Hematopoietic stem cells and the genetics of aging
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 1
Cellular memory
and hematopoietic stem cell aging

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Submitted
Abstract

Hematopoietic stem cells (HSCs) balance self-renewal and differentiation in order to sustain lifelong blood production and simultaneously maintain the HSC pool. However, there is clear evidence that HSCs are subject to quantitative and qualitative exhaustion. In this review, we briefly discuss several known aspects of the stem cell aging process, including DNA damage, telomere shortening, and oxidative stress. Besides these known players there is increasing evidence that higher order chromatin structure, largely defined by the histone code and affecting transcriptional activity, is important. A model is suggested how epigenetic regulation of gene transcription by modulation of the chromatin structure in stem cells can account for regulation of the aging program.

Stem cells and aging

Every embryonic and adult stem cell is capable of both self-renewal and differentiation. Through the poorly understood process of asymmetric partition of cellular constituents, a single cell division can result in the formation of both a newly formed stem cell and a more differentiated progenitor cell\(^1\). Differentiation often interfaces with proliferation, enabling a single stem cell to produce enormous numbers of fully differentiated, post-mitotic, tissue-specific end cells. For example, few hematopoietic stem cells (only accounting for <0.05% of the total bone marrow cells) produce billions of blood cells each day\(^2\)\(^-\)\(^9\). All differentiated cells have a limited life span. This life span may range from several hours (neutrophilic granulocytes, epithelial cells in the small intestines), multiple days (platelets, red blood cells, skin keratinocytes), to many years (lymphocyte subsets). The finite life span of somatic cells and their consequential loss is compensated for through the production of new cells by stem cells. Evolutionary more simple organisms, such as C. elegans and D. melanogaster, are (almost) exclusively post-mitotic, and are not believed to contain somatic stem cells. The life span of these species is largely accounted for by the collective life span of all (most) of its individual cells. It is tempting to speculate that acquisition of adult stem cells during evolution has resulted in a major extension of organismal life span. Along these lines, it is reasonable to argue that the sole function of adult stem cells is to rejuvenate aged tissue. Let us first define the process of aging as we will discuss it in this review. We propose that aging must be a continuous process that already starts in utero. After the second cell division of the zygote there is commitment of both daughter cells to develop into certain lineages. Already at this early stage in life there is loss of cell potential. In the context of this work we define aging as the gradual loss of cell potential. We will focus our discussion on the hematopoietic stem cell system,
as this model is best understood and amenable to careful experimental perturbation. Importantly, however, we believe that molecular mechanisms that specify HSC aging are also operating in other cell systems.

**Hematopoietic stem cells and aging**

Hematopoietic stem cells (HSCs) reside in the bone marrow (BM) and provide lifelong production of progenitors and peripheral blood cells. Simultaneously, HSCs must be able to maintain the stem cell pool by self-renewal divisions. In stem cell homeostasis a delicate balance exists between self-renewal and terminal differentiation, since excessive self-renewal may initiate cancer (i.e., leukemia), and increased differentiation ultimately may lead to premature exhaustion of the stem cell pool. It is likely that during replicative stress (which can be experimentally induced by serial transplantation, but may also result from normal aging) this balance weighs in favor of terminal differentiation, resulting in exhaustion of the HSC pool. An array of different assays are developed to assess stem cell potential, multiple of which should be used in order to claim true stem cell activity\textsuperscript{10,11}. Under normal conditions the hematopoietic stem cell pool is large enough to provide an organism with a sufficient number of committed progenitors to ensure homeostasis, even after serious bleeding or chemotherapy. Old people, or mice for that matter, typically do not die because they run out of HSCs. This suggests that the hematopoietic stem cell compartment does not substantially deteriorate during aging.

In contrast, it has been speculated that stem cell quality decreases with each self-renewal division\textsuperscript{12}. This model suggested that during serial transplantation and repetitive rounds of chemotherapy, but also during normal aging, stem cell quality deteriorates.

Most studies addressing the issue of HSC aging have used serial transplantations. The transplanted cell population must provide radioprotection to rescue the lethally irradiated recipient and must be able to provide permanent long-term engraftment. Upon serial transplantation, HSCs undergo replicative stress and are in this way challenged to rescue lethally irradiated recipients, providing them with sufficient HSCs and multilineage reconstitution. It is argued that the replicative stress HSCs undergo with each serial transplantation induces accelerated loss of stem cell potential (aging). It has been documented that HSCs can outlive their original donor upon repeated serial transplantation in lethally irradiated recipients\textsuperscript{13}. However, multiple studies have shown that serial transplantations can only be carried out for a restricted number of passages, suggesting stem cell exhaustion\textsuperscript{13-22}. In addition, functional decline of HSCs increased with repeated serial transfers\textsuperscript{14,15,18,20,23,24}. In a competitive repopulation assay, serially transplanted stem cells showed impaired self-renewal, already after the first transplantation\textsuperscript{20,25}. 
Chapter 1

The competitive repopulation assay is commonly used to test the quality and to calculate the quantity of (treated) HSCs compared with freshly isolated HSCs. The two populations are co-transplanted (competed) in lethally irradiated recipients. At different time points after transplantation the contribution of both populations to hematopoiesis is tested, mostly by assessing chimerism levels with flowcytometry \(^6,26\). HSCs showed limited self-renewal when either purified HSCs \(^{25,27}\) or unfractionated BM cells were used \(^{20,21,27}\). Purified HSCs are mostly selected based on their cell surface markers. Generally, in situations where stem cells are isolated from normal, unperturbed mice, there is a good correlation between phenotype and in vivo reconstitution potential. However, there is substantial evidence that this stem cell phenotype changes after transplantation and other perturbations \(^{10,28,29}\). Our group has shown that during normal hematopoiesis the stem cell compartment is subject to aging, since freshly isolated stem cells from young donors were able to engraft aged recipients and contribute to multilineage reconstitution \(^{27}\).

An issue that has frequently been addressed is whether stem cell aging is regulated by intrinsic or extrinsic factors. Stem cells are associated with stromal cells, which not only provide structural support and maintain the position of the stem cells, but also secrete various cytokines \(^{30-34}\). The profile of cytokine production changes during aging. Moreover, it has been suggested that early after transplantation the old microenvironment plays a more dominant role in determining the numbers of various lineages than does the age of the HSC \(^{35}\). Recently it has been shown that increasing the number of osteoblasts in the stem cell niche resulted in a parallel increase in the number of HSCs \(^{36}\), indicating an important role for the microenvironment in controlling stem cell self-renewal and differentiation. In vitro experiments demonstrated that HSC function was supported though the Notch pathway. Expression of a constitutively active form of Notch1 in murine BM progenitors can lead to increased HSC self-renewal \(^{37}\). In addition, Notch signaling was active in HSCs in vivo and downregulated as HSCs differentiated. In concordance, inhibition of Notch signaling led to accelerated differentiation of HSCs in vitro and depletion of HSCs in vivo. Intact Notch signaling was required for Wnt-mediated maintenance of undifferentiated HSCs \(^{38}\).

Interestingly, recent studies have identified the Wnt signaling pathway as a regulator of HSC homeostasis \(^{39}\). The recent findings that osteoblasts are an important regulatory component of the HSC microenvironment, and that elements of the Wnt signaling pathway can influence osteoblast frequency, raise the possibility that Wnt signaling may influence HSC function indirectly through the niche \(^{40}\).

If stem cell aging were largely extrinsically regulated one might contemplate studies searching for humoral factors that potentially could interfere in this process. However, evidence from mouse studies shows that the aging program is largely intrinsically regulated. To assess the genetic component regulating stem cell aging, HSCs characteristics have been studied in different inbred
mouse strains. It is thought that HSCs of the commonly used C57BL/6 (B6) mice are not subject to aging, since stem cell number is increasing upon aging\textsuperscript{41-43} and they can outlive their original donor during serial transplantation\textsuperscript{13}. However, homing properties of B6 HSCs seem to be altered, reducing their ability to engraft recipients\textsuperscript{44}. In contrast, aged DBA/2 (D2) mice do show apparent exhaustion of the stem cell pool\textsuperscript{41,45,46}. Interestingly, the maximum life span of different inbred mouse strains correlates negatively with the percentage progenitors in the S-phase of the cell cycle. For example, progenitor cells from long-lived B6 mice have a relatively low cycling activity, whereas the stem cell pool increases with age and is relatively small. In contrast, D2 mice have a shorter life span than B6 mice, their progenitors show increased cycling activity, and their stem cell pool decreases upon aging and is relatively large\textsuperscript{47}. This suggests that rapidly dividing cells exhaust faster. These genetic differences with respect to cycling activity and stem cell pool are still present when D2 and B6 cells co-exist in the same microenvironment\textsuperscript{45,46}. In addition, differences between the D2 and B6 stem cell compartment appear to be pre-determined and do not change over time\textsuperscript{48}. This strongly suggests intrinsic regulation of the stem cell aging program. What cell-intrinsic mechanisms could possibly confer a form of cellular memory to stem cells?

**Cellular damage and stem cell aging**

**Telomere shortening**

Telomeres, the structures protecting chromosome ends, have received much attention as a potential trigger to induce replicative senescence. Telomere length is largely maintained by the enzyme telomerase. Whereas most somatic stem cells have telomerase activity, this is hardly detectable in differentiated cells\textsuperscript{49}. During in vitro proliferation of human fibroblasts, but also during in vivo aging, a gradual shortening of the average length of telomeres is observed\textsuperscript{50,51}. Furthermore, it has been shown that telomere shortening occurs during serial transplantation of HSCs coinciding with impaired functioning\textsuperscript{52}. The most striking evidence of telomere length being important in regulating cellular aging comes from studies in which the consequences of telomerase-deficiency were investigated. In serial and competitive transplantations, telomerase deficient hematopoietic stem cells showed reduced long-term repopulating capacity, concomitant with an increase in genetic instability compared with wild-type cells\textsuperscript{53}. Normal murine HSCs can be serially transplanted four times, but HSCs of telomerase deficient mice can only be transplanted twice\textsuperscript{54}. In contrast, HSCs from mTERT transgenic mice, in which telomerase is overexpressed and telomere length is preserved, can also only be serially transplanted four times, so other mechanisms must be involved in regulating stem cell exhaustion\textsuperscript{55}. In addition, humans suffering from the rare inherited disorder dyskeratosis congenita have reduced levels of telomerase activity, resulting from a mutated hTR
allele, and shortened telomeres. In these patients, bone marrow failure is the principal cause of death. Interestingly, telomere shortening occurs rapidly in cell lines derived from patients that suffer from premature aging disorders, like Werner syndrome and ataxia telangiectasia. However, it has been suggested that not the telomere length as such, but rather the overall telomere structure is important in cellular senescence. Functional telomere structures help maintain the stability of the genome (prevent cancer) and protect cells against telomere-induced apoptosis or senescence (postpone aging). On the other hand, dysfunctional telomeres lead to genomic instability, which can promote cancer, but also lead to the tumor suppressor mechanisms of apoptosis and senescence, which can promote aging.

**DNA damage**

Replication of the genome during cell division inevitably results in numerous copy errors, but elaborate proofreading and editing mechanisms have evolved to correct these. The appropriate cellular response after detection of DNA damage is an initial attempt at repair, but if damage is too extensive or compromises DNA metabolism, a signaling cascade triggers cellular senescence or death. Maintenance of DNA integrity is vital to proper functioning of every cell. DNA injury may compromise transcription and replication and thereby cause additional mutations, cellular senescence or cell death. In several human disorders, such as xeroderma pigmentosum and Werner syndrome, inborn errors in the DNA repair machinery have dramatic clinical consequences, including tissue specific cancer predisposition and/or segmental progeria. Aging-related phenotypes in mouse models with genome maintenance defects as well as the most commonly described human segmental progeroid syndromes were reviewed by Hasty et al.

Normal aging might be caused in part by inadequately repaired and thus accumulating DNA damage. Properly functioning DNA maintenance pathways will reduce damage, promote repair, or optimize the cellular response to DNA damage to prolong healthy life and delay aging. Actually, telomere shortening as discussed earlier can also be considered as a form of DNA damage, which is not restored in somatic cells. DNA damage can result in HSC exhaustion as was recently suggested by Prasher et al. Ercc1 (a protein essential in nucleotide excision repair) deficient mice were used to examine the effects of DNA repair on the hematopoietic system. Ercc1 mutant mice have decreased responses to hematopoietic stress and showed exhaustion of hematopoietic progenitor activity, suggesting premature senescence of the HSC and progenitor cell compartment.

DNA damage can be induced by oxidative damage, resulting from free radical production. Numerous recent discoveries on both extension of lifespan as well as premature aging in model organisms from yeast to mice consistently support a connection between oxidative metabolism, stress resistance and aging. The aging process may therefore be influenced by energy restriction.
through a reduction in the metabolic ‘rate of living’, ultimately leading to reduced oxidative damage. Increasing life span by prolonged caloric restriction has been demonstrated in yeast, worms, flies, fish, mice and rats\textsuperscript{66}. A large number of long-lived mutants that have been identified in \textit{C. elegans} and \textit{Drosophila} resulted from increased resistance to oxidative stress\textsuperscript{67-69}. Additional evidence for involvement of oxidative damage in regulating cellular life span comes from studies that showed that the proliferative lifespan of in vitro cultured cells is extended when these are grown in low ambient oxygen, 2-3\%, a condition more closely resembling physiologic oxygen levels\textsuperscript{70,71}. This increased life span is attributed to a decrease level of reactive oxygen species (ROS), resulting in reduced oxidative stress. Conversely, increase of intracellular ROS levels through hydrogen peroxide treatment or through the inhibition of ROS scavenging enzymes, such as superoxide dismutase Sod1, causes premature senescence\textsuperscript{72}. Lifelong dietary restriction in mice resulted in increased HSC frequencies and improved HSC functional abilities, strongly suggesting delayed hematopoietic senescence and prevention of HSC aging\textsuperscript{73}. Furthermore, studies in mice deficient for the ‘ataxia telangiectasia mutated’ (\textit{Atm}) gene showed that the self-renewal capacity of HSCs depends on \textit{Atm}-mediated inhibition of oxidative stress. \textit{Atm}-deficient mice showed progressive BM failure resulting from a defect in HSC function that was associated with elevated ROS. Treatment with anti-oxidative agents restored the reconstitutive capacity of \textit{Atm}-deficient HSC, resulting in prevention of bone marrow failure\textsuperscript{74}.

**Protein damage**

Proteins are the basis of all cellular functions such as signal transduction, mitosis, cellular transport systems, chaperone activity, etc., and as such it is conceivable that an age-related increase in oxidative damage to proteins could have important physiological consequences to an organism. Proteins can be modified by multiple reactions involving ROS. Among these reactions, carbonylation has attracted a great deal of attention, due to its irreversible and unrepairable nature. It appears that the classical enzymes involved in ROS detoxification, that is, superoxide dismutases, catalases, and peroxidases, are key members of the cellular defense against protein carbonylation\textsuperscript{75}. Oxidative carbonylation has been identified as one important factor in protein function and removal\textsuperscript{76-78}. A large number of studies have shown that protein carbonylation increases with age\textsuperscript{79,80}. Normally, carbonylated proteins are marked for proteolysis by the proteasome and specific proteases. However, carbonylated proteins are able to escape degradation and form high molecular weight aggregates that accumulate with age. Such carbonylated aggregates can become cytotoxic and have been associated with a large number of age-related disorders, including Alzheimer’s disease, Parkinson’s disease, and cancer\textsuperscript{78,81}. The asymmetrically dividing yeast \textit{S. cerevisiae} has evolved a Sir2p-dependent system that specifically retains carbonylated proteins in the mother
cell compartment during mitotic cytokinesis. Thus, the new progeny which, in contrast to the mother cell, exhibits a full reproductive potential and starts out with a markedly reduced load of damage. In addition, recent studies on asymmetric division in *Drosophila* show involvement of these processes in the stem cell niche. It will be of interest to clarify whether segregation of damaged proteins is a phenomenon that can also be observed in higher eukaryotes. Specifically, it would be highly interesting to assess its role during stem cell self-renewal or generation of the germ cell line.

### Cellular memory

During development of multicellular organisms, cells become different from one another by distinct use of their genetic program in response to transient stimuli, an example being lineage specification in hematopoiesis. Long after such a stimulus has disappeared, cellular memory mechanisms still enable cells to “remember” their chosen fate over many cell divisions. This implies that in order to grow and maintain a specific, lineage-restricted state, particular configurations of gene expression need to be transmitted to daughter cells. Such heritable programs that do not involve mutations of the DNA are referred to as epigenetic alterations. The chromatin structure and its modifications play a fundamental role in establishment and maintenance of epigenetically controlled developmental decisions.

Nucleosomes, the fundamental structure units of chromatin, are comprised of the core histone octamer (H2A, H2B, H3, and H4) and the associated DNA that wraps around these eight histones. Although the crystal structure of a nucleosome core particle has provided considerable insight into the protein-protein and protein-DNA interactions that govern nucleosome structure, little is known about how distinct functional domains of chromatin are established and maintained. The precise organization of chromatin is critical for many cellular processes, including transcription, replication, repair, recombination and chromosome segregation. Dynamic changes in chromatin structure are directly influenced by post-translational modifications of the amino-terminal tails of histones. The packaging of eukaryotic DNA into nucleosomal arrays presents a major obstacle to transcription that must be dealt with in order for the transcriptional machinery to access the DNA template. The discovery of enzyme complexes dedicated to chromatin remodeling, whether by directly modifying histone proteins or by ATP-dependent nucleosomal remodeling complexes, has led to new insights into the mechanism of transcription. Gene expression is determined not only by the availability of combinations of transcription factors, but also by chromatin context. Eukaryotic genomes are often conveniently described as transcriptionally active (euchromatin) or transcriptionally silent (heterochromatin). The existing chromatin structure must be properly
passaged (propagated during DNA replication) to daughter cells to provide cells with cellular memory. During this process nucleosomes are recognized by proteins that recruit histone deacetylases and histone H3-K9 methyltransferases to the adjacent, newly deposited histones. Furthermore, there appear to be differences in the timing of DNA replication at active and silent genes, and differences in the compartmentalization of active and silent genes within the nucleus\textsuperscript{93,94}.

Two antagonizing groups of proteins, Polycomb (PcG) and trithorax (trxG), are required to maintain gene expression patterns of important developmental regulators during cellular proliferation. During development TrxG proteins are transcriptional activators, whereas PcG proteins are transcriptional repressors, and both are very well conserved during evolution in different species. The PcG and TrxG proteins therefore appear to form the molecular basis of cellular memory. The maintenance of cellular memory involves dynamic, regulated interactions between the PcG and TrxG proteins and their many target genes, via Polycomb response elements (PREs)\textsuperscript{84}. Is there any evidence that chromatin remodeling involving PcG or TrxG genes indeed confers memory to stem cells?

**Cellular Memory and Stem Cells**

There are two distinct PcG complexes (PcG repressive complex (PRC) 1 and 2) that associate with chromatin, but the core complexes do not contain proteins that bind DNA in a sequence specific way. Therefore, they must be recruited to specific genes by other mechanisms. Currently, it is thought that PRC2 is initially recruited to DNA sequences that contain PREs. As PRC2 binds to PREs, acetyl groups are removed by histone deacetylases (HDACs), and lysine 27 of histone H3 will be methylated. This creates a binding site for the chromodomain of Polycomb proteins, and subsequent recruitment of PRC1. These PREs are believed to be located within or in the vicinity of gene(s) to be silenced\textsuperscript{95}.

The role of selected PcG proteins in stem cell self-renewal has recently been established. Mel-18 negatively regulates self-renewal of HSCs since its loss leads to an increase of HSCs in G\textsubscript{0}, and to enhanced HSC self-renewal\textsuperscript{96}. Mph1/Rae-28 mutant mice are embryonic lethal as HSC activity in these animals is not sufficient to maintain hematopoiesis during embryonic development\textsuperscript{97}. Bmi-1\textsuperscript{-/-} HSCs derived from fetal liver were not able to contribute to long-term hematopoiesis in competitive repopulation experiments, demonstrating cell autonomous impairment of their self-renewal potential\textsuperscript{98}. In contrast, overexpression of Bmi-1 extends replicative life span of mouse and human fibroblasts and causes lymphomas in transgenic mice\textsuperscript{99}. Heterozygosity for a null allele of Eed caused marked myelo- and lymphoproliferative defects, indicating a negative regulation of cell cycle activity of both lymphoid and myeloid progenitor cells\textsuperscript{100}. Overexpression of Ezh2 in HSCs preserves stem cell potential and prevents HSC exhaustion after serial transplantation.
TrxG proteins form complexes that are involved in general transcriptional processes and therefore their function is not limited to epigenetic maintenance\textsuperscript{101,102}. Four complexes that contain TrxG proteins have been purified from \textit{Drosophila} embryos, all with different chromatin-modifying properties\textsuperscript{103}. Recent data from \textit{Drosophila} studies indicate that TrxG proteins such as Trx and Ash1, rather than being general transcriptional co-activators, specifically function to prevent inappropriate gene silencing mediated by the PcG of transcriptional repressors\textsuperscript{104}. Inappropriately expressed TrxG genes seem to be involved in tumor formation\textsuperscript{105}. For example, the mixed lineage leukemia gene (\textit{MLL}) is involved in 11q23 translocations in acute leukemias\textsuperscript{106-108}. \textit{MLL} was recently shown to be a histone 3 Lysine 4-specific methyltransferase\textsuperscript{109,110}. Studies with \textit{Mll} mutant mice demonstrated an intrinsic requirement for \textit{Mll} in definite hematopoiesis, where it is essential for the generation of HSCs during embryogenesis\textsuperscript{111}.

Pharmacological agents are available that are able to interfere with DNA methylation and histone deacetylation, such as 5aza 2’deoxycytidine (5azaD) and trichostatin A (TSA). 5azaD is a DNA hypomethylating agent, whereas TSA acts as a histone deacetylase inhibitor (HDACI). Cultures of purified human HSCs (CD34\textsuperscript{+}) together with growth factors that induced differentiation normally result in rapid loss of primitive phenotypic properties and repopulation potential. However, when CD34\textsuperscript{+} cells were cultured with both 5azaD and TSA, expansion of primitive phenotypic properties and maintenance of repopulating ability was observed\textsuperscript{112}. These data strongly suggest that modulation of the methylation and acetylation patterns by pharmacological drugs can alter the fate of primitive HSCs. This also suggests that upon differentiation of HSCs, the chromatin structure must change, resulting in different gene expression patterns. Interestingly, when neural stem cells (neurospheres) were treated with 5azaD and TSA and transplanted in lethally irradiated mice, this yielded long-term multilineage and transplantable neurosphere-derived hematopoietic cells, whereas untreated neurospheres did not show any hematopoietic reconstitution\textsuperscript{113}.

Naturally occurring microRNAs (miRNAs) also constitute a powerful route to dynamically silence specific gene expression, so it is conceivable that such mechanisms may induce silencing initiation prior to the heterochromatization process that is mediated by histone methyltransferase-mediated lysine 9 and 27 histone H3 methylation\textsuperscript{114}. This suggests that RNA interference (RNAi) can also function in establishing or maintaining the epigenetic control of gene expression, which is essential for mammalian development. Mutations in the RNAi machinery cause abnormal chromosomal segregation as a result of disrupting heterochromatin\textsuperscript{115}. It has been suggested that miRNAs might function in regulating development and therefore also play a role during cellular aging\textsuperscript{116}. miRNAs that are specifically expressed in hematopoietic cells have already been identified. These miRNA
presumably act by pairing to the mRNAs of their target genes to direct gene silencing processes that are critical for hematopoiesis and maybe also for hematopoietic stem cell self-renewal\textsuperscript{117}.

**Cellular Memory and Aging of Stem Cells**

How can this ‘cellular memory’ contribute to aging? It was already discussed that PcG proteins influence stem cell self-renewal potential. Recently it was suggested that SIR2, a histone deacetylase associates with components of the PRC2 in *Drosophila*\textsuperscript{118}. Furthermore, it has also been suggested earlier that SIR2 is an important player in yeast lifespan by regulating the localization of carbonylated proteins during cell division. In *S. cerevisiae* SIR2 was originally identified for its silencing activity through the creation of specialized chromatin domains. SIR2 influences lifespan in several organisms, such as yeast, *Drosophila*, and *C. elegans*. Whereas SIR2 mutants have a shorter lifespan, overexpression of SIR2 leads to longevity. The mammalian homolog of SIR2, Sirt1, is an NAD-dependent deacetylase\textsuperscript{119} and appears to target many proteins, including p53 and forkhead proteins, which are not histones, resulting in a higher threshold for apoptosis\textsuperscript{120}. Knocking out Sirt1 in mice causes embryonic or postnatal death due to severe developmental problems\textsuperscript{121,122}. Since it has been suggested that repressive functions of SIR2 (by histone deacetylation) are dependent on the PcG genes, and it is known that SIR2 is involved in regulating life span it becomes important to test what effect SIR2 has on expression levels of the PcG proteins, which are known for their involvement in stem cell self-renewal.

It has been suggested previously that during normal aging heterochromatin structure changes\textsuperscript{123,124}. As heterochromatin domains must be regenerated epigenetically each time DNA is repaired or replicated, DNA damage and cell division may be the major perturbing factors triggering heterochromatin loss. Loss of heterochromatin was therefore suggested to reflect the number of cell divisions, or cycles of DNA damage and repair, resulting in multiple subtle changes in gene expression\textsuperscript{124}. For example, age-related gene reactivation on the silenced X chromosome has been observed for several loci\textsuperscript{125,126}. The heterochromatin island hypothesis postulates that repressive chromatin structures are scattered over the genome, reflecting the diverse genomic structures in individuals within a species or among various species. For instance, even though brain cells and hematopoietic cells contain the same DNA sequences, due to transcriptional regulation these cells have their own specialized functions and specific characteristics. This model assumes that dynamic changes in the equilibrium in heterochromatin islands, rather than their simple unfolding or loss is the essential driving force of cellular aging\textsuperscript{123}. Others suggest a general open chromatin structure in stem cells, with many options still present.
(a “promiscuous” beginning). Upon aging or differentiation an increase of heterochromatin can be expected, concomitant with a decrease of multilineage potential. Using gene arrays it has been shown that in HSCs more transcripts are present than in committed progenitors. In addition, transcripts common to both HSCs and neural stem cells were identified, raising the possibility of extended commonality in the molecular ground states of HSCs and neural stem cells.

It is clear that these epigenetic marks (heritable changes in gene function, without changes in DNA sequence) are set throughout embryogenesis and adult life, and that this is an important mechanism to guide proper gene transcription. However, it remains unclear to what extent heterochromatin structure changes during differentiation and aging. Even though epigenetic marks are relatively rigid and stable, we hypothesize that during aging the histone code in stem cells gradually is altered, ultimately resulting in impaired functioning. The different hypotheses for changes in heterochromatin structure during differentiation, i.e. loss of heterochromatin; gain of heterochromatin; re-localization of heterochromatin (Figure 1.1), are probably also processes that are important during stem cell aging. According to the first hypothesis, aging might result from a general loss of heterochromatin, as a consequence of which non-stem cell genes might be transcribed. A second model suggests that in stem cells many transcriptional options are available and during cellular aging these options are gradually shut down. A third model suggests re-localization of the heterochromatin structures, also resulting in different gene expression profiles. Whether and how the heterochromatin structure will alter during stem cell aging will be a new field to explore. We argue that changes in the heterochromatin structure might lead to infidelity of gene transcription, resulting in expression of non-stem cell genes. As a consequence stem cells gradually loose their self-renewal capacity during aging.
Figure 1.1. Possible mechanisms of changes in heterochromatin structure during stem cell aging. Euchromatin is shown as thin black lines. Changes in heterochromatin (black boxes) distribution must be apparent during aging. Because of these changes distinct genes (gray bars) will be transcribed in young and old stem cells, some of which are not necessarily stem cell genes. This loss of control over gene expression profiles could lead to aberrant gene expression. Stem cell aging might result from loss of heterochromatin, a gain of heterochromatin, or re-localization of heterochromatin structures, all of which will result in perturbed gene expression profiles.
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