Genes of Aging

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According to developmental genetics theories, aging is a genetically programmed and controlled continuum of development and maturation. Being dynamic and malleable processes, development and aging are controlled not only by genes but also by environmental and epigenetic influences that predominate in the second half of life. Genetic mutations affect many phenotypes in flies, worms, rodents, and humans which share several diseases or their equivalents, including cancer, neurodegeneration, and infectious disorders as well as their susceptibility to them. Life span and stress resistance are closely linked. Oxidative stress actually constitutes a defined hypothesis of aging in that macromolecule oxidative damage accumulates with age and tends to be associated with life expectancy. DNA methylation, a force in the regulation of gene expression, is also one of the biomarkers of genetic damage. The mitotic clock of aging is marked, if not guided, by telomeres, essential genetic elements stabilizing natural chromosomic ends. The dream of humans to live longer, healthy lives is being tested by attempts to modify longevity in animal models, frequently by dietary manipulation. The quest continues to understand the mechanisms of healthy aging, one of the most compelling areas of research in the 21st century.

"WHY AGE AT ALL?" asks John J. Medina in his book *The Clock of Ages* addressed to a large audience and wistfully summarizing why and how we age, at the same time expressing our desire to wind back the clock. Over 50 years ago, Sir Peter Medawar published his essay, "An Unsolved Biology Problem" (1952), in which he put the question of "why we age at all" into evolutionary context. The basic idea is that natural selection takes place only during the reproductive age for the reproductively fit to survive.[1]

Aging can be characterized, according to Helfand and Rogina,[2] as (1) an inevitable consequence of being a multicellular organism; (2) associated with a random, passive decline in function; (3) leading to a global loss of homeostasis over time; and (4) mortality increasing with age. Drawing from several theories of aging, such as the error-catastrophe, protein modification, somatic mutation and DNA repair, and free radical/mitochondrial DNA theories, this review mainly addresses the developmental and genetic causes of aging, including the search for cellular senescence and cell death genes as the primary genes of aging. The developmental genetics theories consider aging as a genetically programmed and controlled continuum of development and maturation. The first demonstration of the fact that life span is an inherited trait was reported by Pearl et al in 1923.[3] From their observation that ambient temperature controls the life span of *Drosophila*, these authors developed the "rate-of-living" theory: the life span of an individual organism is dependent on (1) its rate of energy utilization (metabolic rate), and (2) genetically determined amount of energy consumed during adult life (metabolic potential).[4] Today, knowledge indicates that aging is a dynamic and malleable process,[5] developmental and aging clocks are controlled not only by genes but also by environmental and epigenetic influences.

We will first discuss studies on the fruit fly (*Drosophila melanogaster*) and worm (*Caenorhabditis elegans*) as models of aging, then the importance of rodent models, and paradigms at the cellular level. Next, we will examine the importance of genes, environment, and epigenetic factors, including oxidative stress, DNA methylation, and attempts to prolong longevity.

DROSOPHILA AND *CAENORHABDITIS* AS MODELS

Clearly, experiments on the fruit fly brought us the notion of aging inheritance, as suggested by Pearl. At 18°C, the *Drosophila* life span is over 4 months, with the reporter β-galactosidase (β-gal) gene being steadily expressed until 70 days. Raising the temperature to 25°C decreases its life span to less than 3 months and produces a rapid fall of β-gal as early as 30 days. This finding introduced the concept of temporal gene expression patterns and the existence of possible aging biomarkers. The fly is also a model for evaluation of the oxidative stress hypothesis, which will be discussed later. *Drosophila* is extremely valuable for demographic and gene expression studies because its complex metabolic process shares about 70% similarity with mammalians, including humans.

Isolation of the long-lived *daf-2* gene (an insulin receptor family member) mutations in *C elegans* in 1993 initiated the current search for genes of aging.[6] The mutant carrier lived twice as long as the wild type. A similar mutation in this insulin-like signaling pathway was observed later in *Drosophila*. In fact, a combination of mutations in the insulin-like receptor and in *Chico*, an insulin-like receptor substrate downstream from the insulin-like receptor was found in flies.[2] Preservation of the insulin-like signaling system is relevant from flies to worms and higher species. By underlining the similarities, O'Kane[7] exposed the reasons for studies in flies and worms based on the capacity to simply perform mutagenesis in the 2 species, to look for mutations that affect the phenotype. Other examples of single gene mutation expanding life span of *Drosophila* are *methuselah* (*mth*) and *I'm not dead yet* (*Indy*). This first coding for a secretion-like receptor, and providing resistance to stress (starvation, high temperature, free radicals. . .) and the Indy gene product is homologous to Krebs cycle intermediates but its expression is a tradeoff between long life and performance such as fecundity.[2,8,9] In addition to their common biology, flies, worms, and humans share several diseases or their equivalents, including cancer, neurodegenera-

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tion, and infectious disorders, as well as their susceptibility to them.

RODENTS

Rodents are valuable models of human diseases, especially since their short life span makes investigation accessible. Nevertheless, several differences must be taken into account. Thus, although both rat and mouse cells can be transformed in vitro, this phenomenon is difficult to reproduce with human cells. Even so, many mouse and rat models are available for the search of genetically defined aging processes. Thus, for instance, the $Kl^{-/-}$ mouse has a shortened life span, infertility, and growth retardation with aging onset appearing as early as 4 weeks of age due to a loss of functional mutation in the *klotho* gene, a single path membrane and protein with homology to β-glucosidase. These mice also present skin atrophy, arteriosclerosis, and osteoporosis together with lipodystrophy.[10] The *Sam* model[10] expresses osteoporosis with amyloidosis, neoplasms, and aging onset before 8 months of age, but the genetic causes have not yet been determined. A relatively short life span is observed with telomerase RNA deficiency in the $mTR^{-/-}$ model. Overexpression of growth hormone is associated with shortening of life span to less than 50% of the controls.

Another interesting rodent model is the one developed by Tyner and colleagues who demonstrated that p53 mutant mice display aging-associated phenotypes, including cutaneous atrophy, osteoporosis, impaired stress tolerance, and reduced longevity.[11] Noticeably, several of these phenotypes are also linked with cardiovascular diseases, such as diminished stress tolerance.[12,13]

CELLULAR MODEL

Much has also been learned about aging through ex vivo cell culture models. The aging process is biologically complex, and the distinction must be made between replicative senescence, old age, and accelerated aging such as progeria.[14] Fibroblasts from the elderly share more molecular determinants with those from progeria than they do with replicative senescence. Markers such as p53 and $p21^{WAF}$ are enhanced in all 3 of these conditions, while cdc42 and CAMK2D are limited to progeric and elderly fibroblasts.[15] The clustering method enabled the demonstration of hierarchical similarities between progeria and old age, but with differences from replicative senescence.[16] Ly et al[17] provided detailed analysis of middle age, old age, and progeria with the demonstration of mitotic misregulation related to aging, particularly affecting G_2-M transition: from 6,000 genes analyzed, 61 (1%) were modified, 25% of them in cell cycle progression, and 31% in extracellular matrix remodeling.

Leonard Hayflick,[18] the same scientist who developed the concept of "replicative senescence" or a finite number of cell divisions, urges us today to clearly distinguish between "aging" (biogerontology) and "age-related disease" (geriatric medicine), including cardiovascular disorders, stroke, and cancer. We concur with him that most of the aging process was not submitted to natural selection in the course of evolution, and is not guided by a defined sequence of gene expressions, such as the one governing early development and sexual maturation. Aging is a process that occurs in all members of a species, supposedly after the time of reproduction, and is distinct from age-related disease, even though at the present time of our species' history, the latter prevails in most members of our society, with cardiovascular mortality, stroke, and cancer accounting for over 90% of deaths in industrialized countries. It is noteworthy that progeria, a rare syndrome, is characterized by the presence of severe hypertension[19] and vascular smooth muscle cell (VSMC) depletion[20] leading to cardiovascular death.[21] Environmental and epigenetic factors predominate in the second half of life. Our own view, which has yet to be submitted to prospective validation, is somewhat divergent as we have realized the relevance of neonatal development as a predictor of outcomes in human cardiovascular diseases, as originally proposed by Barker et al,[22] and as we have discussed recently.[23] The genetic substratum of this developmental risk of cardiovascular diseases, with attention to be paid to the prenatal period, remains to be defined.

GENES OF LIFE SPAN

From the beginning of observations in nematodes and insects, insulin-like growth factor (IGF) genes and their receptors have been related to longevity, as already mentioned. Several models of growth hormone (GH)-deficient mice with reduced IGF-1 levels display delayed aging, although this is difficult to reconcile with the neuroprotective effects of IGF-1, and contrasts with declining GH levels during normal aging.[24] Nevertheless, these studies point to the relation between body size, development, reproduction, and availability of energy resources. Recently, inactivation of the IGF-1 receptor (IGF-1R) gene by homologous recombination was analyzed by the *Cre-lox* strategy.[25] While homozygotes died at birth, $IGF1R^{+/-}$ heterozygotes showed decreased IGF-1R levels, and although they presented a modest 8% growth deficit, significantly outlived their wild-type littermates by a 26% longer life span, a change more pronounced in females. These mice also expressed resistance to oxidative stress, one of the primary culprits promoting aging.

FROM GENETICS TO EPIGENETICS

Candidate gene studies in humans have demonstrated the relevance of major histocompatibility complex genes in the aging process.[26] Other genetic associations may sometimes appear counterintuitive, such as the epsilon 4 allele of apolipoprotein E (ApoE4), which is positively associated with coronary disease and Alzheimer's, yet associated with longevity.[10] Both the epsilon 2 allele and angiotensin-converting enzyme (ACE) gene, usually predisposing to coronary disease, and ApoE2 associated with type III and IV hyperlipidemia have been found in French centenarians.[27] Is it that once one escapes this high risk, gene longevity is assured, or is it a pleiotropic mechanism of the genes affecting longevity? These studies, however, have been questioned in other ethnic groups.[28] Clearly, the genetic component of human longevity is supported by observation of centenarians, their siblings and parents.[29] Linkage association has indicated that when longevity is evaluated as a phenotype it can be localized on Chr 4. Perls and

colleagues[29] suggest that prolongation of the child-bearing period serves as an evolutionary force to select longevity-enabling, since centenarians have their children while in their 40s.[10] Transcriptome studies indicate that aging involves many loci as 1,264 genes exhibit age-dependent changes.[30,31]

In complex genetic traits such as aging, it is evident that genes do not exert their influence in isolation, but do so in strong interaction with the environment similarly to susceptibility genes of many complex disorders, including hypertension, arteriosclerosis, etc.[23] Diet, stress, and pharmacological agents should all be included in these environmental pressures as they are most relevant for the delineation of intrinsic and environmental signals leading to the epigenetic regulation of gene expression.[32,33]

The best-known epigenetic effect is exerted by DNA methylation, a force in the regulation of gene expression throughout evolution and aging. It has been suggested that epigenetic factors are so important that the human genome should be sequenced for the fifth nucleotide, the 5-methyldeoxycytidine residues in CpG islands.[34] While imprinting and epigenetic reprogramming are involved in mammalian development, varying from high to low methylation status in germ cells and through embryonic evolution,[35] genetic silencing in adult cells evokes stability. Nevertheless, aging cells, particularly epithelial cells, display increased frequencies of silencing and multiple loci leading to mosaicism in adults, which is influenced, in turn, by lifestyle and environmental exposures.[36] The consequences of methylation may drive age-related disorders, including atherosclerotic vascular disease,[37] some of which may be linked to methylation-mediated epigenetic defects in the estrogen receptor alpha gene that declines in aging blood vessels.[38] Currently, methylation is one of the biomarkers of genetic damage being studied intensively in cancer epidemiology,[39] and aging-related genes, such as the IGF-2 promoter, appear to be methylated on both alleles during aging and carcinogenesis, in contrast to monoallelic methylation in normal tissues.[40]

An equally relevant environmental impact is that exerted by the cumulative repercussions of oxidative stress. IGF-1R regulates life span over the same time as resistance to oxidative stress in mice.[25] Oxidative stress actually constitutes a defined hypothesis of aging[41] based on the view that macromolecule oxidative damage accumulates with age and tends to be associated with life expectancy.[4] However, whether the link between oxidative stress and aging is direct or not is still under exploration, despite the "rate of living" theory.

DNA damage also leads to cell apoptosis,[42] a tightly regulated genetic process.[43] Direct experiments on Cu,Zn-SOD overexpression have not provided convincing evidence because its capacity to prolong life is expressed only in compromised genetic backgrounds.[44] Recent data have demonstrated that ectopic expression of catalase in *Drosophila* mitochondria increases stress resistance but not longevity.[45] Modification in the level of thioredoxin peroxidase expression has shown both beneficial and detrimental effects on *Drosophila* cell viability.[46] The problem is perhaps that expression has to be targeted to specific tissues, as suggested by targeted neuronal gene expression of reactive oxygen species in motor neurons impacting on *Drosophila* longevity.[47] Recent data have demonstrated that mutation of succinate-CoQ oxidoreductase subunits in the mitochondrial electron transport chain in *C elegans* made the mutants hypersensitive to oxidative stress, leading to precocious aging.[48] Another mutation, of *clk-2* gene in *C elegans* linking cell cycle progression, apoptosis, and telomere cell regulation, affected development, behavior, reproduction, and aging.[49]

Table 1. Quantitative Trait Loci (QTL) of Telomeric Length in Rat Recombinant Inbred Strain

Chromosome	Locus	$r =$	P
3	Cebp1038	0.61	.0002
12	Pai1	−0.47	.006
16	Mit3	0.52	.002
X	Myc s	0.54	.0007

From Hamet P, Tremblay J, Pravenec M, Kren V (2003), unpublished data.

ROLE OF TELOMERES AND TELOMERASE

The "mitotic clock" of aging is marked, if not guided, by telomeric loss. Telomeres[50] are essential genetic elements stabilizing natural chromosomic ends by multiples of TTAGGG repeats. About 31 bp are lost annually in humans, and telomere size is highly heritable ($h^2 = 78\%$), as demonstrated in mono- and dizygotic twins.[51] In rodents, however, the role of telomere length is questioned, mainly since it is excessively long, and it is telomerase activity per se that is important in cell proliferation senescence.[52] We[53] observed an accumulation of shorter telomere restriction fragments (TRF) in the kidneys of spontaneously hypertensive rats (SHR), while Aviv's group[54] related telomeric length to systolic blood pressure in humans, confirming the relevance of investigating this component in the context of hypertension. Modulation of p53 activity is suspected to be the culprit in telomerase upregulation *in vivo* in the aorta and cultured VSMC of SHR.[55] Because of their excessive length as well as the newly-uncovered telomere-independent pathway of immortality,[56,57] telomeres are not a simple indicator of cell turnover in rodents. In rat oligodendrocytes, Lloyd[58] showed that environmental modifiers (incubation with serum, x-rays) have a positive input on the induction of senescence, implicating the $p19^{ARF}$, $p16^{INK4a}$, and $p21^{cip1}$/p53 pathways in a telomere-independent fashion.

We have studied recombinant inbred strains of rats that are F_2 replicas of reciprocal intercrosses between SHR and Brown Norway-lx strains brought to homozygosity status by brother/sister mating for 25 generations and designed for genomic localization of many phenotypes.[59,60] We used telomeric mass ascertained by TRF in these strains to attempt the genomic localization of telomeric length in these F_2 crosses. Total genome scan helped us to establish several quantitative trait loci (QTL) of telomeric length (Table 1).

PROLONGATION OF LONGEVITY

The history-long dream of humans to live longer, healthy lives is being tested by attempts to modify longevity in animal models, frequently by dietary manipulation. It is suggested that diet-genotype interaction may have important influences on healthy aging. For example, the APOE 4 genotype, which is the

most important genetic risk factor for Alzheimer's and cardiovascular diseases, interacts with dietary fat intake in healthy older persons (protective effect with lower dietary fat).[61] Nevertheless, Apo alleles and their relevance in cardiovascular disease and hypertension still remain to be elucidated.[62] The most convincing impact on longevity is that of caloric intake limitation and life extension observed in *Drosophila*. Caloric restriction appears to affect life span extension by mechanisms other than suppression of the GH–IGF-1 axis in rodents. Apparently, genetic background modulates the effect of caloric intake on longevity in mice: there is a significant difference in longevity between ad libitum and caloric restriction in C57BL/6 but not in other mouse strains, including DBA/2 mice.[63] Lifelong restriction of calories does not increase longevity in all mouse genotypes. Longevity can even be modulated by drugs, as seen with 4-phenylbutyrate's lengthening of *Drosophila* life span.[64] Another modulator of life span appears to be stress, for which QTLs have been recently mapped in the rat,[12] indicating the relevance of mutations in heat shock transcription factor. A deficiency of this transcription factor alters redox homeostasis in the heart and increases mitochondrial oxidative damage.[65] Life span and stress resistance are closely linked. Signal transduction pathways should also be explored as potential control mechanisms of life span. Thus, mutation in the *egl-4* gene encoding cyclic guanosine monophosphate (GMP)-dependent protein kinase represses not only body size but also life span through insulin pathway mechanisms.[66]

AGING AND CARDIOVASCULAR DISEASE

There is still the matter of evaluating whether or not cardiovascular diseases are simply a mark of aging, independent of it, a result of accumulation of biological errors or oxidative stress, or the reflection of a longstanding biological defect. Our previous analysis points to the importance of neonatal body size and shape in subsequent development of cardiovascular disease in addition to potential environmental modulators. Neonates from genetically hypertensive rats display abnormalities of apoptosis/proliferation ratio leading to accelerated aging, a possible pathogenetic component of cardiovascular disease development and its complications. We have observed reduced DNA half-life in the heart, kidney, and aorta of SHR.[53] We propose that this accelerated turnover rate of cardiovascular cells may culminate in more rapid organ damage.

CONCLUSION

Many of the hypotheses cited in this review, including the one just mentioned, are still awaiting firm evidence; the discovery of molecular genetic determinants and environmentally relevant modulators, including preventive and therapeutic actions, will help our understanding of the mechanisms of healthy aging. The aging process is one of the most compelling areas of research in the beginning of the 21st century, with profound implications for new medical horizons.

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