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REVIEW

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Genetic regulation of aging

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Abstract Aging of biological systems is a complex process that is controlled by both environmental factors and the genetic constitution of the individual. Although the molecular mechanisms have not been elucidated for any system in detail, it is clear that various genetic traits are involved in the modulation of life span. In particular, the genetic information located in the mitochondria has been identified as a major genetic component. Instabilities of the mitochondrial DNA (mtDNA) lead to mitochondrial dysfunction and increased oxidative stress. In some cases mtDNA instabilities are related to the activity of mobile genetic elements. In addition, nuclear genes appear to be crucially involved in mtDNA maintenance. Furthermore, the initial analysis of a few cloned nuclear genes affecting life span suggests a cellular machinery dealing with various stress situations as a major component involved in the genetic control of aging. This conclusion may hold true for all biological systems and be related to a unified mechanism of aging. However, in the various lineages this mechanism may be superimposed by other species or lineage-specific mechanisms.

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Abbreviations *ROS* Reactive oxygen species

Introduction

Biological aging, as the progressive functional impairment of living beings leading to an increase in age-related mortaliy, is a complex process which even today is far from being understood in its details. In humans, it leads to the accumulation of disabilities and diseases in old age. Understanding of the underlying mechanisms is therefore not only a matter of academic interest but can be expected to open new strategies to deal with the various age-related problems.

In the past two decades, research on aging has provided promising data in this direction, and some basic mechanisms are emerging which explain certain aspects of aging. This review focuses on research into the genetic basis of biological aging. I include systems which are much simpler in their organization than humans. Although some of them also have proved to provide significant clues for specific research into human aging, this type of research should not be viewed only as investigations on model systems with the exclusive ultimate goal of unraveling human aging. Aging research on diverse systems has its own justification and is a prerequisite for the detailed understanding of this central biological process and its evolution. The outcome of these investigations will settle controversies about certain questions (e.g., unified mechanisms vs. species specific mechanisms of aging, programmed vs. nonprogrammed aging) which, partly due to the lack of clear data, are currently found as main topics on the agenda of aging conferences, in publications, and are part of a public discussion. Finally, the consequences of aging research in a broader sense will not be restricted to medical and social areas but will certainly prove to be of significance in a variety of interdisciplinary fields (e.g., agriculture, biotechnology).

Biological aging, the consequence of a genetic program?

There is no doubt about a genetic basis for aging in the diverse biological systems. Only two supporting facts should be mentioned here. First, all living beings are characterized by a species-specific mean and maximum life span. Second, in the various systems individuals are known with a life span which significantly differs from the characteristic life span of the species. They pass this difference on to their progeny.

But what is the genetic basis of aging? Does a genetic program operate and is aging merely a continuation of an earlier developmental program? In other words, is there a well-ordered series of specific events under direct genetic control also in the later periods of life? In relation to these questions a point of common confusion must be addressed. The existence of genes involved in the control of aging has often been used as an argument in favor of an aging program. However, this is not necessarily true because genes may code for factors which modulate a primary, causal mechanism of aging, but this mechanism may not constitute a program in the strict sense. For example, the primary cause of aging may be the accumulation of stochastic damage of biomolecules. In this case, genes coding for repair factors clearly affect the life span, but these genes are not necessarily expressed as part of a specific time-dependent series of events.

In fact, although we do not yet know the detailed mechanisms of aging in any biological system, evolutionary considerations do not support programmed aging. If such a program were to exist, it would have to have evolved, although this would mean a clear disadvantage for the individual because it brings life to an end. It may be argued, as it has been by Weismann [1], that aging is an advantage for a population since it protects from overcrowding, with all the attendant consequences. However, from the evolutionary point of view it is rather unlikely that such a type of "group selection" is favored over selection at the individual level [2]. On the other hand, despite these evolutionary considerations, at least in a few lower biological systems, there is some evidence for a well-ordered series of events leading to senescence (see below). However, the final answer as to whether aging in a given species is a programmed process will be available only after the proximate mechanisms of aging in that species have been elucidated in detail.

The genetic basis of biological aging

In all eukaryotes, the genetic information is located in various cellular compartments. In both autotrophic (plants) and heterotrophic eukaryotes (fungi and animals) by far the greatest part is found in the nucleus and only a much smaller complement in mitochondria. In plants, the plastids, organelles involved in CO_2 assimilation and the conservation of solar energy, also contain genetic information.

The size of the nuclear genome may differ significantly over several orders of magnitude (e.g., 15 Mbp in yeast vs. 3000 Mbp in humans). Investigations over recent years have clearly shown that these differences cannot be explained by the higher organization of a species and the requirement for additional genes. The observed size differences have been found to be due mainly to sequences which are present as repetitive units. The number of these sequences may differ from species to species or even from strain to strain. Interestingly, although repetitive sequences account for a significant portion of the genome of most eukaryotes, only very little is known about their function, and these sequences are thought to represent "selfish" DNA, or "molecular parasites," sequences which appear to be concerned exclusively with their own propagation. However, although no specific function can be attributed to these sequences, their multicopy nature appears to be of significance for the flexibility of the genome since repetitive sequences constitute nucleotide stretches of homology which are the targets of the cellular general recombination system.

The genetic information of mitochondria, the mitochondrial DNA (mtDNA), is several factors of magni-

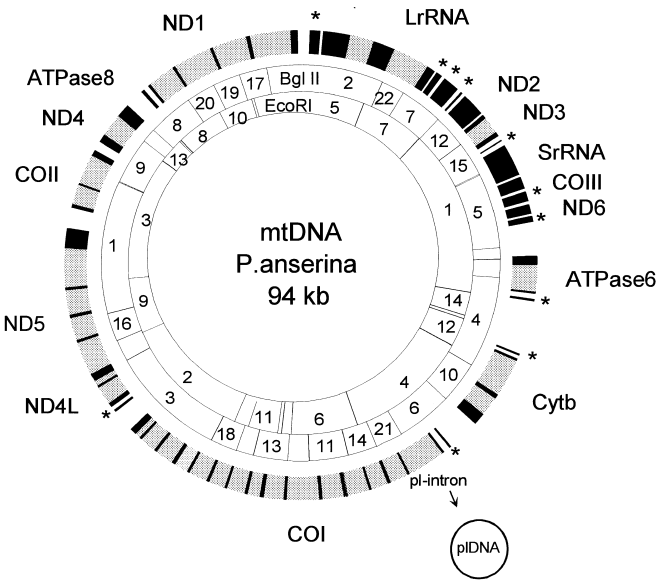


Fig. 1 Physical and genetic map of the mtDNA of *Podospora anserina*. *Inner circle*, the physical map with the recognition sites for endonucleases *Bgl*II and *Eco*RI; *outer circle*, location of the individual genes; *black*, coding sequences for proteins, rRNAs (LrRNA, SrRNA), and tRNAs; *gray*, intron sequences. For simplicity the individual tRNA genes, which appear in clusters, are not indicated individually but by *asterisks*. The position of the pl-intron of the cytochrome oxidase subunit I gene (*COI*), which gives rise to the formation of plDNA is indicated. *COI-III*, genes coding for subunits I, II, and III of cytochrome oxidase, *Cytb* codes for the apocytochrome *b* gene, *ND1-5*, code for subunits of the NADH dehydrogenase, *ATPase6*, *ATPase8* encodes two subunits of the mitochondrial ATP synthase complex. The figure was prepared according to the published complete nucleotide sequence of the *P. anserina* mtDNA [3]

tude smaller than the nuclear genome, and consequently its coding capacity is rather limited. Moreover, the size of the genome may differ significantly in different species (e.g., *Podospora anserina*: 94 kbp; *Homo sapiens*: 16.5 kbp) or even in different strains of the same species. From the nucleotide sequences of several completely sequenced mtDNAs it is clear that these differences, as in the case of the nuclear genome, are generally not due to the presence or absence of additional genes. Basically, all mitochondrial genomes code for a specific gene set (Fig. 1) [3, 4]. One group of genes encodes the RNA components of the mitochondrial protein synthesis apparatus (rRNAs, tRNAs) whereas only a few genes code for proteins. Most of these proteins are part of the energy-generating machinery found in mitochondria. In organisms with larger mtDNAs, additional sequences are found. In particular, intervening sequences (introns) may constitute a significant portion of the mtDNA (Fig. 1). In some cases, these introns code for proteins which are required for the maturation of their own pre-messenger RNA (RNA maturases). In addition, in the past decade it has been shown that some introns code for proteins which allow them to move from one location in the genome to another one. As discussed below, this intron transposition can be of great significance for the aging process.

From the organization of the mitochondrial genome it is apparent that about 90–95% of the genetic information coding for components of this cellular compartment must be located in the nucleus, and the corresponding products need to be transported into mitochondria. The elucidation of the involved apparatus is currently the subject of intensive investigations [5–8] and may be of significance for the complex molecular network involved in the control of the life span.

What is the role of the genetic information in aging? Is the DNA the target of damaging agents and is aging merely the result of molecular damage of this informative biomolecule? Or is the activity of specific age-related genes causally related to aging? The following parts of this review consider these questions and summarize research performed in this field of experimental gerontology.

Genome instabilities and aging

One group of the many aging theories which are prevalent today explains aging as the result of alterations in the genetic information of eukaryotic cells. According to the “somatic mutation theory” [9, 10], random “hits” of genes occurring over the whole life span of an individual lead to the accumulation of defective cells and consequently to cellular death. These “hits” may be the result of an exposure to endogenous or exogenous cell-damaging agents, to irradiation, or may be due to error-prone DNA synthesis. In addition to this type of rather subtle DNA alterations, gross age-related DNA alterations were reported in the past to occur in the various genetic compartments of a cell. Some of these are only loosely correlated to aging processes while others appear to be more tightly linked to primary causes. However, it is not yet clear whether all types of age-related gross reorganizations are the result of stochastic processes, or whether, at least some of them, are part of a well-ordered network of events controlled by the time-dependent activity of certain age-related genes.

Subtle DNA alterations

Subtle DNA alterations have been repeatedly correlated with biological aging. In some investigations, these changes were correlated with oxidative stress and were used to support the idea that oxidative damage is the main contributor or even the cause of biological aging. This theory is an extension of the “free radical theory of aging” which was put forward by Harman [11] and explains aging as the result of the reactions of free radicals with various biomolecules. Molecular damage of biomolecules (e.g., DNA, proteins, lipids), after reaching a particular threshold, are suggested to lead to cellular impairments and consequently to aging. Since not only free radicals lead to this type of damage but also other cellular reactive by-products of the normal metabolism, the theory has been extended to the “oxidative damage theory” which suggests that reactive oxygen species (ROS) or reactive oxygen intermediates are responsible for the accumulation of age-related cellular damage, and that this damage represents an important contributor to biological aging [12].

A large body of evidence has accumulated in recent decades to support this theory, which is extremely attractive because it may relate to a unified mechanism of aging, a mechanism which is conserved in the various biological systems.

Support for the crucial role of ROS-induced DNA damage in aging comes from investigations in which oxidation products of DNA were measured in the urine of rats and humans. These experiments analyzed only a few of all possible oxidation products (e.g., thymine glycol, thymidine glycol) [13]. Further investigations revealed that mtDNA shows a tenfold higher amount of 8-hydroxydeoxyguanosine than nuclear DNA [14]. These data were explained by the fact that mtDNA exposure to ROS is much higher because mitochondria generate rather high amounts of ROS due to leakage of the energy-producing metabolism. In addition, mtDNA is not protected by histones, and the mitochondrial DNA repair system is much less efficient than the corresponding system found in the nucleus. 8-Hydroxydeoxyguanosine, thymine glycol, and thymidine glycol are only a few DNA adducts which are formed but which can be detected by certain techniques. Another type of DNA modifications are I compounds (“indigenous compounds”), which are found to accumulate with age in various tissues of laboratory animals [15–17]. Two types of I components are known: type I components are formed as a

consequence of normal metabolism while type II components are the result of oxidative stress [18, 19].

Gross DNA reorganizations

Nuclear genome Various types of gross, age-dependent instabilities have been reported in the past. Among these, changes in chromosome numbers have been repeatedly described (for review see [20]). In cultured peripheral blood lymphocytes, the frequency of aneuploid nuclei has been found to increase in both sexes. In females, the increase in hypodiploid and hyperdiploid cells is higher than in males. A high proportion of cells are reported to have lost or gained an X chromosome [21, 22]. Most frequently, the loss of the inactive, heterochromatic X chromosome is found [21]. In contrast, other cell types such as ovarian granulosa cells may display neither age-related increases nor decreases in the frequency of aneuploid nuclei [23].

Apart from numerical changes, various types of structural changes of chromosomes are reported to occur during aging in various biological systems. A sixfold increase in the frequency of chromosome aberrations in old kidney cells from mice was reported by Martin et al. [24]. Similar results have been obtained with cultured lymphocytes from human donors of different age [25]. However, conflicting data are also reported. A large survey of peripheral blood lymphocytes from about 500 individuals failed to demonstrate an age-related change in the mean frequency of chromosomal aberrations [26]. Interestingly, increased frequencies of translocations, inversions, and deletions of chromosomal sequences are reported in somatic cells of patients with Werner's syndrome, a model of accelerated aging [27]. Furthermore, fibroblasts from Werner's patients have been shown to undergo replicative senescence and to have elevated homologous recombination frequencies [28]. The recent cloning of the single gene which is mutated in Werner's patients revealed that this gene codes for a component of the DNA maintenance machinery (see below).

In the past few years, another type of structural age-related changes has attracted much interest. These are changes occurring at the ends of chromosomes, the so-called telomeres, which consist of a large number of short direct repeats. It has been found that telomeres of somatic cells propagated in cell culture shorten over time. Various data indicate a crucial role of telomere shortening in replicative senescence, that is, the limited capacity of somatic vertebrate cells to proliferate. First, telomeres of cultured human fibroblasts and other somatic cells are found to shorten during subculturing [29]. Second, a stabilization of telomeres is reported to occur in immortal tumor cells. This stabilization is correlated with the activity of a ribonucleoprotein, "telomerase" [30]. Third, telomere length is greater in generative cells than it is in somatic cells. Furthermore, the chromosome ends of generative cells are stable regardless of the age of the donor [31, 32]. According to these data, the chromosome ends, which cannot be completely replicated by a conventional DNA polymerase, appear to represent the "molecular clock" which was postulated long ago. This clock counts the number of cell divisions of a particular somatic cell. In generative cells there operates an unconventional enzyme, telomerase, consisting of a RNA component and a reverse transcriptase activity. Telomerase is able to extend one strand of the chromosome in the absence of a DNA template using the sequence of its RNA component as a template. At some time during embryogenesis, telomerase becomes repressed in somatic cells, and chromosomes start to shorten with every cell division. After reaching a critical point, the "Hayflick limit,", one or most chromosomes have lost a significant amount of telomeric repeats, and cells stop dividing. Transformation via mutation or via the expression of viral oncogenes allows cells to bypass the Hayflick limit. Transformed cells go on to divide until they reach another checkpoint, termed "crisis." At this point the ends of most chromosomes are shortened beyond a critical limit, and most cells consequently die. However, even at this point, a few cells may escape and become immortalized due to the activation of telomerase and the stabilization of the shortened chromosomes.

The "telomere hypothesis of aging and immortalization" is a very attractive explanation of cellular senescence and may also be of great significance in understanding organismal aging in humans. However, telomere shortening clearly does not account for aging processes in all organisms and may be restricted to higher organisms. In yeast and in the filamentous fungus *Podospora anserina*, two lower eukaryotes in which an early separation into germline and soma does not occur, telomeres have been found to remain constant during aging [33, 34].

Another type of chromosomal age-related DNA reorganizations was analyzed in the past in detail and proposed to be a major cause of aging in humans. The reorganizations occur between tandemly reiterated sequences (for review see [35, 36]). Changes of this type occur during aging of human cell cultures and also in cells isolated from certain human tissues. An age-related loss of sequences coding for ribosomal RNA (rDNA) has been reported to take place in various tissues of mice, beadle dogs, and humans [37–41]. However, conflicting data in other studies show no differences in genomic RNA copy number in heart and liver tissues between mature and senescent mice or during serial passage of human diploid fibroblasts [42]. A critical discussion of the conflicting data on rDNA loss during aging can be found in [35].

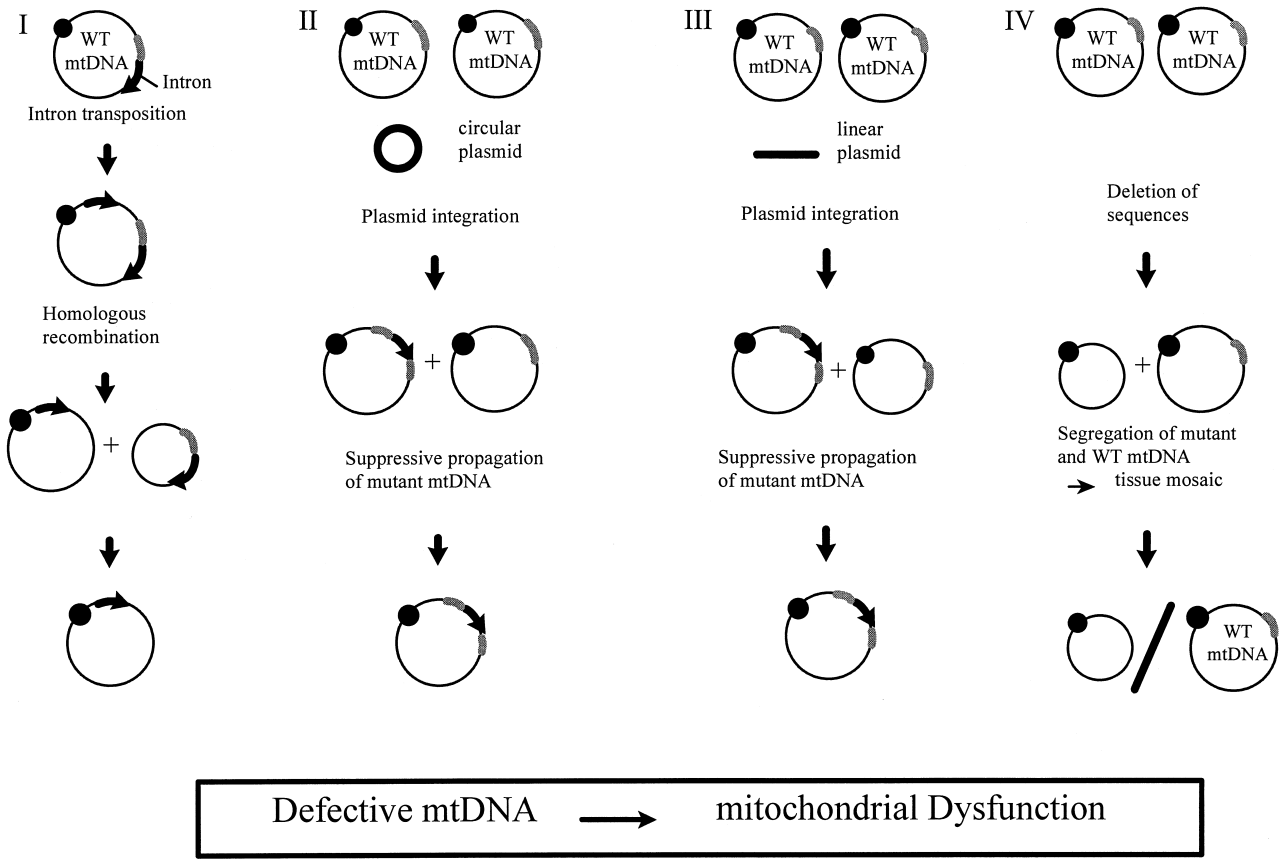
Finally, age-related reorganizations of the nuclear genome are also suggested to be mediated by transposable genetic elements [43]. The effect of these mobile genetic elements may be direct, for example, via the inactivation of essential genes. On the other hand, replicative transposition of a transposon results in the duplication of the element, leaving one copy at the original location and inserting a second copy into another location. Homologous recombination processes between these transposon cop-

ies may lead to gross reorganizations and cellular dysfunction. A number of organisms have been analyzed for the significance of the activity of transposable elements in aging. In some cases, some evidence has emerged that this type of elements indeed contributes to aging processes. In the nematode *Caenorhabditis elegans*, somatic excision of the transposable element Tc1, a transposon present in 30–500 copies per haploid genome, has been found to increase by more than 14-fold during the life span of the analyzed strain [44]. Experiments in fruit flies report a P element transposon induced in males. Decreased life span has been observed in a strain containing 3–17 P elements per genome [45]. A life span reduction has since also been found to occur in strains containing a single somatically active P element [46]. Finally, rat chloroleukemia cells have been shown to die when the number of a transposable element belonging to the abundant long interspersed repetitive sequence family (LINE) increases in the genome [47]. Induction of retrotransposition of this element by UV light or ionizing radiation results in cellular death [48].

Mitochondrial genome. The first clear evidence for a crucial role of genetic traits outside the nucleus in controlling the life span derived from research with the filamentous fungus *Podospora anserina*. This simple eukaryotic microorganism proved an excellent system for genetic investigations of aging processes [49, 50]. An analysis of this type revealed that the onset of senescence, as first described by Rizet [51], is maternally inherited [52]. Subsequently, it has been demonstrated that gross reorganizations occur as *P. anserina* cultures age [53, 54]. These reorganizations are almost quantitative and lead to an impairment of the energy-producing apparatus located in mitochondria (mitochondrial dysfunction) [55]. Reorganizations of the mtDNA have subsequently been identified as occurring in various species. The molecular mechanisms involved in these processes appears different in detail (Fig. 2), but in all systems the consequences are the same, mitochondrial dysfunction (aging, disease).

In wild strains of *P. anserina* reorganization of the mitochondrial DNA is very efficient, and the rate of reorganization appears to be driven by an unusual mobile genetic element, termed plDNA or αSen DNA [56, 57]. In juvenile cultures this element represents the first intron of the gene coding for cytochrome *c* oxidase subunit I

Fig. 2 Simplified scheme showing various types of age-related mtDNA reorganizations. *Gray*, essential genes; *black circles*, origins of replication. *I*, *P. anserina*; reorganization of the mtDNA as consequence of the transposition of a mobile intron (*arrow*). After homologous recombination between duplicated intron sequences two subcircles are formed; only the subcircle containing the origin of replication is retained during senescence. *II*, *III*, *Neurospora spec*; disruption of the essential gene as the consequence of the integration of a circular (*II*) or a linear plasmid (*III*; *arrows*). In both situations the mechanism of suppressive propagation of mutant mtDNA is unclear. *IV*, *Homo sapiens*; deletions of mtDNA and formation of tissue mosaics with wild-type (*WT*) and mutant mtDNA by an unknown molecular mechanism. In all cases defective mtDNA molecules accumulate and lead to mitochondrial dysfunction



[58, 59]. During aging, the intron becomes liberated and amplified and can be isolated as an autonomous circular DNA molecule. Recently, it has been demonstrated that the mobility of the pl-intron appears to proceed via a reverse splicing reaction and by subsequent recombination processes leading to a duplicative transposition of the intron [60]. This process generates two copies of identical intron sequences present in the same mtDNA molecule. Sequences of this type are prone to recombination processes and can account for the gross reorganizations of the mtDNA found in old *P. anserina* cultures. The transposition of the pl-intron appears to be controlled by genetic factors. In particular, a reverse transcriptase encoded by an open reading frame of the intron seems to be crucial [61–64]. However, intron transposition is most likely controlled by other genetic factors as well, factors which may be encoded by nuclear genes (see below).

As described above, the efficient reorganization of the mtDNA is found in all wild strains of *P. anserina*. The efficiency and the rate of reorganization may be affected by various modulators. Some of these appear to be encoded by certain life span affecting nuclear genes. On the other hand, one modulator has been identified in a long-lived mutant in which longevity was demonstrated to be maternally inherited. Detailed genetic and molecular investigations revealed that a linear plasmid, pAL2-1, interacts with the mtDNA reorganizations which are related to the activity of the pl-intron. Plasmid pAL2-1 is an invertron, a genetic element with similarities to genomes of certain DNA viruses (e.g., adenovirus). Importantly, the presence of pAL2-1 delays the wild-type specific age-related mtDNA reorganization of *P. anserina* cultures leading to a 12-fold increase in life span [65–68].

P. anserina is not the only organism in which mtDNA reorganizations have been correlated with aging. First, this type of DNA instability has been demonstrated in various strains of the closely related fungal genus *Neurospora*. In two strains the circular plasmids Mauriceville-1c and Varkud-1c were found to be able to integrate into the standard mtDNA. When the corresponding strains were grown at 37°C instead of 25°C, altered mtDNAs became suppressive and accumulated leading to mitochondrial dysfunction (e.g., deficiencies of cytochromes *b* and aa_3) and senescence [69]. In other strains of *N. intermedia* and *N. crassa*, linear plasmids were correlated with age-related mtDNA reorganizations, mitochondrial dysfunction, and aging. During aging of these strains the two linear plasmids kalilo and maranhar integrate into essential mtDNA sequence (e.g., genes for ribosomal RNAs), leading to integrative inactivation of these sequences. After integration the defective mtDNA molecules become suppressive by a still unknown molecular mechanism [70–72].

The first evidence that age-related mtDNA reorganizations are also found in mammals was derived from an electron microscopic analysis. These investigations reported an age-dependent increase of DNA deletions and/or duplications [73]. With the development of sensitive molecular techniques (polymerase chain reaction), this type of DNA instability was identified in many organisms, including *Drosophila spec, Caenorabditis elegans*, mice, rats, monkeys, and humans [74–82]. In particular, in humans, large deletions are reported to accumulate progressively in various tissues. The highest levels are found in postmitotic tissues such as skeletal and heart muscles and in the brain. These changes are suggested to be important contributors to the aging process. Compared to the almost quantitative progressive accumulation of defective mtDNA molecules in filamentous fungi, only low amounts of specifically altered molecules (up to 10%) are detected in older humans [83]. However, this low figure may be a significant underestimation either due to experimental reasons or because cells in which damaged mtDNAs accumulate over a critical threshold are removed via apoptosis. In any case, mtDNA rearrangements are now believed to play a major role also in human aging [84, 85]. It has been suggested that the accumulation and segregation of mutated mtDNA molecules lead to tissue mosaics, consisting of cells with nonaffected, partially affected, and grossly defective energetic capacities [86, 87]. Mitochondrial dysfunction, as in lower systems, appears to be responsible for functional age-related impairments of human tissues and organs.

Various mechanisms have been proposed to explain the generation and accumulation of mtDNA deletions in humans, including homologous recombination and slip replication [88–91]. The latter may explain the presence of short, direct repeats around the deletion sites. However, a significant number of deletions are reported where no direct repeats can be involved. It therefore remains unanswered whether direct repeats play a significant role at all. Furthermore, experimental evidence has been obtained suggesting that human mtDNA stability is also under the control of specific nuclear genes [92, 93]. The cloning and molecular characterization of these genes will certainly help greatly in elucidating the molecular mechanisms leading to the observed age-related mtDNA instabilities.

Nuclear genes involved in the control of aging

Apart from the experimental investigations described above to elucidate age-related mechanisms by correlating cellular changes which occur during the life span of an organism, another type of approach is currently being followed in various laboratories: the identification, cloning, and molecular characterization of genes which affect the life span. In particular, genes which, after manipulation (e.g., mutation, overexpression), lead to increased life span are attractive candidates because it can be assumed that they normally (unmanipulated) give rise to aging. Only few life span prolonging genes are known and have been analyzed by now to some extent (Table 1).

Saccharomyces cerevisae. A number of genes have been identified in yeast that are expressed differentially over

Table 1 Selection of nuclear genes associated with life prolongation in *Saccharomyces cerevisiae*, *Podospora anserina*, and *Caenorhabditis elegans* (only the genes are indicated which have been initially characterized on the molecular level)

Species	Gene	Type of mutation	Life span increase (%)	Other characterisics	Ref.
S. cerevisiae	*LAG1*	Deletion	50		94
	LAG2	Overexpression	40	Deletion: decreased life span	95
	RAS1	Deletion	30	Overexpression: no effect on life span	96
	RAS2	Overexpression	40	Deletion: decreased life span	96
	SIR4	Nonsense	50	Deletion: no effect on life span	97
P. anserina	gr	Point mutation, splicing deficiency	56	Altered morphology, partial sterilility	98–100
	i		68	Altered morphology, partial sterility	
	viv		164	Altered morphology, partial sterility	99
	gr viv (double mutant)		Immortal	Liberation of a mitochondrial plasmid (plDNA) affected	99, 101
	i viv (double mutant)		Immortal	Amplification of a mitochondrial plasmid (plDNA) affected	99, 101
	AS4		52	Translation factor *EF-1α*	102
C. elegans	*age-1*		70	Increased SOD and catalase activity	103–107
	spe-26		65	Impairment in sperm production	108
	daf-2		100	Dauer formation	109

the life span. Due to the powerful techniques available for manipulating this unicellular eukaryote, the cloned wild-type copies of these genes can be analyzed very easily: gene disruptions, gene deletions, and overexpression of the corresponding genes can be performed routinely and provide the data to determine whether a cloned gene is relevant. This experimental strategy had led to the identification of a number of genes which, after manipulation, turned out to increase life span. Interestingly, not only the knock-out of genes was found to lead to increased life span; in some cases (e.g., *LAG2*, *RAS2*) deletion of the corresponding genes led to a decrease while its overexpression was found to increase the life span significantly.

LAG1 (longevity assurance gene 1) is a gene which, after mutation, leads to an increase in both the mean and the maximum life span, indicating that this gene is involved in determining longevity of yeast. The gene is expressed preferentially in young cells. Cloning and sequencing revealed that *LAG1* codes for a protein with several possible transmembrane domains [94]. No significant homology to any known protein has been found. Interestingly, in Southern blot experiments the cloned *LAG1* probe hybridized to genomic DNA of galago, cow, mouse, salmon, and humans, suggesting that *LAG1* homologues is found over a large variety of different organisms.

LAG2, is another gene which is expressed preferentially in young yeast cells. This codes for a putative protein with a single transmembrane domain [95]. Disruption or deletion of this gene leads to a 40% decreased life span. Overexpression of *LAG2* results in life span extension.

Two other genes, *RAS1* and *RAS2* have been found to affect the life span of *S. cerevisae* [96]. These two homologues of human oncogenes are part of signal transduction pathways in yeast. Only one of the two genes needs to be present for normal growth. Overexpression of *RAS1* has been found to have no effect on the life span of yeast cells while deletion of this gene leads to a 30% increase. On the other hand, overexpression of *RAS2* results in a 30% increase in the life span.

The physiological function of the above yeast genes in controlling longevity is not clear at present. However, the effect of the two *RAS* genes indicate that signal transduction is a part of the complex molecular network involved in the control of this lower eukaryote. Finally, since all of these genes appear to have homologues in humans, it will be interesting to isolate and characterize them to determine whether they are also significant for human aging.

Another gene has recently been shown to be involved in the aging process of yeast. Mutation of *SIR4*, a gene known to be involved in chromatin silencing, was found to lead to an increased life span by 50% [97]. The corresponding mutant was found to be nonsense mutation leading to a truncated protein. A null allele of *SIR4* resulted in a life span decrease. The data of the detailed analysis led to the proposal of a novel locus, *AGE*, which is involved in the control of yeast aging. In young cells this locus is thought to be silenced by the *SIR4* gene product. In old cells *AGE* should be activated.

Podospora anserina. A number of long-lived mutants of *Podospora anserina* were selected in the past either spontaneously or induced by mutagenesis. Only a few of the various fungal genes affecting life span have been characterized to some extent at the molecular level (Table 1). Among these, mutations affecting the translation fidelity have been isolated (for references see [102]). Mutations in *AS4* lead to a significant increase in life span (e.g., *AS4-43*, *mat-:* 52% increase). Recently, this gene was found to encode translation elongation factor EF-1α [102].

A number of other long-lived mutants have been selected as laccase deficiency mutants. Due to the deficien-

cy in this type of phenoloxidases, these mutants are characterized by an altered phenotype [98, 99, 101, 110, 111]. One gene, gerontogene *grisea*, has recently been cloned and initially characterized [100]. Mutation of this gene leads to an 56% increase in mean life span. *Grisea* codes for a putative transcription activator of the ACE1/MAC1/AMT1 family of transcription factors of *S. cerevisiae* and the pathogenic yeast *Candida glabrata* [112–114]. Interestingly, GRISEA appears to be involved in a tight regulation of the cellular copper homeostasis. Since copper is involved in the formation of the hydroxyl radical via Fenton chemistry (Fig. 3) [115], this mutant also relates to a role of oxidative damage as contributor to aging in *P. anserina*. However, it is still too early to draw a complete scenario explaining the role of *grisea* in the control of aging. Clues about this role are expected to arise from the isolation of the target gene(s) which are controlled by transcription activator GRISEA.

In addition, it appears to be of significant interest to clone and characterize another gerontogene, gene *vivax*. Mutation of this gene leads to an 164% increase in life span. The corresponding mutant is, as *grisea*, laccase deficient and characterized by an altered phenotype. Interestingly, double-mutant *gr viv*, which was selected from the progeny of a cross between the two single mutants, displays a synergistic effect. This double-mutant appears to be immortal [101]. Furthermore, the initial characterization of *gr viv* indicates interactions between nuclear-encoded genes and molecular processes occurring in mitochondria since the liberation of the pl-intron is affected leading to a stabilization of the mtDNA. A detailed molecular analyses of the two mutants may thus lead to novel clues about mitochondrial/nuclear interactions involved in the control of aging at least in *P. anserina*, a field of research which also appears important in other systems.

Caenorhabditis elegans. Caenorhabditis elegans is a nematode which has been extensively used to study developmental processes. In recent decades, this system has been used as an important aging model. In particular, genetic and molecular investigations can be performed efficiently on the experimental level. Since the selection of the first nuclear mutant [103] with an increased life span, age-1, a number of life span prolonging mutants have been isolated (Table 1) and analyzed in some detail. The most striking characteristics of these mutants is that they all appear to be more resistant to stress conditions (e.g., ROS, high temperature, UV exposure). Interestingly, in age-1 the cytoplasmic Cu/Zn superoxide dismutase and catalase are expressed at a higher level as in the wild strain [106, 107]. Moreover, mtDNA deletions appear to accumulate at a lower rate than in the wild type [116]. This pleiotropic mutant thus seems to be an attractive system to study age-related nuclear-mitochondrial interactions in a lower animal.

A number of other long-lived mutants of *C. elegans* are known. Among these, spe-26, is a pleiotropic mutant in which sperm production is affected [108]. Finally, some dauer mutations (e.g., *daf-2*), mutations leading to an arrested larval stage, have been found to lead to a significant increase in the life span indicating, that the dauer formation signal transduction pathway affects life span [109].

The cloning and molecular characterization of the *age-1* gene and of the other age-related genes of *C. elegans* will certainly be the challenge of the next years and can be expected to provide new important novel clues about the molecular mechanism of aging in this lower animal model.

Drosophila. The fruit fly is another, more complex animal aging model. Early approaches to elucidate the genetic basis of aging in this system by induction of mutations leading to strains with increased life spans proved to be rather unsuccessful. Subsequent approaches to isolate long-lived strains via selection for late reproduction under well-controlled conditions have been very successful [117, 118]. These strains are characterized by increased resistance to various stress conditions (e.g., dessication, starvation, oxidative stress) [119, 120]. Since these strains are the result of the recombination of whole genomes, and a whole network of genes may be involved, unraveling the molecular mechanisms leading to extended life appears to be rather complicated. However, mapping experiments have identified a major longevity-determining regulatory locus on chromosome III [121, 122].

Another more specific approach to demonstrate the role of genes in the control of the life span was the construction of transgenics in which two genes of the oxidant defense system, coding for superoxide dismutase and catalase, two enzymes involved in the metabolization of oxygen radicals (Fig. 3) were overexpressed. The corresponding flies were characterized by an increased life span of about 30% [123]. These data demonstrate the important role of a defense system against oxidative stress as a part of the molecular network controlling the life span in *Drosophila*.

Humans. Exciting data have recently been reported about the cloning and initial characterization of a nuclear gene affecting the life span of humans [124]. A single gene, *WRN*, was isolated by positional cloning which, when mutated, leads to several symptoms of premature aging including atherosclerosis, osteoporosis, carcinomas, cataracts, and hair graying [125]. Werner's patients have a reduced mean life span of 45–50 years. Sequence analysis of the *WRN* gene led to the conclusion that this gene codes for a DNA helicase, an enzyme involved in DNA replication, recombination, transcription, and repair. In particular, involvement in DNA repair is consistent with the finding that cultured Werner's fibroblasts are characterized by an increased mutation rate [27, 28, 125]. Thus, the *WRN* gene appears to encode a component of the DNA maintenance system crucially involved in controlling the human life span.

Another gene of the same category may be the gene coding for poly(ADP)ribose polymerase (*PARP*). The

level of this enzyme, which appears to be involved in DNA repair, has been shown to be higher in longer-lived species than in short-lived ones. Furthermore, *PARP* activity decreases in mononuclear lymphocytes of human donors of various ages [126].

Nuclear/mitochondrial interactions

As mentioned above, mitochondria are semiautonomous genetic compartments which depend on gene products encoded by the nuclear DNA. In particular, the various genetic factors involved in maintaining the genetic information, replication, transcription, and repair are encoded by genes located in the nucleus. Since the mtDNA has been found to be involved in the genetic control of aging, it can be expected that nuclear-encoded factors functioning in mitochondria are important factors in the complex network involved in the control of aging. Below, a few putative components of this machinery are introduced as potential factors of significance.

MtDNA repair

Although long controversial, it is clear today that mitochondria contain enzymes required for different forms of DNA repair. Moreover, evidence is accumulating that these enzymes are indeed involved in mtDNA repair, although they may also play a role in the degradation of damaged DNA. It appears that the enzymatic apparatus for recombination repair, mismatch repair, and base excision repair exists in mitochondria. However, in comparison to the repair of nuclear DNA, mtDNA repair is rather weak. Moreover, certain types of repair which are used efficiently in the nucleus are totally absent in mitochondria. All the repair enzymes used in mitochondria are encoded by the nuclear DNA. Interestingly, mitochondrial enzymes (e.g., yeast uracil DNA glycosylase, mammalian AP endonuclease) appear to be encoded by different genes than their nuclear counterparts [127, 128]. However, in contrast to what is known for the nuclear compartment, nothing is known about the definitive contribution of this molecular system in respect to aging. In fact, two obvious questions which need to be addressed are whether the efficiency of the mitochondrial repair systems decreases during aging of a given species, and whether differences in the efficiency can be observed in short-lived and long-lived species.

MtDNA replication

It is trivial to mention that the fidelity of the machinery involved in mtDNA replication is of great significance in cellular degeneration. Reduced fidelity necessarily leads to a progressive increase of DNA alterations. Moreover, the mode of replication found in mammals appears to be prone to errors. As suggested by a number of mtDNA deletions located between the two origins of replication of mammalian mtDNA, replication intermediates which consist partially of single-stranded DNA, may be highly susceptible to damage and to erroneous replication processes (e.g., slip replication).

The protein transport machinery of mitochondria

Finally, the last part of the complex network of molecular processes involved in aging which should be noted in this overview is the molecular machinery controlling the transport of nuclear-encoded mitochondrial proteins into this organelle. This process is dependent on well-ordered membrane-bound protein complexes [5–7]. In addition, certain proteins located both in the cytoplasm and in the mitochondrial matrix assist in the corresponding pathway. These proteins, so-called chaperones, are involved in maintaining the protein which needs to be transported across the membranes in a transport-competent conformation [129]. All components of this complex molecular machinery are encoded by nuclear genes and thus are part of the nuclear-mitochondrial interactions which may be important for biological aging. Among the various components of such a system, chaperones belonging to the so-called heat-stress proteins are of particular interest. These proteins which, in addition to elevated temperatures, are induced by various stress conditions (e.g., against toxic substances, oxidative stress), play a key role in protecting biological systems against stress. Some heat-stress proteins are part of the mitochondrial protein transport machinery. Moreover, expression of heat-stress proteins appears to decline during aging [130]. Finally, among the various long-lived strains from various species, most appear to be more resistant to a number of stress conditions. The relevance of these observations is presently far from being understood in detail, but the elucidation of the corresponding network appears to be a key to unraveling the molecular basis of biological aging in general.

Conclusions and perspectives

The accumulation and comparison of data derived from investigations of aging processes in various biological systems allows some general conclusions to be drawn about molecular mechanisms of aging. A rather complex molecular network appears to exist. It also seems that species-specific mechanisms operate in the various systems. However, these species-specific mechanisms may interfere with one or a few basic mechanisms which may be conserved in various species.

One unified mechanism seems to be related to oxidative stress, which, in part, is linked to energy production in mitochondria. As a by-product of this metabolism, ROS are generated, which can lead to a severe damage of biomolecules, including lipids, proteins, and nucleic acids [12, 131–133]. Usually the formation of mitochondri-

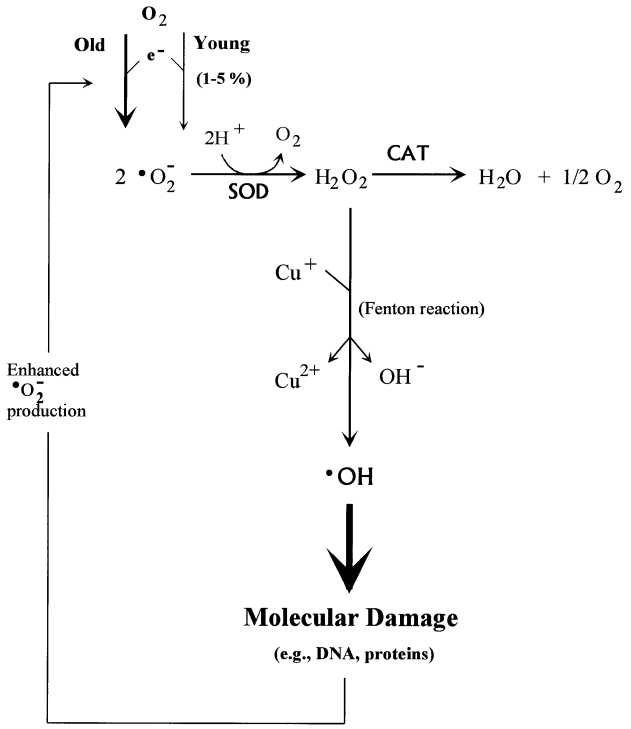


Fig. 3 Cellular formation of the highly reactive hydroxyl radical. In humans about 1–5% of the consumed oxygen leads to the formation of superoxide due to leakage of the electron transport chain. Superoxide dismutase (*SOD*) and catalase (*CAT*), as components of the cellular defense system, metabolize superoxide and hydrogen peroxide, respectively. However, in the presence of transition metals (Cu^{+}, Fe^{2+}), the hydroxyl radical is formed. Molecular damage (e.g., of the mtDNA) subsequently leads to impairment of the respiration chain and as a consequence to enhanced production of superoxide. After reaching a critical threshold molecular damage leads to impairment of cellular functions

al ROS is rather low and results from leakage of the electron transport chain (Fig. 3). In humans, normally only 1–5% of the consumed oxygen leads to the formation of ROS [134]. Moreover, the low levels of these potential hazardous substances can be metabolized by the cellular defense apparatus. However, the production of ROS can be enhanced significantly above a critical threshold when the electron transport chain is impaired (Fig. 3). Impairment of the mitochondrial respiration chain can result from various factors and mechanisms. First, point mutations (e.g., missense mutations) in mitochondrial and nuclear genes may lead to the production of altered proteins. Such proteins, although assembled into the inner mitochondrial membrane, may be affected and lead to increased electron leakage. Second, the accumulation of grossly rearranged mtDNAs (e.g., deletions of sequences coding for proteins) may lead to an unbalanced availability of the components of the respiration chain. Third, an unbalance may also be related to polypeptides which are encoded by the nuclear DNA. The availability of these proteins may be limited in mitochondria for various reasons (e.g., damage of the mitochondrial protein transport machinery). There is enough space in such an scenario for various factors to affect this rather general mechanism. These factors may be encoded by certain age-associated genes as they are currently analyzed in various systems, in particular genes encoding repair functions, factors which metabolize damaging agents, and factors related to the fidelity of DNA replication and of protein synthesis. On the other hand, certain genetic traits (e.g., mobile elements, repetitive sequences) may interfere with this system in such a way that they lead to cellular dysfunction via the generation of various types of DNA instabilities.

It is clear that the notion of this mechanism of aging is yet not mature. However, it represents a concept which can be more specifically approached experimentally in the next years. Indeed, various strategies in this direction are currently being followed. In particular, the identification, cloning, and molecular characterization of specific genes affecting the life span in various species is a promising approach which, in the near future, will certainly provide further clues about the complex molecular network involved in biological aging.

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