Aging, Aging Process and Roles of Genes in Aging

A Dissertation Submitted to the Department of Pharmacy, East West University, in Partial Fulfillment of the Requirements for the Degree of Bachelor of Pharmacy

Submitted By

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Certificate

This is to certify that the thesis on Aging, Aging Process and Roles of Genes in Aging submitted to Department of Pharmacy, East West University, Aftabnagar, Dhaka, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm) was carried out by MOHAMMAD WALIULLAH (ID# 2012-1-70-020) under the guidance and supervision and that no part of this thesis has been submitted for any other degree. We farther certify that all the sources of information are duly acknowledged.

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Declaration by Research Candidate

I MOHAMMAD WALIULLAH hereby declare that the description entitled 'Aging, Aging Process and Roles of Genes in Aging' submitted by me to the Department of Pharmacy, East West University in the partial fulfillment of the requirement for the award of degree of Bachelor of Pharmacy (Honors) is a genuine and authentic record of original research work carried out by me under supervision and guidance of Dr. Repon Kumer Saha, Assistant Professor, Department of Pharmacy, East West University and it has not formed the basis for the award of any other Degree/ Diploma/ Fellowship or other similar title to any candidate of any University.

Signature of Candidate

(MOHAMMAD WALIULLAH)

Date:

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Acknowledgement

All praise to almighty Allah who has given me the ability to complete my B. Pharm thesis and the opportunity to study in this subject.

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I remember the inspiring words of my family members, my classmates and all my well wishers. I say thanks to them for their inspiration during my thesis work.

Abstract

Ageing (British English) or aging (American English) is the process of becoming older. In the narrow sense, the term refers to biological ageing of human beings, animals and other organisms. In the broader sense, ageing can refer to single cells within an organism (cellular senescence) or to the population of a species (population ageing).

In humans, ageing represents the accumulation of changes in a human being over time, encompassing physical, psychological, and social change. Reaction time, for example, may slow with age, while knowledge of world events and wisdom may expand. Ageing 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 die from age-related causes.

The causes of ageing are unknown; current theories are assigned to the damage concept, whereby the accumulation of externally induced damage (such as DNA point mutations) may cause biological systems to fail, or to the programmed ageing concept, whereby internal processes (such as DNA telomere shortening) may cause ageing.

The discovery, in 1934, that calorie restriction can extend lifespan twofold in rats, and the existence of species having negligible senescence and potentially immortal species such as Hydra, have motivated research into delaying and preventing ageing and thus age-related diseases.

Rationale and Objective of the Work

The objective of this work to understand aging and aging process of human and different living creatures and also try to find out whether a single cause (probably cellular or hormonal) lies behind all aging phenomena or whether aging is inherently multi-faceted. Differences in lifespan between species raise critical questions, in this regard. Why is a rodent old at 3 years, a horse old at 35 years and a human old at 80 years? Aren't the cells much the same? Why is it that at age 3 about 30% of rodents have had cancer, whereas at age 85, about 30% of humans have had cancer? Some species (such as lobsters, alligators and sharks) show few signs of aging. Cancer cells, stem cells and human germ cells seem "immortal" when compared to other cells.

Another objective is to find out the roles of genes in aging, what kind’s genes promote aging and what kind’s genes slow down aging. What kinds of mutation to genes abnormal aging.

1. Introduction

Aging is often viewed as an accumulation of changes over time that renders organisms more likely to die. However, neither the nature of these changes nor the causal relationships in aging are understood, nor many related fundamental questions remain unanswered. Has a process that makes organisms more vulnerable and more likely to die evolved? Does it have purpose? What is the cause of aging? What are the associated mechanisms? Can aging be stopped or postponed? How do genomes define lifespan? How is lifespan adjusted during evolution and in response to dietary interventions?

Aging is a syndrome of changes that are deleterious, progressive, universal and thus far irreversible. Aging damage occurs to molecules (DNA, proteins, lipids), to cells and to organs. Physico-chemical properties preclude ideal bio-molecules and perfect biological functions. This inherent imperfectness leads to the generation of damage by every biological process, at all levels, from small molecules to cells. The damage is too numerous to be repaired, is partially invisible to natural selection and manifests as aging.

As each bio-molecule generates specific forms of damage, the cumulative damage is largely non-random and is indirectly encoded in the genome. Accumulation of molecular damage that arises through the imperfections in the molecular machinery of life has long been considered key to the aging process. It is not clear, however, how this damage is generated, whether it is generated purposefully, why it cannot be completely removed from cells, and whether it is stochastic. It is also not known whether damage causes aging or is simply a bystander generated with no influence on the process. Although the idea that cumulative damage causes aging is viewed by many as a truism, nearly every aspect of this idea is questionable, with many researchers discounting damage as a relevant factor altogether.

The effects of aging were traditionally thought to be immutable, particularly evident in the loss of plasticity and cognitive abilities occurring in the aged central nervous system (CNS). However, it is becoming increasingly apparent that extrinsic systemic manipulations such as exercise, caloric restriction, and changing blood composition by heterochronic parabiosis or young plasma administration can partially counteract this age-related loss of plasticity in the aged brain. In this review, we discuss the process of aging and rejuvenation as systemic events. We summarize genetic studies that demonstrate a surprising level of malleability in organismal lifespan, and highlight the potential for systemic manipulations to functionally reverse the effects of aging in the CNS. Thus, systemic manipulations promoting a younger blood composition provide effective strategies to rejuvenate the aged brain. As a consequence, we can now consider reactivating latent plasticity dormant in the aged CNS as a means to rejuvenate regenerative, synaptic, and cognitive functions late in life, with potential implications even for extending lifespan.

Aging at its core can be thought of as a systemic event. Indeed, the effects of aging do not occur in a targeted and isolated manner, but rather functionally alter tissues throughout the body (systemic aging), albeit at different rates. With this in mind, individual tissues exhibit different levels of sensitivity and resilience to aging. In particular, evidence suggests that the central nervous system (CNS) is especially vulnerable to the effects of aging experiencing a gradual loss in the ability to physically and functionally adapt to new experiences with age Consequently, aging in the CNS results in decreased regenerative capacity for repair and impaired maintenance of synaptic and cognitive functions This detrimental influence of aging on the CNS is particularly alarming when considering the role of the CNS in regulating overall homeostasis Functionally, the CNS not only integrates sensory information from the external environment but also responds to changes from within through communication with the systemic environment, collectively regulating important physiological processes including growth, metabolism, and reproduction More recently, the interactions of the CNS with the systemic environment have even been implicated in directly regulating organismal lifespan Thus, the CNS exhibits a unique

duality not reflected in other tissues, serving as both a vulnerable site to the effects of aging while also acting as a potential master regulator of systemic aging itself (Lopez-Otin *et al*. 2013).

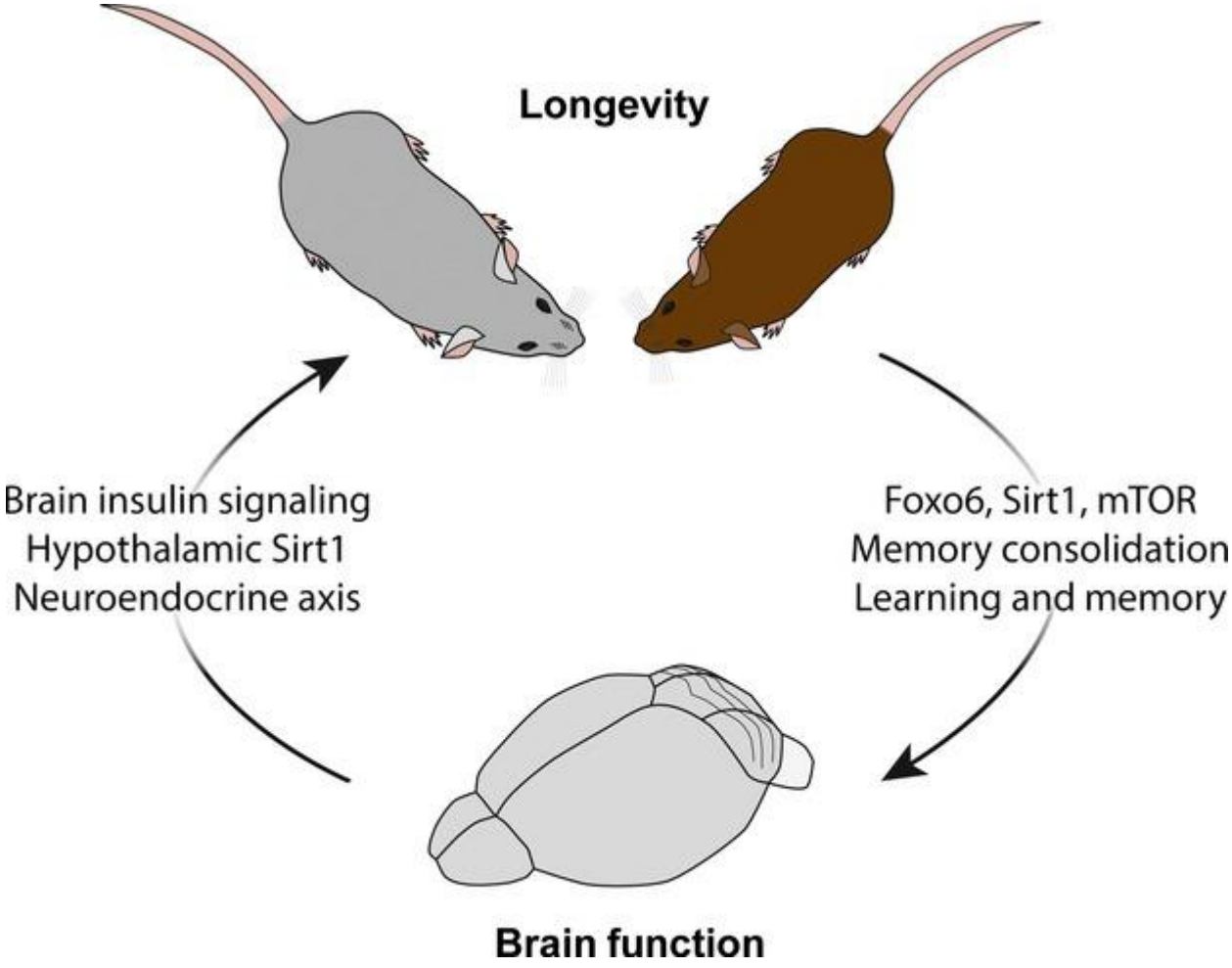

Figure 1.1: Interplay between lifespan regulation and brain function. (Lopez-Otin *et al*. 2013)

Schematic illustration depicts the unique duality of the brain to be both responsive to systemic lifespan regulation as well as serve as a central regulator of lifespan. Genetic studies have identified the FoxO family of transcription factors, Sirtuins and mTOR signaling pathway as molecular regulators that both promote longevity and mediate critical brain functions known to undergo age-related impairments such as learning and memory. Conversely, the brain has also

been demonstrated to promote longevity through neuronal regulation, particularly via the hypothalamus region.

As with many aspects of the aging process, the loss of plasticity in the aged CNS was traditionally thought to be immutable. However, it is becoming increasingly evident that changes in the aged systemic environment through systemic manipulations such as exercise, caloric restriction (CR), and heterochronic parabiosis (in which blood composition is altered by connecting circulatory systems of young and aged animals) can partially counteract the age-related loss of plasticity in the aged CNS. Correspondingly, it is feasible to consider reactivating latent plasticity dormant in the aged CNS as a means to rejuvenate regenerative, synaptic, and cognitive functions late in life, with potential implications for extending lifespan (Merzenich *et al*. 2014).

Population aging is an enormous public health issue and there is clear need for strategies to maximize opportunities for successful aging. Many psychiatric illnesses are increasingly thought to be associated with accelerated aging, therefore emerging data on individual and policy level interventions that alter typical aging trajectories are relevant to mental health practitioners. Although the determinants and definition of successful aging remain controversial, increasing data indicate that psychiatric illnesses directly impact biological aging trajectories and diminish lifestyle, psychological and socio-environmental factors that seem reduce risk of morbidity and mortality. Many interventions designed to enhance the normal course of aging may be adjunctive approaches to management of psychiatric illnesses.

While much of the focus of research on aging has been on the determinants of mortality, illness, and disability, a growing body of work has begun to assess modifiers and interventions to improve upon the usual course of aging. More recently, research has delivered promising data on strategies that might alter the course of typical aging among people with psychiatric disorders, which seem to be associated with accelerated aging. It is important to note that there is yet no consensus definition of successful aging. As such, there is no standard outcome upon which the effectiveness of successful aging strategies could be uniformly gauged. Successful aging strategy

is a potentially modifiable characteristic or intervention that is intended to enhance the functioning of older adults who could be characterized as aging normally.

The insulin/insulin growth factor (IGF) signaling (IIS) cascade is an evolutionarily conserved pathway among diverse species, ranging from yeast to humans. While defects in the insulin signaling pathway lead to insulin resistance and diabetes in rodents and humans, disruption of this signaling pathway has been shown to significantly extend lifespan in *C. elegans*, flies, mice, and humans. These long-lived IIS mutants share some important phenotypic characteristics including reduced insulin signaling, enhanced insulin sensitivity, and reduced serum IGF-1 levels, together with reduced oxidative damage of macromolecules and increased stress resistance. The daf-2/IR/IGFR mutant *C. elegans* live twice as long as wild-type and the longevity phenotype does not need go through the dauer state. PI3K-null adult *C. elegans* are more resistant to oxidative and electrophilic stresses and live remarkably longer under both normal and toxic environments compared to wild-type controls. These effects have been shown to depend on the integrity of the dauer daf-16/FOXO, which has similarity to a family of mammalian forkhead transcriptor factors. The lower level of free radicals in daf-2/IR/IGFR mutants has been shown to be essential for life span extension. Indeed, the gene ctl-1, which encodes a cytosolic catalase, is required for the extension of adult life span by daf-2/IR/IGFR. The expression of mitochondrial Superoxide dismutase 2 (SOD2) is required for the longevity extension caused by mutations decreasing the activity of the Ras/Cyr1/PKA and Sch9 pathways in yeast. Similarly, flies homozygous for chico/IRS null mutant have increased levels of SOD, reduced body size, greatly reduced fecundity, and increased longevity. These findings highlight the central position of oxidative stress in the aging-regulatory machinery and superoxide toxicity plays an important role in aging and death. *C. elegans* proteotoxicity models indicate that the IIS pathway directly links aging to the onset of toxic protein aggregation, and the protective effects are dependent on daf-16/FOXO, as the effects could be abolished by RNAi-mediated depletion of daf-16. Another strong link between insulin/IGF-1 signaling and life span in animal models comes from dietary restriction. Primates maintained on dietary restriction feeding regimens exhibit increased insulin sensitivity and enhanced glucose tolerance. Calorically restricted rats have lower levels of IGF-1 that contributes to the protective effect against age-related pathology

and resistance to p-cristine-induced bladder cancer. Taken together, these results suggest that the IIS pathway is critical for regulating various aging-related disorders and longevity.

The human lifespan is fairly long compared to that of many other animals, but is nevertheless limited with most people living about 80 years and rare individuals reaching 100 years. Yet there are some animals, plants and fungi that can live for several hundred or even several thousand years, and often show negligible senescence. What are the mechanisms underlying great longevity and can we apply such knowledge to enhance the health and longevity of humans? The longest living animals are also among the simplest ones – the ones that are called basal metazoans, a group that includes sponges, corals, jellyfish, comb jellies, hydras, and sea anemones. All of the more advanced animals including humans are bilaterians, and the simplest of these are the flatworms. Basal metazoans typically maintain many pluripotent stem cells that are capable of differentiating into all typesof cells in the body; this gives these animals incredible abilities to grow, regress, regrow and regenerate their bodies as needed. They can become in some cases potentially immortal. However, during the evolution of more complex animal body forms, these abilities were reduced or lost, apparently in an effort to produce complex body structures for sophisticatedfunctions while still avoiding the production of destructive tumors. Nevertheless, there is no direct correlation of increasedbody complexity with reduced lifespan. For example, among the bilaterians, some vertebrates such as tortoises and whales can live more than 200 years and some clams live over 500 years, while the adult roundworm, Caenorhabditis elegans, although only a little more complex in design than flatworms, lacks somatic stem cells and lives only a couple of weeks. The evolution of animals with greater complexity included the development of mechanisms forlimitinglifespan and senescence (Rando, 2006; Pearson and Sánchez Alvarado, 2008; Tanaka and Reddien, 2011; Rink, 2013; Solana, 2013).

Table 1.1: List of major animals discussed in this review (Solana, 2013)

Protozoa	Ciliates (e.g., Paramecium, Tetrahymena, suctorians) Choanoflagellates
Basal Metazoans	Porifera (sponges) Placozoa Ctenophora (comb jellies) Cnidaria (hydras, jellyfish, sea anemones, colonial hydroids, corals) Myxozoa

Table 1.2: Stem cell terminology (Rando, 2006)

Stem cells	Undifferentiated cells that can differentiate into specialized cell types and can divide through mitosis to produce more stem cells, either for a limited number of divisions or potentially as an immortal cell clone.
Unipotent/oligopotent stem cells	Stem cells that can differentiate into only one/few cell types. For example, vertebrate

	muscle satellite cells are stem cells that produce myoblasts that form into muscle cells
Multipotent stem cells	Stem cells that can differentiate into many cell types. An example would be the I-cells of hydras
Pluripotent somatic stem cells	Stem cells that can differentiate into all known somatic cell types of an animal.
Primordial germ cells	The initial cells of a developing germline, which is capable only of forming germ cells (gametogenesis).
Totipotent stem cells	Pluripotent stem cells that also is capable of gametogenesis. Examples would be mammalian zygotes and early embryos. Choanocytes and archeocytes of sponges and neoblasts of planaria may be totipotent or pluripotent, depending on various authors' definitions and descriptions.

Table 1.3: Mechanisms affecting aging and longevity in the simplest animals

(Rando, 2006)

Ciliate Protozoa	asexual clonal immortality asymmetric budding cycling between dedifferentiation and differentiation encystment regeneration
Porifera	asexual reproduction including gemmule formation, budding and stolon formation degeneration and regeneration dissociation and reorganization

Cnidaria	asexual budding clonal colonial growth continuous cellular renewal dedifferentiation of cells dissociation and reorganization regeneration by morphallaxis rejuvenation
Flatworms (Acoelomorpha and Platyhelminthes)	alternating regression and growth asexual reproduction via uneven fission controlled shrinkage during starvation regeneration from body fragments

Some of these phenomena are associated with negligible senescence, and have been largely lost in the evolution of the higher Metazoa; they may be worth exploring in the search for solutions to extend the human life span and avoid the diseases of aging.

2. Materials Used

I have gathered information mainly through internet using ‘PubMed’.

PubMed is a free search engine accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics.

PubMed provides quality control in scientific publishing. Only journals that meet PubMed's scientific standards are indexed.

I have gathered my research material by applying following method

I. Firstly I have entered www.pubmed.gov
II. Then I have entered into Advanced Search option.
III. I have chosen Title as a filter option and put aging or gene
IV. I have chosen MeSH Major Topic as another filter and put aging
V. Then I searched.
VI. I have chosen 2 more filters.
VII. One of them was Article Type. I have chosen ‘Review’.
VIII. Other was Text Availability. I have chosen ‘Free Full Text’.

I have also try to read book and other authentic internet sources for gathering information.

3. Aging Process

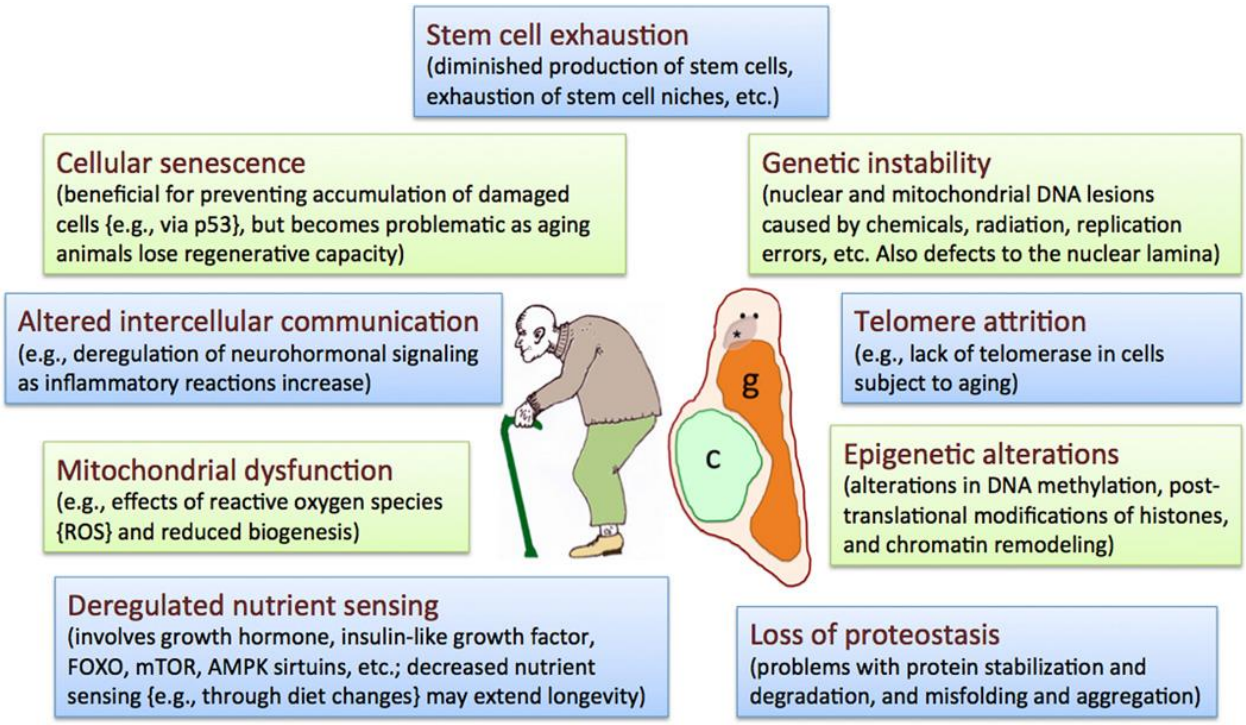

Figure 3.1: The diagram illustrates the nine hallmarks of aging. (Mattson, 2008)

Aging animals often display noticeable changes in external appearance, body shape and posture. The center shows a stereotypical silhouette of an aging person with balding head and stooped posture due to various degenerative diseases. Some aging flatworms can show comparable changes such as a surface head groove (asterisk) and a body deformed by cysts (c). The digestive tract or gut (g) is pushed to the side. The 2 black dots represent the eyes.

Aging of the fully differentiated adult cells results from a combination of mechanisms. Five problems are known to play prominent roles in aging.

Firstly, the genomic DNA and epigenetic problem. Throughout the lifespan, it is inevitable for a cell to encounter a range of challenges and insults from the surrounding environment and within the cell. These insults cause lesions to DNA molecules. The examples of such DNA lesions include point mutation, double-strand break, translocation, and telomere shortening. In order to fix these DNA lesions, cells are equipped with highly elaborate and dedicated DNA repair machineries. Extensive evidence has suggested that increased DNA lesions, and or, weakened DNA repair systems directly contribute to the aging process. In addition to DNA molecules themselves, epigenetic factors are also critical to aging processes. Among these epigenetic factors, histone modifications seem to play particularly important and widespread roles in aging.For example, the reduction of NAD-dependent SIRT6 deacetylase (a member of the sirtuin family) significantly shortens the lifespan in mice; conversely, forced overexpression of this enzyme in mice enhances longevity. The roles of histone modifications in regeneration and stem cell biology have been studied in the planarian Schmidtea mediterranea (Robb and Sánchez Alvarado, 2014).

Secondly, the protein quality control problem. Once proteins are synthesized, they undergo careful folding, which is an uncompromising requirementfor functionally competent proteins and for healthy cells Accordingly, cells ranging from unicellular organisms to humans all have developed tightly-regulated systems that execute the proper folding , and also promptly remove those proteins that are unfolded or misfolded (Jarozs et al., 2010). The maintenance or disruption of such proteostasis directly impacts cellular aging. The importance of proteostasis is further illustrated by the characteristic pathology - aggregation of misfolded proteins - found in several age-related diseases, such as Alzheimer’s disease and Parkinson’s disease (Balch et al., 2008).

Thirdly, the organelle problem. Changes during aging are not restricted to DNA and proteins, but also affect, and are affected by cellular organelles. In the case of the nucleus, the lamina - one of the nuclear envelope components - has been implicated in the aging process. For example,

mutations in genes encoding components of the nuclear lamina have been found to be the cause of several premature aging syndromes. Studies of normal aging cells have demonstrated that lower levels of, or defective lamin A or B subtypes accelerate the aging process. For this reason, nuclear lamina has been used as a biomarker of aging. A recent study of healthy centenarians has shown that nuclear accumulation of prelamin A contributes to longevity by preventing stress-induced DNA damage, further supporting the critical role of nuclear lamina in maintaining healthy aging. Another class of organelle that is associated with cellular aging is mitochondria. Mitochondrial DNA is particularly susceptible to age-dependent mutations, at least in part, due to the DNA repair systems that are less powerful than those used for genomic DNA. Moreover, since mitochondria provide a major level of regulation to a range of intracellular processes such as converting energy substrates into ATP, reactive oxygen species (ROS) metabolism, calcium signaling, and iron homeostasis, it is likely that the dysfunction of mitochondria has major impacts on the molecular processes that contribute to normal aging and age-related diseases (Mattson, 2008; Mallikarjun et al., 2014).

Fourthly, the signaling pathway problem. A number of signaling pathways have been shown to play key roles in cellular aging. Prominent among such age-regulating pathways is the insulin/IGF-1 signaling pathway. The insulin/IGF-1 pathway is important because it regulates energy metabolism, food intake and stress responses. The involvement of the insulin/IGF-I pathway in aging has been documented in a variety of organisms. In hydras, a protein that bears resemblance to mammalian insulin receptor is expressed, and cells expressing such receptors undergo active proliferation in response to mammalian insulin. In Drosophila, an insulin receptor substrate protein CHICO affects cell growth and the loss of this protein extends lifespan significantly. In C. elegans, extensive evidence suggests that the insulin/IGF-1 signaling events directly influence longevity. And in mice, it has been found that proteins that transduce insulin/IGF-1-like signaling have direct roles in determining lifespan. Together, these studies suggest a remarkable conserved role for the insulin/IGF-1 pathway in longevity (Clancy et al., 2001).

Lastly, the oxidative stress problem. Cells generate reactive oxygen species (ROS) during mitochondrial respiration and by the activity of various oxygenases; these include superoxide anion radical, hydrogen peroxide, hydroxyl radical, nitric oxide and peroxynitrite. Such ROS can damage proteins, nucleic acids and cell membranes (lipid peroxidation). However, cells have evolved multiple mechanisms to prevent the formation of, or remove, ROS including the superoxide dismutases (SOD1 and SOD2), glutathione peroxidases, catalase, and glutathione. Such ROS-related mechanisms have been described in hydras and other cnidarians where they are involved in growth, development and reproduction and ROS are involved in injury-induced cell death and activation of regeneration in Hydra, as well as in Drosophila and zebrafish. In essentially all species that age there occurs increased accumulation of oxidatively damage molecules, and reducing this oxidative damage can extend lifespan in experimental animals such as yeast and C. elegans (Labuschagne and Brenkman, 2013; Merksamer et al., 2013).

Interestingly, however, when cells and organisms are subjected to mild intermittent oxidative stress, they become resistant to more severe stress as a result of adaptive responses that bolster their antioxidant defense systems. Such adaptive stress response mechanisms are highly conserved and may be largely responsible for the extension of lifespan by energy restriction and exercise (Mattson, 2012).

3.1. Concepts of Aging

I. **Programmed aging:** The model of programmed aging, first formulated by August Weismann in the 19th century, proposes that aging is a purposeful program and that the death of older individuals in the population benefits subsequent generations. The model implies that this altruistic plan has evolved and been maintained for purpose (e.g., to benefit future generations), involves certain genes, and can possibly be cancelled or postponed. Aging can only be viewed as programmed in the sense that it

involves all genes and the entire metabolism, and is therefore a direct consequence of life.

II. **Hyperfunction:** The hyperfunction aging model suggests that aging is a consequence of over-activity of processes, which continue after the completion of development, and involves an imbalance between cell growth and cell cycle control signals. Hyperfunction resembles programmed aging, although it has not evolved for the purpose of aging, so it has been referred to as quasi-programmed aging. Damage accumulation is explicitly excluded from the theory as a causal or relevant factor (i.e., it may accumulate but is considered irrelevant to the aging process because hyperfunction kills an organism first). What is viewed as hyperfunction and continued development (and any other aspects of dysregulation of cellular processes), however, represent the secondary manifestations of aging that can be easily understood in terms of cumulative damage, especially in cells that are metabolically active, yet unable to divide. What the hyperfunction theory considers as aimless continuation of growth and development is in fact metabolism that sustains life, but inevitably accumulates damage, causing dysfunction. The hyperfunction aging model implies that tight

regulation of the developmental program during adulthood may stop or significantly delay aging.

III. **Evolutionary theory of aging:** According to this model, aging is a consequence of decline in the force of natural selection that occurs late in life, after successful reproduction. The theory proposes that mutations improve genes beneficial in early life, whereas these mutations may inactivate genes that are beneficial later in life. It is further proposed that the genes benefiting young organisms will be selected for even if they are harmful at older ages [16, leading to the concept of antagonistic pleiotropy.

However, the model presented here argues that, over time, the contribution of any gene to cumulative damage will outweigh its beneficial effect, regardless of whether this benefit declines, increases or is constant throughout life. Thus, genes appear to exhibit antagonistic pleiotropy because they both benefit organisms at any time and contribute to cumulative damage over time. Moreover, this argument is not limited to genes and mutations and applies to all metabolites, macromolecules and other purposefully used molecules in the cell. For example, the use of transition metals, such as copper and iron, as protein cofactors is selected during evolution, but they also significantly contribute to cumulative damage due to their reactive nature. Their use made organisms more competitive, but also contributed to accumulation of damage over time. Respiration that produces damaging reactive oxygen species is another example of antagonistic pleiotropy.

IV. **Disposable soma:** The disposable soma theory proposes that organisms balance resources that they invest into reproduction and somatic maintenance, resulting in trade-offs between somatic repair and reproduction. For example, a more significant

investment in reproduction is thought to decrease investment in maintenance thereby shortening lifespan, whereas reduced reproduction allows redistribution of resources towards protection, thereby extending lifespan. The disposable soma theory considers damage accumulation is the key.

V. **Oxidative damage:** This model, also known as the free radical theory of aging, proposes that reactive oxygen species damage biomolecules, causing aging. While oxidative damage may contribute to aging, placing emphasis on just one damage type limits its relevance, and it does not represent the ultimate cause of aging (but represents proximate cause). It may be more relevant to regulating lifespan under

certain conditions, but irrelevant under other conditions. Aging would still occur in the absence of oxygen, and even if there was no such thing as reactive oxygen species. For example, yeast cells grown under anaerobic conditions not only age, but they have a shorter lifespan than cells grown under aerobic conditions.

VI. **DNA damage:** This theory proposes that damage to DNA causes aging. DNA is the only molecular species in the cell that cannot be renewed, and DNA damage clearly contributes to aging. Again, however, DNA damage represents only a subset of cumulative damage. For example, in yeast, daughters of old mother cells have a shorter lifespan, but the lifespan is restored in granddaughters. It appears that much of the damage accumulated in old mother yeast cells is transferred to their daughters, but it is diluted in subsequent generations.

VII. **Damage-centric theories of aging:** Damage-centric models, from simpler ideas such as wear and tear (essentially the classical rusty car analogy) to the advanced concepts that are integrated with evolutionary models, place cumulative damage in the center of the aging process, but they view damage as random and do not explain why damage is inevitable, why it is generated and why it cannot be fully removed. These models do not distinguish between severe and mild damage forms, do not recognize the primacy of genotype in defining damage, and do not logically separate the cause of aging from the control of lifespan. They do not consider damage dilution as a strategy to dilute the damage for which there is no protection and do not view different metabolic states as associated with unique patterns of damage accumulation. Finally, these theories posit that there are many causes of aging, but in fact each damage theory refers to a specific mechanism contributing to the aging process rather than the cause of it.

VIII. **Rate of living:** This model proposes that various species have a relatively constant level of total metabolic output over lifetime. Therefore, organisms with faster metabolic rates age more rapidly. This model is consistent with correlations within mammals, but does not have a clear molecular underpinning and fails to explain why certain organisms with high metabolic rates have longer lifespan than similarly-sized animals (e.g., bats versus mice, birds versus mammals, etc.). It was shown that metabolic rate does not correlate with lifespan when the metabolic rate is normalized to body mass.

3.2. Heterogeneity and imperfectness of biological systems

Accumulation of molecular damage that arises through the imperfections in the molecular machinery of life has long been considered key to the aging process. It is not clear, however, how this damage is generated, whether it is generated purposefully, why it cannot be completely removed from cells, and whether it is stochastic. It is also not known whether damage causes aging or is simply a bystander generated with no influence on the process. Although the idea that cumulative damage causes aging is viewed by many as a truism, nearly every aspect of this idea is questionable, with many researchers discounting damage as a relevant factor altogether.

3.3. The imperfectness model

Cellular life involves (i) the generation of damage as an inevitable consequence of the imperfectness-driven metabolism, and (ii) the removal of damage when it is cleared or diluted, or when cells are renewed

If a cell makes only a few molecules of a certain damage type during its lifetime, this damage will not be “visible” to natural selection, and no genes will evolve to protect against it. Neither

will protection evolve against other minor damage types each represented by several molecules. Moreover, it would be impossible for the cell to deal with all damage forms, as damage is produced by every reaction and interaction in the cell (because all of them are imperfect) and at every level, from the smallest molecules to cells and organs. Therefore, the number of damage forms will always be greater than the number of protective and repair systems.

Although only the more severe types of damage will provide a substrate for natural selection to act on, damage can also be dealt with by cell division. This way, cells can remove severe damage with protection systems and essentially ignore inabundant, slightly deleterious, milder damage forms, which will be diluted when cells divide.

This strategy accommodates imperfectness in biological systems and has been operating for billions of years, defining all cellular life as we know it. It is rooted so deeply in cellular life that it became a part of life itself, similar to the "frozen accident" of the genetic code

Environment also contributes to cumulative damage, but it is not a major factor. Although most damage forms considered in isolation would not result in measurable effects on fitness, together they decrease fitness over time. Moreover, individual damage forms are intimately linked to each other and cumulatively affect the cell that makes them. For example, a damaging posttranslational modification of an enzyme may affect its conformation and catalysis, influencing its catalytic efficiency, degree of promiscuity and macromolecular interactions. In turn, these altered functions would affect other cellular components, propagating the damage.

Cumulative damage is largely not random, and that it is determined by the specific metabolic set up of a cell/organism. That is to say, just as the balance of metabolism is ultimately encoded in the genome, so is the damage that inherently arises from metabolic processes.

Indeed, each enzyme will produce specific forms of damage (rather than any damage), each damage form will exhibit preference with regard to interaction with cellular components, and changes in the metabolic state of a cell will predictably change its damage composition.

Cumulative damage will be primarily defined by genes encoded in the genome (with some contribution from environment, diet and a plethora of other factors), and the damage generated in different species will be related according to phylogeny, changes in life-history traits and lineage-specific adaptations. Just as no two humans are the same, identical cumulative damage patterns do not exist. Cells with similar genotypes and environmental conditions will be characterized by similar damage forms, yet some damage will be unique to each cell. Moreover, damage in a cell/organism changes over time and influences metabolism, further complicating the cumulative damage landscape. Thus, as one defines cumulative damage it is important to consider both damage composition at any given moment and damage accumulation over time.

Thus, although imperfectness inevitably leads to damage, which drives aging, the type and rate of damage accumulation are genetically controlled. Damage is not the cause of aging (imperfectness is) but rather an instrument of evolution to control life-history traits, thereby regulating lifespan.

3.3.1. Features of the imperfectness model of aging

- Cause of aging. Imperfectness (and conceptually related heterogeneity, promiscuity, infidelity) of biological systems represent the ultimate cause of aging.

- Imperfectness of biomolecules. All biomolecules are inherently imperfect as dictated by their physico-chemical properties, e.g., they are built from a limited set of building blocks by error-prone processes.
- Damage from each biomolecule. The by-products and other damage forms are generated by each and every macromolecule and at all levels, from small molecules to cells.
- Damage exceeds the ability to remove or repair it. Damage forms are too numerous to be cleared up, i.e. there is a greater number of damage forms than possible protective systems.
- Inevitable damage. Biological imperfectness is the reason the damage is inevitable.
- Cells clear severe damage and ignore the rest. Damage that decreases fitness is removed by protection systems, whereas slightly deleterious, mild damage is invisible to evolution and cannot be protected from. Instead, cell division and renewal allow dilution of this damage.
- Damage overload in non-renewable cells. The occurrence of non-renewable cells and structures leads to eventual damage overload.
- Non-random damage. Damage is largely non-random: it is determined by composition and rate of metabolism, e.g., an enzyme will generate a specific product and a set of specific byproducts, and not just any damage forms.
- Damage is genetically encoded. Damage and the rate of its generation are indirectly encoded in the genome (through biomolecules that make damage when carrying their direct functions).
- Ever changing cumulative damage. Changes in metabolism (e.g., during speciation, changes in diet or environment, or during aging itself) will lead to the generation of different forms of damage and at different rates.
- Origin of aging. The damage generation/clearance/dilution strategy operated ever since the first cellular organisms and is rooted so deeply in metabolism that it is an essential part of cellular life.
- Mortal and immortal organisms. Organisms may be theoretically immortal if they achieve full equilibrium in cumulative damage, whereas organisms with post-mitotic, non-renewable cells and structures are necessarily mortal.
- Evolution and aging. The cause of aging is not subject to natural selection.

- Evolution of lifespan. Evolution can influence lifespan (and other life-history traits) by optimizing metabolism, thereby adjusting the patterns and rate of accumulation of cumulative damage.

3.4. Aging in individual cells:

Aging studies usually involve research on clonal organisms at the population level, whether referring to a colony of single-celled organisms or an individual multicellular organism (groups of cells that arose asexually from a single cell). Aging can also be studied at the individual cell level, either single-celled organisms or individual cells in a multicellular organism. Single-celled organisms usually divide in a few hours or days. Each daughter cell can begin a potentially immortal cell clone. Even daughter cells of prokaryotes such as bacteria are believed to be potentially immortal; laboratory colonies have been maintained for over 25 years and more than 50,000 generations. However, in some single-celled organisms, the division is uneven. The bacterium, Caulobacter crescentus, begins as a motile swarmer and then attaches to a substrate with a stalk Cell division is asymmetric: the larger stalked "mother" producing a smaller, free-swimming daughter swarmer. Over time, the stalked mother cell shows an accelerating decrease in reproductive rate, indicating that it is undergoing senescence (Ackermann et al., 2003).

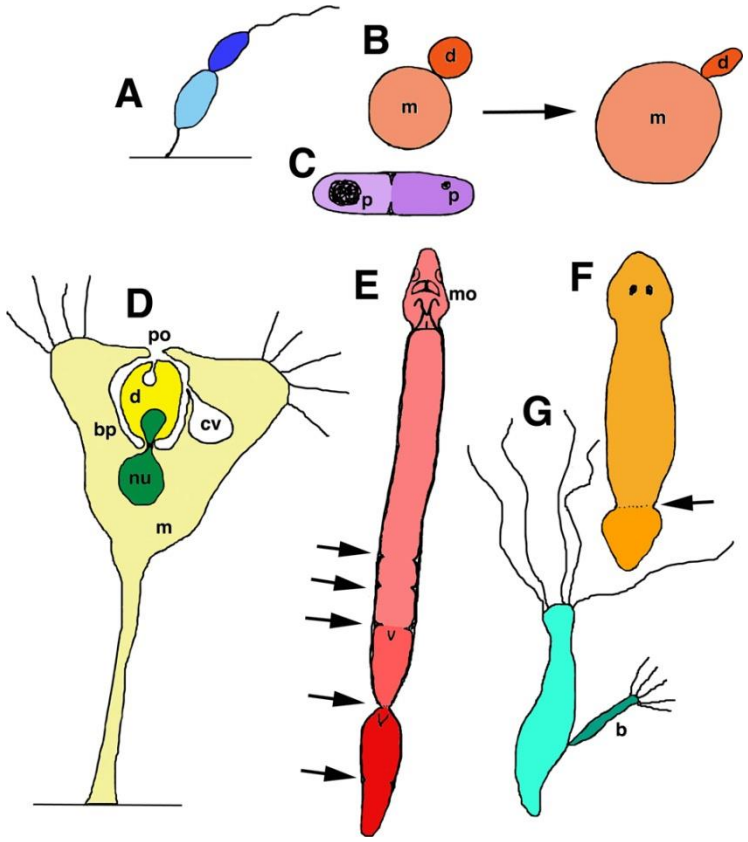

Figure 3.2: Asymmetric asexual reproduction and senescence. (Ackermann et al., 2003)

A–E. These are examples of asymmetric cell/body division where the "mother" shows signs of senescence.A. The sessile, stalked bacterium, Caulobacter crescentus, produces motile swarmer cell progeny. B. Budding yeast (Saccharomyses cerevisiae) mother cells (m) get bigger as they age and produce more ellipsoidal daughters. C. In contrast, fission yeast, such as Schizosaccharomyces pombe, divides symmetrically under normal conditions. But under stress, there is asymmetric accumulation of protein aggregates (p) leading to aging of the cell that retains most of these aggregates. D. Stalked mother cells (m) of some sessile suctorian ciliates, such as Acineta tuberosa and Tokophrya infusionum, produce an offspring in a brood pouch (bp) that is born through a birth pore (po). E. The flatworm, Stenostomum incaudatum, produces a caudal chain of developing

offspring. Arrows show locations rostral to the heads of the forming juveniles (note the pinching in of the digestive tract), where they will separate, eventually, from the mother. mo, mouth. F,G. In contrast to the previous examples, asymmetric asexual reproduction in some planaria by fission and hydras via budding may not lead to increased senescence of the mother. In these cases, rapid cell proliferation continuously replaces all cells of the body in a short time period, thus continuously renewing the "mother" (Ackerman et al., 2003).

The best-known example of asymmetric division associated with cell aging is found in budding yeast. In the common budding yeast, *Saccharomyces cerevisiae*, a mother cell produces a smaller daughter cell as a bud that separates from the mother; the mother cell can only go through a specific number of divisions, depending on the strain, and then dies. Typically, budding yeast have a cell cycle of ~2 hours and have 20–30 generations (of budding). Aging yeast cells show changes reminiscent of human aging: cell size increases and the cell's surface wrinkles as loss of turgor develops. Moreover, bud scars increase; generation (cell cycle) time increases; vacuole size increases and its acidity decreases; protein synthesis, ribosome activity and polysome recruitment decrease; and nucleolar fragmentation appears. In addition, there is increased formation of extrachromosomal ribosomal DNA circles, which are linked to genomic instability; these are preferentially retained in the mother cell. Mother cells also selectively retain those mitochondria with more oxidizing redox potential and higher superoxide levels; thus the mother retains the lower-functioning mitochondria that ultimately help cause age-associated decline in cellular fitness; although as the mother gets older, her daughters inherit progressively lower functioning mitochondria from her) (Jazwinski, 2002; McFaline-Figueroa et al, 2011).

Stated simply, mother cells retain all the 'bad stuff' to assure that their daughters can have a fresh start in life. In contrast to budding yeast, fission yeast such as *Schizosaccharomyces pombe* divide symmetrically, with both daughter cells relatively equal so that each daughter can give rise to a potentially immortal cell clone. However, under stress induced by heat or oxidation, large protein aggregates form and the major aggregatesare segregated into one of the daughters

during cell division. Like the mother cell of budding yeast, the cell with the big protein aggregates tends to get old and die (Coelho et al., 2013; Moseley, 2013).

There are no cases reported of individual single-celled organisms living for years without dividing, althoughdocumenting this would be difficult. The exception though may be organisms that are revived after being in stasis for long periods. The ciliate protozoan, Euplotes neapolitanus, can survive encystment, induced by sudden starvation, for up to 6 months. Goodey (1915) was able to revive protozoa from soil samples that were nearly 50 years old. But the record is probably~34,000 years for bacteria.Similarly, for cells within a multicellular organism; the record may be ~32,000 years for regeneration of fertile plants from seedsofSilene stenophylla found in fruit tissue buried in Siberian permafrost. Viruses also have been revived from Siberian permafrost after ~30,000 years (Legendre et al., 2014).

In vertebrates, some somatic mitotic cells live and function in the G_0 phase of the cell cyclefor many months. Such cells may exhibit a high quality of maintenance of proteome homeostasis (proteostasis), thus minimizing the accumulation of damaged or misfolded proteins. Postmitotic cells such as neurons can survive and function normally for the entire life of the animal, in some cases (e.g., fish, tortoises, whales) for over 200 years. Interestingly, the cellular niche can control the lifespan of these neurons; for example, embryonic neuron precursors from short-lived mice (~18 month life span) that are transplanted into rats (~30 month life span) live twice as long. The neurons arising from the transplanted neuronal precursors (Purkinje cells) remain smaller than the corresponding rat neurons but retain normal polarity and orientation with age; they also show a similar age-dependent loss of dendritic spines with age as seen in the rat neurons. While these long-lived neurons function much as they do in their youth, there are numerous changes that occur with normal aging, especially at the synapses between these neurons (Magrassi et al., 2013).

3.5. Aging in ciliate protozoa:

Aging variesamong protozoa both in the lifespan of individual cells and in that of clonal cultures. Most protozoa reproduce asexually by dividing into two equivalentdaughter cells, but some reproduce by asymmetric budding. As described above for budding yeast, budding protozoa are mother cells that produce a juvenile daughter cell. Some, such as the sessile suctorian ciliates, Acineta tuberosa and Tokophrya infusionum,have endogenous budding, a process that lookssuperficially like vertebrate live birth.The mother cell carries the embryo in a brood sac thatmay be derived from the excretory vacuolar system of the mother cell. She gives birth by forcibly extruding her daughter through the birth pore; the daughter later goes through a metamorphosis, changing from the motile juvenile form to the sessile adult form. Mother cellsof Tokophrya infusionumlive for about 10 days (up to 37 days) and show distinctive signs of progressive senescence. In addition, daughters produced by older mothers tend to show greater variability in life span and in the number of daughters that they produce. Old mother cells display numerous signs of senescence including: enlarged size and sometimes an irregular outline; fewer feeding tentacles that also are less efficient at capturing prey; slower feeding; lower reproduction rate and finally a loss of reproduction; fewer and more peripheral

mitochondria; accumulation of lipid-like pigment granules; large oval or circular areas filled with large, dense particles. Diet also affects the lifespan of the mother: those fed little food with intermittent starvation live twice as long as those fed regularly. Very overfed individuals can turn into giants (180 µm versus 35 µm) that stop reproducing and die young (Rudzinska, 1961; Millecchia and Rudzinska, 1970; Karakashian et al., 1984).

Typically, protozoans divide into two daughter cells of the same size. The daughter cells initially have a juvenile structure while they are reorganizing. In the large, complex ciliate Bursaria, dedifferentiation of the gullet occurs prior to normal cell division and later the two daughter cells differentiate, reforming adult structures. Interestingly, a similar sequence occurs if the organism

is cut into two pieces with a scalpel: dedifferentiation precedes differentiation; the process of encystment is also similar. Thus, the morphogenetic processes of asexual reproduction, regeneration, and encystment in protozoa may be various manifestations of the same event. In the same sense, the somatic mechanisms of aging and regeneration in simple multicellular animals (basal metazoans) may be related to each other. As mentioned above, discussions of aging in most protozoa, where cell division is symmetrical, concerns the aging of the clonal cultures and not the individual organisms, since cells typically divide every few hours or days (e.g., Hungate, 1942; Wichterman, 1964).

Seemingly immortal lines of cultured protozoans are well known. Woodruff (1943) noted that a culture of Paramecium aurelia that was started in 1907 was still viable after 36 years and ~21,800 generations. Similarly, a culture of an amicronucleate strain of Tetrahymena pyriformis started in 1923, was still viable 51 years later. But generally, these immortal lines of protozoa lose most or all of their ability to produce sexual offspring after a certain number of asexual cell divisions (they may fail to complete sexual encounters or if they do, the offspring may die; In some protozoa this clonal aging may produce structural abnormalities; in Euplotes minuta, the number of dorsal ciliary rows decreases gradually after about 500 fissions (i.e., beginning with an individual formed from a sexual encounter, called conjugation), and the clonal culture terminates at ~700 fissions; (Nanney, 1974).

In summary, protozoa can possess several characteristics relevantto aging, including asexual clonal immortality, regeneration, and the ability to cycle between dedifferentiation and differentiation; these features likely predisposed some of them to evolve into the first multi-cellular animals.

3.6. Aging in multi-cellular organisms

Most aging studies look at clonal collections of cells derived from a single cell; this includes multicellular organisms typically derived from a single (usually fertilized) egg cell. In many cases, these cell clones appear to be immortal, showing no signs of aging over many years.

But other multicellular organisms also show this propensity for immortality. In the multicellular fungi, there are some cases of senescence occurring after a short lifetime of about 25 days, but most multicellular fungi appear to be immortal, continuously growing by tip elongation of their filamentous cells, called hyphae. These can form into a largely underground structure called a mycelium (a thallus) that can cover many acres, and individual organisms may live for thousands

of years. Plants grow by cell division of stem cell areas called meristems so that even though, periodically, parts of the plant such as the leaves may show senescence and death, the plant as a whole can live on for many years. In several cases, a single clonal organism, in which an extensive group of above ground trees originates from a single mass of underground roots or rhizomes, has been shown to be living for many thousands of years. For example, Wherry (1972) describes a Box-huckleberry that may be 13,000 years old, and other studies have claimed even greater ages, although some of these claims may be excessive. Nevertheless, even single individual trees can reach great ages – about 5,000 years for the bristlecone pine (Munné-Bosch, 2008).

3.7. Aging in basal metazoans

Five main groups constitute the simplest multicellular animals or metazoans, including the Placozoa, Porifera (sponges),Ctenophora, Cnidaria, and Myxozoa; the simplest metazoans evolved from groups of single-celled protozoans. The simplicity of their multicellularity can be demonstrated readily by breaking them up into individual cells in the laboratory; when this has been done to some placozoans, sponges and cnidarians, the group of cells often can reorganize themselves back into the whole animal, as will be discussed below. Many also can rejuvenate their bodies, as we shall discuss. Placozoans and Porifera (sponges) are the simplest; they show definite differentiation into various cell types with specialized functions, but lack a definitive nervous system. Ctenophores (comb jellies) and Cnidaria (hydras, corals, jellyfish) are a little more complex, having a basic nervous system, and for many cnidarians, simple eyes. Placozoa and Cnidaria may have evolved from ancestors that evolved into the Bilateria - more complex animals with bilateral symmetry, including insects, clams, humans, etc. Bilateria are triploblastic

animals, the embryo showing endoderm, mesoderm and ectoderm, and show a basic bilateral symmetry (lost to some extent in adult echinoderms). The simplest major groupswithin the Bilateria are the flatworms (Platyhelminthes and Acoelomorpha) and they are the first known kinds of animals to evolve a true brain.

Little is known about aging in three of these groups – ctenophores, placozoans, and myxozoans, and they will be covered here only briefly. Myxozoans are small parasites of fish and aquatic invertebrates. They originally were considered protozoa but then phylogenetic analyses showed that they are metazoans, either related to Cnidaria or more likely, to Bilateria. Typically, host animals are infected by spores containing one or more sporoblast cells and one or more polar capsules. The polar capsulescontain a filament that anchors the spore to the host tissue, and resembles the nematocysts (stinging cells) of cnidarians. The spore-producing stage may be a multicellular, often ameboid plasmodium, containing generative cells within the multinucleate plasmodium, although some kinds form into a closed sac or a worm-like organism. The latter

form (Buddenbrockia) has four longitudinal muscle bands reminiscent of nematodes, and appears to be a triploblastic animal like a bilaterian. Little is written about aging of Myxozoa, probably because they are all parasitic and tied to the life cycle of their hosts. Typical infections of fish develop over several months. This may be preceded by an initial infection in an invertebrate such as the tubifex worm, and that also can last several months (Evans et al., 2010; Schnitzler et al., 2012; Ryan et al., 2013).

Ctenophores are an enigmatic group; they may have evolved from ancestors prior to the ones that evolved into Porifera and Placozoa, and may have a nervous system unrelated to that of cnidarians and Bilateria. The ctenophore, Pleurobrachia pileus, has been reared from eggs and maintained in culture for up to 250 days. Ctenophores often have very delicate bodies but can

regenerate parts that are damaged. P. pileus tentacles undergo a continuous regeneration process since they are injured or destroyed easily during feeding. Stem cells are found in restricted areas within the tentacle roots as well as in other main parts of the body and contain genes involved in germline determination and maintenance such as Piwi and Vasa, as described in subsequent sections (Alié et al., 2011).

Placozoans are simple plate-like animals up to 1–2 mm in diameter with anupper and lower epithelium and some cells in between. They typically divide by binary fission every 2–3 days, but also can reproduce asexually by other means, producing smaller daughter animals via floating swarmers that bud off of the dorsal surface, as well as by non-floating spheres or by extension of a long stolon from the dorsal surface. Most cell types in the placozoans retain the ability to divide, and totipotent cells may occur along the margins of the upper and lower epithelia. The body remains very malleable; impaled on a pin, these animals simply flow around the obstruction (Thiemann and Ruthman, 1991; Jakob et al., 2004).

Animals that are disaggregated experimentally will reaggregate and in some cases can reform into a viable animal again. There is little discussion in the literature about aging in placozoans. However, placozoans from old cultures may go through a degenerative phase where they can form into nearly spherical moribund structures (Grell and Benwitz, 1971; Thiemann and Ruthman, 1990).

3.8. Aging in cnidarians:

Among the basal metazoans, the Cnidaria and Ctenophora are the most advanced, possessing a simple nervous system, sense organs (eyes in Cnidaria and statocysts in both), and muscles; in contrast, definitive examples of these structures are lacking in the simplest metazoans, the Porifera and Placozoa. Thus, cnidarians and ctenophores represent an intermediate stage of body complexity between poriferans and placozoans, and the Bilateria, which includes all of the other major phyla of animals. Interestingly, they are also intermediate in theirmaximum lifespans. As described above, there is good evidence that some individual sponges live for more than 10,000 years. In contrast, some individuals in the major groups of Bilateria have lifespans up to about 500 years, and often with negligible senescence (e.g., >500 years for some marine clams, 50–100 years for lobsters, and over 200 years for some vertebrates;.Among the Cnidaria, individual living corals, which are formed by a colony of polyps (an elongate structure with a mouth surrounded by tentacles at the top) that are joined together by a continuous sheet of living tissue (the coenosarc), can be more than 4,000 years old. Perhaps this indicates a reciprocal relationship between maximum ages and body complexity in the evolution of animal aging. Of course it is difficult to study changes in aging of a long-lived creature in the laboratory; but among cnidarians, some sea anemones have been observed to live in captivity for more than 80 years, without obvious signs of aging, budding continuously; apparently, they died of neglect, so they probably would have lived longer if cared for properly. Ashworth and Annandale (1904)

described these latter sea anemones when they were about 50 years old, and noted that they were a little more sensitive to cold and dark compared to young mature sea anemones, and their sexual reproductive fertility seemed to have decreased; however, it was not clear if these differences were due to different rearing conditions. Nevertheless, senescence and death from old age do occur in some Cnidaria. In particular, the individual

polyps in corals or hydrozoans typically have a short life. For example, polyps of the colonial hydroid, Campanularia flexuosa live for 4–8 days at 25°C; ATP content decreases during their lifespan, yet they seem to catch and eat food normally until they die (Strehler and Crowell, 1961). Acropora cytherea corals show growth anomalies in the older, central portions of large colonies; these anomalies are associated with reduced productivity and dysfunctional gametogenes is However, in both of these cases, it is the older polyps within a clonal colony that are dying, and the colony, which is really a single organism with continuous cytoplasm, lives on. But the entire colony of the branching coral, Stylophora pistillata, dies after a few years, and shows decreases in reproduction and growth about 6 months earlier (Finch, 2009; Petralia et al., 2014; Treaster et al., 2013)

Above we discussed the idea of agelessness or immortality in protozoa and sponges. Similarly many cnidarians are likely immortal. This was best illustrated in a study by Martínez (1998) in which individuals of the common polyp-type cnidarian, Hydra vulgaris, were followed for 4 years; during the study period, animals showed no signs of age-related mortality and there were no definitive changes in reproductive rates for either asexual (budding) or sexual reproduction (animals grew testes or eggs normally throughout the study, but were kept isolated). Analyses of reproduction and mortality rates led to predictions that these animals indeed were immortal. One mortality trajectory of Hydra magnipapillata, based on a laboratory study lasting several months, estimated that 5% of adults would be alive after 1400 years. Hydras have a long trunk with a foot or basal disc at one end and a head with mouth surrounded by tentacles at the other end. The cells of the main body column divide continuously and these replace all of the non-dividing, differentiated cells in the hydra within 20 days; thus the hydra's body is renewed continuously - at least 60 times over the 4 years of the Martínez study. Hydras typically maintain a steady adult

size; most excess cells are formed into buds that develop into daughter hydras that separate from their mother; the remaining excess cells are sloughed off of the ends of the hydra. Cell divisions involve 3 cell types; epithelial cells of the 2 body wall layers (ectoderm and endoderm) divide about every 3–4 days, while small interstitial cells (I-cells) from the ectodermal layer divide about once a day to form several specialized cell types: neurons, nematocysts (stinging cells), secretory cells, and gametes. These I-cells then are considered the multipotent stem cells of the hydra. The rejuvenating (regenerative) powers of hydras are amazing. Dissociated hydra cells can reaggregate and form a new hydra, as noted also in the previous sections for some sponges and placozoans. Shimizu et al. (1993) showed that a tiny piece of tissue cut from the trunk of a hydra (Hydra magnipapillata) will reorganize into a 0.2 mm sphere (the animal is about 7 mm long) with only about 270–300 cells, and then finally into a complete mini-hydra less than a mm long. Many factors are probably involved that help guide the cells to reorganize into the hydra shape. For example, Wnt signaling, important in axial patterning in the vertebrate embryo, induces head formation in the hydra. Regeneration of hydra into a new animal is mainly due to a reorganization of existing cells rather than via cellular proliferation; this is called morphallaxis. Thus, the hydra may attain immortality; essentially it keeps itself young by having a highly malleable and renewable body and by replacing its cells continuously. But not all hydras may be immortal. Yoshida et al. (2006) examined aging in 3 species of hydra kept at 10°C to induce and maintain the sexual phase. They found that one species, Hydra magnipapillata, that still reproduced asexually by budding (apparently along with sexual reproduction) at this temperature did not seem to age during the experimental period. But the other two hydra species (mainly Hydra oligactis) showed signs of aging and died off in a few months. The aging hydra showed declines in food capture, contractile movements, and reproduction; tentacles became shorter progressively and there was general atrophy of the body after 60 days. This possible correlation of senescence with sexual reproduction has been noted

for some other cnidarians and we already have mentioned this phenomenon briefly for protozoa above. Yoshida et al. (2006) noted that the I-cells of H. oligactis started producing mainly germ

cells for sexual reproduction, and decreased their production of nerve cells, nematocytes, and gland cells; this explains the decline in food capture as the animals got older. The authors also found changes in gene expression during degeneration and found genes in these hydras that are associated with the aging process of other animals, such as members of the insulin/IGF-I signaling pathway and sir2 (Gold and Jacobs, 2013).

In contrast to the hydra, which is among the simplest of cnidarians, most other kinds of cnidarians have complex body plans and life cycles, with thus many variations in aging and regeneration phenomena. We can only cover some highlights of this variety in this review. Hydras require 3 kinds of cells to continuously regrow their bodies, but in contrast, the marine colonial hydroid, Hydractinia echinata, has I-cells that are true totipotent/pluripotent migratory stem cells, similar to the arrangement that we described for the sponges. In Hydractinia echinata, Polynem (Pln), a POU domain transcription factor gene, is a key regulator of these stem cells, as are similar POU genes in mammalian embryos. These stem cells also have various known cnidarian stem cell markersincluding germline markers, Nanos, Vasa and Piwi, similar to what we described for sponge stem cells. The presence of true stem cells are not definitive in many other cnidarians; in some cases, interstitial cells or amebocytes have been identified as stem cells, but more study of their function is needed.Isolated striated muscle cells from the medusa (jellyfish) stage of Podocoryne can dedifferentiate into a stem cell in the laboratory, and when combined with endoderm cells, can make all of the cell types and form a new, partial medusa structure that can feed, grow, produce gametes, and live for months. Thus, cnidarians may retain a broad ability to create stem cells from various cell types, to regenerate and rejuvenate their bodies; this is similar to what we described above for the stem cell functions of choanocytes and archeocytes in sponges.

The most amazing case of body rejuvenation among cnidaria and possibly among all animals is seen in the life cycle of Turritopsis nutricula. Like many cnidarians, this animal goes through a complex metamorphosis; sexually mature medusae produce mobile larvae (planula stage) that

eventually settle to form a colonial hydroid (polyp) stage, which grows and produces new medusae asexually. In many other Cnidaria with this life style, medusae eventually die after reproducing sexually one or more times. But in Turritopsis nutricula, sexually mature medusae, if stressed in the laboratory, will reverse metamorphose back to the colonial polyp stage that can feed and bud off new medusae. This is analogous to a butterfly changing back into a caterpillar!Here then may be an example of an animal that can achieve immortality by regularly cycling between a complex mature adult stage and a simpler larval stage (Gold and Jacobs, 2013).

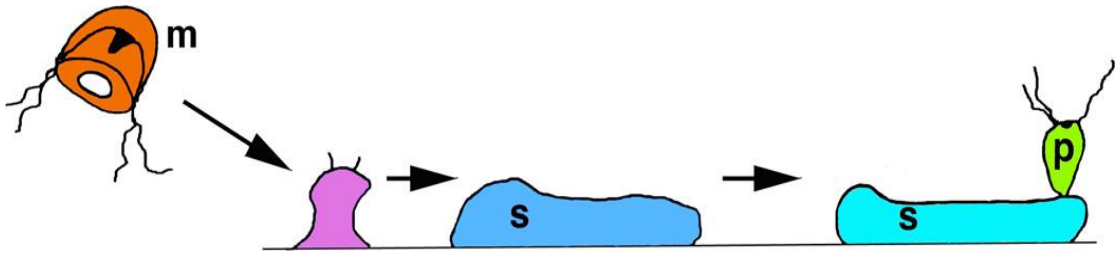

Figure 3.3: An "immortal" jellyfish asexual reproduction by budding. (Gold and Jacobs, 2013).

An "immortal" jellyfish? In the hydrozoan, Turritopsis nutricula (Piraino, 1996), asexual reproduction by budding of hydroid polyps (p) from a stolon (s) follows rejuvenation from the sexual medusoid (jellyfish) stage (m) in a unique mechanism of reverse metamorphosis. In theory, these animals could continue this forever, hydroid colonies producing sexual medusae

that eventually return to the hydroid stage and then repeat the cycle, leading to the popular name in news accounts of "The Immortal Jellyfish.

3.9. Aging in flatworms

Planaria and other flatworms are very important model animals for studying stem cells and regeneration. Flatworms are probably the most primitive of the Bilateria; all are bilaterally symmetrical, but unlike most higher Bilateria (earthworms, clams, insects, humans, etc.), they lack a body cavity (acoelomate). They make up 2 phyla including:1) Platyhelminthes, which includes planaria and other free-living flatworms of marine, freshwater, and land environments, as well as two large groups of parasites, the trematodes or flukes, and the tapeworms; and 2) the Acoelomorpha (a phylum or subphylum recently separated from Platyhelminthes)that includes the acoel flatworms, which have no gut tract. The acoel flatworms may be close to the basal group of the Bilateria, representing the transition between the basal metazoans such as placozoans, sponges and cnidarians, and the more advanced Bilateria such as planaria, insects, humans, etc(Liu et al., 2013; Sikes and Newmark, 2013; Umesono et al., 2013).

Aging in planaria and other flatworms is not well understood. Lifetimes of both individuals and clonal colonies in the laboratory have been variable, probably due both to species and culture differences; a number of studies indicate that many kinds of flatwormscan live for at least several years, maybe in the range of mice and rats (Balázs and Burg, 1962; Haranghy and Balázs, 1964; Mouton et al., 2010).

Individuals of Dugesia tigrina, collected in the wild, were still alive in captivity after 7 years and produced egg capsules annually. Parasitic flatworms such as flukes and tapeworms live in a protected environment in their hosts, and may have even longer lifespans; records of 25 to more than 35 years have been noted reports that a scientist infected with Schistosoma flukes passed

live eggs in his urine for at least 28 years (the life cycle requires an intermediate host), after which he stopped monitoring their passage; eggs were absent when checked after a total of 42 years (Comfort, 1956).

Mouton et al. (2010) found that the free-living flatworm, Macrostomum lignano, ages gradually with an average lifespan of 205 days (maximum is 2.4 years). Signs of old age in these animals include body deformities, grooves in the head region, and liquid-filled cysts, as well as disintegration of gonads in some animals. The size of cysts in this animal does not increase with age; they seem to form as aging animals lose their ability for efficient wound healing, so cyst size may be more dependent on the size of the initial wound rather than animal age. In contrast to M. lignano, Schmidtea polychroa does not show signs of metabolic aging, at least not over a three-year experimental period. After an initial growth phase of five months (sexual maturation by two months), S. polychroa undergoes alternating phases of regressionand growth. Those few animals that did die during the experiment first showed intense regressionand sometimes deformities, prior to death. In addition to these examples, asexual strains have been maintained from several species, including Stenostomum tenuicaudatum, Schmidtea mediterraneaandspecies of Dugesia and these are considered to be immortal lines, as we have describedin previous sections for examples of exclusive asexual reproduction in Protozoa and Cnidaria. Furthermore, individual flatworms may be potentially immortal, as described above for S. polychroa and also for S. mediterranea. Interestingly, the asexual and sexual strains of S. mediterranea differ genetically by a chromosome translocation: animals with the translocation reproduce only asexually by transverse fission and those without it reproduce only sexually. In the wild, there may be different populations of sexual and asexual planarians (Baguñá et al., 1999).

Generally, flatworms can employ three mechanismsto avoid aging: asexual reproduction via fission, controlled shrinkage during starvation, and regeneration from body fragments; all of these are believed to involve a rejuvenation of the animal, as we have discussed in previous sections for other organisms. During fission, the posterior, tail end splits off somewhere in the last two-thirds of the body and both parts regenerate into whole animals. Probably the most elaborate fission mechanism is found in Stenostomum incaudatum. The posterior end of the body starts to differentiate into a miniature worm (called a zooid) and its head and brain start to become distinctive prior to separation from the anterior, typically longer end. Other zooids may

start to grow before the end zooid separates and chains as long as nine zooids may occur. In a four month study, 100% of the anterior ends died, while only 8.5% of the posterior ends died; this phenomenon of differential aging in uneven divisions has been discussed in previous sections for budding yeast and for the protozoan, Tokophrya infusionum; basically the larger "mother" division of the organism grows old and dies eventually, after producing a number of offspring. Dying Stenostomum incaudatum worms stop growing and decrease in size and often show various abnormalities. The most elaborate fission methods are found in Convolutriloba acoels, which in different species produce small juveniles from the posterior end by: 1) transverse fission; 2) transverse fission followed by longitudinal fission of the juvenile into two individuals; or 3) reversed-polarity budding where the two juveniles bud from the posterior end of the mother, developing their heads pointed away from the mother (Sikes and Bely, 2008).

Many flatworms respond to starvation by decreasing in size significantly, while generally maintaining body proportions and integrity. In a few months, the worm can decrease its length 5–10x or more. Its cell number decreases proportionally; for example, a 7 mm Dugesia tigrina with about 520,000 cells can change into a miniature individual, 2 mm long with 60,000 cells, yet proportions and distribution of the different cell types show little change overall, and are similar to those of a juvenile individual. Thus, the starving worm appears to return to its juvenile state. Many flatworms adjust their size by periods of growth and shrinkage to adapt to food availability and other environmental factors (Baguñá and Romero, 1981; Rink, 2013).

Planarians are famous for their capacity to regenerate. For example, Montgomery and Coward (1974) found that a fragment as small as 0.08 mm^3 of an asexual Dugesia dorotocephala (7–12 mm)can regenerate completely. Thisfantastic ability to regenerate depends on the presence of large numbers of pluripotent stem cells called neoblasts. These stem cells are probably present in all kinds of flatworms, including acoels, tapeworms and trematodes. These are small, round cells with little cytoplasm, spread through the mesenchyme around the organs and making up about ¼ of planarian cells. Neoblasts form all of the somatic cells and continuously replace them so that the entire planarian body turns over in a few weeks (Rink, 2013; similar to hydras as discussed in the previous section). This is particularly evident in a study by Wagner et al. (2011), who lethally

irradiated planaria of a sexual strain of Schmidtea mediterranea(because the sexual strain is more tolerant of radiation than asexual ones) and then implanted a single neoblast derived from an asexual strain. Some of these planaria survived and regenerated lost parts and fed, and ultimately were expanded into entire asexual strains. This indicates that the single neoblast transformed the recipient (sexual strain) into an asexually reproducing animal with the donor genotype. More importantly, it shows that an individual neoblast is truly pluripotent, capable of producing all kinds of somatic cells (Baguñá and Romero, 1981;Reddien and Sánchez Alvarado, 2004).

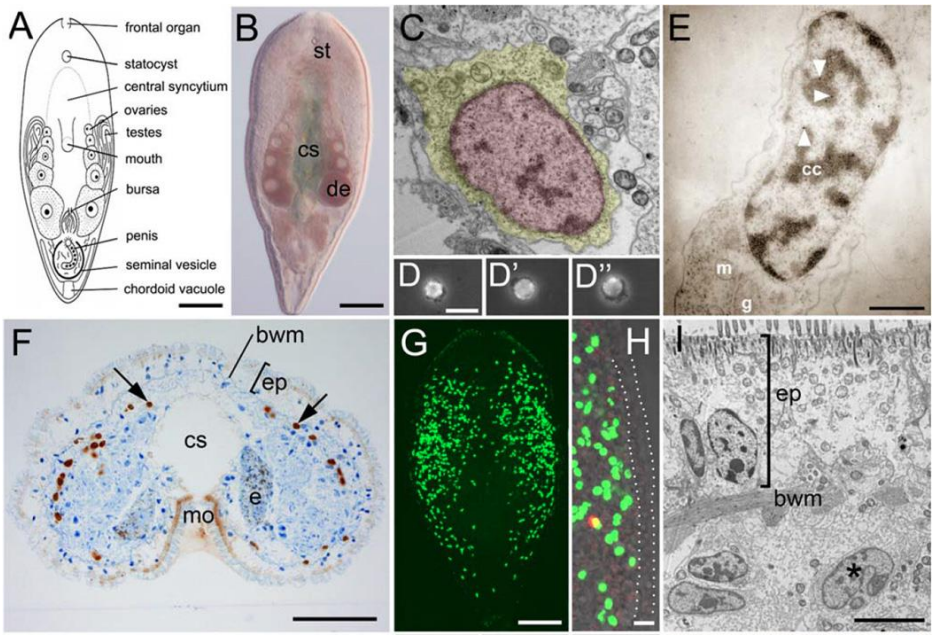

Figure 3.4: The stem cell system of Isodiametra pulchra (Sánchez Alvarado, 2004)

"The stem cell system of Isodiametra pulchra (A,B). Morphology (C–E) and distribution (F–I) of neoblasts. (A) Schematic drawing. (B) Differential interference contrast image. (C) Typical neoblast with nucleus (red) and thin rim of cytoplasm (yellow). (D-D") Macerated BrdU labeled cells show typical neoblast-like morphology (E) BrdU labeled neoblast, as shown by immunogold staining after a 30 min BrdU pulse; arrowheads point to gold particles. (F) Histological cross section; brown spots are BrdU labeled S-phase cells. (G,H) Confocal projection overview (G) and detail of lateral body margin (H) after 30 min BrdU pulse; the red spot in (H) is a mitotic figure. Note that S-phase cells [green fluorescence] were lacking in the epidermis (between dotted lines). (I) Electron microscopic image of a posterior-lateral body margin." "bwm, body wall musculature; cc, condensed chromatin; cs, central syncytium; e, egg; de, developing eggs; ep, epidermis; g, Golgi; m, mitochondria; mo, mouth opening; st, statocyst. Scale bars (A,B,G) 100 μm; (C,E) 1 μm; (D,H) 10 μm; (F) 25 μm; (I) 5 μm."

The molecular genetics of neoblasts and planarian regeneration has been studied widely and we can only discuss it briefly in this review. In addition to the somatic pluripotent neoblasts that we have been discussing, flatworms also have some germline neoblasts; these may cycle at a slower rate than somatic neoblasts and contain the gene nanos, associated with gonad development and germ celldevelopment and regeneration. Interestingly, both sexual and asexual strains have germ cells expressing nanos, so that inhibition of development of mature reproductive organs in asexual strains must occur downstream of nanos function. Generally for animals, viability of populations of germline stem cells is maintained through many cell divisions by overcoming the end-replication problems of chromosomes via telomere elongation, which occurs during embryogenesis. So how do asexual planaria strains maintain the viability of their somatic neoblasts? These cells can produce higher levels of telomerase via alternative splicing of active telomerase so that telomere length is maintained during the cell replication events that occur during fission or regeneration.Thus, this might explain how asexual planaria achieve potential immortality. These neoblasts also express several Piwi homologues, more typically associated with germline stem cells (see discussion in earlier sections of this review); these homologues help to maintain the neoblast genome integrity. Neoblasts also have chromatoid bodies and their numerous associated proteins, classically linked to germ cell function; this isprobably indicative of their close link in evolution. Related to this, we described above how stem cells of sponges and Cnidaria and other groups are involved in both somatic and germ cell lineages. Most likely,

in flatworms of both kinds, acoels and Platyhelminthes, germ cells and pluripotent somatic neoblasts are both derived from totipotent neoblasts that self-renew (Bely and Sikes, 2010; Solana, 2013).

3.10. How does aging in higher animals differ from that of simple animals?

Aging strategies among animals fall roughly into two or three groups, related to cell and tissue maintenance and regeneration, and to aging of the individual cells; proposed mechanisms vary according to the author examined the relative amount of totipotency found in some cell populations among all animal groups, using this to define a population of primordial stem cells that can form all germ and somatic cell types. In early metazoan evolution, these unlimited PriSCs continue into the adult; they include especially the archeocytes of sponges, and the I-cells of many or most cnidaria and neoblasts of flatworms; i.e., probably all of the groups that we have discussed in detail in this review. These PriSCs allow many members of this first group to reproduce by asexual reproduction (fission, budding, etc.), often imparting potential immortality as has been discussed in previous sections; but the PriSCs also can give rise to germ cells for sexual reproduction (however, germ cells also can form earlier in development and be retained into the adult). The second group has restricted PriSCs and includes some annelids (segmented worms) and some molluscs (snails and clams, etc.). In these animals, an early embryonic cell, commonly the 4d blastomere, divides to form primordial germ cells that form adult germ cells, but this blastomere also produces somatic stem cells that continue into the adult (notably as the MPGZ [mesodermal posterior growth zone] of some polychaete annelids), allowing limited pluripotency in the adult animal. The third group has rudimentary PriSCs. includes in this group the oyster, Crassostrea gigas (a mollusk), as well as C. elegans(a nematode), Drosophila (an arthropod), and Xenopus (a frog) and other vertebrates. In these organisms, some early cell stages in embryogenesis gives rise to primordial germ cells and thus adult germ cells, as in the second group, but only some initial somatic cells; thus there are no adult pluripotent somatic stem cells. In fact, numerous studies in fish and amphibians and other vertebrates support the idea that vertebrates normally lack adult pluripotent somatic stem cells.Interestingly, mammalian embryonic stem cells have the same basic molecular determinants of pluripotency that are found

in planarian neoblasts, suggesting that the mechanism is evolutionarily conserved (Önal et al., 2012).

“The germline cycle in freshwater planarians. Freshwater planarians possess a population of stem cells, the so-called neoblasts, which represents the primordial stem cells in these organisms (PriSC). Neoblasts are able to give rise to the germ cells and to somatic cells. Germ cells give rise to oocytes and sperm, which jointly give rise to the zygote. The zygote gives rise to both somatic cells and the PriSCs. The planarian PriSCs have unlimited self-renewal and both germ potential (GP) and somatic potential (SP). Green dots represent the presence of nuage granules and germ plasm components. The vertical blue line represents the position of the germ-to-soma boundary, as classically understood. The horizontal green line represents the proposed position of the germ-to-soma boundary as postulated in the Primordial Stem Cells hypothesis (Solana, 2013)

Thus, there was a trend of reduced incidence of asexual reproduction as more complex metazoans evolved. Within the third group described above, we can recognize two different strategies in the evolution of more complex animal forms from the simple metazoans that show continuous rejuvenation (and potential immortality) in the adult. First, for vertebrates and some other higher metazoans, there is maintenance of some limited rejuvenation abilities in the adult resulting in a moderately long lifespan. In contrast, for C. elegans and some other animals, rejuvenation is restricted to sexual reproduction and the embryo, resulting in a short lifespan and an increase in generations per unit time (Pearson and Sánchez Alvarado, 2008; Tanaka and Reddien, 2011; Rink, 2013; Solana, 2013).

Another way of dividing metazoan reproductive/aging strategies is given by. Shostak argues that basal metazoans have determinativedevelopmentwith narrow potencies, but also, as we have noted, with continuous proliferation of cells followed by asexual reproduction to get rid of the excess cells. Simple metazoans get rid of excess cells, due to proliferation, by asexual reproduction to reduce body size. The best example of this is the hydra as described in a previous section; hydras utilize three kinds of stem cells to proliferate and replace all of the cells of their body in a short time, with excess cells either used to make daughter buds or simply sloughed off

at both ends of the body. In this case at least, two of the three kinds of stem cells, i.e., the ones producing the two epithelial cell layers, appear to have a limited potency. Other relatively simple metazoans such as the nematode C. elegans, also have determinative growth but without cell proliferation in the adults (except for the germline and gametogenesis), and with a consequently short lifespan. This probably is not possible in more complex animalsthat are thus in danger of developing cancer. Onesolution is to constrain proliferation using a more regulative type of development: 1) adult stem cells have asymmetric distribution of DNA strands to constrain steady state populations; 2) a subset of cells can have a count-down timer, possible involving telomere length (capping ends of chromosomes), so that after receiving only freshly-replicated DNA strands for the adult stem cells, precursor cells revert to symmetric division and for a limited number of divisions. In addition, López-Otin et al. (2013) note that some mechanisms promote cellular senescence, which "…protects the organism from cancer but which, in excess, can promote aging." However, strategies to regulate cell and tissue development as a strategy to prevent tumors are not limited to higher metazoans; even planaria can get tumors (as noted in a previous section) and thus have developed molecular mechanisms to avoid them (Oviedo et al., 2008; Pearson and Sánchez Alvarado, 2008).

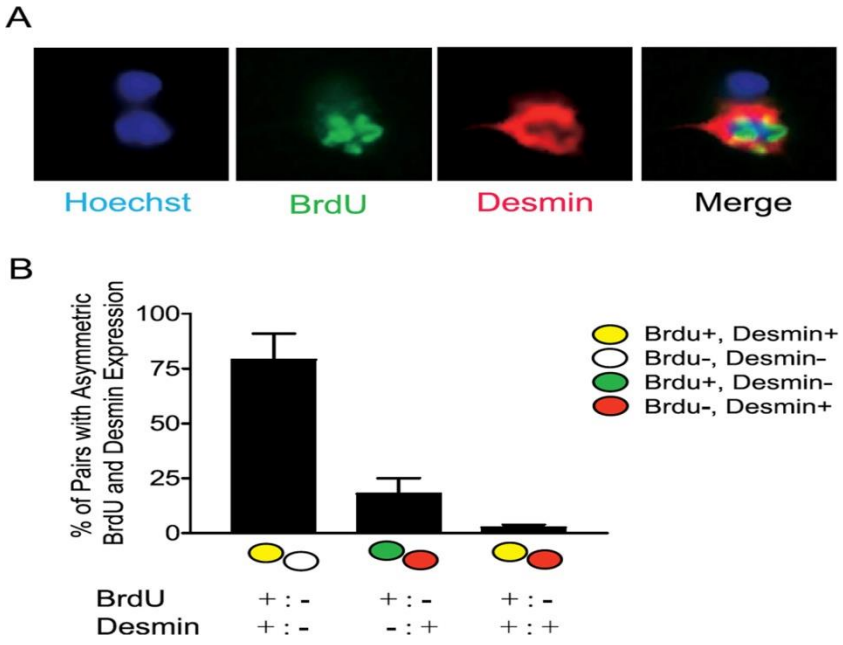

Figure 3.5: Divergent cell fates associated with asymmetric segregation of template strands.

(Oviedo et al., 2008)

(A) Pairs of cells were co-immunostained for BrdU and the myoblast marker Desmin; representative images are shown. (B) The data from experiments as in (A) were quantified. Pairs with Desmin expression and asymmetric BrdU labeling were distinguished based on the pattern of Desmin expression (asymmetric and coincident with BrdU, asymmetric and mutually exclusive with BRdU, or symmetric). The legend for individual cells is shown to the right, and the legends for the cell pairs are shown below. Data represent mean +/− SEM (n=4)." In this study of dividing mouse muscle stem cells (satellite cells) we see that the daughter cells inheriting the older templates (no BrdU) usually retain the more immature phenotype, thus apparently remaining in the stem cell population, while the daughter cells inheriting the younger template (BrdU positive) show that they are forming the differentiated cell type, in this case, the Desmin-containing myoblast.

Within the complex bodies of vertebrates and other higher Metazoa, we can see that different stem cell populations showing unipotency or oligopotency utilize one of two strategies to maintain their cell populations. Either the stem cells have a high quality of proteostasis (maintenance of proteome homeostasis as noted above) and produce differentiated cells with a high turnover/short lifespan (e.g., blood and epithelial cells), or the stem cells have a lower quality of proteostasis but produce differentiated cells with a low or no turnover/long lifespan that show a corresponding higher quality of maintenance of proteostasis. e.g., neurons, cardiomyocytes (Rando, 2006).

Stem cell maintenance in hydras depends on the transcription factor, FoxO, and may account for the potential immortality of hydras. The FoxOgene is expressed at high levels in all threekinds of stems cells of the hydra. FoxO may promote longevity in hydras both by maintaining the stem cells and by supporting innate immune pathways that defend the animal against microbe invasion. FoxO also has been found in other cnidarians, as well as in sponges and placozoans, so it probably is a key longevity factor in basal metazoans. FoxO also has been associated with aging regulation in bilaterians, including C. elegans, Drosophila and humans. Interestingly, in

humans, more polymorphisms in the FOXO3A gene may be associated with people in their 100s compared to those in their 90s or younger. So why does FoxO not promote immortality in humans and other higher bilaterians? As animal life evolved into more complex forms, FoxO seems to have taken on several other functions and has become coupled to pathways involvedmainly with the maintenance of post-mitotic cells as the animal ages (Schaible and Sussman, 2013).

Thus, the groups of basal metazoans and simple flatworms that we have emphasized in this review, and as we have noted previously, have a simple structure that allows them to retain adult pluripotent or totipotent stem cells and to reproduce using both asexual and sexual mechanisms. Adult pluripotency and asexual reproduction seem to work together as a single strategy that is successful mainly for the simplest metazoans, and both are lost in most of the higher metazoans. However, there are exceptions: Isaeva (2011) reviews studies of pluripotent gametogenic stem cells of colonial parasitic rhizocephalan crustaceans (phylum Arthropoda, i.e., the phylum that includes Drosophila) and in colonial ascidians (types of tunicates or sea squirts, i.e., invertebrates in the phylum, Chordata, that includes vertebrates).

3.11. How to Slow Down Aging

- **Hypometabolism and Metabolic Reprogramming:**

Decreasing the rate of metabolism, which may be directly affected by temperature and flux through metabolic pathways, can extend lifespan. For example, the lifespan of fruit flies and nematodes is extended at lower and shortened at higher temperatures. However, energy expenditure is not necessarily proportional to cumulative damage. It may be accompanied by a

more optimized use of energy, thereby decreasing the diversity of damage forms. Physical activity may have this effect. This may also be reflected in differences among cell types (e.g., long-lived cells such as neurons and cardiomyocytes may produce less damage than some other cells due to a more optimized metabolism).

- **Damage dilution:**

Unequal distribution of damage between two daughter cells, or dilution of damage by cell division can extend lifespan. This strategy is used in the budding process in the baker's yeast and iPS-type cell reprogramming, which reset the aging clock.

- **Damage maintenance:**

Damage can be decreased by increased activity of maintenance systems, e.g., DNA repair, proofreading mechanisms during RNA and protein synthesis, protein and metabolite repair pathways, antioxidant and detoxification systems, and autophagy. However, maintenance can only deal with some damage forms and comes at a cost, as these systems themselves generate other damage forms.

- **Lifespan extension by dietary intervention:**

Caloric restriction extends lifespan in a variety of organisms. It may change the metabolic state of a cell/organism, thereby affecting the rate and forms of damage generated. For example, yeast responds to caloric restriction by activating respiration, which generates energy more efficiently,

even though the damage from respiration *per se* may be higher. Lifespan can also be extended by rapamycin, metformin and several other compounds. Rapamycin acts by inhibiting mTOR, which alters the metabolic state and slows down metabolism, thereby changing the damage landscape.

➢ Decreased mortality in late life

One paradox of aging is the decreased mortality in late-life. From the imperfectness perspective, two factors may be relevant. First, any population will consist of sub-cohorts differing in cumulative damage. The individuals with more severe damage will be eliminated earlier, leaving those with less severe damage. Second, cumulative damage will feedback to metabolism, decreasing its rate in late life, thereby decreasing the rate of damage generation. Thus, the paradox of aging subsiding in late life can be explained from both population and individual organism levels.

➢ Organisms that do not age

The imperfectness model suggests that some organisms, even animals, may theoretically be immortal. Damage accumulation may be confined by balancing damage by cell division or replacement of old somatic cells with new cells. For example, some multicellular organisms, such as hydra and planaria, possess efficient cell renewal strategies when growing asexually. Whether they are truly non-aging is unclear, but this possibility is not excluded by the model. Unicellular organisms with truly symmetric divisions do not age, whereas asymmetric division, even in bacteria, will lead to aging.

3.12. Relevance to Psychiatry

There is accumulating evidence for "accelerated aging" in psychiatric disorders. Population-based estimates of the average years of life lost to diseases such as bipolar disorder or schizophrenia are between one and two decades. Although some of this increased mortality is due to suicide, the bulk of lost years of life appears to be attributable to the higher rate of age-associated medical comorbidities, which occur at an earlier age than in the general population. In addition, emerging biomarkers that may enable tracking of biological aging, such as the telomere, have been studied in schizophrenia, depression, and other psychiatric illnesses; there is some indication that these biomarkers indicate a faster rate of biological aging among people with psychiatric illnesses. Psychiatric disorders may have both direct impact on systems that are implicated in aging biology (e.g., immune system) and indirect effects through unhealthy health behaviors (e.g., smoking, sedentary lifestyle) and lower participation in activities that are associated with better functioning in aging (e.g., social and cognitive stimulation). Formal models of progressive lifespan courses of illness have been proposed, such as "neuroprogression" in bipolar disorder or accelerated aging in HIV; these models integrate knowledge about disease course and brain aging. Thus, there is growing recognition of the overlap between the modifiers of long-term outcome in aging and in psychiatric illnesses.

- **Physical activity**

Physical activity contributes to reducing the risk of several diseases associated with aging including cardiovascular disease, metabolic disease, and osteoarthritis. According to the American College of Sports Medicine guidelines, at least 150 minutes of moderate weekly physical activity (30 minutes, 5 days/week) is recommended to obtain health benefits. In a review by Brown, Peiffer, & Martins (2013), the authors describe several epidemiological studies demonstrating a relationship between higher levels of physical activity and lower levels of cognitive decline and/or enhanced cognitive functioning in domains such as verbal memory, executive functioning, attention, and global cognition. One study of 1324 subjects reported that moderate (as opposed to light or vigorous) exercise in mid-life and late-life was associated with a

reduced risk of mild cognitive impairment. Other studies have used more objective measures of physical activity such as actigraph accelerometers (Barnes et al. 2008) or respiratory fitness estimates as assessed by having participants walk on a treadmill and measuring peak oxygen consumption [13]. In these studies, individuals with the highest daytime movement performed better on cognitive functioning tests and individuals with the lowest baseline cardio-respiratory fitness performed the worst on all cognitive tests.

Several randomized controlled studies have attempted to increase physical activity in older adults with encouraging results. For example, Erickson et al. (2011) compared sedentary nondemented older adults participating in a walking program with a group who did only stretching exercises. The walking group demonstrated many positive outcomes including improved cardiovascular fitness as evidenced by a 7.8% increase in VO_2 max, improvement in spatial memory, a 2% increase in hippocampal volume, and an increase in brain-derived neurotrophic factor (BDNF). In a large 24 week intervention program, 150 individuals aged 50 and over with subjective memory complaints and/or mild cognitive impairment were randomized to either an exercise intervention program which consisted of 150 minutes of moderate exercises three days a week over 18 months or a usual care control group. At the completion of the study, the intervention group improved in cognitive function significantly on the Alzheimer Disease Assessment Scale.

A number of studies have examined physical activity interventions in samples of patients with psychiatric illnesses. In a recent meta-analysis of thirty-nine randomized controlled trials across various types of physical activity in people with mental illness, reductions in depression and symptoms of schizophrenia were reported along with improvements in aerobic capacity and quality of life. Other studies have reported similar positive effects of physical activity in patients with depression, anxiety, PTSD and schizophrenia

➢ Cognitive Stimulation/Remediation

The evidence that neuroplasticity is preserved into later life has paved the way for cognitive interventions to attempt to slow or delay the onset of cognitive decline. It has been proposed that cognitively-stimulating activities may delay future decline. Consequently, many older adults are being encouraged to independently engage in daily cognitive stimulating activities such as reading, practicing crossword puzzles, and playing board/card games. There is some evidence to suggest that frequent engagement in cognitively stimulating activities for at least six hours per week may reduce the risk of incident dementia. Research has further suggested that participating in these activities may be effective in altering the rate of cognitive declined in persons diagnosed with dementia. Teasing apart causal effects, as well as which and how frequent/intense cognitive activities must be remains to be established.

In a systematic review of twenty-one cognitive intervention studies ranging from 13 to 242 healthy older adults (mean age 63.5 to 80.2 years), it was concluded that cognitive interventions can be effective in improving various aspects of objective cognitive functioning including memory performance, executive functioning, processing speed, attention, fluid intelligence, and subjective cognitive performance. These interventions varied in design with some addressing working memory by computerized training, teaching memory strategies, improving learning abilities by training metacognitive skills, or improving attention by promoting selective attention tasks[23]. Important questions for the future are whether cognitive interventions generalize to improvements in activities of everyday living (e.g., driving, paying bills, medication management) and whether the mechanism of these interventions is by structural brain changes or alterations in neural activity.

There has been recent interest in cognitive rehabilitation in patients with psychiatric illness, with a particular focus on technology-assisted cognitive training. For example, in patients with schizophrenia, tablets and computerized cognitive training programs have shown some success in improving neuropsychological functioning. Moreover, computerized cognitive training three

times per week for eight consecutive weeks in patients with unipolar depression and bipolar patients in the depressive phase of the disorder has been shown to result in fewer cognitive failures, fewer dysexecutive incidents, and improved neuropsychological scores.

➢ Diet/Nutrition

Dietary and nutritional interventions are among the most studied strategies in animals for extension of the lifespan and prevention of morbidity. Caloric restriction, for example, has been associated with substantially enhanced longevity in rodents, and to a lesser extent in primates. Clinical trials in humans have also shown promise. There is some evidence that obesity is associated with heightened risk for dementia when individuals are followed longitudinally. A recent study found that obesity was related to cognitive impairment in bipolar disorder and schizophrenia.

However, intersection of more specific dietary interventions with cognitive and emotional health has received comparatively less research than cognitive or physical activity interventions described above. A number of large clinical trials evaluating dietary supplements, such as Gingko Biloba and Vitamin D, have not shown significant benefit for cognition. In contrast to isolated supplements, dietary patterns, in particular the Mediterranean diet, have shown associations with reduced rates of depression and lower risk for cognitive decline. Adherence to the Mediterranean diet includes high consumption of fruit and vegetables, high ratio of polyunsaturated to saturated fats and low glycemic load. Frontiers in the understanding of diet and aging include research on the relationship between genetic risk and nutrition, as well as the intersection of the gut microbiome with mood and anxiety symptoms

➢ Complementary and Alternative Medicine

Yoga and meditation are potentially impactful interventions since they can be tailored to ability levels, addressing the needs of those with limited mobility as well as those seeking more challenging physical activity. Although yoga is considered an ancient practice, the research exploring its impact on health outcomes is relatively recent. In one observational, cross-sectional study surveying a sample of 211 women yoga practitioners aged 45-80 years, increased yoga experience predicted higher levels of positive psychological attitudes, mental mastery, subjective vitality, and transcendence (i.e., feelings of oneness with surroundings and unity with the community). The authors found a dose-response effect in that regular and more frequent yoga predicted the highest levels of psychological well-being.

Another promising area where yoga interventions seem to be of benefit is in improving sleep. In a study of older men and women (aged ≥ 60) with insomnia, a 12 week, twice weekly intervention group (n = 59) including yoga postures, meditative yoga, and daily practice of meditative yoga showed significant improvements in several areas including but not limited to overall sleep quality, fatigue, general well-being, depression, anxiety, and stress relative to a control group.

Meditation is also becoming increasingly popular and has shown some associations with reduced age-related cognitive decline. For example, in a cross-sectional study of older adults comparing the cognitive performance of long-term meditators (>10 years, n=20) and non-meditators (n=20), long-term meditators performed significantly better on measures of attention, processing speed, the ability to shift attention, and on tests using distracting factors [37]. Furthermore, in a review paper exploring the effects of meditation on attention, memory, executive functions, and other cognitive measures in older adults and adults suffering from neurodegenerative diseases, meditation techniques revealed a positive effect in several areas including attention, memory, verbal fluency, and cognitive flexibility

➢ Social Engagement

The association between social engagement and health and well-being has been well-documented throughout the lifespan. In many ways, increased age can be considered a risk factor for social withdrawal, as a result of physical decline and retirement. A recent meta-analysis found that social engagement was as strong a protective factor for health as many other established risk factors. Social engagement can be defined as making social and emotional connections with other people such as family/friends and the community (e.g., being an active participant in clubs, religious organizations, volunteer work). In 364 younger (21-59 years), older (60-89 years), and oldest-old (90-97 years) adults participating in the multidisciplinary Louisiana Health Aging Study, it was found that social engagement (indexed by hours spent outside of the home) was significantly associated with self-reported health as assessed by SF-36 physical component scores and a measure of objective health status . Besides the number of relationships and amount of time spent outside of the home, the complexity of one's personal network or the different types of relationships one has acquired has also been deemed as important. In a sample of 2959 Dutch participants aged 54 to 85 assessed at baseline and six times over a twenty-year period, older adults reporting a greater number of relationship types in their social network showed higher global cognitive functioning (as assessed by the Mini-Mental State Exam). Moreover, reductions in network complexity were associated with a decline in cognitive functioning. The authors postulate that being embedded in a variety of different types of relationships exposes an individual to a wider range of activities than those embedded in more homogenous networks.

Several intervention studies have aimed at reducing social isolation in later life by focusing on improving social skills, enhancing social support, increasing opportunities for social contact, and addressing maladaptive social cognition. One such intervention program is The Seniors Centre Without Walls, which provides free educational programming to older adults via telephone to try to address the social needs of older adults who are restricted due to physical, financial, or geographical reasons. Individuals are given a set time to be on the phone with a session leader as well as other participants of the program. Participants of this program reported making friends on the phone and feeling more connected to the community. These participants also said that the program made them feel less lonely, happier, and helped them cope better with depression

In psychiatric disorders, a large number of studies show that social withdrawal, loneliness, and lack of support exacerbate or contribute to psychiatric symptomatology. Most of the focus in psychiatric disorders has been on targeting deficits in skills that arise from the illnesses. There are longstanding data on social skills training in patients with schizophrenia, with evidence for improvements in social isolation, social discomfort, and quality of life post-treatment. Social cognition training is another avenue to enhancing social functioning in schizophrenia.

➢ Positive Psychological Traits

When older adults are asked about what defines successful aging in qualitative studies, a recurring theme is personality variables, such as resilience, adaptability and optimism. Adaptation to disability and losses is at the core of several major theories of successful aging. In recent work, higher scores on a self-reported measure of resilience were associated with a mitigation of the impact of depressive symptoms on subjective successful aging. There is emerging neurobiology of resilience that maps onto work in post-traumatic stress. Similar to resilience, there are striking associations between optimism at mid-life and longevity[53] that remain even after multiple covariates are accounted for. There appears to be a complex genetic basis for these traits and both are heritable, with a large proportion of environmental variation[54]. It is unclear how best to promote resilience and optimism, particularly in the specific context of age related changes, yet there are a number exciting biological and non-pharacologic interventions that are in early phases of development. Less well understood are psychological traits that are specifically associated with aging, such as wisdom or those that extend to the social domain, such as altruism and compassion. Each is associated with positive psychological adjustment in older age samples, and interventions such as intergenerational volunteering that

Taps into these traits appears to have a tangible impact on cognitive and emotional health in older adults as well as the communities they serve.

3.13. Plasticity of aging: interplay between lifespan regulation and brain function

Many of the pro-longevity signaling pathways have more recently also been shown to play important roles in higher level brain function, providing evidence for a connection between the plasticity of aging and the potential plasticity of the aged CNS. For example, FoxO transcription factors have been implicated in regulating CNS function. In particular, FoxO6, which is most highly expressed in the hippocampus, was recently shown to play a role in memory consolidation in mice. The authors found that in the absence of FoxO6, mice were unable to consolidate the memory trace in contextual fear conditioning and novel object recognition tasks. Similarly, Sirt1 was recently also shown to be essential for normal cognitive function and synaptic plasticity in adult mice. Specifically, in the absence of Sirt1, mice displayed deficits in learning and memory processes such as immediate memory, classical conditioning, and spatial learning paradigms, while over-expression of Sirt1 enhanced these behaviors. Furthermore, these

results were validated in an independent study that suggested beneficial effects of Sirt1 on cognition were mediated through a microRNA (miR-134)-dependent mechanism. Lastly, multiple lines of evidence have implicated mTOR signaling as an important molecular pathway for learning and memory. Most of these studies showing the importance of mTOR signaling in memory are elicited via rapamycin treatment. However, there are also genetic studies that validate the conclusion that mTOR signaling plays a role in learning and memory, although many genetic manipulations involve upstream or downstream targets of mTOR as global genetic deletion of mTOR is embryonic lethal. Nonetheless, rapamycin treatment prevents memory consolidation across multiple brain regions including the hippocampus, auditory cortex, gustatory cortex, amygdala, and prefrontal cortex in rodents. Interestingly, in the FoxO and Sirtuin studies mentioned above, the behavioral results were paralleled by changes in synaptic (long-term potentiation and long-term depression) or structural plasticity (spine density) in brain regions such as the hippocampus that are particularly vulnerable to the effects of aging. This suggests a possible parallel role for these signaling pathways in promoting longevity and potentially facilitating functional improvements in age-related cognitive dysfunction. This body of work not only demonstrated that longevity genes regulate critical CNS functions but also

offered the first evidence that just as organismal lifespan is malleable so too could CNS functions prove to be amenable to rejuvenation (Gao *et al*. 2010; Michan *et al*. 2010).

3.13.1. Plasticity of aging: the brain as a central regulator of lifespan

The first evidence for the role of the CNS in controlling systemic aging came from cell-specific manipulations of longevity genes in model organisms such as worms, flies, and mice. For example, restoring insulin signaling in neurons, but not muscle or intestine, was sufficient to increase the lifespan of worms. In flies, manipulation of signaling molecules that impact FoxO activity improved neuronal stress response capabilities to reactive oxygen species and extended overall lifespan. In addition, mutations in genes that affect the ability of gustatory and olfactory

sensory neurons to transmit systemic information about nutrient sources to the CNS, likely affecting metabolism, also regulated lifespan in worms and flies. There is now growing evidence that the same molecular pathways observed to regulate lifespan in the invertebrate CNS are also important regulators of aging in the mammalian CNS. In mice, reduction of brain insulin receptor substrate extended lifespan by 18%, an effect that is nearly identical to the changes in lifespan of mice that have heterozygous deletions of the insulin signaling substrate throughout the entire body. In addition, mice with homozygous deletions of the insulin signaling substrate die in utero; however, mice with homozygous deletion of this substrate specifically in the brain show a 14% increase in lifespan. These results demonstrate that, while complete loss of insulin signaling is detrimental to an organism, maintaining just one copy of this gene specifically in the CNS can significantly influence lifespan regulation promoting organismal longevity even in mammals. Together, these studies suggest that manipulation of longevity genes specifically in the CNS is sufficient to counteract the effects of aging at a systemic level (Bartke 2007).

More recently, aging research in mice has now begun to hone in on a particular region of the brain, the hypothalamus, as the potential CNS mediator of lifespan regulation. The hypothalamus is a region of the brain comprised of a diverse set of nuclei and is located between the thalamus and brainstem. Despite its small size, the hypothalamus receives input from nearly every tissue in the body, and by regulating endocrine signaling via hormones, plays a crucial role in many physiological processes including growth, metabolism, and reproduction to name a few. Thus, the hypothalamus serves as a well-established hub for communication between the CNS and the systemic environment. Indeed, it is the ability to both sense and influence tissues throughout the body that first indicated the hypothalamus as being well poised to regulate systemic aging. For example, studies have shown that brain over-expression of the pro-longevity gene Sirt1, specifically enriched in the hypothalamus, could extend lifespan in male and female mice. Moreover, increased hypothalamic Sirt1 expression also improved age-related phenotypes including improvement of physical activity, oxygen consumption, sleep quality, and body

temperature when compared to age-matched controls. As with invertebrate studies, these data highlight the intimate interplay between pro-longevity mechanisms and CNS-mediated lifespan regulation. Independent studies have now also shown that the hypothalamus can directly regulate systemic aging itself through the immune-neuroendocrine axis. Specifically, investigators showed that reducing inflammation in the hypothalamic immune cells (microglia) could change the hypothalamus response to the systemic environment extending lifespan and reducing aging-associated pathologies. The authors also provide a potential mechanistic link between age-related changes in hypothalamic inflammation and systemic levels of gonadotropin-release hormone. In addition, manipulations of the immune-neuroendocrine axes also yielded significant enhancements in the CNS, including increased neurogenesis and improved cognition in aged mice. While future studies remain necessary to better understand how age-related changes in the hypothalamus influence lifespan regulation, collectively these studies support the hypothesis that the CNS plays a central role in modulating systemic aging (Bartke 2007; Alcedo *et al*. 2013a; Gabuzda and Yankner 2013; Satoh *et al*. 2013; Tang and Cai 2013; Zhang *et al*. 2013).

Taken as a whole, studies in invertebrates and mammals highlight the malleability of the aging process and demonstrate the involvement of the CNS in regulating lifespan, raising the possibility that influencing the communication between the CNS and the systemic environment could prove one effective strategy for counteracting the effects of aging (Tang and Cai 2013).

3.13.2. Reversal of aging: systemic manipulations as mediators of rejuvenation

Manipulating individual genes within the CNS may represent one approach with potential to counteract the effects of aging. However, targeted manipulations of genes, specifically within specialized populations of neurons in regions such as the hypothalamus, prove challenging at this point. While reversal of aging by the CNS appears distant, alternative strategies to rejuvenate

aged tissues through a more systemic approach may prove equally effective in counteracting the aging process. In this manner, broad changes in the aged systemic environment, rather than a central regulator, may provide the means for rejuvenation. Indeed, systemic manipulations such as exercise, CR, and changes in blood composition by heterochronic parabiosis or young plasma administration have already yielded much promise in their ability to combat signs of aging in both peripheral tissues and the CNS. Specifically, to date, these different systemic manipulations have been shown to ameliorate impairments in the regenerative capacity of aged tissues, as well as synaptic plasticity and even cognitive functions in the aged CNS. While exercise, CR, and changes in old blood composition have not been shown to ameliorate all of the same set of age-related impairments or to the same degree, evidence that a systemic approach to rejuvenation is plausible continues to mount. It should be noted that the field of rejuvenation research remains in its infancy and questions such how systemic manipulations can rewind the aging clock remain poorly understood (Kay 2011; Wanisch *et al*. 2013).

➢ Exercise

Physical exercise increases blood delivery to most tissues and leads to changes in the systemic environment. Interestingly, numerous studies have documented rejuvenating effects of exercise on the functional and regenerative capacity of peripheral tissues and CNS in animal models. Moreover, exercise has even been associated with a reduced incidence of developing classical age-related diseases including cardiovascular disease, type II diabetes, osteoporosis, macular degeneration, and dementia. Considering loss of tissue regeneration during aging is thought to contribute to decreased tissue function, it is consistent with the concept of rejuvenation that the beneficial effects of exercise extend to enhancements in adult stem/progenitor cells in old age. Outside the CNS, exercise can promote hematopoiesis (regeneration of blood cells) in the aging systemic environment, and increase the proliferative capacity of aged skeletal muscle stem cells, including satellite and mesenchymal stem cells. Recent studies have shown the effects of exercise on molecular and cellular processes that play major roles in stem cell function both in *in vitro* and *in vivo*. For example, exercise induces autophagy (clearance of cellular debris), which protects hematopoietic stem cells from metabolic stress and contributes to their lifelong maintenance. Exercise is also associated with increased telomere length in humans, which may aid in counteracting the effects of telomere shortening on replicative senescence. On a molecular

level, exercise leads to regulation of telomere-associated genes and microRNA expression, both important for stem cell self-renewal and differentiation (Lee *et al*. 1998; Gangaraju and Lin 2009; Jaskelioff *et al*. 2011).

The beneficial effects of exercise extend beyond peripheral tissues to also include the brain. In particular, increased running in aged mice has been shown to enhance neural progenitor proliferation and neurogenesis to a level comparable to that observed in young animals. Because of the blood–brain barrier, it was traditionally thought that the beneficial effects of exercise on

the CNS were not orchestrated through systemic changes in the periphery. However, recent studies suggest that the effects of exercise are, in part, mediated by changes in the systemic environment. Investigations looking at magnetic resonance imaging (MRI) measurements of cerebral blood volume in the hippocampus have demonstrated that exercise selectively increased the cerebral blood volume of the dentate gyrus, correlating with post-mortem increase in neurogenesis. From a molecular perspective, elevated systemic levels of circulating growth factors such as vascular endothelial growth factor and insulin-like growth factor 1 (IGF-1) in blood elicited by increased exercise have been shown to mediate, in part, enhancements in neurogenesis. Coincidently, circulating levels of IGF-1 decrease with age and the restoration to levels resembling a younger systemic environment up-regulate neurogenesis and improve learning and memory (Lichtenwalner *et al*. 2001; Darnaudery *et al*. 2006).

Together, these studies show that a systemic manipulation such as exercise can rejuvenate adult stem cell function across tissues. In addition, it indicates that targeting the systemic environment could prove to be an effective strategy to reverse the functional impairments of aging on the aged CNS.

➢ Caloric restriction

Another systemic manipulation shown to counteract the age-induced effects on tissue regeneration is CR, a reduction of 20–40% of caloric intake without malnutrition. The ability of CR to counteract aging was initially characterized as an extension of studies investigating the pro-longevity effects of CR on lifespan, a phenomenon conserved across phylogeny. This effect likely results from increasing glucose metabolism, reducing oxidative stress and the ability of cells to counteract DNA damage, as well as influencing aspects of the aging immune and neuroendocrine system. More recently, CR has been shown to rejuvenate tissue regeneration in aged organisms, similar to the effects of exercise. A number of studies have shown rejuvenating effects of CR on the decline of hematopoietic stem cell function. Rejuvenation of regeneration was also observed in skeletal muscle, in which short-term CR in aged animals increased muscle

stem cell availability and activity when compared to ad libitum fed mice. In addition, there are also beneficial effects of CR on intestinal stem cells. Specifically, a 35% increase in intestinal Olfm4-positive progenitor cells was observed in mice under CR compared to ad libitum fed controls, indicating that CR promotes intestinal stem cell self-renewal and preservation (Yilmaz *et al*. 2012).

The effects of both short-term and long-term CR on rejuvenation of regeneration are also found in the CNS. Early studies observed an increase in proliferating cells in the dentate gyrus after a 30% reduction of caloric intake for 3 months in rats and for 2 weeks in mice. However, different from peripheral tissues, this effect of CR on increased cell number in the brain resulted from a reduction in cell death, rather than an increase in cell proliferation. In addition, only Bromodeoxyuridine (BrdU), a marker of cell proliferation, was quantified making it difficult to conclude that this increase in BrdU-positive cells translates into increased levels of neurogenesis. Similar effects were also observed when mice were calorie restricted over an extended duration of 3–11 months, although in this study the authors found that CR led to an increase in glial cells

and not neurons. More recently, prolonged exposure of mice to CR (40% decrease in caloric intake maintained for 10–12 months) was shown to reduce age-related decrease in neural progenitor cell divisions in the aging brain. As a whole, because different experimental paradigms of CR were used and the level of detail with which proliferating cells were characterized varied, the effect of CR on mammalian adult neurogenesis is difficult to ascertain with clarity. Future studies are therefore needed to better understand the effect CR plays in counteracting the age-related decline in adult neurogenesis. Nevertheless, CR illustrates that an additional systemic manipulation independent of exercise can also affect regenerative capacity in the aged brain (Park *et al*. 2013).

➢ Heterochronic parabiosis

Lastly, rejuvenation of tissue regeneration in aging organisms has also been observed after heterochronic parabiosis. First introduced in 1864 by Dr Paul Bert, parabiosis is a surgical procedure by which two animals are physically connected. The procedure and its historical context have previously been described in greater detail. Of particular interest is the effects of the blood of young animals on the physiology and behavior of older animals. Through the use of the heterochronic parabiosis model, it has now been shown that exposure to young blood can rejuvenate the regenerative capacity of peripheral tissues and CNS in aged animals (Conboy *et al*. 2013; Eggel and Wyss-Coray 2014).

Several studies have shown that aged mice exposed to a young systemic environment exhibit reduced signs of biological aging in cardiovascular, skeletal, and digestive systems. In skeletal muscle, old heterochronic parabionts exposed to young blood exhibited increased muscle progenitor cell activity leading to enhanced regeneration and reduced fibrotic responses compared to old isochronic parabionts. Liver tissue isolated from old heterochronic parabionts

was also found to have more youthful levels of hepatocyte rejuvenation. Furthermore, the rejuvenating effects of young blood have also been observed on both cardiovascular and metabolic systems in aging mice, although in these studies the effect of young blood on stem cells function was not investigated. In particular, young blood can reverse age-related cardiac hypertrophy in old heterochronic parabionts and reverse the age-related decline in pancreatic beta cell replication thought to contribute to the development of type II diabetes. Together, these studies show that changing the composition of the aged systemic environment to a more youthful state can reverse the regenerative and functional decline in aged peripheral tissues. Indeed, these studies point to the existence of ‘pro-youthful’ factors in young blood that have begun to be identified in recent studies. For instance, restoring systemic levels of the growth differentiation factor 11 (GDF11), a member of the transforming growth factor β superfamily, to a more youthful state reversed age-related skeletal muscle and cardiovascular impairments in mice. The

authors found that daily administration of recombinant GDF11 into aged mice increased muscle satellite cell frequency and function, as well as improved muscle function. Similarly, treatment of old mice with recombinant GDF11 reversed age-related cardiac hypertrophy. Another study also found that increasing levels of oxytocin, a circulating hormone, in aged mice enhanced muscle regeneration by increasing muscle stem cell activation and proliferation (Elabd *et al*. 2014).

The rejuvenating effects of young blood have now also been observed in the aged CNS of old mice. In a mouse model of demyelination, exposure of old mice to a youthful systemic environment increases myelination in the spinal cord of old heterochronic parabionts by recruiting young peripheral monocytes and promoting differentiation of oligodendrocyte progenitor cells. These findings are of particular significance given that nerve demyelination occurring in the elderly functional impairs muscle strength and sensory discrimination while contributing to axonal degeneration (. In the hippocampus, old heterochronic parabionts exposed to a young systemic environment exhibited increased neurogenesis in the dentate gyrus,

indicated by increased BrdU-positive proliferating cells, Sox2-positive progenitors, and Doublecortin-positive newly born neurons. More recently, an independent study also corroborated the rejuvenating effects of young blood on adult neurogenesis in the SVZ of old heterochronic parabionts. Specifically, they found that young blood increased Ki67-positive proliferating cells in the SVZ, enhanced olfactory neurogenesis, and facilitated vasculature remodeling in neurogenic regions of old mice. Interestingly, the authors found that recombinant GDF11 administration could, in part, mimic the beneficial effects of young blood on neurogenesis and vasculature remodeling. Consistent with other functions of the transforming growth factor-β superfamily, GDF11 appears to have pleiotropic qualities. Indeed, during development, GDF11 participates in patterning of the CNS and has been found to inhibit embryonic neurogenesis. Surprisingly, in the context of aging it seems to promote plasticity of the CNS. Identification of this one factor, GDF11, is an exciting finding; however, the effects of

young blood on rejuvenation of regenerative capacity are not fully recapitulated, suggesting the existence of multiple ‘pro-youthful’ factors. Given the rejuvenating effects of young blood have now been observed in neurogenic zones across multiple brain regions, this supports the possibility of global rejuvenating effects that may extend throughout the aged brain (Laviano 2014).

4. Roles of Genes in Aging

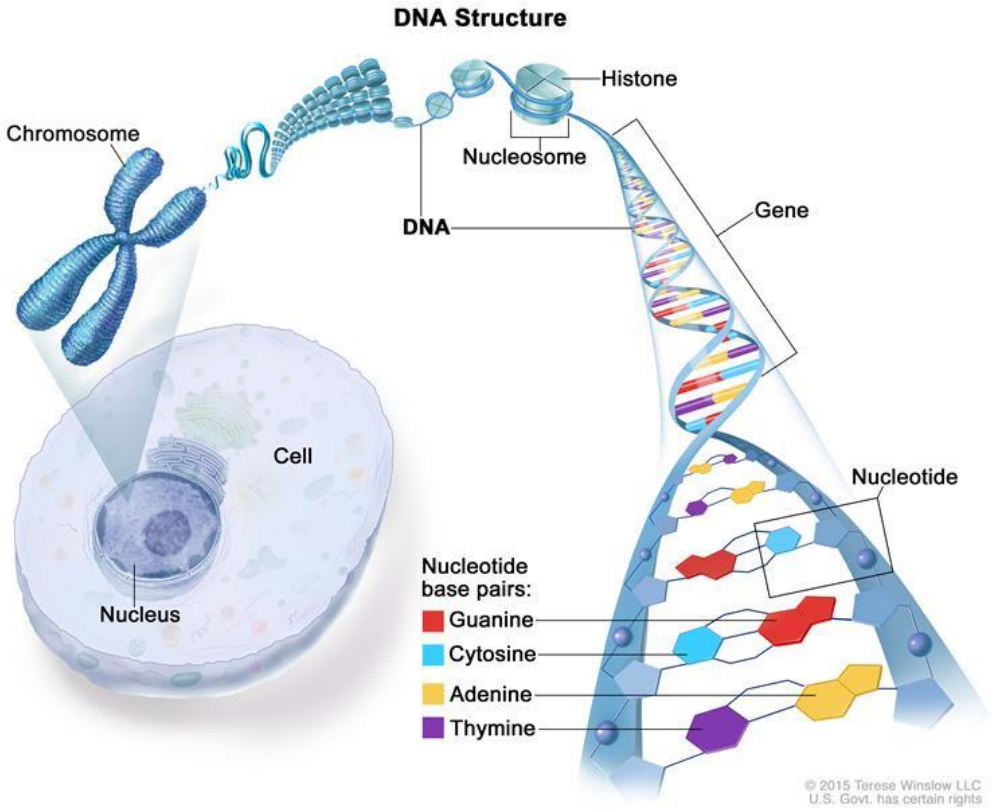

Figure 4.1: Cell, DNA, Gene and Nucleotide Base Pairs (Klass, 1983)

4.1. Is aging in our genes?

Identifying the genes associated with any trait is difficult. First, just locating the gene requires a detailed understanding of the trait, including knowledge of most, if not all, of the contributing factors and pathways related to that trait. Second, scientists must have clear ways of determining

whether the gene suspected to have a relationship with the trait has a direct, indirect, or even no effect on that trait.

Identifying longevity genes is even more complex than determining genes for height or hair color, for example. Scientists do not know all the factors and pathways that contribute to longevity, and measuring a gene's effect on long-lived animals, including humans, would literally take a lifetime! Instead, scientists have identified hundreds of genes that affect longevity in short-lived animal models, like worms and flies. Not all of these genes promote long life. Sometimes, mutating or eliminating a gene increases lifespan, suggesting that the normal function of the gene limits longevity. Findings in animal models point to places for scientists to look for the genes that may influence longevity in humans.

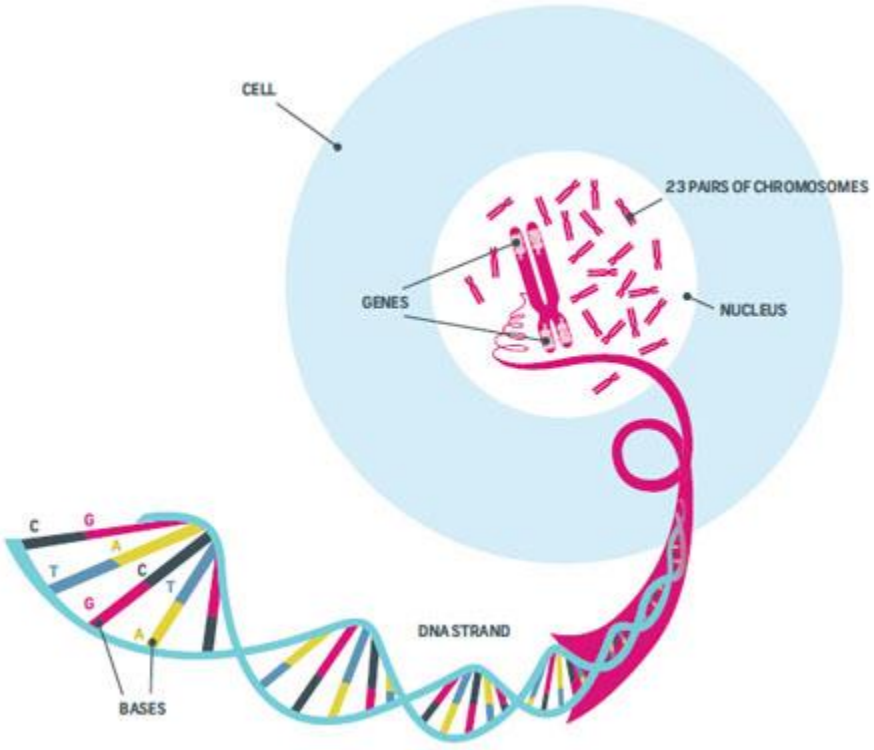

Figure 4.2: Genes and Nucleotide Base Pairs (Walker, 2009)

The human genetic blueprint, or genome, consists of approximately 25,000 genes made up of approximately 3 billion letters (base pairs) of DNA. Base pair sequences: guanine (G) pairs with cytosine (C); adenine (A) pairs with thymine (T).

4.2. Human genes responsible for aging and human gene mutation which causes Aging:

- Any gene that influences the development of a disease is called a disease-susceptibility gene.
- The impacts of disease-susceptibility genes on aging and average lifespan would be much stronger than the impacts of aging genes on maximum lifespan.

- Multiple genes are associated with the aging process.

- Progeria (Hutchinson–Gilford progeria syndrome) is a rare genetic disease with symptoms that resemble the acceleration of the regular aging process.

- Mutation in position 1824 of the **lamin A (LMNA) gene** has been identified as the cause of progeria

- Werner syndrome, also called adult progeria or progenoid syndrome, is another very rare genetic disease characterized by the appearance of premature aging.

- In humans, Werner syndrome is caused by a point mutation in the **WRN gene** on chromosome

- **The LMNA and WRN genes**, which are responsible for progeria and Wener syndrome respectively, cause pathological aging processes, but do not regulate normal aging processes.

- The frequency of genotypes unrelated to lifespan did not differ between younger people and older people in a cross-sectional study.

- However, the frequency of certain genotypes changes with aging.

- A genotype with a high frequency among older people could represent a "longevity genes" that serves to prolong lifespan or to protect against age-related diseases

- In contrast, a genotype with a lower than average frequency among older people could represent an "aging gene" or a "gene resulting in shorter life expectancy".

- A list of genes associated with longevity based on the findings of a cross-sectional study of age difference in genotype frequency.

  Most of these genes are related to a molecular pathway involved in nutrient metabolism, especially lipid or glucose metabolism, or in endocrine regulation (Klass 1983).

4.3. Genes associated with longevity

Gene	Longevity	Relevant biological action	Chromosomal loci
Klotho (KL gene)	+	Insulin sensitivity, modulation of IGF-I and vitamin D	13q12
Silent mating type information regulation 2 homolog 1 (SIRT1)	+	Regulates epigenetic gene silencing and suppresses recombination of rDNA, associated with insulin action/sensitivity	10q21.3
Catalase (CAT)	+	Antioxidant that protects cells from	11p13

		hydrogen peroxide	
Mammalian target of rapamycin (mTOR)	_	Modulates insulin, IGF, and mitogen function	1P36
IGF-I/insulin (FOXO)	_	Transcription factors that take part in cell growth and differentiation	12q23–23
GH	_	Stimulates growth, production of IGF-I	17 q22-q24
TSH β	+	Production of TSH	1p13
Thyrotropin receptor (TSHR)	+	Production of T4 and T3	
CETP	+	Facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins	16q21
APOC-3	+	Inhibits lipoprotein lipase and hepatic lipase	11q23.1-q23.2

Adiponectin (AdipoQ)	+	Modulates glucose and fatty acid metabolism	3q27

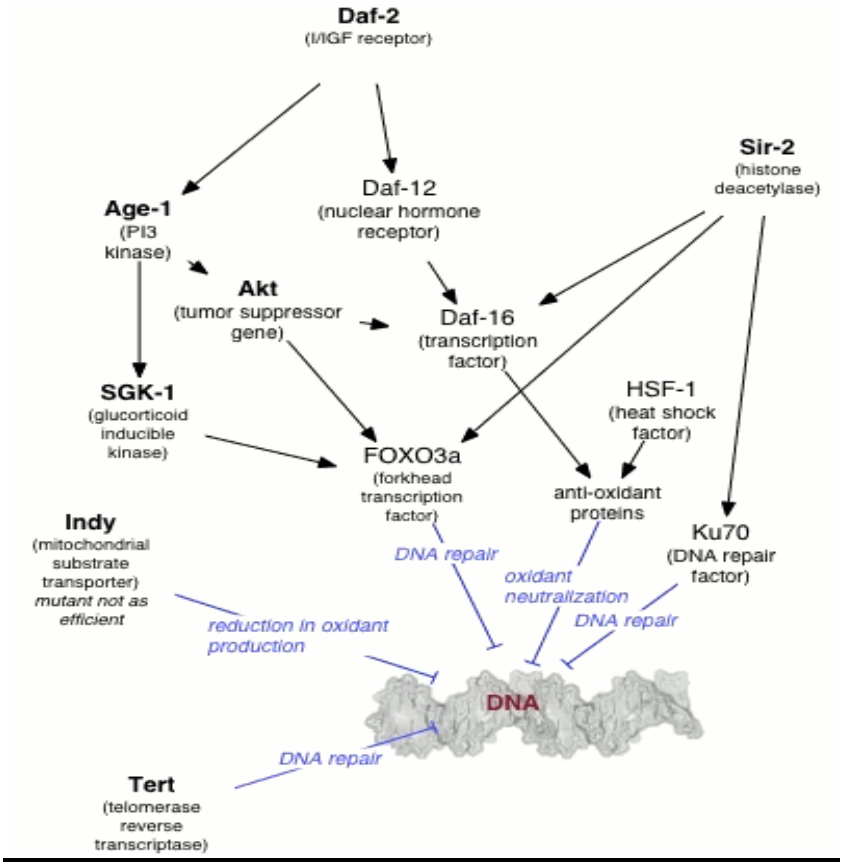

Figure 4.3: Gene mutation and DNA mutation Prolong life (Walker, 2009)

4.4. *C. elegans* mutation and aging:

Loss-of-function mutations in genes in the *C. elegans* insulin signaling pathway (such as **daf-*2* insulin receptor and age-*1* PI3 kinase**) extend life span.

These results indicate that insulin signaling plays an important role in specifying life span.

164 aging-regulated genes are available in *C. elegans.*
164 aging-regulated genes include two insulin-like genes and a ***sir-2* homolog** that increase at the end of life (Walker, 2009).

4.5. How can we find aging genes in humans?

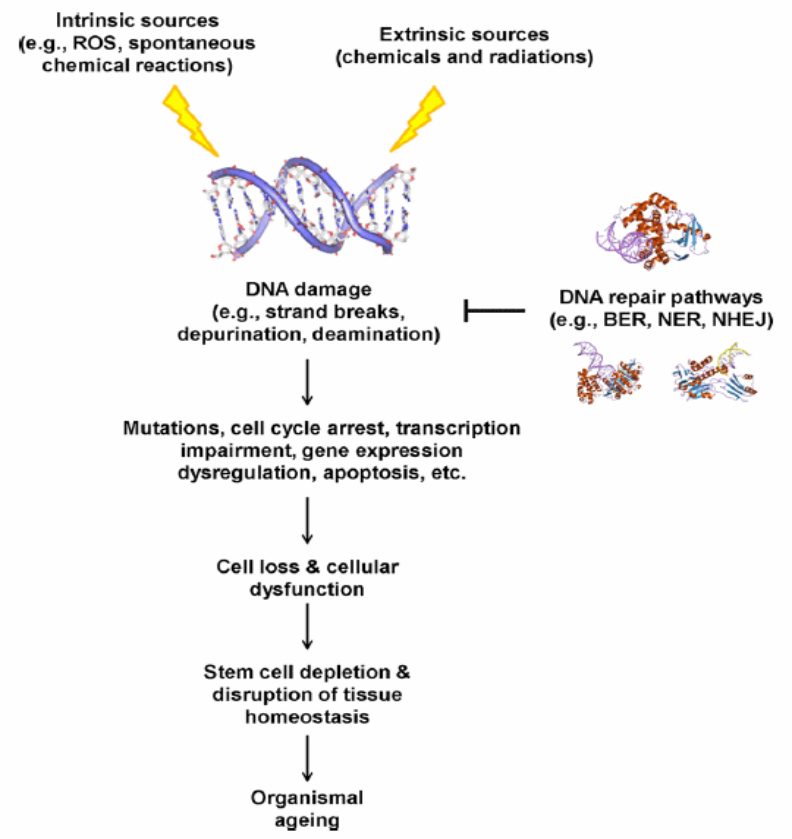

Figure 4.4: DNA Damage and Aging (Klass 1983)

The human genetic blueprint, or genome, consists of approximately 25,000 genes made up of approximately 3 billion letters (base pairs) of DNA. Small deviations in the base pairs naturally occur about once in every 1,000 letters of DNA code, generating small genetic variants. Scientists are finding that some of these variants (polymorphisms) are actually associated with particular traits or chance of developing a specific disease. People with a certain trait, for example, those living past age 100, may be more likely to have one variant of a gene, while people without the same trait may be more likely to have another variant. While it is very difficult to prove that a gene influences aging in humans, a relationship, or "association," may be

inferred based upon whether a genetic variant is found more frequently among successful agers, such as centenarians, compared with groups of people who have an average or short lifespan and health span. Several approaches are used to identify possible genes associated with longevity in humans. In the candidate gene approach, scientists look for genes in humans that serve similar functions in the body as genes already associated with aging in animal models, so-called "homologs" or "orthologs" to animal genes. For instance, after finding longevity genes involved in the insulin/IGF-1 pathway of animal models, researchers look for the comparable genes in the insulin/IGF-1 pathway of humans. Scientists then determine whether the genes are linked to longevity in humans by looking to see if a variant of the genes is prevalent among people who live healthy, long lives but not for people who have an average health span and lifespan. In one NIA-funded project, researchers studied 30 genes associated with the insulin/IGF-1 pathway in humans to see if any variants of those genes were more common in women over 92 years old compared to women who were less than 80 years old. Variants of certain genes—like the *FOXO3a* gene—predominated among long-lived individuals, suggesting a possible role with longer lifespan. This finding provides evidence that, like in animal models, the insulin/IGF-1 pathway has a role in human aging. These genes may be important to future development of therapies to support healthy aging.

Another approach, the genome-wide association study, or GWAS, is particularly productive in finding genes involved in diseases and conditions associated with aging. In this approach, scientists scan the entire genome looking for variants that occur more often among a group with a particular health issue or trait. In one GWAS study, NIH-funded researchers identified genes possibly associated with high and low blood fat levels, cholesterol, and, therefore, risk for coronary artery disease. The data analyzed were collected from Sardinians, a small genetically alike population living off the coast of Italy in the Mediterranean, and from two other international studies. The findings revealed more than 25 genetic variants in 18 genes connected to cholesterol and lipid levels. Seven of the genes were not previously connected to

cholesterol/lipid levels, suggesting that there are possibly other pathways associated with risk for coronary artery disease. Heart disease is a major health issue facing older people. Finding a way to eliminate or lower risk for heart disease could have important ramifications for reducing disability and death from this particular age-related condition.

Scientists are also currently using GWAS to find genes directly associated with aging and longevity. Because the GWAS approach does not require previous knowledge of the function of the gene or its potential relationship with longevity, it could possibly uncover genes involved in cellular processes and pathways that were not previously thought to play roles in aging. Since no single approach can precisely identify each and every gene involved in aging, scientists will use multiple methods, including a combination of the GWAS and candidate gene approaches to identify genes involved in aging.

As scientists continue to explore the genetics of aging, its complexity becomes increasingly evident. Further studies could illustrate the varying ways genes influence longevity. For example, some people who live to a very old age may have genes that better equip them to survive a disease; others may have genes that help them resist getting a disease in the first place. Some genes may accelerate the rate of aging, others may slow it down. Scientists investigating the genetics of aging do not foresee a "Eureka!" moment when one gene is discovered as the

principal factor affecting health and lifespan. It is more likely that we will identify several combinations of many genes that affect aging, each to a small degree.

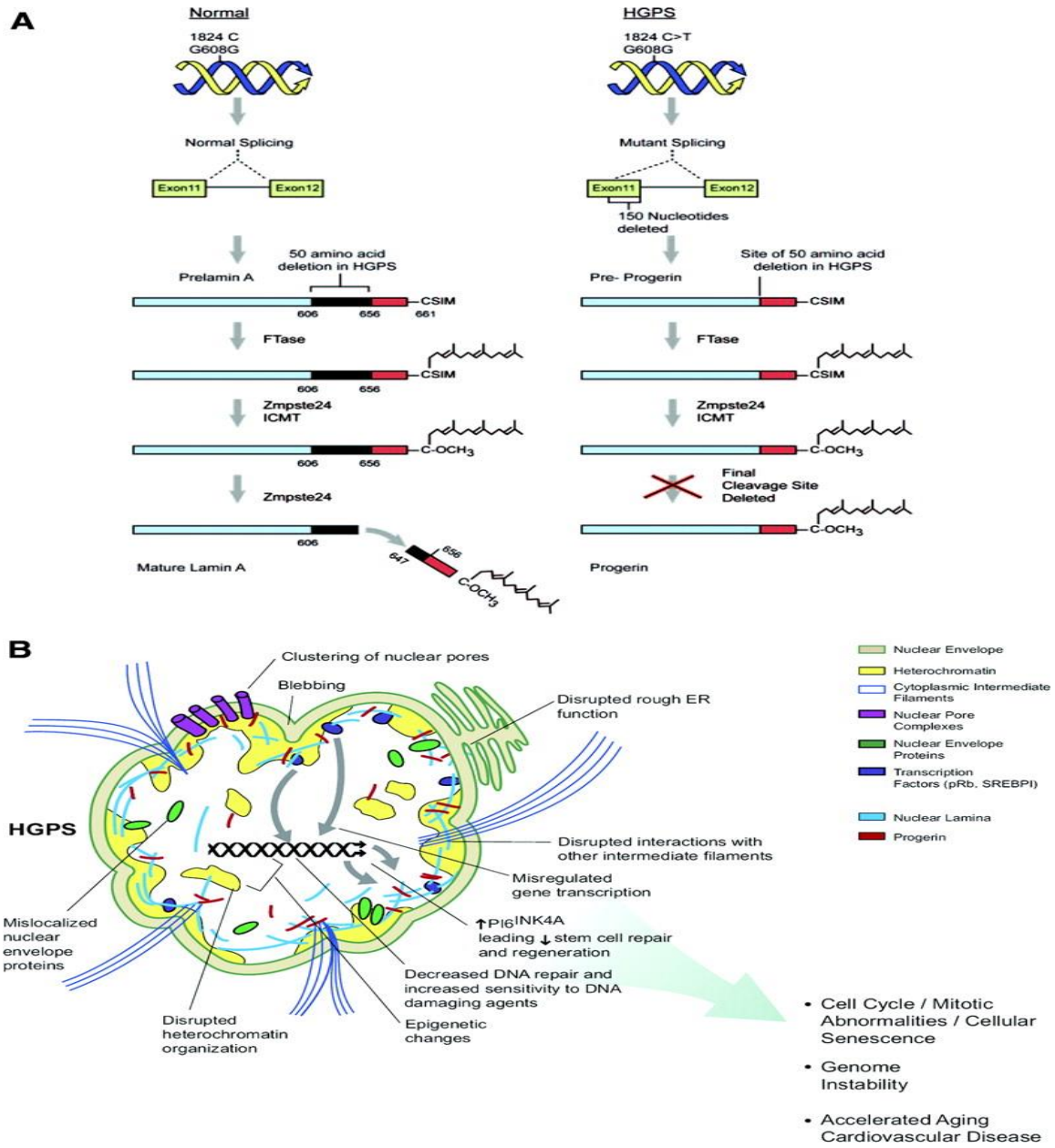

Figure 4.5: Genetic and nuclear defects in Hutchinson-Gilford progeria syndrome (HGPS).

(Walker, 2009)

A, HGPS is caused by a single base change of C to T at position 1824 of the *LMNA* gene. This mutation does not change the encoded amino acid (glycine) but results in the activation of a cryptic splice site 150 nucleotides upstream of the usual exon 11-to-12 splice junction. The lamin A precursor, prelamin A, undergoes a series of posttranslational modifications, including farnesylation at its C-terminal CAAX motif (CSIM in this case) by the enzyme farnesyltransferase (FTase), cleavage of the terminal 3 amino acids (SIM) by Zmpste24, and carboxymethylation by the enzyme isoprenylcysteine carboxymethyltransferase (ICMT). A second and final cleavage by Zmpste24 removes the terminal 15 amino acids and the farnesyl group, allowing mature lamin A to be inserted into the lamina. In contrast, because of its 50–amino acid internal deletion, progerin lacks the second cleavage site and thus remains permanently farnesylated. **B,** Potential mechanisms of disease: HGPS nucleus. It is believed that the permanently farnesylated state of progerin leads to its disruption of the nuclear lamina and multiple other nuclear defects, including blebbed nuclear morphology, altered interactions with other nuclear and cytosolic intermediate filaments, mislocalized nuclear envelope proteins, clustering of nuclear pore complexes, disorganized heterochromatin, epigenetic changes and dramatic dysregulation of gene transcription, increased sensitivity to DNA-damaging agents, and decreased DNA repair capacity. Other potential mechanisms include defective function of the endoplasmic reticulum and decreased stem cell repair and regeneration.

4.6. Pathways of Longevity genes

Most longevity genes identified thus far influence one of three pathways in a cell: insulin/IGF-1, sirtuins, or mTOR.

In the 1980s, scientists discovered the first gene shown to limit lifespan in roundworms, which they named *age-1*. Further investigation revealed that the effects of *age-1* are involved with the

insulin/IGF-1 pathway. When scientists “silenced” the *age-1* gene’s activity, the insulin/IGF-1 pathway’s activity also decreased and the worms lived longer. Since then, many other genes associated with the insulin/IGF-1 pathway have been found to affect the lifespan of fruit flies and

mice, strengthening the hypothesis that the insulin/IGF-1 pathway plays an important role in the aging process. More research is needed to determine if inhibiting this pathway could increase longevity in humans or create insulin-related health problems like diabetes. A recent report suggests that people with a mutation related to the insulin/IGF-1 pathway may have less risk of developing diabetes and cancer.

There is also a great deal of interest in the sirtuin pathway. Sirtuin genes are present in all species and regulate metabolism in the cell. They are crucial for cell activity and cell life. In the 1990s, scientists at the Massachusetts Institute of Technology found that inserting an extra copy of a sirtuin equivalent, called *Sir2*, increased the lifespan of yeast. Extension of lifespan has been replicated in other organisms, including flies and worms. However, studies in mice have yielded conflicting results.

The mTOR pathway—an abbreviation of "mammalian target of rapamycin"—plays a role in aging of yeast, worms, flies, and mice. This pathway controls the cell's rate of protein synthesis, which is important for proper cell function. Researchers have found that inhibiting the pathway in mice genetically or pharmacologically (using rapamycin) leads to increased longevity and improved health span.

4.7. The Future of Aging Research (Epigenetics)

An emerging area of research called "epigenetics" opens the door to a scientific blending of two worlds that for decades were thought of as totally separate—that is nature and nurture, or more specifically genetics and the environment. Epigenetics research looks at how your environment, over time, can affect how your genes work and influence your development, health, and aging.

At the center of this research is the epigenome—chemical modifications, or marks, on our DNA, or in proteins that interact with DNA, that tell it what to do, where to do it, and when to do it.

The marks that make up the epigenome are affected by your lifestyle and environment and may change, for example, based on what you eat and drink, if you smoke, what medicines you take, and what pollutants you encounter. Changes in the epigenome can cause changes in gene activity. Most epigenetic changes are likely harmless, but some could trigger or exacerbate a disease or condition. In some cases, scientists find that these epigenetic changes driven by the environment can be inherited by the offspring.

Identical, maternal twins are ideal for epigenetic research. At birth, twins have nearly the same genetic blueprint; however, over time, they may have fewer identical traits. Careful study of these changes may help scientists better understand environmental and lifestyle's influence on genes.

Epigenetics might also explain variations in lifespan among laboratory mice that are genetically identical and seemingly raised in the exact same environment. Scientists theorize that the difference in theirlifespans may result from a disparity in the amount of nurturing they received when very young. The mice with the shorter lifespan might have been less adept at feeding and, therefore, got less of their mother's milk, or their mother may have licked them less, or they may have slept farther away from the center of the litter. Receiving less nurturing may have influenced their epigenetics, marking the genes that control aging.

As epigenetic research moves forward, scientists hope to answer three key questions:

- How do changes in the epigenome translate into long-term differences in health and aging?
- Do single events influence the epigenome?
- If single events can change the epigenome, does the organism's age (or stage of development) at the time of the change matter?

4.8. Plasticity of aging: lifespan as a genetic program

In the mid-1990s, researchers began to seriously entertain the notion that a single gene could exert significant influence over organismal lifespan. Since then, numerous studies have substantiated the notion that individual genes can determine how long an organism lives, and opened up the possibility that a process as complex as aging could be manipulated at the molecular level. Although many more genes have been identified, the most well-studied gene families shown to influence longevity, either through anti-aging or pro-aging effects, are the insulin receptor signaling pathway, FoxO family of transcription factors, Sirtuins and mechanistic target of rapamycin (mTOR). A significant part of our understanding of these genes and their influence on aging come from genetic studies in model organisms such as yeast, worms, and flies, and have more recently begun to be validated in higher organisms such as mice suggesting the conservation of these pathways for aging across phylogeny (Lopez-Otin *et al*. 2013).

Many of the genes shown to play a role in determining lifespan are involved in cellular processes that contribute to the aging of tissues, including oxidative stress resistance, glucose metabolism, and energy homeostasis, whose regulation ultimately lead to the survival or death of an organism. The first molecular pathway regulating lifespan to be identified was the Insulin/IGF-1/FoxO pathway, an important signaling system that regulates energy homeostasis throughout the body. Whole organism mutations in a number of the insulin signaling pathway genes leading to reduced insulin signaling extend lifespan in worms, flies, and mice. In contrast, factors that act downstream of Insulin/IGF-1 signaling, such as the FoxO transcription factors, when up-regulated, act as pro-longevity signals that extend lifespan and also delay the onset and progression of age-related phenotypes. Another important set of molecular mediators of longevity are the Sirtuins, a collection of histone deacetylase genes involved in oxidative metabolism and stress resistance. Increased expression of several of the Sirtuins, such as Sirt1 and Sirt6, extend lifespan in model organisms from yeast to mice. Lastly, another genetic pathway shown to regulate lifespan is the mTOR pathway. mTOR was originally discovered as a target of the immunosuppressant rapamycin and plays an important role in integrating multiple

signals involved in cellular energy. More recently, reduced activity of the mTOR pathway, often times via rapamycin administration, was also shown to promote longevity in model organisms Collectively, the identification of such individual genes capable of modulating organismal lifespan first challenged long-held dogma of aging as immutable, and introduced the notion that systemic aging could indeed be itself a plastic event (Vellai *et al*. 2003; Jia *et al*. 2004; Kapahi *et al*. 2004; Kaeberlein *et al*. 2005; Harrison *et al*. 2009).

5. Some anti-aging drugs and their mechanism of action

Most of them are Caloric restriction mimetic drugs

- **Metformin:** Metformin is already clinically approved to treat diabetes, and has been used for this indication for the past 40 years. It enhances the sensitivity of insulin receptors on the surface of muscle and fat cells and activates genes that reduce the production of glucose by the liver, thus reducing the risk of non-enzymatic glycation and other age-related damage. Metformin was reported to extend the lifespan of short-lived or genetically cancer-prone mouse strains.

- **Oxaloacetate:** Oxaloacetate is a metabolic intermediate of the citric acid cycle. In the short-lived roundworm *Caenorhabditis elegans*, supplementation with oxoacetate increases the ratio of oxidized to reduced nicotinamide adenine dinucleotide (NAD+NADH) to activate AMPK and FOXO signaling pathways similar to what occurs in calorie restriction. The increase in the NAD+/NADH ratio is due to the reaction of oxaloacetate to malate in the cytoplasm via the enzyme malate dehydrogenase. In mitochondria that have been isolated out of cells and tested in oxaloacetate-enriched medium, this increase can be quite dramatic decrease in the NAD+/NADH ratio has been proposed carbohydrate metabolism-controlled cellular senescence mechanism.

  Because of its parallel effects on these pathways, oxaloacetate was proposed as a calorie restriction mimetic.

- **Rimonabant**: Rimonabant is an anti-obesity drug approved for use in the European Union but rejected approval by the FDA. This is an endocannabinoid-1 receptor blocker. Endocannabinoids are cannabis-like chemicals that stimulate appetite and also regulate energy balance. Overstimulation of the endoannabinoid receptor in the hypothalamus promotes appetite and stimulates lipogenesis. It also blocks the beneficial actions of adiponectin. Rimonabant inhibits these and so it reduces appetite, balances energy, and increases adiponectin, which reduces intra-abdominal fat. It improves lipid profile, glucose tolerance, and waist measurement.

- **2-deoxy-D-glucose:** 2-deoxy-D-glucose was the first agent pursued as a possible CRM. This compound inhibits glycolysis, and can mimic some of the physiological effects of CR, in particular increased insulin sensitivity, reduced glucose levels, reduced body temperature, and other biochemical changes.

- **Rapamycin:** Rapamycin inhibits the mechanistic Target of Rapamycin (mTOR) pathway, might be a Caloric restriction mimetic. Based on the responsiveness of mTORC1 activity to nutrient availability; the fact that mTOR activity is inhibited by Caloric restriction; the fact that genetically inhibiting mTOR signaling extends maximum lifespan in invertebrate animals, and pharmacologically inhibiting mTOR with rapamycin extends maximum lifespan in both invertebrates and mice.

- **Growth hormone (GH):** Low-dose growth hormone treatment for adults with growth hormone deficiency changes the body composition by increasing muscle mass, decreasing fat mass, increasing bone density and muscle strength, improves

cardiovascular parameters (i.e. decrease of LDL cholesterol), and affects the quality of life without significant side effects, the evidence for use of growth hormone as an anti-aging therapy is mixed and based on animal studies.

- **Antioxidant supplements:** Antioxidant supplements such as vitamin C, vitamin E, lipoic acid, carnosine, and N-acetylcysteine, might extend human life. However, combined evidence from several clinical trials suggests that β-carotene supplements and high doses of vitamin E increase mortality rates.

6. Discussion

Aging is a syndrome of changes that are deleterious, progressive, universal and thus far irreversible. Aging damage occurs to molecules (DNA, proteins, lipids), to cells and to organs.Diseases of old age (diseases which increase in frequency with age, such as arthritis, osteoporosis, heart disease, cancer, Alzheimer's Disease, etc.) are often distinguished from aging. But even if the aging process is distinct from the diseases of aging, it is nonetheless true that the damage associated with the aging process increases the probability that diseases of old age will occur.In ancient Rome, the average lifespan was 22 years, but by the mid 1800s the typical North American lived to be 40. Today, people in the most developed countries have an average lifespan of about 80.Maximum lifespan for humans, however, has remained about 115−120 all through known history. The longest documented human lifespan has been for French woman Jean Calment who lived 122.3 years.

In humans and other animals, cellular senescence has been attributed to the shortening of telomeres at each cell division; when telomeres become too short, the cells die. Telomeres have experimentally been shown to shorten with each successive cell division.

FOXO3A is known to have a positive effect on the life expectancy of humans, and is found much more often in people living to 100 and beyond. FOXO3A acts on the sirtuin family of genes which have also been shown to have a significant effect on lifespan, in yeast and in nematodes.

Typically, this involves caloric intake of 60–70% of what an *ad libitum* animal would consume, while still maintaining proper nutrient intake. In rodents, this has been shown to increase lifespan by up to 50%.

The idea that ageing is regulated by reproductive hormones that act in an antagonistic pleiotropic manner via cell cycle signaling, promoting growth and development early in life to achieve reproduction, but later in life, in a futile attempt to maintain reproduction, become dysregulated and drive senescence (dyosis).

It has been established that dogs lose approximately 3.3% of the DNA in their heart muscle cells annually while humans lose approximately 0.6% of their heart muscle DNA each year. These numbers are close to the ratio of the maximum longevities of the two species (120 years vs. 20 years, a 6/1 ratio).

Lipofuscin is formed by a complex reaction that binds fat in the cells to proteins. This waste accumulates in the cells as small granules and increases in size as a person ages.

Metformin is already clinically approved to treat diabetes, and has been used for this indication for the past 40 years. It enhances the sensitivity of insulin receptors on the surface of muscle and fat cells and activates genes that reduce the production of glucose by the liver, thus reducing the risk of non-enzymatic glycation and other age-related damage.

Rapamycin inhibits the mechanistic Target of Rapamycin (mTOR) pathway, might be a Caloric restriction mimetic.

Low-dose growth hormone treatment for adults with growth hormone deficiency changes the body composition by increasing muscle mass, decreasing fat mass, increasing bone density and muscle strength, improves cardiovascular parameters (i.e. decrease of LDL cholesterol), and affects the quality of life without significant side effects, the evidence for use of growth hormone as an anti-aging therapy is mixed and based on animal studies.Another proposed life extension technology would combine existing and predicted future biochemical and genetic techniques. SENS proposes that rejuvenation may be obtained by removing aging damage via the use of stem cells and tissue engineering, removal of telomere-lengthening machinery, allotopic expression of mitochondrial proteins, targeted ablation of cells, immunotherapeutic clearance, and novel lysosomal hydrolases.

While many biogerontologists find these ideas "worthy of discussion" and SENS conferences feature important research in the field, some contend that the alleged benefits are too speculative given the current state of technology, referring to it as "fantasy rather than science".

7. Conclusion

Since the beginning of human history, we have been trying to find ways to stay young. Yet ageing is inevitable, and there seems no way to reverse the process. Each of us is born with an internal biological clock, figuratively speaking, that determines our life span. If we knew how the clock worked, we could understand more about how we age, and eventually, we might find the secret to the mythical fountain of youth.

The human body is made up of cells. Each cell is like a Lego block, and builds various organs for different functions. Cells divide to produce new cells for the growth and repair of body tissues. But cell division is not limitless: on average, human cells can divide only about 50 to 70 times. Afterwards, cells will enter a senescence phase when they no longer divide. At this point, the cells may die, or stay in the body as malfunctioning cells. This causes our bodies to deteriorate and age.

It may seem that, because we cannot control the shortening of our telomeres, we must all grow old eventually. But although it's true that we cannot escape from ageing, we can slow down the process. A small pilot study by the University of California, San Francisco, showed for the first time that lifestyle changes lead to longer telomeres. Individuals on a vegetarian diet, who took moderate exercise and reduced stress by meeting regularly with a social support group, were found to have longer telomeres. This shows that we can actually do something to lengthen our telomeres and slow down ageing.

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References

Ackermann, M., Stearns, S.C., Jenal, U. (2003) Senescence in a bacterium with asymmetric division. Science, 300,1920.

Aitlhadj, L., Sturzenbaum, SR., (2014) Caenorhabditis elegans in regenerative medicine: a simple model for a complex discipline.

Alié, A., Leclere, L., Jager, M., Dayraud, C., Chang, P., Le, Guyader, H., Queinnec, E., Manuel, M., (2011) Somatic stem cells express Piwi and Vasa genes in an adult ctenophore: ancient association of "germline genes" with stemness. Developmental biology, 350, 183–197.

Arumugam, TV., Gleichmann, M., Tang, SC., Mattson, MP., (2006) Hormesis/preconditioning mechanisms, the nervous system and aging. Ageing research reviews, 5, 165–178.

Ashworth, JH., Annandale, N., (1904) Observations on some aged specimens of Sagartia troglodytes and on the duration of life in Coelenterates. Proc Roy Soc Edin, 25, 295–308.

Baguñá, J., Carranza, S., Pala, M., Ribera, C., Giribet, G., A, AM, Ribas, M., Riutort, M., (1999) From morphology and karyology to molecules.New methods for taxonomical identification of asexual populations of freshwater planarians. A tribute to Professor Mario Benazzi. Ital J Zool, 66, 207–214.

Baguñá, J., Romero, R., (1981) Quantitative analysis of cell types during growth, degrowth and regeneration in the planarians Dugesia mediterranea and Dugesia tigrina. Hydrobiologia, 84, 181–194.

Baker, DJ., Dawlaty, MM., Wijshake, T., Jeganathan, KB., Malureanu, L., van, Ree, JH., Crespo-Diaz, R., Reyes, S., Seaburg, L., Shapiro, V., Behfar, A., Terzic, A., van de, Sluis, B., van Deursen, JM.,

(2013) Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan. Nature cell biology, 15, 96–102.

Balázs, A., Burg, M., (1962) Span of life and senescence of Dugesia lugubris. Gerentologia., 6, 227–236.

Balch, WE., Morimoto, RI., Dillin, A., Kelly, JW., (2008) Adapting proteostasis for disease intervention. Science, 319, 916–919.

Bardele, CF., (1970) Budding and metamorphosis in Acineta tuberosa. An electron microscopic study on morphogenesis in Suctoria. J Protozool, 17, 51–70.

Bely, AE., Sikes, JM., (2010) Acoel and platyhelminth models for stem-cell research. Journal of biology, 9, 14.

Bisbee, JW., Francis, JC., Harrison, FW., (1989) Cytological examination of freshwater sponge regeneration from reduction bodies. Trans Amer Micros Soc, 108, 299–303.

Blackstone, NW., (2003) Redox signaling in the growth and development of colonial hydroids. The Journal of experimental biology, 206, 651–658.

Boehm, AM., Khalturin, K., Anton-Erxleben, F., Hemmrich, G., Klostermeier, UC., Lopez-Quintero, JA., Oberg, HH., Puchert, M., Rosenstiel, P., Wittlieb, J., Bosch, TC., (2012) FoxO is a critical regulator of stem cell maintenance in immortal Hydra. Proceedings of the National Academy of Sciences of the United States of America, 109, 19697–19702.

Boehm, AM., Rosenstiel, P., Bosch, TC., (2013) Stem cells and aging from a quasi-immortal point of view. BioEssays : news and reviews in molecular, cellular and developmental biology, 35, 994–1003.

Bosch, TC., (2007) Why polyps regenerate and we don’t: towards a cellular and molecular framework for Hydra regeneration. Developmental biology, 303, 421–433.

Bosch, TCG., (2009) Hydra and the evolution of stem cells. BioEssays : news and reviews in molecular, cellular and developmental biology, 31, 478–486.

Brehm, K., (2010) Echinococcus multilocularis as an experimental model in stem cell research and molecular host-parasite interaction. Parasitology, 137, 537–555.

Brodsky, JL., (2014) The threads that tie protein-folding diseases. Disease models & mechanisms, 7, 3–4.

Butler, PG., Wanamaker, AD., Jr., Scourse, JD., Richardson, CA., Reynolds, DJ., (2013) Variability of marine climate on the North Icelandic Shelf in a 1357-year proxy archive based on growth increments in the bivalve Arctica islandica. Palaeogeography, Paleoclimatology, Pelaeoecology, 373, 141–151.

Brown, BM., Peiffer, JJ., Martins, RN., (2013) Multiple effects of physical activity on molecular and cognitive signs of brain aging: can exercise slow neurodegeneration and delay Alzheimer's disease? Mol Psychiatry, 18(8), 864–874.

Barnes, DE., Yaffe, K., Satariano, WA., Tager, IB., (2003) A longitudinal study of cardiorespiratory fitness and cognitive function in healthy older adults. J Am Geriatr Soc, 51(4), 459–465.

Boettcher, J., Astrom, V., Pahlsson, D., Schenstrom, O., Andersson, G., Carlbring, P., (2014) Internet-based mindfulness treatment for anxiety disorders: a randomized controlled trial. Behav Ther, 45(2), 241–253.

Cabanillas, R., Cadinanos, J., Villameytide, JA., Perez, M., Longo, J., Richard, JM., Alvarez, R., Duran, NS., Illan, R., Gonzalez, DJ., Lopez-Otin, C., (2011) Nestor-Guillermo progeria syndrome: a novel premature aging condition with early onset and chronic development caused by BANF1 mutations. American journal of medical genetics, 155A, 2617–2625.

Carr, M., Leadbeater, BS., Hassan, R., Nelson, M., Baldauf, SL., (2008) Molecular phylogeny of choanoflagellates, the sister group to Metazoa. Proceedings of the National Academy of Sciences of the United States of America, 105, 16641–16646.

Carr, M., Nelson, M., Leadbeater, BS., Baldauf, SL., (2008) Three families of LTR retrotransposons are present in the genome of the choanoflagellate Monosiga brevicollis. Protist, 159, 579–590.

Casselman, A., (2007) Strange but true: the largest organism on earth is a fungus. Sci Amer.

Christopherson, JB., (1924) Longevity of parasitic worms; The term of living existence of Schistosoma haematobium in the human body. The Lancet, 742–743.

Clancy, DJ., Gems, D., Harshman, LG., Oldham, S., Stocker, H., Hafen, E., Leevers, SJ., Partridge, L., (2001) Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. Science, 292, 104–106.

Clay, L., Barral, Y., (2013) New approaches to an age-old problem. Current opinion in biotechnology, 24, 784–789.

Coelho, M., Dereli, A., Haese, A., Kuhn, S., Malinovska, L., DeSantis, ME., Shorter, J., Alberti, S., Gross, T., Tolic-Norrelykke, IM., (2013) Fission yeast does not age under favorable conditions, but does so after stress. Current biology : CB, 23, 1844–1852.

Collins, JJ., III, Wang, B., Lambrus, BG., Tharp, ME., Iyer, H., Newmark, PA., (2013) Adult somatic stem cells in the human parasite Schistosoma mansoni. Nature, 494, 476–479.

Conboy, MJ., Karasov, AO., Rando, TA., (2007) High incidence of non-random template strand segregation and asymmetric fate determination in dividing stem cells and their progeny. PLoS biology, 5, 102.

Crucke, J., (2010) Characterization of the Macrostomum lignano ageing phenotype, Faculty of Sciences -Nematology Section. Ghent, Belgium: Universiteit Gent, 6, 65.

Chang, CK., Hayes, RD., Perera, G., Broadbent, MT., Fernandes, AC., Lee, WE., Hotopf, M., Stewart, R., (2011) Life expectancy at birth for people with serious mental illness and other major disorders from a secondary mental health care case register in London. PloS one, 6(5), 19590.

Chodzko-Zajko, WJ., Proctor, DN., Fiatarone Singh, MA., Minson, CT., Nigg, CR., Salem, GJ., Skinner, JS., (2009) American College of Sports Medicine position stand. Exercise and physical activity for older adults. Med Sci Sports Exerce, 41(7), 1510–1530.

Cabral, P., Meyer, HB., Ames, D., (2011) Effectiveness of yoga therapy as a complementary treatment for major psychiatric disorders: a meta-analysis. Prim Care Companion CNS Disord, 13(4)

Dang, J., Zhang, J., Guo, Z., Lu, W., Cai, J., Shi, Z., Zhang, C., (2014) A Pilot Study of iPad-Assisted Cognitive Training for Schizophrenia. Arch Psychiatr Nurs, 28(3), 197–199.

Dayel, MJ., Alegado, RA., Fairclough, SR., Levin, TC., Nichols, SA., McDonald, K., King, N., (2011) Cell differentiation and morphogenesis in the colony-forming choanoflagellate Salpingoeca rosetta. Developmental biology, 357, 73–82.

De Mulder, K., Kuales, G., Pfister, D., Willems, M., Egger, B., Salvenmoser, W., Thaler, M., Gorny, A-K., Hrouda, M., Borgonie, G., Ladurner, P., (2009) Characterization of the stem cell system of the acoel Isodiametra pulchra. BMC Dev Biol, 9, 1–17.

De Mulder, K., Pfister, D., Kuales, G., Egger, B., Salvenmoser, W., Willems, M., Steger, J., Fauster, K., Micura, R., Borgonie, G., Ladurner, P., (2009) Stem cells are differentially regulated during development, regeneration and homeostasis in flatworms. Developmental biology, 334, 198–212.

Depp, CA., Jeste, DV., (2006) Definitions and predictors of successful aging: a comprehensive review of larger quantitative studies. The American Journal of Geriatric Psychiatry, 14(1), 6–20.

Depp, C., Vahia, IV., Jeste, D., (2010) Successful aging: focus on cognitive and emotional health. Annual Review of Clinical Psychology, 6, 527–550.

Depp, CA., Strassnig, M., Mausbach, BT., Bowie, CR., Wolyniec, P., Thornquist, MH., Luke, JR., McGrath, JA., Pulver, AE., Patterson, TL., (2001) Association of obesity and treated hypertension and diabetes with cognitive ability in bipolar disorder and schizophrenia. Bipolar disorders.

El-Matbouli, M., Fischer-Scherl, T., Hoffmann, RW., (1992) Present knowledge on the life cycle, taxonomy, pathology, and therapy of some Myxosporea spp. important to freshwater fish. Ann Rev Fish Diseases, 367–402.

Eriksson, M., Brown, WT., Gordon, LB., Glynn, MW., Singer, J., Scott, L., Erdos, MR., Robbins, CM., Moses, TY., Berglund, P., Dutra, A., Pak, E., Durkin, S., Csoka, AB., Boehnke, M., Glover, TW., Collins, FS., (2003) Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature, 423, 293–298.

Evans, NM., Holder, MT., Barbeitos, MS., Okamura, B., Cartwright, P., The phylogenetic position of Myxozoa: exploring conflicting signals in phylogenomic and ribosomal data sets. Molecular biology and evolution. 2010;27:2733–2746.

Extavour, CG., Akam, M., (2003) Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. Development, 130, 5869–5884.

Erickson, KI., Voss, MW., Prakash, RS., Basak, C., Szabo, A., Chaddock, L., Kim, JS., Heo, S., Alves, H., White, SM., Wojcicki, TR., Mailey, E., Vieira, VJ., Martin, SA., Pence, BD., Woods, JA., McAuley, E., Kramer, AF., (2011) Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A, 108(7), 3017–3022.

Finch, CE., (2009) Update on slow aging and negligible senescence - a mini-review. Gerontology, 55, 307–313.

Fetzner, MG., Asmundson, GJ., (2014) Aerobic Exercise Reduces Symptoms of Posttraumatic Stress Disorder: A Randomized Controlled Trial. Cogn Behav Ther, 9, 1–13.

Flachsbart, F., Caliebe, A., Kleindorp, R., Blanche, H., von Eller-Eberstein, H., Nikolaus, S., Schreiber, S., Nebel, A., (2009) Association of FOXO3A variation with human longevity confirmed in German centenarians. Proceedings of the National Academy of Sciences of the United States of America, 106, 2700–2705.

Freund, A., Laberge, RM., Demaria, M., Campisi, J., (2012) Lamin B1 loss is a senescence-associated biomarker. Molecular biology of the cell, 23, 2066–2075.

Funayama, N., (2013) The stem cell system in demosponges: suggested involvement of two types of cells: archeocytes (active stem cells) and choanocytes (food-entrapping flagellated cells) Development genes and evolution, 223, 23–38.

Funayama, N., Nakatsukasa, M., Mohri, K., Masuda, Y., Agata, K., (2010) Piwi expression in archeocytes and choanocytes in demosponges: insights into the stem cell system in demosponges. Evolution & development, 12, 275–287.

Gorczynski, P., Faulkner, G., (2010) Exercise therapy for schizophrenia. Cochrane Database Syst Rev, 12(5)

Gershon, H., Gershon, D., (2000) The budding yeast, Saccharomyces cerevisiae, as a model for aging research: a critical review. Mechanisms of ageing and development, 120, 1–22.

Gierer, A., Berking, S., Bode, H., David, CN., Flick, K., Hansmann, G., Schaller, H., Trenkner, E., (1972) Regeneration of hydra from reaggregated cells. Nature: New biology, 239, 98–101.

Gold, DA., Jacobs, DK., (2013) Stem cell dynamics in Cnidaria: are there unifying principles? Development genes and evolution, 223, 53–66.

Goldsmith, ED., (1942) Sexuality of Dugesia tigrina (syn. Planaria maculata) Nature, 150, 351.

Goodey, T., (1915) Note on the remarkable retention of vitality by protozoa from old stored soils. Ann Appl Biol, 1, 395–399.

Geda, YE., Roberts, RO., Knopman, DS., Christianson, TJ., Pankratz, VS., Ivnik, RJ., Boeve, BF., Tangalos, EG., Petersen, RC., Rocca, WA., (2010) Physical exercise, aging, and mild cognitive impairment: a population-based study. Arch Neurol, 57(1), 80–86.

Hughes, TF., Chang, CC., Vander Bilt, J., Ganguli, M., (2010) Engagement in reading and hobbies and risk of incident dementia: the MoVIES project. Am J Alzheimers Dis Other Demen, 25(5), 432–438.

Halpern, J., Cohen, M., Kennedy, G., Reece, J., Cahan, C., Baharav, A., (2014) Yoga for improving sleep quality and quality of life for older adults. Altern Ther Health Med, 20(3), 37–46.

Imayama, I., Ulrich, CM., Alfano, CM., Wang, C., Xiao, L., Wener, MH., Campbell, KL., Duggan, C., Foster-Schubert, KE., Kong, A., (2012) Effects of a caloric restriction weight loss

diet and exercise on inflammatory biomarkers in overweight/obese postmenopausal women: a randomized controlled trial. Cancer research, 72(9), 2314–2326.

Josefsson, T., Lindwall, M., Archer, T., (2014) Physical exercise intervention in depressive disorders: meta-analysis and systematic review. Scand J Med Sci Sports, 24(2), 259–272.

Johnson, DP., Penn, DL., Fredrickson, BL., Kring, AM., Meyer, PS., Catalino, LI., Brantley, M., (2011) A pilot study of loving-kindness meditation for the negative symptoms of schizophrenia. Schizophr , 129(2-3), 137–140.

Kirkpatrick, B., Messias, E., Harvey, PD., Fernandez-Egea, E., Bowie, CR., (2008) Is schizophrenia a syndrome of accelerated aging? Schizophrenia bulletin, 34(6), 1024–1032.

Lautenschlager, NT., Cox, KL., Flicker, L., Foster, JK., van Bockxmeer, FM., Xiao, J., Greenop, KR., Almeida, OP., (2008) Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. JAMA, 300(9), 1027–1037.

Lee, WK., (2013) Effectiveness of computerized cognitive rehabilitation training on symptomatological, neuropsychological and work function in patients with schizophrenia. Asia Pac Psychiatry, 5(2), 90–100.

Martin, P., Kelly, N., Kahana, B., Kahana, E., Willcox, BJ., Willcox, DC., Poon, LW., (2014) Defining Successful Aging: A Tangible or Elusive Concept? The Gerontologist.

Manji, H., Kato, T., Di Prospero, NA., Ness, S., Beal, MF., Krams, M., Chen, G., (2012) Impaired mitochondrial function in psychiatric disorders. Nature Reviews Neuroscience, 13(5), 293–307.

Moore, RC., Moore, DJ., Thompson, W., Vahia, IV., Grant, I., Jeste, DV., (2013) A case-controlled study of successful aging in older adults with HIV. The Journal of clinical psychiatry, 74(5), 417.

Moliver, N., Mika, E., Chartrand, M., Haussmann, R., Khalsa, S., (2013) Yoga experience as a predictor of psychological wellness in women over 45 years. Int J Yoga, 6(1),11–19.

Marciniak, R., Sheardova, K., Cermakova, P., Hudecek, D., Sumec, R., Hort, J., (2014) Effect of meditation on cognitive functions in context of aging and neurodegenerative diseases. Front Behav Neurosci, 8(17)

Nyer, M., Doorley, J., Durham, K., Yeung, AS., Freeman, MP., Mischoulon, D., (2013) What is the role of alternative treatments in late-life depression? Psychiatr Clin North Am, 36(4), 577–596.

Prakash, R., Rastogi, P., Dubey, I., Abhishek, P., Chaudhury, S., Small, BJ., (2012) Long-term concentrative meditation and cognitive performance among older adults. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn, 19(4), 479–494.

Preiss, M., Shatil, E., Cermakova, R., Cimermanova, D., Ram, I., (2013) Personalized cognitive training in unipolar and bipolar disorder: a study of cognitive functioning. Front Hum Neurosci, 7(108)

Piper, MD., Partridge, L., Raubenheimer, D., Simpson, SJ., (2011) Dietary restriction and aging: a unifying perspective. Cell metabolism, 14(2), 154–160.

Pillai, JA., Hall, CB., Dickson, DW., Buschke, H., Lipton, RB., Verghese, J., (2011) Association of crossword puzzle participation with memory decline in persons who develop dementia. J Int Neuropsychol Soc, 17(6) , 1006–1013.

Rosenbaum, S., Tiedemann, A., Sherrington, C., Curtis, J., Ward, PB., (2014) Physical activity interventions for people with mental illness: a systematic review and meta-analysis. J Clin Psychiatry, 31, 31.

Reijnders, J., van Heugten, C, van Boxtel, M., (2013) Cognitive interventions in healthy older adults and people with mild cognitive impairment: a systematic review. Ageing Res Rev, 12(1), 263–275.

Séaacute;nchez-Villegas, A., Delgado-Rodréiacute;guez, M., Alonso, A., Schlatter, J., Lahortiga, F., Majem, LS., Martínez-González, MÁ., (2009) Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort. Archives of General Psychiatry, 66(10), 1090–1098.

Scarmeas, N., Stern, Y., Mayeux, R., Manly, JJ., Schupf, N., Luchsinger, JA., (2009) Mediterranean diet and mild cognitive impairment. Archives of neurology, 66(2),216–225.

Vellas, B., Coley, N., Ousset, PJ., Berrut, G., Dartigues, JF., Dubois, B., Grandjean, H., Pasquier, F., Piette, F., Robert, P., Touchon, J., Garnier, P., Mathiex-Fortunet, H., Andrieu, S., (2012) Long-term use of standardised ginkgo biloba extract for the prevention of Alzheimer's disease (GuidAge): a randomised placebo-controlled trial. The Lancet Neurology, 11(10), 851–859.

Verhoeven, J., Révész, D., Epel, E., Lin, J., Wolkowitz, O., Penninx, B., (2013) Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. Molecular psychiatry.

Wegner, M., Helmich, I., Machado, S., Nardi, AE., Arias-Carrion, O., Budde, H., (2014) Effects of Exercise on Anxiety and Depression Disorders: Review of Meta-Analyses and Neurobiological Mechanisms. CNS Neurol Disord Drug Targets, 11, 11.

Zhang, D., Cheng, L., Craig, DW., Redman, M., Liu, C., (2010) Cerebellar telomere length and psychiatric disorders. Behavior genetics, 40(2), 250–254.