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Molecular Biology of Aging

Review

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Introduction

Is aging the final act in the script of developmental biology? The characteristic changes that are part and parcel of aging appear similar to developmentally regulated programs. But why would aging mechanisms have been evolutionarily selected as advantageous? Indeed, evolutionary biologists might argue that aging occurs by default due to the absence of selection in the postreproductive phase of life. By this view, the aging process is not programmed, but, rather, the detritus of the absence of selection for maintenance (Medawar, 1952; Kirkwood, 1977). However, it is quite reasonable that any mechanisms that sprang up to slow or regulate the pace of aging *would* be selected, because lucky individuals could potentially give rise to more progeny. Therefore, it is reasonable to suppose that life span *extending* processes have been selected and that these can be viewed as an elaboration of development itself. In principle, such extension mechanisms may act to slow or forestall deleterious changes in an organism that progressively lead to death. The life span of an organism, therefore, is the sum of deleterious changes and counteracting repair and maintenance mechanisms that respond to the damage (Figure 1).

A priori, one imagines such longevity mechanisms to be much less complex than those regulating embryonic development. The spatial and temporal constraints on embryonic development are many, while requirements for longevity mechanisms might be much more specific if there were a single process (or a few processes) whose breakdown is the limiting event in longevity (i.e., the Achilles heel).

Aging is defined when two criteria are met. First, the probability of death at any point in time increases with the age of the organism. This statistical definition applies from yeast to mammals and reflects the progressive nature of aging. Second, characteristic changes in phenotype occur in all individuals over time due to the limiting processes.

The phenotypic definition is equally general and is useful in distinguishing the aging process itself from diseases of aging, such as cancer and heart disease. Phenotypes of aging affect all of the individuals in a population, while diseases of aging affect only a subset. Both impact on life span, but in different ways. For example, the many advances in medicine and public health in this century have caused a large increase in the average life span of humans in developed countries. However, because these advances have not altered the aging process itself, there has been no substantial change in the maximum human life span.

Several avenues to studying aging have placed us on the threshold of understanding basic underlying mechanisms. These approaches include the identification of key genes and pathways important in aging; genetic studies of heritable diseases that cause the appearance of premature aging in affected people; physiological experiments that relate the pace of aging to caloric intake; and advances in human genetics, as well as cell and molecular biology leading to an understanding of the basis of many diseases of aging. Strikingly, single gene mutations have been found to significantly extend the life span in *C. elegans*, yeast, and, most recently, *Drosophila*, suggesting that aging may be relatively simple, at least in these organisms. Further, the limited replication potential of human cells in culture has been attributed to a specific mechanism (i.e., the shortening of telomeric ends of chromosomes). An important challenge is now to relate these recent findings to the more complex case of human aging.

In this review, we will discuss several important molecular models of aging that come from current research. These are damage by reactive oxygen species (ROS) generated by metabolism, genome instability, genetically programmed extension mechanisms, cell death, and systemic aging. Questions to be posed include the following. What evidence exists for and against these models? Can more than one of these models apply to aging of different tissues in humans—specifically do organs with continually dividing cells age by the same mechanism as organs that are postmitotic? Finally, is aging amenable to therapeutic intervention, and would such intervention be advisable?

Oxidative Damage

One theory of aging proposes that ROS which are generated by metabolism cause cumulative damage over a lifetime (Harman, 1981). Roughly two to three percent of oxygen taken up is chemically reduced by the addition of single electrons, which are sequentially converted into ROS, including the superoxide anion, hydrogen peroxide, and the hydroxyl radical. ROS have been shown to cause molecular damage relatively indiscriminately to proteins, lipids, and nucleic acids. In addition, specific damage has been observed in the mitochondrial DNA, which we consider below in Genome Instability.

What is the evidence that oxidative damage causes aging? One category of study that is supportive of this view involves animals transgenic for genes encoding antioxidants. Transgenic *Drosophila* overexpressing both Cu/Zn SOD and catalase live 34% longer than controls (Orr and Sohal, 1994). A more recent study shows that expression of human *SOD1* exclusively in *Drosophila* adult motor neurons leads to a 40% extension in life span (Parkes et al., 1998). Further experiments are necessary to clarify the nature of this primary role of motor neurons in life span. Conversely, mice knocked out for either *GPX1* (encoding glutathione peroxidase), *SOD1*,

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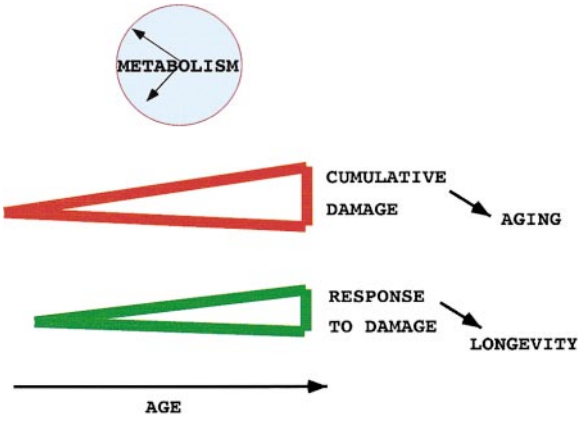

Figure 1. Life Span Is Determined by the Balance of Two Opposing Processes

Metabolism leads to the accumulation of damage (red), thus causing aging. Compensatory responses (green) limit or repair the damage, thus promoting longevity.

SOD2, or *SOD3* do not display a phenotype of rapid aging (Reaume et al., 1996; Ho et al., 1997; Melov et al., 1998); *GPX1*, *SOD1*, and *SOD3* knockout animals appear normal, whereas *SOD2* mice die of cardiac failure at day 8.

Another possible genetic link between oxidative damage and aging is provided by the long-lived *age-1* mutant of *C. elegans* (Klass, 1983; Friedman and Johnson, 1988; Larsen et al., 1995). *age-1* mutants live twice as long as the wild type, have increased levels of SOD and catalase (Larsen, 1993; Vanfleteren and De Vreese, 1996) and are more resistant to oxidative stresses (Larsen, 1993; Vanfleteren and De Vreese, 1996), as well as heat shock (Lithgow et al., 1994) and UV radiation (Murakami and Johnson, 1996). Similarly, the long-lived *Drosophila* mutant *methuselah* is resistant to oxidative stress and also high temperature and starvation (Lin et al., 1998). Mutation in another *C. elegans* gene, *clk-1*, slows development and rhythmic behavior and extends life span (Wong et al., 1995). Interestingly, *clk-1* is a homolog of yeast *CAT5* (Ewbank et al., 1997), a gene involved in synthesis of coenzyme Q, a component of the mitochondrial electron transport chain (Jonassen et al., 1998). Further, *clk-1* mutants contain reduced levels of coenzyme Q (Ewbank et al., 1997), suggesting that all the phenotypes of the *clk-1* mutant, including longevity, may be attributable to a slowing of metabolism.

It is interesting to note that these genetic links between aging and oxidative damage are found in animals in which most adult cells are postmitotic (i.e., *C. elegans* and *Drosophila*). Postmitotic cells may be more susceptible to the cumulative damage of ROS given their inability to replace themselves. In mammals, organs most vulnerable to this mechanism are the brain, heart, and skeletal muscle. The heart does, in fact, apparently experience a reduction in cell number over time, some of which may be due to damage-generated apoptosis (see Cell Death, below). However, the link between ROS and aging in organs consisting of dividing cells is even less clear.

Genome Instability

The importance of specific kinds of genome instability in aging is becoming increasingly apparent. The accumulation of genomic changes (i.e., point mutations [Szilard, 1959], loss of repeated DNA sequences such as ribosomal DNA [rDNA] [Strehler, 1986], rearrangements, and changes in chromosome number [Martin et al., 1985]) have long been proposed as causes of aging. The study of transgenic markers has revealed the accumulation of low levels of mutations with age in mice (Dolle et al., 1997). These changes were observed in the liver, which has mitotic capacity, but not in the brain, which is largely postmitotic. Age-dependent changes have also been observed in the *HPRT* and *HLA-A* genes of peripheral blood lymphocytes in humans (Grist et al., 1992; King et al., 1994). However, the low frequency of these genomic changes, even in old individuals, casts doubt on their importance in aging.

Ribosomal DNA

A strong causal link between genome instability and aging has been established in the model system *S. cerevisiae*. In this system, cell division is asymmetric, giving rise to a large mother cell and a smaller daughter cell. Aging is manifest both by the limited number of cell divisions and the changes in phenotype undergone by mother cells prior to senescence (Barton, 1950; Jazwinski, 1993).

The pace of aging in mother cells is dictated by changes occurring in the ribosomal DNA (rDNA) (Sinclair and Guarente, 1997), which is present in 100–200 tandem copies on chromosome XII. At some point in the middle of the life span of mother cells, a circular copy of rDNA pops out of the genomic array by homologous recombination (Figure 2). This extrachromosomal rDNA circle (ERC) replicates via an origin present in the rDNA sequence during subsequent cell cycles. Importantly, ERCs segregate in a highly biased fashion to mother cells. Thus, daughters are born youthful and ERC free, and mothers experience an exponential buildup in ERCs leading to about 1000 copies in senescent cells. The proliferation of ERCs elicits an apparent enlargement and fragmentation of the nucleolus (Sinclair et al., 1997), the site of rDNA transcription and ribosome assembly. The artificial creation of ERCs, by use of a site-specific recombinase, at an abnormally early age shortens life span, indicating that ERCs are a cause of aging. We imagine that the accumulated ERCs interfere with cell growth by titrating away critical components of the machinery for DNA replication and perhaps transcription.

The initial clue pointing to this mechanism was the genetic control of aging by *SIR4* (Kennedy et al., 1995). This gene, along with *SIR2* and *SIR3*, mediates genomic silencing at telomeres and the silent mating type loci *HML* and *HMR*. Loss-of-function mutations in these genes shorten life span, while a gain-of-function mutation in *SIR4* extends life span. This latter allele causes a relocalization of the SIR2/3/4p complex away from telomeres and *HM* loci to the nucleolus (Kennedy et al., 1997). This mutant constitutively mimics what happens with aging in wild-type cells. These findings suggest that the relocalization of the Sir complex to the nucleolus may be a genetic mechanism to extend life span in wild-type cells. A consequence of this relocalization is the

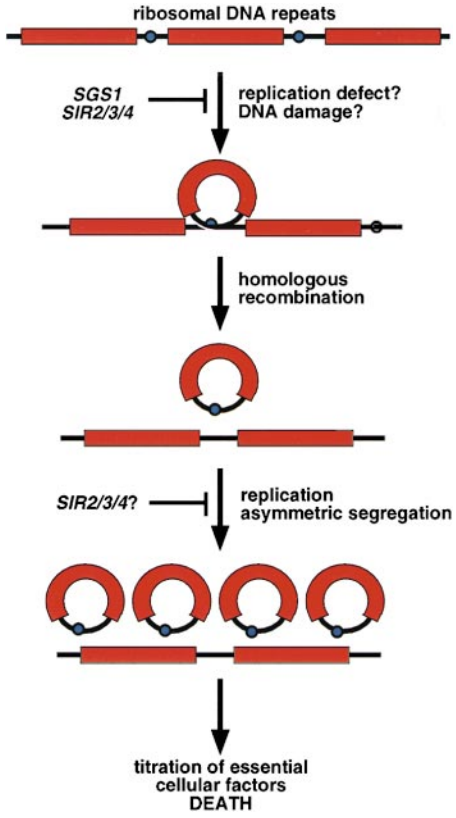

Figure 2. Essential Features of Aging in *Saccharomyces cerevisiae*

Between 150–200 copies of a 9.1 kb unit encoding rRNA is located on chromosome XII. The potentially unstable region is prone to homologous recombination between adjacent repeats at a DNA lesion, perhaps a stalled replication fork or DNA double-strand break. The extrachromosomal rDNA circle (ERC) replicates in ensuing cell cycles via the autonomously replicating sequences in the nontranscribed spacer. ERCs are preferentially held in the mother cell, causing them to accumulate exponentially. Mother cells grow large, and their cell cycle slows. When the ERC copy number reaches between 500 and 1000, the mother cells senesce, possibly due to titration of essential transcription or replication factors. The *SGS1* and *SIR* genes slow the rate of ERC accumulation; *SGS1* presumably limits damage or recombination, and the *SIR* genes act at undefined steps.

evacuation of *HM* loci leading to the expression of a and α mating type genes and the age-related phenotype of sterility (Smeal et al., 1996).

WRN DNA Helicase

Another link between aging and the nucleolus comes from studies of the disease Werner syndrome, which is due to loss-of-function mutations in a single gene, *WRN* (Epstein et al., 1966; Yu et al., 1996). This disease is one of a class of human progerias, which are characterized by shortened life span and the premature onset of phenotypic changes that resemble aging (Martin, 1978) (Table 1). For the model systems described above, the link between the genes identified and aging is clear, because mutations extending life span necessarily identify processes involved in aging. The connection between the mutated genes and normal aging is less certain in the case of the progerias. Figure 3 presents two alternative possibilities for the effects of the progeroid mutations. Consider normal aging as caused by a particular kind of cumulative damage that impinges on a key cellular process to debilitate its output. Normal longevity is promoted by the activity of Gene 1, which slows the accumulation of this damage. The study of a progeria due to mutation in Gene 1 will therefore directly illuminate a step that is the Achilles heel in normal aging. However, a mutation in a Gene 2 encoding a component of the process itself could, in principle, phenocopy rapid aging by limiting the output of the process. In this case, a study of the progeria will not immediately reveal the limiting step in longevity (i.e., the step in which the damage is produced) but may nonetheless identify a relevant process (i.e., the step impacted by the damage).

The *WRN* gene has been cloned and shown to encode a DNA helicase of the RecQ family (Yu et. al, 1996; Gray et al., 1997; Suzuki et al., 1997). Members of this family include *SGS1* of *S. cerevisiae*, *rqh1*$^+$ of *S. pombe*, the gene mutated in Bloom syndrome, *BLM*, and another human homolog *RECQL* (Gangloff et al., 1994; Puranam and Blackshear, 1994; Ellis et al., 1995; Stewart et al., 1997). Studies in yeast show that *SGS1* suppresses recombination in genomic DNA, including the rDNA (Gangloff et al., 1994; Watt et al., 1996). Further, null mutations in *SGS1* suppress the slow growth and rDNA hyperrecombination of *top3* (topoisomerase III) mutants (Gangloff et al., 1994) and decrease the fidelity of mitotic and meiotic chromosome segregation (Watt et al., 1995). Both Bloom and Werner syndromes are characterized by chromosome instability (reviewed in Ellis, 1997). In the case of Bloom syndrome, there are an elevated number of sister chromatid exchanges and varied rearrangements, and patients are prone to all cancers. Werner individuals display more subtle alterations in chromosome stability, but enhanced frequencies of deletions and rearrangements occur.

Strikingly, deletion of the *S. cerevisiae* homolog, *SGS1*, also causes premature aging in that organism (Sinclair et al., 1997). The mutants experience the characteristic fragmentation of the nucleolus and accumulation of ERCs in about half as many cell divisions as the wild type (Sinclair and Guarente, 1997) (Figure 2). Immunostaining reveals that both Sgs1p and the human WRN protein are concentrated in the nucleolus in yeast (Sinclair et al., 1997) and human cells (Gray et al., 1998; Marciniak et al., 1998), reinforcing the possibility that instability of the rDNA is a critical component of aging. In the absence of the Sgs1p helicase, ERCs form more readily, probably due to elevated intrachromosomal recombination.

What could be the biochemical defect in cells lacking *SGS1*-like DNA helicases? Recent findings suggest that a WRN-related protein is required for the formation of DNA replication foci in an vitro assay programmed by a *Xenopus* extract (Yan et al., 1998). A different study shows that the BLM helicase can unwind G quadruplexes in DNA (Sun et al., 1998). One possibility is that these helicases remove secondary structures from the template DNA during replication. In their absense, stalled replication forks may provoke the ensuing recombinational events.

The ERC mechanism of aging may be applicable to mammalian organs consisting of dividing cells. Circular

Table 1. Features of Selected Human Progeroid Syndromes

Progeroid Syndrome	Inheritance	Genetic Defect	Life Span (Approx. Maximum) in Years	Pathologies Possibly Related to Aging	Cellular Changes	Molecular Changes
Werner syndrome	Autosomal recessive	Loss of WRN, a RecQ family helicase.	60	Skin atrophy. Hair graying/loss. Arteriosclerosis. Osteoporosis. Muscle atrophy. Cataracts. Hyperlipidemia. Mild diabetes mellitus, type 2. Hypogonadism. Cancer (sarcomas).	Slow growth. Reduced replicative life span of cultured cells. Chromosome rearrangements. Sensitivity to 4-NQO and camptothecin (topoisomerase I poison)	Increased mutation rate, particularly DNA deletions. Rapid telomere shortening during cellular replicative life span. DNA replication defects (?).
Hutchinson-Gilford progeria	Autosomal dominant (?)	Unknown	25	Skin atrophy. Loss of subcutaneous fat. Hair loss. Arteriosclerosis. Bone loss. Inc. metabolic rate. Growth hormone insensitivity? Hypogonadism.		DNA repair defects?
Down syndrome	Sporadic	Trisomy 21; (sometimes trisomy only of critical regions of chromosome 21).	70	Hair graying/loss. Amyloidosis. Alzheimer-type CNS pathology. Cataracts. Vascular disease. Leukemia. Diabetes mellitus. Hypogonadism.		Elevated amyloid precursor protein (APP) synthesis. DNA repair defects?
Cockayne syndrome	Autosomal recessive	Mutation of CS-B, an ATPase of Swi2 family. Also mutation of CS-A and certain mutations in XP-B, XP-D (helicases in TFII-H), and XP-G.	40 (?)	Loss of subcutaneous fat. Skin photosensitivity. Neurodegeneration. Hypogonadism.	UV sensitivity	Impaired transcription-coupled nucleotide excision repair. Decreased recovery of transcription after irradiation. General RNA polymerase II transcription defect.
Ataxia telangiectasia	Autosomal recessive	Loss of ATM, a protein kinase of the PI-3 kinase family.	40–50	Neurodegeneration. Immunodeficiency. Cancer (leukemias and lymphomas). Occulocutaneous telangiectasias. Progeroid skin and hair changes. Hypogonadism.	Defective cell cycle checkpoint arrest. Reduced replicative life span. Chromosomal rearrangements. Sensitivity to ionizing radiation. Inappropriate apoptosis.	Delayed p53 phosphorylation and accumulation after DNA damage. Short telomeres. Defects in repair of DNA double-strand breaks.

Recent reviews: WS: Goto, 1997, Fry and Loeb, 1998; HG: Brown, 1992; DS: Hayes and Batshaw, 1993; CS: Bohr et al., 1998; AT: Rotman and Shilo, 1998.

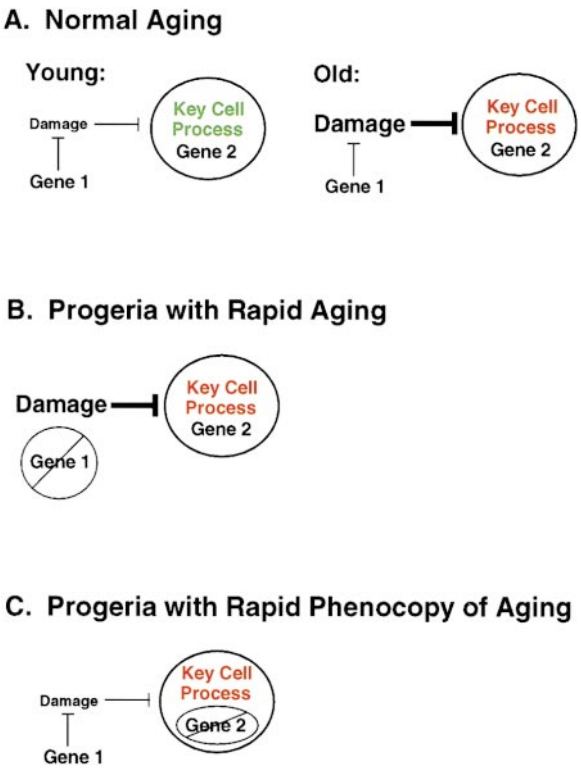

Figure 3. Models for Possible Relationships between Normal Aging and Progeroid Disorders

(A) Normal aging results from the accumulation of damage with age. The damage interferes with a key cellular process critical for longevity, of which Gene 2 is a component. Gene 1 slows the rate of damage accumulation, yielding a normal life span.

(B) A progeroid phenotype results when Gene 1 is inactivated, leading to rapid accumulation of damage and premature inhibition of the key cell process.

(C) Inactivation of Gene 2 leads to decreased activity of the key cell process and a premature aging phenotype. However, there is no effect on the accumulation of damage that normally limits longevity.

DNA has been observed in mammalian cells (reviewed in Gaubatz, 1990). However, although there are a few reports of changes in circular DNA with age in mammals, no dramatic accumulation of circular DNA with age has been observed, and mammalian circular DNA is not known to include rDNA. The strictest analog of yeast mother cells is probably stem cells of mitotically active organs. Although age-related changes have been noted in stem cell numbers and physiology in various studies, it is too early to discern a pattern of changes in rDNA that would be informative.

Telomeres

Telomeres, the repeated DNA sequences at the ends of linear chromosomes, are unable to be fully replicated by DNA polymerases. Thus, they will shorten with cell division unless maintained via telomerase, a ribonucleoprotein enzyme that can add telomeric repeat sequences to chromosome ends. Telomeres indeed shorten during replicative growth of many primary cultures of human cells, which like most somatic tissues lack detectable telomerase activity (Harley et al., 1990). It has thus been proposed that telomere shortening could be a molecular clock that signals the eventual growth arrest, termed replicative senescence, that is observed in all cultured primary human cells. Strong support for this notion has been recently provided by the demonstration that reactivation of telomerase in certain cultured human cells can extend their replicative life span beyond the normal limit (Bodnar et al., 1998; Vaziri and Benchimol, 1998; but see Kiyono et al., 1998). In addition, these apparently immortalized cells have properties indistinguishable from normal, untransformed cells by several phenotypic assays (Jiang et al., 1999; Morales et al., 1999).

There are several findings that relate telomere shortening to aging in vivo. There appears to be a rough correlation between telomere length and age in the human soma, for example in arteries at sites of elevated hemodynamic stress and presumably high cell turnover (Chang and Harley, 1995). It is possible that senescent cells accumulate focally in vivo and because of their altered physiology interfere with the tissues in which they reside. For example, elevated secretion of metalloproteases by senescent fibroblasts could degrade dermal collagen (reviewed in Campisi, 1998). Support for the importance of replicative senescence in human aging has included (1) a weak correlation between in vitro replicative life span and donor age (Martin et al., 1970), (2) correlation between in vitro life span and maximum life span in interspecies comparisons (Rohme, 1981), (3) diminished in vitro replicative life span of cells from people with premature aging syndromes (Faragher et al., 1993), and (4) evidence for the accumulation of senescent cells in elderly individuals (Dimri et al., 1995).

However, other findings and interpretations challenge the sweeping notion that telomere shortening causes aging. First, a recent study that used both cross-sectional and longitudinal samples obtained from the Baltimore Longitudinal Study on Aging indicated that if donor health status and biopsy conditions are controlled, there is no significant correlation between donor age and replicative life span of cultured fibroblasts (Cristofalo et al., 1998). Second, the interspecific correlation can be equally well explained as a correlation with animal size; larger animals generally live longer and might require cells with a greater replicative capacity to produce their larger bodies (Promislow, 1993). Third, the reduced replicative life span of some progeroid cells does not hold for all progerias and might reflect increased cell death or exit from the cell cycle for reasons unrelated to those of wild-type senescent cells. Indeed, WS cells exit the cell cycle before their telomeres are as short as wild-type senescent cells (Schulz et al., 1996) and there are differences in gene expression patterns between senescent wild-type and WS cells (Oshima et al., 1995; Toda et al., 1998). Fourth, the evidence for accumulation of senescent cells in vivo rests largely with the report of cells in elderly skin that express low pH β-galactosidase, an activity that is observed in senescent but not early-passage cultured cells (Dimri et al., 1995). However, the frequency of positive cells in vivo has not been determined. Moreover, there are clear differences between the morphology and gene expression profiles of cultured senescent fibroblasts and those in the skin of elderly individuals (Robbins et al., 1970; Takeda et al., 1992). On the other hand, the finding that $CD28^-$ T cells, which

have shortened telomeres and decreased proliferative capacity in culture, accumulate with age is intriguing (Effros, 1998). More work needs to be done to examine the frequency of senescent cells in vivo, as well as whether sites of pathology are associated with them.

Also of note, telomeres in some mouse species tend to be very long throughout the lifetime of the animals. Telomerase-knockout mice have been constructed and have not yet displayed phenotypes in the first five generations (Blasco et al., 1997). Although highly mitotic tissues (testes, skin, and hematopoietic system) show defects in later generations that may result from chromosomal abnormalities, these changes have no clear relationship to normal aging phenotypes (Lee et al., 1998). It will be of interest to monitor any changes in the early generation animals as they grow older.

Mitochondrial DNA

Genetic instability outside of the nuclear genome might also contribute to aging (reviewed in Lee et al., 1997; Wallace et al., 1998). The mutation rate for mitochondrial DNA (mtDNA) is 10- to 20-fold greater than for nuclear DNA, and it is believed that mtDNA mutations may compromise mitochondrial functions in different ways (Figure 4). First, defects in electron transport and oxidative phosphorylation could lead to declines in ATP levels and the NAD:NADH ratio. Second, defective electron transport might increase free radical production by mitochondria—perhaps enabling a positive feedback loop to produce further mtDNA mutations. Third, and more speculatively, age-associated mitochondrial defects might induce cell death (see Cell Death, below).

There is good evidence for a decline in the function of the mitochondrial electron transport chain with age in humans and other animals, particularly in postmitotic tissues (Boffoli et al., 1994). It is also clear that a variety of mtDNA alterations, including point mutations, deletions, and oxidative modifications increase with age, again preferentially in postmitotic tissues (reviewed in Lee et al., 1997). However, the connection between these two groups of findings is not as certain. Early studies suggested that although mtDNA alterations increased with age, they were quite rare, generally less than one percent of total mtDNA. Cells contain hundreds to thousands of mitochondrial genomes, and studies of hereditary diseases caused by mitochondrial mutations have shown that mutated genomes must accumulate to levels greater than 50%–80% to be apparent clinically (Sciacco et al., 1994).

Two recent findings suggest that age-associated mitochondrial mutations might nonetheless be significant. Using PCR amplification of the entire 16.5 kb mtDNA from skeletal muscle, numerous different deletion mutations are found to accumulate with age, and no one type of mutation predominates (Melov et al., 1995b). Thus, early studies that focused on only a few types of mutations may have underestimated the total number. Moreover, when PCR is performed on just a few or single muscle fibers (rather than on thousands as in earlier studies), deletions can account for a sizable fraction of total mtDNA, suggesting that individual mitochondria may incur unique mutations, which are selectively amplified over wild-type mtDNA (Schwarze et al., 1995; Brierley et al., 1998). The inability of postmitotic tissues to replace cells containing mutant mtDNA with healthy cells might explain a greater incidence of mtDNA mutations in postmitotic versus mitotic tissues. In skeletal muscle, fibers containing high levels of deletions are deficient for cytochrome c oxidase (COX) activity, indicating that the mutant mtDNA is interfering directly with electron transport (Brierley et al., 1998; Kopsidas et al., 1998). Indeed, in situ studies indicate that there are regional differences in COX activity along fibers and these correspond to regions containing deleted mtDNA (Muller-Hocker et al., 1993). Such COX-deficient fibers increase with age, but because they represent only a few percent of total cells it is still not clear that they contribute to the muscle weakening that occurs with normal aging. Another unexplored possibility is that certain mtDNA alterations could have a dominant inhibitory effect on mitochondrial function and thus might not need to accumulate to high levels to be deleterious.

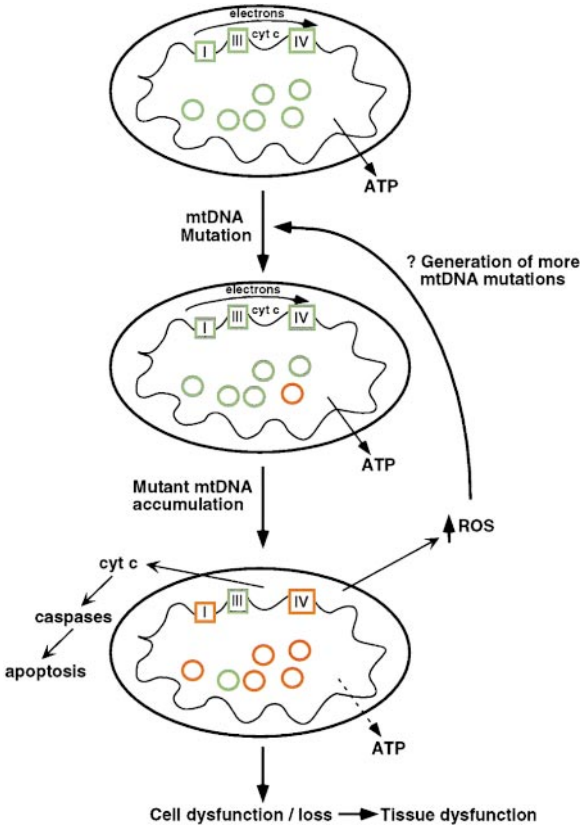

Figure 4. Mitochondrial Theory of Aging

A single mitochondrial genome (mtDNA) incurs a loss-of-function mutation. The mutant genome (red circle) accumulates perhaps due to a replicative advantage compared with wild type (green circle) to a point where mitochondrial and cell activities are compromised. Three types of interference with cell function could occur. First, a decline in electron transport leads to reduced ATP synthesis. The electron transport complexes containing mtDNA-encoded subunits are indicated by roman numerals; complexes I and IV appear most susceptible to age-associated declines in activity (red boxes). Second, increased generation of ROS leads to damage and possibly further mtDNA mutations. Third, cell death is triggered by necrosis or apoptosis, for example by release of cytochrome c. The resulting cell dysfunction or loss could lead to a decline in tissue function.

Even if mtDNA mutations accumulate and contribute to aging, they might not be a primary cause. The *C. elegans age-1* gene encodes a presumptive cytosolic PI-3 kinase (see below), yet mutations in this gene that confer longevity cause mtDNA deletions to accumulate more slowly than wild type (Melov et al., 1995a). This finding suggests that mitochondrial deletions may occur downstream of other aging events that are more directly impacted by the *age-1* mutation.

Genetic Programs

As stated above, the universality of aging phenotypes within a species argues for an underlying genetic program. The redistribution of the Sir complex from telomeres to the nucleolus in yeast is a specific molecular example of how a genetically programmed response can extend life span and also lead to a gradual change in phenotype.

Another example of a genetic pathway that may be relevant in aging comes from *C. elegans*. After hatching, *C. elegans* can proceed through four larval stages directly to adulthood, or they can enter a dormant, juvenile stage known as dauer. Adult development can resume if environmental conditions improve. Which developmental pathway is taken depends primarily on the concentration of a pheromone that indicates environmental stresses, such as starvation, overcrowding, and high temperature. In the dauer state, worms can live up to 6 months, rather than the usual few weeks as adults.

Many genes have been isolated that are involved in this developmental decision. Mutants in these so-called dauer formation (*daf*) genes are either dauer defective (e.g., *daf-16*) or dauer constitutive (e.g., *daf-2* and *age-1*). Strikingly, certain mutations in two of these genes, *age-1* and *daf-2*, permit development to adulthood, but with life span extended 2- to 4-fold (Friedman and Johnson, 1988; Kenyon et al., 1993; Larsen et al., 1995; Tissenbaum and Ruvkun, 1998). This effect has been convincingly interpreted as evidence for a genetic program that influences aging. It would appear that a particular pattern of gene expression and physiology of dauer larvae has been activated in the adult organism. Mutation of *daf-16*, which suppresses dauer-constitutive mutations, also suppresses the life span extension in *daf-2* and *age-1* mutants, providing further evidence for this interpretation (Kenyon et al., 1993; Gottlieb and Ruvkun, 1994).

The cloning of these three *daf* genes has provided insights into the signaling pathway utilized by the worm to regulate dauer development (Figure 5). *daf-2* encodes a nematode member of the family of insulin-like receptors (Kimura et al., 1997), whereas *age-1* encodes a homolog of the p110 catalytic subunit of phosphatidylinositol 3-kinase (PI 3-kinase) that acts downstream of the *daf-2* receptor (Morris et al., 1996). Another gene, *daf-18*, mutations in which can reverse the longevity in certain *age-1* mutants, encodes a PI 3-phosphatase homolog (Ogg and Ruvkun, 1998).

daf-16 encodes a member of the hepatocyte nuclear factor 3/forkhead family of transcriptional activators that is a target of negative regulation by the PI 3-kinase-mediated signaling cascade (Lin et al., 1997; Ogg et al., 1997). In *age-1* and *daf-2* adults, this signal is partially on, allowing adult worms to enjoy an extended life span. The extension of life span may be in part due to the upregulation of a wide array of cellular defenses in the adult (as noted above), which combat oxidative and other damage that occur throughout the lifetime of the worm. The fact that developmental pathways can be manipulated to extend the life of adult worms suggests that genetic programs may regulate the pace of aging more generally.

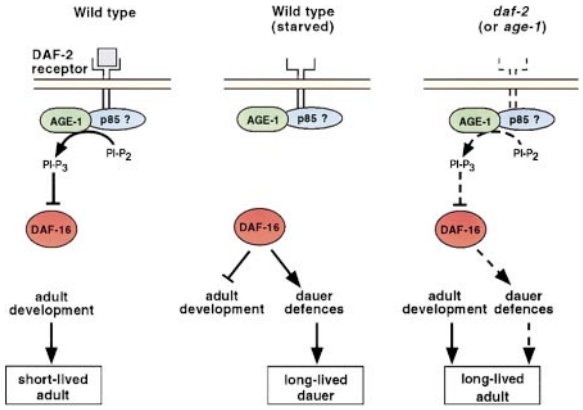

Figure 5. Proposed Mechanism of Life Span Extension by the Dauer Genetic Pathway in Adult *Caenorabditis elegans*

High levels of DAF-2 ligand signal adult development. Under conditions of starvation or overcrowding, increased activity of the DAF-16 forkhead transcription factor triggers dauer development. In certain *daf-2* or *age-1* mutants, a partially active DAF-2/AGE-1 signal results in simultaneous expression of genes involved in adult development and dauer-specific defenses that confer longevity.

Cell Death

Is there a connection between cell death and aging? Cells die via mechanisms that range from necrosis, which is a relatively passive outcome of cell injury, to apoptosis, or programmed cell death, which is an active and ordered process. The function of postmitotic tissues might especially be compromised by cell death, because the lost cells cannot be replaced (although the dogma that heart and brain are truly postmitotic has recently been challenged [Eriksson et al., 1998; Kajstura et al. 1998]). Apoptosis is triggered by a myriad of stimuli, such as steroid hormones, DNA damage, withdrawal of growth factors, or receptor-mediated apoptosis as in clonal deletion of immune cells. This pathway involves activation of a family of proteases, termed caspases, and nucleases, leading to the controlled degradation of cellular structures, followed by removal of the membrane-bound debris by phagocytic cells. There has been speculation that dysregulation of programmed cell death might contribute to aging (reviewed in Warner et al., 1997).

Both apoptotic and necrotic forms of cell death can be mediated by mitochondria (reviewed in Green and Reed, 1998). Given the evidence for changes in mitochondrial function during aging (see Figure 4 and above), one wonders whether these changes contribute to cell death. Mitochondrial events that can mediate cell death

include (1) release of mitochondrial stores of caspase activators including cytochrome c, (2) disruption of the respiratory electron transport chain, and (3) production of ROS. Age-dependent changes in mitochondria might trigger the above mechanisms leading to cell loss. There is as yet no direct evidence for increased release of caspase activators from mitochondria with age, but the activity of electron transport complexes appears to decline (reviewed in Lee et al., 1997) and the rate of ROS production increases with age in many mammals (Sohal et al., 1994).

However, genetic data do not support a role for cell death in aging. For example, studies in *C. elegans* argue against any relationship between programmed cell death and aging. The genetic underpinnings of both processes have been extensively studied in this organism, and there is no apparent overlap between the genes that affect them (reviewed in Hengartner, 1997). In addition, knockout mice for the *bcl2* family proteins, which normally function to inhibit caspase activation and thus apoptosis, do not show clear aging phenotypes, although the *bcl2* knockout does develop some phenotypes that superficially resemble aging (Veis et al., 1993).

Nevertheless, there is correlative evidence in humans and other mammals that cell death is important in certain aspects of aging. One recent study reports that more than a third of ventricular cardiac myocytes are lost during normal aging in human males, although apparently not in females, and the loss of cells is accompanied by hypertrophy of the remaining cells (Olivetti et al., 1995). A similar loss has been observed in rats; in this case the majority of cells die via necrosis, and a substantial number are also lost via apoptosis (Kajstura et al., 1996). Neurodegenerative diseases, for example Alzheimers, are clearly accompanied by extensive loss of neurons. However, the loss of neurons with normal aging may not be as extensive as once thought (reviewed in Morrison and Hof, 1997). Recent studies indicate that the overall number of cortical neurons declines by only 10% with aging, and although particular brain regions do show higher levels of loss, it is not clear that they are responsible for the functional changes that accompany aging (Pakkenberg and Gundersen, 1997). It remains to be demonstrated whether genetic or pharmacological interventions that inhibit cell death can slow cardiac myocyte or neuronal loss and have beneficial effects on organ function.

On the other hand, it has been proposed that an *inability* of certain cells to undergo apoptosis might contribute to aging and diseases of aging. Cultured senescent fibroblasts are resistant to apoptosis, in part due to an inability to downregulate *bcl2*, and this could enable senescent cells to accumulate in vivo with deleterious consequences (Wang, 1995; see Genome Instability, above). There is also evidence from studies in the rat liver that apoptosis helps clear preneoplastic cells (Grasl-Kraupp et al., 1994). An inability of apoptosis to keep pace with increasing numbers of precancerous cells could thus contribute to age-associated elevations in cancer rates. Interestingly, calorically restricted rats increase their hepatocyte apoptosis rates with age more than rats fed ad libitum, with a consequent decline in the incidence of hepatocellular carcinoma (Muskhelishvili et al., 1995). Further studies are necessary before the contribution of cell death to aging can be correctly gauged.

Systemic Control of Aging

Many of the above mechanisms of aging are cell autonomous (i.e., are independent of external signals). However, it is possible, and, in fact, it has been suggested that systemic control of aging may occur (reviewed in Finch, 1990). By this view humoral factors (i.e., endocrine hormones) would coordinate the pace of aging in many different tissues and organs. Further, local mechanisms like cell–cell contact or paracrine hormones could coordinate aging in a proximate compartment of cells. Systemic and cell-autonomous processes are clearly not mutually exclusive; cell-autonomous and systemic aging may coexist or trigger each other.

A recent study in *C. elegans* has demonstrated that the longevity enjoyed by *daf-2* mutant animals is not cell autonomous (Apfeld and Kenyon, 1998). By constructing mosaic animals containing a mixture of *daf-2*(+) and *daf-2*(−) cells, the authors showed that a small number of mutant cells in any of several sublineages conferred a degree of longevity on the entire animal, which contained predominantly *daf-2*(+) cells. This was interpreted to mean that the *daf-2* gene controls the production of a secreted factor that dictates the pace of aging in the animal. In addition, the key determinant of life span was the ratio of *daf-2*(+) to *daf-2*(−) cells, and it did not depend on the presence of mutant cells in any particular lineage. This finding implies that there are not specific adult cells that dictate the rate of aging. Rather, the level of this secreted factor, averaged systemically, sets the life span. In *Drosophila*, a reduction in the activity of the gene *methuselah* extends life span. *methuselah* is homologous to genes encoding G protein–linked transmembrane receptors, raising the possibility that it might also play a role in transducing systemic aging signals (Lin et al., 1998).

Do systemic factors regulate aging in mammals? A genetic argument for a such a role might be provided by the mouse gene *Klotho*, which encodes a humoral factor (Kuro-o et al., 1998). *Klotho* mutants have a shortened life span and develop several pathologies that might have some relationship to aging. In normal human physiology, there are clear examples in which the levels of endocrine factors decline with age (reviewed in Lamberts et al., 1997). Estrogen levels decline sharply at menopause, and estrogen supplementation has been shown to slow several degenerative processes, including skin atrophy, osteoporosis, atherosclerosis, and cognitive decline. Moreover, a recent study suggests that the overall mortality rate declined in postmenopausal women receiving hormonal supplementaton (Grodstein et al., 1997). Other endocrine factors, including growth hormone, testosterone, and DHEA-S (dehydroepiandrosterone sulphate), decline gradually with age. Short-term supplementation with growth hormone has been shown to increase muscle mass, skin thickness, and bone density (Rudman et al., 1990).

While the effects of these hormones on specific organs are apparent, their relation to the aging process

itself, if any, is not yet clear. To our knowledge, there has been no animal study in which hormone supplementation extended maximum life span. However, the recent findings in *C. elegans* provide a basis to believe that humoral factors may turn out to play an important role in at least some aspects of human aging.

Perspective

Recent advances in the study of aging indicate that this process is amenable to molecular analysis and may be relatively simple. The potential of single gene mutations to greatly extend life span in model systems suggests that relatively few limiting cellular or organismal processes control the rate of aging, at least in these species. In mammals, it is noteworthy that a simple dietary regimen can extend life span. Rodents that are calorically restricted live up to twice as long as controls that are fed ad libitum (reviewed in Weindruch, 1996). The restricted animals are about one-third smaller, have a lower body temperature than controls, and are relatively disease free (Weindruch and Sohal, 1997).

What new advances can we expect in the short term? One current limitation in studies of mammalian aging is the paucity of reliable and easily measured biological markers for the aging phenotype. We imagine that the DNA chip arrays that allow gene expression to be assessed across the entire genome will provide useful markers. If a characteristic pattern of changes is demonstrated over the lifetime of, for example, mice, the chip arrays will enable researchers to determine the rate of aging in genetically or physiologically altered animals.

Genome analysis may also identify human polymorphisms that are associated with longevity. So far, there is some evidence for such a link for alleles of *APO-E* (apolipoprotein-E), *ACE* (angiotensinogen-converting enzyme), *HLA-DR* (human leukocyte antigen-DR), and *PAI-1* (plasminogen activator inhibitor) (reviewed in Schachter, 1998). Interestingly, an allele of *WRN* may be associated with a reduced risk of myocardial infarction (Ye et al., 1997). As technologies for rapid assessment of polymorphisms develop, genomic analysis of centenarians may provide a comprehensive list of relevant polymorphic loci.

If only a few key processes are critical in human aging, there may be specific molecular targets that are amenable to therapeutic intervention. One can therefore imagine pharmacological means to slow or even stop aging in one or more organ systems. The fact that adult somatic cells have been used to clone whole animals suggests that aging-related nuclear changes may be reversible (Wilmut et al., 1997; Wakayama et al., 1998). However, it is not clear yet whether a full life span has been given to the clones. Moreover, the small fraction of somatic cells that yield clones may represent rare soma that have escaped aging-related cellular events.

Is it advisable to intervene in human aging? Over the past two centuries, human life expectancy has more than doubled in developed nations due to advances in medicine and public health. Assuming that future intervention will not affect causes of mortality that are independent of the aging process, average life span may be impacted, but probably to a smaller degree than what has occurred in the past 200 years. However, slowing the aging process may increase vitality and quality of life over the entire life span of individuals. In this regard, it is noteworthy that calorically restricted rodents have an extended life span that is relatively free of disease.

For society, the implications of slowing the aging process are more complex. Of course, in an increasingly overpopulated world, it would be important to offset any significant effects on longevity with a compensatory reduction in birth rates. In fact, in many industrialized countries, the current birth rate is sufficiently low to afford zero or negative growth. Most importantly, if the slowing of aging is associated with improved health and productivity of long-lived individuals, there may be a direct benefit to society. In an information-intensive era when societal progress increasingly depends on the knowledge and wisdom of individuals, this benefit should be at a premium.

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