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Can we intervene in human ageing?

Richard G.A. Faragher*, Angela N. Sheerin and Elizabeth L. Ostler

Ageing is a progressive failure of defence and repair processes that produces physiological frailty (the loss of organ reserve with age), loss of homeostasis and eventual death. Over the past ten years exceptional progress has been made in understanding both why the ageing process happens and the mechanisms that are responsible for it. The study of natural mutants that accelerate some, but not all, of the features of the human ageing process has now progressed to a degree that drug trials are either taking place or can be envisaged. Simultaneously, a series of mutations have been identified in different species that confer extended healthy life, indicating that the ageing process is much more malleable than might have been expected and that single interventions have the potential to delay the onset of multiple age-associated conditions. Data generated using these organisms have led to the formulation of a powerful new hypothesis, the 'green theory' of ageing. This proposes that a finite capacity to carry out broad-spectrum detoxification and recycling is the primary mechanistic limit on organismal lifespan. This is turn suggests important new experimental approaches and potential interventions designed to increase healthy lifespan.

Ageing of the human population presents probably the most urgent biomedical challenge facing developed nations today. Although it is possible to age in relatively good health, most people face not just an increased risk of death but also an elevated likelihood of developing a plethora of degenerative conditions and functional impairments. This burden of morbidity is probably the most distressing aspect of old age and it has negative effects at both the level of populations (incurring significant costs on the healthcare system) and individuals (morbidity is a thoroughly miserable experience) (Refs 1, 2, 3).

Faced with this problem, one valid clinical approach has been to focus efforts on the development and evaluation of interventions designed to reduce either the extent of functional impairments or the duration of the morbid period (Refs 4, 5). This approach is commonly known as 'compression of morbidity' and has met with some success. This article does not discuss classical compression of morbidity interventions but instead focuses on research efforts to increase our understanding of how the mechanisms driving human ageing operate. This approach is related to but distinct from compression of morbidity activities and is particularly attractive at the current time because it is evident that, in model organisms, single ageing mechanisms cause multiple morbidities. Thus, it is possible that

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interventions that simultaneously postpone multiple causes of morbidity could be developed and delivered.

Although ageing is sometimes painted in positive terms, the truth of the situation is that morbidity means that growing older is bad news for most people, most of the time (Refs 6, 7). If a proper understanding of the cellular and molecular mechanisms underlying this process can be obtained then the potential healthcare benefits will be exceptionally large. This leads us to pose two rather obvious questions: what is this thing we call 'ageing' and why do we do it at all?

Background to ageing

What is ageing?

The study of the biology of ageing is roughly akin to the study of the consequences of wear and tear or the mechanical properties of worn vehicle parts. As such, a precise and concise definition of ageing is quite difficult to achieve. The best generally accepted one is probably that of Strehler and Mildvan (Ref. 8), who defined ageing as a process that is: universal (i.e. all members of a population of organisms will show it, which distinguishes ageing from infectious disease); progressive (the process is continual and incremental rather than sudden as in the case of 'programmed' organismal death or suicidal reproduction); intrinsic (this distinguishes ageing from death due to outside events); and degenerative (this captures the idea that ageing is associated with both increasing chances of mortality but also an increasing level of morbidity).

Although these criteria are a good start in distinguishing ageing from other pathological processes, they tend to underplay the increased physiological frailty of old organisms compared with their young counterparts. Put simply, aged organisms often die as a result of stresses that young organisms are able to survive. *Saccharomyces cerevisiae*, for example, reproduces asexually but shows an ageing process marked by the eventual cessation of reproductive capacity in older mother cells (Ref. 9). If young and old cultures of yeast are exposed to a stimulus that induces physiological stress (such as ultraviolet radiation), old yeast cells are markedly more prone to die (Ref. 10). Elevated deaths from cold among elderly humans compared with their young counterparts might be an interesting 'real world' example of this type of physiological frailty (Refs 11, 12).

Why do organisms age? The evolutionary rationale

When considering ageing it is important to distinguish between life expectancy and maximum lifespan. The patterns of ageing and mortality seen in human populations illustrate this distinction well. Estimated average life expectancies at birth for members of human populations living under prehistoric conditions are typically of the order of 25–35 years (Refs 13, 14). By contrast, life expectancy at birth in the USA in 2001 was 77.2 years and is predicted to rise. However, there is no evidence that the maximum lifespan of *Homo sapiens* (which appears to be ~120–125 years) has increased over historical time. What has changed is the chance of a given individual reaching an advanced age.

Many other species in the biosphere show similar population dynamics. In the wild, aged organisms are rare because they are rapidly culled by predators or killed by disease. However, when transferred to protected environments ageing changes can be readily observed in a wide variety of species including plants, animals and fungi. What is driving this process?

A compelling explanation for the evolution of ageing is based on the observation that the force of natural selection declines with age. This means that, even in a population of nonageing organisms, there are always far fewer chronologically 'old' ones than 'young' ones around (because the longer any individual has been around the more likely it is to have been killed or eaten). Thus, any mutation that favours early life fecundity will be selected for even if it results in deleterious effects later on in the lifetime (a type of gene action termed 'antagonistic pleiotropy') (Ref. 15). This view of ageing argues against the operation of a 'programme' controlling the ageing of individuals (i.e. the existence of genes that have the sole function of causing ageing). Rather it suggests that ageing will result from the accumulation of a wide range of unrepaired faults that are too energetically costly to repair under normal conditions. 'Disposable soma theory', developed in the 1970s by Kirkwood and Holliday (Ref. 16), draws essentially the

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same conclusions as antagonistic pleiotropy but discusses the evolution of ageing in terms of resource allocation between reproduction and somatic maintenance.

Mechanisms of ageing: whom to study and what to look for?

Since ageing is nothing more than the unprogrammed result of selection for early reproductive success, it presents a 'soft' phenotype by comparison to those seen when single-gene inheritance or discrete clinical entities are studied. There is limited consensus on what 'normal ageing' looks like, which gives rise to two conceptual problems for gerontologists. These may be summarised as: (1) which individuals within a population should be studied to try and uncover ageing mechanisms and (2) what would an ageing mechanism look like if you found it? Before discussing mechanisms that we see as plausible, we offer some initial words of explanation to allow readers to follow our chain of conceptual reasoning in what remains a confused and contested area.

The first problem ('Whom do you study?') has proved divisive for biogerontologists. One approach is to treat ageing as though it is a clinical entity in its own right. Thus, some researchers have attempted to identify a 'pure case of ageing' by screening individuals on the basis of health status prior to study and excluding those who are unhealthy. The result is that the individuals recruited are atypical for the general aged population and show minimal changes in the parameter being studied. The SENIEUR protocol for the study of immunosenescence provides a good example of this type of thinking. Set up around the idea that the study of ageing necessitated study of the healthy aged, it sets strict admission criteria that exclude those who show disease or are on medication. The result has been that many immune deficits that are found in the general elderly population are simply absent in those who meet SENIEUR criteria (Refs 17, 18). Similar problems surrounded attempts to gauge the likelihood that cellular senescence (see below) operates as an ageing mechanism. Lacking markers that could identify senescent cells in vivo, large-scale studies of the in vitro divisional capacity of fibroblast cultures from donors of different ages were undertaken. These show that a small decline occurs with chronological age in unselected populations, which becomes statistically insignificant if cultures from healthy donors are used (Refs 19, 20, 21). These findings seem surprising but are consequences of the design of the studies. If an individual showed an age-related change in factor '*x*' but remained healthy, that would make '*x*' a good means of estimating chronological age but a poor causal mechanism of age-linked decline. The annual cement lines laid down in human teeth are a good example of this type of physiologically passive but strongly age-linked process. This debate is more visible within the literature on human ageing than it is on that concerning ageing in yeast or *Caenorhabditis elegans* (given the difficulty of assessing health status in these species), but it is still present and needs to be factored in when thinking critically about the area. None of the above should be surprising but it continues to cause widespread confusion – possibly because there is confusion within the field concerning the properties an 'ageing mechanism' is expected to possess.

With regard to this latter question, we consider that any candidate ageing mechanism must meet the following criteria: (1) it must be present in vivo; (2) it must be capable of exerting degenerative effects; and (3) altering the rate of the process should alter the rate of organismal ageing. These criteria are easy to write down but often extremely difficult to test experimentally. However, there are examples within the literature that demonstrate what can be achieved.

The oxidative damage hypothesis of ageing is well known within the scientific community and, in a vulgarised form, has helped to sell beauty products. It is less well known that the hypothesis, as usually stated, is probably wrong. In essence the hypothesis postulates that the degenerative changes seen in ageing are the result of damage arising from free radical reactions (Refs 22, 23). This is, in itself, entirely plausible. Reactive oxygen species are generated in vivo and can clearly produce degenerative effects on cells and proteins. However, attempts to alter rates of ageing by altering antioxidant defences have failed to yield convincing results. Two series of studies are particularly pertinent to this question. Richardson and co-workers (Ref. 24) have undertaken a series of 18 distinct genetic

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manipulations in transgenic mice designed to either compromise or enhance antioxidant defences. Of these, only deletion of the *sod1* gene showed any effect on organismal lifespan (reducing mean survival from ~900 to ~730 days). Using a similar strategy, Gems and co-workers (Ref. 25) investigated the role of the *sod* genes in *C. elegans* and saw no major effects on lifespan. These data are not consistent with the hypothesis that the major constraint on organismal survival is the capability to resist oxidative stress.

By contrast, the accumulation of extrachromosomal rDNA circles (ERCs) in *S. cerevisiae* does appear to be a plausible ageing mechanism (Ref. 26). ERCs are formed by homologous recombination between adjacent rDNA repeats in the yeast genome. Such ERCs are asymmetrically inherited by mother cells at cell division and are capable of autonomous replication, increasing ERC levels with age. ERCs are clearly present in yeast, and mutations that increase ERC formation shorten lifespan. Conversely, mutations that reduce ERC formation rates increase lifespan. Although the direct link to degenerative effects is complex (Refs 27, 28), it is clear that ERC formation is probably a cause of ageing in yeast. The only problem is that it doesn't seem to be a cause of ageing in anything else!

Cell and molecular mechanisms of ageing

The 'public' and 'private' divide

There are clearly mechanisms of ageing that are species or lineage specific (sometimes referred to as 'private' ageing mechanisms). Aside from ERC accumulation, perhaps the best example is the toxic effect on female *Drosophila* of seminal fluid components secreted from the accessory gland of the male fruit fly (Ref. 29). Seminal fluid toxicity is a major cause of ageing and death in female fruit flies but is not a serious contender as a human ageing mechanism. However, there are a small class of mechanisms that do appear to be truly multispecies in scope and thus probably evolutionarily conserved (sometimes known as 'public' ageing mechanisms). These form the focus of the sections below.

Insulin/IGF signalling and dietary restriction

Mutants in the insulin/insulin-like growth factor (IGF) signalling pathway (IIS mutants) display significantly extended lifespans compared with wild-type controls in a wide variety of species. These include the *daf-2* mutant in *C. elegans* (a null mutation in the insulin/IGF-1 receptor), *chico* in *Drosophila* (an insulin receptor substrate protein mutant) and a variety of mutations in mice that affect the insulin receptor in selected tissues or global circulating insulin levels and insulin receptor substrates. In addition to being long-lived, IIS mutants show retarded presentation of multiple markers of ageing, including the development of ulcerative dermatitis, bone thinning, increased memory T cell number, and reduced motor coordination (Ref. 30). Thus, it appears that interference in IIS pathways can delay age-associated pathology and morbidity (Ref. 31).

Interestingly, although altered IIS signalling is clearly a conserved longevity assurance mechanism, a cross-species study has shown little evolutionary conservation of the individual genes that are regulated by it (Ref. 32). Instead, it seems that there is conservation of processes. The primary mechanisms conserved between these mutants appear to be altered protein biosynthesis (long-lived mutants show a downregulation of genes involved in mRNA translation) and enhanced phase I/phase II xenobiotic metabolism (there is a marked upregulation of cytochrome p450 genes, short-chain dehydrogenase reductases and glutathione *S*-transferases). These observations, together with the increasingly unsatisfactory nature of the oxidative damage theory of ageing (Ref. 33), have led Gems and co-workers to propose a new hypothesis based on broad-spectrum detoxification. This theory, nicknamed the 'green theory' of ageing (Refs 34, 35), proposes that ageing is the result of macromolecular damage that has accumulated as a result of the action of toxic metabolic byproducts (including, but not restricted to, damage caused by reactive oxygen species). A primary determinant of lifespan is thus the efficiency with which potentially damaging species are either removed or the products of damage are repaired (Fig. 1). The green theory is compatible with antagonistic pleiotropy because phase I/phase II metabolism is an energetically costly process to carry out. Thus, there is likely to have been selective pressure to render it no more efficient than necessary in wild-type organisms.

There is also good evidence (Refs 36, 37, 38, 39, 40) for significant overlap between the pathways

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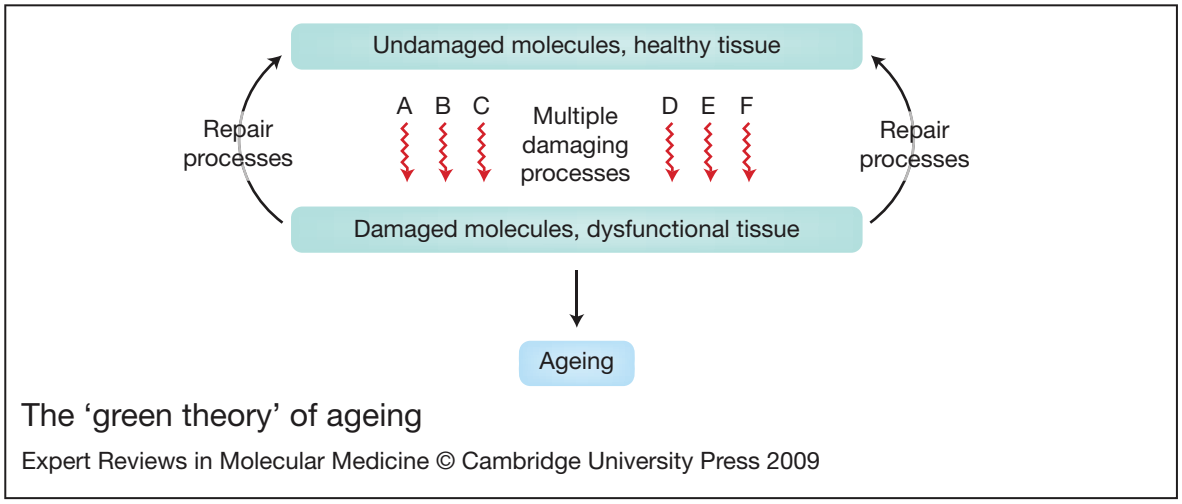


Figure 1. The 'green theory' of ageing. The organism can be visualised as initially composed of undamaged molecular and cellular components. Levels of damage increase in this system as a result of multiple damaging processes including (but not restricted to) oxidative stress. Phase I/phase II xenobiotic metabolism functions as a repair process but is energetically costly.

controlled by IIS signalling and those activated by a regime of calorie or dietary restriction (DR). Animals enter a DR state when they consume significantly fewer calories without malnutrition, a process that results in a significant extension of lifespan. DR appears to be a general anti-ageing mechanism since the state can be triggered in a wide variety of experimental organisms including *Drosophila*, laboratory rodents and primates (Ref. 41). Until recently, it was considered likely that DR mediated its beneficial effects primarily by altering either the production of reactive oxygen species or by enhancing antioxidant defences (Ref. 22). However, since oxidative damage appears increasingly unlikely to be a major ageing mechanism, it seems more plausible that the enhanced survival produced by DR results from entry of the animal into a 'recycling' state marked by repeated cycles of apoptosis and autophagic cell death combined with elevated protein turnover and repair (processes very similar to those invoked by green theory). Similarly, reduction in the activity of the TOR (target of rapamycin) protein by RNA interference (RNAi) or direct inhibition can extend lifespan in yeast, *C. elegans* and *Drosophila* (Refs 42, 43). Since in both yeast and mammalian cells a primary role of TOR is essentially to couple protein synthesis to nutrient availability, inhibition of the enzyme may extend lifespan, at least in part, by mimicking starvation and shifting the emphasis of metabolism more to recycling than de novo protein synthesis.

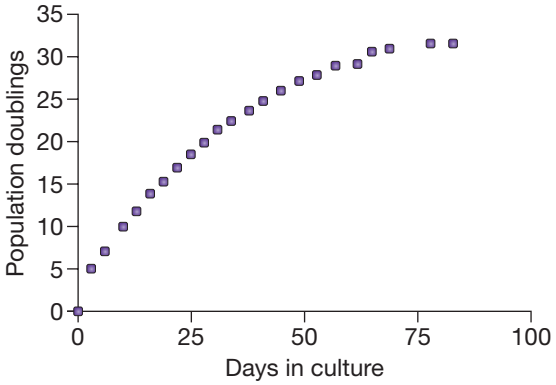
It needs to be stressed that the entry of an organism into a DR state is not without fitness costs. It has recently been shown that aged DR mice exhibit an impaired natural killer cell function, leading to increased mortality in response to viral infection compared with age-matched controls (Refs 44, 45).

Cellular senescence

Progressive accumulation of cells that can no longer divide has also been suggested as a general ageing mechanism. This mechanism is sometimes known as the cell senescence hypothesis (or just as 'replicative senescence') and is relevant only to organisms that turn over somatic cells during life – not to those with a postmitotic soma (such as *C. elegans*). Unlike IIS or DR, replicative senescence was not discovered as the result of in vivo manipulation of lifespan through genetic or environmental means but rather as an in vitro phenomenon limiting the capacity of populations of normal human fibroblasts to continue to expand (Fig. 2). Senescence was proposed as an organismal ageing mechanism when it was first observed in the 1960s. However, only recently has sufficient in vivo work accumulated to render that proposal compelling.

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Senescence of primary human fibroblasts in vitro

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Figure 2. Senescence of primary human fibroblasts in vitro. Growth of a population of primary human diploid fibroblasts (Strain 249.Br). The vertical axis measures cell growth in cumulative population doublings. This generic measure of growth is calculated as: population doublings = ($\log_{10}$ number of cells harvested − $\log_{10}$ number of cells seeded) ÷ $\log_{10}$ 2. Adapted, with permission, from Ref. 46 (© 2008 Biochemical Society).

Senescence is the permanent entry of individual cells into a viable but nondividing state, usually as the result of repeated cell division. The molecular pathways that trigger this process are complex but are now relatively well understood (Fig. 3). For the purposes of this article it is sufficient to say that the controls on the number of times an individual cell may divide before entry into the senescent state occurs are tissue and species specific (Ref. 46). Cell senescence can be observed in vitro in cells from a wide variety of different species and has been proposed to act as an anticancer mechanism (Ref. 47). Considered in these terms, senescence appears to be an example of antagonistic pleiotropy at the process level. In the early part of the organismal lifespan, entry into the senescent state probably prevents the growth of tumours, thus contributing to organismal survival. However, cellular senescence is typically associated with the heavily upregulated secretion of pro-inflammatory factors (e.g. the interleukins IL-6, IL-7, IL-8 and the chemokine CCL8) and similar changes in phenotype (Refs 46, 48, 49), which have the potential to produce degenerative effects. However, for senescence to be a plausible ageing mechanism it must satisfy the criteria given above: first, senescent cells must be present in tissue; and second, those changes must produce deleterious effects. The evidence for this is summarised below.

With regard to the presence of senescent cells in tissue, several studies have now directly demonstrated that they accumulate with in vivo age in the tissues of a variety of species. The most comprehensive of these (Ref. 50) has shown that at least 15% of all dermal cells in very old baboons can be classed as senescent (by immunocytochemical detection of multiple makers of the senescent state). With regard to the effects that the accumulation of senescent cells may have, there is evidence that the accumulation of senescent cells plays a role in liver fibrosis (Ref. 51), in immune dysfunction (Ref. 52), osteoarthritis (Ref. 53) and in the development of atheroma (Ref. 54). In an interesting example of antagonistic pleiotropy, there is also good evidence that if senescent cells form ~10% of the total cell population, the pro-inflammatory factors and matrix-degrading enzymes that they secrete can stimulate nearby premalignant and malignant epithelial cells to proliferate and form tumours (Ref. 55).

Although animals in a DR state show greatly reduced rates of senescent cell accumulation (see below), the best evidence that cellular senescence has the potential to play a causal role in ageing derives from humans suffering from diseases that mimic accelerated or premature ageing (Ref. 56). There are many of these 'progeroid syndromes' but the best known are probably Werner syndrome and Hutchinson–Gilford progeria. Progeria (MCK 176670, Ref. 57) is a sporadic autosomal dominant disorder caused by gain-of-function mutations in the gene coding for nuclear lamin A (*LMNA*). Mature lamin A is formed from a precursor that is first farnesylated in the C-terminal region and then has this region removed by proteolytic cleavage. In progeria, incorrect splicing of the LMNA mRNA occurs. When translated this produces a protein missing the protease cleavage site present in the wild-type protein. As a result, incorrectly processed prelamin A, termed 'progerin' is

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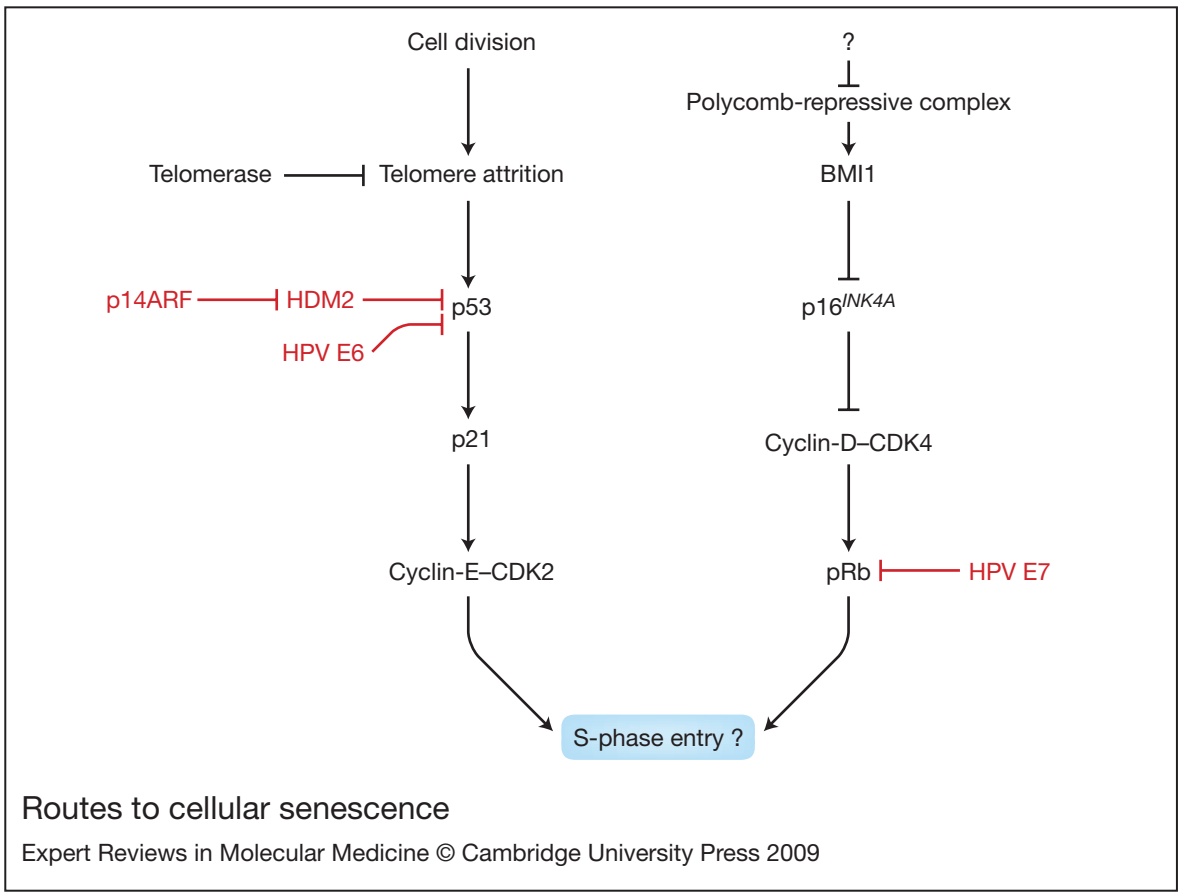


Figure 3. Routes to cellular senescence. The 'pathways' triggering replicative senescence shown here are components of a simplified network. The left side of the diagram essentially summarises components of the network associated with telomere attrition (prevented by the specialised reverse transcriptase telomerase), the tumour suppressor protein p53 and the cyclin-dependent kinase inhibitor p21 ($p21^{CIP1/WAF1}$/CDKN1A). The right side summarises growth suppression associated with the activity of the cyclin-dependent kinase inhibitor $p16^{INK4A}$, which acts on complexes of cyclin D and cyclin-dependent kinase 4 (CDK4). Activity at this locus is partly controlled by the BMI1 proteins (the activity of which is enhanced by EZH2-containing polycomb complexes). EZH2 levels are reduced at senescence leading to general derepression at the *INK4A* locus. These pathways can be interdicted by a variety of gene products including SV40 T antigen, the adenovirus E1A and E1B proteins and the human papilloma virus E6 and E7 proteins (denoted HPV E6 and E7 in the schema). Multiple linkages exist but one of the easiest conceptually is the action of the ARF protein on p53 levels via reduction of HDM2 levels. Useful orientation in this area can be found in Refs 78, 79 and 80. Abbreviations: p14ARF, alternate reading frame *INK4A* locus protein (CDKN2A); BMI1, B-lymphoma Moloney murine leukaemia virus insertion region-1 protein; EZH2, enhancer of zeste, *Drosophila*, homologue 2 histone methyltransferase; HDM2, human homologue of MDM2 (E3 ubiquitin ligase; p53-binding protein); pRb, retinoblastoma protein; SV40, simian virus 40.

formed. This retains an additional 15 amino acid C-terminal sequence including the farnesylated residue and remains anchored at the nuclear membrane. At the level of light microscopy, progerin accumulation is observable in individual fibroblasts. These show highly aberrant nuclear structures and chromosomal positioning as the result of disruption of the nuclear lamina (Ref. 58). Compared with normal controls, populations of such fibroblasts (Refs 59, 60) show hypersensitivity to heat stress, elevated rates of apoptosis hyperproliferation and premature accumulation of senescent cells.

Patients with Werner syndrome (MCK 277700) carry loss-of-function mutations in a gene

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coding for a member of the recQ helicase family (*WRN*/*RECQL2*) and show a wide range of pathologies highly reminiscent of normal ageing. Fibroblasts from these patients show a greatly abbreviated divisional capacity in vitro as the result of accelerated exit from the cell cycle. This accelerated senescence is not observed in cells (such as T cells) from tissues that do not show evidence of clinical involvement. Similar observations have been made with Werner syndrome knockout mice (Ref. 61), suggesting that the accumulation of cells with a pro-inflammatory phenotype is driving the disease. The clinical implications of these findings are discussed below.

Clinical implications

Intervention in the progeroid syndromes

The mechanistic understanding of both Werner syndrome and progeria has now reached the stage when clinical interventions are feasible. Trials aimed at the treatment of progeria in particular are already at an advanced stage. These are based on the use of molecules that block the accumulation of progerin at the nuclear membrane by directly inhibiting the enzyme responsible for farnesylation of the protein. One clinical candidate farnesyltransferase inhibitor in particular, tipifarnib (R115777, Zarnestra), shows dose-dependent prevention of both the onset and progression of cardiovascular disease (the primary cause of death in progeria) when administered orally in a mouse model of the syndrome (Ref. 62). However, since lamin A also undergoes alternative prenylation by geranylgeranyltransferase, inhibition of farnesyltransferase alone may be insufficient to prevent the build-up of progerin. If this should prove to be the case then alternative treatment routes are available. Combination treatment with statins and aminobisphosphonates has recently been shown to inhibit both farnesylation and geranylgeranylation of progerin in a mouse model of the disease (Ref. 63). The prospects for effective treatments for progeria are brighter now than at any time since the disease was first described.

With Werner syndrome, progress has been somewhat slower. The crucial breakthrough from a treatment perspective is the observation by Kipling and co-workers (Ref. 64) that Werner syndrome fibroblasts show activation of p38 mitogen-activated protein (MAP) kinase (probably as a downstream consequence of telomere shortening and replication stress caused by replication fork stalling). Treatment of Werner syndrome fibroblasts with compound SB203580 normalises the lifespan, growth rate and morphology of the cells. SB203580 [4-(4′-fluorophenyl)-2-(4′-methylsulphinylphenyl)-5-(4′-pyridyl) imidazole] is a cell-permeable and semiselective inhibitor of several MAP kinase homologues. There is clear potential for the effects of SB203580 or related compounds to be evaluated in rodent models of Werner syndrome but as yet these trials have not been undertaken. SB203580 itself is not suitable for human use because of issues of selectivity and safety, but p38 MAP kinase is a heavily targeted molecule and many potential alternative inhibitors are available (e.g. RO3201195 and BIRB 796), which may prove to be of clinical utility (Refs 65, 66).

Intervention in the ageing immune system

One sign of the physiological frailty that is the hallmark of ageing is increased susceptibility to infection. The ability to produce naive T cells for release into the peripheral T cell pool is dependent in mammals on the presence of an intact thymus. However, with increasing age the thymus atrophies (Ref. 67). This leads to a dramatic decline in the production and export of T cells bearing new antigen receptor specificities, leading to shrinkage of the overall immune repertoire. Concurrently with this process, replicative senescence within the peripheral T cell pool further compromises the ability to meet an immune challenge. This senescence is driven in part by persistent, often asymptomatic, life-long infection with a variety of viruses such as cytomegalovirus and Epstein–Barr virus (Refs 68, 69). Treatment for this age-related immune dysfunction could potentially take place by a combination of therapies including simple vaccination against the major viruses in childhood (Ref. 70), use of telomerase activators such as TAT2 to retard replicative senescence in T cell populations (Ref. 71), and treatment with IL-7 to enhance thymic function and naive T cell output (Ref. 72).

Insulin/IGF signalling in the clinic?

There is little doubt that the insights into ageing mechanisms that are being obtained using IIS mutants have been some of the most exciting

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research developments of recent years. Interventions based on IIS remain a considerable way off, but it is interesting to note that mutations and particular alleles in genes involved in these pathways appear to correlate with increased healthy life expectancy in humans. It has recently been reported (Ref. 73) that polymorphisms in the *FOXO3A* gene strongly ($P < 0.05$) associate with human longevity. *FOXO* genes are transcription factors that serve as downstream targets of IIS (Ref. 74). Of the Japanese males studied, 75% of those homozygous for a minor allele were healthy compared with 57% of those homozygous for the major *FOXO3A* allele. Health was defined as the absence of several major diseases in combination with both high physical and cognitive function. Study members carrying the minor *FOXO* alleles also had better self-rated health than those carrying the major allele. Other studies have linked variation in IIS in humans to old age survival (Ref. 75) and this will clearly be an area to watch closely in the near future as the links between IIS and healthy human longevity become better understood.

Outstanding research questions

Evolutionary biology: linking species together?

Although antagonistic pleiotropy provides a convincing explanation for the evolution of ageing, there are studies that do not fit altogether smoothly into standard versions of fecundity theory. A case in point concerns recent work conducted on ageing in the guppy (*Poecilia reticulate*). A series of studies have compared guppies that live in high-predation environments with those in low-predation environmental niches in the same river system in Trinidad. Classical resource allocation theory would predict that fish in low-predation environments should display relatively longer lifespan and a reproductive strategy tending more towards the prudent end of the spectrum. By contrast, guppies in high-predation environments should be driven towards prodigal strategies. However, it has been shown that guppies that evolved with predators mature at an earlier age but also show longer lifespans than their counterparts in environments with low extrinsic mortality (Ref. 76). This strongly suggests that our current evolutionary models are incomplete and additional work (e.g. factoring in foraging costs and kin selection) would be very worthwhile.

Extended lifespan and replicative senescence: linking mechanisms together?

There is intense interest in the mechanisms of both IIS- and DR-based lifespan extension. However, very few studies to date have attempted to link these observations to the theory that replicative senescence plays a causal role in ageing. A notable exception to this is the work of Wolf and colleagues (Ref. 77) who established (1) that increasing numbers of cells from the mitotic compartment of the rodent lens became senescent with advancing organismal age and (2) that placing the animals on a DR regime significantly decreased the rate at which senescent cells built up. Thus, at least for DR, it is possible that it exerts its 'anti-ageing' effects by preventing the build up of senescent cells. This is an intriguing possibility and worthy of further study.

No studies have as yet been undertaken to determine the frequency of senescent cells in key tissues in rodents carrying IIS mutations. This represents an important gap in our understanding of how alterations in IIS could lead to extended lifespan. Reduced accumulation of senescent cells in such mutants is quite plausible and could result from altered rates of apoptosis and tissue turnover, from direct effects of altered IIS signalling on pathways governing cell cycle traverse or from autophagy. A reduced rate of senescent cell accumulation would be consistent with extension of lifespan via reduced numbers of senescent cells (and would raise the prospect that increased rates of cell senescence would block healthy lifespan extension in such animals). Alternatively, extended lifespan with normal rates of senescent cell accumulation would, in essence, falsify the hypothesis that lifespan in these animals is limited by the accumulation of senescent cells but would raise the possibility that the systemic environment, rather than cell-intrinsic changes, plays the major role in the extended lifespan phenotype.

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References

1 Khaw, K. (1999) How many, how old, how soon? British Medical Journal 319, 1350-1352
2 NHS National Statistics (2008) Prescriptions Dispensed in the Community – Statistics for 1997 to 2007: England (Bulletin IC 2008 10; ISBN 978-1-84636-223-1), The Information Centre (Government Statistical Service), UK
3 Seshamani, M. and Gray, A. (2004) Ageing and health-care expenditure: the red herring argument revisited. Health Economics 13, 303-314
4 Fries, J.F. (1980) Aging, natural death, and the compression of morbidity. New England Journal of Medicine 303, 130-135
5 Fries, J.F. (2003) Measuring and monitoring success in compressing morbidity. Annals of Internal Medicine 139, 455-459
6 Bowling, A. and Dieppe, P. (2005) What is successful ageing and who should define it? British Medical Journal 331, 1548-1551
7 Strawbridge, W.J., Wallhagen, M.I. and Cohen, R.D. (2002) Successful aging and well-being: self-rated compared with Rowe and Kahn. Gerontologist 42, 727-733
8 Strehler, B.L. and Mildvan, A.S. (1960) General theory of mortality and aging. Science 132, 14-21
9 Powell, C.D., Quain, D.E. and Smart, K.A. (2003) Chitin scar breaks in aged Saccharomyces cerevisiae. Microbiology 149, 3129-3137
10 Kale, S.P. and Jazwinski, S.M. (1996) Differential response to UV stress and DNA damage during the yeast replicative life span. Developmental Genetics 18, 154-160
11 Meyer, K.C. (2004) Lung infections and aging. Ageing Research Reviews 3, 55-67
12 Mercer, J.B. (2003) Cold - an underrated risk factor for health. Environmental Research 92, 8-13
13 Bocquet-Appel, J.P. and Bacro, J.N. (1997) Brief communication: estimates of some demographic parameters in a Neolithic rock-cut chamber (approximately 2000 BC) using iterative techniques for aging and demographic estimators. American Journal of Physical Anthropology 102, 569-575
14 Pietrusewsky, M. et al. (1997) An assessment of health and disease in the prehistoric inhabitants of the Mariana Islands. American Journal of Physical Anthropology 104, 315-342
15 Williams, G.C. (1957) Pleiotropy, natural selection and the evolution of senescence. Evolution 11, 398-411
16 Kirkwood, T.B. and Holliday, R. (1979) The evolution of ageing and longevity. Proceedings of the Royal Society of London. Series B, Biological Sciences 205, 531-546
17 Ligthart, G.H. (2001) The SENIEUR protocol after 16 years: the next step is to study the interaction of ageing and disease. Mechanisms of Ageing and Development 122, 136-140
18 Ershler, W.B. (2001) The value of the SENIEUR protocol: distinction between "ideal ageing" and clinical reality. Mechanisms of Ageing and Development 122, 134-136
19 Martin, G.M., Sprague, C.A. and Epstein, C.J. (1970) Replicative life-span of cultivated human cells. Effects of donor's age, tissue, and genotype. Laboratory Investigation 23, 86-92
20 Goldstein, S. et al. (1978) Chronologic and physiologic age affect replicative life-span of fibroblasts from diabetic, prediabetic, and normal donors. Science 199, 781-782
21 Cristofalo, V.J. et al. (1998) Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proceedings of the National Academy of Sciences of the United States of America 95, 10614-10619
22 Houthoofd, K. and Vanfleteren, J.R. (2006) The longevity effect of dietary restriction in Caenorhabditis elegans. Experimental Gerontology 41, 1026-1031
23 Harman, D. (1991) The aging process: major risk factor for disease and death. Proceedings of the National Academy of Sciences of the United States of America 88, 5360-5363
24 Pérez, V.I. et al. (2009) Is the oxidative stress theory of aging dead? Biochimica et Biophysica Acta Jun 11; [Epub ahead of print]
25 Doonan, R. et al. (2008) Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in Caenorhabditis elegans. Genes and Development 22, 3236-3241
26 Kaberlein, M. and Powers, R.W. (2007) Sir2 and calorie restriction in yeast: a sceptical perspective. Ageing Research Reviews 6, 128-140
27 Falcon, A.A. and Aris, J.P. (2003) Plasmid accumulation reduces life span in Saccharomyces cerevisiae. Journal of Biological Chemistry 278, 41607-41617
28 Jazwinski, S.M. (2004) Yeast replicative lifespan: the mitochondrial connection. FEMS Yeast Research 5, 119-125
29 Barnes, A.I. et al. (2008) Feeding, fecundity and lifespan in female Drosophila melanogaster.

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Proceedings. Biological sciences/The Royal Society 275, 1675-1683
30 Selman, C. et al. (2008) Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. FASEB Journal 22, 807-818
31 Bartke, A. (2008) New findings in gene knockout, mutant and transgenic mice. Experimental Gerontology 43, 4-11
32 Piper, M.D. et al. (2008) Separating cause from effect: how does insulin/IGF signalling control lifespan in worms, flies and mice? Journal of Internal Medicine 263, 179-191
33 McElwee, J.J. et al. (2007) Evolutionary conservation of regulated longevity assurance mechanisms. Genome Biology 8, R132
34 Gems, D. and McElwee, J.J. (2005) Broad spectrum detoxification: the major longevity assurance process regulated by insulin/IGF-1 signaling? Mechanisms of Ageing and Development 126, 381-387
35 Gems, D. (2009) Ageing and oxidants in the nematode Caenorhabditis elegans. In Redox Metabolism and Longevity Relationships in Animals and Plants (SEB Experimental Biology Series Volume 62) (Faragher, R.G., Thornally, P. and Foyer, C.H., eds), pp. 31-47, Taylor & Francis Group, Abingdon, UK
36 Chou, M.W. et al. (1993) Effect of caloric restriction on the metabolic activation of xenobiotics. Mutation Research 295, 223-235
37 Cho, C.G. et al. (2003) Modulation of glutathione and thioredoxin systems by calorie restriction during the aging process. Experimental Gerontology 38, 539-548
38 Spindler, S.R. and Dhahbi, J.M. (2007) Conserved and tissue-specific genic and physiologic responses to caloric restriction and altered IGFI signaling in mitotic and postmitotic tissues. Annual Review of Nutrition 27, 193-217
39 Tóth, M.L. et al. (2008) Longevity pathways converge on autophagy genes to regulate life span in Caenorhabditis elegans. Autophagy 4, 330-338
40 Tsuchiya, T. et al. (2004) Additive regulation of hepatic gene expression by dwarfism and caloric restriction. Physiological Genomics 17, 307-315
41 Mattison, J.A. et al. (2003) Calorie restriction in rhesus monkeys. Experimental Gerontology 38, 35-46
42 Vellai, T. et al. (2003) Influence of TOR kinase on lifespan in C. elegans. Nature 426, 620
43 Kapahi, P. et al. (2004) Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Current Biology 14, 885-890
44 Ritz, B.W. and Gardner, E.M. (2006) Malnutrition and energy restriction differentially affect viral immunity. Journal of Nutrition 136, 1141-1144
45 Ritz, B.W. et al. (2008) Energy restriction impairs natural killer cell function and increases the severity of influenza infection in young adult male C57BL/6 mice. Journal of Nutrition 138, 2269-2275
46 Faragher, R.G.A. (2008) Senescent cells cause ageing: definitely maybe. Biochemist 30, 4-8
47 Campisi, J. and Sedivy, J. (2009) How does proliferative homeostasis change with age? What causes it and how does it contribute to aging? Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 64, 164-166
48 Coppé, J.P. et al. (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biology 6, 2853-2868
49 Kipling, D. et al. (2009) A transcriptomic analysis of the EK1.Br strain of human fibroblastoid keratocytes: the effects of growth, quiescence and senescence. Experimental Eye Research 88, 277-285
50 Herbig, U. et al. (2006) Cellular senescence in aging primates. Science 311, 1257
51 Krizhanovsky, V. et al. (2009) Implications of cellular senescence in tissue damage response, tumor suppression, and stem cell biology. Cold Spring Harbor Symposia on Quantitative Biology 73, 513-522
52 Effros, R.B. (2009) Kleemeier Award Lecture 2008—the canary in the coal mine: telomeres and human healthspan. Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 64, 511-515
53 Loeser, R.F. (2009) Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. Osteoarthritis and Cartilage Mar 12; [Epub ahead of print]
54 Burton, D.G. (2009) Cellular senescence, ageing and disease. Age (Dordrecht, Netherlands) 31, 1-9
55 Krtolica, A. et al. (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proceedings of the National Academy of Sciences of the United States of America 98, 12072-12077
56 Cox, L.S. and Faragher, R.G. (2007) From old organisms to new molecules: integrative biology and therapeutic targets in accelerated human ageing. Cellular and Molecular Life Sciences 64, 2620-2641

Accession information: doi:10.1017/S1462399409001197; Vol. 11; e27; September 2009
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https://doi.org/10.1017/S1462399409001197 Published online by Cambridge University Press

57 Merideth, M.A. et al. (2008) Phenotype and course of Hutchinson-Gilford progeria syndrome. New England Journal of Medicine 358, 592-604
58 Meaburn, K.J. et al. (2007) Primary laminopathy fibroblasts display altered genome organization and apoptosis. Aging Cell 6, 139-153
59 Bridger, J.M. and Kill, I.R. (2004) Aging of Hutchinson-Gilford progeria syndrome fibroblasts is characterised by hyperproliferation and increased apoptosis. Experimental Gerontology 39, 717-724
60 Paradisi, M. et al. (2005) Dermal fibroblasts in Hutchinson-Gilford progeria syndrome with the lamin A G608G mutation have dysmorphic nuclei and are hypersensitive to heat stress. BMC Cell Biology 6, 27
61 Kipling, D. et al. (2004) What can progeroid syndromes tell us about human aging? Science 305, 1426-1431
62 Capell, B.C. et al. (2008) A farnesyltransferase inhibitor prevents both the onset and late progression of cardiovascular disease in a progeria mouse model. Proceedings of the National Academy of Sciences of the United States of America 105, 15902-15907
63 Varela, I. et al. (2008) Combined treatment with statins and aminobisphosphonates extends longevity in a mouse model of human premature aging. Nature Medicine 14, 767-772
64 Davis, T. et al. (2005) Prevention of accelerated cell aging in Werner syndrome using a p38 mitogen-activated protein kinase inhibitor. Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 60, 1386-1393
65 Bagley, M.C. et al. (2008) Microwave-assisted synthesis of 5-aminopyrazol-4-yl ketones and the p38(MAPK) inhibitor RO3 201195 for study in Werner syndrome cells. Bioorganic and Medicinal Chemistry Letters 18, 3745-3748
66 Bagley, M.C. et al. (2006) Microwave-assisted synthesis of N-pyrazole ureas and the p38alpha inhibitor BIRB 796 for study into accelerated cell ageing. Organic and Biomolecular Chemistry 4, 4158-4164
67 Aspinall, R. and Mitchell, W. (2008) Reversal of age-associated thymic atrophy: treatments, delivery, and side effects. Experimental Gerontology 43, 700-705
68 Koch, S. et al. (2007) Cytomegalovirus infection: a driving force in human T cell immunosenescence. Annals of the New York Academy of Sciences 1114, 23-35
69 van Baarle, D. et al. (2008) Progressive telomere shortening of Epstein-Barr virus-specific memory T cells during HIV infection: contributor to exhaustion? Journal of Infectious Diseases 198, 1353-1357
70 Pass, R.F. et al. (2009) Vaccine prevention of maternal cytomegalovirus infection. New England Journal of Medicine 360, 1191-1199
71 Fauce, S.R. et al. (2008) Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. Journal of Immunology 181, 7400-7406
72 Aspinall, R. et al. (2007) Old rhesus macaques treated with interleukin-7 show increased TREC levels and respond well to influenza vaccination. Rejuvenation Research 10, 5-17
73 Willcox, B.J. et al. (2008) FOXO3A genotype is strongly associated with human longevity. Proceedings of the National Academy of Sciences of the United States of America 105, 13987-13992
74 Daitoku, H. and Fukamizu, A. (2007) FOXO transcription factors in the regulatory networks of longevity. Journal of Biochemistry 141, 769-774
75 van Heemst, D. et al. (2005) Reduced insulin/IGF-1 signalling and human longevity. Aging Cell 4, 79-85
76 Reznick, D. et al. (2006) The evolution of senescence and post-reproductive lifespan in guppies (Poecilia reticulata). PLoS Biology 4, e7
77 Li, Y. et al. (1997) Long-term caloric restriction delays age-related decline in proliferation capacity of murine lens epithelial cells in vitro and in vivo. Investigative Ophthalmology and Visual Science 38, 100-107
78 Bracken, A.P. et al. (2007) The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. Genes and Development 21, 525-530
79 Cánepa, E.T. et al. (2007) INK4 proteins, a family of mammalian CDK inhibitors with novel biological functions. IUBMB Life 59, 419-426
80 Fridman, A.L. and Tainsky, M.A. (2008) Critical pathways in cellular senescence and immortalization revealed by gene expression profiling. Oncogene 27, 5975-5987

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Further reading, resources and contacts

Arking, R. (1996) The Biology of Aging: Observations and Principles (3rd edn), Oxford University Press, USA
This probably remains the best general textbook on ageing.

Useful websites dealing specifically with the biology of ageing:

http://www.americanaging.org/ (American Aging Association)
http://www.bsra.org.uk/ (British Society for Research on Ageing)
http://genomics.senescence.info/species/ (AnAge database)

Further insights into ageing can be found at the following UK websites, although the scope of these pages is considerably broader than the biology of ageing alone:

http://ageaction.ncl.ac.uk/ (proceedings of AGEACTION meeting)
http://www.sparc.ac.uk/ (Strategic Promotion of Ageing Research Capacity)

Features associated with this article

Figures

Figure 1. The 'green theory' of ageing.
Figure 2. Senescence of primary human fibroblasts in vitro.
Figure 3. Routes to cellular senescence.

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