes. In industrialized nations, the proportion is higher, reaching 90%.

Table 3.1 – Symptoms of aging at different age ranges and their effects

Age range	Symptom(s)	Effect(s)
Very young age		Ability to hear high-frequency sounds above 20 kHz
Teen ages	Loss of ability to hear high-frequency sounds above 20 kHz	
Late teens to late 20s	Peaking of female fertility	Decline of female fertility thereafter
After 30 to 70	Decrease in mass of human body	After age 70: Damping oscillations
Over 35	Increasing risk for loss of strength in the ciliary muscle of the eyes, leading to difficulty focusing on close objects (presbyopia)	
45-50	Most people experience presbyopia	
About 44-58	Menopause	
Around 50	Hair turns grey	Pattern hair loss affects about 30%–50% of males and 25% of females
60-64	Incidence of osteoarthritis rises to 53%	Only 20% report disabling osteoarthritis at this age
65-74	3% of people have dementia	The spectrum ranges from mild cognitive impairment (MCI) to the neurodegenerative diseases including Alzheimer's disease (AD); Parkinson's disease (PD); and amyotrophic lateral sclerosis (ALS) aka Lou Gehrig's disease. Also cerebrovascular disease (CVD).
Older than 75	Almost 50% of people have hearing loss (presbycusis)	Humans have genetically lost this ability
75-84	19% of people have dementia	

Around 80	50% of all Americans either have a cataract or have had cataract surgery	
Above 80	Nearly 12% have macular degeneration	
Over 85	o 25% of humans experience frailty o 50% of people have dementia	o Muscles have a reduced capacity of responding to exercise or injury and loss of muscle mass and strength (sarcopenia) is common o Maximum oxygen use and maximum heart rate decline o Hand strength and mobility decrease
Other: Memory decline	Declines with age	Not semantic memory or general knowledge such as vocabulary definitions, which typically increases or remains steady until late adulthood
Other: Intelligence decline	Declines with age	Rate of decline varies depending on the type and may in fact remain steady throughout most of the lifespan, dropping suddenly only as people near the end of their lives
Other: Cognitive decline	o Brain changes o After 20 years of age: 10% reduction each decade in total length of the brain's myelinated axons	May be explained in terms of people having different lengths of life
Other: Visual impairment and attendant reduction in communication	Can lead to isolation and possible depression	o Older adults may not experience depression as much as younger adults, o Paradoxically, older adults may have improved mood despite declining physical health
Other: Macular degeneration (vision loss)	Increases with age	

Other: Cataract		Develops over time and seen in older individuals
Other: Glaucoma		Usually develops over time but there are variations some of which have sudden onset
After 105	Age-related risk of death seems to plateau	
115	o Suggested maximum human lifespan. o Exception: The oldest reliably recorded human was the French woman Jeanne Calment who died in 1997 at age 122 (or 124).	

A distinction can be made between "proximal aging" (i.e., age-based effects that come about because of factors in the recent past) and "distal aging" (i.e., age-based differences that can be traced to a cause in a person's early life, such as, for example, childhood poliomyelitis).

Molecular and cellular hallmarks of aging

Aging has been defined as "*a progressive deterioration of physiological function, an intrinsic age-related process of loss of viability and increase in vulnerability*". It is characterized by the declining ability to respond to stress, increased homeostatic imbalance, and increased risk of aging-associated diseases including cancer and heart disease.

A 2013 review assessed aging through the lens of the damage theory (see Chapter 10). It initially proposed nine metabolic "hallmarks" of aging in various organisms (especially mammals) that were later augmented by three others. Including damages caused by the environment, there are 13 hallmarks as set forth in Table 3.2 below:

Table 3.2 – Hallmarks of molecular and cellular aging

Hallmark	Characterization
1. Genomic instability	Mutations accumulated in: o Nuclear DNA o Mitochondrial DNA (mtDNA) o Nuclear lamina
2. Telomeres attrition	Artificial telomerase confers non-cancerous immortality to otherwise mortal cells
3. Epigenetic alterations	o DNA methylation patterns o Post-translational modification of histones o Chromatin remodeling Aging and disease are related to a misregulation of gene expression through impaired methylation patterns from hypo- to hyper-methylation
4. Proteostasis loss	Protein folding and proteolysis
5. Nutrient sensing dysregulation	Relates to: o Growth hormone/Insulin-like growth factor-1 (GH/IGF-1) signaling pathway, which is the most conserved aging-controlling pathway in evolution o Among its targets are the FOXO3/ Sirtuin transcription factors and the mTOR complexes, which are probably responsive to calorie restriction.
6. Mitochondrial dysfunction	Causal link exists between aging and increased mitochondrial production of reactive oxygen species (Note: This is no longer supported by recent research)
7. Cellular senescence	Accumulation of no longer dividing cells in certain tissues (a process induced especially by p16INK4a/Rb and p19ARF/p53 to stop cancerous cells from proliferating)
8. Stem cells exhaustion	Caused by damage factors (such as those listed above)
9. Intercellular communication alteration	Encompasses especially inflammation but also, possibly, other intercellular interactions
10. Inflammaging	Chronic inflammatory phenotype in the elderly in the absence of viral infection. (It is due to over-activation and a decrease in the precision of the innate immune system.)

11. Gut microbiome dysbiosis	Loss of microbial diversity, expansion of enteropathogens, and altered vitamin B12 biosynthesis. (This is correlated with biological age rather than chronological age)
12. Macroautophagy disablement	o Autophagy is the natural, conserved degradation of the cell that removes unnecessary or dysfunctional components. It allows the orderly degradation and recycling of cellular components. It plays a major role in the homeostasis of non-starved cells. Defects in autophagy have been linked to various human diseases, including neurodegeneration and cancer. o Four forms of autophagy have been identified: macroautophagy, microautophagy, chaperone-mediated autophagy, and crinophagy. Macroautophagy is the most thoroughly researched form of autophagy.
13. Environmental damage	o Damage to DNA o Damage to tissues and cells by oxygen (free) radicals (These damages are induced at various levels, some of which not repaired, and thus accumulate with time)

Metabolic pathways involved in aging

There are three main metabolic pathways which can influence the rate of aging (Table 3.3):

Table 3.3 – Metabolic pathways in aging

Metabolic pathway	Influence
1. FOXO3/Sirtuin	Probably responsive to calorie restriction
2. Growth hormone/Insulin-like growth factor1	Signaling
3. Electron transport chain	Activity levels of the chain in mitochondria

It is likely that most of these pathways affect aging separately because targeting them simultaneously leads to additive increases in lifespan.

Evolution of aging

Lifespan, like other phenotypes, is selected for in evolution. Traits that benefit early survival and reproduction will be selected for, even if they contribute to an earlier death. Such a genetic effect is called the "antagonistic pleiotropy effect" (pleiotropy signifying the gene has a double function – enabling reproduction at a young age but costing the organism life expectancy in old age). It is called the "disposable soma effect" when referring to an entire genetic program (the organism diverting limited resources from maintenance to reproduction).

The biological mechanisms which regulate lifespan probably evolved with the first multicellular organisms more than a billion years ago. Aging has its biological roots much earlier than multi-cellularity.

A number of genetic components of aging have been identified using model organisms, ranging from the simple budding yeast *Saccharomyces cerevisiae* to worms such as *Caenorhabditis elegans* and fruit flies (*Drosophila melanogaster*). Study of these organisms has revealed the presence of at least two conserved aging pathways.

Gene expression is imperfectly controlled, and it is possible that random fluctuations in the expression levels of many genes contribute to the aging process, as suggested by a study of such genes in yeast. Individual cells, which are genetically identical, can nonetheless have substantially different responses to outside stimuli, and markedly different lifespans, indicating that epigenetic factors play an important role in gene expression and aging as well as genetic factors.

The ability to repair DNA double-strand breaks declines with aging in humans. A set of rare hereditary (genetics) disorders, each called progeria, has been known for some time. Sufferers exhibit symptoms resembling accelerated aging, including wrinkled skin. The cause of Hutchinson–Gilford progeria syndrome (HGPS) was reported in the journal *Nature* in May 2003. This report suggests that DNA damage, not oxidative stress, is the cause of this form of accelerated aging.

A study indicates that aging may shift activity toward short genes or shorter transcript length and that this can be countered by interventions.

Senescence or biological aging

Senescence (or biological aging) is the gradual deterioration over time of the functional characteristics in living organisms, the resulting effects of which can be delayed. It is considered a by-product of physiology because our cell metabolism creates products that are toxic, we get mutations when we age, and we do not have enough stem cells that regenerate. Why did selection not find and favor mutations in ways that allow us, for example, to regenerate our cells, or to not produce toxic metabolism? Why did menopause evolve? Because natural selection is more efficient on traits that appear early in life. Mutations that have an effect early in life will increase fitness much more than mutations that manifest late. Most people have already reproduced before any disease manifests; this means that parents will pass their alleles to their offsprings before they show any fitness problems, and it is therefore "too late" for selection.

Senescence refers to either "cellular senescence" or "organismal senescence". The latter is aging of the whole organism, involving an increase in death rates and/or a decrease in fecundity with increasing age, at least in the later part of an organism's life cycle. "Actuarial senescence" can also be defined as an increase in mortality and/or a decrease in fecundity with age. The Gompertz–Makeham law of mortality (see Sidebar 3.1) says that the age-dependent component of the mortality rate increases exponentially with age.

The existence of species having negligible senescence and of potentially immortal organisms (such as members of the genus Hydra) together with the discovery in 1934 that calorie restriction can extend rats' lifespans by 50% have motivated research into delaying senescence and, thus, age-related diseases.

Rare human mutations can cause accelerated aging diseases. Also, environmental factors may affect aging (e.g., overexposure to ultraviolet radiation accelerates skin aging). Further, different parts of the body may age at different rates and distinctly, including the brain, the cardiovascular

system, and muscles. Similarly, functions may distinctly decline with aging, including movement control and memory. Two organisms of the same species can also age at different rates, making "biological aging" and "chronological aging" distinct concepts.

The factors proposed to influence biological aging fall into two main categories, programmed and error-related (see next Chapter). Programmed factors follow a biological timetable that might be a continuation of inherent mechanisms that regulate childhood growth and development. This regulation would depend on changes in gene expression that affect the systems responsible for maintenance, repair, and defense responses. Factors causing errors or damage include internal and environmental events that induce cumulative deterioration in one or more organs.

The evolution of senescence

Two theories are used to explain the evolution of senescence, which is the decline in reproduction with age: Non-adaptive and adaptive, The non-adaptive theory assumes that the evolutionary deterioration of human age occurs as a result of accumulation of deleterious mutations in the germ line. These deleterious mutations start expressing themselves late in life, by the time we are weak/wobbly and have already reproduced - this means that natural selection cannot act on them because reproduction has ended. Studies done on *Drosophila melanogaster* have shown an inverse relationship between the mean optimal age at maturity and mutation rates per gene.

Mutation accumulation affects the allocation of energy and time that are directed towards growth and reproduction over the lifetime of an organism - especially the period of reproductive lifespan due to the fact that mutation accumulation accelerates senescence. This means that organisms must reach the optimum age of maturity at a younger age as their reproductive lifespan is shortened with accumulated mutations.

Senescence shrinks chromosomes

Although getting older causes tissues to deteriorate and eventually fail, at a cellular level, senescence is an important process for health. It marks

the permanent, stable end to a cell's replicating ability, inherently tending to prevent cancer (the uncontrollable cell proliferation) but, at the same time, preventing tissues from indefinitely renewing, so, eventually, muscles weaken, bones fracture, and skin wrinkles. At the genomic level, the chromatin of senescent cells changes radically. The normally densely packed heterochromatin at centromeres loosens up as cells age. Also, senescence in some cell types triggers the formation of densely packed heterochromatin foci.

In cells undergoing senescence, chromosomes tend to become more compact, according to a report **published in** Science Advances *(Neretti* et al.*, February 2016).* **This and other chromatin rearrangements noted in the report add to a growing understanding of how the physical structure of chromosomes might contribute to altered gene expression in aging cells.**

Cancer versus cellular senescence

Senescent cells within a multicellular organism can be purged by competition between cells, but this increases the risk of cancer. It leads to an inescapable dilemma between two possibilities—the accumulation of physiologically useless senescent cells or cancer—both of which lead to increasing rates of mortality with age.

Conclusions and take-aways

- **The aging process is a complex interplay of various factors involving genetics, environment, and lifestyle (an eminent example of epi/eco-genetic processes). The** major contributors are: family risk factors (genetics), environment (geographical area and corresponding climatology, and air quality, exposure to ionizing and harmful electromagnetic radiations), and lifestyle (sedentary lifestyle and lack of physical activity, stress, smoking, drinking, and diet).
- **"Aging" is the collection of the early stages of the various age-related diseases. It proceeds in a downward spiral such that the more we age, the more our self-repair functions decline**

and the less able our body is to stop aging. Thus, we age faster and faster!

- **"Senescence" (or biological aging) is the gradual deterioration over time of the functional characteristics in living organisms, the resulting effects of which can be delayed. We can distinguish between "cellular senescence", "organismal senescence", and "actuarial senescence".**
- In the 21st century, researchers are only beginning to investigate the biological basis of aging, even in relatively simple and short-lived organisms, let alone mammals of which little is known.
- Even within mortal species including humans, there are potentially immortal cells (cancer cells and specific stem cells such as germ cells). Further, in artificial cloning, adult cells can be rejuvenated into embryonic status to grow a new tissue or animal without aging.
- Normal human cells die after about 50 cell divisions in laboratory culture ("Hayflick's limit").
- Rare human mutations can cause accelerated aging diseases. Also, environmental factors may affect aging. Further, different parts of the body may age at different rates and distinctly. Similarly, functions may distinctly decline with aging. Two organisms of the same species can also age at different rates, making "biological aging" and "chronological aging" distinct concepts.
- Aging symptoms have been tabulated by age range along with their associated effects. Aging is among the greatest known risk factor for most human diseases.
- A distinction can be made between "proximal aging" (i.e., age-based effects that come about because of factors in the recent past) and "distal aging" (i.e., age-based differences that can be traced to a cause in a person's early life, such as, for example, childhood poliomyelitis).
- Aging has been defined as the progressive deterioration of physiological function, an intrinsic age-related process of loss of viability and increase in vulnerability. It is characterized by the declining ability to respond to stress, increased homeostatic imbalance, and increased risk of aging-associated diseases.
- The 13 hallmarks of aging have been tabulated along with their characterization, including nine metabolic hallmarks.

- The three main metabolic pathways which can influence the rate of aging have likewise been tabulated.
- Aging is a complex interaction of genetics, ecogenetics (chemistry and physiology within our body), and epigenetics (effects of the environment, lifestyle, and behavior). A complete explanation of this phenomenon still eludes us, but has not prevented the formulation of dozens of theories to explain this inevitable fact besetting humanity.
- The rate of aging varies substantially across different species, and this, to a large extent, is genetically based.
- Clonal immortality apart, there are certain species whose individual lifespans stand out among Earth's life-forms. The genetic aspect has also been demonstrated in studies of human centenarians.
- Lifespan, like other phenotypes, is selected for in evolution. Traits that benefit early survival and reproduction will be selected for, even if they contribute to an earlier death (so-called "antagonistic pleiotropy effect"). The "disposable soma effect" refers to an entire genetic program in which the organism diverts limited resources from maintenance to reproduction.
- The biological mechanisms which regulate lifespan probably evolved with the first multicellular organisms more than a billion years ago. Aging has its biological roots much earlier than multi-cellularity.

Sidebar 3.1 – The Gompertz–Makeham law of mortality

The Gompertz–Makeham (GM) law (1825) states that the human death rate is the sum of an age-dependent component (the *Gompertz function*) which increases exponentially with age and an age-independent component (the *Makeham term*). In a protected environment where external causes of death are rare (laboratory conditions, low mortality countries, etc.), the age-independent mortality component is often negligible. In this case the formula simplifies to the Gompertz law of mortality.

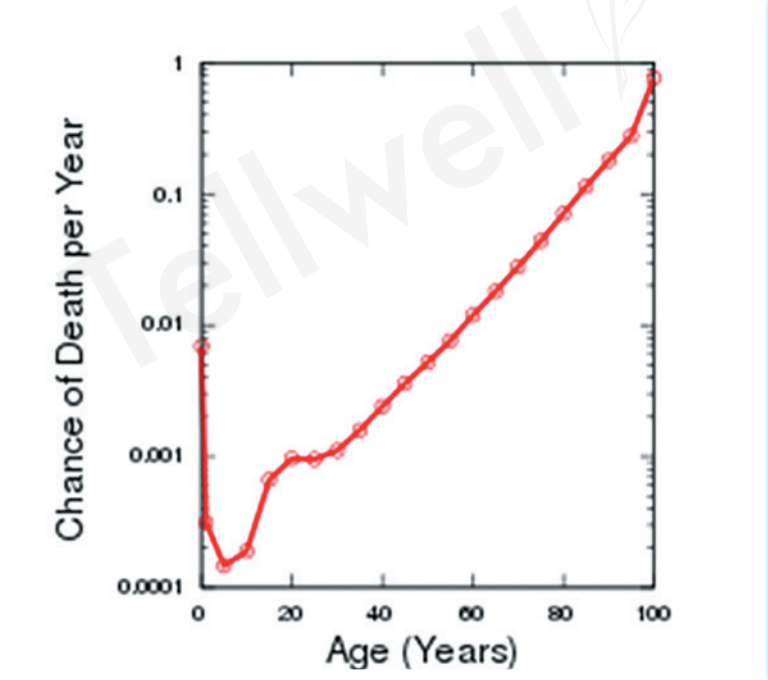
The GM law of mortality describes the age dynamics of human mortality rather accurately in the age window from about 30 to 80 years of age. At more advanced ages, some studies have found that death rates increase

more slowly – a phenomenon known as the *late-life mortality deceleration* – but more recent studies disagree.

The decline in the human mortality rate before the 1950s was mostly due to a decrease in the age-independent (Makeham) mortality component, while the age-dependent (Gompertz) mortality component was surprisingly stable. Since the 1950s, a new mortality trend has started in the form of an unexpected decline in mortality rates at advanced ages and "rectangularization" of the survival curve. There is a doubling of mortality every 8 years.

A study predicts a future in which longevity records will frequently be broken after 2073, with some prediction graphs reaching into the 140s.

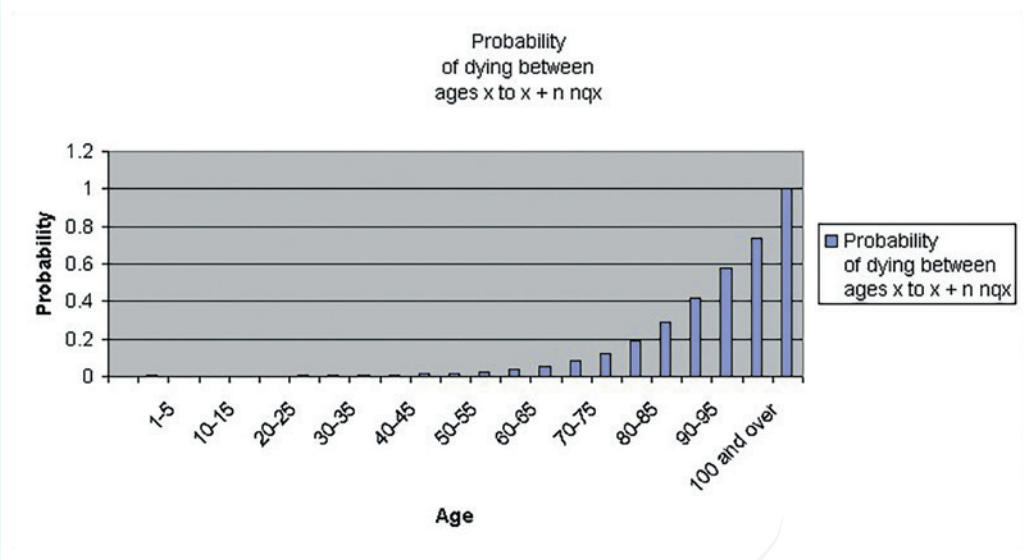
Figure 3.1 - Estimated probability of a person dying at each age for the U.S. in 2003 (Mortality rates increase exponentially with age after age 30)



Source: Wikipedia

Further, Figure 3.2 sets forth the probability of dying between ages x to x+n (every five years from near birth to 100 and over).

Figure 3.2 – Probability of dying between ages x to x+n



4

Understanding the aging process

Contents

Sidebars:

4

Understanding the aging process

Aging is a complex interaction of genetics, ecogenetics (chemistry and physiology within our body), and epigenetics (effects of the environment, lifestyle, and behavior). A complete explanation of this phenomenon still eludes us, but has not prevented the formulation of dozens of theories to explain this inevitable fact besetting humanity. Most such theories will be reviewed in abundant details in Part C of this book.

The rate of aging varies substantially across different species, and this, to a large extent, is genetically based. For example, numerous perennial plants ranging from strawberries and potatoes to willow trees typically produce clones of themselves by vegetative reproduction and are, thus, potentially immortal. On the other hand, annual plants such as wheat and watermelons die each year and reproduce by sexual reproduction. In 2008, it was discovered that inactivation of only two genes in the annual plant *Arabidopsis thaliana* leads to its conversion into a potentially immortal perennial plant. The oldest animals known so far are 15,000-year-old Antarctic sponges, which can reproduce both sexually and clonally.

Clonal immortality apart, there are certain species whose individual lifespans stand out among Earth's life-forms, including the bristlecone pine at 5062 (or 5067) years, invertebrates like the hard clam (known as quahog in New England) at 508 years, the Greenland shark at 400 years, various deep-sea tube worms at over 300 years, fish like the sturgeon and the rockfish, and the sea anemone and lobster. Such organisms are sometimes said to exhibit "negligible senescence". The genetic aspect has also been demonstrated in studies of human centenarians.

Brief overview of the theories of aging

I will outline here only the four major categories of aging theories, reserving Part C for a fuller exposition of the subject.

Programmed theories

Theories dubbed "programmed theories of aging" assert that the human body is "designed" to age so that aging is a natural phenomenon that has been "programmed" into our bodies, following a certain biological timeline. In effect, we are "designed" to age! But, casting aside philosophical or/and religious arguments, all such theories shy away from identifying the "programmer(s)" or "designer(s)".

Within such theories, one may distinguish those that assert that the "on" and "off" genetic switching over time causes aging, without specifying the correspondence between the number and frequency of the switchings with the corresponding aging parameters.

Hormonal theories

Others argue that regular changes in hormones control aging. Again, the identity and number of the effecting hormones and their degree of control on aging has gone silent. In the "age-changing hormonal theory", hormones cause many shifts in organ systems and other functions. The identity, number, mechanism(s), and effectiveness of such proteins need to be further elucidated.

Error theories

So-called "error theories of aging" assert that, over time, cells and tissues simply wear out. There are four variations of this theory including the:

- **Wear-and-tear theory**, which asserts that cells and tissues simply wear out. The manner and duration of the wearing and tearing has been left aside;
- **Rate-of-living theory**, in which aging is inversely related to the organism's consumption of oxygen, that is, the faster an organism uses oxygen, the shorter it lives. However, understandably because of the underlying difficulties, this relationship has not been quantified in terms of the amount of oxygen available, its rate of consumption, and the proximate rate of aging;
- **Cross-linking theory**, which posits that cross-linked proteins accumulate and slow down the body's processes. Again, the quantification of the number and amount of cross-links to the number and quality of the affected body processes have been left aside; and
- **Free radicals theory**, which asserts that free radicals in the environment cause damage to cells, eventually impairing their function. The quantification of the relationship between the quality and quantity of identified radicals to the nature and amount of damage to cells has also been overlooked.

Genetic theory

The "genetic theory of aging" can play a major role in aging. For example, in a mice experiment, Baker *et al.* (2018) found that removing cells containing certain genes from the organs extended the lifespan of the animals by as much as 35%. Whether a similar effect could be applied to humans is not known. There are five variations on this theory based on known facts:

- **Somatic DNA damage theory**: Here, the genetic mutations are known to cause cells to malfunction. While such a causal effect is known, the nature and number of such mutations and the correlated cell malfunction have not been elaborated upon;
- **Longevity genes theory:** This theory is based on so-called longevity genes, which are specific genes that help lengthen lifespan. However,

the identity and number of such genes and the mechanism of their action on aging remain unclear;

- **Cell senescence theory:** It rests on the process of senescence by which cells deteriorate over time. Again, the characteristics, properties, and effects of this phenomenon on aging are not fully comprehended;
- **Telomeres shortening theory:** It is based on the known effect of telomeres (structures at the end of genes) shortening on cell replication (see Sidebar 4.1 for more details); and finally:
- **Stem cells theory**: Stem cells are cells that can become any cell type in the body. This theory holds the promise to repair the damage caused by aging (see Sidebar 4.2).

Biochemical theory

This last category of aging rests on the known fact that no matter what genes we may have inherited, our body is continually undergoing complex biochemical reactions, some of which causing damage and, ultimately, aging of the body. The study of these reactions should help understand how the body changes as it ages. There are five important concepts in the biochemistry of aging on which are based various theories of aging:

- **Age-changing hormonal theory:** Hormones cause many shifts in organ systems and other functions (see also the section above on hormonal theories of aging);
- **Free radicals theory:** Free radicals (unstable oxygen molecules) can damage cells. Which free radicals, what mechanism(s) and the extent of the damage caused are still being studied (see also the section above on error theories of aging);
- **Protein cross-linking theory:** Protein cross-links produce excess sugars in the bloodstream that can cause protein molecules to literally stick together, leading to aging. The identity, number and strength of the cross-links, and the mechanism(s) leading to aging are being researched (see also the section above on error theories of aging);
- **DNA repair theory:** The systems in the body that repair DNA seem to become less effective with age. However, the particulars of the several body systems that repair DNA and the mechanism(s) and effectiveness of their remedial action need to be further elucidated. Lastly:

- **Heat shock proteins theory:** Such proteins help cells survive stress and diminish in numbers with age. The full identity of such proteins, their number and mechanisms of action, and diminution with time require further investigations.

Environmental damage theory

Others assert that aging is caused by the time accumulation of environmental damage to the body's systems without elaborating on the nature and types of damages affecting which body systems and in which manner has likewise not been discussed.

A preliminary classification of the above various aging theories is provided (acronym **PHEGBE**) in Table 4.1:

Table 4.1 – A preliminary classification of aging theories

Aging theory category	Variation(s)	Description
1. Programmed theories		The human body is "designed" to age so that aging is a natural phenomenon that has been "programmed" into our bodies, following a certain biological timeline. In effect, we are "designed" to age!
2. Hormonal theories		Regular changes in hormones control aging.
3. Error theories		Over time, cells and tissues simply wear out.
	3.1 Cross-linking theory	Cross-linked proteins accumulate and slow down the body's processes.
	3.2 Free radicals theory	Free radicals in the environment cause damage to cells, eventually impairing their function.

	3.3 Rate-of-living theory	Aging is inversely related to the organism's consumption of oxygen, that is, the faster an organism uses oxygen, the shorter it lives.
	3.4 Wear-and-tear theory	Cells and tissues simply wear out.
4. Genetic theories		Genes can play a major role in aging.
	4.1 Cell senescence theory	Senescence deteriorates cells over time.
	4.2 Longevity genes theory	These specific genes can help lengthen lifespan.
	4.3 Somatic DNA damage theory	Genetic mutations are known to cause cells to malfunction.
	4.4 Stem cells theory	Stem cells hold promise to repair the damage caused by aging.
	4.5 Telomeres shortening theory	Telomeres shortening affects cell replication.
5. Biochemical theories		No matter what genes we may have inherited, our body is continually undergoing complex biochemical reactions, some of which cause damage and, ultimately, aging in the body.
	5.1 Age-changing hormonal theory	Hormones cause many shifts in organ systems and other functions.
	5.2 DNA repair theory	The systems in the body that repair DNA seem to become less effective with age.
	5.3 Free radicals theory	These unstable oxygen molecules can damage cells.

	5.4 Heat shock proteins theory	Such proteins help cells survive stress and diminish in numbers with age.
	5.5 Protein cross-linking theory	Protein cross-links produce excess sugars in the blood stream that can cause protein molecules to literally stick together, leading to aging.
6. Environmental damage theories		Environmental damage to the body's systems accumulates with time.

Chronological *versus* biological age

"Chronological age" is the number of years you have been alive. On the other hand, "biological age" (also called "physiological age") refers to how old are your cells and tissues, based on physiological evidence. These two ages might not be the same. For a specially healthy and fit individual, the biological age may well be lower than the chronological age. But, for a sedentary individual, chronically ill, or in poor physical condition, the biological age may be higher.

Research suggests that biological age is more accurate than chronological age for predicting the onset of disease and death. Here, I look at chronological *versus* biological aging, how biological age is determined, and how we may be able to lower our biological age. Our chronological age is unchangeable. We were born on a particular day and have spent a certain amount of time on this planet. As much as some people may want it, that cannot be changed. However, some people may look or seem much older or younger than their chronological age; for them, biological age may be significantly different from the chronological measure.

Much of how we age is influenced by genetics and beyond our control. But, research shows that aging can be impacted by external factors (acronym D**EL**), including:

- **D**iet,
- **E**nvironment (where living and working), and

- **L**ifestyle (exercise, stress, sleep, alcohol, and smoking habits, etc.).

Biological age is also affected by these factors and more. While it may predict things like whether we will develop diabetes or dementia, or how soon we will die, it may someday become the more important number on our medical chart. We likely have some control over our biological aging and can even get "younger" by making positive changes. Knowing our biological age may provide an incentive to lead a healthier lifestyle.

How biological age is determined

Our biological age is a measurement of how much life we likely have *left*, based on our physiology at any given time. Changes in our genetic material are key to determining our biological age. Two measures are used:

- **Telomeres:** How much they have shortened.
- **DNA methylation:** How DNA is aging.

Research on telomeres and biological age

One study found that people with shorter telomeres were more likely to have:

- Chronic illness or/and
- A neurodegenerative disorder or/and,
- An early death.

Fortunately, maintaining (or adopting) a healthy lifestyle can actually reverse the aging process by lengthening telomeres (see Chapter 10 discussing telomeres).

Research on DNA methylation and biological age

We have actually many more genes than show up at any given time. Some are turned "on" (or expressed) while others are turned "off". The process that turns genes "on" or "off" is called "methylation". It is the switch that governs their expression. Thus, when certain genes are turned off, such as, for example, being exposed to environmental pollution or an illness, our

immune system may have been altered at the genetic level, and we may get sick more often or be predisposed to certain chronic illnesses.

One study sought to discover whether DNA methylation is an accurate way of predicting age. Looking at methylation rates, it showed that most of the tissue and cell samples studied had the same chronological and biological ages, but some did not. The researchers concluded that certain parts of the body age faster than others. For example, healthy breast tissue can be as much as three years older than the rest of the body. If it is next to cancerous tissue, it is an average of 12 years older. Using the methylation-based method of determining biological age, researchers can determine the risk of breast cancer, thus, being every five years biologically *older* than chronologically entails a breast cancer risk of 15% higher.

Factors that determine biological age

There are multiple options to lower our biological age (these are, generally, the same DEL options):

Physical environments

Physical environment includes where we live, work, and spend significant amounts of time. It determines the amount of air pollution and other contaminants or hazardous materials we are exposed to. Toxins can speed up biological aging. It is possible to take steps to avoid or reduce some of this exposure, which could help reverse the effects. While it may be difficult to cut out some of these hazards, other healthy changes, such as more or better sleep, can reverse the aging caused by pollution.

Diet

Eating a high-nutrient diet can lower the biological age. The difference is more significant in people who:

- Have chronic disease,
- Have a family history of chronic disease,
- Are obese,

- Are older, or
- Have a lower education level.

There is a sex-based difference when it comes to choosing foods: Just under 13% of males think about nutrition data at the grocery store, compared to 27.5% of females.

Other studies on diet and biological age suggest the "ideal diet" is:

- Low in calories,
- Plant-based,
- High in fish,
- A Mediterranean diet.

Exercise

Research into biological aging has revealed a connection between higher activity levels and lower biological age. These studies have shown that:

- Not all studies agree that increased exercise has an impact on biological age. Nonetheless, a growing body of research supports the idea that it does.
- A 2020 study found that people trained with aerobic exercise were almost 5.5 years younger than people who were sedentary.
- A 2021 study on post-menopausal women showed a slowdown in some biomarkers of age and disease, including cancer.
- A 2022 study showed that changing the diet and adding more exercise lowers the biological age more than either dietary changes or increased exercise alone.

Stress

Physical and psychological stress both increase the biological age, but in a way that appears to be reversible. There is a rapid increase in biological age (measured by DNA methylation) during the stressful time. However, it goes back to baseline within a few days of the stressor being removed. Also, people who are emotionally resilient and able to regulate their emotions are able to avoid the aging effects of stress.

Smoking

Smoking has long been known to cause serious health problems and shortens lifespan. It increases biological age. As with stress, though, smoking-related age advancement appears to be reversible.

Sleeping habits

Poor sleep quality has a negative impact on health and longevity, and it has also been found to increase biological age. Adults should get at least seven hours of sleep every night. However, many people do not get this amount of sleep. This may be due to lifestyle factors or sleep disorders. Sleep quality is also important when it comes to our health. The ability to get more and/ or better sleep allows one to reverse the biological aging it caused.

We may be able to improve sleep duration or quality by making simple changes, such as going to bed earlier, turning off screens half-an-hour before bed, or reducing distractions in the bedroom. If unable to get better sleep, you may have a sleep disorder that needs to be diagnosed and treated.

Calculating biological age

There is no simple way to calculate biological age. It takes medical tests and a healthcare provider to accurately determine it. Some websites claim to have biological age calculators, and they may take into account some of the factors that influence age. However, without access to the test results, the age so determined may not be scientifically valid and may not be accurate. Sidebar 4.1 discusses the longevity calculator named "RealAge".

Example of survivors of childhood cancer

A 2024 study conducted by the (U.S.) National Institutes of Health/ National Cancer Institute (NIH/ NCI) of 4117 survivors of childhood cancer, utilizing some of the above markers of biological age, found that "cancer survivors: (a) were 5-16 years older than people who never had cancer, (b) they enjoyed fewer years of good health, and (c) certain toxic treatment regimens exaggerated the gap between biological age and years lived".

Biomarkers of aging and aging clocks

If different individuals age at different rates, then, functional capacity, fecundity, and mortality might be better predicted by biomarkers than by chronological age. However, graying of hair, face aging, skin wrinkles, and other common changes seen with aging are not better indicators of future functionality than chronological age. Biogerontologists are continuing efforts to find and validate biomarkers of aging, but success thus far has been limited.

Biomarkers of aging based on their capability to accurately predict biological age include:

- Basic blood biochemistry and cell counts;
- The epigenetic clock; and
- The transcription aging clocks.

Aging clocks have also been used to evaluate impacts of interventions on humans, including combination therapies. (Note: Levels of CD4 and CD8 memory T-cells and naive T-cells have been used to give good predictions of the expected lifespan of middle-aged mice. This approach has not been applied to humans and it is therefore not known if applicable in this case.)

Regarding the determination of the biological age of different tissues or systems or overall, research and development is ongoing for further biomarkers, detection systems, and software systems. For example, using anatomic magnetic resonance images (MRI), a deep learning language (DLL) software estimated brain age with relatively high accuracy, including detecting early signs of Alzheimer's disease and varying neuroanatomical patterns of neurological aging. A DLL tool was also reported to calculate a person's inflammatory age based on patterns of systemic age-related inflammation.

Conclusions and take-aways

- Aging is a complex interaction of genetics, ecogenetics (chemistry and physiology within our body), and epigenetics (effects of the

environment, lifestyle, and behavior). A complete explanation of this phenomenon still eludes us.

- A brief overview of six major categories of aging theories and their variations was provided and tabulated, including: Programmed theories, hormonal theories, error theories, genetic theory, biochemical theory, and environmental damage theories.
- Chronological age is a measurement of how long you have lived. Biological age is a prediction of how long you have left and how likely you are to become chronically ill. Determining biological age requires medical tests for telomere length and biomarkers of DNA methylation, which is the process by which DNA is changed throughout lifetime.
    - Unlike chronological age, biological age can be changed. Things like diet, physical environments, and lifestyle (exercise, stress levels, sleep quality, and smoking) can affect the biological age. Changing these habits can make a big difference.
    - Commercial tests and online calculators claiming to measure biological age may not be valid.
    - If different individuals age at different rates, then functional capacity fecundity, and mortality might be better predicted by biomarkers than by chronological age.
    - Biomarkers of aging based on their capability to accurately predict chronological age include: Basic blood biochemistry and cell counts; the epigenetic clock; and the transcriptomic aging clocks. Aging clocks have also been used to evaluate impacts of interventions on humans, including combination therapies.
    - Regarding the determination of the biological age of different tissues or systems or overall, research and development is ongoing for further biomarkers, detection systems, and software systems.

Sidebar 4.1 – The longevity calculator

The "RealAge" test is a comprehensive longevity calculator. The questions dig deep into your health and medical history to provide more accurate results. The comprehensive lifestyle recommendations included in the results are very helpful and could actually impact your real age.

Description

- A 20-minute longevity calculator to determine your biological age or "RealAge".
- Gives practical and helpful suggestions and information.
- Test requires an email address.

Pros

- Most comprehensive test available.
- Asks detailed questions about health status and conditions.
- Provides good feedback and resources.

Cons

- Requires e-mail for registration.
- Long test duration.

RealAge is a test that predicts just that: your real age. It is considered a longevity calculator, although it does not actually estimate your life expectancy. Rather, your real age acts as more of a prediction of your life expectancy. The test is split into four sections: health, feelings, diet, and fitness. It takes about 20 minutes to complete, which is longer than other longevity calculators. Results appear after completing the test. They include a list of personalized recommendations for each of the test's four sections. These tips on health, feelings, diet, and fitness are meant to improve your lifestyle. They explain what you need to do and why you need to do them. They also contain links to helpful resources that will help you make the changes, such as quick exercises and high-energy meals to start the day.

Sidebar 4.2 - Mortality variation among species

Different speeds with which mortality increases with age correspond to different maximum life span among species (see Table 4.2 and the Gompertz–Makeham law of mortality in Sidebar 3.1, Figure 3.1):

Table 4.2 – Some elderly age variation with species

Species	Elderly age	Notes
Flat worms	Not biologically immortal	Planarians with apparently limitless telomere regenerative capacity
Jellyfish	Immortal	*Turritopsis dohrnii*
Mouse	3 years	
Hydra genus	Negligible senescence	
Ginko trees	Little effect at 667 years	
Sequoia tree	4,000+ years	
Humans	80+	
Cancer cells	Immortal	

Almost all organisms senesce, including bacteria which have asymmetries between "mother" and "daughter" cells upon cell division - the mother cell experiencing aging while the daughter cell is rejuvenated. There is negligible senescence in some groups, such as the genus Hydra. Planarian flatworms have "apparently limitless telomere regenerative capacity fueled by a population of highly proliferative adult stem cells". These planarians are not biologically immortal, but rather their death rate slowly increases with age. Organisms that are thought to be biologically immortal would, in one instance, be *Turritopsis dohrnii*, also known as the "immortal jellyfish", due to its ability to revert to its youth when it undergoes stress during adulthood. The reproductive system is observed to remain intact, and even the gonads of *Turritopsis dohrnii* are existing. Some species exhibit "negative senescence", in which reproduction capability increases or is stable, and mortality falls with age, resulting from the advantages of increased body size during aging.

5

Medical myths about aging

Contents

5

Medical myths associated with aging

Dispelling the many myths associated with aging seems more pressing than at any point in our evolutionary history. Below, I tackle myths associated with exercise, cognitive ability, sex, and more.

Myths associated with exercise

Physical deterioration is inevitable

This is not entirely untrue. As we age, our body does experience wear and tear from decades of use. Research suggests that merely expecting physical deterioration increases its likelihood. Likewise, perceptions of aging may influence the likelihood of seeking medical attention. However, physical deterioration does not have to be complete and it can be slowed down.

As the World Health Organization (WHO) explains, "*Increased physical activity and improving diet can effectively tackle many of the problems frequently associated with old age*" (namely, reduced strength, increased body fat, high blood pressure, reduced bone density, and others). Expectations regarding aging "*play an important role in the adoption of physically active lifestyles in*

older adults and may influence health outcomes, such as physical function". So, although some deterioration is likely, managing expectations will help individuals make better life choices to maintain physical health and fitness later in life.

Older adults should not exercise

This is untrue as keeping active can boost muscle strength, reduce fat, and improve mental health.

Beyond a certain age, there is no point in exercising

This is also untrue as regular exercise does provide benefits at any age, including reducing the risk or developing or delaying the onset of Alzheimer's disease and other forms of dementia. A study, which involved 1,740 older adults, found that regular exercise was "*associated with a delay in the onset of dementia and Alzheimer's disease*". It remains that the vast majority of older adults can indulge in some form of physical activity.

Myths associated with sleep

Older adults need less (or more) sleep

Some people believe that older adults need more (resp. less) sleep than younger adults, perhaps because of the stereotype that older people enjoy a nap (resp. rise early in the morning). While it is true that older adults have more difficulty getting to sleep and that their sleep tends to be more fragmented, explaining why some older adults need to nap in the day. The (U.S.) Centers for Disease Control & Prevention (CDC&P) state that people aged 61–64 need 7–9 hours, and people aged 65 or older need 7–8 hours of sleep each night. However, older adults can handle sleep deprivation better than young adults in a range of measures, including negative affect, depression, confusion, tension, anger, fatigue, and irritability.

Additionally, as the human body changes with age, it can disrupt the circadian (daily) rhythms, which, in turn, can impact sleep. A disruption of the circadian

rhythms can also influence other aspects of physiology, such as hormone levels, which might also impact sleep.

Aside from circadian disruptions, certain diseases that occur more commonly in older adults (osteoarthritis, osteoporosis) can cause discomfort, which might adversely influence an individual's ability to get to sleep or stay asleep. Similarly, conditions causing shortness of breath, including chronic obstructive pulmonary disease (COPD) and congestive heart failure (CHF) can also make sleeping more challenging.

Certain medications more likely taken by older adults (including beta-blockers, bronchodilators, corticosteroids, decongestants, and diuretics) can also interfere with sleep.

Myths associated with certain medical conditions

Only women get osteoporosis

This is not true. Osteoporosis can affect either sex and people of any age. However, osteoporosis is indeed much more common in older people, white people, and females. The International Osteoporosis Foundation (IOF) estimates that, globally, around 1 in 3 women over 50 has osteoporosis, and about 1 in 5 men will experience a bone fracture related to osteoporosis in their lifetime.

Osteoporosis is inevitable for women as they age

As indicated above, two-thirds of women over 50 do not have osteoporosis. To minimize risks, the (U.S.) National Institute on Aging (NIA) advises people to eat foods rich in calcium and vitamin D and exercise regularly.

Myths associated with a decline in cognitive function

Cognitive decline refers to a gradual decrease in mental functioning with age.

Dementia is inevitable as one ages

While age is a risk factor, it is not a cause of dementia. According to the WHO, the risk of developing dementia increases with age, but it does not affect all older adults. Worldwide, an estimated 5%–8% of people over 60 have dementia, meaning that 92%–95% of people aged 60 or older do not have dementia. In the United States, an estimated 13.9% of people over 71 have dementia, meaning that 86.1% of people over 71 do not have dementia (see my books on dementia and other neurodegenerative diseases).

Cognitive decline is inevitable

This is untrue, regardless of the long-held myth that older adults experience a mental slowing down. Importantly, there are ways to reduce the risk.

Cognitive decline leads to dementia

Contrary to popular opinion, cognitive decline does not necessarily signal the start of dementia. Whereas people who go on to develop dementia tend to experience cognitive decline first, not everyone who experiences cognitive decline will develop dementia. It is estimated that 22.2% of people in the U.S. aged 71 or older experience cognitive decline. Of these, each year, 11.7%–20% develop dementia.

In 2015, the Alzheimer's Association (AA) evaluated the evidence of modifiable risk factors for both cognitive decline and dementia. Their report, presented to the World Dementia Council (WDC), explains that "*there is sufficient evidence to support the link between several modifiable risk factors and a reduced risk for cognitive decline*". They further identified that maintaining regular physical activity and managing classical cardiovascular risk factors (diabetes, obesity, smoking, and high blood pressure) were strongly associated with a reduced risk of cognitive decline. They also found good evidence that a healthful diet and lifelong learning or cognitive training also reduce the risk of cognitive decline.

Myths associated with smoking in aging adults

There is no point giving up smoking now

Whether this is a genuine myth or merely an excuse, some older adults say that there is no point in giving up smoking at "their age." This is not true. As the (U.K.) National Health Service (NHS) clearly explains: "*No matter how long you have smoked for and no matter how many cigarettes you smoke a day, your health will start to improve as soon as you quit. Some health benefits are immediate, some are longer-term, but what matters is that it's never too late*".

Myths associated with sex

Sex is rare or impossible as one ages

Some people believe that older adults lose their ability to enjoy sex and that their sexual organs become unfit for that purpose. While it is true that the risk of erectile dysfunction (ED) and vaginal dryness (VD) increases as people age, for most individuals, these are not insurmountable problems. Medications such as Sildenafil (Viagra) and lubricants or hormone creams can work wonders in many cases. An article in the International Journal of Clinical Practice (IJCP) indicates that around 0.4% of men aged 18–29 experience ED, compared with 11.5% of men aged 60–69, meaning that almost 9 out of 10 men in their 60s do not have ED.

Conclusions and take-aways

- Overall, most of the myths surrounding age seem to center on inevitability – the inevitable gradual crumbling into dust as lives become increasingly unbearable, boring, passionless, and painful. However, although certain aspects of health might decline with age, none of the above is inevitable for everyone.
- A positive psychological outlook on aging can benefit the physical aspects of aging.

PART C

PRIMERS ON THE SCIENTIFIC BASES OF AGING

Introduction to Part C

A good understanding of aging and the associated human diseases cannot be had without a serious preliminary grounding in the basic sciences. The purpose of this Part C is to provide such a grounding through a series of five primers on: Biology; Genetics; Epigenetics; Ecogenetics; and Stem cells. Other useful primers consisting of Toxicology and Toxicogenomics; Pharmacogenetics and Pharmacogenomics; Epidemiology; and Paleopathology would go beyond the scope of this volume.

Chapter 6 begins this series of primers with the all too important field of Biology. After a review of the historical milestones in biology, the main pillars of that discipline will be reviewed - the cell (as the basic unit of life), the genes (the basic units of heredity), evolution, homeostasis (the maintenance of a stable and vital condition), and energy (its consumption, transformation, and regulation of its internal environment are critical to the survival of all organisms). The main tenets of cellular biology will also be reviewed (cells, nucleic acids, peptides, proteins, and enzymes). The all too important Universal Genetic Code will also be discussed.

DNA has a central role in the life process in both supplying the information for the synthesis of proteins and the basis for duplicating this information. Mitosis, fertilization, and meiosis assure the constancy of chromosome number and type in every cell of an individual and in all individuals of the same species. An understanding of mitosis and meiosis will provide the basis for predicting the Laws of Inheritance. In order for inheritance to be accessible for study, however, there must exist differences between individuals. These differences are the result of mutations by substitution of one, two or even three nucleotides for others in the genetic sequence and by gross chromosomal aberrations (deletion, duplication, inversion, and

translocation). Environmental effects (physical and chemical agents, ionizing radiation, and mutagens) will be only briefly mentioned.

The Laws of Mendelian Inheritance will be reviewed utilizing a novel mathematical approach (Hermitian algebra), which I have devised and will introduce and illustrate for this purpose. But, many factors can obscure Mendelian inheritance, including importantly the environment and the fact that more than one gene may affect the same character.

Chapter 7 deals with Evolution, which is defined as "*the change in the heritable characteristics of biological populations over successive generations*". It occurs when the evolutionary processes of "natural selection", "genetic drift", "mutation", and "gene flow" act on genetic variation. This results in certain characteristics becoming more or less common within a population over successive generations. The process of evolution has given rise to biodiversity at every level of biological organization.

The Darwin-Wallace theory of evolution by natural selection was conceived as an explanation for why organisms are adapted to their physical and biological environments. It will be established by four major observable facts about living organisms (acronym **OTDH**): *O*ffspring, *T*raits, *D*ifferential (fitness), and *H*eritability (fitness). The modern evolutionary theory (or synthetic theory) will be presented as a combination of evolution with Mendelian inheritance and population genetics in which the basis for heredity is in DNA molecules that pass information from generation to generation. The processes that change DNA in a population include (acronym **SDMF**): (Natural) *S*election, (Genetic) *D*rift, *M*utation, and (Gene) *F*low. Evolution can occur if there is genetic variation within a population. Variation comes from three different processes (acronym **MRF)**: (Genome) *M*utations, (Genes) *R*eshuffling through sexual reproduction, and (Gene) *F*low (or migration between populations).

Throughout the evolutionary history of life on Earth, existing patterns of biodiversity have been shaped by (acronym **SAE**): *S*peciation, *A*nagenesis, and *E*xtinction. Heredity, the inherited characteristics of organisms, will be sketched as it devolves from organismic evolution through changes in heritable characteristics. Heritable characteristics are passed from one generation to the next via DNA.

Three concepts will be advanced to explain how evolution relates to aging, all having as a starting point Darwin's (1859) evolution theory of "survival of the fittest". The evolution process *opposes* aging, *is neutral* regarding aging, or *promotes* the development of biological mechanisms such as aging that purposely limit lifespans of 'old' organisms". This last concept suggests the possibility of finding *anti-aging agents* that interfere with the aging program and generally *delay aging*.

The next chapter, **Chapter 8**, is concerned with Genetics, a field of biology and the life sciences. It is the study of genes, heredity, and genetic variations in living organisms (bacteria, plants, animals, and humans). Its father, Gregor Johann Mendel, observed that traits are inherited by way of discrete units of inheritance, which we refer to as genes. The earlier theories of inheritance (blending inheritance, inheritance of acquired characteristics, pangenesis inheritance and its revived version) have been essentially invalidated (although some of their aspects are still useful). In discussing Mendel's *first law of segregation*, which states that when a pair of organisms reproduce sexually, their offspring randomly inherit one of the two alleles from each parent, I will introduce a simpler mathematical representation (I call it "Mendel's vector") than the traditional Punnett's square. This vector can be used to represent inheritance as a Hermitian vector multiplication. Mendel's *second law of independent assortment* means that the alleles of different genes get shuffled between parents to form offsprings with many different combinations. However, many traits are not discrete features but are instead continuous features and the products of many genes. The influence of these genes is mediated to varying degrees by the environment so that measurement of the heritability of a trait is relative to its environment.

The various genes and associated genetic processes will be discussed in detail, including: genetic processes; nucleotide sequences; chromosomes crossover; large DNA structural changes (such as duplications, inversions, and deletions of entire regions or chromosomal translocation or accidental exchange of whole parts of sequences between different chromosomes); genetic linkage; gene regulation by transcription factors; and mutations that occur during the process of DNA replication, which impact the phenotype of an organism.

The genetic code and the process of protein formation are described. Even though small (~0.5%), inter-individual genetic divergence between humans is largely responsible for the phenotypic variations observed. Some remarks will be made regarding the field of medical genetics and the paradigm shift from nature-*vs*-nurture to nature-*and*-nurture.

Chapter 9 will cover the field of Epigenetics or the study of cellular and physiological traits inherited by daughter cells, but not caused by changes in the DNA sequence. Three types of epigenetic mechanisms will be discussed: Chromatin remodeling; Histone modification; and DNA Methylation.

There are various applications of epigenetics in medicine and drug development, of particular importance in cancer and cancer treatment. Epigenetic pharmaceuticals could be a replacement or adjuvant therapy for currently accepted treatment methods such as radiation and chemotherapy, or could enhance the effects of these current treatments.

Genome engineering has greatly advanced our understanding of how genes shape phenotypes. However, epigenetic processes also influence how cells use genetic information. The use of targetable chromatin modifiers has ushered in a new era of functional epigenomics with findings that may be translated into therapeutic use. Sidebars will present the Human Epigenome Project, and the International Human Epigenome Consortium.

Ecogenetics, the subject of **Chapter 10,** is an important emerging field. In this context, the Environmental Genome Project (EGP), a part of the Human Genome Project of the U.S. National Institute of Environmental Health Sciences (NIEHS) will be broadly outlined. The different classes of environmental agents with their known ecogenetic variation, the nature of their effect, and the associated ecogenetic factors will also be briefly addressed. The various gene-environment interactions that are important in the etiology of diseases will be reviewed in several cases: Cancer (breast, lung, and gastrointestinal; neurodegenerative diseases (Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis); cardiovascular diseases; type II diabetes; and infectious diseases (malaria and HIV/AIDS).

In **Chapter 11**, Stem cells will be reviewed in their different types (adult or somatic; amniotic; embryonic; extra embryonic mesenchymal; fetal;

hematopoietic; induced; mesenchymal stromal) as well as their properties of self-renewal and potency (toti- or omni, pluri-, oligo, and uni-potency). By a procedure called somatic cell nuclear transfer, stem cells can now be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues (muscles, nerves, etc.).

The uses of stem cells to treat or prevent a disease or condition will be outlined in their applications in regenerative treatment (adipose tissue; bone marrow; cardiac muscle; corneal ulcers; dermis; epidermal tissue; joint tissue; ligaments; nervous system; skin; tendons; and others) and in a number of diseases or conditions (bone marrow transplantation; brain and spinal cord injury; dentistry; diabetes type I; hair cell regrowth; heart and cardiovascular diseases; HIV-AIDS; infertility; neuro- and other -degenerative disorders; vision impairment and blindness; orthopedics; wound healing; and others).

6

Primer on Biology

Content

6 Primer on Biology

The word biology derives from the Greek word βιοξ, *bios*, "life" and the suffix λογία, logia, «study of.» The Latin form of the term first appeared in 1736 when Linnaeus (Carl von Linné) used *biology* in his *Bibliotheca botanica*. It was used again in 1766 in a work entitled *Philosophiae naturalis sive physicae: tomus III, continens geologian, biologian, phytologian generalis*, by Michael Christoph Hanov, a disciple of Christian Wolff. The first German use of the term, *Biologie*, was in a 1771 translation of Linnaeus' work. In 1797, Theodor Georg August Roose used the term in a book, *Grundzüge der Lehre van der Lebenskraft*, in the preface. Karl Friedrich Burdach also used it in 1800 in a more restricted sense of the study of human beings from a morphological, physiological, and psychological perspective (*Propädeutik zum Studien der gesammten Heilkunst*). The term came into its modern usage with the six-volume treatise *Biologie, oder Philosophie der lebenden Nature* (1802–22) by Gottfried Reinhold Treviranus.

Biology can be divided in various ways:

- ***Taxonomic group:*** Zoology, botany, microbiology, that is, what were once seen as the major divisions of life;
- ***Biological organization:*** From cell to molecule to organism to population; and

- ***By approach:*** Field biology, theoretical biology, experimental evolution, and paleontology.

These alternative ways of dividing up the subject can be combined with evolutionary biology to create subfields like Evolutionary Ecology and Evolutionary Developmental Biology.

Important milestones

To place biological concepts in their proper perspective, a brief incursion into their historical background would be helpful. Thus, whereas Natural Philosophy was studied in the ancient civilizations of Mesopotamia, Egypt, India, and China, the origins of modern biology and its approach to the study of nature are traced back to ancient Greece. While the formal study of medicine dates back to Hippocrates (ca. 460 BC – ca. 370 BC), it was Aristotle (384 BC – 322 BC) who contributed most extensively to the development of biology. Especially important are his *History of Animals* and other works where he showed naturalist leanings, and later more empirical works that focused on biological causation and the diversity of life. Theophrasus, Aristotle's successor at the Lyceum, wrote a series of books on Botany that survived as the most important contribution of Antiquity to the plant sciences, even into the Middle Ages. More recent important milestones in the development of biology include:

1632-1723: *Antoine Phillips van Leeuwenhoek*, a Dutch business man and scientist, is considered to be the *Father of Microbiology.* He designed and dramatically improved on the microscope. For the first time, in 1676, using his handcrafted microscopes, he observed and reported on microscopic single-celled organisms (now referred to as microorganisms). He was one of the first people (along with Robert Hooke) to discover cells. His microscopic observations covered muscle fibers, bacteria, spermatozoa, and blood flow in capillaries. His main discoveries include: the *infusoria* (or *protists* in modern zoological classification, in 1674); the bacteria (e.g., large *Selenomonads* from the human mouth, in 1676); the v*acuole* of the cell; the *spermatozoa* (in 1677); the banded pattern of *muscular fibers* (in 1682); and other discoveries. He described in

minute details the disease with which he was afflicted (an uncontrolled movement of the midriff) now known as the *van Leeuwenhoek disease*.

1637-1680: *Jan Swammerdam*, a Dutch physician, biologist, entomologist, and microscopist carried out experiments on muscle contraction. In 1658, he was the first to observe and describe red blood cells (RBC) and to use the microscope in dissections and staining. His techniques remained useful for hundreds of years. He used his entomological observations to bolster his case for epigenesis in his 1669 publication, *Historia Insectorum Generalis* (The Natural History of Insects). He was also a student of anatomy and played a key role in the development of our current understanding of nerve-muscle function by evidencing that the brain, not the circulatory system, was responsible for muscle contraction. This motion or irritation of the nerve alone is necessary to produce muscular motion. The idea that nerve stimulation led to movement had important implications for neuroscience by putting forward the notion that behavior is based on stimuli. He also discovered valves in the lymphatic system, which were later dubbed *Swammerdam valves*. A method he invented for the preparation of hollow human organs was later much employed in anatomy.

1656-1725: Instructed in optics by his compatriot Antoine van Leeuwenhoek, and in collaboration with him and others, Nicolaas Hartsocker co-discovered spermatozoids in 1674. He then postulated the existence of tiny men inside the sperm, which he called *homunculi* or *animalcules* that form part of his *Spermist Theory* -- a theory discarded in later years. He also invented the screw- barrel microscope (*circa* 1694).

1667-1721: *Robert Boyle,* an Irish chemist, now considered as one of the *Founders of Modern Chemistry,* and one of the pioneers of the modern scientific method, co-discovered the *Power- Boyle's Law*, which describes the inversely proportional relationship between the absolute pressure and volume of a gas, if the temperature is kept constant within a closed system. In addition, he contributed several other scientific discoveries including the bubble formation in living tissue due to reduction in ambient pressure (in 1670) and the decompression sickness (in 1670). He published his *Memoirs for the Natural History of the Human Blood* (in 1684) and his *Medicina Hydrostatica* (in 1690). He also described and studied the chemistry of combustion and respiration in physiology.

1707-1788: *Georges-Louis Leclerc, Comte de Buffon,* was a French naturalist. His works influenced the next two generations of naturalists, including Jean-Baptiste Lamarck and George Cuvier. Buffon published thirty-six *quarto* volumes of his *Histoire Naturelle* (Natural History) during his lifetime with additional volumes based on his notes and further research being published in the two decades following his death. It has been said that "Truly, Buffon was the father of all thought in natural history in the second half of the 18th century". He was not an evolutionary biologist, yet he was the father of evolutionism. He was the first person to discuss a large number of evolutionary problems, problems that before him had not been raised by anybody.... he brought them to the attention of the scientific world. Except for Aristotle and Darwin, no other student of organisms (whole animals and plants) has had as far-reaching an influence. He brought the idea of evolution into the realm of science. He developed a concept of the "unity of type", a precursor of comparative anatomy. More than anyone else, he was responsible for the acceptance of a long-time scale for the history of the earth. He was one of the first to imply that you get inheritance from your parents, in a description based on similarities between elephants and mammoths. And yet, he hindered evolution by his frequent endorsement of the immutability of species. He provided a criterion of species and fertility among members of a species that was thought impregnable.

1736: Advances in microscopy also had a profound impact on biological thinking. Meanwhile, taxonomy and classification became the focus of natural historians. *Carl von Linné* (later latinized to Linnaeus) published a basic taxonomy for the natural world in 1735, variations of which have been in use ever since. In the 1750s, he introduced scientific names for all world's species (more than 12,000 species). This is known as the "binomial system" of nomenclature – the *Genus species* system. For humans, he chose *Homo sapiens* (meaning "wise man").

1744-1829: *Jean Baptiste Pierre Antoine de Monet, Chevalier de Lamarck,* a French naturalist, was an early proponent of the idea that evolution occurred and proceeded in accordance with natural laws. Although a premier authority on invertebrate zoology, in the modern era, Lamarck is widely remembered for a theory of *inheritance of acquired characteristics* (called *soft inheritance, Lamarckism* or *use/disuse theory).* He was the

first to present a coherent theory of evolution. He posited that evolution was the result of environmental stress on properties of animals, meaning that the more frequently and rigorously an organ was used, the more complex and efficient it would become, thus adapting the animal to its environment. Lamarck believed that these acquired traits could then be passed on to the animal's offspring, who would further develop and perfect them. His contribution to evolutionary theory represented the first truly cohesive theory of evolution in which an alchemical complexional force drove organisms up a ladder of complexity, and a second environmental force adapted them to local environments through *use and disuse* of characteristics, differentiating them from other organisms. Although he was not the first thinker to advocate organic evolution, he was the first to develop a truly coherent evolutionary theory. He outlined his theories regarding evolution in four seminal works: (a) *Floreal Lecture* (Conference Florale,1800), *(b) Recherches sur l'Organisation des Corps Vivants* (Research on the Organization of Living Bodies, 1802), in which he drew out his theory on evolution, that is, all life was organized in a vertical chain, with gradation between the lowest forms to the highest forms of life, thus demonstrating a path to progressive developments in nature. He stressed two main themes in his biological work: The environment gives rise to changes in animals (examples of evidence of this principle: Blindness in moles, presence of teeth in mammals, absence of teeth in birds); and life was structured in an orderly manner so that many different parts of all bodies make it possible for the organic movements of animals; (c) *Philosophie Zoologique* (Zoological Philosophy, 1809); and (d) *Histoire Naturelle des Animaux sans Vertèbres* (Natural History of Invertebrate Animals), in seven volumes (1815–1822). He used several mechanisms to explain the two forces he saw as comprising evolution: A force driving animals from simple to complex forms in a steady, predictable way based on the fundamental physical principles of alchemy, and a force adapting animals to their local environments and differentiating them from each other. He believed that these forces must be explained as a necessary consequence of basic physical principles, favoring a materialistic attitude toward biology. He also referred to a tendency for organisms to become more complex, moving "up" a ladder of progress. He referred to this phenomenon as *Le Pouvoir de la Vie*

(or The Power of Life) or *La Force qui Tend Sans Cesse à Composer l'Organisation* (or The Force that Perpetually Tends to Make Order). Like many natural historians of his time, Lamarck believed that organisms arose in their simplest forms via ongoing spontaneous generation (a hypothesis later disproved by Pasteur). He also run against the modern chemistry promoted by Lavoisier. Lamarck issued two laws: The First Law states that: "In every animal which has not passed the limit of its development, a more frequent and continuous use of any organ gradually strengthens, develops, enlarges that organ, and gives it a power proportional to the length of time it has been so used, while the permanent disuse of any organ imperceptibly weakens and deteriorates it, and progressively diminishes its functional capacity, until it finally disappears". The first law says little except "an exaggerated generalization of the belief that exercise develops an organ". The Second Law (known as *soft inheritance*) is of greater interest here. It states that: "All the acquisitions or losses wrought by nature on individuals, through the influence of the environment in which their race has long been placed, and hence through the influence of the predominant use or permanent disuse of any organ, are all preserved by reproduction to the new individuals which arise, provided that the acquired modifications are common to both sexes, or at least to the individuals which produce the young. The latter law was widely accepted at the time but has been decisively rejected by modern genetics. However, in the field of epigenetics, there is growing evidence that soft inheritance plays a part in changing some organisms' phenotype: it leaves the DNA unaltered but affects DNA by preventing or stimulating the expression of genes. Some epigenetic changes, such as the methylation of genes, alter the likelihood of DNA transcription and can be produced by changes in behavior and environment. Many epigenetic changes are themselves heritable to a degree. Thus, while DNA itself is not directly altered by the environment and behavior except through selection, the relationship of the genotype to the phenotype can be altered, even across generations, by experience within the lifetime of an individual. This has led to calls for biology to reconsider Lamarckian processes in evolution in light of modern advances in molecular biology.

1776-1847: The German physiologist *Karl Friedrich Burdach* used the term "biologie" in a more restricted sense of the study of human beings from a morphological, physiological, and psychological perspective (*Propädeutik zum Studien der gesammten Heilkunst,* 1800*)*. In 1822, he provided the names *arcuate fasciculus* (due to the arching shape of its longest fibers) and *fasciculus cuneatus* (for the lateral portion of the dorsal funiculars of the spinal cord).

1776-1837: *Gottfried Reinhold Treviranus* published the six-volume treatise *Biologie, oder Philosophie der lebenden Nature,* which ushered in the modern usage of the term biologie.

1809-1882: *Charles Robert Darwin*, an English naturalist and geologist, is best known for his contributions to evolutionary theory (*On The Origin of Species, 1859*). *C*ombining the biogeographical approach of *Humboldt*, the uniformitarian geology of *Lyell*, *Malthus*' writings on population growth, and his own morphological expertise and extensive natural observations, he forged a successful evolutionary theory based on natural selection. Similar reasoning and evidence led *Alfred Russell Wallace* to independently reach the same conclusions. Although it was the subject of controversy (which continues to this day), Darwin's theory quickly spread through the scientific community and soon became a central axiom of the rapidly developing science of biology. Darwin established that all species of life have descended over time from common ancestors. In a joint publication with Wallace, he introduced his scientific theory that this branching pattern of evolution resulted from a process that he called *natural selection*, in which the struggle for existence has a similar effect to the artificial selection involved in selective breeding. Darwin's scientific discovery is the unifying theory of the life sciences, explaining the diversity of life. It established evolutionary descent with modification as the dominant scientific explanation of diversification in nature. He examined human evolution and sexual selection in *The Descent of Man*, and *Selection in Relation to Sex*, followed by *The Expression of Emotions in Men and Animals*. The development of the modern *evolutionary synthesis* from the 1930s to the 1950s, incorporating *natural selection* with *population genetics* and *Mendelian genetics* brought broad scientific consensus that natural

selection was the basic mechanism of evolution. This synthesis set the frame of reference for modern debates and refinements of the theory.

1810-1882: The German physiologist *Theodor Schwann* (considered the *Father of Embryology* and of *Modern Histology*) has many contributions to biology. These include the development of cell theory and the discovery of the *Schwann cells* in the peripheral nervous system, the discovery and study of pepsin (an enzyme essential to digestion), the discovery of muscle contraction, the discovery of the organic nature of yeast, and the invention of the term metabolism. In 1833, together with *Matthias Jakob Schleiden*, and thanks to the work of *Robert Remak* and the pathologist *Rudolf Virchow*, he began promoting the now universal ideas that the cell is the basic unit of organisms and individual cells have all the characteristics of life. However, they opposed the idea that all cells come from the division of other cells. In 1857, with Virchow, he posed the maxim *Omnis cellula e cellula*—that every cell arises from another cell. By the 1860s, cell doctrine became the conventional view of the elementary anatomical composition of plants and animals. Schwann's theory and observations became the foundation of modern histology. He broke away from *vitalism* and worked towards a physico-chemical explanation of life. Through observation and experimentation, he disconfirmed the question of spontaneous generation.

1822-1895: *Louis Pasteur*, a French chemist and microbiologist, is considered to be the *Father of the Germ Theory of Disease* and its application in clinical medicine together with *Ferdinand Cohn* and *Robert Koch*, the *Father of Modern Microbiology*. He is renowned for his discoveries of the principles of vaccination, the germ (microbial) theory of fermentation (initially suggested by *Girolamo Fracastoro, Agostino Bassi*, and *Friedrich Henle* as caused by the growth of micro-organisms), the emergent growth of bacteria in nutrient broths as due not to spontaneous generation but rather biogenesis and pasteurization. He is remembered for his remarkable breakthroughs in the causes and preventions of diseases, and his discoveries have saved countless lives ever since. He reduced mortality from puerperal fever, and created the first vaccines for rabies and anthrax. The notion that a weak form of a disease could cause immunity to the virulent version was not new; this had been known for a long time for smallpox. However, the difference between smallpox

vaccination and anthrax or chicken cholera vaccination was that the weakened form of the latter two disease organisms had been "generated artificially", so a naturally weak form of the disease organism did not need to be found. This discovery revolutionized work in infectious diseases. Pasteur is best known to the general public for his invention of the technique of treating milk and wine to stop bacterial contamination, a process now called "pasteurization". Beverage contamination led him to the idea that microorganisms infecting animals and humans cause disease. He proposed preventing the entry of microorganisms into the human body, leading *Joseph Lister* to develop antiseptic methods in surgery. He also discovered anaerobiosis (called the *Pasteur Effect)* whereby some microorganisms can develop and live without air or oxygen.

1834-1919: *Ernst Heinrich Philipp August Haeckel,* a German biologist, zoologist and physician, discovered, described and named thousands of new species, mapped a genealogical tree relating all forms of life (in his treatise *Tree of Life,*1879), and coined many terms in biology, including *anthropogeny*, *ecology*, *phylum, ontogeny* (or development of form), *phylogeny* (or evolutionary descent)*, stem cell, heterochrony* (or change in timing of embryonic development over the course of evolution), *etc.* This concept has since been refuted in favor of *Karl Ernst von Baer*'s "weak recapitulation hypothesis" where recapitulation means that what is repeated (and built upon) is the ancestral embryonic development process. He promoted and popularized Charles Darwin's work in Germany but did not support natural selection, rather believing in a Lamarckian inheritance of acquired characteristics. His ideas are important to the history of evolutionary theory, his chief interests lying in evolution and life development processes in general, including development of nonrandom forms.

1867: *John Lister* showed that it was possible to reduce the dreadful 43% mortality rate in amputations to 15% through the use of carbolic acid.

1860s: *Ignaz Semmelweis, a* Check physician and surgeon, discovered that the terrible scourge of childbed or puerperal fever could be prevented by having medical students wash their hands before, instead of after birth.

1903: *P.O. Wright* and *A. E. Douglas* discovered that factors in the blood seemed to coat bacteria and make them much more liable to being taken-up by the body's defensive cells.

20th Century: Development of vaccines.

The main pillars of Biology

As a natural science, biology studies life and living organisms (including their structure, growth, function, distribution, evolution, and taxonomy). It includes many branches of which the following are of particular importance here: Aerobiology, anatomy, biochemistry, bioengineering, biomechanics, biophysics, biotechnology, cell biology, developmental biology, ecology, embryology, epigenetics, evolutionary biology, genetics, hematology, histology, integrative biology, microbiology, molecular biology, mycology, neurobiology, parasitology, pharmacology, physiology, structural biology, and virology.

The five main pillars of biology, later discussed, are: cell theory, genetics, evolution, homeostasis, and energy. Biology recognizes:

- ***Cell:*** The basic unit of life (with all cells coming from the division of other cells);
- ***Central dogma:*** A framework for understanding the transfer of sequence information between information-carrying biopolymers, in the most common or general case, in living organisms.
- ***Energy:*** All organisms survive by consuming and transforming energy and by regulating their internal environment.
- ***Evolution:*** The engine that propels the synthesis and creation of new species;
- ***Gene:*** The basic unit of heredity; and
- ***Homeostasis:*** Energy transformation and consumption maintain a stable and vital condition.

Ecology is that sub-discipline that examines how organisms interact with their environment. More recent observations and research on the cell structure, function, and evolution have resulted in the development of epigenetics and

ecogenetics, topics of special interest in this book (see next chapters), as the theory for disease causation and transmission.

Foundations of biology

Now, back to the very foundations of biology.

Cell theory

Cell theory states that the cell is the fundamental unit of life. Every cell in the organism's body derives ultimately from a single cell in a fertilized egg. All cells, whether in unicellular or multicellular organisms, arise from other cells through cell division. All living things are composed of one or more cells or the secreted products of those cells. The cell is also considered to be the basic unit in many pathological processes. In addition, the phenomenon of energy flow occurs in cells in processes that are part of the function known as metabolism. Finally, cells contain hereditary information (DNA) which is passed from cell to cell during cell division.

The central dogma of molecular biology

The "central dogma of molecular biology" is an explanation of the flow of genetic information within a biological system. Note that the word "dogma" should not be literally interpreted as "*a belief that cannot be doubted*" or "an idea for which there was *no reasonable evidence*". Here, according to Crick who advanced it, it "...*could just as well have* (been) *called the 'Central hypothesis' …it was just a catch phrase*".) The simplistic version of it [DNA → RNA → protein pathway] published by James Watson (of the famed Watson-Crick double helix model) does not hold and remains invalid today. The version of it, initially proposed by Francis Crick in 1958, and restated by him in 1970, is more general and remains valid: "*The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred back from protein to either protein or nucleic acid".* It can be summarized in Table 6.1:

Table 6.1 - General transfers of biological sequential information:

The three classes of information transfer suggested by the "central dogma"

General Transfers	Special Transfers	Unknown Transfers
Replication DNA → DNA	***Replication*** RNA → RNA	protein → RNA
Transcription DNA → RNA	***Reverse transcription*** RNA → DNA	protein → DNA
Translation RNA → protein	***Translation*** DNA → protein	protein → protein

Thus, the "dogma" is a framework for understanding the transfer of sequence information between information-carrying biopolymers, in the most common or general case, in living organisms. There are 3 major classes of such biopolymers: DNA and RNA (both nucleic acids), and protein (amino acids). There are 3×3=9 conceivable direct transfers of information that can occur between these. The dogma classes these into 3 groups of 3:

- ***General transfers (column 1):*** There are three general transfers (believed to occur normally in most cells). They describe the normal flow of biological information: DNA information can be copied to DNA (DNA "replication"); DNA information can be copied into RNA ("transcription"); and proteins can be synthesized using the information in m-RNA as a template ("translation");
- ***Special transfers (column 2):*** There are three special transfers that are known to occur, but only under specific conditions in case of some viruses or in a laboratory. They describe DNA being synthesized using: RNA being copied from RNA ("replication'); an RNA template ("reverse transcription"); and proteins synthesized directly from a DNA template without the use of m-RNA ("translation"); and
- ***Unknown transfers (column 3):*** There are three unknown transfers that are believed never to occur. They describe: RNA being synthesized using the primary structure of a protein as a template; DNA synthesized

using the primary structure of a protein as a template; and a protein being copied from a protein,

The biopolymers that comprise DNA, RNA and (poly)peptides are linear polymers (i.e., each monomer is connected to at most two other monomers). The sequence of their monomers effectively encodes information. The transfers of information described by the central dogma ideally are faithful, deterministic transfers wherein one biopolymer's sequence is used as a template for the construction of another biopolymer with a sequence that is entirely dependent on the original biopolymer's sequence.

The processes of replication, transcription, translation and reverse transcription are well known and have been described earlier in this Chapter. Direct translation from DNA to protein has been demonstrated in a cell-free system (i.e. in a test tube), using extracts from *E. coli* that contained ribosomes, but not intact cells. These cell fragments could synthesize proteins from single-stranded DNA templates isolated from other organisms (e.g., mouse or toad), and neomycin was found to enhance this effect. However, it was unclear whether this mechanism of translation corresponded specifically to the genetic code.

Energy

Human life theory posits that the survival of a living organism depends on the continuous input of energy. Chemical reactions that are responsible for its structure and function are tuned to extract energy from substances that act as its food and transform them to help form new cells and sustain them. In this process, molecules of chemical substances that constitute food play two roles: They contain energy that can be transformed for biological chemical reactions, and they develop new molecular structures made up of biomolecules. The organisms responsible for the introduction of energy into an ecosystem are known as producers or autotrophs. Nearly all of these organisms originally draw energy from the Sun. Plants and other phototrophs use solar energy via a process known as photosynthesis to convert raw materials into organic molecules, such as adenosyne tri-phosphate (ATP) whose bonds can be broken to release energy. A few ecosystems, however, depend entirely on energy extracted by chemotrophs from methane, sulfides,

or other non-luminal energy sources. Some of the captured energy is used to produce biomass to sustain life and provide energy for growth and development. The majority of the rest of this energy is lost as heat and waste molecules. The most important processes for converting the energy trapped in chemical substances into energy useful to sustain life are metabolism and cellular respiration.

Evolution theory

Evolution theory posits that life changes and develops through evolution, and that all known life-forms have a common origin. Evolution is now used to explain the great variations of life found on Earth. It further postulates that all organisms on Earth, both living and extinct, have descended from a common ancestor or an ancestral gene pool. This last universal common ancestor (LUCA) of all organisms is believed to have appeared about 3.5 billion years ago. Biologists generally regard the universality and ubiquity of the genetic code as definitive evidence in favor of the theory of universal common descent for all bacteria, archaea, and eukaryotes. The evolutionary history of the species, which describes the characteristics of the various species from which it descended, together with its genealogical relationship to every other species is known as its phylogeny. Widely varied approaches to biology generate information about phylogeny. These include the comparisons of DNA sequences conducted within molecular biology or genomics, and comparisons of fossils or other records of ancient organisms in paleontology. Biologists organize and analyze evolutionary relationships through various methods, including phylogenetics, phenetics, and cladistics. (See Chapter 7 for more discussion.)

Gene

The discovery of the physical representation of heredity came along with evolutionary principles and population genetics. In the 1940s and early 1950s, experiments pointed to DNA as the component of chromosomes that held the trait-carrying units that had become known as genes. A focus on new kinds of model organisms such as viruses and bacteria, along with the discovery of the double helical structure of DNA in 1953, marked the

transition to the era of molecular genetics. From the 1950s to the present times, biology has been vastly extended in the molecular domain. The genetic code was cracked by *Har Gobind Khorana*, *Robert W. Holley* and *Marshall Warren Nirenberg* after DNA was understood to contain codons. Finally, the *Human Genome Project* (HGP) was launched in 1990 with the goal of mapping the general human genome. This project was essentially completed in 2003, with further analysis still being published. It was the first step in a globalized effort to incorporate accumulated knowledge of biology into a functional, molecular definition of the human body and the bodies of other organisms.

Genetics is the science of genes, heredity, and the variation of organisms. Genes encode the information necessary for synthesizing proteins, which in turn play a central role in influencing the final phenotype of the organism. In modern research, genetics provides important tools in the investigation of the function of a particular gene, or the analysis of genetic interactions. Within organisms, genetic information generally is carried in chromosomes, where it is represented in the chemical structure of particular DNA molecules. In genetics theory, genes are the primary units of inheritance in all organisms. A gene is a unit of heredity and corresponds to a region of DNA that influences the form or function of an organism in specific ways. All organisms, from bacteria to animals, share the same basic machinery that copies and translates DNA into proteins. Cells transcribe a DNA gene into an RNA version of the gene, and a ribosome then translates the RNA into a protein, a sequence of amino-acids. The translation code from RNA codon to amino acid is the same for most organisms, but slightly different for some. For example, a sequence of DNA that codes for insulin in humans also codes for insulin when inserted into other organisms, such as plants. DNA usually occurs as linear chromosomes in eukaryotes, and circular chromosomes in prokaryotes. A chromosome is an organized structure consisting of DNA and histones. The set of chromosomes in a cell and any other hereditary information found in the mitochondria, chloroplasts, or other locations is collectively known as its genome. In eukaryotes, genomic DNA is located in the cell nucleus, along with small amounts in mitochondria and chloroplasts. In prokaryotes, the DNA is held within an irregularly shaped body in the cytoplasm called the nucleoli. The genetic information in a genome is held within genes, and

the complete assemblage of this information in an organism is called its genotype.

Homeostasis

Homeostasis is the ability of an open system to regulate its internal environment to maintain stable conditions by means of multiple dynamic equilibrium adjustments controlled by interrelated regulation mechanisms. All living organisms, whether unicellular or multicellular, exhibit homeostasis. To maintain dynamic equilibrium and effectively carry out certain functions, a system must detect and respond to perturbations. After the detection of a perturbation, a biological system normally responds through negative feedback. This means stabilizing conditions by either reducing or increasing the activity of an organ or system. One example is the release of glucagon when sugar levels are too low.

Despite the profound advances made over recent decades in our understanding of life's fundamental processes, some basic problems have remained unresolved. One such problem is the **biological basis of aging**. At present, **there is no consensual view on the underlying cause of aging.**

Cellular biology

Cells

A human being can theoretically be resolved into his/her constituent cells. Although no one cell type is exactly like all others, cells do have many common structural and functional features which are traditionally shown through the concept of a generalized cell. Distinguishing features include the shape, size, internal structure, composition, and function of the various cell types. Almost all cells are highly differentiated, have specific functions, and are made up of three main regions or parts – a *nucleus*, a *plasma* (or cell) *membrane*, and a *cytoplasm*. The nucleus is usually located near the center of the cell. It is surrounded by the semifluid cytoplasm. The cytoplasm, in turn, is surrounded by the plasma membrane, which forms the outer cell boundary.

The plasma membrane has a core of two lipid layers (phospholipids and cholesterol) in which protein molecules (some of which may be enzymes) float. Many of the proteins mounted on the cell exterior are receptors, or binding sites, for hormones or other chemical messengers. Most proteins that span the membrane are involved in transport functions. Branching sugar groups (called glycoproteins) are attached to most of the proteins abutting the extracellular space. They act as receptors that certain bacteria, viruses, or toxins can bind to, and play a role in cell-to-cell interactions. For example, definite changes in glycoproteins occur in cells that are being transformed into cancer cells.

The cytoplasm is the site of most cellular activities (the "factory area" of the cell). It has three major components: the *cytosol, organelles,* and *inclusions*. The cytosol dissolves the other elements, nutrients, and other solutes. The organelles are the metabolic machinery of the cell, each organelle-type carrying out a specific function for the cell as a whole, some synthesizing proteins, others packaging these proteins, and so on. The inclusions are not functioning units, but chemical substances such as stored nutrients or cell products (fat droplets, glycogen granules, melanin pigments, mucus and other excretory products, and various kinds of crystals).

The cytoplasm and the nucleus contain other important chemical constituents, many of which belong to another class of large molecules, the proteins, which are made of one or more folded filaments called polypeptides. A polypeptide is a linear series of equally spaced elements called amino acids.

The principal physical characteristics of cell constituents are summarized in Table 6.2 below:

Table 6.2 - Physical characteristics of cell constituents

Cellular properties	Quantity
Cell diameter	$12x10^{-8}$ m (= 0.12 µm = 120 nm)
Number of constituent cells per adult individual	10^{14} (one hundred thousand billion)
Amount of DNA per human cell	$6x10^{-12}$ grams (six one-thousand-billionth)

DNA strand diameter/length	$2x10^{-9}$ m (=2 nm), almost endlessly long
Nucleotide pair length	$0.34x10^{-9}$ m (0.34 nm)
Number of nucleotide pairs in a single human cell	$6x10^{9}$ (six billion)
Length of longest chromosome	6 cm
Diameter of a nucleus containing all 46 chromosomes	$1x10^{-4}$ mm (=10^{-2} nm)

Note: 1 meter [m]=10^2 centimeter [cm]=10^3 millimeters [mm]=10^6 microns [μm]= 10^9 nanometers [nm]

Only cells that are potentially able to reproduce possess a nucleus (the cell's control center). The nucleus has two distinct regions or structures: the *nuclear membrane* (or envelope) and the *nucleoplasm*. The nuclear membrane is a double membrane barrier bounding the nucleus. It is selectively permeable, but passage of material through it is much freer than elsewhere because of its relatively large pores. Also it encloses the nucleoplasm in which the nucleoli and chromatin are suspended. Nucleoli are sites where ribosomes are assembled to serve as actual sites of protein synthesis as per DNA's specific instructions. When a cell is not dividing, its DNA is combined with protein to form chromatin that is scattered throughout the nucleus. When a cell is dividing to form two daughter cells, the chromatin threads coil and condense to form chromosomes. DNA is organized in chromosomes.

Nucleic Acids

Nucleic acids play a fundamental role. They make up the genes, which provide the basic blueprint of life and do this entirely by dictating the proteins' structure. Composed of carbon, oxygen, hydrogen, nitrogen and phosphorous atoms, nucleic acids are the largest biological molecules in the body. Notwithstanding their diversity, all cells contain the same amount and type of DNA. A cell that has lost or ejected its nucleus (for whatever reason) is literally programmed to death. Whereas every cell contains all of the same information, it uses but a fraction of it to develop its specific activities. Per human cell, the amount of DNA is very small (see Table 6.2 above), but the

information contained therein is enormous and sufficient for the purpose of directing the synthesis of a human individual.

The building blocks of nucleic acids, the *nucleotides*, are quite complex. Each consists of three basic parts: a nitrogen-containing base, a pentose sugar, and a phosphate group. The bases come in five varieties: Adenine (A), Guanine (G), Thymine (T), Cytosine (C), and Uracil (U). A and G are large, two-ring bases, whereas the others are smaller, single-ring structures. The nucleotides are named according to the base they contain, for example, A-containing bases are adenine nucleotides.

The two major kinds of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA and RNA differ in many respects. DNA is the generic material found within the cell nucleus. It has two fundamental roles: It replicates itself exactly before a cell divides, thus ensuring that the genetic information in every body daughter cell is identical; and it provides the instructions for building every protein in the body. RNA is located outside the nucleus, and can be considered the "molecular slave" of DNA, that is, RNA carries out the orders for protein synthesis issued by DNA.

Although both DNA and RNA are formed by the joining together of nucleotides, their final structures are different. A DNA molecule is usually a very long double strand, each strand (or *chromatid*) being made of a series of sequentially attached nucleotides (A, G, T, and C) and its sugar is deoxyribose. The two strands carry substantially the same information in complementary forms and are strictly paired along their length. Structural reasons restrict which nucleotides can be opposite each other in the complementary strands according to certain *Rules of Complementarity*: A faces T and vice versa with the total quantity of A being equal to that of T, and likewise for G and C. The nucleotide pairs AT, TA, GC and CG are the only allowable combinations. The two strands are held together by hydrogen bonds between the bases so that a ladder-like molecule is formed. Alternating sugar and phosphate molecules form the uprights or backbones of the ladder, and each "rung" is formed of two joined bases (one base pair). The whole molecule is then coiled into a spiral staircase-like structure called a double helix.

Whereas DNA is double stranded, RNA molecules are single nucleotide strands. RNA bases are: Adenine (A), Guanine (G), Uracil (U), and Cytosine (C), that is the same as for DNA except that Uracil (U) has been substituted for Thymine (T) with the same pairing properties (AU, UA, GC and CG). Its sugar is ribose instead of deoxyribose.

There are twenty main types of amino acids called *standard or canonical amino acids* (Table 6.3). All amino acids have an amino group, which gives them basic properties, and an acid group, which allows them to act as acids. Each amino acid is made of one short common element that has one end with acidic properties and another with alkaline properties. Amino acids attach to one another to form polypeptide chains, an acidic end uniting with the alkaline end of another amino acid element, and so on.

Polypeptides

Amino acids are joined together in chains, which form large, complex protein molecules that contain from 50 to thousands of amino acids. Amino acid chains containing fewer than 50 amino acids are called *polypeptides*. Polypeptides found in the human body can be formed by as few as a dozen or as many as a few hundred amino acids. The number, types, and sequences of the amino acids in a polypeptide chain determine its properties. A polypeptide tends to assume a given shape by folding into a three-dimensional way determined by the physicochemical properties of its constituent amino acids.

Table 6.3 - The twenty standard (canonical) amino acids

Amino Acid	Symbol	Amino acid	Symbol
Alanine (Ala)	A	**Leucine (Leu)**	L
Arginine (Arg)	R	**Lysine (Lys)**	K
Asparagine (Asn)	N	**Methionine (Met)**	M
Aspartic acid (Asp)	D	**Phenylalanine (Phe)**	F
Cysteine (Cys)	C	**Proline (Pro)**	P
Glutamic acid (Glu)	E	**Serine (Ser)**	S
Glutamine (Glu)	Q	**Threonine (Thr)**	T
Glycine (Gly)	G	**Tryptophan (Try)**	W
Histidine (His)	H	**Tyrosine (Tyr)**	Y
Isoleucine (Ile)	L	**Valine (Val)**	V

Note: Do not confuse the amino acids A, G, T, C with the bases denoted by the same symbols

Proteins and enzymes

Proteins

Proteins account for over 50% of the organic matter in the body, and they have the most varied functions of the organic molecules. Some are construction materials, others play vital roles in cell function. Like carbohydrates and lipids, proteins contain carbon, oxygen, and hydrogen. In addition, they contain nitrogen and sometimes sulfur atoms as well. The shapes of protein molecules differ widely. The number of different protein molecules in existence is enormous, probably in the hundreds of thousands for an organism like man. Some are very abundant in certain tissues, others (for example, regulatory proteins) are represented by a few molecules found in relatively few cells of the body. As a consequence of their different physicochemical properties and shape, every protein has a different function. Some protein functions involve transfer or storage of a variety of chemical substances (Table 6.4). The building blocks of proteins are the amino acids reviewed previously.

Based on their overall shape and structure, proteins are classed as either fibrous or globular proteins. The stand-like fibrous proteins, also called structural proteins, appear most often in body structures together and for providing strength in certain body tissues. Globular proteins are mobile, generally spherical molecules that play crucial roles in virtually all biological processes. Because they do things rather than just form structures, they are also called functional proteins. Some (antibodies) help to provide immunity, others (hormones) help to regulate growth and development; still others, called enzymes (see below) are biological catalysts that regulate essentially every chemical reaction that goes on within the body.

Table 6.4 - Certain protein functions

Protein(s)	Function	Protein(s)	Function
Hemoglobin, Myoglobin	Take up, store, transport, and release oxygen	**Collagen**	Role in connective tissue
Ferredoxin	Similar with iron	**Elastin**	Role in elastic fibers
Haptoglobin	Binds hemoglobin	**Gamma globulins**	Bind with specific foreign substances (antigens) to protect against them and facilitate their capture and elimination

Enzymes

Enzymes form the most common type of proteins. They catalyze all the chemical reactions that occur in the body. In this capacity, they are capable of increasing enormously the rate of some special reactions. Although those reactions might exist even in the absence of catalysis, their rates may be so slow as to be practically meaningless. In general, there is a specific enzyme for each step of each of the many biochemical processes that go on in the body. Enzymes are thus responsible for the metabolism of all small molecules, namely the two complementary types of reactions: degradative reactions that free the chemical energy contained in various compounds and make it available for other processes, and synthetic reactions needed to transform molecules available in the environment into those necessary

for growth and reproduction of cells. There are many thousands of different enzymes in a cell, each with some metabolic function.

The Universal Genetic Code and protein synthesis

The Universal Genetic Code

Twenty-four amino acids are naturally incorporated into polypeptides (this is the number of all possible combinations of the four bases, A, G, T, and C taken three at a time = n!/(n-r)! = 24, where n=4, r=3 and ! is the factorial symbol). They are called proteinogenic (that is, used to build proteins) or natural or else canonical amino acids. Of these, 20 are encoded by the Universal Genetic Code (UGC), three are chain ends of which two, selenocysteine and pyrrolysine, are incorporated into proteins by unique synthetic mechanisms, and one is for the initiation of the nucleotide. Aside from these proteinogenic amino acids, there are many other amino acids that are called non-proteinogenic. Those either are not found in proteins or are not produced directly and in isolation by standard cellular machinery.

The information for making a given protein is coded in a DNA nucleotide sequence, which is converted into a unique corresponding sequence of amino acids according to the genetic code. Here, any amino acid is formed by three adjacent nucleotides (or triplets) made up of three of the four base letters (A, G, T, C). There are (4x4x4=) 64 possible different triplets, 61 of which actually determine amino acids and the remaining three determining the termination of the polypeptide chain. There are, however, only 20 canonical amino acids with each of these coded by one to six triplets. *(Note: There are actually 24 permutations of n=4 objects taken r=3 at a time according to the formula: n!/(n-r)!, where the symbol"!"stands for factorial, that is: (1x2x3x4)/(1)=24, of which 1 is for beginning the sequencing and 3 are for terminating it, leaving 20).* These triplets are "read" in sequence and converted by the machinery of protein synthesis into the corresponding amino acids. Tables 6.5 provide the genetic code for the bacterium *Escherichia.coli (E. coli),* which is probably little different from man. This close similarity, even identity, between the genetic codes of many of the basic phenomena to all living organisms points to the unity of life. Table 6.5 (a) shows the 64 possible

triplets. When permutations of the same base letters in these triplets are removed (the grey shaded areas in the Table), the 20 canonical amino acids (the unshaded areas) are evidenced. The existence of only four base letters (A, G, T, C) is a necessary and sufficient condition for the existence of only 20 canonical amino acids.

Table 6.5 (a) - All the possible triplet combinations of the four bases (A, G, T, C)

Base	A	G	T	C	Base
A	AAA	AGA	ATA	ACA	**A**
	AAG	AGG	ATG	ACG	**G**
	AAT	AGT	ATT	ACT	**T**
	AAC	AGC	ATC	ACC	**C**
G	GAA	GGA	GTA	GCA	**A**
	GAG	GGG	GTG	GCG	**G**
	GAT	GGT	GTT	GCT	**T**
	GAC	GGC	GTC	GCC	**C**
T	TAA	TGA	TTA	TCA	**A**
	TAG	TGG	TTG	TCG	**G**
	TAT	TGT	TTT	TCT	**T**
	TAC	TGC	TTC	TCC	**C**
C	CAA	CGA	CTA	CCA	**A**
	CAG	CGG	CTG	CCG	**G**
	CAT	CGT	CTT	CCT	**T**
	CAC	CGC	CTC	CCC	**C**

Table 6.5 (b) below further shows that the 20 canonical amino acids are synthesized by one to six amino acids (as the numerals in the Table entries indicate). For example, for DNA, the amino acid Glycine is coded by CCA, CCG, CCT, and CCC. Removing the codons that are duplicated elsewhere in the Table, leaves only CCC.

Table 6.5 (b) - The Genetic Code for the bacterium *Escherichia.coli*

Base	A	G	T	C	Base
A	Phenylalanine (1)	Serine (1)	Tyrosine (1)	Cysteine (1)	**A**
	Phenylalanine (2)	Serine (2)	Tyrosine (2)	Cysteine (2)	**G**
	Leucine (1)	Serine (3)	Chain end (1)	Chain end (3)	**T**
	Leucine (2)	Serine (4)	Chain end (2)	Tryptophan (1)	**C**
G	Leucine (3)	Proline (1)	Histidine (1)	Arginine (1)	**A**
	Leucine (4)	Proline (2)	Histidine (2)	Arginine (2)	**G**
	Leucine (5)	Proline (3)	Glutamine (1)	Arginine (3)	**T**
	Leucine (6)	Proline (4)	Glutamine (2)	Arginine (4)	**C**
T	Isoleucine (1)	Threonine (1)	Asparagine (1)	Serine (5)	**A**
	Isoleucine (2)	Threonine (2)	Asparagine (2)	Serine (6)	**G**
	Isoleucine (3)	Threonine (3)	Lysine (1)	Arginine (5)	**T**
	Methionine (1)	Threonine (4)	Lysine (2)	Arginine (6)	**C**
C	Valine (1)	Alanine (1)	Aspartic acid (1)	Glycine (1)	**A**
	Valine (2)	Alanine (2)	Aspartic acid (2)	Glycine (2)	**G**
	Valine (3)	Alanine (3)	Glutamic acid (1)	Glycine (3)	**T**
	Valine (4)	Alanine (4)	Glutamic acid (2)	Glycine (4)	**C**

Source: Based in part on Crick, and Cavalli-Sforza/Bodmer

Ribonucleic acids (RNA) are similar to DNA. They differ mainly in the following: (a) The sugar component of the nucleotide, ribose, which is analogous to the deoxyribose in DNA; (b) The nucleotide Thymine (T) has been replaced by Uracil (U) in Tables 6.5; and (c) they occur as relatively short single strands.

Protein synthesis and the three kinds of RNA

The structural units (monomers) that make up proteins are the amino acids. They join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins. These polymers are linear

and unbranched, with each amino acid within the chain attached to two neighboring amino acids. Proteins cannot reproduce themselves.

Protein synthesis has been reproduced in the laboratory and been shown to depend on three kinds of RNA: messenger (m-RNA), transfer (t-RNA), and ribosomal (r-RNA), each having a specific role to play in carrying out DNA's instructions for building proteins:

- ***Messenger RNA (m-RNA)*:** DNA does not act directly, but through an intermediary form of ribonucleic acid called messenger RNA (denoted m-RNA). m-RNA is formed by the action of an enzyme, RNA polymerase, which copies the sequence from one strand of DNA and forms an RNA strand of the complementary sequence. Only one of the two DNA strands is generally active as a "template" for m-RNA synthesis, the other DNA strand remaining un-copied. The m-RNA sequence is identical to that in the uncoupled strand of DNA except that T is replaced by U. m-RNA remains single stranded. One molecule or strand can generally be used for the manufacture of a large number of protein molecules, each with the amino acid sequence corresponding to the m-RNA's nucleotide sequence. The number of nucleotides in an m-RNA strand is three times the number of amino acids in the polypeptide chain copied from it plus three nucleotides to indicate the termination of the chain, and possibly, another triplet for its initiation.
- ***Transfer RNA (t-RNA)*:** Before amino acids are joined to form a polypeptide chain, they must be "activated" by the attachment of a special phosphoric acid group. They are then attached to another type of RNA called transfer RNA (denoted t-RNA). There are as many varieties of t-RNA molecules as there are triplets that can determine amino acids in the code. The attachment of an amino acid to its corresponding t-RNA is directed by a specific enzyme. The t-RNA carries at one end the activated amino acid and, at a special position, has a triplet of nucleotides complementary to the triplet code for its amino acid. This latter triplet, which is part of the m-RNA, is often called a codon and that on the t-RNA the anticodon. The "Principle of Pairing Complementarity" is used for the recognition by the t-RNA of its appropriate triplet on the m-RNA.

- ***Ribosomal RNA (r-RNA)*:** Alignment of the t-RNA and the m-RNA and the progression of polypeptide synthesis along the m-RNA, which is "read" from beginning to end, is mediated by special particles, the ribosomes. These are present in the cell in large numbers. A ribosome is made from a third form of ribonucleic acid called ribosomal RNA together with a large number of protein molecules (20 or more).

The process of making proteins proceeds in two steps. First, information is transferred from DNA to m-RNA through the synthesis of m-RNA on the DNA template; this is called "transcription". Second, the synthesis of proteins from m-RNA, called "translation", involves the step-by-step addition of amino acids to a growing protein chain by a ribozyme that is called a ribosome. The order in which the amino acids are added is read through the genetic code from an m-RNA template, which is an RNA copy of one of the organism's genes.

Regulation of protein synthesis by adaptation to the living environment

Regulation of protein synthesis allows for adaptation to the living environment. Only when a certain substance, for example, a sugar, is present in the environment is it worthwhile for a cell to develop the enzymes needed to utilize the substance (enzyme induction). At other times, when a certain end product of a reaction chain, necessary for growth (for example, some amino acid), is present in the environment, cells can cease making it and the enzymes necessary for its synthesis (enzyme repression). Mechanisms have so developed that most proteins are made only when they are useful. Some such mechanisms have been shown to operate in microorganisms during transcription, that is, by stopping the production of the specific m-RNA when the proteins coded by it are not needed.

The presence or absence of the chemical substances in the environment is converted to the DNA by other specific"regulatory" proteins coded for by other DNA segments.

The regulation of protein synthesis is at the basis of differentiation, which is the development of different cells and tissues. Various proteins are formed

and, correspondingly, various portions of the DNA are active in different tissues and cells at different times.

DNA duplication and cell mitosis

DNA has a central role in the life process in both supplying the information for the synthesis of proteins and the basis for duplicating this information. In the presence of nucleotide precursors and an enzyme, DNA-polymerase, an identical copy of the DNA molecule can be formed by the production of strands complementary to those in the original "template" DNA. If the process takes place in the presence of just one strand, then, only the complementary strand is produced. When, however, a double strand is copied, two double strands are formed. Two new cells can thus be formed from an old one, and each will have the same amount of information for building the basic cellular materials. It is clearly essential that each daughter cell receive a complete complement of DNA by a mechanism called *mitosis*. Mitosis, the process of cell reproduction, assures an equal distribution of chromosomes to each daughter cell.

The DNA is contained in the nucleus of the cell in parcels consisting of long filaments wrapped in a protein matrix. The parcels are called chromosomes (or "colored bodies" coming from staining properties) and in spite of a certain rigidity due to its double strandedness, DNA must usually be folded many, many times within the chromosomes. The human cell has 46 chromosomes. In a cell that is not actively reproducing, the chromosomes are probably uncoiled and indistinguishable under a light microscope. When a cell is preparing for reproduction, the chromosomes undergo coiling and structural reorganization, as a consequence of which it becomes much shorter and thicker under the light microscope. Each human chromosome has a characteristic shape and size, generally similar in every cell and individual.

Mitosis proceeds along four phases called: (a) "prophase" (the cell at the beginning of mitosis), (b) "metaphase", (new cell poles are formed, the nuclear membrane has dissolved, the chromosomes have divided, and the centromeres – the parts of the chromosomes that contain less DNA- have not divided into two moieties), (c) "anaphase" (migration toward the poles),

and (d) "telophase" (separation and formation of two new cells identical to the original one).

Sidebar 6.1 provides a synopsis of evolutionary or Darwinian medicine.

Mutation and selection

Mitosis (division), meiosis (reduction), and fertilization assure the constancy of chromosome number and type in every cell of an individual and in all individuals of the same species. An understanding of mitosis and meiosis provides the basis for predicting the "Laws of Inheritance". In order for inheritance to be accessible for study, however, there must exist differences between individuals.

DNA is the carrier and transmitter of the information needed to make cells and individuals. Differences between individuals that are transmissible to their progeny, that is inheritable, must therefore be due to differences in DNA. It is clear that if a change can occur in DNA and the changed DNA is copied, all the descendants made by the new DNA will carry and transmit the new DNA form. DNA is therefore a hereditary substance and changes in it will be inherited. If the change in DNA affects the types of proteins that can be made, the effect of the change also will be inherited. A heritable change is called a *mutation*. If a mutation takes place in a cell destined to become a gamete, then it may be transmitted to the progeny. If it happens in a cell not destined to become a gamete, a somatic cell, the effects are limited to the individual, and the change is called a somatic mutation.

The fact that our chromosome set is normally double, man being a diploid organism, means that almost all of our genes are represented twice. If one of two homologous genes is normal but the other has been affected by a mutation, providing that one normal gene is sufficient for normal function, the individual is protected against the possible deleterious effects of the mutation. This is perhaps the main reason why it is advantageous for an organism to be diploid.

Most mutations are deleterious, though many have little effect and some may turn out to be advantageous. The mutations are detrimental because

of two reasons: (a) They are random changes in a functional structure that make the structure less efficient or even totally inadequate, and (b) because of our long evolutionary history, many of the possible changes that turned out to be advantageous have already been incorporated. Further, the frequency of mutations is low, although mutagenic agents can increase this pace. Under normal conditions, this frequency is on the order of one in a billion or more per generation. It varies, however, considerably among organisms and among genes and depends to some extent on the prevailing environmental conditions (see above Section).

The changes that can occur in DNA and their probable consequences are:

Substitution of one nucleotide for another

For example, consider the sequence [DNA: TTT ACG TAG] that specifies the riptide [lysine-cysteine-isoleucine] as seen from the Universal Genetic Code in Table 6.5(b)]. Changing the second nucleotide T to G results in the new sequence [DNA*: TGT ACG TAG] that synthesizes the tripeptide [threonine-cysteine-isoleucine]. Thus, the Universal Genetic Code allows the prediction of the tripeptide resulting from the substitution of one nucleotide in any given original tripeptide. Note, however, that a nucleotide substitution does not always lead to an amino acid substitution because many triplets may correspond to the same amino acid as can be readily verified from Table 6.5(b). However, an amino acid change must involve the substitution of at least one nucleotide. Practically, however, all mutations that have been observed in man have involved only one nucleotide substitution.

The substitution of one nucleotide for another is often called "point mutation" or "gene mutation".

Insertion or deletion of one nucleotide

Because of the mechanism of translation from m-RNA into protein (second step in protein synthesis), the insertion or deletion of one or more nucleotides has much more drastic consequences than a substitution. A polypeptide is usually made up of a sequence of at least a hundred amino acids. If there

is a deletion or insertion of one nucleotide in a DNA or RNA sequence, the message will be changed completely from that point onwards. This is because nucleotides are read successively in groups of three, so that the insertion or deletion of one will change completely the translation into amino acids. For example, consider the original DNA sequence [DNA: AAA ACG AAA CCG AAG CAT CTT …] which, according to Table 6.5(b), reads in the protein language as [phenylalanine-cysteine-phenylalanine-glycine-phenylalanine-valine-glutamic acid], a peptide of seven amino acids. Now, assume that the fourth nucleotide is deleted. The message becomes: [DNA*: AAA CGA ACC AAG ATC TT..], which reads in protein language as [phenylalanine-alanine -tryptophan-phenylalanine-chain end]. After the first triplet phenylalanine, which is not affected, all subsequent amino acids are changed. In addition, the sixth triplet (chain end) means that a peptide of only five amino acids will be produced instead of the presumably much longer one originally specified. This type of change is called a "frameshift mutation".

Insertion or deletion of two nucleotides

In the example above, assume that the third and the fourth nucleotides are inserted or deleted. The resulting sequence will be [DNA*: AAC GAC AAA TT....] which reads in protein language as [leucine-leucine-phenylalanine-...], an entirely different sequence.

Insertion or deletion of three nucleotides

If a deletion or insertion affects three nucleotides in a sequence, frameshift does not occur and there will only be a gap with perhaps the replacement of an amino acid in the gap. Deletion or insertion of three nucleotides not in a sequence leads to a profound alteration. For example, if the second and third nucleotides in the first triplet and the first nucleotide in the second triplet are deleted, the resulting DNA sequence is [DNA*: ACG ACG AAT C...] or in protein language [leucine-leucine-leucine-...], a completely different sequence.

As a first approximation, we call a gene that segment of DNA that codes for a given protein or polypeptide chain; however, not all DNA segments code for proteins.

Chromosomal aberrations

Gross changes in a DNA sequence involving the loss, addition, or displacement of a major part of a chromosome or chromosomes are referred to as "chromosomal aberrations" They are large enough to be seen under the light microscope as a change in the shape or size of the affected chromosome. There are four major types: Deletion, duplication, inversion, and translocation (see Table 6.6).

Table 6.6 - Chromosomal aberrations

Aberration	Initial chromosome	Modified chromosome	Notes
Deletion	ABCD[EFGHIJ]KLMN	ABCDKLMN	Can also be in genes. Have most drastic effects
Duplication	ABCDEFGHIJKLMN	ABCDEF[CDEF]GHIJKL	Tandem duplication. Duplication can be anywhere else on same or another chromosome
Inversion	ABCDEFGHIJKLMN	ABCD[GFE]HIJKLMN	180 deg chromosome rotation
Translocation	A: ABCDEFGHOJKLMN B: ABCDEFGHIJKLMN	A: abcdefghijkLMN B: ABCDEFGHIJKlmn	Chromosome segment transferred from one chromosome to another. Example: Down's syndrome(*)

(*) Down's syndrome can be caused by translocation of part of chromosome 21 to another chromosome as well as by nondisjunction

Chromosome aberrations, especially aneuploidy, cause a considerable unbalance (lethality, serious pathology) of the organism's make-up. Some

of the most extreme congenital malformations are a consequence of chromosomal changes.

Environmental effects

There are many physical, chemical, and ionizing radiation effects that increase the rate of mutations:

Physical agents

Temperature, humidity, pressure.

Chemical Agents

There are many chemical agents that are known to have mutagenic action. They usually either react chemically with DNA, or are "analogues" of essential parts of the nucleotides, that is, they are sufficiently similar to nucleotides to be incorporated into DNA in place of ordinary nucleotides. These chemical agents then have abnormal pairing relationships with other nucleotides and thus lead to errors during duplication. In order to be effective, mutagens must be able to reach the gonads, the organs in which the formation of germ cells take place. Many mutagens active *in vitro*, for example caffein, are probably not dangerous for man because they do not reach the gonads in sufficient concentration.

Ionizing Radiation

Ionizing radiation increases the mutation rate. More generally, any radiation of sufficient energy to determine chemical changes in DNA can give rise to mutations. Ultraviolet radiation is a potent mutagen, especially at a wavelength of around 200 millimicrons, which is that maximally absorbed by nucleic acids. This wavelength is, however, almost absent in solar radiation by the time it reaches the earth's surface. In any case, our germ cells are generally screened from this type of light. On the other hand, X-rays, if sufficiently hard, can penetrate the gonads and increase mutation rates over their spontaneous level.

Other mutagens

Mutagenic agents are relatively unspecific in that they do not affect just one gene, but tend to affect all genes almost equally. Others, however, tend to attack only certain nucleotides, changing them into others. As the number of sites in the total DNA of an organism that such specialized mutagens can attack is large, they cannot lead to any great specificity of action in terms of which genes are affected or the way in which they are affected.

The laws of Mendelian inheritance

From the facts of meiosis and fertilization, we know that:

- Apart from rare exceptions, every individual derives one chromosome of a pair and, hence, one gene of each pair, from one parent and the other chromosome, or gene, from the other parent. All autosomes (non-sex chromosomes) behave in this manner.
- Sex chromosomes have special rules of inheritance.
- Almost without exception, which particular chromosome enters a gamete is determined at random and therefore either chromosome may enter a particular gamete with equal probability.

A basic assumption of Mendelian inheritance is that "*male gametes fertilize female gametes independently of the particular chromosomes or genes that each of them contains"*. The expected outcome of any cross can be predicted as a function of the genotypes of the males as follows. Define the two-dimensional genotype "vector", **G**, in the case of either parent, where the gametes for either male (sperm) or female (egg) can either be denoted G or g without distinction. This vector can take any of the following three forms *(should have been drawn in its vertical form but, for convenience, is provided here by its transpose or horizontal form)*:

(G G); (G g) or (g G); (g g).

All the combinations of male and female gametes resulting from fertilization can then be obtained. The genotype of a zygote is derived by combining the genes of the two gametes. The proportion of zygotes having each combination will be the product of the relative frequencies of each type

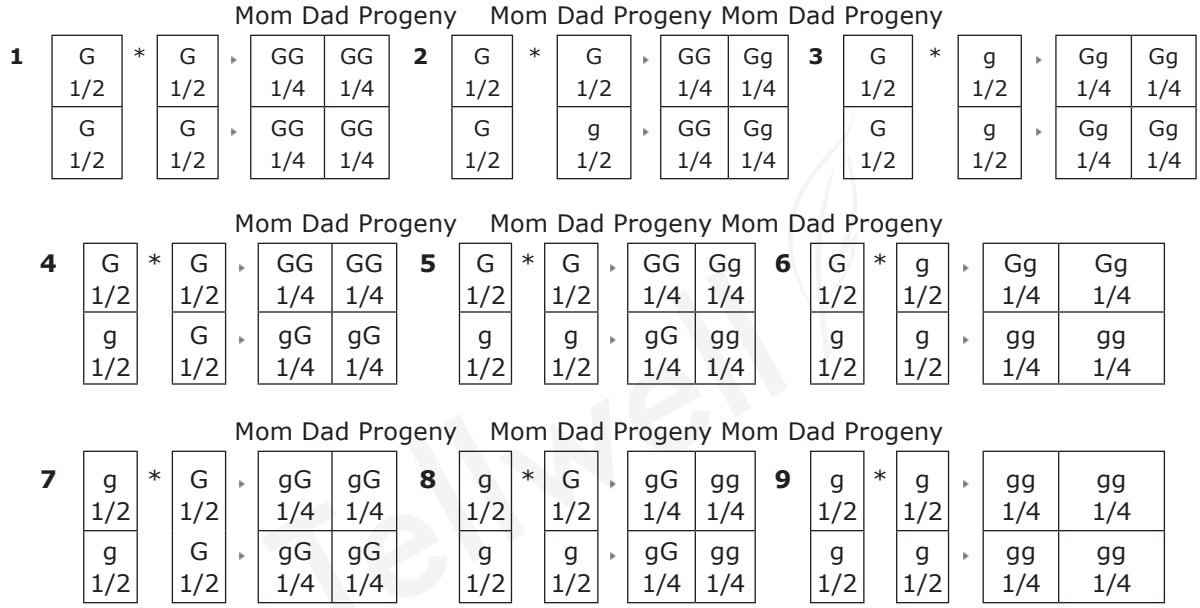
of gamete in each mate. These are easily obtained utilizing the concept of Hermitian product, *, yielding the two-dimensional genotype "square" matrix (Fymat 2017):

G * G= M.

There are nine such matrices of which six only are different (see Table 6.7).

Table 6.7 - All possible combinations of gametes

Gamete*Gamete=>Zygote Gamete*Gamete=>Zygote Gamete*Gamete=>Zygote



Note the following properties: (a) gG = Gg as it is immaterial whether G or g comes from the father or the mother; (b) **2** and **4** are the transpose of one another, and likewise for **3** and **7,** and **6** and **8**; (c) **3** and **7** are additionally identical; (d) In each genotype matrix above (**1** through 9), it is clear what gamete matings form what zygotes and conversely; and (e) In the progenies, there are at a maximum only three possible combinations with corresponding relative frequencies as listed below:

GG	gG + Gg	gg
1/4	1/2	1/4

It is further possible to determine the corresponding phenotypes and their respective proportions. For example, if G is fully dominant over g, the

dominant phenotype which is displayed by all individuals of genotypes GG, Gg, and gG, occurs among the progeny of the cross Gg x Gg (**5** above) with a frequency of (1/4 + 1/4+1/4=) 3/4, and the recessive phenotype displayed by gg individuals occurs with a frequency of 1/4. The phenotypes corresponding to different crosses of either homozygotes or heterozygotes, and their frequencies, can be readily ascertained from the other matrices (**1** through **4** and **6** through **8**). In practice, random sampling effects will generally lead to observed proportions that may differ somewhat from the above expected ones.

Example pattern of inheritance

Except for rare traits, a complete study of the genetic transmission of a trait (phenotype) theoretically requires the analysis of how it is transmitted in all possible matings. Here, we shall consider the case of an autosomal X-Linked recessive inheritance, as is the case for Hemophilia A, a hereditary disease in which blood coagulation is severely delayed. If X^h represents a very rare X-chromosome carrying the corresponding recessive gene, the affected offsprings (all X^hY males and all X^hX^h females) are denoted by the grey-shaded areas in Table 6.8**.** The results in mating types **2** and **4** are most characteristic for an X-linked recessive, whereas those in mating types **3**, **5** and **6** are rarely if ever observed. Specifically, in mating **2**, unaffected heterozygous "carrier" females XX^h and normal males XY produce normal females half of which are carriers $(1/2)XX^h$ and normal males half of which $(1/2)X^hY$ are affected.

Table 6.8 – Example patterns for X-linked recessive inheritance – Case of Hemophilia A

Mating	Parents		OffSprings			
Type	Female	Male	Fem	ale	Ma	le
1	XX	XY	XX		XY	
2	XX^h	XY	1/2XX	$1/2XX^h$	1/2XY	$1/2X^hY$
3	X^hX^h	XY	XX^h		X^hY	
4	XX	X^hY	XX^h		XY	
5	XX^h	X^hY	$1/2XX^h$	$1/2X^hX^h$	1/2XY	$1/2X^hY$
6	X^hX^h	X^hY	X^hX^h		X^hY	

Reference: The Genetics of Human Populations by L. L. Cavalli-Sforza and W. F. Bodmer, 1971

The gene concept

Many factors can obscure Mendelian inheritance. For example, the environment may lead to phenotype variations that may make it difficult or impossible to distinguish the phenotypes corresponding to different genotypes. Another serious disturbance is that more than one gene may affect the same character. The gene can be thought of as a segment of DNA that directs the synthesis of a given polypeptide or protein, however, this definition meets with difficulties: (a) Some DNA regions may not synthesize proteins; (b) Some are also believed to synthesize only r-RNA or t-RNA; (c) There are various kinds of genes; and (d) Some DNA regions code for several different proteins in a block. More generally, therefore, *a gene is more than a chromosome region that synthesizes a given polypeptide*. It is now defined as a DNA segment recognizable by its specific function.

Evolutionary biology

A brief history

1849: *Charles Darwin* proposed the idea of evolution by natural selection.

1930s-1940s: Development of the modern synthesis and establishment of evolutionary biology as an independent academic discipline.

Evolutionary biology and evolutionary developmental biology

Evolutionary biology "studies the evolutionary processes that produced the diversity of life on Earth, starting from a single common ancestor... including (a) natural selection, (b) common descent, and (c) speciation". It "...*merges several previously unrelated fields including ecology, genetics, genetic architecture of adaptation, molecular evolution, and the different forces that contribute to evolution including biogeography, sexual selection, and genetic drift"*. The newer field of Evolutionary Developmental Biology ("evo-devo") investigates how embryonic development is controlled.

Evolutionary biology researchers try to explain phenomena that were poorly accounted for in the modern evolutionary synthesis including:

- Speciation, the evolution of sexual reproduction, cooperation and aging, and evolvability;
- Paleobiology;
- Phylogenetics;
- The genetic architecture of interesting evolutionary phenomena such as adaptation and speciation.

They seek answers to questions such as how many genes are involved, how large are the effects of each gene, how interdependent are the effects of different genes, what do the genes do, and what changes happen to them (e.g., point mutations vs. gene duplication or even genome duplication). They try to reconcile the high heritability seen in twin studies with the difficulty in finding which genes are responsible for this heritability using genome wide association studies (GWAS).

Current research topics

Current research in evolutionary biology covers diverse topics and incorporates ideas from diverse areas, such as molecular genetics and computer science such as:

- To study poorly accounted phenomena in the modern evolutionary synthesis, including speciation, the evolution of sexual reproduction, the evolution of cooperation, the evolution of aging, and evolvavability;

- To answers evolutionary questions such as paleobiology, systematics, and phylogenetics;

- To determine the genetic architecture of interesting evolutionary phenomena such as adaptation and speciation, for example, how many genes are involved, how large are the effects of each gene, how interdependent are the effects of different genes, what do the genes do, and what changes happen to them (e.g., point mutations vs. gene duplication or even genome duplication);

- To reconcile the high heritability seen in twin studies with the difficulty in finding which genes are responsible for this heritability using GWAS;

    - To develop a theory of molecular evolution including which genes have been under strong selection by detecting selective sweeps;
    - To determine the relative importance of the forces that shape evolution including natural selection, sexual selection, genetic drift, developmental constraints, mutation bias and biogeography; and
    - To investigate how developmental processes work, and compare them in different organisms to determine how they evolved.

Conclusions and take-aways

- After a review of historical milestones, we summarized the main pillars of biology: the cell (as the basic unit of life), the genes (the basic unit of heredity), evolution, homeostasis (the maintenance of a stable and vital condition), and energy (its consumption, transformation, and regulation of its internal environment are critical to the survival of all organisms).
- The main tenets of cellular biology were reviewed (cells, nucleic acids, peptides, proteins and enzymes). The Universal Genetic Code was discussed, including how all possible combinations taken three at a time from permutations of the four DNA bases (A, G, T, C) provide the 20 canonical amino acids with each of these coded by one to six triplets, as exemplified by the genetic code for the bacterium *Escherichia.coli.* Protein synthesis by the three kinds of RNA (messenger, transfer, ribosomal) and its regulation by adaptation to the environment was shown as the basis of differentiation, which is the development of different cells and tissues.
- DNA has a central role in the life process in both supplying the information for the synthesis of proteins and the basis for duplicating this information. Mitosis, fertilization, and meiosis assure the constancy of chromosome number and type in every cell of an individual and in all individuals of the same species. An understanding of mitosis and meiosis provides the basis for predicting the Laws of Inheritance. In order for inheritance to be accessible for study, however, there must

exist differences between individuals. These differences are the result of mutations by substitution of one, two or even three nucleotides for others in the genetic sequence and by gross chromosomal aberrations (deletion, duplication, inversion, translocation).

- Environmental effects (physical and chemical agents, ionizing radiation, and mutagens) were only briefly mentioned. The Laws of Mendelian Inheritance were reviewed utilizing a novel mathematical approach (Hermitian algebra) introduced by this author, and illustrated with an example pattern of inheritance (autosomal X-linked recessive in Hemophilia A). But, many factors can obscure Mendelian inheritance including, importantly, the environment and the fact that more than one gene may affect the same character.

➢ Some remarks have been provided on the field of evolutionary biology and evolutionary developmental biology (so named "evo-devo"). This is the subfield of biology that studies the evolutionary processes that produced the diversity of life on Earth, starting from a single common ancestor. Modern research tries to explain phenomena that were poorly accounted for in the modern evolutionary synthesis. Current research topics in this field have been briefly described.

Sidebar 6.1 – Evolutionary or Darwininan medicine

Modern medicine has modeled itself after mechanical physics, deriving from Galileo, Newton, and Descartes. As a result, its concepts are mechanistic, materialistic, reductionistic, linear-causal, and deterministic (i.e., able to make precise predictions). Its explanations of diseases is through their symptoms, signs, risks, and cause(s) in materialistic (i.e., anatomical or structural) changes within the body that have been wrought directly (linearly) by infections, inflammations, toxic or traumatic agents, etc. It thus has focused on the molecular and physiological mechanisms underlying health and disease. By contrast, evolutionary or Darwinian medicine applies modern evolutionary theory to the same goal by focusing on why evolution has shaped these mechanisms in ways that may leave us susceptible to disease. The evolutionary approach has driven important advances in our understanding of cancer, autoimmune diseases, and anatomy.

A brief history

In 1859: *Charles Darwin wrote his celebrated volume* "On the Origin of Species by Means of Natural Selection or the Preservation of Favored Races in the Struggle for Life"*.*

In 1871: *Darwin further wrote* "The Descent of Man and Selection in Relation to Sex". *Neither of these two volumes discussed their implications for medicine although biologists quickly* appreciated the "germ theory of disease" and its implications for understanding the evolution of pathogens as well as an organism's need to defend against them. In turn, Medicine ignored evolution, and, like in the hard sciences, focused instead upon proximate mechanical causes.

In the 1950s: *George C. Williams* applied for the first time the theory of evolution to health in the context of senescence.

In the 1950s: *John Bowlby* explained the disturbed child development from an evolutionary perspective upon attachment.

In the 1960s: *Nikolaas Tinbergen* laid the cornerstone of modern ethology, sociobiology, and transdisciplinarity in the Human Sciences by summarizing causation and ontogeny as the "proximate mechanisms" while adaptation and phylogeny are the "ultimate (evolutionary) mechanisms".

In 1980: *Paul Ewald* published his paper on "*Evolutionary Biology and the Treatment of Signs and Symptoms of Infectious Disease*".

In 1981: G.C. Williams and *Randolph M. Nesse* published "*The Dawn of Darwinian Medicine*" and, later, the book "*Why We Get Sick"* (published in the U.K. as "*Evolution and healing"*).

In 2008: An online journal started: "Evolution and Medicine Review".

In 2010: Randolph M. Nesse articulated the relevance of evolution to medicine, thus ***"****All biological traits need two kinds of explanation, both proximate and evolutionary. The proximate explanation for a disease describes what is wrong in the bodily mechanism of individuals*

affected by it. An evolutionary explanation is completely different. Instead of explaining why people are different, it explains why we are all the same in ways that leave us vulnerable to disease. Why do we all have wisdom teeth, an appendix, and cells that can divide out of control?

Core principles of evolutionary medicine

There are 14 core principles (1-14) that can be further grouped into five general categories (I-V): question framing, evolution I and II (with II involving a higher level of complexity), evolutionary trade-offs, reasons for vulnerability, and culture. Additional information regarding these principles may be found in Table 6.9 below:

Table 6.9 - Core principles of evolutionary (Darwinian) medicine

Category/Topic	Core Principle
I.1. Question framing	Proximate (mechanistic) and ultimate (evolutionary) explanations are needed to provide a full biological understanding of traits, including those that increase vulnerability to disease.
II.2. Evolution I: Processes	All evolutionary processes (natural selection, genetic drift, mutation, migration and non-random mating) are important for understanding traits and disease.
II.3. Evolution I: Reproductive success	Natural selection maximizes reproductive success, sometimes at the expense of health and longevity.
II.4. Evolution I: Sexual selection	Sexual selection shapes traits that result in different health risks between sexes.
II.5. Evolution I: Constraints	Several constraints inhibit the capacity of natural selection to shape traits that are hypothetically optimal for health.
II.6. Evolution II: Levels of selection	Vulnerabilities to disease can result when selection has opposing effects at different levels (e.g. genetic elements, cells, organisms, kin, and other levels).
II.7. Evolution II: Phylogeny	Tracing phylogenetic relationships for species, populations, traits or pathogens can provide insights into health and disease.
II.8. Evolution II: Co-evolution	Co-evolution among species can influence health and disease (e.g. evolutionary arms races and mutualistic relationships such as those seen in the microbiome).

II.9. Evolution II: Plasticity	Environmental factors can shift developmental trajectories in ways that influence health. The plasticity of these trajectories can be the product of evolved adaptive mechanisms.
III.10. Evolutionary Trade-Offs: Changes	Evolutionary changes in one trait that improve fitness can be linked to changes in other traits that decrease fitness.
III.11. Evolutionary Trade-Offs: Life History Theory	Life history traits, such as age at first reproduction, reproductive lifespan, and rate of senescence, are shaped by evolution and have implications for health and disease.
IV.12. Reasons for Vulnerability: Defenses	Many signs and symptoms of disease (e.g. fever) are useful defenses, which can be pathological if dysregulated.
IV.13. Reasons for Vulnerability: Mismatch	Disease risks can be altered for organisms living in environments that differ from those in which their ancestors evolved.
V.14. Culture: Cultural practices	Cultural practices can influence the evolution of humans and other species (including pathogens), in ways that can affect health and disease (e.g. antibiotic use, birth practices, life style, diet, etc.).

Source: Adapted from Wikipedia

Diseases of civilization

The change of humans from hunters-gatherers to "modern" humans has made them vulnerable to a number of health problems, which have been termed "diseases of civilization". The rapid change from the stone age environment to the modern one has created these problems. This is so because, in light of the slowness of evolution, we are still adapted to stone-age circumstances that no longer apply. This misfit has serious implications for our health such that "…modern environments may cause many diseases and syndromes".

On the evolutionary origin of many diseases/syndromes

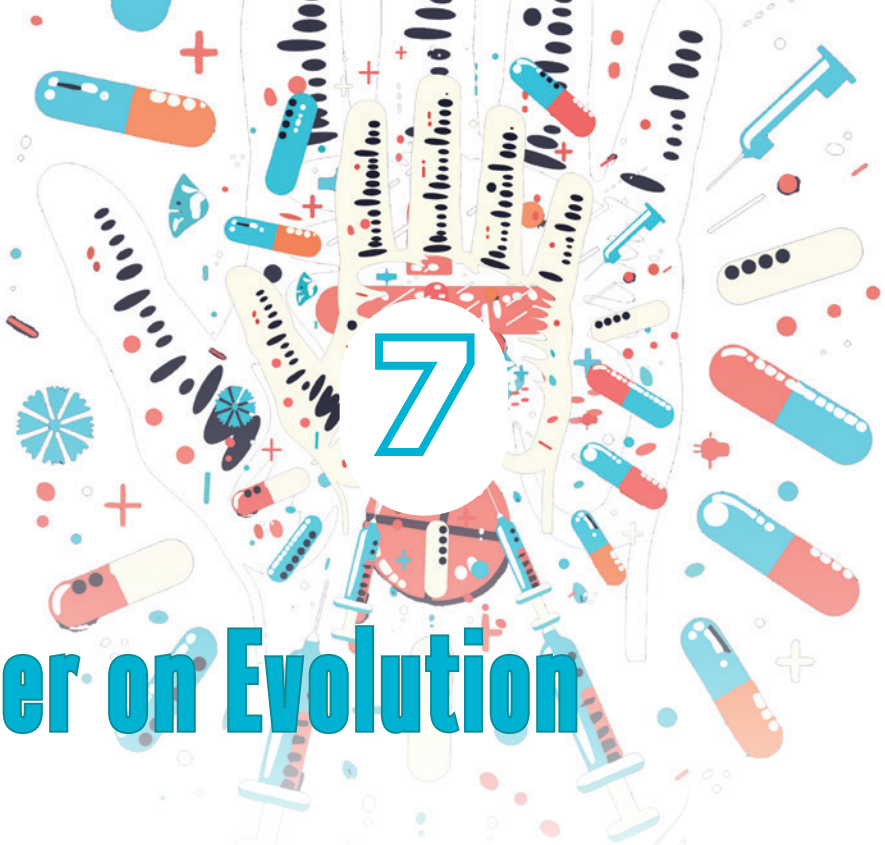
The following is a partial list of diseases that can find their origin in evolution: Atherosclerosis; adipose tissue in human infants; **aging**; Alzheimer's disease; arthritis and chronic inflammatory disease; cough; cystic fibrosis; dental occlusion; diabetes type 2; diarrhea; essential hypertension; fever; gestational hypertension; gout; hemochromatosis; iron deficiency; menarche;

menopause; menstruation; morning sickness; obesity; osteoporosis; phenylketonuria; red blood cell polymorphism disorders; sickle cell anemia; women's reproductive cancers; and psychological abnormalities such as: agoraphobia; anxiety; depression; drug abuse; schizophrenia; unhappiness.

7

Primer on Evolution

Contents



7

Primer on Evolution

Evolution is defined as "*the change in the heritable characteristics of biological populations over successive generations*". It occurs when the evolutionary processes of "natural selection", "genetic drift", "mutation", and "gene flow" act on genetic variation. This results in certain characteristics becoming more or less common within a population over successive generations. The process of evolution has given rise to biodiversity at every level of biological organization.

Inquiry into the evolution of aging aims to explain why a detrimental process such as aging would evolve, and why there is so much variability in the lifespans of organisms. The classical theories of evolution (mutation accumulation, antagonistic pleiotropy, and disposable soma) suggest that environmental factors, such as predation, accidents, disease, and/or starvation ensure that most organisms living in natural settings will not live until old age, and so there will be very little pressure to conserve genetic changes that increase longevity. Natural selection will instead strongly favor genes which ensure early maturation and rapid reproduction, and the selection for genetic traits which promote molecular and cellular self-maintenance will decline with age for most organisms.

Evolution by natural selection

Natural selection is a process that allows organisms to better adapt to the environment - the survival of the fittest who are predicted to produce more offsprings. It acts on life history traits in order to optimize reproductive success and lifetime fitness. In this context, fitness refers to how likely an organism is to survive and reproduce based on the environment and relative to other individuals in the population. Organisms put energy into growth, reproduction, and maintenance by following a particular pattern which changes throughout their lifetime due to the trade-offs that exist between the different energy allocations. However, natural selection is not so effective on organisms as they age.

Different than *individual selection*, *group selection* focuses on the group. It is based on the idea that all members of a given group will either succeed or fail together depending on the circumstances. This mechanism sets them apart from other groups of its own species.

Darwin-Wallace theory

The theory of evolution by natural selection was conceived independently by Charles Darwin and Alfred Russell Wallace in the mid-19th century as an explanation for why organisms are adapted to their physical and biological environments. It was first set out in detail in Darwin's book *On the Origin of Species*.

Evolution by natural selection is established by four major observable facts about living organisms (acronym **OTDH**):

- ***O*ffspring:** More offspring are often produced than can possibly survive;
- ***T*raits:** Traits vary among individuals with respect to their morphology, physiology, and behavior;
- ***D*ifferential fitness:** Different traits confer different rates of survival and reproduction; and
- ***H*eritability fitness:** Traits can be passed from generation to generation. In successive generations, members of a population are

therefore more likely to be replaced by the offspring of parents with favorable characteristics for that environment.

Synthetic theory

In the early 20th century, competing ideas of evolution were refuted and evolution was combined with Mendelian inheritance and population genetics to give rise to modern evolutionary theory. In this synthesis, the basis for heredity is in DNA molecules that pass information from generation to generation. The processes that change DNA in a population include (acronym **SDMF**):

- **Natural *S*election,**
- **Genetic *D*rift,**
- ***M*utation,** and
- **Gene *F*low.**

All life on Earth—including humanity—shares a last universal common ancestor (LUCA), which lived approximately 3.5–3.8 billion years ago. The fossil record includes a progression from early biogenic graphite to microbial mat fossils to fossilized multicellular organisms. Throughout the evolutionary history of life on Earth, existing patterns of biodiversity have been shaped by (acronym **SAE)**:

- ***S*peciation:** These are repeated formations of new species,
- ***A*nagenesis:** These are changes within species (anagenesis), and
- ***E*xtinction:** The loss of species.

Morphological and biochemical traits tend to be more similar among species that share a more recent common ancestor, which historically was used to reconstruct phylogenetic trees, although direct comparison of genetic sequences is a more common method today.

Evolutionary biologists have continued to study various aspects of evolution by forming and testing hypotheses as well as constructing theories based on evidence from the field, the laboratory, and on data generated by the methods of mathematical and theoretical biology. Their discoveries have

influenced not just the development of biology and medicine (but also other fields including agriculture and computer science).

The evolutionary process

Evolvability is the concept that a species should profit from faster genetic adaptation to its present environment. It argues that eliminating old individuals might benefit the species overall. A discussion of the evolutionary process begins with the following two seminal facts:

- Different species have different lifespans up to immortality; and
- Aging is a difficult subject for experimental investigation,

which require theoretical explanations. Three concepts have been advanced to explain how evolution relates to aging, all having as a starting point Darwin's (1859) evolution theory of "survival of the fittest":

- "**The evolution process *opposes* aging**" because aging decreases an organism's ability to survive and reproduce, but must be fundamentally limited when it comes to aging (similarly, there are many laws of physics and chemistry that cannot be overcome by the evolution process). Unfortunately, this theory explains neither of the above two facts.
- "**The evolution process *is neutral* regarding aging**" (Medawar and William, 1950s). Survival of the fittest only applies to young organisms and species did not evolve and retain the capability for living longer than a species-specific age. This theory may explain only the first fact.
- "**The evolution process *promotes* the development of biological mechanisms such as aging that purposely limit lifespans of 'old' organisms**" (multiple authors, 1980s). Purposely limited lifespans have long-term *population* benefits (e.g., avoiding species extinction) although they are neutral or adverse for *individuals.* Such a view has led to modern *programmed aging* theories in which aging is the manifestation of a biological program or suicide mechanism. Unlike the above two theories, it suggests the possibility of finding ***anti-aging agents*** that interfere with the aging program and generally *delay aging*. Experimental and observational evidence suggest that

programmed theories may be the correct ones and explain the two seminal facts.

Alternatively, as applied to humans and most other mammals, there are two main aging theories:

- **Non-programmed theories** (also known as 'passive' or 'non-adaptive' aging theories): They contend that a limited life span is entirely adverse and that aging is not genetically programmed for the purpose of causing deterioration or death. They have difficulty explaining many observations but are more compatible with older evolutionary mechanics concepts. They encompass the first two concepts outlined above.
- **Programmed aging theories** (also known as 'active' or 'adaptive' aging theories): They propose that mammals purposely deteriorate with age because an internally limited life span provides evolutionary benefits. They provide a better match to observations, but are based on newer concepts regarding evolution mechanisms. They include the third concept above.

Organismic evolution or heredity

Heredity, the inherited characteristics of organisms, devolves from organismic evolution through changes in heritable characteristics. Inherited traits are controlled by genes and the complete set of genes within an organism's genome (genetic material) is called its *genotype*.

On the other hand, the complete set of observable traits that make up the structure and behavior of an organism is called its *phenotype.* Some of these traits come from the interaction of its genotype with the environment while others are neutral. Further, some observable characteristics are not inherited. In addition, some phenotypes may be due to differences in genotypic variation.

The DNA molecule

Heritable characteristics are passed from one generation to the next via DNA, a molecule that encodes genetic information. DNA is a long biopolymer composed of four types of *bases*. The sequence of bases along a particular DNA molecule specifies the genetic information, in a manner similar to a sequence of letters spelling out a sentence. Before a cell divides, the DNA is copied, so that each of the resulting two cells will inherit the DNA sequence. (For more details, refer to Chapter 6.)

Portions of a DNA molecule that specify a single functional unit are called *genes*; different genes have different sequences of bases.

Within cells, each long strand of DNA is called a *chromosome.* The specific location of a DNA sequence within a chromosome is known as a *locus*. If the DNA sequence at a locus varies between individuals, the different forms of this sequence are called *alleles*.

DNA sequences can change through mutations, producing new alleles. If a mutation occurs within a gene, the new allele may affect the trait that the gene controls, altering the phenotype of the organism. However, while this simple correspondence between an allele and a trait works in some cases, most traits are influenced by multiple genes in a quantitative or epistatic manner.

Sources of evolutionary variation

Evolution can occur if there is genetic variation within a population. Variation comes from three different processes (acronym **MRFE**):

- **Genome *M*utations**,
- **Genes *R*eshuffling** through sexual reproduction,
- **Gene *F*low** (or migration between populations). And
- ***E*pigenetics.**

Despite the constant introduction of new variation(s) through mutation(s), reshuffling, and gene flow, most of the genome of a species is very similar among all individuals of that species. However, discoveries in the field of

evolutionary developmental biology have demonstrated that even relatively small differences in genotype can lead to dramatic differences in phenotype both within and between species.

An individual organism's phenotype results from both its genotype and the influence of the environment it has lived in. The modern evolutionary synthesis (see Section above) defines evolution as the change over time in this genetic variation. The frequency of one particular allele will become more or less prevalent relative to other forms of that gene. Variation disappears when a new allele reaches the point of fixation—when it either disappears from the population or replaces the ancestral allele entirely.

Mutations

Mutations are changes in the DNA sequence of a cell's genome; they are the ultimate source of genetic variation in all organisms. When they occur, they may alter the product of a gene, or prevent the gene from functioning, or have no effect. A small percentage of the total mutations in the coding regions of protein-coding genes confer a fitness benefit. Some, in other parts of the genome, are deleterious, but the vast majority are neutral.

Mutations can involve large sections of a chromosome becoming duplicated (usually by genetic recombination), which can introduce extra copies of a gene into a genome. Extra copies of genes are a major source of the raw material needed for new genes to evolve. This is important because most new genes evolve within gene families from pre-existing genes that share common ancestors.

New genes can be generated from an ancestral gene when a duplicate copy mutates and acquires a new function. This process is easier once a gene has been duplicated because it increases the redundancy of the system; one gene in the pair can acquire a new function while the other copy continues to perform its original function. Other types of mutations can even generate entirely new genes from previously noncoding DNA, a phenomenon termed *de novo* gene birth.

The generation of new genes can also involve small parts of several genes being duplicated, with these fragments then recombining to form new combinations with new functions (*exon shuffling*). When new genes are assembled from shuffling pre-existing parts, domains act as modules with simple independent functions, which can be mixed together to produce new combinations with new and complex functions.

Sex and recombination

In asexual organisms, genes are inherited together, or linked, as they cannot mix with genes of other organisms during reproduction. In contrast, the offspring of sexual organisms contain random mixtures of their parents' chromosomes that are produced through independent assortment.

In a related process called *homologous recombination*, sexual organisms exchange DNA between two matching chromosomes. Recombination and reassortment do not alter allele frequencies, but instead change which alleles are associated with each other, producing offspring with new combinations of alleles. Sex usually increases genetic variation and may increase the rate of evolution.

Any individual who reproduces sexually can only pass on 50% of its genes to any individual offspring, with even less passed on as each new generation passes. Yet sexual reproduction is the more common means of reproduction among eukaryotes and multicellular organisms.

Gene flow

Gene flow is the exchange of genes between populations and between species. It can therefore be a source of variation that is new to a population or to a species. Gene flow can be caused by the movement of individuals between separate populations of organisms.

Gene transfer between species includes the formation of hybrid organisms and horizontal gene transfer. *Horizontal gene transfer* is the transfer of genetic material from one organism to another organism that is not its offspring; this is most common among bacteria. In medicine, this contributes to the

spread of antibiotic resistance, as when one bacteria acquires resistance genes it can rapidly transfer them to other species. Viruses can also carry DNA between organisms, allowing transfer of genes even across biological domains.

Epigenetics

Some heritable changes cannot be explained by changes to the sequence of nucleotides in the DNA. These phenomena are classed as epigenetic inheritance systems.

Epigenetic inheritance systems have been discovered at the organismic level in the following four areas (see Chapter 9 for more details):

- DNA methylation marking chromatin,
- Self-sustaining metabolic loops,
- Gene silencing by RNA interference, and the three-dimensional
- Conformation of proteins (such as prions).

Other examples of heritability in evolution that are not under the direct control of genes include the inheritance of cultural traits and symbiogenesis.

Mortality

Mortality is the number of deaths, in a particular group, over a specific time period. There are two types of mortality: "intrinsic" and "extrinsic". Intrinsic mortality is defined as mortality due to aging, the physiological decline due to innate processes, whereas extrinsic mortality is the result of environmental factors such as, for example, predation, starvation, accidents, and others.

When examining body-size vs. lifespan relationship, one also observes that predatory mammals tend to live longer than prey mammals in a controlled environment (such as a zoo or a nature reserve). The explanation for the long lifespans of primates (such as humans, monkeys, and apes) relative to body size is that they manage to achieve lower extrinsic mortality due to their intelligence.

Potential immortality of the germ line

Individual organisms are ordinarily mortal - they age and die, while the germ lines which connect successive generations are potentially immortal. The basis for this difference is a fundamental problem in biology.

Conclusions and take-aways

- Evolution is defined as "*the change in the heritable characteristics of biological populations over successive generations*". It occurs when the evolutionary processes of "natural selection", "genetic drift", "mutation", and "gene flow" act on genetic variation. This results in certain characteristics becoming more or less common within a population over successive generations. The process of evolution has given rise to biodiversity at every level of biological organization.
- The Darwin-Wallace theory of evolution by natural selection was conceived as an explanation for why organisms are adapted to their physical and biological environments. It is established by four major observable facts about living organisms (acronym **OTDH**): **O**ffspring, **T**raits, **D**ifferential (fitness), and **H**eritability (fitness).
- The modern evolutionary theory (or synthetic theory) is a combination of evolution with Mendelian inheritance and population genetics in which the basis for heredity is in DNA molecules that pass information from generation to generation. The processes that change DNA in a population include (acronym **SDMF**): (Natural) **S**election, (Genetic) **D**rift, **M**utation, and (Gene) **F**low.
- Throughout the evolutionary history of life on Earth, existing patterns of biodiversity have been shaped by (acronym **SAE)**: **S**peciation, **A**nagenesis, and **E**xtinction.
- Heredity, the inherited characteristics of organisms, devolves from organismic evolution through changes in heritable characteristics. Inherited traits are controlled by genes. The complete set of genes within an organism's genome (genetic material) is called its *genotype* and the complete set of observable traits that make up the structure and behavior of an organism is called its *phenotype.*

- Some phenotypes come from the interaction of the genotype with the environment while others are neutral. Further, some observable characteristics are not inherited. In addition, some phenotypes may be due to differences in genotypic variation.
- Heritable characteristics are passed from one generation to the next via DNA, a molecule that encodes genetic information. Portions of a DNA molecule that specify a single functional unit are called *genes*; different genes have different sequences of *bases*. Within cells, each long strand of DNA is called a *chromosome.* The specific location of a DNA sequence within a chromosome is known as a *locus*. If the DNA sequence at a locus varies between individuals, the different forms of this sequence are called *alleles*.
- Evolution can occur if there is genetic variation within a population. Variation comes from three different processes (acronym **MRF**): (Genome) **M**utations, (Genes) **R**eshuffling through sexual reproduction, and (Gene) **F**low (or migration between populations).
- Despite the constant introduction of new variation(s) through mutation(s) and gene flow, most of the genome of a species is very similar among all individuals of that species. Nonetheless, even relatively small differences in genotype can lead to dramatic differences in phenotype both within and between species.
- A discussion of the evolutionary process begins with the following two seminal facts: Different species have different lifespans up to immortality; and aging is a difficult subject for experimental investigation, which requires theoretical explanations. Three concepts have been advanced to explain how evolution relates to aging, all having as a starting point Darwin's (1859) evolution theory of "survival of the fittest". The evolution process *opposes* aging, *is neutral* regarding aging, or *promotes* the development of biological mechanisms such as aging that purposely limit lifespans of 'old' organisms". This last concept suggests the possibility of finding *anti-aging agents* that interfere with the aging program and generally *delay aging*.
- As applied to humans and most other mammals, there are two main aging theories: Non-programmed theories (also known as 'passive' or 'non-adaptive' aging theories) ad programmed aging theories (also known as 'active' or 'adaptive' aging theories).

- An individual organism's phenotype results from both its genotype and the influence of the environment it has lived in.
- Mutations are changes in the DNA sequence of a cell's genome; they are the ultimate source of genetic variation in all organisms. They may alter the product of a gene, prevent the gene from functioning, or else have no effect. A small percentage of the total mutations confer a fitness benefit while others are deleterious but the vast majority are neutral. Mutations can involve large sections of a chromosome becoming duplicated (usually by genetic recombination).
- New genes can be generated from an ancestral gene when a duplicate copy mutates and acquires a new function. Other types of mutations can even generate entirely new genes from previously noncoding DNA, a phenomenon termed *de novo* gene birth. The generation of new genes can also involve small parts of several genes being duplicated (*exon shuffling*).
- In asexual organisms, genes are inherited together, or linked, as they cannot mix with genes of other organisms during reproduction. In contrast, the offspring of sexual organisms contain random mixtures of their parents' chromosomes that are produced through independent assortment.
- In a related process called *homologous recombination*, sexual organisms exchange DNA between two matching chromosomes. Sex usually increases genetic variation and may increase the rate of evolution.
- Any individual who reproduces sexually can only pass on 50% of its genes to any individual offspring, with even less passed on as each new generation passes. Yet sexual reproduction is the more common means of reproduction among eukaryotes and multicellular organisms.
- Gene flow is the exchange of genes between populations and between species. It can be a source of variation that is new to a population or to a species and can be caused by the movement of individuals between separate populations of organisms.
- Gene transfer between species includes the formation of hybrid organisms and horizontal gene transfer (the transfer of genetic material from one organism to another organism that is not its offspring). In medicine, this contributes to the spread of antibiotic resistance. When one bacteria acquires resistance genes, it can rapidly transfer them

to other species. Viruses can also carry DNA between organisms, allowing transfer of genes even across biological domains.

- Epigenetic inheritance systems have been discovered at the organismic level in four areas: DNA methylation marking chromatin, self-sustaining metabolic loops, gene silencing by RNA interference, and the three-dimensional conformation of proteins (such as prions).
- Mortality is the number of deaths, in a particular group, over a specific time period. There are two types of mortality: "intrinsic" and "extrinsic". Intrinsic mortality is defined as mortality due to aging, the physiological decline due to innate processes, whereas extrinsic mortality is the result of environmental factors.
- The long lifespans of primates (such as humans, monkeys, and apes) relative to body size is that they manage to achieve lower extrinsic mortality due to their intelligence.
- Individual organisms are ordinarily mortal - they age and die, while the germ lines which connect successive generations are potentially immortal. The basis for this difference is a fundamental problem in biology.

Sidebar 7.1 – Progeroid syndromes

Progeroid syndromes are genetic diseases that are linked to premature aging. They are characterized by having features that resemble those of physiological aging such as hair loss and cardiovascular disease.

Progeria

Progeria is a single-gene genetic disease that causes acceleration of many or most symptoms of aging during childhood. It affects about 1 in 4-8 million births. Those who have this disease are known for failure to thrive and have a series of symptoms that cause abnormalities in the joints, hair, skin, eyes, and face. Most who have the disease only live to about age 13. Although the term progeria applies strictly speaking to all diseases characterized by premature aging symptoms, and is often used as such, it is often applied specifically in reference to Hutchinson–Gilford progeria syndrome (HGPS).

HGPS is caused by a point mutation in the gene that encodes lamin A protein. Lamin A promotes genetic stability by maintaining levels of proteins that have key roles in non-homologous end joining and homologous recombination. In HGPS, the inability to adequately repair DNA damages due to defective A-type lamin may cause aspects of laminopathy-based premature aging.

Children diagnosed with HGPS develop prominent facial features such as a small face, thin lips, small chin, and protruding ears. Although progeria can cause physical abnormalities in a child, it does not impact their motor skills or intellectual advancement. Those who have HGPS are prone to suffer from neurological and cardiovascular disorders.

Werner's syndrome

Werner's syndrome (WS), also known as "adult progeria", is another single-gene genetic disease. It is caused by a mutation in the WRN gene. It affects about 1 in 200,000 people in the U.S. This syndrome starts to affect individuals during the teenage years, preventing them from growing at puberty. Once the individual reaches the twenties, there is generally a change in hair color, skin, and voice, an increased risk for cataracts, type 2 diabetes, different types of cancers, and atherosclerosis. This condition can also affect the weight distribution between the arms, legs, and torso. The average life expectancy of someone with this disease is around 46 years.

There are four common traits of Werner's syndrome:

- Cataracts in both eyes,
- Changes in skin similar to scleroderma,
- Short stature, and
- Early graying and loss of hair.

The finding that the WRN protein interacts with DNA-PKcs and the Ku protein complex, combined with evidence that WRN deficient cells produce extensive deletions at sites of joining of non-homologous DNA ends, suggests a role for the WRN protein in the DNA repair process of non-homologous end joining. The WRN protein also appears to play a role in resolving recombination intermediate structures during homologous recombinational repair of DNA double-strand breaks.

Other progeroid syndromes

https://en.wikipedia.org/wiki/Bloom_syndrome

There are three other progeroid syndromes:

Bloom's syndrome (BS): This is a rare autosomal recessive disorder that is characterized by short stature, chromosomal instability, predisposition to cancer, and sun-sensitive skin. Those with BS can also have learning disabilities and have an increased risk of developing chronic obstructive pulmonary disease (COPD) and disease.

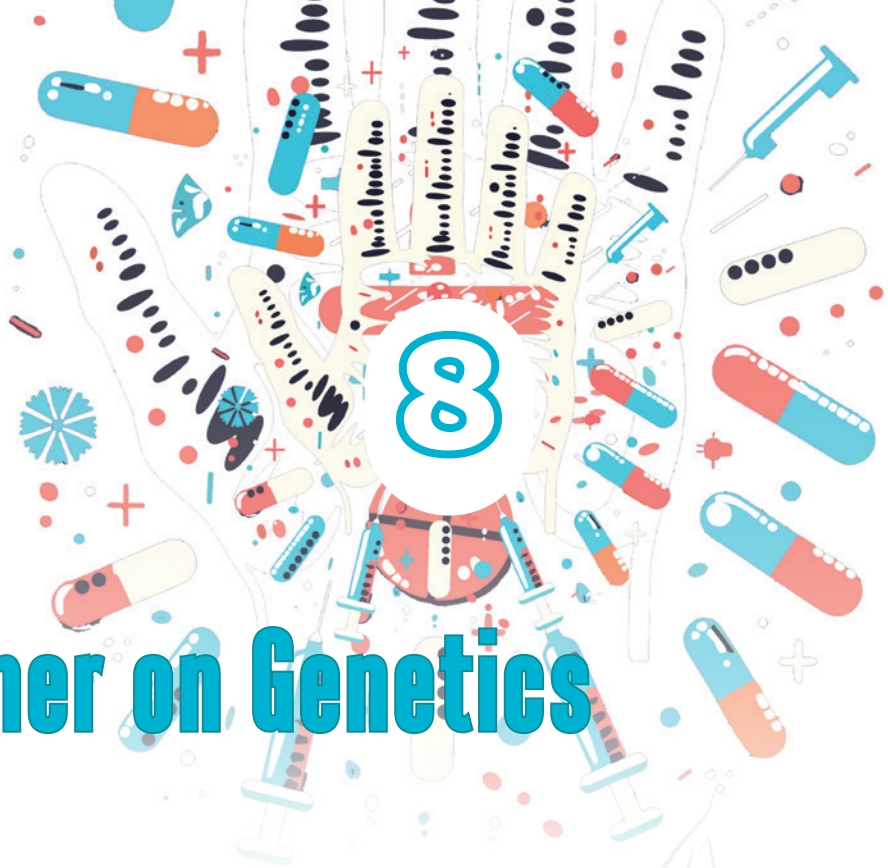
Cockayne's syndrome (CS): This is a homozygous or heterozygous mutation that results in short stature, abnormalities in head size, and slow growth and development.

Rothmund–Thomson syndrome (RTS): This is a rare autosomal recessive disorder that affects the skin. It is characterized by the sparse hair, juvenile cataracts, skeletal abnormalities, and stunted growth.

8

Primer on Genetics

Contents



8 Primer on Genetics

Genetics is the study of genes, genetic variation, and heredity in living organisms (bacteria, plants, animals and, of special interest to us, humans). It is an important branch in biology because heredity is vital to organisms' evolution. Gregor Mendel, a Moravian Augustinian friar working in 19th century in Brno, was the first to study genetics scientifically. He studied *trait inheritance* - patterns in the way traits are handed down from parents to offspring over time. He observed that organisms (pea plants) inherit traits by way of discrete *units of inheritance*. (This term, still used today, is a somewhat ambiguous definition of what is referred to as a gene.) He also observed that traits are inherited by way of discrete "units of inheritance", which we refer to as *genes*. This observation suggested that heredity was *particulate* (i.e., not acquired).

Trait inheritance and molecular inheritance mechanisms of genes are still primary principles of genetics in the 21st century, but modern genetics has expanded to study the function and behavior of genes.

Gene structure and function, variation, and distribution are studied within the context of the cell, the organism (e.g. dominance), and within the context of a population. Genetics has given rise to a number of subfields, including molecular genetics, epigenetics, ecogenetics, and population genetics.

Organisms studied within the broad field span the domains of life (archaea, bacteria, and eukarya).

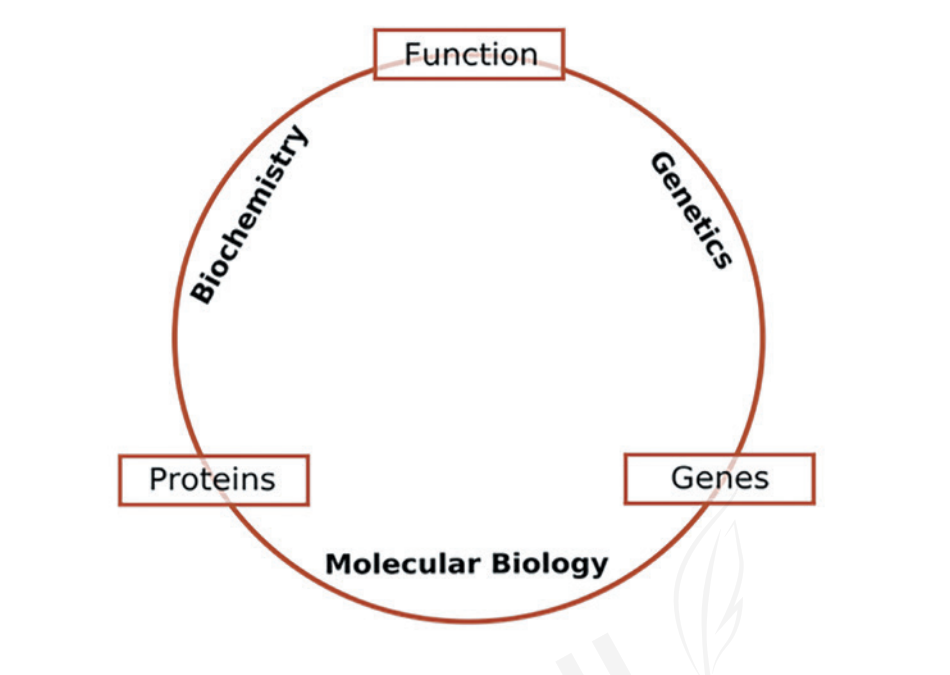
The schematic relationship between genetics, molecular biology, and biochemistry is illustrated in Figure 8.1.

Genes

Gene definition

The modern working definition of a gene is a portion (or sequence) of DNA that codes for a known cellular function or process (e.g., the function "make melanin molecules"). The following metaphors have been found useful in describing genes: gene ~ "word"; nucleotides (molecules) that make up genes ~ "letters" (a single gene may have a small or a large number of nucleotides in the same way that a word may include a small or a large number of letters). All genes must have nucleotides in the same way that all words are made up of letters. A single gene often interacts with neighboring genes to produce a cellular function and can even be ineffectual without those neighboring genes in the same way that a "word" may have meaning only in the context of a "sentence". Similarly, in the same way that a string of letters can be put together without forming a word (e.g. abeghkl), so can a series of nucleotides be put together without forming a gene (non-coding regions of DNA). Gene structure, function, variation, and distribution are studied within the context of the cell, the organism and, beyond that, the population.

Figure 8.1 - Schematic relationship between genetics, biochemistry and molecular biology



Genetic processes

Genetic processes work in symbiosis with an organism's environment and experiences to influence development and behavior, often referred to in the past as "nature *versus* nurture", but more accurately as "nature *and* nurture". The intra- or extra-cellular environment of a cell or organism may switch gene transcription on or off. (A classic example is that of two seeds of genetically identical corn, one placed in a temperate climate and the other in an arid climate. While the average height of the two corn stalks may be genetically determined to be equal, the one in the arid climate only grows to half the height of the one in the temperate climate, due to lack of water and nutrients in its environment.)

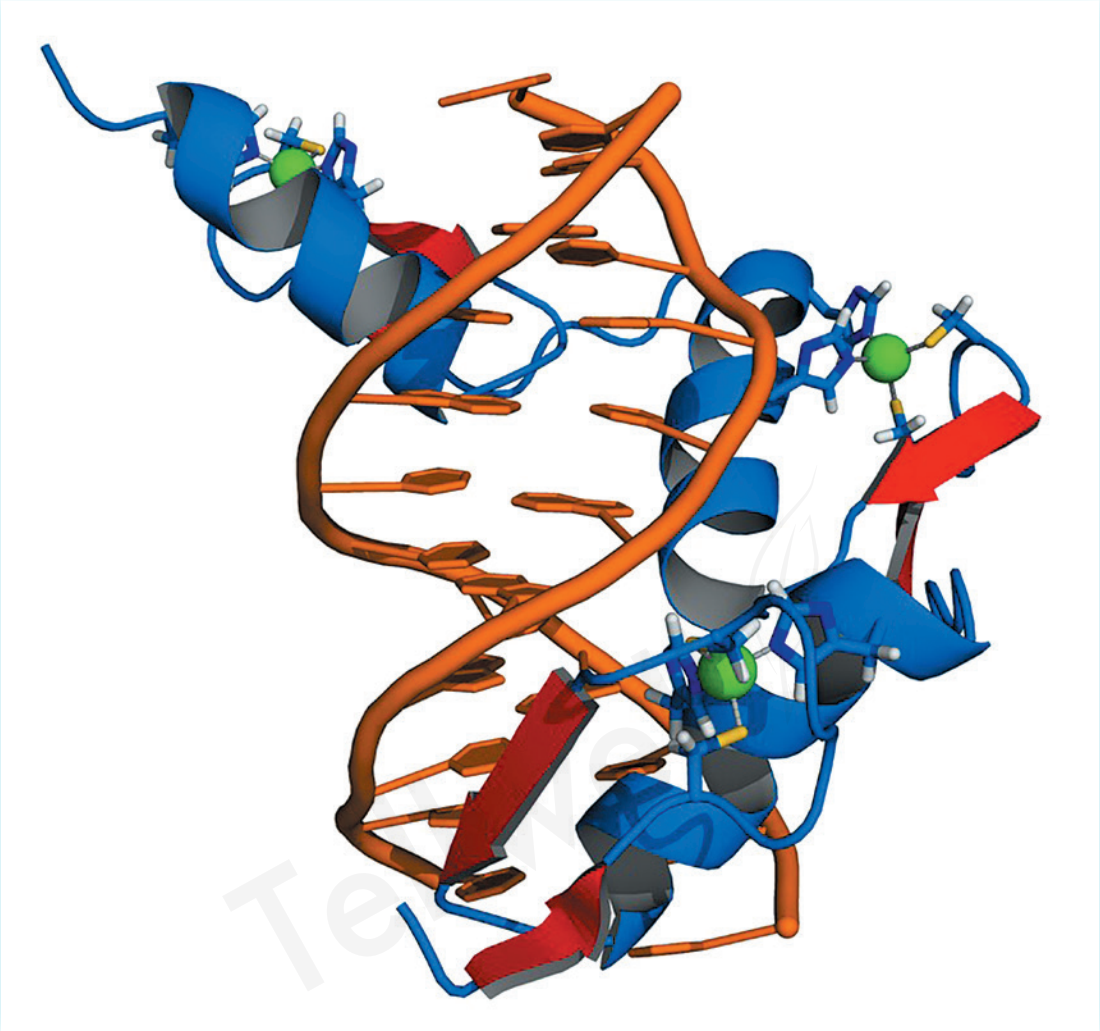
The sequence of nucleotides in a gene is read and translated by a cell to produce a chain of amino acids, which in turn folds into a protein. [A quick heuristic that is often used, but is not always true, is **OGOP** (**O**ne **G**ene, **O**ne **P**rotein), meaning a single gene codes for a single protein type in a cell.] The order of amino acids in a protein corresponds to the order of

nucleotides in the gene. This relationship between nucleotide sequence and amino acid sequence is known as the Genetic Code (discussed in Chapter 7 and further below). The amino acids in a protein determine how it folds into its unique three-dimensional shape, a structure that is ultimately responsible for the protein's function. Proteins carry out many of the functions needed for cells to live. A change to the DNA in a gene can alter a protein's amino acid sequence, thereby changing its shape and function and rendering the protein ineffective or even malignant, e.g., sickle cell anemia (SCA). Changes to genes are called *mutations* (see below).

Gene regulation

The genome of a given organism contains thousands of genes, but not all these genes need to be active at any given moment. A gene is expressed when it is being transcribed into m-RNA. There exist many cellular methods of controlling the *expression* of genes such that proteins are produced only when needed by the cell. *Transcription factors* (TF) (Figure 8.2) are regulatory proteins that bind to DNA to either promote or inhibit the transcription of a gene. Within the genome of *Escherichia coli* bacteria, for example, there exists a series of genes necessary for the synthesis of the amino acid tryptophan.

Figure 8.2 – Gene transcription factors bind to DNA to influence the transcription of associated genes



Differences in gene expression are especially clear within multicellular organisms, where all cells contain the same genome but have very different structures and behaviors due to the expression of different sets of genes. All the cells in a multicellular organism derive from a single cell, differentiating into variant cell types in response to external and inter-cellular signals and gradually establishing different patterns of gene expression to create different behaviors. As no single gene is responsible for the development of structures within multicellular organisms, these patterns arise from the complex interactions between many cells.

Within eukaryotes, there exist structural features of chromatin that influence the transcription of genes, often in the form of modifications to DNA and chromatin that are stably inherited by daughter cells. These features are called *epigenetic* because they exist "on top" of the DNA sequence and retain inheritance from one cell generation to the next. Because of epigenetic features, different cell types grown within the same medium can retain very different properties. Although epigenetic features are generally dynamic over the course of development, some, like the phenomenon of *paramutation*, have multigenerational inheritance and exist as rare exceptions to the general rule of DNA as the basis for inheritance. More on this topic in Chapter 9.

Gene mutations and genetic change

During the process of DNA replication, errors occasionally occur in the polymerization of the second strand. These errors, called *mutations*, can have an impact on the phenotype of an organism, especially if they occur within the protein coding sequence of a gene. Error rates are usually very low—1 error in every 10–100 million bases—due to the "proof-reading" ability of DNA polymerases. Processes that increase the rate of changes in DNA are called mutagenic; mutagenic chemicals promote errors in DNA replication, often by interfering with the structure of base-pairing, while UV radiation induces mutations by causing damage to the DNA structure. Chemical damage to DNA occurs naturally as well and cells use DNA repair mechanisms to repair mismatches and breaks. The repair does not, however, always restore the original sequence. A particularly important source of DNA damages appears to be reactive oxygen species (ROS) produced by cellular aerobic respiration, and these can lead to mutations.

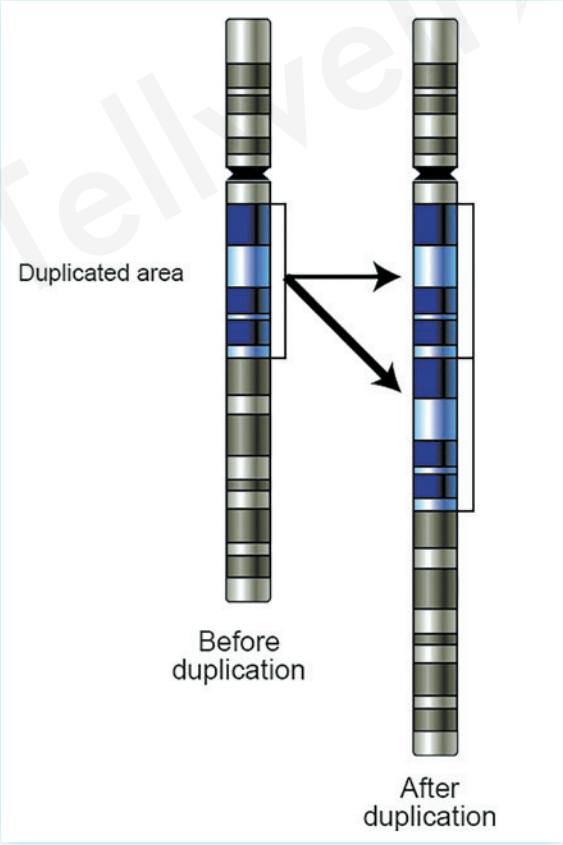
In organisms that use chromosomal crossover to exchange DNA and recombine genes, errors in alignment during meiosis can also cause mutations. Errors in crossover are especially likely when similar sequences cause partner chromosomes to adopt a mistaken alignment; this makes some regions in genomes more prone to mutating in this way. These errors create large structural changes in DNA sequence – *duplications, inversions, deletions* of entire regions – or the accidental exchange of whole parts of sequences between different chromosomes (*chromosomal translocation*).

Gene duplication allows diversification by providing redundancy, that is one gene can mutate and lose its original function without harming the organism (Figure 8.3).

Genetic variations

Genetic variation is the small percentage (~0.5%) of inter-individual genetic divergence that exists between humans - yet, this small variation is largely responsible for the phenotypic variations observed in humans (anthropometric measurements, eye or hair color, etc.). Its study has been permitted by the availability of the large data sets obtained by the Human Genome Project (HGP), the 1000 Genomes Project, and the International HapMap Project (IHP), which have provided a comprehensive catalogue of genetic variation in humans. This has facilitated analyses of the role of genetic variation in modulating various phenotypes and the study of disease susceptibilities.

Figure 8.3 - Gene duplication



Classes of genetic variation

Genetic variants are classified in terms of size and frequency. They vary from large to smaller scale variation. An example of large scale variation is *trisomy* (or addition of a chromosome pair in a diploid organism) such as exists in chromosome 21 in Down's Syndrome (DS). On a smaller scale, we find "Single Nucleotide Variants" (SNVs) (single base substitutions), which are themselves observed with varying frequencies in populations: rare (<1%), uncommon (1%-5%), and common (>5%). The latter, also called *Single Nucleotide Polymorphisms* (SNPs) are important markers in genomic mapping strategies.

Frequency-effect relationship

The variant frequency observed in the case of monogenic diseases was observed to be very rare as these variants are subjected to heavy selective pressure and, thus, are not likely to be propagated in subsequent generations. This is the opposite situation in the case of polygenic and complex diseases where SNPs have been subjected to low selective pressure and thus have reached high frequencies in global populations. This is at the basis of the **CDCV** concept (**C**ommon **D**isease, **C**ommon **V**ariant). As a consequence, SNPs have been established as the genomic marker of choice for many genomic studies.

Linkage disequilibrium

According to Mendel's Second Law of Independent Assortment (see below), in the process of inheritance, the alleles of different genes get shuffled between parents to form offspring(s) with many different combinations. However, some genes do not assort independently (a process called *genetic linkage*). This process applies to variants separated by considerable distances or even located on different chromosomes. However, SNP alleles (present every ~ 300 bases) tend to be inherited together as clusters known as "haplotype blocks". The International HapMap Project (IHP) has created a comprehensive map of haplotype blocks, which in turn permitted the reliable prediction of variant genotypes. This prediction allowed a vastly expanded

genomic coverage without the need of genotyping millions of supplemental markers all the while sparing the associated cost.

Chromosomal crossover and gene linkage

The diploid nature of chromosomes allows for genes on different chromosomes to assort independently or be separated from their homologous pair during sexual reproduction wherein haploid gametes are formed. In this way new combinations of genes can occur in the offspring of a mating pair. Genes on the same chromosome would theoretically never recombine. However, they do via the cellular process of "chromosomal crossover". During crossover, chromosomes exchange stretches of DNA, effectively shuffling the gene alleles between the chromosomes. This process of chromosomal crossover generally occurs during meiosis, a series of cell divisions that creates haploid cells.

The probability of chromosomal crossover occurring between two given points on the chromosome is related to the distance between the points. For an arbitrarily long distance, the probability of crossover is high enough that the inheritance of the genes is effectively uncorrelated. For genes that are closer together, however, the lower probability of crossover means that the genes demonstrate genetic linkage; alleles for the two genes tend to be inherited together. The amounts of linkage between a series of genes can be combined to form a linear linkage map that roughly describes the arrangement of the genes along the chromosome.

However, when tryptophan is already available to the cell, these genes for tryptophan synthesis are no longer needed. The presence of tryptophan directly affects the activity of the genes—tryptophan molecules bind to the tryptophan repressor (a transcription factor), changing the repressor's structure such that the repressor binds to the genes. The tryptophan repressor blocks the transcription and expression of the genes, thereby creating negative feedback regulation of the tryptophan synthesis process.

The Genetic Code

Genes generally express their functional effect through the production of proteins, which are complex molecules responsible for most functions in the cell. Proteins are made up of one or more polypeptide chains, each of which is composed of a sequence of amino acids, and the DNA sequence of a gene (through an RNA intermediate) is used to produce a specific amino acid sequence. This process begins with the production of an RNA molecule with a sequence matching the gene's DNA sequence, a process called *transcription*. This messenger RNA molecule is then used to produce a corresponding amino acid sequence through a process called *translation*. Each group of three nucleotides in the sequence, called a *codon*, corresponds either to one of the twenty possible amino acids in a protein or an instruction to end the amino acid sequence; this correspondence is called the Genetic Code (Figure 8.4). The flow of information is unidirectional: Information is transferred from nucleotide sequences into the amino acid sequence of proteins, but it never transfers from protein back into the sequence of DNA—a phenomenon Francis Crick called the "central dogma of molecular biology" (a misnomer as discussed elsewhere in this book).

Figure 8.4 - The Genetic Code and protein synthesis

Using a triplet code, through a messenger intermediary, RNA, DNA translates into a protein

A single nucleotide difference within DNA can cause a change in the amino acid sequence of a protein. Because protein structures are the result of

their amino acid sequences, some changes can dramatically change the properties of a protein by destabilizing the structure or changing the surface of the protein in a way that changes its interaction with other proteins and molecules. For example, sickle cell anemia (SCA) is a human genetic disease that results from a single base difference within the coding region for the β-globin section of hemoglobin, causing a single amino acid change that changes hemoglobin's physical properties. Sickle-cell versions of hemoglobin stick to themselves, stacking to form fibers that distort the shape of red blood cells carrying the protein. These sickle-shaped cells no longer flow smoothly through blood vessels, having a tendency to clog or degrade, causing the medical problems associated with this disease.

Some genes are transcribed into RNA but are not translated into protein products—such RNA molecules are called *non-coding RNA*. In some cases, these products fold into structures which are involved in critical cell functions (e.g., ribosomal RNA, r-RNA; and transfer RNA, t-RNA). RNA can also have a regulatory effect through hybridization interactions with other RNA molecules (e.g. micro-RNA).

Molecular basis for inheritance

DNA and chromosomes

The molecular basis for genes is deoxyribonucleic acid (DNA). DNA is composed of a chain of nucleotides, of which there are four types: Adenine (A), Cytosine (C), Guanine (G), and Thiamine (T). Genetic information exists in the sequence of these nucleotides, and genes exist as stretches of that sequence along the DNA chain. Viruses are the only exception to this rule—sometimes viruses use the very similar molecule RNA instead of DNA as their genetic material. Viruses cannot reproduce without a host and are unaffected by many genetic processes, so tend not to be considered living organisms.

DNA normally exists as a double-stranded molecule, coiled into the shape of a double helix (Figure 8.5). Each nucleotide in DNA preferentially pairs with its partner nucleotide on the opposite strand: A pairs with T, and C pairs

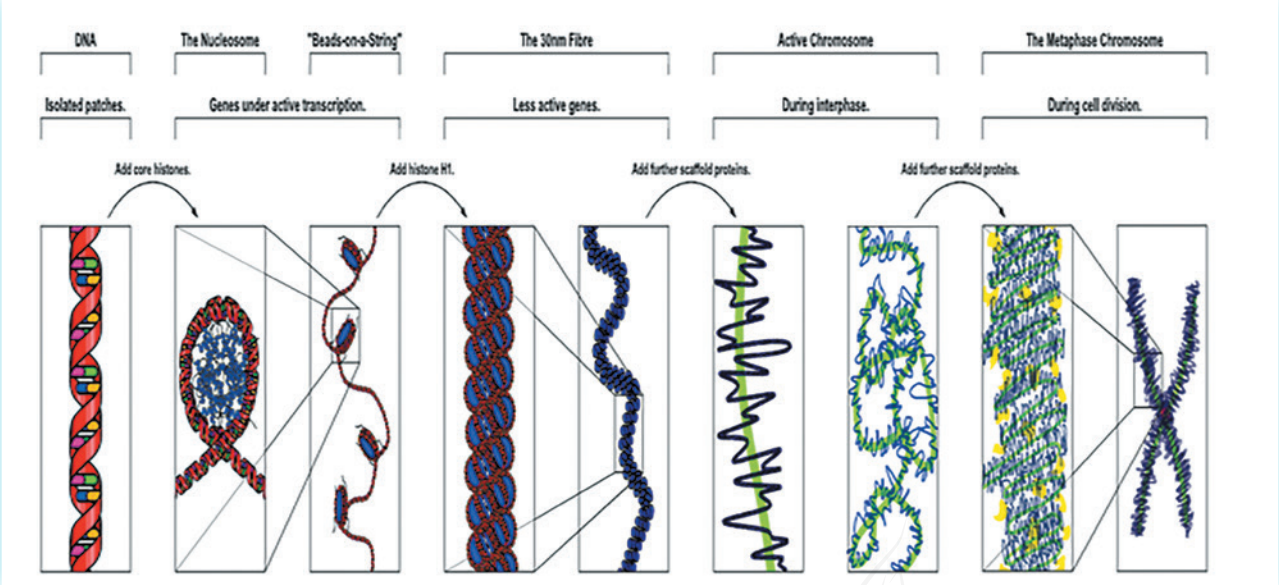
with G. Thus, in its two-stranded form, each strand effectively contains all the necessary information, redundant with its partner strand. This structure of DNA is the physical basis for inheritance: DNA *replication* replicates the genetic information by splitting the strands and using each strand as a template for synthesis of a new partner strand.

Genes are arranged linearly in long chains of DNA base-pair sequences. In bacteria, each cell usually contains a single circular *genophore*, while eukaryotic organisms (such as plants and animals) have their DNA arranged in multiple linear chromosomes. These DNA strands are often extremely long; the longest human chromosome, for example, is about 247 million base pairs in length. The DNA of a chromosome is associated with structural proteins that organize, compact, and control access to the DNA, forming a material called *chromatin.* In eukaryotes, chromatin is usually composed of *nucleosomes*, segments of DNA wound around cores of *histone* proteins. The full set of hereditary material in an organism (usually the combined DNA sequences of all chromosomes) is called the *genome*. The major structures in DNA compaction (DNA, the nucleosome, the 10 nm "beads-on-a-string" fibre, the 30 nm fiber and the metaphase chromosome) are illustrated in Figure 8.5.

In general terms, there are three levels of chromatin organization:

- DNA wraps around histone proteins forming nucleosomes; the "beads on a string" structure (*euchromatin*):
- Multiple histones wrap into a 30-nanometer (nm) fibre consisting of nucleosome arrays in their most compact form (*heterochromatin*). (Definitively established to exist *in vitro*, the 30-nm fibre was not seen in recent X-ray studies of human mitotic chromosomes); and
- Higher-level DNA packaging of the 30-nm fibre into the metaphase chromosome (during mitosis and meiosis).

Figure 8.5 - The major structures in DNA compaction



Source: Wikipedia

There are, however, many cells that do not follow this organization. (For example, spermatozoa and avian red blood cells have more tightly packed chromatin than most eukaryotic cells, and trypanosomatid protozoa do not condense their chromatin into visible chromosomes for mitosis.)

The proposed structure of the 30-nm chromatin filament for DNA repeat length per nucleosomes ranges from 177 to 207 base pairs (bp). Linker DNA is represented in yellow and nucleosomal DNA in pink (Figure 8.6). The structure of the nucleosome is illustrated in Figure 8.7 by a cartoon.

While haploid organisms have only one copy of each chromosome, most animals and many plants are diploid, containing two of each chromosome and thus two copies of every gene. The two alleles for a gene are located on identical loci of the two homologous chromosomes, each allele inherited from a different parent.

Figure 8.6 - A proposed structure for the 30-nm chromatin filament of DNA

Source: Wikipedia

Many species have so-called sex chromosomes that determine the gender of each organism.In humans and many other animals, the Y-chromosome contains the gene that triggers the development of the specifically male characteristics. In evolution, this chromosome has lost most of its content and also most of its genes, while the X-chromosome is similar to the other chromosomes and contains many genes. The X and Y chromosomes form a strongly heterogeneous pair.

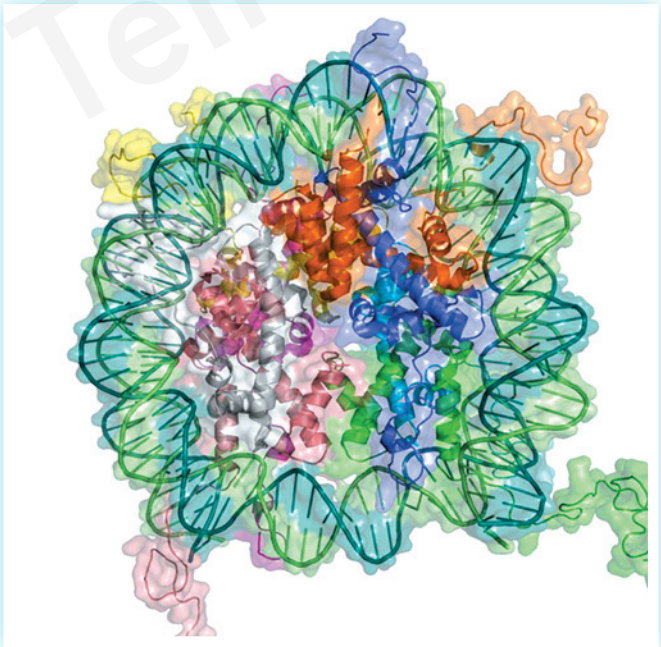
Sexual reproduction

When cells divide, their full genome is copied and each daughter cell inherits one copy. This process, called *mitosis*, is the simplest form of reproduction and is the basis for asexual reproduction. Asexual reproduction can also occur in multicellular organisms, producing offspring that inherit their genome from a single parent. Offspring that are genetically identical to their parents are called *clones*.

Eukaryotic organisms often use sexual reproduction to generate offspring that contain a mixture of genetic material inherited from two different parents. The process of sexual reproduction alternates between forms that contain single copies of the genome (*haploid*) and double copies (*diploid*). Haploid cells fuse and combine genetic material to create a diploid cell with paired chromosomes. Diploid organisms form haploids by dividing, without replicating their DNA, to create daughter cells that randomly inherit one of each pair of chromosomes. Most animals and many plants are diploid for most of their lifespan, with the haploid form reduced to single cell gametes such as sperm or eggs.

Although they do not use the haploid/diploid method of sexual reproduction, bacteria have many methods of acquiring new genetic information. Some bacteria can undergo *conjugation*, transferring a small circular piece of DNA to another bacterium. Bacteria can also take up raw DNA fragments found in the environment and integrate them into their genomes, a phenomenon known as *transformation.* These processes result in horizontal gene transfer, transmitting fragments of genetic information between organisms that would be otherwise unrelated.

Figure 8.7 - A cartoon representation of the nucleosome structure



Source: Wikipedia

Genetic theories

Historical theories

The observation that living things inherit traits from their parents has been used since prehistoric times to improve crop plants and animals through selective breeding. The modern science of genetics, seeking to understand this process, began with the work of the Augustinian friar *Gregor Mendel* in the mid-19th century.

Prior to Mendel, *Imre Festetics*, a Hungarian noble, who lived in Kőszeg before Mendel, was the first who used the word *genetic* in hereditarian context. He described several rules of biological inheritance in his work: "The Genetic Laws of Nature" (Die genetischen Gesetze der Natur, 1819). His second law is the same as that which Mendel published. In his third law, he developed the basic principles of mutation (he can be considered a forerunner of *Hugo de Vries)*. Festetics argued that changes observed in the generation of farm animals, plants, and humans are the result of scientific laws. He empirically deduced that organisms inherit their characteristics, not acquire them. He also recognized recessive traits and inherent variation by postulating that traits of past generations could reappear later, and organisms could produce progeny with different attributes. These observations represent an important prelude to Mendel's theory of particulate inheritance insofar as it features a transition of heredity from its status as myth to that of a scientific discipline, by providing a fundamental theoretical basis for genetics in the twentieth century.

Other theories of inheritance preceded Mendel's work. A popular theory during the 19th century, and implied by Charles Darwin's 1859 "On the Origin of Species", was blending inheritance: The idea that individuals inherit a smooth blend of traits from their parents. Mendel's work provided examples where traits were definitely not blended after hybridization, showing that traits are produced by combinations of distinct genes rather than a continuous blend. Blending of traits in the progeny is now explained by the action of multiple genes with quantitative effects.

Another theory that had some support at that time was the "inheritance of acquired characteristics": The belief that individuals inherit traits strengthened by their parents. This theory (commonly associated with *Jean-Baptiste Lamarck*) is now known to be wrong—the experiences of individuals do not affect the genes they pass to their children although some of its aspects are now being accepted.

Other theories included Darwin's pangenesis (which had both acquired and inherited aspects) and *Francis Galton*'s reformulation of pangenesis as both particulate and inherited.

Earlier theories of inheritance have been essentially invalidated (although some of their aspects are still useful), specifically:

- **Blending inheritance theory:** It posited that individuals inherit a smooth blend of traits from their parents. However, Mendel's work provided examples where traits were definitely not blended after hybridization, thus showing that traits are produced by combinations of distinct genes rather than a continuous blend. However, blending of traits in the progeny is now explained by the action of multiple genes with quantitative effects;
- **Inheritance of acquired characteristics:** In this theory, proposed by *Jean-Baptiste Lamarck,* individuals inherit traits strengthened by their parents. Although it had some support in its time, the theory has since proven to be mostly false. However, epigenetics is reviving some aspects of it, particularly the experiences of individuals affecting the genes they pass to their children;
- **Pangenesis inheritance:** Proposed by *Charles Darwin,* this theory had both acquired and inherited aspects; and
- **Revised pangenesis inheritance:** Reformulated by *Francis Gallon,* this theory has both particulate and inherited aspects.

Genetics has given rise to a number of sub-fields including population genetics, epigenetics (Chapter 9) and ecogenetics (Chapter 10).

Modern theories

A brief history of Mendelian and molecular genetics

In 1865: Modern genetics started when *Mendel* presented his paper "*Versuche über Pflanzenhybriden*" ("Experiments on Plant Hybridization") to the *Naturforschender Verein* ("Society for Research in Nature") in Brünn, in which he traced the inheritance patterns of certain traits in pea plants and further described them mathematically. Although this pattern of inheritance could only be observed for a few traits, many traits could be explained through simple rules and ratios.

In 1905: *Bateson*, a proponent of Mendel's work, coined the word *genetics* (from the Greek word *genesis*—γένεσις, «origin»), predating the noun first used in a biological sense in 1860.

In 1906: Bateson popularized the usage of the word *genetics* to describe the study of inheritance in his inaugural address to the Third International Conference on Plant Hybridization in London, England.

In 1911: *Thomas Hunt Morgan* argued that genes are located on chromosomes, based on observations of a sex-linked white eye mutation in fruit flies.

In 1913: Morgan's student, *Alfred Sturtevant*, used the phenomenon of genetic linkage to show that genes are arranged linearly on the chromosome. Although genes were known to exist on chromosomes and chromosomes are composed of both protein and DNA, scientists did not know which of these is responsible for inheritance.

In 1928: *Frederic Griffith* discovered experimentally the phenomenon of transformation: dead bacteria could transfer genetic material to "transform" other still-living bacteria.

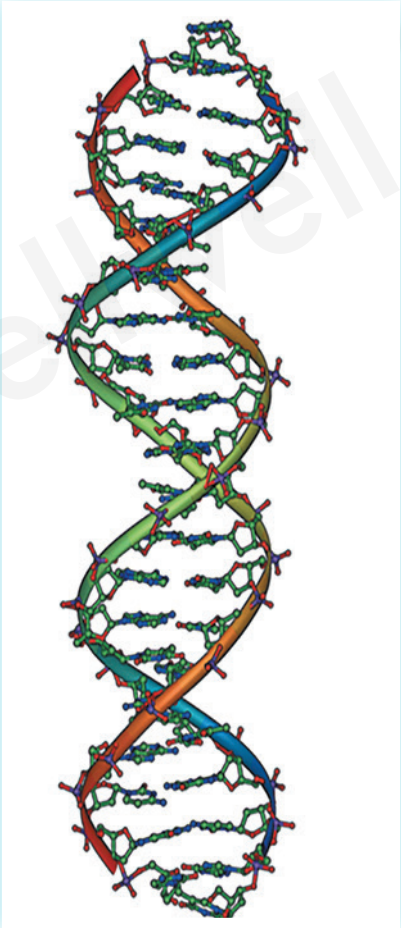
In 1943: In his work on the single-celled alga *Acetabularia, Hammerling* established the role of the nucleus as the repository of genetic information in eukaryotes.

In 1944: *Oswald Theodore Avery, Colin McLeod* and *Maclyn McCarthy* identified DNA as the molecule responsible for inheritance.

In 1952: The *Hershey-Chase* experiment confirmed that DNA (rather than protein) is the genetic material of the viruses that infect bacteria, providing further evidence that DNA is the molecule responsible for inheritance.

In 1953: *James D. Watson* and *Francis Crick* determined the structure of DNA using the X-ray crystallography work of *Rosalind Franklin* and *Maurice Wilkins* that indicated DNA had a helical structure. Their double-helix model had two strands of DNA with the nucleotides pointing inward, each matching a complementary nucleotide on the other strand to form what looks like rungs on a twisted ladder. This structure showed that genetic information exists in the sequence of nucleotides on each strand of DNA. The structure also suggested a simple method for replication: if the strands are separated, new partner strands can be reconstructed for each based on the sequence of the old strand. This property is what gives DNA its semi-conservative nature where one strand of new DNA originates from an original parent strand (Figure 8.8).

Figure 8.8 - The DNA double helical structure



Source: Wikipedia

In 1977: *Frederic Sanger* discovered chain termination DNA sequencing. This technology allows reading the nucleotide sequence of a DNA molecule.

In 1983: *Kary Banks* developed the polymerase chain reaction (PCR), providing a quick way to isolate and amplify a specific section of DNA from a mixture.

In 1987-2001: CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) was independently discovered in Japan (1987), the Netherlands (1993) and Spain (1998-2001). The discovery is based on clustered DNA repeats arranged consecutively along DNA.

In 2003: The efforts of the *Human Genome Project* (HGP), within the U.S. Department of Energy, and the National Institutes of Health, and a parallel private effort by private industry (*Celera Genomics)* led to the sequencing of the human genome.

Although the structure of DNA showed how inheritance works, it was still not known how DNA influences the behavior of cells. In the following years, scientists tried to understand how DNA controls the process of protein synthesis. It was discovered that the cell uses DNA as a template to create matching messenger RNA (m-RNA), which are molecules with nucleotides very similar to DNA. The nucleotide sequence of a m-RNA is used to create an amino acid sequence in protein; this translation between nucleotide sequences and amino acid sequences is known as the genetic code (see above).

Mendelian genetics

Modern genetics started with Mendel's studies of the nature of inheritance in plants. In his paper "*Versuche über Pflanzenhybriden*" ("Experiments on Plant Hybridization"), presented in 1865 to the *Naturforschender Verein* (Society for Research in Nature) in Brno. In it, he traced the inheritance patterns of certain traits in pea plants and described them mathematically. Although this pattern of inheritance could only be observed for a few traits, Mendel's work suggested that heredity was particulate, not acquired, and that the inheritance patterns of many traits could be explained through simple rules and ratios.

The importance of Mendel's work did not gain wide understanding until 1900, after his death, when Hugo de Vries and other scientists rediscovered his research. William Bateson, a proponent of Mendel's work, coined the word genetics in 1905. (The adjective genetic, derived from the Greek word genesis—γένεσις, «origin», predates the noun and was first used in a biological sense in 1860.)

Bateson both acted as a mentor and was aided significantly by the work of other scientists from Newnham College at Cambridge, specifically the work of *Becky Saunders, Nora Darwin Barlow*, and *Muriel Wheldale Onslow*. Bateson popularized the usage of the word genetics to describe the study of inheritance in his inaugural address to the Third International Conference on Plant Hybridization in London in 1906.

After the rediscovery of Mendel's work, scientists tried to determine which molecules in the cell were responsible for inheritance. In 1900, *Nettie Stevens* began studying the mealworm. Over the next 11 years, she discovered that females only had the X-chromosome and males had both X- and Y-chromosomes. She was able to conclude that sex is a chromosomal factor and is determined by the male. In 1911, *Thomas Hunt Morgan* argued that genes are on chromosomes, based on observations of a sex-linked white eye mutation in fruit flies. In 1913, his student *Alfred Sturtevant* used the phenomenon of genetic linkage to show that genes are arranged linearly on the chromosome.

Molecular genetics

Although genes were known to exist on chromosomes and chromosomes are composed of both protein and DNA, scientists did not know which of the two is responsible for inheritance. In 1928, *Frederick Griffith* discovered the phenomenon of transformation: Dead bacteria could transfer genetic material to "transform" other still-living bacteria. Sixteen years later, in 1944, the *Avery–MacLeod–McCarty* experiment identified DNA as the molecule responsible for transformation.

The role of the nucleus as the repository of genetic information in eukaryotes had been established by *Hämmerling* in 1943 in his work on the single celled

alga *Acetabularia*. The *Hershey–Chase* experiment in 1952 confirmed that DNA (rather than protein) is the genetic material of the viruses that infect bacteria, providing further evidence that DNA is the molecule responsible for inheritance.

James Watson and *Francis Crick* determined the structure of DNA in 1953, using the X-ray crystallography work of *Rosalind Franklin* and *Maurice Wilkins* that indicated DNA has a helical structure (i.e., shaped like a corkscrew). Their double-helix model had two strands of DNA with the nucleotides pointing inward, each matching a complementary nucleotide on the other strand to form what look like rungs on a twisted ladder. This structure showed that genetic information exists in the sequence of nucleotides on each strand of DNA. The structure also suggested a simple method for replication: If the strands are separated, new partner strands can be reconstructed for each based on the sequence of the old strand. This property is what gives DNA its semi-conservative nature where one strand of new DNA is from an original parent strand.

Although the structure of DNA showed how inheritance works, it was still not known how DNA influences the behavior of cells. In the following years, scientists tried to understand how DNA controls the process of protein production. It was discovered that the cell uses DNA as a template to create matching messenger RNA, molecules with nucleotides very similar to DNA. The nucleotide sequence of a messenger RNA is used to create an amino acid sequence in protein; this translation between nucleotide sequences and amino acid sequences is known as the genetic code.

With the newfound molecular understanding of inheritance came an explosion of research. A notable theory arose from *Tomoko Ohta* in 1973 with her amendment to the neutral theory of molecular evolution through publishing the nearly neutral theory of molecular evolution. In this theory, she stressed the importance of natural selection and the environment to the rate at which genetic evolution occurs. One important development was chain-termination DNA sequencing in 1977 by Frederick Sanger. This technology allows scientists to read the nucleotide sequence of a DNA molecule. In 1983, *Kary Banks Mullis* developed the polymerase chain reaction, providing a quick way to isolate and amplify a specific section of DNA from a mixture.

The efforts of the (U.S.) Department of Energy (DOE), National Institute of Health (NIH), Human Genome Project (HGP), and parallel private efforts by Celera Genomics led to the sequencing of the human genome in 2003.

Features of inheritance

Discrete inheritance and Mendel's Laws

At its most fundamental level, inheritance in organisms occurs by passing discrete heritable units, the genes, from parents to progeny. In his experiments studying the trait for flower color, Mendel observed that the flowers of each pea plant were either purple or white—but never an intermediate between the two colors. These different discrete versions of the same gene are called *alleles*.

In the case of the pea, which is a diploid species, each individual plant has two copies of each gene, one copy inherited from each parent. Many species, including humans, have this pattern of inheritance. Diploid organisms with two copies of the same allele of a given gene are called *homozygous* at that gene locus, while organisms with two different alleles of a given gene are called *heterozygous*.

The set of alleles for a given organism is called its *genotype*, while the observable traits of the organism are called its *phenotype*. When organisms are heterozygous at a gene, often one allele is called *dominant* as its qualities dominate the phenotype of the organism, while the other allele is called *recessive* as its qualities recede and are not observed. Some alleles do not have complete dominance and instead have incomplete dominance by expressing an intermediate phenotype, or co-dominance by expressing both alleles at once.

Single gene interactions: Mendel's First Law of Segregation

When a pair of organisms reproduce sexually, their offspring randomly inherit one of the two alleles from each parent. These observations of

discrete inheritance and the segregation of alleles are collectively known as *Mendel's First Law of Segregation*. It is illustrated in Figure 8.9.

One of the common diagrams used to predict the result of cross-breeding is the (2 x 2)-square matrix, **P**, or **Punnett's Square**. A simpler alternative to the Punnett's square is the (1 x 2)-(vertical)vector (Fymat, 2020) which he called **Mendel's vector**):

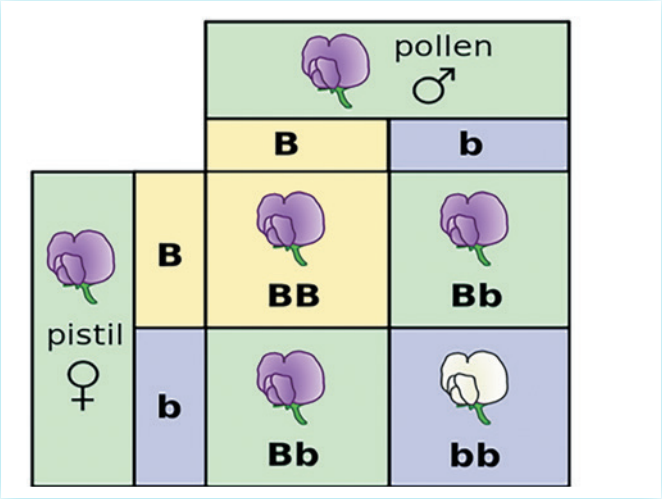
$$\mathbf{B}^{\mathbf{T}} = (B\ b)^{T},$$

where B is the dominant allele, b is the recessive allele, and the superscript T denotes the mathematical transpose of **B** (horizontal vector). **M** is merely the Hermitian vector product (denoted by the asterisk symbol, *, of $\mathbf{B}^{\mathbf{T}}$ and **B**:

$$\mathbf{B}^{\mathbf{T}} * \mathbf{B} = \mathbf{P}, \text{ that is: } \mathbf{B}^{\mathbf{T}} * B = (B\ b)^{T} * \begin{matrix}(B) \\ (b)\end{matrix} = \begin{matrix}(BB\ Bb) \\ (bB\ bb)\end{matrix}$$

Since the order in which the alleles are inherited (first from father or from mother) is irrelevant, it follows that: Bb = bB, making **P** a symmetrical matrix with only three independent elements (BB, bb, and bB or Bb). The above formalism could be easily extended to the genetic inheritance of several generations as a simple product of Hermitian vectors (Fymat, 2020).

Figure 8.9 – Punnett's Square depicting two pea plants heterozygous for purple (B) and white (b) blossoms



(B=Dominant; b= Recessive)

Source: Wikipedia

Multiple gene interactions: Mendel's Second Law of Independent Assortment

Organisms have thousands of genes and, in sexually reproducing organisms, these genes generally assort independently of each other. This means that the inheritance of an allele for yellow or green pea color is unrelated to the inheritance of alleles for white or purple flowers. This phenomenon, known as *Mendel's Second Law of Independent Assortment*, means that the alleles of different genes get shuffled between parents to form offspring(s) with many different combinations. (Some genes do not assort independently, demonstrating *genetic linkage*, a topic discussed earlier in this chapter).

Often, different genes can interact in a way that influences the same trait. This interaction between genes is called *epistasis*, with the second gene epistatic to the first.

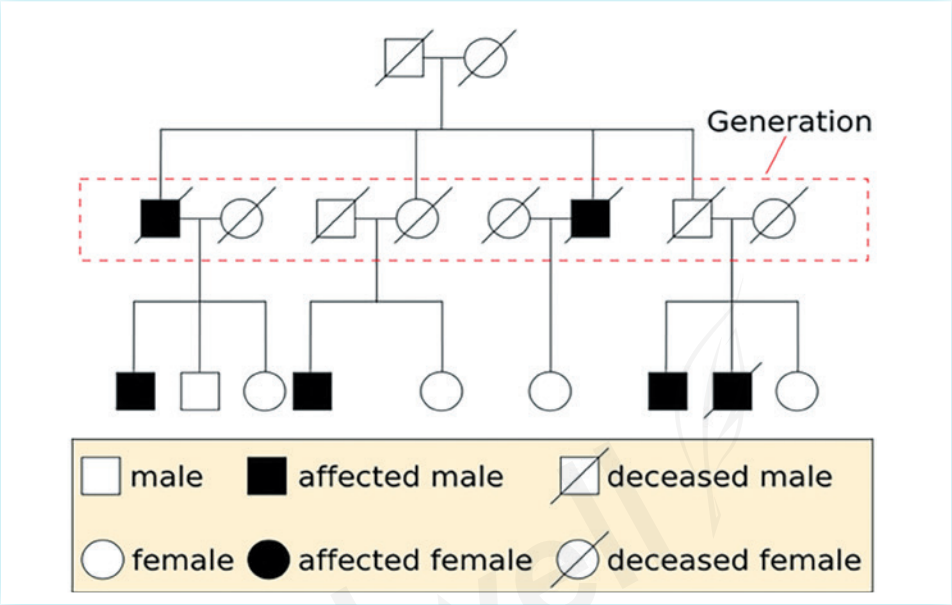
Many traits are not discrete features but are instead continuous features (e.g. human height and skin color). These complex traits are products of many genes. The influence of these genes is mediated, to varying degrees, by the environment an organism has experienced. The degree to which an organism's genes contribute to a complex trait is called *heritability*. Measurement of the heritability of a trait is relative—in a more variable environment, the environment has a bigger influence on the total variation of the trait. For example, human height is a trait with complex causes. It has a heritability of 89% in the United States. In Nigeria, however, where people experience a more variable access to good nutrition and health care, height has a heritability of only 62%.

Pedigree charts

Geneticists use diagrams and symbols to describe inheritance. A gene is represented by one or a few letters. Often a "+" symbol is used to mark the usual, non-mutant allele for a gene. In fertilization and breeding experiments (and especially when discussing Mendel's laws) the parents are referred to as the "P" generation and the offspring as the "F1" (first filial) generation. When the F1 offspring mate with each other, the offspring are called the "F2" (second filial) generation.

When studying human genetic diseases, geneticists often use *pedigree charts* to represent the inheritance of traits. These charts map the inheritance of a trait in a family tree (Figure 8.10).

Figure 8.10 - Genetic pedigree charts to help track the inheritance patterns of traits



Source: Wikipedia

Natural selection and evolution

Mutations alter an organism's genotype and occasionally this causes different phenotypes to appear. Most mutations have little effect on an organism's phenotype, health, or reproductive fitness. Mutations that do have an effect are usually deleterious, but occasionally some can be beneficial.

Population genetics studies the distribution of genetic differences within populations and how these distributions change over time. Changes in the frequency of an allele in a population are mainly influenced by natural selection, where a given allele provides a selective or reproductive advantage to the organism, as well as other factors such as mutation, genetic drift, artificial selection, and migration.

Over many generations, the genomes of organisms can change significantly, resulting in evolution. In the process called *adaptation*, selection for beneficial mutations can cause a species to evolve into forms better able to survive in their environment. New species are formed through the process of *speciation*, often caused by geographical separations that prevent populations from exchanging genes with each other. The application of genetic principles to the study of population biology and evolution is known as the "modern synthesis".

By comparing the homology between the genomes of different species, it is possible to calculate the evolutionary distance between them and when they may have diverged. Genetic comparisons are generally considered a more accurate method of characterizing the relatedness between species than the comparison of phenotypic characteristics. The evolutionary distances between species can be used to form evolutionary trees; these trees represent the common descent and divergence of species over time, although they do not show the transfer of genetic material between unrelated species (known as horizontal gene transfer, which is most common in bacteria).

Nature *and* nurture

In his 1869 book *"Hereditary Genius: An Inquiry Into Its Laws and Consequences", Francis Galton* (the cousin of Charles Darwin) spawned the "Nature *versus* Nurture debate" that lingers to this day. Although genes contain all the information an organism uses to function, the environment plays an important role in determining the ultimate phenotypes an organism displays. This is the complementary relationship often referred to as "nature *and* nurture".

The phenotype of an organism depends on the interaction of genes and the environment. For example, the environment plays a major role in effects of the human genetic disease known as phenylketonuria (PKU). The mutation that causes phenylketonuria disrupts the ability of the body to break down the amino acid phenylanaline, causing a toxic build-up of an intermediate molecule that, in turn, causes severe symptoms of progressive mental retardation and seizures. However, if someone with the phenylketonuria

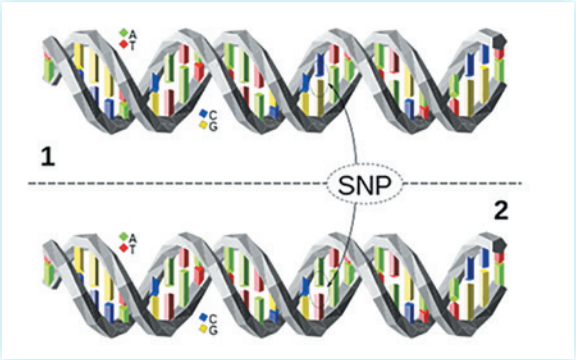
mutation follows a strict diet that avoids this amino acid, they remain normal and healthy.

A popular method in determining how genes and environment contribute to a phenotype is by studying identical and fraternal twins or siblings of multiple births. Because identical siblings come from the same zygote, they are genetically the same. Fraternal siblings are as genetically different from one another as normal siblings. By analyzing statistics on how often a twin of a set has a certain disorder compared to other sets of twins, it can be determined whether that disorder is caused by genetic or environmental factors (i.e. whether it has "nature" or "nurture" causes). One famous example is the multiple birth study of the Genain quadruplets, who were identical quadruplets all diagnosed with schizophrenia.

The Genome-Wide Association Studies (GWAS) now use DNA chips with 1 million or more markers (or Single Nucleotide Polymorphisms, SNP) scattered across the genome (Figure 8.11). Future DNA chips may include even larger numbers of SNPs. GWAS have now been performed for hundreds of different traits. These have identified SNPs that influence blood pressure, breast cancer, height, and many other traits, including intelligence (IQ score). Novel genes have been identified that may not have been predicted otherwise. These studies will be complemented by sequencing whole genomes to identify not only SNPs, but also rare genetic variants.

It seems the pendulum has swung toward nurture in the nature-nurture debate originated by Galton. Nonetheless, no trait is completely genetically determined and environment modifiers have a role for all traits.

Figure 8.11 - Single nucleotide polymorphisms



For more information, the reader is referred to my book "The Odyssey of Humanity's Diseases – Volume 1", particularly its Chapter 10 where the following topics are additionally discussed: Personal genomics and its use in predictive and precision medicine; whole genome sequencing with application to cancers; and gene editing – CRISPR, which lie beyond the scope of the current volume.

Natural selection and evolution

Mutations alter an organism's genotype and occasionally this causes different phenotypes to appear. Most mutations have little effect on an organism's phenotype, health, or reproductive fitness. Those that do have an effect are usually detrimental, but occasionally some can be beneficial. Studies in the fly *Drosophila melanogaster* suggest that if a mutation changes a protein produced by a gene, about 70% of these mutations are harmful with the remainder being either neutral or weakly beneficial. Studies of the distribution of genetic differences within populations and how these distributions change over time hav been carried out.

Changes in the frequency of an allele in a population are mainly influenced by natural selection, where a given allele provides a selective or reproductive advantage to the organism, as well as other factors such as mutation, genetic drift, genetic hitchhiking, artificial selection, and migration.

Over many generations, the genomes of organisms can change significantly, resulting in evolution. In the process called 'adaptation', selection for beneficial mutations can cause a species to evolve into forms better able to survive in their environment. New species are formed through the process of 'speciation', often caused by geographical separations that prevent populations from exchanging genes with each other.

By comparing the homology between different species' genomes, it is possible to calculate the 'evolutionary distance' between them and when they may have diverged. Genetic comparisons are generally considered a more accurate method of characterizing the relatedness between species than the comparison of phenotypic characteristics. The evolutionary distances between species can be used to form 'evolutionary trees'; these trees

represent the common descent and divergence of species over time, although they do not show the transfer of genetic material between unrelated species (known as 'horizontal gene transfer' and most common in bacteria).

Genetic components of aging

A number of genetic components of aging have been identified using model organisms, ranging from the simple budding yeast *Saccharomyces cerevisiae* to worms such as *Caenorhabditis elegans* and fruit flies *Drosophila melanogaster*. Study of these organisms has revealed the presence of at least two conserved aging pathways.

Gene expression is imperfectly controlled, and it is possible that random fluctuations in the expression levels of many genes contribute to the aging process as suggested by a study of such genes in yeast. Individual cells, which are genetically identical, nonetheless can have substantially different responses to outside stimuli, and markedly different lifespans, indicating the epigenetic factors play an important role in gene expression and aging as well as genetic factors. There is research into epigenetics of aging (see next Chapter).

The ability to repair DNA double-strand breaks declines with aging in mice and humans. A set of rare hereditary (genetics) disorders (each called progeria) has been known for some time. Sufferers exhibit symptoms resembling accelerated aging (see earlier discussion). A study indicates that aging may shift activity toward short genes or shorter transcript length and that this can be countered by interventions.

Conclusions and take-aways

- Genetics, a field of biology and the life sciences, is the study of genes, heredity, and genetic variations in living organisms (bacteria, plants, animals and, of special interest to us, humans). Traits are inherited by way of discrete units of inheritance, which we refer to as genes – a particulate characteristic (i.e., not acquired).

- *Trait* inheritance and *molecular* inheritance mechanisms of genes are still primary principles of genetics in the 21st century, but modern genetics has expanded to study the function and behavior of genes.
- Genes were discussed in abundant details including: Structure and function, variation, and distribution are studied within the context of the cell, the organism (e.g. dominance), and within the context of a population; genetic processes that work in symbiosis with an organism's environment and experiences to influence development and behavior; nucleotide sequences that produce amino acids chains that can fold into proteins; chromosomes that can crossover and exchange stretches of DNA, effectively shuffling the gene alleles between the chromosomes causing errors that create large structural changes in DNA sequence such as duplications, inversions, and deletions of entire regions or chromosomal translocation (the accidental exchange of whole parts of sequences between different chromosomes); genetic linkage for genes that are closer together; gene regulation by transcription factors; and mutations that occur during the process of DNA replication, which impact the phenotype of an organism.
- Genetics has given rise to a number of subfields, including molecular genetics, epigenetics, ecogenetics, and population genetics. Organisms studied within the broad field span the domains of life (archaea, bacteria, and eukarya).
- The genetic code and the process of protein formation was described. Even though small (~0.5%), inter-individual genetic divergence between humans is largely responsible for the phenotypic variations observed. It was the subject of the Human Genome Project (HGP), the International HapMap Project (IHP), and the 1000 Genomes Project. The classes of genetic variation were also discussed.
- The molecular basis for inheritance was reviewed including the major structures in DNA compaction as well as sexual reproduction, and natural selection and evolution. The field of medical genetics, which seeks to understand how genetic variation relates to human health and disease, was then cursorily introduced.
- The paradigm shift from nature-*vs*-nurture to nature-*and*-nurture was outlined for, although genes contain all the information an organism uses to function, the environment does play an important role in

determining the ultimate phenotypes an organism displays as no trait is completely genetically determined and environment modifiers have a role for all traits.

- Genetic processes work in combination with an organism's environment and experiences to influence development and behavior, often referred to as 'nature versus nurture'.
- The Avery–MacLeod–McCarty experiment identified DNA as the molecule responsible for transformation and Hämmerling established the role of the nucleus as the repository of genetic information in eukaryotes. The Hershey–Chase experiment confirmed that DNA (rather than protein) is the genetic material of the viruses that infect bacteria, providing further evidence that DNA is the molecule responsible for inheritance.
- James Watson and Francis Crick determined the structure of DNA using the X-ray crystallography work of Rosalind Franklin and Maurice Wilkins that indicated DNA has a double-helical structure. This structure showed that genetic information exists in the sequence of nucleotides on each strand of DNA. It also suggested a simple method for replication.
- In the following years, scientists showed how inheritance works and how DNA controls the process of protein production. It was discovered that the cell uses DNA as a template to create matching messenger RNA, molecules with nucleotides very similar to DNA.
- The earlier theories of inheritance (blending inheritance, inheritance of acquired characteristics, pangenesis inheritance and its revived version) have been essentially invalidated (although some of their aspects are still useful). The historical genetical theories has been summarized from Mendel, to Imre Festetics, Darwin, de Vries, Bateson, Newnham, Saunders, Darwin Barlow, Wheldale-Onslow, Stevens, Morgan, Sturtevant, and others.
- Mendel's first law of segregation states that when a pair of organisms reproduce sexually, their offspring randomly inherit one of the two alleles from each parent. Within this context, Fymat introduced a simpler mathematical representation to the traditional Punnett's square, namely what he dubbed "Mendel's vector", which can be used to represent inheritance as a Hermitian vector multiplication.

This formalism could be easily extended to the genetic inheritance of several generations as a simple product of Hermitian vectors.

- Mendel's second law of independent assortment means that the alleles of different genes get shuffled between parents to form offspring with many different combinations. However, many traits are not discrete features but are instead continuous features and the products of many genes. The influence of these genes is mediated, to varying degrees, by the environment so that measurement of the heritability of a trait is relative to its environment.
- Modern Mendelian genetics suggested that heredity was particulate, not acquired, and that the inheritance patterns of many traits could be explained through simple rules and ratios. After the rediscovery of Mendel's work, scientists determined which molecules in the cell were responsible for inheritance.
- With the newfound molecular understanding of inheritance came an explosion of research. One important development was chain-termination DNA sequencing by Frederick Sanger, the polymerase chain reaction by Kary Banks Mullis, and the sequencing of the human genome.
- During the process of DNA replication, errors (called mutations) occasionally occur in the polymerization of the second strand, affecting the phenotype of an organism, especially if they occur within the protein coding sequence of a gene. Chemical damage to DNA occurs naturally as well and cells use DNA repair mechanisms to repair mismatches and breaks. The repair does not, however, always restore the original sequence.
- A particularly important source of DNA damages appears to be reactive oxygen species (ROS) produced by cellular aerobic respiration, and these can lead to mutations. In organisms that use chromosomal crossover to exchange DNA and recombine genes, errors in alignment during meiosis can also cause mutations.
- Mutations alter an organism's genotype, occasionally causing different phenotypes to appear. Most mutations have little effect on an organism's phenotype, health, or reproductive fitness.
- Changes in the frequency of an allele in a population are mainly influenced by natural selection, where a given allele provides a selective

or reproductive advantage to the organism, as well as other factors such as mutation, genetic drift, genetic hitchhiking, artificial selection and migration.

- Over many generations, the genomes of organisms can change significantly, resulting in evolution. In the process called 'adaptation', selection for beneficial mutations can cause a species to evolve into forms better able to survive in their environment. New species are formed through the process of 'speciation'.
- By comparing the homology between different species' genomes, it is possible to calculate the 'evolutionary distance' between them and when they may have diverged.
- A number of genetic components of aging have been identified. Study of these organisms has revealed the presence of at least two conserved aging pathways.
- Gene expression is imperfectly controlled, and it is possible that random fluctuations in the expression levels of many genes contribute to the aging process. Epigenetic factors play an important role in gene expression and aging as well as genetic factors.
- The ability to repair DNA double-strand breaks declines with aging. Progeria sufferers exhibit symptoms resembling accelerated aging. Aging may shift activity toward short genes or shorter transcript length, which can be countered by interventions.

Sidebar 8.1 – A brief guide to Genomics

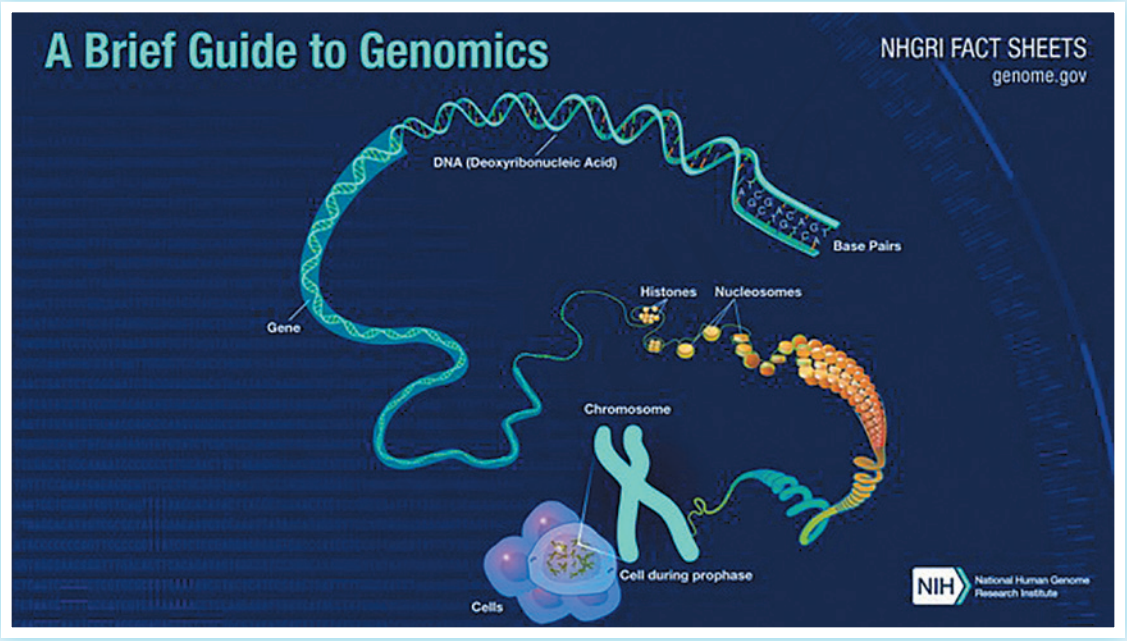
Genomics is the study of all of a person's genes (the genome), including interactions of those genes with each other and with the person's environment.

What is DNA?

As already indicated earlier in this chapter, deoxyribonucleic acid (DNA) is the chemical compound that contains the instructions needed to develop and direct the activities of nearly all living organisms. DNA molecules are made of two twisting, paired strands, often referred to as a double helix. Each DNA strand is made of four chemical units, called nucleotide bases, which comprise the genetic "alphabet". The bases are adenine (A), thymine (T),

guanine (G), and cytosine (C). Bases on opposite strands pair specifically: an A always pairs with a T; a C always pairs with a G. The order of the As, Ts, Cs and Gs determines the meaning of the information encoded in that part of the DNA molecule just as the order of letters determines the meaning of a word. With its four-letter language, DNA contains the information needed to build the entire human body. A gene traditionally refers to the unit of DNA that carries the instructions for making a specific protein or set of proteins. Each of the estimated 20,000 to 25,000 genes in the human genome codes for an average of three proteins.

Figure 8. 12 – A brief guide to Genomics



Source: (U.S.) National Institute of Health NationaL Human Genome Research Institute

What is a genome?

An organism's complete set of DNA is called its genome. Virtually every single cell in the body contains a complete copy of the approximately 3 billion DNA base pairs, or letters, that make up the human genome.

Located on 23 pairs of chromosomes packed into the nucleus of a human cell, genes direct the production of proteins with the assistance of enzymes

and messenger molecules. Specifically, an enzyme copies the information in a gene's DNA into a molecule called messenger ribonucleic acid (mRNA). The mRNA travels out of the nucleus and into the cell's cytoplasm, where the mRNA is read by a tiny molecular machine called a ribosome, and the information is used to link together small molecules called amino acids in the right order to form a specific protein.

Proteins make up body structures like organs and tissue, as well as control chemical reactions and carry signals between cells. If a cell's DNA is mutated, an abnormal protein may be produced, which can disrupt the body's usual processes and lead to a disease such as cancer.

What is DNA sequencing?

Sequencing simply means determining the exact order of the bases in a strand of DNA. Because bases exist as pairs, and the identity of one of the bases in the pair determines the other member of the pair, researchers do not have to report both bases of the pair.

In the most common type of sequencing used today, called *sequencing by synthesis*, DNA polymerase (the enzyme in cells that synthesizes DNA) is used to generate a new strand of DNA from a strand of interest. In the sequencing reaction, the enzyme incorporates into the new DNA strand individual nucleotides that have been chemically tagged with a fluorescent label. As this happens, the nucleotide is excited by a light source, and a fluorescent signal is emitted and detected. The signal is different depending on which of the four nucleotides was incorporated. This method can generate 'reads' of 125 nucleotides in a row and billions of reads at a time.

To assemble the sequence of all the bases in a large piece of DNA such as a gene, researchers need to read the sequence of overlapping segments. This allows the longer sequence to be assembled from shorter pieces, somewhat like putting together a linear jigsaw puzzle. In this process, each base has to be read not just once, but at least several times in the overlapping segments to ensure accuracy. Researchers can use DNA sequencing to search for genetic variations and/or mutations that may play a role in the development or progression of a disease. The disease-causing change may be as small

as the substitution, deletion, or addition of a single base pair or as large as a deletion of thousands of bases.

What is the Human Genome Project (HGP)?

The Human Genome Project (HGP), which was led at the National Institutes of Health (NIH) by the National Human Genome Research Institute (HGRI), produced a very high-quality version of the human genome sequence that is freely available in public databases. That international project was successfully completed in April 2003. The sequence is not that of one person, but is a composite derived from several individuals. It is a "representative" or generic sequence ensuring anonymity of the DNA donors.

The HGP was designed to generate a resource that could be used for a broad range of biomedical studies. One such use is to look for the genetic variations that increase risk of specific diseases, such as cancer, or to look for the type of genetic mutations frequently seen in cancerous cells. More research can then be done to fully understand how the genome functions and to discover the genetic basis for health and disease.

What are the implications for medical science?

Virtually every human ailment has some basis in our genes. Until recently, doctors were able to take the study of genes, or genetics, into consideration only in cases of birth defects and a limited set of other diseases. These were conditions, such as sickle cell anemia (SCA), which have very simple, predictable inheritance patterns because each is caused by a change in a single gene.

With the vast trove of data about human DNA generated by the HGP and other genomic research, scientists and clinicians have more powerful tools to study the role that multiple genetic factors acting together and with the environment play in much more complex diseases. These diseases, such as cancer, diabetes, and cardiovascular disease constitute the majority of health problems in the United States. Genome-based research is already enabling medical researchers to develop improved diagnostics, more effective therapeutic strategies, evidence-based approaches for demonstrating clinical efficacy, and better decision-making tools for patients and providers.

Ultimately, it appears inevitable that treatments will be tailored to a patient's particular genomic makeup. Thus, the role of genetics in health care is starting to change profoundly and the first examples of the era of genomic medicine are upon us.

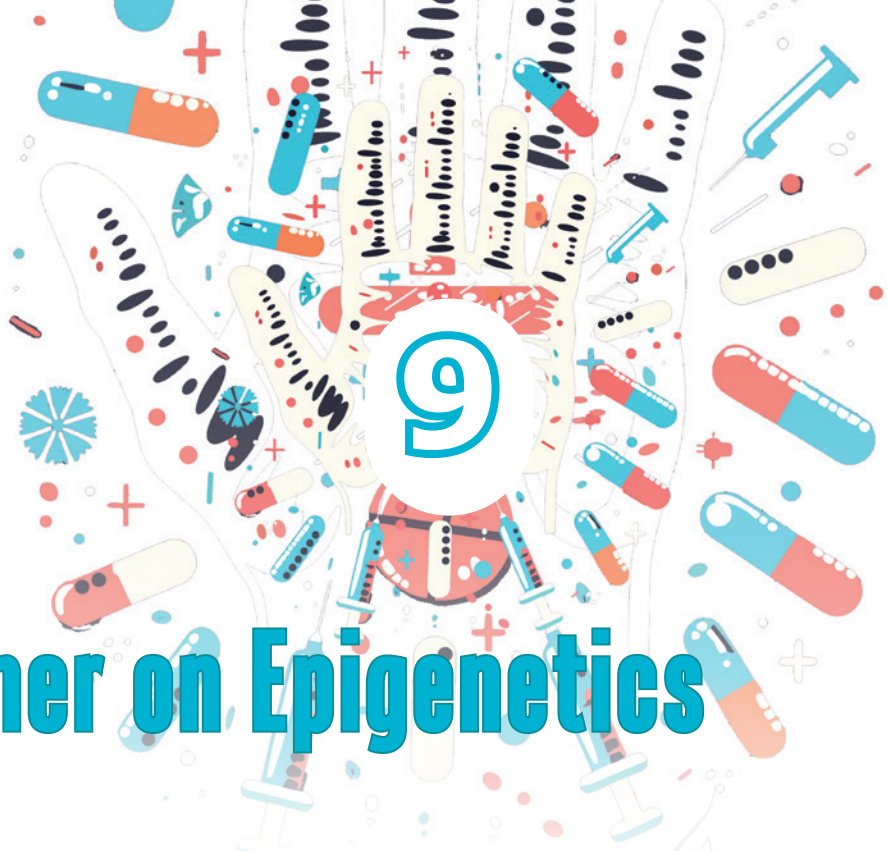
It is important to realize, however, that it often takes considerable time, effort, and funding to move discoveries from the scientific laboratory into the medical clinic. Most new drugs based on genome-based research are estimated to be at least 10 to 15 years away, though recent genome-driven efforts in lipid-lowering therapy have considerably shortened that interval. According to biotechnology experts, it usually takes more than a decade for a company to conduct the kinds of clinical studies needed to receive approval from the Food and Drug Administration (FDA).

Screening and diagnostic tests, however, are here. Rapid progress is also being made in the emerging field of pharmacogenomics, which involves using information about a patient's genetic make-up to better tailor drug therapy to their individual needs. Clearly, genetics remains just one of several factors that contribute to people's risk of developing most common diseases. Diet, lifestyle, and environmental exposures also come into play for many conditions, including many types of cancer. Still, a deeper understanding of genetics will shed light on more than just hereditary risks by revealing the basic components of cells and, ultimately, explaining how all the various elements work together to affect the human body in both health and disease.

9

Primer on Epigenetics

Contents



9

Primer on Epigenetics

Until recently, it was believed that our genes dictate our destiny and that we are slated for the **diseases** that will ultimately beset us based upon the pre-wired indecipherable code written in stone in our genetic material. The burgeoning field of epigenetics, however, is overturning these tenets, and ushering in a school of thought where nurture, not nature, is seen to be the predominant influence when it comes to genetic expression and our freedom from, or affliction by, chronic disease.

It had been generally accepted that evidence for inherited predisposition to a disease automatically implies involvement of DNA sequence variations (polymorphisms, mutations). Although this research program has been very productive in classical genetic disorders such as sickle cell anemia (SCA) and Huntington's disease (HD), application of the DNA sequence-oriented paradigm to complex non-Mendelian diseases such as schizophrenia, asthma, diabetes, multiple sclerosis (MS; see my book), among numerous others, has not led to significant breakthroughs thus far.

Epigenetic theories of complex diseases are usually based on two simple postulates:

- Along with the DNA sequences, at least some epigenetic factors (DNA and chromatin modification) are transmitted from one generation to another, the former accounting for heritability of some traits, and

- Epigenetic factors play a critical role in the regulation of various genomic functions in the cell.

Thus, putative epigenetic misregulation of genes, more so than DNA sequence-based ones, can explain a series of universal non-Mendelian features including: (a) Environmental effects; (b) discordance of monozygotic twins; (c) differential susceptibility in males and females; (d) parental origin effects; (e) in some complex diseases, fluctuating course of, and even recovery from, disease; (f) presence of familial and sporadic cases, and (g) unclear mode of inheritance. It is expected that identification of epigenetic changes in human diseases will provide the basis for new, epigenetic modification-based therapeutic approaches.

The (U.S.) Centers for Disease Control & Prevention (CDC&P) state that genetics accounts for only ~ 10% of the disease burden, with the remaining 90% owing to environmental variables. An article published in the *Public Library of Science One (PLoS One)* entitled "*Genetic factors are not the major causes of* ***chronic diseases***" echoes these claims, citing that chronic diseases' origin is only 16.4% genetic, and 84.6% environmental. These concepts make sense in light of research on the "exposome", the cumulative measure of all the environmental insults an individual incurs during their life course that determine susceptibility to disease. Nonetheless, contrary to claims uttered by some that epigenetics ushered in the demise of genetics, genetics is still an important factor *albeit* much less predominant than previously believed.

We inherit one allele, or variant, of each gene from our mother and the other from our father. If the result of epigenetic processes is "imprinting", a phenomenon in which one of the two alleles of a gene pair is turned off, this can generate a deleterious health outcome if the expressed allele is defective or increases our susceptibility to infections or toxicants. Studies link cancers of nearly all types, neurobehavioral and cognitive dysfunction, **respiratory illnesses**, **autoimmune disorders**, reproductive anomalies, and c**ardiovascular disease** to epigenetic mechanisms. For example, the cardiac antiarrhythmic drug Procainamide and the antihypertensive agent Hydralazine can cause lupus in some people by causing aberrant patterns of DNA methylation and disrupting signaling pathways.

Pharmaceuticals, however, are not the only agents that can induce epigenetic disturbances. Whether one is born via vaginal birth or Cesarean section, breastfed or bottle-fed, raised with a pet in the house, or infected with certain childhood illnesses, all these factors influence one's epigenetic expression. Whether one is sedentary, prays, smokes, mediates, does yoga, has an extensive network of social support or is alienated from one's community—all of one's lifestyle choices play into one's risk for disease operating through mechanisms of epigenetics.

In delineating the totality of exposures to which an individual is subjected over their lifetime, the exposome can be subdivided into three overlapping and intertwined domains:

- **Internal environment:** It is comprised of processes innate to the body which impinge on the cellular milieu. This encompasses hormones and other cellular messengers, oxidative stress, inflammation, lipid peroxidation, bodily morphology, the **gut microbiota**, **aging,** and biochemical stress;
- **Specific external environment:** It consists of exposures including pathogens, radiation, chemical contaminants and **pollutants**, and medical interventions, as well as dietary, lifestyle, and occupational elements (Wild, 2012); and
- **General exterior environment:** This is at an even broader sociocultural and ecological level which may circumscribe factors such as psychological stress, socioeconomic status, geopolitical variables, educational attainment, urban/rural residence, and climate (Wild, 2012).

Defining epigenetics

Epigenetics is the study of the physiological mechanisms that silence or activate genes. It encompasses processes which alter gene function without changing the sequence of nucleotide base pairs in our DNA. Translated literally, it means: In addition to changes in genetic sequence, epigenetics includes processes such as "methylation", "acetylation", "phosphorylation", "sumolyation", and "ubiquitylation" (see further schizophrenia, asthma,

diabetes, multiple sclerosis descriptions below) that can be transmitted to daughter cells upon cell division

Epigenetics *(epi*-from the Greek word επι meaning over, outside of, on top off, around + genetics) is thus the study of cellular and physiological traits inherited by daughter cells, but not caused by changes in the DNA sequence. It is the study of stable, long-term alterations in the heritable transcriptional potential of a cell. Unlike genetics, which is based on changes to the DNA sequence (the genotype), in epigenetics, the changes in gene expression (or cellular phenotype) have other causes.

The term epigenetics also refers to the changes themselves, the relevant changes to the genome that do not involve a change in the nucleotide sequence. Important examples of mechanisms that produce such changes are DNA methylation and histone modification, each of which alters how genes are expressed without altering the underlying DNA sequence, as further described in later sections. Gene expression can be controlled through the action of "repressor proteins" that attach to "silencer regions" of the DNA. These epigenetic changes may last through cell divisions for the duration of the cell's life, and may also last for multiple generations even though they do not involve changes in the underlying DNA sequence of the organism**;** instead, non-genetic factors cause the organism's genes to behave (or "express themselves") differently.

Epigenetics can be divided into "predetermined" and "probabilistic" components. Predetermined epigenesis is a unidirectional movement from structural development in DNA to the functional maturation of the protein. Predetermined here means that development is scripted and predictable. Probabilistic epigenesis, on the other hand, is a bidirectional structure-function development with experiences and external molding development.

A brief history

In 1942: *C. H. Waddington* coined the word "epigenetics" at a time when the physical nature of genes and their role in heredity was not yet known. He used it as a conceptual model of how genes might interact with their surroundings to produce a phenotype. He used the phrase

"*epigenetic landscape*" as a metaphor for biological development. He held that cell fates were established in development like a marble rolls down a slope to the point of lowest local elevation. He suggested visualizing increasing irreversibility of cell type differentiation as ridges rising between the valleys where the marble (cells) are traveling. **In the 1960s:** *Paul Winterbert* advanced a radical epigenetic view that described his position on life processes and living in his trilogy *"Le vivant créateur de son évolution"* (The living being as the creator of his own evolution) (1962), *"Le développement du vivant par lui-même"* (The self- development of the living being) (1963), and *"L'existence délivrée de l'existentialisme"* (1964) (Existence freed from existentialism). **In 1968:** *Erik Erikson* advanced the "*epigenetic principle*" to encompass the notion that we develop through an unfolding of our personality in predetermined stages, and that our environment and surrounding culture influence how we progress through these stages. In relation to our socio- cultural settings, this biological unfolding is done in stages of psychological development where progress through each stage is in part determined by our success, or lack of success, in all the previous stages.**In 1990:** *Robin Holiday* defined epigenetics as *"the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms"*, that is, it describes anything other than DNA that influences the development of an organism. **In 1996:** *Arthur D. Riggs, V.E.A. Russo* and *R.A. Marthiessen* defined epigenetics as "the study of mitotically and meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence*".* **In 2003:** *Gilbert Gottlieb* proposed a "probabilistic epigenesis" that encompasses all of the possible developing factors on an organism and how they not only influence the organism and each other but how the organism also influences its own development.**In 2003 and beyond:** The (U.S.) National Institute of Health's (NIH) "Roadmap to the Epigenomics Project" uses the following definition: "...*For purposes of this program, epigenetics refers to both heritable changes in gene activity and expression (in the progeny of cells or of individuals) and also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable".* **In 2007:** *Sir Adrian Bird* redefined epigenetics without the constraint of requiring heritability as

"the structural adaptation of chromosomal regions so as to register, signal, or perpetuate activity states". The reason is that epigenetics has been used to describe processes such as histone modification that are not heritable. This definition would include transient modifications associated with DNA repair or cell-cycle phases as well as stable changes maintained across multiple cell generations, but exclude others such as templating of membrane architecture and prions unless they impinge on chromosome function. Such redefinitions however are not universally accepted and are still subject to dispute.**In 2009:** *S. L. Berger, T. Kouzarides, R. Shiekhattar,* and *A. Shilatifard* (2009) provided a consensus definition of the epigenetic trait as a *"stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence".*

Epigenetic evidence in humans

Twin studies

Epigenetic changes have been observed to occur in response to environmental exposure. In the case of humans with different environmental exposures, monozygotic (identical) twins were epigenetically indistinguishable during their early years, while older twins had remarkable differences in the overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation. The twin pairs who had spent less of their lifetime together and/or had greater differences in their medical histories were those who showed the largest such differences.

Recent studies involving both dizygotic (not identical) and monozygotic twins have produced some evidence of epigenetic influence in humans. Direct comparisons between identical twins constitute the ideal experimental model for testing environmental epigenetics, because DNA sequence differences that would be abundant in a singleton-based study do not interfere with the analysis. Research has shown that a difference in the environment can produce long-term epigenetic effects, and different developmental monozygotic twin subtypes may be different with respect to their susceptibility to be discordant from an epigenetic point of view.

One of the first high-throughput studies of epigenetic differences between monozygotic twins focused in comparing global and locus-specific changes in DNA methylation and histone modifications in a sample of 40 monozygotic twin pairs. In this case, only healthy twin pairs were studied, but a wide range of ages was represented, between 3 and 74 years. One of the major conclusions from this study was that there is an age-dependent accumulation of epigenetic differences between the two siblings of twin pairs. This accumulation suggests the existence of "epigenetic drift". A more recent study, where 114 monozygotic twins and 80 dizygotic twins were analyzed for the "DNA methylation" status of around 6,000 unique genomic regions, concluded that epigenetic similarity at the time of blastocyst splitting may also contribute to phenotypic similarities in monozygotic co-twins. This supports the notion that the microenvironment at early stages of embryonic development can be quite important for the establishment of epigenetic marks.

Genomic imprinting

Some human disorders are associated with "genomic imprinting", a phenomenon in mammals where the father and mother contribute different epigenetic patterns for specific genomic loci in their germ cells. The best-known cases of imprinting in human disorders are those in Angelman, Prader-Willi, and Beckwith-Wiedemann syndromes. The former two syndromes can be produced by the same genetic mutation (chromosome 15q partial deletion), and the particular syndrome that will develop depends on whether the mutation is inherited from the child's mother or father. This is due to the presence of genomic imprinting in the region. The latter syndrome is often caused by abnormalities in maternal genomic imprinting of a region on chromosome 11.

Transgenerational inheritance

In the Overkalix study, Marcus Pembrey and colleagues observed that the paternal (but not maternal) grandsons of Swedish men who were exposed during preadolescence to famine in the 19th century were less likely to die of cardiovascular disease. If food was plentiful, then, diabetes mortality in

the grandchildren increased, suggesting that this was a "transgenerational epigenetic inheritance". The opposite effect was observed for females—the paternal (but not maternal) grand daughters of women who experienced famine while in the womb (and therefore while their eggs were being formed) lived shorter lives on average. Similar transgenerational effects were observed during the 1944 Dutch Famine. Such transgenerational inheritance traits were noted and studied in the case of these two famines because, especially in the case of the Dutch Famine, accurate records were kept over long periods of time for both male and female descendants. No doubt such observations could have been made in the previous numerous famines except for the lack of the required meticulous records.

Epigenetic changes

Epigenetic changes can modify the activation of certain genes, but not the sequence of DNA. Additionally, the chromatin proteins associated with DNA may be activated or silenced. This is why the differentiated cells in a multi-cellular organism express only the genes that are necessary for their own activity.

Epigenetic changes are preserved when cells divide. Most epigenetic changes only occur within the course of one individual organism's lifetime, but, if gene inactivation occurs in a sperm or egg cell that results in fertilization, then some epigenetic changes can be transferred to the next generation. This raises the question of whether or not epigenetic changes in an organism can alter the basic structure of its DNA.

Specific epigenetic processes are multiple and varied: Bookmarking; carcinogenesis progress; cloning technical limitations; gene silencing; heterochromatin; histone modification regulation; imprinting; maternal effects; paramutation; pathogenesis technical limitations; position effect; reprogramming; teratogens effects; transvection; and X-chromosome inactivation.

DNA damages can also cause epigenetic changes. They are very frequent, occurring on average about 10,000 times a day per cell of the human body. These damages are largely repaired, but at the site of a DNA repair,

epigenetic changes can remain. DNA damaging chemicals, such as benzene, hydroquinone, styrene, carbon tetrachloride, and trichloroethylene cause considerable hypomethylation of DNA.

Foods are known to alter the epigenetics of rats on different diets. Some food components epigenetically increase the levels of DNA repair enzymes while others can reduce DNA damage, such as soy isoflavones and bilberry anthocyanins.

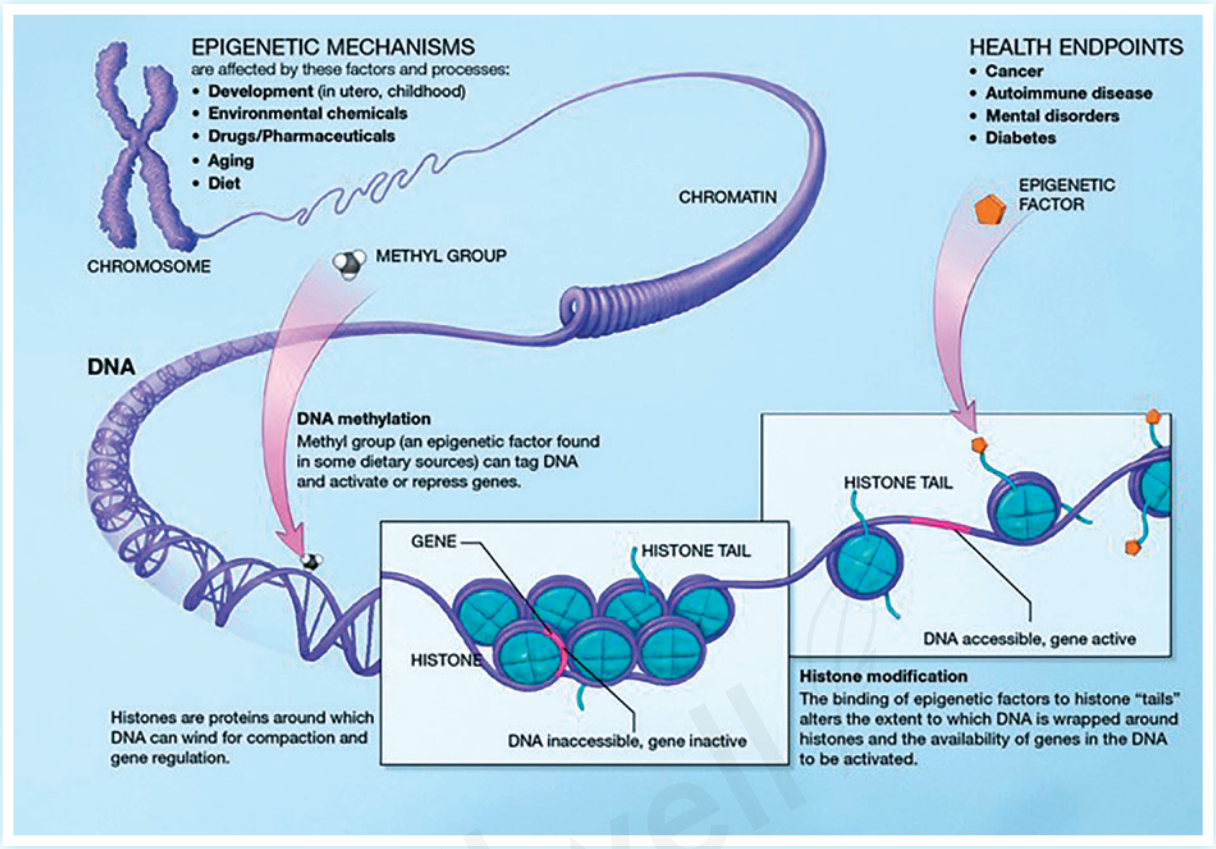
Epigenetic mechanisms

Several types of epigenetic inheritance systems may play a role in what has become known as "cell memory". (Note, however, that not all of these are universally accepted as examples of epigenetics.**)** Figure 9.1 is a sketch of the various epigenetic mechanisms and their health endpoints. These mechanisms are affected by the following factors and programs, including: development *in utero* and childhood, environmental chemicals, drugs/ pharmaceuticals, aging, and diet.

Chromatin remodeling

Because chromatin remodeling (and other mechanisms such as DNA methylation discussed in the next subsection) play such a central role in many types of epigenetic inheritance, the word "epigenetics" is sometimes, *albeit* misleadingly, used as a synonym for these processes. Chromatin remodeling is not always inherited and not all epigenetic inheritance involves chromatin remodeling. It is accomplished through two main mechanisms:

Figure 9.1 - Epigenetic mechanisms and health end points



- **Post-translational modification of the amino acids that make up histone proteins:** Histone proteins are made up of long chains of amino acids. If the amino acids that are in the chain are changed, the shape of the histone might be modified. DNA is not completely unwound during replication. It is possible, then, that the modified histones may be carried into each new copy of the DNA. Once there, these histones may act as templates, initiating the surrounding new histones to be shaped in the new manner. By altering the shape of the histones around them, these modified histones would ensure that a lineage-specific transcription program is maintained after cell division.
- **Addition of methyl groups to the DNA, mostly at CpG sites, to convert cytosyne to 5-methylcytosine:** 5-Methylcytosine performs much like a regular cytosine, pairing with a guanine in double-stranded DNA. However, some areas of the genome are methylated more heavily than others, and highly methylated areas tend to be less transcriptionally active, through a mechanism not fully understood.

Methylation of cytosines can also persist from the germ line of one of the parents into the zygote, marking the chromosome as being inherited from one parent or the other (genetic imprinting).

DNA methylation

In DNA methylation, a methyl group (an epigenetic factor found in some dietary sources) can tag DNA and activate or repress genes. Much is known about the mechanism of heritability of "DNA methylation" state during cell division and differentiation. The attachment of simple methyl group tags to DNA molecules can repress transcription of a gene when it occurs in the region of a gene promoter. This simple methyl group, or a carbon bound to three hydrogen molecules, effectively turns the gene off. Heritability of methylation state depends on certain enzymes, such as the DNA methyltransferase (DNAMT), that have a higher affinity for 5-methylcytosine than for cytosine. If this enzyme reaches a "hemimethylated" portion of DNA (where 5-methylcytosine is in only one of the two DNA strands) the enzyme will methylate the other half.

DNA methylation patterns are known to be established and modified in response to environmental factors by a complex interplay of at least three independent DNA methyltransferases (DNAMT1, DNAMT3A, and DNAMT3B). DNAMT1 is often referred to as the 'maintenance' methyltransferase. It is essential for proper embryonic development, imprinting, and X-inactivation. To emphasize the difference of this molecular mechanism of inheritance from the canonical Watson-Crick base-pairing mechanism of transmission of genetic information, the term "epigenetic templating**"** was introduced. Furthermore, in addition to the maintenance and transmission of methylated DNA states, the same principle could work in the maintenance and transmission of histone modifications.

Variation in methylation states of DNA can alter gene expression levels significantly. Methylation variation usually occurs through the action of DNA methylases. When the change is heritable, it is considered epigenetic. When the change in information status is not heritable, it would be a "somatic epitype". The effective information content has been changed by means of

the actions of a protein or proteins on DNA, but the primary DNA sequence is not altered.

Histone modifications

Post-translational modifications of histone proteins is another epigenetic process. Mechanisms of heritability of histone state are not well understood. Histones are proteins around which DNA can wind for compaction and gene regulation. In other words, histones help to package and condense the DNA double helix into the cell nucleus in a complex called chromatin (discussed above), which can be modified by enzymes, acetyl groups, and forms of RNA called small interfering RNAs (si-RNA) and microRNAs (mi-RNA). These chemical modifications of chromatin influence its three-dimensional structure, which in turn governs its accessibility for DNA transcription and dictates whether genes are expressed or not. Although "histone modifications" occur throughout the entire sequence, the unstructured N-termini of histones (called "histone tails") are particularly highly modified. These modifications include:

- ***Acetylation:*** Acetylation is the formation of an acetyl derivative (acetyl is the atom grouping CH_3CO, an acetic acid molecule from which the hydroxyl group has been removed). It is the most highly studied. It has a tendency to be associated with "active" transcription and is biophysical in nature. Because it normally has a positively charged nitrogen at its end, lysine can bind the negatively charged phosphates of the DNA backbone. The acetylation event converts the positively charged amine group on the side chain into a neutral amide linkage. This removes the positive charge, thus loosening the DNA from the histone. When this occurs, complexes and other transcriptional factors can bind to the DNA and allow transcription to occur. This is the "cis" model of epigenetic function. In other words, changes to the histone tails have a direct effect on the DNA itself. Another model of epigenetic function is the "trans" model in which changes to the histone tails act indirectly on the DNA. Further, acetylation at one position is likely to function differently from acetylation at another position. The idea

that multiple dynamic modifications regulate gene transcription in a systematic and reproducible way is called the "histone code".

- ***Citrullination* (or *deimination*):** This is the conversion of the amino acid arginine in a protein into the amino acid citrulline. Citrulline is not one of the 20 standard amino acids encoded by DNA in the genetic code. Instead, it is the result of a post-translation modification. Citrullination is distinct from the formation of the free amino acid citrulline as part of the urea cycle or as a byproduct of enzymes of the nitric oxide synthase family.
- ***Methylation*:** The addition of methyl groups (methyl is the radical - CH_3). It bears the idea that modifications act as docking modules for related factors. It is a chemical endogenous damage to DNA and an important regulator of gene transcription.
- ***Phosphorylation*:** The addition of phosphate to an organic compound such as glucose to produce glucose monophosphate through the action of a nontransferable (phosphorylase) or kinase.
- ***Tribulation*:** The chemical transformation into a ribbon – a radical formed by loss of the acetylene OH group from either of two cyclic forms of ribose (yielding riboflavin andrybopyranosyl compounds) by combination with an H of -nH- or -CH group.
- ***Sumoylation*: S**mall Ubiquitin-like m**o**difier (or sumo) proteins are a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their function. Sumoylation is a post-translationial modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle. Sumo proteins are similar to ubiquitin, and sumoylation is directed by an enzymatic cascade analogous to that involved in ubiquitination (see below). In contrast to ubiquitin, sumo is not used to tag proteins for degradation. Mature sumo is produced when the last four amino acids of the C-terminus have been cleaved off to allow formation of an isopeptide bond between the C-terminal glycine residue of sumo and an acceptor lysine on the target protein. Sumo family members often have dissimilar names; the sumo homologue in yeast, for example, is called SMT3 (suppressor of mif two 3). Several pseudogenes have been reported for this gene.

- ***Ubiquitination*:** Ubiquitin is a small (8.5 kDa) regulatory protein found in most tissues of eukaryotic organisms, i.e. it occurs ubiquitously. It was discovered in 1975 by *Gideon Goldstein* and further characterized throughout the 1970s and 1980s. There are four genes in the human genome code for ubiquitin: UBB, UBS, UBA52 and RPS27A. The addition of ubiquitin to a substrate protein is called ubiquitination (or less frequently ubiquitylation). Ubiquitination affects proteins in many ways: it can mark them for degradation via the proteasome, alter their cellular location, affect their activity, and promote or prevent protein interactions. Ubiquitination involves three main steps: activation, conjugation, and ligation, performed by ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and uniquitin ligases (E3s), respectively. The result of this sequential cascade is to bind ubiquitin to lysine residues on the protein substrate via an isopeptide bond, cystein residues through a thioester bond, serine and threonine residues through an ester bond, or the amino group of the protein's N-terminus via a peptide bond.

In histone modifications, the binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated.

Epigenetic effects

Prions

In general, proteins fold into discrete units that perform distinct cellular functions, but some proteins are also capable of forming an infectious conformational state known as a "prion". Although often viewed in the context of infectious diseases, prions are more loosely defined by their ability to catalytically convert other native state versions of the same protein to an infectious conformational state. It is in this latter sense that they can be viewed as epigenetic agents capable of inducing a phenotypic change without a modification of the genome.

Fungal prions are considered by some to be epigenetic because the infectious phenotype caused by the prion can be inherited without modification of the genome.

RNA and micro-RNA

Sometimes a gene, after being turned on, transcribes a product that (directly or indirectly) maintains the activity of that gene. RNA signaling includes differential recruitment of a hierarchy of generic chromatin modifying complexes and DNA methyltransferases to specific loci by RNAs during differentiation and development. Other epigenetic changes are mediated by the production of different splice forms of RNA, or by formation of double-stranded RNA. Descendants of the cell in which the gene was turned on will inherit this activity, even if the original stimulus for gene activation is no longer present. These genes are often turned on or off by signal transduction, although in some systems RNA may spread directly to other cells or nuclei by diffusion. A large amount of RNA and protein is contributed to the zygote by the mother during oogenesis or via nurse cells resulting in maternal effects phenotypes. A smaller quantity of sperm RNA is transmitted from the father, but there is recent evidence that this epigenetic information can lead to visible changes in several generations of offspring.

Micro-RNAs (mi-RNAs) are members of non-coding RNAs that range in size from 17 to 25 nucleotides. They regulate a large variety of biological functions. About 2000 mi-RNAs have so far been discovered in humans. It appears that about 60% of human protein coding genes are regulated by mi-RNAs. Many mi-RNAs are epigenetically regulated. About 50% of mi-RNA genes are associated with CpG islands that may be repressed by epigenetic methylation. Other mi-RNAs are epigenetically regulated by either histone modification or by combined DNA methylation and histone modification.

Epigenetic inheritance

Somatic epigenetic inheritance through epigenetic modifications, particularly through DNA methylation and chromatin remodeling, is very important in the development of multicellular eukaryotic organisms. The genome sequence

is static (with some notable exceptions), but cells differentiate into many different types, which perform different functions, and respond differently to the environment and intercellular signaling. Thus, as individuals develop, morphogens activate or silence genes in an epigenetically heritable fashion, giving cells a "memory".

In mammals, most cells terminally differentiate, with only stem cells retaining the ability to differentiate into several cell types ("totipotency" and "multipotency"). Some stem cells continue producing new differentiated cells throughout life, such as in neurogenesis, but mammals are not able to respond to loss of some tissues, for example, the inability to regenerate limbs, of which some other animals are capable. Unlike animals, plant cells do not terminally differentiate, remaining totipotent with the ability to give rise to a new individual plant. While plants do utilize many of the same epigenetic mechanisms as animals, such as chromatin remodeling, it has been hypothesized that some kinds of plant cells do not use or require "cellular memories", resetting their gene expression patterns using positional information from the environment and surrounding cells to determine their fate.

Transgenerational inheritance of epigenetic changes

Scientists formerly speculated that epigenetic changes disappear with each new generation during gametogenesis (the formation of sperm and ovum), and after fertilization. However, this theory was experimentally challenged.

Endocrine disruptors trigger infertility in future generations

The first challenge came with the demonstration that transient exposure of pregnant rats to the insecticide methoxychlor, an estrogenic compound, or the fungicide vinclozolin, an anti-androgenic compound, resulted in increased incidence of male infertility and decreased sperm production and viability in 90% of the males of four subsequent generations that were tracked.

Most notably, these reproductive effects were associated with derangements in DNA methylation patterns in the germ line, suggesting that epigenetic changes are passed on to future generations. The authors concluded, "*The*

ability of an environmental factor (for example, endocrine disruptor) to reprogram the germ line and to promote a transgenerational disease state has significant implications for evolutionary biology and disease etiology". (This may perhaps suggest that the endocrine-disrupting, fragrance-laden personal care products and commercial cleaning supplies to which we are all exposed may trigger fertility problems in multiple future generations.)

Parental experience shapes traits of offspring

In addition, traumatic experiences may be transmitted to future generations via epigenetics as a way to inform progeny about salient information needed for their survival. In one study, researchers wafted the **cherry**-like chemical acetophenone into the chambers of mice while administering electric shocks, conditioning the mice to fear the scent. This reaction was passed onto two successive generations, which shuddered significantly more in the presence of acetophenone despite never having encountered it compared to descendants of mice that had not received this conditioning (Anway *et al.,* 2005).

The study suggests that certain characteristics of the parental sensory environment experienced before conception can remodel the sensory nervous system and neuroanatomy in subsequently conceived generations. Alterations in brain structures that process olfactory stimuli were observed, as well as enhanced representation of the receptor that perceives the odor compared to control mice and their progeny. These changes were conveyed by epigenetic mechanisms, as illustrated by evidence that the acetophenone-sensing genes in fearful mice were hypomethylated, which may have enhanced expression of odorant-receptor genes during development leading to acetophenone sensitivity (Dias and Ressler, 2014).

On the existence of non-Mendelian transgenerational inheritance

Taken cumulatively, the aforementioned research challenges traditional Mendelian laws of genetics, which postulate that genetic inheritance occurs exclusively through sexual reproduction and that traits are passed to offspring through the chromosomes contained in germ line cells, and never through

somatic (bodily) cells. Effectively, this proves the existence of non-Mendelian transgenerational inheritance, where traits separate from chromosomal genes are transmitted to progeny, resulting in persistent phenotypes that endure across generations (Lim and Brunet, 2013).

(Note: This research imparts new meaning to the principle of seven generation stewardship taught by Native Americans, which mandates that we consider the welfare of seven generations to come in each of our decisions. Not only should we embody this approach in practices of environmental sustainability, but we would be wise to consider how the conditions to which we subject our bodies—the pollution and toxicants which permeate the landscape and pervade our bodies, the nutrient-devoid soil that engenders micronutrient-poor food, the disruptions to our **circadian rhythm** due to the ubiquity of electronic devices, our divorce from nature and the demise of our tribal affiliations—may translate into ill health effects and diminished quality of life for a previously unfathomed number of subsequent generations.)

Hazards of modern agriculture, the industrial revolution, and contemporary living are the "*known or suspected drivers behind epigenetic processes… including heavy metals, pesticides, diesel exhaust, tobacco smoke, polycyclic aromatic hydrocarbons, hormones, radioactivity, viruses, bacteria, and basic nutrients*" (Weinhold 2006, p.A160). Serendipitously, however, many inputs such as exercise, mindfulness, and bioactive components in fruits and vegetables such as Sulforaphane in cruciferous vegetables, Resveratrol from red grapes, Genistein from soy, Diallyl sulphide from garlic, Curcumin from turmeric, Betaine from beets, and green tea Catechin can favorably modify epigenetic phenomena "either by directly inhibiting enzymes that catalyze DNA methylation or histone modifications, or by altering the availability of substrates necessary for those enzymatic reactions" (Choi and Friso, 2010, p. 8).

This quintessentially underscores that the air we breathe, the food we eat, the thoughts we allow, the toxins to which we are exposed, and the experiences we undergo may persevere in our descendants and remain in our progeny long after we are gone. We must be cognizant of the effects of our actions, as they elicit a ripple effect through the proverbial sands of time.

The human experience of famine and tragedy spans generations

The mouse study discussed earlier, which illustrates how germ cells (egg and sperm) exhibit dynamic plasticity and adaptability in response to environmental signals, is mirrored by human studies as shown below.

Exposure to starvation and the Dutch famine

Exposures to certain stressors such as starvation during the gestational period are associated with poor health outcomes for offspring. Women who undergo famine before conception of their offspring have been demonstrated to give birth to children with lower self-reported mental health and quality of life, (see, for example, Stein *et al.*, 2009).

Studies similarly highlight that, "*Maternal famine exposure around the time of conception has been related to prevalence of major affective disorders, antisocial personality disorders,* ***schizophrenia****, decreased intracranial volume, and congenital abnormalities of the central nervous system*" (Stein *et al.*, 2009). Gestational exposure to the Dutch famine of the mid-twentieth century is also associated with lower perceived health (Roseboom *et al.*, 2003), as well as enhanced incidence of cardiovascular disease, hypertension, and obesity in offspring (Stein *et al,.* 2009). Maternal undernourishment during pregnancy leads to neonatal adiposity, which is a predictor of future obesity (Badon *et al.*, 2010) in the grandchildren (Veenendaal *et al.,* 2013).

Exposure to stress and the Holocaust

The impact of epigenetics is also exemplified by research on the intergenerational effects of trauma, which illuminate the observational fact that descendants of people who survived the Holocaust exhibit abnormal stress hormone profiles, and low cortisol production in particular (Yehuda and Bierer, 2008). Because of their impaired cortisol response and altered stress reactivity, children of Holocaust survivors are often at enhanced risk for post-traumatic stress disorder (PTSD), anxiety, and **depression** (Aviad-Wilcheck *et al.,* 2013).

Exposure to intimate partner violence during pregnancy

Intrauterine exposure to maternal stress in the form of intimate partner violence during pregnancy can also lead to changes in the methylation status of the glucocorticoid receptor (GCR) of their adolescent offspring (Radke *et al*, 2011). These studies suggest that an individual's experience of trauma can predispose their descendants to mental illness, behavioral problems, and psychological abnormalities due to "*transgenerational epigenetic programming of genes operating in the hypothalamic-pituitary-adrenal (HPA) axis*", a complex set of interactions among endocrine glands which determine stress response and resilience (Radke *et al.*, 2011).

Body cells (exosomes) pass genetic information directly into sperm cells

In addition to the above instances, studies are evidencing that genetic information can be transferred through the germ line cells of a species in real time. These paradigm-shifting findings overturn conventional logic, which postulates that genetic change occurs over the protracted time scale of hundreds of thousands or even millions of years.

Further, in a relatively recent study, exosomes were found to be the medium through which information was transferred from somatic cells to gametes. This experiment entailed xenotransplantation, a process where living cells from one species are grafted into a recipient of another species. Specifically, human melanoma tumor cells genetically engineered to express genes for a fluorescent tracer enzyme called EGFP-encoding plasmid were transplanted into mice. The experimenters found that information-containing molecules containing the EGFP tracer were released into the animals' blood (Cossetti *et al.,* 2014). Exosomes, or "specialized membranous nano-sized vesicles derived from endocytic compartments that are released by many cell types" were found among the EGFP trackable molecules (Zomer *et al.,* 2010).

Exosomes, which are synthesized by all plant and animal cells, contain distinct protein repertoires and are created when inward budding occurs from the membrane of multivesicular bodies (MVBs), a type of organelle that serves as a membrane-bound sorting compartment within eukaryotic

cells (Zomer *et al.,* 2010). Exosomes contain mi-RNA and small RNA, types of non-coding RNA involved in regulating gene expression. In this study, exosomes delivered RNAs to mature sperm cells (spermatozoa) and remained stored there (Cossetti *et al.,* 2014). This kind of RNA can behave as a "transgenerational determinant of inheritable epigenetic variations and that spermatozoal RNA can carry and deliver information that cause phenotypic variations in the progeny" (Cossetti *et al.,* 2014). In other words, the RNA carried to sperm cells by exosomes can preside over gene expression in a way that changes the observable traits and disease risk of the offspring as well as its morphology, development, and physiology. This study was the first to elucidate RNA-mediated transfer of information from somatic to germ cells, which fundamentally overturns what is known as the "Weismann's barrier". The Weismann's barrier is a principle which states that the movement of hereditary information from genes to body cells is unidirectional, and that the information transmitted by egg and sperm to future generations remains independent of somatic cells and parental experience (Cossetti *et al.,* 2014).

Further, this may bear implications for cancer risk (see section below), as exosomes contain vast amounts of genetic information which can be a source of lateral gene transfer (Balaj *et al.,* 2011); they are also abundantly liberated from tumor cells (Azmi *et al.*, 2013). This can be reconciled with the fact that exosome-resembling vesicles have been observed in various mammals (Cossetti *et al.,* 2014), including humans, in close proximity to sperm in anatomical structures such as the epididymis as well as in seminal fluid (Poliakov *et al.*, 2009). These exosomes may thereafter be propagated to future generations with fertilization and augment cancer risk in the offspring (Cheng *et al*., 2004).

It can be concluded therefore that sperm cells can act as the final repositories of somatic cell-derived information, which suggests that epigenetic insults to our body cells can be relayed to future generations. This notion is confirmatory of the evolutionary theory of "soft inheritance" proposed by French naturalist Jean-Baptiste Lamarck, whereby characteristics acquired over the life of an organism are transmitted to offspring, a concept which modern genetics previously rejected before the field of epigenetics arrived on the scene. In this way, the sperm cells are able to spontaneously assimilate exogenous DNA and RNA molecules, behaving both as vector of their native genome

and of extrachromosomal foreign genetic material, which is "then delivered to oocytes at fertilization with the ensuing generation of phenotypically modified animals" (Cossetti *et al.,* 2014).

In summary, the past of our ancestors lives on through us: Groundbreaking research illustrates how parental experience is not only epigenetically imprinted onto offspring, but onto an unprecedented number of future generations. Rather than occurring over the elongated time scale of millions of years, genetic change can transpire in real biological time through nanoparticles known as exosomes.

Epigenetic changes endure longer than ever predicted

In a recent study, nematode worms were manipulated to harbor a transgene for a fluorescent protein, which made the worms glow under **ultraviolet light** when the gene was activated (Klosin *et al.*, 2017). When the worms were incubated under the ambient temperature of 20° Celsius (68° Fahrenheit), negligible glowing was observed, indicating low activity of the transgene. However, transferring the worms to a warmer climate of 25°C (77° F) stimulated expression of the gene, as the worms glowed brightly.

In addition, this temperature-induced alteration in gene expression was found to persist for at least 14 generations, representing the preservation of epigenetic memories of environmental change across an unprecedented number of generations. In other words, the worms transmitted memories of past environmental conditions to their descendants, through the vehicle of epigenetic change, as a way to prepare their offspring for prevailing environmental conditions and ensure their survivability. Whether these conclusions may apply to humans has not yet been proposed or demonstrated.

Applications

In Evolution

Epigenetics can impact evolution when epigenetic changes are heritable. Two important ways in which epigenetic inheritance can be different from

traditional genetic inheritance, with important consequences for evolution, are that (a) rates of epimutation can be much faster than rates of mutation, and (b) the epimutations are more easily reversible. An epigenetically inherited element can be good enough for short-term adaptation that allows the lineage to survive long enough for mutation and/or recombination to genetically assimilate the adaptive phenotypic change. The existence of this possibility increases the evolvability of a species.

In Medicine

Epigenetics has many and varied potential medical applications as it tends to be multi-dimensional in nature. Congenital genetic disease is well understood and it is clear that epigenetics can play a role, for example, in the syndromes considered above. These are normal genetic diseases caused by gene deletions or inactivations. They are unusually common because individuals are essentially hemizygous by genomic imprinting, and therefore a single gene knock-out is sufficient to cause the disease where most cases would require both copies to be knocked out.

Epigenetics has the potential to explain mechanisms of aging, human development, and the origins of cancer, heart disease, mental illness, as well as several other conditions. Some investigators even think that epigenetics may ultimately turn out to have a greater role in disease than genetics.

Epigenetics and cancer

A variety of compounds are considered as epigenetic carcinogens (Fymat 2107a), They result in an increased incidence of tumors, but they do not show mutagen activity. Examples include: diethylstilbestrol, arsenite, hexachlorobenzene, and nickel compounds.

Teratogen effects

Many teratogens exert specific effects on the fetus by epigenetic mechanisms. While epigenetic effects may preserve the effect of a teratogen such as diethylstilbestrol throughout the life of an affected child, the possibility of

birth defects resulting from exposure of fathers or in second and succeeding generations of offspring has generally been rejected on theoretical grounds and for lack of evidence. However, a range of male-mediated abnormalities have been demonstrated, and more are likely to exist. The (U.S.) Food & Drug Administration (FDA) label information for *Vidaza*, a formulation of 5-azacitidine (an unmethylatable analog of cytidine that causes hypomethylation when incorporated into DNA) states that "men should be advised not to father a child" while using the drug, citing evidence in treated male mice of reduced fertility, increased embryo loss, and abnormal embryo development. In rats, endocrine differences were observed in offspring of males exposed to morphine. In mice, second generation effects of diethylstilbesterol have been described occurring by epigenetic mechanisms.

Recent studies have shown that the Mixed Lineage Leukemia (MLL) gene causes leukemia by rearranging and fusing with other genes in different chromosomes, which is a process under epigenetic control. Other investigations have concluded that alterations in histone acetylation and DNA methylation occur in various genes influencing prostate cancer. Gene expression in the prostate can be modulated by nutrition and lifestyle changes.

DNA methylation

DNA methylation is an important regulator of gene transcription and a large body of evidence has demonstrated that aberrant DNA methylation is associated with unscheduled gene silencing, and the genes with high levels of 5-methylcytosine in their promoter region are transcriptionally silent. DNA methylation is essential during embryonic development and, in somatic cells, patterns of DNA methylation are in general transmitted to daughter cells with a high fidelity. Aberrant DNA methylation patterns have been associated with a large number of human malignancies and found in two distinct forms: hypermethylation and hypomethylation compared to normal tissue. Hypermethylation is one of the major epigenetic modifications that repress transcription via promoter region of tumor suppressor genes. It typically occurs at CpG islands in the promoter region and is associated with gene inactivation. Global hypomethylation has also been implicated in the development and progression of cancer through different mechanisms.

DNA repair epigenetics

Germ line (familial) mutations have been identified in 34 different DNA repair genes that cause a high risk of cancer, including, for example BRCA1 and ATM. However, cancers caused by such germ line mutations make up only a very small proportion of cancers. For instance, germ line mutations cause only 2% to 5% of colon cancer cases.

Epigenetic reductions in expression of DNA repair genes, however, are very frequent in sporadic (non-germ line) cancers, as shown among some representative cancers in Table 9.1, while mutations in DNA repair genes in sporadic cancer are very rare.

Table 9.1 - Frequency of Epigenetic Changes (CpG Island Methylation) in DNA Repair Genes in Sporadic Cancers

Cancer	Gene	Epigeneticchange	Frequency
Breast	BRCA1	CpGislandmethylation	13%
	WRN	CpGislandmethylation	17%
Ovarian	WRN	CpGislandmethylation	36%
	BRCA1	CpGislandmethylation	5%–30%
	FANCF	CpGislandmethylation	21%
	RAD51C	CpGislandmethylation	3%
Colorectal	MGMT	CpGislandmethylation	40%–90%
	WRN	CpGislandmethylation	38%
	MLH1	CpGislandmethylation	2%–65%
	MSH2	CpGislandmethylation	13%
	ERCC1	epigenetictypeunknown	100%
	Xpf	epigenetictypeunknown	55%
Head&Neck	MGMT	CpGislandmethylation	35%–57%
	MLH1	CpGislandmethylation	27%–33%
	NEIL1	CpGislandmethylation	62%
	FANCB	CpGislandmethylation	46%
	MSH4	CpGislandmethylation	46%
	ATM	CpGislandmethylation	25%

Deficiencies in expression of DNA repair genes cause increased mutation rates and genome instability, which is likely the main underlying cause of the genetic alterations leading to cancer. In fact, the first event in many

sporadic neoplasias is a heritable alteration that affects genetic instability and epigenetic defects in DNA repair are somatically heritable.

Cancer treatment

Current research has shown that epigenetic pharmaceuticals could be a replacement or adjuvant therapy for currently accepted treatment methods such as radiation and chemotherapy, or could enhance the effects of these current treatments. It has been shown that the epigenetic control of the proto-onco regions and the tumor suppressor sequences by conformational changes in histones directly affects the formation and progression of cancer. Epigenetics also has the factor of reversibility, a characteristic that other cancer treatments do not offer (Fymat (2017a).

Drug development has focused mainly on Histone AcetylTransferase (HAT) and Histone DeACetylase (HDAC), and has included the introduction to the market of the new pharmaceutical *Vorinostat,* an HDAC inhibitor. HDAC has been shown to play an integral role in the progression of oral squamous cancer. Current front-runner candidates for new drug targets are Histone Lysine MethylTransferase (HLMT) and Protein Arginine Methyl Transferase (PAMT).

Mutation genetics of cardiovascular diseases

At the 2014 Annual Meeting of the American Heart Association (AHA), *Dr. Robert Califf,* Cardiologist at Duke University, presented the results of a study sponsored by Merck Pharmaceuticals (manufacturer of the drug *Vytorin*). The study was a six-year, double-blind, randomized clinical study in which participated 18,000 patients who just had heart attacks or episodes of severe chest pain requiring hospital visit(s). The conclusions were that: (1) The use of the statin drug (Simvastatin) alone lowered the Low Density Lipoprotein (LDL) to LDL=69, whereas (2) the combined drug Vytorin [=Simvastatin + Ezetimibe (Zetia)] led to LDL=59, with 6.4% fewer cardiac events with Vytorin as compared with Simvastatin alone, and no side effects observed. This study raises questions on current Cholesterol Guidelines. When compared with a similar study in 2006 that included 750 participants with high cholesterol

levels in which Ezetimibe seemed useless, this latter study looked at the more convenient surrogate of plaque build-up in the carotid artery. The question then arises: Could statin dose be reduced with addition of Ezetimibe so as to counteract the side effects of the statin? (Fymat 2017b).

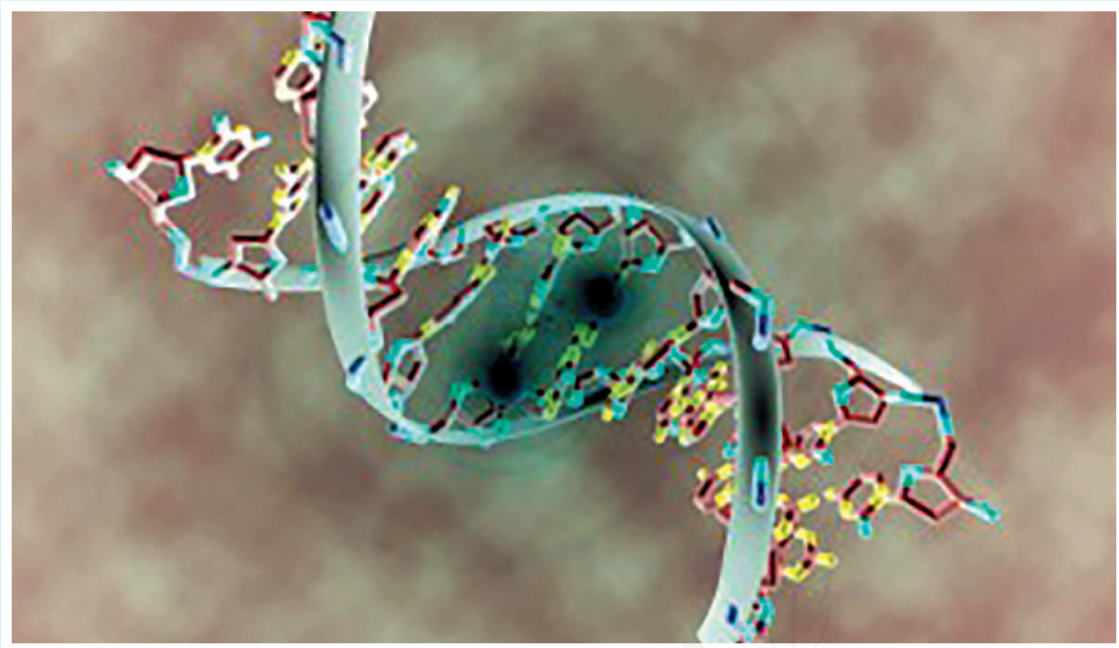
The above results were independently confirmed by two subsequent research studies. In the study by *Dr. Brian A. Ference*, Wayne State University, gene mutations mimicked the effect statins had on heart disease risk for a given reduction in cholesterol. In the other study by Dr. Khatiresen, mutations displaced one copy of the cholesterol absorption gene, producing the same effect as that produced by Ezetimibe (50% reduction in cholesterol), and an LDL reduction of 12 mg/dl of blood. The mutation which gave people the equivalent of a lifetime exposure to Ezetimibe, reduced the heart attack rate by 50%.

It is here speculated that mutation genetics may produce similar effects in other diseases, particularly cancer.

Functional epigenomics – Engineering the epigenome

Our desire to manipulate genomes is not new. Selective breeding, genetic and, more recently, genome engineering have greatly advanced our understanding of how genes shape phenotypes. However, epigenetic processes also influence how cells use genetic information. Like pointing out sections of a book with colored tags or Post-Its, the cell physically sticks chemical tags onto the genome, labeling features such as genes or regulatory elements. To date, millions of these tags ("chromatin marks") have been profiled across different tissues and cell types through international efforts. Yet, until recently, we were not able to assess the influence of individual marks on gene activity because it was only possible to alter chromatin marks globally through mutational approaches or pharmacological inhibition. Emerging technologies for epigenome engineering now make it possible to interrogate the function of individual chromatin marks by adding them to, or removing them from, single locations of interest in the genome. Thus, the use of targetable chromatin modifiers has ushered in a new era of functional epigenomics (Figure 9.2).

Figure 9.2 - Illustrating epigenome engineering with chromatin marks



Targeted modification is achieved by fusing an existing chromatin modifying enzyme (or a functional part of such an enzyme) to a programmable DNA binding domain. Although programmable DNA binding domains have been around for some time, it is only recently that it has been made considerably easier to generate a targetable protein in the laboratory. The chromatin-modifying enzyme of choice is simply fused to the catalytically-inactive Cas9-protein (dCas9). dCas9 is then targeted to a specific genomic locus via a separate, synthetic RNA molecule known as the guide RNA (g-RNA). The base sequence of the g-RNA thus determines the DNA binding specificity of the fusion protein. A range of chromatin modifiers have already been engineered in this way, allowing researchers to address some fundamental questions concerning the functions of individual chromatin marks.

It has long been unclear which of the large number of catalogued chromatin marks possess real gene-regulatory capabilities. Evidence to date has mainly been based on statistical correlation of chromatin marks with expression levels of associated genes. While a causal role of some chromatin marks in transcriptional regulation could be convincingly demonstrated for a few model

loci, it has long been unknown whether these specific findings extended to the vast remainder of the eukaryotic genome.

By directing chromatin modifiers to a range of sites at different genomic loci and measuring resulting changes in transcription of associated candidate genes, a number of functional chromatin marks have now been identified. For example, removal of methylation from lysine4 of histone H3 at putative enhancers and promoters with dCas9-LSD results in down-regulation of proximal genes, while addition of histone acetylation using dCas9-p300 has the opposite effect.

Collectively, these pioneering studies show that manipulation of individual chromatin marks at relevant sites can significantly alter levels of transcription, and that this effect depends both on the enzymatic activity of the chromatin modifier and its guided binding to the target site. However, the biological relevance of these engineering efforts must still be established. Measuring changes in protein levels or phenotypic changes in addition to changes in m-RNA levels, or comparing engineered gene expression to physiological levels of activity should ensure that changing specific chromatin marks can indeed influence cellular behavior.

The use of targetable chromatin modifiers has ushered in a new era of functional epigenomics. We anticipate that it will soon be possible to dissect the effect of altering combinations of chromatin marks by using different targeting platforms that can function independently in the same cell. The ease of targeting chromatin modifiers through an RNA-based DNA binding mechanism will further enable the unbiased discovery of functional marks using screening approaches. Many potentially functional marks could thus be interrogated in a single experiment, and only sites where chromatin modifications have significant impact—not only on transcription, but also on phenotype—may be identified in this way. Eventually, some of the findings may be translated into therapeutic use by adopting epigenetic engineering technologies to *in vivo* situations. There are already first indications that this may be feasible, provided of course that reagents can be delivered into cells efficiently.

RNA Epigenetics

DNA is not the only decorated nucleic acid in the cell. Modifications to RNA molecules are much more common and are critical for regulating diverse biological processes. For years, researchers described DNA and RNA as linear chains of four building blocks—the nucleotides A, G, C, and T for DNA; and A, G, C, and U for RNA. But these information molecules are much more than their core sequences. A variety of chemical modifications decorate the nucleic acids, increasing the alphabet of DNA to about a dozen known nucleotide variants. The alphabet of RNA is even more impressive, consisting of at least 140 alternative nucleotide forms. The different building blocks can affect the complementarity of the RNA molecules, alter their structure, and enable the binding of specific proteins that mediate various biochemical and cellular outcomes.

The large size of RNA's vocabulary relative to that of DNA's is not surprising. DNA is involved mainly with genetic information storage, while RNA molecules—messenger RNA (m-RNA), ribosomal RNA (r-RNA), transfer RNA (t-RNA), micro RNA (mi-RNA), and others—are engaged in diverse structural, catalytic, and regulatory activities, in addition to translating genes into proteins. RNA's multitasking prowess, at the heart of the "RNA World Hypothesis", implicates RNA as the first molecule of life, which likely spurred the evolution of numerous modified nucleotides. This enabled the diversified complementarity and secondary structures that allow RNA species to specifically interact with other components of the cellular machinery such as DNA and proteins.

Methylating RNA

The nucleotide building blocks of RNA contain pyrimidine or purine rings, and each position of these rings can be chemically altered by the addition of various chemical groups. Most frequently, a methyl (–CH3) group is tacked on to the outside of the ring. Other chemical additions such as acetyl, isopentenyl, and threonylcarbamoyl are also found added to RNA bases (Figure 9.3).

As already indicated, at least 140 modified RNA nucleotide variants have been identified. Among these, the most common and most prevalent epigenetic mark in eukaryotic m-RNA is the methylation of adenosine on the N6 position (m6A). This epigenetic mark is laid down by a "writer" protein complex that includes three well-characterized components: METTL-3, -14 and WTAP. The reverse process of RNA demethylation is performed by "erasers" such as the enzymes FTO and ALKBH5 (Figure 9.4).

Figure 9.3 -RNA methylation dynamics

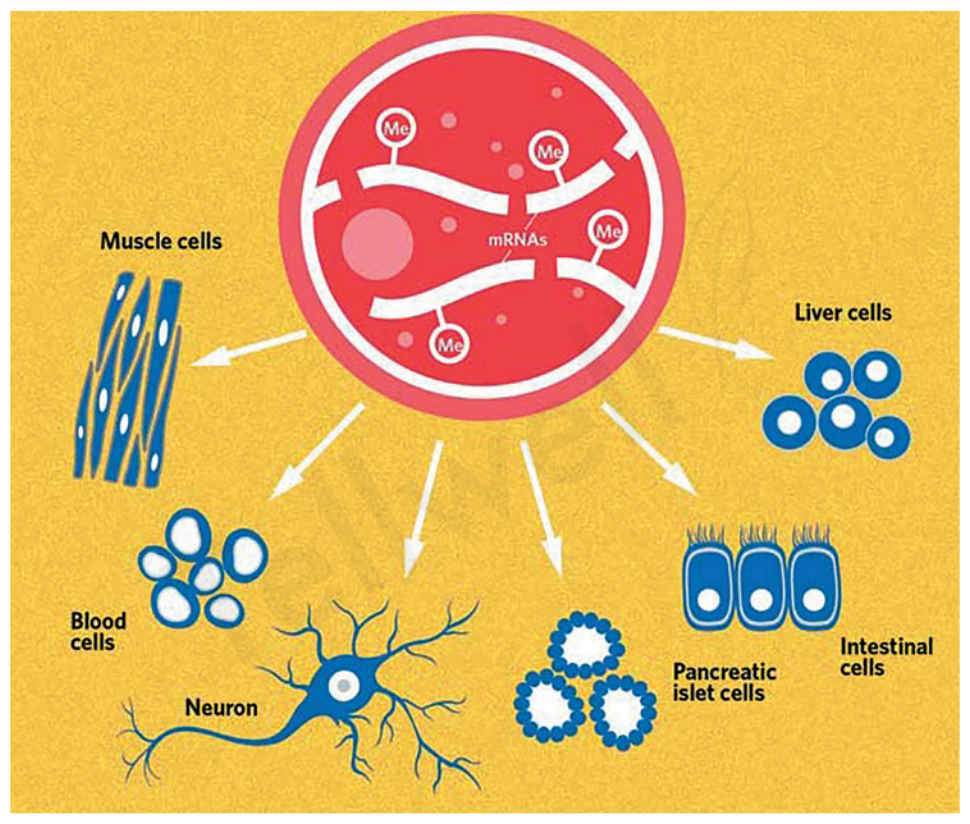
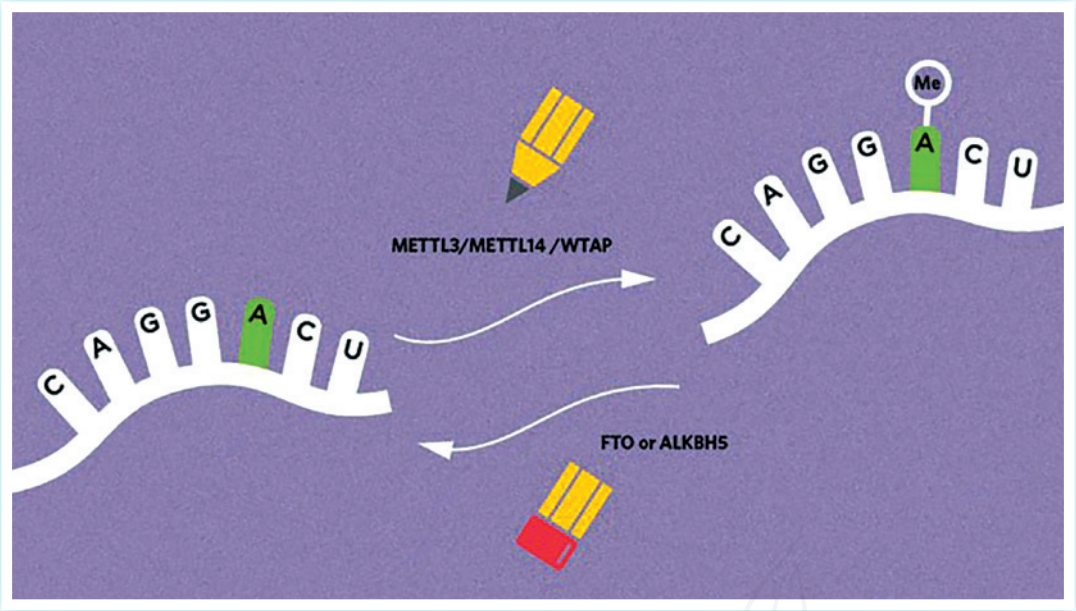


Figure 9.4 - Methylation of adenosine on the N6 position



More than 12,000 methylated sites in m-RNA molecules have been derived from approximately 7,000 protein-coding genes. This clearly indicates the importance of m6A decoration in regulating the expression of diverse transcripts. It plays diverse roles in regulating cellular function, starting with basic processes such as gene expression, translation, and alternative splicing.

RNA epigenetics in action

Understanding the molecular mechanisms by which m6A regulation controls RNA stability, translation efficiency, and alternative splicing is helping researchers decipher the importance of this new epigenetic mark in physiological and pathological processes. Other work has hinted at m6A's role in the regulation of circadian rhythms. Researchers identified m6A sites on many transcripts of genes involved in the regulation of daily cycles.

Figure 9.5 - Illustrating newly transcribed messenger RNA exiting the nucleus via nuclear pores

Ref: Benjamin Capillo, Science Source

It is quickly becoming clear that m6A decoration has diverse cellular and physiological functions. But, perhaps, the best illustration of its critical ability to precisely control processes at the cellular level is its involvement in early embryogenesis. Cell-fate decisions are coordinated by alterations in global gene expression, which are orchestrated by epigenetic regulation. Well-established epigenetic marks, such as

DNA methylation and histone modifications, are known to mediate embryonic stem cell (ESC) cell-fate decisions, and it turns out that m6A modification is no different. Dynamic m6A RNA markings herald the era of tripartite epigenetics where modifications of DNA, RNA, and proteins join hands to fine-tune gene expression and to execute prompt and precise responses to environmental stimuli and stresses. Indeed, m6A is just one of 140 modified RNA nucleotides that likely affect the function of the nucleic acid messenger and key cellular actor in diverse ways. Molecular approaches are paving the way for the study of additional RNA modifications (Figure 9.5).

Beyond m-RNA

While m6A methylation is most prevalent on mRNAs, this mark also decorates other RNA species. It is well established, for example, that m6A is abundant on r-RNAs, t-RNAs, and small nuclear RNAs (sn-RNAs), which mediate splicing and other RNA processing and protein synthesis reactions.

As the list of RNA epigenetic marks continues to expand, researchers will gain a clearer picture of how diverse cellular processes are regulated. The extremely large repertoire of such modifications is expected to reveal various RNA marks analogous to the known DNA and histone epigenetic marks, and the various modifications of DNA, RNA, and proteins can enrich the language that allows the development, adaptation, and diversity of complex organisms.

Conclusions and take-aways

- Epigenetics is the study of cellular and physiological traits inherited by daughter cells, but not caused by changes in the DNA sequence. Thus, unlike genetics, which is based on changes to the DNA sequence (the genotype), in epigenetics, the changes in gene expression or cellular phenotype have other causes. The term epigenetics also refers to the changes in the genome that do not involve a change in the nucleotide sequence.
- The epigenetic evidence in the case of humans came from (a) twin studies, (b) genomic imprinting, and (c) transgenerational inheritance.
- Epigenetic changes do take place and can modify the activation of certain genes, but not the sequence of DNA. Additionally, the chromatin proteins associated with DNA may be activated or silenced. The changes are preserved when cells divide. Whereas most epigenetic changes only occur within the course of one individual organism's lifetime, they can be transferred to the next generation if gene inactivation occurs in a sperm or egg cell.
- Three types of epigenetic mechanisms have been discussed (a) chromatin remodeling, (b) histone modifications, and (c) DNA methylation. Chromatin remodeling is accomplished through two main mechanisms: post-translational modification of the amino acids that

make up histone protein, and addition of methyl groups to the DNA. Histone modifications occur throughout the entire sequence and include: acetylation, citrullination, methylation, phosphorylation, ribosylation, sumoylation, and ubiquitylation. DNA methylation patterns are known to be established and modified in response to environmental factors. Its molecular mechanism of inheritance is different from the canonical Watson-Crick base-pairing mechanism.

- There are various applications of epigenetics in medicine and drug development, of particular importance in cancer and cancer treatment. Epigenetic pharmaceuticals could be a replacement or adjuvant therapy for currently accepted treatment methods such as radiation and chemotherapy, or could enhance the effects of these current treatments.
- Genome engineering has greatly advanced our understanding of how genes shape phenotypes. However, epigenetic processes also influence how cells use genetic information.
- Modifications to RNA molecules are common and are critical for regulating diverse biological processes. A variety of chemical modifications decorate the nucleic acids, increasing the alphabet of DNA to about a dozen known nucleotide variants. By contrast, the alphabet of RNA is even more impressive, consisting of at least 140 alternative nucleotide forms.
- Among the 140 modified RNA nucleotide variants that have been identified on m-RNA, the most common and most prevalent epigenetic mark in eukaryotic m-RNA is the methylation of adenosine on the N6 position (m6A). More than 12,000 methylated sites in m-RNA molecules have been derived from approximately 7,000 protein-coding genes. This clearly indicates the importance of m6A decoration in regulating the expression of diverse transcripts. It plays diverse roles in regulating cellular function, starting with basic processes such as gene expression, translation, and alternative splicing.
- Understanding the molecular mechanisms by which m6A regulation controls RNA stability, translation efficiency and alternative splicing is helping researchers decipher the importance of this new epigenetic mark in physiological and pathological processes, most importantly in early embryogenesis.

- As the list of RNA epigenetic marks continues to expand, researchers will gain a clearer picture of how diverse cellular processes are regulated. The extremely large repertoire of such modifications will enrich the language that allows the development, adaptation, and diversity of complex organisms.

Sidebars 9.1 and 9.2 respectively describe the Human Epigenome Project, and the International Human Epigenome Consortium.

Sidebar 9.1- The Human Epigenome Project

Aim

The Human Epigenome Project (HEP) is a multinational scientific research project, with the stated aim to "*identify, catalog, and interpret genome-wide DNA methylation patterns of all human genes in all major tissues*". It is financed by government funds as well as private investment, via a consortium of genetic research organizations. It is important to emphasize that there is not one "epigenome," but rather many different "epigenomes" that define normal and disease states. Deciphering these epigenomes will tell us not only how the genome is packaged, but, more importantly, will also indicate how it is functionally organized into states that allow for differential output in specific tissues.

Component organizations

The HEP consortium is made up of the following organizations:

- The Wellcome Trust Sanger Institute – UK,
- Epigenomics AG – Germany/USA, and
- The Centre National de Génotypage – France.

As powerful as the Human Genome Project (HGP) was, defining the exact sequence of almost all of the 109 bases in DNA, it does not predict how the genome is packaged in chromosomes and chromatin to provide for the differential expression of genes, which is essential for organismal development and differentiation. This key function is provided by epigenetic

processes that initiate and maintain heritable patterns of gene expression without changing the sequence of the genome. HEP will offer such information of direct relevance to public health because of the acknowledged role of epigenetics, which will be of importance to such pathologic processes as **aging**, mental health (e.g., Rett's syndrome), cancer, among many others. However, several issues need to be resolved such as: How many epigenomes should be sequenced and at what level of resolution? Which of the bewildering array of histone modifications should be examined? How will the project be coordinated? Is the technology ready?

Goal

The goal of the HEP is to identify all the chemical changes and relationships among chromatin constituents that provide function to the DNA code. This will allow a fuller understanding of normal development, **aging**, abnormal gene control in cancer, and other diseases as well as the role of the environment in human health. It is becoming evident that cancer cannot be comprehensively understood or addressed in the clinic unless the scope of the epigenetic effect on cancer is revealed. In addition to this role, the HEP would also be relevant to many other scientific areas, including stem cell therapies, developmental biology, neurologic disorders, and a host of other diseases.

Technologies

Technologies designed to examine epigenetic phenomena at the genomic level have shown remarkable progress over the last few years. These include:

- ***Chromatin immunoprecipitation/chip methodology*:** Used to examine histone modifications;
- ***High-throughput DNA methylation analysis*:**
- ***Reference epigenomes*:** The existence of many epigenomes requires the definition of a subset of epigenomes to be determined at a high level of resolution. In addition, a second set of samples would be analyzed at lower resolution.

Our growing knowledge of epigenetic mechanisms suggests that this complex network of molecular signals that regulate gene activity has vast implications

for understanding the initiation, growth, and spread of cancer and other diseases.

Sidebar 9.2 – The International Human Epigenome Consortium

Aim

The International Human Epigenome Consortium (IHEC), not to be confused with the Human Epigenome Project (HEP, see Sidebar 9.1), is a scientific organization, founded in 2010, that helps to coordinate global efforts in the field of epigenomics. Specifically, IHEC member organizations are engaged in efforts to generate at least 1,000 reference (baseline) human epigenomes from different types of normal and disease-related human cell types.

Structure and funding

IHEC's operations are funded by its full members (national and regional scientific funding agencies), and staffed largely on a volunteer basis by scientists and other experts from participating funding agencies and epigenome mapping projects. Full member countries each agree to invest at least US $10 million in IHEC-aligned epigenome mapping activities.

Current full members of IHEC are:

- Canadian Institutes for Health Research (CIHR), (Canada);
- European Union (EU);
- European Institute of Oncology (EIO);
- FIRC Institute of Molecular Oncology Foundation (IMOF);
- Italy Institute of Technology, Center for Genomic Science (IIT-CGS), (Italy);
- Germany Federal Ministry of Education and Research - Projects Management Agency (FMER-PMA), German Aerospace Center (Germany);
- Japan Science and Technology Agency (JSTA), (Japan);
- South Korea National Institute of Health (NIH); and

- U.S. National Institutes of Health's Roadmap Epigenomics Program (NIH-REP).

In addition, IHEC Member Observers are organizations that have not yet made a full financial contribution to the project, but whose members provide time and expertise. Current IHEC Member Observers are:

- France National Agency of Research (NAR);
- Australian National Health and Medical Research Council (NHMRC);
- UK Founders Alliance, Medical Research Council, Biotechnology and Biological Sciences Research Council, Cancer Research (FA-MRC BBSRC RC); and Wellcome Trust.

Oversight of the IHEC is provided by an Executive Committee, whose members are nominated by Full Member organizations. This Committee works closely with an International Scientific Steering Committee, whose members are the scientific leaders of participating projects and other leaders in the field of epigenetics, as well as a Data Coordination Center DCC). Additional expertise is contributed by workgroups composed of members of participating research projects.

IHEC interacts and coordinates its efforts with other large-scale international genomics projects, such as the International Cancer Genome Consortium (ICGC) and ENCODE. Committee and workgroup members, as well as other individuals involved in IHEC, meet annually at an event hosted by member countries on a rotating basis. Most meetings are hosted in conjunction with a scientific symposium, some of which are open to non-IHEC scientists and sometimes members of the public.

Goals

The ultimate objective of IHEC is to determine how the epigenome has shaped human populations over generations and in response to the environment. The first phase of IHEC's operations involves coordinating the production of at least 1,000 reference epigenomes from healthy and diseased human cells, as well as a limited number of model organisms relevant to specific human diseases. The initial focus is on cellular states including stemness, immortality, proliferation, differentiation, senescence, and stress. The

reference epigenome for each sample comprises high resolution maps of DNA methylation and key regulatory histone modifications, with corresponding information about the type and expression level of all transcribed genes (protein coding as well as non-coding/small RNAs). The data produced are made freely available to the research community. In addition, participating research projects are engaged in developing new epigenomics and associated bioinformatics methods.

10

Primer on Ecogenetics

Contents

10 Primer on Ecogenetics

A brief history of Ecogenetics

In 1546: *Girolamo Fracastoro* proposed that some diseases might be spread by invisible *seminaria contagium,* seeds of contagion that could be spread from person to person by touch or through the air.

In 1654: *Antoine Philips van Leeuwenhoek* handcrafted microscopes and was the first to observe and describe single-celled organisms, which he originally called *animalcules (*now referred to as microorganisms). He was also the first to record microscopic observations of muscle, bacteria, spermatozoa, and blood flow in capillaries. He is commonly known as the "Father of Microbiology" and considered to be the first microbiologist.

In the 1880s-1890s: *Louis Pasteur* discovered the principles of vaccination, microbial fermentation, pasteurization, and the causes and preventions of diseases. He reduced mortality from puerperal fever and created the first vaccines for rabies and anthrax. His discoveries provided direct support for the germ theory of disease and its application to clinical medicine. He is regarded as one of the three main founders of bacteriology together with *Robert Heinrich Herman Koch* and *Ferdinand Julius Cohn*. (see below).

In the mid- to-late 19th century: *Robert Koch* is known for identifying the specific causative agents for tuberculosis, cholera, and anthrax, and for giving experimental support for the concept of infectious diseases. The "Koch's postulates" are generalized principles linking specific microorganisms to specific diseases.

In the 1870s: *Ferdinand Cohn* studied bacteria. His classification of bacteria into four groups based on shape (spherical, short rod, thread, and spiral) is still in use today. He was the first to show that *Bacillus* can change from a vegetative state to an endospore state when subjected to an environment deleterious to the vegetative state.

In early 20th century: *Sir Archibald Garrod* elucidated the role of inherited metabolic variation in some rare genetic diseases and suggested that in some people components of certain foods and drugs may set off highly abnormal reactions because of a specific hereditary susceptibility.

In 1938: *J. B. S. Haldane* noted that "potter's bronchitis" might be caused by work-related environmental exposures in those potters with a "constitutional" predisposition to injury to the respiratory passages.

In 1941: *Beadle* and *Tatum* introduced the "one gene-one enzyme" concept to provide the conceptual and methodological framework to study gene action.

In 1949: *J. B. S. Haldane* suggested that heterozygotes are fitter than normal, that is are more resistant to attacks by the sporozoa which cause malaria. He also linked malaria with sickle cell anemia (SCA) and β-thalassemia.

In the 1950s: Rare adverse reactions to therapeutic drugs were shown to occur in individuals who had inherited an otherwise innocuous variant enzyme when exposed to the standard dose of a drug that required the breakdown of the enzyme for its metabolic breakdown.

In the 1950s: The science of pharmacogenetics was born following the discovery of heritable variations of plasma cholinesterase or primaquine-induced hemolysis [now known as *glucose- 6-phosphate dehydrogenase (G6PD)* deficiency].

In 1956: *Carson et al* showed that the adverse response (hemolytic anemia) to the anti-malarial drug, Primaquine, is inherited as an X-chromosome-recessive trait caused by deficiency of the enzyme G6PD deficiency.

In 1956: *Williams* reinforced the Garrodian concept of biochemical individuality with notable emphasis on differences in nutrition and metabolism.

In 1957: *Beutler* evidenced the appearance of hemolytic anemia in some African-American soldiers during World War II after taking the anti-malarial drug Primaquine.

In 1957: *Motulsky* posited that "genetically-conditioned drug reactions not only are of practical significance, but may be considered pertinent models for demonstrating the interaction of heredity and environment in the pathogenesis of disease". He also outlined the confluence of genetics, biochemistry, and pharmacology and defined drugs as specific environmental agents that trigger disease (the adverse reaction to the drug) only in genetically susceptible individuals.

In 1959: The heritable variations of plasma cholinesterase or primaquine-induced hemolysis, now known as glucose-6-phosphate dehydrogenase (G6PD) deficiency, paved the way for the rapid development of the field of Pharmacogenetics.

In 1971: *Brewer* posited that genetic variations would be expected to affect the body's response to any kind of environmental and xenobiotic (chemical) agent, not just drugs, and other environmental agents and exposures. He also introduced the term "Ecogenetics".

In 1998: *Eaton et al* concluded that, unlike major genes associated with specific diseases, "susceptibility genes" are neither necessary nor sufficient to cause disease, but modify the risk when there is appropriate exposure.

In 2004: *Kelada et al* showed that, in the case of specific diseases, the susceptibility genes do not differ from the major genes that cause the disease but, rather, induce a shift in the dose-response relationship.

The Environmental Genome Project

In 1997, as part of the Human Genome Project (HGP), the (U.S.) National Institute of Environmental Health Sciences (NIEHS) started the Environmental Genome Project (EGP) -- a comprehensive effort to identify "polymorphisms" in genes involved in environmentally-induced diseases. This is a multi-disciplinary collaborative effort focused on examining the relationships between environmental exposures, inter-individual sequence variation in human genes, and disease risk in U.S. populations. The key objective

is to identify alleles that confer susceptibility to the adverse effects of environmental agents. Overall, EGP will provide the ability to understand the combination of environmental and genetic components of important human diseases [cardiovascular disease, cancer, neurological disorders (ND), asthma, etc.] and to identify and protect "at risk" groups.

The EGP devolves into three phases as summarized in Table 10.1:

Table 10.1 - The Environmental Genome Project

Project phase	Objective & Goals	Accomplishments
1	Candidate gene re-sequencing: o Develop a sample repository o Re-sequence 200 candidate genes o Develop new technologies for variant identification o Develop databases of polymorphic variations o Consider ethical, legal, and social implications	o 647 candidate "environmental response" genes were identified and re-sequenced (all promotor, intronic and exonic regions) to identify common single nucleotide polymorphisms (SNPs) o Genes included those involved in xenobiotic metabolism, DNA repair, cell cycle regulation, cell death, oxidative mechanism, signal transduction, and immune and inflammatory response.
2	Exome re-sequencing: o Functional studies of allelic variants o Initiate population-based studies o Refine databases	o The functional implications of polymorphisms in both the coding and regulatory regions of these genes are studied

3	o Implement genetic epidemiology studies o Develop animal models o Develop cell models o Understand dose-response relationship o Risk assessment	o Animal and cell models developed to investigate how a risk for a specific disease is altered by a particular genotype and environmental exposure

Source: Adapted and augmented from "Gene-Environment Interactions" edited by Lucio G. Costa and David L. Eaton, 2006

Genetic polymorphisms

A "genetic polymorphism" defines monogenic traits that exist in the normal population in at least two phenotypes, neither of which is rare, with the frequency of the specific genetic variation affecting 1% or more of the population. Polymorphisms may be:

- **Non-functional** (i.e., silent or ineffective) although important nonetheless for they may be either in linkage disequilibrium with other functional polymorphisms or associated with still unknown functions; or
- **Functional** if they result in altered function, stability, and/or level of expression of the resulting protein. Functional polymorphisms include:
  - Point mutations in the coding region of the gene, resulting in amino acid substitutions that in turn affect protein function or stability;
  - Duplicated genes, which result in higher protein levels;
  - Completely or partially deleted genes, resulting in no gene product; or
  - Splice-site variants that result in truncated or alternatively spliced protein products.

Mutations in the regulatory regions of genes may affect the level of protein expression, and mutations in other noncoding regions may affect m-RNA stability or splicing.

Most DNA polymorphisms occur as single-nucleotide polymorphism**s** (SNPs). Nearly 20 million of them have been identified in the 2003 NIH SNP database of which 800,000 are considered frequent (http://www.ncbi.nlm.nih.gov/

SNP/snp_summary.cgi). Of these, 120,000 are located in "exons" – so-called "complementary SNPs" (or cSNPs) of which 50,000 are expected to be functional. However, the number of SNPs affecting protein function, including level of expression, is believed to be much greater and will include many polymorphisms outside exons at the exon-"intron" boundaries ("splice-site variants") or in regulatory protein binding sites (*cis* elements).

More recent studies have demonstrated that SNPs located in introns far removed from any functional aspect of a gene (called "deep intronic variants") may also have functional significance as does the redundant third base in many codons even if it does not result in a change in amino acid.

Thus, a major challenge in the field of ecogenetics is the correct identification of genetic variation that is biologically meaningful.

Classes of environmental agents with known ecogenetic variation

Examples abound of genetic variation in responses to various types of environmental agents. Some are summarized in Table 10.2.

Table 10.2 - Classes of environmental agents with known ecogenetic variation

Class of agent	Nature of effect	Ecogenetic factors
1. INFECTIOUS DISEASES	o Infections, autoimmune disorders, malaria, ankylosing spondylitis o Metabolic disorders	o Defects in cellular or humoral immunity, HbS, G6PD, thalassemia, HLA-B27 o Metabolic problems
2. PHARMACEUTICAL AGENTS	o Biotransformation o Target site susceptibility o Toxicity from chemotherapy	o Acetylation, CYP variants o G6PD deficiency o Thiopurine methyltransferase
3. METABOLISM	o Metabolic disorders	

4. DIET & NUTRITION	o Malnutrition o Lactose intolerance o Favism o Hyperhomocysteinemia o Dyslipidemia o Alcohol sensitivity	o Metabolic problems
5. FOODSTUFFS	o Lactose intolerance o Celiac disease o Nuts intolerance o Hemolysis from fava bean ingestion o Atherosclerosis o Thyroid goiter o Nutritional disorders	o Intestinal lactase turned off at weaning in most humans o Sensitivity to wheat gluten o Allergies (may be fatal) o G6PD deficiency o Hyperlipidemia o Hyperhomocysteinemia o Phenylthiocarbamide nontasters o Phenylketonuria, ornithine transcarbamylase deficiency, hypophosphatemic ricketts, some rare single gene disorders
6. FOOD SUPPLEMENTS	o Iron deposition diseases o Food additives	o Iron absorption increases hematochromatosis or thalassemia gene
7. ENVIRONMENTAL / CHEMICAL AGENTS: o Inhaled pollutants o Metal poisoning o Pesticides o Occupational exposure (ionizing radiation)	o Emphysema o Lung cancer o Bladder cancer o Minamita neurologic disease o Neurotoxicity o X-and higher energy radiation	o a-1 Antitrypsin deficiency o Arythydrocarbon hydroxilase induction and CYP variants o Nicotine metabolism acetylation differences o Organic mercury ingestion (?) o Paraoxonase (PONI) variation o DNA damage, cancer induction
8. PHYSICAL AGENTS	o Tolerance for heat, cold, humidity, motion, sunlight	o Mechanisms unspecified, UV DNA damage repair

9. LIFESTYLE & BEHAVIORAL AGENTS: o Behavior o Stress:	o Stimulants – Caffeine (wakefulness) o Alcohol (Flushing syndrome) o Drugs of abuse o Metabolic disorders o Smoking o Sexually transmitted infections o Lack of physical exercise	o Uncertain o Aldehyde dehydrogenase deficiency o Metabolic disorders o Lung cancer o HIV/AIDS, syphilis, gonorrhea

Source: Adapted and augmented from Ommen and Motulsky (1978), Motulsky (2002, 2006), Lampe and Potter (2006), Saxon (2006)

Biotransformation enzymes

A major area of focus in pharmacogenetics and ecogenetics research to date is the role of enzymes in processing toxic substances. There are many enzymes in the body that participate in the metabolism and elimination of endogenous compounds and xenobiotics. These biotransformations usually aid in the ultimate elimination of such compounds although, in some cases, the reactions bioactivate parent compounds. Typically, biotransformation reactions are categorized under three processes as phase I, phase II, and phase III:

- ***Phase I reactions:*** They are generally oxidative, and usually increase by a small amount the hydrophilicity of the original compound;
- ***Phase II reactions:*** They usually greatly increase the hydrophilicity of the original compound or the compound modified by a phase I reaction promoting the excretion of the compound in urine or feces (via the bile);
- ***Phase III processes:*** They represent carrier-mediated transport that facilitates the movement across biological membranes of the water-soluble products formed by phase I and phase II reactions.

Individual differences in the biotransformation enzymes that bioactivate or detoxify pharmaceutical drugs can limit their efficacy, or lead to severe

adverse reactions. Because of the need to recall several widely utilized drugs due to unexpected severe toxic effects, pharmacogenetic research is now focused toward the definition of individualized therapies that take into account individuals' genetic makeup. In addition, evidence is emerging that these same biotransformation enzymes can also modulate an individual's susceptibility to environmental and occupational toxicants – the specific area of ecogenetics research.

The toxic effects of many chemicals result from the ability of the activated chemical to damage DNA. DNA damage from both endogenous and exogenous sources occurs frequently in every living cell in our bodies. Fortunately, our cells possess remarkably efficient repair processes that remove and correct such DNA damage.

Biotransformation enzymes responsible for oxidative metabolism

Phase I enzymes

Phase I enzymes refer to that class of enzymes that participate in oxidative processes of a host of drugs and xenobiotics including:

- ***Cytochromes* (CYP):** In the case of xenobiotics, the most widely studied enzymes are the cytochromes P450 (CYP). There are 57 CYP proteins that are encoded in the human genome, which are involved in the metabolism of almost every known chemical. They can bind to and damage DNA, potentially increasing the risk of cancer. Indeed, over 90% of all known chemical carcinogens are not directly carcinogenic but require metabolic activation (usually via one or more CYPs) to exert their carcinogenic effects. Thus, it is perhaps not surprising that subtle genetic differences in the rates at which one enzyme activates a "procarcinogen" to the ultimate "carcinogen" might contribute to important differences in susceptibility to cancer causing chemicals.
- ***Flavin-dependent mono-oxygenases (FMOs):*** FMOs are a related, much smaller family of enzymes than CYP. They participate in oxidation of xenobiotics that contain a nitrogen or sulfur atom. Several of the

FMOs are also polymorphic and these genetic differences can contribute to variability in how people respond to certain drugs and chemicals.

Websites cataloging mutations for CYPs and FMOs are maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee: http://www.imm.ki.se/CYPalleles/htm and the Medical Research Council Human Genetics Unit, respectively.

Phase II enzymes

Although the rate of activation of a relatively nontoxic chemical to a highly reactive, toxic intermediate via a CYP or FMO enzyme is certainly important, fortunately, the body possesses other enzymes that are able to intercept and detoxify reactive intermediates. The Phase II biotransformation enzymes provide an important mean of protecting cells from both endogenous and exogenous chemicals. These include the family of transferases (glutathione S-, glucuronosyl, sulfo-) and various esterases and hydrolases. It is important to understand all of the biotransformation pathways that might be acting on a given chemical in order to assess the overall ratio of the rates of activation to detoxification of a given chemical.

Enzymes involved in the repair of damaged DNA

Loss of DNA repair capacity is associated with a variety of chronic diseases, especially some forms of cancer. There is much interest in identifying common genetic polymorphisms in DNA repair capacity that might cause slight alterations in the efficiency or/and accuracy of DNA repair. Mutations in such enzymes that lead to inefficient DNA repair may represent a significant genetic susceptibility risk factor for exposure to mutagens and carcinogens.

Xenobiotic metabolism and disease risk

Polymorphisms in the genes encoding for CYP and FMO family of enzymes contribute to inter-individual differences in the biological effects of xenobiotics by affecting the peak concentration and duration of exposure of the parent molecules or active metabolites in the body.

The most penetrant mutations are associated with the metabolic clearance of drugs (e.g., warfarin-CYP2C9; nortrityline; tacrolimus-CYP3A5) and these

may be the targets of prospective pharmacogenetic testing in order to improve drug safety and efficacy. In theory, the very same mutations could affect the metabolic clearance of some non-therapeutic xenobiotics and their associated toxicity.

Environmental factors will also modify the impact of many high- and low-penetrance gene mutations by affecting the synthesis, degradation, or function of the polymorphic enzyme as well as secondary enzymes that catalyze the same or parallel metabolic event.

DNA repair enzymes

Ever since the origin of DNA-based life, genomes have been subjected to environmental stresses. Every second, the genome of each of our cells is altered, broken, and reassembled. The survival of cells, humans, and species depends on mechanisms to repair this damage and reconstitute genomes.

Evolution has been at work for billions of years to produce exquisite DNA repair systems that patrol the genome, fixing or replacing damaged, altered, and miscoded nucleotides. The ability of the cell to maintain its genetic integrity is crucial for, without this ability, there would ensue a cascade of mutations reaching an error threshold at which point the genetic information could no longer be maintained. Unrepaired DNA damage can reduce the overall fitness of a cell, triggering cell cycle arrest, apoptosis, unchecked growth, or other diminished functionality. Therefore, loss of any of these repair pathways in humans can result in mutations, cancer and death. Variability in the ability of these repair systems to perform may ultimately lead to an increase in mutations in somatic cells (any cells that are not egg or sperm) and to a higher risk for disease. Polymorphisms in DNA repair enzymes may increase the risk of disease.

If the tens to hundreds of thousands of damaging alterations (see below) that occur in each cell each day are not repaired, these altered, damaged, and miscoded nucleotides can result in somatic mutations. In order to correct this damage before it affects cellular functionality or triggers apoptosis, the cell has evolved multiple repair mechanisms (reversal of damage, base excision repair, nucleotide excision repair, mismatch repair, recombination

with restoration of DNA sequences, and bypass of lesions by special DNA polymerases). Genetic variations in the human population can affect the efficiency and accuracy of these repair mechanisms and can lead to greater disease susceptibility.

DNA damage

There are two main categories of DNA damage: exogenous (environmental) and endogenous (internal, spontaneous). With all of this damage occurring within the cell, it is no wonder that drugs that reduce the damage load on cells, such as anti-oxidants, are being promulgated for cancer prevention.

Exogenous DNA damage

Exogenous DNA damage can be caused by many environmental agents, including (a) natural chemicals found in food (e.g., aflatoxins); (b) synthetic (human-made) chemicals (e.g., benzopyrine found in cigarette smoke); and (c) chemicals used in chemotherapy of cancer (e.g., cisplatin); (d) exposure to UV radiation produced naturally by the sun or artificially by tanning booth lamps; and (e) ionizing radiation such as γ-rays and X-rays (during diagnosis and therapeutic treatment; occupational exposure).

The damages result in (a) the chemical instability of DNA, which can manifest as depurination and depyrimidation events resulting in the loss of a base from the DNA strand. It can be estimated that 10,000 bases per cell per day are lost spontaneously and subsequently repaired; and (b) the production of reactive molecules by normal cellular processes. The damage in cells by reactive oxygen (e.g., hydroxyl radicals, superoxide anion) is likewise estimated to be 10,000 events per cell per day.

Endogenous DNA damage

Endogenous DNA damage is caused by chemical alterations such as methylation, and incorporation of incorrect bases during DNA synthesis.

Diseases linked to nonfunctional DNA repair systems

Various diseases are caused by nonfunctional DNA repair systems, including:

- ***Xeroderma pigmentosa (XP)*:** A rare inherited DNA repair disease presents with a high incidence of skin cancers. Individuals with XP have a 1000-fold increase in risk of skin cancer after exposure to sunlight.
- ***Other diseases where mutations in DNA repair enzymes have been linked to a high incidence of cancer:*** These include:
- ***Ataxia telangectasia (AT);***
- ***Fanconi's anemia (FA);***
- ***Hereditary non-polyposis coli (HNPC);***
- ***Bloom's syndrome (BS);***
- ***Inherited forms of breast cancer:*** With mutations in BRCA1 and BRCA2; and
- ***Werner's Syndrome (WS):*** A disease of premature aging characterized by a high incidence of unusual cancers.

Fortunately, most inherited DNA repair diseases are rare and require that the individual be homozygous for the polymorphisms. However, heterozygotes who have a single mutated allele are much more common in the population and also suffer from a reduction in expression that may render them susceptible to environmentally-mediated DNA damage.

In the case of cancer chemotherapy, pre-existing drug-resistant mutants may diminish the overall effectiveness of chemotherapeutic agents. These drug-resistant mutants would have a replicative advantage and could repopulate the tumor. This provides one mechanism by which tumors routinely acquire drug-resistance to a single chemotherapeutic agent, and provide a mechanistic explanation for the efficiency of, and frequent necessity for, multiple drug therapy in cancer treatment. If the accumulation of mutations is directly correlated with tumor progression, then, inhibiting mutation accumulation may delay carcinogenesis. By exploring ways to reduce the high amount of DNA damage occurring within the cell, we may successfully prevent cancer by delaying onset to the point that death from other causes occurs first. Further, future treatments involving gene therapy may be able to specifically

target repair system genes that are malfunctioning, enhancing their activities and reducing the mutational load (Fymat 2017).

Gene-environment interaction in the etiology of diseases

All the diseases considered below clearly have a genetic and an environmental component to their etiology. The complexity of the diseases poses substantial difficulties in establishing clear-cut associations. Most often, the end point is the result of an array of multifactorial aspects involving both the individual's genome and the environment. In some instances, gene-environment (GE) interactions must be enlarged to gene-gene-environment interactions (GGE).

Well over 100 types of cancer have been observed to occur in humans, each with its own unique constellation of risk and protective factors. However, no matter how strong a particular risk factor might be, whether an environmental exposure such as tobacco or alcohol, or an inherited predisposition to cancer, it is quite rare that one factor completely determines the development of cancer. For example, the vast majority of regular cigarette smokers never develop lung cancer, and a sizable number of women who inherit a mutation in the BRCA1 gene do not develop breast cancer over their lifetimes.

Clearly, multiple factors must therefore play a role in causing a normal cell to develop the myriad genetic abnormalities characteristic of the malignant phenotype. In some instances, risk factors may act through different biological pathways, and are therefore said to act independently of each other. In other instances, the effect of one risk factor may depend on the presence of another risk factor, that is, they may interact. The interacting risk factors might both be environmental in nature. In other instances, environmental risk factors may interact with genetic factors in modifying disease risk. Finally, multiple gene products typically act in complex metabolic pathways, and they may interact with each other in determining disease risk (e.g., gene-gene interactions). Notwithstanding the wide variation in etiology of different cancers, it is important to consider each tissue or organ system in its own particular context.

A person's likelihood of developing a particular cancer is determined largely by a complex interplay of risk factors involving environmental exposures,

lifestyle factors, and inherited susceptibility. The challenge is to understand in sufficient detail how these specific factors interact in causing (or preventing) these cancers so that more effective prevention and early detection programs can be developed and applied toward persons at highest risk of the disease. (Further, let us recall here, this author's admonition that "risk is not cause, and risk management is not cure, only palliation".)

Lung Cancer

Ecogenetics of lung cancer

In the ecogenetics of lung cancer, the main environmental "cause", tobacco, is well established as a very strong risk factor (current smokers experience 10-17 times the incidence among never-smokers). Indeed, most cases (70%-90%) of lung cancers are caused by tobacco smoking. Nonetheless, family history is also a risk factor suggesting the presence of a genetic component to risk.

Genetic contributions to lung cancer are complicated with multiple genes involved in complex metabolic pathways for numerous carcinogens in tobacco smoke. Gene-environment interactions are even more complex as they include: Genetic differences in the internal processing of carcinogens and reactive oxygen; the combined effects of multiple genes; relationships between genes and intermediate markers for disease; influences of ethnic background and gender; and influences of both endogenous and exogenous exposures on gene expression and protein function.

Many studies have sought to determine whether inter-individual differences in ability to transform carcinogens and reactive oxygen in tobacco smoke determine susceptibility to tobacco-related lung cancer.

Cigarette smoke contains thousands of compounds, including 30-50 known carcinogens as well as reactive oxygen compounds that can damage DNA. Most tobacco carcinogens are inhaled as less harmful procarcinogens; they are activated by Phase I enzymes (primarily CYP450) to metabolites capable of reacting with DNA and beginning carcinogenesis. Carcinogenesis is prevented when reactive carcinogens are detoxified by Phase II enzymes

(e.g., glutathione S-transferases) or DNA damage is repaired by DNA repair enzymes. Two classes of carcinogens important in lung cancer are polycyclic aromatic hydrocarbons (PAHs) and tobacco-specific N-nitrosamines (TSNA). Tobacco smoke also contains free oxygen as well as compounds that generate reactive oxygen species (ROS) that can damage DNA (Fymat 2017 c-i; 2018a).

Combined effects of biotransformation enzymes

Given the complexity of tobacco smoke metabolism, measurement of one isolated enzyme at a time may provide limited insight into the underlying biology of lung cancer. Unfortunately, few studies of combined effects or interactions of multiple polymorphisms have been reported because they require very large study populations. However, there are reasons to believe that glutathione S-transferases (GSTs), at least, may influence prognosis and survival particularly because several chemotherapeutic drugs are detoxified by GSTs. Since chemotherapy is increasingly used to treat lung cancer, differential ability to metabolize these drugs could influence response to therapy and corresponding survival.

Clinical epidemiology

Clinical epidemiology has contributed considerably to progress in understanding the molecular basis for differences in susceptibility to lung cancer among smokers. This progress has gone hand-in-hand with advances in laboratory methods and progress in understanding study design and analytical methods most suitable for gene-environment studies.

Gastrointestinal cancers

Cancers arise in the gastrointestinal (GI) tract more commonly than in any other organ system. Moreover, these tumors are the leading cause of cancer-related death in the world. While GI tract cancers are uniformly important contributors to morbidity and mortality throughout the world, the relative contributions of cancers arising in the esophagus, stomach, and colon/rectum vary markedly in different populations (Table 10.3):

Table 10.3 - Annual incidence* of gastro-intestinal tract cancers by site of origin and geographic region (1990)

	Esoph agus		Stom ach		Colon/ Rectum		% GI Cancers		All Cancers	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
East Asia	21.6	9.9	43.6	19	13.3	10.2	43.8	37.1	179.2	105.3
Japan	9.5	1.6	77.9	33.3	39.5	24.6	46.8	35.7	270.9	166.8
North America	5.2	1.4	8.4	4	44.3	32.8	15.7	13.8	369.9	277.5

**Per 100,000, age adjusted to world standard population*
Source: Parkin et al (1999)

The prognosis of persons diagnosed with cancers arising in the GI tract also varies widely by site of origin. Esophageal cancers remain one of the most deadly neoplasms with median survival less than 12 months, 5-year relative survival of 14.3%, and little evidence that more modern treatment techniques yield significant survival advantages. Stomach cancer is a disease that also confers a poor prognosis, although not as dismal as esophageal cancer: 5-year survival is 23.5%. On the other hand, 5-year survival for colon cancer is relatively good: 63.4%.

A key challenge in the study of the ecogenetics of the GI system as well as other organ sites lies in identifying the important genes and related risk factors to study. The advent of high-throughput genotyping methods has helped immensely; for example, large-scale-array-based studies of DNA and protein expression has brought a powerful new set of tools to the study of gene-environment interactions.

Neurodegenerative diseases

Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS or *Lou Gehrig's disease)* are relatively common neurodegenerative diseases (NDD) of late onset. They are characterized by the progressive loss of specific groups of neurons, resulting in cognitive or motor function deficits. [Multiple sclerosis (MS), an important degenerative disease of concern, is generally considered to be an autoimmune disease, probably with a viral etiology.] While mutations in specific genes causally related to the familial forms of AD, PD and ALS have been identified, the causes of the

sporadic forms of these diseases remain largely unknown (Fymat, 2017-8). Like many other complex diseases, they probably result from numerous complex interactions between genes and the environment but these, too, remain mostly unidentified. Interestingly, there is much evidence suggesting that these three diseases may share common pathological mechanisms and hence there may also be overlap in their causal gene-disease associations and gene-environment interactions (Fymat 2017j; 2018 b, d-g).

Alzheimer's disease

Researchers increasingly believe that much of the damage in AD is done when naturally occurring proteins fold into the wrong shape and clump together into harmful structures known as amyloid fibrils, as illustrated in Figure 10.1. Once these begin forming, they can start a "chain reaction" which rapidly kills off brain cells (see also Fymat, 2017g; 2018 d, e, g).

The primary clinical manifestation of AD is dementia, an accelerated loss of cognitive function beyond that due to normal aging. Alterations in mood and behavior often accompany the onset of dementia followed in time by memory loss, disorientation, and aphasia. In AD, the hippocampus and cerebral cortex are severely affected. Pathologically, there are two hallmarks in affected tissues (senile or neuritic plaques in blood vessels and neurons, and the occurrence of neurofibrillary tangles that accumulate in the cytoplasm of affected neurons).

We distinguish between the "familial" AD and the "sporadic" AD. The candidate genes for familial AD are the four major loci (APP; PSI, which codes for presenilin 1; PS2, which codes for presenilin 2; and Apolipoprotein E or *ApoE*) and those for sporadic AD are the three common *ApoE* alleles (ε2, ε3, and ε4). Both familial and sporadic *ApoE* have been clearly and reproductively linked to AD. In order for *ApoE* genotype to predispose to AD, some additional genetic or environmental risk factors must be present as well. It has been reported, but not generally agreed upon, that the relationship of *ApoE*-genotype and total cholesterol, as well as age and gender, indicate a gene-environment interaction. There are some epidemiologic reports of an association between head injury and AD. It has lastly been shown that the effect of smoking on AD risk may be dependent upon *ApoE*-genotype as well.

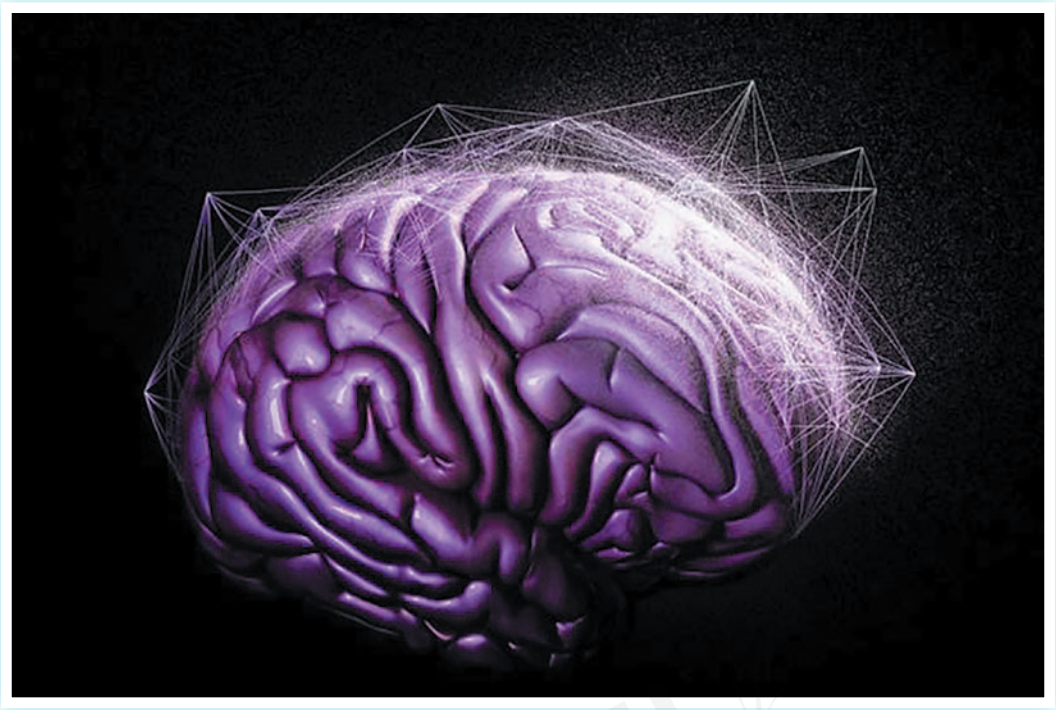
Recently (Journal: *Nature Structure and Molecular Biology*, February 2015), researchers at the Cambridge University and the Karolinska Institute in Sweden discovered that a naturally occurring molecule found in the lungs binds to the surface of the fibrils, preventing them from sticking together. Such molecules might eventually be turned into drugs that could be given to people before symptoms appear to stop the disease from progressing. They can theoretically arrest the build-up of the deadly protein tangles, which are thought to be crucial in the progression of the disease. Thus, improving the body's natural defenses could hold AD at bay in a discovery that paves the way for new treatments. In particular, AD could be halted in its early stages, raising the prospect of "statin-like drugs" to stave off the disease. Nonetheless, further studies are needed before clinical trials could begin, which themselves would take at least a decade.

It has even been suggested that arthritis, multiple sclerosis and even Alzheimer's and Parkinson's could all be treatable with a single "marvel" pill. Laboratory studies suggest that a compound discarded years ago by a drug company might block the damaging inflammation caused by a host of serious diseases. Human trials are being planned.

Parkinson's disease

Parkinson's disease (PD) is a movement disorder characterized by the loss of dopamine-producing neurons in the *substantia negra* region of the mid-brain. Like AD, PD can be manifest as both a rare familial form and a more common sporadic form of the disease. Familial PD onset is typically before age 50, whereas the sporadic form occurs predominantly at age 60 and older. There is a modest difference in the incidence of PD with a 1.5-3-fold male preponderance. Like AD, PD is also a disease of toxic protein aggregation (Fymat 2017j; 2018 f-g).

Figure 10.1 - Illustrating the amyloid fibrils in Alzheimer's disease



The candidate genes for familial PD are *PARK1-PARK10,* whereas *PARK1* (α-synuclein) alone seems to be associated with sporadic PD.

Environmental factors have long been considered to be fundamentally important in PD on the basis of two important findings: Twin studies have not identified a major role for genetics in sporadic PD, and a contaminant of synthetic heroin (called MPTP) was shown to be causally related to the development of chronic PD. Even though pesticides (particularly the herbicide paraquat) have a structural similarity to MPTP, their relationship with PD has not been clearly established. New studies could further elucidate their role as well as that of metals. Interestingly, smoking has consistently been shown to be associated with a decreased risk of PD (40% less for ever smokers, 20% less for past smokers, and ~ 60% less for current smokers).

Amyotrophic lateral sclerosis (*Lou Gehrig's disease*)

Amyotrophic Lateral Sclerosis (ALS) is a disease of the motor neurons of the anterior horns of the spinal cord and in the cerebral cortex. As with AD and PD, there are both familial and sporadic forms. ALS appears to be more common in men, and onset usually occurs in the middle to later years of

life. The candidate genes are mutations in the CuZnSOD-gene (*SOD1*). The gene-environment interaction in ALS, if it exists, remains to be uncovered.

A summary of the genes implicated in both the familial and sporadic forms of AD, PD and ALS is provided in Table 10.4.

Table 10.4 - Genes implicated in both the familial and sporadic forms of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis

Disease	Gene	Familial phenotype	Sporadic phenotype
Alzheimer's	*ApoE*	Lower age of onset with ε4 allele(s)	Increased disease risk
Parkinson's	*PARK1* *PARK2* *PARK5*	o Autosomal dominant disease o Autosomal dominant disease o Autosomal dominant disease	o Possible Rep1 polymorphism effect o Unclear. Possible role for promoter polymorphisms o Lower disease risk among 18Y vs 18S carriers
Amyotrophic Lateral Sclerosis	*SOD1*	Young onset	Unclear: remains to be determined

Source: From S. N. Kelada, Harvey Checkoway and Lucio G. Costa (2006)

Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of illness and death and, thus. a major public health concern in the United States. It is manifest as a variety of clinical conditions, including its most common form coronary heart disease (CHD), which itself includes myocardial infarction (MI) or heart attack, unstable angina, venous thrombosis, and peripheral vascular disease (PVD). CHD is influenced by genetic factors among both men and women at younger ages, although the effects may decrease at older ages.

The pathophysiological processes underlying these clinical events are also varied, and include lipid metabolism, inflammatory responses, oxidative stress, endothelial function, platelet function, thrombosis and hemostasis, homocysteine metabolism, insulin sensitivity, and hypertension, as reflected

by the many intermediate phenotypes that can often be used as biomarkers for disease risk. The etiology of CVD is extremely complex.

Each process underlying CVD risk is regulated by structural proteins, enzymes, receptors, and ligands, all of which are encoded by genes. Studies have demonstrated that cholesterol, high blood pressure, and cigarette smoking are a necessary but not sufficient condition for developing CHD. Whereas these factors explain the occurrence of CHD in 80%-90% of patients, most individuals who have these risk factors do not develop CHD. Further, even though environmental and behavioral risk factors for CVD, including dietary fat, smoking, alcohol consumption, and lower exercise levels are well established, these may be genetically influenced. Thus, most of CVD is likely the result of environmental exposure acting on a background of inherent susceptibility.

Perhaps one of the more important environmental risk factors that could interact with genetic susceptibility for CVD is cigarette smoking. Smoking approximately doubles the risk of CHD probably through several different mechanisms, including direct damage of the vascular endothelium, perturbing lipid metabolism, and inducing inflammatory response. Identifying whether there are subsets of cigarette smokers who are at increased CVD risk due to genetic susceptibility is of considerable interest.

The contribution of specific inherited susceptibility factors to CVD risk has been delineated for only a few, relatively large settings. For example, familial hypercholosterolemia is caused by mutations in the low density lipoprotein receptor (LDL-R) and is associated with substantially increased risk of CVD. Similarly, other single gene defects are known to cause hypertension and arrhythmias. These genetic disorders however explain only a small proportion of CVD in the general population. The reason is that CVD, like other complex diseases, is a consequence of many intermediate biochemical and physiological systems that interact nonlinearly, are population specific, and change over time. Equal weight must be placed on understanding the environmental risk factors and how these interact with genetic variants to influence CVD risk. In addition to increasing our understanding of disease etiology, examining associations between combinations of environmental risk factors and genetic variants will potentially enhance the predictive value of

risk factor information, and may inform development of preventive strategies and effective therapeutic measures.

Lastly, it has been known for decades that individual inter-variation exists in the efficacy of pharmaceutical agents and a substantial component of such variation is due to genetic factors. This points to the requirement of determining whether clinical prescribing practices will need to be altered for individuals carrying genotypes that influence the actions and metabolism of particular anti-hypertensive medications. Assessment of gene-drug interactions will be particularly important.

Diabetes

Diabetes results from genetic imbalances and environmental insults (e.g., exposure to arsenic or lifestyle factors) or interactions between the two. Other important and well established risk factors for type 2 diabetes (T2D) include physical inactivity, dietary fat intake, obesity (particularly central or visceral adiposity), family history of diabetes, and gestational diabetes mellitus (GDM). Changing lifestyle factors (weight loss, improved diet, and increased physical activity) result in a decreased risk. There is also limited evidence for environmental contaminants and occupational exposures in disease development.

Diabetes is one of the most costly and burdensome chronic diseases of our time (in the United States in 2002: $132 billion). Diabetic complications are a significant cause of morbidity (major cause of amputations and blindness) and mortality. Diabetics are also at significantly increased risk for CHD, PVD, and stroke. GDM refers to a diverse group of metabolic diseases characterized by chronic elevation of plasma glucose:

- ***Type I diabetes*** **(T1D)** (previously referred to as "juvenile-onset" or "insulin-dependent DM"): It accounts for less than 10% of all DM cases, and is characterized by failure of the pancreatic β- cells due to autoimmune destruction, and hyperglycemia (elevated plasma glucose levels);
- ***Type II diabetes*** **(T2D)** (previously referred to as "adult-onset" or "non-insulin-dependent DM"): It accounts for 90%-95% of all DM cases. Its metabolic hallmarks are: insulin resistance, impaired pancreatic

β-cell function, and increased hepatic glucose production resulting in hyperglycemia. In addition, people with T2D are usually overweight.

- ***Maturity-onset diabetes of the young*** (MODY): It includes several rare monogenic forms of type II diabetes that account for 1%-5% of T2D cases. MODY is genetically heterogeneous, characterized by an autosomal dominant mode of inheritance, an early age of onset (usually less than 25 years of age), and abnormal pancreatic β-cell function, but not insulin resistance. In contrast to people with T2D, MODY subjects are typically lean.

It is the chronic complications, including accelerated macrovascular disease (particularly ischemic heart disease, stroke, and gangrene) and microvascular disease (particularly renopathy, neuropathy, and nephropathy) that reduce the life expectancy and confer the enormous public health burden associated with diabetes.

Genetic Influences

Diabetes includes a strong genetic component, however, little is known regarding specific genes that increase susceptibility to this disease. Regarding type 2 DM, a variety of studies indicate that genetic factors make a major contribution to its development. However, the genetic basis of this disease still remains not well understood. From studies of twins, it is known that concordance for monozygous (MZ) twins (twins who are genetically identical as they share 100% of the same genes) is 50-92% whereas for dizygous (DZ) twins (twins who are genetically alike as non-twin siblings but share only 50% of the same genes) is lower. The greater concordance among MZ compared to DZ twins is consistent with genetic influences. However, the incomplete concordance suggests a role for environmental factors as well. Also, mutations in pancreatic β-cell genes have been linked to specific MODY subtypes. Today, only 11% of MODY (referred to as MODY X) cases have no known genetic cause.

Environmental influences

There is a positive but not strong association between occupational exposure to arsenic including arsenic in drinking water and risk of T2D. However, this

association is presently too limited to draw any firm conclusion. Likewise for exposure to tetrachlorodibenzo-p-dioxin (TCDD).

Gene-environment interactions

The dramatic rise in the prevalence of T2D likely reflects interactions between genetic and environmental factors, particularly lifestyle factors. It is likely that only those with high-risk genetic profiles who are exposed to a high-risk environment (e.g., lack of physical activity and/or excessive caloric intake) will develop the disease. The interaction suggests that environmental factors modify the molecular function of the gene or product, that is the environmental dependence on gene expression or, alternatively, a particular genetic response to an environmental factor. Nonetheless, despite the belief that gene-environment interactions are important in the development of T2D, direct evidence for interactions between functional gene polymorphisms and environmental factors is lacking.

As evidenced in the case of the Dutch Famine (see Chapter 9), T2D emerges in populations transitioning from vigorous activity and subsistence nutrition to inactivity, over nutrition, and consequent obesity (the so-called "thrifty gene hypothesis"). This would be universally the case for populations that had survived periods of marked food scarcity in their history. From an evolutionary standpoint, the thrifty gene hypothesis speculates that a genetic predisposition to obesity and diabetes would be advantageous in an evolutionary sense in times of food scarcity by promoting the efficient retention of energy stores in the form of adipose tissue but would become disadvantageous in times of relative food abundance and low energy expenditures. Such populations would become enriched for these thrifty genes and thus susceptible to developing diabetes. The thrifty gene hypothesis also explains the increases in T2D among many aboriginal populations undergoing Westernization. It is likely that the genetic basis of the thrifty genotype is due to polymorphisms at multiple sites rather than a single abnormality.

Family history ecogenetics

Because an individual's family history information reflects both genetic and environmental factors, it too may serve as a unique measure of GE interactions. Many studies have evaluated the risk of T2D associated with

a positive family history of diabetes. Briefly, most studies report a two- to six-fold increased risk associated with a positive family history (first degree relative affected) compared to a negative family history, and that the risk is greater when both parents are affected. Associations with family history are consistently demonstrated in different ethnic groups and regardless of study design. Further, the risk associated with family history appears to be independent of other known risk factors including age, body mass index, glucose status, and smoking. Some studies suggest that having a mother with diabetes confers a greater risk than if the father has diabetes, and could be consistent with mitochondrial DNA (m-DNA) inheritance. However, the relative importance of maternal versus paternal diabetes is unclear and differences in risk may reflect bias.

Infectious diseases

Infectious diseases are random in nature and have a potentially devastating impact. (The history of these diseases has been recalled in Chapter 9, particularly regarding the discoveries of Girolamo Fracastoro, Antoine Philipps von Leeuwenhoek, Louis Pasteur, Robert Koch, and Ferdinand Cohn.) Of all the subdisciplines of ecogenetics, host susceptibility to infectious disease is one of the most challenging owing to multiple complex interacting factors (polygenic traits, host's genetic polymorphisms, environmental variability, pathogens carrying their own genome and adapting to conditions within their hosts). With the advent of whole genome sequencing, we are now poised for a major advance in our understanding of susceptibility to infectious diseases. We will cover here only two prominent human pathogens, malaria and AIDS, which are rich examples of infectious disease ecogenetics.

Malaria

Malaria is probably the first infectious illness for which host genetics were shown to play a role in susceptibility and resistance. It is caused by protozoan parasites of the genus *Plasmodium (P. vivax, P. falciparum, P. ovale, and P. malariae)* transmitted by the mosquitoes that infest tropical regions.

- ***P. vivax* and *P. falciparum*:** Most serious health threats; and
- ***P. falciparum*:** By far, the most lethal. Occurs principally in Africa where ~ 90% of malaria deaths occur.

Malaria is spread by an insect vector, the Anopheles mosquito, which can transmit very effectively even when a person is too sick to get out of bed. Here, high parasite titers in the blood are crucial for effective transmission, increasing the odds that each mosquito bite will also transmit the pathogen. Ample time for transmission and high pathogen titers can promote pathogen success even though millions of persons are killed each year (Fymat, 2017a, b; 2018c).

The global incidence of the world's deadliest infectious diseases is summarized in Table 10.5:

Table 10.5 - Global incidence of the world's deadliest infectious diseases

Pathogen	# People infected	# People with active disease	# Deaths annually
Malaria	300-500 million (2002)	--	1.5 million (2002)
HIV	40 million (2003)	--	3 million (2003)
Tuberculosis	1.86 billion (1999)	8.8 million (2002)	2 million (2003)

Source: David R. Sherman (2006)

Hemoglobin (Hb) and its variants (HbS, HbC, and HbE) are known to confer protection against malaria.

Worldwide human activities often exacerbate the malaria pandemic: generation of drug-resistant parasites and insecticide-resistant mosquitoes as well as war, climate change, population expansion, and migration.

HIV/AIDS

Acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). It spreads when body fluids such as blood or semen from an HIV-infected individual enter someone who is uninfected. Specific high-risk behaviors are necessary to fuel the AIDS pandemic, especially unprotected sex, intravenous drug use and, in countries with poor surveillance, transfusions with tainted blood. In addition, perinatal transmission is very widespread. More than 15% of all AIDS deaths occur in children under 15 years old.

HIV infects cells of the autoimmune system that express the surface protein CD4, primarily T-lymphocytes, but also macrophages and monocytes. These cells normally form the host defense against infectious disease. On entry, HIV subverts the host cellular machinery for its own reproduction until the cell is killed. Despite a massive immunological onslaught, it typically takes about a decade, and in some individuals 15 years or more, before T-cells levels decline substantially and the immune system falters, signaling the onset of AIDS disease. Once T-cells levels drop sufficiently, the person becomes susceptible to a variety of other infectious agents and eventually succumbs.

Certain infections that plague AIDS patients, but are rarely encountered in immunocompetent individuals, are considered AIDS-defining illnesses. These include *Pneumocystis carini* pneumonia (PCC), oral and systemic candidiasis, cytomegalovirus, lymphoma, and Kaposi sarcoma. Globally, tuberculosis (TB) is much too prevalent to be considered an AIDS-defining illness, but the synergy between TB and AIDS is especially sinister. TB is responsible for almost 10% of AIDS-related deaths, far more than any other infectious agent.

The genes implicated in resistance or susceptibility to HIV are summarized in Table 13.6:

Table 10.6 - Genes implicated in resistance or susceptibility to HIV

Gene	Allele	European	African	East Asian	Characteristics
CCR5	Wild type 632	0.86-0.96 0.044-0.14	1 0.0	1 0.0	Susceptible Resistant
CCR2	Wild type 64I	0.9 0.1	0.72 0.23	0.75 0.25	Susceptible Resistant
SDF1	Wild type 3'A	0.79 0.21	0.98 0.02	0.74 0.26	Susceptible Resistant
CCR5P	P1 P2 P3 P4	0.56 0.09 0.14 0.35		0.44 0.23 0.15	Susceptible

Source: O'Brien and Moore (2000)

Genetics versus epigenetics and ecogenetics

It is of interest to contrast, at least in part, genetics with epigenetics and ecogenetics. This is the tentative purpose of Table 10.7 below.

Table 10.7 - Comparison between genetics, epigenetics, and ecogenetics

Property	Genetics	Epigenetics	Ecogenetics
Type of study	o Genes and heredity o Genetic variations in living organisms (bacteria, plants, animals, humans) o Relationship with biochemistry and molecular biology	o Cellular and physiological traits inherited by daughter cells but *not* caused by DNA changes o Relevant changes to the genome that do not involve a change in the nucleotide sequence	Genes-environment and genes-genes-environment interactions
Characteristic		Can be divided into "predetermined" and "probabilistic" epigenesis	

Processes	o Gene linkage o Gene regulation o Gene mutation o Replication o Duplication o Inversion o Deletions o Chromosomal cross-over o Chromosomal translocation	o Genetic drift o Genomic imprinting o Transgenerational inheritance o Bookmarking o Carcinogenesis progress o Cloning limitations o Gene silencing o Heterochromatin o Histone modifications regulations o Imprinting o Maternal effects o Paramutation o Pathogenesis limitations o Position effect o Reprogramming o Teratogen effects o Transvection o X-chromosome inactivation	o Family history ecogenetics o Infectious diseases (malaria; HIV/AIDS) o Neurodegenerative diseases (Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis aka *Lou Gehring disease*), cardiovascular diseases, Type 2 diabetes)

Mechanisms	o Frequency-Effect relationship o Linkage disequilibrium	o DNA methylation. Also applies to RNA Epigenetics o Histone modification (acetylation, citrillination, methylation, phosphorylation, ribosylation, summoylation, ubiquitylation) o Chromatin remodeling (post-translational modifications of amino-acids that make up histone proteins, addition of methyl groups to DNA)	o Biotransformation enzymes' role in processing toxic substances (repair of damaged DNA: endogenous, exogenous; xenobiotic metabolism and disease risk) o Non-functional DNA repair systems causing diseases
Control		Through action of repressor genes that attach to silencer regions of DNA	
Agents		o Prions o RNA o Micro-RNA	o Infectious diseases o Pharmaceutical agents o Metabolism o Diet and nutrition o Foodstuffs o Food supplements o Environmental/ chemical agents o Physical agents o Lifestyle and behavioral agents

Changes	o Genetic variations	o Last through cell divisions for duration of the cell's life o Can be transferred to next generations o Can modify action of certain genes (not DNA) o Can be caused by DNA changes and by food/diet	
Inheritance	Inheritance theories: o Mendel's single gene o Laws of discrete inheritance o Law of segregation o Law of independent assortment for multiple genes	o "Cell memory"	
Applications	o Evolution o Nature *and* Nurture	o Evolution o Cancer o Teratogen effects o Cardiovascular diseases o etc.	o Vaccination o Microbial fermentation o Pasteurization o Disease prevention o Clinical medicine
Functional	o Molecular basis of inheritance o Natural selection	o Engineered Epigenome	Causative agents for tuberculosis, cholera, anthrax

Linkages			o Organisms to specific diseases o Diseases due to environmental exposure o Some food components and drugs to abnormal reactions because of specific hereditary susceptibility o Rare adverse reactions to standard drug dose that required the breakdown of certain enzymes o Adverse response to anti-malarial drug (*primaquine*) because of inherited X-chromosome recessive trait caused by G6PD deficiency o Hemolytic anemia caused by *primaquine* o Exposure to any kind of environmental and xenobiotic (chemical) agents

Conclusions and take-aways

- A historical perspective has provided the appropriate background against which to study the important emerging field of ecogenetics. In this context, the Environmental Genome Project (EGP), a part of the Human Genome Project of the (U.S.) National Institute of

Environmental Health Sciences (NIEHS) has been broadly outlined. EGP is a collaborative effort focused on examining how environmental exposures and variations in human genes between individuals influence the risk of disease.

- A major challenge in the field of ecogenetics is the correct identification of genetic variation that is biologically meaningful, and a major area of focus is the role of enzymes in processing and eliminating toxic substances. These biotransformation enzymes can also modulate an individual's susceptibility to environmental and occupational toxicants, and are responsible for oxidative metabolism and repair of DNA damage. They are important in understanding the various diseases linked to nonfunctional DNA repair systems.
- The different classes of environmental agents with their known ecogenetic variation, the nature of their effect, and the associated ecogenetic factors have been briefly addressed.
- The various gene-environment interactions that are important in the etiology of diseases have been reviewed in several cases: cancer (including breast, lung, and gastrointestinal); neurodegenerative diseases (including Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis or *Lou Gehrig's disease*); cardiovascular disease; diabetes; and infectious diseases, including malaria and HIV/AIDS.
- A person's likelihood of developing a particular cancer is determined largely by a complex interplay of risk factors involving environmental exposures, lifestyle factors, and inherited susceptibility. The challenge is to understand in sufficient detail how these specific factors interact in causing (or preventing) these cancers, so that more effective prevention and early detection programs can be developed and applied toward persons at highest risk of the disease.
- Neurodegenerative diseases (NDD) have a late onset and are characterized by the progressive loss of specific groups of neurons, resulting in cognitive or motor function deficits. Whereas multiple sclerosis (MS) is an important degenerative disease, it is generally considered to be an autoimmune disease, probably with a viral etiology.
- The pathophysiological processes underlying cardiovascular disease (CVD) events are varied, and include lipid metabolism, inflammatory responses, oxidative stress, endothelial function, platelet function,

thrombosis and hemostasis, homocysteine metabolism, insulin sensitivity, and hypertension. Each process underlying CVD risk is regulated by structural proteins, enzymes, receptors, and ligands, all of which are encoded by genes. Environmental and behavioral risk factors for CVD, including dietary fat, smoking, alcohol consumption, and lower exercise levels may be genetically influenced. Thus, CVD is likely the result of environmental exposure acting on a background of inherent susceptibility. Lastly, individual inter-variation points to the requirement of determining whether clinical prescribing practices need to be altered for individuals carrying genotypes that influence the actions and metabolism of particular anti-hypertensive medications. Assessment of gene-drug interactions are particularly important.

- Diabetes results from genetic imbalances, environmental insults (e.g., exposure to arsenic or lifestyle factors) or interactions between the two. Other important and well established risk factors for type 2 diabetes include physical inactivity, dietary fat intake, obesity (particularly central or visceral adiposity), family history of diabetes, and gestational diabetes mellitus. Changing lifestyle factors (weight loss, improved diet, and increased physical activity) results in a decreased risk. It is the chronic complications, including accelerated macrovascular disease (particularly ischemic heart disease, stroke, and gangrene) and microvascular disease (particularly renopathy, neuropathy, and nephropathy) that reduce life expectancy. Type 2 diabetes emerges in populations transitioning from vigorous activity and subsistence nutrition to inactivity, over nutrition and consequent obesity (the so-called "thrifty gene hypothesis"). This would be universally the case for populations that had survived periods of marked food scarcity in their history and among many aboriginal populations undergoing or having undergone Westernization.
- Infectious diseases are random in nature and have a potentially devastating impact. With the advent of whole genome sequencing, we are now poised for a major advance in our understanding of susceptibility to infectious diseases. Malaria is probably the first infectious illness for which host genetics plays a role in susceptibility and resistance. It is spread by an insect vector, the Anopheles mosquito, which can

transmit very effectively even when a person is too sick to get out of bed.

- Acquired immunodeficiency syndrome is caused by the human immunodeficiency virus. It spreads when body fluids such as blood or semen from an HIV-infected individual enter someone who is uninfected. HIV infects cells of the autoimmune system that express the surface protein CD4, primarily T-lymphocytes, but also macrophages and monocytes. These cells normally form the main host defense against infectious disease. Globally, tuberculosis is much too prevalent to be considered an AIDS-defining illness, but the synergy between tuberculosis and AIDS is especially sinister.
- A tabular comparison was provided of genetics versus epigenetics versus ecogenetics.

11

Primer on Stem cells

Contents

11

Primer on Stem cells

Stem cells (SC) are cells that can differentiate into other cell types and, like other cells, renew themselves by dividing and producing more of them. There are two broad types: 'embryonic' and 'adult'. In a developing embryo, SCs can differentiate into all the specialized cells (ectoderm, endoderm, mesoderm) and maintain the normal turnover of regenerative organs (blood, skin, intestinal tissues, etc.). In an adult organism, SCs together with 'progenitor' cells (PC) replenish tissues, acting as a repair system for the body. PC are non-SCs that cannot self-renew. Adult stem cells (ASC) are frequently used in various medical therapies.

Properties of stem cells

By definition, a SC is required to possess two properties:

- ***Self-Renewal:*** The ability of going through numerous cell division cycles while maintaining their undifferentiated state by two different mechanisms:
  - ***Obligatory asymmetric replication (OAR):*** A SC divides into a mother cell that is identical to the original undifferentiated SC and another daughter cell that is differentiated; and

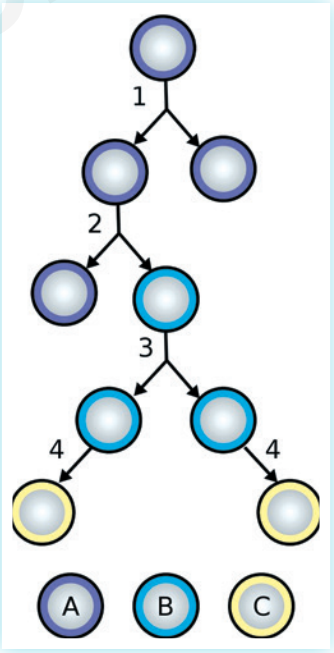
- ***Stochastic differentiation (SD):*** One SC divides into two differentiated daughter cells while another SC differentiates into two daughter cells that are identical to the original SC.

➢ ***Potency:*** The capacity to differentiate into specialized cell types, requiring the SCs to be either:

- ***Totipotent* (or *omnipotent*):** Produced by the fusion of an egg and a sperm, they can differentiate into embryonic and extra-embryonic cell types and can construct a complete and viable organism. The first few divisions of the fertilized egg are also totipotent; or
- ***Pluripotent:*** Derived from any of the three germ layers, they can differentiate into a number of cell types, but only those of a closely related family of cells.
- ***Oligopotent:*** They can differentiate only into a few cell types; or
- ***Unipotent:*** They can produce only their own type but can renew themselves.

The above properties can be illustrated *in vivo* using clonogenic assays, however, that may alter the cells' behavior, making it unclear whether the cells shall behave in a similar manner *in vivo*. Figure 11.1 illustrates stem cell division and differentiation.

Figure 11.1 - Stem cell division and differentiation



(A: stem cell; B: progenitor cell; C: differentiated cell; 1: symmetric stem cell division; 2: asymmetric stem cell division; 3: progenitor division; 4: terminal differentiation)
Source: Wikipedia

By a procedure called somatic cell nuclear transfer (SCNT), SCs can now be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues (muscles, nerves, etc.). This technology has fallen in disfavor because of the possibility of inducing pluripotent stem cells (see below).

Figure 11.2 - Evolution from a fertilized egg to a human fetus

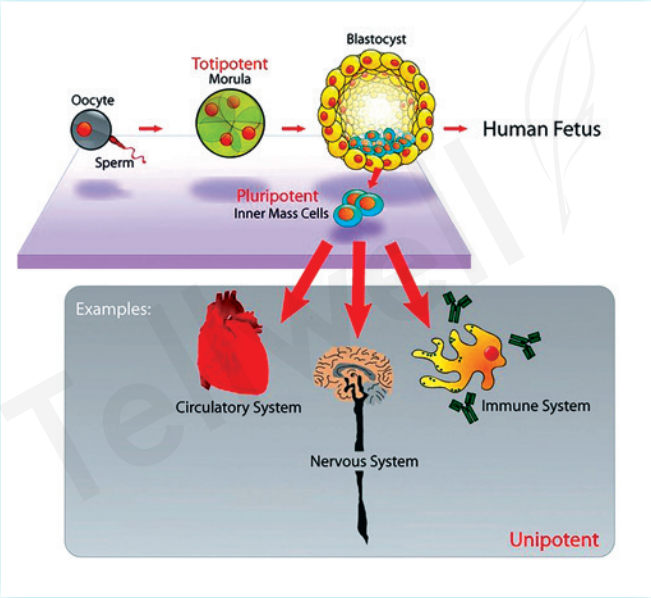


Figure 11.2 illustrates the evolution from a fertilized egg to a human fetus. SCs from the earlier stage of comparison between genetics, epigenetics, and ecogenetics of an an embryo (the morula) are totipotent and able to become all tissues in the body and the extra embryonic placenta. Embryonic stem cells (ESC) originate as inner cell mass (ICM) within a blastocyst. Those from the blastocyst or inner mass cells (IMC) are pluripotent giving rise to unipotent stem cells (USC) that can become any tissue in the body excluding a placenta such as, for example, cells of the circulatory, nervous, and immune systems.

There are four known accessible sources of autologous SCs that offer least risk for harvesting:

- ***Bone marrow:*** Typically from the femur or the iliac crest. It is a rich source of ASCs. That are used for treating such conditions as liver cirrhosis, chronic limb ischemia, end-stage heart failure, etc.
- ***Adipose tissue:*** Fat cells obtained by liposuction.
- ***Blood:*** They are extracted by a procedure called *apheresis* wherein blood is drawn from the donor, passed through a machine that extracts the stem cells, and other portions of the blood returned to the donor.
- *Umbilical cord blood* ***(UCB).***

Types of stem cells

Embryonic stem cells

Formed prior to implantation in the uterus, 4-5 days after fertilization, embryonic stem cells (ESC) about 50-150 cells in number are pluripotent, giving rise to the three germ layers (ectoderm, endoderm, and mesoderm). They can develop into each of the more than 200 cell types of the adult body. However, they do not contribute to the extra-embryonic membranes or to the placenta. They continuously divide and become more specialized. ESCs are also defined by the expression of several transcription factors and cell surface proteins. There are currently no approved treatments using embryonic stem cells. Due to ethical considerations, many nations have moratoria or limitations on either human ESC research or the production of new human ESC lines. Nonetheless, because of their important properties, ESCs remain a theoretically potential source for regenerative medicine and tissue replacement after injury or disease.

Fetal stem cells

There are two types of fetal stem cells:

- ***Fetal proper stem cells:*** They come from the tissue of the fetus proper, and are generally obtained after an abortion. They are not immortal but have a high level of division and are multipotent.
- ***Extra-embryonic fetal stem cells:*** They come from extra-embryonic membranes and are generally not distinguished from adult stem cells (ASC). They are acquired after birth, are not immortal, have a high level of cell division, and are pluripotent.

Adult stem cells

Adult stem cells (ASC), also called somatic stem cells (SSC), maintain and repair the tissue in which they are found. They can also be found in children. They have been successfully used for many years to treat leukemia and related bone/blood cancers. However, unlike ESC, they are not able to differentiate into cells from the three germ layers (ectoderm, endoderm, and mesoderm). They are deemed multipotent.

Amniotic fluid stem cells

Amniotic fluid stem cells (AFSC) are very active, expand extensively without feeders, and are not tumorigenic. They are multipotent and can differentiate into cells of adipogenic, osteogenic, myogenic, endothelial, hepatic, and also neuronal lines. They can be collected for donors or for autologous use.

Induced pluripotent stem cells

As indicated earlier, ASCs are only multipotent. However, using protein transcription factors (PTF), it is possible to genetically reprogram them to create pluripotent SCs (Yamanaka *et al.*, Yu and Thomson) – these are the induced pluripotent stem cells (IPSC). While sharing many similar properties with ESCs (pluripotency, differentiation potential, expression of pluripotency genes, epigenetic patterns, etc.), IPSCs exhibit many differences with them. Thus, for example, the chromatin of IPSCs appears to be more

"closed" or methylated than that of ESCs, raising the question as to whether reprogramming was or could be complete. Nonetheless, IPSCs are viable, providing several therapeutic advantages. Importantly, they potentially could allow the creation of pluripotent stem cells (PSC) for each individual patient. Such patient-specific PSCs could allow screening for side effects before drug treatment, as well as reducing risk of transplantation rejection.

Stem cell lines

To ensure self-renewal, SCs undergo two types of cell division: (1) Symmetric division that gives rise to two identical daughter cells both endowed with stem cell properties; and (2) asymmetric division that produces only one stem cell and a progenitor cell with limited self-renewal potential. In turn, progenitors can go through several rounds of cell division before terminally differentiating into a mature cell.

Stem cell therapy

Stem cell therapy (SCT) is the use of stem cells to treat or prevent a disease or condition. For example, bone marrow transplantation (BMT) is a form of SCT that has been used for many years without controversy. Figure 10.3 is a pictorial showing those diseases and conditions where SCT is promising or emerging, BMT being the most widely used, but some therapies derived from umbilical cord blood (UCBT) - not shown in Figure 11.3 - are also employed.

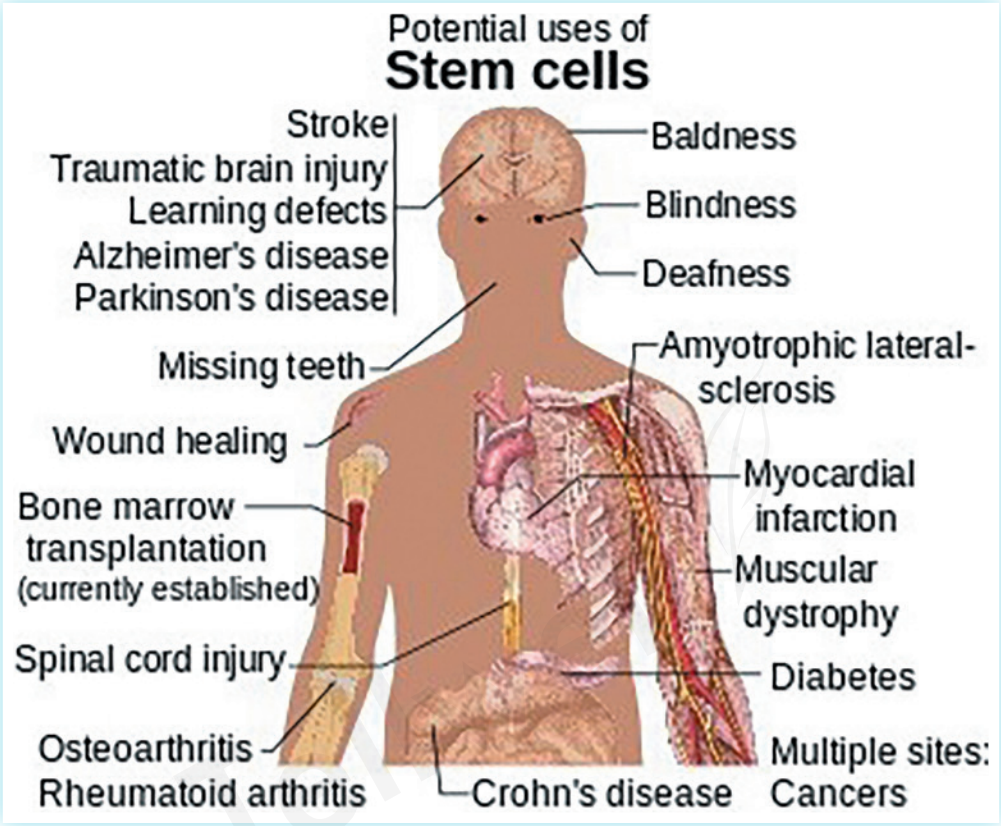
Regenerative treatment models

SCs are thought to mediate repair via five primary mechanisms:

- ***Providing an anti-inflammatory effect;***
- ***Homing on to damaged tissues and recruiting other cells***. These other cells are endothelial progenitor cells that are necessary for tissue growth;
- ***Supporting tissue remodeling over scar formation;***
  - ***Inhibiting apoptosis*;** and

- ***Differentiating into bone, cartilage, tendon, and ligament tissue.***

Figure 11.3 - Pictorial showing the diseases and conditions where stem cell treatment is promising or emerging



Source: Haggstrom Mikael (2014)

To further enrich blood supply to the damaged areas, and consequently promote tissue regeneration, platelet-rich plasma could be used in conjunction with SCT. The efficacy of some SC populations may also be affected by the method of delivery. For instance, to regenerate bone, SCs are often introduced in a scaffold where they produce the minerals necessary for generation of functional bone. SCs have also been shown to have a low immunogenicity due to the relatively low number of MHC molecules found on their surface. In addition, they have been found to secrete chemokines that alter the immune response and promote tolerance of the new tissue. This allows for allogenic treatments to be performed without a high rejection risk.

Advantages

Advantages accruing to SCT are:

- ***Lowering symptoms or conditions of the disease treated;*** and
- ***Allowing patients to reduce their drug intake.***

Disadvantages

Despite the above advantages, there are the following associated disadvantages:

- ***Treatment may require immunosuppression before transplantation:*** This may be required in order to perform a preliminary radiation treatment to kill previous cancerous cells. It may also be required if the patient's immune system may target the stem cells considering them as foreign bodies (this could be avoided using SCs from the same patient);
- ***Pluripotency in certain stem cells could also make it difficult to obtain a specific cell type;***
- ***Not all cells in a population differentiate uniformly.*** This makes it difficult to obtain the exact cell type needed;
- ***Undifferentiated cells can create tissues other than of the desired types;*** and
- ***Pluripotent stem cells can form tumors:*** This is especially the case for ESCs, FSCs and IPSCs.

Diseases and conditions treated

Figure 11.3 is a pictorial of the diseases and conditions where stem cell treatment is either applied or/and being investigated. (Not mentioned there are the following conditions: male infertility when due to the absence of spermatogonial stem cells; female infertility including premature ovarian insufficiency.)

Research is also underway in organogenesis in order to better understand human development and modeling human diseases.

Blindness and vision impairment

Corneal SCs have been successfully transplanted into damaged eyes to stimulate renewal repair, eventually leading to restored vision. In June 2005, using this technique, researchers at the Queen Victoria Hospital in Sussex, England were able to restore the sight of forty people. The SCs were obtained from either the patient, a relative, or even a cadaver.

Bone marrow transplantation

Bone marrow transplantation (BMT) is well established, having been widely practiced over the past 40 years to treat bone cancer, leukemia, and lymphoma. Conventional chemotherapy with cytotoxic agents destroys not only leukemic and neoplastic cells but also the hematopoietic SCs within the bone marrow. To correct the latter serious adverse event (SAE), the cells lost in the host are replaced with a compatible donor's healthy cells, reintroducing functional SCs in the host's body during treatment. The transplanted cells have an additional benefit in that they also generate an immune response that further helps to kill off the cancer cells. However, this process must be managed carefully so as to prevent the occurrence of a graft-versus-host disease (GVHD), the most serious side effect of this treatment.

Brain and spinal cord injury

Stroke and traumatic brain injury (TBI) lead to cell death characterized by loss of oligodendrocytes and neurons within the brain. Clinical and animal studies have been conducted into the use of SCs in cases of spinal cord injury (SCI).

Cancer

Bioengineered immune cells, known as chimeric antigen receptor T-cells (CAR T-cells), have been shown to attack and even cure certain forms of cancer, but lose their efficacy in prolonged attacks on the cancer. It has recently been found that, when boosted with stem cells, these cancer-fighting cells gain renewed fighting vigor. (reference: *Nature* **628:**486-7, 18 April 2024).

Cochlear hair cell regrowth

Success has been reported in re-growing cochlear hair cells with the use of ESCs.

Diabetes type I

Type 1 diabetes patients lose the function of **insulin**-producing beta-cells within the pancreas. In recent laboratory experiments, scientists have been able to coax ESCs to turn into beta-cells, replacing the malfunctioning ones.

Heart

SCT for treatment of myocardial infarction (MI) makes use of autologous bone marrow SCs although other types of adult SCs (e.g., adipose-derived SCs) may also be used. Possible recovery mechanisms include: Generation of heart muscle cells, stimulating growth of new blood vessels to repopulate damaged heart tissue, and secretion of growth factors (GF). However, clinical trials have so far led to only modest improvements in heart function.

HIV/AIDS

Recently scientists have been investigating an approach to treating HIV-1/AIDS, based on the creation of a disease-resistant immune system through transplantation of autologous, gene-modified (HIV-1-resistant) hematopoietic stem and progenitor cells (GM-HSPC).

Infertility

Human ESCs have been stimulated to form spermatozoa-like cells, yet still slightly damaged or malformed. They could potentially treat azospermia. In 2012, oogonial SCs were isolated from adult mouse and human ovaries and demonstrated to be capable of forming mature oocytes, offering the potential to treat infertility.

Neurodegeneration and neurodegenerative diseases

Healthy adult brains contain neural SCs, which divide to maintain general SC numbers, or become PCs.

In healthy laboratory animals, PCs migrate within the brain and function primarily to maintain neuron populations for olfaction (the sense of smell). In these animals, research has been conducted on the effects of SCs on brain degeneration including such brain diseases as Alzheimer's (AD), Parkinson's (PD) and amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), and multiple sclerosis (MS).

Of particular note, pharmacological activation of endogenous neural SCs has been reported to induce neuroprotection and behavioral recovery in adult rat models of neurological disorder.

Orthopedics

Use of MSCs derived from ASCs is under preliminary research for potential orthopedic applications in bone and muscle trauma, cartilage repair, osteoarthritis, intervertebral disc surgery, rotator cuff surgery, musculoskeletal disorders, tissue engineering, and regenerative medicine.

Regrowing teeth

Tooth regeneration has been developed in laboratory animals according to the following steps: (1) SCs are coaxed in the laboratory to produce a tooth bud; (2) implanted in the gums, the tooth bud will rise to a new tooth; (3) the new tooth will fuse with the jawbone and release chemicals that encourage nerves and blood vessels to connect with it. The above process is similar to that when humans grow their original adult teeth. The process is anticipated to last approximately 3 weeks. Many challenges, however, remain before SCs could be a choice for the replacement of missing teeth.

Wound healing

SCs can be used to stimulate the growth of human tissues. A possible method for tissue regeneration in adults is to place adult SC "seeds" inside a tissue bed "soil" in a wound bed and allow the SCs to stimulate differentiation in the tissue bed cells.

Current practice and research directions

Research into stem cells (SC) grew out of findings by Canadian biologists Ernest McCulloch, James Till and Andrew J. Becker at the University of Toronto and the Ontario Cancer Institute in the 1960s. As of 2016, the only established medical therapy using SC is hematopoietic SC transplantation, first performed in 1958 by French oncologist Georges Mathé. Since 1998 however, it has been possible to culture and differentiate human embryonic stem cells (ESC) in SC lines. The process of isolating these cells has been controversial, because it typically results in the destruction of the embryo. Sources for isolating ESCs have been restricted in some European countries and Canada, but others such as the U.K. and China have promoted the research. Somatic cell nuclear transfer (SCNT) is a cloning method that can be used to create a cloned embryo for the use of its embryonic stem cells in stem cell therapy (SCT). In 2006, a Japanese team led by Shinya Yamanaka discovered a method to convert mature body cells back into SC. These were termed induced pluripotent stem cells (iPSC).

Sources of stem cells for regenerative therapy

There are five recognized sources of SCs used for regenerative therapy:

- ***Bone marrow:*** MSCs can differentiate into the cells that make up bone, cartilage, tendons, and ligaments, as well as muscle, neural, and other progenitor tissues. They have been the main type of SCs in the treatment of diseases affecting these tissues. The number of SCs transplanted into damaged tissue may alter the efficacy of treatment. Accordingly, SCs derived from bone marrow aspirates, for instance,

are cultured in specialized laboratories for expansion to millions of cells.

- ***Adipose tissue:*** Adipose-derived SCs also require processing prior to use, but the associated culturing methodology is not as extensive as that for bone marrow-derived cells Additionally, the multi-cellular microenvironment already present makes them the preferred source for autologous transplantation.
- ***Skin and dermis:*** These are of interest because of the ease with which they can be harvested with minimal risk.
- ***Hematopoietic:*** These SCs travel in the blood stream and possess equal differentiating ability as other MSCs with a very non-invasive harvesting technique.
- ***Extra embryonic mesenchymal:*** Research is underway to examine the differentiating capabilities of stem cells found in the umbilical cord, yolk sac, and placenta of different animals. They are thought to have more differentiating ability than their adult counterparts, including the ability to more readily form tissues of endodermal and ectodermal origin.
- ***On embryonic stem cell lines:*** There is widespread controversy over the use of human ESCs because it often requires the destruction of the blastocyst. Opposition is based on philosophical, moral, or religious objections. Other SC research that does not require the destruction of the embryo uses adult stem cells, amniotic stem cells, and induced pluripotent stem cells.

Corneal ulcers repairs

SCs are also in clinical phases for treatment in ophthalmology. Hematopoietic SCs have been used to treat corneal ulcers of different origin. These ulcers were resistant to conventional treatments available, but quickly responded positively to the SC treatment. SCs were also able to restore sight in one eye with retinal detachment.

Epidermal tissue repair

This is particularly important for patients with reduced healing capabilities, like diabetics and those undergoing chemotherapy. In one trial, SCs were injected directly into the wounds. Within a week, full re-epithelialization of the wounds had occurred, compared to minor re-epithelialization in the control wounds. This showed the capabilities of mesenchymal stem cells in the repair of epidermal tissues.

Hard-tissue repair

Bone has a unique and well documented natural healing process that normally is sufficient to repair fractures and other common injuries. Misaligned breaks due to severe trauma, as well as treatments like tumor resections of bone cancer, are prone to improper healing if left to the natural process alone. Scaffolds composed of natural and artificial components are seeded with MSCs and placed in the defect. Within four weeks of placing the scaffold, newly formed bone begins to integrate with the old bone and within 32 weeks, full union is achieved. Further studies are necessary to fully characterize the use of cell-based therapeutics for treatment of bone fractures.

Joint repair

Osteoarthritis is the main cause of joint pain. Natural cartilage regeneration is very limited and no current drug therapies are curative, but rather look to reduce the symptoms associated with the degeneration. Different types of MSCs and other additives are still being researched to find the best type of cell and method for long-term treatment.

Adipose-derived mesenchymal cells are currently the most often used because of the non-invasive harvesting. A recently developed, non-invasive technique consists in injecting MSCs directly into the joint.

Ligament and tendon repairs

Autologous stem cell-based treatments for ligament injury, tendon injury, osteoarthritis, osteochondrosis, and sub-chondral bone cysts have been

commercially available to practicing veterinarians to treat race horses (U.S.: since 2003; U.K. Since 2006). It does not appear that such repairs have been effected on humans. **ESCs** have a better survival rate in the tendon as well as better migrating capabilities to reach all areas of damaged tendon. The overall repair quality is high with good tendon architecture and collagen formation. Nonetheless, long-term studies need to be carried out to examine the long-term efficacy and risks associated with the use of ESCs.

Mesenchymal stromal cells

When transfused within a few hours post-thawing, mesenchymal stromal cells (MSC) show reduced function and decreased efficacy in treating diseases as compared to fresh MSCs (in the so-called "log phase of cell growth). So, cryo-preserved MSCs should be brought back into the cell growth log phase in *in vitro* culture before they are administered for clinical trials or experimental therapies. Re-culturing of MSCs will additionally help the cells in recovering from the shock they get during freezing and thawing.

Muscle and cardiac muscle repairs

Stem cells have successfully been used to ameliorate healing in the heart after myocardial infarction (MI). Adipose and bone marrow derived SCs were removed and induced to a cardiac cell fate before being injected into the heart. The heart was found to have improved contractility and a reduction in the damaged area four weeks after the SCs were applied.

A different trial is underway for a patch made of a porous substance onto which the SCs are "seeded" in order to induce tissue regeneration in heart defects. Tissue was regenerated and the patch was well incorporated into the heart tissue. This is thought to be due, in part, to improved angiogenesis and reduction of inflammation. Although cardiomyocytes were produced from the MSCs, they did not appear to be contractile. Other treatments that induced a cardiac fate in the cells before transplanting had greater success at creating contractile heart tissue.

Nervous system repairs

Spinal cord injuries (SCI) occur in two ways after the trauma: (1) The primary mechanical damage, and (2) the secondary processes of inflammation and scar formation in the days following the trauma. The cells involved in the secondary damage response secrete factors that promote scar formation and inhibit cellular regeneration. MSCs that are induced to a neural cell fate are loaded onto a porous scaffold and are then implanted at the site of injury. The cells and scaffold secrete factors that counteract those secreted by scar forming cells and promote neural regeneration.

Treatments are currently in clinical trials to repair and regenerate peripheral nerves. Peripheral nerves are more likely to be damaged, but the effects of the damage are not as widespread as seen in injuries to the spinal cord. SCs are induced to a neural fate and injected into a severed nerve. The treatment repairs severed nerves. Within four weeks, regeneration of previously damaged stem cells and completely formed nerve bundles can be observed.

Other approved therapies

Another SCT, called *Prochymal*, was conditionally approved in Canada in 2012 for the management of acute GVHD in children who are unresponsive to steroids. It is an allogenic stem therapy based on MSCs derived from the bone marrow of adult donors. MSCs are purified from the marrow, cultured and packaged, with up to 10,000 doses derived from a single donor. The doses are stored frozen until needed.

The (U.S.) Food & Drug Administration (FDA) has also approved five hematopoietic stem-cell products derived from umbilical cord blood for the treatment of blood and immunological diseases.

In 2014, the European Medicines Agency (EMA) recommended approval of limbal stem cells (LSC) for people with severe LSC deficiency due to eye burns.

Drug discovery and personalization

The ability to grow up functional adult tissues indefinitely in culture through directed differentiation creates new opportunities for drug research. Researchers are able to grow up differentiated cell lines and then test new drugs on each cell type to examine possible interactions *in vitro* before performing *in vivo* studies. With the advent of induced pluripotent stem cells (iPSC), treatments are being explored and created. Rather than harvesting embryos or eggs, which are limited, the researchers can remove MSCs with greater ease and greatly reduce the danger due to noninvasive techniques.

SCs are being studied for a number of reasons. The molecules and exosomes released from them are also being studied in an effort to make medications. The paracrine soluble factors (PSF) produced as the SC's secretome have been found to be the predominant mechanism by which SC-based therapies mediate their effects in degenerative, autoimmune and inflammatory diseases.

Conclusions and take-aways

- Stem cells are cells that can differentiate into other cell types and, like other cells, renew themselves by dividing and producing more of them. There are two broad types: embryonic and adult.
- In a developing embryo, stem cells can differentiate into all the specialized cells (ectoderm, endoderm, mesoderm) and maintain the normal turnover of regenerative organs (blood, skin, intestinal tissues, etc.).
- In an adult organism, stem cells together with progenitor cells replenish tissues, acting as a repair system for the body. Progenitor cells are different from stem cells in that they cannot self-renew. Adult stem cells are frequently used in various medical therapies that have been described.
- A stem cell possesses two properties: self-renewal and potency (toti- or omni, pluri-, oligo, and uni-potency). **By a procedure called somatic cell nuclear transfer, stem cells can now be artificially grown and transformed (differentiated) into specialized cell types**

with characteristics consistent with cells of various tissues (muscles, nerves, etc.).

- **The various stem cell types have been described and their respective properties set forth: Embryonic, fetal, adult (or somatic), amniotic, and induced.**
- Stem cell therapy is the use of stem cells to treat or prevent a disease or condition. It has been (or is being) used in regenerative treatment, and in a number of diseases or conditions (**bone marrow transplantation, neuro- and other -degenerative disorders, brain and spinal cord injury, heart and cardiovascular diseases, dentistry; hair cell regrowth; vision impairment and blindness; type I diabetes; orthopedics; wound healing; infertility; HIV-AIDS; and others).**
- **Current practice and research directions have been detailed for the sources of stem cells for regenerative therapy (bone marrow, adipose tissue, skin and dermis, hematopoietic stem cells, and extra embryonic mesenchymal stem cells); embryonic stem cells; mesenchymal stromal cells; and repairs of hard and epidermal tissue, ligament, tendon, and joint, muscle and cardiac muscle, nervous system, corneal ulcers, and others.**
- The ability to grow up functional adult tissues indefinitely in culture through directed differentiation creates new opportunities for drug research, including differentiation of cell lines and testing new drugs on each cell type and their possible interactions.
- Stem cells are also **being studied for a number of other reasons, for example, the molecules and exosomes released from them are also being studied in an effort to make medications.**

PART D

MODERN THEORIES AND HYPOTHESES OF AGING

Introduction to Part D

Five modern theories and hypotheses of biological aging will be discussed in Part D. The various aging theories will be initially classified in **Chapter 12.** More than 30 different theories have so far been been posited to explain the nature of aging and its causes. They fall into two broad categories: Evolutionary and mechanistic. "Evolutionary theories" primarily explain why aging happens, but do not concern themselves with the molecular mechanism(s) that drive the process. They all rest on the basic premise that the force of natural selection declines with age. Evolutionary theories can be explained briefly by: Mutations accumulation, somatic disposition, antagonistic pleiotropy, and selective shadowing. By contrast, "Mechanistic theories" can be divided into theories that propose that aging is "programmed" and "damage accumulation theories".

"Systems theories" include: "Immunologic", "Rate-of-living" (an error theory), and "Neuroendocrinal control mechanisms alterations". "Molecular theories" include phenomena such as: Gene regulation (gene expression), codon restriction, error catastrophe, somatic mutation, genetic material (DNA) damage accumulation, and dysdifferentiation. "Cellular theories" can be categorized as: Telomere theory, free radical theory, apoptosis, stem cell theory, and reproductive cell cycle theory.

There are now dozens of theories of aging to explain this inevitable fact of being human. These have been classified in four categories (acronym **PEGB**): Programmed; Error; Genetics; and Biochemical. "Programmed theories" assert that the human body is "designed" to age so that aging is a natural phenomenon that has been "programmed" into our bodies, following a certain biological timeline. Within such theories, one may distinguish those that assert that the genetic switching on and off over time causes aging. Others

argue that regular changes in hormones control aging. Still others assert that aging is caused by the time accumulation of environmental damage to the body's systems. "Error theories" assert that, over time, cells and tissues simply wear out. Variation of these theories include: "Wear-and-tear theory" in which cells and tissues simply wear out; "Rate- of-living theory" in which aging is inversely related to the organism consumption of oxygen; "Cross-linking theory" which posits that cross-linked proteins accumulate and slow down the body's processes; and "Free radicals theory" which asserts that free radicals in the environment cause damage to cells, eventually, impairing their function and causing aging.

Genetics plays a major role in aging. Variations on this theory include the: "Somatic DNA damage theory" in which genetic mutations are known to cause the malfunctioning of cells and subsequently aging; "Longevity genes theory", based on so-called "longevity genes", which are specific genes that help lengthen lifespan; "Cell senescence theory", which rests on the process of senescence by which cells deteriorate over time and cause aging; "Telomeres shortening theory", which is based on the known effect of telomeres shortening on cell replication; and "Stem cells theory", which holds promise to repair the damage caused by aging.

"Biochemical theories" rest on the known fact that no matter what genes we may have inherited, our body is continually undergoing complex biochemical reactions, some of which causing damage and, ultimately, aging in the body. There are five important concepts: "Free radicals theory", which can damage cells; "Protein cross-linking theory", which produce excess sugars in the bloodstream that can cause protein molecules to literally stick together leading to aging; "DNA repair theory" in which the systems in the body that repair DNA seem to become less effective with age; "Heat shock proteins theory", which help cells survive stress and diminish in numbers with age; and "Age-changing hormonal theory" in which hormones cause many shifts in organ systems and other functions.

Theories of aging affect efforts to understand and find treatments for age-related conditions: Those who believe in the idea that aging is an unavoidable side effect of some necessary function (antagonistic pleiotropy or disposable soma theories) logically tend to believe that attempts to delay aging would

result in unacceptable side effects to the necessary functions. Altering aging is therefore "impossible" and study of aging mechanisms is of only academic interest. On the other hand, those believing in "Default theories" of multiple maintenance mechanisms tend to believe that ways might be found to enhance the operation of some of those mechanisms (e.g., assistance by antioxidants or other agents). Still others who believe in "Programmed aging" suppose that ways might be found to interfere with the operation of the part of the aging mechanism that appears to be common to multiple symptoms, essentially "slowing down the clock" and delaying multiple manifestations. One such effort is an attempt to find a "mimetic" that would "mime" the anti-aging effect of calorie restriction without having to actually radically restrict diet.

Chapter 13 will be more specifically dedicated to Programmed theories. They assert that *aging and death are necessary parts of evolution, not of biology*. The key point is that if biological individuals lived forever, evolution would not exist … and conversely. In this view, since aging is about evolution and not biology, it must be inherent in the organism and not simply a result of environmental factors or disease. Accordingly, aging and death are not a result of wear-and-tear or exposure, but are a programmed, natural, and necessary part of genetics. Some changes can be made based on nutrition, medical care, and other demographic factors, but overall lifespan within species is fairly constant. In short, we are genetically programmed to age and die. That said, aging and dying are inevitable, but there are things we can do to improve our chances of living a long and healthy life.

In **Chapter 14,** the genetic theories of aging are reviewed. Studies have demonstrated that genetics can play a major role in aging and account for much of the variation in aging among people. Particularly, l**ongevity genes** are specific genes that help a person live longer. Telomeres (those small structures at the tips of genes) stabilize DNA as cells divide and get shorter with chronological age. Their length determines the age of a cell and how many more replications it has left.

Chapter 15 is concerned with th**e e**rror theories of aging. There is little evidence that oxygen metabolism, heartbeat, or the number of breaths determine an individual's lifespan. The rate-of-living theory seems to hold

up when smaller species with faster metabolisms (i.e., mice) are compared with larger species with slower metabolisms (i.e., tortoises). However, the theory can only partially explain the differences in life span between species, and it cannot explain the most important factor: What determines lifespan *within* species. Further, there really is no data that slowing the metabolism extends human life. In fact, a slower metabolism would put someone at risk for obesity and other nutritional-related illnesses. The best bet remains a healthy lifestyle with plenty of exercise, a diet with lots of plants, and a positive, relaxed attitude.

No matter what genes we have inherited, our body is continually undergoing complex biochemical reactions. Some of these reactions cause damage and, ultimately, aging in the body. Studying these complex reactions helps understand how the body changes as it ages. Important concepts in the biochemistry of aging are first discussed in **Chapter 16,** which reviews the biochemical theories of aging beginning with important concepts in the biochemistry of aging such as the age-changing hormonal theory; the free radicals theory; the protein cross-linking theory; the DNA repair theory; and the heat shock proteins theory. It will then proceed with the modern biological theories of aging including the programmed theory of aging and its three sub-categories (programmed longevity theory; endocrine theory; and immunological theory), the damage or error theory of aging including its five sub-categories (wear-and-tear theory; rate-of-living theory; cross-linking theory; free radicals theory; and somatic DNA damage theory), and the calorie restriction theory of aging. It will lastly outline the various risks of neurological diseases with increasing age and the role of telomeres.

12

Classifications of the theories of aging

Contents

12

Classifications of the theories of aging

More than 300 different theories of aging have been posited to explain the nature and causes of aging. They fall into two broad categories: Evolutionary and mechanistic. "Evolutionary theories" of aging primarily explain why aging happens, but do not concern themselves with the molecular mechanism(s) that drive the process. They all rest on the basic mechanisms that the force of natural selection declines with age. "Mechanistic theories", on the other hand, can be divided into theories that propose that aging is "programmed", and "damage accumulation" theories that propose aging to be caused by specific molecular changes occurring over time.

Gerontology, the study of aging, is a relatively new science that has made incredible progress over the last 30 years. In the past, scientists looked for a single theory that explained aging, but have realized that aging is a complex interaction of genetics, chemistry, physiology, and behavior. There are now dozens of theories of aging to explain this inevitable fact of being human. These have been classified in four categories: ***P***rogrammed, ***E***rror, ***G***enetic, ***B***iological (acronym **PEGB**), which are briefly presented in Table 12.1 below and further elaborated upon in this chapter. Any of these theories is not

exclusive and a combination of them may be able to explain aging in any given aging circumstance.

In the following sections, I will further elaborate on the above different theoretical concepts.

Evolutionary theories

August Weismann was the first to publish seminal works on early evolutionary theories of aging. In 1881, he offered an explanation of senescence in terms of evolution by natural selection. Thus, in his 1881 lecture titled "The Duration of Life", he proposed that longevity was programmed according to "the need of the species" (Weismann 1891, p. 9). He rejected the idea that an organism's longevity was determined merely by its physiological "construction", arguing instead that evolution could shape longevity according to the dictates of natural selection, as he understood them.

The need for death was an important theme in Weismann's work. He explained that, if not killed by accident, an individual would experience injuries over time. A limited ability to heal such injuries would result in older individuals having lower Darwinian fitness than younger individuals. Older individuals would therefore take limited resources that could be better allocated to younger individuals, thus creating a selective advantage (at the level of the population or group) for dying at old ages.

Weismann proposed limits to somatic cell replication as a mechanism for this inability to heal. In his own language, "*when one or more individuals have provided a sufficient number of successors they themselves, as consumers of nourishment in a constantly increasing degree, are an injury to those successors… natural selection therefore will weed them out*".

Further, in his essays "Life and Death" and "On Heredity", Weismann suggested that once a selective advantage for death had been established, there would be no barrier to selection for any advantageous traits that might trade-off against immortality. The forgoing of immortality might make additional resources available to reproductive cells. He attributed this evolutionary loss of immortality to "panmixia". The central idea of Weismann's theory is that

characters useless to an organism escape the action of natural selection and therefore disappear. In reappraising this theory and its emphasis on the preeminent role that selection plays in the evolution of senescence, Kirkwood and Cremer (1982) assesswd the research in the field of aging from an evolutionary and cellular standpoint. They believed that Weismann's theory and thoughts were "... *more extensive in their scope and more pertinent to current research than is generally recognized*" (p. 101). Weismann's panmixia theory seems to be an anticipation of modern thinking about the evolution of aging.

Evolutionary biology offers a coherent and experimentally supported theory for biological aging. Here we will introduce the literature on the evolutionary biology of aging, starting with formal theory. Over the last forty years this field has developed important empirical foundations. The longest standing data pertaining to the evolutionary biology of aging naturally are its comparative biology. We will touch on the quantitative genetics of aging and its manipulation using experimental evolution. Lastly, we will consider research on the cessation of aging, a recently uncovered phenomenon of great interest from an evolutionary standpoint.Evolutionary theories were first proposed in the late 1940s. We can distinguish therein the following four concepts:

- Antagonistic pleiotropy,
- Mutations accumulation,
- Selective shadow, and
- Somatic disposition.

Antagonistic pleiotropy

Medawar's theory was critiqued and later further developed by George C. Williams in 1957 who noted that senescence may be causing many deaths even if animals are not dying of old age. He began his hypothesis with the idea that aging can cause earlier senescence due to the competitive nature of life. Even a small amount of aging can be fatal, hence, natural selection does indeed care and aging is not cost-free. He eventually proposed his own hypothesis called "antagonistic pleiotropy". Pleiotropy, alone means one mutation that causes multiple effects on phenotype. Antagonistic pleiotropy,

on the other hand, deals with one gene that creates two traits with one being beneficial and the other detrimental. In essence, this refers to genes that offer benefits early in life, but later accumulate a cost. In other words, antagonistic pleiotropy is when the resultant relationship between two traits is negative: One phenotypic trait positively affects current reproduction at the expense of later accelerated senescence, growth, and maintenance. Antagonistic pleiotropy is permanent unless a mutation that modifies the effects of the primary locus occurs.

Now, a single gene may affect multiple traits. Williams suggested that some traits that increase fitness early in life may also have negative effects later in life. But, because many more individuals are alive at young ages than at old ages, even small positive effects early can be strongly selected for, and large negative effects later may be very weakly selected against. He further suggested the following example: Perhaps a gene codes for calcium deposition in bones, which promotes juvenile survival and will therefore be favored by natural selection; however, this same gene promotes calcium deposition in the arteries, causing negative atherosclerotic effects in old age. Thus, harmful biological changes in old age may result from selection for pleiotropic genes that are beneficial early in life but harmful later on. In this case, selection pressure is relatively high when Fisher's reproductive value is high and relatively low when Fisher's reproductive value is low.

Although antagonistic pleiotropy is a prevailing theory today, this is largely by default, and has not been well verified. Research has shown that this is not true for all genes and may be thought of as partial validation of the theory, but it cuts the core premise: that genetic trade-offs are the root cause of aging.

In breeding experiments, Michael R. Rose selected fruit flies for long lifespan. Based on antagonistic pleiotropy, he expected that this would surely reduce their fertility. His team found that they were able to breed flies that lived more than twice as long as the flies they started with but, to their surprise, the long-lived, inbred flies actually laid more eggs than the short-lived flies. This was another setback for the pleiotropy theory, though Rose maintains it may be an experimental artifact.

Mutations accumulation

Accumulation theories of aging suggest that aging is the bodily decline that results from an accumulation of elements, whether introduced into the body from the environment or resulting from cell metabolism. The first modern theory of mammal aging was formulated by Peter Medawar in 1952 as an evolutionary explanation for biological aging and the associated decline in fitness that accompanies it. It was formed in the previous decade with J. B. S. Haldane and his "selective shadow concept". The concept of selective shadow shifted as the conditions that humans now live in include improved quality of victuals, living conditions, and improved healthcare (including modern medicine such as antibiotics and new medical technology).

A few studies in *Drosophila* have shown that the age of expression of novel deleterious mutations, defines the effects they contribute on mortality. Overall, however; although their frequency increases, their effects and variation decreases with age. The theory explains that, in the case where harmful mutations are only expressed later in life, when reproduction has ceased and future survival is increasingly unlikely, these mutations are likely to be unknowingly passed on to future generations. In this situation the force of natural selection will be weak and insufficient to consistently eliminate the mutations. Medawar posited that over time these mutations would accumulate due to genetic drift and lead to the evolution of what is now referred to as aging.

However, there is no theory that explains how these deleterious mutations affect fitness at different ages and the evolution of senescence. Their idea was that aging was a matter of neglect, as nature is a highly competitive place. Almost all animals die in the wild from predators, disease, or accidents, which lower the average age of death. Therefore, there is not much reason why the body should remain fit for the long haul because selection pressure is low for traits that would maintain viability past the time when most animals would have died anyway. Metabolic diseases come along due to the low demand for physical activity in modern civilization compared to times where humans had to forage in the wild for survival. With the selective shadow now shifted, humans must deal with these new selective pressures.

Mutations happen, and they are completely random with respect to a need in the environment and fitness. They can either be:

- **Beneficial:** in which case they increase an organism's fitness,
- **Neutral:** in which case they do not affect an organism's fitness, or
- **Deleterious:** where they negatively affect an organism's fitness.

Previous experiments have shown that most mutation accumulations are deleterious, and just a few are beneficial. Mutations of genes that interact with one another during the developmental process create biological and, thus, phenotypical diversities. Mutations are genetic information that is expressed among organisms via gene expression (the translation of genetic information into a phenotypic character). Evolution is the change in a heritable trait in a population across generations since mutations generate variations in the heritable traits; they are considered the raw material for evolution. Therefore, beneficial mutation accumulations during the developmental processes could generate more phenotypic variations, which increases their gene frequency and affect the capacity of phenotypic evolution.

The onset of Huntington's disease (HD) is (on average) at age 45 and is invariably fatal within 10–20 years. Haldane wondered why the dominant mutation that causes this neurological disease remained in the population, and why natural selection had not eliminated it. He then assumed that, in human prehistory, few survived until age 45. Since few were alive at older ages and their contribution to the next generation was therefore small relative to the large cohorts of younger age groups, the force of selection against such late-acting deleterious mutations was correspondingly small. Therefore, a genetic load of late-acting deleterious mutations could be substantial at mutation–selection balance. Natural selection can support lethal and harmful alleles, if their effects are felt after reproduction. This concept came to be known as the 'selection shadow'.

Medawar formalized this observation in his mutation accumulation theory of aging. "*The force of natural selection weakens with increasing age—even in a theoretically immortal population, provided only that it is exposed to real hazards of mortality. If a genetic disaster... happens late enough in individual life, its consequences may be completely unimportant*". Age-independent

hazards such as predation, disease, and accidents, called 'extrinsic mortality', mean that even a population with negligible senescence will have fewer individuals alive in older age groups.

Selective shadow

Selection shadow is one of the evolutionary theories of aging based on the presumption that selection of an individual generally decreases once they essentially pass the sexual mature phase. As a result, this forms a shadow without the account of sexual fitness, which is no longer considered as an individual ages. This supports the idea, first introduced by Medawar and Haldane, that the force of natural selection declines as a function of age. To quote W. D. Hamilton (1966):

> *"The key conceptual insight that allowed Medawar, Williams, and others to develop the evolutionary theory of aging is based on the notion that the force of natural selection, a measure of how effectively selection acts on survival rate or fecundity as a function of age, declines with progressive age."*

Medawar developed a model that highlights this, showing the decrease in the survival rate of a population as an individual ages, however, the reproduction rate stays constant. The reproduction probability typically peaks during sexual maturity and decreases as an individual ages, while the rest of the population decreases with age as they enter the selection shadow. The model also supports Medawar's theory that due to dangerous and unpredicted conditions in the environment (such as diseases, climate changes, and predators), many individuals die not too long after sexual maturation. Consequently, the probability of an individual surviving and suffering from age-related effects is relatively low.

In the same way, many beneficial mutations are selected against if they have a positive effect on an individual later on in life. For instance, if a beneficial or deleterious mutation occurs only after an individual's reproductive phase, then, it will not affect fitness, which therefore cannot be selected against.

Subsequently, these later mutations and effects are considered to be in the "shadow region" of selection."

Somatic disposition

The somatic mutation theory of aging states that accumulation of mutations in somatic cells is the primary cause of aging. A comparison of somatic mutation rate across several mammal species found that the total number of accumulated mutations at the end of lifespan was roughly equal across a broad range of lifespans. This strong relationship between somatic mutation rate and lifespan across different mammalian species suggests that evolution may constrain somatic mutation rates, perhaps by selection acting on different DNA repair pathways.

The disposable soma theory of aging was proposed in 1977 by Thomas Kirkwood. It suggests that aging occurs due to a strategy in which *"an individual only invests in maintenance of the soma for as long as it has a realistic chance of survival"*. In other words, the body must budget the resources available to it. It uses resources derived from the environment for metabolism, reproduction, and repair and maintenance, and it must compromise when there is a finite supply of resources. The theory states that this compromise causes the body to reallocate energy to the repair function that causes the body to gradually deteriorate with age. A species that uses resources more efficiently will live longer and, therefore, be able to pass on genetic information to the next generation. The demands of reproduction are high, so less effort is invested in repair and maintenance of somatic cells, compared to germ line cells, in order to focus on reproduction and species survival.

A caveat to this theory suggests that this reallocation of energy is based on time instead of limiting resources. This concept focuses on the evolutionary pressure to reproduce in a set optimal time period that is dictated by age and ecological niche. The way that this is successful is through the allocation of time and energy in damage repair at the cellular level, resulting in an accumulation of damage and a decreased lifespan relative to organisms with longer gestation. This concept stems from a comparative analysis of genomic stability in mammalian cells.

One opposing argument is based on the effect of calorie restriction, which lengthens life. However, dietary restriction has not been shown to increase lifetime reproductive success (fitness), because when food availability is lower, reproductive output is also lower. Moreover, calories are not the only resource of possibly limited supply to an organism that could have an effect on multiple dimensions of fitness.

Mechanistic theories of aging

The mechanistic theories include system theories (three concepts), molecular theories (six concepts), and cellular theories (five concepts):

- Systems theories,
- Molecular theories, and
- Cellular theories

Systems theories

They include:

- Immunologic,
- Neuroendocrinal control mechanisms alterations, and
- Rate-of-living (also an error theory).

Immunologic approach

Neuroendocrinal control mechanisms alterations

Rate-of-living

While there may be some validity to the idea that, for various types of specific damage that are by-products of metabolism, all other things being equal, a fast metabolism may reduce lifespan. Actually, one of the earliest aging theories was the 'rate-of-living hypothesis' described by Raymond Pearl in 1928 (based on earlier work by Max Rubner), which states that fast basal metabolic rate corresponds to short maximum lifespans. In general, this theory does not adequately explain the differences in lifespan either

within, or between, species. When correcting for the effects of body size and phylogeny, it was shown that *metabolic rate does not correlate with longevity in mammals or birds*.

Molecular theories

They include phenomena such as:

- Codon restriction,
- Dysdifferentiation,
- Error catastrophe,
- Gene regulation (also a gene expression theory),
- Genetic material (DNA) damage accumulation. and
- Somatic mutation (also a genetic theory).

Codon restriction

Dysdifferentiation

Error catastrophe

Gene regulation

Genetic material (DNA) damage accumulation

DNA damage is distinctly different from mutation, although both are types of error in DNA. DNA damage is an abnormal chemical structure in DNA, while a mutation is a change in the sequence of standard base pairs. DNA damage has been one of the major causes in diseases related to aging. The stability of the genome defined by the cells machinery of repair, damage tolerance, and checkpoint pathways counteracts DNA damage. One hypothesis proposed by physicist Gioacchino Failla in 1958 is that damage accumulation to the DNA causes aging. The hypothesis was developed soon by another physicist Leó Szilárd. This theory has changed over the years as new research has discovered new types of DNA damage and mutations, and several theories of aging argue that DNA damage with or without mutations causes aging.

The theory that DNA damage is the primary cause of aging is based, in part, on evidence in human and mouse that inherited deficiencies in DNA repair

genes often cause accelerated aging. There is also substantial evidence that DNA damage accumulates with age in mammalian tissues, such as those of the brain, muscle, liver, and kidney. One expectation of the theory is that, among species with differing maximum life spans, the capacity to repair DNA damage should correlate with lifespan.

The first experimental test of this idea was by Hart and Setlow who measured the capacity of cells from seven different mammalian species to carry out DNA repair. They found that nucleotide excision repair capability increased systematically with species longevity. This correlation was striking and stimulated a series of 11 additional experiments in different laboratories over succeeding years on the relationship of nucleotide excision repair and life span in mammalian species (see the review by Bernstein and Bernstein). In general, the findings of these studies indicated a good correlation between nucleotide excision repair capacity and life span. Further support for the theory that DNA damage is the primary cause of aging comes from study of Poly ADP ribose polymerases (PARPs). PARPs are enzymes that are activated by DNA strand breaks and play a role in DNA base excision repair. Burkle *et al.* reviewed evidence that PARPs, and especially PARP-1, are involved in maintaining mammalian longevity. The life span of 13 mammalian species correlated with polyADP ribosylation capability measured in mononuclear cells. Furthermore, lymphoblastoid cell lines from peripheral blood lymphocytes of humans over age 100 had a significantly higher poly(ADP-ribosylation capability than control cell lines from younger individuals.

Evidence for the theory that DNA damage is the fundamental cause of aging was first reviewed in 1981 and proposed in 2021 as the underlying cause of aging because of the mechanistic link of DNA damage to nearly every aspect of the aging phenotype. DNA damage-induced epigenetic alterations, such as DNA methylation and many histone modifications, appear to be of particular importance to the aging process.

With respect to specific types of chemical damage caused by metabolism, it is suggested that damage to long-lived biopolymers, such as structural proteins or DNA, caused by ubiquitous chemical agents in the body such as oxygen and sugars, are in part responsible for aging. The damage can include:

- Breakage of biopolymer chains;
- Cross-linking of biopolymers; or
- Chemical attachment of unnatural substituents (haptens) to biopolymers.

Just like DNA mutation and expression have phenotypic effects on organisms, DNA damage and mutation accumulation also have phenotypic consequences in older humans. Damage to macromolecules such as DNA, RNA, and proteins along with the deterioration of tissues and organs are the basis of aging. Species-specific rates of aging are due to deleterious changes which manifest after the reproductive phase. Mitochondrial DNA (mtDNA) regulates cellular metabolism, apoptosis and oxidative stress control. Damage to mtDNA is therefore another contributing factor to phenotypes related to aging. Neurodegeneration and cancer are two factors that manifest with DNA damage; therefore, we need to understand the change in the association between DNA damage and DNA repair as we age in order to be aware of age-related diseases and develop lifestyles that could possibly promote a healthy life span.

The DNA damage theory of aging postulates that DNA damage is ubiquitous in the biological world and is the primary cause of aging. The theory is based on the idea that aging occurs over time due to the damage of the DNA. As an example, studies of mammalian brain and muscle have shown that DNA repair capability is relatively high during early development when cells are dividing mitotically, but declines substantially as cells enter the post-mitotic state.

The effect of reducing expression of DNA repair capability is increased accumulation of DNA damage. This impairs gene transcription and causes the progressive loss of cellular and tissue functions that define aging. As a response to DNA damage, one of the responses triggered by oxidative stress is the activation of the p53. The p53 protein binds to DNA, then stimulates the production of a p21, which is also known as cyclin-dependent kinase inhibitor 1. This ensures that the cell cannot enter the next stage of cell division unless the DNA damage is repaired. However, the p21 cells can trigger apoptosis. Apoptosis (or programmed cell death) is associated with gradual degradation of the immune system, skeletal muscle, and aging-associated malfunction.

Now, under normal aerobic conditions, approximately 4% of the oxygen metabolized by mitochondria is converted to superoxide ion, which can subsequently be converted to hydrogen peroxide, hydroxyl radical and eventually other reactive species including other peroxides and singlet oxygen, which can, in turn, generate free radicals capable of damaging structural proteins and DNA. Certain metal ions found in the body, such as copper and iron, may participate in the process. [Note: In Wilson's disease (WD), a hereditary defect that causes the body to retain copper, some of the symptoms resemble accelerated senescence.] These processes termed oxidative stress (OS) are linked to the potential benefits of dietary polyphenol antioxidants, for example in coffee and tea. However their typically positive effects on lifespans when consumption is moderate have also been explained by effects on autophagy, glucose metabolism, and AMPK.

Sugars, such as glucose and fructose, can react with certain amino acids such as lysine and arginine and certain DNA bases, such as guanine, to produce sugar adducts, in a process called glycation. These adducts can further rearrange to form reactive species, which can then cross-link the structural proteins or DNA to similar biopolymers or other biomolecules such as non-structural proteins. People with diabetes, who have elevated blood sugar, develop senescence-associated disorders much earlier than the general population, but can delay such disorders by rigorous control of their blood sugar levels. There is evidence that sugar damage is linked to oxidant damage in a process termed glycoxidation. Free radicals can damage proteins, lipids or DNA. Glycation mainly damages proteins. Damaged proteins and lipids accumulate in lysosomes as lipofuscin. Chemical damage to structural proteins can lead to loss of function; for example, damage to collagen of blood vessel walls can lead to vessel-wall stiffness and, thus, hypertension, and vessel wall thickening and reactive tissue formation (atherosclerosis); similar processes in the kidney can lead to kidney failure. Damage to enzymes reduces cellular functionality. Lipid peroxidation of the inner mitochondrial membrane reduces the electric potential and the ability to generate energy. It is probably no accident that nearly all of the so-called "accelerated aging diseases" are due to defective DNA repair enzymes.

Lastly, it is believed that the impact of alcohol on aging can be partly explained by alcohol's activation of the HPA axis, which stimulates glucocorticoid secretion, long-term exposure to which produces symptoms of aging.

Somatic mutation

Cellular theories

They include these other phenomena:

- Apoptosis,
- Free radicals (also an error theory, and a biochemical theory),
- Reproductive cell cycle,
- Stem cells (also a genetic theory), and
- Telomeres(also a genetic theory).

Apoptosis

Free radicals theory

Free radicals are reactive molecules produced by cellular and environmental processes. They can damage the elements of the cell such as the cell membrane and the DNA and cause irreversible damage. The free-radical theory of aging proposes that this damage cumulatively degrades the biological function of the cells and impacts the process of aging.

The idea that free radicals are toxic agents was first proposed by Rebeca Gerschman and colleagues in 1945, but came to prominence in 1956 when Denham Harman proposed the free-radical theory of aging. The theory posits that free radicals produced by dissolved oxygen, radiation, cellular respiration and other sources cause damage to the molecular machines in the cell and gradually wear them down. (this is also known as oxidative stress). Aging results from the damage generated by reactive oxygen species (ROS) – the small, highly reactive, oxygen-containing molecules that can damage a complex of cellular components such as fat, proteins, or DNA. They are naturally generated in small amounts during the body's metabolic reactions. These conditions become more common as humans grow older and include diseases related to aging, such as dementia, cancer, and heart

disease. The amount of free radicals in the cell can be reduced with help of antioxidants. But there is a problem that some free radicals are used by organisms as signal molecules and a hyperactive general reduction of free radicals causes more harm than good to the organism. Some time ago, the idea of slowing aging using antioxidants was very popular but now high doses of antioxidants are considered harmful. At present, some scientists try to invent approaches of local suppression of free radicals only in certain places of cells. The efficiency of such approaches remains unclear.

There is substantial evidence to back up this theory. Old animals have larger amounts of oxidized proteins, DNA, and lipids than their younger counterparts.

Reproductive-cell cycle theory

It suggests that aging is regulated by changes in hormonal signaling over the lifespan.

Stem cells theories

Damage and error accumulation in genetic material is always a problem for systems regardless of the age. The stem cells theory of aging postulates that the aging process is the result of the inability of various types of stem cells to continue to replenish the tissues of an organism with functional differentiated cells capable of maintaining that tissue's (or organ's) original function. The number of stem cells in young people is very much higher than in older people and thus creates a better and more efficient replacement mechanism in the young contrary to the old. In other words, *aging is not a matter of the increase in damage, but a matter of failure to replace it* due to a decreased number of stem cells. Stem cells decrease in number and tend to lose the ability to differentiate into progenies or lymphoid lineages and myeloid lineages.

Maintaining the dynamic balance of stem cells pools requires several conditions. Balancing proliferation and quiescence along with homing and self-renewal of hematopoietic stem cells are favoring elements of stem cell pool maintenance while differentiation, mobilization and senescence

are detrimental elements. These detrimental effects will eventually cause apoptosis.

There are also several challenges when it comes to the therapeutic use of stem cells and their ability to replenish organs and tissues. First, different cells may have different lifespans even though they originate from the same stem cells (see T-cells and erythrocytes), meaning that aging can occur differently in cells that have longer lifespans as opposed to the ones with shorter lifespans. Also, continual effort to replace the somatic cells may cause exhaustion of stem cells (see also Chapter 11).

- **Hematopoietic stem cells aging:** Hematopoietic stem cells (HSCs) regenerate the blood system throughout life and maintain homeostasis. DNA strand breaks accumulate in long term HSCs during aging. This accumulation is associated with a broad attenuation of DNA repair and response pathways that depends on HSC quiescence.
- **Hematopoietic stem cells diversity aging:** A study showed that the clonal diversity of stem cells that produce blood cells gets drastically reduced around age 70 to a faster-growing few, substantiating a novel theory of aging which could enable healthy aging.
- **Hematopoietic mosaic loss of the Y-chromosome:** A 2022 study showed that blood cells' loss of the Y-chromosome in a subset of cells, called 'mosaic loss of chromosome Y' (mLOY) and reportedly affecting at least 40% of 70 years-old men to some degree, contributes to fibrosis, heart risks, and mortality in a causal way.

Telomeres theory

Telomeres are recurring nucleotide sequences that protect the ends of chromosomes; they are sensitive to oxidative stress (OS) and degrade during chromosomal replication. Telomerase is a ribonucleotide protein that helps repair and replace degraded telomeres. However, telomerase fails us as we age; it becomes less able to repair telomeres, and our whole body starts falling apart. This means that our cells can no longer divide or divide with errors, and some believe that this contributes to the process of aging.

New research has also shown that there is an association between telomere shortening and mitochondrial dysfunction. Nevertheless, over-expression of

telomerase increases the chances of cancer. If telomeres stay in repair, there is a greater chance of longevity, but there is also more cell division and a greater chance of mutation, which could result in cancer. Therefore, a long-lived cell is just a time bomb! Enhancing telomerase activity is, therefore, not a solution; it only allows the cells to live longer. Naked mole rats have high telomerase activity, they live longer, and were thought by some to never get cancer; and therefore possibly be an exception to this hypothesis. However, naked mole rats do get cancer.

Classification of the theories of aging

The several theories of aging can be classified within four categories (acronym **PEGB**), which are briefly presented in Table 12.1 below and further elaborated upon in this chapter. Any of these theories is not exclusive and a combination of them may be able to explain aging in any given aging circumstance.

Table 12.1 – Theories of aging, principles, and features

Theories of aging	Principles	Features
A. Programmed theories (or Phenoptosis or cellular clock theories)	The human body is designed to age and there is a certain biological timeline that bodies follow.	Aging is an essential and innate part of the biology of humans. It is "programmed" into our body systems. These systems change over time, and these changes cause the symptoms and signs of aging.
1. Programmed longevity	Aging is caused by certain genes switching "on" and "off" over time.	Longevity genes.
2. Endocrine	Regular changes in hormones control aging.	
3. Immunological	The process of human aging is a mild and generalized form of a prolonged autoimmune phenomenon.	The immune system is programmed to decline over time, leaving people more susceptible to diseases and aging.

B. Error theories (or "simple deterioration" or "fundamental limitation")	Aging is caused by environmental damage to the body's systems.	Damage accumulates over time.
1. Wear-and-tear	Cells and tissues simply wear out.	
2. Rate-of-living	People (and other living organisms) have a finite number of breaths, heartbeats, or other measures, and that they will die once they would have used those up.	The faster an organism uses oxygen, the shorter it lives. Slowing one's metabolism does not enhance lifespan.
3. Cross-linking	Aging is due to the DNA damage of "free radicals" acting on cells.	Cross-linked proteins accumulate and slow down the body's processes.
4. Free radicals	Free radicals in the environment cause damage to cells.	Cell damage eventually impairs their function.
C. Genetic theories	Genetics plays a major role in aging.	In one animal study, lifespan was extended by 35%.
1. Somatic DNA damage	Genetic mutations cause cells to malfunction.	
2. Longevity genes	Specific genes that help a person live longer.	
3. Cell senescence.	Process by which cells deteriorate over time.	
4. Telomeres	Structures on the end of DNA that eventually are depleted.	Depletion results in cells ceasing to replicate.
5. Stem cells	Cells that can become any type of cell in the body.	Hold promise to repair damage caused by aging.
D. Biochemical theories	Body is continually undergoing complex biochemical reactions.	Some chemical reactions cause damage and, ultimately, aging.
1. Free radicals	Unstable oxygen molecules that can damage cells.	

2. Protein cross-linking	Excess sugars in the bloodstream can cause protein molecules to literally stick together.	
3. DNA repair	Systems in the body that repair DNA seem to become less effective in older people.	
4. Heat shock proteins	Proteins that help cells survive stress are present in fewer numbers in older people.	
5. Hormones	Change as we age.	Cause many shifts in organ systems and other functions.

Programmed theories of aging

Programmed theories of aging posit that aging is adaptive, normally invoking selection for evolvability or group selection. They assert that the human body is "designed" to age so that aging is a natural phenomenon that has been "programmed" into our bodies, following a certain biological timeline. In effect, we are "designed" to age! But, casting aside philosophical or/ and religious arguments, all such theories shy away from identifying the "programmer(s)" or "designer(s)".

Within such theories, one may distinguish those that assert that the genetic switching on and off over time causes aging, without specifying the correspondence between the number and frequency of the switchings with the corresponding aging parameters. Others argue that regular changes in hormones control aging. Again, the identity and number of the effecting hormones and the degree of control on aging has gone silent. Still others assert that aging is caused by the time accumulation of environmental damage to the body's systems without elaborating on the nature and types of damages affecting which body systems and in which manner has likewise not been discussed.

Programmed maintenance theories

Theories, such as Weismann's "programmed death" theory, suggest that deterioration and death due to aging are a purposeful result of an organism's evolved design, and are referred to as theories of programmed aging or adaptive aging.

By contrast, the programmed maintenance theory based on evolvability suggests that the repair mechanisms are controlled by a common control mechanism capable of sensing conditions, such as calorie restriction, and may be responsible for lifespan in particular species. In this theory, the survival techniques are based on control mechanisms instead of individual maintenance mechanism, which is seen in the non-programmed theory of mammal aging.

A non-programmed theory of mammal aging states that different species possess different capabilities for maintenance and repair. Longer-lived species possess many mechanisms for offsetting damage due to causes such as oxidation, telomere shortening, and other deteriorative processes. Shorter-lived species, having earlier ages of sexual maturity, have less need for longevity and, thus, did not evolve or retain the more-effective repair mechanisms. Damage therefore accumulates more rapidly, resulting in earlier manifestations and a shorter lifespan. Since there are a wide variety of aging manifestations that appear to have very different causes, it is likely that there are many different maintenance and repair functions.

Error theories of aging

These theories assert that, over time, cells and tissues simply wear out. One variation of these theories includes "wear-and-tear theory", which asserts that cells and tissues simply wear out. The manner and duration of the wearing and tearing has been left aside. In a second variation, called the "rate-of-living theory", aging is inversely related to the organism consumption of oxygen, that is, the faster an organism uses oxygen, the shorter it lives. However, understandably because of the underlying difficulties, this relationship has not been quantified in terms of the amount of oxygen available, its rate of consumption, and the proximate rate of aging. A third variation, called

"cross-linking theory", posits that cross-linked proteins accumulate and slow down the body's processes. Again, the quantification of the number and amount of cross-links to the number and quality of the affected body processes has been left aside. A fourth variation named "free radicals theory" asserts that free radicals in the environment cause damage to cells, eventually impairing their function and causing aging. The quantification of the relationship between the quality and quantity of identified radicals to the nature and amount of damage to cells has also been overlooked.

Genetic theories of aging

Genetics plays a major role in aging. For example, in a mice experiment, Baker *et al.* (2018) found that removing cells containing certain genes from the organs extended the lifespan of the animals by as much as 35%. Whether a similar effect could be applied to humans is not known. Variations on this theory are based on known facts. Thus, in one variation called "somatic DNA damage theory", genetic mutations are known to cause cells to malfunction. While such a causal effect is known, the nature and number of such mutations and the correlated cell malfunction(s) have not been elaborated upon. A second variation, called the "longevity genes theory", is based on so-called "longevity genes", which are specific genes that help lengthen lifespan. However, the identity and number of such genes and the mechanism of their action on aging remains unclear. A third variation, called "cell senescence theory", rests on the process of senescence by which cells deteriorate over time. Again, the characteristics, properties, and effects of this phenomenon on aging are not fully understood. The fourth variation, called "telomeres shortening theory", is based on the known effect of telomeres (structures on the end of genes) shortening on cell replication (see Sidebar 10.1 for more details). Finally, in a fifth variation, called "stem cells theory", stem cells (cells that can become any cell type in the body) hold promise to repair the damage caused by aging (see Sidebar 10.2).

Biochemical theories of aging

These theories rest on the known fact that no matter what genes we may have inherited, our body is continually undergoing complex biochemical

reactions, some of which causing damage and, ultimately, aging in the body. The study of these reactions should help understand how the body changes as it ages. There are five important concepts in the biochemistry of aging on which are based various theories of aging. In one, called "free radicals theory", free radicals (unstable oxygen molecules) can damage cells. Which free radicals, what mechanism(s) and the extent of the damage caused are being studied. In a second variation, called "protein cross-linking theory", protein cross-links produce excess sugars in the bloodstream that can cause protein molecules to literally stick together leading to aging. The identity, number and strength of the cross-links, and the mechanism(s) leading to aging are being researched. In a third variation, called "DNA repair theory", the systems in the body that repair DNA seem to become less effective with age. However, the particulars of the several body systems that repair DNA and the mechanism(s) and effectiveness of their remedial action need to be further elucidated. In a fourth variation, called "heat shock proteins theory", such proteins help cells survive stress and diminish in numbers with age. The full identity of such proteins, their number, and mechanisms of action, and diminution with time require further investigations. Lastly, in the fifth variation, called "age-changing hormonal theory", hormones cause many shifts in organ systems and other functions. The identity, number, mechanism(s), and effectiveness of such proteins need to be further studied.

Damage-related factors

In the above theories, several damage-related factors have been mentioned. More specifically, these are:

DNA damage theory of aging

DNA damage is thought to be the common basis of both cancer and aging, and it has been argued that intrinsic causes of DNA damage are the most important causes of aging. We can distinguish the following:

- **Genetic damage:** Aberrant structural alterations of the DNA (that is, DNA damage proper) cause the cells to stop dividing or induce

apoptosis, often affecting stem cell pools and, therefore, hindering regeneration.

- **Mutations** (that is, changes in the DNA sequence): They can cause abnormal gene expression. However, lifelong studies of mice suggest that most mutations happen during embryonic and childhood development when cells divide often, as each cell division is a chance for errors in DNA replication.
- **Epimutations** (the methylation of gene promoter regions or alterations of the DNA scaffolding which regulate gene expression).
- **Genetic instability:** With respect to the annual DNA loss in heart muscles, it is approximately 3.3% in dogs and 0.6% in humans - numbers that are are close to the ratio of the maximum longevities of the two species (120 years vs. 20 years, a 6/1 ratio). The comparative percentage is also similar between dogs and humans for yearly DNA loss in the brain and lymphocytes. As stated by Bernard L. Strehler, "... *genetic damage (particularly gene loss) is almost certainly (or probably the) central cause of aging*".
- **Mitochondrial DNA mutations:** Mice studies have shown that increased levels of somatic mtDNA mutations directly can cause a variety of aging phenotypes. They can lead to respiratory-chain-deficient cells and, thence, to apoptosis and cell loss. A common (perhaps doubtful) assumption is that mitochondrial mutations and dysfunction lead to increased generation of reactive oxygen species (ROS).
- **Mitochondrial activity damage:** Free radicals produced by mitochondrial activity damage cellular components, leading to aging.
- **DNA oxidation and calorie restriction:** Caloric restriction reduces 8-OH-dG DNA damage in organs of aging rats and mice. Thus, reduction of oxidative DNA damage is associated with a slower rate of aging and increased lifespan. In a 2021 review article, Vijg stated the following: "*Based on an abundance of evidence, DNA damage is now considered as the single most important driver of the degenerative processes that collectively cause aging*".

DNA damage causes the cells to stop dividing or induces apoptosis, often affecting stem cell pools and therefore hindering regeneration. However,

lifelong studies of mice suggest that most mutations happen during embryonic and childhood development, when cells divide often, as each cell division is a chance for errors in DNA replication.

Waste accumulation

A buildup of waste products in cells presumably interferes with metabolism. For example, a waste product called lipofuscin is formed by a complex reaction in cells that binds fat to proteins. Autophagy induction can enhance the clearance of toxic intracellular waste associated with neurodegenerative diseases; it has been comprehensively demonstrated to improve lifespan in yeast, worms, flies, rodents and primates. The situation, however, has been complicated by the identification that autophagy up-regulation can also occur during aging.

Wear-and-tear

The general idea that changes associated with aging are the result of chance damage that accumulates over time.

Errors accumulation

This is the idea that aging results from chance events that escape proofreading mechanisms, which gradually damages the genetic code.

Heterochromatin loss

A model of aging.

Cross-linkage

The idea that aging results from the accumulation of cross-linked compounds that interfere with normal cell function.

Free-radicals damage

Free radicals or, more generally, reactive oxygen species or oxidative stress, create damage that may give rise to the symptoms of aging. The effect of calorie restriction may be due to the increased formation of free radicals within the mitochondria, causing a secondary induction of increased antioxidant defense capacity.

Mitochondrial activity

Free radicals produced by mitochondrial activity damage cellular components, leading to aging.

DNA oxidation and calorie restriction

Calorie restriction reduces 8-OH-dG DNA damage in organs of aging rats and mice. Thus, reduction of oxidative DNA damage is associated with a slower rate of aging and increased lifespan.

Conclusions and take-aways

- Aging is a complex interaction of genetics, chemistry, physiology, and behavior. More than 300 different theories of aging have been posited to explain its nature and causes. They fall into two broad categories: Evolutionary and mechanistic.
- Evolutionary theories of aging primarily explain why aging happens, but do not concern themselves with the molecular mechanism(s) that drive the process. They all rest on the basic mechanisms that the force of natural selection declines with age.
- Mechanistic theories can be divided into theories that propose that aging is "programmed", and "damage accumulation theories" that propose aging to be caused by specific molecular changes occurring over time.
- Evolutionary theories can be explained briefly by: Mutations accumulation, somatic disposition, antagonistic pleiotropy, and selective shadowing.

- Systems theories include: Immunologic approach, rate-of-living (an error theory), and neuroendocrinal control mechanisms alterations.
- Molecular theories include phenomena such as: Gene regulation (gene expression), codon restriction, error catastrophe, somatic mutation, genetic material (DNA) damage accumulation, and dysdifferentiation.
- Cellular theories can be categorized as: Telomere theory, free radical theory, apoptosis, stem cell theory, and reproductive cell cycle theory.
- There are now dozens of theories of aging to explain this inevitable fact of being human. These have been classified in four categories (acronym **PEGB**).
- Programmed theories of aging assert that the human body is "designed" to age so that aging is a natural phenomenon that has been "programmed" into our bodies, following a certain biological timeline. Within such theories, one may distinguish those that assert that the genetic switching on and off over time causes aging. Others argue that regular changes in hormones control aging. Still others assert that aging is caused by the time accumulation of environmental damage to the body's systems.
- Error theories of aging assert that, over time, cells and tissues simply wear out. Variation of these theories include: "wear-and-tear theory" in which cells and tissues simply wear out; "rate- of-living theory" in which aging is inversely related to the organism consumption of oxygen; "cross-linking theory" which posits that cross-linked proteins accumulate and slow down the body's processes; and "free radicals theory" which asserts that free radicals in the environment cause damage to cells, eventually, impairing their function and causing aging.
- Genetics plays a major role in aging. Variations on this theory include the: "somatic DNA damage theory" in which genetic mutations are known to cause the malfunctioning of cells and subsequently aging; "longevity genes theory", based on so-called "longevity genes", which are specific genes that help lengthen lifespan; "cell senescence theory", which rests on the process of senescence by which cells deteriorate over time and cause aging; "telomeres shortening theory", which is based on the known effect of telomeres shortening on cell replication; and "stem cells theory", which holds promise to repair the damage caused by aging.

- Biochemical theories of aging rest on the known fact that no matter what genes we may have inherited, our body is continually undergoing complex biochemical reactions, some of which causing damage and, ultimately, aging in the body. There are five important concepts: "free radicals theory", which can damage cells; "protein cross-linking theory", which produce excess sugars in the bloodstream that can cause protein molecules to literally stick together leading to aging; "DNA repair theory" in which the systems in the body that repair DNA seem to become less effective with age; "heat shock proteins theory", which help cells survive stress and diminish in numbers with age; and "age-changing hormonal theory" in which hormones cause many shifts in organ systems and other functions.
- DNA damage is thought to be the common basis of both cancer and aging and the most important cause of aging, including the following types: Genetic damage in which aberrant structural alterations of the DNA cause the cells to stop dividing or induce apoptosis, often affecting stem cell pools and, therefore, hindering regeneration; mutations that can cause abnormal gene expression; epimutations; genetic instability; mitochondrial DNA mutations which can lead to respiratory-chain-deficient cells and, thence, to apoptosis and cell loss; mitochondrial activity damage in which the free radicals produced damage cellular components and lead to aging; and DNA oxidation and calorie restriction. DNA damage is now considered as the single most important driver of the degenerative processes that collectively cause aging".

Theories of aging affect efforts to understand and find treatments for age-related conditions:

- Those who believe in the idea that aging is an unavoidable side effect of some necessary function (antagonistic pleiotropy or disposable soma theories) logically tend to believe that attempts to delay aging would result in unacceptable side effects to the necessary functions. Altering aging is therefore "impossible" and study of aging mechanisms is of only academic interest.
- Those believing in default theories of multiple maintenance mechanisms tend to believe that ways might be found to enhance the operation

of some of those mechanisms. Perhaps they can be assisted by antioxidants or other agents.

- Those who believe in programmed aging suppose that ways might be found to interfere with the operation of the part of the aging mechanism that appears to be common to multiple symptoms, essentially "slowing down the clock" and delaying multiple manifestations. Such effect might be obtained by fooling a sense function. One such effort is an attempt to find a "mimetic" that would "mime" the anti-aging effect of calorie restriction without having to actually radically restrict diet.

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Programmed theories

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Programmed theories of aging

As already indicated in previous chapters, there are numerous theories of aging but no one theory has been entirely accepted. There are two main aging concepts as applied to humans and most other mammals:

- **Programmed aging theories** (also known as 'adaptive' or 'active' aging theories): They propose that mammals purposely deteriorate with age because an internally limited life span provides evolutionary benefits. They offer a better match to observations, but are based on newer concepts regarding evolution mechanisms.
- **Non-programmed theories** (also known as 'passive' or 'non-adaptive' aging theories): They contend that a limited life span is entirely adverse and that aging is not genetically programmed for the purpose of causing deterioration or death. They have difficulty explaining many observations but are more compatible with older evolutionary mechanics concepts.

Aging is important because most people in developed countries now die of age-related diseases. Understanding, preventing, and treating these diseases requires that we understand the aging process.

Introduction

At their extremes, the wide spectrum of aging theories can be categorized into programmed theories – which imply that aging follows a biological timetable, and error theories – which suggest aging occurs due to cumulative damage experienced by organisms. This chapter will only be concerned with programmed theories of aging.

The idea that aging is a purposeful, programmed series of events is intuitively appealing, particularly since the feasibility of modifying life span by manipulating single genes or pathways can be demonstrated. Nonetheless, the case for a non-adaptive basis of aging is strong and now generally accepted in the field. Focusing on the lack of possible evolutionary beneficial effects, I will briefly review in this chapter why the case for programmed aging is a weak one.

Programmed theories assert that aging is an essential and innate part of the biology of humans. It is natural and "programmed" into our body systems. It is a normal developmental process. In other words, the human body is designed to age along a certain biological timeline that it follows and ends in death. Accordingly, death is an inevitable part of being human, otherwise, we would live forever.

Programmed longevity theory

The body is not a machine

In order to understand this concept, it is important to recognize that the body is indeed *not* a machine for, if it were, why do we not live forever? While we like to compare the human body to a machine, this is not a very good comparison. Unlike a machine, which has only the parts it was built with, the human body continually repairs and replaces cells. Believe it or not, every seven years, 90% of the cells in our body are brand new. The human body is an amazing, open, dynamic, and largely self-renewing system, which is why it ages,... unlike a machine!

Aging is about evolution

Technically, there is really no reason why the human body should "wear out" as long as it can repair and renew itself, which it does, *albeit* not perfectly (as stated earlier, only 90% every seven years). Therefore, something other than time must be at play to cause the inevitable effects of aging.

The programmed theory of aging asserts that *aging and death are necessary parts of evolution, not of biology*. If a species did not have the genetic capacity for aging and death, then, it would not be forced to replicate to survive. Individuals in the species would just keep on living until a climate or other change wiped them all out. The key point here is that if biological individuals live forever, evolution would not exist … and conversely.

Aging is programmed

Since aging is about evolution and not biology, it must be inherent in the organism and not simply a result of environmental factors or disease. That means that aging and death, according to this theory, are not a result of wear-and-tear or exposure, but are a programmed, natural, and necessary part of genetics. In short, we are genetically programmed to age and die.

Evidence supporting the theory

The evidence supporting this theory is that there is not a great deal of variation in lifespan within species. But, why do lifespans vary so much between different species of animals? While, on average, humans die around the age of 80, elephants die at around 70 years old, spider monkeys at around 25 years old, and some whales live almost twice as long as humans.

Some changes can be made based on nutrition, medical care, and other demographic factors, but overall lifespan within species is fairly constant. The programmed theory asserts that if aging were due to wear -and-tear, there would be more variation in lifespan within each species. That said, aging and dying are inevitable, but there are things we can do to improve our chances of living a long and healthy life.

Endocrine (hormone) theory

Some experts believe that hormones, which control the function of organs, could be behind the aging process.

Role of the endocrine system

The endocrine system secretes and controls the hormones that regulate many body processes, including metabolism, use of nutrients, excretion, and reproduction. As we age, these systems become less efficient, leading to changes in our body, such as menopause and andropause. The hormone theory of aging states that these changes eventually cause the effects of aging.

Do hormones cause aging?

There is some evidence to support the hormone theory of aging. In one older mice study, researchers removed the pituitary gland that controls much of the endocrine system. They, then, substituted that gland with supplementation of all of the hormones identified in mice. They found that those mice without a pituitary gland lived longer than the control group of mice that did have the gland. They concluded that the pituitary gland must also excrete another, as yet unknown, hormone that negatively impacts aging.

Research on a variety of organisms has shown that mutations that reduce insulin-like growth factor 1 (IGF-1) result in longer lives. But reducing IGF-1 has inconsistent effects on age-related diseases in humans. It reduces the risks for some but increases them for others. Also, growth hormone stimulates the production of IGF-1, which is a strike against supplementing with human growth hormone (HGH) to stop aging.

An intriguing review of studies, published in *Frontiers in Endocrinology* in 2019, noted that subjects on a calorie-restricted diet had a similar endocrine profile to centenarians, both having a favorable HGH/IGF-1 insulin profile. Restricting caloric intake is only one area in which lifestyle modification has been shown to improve hormonal function. Another example of lifestyle

changes improving hormone function is the observation that weight loss and exercise improve insulin sensitivity.

Hormones for antiaging

The concept that hormones or reduced production of hormones might cause aging has also led some to believe that the right amount of certain hormones could be an antiaging elixir. Growth hormone, which is produced by the pituitary gland, helps maintain tissues and organs throughout life. It is also responsible for childhood growth. Synthetic human growth hormone has been studied in this way and promoted by some as a potential fountain of youth, with proponents hoping it can stave off the decline in tissue growth from aging.

While some adults have growth hormone deficiencies and require supplementation, this condition is rare. Research is indecisive on any other potential benefits of HGH. In addition, the use of HGH has many potential side effects, including swelling of the arms and legs, joint and muscle pain, carpal tunnel syndrome, diabetes, hypertension, and an increased risk of colon cancer.

Another aspect to consider is that growth hormone stimulates IGF-1, but some theories posit that a reduction in IGF-1 is beneficial for aging. In this case, adding growth hormone would produce the opposite of the desired effects.

In summary: Hormonal changes are an important part of aging. Whether they control the pace at which aging occurs or are a consequence of other changes in the body is unknown. It is unlikely that hormone substitution in humans will increase lifespan, and it can even be dangerous. Research does not support the use of HGH for antiaging.

Immunological theory

The immunological theory of aging asserts that the process of human aging is a mild and generalized form of a prolonged autoimmune phenomenon. In other words, aging—which involves a highly complex series of processes—is

suspected to be largely controlled by the immune system. The immune system weakens as an organism ages, making the organism unable to fight infections and less able to destroy old and neoplastic cells. This leads to aging and will eventually to death. This theory of aging was developed by Roy Walford in 1969. According to Walford, incorrect immunological procedures are the cause of the process of aging. He also stated that his optimized health regime would allow him to live to 120, although he died of amyotrophic lateral sclerosis (ALS) at age 79.

Basics of the theory

As humans age, they experience changes to almost all physiological functions, including those related to the immune system. Immune function does indeed decrease with age, which contributes to a whole host of well-known issues among seniors, from increased health risks posed by common infections like a cold or the flu to a greater occurrence of chronic inflammatory diseases, such as gout and some types of arthritis.

While the data suggests that changes in immune system function in the elderly could be a symptom of the aging process, proponents of the immunological theory of aging reverse the relationship and rather believe that common symptoms of aging (like chronic disease) are *caused* by changes in the immune system.

The aging immune system

The immune system changes that seem to accompany old age can have a direct impact on a person's longevity. Not only does our immune system protect us against viruses and bacteria, but it also helps identify and remove cancer cells and toxins. As we get older, the potential for these elements to cause damage in our body increases. What triggers these changes in immune system function (and how they develop and progress) is not fully understood. Research suggests that old age-related immune system dysfunction, sometimes known as "inflammaging", may, at least in part, cause and/or explain some of the known aspects of the aging processes. In

fact, chronic inflammation is believed to contribute to a whole host of chronic and terminal diseases from cancer to Alzheimer's disease.

Cellular changes

The immune system is made up of cells, substances, and organs. Thus:

- **Cells:** As we age, critical cells in the immune system decrease in number and become less functional. Those that are of special interest are the class of white blood cells called lymphocytes, which fight invading bacteria and other foreign cells. Lymphocytes fall into two major classes:
  - B-cells: **They** mature in the **b**one marrow. One of their functions is to secrete antibodies in response to infectious agents or antigens.
  - **T-cells: They** develop in the **t**hymus, which shrinks after puberty. There are two subtypes:
    - Cytotoxic T-cells: They attack infected or damaged cells directly.
    - Helper T-cells: They produce powerful chemicals, called lymphokines, which mobilize other immune system substances and cells.

While the number of T-cells remains fairly constant as we age, the portion of them that proliferate and function declines. Furthermore, T-cells destroyed by cancer treatments (chemotherapy, radiation) take longer to renew in older people than they do in younger people. Beyond making us more prone to common viruses and bacterial infections, such immune system changes can have a much greater impact.

- **Organs:** The thymus, spleen, tonsils, bone marrow, and lymphatic system produce, store, and transport cells and substances such as antibodies, interleukins, and interferon.
- **Substances:**
  - **Interleukins:** They (there are more than 20 of them) serve as messengers, relaying signals that regulate the immune response. Some, like interleukin-6, rise with age, and it is speculated that they interfere with the immune response in some way. Others, like

interleukin-2, which stimulates T-cell proliferation, tend to decrease with age.

When it comes to the immunological theory of aging, research points to increasing immunogenetic *diversification* of human cells as the culprit, as opposed to the shifting numbers of cells. This increased diversification or cell mutation in old age may eventually lead to a failure of cell recognition and the breakdown of certain physiological systems, which ultimately triggers autoimmune-like reactions such as chronic inflammation.

Conclusions and take-aways

- There are numerous theories of aging but no one theory has been entirely accepted. At their extremes, the wide spectrum of aging theories can be categorized into programmed theories.
- The idea that aging is a purposeful, programmed series of events is intuitively appealing. Nonetheless, the case for a non-adaptive basis of aging is strong and now generally accepted.
- Programmed theories assert that aging is an essential and innate part of the biology of humans. Accordingly, death is an inevitable part of being human, otherwise, we would live forever.vm
- The body is not a machine for, if it were, why do we not live forever? Every seven years, 90% of the cells in our body are brand new.
- Aging is about evolution. Technically, there is really no reason why the human body should "wear out" as long as it can repair and renew itself, which it does, *albeit* not perfectly. Something other than time must be at play to cause the inevitable effects of aging.
- The programmed theory of aging asserts that *aging and death are necessary parts of evolution, not of biology*. The key point is that if biological individuals live forever, evolution would not exist … and conversely.
- Since aging is about evolution and not biology, it must be inherent in the organism and not simply a result of environmental factors or disease. Accordingly, aging and death are not a result of wear-and-tear or exposure, but are a programmed, natural, and necessary part of genetics. Some changes can be made based on nutrition, medical care,

and other demographic factors, but overall lifespan within species is fairly constant. In short, we are genetically programmed to age and die.

- The programmed theory asserts that if aging were due to wear -and-tear, there would be more variation in lifespan within each species. That said, aging and dying are inevitable, but there are things we can do to improve our chances of living a long and healthy life.
- The endocrine system secretes and controls the hormones that regulate many body processes, including metabolism, use of nutrients, excretion, and reproduction. The hormone theory of aging states that these changes eventually cause the effects of aging.
- Hormonal changes are an important part of aging. Whether they control the pace at which aging occurs or are a consequence of other changes in the body is unknown. It is unlikely that hormone substitution in humans will increase lifespan, and it can even be dangerous.
- The immunological theory of aging asserts that the process of human aging is a mild and generalized form of a prolonged autoimmune phenomenon.
- Research points to increasing immunogenetic *diversification* of human cells as the culprit, as opposed to the shifting numbers of cells. This may eventually lead to a failure of cell recognition and the breakdown of certain physiological systems, which ultimately triggers autoimmune-like reactions such as chronic inflammation.

Sidebar 13.1 – A primer on the immunological system

Our immune system protects our body from infectious germs. Through highly complex and adaptive processes, a healthy immune system is always at work, protecting us from infections by identifying and destroying harmful microorganisms. Our immune system also helps us build immunity so that when we encounter certain invading germs again, we can fight them faster the next time around, often without even getting sick at all.

Recognizing infectious organisms

To do its job, the immune system must understand the difference between a foreign substance and the cells of our own body. Foreign substances can

be referred to as invaders or pathogens and may include microorganisms, such as bacteria, viruses, fungi, and parasites.

The cells and tissues of our body have proteins called self-antigens. Likewise, living organisms that can cause infections do too, though their antigens are not the same. Our immune system "flags" foreign antigens to quickly target the invading microorganisms and destroy them, protecting us from harm.

White blood cells (leukocytes)

White blood cells (WBCs), the cornerstone of the immune system, are called leukocytes. There are a variety of types of leukocytes, each with unique features that work together to protect us from infections. Depending on the leukocyte, it may help the "seek and destroy" function of the immune system by:

- Rapidly recognizing germs.
- Binding to germs.
- Engulfing and surrounding germs.
- Using chemicals contained within them to destroy germs.

Others take time to recognize and respond to infectious microorganisms.

Macrophages, neutrophils, mast cells, and basophils

Macrophages are leukocytes that circulate throughout the blood and tissues, while neutrophils are leukocytes that circulate in the blood, patrolling for new foreign antigens. Invading germs and microorganisms enter the body through different entry points, such as the nostrils or a cut on the surface of the skin. When these particular leukocytes recognize such infectious threats, they send chemical signals that attract other leukocytes to surround, absorb, and destroy these harmful substances.

Macrophages and neutrophils, along with other leukocytes, such as mast cells and basophils, secrete toxins that damage or kill foreign microorganisms, and then they engulf the cellular debris to "clean it up".

Lymphocytes: B- and T-cells

Lymphocytes are a subset of leukocytes. They take longer than other leukocytes to mount a response to an infection, and they build long-term immunity. The two types of lymphocytes are T-cells and B-cells, and they each have different jobs.

- **B-cells:** They are largely responsible for creating specific proteins called antibodies. The antibodies bind to the antigen on the surface of a foreign invader and mark it for destruction by the immune system. B-cells are useful in protecting us against bacterial infections.
  - **Antibodies**: Our body can produce a variety of antibodies. The different types of antibodies work against various types of infections, such as infections of the skin or of the gastrointestinal system. They bind to antigens, forming an "**immune complex"** that is destroyed by the body's leukocytes and their associated chemicals.
  - **Autoantibodies**: Problems occur when the immune system mistakenly manufactures autoantibodies, which are antibodies that fight our own body. This is the hallmark problem of autoimmune diseases, such as thyroid disease, and it happens when the immune system misidentifies self-antigens—our own cells, tissues, and organs—as foreign bodies.
- **T-cells: They** identify antigens on the surface of our own cells. When a tiny microorganism, such as a virus, enters into our cells, our body's "**major histocompatibility complex" (MHC)** can change the surface of our cells, adding new antigens to our own cells. Passing T-cells are alerted to the presence of the infection within our cell because of these altered antigens. T-cells themselves are useful in destroying viruses and cancer cells.

The MHC is quite sophisticated. A tiny microorganism "hiding" inside a human cell would not be recognized—and can wreak havoc. The MHC can bind to fragments of microorganisms within a human cell and carry these fragments to the surface of the cell so that they can be recognized by their new antigens. The antigen molecules on an infected cell and a responding

T-cell bind together to form "**co-stimulatory molecules"**, which mediate an immune response.

Cytokines and chemokines: Lymphocytes can release chemicals called cytokines, which are signaling molecules. There are several types of cytokines involved in the immune response, including:

- Chemokines.
- Interferons.
- Lymphokines.
- Interleukins.

These immune-mediated cytokines can affect lymphocytes, as well as other nearby cells that are not part of the immune system. In doing so, they stimulate an inflammatory response, as well as repair of tissues that may have been harmed by an infectious microorganism.

Immune complexes and the complement system

Part of the body's immune activity involves the "complement system", which is a group of specialized molecules that work in a variety of ways to destroy invaders. For example. It can form a structure called the "membrane attack complex", which punctures the microorganism to destroy it from within by inserting toxic chemicals.

Autoimmune diseases and allergies

We may have recurrent inflammation and an immune response even at times when we do not have an infection. Autoimmune diseases (such as thyroid disease, lupus, or multiple sclerosis) occur when the body's immune system attacks itself. In some types of hypothyroidism, for example, the body can attack cells that produce thyroid hormone, interfering with the production and function of the hormone.

Allergies are an inflammatory response to a non-threatening substance, like pollen or certain foods. These illnesses can develop at least partially as the result of genetic factors, but it is not always clear why someone develops these conditions.

Our genes are the blueprint for our body's cells and tissues. That same blueprint patterns our immune function, including our T-cell receptors, the type of MHC molecules produced, and our antibody response. An overactive immune system can cause recurrent pain, swelling, and may even cause life-threatening allergic reactions.

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Genetic theories of aging

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Genetic theories of aging

Studies have demonstrated that genetics can play a major role in aging. In one study, when researchers removed cells containing certain genes from the organs of mice, they were able to extend the lifespan of the animals by as much as 35%. The meaning of these experiments for humans is not known, but researchers think that genetics account for much of the variation in aging among people.

Genetic theories of aging propose that aging is programmed within each individual's genes. According to this theory, genes dictate cellular longevity. Programmed cell death, or apoptosis, is determined by a "biological clock" via genetic information in the nucleus of the cell. Genes responsible for apoptosis provide an explanation for cell death, but are less applicable to death of an entire organism. An increase in cellular apoptosis may correlate to aging, but is not a 'cause of death'. Environmental factors and genetic mutations can influence gene expression and accelerate aging.

More recently epigenetics have been explored as a contributing factor. The epigenetic clock, which relatively objectively measures the biological age of cells, is useful tool for testing different anti-aging approaches. The most famous epigenetic clock is Horvath's clock, but now already more accurate analogues have appeared.

Somatic theory

Not treated.

Longevity genes

Longevity genes are specific genes that help a person live longer. In the longevity and healthy aging fields, senescence is the decline in health and function associated with aging.

Cell senescence

Senescence (derived from the Latin word *senex,* meaning "old age") literally means "*the process of growing old*" or "t*he process by which cells deteriorate over time*". It is defined as the period of gradual decline that follows the development phase in an organism's life. So senescence in humans would start sometime in the 20s, at the peak of physical strength, and continue for the rest of life. There are several sub-terms that will often come up, including *cellular senescence* and *organismal senescence*.

Cellular senescence

When cells lose the ability to divide because of DNA damage or a shortening of telomeres, they go through a transformation that results in decline or destruction. The cells either self-destruct (called *apoptosis*) or go into a period of decline (called *senescence*). The ultimate end result is cell death, which is a normal part of a biological functioning and occurs regularly in our body.

Cancer cells are thought to be cells that no longer undergo the process of senescence and instead, continue to replicate and cause problems (and tumors). Researchers are working to develop therapies that cause cancer cells to begin aging more like normal cells, that is, to induce normal senescence in these cells as a way of fighting cancer.

Organismal senescence

While cellular senescence may be a good thing because cells are continually replacing themselves, our body will eventually go into a period of decline known as *organismal senescence. T*his process begins when the body is fully developed and is at peak strength, and continues for the rest of life. The accumulated damage to the body begins to interfere with the body's ability to function, causing the effects often associated with aging.

Longevity, healthy aging, and senescence

We cannot yet interrupt the process of senescence as it relates to humans, but some scientists argue that we will never be able to stop it although we can slow it down. The goal, then, is to slow the process of senescence as much as possible by doing the following:

- **Eating a healthy diet:** To provide the body with the nutrients it needs to repair damage and fight off future damage.
- R**educing stress:** To prevent long-term damage from over-exposure to the stress hormones and the physiological state they create.
- A**voiding over exposure to damaging substances (electromagnetic radiation, pollution, toxins):** To help limit the amount of damage to the body's tissues.
- **Exercising more:** To help the body build strong tissues that resist damage.
- **Preventing disease: To k**eep blood pressure, weight, and other risk factors under control.

Telomeres

Each strand of DNA is made up of chromosomes, which carry genetic information. They look like X's (except for the one that determines male sex characteristics, which looks like a Y). At the tip of each point of the X (or Y) is a small structure called a telomere. Telomeres stabilize our DNA as cells divide.

Scientists have discovered that telomeres get shorter with chronological age. Also, shorter telomeres are linked to diseases like dementia and cancer.

The Hayflick's limit of cell division

All cells have a programmed lifespan by which they are synthesized, multiply, and eventually undergo apoptosis (cell death) when they are no longer functional. (It often helps to think of cellular replication as an old-fashioned photocopy machine: the more a cell copies itself, the more blurry and misaligned the image becomes. Over time, the genetic material of the cell (DNA) begins to fracture and the cell itself becomes a pale copy of the original. When this happens, programmed cell death allows a new cell to take over and keep the systems running.

The number of times a cell can divide is bounded by a phenomenon known as the "Hayflick's limit", which describes the action by which the process of division (known as "mitosis") progressively degrades the genetic material, specifically the part of DNA called a telomere. The Hayflick's limit dictates that the average cell will divide between 40 to 60 (or 50 to 70) times before apoptosis.

Understanding telomeres

Chromosomes are thread-like structures located inside the nucleus of a cell. Each chromosome is made of protein and a single molecule of DNA. At each end of a chromosome is a telomere (which people often compare to the plastic tips at the ends of a shoelace). Telomeres are important because they prevent chromosomes from unraveling, sticking to each other, or fusing into a ring. Each time a cell divides, the double-stranded DNA separates in order for the genetic information to be copied. When this happens, the DNA coding is duplicated, but not the telomere. When the copy is complete and mitosis begins, the place where the cell is snipped apart is at the telomere. As such, with each cell generation, the telomere gets shorter and shorter until it can no longer maintain the integrity of the chromosome. It is then that apoptosis occurs. Telomeres will eventually get depleted, resulting in cells ceasing to replicate.

Telomeres' relation to aging and cancer

Scientists can use the length of a telomere to determine the age of a cell and how many more replications it has left. As cellular division slows, it undergoes a progressive deterioration known as senescence, which we commonly refer to as aging. Cellular senescence explains why our organs and tissues begin to change as we grow older. In the end, all of our cells are "mortal" and subject to senescence. All, that is, but one type of cells.

Cancers cells are the one cell type that can truly be considered "immortal". Unlike normal cells, cancer cells do not undergo programmed cell death but can continue to multiply without end. This, in and of itself, disrupts the balance of cellular replication in the body. If one type of cell is allowed to replicate unchecked, it can supplant all others and undermine key biological functions. This is what happens with cancer and why these "immortal" cells can cause disease and death. It is believed that cancer occurs because a genetic mutation can trigger the production of an enzyme, known as telomerase, which prevents telomeres from shortening (see my book on Cancer).

While every cell in the body has the genetic coding to produce telomerase, only certain cells actually need it. Sperm cells, for example, need to switch off telomere shortening in order to make more than 50 copies of themselves; otherwise, pregnancy could never occur.

If a genetic mishap inadvertently turns telomerase production on, it can cause abnormal cells to multiply and form tumors. It is believed that as life expectancy rates continue to grow, the chances of this to occur will not only become greater but eventually become inevitable.

Conclusions and take-aways

- Studies have demonstrated that genetics can play a major role in aging and account for much of the variation in aging among people.
- **Somatic theory.**

- **Longevity genes** are specific genes that help a person live longer. In the longevity and healthy aging fields, senescence is the decline in health and function associated with aging.
- Senescence is the process of growing old or the process by which cells deteriorate over time. It is defined as the period of gradual decline that follows the development phase in an organism's life.
- There are several sub-terms of senescence, including *cellular senescence* and *organismal senescence*.
- We cannot yet interrupt the process of senescence as it relates to humans, but we can slow it down.
- Telomeres are small structures at the tips of genes, which stabilize DNA as cells divide. They get shorter with chronological age. The length of a telomere determines the age of a cell and how many more replications it has left.
- Cellular senescence explains why our organs and tissues begin to change as we grow older. In the end, all but one type of our cells are "mortal" and subject to senescence. Cancers cells are the one cell type that can truly be considered "immortal".
- The number of times a cell can divide is bounded by the "Hayflick's limit", which dictates how many times the average cell will divide (between 40 to 60 or 50 to 70 times) before apoptosis.
- The length of a telomere can be used to determine the age of a cell and how many more replications it has left.
- Cancers cells are the one cell type that can truly be considered "immortal". It is believed that cancer occurs because a genetic mutation can trigger the production of an enzyme (known as telomerase) which prevents telomeres from shortening.
- Stem cells are cells that can become any type of cell in the body and hold promise to repair damage caused by aging.

Sidebar 14.1 - Telomeres

Stretches of DNA called telomeres form protective caps at the ends of chromosomes. They consist of repeated, noncoding nucleotide motifs and associated proteins that are found at the ends of eukaryotic chromosomes,

mediating genome stability and determining cellular lifespan. But as cells divide, the telomeres become gradually shorter, making the protective cap less effective. When the telomeres eventually become too short, the cell stops dividing. Telomere shortening and malfunction have been linked to cell aging and age-related diseases, including cancer.

Scientists have known that RNA species called TERRA (telomeric-repeat-containing RNA) are not translated into proteins but, instead, function as structural components of chromosomes. TERRA accumulates at chromosome ends, signaling that telomeres should be elongated or repaired. TERRA is "*…a class of long noncoding RNAs (lncRNAs) that are transcribed from chromosome ends and regulate telomeric chromatin structure and telomere maintenance through the telomere-extending enzyme telomerase and homology-directed DNA repair"*. However, the mechanisms by which TERRA is recruited to chromosome ends remain poorly defined although it was found that when TERRA reaches the tip of chromosomes, several proteins regulate its association with telomeres. Among these proteins, RAD51 plays a particularly important role. RAD51 is a well-known enzyme that is involved in the repair of broken DNA molecules. The protein also seems to help TERRA stick to telomeric DNA, to form an 'RNA-DNA hybrid' molecule. Researchers also found that short telomeres recruit TERRA much more efficiently than long telomeres.

Given the role of telomeres in health and disease, it will be important to see how the above newly discovered mechanism, which was deduced from observations in living cells and reproduced in test tubes, is regulated in the very complex cellular environment.

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Error theories of aging

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Error theories of aging

Wear-and-tear theory

Stochastic theories of aging are theories suggesting that aging is caused by small changes in the body over time and the body's failure to restore the system and mend the damages to the body. Cells and tissues are injured due to the accumulation of damage over time resulting in the diminished functioning of organs. The notion of accumulated damage was first introduced in 1882 by biologist Dr. August Weismann as the "wear and tear" theory. It suggests that, as an individual ages, body parts such as cells and organs wear out from continued use - the effects being caused by progressive damage to cells and body systems over time. Wearing of the body can be attributable to internal or external causes that eventually lead to an accumulation of insults which surpasses the capacity for repair. The decrease of functioning that is seen as "tear" in the "wear-and-tear" theory is really the result, not the cause, of aging. Due to these internal and external insults, cells lose their ability to regenerate, which ultimately leads to mechanical and chemical exhaustion. Some insults include chemicals in the air, food, or smoke. Other insults may be things such as viruses, trauma, free radicals, cross-linking, and high body temperature.

Essentially, human bodies "wear out" due to use. Once they wear out, they can no longer function correctly. It is sometimes called "simple deterioration theory" or "fundamental limitation theory". Error theories of aging believe

that aging is not something that is programmed to occur, but rather aging is due to a series of "accidents". (A good *albeit* common representation of this theory is that it sees cells as being like socks that only last so long before they become threadbare or get holes. They can patch themselves, like socks, but only so many times before they just do not work anymore.) Perhaps with an increased understanding of genetics, there will be better information on just what causes bodies to age.

Causes of wear-and-tear damage

A wide range of internal and external factors can damage body systems, including exposures that can damage genes:

- **Exposure to radiation** (environmental or/and occupational);
- **Exposure to toxins;** and
- **Exposure to ultraviolet light.**

The effects of the body's own functioning can also cause damage. When the body metabolizes oxygen, free radicals are produced that can cause damage to cells and tissues. There are some cellular systems that do not replace themselves throughout life, such as the nerve cells of the brain. As these cells are lost, function eventually will be lost.

Within cells that continue to divide, the DNA can sustain damage and errors can accumulate. The simple act of dividing, again and again, shortens the telomeres of the chromosomes, eventually resulting in a senescent cell that can no longer divide.

Further, oxidative damage in cells results in cross-linking of proteins, which prevents them from doing the jobs they are intended to do in the cells. Free radicals inside mitochondria (the powerhouses of the cells in our body) injure their cell membranes so they cannot function as well.

Supporting and contrary evidence

It might simply seem that the wear-and-tear theory "makes sense" based on observations, but it is important to compare these gut feelings with what is

scientifically known about the body and aging. Under the microscope, there are some processes that support wear-and-tear as a factor in aging, but several other findings question this process. There is evidence both for and against this theory.

Evidence "for":

- **Matches common perceptions of aging:** Regardless of chronological age, the term "aging" is used to describe the progressive deterioration of a person or object.
- **Fits law of entropy:** The theory fits closely with one of the fundamental laws of chemistry and physics, that of "entropy", which states that all systems tend toward a state of increased entropy or progressive disorganization.
- **Many bodily processes decline with aging:** Visually, there are structural changes with age in human skin and bones. On a cellular level, there are a number of functions which decline with age. Even with a good diet, cells have a decreased ability to absorb nutrients with age.

Evidence "against ":

- **Cells are well-equipped to repair damage:** Our bodies have a tremendous ability to repair the damage. DNA is equipped with DNA-repair genes - such as tumor suppressor genes (TSG) that work to repair genetic damage. In addition, some studies have found that the aging process may be partially or completely reversed by simply changing the microenvironment of cells or certain hormonal factors. Of course, not all damage can be repaired fully, and mistakes in repair may accumulate over time.
- **Humans and other organisms grow stronger from youth to maturity:** In their growth phase, organisms become stronger and stronger. Rather than starting out at the peak of performance, living organisms often start life fragile. They build strength and resilience with age. They are able to repair and replace most broken parts themselves.

- **Organisms vary widely in lifespan rather than all following the same pattern:** There are some circumstances (for example, muscle building from exercise) in which wear-and-tear actually extends life expectancy.
- **Some species die when seemingly at their peak physical condition:** For example, after a long upstream energetic swim, salmons spawn and then die, seemingly at the peak of physical condition.

Rate-of-living theory

The rate-of-living theory of aging states that people (and other living organisms) have a finite number of breaths, heartbeats, or other measures, and that they will die once they would have used those up. (But, do not try to live longer by slowing your metabolism just yet!) While the theory is helpful to explain some aspects of aging, it does not really hold up under modern scientific scrutiny.

There is some evidence, when comparing species, that creatures with faster oxygen metabolisms die younger. For example, tiny mammals with rapid heartbeats metabolize oxygen quickly and have short lifespans, while tortoises, on the other hand, metabolize oxygen very slowly and have long lifespans.

Supporting evidence

The supporting evidence is rather scant. In one study with genetically-engineered mice presenting with a defect in the hypothalamus, researchers found that the defect caused the mice to overexert which, in theory, would "use up" their lifespans faster. However, because the hypothalamus in mice is near the temperature control center, their brains thought their bodies were overheating and so they lowered the mice's core temperatures. The results did show that a drop of .6 degrees Celsius extended the life of the mice by 12%-20%, so the mice did live longer with lower body temperatures.

The problem is we do not know *why* they lived longer. The lower temperature may have slowed the rate of oxygen metabolism, but it may also have changed a number of other systems and processes in the body. So we do not know why the mice lived longer, only that they did, and that is not proof of the rate-of-living theory of aging.

Cross-linking theory

The cross-linking theory proposes that advanced glycation end-products (stable bonds formed by the binding of glucose to proteins) and other aberrant cross-links accumulating in aging tissues is the cause of aging. The cross-linking of proteins disables their biological functions. The hardening of the connective tissue, kidney diseases, and enlargement of the heart are connected to the cross-linking of proteins. Cross-linking of DNA can induce replication errors, and this leads to deformed cells and increases the risk of cancer.

Free radicals theory

Free radicals are a byproduct of normal cell function. When cells create energy, they also produce unstable oxygen molecules. These molecules, called free radicals, have a free electron, which makes the molecule highly unstable. Free radicals bond to other molecules in the body, causing proteins and other essential molecules to not function as they should. While they can be formed through this natural process, free radicals can also be caused by diet, stress, smoking, alcohol, exercise, inflammation drugs, and exposure to the sun or air pollutants.

What are antioxidants?

Antioxidants are substances found in plants that soak up free radicals like sponges and are believed to minimize free radical damage. If the body has plenty of antioxidants available, it can minimize the damage caused by free radicals. There is some evidence that we can only get the full antioxidant benefits from eating real plants and other foods. Supplements appear not to be as effective.

Free radicals and aging

The free radical theory of aging asserts that many of the changes that occur as our bodies age are caused by free radicals. Damage to DNA, protein cross-linking and other changes have been attributed to free radicals. Over time, this damage accumulates and causes us to experience aging.

There is some evidence to support this claim. Studies have shown that increasing the number of antioxidants in the diets of mice and other animals can slow the effects of aging. However, this theory does not fully explain all the changes that occur during aging and it is likely that free radicals are only one part of the aging equation. In fact, more recent research suggests that free radicals may actually be beneficial to the body in some cases and that consuming more antioxidants than we would through food have the opposite intended effect. In one study (in worms) those that were made more free radicals or were treated with free radicals lived longer than other worms. It is not clear if these findings would carry over into humans, but research is beginning to question the conventions of the free radical theory of aging.

Conclusions and take-aways

- There is little evidence that oxygen metabolism, heartbeat, or the number of breaths determine an individual's lifespan.
- The rate-of-living theory seems to hold up when smaller species with faster metabolisms (i.e., mice) are compared with larger species with slower metabolisms (i.e., tortoises). However, the theory can only partially explain the differences in life span between species, and it cannot explain the most important factor: What determines lifespan *within* species.
- It is not known what determines which individuals within a species live the longest.
- There really is not data that slowing the metabolism extends human life. In fact, a slower metabolism would put someone at risk for obesity and other nutritional-related illnesses.
- The best bet remains a healthy lifestyle with plenty of exercise, a diet with lots of plants, and a positive, relaxed attitude.

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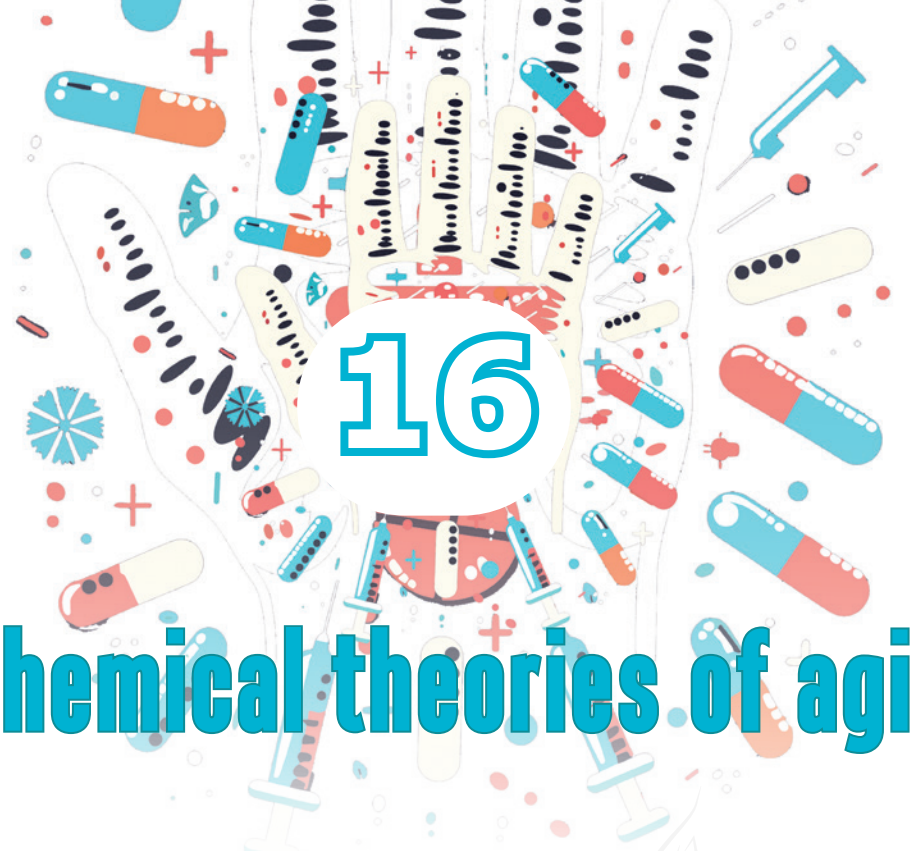
Biochemical theories of aging



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Biochemical theories of aging

No matter what genes we have inherited, our body is continually undergoing complex biochemical reactions. Some of these reactions cause damage and, ultimately, aging in the body. Studying these complex reactions helps understanding how the body changes as it ages. Important concepts in the biochemistry of aging are first discussed below.

Important concepts in the biochemistry of aging

There are five important concepts in the biochemistry of aging on which are based various theories of aging (see also Table 12.1):

Age-changing hormonal theory

Hormones cause many shifts in organ systems and other functions.

Free radicals theory

Damage by free radicals, or more generally reactive oxygen species (or oxidative stress) may give rise to the symptoms we recognize as aging. Calorie restriction reduces 8-OH-dG DNA damage in organs of aging rats and mice. Thus, reduction of oxidative DNA damage is associated with a slower rate of aging and increased lifespan. In a 2021 review article, Vijg

stated that "*Based on an abundance of evidence, DNA damage is now considered as the single most important driver of the degenerative processes that collectively cause aging*". The effect of calorie restriction may be due to increased formation of free radicals within the mitochondria, causing a secondary induction of increased antioxidant defense capacity. Which free radicals, what mechanism(s) and the extent of the damage caused are still being studied;

Protein cross-linking theory

Protein cross-links produce excess sugars in the bloodstream that can cause protein molecules to literally stick together, leading to aging. The identity, number and strength of the cross-links, and the mechanism(s) leading to aging are being researched;

DNA repair theory

DNA damage is thought to be the common basis of both cancer and aging, and it has been argued that intrinsic causes of DNA damage are the most important causes of aging. Genetic damage (aberrant structural alterations of the DNA), mutations (changes in the DNA sequence), and epimutations (methylation of gene promoter regions or alterations of the DNA scaffolding which regulate gene expression), can cause abnormal gene expression. DNA damage causes the cells to stop dividing or induces apoptosis, often affecting stem cell pools and therefore hindering regeneration. However, lifelong studies of mice suggest that most mutations happen during embryonic and childhood development, when cells divide often, as each cell division is a chance for errors in DNA replication. The systems in the body that repair DNA seem to become less effective with age. However, the particulars of the several body systems that repair DNA and the mechanism(s) and effectiveness of their remedial action need to be further elucidated. Lastly:

Heat shock proteins theory

Such proteins help cells survive stress and diminish in numbers with age. The full identity of such proteins, their number, and mechanisms of action, and diminution with time require further investigations.

Modern biological theories of aging

As already indicated, many theories have been proposed to explain the process of aging, but neither of them appears to be fully satisfactory. The traditional aging theories hold that aging is not an adaptation or genetically programmed.

Modern biological theories of aging in humans fall into two main categories: Programmed and damage or error theories.

Programmed theory of aging

The programmed theories imply that aging follows a biological timetable, perhaps a continuation of the one that regulates childhood growth and development. This regulation would depend on changes in gene expression that affect the systems responsible for maintenance, repair, and defense responses. The damage or error theories emphasize environmental assaults to living organisms that induce cumulative damage at various levels as the cause of aging.

The programmed theory has three sub-categories:

- **Programmed longevity:** Aging is the result of a sequential switching on and off of certain genes, with senescence being defined as the time when age-associated deficits are manifested. Dr. Davidovic *et al* have discussed the role of genetic instability in aging and dynamics of the aging process
- **Endocrine theory:** Biological clocks act through hormones to control the pace of aging. Recent studies confirm that aging is hormonally regulated and that the evolutionarily conserved insulin/IGF-1 signaling (IIS) pathway plays a key role in the hormonal regulation of aging.

Dr. van Heemst has discussed the potential mechanism underlying IIS and aging process.

- **Immunological theory:** The immune system is programmed to decline over time, which leads to an increased vulnerability to infectious disease and, thus, aging and death. It is well documented that the effectiveness of the immune system peaks at puberty and gradually declines thereafter with advance in age. For example, as one grows older, antibodies lose their effectiveness, and fewer new diseases can be combated effectively by the body, which causes cellular stress and eventual death. Indeed, dysregulated immune response has been linked to cardiovascular disease, inflammation, Alzheimer's disease (AD), and cancer. Although direct causal relationships have not been established for all these detrimental outcomes, the immune system has been at least indirectly implicated.

Damage or error theory of aging

The damage or error theory of aging includes five sub-categories:

- **Wear-and-tear theory:** Cells and tissues have vital parts that wear out resulting in aging. Like components of an aging car, parts of the body eventually wear out from repeated use, killing them and then the body. So, the wear-and-tear theory of aging was first introduced by Dr. August Weismann, a German biologist, in 1882, it sounds perfectly reasonable to many people even today, because this is what happens to most familiar things around them keeping in mind, however, that the body is not a machine.
- **Rate-of-living theory:** The greater an organism's rate of oxygen basal metabolism, the shorter its life span. The rate-of-living theory of aging while helpful is not completely adequate in explaining the maximum life span. Dr. Rollo proposed a modified version of Pearl's rate-of- living theory emphasizing the hard-wired antagonism of growth (TOR) and stress resistance (FOXO).
- **Cross-linking theory:** The cross-linking theory of aging was proposed by Johan Bjorksten in 1942. According to this theory, an accumulation of cross-linked proteins damages cells and tissues, slowing down

bodily processes resulting in aging. Recent studies show that cross-linking reactions are involved in the age related changes in the studied proteins.

- **Free radicals theory:** This theory, which was first introduced by Dr. Gerschman in 1954, but was developed by Dr. Denham Harman, proposes that superoxide and other free radicals cause damage to the macromolecular components of the cell, giving rise to accumulated damage causing cells and, eventually, organs to stop functioning. The macromolecules such as nucleic acids, lipids, sugars, and proteins are susceptible to free radical attack. Nucleic acids can get additional base or sugar group; break in a single- and double-strand fashion in the backbone and cross link to other molecules. The body does possess some natural antioxidants in the form of enzymes, which help to curb the dangerous build-up of these free radicals, without which cellular death rates would be greatly increased, and subsequent life expectancies would decrease. This theory has been bolstered by experiments in which rodents fed antioxidants achieved greater mean longevity. However, at present, there are some experimental findings which do not agree with this early proposal. The review by Igor Afanas'ev shows that reactive oxygen species (ROS) signaling is probably the most important enzyme/gene pathway responsible for the development of cell senescence and organismal aging and that ROS signaling might be considered as further development of free radical theory of aging.
- **Somatic DNA damage theory:** DNA damages occur continuously in cells of living organisms. While most of these damages are repaired, some accumulate, as the DNA polymerases and other repair mechanisms cannot correct defects as fast as they are apparently produced. In particular, there is evidence for DNA damage accumulation in non-dividing cells of mammals. Genetic mutations occur and accumulate with increasing age, causing cells to deteriorate and malfunction. In particular, damage to mitochondrial DNA might lead to mitochondrial dysfunction. Therefore, aging results from damage to the genetic integrity of the body's cells.

Calorie restriction theory of aging

As stated earlier, since the 1930s, it has been found that restricting calories can extend lifespan in laboratory animals. Many studies were performed to try to elucidate the underlying mechanisms. However, our knowledge remained limited at the genetic and molecular levels until 1990. Recently, Michael Ristow's group has provided evidence that this effect is due to increased formation of free radicals within the mitochondria, causing a secondary induction of increased antioxidant defense capacity. Drs. Shimokawa and Trindade discussed recent findings on restricting calories-related genes or molecules in rodent models, particularly on the roles of fork head box O transcription factors, AMP-activated protein kinase, and sirtuins (particularly SIRT1) in the effects of restricting calories in rodents.

Risks of neurological diseases with increasing age

Some neurological diseases are considered to be at high risk with increasing age, for example, **AD**, which is diagnosed in people over 65 years of age. Discovery of the molecular basis of the processes involved in their pathology or creating and studying aging model systems may help our better understanding the aging processing. In the early stages, the most commonly recognized symptom of AD is inability to acquire new memories. Recent studies show that endogenous neural stem cells in the hippocampus of adult brain may involve in memory function. Consistently, neural stem cell function in the hippocampus decreases with increased aging, but the reasons are still unclear. Dr. Fymat has also posited in 2017 that AD is but a runaway autoimmune disease facilitated by the decrease of brain immunity with advancing age. He has also proposed a path to a cure including a method for regulating that immune system.

On the role of telomeres

It is well-known that telomere maintenance appears to be essential for the prolonged persistence of stem cell function in organs with extensive cell turnover. In 1961, Dr. Hayflick theorized that the human cells ability to divide is limited to approximately 50-times, after which they simply stop dividing

(this is the Hayflick's limit **t**heory of aging). According to telomere theory, telomeres have experimentally been shown to shorten with each successive cell division. Certain cells, such as egg and sperm cells, use telomerase to restore telomeres to the end of their chromosome, insuring that cells can continue to reproduce and promote the survival of the species. But, most adult cells lack this capacity. When the telomeres reach a critical length, the cell stops replicating at an appreciable rate and so dies off, which eventually leads to the death of the entire organism. Telomerase cannot completely prevent telomere shortening after extensive stem cell division either, providing a putative mechanism for the timely limit of stem cell replicative history and subsequent progressive decay in the maintenance of organ homeostasis at old ages. A recent study shows that telomeres shorten with age in neural stem cells of the hippocampus and that telomerase-deficient mice exhibit reduced neurogenesis as well as impaired neuronal differentiation and neuritogenesis. Taken together, these findings indicate the link among brain aging, neural stem cells, and neurological diseases. Dr. Taupin discussed the association of aging with neurogenesis by emphasizing the role of adult neurogenesis in the pathogenesis of neurological diseases.

Overall, while multiple theories of aging have been proposed, currently there is **no consensus** on this issue. Many of the proposed theories interact with each other in a complex way. By understanding and testing the existing and new aging theories, it may be possible to promote successful aging as well as to enhance the lifespan of mankind.

Conclusions and take-aways

- No matter what genes we have inherited, our body is continually undergoing complex biochemical reactions some of which cause damage and, ultimately, aging in the body.
- There are five important concepts in the biochemistry of aging on which are based various theories of aging: Age-changing hormonal theory; free radicals theory; protein cross-linking theory; DNA repair theory; and heat shock proteins theory.
- Modern biological theories of aging in humans fall into two main categories: Programmed and damage or error theories. The

programmed theory of aging has three sub-categories: Programmed longevity: endocrine theory; and immunological theory.

- The damage or error theory of aging includes five sub-categories: Wear and tear theory; rate-of-living theory; cross-linking theory; free radicals theory; and somatic DNA damage theory.
- Restricting calories can extend lifespan in laboratory animals. There is evidence that this effect is due to increased formation of free radicals within the mitochondria, causing a secondary induction of increased antioxidant defense capacity.
- Some neurological diseases are considered to be at high risk with increasing age. Discovery of the molecular basis of the processes involved in their pathology or creating and studying aging model systems may help our better understanding the aging processing. This author has posited in 2017 that AD is but a runaway autoimmune disease facilitated by the decrease of brain immunity with advancing age. He has also proposed a path to a cure including a method for regulating that immune system.
- Telomere maintenance appears to be essential for the prolonged persistence of stem cell function in organs with extensive cell turnover. Telomeres have experimentally been shown to shorten with each successive cell division. Telomerase cannot completely prevent telomere shortening after extensive stem cell division either, providing a putative mechanism for the timely limit of stem cell replicative history and subsequent progressive decay in the maintenance of organ homeostasis at old ages.

PART E

WHAT CAN BE DONE ABOUT AGING?

Introduction to Part E

Part E examines what can be done about aging beginning with **Chapter 17** on anti-aging behaviors. The candidate causes of aging have been narrowed to the following: Mutations accumulation (including nuclear epimutations); extra-cellular aggregations; cross-linkings outside cells; cell defects; and, to a much lesser extent, nuclear mutations. The time of life when age-related changes appear depends on a variety of factors, including: genetics, diet, culture, activity levels, and environmental exposure. In the end, aging is inevitable. Taking care of body and mind, and embracing the changes as they come goes a long way toward a healthier and, hopefully, longer life span. Efforts should be made to prevent the aging of the cardiovascular system, the brain and nervous system, and the bones and muscles.

Health span extension and anti-aging research seek to extend the span of health in the old as well as slow aging or its negative impacts such as physical and mental decline. Measures that may extend life spans may simultaneously also extend health spans. Aging and senescence can be combated vigorously by pursuing one or more of three approaches: Calorie restriction and its genetic emulation; interference with metabolic processes to lessen damage; and alleviation of the molecular damage itself.

Numerous research groups are now working on extending life and combating senescence of old age. The approaches consist of: Hacking the code of life; the coming of age of medical nanobots; gene therapy to combat old age and increasing muscle mass; deleting 238 age-related genes; repairing or replacing worn-out tissues and body parts including using stem cells; optimizing DNA for a longer life; transfusing blood from young persons to older ones; producing anti-aging drugs; growing (or "printing") organs from scratch; editing those gene varieties that prolong life; and others. Other

futuristic and not so futuristic technologies will also be briefly discussed. Further research is certainly needed in each of these approaches.

Chapter 18 examines therapies for retarding or reversing aging. Cellular degeneration may be delayed or even reversed through an experimental type of hyperbaric oxygen therapy. Lengthening human telomeres through treatment (a specialized form of oxygen therapy) appears to reverse some biological markers of aging. However, the long-term effects of the treatment are still unknown as it is not clear how lengthening telomeres might affect aging, chronic illness, and longer life span.

It might be possible to treat aging as a preventable disease, and stave-off related illnesses (cancer, diabetes, cognitive decline, etc.), including: Oxygen therapy; anti-aging hormone supplements; interventions in diet, exercise, and mental outlook; and others. The maximum human life span could be extended by continuing to "avert early and mid-life deaths". Certain lifestyles help *individuals* live longer than they otherwise would. Findings from longevity research could support better health in old age, with fewer age-related diseases and disabilities.

There are three known ways of switching on the longevity genes: Nutritional evolutionary scarcity causing stress; calorie restriction without malnutrition; and optimal exercise simulating evolutionary stressful environments by duping genes into extending the health span. Beyond diet and exercise, other drivers of longevity include long-term loving relationships - one (if not) the most important factor in a long, healthy life is having a close partner. Another protective factor is optimism.

Chapter 19 reviews some of the latest research and developments. Aging is a "global phenomenon" that is occurring fastest in developing countries, including those with large youth populations. It poses social and economic challenges. Among the most urgent concerns of older persons worldwide is income security. It has been argued that population aging has undermined economic development. In the field of sociology and mental health, aging is seen in five different views as: Maturity, decline, life-cycle event, generation, and survival. Positive correlates with aging often include among many others: Economics, employment, marriage, children, education, and sense of control.

The social science of aging includes several theories: Disengagement, activity, selectivity, and continuity.

Chapter 20 answers a few frequently asked questions whereas **Chapter 21** lists some of the available resources.

Finally, **Chapter 22** provides several Guides for healthy aging in order to find healthcare that meets one's needs, not making medication mistakes, and staying on top of health problems. These include: The Guide to Wellness and Prevention; the Guide to Geriatric Syndromes; and the Guide to Age-Friendly Healthcare, which encompasses the five M's: Matters, Medications, Mentation, Mobility, and Multi-complexity.

17

Anti-aging behaviors and human life extension

Contents

17

Anti-aging behaviors and human life extension

Many of the causes of aging that may be happening prematurely can be modified through our behaviors. Here are a few ways to keep your body feeling as young as possible:

- Eating foods loaded with antioxidants to minimize damage caused by free radicals.
- Exercising regularly to limit bone and muscle loss.
- Controlling cholesterol to slow the hardening of the arteries and protecting the heart.
- Practicing mental fitness to keep the brain sharp.

In the end, aging is inevitable. Taking care of body and mind and embracing the changes as they come goes a long way toward a healthier and, hopefully, longer lifespan.

Understanding and extending the lifespan

In order to understand the aging process, it is important to identify those factors that affect the overall lifespan of an organism. In mammals, there

is a progressive physiologic decline with aging that is often accompanied by disease and disability. Understanding the responsible physiological mechanisms and further identifying ways to slow down age-related changes are important. Beyond any gains in lifespan, studies in this area are aimed more importantly at developing interventions to keep older people healthy and free of disease and/or disability as long as possible. Experiments in a number of animal models are providing valuable insights.

Extension of average lifespan of nematodes by pharmacological intervention

It is widely accepted that oxidative stress is a factor in aging. To date, however, it has not been demonstrated convincingly that natural antioxidants such as vitamins C and E or ß-carotene extend lifespan in model experiments with mice, fruit flies, or nematodes (a kind of worm). Varied results have been obtained in genetically altered fruit flies over-expressing either superoxide dismutase (SOD) or SOD and catalase, enzymes that reduce oxidative damage. Now, an artificial compound, EUK-134, which mimics both SOD and catalase activity, has been shown to increase the average lifespan of nematodes by about 50%. EUK-134 also reversed premature aging in a nematode strain subject to elevated oxidative damage. These results strongly suggest that oxidative stress is a major factor in the rate of aging in the nematode, and that this rate can be slowed by pharmacological intervention. It may be that similar compounds could lessen oxidative stress in humans and delay or reduce age-related pathology.

Genetically mimicking calorie restriction significantly extends yeast lifespan

Calorie restriction (CT) has been shown to significantly extend lifespan in a variety of organisms. In organisms studied to date (yeast, nematodes, fruit flies, mice and rats), CR increased both mean and maximum lifespan, as well as significantly reducing signs of disease. In all species examined, the extended longevity and health of the animals was accompanied by changes in the regulation of energy metabolism. Recent research has determined that genetic manipulation of glucose availability, metabolism, and signaling

pathways can mimic the longevity-extending effects of CR in the yeast model. This discovery makes the yeast model of aging and longevity a powerful tool for uncovering the underlying cellular and molecular mechanisms responsible for increased longevity and health span, with a view to developing effective interventions.

Calorie restriction increases neurotrophic factor production in the brain and protects neurons

Beyond extending lifespan, CR also reduces development of age-related cancers, immune and neuroendocrine alterations, and motor dysfunction in rodents. Recent animal model studies of neurodegenerative disorders provide the first evidence that CR can also increase resistance of neurons to age-related and disease-specific stresses. One possible mechanism is that the mild metabolic stress associated with CR induces cells to produce proteins that increase cellular resistance to disease processes. Indeed, CR increases production of one such protein, a neuronal survival factor, BDNF. BDNF signaling in turn plays a central role in the neuroprotective effect of CR. This work suggests that CR may be an effective approach for reducing neuronal damage and neurodegenerative disorders in aging, providing insight into the design of approaches that might mimic CR's beneficial consequences.

Use of gene expression microarrays in aging research

Aging is normally accompanied by changes in expression (or activity) of a large number of genes, but it is not clear which of these changes are critical in the aging process. Gene expression microarrays, which allow profiling the activity of many thousands of genes at once, provide an opportunity to obtain a more complete picture of what these changes are, and to design tests of whether these changes are causally associated with aging. In three recent studies, investigators looked at differences in gene expression patterns in young and old mouse skeletal muscle, liver, and brain tissue and also made several observations on changes brought about by calorie restriction. Though the data analyses are complex, some initial observations are: (1) aging results in lower levels of activity of metabolic and biosynthetic genes; (2) aging is accompanied by patterns of gene expression that are indicative

of stress responses, including inflammatory and oxidative stress; (3) many, but not all, age-related changes in gene expression in mouse liver and skeletal muscle are slowed by caloric restriction; and (4) caloric restriction appears to increase expression of genes for repairing and/or preventing damage to cellular macromolecules. Microarray technology is proving to be an efficient approach to answering longstanding important questions about molecular mechanisms of aging and how these may be manipulated, for example, by calorie restriction. Profiling changes in gene activity may eventually provide useful biomarkers of the aging process itself, markers that might be important in assessing the effectiveness of strategies to retard aging-related processes.

Age-related body changes

The time of your life when age-related changes appear depends on a variety of factors, including:

- **Genetics.**
- **Diet.**
- **Culture.**
- **Activity levels.**
- **Environmental exposure**.

Preventing aging of the cardiovascular system

The heart muscle thickens and blood vessels get stiffer with age, reducing the amount of oxygen available to the brain and body. The breathing capacity declines by as much as 40% between 20 and 70 years of age. To remedy this situation, at least in part, *one should engage in r*egular, sustained exercise to improve heart and lung function at any age.

Preventing aging of the brain and nervous system

As we age, we lose nerve cell structure along with some function of individual nerve cells. Adult nerve cells may reproduce, but experts do not understand the extent of this regeneration. Normal aging of the brain does not include

the severe decline in mental function caused by diseases like Alzheimer's and dementia (see my books on these subjects).

To alleviate this situation, scientists are just beginning to learn how plastic, or adaptable, the brain is. One can improve memory and other brain functions by trying brain exercises and learning new skills, such as dancing or playing a musical instrument.

Preventing aging of the bones and muscles

Bone density diminishes on average beginning at age 35, with an accelerated rate of loss in women who have gone through menopause. To remedy this situation, weight-bearing exercise, like strength training, in addition to walking and running, help maintain bone density. Between the ages of 30 and 70, muscle mass declines more than 20% in men and women if they do not exercise regularly. The same regular exercise that slows bone loss will help maintain muscle mass.

Healthspan and lifespan in an aging society

Healthspan can broadly be defined as the period of one's life during which one is healthy and free of significant diseases or declines of capacities (e.g. of senses, muscle, endurance, and cognition). However, with aging populations, there is a rise of age-related diseases, putting major burdens on healthcare systems as well as contemporary economies and their appendant societal systems.

Healthspan extension

Healthspan extension and anti-aging research seek, therefore, to extend the span of health in the old as well as slow aging or its negative impacts such as physical and mental decline. Modern anti-senescent and regenerative technology with augmented decision-making could help "*responsibly bridge the healthspan-lifespan gap for a future of equitable global well-being*".

Aging is the most prevalent risk factor for chronic disease, frailty, and disability, and it is estimated that there will be over 2 billion persons age > 60

by the year 2050, making it a large global health challenge that demands substantial (and well-orchestrated or efficient) efforts, including interventions that alter and target the inborn aging process.

Biological aging comes with a great cost burden to society, including potentially rising health care costs (also depending on types and costs of treatments). This, along with global quality of life or well-being, highlight the importance of extending healthspans.

Many measures that may extend lifespans may simultaneously also extend healthspans, *albeit* that is not necessarily the case, indicating that "*lifespan can no longer be the sole parameter of interest*" in related research. While recent life expectancy increases were not followed by "parallel" healthspan expansion, awareness of the concept and issues of healthspan lags. Scientists have noted that chronic diseases of aging are increasing and are inflicting untold costs on human quality of life.

Lifespan extension

Lifespan extension is the concept of extending the human lifespan, either modestly through improvements in medicine or dramatically by increasing the maximum lifespan beyond its generally-settled limit of 125 years. Researchers in the area, along with "life extensionists","immortalists", or "longevists" (those who wish to achieve longer lives themselves) postulate that future breakthroughs in tissue rejuvenation, stem cells, regenerative medicine, molecular repair, gene therapy, pharmaceuticals, and organ replacement (such as with artificial organs or xenotransplantations) will eventually enable humans to have indefinite lifespans through complete rejuvenation to a healthy youthful condition (agerasia).

If life extension were to become a possibility, the ethical ramifications would require bioethical debates. Along this line, the sale of purported anti-aging products such as supplements and hormone replacement is a lucrative global industry. However, the use of such hormone products has not been proven to be effective or safe.

Combating aging and senescence

Aging and senescence can be combated vigorously by pursuing one or more of the following three approaches: (1) Calorie restriction and its genetic emulation; (2) Interference with metabolic processes to lessen damage; and (3) Alleviation of the molecular damage itself (acronym: **CMD** for **C**alorie, **M**etabolism, **D**amage).

Calorie restriction

Calorie restriction (CR) is the only non-genetic intervention known to slow down aging in mammals. It lowers the generation of mitochondrial free radicals, toughens their membranes against the free radicals' assault and, above all, reduces the age-related accumulation of mitochondrial DNA mutations. Free radical damage outside the mitochondria is not a directly important cause of aging. CR slows down aging, yet has no consistent effect on the levels of most self-produced antioxidant enzymes. It has been established that there is a fixed degree of life extension (2-3 years according to some, 20-30 years according to others) that can be achieved by manipulating the nutrient sensing pathway - whether by CR or by drugs that trick the body into thinking it is being starved, or else by genetic changes that flip the same "switch".

A recent multi-center, randomized, controlled clinical trial called CALERIE (Comprehensive Assessment of Long Term Effects of Reducing Intake of Energy), sponsored by NIH, tested the effects of CR on metabolism in more than 200 healthy, non-obese patients. The aim was to see whether a CR diet in humans induces some of the same metabolic, hormonal and gene-expression adaptations that are thought to be involved in slowing aging in other species during long term CR. To determine accurately whether the subject participants burn fat, carbohydrate or protein, the amounts of oxygen they inhale and carbon dioxide they exhale are measured together with the amount of nitrous oxide in their urine. At the end of the trial period (2 years), the participants underwent tests related to metabolism and biological markers of aging. Compared to the control group, it was found that: (a) Participants on the diet used energy much more efficiently while sleeping, (b) the reduction in their metabolic rate was greater than would be

expected, (c) all clinical measurements were in line with reduced metabolic rate, and (c) indicated a decrease in damage due to aging. Ongoing monkey studies also hint at longer survival and reduced signs of aging.

While the above results are encouraging, trials should be run over much longer periods of time in order to truly determine whether the trial participants actually lived longer. However, considering the strict diet restrictions during the trial, modifications to trials of longer duration could include some or all of the following: (a) A diet containing antioxidant food to reduce damage from oxygen free radicals (OFR), (b) the use of the drug *Resveratrol* which mimics key aspects of CR, and (c) restricting the diet intermittently (e.g., over a few days every month).

Interference with metabolic processes

Interference with metabolic processes (IMP) requires a clear understanding of the various metabolic disruptions that cause aging, and those that are effects (or secondary causes) that would simply disappear if the underlying primary causes were addressed. Despite considerable effort, progress has been extremely slow owing to the myriad of interacting processes that contribute to aging damage.

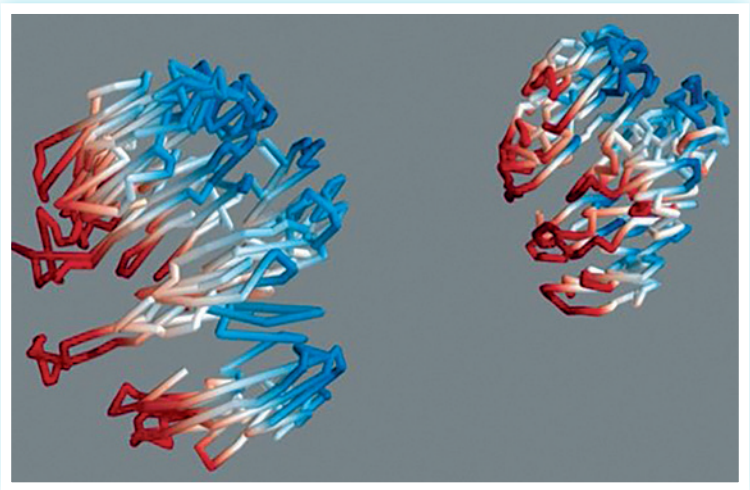
Alleviation of molecular damage

Alleviation of the molecular damage (AMD), itself caused by aging, does not require a complete understanding of all the myriad interacting processes that contribute to aging damage. The real issue is not which metabolic processes cause aging damage in the body, but the damage itself, that is the molecular and cellular lesions that impair the structure and function of body tissues. Aging should, in principle, be just as amenable to modulation and eventual elimination as specific diseases are. It has been argued that the design of therapies should therefore focus on the damage itself and on ways to alleviate the accumulation of damage. However, this is only addressing the symptoms not the root causes of aging, a traditional approach that has festered across all fields of western medicine.

In the AMD approach, the field of candidate causes of aging can thus be narrowed to the following (acronym **MacD** for **M**utations, **a**ggregates, **c**ross-links, **D**efects):

- ***Mutations accumulation:*** This is the disruption of the cellular biochemistry by increasing oxidative stress (OS). It includes: Chromosomal (cause cancer, which is predominantly a consequence of aging); mitochondrial; glycation (warping of proteins by glucose); amyloid; and intra-nucleus events and processes that cause cancer (because non-cancer mutations accumulate too slowly to matter in a normal lifetime). Mutations include nuclear epi-mutations for the case of cancer;
- ***Intra-cellular aggregates:*** These involve lipofuscin;
- ***Extra-cellular aggregates:*** These include beta-amyloid, transthyretin, and other substances of the same general sort;
- ***Cross-links outside cells;***
- ***Cell defects:*** These include death resistance; loss; atrophy; and senescence which produce chemical signals that are dangerous to neighboring cells; and depletion of stem cells, which are essential to healing and maintenance of tissue; and
- ***Nuclear mutations*** (not important to aging).

Figure 17.1 - Showing model of chromosome compaction in aging cells



Source: Neretti et al.(2016)

Figure 17.1 is a still picture from video showing model of chromosome compaction (right)in aging cells.

The end of aging?

Hacking the code of life

Back in the 1800s, people rarely lived past 50, but now, the global average life expectancy has surpassed 70 due, at least in part, to our advances in the fields of medicine and biotechnology. Now, in the midst of a rapidly progressing era of biotechnology, the expansion of our lifetimes might increase faster than ever before. In fact, biotech firms in Silicon Valley have launched programs that are working to extend the human lifespan well past 120 years of age by hacking the "code of life" and thus "solving aging". Since the longest confirmed lifespan to date is 122-years-old, it will be no simple task to bring the whole human race to that level. Soon we may live longer than 120 years and, one scientist (Aubrey de Grey) even claims the first person who will live past 1,000 is probably already alive. While most people accept death as an inevitability of life, he sees it as a "medical problem" that can be solved by science. He analogizes maintaining the human body to maintaining a vintage car, because we, too, are machines — but biological ones. The probability of a 25-year-old dying before his or her 26th birthday is only 0.1 percent! So, statistically speaking, the average person could live 1,000 years if the probability of death could be kept constant instead of skyrocketing with the trials of old age.

Biologist and technologist Craig Venter of the Human Genome Project (HGP) fame compiled a 1 million gene sequences to create a giant database (2020). The extensive data should help biotech researchers determine what makes for a longer, healthier life. Currently, there are several studies testing whether lifestyle choices — spicy foods, *Resveratrol* (the compound found in red wine), mediterranean diets, exercise, etc. — bring about longer lives. Less conventional studies test whether young blood has the potential to regenerate old brains. Or perhaps technology could be the answer, and humans could simply upload their consciousness to machines. Biotech and

biomedical companies working to extend human longevity include BioViva, the Buck Institute for Research on Aging (BIRA), Calico, and others.

A new phase in human evolution

While it is an intriguing prospect that we could see the human lifespan radically increase in our lifetimes, it would completely change the course of human evolution, heralding a "third" evolution era! Nonetheless, the thriving fields of science, biotechnology, and evolution bear exciting possibilities for the future.

The coming of age of medical nanobots

Google's chief futurist, Ray Kurzweil, predicts that humans will start living forever by 2029! Further, according to him, the merging of human intelligence with nonbiological intelligence (or technology), which he terms a "singularity", will happen in 2045. In his words "...*the nonbiological intelligence created in that year will reach a level that's a billion times more powerful than all human intelligence today,...but there will be dramatic changes prior to that*". By the 2020s, we will start using *nanobots* (or nanorobots the size of a blood cell) to complete the job of the immune system, a system that has evolved thousands of years ago when conditions were different. The nanobots — microscopic, self-propelled robots — will act as T-cells, which are blood cells involved in our immune responses (see Sidebar 12.1). Using T-cells to attack cancer cells is already an idea that researchers are using in some cancer immunotherapy but, instead of harnessing the body's own T-cells, Kurzweil wants to send in nanobots to do the job. The above ideas are not so futuristic for, after all, the National Cancer Institute (NCI) supports nanotechnology (Fymat 2016-2018). Witness, the research conducted at the Joslyn Diabetes Center in Connecticut, which has turned-off the fat insulin receptor gene in animals, allowing them to eat large amounts of food without gaining weight or developing diabetes.

The first ever successful gene therapy to combat old age?

Elizabeth Parrish, the CEO of BioViva USA, Inc., a biotechnology company that "aims to provide regenerative medicine to the masses through gene and cell therapies" claims that gene treatment reversed 20 years of one of the underlying causes of aging. Current therapeutics offer only marginal benefits for people suffering from diseases of aging, and lifestyle modifications have only limited impact for treating these diseases. Thus, advances in biotechnology would be the best solution. She also claims to be the first person in history to have successfully reversed one of the hallmark signs of aging with the company's experimental gene therapy. That therapy is designed to protect against muscle mass depletion and stem cell depletion, which are both inherent to aging and age-related diseases. Basically, the therapy is claimed to lengthen the *telomeres*, those protective tips at the end of each DNA strand. At birth, the telomeres are long, but as cells age, the telomeres become shorter and shorter until the DNA starts accumulating damage, making the bodies frail and diseased. Parrish's result has not yet been verified or confirmed by independent researchers.

The company's approach is backed by preclinical evidence—in particular, that from Maria Blasco's group at the Spanish National Cancer Research Center (CNIO) in Madrid. In 2012, Blasco's team reported the results of a telomerase gene therapy in mice. The enzyme telomerase, encoded by the *TERT* gene, lengthens telomeres. They demonstrated that AAV9-TERT gene therapy was sufficient to delay age-related pathologies and extend both median and maximum longevity in mice. Many pathologies were delayed, including cancer. They have since demonstrated that telomerase gene therapy (TGT) can abate certain age-related diseases in mice as well.

Some human diseases are the product of shorter-than-usual telomeres. However, the idea that in the general population relatively short telomeres are bad and relatively long telomeres are good may not be sensical. Telomere length is associated—in opposing directions—with cardiovascular disease and cancer risk. This phenomenon of the cancer-cardiovascular disease trade-off largely defines longevity of contemporary humans. And telomere length is not a good predictor of mortality. After adjusting for age and sex, researchers found that more than a dozen other measurements—from

self-reported health status to C-reactive protein (CRP) levels – were better at predicting five-year mortality. Another potential weakness of the BioViva data is measurement error. The 9% difference between Parrish's before and after telomere lengths is within the measurement error of most laboratories; most telomere-length assays have a variance of 8%. In addition, regarding the other gene therapy Parrish received—the gene encoding the follistatin protein—is supported by human data, at least in the context of people with muscle disorders. There are not yet data demonstrating the effects of follistatin gene therapy (FGT) on aging-related muscle loss. Follistatin inhibits myostatin, which puts the breaks on muscle growth and therefore makes it an attractive therapy for muscular dystrophies (MD). Early clinical trials on six people with Becker muscular dystrophy (BMD), for instance, showed that four of them could walk longer distances after the follistatin gene therapy.

Apparently, the assumption is that extending telomeres is the solution to stop the normal process of aging, which unavoidably ends in death. Yet, the aging process is not dependent on one or two genes, but it is more complicated. During life, we accumulate a series of damages to our DNA, which eventually will impact our bodies. Maybe, it would be wiser if before trying to extend life, we make sure that we can prevent neurodegenerative diseases like Alzheimer's, dementia, etc. (Fymat)

Deleting 238 age-related genes

A decade-long effort of tweaking yeast genomes has led scientists at the Buck Institute for Research on Aging and the University of Washington to discover 238 age-related genes (238ARD) which, when removed, extend the lifespan of yeast by a massive 60%. Many of the genes and genetic pathways involved in the research are also found in humans, so there is a promising possibility that this genetic editing could be replicated in humans. If any of those specific 238 genes were removed, the mother cells underwent an increased number of cell divisions.

Is the elixir of life hiding in ancient bacteria in Siberia?

Ancient bacteria that have survived for 3.5 million years have been discovered in the Siberian permafrost. Dr. Anatoli Brouchkov of Moscow State University first found the bacteria, called *Bacillus F,* on Mamontova Gora in Siberia's Sakha Republic in 2009. After thawing out, the bacteria came right back to life. The researchers found that the **bacteria had survived** untouched in the ice for up to 3.5 million years, and yet appeared remarkably youthful. They possessed some type of natural defense mechanism against aging and deterioration. Initial tests showed that the bacteria can even grant their rejuvenating powers to other organisms: Exposure to extracts isolated from *Bacillus F* dramatically extended the lifespans of fruit flies and mice. As the dosage of *Bacillus F* increased in concentration, fruit fly larvae actually grew larger and faster. The fruit flies also became more resistant to stress like heat shock and ultraviolet radiation, prompting the researchers to suspect that *Bacillus F* somehow stimulates natural mechanisms for repairing damage to DNA and important proteins. Brouchkov announced that his team has now finished decoding the genome of *Bacillus F*. The key question remains what provides the vitality of this bacteria, but it is as complicated as which human genes are responsible for cancer and how to cure it.

The long-living bacteria had an equally extraordinary effect on mouse models. When injected with the extract, mice lived an average of 308 days longer than the control group's average lifespan of 589 days. (an increase of ~ 52%). Epidemiologist Viktor Chernyavsky also claims that the bacteria improved the fertility of mice subjects. He suspects that the bacteria synthesize a compound responsible for their long-lasting vigor, and that it can activate the immune systems of other animals to create a similar effect.

A team of researchers under Professor Sergey Petrov at the Tyumen Scientific Center expanded experiments with *Bacillus F* to other systems, including tiny crustaceans called copepods and human blood cells. The bacteria strengthened the immune systems of the test subjects. Petrov has also begun to test the bacteria's effect on crops, with encouraging results: The crops grew faster, produced a greater yield, and are even more resistant to frost. The bacteria enhanced photosynthesis.

Further research will reveal how exactly these bacteria work their magic, and which genes are responsible.

Other futuristic technologies

Prolonging life may only be the beginning! In the next phase, not just average lifespans but maximum lifespans will rise. Thus:

- ***Worn-out or defective body parts:*** Will be repaired or replaced. Stem cells will upgrade worn-out tissues.
- ***DNA optimization:*** Will be optimized for long life.
- ***Anti-aging drugs****:* Will become routinely available.
- ***Blood transfusions:*** From the young into the old.
- ***Organ growing (***or ***"printing"):*** Organs will be grown from scratch. At the moment, these "organoids" are small, imperfect, and used mainly for drug testing... but that will surely change.
- ***Gene varieties that prolong lives:*** Longevity is known to run in families, which suggests that particular gene varieties prolong life. Modern gene-editing techniques might be used to make crucial, life-extending tweaks to the DNA of those who need them.

The above life-extending biotechnologies, the underlying processes and their proponents are summarized in Table 17.1.

Table 17.1 - Summary of life-prolonging biotechnologies

Biotechnology	Process	Proponent(s)
Hacking the code of life	1 million gene database	Craig Venter
Medical nanobots	Nanobots complementing the immune system	Ray Kurzweil
Gene therapies (telomeres; folistatin)	Elongating telomeres	o Maria Blasco o Elizabeth Parrish
Age-related genes	Gene deletions	o Buck Institute for Aging o University of Washington
Ancient *Bacillus F*	Transplants	o Anatoli Brouchkov o Sergey Petrov

Others	o Stem cells o DNA optimization o Anti-aging drugs o Blood transfusions o Organ growing ("printing") o Life-prolonging gene varieties	

Conclusions and take-aways

- Many of the causes of aging that may be happening prematurely can be modified through our behaviors. In the end, aging is inevitable. Taking care of body and mind and embracing the changes as they come goes a long way toward a healthier and, hopefully, longer lifespan.
- It is not true that older people need less sleep (see also Chapter 5). For a number of reasons, older people have trouble falling asleep and staying asleep. As we age, our bodies change, impacting the length and quality of our sleep. One or more of these factors may apply: Hormones, melatonin, and growth hormone.
- Health conditions and lifestyle can interfere with sleep. Identifying the underlying cause(s) and making appropriate changes may help improve sleep.
- The time of life when age-related changes appear depends on a variety of factors, including: Genetics, diet, culture, activity levels, and environmental exposure.
- Efforts should be made to prevent the aging of the cardiovascular system, the brain and nervous system, and the bones and muscles.
- Healthspan extension and anti-aging research seek to extend the span of health in the old as well as slow aging or its negative impacts such as physical and mental decline. Measures that may extend lifespans may simultaneously also extend healthspans
- Lifespan extension is the concept of extending the human lifespan, either modestly through improvements in medicine or dramatically by increasing the maximum lifespan beyond its generally-settled limit of 125 years.

- If life extension were to become a possibility, the ethical ramifications would require bioethical debates.
- Aging and senescence can be combated vigorously by pursuing one or more of three approaches: Calorie restriction and its genetic emulation (as demonstrated in animals); interference with metabolic processes to lessen damage; and alleviation of the molecular damage itself. In summary, the candidate causes of aging have been narrowed to the following: Mutations accumulation (including nuclear epimutations); extra-cellular aggregations; cross-linkings outside cells; cell defects; and nuclear mutations (not too important).
- Numerous research groups are now working on extending life and combating senescence of old age. The approaches consist of: Hacking the code of life; the coming of age of medical nanobots; gene therapy to combat old age and increasing muscle mass; deleting 238 age-related genes; and unraveling the elixir of life hiding in ancient bacteria in Siberia. Further research is certainly needed in each of these approaches. Other futuristic and not so futuristic technologies have also been briefly discussed: repairing or replacing worn-out tissues and body parts including using stem cells; optimizing DNA for a longer life; transfusing blood from young persons to older ones; producing anti-aging drugs; growing (or "printing") organs from scratch; editing those gene varieties that prolong life; and others.

Sidebar 17.1 - On sleep

There is a myth that older people need less sleep. That is simply not true. All adults need between seven and nine hours of sleep each night. As we age, it gets more difficult to get a good night's sleep. That does not mean we do not still need seven to nine hours. One of the challenges to healthy aging is troubleshooting sleep to ensure that we are getting enough rest for good health.

Sleep Changes in Older Adults

For a number of reasons, older people have trouble falling asleep and staying asleep. As we age, we may notice some of the following:

- Tendency to fall asleep in the early evening and wake up in the early morning.
- Taking longer to fall asleep.
- Sleep is less deep.
- Waking up three or four times a night.
- Frequent nighttime bathroom trips.
- Sleep is not as restful or satisfying.

As we age, our bodies change. These changes impact the length and quality of our sleep. Depending on our situation, one or more of these factors may apply:

- **Hormones:** As we age, our bodies secrete less of two important sleep hormones: melatonin and growth hormone.
- **Melatonin:** It is important because changes in the level of this hormone control our sleep cycle. With less melatonin, many older adults feel sleepy in the early evening and wake up in the early morning. They also may have more trouble falling asleep.
- **Growth hormone:** This is what makes children sleep so deeply. As we age, our body secretes less of this hormone and deep sleep becomes more difficult.
- **Menopause:** This causes a lot of hormonal changes in women, sometimes resulting in night sweats and other symptoms that interfere with sleep.

Interference of health conditions

Health conditions can interfere with sleep. As we age, we are more likely to develop a chronic illness. These illnesses result in changes in our body that interfere with normal sleep. By managing our health condition well, we can minimize this effect. Examples of how some illnesses interfere with sleep are:

- Some health conditions (like arthritis) cause pain, which makes it difficult to fall asleep.

- Other conditions (like diabetes or enlarged prostate) may cause one to use the bathroom frequently during the night, which interrupts deep sleep.
- Heart disease, high blood pressure, and other cardiovascular conditions may cause one to wake suddenly due to breathing difficulties or changes in heart rate.
- Parkinson's disease, Alzheimer's disease, and mental illnesses may cause anxiety that interferes with sleep.

Lifestyle interferences

- As we age, our daily routines change. These changes can affect our sleep. By increasing exercise and time spent outdoors and decreasing napping, we can improve both the length and quality of our sleep.
- Older people get less exercise.
- Sunlight helps our body to produce melatonin, which regulates our sleep cycle. It is recommended to get at least two hours of exposure to bright light each day. If this is difficult, consider using a full-spectrum light indoors.
- While napping can be great, napping more than 20 minutes a day may be interfering with sleep.
- Alcohol, caffeine, and nicotine. These three culprits will wreak havoc on sleep. It is recommended not to use any of these within three hours of going to bed.
- As we age, it is more likely that we are taking one or more medications. These medications can often interfere with sleep. Changing the schedule at which they are taken may be beneficial. Alternatively, it may be possible to replace those medications with others that may have no such side effect. (Some common medications that are known to interfere with sleep include some high blood pressure medications, antidepressants, steroids, some decongestants, and bronchodilators.)

What to do to improve sleep

Identifying the underlying cause(s) and making appropriate changes may help improve sleep. If lack of sleep is due to illness or medication, the possibility of changing the schedule of taking the medication or/and a change in it may

be the solution. Likewise, exercise and sunlight every day may also help sleep. However, if it does not improve, it may be caused by a sleep disorder. Health conditions that prevent a person from falling asleep or staying asleep include sleep apnea and insomnia, which can be treated. Whatever one does, one should not accept being tired as part of getting older.

18

Therapies for retarding or reversing aging

Contents

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Therapies for retarding or reversing aging

Reversing key markers of aging

Lengthening human telomeres through treatment (a specialized form of oxygen therapy) appears to reverse some biological markers of aging, according to a small new study. This first evidence is in need of more research to better understand what that means for chronic illness and longevity.

As cells divide and reproduce in our bodies, they gradually deteriorate, and our mental and physical health decline as a result. In a potential breakthrough for anti-aging research, a recent study (Efrati, 2023) has shown that cellular degeneration may be delayed or even reversed. In this study, an experimental type of hyperbaric oxygen therapy appeared to improve two key markers of biological aging, which had ben linked to age-related diseases: telomeric length and number of senescent cells. Following the treatment, participants had a significant increase in the length of their telomeres (an astounding increase of more than 20%) and a significant decrease in senescent cells. (Note: These claims have not been generally supported and are undergoing independent verification.)

Hyperbaric oxygen therapy (HOT) has previously been used as treatment for a wide variety of conditions, including carbon monoxide poisoning and decompression sickness (as experienced by scuba divers and astronauts). But the therapy used in this study is different from what is commonly available in some specialized clinics.

In the above experiment, the therapy floods the body with oxygen. Five days a week for three months, participants spent 90 minutes inside a compression chamber, breathing 100% oxygen from a mask, with five-minute breaks at regular intervals (every 20 minutes) to breathe normal air at normal levels. The body interprets this abrupt change as a sudden lack of oxygen, creating a biological cascade (a chain reaction) that initiates the generation of new tissue and, more importantly, activates telomeres on the cellular level. Changing the environmental conditions can, in turn, change the basic biology and increase telomere length. However, the long-term effects of the treatment are still unknown as it is not clear how lengthening telomeres might affect aging, chronic illness, and longer lifespan. Nonetheless, the research has shown that telomeres can be measured, variations in their length can be assessed, and it may be possible to control this important marker of aging.

Accordingly, it might be possible to treat aging as a preventable disease, and stave-off related illnesses (cancer, diabetes, cognitive decline, etc.). While the therapy is promising, even these early results suggest that it is not a magic solution to all age-related health issues. Lifestyle factors **DESS** (**D**iet, **E**xercise, **S**leep, **S**tress, etc.) still play a key role in aging and may conceivably counter the beneficial effects of the treatment.

Further, the experimental therapy is promising but expensive, time-consuming, and not yet readily available.

Popular anti-aging hormone supplements

Is DHEA an anti-aging supplement?

Dehydroepiandrosterone (DHEA), also known as androstenolone, is an endogenous steroid hormone precursor and one of the most abundant

circulating steroids in humans. It is produced in the adrenal glands, the gonads, and the brain. It functions as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex steroids both in the gonads and in various other tissues. However, DHEA also has a variety of potential biological effects in its own right, binding to an array of nuclear and cell surface receptors, and acting as a neurosteroid and modulator of neurotrophic factor receptors. It decreases naturally with age. Anti-aging doctors claim that DHEA supplementation can reduce the effects of aging. The claim includes that DHEA supplements can increase muscle mass and even burn fat.

Human growth hormone - A fountain of youth?

An entire industry has been created to sell human growth hormone (HGH) injections as a "cure" for aging. This stemmed from a small study done in the early 1990s. The claim is that HGH can decrease the effects of aging and leave you stronger and feeling younger than ever before. Why all the hype? A year's supply of HGH can cost as much as $15,000 dollars.

Melatonin and anti-aging

Melatonin is an important hormone in our body for regulating sleep. There have been some claims that melatonin can reverse the effects of aging. These claims are founded on a false belief that melatonin levels decrease with aging. However, melatonin has been found useful in a variety of conditions, mostly related to sleep disorders.

Estrogen, menopause, and aging

Estrogen is one of the most studied and prescribed hormones. For years, women were placed on estrogen replacement therapy (ERT) to treat the symptoms of menopause. As more data piled up, the risks and benefits of menopausal replacement therapy became more complicated.

Testosterone and male aging

As men age, testosterone levels decrease. Because of this, there has been a buzz in treating male aging with testosterone. These advertising campaigns overlook two facts: The drop in male testosterone happens gradually and is in no way similar to menopause in women, and supplementing testosterone does not help men who have normal levels for their age.

Adding health years to life

Interventions in diet, exercise, and mental outlook could slow down age-related diseases and aging. It is unclear, however, how much longevity could be increased. Even "super-agers" (individuals born with more favorable tiny genetic differences from other human beings) have an age upper limit. The current record, Jeanne Louise Calment of France, lived until the ripe old age of 122! Nonetheless, based on a still debated "mortality plateau" observed by statisticians, some experts believe that a higher record for our species might still be set, perhaps by the end of this century.

The "mortality plateau" for very old people goes like this: Although the chance of dying in a given year increases with age, it seems to stop increasing and levels off after age 105; beyond that, it becomes basically a random event. This does not necessarily imply that super-agers will live longer lives than before. It is true, however, that the maximum human life expectancy has increased by about three months per year since the mid-1800s or by about 45 years. But, this increase could be explained by fewer early and midlife deaths. Thus, the maximum human life span could be extended by continuing to "avert early and mid-life deaths", which simply increases the pool of people who could (but, not necessarily will) live a really long time.

Pending a definitive assessment of the life span of our species, it is nonetheless clear that certain lifestyles help *individuals* live longer than they otherwise would - including the genetically blessed. According to Harvard researchers, healthy habits add nearly 15 years of life expectancy. Unfortunately,

not enough people can access healthy lifestyles and we are getting sick and dying earlier across economic levels. For example, in the U.S., people under

65 in the richest areas have higher mortality than those in the poorest areas of Europe.

Findings from longevity research could support better health in old age, with fewer age-related diseases and disabilities. Interestingly, many scientists believe that a certain amount and type of stress to induce hormesis can help, thanks to evolution. ("Hormesis" is a process in which various stressors - such as those related to diet and exercise - seem to activate genes that slow down cell growth and aging.)

Switching on the longevity genes

There are three known ways of switching on the longevity genes:

Nutritional evolutionary scarcity causing stress

Stress that is good for longevity can be caused by nutrition. Our bodies still infer a state of evolutionary scarcity if we consume lots of vegetables, switching on the longevity genes. Indeed, such a diet is associated with longer lives. However, becoming a full-fledged vegetarian probably is not necessary. To maximize what longevity experts call "healthspan", at least 50% of protein should come from vegetable sources and the rest mostly from fatty fish while moderating the intake of starchy carbohydrates (potatoes, pasta, etc.) and replacing them with foods such as lentils or extra vegetables, which have more fiber and minerals than refined carbs. Research has shown that older people who routinely devour such starchy carbohydrates may be more likely to become cognitively impaired.

Calorie restriction without malnutrition

Another signal of scarcity that seems to switch on longevity genes is the restriction of all foods. Although water-only fasting over several days can be dangerous, "fasting-mimicking" diets — very low-calorie, five-day eating plans that trick the body into thinking it is fasting while allowing some foods and nutrients — have been shown to be safer and to play a major part in maximizing longevity. Research continues on various fasting regimens, including: avoidance of food during specific windows of the day without dropping overall calorie intake and calorie restriction without malnutrition.

An added benefit simply comes from losing weight. As is known, obesity is a risk factor for inflammation, and chronic, low-grade inflammation can accelerate aging in a process known as "inflammaging".

Optimal exercise simulating evolutionary stressful environments

Optimal exercise can further simulate the evolutionary stressful environments by duping genes into extending the health span.

A 2021 Mayo Clinic research publication suggests an optimal amount of exercise: 2.6 to 4.5 hours per week. Cardio workouts may extend longevity by multiplying mitochondria as mitochondrial dysfunction results in inflammaging in humans.

High-intensity interval training, or HIIT, may be particularly effective in adding to longevity, reversing many age-related differences in how older people synthesize proteins, buffering their mitochondria. Strength training may also partially reverse aspects of aging.

However, two notes of caution: (a) Previous research has shown an association between extreme exercise and health problems, such as premature aging of the heart; and (b) diet and exercise regimens cannot magically undo a lifetime of mistakes.

Beyond diet and exercise

Other drivers of longevity include long-term, loving relationships. In a nearly 80-year long study, researchers found that the most important factor in a long, healthy life was having a close partner. Another protective factor is optimism, which was found to be associated with exceptional longevity.

Conclusions and take-aways

- As cells divide and reproduce in our bodies, they gradually deteriorate, and our mental and physical health decline as a result. Luckily, if independently verified, cellular degeneration may be delayed or even reversed through an experimental type of hyperbaric oxygen therapy.

- Lengthening human telomeres through treatment (a specialized form of oxygen therapy) appears to reverse some biological markers of aging. The body interprets this abrupt change as a sudden lack of oxygen, creating a biological cascade (a chain reaction) that initiates the generation of new tissue and, more importantly, activating and increasing telomeric length at the cellular level. However, the long-term effects of the treatment are still unknown as it is not clear how lengthening telomeres might affect aging, chronic illness, and longer lifespan.
- It might be possible to treat aging as a preventable disease, and stave-off related illnesses (cancer, diabetes, cognitive decline, etc.). While promising, oxygen therapy is not a magic solution to all age-related health issues and it is expensive, time-consuming, and not yet readily available.
- Popular anti-aging hormone supplements include Dehydroepiandrosterone (DHEA), human growth hormone (HGH), melatonin, estrogen, and testosterone.
- Interventions in diet, exercise, and mental outlook could slow down age-related diseases and aging. It is unclear, however, how much longevity could be increased.
- The chance of dying in a given year increases with age, but seems to stop increasing and levels off after age 105 (the "mortality plateau").
- The maximum human life span could be extended by continuing to "avert early and midlife deaths", which simply increases the pool of people who could (but, not necessarily will) live a really long time.
- Certain lifestyles help *individuals* live longer than they otherwise would - including the genetically blessed. Findings from longevity research could support better health in old age, with fewer age-related diseases and disabilities.
- There are three known ways of switching on the longevity genes: Nutritional evolutionary scarcity causing stress, calorie restriction without malnutrition, and optimal exercise simulating evolutionary stressful environments. Optimal exercise can further simulate the evolutionary stressful environments by duping genes into extending the health span.

- Beyond diet and exercise, other drivers of longevity include long-term, loving relationships - one (if not) the most important factor in a long, healthy life is having a close partner. Another protective factor is optimism.

19

Research and latest developments

Contents

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Research and latest developments

Aging research has progressed along different streams. A United Nations Populations Fund (UNFPA) report about aging in the 21st century highlighted the need to " *…develop a new rights-based culture of aging and a change of mindset and societal attitudes towards aging and older persons - from welfare recipients to active, contributing members of society*". The report further stated that this "*…requires, among other things, working towards the development of international human rights instruments, their translation into national laws and regulations, and affirmative measures that challenge age discrimination and recognize older people as autonomous subjects*". Older people can and do make numerous contributions to society.

The facts of aging—what is happening on a biochemical, genetic, and physiological level—remain rich for exploration. The following paragraphs explore only a few of these research areas. Research on aging is dynamic, constantly evolving based on new discoveries. This chapter introduces some key areas of research into the biology of aging, each area being a part of a larger field of scientific inquiry.

The several forms of agism

"Chronological aging" may be distinguished from "social aging" (cultural age-expectations of how people should act as they grow older) and "biological aging" (an organism's physical state as it ages).

Expression of age

Different cultures express age in different ways. The age of an adult human is commonly measured in whole years since the day of birth. Arbitrary divisions set to mark periods of life may include juvenile (from infancy through childhood, pre-adolescence, and adolescence), early adulthood, middle adulthood, and late adulthood. Informal terms include "tweens", "teenagers", "twenty-something", "thirty-something", etc. as well as "denarian", "vicenarian", "tricenarian", "quadragenarian", etc.

Age classification and corresponding specific activities

Most legal systems define a specific age for when an individual is allowed or obliged to do particular activities. These age specifications include voting age, drinking age, age of consent, age of majority, age of criminal responsibility, marriageable age, age of candidacy, and mandatory retirement age.

Admission to a movie, for instance, may depend on age according to a motion picture rating system. A bus fare might be discounted for the young or old. Each nation, government, and non-governmental organization has different ways of classifying age.

Economics of agism

Population aging is the increase in the number and proportion of older people in society. It has three possible causes:

- **Migration;**
- **Longer life expectancy** (decreased death rate); and
- **Decreased birth rate.**

Aging has a significant impact on society. Young people tend to have fewer legal privileges (if they are below the age of majority), are more likely to push for political and social change, develop and adopt new technologies, and need education. Older people have different requirements from society and government and, frequently, have differing values as well, such as for property and pension rights.

In the 21st century, one of the most significant population trends is aging. Currently, over 11% of the world's current population are people aged 60 and older and the UNFPA estimates that by 2050 that number will rise to approximately 22%. Aging has occurred due to development which has enabled better nutrition, sanitation, health care, education, and economic well-being. Consequently, fertility rates have continued to decline and life expectancy has risen. Life expectancy at birth is over 80 now in 33 countries.

Aging is a "global phenomenon", that is occurring fastest in developing countries, including those with large youth populations, and poses social and economic challenges to the work which can be overcome with "*the right set of policies to equip individuals, families and societies to address these challenges and to reap its benefits*".

As life expectancy rises and birth rates decline in developed countries, the median age rises accordingly. The United Nations (UN) indicates this process is taking place in nearly every country in the world. A rising median age can have significant social and economic implications, as the workforce gets progressively older and the number of old workers and retirees grows relative to the number of young workers. Older people generally incur more health-related costs than do younger people in the workplace and can also cost more in worker's compensation and pension liabilities. In most developed countries, an older workforce is somewhat inevitable. In the United States, for instance, the Bureau of Labor Statistics (BLS) estimates that one in four American workers will be 55 or older by 2020.

Among the most urgent concerns of older persons worldwide is income security. This poses challenges for governments with aging populations to ensure investments in pension systems continue to provide economic independence and reduce poverty in old age. These challenges vary for

developing and developed countries. The UNFPA stated that, "…*sustainability of these systems is of particular concern, particularly in developed countries, while social protection and old-age pension coverage remain a challenge for developing countries, where a large proportion of the labour force is found in the informal sector*."

The global economic crisis has increased financial pressure to ensure economic security and access to health care in old age. To elevate this pressure "*social protection floors must be implemented in order to guarantee income security and access to essential health and social services for all older persons and provide a safety net that contributes to the postponement of disability and prevention of impoverishment in old age*".

It has been argued that population aging has undermined economic development and can lead to lower inflation because elderly individuals care especially strongly about the value of their pensions and savings. Evidence suggests that pensions, while making a difference to the well-being of older persons, also benefit entire families, especially in times of crisis when there may be a shortage or loss of employment within households. Due to increasing share of the elderly in the population, health care expenditures will continue to grow relative to the economy in coming decades. This has been considered as a negative phenomenon and effective strategies, like labor productivity enhancement, should be considered to deal with negative consequences of aging.

According to a Yale School of Public Health study, agism costs the United States $63 billion in one year.

Sociological aspects of agism

In the field of sociology and mental health, aging is seen in five different views as:

- **Maturity;**
- **Decline;**
- **Life-cycle event;**

- **Generation;** and
- **Survival.**

Positive correlates with aging often include among many others: Economics, employment, marriage, children, education, and sense of control.

The social science of aging includes:

- **Disengagement theory;**
- **Activity theory;**
- **Selectivity theory;** and
- **Continuity theory.**

Retirement, a common transition faced by the elderly, may have both positive and negative consequences. Some theorists argue there is a need to develop new definitions of aging and, for instance, a bio-techno-social definition of aging has been suggested.

There is a current debate as to whether or not the pursuit of longevity and the postponement of senescence are cost-effective health care goals given the finite health care resources specially because of the accumulated infirmities of old age. Some question the "value" of life beyond a certain age, others state that life can be worthwhile during old age and that longevity should be pursued in association with the attainment of quality of life. Still others claim that postponement of senescence as well as happiness and wisdom can be attained in old age in a large proportion of those who lead healthy lifestyles and remain intellectually active.

Health care demands

With age, inevitable biological changes occur that increase the risk of illness and disability. Thus, UNFPA states that: *"A life-cycle approach to health care – one that starts early, continues through the reproductive years and lasts into old age – is essential for the physical and emotional well-being of older persons, and, indeed, all people. Public policies and programs should additionally address the needs of older impoverished people who cannot afford health care*".

Many societies in Western Europe and Japan have aging populations. While the effects on society are complex, there is a concern about the impact on health care demand. The large number of suggestions in the literature for specific interventions to cope with the expected increase in demand for long-term care in aging societies can be organized under four headings:

- **Improve system performance;**
- **Redesign service delivery;**
- **Support informal caregivers;** and
- **Shift demographic parameters.**

However, the annual growth in national health spending is not mainly due to increasing demand from aging populations, but rather has been driven by rising incomes, costly new medical technology, a shortage of health care workers, and informational asymmetries between providers and patients. A number of health problems become more prevalent as people get older. These include mental health problems as well as physical health problems, especially dementia (see my articles on the dementia coming pandemic).

Thus, for the U.S., it has been estimated that population aging only explains 0.2% of the annual growth rate in medical spending of 4.3% since 1970. In addition, certain reforms to the Medicare system decreased elderly spending on home health care by 12.5% per year between 1996 and 2000.

Self-perception

Beauty standards have evolved over time, and as scientific research in cosmeceuticals has increased, the industry has also expanded; the kinds of products they produce (such as serums and creams) have gradually gained popularity and become a part of many people's personal care routine.

The increase in demand for cosmeceuticals has led scientists to find ingredients for these products in unorthodox places. For example, the secretion of *cryptomphalus aspersa* (or brown garden snail) has been found to have antioxidant properties, increase skin cell proliferation, and increase extracellular proteins such as collagen and fibronectin (important proteins

for cell proliferation). Another substance used to prevent the physical manifestations of aging is OnobotulinumtoxinA, the toxin injected for Botox.

In some cultures, old age is celebrated and honored. In Korea, for example, a special party called "hwangap" is held to celebrate and congratulate an individual for turning 60 years old. In China, respect for elderly is often the basis for how a community is organized and has been at the foundation of Chinese culture and morality for thousands of years. Older people are respected for their wisdom and most important decisions have traditionally not been made without consulting them. This is a similar case for most Asian countries such as the Philippines, Singapore, Thailand, Vietnam, etc.

Positive self-perceptions of aging are associated with better mental and physical health and well-being. They have been correlated with higher well-being and reduced mortality among the elderly. Various reasons have been proposed for this association. People who are objectively healthy may naturally rate their health better than that of their ill counterparts, though this link has been observed even in studies which have controlled for socioeconomic status, psychological functioning and health status. This finding is generally stronger for men than women, though this relationship is not universal across all studies and may only be true in some circumstances.

As people age, subjective health remains relatively stable, even though objective health worsens. In fact, perceived health improves with age when objective health is controlled. This phenomenon is known as the "paradox of aging". It may be a result of social comparison; for instance, the older people get, the more they may consider themselves in better health than their same-aged peers. Elderly people often associate their functional and physical decline with the normal aging process.

One way to help younger people experience what it feels like to be older is through an aging suit. There are several different kinds of suits including the GERT (named as a reference to gerontology), the R70i exoskeleton, and the AGNES (Age Gain Now Empathy Suit) suits. These suits create the feelings of the effects of aging by adding extra weight and increased pressure in certain points like the wrists, ankles and other joints. In addition, the various suits have different ways to impair vision and hearing to simulate the loss of these

senses. To create the loss of feeling in hands that the elderly experience, special gloves are a part of the uniforms. Use of these suits may help to increase the amount of empathy felt for the elderly and could be considered particularly useful for those who are either learning about aging, or those who work with the elderly, such as nurses or care center staff.

Design is another field that could benefit from the empathy these suits may cause. When designers understand what it feels like to have the impairments of old age, they can better design buildings, packaging, or even tools to help with the simple day-to-day tasks that are more difficult with less dexterity. Designing with the elderly in mind may help to reduce the negative feelings that are associated with the loss of abilities that the elderly face.

Healthy aging

The healthy aging framework, proposed by the World Health Organization (WHO) "*operationalizes health as functional ability, which results from the interactions of intrinsic capacity and the environments*".

Intrinsic capacity

Intrinsic capacity (IC) is a construct encompassing people's physical and mental abilities which can be drawn upon during aging. It comprises the domains of:

- **Cognition;**
- **Locomotion;**
- **Vitality/nutrition;**
- **Psychological;** and
- **Sensory (**visual and hearing**).**

A recent study found four "profiles" or "statuses" of IC among older adults, namely:

- **High IC** (43% at baseline),;
- **Low deterioration with impaired locomotion** (17%);

- **High deterioration without cognitive impairment** (22%); and
- **High deterioration with cognitive impairment** (18%).

Over half of the study sample remained in the same status at baseline and follow-up (61%). Around one-fourth of participants transitioned from the high IC to the low deterioration status, and only 3% of the participants improved their status. Interestingly, the probability of improvement was observed in the status of high deterioration. Participants in the latent statuses of low and high levels of deterioration had a significantly higher risk of frailty, disability, and dementia than their high IC counterparts.

Successful aging

The concept of successful aging (SA) can be traced back to the 1950s and was popularized in the 1980s. Traditional definitions have emphasized the absence of physical and cognitive disabilities. In their 1987 article, Rowe and Kahn characterized successful aging as involving three components:

- **Freedom from disease and disability;**
- **High cognitive and physical functioning;** and
- **Social and productive engagement.**

With current knowledge, scientists also started to focus on learning about the effect of spirituality in successful aging. There are some differences in cultures as to which of these components are the most important. Most often, across cultures, social engagement was the most highly rated but depending on the culture the definition of successful aging changes.

Ameta-analys is showed that loneliness carries a higher mortality risk than smoking.

The beneficial effects of **DESS** (Diet, Exercise, Stress, Sleep) have already been discussed earlier. In particular, the Mediterranean diet is credited with lowering the risk of heart disease and early death. The major contributors to mortality risk reduction appear to be a higher consumption of vegetables, fish, fruits, nuts, and monounsaturated fatty acids, such as by consuming olive oil. However, as of 2021, there is insufficient clinical evidence that calorie restriction or any dietary practice affects the process of aging.

Regarding exercise, people who participate in moderate to high levels of physical exercise have a lower mortality rate compared to individuals who are not physically active. The majority of the benefits from exercise are achieved with around 3500 metabolic equivalent (MET) minutes per week. For example, climbing stairs 10 minutes, vacuuming 15 minutes, gardening 20 minutes, running 20 minutes, and walking or bicycling for 25 minutes on a daily basis would together achieve about 3000 MET minutes a week.

From the (U.S.) National Institute on Aging

The following are selected future research directions at the (U.S.) National Institute on Aging (NIA) regarding the biology of aging.

Biological interventions to promote healthy aging

Interventions include:

Counteracting the effects of aging by hormonal and dietary supplements

There are concerns that many middle-aged and older people may be taking hormonal and dietary supplements (estrogen, testosterone, human growth hormone, melatonin, and DHEA) before safety and efficacy of these substances for so-called "anti-aging" purposes have been adequately assessed. Although levels of some hormones may decline with age, maintaining levels that are normal at younger ages may not be needed, or even desirable, as a person grows older. Even if effective, supplementation may entail risks. More research is needed to determine how the biologic action of these hormones changes in older people and to assess whether their replacement will improve health.

Calorie restriction

Calorie restriction (CR) is another biological intervention that may promote healthy aging (see also Sidebar 19.1). Some of CR's effects on longevity have been linked to changes in specific metabolic pathways. Studies are now planned to define the role of energy metabolism and metabolic regulation

in mammalian aging, longevity and age-related disease, and uncover the cellular and molecular mechanisms that may be regulating aging processes, including those affected by CR. Most recently, researchers have identified changes in physiologic function in calorically restricted rhesus monkeys that suggest delays in aging-related decline. At this point, the effects of voluntary CR on lifespan and development of age-related diseases in humans are unknown. Preliminary human intervention studies are being designed to determine whether CR and physical activity differ in their long-term effects on obesity, body composition, and prevention and susceptibility to age-related diseases.

Understanding the genetic basis of aging, longevity, disease, and behavior

Interactions between genetic and environmental factors are major determinants of aging and longevity in many species, including humans. Studies at the NIA and elsewhere have begun to reveal the biologic factors associated with extended longevity in humans and animal models, implicating numerous genes in normal aging processes, age-related pathologies and diseases, and longevity. Some of these genes are associated with dramatic extension of lifespan. Using advanced technology, the NIA plans to accelerate its efforts to discover additional age- and longevity-related genes and to characterize their biological function. A new research initiative will extend studies of longevity-associated genes, changes in gene expression patterns, and the genetic epidemiology of human longevity. The ultimate goal of this effort is to develop interventions to reduce or delay age-related degenerative processes in humans. In addition, revolutionary advances in the fields of quantitative and molecular genetics hold great promise in the search for the genetic determinants of complex behaviors. Studies in humans can help identify the relative contributions of environment and heredity to dementia, cognitive abilities, physical functioning, well being, and social aging. New techniques can track the developmental course of genetic contributions to behavior, identify genetic heterogeneity, and explore genetic links between the normal and abnormal. Basic research will explore error accumulation in DNA with age and how the cell repairs such damage.

Exploring the potential of adult stem cells and cell replacement in aging

Stem cells in adult human tissues retain the capacity for self-renewal and the potential to become many of the cell types in the human body (see Chapter 11). This capacity holds enormous potential for cell replacement or tissue repair therapy in many degenerative diseases of aging, including Alzheimer's disease (AD), Parkinson's disease (PD), stroke, myocardial infarction, musculoskeletal disorders, immune system dysfunction, and diabetes. Emerging research findings suggest that it may be possible to harness the multipotential nature of adult stem cells to maintain tissue structure and function in aging. Much remains to be learned, however, about the basic biology of stem cells in animal models before effective cell therapy can be realized. The NIA is developing a research initiative on changes in stem cells and their environment with aging in animal models and in human nonfetal tissues. This research initiative will complement as well as encourage collaboration with other components of NIH.

From the American Federation for Aging Research

Epigenetic alterations

The genome is more than a long sequence of DNA letters. DNA strands are wound around spools of protein called histones, and both DNA and histones can have various chemical handles, cranks, and levers attached to them to help turn genes on or off. These handles, cranks, and levers comprise the epigenome.

The epigenome changes with age--levers are lost, added inappropriately, or shifted around. As a result, precise coordination of gene activity can be compromised. One particularly well-studied group of molecules that influence the epigenome is the sirtuins, molecules that remove one type of epigenetic handle. Interestingly, the epigenome can be modified by diet, other lifestyle factors, and pharmaceuticals.

Evidence that the epigenome affects aging comes mostly from the study of yeast, worms, and flies. However, dietary restriction in mice slows epigenetic

changes, and when mice are made deficient in one of the seven mouse sirtuins, they show signs of accelerated aging. Moreover, when that same sirtuin is superabundant, male mice live longer.

Loss of proteostasis

The main job of genes is to make proteins, which are the heart and soul of cell biology. Proteins regulate virtually all chemical reactions and provide cell structure. Protein homeostasis, or proteostasis, is the maintenance of all proteins in their original form and abundance.

In order to perform their duties, proteins must be folded in precise, complex shapes like origami. However, with age proteins are damaged by normal cellular process and when damaged begin to misfold. Misfolded proteins not only fail to perform their normal job, they can clump together, and become toxic. Alzheimer's disease may be an example of an age-related disease caused by protein misfolding.

The importance of maintaining proteostasis can be seen in the elaborate cellular systems for maintaining it: There are specialized molecular devices to repair and refold damaged proteins as well as to degrade irretrievably damaged proteins and replace them.

Several pieces of evidence highlight the role of proteostasis in aging: Misfolded proteins increase with age; protein misfolding occurs in the brain and muscle of Alzheimer's patients; both genetic and drug-induced enhancement of protein quality control will extend life in mice.

Dysregulated nutrient sensing

When nutrients are abundant, animals, including humans, grow and reproduce--the evolutionary imperative. When nutrients are scarce, evolution has designed animals to focus on maintenance and repair.

Studies have been designed to inhibit the signaling of nutrient abundance by reducing food, by fooling the body into thinking fewer nutrients are available

with drugs such as Rapamycin and by inhibiting the signals of insulin or its close relative, the insulin-like growth factor.

All of these strategies enhance health and longevity in mice and other species.

Mitochondrial dysfunction

Mitochondria—often called the "powerhouses of the cell"— are places where most of the cells' energy is produced. Unfortunately mitochondria also produce most of the free radicals or reactive oxygen species (ROS) in the cells.

As ROS damage nearly any molecule they touch, for many years it was thought that ROS were the major culprit behind aging and that minimizing them would lead to longer health and life. However, in the past decade, it was discovered that sometimes lowering ROS had no impact on health. Moreover, sometimes, actually increasing ROS by inhibiting mitochondrial function, seemed beneficial. The newer thinking is that ROS are important in signaling cellular stress.

Cells, organs, and tissues that sense stress increase their maintenance and repair processes in response to the stress. Current thinking suggests that ROS production should be not too much, not too little, just the right amount.

Cellular senescence

Cells that once replicated vigorously but have now entered a permanent non-dividing state are called senescent cells. They accumulate with age. Alas, these cells do not die but persist and secrete damaging molecules into the surrounding area.

Telomere attrition is one cause of cellular senescence, although other types of damage can also trigger this state. For years, it was debated whether senescent cells contributed to aging or were simply a protective mechanism against the development of cancer.

Recent work, in which mice were genetically engineered so that researchers could eliminate many of their senescent cells, has clearly shown many health benefits, including longer life. Work is now underway to identify drugs that target senescent cells for destruction.

Stem cell exhaustion

The ability of tissues and organs to regenerate and repair damage is critical to maintaining health. Bodies' ability to regenerate tissues and organs depends on healthy stem cells--the ultimate source of new cells in virtually every tissue.

Healthy stem cells must replicate when required, but not otherwise. The replication ability of stem cells--and their ability to replicate only when needed--declines with age.

Several labs have now shown that stem cell function can be resuscitated by external factors such as the as-yet-unidentified rejuvenating factor(s) found in the blood of young mice or humans, opening the door for possible pharmacological prolongation of stem cell health.

Altered intercellular communication

Although a number of other hallmarks of aging focus on processes that lead to deterioration of cells, appropriate communication among cells and tissues is also important to maintaining health. Hormones, for instance, are one way cells communicate. Hormones produced in the brain alter the way cells behave in the rest of the body and vice versa. The liver might chemically tell the brain to reduce hormone production or nerve cells that signal pain can chemically alert the immune system in the rest of the body. In relation to aging, perhaps the most important loss of appropriate communication in bodies is the low-level, chronic inflammation that occurs as age advances.

In youth, inflammation is mainly a response to injury that is turned off once the injury heals. In later life, low-level inflammation is not injury-related, but constant. Moreover, this inflammation is damaging to surrounding tissue.

Although the cause of age-related inflammation is unclear, considerable evidence points to senescent cells as the culprit.

Restoring proper intercellular communication could extend health by reducing chronic age-related inflammation. Additionally, investigators are studying how intercellular communication influences the rejuvenating properties of young blood; studies lend evidence that the blood of young animals contains molecules that can actually rejuvenate damaged heart, brain, and muscle in older adult animals.

Genomic instability

Each cell in the body--except red blood cells--contains the string of 3 billion DNA letters that defines the individual genome. Proper functioning of the genome is largely responsible for the smooth running of the body. However, the genome is under constant attack from both external sources such as radiation or pollution and internal sources such as oxygen free radicals.

By one estimate the DNA in each of the cells is damaged up to 1 million times per day. Fortunately, DNA also encodes a number of processes that detect and repair virtually all of this damage. Still, repair is not perfect and, with advancing age, damage to the genome accumulates. Cancer is one result of unrepaired DNA damage. In both humans and mice, individuals with compromised DNA repair processes show multiple signs of accelerated aging and that therapies such as dietary restriction reduce the rate of DNA damage accumulation: this gives evidence that genomic accumulation is fundamental to aging.

Telomere attrition

One specific type of genomic instability is telomere attrition. Telomeres are repetitive sequences of DNA that protect the ends of chromosomes and prevent them from being mistaken for broken DNA strands.

Telomere attrition, or shortening, is a specific type of DNA damage to the ends of chromosomes. Normal cell division shortens telomeres as do other

processes that damage DNA. When telomeres reach a critically short length, cells sense it and permanently turn off their replication machinery.

An enzyme called telomerase, which is turned off in most adult cells, can prevent telomere shortening and even restore telomere length. Evidence linking telomere attrition to aging is that telomeres shorten with age in both people and mice. Mice genetically engineered to lack telomerase have shown some symptoms of premature aging, and mice engineered to express higher levels of telomerase than normal have been reported to live longer.

As biomedical research on healthy aging continues to evolve, these hallmarks provide a foundation for our knowledge of the basic biology of aging.

Conclusions and take-aways

- Chronological aging may be distinguished from social aging (cultural age-expectations of how people should act as they grow older) and biological aging (an organism's physical state as it ages).
- Different cultures express age in different ways with arbitrary divisions set to mark periods of life.
- Most legal systems define age specifications for various activities of life phases.
- Population aging is the increase in the number and proportion of older people in society. It has three possible causes: Migration, longer life expectancy, and decreased birth rate.
- Aging has a significant impact on society.
- Aging is a "global phenomenon", that is occurring fastest in developing countries, including those with large youth populations, and poses social and economic challenges.
- As life expectancy rises and birth rates decline in developed countries, the median age rises accordingly. This process is taking place in nearly every country in the world.
- Among the most urgent concerns of older persons worldwide is income security. The global economic crisis has increased financial pressure to ensure economic security and access to health care in old age.

- It has been argued that population aging has undermined economic development and can lead to lower inflation because elderly individuals care especially strongly about the value of their pensions and savings.
- In the field of sociology and mental health, aging is seen in five different views as: Maturity, decline, life-cycle event, generation, and survival.
- Positive correlates with aging often include among many others: Economics, employment, marriage, children, education, and sense of control.
- The social science of aging includes several theories: Disengagement, activity, selectivity, and continuity.
- There is a current debate as to whether or not the pursuit of longevity and the postponement of senescence are cost-effective health care goals given the finite health care resources, specially because of the accumulated infirmities of old age.
- The large number of suggestions for specific interventions to cope with the expected increase in demand for long-term care in aging societies can be organized under four headings: Improve system performance, redesign service delivery, support informal caregivers, and shift demographic parameters.
- However, the annual growth in national health spending is not mainly due to increasing demand from aging populations, but rather has been driven by rising incomes, costly new medical technology, a shortage of health care workers, and informational asymmetries between providers and patients. decreased elderly spending on home health care by 12.5% per year between 1996 and 2000.
- Beauty standards have evolved over time, and as scientific research in cosmeceuticals has increased, the industry has also expanded; the kinds of products they produce (such as serums and creams) have gradually gained popularity and become a part of many people's personal care routine.
- Positive self-perceptions of aging are associated with better mental and physical health and well-being. They have been correlated with higher well-being and reduced mortality among the elderly.
- As people age, subjective health remains relatively stable, even though objective health worsens.

- The World Health Organization's healthy aging framework operationalizes health as functional ability, which results from the interactions of intrinsic capacity [Cognition, locomotion, vitality/nutrition, psychological, and sensory (visual and hearing)] and the environments.
- A recent study found four intrinsic capacity profiles: High, low deterioration with impaired locomotion, high deterioration without cognitive impairment, and high deterioration with cognitive impairment.
- Traditional definitions of successful aging emphasize the absence of physical and cognitive disabilities. They involve three components: Freedom from disease and disability, high cognitive and physical functioning, and social and productive engagement.
- With current knowledge, scientists also started to focus on learning about the effect of spirituality in successful aging.
- A meta-analysis showed that loneliness carries a higher mortality risk than smoking.
- The beneficial effects of **DESS** (Diet, Exercise, Stress, Sleep) have already been discussed earlier. However, there is insufficient clinical evidence that calorie restriction or any dietary practice affects the process of aging. Regarding exercise, people who participate in moderate to high levels of physical exercise have a lower mortality rate compared to individuals who are not physically active.
- Evidence that the epigenome affects aging comes mostly from the study of yeast, worms, and flies. However, dietary restriction in mice slows epigenetic changes and signs of accelerated aging.
- Protein homeostasis, or proteostasis, is the maintenance of all proteins in their original form and abundance. Misfolded proteins not only fail to perform their normal job, they can clump together, and become toxic. Several pieces of evidence highlight the role of proteostasis in aging
- When nutrients are abundant, animals, including humans, grow and reproduce--the evolutionary imperative. All strategies to deregulate nutrient sensing enhance health and longevity in mice and other species.
- Mitochondria produce most of the free radicals or reactive oxygen species (ROS) in the cells. Current thinking suggests that ROS production should be not too much, not too little, just the right amount.

- Senescent cells do not die but persist and secrete damaging molecules into the surrounding area. Telomere attrition is one cause of cellular senescence, although other types of damage can also trigger this state.
- Healthy stem cells must replicate when required, but not otherwise. The replication ability of stem cells--and their ability to replicate only when needed--declines with age.
- Although a number of hallmarks of aging focus on processes that lead to deterioration of cells, appropriate communication among cells and tissues is important to maintaining health. Hormones, for instance, are one way cells communicate. In relation to aging, perhaps the most important loss of appropriate communication in bodies is the low-level, chronic inflammation that occurs as age advances. Although the cause of age-related inflammation is unclear, considerable evidence points to senescent cells as the culprit.
- Restoring proper intercellular communication could extend health by reducing chronic age-related inflammation.
- Proper functioning of the genome is largely responsible for the smooth running of the body. However, the genome is under constant attack from both external sources such as radiation or pollution and internal sources such as oxygen free radicals. With advancing age, damage to the genome accumulates. Therapies such as dietary restriction reduce the rate of DNA damage accumulation.
- Telomeres are repetitive sequences of DNA that protect the ends of chromosomes and prevent them from being mistaken for broken DNA strands. One specific type of genomic instability is telomere attrition or shortening. Normal cell division shortens telomeres as do other processes that damage DNA. When telomeres reach a critically short length, cells sense it and permanently turn off their replication machinery. An enzyme called telomerase, which is turned off in most adult cells, can prevent telomere shortening and even restore telomere length.

Sidebar 19.1 – Medical effects of dietary restriction

Calorie restriction (or caloric restriction, or energy restriction) is a dietary regimen that reduces calorie intake without incurring malnutrition or a reduction in essential nutrients. In a number of species (yeast, fish, rodents, dogs, and others), calorie restriction without malnutrition has been shown to slow the biological aging process, resulting in longer maintenance of youthful health and an increase in both median and maximum lifespan. However, despite research extending over 70 years, the mechanism by which caloric restriction works is still not well understood. Some explanations include reduced core body temperature, reduced cellular divisions, lower metabolic rates, reduced production of free radicals, reduced DNA damage, and hormesis (the process in which various stressors - such as those related to diet and exercise - seem to activate genes that slow down cell growth and aging).

Using rhesus monkeys (which harbor 93% of the human genome), a collaboration of the U.S. National Institute on Aging (NIA) and the University of Wisconsin found that calorie restriction without malnutrition extended lifespan and delayed the onset of age-related disorders. In humans, the following must be noted regarding calorie restriction with sufficient nutrition:

Life-extending effect

Not shown to be universal.

Long-term health effects

Unknown.

Risks of malnutrition

If a restricted diet is not designed to include essential nutrients, malnutrition may result in serious deleterious effects, as shown in the Minnesota Starvation Experiment (MSE). This study was conducted during World War II on a group of lean men, who restricted their calorie intake by 45% for 6 months, and composed roughly 90% of their diet with carbohydrates. As expected, this

malnutrition resulted in many positive metabolic adaptations (e.g. decreased body fat, blood pressure, improved lipid profile, low serum T3 concentration, decreased resting heart rate, and whole-body resting energy expenditure), but also caused a wide range of negative effects (such as anemia, lower extremity edema, muscle wasting, weakness, neurological deficits, dizziness, irritability, lethargy, and depression).

Musculoskeletal losses

Short-term studies in humans report loss of muscle mass and strength and reduced bone mineral density (BMD), However, whether or not the reduction in BMD actually harms bone health is unclear. In a study in premenopausal women, BMD after weight loss was higher when normalized for body weight. Reduced BMD is also observed in humans undergoing long-term calorie restriction with adequate nutrition, but no fractures have been reported and the reduction in BMD was not associated with deleterious changes in bone microarchitecture.

Lower-than-normal body mass index, high mortality

Calorie restriction diets typically lead to reduced body weight, yet reduced weight can come from other causes and is not in itself necessarily healthy. In some studies, low body weight has been associated with increased mortality, particularly in late middle-aged or elderly subjects. Low body weight in the elderly can be caused by pathological conditions associated with aging and predisposing to higher mortality such as cancer, chronic obstructive pulmonary disorder (COPD) or depression) or of the cachexia (wasting syndrome) and sarcopenia (loss of muscle mass, structure, and function).

Case of young or pregnant individuals

Long-term caloric restriction at a level sufficient for slowing the aging process is generally not recommended in children, adolescents, and young adults because this type of diet may interfere with natural physical growth. In addition, mental development and physical changes to the brain take place in late adolescence and early adulthood that could be negatively affected

by severe caloric restriction. Pregnant women and women trying to become pregnant are advised not to practice calorie restriction, because low BMI may result in ovulatory dysfunction (infertility), and underweight mothers are more prone to preterm delivery.

Miscellaneous concerns

It has also been noted that people losing weight on such diets risk developing cold sensitivity, menstrual irregularities, and even infertility and hormonal changes.

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Frequently asked questions

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20 Frequently asked questions

Genetics and genomics

What are genes?

Genes are units of heredity that carry the instructions for making proteins. In turn, proteins direct the activities of cells and functions of the body. Examples of genetic or inherited disorders include cystic fibrosis (CF), Huntington's disease (HD), and phenylketonuria (PKU).

What is the difference between genetics and genomics?

Genetics is a term that refers to the study of genes and their roles in inheritance, i.e. the way that certain traits or conditions are passed down from one generation to another. Genetics involves scientific studies of genes and their effects.

Genomics is a more recent term that describes the study of all of a person's genes (the genome), including interactions of those genes with each other and with the person's environment. Genomics includes the scientific study of complex diseases such as heart disease, asthma, diabetes, and cancer because these diseases are typically caused more by a combination of

genetic and environmental factors than by individual genes. Genomics is offering new possibilities for therapies and treatments for some complex diseases, as well as new diagnostic methods.

Why are genetics and genomics important to my health?

Genetics and genomics both play roles in health and disease. Genetics helps individuals and families learn about how conditions such as sickle cell anemia (SCA) and cystic fibrosis (CF) are inherited in families, what screening and testing options are available, and, for some genetic conditions, what treatments are available.

Genomics is helping researchers discover why some people get sick from certain infections, environmental factors, and behaviors, while others do not. For example, there are some people who exercise their whole lives, eat a healthy diet, have regular medical checkups, and die of a heart attack at age 40. There are also people who smoke, never exercise, eat unhealthy foods and live to be 100. Genomics may hold the key to understanding these differences.

Apart from accidents (such as falls, motor vehicle accidents or poisoning), genomic factors play a role in nine of the ten leading causes of death in the United States (for example, heart disease, cancer and diabetes. All human beings are 99.9% identical in their genetic makeup. Differences in the remaining 0.1% hold important clues about the causes of diseases. Gaining a better understanding of the interactions between genes and the environment by means of genomics is helping researchers find better ways to improve health and prevent disease in people who carry genetic predispositions to developing certain diseases.

Why are genetics and genomics important to my family's health?

Understanding more about diseases caused by a single gene (using genetics) and complex diseases caused by multiple genes and environmental factors (using genomics) can lead to earlier diagnoses, interventions, and targeted treatments. A person's health is influenced by his/her family history and

shared environmental factors. This makes family history an important, personalized tool that can help identify many of the causative factors for conditions that also have a genetic component. The family history can serve as the cornerstone for learning about genetic and genomic conditions in a family, and for developing individualized approaches to disease prevention, intervention, and treatment.

What are some of the new genetic and genomic techniques and technologies?

Proteomics

This is a large-scale analysis of all the proteins in an organism, tissue type, or cell (called the proteome). Proteomics can be used to reveal specific, abnormal proteins that lead to diseases, such as certain forms of cancer.

Pharmacogenetics and Pharmacogenomics

Pharmacogenetics is the field of study dealing with the variability of responses to medications due to variation in single genes. Pharmacogenetics takes into account a person's genetic information regarding specific drug receptors and how drugs are transported and metabolized by the body. The goal of pharmacogenetics is to create an individualized drug therapy that allows for the best choice and dose of drugs.

Pharmacogenomics is similar to pharmacogenetics, except that it typically involves the search for variations in multiple genes that are associated with variability in drug response. Since pharmacogenomics is one of the large-scale "omic" technologies, it can examine the entirety of the genome, rather than just single genes. Pharmacogenomic studies may also examine genetic variation among large groups of people (populations), for example, in order to see how different drugs might affect different racial or ethnic groups.

Pharmacogenetic and pharmacogenomic studies are leading to drugs that can be tailor-made for individuals, and adapted to each person's particular genetic makeup. Although a person's environment, diet, age, lifestyle, and state of health can also influence that person's response to medicines, understanding an individual's genetic makeup is key to creating personalized

drugs that work better and have fewer side effects than the one-size-fits-all drugs that are common today.

Stem cell therapy

Stem cells have two important characteristics: (a) They are unspecialized cells that can develop into various specialized body cells, and (b) they are able to stay in their unspecialized state and make copies of themselves. 'Embryonic' stem cells come from the embryo at a very early stage in development (the blastocyst stage). The stem cells in the blastocyst go on to develop all of the cells in the complete organism. 'Adult' stem cells come from more fully developed tissues, like umbilical cord blood in newborns, circulating blood, bone marrow or skin.

Medical researchers are investigating the use of stem cells to repair or replace damaged body tissues, similar to whole organ transplants. Embryonic stem cells from the blastocyst have the ability to develop into every type of tissue (skin, liver, kidney, blood, etc.) found in an adult human. Adult stem cells are more limited in their potential (for example, stem cells from liver may only develop into more liver cells). In organ transplants, when tissues from a donor are placed into the body of a patient, there is the possibility that the patient's immune system may react and reject the donated tissue as "foreign." However, by using stem cells, there may be less risk of this immune rejection, and the therapy may be more successful.

Stem cells have been used in experiments to form cells of the bone marrow, heart, blood vessels, and muscle. Since the 1990's, umbilical cord blood stem cells have been used to treat heart and other physical problems in children who have rare metabolic conditions, or to treat children with certain anemias and leukemias.

Cloning

Cloning can refer to genes, cells, or whole organisms. In the case of a cell, a clone refers to any genetically identical cell in a population that comes from a single, common ancestor. Gene cloning involves manipulations to make multiple identical copies of a single gene from the same ancestor gene. Cloning an organism means making a genetically identical copy of all of the

cells, tissues, and organs that make up the organism. There are two major types of cloning that may relate to humans or other animals: therapeutic cloning and reproductive cloning.

Therapeutic cloning involves growing cloned cells or tissues from an individual, such as new liver tissue for a patient with a liver disease. Reproductive cloning is a related process used to generate an entire animal that has the same nuclear DNA as another currently or previously existing animal.

The wear-and-tear theory

Who created the wear-and-tear theory?

The wear-and-tear theory was first proposed scientifically in 1882 by German biologist Dr. August Weismann. The theory is deeply ingrained in human thinking, and it is the theory often heard expressed in conversations and culture.

What are examples of the wear-and-tear theory?

Examples in our bodies include wrinkles and other skin changes that accrue with time, or arthritic joints that become more stiff and painful as we age. However, aging research continues to seek answers for why people age and die.

What is a key difference between the wear-and-tear theory and the cellular-aging theory?

The cellular-aging theory is based on the idea of senescence, by which cells lose the ability to reproduce and grow. It is an area of focused study in the age of molecular medicine, which is producing new insights. Wear-and-tear theory aligns more with a view that bodies simply "break down" over time.

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Resources

International

World Health Organization (WHO):
www.who.int

(U.S.) Government institutions

Centers for Medicare and Medicaid Services:
www.medicare.gov

Administration on Aging (AoA):
www.eldercare.gov
800-677-1116
The AoA offers mental health services, transportation, nutritional programs, senior health programs, benefits counseling, services for family caregivers, and elder abuse prevention programs.

Centers for Disease Control & Prevention (CDC&P):
www.cdc.gov

National Cancer Institute (NCI):
www.nih.nci.gov

National Institute on Aging (NIA):
www.nih.nia.gov

National Institute on Deafness and Other Communications Disorders (NIDOCD):
www.nidocd.nih.gov

National Institutes of Health (2019):
www.nih.gov
For 50 years, the NIA has led a broad scientific research effort "to understand the nature of aging and to extend the healthy, active years of life". NIA is also the primary federal agency supporting and conducting Alzheimer's disease and related dementias research.

Onco-Aging Consortium (OAC):
https://www.cancer.gov/about-nci/organization/dcb/research-programs/oac
The OAC is a joint NCI and NIA program. It was established "to support research addressing key questions regarding how hallmarks of aging lead to impaired cellular activities and alterations in the microenvironment that contribute to the development and outgrowth of cancer-initiating cells".

Professional organizations

American Academy of Sleep Medicine (AASM):
www.aasm.org

American Cancer Society (ACS):
www.acs.org

American Federation for Aging Research (AFAR):
www.afar.org
AFAR provides trustworthy "information on the basic biology of aging, its connection to age-related diseases, and the socioeconomic benefits of living healthier, longer".

Others

The Buck Institute for Research on Aging:
8001 Redwood Blvd.
Novato, CA 94945-1400
www.buckinstitute.org

It is an independent biomedical research institute that researches aging and age-related disease with mission to extend the healthy years of life. It is one of nine centers for aging research of the Glenn Foundation for Medical Research. Founded in 1999, it is the world's first institute founded primarily to study intervention into the aging process. Its research program has ten focus areas related to geriatrics and longevity.

It coordinates stem cell research with the University of California's Davis and Merced campuses. In 2015, California researchers opened the world's largest publicly available stem cell bank, with samples stored at the Buck Institute. The initial bank had 300 stem cell lines, with an ultimate goal of 9,000 lines. Through a separate commercial entity (Unity Biotechnology First Company), it aims to develop medicines to treat age-related diseases and boost healthy lifespans.

Glenn Foundation for Medical Research:
Founded in 1965, the mission of the Glenn Foundation For Medical Research is "to extend the healthy years of life through research on mechanisms of biology that govern normal human aging and its related physiological decline, with the objective of translating research into interventions that will extend healthspan with lifespan".

Mayo Foundation:
Health in Aging Foundation:
www.healthinaging.org
40 Fulton St., Suite 809, New York, NY 10038
Phone: 212.308.1414

Life Extension Advocacy Foundation (LEAF):
www.leaf.org

National Sleep Foundation (NSF):
www.thensf.org

NSF is an independent non-profit, dedicated to improving overall health and well-being by advancing sleep health. It conducts groundbreaking research and uses proven methods to investigate how cultural behavior and the latest trends are impacting the state of sleep.

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Guides for healthy aging

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Guide to healthy aging

Today, 12 out of every 100 people in the U.S. are age 65 or older, and older adults make up the fastest growing part of our population. Americans are leading longer, healthier lives and we need to seize on this opportunity by adjusting our systems and policies. The following guide can help to enjoy better health and greater independence in later life.

Finding healthcare that meets needs

- Special health and other programs in the community that are just for older adults.
- A local geriatrician.
- Medicare & Medicaid Services offer healthcare benefits to older Americans.
- The federal Administration on Aging (AoA) offers a wide range of services for older adults in every State.

Not making medication mistakes

Many older adults take prescription medications, over-the counter drugs, vitamins, and other supplements such as herbs or home remedies, every day. Taking lots of different pills can cause side effects and problems. It is very important that your healthcare provider, pharmacist, and others who care for you know every medication or pill you are taking including for each item, the number, dose, and frequency taken. Always check with your healthcare provider or your pharmacist before taking any new medicines of any kind.

Take all medicines as directed, and tell your healthcare provider right away if a medication or pill seems to be causing any problems or side effects. Ask if there is any way to take care of your health problems without having to take pills or medicine. Never borrow or take any pills or medications that were meant for someone else.

Staying on top of health problems

- Get your blood pressure checked at least once a year. High blood pressure can cause heart disease, kidney problems, blindness, and other health problems.
- **G**et a cholesterol test at least every five years. This fat can cause heart disease, strokes, and other health problems. If heart disease or diabetes runs in your family, you should have your cholesterol checked more often.
- Get checked for diabetes, especially if you are hungry or thirsty all the time, are overweight, or find that you have to urinate often. These problems could all be signs of diabetes.

Guide to wellness and prevention

Staying on top of the health conditions and concerns faced after age 65 is an important part of the forward momentum that comes with aging. There are steps that can be taken in advance to maintain overall well-being and making sure needs and preferences still fit into care.

"Preventive health" is the medical term for care that helps us protect, promote, and maintain health and well-being. Important information about wellness, preventative health, and other issues is available to help keep us healthy and safe for as long as possible.

Illness prevention

Illness prevention is often better than having to rely on treatment. Steps to prevent some illnesses include: Get screening tests and vaccinations and living a healthy lifestyle.

Lifestyle management

Lifestyle management and changes can help an older adult prevent diseases from developing or getting worse. Examples include:

- **Quitting smoking:** Smoking is still the most preventable cause of death in the United States. Quitting can:
  - Help a person live longer.
  - Lower the risk of heart disease and cancers.
  - Improve lung health and blood circulation.

  A plan for quitting includes some of the following:
    - Setting a date for the last cigarette. On that day, getting rid of all tobacco and anything related to it.
    - Getting nicotine replacement therapy if smoking more than 10 cigarettes a day (using, for example, a nicotine patch, gum, nasal spray, or inhaler) to reduce the physical cravings for nicotine.
    - Getting eventually Bupropion (Wellbutrin, Zyban), Varenicline (Chantix), or similar medications alone or with nicotine replacement therapy.
    - Joining a support group, and getting counseling.
    - Avoiding people or situations that make it tempting to smoke.
    - After quitting, switching from coffee and alcoholic drinks to juices or water.; taking walks instead of coffee breaks; chewing gum or sucking on hard candy to deal with cravings.

- **Increasing physical activity:** Physical activity is one of the best ways to remain healthy and independent. Activity helps prevent and treat many diseases. There are three types of activity:
  - **Aerobic activity:** 30 minutes a day of moderate activity (such as brisk walking). Do this at least 10 minutes at a time and spread it throughout the week. If the activity is vigorous (hard to do) the person can do 75 to 150 minutes of exercise a week.
  - **Muscle strength training: B**y activities like lifting weights, or using resistance bands, or machines. Use all major muscle groups on two separate days a week.
  - **Balance improvement:** Doing exercises such as Tai-chi.

  Medical advice should be sought prior to engaging in such activities, particularly in the presence of medical and heart problems.

- **Eating a healthy diet:** A healthy diet primarily includes:
  - Whole grains and legumes, like beans and soy.
  - Vegetables, fruits, nuts, and seeds.
  - Fish or lean meat and poultry.
  - Low-fat dairy foods.
  - Avoiding foods that are high in salt, fat, or sugar, such as fatty meats, fried food, and highly processed foods.

  If a person needs to gain or lose weight, advice from a healthcare, dietitian or nutritionist may be sought after.

- **Limiting alcohol use:** Older adults have a higher risk of problems with alcohol:
  - Changes in older adults' body affect the impact of alcohol, causing higher alcohol concentrations.
  - Alcohol can interact with many medications. It also can make many medical conditions worse, including:
    - High blood pressure.
    - Dementia or other problems with memory, thinking, and learning.
    - Liver disease.
    - Inability to control urine flow.
    - Stomach bleeding.

- Sleep problems.
- Balance, leading to falls.

Taking steps to prevent accidents

Accidents are the leading cause of injury among older adults. Common accidents that can cause serious injury or death include falls, home hazards, and car crashes.

- **Falls:** An unexpected fall can cause serious injury, resulting in hospitalization, surgery, care in a rehabilitation facility, or even long-term nursing home care. Falls often are the first step to losing independence. To lower the risk of falling and avoiding a fall, providers will:
  - Evaluate balance, walking ability and speed, bone health, blood pressure, heart health.
  - Look for physical disabilities.
  - Check vision and hearing.
  - Check medications and suggest changes, if needed.
  - Work on strength and balance or a falls-prevention or other exercise program.
  - Making changes in the home to make it safer (hand rails and grab bars, bright lighting, and removal of loose rugs and electrical cords).
- **Hazards in the home:** The risks of accidental injury could be lowered by:
  - Lowering the water temperature in the hot-water heater to prevent serious burns.
  - Installing smoke and carbon monoxide detectors.
  - Installing alarms and automatic shut-off features on appliances.
  - Removing or safely storing guns.
  - Knowing how to use home medical equipment, such as oxygen.
- **Car crashes:** Car crashes are a leading cause of accidental death in adults up to age 75. The crash rate for older drivers is higher than for any other age groups except for drivers under 25. To reduce the risks of a car crash:
  - Always wear seat belts.

- Have regular driving tests.
- Take a refresher driving course.
- Check vision regularly and wear the right eyeglasses.
- Don't drink and drive.
- Don't use a cell phone or text while driving.

Knowing when to stop driving is hard because of the loss of independence. Some people with health conditions should not drive. These conditions include: severe vision or hearing loss, dementia, and uncontrolled seizures.

Many older adults also reduce their risk by not driving at night, in poorly-lit streets, on busy highways, or in bad weather.

Keeping a healthy mouth

Older adults can take several steps to a healthy mouth:

- Treat unhealthy or missing teeth or diseased gums because they can make it difficult to eat normally. Untreated problems can lead to poor nutrition.
- Treat dry mouth, which many older adults have. The condition may be due to medications or lack of hydration and can cause or worsen tooth problems.
- Visit the dentist at least twice a year to have teeth and mouth cleaned and examined.
- Brush and floss regularly.
- Do any rinses the dentist may recommend to help with dry mouth.
- Discuss the need for dentures or dental implants, if needed.

Taking care of skin

Older adults need an annual screening by a dermatologist to find skin cancers. These cancers have increased over time.

Fair-skinned people, and people who have had a lot of time in the sun are at higher risk of getting skin cancer. However, darker skinned people also get skin cancer.

Do not wait for the annual skin check, if you notice:

- A change in size, shape, color, or borders of a mole.
- A change in appearance of the skin;
- New unexplained area of discoloration, scaliness, or roughness.
- New appearance of a nodule or pale, sunken area.

Limit the amount of time in direct sunlight. Also, wear protective clothing and sunscreen (at least a SPF 30).

Caution about baby aspirin

Taking a baby aspirin (81mg) every day can reduce the risk of a cardiovascular problem, like a heart attack or stroke for some adults. It is best for older adults who:

- Are under age 70.
- Have other risks for heart disease, such as smoking, high blood pressure, or strong family history.

But, aspirin is no longer routinely recommended for older adults in general as it can increase the risk of serious bleeding.

Guide to prevention and vaccinations

Vaccines available to protect older adults

The following vaccines are recommended for all older adults:

- Flu vaccine.
- Pneumococcal vaccines.
- Shingles vaccine.
- COVID-19 vaccine.
- Tetanus, diphtheria and pertussis vaccine.
- Additional vaccinations for older adults with certain health conditions or risk factors, or who are traveling internationally.

The vaccines below are also recommended for certain groups of people at risk for other vaccine-preventable diseases:

- **Hepatitis A** is recommended for adults with liver disease, men who have sex with men, adults who use injection or non-injection drugs, those who are homeless, and those who are traveling to areas with high levels of Hepatitis A.
- **Hepatitis B** is recommended for adults with kidney failure or on dialysis, those with liver disease, Hepatitis C or HIV infection, multiple sex partners, those who use injection drugs, are incarcerated, or who are traveling to areas with high levels of Hepatitis B. Hepatitis B vaccine is also considered for older adults with diabetes.
- **Meningococcal vaccine** is recommended for adults whose spleen has been removed or damaged (such as in sickle cell disease).
- **Haemophilus influenza type B** is also recommended for adults whose spleen has been removed or damaged (such as in sickle cell disease), as well as for adults who have had a bone marrow transplant.
- **Measles:** People born before 1957 are presumed to be immune and do not require measles vaccination. However, measles vaccine could be considered in people born before 1957 who are at high risk of exposure through work or travel. People born during or after 1957 who do not have evidence of immunity to measles (through blood tests or immunization records) should be vaccinated.

A questionnaire by the Centers for Disease Control & Prevention (CDC & P) can help find which vaccines are recommended for any given situation.

Frequency of vaccinations

The tables below show the recommended schedule for older adults.

Table 22.1 - Vaccine schedule for adults 65 and older

Vaccine	Schedule
Influenza (Flu)	1 dose every year
Tetanus, diphtheria, and pertussis (Tdap)	1 dose, then tetanus-diphtheria (Td) booster every 10 years
Shingles *Zoster recombinant (Shingrix®)** *A shingles vaccine called zoster vaccine live (Zostavax) is no longer available for use in the United States. If you had Zostavax in the past, you should still get Shingrix.	2 doses (2-6 months apart)
COVID-19	As recommended

Table 22.2 - Pneumoccocal vaccine schedule for adults 65 and older

Category	Schedule
Adults 65+ who have not previously received any pneumococcal vaccine or their pneumococcal vaccination history is unknown	One dose of PCV20 OR One dose of PCV15 followed by one dose of PPSV23 at least 1 year later
Adults 65+ who have received PPSV23 but who have not previously received any pneumococcal conjugate vaccine (PCV13, PCV15, or PCV20)	One dose of PCV15 or PCV20 a year after receiving PPSV23
Adults 65+ who have previously received only PCV13	One dose of PPSV23 at least 1 year later

Vaccines side effects

Getting a vaccine is much safer than getting the disease the vaccine prevents. Serious side effects from vaccines are extremely rare. Only 1-2 people out of every 1 million people vaccinated will have a severe allergic reaction. Common side effects are mild and go away quickly on their own. These side effects are a sign that the immune system is responding to the vaccine as it should! Common side effects of vaccines include:

- Pain, swelling, or redness where the shot was given.
- Mild fever.
- Chills.
- Feeling tired.
- Headache.
- Muscle and joint aches.

Guide to geriatric syndromes

Most populations of the developed world continue to live longer and healthier lives but, as individuals age, they become more likely to develop different kinds of health problems. These "geriatric syndromes" are problems that usually have more than one cause and involve many parts of the body. Geriatricians and other geriatrics healthcare professionals can play an important role in diagnosing and managing these syndromes. There are many treatments available for these conditions that can help in maintaining independence and quality of life. The following are examples of such syndromes and what should be done about them, including informing the healthcare provider tram:

Bladder control problems

Lack of bladder control, or "urinary incontinence", can lead to problems such as falls, depression, and isolation. Fortunately, in most cases, incontinence can be cured or greatly improved with treatment.

Delirium

Many older adults who go to the emergency room or are admitted to the hospital develop delirium - a state of sudden confusion. Delirium is a medical emergency, similar to chest pain. The older adult, caregiver, family and friends should become aware of the signs of delirium and seek medical attention right away at the first sign of any sudden changes in mental function.

Dementia

Dementia, including Alzheimer's disease and many other types are significant memory problem that affect the ability to carry out usual tasks. Various tests can help determine whether on might have dementia and what type it might be. There are treatments that can improve function and slow down the disease. (For more details, see my books on these subjects as listed in the Chapter: References).

Falls

Falls are a leading cause of serious injury in older people. There are many risk factors for falling, including safety hazards in the home, medication side effects, walking and vision problems, dizziness, arthritis, weakness, and malnutrition. Like other geriatric syndromes, falls usually have more than one cause. There are many treatments, such as exercise and physical therapy, that can help improve gait and walking and prevent falls.

Osteoporosis

Osteoporosis, or "thinning bones," is a condition that makes the bones of older adults more fragile and easy to break. Women 65 and older, and men over age 70, should get a bone mass density (BMD) test. Increased calcium and vitamin D intake, strength training exercises, and weight-bearing exercises such as walking are important to keeping the bones healthy. Medications or other treatments may also recommended.

Sleep problems

Sleep problems can affect the quality of life and can contribute to falls, injuries, and other health problems. Again, there are available treatments (pharmaceutical and otherwise).

Weight loss

Weight loss is a very common problem in older adults. It can be caused by the diminished sense of taste that comes with aging, or it can be a suggestion of an underlying serious medical problem. No matter the cause, weight loss can lead to other problems, such as weakness, falls, and bone disorders.

Guide to age-friendly healthcare

A healthcare system is "age friendly" when it is expertly designed to coordinate all care with advancing age, while also making sure personal needs, values, and preferences are at the heart of that care. Age-friendly health systems pay particular attention to:

- Providing older adults the best care possible.
- Reducing some of the specific harms older adults face more often than others.
- Ensuring older adults, their families and caregivers are satisfied with care.
- Improving the value of care for all, including the professionals who make that care possible.

Age-friendly health systems achieve their goals by focusing on what geriatrics specialists call the "4Ms" that make their expert care so unique. These 4Ms include:

- Knowing what Matters to older adults when it comes to making healthcare decisions.
- Reviewing, reducing, and removing Medications that may cause harm or are unnecessary.
- Improving care for the Mentation (or the Mind) by addressing critical problems like dementia, delirium, and depression.
- Promoting Mobility.
- Multi-complexity (or the need to manage multiple health conditions at once).

The above Guides have been mostly compiled by the Health in Aging Foundation to which the reader is referred for more details.

Conclusions and take-aways

- Several guides have been offered for healthy aging in order to find healthcare that meets one's needs, not making medication mistakes, and staying on top of health problems.
- The Guide to wellness and prevention emphasizes illness prevention, lifestyle management, taking steps to prevent accidents and car crashes, keeping a healthy mouth, taking care of skin and caution about baby aspirin.
- The Guide to prevention is mainly about vaccination showing vaccines available and recommended for all older adults (flu vaccine, pneumococcal vaccines, shingles vaccine, COVID-19 vaccine., tetanus, diphtheria and pertussis vaccine, and additional vaccinations. Recommended vaccines for certain groups of people at risk for other vaccine-preventable diseases include: Hepatitis A and B, meningococcal vaccine, Haemophilus influenza type b, and measles. A questionnaire by the CDC & P can help find which vaccines are recommended for any given situation.
- The Guide to geriatric syndromes that usually have more than one cause and involve many parts of the body included: Bladder control problems, delirium, dementia, falls, osteoporosis, sleep problems, and weight loss.
- The Guide to age-friendly healthcare encompasses the five M's: Matters, Medications, Mentation, Mobility, and Multi-complexity.
- Sidebar 22.1 offers 10 tips for aging well; sidebar 22.2 discusses the important subject of drugs and aging for 7 seven categories of drugs that may affect memory in the elderly; and sidebar 22.3 covers topics in caregiving.

Sidebar 22.1 - Tips for aging well

Simply living longer is not enough. What we really want is to live longer well, staying healthy enough to continue doing the things we love. While having good genes certainly helps, a growing body of research suggests that how well you age depends largely on you and what you do. Fortunately, research also finds that it is never too late to make changes that can help

you live a longer and healthier life. Th following are 10 tips for living longer and better:

1. **Eat a rainbow:** You need fewer calories when you get older, so choose nutrient-rich foods like brightly colored fruits and vegetables. Eat a range of colors— the more varied, the wider the range of nutrients you are likely to get. The longest-lived and healthiest people in the world eat primarily a whole-food, plant-based diet. Vegetables. legumes, fruits, nuts, and seeds form the basis of this diet. Limit red meat, dairy, and other animal products. Choose whole grains over the refined stuff.
2. **Sidestep falls:** Walking at least 30 minutes a day, three times a week, can help you stay physically fit and mentally sharp, strengthen your bones, lift your spirits—and lower your risk of falls. Aim for about 7,500 steps per day for the most benefits. Aim to bring in more activity into your normal daily routine (such as parking farther away from the store, or taking the stairs instead of the elevator). Preventing falls is important because falls are a leading cause of fractures, other serious injuries, and death among older adults. Bicycling, dancing, and jogging are also good weight-bearing exercises that can help strengthen your bones. In addition to exercising, get plenty of bone-healthy calcium (from food sources) and vitamin D daily.
3. **Toast with a smaller glass:** The amount of alcohol that is safe to drink changes as we get older. Adults over age 65 who are healthy and do not take medications should not have more than 3 drinks on a given day or 7 drinks in a week. (A drink is 1.5 oz of hard liquor, 6 oz of wine, or 12 oz of beer.) If you have a health problem or take certain medications, you may need to drink less or not at all. Since alcohol can interact with certain drugs, ask your healthcare professional whether any alcohol is safe for you.
4. **Know the low down on sleep in later life:** Contrary to popular belief, older people do not need less sleep than younger adults. New recommendations from the National Sleep Foundation (NSF) suggest 7 to 8 hours of shut-eye a night. People with sleep apnea stop breathing briefly, but repeatedly, while sleeping. Among other

things, untreated sleep apnea can increase the risk of developing heart disease. (see also Sidebar 22.2.)

5. **Flatten your (virtual) opponent, sharpen your mind:** Conquering your adversary in a complex computer game, joining a discussion club, learning a new language, and engaging in social give-and-take with other people can all help keep your brain sharp, studies suggest.
6. **Enjoy safer sex:** Older adults are having sex more often and enjoying it more, research finds. Unfortunately, more older people are also being diagnosed with sexually transmitted diseases. To protect yourself, use a condom and a lubricant every time you have sex until you are in a monogamous relationship with someone whose sexual history you know.
7. **Get your medications checked:** When you visit your healthcare professional, bring all of the prescription and over-the-counter medications, vitamins, herbs, and supplements you take. You can also bring a complete list that notes the names of each, the doses you take, why you take them, and how often you take them. Ask your healthcare provider to review everything you brought or put on your list. Your provider should make sure they are safe for you to take, and that they do not interact in harmful ways. The older you are, and the more medicines you take, the more likely you are to experience medication side effects, even from drugs bought over-the-counter.
8. **Speak up when you feel down or anxious:** Roughly 1 in 5 older adults suffers from depression or anxiety. Lingering sadness, tiredness, loss of appetite or pleasure from things you once enjoyed, difficultly sleeping, worry, irritability, and wanting to be alone much of the time can all be signs that you need help. Tell your healthcare professional right away. There are many good treatments for these problems.
9. **Get your shots:** Must-have vaccines for seniors include those that protect against pneumonia, tetanus/ diphtheria, shingles, and the flu, which kills thousands of older adults in the US every year.
10. **Find the right healthcare professional and make the most of your visits:** See your healthcare professional regularly, answer questions frankly, ask any questions you have, and follow your

provider's advice. If you have multiple, chronic health problems, your best bet may be to see a geriatrics healthcare professional.

Sidebar 22.2 – Drugs and aging

For a long time, forgetfulness, brain fog, and mental confusion were considered as normal parts of aging. However, we now know that memory loss as we get older is by no means inevitable. In fact, according to the Centers for Disease Control & Prevention (CDC). routine memory, skills, and knowledge may even improve with age.

Most people are familiar with at least some of the things that can impair memory, including alcohol, drug abuse, heavy cigarette smoking, head injuries, stroke, sleep deprivation, severe stress, vitamin B12 deficiency, and illnesses such as Alzheimer's disease and depression. But what many people do not realize is that a number of commonly prescribed drugs also can interfere with memory. (For a treatise on memory, see Fymat, 2023). Here, I will dwell on seven classes of drugs that may cause memory loss and explore alternative treatment options. I will also dwell on other medications of concern. Drugs that affect short-term memory loss (events within the last 30 seconds) can interfere with a person's ability to focus and process information. These medications disrupt so-called messenger pathways in the brain, changing the short-term memory processing. Fortunately, once the offending medications are stopped, short-term memory improves. On the other hand, drugs that affect working memory (events taking place at any time beyond the first 30 seconds) can interfere with neurotransmitters in the brain (these are the body's chemical messengers that help one to think, move, breathe, and function generally. This effect on working memory is compounded when multiple drugs are taken concurrently, at high doses, and used for long periods of time. Further, certain medications can affect both short- and long-term memory, while others may affect only one. Still further, taking multiple medications (a practice known as polypharmacy) has been linked to lower memory function in older adults, as well as an increased risk for delirium.

A summary of those drugs that cause memory loss follows:

1. Antianxiety drugs (benzodiazepines)

- **Why are they prescribed?** To treat a variety of anxiety disorders, agitation, seizures, delirium, and muscle spasms. Because they have a sedative effect, they are sometimes used to treat insomnia and the anxiety that can accompany depression.
  Examples: Alprazolam (Xanax); Chlordiazepoxide; Clonazepam (Klonopin); Diazepam (Valium); Flurazepam; Lorazepam (Ativan); Midazolam; Quazepam (Doral); Temazepam (Restoril); and Triazolam (Halcion).

- **How is memory affected?** Benzodiazepines dampen activity in key parts of the brain, including those involved in the transfer of events from short-term to long-term memory. In fact, they're used in anesthesia for this very reason.
- **Alternatives:** Benzodiazepines should be prescribed only rarely in older adults, and then only for short periods of time. It takes older people much longer than younger people to flush these drugs out of their bodies, and the ensuing buildup puts older adults at higher risk for not just memory loss but delirium, falls, fractures and motor vehicle accidents. Further, they are addicting. Alternatives may be available, e.g. if prescribed in case of insomnia, the first line of treatment is cognitive behavioral therapy for insomnia (CBT-I) and an antidepressant might be able to treat anxiety.
- **Caution:** Sudden withdrawal of these medicines can trigger serious side effects, so a health professional should always monitor the process.

2. Anticonvulsant drugs

- **Why are they prescribed?** To limit seizures by dampening the flow of signals within the central nervous system (CNS). Also to treat nerve pain, bipolar disorder, mood disorders, and mania. They can also be sedative.
  Examples: Carbamazepine (Tegretol); Gabapentin (Neurontin); Lamotrigine (Lamictal); Levetiracetam (Keppra); Oxcarbazepine

(Trileptal); Pregabalin (Lyrica); Rufinamide (Banzel); Topiramate (Topamax); Valproic acid (Depakote); Phenobarbital (Luminal); Primidone (Mysoline); and Zonisamide (Zonegran).

- **How is memory affected?** It may be difficult to separate what is a true cognitive decline from simple sedation.
- **Alternatives:** For seizures, Phenytoin (Dilantin) at lower doses has less of an impact on memory. For chronic nerve pain, Venlafaxine (Effexor) spares memory.

3. Tricyclic antidepressants

- **Why are they prescribed?** To treat depression, anxiety disorders, obsessive-compulsive disorder, and nerve-related pain.

  Examples: Carbamazepine (Tegretol); Gabapentin (N eurontin); Lamotrigine (Lamictal); Levetiracetam (Keppra); Oxcarbazepine (Trileptal); Pregabalin (Lyrica); Rufinamide (Banzel); Topiramate (Topamax); Valproic acid (Depakote); Phenobarbital (Luminal); Primidone (Mysoline); and Zonisamide (Zonegran).

- **How is memory affected?** Anticonvulsants are believed to limit seizures by dampening the flow of signals within the central nervous system (CNS). Drugs that depress signaling in the CNS can cause memory loss.
- **Alternatives:** Many patients with seizures do well on Phenytoin (Dilantin), which, at lower doses, has less of an impact on memory. Patients with chronic nerve pain find that Venlafaxine (Effexor) – which also spares memory – alleviates their pain.

4. Narcotic painkillers (opioids)

- **Why are they prescribed?** To relieve moderate to severe pain from surgery or injuries; in some instances, also to treat chronic pain.
  Examples: Fentanyl (available as a patch); Hydrocodone (Vicodin); Hydromorphone (Dilaudid, Exalgo); Morphine and Oxycodone (Oxycontin). These drugs come in many different forms, including tablets, solutions for injection, transdermal patches and suppositories.

- **How is memory affected?** These drugs stem the flow of pain signals within the central nervous system and by blunting one's emotional reaction to pain. Both of these actions are mediated by chemical messengers that are also involved in many aspects of cognition, so their use can interfere with long- and short-term memory, especially when used for extended periods of time. There is also a link between opioid use and dementia in older adults.
- **Alternatives:** In patients under the age of 50 years, nonsteroidal anti-inflammatory drugs (NSAIDs) are the frontline therapy for pain. Unfortunately, NSAID therapy is less appropriate for older patients, who have a much higher risk of gastrointestinal bleeding (the risk goes up with the dosage and duration of treatment). Acetaminophen (Tylenol) may be another option, but again, there are risks, side effects, and drug interactions for all medications.

5. Sleeping aids (nonbenzodiazepine sedative-hypnotics; or "Z" drugs)

- **Why are they prescribed?** To treat insomnia and other sleep problems and also for mild anxiety.
  Examples: Eszopiclone (Lunesta); Zaleplon (Sonata); and Zolpidem (Ambien).

- **How is memory affected?** Although molecularly distinct from benzodiazepines (see No. 1, above), they act on many of the same brain pathways and chemical messengers, producing similar side effects and problems with addiction and withdrawal. They also can cause amnesia and sometimes trigger dangerous or strange behaviors.
- **Alternatives:** Melatonin (to reestablish healthy sleep patterns); Dimethylhistamin HCL 25 mg x2 (sold over the counter); and cognitive behavioral therapy for insomnia (CBT-I) - the first-line treatment for the sleep disorder.

6. Incontinence drugs (anticholinergics)

- **Why are they prescribed?** To relieve symptoms of overactive bladder and reduce episodes of urge incontinence.

Examples: Darifenacin (Enablex); Oxybutynin (Ditropan XL; Oxytrol for Women sold over the counter.); Solifenacin (Vesicare); Tolterodine (Detrol); and Trospium (Sanctura). Another oxybutynin product,

- **How is memory affected?** They affect long-term memory, are associated with an increased risk of dementia, which can persist even after the medication has been discontinued.

  Reason: These drugs block the action of acetylcholine, a neurotransmitter that mediates all sorts of functions in the body. In the bladder, anticholinergics prevent involuntary contractions of the muscles that control urine flow. In the brain, they inhibit activity in the memory and learning centers. The risk of memory loss is heightened when the drugs are taken for more than a short time or used with other anticholinergic drugs. Older adults are particularly vulnerable to the other adverse effects of anticholinergic drugs, including constipation (which, in turn, can cause urinary incontinence), blurred vision, dizziness, anxiety, depression and hallucinations.

- **Alternatives:** Simple lifestyle changes (cutting back on caffeinated and alcoholic beverages, drinking less before bedtime and doing exercises to strengthen the pelvic muscles that help control urination), under-garment items..
  Note: Some urologists are treating overactive bladder with Botox injections to help the muscle relax.

7. Antihistamines (first generation)

- **Why are they prescribed?** To relieve or prevent allergy symptoms or symptoms of the common cold. Some antihistamines are also used to prevent motion sickness, nausea, vomiting and dizziness, and to treat anxiety or insomnia.
- **Examples:** Brompheniramine (Dimetane); Chlorpheniramine (Chlor-Trimeton); Clemastine (Tavist); Diphenhydramine (Benadryl); Promethazine (Phenergan); and Hydroxyzine (Vistaril).
- **How is memory affected?** These medications [prescription and over-the-counter (OTC)] inhibit the action of acetylcholine, a chemical

messenger that mediates a wide range of functions in the body. In the brain, they inhibit activity in the memory and learning centers.

- **Alternatives:** Newer-generation antihistamines such as Loratadine (Claritin) and Cetirizine (Zyrtec). These are better tolerated by older patients and do not present the same risks to memory and cognition.

8. Other medications worth noting

- **Beta-blockers:** While there is no evidence that beta-blockers (prescribed for heart failure, angina, certain heart rhythm disorders and sometimes high blood pressure) contribute to long-term cognitive decline or dementia, they can make some people feel "fatigued," "foggy" and "not their sharpest".
- **Cannabinoids:** May have an effect on cognition. Long-term cannabis users show cognitive deficits, as well as memory and attention problems.
- **Corticosteroids:** These anti-inflammatory drugs (used to treat rheumatoid arthritis, lupus, and other conditions), can cause confusion and memory loss in patients on high doses. They can also trigger depression.
- **Heartburn medications:** Medications commonly used to treat gastroesophageal reflux disease (GERD), heartburn, and peptic ulcers have an increased risk of dementia. The OTC proton-pump inhibitor should also use medication in moderation and for short amounts of time. (not more than two weeks at a time).

Table 22.3 summarizes the drugs that may cause short-term and long-term memory loss as well as dementia-like symptoms:

Table 22.3 – Medications affecting short- and long-term memory and dementia-like symptoms

Drug class	Short-term memory loss	Long-term memory loss	Dementia-like symptoms
Antianxiety (benzodiazepines)	X	X	
Anticonvulsants	X		

Antidepressants (tricyclic)	X	X	
Painkillers (narcotics; opioids)	X		
Sleeping aids (nonbenzodiazepine or "Z" drugs)	X	X	X
Anticholinergics (incontinence drugs)	X	X	X
Antihistamines (first generation)		X	X

Reference: Zachary Cox, AARP research (2016)

Sidebar 22.3 – Topics in caregiving

The Health in Aging Foundation offers over 25 topics, giving guidance and providing clear, practical instructions for dealing with common caregiving problems, including:

- **Advance directives:** Sometimes, decisions about medical treatments must be made when the older person is too sick or is unable to express his or her own wishes.
- **Bone weakness:** As people age, their bones begin to thin and lose strength. Bone thinning is called osteopenia. Severe bone thinning to the point where a person is at a high risk for broken bones (fractures) is called osteoporosis.
- **Breathing problems:** As people age, certain activities, such as going up a flight of stairs, can make them slightly winded or out of breath. This may be because they are out of condition due to physical inactivity.
- **Caregiving:** Caring for an older person at home requires a team of people with different skills and perspectives. Doctors, nurses, social workers, and clergy all make important, specialized contributions, but family members or friends give the day-to-day care.

- **Choosing a nursing home:** A nursing home becomes a valuable option when the amount of medical care and nursing attention the older person needs cannot be provided at home.
- **Communications problems:** Communication problems can be frustrating for the older person and his/her caregiver. The older person may become upset because of not being able to hear or understand what others are saying, or because of having trouble expressing thoughts.
- **Constipation:** Constipation is passage of small amounts of hard, dry bowel movements, usually fewer than three times a week. As a rule, if more than three days pass without a bowel movement (also called stool), the intestinal contents may harden, and a person may have difficulty or even pain during elimination.
- **Dental problems:** Dental problems are among the most common health problems experienced by older adults. In fact, people over 65 with natural teeth have more tooth decay than any other age group and thus continue to need a yearly visit to the dentist.
- **Depression:** Older people often have life changes that lead to feeling depressed. Some people go through physical changes affecting their eyesight, hearing, or how well they can move. Others have changes in their health that are treated with many medicines. Still others have changes in their ability to think and remember.
- **Diarrhea:** Diarrhea is the passing of three or more loose or watery stools per day, or a definite decrease in consistency and increase in frequency of bowel movements based upon what is usual for the individual.
- **Hearing problems:** Fifty percent of Americans over 65 suffer from hearing loss, although it is more common in older men than in older women. Problems can be small (missing certain sounds) or large (not hearing at all).
- **Helping with recovery from illness:** Taking care of a family member who is recovering from surgery or an illness can be very demanding for both the caregiver and the person who receives care.
- **Incontinence:** Urinary incontinence, or involuntary leaking of urine, is a problem for at least 30% of people over age 60. It is more common

in women than in men and can range from occasional dribbling to total loss of bladder control.

- **Memory problems:** In our busy lives, all of us forget things at one time or another. As we age, this can happen more frequently and can be a natural part of the aging process.
- **Mobility problems:** The changes that occur with aging can lead to problems with a person's ability to move around, or mobility. Mobility problems may be unsteadiness while walking, difficulty getting in and out of a chair, or falls.
- **Pain:** Some people think that pain is natural with aging. Others may believe that older people are "just complaining" if they are not clear in explaining the cause or nature of their pain.
- **Problems of daily living:** Many older people experience problems in daily living because of chronic illnesses or health-related disabilities. Those difficulties restrict their ability to perform self-care.
- **Skin problems:** Wrinkles and age spots are normal skin conditions that occur as a person ages. These are natural processes. Nevertheless, some elderly people can develop skin disorders that give rise to serious medical problems.
- **Sleep problems:** People of all ages can have trouble falling asleep or staying asleep or have poor quality sleep (insomnia). There are many possible causes including stress, changes in schedule, diet, or as a side effect of medicines.
- **Using medicines safely:** People age 65 and over buy more than 30 percent of all prescription medicines and 40 percent of all nonprescription (over-the-counter) medicines sold in this country.
- Vision problems: Growing older does not always lead to poor vision. However, age can bring about changes that might affect the eyes and vision.

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Glossary

A

Acetylation: The formation of an acetyl derivative (acetyl is the atom grouping CH_3CO, an acetic acid molecule from which the hydroxyl group has been removed).

Adaptive aging: A process supposed to have emerged during evolution as a series of germ line mutations that were selected on the basis of a particular gain in fitness. Accordingly, each of us has a biological clock set to go off at a particular time to signal our bodies first to age and then to die.

Age:

Biological (or **physiological):** Refers to how old one's cells and tissues are based on physiological evidence. It is more accurate than chronological age for predicting the onset of disease and death.

Chronological: The number of years one has been alive.

Agerasia: The search for an indefinite lifespan through complete rejuvenation to a healthy youthful condition.

Aging: The process of becoming older. The progressive deterioration of physiological function, an intrinsic age-related process of loss of viability and increase in vulnerability.

Distal aging: The age-based differences that can be traced to a cause in a person's early life.

Proximal aging: The age-based effects that come about because of factors in the recent past.

Aging theories: Theories that purport to explain the phenomenon of aging:

Biochemical theories: No matter what genes we may have inherited, our body is continually undergoing complex biochemical reactions, some of which cause damage and, ultimately, aging in the body.

- **Age-changing hormonal theory:** Hormones cause many shifts in organ systems and other functions.
- **DNA repair theory:** The systems in the body that repair DNA seem to become less effective with age.
- **Free radicals theory:** These unstable oxygen molecules can damage cells.
- **Heat shock proteins theory:** Such proteins help cells survive stress and diminish in numbers with age.
- **Protein cross-linking theory:** Protein cross-links produce excess sugars in the blood stream that can cause protein molecules to literally stick together, leading to aging.

Error theories: Over time, cells and tissues simply wear out. There are the following variations:

- **Cross-linking theory:** Cross-linked proteins accumulate and slow down the body's processes.
- **Free radicals theory:** Free radicals in the environment cause damage to cells, eventually impairing their function.
- **Rate of living theory:** Aging is inversely related to the organism's consumption of oxygen, that is, the faster an organism uses oxygen, the shorter it lives.
- **Wear-and-tear theory:** Cells and tissues simply wear out.

Genetic theories: Genes can play a major role in aging:

- **Cell senescence theory:** The process of senescence deteriorates cells over time.
- **Longevity genes theory:** Longevity genes are specific genes that help lengthen lifespan.
- **Somatic DNA damage theory:** Genetic mutations are known to cause cells to malfunction.
- **Stem cells theory:** Stem cells hold promise to repair the damage caused by aging.
- **Telomeres shortening theory:** Telomeres shortening affects cell replication.

Hormonal theories: Regular changes in hormones control aging.

Programmed aging: The human body is "designed" to age so that aging is a natural phenomenon that has been "programmed" into our bodies, following a certain biological timeline. In effect, we are "designed" to age!

Alleles: The different forms of a DNA sequence at a locus when it varies between individuals.

Anagenesis: Changes within species.

Antagonistic pleiotropy: A concept described by George C. Williams in which the same gene variant controls a phenotypic trait with beneficial effects at an early age and adverse effects later.

Apheresis*:* A procedure in which blood is drawn from the donor, passed through a machine that extracts the stem cells, and other portions of the blood returned to the donor.

Apoptosis*:* The process by which cells self-destruct.

Autophagy: The natural, conserved degradation of the cell that removes unnecessary or dysfunctional components, allowing the orderly degradation and recycling of cellular components. It plays a major role in the homeostasis of non-starved cells. Defects in autophagy have been linked to various human diseases, including neurodegeneration and cancer. Four forms of autophagy have been identified: macroautophagy, microautophagy, chaperone-mediated autophagy, and crinophagy.

Autotrophs: The organisms responsible for the introduction of energy into an ecosystem. Nearly all of these organisms originally draw energy from the Sun. Phototrophs use solar energy via a process known as photosynthesis to convert raw materials into organic molecules. A few ecosystems depend entirely on energy extracted by chemotrophs from methane, sulfides, or other non-luminal energy sources. The most important processes for converting the energy trapped in chemical substances into energy useful to sustain life are metabolism and cellular respiration.

B

Biogerontology: A sub-field of gerontology concerned with the biological aging process, its evolutionary origins, and potential means to intervene in the process.

Biomedical gerontology (or **experimental gerontology** and **life extension**): A sub-discipline of biogerontology endeavoring to slow, prevent, and even reverse aging in both humans and animals.

C

Calorie restriction (or caloric restriction, or energy restriction): Adietary regimen that reduces calorie intake without incurring malnutrition or a reduction in essential nutrients.

Cataract: Occurs when the lens of the eye becomes cloudy making vision blurry and eventually causing blindness if untreated.

Central dogma of molecular biology: An explanation of the flow of genetic information within a biological system. Note that the word "dogma" should not be literally interpreted - it is just a catch phrase.

Chromosome: A long strand of DNA.

Citrullination (or **deimination):** The conversion of the amino acid arginine in a protein into the amino acid citrulline. It is distinct from the formation of the free amino acid citrulline as part of the urea cycle or as a byproduct of enzymes of the nitric oxide synthase family.

D

Dehydroepiandrosterone (DHEA), also known as androstenolone, is an endogenous steroid hormone precursor and one of the most abundant circulating steroids in humans. It is produced in the adrenal glands, the gonads, and the brain. It functions as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex steroids both in the gonads and in various other tissues. However, DHEA also has a variety of potential biological effects in its own right, binding to an array of nuclear and cell surface receptors, and acting as a neurosteroid and modulator of neurotrophic factor receptors. It decreases naturally with age. Anti-aging doctors claim that DHEA supplementation can reduce the effects of aging, including increasing muscle mass and even burning fat.

Diptera (Gr. δι- di- «two», and πτερόν pteron «wing».): A large order containing an estimated 1,000,000 species including horse-flies,

crane flies, hoverflies, mosquitoes and others, although only about 125,000 species have been described. Insects of this order use only a single pair of wings to fly, the hindwings having evolved into advanced mechanosensory organs known as halteres, which act as high-speed sensors of rotational movement and allow dipterans to perform advanced aerobatics.

Disposable somatic effect: Refers to an entire genetic program (the organism diverting limited resources from maintenance to reproduction (see also "antagonistic pleiotropy").

Distal aging: Age-based differences that can be traced to a cause in a person's early life, such as childhood poliomyelitis.

DNA methylation: An important regulator of gene transcription.

E

Epigenetic clock: A biomarker of aging capable of predicting human chronological age.

Epigenetic inheritance systems: The heritable changes that cannot be explained by changes to the sequence of nucleotides in the DNA.

Epigenetics: The study of cellular and physiological traits inherited by daughter cells, but not caused by changes in the DNA sequence. It is the study of stable, long-term alterations in the heritable transcriptional potential of a cell. Unlike genetics, which is based on changes to the DNA sequence (the genotype), in epigenetics, the changes in gene expression (or cellular phenotype) have other causes.

Predetermined: A unidirectional movement from structural development in DNA to the functional maturation of the protein. It means that development is scripted and predictable.

Probabilistic: A bidirectional structure-function development with experiences and external molding development.

Epimutation: The methylation of gene promoter regions or alterations of the DNA scaffolding which regulate gene expression,

Evolution: The change in the heritable characteristics of biological populations over successive generations.

By natural selection: An explanation for why organisms are adapted to their physical and biological environments.

Evolutionary synthesis: The change over time in genetic variation.

Exon shuffling: The generation of new genes involving small parts of several genes being duplicated, with these fragments then recombining to form new combinations with new functions. (See also *de novo* gene birth.)

Exposome: The cumulative measure of all the environmental insults an individual incurs during their life course that determine susceptibility to disease.

Extinction: The loss of species.

F

Fitness: Refers to how likely an organism is to survive and reproduce.

- **Differential:** Different traits confer different rates of survival and reproduction.
- **Heritability:** Traits can be passed from generation to generation.

Frailty: A syndrome of decreased strength, physical activity, physical performance and energy.

Free radicals: Reactive molecules produced by cellular and environmental processes, that can damage the elements of the cell such as the cell membrane and DNA and cause irreversible damage. A byproduct of normal cell function. When cells create energy, they also produce unstable oxygen molecules.

G

Gametogenesis: The formation of sperm and ovum.

Gene birth *de novo*: Entirely new genes generated from mutations from previously noncoding DNA. (See also exon shuffling.)

Gene flow: The exchange of genes between populations and between species.

Gene reshuffling: Can be caused by sexual reproduction.

Genetic damage: Aberrant structural alterations of the DNA.

Genetic polymorphism: Defines monogenic traits that exist in the normal population in at least two phenotypes, neither of which is rare, with the

frequency of the specific genetic variation affecting 1% or more of the population.

Gene transfer: The transfer of genes between species, including the formation of hybrid organisms and horizontal gene transfer.

Horizontal gene transfer: The transfer of genetic material from one organism to another organism that is not its offspring.

Genomic imprinting: A phenomenon in mammals where the father and mother contribute different epigenetic patterns for specific genomic loci in their germ cells.

Genotype (or **genetic material):** The complete set of genes within an organism's genome. (See also phenotype.)

Geriatrics: A field of medicine that studies the treatment of existing disease in aging people rather than the treatment of aging itself.

Germ cells: The gametes such as egg cells and sperm cells.

Germ plasm theory (at one time also known as Weismannism): According to this theory, inheritance (in a multicellular animal) only takes place by means of the germ cells—the gametes such as egg cells and sperm cells.

Gerontology: A sub-field of gerontology concerned with the biological aging process, its evolutionary origins, and potential means to intervene in the process. It involves interdisciplinary research on the causes, effects, and mechanisms of biological aging.

Geroscience: The science focused on preventing diseases of aging and prolonging the 'healthspan' over which an individual lives without serious illness.

Glaucoma: A common visual disease that appears in older adults. It is caused by damage to the optic nerve causing vision loss. There are a few procedures but there is no cure or fix for the damage once it has happened. Prevention is the best measure.

Glycation: A process in which sugars such as glucose and fructose can react with certain amino acids such as lysine and arginine and certain DNA bases such as guanine to produce sugar adducts. It mainly damages proteins.

Glycoxidation: A process in which sugar damage is linked to oxidant damage.

Gompertz–Makeham law of mortality: The age-dependent component of the mortality rate increases exponentially with age.

H

Hayflick's limit: The maximal number of human cell divisions **(**about 50) in laboratory culture. It was discovered in 1961 by Leonard Hayflick.

Healthspan: The period of one's life that one is healthy, such as free of significant diseases or declines of capacities.

Heredity: The inherited characteristics of organisms, which devolves from organismic evolution through changes in heritable characteristics.

Hippuric acid (Gr. hippos, horse, ouron, urine): A carboxylic acid and organic compound formed from the combination of benzoic acid and glycine. Levels of hippuric acid rise with the consumption of phenolic compounds (such as in fruit juice, tea and wine). The phenols are first converted to benzoic acid, then to hippuric acid, and excreted in urine.

Histones: The spools of protein around which The genome is more than a long sequences of DNA letters are wound. Both DNA and histones can have various chemical handles, cranks, and levers attached to them to help turn genes on or off. These handles, cranks, and levers comprise the epigenome.

Homeostasis: The ability of an open system to regulate its internal environment to maintain stable conditions by means of multiple dynamic equilibrium adjustments controlled by interrelated regulation mechanisms. All living organisms, whether unicellular or multicellular, exhibit homeostasis.

Homologous recombination: The process wherein sexual organisms exchange DNA between two matching chromosomes.

Hormesis: A process in which various stressors - such as those related to diet and exercise - seem to activate genes that slow down cell growth and aging.

I

Imprinting: A phenomenon in which one of the two alleles of a gene pair is turned off,

Inflammaging: A chronic inflammatory phenotype in the elderly in the absence of viral infection and in the obese. It is due to over-activation and a decrease in the precision of the innate immune system. Obesity is

a risk factor for inflammation, and this chronic, low-grade inflammation can accelerate aging.

Interleukins: They (there are more than 20 of them) serve as messengers, relaying signals that regulate the immune response.

Iteroparity (from the Latin *itero*, to repeat, and *pario*, to beget)**:** A reproductive strategy available to living organisms characterized by multiple reproductive cycles over the course of their lifetime. Iteroparity can be further divided into continuous iteroparity (primates including humans and chimpanzees) and seasonal iteroparity (birds, dogs, etc.) Some botanists use the parallel terms "monocarpy" and "polycarpy". (See also semelparity.)

L

Lipofuscin: A waste product formed by a complex reaction in cells that binds fat to proteins.

Locus: The specific location of a DNA sequence within a chromosome.

Longevity genes: Specific genes that help a person live longer.

M

Macroautophagy: The most thoroughly researched form of autophagy. (See Autophagy.)

Macular degeneration: A degeneration caused by systemic changes in the circulation of waste products and by growth of abnormal vessels around the retina. It lads to vision loss that increases with age.

Meiosis: A special type of cell division of germ cells and apicomplexans in sexually-reproducing organisms that produces the gametes, the sperm or egg cells. It involves two rounds of division that ultimately result in four cells, each with only one copy of each chromosome (haploid). Additionally, prior to the division, genetic material from the paternal and maternal copies of each chromosome is crossed over, creating new combinations of code on each chromosome. Later on, during fertilization, the haploid cells produced by meiosis from a male and a female will fuse to create a zygote, a cell with two copies of each chromosome again.

Methylation: The process that turns genes "on" or "off". It is the switch that governs their expression. The addition of methyl groups (methyl is the radical - CH_3).

Mitosis: The process of cell division. It assures an equal distribution of chromosomes to each daughter cell. It proceeds along four phases called "prophase" (the cell at the beginning of mitosis), "metaphase" (new cell poles are formed, the nuclear membrane has dissolved, the chromosomes have divided, and the centromeres – the parts of the chromosomes that contain less DNA- have not divided into two moieties), "anaphase" (migration toward the poles), and "telophase" (separation and formation of two new cells identical to the original one).

Mutagenic: Processes that increase the rate of changes in DNA.

Mutagenic chemicals: Chemicals that promote errors in DNA replication, often by interfering with the structure of base-pairing, while UV radiation induces mutations by causing damage to the DNA structure.

Mutations: The changes in the DNA sequence of a cell's genome; they are the ultimate source of genetic variation in all organisms.

P

Panmixia: The phenomenon in which a phenotype that becomes useless during evolution tends to regress since natural selection ceases to operate upon it.

PARP (Poly ADP ribose polymerases): Enzymes that are activated by DNA strand breaks. They play a role in DNA base excision repair.

Phenoptosis: A special suicide program for the whole organisms. The cellular clock theories of aging.

Phenotype: The complete set of observable traits that make up the structure and behavior of an organism. (See also genotype.)

Phosphorylation: The addition of phosphate to an organic compound such as glucose to produce glucose monophosphate through the action of a nontransferable (phosphorylase) or kinase.

Phylogeny: The evolutionary history of species, which describes the characteristics of the various species from which it descended together with its genealogical relationship to every other species.

Pleiotropy: Signifying a gene has a double function – enabling reproduction at a young age but costing the organism life expectancy in old age.

Antagonistic pleiotropy effect: Signifies that the gene has a double function – enabling reproduction at a young age but costing the organism's life expectancy in old age. Traits that benefit early survival and reproduction will be selected for, even if they contribute to an earlier death.

Disposable soma effect: Refers to an entire genetic program wherein the organism diverts limited resources from maintenance to reproduction.

Polypeptide: A linear series of 50 or less equally spaced elements called amino acids**.** The number, types, and sequences of the amino acids in a polypeptide chain determine its properties. A polypeptide tends to assume a given shape by folding into a three-dimensional way determined by the physicochemical properties of its constituent amino acids.

Population aging: The increase in the number and proportion of older people in society.

Presbycusis: Hearing loss inhibiting spoken communication.

Presbyopia: The difficulty of focusing on close objects caused by harde ning of the lens due to decreasing levels of alpha-crystallin, a process which may be sped-up by higher temperatures.

Preventive health: The medical term for care that helps us protect, promote, and maintain health and well-being.

Prion: An infectious conformational state formed by some proteins. Although often viewed in the context of infectious diseases, prions are more loosely defined by their ability to catalytically convert other native state versions of the same protein to an infectious conformational state.

Progeria: Progeria is a single-gene hereditary genetic disease that causes acceleration of many or most symptoms of aging during childhood. It affects about 1 in 4-8 million births. It is a specific type of progeroid syndrome, also known as Hutchinson–Gilford syndrome or Hutchinson–**Gilford progeroid syndrome** (HGPS). A single gene mutation is responsible for causing progeria. The gene, known as lamin A (LMNA), makes a protein necessary for holding the nucleus of the cell together. When this gene gets mutated, an abnormal form of lamin A protein called

progerin is produced. Progeroid syndromes are a group of diseases that causes individuals to age faster than usual, leading to them appearing older than they actually are. Patients born with progeria typically live to an age of mid-teens to early twenties. Severe cardiovascular complications usually develop by puberty, later on resulting in death.

Proteostasis (or protein homeostasis): The maintenance of all proteins in their original form and abundance.

Proteolysis: The breakdown of proteins into smaller polypeptides or amino acids. Catalyzed, the hydrolysis of peptide bonds is extremely slow, taking hundreds of years. Proteolysis is typically catalyzed by cellular enzymes called proteases, but may also occur by intra-molecular digestion.

Proteostasis: Protein folding and proteolysis.

Proximal aging: Age-based effects that come about because of factors in the recent past.

R

Rate of-living hypothesis: A hypothesis' described by Raymond Pearl in 1928 (based on earlier work by Max Rubner), which states that fast basal metabolic rate corresponds to short maximum life span.

Reactive oxygen species: In chemistry and biology, these are highly reactive chemicals formed from diatomic oxygen (O2), water, and hydrogen peroxide. Some prominent ROS are hydroperoxide (O2H), superoxide (O2-), hydroxyl radical (OH.), and singlet oxygen. They are pervasive because they are readily produced from O2, which is abundant. They are important in many ways, both beneficial and otherwise. They function as signals, that turn on and off biological functions. They are intermediates in the redox behavior of O2, which is central to fuel cells. They are central to the photodegradation of organic pollutants in the atmosphere. Most often however, they are discussed in a biological context, ranging from their effects on aging and their role in causing dangerous genetic mutations.

S

Sarcopenia: Loss of muscle mass and strength.

Selection shadow: A consent in which a genetic load of late-acting deleterious mutations could be substantial at mutation–selection balance.

Semelparity (from Sémélé, a strikingly beautiful princess of the ancient Greek city of Thebe and the lover of Zeus, the king of the Greek gods): A reproductive strategy available to living organisms characterized by a single reproductive episode before death. (See also iteroparity.)

Senescence (or **biological aging):** The gradual deterioration over time of the functional characteristics in living organisms. The resulting effects of senescence can be delayed.

Actuarial: An increase in mortality and/or a decrease in fecundity with age.

Cellular: The accumulation of no longer dividing cells in certain tissues.

Organismal: Involves an increase in death rates and/or a decrease in fecundity with increasing age, at least in the later part of an organism's life cycle.

Somatic cells: Other cells of the body (other than gametes).

Somatic cell nuclear transfer: A cloning method that can be used to create a cloned embryo for the use of its embryonic stem cells in stem cell therapy. Here, somatic cells can be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues (muscles, nerves, etc.). This technology has fallen in disfavor because of the possibility of inducing pluripotent stem cells.

Speciation: Repeated formations of new species.

Stem cells: Undifferentiated or partially differentiated cells in multicellular organisms. They can change into various types of cells and proliferate indefinitely to produce more of the same stem cell. They are the earliest type of cell in a cell lineage. They are found in both embryonic and adult organisms, but they have slightly different properties in each.

Multipotent cells: Stem cells that can only differentiate into a few cell types. or one type of cell.

Pluripotent cells: Stem cells that can eventually differentiate into all of the body's cell types.

Precursor or blast cells:Stem cells usually committed to differentiating into one cell type.

Progenitor cells: Stem cells that cannot divide indefinitely.

Unipotent cells: Stem cells that can only differentiate into one type of cell.

Sumoylation: A post-translationial modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle.

T

Telomerase: An enzyme, which prevents telomeres from shortening.

Telomeres: Stretches of DNA forming protective caps at the ends of chromosomes. They are repeated, noncoding nucleotide motifs and associated proteins found at the ends of eukaryotic chromosomes—mediate genome stability and determine cellular lifespan.

Thrifty gene hypothesis: It speculates that a genetic predisposition to obesity and diabetes would be advantageous in an evolutionary sense in times of food scarcity by promoting the efficient retention of energy stores in the form of adipose tissue but would become disadvantageous in times of relative food abundance and low energy expenditures. Such populations would become enriched for these thrifty genes and thus susceptible to developing diabetes. The thrifty gene hypothesis also explains the increases in type 2 diabetes among many aboriginal populations undergoing Westernization. It is likely that the genetic basis of the thrifty genotype is due to polymorphisms at multiple sites rather than a single abnormality.

Trait inheritance: Patterns in the way traits are handed down from parents to offspring over time.

Tribulation: The chemical transformation into a ribbon – a radical formed by loss of the acetylene OH group from either of two cyclic forms of ribose (yielding riboflavin andrybopyranosyl compounds) by combination with an H of -nH- or -CH group.

U

Ubiquitination: It can mark proteins for degradation via the proteasome, alter their cellular location, affect their activity, and promote or prevent protein interactions. It involves three main steps: activation, conjugation, and ligation.

V

Panmixia: A central idea in Weismann's theory of aging as the evolutionary loss of immortality in which an organism does not escape the action of natural selection and therefore disappears. Once a selective advantage for death had been established, there would be no barrier to selection for any advantageous traits that might trade-off against immortality. The forgoing of immortality might make additional resources available to reproductive cells.

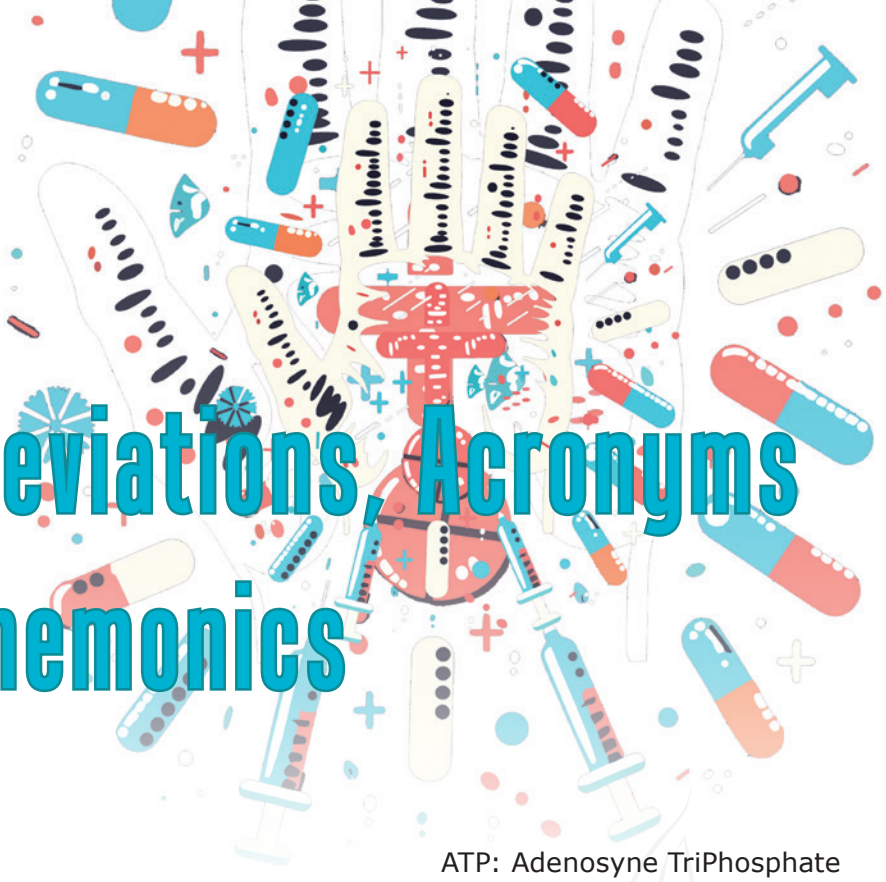
W

Wear-and-tear theory of aging (or simple deterioration theory or fundamental limitation theory): A theory of aging that asserts that the effects of aging are caused by progressive damage to cells and body systems over time.

Weismann's barrier: The idea that germ line cells contain information that passes to each generation unaffected by experience and independent of the somatic (body) cells. Germ cells produce somatic cells but are not affected by anything the somatic cells learn or, therefore, any ability an individual acquires during its life.

X

Xenotransplantation: A process where living cells from one species are grafted into a recipient of another species.



Abbreviations, Acronyms & Mnemonics

A

A: Adenine
AA: Alzheimer's Association
AASM: American Academy of Sleep Medicine
AD: Alzheimer's Disease
ADD: AD Dementia
AE: Adverse Event
AFAR: American Federation for Aging Research
AFSC: Amniotic Fluid SC
AGNES: Age Gain Now Empathy Suit
AHA: American Heart Association
Ala: Alanine
ALL: Acute Lymphocytic Leukemia
ALS: Amyotrophic Lateral Sclerosis
ANHMRC: (Australia) Australia National Health &Medical Research Council
AoA: (U.S.) Administration on Aging
APS: American Philosophical Society
ARD: Age-Related Diseases
Arg: Arginine
AS: Angelman's Syndrome
ASC: Adult SC
Asn: Asparagine
AT: Ataxia Telangectasia
ATP: Adenosyne TriPhosphate

B

BAC: Bacterial Artificial Chromosomes
BEST: Both Ends Sequencing Technology
BIRA: (U.S.) Buck Institute for Research on Aging
BLS: (U.S.) Bureau of Labor Statistics
BMD: Becker's MD
BMD: Bone Mineral Density
BMI: Body Mass Index
BMT: Bone Marrow Transplantation
BRCA: Breast Cancer
BS: Bloom's Syndrome
BWS: Beckwith-Wiedemann Syndrome

C

C: Cytosine
CALERIE: Comprehensive Assessment of Long Term Effects of Reducing Intake of Energy
CAR-T: Chimeric Antigen Receptor -T cells
Cas: CRISPR Associated Systems
CBT-I: Cognitive Behavioral Therapy for Insomnia
CD: Crohn's Disease

CDC&P: (U.S.) Centers for Disease Control & Prevention
CDCV: Common Disease Common Variant
CF: Cystic Fibrosis
CGS: Center for Genome Science
CHD: Coronary Heart Disease
ChIP: Chromatin Immuno Precipitation
CIHR: Canadian Institute for Health Research
CLL: Chronic Lymphocytic Leukemia
CMD: Calorie, Metabolism, Damage
CMMS: (U.S.) Centers for Medicare & Medicaid Services
c-NSP: Complementary SNP
COPD: Chronic Obstructive Pulmonary Disease
CR: **Calorie Restriction**
CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
CRP: C-Reactive Protein
CS: Cockayne's Syndrome
CST: Capillary Sequencing Technology
CVD: CerebroVascular Disease
CVDD: CVD Dementia
CYP: Cytochromes
Cys: Cystine

D

DBSST: Double Barrel Shotgun Sequencing Technology
DCC: Data Coordination Center
DD: Degenerative Diseases/Disorders
DEL: Diet, Exercise, Lifestyle
DESS: Diet, Exercise, Stress, Sleep
DHEA: DeHydroEpiAndrosterone
DLL: Deep Learning Language
DM: Diabetes Mellitus (or Juvenile Diabetes)
DNA: DeoxyriboNucleic Acid
  mDNA: messenger DNA
  mtDNA: mitochondrial-DNA
DNAMT: DNA Methyltransferase
DNANT: DNA Nanoball Technology
DOE: (U.S.) Department of Energy
DR: Direct Repeats
DS: Down's Syndrome
DTC: Direct-to-Consumers
DZ: Dizygous

E

EBV: Epstein-Barr Virus
E.coli: Escherichia Coli
ED: Erectile Dysfunction
EDTA: EthyleneDiamineTetraacetic Acid
EGP: (U.S.) Environmental Genome Project
EIO: European Institute of Oncology
EMA: European Medicines Agency
ERT: Estrogen Replacement Therapy
ESC: Embryonic SC
EU: European Union
Evo-Devo: Evolutionary-Developmental Biology

F

FA: Fanconi's Anemia
FA-MRC BBSRC RC: (U.K.) Founders Alliance, Medical Research Council, Biotechnology and Biological Sciences Research Council, Cancer Research
FDA: (U.S.) Food & Drug Administration
FGT: Follistatin Gene Therapy
FMER-PMA: Germany Federal Ministry of Education & Research, Project Management Agency
FMO: Flavin-dependent Mono Oxygenase
FNAR: (France) French National Agency of Research
FSC: Fetal Stem Cells
FSGS: Focal Segmental Glomerulosclerosis
FT: Fluorophore Technology

G

G: Guanine
GCR: Gluco Corticoid Receptor

GDM: Gestational Diabetes Mellitus
GE: Gene-Environment
GEL: Genetics, Environment, Lifestyle
GERD: GastroEsophageal Reflux Disease
GERT: Gerontology Reference Suit
GGE: Gene-Gene-Environment
GH/IGF-1: Growth Hormone/Insulin-like Growth Factor-1
GI: Gastro-Intestinal
GINA: Genetic Information Non-Discrimination Act
GlY: Glycine
Glu: Glutamic Acid
GM: Gompertz–Makeham (law of mortality)
GST: Glutathione S-Transferase
GVHD: Graft-Versus-Host Disease
GWAS: Genome Wide Association Studies
G6PD: Glucose-6-Phosphate Dehydrogenase

H

H: Histidine
HAT: Histone Acetyl Transferase
Hb: Hemoglobin
HCANC: Human Cytochrome Allele Nomenclature Committee
HD: Huntington's Disease
HDAC: Histone De Acetylase Transferase
HDAT: Histone De-Acetyl Transferase
HEP: (U.S.) Human Epigenome Project
HGH: Human Growth Hormone
HGP: (U.S.) Human Genographic Project
HGP: (U.S.) Human Genome Project
HGPS: Hutchinson–Gilford Progeroid Syndrome
HHC: Hyper Homo Cystenemia
HIIT: High-Intensity Interval Training
HIV/AIDS: Human Immunodeficiency Virus/Acquired Immuno Deficiency Syndrome
HLMT: Histone Lysine Methyl Transferase
HNPC: Hereditary Non-Polyposis Coli
HOT: Hyperbaric Oxygen Therapy
HPA: Hypothalamic-Pituitary-Adrenal axis
HSC: Hematopoietic SC
HZ: Heterozygous

I

IC: Intrinsic Capacity
ICGC: International Cancer Genome Consortium
IGF-1: Insulin-like Growth Factor 1
IHEC: International Human Epigenome Consortium
IHP: International Haplogroup Project
IHP: International HapMap Project
IJCP: International Journal of Clinical Practice
IIT: Italian Institute of Oncology
Ile: Isoleucine
IMC: Inner Mass Cells
IMOF: Institute of Molecular Oncology Foundation
IMP: **Interference with Metabolic Processes**
IOF: International Osteoporosis Foundation
iPSC: induced PSC
ITT-CGS: (Italy) Italian Institute of Technology, Center for Genomic Science

J

JSTA: Japan Science and Technology Institute

L

LDL: Low Density Lipoprotein
LDL-R: LDL-Receptor
Leu: Leucine
LMNA: Lamin A (a gene)
LSC: Limbal SC
LUCA: Last Universal Common Ancestor
Lys: Lysine

M

M**acD: Mutations, aggregates, cross-links, Defects**
Mb: Megabase
MCI: Mild Cognitive Impairment
MD: Macular Degeneration
MD: Muscular Dystrophy
MET: Metabolic Equivalent
Met: Methionine
MI: Myocardial Infarction **10.**16
MLL: Mixed Lineage Leukemia
mLOY: mosaic Loss Of chromosome Y
MODY: Maturity Onset Diabetes of the Young
MRFE: (Genome) Mutations, (Genes) Reshuffling, (Gene) Flow, Epigenetics
MRI: Magnetic Resonance Imaging
MS: Multiple Sclerosis
MSC: Mesenchymal SC
MSE: Minnesota Starvation Experiment
MSS: Micro Satellite Stable
MVB: Multi Vesticular Bodies
MZ: Monozygous
M5: **Matters, Medications, Mentation, Mobility, Multi-complexity**

N

NAR: (France) National Research Agency
NAS: (U.S.) National Academy of Sciences
NCI: (U.S.) National Cancer Institute
ND: Neurological Diseases/Disorders
NDD: Neuro-Degenerative Diseases/Disorders NHGRI: (U.S.) National Human Genome Research Institute
NHMR: (U.S.) National Health Medical Research Council
NHS: (U.K.) National Health Service
NIDOCD: (U.S.) National Institute on Deafness and Other Communications Disorders
NIEHS: (U.S.) National Institute of Environmental Health Sciences
NIH: (South Korea) National Institute of Health
NIH: (U.S.) National Institutes of Health
NIH/NIA: (U.S.) National Institutes of Health, National Institute on Aging
NIH-REP: (U.S.) National Institutes of Health, Roadmap Epigenome Project
NMDP: National Marrow Donor Program
NMR: Nuclear Magnetic Resonance
NSF: National Sleep Foundation
NT: Nanopore Technology

O

OAR: Obligatory Asymmetric Replication
OFR: O**xygen Free Radicals**
OGOP: One Gene, One Protein
OS: Oxidative Stress
OTC: Over-The-Counter
OTDH: Offspring, Traits, Differential, Heritability

P

PAH: Polycyclic Aromatic Hydrocarbons
PAMT: Protein Arginine Methyl Transferase
PARP: Poly ADP Ribose Polymerases
PCP: Pneumocystis Carini Pneumonia
PCR: Polymerase Chain Reaction
PD: Parkinson's Disease
PD: Programmed Death
PDD: PD Dementia
PEGB: Programmed, Error, Genetic, Biological
PG: Pluripotency Gene
PGEP: Personal Genetics Education Project
PGP: Personal Genome Project
Phe: Phenylalanine
PHEGBE: Programmed, Hormonal, Error, Genetic, Biological, Environmental
PKD: Polycystic Kidney Disease
PKU: PhenylKetonUria
PLoS: *Public Library of Science*
PMER: (Germany) Federal Ministry of Education &Research - Projects

Management Agency, *German Aerospace Center*
Pro: Proline
PSC: Pluripotent SC
PSF: Paracrine soluble factors
PST: Pyro Sequencing Technology
PTF: Protein Transcription Factors
PTSD: Post-Traumatic Stress Disorder
PVD: Peripheral Vascular Disease **10.**16
PWS: Prader-Willi Syndrome

R

RBC: Red Blood Cells
RNA: RiboNucleic Acid
- g-RNA: Guide-RNA
- m-RNA: Messenger-RNA
- mi-RNA: Micro-RNA
- mi-RNA: Mitochondrial-RNA
- r-RNA: Ribosomal-RNA
- sg-RNA: Single Guide RNA
- si-RNA: Small-Interfering RNA
- sn-RNA: Small Nuclear-RNA
- t-RNA: Transfer-RNA

ROS: Reactive Oxygen Species
RTS: Rothmund–Thomson Syndrome

S

SA: Successful Aging
SAE: Speciation, Anagenesis, Extinction
SAE: Serious AE
SC: Stem Cells
- ESC: Embryonic SC
- PSC: Pluripotent SC
- SSC: Somatic (or adult) SC
- TSC: Totipotent SC

SCA: Sickle Cell Anemia
SCGS: Single Cell Genome Sequencing
SCI: Spinal Cord Injury
SCNT: Somatic Cell Nuclear Transfer
SCT: SC Therapy
SD: Stochastic Differentiation
SDMF: (Natural) Selection, (Genetic) Drift, Mutation, (Gene) Flow
Ser: Serine
SMRTST: Single Molecule Real Time Sequencing Technology
SNP: Single Nucleotide Polymorphisms
SNV: Single Nucleotide Variant
SOD: SuperOxide Dismutase
SST: Shotgun Sequencing Technology
SUMO: Small Ubiquitin-Like Modifiers

T

T: Thymine
TB: Tuberculosis
TBI: Traumatic Brain Injury
TCBD: TetraChlorodiBenzo-p-Dioxin
Td: Tetanus-diphtheria vaccine
Tdap: Tetanus-diphtheria and pertussis vaccine
TERRA: Telomeric-Repeat-containing RNA
TF: Transcription Factors
TGT: Telomerase Gene Therapy
Thr: Threonine
Try: Tryptophan
TSG: Tumor Suppressor Genes
TSNA: Tobacco-Specific N-Nitrosamines
Tyr: Tyrosine
T(1, 2, 3)D: Type (1, 2, 3) Diabetes

U

U: Uracil
UCB: Umbilical Cord Blood
UCBT: UCB Therapy
UGC: Universal Genetic Code
UKFA-MRC-BBSRC-RC-CR-WT: (U.K.) Founders Alliance, Medical Research Council, Biotechnology and Biological Sciences Research Council, Cancer Research, Wellcome Trust
UN: United Nations
UNFPA: Populations Fund
USC: Unipotent SC
UV: Ultra-Violet

V

Val: Valine
VD: Vaginal Dryness

W

WD: Wilson's Disease
WDC: World Dementia Council
WGS: Whole Genome Sequencing
WHO: World Health Organization
WS: Werner's Syndrome

X

XP: Xeroderma Pigmentosa

Y

YAC: Yeast Artificial Chromosomes

Diseases/ Disorders & Syndromes

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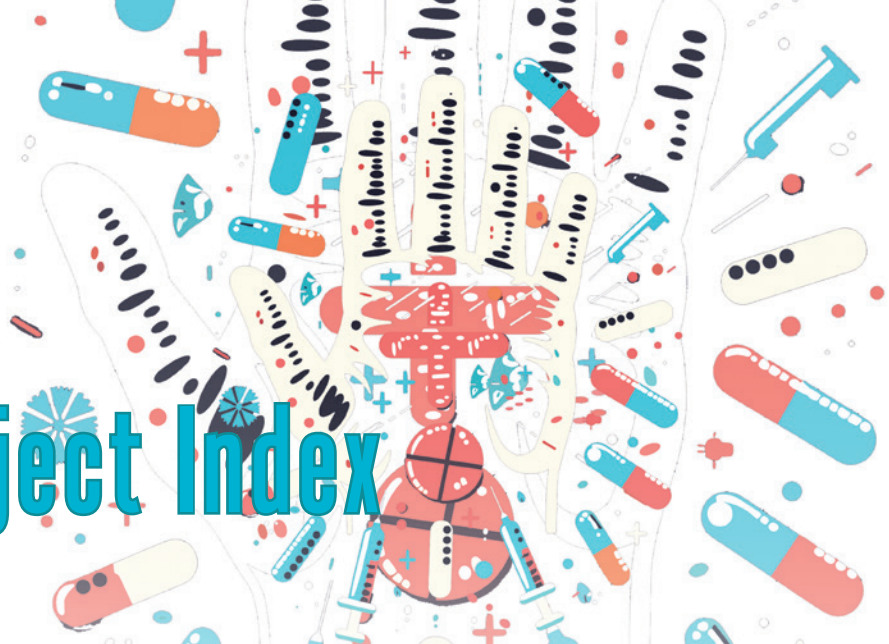

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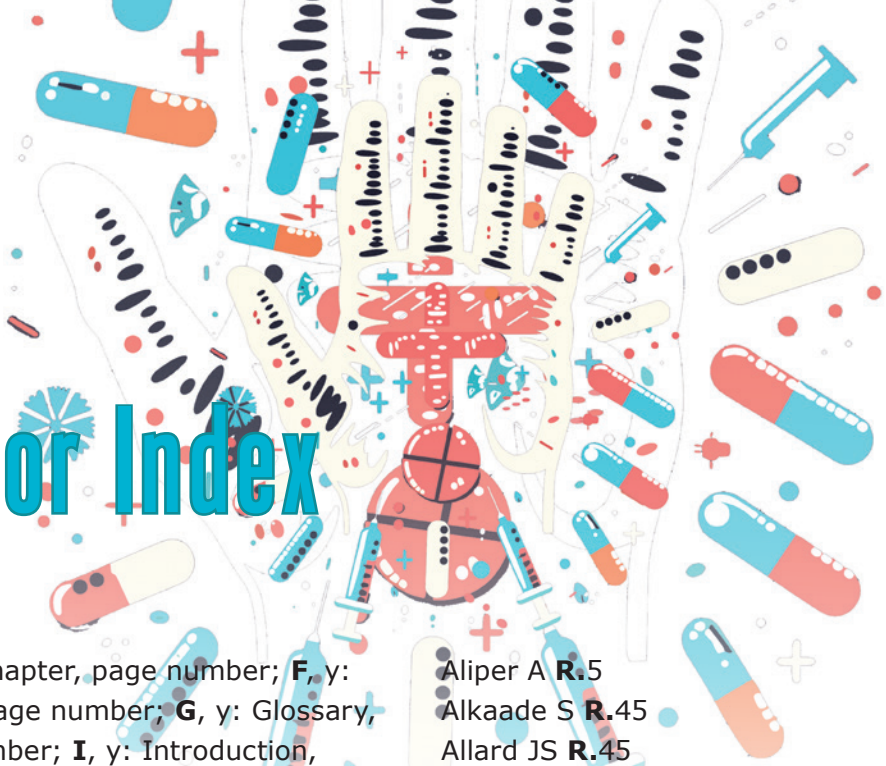

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