

## REVIEW

Epigenetic regulation of aging stem cellsEA Pollina<sup>1,2</sup> and A Brunet<sup>1,2</sup><sup>1</sup>Department of Genetics, Stanford University, Stanford, CA, USA and <sup>2</sup>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

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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<sup>+</sup>, Lin<sup>-</sup>, Sca1<sup>+</sup>) 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<sup>+</sup> 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 progenitor 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*

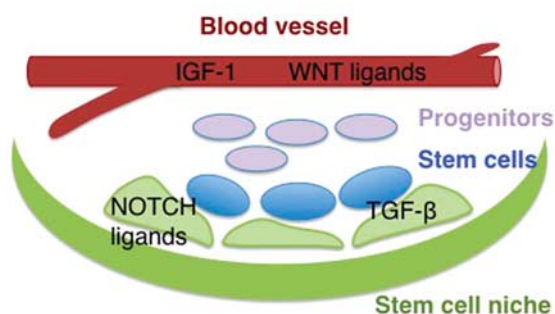
Given the decline in stem cell function with age, an important question regards the mechanisms underlying adult stem cell homeostasis. While this review will focus on stem cell aging in mammals, it is important to note that elegant studies in invertebrates have highlighted the changes in stem cell and 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- $\beta$ 1 (TGF- $\beta$ 1). TGF- $\beta$ 1 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.

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- $\beta$  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,

Irreversible changes	Reversible changes
DNA damage	Signaling pathways
Telomere erosion	Transcription factor activity
Mitochondrial dysfunction	Chromatin state
	DNA Methylation



**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.

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.

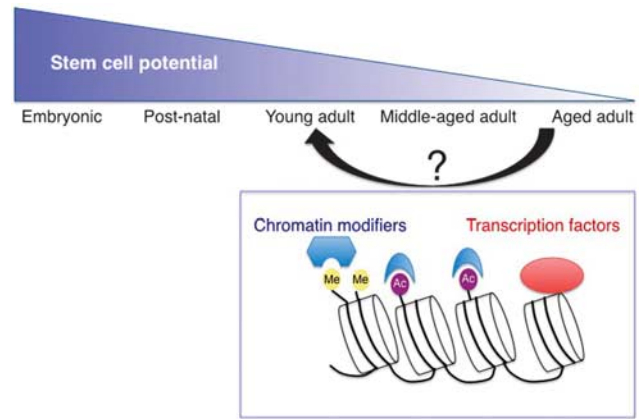
*Epigenetic changes: a pivotal mechanism for stem cell regulation during aging?*

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



**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.

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<sup>+</sup>, Lin<sup>-</sup>, Sca1<sup>+</sup> (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<sup>+</sup>, Lin<sup>-</sup>, Sca1<sup>+</sup>, Flk2<sup>-</sup>, CD34<sup>-</sup>) 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

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 (IL7R $\alpha^-$ /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.

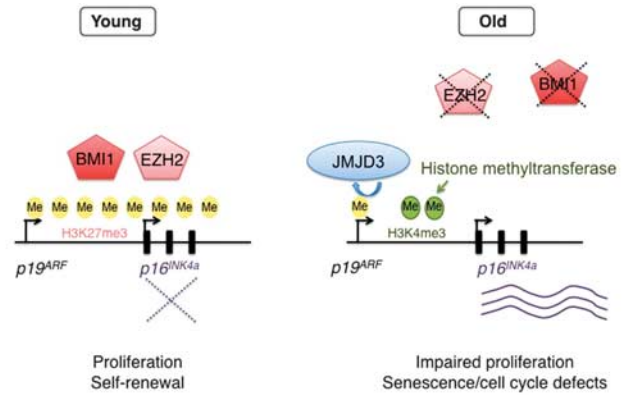
*BMI1 controls stem cells via the key 'aging locus' p16<sup>INK4a</sup>/p19<sup>ARF</sup>.* BMI1 function in young adult HSC and NSC self-renewal is mediated, in large part, through its transcriptional repression of the p16<sup>INK4a</sup>/p19<sup>ARF</sup> aging locus (Jacobs *et al.*, 1999). p16<sup>INK4a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



**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.

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).

**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*<sup>-/-</sup> 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*<sup>-/-</sup> 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*<sup>-/-</sup> 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<sup>INK4a</sup>/p19<sup>ARF</sup>* 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<sup>INK4a</sup>/p19<sup>ARF</sup>* 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<sup>Ink4a</sup>/p19<sup>Arf</sup>* 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 multi-subunit 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<sup>2+</sup> as a cofactor, whereas class III HDACs, also known as Sirtuins, require NAD<sup>+</sup> 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 *Chp*<sup>+/-</sup> 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<sup>INK4a</sup>/p19<sup>ARF</sup>* 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 as *p16<sup>INK4a</sup>/p19<sup>ARF</sup>*, that limit stem cell function.

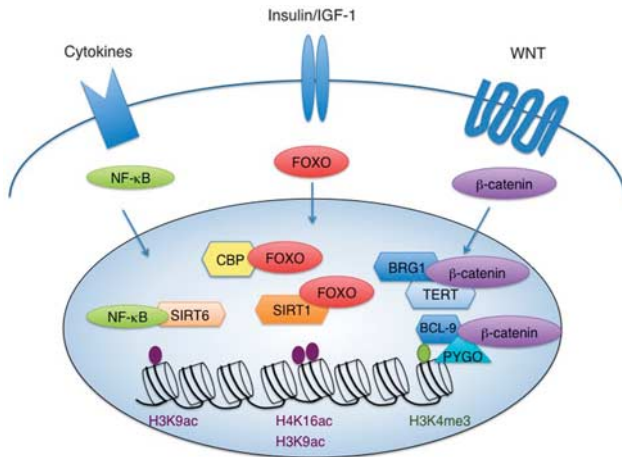
#### **Transcriptional regulators and chromatin states in aging stem cells**

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*<sup>-/-</sup> 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*<sup>-/-</sup> 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- $\kappa$ B 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).  $\beta$ -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  $\beta$ -catenin ortholog *bar-1* decreases organismal lifespan and oxidative stress resistance (Essers *et al.*, 2005). The role of  $\beta$ -catenin in adult stem cell function may be mediated through its activity at chromatin, as  $\beta$ -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  $\beta$ -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,  $\beta$ -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

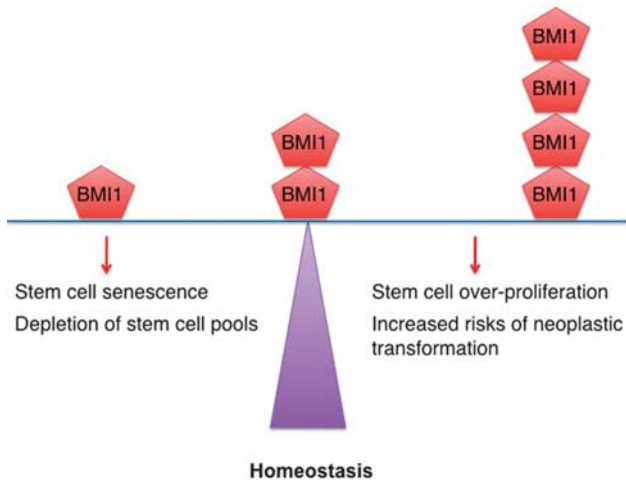
prominent role in stem cell proliferation independent of its reverse transcriptase activity, TERT is a pivotal component of the transcriptional complex downstream of WNT signaling (Choi *et al.*, 2008; Park *et al.*, 2009). In gastrointestinal tract stem cells, TERT interacts with the chromatin remodeling protein BRG1 and with  $\beta$ -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<sup>CIP1</sup>* 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- $\beta$  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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### References

- Adler AS, Sinha S, Kawahara TL, Zhang JY, Segal E, Chang HY. (2007). Motif module map reveals enforcement of aging by continual NF-kappaB activity. *Genes Dev* **21**: 3244–3257.
- Agger K, Cloos PA, Rudkjaer L, Williams K, Andersen G, Christensen J *et al.* (2009). The H3K27me3 demethylase JMJD3 contributes to the activation of the INK4A-ARF locus in response to oncogene- and stress-induced senescence. *Genes Dev* **23**: 1171–1176.
- Ahlenius H, Visan V, Kokaia M, Lindvall O, Kokaia Z. (2009). Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain. *J Neurosci* **29**: 4408–4419.
- Anselmi CV, Malovini A, Roncarati R, Novelli V, Villa F, Condorelli G *et al.* (2009). Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res* **12**: 95–104.
- Anthony TE, Klein C, Fishell G, Heintz N. (2004). Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron* **41**: 881–890.
- Arantes-Oliveira N, Apfeld J, Dillin A, Kenyon C. (2002). Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* **295**: 502–505.
- Attema JL, Pronk CJ, Norddahl GL, Nygren JM, Bryder D. (2009). Hematopoietic stem cell ageing is uncoupled from p16 INK4A-mediated senescence. *Oncogene* **28**: 2238–2243.
- Banks AS, Kon N, Knight C, Matsumoto M, Gutierrez-Juarez R, Rossetti L *et al.* (2008). SirT1 gain of function increases energy efficiency and prevents diabetes in mice. *Cell Metab* **8**: 333–341.
- Barker N, Hurlstone A, Musisi H, Miles A, Bienz M, Clevers H. (2001). The chromatin remodelling factor Brg-1 interacts with beta-catenin to promote target gene activation. *EMBO J* **20**: 4935–4943.
- Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M *et al.* (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**: 1003–1007.
- Barradas M, Anderton E, Acosta JC, Li S, Banito A, Rodriguez-Niedenfuhr M *et al.* (2009). Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS. *Genes Dev* **23**: 1177–1182.
- Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. (1992). Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci USA* **89**: 2804–2808.
- Beckervordersandforth R, Tripathi P, Ninkovic J, Bayam E, Lepier A, Stempfhuber B *et al.* (2010). *In vivo* fate mapping and expression analysis reveals molecular hallmarks of prospectively isolated adult neural stem cells. *Cell Stem Cell* **7**: 744–758.
- Beeraman I, Bhattacharya D, Zandi S, Sigvardsson M, Weissman IL, Bryder D *et al.* (2010a). Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. *Proc Natl Acad Sci USA* **107**: 5465–5470.
- Beeraman I, Maloney WJ, Weissmann IL, Rossi DJ. (2010b). Stem cells and the aging hematopoietic system. *Curr Opin Immunol* **22**: 500–506.
- Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S *et al.* (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* **114**: 763–776.
- Berger SL, Kouzarides T, Shiekhhattar R, Shilatifard A. (2009). An operational definition of epigenetics. *Genes Dev* **23**: 781–783.
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J *et al.* (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**: 315–326.

- Biteau B, Hochmuth CE, Jasper H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut. *Cell Stem Cell* **3**: 442–455.
- Biteau B, Karpac J, Supoyo S, Degennaro M, Lehmann R, Jasper H. (2010). Lifespan extension by preserving proliferative homeostasis in *Drosophila*. *PLoS Genet* **6**: e1001159.
- Blanpain C, Horsley V, Fuchs E. (2007). Epithelial stem cells: turning over new leaves. *Cell* **128**: 445–458.
- Blasco MA. (2007). The epigenetic regulation of mammalian telomeres. *Nat Rev Genet* **8**: 299–309.
- Bockhold KJ, Rosenblatt JD, Partridge TA. (1998). Aging normal and dystrophic mouse muscle: analysis of myogenicity in cultures of living single fibers. *Muscle Nerve* **21**: 173–183.
- Bondolfi L, Ermini F, Long JM, Ingram DK, Jucker M. (2004). Impact of age and caloric restriction on neurogenesis in the dentate gyrus of C57BL/6 mice. *Neurobiol Aging* **25**: 333–340.
- Bonnet D, Dick JE. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* **3**: 730–737.
- Bouchard C, Lee S, Paulus-Hock V, Loddenkemper C, Eilers M, Schmitt CA. (2007). FoxO transcription factors suppress Myc-driven lymphomagenesis via direct activation of Arf. *Genes Dev* **21**: 2775–2787.
- Boyle M, Wong C, Rocha M, Jones DL. (2007). Decline in self-renewal factors contributes to aging of the stem cell niche in the *Drosophila* testis. *Cell Stem Cell* **1**: 470–478.
- Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C *et al.* (2007). Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* **317**: 807–810.
- Brack AS, Murphy-Seiler F, Hanifi J, Deka J, Eyckerman S, Keller C *et al.* (2009). BCL9 is an essential component of canonical Wnt signaling that mediates the differentiation of myogenic progenitors during muscle regeneration. *Dev Biol* **335**: 93–105.
- Bracken AP, Kleine-Kohlbrecher D, Dietrich N, Pasini D, Gargiulo G, Beekman C *et al.* (2007). The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. *Genes Dev* **21**: 525–530.
- Breton-Provencher V, Lemasson M, Peralta III MR, Saghatelian A. (2009). Interneurons produced in adulthood are required for the normal functioning of the olfactory bulb network and for the execution of selected olfactory behaviors. *J Neurosci* **29**: 15245–15257.
- Bruggeman SW, Hulsman D, Tanger E, Buckle T, Blom M, Zevenhoven J *et al.* (2007). Bmi1 controls tumor development in an Ink4a/Arf-independent manner in a mouse model for glioma. *Cancer Cell* **12**: 328–341.
- Bruggeman SW, Valk-Lingbeek ME, van der Stoop PP, Jacobs JJ, Kieboom K, Tanger E *et al.* (2005). Ink4a and Arf differentially affect cell proliferation and neural stem cell self-renewal in Bmi1-deficient mice. *Genes Dev* **19**: 1438–1443.
- Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y *et al.* (2004). Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303**: 2011–2015.
- Bug G, Gul H, Schwarz K, Pfeifer H, Kampmann M, Zheng X *et al.* (2005). Valproic acid stimulates proliferation and self-renewal of hematopoietic stem cells. *Cancer Res* **65**: 2537–2541.
- Buszczak M, Spradling AC. (2006). Searching chromatin for stem cell identity. *Cell* **125**: 233–236.
- Cameron HA, McKay RD. (1999). Restoring production of hippocampal neurons in old age. *Nat Neurosci* **2**: 894–897.
- Carlson ME, Conboy IM. (2007). Loss of stem cell regenerative capacity within aged niches. *Aging Cell* **6**: 371–382.
- Carlson ME, Hsu M, Conboy IM. (2008). Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. *Nature* **454**: 528–532.
- Carlson ME, Suetta C, Conboy MJ, Aagaard P, Mackey A, Kjaer M *et al.* (2009). Molecular aging and rejuvenation of human muscle stem cells. *EMBO Mol Med* **1**: 381–391.
- Cavalli G, Paro R. (1998). The *Drosophila* Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. *Cell* **93**: 505–518.
- Challen GA, Boles NC, Chambers SM, Goodell MA. (2010). Distinct hematopoietic stem cell subtypes are differentially regulated by TGF-beta1. *Cell Stem Cell* **6**: 265–278.
- Chambers SM, Shaw CA, Gatz C, Fisk CJ, Donehower LA, Goodell MA. (2007). Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol* **5**: e201.
- Chen J, Luo Q, Yuan Y, Huang X, Cai W, Li C *et al.* (2010). Pygo2 associates with MLL2 histone methyltransferase (HMT) and GCN5 histone acetyltransferase (HAT) complexes to augment Wnt target gene expression and breast cancer stem-like cell expansion. *Mol Cell Biol* **30**: 5621–5635.
- Chen S, Whetstone JR, Ghosh S, Hanover JA, Gali RR, Grosu P *et al.* (2009). The conserved NAD(H)-dependent corepressor CTBP-1 regulates *Caenorhabditis elegans* life span. *Proc Natl Acad Sci USA* **106**: 1496–1501.
- Cheshier SH, Morrison SJ, Liao X, Weissman IL. (1999). *In vivo* proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. *Proc Natl Acad Sci USA* **96**: 3120–3125.
- Cheung I, Shulha HP, Jiang Y, Matevosian A, Wang J, Weng Z *et al.* (2010). Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. *Proc Natl Acad Sci USA* **107**: 8824–8829.
- Cho RH, Sieburg HB, Muller-Sieburg CE. (2008). A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. *Blood* **111**: 5553–5561.
- Choi J, Southworth LK, Sarin KY, Venteicher AS, Ma W, Chang W *et al.* (2008). TERT promotes epithelial proliferation through transcriptional control of a Myc- and Wnt-related developmental program. *PLoS Genet* **4**: e10.
- Chuikov S, Levi BP, Smith ML, Morrison SJ. (2010). Prdm16 promotes stem cell maintenance in multiple tissues, partly by regulating oxidative stress. *Nat Cell Biol* **12**: 999–1006.
- Clelland CD, Choi M, Romberg C, Clemenson Jr GD, Fagniere A, Tiers P *et al.* (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* **325**: 210–213.
- Conboy IM, Conboy MJ, Smythe GM, Rando TA. (2003). Notch-mediated restoration of regenerative potential to aged muscle. *Science* **302**: 1575–1577.
- Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* **433**: 760–764.
- Conboy IM, Rando TA. (2005). Aging, stem cells and tissue regeneration: lessons from muscle. *Cell Cycle* **4**: 407–410.
- Corbu A, Scaramozza A, Badiali-DeGiorgi L, Tarantino L, Papa V, Rinaldi R *et al.* (2010). Satellite cell characterization from aging human muscle. *Neurol Res* **32**: 63–72.
- Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL. (2003). Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes Dev* **17**: 3029–3035.
- Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wickelso C *et al.* (2007). Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science* **315**: 1243–1249.
- Daitoku H, Hatta M, Matsuzaki H, Aratani S, Ohshima T, Miyagishi M *et al.* (2004). Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc Natl Acad Sci USA* **101**: 10042–10047.
- Dang W, Steffen KK, Perry R, Dorsey JA, Johnson FB, Shilatifard A *et al.* (2009). Histone H4 lysine 16 acetylation regulates cellular lifespan. *Nature* **459**: 802–807.
- de Haan G, Nijhof W, Van Zant G. (1997). Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: correlation between lifespan and cycling activity. *Blood* **89**: 1543–1550.
- de Haan G, Van Zant G. (1999). Dynamic changes in mouse hematopoietic stem cell numbers during aging. *Blood* **93**: 3294–3301.

- Deka J, Wiedemann N, Anderle P, Murphy-Seiler F, Bultinck J, Eyckerman S *et al.* (2010). Bcl9/Bcl9l are critical for Wnt-mediated regulation of stem cell traits in colon epithelium and adenocarcinomas. *Cancer Res* **70**: 6619–6628.
- Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**: 703–716.
- Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ *et al.* (2003). *in vitro* propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev* **17**: 1253–1270.
- Dumble M, Moore L, Chambers SM, Geiger H, Van Zant G, Goodell MA *et al.* (2007). The impact of altered p53 dosage on hematopoietic stem cell dynamics during aging. *Blood* **109**: 1736–1742.
- Dykstra B, Kent D, Bowie M, McCaffrey L, Hamilton M, Lyons K *et al.* (2007). Long-term propagation of distinct hematopoietic differentiation programs *in vivo*. *Cell Stem Cell* **1**: 218–229.
- Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S. (2004). Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *J Neurosci* **24**: 8354–8365.
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA *et al.* (1998). Neurogenesis in the adult human hippocampus. *Nat Med* **4**: 1313–1317.
- Essers MA, de Vries-Smits LM, Barker N, Polderman PE, Burgering BM, Korswagen HC. (2005). Functional interaction between beta-catenin and FOXO in oxidative stress signaling. *Science* **308**: 1181–1184.
- Fasano CA, Dimos JT, Ivanova NB, Lowry N, Lemischka IR, Temple S. (2007). shRNA knockdown of Bmi-1 reveals a critical role for p21-Rb pathway in NSC self-renewal during development. *Cell Stem Cell* **1**: 87–99.
- Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC *et al.* (2008). Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. *Cell Metab* **8**: 347–358.
- Fiedler M, Sanchez-Barrena MJ, Nekrasov M, Mieszczynek J, Rybin V, Muller J *et al.* (2008). Decoding of methylated histone H3 tail by the Pygo-BCL9 Wnt signaling complex. *Mol Cell* **30**: 507–518.
- Flachsbart F, Caliebe A, Kleindorfer R, Blanche H, von Eller-Eberstein H, Nikolaus S *et al.* (2009). Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA* **106**: 2700–2705.
- Flores I, Cayuela ML, Blasco MA. (2005). Effects of telomerase and telomere length on epidermal stem cell behavior. *Science* **309**: 1253–1256.
- Frescas D, Valenti L, Accili D. (2005). Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenic genes. *J Biol Chem* **280**: 20589–20595.
- Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T. (2003). The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J Biol Chem* **278**: 4035–4040.
- Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J. (1998). Multipotent progenitor cells in the adult dentate gyrus. *J Neurobiol* **36**: 249–266.
- Gheusi G, Cremer H, McLean H, Chazal G, Vincent JD, Lledo PM. (2000). Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. *Proc Natl Acad Sci USA* **97**: 1823–1828.
- Giannakou ME, Goss M, Junger MA, Hafen E, Leivers SJ, Partridge L. (2004). Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* **305**: 361.
- Gibson MC, Schultz E. (1983). Age-related differences in absolute numbers of skeletal muscle satellite cells. *Muscle Nerve* **6**: 574–580.
- Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M *et al.* (2007). ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* **1**: 555–567.
- Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J Exp Med* **183**: 1797–1806.
- Goodman RH, Smolik S. (2000). CBP/p300 in cell growth, transformation, and development. *Genes Dev* **14**: 1553–1577.
- Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. (1999). Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci* **2**: 260–265.
- Greer EL, Brunet A. (2005). FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene* **24**: 7410–7425.
- Greer EL, Maures TJ, Hauswirth AG, Green EM, Leeman DS, Maro GS *et al.* (2010). Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in *C. elegans*. *Nature* **466**: 383–387.
- Grewal SI, Klar AJ. (1996). Chromosomal inheritance of epigenetic states in fission yeast during mitosis and meiosis. *Cell* **86**: 95–101.
- Gu B, Sun P, Yuan Y, Moraes RC, Li A, Teng A *et al.* (2009). Pygo2 expands mammary progenitor cells by facilitating histone H3 K4 methylation. *J Cell Biol* **185**: 811–826.
- Guerretaz LM, Johnson SA, Cambier JC. (2008). Acquired hematopoietic stem cell defects determine B-cell repertoire changes associated with aging. *Proc Natl Acad Sci USA* **105**: 11898–11902.
- Haigis MC, Guarente LP. (2006). Mammalian sirtuins—emerging roles in physiology, aging, and calorie restriction. *Genes Dev* **20**: 2913–2921.
- Hatta M, Cirillo LA. (2007). Chromatin opening and stable perturbation of core histone:DNA contacts by FoxO1. *J Biol Chem* **282**: 35583–35593.
- Hattiangady B, Shetty AK. (2008). Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus. *Neurobiol Aging* **29**: 129–147.
- Hayden MS, Ghosh S. (2008). Shared principles in NF-kappaB signaling. *Cell* **132**: 344–362.
- Hecht A, Vlemminckx K, Stemmler MP, van Roy F, Kemler R. (2000). The p300/CBP acetyltransferases function as transcriptional coactivators of beta-catenin in vertebrates. *EMBO J* **19**: 1839–1850.
- Hennighausen L, Robinson GW. (2005). Information networks in the mammary gland. *Nat Rev Mol Cell Biol* **6**: 715–725.
- Hirabayashi Y, Suzuki N, Tsuboi M, Endo TA, Toyoda T, Shinga J *et al.* (2009). Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. *Neuron* **63**: 600–613.
- Hisahara S, Chiba S, Matsumoto H, Tanno M, Yagi H, Shimohama S *et al.* (2008). Histone deacetylase SIRT1 modulates neuronal differentiation by its nuclear translocation. *Proc Natl Acad Sci USA* **105**: 15599–15604.
- Ho L, Crabtree GR. (2010). Chromatin remodelling during development. *Nature* **463**: 474–484.
- Ho L, Ronan JL, Wu J, Staahl BT, Chen L, Kuo A *et al.* (2009). An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. *Proc Natl Acad Sci USA* **106**: 5181–5186.
- Holland EC, Li Y, Celestino J, Dai C, Schaefer L, Sawaya RA *et al.* (2000). Astrocytes give rise to oligodendrogliomas and astrocytomas after gene transfer of polyoma virus middle T antigen *in vivo*. *Am J Pathol* **157**: 1031–1037.
- Hsieh J, Nakashima K, Kuwabara T, Mejia E, Gage FH. (2004). Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. *Proc Natl Acad Sci USA* **101**: 16659–16664.
- Hu MC, Lee DF, Xia W, Golfman LS, Ou-Yang F, Yang JY *et al.* (2004). IkappaB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. *Cell* **117**: 225–237.
- Hwangbo DS, Gershman B, Tu MP, Palmer M, Tatar M. (2004). *Drosophila* dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* **429**: 562–566.
- Imai S, Armstrong CM, Kaeberlein M, Guarente L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403**: 795–800.



- Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M *et al.* (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci* **11**: 1153–1161.
- Iwama A, Oguro H, Negishi M, Kato Y, Morita Y, Tsukui H *et al.* (2004). Enhanced self-renewal of hematopoietic stem cells mediated by the polycomb gene product Bmi-1. *Immunity* **21**: 843–851.
- Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S *et al.* (2006). PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron* **51**: 187–199.
- Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. (1999). The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* **397**: 164–168.
- Jaenisch R, Bird A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* **33**(Suppl): 245–254.
- Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM *et al.* (2006). Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature* **443**: 421–426.
- Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC *et al.* (2011). Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature* **469**: 102–106.
- Jawerka M, Colak D, Dimou L, Spiller C, Lager S, Montgomery RL *et al.* (2010). The specific role of histone deacetylase 2 in adult neurogenesis. *Neuron Glia Biol* **6**: 93–107.
- Jepsen K, Solum D, Zhou T, McEvelly RJ, Kim HJ, Glass CK *et al.* (2007). SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. *Nature* **450**: 415–419.
- Jones DL. (2007). Aging and the germ line: where mortality and immortality meet. *Stem Cell Rev* **3**: 192–200.
- Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N *et al.* (1998). Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet* **19**: 187–191.
- Jude CD, Climer L, Xu D, Artinger E, Fisher JK, Ernst P. (2007). Unique and independent roles for MLL in adult hematopoietic stem cells and progenitors. *Cell Stem Cell* **1**: 324–337.
- Kaeberlein M, McVey M, Guarente L. (1999). The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev* **13**: 2570–2580.
- Kamminga LM, Bystrykh LV, de Boer A, Houwer S, Douma J, Weersing E *et al.* (2006). The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. *Blood* **107**: 2170–2179.
- Kamminga LM, van Os R, Ausema A, Noach EJ, Weersing E, Dontje B *et al.* (2005). Impaired hematopoietic stem cell functioning after serial transplantation and during normal aging. *Stem Cells* **23**: 82–92.
- Kaplan MS, Bell DH. (1984). Mitotic neuroblasts in the 9-day-old and 11-month-old rodent hippocampus. *J Neurosci* **4**: 1429–1441.
- Katsumoto T, Aikawa Y, Iwama A, Ueda S, Ichikawa H, Ochiya T *et al.* (2006). MOZ is essential for maintenance of hematopoietic stem cells. *Genes Dev* **20**: 1321–1330.
- Kawahara TL, Michishita E, Adler AS, Damian M, Berber E, Lin M *et al.* (2009). SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. *Cell* **136**: 62–74.
- Kawakami K, Nakamura A, Ishigami A, Goto S, Takahashi R. (2009). Age-related difference of site-specific histone modifications in rat liver. *Biogerontology* **10**: 415–421.
- Kee N, Teixeira CM, Wang AH, Frankland PW. (2007). Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat Neurosci* **10**: 355–362.
- Kempermann G, Brandon EP, Gage FH. (1998a). Environmental stimulation of 129/SvJ mice causes increased cell proliferation and neurogenesis in the adult dentate gyrus. *Curr Biol* **8**: 939–942.
- Kempermann G, Gast D, Gage FH. (2002). Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol* **52**: 135–143.
- Kempermann G, Kuhn HG, Gage FH. (1998b). Experience-induced neurogenesis in the senescent dentate gyrus. *J Neurosci* **18**: 3206–3212.
- Ketel CS, Andersen EF, Vargas ML, Suh J, Strome S, Simon JA. (2005). Subunit contributions to histone methyltransferase activities of fly and worm polycomb group complexes. *Mol Cell Biol* **25**: 6857–6868.
- Kim HS, Xiao C, Wang RH, Lahusen T, Xu X, Vassilopoulos A *et al.* (2010). Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. *Cell Metab* **12**: 224–236.
- Kim M, Moon HB, Spangrude GJ. (2003). Major age-related changes of mouse hematopoietic stem/progenitor cells. *Ann N Y Acad Sci* **996**: 195–208.
- Kordon EC, Smith GH. (1998). An entire functional mammary gland may comprise the progeny from a single cell. *Development* **125**: 1921–1930.
- Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, Su L *et al.* (2004). Ink4a/Arf expression is a biomarker of aging. *J Clin Invest* **114**: 1299–1307.
- Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J *et al.* (2006). Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* **442**: 818–822.
- Kronenberg G, Bick-Sander A, Bunk E, Wolf C, Ehninger D, Kempermann G. (2006). Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus. *Neurobiol Aging* **27**: 1505–1513.
- Kuhn HG, Dickinson-Anson H, Gage FH. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* **16**: 2027–2033.
- Kukekov VG, Laywell ED, Suslov O, Davies K, Scheffler B, Thomas LB *et al.* (1999). Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain. *Exp Neurol* **156**: 333–344.
- Kung AL, Rebel VI, Bronson RT, Ch'ng LE, Sieff CA, Livingston DM *et al.* (2000). Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev* **14**: 272–277.
- Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. (2002). Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev* **16**: 2893–2905.
- Lagace DC, Whitman MC, Noonan MA, Ables JL, DeCarolis NA, Arguello AA *et al.* (2007). Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *J Neurosci* **27**: 12623–12629.
- Lee CK, Weindruch R, Prolla TA. (2000). Gene-expression profile of the ageing brain in mice. *Nat Genet* **25**: 294–297.
- Lee KK, Workman JL. (2007). Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol* **8**: 284–295.
- Lessard J, Sauvageau G. (2003). Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* **423**: 255–260.
- Lessard J, Wu JI, Ranish JA, Wan M, Winslow MM, Staahl BT *et al.* (2007). An essential switch in subunit composition of a chromatin remodeling complex during neural development. *Neuron* **55**: 201–215.
- Lewis PD. (1968). Mitotic activity in the primate subependymal layer and the genesis of gliomas. *Nature* **217**: 974–975.
- Li J, Ebata A, Dong Y, Rizki G, Iwata T, Lee SS. (2008). Caenorhabditis elegans HCF-1 functions in longevity maintenance as a DAF-16 regulator. *PLoS Biol* **6**: e233.
- Li Y, Wang WJ, Cao H, Lu J, Wu C, Hu FY *et al.* (2009). Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. *Hum Mol Genet* **18**: 4897–4904.
- Liang Y, Van Zant G, Szilvassy SJ. (2005). Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. *Blood* **106**: 1479–1487.
- Lichtenwalner RJ, Forbes ME, Bennett SA, Lynch CD, Sonntag WE, Riddle DR. (2001). Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis. *Neuroscience* **107**: 603–613.

- Lim DA, Cha S, Mayo MC, Chen MH, Keles E, VandenBerg S *et al.* (2007). Relationship of glioblastoma multiforme to neural stem cell regions predicts invasive and multifocal tumor phenotype. *Neuro Oncol* **9**: 424–429.
- Lim DA, Huang YC, Swigut T, Mirick AL, Garcia-Verdugo JM, Wysocka J *et al.* (2009). Chromatin remodelling factor Mll1 is essential for neurogenesis from postnatal neural stem cells. *Nature* **458**: 529–533.
- Lin K, Dorman JB, Rodan A, Kenyon C. (1997). Daf-16: an HNF-3/ forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* **278**: 1319–1322.
- Liu H, Fergusson MM, Castilho RM, Liu J, Cao L, Chen J *et al.* (2007). Augmented Wnt signaling in a mammalian model of accelerated aging. *Science* **317**: 803–806.
- Liu HK, Belz T, Bock D, Takacs A, Wu H, Lichter P *et al.* (2008). The nuclear receptor tailless is required for neurogenesis in the adult subventricular zone. *Genes Dev* **22**: 2473–2478.
- Liu HK, Wang Y, Belz T, Bock D, Takacs A, Radlwimmer B *et al.* (2010). The nuclear receptor tailless induces long-term neural stem cell expansion and brain tumor initiation. *Genes Dev* **24**: 683–695.
- Liu J, Cao L, Chen J, Song S, Lee IH, Quijano C *et al.* (2009). Bmi1 regulates mitochondrial function and the DNA damage response pathway. *Nature* **459**: 387–392.
- Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW *et al.* (2006). Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res* **66**: 6063–6071.
- Lois C, Alvarez-Buylla A. (1993). Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci USA* **90**: 2074–2077.
- Longo VD, Kennedy BK. (2006). Sirtuins in aging and age-related disease. *Cell* **126**: 257–268.
- Lowe SW, Sherr CJ. (2003). Tumor suppression by Ink4a-Arf: progress and puzzles. *Curr Opin Genet Dev* **13**: 77–83.
- Lugert S, Basak O, Knuckles P, Haussler U, Fabel K, Gotz M *et al.* (2010). Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. *Cell Stem Cell* **6**: 445–456.
- Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N *et al.* (2009). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* **323**: 1074–1077.
- Ma DK, Marchetto MC, Guo JU, Ming GL, Gage FH, Song H. (2010). Epigenetic choreographers of neurogenesis in the adult mammalian brain. *Nat Neurosci* **13**: 1338–1344.
- Major MB, Roberts BS, Berndt JD, Marine S, Anastas J, Chung N *et al.* (2008). New regulators of Wnt/beta-catenin signaling revealed by integrative molecular screening. *Sci Signal* **1**: ra12.
- Martin C, Zhang Y. (2007). Mechanisms of epigenetic inheritance. *Curr Opin Cell Biol* **19**: 266–272.
- Maslov AY, Barone TA, Plunkett RJ, Pruitt SC. (2004). Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J Neurosci* **24**: 1726–1733.
- Matsuzaki H, Daitoku H, Hatta M, Aoyama H, Yoshimochi K, Fukamizu A. (2005). Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. *Proc Natl Acad Sci USA* **102**: 11278–11283.
- McMahon KA, Hiew SY, Hadjur S, Veiga-Fernandes H, Menzel U, Price AJ *et al.* (2007). Mll has a critical role in fetal and adult hematopoietic stem cell self-renewal. *Cell Stem Cell* **1**: 338–345.
- Merkle FT, Mirzadeh Z, Alvarez-Buylla A. (2007). Mosaic organization of neural stem cells in the adult brain. *Science* **317**: 381–384.
- Merson TD, Dixon MP, Collin C, Rietze RL, Bartlett PF, Thomas T *et al.* (2006). The transcriptional coactivator Querkopf controls adult neurogenesis. *J Neurosci* **26**: 11359–11370.
- Michallet M, Philip T, Philip I, Godinot H, Sebban C, Salles G *et al.* (2000). Transplantation with selected autologous peripheral blood CD34+Thy1+ hematopoietic stem cells (HSCs) in multiple myeloma: impact of HSC dose on engraftment, safety, and immune reconstitution. *Exp Hematol* **28**: 858–870.
- Michan S, Sinclair D. (2007). Sirtuins in mammals: insights into their biological function. *Biochem J* **404**: 1–13.
- Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M *et al.* (2008). SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* **452**: 492–496.
- Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S *et al.* (2007). Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* **1**: 101–112.
- Miyamoto K, Miyamoto T, Kato R, Yoshimura A, Motoyama N, Suda T. (2008). FoxO3a regulates hematopoietic homeostasis through a negative feedback pathway in conditions of stress or aging. *Blood* **112**: 4485–4493.
- Molofsky AV, He S, Bydon M, Morrison SJ, Pardal R. (2005). Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16Ink4a and p19Arf senescence pathways. *Genes Dev* **19**: 1432–1437.
- Molofsky AV, Pardal R, Iwashita T, Park IK, Clarke MF, Morrison SJ. (2003). Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* **425**: 962–967.
- Molofsky AV, Slutsky SG, Joseph NM, He S, Pardal R, Krishnamurthy J *et al.* (2006). Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. *Nature* **443**: 448–452.
- Monaghan AP, Grau E, Bock D, Schutz G. (1995). The mouse homolog of the orphan nuclear receptor tailless is expressed in the developing forebrain. *Development* **121**: 839–853.
- Monje ML, Toda H, Palmer TD. (2003). Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **302**: 1760–1765.
- Morgan DE, Crittenden SL, Kimble J. (2010). The *C. elegans* adult male germline: stem cells and sexual dimorphism. *Dev Biol* **346**: 204–214.
- Morgan JE, Partridge TA. (2003). Muscle satellite cells. *Int J Biochem Cell Biol* **35**: 1151–1156.
- Morita Y, Ema H, Nakauchi H. (2010). Heterogeneity and hierarchy within the most primitive hematopoietic stem cell compartment. *J Exp Med* **207**: 1173–1182.
- Morrison SJ, Spradling AC. (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**: 598–611.
- Morrison SJ, Uchida N, Weissman IL. (1995). The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol* **11**: 35–71.
- Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. (1996). The aging of hematopoietic stem cells. *Nat Med* **2**: 1011–1016.
- Morrison SJ, Weissman IL. (1994). The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* **1**: 661–673.
- Morshead CM, Craig CG, van der Kooy D. (1998). *in vivo* clonal analyses reveal the properties of endogenous neural stem cell proliferation in the adult mammalian forebrain. *Development* **125**: 2251–2261.
- Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D *et al.* (1994). Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* **13**: 1071–1082.
- Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L *et al.* (2006). Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **124**: 315–329.
- Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W *et al.* (2004). Mammalian SIRT1 represses forkhead transcription factors. *Cell* **116**: 551–563.
- Muller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B *et al.* (2002). Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell* **111**: 197–208.
- Musaro A, Giacinti C, Borsellino G, Dobrowolny G, Pelosi L, Cairns L *et al.* (2004). Stem cell-mediated muscle regeneration is enhanced by local isoform of insulin-like growth factor 1. *Proc Natl Acad Sci USA* **101**: 1206–1210.
- Naka K, Hoshii T, Muraguchi T, Tadokoro Y, Ooshio T, Kondo Y *et al.* (2010). TGF-beta-FOXO signalling maintains

- leukaemia-initiating cells in chronic myeloid leukaemia. *Nature* **463**: 676–680.
- Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN *et al.* (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**: 386–389.
- Narala SR, Allsopp RC, Wells TB, Zhang G, Prasad P, Coussens MJ *et al.* (2008). SIRT1 acts as a nutrient-sensitive growth suppressor and its loss is associated with increased AMPK and telomerase activity. *Mol Biol Cell* **19**: 1210–1219.
- Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, Myers MP *et al.* (2006). A novel role for high-mobility group proteins in cellular senescence and heterochromatin formation. *Cell* **126**: 503–514.
- Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe T, Liu C *et al.* (2007). DNA repair is limiting for haematopoietic stem cells during ageing. *Nature* **447**: 686–690.
- Nishimura EK, Granter SR, Fisher DE. (2005). Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. *Science* **307**: 720–724.
- Nishino J, Kim I, Chada K, Morrison SJ. (2008). Hmga2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf expression. *Cell* **135**: 227–239.
- Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J, Park SK *et al.* (2008). SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell* **135**: 907–918.
- Ogawa T, Kitagawa M, Hirokawa K. (2000). Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. *Mech Ageing Dev* **117**: 57–68.
- Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA *et al.* (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389**: 994–999.
- Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87**: 953–959.
- Oguro H, Iwama A, Morita Y, Kamijo T, van Lohuizen M, Nakauchi H. (2006). Differential impact of Ink4a and Arf on hematopoietic stem cells and their bone marrow microenvironment in Bmi1-deficient mice. *J Exp Med* **203**: 2247–2253.
- Oguro H, Yuan J, Ichikawa H, Ikawa T, Yamazaki S, Kawamoto H *et al.* (2010). Poised lineage specification in multipotential hematopoietic stem and progenitor cells by the polycomb protein Bmi1. *Cell Stem Cell* **6**: 279–286.
- Oh YH, Conard RA. (1972). Effect of aging on histone acetylation of the normal and regenerating rat liver. *Life Sci* **11**: 1207–1214.
- Osawa M, Hanada K, Hamada H, Nakauchi H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* **273**: 242–245.
- Ou X, Chae HD, Wang RH, Shelley WC, Cooper S, Taylor T *et al.* (2010). SIRT1 deficiency compromises mouse embryonic stem cell hematopoietic differentiation, and embryonic and adult hematopoiesis in the mouse. *Blood* **117**: 440–450.
- Paik JH, Ding Z, Narurkar R, Ramkissoon S, Muller F, Kamoun WS *et al.* (2009). FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. *Cell Stem Cell* **5**: 540–553.
- Paik JH, Kollipara R, Chu G, Ji H, Xiao Y, Ding Z *et al.* (2007). FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell* **128**: 309–323.
- Palmer TD, Takahashi J, Gage FH. (1997). The adult rat hippocampus contains primordial neural stem cells. *Mol Cell Neurosci* **8**: 389–404.
- Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci* **17**: 3727–3738.
- Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL *et al.* (2003). Bmi-1 is required for maintenance of adult self-renewing hematopoietic stem cells. *Nature* **423**: 302–305.
- Park JI, Venteicher AS, Hong JY, Choi J, Jun S, Shkreli M *et al.* (2009). Telomerase modulates Wnt signalling by association with target gene chromatin. *Nature* **460**: 66–72.
- Pastrana E, Cheng LC, Doetsch F. (2009). Simultaneous prospective purification of adult subventricular zone neural stem cells and their progeny. *Proc Natl Acad Sci USA* **106**: 6387–6392.
- Pawlikowska L, Hu D, Huntsman S, Sung A, Chu C, Chen J *et al.* (2009). Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Ageing Cell* **8**: 460–472.
- Pearce DJ, Anjos-Afonso F, Ridler CM, Eddaoudi A, Bonnet D. (2007). Age-dependent increase in side population distribution within hematopoiesis: implications for our understanding of the mechanism of aging. *Stem Cells* **25**: 828–835.
- Peinado MA, Quesada A, Pedrosa JA, Torres MI, Martinez M, Esteban FJ *et al.* (1998). Quantitative and ultrastructural changes in glia and pericytes in the parietal cortex of the aging rat. *Microsc Res Technol* **43**: 34–42.
- Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC *et al.* (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science* **328**: 753–756.
- Perez-Campo FM, Borrow J, Kouskoff V, Lacaud G. (2009). The histone acetyl transferase activity of monocytic leukemia zinc finger is critical for the proliferation of hematopoietic precursors. *Blood* **113**: 4866–4874.
- Prozorovski T, Schulze-Topphoff U, Glumm R, Baumgart J, Schroter F, Ninnemann O *et al.* (2008). Sirt1 contributes critically to the redox-dependent fate of neural progenitors. *Nat Cell Biol* **10**: 385–394.
- Ramadori G, Fujikawa T, Fukuda M, Anderson J, Morgan DA, Mostoslavsky R *et al.* (2010). SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity. *Cell Metab* **12**: 78–87.
- Rando TA. (2005). The adult muscle stem cell comes of age. *Nat Med* **11**: 829–831.
- Rando TA. (2006). Stem cells, ageing and the quest for immortality. *Nature* **441**: 1080–1086.
- Rastelli L, Chan CS, Pirrotta V. (1993). Related chromosome binding sites for zeste, suppressors of zeste and Polycomb group proteins in *Drosophila* and their dependence on enhancer of zeste function. *EMBO J* **12**: 1513–1522.
- Rebel VI, Kung AL, Tanner EA, Yang H, Bronson RT, Livingston DM. (2002). Distinct roles for CREB-binding protein and p300 in hematopoietic stem cell self-renewal. *Proc Natl Acad Sci USA* **99**: 14789–14794.
- Renault V, Thornell LE, Eriksson PO, Butler-Browne G, Mouly V. (2002). Regenerative potential of human skeletal muscle during aging. *Ageing Cell* **1**: 132–139.
- Renault VM, Rafalski VA, Morgan AA, Salih DA, Brett JO, Webb AE *et al.* (2009). FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell* **5**: 527–539.
- Reya T, Clevers H. (2005). Wnt signalling in stem cells and cancer. *Nature* **434**: 843–850.
- Rietze RL, Valcanis H, Brooker GF, Thomas T, Voss AK, Bartlett PF. (2001). Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature* **412**: 736–739.
- Rine J, Strathern JN, Hicks JB, Herskowitz I. (1979). A suppressor of mating-type locus mutations in *Saccharomyces cerevisiae*: evidence for and identification of cryptic mating-type loci. *Genetics* **93**: 877–901.
- Ringrose L, Paro R. (2007). Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development* **134**: 223–232.
- Rogina B, Helfand SL. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci USA* **101**: 15998–16003.
- Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. (2007a). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* **447**: 725–729.



- Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ *et al.* (2005). Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci USA* **102**: 9194–9199.
- Rossi DJ, Jamieson CH, Weissman IL. (2008). Stems cells and the pathways to aging and cancer. *Cell* **132**: 681–696.
- Rossi DJ, Seita J, Czechowicz A, Bhattacharya D, Bryder D, Weissman IL. (2007b). Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging. *Cell Cycle* **6**: 2371–2376.
- Roy K, Kuznicki K, Wu Q, Sun Z, Bock D, Schutz G *et al.* (2004). The *Tlx* gene regulates the timing of neurogenesis in the cortex. *J Neurosci* **24**: 8333–8345.
- Roy NS, Benraiss A, Wang S, Fraser RA, Goodman R, Couldwell WT *et al.* (2000). Promoter-targeted selection and isolation of neural progenitor cells from the adult human ventricular zone. *J Neurosci Res* **59**: 321–331.
- Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C *et al.* (1999). Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* **96**: 701–712.
- Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM. (2008). Self-renewal and expansion of single transplanted muscle stem cells. *Nature* **456**: 502–506.
- Sahin E, Depinho RA. (2010). Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature* **464**: 520–528.
- Sanai N, Alvarez-Buylla A, Berger MS. (2005). Neural stem cells and the origin of gliomas. *N Engl J Med* **353**: 811–822.
- Sanai N, Tramontin AD, Quinones-Hinojosa A, Barbaro NM, Gupta N, Kunwar S *et al.* (2004). Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**: 740–744.
- Sarin KY, Cheung P, Gilison D, Lee E, Tennen RI, Wang E *et al.* (2005). Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature* **436**: 1048–1052.
- Scheller M, Huelsen J, Rosenbauer F, Taketo MM, Birchmeier W, Tenen DG *et al.* (2006). Hematopoietic stem cell and multilineage defects generated by constitutive beta-catenin activation. *Nat Immunol* **7**: 1037–1047.
- Schultz E, Gibson MC, Champion T. (1978). Satellite cells are mitotically quiescent in mature mouse muscle: an EM and radioautographic study. *J Exp Zool* **206**: 451–456.
- Schultz E, Lipton BH. (1982). Skeletal muscle satellite cells: changes in proliferation potential as a function of age. *Mech Ageing Dev* **20**: 377–383.
- Sen GL, Reuter JA, Webster DE, Zhu L, Khavari PA. (2010). DNMT1 maintains progenitor function in self-renewing somatic tissue. *Nature* **463**: 563–567.
- Sen GL, Webster DE, Barragan DI, Chang HY, Khavari PA. (2008). Control of differentiation in a self-renewing mammalian tissue by the histone demethylase JMJD3. *Genes Dev* **22**: 1865–1870.
- Sgarra R, Furlan C, Zammitti S, Lo Sardo A, Maurizio E, Di Bernardo J *et al.* (2008). Interaction proteomics of the HMGA chromatin architectural factors. *Proteomics* **8**: 4721–4732.
- Sgarra R, Tessari MA, Di Bernardo J, Rustighi A, Zago P, Liberatori S *et al.* (2005). Discovering high mobility group A molecular partners in tumour cells. *Proteomics* **5**: 1494–1506.
- Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML *et al.* (2006). Generation of a functional mammary gland from a single stem cell. *Nature* **439**: 84–88.
- Sharpless NE, DePinho RA. (2007). How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* **8**: 703–713.
- Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ *et al.* (2008). Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. *Nat Neurosci* **11**: 1024–1034.
- Shi Y, Chichung Lie D, Taupin P, Nakashima K, Ray J, Yu RT *et al.* (2004). Expression and function of orphan nuclear receptor *TLX* in adult neural stem cells. *Nature* **427**: 78–83.
- Shi Y. (2007). Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat Rev Genet* **8**: 829–833.
- Shizuru JA, Negrin RS, Weissman IL. (2005). Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. *Annu Rev Med* **56**: 509–538.
- Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature* **410**: 372–376.
- Siebold AP, Banerjee R, Tie F, Kiss DL, Moskowitz J, Harte PJ. (2010). Polycomb repressive complex 2 and Trithorax modulate *Drosophila* longevity and stress resistance. *Proc Natl Acad Sci USA* **107**: 169–174.
- Siebzehnrubl FA, Buslei R, Eyupoglu IY, Seufert S, Hahnen E, Blumens I. (2007). Histone deacetylase inhibitors increase neuronal differentiation in adult forebrain precursor cells. *Exp Brain Res* **176**: 672–678.
- Song HJ, Stevens CF, Gage FH. (2002). Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat Neurosci* **5**: 438–445.
- Song Z, Ju Z, Rudolph KL. (2009). Cell intrinsic and extrinsic mechanisms of stem cell aging depend on telomere status. *Exp Gerontol* **44**: 75–82.
- Spangrude GJ, Heimfeld S, Weissman IL. (1988). Purification and characterization of mouse hematopoietic stem cells. *Science* **241**: 58–62.
- Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D *et al.* (2006). Purification and unique properties of mammary epithelial stem cells. *Nature* **439**: 993–997.
- Su IH, Basavaraj A, Krutchinsky AN, Hobert O, Ullrich A, Chait BT *et al.* (2003). Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement. *Nat Immunol* **4**: 124–131.
- Su X, Paris M, Gi YJ, Tsai KY, Cho MS, Lin YL *et al.* (2009). TAp63 prevents premature aging by promoting adult stem cell maintenance. *Cell Stem Cell* **5**: 64–75.
- Sudo K, Ema H, Morita Y, Nakauchi H. (2000). Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med* **192**: 1273–1280.
- Sun G, Alzayady K, Stewart R, Ye P, Yang S, Li W *et al.* (2010). Histone demethylase LSD1 regulates neural stem cell proliferation. *Mol Cell Biol* **30**: 1997–2005.
- Sun G, Yu RT, Evans RM, Shi Y. (2007). Orphan nuclear receptor *TLX* recruits histone deacetylases to repress transcription and regulate neural stem cell proliferation. *Proc Natl Acad Sci USA* **104**: 15282–15287.
- Taraldsrud E, Groggaard HK, Solheim S, Lunde K, Floisand Y, Arnesen H *et al.* (2009). Age and stress related phenotypical changes in bone marrow CD34+ cells. *Scand J Clin Lab Invest* **69**: 79–84.
- Thomas T, Corcoran LM, Gugasyan R, Dixon MP, Brodnicki T, Nutt SL *et al.* (2006). Monocytic leukemia zinc finger protein is essential for the development of long-term reconstituting hematopoietic stem cells. *Genes Dev* **20**: 1175–1186.
- Thomas T, Voss AK, Chowdhury K, Gruss P. (2000). Querkopf, a MYST family histone acetyltransferase, is required for normal cerebral cortex development. *Development* **127**: 2537–2548.
- Tissenbaum HA, Guarente L. (2001). Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* **410**: 227–230.
- Tothova Z, Gilliland DG. (2007). FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. *Cell Stem Cell* **1**: 140–152.
- Tropepe V, Craig CG, Morshead CM, van der Kooy D. (1997). Transforming growth factor-alpha null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. *J Neurosci* **17**: 7850–7859.
- Trowbridge JJ, Snow JW, Kim J, Orkin SH. (2009). DNA methyltransferase 1 is essential for and uniquely regulates hematopoietic stem and progenitor cells. *Cell Stem Cell* **5**: 442–449.
- Trowbridge JJ, Orkin SH. (2010). DNA methylation in adult stem cells: new insights into self-renewal. *Epigenetics* **5**: 189–193.
- Uchida N, Tsukamoto A, He D, Frieria AM, Scollay R, Weissman IL. (1998). High doses of purified stem cells cause early hematopoietic recovery in syngeneic and allogeneic hosts. *J Clin Invest* **101**: 961–966.

- Valk-Lingbeek ME, Bruggeman SW, van Lohuizen M. (2004). Stem cells and cancer; the polycomb connection. *Cell* **118**: 409–418.
- van der Flier LG, Clevers H. (2009). Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* **71**: 241–260.
- van der Heide LP, Smidt MP. (2005). Regulation of FoxO activity by CBP/p300-mediated acetylation. *Trends Biochem Sci* **30**: 81–86.
- van der Horst A, Tertoolen LG, de Vries-Smits LM, Frye RA, Medema RH, Burgering BM. (2004). FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2-(SIRT1). *J Biol Chem* **279**: 28873–28879.
- van der Lugt NM, Domen J, Linders K, van Roon M, Robanus-Maandag E, te Riele H *et al.* (1994). Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. *Genes Dev* **8**: 757–769.
- Vaquero A, Loyola A, Reinberg D. (2003). The constantly changing face of chromatin. *Sci Aging Knowledge Environ* **2003**: RE4.
- Vaquero A, Scher M, Lee D, Erdjument-Bromage H, Tempst P, Reinberg D. (2004). Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell* **16**: 93–105.
- Vick NA, Lin MJ, Bigner DD. (1977). The role of the subependymal plate in glial tumorigenesis. *Acta Neuropathol* **40**: 63–71.
- Vidanes GM, Bonilla CY, Toczyski DP. (2005). Complicated tails: histone modifications and the DNA damage response. *Cell* **121**: 973–976.
- Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C *et al.* (2006). The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* **439**: 871–874.
- Visvader JE. (2009). Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes Dev* **23**: 2563–2577.
- Voog J, Jones DL. (2010). Stem cells and the niche: a dynamic duo. *Cell Stem Cell* **6**: 103–115.
- Wagers AJ, Christensen JL, Weissman IL. (2002). Cell fate determination from stem cells. *Gene Ther* **9**: 606–612.
- Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS *et al.* (2004). Role of histone H2A ubiquitination in Polycomb silencing. *Nature* **431**: 873–878.
- Waterstrat A, Oakley E, Miller A, Swiderski C, Liang Y, Van Zant G. (2008) In: Rudolph KL (ed). *Telomeres and Telomerase in Ageing, Disease, and Cancer*. Springer: Berlin-Heidelberg, 111–140.
- Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M. *et al.* (2004). Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **430**: 686–689.
- Wu H, Coskun V, Tao J, Xie W, Ge W, Yoshikawa K *et al.* (2010). Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. *Science* **329**: 444–448.
- Xing Z, Ryan MA, Daria D, Nattamai KJ, Van Zant G, Wang L *et al.* (2006). Increased hematopoietic stem cell mobilization in aged mice. *Blood* **108**: 2190–2197.
- Yalcin S, Zhang X, Luciano JP, Mungamuri SK, Marinkovic D, Vercherat C *et al.* (2008). Foxo3 is essential for the regulation of ataxia telangiectasia mutated and oxidative stress-mediated homeostasis of hematopoietic stem cells. *J Biol Chem* **283**: 25692–25705.
- Yang XJ, Seto E. (2007). HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* **26**: 5310–5318.
- Zammit PS, Heslop L, Hudon V, Rosenblatt JD, Tajbakhsh S, Buckingham ME *et al.* (2002). Kinetics of myoblast proliferation show that resident satellite cells are competent to fully regenerate skeletal muscle fibers. *Exp Cell Res* **281**: 39–49.
- Zencak D, Lingbeek M, Kostic C, Tekaya M, Tanger E, Hornfeld D *et al.* (2005). Bmi1 loss produces an increase in astroglial cells and a decrease in neural stem cell population and proliferation. *J Neurosci* **25**: 5774–5783.
- Zhang CL, Zou Y, He W, Gage FH, Evans RM. (2008). A role for adult TLX-positive neural stem cells in learning and behaviour. *Nature* **451**: 1004–1007.
- Zhang R, Zhang Z, Zhang C, Zhang L, Robin A, Wang Y *et al.* (2004). Stroke transiently increases subventricular zone cell division from asymmetric to symmetric and increases neuronal differentiation in the adult rat. *J Neurosci* **24**: 5810–5815.
- Zhao C, Deng W, Gage FH. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell* **132**: 645–660.
- Zhao X, Ueba T, Christie BR, Barkho B, McConnell MJ, Nakashima K *et al.* (2003). Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. *Proc Natl Acad Sci USA* **100**: 6777–6782.
- Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD *et al.* (2010). The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. *Cell* **140**: 280–293.
- Zhu Y, Guignard F, Zhao D, Liu L, Burns DK, Mason RP *et al.* (2005). Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer Cell* **8**: 119–130.
- Zindy F, Quelle DE, Roussel MF, Sherr CJ. (1997). Expression of the p16INK4a tumor suppressor versus other INK4 family members during mouse development and aging. *Oncogene* **15**: 203–211.