Longevity, Genes, and Aging

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Until recently, biogerontology was a backwater of biology, but progress in the qualitative and quantitative genetic analysis of longevity has led to a revolution in aging research. This research has revealed that extended longevity is frequently associated with enhanced metabolic capacity and response to stress. Moreover, it suggests that there are multiple mechanisms of aging. Because of its complexity, the aging process takes us into the realm of integrative biology, and thus, biogerontology should prove instrumental in deciphering the functional and regulatory circuitry of the sequenced genome.

"You can't study aging; it just happens," confided a prominent molecular biologist in 1987. But everything just happens, from sunspots to cell division. The statement of the molecular biologist likely reflects the deep frustration many have felt about a complex biological process that is characterized by increasing disorder and decline. The difficulty is compounded by the quantitative nature of the phenotype; a population must be studied to obtain meaningful data.

Without denying the importance of environmental influences in aging, biogerontologists have long argued that genes play a part in this process. Two major developments form the underpinning of the genetics of aging. The first of these arose from efforts to test evolutionary theories of aging (*1*). Fruit flies that displayed extended longevity were obtained after many generations of selection for late reproduction. The success of this selection firmly established the participation of genes in aging and subsequently showed longevity to be a polygenic character. The second validation of genetic analysis in aging research came from studies in which single genes were found to modulate life-span. Screening for mutants of the worm *Caenorhabditis elegans* that displayed increased life-spans led to the first identification of a longevity gene (*2*); this gene, *age-1*, remains to be cloned. This lack of molecular identity was circumvented in *Saccharomyces cerevisiae* when the expression of an activated form of the small guanosine triphosphatase (GTPase) Ras was shown to extend longevity (*3*). These developments initiated a growing stream of results in the qualitative (Mendelian) and quantitative genetics of aging.

The genetic determination of life-span should not be equated with the action of a genetic program. The operation of such a program from birth to death is not likely, nor is it sustained by evolutionary theory (*4*). Aging is a stochastic process (*4*). It is genetically determined in the sense that the genetic constitution determines its course. Because it encompasses stereotypic biochemical responses to particular cellular states, aging may superficially appear to be programmed.

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Saccharomyces cerevisiae

In yeast (*5*), life-span is more accurately characterized by the number of progeny an individual cell produces than by chronological age (*6*). The mother cell dies after a finite number of divisions, as depicted by the cell spiral (Fig. 1). Inherent in this model is the concept of molecular memory, which has been proposed to reside in the regulatory states of chromatin (*7*). An epigenetic change or an environmental factor would be part of a stochastic trigger that sets the aging process in motion (*7*).

The genetic analysis of yeast aging was initiated by the cloning of genes that are differentially expressed during the life-span; such differential expression constitutes a secondary phenotype for a longevity gene (*8*). [Other than *age-1*, all longevity-determining genes have been found by first examining surrogate (secondary) phenotypes rather than life-span itself.] This approach ultimately provided more than a dozen candidate genes with expression profiles that change in various ways. Among these was *LAG1*, a yeast gene that determines both mean and maximum life-span (*9*). The protein Lag1p has several potential transmembrane domains, and *LAC1*, a virtual copy of *LAG1* on a different chromosome, also determines longevity (*10*).

Two other genes that are differentially expressed during the yeast life-span are *RAS1* and *RAS2*. *RAS1* has a life-shortening effect, whereas *RAS2* extends longevity, indicating that the genes have discrete roles in yeast physiology (*11*). The effect of *RAS2* on longevity appears to be mediated by a pathway distinct from the interaction of Ras2p with adenyl cyclase (*11*). Indeed, overstimulation of adenyl cyclase has the opposite effect, a marked reduction in longevity.

These *RAS* studies suggest that an optimal amount of Ras2p activity is necessary for maximal longevity. Increasing the amount of an activated equivalent of Ras2p in the cell beyond a certain threshold reverses its life-extending effect (*3*). This biphasic effect can be ascribed to the opposing effects on longevity of stimulation of the adenyl cyclase pathway and of the new *RAS2* pathway (*11*). The quantitative effect on life-span of a particular amount of Ras2p likely depends on the genetic background and the environment. *RAS2* is very pleiotropic (*12*) and interacts with many other genes. [Ras1p weakly stimulates adenyl cyclase (*12*) and is always present to partially fulfill Ras2p's role.] These interactions and environmental and epigenetic exigencies result in the establishment of homeostasis, which is difficult to circumvent.

RAS2 provides an interface between the cell and the environment. Ras2p participates in sensing the nutritional status of the organism and in responding to stresses (*12*), such as starvation, crowding, ultraviolet (UV) light, and heat shock. Indeed, Ras2p controls or modifies most or all stress responses (*12–14*), including oxidative stress, to which the inflammatory response in mammals is related through p38 kinase (*15*). The response to UV light is independent of DNA damage (*13*). The resistance of yeasts to sublethal doses of UV light increases through mid-life and then plummets, whereas resistance to ethylmethane sulfonate, an alkylating agent, shows a steady decline (*16*). The peak UV light resistance coincides with a peak of *RAS2* expression during the life-span (*16*). These observations suggest that *RAS2* expression may be the molecular basis for an age-specific phenotype, and they raise the possibility that active life maintenance processes are operative through middle age, after which the organism survives on a dwindling capacity for life maintenance. *RAS2* may also play an important role in determining the regulatory states of chromatin; such a role is intimated by the impact of *RAS2* on sporulation (*12*) and by the deduced interaction of this gene with *RAP1*, a gene involved in transcriptional regulation (*17*).

Extension of life by *RAS2* overexpression postpones senescence in yeast, as evidenced by the pronounced delay in the characteristic increase in generation time during the life-span (*11*). These long-lived yeasts show a marked increase in metabolic capacity and efficiency (*5*). The role of

RAS2 in life maintenance is likely to be at least partially a result of this effect. This mechanism of *RAS2* action is supported by petite mutants, which lack intact mitochondria and reveal a genetic interaction between *RAS2* and *PHB1* found by examination of the effects on longevity of mutants of these genes (*18*). Phb1p shares sequence motifs with Ras-GTPase activating proteins (Ras-GAPs) (*19*), and *PHB1* is upstream of *RAS2* in a genetic pathway, which suggests that it acts as a GAP to down-regulate Ras2p activity (*18*). *PHB1* is differentially expressed during the life-span (*18*) and is highly conserved between yeast and humans (*20*).

Mammalian somatic cells have a limited population-doubling capacity in tissue culture; as they exhaust this doubling capacity, they undergo a gradual attrition in the telomere DNA sequences that cap the ends of chromosomes. This attrition has been proposed to result in changes in the transcriptional status (that is, silencing) of subtelomeric genes (*21*). In contrast, yeast cells do not display telomere shortening during their life-span (*22*), although changes in transcriptional silencing may still occur during yeast aging. Indeed, some evidence suggests that the *CDC7* gene, an antisilencer (*23*), may be involved in determining longevity (*24*). Further support for this notion comes from the implication of the *SIR4* gene, a component of the silencing apparatus (*25*), in yeast aging (*26*). *SIR4* was among four genes (the *UTH* genes) identified by selecting for survival of haploid cells on sporulation medium (a secondary phenotype) and screening them for mutants that displayed extended longevity. Mutations in *SIR4* relieve transcriptional silencing. The *sir4-42* mutant extends life-span, unlike other mutants in *SIR4*, which curtail it. On the basis of this difference between *sir4-42* and other *sir4* mutants, it has been proposed that there is a specific locus (*AGE*) in the yeast genome that actively promotes aging and is silenced specifically by *sir4-42* (*26*). The other *uth* mutants have not been fully characterized; however, mutations in all the *UTH* genes result in enhanced resistance to a variety of environmental stresses.

Recent studies support the hypothesis that changes in silencing may be important in normal yeast aging. An age-dependent decrease in transcriptional silencing was detected near at least one telomere; however, no significant change was detected at another telomere (*27*). By extension, loss of silencing near telomeres may well occur in postmitotic mammalian cells, in which telomere attrition does not happen. Loss of silencing may also occur at the silent mating-type locus *HMRa* before yeasts die, as suggested by the presence of *HMRa* messenger RNA in dead cells (*28*). This finding could explain the observation that yeasts lose mating ability as they age (*29*), because the expression of the silent mating-type loci would make them phenotypically diploid and thus unable to mate. The enhanced response to stress and life extension caused by the loss of silencing induced by *sir4-42* (*26*) likely reflects partial, though not complete (*28*), activation of the sporulation pathway and adaptation to the stationary phase. The gradual loss of silencing at telomeres (*27*), and perhaps at other heterochromatic regions, may not provide this fortunate combination, and the result may be a progressive and self-accelerating dysregulation of genes that disturbs cellular homeostasis. [There is no selection for a "positive" outcome here, because old yeasts have escaped the force of natural selection by contributing little to the gene pool of subsequent generations (*5*).]

Caenorhabditis elegans

In the nematode (*30*), the genetic approach has yielded much physiological information on aging and has identified regions of the genome that contain longevity genes. Studies with recombinant-inbred (RI) strains have shown that aging begins after development is completed, mainly during the postreproductive period (*31*). The RI strains lose motor ability at a rate that is inversely proportional to their longevity. Thus, longer life is characterized by a longer period of activity—that is, by a greater metabolic capacity. Two different methodologies have been used to map the quantitative trait loci (QTLs) associated with extended longevity in RI strains (*32*); at least five genomic regions appear to be associated with longevity.

Major new insight into nematode aging has come from the implication of *daf* genes in this process. These genes control a program that results in the formation of a dispersal form, the dauer larva. Environmental cues (such as insufficient food, crowding, and heat) lead to this "time out" from development. Inactivation of the *daf-2* gene product results in constitutive dauer formation. If *daf-2*$^-$ worms are allowed to develop into adults and are then shifted to a nonpermissive temperature, they exhibit an increase in life-span with no overt signs of dauer formation (*33*); this effect is entirely suppressed by a mutation in *daf-16*. This result shows that *daf-16* lies downstream of *daf-2* for adult longevity, just as it

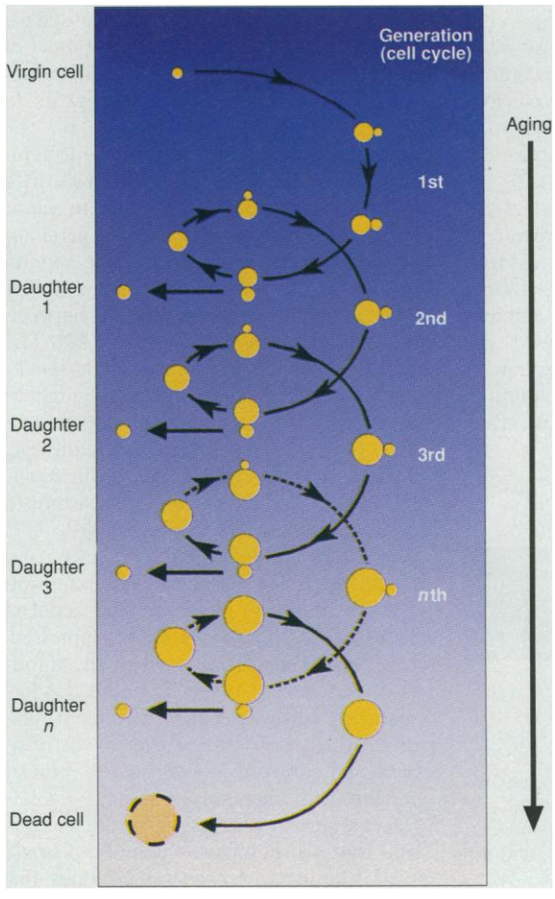

Fig. 1. The cell spiral in *S. cerevisiae*. A virgin cell that has never divided enters the spiral and divides a number of times. The daughters it produces enter the spiral at the top. The probability that the mother continues downward decreases at each turn of the spiral, and she ultimately ceases dividing and dies. [Adapted from (*24*) by permission of the publisher, Elsevier Science Inc.]

does for dauer formation.

Nine additional *daf* genes have been tested, but only *daf-12*, *daf-18*, and *daf-23* are involved in aging (*34*). All of the genes that play a role in longevity are part of the *daf-2* branch of the dauer pathway. *daf-12* has a synergistic effect with *daf-2* (*34*). These studies allow the elaboration of a *daf* pathway for adult longevity in *C. elegans* (Fig. 2) that roughly parallels the dauer pathway. Only the *daf-12* gene has been cloned; it encodes a zinc finger protein that may belong to the superfamily that includes thyroid, steroid, vitamin D, and retinoic acid receptors (*35*). Not surprisingly, the physiological response to environmental cues involved in dauer formation is mediated by signal transduction pathways.

There are striking similarities between *daf-2* and *age-1* mutants. Both show an enhanced intrinsic thermal tolerance (*36*). In addition, *age-1* mutants display an increase in induced thermal tolerance, relative to the wild type, as well as a modest and variable extension of mean life-span on prior exposure to sublethal temperatures (*36*). It is not clear that the resistance to heat is the only affected end point; heat shock proteins are induced by other stresses as well (*37*). These findings underscore the often simultaneous appearance of stress resistance in extended longevity.

The prototypic longevity gene, *age-1*, has been assigned to the *daf* pathway for adult longevity (*34*). The dauer pathway controls a metabolic adjustment in response to environmental stress. Given the placement of *age-1* in the *daf* pathway, it is interesting that food restriction of *age-1* mutants results in a further extension of life-span (*38*). Thus, manipulation of the *daf* pathway in adults is somehow divorced from the environmental cue that results in dauer formation. Food restriction and lower ambient temperature increase longevity in wild-type *C. elegans*, concomitant with a lower rate of loss of motor activity similar to that found in the RI strains (*39*). These regimens affect metabolic activity, and they apparently are located higher in the genetic hierarchy of life maintenance and affect the activity of a greater number of genes than do the *daf* manipulations discussed here.

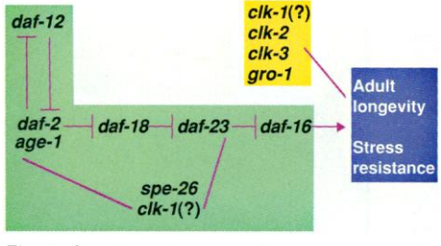

Fig. 2. Genetic pathways in *C. elegans* longevity. The *clk* genes and *gro-1* show complicated mutual interactions. The question mark (?) denotes conflicting information. The genes *spe-26* and *clk-1* may reside anywhere between the two points in the designated pathway (*44*), and the precise location of *age-1* is not certain.

The *age-1* mutant does not alter the growth rate or the timing of developmental processes, and thus it is not a mutation in a "clock" gene (*2*). In contrast, the *clk-1* mutant exhibits certain properties that would be expected of such a gene (*40*). This gene is highly pleiotropic and affects the length of embryonic and postembryonic development, growth rate, cell cycle time, and defecation, swimming, and pumping (feeding) cycles. Some alleles also extend adult life-span. Mutants in *clk-1* show a maternal effect, such that the mutant phenotype is only expressed in the progeny when the parent is homozygous for the *clk-1* mutant. For at least one of the *clk-1* alleles, there is a striking alteration in the animals' response to temperature. Moreover, *clk-1* mutant embryos develop at a rate characteristic of their temperature until the two-cell stage. The variability in the timing of many of the events affected by *clk-1* is much greater in the mutant than in the wild type. Many of these features suggest that *clk-1* may act through an epigenetic mechanism such as genome imprinting. Indeed, the properties of this gene, including maternal rescue for life-span, underscore the importance of the developmental environment for subsequent events. The *clk-1* gene appears to control metabolic adjustments in the nematode, particularly in response to temperature. It likely performs this role in a regulatory capacity, as *RAS* does in yeast.

Particular alleles of *spe-26*, a gene required for sperm production, also extend life-span in the worm (*41*). Recent studies argue against a reciprocal relation between gamete production and longevity (*42*). The effect of *spe-26* on life-span is allele-specific. This gene encodes a protein similar to the actin-associated proteins kelch and scruin (*43*). The *spe-26* gene is pleiotropic; mutants in this gene, as well as in *age-1*, *daf-2*, *daf-23*, and *clk-1*, are more resistant to UV light than is the wild type (*44*). This UV resistance is suppressed by mutations in *daf-16*, which also suppresses life extension by these mutants. However, the reduced fertility of *spe-26* mutants is not suppressed. These results suggest that all of these longevity genes feed into a common *daf-16*–dependent pathway; hence, the identification of this gene, and of the downstream events it controls, constitutes a crucial question in the genetics of aging.

Some caution is necessary before we conclude that all these genes describe a single global genetic pathway that determines longevity in *C. elegans*. Other studies show no suppression of the extended longevity of the *clk-1* mutant by a *daf-16* mutant (*45*): The life-spans of *daf-2*; *clk-1* double mutants are six times that of the wild type, and three additional longevity gene mutants—*clk-2*, *clk-3*, and *gro-1*—show complex interactions with each other and with *clk-1*.

Drosophila

Effects of single genes in *Drosophila* that result in extended longevity are difficult to find (*46*), perhaps because of the lack of a dispersal form (similar to the yeast spore or the *C. elegans* dauer) that engages the entire organism in a profound response to environmental stress. However, selection for extended longevity has been successful, as indicated earlier. High larval density is necessary for success when selection is for late reproduction; the stress of crowding may derepress genes that are then more easily selected. Indeed, this stress, which includes restriction of food intake and is similar to the signal for dauer formation in the nematode, appears to be required for expression of the extended-longevity phenotype in some selected lines (*47*). The density effect on longevity is exerted during the third larval instar, a period during which epigenetic events can affect the future adult form. This fact and other clues point to the interpretation of direct environmental cues by the neuroendocrine axis in setting physiological thresholds and limits for longevity. The long-lived flies exhibit a markedly increased metabolic capacity at any temperature [as measured in terms of lifetime oxygen consumption or total egg production (*48*)], and they are more persistent fliers (*49*).

Interestingly, the extended-longevity lines (*1*) differ substantially in their physiology. In one group, the flies are more resistant to oxidative stress, likely because of the enhanced activities of several antioxidant enzymes, but their resistance to starvation and desiccation is low (*50*). They have an increased glycogen content, but their lipid is lower than in controls (*50*). In the other group, the flies display enhanced resistance to starvation, desiccation, ethanol vapor, and heat (*51*). They have a high lipid content, which explains their starvation resistance (*51*); they also have a higher glycogen content, which explains their greater flight duration (*51*). Selection for either starvation or desiccation resistance resulted in further gains in the respective stress resistance as well as in longevity (*52*); the responses to the two stresses were partly independent of each other. Thus, there are multiple mechanisms of aging and more than one route to extended longevity.

In comparing C. *elegans* and *Drosophila*, it is possible to discern a shift from the determination of longevity as a function of components of developmental programs (C. *elegans*) to the impact on aging of epigenetic effects during development (*Drosophila*). This shift is paralleled by the interposition of the neuroendocrine system in the response of the organism to environmental cues. The final step in this progression may be the calorically restricted animal. Caloric restriction is the only widely validated method for the extension of life-span and postponement of senescence in mammals (53). It results in major metabolic changes and in the preservation of stress responses. The power of caloric restriction is attested by its ability to delay the appearance of spontaneous tumors in p53 knockout mice (54). The profound and early hormonal changes associated with caloric restriction suggest that the neuroendocrine system is a mediator (53). In different phyla, the caloric restriction mediators may differ, but the effectors may be the same.

The studies recounted above all point to the importance of metabolic capacity and stress responses in aging. Oxidative stress appears particularly important, especially because oxidative damage increases substantially with age (55). The impact of such damage on aging was assessed by creating transgenic fruit flies that carried ectopic copies of the genes coding for the antioxidants superoxide dismutase (SOD) or catalase. Little or no effect on mean life-span, and no effect on maximum life-span, was observed, indicating no change in intrinsic aging. However, when expression of the two enzymes was combined in an appropriate ratio in transgenic *Drosophila*, a 30% increase in mean and maximum life-span was detected in some lines, with a corresponding decrease in mortality rate (55). Despite greater physical activity and oxygen consumption, the flies suffered less oxidative damage to protein, DNA, and enzymes (55). These data are consistent with the notion that oxidative stress is a determinant of longevity. However, some caution is needed in interpretation. Only 50% of normal SOD activity and 14% of normal catalase activity are necessary for a normal life (56). Thus, the excess enzymatic activities in the transgenic flies may do more than simply protect them from oxidative damage.

There is some evidence for gene dysregulation during aging in *Drosophila*; heat shock results in the synthesis of about four times as many different peptides in old flies as in younger flies (57). Some of these proteins are induced even in the absence of thermal stress. Thus, epigenetic changes may play a role during aging. There is reason to suspect that in fruit flies the population is stratified, such that individuals reside in one of several epigenetic states. The plateau and reduction in the mortality rate in the oldest flies is most readily explained by such a phenomenon, given the virtual absence of genetic and environmental factors to account for it (58).

Mammals

The achievements of genetics in the analysis of aging in mammals are less impressive than in other organisms, although this situation is changing. Studies with mice have placed emphasis on immune function. Congenic and back-cross mice have been used to define a role for the *H-2* locus in determining longevity. These studies have been supplemented by the analysis of multiple chromosomal regions in RI mice (59). This work has revealed large effects of heterozygosity, multiple gene interactions, environmental effects, and the association with longevity of nearly one-half of the genomic regions tested (in part the result of statistical difficulties with this approach), although multiple-regression models involving six genetic regions correlated best with life-span. Longer-lived and shorter-lived lines of mice have been selected by using high and low early-life antibody response to sheep red blood cells as a surrogate phenotype. High immune responsiveness was positively correlated with life-span, and both were regulated by a small number of gene loci (60). Closer scrutiny of the results suggests that the detrimental effect of low antibody responsiveness is more obvious than are the benefits of high responsiveness. These studies are weakened by the rather short life-span of the mice. It is perhaps not surprising that selection for immune responsiveness would result in interline separation for longevity; the immune system is the first line of defense against environmental insults in mammals (61).

Immune function has been implicated in the genetics of aging in humans in studies of the *HLA* locus (62). The *APOE* and *ACE* loci have also been associated with human longevity in a study of French centenarians (63). The apoE4 isoform of apolipoprotein E is correlated with an increased susceptibility to both coronary heart disease and Alzheimer's disease. It is thus not difficult to understand that the presence of apoE4 is associated with shorter life expectancy. The increased frequency of the apoE2 isoform in longevous individuals is unexpected, given its hypertriglyceridemic effect. The increased representation of the *ACE/DD* genotype in centenarians is even more surprising, in view of its association with myocardial infarction.

The association of *APOE* with longevity raises important issues. When we uncover human longevity genes, are we actually defining genes that affect susceptibility to disease (and if so, are the affected susceptibilities only for diseases with an age-dependent onset)? How does this question relate to the elucidation of the intrinsic aging process? If we accept that human aging is a functional decline that involves an increase in the predisposition to disease, our quandary becomes less disturbing. We can arrive at generalizations that have wider applicability to the aging process through an analysis of the factors that predispose people to age-related disease. These considerations are particularly relevant to the study of premature aging syndromes of humans (64).

There is some controversy as to whether the so-called premature aging syndromes are representative of normal aging. Although people with these disorders display some or many of the manifestations of aging at an accelerated rate, in no case do they present with all of the features of normal aging; hence, these syndromes are termed segmental. Moreover, some of the symptoms displayed clearly differ from normal aging; some prefer to call these genetic disorders caricatures of aging. Three of these disorders appear in many ways to be related. Cockayne syndrome can result from mutations in DNA helicases encoded by the *ERCC-6* gene or the *ERCC-3* gene in complementation groups B and C, respectively, which cause a defect in the repair of UV light–induced DNA damage in transcriptionally active DNA (65). Ataxia telangiectasia is caused by a mutation in the

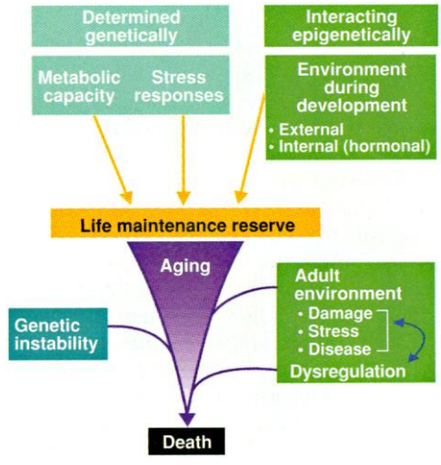

Fig. 3. Determinants of aging and longevity. Physiological relations, not molecular mechanisms, are shown. Metabolic capacity, stress responses, and environment during development all contribute to the life maintenance reserve, where they interact. Genetic instability is genetically determined, results in genetic alterations, and is influenced by environmental factors. Aging plays itself out at all levels of biological organization; it is not possible to separate cellular changes from organismal aging.

ATM gene that encodes a protein with homology to phosphatidylinositol kinases (*66*). The closest homolog of *ATM* is the yeast gene *TEL1*, which is involved in telomere metabolism (*67*). Other homologs include DNA-dependent protein kinase and *MEC1*, a yeast checkpoint control gene. Werner syndrome expresses a mutator phenotype, elevated rates of nonhomologous recombination, rapid telomere shortening, and decreased repair of telomeres; it is caused by a mutant DNA helicase (*68*). All three disorders are characterized by genetic instability of somatic cells. The association of repair, recombination, transcription, telomere metabolism, and helicase activity with these premature aging syndromes suggests a profound effect on gene expression that could cause pronounced genetic and epigenetic changes in somatic cells, resulting in cellular degeneration.

What Is Genetics Telling Us About Aging?

The life maintenance reserve is a genetically determined functional potential that allows the organism to survive, at least to reproductive maturity (Fig. 3). Exhaustion of this reserve is a stochastic phenomenon with a probability that increases with age. There are two physiologic antecedents of life maintenance reserve: metabolic capacity and stress responses. The expansion of metabolic capacity results in the generation of oxidants, which demands the elaboration of the oxidative stress response; thus, the response to oxidative stress is a primary stress response. The mitochondrial genome is especially vulnerable to oxidative damage and suffers deletions during aging (*69*). The life maintenance reserve is modulated by epigenetic factors; the environment during development is of fundamental importance. This environment can be clearly external, as in the nematode. It can also be the internal, especially the hormonal, milieu encountered by the fetus in the womb. The postdevelopmental environment exerts its effect—usually, though not always, promoting aging—through damage, stress, and disease.

As a somatic cell progresses through its life-span, an epigenetic change in the regulatory states of chromatin set up during development becomes more probable. Deficits in the silencing apparatus lead to small changes (either increases or decreases) in silencing. Beyond a certain threshold, this becomes a self-accelerating process, resulting in gene dysregulation. (Dysregulation can occur at other levels of gene expression as well.) Stress further accentuates this process; indeed, there is a reciprocal relation between environmental insult and dysregulation, one accentuating the other. Stress, particularly oxidative stress, has a profound effect on telomere metabolism and cellular senescence (*70*). The resulting telomere changes are likely to affect the transcriptional status of subtelomeric genes, and they may also induce checkpoint controls. The age-dependent, stochastic loss of silencing described in mice (*71*) illustrates epigenetic changes in vivo that are likely caused by dysregulation.

The increase in stress responses as a function of age in the absence of overt stress may reflect gene dysregulation, although it is difficult to rule out a compensatory response to covert stress. Such chronic stress has deleterious effects in its own right and has been described for the acute-phase inflammatory response (*72*). The chronic response is associated with an inability to mount a full-blown response to acute stress, and it obviously constitutes an aging deficit. An age-dependent increase in interleukin-6 production may be the cause of this chronic elevation of the inflammatory response. Caloric restriction largely prevents this increase (*73*). These epigenetic phenomena, along with somatic genetic instability, are likely to be a prime cause of cellular degeneration with aging, which further results in the degenerative disorders characteristic of aging. Thus, the factors that affect aging are genetic, epigenetic, and environmental, and the limiting factors for longevity are metabolic capacity, efficiency of stress responses, and dysregulation. The rapid progress in the genetics of aging will provide critical tests of the speculative view of aging sketched out here.

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Oxidative Stress, Caloric Restriction, and Aging

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Under normal physiological conditions, the use of oxygen by cells of aerobic organisms generates potentially deleterious reactive oxygen metabolites. A chronic state of oxidative stress exists in cells because of an imbalance between prooxidants and antioxidants. The amount of oxidative damage increases as an organism ages and is postulated to be a major causal factor of senescence. Support for this hypothesis includes the following observations: (i) Overexpression of antioxidative enzymes retards the age-related accrual of oxidative damage and extends the maximum life-span of transgenic *Drosophila melanogaster*. (ii) Variations in longevity among different species inversely correlate with the rates of mitochondrial generation of the superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide. (iii) Restriction of caloric intake lowers steady-state levels of oxidative stress and damage, retards age-associated changes, and extends the maximum life-span in mammals.

A common feature of the life cycle of virtually all multicellular organisms is the progressive decline in the efficiency of various physiological processes once the reproductive phase of life is over. A variety of strategies and models have been used to understand the nature of the mechanisms underlying the phenomenon of senescence. Frequently the purported explanations or hypotheses deal with the manifestations of aging, which are unlikely to be self-initiating, rather than with a more fundamental underlying cause accounting for the plethora of changes associated with senescence. To elucidate the mechanisms of aging, any causal hypothesis should explain the following three conditions: (i) why organisms undergo progressive and irreversible physiological decline in the latter part of life, (ii) why the life expectancy or rate of aging varies within and among species, and (iii) why experimental regimens such as caloric restriction delay the onset of a variety of age-associated physiological and pathological changes and extend the average and maximum life-span of animals. A mechanistic understanding of the effects of caloric restriction is important because of the efficacy of this regimen in the prolongation of the maximum life-span of mammals and because of its implications for human health.

A hypothesis ascribing one cause for aging postulates that the senescence-associated loss of functional capacity is due to the accumulation of molecular oxidative damage (*1–4*). This hypothesis is based on the fact that oxygen is potentially a toxic substance, and its use by aerobes, although necessary for their immediate survival, also may be hazardous to their long-term existence. The phenomenon of oxygen toxicity, sometimes referred to as the "oxygen paradox," is inherent in the atomic structure of oxygen. Molecular oxygen is a biradical that upon single electron additions sequentially generates the partially reduced molecules $O_2^{\cdot-}$, H_2O_2, and ·OH, which by further reactions can generate an array of additional reactive oxygen metabolites (ROMs) and cause extensive oxidative damage to biological macromolecules (*5–11*). This damage manifests as the peroxidation of membrane polyunsaturated fatty acid chains, modification of DNA (including base alterations, single-strand breaks, sister chromatid exchanges, and DNA-protein cross-links), and carbonylation and loss of sulfhydryls in proteins, among other changes. Carbonyl modifications of proteins occur in certain amino acid residues present near transition metal–binding sites and have been shown convincingly to cause enzymatic inactivation and enhance the likelihood of proteolysis (*2*).

There are several indications that the oxidant challenge to aerobic cells is not trivial. It is estimated that ~2 to 3% of the oxygen consumed by aerobic cells is diverted to the generation of $O_2^{\cdot-}$ and H_2O_2 (*8*). A typical cell in the rat may undergo 100,000 ROM attacks on DNA per day (*3*), and under steady-state conditions, ~10% of protein molecules may exhibit carbonyl modifications (*2*). The presence of the products of ROM interactions with macromolecules under steady-state conditions has led to the concept that antioxidative defenses are not fully efficient, that cells are chronically under oxidative stress, and that aging is a consequence of oxidative damage.

The Oxidative Stress Hypothesis of Aging

The basic tenet of the oxidative stress hypothesis is that senescence-related loss of function is due to the progressive and irreversible accrual of molecular oxidative damage (*1–4*). The predictions of this hypothesis, in the context of the above-stated

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