2 The biology of ageing

A primer

JOÃO PEDRO DE MAGALHÃES

OVERVIEW

This chapter introduces key biological concepts of ageing. First, it defines ageing and presents the main features of human ageing, followed by a consideration of evolutionary models of ageing. Causes of variation in ageing (genetic and dietary) are reviewed, before examining biological theories of the causes of ageing.

Introduction

Thanks to technological progress in different areas, including biomedical breakthroughs in preventing and treating infectious diseases, longevity has been increasing dramatically for decades. The life expectancy at birth in the UK for boys and girls rose, respectively, from 45 and 49 years in 1901 to 75 and 80 in 1999 with similar figures reported for other industrialized nations (see Chapter 1 for further discussion). A direct consequence is a steady increase in the proportion of people living to an age where their health and well-being are restricted by ageing. By the year 2050, it is estimated that the percentage of people in the UK over the age of 65 will rise to over 25 per cent, compared to 14 per cent in 2004 (Smith, 2004).

The greying of the population, discussed elsewhere (see Chapter 1), implies major medical and societal changes. Although ageing is no longer considered by health professionals as a direct cause of death (Hayflick, 1994), the major killers in industrialized nations are now age-related diseases like cancer, diseases of the heart and

neurodegenerative diseases. The study of the biological mechanisms of ageing is thus not merely a topic of scientific curiosity, but a crucial area of research throughout the twenty-first century. In recent years, considerable progress has been made in understanding the biology of ageing. This chapter aims to describe the major observations, theories and hypotheses of *biogerontology*, the field of research that studies ageing from a biological perspective. A brief discussion on the prospects of developing therapies that delay the process of ageing and an overview of future critical areas in biogerontology are also included.

What is ageing?

Human ageing entails multiple changes at different levels. Some, like wrinkles or grey hair, are more visible than others. Age-related changes also make themselves felt at the functional and physiological level. By and large, most functions begin to decline linearly after reaching peak performance in the third decade of life (Strehler, 1999). It is common knowledge that the ability of adults to perform physical tasks declines with age. Due to loss of muscle and bone mass, ageing is also characterized by weight loss. Though there is considerable individual variability and no two people age alike, other physiological and functional hallmarks of ageing include a gradual reduction in height, a lower metabolic rate, longer reaction times and decreased sexual activity (see Chapter 10). In women, menopause or reproductive senescence is an inevitable consequence of old age. Functional declines in kidney, pulmonary and immune functions are also frequent. Lastly, as discussed in Chapter 7, a major concern of older adults is mental health since memory and cognitive impairment are associated with human ageing even in the absence of disease (Arking, 2006; Finch, 1990; Hayflick, 1994).

Although not all physiological changes lead to pathology, ultimately functional and physiological changes render people more susceptible to a number of diseases (Figure 2.1). Ageing has been defined as an intrinsic, inevitable and irreversible age-related process of loss of viability and increase in vulnerability (Comfort, 1964). Practically any system, tissue or organ can fail because of ageing (Austad, 1997b; Strehler, 1999). The heart, a critical organ with little room for error or rest, is the organ that most often fails. For people over the age of 85, diseases of the heart are the major cause of death, responsible for almost 40 per cent of all deaths, followed by cancer, cerebrovascular diseases, neurodegenerative diseases like Alzheimer's and Parkinson's disease, infectious diseases and diabetes (Heron, Hoyert, Jiaquan *et al.*, 2008). (Health issues of older adults are further discussed in the following two chapters of this book.)

It is well known that women live longer than men, even though it does not appear that women age more slowly. At virtually all ages, women have lower mortality rates for the major causes of death, including heart diseases and cancer. It is as

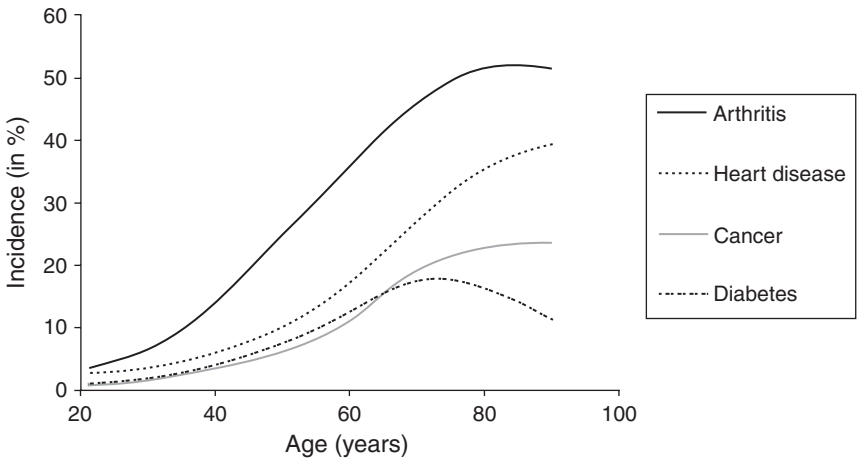

Figure 2.1 Prevalence of selected chronic conditions as a function of age. Values are expressed in percentage for the US population (2002–03 data set). *Source:* Centers for Disease Control and Prevention, 2008.

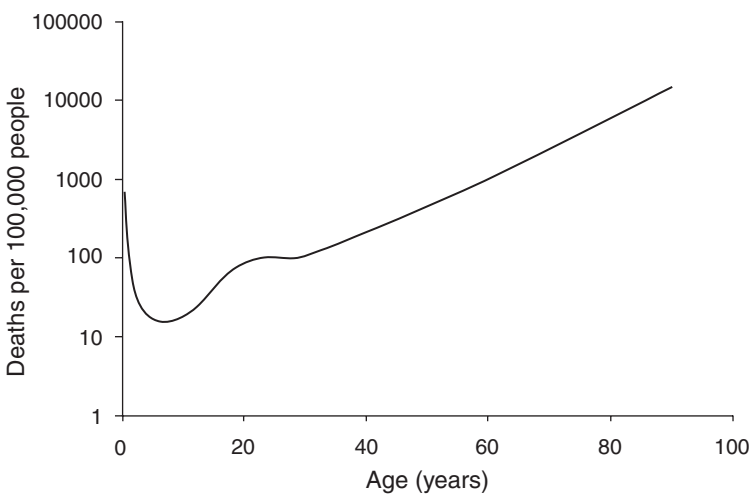

Figure 2.2 Mortality rates as a function of age. Values are expressed in deaths per 100,000 people, plotted on a logarithmic scale, for the 2002 US population. *Source:* Centers for Disease Control and Prevention, 2008.

if women were more robust than men. The causes for these gender differences remain a mystery (Austad, 2006). Paradoxically, it is conventional wisdom that women have poorer health than men, though sex differences in morbidity suggest the situation to be more complex than conventional wisdom suggests, with strong variation observed across the lifespan (Macintyre, Hunt and Sweeting, 1996).

Because ageing increases the morbidity of many diseases, one of the defining features of ageing is an increased probability of death with age (Figure 2.2). In fact, ageing has been defined as the collection of changes that render human beings

progressively more likely to die (Medawar, 1952). As is clear from Figure 2.2, human mortality rates increase exponentially after about age 30. Specifically, the probability of dying for a given individual over the age of 30 doubles approximately every 8 years (Finch, 1990). The exponential increase in mortality with age is a hallmark of ageing, also observed in many other species and in animal models of biomedical research. The rate at which mortality increases with age in a given population can also be used as an estimate of the rate of ageing. For humans the 8-year mortality doubling time seems to be relatively constant across populations (Finch, 1990), though in animal models this value can vary substantially between species and even strains of the same species and can be used to test for the effects on the process of ageing of a given gene or intervention, as further discussed below.

Thus, human ageing encompasses physiological changes that typically lead to a functional decline with age, which in turn leads to a loss of the equilibrium between different physiological systems and their capacity to respond to environmental challenges, also known as *homeostasis*. As organs' functions are progressively impaired, this results in an increased susceptibility to most diseases and it is this that gives rise to the exponential increase in mortality. Ageing can thus be defined as a progressive deterioration of physiological function, accompanied by an increase in vulnerability and mortality with age. The basic process of ageing, or *senescence* as some call it, can be defined as the underlying process synchronizing the progression of the different components of ageing and is the main focus of research in biogerontology.

The evolutionary theory of ageing

As detailed above, biological ageing implies frailty, progressive functional decline and ultimately death. One puzzle of biogerontology is why, in light of evolution and the apparent disadvantage to organisms that ageing represents, is there an ageing process at all? One of the first people to address this apparent paradox was August Weismann. He proposed the hypothesis known as *group selection*, which states that ageing evolved because it benefits the species or group, even if it is detrimental to the individual (Weismann, 1891). Weismann later abandoned this concept and instead advocated that organisms that segregate *germ* and *soma* – respectively, reproductive cells and all other cells – must invest more resources to reproduce rather than to maintain the soma and ageing is the consequence of organisms renouncing the soma. Unlike group selection, which today is dismissed by most authorities, the idea of soma–germ segregation playing a role in the evolution of ageing has remained until now. It was later revised to become the *disposable soma theory*, which states that there is a trade-off between investment in soma

maintenance and reproduction, such that the body declines so that reproductive capacity can be maintained (Kirkwood, 1977).

Arguably, the first model of the evolution of ageing was put forward by Peter Medawar more than half a century after Weismann's work. Inspired by earlier work from the evolutionary biologists Fisher and Haldane, Medawar assumed that, because the greatest contribution to create a new generation comes from young, not old organisms, the force of natural selection fades with age (Medawar, 1952). In the wild, the mortality of most animals is very high. Hence, if the probability of a mouse reaching age 5 is only 0.01 per cent, then there is little evolutionary pressure to select for gene variants, called *alleles*, which maintain function until age 5. Furthermore, a deleterious mutation that is harmful or even lethal at age 5 will not be selected against because few if any animals will ever reach such age, allowing late-acting hazardous alleles to persist in the population. This theory became known as the *mutation accumulation theory* (Hamilton, 1966; Kirkwood and Austad, 2000; Rose, 1991). Shortly afterwards, a complementary theory was developed by George Williams (1957). This argued that a gene that increases the chances of the survival of a mouse to reproductive age will be favoured even if the same gene produces lethal effects at age 5. As a result, genes with beneficial effects at early ages but harmful at later ones will be favoured by natural selection. Because genes with opposing effects are known as *pleiotropic*, this theory became known as the *antagonistic pleiotropy theory* (Williams, 1957).

Therefore, two main models exist for explaining the evolution of ageing: one in which harmful late-acting mutations accumulate because the force of natural selection is weaker at later ages, and the other in which harmful late-acting mutations accumulate because they are beneficial at earlier ages. Both theories, together with the mathematical models of Hamilton (1966), compose the classical evolutionary theory of ageing. The theory and models are largely supported by experimental evidence (Kirkwood and Austad, 2000; Rose, 1991). A few findings are incompatible with the theory, however. The observation that some animals might not age at all, or at least fail to exhibit the typical hallmarks of ageing even after decades of study (Finch, 1990), conflicts with the predictions of Hamilton (1966), and suggests that some refinements can still be made to the theory (Vaupel, Baudisch, Dolling *et al.*, 2004). Nonetheless, the evolutionary theory of ageing is one of the theoretical landmarks of biogerontology.

Life history theory

The evolutionary theory of ageing is part of a larger theoretical framework known as life history theory. This addresses the changes organisms undergo from conception to death, focusing particularly on the schedule of reproduction and

Table 2.1 Diversity of mammalian life histories obtained from the AnAge database (derived from: http://genomics.senescence.info/species/)

Organism (species)	Longevity (years)	Maturity (years)	Body mass (kilograms)
Human (*Homo sapiens*)	122.5	13	60
Chimpanzee (*Pan troglodytes*)	59.4	9	45
Rhesus monkey (*Macaca mulatta*)	40	5	8
Marmoset (*Callithrix jacchus*)	16.5	1.25	0.25
Mouse (*Mus musculus*)	4	0.15	0.02
Naked mole rat (*Heterocephalus glaber*)	28.3	0.75	0.03
Shrew (*Crocidura russula*)	4	0.2	0.01
Bat (*Myotis lucifugus*)	34	<1	0.01
Elephant (*Elephas maximus*)	65.5	9	3000
Sheep (*Ovis aries*)	22.8	2	110
Horse (*Equus caballus*)	57	3	250
Bowhead whale (*Balaena mysticetus*)	211	22.5	100,000
Cat (*Felis catus*)	>30	1	4
Opossum (*Didelphis virginiana*)	6.6	0.5	3

survival (Stearns, 1992). An extraordinary diversity of lifespans and ageing phenotypes – the characteristics of an organism as determined by both genes and the environment – is found among the world's species. Some organisms, like certain turtles and rockfishes, do not exhibit signs of ageing for many decades and are known as cases of *negligible senescence* (Finch, 1990). On the other hand, even vertebrates can exhibit extremely short lifespans, such as certain African annual fishes that in the laboratory do not live more than 12 weeks and exhibit signs of an extremely fast ageing process (Valdesalici and Cellerino, 2003). Intriguingly, some organisms are *semelparous*, which means they reproduce only once (also called 'big bang reproduction'), in contrast to *iteroparous* species like humans that can reproduce multiple times across the lifespan. The classical example of a semelparous species is the salmon, which dies shortly after spawning, exhibiting several pathologies. Castrated animals live longer and a hormonal cascade appears to trigger both reproduction and degeneration leading to death (Finch, 1990; Gosden, 1996; Hayflick, 1994).

Even similar species such as mammals can exhibit marked differences in longevity (Table 2.1) and ageing (Austad, 1997a, 1997b). Among mammals, only a few marsupials, like certain species of the mouse-like genus *Antechinus*, exhibit semelparity that is also triggered by hormonal changes. No mammalian species is known to avoid ageing (Finch, 1990; Gosden, 1996). Nonetheless, mammals have a wide range of lifespans from short-lived species like mice, which do not commonly live more than 4 years even in protective environments and exhibit marked

signs of ageing, to the estimated 211 years one bowhead whale had lived by the time it was killed by Alaskan Inuit (George *et al.*, 1999). Humans are the second longest-lived mammal and there is abundant evidence that longevity evolved in the primate lineage leading to humans (Finch, 1990). Understanding these differences in longevity and how it evolved could provide important insights about the biology of ageing.

Life history theory provides a structure to understand these differences in *lifespan* and *longevity* – which are used herein interchangeably, though often lifespan means average lifespan while longevity represents maximum lifespan. Of interest is the concept of *r*- and *K*-*selections* (MacArthur and Wilson, 1967). In this model, animals in hazardous environments and thus with high mortality will maximize reproduction and thus be *r*-selected while organisms in non-hazardous environments will maximize performance under crowded conditions and be *K*-selected. In practice, *r*-selection will favour rapid development, small body size and a short lifespan while *K*-selection will favour delayed development, larger body sizes and a longer lifespan (Austad, 1997a). Humans and whales are examples of *K*-selection while mice are an example of *r*-selection (Table 2.1). Although the *r*- and *K*-selection model is now recognized as an oversimplification and more complex models exist, it can be useful to interpret life history events and strategies in the context of biogerontology.

By relating the environments under which organisms evolve to their life history, evolutionary theory provides an understanding of why different species evolve different lifespans and ageing phenotypes. There is a great interest, however, in understanding the exact genetic and physiological mechanisms underlying species differences in longevity and ageing, in particular differences between humans and related species, as these may provide clues about the human ageing process. To study species differences in ageing, researchers can compare species with different lifespans and attempt to correlate some factor or trait under study, such as the rate a given change occurs with age or potentially important functions like repair mechanisms, with the longevity of the species. Some of the mechanistic theories of ageing detailed below have been tested by such comparative approaches. Life history theory and the evolutionary theory of ageing in particular provide the theoretical framework for interpreting such experiments.

The plasticity of ageing

Ageing is thus not the immutable feature of all living things that people generally assume it to be. As mentioned above, some species appear to avoid it. Besides, differences in genes and environments can give rise to marked differences in

ageing between individuals of the same species. In particular, experiments in model organisms have demonstrated that ageing can be manipulated by genetic and environmental factors. Understanding the mechanisms involved might allow human ageing to be manipulated. As such, the discoveries detailed below on the plasticity of ageing are among the most important in our understanding of the biology of ageing.

Caloric restriction

In the 1930s, Clive McCay and colleagues, guided by earlier findings, tested the idea that growth retardation could extend lifespan. They showed in a carefully conducted study that it is possible to extend the lifespan of rats by limiting the amount of calories in their diet while maintaining normal amounts of other nutrients (McCay, Crowell and Maynard, 1935). This phenomenon became known as caloric restriction (CR), or calorie restriction as some call it. Interpreting experimental results is seldom straightforward in biogerontology. In the same way that women live longer than men but do not age slower, the health, mortality and consequently average lifespan of laboratory animals can be manipulated by interventions without any effect on the ageing process. This is a crucial but often misunderstood issue in interpreting experimental results because it means that genes or interventions that affect the lifespan of animals are not necessarily influencing the process of ageing. From studies in mice and rats, not only can CR increase longevity by up to 50 per cent in certain strains but it also delays physiological ageing, postpones or diminishes the morbidity of most age-related diseases and decreases the rate of exponential increase of mortality (Masoro, 2005; Weindruch and Walford, 1988). Therefore, at least in some cases, it appears that the process of ageing is delayed by CR.

As the first robust method to extend lifespan and modulate ageing, CR has been intensively studied for decades and has been shown to increase lifespan in many organisms, including mice, rats, flies, worms and yeast. There are exceptions, however. CR does not appear to extend the lifespan of the housefly (Cooper, Mockett, Sohal *et al.*, 2004). There is also variation in the effects of CR on different strains of the same species. CR does not appear to extend average lifespan in wild-derived mice, even though it might slightly increase maximum lifespan and protect against cancer (Harper, Leathers and Austad, 2006). Because wild-derived mice are genetically more heterogeneous than laboratory strains, it could be that different individuals respond to CR in different ways and only a fraction of animals benefit from it. Also, in spite of its advantages, CR has been shown to have negative side effects, such as rendering mice more susceptible to infection (Gardner, 2005). As

predicted by McCay, animals under CR are normally smaller than controls. Lastly, not much is known about the mechanisms by which CR extends lifespan, though a few mechanistic hypotheses are discussed further below.

Whether CR might delay human ageing remains a subject of intense debate. Ongoing studies in rhesus monkeys suggest that CR delays the onset of age-related diseases (Colman *et al.*, 2009). There are no long-term studies of CR in humans, however. In spite of some practitioners, CR has considerable side effects, such as mental stress and sexual dysfunctions (Dirks and Leeuwenburgh, 2006). Short-term studies have demonstrated beneficial effects of CR, such as in reducing the risk of atherosclerosis (Fontana, Meyer, Klein *et al.*, 2004). Of course, limiting calorie intake may be beneficial to health, as is conventional wisdom particularly in our societies struck by an 'obesity epidemic', without necessarily delaying the ageing process. Possibly, CR has benefits for some people, but not for all as it is unlikely that lean people will benefit from such extreme diet (Fontana and Klein, 2007). Whether CR can delay human ageing will likely generate more heat than light for years to come.

Human progeroid syndromes

One of the most fascinating observations in the biology of ageing is the accelerated ageing phenotype observed in rare patients suffering from what are known as progeroid syndromes. Many diseases can result in progeroid features (signs of early ageing such as an early greying of hair or premature cognitive impairment), but only a few diseases lead to a multitude of these features. Among such diseases are *Werner*, *Cockayne* and *Hutchinson–Gilford syndromes* (Martin, 1978; Martin and Oshima, 2000). Werner syndrome is the best studied of these. In spite of a short stature, patients with this disease tend to develop normally, and typically the first symptom is the absence of the normal teenage growth spurt. As the disease progresses, however, patients develop at an early age several characteristics resembling typical ageing such as cataracts, grey hair and aged skin (Martin, 1982). Patients also abnormally develop early age-related diseases like diabetes, osteoporosis, baldness and atherosclerosis, and their average lifespan is only 47–48 years (Goto, 1997; Martin and Oshima, 2000). Martin (1982), in fact, estimated that Werner syndrome patients prematurely develop roughly half of all ageing characteristics. Werner syndrome is the result of mutations in a single gene, the Werner syndrome gene (*WRN*) (Yu *et al.*, 1996), and demonstrates how disruption of a single gene can modulate multiple age-related changes and pathologies (Goto, 1997). No other known disease mimics ageing as faithfully as Werner syndrome

does, though patients with Hutchinson–Gilford and Cockayne syndromes develop multiple signs of premature ageing. Contrary to patients with Werner syndrome who normally reach adulthood, patients with Hutchinson–Gilford or Cockayne syndrome are almost exclusively children.

Genetic manipulations

It is much easier to stop a machine than it is to make it work longer. A small flaw in a critical component of a large machine can break it down over time, but in order to make a machine operate longer many key components need to be made more robust. Therefore, the discovery that alterations in single genes could increase lifespan and delay ageing was not only startling but contrary to many previously held views (Johnson, 2002). Thomas Johnson and colleagues were the first to show that ageing could be regulated by single genes. Working on the roundworm nematode *Caenorhabditis elegans*, they showed that different alleles could result in worms living up to twice as long (Friedman and Johnson, 1988). They called the gene *age-1*. A few years later, Cynthia Kenyon and colleagues showed that mutations in *daf-2* could also double the lifespan of worms (Kenyon, Chang, Gensch *et al.*, 1993). For mutations in *daf-2* to extend lifespan, the activity of a second gene, *daf-16*, is required. Both *daf-2* and *daf-16* were named because of their involvement in a developmental arrest form of worms called *dauer*, induced in times of starvation or crowding and known to be long-lived. Because *daf-2* mutants develop normally into adulthood, however, Kenyon's results indicated the existence of a regulated life extension mechanism. A few years later, Gary Ruvkun and colleagues showed that *age-1*, *daf-2* and *daf-16* encode, respectively, a signalling protein of the phosphoinositide-3-kinase family, a member of the insulin receptor family and a member of the forkhead family of transcription factors involved in development. These three genes – as well as many others – have since been shown to play roles in a pathway known as the insulin/insulin-like growth factor 1 (IGF1) signalling pathway. This pathway encompasses hormonal signals that trigger a signal transduction cascade regulating ageing not only in worms but also in flies and, as detailed below, mice (Finch and Ruvkun, 2001; Kenyon, 2010; Tatar, Bartke and Antebi, 2003).

The work of Johnson, Kenyon, Ruvkun and others opened the door for remarkable progress on the genetics of ageing. For a number of reasons outside the scope of this chapter, but including short generation times and high fecundity as well as the availability of tools for genetic manipulation, by far the four most widely used model organisms for genetic studies of ageing are yeast, fruit flies, roundworms and mice. Not surprisingly, the vast majority of genes shown to modulate ageing

Table 2.2 Genes associated with ageing and/or longevity per model organism obtained from the GenAge database (derived from: http://genomics.senescence.info/genes/)

Organism (species)	Number of genes
Roundworm (*Caenorhabditis elegans*)	555
Baker's yeast (*Saccharomyces cerevisiae*)	87
Fruit fly (*Drosophila melanogaster*)	75
Mouse (*Mus musculus*)	68
Filamentous fungus (*Podospora anserina*)	2
Golden hamster (*Mesocricetus auratus*)	1
Fission yeast (*Schizosaccharomyces pombe*)	1

and/or longevity have been identified in these organisms (Table 2.2). In worms, thanks to high-throughput technologies that allow researchers to screen for the effects on longevity of hundreds of genes simultaneously, over 500 genes have been shown to modulate longevity. Many of these ageing-associated genes appear to be related to common pathways, such as the insulin/IGF1 pathway which is the best studied pathway in the context of ageing (Guarente and Kenyon, 2000; Kenyon, 2010).

In mice, there are several genes that when individually disrupted result in a phenotype resembling accelerated ageing. Of even greater interest, a handful of genes have been shown to increase lifespan in mice, most of which are related to the mammalian equivalent of the insulin/IGF1 pathway. The first of these genes was identified somewhat serendipitously by Andrzej Bartke and colleagues. While working on a mouse strain called the Ames dwarf mouse, which has a small body size due to a lack of growth hormone, Bartke and colleagues noticed that animals were living longer than expected. Intrigued, they conducted a careful study in which they showed that indeed the Ames dwarf mice can live roughly 50 per cent longer than controls (Brown-Borg, Borg, Meliska and Bartke, 1996). Ames dwarf mice are the result of a mutation in a single gene called *Prop1*, which is essential for the production of growth hormone and prolactin in the pituitary gland. Growth hormone (GH) stimulates production of IGF1 (Hammerman, 1987), suggesting that similar endocrine pathways may regulate ageing in invertebrates and in mammals (Guarente and Kenyon, 2000; Kenyon, 2010). Several mutations in other genes that result in a disruption of what is known as the GH/IGF1 axis have since been implicated in mouse ageing (Tatar *et al.*, 2003). Because animals under CR are also smaller than controls and have decreased GH/IGF1 signalling, one emergent hypothesis is that the GH/IGF1 axis plays a causative role in CR (Bartke *et al.*, 2001; de Magalhães, 2005). Mutations in human homologues of *Prop1*, as

well as in homologues of other genes in the GH/IGF1 axis, have been identified in patients. The results from human studies, however, are far less clear than those from mice, with some mutations apparently increasing lifespan and others appearing to be detrimental to health (Laron, 2005). Because mouse studies are conducted in highly controlled environments and in homogeneous genetic backgrounds, while human populations are much more diverse, it could be that mutations in the same gene have different effects on different individuals depending on environment and genetics. Supporting this notion, *Prop1* mutations in some mouse genetic backgrounds are lethal (Nasonkin *et al.*, 2004).

In addition to studying specific genetic diseases in humans, it is also possible to search for longevity effects of genes in human populations. In longevity association studies the frequency of alleles in a given population is determined. Alleles that confer an advantage to longevity will be relatively more abundant in older people, such as centenarians, than in younger individuals. One of the first of such studies focused on apolipoprotein E (*APOE*), since this gene was a known risk factor in cardiovascular disease. Indeed, an association between certain *APOE* alleles and longevity was reported (Schächter *et al.*, 1994), though because *APOE* is involved in cardiovascular diseases it might not necessarily play a causative role in the ageing process. Many other such studies have been conducted in human populations, including studies suggesting an association between alleles in the IGF1 receptor and longevity, potentially linking findings in model organisms to humans (Suh *et al.*, 2008). Though results from model organisms are not always relevant to human biology, it is plausible that some genes associated with ageing in model organisms also regulate human ageing.

Mechanistic theories of ageing

Until recently, many questioned whether there is a basic process of ageing at all (Peto and Doll, 1997). Perhaps what we call 'ageing' is merely the outcome of various debilitating diseases and processes running in parallel and overlapping each other across the body's systems (Holliday, 1995; Masoro, 2006). This notion that there is no underlying process of ageing has now been put to rest, thanks partly to the breakthroughs at the genetic level in model organisms demonstrating how the process of ageing can be regulated as a whole by manipulating single genes, as detailed above. The observation that similar species can age at remarkably different paces also suggests the existence of synchronizing mechanisms of ageing (de Magalhães, 2003). Possibly the most consensual view is that there are indeed processes capable of synchronizing and driving the progression of functional and physiological age-related debilitation as well as age-related diseases (de Magalhães, 2003; Miller, 1999). Though there could even be a single unifying process driving

ageing, it also appears plausible that each individual organ system has unique degenerative processes or constraints that also contribute, with varying degrees, to its debilitation and pathological progression. At the extreme, the possibility exists that a few age-related changes progress largely independently of the process of ageing with tooth erosion as a key example of what is labelled as *mechanical senescence*, or age-related changes that are the result of worn-out body parts. One of the fundamental steps in the biology of ageing is hence the shift from describing age-related changes to understanding them and ascertaining which changes trigger which, what is the underlying mechanism or mechanisms of ageing driving this process, and synchronizing it across tissues and organs.

So far, only major physiological changes have been discussed herein. Each organ, however, is made of cells composed of smaller organelles in turn made of molecules, mostly proteins that are programmed from the genome. Multiple changes have also been observed with age on cells and molecules, including on the DNA itself (Arking, 2006; Comfort, 1964; Finch, 1990; Hayflick, 1994; Strehler, 1999). Considering this multitude of age-related changes occurring at different levels, one of the major quests in the biology of ageing has been to determine which change, process or mechanism causes the ageing process. Many hypotheses have been put forward throughout the centuries, dating back to Aristotle's treatise *On Longevity and Shortness of Life* in 350 BC, as to why we age. Medvedev (1990) catalogues these hypotheses and concluded that there are over 300 different explanations of ageing, even though many could be grouped into common themes.

There are intrinsic difficulties in testing theories of ageing. As discussed above, interpreting results is often controversial and ageing studies are expensive and time-consuming, even in animal models. It is also possible that some mechanisms of ageing are conserved across species, while others are not (Martin, Austad and Johnson, 1996). Besides, discriminating between causes and effects of a complex biological process such as ageing is problematic, even if the new genetic technologies have opened up new opportunities to move beyond correlative studies. Although the search for a causal factor in ageing remains fruitless, if indeed we could identify a unifying process of ageing it would be remarkable, and a critical step for developing anti-ageing therapies (as discussed further below). What follows is a succinct, non-exhaustive description of the most influential mechanistic theories of ageing.

Rate of living theory

The rate of living theory emerged from the work of Max Rubner, who showed in 1908 that long-lived animals are, on average, bigger and have lower metabolic

rates than smaller, shorter-lived ones (Rubner, 1908). Rubner's *energy consumption hypothesis*, as it is called, states that animals are born with some form of substance or capacity and the faster they use it, the faster they will degenerate and die (Austad, 1997b; Hayflick, 1994). Raymond Pearl later extended Rubner's work to develop the rate of living theory, which is the simple idea that ageing is determined by the pace at which life is lived. The faster the metabolic rate, the faster the biochemical activity and the faster an organism will age (Pearl, 1928). The rate of living theory is intuitive as many apparent similarities can be found with machines (e.g. the higher mileage a car has, the more likely it is in poor condition). In the past decades, many researchers have tested Pearl's theory. Because CR limits energy intake, the rate of living theory has been suggested to explain the life-extending effects of CR. Some studies in rodents, however, suggest that CR can extend lifespan without reducing metabolic rate. Moreover, most experimental results have been contrary to the rate of living theory (Masoro, 2005). For example, in a classic experiment, rats kept at a cooler temperature consumed 44 per cent more food and yet did not live less long than controls (Holloszy and Smith, 1986). It has also been reported that individual mice with higher metabolic rates tend to live longer (Speakman *et al.*, 2004).

Rubner's observation that species with lower metabolic rates live longer has come under attack as well. Although it is well established that bigger species tend to have longer lifespans, this is likely due to ecological constraints such as bigger animals being less prone to predation (de Magalhães, Costa and Church, 2007; Stearns, 1992). In addition, there are notable exceptions. Bats and birds are, like mice, typically small animals with high metabolic rates and yet can live much longer than mice (Austad, 1997b). As the final nail in the coffin of the rate of living theory, studies using modern statistical methods suggested that metabolic rate does not, in fact, correlate with the longevity of mammals, including primates (de Magalhães *et al.*, 2007). Although energy metabolism might play a role in ageing, for instance as part of the GH/IGF1 axis, the rate of living theory as proposed by Rubner and Pearl has now, after a long life, been put to rest.

Damage-based theories of ageing

As the name implies, damage-based theories revolve around the notion that continuous damage accumulates throughout life and causes ageing. One or more sources of damage may exist, including by-products of metabolism. Contrary to the 'wear and tear' of inanimate objects, however, it is now largely recognized that ageing in higher organisms, which tend to have a high turnover of most cells and molecules, is not primarily the result of damage to irreplaceable body parts. Even tooth erosion, rather than the wearing out of teeth, can be seen as their lack of replacement

(Williams, 1957). Therefore, modern theories recognize that damage can often be counteracted by repair or replacement systems, even if these are imperfect.

Arguably, the most influential of the damage-based theories and perhaps the most intensively studied mechanistic theory of ageing has been the *free radical theory*. Proposed in 1956 by Denham Harman, the free radical theory argues that ageing is caused by damage from free radicals (Harman, 1956). Free radicals and oxidants, also called reactive oxygen species (ROS), are highly reactive molecules that can damage all sorts of cellular components. ROS are produced in *mitochondria*, which are the cell's powerhouses that convert oxygen and nutrients into energy and thus ROS are a normal by-product of cellular metabolism. The rate of free radical production is linked to metabolic rate and led to the association between the free radical and rate of living theories, which some authors see as one (Harman, 1981; Sohal, Mockett and Orr, 2002). Although cells possess several antioxidants (molecules that protect against oxidation such as vitamins C and E and enzymes like catalase and superoxide dismutase), oxidative damage has been shown to increase with age in several tissues, thus supporting the free radical theory (Beckman and Ames, 1998).

In the same way that oxidative damage has been hypothesized slowly to accumulate with age, evidence has been gathering against the free radical theory of ageing. Even if the outcome is not fatal yet, the free radical theory is becoming progressively more vulnerable to critics. Because certain antioxidants such as vitamins can be purified and ingested, their effects on lifespan can be tested in model systems, yet the results of decades of research have been far from convincing. The subset of studies in rodents that were able to increase average lifespan did so to a small degree (Hagen *et al.*, 2002), making the results difficult to interpret. It is widely recognized, in fact, that there is no evidence that antioxidants taken as dietary supplements influence the ageing process (Arking, 2006; Hayflick, 1994; Olshansky, Hayflick and Carnes, 2002). Furthermore, even though some studies have found correlations between antioxidant levels and species longevity, there are also contradictory findings (Finch, 1990; Sohal *et al.*, 2002).

The main reason, however, why doubts have been cast on the free radical theory is the number of contradictory results from genetic experiments. Particularly in mice, several strains have now been produced with altered levels of expression of antioxidant enzymes, yet the results have, as a rule, failed to support the free radical theory (de Magalhães, 2005; Lapointe and Hekimi, 2009; Sohal *et al.*, 2002). One strain of mice with catalase expressed in mitochondria lived 18 per cent longer but did not appear to age slower, and most likely the extended lifespan derived from a decrease in cardiac diseases across the entire lifespan (Schriner *et al.*, 2005). On the other hand, several mouse strains with altered antioxidant systems failed to support the free radical theory. In particular, one ingenious experiment raised doubts about the free radical theory. Like humans, mice have two copies of each chromosome

plus two sex chromosomes. Because antioxidant enzymes are often crucial for organisms, disrupting both copies of a gene can be lethal. Arlan Richardson and colleagues created a mouse strain with only one copy of superoxide dismutase disrupted, thus lowering the enzyme's activity without completely eliminating it. The resulting heterozygous mice showed increased levels of oxidative damage with age when compared to controls but did not show differences in lifespan or ageing (Van Remmen *et al.*, 2003). Overall, although ROS might well play a role in age-related pathologies, there is little evidence that they are at the root of the ageing process.

One crucial molecule in life is DNA. Given its central role in directing cellular functions and in the creation of the cell itself, damage to the DNA can be catastrophic. The physicist Leo Szilard first proposed the idea that a slow build-up of damage in the DNA could gradually disrupt normal cellular functions and result in ageing (Szilard, 1959). Several studies have shown that indeed DNA mutations and lesions are observed at a higher frequency in at least some tissues of older animals, including mammals (Dolle *et al.*, 1997; Esposito *et al.*, 1989). A correlation between DNA repair and the lifespan of mammalian species has also been reported (Hart and Setlow, 1974).

The Werner syndrome gene, *WRN*, is involved in DNA repair. All genes associated with the three principal progeroid syndromes mentioned above, in fact, appear to have some function in DNA metabolism and repair (Martin and Oshima, 2000). To test further whether changes to the DNA are a cause or effect of ageing, a number of mouse strains with modified DNA repair pathways have been created. Many, though not all, mouse strains with disrupted DNA repair exhibit evidence of accelerated ageing, as predicted by the theory (de Magalhães, 2005; Hasty, Campisi, Hoeijmakers *et al.*, 2003). On the other hand, it has proven difficult to extend the lifespan of mice through optimization or enhancement of DNA repair systems, an essential proof of the theory. Mice overexpressing DNA repair enzymes have in a few cases been shown to have a lower cancer incidence but not an increased lifespan (Garcia-Cao *et al.*, 2002; Zhou *et al.*, 2001). One study reported a 16 per cent increase in average lifespan through generation of mice with extra copies of two DNA repair genes, but it was not obvious whether ageing was delayed (Matheu *et al.*, 2007). In conclusion, much work remains to prove this hypothesis, but it is widely accepted that changes to the DNA could play a role in the process of ageing.

In addition to the two aforementioned theories, there are many others, including variants and combinations. Because ROS can damage DNA and one major source of ROS is the mitochondrion, which possesses its own genome, an overlapping theory is the idea that ROS-inflicted damage to the mitochondrial DNA drives ageing (Harman, 1972). Interestingly, mice with a defect in mitochondrial DNA

repair accumulate damage faster and exhibit signs of accelerated ageing, even if this does not appear to be related to oxidative stress (Kujoth *et al.*, 2005), and the idea that mutations to mitochondrial DNA contribute to ageing and age-related diseases remains a powerful one (Wallace, 1992).

Cellular senescence and organismal ageing

In 1961, and contradicting what was thought at the time, Leonard Hayflick and Paul Moorhead discovered that human cells can only divide a finite number of times in culture (Hayflick and Moorhead, 1961). Because studying cells *in vitro* is easier than studying animals or humans, this phenomenon, termed *replicative senescence* (RS), has been intensively studied for decades. One major hypothesis in biogerontology is that in whole organisms cells that exit the cell cycle and become senescent contribute to ageing and age-related diseases. Indeed, senescent cells have been shown to accumulate with age in certain tissues (Campisi, 2005). Moreover, many accelerated ageing diseases in mice and men, including Werner syndrome, have effects on RS, and it has been argued that senescent cells play a critical role in progeroid syndromes (Kipling, Davis, Ostler *et al.*, 2004).

At least in some cell types, RS is caused by the wearing off of the tips of chromosomes, called *telomeres*. Telomeres shorten as cells replicate and when critically short, trigger signalling pathways that result in senescence. Although other mechanisms exist, including oxidative damage from ROS, telomere shortening is considered by some authorities as the primary mechanism of RS and has been intensively studied. An enzyme called *telomerase* can elongate the telomeres and, though most human cell types do not have active telomerase, cells with telomerase activity can avoid RS (Campisi, 2005; de Magalhães, 2004; Wright and Shay, 2001). Much research has focused on RS, telomeres and telomerase. At present, their relevance to the ageing process is unclear, though there is abundant evidence that RS and telomere shortening are anti-cancer mechanisms (Campisi, 2005; de Magalhães, 2004). Mice engineered to express higher levels of telomerase have a higher cancer incidence and thus a shorter lifespan (Artandi *et al.*, 2002). In cancer-resistant genetic backgrounds, however, telomerase overexpression increased lifespan by up to 26 per cent, even if it was not clear whether ageing was delayed (Tomas-Loba *et al.*, 2008). For many decades it was thought that cells obtained from older donors could divide fewer times than cells from younger donors, an idea that has now been disproved (Cristofalo, Allen, Pignolo *et al.*, 1998). It was also thought that cells from long-lived animals could divide more times, yet it now appears that this solely reflects the fact that long-lived animals tend to be bigger (Lorenzini,

Tresini, Austad *et al.*, 2005). Humans also have relatively short telomeres when compared to other primates and mice and yet live longer (Steinert, White, Zou *et al.*, 2002).

Programmed theories of ageing

At the root of programmed theories of ageing is the idea that ageing is not a result of random or stochastic processes but rather it is driven by genetically regulated processes or predetermined mechanisms, even if these can be influenced to some degree by environmental factors and ultimately cause certain forms of damage. Semelparous species like the salmon demonstrate how the endocrine system can drive degeneration and ultimately lead to death in a well-timed, predetermined manner. Because the concentrations of key hormones like growth hormone (GH) and insulin-like growth factor 1 (IGF1) decline steadily with age (Hammerman, 1987), one influential mechanistic theory of ageing for decades has been the *endocrine theory of ageing*, the idea that hormonal changes drive ageing (Gosden, 1996). Initially, it was thought that increasing the concentration of GH at older ages would restore youthfulness. In reality, this is not the case and mice with high levels of GH tend to have shorter lifespans and maybe even age faster (Bartke, 2003). As discussed above, the results from CR and genetic mutations suggest that an attenuation of the GH/IGF1 axis increases lifespan. CR may also be, at least partly, explained by changes to the GH/IGF1 axis (Bartke *et al.*, 2001; de Magalhães, 2005; Masoro, 2005). Therefore, hormones appear to play some role in the ageing process. However, it is not clear yet how widespread this role is and which downstream mechanisms are involved.

As mentioned above, worms can enter a long-lived, developmentally arrested stage called dauer (Klass and Hirsh, 1976). There are also many other examples of delayed development leading to a longer lifespan, suggesting that at least in some species ageing can be a part of the genetic programme controlling development. In other words, as an organism progresses through its developmental stages, it triggers the process of ageing. This idea that developmental mechanisms can regulate ageing, or many aspects of it, is known as the *developmental theory of ageing* (de Magalhães and Church, 2005), an extreme form of antagonistic pleiotropy. The salmon is a good example, but the marine mollusc *Phestilla sibogae* is an even better illustration: in these animals, the length of larval life is determined by a chance encounter with a stimulus that causes metamorphosis, yet the length of post-larval life is unaffected by the length of time it takes the larva to metamorphose, as if ageing were suspended during the larval time (Miller and Hadfield, 1990). Moreover, as mentioned above, there is a robust correlation between the age

at maturity and longevity (de Magalhães *et al.*, 2007). Although the developmental theory of ageing explains many observations, and may be linked to endocrine changes that regulate both development and ageing, just like the endocrine theory, it lacks concrete details of how developmental mechanisms could influence age-related changes at the cellular and molecular levels.

Other programmed theories focus on specific organs, such as the brain, from which neuroendocrine signals that could regulate ageing originate (Mattson, Duan and Maswood, 2002). Overall, programmed theories of ageing such as those based on developmental processes, like the endocrine theory, provide an alternative view to damage-based theories that manage to explain many observations in biogerontology, even if they fail fully to explain the process of ageing.

In conclusion, it is well established that cells undergo changes as humans and animals grow older that may well play a role in ageing. Though senescent cells might contribute to ageing at least in some tissues with high cellular turnover, the idea that merely lengthening telomeres with telomerase will fend off the effects of ageing has now been disproved.

Anti-ageing medicine

The greying population raises the need not only further to advance our understanding of ageing but even to develop therapies that delay the ageing process. One of the most pervasive public misconceptions about biogerontology is the idea that the goal of anti-ageing medicine is to make old people live longer by merely extending life and consequently extending age-related debilitation and suffering. This is known as the *Tithonus error*. In Greek mythology, Tithonus was a mortal to whom Zeus conceded immortality but not eternal youth, rendering Tithonus increasingly debilitated and demented as he aged. Contrary to the immortality granted to Tithonus, the goal of biogerontology is to extend healthy lifespan by postponing disease and extending the healthy period of life. Rather than focus on specific age-related diseases or changes, the goal of biogerontology is also to delay the process of ageing as a whole and not just its individual manifestations.

Historically (efforts to combat ageing date back to at least 3500 BC), anti-ageing medicine has had a poor reputation. A countless number of treatments, diets and supplements hailed as 'anti-ageing' have failed to live up to their reputation. Hormonal treatments to replenish the concentrations of key hormones to youthful levels were an early anti-ageing promise. Although much progress has been made since early last century when patients received testicular grafts from young animals as a rejuvenation method (Gosden, 1996), hormonal therapies and in particular GH supplements continue to be widely touted as anti-ageing. As

mentioned above, however, higher GH concentrations seem to foster ageing in rodents, indicating that GH supplements are not the 'fountain of youth' (Olshansky *et al.*, 2002). Other anti-ageing products have been inspired by mechanistic theories of ageing. Antioxidants aim to counteract the effects of ROS and prevent the accumulation of oxidative damage and many are sold as dietary supplements. Unfortunately, even if some antioxidants such as vitamins might be healthy, they have never been shown to delay ageing in animal models (Olshansky *et al.*, 2002). Because telomere shortening can trigger cellular ageing, telomerase activators have also been suggested as a method to counter the effects of ageing. Considering that telomere shortening appears to be mostly an anti-cancer mechanism, and telomerase activation contributes to cancer in animal models, there is no evidence that elongating telomeres will delay ageing and might not even be healthy.

As aforementioned, the only intervention that might delay human ageing is CR, though because of its side effects it has failed to capture widespread public enthusiasm. One long-sought breakthrough is the development of CR mimetics, products that provide the beneficial actions of CR without its side effects. One candidate CR mimetic is *resveratrol*, a compound found in certain plants. Though resveratrol was known to have possible beneficial effects in preventing cardiovascular disease (Mizutani, Ikeda and Yamori, 2000), it came to the attention of biogerontology due to its capacity to activate a family of proteins called *sirtuins*, which had previously been shown to play a role in ageing and CR in yeast (Howitz *et al.*, 2003). Although resveratrol was reported to improve the health and survival of mice on a high-calorie diet, raising the possibility that it could be used to treat obesity-related disorders and diseases of ageing, it later failed to increase the lifespan of normal mice (Pearson *et al.*, 2008). Rapamycin – which inhibits the target of rapamycin (TOR) pathway also associated with ageing and CR in model organisms – extended the lifespan of middle-aged mice, suggesting that interventions targeting this pathway may be developed to treat or prevent age-related diseases (Harrison *et al.*, 2009). Additional compounds that modulate the activity of genes associated with CR have been and will be the subject of research for decades to come.

In conclusion, there is presently no proven way to delay, even slightly, the human ageing process. As is common knowledge, some lifestyles and diets are healthy and will increase one's chances of reaching old age, but there is no evidence that any of them will affect the process of ageing. CR might delay ageing in some people, but its effects may be less impressive than in model organisms. Nonetheless, the plasticity of ageing opens numerous opportunities for developing interventions that researchers and companies will no doubt explore. The quest for the 'fountain of youth' continues.

The post-genome future of ageing research

Technology has always driven biomedical research and many breakthroughs in the biology of ageing, such as the genetic experiments detailed above, were made possible by technological advances. At the dawn of the twenty-first century, the sequencing of genomes in particular opened a new era of biological research that is bound to lead to remarkable progress in the biology and genetics of ageing (de Magalhães, Finch and Janssens, 2010). Genetic association studies of longevity can now be done at a whole-genome level, testing all genes in the genome simultaneously for associations with longevity rather than by focusing on a few candidates, and genetic screens can be performed in model organisms in large scale to search for new genes regulating ageing. The expression of thousands of genes can also be determined in parallel thanks to post-genome technologies like microarrays, which allow changes in ageing and age-related diseases to be characterized with unprecedented accuracy. To find clues about which genes may be involved in the evolution of longevity in humans and other animals, the genomes of several organisms with different lifespans can be compared and analysed (de Magalhães *et al.*, 2010).

This scaling up in the amount of data and information that can be gathered about ageing leads to many new opportunities but also presents difficulties. One of the main challenges of post-genome research is to manage and integrate the massive amounts of data being generated to provide useful biological insights. Advances in bioinformatics allow data to be managed and archived in databases and made available online to researchers. New methods also need to be developed to integrate the different data sets. Studies of networks of genes known to impact on ageing have been used to infer how genes interact to drive ageing (de Magalhães and Toussaint, 2004). Developing integrative models of age-related changes and ageing mechanisms at different biological levels will be crucial to deepen our understanding of ageing (Arking, 2006; de Magalhães *et al.*, 2010).

Concluding remarks

Biogerontology came a long way in the twentieth century by providing accurate descriptions of multiple aspects of biological ageing, giving rise to an evolutionary explanation for the existence of ageing and showing the surprising plasticity of the ageing process and how it can be manipulated by dietary and genetic interventions. It is now established that indeed there is an ageing process and if we could understand its mechanisms and even manipulate it, this would result in

unprecedented benefits to medicine and society. Unfortunately, and in spite of the many mechanistic theories that have been put forward, the mechanisms of ageing remain shrouded in mystery. It is plausible that more than one mechanistic theory of ageing is correct. Age changes to one tissue may also influence changes in another one, so it will be crucial to determine which mechanisms are essential for different tissues and how they influence each other. The challenge to biogerontology in the twenty-first century is to identify which are the most important mechanisms and genes and how they interact with each other, with the environment and with different biological levels of organization to determine the ageing phenotype.

Summary

Biogerontology is the field that studies ageing from a biological perspective. Human ageing entails numerous biological changes, which can be summarized as various physiological changes that result in functional loss or decline, leading to increased risk of disease; echoing other species, human mortality increases exponentially after 30 years of age. The evolutionary theory of ageing argues that senescence occurs because of the waning force of natural selection with age since only a small fraction of animals reach old age. Based on this premise, ageing can evolve either because mutations harmful at late ages accumulate in species (the mutation accumulation theory) or because the same mutations that are detrimental in later life confer evolutionary advantages in younger animals (the antagonistic pleiotropy theory). These theories explain much, but not all, of the evidence and inform life history (the study of changes across the lifespan, with particular reference to survival and reproduction). The latter indicates that lifespan varies considerably between species, in large part due to the evolutionary pressures placed on them by their habitat. Within species, typically biomedical model organisms but also humans, a variety of genetic and environmental factors can alter the process of ageing. Caloric restriction can increase lifespan in some (but not all) species, though often with undesirable side effects. Genes also play a key role, witnessed *in extremis* in human progeroid syndromes, but also in genes shown to modulate longevity in model organisms. Current research indicates many of these genes are concerned with the growth hormone/insulin/IGF1 pathway. Overall, the evidence supports there being processes capable of synchronizing and to a large extent regulating ageing. Models to explain this can be grouped into the rate of living theory and damage-based theories, including theories linking free radical damage and DNA mutations to ageing, models of cellular senescence and programmed theories of ageing. All have their limitations and/or lack complete supporting evidence. Likewise, biological methods of halting or retarding the ageing process have met

with no lasting success. However, post-genome research promises much in this regard.

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