REVIEW

# Stem cells and healthy aging

Margaret A. Goodell[1]* and Thomas A. Rando[2]*

**Research into stem cells and aging aims to understand how stem cells maintain tissue health, what mechanisms ultimately lead to decline in stem cell function with age, and how the regenerative capacity of somatic stem cells can be enhanced to promote healthy aging. Here, we explore the effects of aging on stem cells in different tissues. Recent research has focused on the ways that genetic mutations, epigenetic changes, and the extrinsic environmental milieu influence stem cell functionality over time. We describe each of these three factors, the ways in which they interact, and how these interactions decrease stem cell health over time. We are optimistic that a better understanding of these changes will uncover potential strategies to enhance stem cell function and increase tissue resiliency into old age.**

The aging process remains one of the central mysteries of biology, both from an evolutionary perspective (why we age) and from a mechanistic perspective (how we age). Organismal aging is the failure of an integrated system that balances genetic programs for survival and reproduction. As reflected in the "disposable soma" theory (*1*), resources that are available to an organism are allocated either to survival or to reproduction, both of which are essential for the propagation of the species as evolved in the wild. However, additional factors come into play when species are protected from extrinsic causes of mortality (such as predation, starvation, and exposure), as is the case for modern *Homo sapiens* as well as animals on farms, in zoos, and in the laboratory. In those cases, individuals within the species are more likely to live far beyond the ages of their wild counterparts, allowing the emergence of phenotypes of aging and age-related diseases that would rarely if ever be manifest in the wild; we consider this "protected aging."

One of the central features of protected aging is prolonged survival beyond the ages of peak reproductive fitness (Fig. 1). The fact that individual members of any species rarely live beyond this stage in the wild means that there would have been no evolutionary pressure to select for genetic mechanisms to assure maintenance of somatic tissues into old age. The homeostatic mechanisms that are necessary to preserve function throughout life are thus predicted to lose robustness over time. Furthermore, for most species, growth-suppressive mechanisms take over from growth-promoting mechanisms around the time of reproductive maturity. This critical transition, and the perpetuation of growth-suppressive mechanisms beyond it, likely account for the progressive degradation of tissue function that characterizes protected aging. As such, what is generally called "aging" represents the intersection of a gradually failing system selected for early growth and reproductive fitness with the cumulative effects of growth-suppressive mechanisms and acquired somatic insults. Modulation of any aspect of this network could potentially accelerate or decelerate the process of aging.

The ability of an organism to ensure healthy function during adult life depends on homeostatic mechanisms. In many organs of mature vertebrates, resident stem cells participate in tissue maintenance and regeneration after injury, with variations in these roles across different tissues. For example, neural stem cells (NSCs) are important for ongoing generation of new neurons in specific regions of the brain but play a limited role in damage repair. In contrast, skeletal muscle stem cells (MuSCs, or satellite cells) play a minimal role in muscle maintenance but vigorously engage in regeneration after injury. Hematopoietic stem cells (HSCs) and intestinal stem cells (ISCs) do both, contributing to ongoing production of differentiated cells and also repairing tissue after injury.

During protected aging, the extent to which stem cells continue to maintain their cognate tissues depends on their own health. Although stem cells have characteristics (e.g., turnover rate, a specialized niche) that may protect them from insults associated with aging, data also indicate that stem cells deteriorate with age (*2*). Furthermore, any aberrations in stem cells may be carried forward into their differentiated progeny, contributing to tissue aging. As such, the question remains as to how effectively stem cell populations maintain tissue health, what the limitations of that capacity are, and what the mechanisms are that ultimately lead to decline in stem cell function. Research in stem cells and aging is geared toward these questions, with one long-term goal being the maintenance or restoration of youthful characteristics in aged somatic stem cells to promote healthy tissue aging. Here, we focus on three major areas of recent research in stem cell aging: genetic mutations, epigenetic changes, and extrinsic factors. We also consider how these influences are interrelated, and how in the future we might be able to enhance stem cell function and increase tissue resiliency into old age by modulating these factors.

## Somatic mutations, stem cells, and age

For decades, we have understood that environmental insults such as irradiation and xenobiotic exposure can lead to accumulation of somatic mutations in a variety of tissues. Indeed, this

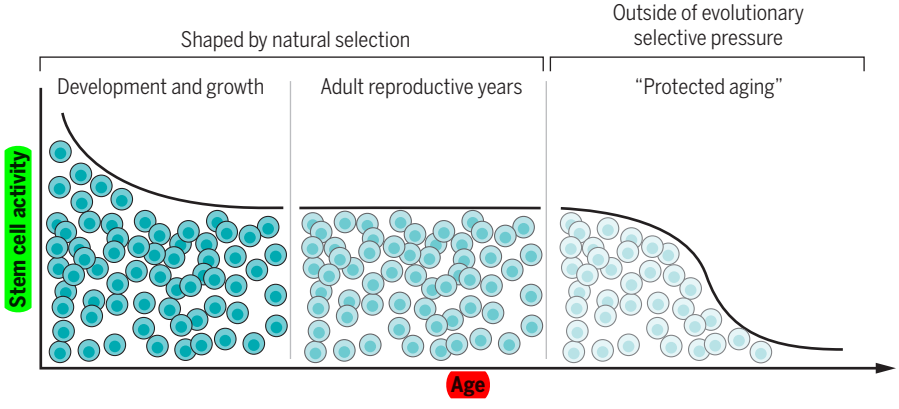


**Fig. 1. Model of stem cell use over the life span.** During embryogenesis and organismal growth, stem cells are highly active and contribute to tissue formation and growth. During the prime reproductive phase, growth is suppressed. Stem cells maintain and repair tissues. Properties of stem cells during these first two phases would be subject to forces of natural selection, because these phases of survival and reproduction would be critical to the propagation of the species. Beyond the period of reproductive maturity as fecundity declines, which is also the period of "protected aging," cell and tissue functions are predicted to be under little or no evolutionary pressure, both because they are predicted to have negligible effect on species survival and also because, in the wild, survival beyond this point markedly diminishes. It is during this phase that stem cell functionality (although not necessarily stem cell number) declines in most tissues, in some cases precipitously.

[1]Stem Cells and Regenerative Medicine Center, Center for Cell and Gene Therapy, and Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA. [2]Glenn Center for the Biology of Aging and Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305, USA, and Center for Regenerative Rehabilitation, Veterans Administration Palo Alto Health Care System, Palo Alto, CA 94304, USA.
*Corresponding author. E-mail: goodell@bcm.edu (M.A.G.); rando@stanford.edu (T.A.R.)

concept underlies views of increased cancer incidence with age. However, it has remained unclear how the accumulation of somatic mutations affects either organismal aging or aging of stem cells. Recent work from the hematopoietic system has begun to shed some light on these issues.

If somatically acquired mutations are rare, most cells in the peripheral blood should be virtually identical in their genome sequence; hence, blood can be used to assess the "germline status" of an individual's genetic complement. However, deep genome sequencing studies investigating mutations that contributed to leukemia development revealed that normal blood cells often harbor passenger mutations (unrelated to the leukemia) much more frequently than expected (*3*). These data indicate that blood progenitors acquire random mutations constantly, on the order of 10 mutations per HSC per year, many of which then appear in small fractions of differentiated blood progeny (*3*). When this deep sequencing approach was extended to blood samples from tens of thousands of individuals across many ages, a striking pattern arose: Specific somatically acquired genetic variants were often present in a large fraction of blood cells (Fig. 2), up to 70% in some cases (*4*–*7*). Although blood cells were thought to be generated from ~1000 active stem cells in young adults (*8*), the explanation for the high proportion of blood cells with particular somatic mutations was that a single stem cell clone was dominating the generation of the peripheral blood in some individuals. This collapse of highly polyclonal into quasi-monoclonal hematopoiesis increases with age; between 5 and 20% of 70-year-olds show clear evidence of this state, and almost all individuals above the age of 90 are estimated to have a single dominant stem cell clone generating a substantial proportion of their blood (*9*).

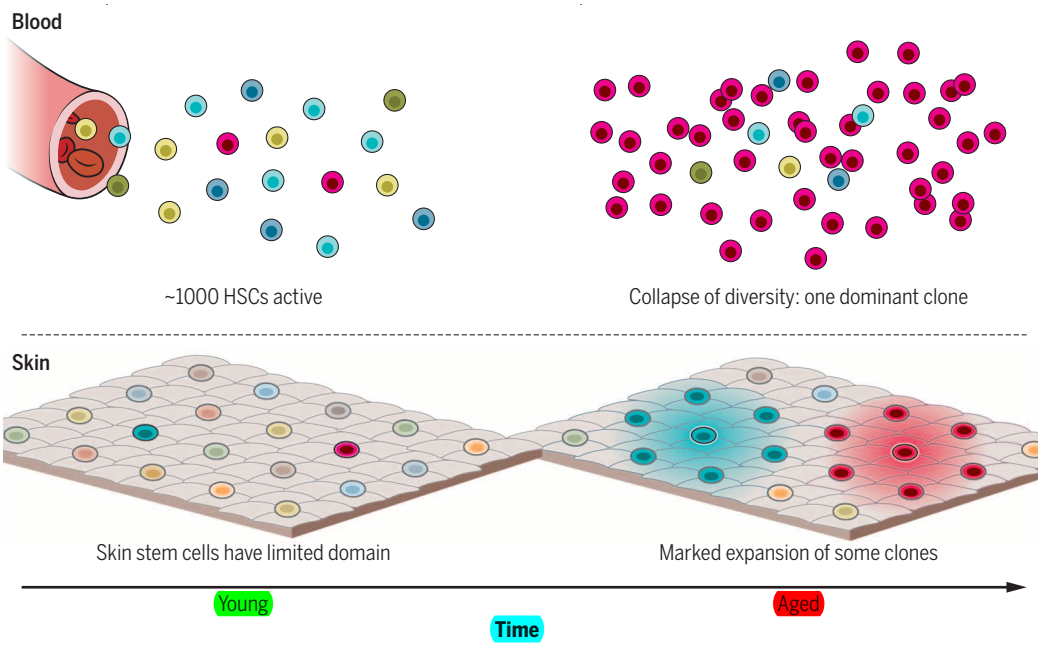


**Fig. 2. Stem cell diversity and dynamics with age.** Top: Peripheral blood from young individuals is generated from around 1000 active stem cells. By the age of 70, the clonal diversity collapses, resulting in dominance of one HSC clone, such that about 20% of individuals have one clone that dominates 20 to 80% of blood cell production. Bottom: Representation of the surface of skin. Young skin is continuously replenished from stem cells, each with a highly restricted domain (represented by circles). Random mutations generate small variations across the surface in terms of stem cells and their progeny (colored circles). With time, some clones expand markedly, resulting in clonally derived patches with a common set of genetic variants (*18*).

The notion of clonal collapse is revolutionizing our view of HSC dynamics with age, and there is intense interest in understanding the potential mechanisms of this phenomenon. One clue has come from examining the genes that are most commonly mutated in these large clones. Mutations in about 20 genes are recurrently associated with clonal hematopoiesis. The top two, *DNMT3A* and *TET2*, are epigenetic regulators that control DNA methylation status. Both are frequently mutated across many hematologic malignancies, and their loss leads to increased numbers of stem and progenitor cells (promoting self-renewal) while hindering their ability to differentiate in mouse models (*10*–*13*), although the precise mechanisms through which these mutations act is not well understood. The third recurrently mutated gene, *TP53*, is the most frequently mutated gene across all cancers (*14*) and its product, p53, is considered the "guardian of the genome" because of its central role in regulating cellular responses to stress and DNA damage. In mice, *Tp53* has been shown to regulate HSC proliferation (*15*) and its deficiency leads to age-related stem cell expansion (*16*). With each HSC acquiring around 10 mutations per year, any individual stem cell will harbor 700 to 800 mutations in later decades of life (*3*). Most of these mutations will be neutral, although some will have a deleterious effect, potentially leading to the arrest or elimination of those cells. Thus, mutations seen in advanced age are those compatible with the cell's long-term survival.

Together, data from humans and mice suggest that acquired somatic mutations can confer a growth or survival advantage to the target stem cell, enabling its expansion and leading to a preponderance of its progeny in the blood with age. What are the long-term health implications? The vast majority of individuals who exhibit clonal hematopoiesis will not develop hematologic malignancy during their remaining life span. Nonetheless, they are at significantly higher risk of developing age-associated blood diseases such as myelodysplastic syndrome, aplastic anemia, and leukemias (*5*) associated with the acquisition of additional mutations. Moreover, individuals with clonal hematopoiesis are at higher risk for earlier mortality when all causes are considered, with myocardial infarctions and strokes having the strongest association (*5*). Furthermore, aging-associated diseases such as type 2 diabetes have also been correlated with blood-based genomic aberrations (*17*). The mechanisms behind these correlations are not understood, but mutations in stem cell regulators likely affect the function of their progeny; HSCs are continuously generating an array of immune cells, platelets, and red blood cells, all of which have an impact on disease resistance, inflammation, clotting, and tissue oxygenation. Because the blood system supports all tissues in the organism, any age-related impairment of stem cells manifest in their progeny could conceivably affect distant tissues and therefore healthy aging.

Is there evidence for aging-associated clonal expansion of stem cells in other tissues, and does this affect tissue heath? This phenomenon, although not yet examined on a large scale, is known to occur in other tissues. For example, stem cells are generally responsible for generating the differentiated epithelial cells in a very restricted surface area of young skin. However, in normal aged Sun-exposed skin, marked expansion of clones associated with specific mutations—including *TP53* and the stem cell regulator *NOTCH1*—occurs well beyond those original boundaries (*18*). Remarkably, almost 20% of normal skin cells have these potent expansion-promoting *NOTCH1* mutations. The relatively low incidence of cancer acquisition despite the high frequency of clones bearing cancer-associated mutations is a testament to the mechanisms that restrain

malignancy development. The effect of these expanded mutant clones on age-related changes of tissue function (including the critical barrier function of the skin), and on organismal health generally, is unknown but warrants investigation, given the striking associations of clonal collapse in the hematopoietic system.

Together, these and other studies demonstrate that somatic mutations that arise in stem cells confer an advantage that leads to their expansion within a tissue over many years. Many of the somatic mutations repeatedly observed are associated with cancer, and this may underlie the correlation between tissue-specific cancer incidence and stem cell proliferation (*19*). More important, we expect that clonal dominance in the blood, and possibly in other tissues, can have broad effects on healthy aging of the cognate tissues.

## Epigenetic erosion with age

Epigenetic regulation refers to the mechanisms, mainly DNA methylation and histone modifications, that license regions of the genome for expression while shutting down others. There has been great interest in understanding the extent to which erosion of these genome-scale regulatory mechanisms leads to dysregulated control of gene expression, contributing to the decline of stem cell and tissue function with age. Genetic evidence in model organisms has supported the notion that aberrant epigenetic regulation affects organismal aging. For example, in *Caenorhabditis elegans*, a genetic screen revealed that loss of function of *Wdr5*—a gene encoding a histone methyltransferase that leads to trimethylation of lysine 4 on histone 3 (designated as H3K4me3 and generally considered to be a mark of expressed genes)—led to extended life span (*20*). Although H3K4me3 generally marks promoters, the histone mark covers the entire coding unit at a subset of genes controlling cellular identity; this pattern is associated with very high gene expression and transcriptional elongation (*21*). However, research is needed to understand why reduction of H3K4me3 should be correlated with longer life span. Similar efforts in yeast have shown that lower levels of another histone mark, H3K36me3, reduced replicative life span, whereas ablating genes that diminish the mark increased yeast life span (*22*). Reduction of the H3K36me3 mark was associated with transcriptional infidelity and cryptic transcripts.

How do these and other findings relate to aging in mammalian stem cells? Although not extensively examined, current data support the concept that epigenetic regulation erodes in aging stem cells. In concordance with the *C. elegans* findings, H3K4me3 tends to increase in aging HSCs, particularly on the genes involved in maintaining HSC identity (*23*). In both HSCs and MuSCs, the repressive H3K27me3 mark increased with age (*23*, *24*). In HSCs, this increase was associated with repression of some genes that direct specific differentiation programs such as the lymphoid fate, known to diminish with age. In MuSCs, the increase was associated with repression of genes encoding histone genes themselves (*24*); this finding is of interest because of the relationship between histone gene expression and yeast replicative life span (*25*). Interestingly, mesenchymal stem cells (MSCs) from aged individuals show a decline in H3K9me3, a mark associated with proper maintenance of heterochromatin. Loss of this mark is also found in MSCs harboring the Werner's progeria syndrome mutation (*26*), again linking epigenetic erosion with aging.

Finally, age-related changes in DNA methylation have been examined in HSCs. DNA methylation was decreased at genes associated with the promotion of self-renewal and was increased near genes associated with differentiation (*23*, *27*). Polycomb proteins are factors generally involved in gene silencing. Regions with histones bearing Polycomb-associated marks (H3K27me3) tended to become hypermethylated, a phenomenon noted previously in other tissues in mouse and human (*28*). Changes in methylation may play a role in inhibiting the expression of tumor suppressor genes, thereby increasing the possibility of malignant transformation.

Overall, these epigenetic changes in aging stem cells are consistent with the functional deficits that have been repeatedly observed. With aging, HSCs appear to increase in numbers but simultaneously lose differentiation capacity (*23*, *27*). Nonetheless, the extent to which these changes are correlative versus causal is not yet clear and merits further exploration. In HSCs and MuSCs, the precise epigenetic regulation observed at young ages appears to drift. This drift is aligned with the general aging process: The identity and general function of the stem cells remain the same, but they cannot regenerate their cognate tissues quite as well as they did before.

## Extrinsic factors affect aging stem cells

The influence of the local and systemic environment on stem cell function during protected aging has been demonstrated by exposing young stem cells to an aged environment, and vice versa. These studies have used strategies such as heterochronic transplantation, in which cells derived from a donor of one age are transplanted into a recipient of a different age, or heterochronic parabiosis, in which two mice of different ages are adjoined to create a shared circulatory system, thus exposing cells in one animal to the systemic environment of the other (*29*) (Fig. 3). When young stem cells were subjected to an aged systemic milieu by heterochronic

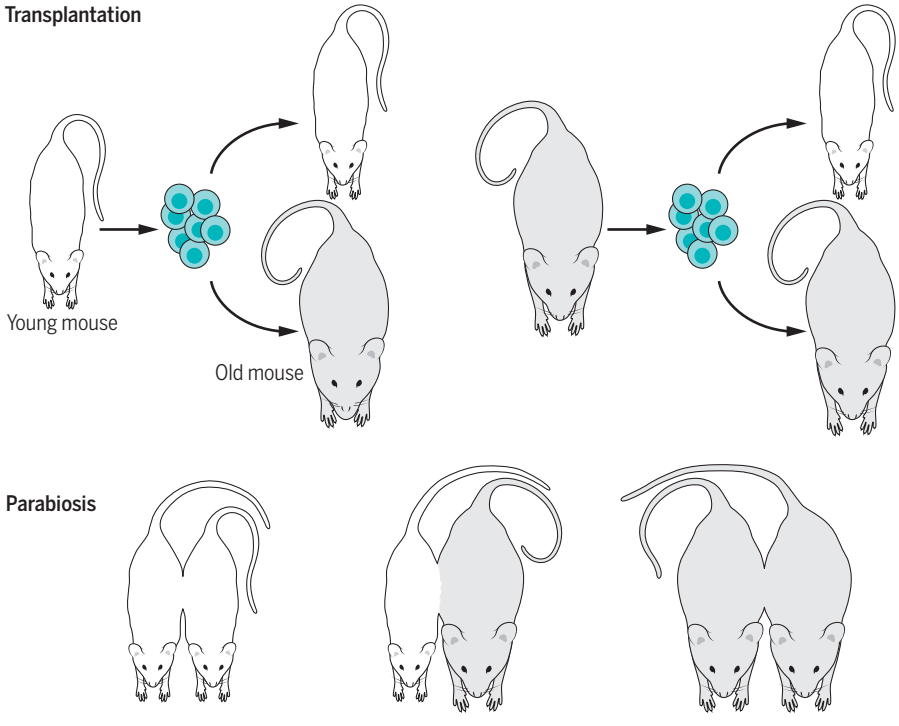


**Fig. 3. Intrinsic and extrinsic factors influence age-related changes in stem cell function.** Distinguishing cell-intrinsic changes from cell-extrinsic changes (e.g., arising from the cellular environment) in cell function has been aided by heterochronic studies. In heterochronic transplantation, stem cells isolated from either young or old donors are transplanted into young or old hosts, and cellular function is then analyzed in these four-way comparisons (young into young, young into old, old into young, old into old). In parabiosis, animals are joined to promote the development of a single, shared circulatory system, thus exposing cells in one animal to the systemic environment of the other animal. In this case, the cellular functions in young or old partners in the heterochronic pairs (young-to-old) are compared to those in isochronic pairs (young-to-young and old-to-old).

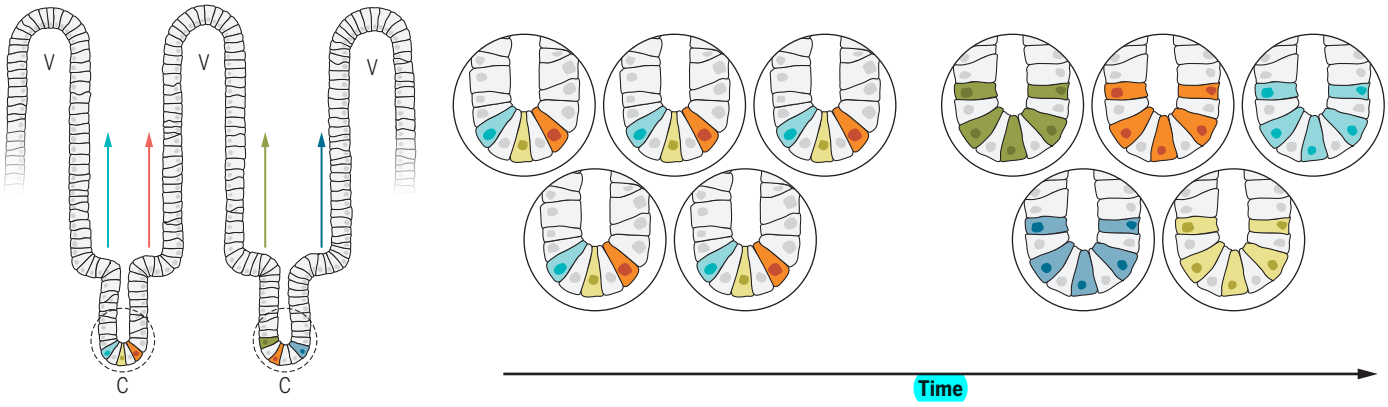


**Fig. 4. Neutral drift of stem cell populations.** Left: The small intestine comprises villi (v) containing differentiated cells that are replenished (arrows) from the progeny of stem cells residing in the crypts (c). Right: When crypts are viewed from above, en face (as if cut on the dashed line), several stem cell domains (each represented by a different color) are present. With age, the crypt stem cells continuously compete with each other, such that over time, all the stem cells in an individual crypt originate from one stem cell. These presumptions are based on mouse data (*44*). Certain genetic alterations and environmental conditions can accelerate this process.

parabiosis, they exhibited functional decline that resembled accelerated aging (*30*, *31*). On the other hand, the converse was also true: Aged cells placed in a young environment or exposed to a youthful systemic milieu exhibited more youthful characteristics, suggesting that it may be possible to ameliorate certain aging features. These findings have led to the search for "age-promoting" factors in old blood (see below) and for "youth-promoting" factors in young blood (*32*–*34*) [but see also (*35*)]. Together, these studies demonstrate that stem cells are profoundly influenced by their environment and imply that blood-borne factors may be responsible for at least some of the age-associated declines in stem cell functionality.

Efforts to identify specific aging-associated circulating factors have repeatedly highlighted pro-inflammatory molecules, such as cytokines in the blood, as key drivers of cell and tissue aging (*36*). One of the first "aging factors" identified by heterochronic parabiotic studies was the cytokine CCL11 (*31*). The levels of this protein increase with age in the blood, and administration of this cytokine into the circulation of young animals led to a decline in NSC activity, as occurs during normal aging. Likewise, the related inflammatory cytokine Rantes was found to be elevated in the HSC niche with age and to contribute to the age-related myeloid skewing in the hematopoietic lineage (*37*).

In addition to cytokines, other immune system–associated molecules have been shown to change with age and promote aging phenotypes. Plasma levels of the complement protein C1q were shown to increase with age and to promote age-related MuSC decline by activation of the Wnt signaling pathway (*38*, *39*). Similarly, β2-microglobulin, a component of the major histocompatibility complex, was found to be elevated in the blood of aged mice and to contribute to an age-related decline in NSC function (*40*). The increase in proinflammatory factors, along with the concomitant decrease in factors that promote tissue repair, likely contributes to aging phenotypes in many tissues.

## Somatic natural selection of stem cells

From the studies discussed above, we can appreciate at least three key influences on somatic stem cells within an aging organism. Stem cells acquire many somatic mutations over time, they experience epigenetic drift, and they are bathed in a broader milieu that can negatively influence function. Among the cells in any given stem cell population, each cell brings its unique characteristics (e.g., mutations) and experiences (e.g., exposure to local cytokines) to the evolving adaptive landscape. We propose that these forces interact over time to result in selective pressure on individual stem cells: Stem cells that have acquired, through somatic mutation or epigenetic drift, the characteristics best adapted to the aged environmental milieu will become enriched in the population, as a result of Darwinian-like natural selection that occurs in vivo during aging ("somatic natural selection"). These stem cells have characteristics that confer optimal survival in the protected aging environment, regardless of other functional capabilities. Any detrimental characteristics they have assumed will be conveyed to the tissue via their progeny, in proportion to their relative abundance.

This view of clonal dynamics has both theoretical and experimental foundations. Mathematical modeling of stem cell populations predicts that constant competition in a closed environment will lead to domination by one stem cell, even in the absence of any selection (so-called "neutral drift") (*41*), analogous to population drift of species leading to fixation of traits even without selection, particularly in small populations (*42*). Moreover, certain mutations or environmental pressures should accelerate this phenomenon, selecting for stem cells with particular adaptions [akin to a changed fitness landscape (*43*)].

Studies of the murine small intestine have borne out these ideas. Differentiated cells of the intestinal villi are continuously replenished by stem cells that reside at their bases in specialized crypts. Each crypt contains several stem cells that compete to populate the adjacent villi. Even in the absence of selection, individual stem cells overtake an entire crypt in a completely random fashion, as predicted by neutral drift theory (Fig. 4) (*44*, *45*). Mutations that even marginally increase proliferation, such as in the *K-ras* oncogene, accelerate crypt clonality (*46*). In specific environmental contexts, some mutations appear particularly adaptive. Stem cells with a *Tp53* mutation have no advantage in a normal crypt, but in an inflammatory environment that mimics colitis, the *Tp53*-mutant stem cells rapidly take over and their progeny dominate production of the entire crypt (*47*).

Although not established experimentally, we speculate that similar forces lead to the emergence of clonal dominance as a feature of the aging hematopoietic system (Fig. 2). This hypothesis would predict that few of the acquired mutations in HSCs would confer an advantage in the young environment, allowing hematopoiesis to remain highly polyclonal. By contrast, the same mutations, compounded by epigenetic drift, could confer a distinct survival and/or proliferative advantage in the changing milieu of age, allowing for the expansion of specific HSCs and clonal dominance. Indeed, in young mice, *Tp53* knockout HSCs have no particular advantage, but in old hosts they expand relative to their normal counterparts (*16*). We speculate that *DNMT3A* and *TET2* mutations may similarly confer an advantage in the aging environment. This view is supported by the observation that some mutations associated with clonal hematopoiesis (e.g., in splicing factors) only become prevalent after the seventh decade (*5*, *7*, *9*), which suggests that the aging environment is particularly important for the emergence of HSC clones with these mutations. This model of continuous Darwinian

selection acting on variants across a population in a changing landscape also applies to cancer development, an inherently aging-associated phenomenon (*48*).

Considered more broadly, highly dynamic tissues such as the gut and bone marrow have stem cells that, in effect, continuously compete with each other. Thus, any cell-intrinsic change, genetic or otherwise, that confers a growth or survival advantage may lead to predominance of particular stem cells (Fig. 5). This is important because the selective environment (adaptive landscape) changes with age. In aged organisms, factors such as systemic inflammation may offer an advantage to stem cells with particular characteristics. The stem cells that respond best in an aged or injured environment may not be the most effective at regenerating healthy tissue if their progeny also bear somatic mutations or exhibit epigenetic drift. These general principles are likely in effect throughout many tissues, albeit manifesting differently depending on factors such as tissue turnover rate, local interactions among stem cells, physical constraints on cell interchange, and the magnitude of alterations in the adaptive landscape. For example, in tissues such as skeletal muscle, in which there is thought to be lower stem cell interchange and tissue turnover, internal competition and selective forces may be less important. Even in the gut, stem cell competition occurs almost exclusively within, and not between, individual crypts. Thus, gaining a broader understanding of the role that somatic stem cell competition plays across many tissues will be important in the future.

Finally, how do the environment and somatic mutations interact together with epigenetic modulation? Epigenetic drift likely occurs even in the absence of particular somatic mutations, driven by, and promoting adaption to, the changing environmental milieu. The frequent selection for mutations in epigenetic regulators (e.g., *DNMT3A* and *TET2*) may suggest that these afford a degree of epigenetic plasticity that hastens adaption to the aged environment.

## Conclusions

Taken together, recent studies on somatic mutations, epigenetic drift, and the environmental influences on stem cells usher in a new view of aging and the challenges to preserving healthy tissue function over time. After organismal growth ceases, stem cells effectively maintain tissues through the peak reproductive years. Subsequently, there are no effective mechanisms that have been evolutionarily selected to preclude the gradual loss of cell and tissue health. Instead, a variety of genetic, epigenetic, and environmental factors allow drift until the aging environment acts strongly enough to select for particular (usually detrimental) characteristics (Fig. 5). The mutant stem cells that accumulate with age are not causes of aging per se; they simply exploit the aged environment to become dominant. In turn, the functional deficiencies they confer on their progeny contribute to the phenotypes associated with aging.

With these views, what strategies or interventions can be envisioned to extend healthspan by targeting stem cells? Two approaches emerge naturally from the studies discussed. First, it seems as if the phenotypes of aging stem cells may be at least partially reversible. As noted above, heterochronic parabiosis studies suggest that factors in young blood might partially ameliorate the functional deficits of aged stem cells (*30*, *31*). Furthermore, the injection of plasma from young mice into the circulation of aged mice has recently been shown to induce a more youthful state of cells in the brain of the old animal (*32*). These findings indicate that at least some aspects of cellular aging may be reversible, perhaps through reprogramming of the epigenome (*49*). Indeed, interventions that clearly extend organismal life span even when applied late in life, such as rapamycin treatment (*50*), may enhance stem cell function in aged animals. As such, it may be that treatments that directly enhance the function of aged stem cells do so by acting on the epigenome to adopt a more youthful state.

Is it possible to reduce the acquisition of somatic mutations? Most are probably the inevitable consequence of cell division and deamination events that result in C→T transitions (*51*, *52*).

Assuming we cannot eliminate mutations altogether, another approach would be to alter the adaptive landscape so as to select for more functional cells. By the time that the protected aging phase begins, stem cells will already have acquired a burden of somatic mutations (a largely inevitable consequence of cell division) and have drifted epigenetically. We suggest that monitoring changes and modulating the environment early—such as reducing inflammatory mediators and otherwise slowing the transition of the systemic environment that occurs with age—may limit the development of clonal dominance, allowing the polyclonal state to be sustained longer (Fig. 5). Alternatively, providing a new adaptive landscape in which different stem cell variants are better adapted to thrive could likewise contribute to the maintenance of tissue health. Along these lines, it is interesting that genetic manipulations early in life that preserve proliferative homeostasis of gut stem cells in *Drosophila* lead to life-span extension (*53*). It may be that early life treatments that extend life span, such as caloric restriction, do so in part by altering the adaptive landscape to prevent detrimental clonal dominance and preserve tissue function. We are optimistic that better understanding of

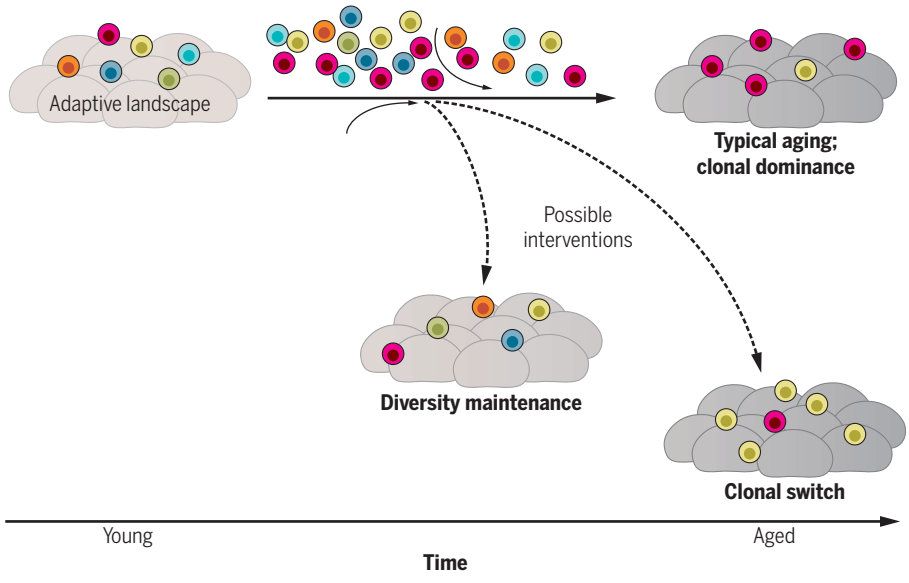


**Fig. 5. Model of age-related selection for stem cells with new characteristics and potential outcomes.** In young individuals, a polyclonal population of HSCs gives rise to a heterogeneous population of blood cells. With age, stem cells acquire somatic mutations and experience epigenetic drift. Concurrently, attrition ("collapse") of some clones occurs and the adaptive landscape gradually shifts. This may be caused in part by changes in humoral factors (such as circulating cytokines or inflammatory factors) or changes in the cellular environment that regulates the stem cells, the so-called niche (change could occur in niche composition or behavior). The result of a changing landscape and clonal attrition may be a population bottleneck that provides an opportunity for clones with a selective advantage to expand. Ultimately, this leads to quasi-monoclonality and to the dominance of particular clones. In principle, it could be possible to intervene to alter the forces that drive toward deleterious quasi-monoclonality (dashed arrows). Any intervention that suppresses the change in the adaptive landscape would tend to preserve the healthy polyclonality (diversity maintenance) of youth. Likewise, understanding any growth or survival advantages conferred upon HSCs with "nondeleterious" somatic mutations or epigenetic changes could allow for a rational modulation ("clonal switch") of the adaptive landscape to select for those clones rather than deleterious clones.

the mechanisms of stem cell dysregulation and selection with age will enable new rational interventions based on these principles.

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REVIEW

# Mitochondrial dysfunction and longevity in animals: Untangling the knot

**Ying Wang and Siegfried Hekimi***

**Mitochondria generate adenosine 5′-triphosphate (ATP) and are a source of potentially toxic reactive oxygen species (ROS). It has been suggested that the gradual mitochondrial dysfunction that is observed to accompany aging could in fact be causal to the aging process. Here we review findings that suggest that age-dependent mitochondrial dysfunction is not sufficient to limit life span. Furthermore, mitochondrial ROS are not always deleterious and can even stimulate pro-longevity pathways. Thus, mitochondrial dysfunction plays a complex role in regulating longevity.**

The primary and most essential function of mitochondria is to produce energy for the cell. The oldest explanation for aging, the rate-of-living theory, postulates that aging and life span are regulated by the rate of energy metabolism, with lower rates leading to longer life spans. However, the appeal of the rate-of-living theory has been weakened by its failure to accurately predict the observed relationships between energy expenditure and life span. This is not to say that mitochondrial and energy metabolism don't play a crucial role in aging, but their relationship to aging might not be simple. Mitochondria do much more than produce energy. Particularly relevant to aging, the mitochondrial electron transport chain (ETC) leaks electrons and generates reactive oxygen species (ROS) during normal respiration. Thus, a potentially harmful elevation of ROS production occurs when the ETC function is perturbed. The mitochondrial free-radical theory of aging posits that biological aging results from the production of ROS and the ensuing damage. However, direct manipulation of cellular ROS levels within the biologically meaningful range does not accelerate aging or decrease life span. Here we review the relationships between normal mitochondrial function, mitochondrial dysfunction, ROS generation, and life span.

## Deleterious mitochondrial dysfunction

Numerous studies have described damage to mitochondria in aged cells and organisms, including in human samples. This damage includes a gradual decline in respiratory chain capacity, decreased activities of individual ETC complexes, elevated oxidative damage, decreased mitochondrial content, morphological abnormalities in mitochondrial structure, and increased fragility of aged mitochondria during experimental isolation (Fig. 1) (*1*). In exploring the implications of these observations for the aging process, a key question is whether the observed damage and dysfunction are severe enough to cause the other degenerative phenotypes of aging.

There is no doubt that mitochondrial dysfunction can severely damage the organism. Human patients with mutations in mitochondrial DNA (mtDNA) or in nuclear genes coding for proteins that function in the mitochondrial ETC are generally severely affected. They often show multisystem disorders that include myopathy, encephalopathy, stroke, and hearing loss (*2*). Most mitochondrial disorders present with neurological and muscular symptoms. It is thus generally postulated that cells with high energy demands, such as those in the central nervous system and muscles, are more susceptible to the reduced energy output of defective mitochondria and are consequently more strongly affected by mitochondrial impairment. There is, however, considerable clinical variability among mitochondrial disease patients, and some mutations only affect particular tissues, reflecting a diversity of distinct disease mechanisms that are still poorly understood. To understand these conditions, a variety of mouse knockout (KO) models have been developed for nuclear-encoded mitochondrial proteins (*3*). These include mutants carrying KO mutations in genes that are required for the assembly and function of ETC complexes, mutants with defects in the production of mobile electron carriers [cytochrome c and ubiquinone (UQ)], and mutants lacking necessary factors for the maintenance of mitochondrial dynamics or the integrity of mtDNA. In virtually every case, complete germline KO causes embryonic to perinatal lethality. Tissue-specific conditional KOs, mostly targeted to neurons or muscles, result in abnormal mitochondria with severe deficits in respiratory chain function, giving rise to a variety of disease phenotypes. Most show severe progressive loss of tissue function, such as progressive skeletal muscle weakening, movement impairment, and neurobehavioral abnormalities. All result in death within the first year of life,

Department of Biology, McGill University, Montreal, Quebec H3A 1B1, Canada.
*Corresponding author. E-mail: siegfried.hekimi@mcgill.ca

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