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REVIEW

Epigenetic regulation of aging stem cells

EA Pollina[1,2] and A Brunet[1,2]

[1]Department of Genetics, Stanford University, Stanford, CA, USA and [2]Cancer Biology Program, Stanford University, Stanford, CA, USA

The function of adult tissue-specific stem cells declines with age, which may contribute to the physiological decline in tissue homeostasis and the increased risk of neoplasm during aging. Old stem cells can be 'rejuvenated' by environmental stimuli in some cases, raising the possibility that a subset of age-dependent stem cell changes is regulated by reversible mechanisms. Epigenetic regulators are good candidates for such mechanisms, as they provide a versatile checkpoint to mediate plastic changes in gene expression and have recently been found to control organismal longevity. Here, we review the importance of chromatin regulation in adult stem cell compartments. We particularly focus on the roles of chromatin-modifying complexes and transcription factors that directly impact chromatin in aging stem cells. Understanding the regulation of chromatin states in adult stem cells is likely to have important implications for identifying avenues to maintain the homeostatic balance between sustained function and neoplastic transformation of aging stem cells.
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Adult stem cells in tissue maintenance

Aging is characterized by a progressive decline in the physiology and function of adult tissues. In addition to changes in the biology of postmitotic cells, aspects of mammalian tissue aging may be attributable to a loss of regenerative capacity of adult stem cells. Unlike differentiated cells, adult tissue-specific stem cells retain at least a portion of the plasticity of their embryonic counterparts: adult stem cells can both self-renew and differentiate into at least one other cell type within a committed lineage. Specialized stem cell niches comprised of differentiated cells, blood vessels and extracellular matrix support adult stem cells and provide survival, differentiation and/or self-renewal cues (Wagers *et al*., 2002; Morrison and Spradling, 2008; Voog and Jones, 2010). Adult stem cell niches have now been identified in most adult tissues in mammals, including the highly regenerative blood (Morrison and Weissman, 1994, Morrison *et al*., 1995), intestine (Barker *et al*., 2007), skin (Blanpain *et al*., 2007) and mammary gland (Visvader, 2009), as well as the less regenerative skeletal and cardiac muscle (Beltrami *et al*., 2003; Morgan and Partridge, 2003; Rando, 2005) and brain (Lois and Alvarez-Buylla, 1993; Morshead *et al*., 1994; Palmer *et al*., 1997; Gage *et al*., 1998; Doetsch *et al*., 1999).

Adult tissue stem cells play important roles in overall tissue homeostasis and repair in response to injury. The contribution of adult stem cells to tissue maintenance depends on the properties of the tissue itself. Tissues with continuous high turnover, such as the blood and gut, rely heavily on robust stem cell pools (Morrison *et al*., 1995; Rando, 2006; van der Flier and Clevers, 2009). For example, stem cells present in the bone marrow are the source for continuously replenished erythrocytes, platelets and leukocytes in the blood of humans and rodents (Spangrude *et al*., 1988; Baum *et al*., 1992; Osawa *et al*., 1996; Uchida *et al*., 1998; Michallet *et al*., 2000; Shizuru *et al*., 2005). Intestinal stem cells are the primary source of new epithelial cells in intestinal crypts of humans and mice, and these crypts undergo complete turnover in 4–5 days (van der Flier and Clevers, 2009). Similarly, stem cells of mammary gland contribute to cyclic bouts of tissue regeneration during specific phases of the ovarian cycle and pregnancy in humans and mice (Kordon and Smith, 1998; Dontu *et al*., 2003; Hennighausen and Robinson, 2005; Liu *et al*., 2006; Shackleton *et al*., 2006; Stingl *et al*., 2006; Blanpain *et al*., 2007; Ginestier *et al*., 2007).

In tissues with notably less cell turnover, adult stem cells play important roles in response to environmental stimuli. For example, muscle stem cells (satellite cells) are required for the regeneration of myofibers following injury or transplantation in humans and mice (Schultz *et al*., 1978; Zammit *et al*., 2002; Morgan and Partridge, 2003; Conboy and Rando, 2005; Sacco *et al*., 2008; Corbu *et al*., 2010). Even for tissues with low turnover and regenerative capacity in response to injury, such as the brain, stem cells may play important roles in the adaptive nature of the tissue. In the adult rodent brain, neural stem cells (NSCs) reside in two main niches, the subventricular zone (SVZ) and the dentate

Correspondence: Dr A Brunet, Department of Genetics, Stanford University, 300 Pasteur Drive, Alway M336, Stanford, CA 94305, USA.
E-mail: anne.brunet@stanford.edu
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gyrus of the hippocampus (Doetsch *et al*., 1999; Anthony *et al*., 2004; Zhao *et al*., 2008). NSCs in the hippocampus give rise to new granule layer neurons that integrate into functional neuronal circuits (Kaplan and Bell 1984; Kempermann *et al*., 1998a; Gould *et al*., 1999; Song *et al*., 2002; Lagace *et al*., 2007) and are critical for such cognitive functions as learning and memory formation (Shors *et al*., 2001; Kee *et al*., 2007; Imayoshi *et al*., 2008; Zhang *et al*., 2008; Clelland *et al*., 2009). Deep layer neurons of the olfactory bulb are continuously replaced by neurons generated from the NSCs in the SVZ and play essential roles in odor discrimination and odor memory (Gheusi *et al*., 2000; Enwere *et al*., 2004; Lagace *et al*., 2007; Breton-Provencher *et al*., 2009). In humans, adult neural progenitors have been identified in the dentate gyrus and lateral ventricles (Eriksson *et al*., 1998; Kukekov *et al*., 1999; Roy *et al*., 2000; Sanai *et al*., 2004; Curtis *et al*., 2007), although their role in cognition has not been established. Adult stem cells are thus critical for tissue regeneration, response to injury and tissue plasticity in adult mammals. In this review, we discuss the biology of aging stem cells in mammals, with particular focus on hematopoietic and neural stem cells as key examples of adult stem cells in tissues with vastly different regenerative properties.

Stem cells and aging

Over the course of organismal lifespan, adult stem cells face the challenge of maintaining an undifferentiated, yet committed, state that is primed to respond to the environment. Fully functional adult stem cells must remain cells with options. In the process of differentiation, adult stem cells have the option to adopt one of typically several different cell fates. In the process of self-renewal, stem cells can divide symmetrically to rapidly produce more cells or asymmetrically to maintain a population of multipotent stem cells and produce differentiated cells. For example, under basal conditions, most stem and progenitor divisions are asymmetric in the adult rodent brain (Morshead *et al*., 1998). In contrast, in response to stroke or seizure, there is evidence for an increase of symmetric divisions that represents a key stem cell response to injury (Parent *et al*., 1997; Zhang *et al*., 2004; Lugert *et al*., 2010). Throughout life, adult stem cells are subject to the environmental stresses and intracellular damages that accompany the aging process. A fundamental question is whether stem cells progressively lose their potential to self-renew and properly differentiate during organismal aging, and if so, whether these defects are entirely irreversible.

Defects in number in aging stem cells

The number of adult stem cells is affected by aging, although the directionality of this change is variable. In some tissues (for example, blood), stem cells have been reported to increase in number with age, whereas in other tissues (for example, brain and muscle) stem cells display an age-dependent decrease in number. Even within the same tissue, studies addressing stem cell number have reported different results. The underlying reasons for conflicting reports are still unclear, but may relate to differences in the experimental assays used to define, isolate and quantify stem cells, as well as differences in genetic background. Indeed, how age influences the number of hematopoietic stem cells (HSCs) in mice has been subject to much debate. Early studies comparing HSC number in short- and long-lived mouse strains using *in vitro* cobblestone-forming assays reported an age-dependent decrease in HSC number in short-lived mouse strains (CH3/He, CBA/J and DBA/2), but an increase in HSC number in the long-lived C57BL/6 mouse strain (de Haan *et al*., 1997; de Haan and Van Zant, 1999). Likewise, quantification of HSCs by fluorescence-activated cell sorting (FACS) using cell surface markers indicate that the frequency of cells expressing stem and progenitor markers (KLS; c-Kit$^+$, Lin$^-$, Sca1$^+$) increases with age in C57BL/6 mice (Sudo *et al*., 2000; Kim *et al*., 2003; Rossi *et al*., 2005; Pearce *et al*., 2007). The observed increase in HSCs with age is due to specific expansion of the HSC compartment capable of long-term reconstitution of the hematopoietic system in a recipient mouse, termed LT-HSCs (Rossi *et al*., 2005; Chambers *et al*., 2007; Pearce *et al*., 2007). Notably, the majority of LT-HSCs are relatively quiescent and do not undergo major changes in cell cycle status with age, suggesting that HSC expansion is not caused by substantial changes in HSC cycling (Cheshier *et al*., 1999; Sudo *et al*., 2000; Chambers *et al*., 2007; Rossi *et al*., 2007b). Although FACS-based analysis and *in vitro* assays might characterize different populations of stem cells—perhaps more committed progenitors in the case of *in vitro* assays—these data raise the possibility that the genetic background of mouse strains influences the proliferative control of HSCs and their progeny during aging (de Haan *et al*., 1997; de Haan and Van Zant, 1999). In humans, age-dependent changes in hematopoietic stem and progenitor cell number also differ depending on the study. Early reports examining changes to whole bone marrow (Ogawa *et al*., 2000) or a heterogeneous CD34$^+$ cell population (Waterstrat *et al*., 2008) indicated a decrease in progenitor cell frequency. However, the most recent studies of more pure cell fractions report higher numbers of primitive stem and progenitor cells with age (Taraldsrud *et al*., 2009; Beerman *et al*., 2010b). Thus, although there is currently no strong consensus in the field, studies using increasingly homogenous populations suggest an age-dependent increase in HSCs in humans and some mouse strains.

In the brain, age-dependent changes in the number of NSCs appear to be region-specific and the directionality of the change also remains controversial. Studies examining NSC number both by long-term label retention assays as well as by staining for putative stem/progenitor markers indicate decreases in the neural stem/progenitor pools in the adult SVZ of rodents (Maslov *et al*., 2004; Molofsky *et al*., 2006; Ahlenius

et al., 2009). In contrast, in the adult hippocampus of rats, the numbers of neural stem and progenitors, defined by expression of markers SOX2 and GFAP, have been reported to remain relatively constant with age (Hattiangady and Shetty, 2008). However, recent studies in mice using more specific stem cell markers, such as HES5, report a slight decrease in hippocampal NSC numbers with age (Lugert *et al*., 2010). These differences may be due, in part, to the technical challenge of defining a pure population of NSCs, which is distinguishable from the population of progenitor progeny. As knowledge of specific markers for distinct subpopulations of stem and progenitor cells in adult neurogenic regions increases (Pastrana *et al*., 2009; Beckervordersandforth *et al*., 2010), such discrepancies will likely be resolved. Whether there are age-dependent changes in adult neural stem and progenitor cells in human brains has not yet been investigated.

Different age-related changes to stem cell number have also been reported in other tissue-specific stem cells. In human muscle, *in vivo* studies quantifying numbers of muscle satellite cells by immunostaining for panels of markers indicate modest decreases in number (Renault *et al*., 2002; Carlson *et al*., 2009). Studies in rodents, however, show opposite results, with increased numbers of muscle satellite cells during aging in rats (Gibson and Schultz, 1983) and either a decrease (Bockhold *et al*., 1998) or no significant difference in aging mice (Conboy *et al*., 2003). Changes in muscle satellite cell number with age may depend on the species and genetic background, the type of muscle assessed and the age at which the measurements are carried out. Finally, in the hair follicle, age-dependent hair graying is associated with depleted numbers of melanocyte stem cells in humans, as assessed by decreased immunostaining for melanocyte markers in aged human scalp samples (Nishimura *et al*., 2005). Alterations in the numbers of stem cells during aging suggest that change to self-renewal programs is a common characteristic of aging in stem cells, although the mechanisms of deregulation may be species- and tissue-specific.

Functional decline in aging stem cells

Despite disputed differences between tissues with regard to changes in the numbers of stem cells with age, the decline in stem cell function—including the ability to repopulate a tissue after injury, the ability to proliferate in response to external stimuli and the ability to differentiate into multiple cell types—is shared among all adult stem cell compartments.

The functional decline of hematopoietic stem and progenitors cells with age has been well documented. HSCs isolated from older mice show defects in mobilization and homing to bone marrow (Morrison *et al*., 1996; Kim *et al*., 2003; Liang *et al*., 2005; Xing *et al*., 2006). In competitive transplantation assays, a rigorous test for both self-renewal and multipotency during which donor HSCs are co-injected with wild-type bone marrow and assessed for their ability to regenerate all blood lineages, aged HSCs shows defect in long-term reconstitution of the immune system (Sudo *et al*., 2000; Kamminga *et al*., 2005; Rossi *et al*., 2005; Chambers *et al*., 2007). Aged HSCs also give rise to more cells of myeloid lineage at the expense of lymphoid fates (Sudo *et al*., 2000; Kim *et al*., 2003; Rossi *et al*., 2005; Cho *et al*., 2008; Guerrettaz *et al*., 2008). This myeloid bias in aging HSC populations has been explained by alterations in the subcomposition of the HSC pool, which is thought to contain clones with pre-determined differentiation bias (Dykstra *et al*., 2007; Cho *et al*., 2008; Beerman *et al*., 2010a; Morita *et al*., 2010). The frequency of myeloid-biased HSC clones, distinguished by their high expression of marker SLAMF1/CD150 (Beerman *et al*., 2010a; Challen *et al*., 2010; Morita *et al*., 2010), increases with age, although the mechanism of this expansion is not yet fully understood. Myeloid-biased cells do not appear to cycle more rapidly than clones giving rise to balanced numbers of lymphoid and myeloid cells (Beerman *et al*., 2010a). However, modest increased cycling of lymphoid-biased clones relative to myeloid-biased clones has been reported (Challen *et al*., 2010). Aged clones also maintain their surface phenotype and differentiation bias during serial transplantation (Cho *et al*., 2008; Beerman *et al*., 2010a; Challen *et al*., 2010), suggesting that conversion between clonal types does not contribute to age-dependent fluctuations in clonal subtype. However, the enhanced expansion of myeloid-biased clones with age in the DB/2 strain compared with C57BL/6 indicates a genetic component for clonal regulation (Cho *et al*., 2008). Notably, although HSC subclones maintain many of their properties, defects of both myeloid and balanced HSC clones in competitive transplantation assays suggest that there may also be functional alterations within clonal subtypes during aging (Beerman *et al*., 2010a).

In the nervous system, the ability of NSCs to produce new neurons (neurogenesis) declines with age (Kuhn *et al*., 1996; Tropepe *et al*., 1997; Bondolfi *et al*., 2004; Enwere *et al*., 2004; Hattiangady and Shetty, 2008). Instead, aging is accompanied by increased production of astrocytes and elevated expression of astrocyte-specific genes in the brain, indicating a loss of multipotentiality of stem/progenitor cells and astroglial lineage skewing (Peinado *et al*., 1998; Lee *et al*., 2000; Bondolfi *et al*., 2004). On the basis of findings in the HSC field, it is tempting to speculate that age-dependent changes to the subcomposition of a potentially heterogeneous pool of NSCs (Merkle *et al*., 2007) may partially account for alterations in NSC differentiation potential.

During aging in rodents and humans, muscle satellite cells also display impaired activation following insults, resulting in decreased muscle regeneration after injury or exercise (Conboy *et al*., 2003; Carlson and Conboy, 2007; Carlson *et al*., 2008, 2009). Muscle satellite cells isolated from aged rats produce fewer progeny when propagated *in vitro*, suggesting impaired proliferative capacity outside the niche (Schultz and Lipton, 1982). Similarly, the increased tendency of satellite cells to convert from myogenic to fibroblastic lineages in aged

muscles contributes to enhanced muscle fibrosis with age (Brack *et al*., 2007). Thus, although aging may influence stem cell number in a variety of ways, it is accompanied by a striking decrease in stem cell function in all tissue types.

Mechanisms underlying adult stem cell decline

Given the decline in stem cell function with age, an important question regards the mechanisms underlying adult stem cell homeostasis. While this review will focus on stem cell aging in mammals, it is important to note that elegant studies in invertebrates have highlighted the changes in stem cell and niche during aging, and the mechanisms underlying some of these changes (Arantes-Oliveira *et al*., 2002; Boyle *et al*., 2007; Jones, 2007; Biteau *et al*., 2008). In mammals, age-related changes to stem cells and their niches may be broadly grouped into two classes: those that are irreversible versus reversible in nature (Figure 1). Irreversible damages to aging stem cells include intrinsic changes, such as accumulated nuclear and mitochondrial DNA damage and telomere shortening, and have been extensively reviewed elsewhere (Rudolph *et al*., 1999; Sharpless and DePinho, 2007; Rossi *et al*., 2008; Song *et al*., 2009; Sahin and Depinho, 2010). In contrast, other changes during aging, such as systemic and local signaling changes, may be reversible. The extent to which irreversible versus reversible cell-intrinsic or environmental factors contribute to stem cell aging is likely to be tissue dependent. For example, age-related changes to HSCs are thought to be primarily cell intrinsic. Indeed, transplantation of HSCs from old donors into a young microenvironment does not reverse the age-dependent effects on increased HSC number or myeloid bias (Rossi *et al*., 2005; Pearce *et al*., 2007). The accumulation of DNA damage markers in aging HSCs and defects in HSC function in mice mutant for certain DNA repair pathways suggest that irreversible DNA damage may be a key contributor to HSC aging (Nijnik *et al*., 2007; Rossi *et al*., 2007a). However, HSC clonal subtypes are differentially responsive to a key factor involved in paracrine signaling, transforming growth factor-β1 (TGF-β1). TGF-β1 stimulates myeloid-biased HSC clones while inhibiting lymphoid-biased clones (Challen *et al*., 2010), indicating that mechanisms of clonal expansion may also be environmentally controlled.

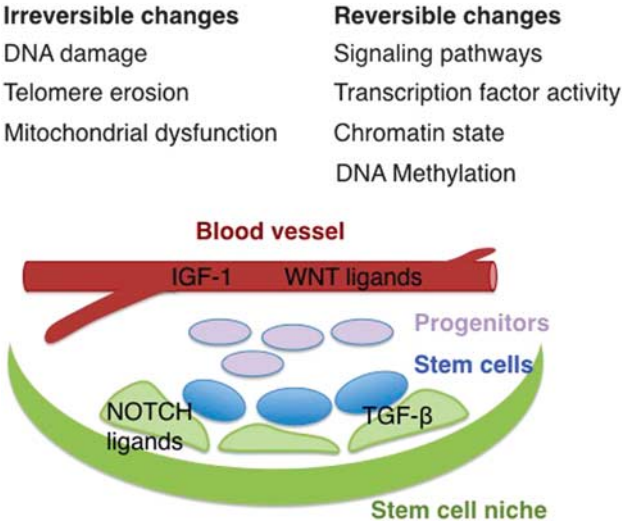

Figure 1 Mechanisms of stem cell aging. Stem cell aging is likely due to a combination of intrinsic (irreversible) and extrinsic (reversible) changes. This review focuses on the reversible changes and how they can be integrated in stem cells. Systemic circulating factors or factors secreted by the local stem cell niche can affect stem cell function in a reversible manner by influencing signal-transduction pathways, chromatin states and transcription factor function.

The function of other adult stem cells, such as muscle or brain, is strongly influenced by environmental factors during aging, suggesting that age-dependent changes to stem cells may be reversible in nature in these tissues. The reversibility of age-related defects in muscle satellite cells have been elegantly shown by heterochronic parabiosis experiments, in which the circulatory system of old and young animals is joined (Conboy *et al*., 2005; Brack *et al*., 2007). Muscle satellite cells isolated from aging mice or humans fail to upregulate the NOTCH ligand DELTA and have increased signaling through the TGF-β pathway, both of which lead to impaired stem cell activation and defective proliferation following injury (Conboy *et al*., 2003; Carlson *et al*., 2008, 2009). However, circulating factors from the blood of young mice can restore NOTCH signaling and proliferative potential in satellite cells from old mice (Conboy *et al*., 2005). Proliferation defects of old human satellite cells can also be partially restored when cultured in the presence of NOTCH inhibitors (Carlson *et al*., 2009). Consistently, systemic increases in WNT signaling owing to constitutive loss of the WNT antagonist KLOTHO leads to increased senescence in stem cells from intestinal crypts and depletions in the pools of adult HSCs and epidermal stem cells (Liu *et al*., 2007). The consequences of altered WNT signaling on adult stem cells can be reversed; parabiosis or addition of WNT inhibitors to satellite cells from old mice revert the age-dependent myogenic to fibroblastic conversion (Brack *et al*., 2007).

Other systemic factors that can reversibly regulate stem cell function during aging include cytokines and stress hormones. For example, reducing circulating levels of corticosteroids in adult rats by adrenalectomy has been shown to restore neural progenitor proliferation and neurogenesis (Cameron and McKay, 1999) and increasing the declining levels of insulin growth factor 1 (IGF-1) in aged rodents promotes adult neurogenesis and stem-cell-dependent muscle regeneration (Lichtenwalner *et al*., 2001; Musaro *et al*., 2004). In further support of reversible alterations to stem cell function in some tissues, changes in external stimuli, such as exposure to an enriched social environment (Kempermann *et al*., 1998b, 2002) or physical exercise (Kronenberg *et al*., 2006; Lugert *et al*., 2010), improve age-related declines in NSC proliferation and neurogenesis. Taken together,

these observations suggest that a variety of environmental signals impact intracellular mechanisms that can restore the potential of some adult stem cell types during organismal aging.

Epigenetic changes: a pivotal mechanism for stem cell regulation during aging?

Given evidence for stem cell rejuvenation, it is interesting to consider that epigenetic changes within adult stem cells in response to environmental cues are important to regulate stem cell function. 'Epigenetics,' in the strict definition of the term, is the study of phenotypic or gene expression patterns heritable through cell division that are independent of DNA sequence (Berger *et al*., 2009). Epigenetics has also been defined more broadly as the dynamic regulation of gene expression by sequence-independent mechanisms, including changes in DNA methylation and histone modifications (Jaenisch and Bird, 2003; Vaquero *et al*., 2003; Ma *et al*., 2010). In this review, we discuss epigenetic regulation of adult stem cells in the broad sense of the term, with specific focus on the regulation of chromatin state by chromatin-modifying complexes and transcription factors that interact with chromatin. We propose that control of chromatin state is a pivotal means by which stem cells integrate environmental stimuli to trigger appropriate cell fates. Changes to chromatin are thought to be reversible and are thus ideally situated to be molecular effectors of stem cell rejuvenation (Figure 2). The fact that chromatin changes can themselves be mitotically inherited (Grewal and Klar, 1996; Cavalli and Paro, 1998; Martin and Zhang, 2007) raises the possibility that an additional level of regulation in aging stem cells is the heritability of environmentally induced chromatin changes in parent stem cells to daughter cells.

This review will focus primarily on changes in histone modifications. However, we also note that regulators of DNA methylation are required for the function of a variety of adult stem cells (Zhao *et al*., 2003; Ma *et al*., 2009; Trowbridge *et al*., 2009; Sen *et al*., 2010; Trowbridge and Orkin, 2010; Wu *et al*., 2010), and that they complex with chromatin modifiers to elicit changes in chromatin state (Jones *et al*., 1998; Nan *et al*., 1998; Fuks *et al*., 2003). In addition, chromatin remodeling factors are also important for stem and progenitor cell function (Lessard *et al*., 2007; Ho *et al*., 2009; Ho and Crabtree, 2010), suggesting that several epigenetic mechanisms could coordinately control adult stem cell gene expression programs during organismal aging.

Chromatin modifiers in aging stem cells

Well-regulated maintenance of chromatin—and thereby access to genes controlling self-renewal, differentiation, cellular metabolism, DNA damage repair and response to oxidative stress—is likely to be critical for the sustained potential of adult stem cells. Gene expression studies in populations of aging stem cells show alterations to stem cell transcriptomes with age, although whether such changes are causal for stem cell decline has not yet been established. In one study of aging HSCs (identified by the side population method together with the surface phenotype c-Kit$^+$, Lin$^-$, Sca1$^+$ (SP-KLS); Goodell *et al*., 1996), genes identified as age-regulated were shown to map to physical clusters in the genome, indicating that stem cell aging is associated with global changes in genome structure and accessibility (Chambers *et al*., 2007). However, another study of gene expression using more purified HSC populations (identified by surface markers for LT-HSCs: c-Kit$^+$, Lin$^-$, Sca1$^+$, Flk2$^-$, CD34$^-$) revealed far fewer changes to the HSC transcriptome with age (Rossi *et al*., 2005). Likely, many age-dependent expression changes occur in more committed progenitors. Nevertheless, both studies identify age-dependent alterations in the expression of select modifiers of chromatin state (Rossi *et al*., 2005; Chambers *et al*., 2007). Interestingly, chromatin modifiers have recently been found to control longevity in model organisms such as yeast, flies and worms (Li *et al*., 2008; Chen *et al*., 2009; Dang *et al*., 2009; Greer *et al*., 2010; Siebold *et al*., 2010). In addition, chromatin modifiers have been implicated in the control of a number of cellular processes that may contribute to longevity, including DNA damage repair, telomere maintenance and cellular metabolism (Vidanes *et al*., 2005; Longo and Kennedy, 2006; Blasco, 2007). The role for chromatin modifiers in organismal longevity and adult stem cells underscores the importance of chromatin maintenance in sustaining cellular and tissue integrity throughout life. Alterations in expression, activity or interaction between molecules that program

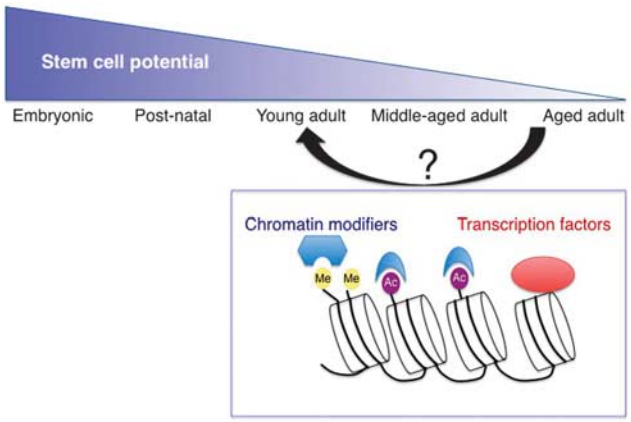

Figure 2 Stem cell potential declines with age. During aging, tissue-specific stem cells lose their potential to regenerate tissues after damage because of decreased proliferation and differentiation potential. An important question is whether reversible chromatin changes could underlie this decline in tissue-specific stem cells. Chromatin modifiers and transcription factors may play an important role in restoring the regenerative capacity of old stem cells. Me: methylation of lysine residues on histones; Ac: acetylation of lysine residues on histones.

chromatin states are likely to contribute to observed declines in adult stem cell potential with organismal age.

Polycomb and trithorax histone methyltransferase complexes in aging stem cells

Polycomb group (PcG) and trithorax group (TrxG) complexes, which direct methylation of specific lysine residues on histones, have antagonistic functions during development (Buszczak and Spradling, 2006). TrxG complexes catalyze methylation of the activating mark tri-methyl lysine 4 of histone H3 (H3K4me3), which promotes gene expression. PcG proteins control levels of the repressive mark tri-methyl lysine 27 of histone H3 (H3K27me3), which inhibits gene expression (Ringrose and Paro, 2007). Members of both complexes have been implicated in organismal longevity (Greer *et al*., 2010; Siebold *et al*., 2010) and adult stem cell regulation (Molofsky *et al*., 2003; Park *et al*., 2003; Jude *et al*., 2007; McMahon *et al*., 2007; Lim *et al*., 2009), indicating that interplay between PcG and TrxG complexes may mediate transcriptional regulation of genes critical for adult stem cell function throughout an organism's lifespan.

The PcG protein BMI1 regulates adult stem cell self-renewal. The best-characterized chromatin regulator of adult stem cells is BMI1, a structural member of the polycomb repressive complex 1 (PRC1). PRC1 has been reported to bind to the repressive H3K27me3 mark and act both as a recruitment factor for polycomb repressive complex 2 (PRC2) (Rastelli *et al*., 1993) and as an E3 ligase catalyzing monoubiquitination of the repressive histone H2A lysine 119 (Wang *et al*., 2004). BMI1 has emerged an age-dependent regulator of adult hematopoietic and neural systems. *Bmi1*-deficient mice die pre-maturely with signs of growth retardation, neurological abnormalities manifested by extreme ataxia and progressive decline of the hematopoietic system, manifested by hypoplasia of bone marrow and defects in lymphoid and myeloid lineages (van der Lugt *et al*., 1994; Park *et al*., 2003). Constitutive deletion of *Bmi1* does not affect numbers of fetal liver HSCs, but does result in a decrease in the frequency of HSCs isolated in young adult mice (van der Lugt *et al*., 1994; Park *et al*., 2003). However, BMI1 is necessary for maintaining the proliferative potential of both embryonic and adult stem cells, as *Bmi1*-deficient HSCs isolated from embryonic livers (Lessard and Sauvageau, 2003; Park *et al*., 2003) and young adult mice (Oguro *et al*., 2006) show reduced long-term repopulating activity in competitive transplantation assays. These results suggest that BMI1 is critical for the self-renewal of HSCs, but that other PcG genes are likely to compensate for *Bmi1* loss to promote initial specification of an HSC pool during development.

In the nervous system, BMI1 is also required for the self-renewal of adult NSCs. Both constitutive deletion and acute knockdown of *Bmi1* result in impaired self-renewal of cultured NSCs isolated from young adult mice (Molofsky *et al*., 2003; Fasano *et al*., 2007). The effect of *Bmi1* knockdown on NSCs is exacerbated if NSCs are isolated from adult as opposed to embryonic and postnatal mice (Fasano *et al*., 2007). *In vivo*, *Bmi1* deficiency causes a decrease in the numbers of proliferating, bromodeoxyuridine-positive SVZ cells (neural progenitors) without affecting apoptosis (Molofsky *et al*., 2003; Zencak *et al*., 2005). Although the effects of a conditional *Bmi1* deletion in adult NSCs have not yet been reported, cell-autonomous loss of *Bmi1* is likely to cause self-renewal defects that are amplified by aging.

In addition to modulating the self-renewal of stem cells, BMI1 regulates stem cell differentiation potential in both HSCs and NSCs. Loss of *Bmi1* does not block the differentiation of more committed hematopoietic progenitors (Jacobs *et al*., 1999; Iwama *et al*., 2004), but affects the ability of stem and early progenitors to retain all cell fate choices. In culture, HSCs from young adult *Bmi1*-deficient mice have reduced multi-lineage potential compared with wild-type HSCs when assessed at early passage (Iwama *et al*., 2004). *Bmi1*'s effects on HSC differentiation have been linked to its effects on chromatin state. In a mixed population of HSCs and multipotent progenitors (IL7Rα^-/KLS), BMI1 binds at genomic loci that are marked by both repressive H3K27me3 and active H3K4me3 (Oguro *et al*., 2010), a 'bivalent' chromatin state associated with genes that are poised to be expressed during differentiation (Bernstein *et al*., 2006). Constitutive loss of *Bmi1* in the HSC/multipotent progenitor population results in a reduction in H3K27me3 binding, de-repression of B-cell lineage factors and consequent increase in B-lymphopoiesis (Oguro *et al*., 2010). Thus, BMI1 is a promising candidate for the regulation of HSC differentiation potential during aging. However, the importance of BMI1 in establishing and maintaining the age-dependent bias of myeloid versus lymphoid HSC clonal subtypes is currently unknown.

On the basis of its role in HSCs, BMI1 may be a key mediator for the resolution of repressed bivalent loci during differentiation of other adult stem cell types. Consistently, BMI1 also controls the differentiation potential of adult NSCs (Zencak *et al*., 2005; Bruggeman *et al*., 2007). In young adult mice, constitutive deletion of *Bmi1* triggers increased glial cell production *in vivo* (Zencak *et al*., 2005) and decreased neurogenic capacity of cultured adult NSCs after serial passaging (Bruggeman *et al*., 2007). This increase in astrocytes phenocopies the increase in astrocyte production known to occur in the brain during aging (Bondolfi *et al*., 2004), raising the possibility that altered activity of BMI1 during aging contributes to a loss of stem cell multipotency.

BMI1 controls stem cells via the key 'aging locus' $p16^{INK4a}/p19^{ARF}$. BMI1 function in young adult HSC and NSC self-renewal is mediated, in large part, through its transcriptional repression of the $p16^{INK4a}/p19^{ARF}$ aging locus (Jacobs *et al*., 1999). $p16^{INK4a}$ inhibits CYCLIN-D/CDK4/6 complexes to control cell cycle and senescence, whereas $p19^{ARF}$ contributes to cell cycle control, senescence and apoptosis through the

regulation of p53 (Lowe and Sherr, 2003). Notably, $p16^{INK4a}$ and $p19^{ARF}$ expression increase with age in a variety of tissues in rodents and humans (Zindy *et al*., 1997; Krishnamurthy *et al*., 2004), including adult stem cell niches. Expression of $p16^{INK4a}$ has been shown to increase in uncultured pools of adult SVZ cells (Molofsky *et al*., 2006; Nishino *et al*., 2008) and LT-HSCs isolated from old mice (Janzen *et al*., 2006). However, expression studies in single cells with the HSC immunophenotype Lin^-, $IL7R\alpha^-$, $Slamf1^+$ have revealed that $p16^{INK4a}$ upregulation with aging may be a rare event occurring in only a few cells of an isolated population of HSCs (Attema *et al*., 2009). The reasons for these conflicting reports may be due to differences in the immunophenotype of reported HSCs, slight differences in the ages and to the technologies used to assess expression level (single-cell versus conventional population-based reverse transcriptase followed by quantitative PCR).

Despite discrepancies regarding $p16^{INK4a}$ regulation during physiological aging of some stem cell types, $p16^{INK4a}$ has an inhibitory role on stem cell function. Constitutive deletion of $p16^{INK4a}$ increases the numbers of long- and short-term HSCs isolated from the bone marrow of old, but not young mice (Janzen *et al*., 2006). Under conditions of transplant-induced stress, loss of $p16^{INK4a}$ improves serial repopulating ability, proliferation and resistance to apoptosis to a greater degree in old as opposed to younger animals (Janzen *et al*., 2006). Similarly, $p16^{INK4a}$ deficiency rescues age-dependent defects in multipotent neurosphere formation and self-renewal *in vitro* (Molofsky *et al*., 2006). Although de-repression of $p16^{INK4a}$ or $p19^{ARF}$ has not yet been shown to cause stem cell senescence *in vivo*, repression of the $p16^{INK4a}/p19^{ARF}$ locus by BMI1 is likely critical for sustained adult stem cell self-renewal.

Genetic experiments suggest that in HSCs, $p16^{INK4a}$ is the dominant mediator of BMI1's effects on stem cell proliferation. Deletion of the entire $p16^{INK4a}/p19^{ARF}$ locus, but not that of $p19^{ARF}$ alone, can mostly rescue the effect of *Bmi1* deficiency on HSC self-renewal in long-term competitive repopulation assays (Oguro *et al*., 2006). $p19^{ARF}$ may be a more critical target in adult NSCs, as $p19^{ARF}$ deletion partially rescues self-renewal defects caused by *Bmi1* deficiency, although to a lesser extent than deletion of the entire $p16^{INK4a}/p19^{ARF}$ locus (Bruggeman *et al*., 2005; Molofsky *et al*., 2005). In contrast to chronic *Bmi1* loss, acute RNA interference-mediated knockdown of *Bmi1* in NSC cultures from young adult mice does not lead to an increase in $p16^{INK4a}$ or $p19^{ARF}$ expression, but results in altered expression of another cell cycle inhibitor, $p21^{CIP1}$, which can rescue the antiproliferative phenotype of *Bmi1* knockdown (Fasano *et al*., 2007). Thus, acute loss of *Bmi1* is likely not sufficient to induce rapid changes to the chromatin state of the $p16^{INK4a}/p19^{ARF}$ locus, whereas constitutive deletion of *Bmi1* may result in gradually accumulating and stably maintained activating chromatin marks, such as H3K4me3 or histone acetylation, at the $p16^{INK4a}/p19^{ARF}$ locus (Figure 3). Whether BMI1 directly binds to the $p16^{INK4a}/p19^{ARF}$ genomic locus has not yet been shown in adult NSC populations. However, in mouse and human fibroblasts, direct binding of BMI1 to the $p16^{INK4a}/p19^{ARF}$ promoter triggers an increase in the repressive mark H3K27me3 at this locus (Bracken *et al*., 2007), suggesting that BMI1 may affect changes in chromatin state in a similar manner in adult NSCs (Bracken *et al*., 2007).

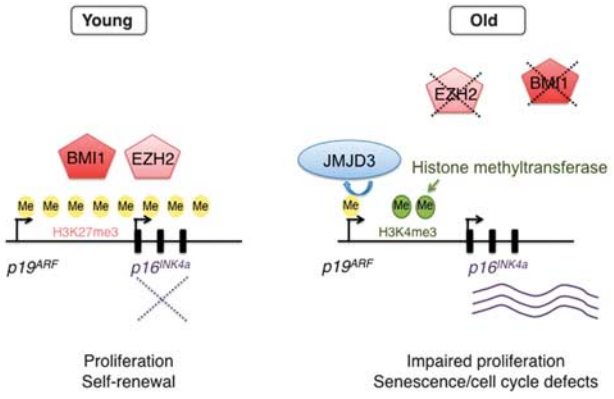

Figure 3 A potential model for epigenetic regulation of the *Ink4a/Arf* locus in aging neural stem cells. In young neural stem cells, the $p16^{INK4a}/p19^{ARF}$ locus is marked by repressive H3K27me3, which is deposited and maintained by Polycomb complex members EZH2 and BMI1. The $p16^{INK4a}/p19^{ARF}$ locus is transcriptionally repressed, allowing young stem cell self-renewal. By contrast, in old stem cells, the activity and/or levels of BMI1 and EZH2 decrease and the levels of the H3K27me3 demethylases such as JMJD3 increase, leading to the loss of this repressive mark. The $p16^{INK4a}/p19^{ARF}$ locus is de-repressed, leading to cell cycle arrest and senescence of aged stem cells. Me: methylation of lysine residues on histones; yellow circles: H3K27me3; green circles: H3K4me3.

Additional targets of Bmi1 in aging stem cells. The expression of *Bmi1* itself does not change significantly in isolated HSC and NSC populations during aging (Janzen *et al*., 2006; Molofsky *et al*., 2006). By contrast, BMI1's role in maintaining self-renewal and multipotency notably declines during aging, arguing for altered activity of BMI1 at yet unidentified targets. Indeed, growing evidence suggests additional age-related targets for BMI1 in addition to $p16^{INK4a}/p19^{ARF}$. Overexpression of *Bmi1* in HSCs isolated from $p19^{ARF}$ mutant mice and $p16^{INK4a}/p19^{ARF}$ compound mutant mice can still enhance multipotency of HSCs *in vitro* (Iwama *et al*., 2004; Oguro *et al*., 2006). Furthermore, BMI1 plays a non-cell autonomous role in the bone marrow microenvironment that does not depend on $p16^{INK4a}$ or $p19^{ARF}$ (Oguro *et al*., 2006). Similarly, deletion of the entire $p16^{INK4a}/p19^{ARF}$ locus in $Bmi1^{-/-}$ mice does not completely rescue NSC defects in self-renewal capacity (Bruggeman *et al*., 2005; Molofsky *et al*., 2005).

The $p16^{INK4a}/p19^{ARF}$-independent requirement for BMI1 in adult stem cell populations may be due to BMI1's ability to regulate the DNA damage response pathway via repression of the cell cycle checkpoint

protein CHK2 (Liu *et al*., 2009). Deletion of *Chk2* in *Bmi1*$^{-/-}$ mice restores hematopoietic stem and progenitor cell function and enhances progenitor cell proliferation (Liu *et al*., 2009). Whether altered BMI1 function during aging may partly explain the observed accumulation of DNA damage markers in aging stem cell populations (Rossi *et al*., 2007a) is not yet known, but BMI1 may provide an important molecular link between chromatin regulation and response to DNA damage. Interestingly, loss of *Chk2* also rescues the premature aging phenotype of *Bmi1*$^{-/-}$ mice and thereby links improved progenitor cell function with overall extended organismal lifespan (Liu *et al*., 2009). These findings suggest that modulators of chromatin state, such as BMI1, are pivotal for maintaining the ability of adult stem cells to integrate and respond to environmental stresses during aging.

The PcG protein EZH2 in aging stem cells. Another PcG gene implicated in adult stem cell function during aging is *enhancer of zeste 2* (*Ezh2*), which comprises the methyltransferase activity of PRC2 (Kuzmichev *et al*., 2002; Muller *et al*., 2002; Ketel *et al*., 2005). In the hematopoietic system, *Ezh2* does not appear to be required for stem and early progenitor cell function under normal steady-state conditions, as deletion of *Ezh2* in all adult hematopoietic lineages causes a block in B-cell lineage production, but not in other lineages (Su *et al*., 2003). However, lentiviral-mediated overexpression of *Ezh2* in transplanted bone marrow of young mice enhances long-term repopulating capacity of HSCs upon serial transplantation (Kamminga *et al*., 2006). Compensation by the related protein EZH1 may allow normal function of *Ezh2*-deficient HSCs under basal conditions. By contrast, transplantation, which requires the rapid expansion and sustained self-renewal of HSCs, reveals the dependency of HSCs on *Ezh2*. Notably, *Ezh2* expression is decreased in LT-HSCs isolated from aged as opposed to young adult mice (Rossi *et al*., 2005; Attema *et al*., 2009). The decrease in *Ezh2* expression in HSC does not seem to elicit changes in the histone marks H3K4me3 and H3K27me3 on $p16^{INK4a}/p19^{ARF}$ locus in a subset of HSCs (Attema *et al*., 2009). Nevertheless, *Ezh2* downregulation in mouse and human fibroblasts leads to decreased binding of BMI1 and other PcG members at the $p16^{INK4a}/p19^{ARF}$ locus in response to stress stimuli or senescence (Bracken *et al*., 2007). EZH2 may thus orchestrate chromatin control at additional repressed targets in HSCs and other adult stem cell types.

BMI1 and EZH2 are the main PcG components implicated in aging stem cell function thus far. However, other members of the PcG complexes have been shown to control stem cell properties, such as differentiation potential, during development. For example, both RNA interference-mediated knockdown of PRC2 component *Eed* in culture and conditional loss of the PRC1 component *Ring1B* in the nervous system result in increased production of neurons at the expense of astrocytes in developing neocortical progenitors (Hirabayashi *et al*., 2009). Although similar studies in adult NSCs have not yet been performed, misregulation of *Eed* and *Ring1B* in adults may contribute to deregulation of the neurogenic competence of adult NSCs with age. Given recent progress in understanding the role and interaction of PcG components, it will be especially interesting to examine the interaction of PRC1 and PRC2 in a variety of aging adult stem cell types.

PcG proteins and DNA methylation in control of stem cell differentiation. PcG proteins also regulate adult stem cells through their interaction with DNA methyl modifiers. In mice, deletion of the DNA methyltransferase 3a (*Dnmt3a*) causes a defect in neuronal differentiation of postnatal NSCs. Genome-wide mapping of DNMT3A binding and H3K27me3 in NSCs reveals that loss of DNMT3A binding results in an increase in H3K27me3 levels and a decrease in the expression of genes critical for promoting neuronal differentiation (Wu *et al*., 2010). Moreover, the DNA-methylating activity of DNMT3A is required for the inhibition of PcG binding at neuronal genes (Wu *et al*., 2010). Interestingly, EZH2 has been shown to recruit DNA methyltransferases to promote *de novo* DNA methylation (Vire *et al*., 2006), suggesting that EZH2 may be critical for promoting neuronal differentiation of NSCs by initiating the attenuation of its own repression of differentiation-specific genes. These findings illustrate the versatility of PcG-mediated mechanisms in adult stem cell control and underscore the importance of sustained PcG function in adult stem cells with age.

The TrxG protein MLL1 in adult stem cell regulation. TrxG proteins promote transcriptional activation through deposition and maintenance of the activating chromatin mark H3K4me3. In *Caenorhabditis elegans* (*C. elegans*), mutations in TrxG complex members extend organismal longevity in a manner dependent on the germline (Greer *et al*., 2010). The germline contains the only stem-like cells of the postmitotic nematode (Morgan *et al*., 2010), raising the intriguing possibility that chromatin regulation by TrxG in stem cells is critical for controlling age-related phenotypes. TrxG proteins also regulate the potential of adult stem cells in mammals. One notable example is *mixed-lineage leukemia-1* (*Mll1*), whose function is critical for adult HSC and NSC function. Conditional deletion of *Mll1* in hematopoietic lineages using either a *Vav-Cre* or an inducible *Mx1-Cre* model causes a depletion of HSCs and common lineage progenitors due to an increase in the number of cycling HSCs and depletion of quiescent HSC reserves (Jude *et al*., 2007; McMahon *et al*., 2007). *Mll1*-deficient HSCs cannot self-renew and fail to contribute to immune reconstitution in transplantation assays (Jude *et al*., 2007; McMahon *et al*., 2007). Loss of *Mll1* is not compensated for by expression of close paralogs *Mll2* and *Mll3* in stem and early progenitors, suggesting an essential role for *Mll1*-containing complexes in uncommitted cells (Jude *et al*., 2007). The mechanism for MLL1's specific effects on proliferation

of stem and early progenitor cells has not yet been determined, but MLL1 likely methylates distinct target genes in stem versus more differentiated cells. It is surprising to consider that loss of the TrxG gene *Mll1* as well as loss of PcG genes show similar—rather than opposite—defects on stem cell self-renewal, which argues that appropriate regulation of active versus repressive histone methylation and/or a careful balance of these proteins is required for control of proliferation in adult HSCs. Perturbation of this balance during the aging process may account for altered numbers of HSCs.

In contrast to HSCs, deletion of *Mll1* in adult NSCs does not affect self-renewal, but rather alters multilineage potential of NSCs. Conditional deletion of *Mll1* in brain astrocytes and NSCs using the GFAP-Cre promoter results in a defect in neurogenesis, but not gliogenesis, *in vivo* and *in vitro* and is linked to altered transcription of the proneural gene *Dlx2* (Lim *et al*., 2009). Loss of *Mll1* in differentiating NSCs in culture does not affect levels of the H3K4me3 mark, but does cause an increase in H3K27me3 levels on the *Dlx2* promoter and thereby generation of a bivalent H3K4me3/H3K27me3 region that correlates with low levels of transcription (Lim *et al*., 2009). These results suggest that MLL1 functions to recruit H3K27me3 demethylases necessary to remove repressive histone marks on pro-neural promoters, or that in the absence of MLL1, H3K27me3 methyltransferases are more active at certain promoters. The role for *Mll1* specifically in neural generation raises the possibility that MLL1 activity on its targets may contribute to the observed decrease in neurogenesis with age. A global examination of MLL1 targets in young adult and aging NSC populations will be useful for elucidating the mechanism of this specific phenotype. Owing to the premature death of *Mll1* brain-specific knockout mice (Lim *et al*., 2009), the activity of MLL1 in aging stem cell populations has not yet been explored. Interestingly, in the human cortex, H3K4me3 levels increase at a subset of promoters during aging (Cheung *et al*., 2010). Although not specifically a stem cell phenomenon, this observation suggests that changes to the activity of MLL1 and other methyltransferases controlling active chromatin regions in stem cells may lead to deregulated gene expression with age.

Histone demethylases as regulators of reversible chromatin state

Histone demethylases can reverse the effects of methyltransferases by removing methyl groups at specific lysine residues on histones (Shi, 2007), but their role in adult stem cells is still relatively unexplored. However, a few key examples highlight potentially critical functions for these molecules in stem cell maintenance during aging and illustrate the dynamic nature of chromatin changes. As a primary example, the H3K27me3 histone lysine demethylase JMJD3 functions to mediate stem cell differentiation potential. In undifferentiated human keratinocytes, a form of unipotent adult stem cell, JMJD3 is required to remove repressive H3K27me3 marks at pro-differentiation genes (Sen *et al*., 2008). Consistently, overexpression of *JMJD3* drives premature keratinocyte differentiation (Sen *et al*., 2008). Similarly, in the developing nervous system of mice, JMJD3 promotes differentiation upon induction of retinoic acid signaling, and its suppression is required for the maintenance of an uncommitted stem cell state (Jepsen *et al*., 2007). The role for JMJD3 in adult NSCs has not yet been determined, but altered JMJD3 activity in aging NSC populations may promote loss of a stem cell state by inappropriate initiation of differentiation programs or by de-repression of the $p16^{Ink4a}/p19^{Arf}$ locus, as has been reported in fibroblasts (Agger *et al*., 2009; Barradas *et al*., 2009). Alternatively, failure of aged stem cells to induce *Jmjd3* may render NSCs less responsive to differentiation signals and thereby contribute to decreased neurogenic potential of stem/progenitors during aging. A second example is the H3K4me1 and H3K4me2 histone lysine demethylase LSD1, which has been reported to control the proliferation of adult NSCs *in vitro* and *in vivo* (Sun *et al*., 2010). Chemical inhibition of LSD1 or acute RNA interference-mediated knockdown of LSD1 in the adult hippocampus causes decreases in neural stem and progenitor proliferation (Sun *et al*., 2010). Further examination of histone lysine demethylase activity, and the interplay between methyltransferases and demethylases, is likely to reveal novel mechanisms of stem cell plasticity through reversible regulation of chromatin states (Figure 3).

Histone acetylation in aging stem cells

Histone acetylation, which is associated with a transcriptionally permissive state, is controlled by the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs are diverse in their structures and substrates and are often multisubunit complexes (Lee and Workman, 2007). HDACs can be divided into several classes based on sequence homology and cofactor dependency: class I, II and IV HDACs are classical HDACs requiring Zn^{2+} as a cofactor, whereas class III HDACs, also known as Sirtuins, require NAD^{+} as a cofactor (Imai *et al*., 2000; Haigis and Guarente, 2006; Yang and Seto, 2007). Control of histone acetylation is associated with longevity regulation in lower organisms (Kaeberlein *et al*., 1999; Tissenbaum and Guarente, 2001; Rogina and Helfand, 2004; Wood *et al*., 2004; Dang *et al*., 2009), and changes in histone acetylation in tissues, such as the brain and liver, correlate with age-dependent declines in tissue function (Oh and Conard, 1972; Shen *et al*., 2008; Kawakami *et al*., 2009; Peleg *et al*., 2010). However, the importance of histone acetylation in aging stem cells is less well studied. This section will highlight recently discovered roles for histone-acetyl modifiers in stem cell compartments and speculate on their contribution to adult stem cell function during the process of organismal aging.

HATs: CBP/p300 and MYSTS in adult stem cells. Transcriptional co-regulator CREB binding protein (CBP) and its paralog p300 are HATs with numerous roles in development and tumorigenesis (Ogryzko *et al.*, 1996; Goodman and Smolik, 2000). In the hematopoietic system, CBP has been reported to regulate stem and progenitor cell proliferation and differentiation during aging. Mice with monoallelic loss of CBP have defects in bone marrow cellularity and hematopoietic lineage differentiation and display an age-dependent increase in hematopoietic malignancies (Kung *et al.*, 2000). HSC numbers, as estimated by whole bone marrow transplantation assays, are reported to decrease in old $Cbp^{+/-}$ mice relative to young mice (Rebel *et al.*, 2002). Although detailed analysis of purified stem versus progenitor populations has not been performed, these studies suggest that CBP may be necessary for proper HSC self-renewal during aging. Members of the MYST family of HATs have likewise been implicated in tissue-specific stem cell function. For example, MYST3/MOZ is necessary for self-renewal of embryonic HSCs, as shown by decreased numbers of stem and early progenitors and decreased long-term repopulating capacity of HSCs in *Moz* mutants (Katsumoto *et al.*, 2006; Thomas *et al.*, 2006; Perez-Campo *et al.*, 2009). The role of MOZ in adult HSCs has not yet been determined due to embryonic lethality of *Moz*-deficient mouse mutants, but MOZ is likely to play a similar role in adult HSC self-renewal. In NSCs, MYST4/QUERKOPF has a role in regulating neurogenic potential and proliferation during development and in the adult SVZ (Thomas *et al.*, 2000; Rietze *et al.*, 2001; Merson *et al.*, 2006). Mice with a hypomorphic *Querkopf* allele show modestly reduced numbers of long-term label retaining cells in the adult SVZ, reduced proliferation of neurospheres in culture and impaired neuronal differentiation *in vitro* and *in vivo* leading to reduced numbers of olfactory interneurons in adults (Thomas *et al.*, 2000; Rietze *et al.*, 2001; Merson *et al.*, 2006). It will be informative to investigate the role of MYST4/QUERKOPF in controlling histone and protein acetylation in NSCs and to further explore if MYST4/QUERKOPF expression or targeting changes during aging.

Class I and II HDACs control stem cell proliferation and differentiation. Class I and II HDACs regulate adult stem and progenitor differentiation in both the adult hippocampus (Hsieh *et al.*, 2004) and the postnatal SVZ (Siebzehnrubl *et al.*, 2007). In hippocampal neural progenitors isolated from adult rats, global levels of histone acetylation on histone H3 and H4 decrease over the course of differentiation, with higher levels in neurons as opposed to astrocytes or oligodendrocytes (Hsieh *et al.*, 2004). Pharmacological inhibition of class I and II HDACs by valproic acid *in vivo* and in culture decreases proliferation of adult hippocampal NSCs, while promoting differentiation to a neuronal fate (Hsieh *et al.*, 2004). The role of specific HDACs is currently under investigation. Knockdown of *Hdac3*, *Hdac5* and *Hdac7* by RNA interference in adult NSC cultures reduces the proliferation of these cells (Sun *et al.*, 2007). In contrast, HDAC2 plays a more critical role during the transition from proliferative progenitor to differentiated neuron in the adult brain. Indeed, deletion of *Hdac2 in vivo* triggers increased proliferation and prolonged expression of NSC markers, such as SOX2, in maturing neurons during adult neurogenesis (Jawerka *et al.*, 2010). Differences in the functional phenotypes caused by the deficiencies of different HDACs emphasize the need for a more complete understanding of HDAC specificity in adult NSCs. Contrary to what is observed in NSCs, inhibition of HDAC activity by the addition of valproic acid increases proliferation of HSCs without affecting differentiation (Bug *et al.*, 2005), suggesting that the regulation and importance of histone acetylation is specific to stem cell types.

Role of class III HDACs (Sirtuins) in longevity and adult stem cells. Class III HDACs, also known as Sirtuins, regulate longevity in invertebrates and coordinate cellular responses to oxidative stress and DNA damage in mammals (Haigis and Guarente, 2006; Longo and Kennedy, 2006; Michan and Sinclair, 2007). Although limited studies have been performed on the relative function of each Sirtuin in adult stem cell compartments, these deacetylases may control some aspects of stem cell function through modulation of specific histone residues.

SIRT1, one of the seven mammalian Sirtuins, is the mammalian ortholog of yeast protein SIR2 (Rine *et al.*, 1979), which extends lifespan in yeast, worms and flies (Kaeberlein *et al.*, 1999; Tissenbaum and Guarente, 2001; Rogina and Helfand, 2004; Wood *et al.*, 2004). SIRT1 deacetylates histone H3 lysine 9 (H3K9ac) and histone H4 lysine 16 (H4K16ac) *in vitro* and in human cells (Imai *et al.*, 2000; Vaquero *et al.*, 2004). SIRT1 regulates HSC differentiation and self-renewal in the context of altered environmental conditions. Constitutive deletion of *Sirt1* does not alter numbers of HSCs or multipotent progenitors isolated from young adult mice under basal conditions (Narala *et al.*, 2008). However, loss of *Sirt1* confers a growth advantage in culture to HSCs under conditions of nutrient limitation (Narala *et al.*, 2008), suggesting that SIRT1 negatively regulates HSC self-renewal under some conditions. In a different mouse model for *Sirt1* constitutive deletion, SIRT1 promotes HSC self-renewal in low oxygen when assessed at early ages (Ou *et al.*, 2010). Defects in HSC self-renewal under both basal and low oxygen conditions are exacerbated in older mice compared with young adults (Ou *et al.*, 2010). The role of SIRT1 in adult NSCs has not yet been characterized, but SIRT1 plays a role in directing cell fate decisions of embryonic neural progenitors in response to different extracellular conditions (Hisahara *et al.*, 2008; Prozorovski *et al.*, 2008). In the presence of low amounts of growth factors, SIRT1 promotes neuronal differentiation in cultures of murine neural

progenitors, and acute deletion of *Sirt1* at embryonic stages results in fewer neurons *in vivo* (Hisahara *et al*., 2008). However, under conditions of mild oxidative stress, SIRT1 contributes to decreased production of neurons and increased astrocytes, an effect linked to SIRT1's effects on H3K9ac levels of proneural gene *Mash1*/*Ascl1* (Prozorovski *et al*., 2008). In the context of increased oxidative stress in the brain during aging or disease (Monje *et al*., 2003), misregulation of SIRT1 may contribute to the decrease in neurogenesis with age. In tissue-specific stem cells, SIRT1 may modulate chromatin state to coordinate stem cell responses to stress stimuli and DNA damage. Indeed, in murine embryonic stem cells, SIRT1 is important to maintain the genomic stability following DNA damage or oxidative stress (Oberdoerffer *et al*., 2008). Interestingly, a majority of SIRT1-bound genes that are deregulated in embryonic stem cells following oxidative damage also change with age in the adult neocortex, and the expression of these genes can be reverted upon *Sirt1* overexpression in the mouse brain (Oberdoerffer *et al*., 2008). Taken together, these studies reveal a critical role for SIRT1 in mediating stem cell response to environment, a role that may become more crucial with age.

SIRT6 is another promising candidate for the control of adult stem cell chromatin during aging. Constitutive deletion of *Sirt6* causes dramatically reduced lifespan in mice accompanied by features of premature aging (Mostoslavsky *et al*., 2006), implicating proper SIRT6 function in the prevention of tissue aging. In human fibroblasts, SIRT6 deacetylates H3K9ac at telomeric chromatin, and this function is critical for telomere maintenance and prevention of a senescent state (Michishita *et al*., 2008). Outside of telomeric regions, SIRT6 also deacetylates histones at the promoters of specific genes (Kawahara *et al*., 2009), raising the possibility that SIRT6 modulates age-related gene expression programs. *Sirt6* expression remains constant during aging in a variety of whole tissue extracts (Kawahara *et al*., 2009). However, decreased expression or altered activity of SIRT6, specifically in stem cell compartments, may impair stem cell response to stresses or signaling. The role of other Sirtuins in adult stem cells is still relatively unexplored. Expression of *Sirt2*, *Sirt3* and *Sirt7* was shown to decrease with age in the HSC compartment (SP-KLS) (Chambers *et al*., 2007), although not in highly purified LT-HSCs (Rossi *et al*., 2005), suggesting that changes in expression of these Sirtuins occur in more committed progenitors. Understanding the role of Sirtuins in aging stem cells will likely provide insights into the mechanisms that regulate stem cell homeostasis, particularly in response to stress stimuli. In addition, the ability of Sirtuins to regulate both organismal energy metabolism (Banks *et al*., 2008; Feige *et al*., 2008; Kim *et al*., 2010; Ramadori *et al*., 2010; Zhong *et al*., 2010) and chromatin may be particularly important for the response of adult stem cells to environmental stimuli such as dietary restriction that are known to promote longevity and delay signs of aging.

Chromatin structure in adult stem cells

The chromatin binding and structural protein high-mobility group protein A 2 (HMGA2) regulates adult NSCs during organismal aging (Nishino *et al*., 2008). NSCs isolated from the SVZ of *Hmga2*-deficient mice show defects in self-renewal *in vitro* and decreased proliferation in the SVZ *in vivo*, effects mediated through the $p16^{INK4a}/p19^{ARF}$ locus (Nishino *et al*., 2008). Decreased NSC proliferation in the adult SVZ with age may thus be due, in part, to the observed decrease in the expression of *Hmga2* in freshly isolated SVZ cells, which are enriched in neural stem and progenitors (Nishino *et al*., 2008). Although the role of HMGA2 in modulating chromatin structure in adult stem cells has not yet been addressed, studies in human fibroblasts suggest that HMGA2 alters chromatin compaction (Narita *et al*., 2006) or may complex with other structural chromatin-associated proteins (Sgarra *et al*., 2005, 2008). Likely, decreasing levels of *Hmga2* during aging (Nishino *et al*., 2008) results in altered chromatin compaction that either silences key stem cell proliferation genes or inappropriately activates genes, such $p16^{INK4a}/p19^{ARF}$, that limit stem cell function.

Transcriptional regulators and chromatin states in aging stem cells

Tissue-specific transcription factors control adult stem cell maintenance through regulation of stem cell differentiation, self-renewal and response to environmental stimuli and damage (Chambers *et al*., 2007; Dumble *et al*., 2007; Su *et al*., 2009; Chuikov *et al*., 2010). Altered activity of transcription factors in adult stem cell compartments is linked to premature aging phenotypes (Chambers *et al*., 2007; Dumble *et al*., 2007; Su *et al*., 2009). For example, conditional deletion of transcriptional regulator *Tap63* in epidermal stem and progenitors results in decreased lifespan, accompanied by stem cell senescence, skin atrophy and impaired wound healing (Su *et al*., 2009). Transcription factors also direct chromatin states by recruitment of chromatin-modifying complexes. Activities of specific transcription factors may thus be an additional layer of chromatin regulation in adult stem cells. Transcription factor/chromatin interactions also represent an important mechanism by which adult stem cells respond to signaling changes in the environment during aging (Figure 4). This section will highlight several examples of transcription factors associated with longevity signaling pathways and their chromatin interactions in adult stem cells, with attention to how such interactions may serve as environmental sensors.

FOXO transcription factors in adult stem cells: integrating insulin signaling with chromatin states?

The FOXO family of transcription factors plays conserved roles in organismal longevity and has recently emerged as a key regulator of adult stem cell pools. All FOXO isoforms (FOXO1, FOXO3, FOXO4 and

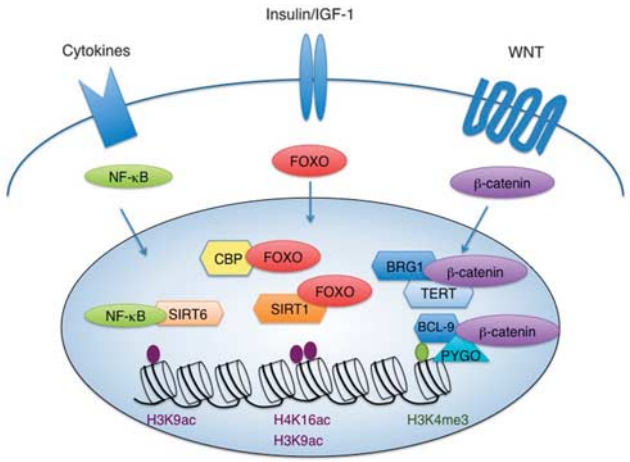

Figure 4 Signaling pathways and transcription factors involved in stem cell maintenance and their connection with chromatin modifiers. A number of signaling pathways regulate adult stem cells during aging, including cytokine, insulin/insulin growth factor 1 (IGF-1) and WNT pathways. Transcription factors downstream of each pathway have been found to interact with a number of chromatin regulators, including histone acetylases (for example, CBP), histone deacetylases (for example, SIRT1 and SIRT6), chromatin remodeling proteins (for example, BRG1) and histone methyl readers (for example, PYGO2 and BCL9).

FOXO6) respond to insulin/growth factor signaling and stress stimuli to coordinate various cell responses, including proliferation, cell cycle arrest, resistance to oxidative stress, cellular metabolism and differentiation (Greer and Brunet, 2005). In invertebrates, FOXO transcription factors are important for longevity downstream of insulin signaling (Lin *et al*., 1997; Ogg *et al*., 1997; Giannakou *et al*., 2004; Hwangbo *et al*., 2004). Interestingly, polymorphisms in the *FOXO3* gene are associated with extreme longevity in humans (Anselmi *et al*., 2009; Flachsbart *et al*., 2009; Li *et al*., 2009; Pawlikowska *et al*., 2009). In adult HSCs and NSCs, FOXO factors cooperate to regulate stem cell self-renewal and homeostasis. Conditional deletion of *FoxO1*, *FoxO3* and *FoxO4* in the adult hematopoietic system, as well as germline deletion of the single *FoxO3* gene, trigger a loss in long-term repopulating capacity of HSCs due to enhanced exit from quiescence, increased apoptosis and increased levels of reactive oxygen species (Miyamoto *et al*., 2007; Tothova and Gilliland 2007; Yalcin *et al*., 2008). FOXO transcription factors are also necessary for the maintenance of adult NSCs (Paik *et al*., 2009; Renault *et al*., 2009). Concomitant deletion of *FoxO1*, *FoxO3* and *FoxO4* in the brain leads to a depletion of the NSC pools in adult mice (Paik *et al*., 2009). Highlighting the importance of the FOXO3 isoform in NSCs, mice with germline- and brain-specific deletion of *FoxO3* alone show significant decreases in long-term label retaining cells, the proposed quiescent pool of NSCs (Renault *et al*., 2009). FOXO factors also function to control the neurogenic potential of NSCs (Paik *et al*., 2009; Renault *et al*., 2009). $FoxO3^{-/-}$ NSCs generate fewer multipotent neurospheres *in vitro*, with a specific defect in generation of neurons (Renault *et al*., 2009), whereas *FoxO1*/*FoxO3*/*FoxO4* compound mutant mice show decreased adult neurogenesis in the SVZ (Paik *et al*., 2009). Notably, proper function of FOXO factors appears to become more critical with age. $FoxO3^{-/-}$ HSCs show more significantly reduced long-term repopulation at middle age than at younger ages (Miyamoto *et al*., 2007). Sustained FOXO function is thus critical for maintaining stem cell sensitivity to the environment during aging.

How FOXO transcription factors connect with chromatin states to regulate adult HSCs and NSCs is not yet known, but evidence obtained in cultured cell systems highlights direct interactions between FOXO transcription factors and chromatin regulators (Figure 4). Under conditions of oxidative stress, the class III HDAC SIRT1 deacetylates and complexes with FOXO family members to increase cell quiescence and cellular stress resistance and to decrease apoptosis (Brunet *et al*., 2004; Daitoku *et al*., 2004; Motta *et al*., 2004; van der Horst *et al*., 2004; Frescas *et al*., 2005). In addition, FOXO factors have been shown to interact with the HAT CBP/p300 (Matsuzaki *et al*., 2005; van der Heide and Smidt 2005). Interaction with these chromatin regulators may thus alter histone acetylation, and as a consequence histone methylation, at key FOXO target genes in stem cells. Moreover, some FOXO family members may directly alter chromatin structure. FOXO1 is sufficient to decondense chromatin arrays *in vitro*, suggesting that FOXO1 can act directly as a 'pioneer factor' to facilitate opening of chromatin (Hatta and Cirillo, 2007). In response to changes in insulin signaling, FOXO transcription factors could facilitate access to essential genes enhancing stem cell self-renewal and differentiation by opening chromatin and/or increasing permissive histone acetylation. At the organismal level, FOXO factors may provide a critical link between signaling pathways that regulate overall energy metabolism and chromatin regulation, which could be particularly important in aging stem cells.

The pro-aging NF-κB transcription factors and histone acetylation

The nuclear factor-κB (NF-κB) family of transcription factors is activated by inflammatory cytokines to regulate targets involved in processes such as apoptosis, cellular senescence and immune function (Hayden and Ghosh, 2008). Genes that display age-related changes in expression are enriched for the presence of NF-κB binding sites in their regulatory region, and blocking NF-κB is sufficient to reverse aging phenotypes in the skin of mice (Adler *et al*., 2007). Like FOXO factors, NF-κB may integrate environmental stimuli with chromatin state changes and transcriptional responses in aging adult stem cells (Figure 4). In cultured cell lines, NF-κB member RELA (p65) physically interacts with and recruits the HDAC SIRT6 to target genes following stimulation by external signals such as oxidative stress or cytokines (Kawahara *et al*., 2009). Deletion of *Sirt6* promotes RELA binding to its targets and RELA-dependent apoptosis and senescence (Kawahara *et al*.,

2009). Whether SIRT6 and RELA interact *in vivo* in adult stem cell populations has not yet been determined. However, increased NF-κB signaling has been documented in aging HSCs and epithelial progenitor cells (Adler *et al*., 2007; Chambers *et al*., 2007), suggesting interactions between RELA, SIRT6 and chromatin acetylation may be altered over the course of stem cell aging.

Transcriptional regulators downstream of WNT signaling in chromatin regulation and stem cell function

Transcriptional regulators downstream of the WNT pathway, a major signaling pathway controlling stem and progenitor cell function across a variety of tissues (Reya and Clevers, 2005), interact with multiple chromatin-modifying complexes to alter target gene chromatin (Figure 4). β-catenin, the primary downstream target of canonical WNT signaling, has roles in promoting organismal longevity and regulating stem cell function (Essers *et al*., 2005; Reya and Clevers, 2005; Scheller *et al*., 2006). In *C. elegans*, deletion of β-catenin ortholog *bar-1* decreases organismal lifespan and oxidative stress resistance (Essers *et al*., 2005). The role of β-catenin in adult stem cell function may be mediated through its activity at chromatin, as β-catenin complexes with chromatin remodelers such as SMARCA4/BRG1 (Barker *et al*., 2001; Major *et al*., 2008) and HATs such as CBP (Hecht *et al*., 2000). In addition, the roles for transcriptional cofactors of β-catenin, BCL9/BCL9L and PYGO2, in adult stem cell proliferation have been associated with their function as readers of histone marks H3K4me2 and H3K4me3 (Fiedler *et al*., 2008; Chen *et al*., 2010). BCL9 and homolog BCL9L are necessary for adult stem cell-mediated regeneration of muscle and colon epithelium (Brack *et al*., 2009; Deka *et al*., 2010), whereas PYGO2 promotes self-renewal of mammary progenitor cells through regulation of cell cycle progression (Gu *et al*., 2009; Chen *et al*., 2010). In mammary progenitors, PYGO2 binds to H3K4me2 and H3K4me3 and recruits histone methyltransferases to catalyze this active mark at WNT target genes (Gu *et al*., 2009; Chen *et al*., 2010). In other adult stem cells, β-catenin complexes likely control active chromatin marks in response to WNT signaling.

The reverse transcriptase of the telomerase complex, TERT, has also recently been found to act downstream of the WNT pathway and to interact with chromatin modifiers to regulate stem cell function. Canonically, TERT and the RNA component of the telomerase enzyme, TERC, function to add telomeric repeats to the ends of chromosomes to prevent telomere loss during cell division (Rudolph *et al*., 1999). In mice, deletion of *Terc* results in decreased lifespan after several generations (Rudolph *et al*., 1999), implicating telomerase components as longevity factors. Interestingly, distinct from its action in telomere maintenance, TERT regulates the mobilization and proliferation of epidermal stem cells in the hair follicle (Flores *et al*., 2005; Sarin *et al*., 2005). Consistent with its prominent role in stem cell proliferation independent of its reverse transcriptase activity, TERT is a pivotal component of the transcriptional complex downstream of WNT signaling (Choi *et al*., 2008; Park *et al*., 2009). In gastrointestinal tract stem cells, TERT interacts with the chromatin remodeling protein BRG1 and with β-catenin to activate essential WNT target genes (Park *et al*., 2009). It is tempting to speculate that modulating the activity of transcriptional regulators downstream of the WNT signaling pathway could reverse some of the effects of age-dependent changes in WNT signaling (Liu *et al*., 2007) on internal stem cell state.

The nuclear receptor TLX recruits chromatin modifiers to regulate adult NSCs

The interaction of transcription factors and chromatin in adult stem cells is exemplified by the orphan nuclear receptor tailless (NRE21/TLX), which regulates self-renewal and differentiation of adult NSCs. *Tlx* is specifically expressed in the adult brain in both the adult hippocampus and SVZ (Monaghan *et al*., 1995; Roy *et al*., 2004; Shi *et al*., 2004) and may mark the relatively quiescent population of SVZ stem cells (Liu *et al*., 2008). NSCs isolated from young adult mice with a constitutive deletion of *Tlx* show defects in self-renewal *in vitro* and *in vivo* and spontaneous differentiation along the astrocytic lineage due to TLX-mediated repression of pro-astrocytic genes (Shi *et al*., 2004). Inducible deletion of *Tlx* in adults reduces adult neurogenesis (Liu *et al*., 2008; Zhang *et al*., 2008), thus underscoring a role for TLX in adult NSCs independent of its function in development. The role for TLX in self-renewal and differentiation of NSCs has been linked to its interactions with chromatin modifiers HDAC3, HDAC5 and histone demethylase LSD1 (Shi *et al*., 2004). In cultured neurospheres, TLX complexes with and recruits these modifiers to the promoters of the antiproliferative genes $p21^{CIP1}$ and *Pten*, thereby reducing histone acetylation and methylation levels and promoting NSC proliferation (Sun *et al*., 2007, 2010). Adult NSCs overexpressing *Tlx in vivo* show enhanced expression of *Bmi1* (Liu *et al*., 2010), suggesting that TLX may also impact chromatin state by altering expression of additional chromatin modifiers. Whether TLX is deregulated during aging is not yet known, but overexpression of *Tlx* in SVZ NSCs reduces the decline in stem cell proliferation in old mice (Liu *et al*., 2010). Although TLX is believed to be mostly a cell-intrinsic regulator, this transcription factor may integrate environmental signals to regulate chromatin states and stem cell function during aging.

Chromatin, aging stem cells and tumor development

Adult stem cells and their progeny may be the cells of origin for some cancers, notably age-dependent cancers. This observation underscores the crucial importance of keeping stem cell proliferation in check during aging. For example, in chronic myeloid leukemia, rare populations of self-renewing leukemia clones have

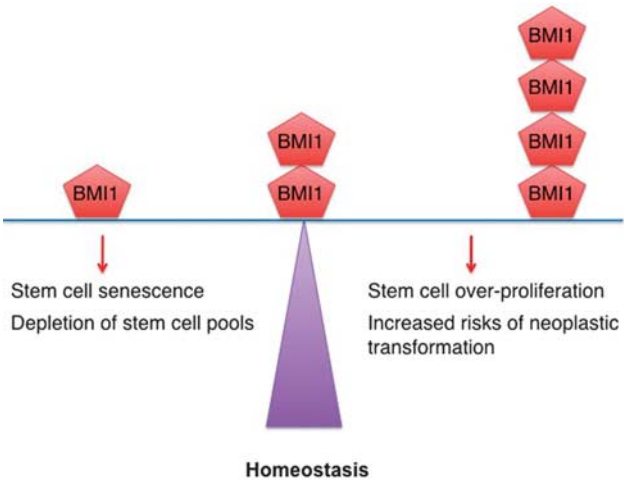

Figure 5 Balance between stem cell self-renewal and transformation during aging. Chromatin regulators (for example, BMI1) that normally maintain stem cell self-renewal and prevent age-dependent stem cell depletion have also been found to promote tumorigenesis when overexpressed. Understanding the mechanistic balance that allows stem cell self-renewal while preventing neoplastic transformation will be crucial for identifying ways to maintain the proper homeostasis of aging stem cells.

similar expression profiles and markers of normal HSCs (Krivtsov *et al*., 2006). In other hematopoietic malignancies, an initiating lesion in self-renewing stem cells may progress to malignancy when coupled with increased mutagenic events in the more rapidly expanding progenitor pool (Bonnet and Dick 1997; Cozzio *et al*., 2003; Rossi *et al*., 2008). Similarly, adult SVZ NSCs have been proposed as the origin of gliomas and astrocytomas (Lewis 1968; Vick *et al*., 1977; Holland *et al*., 2000; Sanai *et al*., 2005; Zhu *et al*., 2005; Jackson *et al*., 2006). Brain tumor cells share both anatomical location with SVZ niches and similar properties, including dependence on signaling pathways regulating normal NSC self-renewal (Jackson *et al*., 2006; Lim *et al*., 2007). Thus, identifying ways of reactivating the self-renewal and/or functional capacities of aging stem cells may in fact increase the risk of neoplastic transformation for these cells.

Regulation of chromatin state is likely a key factor in the balance between the maintenance of functional stem cell pools during aging and the risk of transformation of these cells (Figure 5). Chromatin regulators that maintain adult stem function have often been found to promote tumorigenesis. For example, PcG group proteins BMI1 and EZH2 have both been strongly implicated in cancer progression and are overexpressed in a variety of cancer types (Valk-Lingbeek *et al*., 2004). BMI1 is required for proliferation of leukemia stem cells (Lessard and Sauvageau, 2003) and transformation in a mouse model of glioma (Bruggeman *et al*., 2007). Transcriptional regulators of stem cell chromatin have likewise been implicated in promoting neoplasm. Overexpression of the nuclear receptor *Tlx* in NSCs induces the formation of glioma-like lesions that progress to full gliomas upon secondary loss of the tumor suppressor p53 (Liu *et al*., 2010). The role of other transcription factors, such as FOXOs, in the balance between stem cell maintenance and tumor development appears to be more intricate. FOXO factors, which are necessary for adult stem cell maintenance and self-renewal, also promote tumor suppression (Hu *et al*., 2004; Bouchard *et al*., 2007; Paik *et al*., 2007). Transcription factors like FOXOs that are involved in maintaining stem cell quiescence (Miyamoto *et al*., 2008; Paik *et al*., 2009; Renault *et al*., 2009) may prevent both the premature depletion of stem cell reservoirs and neoplastic transformation by limiting the generation of rapidly proliferating progenitors, which could be at greater risk for unrestrained growth. Nevertheless, recent studies indicate that FOXO3, in cooperation with TGF-β signaling, is required for sustained tumor-initiating potential of leukemia stem cells through suppression of apoptosis (Naka *et al*., 2010). These results suggest that FOXO3's role in normal stem cell self-renewal may become deleterious in the context of a strong transforming oncogene. These specific examples highlight how both chromatin regulators and transcription factors could play an important role in a trade-off between self-renewal of normal stem cells and that of aberrant tumor-initiating cells.

Future directions

During the course of organismal aging, stem cells of diverse adult tissues undergo striking changes in the regulation of self-renewal and multi-lineage differentiation potential. In this review, we have highlighted the role of chromatin state in the control of adult stem cell function during aging. Although mostly circumstantial, evidence is mounting for specific roles of histone regulators and for factors that alter chromatin compaction in maintaining adult stem cell potential. The coordinated control of chromatin states by histone modifiers and by proteins that alter the global chromatin compaction will be a fascinating avenue for further investigation. Such studies will particularly benefit from genome-wide examination of histone modifications and chromatin structure in young and old stem cell populations. As technologies for quantitatively assessing genome-wide gene expression and chromatin landscapes improve, profiling the rare populations of adult stem cells will become increasingly feasible.

Whether changes to stem cell pools are actually responsible for tissue failure during aging is only beginning to be addressed. The majority of studies addressing the mechanisms underlying stem cell aging measure the function of young and old stem cells in surrogate stem cell assays, such as competitive transplantation for HSCs, neurosphere formation or long-term label-retention assays for NSCs. Certainly, age-dependent defects in stem cell function in such assays correlate with observed changes to tissue function with aging, including decline in cognitive capacity, muscle atrophy and susceptibility to immune system disorders and cancer. However, the causative relationship between stem cell function and tissue homeostasis

during aging is not yet fully understood. Recent evidence suggests that inducible reactivation of the enzyme telomerase is sufficient to restore both the proliferation and neuronal differentiation of NSCs in the adult brain and improve defects in olfactory dysfunction, which is linked with NSC decline, in late-generation telomerase-deficient mice (Jaskelioff *et al*., 2011). It will be interesting to determine whether the modulation of specific chromatin regulators can restore the proliferative and multipotential properties of stem cells and thereby ameliorate age-dependent tissue decline. Moreover, the relationship between stem cell function and organismal longevity has yet to be rigorously addressed in mammals, although the importance of specific tissue-stem cells (gut stem cells) in organismal longevity has been revealed in invertebrates (Biteau *et al*., 2010). As the mechanisms underlying age-dependent stem cell decline are better understood, studying the effects of manipulating stem cell function on overall organismal lifespan and healthspan will be an attainable goal.

The role of chromatin regulators and transcription factors that affect stem cell maintenance in tumor development indicates that manipulating adult stem cell function with age must be carried out cautiously, given increased risks of cellular transformation. However, studies on the effect of exercise, environmental enrichment and parabiosis on old stem cells have revealed that stem cells rejuvenation can be achieved without the acquisition of neoplastic properties. Elucidating the synergistic or antagonistic roles of different chromatin regulators, their primary targets and the external signaling pathways that regulate these modifiers will be essential for restoring regenerative potential to aging adult stem cells in a controlled manner. Tapping into the regenerative potential of dormant endogenous stem cells will be a promising avenue to prevent and treat a number of age-dependent diseases characterized by tissue degeneration. Moreover, the recent ability to generate *in vitro* pluripotent stem cells from adult patients has opened exciting new paths for exogenous stem cell therapies to treat age-dependent diseases. Thus, understanding how age influences stem cell properties and whether epigenetic pathways identified in mice also apply to humans will be critical steps in implementing new therapies.

Conflict of interest

The authors declare no conflict of interest.

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