Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation

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Abstract | The regulation of stem cell self-renewal must balance the regenerative needs of tissues that persist throughout life with the potential for cell overgrowth, transformation and cancer. Here, we attempt to deconstruct the relationship that exists between cell-cycle progression and the self-renewal versus commitment cell-fate decision in embryonic and adult stem cells. Recent genetic studies in mice have provided insights into the regulation of the cell cycle in stem cells, including its potential modulation by the stem cell niche. Although the dynamics of the embryonic and adult stem cell cycles are profoundly dissimilar, we suggest that shared principles underlie the governance of this important decision point in diverse stem cell types.

Embryonic stem cell

ES cell. Cell lines that are derived from early-stage embryos (blastocysts). These cells can differentiate into all somatic lineages (pluripotent) and are capable of self-renewal when grown under the proper culture conditions.

Quiescence

For a cell, quiescence is the property of not being in the cell cycle. Most adult stem cell populations are maintained in this resting state.

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Complex biology and extraordinary clinical potential make stem cell biology an area of intense study. The long-term goals of this research range from a deeper understanding of cell-lineage determination and tissue organization to cellular therapeutics for degenerative diseases. Adult stem cells maintain tissue function and integrity throughout an organism's lifespan by replacing differentiated cells as they are lost because of attrition or damage^{1,2}. In order to perform this function, stem cells are endowed with a unique combination of pluri- or multilineage developmental potential — which is generally restricted to the lineages that comprise the tissue of origin — and the capacity to undergo self-renewing divisions. The loss of stem cell self-renewal capacity underlies certain degenerative diseases and is probably one component of the ageing process^{3,4}.

Although necessary, the capacity for self-renewal presents a risk to the organism on several levels. If not properly controlled, these self-renewing cells have the potential to generate large numbers of descendent cells that would disrupt tissue architecture. More concerning for long-lived organisms is the fact that self-renewing, highly undifferentiated stem cells provide a potential substrate for malignant transformation⁵. However, inadequate self-renewal results in tissue or organ failure, with dire consequences for the organism.

Because the process of self-renewal requires the coordination of cell-cycle progression and cell-fate choices (for example, commitment versus self-renewal), any effort to understand the regulation of stem cell self-renewal should include an analysis of the relationship between these processes⁶. In this Review, we explore this relationship between cell-cycle progression and the maintenance of mammalian stem cell function in both embryonic stem (ES) cells and adult stem cells. We begin by discussing the unique nature of the embryonic stem cell cycle and how their distinctive cell-cycle features are important mediators of self-renewal. We then explore the dynamics of the adult stem cell cycle, which differs greatly from that of ES cells. Our focus is on genetic studies in mice that reveal the importance of stem cell quiescence in the maintenance of stem cell function and provide insights into the molecular mechanisms that regulate progression through the cell cycle. Our synthesis of the findings from these studies leads us to identify commonalities in the approaches taken by embryonic and adult stem cells to solving the problem of self renewal. In addition, other genetic studies have begun to reveal how the role of the stem cell niche in the maintenance of stem cell function is related to the regulation of the cell cycle. Against this background, we conclude by discussing the relative importance of the active and passive mechanisms of developmental blockade that characterize stem cell self-renewal.

Cell-cycle regulation in embryonic stem cells

Overview of the cell cycle in ES cells. Murine embryonic stem (mES) cell growth in culture is characterized by extraordinarily rapid proliferation and a short cell cycle (11–16 hours), primarily owing to a reduction in the

Niche

A specific anatomic location composed of cellular and extracellular constituents that regulates how stem cells participate in tissue generation, maintenance and repair. It is a basic unit of tissue physiology, integrating signals that mediate the balanced response of stem cells to the needs of organisms.

duration of G1 phase (see BOX 1 for an overview of cellcycle regulation in general)⁷⁻¹¹. Whereas cyclin E– $CDK2$ activity is periodic in somatic cells, peaking at the G1 to S transition, mES cells have constitutive cyclin E–CDK2 activity that is independent of cell-cycle phase (FIG. 1). This effectively omits the early G1 phase by bypassing the restriction point (R point, see BOX 1) that separates early G1 from late G1. Furthermore, mES cells express low levels of the D-type cyclins and have almost no detectable CDK4-associated kinase activity.

Maintenance of mES cells generally requires the cytokine leukaemia inhibitory factor [\(LIF\)](http://www.expasy.org/uniprot/P09056). When LIF is withdrawn, mES cells lose their ability to self-renew and commit to specification and differentiation. Interestingly, LIF-withdrawal also induces a dramatic change in cellcycle control in these cells. Following LIF withdrawal,

Box 1 | The cell cycle

Cell proliferation occurs through a series of stages that are collectively termed the cell cycle. Classically, the cell cycle has been divided into four phases that are organized around the synthesis (S) phase and mitotic segregation (M) phase of the genome with two intervening gap phases (G1 and G2) preceding S and M phases, respectively. Progression through the cell cycle is highly regulated, particularly at the transitions from G1 phase to S phase and from G2 phase to M phase. These two 'checkpoints' assess cellintrinsic signals (for example, the integrity of the genome) and are governed by the cyclin dependent kinases CDK2 and CDK1, respectively.

In addition to these cell-intrinsic checkpoints, a combination of intrinsic and extrinsic signals regulate the passage from early to late G1 phase in most cells. This transition is called the restriction (R) point, and divides the G1 phase of the cell cycle into the mitogen-dependent early

 $G1$ phase and the mitogen-independent late $G1$ phase. In general, cells must be stimulated by mitogenic signals (for example, soluble growth factors) to traverse the G1 phase and enter into the cell cycle. The R point represents the 'point of no return' for the cell, after which the cell has committed to enter the cell cycle and mitogenic stimuli are no longer required. In the absence of mitogenic stimulation, cells can exit from the cell cycle during early G1 phase and enter a dormant, or quiescent, state called the G0 phase that is characterized by small cellular size and low metabolic activity.

Late G1 phase is characterized by mitogen-independent activation of cyclin E-CDK2 complex activity and concomitant hyperphosphorylation and inactivation of the Retinoblastoma tumour suppressor protein (RB). The transition from early to late G1 is mainly regulated by the D-type cyclins and their enzymatic counterparts, CDK4 and CDK6. Cyclin D–CDK4 and cyclin D–CDK6 complexes function through enzymatic and non-enzymatic mechanisms to partially inactivate Rb and activate expression of cyclin E. Upon reaching a threshold level of cyclin E-CDK2 activity, RB becomes fully inactivated by hyperphosphorylation and cyclin D–CDK4 and cyclin D–CDK6 activity is no longer required for the G1/S transition to occur.

Two families of cyclin-dependent kinase inhibitors (CDKIs) regulate the transition through G1 phase. In particular, members of the Ink4 family are direct inhibitors of the early G1 cyclin D-CDK4 and cyclin D-CDK6 complexes, and members of the CIP/KIP family (p21^{CIP}, p27^{KIP1}, p57^{KIP2}) are direct inhibitors of the late G1 cyclin E-CDK2 complexes. These inhibitors generally slow or prevent the transition through the cell cycle by blocking these activities. Paradoxically, p27 can actually enable the formation of cyclin D–CDK4 complexes, presumably by stimulating the cyclin D‑mediated transition to late G1 while, at the same time, preventing the transition across the R point by inhibiting cyclin E-CDK2 activity.

cyclin E expression comes under the control of the retinoblastoma protein (RB)-related family of pocket proteins and, therefore, requires the mitogen-induced activity of c[yclin D–CDK](http://www.expasy.org/uniprot/P30285)4 or cyclin D[–CDK6](http://www.expasy.org/uniprot/Q64261) complexes¹² (FIG. 1). This change causes mES cells to acquire the early G1 phase of the cell cycle.

MAPK signalling and ES cell differentiation. In most somatic cells, proliferation is dependent on mitogen activated protein kinase (MAPK) signalling, which facilitates the transition through the early G1 phase of the cell cycle13,14. MAPK signalling in somatic cells — particularly prolonged MAPK signalling — is also a potent inducer of differentiation and, in this way, links proliferation and developmental progression in somatic cells^{15,16}. The absence of early G1 from mES cells allows them to avoid the differentiation-inducing effects of certain mitogenic signalling pathways that are active during early G1 in other cells. For instance, signalling through the MAPK pathway inhibits mES cell self-renewal and promotes commitment to differentiation^{8,17}. Growing cells in the presence of MAPK inhibitors reinforces this concept because it enhances self-renewal of mES cells by preventing their differentiation. Furthermore, *Erk2* (also known as *[Mapk1](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=26413&ordinalpos=5&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*) null ES cell lines (*Erk2*–/–) have defects in their ability to undergo differentiation¹⁸. As MAPK signalling is the primary (although not exclusive) mechanism by which cyclin D expression is induced, dependence on elevated cyclin D expression — and therefore MAPK signalling — to proceed through the cell cycle would probably make mES cells more vulnerable to MAPK-induced differentiation.

RB is hyperphosphorylated in self-renewing ES cells. Further evidence that the R point is essentially absent from mES cells and is necessary for their differentiation comes from studies on the RB-related family of pocket proteins in ES cells. Throughout the cell cycle of mES cells, RB is hyperphosphorylated and therefore inactive^{7,8}. Because RB activity is a defining component of the R point, the lack of active RB suggests that these cells lack an R point, presumably owing to constitutive cyclin E-CDK2 activity. Furthermore, mES cells that lack all three members of the *Rb* gene family (*[Rb](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=19645&ordinalpos=2&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*, *[p107](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=19650&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)* and *[p130](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=19651&ordinalpos=6&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*) are incapable of undergoing proper differentiation and cell-cycle withdrawal¹⁹. Interestingly, single and double knockout ES cells showed no defect in differentiation, demonstrating the ability of RB family members to compensate for one another in this setting.

These data strongly link the acquisition of the R point (through the loss of constitutive cyclin E–CDK activity and accumulation of hypophosphorylated RB) with the capacity to undergo multilineage differentiation. This is consistent with the concept that early G1 phase is the main window during which extracellular stimuli (for example, growth factors and morphogens) can affect cellfate decisions, particularly the decision to enter the cell cycle and/or commit to differentiation^{20,21}. In summary, the mES cell cycle seems to lack the mitogen-dependent early G1 phase of the cell cycle owing to constitutive cyclin E–CDK2 activity. By eliminating the requirement

Nature Reviews | **Genetics** Figure 1 | **The cell cycle in embryonic stem cells.** The cell cycle of embryonic stem (ES) cells is shortened relative to that of most other cells (~11–16 hours as opposed to ~24 hours). An abbreviated G1 phase is responsible for the difference in cell-cycle length. For most cells, the transition through early G1 phase requires the mitogeninduced accumulation of cyclin D, resulting in the hyperphosphorylation of the retinoblastoma tumour suppressor protein (RB) by cyclin D–CDK4 or cyclin D–CDK6 complexes (D/4,6). Inactivation of RB by hyperphosphorylation results in the mitogen-independent activity of cyclin E–CDK2 complexes, the defining characteristic of late G1 phase. In ES cells, cyclin E-CDK2 (E/2) is constitutively active throughout the cell cycle, which allows the transition of ES cells from M phase directly to late G1. The resulting absence of the cyclin D-dependent early G1 phase shortens the G1 phase and the entire cell cycle. Upon commitment of ES cells, the cell-cycle length is extended as cyclin E-CDK2 activity comes under the control of cyclin D–CDK4 and phosphorylated RB. + refers to cyclin–CDK activity: +/-, negligible; +, low; ++, intermediate; +++, high.

for MAPK-induced cyclin D expression, ES cells uncouple cell-cycle traversal from differentiation, allowing for efficient *in vitro* self-renewal.

Morphogens

A small set of secreted, developmental-regulatory signalling molecules that have the unique property of graded activity. That is to say, these molecules tend to form concentration gradients and can have different biological effects at different concentrations.

Comparison to human ES cells. Although less is known about the cell-cycle regulation of human ES (hES) than mES cells, some similarities and differences have been identified. Even though hES cells are grown under different conditions than mES cells (because hES cells do not respond to LIF), both hES cells and mES cells have a shortened cell cycle owing to the truncation of G1 (Ref. 10). The molecular regulation of the transition through G1 is less well defined in hES and primate ES (pES) than it is in mES cells. The expression of cyclin D2 and *CDK4* seem to be upregulated upon entry into G1, suggesting that these cells might be dependent on

D‑type cyclins; however, no functional dependence on this activity has been shown and the expression of cyclin E was not assessed throughout the cell cycle¹⁰. Conversely, pES cells are similar to mES cells in terms of having cell-cycle-independent expression of cyclin E, constitutive hyperphosphorylation of RB and serum and MAPK-independent cell-cycle progression⁹. Although there are some differences in the regulation of the cell cycle between hES, pES and mES cells, self-renewal of the three cell lines is characterized by a shortened early G1 phase.

Cell-cycle regulation in adult stem cells

Adult stem cell quiescence. In contrast to ES cells, a hallmark feature of adult stem cells is their relative proliferative quiescence. Haematopoietic stem cells (HSCs), which are the most extensively studied adult stem cell population in both humans and mice (BOX 2), are largely in the G0 or G1 phase of the cell cycle and, of these cells, the large majority have exited the cell cycle completely. Approximately 75% of the most primitive long-term repopulating haematopoietic stem cells (LT-HSCs) are resting in G0(Ref. 22). Interestingly, primate HSCs seem to be even more quiescent than murine HSCs²³.

It is widely accepted that the quiescent state is a functionally important characteristic of adult stem cells. This view has developed largely from experience with the haematopoietic system. Although these cells are often considered immortal, HSC function clearly has limitations and these limitations are frequently hastened by proliferative stress^{24,25}. For example, HSCs are capable of reconstituting the haematopoietic system following transplantation. However, after serial transplantation, HSCs gradually decline and are eventually exhausted²⁶⁻²⁹ (BOX 2).

The link between proliferation and self-renewal of HSCs has been further explored using mouse strains that have intrinsically different life spans. The proliferative rate of HSCs in these various mouse strains was strongly anti-correlated with the maximum longevity of the strain^{30,31}. Furthermore, HSCs that are derived from young animals from shorter-lived mouse strains with more proliferative HSCs (for example, the DBA/2 strain) reconstituted the haematopoietic system more efficiently than HSCs that are derived from young animals from longer-lived strains with less proliferative HSCs (for example, the C57B6 strain). However, HSCs from old animals of these strains showed the opposite result, suggesting that the HSCs from faster cycling animals might become functionally exhausted more rapidly than those in animals with slower cycling HSCs.

Proliferation results in stem cell exhaustion. Support for the suggestion that proliferation can lead to the exhaustion of stem cell function comes from a number of genetic models in which there is increased proliferation of stem cells or a stem cell-containing primitive cell population (TABLE 1). In most of these models, the result is long-term loss of stem cells and increased susceptibility to stress-induced exhaustion. One of the first publications to suggest that stem cell proliferation itself results in

The study of adult stem cells is hampered by a number of factors including low abundance, dependence on functional assays, lack of clear methods to identify them and the inability to maintain their 'stemness' in culture. Although certainly imperfect, the haematopoietic system has proven the most tractable tissue in adult mammals in which to study stem cell function. Two of the most important reasons for this have been that the cellular components of the haematopoietic system have been identified and can be analysed or purified based on their cell-surface markers, and various functional assays have been developed for the detailed study of haematopoietic progenitors and stem cells.

The cellular components of the haematopoietic system have been identified

Over the past several years, most cellular components of the primitive and mature haematopoietic compartments have been defined¹¹². The major breakthrough that enabled this is flow cytometry using monoclonal antibodies directed at cell-surface markers. Multipotential and lineage-restricted progenitors as well as haematopoietic stem cells can be highly purified based on a particular constellation of cell-surface markers. For instance, most (if not all) bone-marrow cells that express the proteins C-KIT and SCA1 and lack any of a set of lineage-specific markers on their cell surface are multipotential cells, including long-term repopulating stem cells (LT-HSCs), short-term repopulating stem cells (ST-HSCs) and multipotential progenitors (MPPs). LT-HSCs can be more precisely identified within this population by excluding cells that express [CD34](http://www.expasy.org/uniprot/Q64314) and FLT3, or including only those cells that express [CD150](http://www.expasy.org/uniprot/Q9QUM4) (REFS 94,113-115). This constellation of cell-surface markers is referred to as a cell's immunophenotype, and is often used as a means of purifying specific cell populations such as LT-HSCs. Another approach to the identification and purification of adult stem cells relies on their ability to efflux particular dyes such as Hoechst 33342 or Rhodamine 123 (Refs 116,117). For instance, when bone-marrow cells are stained with Hoechst 33342, flow cytometric analysis identifies a small population of cells with low fluorescence, owing to efflux of the dye. These 'side population' cells are highly enriched for HSCs.

Assays to assess haematopoietic stem cell function

Although the immunophenotype is useful for the analysis and purification of haematopoietic cell populations, the only way to truly assess the function of a cell population is through functional assays. Various functional assays have been developed that test different aspects of haematopoietic stem cell (HSC) and progenitor cell functions. For instance, the colony forming cell (CFC) assay plates cells at low density in a semi-solid medium in the presence of a particular cocktail of cytokines and then counts the number and type of colonies that form. The CFC assay tests the progenitor number and identity and has been a mainstay of haematopoiesis research for decades. There are several tests of HSC function, including the competitive repopulation assay, which tests the ability of an experimental cell population (for example, from a knockout animal) to provide long-term bone-marrow engraftment in the presence of a congenic control population of bone-marrow cells (distinguished by cell-surface markers). To assess the number of stem cells, limiting dilution transplantation studies are performed and the number of LT-HSCs is back-calculated using Poisson statistics. Another important assay is the serial transplantation assay. This assay is used to test the ability of HSCs to undergo self-renewal; the recipient of a bone-marrow transplant, Recipient 1 (whose own HSCs were completely ablated before the transplant), can only serve as a successful donor for a subsequent (serial) transplant if the HSCs from the original donor, Donor 1, were able to undergo substantial self-renewal within Recipient 1 following the original transplant. The number of serial transplants that the original donor's bone marrow can perform successfully is a measure of the self-renewal capacity of its HSCs.

Table 1 | A summary of the main phenotypes of the genetic models discussed in the text

CRA, competitive repopulation assay; HSC, haematopoeitic stem cell; KO, knockout; NSC, neuronal stem cell; ROS, reactive oxygen species.

exhaustion of stem cell function reported the HSC phenotype from *p21* (also known as *[Cdkn1a](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=12575&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*) knockout animals. p21 is a cyclin-dependent kinase inhibitor (CDKI) that is known to inhibit cyclin E-CDK2 activity, and is an important downstream target of the *[p53](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=22059&ordinalpos=2&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)* tumoursuppressor gene^{32,33}. In $p21^{-/-}$ animals, the proportion of primitive haematopoietic cells in the G0 phase of the cell cycle was reduced, and the number of very primitive cells that gave rise to long-term multipotent colonies in culture was increased³⁴. Counter-intuitively, bone marrow in *p21*–/– animals was more rapidly exhausted as compared with wild type, both by serial transplantation and repeated administration of the myelotoxic agent 5-fluorouracil (5-FU). Analysis of neurosphere number and serial replating produced similar results and also demonstrated the loss of neurosphere-initiating cells with ageing³⁵. Interestingly, $p21^{-/-}$ animals on the C57B6 background had a substantially less impressive HSC phenotype, although certain aspects of the phenotype are maintained³⁶. These variations in the $p21^{-/-}$ phenotype could be a result of the strain-dependent cell-cycle differences in stem cell populations discussed above.

HSCs that are null for growth factor independent 1 (*[Gfi1](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=14581&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*), phosphatase and tensin homologue (*[Pten](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=19211&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*) and forkhead box (*Foxo*) proteins 1, 3 and 4 all show a similar phenotype to that of *p21*–/– HSCs with regard to cell cycling and exhaustion. In young mice, *Gfi1*–/– HSCs are increased in number (as determined by their immunophenotype) and are more proliferative than their wild-type counterparts³⁷. However, in the competitive repopulation assays (CRAs), the *Gfi1*–/– HSCs performed poorly — only partially reconstituting peripheral blood even at high cell doses. However, in the absence of competitor bone marrow they were able to reconstitute all lineages. These results demonstrate that although *Gfi1*–/– HSCs have stem cell potential, they are profoundly deficient when compared with wild-type HSCs. The authors demonstrated that *Gfi1*–/– HSCs expressed the

Neurosphere

A cluster of neurogenic cells that is generated from a single neural stem cell or progenitor cell when they are cultured in a semi-solid medium that contains appropriate neurotrophic growth factors.

Intestinal polyposis

A condition that is

characterized by the presence of a multitude of benign polyps within the intestine; although most polyps are benign, these intestinal polyps have the potential to become malignant. *p21* gene at a fraction of the level of wild-type HSCs, providing a plausible mechanism for the HSC defect seen in *Gfi1*–/– animals (FIG. 2).

The *P[ten](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=5728&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)* gene, which encodes a negative regulator of phosphatidylinositol 3-kinase (PI3K) signalling, is a tumour-suppressor gene that is commonly deleted in human malignancies³⁸. Conditional deletion of *Pten* from adult HSCs induced the expansion of immunophenotypic HSCs over the course of 5–7 days, but eventually resulted in the depletion of immunophenotypic HSCs by 24–39 days post-deletion^{39,40}. In addition, *Pten^{-/-}* HSCs were unable to provide long-term reconstitution in competitive repopulation assays (CRAs). Paradoxically, *Pten* deletion also resulted in myeloproliferative disease and, eventually, transplantable leukaemias. The simplest explanation of these data is that PTEN is necessary for the maintenance of quiescence in the HSC compartment; upon losing PTEN, HSCs enter the cell cycle causing short-term myeloproliferative disease that ends in HSC exhaustion by approximately one month. A small subset of *Pten*–/– HSCs might avoid exhaustion and acquire further mutations that result in uncontrolled proliferation and frank leukaemia. The fact that *Pten* deletion results in intestinal polyposis suggests that it might also regulate intestinal stem cell cycling⁴¹.

The FoxO family of transcription factors (TFs) are known to regulate a number of cellular processes including cell-cycle arrest and resistance to cell stress. Although individual *Foxo* knockouts had no haematopoietic phenotype, an inducible triple knockout animal — *Foxo1*/*3*/*4*flox/flox in *Mx1*-Cre mice (Cre recombinase under the control of the interferon-inducible *Mx1* promoter) — did reveal haematopoietic defects⁴². As with the preceding models, inducible deletion of *Foxo1*, *3* and *4* results in increased cycling of the primitive

number of genetic perturbations result in adult stem cell proliferation. The majority of Figure 2 | **Loss of quiescence results in loss of function in adult stem cells.** A those that have been described also result in the loss of stem cell function over time. The first demonstration of this relationship was the $p21^{-/-}$ animal in which the proliferative rate of two stem populations (haematopoietic stem cells (HSCs) and neural stem cells) is increased and their self-renewal capacity seems to be reduced. Similarly, *Gfi1^{-/-}* HSCs are more proliferative but compete poorly with wild-type HSCs in competitive repopulation assays. Gfi1 has been shown to regulate the expression of *p21*, providing a potential mechanistic explanation for the phenotype. The deletion of three forkhead box protein (FoxO) family members resulted in reactive oxygen species (ROS)-dependent increase in HSC proliferation with eventual HSC exhaustion. Pten^{-/-} HSCs are also hyperproliferative and subject to exhaustion through a rapamycin-sensitive mechanism downstream of phosphatidylinositol 3-kinase. Myeloid Elf1-like (Mef) is a negative regulator of quiescence. Gfi1, growth factor independant 1; PTEN, phosphatase and tensin homologue.

lineage– /c-Kit+/Sca1+ immunophenotype (LKS) population (which includes LT-HSCs, short-term repopulating HSCs (ST-HSCs) and multipotent progenitors (MPPs)) and a reduction in the number of immunophenotypic LT-HSCs. In addition to being decreased in number and increased in cell-cycle entry, the *[Foxo1,](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=56458&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum) [Foxo3](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=219038&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)* and *[Foxo4](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=54601&ordinalpos=2&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)* null HSCs accumulated elevated levels of reactive oxygen species (ROS). The functional relevance of this was demonstrated by the almost complete reversal of the phenotype by treating animals with the antioxidant *N*-acetylcysteine. These data suggest that ROS are important regulators of HSC quiescence and maintenance. More recently, similar data were reported for HSCs from *[Foxo3a](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=56484&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)* single knockout mice. In particular, maintenance of the HSC pool was defective in *Foxo3a*–/– animals concomitant with increased ROS, increased activation of p38 and defective maintenance within the HSC population⁴³. As PI3K is known to inhibit the activity of members of the FOXO family, and PTEN is a negative regulator of PI3K, PTEN might regulate stem cell function by indirectly activating FOXO TFs⁴⁴ (FIG. 2).

Negative regulation of quiescence. Another genetic model that illustrates the relationship between adult stem cell quiescence and the maintenance of stem cell function is the conditional knockout of the TF myeloid Elf1-like (Mef, also known as [Elf4](http://www.expasy.org/uniprot/Q9Z2U4)) (FIG. 2). Following the conditional deletion of *Mef* from the adult haematopoietic system, immunophenotypic HSCs (LSK34– Flk2– and LSK-SP bone marrow cells) increase in number and HSC function is increased in a cell autonomous manner⁴⁵. These increases in HSC number and function coincide with increased quiescence of the primitive LKS population. Furthermore, *Mef^{-/-}* HSCs recovered more rapidly from myeloablative doses of 5-FU, apparently owing to their reduced sensitivity to the cell-cycle-specific toxicity of 5‑FU and their expanded population of quiescent primitive haematopoietic cells, including HSCs. Taken together, these data suggest that MEF is an important negative regulator of HSC quiescence and its deletion results in increased quiescence and the paradoxical HSC expansion that results from reduced HSC exhaustion.

Stem cell exhaustion: exceptions to the rule. The preponderance of evidence supports the concept that stem cell proliferation is detrimental to the long-term function of adult stem cells, particularly in the case of HSCs. However, there are genetic models in which HSC proliferation does not seem to result in stem cell exhaustion. Careful consideration of these atypical cases might provide some insight into the mechanistic relationship between the cell cycle and stem cell function.

The *p18INK4c* gene (also known as *[cdkn2c](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=12580&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*) encodes a member of the INK4 family of cyclin D–CDK4 and cyclin D–CDK6-specific CDKIs. Unlike the case of the *p21* knockout, *p18INK4c*–/– animals have increased numbers of immunophenotypic HSCs and increased HSC function — as defined by the CRA — even in the face of increased HSC proliferation^{46,47}. The increased HSC function was maintained after a secondary transplant, indicating that these HSCs were not exhausted

cell cycle consists of the period of mitogen-dependent stimulation of cyclin D–CDK4 activity (represented by taps Figure 3 | **A possible mechanism linking early G1 transit with commitment or self-renewal.** Early G1 phase of the into the Proliferation barrels), hyperphosphorylation of RB and activation of cyclin E–CDK2. When the cell reaches a threshold of cyclin E–CDK2 activation at the R point (larger arrows), cell-cycle progression occurs independently of external inputs. Certain proliferative stimuli (for example, mitogen activated protein kinase (MAPK) signalling) simultaneously induce developmental commitment (represented by a tap into the Commitment barrels). This model postulates that if the commitment barrel reaches its threshold, the cell is committed to development and, therefore, is unable to undergo self-renewal. However, stimuli that preferentially stimulate proliferation without pushing the cell toward commitment (for example, morphogen signalling and HoxB4) and those that lower the R point threshold of cyclin E–cdk2 activity (for example, the *p18* knockout and *Hoxb4* overexpression) promote self-renewal. D, cyclin D–CDK4 activity; ?, undefined downstream mediators of MAPK signalling that induce differentiation.

following the stress of transplantation. Therefore, loss of p18INK4c enables preservation or expansion of HSCs despite increased proliferation. One attractive interpretation is that because the different CDKIs regulate specific transition points in the cell cycle; the absence of p18INK4c might facilitate the transition through a stage of the cell cycle during which HSCs are susceptible to exhaustion (by commitment to differentiation, senescence or apoptosis), whereas that stage is intact in the $p21^{-/-}$ animals (FIG. 3). In other words, the absence of p18INK4c might permit the rapid transition to late G1, perhaps by lowering the threshold of cyclin D-CDK4 activity that is required to activate cyclin E–CDK2 and enter late G1 phase.

Another model in which HSC developmental potential is maintained in the face of increased proliferation is the overexpression of *[Hoxb4](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=15412&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*. The HoxB4 protein is a member of the homeobox family of developmentalregulatory TFs, and is expressed in primitive haematopoietic cells including HSCs. Although its role in normal haematopoiesis is unclear owing to the redundant nature of Hox genes, enforced expression of *Hoxb4* results in extraordinary *in vitro* and *in vivo* expansion of murine and human HSCs⁴⁸⁻⁵³. The *in vivo* expansion provides a competitive advantage for *Hoxb4*-expressing HSCs in CRAs and results in the complete reconstitution of the HSC compartment following bone-marrow transplantation (BMT), as compared with the partial reconstitution seen following BMT with wild-type HSCs. HoxB4 also seems to be a crucial regulator of the transition from primitive to definitive haematopoiesis, potentially regulating the expansion of definitive HSCs in the fetal liver54–56. In culture, HoxB4 does not seem to shorten the cell cycle *per se*, but rather maintains a relatively rapid proliferative rate throughout the culture period, preventing the extension of the cell cycle that normally occurs as primitive haematopoietic cells are cultured for several days⁵⁷.

Although few downstream target genes are known for Hox family genes, cell-cycle regulatory genes seem to be relatively common direct and indirect Hox targets, particularly regulators of the transition through

Senescence

The irreversible exit from the cell cycle. This is often caused by passaging through many rounds of cellular proliferation (replicative senescence) or certain forms of cellular stress and is often linked to ageing.

the G158,59. HoxB4 in particular has been shown to upregulate the expression of cyclin D2, D3 and E in primitive haematopoietic cells, consistent with its effects on the cell cycle⁶⁰. Although the specific mechanisms by which *Hox* genes regulate development are uncertain, it is clear that they can regulate organ size, probably through direct and indirect regulation of the G1 phase of the cell cycle.

In summary, several *Hox* genes, particularly *Hoxb4*, are capable of expanding HSCs both *in vitro* and *in vivo* without compromising their function. Although the mechanism responsible for this effect is unknown, the ability to maintain a short cell cycle and their known ability to stimulate the expression of early G1 regulators is consistent with a role in facilitating the transition from early to late G1 (FIG. 3).

Cell cycle-mediated regulation of cell fate: possible

mechanisms. Owing to limitations in the data that are currently available, it is not possible to construct a detailed model depicting the regulation of adult stem cell function by the various cell-cycle regulatory proteins. However, we can make some general statements that are consistent with the currently published data described above.

First, most genes that regulate the entry of quiescent HSCs into the cell cycle (for example, *p21*, *Gfi1*, *Pten*, *Mef* and members of the *Foxo* family) also have an effect on stem cell function. Because the G1 phase of the cell cycle — particularly the early G1 phase — seems to be a sensitive period during which cell-fate decisions are made20,21,61,62, genes that prevent HSCs from entering the cell cycle will tend to preserve their long-term function by reducing their exposure to exhaustion-inducing stimuli in early G1 phase. By contrast, genes that stimulate cell-cycle entry will increase exposure to the potentially perilous early G1 phase. In particular, the dependence on prolonged signalling through MAPK pathways to promote G1 progression might result in differentiation, senescence or apoptosis, rather than stem cell self-renewal¹⁵

Second, genes that facilitate the transition through the G1 phase of the cell cycle, particularly through early G1, might have an impact on the likelihood of particular cell-fate decisions by altering the time spent in the sensitive period. For instance, p18 might normally increase the barrier to transitioning from early to late G1 by blocking cyclin D-CDK4 activity and therefore its absence could increase the self-renewal of stem cells by facilitating that transition. Similarly, HoxB4 might prevent the extension of G1 that normally occurs in differentiating stem cells, thereby reducing the likelihood of differentiation. Conversely, p21 might regulate the transition from late G1 to S phase, in which case the absence of p21 would increase cycling without affecting the duration of early G1. Because they retain a relatively normal early G1 phase and are more actively cycling, *p21*–/– cells would be more exposed to early G1 signals that induce exhaustion (for example, MAPK).

Third, the re-entry of cells into G1 phase of the cell cycle from G0 might occur near the R point, effectively bypassing a portion of early G1. This would provide another potential mechanism (bypassing a portion of early G1) by which stem cell quiescence might enhance stem cell self-renewal.

Although embryonic and adult stem cell-cycle dynamics are highly divergent, we postulate that they might represent different ways to achieve the goal of selfrenewal by avoiding the early G1 phase of the cell cycle. ES cells accomplish this by maintaining constitutive cyclin E-CDK2 activity, thereby eliminating or greatly shortening early G1 and increasing the rate of proliferation. Conversely, adult stem cells are maintained in the quiescent state and therefore rarely transit through early G1. In addition, the microenvironmental niche of the adult stem cell might provide crucial signals that facilitate the transition through early G1 without inducing exhaustion.

Responding to ageing and stress

The response of adult stem cells to stress and ageing typically results in the diminution of stem cell function. Certain aspects of these phenomena seem to involve cellcycle regulation or cell-cycle regulators. For instance, p16 is an important regulator of cellular senescence — a state of permanent cell-cycle withdrawal63. The expression of *p16* is relatively low in the HSCs of young mice and is upregulated with ageing or in response to cellular stresses, including BMT and ROS^{4,64}. In wildtype animals, the number of immunophenotypic HSCs increases with age but HSC function is impaired. In particular, the HSC compartment from old animals is more rapidly exhausted by serial transplantation than that of young animals. Interestingly, ageing of *p16*–/– HSCs has the opposite effect, with *p16*–/– HSCs from old animals substantially outperforming young *p16*–/– HSCs in serial transplantation assays4 . In fact, old *p16*–/– HSCs perform as well as young wild-type HSCs in this assay. This result demonstrates that although immunophenotypic HSCs accumulate to a greater extent in older mice, their functional capacity is compromised in a p16-dependent manner. Similar results were obtained in studies of *p16*–/– neuronal stem cells and pancreatic islets, revealing a generalizable role for p16 in the regulation of stem cell and progenitor cell ageing65,66.

Also of note was the fact that young *p16*–/– HSCs were more rapidly depleted by serial transplantation than were young wild-type HSCs. Because *p16* expression is induced in response to serial transplantation of young (and old) HSCs, *p16* expression might protect HSC function in the setting of proliferative stress. Although the presence of the *p16* gene had no effect on 5-bromo-2-deoxyuridine incorporation 6 weeks after transplantation of young HSCs, *p16* expression might protect HSCs from the intense proliferative stress in the immediate days and weeks following transplantation by maintaining a population in the quiescent state.

In summary, *p16* expression constrains the number and function of HSCs in old wild-type animals, probably in an effort to prevent leukaemias and lymphomas from developing through the induction of senescence. This tumour-suppressive function of p16 is an evolved

Nature Reviews | **Genetics** within a particular microenvironmental niche. This niche is mainly composed of the local cell populations and the Figure 4 | **A model of niche regulation of stem cell proliferation and cell-fate decisions.** Adult stem cells reside secreted and cell-surface-bound molecules that these cells generate. In addition, the metabolic state of the niche (for example, the oxygen tension) is another local parameter that will affect the behaviour of stem cells. Among the microenvironmental components that are thought to be important regulators of stem cell function are morphogens (including Notch, Wnt and Hedgehog), cell–cell and cell–extracellular matrix (ECM) adhesion molecules (for example, cadherins and integrins) and hypoxia. These components might influence the entry of cells into the cell cycle (**a**), the mechanisms of proliferative stimulation (**b**) and the imposition of developmental blockade by Polycomb-group-complex gene silencing or translational repression (**c**). D, cyclin D–CDK4 activity; frz, frizzled; N-cad, N-cadherin; Trx, Trithorax.

balance between two important needs of the organism — long-term HSC function versus cancer prevention. However, p16 activity seems to prevent the exhaustion of young HSCs in the setting of proliferative stress, possibly by restraining cell-cycle progression in its more typical role as a cell-cycle inhibitor.

The role of the niche

It has become increasingly clear that the microenvironmental niche of the adult stem cell is an important regulator of its function, particularly cell-fate decisions such as self-renewal and commitment⁶⁷⁻⁷⁶. A number of genetic models implicate niche residence and niche evacuation as important regulators of stem cell phenotype (FIG. 4).

Endosteal niche

A location in the bone marrow where HSCs localize adjacent to osteoblasts. Osteoblasts regulate HSC function through secreted signalling molecules and direct cell–cell contact.

Induction of quiescence. One potential mechanism by which the stem cell niche maintains stem cell function is by the induction of quiescence. As discussed above, there is a strong correlation between stem cell proliferation and the loss of stem cell function over time. Evidence is accumulating that implicates the niche in the maintenance of stem cell quiescence. For instance, the Rho GTPase CDC42 regulates a number of cellular activities including actin polymerization and cell adhesion. Conditional knockout of *Cdc42* resulted in increased cycling of primitive haematopoietic cells (with the LKS immunophenotype) and a reduction in the number of LT-HSCs⁷⁷. Further analyses into the molecular and cellular mechanisms responsible for the phenotype suggested that the primary defect in these cells was one of cell adhesion to the niche, as evidenced by poor homing to the endosteal niche following transplantation, and reduced adhesion to and migration through stromal cells *in vitro.* The reduced adhesion and migration phenotype correlated with aberrant actin organization, a mechanism that is consistent with the known function of CDC42. These data suggest that the HSC phenotype in *Cdc42*–/– animals is primarily a result of aberrant niche localization.

The phenotype of *[Tie2](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=21687&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*–/– HSCs is also suggestive of a role for the niche in the imposition of quiescence on HSCs78. Tie2 (also known as TEK), a transmembrane tyrosine kinase receptor for the ANG family of soluble ligands, is expressed on the cell surface of HSCs and endothelial cells. *Tie2^{-/-}* HSCs are more actively cycling and are more rapidly depleted both *in vitro* and *in vivo*. Arai et al. demonstrated that TIE2 was required for the maintenance of HSCs *in vivo* and *in vitro*. The inhibition of TIE2-ANG1 interaction (by the addition of TIE2-Fc chimeric protein) in co-cultures of HSCs and bone marrow stromal cells prevented the burrowing of HSCs under the stromal cells (known as cobblestoning), a behaviour that is characteristic of the very primitive haematopoietic cells that initiate long-term haematopoietic cultures. Conversely, activation of Tie2 signalling by exogenous Ang1 resulted in increased cobblestoning, increased expression of N‑cadherin (a key mediator of HSC– osteoblast interactions *in vivo*) and reduced expression of genes associated with HSC mobilization. These data also suggest that the adherent cobblestoning phenotype induced by TIE2–ANG1 signalling precedes, and probably causes, HSC quiescence.

Although direct causality can be difficult to demonstrate, these data suggest that the association of HSCs with their niche is crucial for the imposition of quiescence and long-term functional maintenance of HSCs. Niche occupancy, therefore, is a likely mechanism by which HSC function is regulated in the wild-type situation.

Prevention of differentiation. To maintain their numbers, adult stem cells must occasionally enter the cell cycle in order to undergo self-renewing cell divisions. Another potential mechanism by which the microenvironment of the stem cell niche might prevent stem cell exhaustion is encouraging self-renewal by preventing commitment to differentiation. As discussed above and elsewhere, most proliferative signals seem to concomitantly stimulate the commitment to differentiation. For instance, through the regulation of D-type cyclins and the c-Myc TF, MAPK signalling is a crucial regulator of progression through early G1 (REFS 14,79). However, MAPK signalling, especially prolonged MAPK signalling, is also implicated in the commitment of many cells to differentiation^{15,16}. c-myc is another proliferative signal that can result in stem cell commitment, probably by inducing egress from the niche⁸⁰.

Whereas ES cells seem to avoid MAPK-induced commitment by proceeding directly to late G1 through the constitutive activation of cyclin E-CDK2, the adult stem cell cycle does not have this option. Rather than being perpetually in cycle, adult stem cells are typically quiescent and presumably re-enter the cell cycle in the early G1 phase. However, morphogens within the niche might facilitate the transition through early G1 with little or no dependence on MAPK signalling. As discussed above, MAPK signalling stimulates cell-cycle progression mainly by activating the expression of D-type cyclins while concomitantly activating differentiation, often through unknown mechanisms. Furthermore, MAPK signalling can induce *c*-*Myc* expression, which is crucial for progenitor expansion but results in the loss of stem cells from the niche.

Signalling by morphogens, particularly through the Wnt, Notch and Hedgehog pathways, is known to be important for the self-renewal of many adult stem cell types⁸¹⁻⁸⁸. Although the expression of these signalling molecules is considered to be highly restricted, various stem cell niches have been shown to express some combination of these three morphogen families. Each of these signalling pathways has been directly demonstrated to shorten the G1 phase of the cell cycle in particular contexts⁸⁹⁻⁹¹. One intriguing hypothesis is that morphogen signalling in the niche provides a cytokine and MAPK-independent mechanism, by which stem cells can be induced to enter the cell cycle, that does not simultaneously stimulate the cell to commit to differentiation. Finally, although a clear relationship between the niche and gene silencing mediated by Polycomb group (PcG) proteins (see below) has not been demonstrated, the morphogen Hedgehog has been shown to induce the expression of *[Bmi1](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=12151&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum)*, which encodes a Polycombcomplex protein, and stimulate mammary stem cell self-renewal in a BMI1-dependent manner⁹². Although these data are by no means definitive, niche-dependent signalling (for example, by morphogens) might be able to directly silence the expression of developmental regulatory genes and, thereby, prevent stem cell commitment and differentiation.

Recent data suggest that HSCs can exist in two different niches within the bone marrow: one adjacent to osteoblasts (endosteal) and the other adjacent to endothelial cells (vascular)93–95. However, the two dimensional nature of the experimental approaches that have characterized this phenomenon make it difficult to clearly demonstrate that these are separate niches. Therefore, although stem cells have been identified at locations near bone and blood vessels, whether these are distinctive sites, whether they each serve as true niches and whether stem cells occupying these sites are comparable are important questions that remain to be answered experimentally.

Self-renewal: active versus passive mechanisms

There are two fundamental ways in which cell division can result in self-renewal rather than commitment and differentiation. Commitment and differentiation can be actively prevented in self-renewing cells, or self-renewal might be the result of proliferative stimuli that fail to concomitantly stimulate the commitment and differentiation programme. The latter hypothesis presupposes that most proliferative signals do, in fact, concomitantly stimulate differentiation and much of the data presented above support this hypothesis. However, there are data that suggest that stem cell differentiation is being actively suppressed in self-renewing cells. The strongest evidence for this concept comes from the study of the epigenetic regulation of developmental regulatory genes in ES cells (reviewed in Ref. 96). Genome-wide analyses in ES cells have revealed a unique chromatin state at many developmental genes, characterized by coincident activating and silencing histone modifications⁹⁷. Most of these 'bivalent' genes are

Bone marrow stromal cells

Non-haematopoietic cellular components of the bone marrow that regulate haematopoiesis and constitute part of the HSC niche.

Polycomb group (PcG) proteins

A family of developmentally important proteins that silence gene expression though a chromatin-mediated mechanism. The Polycomb group proteins can function as an epigenetic mechanism that remembers and imposes the transcriptionally silenced state.

Nature Reviews | **Genetics** Figure 5 | **Polycomb-mediated silencing of developmental genes imposes self-renewal by actively blocking stem cell differentiation.** Components of the Polycomb group (PcG) system of gene-silencing complexes are crucial mediators of embryonic stem (ES) cell and adult stem cell self-renewal. In ES cells, PcG complexes directly silence the expression of developmental regulatory genes that, when expressed, tend to promote stem cell differentiation. When the PcG genes are inactivated, ES cells upregulate these developmental-regulatory genes and become difficult to maintain in the undifferentiated state. Although not directly shown, this mechanism (along with *p16* silencing) might also explain the role of PcG components in adult stem cell self-renewal. We speculate that one mechanism by which MAPK signalling induces stem cell commitment could be through the inhibition of PcG-mediated silencing of developmental gene expression. Ecto, ectoderm; Mes-endo, mesendoderm; Trx, Trithorax.

transcriptionally inactive and are concomitantly bound by the pluripotency factors OCT4, NANOG and SOX2 (Refs 98–100). The chromatin state of many of these bivalent genes resolves into a univalent state, either activated or silenced, upon differentiation¹⁰¹ (FIG. 5). Importantly, PcG silencing plays an important part in the silencing of bivalent genes as the partial inactivation of the PcG system in mES cells results in increased expression of bivalent genes and a propensity to differentiate¹⁰⁰.

The PcG system also seems to be crucial for adult stem cell self-renewal. Neural and haematopoietic stem cells are dependent on the PcG gene *Bmi1* for selfrenewal in serial transplantation (replating) assays owing to accelerated differentiation and the induction of senescence^{102,103}. Leukaemic stem cells are also dependent on BMI1 function as leukaemic cells from *Bmi1*–/– animals undergo differentiation, proliferation arrest and apoptosis, thereby rendering them nonfunctional in a transplant model¹⁰⁴. These data are consistent with the role of PcG complexes in preventing differentiation and also reveal a role in the regulation of stem cell senescence by silencing the senescence-inducing $CDKI$ p16.

The relative importance of active silencing of developmental genes versus passively avoiding the commitment to differentiation is unclear. The demonstration that gene silencing by PcG complexes is important for ES and adult stem cell self-renewal suggests that self-renewing cells must actively repress the expression of developmental genes that tend to drive the developmental process forward, and argues for the more active mechanism. Indeed, active suppression of differentiaton by niche signals has been documented in the regulation of germ stem cells in the *Drosophila melanogaster* ovary, in which bone morphogenic protein signals inhibit expression of the transcriptional activator *bag of [marbles](http://flybase.bio.indiana.edu/reports/FBgn0000158.html)*, a potent inducer of stem cell differentiation¹⁰⁵. Numerous other mechanisms are also emerging for suppression of differentiation in stem cells, including the translational repression by the products of

[piwi](http://flybase.bio.indiana.edu/reports/FBgn0004872.html) and *[pumilio](http://flybase.bio.indiana.edu/reports/FBgn0003165.html)* and a likely role for microRNAs¹⁰⁶⁻¹⁰⁸. Alternatively, the niche might facilitate the transition through early G1 phase of the cell cycle, avoiding the need for the strong proliferative stimuli that also tend to induce differentiation (for example, cytokine and growth-factor signalling through MAPK). Clearly, these two models are not mutually exclusive. For instance, specific signals might induce PcG-mediated silencing of developmental genes whereas MAPK-mediated signalling might inactivate the PcG-mediated silencing mechanism, resulting in developmental gene expression and stem cell commitment.

Future directions

Cell-cycle dynamics differ greatly between embryonic and adult stem cells — ES cells cycle rapidly whereas adult stem cells are highly quiescent. We have postulated in this Review that this might reflect two different ways of achieving the same goal: self-renewal. Early G1 is a crucial period of cell-fate decision-making in response to extracellular signals. ES cells either avoid this phase altogether (mES cells) or profoundly shorten it (hES cells). Conversely, adult stem cells are maintained in a quiescent state and therefore only rarely transit through early G1. Further, the microenvironmental niche of the adult stem cell might provide crucial signals (for example, morphogens) that facilitate the transition through early G1 without inducing differentiation.

There are many questions that remain regarding the regulation of stem cell self-renewal, particularly the relationship between cell-cycle progression and cellfate decision-making. First and foremost, elucidating the cell fates that constitute stem cell exhaustion will be an important step towards delineating the mechanisms involved. It is likely that exhaustion is actually a combination of differentiation, senescence, apoptosis and niche evacuation, each of which can be independently regulated. Furthermore, other proliferation-dependent stimuli (for example, telomere shortening) might play an important part in regulating the process. In addition, we have speculated that morphogens can be important niche-dependent signals that facilitate the transition through G1 in adult stem cells. It is clear that the outcome of morphogen signalling is highly context dependent, varying on the basis of the developmental state, temporal factors and concomitant signalling influences. Recently, inappropriate Wnt signalling was implicated in ageing through the senescence pathway^{109,110}. Elucidating the function of morphogens is an area of intense investigation that will increasingly need to consider the role of the cellular context. The roles of hypoxia in the stem cell microenvironment and ROS in signal transduction are also of great interest and might be a key component of the stem cell niche^{69,111}. Finally, it will be crucially important to identify the upstream signalling mechanisms that inactivate the Polycomb or translational repressor-mediated silencing of developmental-regulatory genes that seem to be central to the self-renewal versus commitment decision.

The study of stem cells is profoundly linked to hopes for future therapeutic benefits, including the use of stem cells or their derivatives as regenerative cellular therapies and identifying molecular targets for cancer treatments. A deeper understanding of the mechanisms that link the cell cycle and developmental progression — particularly in human stem cell populations — will facilitate efforts to develop new methods to expand adult stem cells *in vitro*, a key step toward the goal of using cellular therapeutics to replace damaged, diseased or aged tissues. Also, some cancers might be resistant to current treatments, perhaps because they target the cells that constitute the bulk of the tumour but spare the tumour-initiating cancer stem cells that are capable of regrowing and metastasizing⁵. The molecular mechanisms that cancer stem cells use to undergo self-renewing cell divisions, particularly mechanisms that differ from those used by normal adult stem cells, will serve as prime targets for the design of new cancer therapeutics.

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