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Genetic and epigenetic regulation of aging

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Many age-associated conditions, such as the decrease in regenerative capacity of tissues, appear to be determined by a decline in the function of specific somatic stem cells. Although it is obvious that the genotype determines the average lifespan of different species, the variation in lifespan of individuals within a species seems to be more affected by the accumulation over time of molecular errors that compromise adult stem cell function. These molecular alterations can occur at both the genetic and epigenetic levels and depend on hereditary, environmental, and stochastic factors. This complex multifactorial mixture determines characteristics, such as longevity and a healthy life, that are central concerns of human existence.

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Introduction

The process of aging in humans, understood as the loss of corporal functions accompanied by a general degeneration of cells and tissues, most likely arises from the progressive decay of adult stem cells' potential to maintain correct tissular homeostasis [1,2]. The factors involved in the process and the reasons for its occurrence have been a matter of debate for decades. It is indisputable that the genotype determines the variation in average maximum lifespan between species: for example, some organisms, such as the nematode *C. elegans*, live less than one month while others, such as giant tortoises, can live for hundreds of years [3]. However, the variation in lifespan among individuals of the same species seems to be more strongly affected by the accumulation over time of molecular errors that compromise adult stem cell function than by specific genetic programs [2,4]. These molecular alterations can occur at both the genetic and epigenetic levels and depend on the genotype (intrinsic factors), the environment (extrinsic factors), and stochastic (undetermined) factors. Thus, species-specific genotypes may determine the general program of ontogenic development and the maximum lifespan of the species while the intraspecies-specific peculiarities of the process of aging are determined by a complex multifactorial combination of genetic, environmental, and stochastic factors, whose relative contributions are yet to be fully elucidated (Figure 1). In our species, this combination governs characteristics, such as longevity and healthy life that are central to human existence.

This article reviews the types of genetic and epigenetic alterations that accumulate over time, their potential to affect somatic stem cell function, and the hereditary, environmental, and stochastic factors involved in their establishment.

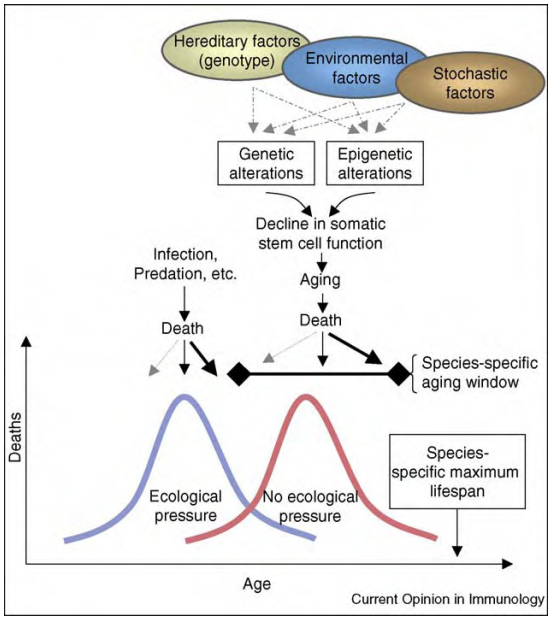
The role of genetic factors in aging

The role of genetic factors in aging has many facets. One concerns the fact that specific combinations of genes (species-specific genotypes) determine the general order of magnitude of the lifespan. This is demonstrated by the wide variation in the average lifespan of different species and is also consistent with the dramatic changes in lifespan observed as a result of the alteration of a single gene, as occurs in human progeroid syndromes [5]. A second facet concerns the impact of hereditary factors on the variation in intraspecies lifespan. Studies of twin siblings have shown that 20–30% of the overall variation in lifespan is due to hereditary factors [6], which is in line with the wide range of genetic variants involved in aging and age-related diseases that have been described in genome-association studies in centenarians [5]. A third facet involves alterations of the sequences of both genomic and mitochondrial DNA, which are at least partly responsible for the decay of somatic stem cell function over time [2,4]. The accumulation of these alterations depends not only on the efficiency of repair, which depends, in turn, on the genotypic factors mentioned above, but also on their rate of accumulation, which is governed by environmental and, probably, stochastic factors.

The role of specific combinations of genes in determining a first general level of lifespan: the example of progeroid syndromes

The great variation in the average lifespan of different species unambiguously indicates that the maximum lifespan of a species is determined by its species-specific genotype (Figure 1). Consistent with a role for genetic factors in aging, mutations or variants of several genes are associated with progeroid syndromes in humans (reviewed

Figure 1



A schematic representation of how genetic factors might affect aging and lifespan. The curves represent the number of deaths as a function of the age of the individual under strong ecological pressure (blue) and without ecological pressure (red). The species-specific genotype determines the maximum lifespan. Most individuals under strong ecological pressure do not age because they die early in life from infection, predation, and other external factors. However, individuals that do not experience any ecological pressure easily reach the species-specific window of aging. The time when a specific individual dies within this window depends on the extent of accumulation of genetic and epigenetic molecular alterations, which in turn depends on hereditary, environmental, and stochastic factors.

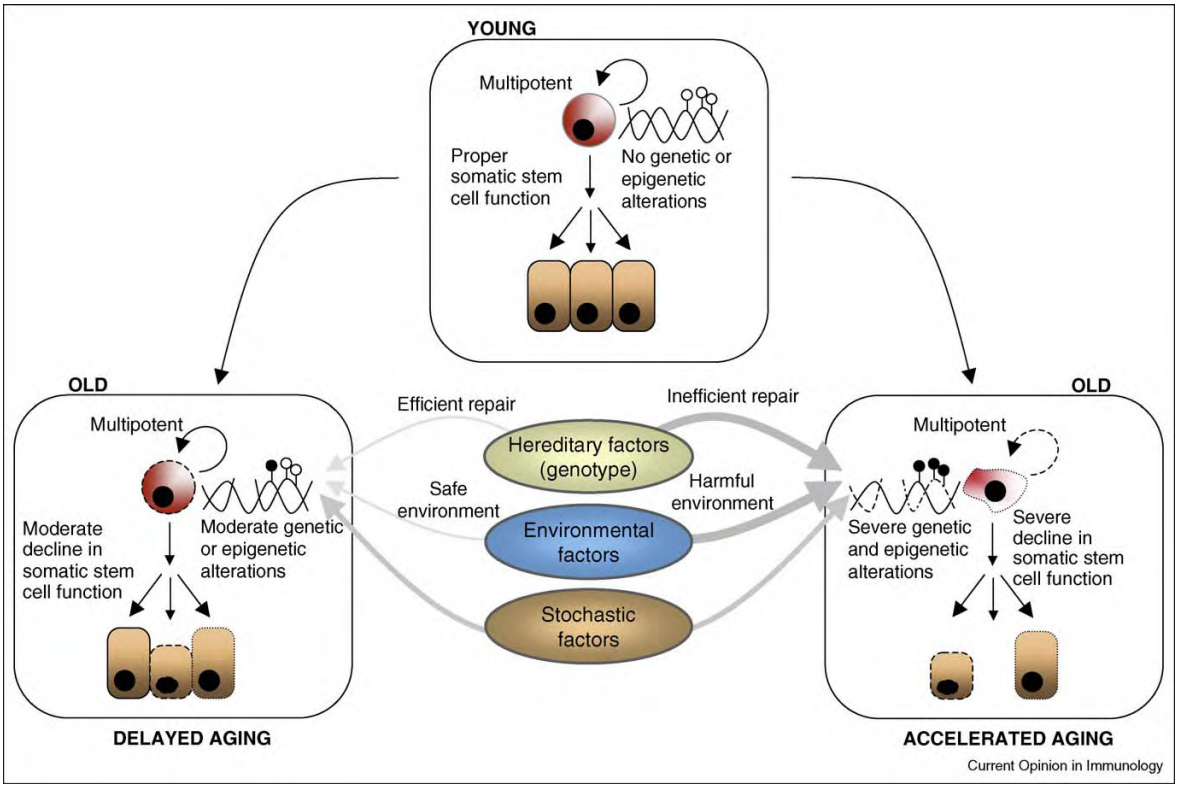
in [5,7]), and may affect one or more age-related phenotypes [5]. As no progeroid syndrome exhibits all the features usually associated with physiological aging they are known as 'segmental aging syndromes'. In humans, they may be caused primarily by mutations in DNA repair genes, giving rise to syndromes such as those of Werner Rothmund–Thomson, Bloom and Cockayne, and mutations in genes affecting the nuclear lanins, which cause conditions such as Hutchinson–Gilford progeria syndrome and restrictive dermopathy [8]. The best studied syndromes in humans are Werner syndrome, caused by mutation in a member (*WRN*) of the RecQ family of helicases, which are involved in DNA repair, and the Hutchinson–Gilford progeria syndrome, which is caused by mutations in the LMNA gene that cause aberrant processing of the nuclear envelope protein lamin A and, consequently, alterations of nuclear morphology [9]. Patients with Werner syndrome exhibit several age-related traits, including type 2 diabetes mellitus, arteriosclerosis, osteoporosis, ocular cataracts, neoplasia, skin atrophy and hair loss, among others [5]. Patients with Hutchinson–Gilford progeria syndrome also exhibit many age-related symptoms, for example, osteoporosis, skin atrophy, and alopecia, although these patients are not more affected by cancer [5].

The impact of a single gene alteration in aging is also demonstrated by several animal models of human progeroid syndromes. Mice that are not able to process lamin A, either by targeted mutation at the LMNA gene [10] or by deleting *Zmpste24* (the metalloprotease that processes prelamin A) [11], acquire a Hutchinson–Gilford progeria-like phenotype. Prenylated forms of prelamin A and its truncated form, progerin, are known to cause nuclear alterations in this syndrome. Interestingly, results from animal models show that inhibiting prelamin A or progerin prenylation by treatments with either farnesyltransferase inhibitors [12] or a combination of statins and aminobisphosphonates [13••] markedly ameliorate many of the Hutchinson–Gilford progeria syndrome-like phenotypes and improve growth and survival. This suggests a promising new direction for the treatments of patients suffering from premature aging syndromes. Progeroid syndromes are a good example of how genetic factors affect aging, and are also a valuable tool for studying normal human aging.

Genetic variants among individuals affect aging and lifespan

The relationship between specific genetic variants and aging has customarily been investigated in association studies using candidate genes in centenarian populations. Examples of genetic variants associated with aging that have been identified by this strategy include polymorphisms at PON1, IGF1 receptor and PI3K genes, and APOC3 [14] (extensively reviewed in [5]). Furthermore, a genome-wide analysis of North American centenarians identified a locus on chromosome 4 [15], although this association has not been confirmed in other populations [16]. In addition, a large number of genome-wide case–control studies of association have identified many genetic variants linked to age-related diseases. Examples include the genetic variation in APOE and PCDH11X associated with Alzheimer's disease, functional SNPs in CD244 associated with rheumatoid arthritis and type 2 diabetes (extensively reviewed elsewhere [17]). Other useful approaches that have enabled age-related genetic variants to be identified include families enriched in long-living members, twin pair, and longitudinal studies (reviewed in [18]). Although association studies are useful for identifying age-related loci they require further development if they are to be able to demonstrate functional relationships with the aging phenotype. There are various ways by which a particular epigenetic variant could be associated with longevity. The proposed mechanisms include a role in metabolism or in DNA repair [5], among others.

Figure 2



A model of how genetic and epigenetic factors can affect aging. Young adult stem cells present no alterations in either the genetic or epigenetic levels and so there is proper stem cell function and, consequently, tissue regeneration. Genotypes of low efficiency in repairing genetic or epigenetic (represented as lollipops over the structure of the DNA) defects or in maintaining epigenetic stability accompanied by harmful environmental exposures can accelerate the accumulation of molecular alterations at the genetic and the epigenetic levels, which, in turn, can accelerate the aging process. On the other hand, genotypes that are highly efficient in repairing genetic and epigenetic defects and in maintaining epigenetic stability accompanied by harmless environmental exposures can slow the accumulation of molecular alterations at the genetic and epigenetic levels, which, in turn, can delay the aging process.

DNA alteration over time and aging: DNA damage and telomere length

Some aspects of mammalian aging result from an age-associated decline in the replicative function of certain regenerative cells, that is, adult or somatic stem cells [2] (Figure 2). Although the number of somatic stem cells does not necessarily decrease during aging, their function — understood as the ability to produce the correct proportion of differentiated cells for proper tissular function — does decline [2]. One of the major mechanisms postulated as being a cause of decline in somatic stem cell function during aging is the accumulation of unrepaired DNA and chromosomal damage [4] (Figure 2). Consistent with this hypothesis, mutations in genes involved in DNA repair cause premature aging in humans [19] and mice deficient in DNA repair genes present altered hematopoietic stem cell function [20]. The accumulation of DNA damage over time is caused by the decline in the repair capability of stochastic genetic alterations (e.g. mis-incorporations) and DNA deterioration driven by oxidative stress, toxic byproducts, and other harmful exogenous factors, such as radiation [8]. These alterations can affect genomic and mitochondrial DNA, although the role of mitochondrial DNA mutations in aging is under debate [21]. Thus, the extent of accumulated interindividual DNA damage depends on the degree of exposure to DNA-damaging agents (external factors) and the intraindividual ability to repair these alterations (the intrinsic or hereditary factors described in the previous section). This is exemplified by a mutation in the gene encoding the oxidative stress response protein $p66^{shc}$ that extends lifespan in mice and enhances resistance to apoptosis following oxidative stress in *in vitro*-cultured cells [22•].

It has also been proposed that there is a relationship between telomere shortening and somatic stem cell decline during aging [23]. Certainly, telomere length is associated with age-related diseases in humans [24] and patients displaying syndromes of accelerated aging exhibit a higher rate of telomere attrition and marked chromosomal instability [23]. Consistent with this, telomere dynamics are important for hematopoietic stem cells [22•], telomere shortening impairs adult stem cell function [25], telomerase-deficient mice have short telomeres and age prematurely [23] and, most strikingly, cancer-resistant mice overexpressing telomerase have long telomeres and delayed aging [26••].

The molecular mechanisms by which DNA sequence alterations occurring over time are involved in somatic stem cell-dependent aging remain to be fully elucidated. One possibility is that telomere shortening and/or unrepaired DNA activate(s) the canonical DNA damage response pathway that prompts p53 to initiate apoptosis or replicative senescence [23]. However, evidence for the role of senescence in aging needs confirmation [2,23].

The role of epigenetics in aging

The term *epigenetics*, which was originally coined to define how genotypes give rise to phenotypes through programed changes during development [27••], today refers to the study of stable genetic modifications that result in changes in gene expression and function without a corresponding alteration in DNA sequence. The best-known epigenetic modifications are DNA methylation and histone post-transcriptional modifications, including methylation, acetylation, ubiquitination, and phosphorylation [28]. Epigenetic modifications are essential for the normal growth and development of superior organisms [29] and their alterations are associated with various pathologies, including cancer [30].

A relationship between epigenetics and aging was first observed over 40 years ago in a study of spawning humpbacked salmon that showed a global decrease of genomic DNA methylation with age [31]. This decrease was subsequently observed in other species including humans [32]. In addition to global hypomethylation, a number of specific loci are known to become hypermethylated during aging. Examples include the increase of methylation in ribosomal DNA hypermethylated clusters in liver and germ cells of senescent rats [33] and, in humans, CpG island promoter hypermethylation in the tumor suppressor genes lysyl oxidase (LOX), p16INK4a, runt-related transcription factor 3 (RUNX3), and TPA-inducible gene 1 (TIG1) in various tissues [34,35]. Intriguingly, the two best-known epigenetic alterations occurring in aging — global DNA hypomethylation and aberrant promoter hypermethylation — also occur in cancer [30]. This suggests that the accumulation of epigenetic alterations during aging may directly contribute to malignant transformation, although this hypothesis needs further evaluation.

Factors affecting epigenetic variation over time and their functional consequences

The accumulation of epigenetic variation over time depends on hereditary, environmental, and stochastic factors and their relative contribution has been a matter of debate for decades (Figure 2). In humans, results of twin studies have been the gold standard for distinguishing genetic from nongenetic factors. The rationale underlying classical twin studies is the assumption that monozygotic (MZ) twins are genetically identical, whereas dizygotic (DZ) twins share 50% of their segregating genes on average and are as genetically similar as ordinary siblings [36]. Despite being almost genetically identical, MZ twin pairs often diverge phenotypically over time, for example with respect to their susceptibility to disease and to a wide range of anthropomorphic features [36]. It has been proposed that phenotypic discordance between MZ twins can arise from the influence of epigenetic factors that change over the lifetime of individual organisms.

As initially hypothesized, MZ twins present numerous epigenetic differences that, in some cases, are associated with specific behavioral and physical phenotypic features. These include skewed X-chromosome inactivation associated with hemophilia, nonsyndromic cleft lip, autism, bipolar disorder, discordant imprinting associated with Beckwith–Wiedemann syndrome and Silver–Russell syndrome and, possibly, with body weight (reviewed in [35]). Other examples include DNA methylation changes in single-copy nonimprinted genes associated with risk-taking behavior, bipolar disorder, type 2 diabetes and aging, bird weight, psychosis, and caudal duplication (reviewed in [35]). We have analyzed global epigenetic differences in different-aged MZ twins and shown that elderly MZ twin pairs living apart from their biological families and with many phenotypic differences presented more epigenetic differences than young phenotypically similar MZ twin pairs living in the same household with their biological parents [37••]. We also found that although most of the epigenetic changes occurred in nonfunctional and repetitive DNA elements, MZ twins had significantly different gene expression phenotypes [37••], as previously noted by other authors [38–40]. Although the study design was not capable of determining whether epigenetic changes in MZ twins were functionally relevant or related to disease discordance, the apparent accumulation of epigenetic modifications with age is consistent with the idea that age-related loss of normal epigenetic patterns is a mechanism for late onset of common human diseases [41]. Thus, on the basis of epigenetic differences between different-aged MZ twins, our results suggested that intraindividual epigenetic changes do occur over time. This was corroborated in a

longitudinal study of DNA methylation patterns, in which successive DNA samples were collected over 10 years apart from two populations each of more than 100 individuals [42•]. Further evidence of the epigenetic differences between twin siblings was recently reported by Kaminsky *et al.* [43••], wherein a genome-wide analysis of DNA methylation patterns in MZ and DZ twins revealed significant epigenetic differences in both groups.

Longitudinal studies and MZ twin data demonstrate that intraindividual epigenetic changes occur over time, so next we need to know how these changes are produced. One possibility is that they depend on genetic factors (i.e. genes coding DNA methyltransferases) and thus the individual genotypes determine the extent of these epigenetic variations over time. This possibility is supported by the lower intra-pair than inter-individual epigenetic differences observed in twin studies [37••], the familial clustering of methylation changes observed in longitudinal studies [41] and by the fact that DZ twins feature more genome-wide [43••] and locus-specific [44] DNA methylation differences than do MZ twins. A second possibility is that environmental exposures affect epigenetic patterns over time. Indeed, the effect of the environment on epigenetic factors has been widely reported (reviewed in [45]). Examples of this include the promoter hypermethylation of tumor suppressor genes that occur in nontumorigenic lung tissues of smokers but not in the corresponding tissue of nonsmokers, the effect of diet on DNA methylation levels at particular loci in animals and humans, *in utero* dietary conditions associated with promoter hypermethylation of the estrogen receptor gene, DNA methylation changes associated with exposure to metal ions such as chromium, cadmium, and nickel, epigenetic downregulation of genes involved in pancreatic β-cell function in abnormal intrauterine environments, specific DNA methylation profiles of offspring associated with maternal diet, and even with maternal behavior (reviewed in [35]). A final possibility is that there are epigenetic changes, such as DNA methylation-associated epigenetic differences in isogenic animals living under the same environmental conditions [46] that cannot be explained solely by genetic or environmental effects. Consistent with this, isogenic laboratory animals maintained under identical environmental conditions exhibit significant phenotypic differences [47], including marked differences in lifespan [48]. Such phenotypic variability has long been believed to depend on a so-called 'third component', of epigenetic origin, that was independent of the environment and significantly contributed to the creation of random biological variability [47].

The relative contribution of genetic, environmental, and stochastic factors to the epigenetic changes occurring over time is unclear (Figure 2). The similar concordance of some psychic aptitudes between MZ twins reared apart or together [49] suggests that, if they depend on epigenetic factors, stochastic events are the most important. In contrast, the importance of environmental factors is exemplified by the pronounced environment-dependent differences between aging phenotypes of MZ human twins [37••,50,51] and is clearly evident from the discordant environment-dependent patterns in cancer — probably the best-known epigenetic disease [30] — among MZ twins revealed by large cohort studies [52••,53].

It is also possible that the relative influence of the factors differs between genomic regions. Consistent with this hypothesis, most of the environment-related changes in phenotypic expression between MZ twins preferentially occur in heterochromatic, gene-poor regions [39,40], which, interestingly, are the regions where most epigenetic differences were found in environment-dependent phenotypically discordant MZ twins [37••]. In line with this, the IGF2/H19 locus, whose epigenetic variation primarily depends on genetic factors, is resistant to age-related methylation changes [44]. The DNA regions where epigenetic variation occurs over time are important with regard to their functionality. Although global [37••] and genome-wide [43••] approaches suggest that epigenetic differences between MZ twins primarily occur outside functional elements, the discordant expression phenotypes of MZ twins [39] and the relationship between environment-dependent epigenetic alterations and cancer [45] suggest that they could have significant functional implications. Further studies are needed to determine the functional components of epigenetic variation over time and their possible role in establishing the aging phenotype.

Trans-generational transmission of epigenetic signatures

The observed heritability of some epigenetic signatures in animal models [54•] and humans [43••,44] suggests that the epigenotype, or at least a part of it, can be transmitted from generation to generation. If this is true, then we need to discover how the epigenetic signatures are transmitted to the next generation. One possibility is that the process depends on genetic configurations directly affecting epigenetic factors (e.g. DNA methyltransferases). In this case, the epigenotype of the offspring would resemble that of its progenitors by the direct action of the epigenotype-associated inherited genotype. The dependence on genetic factors is also supported by the association between methylation at the IGF2/H19 locus with SNPs in *cis* in DZ twins. Another possibility is that epigenetic marks can be directly transmitted to the next generation. This is supported by the study of Kaminsky *et al.* [43••], in which they found that epigenetic differences between outbred mice were not significantly associated with variation in the DNA sequence. The authors used this finding, among others, to argue that DZ twins have more epigenetic differences than MZ twins because

they originate from two separate zygotes with distinct epigenetic profiles, whereas MZ twins develop from the same zygote, and so should possess similar epigenomes at the time of blastocyst splitting. It is even possible that genetic-dependent and direct transmission of epigenetic signatures occur simultaneously (i.e. some DNA regions are directed by one mechanism and other regions by another). Although the possibility that epigenetic marks can be transmitted down the generations is exciting, in the case of DNA methylation the molecular mechanisms involved in the process are still unclear. Indeed, most genomic DNA methylation is erased during embryonic development [55], which implies that molecular mechanisms other than this must participate in the process. It is also important to know whether the transmission of epigenetic marks occurs at the genome-wide scale or whether just stable locus-specific marks are transmitted. This is important because if the process occurs at a genome-wide level then epigenetic changes over time in germinal cells could be transmitted to the next generation, which would obviously result in epigenomic collapse in only a few generations. In addressing this matter, Teixeira *et al.* recently showed that genetically induced epigenetic alterations can be transmitted to the next generation but are corrected in successive generations by an RNAi-mediated mechanism [56].

Concluding remarks and perspectives

In conclusion, the aging phenotype primarily results from the decline of the capacity of adult stem cells to regenerate tissues and organs. The great variation in lifespan within isogenic individuals of the same species suggests that this decline is affected more by the accumulation over time of molecular errors that compromise adult stem cell function than by specific genetic programs. These molecular alterations occur at both the genetic and epigenetic levels and depend on hereditary, environmental, and stochastic factors. Thus, genetic and epigenetic alterations during aging result from a complex combination of hereditary, environmental, and stochastic variables with a still unknown relative contribution. Further studies are needed to establish the magnitude of the contribution of each component to genetic and epigenetic variation over time, to determine the molecular mechanism involved in the transmission of epigenetic patterns between generations, to assess their functional role and to identify the DNA regions in which they occur. We are on the brink of gaining important insights into these aspects from the epigenome-wide information generated by the application of the new technologies of ultra-deep sequencing to large cohorts of accurately phenotypically annotated MZ and DZ twins.

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