Gerontology Information

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Introduction

Aging and death do seem to be what Nature has planned for us. But what if we have other plans?

- Bernard Strehler

This section consists of a series of scientific essays on the biology of aging, also called biogerontology. They provide an overview of the science, methods, challenges and discoveries from research on aging and offer a summary of our current understanding of human aging. Although essays for the non-specialist are available elsewhere on **senescence.info**, I tried to make this section generally accessible to anyone with some basic background in biology, including undergraduates. Some essays are more detailed than others, however, and there is the occasional discussion that can be quite technical. A glossary is available to help readers. Since these essays review the most important aspects of the biology of aging, they could also be useful for researchers. (I use them myself as a reference and recommend them to my students.) These essays were also the basis of a review of mine that serves as an introduction to the biology of aging and may be useful to readers seeking a briefer overview of gerontology (de Magalhaes, 2011). A few individual essays have also served as basis for my own publications and obviously inform and are informed extensively by my papers.

I start this section with some definitions of basic terms and concepts and an overview of what is human aging, which I recommend that you read in order to better understand the other essays. In fact, the essays follow a logical sequence which I recommend that you follow, particularly if you are unfamiliar with the biology of aging. Of course, many readers access just one essay of interest, and cross-links between essays help readers navigate through different topics. Still, readers familiar with general observations related to the model systems used in aging research and the genetics of aging will find it easier to understand the entire section, so I recommend at least reading those two essays--plus the basic definitions--if you are unfamiliar with the field.

Some of these essays are based on my academic publications. I tend to cite my papers often for the simple reason that I am more familiar with them, but I try to provide a general perspective on various findings and theories even if I do not agree with them. Further references (about 1,000 of them) are cited in the bibliography in case you want further details concerning the experiments described or ideas presented.

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1. What Is Aging?

In this essay I attempt to define aging. The different components of human aging are succinctly reviewed and several other key concepts in gerontology are defined.

Sections

Keywords: ageing, aging traits, biomarkers, demography, older people

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Although everyone is familiar with aging, defining it is not so straightforward. Aging can simply refer to the passage of time and can even have a positive connotation as in "aging wine." In the context of **senescence.info**, and unless otherwise noted, the term "aging" refers to the biological process of growing older in a deleterious sense, what some authors call "senescence" (Williams, 1957; Comfort, 1964; Finch, 1990). (Personally, I actually prefer the term "senescence." If this were an academic book, I would be tempted to use the term "senescence." Being a website with visitors from various backgrounds, I think the term "aging" is more accessible; "senescence" now also frequently refers to cellular senescence.) Aging is one of the most complex biological processes, whose definition is intrinsically related to its phenotype, as developed below.

Demographic Measurements of Aging

Aging has been defined as the collection of changes that render human beings progressively more likely to die (Medawar, 1952). Indeed, one hallmark of aging in humans and in many other species is an age-related increase in mortality rates shortly after maturity (Fig. 1).

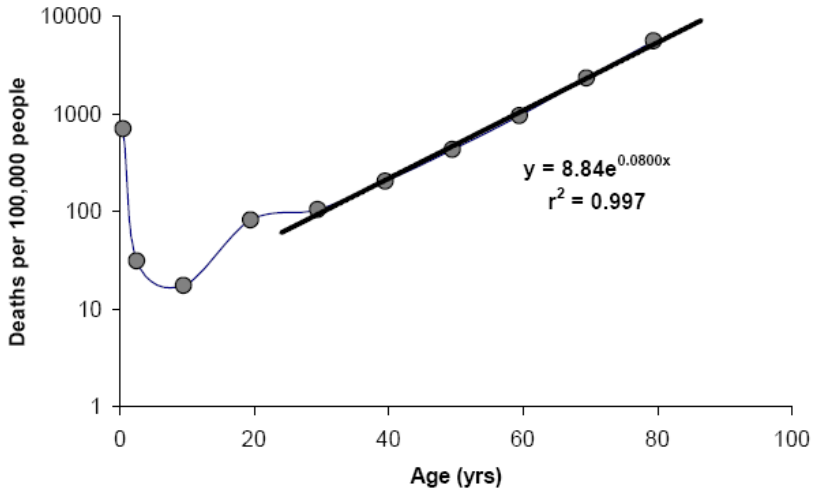

Figure 1: Mortality rates, expressed in deaths per 100,000 people, as a function of age for the 2002 US population. The black line represents the Gompertz function extrapolated from the mortality rates after maturity. Source: CDC/NCHS, National Vital Statistics System, Mortality.

Mathematically, aging can be quantified from mortality curves such as that in **Figure 1**. There are several mathematical functions that can be used (Wilson, 1994; Strehler, 1999, pp. 103-124). The simplest, most widely used method is based on the Gompertz function (Finch, 1990, pp. 13-22; Strehler, 1999, pp. 111-113):

$$m(t) = Ae^{Gt}$$

Being $m(t)$ the mortality rate as a function of time or age (t); A is the extrapolated constant to birth or maturity, and G is the exponential (Gompertz) mortality rate coefficient. From **Figure 1** it is possible then to estimate the Gompertz equation by performing a simple regression analysis after maturity: $m(t) = 8.84e^{0.0800t}$ with $r^2 = 0.997$. From this equation--or even sometimes from the mortality plot--we can derive the initial mortality rate (IMR), which is the mortality rate independent of aging, often calculated from the mortality rate prior to its exponential increase with age; in this case, $IMR = 0.0002/year$ since that is the mortality rate at ages 10-20. Another important variable derived from the Gompertz equation is the mortality rate doubling time (MRDT) given by $MRDT = 0.693/G$ (Finch, 1990, pp. 22-24). Hence, $MRDT = 0.693/0.0800 = 8.66$ years. In fact, human populations tend to have a MRDT around 8 years. This means that after our sexual peak, at roughly age 30, our chances of dying double approximately every 8 years.

Demographic measurements of aging, such as the MRDT, may then serve as estimates of the rate of aging. Changes in the MRDT are expected to reflect changes in the rate of aging, but the same is not true for the IMR (Finch, 1990; Finch and Pike, 1996; de Magalhaes et al., 2005). For example, the life expectancy at birth increased considerably in the past 100 year. In the US, it jumped from 47.3 years in 1900 to 77.3 years in 2002 (National Center for Health Statistics, Data Warehouse on Trends in Health and Aging). Nonetheless, the rate of aging and the MRDT are thought to have remained unaltered for thousands of years (Finch, 1990; Hayflick, 1994). What happened last century was that the IMR, which is not affected by the aging rate, was lowered due to breakthroughs in different areas, such as in the war against infectious diseases, thus lowering mortality rates across the entire lifespan and increasing the life expectancy. Because the increase in life expectancy was due to changes in the IMR independent of changes in aging rates is also the reason why the average lifespan of humans may be reaching a plateau. The only way to considerably increase human longevity in the future is to retard the aging process itself (Olshansky et al., 1990; Butler et al., 2008).

The way changes in the IMR and in the MRDT affect lifespan yet only changes in the MRDT reflect changes in aging rates means that changes in lifespan, for example due to feeding animals a particular anti-aging drug, may not reflect changes in the rate of aging. This is a crucial concept to correctly interpret experimental results in gerontology. For experiments in, for instance, animal models to be relevant to aging it is therefore imperative to discriminate between interventions affecting the aging process (i.e., the MRDT) and interventions affecting health (i.e., the IMR), as argued by many others (Hayflick, 2000; Pletcher et al., 2000). To determine whether rate of aging is affected one tool researchers have at their disposal is then calculating the MRDT and IMR (Pletcher et al., 2000; de Magalhaes et al., 2005). Indeed, such demographic measurements have been employed to determine whether genetic and dietary manipulations of lifespan in rodents modified or not the aging process (de Magalhaes et al., 2005). Moreover, and being the IMR independent of aging, the conditions by which aging is studied should be ideal environmental conditions in order to minimize the IMR and allow us to better focus on the aging process (Strehler, 1986). Lastly, demographic measurements are also useful for comparisons between species, as further discussed elsewhere.

It is common knowledge that women have a longer life expectancy than men. Pre-menopausal hormonal protection might contribute to this. Women have a lower IMR than men but the MRDT is similar for men and women[1]. This indicates that women do not age slower than men. Rather, women

[1] Although there are published papers on this, I actually made the calculations based on mortality data for the US population (Hayflick, 1994, pp. 73-76).

appear better protected against many major diseases at all ages (Austad, 2006). Men are the sicker sex (Zuk, 2009). Interestingly, there is some anecdotal evidence that, after menopause, women may suffer more from aging than men. Eunuchs also appear to live slightly longer than men (Hamilton and Mestler, 1969); a reduction in IMR due to hormonal alterations may be at the origin of this phenomenon (Grossman, 1984).

As mentioned above, human mortality rates begin to climb exponentially after about age 30. One peculiar phenomenon, however, is that this rate of increase of mortality actually levels off after about age 65 (Vaupel et al., 1998), and this has been reported in other species too. This is probably due, however, to statistics and heterogeneity--e.g., if a population is made up of two sub-populations with different rates of aging eventually only slower aging individuals will remain--rather than any unknown biological process (Vaupel et al., 1979; Partridge and Mangel, 1999; Rossolini and Piantanelli, 2001).

Pathological and Physiological Age-Related Changes

Aging can also be defined as a progressive functional decline, or a gradual deterioration of physiological function with age, including a decrease in fecundity (Partridge and Mangel, 1999), or the intrinsic, inevitable, and irreversible age-related process of loss of viability and increase in vulnerability (Comfort, 1964). Clearly, human aging is associated with a wide range of physiological changes that not only make us more susceptible to death but limit our normal functions and render us more susceptible to a number of diseases. The purpose of **senescence.info** is not to describe all age-related changes and pathologies typical of old age, as there are excellent resources on the topic (Craik and Salthouse, 1992; Spence, 1995; Timiras, 2002), including our lab's Digital Ageing Atlas. Nonetheless, a brief inspection of the most important physiological changes that occur with age and the pathological consequences of these changes is useful to understand aging.

In humans, albeit some functions like hearing and flexibility begin to deteriorate early in life (Bowen and Atwood, 2004), most of our body's functional decline tends to begin after the sexual peak, roughly at age 19. Contrary to demographic measurements of aging that show mortality rates increasing exponentially, the human functional decline tends to be linear (Strehler, 1999). Succinctly, aging is characterized by changes in appearance, such as a gradual reduction in height and weight loss due to loss of muscle and bone mass, a lower metabolic rate, longer reaction times, declines in certain memory functions, declines in sexual activity--and menopause in women--, a functional decline in audition, olfaction, and vision, declines in kidney, pulmonary, and immune functions, declines in exercise performance, and multiple endocrine changes (Craik and Salthouse, 1992; Hayflick, 1994, pp. 137-186; Spence, 1995). Although the immune system deteriorates with age, called immunosenescence, a major hallmark of aging is an increase in inflammation levels, reflected in higher levels of circulating proinflammatory cytokines and that may contribute to several age-related disorders such as Alzheimer's disease, atherosclerosis and arthritis (Franceschi et al., 2000; Bruunsgaard et al., 2001). Some age-related changes, such as presbyopia, also called farsightedness, which is caused by the continuous growth of the eyes' lenses and appears to be universal of human aging (Finch, 1990, pp. 158-159; Hayflick, 1994, p. 179), and menopause, are inevitable yet the incidence of most age-related changes vary considerably between individuals.

The phenotype of human aging is one in which practically any system, tissue or organ can fail (Austad, 1997a; Strehler, 1999). This indicates an intrinsic phenomenon affecting the whole organism and leading to the "weakest link" failing, resulting in death. Interestingly, studies in supercentenarians--i.e., people over 110 years of age--suggest that these individuals age uniformly. In other words, one thing that makes supercentenarians unique is the fact they do not have one debilitating organ or system that results in death; they do not have a "weakest link." Supercentenarians are nonetheless extremely frail and debilitated, showing multiple pathologies (Coles, 2004). Likewise, one "autopsy study" in centenarians revealed that all, even those described as healthy before death, had an acute organic failure causing death. These results also suggest that the idea that people can die of "old age" is incorrect (Berzlanovich et al., 2005).

Clearly, the incidence of a number of pathologies increases with age (Fig. 2). These include type 2 diabetes, heart disease, cancer, arthritis, and kidney disease. Also note how the incidence of some pathologies, like sinusitis, remains relatively constant with age, while the incidence of others, like asthma, even decline. Therefore, it is important to stress that aging is not merely a collection of diseases. With age we become more susceptible to certain diseases, but as described above we also become more likely to die, frailer, and endure a number of physiological changes, not all of which lead to pathology.

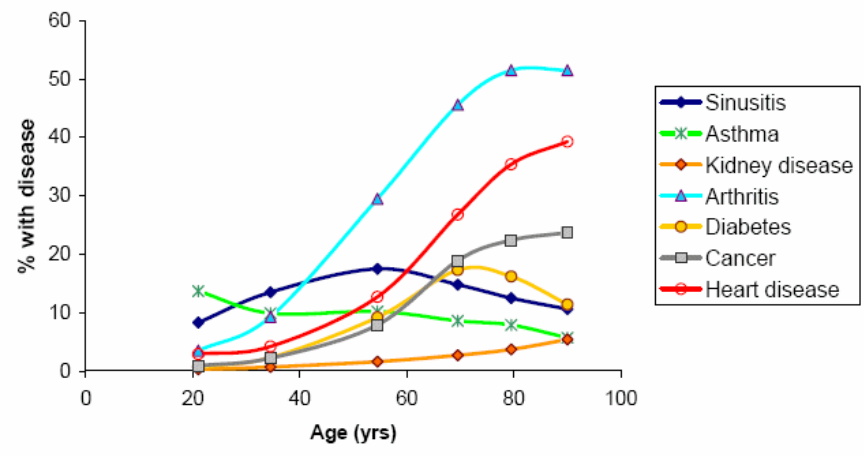

Figure 2: Prevalence of selected chronic conditions, expressed in percentages, as a function of age for the US population (2002-2003 dataset). All forms of cancer and heart disease are featured. Source: National Center for Health Statistics, Data Warehouse on Trends in Health and Aging.

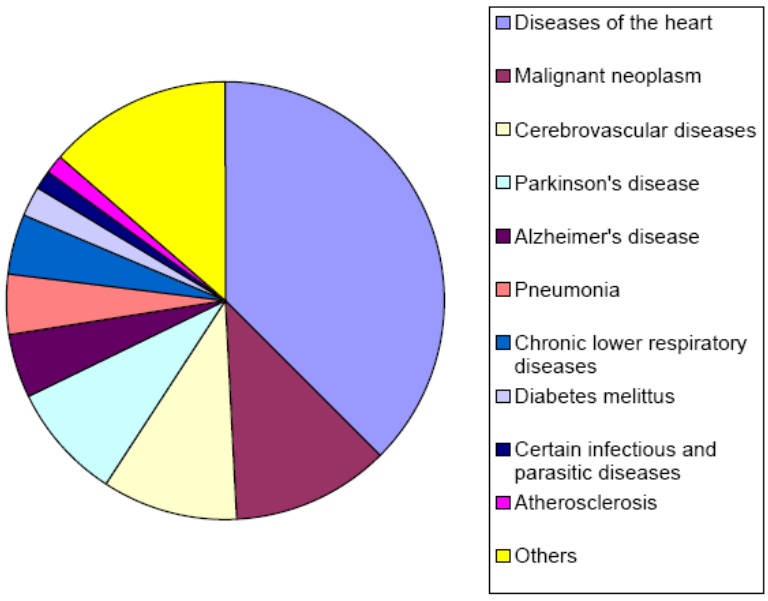

Figure 3: Death by underlying or multiple cause, expressed in rates per 100,000 people, as a function of age for the 2001 US population aged 85 and older. Source: National Center for Health Statistics, Data Warehouse on Trends in Health and Aging.

	45-54 years		Over 85 years	
Cause of death	**Incidence**	**% of deaths**	**Incidence**	**% of deaths**
Diseases of the heart	92.8	21.66%	5607.5	37.48%
Malignant neoplasm	126.3	29.48%	1747	11.68%
Cerebrovascular diseases	15.1	3.52%	1485.2	9.93%
Parkinson's disease	0.1	0.02%	1312.8	8.77%
Alzheimer's disease	0.2	0.05%	703.2	4.70%
Pneumonia	4.6	1.07%	676.5	4.52%
Chronic lower respiratory diseases	8.5	1.98%	638.2	4.27%
Diabetes mellitus	13.6	3.17%	318.6	2.13%
Certain infectious and parasitic diseases	22.9	5.35%	243.8	1.63%
Atherosclerosis	0.5	0.12%	177.3	1.19%
Others	143.8	33.57%	2050.9	13.71%

Table 1: Death by underlying or multiple cause, expressed in rates per 100,000 people or in percentage of the total deaths, for the 2001 US population in two age groups: 45-54 years and 85 years of age and older. Source: National Center for Health Statistics, Data Warehouse on Trends in Health and Aging.

Figure 3 shows the most important causes of death in the elderly. Not surprisingly, heart diseases are the number one cause of death in people aged 85 and older, followed by cancer, cerebrovascular diseases, Parkinson's and Alzheimer's diseases, pneumonia, and chronic lower respiratory diseases. While diseases like cancer and heart diseases are major causes of death at all ages, other diseases, like Parkinson's and Alzheimer's, only become significant at old age (Table 1). Lastly, it is important to note that an understanding of the physiology and pathology of aging is important to assess the relevance of model organisms for the study of human aging, as mentioned elsewhere.

Despite all the physiological and pathological changes, there is still no accurate way to quantify how aged someone is. Despite decades of research, and even though it is clear that different people age at different paces, the most accurate method to determine the biological age of someone is still chronological age. This is a major problem for studying aging and there have been ongoing efforts to determine a better way to quantify aging for years (reviewed in Balin, 1994).

Basic Definitions in Gerontology

Given the description of the human aging phenotype detailed above, I will define the basic terms that are used in **senescence.info**. To sum it up, aging is a complex process composed of several features:

1) an exponential increase in mortality with age;
2) physiological changes that typically lead to a functional decline with age;
3) increased susceptibility to certain diseases with age.

So, I define aging as a progressive deterioration of physiological function, an intrinsic age-related process of loss of viability and increase in vulnerability.

Gerontology is the branch of biomedical sciences that studies aging. In **senescence.info**, gerontology normally refers to the study of the biological process of aging, not its medical consequences. Generally, I use geriatrics to refer specifically to the medical study of diseases and problems of the elderly. Technically, gerontology includes both the biological and the medical branches of the study of aging, but since **senescence.info** is written in the context of the biology of aging, gerontology usually refers to the study of the biological aspects of aging, unless otherwise specified. Biogerontology refers specifically to the biological study of aging and is also used, usually interchangeably, with gerontology.

Life expectancy is how long, on average, an organism can be expected to live. Longevity is the period of time an organism is expected to live under ideal circumstances. Lifespan is defined as the period of time in which the life events of a species or sub-species (e.g., a strain or population) typically occur. Lifespan and longevity can sometimes be used interchangeably, though they have slightly different meanings. For humans, lifespan and longevity are about the same in industrial nations, but when studying species in the wild, one can expect that lifespan will be lower than longevity since feral conditions are certainly not ideal for assessing longevity. For most purposes, life expectancy, average longevity, and average lifespan have the same meaning. Maximum longevity and maximum lifespan are the maximum amount of time animals of a given species or sub-species can live--typically, the record longevity for that species. The maximum longevity of humans is 122 years, recorded by the late Jeanne Calment (Allard et al., 1998).

For a quick reference on terms and definitions, you may always consult the glossary.

2. Some Animals Age, Others May Not

Previously, I described the major features of the human aging process. In the amazing biodiversity of our planet, however, we can find diverse forms of aging, many of which are fascinating. In this essay I describe the aging phenotypes observed in an array of species and how these compare to human aging. By studying the aging process of other animals and comparing the way different species age, it may be possible to gather clues about the human aging process and how to delay it, as further detailed elsewhere.

Sections

Keywords: ageing, biogerontology, comparative biology, immortal germplasm, life span, microbes, nature, phylogeny, vertebrates

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This essay follows a phylogenetic perspective, starting in the species most distant from us (Figure 1). As the we move to species evolutionary and biologically closer to us, I increase the detail in which I describe them. Many of the species mentioned in this essay are present in our lab's AnAge database, the benchmark aging and longevity database in animals (de Magalhaes and Costa, 2009), and further information and quantitative data on the species mentioned below is likely available.

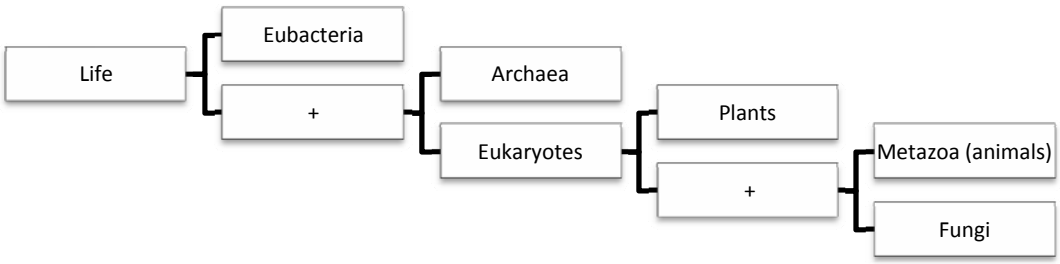

Figure 1: Simplified tree of the evolution of life on earth. (Adapted from Maddison and Schulz, 2004.)

Primordial Life Forms

The species further from us are prokaryotic microorganisms (Maddison and Schulz, 2004). Bacteria, for instance, are unicellular organisms that reproduce by cell division or fission; normally a mother cell divides into two equal daughter cells (Brock, 1997). Since we cannot distinguish between progenitor and offspring it was thought that these organisms do not age. Protected from violent death, bacteria have until recently been thought to be immortal. However, senescence has been reported in a bacterium with asymmetric division, *Caulobacter crescentus*, in the form of a decrease in reproductive output with age (Ackermann et al., 2003). *Escherichia coli* cells growth-arrested by nutrient depletion have also been studied in the context of aging since they lose their ability to recover

and reproduce (reviewed in Nystrom, 2002). Evidence that division in *E. coli* is functionally asymmetrical has been suggested as evidence of aging in this species. Briefly, *E. coli* divides in the form of a rod and the cell that inherits the oldest end, or pole, exhibits a diminished growth rate, decreased offspring production, and an increased incidence of death (Stewart et al., 2005). On the other hand, certain bacteria can form spores and remain in a state of suspended animation for years with one study reporting the growth of a bacterium with 250 million years (Vreeland et al., 2000). Nevertheless, and despite the basic cellular functions being the same for all living organisms, bacteria's constitution and physiology have many differences when compared to human cells--e.g., bacteria have circular chromosomes, do not have mitochondria, etc. So while these observations are intellectually stimulating, their relevance to understanding human aging is somewhat dubious.

When observing eukaryotic species we start to witness sex and its implications: sexual genetic recombination can be seen as a re-adaptation of the genome to the environment with the exchange of genetic information being advantageous in terms of increasing diversity. Sex also appears to be related to the emergence of aging in many evolutionary lineages. Beginning with ciliates, species exist in which individual cells are "destined to die," a process resembling the programmed death of the organism that we will also witness ahead in animals. An example is the protozoan *Tokophrya* (Karakashian et al., 1984): it reproduces by internal "budding," an asymmetric mitotic division, yet it is born without any means to dispose of wastes, which makes individual death inevitable. This form of aging resulting from mechanical limitations in the design of organisms has be called "mechanical senescence" (Comfort, 1964). In addition, asexually reproducing clones show another form of aging, called clonal or replicative senescence in that they stop proliferating; interestingly, cells born from older individuals have shorter lifespans. *Paramecium* and *Tetrahymena* are two other well-studied species in gerontology (Nanney, 1974). They can reproduce asexually for hundreds of generations but eventually reach clonal extinction--though perhaps not all strains in the case of *Tetrahymena*. In *Paramecium* it has been demonstrated that sex--the two cells fuse, exchange genetic material and then segregate--can rejuvenate individual cells (Smith-Sonneborn, 1987; Bell, 1988). Finally, numerous species among ciliates and other protista show no evidence of aging or clonal senescence; examples are found among the taxa Amoebina, Cryptomonadina, Phytomonadina, Sarcosporidia, and Radiolaria (Finch, 1990, p. 228). Lacking a sexual phase many species can proliferate with no detectable clonal senescence.

Plants (kingdom: Plantae)

Defining when life begins and ends is quite easy for us humans. Life begins at birth--or at conception or when the central nervous system is formed--and ends at brain death. But when life and individuality begin in other kingdoms is harder to define. When I was younger, and still had free time, I sometimes did gardening with my mother. As many are aware, for many species of plants if you cut a graft, put it in water, and then plant it in the soil, you will have a new plant. Similarly, there are species in the plant kingdom (Plantae) in which trees are generated on the roots of another tree. If we have two trees connected by the roots in which one of the trees originated from the roots of another, is this the same tree or are there two trees? And when the first tree dies, is the second tree now another tree or is it still the same tree? Aging in plants is thus difficult to define. This process of vegetative reproduction can, in some species, be done (in theory) eternally; grafting is widely used is agriculture and many species--tulips (*Tulipa clusiana*), saffron crocus (*Crocus sativa*), and banana (*Musa sapientum*)--are examples of how it can be done for hundreds if not thousands of years (Cook, 1983; Finch, 1990, p. 229). Cases of species undergoing clonal reproduction for over 10,000 years have been reported with one species (*Lomatia tasmanica*) dated to be at least 43,600 years (reviewed in Munne-Bosch, 2008). Species exist, however, in which vegetative reproduction shows decreasing vigor with time. For example sugarcane and citrus show limited clonality in cuttings but other factors besides aging, such as viruses, can be involved (Finch, 1990, p. 230).

Another unique feature of plants is that, in general and unlike animals, they segregate their germ cells from the soma shortly before reproduction. In addition, there is an enormous diversity of plants and many seem capable of living thousands of years. Asexual, agametic reproduction is highly spread

among higher and lower plants (Watkinson and White, 1985). Examples include the genera *Rubis*, *Hieracium*, *Poa*, and *Taraxacum* (Cook, 1983).

There are also many plant species that show clear signs of senescence. Some, such as bamboo, reproduce, age, and die at well defined times indicating a pre-programmed mechanism. Bamboo are then considered semelparous, meaning they reproduce only once, in contrast to iteroparous species like humans that can reproduce more than once during their lifetime. Also note that semelparous species can be long-lived. For example, plants of the genus *Agave* can take 100 years to mature and then suddenly die, which can also be called "big bang" reproduction (Finch, 1990, p. 101). These events are hormonally triggered and, for example, depending on the species of bamboo can take between 7 and 120 years (Janzen, 1976). Many species of plants also show a functional decline with time, indicating a slow aging rate. Examples are well known in agriculture such as apple, orange, and other fruit trees. In some cases, such as in citrus, vegetative reproduction by cutting can lead to a sort of rejuvenation (Finch, 1990, p. 127). Our knowledge of how hormones control these events is limited but it can be that the hormones produced by roots or growing tips influence the process.

Finally, probably the best described example of a non-aging plant is the bristlecone pine. This tree grows on high rocky ground practically without predators and has been estimated to live up to 4,713 years. Studies have shown an absence of MRDT and no declines in reproductive output with age (Lanner and Connor, 2001).

Fungus (kingdom: Fungi)

Saccharomyces cerevisiae is a yeast that reproduces by budding divisions (Jazwinski, 1990). A new cell forms as a small outgrowth of the mother cell, the bud enlarges and then separates, leaving a scar behind. Since each cell can only do this process a limited number of times it has been argued that this is a form of aging. In addition, chronological lifespan can also be quantified and used as an estimate of aging. A favorite model of researchers, the "aging process" of this yeast has been studied in detail. One possible cause of senescence might be ribosomal DNA circles (Sinclair and Guarente, 1997; Sinclair, 2002). Around the middle of the lifespan a circular copy of the rDNA pops out of the genome and begins replicating, eventually leading to the mother cell's death. Since daughter cells are born without this rDNA, perhaps that is what makes them young. An alternative hypothesis is a role of the mitochondrion, the cell's powerhouse, in the aging of *S. cerevisiae* (Jazwinski, 2005). Another yeast, *S. pombe*, reproduces by fission but it shows asymmetrical cell division and replicative senescence (Barker and Walmsley, 1999). *Pseudospora* is a filamentous ascomycete that has also been the subject of gerontological research. Its lifespan is limited and, although the mechanism has not yet been fully elucidated, it can involve a process involving copper levels that in turn impact on the energy transduction apparatus of mitochondria as well as reactive oxygen species production (Osiewacz and Borghouts, 2000). Probably the oldest known fungus is the *Armillaria ostoyae*. Some authors believe a giant fungus of this species at the Malheur National Forest (US) can be over 2,400 years and is the biggest organism on earth (Ferguson et al., 2003).

Animals (kingdom: Animalia or Metazoa)

Like in plants, vegetative propagation may occur among some animals, although usually coupled with sexual reproduction. *Campanularia* reproducing asexually have been claimed not to age despite showing a cycle of growth and involution (Brock and Strehler, 1963). *Hydra* and *Cyanea capillata* have also been claimed by some authors not to show senescence (Brock and Strehler, 1963; Martinez, 1998), despite contrasting opinions of others (Bell, 1988). Sponges and corals are other good examples of asexual vegetative reproduction leading to long lifespans--over 200 years in the case of corals and perhaps thousands of years in the case of sponges. Some evidence, however, supports the idea that corals age (Finch, 1990, p. 233). Still, corals have high infant mortality rates and maybe a decrease in mortality with age, which makes them a fascinating case (Finch, 1990, p. 242).

Worms vary much between asexually and sexually reproducing species and many species have alternating cycles of both--like in certain plants, a process often dependent on diet and temperature. One flatworm, *Stenostomum tenuicauda*, produced a calculated number of 1,000 asexual generations for 11 years and *Ctenodrilus monostylos* was kept reproducing by fission for 60 years. Asexually reproducing parasitic worms must have reproduced asexually for decades (Moore, 1981; Finch, 1990, p. 235). Finally, protochordates also reproduce asexually. Ascidians reproduce by fission or budding on a regular basis. For example, in *Perophora viridis* buds originate in certain lymphocytes that are, in effect, stem cells capable of creating a full organism with heart, neural structures, etc. (Finch, 1990, p. 236).

One of the most widely studied species in the world is the soil nematode *Caernohabditis elegans*, composed mostly of post-mitotic cells (Murakami et al., 2000). This species has a very short lifespan--days to weeks--but it can be radically extended by making the animal enter an alternative developmental pathway called *dauer*. This pathway consists of a developmental arrest and is normally activated when animals are starved or under crowded conditions; it delays development leading to an increased adult phase (Klass and Hirsh, 1976). Certain genetic interventions can also induce this *dauer* phase. In fact, the first gene ever shown to retard aging (*age-1*) was identified in *C. elegans* (Johnson, 1990). It is interesting to note the contrast between the short-lived *C. elegans* and other parasitic worms like *Necator americanus* and *Ancylostoma duodenale* that can live up to 15 years (Finch, 1990, pp. 215-216). Another nematode worm, *Strongyloides ratti*, has both a short-lived free-living (~5 days) form and a parasitic form that can live over one year inside a host (Gardner et al., 2006). While these differences in lifespan are dramatic, I should note that the two forms are quite different morphologically and physiologically. The ability of a single genome to give rise to two or more morphologies is called polyphenism.

Figure 2: Red sea urchin (*Strongylocentrotus franciscanus*), which has been estimated to live up to 200 years and is considered a species with negligible senescence. Source: U.S. Fish and Wildlife Service.

Rotifers are minute aquatic multicellular organisms that have short lifespans ranging from a few days to months; males, unlike females, typically lack excretory systems and therefore have even shorter lifespans (Finch, 1990, p. 72). Again, this can be seen as a form of mechanical senescence.

One of the animal species with the longest longevity is an invertebrate tubeworm called *Lamellibrachia*. In contrast to the rapid growth and much shorter lifespans of similar species living in habitats richer in nutrients, animals living around hydrocarbon seeps grow very slowly and have longevities estimated to be between 170 and 250 years (Bergquist et al., 2000). Other long-lived invertebrate species include the bivalve mollusk ocean quahog (*Arctica islandica*) that has been estimated to live up to 400 years (Abele et al., 2008), and the red sea urchin (*Strongylocentrotus franciscanus*; Fig. 2) that may live up to 200 years (Ebert and Southon, 2003). These three species appear not to age and are referred to as species with negligible senescence (Finch, 1990). The AnAge database includes a list of the longest-lived animals and a list of species with negligible senescence.

In general (but see below), insects are short-lived. Nonetheless, we find a staggering diversity of aging phenotypes among them. A common mechanical senescence process in insects involves aphagy (Weismann, 1891), or the inability to ingest a complete meal as an adult, generally related to defective mouthparts or a defective gut. Numerous examples exist among insects and include animals like mayflies and species in Ephemeroptera, Neoptera, Coleoptera, etc. (Finch, 1990, pp. 584-585). Many insects and other members of Arthropoda are therefore semelparous. Insects, of course, have a much different body plan than ours. For example, the fruit fly *Drosophila*, a common model of aging, is mostly composed of post-mitotic tissues and fragile organs, making it susceptible to wear and tear that affects irreplaceable organs and/or tissues.

No mentioning of insects is complete without ants (Finch, 1990, pp. 67-72). Ants, like many other social insects such as honey bees, show polyphenism. Not only are ant castes morphologically different, but ants have two distinct aging phenotypes in workers and queens. What makes them fascinating is the fact that they all share the same genome and both are probably postmitotic, except perhaps the gut and sex cells. Queens can live over 15 years, 100 times more than the workers. The difference between workers and queens is that queens have specialized feeding; for example, larvae destined to become queens are fed ten times more often. In addition, queens suffer little mechanical abrasion and are constantly groomed by the workers. Also interesting are the large differences in lifespan between workers in the same colony depending on seasons. Hormonal levels dependant on the amount of activity needed by the colony appear to play a role in this. There are also exceptional cases of worker ants living over 5 years but these are rare. In fact, while workers show a clear acceleration of mortality, queens die very rapidly after their sperm stocks are exhausted, sometimes even assassinated by the workers. Therefore, queens may well feature negligible senescence.

Apart from mechanical senescence, other processes can lead to rapid aging and/or sudden death after reproduction. Hormone-driven processes are common in many lower species. Some octopus species do not eat after spawning, which is driven by hormones (Wodinsky, 1977). Spiders such as *Frontinella pyramitela* have short lifespans and increased feeding leads to increased reproduction and earlier death (Austad, 1989). On the other end of the lifespan spectrum, female tarantulas can live more than 25 years without showing clear signs of aging (Finch, 1990, p. 78). Other arthropods with indefinite lifespan include lobsters, whose molting leads to the replacement of hard tissues avoiding wear and tear and leading to continual growth (Finch, 1990, p. 215). Among Echinodermata, sea urchins (as mentioned above) and other starfish are claimed to be able to live more than 40 years, showing decreasing mortality with size (Finch, 1990, p. 216). In mollusks, certain octopus species also show no signs of senescence (Arnold and Carlson, 1986).

Chordates (phylum: Chordata)

Moving closer to humans, tunicates are primitive aquatic animals, members of Urochordata. They have several asexual and sexual reproductive cycles and are typically short-lived (Finch, 1990, p. 236). On the other hand, hagfishes can show continued growth and very slow, if any, senescence as well as continued *de novo* oogenesis in adults; their estimated maximum lifespan is about 40 years (Finch, 1990, pp. 216-217). Despite many body plan similarities with hagfishes, lampreys are a completely different story. Lampreys are semelparous and show "big bang" reproduction followed by rapid senescence. In fact, adult lampreys typically do not eat and do not replace their oocytes.

Jawed fishes are considered the most modern of fishes. Ray-finned fishes such as sturgeons can have very long lifespans, exceeding a century (Finch, 1990, pp. 216-217). Female sturgeons, however, may have a finite ovarian oocyte stock (Finch, 1990, pp. 134-135). Teleosts such as rockfishes also live very long, show no signs of reproductive senescence and grow continuously, albeit slowly (Fig. 3A). Some rockfishes, such as *Sebastes aleutianus* have been estimated to live over 200 years and show no signs of aging (Cailliet et al., 2001). Sharks are often claimed to show no senescence or cancer (Lane and Comac, 1992), but despite low cancer rates, cancer can appear and females may show reproductive senescence. On the other end of the spectrum, some fishes have very short lifespans. Certain annual African fishes such as of the genus *Nothobranchius* do not commonly live more than 12 weeks even in protected environments and exhibit signs of an extremely fast aging process (Valdesalici and Cellerino, 2003).

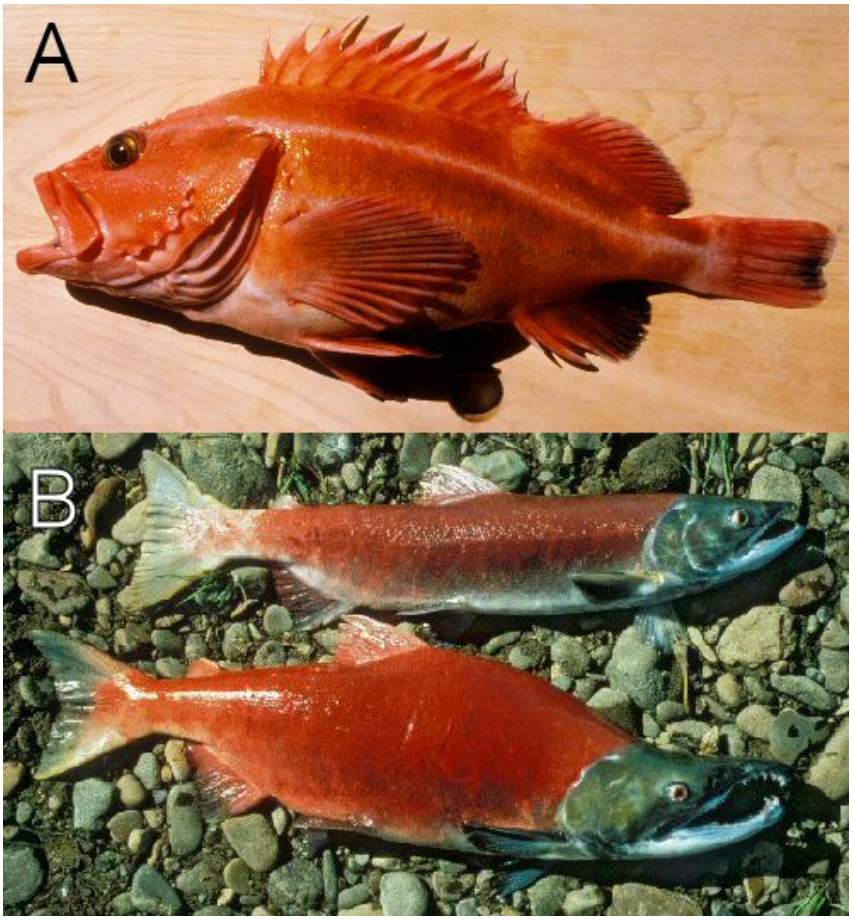

Figure 3: Two fishes with completely different life-history strategies and aging phenotypes. A: Yellow rockfish (*Sebastes reedi*), which has been estimated to live nearly a century. B: Sockeye salmon (*Oncorhynchus nerka*), which dies shortly after spawning. Source: U.S. Fish and Wildlife Service.

A famous fish in gerontology is the salmon (Fig. 3B), a teleost, and its rapid senescence following reproduction is a landmark phenomenon. Typically salmon are born in rivers and lakes, migrate to the ocean until returning to spawn in fresh water and die shortly after. During the reproductive period, a hormonal cascade causes animals to stop eating, develop various pathologies, and eventually die (Finch, 1990, pp. 83-90). Castrated fish can live twice as long but eventually show the usual hormonal-based changes (Robertson, 1961). One fascinating facet of salmon senescence is that the salmon has a particular mussel parasite that can extend the salmon's lifespan by affecting the

hormonal program of accelerated senescence (Ziuganov, 2005). Another remarkable observation is that in some normally semelparous species of salmon also exhibit iteroparous life histories; male jacks and parr mature early and parr can survive reproduction and mate again (Unwin et al., 1999).

Certain salmoniforms show conditional semelparity. The steelhead trout *Oncorhynchus mykiss* is a good example; just like typical salmons, these animals have to spawn in rivers, but some animals are able to return to the sea and that way can survive to reproduce another year (Finch, 1990, p. 92). Another interesting case is the plaice (*Pleuronectes platessa*), a flounder-like fish; the female continually grows and shows no signs of aging while the male ages and dies (Bidder, 1932; Finch, 1990, p. 240). In fact, partly based on this species, Bidder proposed that cessation of growth leads to the onset of aging. Despite being true for many species, exceptions exist. A major open question is whether growth cessation is caused by hormonal changes or by intrinsic cellular limitations.

Ending the marine examples I want to mention a phenomenon quite common among fishes, which is also observed in many invertebrates like certain insects, including flies and worms typically used in aging research. The walleye (*Stizostedion vitreum*) is a fish found in North American freshwaters. Depending on the temperature of its environment, its lifespan can increase five-fold while also delaying maturation and overall development (Gosden, 1996, p. 107; Mangel and Abrahams, 2001). A delayed maturity and increased lifespan as a result of a lower ambient temperature is common in many ectotherms (see below).

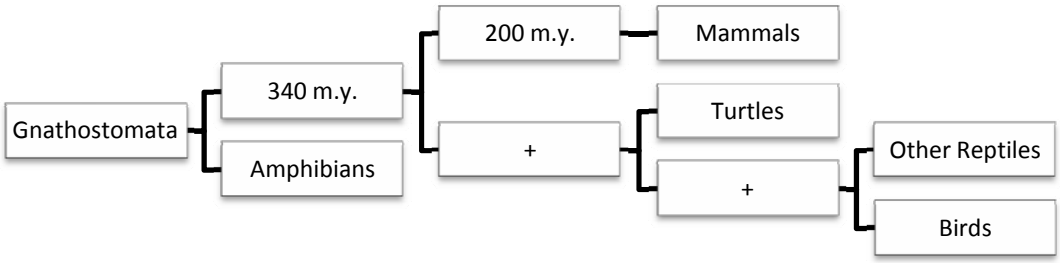

Figure 4: Simplified tree of the evolution of mammals and closely related taxa. Most modern fishes are in Gnathostomata with the exception of sarcopterygian fishes, which are not shown. (Adapted from de Magalhaes and Toussaint, 2002; Maddison and Schulz, 2004.)

Approaching mammals (Fig. 4), amphibians have no reported cases of "big bang" reproduction, though some species are short-lived showing signs of gradual aging (Kara, 1994; Smirina, 1994). Overall, and although there are not many detailed studies of aging in amphibians, amphibians appear to be longer lived than mammals of the same size. In fact, aging's incidence and intensity appear to be lower in amphibians when compared to mammals (Finch, 1990, pp. 219-221; de Magalhaes and Toussaint, 2002). Studies in amphibians have reported possible negligible senescence in frogs (Brocas and Verzar, 1961; Plytycz et al., 1995a) and toads (Plytycz and Bigaj, 1993; Plytycz et al., 1995b), but this has not been proven. Like for many other species, temperature is a major factor in determining life history traits. Typically, animals in northern or mountain regions tend to live longer and, usually, mature later, though hibernation could be a factor too (Smirina, 1994). Reports of some age-related declines have been observed in small species such as frogs (Perez-Campo et al., 1993), *Rana temporaria* (Plytycz et al., 1995a), and the tiger salamander (Townes-Anderson et al., 1998). Other salamanders such as *Andrias japonicus* live at least 55 years and show no reproductive senescence or decrease in fitness with age. Bullfrogs (*Rana catesbeiana*) show an increase in fitness with age as older females prefer older and larger males. The longevity record for the class belongs to the olm, a blind subterranean animal that has been predicted to live over 100 years and may be a case of negligible senescence (Voituron et al., 2011). A laboratory favorite is *Xenopus laevis*: it lives over 15 years and shows few signs of senescence. Oogonial proliferation in amphibians occurs after maturity

but there are no reports at advanced ages. Nonetheless, it is possible that some amphibian species feature negligible senescence including neurogenesis and oogenesis in adulthood. Teeth in amphibians are polyphyodont, meaning animals develop several sets of teeth successively throughout life (reviewed in Kara, 1994).

Like amphibians, reptiles not show "big bang" reproduction (reviewed in Patnaik, 1994), and tend to show a lower incidence and intensity of aging than most mammals (Finch, 1990, pp. 219-221; de Magalhaes and Toussaint, 2002). Some reptilian species show signs of aging comparable to what is observed in mammals (Majhi et al., 2000; Jena et al., 2002; Olsson and Shine, 2002). Unlike some animals, like many fishes, that grow continuously throughout their lives, reptiles tend to grow slower at older ages, in both short- and long-lived species. Like amphibians, most reptiles feature polyphyodonty (Patnaik, 1994). Several species of reptiles, particularly turtles, appear to feature negligible senescence and very long lifespans. Blanding's turtle (*Emydoidea blandingii*) has been shown to increase survival and reproductive output over a 75-year period (Congdon et al., 2001), and similar results have been reported for the eastern box turtle (*Terrapene carolina*; Fig. 5A; Miller, 2001). Marion's tortoise (*Geochelone gigantia*) is claimed to have lifespans over 150 years, which is uncertain but possible since some captive turtles live up to 70 years. An increase in mortality was found in wild *Geochelone* but extrinsic factors might be involved. The Galapagos tortoise (*Geochelone nigra*) also appears to be long-lived with a possible record longevity of 177 years (Fig. 5B). Some snakes might also escape senescence; many species actually lay more eggs as they increase in size with age; for instance, *Natrix maura* ceases to grow but animals can live beyond that point with no detectable increases in mortality. Despite some species having a sexual peak, reproductive senescence has not been convincingly reported in reptilians, though further studies are necessary. For example, alligators have been reported to exhibit some evidence of reproductive senescence but the results are inconclusive (Finch, 1990, pp. 144-145). Oogenesis in adulthood has been reported for some species yet again further studies of reproduction across the lifespan are necessary. Although the evidence is limited, it appears that reptilians and amphibians show a less intense aging phenotype than mammals with many species failing to show a characteristic maximum lifespan.

Figure 5: Two examples of long-lived turtles that show no signs of aging. A: Eastern box turtle, which can live up to 138 years. B: Galapagos tortoise, which can live up to 177 years. Sources: Ryan Hagerty (A) and Paul Guther (B), U.S. Fish and Wildlife Service.

Evolving, as mammals, from reptiles (Fig. 4), birds are another taxon whose aging phenotype is worth appreciating (Finch, 1990, pp. 144-150). Like amphibians and reptiles, birds are not known to exhibit rapid senescence or semelparity. Certain species show a definitive trend of accelerating mortality with age, being gallinaceous birds (order: Galliformes) the most extreme example (Ottinger, 2001). Other species, however, show very slow increases in mortality with age and some long-lived species show an increase in parenting success with age. Species with very long lifespans and little signs of reproductive senescence are common: the Andean condor (*Vultur gryphus*) is capable of living up to 75 years and is one the longest lived birds; no senescence has been reported but detailed studies are lacking. Other species such as *Fulmarus glacialis* and *Sterna paradisaea* also show little signs senescence (Gosden, 1996, pp. 55-56). From mathematical calculations it has been proposed that these long-lived birds must show some mortality increase, apart perhaps from condors. Parrots and

cockatoos (order: Psittaciformes) are also known to be long-lived with anecdotal reports of animals living over 100 years, though detailed studies are lacking. Comparing birds with mammals, some authors suggest that birds age slower than mammals (Holmes and Austad, 1995; Holmes et al., 2001; Holmes and Ottinger, 2003). Nonetheless, there are no verified reports of birds with negligible senescence or animals living over 100 years. Birds do not have continuous growth.

Before moving to mammals there is just one last group of species worth mentioning. These are species with a clearly defined finite lifespan but that might not age at all (Finch, 1990, pp. 222-226). Species with a very high IMR are expected to be short-lived, yet senescence might not necessarily occur. (A high extrinsic mortality--i.e., high IMR--is expected to lead to the evolution of aging, but this may not be an universal rule.) Exemplifying, in certain fishes, such as *Cynolebia* or *Nothobranchius*, adults die soon after spawning in the wild but their lifespan can be increased several fold in the laboratory where a gradual increase in mortality is witnessed. So, species might exist in which the high mortality masks an absence of senescence. *Chiton tuberculatus* is a marine mollusk that is an excellent case of what I call ecological senescence: at age four animals show a large increase in mortality but this increase appears to derive from continual growth and the subsequent need to find new habitats where animals are more exposed to predation. Phenomena along these lines may cause increased mortality rates in other species with negligible senescence but continual growth. One last example is the saguaro cactus (*Cereus giganteus*); it shows a MRDT of 18 years but this is probably due to extrinsic factors such as accumulation of exogenous damage. Species showing no senescence and continuous growth might also be affected by ecological senescence; increasing size might lead, for example, to changes in diet that cause an increase in mortality.

Mammals (class: Mammalia)

One of the few reported mammalian species with "big bang" reproduction and semelparity is a marsupial called *Antechinus stuartii*, a type Australian mouse (Finch, 1990, pp. 95-98; Gosden, 1996, pp. 13-30). During the annual mating season, males become "intoxicated" with sex hormones. They have such an increased libido that they are unable to eat and eventually die of sexual stress. The endocrine changes even affect the immune system so that more energy is available for reproduction. Just like in the salmon, castrating the males also increases their lifespan by two to three-fold. Other small mammals such as the well-known lemmings also show seasonal population crashes or, as in voles (*Microtus townsendii*), high mortality in the spring, probably dependant on food resources yet these are not considered semelparous.

Figure 6: The little brown bat (*Myotis lucifugus*), despite its small size--they weight about 10 grams--can live up to 34 years. Source: Don Pfritzer, U.S. Fish and Wildlife Service.

Placental mammals, known as eutherians, show a large diversity in average and maximum lifespan (Finch, 1990, pp. 122-123). Some rodents, such as *Mus musculus* or *Rattus norvegicus* have short lifespans rarely exceeding 4 years. In contrast, the longest-lived rodent is the naked mole-rat with a

record longevity of 28 years and is also fascinating in being exceptionally resistant to cancer (Buffenstein, 2005 & 2008). There are no recorded species of mammals with negligible senescence and it is unlikely that any exist. So far, all studied mammals featured reproductive senescence (Cohen, 2004), an increased mortality with age, and evidence of functional decline with age. The longest-lived mammal known is the bowhead whale; an individual with 211 years was reported in one study (George et al., 1999). Cetaceans, in general, appear to be long-lived with several anecdotal claims of animals living over 100 years. Elephants also show long lifespans and might live over 70 years (Finch, 1990, p. 152). Finally, bats are one interesting order of species (Chiroptera) for, despite their small size, they can live over 30 years and have a low MRDT (Fig. 6; Jurgens and Prothero, 1987; Austad and Fischer, 1991).

Apart humans, other primates can be long-lived, though none are as long-lived as we are. Rhesus monkeys (*Macaca mulata*) can live over 35 years and 25/30 year-old animals tend to display the age-related patterns found in a 50/60 year-old human. Interestingly, rhesus monkeys have a MRDT similar, if not superior, to that of humans, showing that MRDT values are not perfect estimates of the rate of physiological aging. Chimpanzees (*Pan troglodytes*), our closest relatives, can live over 60 years and, although their aging process has not been studied in detail, they show age-related changes typical of humans at considerably earlier ages. Their MRDT is about 8 years, so is about the same as humans (Hill et al., 2001). It could be that chimpanzees age at the same pace as humans but the onset of aging occurs sooner in them (de Magalhaes, 2006). As for humans (*Homo sapiens*), our MRDT is about 8 years and our maximum lifespan is 122 years, as detailed elsewhere. On the other hand, some primates can be shorter-lived and exhibit a fast rate of aging--though not as fast as rodents. These tend to be species more distant from humans, as primates biologically and evolutionary closer to humans tend to be long-lived since it is thought that longevity increased in the lineage leading to humans (Cutler, 1979). Examples of short-lived primates include marmosets (genus *Callithrix*), dwarf and mouse lemurs (genera *Cheirogaleus* and *Microcebus*), tarsiers (genus *Tarsius*), and animals of the Galagonidae family (Austad, 1997c). These animals tend not to live more than 20 years, show age-related changes in their second decade of life, and have short life cycles attaining sexual maturity in less than 2-3 years (Bons et al., 1992; Austad, 1997c; Harada et al., 1999).

A well-documented mechanical senescence process in mammals is tooth erosion (Finch, 1990, pp. 196-202), which is a major problem for several species such as hippopotamus, horses, elephants, etc. Long-lived species evolved creative mechanisms to cope with this. For instance, elephants have up to six sets of molars. Still, and although the Nabarlek (*Petrogale concinna*) could be an exception, mammals are not known to be polyphyodont.

One of the most interesting features of mammalian aging is that its phenotype is similar in most species (Finch, 1990, p. 619; Miller, 1999). Female reproductive senescence at mid-life, osteoporosis, arthritis, vascular lesions, cataracts, etc. are quite common among well-studied mammals. Despite some exceptions, such as certain marsupials like *Antechinus*, the pathophysiology of aging is remarkably similar in mammals which has implications for our understanding of genetic mechanisms of aging, as discussed elsewhere.

2.1 Comparative Biology of Aging

The reasons for some animals being long-lived and others short-lived, and, in a word, causes of the length and brevity of life call for investigation.

- Aristotle, 350 BC

After witnessing the amazing diversity of aging phenotypes and lifespans found in Nature, a key question is whether there are any trends in the way animal age. In this essay, I briefly examine the main factors associated with longevity in animals. Some of the trends observed can inform our knowledge of aging mechanisms. Recent advances in genomics and how these may contribute to unravel the biodiversity of aging are also discussed below.

Keywords: ageing, allometric scaling, allometry of life, evolutionary genomics, life span

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Why cannot a mouse live more than 5 years while humans and whales can live over 100? Why do some animals appear not to suffer from aging? In the end, why do different species age at different paces? Many researchers have asked these questions (Austad, 1997a & 2005; Warner et al., 2002; de Magalhaes, 2003). So far, and even though this topic will be discussed in other essays, including at the genetic level, the answer has eluded us. Nonetheless, there some general trends have been reported and some factors shown to correlate with maximum lifespan.

As mentioned before, quantifying aging is a difficult, controversial task. Quantifying the rate of aging for a given species can be done through the MRDT, but there are some caveats: MRDT calculations are available for only a fraction of species and, as shown before, are not perfect estimates of rate of aging. Consequently, most researchers use maximum lifespan (*tmax*) as an estimate of rate of aging. It has been argued that *tmax* represents the genetic potential for longevity of each species and is related to a species' rate of aging (Cutler, 1979; Allman et al., 1993; Finch and Pike, 1996). Even though there are potential problems in using *tmax* to estimate rate of aging, such as biases caused by different in sample sizes, *tmax* remains the best and most widely available measurement to quantify rate of aging (de Magalhaes, 2006).

One factor that correlates with maximum lifespan is body size. Clearly, the typical adult body mass for a species correlates with *tmax* (Fig. 1). In other words, larger animals live, on average, longer than smaller animals, as shown and debated by a large number of authors (Calder, 1984; Schmidt-Nielsen, 1984; Promislow, 1993; Austad, 2005; de Magalhaes et al., 2007a). The logarithmic relation between *tmax* and body mass (*M*), also called the allometry of lifespan, has been the subject of intense scrutiny. From Figure 1, we can obtain the equation: $tmax = 5.58M^{0.146}$ with $r^2 = 0.340$. The squared Pearson correlation coefficient (r) suggests that body mass explains 58% of the variation in *tmax*. Clearly, there are many exceptions to this correlation. One exception are bats that live a lot longer than expected for their body size (Austad and Fischer, 1991; Austad, 2005; de Magalhaes et al., 2007a). Likewise, when compared to mammals, birds live longer than expected for their body size.

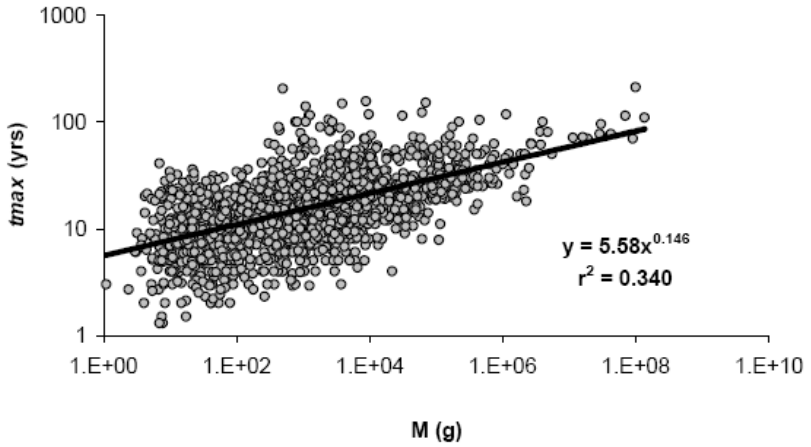

Figure 1: Correlation between maximum lifespan (*tmax*) and typical adult body mass (*M*) using all species (n = 1,701) present in AnAge build 8. Plotted on a logarithmic scale.

At present, the simplest and most likely explanation for the allometry of lifespan is related to ecological constraints: smaller animals tend to be more prone to predation and thus are expected to have higher extrinsic mortality rates, a shorter *tmax*, and a faster aging process--as debated ahead in more detail. For example, the ability to fly gives most birds and bats the capacity to evade predators. Consequently, it seems that body mass is a determinant of ecological opportunities and habitat that impacts on mortality, which consequently influences the evolution of longevity and aging (Stearns, 1992). To date, there is no evidence to suggest that some unknown physiological affects aging in a way proportional to body mass.

Experimentally, the impact of body mass on *tmax* is relevant because it can bias comparative studies of aging (Promislow, 1993; Speakman, 2005). Researchers trying to identify factors that correlate with *tmax* must eliminate the effects of body mass from their calculations, which can be done with certain statistical procedures. In fact, body mass appears to correlate with many life history events besides maximum lifespan: gestation period, time to maturity, etc. Therefore, researchers studying whether a given factor correlates with *tmax* or not must play close attention to the impact of body size. As I discuss in the context of theories of aging, this has not always been done, however, sometimes resulting in erroneous interpretations of experimental results.

Brain mass also correlates with *tmax*, even after correcting for the biases caused by body mass. This is particularly true in primates (Allman et al., 1993). The way brain mass appears to be a better predictor of longevity than body mass is probably due to less variation in brain mass (Lindstedt and Calder, 1981). Therefore, even though it can be argued that this relationship shows the influence of the brain on longevity, it does not prove that the causes of aging are located in the brain. In fact, the size of other organs also correlate with *tmax*, in some cases more strongly than brain size (Austad and Fischer, 1992). Besides, ecological explanations are also possible in that maybe animals with bigger brains are better at escaping predators for a number of reasons.

Even though bigger species tend to be longer-lived than smaller ones, it is interesting to note that there are a number of cases in which smaller animals within a given species live longer in captivity. These include mice, rats, horses, and dogs (Miller, 1999; Miller et al., 2002a; Rollo, 2002). For example, it is well-known that smaller breeds of dogs live longer. Interestingly, it has been argued that "little people" may also be longer-lived (Krzisnik et al., 1999). Therefore, it appears that while on one hand bigger species tend to be longer-lived, within a given species smaller individuals--in protected environments--tend to live longer. The possible physiological and genetic reasons for the latter phenomenon and implications for our understanding of aging are debated in another essay.

Another relationship long studied in gerontology is Kleiber's rule that relates maximum lifespan with metabolic rate (Kleiber, 1975; Gosden, 1996, pp. 103-110). (Kleiber's rule actually originates in a theory of aging called the "rate of living theory," which is discussed in more detail elsewhere.) It can be argued, for instance, that reptilians and amphibians live longer because they have decreased metabolic rates since they are cold-blooded animals. Similarly, if the metabolic rate, the rate at which reactions occur in cells is higher in, for instance, mice than in humans then maybe that is why mice live less than humans (Prinzinger, 2005).

Despite its intuitive nature, there is no evidence that metabolic rates influence aging in endotherms like birds and mammals. First of all, there are gross exceptions: bats and birds live longer than what would be expected for their metabolic rates. In addition, marsupials live less than eutherians and yet have lower body temperatures, which implies a lower metabolic rate (Austad, 1997a, pp. 88-90). Another problem is related to body size. Metabolic rates are often estimated by measuring oxygen consumption at rest. Clearly, an elephant will breath in more oxygen than a mouse, so it is necessary to correct for body mass. Failure to do so will result in oxygen consumption being associated with *tmax* incorrectly--i.e., due to its relation to body mass which in turn correlates with *tmax*. When the effect of body mass is correctly eliminated from metabolic rates metabolic rates do not appear to correlate with *tmax*. In fact, recent results suggest that metabolic rates are not associated with *tmax* in mammals or birds after correcting for the effects of body mass using the most state-of-the-art statistical methods (de Magalhaes et al., 2007a). The exact methodology of these calculations can be attacked--e.g., because to the way metabolic rates are corrected for body mass or even the way *tmax* records are obtained. Nonetheless, there are no results in which metabolic rates are correctly adjusted for body mass that show a correlation between metabolic rates and maximum lifespan in mammals or birds. Kleiber's rule is thus mostly discarded now.

Partly related to metabolic rates, a point of debate is whether hibernating species live longer than non-hibernating species. So far the results are mixed, but some results suggest hibernating animals may live longer (see, for instance, Lyman et al., 1981; Brunet-Rossinni and Austad, 2004; Turbill et al., 2011), which could suggest that a period of metabolic torpor could increase lifespan. On the other hand, it can be argued that spending a fraction of the year in hiding, during which time mortality is presumably low, contributes to the observed longer lifespan in hibernating animals.

Growth and development are two other factors that correlate with *tmax*. Independently of body mass, age at sexual maturity correlates with average and maximum adult lifespan in many taxa, including in mammals (Charnov, 1993; Prothero, 1993; de Magalhaes et al., 2007a). In other words, the longer it takes for a given mammal to reach sexual maturity, the longer it will live afterwards. There are some exceptions, however, such as the male Anthechinus which is mentioned elsewhere. One hypothesis is that there is a mechanistic link between pace of development and pace of aging, as discussed in another essay. It is also worth mentioning that each organism's body-plan is largely determined by its genetic program, and the body-plan can have a powerful influence on longevity, as shown by aphagy in some insects or the semelparity of species like the salmon. Different species could well be influenced by development in different ways: the relation (adult phase)/(total lifespan) shows a wide variation, which is in accordance with the several aging phenotypes found in nature. So development and its consequential body-plan can influence aging to different degrees. The body-plan of mammals, for instance, may place indirect constraints on adult life but this could be regarded as a by-product of development. That said, age at maturity correlates strongly with *tmax* in mammals which hints that common regulatory mechanisms could be involved (de Magalhaes et al., 2007a). Though not as strongly, growth rates also correlate negatively with *tmax*; in other words, species that grow slower tend to live longer (de Magalhaes et al., 2007a). Likewise, growth rates correlate negatively with demographic rate of aging--not MRDT but a similar parameter estimated from the Weibull model (Ricklefs, 2010). On the other hand, for evolutionary reasons, development can be timed similarly to aging even if the relation between development and aging in mammals is indirect and minimal (Miller, 1999). Therefore, the causes for the relationship between developmental time and longevity remain a subject of debate, though it is clear that there is a strong correlation between them.

Recently, the declining costs of DNA sequencing have led to increasingly more powerful approaches in comparative genomics (reviewed in de Magalhaes et al., 2010). Sequencing the genome of an organism is no longer a large-scale endeavour and the genomes of hundreds of species are currently being sequenced. This is exemplified in the sequencing of the long-lived naked mole-rat (Kim et al., 2011). The transcriptome (i.e., RNA) can also be sequenced in a cost-effective fashion to obtain, for example, measures of gene expression levels. Again using the naked mole-rat as an example, transcriptome sequencing revealed that genes associated with oxidoreduction and mitochondria were expressed at higher levels in naked mole-rats when compared to mice, which may contribute to the naked mole-rat's longevity (Yu et al., 2011). The availability of multiple mammalian genomes also opens the door to try to identify gene features associated with longevity. For example, methionine residues in mitochondrially encoded proteins appear to be enriched in short-lived species (Aledo et al., 2011) and cysteine residues appear to be depleted (Moosmann and Behl, 2008). Comparisons between nuclear genomes across species with different lifespans can also focus on identifying genes with patterns of evolution associated with longevity (de Magalhaes and Church, 2007). One genome-wide scan for genes associated with the evolution of longevity in mammals found evidence that proteins involved in protein degradation, a process associated with aging, are under selection in lineages where longevity increased (Li and de Magalhaes, 2012). Given the explosion of genomic data, these approaches are bound to become more powerful and reveal specific genes and patterns associated with longevity. To facilitate comparative studies of aging, including in genomics, our lab has developed the AnAge database which features thousands of longevity records for animals (reviewed in de Magalhaes et al., 2009b). As discussed elsewhere, I think this shift from comparing physiological traits into digital biology will have a major impact in furthering our knowledge of mechanisms of aging.

3. The Evolutionary Theory of Aging

Senescence has no function--it is the subversion of function.

- Alex Comfort

Because aging increases an organism's vulnerability and ultimately leads to its death, as detailed before, it is apparently in contradiction with Darwin's evolutionary theory. After all, how could evolution favor a process that, as happens in most animals, gradually increases mortality and decreases reproductive capacity? How could genes that cause aging evolve? This essay presents and discusses the most important evolutionary models for how aging may have evolved.

Sections

Keywords: ageing, biogerontology, evolutionary biology, genetic dustbin, genotype, immortal germplasm

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Classical Evolutionary Theories of Aging

Although the oldest written argument on the evolution of aging is the work of Russel Wallace (reviewed in Rose, 1991), the problem of how aging evolved was first debated by August Weismann (Weismann, 1891). Weismann's initial hypothesis was that aging evolved to the advantage of the species (e.g., by replacing worn out individuals with younger ones), not the individual, a theory known as group selection. Later, Weismann dropped this concept and instead suggested that aging evolved because organisms that segregate germ and soma must invest additional resources to reproduce instead of maintaining the soma, and this renunciation of the soma results in aging. Weismann's ideas were later comprised by Thomas Kirkwood to become the disposable soma theory, which states that organisms must reach a balance between the resources they invest in soma maintenance and reproduction (Kirkwood, 1977). The disposable soma theory predicts that aging occurs due to the accumulation of damage during life and that multiple defensive or repair mechanisms contribute to aging (Kirkwood and Austad, 2000). But the two key concepts that mark the evolutionary theory of aging came before that.

Drawing on the theories from Weismann and others, like evolutionary biologists Fisher and Haldane, Peter Medawar developed one of such key ideas. The basic observation is that the force of natural selection declines with age (Medawar, 1952). Since all organisms eventually die of diseases, accidents, predation, etc., genes--or gene variants called alleles--beneficial early in life are favored by natural selection over genes beneficial late in life. Exemplifying, imagine a species with an average longevity of 2 years (Fig. 1). There is little evolutionary advantage in having beneficial genes at age 10 because only a small fraction (+/- 3%) of the population will reach that age. On the contrary, genes that are beneficial at age 1 will be strongly selected for by evolution. Following the same reasoning, a gene that kills organisms at age 20 will have little impact on organisms bearing it since very few (+/- 0.08%) will reach such advanced ages and therefore such gene will likely not be eliminated by natural selection. In other words, the greatest contribution to create a new generation comes from young, not

old organisms and so the power of natural selection fades with age, making it possible for hazardous late-acting genes to exist (reviewed in Hamilton, 1966; Rose, 1991; Charlesworth, 1993 & 2000).

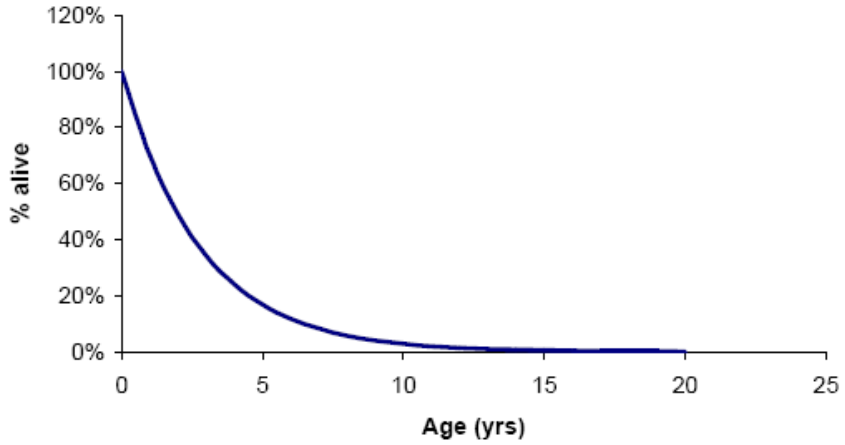

Figure 1: Survival curve showing the percentage of organisms alive at a given age for a hypothetical population assuming a constant mortality rate across the entire lifespan--i.e., no aging.

Another important work was the antagonistic pleiotropy model of George Williams. Since natural selection is weaker at later ages, as demonstrated by Medawar, then perhaps some genes are beneficial at earlier ages but harmful at later ages. Such genes with opposite effects are called pleiotropic genes (Williams, 1957). For example, and using the population of Figure 1, a gene that increases survival to reproductive age or reproductive output will be favored by natural selection, even if it decreases the changes of dying at age, say, 10. Hence, harmful late-acting genes can remain in a population if they have a benefitial effect early in life--e.g., by increasing fitness at early ages or increasing reproductive success. One example are the costly sexual ornaments of male birds that are crucial to attract females, and hence pass one's genes to the next generation, but can be considered handicaps--e.g., male peacocks are limited in their movements which can hinder their ability to escape predators (Zahavi, 1975). Together with Hamilton's mathematical models (Hamilton, 1966), the above models make up the classical evolutionary theory of aging.

Therefore, the evolutionary theory of aging proposes two models for how aging can evolve. One derives from Medawar's ideas in which genetic drift and mutation accumulation lead to the loss of late-acting beneficial genes or to the appearance of late-acting harmful genes. In Williams's model, aging evolves due to the pleiotropic effect of some genes that are beneficial early in life and then harmful at later ages. At present, both theories are widely accepted and they are not mutually exclusive (Gavrilov and Gavrilova, 2002). Some results conducted in *Drosophila*, a fruit fly widely used in aging studies, hint that Medawar's theory of mutation accumulation might be more prevalent over Williams's antagonistic pleiotropy hypothesis (Charlesworth and Hughes, 1996; Hughes et al., 2002), but conflicting results exit (Rose et al., 2002).

Life History Theory

The evolutionary theory of aging is actually part of a broader theoretical framework called life history theory. Life history studies the changes organisms undergo from conception to death, but focuses particularly on the schedule of reproduction and survival (Stearns, 1992; Charnov, 1993). One life history model useful for gerontologists is the concept of *r* and *K* selection that was formally proposed by Robert MacArthur and Edward Wilson (MacArthur and Wilson, 1967; Pianka, 1970; Austad, 1997b). Even though the *r* and *K* selection model is widely recognized as a simplification, it can be useful to interpret certain life history events. In brief, *r*-selection is the density-independent

component of natural selection, which in practice refers to reproductive rate, while *K*-selection is density dependent, referring to the biggest population resources can sustain. Organisms in hazardous environments will maximize reproduction and thus be *r*-selected while organisms in non-hazardous environments will maximize their performance under crowded conditions and thus be *K*-selected. Therefore, *r*-selection will favor rapid development, small body sizes, and a short lifespan while *K*-selection will favor delayed development, larger body sizes, and a longer lifespan (Austad, 1997b). For instance, humans, whales, or elephants are *K*-selected while mice and voles are *r*-selected. If we consider the wide range of lifespans among animals (including mammals), as well as factors correlating with longevity, *r* and *K* selection provide a useful model to begin understanding such variation.

Empirical Evidence For and Against the Evolutionary Theory of Aging

The evolutionary theory of aging is supported by abundant experimental evidence (reviewed in Rose, 1991). In two classical experiments, researchers were able to delay aging in *Drosophila* by only allowing older flies to reproduce (Luckinbill and Clare, 1985; Rose, 1989 & 1991). This way, the force of natural selection would no longer decrease with age and, as predicted by the theory, lifespan was extended and aging delayed. A more recent experiment revealed that selection for longevity also affected reproductive effort, supporting the antagonistic pleiotropy theory (Hunt et al., 2006). Also in accordance with the theory, Steven Austad observed that opossums, a North American marsupial, living in a predator-free island reproduced later than animals of the same species on the more hazardous mainland. As determined by collagen elasticity, these animals appeared to age slower than the continental opossum (Austad, 1988; Austad, 1997a). Overall, for the majority of species studied, the classical models of Medawar and Williams based on the fading force of natural selection appear to explain the observations. As detailed below, however, there are some exceptions.

One extreme example among life history strategies are animals that reproduce only once, as mentioned earlier. Semelparous species appear to fit life history theory as examples of ecological adaptation to certain life history conditions. For example, if due to high extrinsic mortality attaining reproductive maturity is unusually difficult and not likely to be repeated more than once (Austad, 1997a, p. 117). Another scenario is one in which mortality is significantly lower in juveniles than in adults--e.g., due to different habitats or predators--and so evolution will favor organisms that spend most of their lifespan as juveniles. An example of the latter is *Dolania americana*, a mayfly with a lifespan of 2 years in which the adult stage lasts ~2 hours (McKinney and McNamara, 1991, pp. 194-196). On the other hand, there is evidence that some cases of semelparity might be a result of group selection. The idea that aging evolved for a purpose goes against classical evolutionary models of aging, but perhaps some cases could be considered as such (reviewed in Bowles, 2000; Goldsmith, 2004; Longo et al., 2005). For instance, adult moths mimic the movements of the juvenile forms presumably to attract predators and there are a few documented cases of insects in which the offspring eats its mother (Hayflick, 1994, pp. 26 & 215). Therefore, while evidence of group selection is largely absent for most species, such as mammals, we cannot dismiss it from playing a role in the evolution of aging of a subset of short-lived species.

There is some experimental evidence against the evolutionary theory of aging. Although Medawar suggested that aging was controlled by a few, key physiological processes (Medawar, 1955), modern evolutionary theory of aging argues that aging is multifactorial (Rose, 1991; Kirkwood and Austad, 2000). In other words, the evolutionary theory of aging postulates that numerous small-effect genes, rather than a few strong-effect ones, are involved in aging. Hence, the way single gene knock-outs have been shown to delay aging in animals, which is detailed elsewhere, was in contradiction with at least some aspects of the evolutionary theory of aging (reviewed in Johnson, 2002). In addition, some genetic manipulations appear to delay aging while not affecting reproduction (Dillin et al., 2002; Marden et al., 2003; Simon et al., 2003), which also contradicts the evolutionary theory of aging. Eusocial animals like ants that have a single reproductive female also provide evidence against trade-offs between longevity and reproduction; one study even found that mating increases longevity in ant queens (Schrempf et al., 2005). Lastly, it was shown in guppies that animals with higher extrinsic

mortality rates evolved earlier maturity and invested more in reproduction, as expected, but do not have an earlier onset of demographic or reproductive aging, which contradicts the evolutionary theory of aging (Reznick et al., 2004). In addition, the classical evolutionary theory of aging does not explain why aging, a phenotype that escaped natural selection, is so similar among mammals, as described before.

One of the most intriguing phenotypes in the biology of aging are animals that appear not to age, as previously discussed. Studies conducted both in captivity and in the wild have shown that several species of fishes, amphibians, and reptiles, to name only vertebrates, fail to show signs of aging. Of course, these animals have only been studied for a limited amount of time. Still, it is surprising to find that, in a 50-year study, female Blanding's turtles increased both survivorship and reproductive output with age (Congdon et al., 2001). Another 38-year field study suggested that older female Painted turtles, when compared to younger animals, feature increased reproductive output and offspring quality while maintaining survivorship (Congdon et al., 2003). Classical evolutionary models of aging predict that all species eventually age (Hamilton, 1966). It has also been argued that since bacteria age, all other organisms must age (Stewart et al., 2005). This idea seems a bit counter-intuitive since it assumes that complex species must age if less complex species age; it seems more logical that highly complex species have a greater capacity to replace their components, such as cells, and hence avoid aging. Nonetheless, the observations clearly suggest some species may not age, which is in contradiction with the evolutionary theory of aging. It is also difficult to reconcile the disposable soma theory with these observations of increased reproduction and survival with age. Moreover, some have argued that germ cells in mammals could originate in somatic cell precursors (Bukovsky et al., 2005), so the soma-germ discrimination may be overly simplistic.

In conclusion, the evolutionary theory of aging offers a theoretical framework that explains many--perhaps most--observations and remains a major theoretical landmark in gerontology. The theory offers clues as to the evolutionary mechanisms and the events leading to the evolution of aging, yet it does not offer a complete picture on the evolution of aging across different species. Moreover, the evolutionary theory of aging can be harmful by imposing limitations on aging studies (Gavrilov and Gavrilova, 2002). As it stands, the evolutionary theory of aging cannot be safely used to make predictions on the biology of aging (Le Bourg, 2001). Evolutionary theories of aging are not predictive, they are descriptive. For instance, it has been argued that non-aging animals, especially those that increase size and fertility with age, may be favored by natural selection, thus contradicting the classical evolutionary theory of aging (Vaupel et al., 2004). In species with a high infant mortality and long generation times, an adult animal is precious and worth preserving; if reproductive output increases with age, natural selection will favor preservation rather than immediate reproduction. The impact of intergenerational transfers--e.g., nurturing--has also been suggested as an important factor that must be taken into consideration (Lee, 2003). Therefore, new evolutionary models of aging continue to be proposed and the evolutionary theory of aging will certainly continue to evolve. Besides, a major open question concerns the precise genetic mechanisms and specific genes underlying the evolution of aging and species differences in aging, as discussed elsewhere.

The Unique Evolution of Mammalian Aging

Since humans are mammals, of special interest is the evolution of mammalian aging. As mentioned before, the aging phenotype of mammals has some common features that may be a result of unique evolutionary events. What follows is a particular model that aims to explain the evolution of aging in mammals based on the classical evolutionary models of aging.

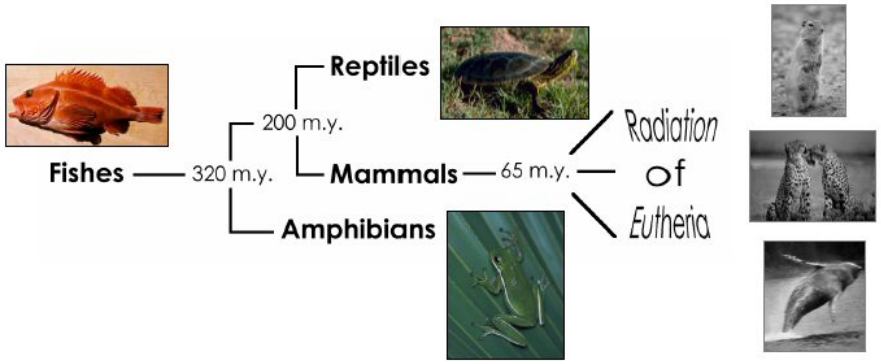

Figure 2: Overview of the evolution of mammals and closely-related taxa. Lines are not to scale. (Adapted from Hedges, 2002; Maddison and Schulz, 2004.) Images source: green tree frog (Jane Rohling), Western painted turtle (Gary Stolz), ground squirrel (John and Karen Hollingsworth), humpback whale (Robin Hunter), cheetah (Gary Stolz), U.S. Fish and Wildlife Service.

Mammals evolved from reptiles (Fig. 2), a taxon with many apparently non-aging species (e.g., Congdon et al., 2001 & 2003). On the contrary, and as described before, all known mammals age. In fact, the intensity and incidence of aging appears to be higher in mammals than in reptiles. This is surprising since mammals can be long-lived. The long lifespan of mammals also suggests the incidence of aging in mammals is not an accident, as proved by the number of old mammals that can be found in the wild (Nesse, 1988; Spencer and Promislow, 2002); in particular among long-lived mammals aging does limit somewhat the natural lives of animals (Turbill and Ruf, 2010). Moreover, a careful analysis of the aging phenotype of mammals and reptiles reveals an extraordinary contrast (Table 1). For example, reproductive senescence, in the form of no oocyte regeneration, is thought to occur in all studied mammals, but not in reptiles. Continuous tooth development is another common feature of reptiles absent from nearly all mammals. Therefore, some authors have found it bizarre that all studied mammals feature aging when more primitive species such as fishes and reptiles appear to avoid it (Hayflick, 1994, p. 23). Others too have wondered why some mammals can be found senescent in the wild (Finch, 1990).

Mammals	**Reptiles**
Increase in mortality with age	No increase in mortality with age
No oocyte regeneration	Oocyte regeneration
Limited tissue regeneration	Limb regeneration
Two sets of teeth	Continuous tooth replacement

Table 1: General observations of the aging phenotype across the *Mammalia* and *Reptilia* classes (de Magalhaes and Toussaint, 2002).

One hypothesis is that the evolution aging in mammals is a unique event shaped by *r*-selection during the dinosaur's rule. During the first two thirds of mammalian history, when the dinosaurs and large reptilians ruled the earth, mammals were small nocturnal animals about the size or even smaller than modern mice and rats (Rougier and Novacek, 1998). Certainly, these early were on the bottom of the

food chain, meaning high mortalities that, as predicted by classical evolutionary models of aging, fostered the evolution of a rapid aging phenotype (Fig. 3). During this period, it is possible that selection for early reproduction rather than survival shaped mammalian aging whose effects last until today. In other words, the large evolutionary time as small, short-lived animals allowed aging to develop an intensity in mammals not seen in reptiles with similar average lifespans or body sizes (de Magalhaes and Toussaint, 2002).

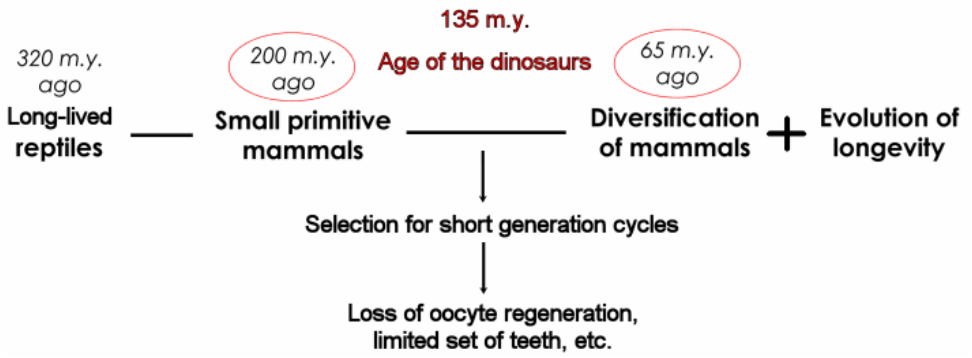

Figure 3: Model for the evolution of mammalian aging (de Magalhaes and Toussaint, 2002).

The process by which this fostering of aging occurred is opened to speculation: it could have been genetic drift caused by decreased evolutionary pressure at later ages, it could have been mutation accumulation, it could have been antagonistic pleiotropy, etc. Once the dinosaurs disappeared, ~65 million years ago, mammals took over the world and, in some species such as humans, whales, and elephants, longevity could evolve. Nonetheless, the consequences of this phenomenon of *r*-selection in early mammals are visible in modern mammals (Table 1). This hypothesis explains why reptiles feature apparently non-aging organisms and some traits associated with long lifespans, such as oocyte regeneration, that are absent from all studied mammals and why, in contrast, even whales, the longest-lived mammal, reach menopause and age. Moreover, this model may help explain why mammals lost part of their tissue regeneration capacity when compared to, for instance, amphibians and reptiles (Brockes et al., 2001). It also explains why there are so many similarities in the aging process of mammals since it is postulated that the aging process of mammals has, to some degree, a common origin. Lastly, it is possible that reptiles feature unique mechanisms to delay aging and age-related debilitation, which makes reptiles an intriguing model to study aging, as discussed elsewhere.

A Few Reading Suggestions on Evolutionary Biology

Darwin, Charles; "On the Origin of Species" (1859). Make sure you get the first edition as it is considered the best. There is an online version available at the classics section of the Online Literature Library.

Dawkins, Richard; "The Blind Watchmaker" (1986).

Dawkins, Richard; "The Selfish Gene" (1989).

Ridley, Matt; "The Red Queen: Sex and the Evolution of Human Nature" (1995).

4. Human Aging Model Systems

I want to know why we, humans, age and how we can fight and ultimately cure human aging. The goal of biomedical research is to improve the human condition and great emphasis is placed on translating findings into human applications. As such, gerontology must be more than curiosity-driven, even though the use of model organisms is inevitable. In this essay, I present the different model systems used to study human aging and debate their strengths and pitfalls.

Sections

Keywords: ageing, biogerontology, insects, Homo sapiens, non-human primates, translational science, vertebrates

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One major difficulty in studying human aging is its duration, particularly because researchers themselves are aging and have a limited lifespan. Because researchers cannot conduct experiments in humans in the same way drugs aimed at cancer or AIDS can be tested in clinical trials, most biogerontologists resort to model systems and then extrapolate data from these different models into humans. The choice of models, however, is diverse and highly controversial (Gershon and Gershon, 2000b; Partridge and Gems, 2007). The major model systems used to study human aging are: 1) human cells; 2) unicellular organisms such as the yeast *Saccharomyces cerevisiae*; 3) the roundworm *Caenorhabditis elegans* 4) the fruit fly *Drosophila melanogaster* 5) rodents such as mice (*Mus musculus*) and rats (*Rattus norvegicus*). The small size and short life cycles of these organisms--even mice do not commonly live more than 4 years--make them inexpensive subjects for aging studies, and the ability to genetically manipulate them gives researchers ample opportunities to test their theories and unravel molecular and genetic mechanisms of aging. Nonetheless, the employment of inadequate models to study human aging can be catastrophic for research since it can shift the focus of gerontology to pathways that, even though relevant in a certain model, may be irrelevant in humans.

Studying Human Aging in Humans

Because the human aging process takes decades to develop it is virtually impossible to study it in vivo. Researchers can describe human aging and investigate age-related pathologies, as mentioned before. Longitudinal studies follow individuals throughout their lives while cross-sectional studies compare young and old individuals. Both types of studies are observational, not mechanistic. There are also genetic studies of longevity in humans (Puca et al., 2001; Perls et al., 2002; Perls 2006), but as detailed elsewhere longevity is only a proxy for aging and the true relevance of these studies to understand the human aging process remains to be established.

One major model system of human aging are human cells, which have as major advantage the fact that researchers can concentrate on human biology. It is reasonable to assume that human aging has a cellular basis (de Magalhaes, 2004; de Magalhaes and Faragher, 2008). Nonetheless, cellular models and the methods used to study them have several caveats, and so in vitro results may not be representative of what happens in vivo (Mondello et al., 1999). Succinctly, the major argument against cellular models is that most cellular models of aging, such as replicative senescence, are based on measurements of cell proliferation which are not necessarily a measurement of vitality (Cristofalo, 2001). Cancer, for instance, is derived from rapid, uncontrolled cellular proliferation. Other methods exist to measure aging in cells, such as stress resistance, but the relevance of these methods to

organismal aging remains to be demonstrated (de Magalhaes, 2004). Cellular models of aging will not be covered in detail here as they are discussed in detail in another essay.

Studying Human Aging in Model Organisms

Human studies should always be preferred, but model organisms are simply unavoidable in gerontology. Much of what we know about aging today derives from organisms like yeast, mice, rats, fruit flies, and roundworms (Fig. 1). These traditional biomedical model organisms benefit from having widespread resources, reagents and protocols that allow studies to be conducted in a faster and cheaper way. Scientifically, there are also advantages, such as the possibility of employing large-scale genetic screens and functional genomics (for a review de Magalhaes, 2009), that are not possible in humans or in other non-traditional model organisms. To give an example, thousands of genes (Hamilton et al., 2005) and drugs (Petrascheck et al., 2007) can be screened for effects on worm lifespan.

The downside of model organisms, of course, is that it is nearly impossible to tell whether an organism is representative of human aging or not. It has been argued that similar mechanisms operate across many species (Longo, 1999; Longo and Fabrizio, 2002), while others have proposed that some aging mechanisms (called "public") are common to all species while others are unique ("private") of each species (Martin et al., 1996). Since the basic blocks of life are common to most known species, common pathways might be involved in aging across phylogeny (Tissenbaum and Guarente, 2002). Could it be that the weakest pathway succumbing to senescence is the same in all organisms? Such hypothesis is hard to believe based on the huge diversity of aging phenotypes found in Nature, and certain animals appear to age for different causes than us. For example, the male Australian mouse (*Antechinus stuartii*) has a bizarre aging phenotype, as mentioned elsewhere. The rapid death following reproduction observed in *Antechinus* and in other species is much different from the gradual waning of humans and so *Antechinus* is not a good model of human aging as different mechanisms are likely involved. The traditional biomedical model organisms (Fig. 1) all exhibit a gradual decline but the question remains of whether they are accurate paradigms of human aging.

Figure 1: Major model organisms of aging: yeast (top left), roundworms (top right), fruit flies (bottom left), and mice (bottom, right). The picture of *C. elegans* is used with permission from the copyright holders Juergen Berger and Ralf Sommer, Max-Planck-Institute for Developmental Biology, Tübingen, Germany. The picture of a fruit fly was taken by André Karwath and the picture of *S. cerevisiae* was taken by Maxim Zakhartsev and Doris Petroi, International University Bremen, Germany.

Even in animals that age gradually there is no *a priori* reason to expect them to age for the same causes and mechanisms as humans (Gershon and Gershon, 2000a). One problem is that all models organisms are considerably shorter-lived than humans and were developed for laboratory research

based on their high fertility. Not only this means that different evolutionary processes acted on these organisms and on humans (see Austad, 1997b for arguments), but selection for fertility may have also selected for short lifespans in laboratory strains that generate biases in aging studies. In other words, it has been argued that the life-extending gene variants found in these organisms may be simply restoring lifespan to what is normally found in the wild (Spencer and Promislow, 2002). The fact that wild-derived mouse strains take longer to reach sexual maturity and live significantly longer than common laboratory strains supports this view (Miller et al., 2002b). On the other hand, worm strains (N2) seem to have been adapted to lab conditions (Chen et al., 2006), yet even wild-derived nematodes are long-lived under CR (Sutphin and Kaeberlein, 2008). Interestingly, CR extends the maximum lifespan of wild-derived mice but not average lifespan, suggesting that genotype impacts on CR effects (Harper et al., 2006). A related problem is that laboratory strains are often genetically homogeneous, which provides more consistent results, but also gives rise to discrepancies between strains on the effects of genes or interventions on aging (Partridge and Gems, 2007).

Human physiology can be much different than that of model organisms. Clearly, the phenotypes of aging in yeast and humans are totally unalike (Gershon and Gershon, 2000b). Not even researchers working on yeast expect yeast aging to be a perfect mirror of human aging (Sinclair, 2002; Jazwinski, 2005), and some of the same criticisms aimed above at human cells can be extrapolated to yeast. *Drosophila* and *C. elegans* are mostly composed of post-mitotic cells, which means that not only they do not normally have cancer or many other age-related diseases, but it raises doubts on how valid results obtained in these animals are when extrapolated to humans. It is possible that some general pathways of aging are conserved between all organisms, as discussed below, but certainly not all age-related changes overlap. At present, it is impossible to tell for sure if a given model organism is accurate or how accurate it is. It does appear reasonable to assume that species evolutionary closer to humans, such as mice, have higher chances of being representative of human biology than evolutionary distant species such as yeast.

It can be argued that even if the aging process is not the same in distant organisms and humans, studies in lower life forms can help us understand the dynamics and structure of human aging. If aging is the corruption of life, then understanding how life itself works may help us understand aging. Much of what we know about the biochemistry of life--e.g., DNA replication and repair--came from studies in lower life forms such as bacteria and yeast. Hundreds of genes that modulate aging have been identified in model organisms (de Magalhaes et al., 2009a), such as in yeast (Jazwinski, 2001), *C. elegans* (Johnson, 2002), *Drosophila* (Tower, 2000), and mice (Liang et al., 2003; Hasty and Vijg, 2004). These findings can serve as the basis for research into mammals and humans (Sinclair, 2002; Tissenbaum and Guarente, 2002; Butler et al., 2003; Warner, 2003; Liu et al., 2005). Some of these findings, such as CR and the insulin-signalling pathway (Longo and Fabrizio, 2002; Butler et al., 2003), appear to be conserved between different species while others have not been demonstrated in mammals (see below). Therefore, some genes and mechanisms of aging found in model organisms may turn out to be relevant to humans, though studies on the mechanisms of aging in lower life forms must be corroborated in mammalian models before extrapolating into human aging. For example, some pathways and genes may be conserved between invertebrates and humans, but functions may be different and the higher complexity of mammals means that even if functions are conserved human pathways will have unique features not present in lower life forms. Gene expression analyses of longevity-assurance mechanisms in flies, worms and mice reveal some, yet few, overlapping pathways (McElwee et al., 2007). Similarities in pathways related to aging in different organisms could also be due to similar selection processes that derive from domestication (Reznick, 2005). Clearly, explaining human aging based on research in model organisms, even mice, is problematic.

The telomeres, described in more detail elsewhere, are the perfect example of the limitations of model systems in aging research. Clearly, they are major players in cellular senescence in human cells as well as other organisms. Telomere dysfunction in *S. cerevisiae* due to mutations in telomerase, an enzyme critical for telomere maintenance, leads to senescence (Lundblad and Szostak, 1989; Lowell and Pillus, 1998). In *C. elegans*, telomerase defects appear to result in sterility after a certain number of generations (Ahmed and Hodgkin, 2000), but a role of telomeres in its aging process has not been

established. In *Drosophila*, telomere maintenance does not appear to involve telomerase, though other proteins involved in telomere maintenance may overlap with those of humans (Cenci et al., 2005). Telomerase-deficient mice are normal up to four generations and then show some signs of accelerated aging (Blasco et al., 1997; Rudolph et al., 1999), but telomerase overexpression does not alter aging in mice (Artandi et al., 2002). On the other hand, telomerase dysfunction in humans causes a disease called dyskeratosis congenita, which shares some features with the sixth generation telomerase-deficient mice (Marrone and Mason, 2003). It is therefore nearly impossible to determine the impact of the telomeres and telomerase in human aging based on model systems. Even between mice and humans, there are similarities but also differences in the biological outcomes of telomerase deficiency.

Although aging in different mammals is often phenotypically similar, as mentioned before, even eutherians may have different mechanisms of aging. As argued by others (Reznick, 2005), candidate genes emerging from a given model organism are an answer, not the one and only answer. Nonetheless, the bulk of research in mammals is based in short-lived animals like mice and rats whose evolutionary forces acting on aging may have been different from those acting in humans (Austad, 1997b). Besides, there are numerous examples of research in animals, including mammals, being inadequate to humans (Pound et al., 2004). The phenotypic differences mentioned above between mice and humans due to telomerase deficiency are a perfect example. As proven time and time again in other biomedical fields, what occurs in the aging process of other mammals may not be representative of human biology (reviewed in Davenport, 2003). Therefore, if we base our understanding of human aging on model organisms, even mammalian models, we must be careful about extrapolating findings to humans.

Unfortunately, there is no simple solution to the issues mentioned above. Gerontology continues to be dependent on model systems. While these have provided--and will continue to provide--clues regarding mechanisms of aging and even targets for drug discovery with potential human applications (reviewed in de Magalhaes et al., 2012), findings from model organisms must be interpreted with caution. One of the reasons why the mechanisms responsible for human aging remain largely a mystery is the lack of unambiguous models where scientists can test their hypotheses and the controversy relating to the interpretation of findings in current model organisms.

Underrated Models of Aging: Reptiles, Naked Mole-Rats and Whales

One of the problems of research on aging is that it is based on only a handful of models. Definitely, more models of aging would be invaluable to gerontology, particularly models biologically closer to humans. For example, employing other primate models in the study of aging could open new opportunities for research on aging because we are biologically closer to them; some primate species, such as marmosets, have been shown to age considerably faster than humans (Austad, 1997c; Tardif et al., 2011). It is not simple, however, to develop new models for studying aging or new biomedical models for that matter. As mentioned above, a variety of resources such as protocols and reagents are available for traditional biomedical models and developing such resources for a new model organism costs time and money. It would be worthwhile investment, though, and marmosets for instance are being developed as a model for aging (Tardif et al., 2011).

Previously, I argued that reptiles, in general, age slower than mammals. Not only reptiles age slower than size-equivalent mammals, but several reptilian families feature apparently non-aging animals with traits associated with long lifespans such as continuous tooth replacement. As such, it is possible that mammals lack certain anti-aging mechanisms that are present in some or all reptiles (de Magalhaes and Toussaint, 2002). A few studies in reptiles have already began to explore mechanisms that may contribute to the delayed reptilian aging (see Lutz et al., 2003). For instance, neurogenesis is predominant in reptiles (Font et al., 2001). Other studies found unique traits in reptiles that could be useful to humans: crocodiles have been shown to possess novel antimicrobial peptides (Shaharabany et al., 1999). Clearly, long-lived reptiles are an underestimated model for the study of aging and more attention should be given to study reptilian aging or the apparent absence of aging of some species.

Other long-lived animals could be extremely useful models of aging not so much to discover what causes aging but to investigate how we can fight it. In other words, traditional biomedical models provide clues about the mechanisms of aging and disease, yet an unexplored paradigm in biomedical research is the use of disease-resistant organisms to identify genes, mechanisms and processes that protect against disease. As mentioned before, some amphibians and fishes also feature negligible senescence (reviewed in Finch, 1990; Cailliet et al., 2001). Since amphibians and fishes are evolutionary further from us than reptiles, they are not, *a priori*, better models but can still prove useful. For example, amphibians can regenerate entire limbs while mammalian tissues, such as muscle, can only regenerate as isolated entities (reviewed in Brockes et al., 2001; Carlson, 2003). Lastly, birds too have been proposed as potentially useful models for understanding human aging due to their slow aging rates when compared to size-equivalent mammals (Holmes and Austad, 1995; Holmes et al., 2001). With the emerging age of genomics, employing these long-lived species in studies of aging is becoming a reality (de Magalhaes et al., 2010), for example via bioinformatics analyses (de Magalhaes and Toussaint, 2004b).

Long-lived mammals, although none avoid aging completely, may also prove extremely useful to unravel the mechanisms of longevity and healthy aging. In 2007, myself and others proposed the sequencing of long-lived mammals and specifically of the naked mole-rat (*Heterocephalus glaber*), the white-face capuchin monkey (*Cebus capucinus*) and the bowhead whale (*Balaena mysticetus*) (de Magalhaes et al., 2007b). Genome and RNA sequencing of the naked mole-rat has since revealed insights and candidate genes regarding the evolution of longevity (Kim et al., 2011; Yu et al., 2011). In particular, it appears that genes associated with oxidoreduction and mitochondria are expressed at higher levels in the naked mole-rat which might contribute to their long lifespan (Yu et al., 2011). Naked mole-rats also exhibit an extraordinary resistance to cancer (Buffenstein, 2008), and studies are beginning to unravel the anti-tumour mechanisms of naked mole-rat cells (Seluanov et al., 2009). For example, they are more resistant to experimental tumorigenesis with oncogenes (Liang et al., 2010). As the longest-lived mammal, bowhead whales are equally fascinating and, because of their massive size, whales must have evolved anti-tumor mechanisms not present in humans (reviewed in Caulin and Maley, 2011). Though for obvious reasons not easy to study experimentally, bioinformatics and genomics can provide clues about the genetic and molecular mechanisms that allow whales to live longer than humans and resist cancer (de Magalhaes et al., 2007b). Cellular studies could also be useful for studying long-lived species whose study as organisms would be practically impossible (Austad, 2001).

It may be asked: why learn from species that are behind us in the race for immortality? It has been proposed that species without detectable senescence can "teach" us much more than species with short lifespans (Strehler, 1986). Even in species such as whales, elephants or naked mole-rats their evolution of longevity might have followed different pathways than in humans; studies in these species can lead to the discovery of new ways to combat against age-related diseases and, crucially, in the fight against aging itself, the only disease we all suffer from. The current explosion in genomics also makes large-scale studies possible, including in comparative genomics. Research on several of these species can then allow hypothesis to be tested in traditional model systems, for example by creating cell lines or mice with genes from long-lived species that if successful can then be extrapolated to humans. Ultimately, animal models of resistance to disease and aging may prove valuable for human disease prevention and aging retardation.

4.1 Cellular Senescence

Because cells are the fundamental building blocks of our bodies, it is logical to assume that cellular changes contribute to the aging process. In this essay I review the methods used to study cellular aging in vitro, in particular replicative senescence, and debate whether these findings could be related to organismal aging.

Sections

Keywords: ageing, cell immortality, cell immortalization, cellular immortality, cytogerontology, Hayflick phenomenon

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The Hayflick Limit

In 1961, and in contradiction to what was thought at the time, Leonard Hayflick and Paul Moorhead discovered that human cells derived from embryonic tissues could only divide a finite number of times in culture (Hayflick and Moorhead, 1961). They divided the stages of cell culture into Phases I-III. Phase I is the primary culture, when cells from the explant multiply to cover the surface of the culture flask--most cell types grow in the lab attached to a solid surface. Phase II represents the period when cells divide in culture. Briefly, once cells cover a flask's surface, they stop multiplying. For cell growth to continue, the cells must be subcultivated. To do so, one removes the culture's medium and adds a digestive enzyme called trypsin that dissolves the substances binding cells together. After adding growth medium and pipetting one obtains the cells in a homogeneous suspension that are then divided by two--or more--new flasks. Cells then attach to the new flasks' surface and start dividing once again until a new subcultivation is required. Most cells divide vigorously and can often be subcultivated in a matter of a few days. After several months, however, cells start dividing slower, which marks the beginning of Phase III. Eventually cells stop dividing at all, though they may or may not die (reviewed in Hayflick, 1985 & 1994). Hayflick and Moorhead noticed that cultures stopped dividing after an average of 50 cumulative population doublings (CPDs)--splitting one flask of cells into two new flasks of the same size increases the CPDs by one, splitting by four flasks increases the CPDs by two and so on. This phenomenon of growth arrest after a period of apparently normal cell proliferation is known as the Hayflick limit, Phase III phenomenon, or, as it will be called herein, replicative senescence (RS).

Hayflick and Moorhead worked with fibroblasts, a cell type found in connective tissue widely used in research, but RS has been found in other cell types: keratinocytes, endothelial cells, lymphocytes, adrenocortical cells, vascular smooth muscle cells, chondrocytes, etc. In addition, RS is observed in cells derived from embryonic tissues, in cells from adults of all ages, and in cells taken from many animals: mice, chickens, Galapagos tortoises, etc. (reviewed in Hayflick, 1994). The number of CPDs cells undergo in culture varies considerably between cell types and species. Early results suggested a relation between CPDs cells could endure and the longevity of the species from which the cells were derived. For example, cells from the Galapagos tortoise, which--as described--can live over a century, divide about 110 times (Goldstein, 1974), while mouse cells divide roughly 15 times (Stanley et al., 1975; Rohme, 1981)--but see more recent studies below. In addition, cells taken from patients with progeroid syndromes such as Werner syndrome (WS)--described elsewhere--endure far fewer CPDs than normal cells (Salk et al., 1981). Exceptions exist and certain cell lines can divide indefinitely

without reaching RS. These are said to be "immortal" and include embryonic germ cells and most cell lines derived from tumors, such as HeLa cells (Brunmark et al., 1986; Chen and Yu, 1994; Pera et al., 2000). Some types of rat cells have also been claimed as capable of evading RS (Mathon et al., 2001; Tang et al., 2001), and more recently some mouse cells have been found to be immortal under certain culture conditions (Parrinello et al., 2003), as detailed below.

Biomarkers of Cell Senescence

The discovery of RS sparked considerable interest and the phenotype of cell senescence in human fibroblasts has been characterized by a series of features, termed biomarkers d'Adda di Fagagna, 2007). The most obvious biomarker is growth arrest, i.e., cells stop dividing, which can be detected by different methods. Even vigorously dividing cultures are heterogeneous and contain a percentage of growth-arrested cells; this percentage progressively increases until all cells in the population are quiescent, that is, they have stopped dividing (Cristofalo and Sharf, 1973; Smith and Whitney, 1980); interestingly, the percentage of growth-arrested cells is higher in cells from patients with progeroid syndromes, such as WS cells, when compared with normal cells at the same CPD (Kill et al., 1994). Senescent cells are growth arrested in the transition from phase G1 to phase S of the cell cycle (Sherwood et al., 1988). The growth arrest in RS is irreversible in the sense that growth factors cannot stimulate the cells to divide (reviewed in Cristofalo and Pignolo, 1993), even though senescent cells can remain metabolically active for long periods of time (reviewed in Goldstein, 1990).

Another important biomarker is cellular morphology (Fig. 1). While cells age in vitro they endure progressive morphological changes (e.g., Bayreuther et al., 1988). Briefly, senescent cells are bigger and a senescent population has more diverse morphotypes than cells at earlier CPDs. In fact, a confluent senescent culture has a smaller cellular density than a confluent young culture, though this also occurs because senescent cells are more sensitive to cell-cell contact inhibition.

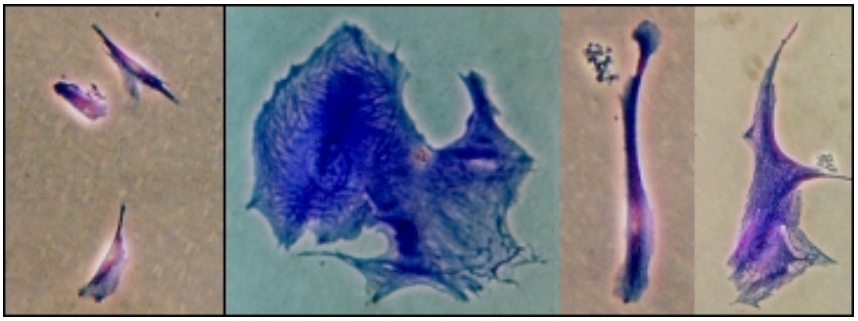

Figure 1: Normal human fibroblasts (left) and fibroblasts showing a senescent morphology (three cells on the right). Notice the common elongated morphology of senescent cells.

One widely-used marker of RS is senescence-associated β-galactosidase (SA β-gal) activity. The enzyme β-galactosidase, a lysosomal hydrolase, is normally active at pH 4, but in senescent cells it often happens for β-galactosidase to be active at pH 6 which can be detected with a simple biochemical assay. Both in vitro and in vivo, the percentage of cells positive for SA β-gal increases with, respectively, CPDs and age (Dimri et al., 1995). Conversely, in immortal cell lines, such as HeLa tumor cells, the percentage of cells positive for SA β-gal does not correlate with CPDs. The increase in SA β-gal also correlates with the appearance of the senescent morphotypes (Toussaint et al., 2000). Lysosomes are organelles that break down cellular junk. Early reports showed that lysosomes increase in number and size in senescent cells (Robbins et al., 1970; Brunk et al., 1973). SA β-gal appears to be a result of increased lysosomal activity at a suboptimal pH, which becomes detectable in senescent cells due to an increase in lysosomal content (Kurz et al., 2000). Other results also suggest that during in vitro aging increased autophagy--i.e., digestion of the cell's organelles;

discussed elsewhere--may be associated with an increase of lysosomal mass and SA β-gal (Gerland et al., 2003).

Normal human cells are diploid, which means they have two copies of each chromosome. With each subcultivation the percentage of polyploid cells--i.e., with three or more copies of chromosomes--has been shown to increase (Matsumura, 1980). Deletions in the mitochondrial DNA (mtDNA) have also been observed both during RS (Dumont et al., 2000a) and during aging in vivo, at least in some tissues (Corral-Debrinski et al., 1992; Yang et al., 1994; Liu et al., 1998).

The expression levels of several genes change during in vitro cellular aging (reviewed in Cristofalo et al., 1998a; Campisi and d'Adda di Fagagna, 2007). One important type of gene overexpressed in senescent cells are inflammatory regulators like interleukin 6 (IL6) (Shelton et al., 1999; de Magalhaes et al., 2004); some studies support a role for proinflammatory proteins secreted by senescent cells in driving senescence, which may lead to positive feedback loops and to senescence induction in normal cells near senescent cells (Acosta et al., 2008; Kuilman et al., 2008; Freund et al., 2010). Senescent cells also display an increased activity of metalloproteinases which degrade the extracellular matrix (reviewed in Campisi, 1999). On the other hand, senescent cells have a decreased ability to express heat shock proteins (Choi et al., 1990; Bonelli et al., 1999).

Telomeres are non-coding regions at the tips of chromosomes. In vertebrates, they are composed of repeated sequences of TTAGGG (Moyzis et al., 1988; Meyne et al., 1989). During in vitro aging, the telomeres shorten gradually in each subcultivation (Harley et al., 1990). The same process might occur in vivo too (Hastie et al., 1990; Lindsey et al., 1991; Allsopp et al., 1992). Telomere shortening is the primary cause of RS in human fibroblasts, and given their importance, telomeres and their role in aging are discussed in detail in another essay.

Stress-Induced Premature Senescence

Assuming human fibroblasts endure 50 CPDs, 2^{50} is more than enough cells for several lifetimes (Hayflick, 1994). However, a number of factors can accelerate and/or trigger cell senescence, one of which is oxidative stress. Normally, cell culture conditions include 20% oxygen (O_2) and these were the conditions initially used by Hayflick and Moorhead and most subsequent studies. When human fibroblasts are cultured at 3% O_2, which is closer to physiological conditions, they achieve a further 20 CPDs (Chen et al., 1995). Conversely, different types of human cells cultured above 20% O_2 display a reduced growth rate and endure fewer CPDs (Horikoshi et al., 1986 & 1991; von Zglinicki et al., 1995). If O_2 is above 50%, in fact, it becomes cytotoxic (Horikoshi et al., 1991). The way subcytotoxic stress can accelerate the appearance of the senescent phenotype in cells has been deemed as another form of cellular senescence called stress-induced premature senescence (SIPS; see Fig. 2) (Brack et al., 2000).

Figure 2: Schematic drawing of SIPS. Source: Ageing and Stress Group, University of Namur, Belgium.

Not surprisingly, depending on the dose of stressor used, a cell population will react in different ways. For instance, a high cytotoxic dosage will cause such an amount of damage that cellular biochemical activities decrease leading to cellular death by necrosis. The level of damage sustained by cells determines whether programmed cell death--apoptosis--can unfold or, if the damage is lower, senescence. Since a cellular population is not homogeneous, the dosage of the stressor will shift the percentage of cells executing each of the possible programs depending on the amount of stress, respectively from no stress to high stress: cellular proliferation, senescence, apoptosis, and necrosis (reviewed in Toussaint et al., 2002a).

In addition to O_2, other sources of oxidative damage, such as H_2O_2 and tert-butylhydroperoxide, and other stressors--e.g., ethanol, ionizing radiations, and mitomycin C--can induce SIPS in many types of proliferative cells such as lung and skin fibroblasts, endothelial cells, melanocytes, and retinal pigment epithelial cells (reviewed in Toussaint et al., 2002b; Dierick et al., 2003). The list of stressors that can cause SIPS is constantly growing. Instead of chronic stress, SIPS can be induced based on a single or repeated short exposure(s) to stressors. Oncogenes such as *ras* can also induce senescence (Serrano et al., 1997). As discussed below, because organisms and cells are constantly being exposed to stressors, senescent cells in vivo may derive not only from cell divisions but from cells being exposed to stress.

Senescent Cells, Stress and Organismal Aging

The connection between organismal aging and cell senescence remains a subject of controversy, in spite of decades of study (reviewed in Hayflick, 1994; de Magalhaes, 2004; Campisi, 2005; Campisi and d'Adda di Fagagna, 2007; Jeyapalan and Sedivy, 2008). Below I present and discuss some of the key arguments for and against a role of RS and senescent cells in human aging.

At least *post partum*, there is no relation between the number of CPDs cells can endure and the age of the donor (Cristofalo et al., 1998b). Chances are previous studies showing otherwise were biased (Cristofalo, 2001). Likewise, one study in centenarians failed to find differences in the CPDs cells taken from centenarians could endure when compared to cells from young donors (Tesco et al., 1998). As mentioned above, cells at birth from patients with certain progeroid syndromes have fewer divisions than cells from healthy controls. This, however, might be a result of increased cell death or exit from the cell cycle for reasons unrelated to RS (Johnson et al., 1999). In fact, senescent cells from patients with Werner's syndrome have different patterns of gene expression (Oshima et al., 1995; Toda et al., 1998) and biomarkers of senescence (Schulz et al., 1996); similar findings have been reported in Hutchinson-Gilford progeria (Park et al., 2001). I should also point out that a caveat of comparing CPDs is that when cell lines are derived from people, the selected cells are those that grow because people, even very old people, never run out of proliferating cells (Tesco et al., 1998; Cristofalo, 2001). As such, these studies would not detect, say, differences in the proportion of proliferating cells.

Although a relation between a species' longevity and the CPDs its cells can endure in vitro exists, it is debatable if this is related to aging. For one, optimal culture conditions vary from species to species. As an example, O_2 partial pressure can affect cellular proliferation and there is evidence that O_2 limits the replicative capacity of mouse fibroblasts as these are immortal under low O_2 (Parrinello et al., 2003). As such, comparisons between different species may be biased due to intra-species differences in O_2 sensitivity (Toussaint et al., 2002b). In addition, due to the positive correlation between body size and longevity--mentioned before--, perhaps cells taken from long-lived animals endure more CPDs because of differences in size, not due to differences in longevity, as supported by results using more sophisticated methods (Lorenzini et al., 2005; Seluanov et al., 2008).

Senescent cells and senescence-associated biomarkers can be found in various human tissues in vivo associated with both aging and pathology (Paradis et al., 2001; Going et al., 2002; Minamino et al., 2003; Campisi, 2005; Jeyapalan and Sedivy, 2008). Interestingly, stress-prone tissues appear to be the most affected. For example, fibroblasts cultured from distal lower extremities of patients with venous

reflux, which precedes the development of venous ulcers, display characteristics of senescent cells (Mendez et al., 1998). Similar results also relate cellular senescence to atherosclerosis (Minamino et al., 2002) and benign prostatic hyperplasia, a common age-related male pathology (Castro et al., 2003). One study found that the number of senescent fibroblasts increases exponentially with age in the skin of baboons and senescent cells are >15% of all cells in very old animals (Herbig et al., 2006). In the mouse liver, one study estimated that over 20% of hepatocytes were potentially senescent (Panda et al., 2008). Senescent cells have been found in other mouse tissues too, though possibly through telomere-independent mechanisms (Wang et al., 2009). Markers of cell senescence, such as $p16^{INK4a}$ which is discussed elsewhere, have been found in mouse brain (Molofsky et al., 2006) and pancreas (Krishnamurthy et al., 2006), potentially contributing to age-dependent decline in regeneration.

Because senescent cells can secrete proinflammatory cytokines and other factors that disrupt the tissue microenvironment, they may contribute to disruption of cell and tissue function. Even a small percentage of senescent cells, in fact, may interfere with tissue homeostasis and function (Shay and Wright, 2000). Indeed, some evidence exist that senescent cells contribute to age-related pathologies such as osteoarthritis (Martin and Buckwalter, 2002; Price et al., 2002) and to skin aging (Giacomoni et al., 2000). Repeated stimulation of WI-38 human fibroblasts with pro-inflammatory cytokines interleukin-1 α (IL-1α) or tumor necrosis factor-α (TNF-α) induces SIPS (Dumont et al., 2000b). These cytokines' circulating levels increase in vivo (reviewed in Lio et al., 2003), favoring inflammation and perhaps contributing to SIPS in vivo; senescent cells might then also contribute to increase inflammatory levels, creating a positive feedback loop.

One high-profile study reported that clearance of senescent cells delays aging-associated disorders in mice (Baker et al., 2011). It is important to note that this study was conducted in progeroid mice that exhibit accelerated aging. The animals have a defect in a cell cycle regulator called BubR1 and hence accumulate more senescent cells, in particular in the tissues the study focused on. So the pathological mechanisms in these mice may be different than during normal aging. It should also be noted that clearance of senescent cells did not extend lifespan, which Baker et al. claim is due to their mice mice dying primarily of heart disease which is not affected by the treatment. Therefore, while the study provides evidence that senescent cells can cause age-related phenotypes, they seem to only contribute to a subset of these. As such, whether this treatment will extend lifespan in normal mice is dubious, but hopefully this will be tested empirically.

Clearly, senescent cells can be found in vivo without telomere shortening (Melk et al., 2003). Since cells taken from old donors do not endure less CPDs, one hypothesis is that senescent cells in vivo are not widely caused by shortening telomeres but instead by various stressors and insults. Exemplifying, studies in centenarians have raised doubts on whether telomere shortening occurs in vivo and whether senescence-associated genes in vitro are also differentially expressed in vivo (Mondello et al., 1999). Besides, some data indicate that chronic stressors may accelerate risk of a host of age-related diseases by prematurely aging the immune response (Kiecolt-Glaser et al., 2003). Lastly, as hinted by the above mentioned results on the impact of O_2 in cell proliferation, RS for many cell lines in vitro and in vivo might instead be better defined as SIPS resulting from oxidative stress.

A relationship appears to exist between stress resistance and aging. In model organisms, extended longevity is often associated with increased stress resistance (reviewed in Longo, 1999). Concisely, manipulations in *C. elegans* that extend longevity show a strong correlation with resistance to stress (Murakami et al., 2000). In *Drosophila* too some mutations can increase longevity and augment stress resistance (Lin et al., 1998). Cell lines from long-lived mouse strains are also stress resistant (Salmon et al., 2005) and stress resistance in vitro correlates with mammalian longevity, at least for some stressors (Kapahi et al., 1999; Harper et al., 2007); similar results have been observed in birds (Harper et al., 2011). Cells from older individuals are more susceptible to stress and exhibit higher levels of biomarkers of senescence in general (Dekker et al., 2009). Finally, cells taken from patients with progeroid syndromes are more susceptible to stress (Gebhart et al., 1988), as not surprisingly are late passage fibroblasts (Yuan et al., 1996). Of course, it is not known whether these relationships are

causal or not. Nevertheless, aging and stress resistance appear to be inversely related and so an association between cellular stress resistance and organismal aging is a possibility.

There is no doubt that changes occur with age at a cellular level. Some genetic interventions regulating aging appear to influence tissue homeostasis by affecting senescence, cell proliferation, and cell death (reviewed in de Magalhaes and Faragher, 2008), as detailed in the context of the endocrine theory of aging. Results from mice suggest that systemic factors can influence aging, but only to some degree, showing that intrinsic cellular mechanisms likely play a role in aging (Conboy et al., 2005). In some tissues, such as the immune system, decreased proliferative ability may play a role in age-related degeneration (reviewed in Effros, 1996). Successive transplants of spleen and bone marrow yielded far from conclusive results but it appears that a slight decrease in proliferative ability does occur in vivo in spite the cells having had to divide much more than 50 times (Strehler, 1999, p. 53). Therefore, mechanisms of aging intrinsic to cells no doubt exist (de Magalhaes, 2004). These may be related to the senescent phenotype but no doubt other processes too. It was initially reported that cells from older donors have a slower proliferative capacity (Waters and Walford, 1970; Hayflick, 1994). This effect, known as the latent period, occurs because fewer cells are in the replication cycle, not because they take longer to divide (Ponten et al., 1983; Karatza et al., 1984), but it has also been under attack (Cristofalo et al., 1998b). One study argued that cells ceasing division is not relevant to aging. Instead, altered gene expression, resulting from quality control defects that allow errors to accumulate as cells divide, leads to cells with diminished function (Ly et al., 2000).

Overall, it is clear that RS is not a faithful model of aging changes occurring in vivo (Gershon and Gershon, 2000a for arguments). In fact, RS is likely an anti-cancer mechanism (Wynford-Thomas, 1999), as further debated elsewhere. That is not to say, however, that senescent cells do not play a role in aging. One hypothesis is that while RS evolved as an anti-cancer mechanism, the accumulation of senescent cells contributes to aging Sedivy, 2008). While there is little evidence to suggest that cells running out of divisions are a major factor in aging, it is possible that stress and various insults trigger cell senescence in vivo. Even a small fraction of senescent cells in organs may impair tissue renewal and homeostasis, decrease organ function, and contribute to the aging phenotype. Although there is no evidence that accumulating senescent cells *per se* are a cause of aging, they may well contribute to age-related pathologies or at the very least reflect damage to tissues. Because telomere shortening is the main cause of RS in human fibroblasts, this topic is further debated in context of the role of telomeres in aging.

Stem Cells and Germ Cells

Stem cells are found in different places throughout the body and participate in tissue homeostasis by replacing differentiated cells that die; due to their high place in tissues' hierarchy, stem cells are promising subjects for study in the context of aging. Some human stem cells can express telomerase (Chiu et al., 1996; Sugihara et al., 1999), indicating that the most actively dividing cell lines in the body overcome telomere shortening--though somatic stem cells can show senescence in vitro (Smith and Schofield, 1994) and in vivo (Martin et al., 2000). Interestingly, a correlation between mean telomeres and age is found in the first two decades for muscle satellite cells--a type of muscle stem cell--but not afterwards (Decary et al., 1997). Consequently, one hypothesis is that somatic cells can only divide a limited amount of times but are constantly being replenished by stem cells. Interestingly, one study found a correlation between stem cell turnover and mice lifespan (de Haan and Van Zant, 1999), meaning that perhaps stem cell senescence influences organismic senescence (Snyder and Loring, 2005). Mechanisms of stem cell aging are also of great interest and have been linked to various processes, including DNA damage (Rossi et al., 2007; Freitas and de Magalhaes, 2011) and telomeres. At present this is only one hypothesis but no doubt unraveling the role of stem cells in aging is a major avenue for future research. As mentioned elsewhere, stem cells may also have anti-aging applications.

As previously mentioned, the doctrine of the immortal germplasm claims that germ cells are immortal and can divide forever (Weismann, 1891; Kirkwood, 1977). A prediction of such hypothesis is that the

germ cells should have increased stress resistance and repair mechanisms (Kirkwood, 1977). Experimental evidence, however, is contradictory: the soma of *Drosophila* has been reported to be more sensitive to mutagens (Vogel and Zijlstra, 1987); increased DNA repair has been documented in male mice germ cells (Walter et al., 1994), but using ionizing radiation no difference in sensibility was found between mice male germ cells and bone marrow (van Loon et al., 1993). It has also been proposed that meiosis and gametogenesis can have recombinational and other genetic events that contribute to a rejuvenation not possible in differentiated somatic cells (Medvedev, 1981; Holliday, 1984), yet little or no evidence exists to support such claims. Furthermore, the common notion that germ cells have improved DNA repair mechanisms and thus avoid aging is itself debatable (reviewed in Walter et al., 2003).

4.11 Telomeres and Telomerase

Telomere shortening causes cellular senescence, making it a major candidate mechanism for a role in aging and a target for anti-aging interventions. In this essay, I review current knowledge on telomere biology and discuss the possible role of telomeres and telomerase in human aging and in cancer.

Sections

Keywords: ageing, biogerontology, cell aging clock, cytogerontology, terminal restriction fragments, TRF

Telomere Shortening As the Timekeeper of Cells

Early studies by Hermann Muller and Barbara McClintock showed that the ends of chromosomes are capped by a structure called the telomere to prevent chromosome fusions (Muller, 1938; McClintock, 1941). In the 1970's, as the mechanisms behind DNA replication were becoming better understood, it became clear that DNA polymerase, the enzyme responsible for DNA replication, could not fully synthesize the 3' end of linear DNA (Fig. 1). In 1972, James Watson called this the end-replication problem (Watson, 1972). At about the same time, in a Moscow subway station, Alexey Olovnikov also recognized Watson's problem in an analogy between the track that represented the DNA and the train that represented DNA polymerase. Yet Olovnikov went further to propose that the end-replication problem would result in telomere shortening with each round of replication and that, because the DNA is replicated during cell division, this mechanism could be the cause of replicative senescence (RS) (Olovnikov, 1971 & 1973). Soon after, studies by Leonard Hayflick and colleagues found that the nucleus controls RS (Wright and Hayflick, 1975; Hayflick, 1994).

Olovkikov's model turned out to be incredibly accurate. Telomere shortening is now considered the main causal mechanism of RS and telomere length is the molecular clock that counts the cumulative population doublings (CPDs) cells can endure (reviewed in Wright and Shay, 2001). Although it was previously known that telomere shortening occurs in each subcultivation (Harley et al., 1990), the key finding relating the telomeres to RS was made in 1998 by scientists from Geron Corporation. Telomerase is a reverse-transcriptase enzyme that elongates the telomeres and thus corrects the normal telomere erosion (Greider and Blackburn, 1985). It has two components: an RNA component (Feng et al., 1995) and a catalytic subunit (Nakamura et al., 1997). Telomerase activity parallels expression of the catalytic subunit (hTERT) and ectopic hTERT expression is sufficient to restore telomerase activity in human cells (Counter et al., 1998). Telomerase activity was shown in immortal cell lines (Counter et al., 1992). But the definitive breakthrough came when it was shown that expression of hTERT in human cells avoids RS (Bodnar et al., 1998). Human fibroblasts immortalized with hTERT divide vigorously, do not show increased staining for SA β-gal--a marker of cell senescence--, and do not show signs of neoplastic transformation (Jiang et al., 1999; Morales et al., 1999). Even expression of hTERT in late passage fibroblasts appears to reverse the loss of function characteristic of pre-senescent cells (Funk et al., 2000).

Transient expression of hTERT for 7 CPDs in human fibroblasts elongated the shortest telomeres by 2.5 kilobase pairs (kbp). Afterwards, these cells divided for roughly 50 CPDs with a telomere shortening of 50 bp per division before reaching RS. These results strongly argued that telomere

length, not hTERT expression, is the key to bypass RS (Steinert et al., 2000) and established telomere length as the clock that keeps track of CPDs and gives rise to RS and the Hayflick limit.

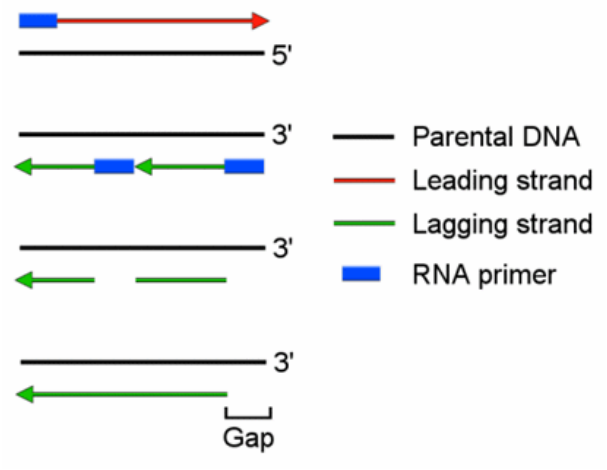

Figure 1: DNA polymerase requires an RNA primer to initiate synthesis in the 5'-3' direction. At the end of a linear chromosome, DNA polymerase can synthesize the leading strand until the end of the chromosome. In the lagging strand, however, DNA polymerase's synthesis is based on a series of fragments, called Okazaki, each requiring an RNA primer. Without DNA to serve as template for a new primer, the replication machinery is unable to synthesize the sequence complementary to the final primer event. The result is an end-replication problem in which sequence is lost at each round of DNA replication.

Telomerase is not the only mechanism capable of elongating the telomeres. There are several immortal telomerase-negative cell lines with typically a great variety of telomere lengths (e.g., Bryan et al., 1995). Although the exact mechanisms behind what is called alternative lengthening of telomeres remain largely unknown, recombinational processes may be involved (McEachern and Blackburn, 1996; Dunham et al., 2000). Still, either using telomerase or not, all known immortal cell lines must stabilize their telomeres (reviewed in Colgin and Reddel, 1999; Stewart and Weinberg, 2000). Unicellular eukaryotes must also stabilize their telomeres. For example, defects in telomere replication have been shown to trigger senescence in yeast (Lundblad and Szostak, 1989) and in the protozoan Tetrahymena (Yu et al., 1990).

How Telomere Dysfunction Induces Cellular Senescence

Although telomere length regulates RS and can be seen as a mitotic clock, the mechanisms involved are more complex than they may seem at first. This section and the next provide a fairly detailed and technical discussed of the molecular mechanisms involved; the final section in this essay discusses the potential role of telomeres and telomerase in aging and cancer.

Telomere length is neither the only nor the ultimate timekeeper of cells (reviewed in Blackburn, 2000). During telomerase-immortalization of human cell lines, several researchers noticed that immortalized cells had shorter telomeres than growth arrested controls (Ducray et al., 1999; Zhu et al., 1999). Surprisingly, these immortalized cells featured less chromosome fusions, which are the most noticeable outcome of short telomeres (Hande et al., 1999). Similarly, it was noticed in yeast that certain telomerase-negative strains would senesce with longer telomeres than immortal telomerase-positive strains (Prescott and Blackburn, 1997; Roy et al., 1998). Since telomere length alone could not explain these observations, other players had to be involved.

Using electron microscopy, it was revealed that telomeres are not linear, but instead appear to form duplex loops, called t-loops (Fig. 2). Crucial in these loops are the telomeric repeat-binding factors

TRF1 (van Steensel and de Lange, 1997) and TRF2 (Smogorzewska et al., 2000). In particular TRF2 can remodel linear telomeric DNA into t-loops (Griffith et al., 1999). Although not completely understood, the prevailing hypothesis is that these loops stabilize or cap the telomeres. Capping may protect the telomeres from being recognized as DNA damage. TRF2 protects telomeres (van Steensel et al., 1998): inhibition of TRF2 induces apoptotic cell death (Karlseder et al., 1999) while overexpression of TRF2 reduces the senescent checkpoint of cells in terms of telomere length (Karlseder et al., 2002). These results suggest that telomere capping, not just telomere length, is crucial in avoiding telomere dysfunction and preventing cell senescence. Results showing that telomerase disruption can slow cell proliferation and alter the 3' single-stranded telomeric overhang without telomere shortening support this view (Masutomi et al., 2003). One plausible hypothesis is that telomere shortening may destabilize or even prevent the capping of telomeres, leading to RS (Shay, 1999).

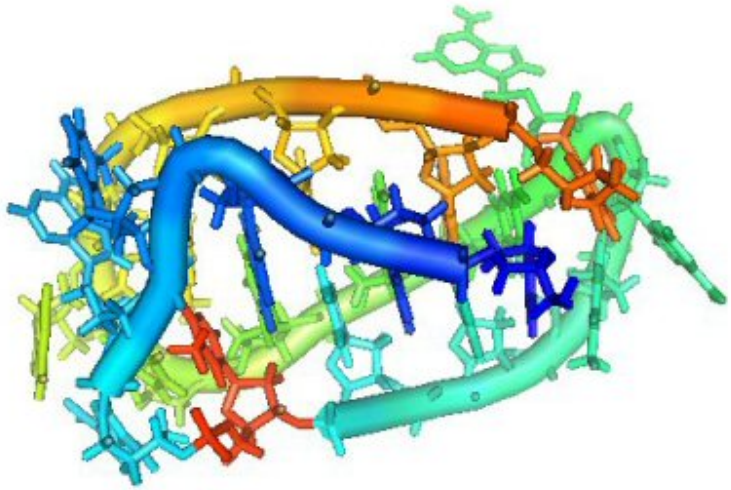

Figure 2: Telomeric structure forming a loop that caps the end of telomeres. Rendered using PyMOL.

Whether the end-replication problem alone is responsible for telomere shortening is still under debate. RS can occur in human fibroblasts--often referred to as human diploid fibroblasts or HDFs--in the absence of cell division and short telomeres. Cells kept confluent for long periods of time--up to 12 weeks--exit the cell cycle. The small proportion of cells that continue dividing endured fewer CPDs than normal presumably due to compensatory cycling (Munro et al., 2001). Although quiescent cells do not appear to lose telomeres (von Zglinicki, 2000), cells endure an accelerated telomere shortening following extensive periods of confluency (Sitte et al., 1998). One hypothesis is that telomere dysfunction occurs in confluent cells despite lack of telomere shortening. Therefore, the end-replication problem as a model to explain telomere shortening may not be entirely correct. Telomeres end in a single-stranded G-rich 3'-overhang, presumably as a result of C-rich strand degradation during telomere processing (Wellinger et al., 1996; Makarov et al., 1997). Some results suggest that erosion of the overhang occurs at cell senescence and is prevented by telomerase expression. Progressive erosion appears to be a result of cell division and not an effect of RS (Stewart et al., 2003). As such, the exact molecular mechanisms behind telomere shortening and dysfunction remain undetermined.

Since a normal human diploid cell contains 92 telomeres, another issue is whether it is mean telomere length or the shortest telomere to trigger RS. Evidence from mice indicates that the shortest telomeres, not mean telomere length, are responsible for inducing RS (Hemann et al., 2001). Yet one study in human fibroblasts found that the onset of RS shows a better correlation with mean telomere length than with the shortest telomere (Martens et al., 2000).

Uncapped Telomeres Recognized as DNA Damage

Even before hTERT-derived immortalization, it was possible to immortalize human fibroblasts using viral genes such as the simian virus 40 (SV40) T-antigen, E1A and E1B from adenovirus, or the human papillomavirus E6 and E7 genes. The E1B and E6 proteins bind and inactivate the tumor suppressor protein p53 while E1A and E7 bind and inactivate the retinoblastoma protein (pRb) (Dyson et al., 1989; Werness et al., 1990). Immortalization requires E6 and E7 or E1A plus E1B, so both p53 and pRb must be inactive. SV40 immortalization is also dependent on inactivation of both p53 and pRb (Shay et al., 1991). These findings led to the present concept that two pathways are responsible for inducing cell senescence (Fig. 3). Confirming these suspicions, inhibition of p53 and pRb by antisense technology caused cells to endure 50 CPDs more than normal (Hara et al., 1991).

In agreement with its anti-oncogenic profile, pRb is a central regulator of cell cycle progression and its state of phosphorylation determines cell cycle regulation (Buchkovich et al., 1989; reviewed in Herwig and Strauss, 1997). Hyperphosphorylated pRb allows the cell cycle to proceed while hypophosphorylated pRb prevents cell cycle progression. Presumably, pRb operates through inactivation of the E2F family of transcription factors, responsible for transcription of several genes involved in G1/S transition and DNA synthesis (Weintraub et al., 1992; Campisi, 1999). Briefly, the phosphorylation of pRb is dependent on cyclin-dependent kinases (CDKs) that govern the progression through the various phases of the cell cycle (reviewed in Lees, 1995). Inactivation of the G1 CDKs, responsible for the phosphorylation of pRb, prevents transition from phase G1 to phase S and blocks the cell cycle, originating, for example, RS.

Cyclin-dependent kinase inhibitors (CDKIs), as the name implies, inhibit the activity of CDKs. One of such proteins is $p16^{INK4a}$, that disrupts and inhibits the activities of CDK4 and CDK6, thus preventing cell cycle progression (Hara et al., 1996). Exogenous expression of $p16^{INK4a}$ induces RS in young cells and in immortal cells without p53 activity (McConnell et al., 1998; Vogt et al., 1998). In addition, immortalization may also be achieved by disruption of both $p16^{INK4a}$ and p53 (Rogan et al., 1995). pRb is needed for growth suppression mediated by $p16^{INK4a}$ (Medema et al., 1995). These results suggest that $p16^{INK4a}$ acts upstream of pRb in regulating RS.

Another important CDKI is $p21^{WAF1}$, which also has the ability to block the cell cycle by inhibiting CDK2, CDK4, and CDK6 and thus preventing pRb phosphorylation (Harper et al., 1993 & 1995). $p21^{WAF1}$ can induce senescence independently of $p16^{INK4a}$ (McConnell et al., 1998; Vogt et al., 1998). Since $p21^{WAF1}$ expression levels increase in pre-senescent cells--i.e., before $p16^{INK4a}$ overexpression--, $p21^{WAF1}$ likely triggers senescence before $p16^{INK4a}$ (Tahara et al., 1995; Alcorta et al., 1996; Wong and Riabowol, 1996; Dulic et al., 2000). In contrast, $p16^{INK4a}$ remains overexpressed in senescent cells while $p21^{WAF1}$ levels wane (Stein and Dulic, 1998).

$p21^{WAF1}$ is induced by p53 to trigger RS (reviewed in Wang et al., 2003). Briefly, p53 is a transcription factor capable of acting both as a transcriptional activator and suppressor. Overexpression of p53 leads to cell cycle arrest or apoptosis (Sugrue et al., 1997). The induction of p53 by DNA-damaging agents led to the suggestion that p53 is a checkpoint factor that prevents cells from accumulating mutations by inducing apoptosis or growth arrest (reviewed in Ko and Prives, 1996). p53 may help maintain genetic stability (see Linke et al., 2003 for arguments). Increased levels of p53 have been associated with critically short telomeres (Vaziri and Benchimol, 1996; Gonzalez-Suarez et al., 2000). Thus p53 is probably responsible for recognizing dysfunctional telomeres--e.g., critically short telomeres--as DNA damage and triggering RS. Indeed, activation of p53 occurs as cells approach senescence (Atadja et al., 1995; Kulju and Lehman, 1995; Bond et al., 1996). Therefore, p53 appears to be the major initiator of senescence, while $p16^{INK4a}$ presumably maintains senescence (Alcorta et al., 1996; Dulic et al., 2000; Serrano and Blasco, 2001; Wang et al., 2003). Another possibility is that $p16^{INK4a}$ serves as a second barrier to prevent growth of cells with significantly damaged DNA d'Adda di Fagagna, 2007).

$p16^{INK4a}$ appears to be important in the response to DNA damage (Robles and Adami, 1998; Shapiro et al., 1998; te Poele et al., 2002) and other stimuli like oncogenic signals (Serrano et al., 1997). The immortalization of human epithelial cells requires inactivation of $p16^{INK4a}$--or E7 expression to inhibit pRb--in addition to hTERT activity (Kiyono et al., 1998). Since under 2% O_2 epithelial cells can be immortalized with hTERT activity alone, it appears that stressful culture conditions may activate $p16^{INK4a}$ and induce senescence independently of the telomeres (Ramirez et al., 2001; Rheinwald et al., 2002). Lastly, $p16^{INK4a}$ does not appear to be involved in RS of at least some mouse cells (Smogorzewska and de Lange, 2002), indicating that the regulation of RS and telomere dysfunction in mouse and human cell lines is different, as suggested by others (Hamad et al., 2002; Kim et al., 2002). (RS in mouse cells will not be discussed in detail here.)

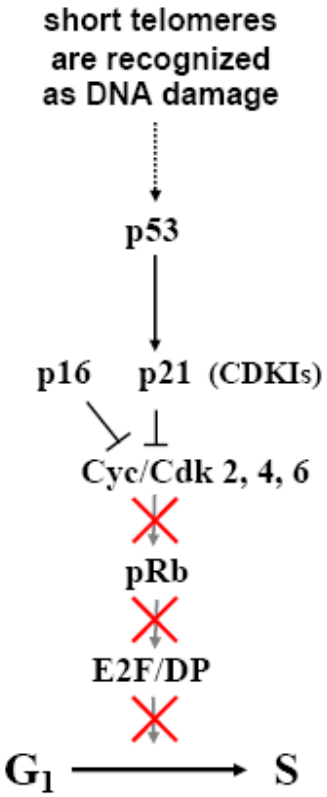

Figure 3: Simplistic overview of the signal transduction from critically short telomeres to irreversible growth arrest at the G1/S transition of the cell cycle. Telomere dysfunction causes an activation of DNA damage response pathways, such as an activation of p53. p53 in turn activates $p21^{WAF1}$ that blocks the actions of several CDKs preventing the phosphorylation of pRb. Without hyperphosphorylated pRb several critical genes in the G1/S transition are not transcribed, blocking the cell cycle. Adapted from (de Magalhaes, 2004).

Although Figure 3 and the discussion above provide an overview of the current knowledge of cell cycle regulation, it is likely other players exist. For instance, p53 itself may be upregulated. Although the issue is controversial, some evidence indicates that the *ATM* gene, or other players involved in DNA damage response, may be the "sensor" that detects telomere dysfunction and then regulates p53 (Vaziri et al., 1997; Rouse and Jackson, 2002). Other results suggest that a novel transcriptional element regulates cyclin D1, and possibly other senescence-associated genes, in senescence cells (Berardi et al., 2003).

Immortalization with viral proteins is not as simple as it may seem at first. Infection of human fibroblasts with viral oncogenes results in an extended replicative lifespan after which cells enter a stage called crisis (reviewed in Goldstein, 1990; McCormick and Campisi, 1991; Wei and Sedivy, 1999). During crisis, cells proliferate but the proportion of cells entering apoptosis gradually increases and thus cell numbers eventually diminish (Macera-Bloch et al., 2002). Since both p53 and pRb/$p16^{INK4a}$ pathways are inactive and chromosomal instability and fusions are abundant, crisis is thought to emerge due to extremely short telomeres. Occasionally, immortal cells emerge from crisis with stabilized telomeres, normally involving telomerase activation (reviewed in Stewart and Weinberg, 2000; Mathon and Lloyd, 2001). In a sense, crisis can be seen as the ultimate consequence of telomere dysfunction since it occurs when the mechanisms that respond to short telomeres, like p53 and pRb, are inactive.

One last point is that even assuming that the p53 and pRb/$p16^{INK4a}$ pathways explain RS, they do not entirely explain the gradual aging of cells in culture. One hypothesis is that cell populations become more heterogeneous as they age. For example, since the percentage of cells actively dividing decreases with CPD, it is normal that the cell population as a whole ages, without changes other than more cells entering RS.

Overall, whatever changes occur during telomere dysfunction, the mechanisms triggering growth arrest appear to involve DNA damage pathways. As such, the most likely explanation is that dysfunctional telomeres are recognized as DNA damage and repairing the short telomeres leads to chromosome fusions. Although unidentified genes may also be involved (e.g., Blasco and Hahn, 2003; Yawata et al., 2003), the most widely accepted hypothesis is that the p53 and pRb/$p16^{INK4a}$ pathways collaborate to stop cellular proliferation derived from telomere shortening in normal human fibroblasts (Fig. 3). Probably, the p53 pathway involving $p21^{WAF1}$ is activated beforehand, while $p16^{INK4a}$ prevails under strong physiological stimuli or stress and to maintain cells growth arrested, a state also called quiescence.

Aging, Cancer, and the Telomeres

The role of telomeres in RS has led to suggestions that telomerase can be used as an anti-aging therapy (reviewed in Fossel, 1996; Blasco, 2005; Shawi and Autexier, 2008). As mentioned before, however, the relation between RS and organismal aging is controversial. Whether telomere shortening plays a role in human aging is a hotly-debated issue, as reviewed below.

Most, not all, human somatic tissues have no detectable telomerase activity (reviewed in Collins and Mitchell, 2002). In the bone marrow, hematopoietic cells express telomerase. Telomerase activity is higher in primitive progenitor cells and then downregulated during proliferation and differentiation (Chiu et al., 1996). Other reports associate, normally low, levels of telomerase activity with human stem cells (Sugihara et al., 1999), though probably not mesenchymal stem cells (Zimmermann et al., 2003). Telomerase activity has been detected in some highly proliferating normal human somatic cells; for instance, in skin cells (Harle-Bachor and Boukamp, 1996; Taylor et al., 1996), immune system cells (Counter et al., 1995; Morrison et al., 1996), and colorectal tissues (Tahara et al., 1999). A decline in telomerase activity was reported in blood mononuclear cells with age (Iwama et al., 1998). Human germ cells have been found to express hTERT (Kilian et al., 1997).

As with replicative potential, telomere length in vivo is very heterogeneous (Serra and von Zglinicki, 2002; Takubo et al., 2002). Telomere shortening in vivo has been reported in liver cells (Aikata et al., 2000), lymphocytes (Pan et al., 1997), skin cells (Lindsey et al., 1991), blood (Iwama et al., 1998), and colon mucosa (Hastie et al., 1990). For example, telomere shortening appears to impact on the function of immune T cells and telomerase activators can restore a more youthful functional profile (reviewed in Effros, 2009). Other studies found weak correlations between donor age and telomere length (Allsopp et al., 1992; Kammori et al., 2002; Njajou et al., 2007), while some studies found no correlation at all (Mondello et al., 1999; Renault et al., 2002; Serra and von Zglinicki, 2002; Takubo et

al., 2002; Nwosu et al., 2005). Long telomeres have been found in cells from centenarians (Franceschi et al., 1999). Taken as a whole, these results indicate that telomere length varies widely between individuals and between different tissues, and that telomere shortening may occur in some tissues in vivo in association with certain pathologies and with age; this is similar to what is observed for senescent cells. An association between telomere length and mortality has been reported in people aged 60 and over (Cawthon et al., 2003), and telomere shortening appears to be accelerated in people living more stressful lives (Epel et al., 2004). While these results support the idea that telomere shortening is a marker of stress and age-related pathology, they do not prove that telomere shortening is a causal factor in aging. Lastly, although telomerase may prevent the accelerated clonal senescence of Werner's syndrome cells (Wyllie et al., 2000), it does not appear to fully reverse the WS phenotype (Choi et al., 2001).

No connection appears to exist between mean telomere length of cells and longevity of mammalian species. Of all studied primates, humans appear to have the shortest telomeres and the longest lifespan (Kakuo et al., 1999; Steinert et al., 2002). Mice also have long telomeres and feature high telomerase activity in many organs, in contrast to humans (Prowse and Greider, 1995). Interestingly, inbred mice have long (Kipling and Cooke, 1990) while wild mice have short telomeres, suggesting telomere length does not affect organismal longevity (Hemann and Greider, 2000). In rodents, telomerase activity correlates negatively with lifespan but does not correlate with longevity (Seluanov et al., 2007). The largest comparative study of telomeres and telomerase, involving over 60 mammalian species, found that smaller, short-lived species tend to have long telomeres and high levels of telomerase. This suggests that short telomeres and suppression of telomerase are necessary for the evolution of large body sizes and longevity, presumably by suppressing cancer (Gomes et al., 2011).

Though mean telomere length at birth does not correlate with longevity in birds, rate of telomere shortening in erythrocytes was reported to inversely correlate with bird longevity. Telomere shortening in a variety of tissues was also reported to correlate, though to a lesser extent, with mammalian longevity (Haussmann et al., 2003; Vleck et al., 2003). In fact, a correlation between erythrocyte longevity and organismal longevity was previously reported, suggesting that cells, in this case erythrocyte stem cells, from long-lived animals divide fewer times (Rohme, 1981). One study in rodents, however, failed to find evidence of a correlation between rate of telomere shortening in vitro and longevity (Seluanov et al., 2008).

Mice overexpressing telomerase have a higher cancer incidence and hence a shorter lifespan (Artandi et al., 2002). But mice lacking telomerase were viable up to six generations. Telomeres gradually shortened and cells from animals of generation four displayed aneuploidy and other chromosomal aberrations. Abnormalities were observed as early as in the third generation and late-generation animals showed a few signs of accelerated aging (Blasco et al., 1997; Rudolph et al., 1999); it is controversial whether these animals are aging faster or merely developing a variety of pathologies. All in all, these results suggest that telomerase activity could be crucial for the normal functioning of highly proliferative organs in mice (Lee et al., 1998). Nonetheless, telomere length and/or telomerase activity do not explain why humans age slower than other primates and live so much longer than mice. They may help explain, however, why mice have a much higher cancer incidence than humans (Blasco, 2005).

Telomerase expression has been found in lobsters, a species in which aging remains undetected (Klapper et al., 1998), though it could be due to molting. On the other hand, in the frog *Xenopus laevis*, another animal with a slow rate of aging (Brocas and Verzar, 1961), not only a great variation in telomere length has been observed (Bassham et al., 1998), but telomere length can diminish from parents to offspring with no detectable consequences and despite telomerase activity in germ cells (Mantell and Greider, 1994). The way telomere length does not impact on the life history of cloned animals is also in contradiction with a role of telomeres in aging. For instance, scientists took cells from a 17-year old bull and allowed them to divide (Kubota et al., 2000); they then used cells at different stages of their replicative lifespan to create clones and, surprisingly, it appears that the older cells with shorter telomeres are more efficient for generating clones. It would be interesting to know

the longevity of these clones as well as that of cloned calves with extended telomeres (Lanza et al., 2000). Overall, maybe telomeres are the cellular clock, but judging from these results telomere length is not a major determinant of the aging process.

As with RS, telomere shortening appears to be a tumor suppressor mechanism (de Magalhaes, 2004; Campisi, 2005; Deng et al., 2008). Tumor development is dependent on telomere stabilization, normally by telomerase (Chen et al., 2000). For example, telomerase activation has been associated with skin malignancy as a result of exposure to ultraviolet radiation (Ueda et al., 1997). In contrast, telomerase inhibition can induce senescence in some cancer cells (Shammas et al., 1999). Knocking-out telomerase in mice through deletion of its RNA component, while not preventing cancer (Blasco et al., 1997; Rudolph et al., 1999), appears to increase cancer resistance (Gonzalez-Suarez et al., 2000; Rudolph et al., 2001). On the other hand, telomerase overexpression in mice promoted cancer development (Gonzalez-Suarez et al., 2001; Artandi et al., 2002). In addition, the connection between telomere signaling pathways and cancer is obvious (reviewed in Fearon, 1997). The human Li-Fraumeni syndrome has been associated with mutations in p53 and is characterized by increased cancer incidence (reviewed in Varley et al., 1997). Human germline mutations in p53 are also associated with a major cancer risk (Hwang et al., 2003). Retinoblastoma is also recognized as hereditary cancer (Murphree and Benedict, 1984; Goodrich and Lee, 1993). Germline mutations in $p16^{INK4a}$ have too been implicated in familial melanoma (Hussussian et al., 1994).

More debatable is the role of telomeres in animal aging (de Magalhaes and Toussaint, 2004a). As mentioned elsewhere, senescent cells likely accumulate in some tissues and may contribute to organ disfunction yet telomere-independent mechanisms may play a more prominent role. Some genetic interventions that alter aging appear to influence tissue homeostasis by affecting senescence, cell proliferation, and cell death, yet such evidence is circumstantial (reviewed in de Magalhaes and Faragher, 2008). Evidence from genetic manipulation experiments of players involved in telomeric signal transduction (Fig. 3) is mixed (reviewed in de Magalhaes, 2004). Increasing the dosage in mice of INK4a/ARF (the gene coding the mouse homolog of $p16^{INK4a}$) offers resistance against cancer but does not affect aging (Matheu et al., 2004). There is some evidence that p53 may influence aging in mice (Donehower, 2002), as debated elsewhere, but it is not clear the same is true for humans. Likewise, disruption of p63, a homologue of p53, appears to accelerate aging (Keyes et al., 2005), yet human defects in p63 do not (Celli et al., 1999). Mouse strains with increased levels of p53 and INK4a/ARF are long-lived (Matheu et al., 2007), though it is unclear whether their aging process is altered--as defined before. Arguably the strongest evidence for a role of telomerase in aging comes from telomerase overexpressing mice also engineered to resist cancer via enhanced expression of p53 and INK4a/ARF as these are long-lived (Tomas-Loba et al., 2008). Even though it is not clear whether aging is delayed in these animals or the exact mechanisms, these findings do point towards some level of protection from age-related degeneration via optimization of pathways associated with telomeres and RS. It should be noted, however, that telomerase may have functions independent of telomere elongation, such as in protecting mitochondria from stress (Ahmed et al., 2008). Another study showed that telomerase reactivation reverses degeneration in mice (Jaskelioff et al., 2011). However, this study was conducted in animals that have no telomerase to begin with and thus develop a number of pathologies. Benefits from reactivating telomerase in mice that become sick for lack of telomerase are hardly surprising or noteworthy.

Dyskeratosis congenita is an inherited disease involving skin and bone marrow failure (reviewed in Marrone and Mason, 2003). It is caused by a mutation in the *DKC1* gene. Intriguingly, the protein encoded by *DKC1*, dyskerin, is a component of telomerase. Mutations in the RNA component of telomerase are associated with an autosomal dominant form of dyskeratosis congenita (Vulliamy et al., 2001). Families with this form of the disease are more severely affected in later generations, suggesting telomere shortening could be involved. Features of dyskeratosis congenita include bone marrow failure, which is the most usual cause of death, abnormal skin pigmentation, leukoplakia and nail dystrophy (Knight et al., 1998). The role of stem cells has also been suggested (Mason, 2003). As judged from the phenotype of dyskeratosis congenita, telomeres are crucial in rapidly proliferating tissues but it is unclear whether telomere shortening is involved in human aging.

In conclusion, it is unquestionable that cellular senescence and telomere biology are important in cancer and may be suitable to develop anti-cancer treatments (reviewed in Campisi et al., 2001; Blasco and Hahn, 2003; Hahn, 2003; Lee and Schmitt, 2003; Wang et al., 2003). Whether these can aid in understanding human aging is unknown. Hopefully readers can make their own mind from the aforementioned discussion. My personal opinion is that cellular senescence, primarily caused by stress but to some degree perhaps also by telomere shortening, can contribute to aging and age-related diseases. Indeed, a genetic variant of telomerase has been associated with longer telomeres and exceptional human longevity (Atzmon et al., 2010). Having said that, I am not convinced by the empirical evidence that telomere shortening and cell senescence are causes of aging. They may be contributors or intermediaries, for example by enhancing the effects of other types of molecular damage, but I see little evidence that targeting the telomeres and/or telomerase by itself will have much effect on human aging even if it might be helpful in the case of some specific pathologies. I also think that cellular studies are crucial to gerontology, yet so much focus on measuring cellular proliferation does not appear to me to be the best approach, as mentioned before. Other methodologies are desperately needed to assess the role of cellular changes in organismal aging.

5. Is Aging Genetic or Is It Wear and Tear?

Aging has been compared to the natural decay of materials and objects. In this essay, I debate the essence of the aging process and hope to demonstrate that, despite any initial appearances, human aging is not simply wear and tear. The second law of thermodynamics is not applicable to aging. Genes can regulate the aging process, even though the environment is also important.

Sections

Keywords: ageing, aging genes, biogerontology, genotype, heritability, longevity genes

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Aging Is Not Wear and Tear

Tooth erosion is a frequent hallmark of aging among different organisms, particularly in mammals (Finch, 1990, pp. 196-202). At a first glance, it would appear that tooth erosion, like many other age-related changes like skin aging, bone aging, etc., is a mere result of wear and tear, similar to the deterioration of materials and objects. However, to quote George Williams (Williams, 1957): "The senescence of human teeth consists not of their wearing out but of their lack of replacement when worn out." The same argument can be made of menopause and female reproductive senescence in general. In mammals, females reach reproductive senescence ultimately because of their lack of oogenesis as adults. Hence aging is not merely a consequence of wear and tear, but rather a consequence of a lack of replacement of the parts. All molecules and cells that compose our body can individually age, but it is the inefficient or lack of replacement of these building blocks that leads to aging.

As detailed before, the teeth of sharks also suffer from wear and tear, but they evolved a mechanism to cope with this problem: they can replace their teeth throughout life, and many other species possess this ability. In some mammals, like rabbits and many rodents, the teeth grow continuously, as an attempt to mitigate the increased wear and tear from their food habits that involve chewing, but this is not a viable long-term solution. Long-lived mammals like elephants have more than the two sets of teeth. So while wear and tear do contribute to the erosion of individual teeth, the ultimate cause of tooth erosion is in the genetic program of animals that determines their body plan and its inherited limitations. From this perspective, aging is ultimately genetic. In fact, through genetic and tissue engineering scientists are trying to create new teeth in the gums of patients (Chai and Slavkin, 2003). Moreover, and unlike objects, higher organisms endure an extraordinary period of development in which their abilities, functions and capacity to maintain homeostasis are greatly increased. To quote George Williams again: "It is indeed remarkable that after a seemingly miraculous feat of morphogenesis a complex metazoan should be unable to perform the much simpler task of merely maintaining what is already formed."

In summary, it is now largely recognized that, contrary to the wear and tear of inanimate objects, aging in higher organisms is not primarily the result of damage to irreplaceable body parts. Certainly, molecules and cells can suffer from damage akin to wear and tear. Unlike objects, however, animals can replace most of their cells and molecules, and often have a high turnover of components exposed to environmental insults. In other words, complex biological systems are dynamic and have the ability to repair and regenerate their damaged components. Even for components that cannot be replaced, like mammalian teeth, their degeneration can be seen not just as mechanical senescence but as limitations of the genetic program. I should note that there are differences in interpretation of aging changes

which influence the way different researchers interpret the essence of aging; as discussed elsewhere, some authors see aging as genetic in nature while others see it as a build-up of damage counteracted by genetically-regulated mechanisms. Nonetheless, as detailed below, it is clear now that aging has a strong genetic component and it is not merely wear and tear.

Multiple versus Unifying Mechanisms of Aging

Some have argued that aging has multiple origins and is a mere combination of age-related changes and diseases each timed by independent clocks (Olson, 1987). For instance, some experts have defended that aging derives from the failure of multiple maintenance mechanisms and that there is no basic aging process at all (Holliday, 1995; Peto and Doll, 1997). On the other hand, some defend that aging is genetically programmed (e.g., Longo et al., 2005). Clearly, some species age due to a precise, uniform genetic clock (Prinzinger, 2005). Semelparous species such as the salmon are such an example, as described before. In these organisms, aging and death follow a very specific, well-timed program analogous to development (Austad, 2004). But humans show a gradual aging process, not sudden death, so the mechanisms of aging in semelparous species may not be similar to what happens in humans.

Species (estimated last common ancestral)	MRDT in years	Observations
Humans	7.6 – 8.9	
Chimpanzees (5.4 Mya)	Similar to humans	Our closest relatives, whose onset of aging occurs considerably earlier than in humans
Old world monkeys (23 Mya)	3.5-4.8 (baboons), 15 (rhesus macaques)	Some primates appear to age about twice as fast as humans
Other non-primates like marmosets, tarsiers, and dwarf and mouse lemurs (60 Mya)		Age considerably faster than humans, showing signs of aging in their second decade of life
Mice and rats (91 Mya)	0.3	Two of the fastest aging mammals
Some common mammals from farm to domestic animals (92 Mya)	3 (dog), 4 (horse), 1.5 (sheep)	
Long-lived mammals such as elephants and whales (92 Mya)	8 (elephants)	
Slowly aging reptiles (200 Mya)	Often no MRDT detected	Some reptiles appear not to age

Table 1: Last common ancestral represents estimates--often still under debate--of when a species and humans split, in millions of years ago (Mya). (See Finch, 1990; Hill et al., 2001; Bronikowski et al., 2002; de Magalhaes and Toussaint, 2002; Hedges, 2002; de Magalhaes, 2006.)

Although the MRDT is only an approximation of the rate of aging, as described before, it varies widely among similar species (Table 1). Even the pace and/or onset of age-related changes can be remarkably different between similar species, as described elsewhere. On the other hand, the MRDT is relatively constant among human populations, even under different environmental conditions (Finch, 1990). This robustness of the aging process suggests a strong genetic component. Independently of environmental conditions, a mouse will age 25-30 times faster than a human being. Why does a mouse age in a few years while humans take over a decade just to reach maturity? The reasons why species age at different paces must be located in the genome. Even though nutrition and exercise can make you live longer and attenuate certain age-related diseases, as discussed elsewhere, you will not be able to live as along as a Galapagos tortoise because humans are genetically determined to age within a given blueprint. That is not to say that aging evolved for an evolutionary purpose, a topic debated elsewhere, just like cancer has a strong genetic basis but it did not evolve for a purpose. What it means is that there are genetic factors determining the pace of aging of different

species. In other words, aging is programmed in our genes. Possibly, there is a molecular clock--or processes akin to a clock--regulating the aging process (de Magalhaes, 2003). This is further supported by the synchronization of life events among mammals (Finch, 1990, pp. 150-202 & 619; de Magalhaes and Church, 2005). In fact, the aging process of mammals often appears as the same process only timed at different rates.

Some gerontologists that defend multifactorial causes of aging previously argued against a molecular clock regulating cellular senescence (e.g., Holliday, 1995). Now we know that indeed such a clock exists, as detailed in another essay. A complex cascade of events such as cellular senescence can be regulated by a few genes such as telomerase (Bodnar et al., 1998; Wright and Shay, 2001; de Magalhaes, 2004), as described elsewhere. While no doubt organismal aging is more complex and under the regulation of more genes than cellular senescence, the point is that aging is not merely random deterioration but must entail mechanisms under the regulation of the genome; whether these mechanisms actively cause aging, like certain mechanisms cause cell senescence, or merely regulate systems, such as repair processes, that end up modulating aging is discussed elsewhere. Like for other continuous traits--phenotypes that can be measured on a quantitative scale--, aging is multifactorial in the sense that it is influenced by many genes interacting with the environment. Indeed, it has been long acknowledged that extrinsic factors are important in aging (Rowe and Kahn, 1987). What has surprised scientists in recent years is the extent and number of genes that can influence aging, as detailed below.

How Genes Can Regulate the Aging Process

The greatest evidence in favor of seeing aging as having a unifying core is the way single genes can modulate the aging process. According to the GenAge database, hundreds of genes that when individually manipulated can alter aging and/or lifespan have been identified in model organisms. The plasticity of lifespans in invertebrates shows how one or a few genes can regulate the entire aging process (Lin et al., 1998; Vanfleteren and Braeckman, 1999; Benard and Hekimi, 2002; Johnson, 2002; Kenyon, 2010). For example, in worms, single gene mutations can extend lifespan by almost 10-fold (Ayyadevara et al., 2008). In mice, there are several examples of single genes that can extend longevity, in some cases by around 50%, increase the MRDT, and delay the onset of multiple age-related changes and diseases (Liang et al., 2003; de Magalhaes et al., 2005). These results clearly argue that genetic mechanisms can, up to a certain degree, regulate aging in mammals. (These genes and their mechanisms are further detailed elsewhere.) Human studies have shown a significant degree of heritability of longevity, in particular at later ages (Hjelmborg et al., 2006). Studies in centenarians, in fact, have shown that the offspring of long-lived parents are protected against age-related diseases (for example see Atzmon et al., 2004). Genetic variants (alleles) in human homologs of genes shown to regulate aging in model organisms have also been associated with human longevity (Bonafe et al., 2003; Suh et al., 2008), albeit effects as marked as those observed in model organisms have not been observed in humans. Lastly, it has been argued that the rapid evolution of longevity in the human lineage indicates that maybe a small number of genes are able to regulate the pace of aging (Cutler, 1975).

One of the most intriguing phenotypes in the biology of aging is the accelerated aging witnessed in humans and animals as a result of certain mutations. Progeroid syndromes, as they are called, are rare genetic diseases that originate a phenotype that resembles accelerated aging. The three most studied such syndromes are Werner's (WS), Cockayne, and Hutchinson-Gilford's syndrome (Martin, 1978; Martin and Oshima, 2000). Though patients with Down syndrome or trisomy 21 often also exhibit progeroid features (Martin, 1978; Raji and Rao, 1998), this disease has not gathered as much attention from the perspective of aging research as the other three syndromes named above. In particular patients with WS exhibit striking features resembling accelerated aging and show an early onset--compared to normal aging--of multiple age-related diseases like diabetes, cataracts, osteoporosis, baldness, and atherosclerosis (Goto, 1997; Fig. 1). Though differences exist in terms of pathology, what most markedly distinguishes these syndromes is age of onset with Hutchinson-Gilford's and Cockayne syndrome almost exclusively affecting children while WS patients normally reach

adulthood. There are also five reported cases of a neonatal form of progeria called Wiedemann-Rautenstrauch syndrome, in which babies appear to be born old, but further research is needed to confirm or dismiss such cases as accelerated aging (Rodriguez et al., 1999; Arboleda et al., 2007).

George Martin suggested that WS mimics about 50% of aging characteristics: early cataracts, old skin, gray hair, etc., but not brain aging (Martin, 1982; Gosden, 1996, p. 126). This is a high proportion since it is not clear that these diseases are indeed accelerated aging. Moreover, the WS phenotype tends to affect tissues where *WRN*, the gene in which mutations result in WS (Yu et al., 1997), is expressed (Motonaga et al., 2002), so it makes sense that not all organs display signs of accelerated aging in WS. Such diseases demonstrate the hierarchical essence of aging in which a single gene can regulate a vast array of complex age-related changes. (Further details concerning the underlying molecular mechanisms of these diseases are presented in another essay.)

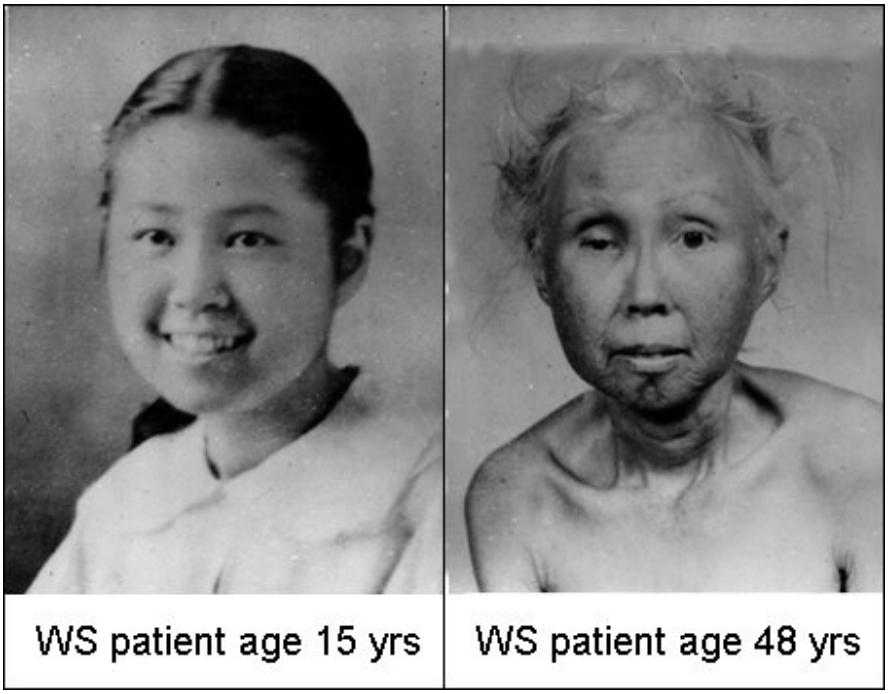

Figure 1: A Werner syndrome patient. Source: the University of Washington Werner syndrome Home Page.

One key discovery in the biology of aging was made in 1935, following earlier findings (Osborne et al., 1917), by veterinary nutritionist Clive McCay and colleagues. As previously mentioned, they discovered they could slow aging in laboratory rats just by making them eat less calories while maintaining normal levels of proteins, vitamins, and minerals (McCay et al., 1935). This process became known as caloric restriction (CR) and appears to work in many animals; it has been particularly well-studied in mice. From mice, we know that CR not only increases longevity by up to 50% but it also postpones or diminishes the incidence of most age-related diseases, decreases the rate of aging (de Magalhaes et al., 2005), and delays development (reviewed in Weindruch and Walford, 1988). Doubts have for long existed on whether CR results from some technical artifact. Even so, CR remains the most impressive way to delay aging in mammals, particularly since it derives from a quite simple intervention. Like WS, CR demonstrates how it is possible to delay the aging process as a whole, suggesting that aging has a unifying clock. (The mechanisms of CR are still under debate but are further discussed in another essay.)

Recent large-scale gene expression studies have revealed a degree of coordination in age-related changes in gene expression; in mice different tissues age in a coordinated fashion so that a given mouse may exhibit rapid aging while another ages slowly across multiple tissues (Zahn et al., 2007). This suggest the existence of common or synchronizing mechanisms, or at least systemic factors, in the aging phenotype. On the other hand, individual organs have some unique gene expression changes with age (Zahn et al., 2007; de Magalhaes et al., 2009b). Besides, only a small percentage of genes (<5%) are expressed in all tissues (Su et al., 2004) which suggests that aging may have multiple modulators in individual tissues. For example, even if one particular type of damage (e.g., DNA damage) is the underlying cause of aging different tissues will respond differently and be affected in different ways.

Like many others (Miller, 1999), I think there is a fundamental process that gives rise to aging. There is a uniform, unifying genetic-based core that synchronizes most facets of aging. Nonetheless, it is plausible--even likely--that some age-related changes and diseases are independent of this core process; causes of disease and causes of aging can be different. For example, Machado-Joseph is a neurodegenerative genetic disease with a typical adult onset that results from a single gene defect that appears to result in a toxic form of the protein (Sequeiros et al., 1994). Similarly, many genes can influence individual age-related changes (Martin, 1982). Furthermore, there is a great variability in age-related changes among individuals, suggesting that lifestyle can influence aging to some degree (Finch, 1990, pp. 317-352). Nonetheless, it is interesting to note that, in worms, retarding aging also reduces aberrant protein aggregation as observed in neurodegenerative diseases like Alzheimer's disease (Morley et al., 2002; Cohen et al., 2006). Even for diseases that involve a toxic gain of function, the length of time until the disease develops often correlates with the organism's lifespan, possibly because such diseases are rarely the result of a single defect and thus can be influenced by a number of processes and additional insults (Morimoto, 2006). In conclusion, all the hundreds of genes identified to regulate aging provide strong evidence that aging has a strong genetic basis and that indeed a basic aging process exists. There may even be a single mechanistic clock, though this is not yet proven. Nonetheless, age-related changes in individual organs may be subject to unique constraints, both environmental and genetic.

Although the broad aim of my work is to unravel the aging process, I believe that focusing on these fundamental causes of aging, the genetic basis for differences in rate of aging between similar species and between individuals, is the most appropriate strategy (de Magalhaes, 2003), an idea long defended by many others (Comfort, 1968). The proportion of diseases that are independent of--or largely unaffected by--the fundamental cause(s) of aging seem low, but I cannot exclude that the impact of such fundamental mechanism(s) is overestimated. Besides, no doubt independent causes and risk factors (including environmental factors) contribute to the development of age-related diseases, even if influenced by unifying aging mechanisms. Of course, humans do not appear to have death genes like the salmon, so no doubt many genes interacting with each other are involved in aging; yet elucidating the genes and mechanisms controlling aging is paramount to develop interventions that delay aging and ultimately to cure aging. Candidate mechanisms of aging are discussed elsewhere.

As mentioned before, more resources are aimed at trying to cure age-related diseases than aging, or senescence, itself. This is partly due to the belief that aging is a complex, difficult to understand process that has eluded generations of gerontologists. Such way of thinking may lead to complacency and unambitious objectives. On the other hand, if one sees aging as caused by a unifying mechanism objectives become more ambitious; CR and recent findings in the genetics of aging prove that aging can be manipulated in model organisms, and the prospects of developing drugs that delay aging is excellent (de Magalhaes et al., 2012). That said, just because aging has a genetic core does not mean that curing it will be easy. Naturally occurring genetic variants in mice (and possibly in humans too) can delay aging, but only to a certain point. Therefore, even when we identify the genetic mechanisms behind human aging, curing aging will be an Herculean task.

6. Why Do We Age?

We do not see the world as it is, we see the world as we are.

- The Talmud

Keywords: ageing, causes of aging, genetics, science of aging, why do people age, why we get old

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Over the years, many theories have emerged to explain what process or mechanism drives aging (reviewed in Medvedev, 1990; Weinert and Timiras, 2003). In fact, almost every important discovery in molecular or cellular biology has led to a new family of theories of aging. Most theories of aging have old origins, but the inherent difficulties of studying human aging--such as the lack of adequate models--make testing these theories a difficult, lengthy, and expensive process. Moreover, interpreting the results, for example from longevity studies, is frequently controversial; discriminating between causes and effects of aging is often impossible. That is why, at present, no consensus exists over what causes aging, what determines rate of aging across mammals, or what changes occur in humans from age 30 to 70 to increase the chances of dying by over 30-fold. Nevertheless, some theories have gathered more empirical support than others and this essay aims to present and discuss them. As always in **senescence.info**, I try to provide a balanced view, even though I of course give more emphasis to the most prominent theories. Like in other fields with little empirical evidence and many conflicting theories, biogerontology is tainted by scientific dogmatism and, even though I have my own favorite theories of aging, I try to follow an open-minded skepticism regarding this crucial yet controversial topic (de Magalhaes, 2005a).

Aging is a largely mysterious process. The aging process may derive from changes occurring in parallel in different tissues due to intrinsic cellular mechanisms or changes in one tissue may be predominant. Some authors argue that aging is located within one tissue such as the brain (e.g., Mattson et al., 2002) while others defend that aging originates in all tissues (e.g., Kowald and Kirkwood, 1994). Some researchers even argue that one type of cells such as bone marrow stem cells may be crucial (Geiger and Van Zant, 2002; Van Zant and Liang, 2003). "Big bang" reproduction demonstrates how one particular system, often the endocrine system, can regulate aging (see Gosden, 1996 for arguments). Results from the model system *C. elegans* indicate that a few lineages in mosaic organisms confer longevity (Apfeld and Kenyon, 1998; Hsin and Kenyon, 1999; Lin et al., 2001; Arantes-Oliveira et al., 2002; Patel et al., 2002), perhaps due to endocrine signals (Wolkow et al., 2000; Ch'ng et al., 2008). Neurons and the brain might play a regulatory role in aging (Bauer et al., 2005; Bishop and Guarente, 2007), at least to some degree. Some results from mice also suggest the existence of systemic factors in aging, but only to some degree (Conboy et al., 2005). On the other hand, as mentioned previously, it appears that intrinsic changes occur in human cells as we age (de Magalhaes, 2004). Although this debate has not been settled yet, it appears that intrinsic cellular mechanisms play a role in aging, though these can be modulated by extracellular factors like hormones.

There are many types of theories of aging. I could have divided this section in many different ways, but I think it makes sense to divide it into damage-based and programmed theories of aging. Damage-based theories, as the name implies, defend that aging results from a continuous process of damage accumulation originating in by-products of metabolism; in other words, a certain form of damage accumulates throughout the entire lifespan and causes most aspects of what I previously defined as aging. Typically, this damage is a by-product of normal cellular processes, or a consequence of inefficient repair systems. On the other hand, programmed theories of aging argue that aging is not a result of random or stochastic process but rather driven by genetically regulated processes.

As argued elsewhere, aging has a strong genetic component. Even damage-based theories of aging recognize that certain genetic factors, such as defensive or protective genes, play a role in aging (Kirkwood and Austad, 2000). Likewise, programmed theories of aging recognize that some forms of damage contribute to aging and that environmental factors influence the outcome of aging to some degree. So the difference between these two camps lies in the underlying mechanism: damage-based theories of aging argue that aging is predominantly a result of interactions with the environment (e.g., Holliday, 2004) and/or damage from chemical reactions (e.g., Baynes, 2000), while programmed theories argue that aging is predetermined and occurs on a fixed schedule triggered by genetic programs. Others have suggested similar segregations of theories of aging (e.g., Cutler, 1979). For instance, it has been proposed that aging could be: 1) a result of extrinsic or intrinsic factors that cause an accumulation of damage; or 2) that aging is a result of changes in gene expression that are either programmed or derived from DNA structural changes (Campisi, 2000). As will become apparent, however, a certain amount of overlap between theories of aging is possible.

One of the major problems in developing a coherent aging theory is separating causes from effects. As any statistician will tell you: "Correlation does not mean causation." Just because two processes parallel each other we cannot imply a causal relation in any direction. Therefore, it is extraordinarily difficult to predict which, if any, mechanistic theory of aging is correct. One way to infer the impact on aging of the pathways described here is using a system-level approach. By perturbing each component of a pathway under study and integrating the observed effects it is possible to discriminate causes from effects and formulate new hypotheses (de Magalhaes and Toussaint, 2004b). While interpreting theories of aging I try to follow a system-biology approach based on, if any, published perturbations of the pathway's components. Perturbations typically refer to genetic manipulations and more details concerning many of the genes discussed are available at the GenAge database. Epistemology in the field of aging is crucial and I think genetic manipulations offer ample, usually unambiguous, evidence with which to understand theories of aging.

Unfortunately, the inevitable conclusion of this section is that the jury is still out regarding mechanisms of aging. Although the search for a pacemaker of age-related changes continues, the bottom line is that all proposed mechanisms can be upregulated by some other--unknown or not--mechanism. The large number of aging theories is proof that our understanding of aging is still far from perfect; to quote David Rollo: "In any field of science, the true degree of understanding is inversely proportional to the number of explanatory theories that prevail." Even so, and since there are more doubts than answers in gerontology, we should not discard these theories easily. Life, and marveling life and death as we do in gerontology, is a game of probabilities. Some theories have gathered more evidence than others and hence may be more promising foci for future research and for developing anti-aging interventions. So please read on the different theories of aging and hopefully you can conjure better theories or determine ways to better test the current theories experimentally.

6.1 Damage-Based Theories of Aging

One class of theories of aging is based on the concept that damage, either due to normal toxic by-products of metabolism or inefficient repair/defensive systems, accumulates throughout the entire lifespan and causes aging. In this essay, I present and review the most important of these damage-based theories.

Sections

Keywords: ageing, anti-oxidants, biogerontology, error catastrophe theory of aging, hypotheses, life span

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The general idea behind damage-based theories of aging is that a slow build-up of damage, perhaps even from conception (Gavrilov and Gavrilova, 2001), eventually leads to failure of the system which can be seen as failure of a critical organ like the heart or the whole body. It is useful to point out, however, that some authors (e.g., Olson, 1987; Holliday, 2004; Kirkwood, 2005) defend that aging is a result of many forms of damage accumulation, and hence that aging is due to an overlap of the mechanistic theories of aging described below.

Orgel's Hypothesis, Protein Damage and Autophagy

Aging has long been seen as a result of errors of many kind. An early attempt to develop a theory engulfing the genetic and protein machineries was Orgel's hypothesis (Orgel, 1963). Essentially, his idea was that errors in transcription from DNA lead to errors in proteins which build-up over time and cause more errors in transcription, creating an amplifying loop that eventually kills the cell and leads to aging. Errors in DNA repair would also affect the accuracy of the flow of information in cells (Orgel, 1973). Indeed, damaged proteins accumulate with age, and enzymes lose catalytic activity with age (Gershon and Gershon, 1970). This can lead to cellular dysfunction and accumulation of other forms of damage. On the other hand, Orgel's hypothesis has been regarded as unlikely to be correct for various reasons: feeding abnormal amino acids to animals to increase the number of errors in proteins does not result in a shorter lifespan (Strehler, 1999, p. 293); errors in macromolecular synthesis also do not appear to increase with age (Rabinovitch and Martin, 1982); *in vitro* aging cultured fibroblasts do not have increased protein errors (Harley et al., 1980)--and, in fact, cellular senescence appears to be caused by other mechanisms. Presently, Orgel's hypothesis is largely discarded.

Even though Orgel's hypothesis failed the test of time, some age-related diseases could be due to protein defects and accumulating protein errors (e.g., see Lee et al., 2006; Morimoto, 2006) and a role for protein dysfunction in aging is a possibility. Proteasomes are protein complexes that degrade other proteins; their expression decreases with age (Lee et al., 1999) and this has been implicated as a factor contributing to aging (Friguet et al., 2000). Also, the half-life of proteins is longer in older animals (Friguet et al., 2000). One study found evidence that proteins involved in protein degradation are under selection in lineages where longevity increased (Li and de Magalhaes, 2012). Two studies found evidence that protein stability and protein homeostasis are enhanced in long-lived bats and in the naked mole-rat when compared to mice (Perez et al., 2009; Salmon et al., 2009), yet another study found no evidence that protein repair and recycling are correlated with longevity in 15 species of birds

and mammals (Salway et al., 2011). Therefore, the results are not conclusive but this is an area where further studies are warranted.

Autophagy is a process by which the cell digests its own organelles and components. Recent studies, in particular genetic manipulations in model organisms, point towards a role of autophagy in aging (reviewed in Cuervo et al., 2005; Rubinsztein et al., 2011). In flies, disruption of autophagy shortens (Juhasz et al., 2007) while enhanced autophagy increase lifespan (Simonsen et al., 2008). Manipulation of autophagy-related genes has also been associated with longevity in yeast (Tang et al., 2008). There is in fact evidence that longevity-associated pathways, such as GH/IGF1 that is detailed elsewhere and TOR (which some anti-aging interventions target), influence autophagy (Toth et al., 2008; Salminen and Kaarniranta, 2009; Kamada et al., 2010; Neufeld, 2010), though the causality of these links remains to be established since autophagy is related to other processes too. Dysfunction of autophagy has also been linked to neurodegenerative disorders (Wong and Cuervo, 2010). In mouse liver, autophagy declines with age and its maintenance through genetic manipulation can improve the ability of cells to handle protein damage, resulting in lower levels of damaged proteins and improved organ function (Zhang and Cuervo, 2008). While a lot of works remains to elucidate the role of autophagy in aging, it does appear that protein homeostasis is important for longevity and its dysfunction could contribute to aging (Morimoto and Cuervo, 2009).

Energy Metabolism and Aging

In 1908, physiologist Max Rubner discovered a relationship between metabolic rate, body size, and longevity. In brief, long-lived animal species are on average bigger--as detailed before--and spend fewer calories per gram of body mass than smaller, short-lived species. The energy consumption hypothesis states that animals are born with a limited amount of some substance, potential energy, or physiological capacity and the faster they use it, the faster they will die (Hayflick, 1994). Later, this hypothesis evolved into the rate of living theory: the faster the metabolic rate, the faster the biochemical activity, the faster an organism will age. In other words, aging results from the pace at which life is lived (Pearl, 1928). This hypothesis is in accordance with the life history traits of mammals in which a long lifespan is associated with delayed development and slow reproductive rates (reviewed in Austad, 1997a & 1997b).

As previously mentioned, caloric restriction (CR) is one of the most important discoveries in aging research. Although the mechanisms behind CR remain a subject of discussion (see below), since it involves a decrease in calories, one hypothesis put forward by George Sacher is that maybe CR works by delaying metabolic rates, in accordance with the energy consumption hypothesis (reviewed in Masoro, 2005). Body temperature is crucial to determine metabolic rate since the rate of chemical reactions rises with temperature. One common feature of animals, such as mice, rats, and monkeys, under CR is a lower body temperature (Weindruch and Walford, 1988; Ramsey et al., 2000), which is consistent with the energy consumption hypothesis. On the other hand, some studies in rodents suggest that CR can extend lifespan without reducing metabolic rate (Masoro 2005). For example, some evidence indicates that mice under CR burn the same amount of energy as controls, suggesting they have similar metabolic rates. These studies, however, remain controversial in the way metabolic rate is normalized to metabolic mass (McCarter and Palmer, 1992). An alternative hypothesis is that CR shifts metabolic pathways (Duffy et al., 1990). More recent results suggest that previous studies used unreliable estimates of metabolic mass in their calculations and indeed CR changes metabolic rates, supporting the rate of living hypothesis (Greenberg and Boozer, 2000), yet the debate has not been settled.

Several experiments have cast doubts on the energy consumption hypothesis. For instance, rats kept at lower temperatures eat 44% more than control mice and yet do not age faster (Holloszy and Smith, 1986). In fact, mice with higher metabolic rates may live slightly longer (Speakman et al., 2004). Mutations in the tau protein in hamsters increase metabolic rates and extends lifespan (Oklejewicz and Daan, 2002). Lastly, as detailed before, metabolic rates, when correctly normalized for body size, do not correlate with longevity in mammals (de Magalhaes et al., 2007a). Despite its intuitive concept,

the rate of living theory is practically dead. Based on observations from CR, it is likely that energy metabolism plays a role in aging but, as described below, it is not clear how this occurs. One hypothesis is that energy metabolism is linked to insulin-signaling, as mentioned ahead.

The Free Radical Theory of Aging

Free radicals and oxidants--such as singlet oxygen that is not a free radical--are commonly called reactive oxygen species (ROS) and are such highly reactive molecules that they can damage all sorts of cellular components (Fig. 1). ROS can originate from exogenous sources, such as ultraviolet (UV) and ionizing radiations, and from several intracellular sources. The idea that free radicals are toxic agents was first suggested by Rebeca Gerschman and colleagues (Gerschman et al., 1954). In 1956, Denham Harman developed the free radical theory of aging (Harman, 1956; Harman, 1981). Since oxidative damage of many types accumulate with age (e.g., Ames et al., 1993), the free radical theory of aging simply argues that aging results from the damage generated by ROS (reviewed in Beckman and Ames, 1998).

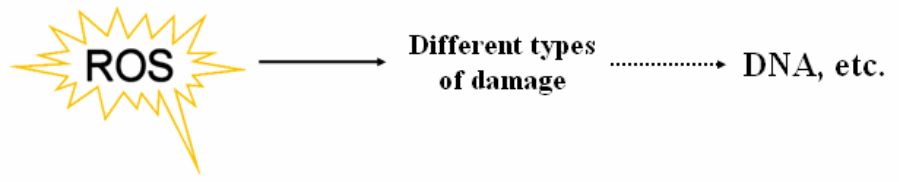

Figure 1: ROS or reactive oxygen species can be formed by different processes including normal cell metabolic processes. Due to their high reactivity, ROS can damage other molecules and cell structures. The free radical theory of aging argues that oxidative damage accumulates with age and drives the aging process.

To protect against oxidation there are many different types of antioxidants, from vitamins C and E to enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. Briefly, antioxidant enzymes are capable of degrading ROS into inert compounds through a series of chemical reactions (Ames et al., 1981; Ames et al., 1993). The simple existence of enzymes to prevent damage by ROS is a strong indicator that ROS are biologically important, dangerous molecules (de Magalhaes and Church, 2006).

Most experimental evidence in favor of the free radical theory of aging comes from invertebrate models of aging. Transgenic fruit flies, *Drosophila melanogaster*, overexpressing the cytoplasmic form of SOD, called Cu/ZnSOD or SOD1, and catalase have a 34% increase in average longevity and a delayed aging process (Orr and Sohal, 1994). More recent findings, however, suggest that the influence of SOD1 and catalase in *Drosophila* aging may had been overestimated because the authors only took into account short-lived strains (Orr et al., 2003). Overexpressing bovine SOD2, the mitochondrial form of SOD, also called MnSOD, in *Drosophila* slightly extends average longevity but does not delay aging (Fleming et al., 1992). Also in *Drosophila*, expression of SOD1 in motor neurons increases longevity by 40% (Parkes et al., 1998) and overexpression in neurons of thioredoxin, a protein involved in reduction-oxidation (redox) reactions that can act as an antioxidant, increased lifespan by 15% (Umeda-Kameyama et al., 2007). Certain long-lived strains of both *Drosophila* (Rose, 1989; Hari et al., 1998) and the nematode worm *Caenorhabditis elegans* (Larsen, 1993) have increased levels of antioxidant enzymes. On the other hand, evidence against the free radical theory has also emerged from invertebrate models. Briefly, deletion of SOD2 in *C. elegans* surprisingly extends lifespan (Van Raamsdonk and Hekimi, 2009), and long-lived ant queens actually have lower levels of SOD1 (Parker et al., 2004). Overexpression of SOD2 and catalase decreased the mitochondrial ROS release and increased resistance to oxidative stress in *Drosophila* yet decreased lifespan (Bayne et al., 2005).

In addition to antioxidants, some enzymes catalyze the repair caused by ROS. One of such enzymes is methionine sulfoxide reductase A (MSRA), which catalyzes the repair of protein-bound methionine residues oxidized by ROS. Overexpression of MSRA in the nervous system of *Drosophila* increases longevity (Ruan et al., 2002) while mice without MSRA have a decreased longevity of about 40% (Moskovitz et al., 2001). Whether the aging process is affected remains to be seen (de Magalhaes et al., 2005), although the results from *Drosophila* suggest that age-related decline is also delayed by MSRA overexpression. Another enzyme that repairs oxidative damage is 8-oxo-dGTPase, which repairs 8-oxo-7,8-dihydroguanine, an abundant and mutagenic form of oxidative DNA damage. But contrary to the results involving MSRA, when researchers knocked out the gene responsible for 8-oxo-dGTPase, although the mutated mice had an increased cancer incidence, their aging phenotype did not appear altered (Tsuzuki et al., 2001).

Targeted mutation of $p66^{shc}$ in mice has been reported to increase longevity by about 30%, inducing resistance to oxidative damage, and maybe delaying aging (Migliaccio et al., 1999). Although the exact function of $p66^{shc}$ remains unclear, some evidence suggests it may be related to intracellular oxidants and apoptosis (Nemoto and Finkel, 2002; Trinei et al., 2002; Napoli et al., 2003). Also, transgenic mice overexpressing the human thioredoxin gene featured an increased resistance to oxidative stress and an extended longevity of 35% (Mitsui et al., 2002). Like $p66^{shc}$, mammalian thioredoxin regulates the redox content of cells and is thought to have anti-apoptotic effects (Saitoh et al., 1998; Kwon et al., 2003). More recent results suggest modest effects of thioredoxin overexpression on the lifespan of male mice and no effects on the lifespan of females (Perez et al., 2011). Neither $p66^{shc}$ nor thioredoxin are "traditional" antioxidants, so these findings could be unrelated to the free radical theory of aging but rather, for instance, tissue homeostasis. Mice with extra catalase in their mitochondria lived 18% more than controls and were less likely to develop cataracts, but they did not appear to age slower and their extended lifespan appeared to derive from a decrease in cardiac diseases throughout the entire lifespan (Schriner et al., 2005). Lastly, the phenotype witnessed in a strain called senescence-accelerated mice may be related to free radical damage (Edamatsu et al., 1995; Mori et al., 1998).

Experiments in feeding mice antioxidants--either a single compound or a combination of compounds--were able to decrease oxidative damage and increase average longevity but none of them clearly delayed aging (Harman, 1968; Comfort et al., 1971; Heidrick et al., 1984; Saito et al., 1998; Holloszy, 1998; Quick et al., 2008), while other studies did not conclude that feeding mice antioxidants increases longevity (e.g., Lipman et al., 1998). Several attempts have been made to overexpress or knock-out antioxidants in mice, but the results have been largely disappointing (Sohal et al., 2002; de Magalhaes, 2005a; de Magalhaes and Church, 2006; Lapointe and Hekimi, 2010). Often animals do not show any differences in their aging phenotype when compared to controls (Reaume et al., 1996; Ho et al., 1997; Schriner et al., 2000). In one of the most elegant experiments to test the free radical theory of aging, knockout mice heterozygous for SOD2 showed increased oxidative damage at a cellular and molecular level but did not show significant changes in longevity or rate of aging (Van Remmen et al., 2003). Ubiquitous overexpression of SOD1 in mice also failed to increase longevity (Huang et al., 2000). These results suggest that antioxidant proteins are already optimized in mammals. Indeed, correlations between rate of aging and antioxidant levels in mammals are, if they exist, very weak (reviewed in Finch, 1990; Sohal and Weindruch, 1996). Some studies found correlations between the levels of certain antioxidants and longevity in mammals, but failed to find any consensus (Tolmasoff et al., 1980; Ames et al., 1981; Cutler, 1985; Sohal et al., 1990). The long-lived naked mole-rat does not appear to have higher levels of antioxidants when compared to mice (Andziak et al., 2005). The way antioxidants can increase longevity but do not affect rate of aging suggests that antioxidants may be healthy but do not affect the aging process, as debated elsewhere.

Although ROS can have several sources, some argue that ROS originating in the cellular metabolism which takes place in the cell's energy source, the mitochondrion, are the source of damage that drives aging. Since ROS are a result of cellular metabolism, the free radical theory of aging has been associated with the rate of living theory (Harman, 1981). One mechanism proposed for CR is that animals under CR produce less ROS and therefore age slower (Weindruch, 1996; Masoro, 2005), but

since the rate of living theory seems out-of-favor this perspective will not be further discussed here. An alternative hypothesis is that the rate of mitochondrial ROS generation, independently of metabolic rates or antioxidant levels, may act as a longevity determinant (Sohal and Brunk, 1992; Barja, 2002). Some results suggest that the rate of ROS generated in the mitochondria of post-mitotic tissues helps explain the differences in lifespan among some animals, particularly among mammals (Ku et al., 1993; Barja and Herrero, 2000; Sohal et al., 2002; Lambert et al., 2007) and between birds and mammals (reviewed in Barja, 2002). One pitfall of these studies is that technical limitations exist in measuring ROS production in isolated mitochondria. For example, none of these studies measures the levels of hydroxyl radical, the most reactive and destructive of the ROS; often, hydrogen peroxide and superoxide anion are measured since they can react to give the hydroxyl radical. Even so, such studies may not be representative of what actually occurs. Moreover, two studies in *Drosophila* found that lowering ROS leakage from the mitochondria either did not result in a longer lifespan (Miwa et al., 2004) or even resulted in a shorter lifespan (Bayne et al., 2005). On the other hand, comparative genomics studies have revealed several associations between features of mitochondrial DNA (mtDNA) across species and longevity (de Magalhaes, 2005b; Moosmann and Behl, 2008; Nabholz et al., 2008; Aledo et al., 2011), suggesting that changes to mitochondrial proteins may be involved in the evolution of long lifespans, though further studies are necessary to elucidate the mechanisms involved.

Several pathologies in mice and humans derive from mutations affecting the mitochondrion, which often involve an increase in ROS leakage from the mitochondrion (Pitkanen and Robinson, 1996; Wallace, 1999; DiMauro and Schon, 2003). Yet these pathologies do not result in an accelerated aging phenotype, but frequently result in diseases of the central nervous system (Martin, 1978). One example is Friedreich's ataxia which appears to result from increased oxidative stress in mitochondria and does not resemble accelerated aging (Rotig et al., 1997; Wong et al., 1999). Deficiency of the mitochondrial complex I has been reported in a variety of pathologies such as neurodegenerative disorders (reviewed in Robinson, 1998). Cytochrome c deficiency has also been associated with neurodegenerative disorders (reviewed in DiMauro and Schon, 2003) as has selective vitamin E deficiency (Burck et al., 1981). Rats selected for high oxidative stress develop cataracts from an early age (Marsili et al., 2004); other pathologies, such as heart changes and brain dysfunctions, have been described in these animals (Salganik et al., 1994), yet it is unclear whether they can be considered progeroid. Perhaps ROS are involved in some pathologies involving post-mitotic cells, such as neurons. Another hypothesis is that mitochondrial diseases affect mainly the central nervous system due to its high energy usage (Parker, 1990 for arguments). There is some evidence of a mitochondrial optimization in the human lineage to delay degenerative diseases, but not necessarily aging (de Magalhaes, 2005b). Interestingly, both *Drosophila* and *C. elegans* are mostly composed of post-mitotic cells, which can explain why results from these invertebrates are more supportive of the free radical theory of aging than results from mice.

Although it is undeniable that ROS play a role in several pathologies, including age-related pathologies like cataracts (Wolf et al., 2005), the exact influence of ROS in mammalian aging is debatable. It is plausible that ROS play a role in age-related degeneration of energy-rich tissues such as the brain. One study found that superoxide dismutase/catalase mimetics prevented cognitive defects and oxidative stress in aged mice (Clausen et al., 2010). A similar study found that a SOD mimetic administered from middle age attenuated oxidative stress, improved cognitive performance, and extended lifespan by 11% (Quick et al., 2008). In conclusion, there is little direct evidence that ROS influence mammalian aging except perhaps in specific tissues such as the brain. Lastly, a changing paradigm is that ROS are not only damaging compounds but crucial in many cellular functions and thus it is the deregulation of pathways managing ROS that can contribute to aging rather than merely damage accumulation with age (de Magalhaes and Church, 2006).

The DNA Damage Theory of Aging

The DNA, due to its central role in life, was bound to be implicated in aging. One hypothesis then is that damage accumulation to the DNA causes aging, as first proposed by Failla in 1958 (Failla, 1958)

and soon after developed by physicist Leo Szilard (Szilard, 1959). The theory has changed over the years as new types of DNA damage and mutation are discovered, and several theories of aging argue that DNA damage and/or mutation accumulation causes aging (reviewed in Gensler and Bernstein, 1981; Vijg and Dolle, 2002; Hoeijmakers, 2009; Freitas and de Magalhaes, 2011). Because DNA damage is seen as a broader theoretical framework than mutations, and DNA damage can lead to mutations, the current focus is on DNA damage and thus the theory herein is referred to as DNA damage theory of aging.

It is well-established that DNA mutations/alterations--many often irreversible--and chromosomal abnormalities increase with age in mice (Martin et al., 1985; Dolle et al., 1997; Vijg, 2000; Dolle and Vijg, 2002) and humans (e.g., Esposito et al., 1989; Lu et al., 2004). Experiments in mice also suggest that DNA damage accumulates with age in some types of stem cells and may contribute to loss of function with age (Rossi et al., 2007). Long-lived mutant mice and animals under CR seem to have a lower mutation frequency, at least in some tissues (Garcia et al., 2008). Similarly, longevity of worm strains correlates with DNA repair capacity (Hyun et al., 2008). It is impossible, however, to tell whether these changes are effects or causes of aging. Correlations have been found between DNA repair mechanisms and rate of aging in some mammalian species (Hart and Setlow, 1974; Grube and Burkle, 1992; Cortopassi and Wang, 1996). In theory, even a slight increase in DNA repair rate over a large period of time and hundreds of cell divisions will have major consequences and could contribute to determine rate of aging. On the other hand, it has been argued that such correlations may be an artifact of long-lived species being on average bigger (Promislow, 1994).

As mentioned elsewhere, progeroid syndromes are rare genetic diseases that appear to be accelerated aging. Interestingly, the most impressive progeroid syndromes, Werner's, Hutchinson-Gilford's, and Cockayne syndrome originate in genes that are related to DNA repair/metabolism (Martin and Oshima, 2000; de Magalhaes, 2005a; Freitas and de Magalhaes, 2011). Werner's syndrome (WS) originates in a recessive mutation in a gene, *WRN*, encoding a RecQ helicase (Yu et al., 1996; Gray et al., 1997). Since WRN is unique among its protein family in also possessing an exonuclease activity (Huang et al., 1998), it seems to be involved in DNA repair. Although the exact functions of WRN remain a subject of debate, it is undeniable that WRN plays a role in DNA biology, particularly in solving unusual DNA structures (reviewed in Shen and Loeb, 2000; Bohr et al., 2002; Fry, 2002). In fact, cells taken from patients with WS have increased genomic instability (Fukuchi et al., 1989). Topoisomerases are enzymes that regulate the supercoiling in duplex DNA. WS cells are hypersensitive to topoisomerase inhibitors (Pichierri et al., 2000). As such, WS is an indicator that alterations in the DNA over time play a role in aging.

As with WRN, the protein whose mutation causes Hutchinson-Gilford's syndrome is also a nuclear protein: lamin A/C (Eriksson et al., 2003). Recent results also suggest that some atypical cases of WS may be derived from mutations in lamin A/C (Chen et al., 2003). The exact functions of lamin A/C remain unknown, but it appears to be involved in the biology of the inner nuclear membrane. Some evidence suggests that the DNA machinery is impaired in Hutchinson-Gilford's syndrome (Wang et al., 1991; Sugita et al., 1995), again suggesting that changes in the DNA are important in these diseases and, maybe, in normal aging. The protein involved in Cockayne Syndrome Type I participates in transcription and DNA metabolism (Henning et al., 1995). Other progeroid syndromes exist, though the classification is subjective. For example, Nijmegen breakage syndrome, which derives from a mutated DNA double-strand break repair protein (Carney et al., 1998; Matsuura et al., 1998; Varon et al., 1998), has been considered as progeroid (Martin and Oshima, 2000).

Ample mouse models of accelerated aging have implicated genes involved in DNA repair such as the mouse homologues of xeroderma pigmentosum, group D (de Boer et al., 2002), ataxia telangiectasia mutated or ATM (Wong et al., 2003), p53 (Donehower et al., 1992; Donehower, 2002; Tyner et al., 2002; Cao et al., 2003), and Ercc1 (Weeda et al., 1997). Thus many progeroid syndromes in mice involve the DNA machinery (Hasty et al., 2003; de Magalhaes, 2005a). (I should note that most of the aforementioned genes, as well as other genes implicated in accelerated aging in mice, are described in the GenAge database.) Taken together, results from progeroid syndromes in mice and man support the

DNA damage theory of aging. One hypothesis is that DNA damage accumulation with age triggers cellular signalling pathways, such as apoptosis, that result in a faster depletion of stem cells which in turn contributes to accelerated aging (Freitas and de Magalhaes, 2011).

In spite of the progeroid syndromes described above, some genetic manipulations in mice have failed to support the theory. Mice deficient in Pms2, a DNA repair protein, had elevated mutation levels in multiple tissues yet did not appear to age faster than controls (Narayanan et al., 1997). Embryos of mice and flies irradiated with x-rays do not age faster (reviewed in Cosgrove et al., 1993; Strehler, 1999), though one report argued that Chernobyl victims do (Polyukhov et al., 2000). Certain mutations in DNA repair proteins, such as p53 in humans (Varley et al., 1997), despite affecting longevity and increasing cancer incidence, fail to accelerate aging.

An emerging hypothesis is that only specific types of DNA changes are crucial in aging, which would explain why mutations in some DNA repair genes affect aging while others do not. One study systematically analyzed DNA repair genes associated or not with aging and found that genes involved in non-homologous end joining are more often related to aging (Freitas et al., 2011). Emerging evidence also suggests that DNA damage that contributes to mutations and/or chromosomal aberrations increases the risk of cancer while DNA damage that interferes with transcription appears to contribute to aging possibly via effects on cellular aging and cell signalling (Hoeijmakers, 2009). Taken together, the results from manipulations of DNA repair pathways in mice suggest that disruption of specific pathways, such as nucleotide excision repair and non-homologous end joining, is more strongly associated with premature aging phenotypes and may thus be more important to aging (Freitas and de Magalhaes, 2011).

If the DNA damage theory of aging is correct, then it should be possible to delay aging in mice by enhancing or optimizing DNA repair mechanisms. Unfortunately, and in spite of numerous efforts (reviewed in de Magalhaes, 2005a), this crucial piece of evidence is still lacking. For example, mice overexpressing a DNA repair gene called *MGMT* had a lower cancer incidence but did not age slower (Zhou et al., 2001). Arguably the most compelling evidence comes from mice with extra copies of tumour suppressors. Mice with extra copies of p53 and INK4a/ARF--whose functions are described elsewhere--lived 16% longer than controls but it was not clear if aging was delayed (Matheu et al., 2007). Interestingly, overexpressing telomerase in mice with enhanced expression of p53 and INK4a/ARF, which are cancer-resistant, results in an increase in lifespan up to 40% (Tomas-Loba et al., 2008). Whether aging is delayed in these animals or even if DNA repair is improved is unclear but these findings do suggest some level of protection from age-related degeneration via optimization of pathways associated with cancer and DNA damage responses.

One possibility is that ROS damage to DNA plays a role in aging. Some circumstantial evidence exists in favor of such hypothesis (Hamilton et al., 2001), yet given the aforementioned concerns regarding the role of ROS in aging this appears unlikely, except perhaps in specific tissues like the brain. Even though damage from free radicals to nuclear DNA remains an unproven cause of aging, since ROS originate in the mitochondrion, and since mitochondria possess their own genome, many advocates of the free radical theory of aging consider that oxidative damage to mitochondria and to the mtDNA is more important (Harman, 1972; Linnane et al., 1989; de Grey, 1997; Barja, 2002). Indeed, some evidence exists that under CR oxidative damage to mtDNA is more important than oxidative damage to nuclear DNA (reviewed in Barja, 2002). Mutations in mtDNA tend to accumulate with age in some tissues (Corral-Debrinski et al., 1992; Yang et al., 1994; Tanhauser and Laipis, 1995; Liu et al., 1998), though not necessarily caused by ROS (reviewed in Larsson, 2010). Likewise, nuclear mutations have been suggested to contribute to mitochondrial dysfunction (Hayashi et al., 1994). One study found that accumulating mutations to mitochondrial DNA are also unlikely to drive stem cell aging (Norddahl et al., 2011). At present, and despite contradictory evidence in favor (Khaidakov et al., 2003 for arguments) and against the theory (Rasmussen et al., 2003 for arguments), current technology does not appear capable of assessing the true relevance of damage to mtDNA in aging (Lightowlers et al., 1999; DiMauro et al., 2002).

Interestingly, disruption of the mitochondrial DNA polymerase resulted in an accelerated aging phenotype, for the first time directly implicating the mtDNA in aging (Trifunovic et al., 2004). This appears to be unrelated to oxidative damage, however, and instead result from increased apoptosis and accumulated mtDNA damage (Kujoth et al., 2005; Trifunovic et al., 2005). On the other hand, a mitochondrial mutator mouse with much higher mutation frequency than normal mice did not exhibit signs of accelerated aging, though it also failed to show increased levels of mitochondrial deletions (Vermulst et al., 2007). Mice with a mutation in a mtDNA helicase accumulate mitochondrial deletions and develop progressive external ophthalmoplegia, but do not age faster (Tyynismaa et al., 2005). Mutations in mitochondrial DNA polymerase in humans result in mitochondrial disorders that, as in the context of the free radical theory of aging, typically affect the nervous system (Van Goethem et al., 2001; Tang et al., 2011), though other pathologies such as infertility (Rovio et al., 2001) have also been reported. As such, mtDNA may play a role in age-related diseases and aging (Wallace, 1992), though further research remains to confirm such hypothesis and elucidate the exact mechanisms involved.

Animal cloning involving somatic cells to create new organisms is an interesting technique for gerontologists (e.g., Lanza et al., 2000; Yang and Tian, 2000). Clones from adult frogs do not show signs that differentiation affects the genome (Gurdon et al., 1975). Dolly was "created" by transferring the DNA-containing nucleus of a post-mitotic mammary cell into an egg and from there a whole new organism was formed. We know Dolly had some genetic (Shiels et al., 1999) and--possibly more crucially--epigenetic defects (Young et al., 2001), so maybe her arthritis and the pathologies leading to her death are a result of damage present in the DNA. Nonetheless, she was remarkably "normal," having endured a complete developmental process and being fertile (Wilmut et al., 1997). Moreover, mice have been cloned for six generations without apparent harm (Wakayama et al., 2000). Perhaps the highly proliferative nature of the embryo can, by recombination, dilute the errors present in the DNA, but results from cloning experiments suggest that at least some cells in the body do not accumulate great amounts of DNA damage. It would be interesting to further study the longevity of cloned animals.

If progeroid syndromes represent a phenotype of accelerated aging then changes in DNA over time most likely play a role in aging, possibly through effects on cell dysfunction and loss that may involve stem cells (Freitas and de Magalhaes, 2011). Since many genetic perturbations affecting DNA repair do not influence aging, it is doubtful overall DNA repair is related to aging or that DNA damage accumulation alone drives aging. Understanding which aspects, if any, of DNA biology play a role in aging remains a great challenge in gerontology. Moreover, the next step to give strength to the DNA damage theory of aging would be to delay aging in mice based on enhanced DNA repair systems, but that has so far eluded researchers. In conclusion, changes in DNA over time may play an important role in aging, yet the essence of those changes and the exact mechanisms involved remain to be determined.

6.2 Programmed Theories of Aging

In addition to damage-based theories, a second class of theories of aging defends that aging is a genetically-determined, programmed process. In this essay, I present and review the most important concepts and theories in this context.

Sections

Keywords: ageing, aging clock, biogerontology, deterministic theories of aging, endocrinology, GHR, hormone theory of aging, pre-programmed aging

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For decades the idea that aging is programmed has been debated, and it was given new impetus by the extraordinary discoveries in the genetics of aging. The observation that single genes can modulate longevity and, to some degree, regulate the process of aging in model systems supports the idea that aging is to some degree programmed. For example, in yeast, transient expression of a single transcription factor can rejuvenate cells and extend lifespan (Unal et al., 2011). Of course, the same may not hold true for more complex organisms, like vertebrates, and discussed below are some of the proposed models of aging based on the concept of programmed aging.

Though there have been arguments in favor of seeing aging as part of an altruistic predetermined plan that serves a purpose (Longo et al., 2005), the idea that aging evolved for a reason--also known as group selection--is largely out-of-favor in modern gerontology. As mentioned elsewhere, in the vast majority of species, aging does not appear to be programmed in the sense that it serves a purpose (Austad, 2004). Therefore, in this essay, I do not imply any evolutionary purpose for aging; by programmed I mean in the sense of following a predetermined set of instructions, like in the result of gene action.

The Endocrine System as the Pacemaker of Aging

The idea that hormonal changes drive aging is decades-old (reviewed in Gosden, 1996). Since the levels of certain hormones like growth hormone (GH) (Ho et al., 1987) and its downstream target insulin-like growth factor I (IGF-1) (Hammerman, 1987) decline with age, an old idea is that such changes cause aging. Because to some degree the brain regulates endocrine changes (e.g., GH production), a major variant of hormone-based theories of aging is the neuroendocrine theory of aging that posits that the brain acts as the master clock of each life stage via hormonal changes Timiras, 2003). Given the intuitive nature of such endocrine-based theories, even today many anti-aging products aim to increase the levels of these hormones in older people. As briefly discussed before and further detailed below, however, it appears that restoring hormonal levels to youthful levels does not fight aging and increasing GH and IGF-1 levels may even accelerate the aging process. Still, it is possible that the endocrine system influences aging, as discussed below.

Many experiments using different model organisms associate the insulin/insulin-like pathway with aging (Lin et al., 2000; Clancy et al., 2001; Kenyon, 2010). As mentioned earlier, smaller mice, rats, horses, and dogs appear to live longer and this could be related to lower levels of IGF-1 (Miller, 1999; Miller et al., 2002a; Rollo, 2002). Moreover, a number of long-lived genetic mutants have decreased GH/IGF-1 signaling. Mice homozygous for *Pit1* have lower GH and IGF-1 levels; they are dwarf, live about 40% longer with a longer maximum lifespan, and their aging process appears to be delayed (Flurkey et al., 2001). Mice mutant for *Prop1*, a transcription factor that regulates *Pit1*, live 50%

longer (Brown-Borg et al., 1996). Likewise, mice overexpressing bovine growth hormone appear to age faster (Bartke, 2003). Interestingly, some studies suggest that genetic variants of genes in the insulin/insulin-like pathway pathway are associated with human longevity (Bonafe et al., 2003; Suh et al., 2008). Human patients with a mutated *Prop1* might live slightly longer (Bartke et al., 2001a), though patients with deficiencies in GH and IGF-1 often show signs of early aging even if their lifespan may actually be increased (Laron, 2005). Besides, some untreated patients with GH deficiency have a reduced longevity (Besson et al., 2003). Patients with a GH receptor deficiency have greatly decreased mortality from cancer and type 2 diabetes, though cardiac disease mortality appears to be increased and overall mortality does not appear to change (Guevara-Aguirre et al., 2011). Nonetheless, it is clear that neuroendocrine systems can impact on aging and possibly on human aging as well (Bartke, 2005; de Magalhaes, 2005a). As another example, mutations in the *klotho* gene, which acts as a circulating hormone, appear to accelerate the aging process (Kuro-o et al., 1997). In contrast, overexpression of *klotho* extend lifespan by about 30%. The functions of *klotho* are largely unknown but it could be related to insulin/IGF-1 signaling (Kurosu et al., 2005). (Please consult the GenAge database for more information on most of the aforementioned genes and others related to the GH/IGF-1 axis.)

It is interest to note that hormonal changes appear to play a role in CR. CR clearly induces various hormonal alterations in rodents, such as decreasing plasma levels of insulin (Masoro et al., 1992) and IGF-1 (Breese et al., 1991); an increase in GH secretory dynamics has also been observed though it could be a compensatory mechanism (Sonntag et al., 1999). Interestingly, several genes have been identified in model organisms whose effects appear to mimic CR. The best example is probably the urokinase-type plasminogen activator. Overexpression of this gene in the brain of mice causes a decrease in appetite, resulting in a 20% decrease in food consumption and body mass, and a 20% increase in longevity (Miskin and Masos, 1997). Other genes appear to result in a phenotype similar to CR, generally by affecting body size, GH and IGF-1, and body temperature (reviewed in Bartke et al., 2001a). The best example is the GH receptor whose disruption in mice interferes with the life-extending effects of CR (Bonkowski et al., 2006). Some of the genes mentioned above may also mimic CR to some degree, though by combining CR and mutations in one of these genes--the *Prop1* gene--, an even greater increase in longevity was observed, suggesting that distinct mechanisms may be at work (Bartke et al., 2001b). Recent results suggest that although the growth hormone/IGF-1 pathway is involved in CR, other mechanisms might also operate (Shimokawa et al., 2003). Whatever the exact mechanisms, CR appears to operate through a neuroendocrine signaling cascade of which the GH/IGF-1 axis is a pivotal, though probably not the only, component (Masoro, 2005). These results hence link some aspects of energy metabolism to aging via the GH/IGF-1 axis (reviewed in Tatar et al., 2003; Berner and Stern, 2004; de Magalhaes, 2005a).

The exact mechanisms by which GH, IGF-1 and other hormones impact on aging remain unknown. Several possibilities exist (reviewed in Berner and Stern, 2004). It has been proposed that the GH/IGF-1 axis regulates antioxidants (Brown-Borg et al., 2005). Another hypothesis is that since GH and IGF-1 and mitogens and trigger cell division, lower levels of the GH/IGF-1 axis decrease cellular replication that may impact on some sort of cellular clock (Sonntag et al., 1999; Bowen and Atwood, 2004; de Magalhaes and Faragher, 2008). Similarly, maybe the GH/IGF-1 axis impacts on cellular processes like apoptosis and/or stress resistance (Sapolsky et al., 1986). As mentioned below, maybe hormonal changes regulate aging as indirect consequences of the developmental program. The jury is still out.

Overall, the GH/IGF-1 axis and associated neuroendocrine mechanisms--some of which are probably still unknown--appear to influence mammalian aging. How exactly this happens is not known and the signal transduction involved in the aging effects of the GH/IGF-1 axis remains largely a mystery. It is clear, however, that early theories defending that hormone changes with age drive aging were incorrect. If anything, decreasing GH/IGF-1 signalling increases lifespan, not the opposite. Nonetheless, as argued by others Timiras, 2003), the results implicating neuroendocrine mechanisms in aging suggest a certain level of coordination of aging changes.

The Developmental Theory of Aging

As mentioned previously, the *dauer* pathway in *C. elegans* is an alternative developmental pathway that results in a significant life-extension (Klass and Hirsh, 1976). In the *dauer* pathway, which can be activated by starvation and hence may be analogous to CR, there is a developmental arrest, which suggests that, at least in this model system, aging and development are coupled (Johnson et al., 1984). Further genes influencing lifespan in *C. elegans* confirm a linkage between the timing of development and the timing of aging (Lakowski and Hekimi, 1996; Chen et al., 2007). In insects too arrested development due to environmental factors has been suggested to slow or even stop aging (Tatar and Yin, 2001). Other examples exist (reviewed in Brakefield et al., 2005): in the marine mollusk *Phestilla sibogae*, the length of larval life is determined by a chance encounter with a stimulus that causes metamorphosis. Interestingly, the duration of post-larval life is unaffected by the length of the time it takes the larva to metamorphose. In other words, during the developmental hiatus from the onset of larval metamorphic competence to metamorphosis, aging is suspended (Miller and Hadfield, 1990). Similarly, semelparous species like the salmon, described earlier, clearly argue that developmental programs can cause aging, or a phenotype resembling aging, and death (de Magalhaes and Church, 2005). Lastly, as mentioned before, there is a correlation in higher animals, including in mammals (Fig. 1), between the time it takes to reach sexual maturity and how long, on average, they live afterwards (Charnov, 1993; de Magalhaes et al., 2007a). This could be due to similar extrinsic mortality rates acting on animals, however, and may thus be a product of co-evolution rather than a causal relation.

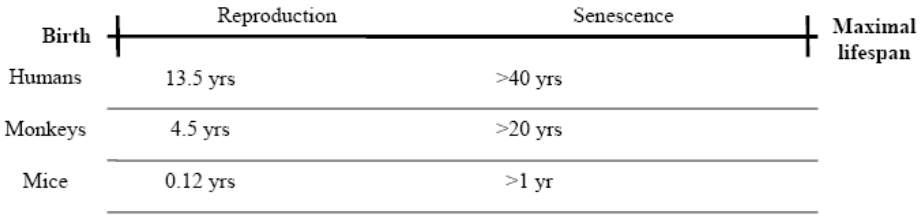

Figure 1: The life history events of mammals, such as development, reproduction, and aging, typically occur in proportion to the entire lifespan. (Adapted from de Magalhaes and Sandberg, 2005.)

Of course, invertebrates are distant animal models and these findings may not be representative of human biology, but they demonstrate how, at least in some species, aging is to a large degree a result of the genetic program that also controls development. The developmental theory of aging--also called DevAge--defends that aging is a result of development, that aging and development are regulated by the same genetic mechanisms and processes (Medvedev, 1990; Kanungo, 1994; Zwaan, 2003; Bowen and Atwood, 2004; de Magalhaes and Church, 2005). Another way of looking at aging from this perspective is considering the idea that damage only begins accumulating after developmental processes are completes and it is this developmentally-triggered damage that causes most aspects of aging.

Although it can be argued that, in some species, aging is a direct product of evolution, as debated before, such possibility appears unlikely in higher animals, such as mammals that rear their offspring. Instead, one idea is that aging is an unintended product of evolution, an unintended product of selection acting on development. Evolution does not favor long life. Rather, evolution optimizes developmental mechanisms for reproduction. Once an organism has passed its genes to the next generation maybe evolution gives up on it and the same genes responsible for the growth and maturation of that organism will inadvertently end up killing it (de Magalhaes and Church, 2005). Evolutionary, this can be seen as a form of antagonistic pleiotropy (Williams, 1957), one in which alleles beneficial early in life are harmful late in life.

The insulin/insulin-like pathway described above appears to play a role in animals entering or not the *dauer* pathway (e.g., Wolkow et al., 2000; Lin et al., 2001). As mentioned above, endocrine regulation appears to have an effect on aging, while indirectly affecting growth and maturation. The way neuroendocrine systems limit longevity suggests a link between reproduction and lifespan (Mobbs, 2004). Thus, maybe some hormones like GH and genes involved in insulin-like signaling regulate growth and development early in life and later contribute to aging (de Magalhaes and Church, 2005). Neuroendocrine mechanisms controlling development may thus extend after maturation and results in a regulatory cascade that result in age-related changes (Finch, 1976). Early studies showed that CR stunted growth and sexual development (McCay et al., 1935), though the extent of which depends on the severity of the CR used. Interestingly, high nutrition may accelerate maturation and decrease lifespan in ground squirrels (Harvey and Zammuto, 1985). Therefore, maybe seeing aging as a consequence of development links the impact of the endocrine system on aging and on CR.

The way mammalian aging is similar in different species, sometimes appearing as the same process only timed at different paces, has puzzled researchers (Finch, 1990; Miller, 1999). If the timing of development is linked at a mechanistic and genetic level to the rate of aging in mammals that would explain the plasticity of the aging process in mammals and how a process escaping natural selection is so similar among them. Assuming a link between the genetic mechanisms regulating development and aging would also explain how aging has changed so rapidly in primates (Cutler, 1979; Allman et al., 1993). Hence, one hypothesis is that, probably driven by an extended brain development (Cutler, 1979; Allman et al., 1993; Kaplan and Robson, 2002; Lee, 2003), hominid evolution led to an extension of development which in turn led to a delay of aging.

While the developmental theory of aging is theoretically sound, it lacks many concrete details for how developmental mechanisms could influence age-related changes. Some theoretical models exist, like for brain aging (de Magalhaes and Sandberg, 2005), but many details remain unclear. Also, it is likely that at least some age-related changes are the result of an accumulation of some toxic by-product of metabolism, so an overlap between theories of aging may exist. In the end, the developmental theory of aging argues that the bulk of the aging phenotype is due to the indirect actions of developmental mechanisms. Further research is necessary to test and elucidate this hypothesis and, more broadly, unravel the causal mechanisms of aging.

7 Curing Aging and the Science of Immortality

No problem can stand the assault of sustained thinking.

- Voltaire

Previously, I argued how real anti-aging medicine does not yet exist. In this speculative essay, I debate how gerontology may progress with the aim of developing true anti-aging therapies that not only considerably extend lifespan and delay human aging but may eventually cure aging.

Sections

Keywords: ageing, biogerontology, biomedical gerontology, ending aging, functional genomics, immortalism, life-extension, neurodegeneration, neuroscience, pharmacogenomics, rejuvenation, translational science

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A Roadmap to Developing a Cure for Aging

As previously mentioned, I care about understanding the aging process for human benefit, to develop biomedical interventions that can delay aging in people and improve their health. No doubt curing aging is an Herculean task. The major age-related diseases like cancer, heart disease and neurodegenerative diseases are still incurable and everyone becomes frail with age. It is possible that known aging-related genes and life-extending interventions, such as CR, can be used to develop anti-aging therapies, as debated before. The prospects for drug discovery in the field of aging are extremely promising (reviewed in de Magalhaes et al., 2012). However, even in the best case scenario that we can develop therapies that mimic CR and the effects of genes on aging observed in model organisms, such therapies will not cure aging and will not radically improve our lifespan. For example, in rodents, extending lifespan is possible up to 50%, as discussed elsewhere, which would be extraordinary if applicable to humans but still far from an aging cure. Therefore, while we can manipulate aging in model systems, including mammals, there is still a long road ahead. Driven by technology, however, we may be on the verge of a biotechnology and medical revolution in which we shift from observers of Nature to architects. So what scientific approaches are more suited to cure aging? Is it even possible to cure aging?

Given the complexity of aging, many have questioned whether curing aging is even realistic (Warner et al., 2005; Olshansky et al., 2006; Holliday, 2009). There is no scientific reason, however, to think that aging cannot be cured. After all, curing aging does not violate any law of physics. There are even reasons to be optimistic, like the fact we can reverse some forms of cellular aging in vitro, including in human cells via telomerase. It is also possible to rejuvenate yeast by expressing one single transcription factor (Unal et al., 2011). Importantly, some species live much longer than humans do, and some even appear not to age. If Nature can solve the problem of aging, there is no reason to think we cannot do the same thing. This is akin to developing heavier than air flying machines which was in fact partly inspired by birds. As detailed elsewhere, the process of aging is surprisingly plastic and can be manipulated by genetic and environmental interventions. Stopping or reversing aging is no doubt

much harder than slowing aging, but it is not impossible. How difficult it is exactly? I am hopeful we can find out in the coming decades.

Unfortunately, the development of true anti-aging interventions is hindered by the little we know about the mechanisms of aging. Others have argued that we do not need to learn how a car works in order to drive it, and so maybe we do not need to learn everything about aging in order to cure it (de Grey, 2003). As discussed elsewhere, I am less optimistic as I think a better analogy is: when a car breaks down we need to know a lot about how it works in order to fix it. As detailed below, I think we need to increase our knowledge not only of aging, but of life itself in order to decisively intervene on aging. But what exactly do we need to know?

With curing aging as the ultimate goal, and based on the model systems available, I believe there are three general areas we must tackle:

1. Increase our knowledge of the mechanisms of aging
2. Develop a deeper understanding of biology, and specifically of the machines of life and their interactions (which can be seen as a broader goal of the above point)
3. Develop new methods to modify biological processes in vivo

Understanding the Process of Aging

Opinions diverge, and many different strategies can be employed to study aging, yet I feel that the two most important questions in gerontology are: 1) What controls the rate of aging among mammals? This can refer to genetic differences between individuals (i.e., different people) but the major question to is: Why does a mouse age 30 times faster than a human being? 2) What changes in a person from age 30 to age 70 to increase the chance of dying by roughly 30-fold? Addressing these two questions would give us the basic knowledge to start thinking about therapies against the aging process as a whole. By knowing which mechanisms control the pace of age-related debilitation we will know which pathways we need to target to delay aging. Likewise, by identifying the differences between young and old persons that so markedly increase the mortality we may find mechanisms that we can target through therapies, even if discriminating between causes and effects of aging will continue to prove troublesome.

At present, we know very little about both 1) and 2). The little we know about the changes people endure as they age come from studies at the level of tissues and organs, as described before. That is, we know of specific age-related changes and functional declines but we do not know why those happen, what are the underlying mechanisms causing those events or how changes at different biological levels (i.e., molecular, cellular, tissue, etc.) influence each other. At a cellular and genetic level, our knowledge of the aging process has grown in recent decades but it still limited and controversial. For example, our knowledge of the role of cellular changes in aging remains a subject of controversy. Related to 1) progress has been made in model organisms in understanding the genetic regulators of aging within species. Hundreds of genes have been shown to modulate aging in model systems in recent years, though much work remains to understand how they interact with each other, how they work as a whole to influence the aging phenotype. To facilitate such studies our lab has developed the GenAge database of aging-related genes (reviewed in de Magalhaes et al., 2009b). However, the effects of these aging-related genes are modest when compared to species differences in aging of which we know almost nothing about in terms of mechanisms. Some evidence from comparative genomics actually suggest that genes regulating aging within species, such as the GH/IGF-1 axis, are highly evolutionary conserved and are unlikely to determine species in aging (de Magalhaes and Church, 2007). Not surprisingly, the focus of our lab has been largely on 1) and 2). For example, to help address 2) we developed the Digital Ageing Atlas. Overall, I think gerontologists must first begin to answer these questions before we can start devising more powerful anti-aging therapies (Fig. 1).

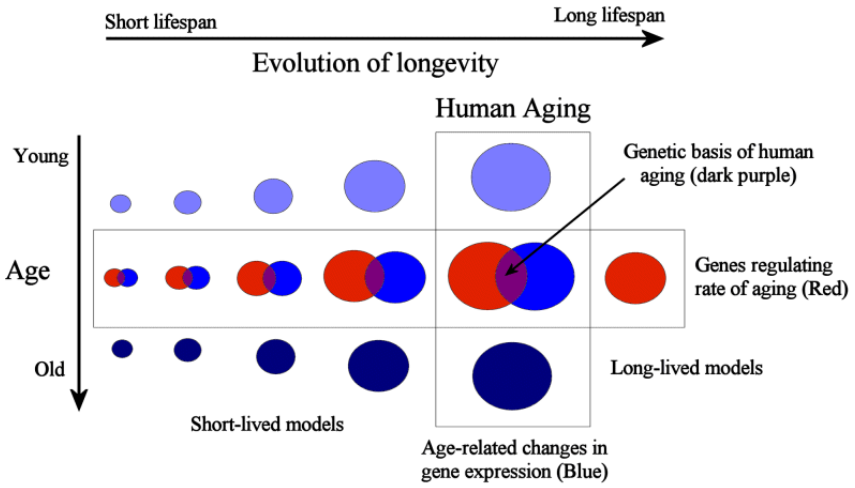

Figure 1: Methodologies for studying human aging. Variation is the basis for studying any phenomena and aging is no exception. On one hand we may use a comparative biology approach to understand why different species age at different paces (and to a lesser degree study differences in longevity between individuals of the same species). In parallel, we may study the changes people, or animals, endure while they age (de Magalhaes and Toussaint, 2004b). Facilitating such studies are a variety of high-throughput -omics technologies (reviewed in de Magalhaes, 2009). With next-generation sequencing we can sequence hundreds of genomes as well as study the expression of thousands of genes as humans, or animals, age (reviewed in de Magalhaes et al., 2010). Notice how the area of the circles decreases as we study species progressively more distant to humans, since it is expected that species evolutionary more distant from humans are less likely to share mechanisms of aging that are relevant in humans.

One crucial aspect of research on aging, which is sometimes overlooked by researchers including myself, is that our work should deal with human aging. Aging in model organisms is irrelevant if it is not applicable to humans. Some mechanisms of aging identified in model organisms may be relevant to human aging while others may not, but discriminating between the two is often impossible, as argued elsewhere. As such, and while no doubt model systems will continue to be of paramount importance for research on aging, it is imperative we keep a skeptical mind when analyzing data from model organisms, particularly non-mammalian models (de Magalhaes and Toussaint, 2002 & 2004b).

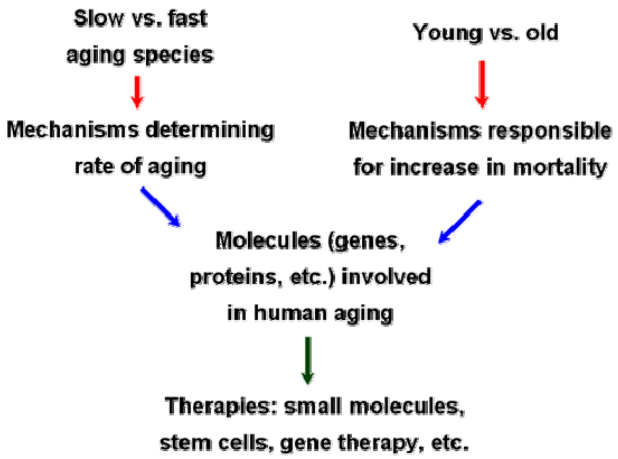

Figure 2: Steps necessary to gain enough information about aging to start developing a cure. On one hand, we must identify therapeutic targets by studying why we become frailer with age and/or why we age slower than most other mammals. Then we must develop technologies capable of targeting the molecules, cells, or tissues necessary to revert aging, as detailed ahead.

Once we know more about which mechanisms to target for therapeutic purposes, we can consider the development of therapies that delay, stop or reverse the aging process (Fig. 2). It may be seen as speculative to consider such ambitious anti-aging therapies at present, since we know little about what interventions will be necessary, but a few ideas are given below and elsewhere. I am optimistic that as researchers address the two questions mentioned above, this will open the door to the development of true anti-aging therapies capable of radically extending lifespan. As detailed below, however, these advances must be put in context with other advances in the life sciences.

Genomics and the Promise of Digital Biology

. . . the general who wins a battle makes many calculations . . .

- Sun Tzu

One of the major problems of biology is that it is highly unpredictable. For example, the rate of success of drugs in clinical trials is only 20% (DiMasi et al., 2010). The reason for this is that biology is intrinsically complex and thus, even with promising pre-clinical results from cells and model organisms, most drugs tested in humans do not behave the way scientists and clinicians predict--and often have unpredictable negative side-effects. Likewise, engineering biology, even in lower organisms, is still very limited, mostly due to our incomplete knowledge of biological systems (Heinemann and Panke, 2006). Therefore, a much deeper understanding of biology is necessary in order to develop interventions on aging as well as other diseases and processes. Fortunately, some emerging technologies may allow us to tackle the complexity of life.

In a sense, the human genome has all the information we need to know about aging, and we may have the secret of immortality in the genomes of animals that appear not to age. The problem is that the secret is encrypted and many facets of the genome remain a mystery. For example, at present almost half of the ~20,000 human genes have been poorly studied. In addition, emerging layers of gene regulation, like microRNAs, remain largely unexplored. The ongoing genomic revolution and in particular the development of next-generation sequencing technologies, however, has the promise to turn biology into a mathematical problem and decipher the human genome (de Magalhaes et al., 2010). Our capacity to generate data in a genome-wide fashion is increasing at an astonishing pace, even faster than computers increase in power. This leads to the emerging paradigm of digital biology in which biological systems are treated as information systems that can be studied by a combination of bioinformatic and mathematical approaches. Large-scale consortia are surveying phenotypes at a genome-wide basis and, for example, ongoing efforts aim to develop and characterize mouse mutants of all protein-coding genes (Collins et al., 2007; Morgan et al., 2010). Other large-scale efforts, such as ENCODE which aims to identify all functional elements in the human genome (Birney et al., 2007), will no doubt further our understanding of the genome. These combined efforts hold great promise to increase our predictive power in medicine and thus lead a more precise targeting of biological systems to preserve health and fight disease.

I am convinced that genomics and bioinformatics will play a major role in deciphering the process of aging and perhaps the secret of immortality. Of course that major hurdles will need to be overcome. The genomes of certain viruses have been sequenced years ago and we still cannot cure the diseases associated with them, as discussed ahead. Besides, the genetic differences that determine rate of aging across and within species are likely very subtle. For instance, humans and chimpanzees have about the same set of genes and it is thought that subtle differences in proteins or in transcriptional regions determine the differences between chimpanzees and humans (Carroll, 2003; Olson and Varki, 2003; de Magalhaes and Church, 2007). Consequently, subtle genetic differences are also expected to determine rate of aging, and finding these in the billions of base pairs that make up a genome will be a monumental task (de Magalhaes, 2003; de Magalhaes and Toussaint, 2004b). Eventually, however, the genome holds all answers; all we need to do is generate the data to ask the right questions (Fig. 3).

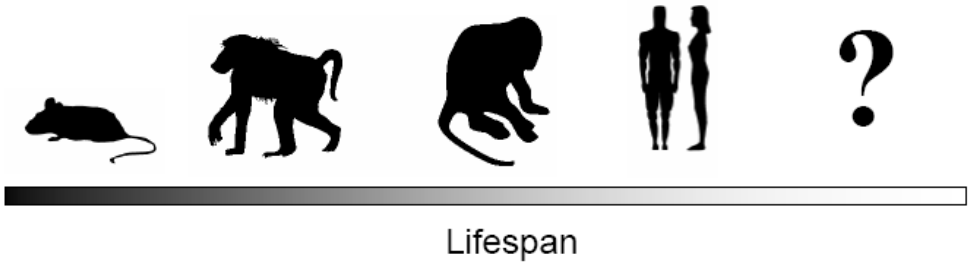

Figure 3: If we can understand the genetic factors that determine the rate of aging among similar species, like primates, then it may be possible to develop interventions that extend the human lifespan even further. To quote Leslie Orgel: "Evolution is cleverer than you are," so identifying the tricks evolution uses to extend lifespan may have biomedical applications. Figure rendered using the animal fonts by Alan Carr.

Fighting Aging: The Road Ahead

By the year 2030, we will have (1) developed a complete model of all human cell types, obviating the need for many laboratory experiments [by doing computer simulations instead]; (2) lowered the cost of doing a complete genomic sequence for an human individual to less than $1,000 each; and (3) catalogued all the genes involved in aging. Therefore, human clinical trials to extend lifespan could already be underway by this date.

- Francis Collins

In silico studies will be one of the major approaches for determining the causes of aging and developing interventions. Some immortalists argue that the key to solve human aging is in computers and artificial intelligence, not in biology; i.e., building computers smarter than us capable of solving the problems we cannot solve. I am not so enthusiastic but agree that solving aging will be partly based on computational biology (de Magalhaes and Toussaint, 2004b). If genomics and bioinformatics lead to the deeper understanding of biology described above then we will be able to build computer models of human cells and better develop interventions. The emerging field of systems biology, which combines modelling, large-scale -omics technologies, bioinformatics and experiments holds great promise, even if much work remains to make truly predictive models (Kitano, 2002; de Magalhaes, 2009; Cevenini et al., 2010). Ultimately, the aim is to build models of biological systems, including aging, that are accurate enough to make predictions about manipulations of components of the system (e.g., which gene target is more power for drug development), predictions about spatio-temporal changes in the system and how these can be modulated by drugs and other interventions, etc. At present, systems and synthetic biology are still at a very early stage and restricted to very simple models and gene circuits, but when looking decades ahead the potential to model the whole aging process and identify how it can be retarded, stopped and even reversed certainly exist.

I'll live forever or die trying.

- Anonymous

There is considerable evidence that aging is not irreversible. At the molecular and cellular level this certainly appears to be the case. In stem cells, self-renewal can be reinstated by suppression of certain

factors (Wang et al., 2011). The fact a number of aging changes seem to be due to signaling pathways is encouraging because it means these may be reversible. For example, senescence in T cells appears to be regulated by signaling pathways that are reversible (Di Mitri et al., 2011). As detailed elsewhere, with four factors it is possible to rejuvenate cells from centenarians and induce pluripotency (Lapasset et al., 2011). Similarly, there is evidence that systemic factors are important in aging. Transplanting young ovaries to old mice slightly extends lifespan (Mason et al., 2009). Both factors intrinsic to cells and extrinsic factors affect muscle regeneration (Carlson et al., 2008). Blood levels of a chemokine can also negatively regulate neurogenesis (Villeda et al., 2011). Taken together, this argues that with the right information aging can be reversed.

No doubt I am optimistic about the prospect of radically increasing our lifespan, but I am also aware of the numerous problems involved. While I think that there are genetic factors that make up a unifying core of human aging, it is impossible to say how many genes are involved. The fact that no human (or mammal) can avoid aging completely or even live much longer (e.g., a human living to 200 years) than average shows that curing aging cannot be achieved by changing one or a few genes. It is possible that some age-related changes are largely independent of the aging process, as mentioned elsewhere. Maybe some age-related pathologies are the result of late-acting genes. In fact, if late-acting deleterious genes do exist, then it is possible that there are deleterious genes affecting humans after our maximum lifespan--say, after 300 years. These could result in a disease or even in some form of mechanical senescence. I call these genes whose effects are deleterious after our present maximum lifespan post-mortem lethal genes (Magalhaes, 1999). Take as an example the diseases that result from the levels of a given defective protein passing a certain threshold, like mad cow disease or familial amyloidotic polyneuropathy, which can be the result of a long-term accumulation of a defective protein or a slow-acting infectious agent. Maybe if we increase our maximum lifespan we will also increase the number of people affected by this kind of diseases.

Overall, understanding the mechanisms of aging and deciphering the genome will be monumental tasks. Still, I am confident that we will be able to elucidate all the genetic mechanisms that drive aging within my own lifetime via the combination of approaches mentioned earlier (Figs. 1 and 2). I am equally confident that an in-depth characterization of biology will be possible in the coming decades which will lead to computer models of all the players involved and their interactions which can then be used to make predictions about interventions. This is why the focus of my lab is on increasing our knowledge of aging in particular using genomic approaches. A crucial issue, however, is that even if we can predict which genes to manipulate to avoid aging we will still have to "order" our cells not to age. This is a key hurdle in my opinion and how to manipulate aging in vivo is the subject of my next essay.

We, alone on earth, can revolt against the selfish replicators.

- Richard Dawkins

The Importance of the Brain in Anti-Aging Research

One topic I should emphasize is brain aging. Theoretically, the only organ that cannot be replaced is the brain; lifespan is equivalent to brainspan. Following an earlier discussion, it is open to debate whether aging is caused by factors that do not have their origin in the brain. Perhaps our brain just ages because the other organs in the body can no longer support it (but see below). If we could change the body at regular intervals to keep it always young, it might happen that our brain would never age. (White et al., 1996). Notice the fact that I call it "body transplants" and not "brain" or "head transplants" because size does not matter here; the brain is us and can never be changed, yet the body can, and therefore it is the body that is transplanted. Of course, body transplants, are a difficult, expensive, even far-fetched technique that at the moment remains in the realms of science fiction.)

It is also possible, though speculative, that future developments in cybernetics, artificial organs and therapeutic cloning will make it possible to replace all other organs besides the brain. But even if we could develop replacement organs for our most vital organs, this appears to be a difficult, dangerous, and unpredictable approach. Also, many theories center aging on post-mitotic tissues such as neurons, so the idea that we could avoid brain aging by replacing or rejuvenating the rest of the body may not be correct. One study found that centenarians, when compared to elderly non-centenarian subjects, exhibit a lower prevalence of cancer but actually a higher prevalence of cerebro-degenerative pathologies (Motta et al., 2010). For now, we must focus on trying to discover a way to stop aging in all the body, having, of course, the brain as top priority. Although intervening in the brain is harder than in most organs, in part because of the blood-brain barrier, there is some cause for optimism. For example, in mice transplanted neurons have been shown to reconstitute complex neuronal circuitry (Czupryn et al., 2011).

Short-term memory loss, personality and cognitive changes with age, dementia, general decline of the nervous system and senses, and many other changes are likely to occur with aging (Craik and Salthouse, 1992; Hayflick, 1994, pp. 161-166; Zec, 1995). Until recently, it was thought that neuronal loss, due to the accumulation of damage--such as oxidative damage--was the main cause of brain aging. Nowadays, it appears that neurons can remain relatively healthy through life, except in pathological states (Morrison and Hof, 1997). Some evidence also suggests that neurons can emerge in adult brains, perhaps originating from neural stem cells (Alvarez-Buylla and Garcia-Verdugo, 2002). The idea of replicating neurons dates back many years; Joseph Altman reported replicating neurons in rats decades ago (Altman and Das, 1965), and Fernando Nottebohm reported brain rejuvenation in birds (Nottebohm, 1989). Some evidence suggests that new neurons can appear in adult monkeys, in an area of the brain called hippocampus which is used for long-term memory (Gould et al., 1999). Similar results have been reported in humans (Eriksson et al., 1998). Overall, instead of seeing brain aging as a mere consequence of the death of neurons, it appears that, even without neuronal death, biochemical and structural changes compromise neuron function (Teter and Finch, 2004). With age, what changes is the wiring, the complex network of connections between cells (Gopnik et al., 2000). It has even been suggested that brain aging is an extension of brain development (de Magalhaes and Sandberg, 2005), in line with a linkage between development and aging. The debate of whether aging is a result of damage accumulation or of programmed events also extends to brain aging, though given the relevance of the brain, understanding its mechanisms of aging are of prime importance.

7.1 Strategies for Engineered Negligible Senescence

Strategies for Engineered Negligible Senescence or SENS is a proposal by Aubrey de Grey to cure aging. SENS has gathered widespread media and public attention, yet it has also been vehemently attacked by a number of experts. This essay briefly presents SENS and the arguments from both sides on its merits and drawbacks.

Keywords: ageing, biogerontology, biomedical gerontology, ending aging, human regeneration, immortality, radical life-extension, regenerative medicine, rejuvenation biotechnologies

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About 10 years ago, Aubrey de Grey proposed the SENS approach with the goal of curing aging (de Grey et al., 2002a; de Grey, 2003; de Grey and Rae, 2008). Briefly, his proposal is that even if we do not know the underlying mechanisms of aging, if we can engineer the reversal of all the major molecular and cellular changes that occur with age, we will be able to reverse aging and rejuvenate ourselves. The proposal includes addressing seven forms of molecular or cellular damage, based on earlier works (Holliday, 1995), that accumulate with age:

- Cell loss, tissue atrophy
- Nuclear [epi]mutations (only cancer matters)
- Mutant mitochondria
- Death-resistant cells
- Tissue stiffening
- Extracellular aggregates
- Intracellular aggregates

de Grey then proposes a number of creative solutions to reverse the above forms of damage. The solutions, however, are based on technologies and therapies that are not yet available or at least not yet developed to do what is necessary for SENS. These include stem cells to replace old cells, the application of enzymes--including bacterial enzymes--to degrade certain forms of cellular junk, gene therapy technologies that allow the incorporation of transgenes with a high efficiency and viability, and the ablation of old cells.

The idea that curing aging may be possible before we can fully answer the main issues in the field is original but controversial. de Grey assumes we can understand the changes that occur with age well enough to develop therapies, even if the causality of these is not yet established. Like previously discussed, many pharmaceutical interventions work as intended even though we do not know why. Some anti-aging therapies may follow the same principle: for instance, maybe we do not need to know the exact functions a given gene shown to retard aging in model systems to develop anti-aging drugs by targeting that gene, as discussed earlier. Likewise, perhaps we do not need to know the mechanisms leading to damage in order to repair it to preserve health. SENS, however, promises radical results: a cure for aging within a relatively short time of a couple of decades. Even though it is plausible that if the technologies in which SENS is based on--e.g., stem cells, tissue engineering, and gene therapy--reach a high level of sophistication then anti-aging research will greatly benefit, the idea that we can cure a complex process like aging without knowing its underlying mechanisms is, of course, debatable and SENS has been criticized by numerous scientists (Warner et al., 2005; Warner, 2006; Holliday, 2009).

One key criticism of SENS comes from analogies with other medical problems like cancer. Since US President Nixon declared the war on cancer in 1971, the R&D spending on cancer has been estimated at $100-$300 billion. Some new treatments have been developed but while overall cancer survival has increased in recent decades this is mostly due to early detection rather some "magic bullet"

(Lakdawalla et al., 2010). From a engineer's perspective, however, cancer is a simple problem: all we need to do to cure it is the ablation of cancer cells. This is similar to the ablation of death-resistant cells in the SENS proposal. In the case of SENS, de Grey proposes that ablation of cells may be achieved by making unwanted cells commit suicide or stimulating the immune system to kill them. The issue then is: if the ablation of unwanted cells were so simple, then why have decades of cancer research, with way more money than is available to aging research, failed to do so? Essentially the problem is that developing therapies that destroy harmful cells but not normal cells is technically very challenging and has not been solved yet in spite of a massive investment and research effort. As a side note, de Grey's proposal to target cancer actually involves restricting telomere elongation in the whole body by deleting the genes responsible and then, because telomere elongation is necessary for self-renewal in some tissues, use cell therapy and tissue engineering to keep organs healthy (de Grey, 2005b).

It can be argued that we still do not have the necessary technologies to implement SENS or cure cancer but are now on the verge of developing them. On the other hand, critics of SENS point out that these engineering achievements are actually very difficult to achieve due to intrinsic problems and limitations of of biomedical research. There are still few success stories of engineering in biology, even in lower organisms, in part due to our incomplete knowledge of biological systems (Heinemann and Panke, 2006). As such, critics of SENS argue that all individual components of the SENS proposal are exceptionally optimistic (Warner et al., 2005; Holliday, 2009). A related issue is that interventions in medicine, including stem cell therapies and others that are essential for SENS, are intrinsically difficult to develop and perfect. As mentioned elsewhere, the rate of success of drugs in clinical trials is only 20% (DiMasi et al., 2010), which is due to the intrinsic complexity and unpredictability of biological systems. As such, critics of SENS argue that the chances that each SENS component will be successful are actually very low and the SENS agenda, to quote a group of gerontologists who criticized it, "is so far from plausible that it commands no respect at all within the informed scientific community" (Warner et al., 2005). Indeed, as Warner et al. point out, none of de Grey's proposals to tackle the seven forms of damage has been shown to extend lifespan even in simple model organisms. The relevance of these seven types of damage to aging is far from proven and other processes that are not tackled in SENS may prove important to aging (Warner, 2006). de Grey responded to the above criticisms (de Grey, 2005c & 2006). Briefly, he argues that the effects of tackling the seven forms of damage can only be correctly evaluated when tested together rather than individually. de Grey also argues that Warner et al. are being pessimistic about progress of technology and that indeed relevant technologies for SENS are forthcoming. A final point is that even before curing aging we may reach a stage, which de Grey calls "anti-aging escape velocity," where successive medical advances postpone aging faster than time is passing (de Grey et al., 2002b).

Even though SENS is controversial and polarizing, it can be argued it has had positive effects in the field by fostering discussion of key issues and, given that de Grey is arguably the most famous gerontologist in the world, raising awareness to research on aging and life-extension. de Grey's advocacy and optimistic vision is no doubt important to attract and excite young scientists and students to study aging and try to develop interventions to extend human lifespan. The public interest generated by SENS can be argued to even influence funding policy and thus have a positive influence for the field (de Grey, 2005d). Projects originating in SENS, such as the Mprize for longevity or rejuvenation breakthroughs in mice, also foster development of life-extension therapies. The SENS foundation also provides funding for a number of high-risk, high-reward technologies and approaches. Critics of SENS, however, point out that numerous false anti-aging claims throughout the centuries have given the field a poor reputation and that SENS will further erode the public's confidence in gerontology; the attention given to the SENS agenda may also divert resources for what they think are more promising research foci (Warner et al., 2005). Because of its controversial nature, however, SENS does not at the time of writing receive funding from traditional funding bodies, such as government agencies and charities, and so it is unclear if indeed SENS is diverting funds from other researchers or instead generating new sources of funding (e.g., private donations) that would normally not be available to traditional gerontologists.

August Weismann wrote: "The complex processes of life can only be followed by degrees, and we can only hope to solve the great problem by attacking it from all sides." In a field like gerontology where proven facts are rare, it is important to invest a certain amount of resources in unorthodox practical research; reasonable ideas that go against some of the most popular theories can also be successful and History proves it. When Einstein thought and developed his ideas trying to solve the paradoxes relating light speed and confronting Newton's and Maxwell's laws, he was unaware that others were thinking about the same problems; this was a blessing, for the others were heading in wrong directions and could have clouded his thoughts. Therefore, large advances in science and technology are often due to individuals following what others think is an impossible vision and investing some aging research funds in risky ideas is clearly important (Strehler, 1986). Even if the components of SENS are regarded by many--possibly most--scientists as optimistic and by some even as unrealistic, positive outcomes may emerge from developing such approaches, even if falling short of curing aging.

I discussed above--hopefully in a balanced and objective way--the different arguments, strengths and weaknesses regarding SENS and hopefully readers can now make up their own decisions about it or dig deeper into the cited references and the links below. Still, it would be cowardly of me not to explicitly mention my personal opinion on this issue, and many have asked me about it, so I will make it clear. I have no doubt that SENS is highly optimistic. Part of the problem is that SENS, like the proposals of futurist Ray Kurzweil (Kurzweil and Grossman, 2004; Grossman, 2005), depends on technologies that have not been developed yet and thus may or may not pan out. The unpredictability of biological systems, in particular, is a key issue to render SENS very optimistic in my eyes. I personally think that a much deeper understanding of biology, and specifically of the machines of life, is essential to develop the level of sophistication in biomedical research (including pharmacology, synthetic biology and regenerative medicine) required for curing aging. Hence, the focus of my work is not on SENS but on increasing our knowledge of aging and on genomics, as discussed elsewhere. I also think a better understanding of the mechanisms of aging is important to develop more specific therapies--the types of damage in SENS are quite broad--that will have a higher chance of success. On the other hand, and unlike many gerontologists, I aim to cure aging, and not surprisingly I disagree with the many SENS critics who reject any possibility to prevent aging indefinitely or reverse aging (Warner et al., 2005). It may be extraordinarily hard to cure aging, but there is no scientific reason to think it is impossible, as I discuss elsewhere. de Grey has, in fact, tackled one of the major issues in getting more attention and funding to anti-aging research: he attacked the view that aging is untreatable. Since I have no doubt that increasing public understanding and support is of prime importance for us to cure aging, the SENS initiative has, in my opinion, been very positive because of the momentum it generates, of how it raises awareness and rallies the field. Probably SENS will not cure aging but it may create the opportunities for others to cure it.

A Few Links on the SENS Debate

The BBC, in 2004, featured two contrasting articles: 'We will be able to live to 1,000' by de Grey, and 'Don't fall for the cult of immortality' by Olshansky.

SENS Foundation; official website of SENS.

Technology Review: Is Defeating Aging Only a Dream?; also known as "SENS Challenge", it features submissions aimed at disproving SENS as well as de Grey's rebuttals and the remarks of an independent jury.

Godseed

In this essay, I focus on the crucial issue of how to develop therapies to delay and eventually cure aging in adult human beings after we understand the causes of aging. Imagine that we know all the genetic and molecular mechanisms of aging, that we address the questions I previously argued as crucial for the field. Since germ therapy is too late for everyone alive, we have to correct the genotype of a significant number of our somatic cells or replace them by new ones. This is one of the most difficult steps in fighting aging and it is worth a more detailed discussion.

Sections

Keywords: ageing, biogerontology, elixir of life, elixir vitae, engineered longevity, eternal youth, fountain of youth, life-extension, regenerative medicine, pharmacogenomics

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From the Mechanisms of Aging to a Cure

Although so far the underlying mechanisms of aging remain largely a mystery, as detailed elsewhere, it is reasonable to expect that we will eventually understand the human aging process. Possibly during this century, we will know which genes determine rate of aging and we will know what changes occur in a human being from ages 30 to 70 to increase the chances of dying by over 30-fold. Yet even if researchers fully unravel the aging process, even if researchers identify the causal molecular and cellular mechanisms responsible for human aging, such breakthroughs will not necessarily lead to a cure for aging. After all, HIV was identified as the cause of AIDS roughly 30 years ago and, though there has been progress and new treatments, we still cannot cure AIDS (Gallo and Montagnier, 2003). To delay aging, not to mention to stop or reverse the human aging process, will be a monumental task.

A disease, any type of disease, is a time-dependent change in the body that leads to discomfort, pain, or even death. Therapies aim to delay, stop, or reverse those changes from occurring either by large-scale interventions, such as surgery, or by transmitting the necessary information to the body. For example, a bacterial infection may be reversed by penicillin, which can be seen as an information vector that disrupts the bacterial wall, thus killing the bacteria and reversing the disease state. Though they have a physical, biochemical basis, most pharmaceutical interventions can be seen as information vectors transmitting simple instructions that are intended to delay, stop, or reverse the time-dependent changes related to a given pathology. Not only antibiotics, but painkillers, corticosteroids, anti-depressants, and a thousand more products fit this description. Yet present therapies transmit relatively simple instructions: a painkiller may "tell" the brain to reduce its transmission of pain information and a corticosteroid may "tell" the immune system to diminish its response. Curing aging, however, will no doubt require the transmission of much larger amounts of information to the body.

Aging is a "sexually transmitted, terminal disease" that can be defined as a number of time-dependent changes in the body that lead to discomfort, pain, and eventually death. We still do not know whether aging derives from changes in a specific organ or if there are intrinsic age-related changes in each tissue that are independent of one another, as discussed elsewhere. Maybe there are organs that age faster than others or that are more important. For instance, it is clear that aging of the brain must be a top priority, as debated previously. Nonetheless, it appears that in order to cure aging we will need to

target multiple types of cells and possibly address different types of molecular damage and malfunction. That is why, in spite of progress in artificial organs (e.g., Suga et al., 2011), organ transplants are unlikely to be the solution for aging, and at least not a definitive cure. The future of medicine is not in large-scale interventions but in smaller, less invasive but more precise therapies. The solution to aging is not in addressing individual age-related pathologies but rather in information vectors able to instruct our body to become young again.

To slow, stop, and reverse human aging we will likely require three steps: 1) remove damaged or inactive molecules and cells; 2) restore function to several molecules and cells by repair or replacement; 3) modify the genetic program to prevent the aging process from continuing. These interventions are what we will most likely need to balance the body's chemical reactions and molecular structural changes that become disrupted as we age. But how can we transmit such massive amounts of information to our body?

Instructing the Human Body with Drugs

Most pharmaceutical interventions are composed of compounds or biomolecules usually transmitting a single signal to the body: acetyl-salicylic acid, also known as aspirin, the anti-depressant fluxetine, hormones like growth hormone, etc. Novel developments in chemical genetics, chemogenomics and high-throughput screening are allowing the development of small molecules that target specific genes and pathways, including in the context of aging (de Magalhaes et al., 2012). A number of small molecules can activate or repress specific proteins (Peterson et al., 2000; Kuruvilla et al., 2002). With advances in technology it might be possible to develop compounds that target all or at least most genes in the human genome. If so, this would be a tremendous step in biomedical research in general and particularly in the development of therapies against disease, such as aging. Nonetheless, the number of human genome targets of approved drugs is in the hundreds with many drugs having multiple targets while others have unknown mechanisms of action (Overington et al., 2006). Besides, certain types of protein may not be amenable to drug development and the recent woes of the pharmaceutical industry (e.g., Sams-Dodd, 2005) suggest that developing specific drugs targeting most human genes is unlikely to occur in the foreseeable future.

Another problem with drugs is that the simple instructions these compounds deliver to our cells are unlikely to be adequate to cure aging. Assuming aging has, to a large degree, a genetic basis, as debated before, then to cure aging will require technologies that are not yet available. To give an example, there are dozens of inherited diseases originating in single genes for which there is no cure simply because we lack the technologies to turn on and off human these proteins in vivo. Since curing aging will require us to transmit large amounts of information to the body, new technologies will be necessary. Below, I will first give a brief overview of the most promising technologies to address this problem: gene therapy and single-gene interventions, cell therapy and stem cells, and nanotechnology. Afterwards, I will attempt to foresee how we can cure aging based on these technologies and what breakthroughs are still necessary.

Gene Therapy

Gene therapy has been hailed as a major tool to deliver information, in this case genes, to the human body (Lyon and Gorner, 1995; Kay, 2011). Although genes can be injected directly into the body (Symes et al., 1999; Bersell et al., 2009), most gene therapy methods involve the use of a vector for the specific purpose of inserting DNA into cells. Viruses are the most widely used vector and several experiments have already shown the power of this technology. In one exciting discovery, virus-induced expression of IGF-1, a growth factor mentioned in another essay, reversed age-related changes in the skeletal muscle of mice; increases of almost 30% in strength were observed in treated old animals when compared to controls (Barton-Davis et al., 1998). If aging may be reversed by the expression of key genes then gene therapy holds great promise. Neuronal death has also been delayed by the introduction of a single gene using the herpes virus (Antonawich et al., 1999), reversal of age-

associated neural atrophy was achieved in monkeys by gene therapy (Smith et al., 1999), and the phenotype of patients with hemophilia was improved with gene therapy (Nathwani et al., 2011).

Gene therapy is promising but limited in scope due to the inherited "bandwidth" constraints of the technique. Large-scale genetic engineering is already possible in embryos (Chan et al., 2001) and maybe our grandchildren will be born without aging. But since germ therapy is too late for anyone reading these lines, present-day gene therapy has a number of limitations. The main one is that viruses cannot deliver much genetic information. A typical virus can carry up to a few thousand base pairs with some viruses being able to in the dozens of thousands base pairs. This pales in comparison to the three billion base pairs in the human genome, though of course over 90% of the genome is "junk." Maybe it is possible to use a combination of viruses but there are other problems. Viral vectors can stably integrate the desired gene into the target cell's genome but the gene's integration may occur at oncogenes, causing cancer. Efficiency of gene therapy is also low, meaning that only a small percentage of target cells are usually affected. An immune response against viruses or transgenes may also occur and is a major problem in gene therapy (reviewed in Kay, 2011). The immune response could even be fatal as in the famous case of Jesse Gelsinger. At present, virus-based gene therapy does not appear adequate to cure aging for not only is its safety dubious but its efficiency is low and the amount of genetic information viruses can carry appears largely insufficient.

In addition to viruses, it has also been proposed that certain bacteria can act as vectors in gene therapy. The major advantage being that bacteria can transport larger amounts of information and still be able to change the genome (Theys et al., 2003). As with viral-induced gene therapy, the immune response is a major problem. Some promising results have emerged from cancer treatments but it is dubious bacterial-based vectors will become a solution to aging within a near future due to safety concerns. Non-viral synthetic vectors for targeted gene delivery have also shown promise in targeting cancer (e.g., Zhou et al., 2011), but much work remains to optimize these vectors for clinical studies and increase their "bandwidth".

The above examples of gene therapy entail using viral vectors to express certain genes. In parallel, RNA interference or RNAi can be used to inactivate gene. Tiny double-stranded molecules of RNA can be designed to block a given target gene (Tuschl, 2002). For example, it has been proposed that blocking the action of the gene responsible for Huntington's disease may prevent the onset of this disease. RNAi can thus be seen as another type of information that can be delivered to the body, though developing suitable delivery methods remains a major hurdle. RNA oligonucleotides can be injected directly or a vector--often a virus--can be used to transmit the RNAi to the body (Davidson and McCray, 2011). Of course there are limitations in the use of RNAi but if specific genes have to be turned off at specific times to cure aging, RNAi appears a promising solution. For instance, oncogenes appear to be activated during aging, so it is possible that genes fostering aging, gradually leading to a decrease in viability, emerge during aging. For these, RNAi and "classical" single-molecule-based pharmaceutical interventions appear a viable solution (Haseltine, 2004).

Cell Therapy and Stem Cells

Gene therapy and RNAi are limited by their low efficiency and by the low number of genes they can affect in cells. One way to overcome this limitation is by replacing the cells themselves, a process known as cell therapy. Cells can be genetically engineered in vitro prior to be used for treatments. Since there are few theoretical restrictions as to the number of genetic modifications cells can endure before being injected into the body, cell therapy has a greater "bandwidth." For example, in an experiment aimed at treating the immunodeficiency disease SCID-X1, cells from the immune system were extracted from a patient, genetically engineered, and inserted back again with encouraging results (Cavazzana-Calvo et al., 2000); the same procedure has also been proposed to treat AIDS (Kohn et al., 1999). A number of technical hurdles remain, though, since creating and engineering cells for treatments is a complex process that still requires much more research.

One growing area of great medical potential involves stem cells (Snyder and Loring, 2005). A stem cell is a sort of "unprogrammed" cell that has the potential to become any type of cell in the organ or even in the adult body. As detailed elsewhere, aging has been linked to an age-related inability of stem cells to replenish mature cells and so therapeutic interventions that enhance stem cell functional capacity might ameliorate the age-associated atrophies of several organ systems (Donehower, 2002). More importantly, there are now techniques available to create patient-specific undifferentiated stem cells. With nuclear transfer techniques such as those that created Dolly (Wilmut et al., 1997), it is now possible to generate embryonic stem cells from an adult (Cibelli et al., 2002; Hwang et al., 2004 & 2005). In theory, it is possible to genetically modify these cells according to needs, differentiate them into the necessary tissue or organ and then implant them to treat age-related diseases, a procedure called therapeutic cloning (Cibelli et al., 1998; Lanza et al., 1999). Since these cells are genetically equal to the patient's there are few or no problems of immune incompatibility. Moreover, to avoid ethical concerns regarding the use embryonic stem-cell research, one technique called induced pluripotency (iPS) allows adult cells to be transformed into pluripotent cells using only four defined factors; in theory such iPS cells can be later derived into any type of tissue (Takahashi and Yamanaka, 2006). This revolutionary technique permits the generation of embryonic stem cells from an adult. iPS appears to rejuvenate cells to some degree (Suhr et al., 2010) and even cells from centenarians appear to be rejuvenated (Lapasset et al., 2011).

The ability of stem cells to regenerate virtually all types of tissues holds great promise (Krause et al., 2001). In theory, it is possible to create practically all components of a human being in the lab and then replace the patient's organs and tissues one by one. For example, stem cells have been used with success against heart disease (Orlic et al., 2001; Bolli et al., 2011) and to repair damage to the brain (Bjorklund and Lindvall, 2000) and spinal cord (Liu et al., 2000). Blood- and marrow-derived stem cells have been used successfully in some autoimmune and cardiovascular diseases (Burt et al., 2008). Besides, stem cells are incredibly versatile: transplantation of mesenchymal stem cells into the bone marrow shows that they can travel through the body and become bone or muscle cells depending on the needs (Horwitz et al., 1999). Interestingly, mesenchymal stem cells transplanted from young donors extends lifespan in mice (Shen et al., 2011). Taken together, these experiments demonstrate how a small subset of cells can impact on whole organs by fostering regeneration, how a few tiny cells can transmit massive amounts of information to the human body.

Harvesting and/or preparing stem cells for treatments is complex and much work remains to optimize protocols and avoid side-effects, so stem cells are not yet suitable for anti-aging treatments. Therefore, much more research is necessary but the basics for using these techniques are known and we can expect more practical applications to emerge in a near future. The ability stem cells have to sprout regeneration, repair tissues, and release tailor-made factors makes them excellent candidates for anti-aging therapies.

Nanotechnology

An adult human, once a tiny cell, is a self-assembling machine made of trillions of microscopic components. Roughly, a human being consists of ~7 x 10^{27} atoms and ~10^{5} different molecular species, mostly proteins. Genes and proteins can be seen as organic nanostructures working with molecular precision to form complex components, such as cells. The concept of nanotechnology, first proposed by Richard Feynman and later developed by the pioneering work of Eric Drexler, is that our ability to manipulate matter and energy at smaller scales--one billionth of a unit is called a nano--will increase until we reach and surpass our own biological nanostructures (Drexler, 1986; Wiley, 2005). One key concept in nanotechnology is the molecular assembler, a machine capable of assembling other molecules given a set of instructions and the necessary resources. Ribosomes, the sites where proteins are built based on the instructions of the genes, are the only known molecular assemblers. A man-made molecular assembler capable of building molecule-scale machines to guide specific chemical reactions would allow the construction of devices with atomic precision capable of a myriad of functions.

In theory, nanostructures can be built to drive chemical reactions capable of reversing aging by reversing chemical reactions and damage that occur as we age. The goal of nanotech-based therapies would be to build the necessary nanostructures to reverse age-related changes with the minimal perturbation. For example, damage to the DNA increases with age. Even though it is debatable whether this is an effect or a cause of aging, it appears likely that if we could build nanostructures to reverse these changes it could at least prevent some age-related pathologies like cancer. Cells already features several of these nanostructures as part of their DNA repair machinery. Enhancing it with novel nanostructures could thus reverse this form of damage. The applications of nanotechnology are multiple and it is not possible to describe them all here, but one possible application would be to design bacteria, viruses, or even stem cells to perform large-scale gene therapy without being attacked by the immune system. For example, by taking the viral nanostructures for integrating foreign DNA into host cells and apply them to stem cells (Freitas, 2003). Simple nanofactories have also been proposed to fight disease (Leduc et al., 2007). One more advanced proposal is the design of nanites, submicroscopic robots that could fit inside cells and perform medical functions from sensory functions to killing viruses and even repairing macromolecular damage (Wiley, 2005).

Nanotechnology holds great expectations and promises (Kurzweil and Grossman, 2004). The greatest problem is that, so far, nanotechnology, at the level described above, is almost exclusively theoretical without any clinical or medical trials. Even so, nanomachines aimed at correcting molecular defects for which there is no natural tool--e.g., removal of lipofuscin, also called age-pigment--may be necessary (Freitas, 1999).

Godseed: Changing the Soul of Man

The godseed will take over the programming of the DNA.

- David Zindell in "Neverness"

The ultimate aim of research on aging is to create what David Zindell called godseed, a molecular entity capable of reversing the molecular and cellular changes that occur as we age and capable of changing the genome of our cells to prevent aging from happening again. Initially, the godseed will need to transmit a signal to drive regeneration, as happens in some of the apparently non-aging animals described before. It may even be the case that tissue regeneration will eliminate damaged molecules and senescent cells while at the same time restoring function. Otherwise the godseed will have to include ways of eliminating nonfunctional nanostructures and cells while at the same time restoring youthful vigor. Afterwards, the regenerated tissue will need to be prevented from aging again, probably by including the necessary instructions in the godseed together with the instructions ordering regeneration (Fig. 1).

From a technological perspective, the godseed may well entail a combination of the techniques presented previously: a mix of RNAi to prevent the expression of certain genes and gene therapy either in vivo or ex vivo to express other types of genes and create the stem cells that will transmit the massive amounts of information necessary. The goal is to "tell" the body to regenerate. In addition, even if we do not know in detail how to reverse all age-related changes and pathologies, we may address specific pathologies through conventional therapies. For instance, to rejuvenate the immune system we will need to prevent the thymus from degenerating and so specific interventions will be necessary. Eventually, novel nanostructures may allow us to reverse specific age-related degenerative changes (Freitas, 1999). Yet we will not need mature nanotechnology for building the godseed. It is impossible to say if man-built molecular assemblers will emerge in 10, 50, or 500 years from now, so we should not, and need not, depend upon nanotechnology to cure aging. As such, the core of the godseed will likely consist of genetically-modified stem cells.

One specific case is the brain, the source of our consciousness. Again, the primary strategy should be to foster regeneration and the reversal of age-related changes. Though future technological developments are hard to predict, it appears dangerous to use viruses or bacteria as vectors for gene therapy in the brain, so again stem cells hold the greatest promise. Though these have "bandwidth" limits, non-invasive methods to express exogenous genes in the brain are being developed and may be useful to express specific critical genes (Shi and Pardridge, 2000). Exosomes vesicles are one emerging area of research and have already been used to deliver RNAi to the mouse brain (Alvarez-Erviti et al., 2011).

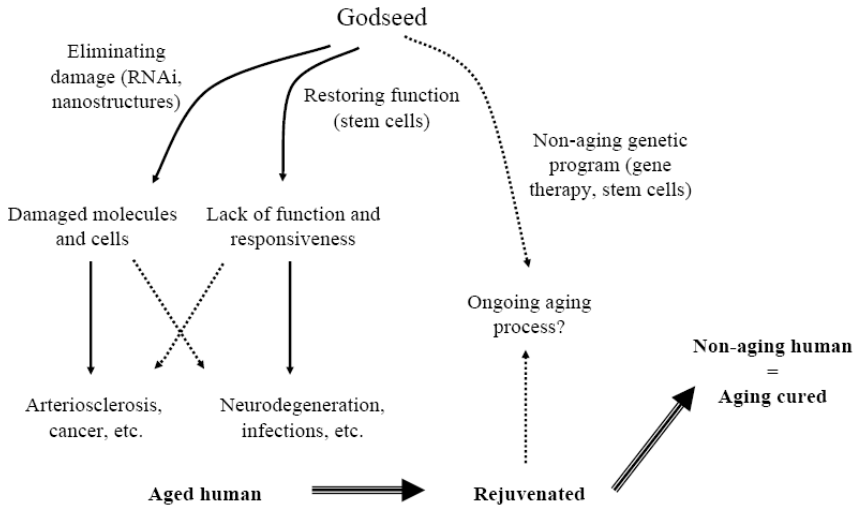

Figure 1: A human is aged because of a decrease in the functional capacity of the body plus the accumulation of damage--though both could be linked. Both these processes occur at a molecular and cellular level and lead to age-related pathologies either together or independently. To reverse aging, the godseed will first need to eliminate the damaged structures: RNAi may prevent these from being produced while nanostructures from drugs to biomolecules may eliminate the damage. For restoring function the most promising method appears to involve stem cells, which can also contribute to eliminate damage. Once the body has been rejuvenated it may be necessary to prevent aging from occurring again. If the genetic program was not changed in the previous phase, then the godseed will need to modify the genetic program either by gene therapy, stem cells, or even novel nanostructures. Afterwards, aging is cured.

To design and control the godseed we will need massive information systems: synthetic biology and whole genome engineering aim to allow us to program cells and genomes as if they were computers (Hasty et al., 2002). The goal is to convert information into gene networks designed to perform any task conceivable. Research is already been conducted in how to program stem cells to suit our needs. As mentioned earlier, several species such as reptiles, lobsters, and birds feature advanced regenerative capacities and appear not to age. Deriving information from these species to devise ways to re-built the human genome to avoid aging may be feasible (de Magalhaes and Toussaint, 2004b). In another example, work is being conducted to attempt to implement the advanced regenerative capacity of amphibians in mammals (Brockes et al., 2001). Synthetic biology and information systems will be the "glue" that binds all these fields together and allow us to design, regulate, and apply the godseed.

In a sense, to cure aging we will need to increase the bandwidth with which we send information to our cells. godseed need not be anything besides present technologies with more powerful and sophisticated features. If most of these technologies already exist what remains is an engineering problem of making them work according to our needs. Namely, we must: 1) develop therapies based on stem cells for tissue regeneration; 2) implement synthetic biology to control stem cells; 3) improve the safety, efficiency and accuracy of RNAi and gene therapy; 4) learn more about regeneration and what signals are involved in each tissue. Lastly, to apply whole genome engineering to aging we need

to know, of course, where to act. That is, what causes aging in humans, what makes us gradually weaker and more vulnerable, but that is not the subject of this article--it is discussed elsewhere.

The most promising strategy to cure aging is to stimulate the body's own regenerative capacities, to "tell" the body not to age. As discussed elsewhere, biology is becoming an information science and intervening in aging is primarily a question of transmitting the right information to the body. godseed may well be stem cells engineered by synthetic biology and coupled to nanostructures. It may also be worthwhile to modify stem cells to change the DNA, which may be necessary to avoid aging in non-dividing tissues. Ironically, we could even make the godseed, or some of its nanostructures, endure senescence after the body is rejuvenated. Most of the molecular mechanisms for such functions, although not completely understood, exist. What is necessary is research to solve all the engineering problems we still face. The godseed is not just a utopia but an achievable goal that we can build within a, hopefully, reasonable future. We would then become gods of our bodies, making aging a sad past tale.

Notice: This is an adapted and updated version of a chapter entitled "The Dream of Elixir Vitae" that appeared in the book The Scientific Conquest of Death: Essays on Infinite Lifespans.

Glossary

Non-exhaustive list of definitions of terms used in gerontology and in **senescence.info**.

Aging: a progressive deterioration of physiological function, an intrinsic age-related process of loss of viability and increase in vulnerability. In humans, aging is characterized by a complex phenotype.

Allele: one of two or more variant forms of a gene.

Aphagy: inability to feed oneself due to anatomical deficiencies, common in the adult phase of some species of animals.

Apoptosis: programmed cell death.

Antagonistic pleiotropy: theory by George Williams that explains the existence of aging by the existence of genes beneficial early in life but harmful at later stages.

Autophagy: digestion of the cell's own components; it has been implicated in aging.

Biogerontology: the scientific study of the biological process of aging.

Caloric restriction (CR): diet regime consisting of eating considerably fewer calories that has been considered as a potential method to delay aging.

Cellular or clonal senescence: see replicative senescence.

Cellular immortality: the ability of certain cell populations, like most cancer cells, to divide indefinitely in culture.

Demography: the statistical study of populations; human aging is characterized by demographic aging.

Developmental theory of aging (DevAge): theory arguing that aging is an extension of developmental mechanisms.

Diphyodont: an animal that develops two successive sets of teeth, common in most mammals.

Diploid: a cell with two sets of chromosomes.

Dyskeratosis congenita: genetic disease caused by defects in the dyskerin protein, one of the components of telomerase.

Disposable soma theory: theory by Thomas Kirkwood that explains the existence of aging by the allocation of resources from somatic maintenance to reproduction.

Ectotherm: a cold-blooded animal, such as a reptile, fish, or amphibian, whose body temperature is mostly determined by the surrounding environment.

Endocrine system: group of hormone-producing glands and their secretions (hormones); the endocrine system have been implicated in aging.

Endotherm: a warm-blooded animal, like a bird and mammal, capable of regulating its internal temperature.

Epigenetics: study of heritable changes in a phenotype that are not due to alterations in the DNA sequence but rather due to chemical changes of the DNA and associated proteins.

Eutherian: a placental mammal. All mammals are eutherians with the exception of marsupials and monotremes.

Exonuclease: enzyme that cleaves nucleotides from one end of a strand of nucleic acid.

Free radical theory of aging: theory by Denham Harman that argues that aging is a result of damage accumulation caused by reactive oxygen species.

Gene: DNA sequence that encodes a protein and represents the basic unit of inheritance.

Genetics: the study of heredity--i.e., the passing of characteristics from one generation to another--and of variation of inherited characteristics. Aging has a strong genetic component.

Genomics: the study of an organism's genome.

Genome: the full DNA sequence of an organism.

Genotype: genetic makeup of a given organism, usually related to a given characteristic.

Geriatrics: the medical study of diseases and problems of the elderly.

Germ cells: the reproductive cells which contain the genetic material passed on to the offspring.

Gerontology: the scientific study of the aging process and old age. In the context of **senescence.info**, gerontology refers to the biological study of aging and old age, also called biogerontology.

Hayflick limit: the inability of cells to replicative indefinitely in culture.

Helicase: an enzyme that unwinds the DNA helix.

IMR: initial mortality rate. The age-independent mortality rate obtained from the Gompertz equation.

Iteroparous: an organism that may reproduce more than once during its lifespan.

Life expectancy: how long, on average, an animal can be expected to live. Can be used interchangeably with average lifespan and average longevity.

Life history: the changes organisms undergo from conception to death, focusing particularly on the schedule of reproduction and survival.

Lifespan: the period of time in which the life events of a species or sub-species (e.g., a strain or population) typically occur. Can sometimes be used interchangeably with longevity even though they have slightly different meanings.

Longevity: the period of time an organism is expected to live under ideal circumstances. Can sometimes be used interchangeably with lifespan even though they have slightly different meanings.

Maximum lifespan (*tmax*): the maximum period of time organisms of a given species or sub-species (e.g., a strain or population) can live. Usually refers to the longevity of the longest-lived individual of a given species or sub-species.

Mechanical senescence: age-related changes that are a consequence of mechanical usage.

Mitochondrion: cellular organelle that produces most of the cell's energy.

MRDT: mortality rate doubling time. The time required for the mortality rate to double. Inferred from the Gompertz equation.

Mutation: change in the DNA sequence of an organism or cell.

Mutation accumulation theory: theory by Peter Medawar that explains the existence of aging by the accumulation of mutations with harmful effects at later ages.

Negligible senescence: organisms in which the aging process has not been detected in spite of detailed studies, as observed in some animals.

Oocyte: a female gametocyte that develops into an ovum after two meiotic divisions.

Oogenesis: formation of new oocytes.

Oxidative stress: damage caused by reactive oxygen species; oxidative stress has been implicated in aging.

Phenotype: the characteristics of an organism as determined by both genetic makeup and environmental influences.

Phylogeny: the evolutionary development and history of a species or taxonomic group of species.

Polyphenism: the ability of a single genome to give rise to two or more morphologies.

Polyphyodont: an animal that develops several sets of teeth successively throughout its life, as observed in many species.

Polyploid: a cell with three or more sets of chromosomes.

Progeria: genetic disease resembling accelerated aging which typically affects children. Also called Hutchinson-Gilford syndrome.

Progeroid: a phenotype with features resembling accelerated aging.

Quiescent: in cell biology, a quiescent cell is one that is not dividing.

Rate of living theory: theory that argues that lifespan inversely correlates with metabolic rates.

Reactive oxygen species (ROS): any of a number of highly reactive forms of oxygen that are potential sources of damage; damage caused by ROS has been implicated in aging.

Replicative senescence: irreversible cessation of cell division of normally proliferating cells. It is also characterized by various biomarkers and can or not be accompanied by cell death.

Semelparous: organisms that reproduce only once, usually followed by death, as observed in several animals.

Senescence: the fundamental process of aging or aging itself. Can also refer to cellular aging in some contexts.

Senescent cell: normally dividing cell that is irreversibly growth arrested and exhibits a number of other biomarkers associated with cellular senescence.

Soma: the entire body of an organism with exception of the germ cells.

Stem cell: an undifferentiated cell that can divide, differentiate into specialized cells, and can self-renew to give rise to more stem cells.

Strategies for engineered negligible senescence (SENS): a proposal by Aubrey de Grey that details how by reversing seven forms of cellular and molecular age-related changes will allow us to cure aging.

Stress-induced premature senescence (SIPS): irreversible cell cycle arrest and associated cell phenotypes as the result of subcytotoxic stress.

Supercentenarian: someone 110 years of age or older.

Topoisomerase: an enzyme that regulate the supercoiling structure of the DNA.

Taxon (plural: taxa): a taxonomic group of any rank.

Telomeres: the long end sequences of a DNA strand occurring at the tip of the chromosomes that play a key role in replicative senescence.

Telomerase: enzyme that adds telomeric sequences to the telomeres and has been associated with cellular immortality.

Trait: a particular characteristic of an organism that can have different phenotypes.

Werner syndrome (WS): genetic disease resembling accelerated aging; it typically has an adult onset.

Bibliography

Listed below are all the roughly 1,000 references cited in **senescence.info**. I often cite my own academic publications for the simple reason that I am more familiar with them, though I always aim to provide a broad overview of the literature on any given topic. For the sake of brevity I do not cite every single paper related to a given topic, and I frequently cite review papers and books--yet I try to cite the most important primary research papers. Lastly, please see my list of links and books for additional sources of information.

Abele, D., Strahl, J., Brey, T., and Philipp, E. E. (2008). "Imperceptible senescence: ageing in the ocean quahog Arctica islandica." *Free Radic Res* **42**(5):474-480. PubMed
Ackermann, M., Stearns, S. C., and Jenal, U. (2003). "Senescence in a bacterium with asymmetric division." *Science* **300**(5627):1920. PubMed
Acosta, J. C., O'Loghlen, A., Banito, A., Guijarro, M. V., Augert, A., Raguz, S., Fumagalli, M., Da Costa, M., Brown, C., Popov, N., *et al.* (2008). "Chemokine signaling via the CXCR2 receptor reinforces senescence." *Cell* **133**(6):1006-1018. PubMed
Adelman, R. C. (1998). "The Alzheimerization of aging: a brief update." *Exp Gerontol* **33**(1-2):155-157. PubMed
Ahmed, S., and Hodgkin, J. (2000). "MRT-2 checkpoint protein is required for germline immortality and telomere replication in C. elegans." *Nature* **403**(6766):159-164. PubMed
Ahmed, S., Passos, J. F., Birket, M. J., Beckmann, T., Brings, S., Peters, H., Birch-Machin, M. A., von Zglinicki, T., and Saretzki, G. (2008). "Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress." *J Cell Sci* **121**(Pt 7):1046-1053. PubMed
Aikata, H., Takaishi, H., Kawakami, Y., Takahashi, S., Kitamoto, M., Nakanishi, T., Nakamura, Y., Shimamoto, F., Kajiyama, G., and Ide, T. (2000). "Telomere reduction in human liver tissues with age and chronic inflammation." *Exp Cell Res* **256**(2):578-582. PubMed
Alcendor, R. R., Gao, S., Zhai, P., Zablocki, D., Holle, E., Yu, X., Tian, B., Wagner, T., Vatner, S. F., and Sadoshima, J. (2007). "Sirt1 regulates aging and resistance to oxidative stress in the heart." *Circ Res* **100**(10):1512-1521. PubMed
Alcorta, D. A., Xiong, Y., Phelps, D., Hannon, G., Beach, D., and Barrett, J. C. (1996). "Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts." *Proc Natl Acad Sci U S A* **93**(24):13742-13747. PubMed
Aledo, J. C., Li, Y., de Magalhaes, J. P., Ruiz-Camacho, M., and Perez-Claros, J. A. (2011). "Mitochondrially encoded methionine is inversely related to longevity in mammals." *Aging Cell* **10**(2):198-207. PubMed
Allard, M., Lebre, V., Robine, J., and Calment, J. (1998). *Jeanne Calment: From Van Gogh's time to ours: 122 extraordinary years*. W.H. Freeman & Co., New York.
Allman, J., McLaughlin, T., and Hakeem, A. (1993). "Brain weight and life-span in primate species." *Proc Natl Acad Sci U S A* **90**(1):118-122. PubMed
Allsopp, R. C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E. V., Futcher, A. B., Greider, C. W., and Harley, C. B. (1992). "Telomere length predicts replicative capacity of human fibroblasts." *Proc Natl Acad Sci U S A* **89**(21):10114-10118. PubMed
Altman, J., and Das, G. D. (1965). "Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats." *J Comp Neurol* **124**(3):319-335. PubMed
Alvarez-Buylla, A., and Garcia-Verdugo, J. M. (2002). "Neurogenesis in adult subventricular zone." *J Neurosci* **22**(3):629-634. PubMed
Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., and Wood, M. J. (2011). "Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes." *Nat Biotechnol* **29**(4):341-345. PubMed
Ames, B. N., Cathcart, R., Schwiers, E., and Hochstein, P. (1981). "Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis." *Proc Natl Acad Sci U S A* **78**(11):6858-6862. PubMed
Ames, B. N., Shigenaga, M. K., and Hagen, T. M. (1993). "Oxidants, antioxidants, and the degenerative diseases of aging." *Proc Natl Acad Sci U S A* **90**(17):7915-7922. PubMed
Andziak, B., O'Connor T, P., and Buffenstein, R. (2005). "Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat." *Mech Ageing Dev* **126**(11):1206-1212. PubMed
Anisimov, V. N., Zavarzina, N. Y., Zabezhinski, M. A., Popovich, I. G., Zimina, O. A., Shtylick, A. V., Arutjunyan, A. V., Oparina, T. I., Prokopenko, V. M., Mikhalski, A. I., *et al.* (2001). "Melatonin increases both life span and tumor incidence in female CBA mice." *J Gerontol A Biol Sci Med Sci* **56**(7):B311-323. PubMed
Anisimov, V. N., Berstein, L. M., Egormin, P. A., Piskunova, T. S., Popovich, I. G., Zabezhinski, M. A., Kovalenko, I. G., Poroshina, T. E., Semenchenko, A. V., Provinciali, M., *et al.* (2005). "Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice." *Exp Gerontol* **40**(8-9):685-693. PubMed
Antonawich, F. J., Federoff, H. J., and Davis, J. N. (1999). "BCL-2 transduction, using a herpes simplex virus amplicon, protects hippocampal neurons from transient global ischemia." *Exp Neurol* **156**(1):130-137. PubMed
Apfeld, J., and Kenyon, C. (1998). "Cell nonautonomy of C. elegans daf-2 function in the regulation of diapause and life span." *Cell* **95**(2):199-210. PubMed
Arantes-Oliveira, N., Apfeld, J., Dillin, A., and Kenyon, C. (2002). "Regulation of life-span by germ-line stem cells in Caenorhabditis elegans." *Science* **295**(5554):502-505. PubMed
Arboleda, G., Ramirez, N., and Arboleda, H. (2007). "The neonatal progeroid syndrome (Wiedemann-Rautenstrauch): a model for the study of human aging?" *Exp Gerontol* **42**(10):939-943. PubMed
Arking, D. E., Krebsova, A., Macek, M., Sr., Macek, M., Jr., Arking, A., Mian, I. S., Fried, L., Hamosh, A., Dey, S., McIntosh, I., *et al.* (2002). "Association of human aging with a functional variant of klotho." *Proc Natl Acad Sci U S A* **99**(2):856-861. PubMed
Arnold, J. M., and Carlson, B. A. (1986). "Living Nautilus embryos: Preliminary observations." *Science* **232**:73-76.
Artandi, S. E., Alson, S., Tietze, M. K., Sharpless, N. E., Ye, S., Greenberg, R. A., Castrillon, D. H., Horner, J. W., Weiler, S. R., Carrasco, R. D., *et al.* (2002). "Constitutive telomerase expression promotes mammary carcinomas in aging mice." *Proc Natl Acad Sci U S A* **99**(12):8191-8196. PubMed
Atadja, P., Wong, H., Garkavtsev, I., Veillette, C., and Riabowol, K. (1995). "Increased activity of p53 in senescing fibroblasts." *Proc Natl Acad Sci U S A* **92**(18):8348-8352. PubMed
Atzmon, G., Schechter, C., Greiner, W., Davidson, D., Rennert, G., and Barzilai, N. (2004). "Clinical phenotype of families with longevity." *J Am Geriatr Soc* **52**(2):274-277. PubMed
Atzmon, G., Cho, M., Cawthon, R. M., Budagov, T., Katz, M., Yang, X., Siegel, G., Bergman, A., Huffman, D. M., Schechter, C. B., *et al.* (2010). "Evolution in health and medicine Sackler colloquium: Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians." *Proc Natl Acad Sci U S A* **107 Suppl 1**:1710-1717. PubMed
Austad, S. N. (1988). "The adaptable opossum." *Sci Am* (Feb.):98-104.
Austad, S. N. (1989). "Life extension by dietary restriction in the bowl and doily spider, Frontinella pyramitela." *Exp Gerontol* **24**(1):83-92. PubMed
Austad, S. N., and Fischer, K. E. (1991). "Mammalian aging, metabolism, and ecology: evidence from the bats and marsupials." *J Gerontol* **46**(2):B47-53. PubMed
Austad, S. N., and Fischer, K. E. (1992). "Primate longevity: Its place in the mammalian scheme." *Am J Primatol* **28**(4):251-261.
Austad, S. N. (1997a). *Why We Age: What Science Is Discovering about the Body's Journey through Life*. John Wiley & Sons, New York.
Austad, S. N. (1997b). "Comparative aging and life histories in mammals." *Exp Gerontol* **32**(1-2):23-38. PubMed
Austad, S. N. (1997c). "Small nonhuman primates as potential models of human aging." *Ilar J* **38**(3):142-147. PubMed
Austad, S. N. (2001). "An experimental paradigm for the study of slowly aging organisms." *Exp Gerontol* **36**(4-6):599-605. PubMed
Austad, S. N. (2004). "Is aging programed?" *Aging Cell* **3**(5):249-251. PubMed
Austad, S. N. (2005). "Diverse aging rates in metazoans: targets for functional genomics." *Mech Ageing Dev* **126**(1):43-49. PubMed
Austad, S. N. (2006). "Why women live longer than men: sex differences in longevity." *Gend Med* **3**(2):79-92. PubMed
Ayyadevara, S., Alla, R., Thaden, J. J., and Shmookler Reis, R. J. (2008). "Remarkable longevity and stress resistance of nematode PI3K-null mutants." *Aging Cell* **7**(1):13-22. PubMed
Baker, D. J., Wijshake, T., Tchkonia, T., LeBrasseur, N. K., Childs, B. G., van de Sluis, B., Kirkland, J. L., and van Deursen, J. M. (2011). "Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders." *Nature* **479**(7372):232-236. PubMed
Balin, A. K. E. (1994). *Practical Handbook of Biologic Age Determination*. CRC Press, Boca Raton, FL.
Barja, G., and Herrero, A. (2000). "Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals." *Faseb J* **14**(2):312-318. PubMed
Barja, G. (2002). "Endogenous oxidative stress: relationship to aging, longevity and caloric restriction." *Ageing Res Rev* **1**(3):397-411. PubMed
Barker, M. G., and Walmsley, R. M. (1999). "Replicative ageing in the fission yeast Schizosaccharomyces pombe." *Yeast* **15**(14):1511-1518. PubMed
Bartke, A., Coschigano, K., Kopchick, J., Chandrashekar, V., Mattison, J., Kinney, B., and Hauck, S. (2001a). "Genes that prolong life: relationships of growth hormone and growth to aging and life span." *J Gerontol A Biol Sci Med Sci* **56**(8):B340-349. PubMed
Bartke, A., Wright, J. C., Mattison, J. A., Ingram, D. K., Miller, R. A., and Roth, G. S. (2001b). "Extending the lifespan of long-lived mice." *Nature* **414**(6862):412. PubMed
Bartke, A. (2003). "Can growth hormone (GH) accelerate aging? Evidence from GH-transgenic mice." *Neuroendocrinology* **78**(4):210-216. PubMed
Bartke, A. (2005). "Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging." *Endocrinology* **146**(9):3718-3723. PubMed
Barton-Davis, E. R., Shoturma, D. I., Musaro, A., Rosenthal, N., and Sweeney, H. L. (1998). "Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function." *Proc Natl Acad Sci U S A* **95**(26):15603-15607. PubMed
Bass, T. M., Weinkove, D., Houthoofd, K., Gems, D., and Partridge, L. (2007). "Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans." *Mech Ageing Dev* **128**(10):546-552. PubMed
Bassham, S., Beam, A., and Shampay, J. (1998). "Telomere variation in Xenopus laevis." *Mol Cell Biol* **18**(1):269-275. PubMed
Bastianetto, S., and Quirion, R. (2002). "Natural extracts as possible protective agents of brain aging." *Neurobiol Aging* **23**(5):891-897. PubMed

Bauer, J. H., Poon, P. C., Glatt-Deeley, H., Abrams, J. M., and Helfand, S. L. (2005). "Neuronal expression of p53 dominant-negative proteins in adult Drosophila melanogaster extends life span." *Curr Biol* **15**(22):2063-2068. PubMed
Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., *et al.* (2006). "Resveratrol improves health and survival of mice on a high-calorie diet." *Nature* **444**(7117):337-342. PubMed
Baur, J. A., and Sinclair, D. A. (2006). "Therapeutic potential of resveratrol: the in vivo evidence." *Nat Rev Drug Discov* **5**(6):493-506. PubMed
Bayne, A. C., Mockett, R. J., Orr, W. C., and Sohal, R. S. (2005). "Enhanced catabolism of mitochondrial superoxide/hydrogen peroxide and aging in transgenic Drosophila." *Biochem J* **391**(Pt 2):277-284. PubMed
Baynes, J. W. (2000). "From life to death--the struggle between chemistry and biology during aging: the Maillard reaction as an amplifier of genomic damage." *Biogerontology* **1**(3):235-246. PubMed
Bayreuther, K., Rodemann, H. P., Hommel, R., Dittmann, K., Albiez, M., and Francz, P. I. (1988). "Human skin fibroblasts in vitro differentiate along a terminal cell lineage." *Proc Natl Acad Sci U S A* **85**(14):5112-5116. PubMed
Beckman, K. B., and Ames, B. N. (1998). "The free radical theory of aging matures." *Physiol Rev* **78**(2):547-581. PubMed
Bell, G. (1988). *Sex and Death in Protozoa. The History of an Obsession*. Cambridge University Press, Cambridge.
Benard, C., and Hekimi, S. (2002). "Long-lived mutants, the rate of aging, telomeres and the germline in Caenorhabditis elegans." *Mech Ageing Dev* **123**(8):869-880. PubMed
Berardi, P., Meyyappan, M., and Riabowol, K. T. (2003). "A novel transcriptional inhibitory element differentially regulates the cyclin D1 gene in senescent cells." *J Biol Chem* **278**(9):7510-7519. PubMed
Bergquist, D. C., Williams, F. M., and Fisher, C. R. (2000). "Longevity record for deep-sea invertebrate." *Nature* **403**(6769):499-500. PubMed
Berner, Y. N., and Stern, F. (2004). "Energy restriction controls aging through neuroendocrine signal transduction." *Ageing Res Rev* **3**(2):189-198. PubMed
Bersell, K., Arab, S., Haring, B., and Kuhn, B. (2009). "Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury." *Cell* **138**(2):257-270. PubMed
Berzlanovich, A. M., Keil, W., Waldhoer, T., Sim, E., Fasching, P., and Fazeny-Dorner, B. (2005). "Do centenarians die healthy? An autopsy study." *J Gerontol A Biol Sci Med Sci* **60**(7):862-865. PubMed
Besson, A., Salemi, S., Gallati, S., Jenal, A., Horn, R., Mullis, P. S., and Mullis, P. E. (2003). "Reduced longevity in untreated patients with isolated growth hormone deficiency." *J Clin Endocrinol Metab* **88**(8):3664-3667. PubMed
Bidder, G. P. (1932). "Senescence." *Brit Med J* **2**:583-585.
Birney, E., Stamatoyannopoulos, J. A., Dutta, A., Guigo, R., Gingeras, T. R., Margulies, E. H., Weng, Z., Snyder, M., Dermitzakis, E. T., Thurman, R. E., *et al.* (2007). "Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project." *Nature* **447**(7146):799-816. PubMed
Bishop, N. A., and Guarente, L. (2007). "Two neurons mediate diet-restriction-induced longevity in C. elegans." *Nature* **447**(7144):545-549. PubMed
Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., and Gluud, C. (2007). "Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis." *Jama* **297**(8):842-857. PubMed
Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., and Gluud, C. (2008). "Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases." *Cochrane Database Syst Rev* (2):CD007176. PubMed
Bjorklund, A., and Lindvall, O. (2000). "Self-repair in the brain." *Nature* **405**(6789):892-893, 895. PubMed
Blackburn, E. H. (2000). "Telomere states and cell fates." *Nature* **408**(6808):53-56. PubMed
Blackman, M. R., Sorkin, J. D., Munzer, T., Bellantoni, M. F., Busby-Whitehead, J., Stevens, T. E., Jayme, J., O'Connor, K. G., Christmas, C., Tobin, J. D., *et al.* (2002). "Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial." *Jama* **288**(18):2282-2292. PubMed
Blasco, M. A., Lee, H. W., Hande, M. P., Samper, E., Lansdorp, P. M., DePinho, R. A., and Greider, C. W. (1997). "Telomere shortening and tumor formation by mouse cells lacking telomerase RNA." *Cell* **91**(1):25-34. PubMed
Blasco, M. A., and Hahn, W. C. (2003). "Evolving views of telomerase and cancer." *Trends Cell Biol* **13**(6):289-294. PubMed
Blasco, M. A. (2005). "Telomeres and human disease: ageing, cancer and beyond." *Nat Rev Genet* **6**(8):611-622. PubMed
Bodkin, N. L., Alexander, T. M., Ortmeyer, H. K., Johnson, E., and Hansen, B. C. (2003). "Mortality and morbidity in laboratory-maintained Rhesus monkeys and effects of long-term dietary restriction." *J Gerontol A Biol Sci Med Sci* **58**(3):212-219. PubMed
Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., Harley, C. B., Shay, J. W., Lichtsteiner, S., and Wright, W. E. (1998). "Extension of life-span by introduction of telomerase into normal human cells." *Science* **279**(5349):349-352. PubMed
Bohr, V. A., Brosh, R. M., Jr., von Kobbe, C., Opresko, P., and Karmakar, P. (2002). "Pathways defective in the human premature aging disease Werner syndrome." *Biogerontology* **3**(1-2):89-94. PubMed
Bolli, R., Chugh, A. R., D'Amario, D., Loughran, J. H., Stoddard, M. F., Ikram, S., Beache, G. M., Wagner, S. G., Leri, A., Hosoda, T., *et al.* (2011). "Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial." *Lancet* **378**(9806):1847-1857. PubMed
Bonafe, M., Barbieri, M., Marchegiani, F., Olivieri, F., Ragno, E., Giampieri, C., Mugianesi, E., Centurelli, M., Franceschi, C., and Paolisso, G. (2003). "Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionarily conserved mechanism of life span control." *J Clin Endocrinol Metab* **88**(7):3299-3304. PubMed
Bond, J., Haughton, M., Blaydes, J., Gire, V., Wynford-Thomas, D., and Wyllie, F. (1996). "Evidence that transcriptional activation by p53 plays a direct role in the induction of cellular senescence." *Oncogene* **13**(10):2097-2104. PubMed
Bonelli, M. A., Alfieri, R. R., Petronini, P. G., Brigotti, M., Campanini, C., and Borghetti, A. F. (1999). "Attenuated expression of 70-kDa heat shock protein in WI-38 human fibroblasts during aging in vitro." *Exp Cell Res* **252**(1):20-32. PubMed
Bonkowski, M. S., Rocha, J. S., Masternak, M. M., Al Regaiey, K. A., and Bartke, A. (2006). "Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction." *Proc Natl Acad Sci U S A* **103**(20):7901-7905. PubMed
Bons, N., Mestre, N., and Petter, A. (1992). "Senile plaques and neurofibrillary changes in the brain of an aged lemurian primate, Microcebus murinus." *Neurobiol Aging* **13**(1):99-105. PubMed
Bowen, R. L., and Atwood, C. S. (2004). "Living and dying for sex. A theory of aging based on the modulation of cell cycle signaling by reproductive hormones." *Gerontology* **50**(5):265-290. PubMed
Bowles, J. (2000). "Shattered: Medawar's test tubes and their enduring legacy of chaos." *Med Hypotheses* **54**(2):326-339. PubMed
Brack, C., Lithgow, G., Osiewacz, H., and Toussaint, O. (2000). "EMBO WORKSHOP REPORT: Molecular and cellular gerontology Serpiano, Switzerland, September 18-22, 1999." *Embo J* **19**(9):1929-1934. PubMed
Brakefield, P. M., Gems, D., Cowen, T., Christensen, K., Grubeck-Loebenstein, B., Keller, L., Oeppen, J., Rodriguez-Pena, A., Stazi, M. A., Tatar, M., *et al.* (2005). "What are the effects of maternal and pre-adult environments on ageing in humans, and are there lessons from animal models?" *Mech Ageing Dev* **126**(3):431-438. PubMed
Breese, C. R., Ingram, R. L., and Sonntag, W. E. (1991). "Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins." *J Gerontol* **46**(5):B180-187. PubMed
Brocas, J., and Verzar, F. (1961). "The aging of Xenopus laevis, a South African frog." *Gerontologia* **5**:228-240. PubMed
Brock, M. A., and Strehler, B. L. (1963). "Studies on the comparative physiology of aging. IV. Age and mortality of some marine Cnidaria in the laboratory." *J Gerontol* **18**:23-28. PubMed
Brock, T. D. (1997). *Biology of Microorganisms*. Prentice Hall & IBD, New Jersey.
Brockes, J. P., Kumar, A., and Velloso, C. P. (2001). "Regeneration as an evolutionary variable." *J Anat* **199**(Pt 1-2):3-11. PubMed
Bronikowski, A. M., Alberts, S. C., Altmann, J., Packer, C., Carey, K. D., and Tatar, M. (2002). "The aging baboon: comparative demography in a non-human primate." *Proc Natl Acad Sci U S A* **99**(14):9591-9595. PubMed
Brown-Borg, H. M., Borg, K. E., Meliska, C. J., and Bartke, A. (1996). "Dwarf mice and the ageing process." *Nature* **384**(6604):33. PubMed
Brown-Borg, H. M., Rakoczy, S. G., and Uthus, E. O. (2005). "Growth hormone alters methionine and glutathione metabolism in Ames dwarf mice." *Mech Ageing Dev* **126**(3):389-398. PubMed
Brunet-Rossinni, A. K., and Austad, S. N. (2004). "Ageing studies on bats: a review." *Biogerontology* **5**(4):211-222. PubMed
Brunk, U., Ericsson, J. L., Ponten, J., and Westermark, B. (1973). "Residual bodies and "aging" in cultured human glia cells. Effect of entrance into phase 3 and prolonged periods of confluence." *Exp Eye Res* **79**(1):1-14. PubMed
Brunmark, A., Collins, V. P., Thaw, H., and Brunk, U. (1986). "Lipofuscin accumulation in cultured non-dividing cells as a function of time and oxygen tension." *Scan Electron Microsc* **1**:189-192. PubMed
Bruunsgaard, H., Pedersen, M., and Pedersen, B. K. (2001). "Aging and proinflammatory cytokines." *Curr Opin Hematol* **8**(3):131-136. PubMed
Bryan, T. M., Englezou, A., Gupta, J., Bacchetti, S., and Reddel, R. R. (1995). "Telomere elongation in immortal human cells without detectable telomerase activity." *Embo J* **14**(17):4240-4248. PubMed
Buchkovich, K., Duffy, L. A., and Harlow, E. (1989). "The retinoblastoma protein is phosphorylated during specific phases of the cell cycle." *Cell* **58**(6):1097-1105. PubMed
Buffenstein, R. (2005). "The naked mole-rat: a new long-living model for human aging research." *J Gerontol A Biol Sci Med Sci* **60**(11):1369-1377. PubMed
Buffenstein, R. (2008). "Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species." *J Comp Physiol B* **178**(4):439-445. PubMed
Bukovsky, A., Caudle, M. R., Svetlikova, M., Wimalasena, J., Ayala, M. E., and Dominguez, R. (2005). "Oogenesis in adult mammals, including humans: a review." *Endocrine* **26**(3):301-316. PubMed
Burck, U., Goebel, H. H., Kuhlendahl, H. D., Meier, C., and Goebel, K. M. (1981). "Neuromyopathy and vitamin E deficiency in man." *Neuropediatrics* **12**(3):267-278. PubMed
Burnett, C., Valentini, S., Cabreiro, F., Goss, M., Somogyvari, M., Piper, M. D., Hoddinott, M., Sutphin, G. L., Leko, V., McElwee, J. J., *et al.* (2011). "Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila." *Nature* **477**(7365):482-485. PubMed
Burt, R. K., Loh, Y., Pearce, W., Beohar, N., Barr, W. G., Craig, R., Wen, Y., Rapp, J. A., and Kessler, J. (2008). "Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases." *Jama* **299**(8):925-936. PubMed

Butler, R. N., Austad, S. N., Barzilai, N., Braun, A., Helfand, S., Larsen, P. L., McCormick, A. M., Perls, T. T., Shuldiner, A. R., Sprott, R. L., *et al.* (2003). "Longevity genes: from primitive organisms to humans." *J Gerontol A Biol Sci Med Sci* **58**(7):581-584. PubMed
Butler, R. N., Miller, R. A., Perry, D., Carnes, B. A., Williams, T. F., Cassel, C., Brody, J., Bernard, M. A., Partridge, L., Kirkwood, T., *et al.* (2008). "New model of health promotion and disease prevention for the 21st century." *Bmj* **337**:a399. PubMed
Cailliet, G. M., Andrews, A. H., Burton, E. J., Watters, D. L., Kline, D. E., and Ferry-Graham, L. A. (2001). "Age determination and validation studies of marine fishes: do deep- dwellers live longer?" *Exp Gerontol* **36**(4-6):739-764. PubMed
Calder, W. A. (1984). *Size, Function, and Life History*. Harvard University Press, Cambridge.
Campisi, J. (1999). "Replicative senescence and immortalization." In: *The molecular basis of cell cycle and growth control*, Stein, G. S., Baserga, A., Giordano, A. and Denhardt, D. T. (ed.).Â Wiley-Liss, New York, 348-373.
Campisi, J. (2000). "Aging, chromatin, and food restriction--connecting the dots." *Science* **289**(5487):2062-2063. PubMed
Campisi, J., Kim, S. H., Lim, C. S., and Rubio, M. (2001). "Cellular senescence, cancer and aging: the telomere connection." *Exp Gerontol* **36**(10):1619-1637. PubMed
Campisi, J. (2005). "Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors." *Cell* **120**(4):513-522. PubMed
Campisi, J., and d'Adda di Fagagna, F. (2007). "Cellular senescence: when bad things happen to good cells." *Nat Rev Mol Cell Biol* **8**(9):729-740. PubMed
Cao, L., Li, W., Kim, S., Brodie, S. G., and Deng, C. X. (2003). "Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform." *Genes Dev* **17**(2):201-213. PubMed
Caplan, A. L. (2005). "Death as an unnatural process. Why is it wrong to seek a cure for aging?" *EMBO Rep* **6 Spec No**:S72-75. PubMed
Carlson, B. M. (2003). "Muscle regeneration in amphibians and mammals: passing the torch." *Dev Dyn* **226**(2):167-181. PubMed
Carlson, M. E., Hsu, M., and Conboy, I. M. (2008). "Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells." *Nature* **454**(7203):528-532. PubMed
Carney, J. P., Maser, R. S., Olivares, H., Davis, E. M., Le Beau, M., Yates, J. R., 3rd, Hays, L., Morgan, W. F., and Petrini, J. H. (1998). "The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response." *Cell* **93**(3):477-486. PubMed
Carroll, S. B. (2003). "Genetics and the making of Homo sapiens." *Nature* **422**(6934):849-857. PubMed
Castro, P., Giri, D., Lamb, D., and Ittmann, M. (2003). "Cellular senescence in the pathogenesis of benign prostatic hyperplasia." *Prostate* **55**(1):30-38. PubMed
Caulin, A. F., and Maley, C. C. (2011). "Peto's Paradox: evolution's prescription for cancer prevention." *Trends Ecol Evol* **26**(4):175-182. PubMed
Cavazzana-Calvo, M., Hacein-Bey, S., de Saint Basile, G., Gross, F., Yvon, E., Nusbaum, P., Selz, F., Hue, C., Certain, S., Casanova, J. L., *et al.* (2000). "Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease." *Science* **288**(5466):669-672. PubMed
Cawthon, R. M., Smith, K. R., O'Brien, E., Sivatchenko, A., and Kerber, R. A. (2003). "Association between telomere length in blood and mortality in people aged 60 years or older." *Lancet* **361**(9355):393-395. PubMed
Celli, J., Duijf, P., Hamel, B. C., Bamshad, M., Kramer, B., Smits, A. P., Newbury-Ecob, R., Hennekam, R. C., Van Buggenhout, G., van Haeringen, A., *et al.* (1999). "Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome." *Cell* **99**(2):143-153. PubMed
Cenci, G., Ciapponi, L., and Gatti, M. (2005). "The mechanism of telomere protection: a comparison between Drosophila and humans." *Chromosoma* **114**(3):135-145. PubMed
Cevenini, E., Bellavista, E., Tieri, P., Castellani, G., Lescai, F., Francesconi, M., Mishto, M., Santoro, A., Valensin, S., Salvioli, S., *et al.* (2010). "Systems biology and longevity: an emerging approach to identify innovative anti-aging targets and strategies." *Curr Pharm Des* **16**(7):802-813. PubMed
Chai, Y., and Slavkin, H. C. (2003). "Prospects for tooth regeneration in the 21st century: a perspective." *Microsc Res Tech* **60**(5):469-479. PubMed
Chan, A. W., Chong, K. Y., Martinovich, C., Simerly, C., and Schatten, G. (2001). "Transgenic monkeys produced by retroviral gene transfer into mature oocytes." *Science* **291**(5502):309-312. PubMed
Charlesworth, B. (1993). *Evolution in Age-Structured Populations*. Cambridge University Press, Cambridge.
Charlesworth, B., and Hughes, K. A. (1996). "Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence." *Proc Natl Acad Sci U S A* **93**(12):6140-6145. PubMed
Charlesworth, B. (2000). "Fisher, Medawar, Hamilton and the evolution of aging." *Genetics* **156**(3):927-931. PubMed
Charnov, E. L. (1993). *Life History Invariants: Some Explorations of Symmetry in Evolutionary Ecology*. Oxford University Press, Oxford.
Chen, J. J., and Yu, B. P. (1994). "Alterations in mitochondrial membrane fluidity by lipid peroxidation products." *Free Radical Biol Med* **17**(5):411-418. PubMed
Chen, Q., Fischer, A., Reagan, J. D., Yan, L. J., and Ames, B. N. (1995). "Oxidative DNA damage and senescence of human diploid fibroblast cells." *Proc Natl Acad Sci U S A* **92**(10):4337-4341. PubMed
Chen, H. J., Liang, C. L., Lu, K., Lin, J. W., and Cho, C. L. (2000). "Implication of telomerase activity and alternations of telomere length in the histologic characteristics of intracranial meningiomas." *Cancer* **89**(10):2092-2098. PubMed
Chen, L., Lee, L., Kudlow, B. A., Dos Santos, H. G., Sletvold, O., Shafeghati, Y., Botha, E. G., Garg, A., Hanson, N. B., Martin, G. M., *et al.* (2003). "LMNA mutations in atypical Werner's syndrome." *Lancet* **362**(9382):440-445. PubMed
Chen, J., Lewis, E. E., Carey, J. R., Caswell, H., and Caswell-Chen, E. P. (2006). "The ecology and biodemography of Caenorhabditis elegans." *Exp Gerontol* **41**(10):1059-1065. PubMed
Chen, D., Pan, K. Z., Palter, J. E., and Kapahi, P. (2007). "Longevity determined by developmental arrest genes in Caenorhabditis elegans." *Aging Cell* **6**(4):525-533. PubMed
Chiu, C. P., Dragowska, W., Kim, N. W., Vaziri, H., Yui, J., Thomas, T. E., Harley, C. B., and Lansdorp, P. M. (1996). "Differential expression of telomerase activity in hematopoietic progenitors from adult human bone marrow." *Stem Cells* **14**(2):239-248. PubMed
Ch'ng, Q., Sieburth, D., and Kaplan, J. M. (2008). "Profiling synaptic proteins identifies regulators of insulin secretion and lifespan." *PLoS Genet* **4**(11):e1000283. PubMed
Choi, H. S., Lin, Z., Li, B. S., and Liu, A. Y. (1990). "Age-dependent decrease in the heat-inducible DNA sequence-specific binding activity in human diploid fibroblasts." *J Biol Chem* **265**(29):18005-18011. PubMed
Choi, D., Whittier, P. S., Oshima, J., and Funk, W. D. (2001). "Telomerase expression prevents replicative senescence but does not fully reset mRNA expression patterns in Werner syndrome cell strains." *Faseb J* **15**(6):1014-1020. PubMed
Cibelli, J. B., Stice, S. L., Golueke, P. J., Kane, J. J., Jerry, J., Blackwell, C., Ponce de Leon, F. A., and Robl, J. M. (1998). "Cloned transgenic calves produced from nonquiescent fetal fibroblasts." *Science* **280**(5367):1256-1258. PubMed
Cibelli, J. B., Lanza, R. P., West, M. D., and Ezzell, C. (2002). "The first human cloned embryo." *Sci Am* **286**(1):44-51. PubMed
Clancy, D. J., Gems, D., Harshman, L. G., Oldham, S., Stocker, H., Hafen, E., Leevers, S. J., and Partridge, L. (2001). "Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein." *Science* **292**(5514):104-106. PubMed
Clausen, A., Doctrow, S., and Baudry, M. (2010). "Prevention of cognitive deficits and brain oxidative stress with superoxide dismutase/catalase mimetics in aged mice." *Neurobiol Aging* **31**(3):425-433. PubMed
Cohen, A. A. (2004). "Female post-reproductive lifespan: a general mammalian trait." *Biol Rev Camb Philos Soc* **79**(4):733-750. PubMed
Cohen, H. Y., Miller, C., Bitterman, K. J., Wall, N. R., Hekking, B., Kessler, B., Howitz, K. T., Gorospe, M., de Cabo, R., and Sinclair, D. A. (2004). "Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase." *Science* **305**(5682):390-392. PubMed
Cohen, E., Bieschke, J., Perciavalle, R. M., Kelly, J. W., and Dillin, A. (2006). "Opposing activities protect against age-onset proteotoxicity." *Science* **313**(5793):1604-1610. PubMed
Coles, L. S. (2004). "Demographics of human supercentenarians and the implications for longevity medicine." *Ann N Y Acad Sci* **1019**:490-495. PubMed
Colgin, L. M., and Reddel, R. R. (1999). "Telomere maintenance mechanisms and cellular immortalization." *Curr Opin Genet Dev* **9**(1):97-103. PubMed
Collins, K., and Mitchell, J. R. (2002). "Telomerase in the human organism." *Oncogene* **21**(4):564-579. PubMed
Collins, F. S., Rossant, J., and Wurst, W. (2007). "A mouse for all reasons." *Cell* **128**(1):9-13. PubMed
Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T. M., Allison, D. B., Cruzen, C., Simmons, H. A., Kemnitz, J. W., *et al.* (2009). "Caloric restriction delays disease onset and mortality in rhesus monkeys." *Science* **325**(5937):201-204. PubMed
Comfort, A. (1964). *Ageing: The Biology of Senescence*. Routledge & Kegan Paul, London.
Comfort, A. (1968). "Feasibility in age research." *Nature* **217**(5126):320-322. PubMed
Comfort, A., Youhotsky-Gore, I., and Pathmanathan, K. (1971). "Effect of ethoxyquin on the longevity of C3H mice." *Nature* **229**(5282):254-255. PubMed
Conboy, I. M., Conboy, M. J., Wagers, A. J., Girma, E. R., Weissman, I. L., and Rando, T. A. (2005). "Rejuvenation of aged progenitor cells by exposure to a young systemic environment." *Nature* **433**(7027):760-764. PubMed
Congdon, J. D., Nagle, R. D., Kinney, O. M., and van Loben Sels, R. C. (2001). "Hypotheses of aging in a long-lived vertebrate, Blanding's turtle (Emydoidea blandingii)." *Exp Gerontol* **36**(4-6):813-827. PubMed
Congdon, J. D., Nagle, R. D., Kinney, O. M., van Loben Sels, R. C., Quinter, T., and Tinkle, D. W. (2003). "Testing hypotheses of aging in long-lived painted turtles (Chrysemys picta)." *Exp Gerontol* **38**(7):765-772. PubMed
Cook, R. E. (1983). "Clonal plant populations." *Am Sci* **71**:244-253.
Cooper, T. M., Mockett, R. J., Sohal, B. H., Sohal, R. S., and Orr, W. C. (2004). "Effect of caloric restriction on life span of the housefly, Musca domestica." *Faseb J* **18**(13):1591-1593. PubMed
Corral-Debrinski, M., Horton, T., Lott, M. T., Shoffner, J. M., Beal, M. F., and Wallace, D. C. (1992). "Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age." *Nat Genet* **2**(4):324-329. PubMed
Cortopassi, G. A., and Wang, E. (1996). "There is substantial agreement among interspecies estimates of DNA repair activity." *Mech Ageing Dev* **91**(3):211-218. PubMed
Coschigano, K. T., Clemmons, D., Bellush, L. L., and Kopchick, J. J. (2000). "Assessment of growth parameters and life span of GHR/BP gene-disrupted mice." *Endocrinology* **141**(7):2608-2613. PubMed
Cosgrove, G. E., Selby, P. B., Upton, A. C., Mitchell, T. J., Steele, M. H., and Russell, W. L. (1993). "Lifespan and autopsy findings in the first-generation offspring of X-irradiated male mice." *Mutat Res* **319**(1):71-79. PubMed
Counter, C. M., Avilion, A. A., LeFeuvre, C. E., Stewart, N. G., Greider, C. W., Harley, C. B., and Bacchetti, S. (1992). "Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity." *Embo J* **11**(5):1921-1929. PubMed

Counter, C. M., Gupta, J., Harley, C. B., Leber, B., and Bacchetti, S. (1995). "Telomerase activity in normal leukocytes and in hematologic malignancies." *Blood* **85**(9):2315-2320. PubMed
Counter, C. M., Meyerson, M., Eaton, E. N., Ellisen, L. W., Caddle, S. D., Haber, D. A., and Weinberg, R. A. (1998). "Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase." *Oncogene* **16**(9):1217-1222. PubMed
Craik, F. I. M., and Salthouse, T. A. (1992). *Handbook of Aging and Cognition*. Erlbaum, Hillsdale.
Cristofalo, V. J., and Sharf, B. B. (1973). "Cellular senescence and DNA synthesis. Thymidine incorporation as a measure of population age in human diploid cells." *Exp Cell Res* **76**(2):419-427. PubMed
Cristofalo, V. J., and Pignolo, R. J. (1993). "Replicative senescence of human fibroblast-like cells in culture." *Physiol Rev* **73**(3):617-638. PubMed
Cristofalo, V. J., Volker, C., Francis, M. K., and Tresini, M. (1998a). "Age-dependent modifications of gene expression in human fibroblasts." *Crit Rev Eukaryot Gene Expr* **8**(1):43-80. PubMed
Cristofalo, V. J., Allen, R. G., Pignolo, R. J., Martin, B. G., and Beck, J. C. (1998b). "Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation." *Proc Natl Acad Sci U S A* **95**(18):10614-10619. PubMed
Cristofalo, V. (2001). ""I no longer believe that cell death is programmed. ..", an interview with Vincent Cristofalo." *Biogerontology* **2**(4):283-290. PubMed
Cuervo, A. M., Bergamini, E., Brunk, U. T., Droge, W., Ffrench, M., and Terman, A. (2005). "Autophagy and aging: the importance of maintaining "clean" cells." *Autophagy* **1**(3):131-140. PubMed
Cutler, R. G. (1975). "Evolution of human longevity and the genetic complexity governing aging rate." *Proc Natl Acad Sci U S A* **72**(11):4664-4668. PubMed
Cutler, R. G. (1979). "Evolution of human longevity: a critical overview." *Mech Ageing Dev* **9**(3-4):337-354. PubMed
Cutler, R. G. (1985). "Antioxidants and longevity of mammalian species." *Basic Life Sci* **35**:15-73. PubMed
Czupryn, A., Zhou, Y. D., Chen, X., McNay, D., Anderson, M. P., Flier, J. S., and Macklis, J. D. (2011). "Transplanted hypothalamic neurons restore leptin signaling and ameliorate obesity in db/db mice." *Science* **334**(6059):1133-1137. PubMed
Das, M., Gabriely, I., and Barzilai, N. (2004). "Caloric restriction, body fat and ageing in experimental models." *Obes Rev* **5**(1):13-19. PubMed
Davenport, R. J. (2003). "Power to the people." *Sci Aging Knowledge Environ* **2003**(50):NS8. PubMed
Davidson, B. L., and McCray, P. B. (2011). "Current prospects for RNA interference-based therapies." *Nat Rev Genet* **12**(5):329-340. PubMed
Davis, J. K. (2004). "Collective suttee: is it unjust to develop life extension if it will not be possible to provide it to everyone?" *Ann N Y Acad Sci* **1019**:535-541. PubMed
Davis, J. K. (2005). "Life-extension and the Malthusian Objection." *J Med Philos* **30**(1):27-44. PubMed
de Boer, J., Andressoo, J. O., de Wit, J., Huijmans, J., Beems, R. B., van Steeg, H., Weeda, G., van der Horst, G. T., van Leeuwen, W., Themmen, A. P., *et al.* (2002). "Premature aging in mice deficient in DNA repair and transcription." *Science* **296**(5571):1276-1279. PubMed
de Grey, A. D. (1997). "A proposed refinement of the mitochondrial free radical theory of aging." *Bioessays* **19**(2):161-166. PubMed
de Grey, A. D., Ames, B. N., Andersen, J. K., Bartke, A., Campisi, J., Heward, C. B., McCarter, R. J., and Stock, G. (2002a). "Time to talk SENS: critiquing the immutability of human aging." *Ann N Y Acad Sci* **959**:452-462; discussion 463-455. PubMed
de Grey, A. D., Baynes, J. W., Berd, D., Heward, C. B., Pawelec, G., and Stock, G. (2002b). "Is human aging still mysterious enough to be left only to scientists?" *Bioessays* **24**(7):667-676. PubMed
de Grey, A. D. (2003). "An engineer's approach to the development of real anti-aging medicine." *Sci Aging Knowledge Environ* **2003**(1):VP1. PubMed
de Grey, A. D. (2005a). "The unfortunate influence of the weather on the rate of ageing: why human caloric restriction or its emulation may only extend life expectancy by 2-3 years." *Gerontology* **51**(2):73-82. PubMed
de Grey, A. D. (2005b). "Whole-body interdiction of lengthening of telomeres: a proposal for cancer prevention." *Front Biosci* **10**:2420-2429. PubMed
de Grey, A. D. (2005c). "Like it or not, life-extension research extends beyond biogerontology." *EMBO Rep* **6**(11):1000. PubMed
de Grey, A. D. (2005d). "Resistance to debate on how to postpone ageing is delaying progress and costing lives. Open discussions in the biogerontology community would attract public interest and influence funding policy." *EMBO Rep* **6 Spec No**:S49-53. PubMed
de Grey, A. D. (2006). "Is SENS a farrago?" *Rejuvenation Res* **9**(4):436-439. PubMed
de Grey, A., and Rae, M. (2008). *Ending Aging: The Rejuvenation Breakthroughs that Could Reverse Human Aging in Our Lifetime*. St. Martin's Press, New York.
de Haan, G., and Van Zant, G. (1999). "Genetic analysis of hemopoietic cell cycling in mice suggests its involvement in organismal life span." *Faseb J* **13**(6):707-713. PubMed
de Jesus, B. B., Schneeberger, K., Vera, E., Tejera, A., Harley, C. B., and Blasco, M. A. (2011). "The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence." *Aging Cell* **10**(4):604-621. PubMed
de Magalhaes, J. P., and Toussaint, O. (2002). "The evolution of mammalian aging." *Exp Gerontol* **37**(6):769-775. PubMed
de Magalhaes, J. P. (2003). "Is mammalian aging genetically controlled?" *Biogerontology* **4**(2):119-120. PubMed
de Magalhaes, J. P. (2004). "From cells to ageing: a review of models and mechanisms of cellular senescence and their impact on human ageing." *Exp Cell Res* **300**(1):1-10. PubMed
de Magalhaes, J. P., Chainiaux, F., de Longueville, F., Mainfroid, V., Migeot, V., Marcq, L., Remacle, J., Salmon, M., and Toussaint, O. (2004). "Gene expression and regulation in H2O2-induced premature senescence of human foreskin fibroblasts expressing or not telomerase." *Exp Gerontol* **39**(9):1379-1389. PubMed
de Magalhaes, J. P., and Toussaint, O. (2004a). "Telomeres and telomerase: a modern fountain of youth?" *Rejuvenation Res* **7**(2):126-133. PubMed
de Magalhaes, J. P., and Toussaint, O. (2004b). "How bioinformatics can help reverse engineer human aging." *Ageing Res Rev* **3**(2):125-141. PubMed
de Magalhaes, J. P. (2005a). "Open-minded scepticism: inferring the causal mechanisms of human ageing from genetic perturbations." *Ageing Res Rev* **4**(1):1-22. PubMed
de Magalhaes, J. P. (2005b). "Human disease-associated mitochondrial mutations fixed in nonhuman primates." *J Mol Evol* **61**(4):491-497. PubMed
de Magalhaes, J. P., Cabral, J. A., and Magalhaes, D. (2005). "The influence of genes on the aging process of mice: a statistical assessment of the genetics of aging." *Genetics* **169**(1):265-274. PubMed
de Magalhaes, J. P., and Church, G. M. (2005). "Genomes optimize reproduction: aging as a consequence of the developmental program." *Physiology (Bethesda)* **20**:252-259. PubMed
de Magalhaes, J. P., and Sandberg, A. (2005). "Cognitive aging as an extension of brain development: A model linking learning, brain plasticity, and neurodegeneration." *Mech Ageing Dev* **126**(10):1026-1033. PubMed
de Magalhaes, J. P. (2006). "Species selection in comparative studies of aging and antiaging research." In: *Handbook of Models for Human Aging*, Conn, P. M. (ed.).Â Elsevier Academic Press, Burlington, MA, 9-20.
de Magalhaes, J. P., and Church, G. M. (2006). "Cells discover fire: employing reactive oxygen species in development and consequences for aging." *Exp Gerontol* **41**(1):1-10. PubMed
de Magalhaes, J. P., and Church, G. M. (2007). "Analyses of human-chimpanzee orthologous gene pairs to explore evolutionary hypotheses of aging." *Mech Ageing Dev* **128**(5-6):355-364. PubMed
de Magalhaes, J. P., Costa, J., and Church, G. M. (2007a). "An analysis of the relationship between metabolism, developmental schedules, and longevity using phylogenetic independent contrasts." *J Gerontol A Biol Sci Med Sci* **62**(2):149-160. PubMed
de Magalhaes, J. P., Sedivy, J. M., Finch, C. E., Austad, S. N., and Church, G. M. (2007b). "A proposal to sequence genomes of unique interest for research on aging." *J Gerontol A Biol Sci Med Sci* **62**(6):583-584. PubMed
de Magalhaes, J. P., and Faragher, R. G. (2008). "Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity." *Bioessays* **30**(6):567-578. PubMed
de Magalhaes, J. P. (2009). "Aging Research in the Post-Genome Era: New Technologies for an Old Problem." In: *Redox Metabolism and Longevity Relationships in Animals and Plants*, Foyer, C. H., Faragher, R. and Thornalley, P. J. (ed.).Â Taylor and Francis, New York and Abingdon, 99-115.
de Magalhaes, J. P., and Costa, J. (2009). "A database of vertebrate longevity records and their relation to other life-history traits." *J Evol Biol* **22**(8):1770-1774. PubMed
de Magalhaes, J. P., Budovsky, A., Lehmann, G., Costa, J., Li, Y., Fraifeld, V., and Church, G. M. (2009a). "The Human Ageing Genomic Resources: online databases and tools for biogerontologists." *Aging Cell* **8**(1):65-72. PubMed
de Magalhaes, J. P., Curado, J., and Church, G. M. (2009b). "Meta-analysis of age-related gene expression profiles identifies common signatures of aging." *Bioinformatics* **25**(7):875-881. PubMed
de Magalhaes, J. P., Finch, C. E., and Janssens, G. (2010). "Next-generation sequencing in aging research: emerging applications, problems, pitfalls and possible solutions." *Ageing Res Rev* **9**(3):315-323. PubMed
de Magalhaes, J. P. (2011). "The biology of ageing: A primer." In: *An Introduction to Gerontology*, Stuart-Hamilton, I. (ed.).Â Cambridge University Press, Cambridge, 21-47.
de Magalhaes, J. P., Wuttke, D., Wood, S. H., Plank, M., and Vora, C. (2012). "Genome-environment interactions that modulate aging: powerful targets for drug discovery." *Pharmacol Rev* **64**(1):88-101. PubMed
Decary, S., Mouly, V., Hamida, C. B., Sautet, A., Barbet, J. P., and Butler-Browne, G. S. (1997). "Replicative potential and telomere length in human skeletal muscle: implications for satellite cell-mediated gene therapy." *Hum Gene Ther* **8**(12):1429-1438. PubMed
Dekker, P., Maier, A. B., van Heemst, D., de Koning-Treurniet, C., Blom, J., Dirks, R. W., Tanke, H. J., and Westendorp, R. G. (2009). "Stress-induced responses of human skin fibroblasts in vitro reflect human longevity." *Aging Cell* **8**(5):595-603. PubMed
Deng, Y., Chan, S. S., and Chang, S. (2008). "Telomere dysfunction and tumour suppression: the senescence connection." *Nat Rev Cancer* **8**(6):450-458. PubMed
Di Mitri, D., Azevedo, R. I., Henson, S. M., Libri, V., Riddell, N. E., Macaulay, R., Kipling, D., Soares, M. V., Battistini, L., and Akbar, A. N. (2011). "Reversible senescence in human CD4+CD45RA+CD27- memory T cells." *J Immunol* **187**(5):2093-2100. PubMed
Dierick, Frippiat, Salmon, Chainiaux, and Toussaint (2003). "Cells, stress and tissue ageing." In: *Biology of Aging and its Modulation*, Rattan, S. I. S. (ed.).Â Kluwer, Amsterdam, 101-125.
Dillin, A., Crawford, D. K., and Kenyon, C. (2002). "Timing requirements for insulin/IGF-1 signaling in C. elegans." *Science* **298**(5594):830-834. PubMed
DiMasi, J. A., Feldman, L., Seckler, A., and Wilson, A. (2010). "Trends in risks associated with new drug development: success rates for investigational drugs." *Clin Pharmacol Ther* **87**(3):272-277. PubMed
DiMauro, S., Tanji, K., Bonilla, E., Pallotti, F., and Schon, E. A. (2002). "Mitochondrial abnormalities in muscle and other aging cells: classification, causes, and effects." *Muscle Nerve* **26**(5):597-607. PubMed
DiMauro, S., and Schon, E. A. (2003). "Mitochondrial respiratory-chain diseases." *N Engl J Med* **348**(26):2656-2668. PubMed

Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E. E., Linskens, M., Rubelj, I., Pereira-Smith, O., *et al.* (1995). "A biomarker that identifies senescent human cells in culture and in aging skin in vivo." *Proc Natl Acad Sci U S A* **92**(20):9363-9367. PubMed
Dirks, A. J., and Leeuwenburgh, C. (2006). "Caloric restriction in humans: potential pitfalls and health concerns." *Mech Ageing Dev* **127**(1):1-7. PubMed
Dolle, M. E., Giese, H., Hopkins, C. L., Martus, H. J., Hausdorff, J. M., and Vijg, J. (1997). "Rapid accumulation of genome rearrangements in liver but not in brain of old mice." *Nat Genet* **17**(4):431-434. PubMed
Dolle, M. E., and Vijg, J. (2002). "Genome dynamics in aging mice." *Genome Res* **12**(11):1732-1738. PubMed
Dominguez, L. J., Barbagallo, M., and Morley, J. E. (2009). "Anti-aging medicine: pitfalls and hopes." *Aging Male* **12**(1):13-20. PubMed
Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., and Bradley, A. (1992). "Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours." *Nature* **356**(6366):215-221. PubMed
Donehower, L. A. (2002). "Does p53 affect organismal aging?" *J Cell Physiol* **192**(1):23-33. PubMed
Drexler, E. K. (1986). *Engines of Creation*. Anchor Press, Garden City.
Ducray, C., Pommier, J. P., Martins, L., Boussin, F. D., and Sabatier, L. (1999). "Telomere dynamics, end-to-end fusions and telomerase activation during the human fibroblast immortalization process." *Oncogene* **18**(29):4211-4223. PubMed
Duffy, P. H., Feuers, R., Nakamura, K. D., Leakey, J., and Hart, R. W. (1990). "Effect of chronic caloric restriction on the synchronization of various physiological measures in old female Fischer 344 rats." *Chronobiol Int* **7**(2):113-124. PubMed
Dulic, V., Beney, G. E., Frebourg, G., Drullinger, L. F., and Stein, G. H. (2000). "Uncoupling between phenotypic senescence and cell cycle arrest in aging p21-deficient fibroblasts." *Mol Cell Biol* **20**(18):6741-6754. PubMed
Dumont, P., Burton, M., Chen, Q. M., Gonos, E. S., Frippiat, C., Mazarati, J. B., Eliaers, F., Remacle, J., and Toussaint, O. (2000a). "Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast." *Free Radical Biol Med* **28**(3):361-373. PubMed
Dumont, P., Balbeur, L., Remacle, J., and Toussaint, O. (2000b). "Appearance of biomarkers of in vitro ageing after successive stimulation of WI-38 fibroblasts with IL-1alpha and TNF-alpha: senescence associated beta-galactosidase activity and morphotype transition." *J Anat* **197 Pt 4**:529-537. PubMed
Dunham, M. A., Neumann, A. A., Fasching, C. L., and Reddel, R. R. (2000). "Telomere maintenance by recombination in human cells." *Nat Genet* **26**(4):447-450. PubMed
Dyson, N., Howley, P. M., Munger, K., and Harlow, E. (1989). "The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product." *Science* **243**(4893):934-937. PubMed
Ebert, T. A., and Southon, J. R. (2003). " Red sea urchins (Strongylocentrotus franciscanus) can live over 100 years: confirmation with A-bomb 14carbon." *Fish Bull* **101**(4):915-922.
Edamatsu, R., Mori, A., and Packer, L. (1995). "The spin-trap N-tert-alpha-phenyl-butylnitrone prolongs the life span of the senescence accelerated mouse." *Biochem Biophys Res Commun* **211**(3):847-849. PubMed
Effros, R. B. (1996). "Insights on immunological aging derived from the T lymphocyte cellular senescence model." *Exp Gerontol* **31**(1-2):21-27. PubMed
Effros, R. B. (2009). "Kleemeier Award Lecture 2008--the canary in the coal mine: telomeres and human healthspan." *J Gerontol A Biol Sci Med Sci* **64**(5):511-515. PubMed
Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., and Cawthon, R. M. (2004). "Accelerated telomere shortening in response to life stress." *Proc Natl Acad Sci U S A* **101**(49):17312-17315. PubMed
Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., and Gage, F. H. (1998). "Neurogenesis in the adult human hippocampus." *Nat Med* **4**(11):1313-1317. PubMed
Eriksson, M., Brown, W. T., Gordon, L. B., Glynn, M. W., Singer, J., Scott, L., Erdos, M. R., Robbins, C. M., Moses, T. Y., Berglund, P., *et al.* (2003). "Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome." *Nature* **423**(6937):293-298. PubMed
Esposito, D., Fassina, G., Szabo, P., De Angelis, P., Rodgers, L., Weksler, M., and Siniscalco, M. (1989). "Chromosomes of older humans are more prone to aminopterine-induced breakage." *Proc Natl Acad Sci U S A* **86**(4):1302-1306. PubMed
Failla, G. (1958). "The aging process and cancerogenesis." *Ann N Y Acad Sci* **71**(6):1124-1140. PubMed
Farrelly, C. (2010). "Why aging research? The moral imperative to retard human aging." *Ann N Y Acad Sci* **1197**:1-8. PubMed
Fearon, E. R. (1997). "Human cancer syndromes: clues to the origin and nature of cancer." *Science* **278**(5340):1043-1050. PubMed
Feng, J., Funk, W. D., Wang, S. S., Weinrich, S. L., Avilion, A. A., Chiu, C. P., Adams, R. R., Chang, E., Allsopp, R. C., Yu, J., *et al.* (1995). "The RNA component of human telomerase." *Science* **269**(5228):1236-1241. PubMed
Ferguson, B. A., Dreisbach, T. A., Parks, C. G., Filip, G. M., and Schmitt, C. L. (2003). "Coarse-scale population structure of pathogenic Armillaria species in a mixed-conifer forest in the Blue Mountains of northeast Oregon." *Can J For Res* **33**(4):612-623.
Finch, C. E. (1976). "The regulation of physiological changes during mammalian aging." *Q Rev Biol* **51**(1):49-83. PubMed
Finch, C. E. (1990). *Longevity, Senescence, and the Genome*. The University of Chicago Press, Chicago and London.
Finch, C. E., and Pike, M. C. (1996). "Maximum life span predictions from the Gompertz mortality model." *J Gerontol A Biol Sci Med Sci* **51**(3):B183-194. PubMed
Fleming, J. E., Reveillaud, I., and Niedzwiecki, A. (1992). "Role of oxidative stress in Drosophila aging." *Mutat Res* **275**(3-6):267-279. PubMed
Flurkey, K., Papaconstantinou, J., Miller, R. A., and Harrison, D. E. (2001). "Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production." *Proc Natl Acad Sci U S A* **98**(12):6736-6741. PubMed
Font, E., Desfilis, E., Perez-Canellas, M. M., and Garcia-Verdugo, J. M. (2001). "Neurogenesis and neuronal regeneration in the adult reptilian brain." *Brain Behav Evol* **58**(5):276-295. PubMed
Fontana, L., Meyer, T. E., Klein, S., and Holloszy, J. O. (2004). "Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans." *Proc Natl Acad Sci U S A* **101**(17):6659-6663. PubMed
Fontana, L., and Klein, S. (2007). "Aging, adiposity, and calorie restriction." *Jama* **297**(9):986-994. PubMed
Fontana, L., Partridge, L., and Longo, V. D. (2010). "Extending healthy life span--from yeast to humans." *Science* **328**(5976):321-326. PubMed
Forster, M. J., Morris, P., and Sohal, R. S. (2003). "Genotype and age influence the effect of caloric intake on mortality in mice." *Faseb J* **17**(6):690-692. PubMed
Fossel, M. (1996). *Reversing Human Aging*. William Morrow and Company, New York.
Franceschi, C., Mondello, C., Bonafe, M., Valensin, S., Sansoni, P., and Sorbi, S. (1999). "Long telomeres and well preserved proliferative vigor in cells from centenarians: a contribution to longevity?" *Aging (Milano)* **11**(2):69-72. PubMed
Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., and De Benedictis, G. (2000). "Inflamm-aging. An evolutionary perspective on immunosenescence." *Ann N Y Acad Sci* **908**:244-254. PubMed
Freitas, R. (1999). *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience, Georgetown.
Freitas, R. (2003). *Nanomedicine, Volume IIA: Biocompatibility*. Landes Bioscience, Georgetown.
Freitas, A. A., and de Magalhaes, J. P. (2011). "A review and appraisal of the DNA damage theory of ageing." *Mutat Res* **728**(1-2):12-22. PubMed
Freitas, A. A., Vasieva, O., and de Magalhaes, J. P. (2011). "A data mining approach for classifying DNA repair genes into ageing-related or non-ageing-related." *BMC Genomics* **12**:27. PubMed
Freund, A., Orjalo, A. V., Desprez, P. Y., and Campisi, J. (2010). "Inflammatory networks during cellular senescence: causes and consequences." *Trends Mol Med* **16**(5):238-246. PubMed
Friguet, B., Bulteau, A. L., Chondrogianni, N., Conconi, M., and Petropoulos, I. (2000). "Protein degradation by the proteasome and its implications in aging." *Ann N Y Acad Sci* **908**:143-154. PubMed
Froy, O., and Miskin, R. (2007). "The interrelations among feeding, circadian rhythms and ageing." *Prog Neurobiol* **82**(3):142-150. PubMed
Fry, M. (2002). "The Werner syndrome helicase-nuclease--one protein, many mysteries." *Sci Aging Knowledge Environ* **2002**(13):re2. PubMed
Fujioka, K., Greenway, F., Sheard, J., and Ying, Y. (2006). "The effects of grapefruit on weight and insulin resistance: relationship to the metabolic syndrome." *J Med Food* **9**(1):49-54. PubMed
Fukuchi, K., Martin, G. M., and Monnat, R. J., Jr. (1989). "Mutator phenotype of Werner syndrome is characterized by extensive deletions." *Proc Natl Acad Sci U S A* **86**(15):5893-5897. PubMed
Funk, W. D., Wang, C. K., Shelton, D. N., Harley, C. B., Pagon, G. D., and Hoeffler, W. K. (2000). "Telomerase expression restores dermal integrity to in vitro-aged fibroblasts in a reconstituted skin model." *Exp Cell Res* **258**(2):270-278. PubMed
Gallo, R. C., and Montagnier, L. (2003). "The discovery of HIV as the cause of AIDS." *N Engl J Med* **349**(24):2283-2285. PubMed
Garcia, A. M., Busuttil, R. A., Calder, R. B., Dolle, M. E., Diaz, V., McMahan, C. A., Bartke, A., Nelson, J., Reddick, R., and Vijg, J. (2008). "Effect of Ames dwarfism and caloric restriction on spontaneous DNA mutation frequency in different mouse tissues." *Mech Ageing Dev* **129**(9):528-533. PubMed
Gardner, E. M. (2005). "Caloric restriction decreases survival of aged mice in response to primary influenza infection." *J Gerontol A Biol Sci Med Sci* **60**(6):688-694. PubMed
Gardner, M. P., Gems, D., and Viney, M. E. (2006). "Extraordinary plasticity in aging in Strongyloides ratti implies a gene-regulatory mechanism of lifespan evolution." *Aging Cell* **5**(4):315-323. PubMed
Gavrilov, L. A., and Gavrilova, N. S. (2001). "The reliability theory of aging and longevity." *J Theor Biol* **213**(4):527-545. PubMed
Gavrilov, L. A., and Gavrilova, N. S. (2002). "Evolutionary theories of aging and longevity." *ScientificWorldJournal* **2**:339-356. PubMed
Gebhart, E., Bauer, R., Raub, U., Schinzel, M., Ruprecht, K. W., and Jonas, J. B. (1988). "Spontaneous and induced chromosomal instability in Werner syndrome." *Hum Genet* **80**(2):135-139. PubMed
Geiger, H., and Van Zant, G. (2002). "The aging of lympho-hematopoietic stem cells." *Nat Immunol* **3**(4):329-333. PubMed
Gems, D. (2011). "Tragedy and delight: the ethics of decelerated ageing." *Philos Trans R Soc Lond B Biol Sci* **366**(1561):108-112. PubMed
Gensler, H. L., and Bernstein, H. (1981). "DNA damage as the primary cause of aging." *Q Rev Biol* **56**(3):279-303. PubMed
George, J. C., Bada, J., WZeh, J., Brown, S. E., O'Hara, T., and Suydam, R. (1999). "Age and growth estimates of bowhead whales (Balaena mysticetus) via aspartic acid racemization." *Can J Zool* **77**:571-580.
Gerland, L. M., Peyrol, S., Lallemand, C., Branche, R., Magaud, J. P., and Ffrench, M. (2003). "Association of increased autophagic inclusions labeled for beta-galactosidase with fibroblastic aging." *Exp Gerontol* **38**(8):887-895. PubMed
Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P., and Fenn, W. O. (1954). "Oxygen poisoning and x-irradiation: a mechanism in common." *Science* **119**(3097):623-626. PubMed

Gershon, H., and Gershon, D. (1970). "Detection of inactive enzyme molecules in ageing organisms." *Nature* **227**(5264):1214-1217. PubMed
Gershon, H., and Gershon, D. (2000a). "Paradigms in aging research: a critical review and assessment." *Mech Ageing Dev* **117**(1-3):21-28. PubMed
Gershon, H., and Gershon, D. (2000b). "The budding yeast, Saccharomyces cerevisiae, as a model for aging research: a critical review." *Mech Ageing Dev* **120**(1-3):1-22. PubMed
Giacomoni, P. U., Declercq, L., Hellemans, L., and Maes, D. (2000). "Aging of human skin: review of a mechanistic model and first experimental data." *IUBMB Life* **49**(4):259-263. PubMed
Going, J. J., Stuart, R. C., Downie, M., Fletcher-Monaghan, A. J., and Keith, W. N. (2002). "'Senescence-associated' beta-galactosidase activity in the upper gastrointestinal tract." *J Pathol* **196**(4):394-400. PubMed
Goldsmith, T. C. (2004). "Aging as an evolved characteristic - Weismann's theory reconsidered." *Med Hypotheses* **62**(2):304-308. PubMed
Goldstein, S. (1974). "Aging in vitro. Growth of cultured cells from the Galapagos tortoise." *Exp Cell Res* **83**(2):297-302. PubMed
Goldstein, S. (1990). "Replicative senescence: the human fibroblast comes of age." *Science* **249**(4973):1129-1133. PubMed
Gomes, N. M., Ryder, O. A., Houck, M. L., Charter, S. J., Walker, W., Forsyth, N. R., Austad, S. N., Venditti, C., Pagel, M., Shay, J. W., *et al.* (2011). "Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination." *Aging Cell* **10**(5):761-768. PubMed
Gonzalez-Suarez, E., Samper, E., Flores, J. M., and Blasco, M. A. (2000). "Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis." *Nat Genet* **26**(1):114-117. PubMed
Gonzalez-Suarez, E., Samper, E., Ramirez, A., Flores, J. M., Martin-Caballero, J., Jorcano, J. L., and Blasco, M. A. (2001). "Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes." *Embo J* **20**(11):2619-2630. PubMed
Goodrich, D. W., and Lee, W. H. (1993). "Molecular characterization of the retinoblastoma susceptibility gene." *Biochim Biophys Acta* **1155**(1):43-61. PubMed
Gopnik, A., Meltzoff, A., and Kuhl, P. (2000). *The Scientist in the Crib: What Early Learning Tells Us About the Mind*. HarperCollins, New York.
Gosden, R. (1996). *Cheating Time*. W. H. Freeman & Company, New York.
Goto, M. (1997). "Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing." *Mech Ageing Dev* **98**(3):239-254. PubMed
Gould, E., Reeves, A. J., Graziano, M. S., and Gross, C. G. (1999). "Neurogenesis in the neocortex of adult primates." *Science* **286**(5439):548-552. PubMed
Gray, M. D., Shen, J. C., Kamath-Loeb, A. S., Blank, A., Sopher, B. L., Martin, G. M., Oshima, J., and Loeb, L. A. (1997). "The Werner syndrome protein is a DNA helicase." *Nat Genet* **17**(1):100-103. PubMed
Greenberg, J. A., and Boozer, C. N. (2000). "Metabolic mass, metabolic rate, caloric restriction, and aging in male Fischer 344 rats." *Mech Ageing Dev* **113**(1):37-48. PubMed
Greider, C. W., and Blackburn, E. H. (1985). "Identification of a specific telomere terminal transferase activity in Tetrahymena extracts." *Cell* **43**(2 Pt 1):405-413. PubMed
Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H., and de Lange, T. (1999). "Mammalian telomeres end in a large duplex loop." *Cell* **97**(4):503-514. PubMed
Grossman, C. J. (1984). "Regulation of the immune system by sex steroids." *Endocr Rev* **5**(3):435-455. PubMed
Grossman, T. (2005). "Latest advances in antiaging medicine." *Keio J Med* **54**(2):85-94. PubMed
Grube, K., and Burkle, A. (1992). "Poly(ADP-ribose) polymerase activity in mononuclear leukocytes of 13 mammalian species correlates with species-specific life span." *Proc Natl Acad Sci U S A* **89**(24):11759-11763. PubMed
Guevara-Aguirre, J., Balasubramanian, P., Guevara-Aguirre, M., Wei, M., Madia, F., Cheng, C. W., Hwang, D., Martin-Montalvo, A., Saavedra, J., Ingles, S., *et al.* (2011). "Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans." *Sci Transl Med* **3**(70):70ra13. PubMed
Gurdon, J. B., Laskey, R. A., and Reeves, O. R. (1975). "The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs." *J Embryol Exp Morphol* **34**(1):93-112. PubMed
Hahn, W. C. (2003). "Role of telomeres and telomerase in the pathogenesis of human cancer." *J Clin Oncol* **21**(10):2034-2043. PubMed
Haigh, R., and Bagaric, M. (2002). "Immortality and sentencing law." *The Journal of Philosophy, Science & Law* **2**
Hamad, N. M., Elconin, J. H., Karnoub, A. E., Bai, W., Rich, J. N., Abraham, R. T., Der, C. J., and Counter, C. M. (2002). "Distinct requirements for Ras oncogenesis in human versus mouse cells." *Genes Dev* **16**(16):2045-2057. PubMed
Hamilton, W. D. (1966). "The moulding of senescence by natural selection." *J Theor Biol* **12**(1):12-45. PubMed
Hamilton, J. B., and Mestler, G. E. (1969). "Mortality and survival: comparison of eunuchs with intact men and women in a mentally retarded population." *J Gerontol* **24**(4):395-411. PubMed
Hamilton, M. L., Van Remmen, H., Drake, J. A., Yang, H., Guo, Z. M., Kewitt, K., Walter, C. A., and Richardson, A. (2001). "Does oxidative damage to DNA increase with age?" *Proc Natl Acad Sci U S A* **98**(18):10469-10474. PubMed
Hamilton, B., Dong, Y., Shindo, M., Liu, W., Odell, I., Ruvkun, G., and Lee, S. S. (2005). "A systematic RNAi screen for longevity genes in C. elegans." *Genes Dev* **19**(13):1544-1555. PubMed
Hammerman, M. R. (1987). "Insulin-like growth factors and aging." *Endocrinol Metab Clin North Am* **16**(4):995-1011. PubMed
Hande, M. P., Samper, E., Lansdorp, P., and Blasco, M. A. (1999). "Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice." *J Cell Biol* **144**(4):589-601. PubMed
Hara, E., Tsurui, H., Shinozaki, A., Nakada, S., and Oda, K. (1991). "Cooperative effect of antisense-Rb and antisense-p53 oligomers on the extension of life span in human diploid fibroblasts, TIG-1." *Biochem Biophys Res Commun* **179**(1):528-534. PubMed
Hara, E., Smith, R., Parry, D., Tahara, H., Stone, S., and Peters, G. (1996). "Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence." *Mol Cell Biol* **16**(3):859-867. PubMed
Harada, T., Tokuriki, M., and Tanioka, Y. (1999). "Age-related changes in the brainstem auditory evoked potentials of the marmoset." *Hear Res* **128**(1-2):119-124. PubMed
Hari, R., Burde, V., and Arking, R. (1998). "Immunological confirmation of elevated levels of CuZn superoxide dismutase protein in an artificially selected long-lived strain of Drosophila melanogaster." *Exp Gerontol* **33**(3):227-237. PubMed
Harle-Bachor, C., and Boukamp, P. (1996). "Telomerase activity in the regenerative basal layer of the epidermis inhuman skin and in immortal and carcinoma-derived skin keratinocytes." *Proc Natl Acad Sci U S A* **93**(13):6476-6481. PubMed
Harley, C. B., Pollard, J. W., Chamberlain, J. W., Stanners, C. P., and Goldstein, S. (1980). "Protein synthetic errors do not increase during aging of cultured human fibroblasts." *Proc Natl Acad Sci U S A* **77**(4):1885-1889. PubMed
Harley, C. B., Futcher, A. B., and Greider, C. W. (1990). "Telomeres shorten during ageing of human fibroblasts." *Nature* **345**(6274):458-460. PubMed
Harley, C. B., Liu, W., Blasco, M., Vera, E., Andrews, W. H., Briggs, L. A., and Raffaele, J. M. (2011). "A natural product telomerase activator as part of a health maintenance program." *Rejuvenation Res* **14**(1):45-56. PubMed
Harman, D. (1956). "Aging: a theory based on free radical and radiation chemistry." *J Gerontol* **11**(3):298-300. PubMed
Harman, D. (1968). "Free radical theory of aging: effect of free radical reaction inhibitors on the mortality rate of male LAF mice." *J Gerontol* **23**(4):476-482. PubMed
Harman, D. (1972). "The biologic clock: the mitochondria?" *J Am Geriatr Soc* **20**(4):145-147. PubMed
Harman, D. (1981). "The aging process." *Proc Natl Acad Sci U S A* **78**(11):7124-7128. PubMed
Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993). "The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases." *Cell* **75**(4):805-816. PubMed
Harper, J. W., Elledge, S. J., Keyomarsi, K., Dynlacht, B., Tsai, L. H., Zhang, P., Dobrowolski, S., Bai, C., Connell-Crowley, L., Swindell, E., *et al.* (1995). "Inhibition of cyclin-dependent kinases by p21." *Mol Biol Cell* **6**(4):387-400. PubMed
Harper, J. M., Leathers, C. W., and Austad, S. N. (2006). "Does caloric restriction extend life in wild mice?" *Aging Cell* **5**(6):441-449. PubMed
Harper, J. M., Salmon, A. B., Leiser, S. F., Galecki, A. T., and Miller, R. A. (2007). "Skin-derived fibroblasts from long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor rotenone." *Aging Cell* **6**(1):1-13. PubMed
Harper, J. M., Wang, M., Galecki, A. T., Ro, J., Williams, J. B., and Miller, R. A. (2011). "Fibroblasts from long-lived bird species are resistant to multiple forms of stress." *J Exp Biol* **214**(Pt 11):1902-1910. PubMed
Harris, J. (2004). "Immortal ethics." *Ann N Y Acad Sci* **1019**:527-534. PubMed
Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., Nadon, N. L., Wilkinson, J. E., Frenkel, K., Carter, C. S., *et al.* (2009). "Rapamycin fed late in life extends lifespan in genetically heterogeneous mice." *Nature* **460**(7253):392-395. PubMed
Hart, R. W., and Setlow, R. B. (1974). "Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species." *Proc Natl Acad Sci U S A* **71**(6):2169-2173. PubMed
Harvey, P. H., and Zammuto, R. M. (1985). "Patterns of mortality and age at first reproduction in natural populations of mammals." *Nature* **315**(6017):319-320. PubMed
Haseltine, W. A. (2004). "An interview with William A. Haseltine." *Rejuvenation Res* **7**(3):229-231. PubMed
Hastie, N. D., Dempster, M., Dunlop, M. G., Thompson, A. M., Green, D. K., and Allshire, R. C. (1990). "Telomere reduction in human colorectal carcinoma and with ageing." *Nature* **346**(6287):866-868. PubMed
Hasty, J., McMillen, D., and Collins, J. J. (2002). "Engineered gene circuits." *Nature* **420**(6912):224-230. PubMed
Hasty, P., Campisi, J., Hoeijmakers, J., van Steeg, H., and Vijg, J. (2003). "Aging and genome maintenance: lessons from the mouse?" *Science* **299**(5611):1355-1359. PubMed
Hasty, P., and Vijg, J. (2004). "Accelerating aging by mouse reverse genetics: a rational approach to understanding longevity." *Aging Cell* **3**(2):55-65. PubMed
Haussmann, M. F., Winkler, D. W., O'Reilly, K. M., Huntington, C. E., Nisbet, I. C., and Vleck, C. M. (2003). "Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones." *Proc R Soc Lond B Biol Sci* **270**(1522):1387-1392. PubMed
Hayashi, J., Ohta, S., Kagawa, Y., Kondo, H., Kaneda, H., Yonekawa, H., Takai, D., and Miyabayashi, S. (1994). "Nuclear but not mitochondrial genome involvement in human age-related mitochondrial dysfunction. Functional integrity of mitochondrial DNA from aged subjects." *J Biol Chem* **269**(9):6878-6883. PubMed
Hayflick, L., and Moorhead, P. S. (1961). "The serial cultivation of human diploid cell strains." *Exp Cell Res* **25**:585-621. PubMed
Hayflick, L. (1985). "The cell biology of aging." *Clin Geriatr Med* **1**(1):15-27. PubMed
Hayflick, L. (1994). *How and Why We Age*. Ballantine Books, New York.
Hayflick, L. (2000). "The future of ageing." *Nature* **408**(6809):267-269. PubMed
Hayflick, L. (2004). ""Anti-aging" is an oxymoron." *J Gerontol A Biol Sci Med Sci* **59**(6):B573-578. PubMed

Hedges, S. B. (2002). "The origin and evolution of model organisms." *Nat Rev Genet* **3**(11):838-849. PubMed
Heidrick, M. L., Hendricks, L. C., and Cook, D. E. (1984). "Effect of dietary 2-mercaptoethanol on the life span, immune system, tumor incidence and lipid peroxidation damage in spleen lymphocytes of aging BC3F1 mice." *Mech Ageing Dev* **27**(3):341-358. PubMed
Heilbronn, L. K., de Jonge, L., Frisard, M. I., DeLany, J. P., Larson-Meyer, D. E., Rood, J., Nguyen, T., Martin, C. K., Volaufova, J., Most, M. M., *et al.* (2006). "Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial." *Jama* **295**(13):1539-1548. PubMed
Heinemann, M., and Panke, S. (2006). "Synthetic biology--putting engineering into biology." *Bioinformatics* **22**(22):2790-2799. PubMed
Hemann, M. T., and Greider, C. W. (2000). "Wild-derived inbred mouse strains have short telomeres." *Nucleic Acids Res* **28**(22):4474-4478. PubMed
Hemann, M. T., Strong, M. A., Hao, L. Y., and Greider, C. W. (2001). "The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability." *Cell* **107**(1):67-77. PubMed
Henning, K. A., Li, L., Iyer, N., McDaniel, L. D., Reagan, M. S., Legerski, R., Schultz, R. A., Stefanini, M., Lehmann, A. R., Mayne, L. V., *et al.* (1995). "The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH." *Cell* **82**(4):555-564. PubMed
Herbig, U., Ferreira, M., Condel, L., Carey, D., and Sedivy, J. M. (2006). "Cellular senescence in aging primates." *Science* **311**(5765):1257. PubMed
Herranz, D., MuÃ±oz-Martin, M., CaÃ±amero, M., Mulero, F., Martinez-Pastor, B., Fernandez-Capetillo, O., and Serrano, M. (2010). "Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer." *Nat Commun* **1**:3.
Herwig, S., and Strauss, M. (1997). "The retinoblastoma protein: a master regulator of cell cycle, differentiation and apoptosis." *Eur J Biochem* **246**(3):581-601. PubMed
Hill, K., Boesch, C., Goodall, J., Pusey, A., Williams, J., and Wrangham, R. (2001). "Mortality rates among wild chimpanzees." *J Hum Evol* **40**(5):437-450. PubMed
Hjelmborg, v. J., Iachine, I., Skytthe, A., Vaupel, J. W., McGue, M., Koskenvuo, M., Kaprio, J., Pedersen, N. L., and Christensen, K. (2006). "Genetic influence on human lifespan and longevity." *Hum Genet* **119**(3):312-321. PubMed
Ho, K. Y., Evans, W. S., Blizzard, R. M., Veldhuis, J. D., Merriam, G. R., Samojlik, E., Furlanetto, R., Rogol, A. D., Kaiser, D. L., and Thorner, M. O. (1987). "Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations." *J Clin Endocrinol Metab* **64**(1):51-58. PubMed
Ho, Y. S., Magnenat, J. L., Bronson, R. T., Cao, J., Gargano, M., Sugawara, M., and Funk, C. D. (1997). "Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia." *J Biol Chem* **272**(26):16644-16651. PubMed
Hoeijmakers, J. H. (2009). "DNA damage, aging, and cancer." *N Engl J Med* **361**(15):1475-1485. PubMed
Holliday, R. (1984). "The biological significance of meiosis." *Symp Soc Exp Biol* **38**:381-394. PubMed
Holliday, R. (1995). *Understanding Ageing*. Cambridge University Press, Cambridge.
Holliday, R. (2004). "The multiple and irreversible causes of aging." *J Gerontol A Biol Sci Med Sci* **59**(6):B568-572. PubMed
Holliday, R. (2009). "The extreme arrogance of anti-aging medicine." *Biogerontology* **10**(2):223-228. PubMed
Holloszy, J. O., and Smith, E. K. (1986). "Longevity of cold-exposed rats: a reevaluation of the "rate-of-living theory"." *J Appl Physiol* **61**(5):1656-1660. PubMed
Holloszy, J. O., and Schechtman, K. B. (1991). "Interaction between exercise and food restriction: effects on longevity of male rats." *J Appl Physiol* **70**(4):1529-1535. PubMed
Holloszy, J. O. (1997). "Mortality rate and longevity of food-restricted exercising male rats: a reevaluation." *J Appl Physiol* **82**(2):399-403. PubMed
Holloszy, J. O. (1998). "Longevity of exercising male rats: effect of an antioxidant supplemented diet." *Mech Ageing Dev* **100**(3):211-219. PubMed
Holloszy, J. O. (2000). "The biology of aging." *Mayo Clin Proc* **75 Suppl**:S3-8; discussion S8-9. PubMed
Holmes, D. J., and Austad, S. N. (1995). "Birds as animal models for the comparative biology of aging: a prospectus." *J Gerontol A Biol Sci Med Sci* **50**(2):B59-66. PubMed
Holmes, D. J., Fluckiger, R., and Austad, S. N. (2001). "Comparative biology of aging in birds: an update." *Exp Gerontol* **36**(4-6):869-883. PubMed
Holmes, D. J., and Ottinger, M. A. (2003). "Birds as long-lived animal models for the study of aging." *Exp Gerontol* **38**(11-12):1365-1375. PubMed
Hopkin, K. (2003). "Dietary drawbacks." *Sci Aging Knowledge Environ* **2003**(8):NS4. PubMed
Horikoshi, T., Balin, A. K., and Carter, D. M. (1986). "Effect of oxygen on the growth of human epidermal keratinocytes." *J Invest Dermatol* **86**(4):424-427. PubMed
Horikoshi, T., Balin, A. K., and Carter, D. M. (1991). "Effects of oxygen tension on the growth and pigmentation of normal human melanocytes." *J Invest Dermatol* **96**(6):841-844. PubMed
Horwitz, E. M., Prockop, D. J., Fitzpatrick, L. A., Koo, W. W., Gordon, P. L., Neel, M., Sussman, M., Orchard, P., Marx, J. C., Pyeritz, R. E., *et al.* (1999). "Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta." *Nat Med* **5**(3):309-313. PubMed
Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung, P., Kisielewski, A., Zhang, L. L., *et al.* (2003). "Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan." *Nature* **425**(6954):191-196. PubMed
Hsin, H., and Kenyon, C. (1999). "Signals from the reproductive system regulate the lifespan of C. elegans." *Nature* **399**(6734):362-366. PubMed
Huang, S., Li, B., Gray, M. D., Oshima, J., Mian, I. S., and Campisi, J. (1998). "The premature ageing syndrome protein, WRN, is a 3'-->5' exonuclease." *Nat Genet* **20**(2):114-116. PubMed
Huang, T. T., Carlson, E. J., Gillespie, A. M., Shi, Y., and Epstein, C. J. (2000). "Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice." *J Gerontol A Biol Sci Med Sci* **55**(1):B5-9. PubMed
Hughes, K. A., Alipaz, J. A., Drnevich, J. M., and Reynolds, R. M. (2002). "A test of evolutionary theories of aging." *Proc Natl Acad Sci U S A* **99**(22):14286-14291. PubMed
Hunt, J., Jennions, M. D., Spyrou, N., and Brooks, R. (2006). "Artificial selection on male longevity influences age-dependent reproductive effort in the black field cricket Teleogryllus commodus." *Am Nat* **168**(3):E72-86. PubMed
Hussussian, C. J., Struewing, J. P., Goldstein, A. M., Higgins, P. A., Ally, D. S., Sheahan, M. D., Clark, W. H., Jr., Tucker, M. A., and Dracopoli, N. C. (1994). "Germline p16 mutations in familial melanoma." *Nat Genet* **8**(1):15-21. PubMed
Hwang, S. J., Lozano, G., Amos, C. I., and Strong, L. C. (2003). "Germline p53 mutations in a cohort with childhood sarcoma: sex differences in cancer risk." *Am J Hum Genet* **72**(4):975-983. PubMed
Hwang, W. S., Ryu, Y. J., Park, J. H., Park, E. S., Lee, E. G., Koo, J. M., Jeon, H. Y., Lee, B. C., Kang, S. K., Kim, S. J., *et al.* (2004). "Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst." *Science* **303**(5664):1669-1674. PubMed
Hwang, W. S., Roh, S. I., Lee, B. C., Kang, S. K., Kwon, D. K., Kim, S., Kim, S. J., Park, S. W., Kwon, H. S., Lee, C. K., *et al.* (2005). "Patient-specific embryonic stem cells derived from human SCNT blastocysts." *Science* **308**(5729):1777-1783. PubMed
Hyun, M., Lee, J., Lee, K., May, A., Bohr, V. A., and Ahn, B. (2008). "Longevity and resistance to stress correlate with DNA repair capacity in Caenorhabditis elegans." *Nucleic Acids Res* **36**(4):1380-1389. PubMed
Ingram, D. K., Roth, G. S., Lane, M. A., Ottinger, M. A., Zou, S., de Cabo, R., and Mattison, J. A. (2006). "The potential for dietary restriction to increase longevity in humans: extrapolation from monkey studies." *Biogerontology* **7**(3):143-148. PubMed
Inoue, S., Koya-Miyata, S., Ushio, S., Iwaki, K., Ikeda, M., and Kurimoto, M. (2003). "Royal Jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage." *Exp Gerontol* **38**(9):965-969. PubMed
Iwama, H., Ohyashiki, K., Ohyashiki, J. H., Hayashi, S., Yahata, N., Ando, K., Toyama, K., Hoshika, A., Takasaki, M., Mori, M., *et al.* (1998). "Telomeric length and telomerase activity vary with age in peripheral blood cells obtained from normal individuals." *Hum Genet* **102**(4):397-402. PubMed
Ja, W. W., West, A. P., Jr., Delker, S. L., Bjorkman, P. J., Benzer, S., and Roberts, R. W. (2007). "Extension of Drosophila melanogaster life span with a GPCR peptide inhibitor." *Nat Chem Biol* **3**(7):415-419. PubMed
Jahng, J. W., Kim, J. G., Kim, H. J., Kim, B. T., Kang, D. W., and Lee, J. H. (2007). "Chronic food restriction in young rats results in depression- and anxiety-like behaviors with decreased expression of serotonin reuptake transporter." *Brain Res* **1150**:100-107. PubMed
Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., *et al.* (1997). "Cancer chemopreventive activity of resveratrol, a natural product derived from grapes." *Science* **275**(5297):218-220. PubMed
Janzen, D. H. (1976). "Why bamboos wait so long to flower." *Ann Rev Ecol Sys* **7**:347-391.
Jaskelioff, M., Muller, F. L., Paik, J. H., Thomas, E., Jiang, S., Adams, A. C., Sahin, E., Kost-Alimova, M., Protopopov, A., Cadinanos, J., *et al.* (2011). "Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice." *Nature* **469**(7328):102-106. PubMed
Jazwinski, S. M. (1990). "Aging and senescence of the budding yeast Saccharomyces cerevisiae." *Mol Microbiol* **4**(3):337-343. PubMed
Jazwinski, S. M. (2001). "New clues to old yeast." *Mech Ageing Dev* **122**(9):865-882. PubMed
Jazwinski, S. M. (2005). "Yeast longevity and aging--the mitochondrial connection." *Mech Ageing Dev* **126**(2):243-248. PubMed
Jena, B. S., Nayak, S. B., and Patnaik, B. K. (2002). "Age-related effect of aluminium on the catalase activities of the brains of two species of poikilothermic vertebrates." *Gerontology* **48**(1):34-38. PubMed
Jeyapalan, J. C., and Sedivy, J. M. (2008). "Cellular senescence and organismal aging." *Mech Ageing Dev* **129**(7-8):467-474. PubMed
Jiang, X. R., Jimenez, G., Chang, E., Frolkis, M., Kusler, B., Sage, M., Beeche, M., Bodnar, A. G., Wahl, G. M., Tlsty, T. D., *et al.* (1999). "Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype." *Nat Genet* **21**(1):111-114. PubMed
Johnson, T. E., Mitchell, D. H., Kline, S., Kemal, R., and Foy, J. (1984). "Arresting development arrests aging in the nematode Caenorhabditis elegans." *Mech Ageing Dev* **28**(1):23-40. PubMed
Johnson, T. E. (1990). "Increased life-span of age-1 mutants in Caenorhabditis elegans and lower Gompertz rate of aging." *Science* **249**(4971):908-912. PubMed
Johnson, F. B., Sinclair, D. A., and Guarente, L. (1999). "Molecular biology of aging." *Cell* **96**(2):291-302. PubMed
Johnson, T. E. (2002). "A personal retrospective on the genetics of aging." *Biogerontology* **3**(1-2):7-12. PubMed
Jolly, C. A., Muthukumar, A., Avula, C. P., Troyer, D., and Fernandes, G. (2001). "Life span is prolonged in food-restricted autoimmune-prone (NZB x NZW)F(1) mice fed a diet enriched with (n-3) fatty acids." *J Nutr* **131**(10):2753-2760. PubMed
Juhasz, G., Erdi, B., Sass, M., and Neufeld, T. P. (2007). "Atg7-dependent autophagy promotes neuronal health, stress tolerance, and longevity but is dispensable for metamorphosis in Drosophila." *Genes Dev* **21**(23):3061-3066. PubMed
Jurgens, K. D., and Prothero, J. (1987). "Scaling of maximal lifespan in bats." *Comp Biochem Physiol A* **88**(2):361-367. PubMed
Kakuo, S., Asaoka, K., and Ide, T. (1999). "Human is a unique species among primates in terms of telomere length." *Biochem Biophys Res Commun* **263**(2):308-314. PubMed

Kamada, Y., Yoshino, K., Kondo, C., Kawamata, T., Oshiro, N., Yonezawa, K., and Ohsumi, Y. (2010). "Tor directly controls the Atg1 kinase complex to regulate autophagy." *Mol Cell Biol* **30**(4):1049-1058. PubMed
Kammori, M., Nakamura, K. I., Kawahara, M., Mimura, Y., Kaminishi, M., and Takubo, K. (2002). "Telomere shortening with aging in human thyroid and parathyroid tissue." *Exp Gerontol* **37**(4):513-521. PubMed
Kanungo, M. S. (1994). *Genes and Aging*. Cambridge University Press, Cambridge, UK.
Kapahi, P., Boulton, M. E., and Kirkwood, T. B. (1999). "Positive correlation between mammalian life span and cellular resistance to stress." *Free Radic Biol Med* **26**(5-6):495-500. PubMed
Kaplan, H. S., and Robson, A. J. (2002). "The emergence of humans: the coevolution of intelligence and longevity with intergenerational transfers." *Proc Natl Acad Sci U S A* **99**(15):10221-10226. PubMed
Kara, T. C. (1994). "Ageing in amphibians." *Gerontology* **40**(2-4):161-173. PubMed
Karakashian, S. J., Lanners, H. N., and Rudzinska, M. A. (1984). "Cellular and clonal aging in the suctorian protozoan Tokophrya infusionum." *Mech Ageing Dev* **26**(2-3):217-229. PubMed
Karasek, M. (2004). "Melatonin, human aging, and age-related diseases." *Exp Gerontol* **39**(11-12):1723-1729. PubMed
Karatza, C., Stein, W. D., and Shall, S. (1984). "Kinetics of in vitro ageing of mouse embryo fibroblasts." *J Cell Sci* **65**:163-175. PubMed
Karlseder, J., Broccoli, D., Dai, Y., Hardy, S., and de Lange, T. (1999). "p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2." *Science* **283**(5406):1321-1325. PubMed
Karlseder, J., Smogorzewska, A., and de Lange, T. (2002). "Senescence induced by altered telomere state, not telomere loss." *Science* **295**(5564):2446-2449. PubMed
Kay, M. A. (2011). "State-of-the-art gene-based therapies: the road ahead." *Nat Rev Genet* **12**(5):316-328. PubMed
Kealy, R. D., Lawler, D. F., Ballam, J. M., Mantz, S. L., Biery, D. N., Greeley, E. H., Lust, G., Segre, M., Smith, G. K., and Stowe, H. D. (2002). "Effects of diet restriction on life span and age-related changes in dogs." *J Am Vet Med Assoc* **220**(9):1315-1320. PubMed
Keaney, M., and Gems, D. (2003). "No increase in lifespan in Caenorhabditis elegans upon treatment with the superoxide dismutase mimetic EUK-8." *Free Radic Biol Med* **34**(2):277-282. PubMed
Keaney, M., Matthijssens, F., Sharpe, M., Vanfleteren, J., and Gems, D. (2004). "Superoxide dismutase mimetics elevate superoxide dismutase activity in vivo but do not retard aging in the nematode Caenorhabditis elegans." *Free Radic Biol Med* **37**(2):239-250. PubMed
Kenyon, C. J. (2010). "The genetics of ageing." *Nature* **464**(7288):504-512. PubMed
Keyes, W. M., Wu, Y., Vogel, H., Guo, X., Lowe, S. W., and Mills, A. A. (2005). "p63 deficiency activates a program of cellular senescence and leads to accelerated aging." *Genes Dev* **19**(17):1986-1999. PubMed
Khaidakov, M., Heflich, R. H., Manjanatha, M. G., Myers, M. B., and Aidoo, A. (2003). "Accumulation of point mutations in mitochondrial DNA of aging mice." *Mutat Res* **526**(1-2):1-7. PubMed
Khansari, D. N., and Gustad, T. (1991). "Effects of long-term, low-dose growth hormone therapy on immune function and life expectancy of mice." *Mech Ageing Dev* **57**(1):87-100. PubMed
Khaw, K. T., Wareham, N., Bingham, S., Welch, A., Luben, R., and Day, N. (2008). "Combined impact of health behaviours and mortality in men and women: the EPIC-Norfolk prospective population study." *PLoS Med* **5**(1):e12. PubMed
Kiecolt-Glaser, J. K., Preacher, K. J., MacCallum, R. C., Atkinson, C., Malarkey, W. B., and Glaser, R. (2003). "Chronic stress and age-related increases in the proinflammatory cytokine IL-6." *Proc Natl Acad Sci U S A* **100**(15):9090-9095. PubMed
Kilian, A., Bowtell, D. D., Abud, H. E., Hime, G. R., Venter, D. J., Keese, P. K., Duncan, E. L., Reddel, R. R., and Jefferson, R. A. (1997). "Isolation of a candidate human telomerase catalytic subunit gene, which reveals complex splicing patterns in different cell types." *Hum Mol Genet* **6**(12):2011-2019. PubMed
Kill, I. R., Faragher, R. G., Lawrence, K., and Shall, S. (1994). "The expression of proliferation-dependent antigens during the lifespan of normal and progeroid human fibroblasts in culture." *J Cell Sci* **107 (Pt 2)**:571-579. PubMed
Kim, H., You, S., Farris, J., Kong, B. W., Christman, S. A., Foster, L. K., and Foster, D. N. (2002). "Expression profiles of p53-, p16(INK4a)-, and telomere-regulating genes in replicative senescent primary human, mouse, and chicken fibroblast cells." *Exp Cell Res* **272**(2):199-208. PubMed
Kim, E. B., Fang, X., Fushan, A. A., Huang, Z., Lobanov, A. V., Han, L., Marino, S. M., Sun, X., Turanov, A. A., Yang, P., *et al.* (2011). "Genome sequencing reveals insights into physiology and longevity of the naked mole rat." *Nature* **479**(7372):223-227. PubMed
King, D. E., Mainous, A. G., 3rd, and Geesey, M. E. (2007). "Turning back the clock: adopting a healthy lifestyle in middle age." *Am J Med* **120**(7):598-603. PubMed
Kipling, D., and Cooke, H. J. (1990). "Hypervariable ultra-long telomeres in mice." *Nature* **347**(6291):400-402. PubMed
Kirkwood, T. B. (1977). "Evolution of ageing." *Nature* **270**(5635):301-304. PubMed
Kirkwood, T. B., and Austad, S. N. (2000). "Why do we age?" *Nature* **408**(6809):233-238. PubMed
Kirkwood, T. B. (2005). "Understanding the odd science of aging." *Cell* **120**(4):437-447. PubMed
Kitano, H. (2002). "Computational systems biology." *Nature* **420**(6912):206-210. PubMed
Kiviniemi, T. O., Saraste, A., Toikka, J. O., Saraste, M., Raitakari, O. T., Parkka, J. P., Lehtimaki, T., Hartiala, J. J., Viikari, J., and Koskenvuo, J. W. (2007). "A moderate dose of red wine, but not de-alcoholized red wine increases coronary flow reserve." *AtherosclerosisÂ* PubMed
Kiyono, T., Foster, S. A., Koop, J. I., McDougall, J. K., Galloway, D. A., and Klingelhutz, A. J. (1998). "Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells." *Nature* **396**(6706):84-88. PubMed
Klapper, W., Kuhne, K., Singh, K. K., Heidorn, K., Parwaresch, R., and Krupp, G. (1998). "Longevity of lobsters is linked to ubiquitous telomerase expression." *FEBS Lett* **439**(1-2):143-146. PubMed
Klass, M., and Hirsh, D. (1976). "Non-ageing developmental variant of Caenorhabditis elegans." *Nature* **260**(5551):523-525. PubMed
Kleiber, M. (1975). "Metabolic turnover rate: a physiological meaning of the metabolic rate per unit body weight." *J Theor Biol* **53**(1):199-204. PubMed
Knight, S., Vulliamy, T., Copplestone, A., Gluckman, E., Mason, P., and Dokal, I. (1998). "Dyskeratosis Congenita (DC) Registry: identification of new features of DC." *Br J Haematol* **103**(4):990-996. PubMed
Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., and Nathan, D. M. (2002). "Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin." *N Engl J Med* **346**(6):393-403. PubMed
Ko, L. J., and Prives, C. (1996). "p53: puzzle and paradigm." *Genes Dev* **10**(9):1054-1072. PubMed
Kohn, D. B., Bauer, G., Rice, C. R., Rothschild, J. C., Carbonaro, D. A., Valdez, P., Hao, Q., Zhou, C., Bahner, I., Kearns, K., *et al.* (1999). "A clinical trial of retroviral-mediated transfer of a rev-responsive element decoy gene into CD34(+) cells from the bone marrow of human immunodeficiency virus-1-infected children." *Blood* **94**(1):368-371. PubMed
Kondratov, R. V. (2007). "A role of the circadian system and circadian proteins in aging." *Ageing Res Rev* **6**(1):12-27. PubMed
Kopp, P. (1998). "Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'?" *Eur J Endocrinol* **138**(6):619-620. PubMed
Kowald, A., and Kirkwood, T. B. (1994). "Towards a network theory of ageing: a model combining the free radical theory and the protein error theory." *J Theor Biol* **168**(1):75-94. PubMed
Krause, D. S., Theise, N. D., Collector, M. I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S., and Sharkis, S. J. (2001). "Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell." *Cell* **105**(3):369-377. PubMed
Krishnamurthy, J., Ramsey, M. R., Ligon, K. L., Torrice, C., Koh, A., Bonner-Weir, S., and Sharpless, N. E. (2006). "p16INK4a induces an age-dependent decline in islet regenerative potential." *Nature* **443**(7110):453-457. PubMed
Krzisnik, C., Kolacio, Z., and Battelino, T. (1999). "The "Little People" of the island of Krk - Revisited. Etiology of hypopituitarism revealed." *J Endocr Genet* **1**(1):9-19.
Ku, H. H., Brunk, U. T., and Sohal, R. S. (1993). "Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species." *Free Radic Biol Med* **15**(6):621-627. PubMed
Kubota, C., Yamakuchi, H., Todoroki, J., Mizoshita, K., Tabara, N., Barber, M., and Yang, X. (2000). "Six cloned calves produced from adult fibroblast cells after long-term culture." *Proc Natl Acad Sci U S A* **97**(3):990-995. PubMed
Kuilman, T., Michaloglou, C., Vredeveld, L. C., Douma, S., van Doorn, R., Desmet, C. J., Aarden, L. A., Mooi, W. J., and Peeper, D. S. (2008). "Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network." *Cell* **133**(6):1019-1031. PubMed
Kujoth, G. C., Hiona, A., Pugh, T. D., Someya, S., Panzer, K., Wohlgemuth, S. E., Hofer, T., Seo, A. Y., Sullivan, R., Jobling, W. A., *et al.* (2005). "Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging." *Science* **309**(5733):481-484. PubMed
Kulju, K. S., and Lehman, J. M. (1995). "Increased p53 protein associated with aging in human diploid fibroblasts." *Exp Cell Res* **217**(2):336-345. PubMed
Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Ohyama, Y., Kurabayashi, M., Kaname, T., Kume, E., *et al.* (1997). "Mutation of the mouse klotho gene leads to a syndrome resembling ageing." *Nature* **390**(6655):45-51. PubMed
Kurosu, H., Yamamoto, M., Clark, J. D., Pastor, J. V., Nandi, A., Gurnani, P., McGuinness, O. P., Chikuda, H., Yamaguchi, M., Kawaguchi, H., *et al.* (2005). "Suppression of aging in mice by the hormone Klotho." *Science* **309**(5742):1829-1833. PubMed
Kuruvilla, F. G., Shamji, A. F., Sternson, S. M., Hergenrother, P. J., and Schreiber, S. L. (2002). "Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays." *Nature* **416**(6881):653-657. PubMed
Kurz, D. J., Decary, S., Hong, Y., and Erusalimsky, J. D. (2000). "Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells." *J Cell Sci* **113 (Pt 20)**:3613-3622. PubMed
Kurzweil, R., and Grossman, T. (2004). *Fantastic Voyage: Live Long Enough to Live Forever*. Rodale, Emmaus, PA.
Kwon, Y. W., Masutani, H., Nakamura, H., Ishii, Y., and Yodoi, J. (2003). "Redox regulation of cell growth and cell death." *Biol Chem* **384**(7):991-996. PubMed
Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., *et al.* (2006). "Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha." *Cell* **127**(6):1109-1122. PubMed
Lakdawalla, D. N., Sun, E. C., Jena, A. B., Reyes, C. M., Goldman, D. P., and Philipson, T. J. (2010). "An economic evaluation of the war on cancer." *J Health Econ* **29**(3):333-346. PubMed
Lakowski, B., and Hekimi, S. (1996). "Determination of life-span in Caenorhabditis elegans by four clock genes." *Science* **272**(5264):1010-1013. PubMed
Lambert, A. J., Boysen, H. M., Buckingham, J. A., Yang, T., Podlutsky, A., Austad, S. N., Kunz, T. H., Buffenstein, R., and Brand, M. D. (2007). "Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms." *Aging Cell* **6**(5):607-618. PubMed

Lane, I. W., and Comac, L. (1992). *Sharks Don't Get Cancer*. Avery Penguin Putnam, New York.
Lanner, R. M., and Connor, K. F. (2001). "Does bristlecone pine senesce?" *Exp Gerontol* **36**(4-6):675-685. PubMed
Lanza, R. P., Cibelli, J. B., and West, M. D. (1999). "Human therapeutic cloning." *Nat Med* **5**(9):975-977. PubMed
Lanza, R. P., Cibelli, J. B., Blackwell, C., Cristofalo, V. J., Francis, M. K., Baerlocher, G. M., Mak, J., Schertzer, M., Chavez, E. A., Sawyer, N., *et al.* (2000). "Extension of cell life-span and telomere length in animals cloned from senescent somatic cells." *Science* **288**(5466):665-669. PubMed
Lapasset, L., Milhavet, O., Prieur, A., Besnard, E., Babled, A., Ait-Hamou, N., Leschik, J., Pellestor, F., Ramirez, J. M., De Vos, J., *et al.* (2011). "Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state." *Genes Dev* **25**(21):2248-2253. PubMed
Lapointe, J., and Hekimi, S. (2010). "When a theory of aging ages badly." *Cell Mol Life Sci* **67**(1):1-8. PubMed
Laron, Z. (2005). "Do deficiencies in growth hormone and insulin-like growth factor-1 (IGF-1) shorten or prolong longevity?" *Mech Ageing Dev* **126**(2):305-307. PubMed
Larsen, P. L. (1993). "Aging and resistance to oxidative damage in Caenorhabditis elegans." *Proc Natl Acad Sci U S A* **90**(19):8905-8909. PubMed
Larsson, N. G. (2010). "Somatic mitochondrial DNA mutations in mammalian aging." *Annu Rev Biochem* **79**:683-706. PubMed
Le Bourg, E. (2001). "A mini-review of the evolutionary theories of aging. Is it the time to accept them?" *Demographic Research* **4**(1)
Leduc, P. R., Wong, M. S., Ferreira, P. M., Groff, R. E., Haslinger, K., Koonce, M. P., Lee, W. Y., Love, J. C., McCammon, J. A., Monteiro-Riviere, N. A., *et al.* (2007). "Towards an in vivo biologically inspired nanofactory." *Nat Nanotechnol* **2**(1):3-7. PubMed
Lee, H. W., Blasco, M. A., Gottlieb, G. J., Horner, J. W., 2nd, Greider, C. W., and DePinho, R. A. (1998). "Essential role of mouse telomerase in highly proliferative organs." *Nature* **392**(6676):569-574. PubMed
Lee, C. K., Klopp, R. G., Weindruch, R., and Prolla, T. A. (1999). "Gene expression profile of aging and its retardation by caloric restriction." *Science* **285**(5432):1390-1393. PubMed
Lee, R. D. (2003). "Rethinking the evolutionary theory of aging: transfers, not births, shape senescence in social species." *Proc Natl Acad Sci U S A* **100**(16):9637-9642. PubMed
Lee, S., and Schmitt, C. A. (2003). "Chemotherapy response and resistance." *Curr Opin Genet Dev* **13**(1):90-96. PubMed
Lee, J. W., Beebe, K., Nangle, L. A., Jang, J., Longo-Guess, C. M., Cook, S. A., Davisson, M. T., Sundberg, J. P., Schimmel, P., and Ackerman, S. L. (2006). "Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration." *Nature* **443**(7107):50-55. PubMed
Lees, E. (1995). "Cyclin dependent kinase regulation." *Curr Opin Cell Biol* **7**(6):773-780. PubMed
Li, Y., and de Magalhaes, J. P. (2012). "Accelerated protein evolution analysis reveals genes and pathways associated with the evolution of mammalian longevity." *Age (Dordr)*, in press.Â PubMed
Liang, H., Masoro, E. J., Nelson, J. F., Strong, R., McMahan, C. A., and Richardson, A. (2003). "Genetic mouse models of extended lifespan." *Exp Gerontol* **38**(11-12):1353-1364. PubMed
Liang, S., Mele, J., Wu, Y., Buffenstein, R., and Hornsby, P. J. (2010). "Resistance to experimental tumorigenesis in cells of a long-lived mammal, the naked mole-rat (Heterocephalus glaber)." *Aging Cell* **9**(4):626-635. PubMed
Lightowlers, R. N., Jacobs, H. T., and Kajander, O. A. (1999). "Mitochondrial DNA--all things bad?" *Trends Genet* **15**(3):91-93. PubMed
Lin, Y. J., Seroude, L., and Benzer, S. (1998). "Extended life-span and stress resistance in the Drosophila mutant methuselah." *Science* **282**(5390):943-946. PubMed
Lin, S. J., Defossez, P. A., and Guarente, L. (2000). "Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae." *Science* **289**(5487):2126-2128. PubMed
Lin, K., Hsin, H., Libina, N., and Kenyon, C. (2001). "Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling." *Nat Genet* **28**(2):139-145. PubMed
Lindsey, J., McGill, N. I., Lindsey, L. A., Green, D. K., and Cooke, H. J. (1991). "In vivo loss of telomeric repeats with age in humans." *Mutat Res* **256**(1):45-48. PubMed
Lindstedt, S. L., and Calder, W. A. (1981). "Body size, physiological time, and longevity of homeothermic animals." *Q Rev Biol* **56**(1):1-16.
Linke, S. P., Sengupta, S., Khabie, N., Jeffries, B. A., Buchhop, S., Miska, S., Henning, W., Pedeux, R., Wang, X. W., Hofseth, L. J., *et al.* (2003). "p53 interacts with hRAD51 and hRAD54, and directly modulates homologous recombination." *Cancer Res* **63**(10):2596-2605. PubMed
Linnane, A. W., Marzuki, S., Ozawa, T., and Tanaka, M. (1989). "Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases." *Lancet* **1**(8639):642-645. PubMed
Lio, D., Scola, L., Crivello, A., Colonna-Romano, G., Candore, G., Bonafe, M., Cavallone, L., Marchegiani, F., Olivieri, F., Franceschi, C., *et al.* (2003). "Inflammation, genetics, and longevity: further studies on the protective effects in men of IL-10 -1082 promoter SNP and its interaction with TNF-alpha -308 promoter SNP." *J Med Genet* **40**(4):296-299. PubMed
Lipman, R. D., Smith, D. E., Bronson, R. T., and Blumberg, J. (1995). "Is late-life caloric restriction beneficial?" *Aging (Milano)* **7**(2):136-139. PubMed
Lipman, R. D., Bronson, R. T., Wu, D., Smith, D. E., Prior, R., Cao, G., Han, S. N., Martin, K. R., Meydani, S. N., and Meydani, M. (1998). "Disease incidence and longevity are unaltered by dietary antioxidant supplementation initiated during middle age in C57BL/6 mice." *Mech Ageing Dev* **103**(3):269-284. PubMed
Liu, V. W., Zhang, C., and Nagley, P. (1998). "Mutations in mitochondrial DNA accumulate differentially in three different human tissues during ageing." *Nucleic Acids Res* **26**(5):1268-1275. PubMed
Liu, S., Qu, Y., Stewart, T. J., Howard, M. J., Chakrabortty, S., Holekamp, T. F., and McDonald, J. W. (2000). "Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation." *Proc Natl Acad Sci U S A* **97**(11):6126-6131. PubMed
Liu, X., Jiang, N., Hughes, B., Bigras, E., Shoubridge, E., and Hekimi, S. (2005). "Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice." *Genes Dev* **19**(20):2424-2434. PubMed
Liu, H., Bravata, D. M., Olkin, I., Nayak, S., Roberts, B., Garber, A. M., and Hoffman, A. R. (2007). "Systematic review: the safety and efficacy of growth hormone in the healthy elderly." *Ann Intern Med* **146**(2):104-115. PubMed
Longo, V. D. (1999). "Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells." *Neurobiol Aging* **20**(5):479-486. PubMed
Longo, V. D., and Fabrizio, P. (2002). "Regulation of longevity and stress resistance: a molecular strategy conserved from yeast to humans?" *Cell Mol Life Sci* **59**(6):903-908. PubMed
Longo, V. D., Mitteldorf, J., and Skulachev, V. P. (2005). "Programmed and altruistic ageing." *Nat Rev Genet* **6**(11):866-872. PubMed
Lorenzini, A., Tresini, M., Austad, S. N., and Cristofalo, V. J. (2005). "Cellular replicative capacity correlates primarily with species body mass not longevity." *Mech Ageing Dev* **126**(10):1130-1133. PubMed
Lowell, J. E., and Pillus, L. (1998). "Telomere tales: chromatin, telomerase and telomere function in Saccharomyces cerevisiae." *Cell Mol Life Sci* **54**(1):32-49. PubMed
Lu, T., Pan, Y., Kao, S. Y., Li, C., Kohane, I., Chan, J., and Yankner, B. A. (2004). "Gene regulation and DNA damage in the ageing human brain." *Nature* **429**(6994):883-891. PubMed
Lucke, J. C., Diedrichs, P. C., Partridge, B., and Hall, W. D. (2009). "Anticipating the anti-ageing pill. Lessons from the history of the oral contraceptive pill and hormone replacement therapy." *EMBO Rep* **10**(2):108-113. PubMed
Luckinbill, L. S., and Clare, M. J. (1985). "Selection for life span in Drosophila melanogaster." *Heredity* **55 (Pt 1)**:9-18. PubMed
Lundblad, V., and Szostak, J. W. (1989). "A mutant with a defect in telomere elongation leads to senescence in yeast." *Cell* **57**(4):633-643. PubMed
Lutz, P. L., Prentice, H. M., and Milton, S. L. (2003). "Is turtle longevity linked to enhanced mechanisms for surviving brain anoxia and reoxygenation?" *Exp Gerontol* **38**(7):797-800. PubMed
Ly, D. H., Lockhart, D. J., Lerner, R. A., and Schultz, P. G. (2000). "Mitotic misregulation and human aging." *Science* **287**(5462):2486-2492. PubMed
Lyman, C. P., O'Brien, R. C., Greene, G. C., and Papafrangos, E. D. (1981). "Hibernation and longevity in the Turkish hamster Mesocricetus brandti." *Science* **212**(4495):668-670. PubMed
Lyon, J., and Gorner, P. (1995). *Altered Fates: Gene Therapy and the Retooling of Human Life*. W. W. Norton, New York.
MacArthur, R. H., and Wilson, E. O. (1967). *The Theory of Island Biogeography*. Princeton University Press, Princeton.
Macera-Bloch, L., Houghton, J., Lenahan, M., Jha, K. K., and Ozer, H. L. (2002). "Termination of lifespan of SV40-transformed human fibroblasts in crisis is due to apoptosis." *J Cell Physiol* **190**(3):332-344. PubMed
Maddison, D. R., and Schulz, K. S. (2004). "The Tree of Life Web Project." http://tolweb.org
Magalhaes, J. P. (1999). "Post-mortem lethal genes." *Longevity Report* (69):6.
Majhi, S., Jena, B. S., and Patnaik, B. K. (2000). "Effect of age on lipid peroxides, lipofuscin and ascorbic acid contents of the lungs of male garden lizard." *Comp Biochem Physiol C Toxicol Pharmacol* **126**(3):293-298. PubMed
Makarov, V. L., Hirose, Y., and Langmore, J. P. (1997). "Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening." *Cell* **88**(5):657-666. PubMed
Mangel, M., and Abrahams, M. V. (2001). "Age and longevity in fish, with consideration of the ferox trout." *Exp Gerontol* **36**(4-6):765-790. PubMed
Mantell, L. L., and Greider, C. W. (1994). "Telomerase activity in germline and embryonic cells of Xenopus." *Embo J* **13**(13):3211-3217. PubMed
Marden, J. H., Rogina, B., Montooth, K. L., and Helfand, S. L. (2003). "Conditional tradeoffs between aging and organismal performance of Indy long-lived mutant flies." *Proc Natl Acad Sci U S A* **100**(6):3369-3373. PubMed
Marrone, A., and Mason, P. J. (2003). "Dyskeratosis congenita." *Cell Mol Life Sci* **60**(3):507-517. PubMed
Marsili, S., Salganik, R. I., Albright, C. D., Freel, C. D., Johnsen, S., Peiffer, R. L., and Costello, M. J. (2004). "Cataract formation in a strain of rats selected for high oxidative stress." *Exp Eye Res* **79**(5):595-612. PubMed
Martens, U. M., Chavez, E. A., Poon, S. S., Schmoor, C., and Lansdorp, P. M. (2000). "Accumulation of short telomeres in human fibroblasts prior to replicative senescence." *Exp Cell Res* **256**(1):291-299. PubMed
Martin, G. M. (1978). "Genetic syndromes in man with potential relevance to the pathobiology of aging." *Birth Defects Orig Artic Ser* **14**(1):5-39. PubMed
Martin, G. M. (1982). "Syndromes of accelerated aging." *Natl Cancer Inst Monogr* **60**:241-247. PubMed
Martin, G. M., Smith, A. C., Ketterer, D. J., Ogburn, C. E., and Disteche, C. M. (1985). "Increased chromosomal aberrations in first metaphases of cells isolated from the kidneys of aged mice." *Isr J Med Sci* **21**(3):296-301. PubMed
Martin, G. M., Austad, S. N., and Johnson, T. E. (1996). "Genetic analysis of ageing: role of oxidative damage and environmental stresses." *Nat Genet* **13**(1):25-34. PubMed
Martin, G. M., and Oshima, J. (2000). "Lessons from human progeroid syndromes." *Nature* **408**(6809):263-266. PubMed

Martin, K., Potten, C. S., and Kirkwood, T. B. (2000). "Age-related changes in irradiation-induced apoptosis and expression of p21 and p53 in crypt stem cells of murine intestine." *Ann N Y Acad Sci* **908**:315-318. PubMed
Martin, J. A., and Buckwalter, J. A. (2002). "Human chondrocyte senescence and osteoarthritis." *Biorheology* **39**(1-2):145-152. PubMed
Martinez, D. E. (1998). "Mortality patterns suggest lack of senescence in hydra." *Exp Gerontol* **33**(3):217-225. PubMed
Mason, P. J. (2003). "Stem cells, telomerase and dyskeratosis congenita." *Bioessays* **25**(2):126-133. PubMed
Mason, J. B., Cargill, S. L., Anderson, G. B., and Carey, J. R. (2009). "Transplantation of young ovaries to old mice increased life span in transplant recipients." *J Gerontol A Biol Sci Med Sci* **64**(12):1207-1211. PubMed
Masoro, E. J., McCarter, R. J., Katz, M. S., and McMahan, C. A. (1992). "Dietary restriction alters characteristics of glucose fuel use." *J Gerontol* **47**(6):B202-208. PubMed
Masoro, E. J. (2005). "Overview of caloric restriction and ageing." *Mech Ageing Dev* **126**(9):913-922. PubMed
Masutomi, K., Yu, E. Y., Khurts, S., Ben-Porath, I., Currier, J. L., Metz, G. B., Brooks, M. W., Kaneko, S., Murakami, S., DeCaprio, J. A., *et al.* (2003). "Telomerase maintains telomere structure in normal human cells." *Cell* **114**(2):241-253. PubMed
Matheu, A., Pantoja, C., Efeyan, A., Criado, L. M., Martin-Caballero, J., Flores, J. M., Klatt, P., and Serrano, M. (2004). "Increased gene dosage of Ink4a/Arf results in cancer resistance and normal aging." *Genes Dev* **18**(22):2736-2746. PubMed
Matheu, A., Maraver, A., Klatt, P., Flores, I., Garcia-Cao, I., Borras, C., Flores, J. M., Vina, J., Blasco, M. A., and Serrano, M. (2007). "Delayed ageing through damage protection by the Arf/p53 pathway." *Nature* **448**(7151):375-379. PubMed
Mathon, N. F., and Lloyd, A. C. (2001). "Cell senescence and cancer." *Nat Rev Cancer* **1**(3):203-213. PubMed
Mathon, N. F., Malcolm, D. S., Harrisingh, M. C., Cheng, L., and Lloyd, A. C. (2001). "Lack of replicative senescence in normal rodent glia." *Science* **291**(5505):872-875. PubMed
Matsumura, T. (1980). "Multinucleation and polyploidization of aging human cells in culture." *Adv Exp Med Biol* **129**:31-38. PubMed
Matsuura, S., Tauchi, H., Nakamura, A., Kondo, N., Sakamoto, S., Endo, S., Smeets, D., Solder, B., Belohradsky, B. H., Der Kaloustian, V. M., *et al.* (1998). "Positional cloning of the gene for Nijmegen breakage syndrome." *Nat Genet* **19**(2):179-181. PubMed
Mattson, M. P., Duan, W., and Maswood, N. (2002). "How does the brain control lifespan?" *Ageing Res Rev* **1**(2):155-165. PubMed
Mattson, M. P., Cutler, R. G., and Camandola, S. (2007). "Energy intake and amyotrophic lateral sclerosis." *Neuromolecular Med* **9**(1):17-20. PubMed
McCarter, R. J., and Palmer, J. (1992). "Energy metabolism and aging: a lifelong study of Fischer 344 rats." *Am J Physiol* **263**(3 Pt 1):E448-452. PubMed
McCay, C. M., Crowell, M. F., and Maynard, L. A. (1935). "The effect of retarded growth upon length of the life span and upon the ultimate body size." *J Nutrit* **10**(1):63-75.
McClintock, B. (1941). "The stability of broken ends of chromosomes in Zea mays." *Genetics* **26**:234-282.
McConnell, B. B., Starborg, M., Brookes, S., and Peters, G. (1998). "Inhibitors of cyclin-dependent kinases induce features of replicative senescence in early passage human diploid fibroblasts." *Curr Biol* **8**(6):351-354. PubMed
McCormick, A., and Campisi, J. (1991). "Cellular aging and senescence." *Curr Opin Cell Biol* **3**(2):230-234. PubMed
McEachern, M. J., and Blackburn, E. H. (1996). "Cap-prevented recombination between terminal telomeric repeat arrays (telomere CPR) maintains telomeres in Kluyveromyces lactis lacking telomerase." *Genes Dev* **10**(14):1822-1834. PubMed
McElwee, J. J., Schuster, E., Blanc, E., Piper, M. D., Thomas, J. H., Patel, D. S., Selman, C., Withers, D. J., Thornton, J. M., Partridge, L., *et al.* (2007). "Evolutionary conservation of regulated longevity assurance mechanisms." *Genome Biol* **8**(7):R132. PubMed
McKinney, M. L., and McNamara, K. J. (1991). *Heterochrony: The Evolution of Ontogeny*. Plenum, New York.
Medawar, P. B. (1952). *An Unsolved Problem of Biology*. H. K. Lewis, London.
Medawar, P. (1955). "The definition and measurement of senescence." In: *Ciba Foundation Colloquia on Ageing, Volume I*, Wolstenholme, G. E. W. (ed.).Â J. & A. Churchill, London, 4-15.
Medema, R. H., Herrera, R. E., Lam, F., and Weinberg, R. A. (1995). "Growth suppression by p16ink4 requires functional retinoblastoma protein." *Proc Natl Acad Sci U S A* **92**(14):6289-6293. PubMed
Medvedev, Z. A. (1981). "On the immortality of the germ line: genetic and biochemical mechanism. A review." *Mech Ageing Dev* **17**(4):331-359. PubMed
Medvedev, Z. A. (1990). "An attempt at a rational classification of theories of ageing." *Biol Rev Camb Philos Soc* **65**(3):375-398. PubMed
Melk, A., Kittikowit, W., Sandhu, I., Halloran, K. M., Grimm, P., Schmidt, B. M., and Halloran, P. F. (2003). "Cell senescence in rat kidneys in vivo increases with growth and age despite lack of telomere shortening." *Kidney Int* **63**(6):2134-2143. PubMed
Melov, S., Ravenscroft, J., Malik, S., Gill, M. S., Walker, D. W., Clayton, P. E., Wallace, D. C., Malfroy, B., Doctrow, S. R., and Lithgow, G. J. (2000). "Extension of life-span with superoxide dismutase/catalase mimetics." *Science* **289**(5484):1567-1569. PubMed
Mendez, M. V., Stanley, A., Phillips, T., Murphy, M., Menzoian, J. O., and Park, H. Y. (1998). "Fibroblasts cultured from distal lower extremities in patients with venous reflux display cellular characteristics of senescence." *J Vasc Surg* **28**(6):1040-1050. PubMed
Meyer, T. E., Kovacs, S. J., Ehsani, A. A., Klein, S., Holloszy, J. O., and Fontana, L. (2006). "Long-term caloric restriction ameliorates the decline in diastolic function in humans." *J Am Coll Cardiol* **47**(2):398-402. PubMed
Meyne, J., Ratliff, R. L., and Moyzis, R. K. (1989). "Conservation of the human telomere sequence (TTAGGG)n among vertebrates." *Proc Natl Acad Sci U S A* **86**(18):7049-7053. PubMed
Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P. P., Lanfrancone, L., and Pelicci, P. G. (1999). "The p66shc adaptor protein controls oxidative stress response and life span in mammals." *Nature* **402**(6759):309-313. PubMed
Miller, S. E., and Hadfield, M. G. (1990). "Developmental arrest during larval life and life-span extension in a marine mollusc." *Science* **248**(4953):356-358.
Miller, R. A. (1999). "Kleemeier award lecture: are there genes for aging?" *J Gerontol A Biol Sci Med Sci* **54**(7):B297-307. PubMed
Miller, J. K. (2001). "Escaping senescence: demographic data from the three-toed box turtle (Terrapene carolina triunguis)." *Exp Gerontol* **36**(4-6):829-832. PubMed
Miller, R. A., Harper, J. M., Galecki, A., and Burke, D. T. (2002a). "Big mice die young: early life body weight predicts longevity in genetically heterogeneous mice." *Aging Cell* **1**(1):22-29. PubMed
Miller, R. A., Harper, J. M., Dysko, R. C., Durkee, S. J., and Austad, S. N. (2002b). "Longer life spans and delayed maturation in wild-derived mice." *Exp Biol Med (Maywood)* **227**(7):500-508. PubMed
Miller, R. A. (2005). "Genetic approaches to the study of aging." *J Am Geriatr Soc* **53**(9 Suppl):S284-286. PubMed
Miller, R. A., Harrison, D. E., Astle, C. M., Baur, J. A., Boyd, A. R., de Cabo, R., Fernandez, E., Flurkey, K., Javors, M. A., Nelson, J. F., *et al.* (2011). "Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice." *J Gerontol A Biol Sci Med Sci* **66**(2):191-201. PubMed
Milne, J. C., Lambert, P. D., Schenk, S., Carney, D. P., Smith, J. J., Gagne, D. J., Jin, L., Boss, O., Perni, R. B., Vu, C. B., *et al.* (2007). "Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes." *Nature* **450**(7170):712-716. PubMed
Minamino, T., Miyauchi, H., Yoshida, T., Ishida, Y., Yoshida, H., and Komuro, I. (2002). "Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction." *Circulation* **105**(13):1541-1544. PubMed
Minamino, T., Yoshida, T., Tateno, K., Miyauchi, H., Zou, Y., Toko, H., and Komuro, I. (2003). "Ras induces vascular smooth muscle cell senescence and inflammation in human atherosclerosis." *Circulation* **108**(18):2264-2269. PubMed
Miskin, R., and Masos, T. (1997). "Transgenic mice overexpressing urokinase-type plasminogen activator in the brain exhibit reduced food consumption, body weight and size, and increased longevity." *J Gerontol A Biol Sci Med Sci* **52**(2):B118-124. PubMed
Mitsui, A., Hamuro, J., Nakamura, H., Kondo, N., Hirabayashi, Y., Ishizaki-Koizumi, S., Hirakawa, T., Inoue, T., and Yodoi, J. (2002). "Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span." *Antioxid Redox Signal* **4**(4):693-696. PubMed
Miwa, S., Riyahi, K., Partridge, L., and Brand, M. D. (2004). "Lack of correlation between mitochondrial reactive oxygen species production and life span in Drosophila." *Ann N Y Acad Sci* **1019**:388-391. PubMed
Mizutani, K., Ikeda, K., and Yamori, Y. (2000). "Resveratrol inhibits AGEs-induced proliferation and collagen synthesis activity in vascular smooth muscle cells from stroke-prone spontaneously hypertensive rats." *Biochem Biophys Res Commun* **274**(1):61-67. PubMed
Mobbs, C. V. (2004). "Not wisely but too well: aging as a cost of neuroendocrine activity." *Sci Aging Knowledge Environ* **2004**(35):pe33. PubMed
Mokni, M., Elkahoui, S., Limam, F., Amri, M., and Aouani, E. (2007). "Effect of resveratrol on antioxidant enzyme activities in the brain of healthy rat." *Neurochem Res* **32**(6):981-987. PubMed
Molofsky, A. V., Slutsky, S. G., Joseph, N. M., He, S., Pardal, R., Krishnamurthy, J., Sharpless, N. E., and Morrison, S. J. (2006). "Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing." *Nature* **443**(7110):448-452. PubMed
Mondello, C., Petropoulou, C., Monti, D., Gonos, E. S., Franceschi, C., and Nuzzo, F. (1999). "Telomere length in fibroblasts and blood cells from healthy centenarians." *Exp Cell Res* **248**(1):234-242. PubMed
Monroe, K. R., Murphy, S. P., Kolonel, L. N., and Pike, M. C. (2007). "Prospective study of grapefruit intake and risk of breast cancer in postmenopausal women: the Multiethnic Cohort Study." *Br J Cancer* **97**(3):440-445. PubMed
Moore, J. (1981). "Asexual reproduction and environmental predictability in cestodes (Cyclopdyllidea: Taeniidae)." *Evolution* **35**:723-741.
Moosmann, B., and Behl, C. (2008). "Mitochondrially encoded cysteine predicts animal lifespan." *Aging Cell* **7**(1):32-46. PubMed
Morales, C. P., Holt, S. E., Ouellette, M., Kaur, K. J., Yan, Y., Wilson, K. S., White, M. A., Wright, W. E., and Shay, J. W. (1999). "Absence of cancer-associated changes in human fibroblasts immortalized with telomerase." *Nat Genet* **21**(1):115-118. PubMed
Morgan, H., Beck, T., Blake, A., Gates, H., Adams, N., Debouzy, G., Leblanc, S., Lengger, C., Maier, H., Melvin, D., *et al.* (2010). "EuroPhenome: a repository for high-throughput mouse phenotyping data." *Nucleic Acids Res* **38**(Database issue):D577-585. PubMed
Mori, A., Utsumi, K., Liu, J., and Hosokawa, M. (1998). "Oxidative damage in the senescence-accelerated mouse." *Ann N Y Acad Sci* **854**:239-250. PubMed
Morimoto, R. I. (2006). "Stress, aging, and neurodegenerative disease." *N Engl J Med* **355**(21):2254-2255. PubMed
Morimoto, R. I., and Cuervo, A. M. (2009). "Protein homeostasis and aging: taking care of proteins from the cradle to the grave." *J Gerontol A Biol Sci Med Sci* **64**(2):167-170. PubMed
Morley, J. F., Brignull, H. R., Weyers, J. J., and Morimoto, R. I. (2002). "The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in Caenorhabditis elegans." *Proc Natl Acad Sci U S A* **99**(16):10417-10422. PubMed

Morrison, S. J., Prowse, K. R., Ho, P., and Weissman, I. L. (1996). "Telomerase activity in hematopoietic cells is associated with self-renewal potential." *Immunity* **5**(3):207-216. PubMed
Morrison, J. H., and Hof, P. R. (1997). "Life and death of neurons in the aging brain." *Science* **278**(5337):412-419. PubMed
Moskovitz, J., Bar-Noy, S., Williams, W. M., Requena, J., Berlett, B. S., and Stadtman, E. R. (2001). "Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals." *Proc Natl Acad Sci U S A* **98**(23):12920-12925. PubMed
Motonaga, K., Itoh, M., Hachiya, Y., Endo, A., Kato, K., Ishikura, H., Saito, Y., Mori, S., Takashima, S., and Goto, Y. (2002). "Age related expression of Werner's syndrome protein in selected tissues and coexpression of transcription factors." *J Clin Pathol* **55**(3):195-199. PubMed
Motta, M., Bennati, E., Vacante, M., Stanta, G., Cardillo, E., Malaguarnera, M., and Giarelli, L. (2010). "Autopsy reports in extreme longevity." *Arch Gerontol Geriatr* **50**(1):48-50. PubMed
Moyzis, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., Meyne, J., Ratliff, R. L., and Wu, J. R. (1988). "A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes." *Proc Natl Acad Sci U S A* **85**(18):6622-6626. PubMed
Muller, H. J. (1938). "The remaking of chromosomes." *Collecting Net* **13**(8):182-198.
Munne-Bosch, S. (2008). "Do perennials really senesce?" *Trends Plant Sci* **13**(5):216-220. PubMed
Munro, J., Steeghs, K., Morrison, V., Ireland, H., and Parkinson, E. K. (2001). "Human fibroblast replicative senescence can occur in the absence of extensive cell division and short telomeres." *Oncogene* **20**(27):3541-3552. PubMed
Murakami, S., Tedesco, P. M., Cypser, J. R., and Johnson, T. E. (2000). "Molecular genetic mechanisms of life span manipulation in Caenorhabditis elegans." *Ann N Y Acad Sci* **908**:40-49. PubMed
Murphree, A. L., and Benedict, W. F. (1984). "Retinoblastoma: clues to human oncogenesis." *Science* **223**(4640):1028-1033. PubMed
Murphy, K. M., and Topel, R. H. (2006). "The value of health and longevity." *Journal of Political Economy* **114**(5):871-904.
Nabholz, B., Glemin, S., and Galtier, N. (2008). "Strong variations of mitochondrial mutation rate across mammals--the longevity hypothesis." *Mol Biol Evol* **25**(1):120-130. PubMed
Nair, K. S., Rizza, R. A., O'Brien, P., Dhatariya, K., Short, K. R., Nehra, A., Vittone, J. L., Klee, G. G., Basu, A., Basu, R., *et al.* (2006). "DHEA in elderly women and DHEA or testosterone in elderly men." *N Engl J Med* **355**(16):1647-1659. PubMed
Nakamura, T. M., Morin, G. B., Chapman, K. B., Weinrich, S. L., Andrews, W. H., Lingner, J., Harley, C. B., and Cech, T. R. (1997). "Telomerase catalytic subunit homologs from fission yeast and human." *Science* **277**(5328):955-959. PubMed
Nanney, D. L. (1974). "Aging and long-term temporal regulation in ciliated protozoa. A critical review." *Mech Ageing Dev* **3**(2):81-105. PubMed
Napoli, C., Martin-Padura, I., de Nigris, F., Giorgio, M., Mansueto, G., Somma, P., Condorelli, M., Sica, G., De Rosa, G., and Pelicci, P. (2003). "Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet." *Proc Natl Acad Sci U S A* **100**(4):2112-2116. PubMed
Narayanan, L., Fritzell, J. A., Baker, S. M., Liskay, R. M., and Glazer, P. M. (1997). "Elevated levels of mutation in multiple tissues of mice deficient in the DNA mismatch repair gene Pms2." *Proc Natl Acad Sci U S A* **94**(7):3122-3127. PubMed
Nathwani, A. C., Tuddenham, E. G., Rangarajan, S., Rosales, C., McIntosh, J., Linch, D. C., Chowdary, P., Riddell, A., Pie, A. J., Harrington, C., *et al.* (2011). "Adenovirus-associated virus vector-mediated gene transfer in hemophilia B." *N Engl J Med* **365**(25):2357-2365. PubMed
Nemoto, S., and Finkel, T. (2002). "Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway." *Science* **295**(5564):2450-2452. PubMed
Nesse, R. M. (1988). "Life table tests of evolutionary theories of senescence." *Exp Gerontol* **23**(6):445-453. PubMed
Neufeld, T. P. (2010). "TOR-dependent control of autophagy: biting the hand that feeds." *Curr Opin Cell Biol* **22**(2):157-168. PubMed
Njajou, O. T., Cawthon, R. M., Damcott, C. M., Wu, S. H., Ott, S., Garant, M. J., Blackburn, E. H., Mitchell, B. D., Shuldiner, A. R., and Hsueh, W. C. (2007). "Telomere length is paternally inherited and is associated with parental lifespan." *Proc Natl Acad Sci U S A* **104**(29):12135-12139. PubMed
Norddahl, G. L., Pronk, C. J., Wahlestedt, M., Sten, G., Nygren, J. M., Ugale, A., Sigvardsson, M., and Bryder, D. (2011). "Accumulating mitochondrial DNA mutations drive premature hematopoietic aging phenotypes distinct from physiological stem cell aging." *Cell Stem Cell* **8**(5):499-510. PubMed
Nottebohm, F. (1989). "From bird song to neurogenesis." *Sci Am* **260**(2):74-79. PubMed
Nwosu, B. U., Nilsson, O., Mitchum, R. D., Jr., Coco, M., Barnes, K. M., and Baron, J. (2005). "Lack of telomere shortening with age in mouse resting zone chondrocytes." *Horm Res* **63**(3):125-128. PubMed
Nystrom, T. (2002). "Translational fidelity, protein oxidation, and senescence: lessons from bacteria." *Ageing Res Rev* **1**(4):693-703. PubMed
Oklejewicz, M., and Daan, S. (2002). "Enhanced longevity in tau mutant Syrian hamsters, Mesocricetus auratus." *J Biol Rhythms* **17**(3):210-216. PubMed
Olovnikov, A. M. (1971). "[Principle of marginotomy in template synthesis of polynucleotides]." *Dokl Akad Nauk SSSR* **201**(6):1496-1499. PubMed
Olovnikov, A. M. (1973). "A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon." *J Theor Biol* **41**(1):181-190. PubMed
Olshansky, S. J., Carnes, B. A., and Cassel, C. (1990). "In search of Methuselah: estimating the upper limits to human longevity." *Science* **250**(4981):634-640. PubMed
Olshansky, S. J., Hayflick, L., and Carnes, B. A. (2002). "No truth to the fountain of youth." *Sci Am* **286**(6):92-95. PubMed
Olshansky, S. J., Perry, D., Miller, R. A., and Butler, R. N. (2006). "In pursuit of the longevity dividend." *The Scientist* **20**:28-36.
Olson, C. B. (1987). "A review of why and how we age: a defense of multifactorial aging." *Mech Ageing Dev* **41**(1-2):1-28. PubMed
Olson, M. V., and Varki, A. (2003). "Sequencing the chimpanzee genome: insights into human evolution and disease." *Nat Rev Genet* **4**(1):20-28. PubMed
Olsson, M., and Shine, R. (2002). "Growth to death in lizards." *Evolution Int J Org Evolution* **56**(9):1867-1870. PubMed
Orgel, L. E. (1963). "The maintenance of the accuracy of protein synthesis and its relevance to ageing." *Proc Natl Acad Sci U S A* **49**:517-521. PubMed
Orgel, L. E. (1973). "Ageing of clones of mammalian cells." *Nature* **243**(5408):441-445. PubMed
Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S. M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D. M., *et al.* (2001). "Bone marrow cells regenerate infarcted myocardium." *Nature* **410**(6829):701-705. PubMed
Orr, W. C., and Sohal, R. S. (1994). "Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster." *Science* **263**(5150):1128-1130. PubMed
Orr, W. C., Mockett, R. J., Benes, J. J., and Sohal, R. S. (2003). "Effects of overexpression of copper-zinc and manganese superoxide dismutases, catalase, and thioredoxin reductase genes on longevity in Drosophila melanogaster." *J Biol Chem* **278**(29):26418-26422. PubMed
Osborne, T. B., Mendel, L. B., and Fey, E. L. (1917). "The effect of retardation of growth upon the breeding period and duration of life in rats." *Science* **45**:294-295.
Oshima, J., Campisi, J., Tannock, T. C., and Martin, G. M. (1995). "Regulation of c-fos expression in senescing Werner syndrome fibroblasts differs from that observed in senescing fibroblasts from normal donors." *J Cell Physiol* **162**(2):277-283. PubMed
Osiewacz, H. D., and Borghouts, C. (2000). "Mitochondrial oxidative stress and aging in the filamentous fungus Podospora anserina." *Ann N Y Acad Sci* **908**:31-39. PubMed
Ottinger, M. A. (2001). "Quail and other short-lived birds." *Exp Gerontol* **36**(4-6):859-868. PubMed
Outeiro, T. F., Kontopoulos, E., Altmann, S. M., Kufareva, I., Strathearn, K. E., Amore, A. M., Volk, C. B., Maxwell, M. M., Rochet, J. C., McLean, P. J., *et al.* (2007). "Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease." *Science* **317**(5837):516-519. PubMed
Overington, J. P., Al-Lazikani, B., and Hopkins, A. L. (2006). "How many drug targets are there?" *Nat Rev Drug Discov* **5**(12):993-996. PubMed
Pan, C., Xue, B. H., Ellis, T. M., Peace, D. J., and Diaz, M. O. (1997). "Changes in telomerase activity and telomere length during human T lymphocyte senescence." *Exp Cell Res* **231**(2):346-353. PubMed
Panda, S., Isbatan, A., and Adami, G. R. (2008). "Modification of the ATM/ATR directed DNA damage response state with aging and long after hepatocyte senescence induction in vivo." *Mech Ageing Dev* **129**(6):332-340. PubMed
Paradis, V., Youssef, N., Dargere, D., Ba, N., Bonvoust, F., Deschatrette, J., and Bedossa, P. (2001). "Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas." *Hum Pathol* **32**(3):327-332. PubMed
Park, W. Y., Hwang, C. I., Kang, M. J., Seo, J. Y., Chung, J. H., Kim, Y. S., Lee, J. H., Kim, H., Kim, K. A., Yoo, H. J., *et al.* (2001). "Gene profile of replicative senescence is different from progeria or elderly donor." *Biochem Biophys Res Commun* **282**(4):934-939. PubMed
Park, S. Y., Murphy, S. P., Wilkens, L. R., Henderson, B. E., and Kolonel, L. N. (2011). "Multivitamin use and the risk of mortality and cancer incidence: the multiethnic cohort study." *Am J Epidemiol* **173**(8):906-914. PubMed
Parker, S. T. (1990). "Why big brains are so rare: energy costs of intelligence and brain size in anthropoid primates." In: *Language and Intelligence in Monkeys and Apes*, Parker, S. T. and Gibson, K. R. (ed.).Â Cambridge University Press, Cambridge, 129-156.
Parker, J. D., Parker, K. M., Sohal, B. H., Sohal, R. S., and Keller, L. (2004). "Decreased expression of Cu-Zn superoxide dismutase 1 in ants with extreme lifespan." *Proc Natl Acad Sci U S A* **101**(10):3486-3489. PubMed
Parkes, T. L., Elia, A. J., Dickinson, D., Hilliker, A. J., Phillips, J. P., and Boulianne, G. L. (1998). "Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons." *Nat Genet* **19**(2):171-174. PubMed
Parrinello, S., Samper, E., Krtolica, A., Goldstein, J., Melov, S., and Campisi, J. (2003). "Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts." *Nat Cell Biol* **5**(8):741-747. PubMed
Partridge, L., and Mangel, M. (1999). "Messages from mortality: the evolution of death rates in the old." *Trends in Ecology and Evolution* **14**(11):438-442. PubMed
Partridge, L., and Gems, D. (2007). "Benchmarks for ageing studies." *Nature* **450**(7167):165-167. PubMed
Patel, M. N., Knight, C. G., Karageorgi, C., and Leroi, A. M. (2002). "Evolution of germ-line signals that regulate growth and aging in nematodes." *Proc Natl Acad Sci U S A* **99**(2):769-774. PubMed
Patnaik, B. K. (1994). "Ageing in reptiles." *Gerontology* **40**(2-4):200-220. PubMed
Pearl, R. (1928). *The Rate of Living*. Knopf, New York.
Pearson, K. J., Baur, J. A., Lewis, K. N., Peshkin, L., Price, N. L., Labinskyy, N., Swindell, W. R., Kamara, D., Minor, R. K., Perez, E., *et al.* (2008). "Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span." *Cell Metab* **8**(2):157-168. PubMed
Pera, M. F., Reubinoff, B., and Trounson, A. (2000). "Human embryonic stem cells." *J Cell Sci* **113 (Pt 1)**:5-10. PubMed

Perez, V. I., Buffenstein, R., Masamsetti, V., Leonard, S., Salmon, A. B., Mele, J., Andziak, B., Yang, T., Edrey, Y., Friguet, B., *et al.* (2009). "Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat." *Proc Natl Acad Sci U S A* **106**(9):3059-3064. PubMed
Perez, V. I., Cortez, L. A., Lew, C. M., Rodriguez, M., Webb, C. R., Van Remmen, H., Chaudhuri, A., Qi, W., Lee, S., Bokov, A., *et al.* (2011). "Thioredoxin 1 overexpression extends mainly the earlier part of life span in mice." *J Gerontol A Biol Sci Med Sci* **66**(12):1286-1299. PubMed
Perez-Campo, R., Lopez-Torres, M., Rojas, C., Cadenas, S., and Barja de Quiroga, G. (1993). "Lung glutathione reductase induction in aging catalase-depleted frogs correlates with early survival throughout the life span." *Mech Ageing Dev* **67**(1-2):115-127. PubMed
Perls, T., Kunkel, L. M., and Puca, A. A. (2002). "The genetics of exceptional human longevity." *J Am Geriatr Soc* **50**(2):359-368. PubMed
Perls, T. T. (2006). "The different paths to 100." *Am J Clin Nutr* **83**(2):484S-487S. PubMed
Pervaiz, S. (2003). "Resveratrol: from grapevines to mammalian biology." *Faseb J* **17**(14):1975-1985. PubMed
Peterson, R. T., Link, B. A., Dowling, J. E., and Schreiber, S. L. (2000). "Small molecule developmental screens reveal the logic and timing of vertebrate development." *Proc Natl Acad Sci U S A* **97**(24):12965-12969. PubMed
Peto, R., and Doll, R. (1997). "There is no such thing as aging." *Bmj* **315**(7115):1030-1032. PubMed
Petrascheck, M., Ye, X., and Buck, L. B. (2007). "An antidepressant that extends lifespan in adult Caenorhabditis elegans." *Nature* **450**(7169):553-556. PubMed
Phelan, J. P., and Rose, M. R. (2005). "Why dietary restriction substantially increases longevity in animal models but won't in humans." *Ageing Res Rev* **4**(3):339-350. PubMed
Pianka, E. R. (1970). "On r and K selection." *Am Nat* **104**(940):592-597.
Pichierri, P., Franchitto, A., Mosesso, P., Proietti de Santis, L., Balajee, A. S., and Palitti, F. (2000). "Werner's syndrome lymphoblastoid cells are hypersensitive to topoisomerase II inhibitors in the G2 phase of the cell cycle." *Mutat Res* **459**(2):123-133. PubMed
Pijnenburg, M. A., and Leget, C. (2007). "Who wants to live forever? Three arguments against extending the human lifespan." *J Med Ethics* **33**(10):585-587. PubMed
Pitkanen, S., and Robinson, B. H. (1996). "Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase." *J Clin Invest* **98**(2):345-351. PubMed
Pletcher, S. D., Khazaeli, A. A., and Curtsinger, J. W. (2000). "Why do life spans differ? Partitioning mean longevity differences in terms of age-specific mortality parameters." *J Gerontol A Biol Sci Med Sci* **55**(8):B381-389. PubMed
Plytycz, B., and Bigaj, J. (1993). "Studies on the growth and longevity of the yellow-bellied toad, Bombina variegata, in natural environments." *Amphibia-Reptilia* **14**:35-44.
Plytycz, B., Mika, J., and Bigaj, J. (1995a). "Age-dependent changes in thymuses in the European common frog, Rana temporaria." *J Exp Zool* **273**(6):451-460. PubMed
Plytycz, B., Jozkowicz, A., Chadzinska, M., and Bigaj, J. (1995b). "Longevity of yellow-bellied toads (Bombina variegata) and the efficiency of their immune system." *Naturschutzreport* **11**:77-84.
Poeggeler, B. (2005). "Melatonin, aging, and age-related diseases: perspectives for prevention, intervention, and therapy." *Endocrine* **27**(2):201-212. PubMed
Polyukhov, A. M., Kobsar, I. V., Grebelnik, V. I., and Voitenko, V. P. (2000). "The accelerated occurrence of age-related changes of organism in Chernobyl workers: a radiation-induced progeroid syndrome?" *Exp Gerontol* **35**(1):105-115. PubMed
Ponten, J., Stein, W. D., and Shall, S. (1983). "A quantitative analysis of the aging of human glial cells in culture." *J Cell Physiol* **117**(3):342-352. PubMed
Post, S. G. (2004). "Anti-aging medicine: the history: establishing an appropriate ethical framework: the moral conversation around the goal of prolongevity." *J Gerontol A Biol Sci Med Sci* **59**(6):B534-539. PubMed
Pound, P., Ebrahim, S., Sandercock, P., Bracken, M. B., and Roberts, I. (2004). "Where is the evidence that animal research benefits humans?" *Bmj* **328**(7438):514-517. PubMed
Prescott, J., and Blackburn, E. H. (1997). "Telomerase RNA mutations in Saccharomyces cerevisiae alter telomerase action and reveal nonprocessivity in vivo and in vitro." *Genes Dev* **11**(4):528-540. PubMed
Price, J. S., Waters, J. G., Darrah, C., Pennington, C., Edwards, D. R., Donell, S. T., and Clark, I. M. (2002). "The role of chondrocyte senescence in osteoarthritis." *Aging Cell* **1**(1):57-65. PubMed
Prinzinger, R. (2005). "Programmed ageing: the theory of maximal metabolic scope. How does the biological clock tick?" *EMBO Rep* **6 Spec No**:S14-19. PubMed
Promislow, D. E. (1993). "On size and survival: progress and pitfalls in the allometry of life span." *J Gerontol* **48**(4):B115-123. PubMed
Promislow, D. E. (1994). "DNA repair and the evolution of longevity: a critical analysis." *J Theor Biol* **170**(3):291-300. PubMed
Prothero, J. (1993). "Adult life span as a function of age at maturity." *Exp Gerontol* **28**(6):529-536. PubMed
Prowse, K. R., and Greider, C. W. (1995). "Developmental and tissue-specific regulation of mouse telomerase and telomere length." *Proc Natl Acad Sci U S A* **92**(11):4818-4822. PubMed
Puca, A. A., Daly, M. J., Brewster, S. J., Matise, T. C., Barrett, J., Shea-Drinkwater, M., Kang, S., Joyce, E., Nicoli, J., Benson, E., *et al.* (2001). "A genome-wide scan for linkage to human exceptional longevity identifies a locus on chromosome 4." *Proc Natl Acad Sci U S A* **98**(18):10505-10508. PubMed
Quick, K. L., Ali, S. S., Arch, R., Xiong, C., Wozniak, D., and Dugan, L. L. (2008). "A carboxyfullerene SOD mimetic improves cognition and extends the lifespan of mice." *Neurobiol Aging* **29**(1):117-128. PubMed
Rabinovitch, P. S., and Martin, G. M. (1982). "Encephalomyocarditis virus as a probe of errors in macromolecular synthesis in aging mice." *Mech Ageing Dev* **20**(2):155-163. PubMed
Racette, S. B., Weiss, E. P., Villareal, D. T., Arif, H., Steger-May, K., Schechtman, K. B., Fontana, L., Klein, S., and Holloszy, J. O. (2006). "One year of caloric restriction in humans: feasibility and effects on body composition and abdominal adipose tissue." *J Gerontol A Biol Sci Med Sci* **61**(9):943-950. PubMed
Raji, N. S., and Rao, K. S. (1998). "Trisomy 21 and accelerated aging: DNA-repair parameters in peripheral lymphocytes of Down's syndrome patients." *Mech Ageing Dev* **100**(1):85-101. PubMed
Ramirez, R. D., Morales, C. P., Herbert, B. S., Rohde, J. M., Passons, C., Shay, J. W., and Wright, W. E. (2001). "Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions." *Genes Dev* **15**(4):398-403. PubMed
Ramsey, J. J., Colman, R. J., Binkley, N. C., Christensen, J. D., Gresl, T. A., Kemnitz, J. W., and Weindruch, R. (2000). "Dietary restriction and aging in rhesus monkeys: the University of Wisconsin study." *Exp Gerontol* **35**(9-10):1131-1149. PubMed
Rasmussen, U. F., Krustrup, P., Kjaer, M., and Rasmussen, H. N. (2003). "Experimental evidence against the mitochondrial theory of aging A study of isolated human skeletal muscle mitochondria." *Exp Gerontol* **38**(8):877-886. PubMed
Reaume, A. G., Elliott, J. L., Hoffman, E. K., Kowall, N. W., Ferrante, R. J., Siwek, D. F., Wilcox, H. M., Flood, D. G., Beal, M. F., Brown, R. H., Jr., *et al.* (1996). "Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury." *Nat Genet* **13**(1):43-47. PubMed
Renault, V., Thornell, L. E., Eriksson, P. O., Butler-Browne, G., Mouly, V., and Thorne, L. E. (2002). "Regenerative potential of human skeletal muscle during aging." *Aging Cell* **1**(2):132-139. PubMed
Reznick, D. N., Bryant, M. J., Roff, D., Ghalambor, C. K., and Ghalambor, D. E. (2004). "Effect of extrinsic mortality on the evolution of senescence in guppies." *Nature* **431**(7012):1095-1099. PubMed
Reznick, D. N. (2005). "The genetic basis of aging: an evolutionary biologist's perspective." *Sci Aging Knowledge Environ* **2005**(11):pe7. PubMed
Rheinwald, J. G., Hahn, W. C., Ramsey, M. R., Wu, J. Y., Guo, Z., Tsao, H., De Luca, M., Catricala, C., and O'Toole, K. M. (2002). "A two-stage, p16(INK4A)- and p53-dependent keratinocyte senescence mechanism that limits replicative potential independent of telomere status." *Mol Cell Biol* **22**(14):5157-5172. PubMed
Ricklefs, R. E. (2010). "Life-history connections to rates of aging in terrestrial vertebrates." *Proc Natl Acad Sci U S A* **107**(22):10314-10319. PubMed
Robbins, E., Levine, E. M., and Eagle, H. (1970). "Morphologic changes accompanying senescence of cultured human diploid cells." *J Exp Med* **131**(6):1211-1222. PubMed
Robertson, O. H. (1961). "Prolongation of the life span of kokanee salmon (*Oncorhynchus nerka kennerlyi*) by castration before beginning of gonad development." *Proc Natl Acad Sci U S A* **47**(4):609-621.
Robinson, B. H. (1998). "Human complex I deficiency: clinical spectrum and involvement of oxygen free radicals in the pathogenicity of the defect." *Biochim Biophys Acta* **1364**(2):271-286. PubMed
Robles, S. J., and Adami, G. R. (1998). "Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts." *Oncogene* **16**(9):1113-1123. PubMed
Rodriguez, J. I., Perez-Alonso, P., Funes, R., and Perez-Rodriguez, J. (1999). "Lethal neonatal Hutchinson-Gilford progeria syndrome." *Am J Med Genet* **82**(3):242-248. PubMed
Rogan, E. M., Bryan, T. M., Hukku, B., Maclean, K., Chang, A. C., Moy, E. L., Englezou, A., Warneford, S. G., Dalla-Pozza, L., and Reddel, R. R. (1995). "Alterations in p53 and p16INK4 expression and telomere length during spontaneous immortalization of Li-Fraumeni syndrome fibroblasts." *Mol Cell Biol* **15**(9):4745-4753. PubMed
Rohme, D. (1981). "Evidence for a relationship between longevity of mammalian species and life spans of normal fibroblasts in vitro and erythrocytes in vivo." *Proc Natl Acad Sci U S A* **78**(8):5009-5013. PubMed
Rollo, C. D. (2002). "Growth negatively impacts the life span of mammals." *Evol Dev* **4**(1):55-61. PubMed
Rose, M. R. (1989). "Genetics of increased lifespan in Drosophila." *Bioessays* **11**(5):132-135. PubMed
Rose, M. R. (1991). *Evolutionary Biology of Aging*. Oxford University Press, New York.
Rose, M. R., Drapeau, M. D., Yazdi, P. G., Shah, K. H., Moise, D. B., Thakar, R. R., Rauser, C. L., and Mueller, L. D. (2002). "Evolution of late-life mortality in Drosophila melanogaster." *Evolution Int J Org Evolution* **56**(10):1982-1991. PubMed
Rossi, D. J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., and Weissman, I. L. (2007). "Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age." *Nature* **447**(7145):725-729. PubMed
Rossolini, G., and Piantanelli, L. (2001). "Mathematical modeling of the aging processes and the mechanisms of mortality: paramount role of heterogeneity." *Exp Gerontol* **36**(8):1277-1288. PubMed
Rotig, A., de Lonlay, P., Chretien, D., Foury, F., Koenig, M., Sidi, D., Munnich, A., and Rustin, P. (1997). "Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia." *Nat Genet* **17**(2):215-217. PubMed
Rougier, G. W., and Novacek, M. J. (1998). "Early mammals: teeth, jaws and finally a skeleton!" *Curr Biol* **8**(8):R284-287. PubMed
Rouse, J., and Jackson, S. P. (2002). "Interfaces between the detection, signaling, and repair of DNA damage." *Science* **297**(5581):547-551. PubMed

Rovio, A. T., Marchington, D. R., Donat, S., Schuppe, H. C., Abel, J., Fritsche, E., Elliott, D. J., Laippala, P., Ahola, A. L., McNay, D., *et al.* (2001). "Mutations at the mitochondrial DNA polymerase (POLG) locus associated with male infertility." *Nat Genet* **29**(3):261-262. PubMed
Rowe, J. W., and Kahn, R. L. (1987). "Human aging: usual and successful." *Science* **237**(4811):143-149. PubMed
Roy, J., Fulton, T. B., and Blackburn, E. H. (1998). "Specific telomerase RNA residues distant from the template are essential for telomerase function." *Genes Dev* **12**(20):3286-3300. PubMed
Ruan, H., Tang, X. D., Chen, M. L., Joiner, M. L., Sun, G., Brot, N., Weissbach, H., Heinemann, S. H., Iverson, L., Wu, C. F., *et al.* (2002). "High-quality life extension by the enzyme peptide methionine sulfoxide reductase." *Proc Natl Acad Sci U S A* **99**(5):2748-2753. PubMed
Rubinsztein, D. C., Marino, G., and Kroemer, G. (2011). "Autophagy and aging." *Cell* **146**(5):682-695. PubMed
Rudolph, K. L., Chang, S., Lee, H. W., Blasco, M., Gottlieb, G. J., Greider, C., and DePinho, R. A. (1999). "Longevity, stress response, and cancer in aging telomerase-deficient mice." *Cell* **96**(5):701-712. PubMed
Rudolph, K. L., Millard, M., Bosenberg, M. W., and DePinho, R. A. (2001). "Telomere dysfunction and evolution of intestinal carcinoma in mice and humans." *Nat Genet* **28**(2):155-159. PubMed
Saito, K., Yoshioka, H., and Cutler, R. G. (1998). "A spin trap, N-tert-butyl-alpha-phenylnitrone extends the life span of mice." *Biosci Biotechnol Biochem* **62**(4):792-794. PubMed
Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K., and Ichijo, H. (1998). "Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1." *Embo J* **17**(9):2596-2606. PubMed
Salganik, R. I., Solovyova, N. A., Dikalov, S. I., Grishaeva, O. N., Semenova, L. A., and Popovsky, A. V. (1994). "Inherited enhancement of hydroxyl radical generation and lipid peroxidation in the S strain rats results in DNA rearrangements, degenerative diseases, and premature aging." *Biochem Biophys Res Commun* **199**(2):726-733. PubMed
Salk, D., Bryant, E., Au, K., Hoehn, H., and Martin, G. M. (1981). "Systematic growth studies, cocultivation, and cell hybridization studies of Werner syndrome cultured skin fibroblasts." *Hum Genet* **58**(3):310-316. PubMed
Salminen, A., and Kaarniranta, K. (2009). "SIRT1: regulation of longevity via autophagy." *Cell Signal* **21**(9):1356-1360. PubMed
Salmon, A. B., Murakami, S., Bartke, A., Kopchick, J., Yasumura, K., and Miller, R. A. (2005). "Fibroblast cell lines from young adult mice of long-lived mutant strains are resistant to multiple forms of stress." *Am J Physiol Endocrinol Metab* **289**(1):E23-29. PubMed
Salmon, A. B., Leonard, S., Masamsetti, V., Pierce, A., Podlutsky, A. J., Podlutskaya, N., Richardson, A., Austad, S. N., and Chaudhuri, A. R. (2009). "The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis." *Faseb J* **23**(7):2317-2326. PubMed
Salway, K. D., Page, M. M., Faure, P. A., Burness, G., and Stuart, J. A. (2011). "Enhanced protein repair and recycling are not correlated with longevity in 15 vertebrate endotherm species." *Age (Dordr)* **33**(1):33-47. PubMed
Sams-Dodd, F. (2005). "Target-based drug discovery: is something wrong?" *Drug Discov Today* **10**(2):139-147. PubMed
Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1986). "The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis." *Endocr Rev* **7**(3):284-301. PubMed
Schmidt-Nielsen, K. (1984). *Scaling: Why is Animal Size So Important?* Cambridge Univ. Press, Cambridge.
Schneider, E. L. (1999). "Aging in the third millennium." *Science* **283**(5403):796-797. PubMed
Schrempf, A., Heinze, J., and Cremer, S. (2005). "Sexual cooperation: mating increases longevity in ant queens." *Curr Biol* **15**(3):267-270. PubMed
Schriner, S. E., Ogburn, C. E., Smith, A. C., Newcomb, T. G., Ladiges, W. C., Dolle, M. E., Vijg, J., Fukuchi, K., and Martin, G. M. (2000). "Levels of dna damage are unaltered in mice overexpressing human catalase in nuclei." *Free Radic Biol Med* **29**(7):664-673. PubMed
Schriner, S. E., Linford, N. J., Martin, G. M., Treuting, P., Ogburn, C. E., Emond, M., Coskun, P. E., Ladiges, W., Wolf, N., Van Remmen, H., *et al.* (2005). "Extension of murine life span by overexpression of catalase targeted to mitochondria." *Science* **308**(5730):1909-1911. PubMed
Schulz, V. P., Zakian, V. A., Ogburn, C. E., McKay, J., Jarzebowicz, A. A., Edland, S. D., and Martin, G. M. (1996). "Accelerated loss of telomeric repeats may not explain accelerated replicative decline of Werner syndrome cells." *Hum Genet* **97**(6):750-754. PubMed
Selman, C., McLaren, J. S., Meyer, C., Duncan, J. S., Redman, P., Collins, A. R., Duthie, G. G., and Speakman, J. R. (2006). "Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes." *Mech Ageing Dev* **127**(12):897-904. PubMed
Seluanov, A., Chen, Z., Hine, C., Sasahara, T. H., Ribeiro, A. A., Catania, K. C., Presgraves, D. C., and Gorbunova, V. (2007). "Telomerase activity coevolves with body mass not lifespan." *Aging Cell* **6**(1):45-52. PubMed
Seluanov, A., Hine, C., Bozzella, M., Hall, A., Sasahara, T. H., Ribeiro, A. A., Catania, K. C., Presgraves, D. C., and Gorbunova, V. (2008). "Distinct tumor suppressor mechanisms evolve in rodent species that differ in size and lifespan." *Aging Cell* **7**(6):813-823. PubMed
Seluanov, A., Hine, C., Azpurua, J., Feigenson, M., Bozzella, M., Mao, Z., Catania, K. C., and Gorbunova, V. (2009). "Hypersensitivity to contact inhibition provides a clue to cancer resistance of naked mole-rat." *Proc Natl Acad Sci U S A* **106**(46):19352-19357. PubMed
Sequeiros, J., Silveira, I., Maciel, P., Coutinho, P., Manaia, A., Gaspar, C., Burlet, P., Loureiro, L., Guimaraes, J., Tanaka, H., *et al.* (1994). "Genetic linkage studies of Machado-Joseph disease with chromosome 14q STRPs in 16 Portuguese-Azorean kindreds." *Genomics* **21**(3):645-648. PubMed
Serra, V., and von Zglinicki, T. (2002). "Human fibroblasts in vitro senesce with a donor-specific telomere length." *FEBS Lett* **516**(1-3):71-74. PubMed
Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., and Lowe, S. W. (1997). "Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a." *Cell* **88**(5):593-602. PubMed
Serrano, M., and Blasco, M. A. (2001). "Putting the stress on senescence." *Curr Opin Cell Biol* **13**(6):748-753. PubMed
Shaharabany, M., Gollop, N., Ravin, S., Golomb, E., DeMarco, L., Ferreira, P. C., Boson, W. L., and Friedman, E. (1999). "Naturally occurring antibacterial activities of avian and crocodile tissues." *J Antimicrob Chemother* **44**(3):416-418. PubMed
Shammas, M. A., Simmons, C. G., Corey, D. R., and Shmookler Reis, R. J. (1999). "Telomerase inhibition by peptide nucleic acids reverses 'immortality' of transformed human cells." *Oncogene* **18**(46):6191-6200. PubMed
Shapiro, G. I., Edwards, C. D., Ewen, M. E., and Rollins, B. J. (1998). "p16INK4A participates in a G1 arrest checkpoint in response to DNA damage." *Mol Cell Biol* **18**(1):378-387. PubMed
Shawi, M., and Autexier, C. (2008). "Telomerase, senescence and ageing." *Mech Ageing Dev* **129**(1-2):3-10. PubMed
Shay, J. W., Pereira-Smith, O. M., and Wright, W. E. (1991). "A role for both RB and p53 in the regulation of human cellular senescence." *Exp Cell Res* **196**(1):33-39. PubMed
Shay, J. W. (1999). "At the end of the millennium, a view of the end." *Nat Genet* **23**(4):382-383. PubMed
Shay, J. W., and Wright, W. E. (2000). "Hayflick, his limit, and cellular ageing." *Nat Rev Mol Cell Biol* **1**(1):72-76. PubMed
Shelton, D. N., Chang, E., Whittier, P. S., Choi, D., and Funk, W. D. (1999). "Microarray analysis of replicative senescence." *Curr Biol* **9**(17):939-945. PubMed
Shen, J. C., and Loeb, L. A. (2000). "The Werner syndrome gene: the molecular basis of RecQ helicase-deficiency diseases." *Trends Genet* **16**(5):213-220. PubMed
Shen, J., Tsai, Y.-T., DiMarco, N. M., Long, M. A., Sun, X., and Tang, L. (2011). "Transplantation of mesenchymal stem cells from young donors delays aging in mice." *Scientific Reports* **1**:67.
Sherwood, S. W., Rush, D., Ellsworth, J. L., and Schimke, R. T. (1988). "Defining cellular senescence in IMR-90 cells: a flow cytometric analysis." *Proc Natl Acad Sci U S A* **85**(23):9086-9090. PubMed
Shi, N., and Pardridge, W. M. (2000). "Noninvasive gene targeting to the brain." *Proc Natl Acad Sci U S A* **97**(13):7567-7572. PubMed
Shiels, P. G., Kind, A. J., Campbell, K. H., Waddington, D., Wilmut, I., Colman, A., and Schnieke, A. E. (1999). "Analysis of telomere lengths in cloned sheep." *Nature* **399**(6734):316-317. PubMed
Shimokawa, I., Higami, Y., Tsuchiya, T., Otani, H., Komatsu, T., Chiba, T., and Yamaza, H. (2003). "Life span extension by reduction of the growth hormone-insulin-like growth factor-1 axis: relation to caloric restriction." *Faseb J* **17**(9):1108-1109. PubMed
Simon, A. F., Shih, C., Mack, A., and Benzer, S. (2003). "Steroid control of longevity in Drosophila melanogaster." *Science* **299**(5611):1407-1410. PubMed
Simonsen, A., Cumming, R. C., Brech, A., Isakson, P., Schubert, D. R., and Finley, K. D. (2008). "Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult Drosophila." *Autophagy* **4**(2):176-184. PubMed
Sinclair, D. A., and Guarente, L. (1997). "Extrachromosomal rDNA circles--a cause of aging in yeast." *Cell* **91**(7):1033-1042. PubMed
Sinclair, D. A. (2002). "Paradigms and pitfalls of yeast longevity research." *Mech Ageing Dev* **123**(8):857-867. PubMed
Sitte, N., Saretzki, G., and von Zglinicki, T. (1998). "Accelerated telomere shortening in fibroblasts after extended periods of confluency." *Free Radic Biol Med* **24**(6):885-893. PubMed
Smirina, E. M. (1994). "Age determination and longevity in amphibians." *Gerontology* **40**(2-4):133-146. PubMed
Smith, J. R., and Whitney, R. G. (1980). "Intraclonal variation in proliferative potential of human diploid fibroblasts: stochastic mechanism for cellular aging." *Science* **207**(4426):82-84. PubMed
Smith, J., and Schofield, P. N. (1994). "The effects of fibroblast growth factors in long-term primary culture of dystrophic (mdx) mouse muscle myoblasts." *Exp Cell Res* **210**(1):86-93. PubMed
Smith, D. E., Roberts, J., Gage, F. H., and Tuszynski, M. H. (1999). "Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy." *Proc Natl Acad Sci U S A* **96**(19):10893-10898. PubMed
Smith-Sonneborn, J. (1987). "Longevity in the protozoa." *Basic Life Sci* **42**:101-109. PubMed
Smogorzewska, A., van Steensel, B., Bianchi, A., Oelmann, S., Schaefer, M. R., Schnapp, G., and de Lange, T. (2000). "Control of human telomere length by TRF1 and TRF2." *Mol Cell Biol* **20**(5):1659-1668. PubMed
Smogorzewska, A., and de Lange, T. (2002). "Different telomere damage signaling pathways in human and mouse cells." *Embo J* **21**(16):4338-4348. PubMed
Snyder, E. Y., and Loring, J. F. (2005). "A role for stem cell biology in the physiological and pathological aspects of aging." *J Am Geriatr Soc* **53**(9 Suppl):S287-291. PubMed
Sohal, R. S., Sohal, B. H., and Brunk, U. T. (1990). "Relationship between antioxidant defenses and longevity in different mammalian species." *Mech Ageing Dev* **53**(3):217-227. PubMed
Sohal, R. S., and Brunk, U. T. (1992). "Mitochondrial production of pro-oxidants and cellular senescence." *Mutat Res* **275**(3-6):295-304. PubMed
Sohal, R. S., and Weindruch, R. (1996). "Oxidative stress, caloric restriction, and aging." *Science* **273**(5271):59-63. PubMed
Sohal, R. S., Mockett, R. J., and Orr, W. C. (2002). "Mechanisms of aging: an appraisal of the oxidative stress hypothesis." *Free Radic Biol Med* **33**(5):575-586. PubMed
Sonntag, W. E., Lynch, C. D., Cefalu, W. T., Ingram, R. L., Bennett, S. A., Thornton, P. L., and Khan, A. S. (1999). "Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals." *J Gerontol A Biol Sci Med Sci* **54**(12):B521-538. PubMed
Speakman, J. R., Talbot, D. A., Selman, C., Snart, S., McLaren, J. S., Redman, P., Krol, E., Jackson, D. M., Johnson, M. S., and Brand, M. D. (2004). "Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer." *Aging Cell* **3**(3):87-95. PubMed

Speakman, J. R. (2005). "Correlations between physiology and lifespan--two widely ignored problems with comparative studies." *Aging Cell* **4**(4):167-175. PubMed
Spence, A. P. (1995). *Biology of Human Aging*. Prentice Hall, Englewood Cliffs, NJ.
Spencer, C. C., and Promislow, D. E. (2002). "Genes, culture, and aging flies--what the lab can and cannot tell us about natural genetic variation for senescence." *Sci Aging Knowledge Environ* **2002**(20):pe6. PubMed
Spencer, C. C., and Promislow, D. E. (2002). "Biologists finally horn in on senescence in the wild." *Sci Aging Knowledge Environ* **2002**(47):pe19. PubMed
Spindler, S. R. (2005). "Rapid and reversible induction of the longevity, anticancer and genomic effects of caloric restriction." *Mech Ageing Dev* **126**(9):960-966. PubMed
Stanley, J. F., Pye, D., and MacGregor, A. (1975). "Comparison of doubling numbers attained by cultured animal cells with life span of species." *Nature* **255**(5504):158-159. PubMed
Stearns, S. C. (1992). *The Evolution of Life Histories*. Oxford University Press, Oxford.
Stein, G. H., and Dulic, V. (1998). "Molecular mechanisms for the senescent cell cycle arrest." *J Investig Dermatol Symp Proc* **3**(1):14-18. PubMed
Steinert, S., Shay, J. W., and Wright, W. E. (2000). "Transient expression of human telomerase extends the life span of normal human fibroblasts." *Biochem Biophys Res Commun* **273**(3):1095-1098. PubMed
Steinert, S., White, D. M., Zou, Y., Shay, J. W., and Wright, W. E. (2002). "Telomere biology and cellular aging in nonhuman primate cells." *Exp Cell Res* **272**(2):146-152. PubMed
Stewart, S. A., and Weinberg, R. A. (2000). "Telomerase and human tumorigenesis." *Semin Cancer Biol* **10**(6):399-406. PubMed
Stewart, S. A., Ben-Porath, I., Carey, V. J., O'Connor, B. F., Hahn, W. C., and Weinberg, R. A. (2003). "Erosion of the telomeric single-strand overhang at replicative senescence." *Nat Genet* **33**(4):492-496. PubMed
Stewart, E. J., Madden, R., Paul, G., and Taddei, F. (2005). "Aging and death in an organism that reproduces by morphologically symmetric division." *PLoS Biol* **3**(2):e45. PubMed
Stock, G. B. (2004). "The pitfalls of planning for demographic change." *Ann N Y Acad Sci* **1019**:546-551. PubMed
Strehler, B. L. (1986). "Genetic instability as the primary cause of human aging." *Exp Gerontol* **21**(4-5):283-319. PubMed
Strehler, B. L. (1999). *Time, Cells, and Aging*. Demetriades Brothers, Larnaca.
Su, A. I., Wiltshire, T., Batalov, S., Lapp, H., Ching, K. A., Block, D., Zhang, J., Soden, R., Hayakawa, M., Kreiman, G., *et al.* (2004). "A gene atlas of the mouse and human protein-encoding transcriptomes." *Proc Natl Acad Sci U S A* **101**(16):6062-6067. PubMed
Suga, H., Kadoshima, T., Minaguchi, M., Ohgushi, M., Soen, M., Nakano, T., Takata, N., Wataya, T., Muguruma, K., Miyoshi, H., *et al.* (2011). "Self-formation of functional adenohypophysis in three-dimensional culture." *Nature* **480**(7375):57-62. PubMed
Sugihara, M., Ohshima, K., Nakamura, H., Suzumiya, J., Nakayama, Y., Kanda, M., Haraoka, S., and Kikuchi, M. (1999). "Decreased expression of telomerase-associated RNAs in the proliferation of stem cells in comparison with continuous expression in malignant tumors." *Int J Oncol* **15**(6):1075-1080. PubMed
Sugita, K., Suzuki, N., Fujii, K., and Niimi, H. (1995). "Reduction of unscheduled DNA synthesis and plasminogen activator activity in Hutchinson-Gilford fibroblasts during passaging in vitro: partial correction by interferon-beta." *Mutat Res* **316**(3):133-138. PubMed
Sugrue, M. M., Shin, D. Y., Lee, S. W., and Aaronson, S. A. (1997). "Wild-type p53 triggers a rapid senescence program in human tumor cells lacking functional p53." *Proc Natl Acad Sci U S A* **94**(18):9648-9653. PubMed
Suh, Y., Atzmon, G., Cho, M. O., Hwang, D., Liu, B., Leahy, D. J., Barzilai, N., and Cohen, P. (2008). "Functionally significant insulin-like growth factor I receptor mutations in centenarians." *Proc Natl Acad Sci U S A* **105**(9):3438-3442. PubMed
Suhr, S. T., Chang, E. A., Tjong, J., Alcasid, N., Perkins, G. A., Goissis, M. D., Ellisman, M. H., Perez, G. I., and Cibelli, J. B. (2010). "Mitochondrial rejuvenation after induced pluripotency." *PLoS One* **5**(11):e14095. PubMed
Sun, D., Muthukumar, A. R., Lawrence, R. A., and Fernandes, G. (2001). "Effects of calorie restriction on polymicrobial peritonitis induced by cecum ligation and puncture in young C57BL/6 mice." *Clin Diagn Lab Immunol* **8**(5):1003-1011. PubMed
Sutphin, G. L., and Kaeberlein, M. (2008). "Dietary restriction by bacterial deprivation increases life span in wild-derived nematodes." *Exp Gerontol* **43**(3):130-135. PubMed
Symes, J. F., Losordo, D. W., Vale, P. R., Lathi, K. G., Esakof, D. D., Mayskiy, M., and Isner, J. M. (1999). "Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease." *Ann Thorac Surg* **68**(3):830-836; discussion 836-837. PubMed
Szilard, L. (1959). "On the nature of the aging process." *Proc Natl Acad Sci U S A* **45**(1):30-45.
Tahara, H., Sato, E., Noda, A., and Ide, T. (1995). "Increase in expression level of p21sdi1/cip1/waf1 with increasing division age in both normal and SV40-transformed human fibroblasts." *Oncogene* **10**(5):835-840. PubMed
Tahara, H., Yasui, W., Tahara, E., Fujimoto, J., Ito, K., Tamai, K., Nakayama, J., Ishikawa, F., and Ide, T. (1999). "Immuno-histochemical detection of human telomerase catalytic component, hTERT, in human colorectal tumor and non-tumor tissue sections." *Oncogene* **18**(8):1561-1567. PubMed
Takahashi, K., and Yamanaka, S. (2006). "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors." *Cell* **126**(4):663-676. PubMed
Takubo, K., Izumiyama-Shimomura, N., Honma, N., Sawabe, M., Arai, T., Kato, M., Oshimura, M., and Nakamura, K. I. (2002). "Telomere lengths are characteristic in each human individual." *Exp Gerontol* **37**(4):523-531. PubMed
Tang, D. G., Tokumoto, Y. M., Apperly, J. A., Lloyd, A. C., and Raff, M. C. (2001). "Lack of replicative senescence in cultured rat oligodendrocyte precursor cells." *Science* **291**(5505):868-871. PubMed
Tang, F., Watkins, J. W., Bermudez, M., Gray, R., Gaban, A., Portie, K., Grace, S., Kleve, M., and Craciun, G. (2008). "A life-span extending form of autophagy employs the vacuole-vacuole fusion machinery." *Autophagy* **4**(7):874-886. PubMed
Tang, S., Wang, J., Lee, N. C., Milone, M., Halberg, M. C., Schmitt, E. S., Craigen, W. J., Zhang, W., and Wong, L. J. (2011). "Mitochondrial DNA polymerase gamma mutations: an ever expanding molecular and clinical spectrum." *J Med Genet* **48**(10):669-681. PubMed
Tanhauser, S. M., and Laipis, P. J. (1995). "Multiple deletions are detectable in mitochondrial DNA of aging mice." *J Biol Chem* **270**(42):24769-24775. PubMed
Tardif, S. D., Mansfield, K. G., Ratnam, R., Ross, C. N., and Ziegler, T. E. (2011). "The marmoset as a model of aging and age-related diseases." *Ilar J* **52**(1):54-65. PubMed
Tatar, M., and Yin, C. (2001). "Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms." *Exp Gerontol* **36**(4-6):723-738. PubMed
Tatar, M., Bartke, A., and Antebi, A. (2003). "The endocrine regulation of aging by insulin-like signals." *Science* **299**(5611):1346-1351. PubMed
Taylor, R. S., Ramirez, R. D., Ogoshi, M., Chaffins, M., Piatyszek, M. A., and Shay, J. W. (1996). "Detection of telomerase activity in malignant and nonmalignant skin conditions." *J Invest Dermatol* **106**(4):759-765. PubMed
te Poele, R. H., Okorokov, A. L., Jardine, L., Cummings, J., and Joel, S. P. (2002). "DNA damage is able to induce senescence in tumor cells in vitro and in vivo." *Cancer Res* **62**(6):1876-1883. PubMed
Tesco, G., Vergelli, M., Grassilli, E., Salomoni, P., Bellesia, E., Sikora, E., Radziszewska, E., Barbieri, D., Latorraca, S., Fagiolo, U., *et al.* (1998). "Growth properties and growth factor responsiveness in skin fibroblasts from centenarians." *Biochem Biophys Res Commun* **244**(3):912-916. PubMed
Teter, B., and Finch, C. E. (2004). "Caliban's heritance and the genetics of neuronal aging." *Trends Neurosci* **27**(10):627-632. PubMed
Theys, J., Barbe, S., Landuyt, W., Nuyts, S., Van Mellaert, L., Wouters, B., Anne, J., and Lambin, P. (2003). "Tumor-specific gene delivery using genetically engineered bacteria." *Curr Gene Ther* **3**(3):207-221. PubMed
Timiras, P. S. (2002). *Physiological Basis of Aging and Geriatrics*. CRC Press, Boca Raton, FL.
Timmers, S., Konings, E., Bilet, L., Houtkooper, R. H., van de Weijer, T., Goossens, G. H., Hoeks, J., van der Krieken, S., Ryu, D., Kersten, S., *et al.* (2011). "Calorie Restriction-like Effects of 30 Days of Resveratrol Supplementation on Energy Metabolism and Metabolic Profile in Obese Humans." *Cell Metab* **14**(5):612-622. PubMed
Tissenbaum, H. A., and Guarente, L. (2002). "Model organisms as a guide to mammalian aging." *Dev Cell* **2**(1):9-19. PubMed
Toda, T., Satoh, M., Sugimoto, M., Goto, M., Furuichi, Y., and Kimura, N. (1998). "A comparative analysis of the proteins between the fibroblasts from Werner's syndrome patients and age-matched normal individuals using two-dimensional gel electrophoresis." *Mech Ageing Dev* **100**(2):133-143. PubMed
Tolmasoff, J. M., Ono, T., and Cutler, R. G. (1980). "Superoxide dismutase: correlation with life-span and specific metabolic rate in primate species." *Proc Natl Acad Sci U S A* **77**(5):2777-2781. PubMed
Tomas-Loba, A., Flores, I., Fernandez-Marcos, P. J., Cayuela, M. L., Maraver, A., Tejera, A., Borras, C., Matheu, A., Klatt, P., Flores, J. M., *et al.* (2008). "Telomerase reverse transcriptase delays aging in cancer-resistant mice." *Cell* **135**(4):609-622. PubMed
Toth, M. L., Sigmond, T., Borsos, E., Barna, J., Erdelyi, P., Takacs-Vellai, K., Orosz, L., Kovacs, A. L., Csikos, G., Sass, M., *et al.* (2008). "Longevity pathways converge on autophagy genes to regulate life span in Caenorhabditis elegans." *Autophagy* **4**(3):330-338. PubMed
Toussaint, O., Dumont, P., Dierick, J. F., Pascal, T., Frippiat, C., Chainiaux, F., Sluse, F., Eliaers, F., and Remacle, J. (2000). "Stress-induced premature senescence. Essence of life, evolution, stress, and aging." *Ann N Y Acad Sci* **908**:85-98. PubMed
Toussaint, O., Dumont, P., Remacle, J., Dierick, J. F., Pascal, T., Frippiat, C., Magalhaes, J. P., Zdanov, S., and Chainiaux, F. (2002a). "Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions?" *ScientificWorldJournal* **2**(1):230-247. PubMed
Toussaint, O., Remacle, J., Dierick, J. F., Pascal, T., Frippiat, C., Zdanov, S., Magalhaes, J. P., Royer, V., and Chainiaux, F. (2002b). "From the Hayflick mosaic to the mosaics of ageing. Role of stress-induced premature senescence in human ageing." *Int J Biochem Cell Biol* **34**(11):1415-1429. PubMed
Tower, J. (2000). "Transgenic methods for increasing Drosophila life span." *Mech Ageing Dev* **118**(1-2):1-14. PubMed
Townes-Anderson, E., Colantonio, A., and St Jules, R. S. (1998). "Age-related changes in the tiger salamander retina." *Exp Eye Res* **66**(5):653-667. PubMed
Tredici, G., Miloso, M., Nicolini, G., Galbiati, S., Cavaletti, G., and Bertelli, A. (1999). "Resveratrol, map kinases and neuronal cells: might wine be a neuroprotectant?" *Drugs Exp Clin Res* **25**(2-3):99-103. PubMed
Trewavas, A. (2002). "Malthus foiled again and again." *Nature* **418**(6898):668-670. PubMed
Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J. N., Rovio, A. T., Bruder, C. E., Bohlooly, Y. M., Gidlof, S., Oldfors, A., Wibom, R., *et al.* (2004). "Premature ageing in mice expressing defective mitochondrial DNA polymerase." *Nature* **429**(6990):417-423. PubMed
Trifunovic, A., Hansson, A., Wredenberg, A., Rovio, A. T., Dufour, E., Khvorostov, I., Spelbrink, J. N., Wibom, R., Jacobs, H. T., and Larsson, N. G. (2005). "Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production." *Proc Natl Acad Sci U S A* **102**(50):17993-17998. PubMed

Trinei, M., Giorgio, M., Cicalese, A., Barozzi, S., Ventura, A., Migliaccio, E., Milia, E., Padura, I. M., Raker, V. A., Maccarana, M., *et al.* (2002). "A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis." *Oncogene* **21**(24):3872-3878. PubMed
Tsuzuki, T., Egashira, A., Igarashi, H., Iwakuma, T., Nakatsuru, Y., Tominaga, Y., Kawate, H., Nakao, K., Nakamura, K., Ide, F., *et al.* (2001). "Spontaneous tumorigenesis in mice defective in the MTH1 gene encoding 8-oxo-dGTPase." *Proc Natl Acad Sci U S A* **98**(20):11456-11461. PubMed
Turbill, C., and Ruf, T. (2010). "Senescence is more important in the natural lives of long- than short-lived mammals." *PLoS One* **5**(8):e12019. PubMed
Turbill, C., Bieber, C., and Ruf, T. (2011). "Hibernation is associated with increased survival and the evolution of slow life histories among mammals." *Proc Biol Sci* **278**(1723):3355-3363. PubMed
Tuschl, T. (2002). "Expanding small RNA interference." *Nat Biotechnol* **20**(5):446-448. PubMed
Tyner, S. D., Venkatachalam, S., Choi, J., Jones, S., Ghebranious, N., Igelmann, H., Lu, X., Soron, G., Cooper, B., Brayton, C., *et al.* (2002). "p53 mutant mice that display early ageing-associated phenotypes." *Nature* **415**(6867):45-53. PubMed
Tyynismaa, H., Mjosund, K. P., Wanrooij, S., Lappalainen, I., Ylikallio, E., Jalanko, A., Spelbrink, J. N., Paetau, A., and Suomalainen, A. (2005). "Mutant mitochondrial helicase Twinkle causes multiple mtDNA deletions and a late-onset mitochondrial disease in mice." *Proc Natl Acad Sci U S A* **102**(49):17687-17692. PubMed
Ueda, M., Ouhtit, A., Bito, T., Nakazawa, K., Lubbe, J., Ichihashi, M., Yamasaki, H., and Nakazawa, H. (1997). "Evidence for UV-associated activation of telomerase in human skin." *Cancer Res* **57**(3):370-374. PubMed
Umeda-Kameyama, Y., Tsuda, M., Ohkura, C., Matsuo, T., Namba, Y., Ohuchi, Y., and Aigaki, T. (2007). "Thioredoxin suppresses Parkin-associated endothelin receptor-like receptor-induced neurotoxicity and extends longevity in Drosophila." *J Biol Chem* **282**(15):11180-11187. PubMed
Unal, E., Kinde, B., and Amon, A. (2011). "Gametogenesis eliminates age-induced cellular damage and resets life span in yeast." *Science* **332**(6037):1554-1557. PubMed
Unwin, M. J., Kinnison, M. T., and Quinn, T. P. (1999). "Exceptions to semelparity: postmaturation survival, morphology, and energetics of male chinook salmon (Oncorhynchus tshawytscha)." *Can J Fish Aquat Sci*, **56**(7):1172-1181.
Valdesalici, S., and Cellerino, A. (2003). "Extremely short lifespan in the annual fish Nothobranchius furzeri." *Proc Biol Sci* **270 Suppl 2**:S189-191. PubMed
Van Goethem, G., Dermaut, B., Lofgren, A., Martin, J. J., and Van Broeckhoven, C. (2001). "Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions." *Nat Genet* **28**(3):211-212. PubMed
van Loon, A. A., Sonneveld, E., Hoogerbrugge, J., van der Schans, G. P., Grootegoed, J. A., Lohman, P. H., and Baan, R. A. (1993). "Induction and repair of DNA single-strand breaks and DNA base damage at different cellular stages of spermatogenesis of the hamster upon in vitro exposure to ionizing radiation." *Mutat Res* **294**(2):139-148. PubMed
Van Raamsdonk, J. M., and Hekimi, S. (2009). "Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in Caenorhabditis elegans." *PLoS Genet* **5**(2):e1000361. PubMed
Van Remmen, H., Ikeno, Y., Hamilton, M., Pahlavani, M., Wolf, N., Thorpe, S. R., Alderson, N. L., Baynes, J. W., Epstein, C. J., Huang, T. T., *et al.* (2003). "Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging." *Physiol Genomics* **16**(1):29-37. PubMed
van Steensel, B., and de Lange, T. (1997). "Control of telomere length by the human telomeric protein TRF1." *Nature* **385**(6618):740-743. PubMed
van Steensel, B., Smogorzewska, A., and de Lange, T. (1998). "TRF2 protects human telomeres from end-to-end fusions." *Cell* **92**(3):401-413. PubMed
Van Zant, G., and Liang, Y. (2003). "The role of stem cells in aging." *Exp Hematol* **31**(8):659-672. PubMed
Vanfleteren, J. R., and Braeckman, B. P. (1999). "Mechanisms of life span determination in Caenorhabditis elegans." *Neurobiol Aging* **20**(5):487-502. PubMed
Varley, J. M., Evans, D. G., and Birch, J. M. (1997). "Li-Fraumeni syndrome--a molecular and clinical review." *Br J Cancer* **76**(1):1-14. PubMed
Varon, R., Vissinga, C., Platzer, M., Cerosaletti, K. M., Chrzanowska, K. H., Saar, K., Beckmann, G., Seemanova, E., Cooper, P. R., Nowak, N. J., *et al.* (1998). "Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome." *Cell* **93**(3):467-476. PubMed
Vaupel, J. W., Manton, K. G., and Stallard, E. (1979). "The impact of heterogeneity in individual frailty on the dynamics of mortality." *Demography* **16**(3):439-454. PubMed
Vaupel, J. W., Carey, J. R., Christensen, K., Johnson, T. E., Yashin, A. I., Holm, N. V., Iachine, I. A., Kannisto, V., Khazaeli, A. A., Liedo, P., *et al.* (1998). "Biodemographic trajectories of longevity." *Science* **280**(5365):855-860. PubMed
Vaupel, J. W., Baudisch, A., Dolling, M., Roach, D. A., and Gampe, J. (2004). "The case for negative senescence." *Theor Popul Biol* **65**(4):339-351. PubMed
Vaziri, H., and Benchimol, S. (1996). "From telomere loss to p53 induction and activation of a DNA-damage pathway at senescence: the telomere loss/DNA damage model of cell aging." *Exp Gerontol* **31**(1-2):295-301. PubMed
Vaziri, H., West, M. D., Allsopp, R. C., Davison, T. S., Wu, Y. S., Arrowsmith, C. H., Poirier, G. G., and Benchimol, S. (1997). "ATM-dependent telomere loss in aging human diploid fibroblasts and DNA damage lead to the post-translational activation of p53 protein involving poly(ADP-ribose) polymerase." *Embo J* **16**(19):6018-6033. PubMed
Vermulst, M., Bielas, J. H., Kujoth, G. C., Ladiges, W. C., Rabinovitch, P. S., Prolla, T. A., and Loeb, L. A. (2007). "Mitochondrial point mutations do not limit the natural lifespan of mice." *Nat Genet* **39**(4):540-543. PubMed
Vijg, J. (2000). "Somatic mutations and aging: a re-evaluation." *Mutat Res* **447**(1):117-135. PubMed
Vijg, J., and Dolle, M. E. (2002). "Large genome rearrangements as a primary cause of aging." *Mech Ageing Dev* **123**(8):907-915. PubMed
Villeda, S. A., Luo, J., Mosher, K. I., Zou, B., Britschgi, M., Bieri, G., Stan, T. M., Fainberg, N., Ding, Z., Eggel, A., *et al.* (2011). "The ageing systemic milieu negatively regulates neurogenesis and cognitive function." *Nature* **477**(7362):90-94. PubMed
Viswanathan, M., and Guarente, L. (2011). "Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes." *Nature* **477**(7365):E1-2. PubMed
Vleck, C. M., Haussmann, M. F., and Vleck, D. (2003). "The natural history of telomeres: tools for aging animals and exploring the aging process." *Exp Gerontol* **38**(7):791-795. PubMed
Vogel, E. W., and Zijlstra, J. A. (1987). "Somatic cell mutagenicity in Drosophila melanogaster in comparison with genetic damage in early germ-cell stages." *Mutat Res* **180**(2):189-200. PubMed
Vogt, M., Haggblom, C., Yeargin, J., Christiansen-Weber, T., and Haas, M. (1998). "Independent induction of senescence by p16INK4a and p21CIP1 in spontaneously immortalized human fibroblasts." *Cell Growth Differ* **9**(2):139-146. PubMed
Voituron, Y., de Fraipont, M., Issartel, J., Guillaume, O., and Clobert, J. (2011). "Extreme lifespan of the human fish (Proteus anguinus): a challenge for ageing mechanisms." *Biol Lett* **7**(1):105-107. PubMed
von Zglinicki, T., Saretzki, G., Docke, W., and Lotze, C. (1995). "Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence?" *Exp Cell Res* **220**(1):186-193. PubMed
von Zglinicki, T. (2000). "Role of oxidative stress in telomere length regulation and replicative senescence." *Ann N Y Acad Sci* **908**:99-110. PubMed
Vreeland, R. H., Rosenzweig, W. D., and Powers, D. W. (2000). "Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal." *Nature* **407**(6806):897-900. PubMed
Vulliamy, T., Marrone, A., Goldman, F., Dearlove, A., Bessler, M., Mason, P. J., and Dokal, I. (2001). "The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita." *Nature* **413**(6854):432-435. PubMed
Wakayama, T., Shinkai, Y., Tamashiro, K. L., Niida, H., Blanchard, D. C., Blanchard, R. J., Ogura, A., Tanemura, K., Tachibana, M., Perry, A. C., *et al.* (2000). "Cloning of mice to six generations." *Nature* **407**(6802):318-319. PubMed
Wallace, D. C. (1992). "Mitochondrial genetics: a paradigm for aging and degenerative diseases?" *Science* **256**(5057):628-632. PubMed
Wallace, D. C. (1999). "Mitochondrial diseases in man and mouse." *Science* **283**(5407):1482-1488. PubMed
Walter, C. A., Lu, J., Bhakta, M., Zhou, Z. Q., Thompson, L. H., and McCarrey, J. R. (1994). "Testis and somatic Xrcc-1 DNA repair gene expression." *Somat Cell Mol Genet* **20**(6):451-461. PubMed
Walter, C. A., Walter, R. B., and McCarrey, J. R. (2003). "Germline genomes--a biological fountain of youth?" *Sci SAGE KE* **2003**(8):PE4. PubMed
Wang, S. M., Nishigori, C., Yagi, T., and Takebe, H. (1991). "Reduced DNA repair in progeria cells and effects of gamma-ray irradiation on UV-induced unscheduled DNA synthesis in normal and progeria cells." *Mutat Res* **256**(1):59-66. PubMed
Wang, X., Tsao, S. W., Wong, Y. C., and Cheung, A. L. (2003). "Induction of senescent-like growth arrest as a new target in anticancer treatment." *Curr Cancer Drug Targets* **3**(2):153-159. PubMed
Wang, C., Jurk, D., Maddick, M., Nelson, G., Martin-Ruiz, C., and von Zglinicki, T. (2009). "DNA damage response and cellular senescence in tissues of aging mice." *Aging Cell* **8**(3):311-323. PubMed
Wang, J., Geesman, G. J., Hostikka, S. L., Atallah, M., Blackwell, B., Lee, E., Cook, P. J., Pasaniuc, B., Shariat, G., Halperin, E., *et al.* (2011). "Inhibition of activated pericentromeric SINE/Alu repeat transcription in senescent human adult stem cells reinstates self-renewal." *Cell Cycle* **10**(17):3016-3030. PubMed
Warner, H. R. (2001). "Support for basic gerontological research in the USA." *Exp Gerontol* **36**(3):403-412. PubMed
Warner, H., Miller, R. A., and Carrington, J. (2002). "Meeting report--National Institute on Aging Workshop on the Comparative Biology of Aging." *Sci Aging Knowledge Environ* **2002**(17):pe5. PubMed
Warner, H. R. (2003). "Subfield history: use of model organisms in the search for human aging genes." *Sci Aging Knowl Environ* **2003**(6):RE1. PubMed
Warner, H., Anderson, J., Austad, S., Bergamini, E., Bredesen, D., Butler, R., Carnes, B. A., Clark, B. F., Cristofalo, V., Faulkner, J., *et al.* (2005). "Science fact and the SENS agenda. What can we reasonably expect from ageing research?" *EMBO Rep* **6**(11):1006-1008. PubMed
Warner, H. R. (2006). "Scientific and ethical concerns regarding engineering human longevity." *Rejuvenation Res* **9**(4):440-442; discussion 443-445. PubMed
Waters, H., and Walford, R. L. (1970). "Latent period for outgrowth of human skin explants as a function of age." *J Gerontol* **25**(4):381-383. PubMed
Watkinson, A. R., and White, J. (1985). "Some life-history consequences of modular construction in plants." *Phil Trans Ro Soc* **313**:31-51.
Watson, J. D. (1972). "Origin of concatemeric T7 DNA." *Nat New Biol* **239**(94):197-201. PubMed
Weeda, G., Donker, I., de Wit, J., Morreau, H., Janssens, R., Vissers, C. J., Nigg, A., van Steeg, H., Bootsma, D., and Hoeijmakers, J. H. (1997). "Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence." *Curr Biol* **7**(6):427-439. PubMed
Wei, W., and Sedivy, J. M. (1999). "Differentiation between senescence (M1) and crisis (M2) in human fibroblast cultures." *Exp Cell Res* **253**(2):519-522. PubMed
Weindruch, R., Walford, R. L., Fligiel, S., and Guthrie, D. (1986). "The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake." *J Nutr* **116**(4):641-654. PubMed
Weindruch, R., and Walford, R. L. (1988). *The Retardation of Aging and Disease by Dietary Restriction*. C. C. Thomas, Springfield.

Weindruch, R. (1996). "The retardation of aging by caloric restriction: studies in rodents and primates." *Toxicol Pathol* **24**(6):742-745. PubMed
Weinert, B. T., and Timiras, P. S. (2003). "Invited review: Theories of aging." *J Appl Physiol* **95**(4):1706-1716. PubMed
Weintraub, S. J., Prater, C. A., and Dean, D. C. (1992). "Retinoblastoma protein switches the E2F site from positive to negative element." *Nature* **358**(6383):259-261. PubMed
Weismann, A. (1891). *On Heredity*. Claredon Press, Oxford.
Wellinger, R. J., Ethier, K., Labrecque, P., and Zakian, V. A. (1996). "Evidence for a new step in telomere maintenance." *Cell* **85**(3):423-433. PubMed
Werness, B. A., Levine, A. J., and Howley, P. M. (1990). "Association of human papillomavirus types 16 and 18 E6 proteins with p53." *Science* **248**(4951):76-79. PubMed
White, R. J., Albin, M. S., Verdura, J., Takaoka, Y., Massopust, L. C., Wolin, L. R., Locke, G. E., Taslitz, N., and Yashon, D. (1996). "The isolation and transplantation of the brain. An historical perspective emphasizing the surgical solutions to the design of these classical models." *Neurol Res* **18**(3):194-203. PubMed
Whitlock, G., Lewington, S., Sherliker, P., Clarke, R., Emberson, J., Halsey, J., Qizilbash, N., Collins, R., and Peto, R. (2009). "Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies." *Lancet* **373**(9669):1083-1096. PubMed
Wiley, C. (2005). "Nanotechnology and molecular homeostasis." *J Am Geriatr Soc* **53**(9 Suppl):S295-298. PubMed
Willcox, D. C., Willcox, B. J., Todoriki, H., Curb, J. D., and Suzuki, M. (2006). "Caloric restriction and human longevity: what can we learn from the Okinawans?" *Biogerontology* **7**(3):173-177. PubMed
Williams, G. C. (1957). "Pleiotropy, natural selection, and the evolution of senescence." *Evolution* **11**:398-411.
Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., and Campbell, K. H. (1997). "Viable offspring derived from fetal and adult mammalian cells." *Nature* **385**(6619):810-813. PubMed
Wilson, D. L. (1994). "The analysis of survival (mortality) data: fitting Gompertz, Weibull, and logistic functions." *Mech Ageing Dev* **74**(1-2):15-33. PubMed
Witte, A. V., Fobker, M., Gellner, R., Knecht, S., and Floel, A. (2009). "Caloric restriction improves memory in elderly humans." *Proc Natl Acad Sci U S A* **106**(4):1255-1260. PubMed
Wodinsky, J. (1977). "Hormonal inhibition of feeding of death in octopus. Control by optic gland secretion." *Science* **198**:948-951.
Wolf, N., Penn, P., Pendergrass, W., Van Remmen, H., Bartke, A., Rabinovitch, P., and Martin, G. M. (2005). "Age-related cataract progression in five mouse models for anti-oxidant protection or hormonal influence." *Exp Eye Res* **81**(3):276-285. PubMed
Wolkow, C. A., Kimura, K. D., Lee, M. S., and Ruvkun, G. (2000). "Regulation of C. elegans life-span by insulinlike signaling in the nervous system." *Science* **290**(5489):147-150. PubMed
Wong, H., and Riabowol, K. (1996). "Differential CDK-inhibitor gene expression in aging human diploid fibroblasts." *Exp Gerontol* **31**(1-2):311-325. PubMed
Wong, A., Yang, J., Cavadini, P., Gellera, C., Lonnerdal, B., Taroni, F., and Cortopassi, G. (1999). "The Friedreich's ataxia mutation confers cellular sensitivity to oxidant stress which is rescued by chelators of iron and calcium and inhibitors of apoptosis." *Hum Mol Genet* **8**(3):425-430. PubMed
Wong, K. K., Maser, R. S., Bachoo, R. M., Menon, J., Carrasco, D. R., Gu, Y., Alt, F. W., and DePinho, R. A. (2003). "Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing." *Nature* **421**(6923):643-648. PubMed
Wong, E., and Cuervo, A. M. (2010). "Autophagy gone awry in neurodegenerative diseases." *Nat Neurosci* **13**(7):805-811. PubMed
Wood, J. G., Rogina, B., Lavu, S., Howitz, K., Helfand, S. L., Tatar, M., and Sinclair, D. (2004). "Sirtuin activators mimic caloric restriction and delay ageing in metazoans." *Nature* **430**(7000):686-689. PubMed
Wright, W. E., and Hayflick, L. (1975). "Nuclear control of cellular aging demonstrated by hybridization of anucleate and whole cultured normal human fibroblasts." *Exp Cell Res* **96**(1):113-121. PubMed
Wright, W. E., and Shay, J. W. (2001). "Cellular senescence as a tumor-protection mechanism: the essential role of counting." *Curr Opin Genet Dev* **11**(1):98-103. PubMed
Wyllie, F. S., Jones, C. J., Skinner, J. W., Haughton, M. F., Wallis, C., Wynford-Thomas, D., Faragher, R. G., and Kipling, D. (2000). "Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts." *Nat Genet* **24**(1):16-17. PubMed
Wynford-Thomas, D. (1999). "Cellular senescence and cancer." *J Pathol* **187**(1):100-111. PubMed
Yang, J. H., Lee, H. C., Lin, K. J., and Wei, Y. H. (1994). "A specific 4977-bp deletion of mitochondrial DNA in human ageing skin." *Arch Dermatol Res* **286**(7):386-390. PubMed
Yang, X., and Tian, X. C. (2000). "Cloning adult animals - what is the genetic age of the clones?" *Cloning* **2**(3):123-128. PubMed
Yang, H., Baur, J. A., Chen, A., Miller, C., and Sinclair, D. A. (2007). "Design and synthesis of compounds that extend yeast replicative lifespan." *Aging Cell* **6**(1):35-43. PubMed
Yawata, T., Kamino, H., Kugoh, H., Katoh, M., Nomura, N., Oishi, M., Horikawa, I., Barrett, J. C., and Oshimura, M. (2003). "Identification of a </= 600-kb region on human chromosome 1q42.3 inducing cellular senescence." *Oncogene* **22**(2):281-290. PubMed
Young, L. E., Fernandes, K., McEvoy, T. G., Butterwith, S. C., Gutierrez, C. G., Carolan, C., Broadbent, P. J., Robinson, J. J., Wilmut, I., and Sinclair, K. D. (2001). "Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture." *Nat Genet* **27**(2):153-154. PubMed
Yu, G. L., Bradley, J. D., Attardi, L. D., and Blackburn, E. H. (1990). "In vivo alteration of telomere sequences and senescence caused by mutated Tetrahymena telomerase RNAs." *Nature* **344**(6262):126-132. PubMed
Yu, C. E., Oshima, J., Fu, Y. H., Wijsman, E. M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S., *et al.* (1996). "Positional cloning of the Werner's syndrome gene." *Science* **272**(5259):258-262. PubMed
Yu, C. E., Oshima, J., Wijsman, E. M., Nakura, J., Miki, T., Piussan, C., Matthews, S., Fu, Y. H., Mulligan, J., Martin, G. M., *et al.* (1997). "Mutations in the consensus helicase domains of the Werner syndrome gene. Werner's Syndrome Collaborative Group." *Am J Hum Genet* **60**(2):330-341. PubMed
Yu, C., Li, Y., Holmes, A., Szafranski, K., Faulkes, C. G., Coen, C. W., Buffenstein, R., Platzer, M., de Magalhaes, J. P., and Church, G. M. (2011). "RNA sequencing reveals differential expression of mitochondrial and oxidation reduction genes in the long-lived naked mole-rat when compared to mice." *PLoS One* **6**(11):e26729. PubMed
Yuan, H., Kaneko, T., and Matsuo, M. (1996). "Increased susceptibility of late passage human diploid fibroblasts to oxidative stress." *Exp Gerontol* **31**(4):465-474. PubMed
Zahavi, A. (1975). "Mate selection-a selection for a handicap." *J Theor Biol* **53**(1):205-214. PubMed
Zahn, J. M., Poosala, S., Owen, A. B., Ingram, D. K., Lustig, A., Carter, A., Weeraratna, A. T., Taub, D. D., Gorospe, M., Mazan-Mamczarz, K., *et al.* (2007). "AGEMAP: a gene expression database for aging in mice." *PLoS Genet* **3**(11):e201. PubMed
Zec, R. F. (1995). "The neuropsychology of aging." *Exp Gerontol* **30**(3-4):431-442. PubMed
Zhang, C., and Cuervo, A. M. (2008). "Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function." *Nat Med* **14**(9):959-965. PubMed
Zhao, Z. Y., Xie, Y., Fu, Y. R., Bogdan, A., and Touitou, Y. (2002). "Aging and the circadian rhythm of melatonin: a cross-sectional study of Chinese subjects 30-110 yr of age." *Chronobiol Int* **19**(6):1171-1182. PubMed
Zhou, Z. Q., Manguino, D., Kewitt, K., Intano, G. W., McMahan, C. A., Herbert, D. C., Hanes, M., Reddick, R., Ikeno, Y., and Walter, C. A. (2001). "Spontaneous hepatocellular carcinoma is reduced in transgenic mice overexpressing human O6- methylguanine-DNA methyltransferase." *Proc Natl Acad Sci U S A* **98**(22):12566-12571. PubMed
Zhou, J., Liu, J., Cheng, C. J., Patel, T. R., Weller, C. E., Piepmeier, J. M., Jiang, Z., and Saltzman, W. M. (2011). "Biodegradable poly(amine-co-ester) terpolymers for targeted gene delivery." *Nat Mater* **11**(1):82-90. PubMed
Zhu, J., Wang, H., Bishop, J. M., and Blackburn, E. H. (1999). "Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening." *Proc Natl Acad Sci U S A* **96**(7):3723-3728. PubMed
Zimmermann, S., Voss, M., Kaiser, S., Kapp, U., Waller, C. F., and Martens, U. M. (2003). "Lack of telomerase activity in human mesenchymal stem cells." *Leukemia* **17**(6):1146-1149. PubMed
Ziuganov, V. V. (2005). "A long-lived parasite extending the host life span: the pearl mussel Margaritifera margaritifera elongates host life by turns out the program of accelerated senescence in salmon Salmo salar." *Dokl Biol Sci* **403**:291-294. PubMed
Zuk, M. (2009). "The sicker sex." *PLoS Pathog* **5**(1):e1000267. PubMed
Zwaan, B. J. (2003). "Linking development and aging." *Sci Aging Knowledge Environ* **2003**(47):pe32. PubMed