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## Regulation of adult stem cell quiescence and its functions in the maintenance of tissue integrity

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### Abstract

Quiescent adult stem cells are important for mammalian tissues, where they act as a reserve that supports normal tissue turnover and can mount a regenerative response following acute injuries. Quiescent stem cells are well-established in certain tissues, such as skeletal muscle, brain, and bone marrow. The quiescent state is actively maintained and is essential for long-term maintenance of stem cell pools. In this Review, we discuss the importance of actively maintaining a functional pool of quiescent adult stem cells, including hematopoietic stem cells (HSCs), skeletal- muscle stem cells (MuSCs), neural stem cells (NSCs), hair follicle stem cells (HFSCs), and mesenchymal stem cells (MSCs) such as fibro-adipogenic progenitors (FAPs) to ensure tissue maintenance and repair. We discuss the molecular mechanisms that regulate the entry into, maintenance of, and exit from the quiescent state in mice. Recent studies revealed that quiescent stem cells have a discordance between RNA and protein levels, indicating the importance of post-transcriptional mechanisms, such as alternative polyadenylation, alternative splicing, and translation repression, in the control of stem cell quiescence. Understanding how these mechanisms guide stem cell function during homeostasis and regeneration has important implications for regenerative medicine.

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## Introduction

Stem cells have the ability to proliferate, to self-renew (produce new stem cells), or to differentiate [G] into different cell types, thereby offering great potential for regenerative medicine<sup>1,2</sup>. First described in the 19<sup>th</sup> century, stem cells include embryonic stem cells [G], germline stem cells [G], induced pluripotent stem cells [G], and adult stem cells [G], which have key roles in the maintenance and regeneration of tissues<sup>3,4</sup>. Tissues that undergo high cell turnover, such as the blood and the epithelium of the gut, maintain populations of continuously proliferating adult stem cells and progenitor cells to generate differentiating progeny<sup>5</sup>. However, many tissues also maintain populations of stem cells in a quiescent state, to support the cycling progenitors or in reserve for tissue injuries (Figure 1a).

Quiescence is a state of prolonged and reversible cell cycle exit, which is different from the irreversible cell-cycle exit that characterizes terminal cell differentiation or cell senescence<sup>2,6</sup>. Some differentiated cells can de-differentiate and proliferate to contribute to tissue repair (such reversible exit from the cell cycle is observed in endothelial cells and hepatocytes), but do not fit the stem cell paradigm of self-renewal<sup>7</sup>. Quiescent stem cells act as a reserve for producing progenitor cells that sustain tissue homeostasis and tissue regeneration in response to injury<sup>2,8</sup>. Among the best studied quiescent stem cell models are hematopoietic stem cells (HSCs), which are found in the bone marrow, skeletal muscle stem cells (MuSCs), neural stem cells (NSCs), hair follicle stem cells (HFSCs), and mesenchymal stem cells (MSCs), which include those in bone marrow, teeth, and the muscle-resident fibro-adipogenic progenitors (FAPs) (Figure 1a)<sup>2,9,10</sup>. Quiescent adult stem cells have recently been described in the epithelium of the gut and the mammary gland<sup>11–13</sup>, and may exist in other tissues as well. Germline stem cells in the ovary constitute a unique quiescent stem cell population that can give rise to totipotent stem cells post-fertilization.

A common feature of quiescent stem cells is their high potential for engraftment and self-renewal following transplantation compared to their cycling progeny, which is rapidly lost when these stem cells are cultured *ex vivo*<sup>14–18</sup>. This technical limitation poses a barrier to the expansion of quiescent stem cells for autologous stem cell transplantation therapies [G]. Thus, understanding how quiescent stem cells are maintained in tissues is a topic of intense study. Quiescent stem cells have long been considered to be in an inactive state<sup>2</sup>. However, recent genetic studies have revealed many signalling pathways that must be active to maintain the quiescent state. When these pathways are disrupted, stem cells become activated prematurely and often fail to self-renew, leading to depletion of the stem cell pool and a loss of regeneration potential *in vivo*<sup>19–23</sup>. Moreover, many of these pathways are deregulated in ageing<sup>24</sup> (Supplementary Table 1). Recent studies have focused on understanding how the mechanisms that control quiescence maintenance, exit, and re-entry are regulated. Much effort has gone into characterizing the transcriptional networks that regulate stem cell function and identity<sup>25–32</sup>. However, transcriptional networks are only one component of the control of stem cell function and regulation of the quiescent state<sup>33</sup>. Post-transcriptional regulatory mechanisms also play a key part. For example, quiescent MuSCs, HSCs, HFSCs, and NSCs all express genes that are crucial for stem cell activation [G] at the RNA level, but protein synthesis and accumulation are repressed to prevent

activation<sup>34–37</sup>. Such mechanisms are thought to establish a poised state that enables more rapid activation when required<sup>38</sup>.

In this review, we first describe key features of quiescent stem cells and the biological importance of stem cell quiescence. We discuss the different stem cell processes that enable the function of quiescent stem cells, and many of the genes that control these processes. We then discuss recent findings of post-transcriptional regulatory mechanisms that control the quiescent state in the context of tissue homeostasis and regeneration.

## Key features of quiescent stem cells

Quiescent stem cells have several features that distinguish them from their activated progeny and other proliferating stem cells (Figure 1b). This includes a lower level of activity, a reduced cell size, reduced mitochondrial content, and lower levels of biomolecules such as RNA and protein.

### Gene and protein expression signatures

Typically, quiescent stem cells are smaller than non-quiescent stem cells and have low RNA and protein content<sup>39–41</sup>. This coincides with low levels of mRNA transcription, rather than high levels of mRNA turnover, as indicated by the low levels of Ser2 phosphorylation at the C-terminal domain of RNA polymerase II — a marker of transcript elongation — in quiescent stem cells<sup>42</sup>. Nevertheless, transcription does occur in quiescent stem cells *in vivo*<sup>43</sup>.

Quiescent stem cells are marked by low protein and RNA levels of cyclin A2, cyclin B1, and cyclin E2, which are the key drivers of the cell cycle, and by high levels of the cyclin-dependent kinase inhibitors CDKN1A (p21 or CIP1), CDKN1B (p27 or KIP1), and CDKN1C (p57 or KIP2), which function as inhibitors of cyclins and thus block cell cycle transitions<sup>2,44,45</sup>. Cycling cells progressing through G1 normally accumulate cyclin D, which partners with cyclin-dependent kinase 4 (CDK4) or CDK6 to inhibit retinoblastoma-associated protein (Rb; also known as RB1) and enable S-phase entry<sup>46</sup>. Strikingly, cyclin D1 is expressed in quiescent HSCs and MuSCs and may function in a cell-cycle independent manner in these quiescent cells<sup>40,47</sup>.

In different stem cell types, distinct transcription factors marking the quiescent state have been identified. Quiescent MuSCs express PAX7, the level of which decreases upon MuSC activation<sup>48</sup>. Quiescent HFSCs express TCF3 (also known as TCF7L1) and TCF4 (also known as TCF7L2), which are lost upon HFSC activation<sup>49</sup>. HOXB5 and TCF15 expression mark quiescent HSCs<sup>27,50</sup>.

### Epigenetic signatures

Quiescent stem cells have distinct patterns of epigenetic modifications and of heterochromatin. Quiescent MuSCs, NSCs and HFSCs can be identified in electron microscopy micrographs by their nuclei filled with dense heterochromatin<sup>51–53</sup>. Many genes in quiescent stem cells are characterized by bivalent chromatin, which includes both gene-repressive and permissive histone modifications<sup>54–57</sup>, suggesting that these genes are

poised for activation<sup>38</sup>. Quiescent stem cells express regulators of histone modifications. For example, Trithorax group [G] histone methyltransferases are expressed in quiescent HSCs and MuSCs to maintain histone H3 Lys4 tri-methylation (a gene-expression permissive modification)<sup>58–60</sup>. In addition, DNA methylation patterns are dynamically regulated in quiescent HSCs and MuSCs<sup>61,62</sup>.

### Low metabolic activity.

Quiescent HSCs, MuSCs, NSCs, and HFSCs all have lower energy demands than cycling cells and correspondingly low mitochondrial activity<sup>63–66</sup> (Figure 1b). Subsets of HSCs with low mitochondrial activity are more quiescent compared to subsets of HSCs with high mitochondrial activity, and show better long term engraftment<sup>67</sup>. When HSCs and HFSCs undergo activation, they increase mitochondria biosynthesis and gradually increase oxidative phosphorylation<sup>66,67</sup>. Quiescent stem cells reside in niches with low access to oxygen<sup>68</sup>. Accordingly, they rely on glycolysis and fatty acid oxidation to satisfy their low energy demands<sup>69–72</sup>. Moreover, quiescent stem cells maintain low levels of mTOR complex 1 (mTORC1) signaling<sup>73–75</sup>, and nutrient-sensing pathways such as the AMPK and LKB1 pathways are vital for accurate balance between quiescence and activation of MuSCs and HSCs<sup>76–79</sup>.

### Altered DNA damage repair.

Quiescent stem cells do not undergo DNA replication and thus have no access to high-fidelity DNA repair by homologous recombination, which generally requires a sister chromatid as template for repair<sup>80,81</sup>. Instead, quiescent MuSCs, HSCs, HFSCs, and NSCs use the cell-cycle-independent, but error-prone process of non-homologous end-joining [G]<sup>81–85</sup>. Nevertheless, DNA damage does accumulate with age in quiescent stem cells, as shown in HSCs and MuSCs<sup>86–89</sup>. DNA damage in aged stem cells can be repaired by exposing the aged cells to a young systemic environment, due to a shared circulation after heterochronic parabiosis, or upon stem cell activation<sup>89,90</sup>. Given the importance of DNA repair, it is not surprising that deletion of the gene encoding checkpoint kinase ATR [G] results in apoptosis of activated HSCs, NSCs, and HFSCs<sup>91–93</sup>. By contrast, MuSCs lacking ATR spontaneously undergo activation in the absence of injury<sup>94</sup>. In quiescent MuSCs, ATR activity is triggered by DNA–RNA hybrids, resulting in stabilization of cyclin F and quiescence maintenance<sup>94</sup>.

### Stem cell survival.

The quiescent state itself also affects the exposure to stress. For example, quiescent stem cells are protected from replication-induced DNA damage, and their low mitochondrial content limits the exposure to reactive oxygen species (ROS). Resilient subpopulations of quiescent MuSCs, HSCs, and NSCs can survive irradiation or regeneration stress<sup>95–97</sup>. Nevertheless, quiescent stem cells are still subject to a host of environmental stresses and their ability to withstand them may be limited by their low levels of gene expression and metabolism. Moreover, the complex changes that occur during stem cell activation render the cells vulnerable to damage. For example, mitochondrial biosynthesis upon activation poses additional risks for oxidative stress, and ramping up protein synthesis increases

the risk of endoplasmic-reticulum stress<sup>98–103</sup>. Quiescent MuSCs and HSCs have stress response pathways, which are key for maintaining the quiescent state<sup>104,105</sup>. Whether quiescent stem cells possess unique mechanisms that actively promote survival remains largely unexplored.

## Key processes related to stem cell quiescence

For quiescent stem cells to contribute to tissue homeostasis and repair, they depend on the timely execution of multiple distinct processes. During homeostasis, the stem cells maintain the quiescent state. Nevertheless, a low level of stem cell activation does occur and this contributes to normal homeostasis by supplying the progeny to sustain tissue turnover. Following tissue injury, the cells must rapidly exit the quiescent state in order to activate and enter the cell cycle to drive the repair process. When tissue repair is underway, a subset of stem cells must re-establish the quiescent stem cell pool in a process. This process of re-quiescence ensures the establishment of a stem cell reserve population for future tissue needs. During all of these processes, the cells must devote resources to survival mechanisms in the face of stress and accumulating damage.

### Maintenance of stem cell quiescence

The stem cell niche triggers signaling pathways to maintain quiescence, which is instrumental to preservation of the quiescent stem cell pool. For example, a common signaling pathway for maintaining quiescence is the Delta–Notch signaling, in which Delta ligands presented by the niche trigger Notch signaling in the stem cell (Figure 2). Loss of the receptor NOTCH2 or of RBPJ, the downstream effector of Notch signaling, causes activation of MuSCs, NSCs, and HSCs<sup>19–23</sup>. Moreover, the Notch target genes HES1 and HEY1, which encode transcription factors, are key to quiescence maintenance in all three stem cell types<sup>106–108</sup>. By contrast, in HFSCs, loss of HES1 has no effect in the telogen phase of the hair growth cycle when the HFSCs are quiescent, but it severely limits the anagen phase when the HFSCs are actively cycling, suggesting that HES1 has an important role in HFSC activation<sup>109</sup>. More surprisingly, deletion of *Rbpj* in HFSCs does not affect quiescence or activation, but results in activation of a neighboring quiescent population of melanocyte stem cells through paracrine retinoic acid signaling<sup>110</sup>. In addition, there are signaling pathways unique to each stem cell type (Supplementary Table 2). For example, HSCs respond to thrombopoietin signals from the bone marrow niche, whereas NSCs respond to neurotransmitters.

In addition to signaling from the niche, quiescent stem cells use cell-intrinsic inhibitory mechanisms to prevent activation in the absence of triggering cues<sup>38</sup> (Figure 2a). As CDK inhibitors block the G1 cyclins (cyclin D and cyclin E)<sup>111</sup>, it is not surprising that these proteins are essential to maintaining a quiescent state in stem cells (Figure 2a,3a). Deletion of the genes encoding p21, p27 or p57 result in spontaneous activation of HSCs, NSCs, MuSCs, and HFSCs<sup>12,112–119</sup>. Quiescent stem cells must avoid the restriction point, from which the cell is fully committed to complete the cell cycle. The restriction point is defined by Rb phosphorylation, which relieves the inhibition of Rb on E2F (Figure 3a)<sup>111</sup>. However, there is a short window after Rb inactivation during which stress can force the cell back into

quiescence, and this window is dependent on the anaphase promoting complex/cyclosome (APC/C)<sup>120</sup>. Interestingly, the restriction point may be differentially regulated in different quiescent stem cell types. Deletion of *Rb1* results in MuSC and HSC activation<sup>121–123</sup>, but has no effect in NSCs<sup>124</sup>. Deletion of APC/C results in HSC activation<sup>125</sup>, but while this lowers NSC quiescence, these cells cannot complete the cell cycle and consequently undergo apoptosis<sup>126</sup>.

Quiescent stem cells express high levels of p53<sup>88,127,128</sup>, which, in somatic cells, can trigger cell cycle exit or cell death in response to stress. Germline deletion of p53 causes aberrant activation of NSCs and HSCs<sup>127,128</sup>. In HSCs, this activation is due to the loss of the cell cycle repressors Necdin and GFI-1 (Ref.<sup>127</sup>), or owing to the indirect loss of p53 through the loss of OCIAD1, an inhibitor of p53 degradation<sup>129</sup>. In quiescent MuSCs, p53 is stabilized because Notch signaling represses MDM2 expression<sup>88</sup>. When MuSCs undergo activation, they lose Notch signaling and p53 level decreases (Figure 2). Upon activation, MuSCs that lack p53 have a higher propensity to undergo apoptosis, which can be prevented by pharmacological stabilization of p53 (Ref.<sup>88</sup>). This is in contrast to other stem cell types, where p53 stabilization induces cell death<sup>83,129–131</sup>.

### Activation and exit from quiescence

Quiescent stem cells retain the capacity to undergo activation and enter the cell cycle in response to triggers such as acute injuries. Stem cell activation is disrupted during aging (BOX 1), resulting in impaired regeneration and highlighting the importance of this process for quiescent stem cell function. Activation is the result of loss of niche signals that maintain quiescence and the inhibitory signals that prevent activation, combined with activating signals and mitogenic and non-mitogenic signals that stimulate the cell cycle (Figure 2b). In preparation for cell cycle entry, the cells first complete a growth phase, which enables them to acquire sufficient biomass and volume to undergo DNA replication. Whether quiescent stem cells must overcome a size threshold to fully activate is unclear. Nevertheless, quiescent HSCs, NSCs, and MuSCs are smaller than their activated progeny and, in somatic cells there is a correlation between cell size and cell cycle speed<sup>39,41,73,132</sup>.

It is striking that the first stem cell division upon their activation requires up to two days — considerably longer than subsequent cell divisions, which take about half a day<sup>88,133,134</sup>. It is unclear whether this extended time is due to lengthening of the G1 phase, or whether the cells transition through several uncharacterized sub-phases of quiescence, similar to the different stages of the cell cycle (Figure 3). Although the process of activation generally concludes with progression into the cell cycle, HSCs and MuSCs can progress to differentiation without first entering the cell cycle<sup>19,135</sup>.

Activation of stem cells also adjusts their metabolism to meet the energetic demands of proliferation. This adjustment includes a switch from fatty acid oxidation and glycolysis to mitochondrial respiration, which requires the biosynthesis of mitochondria<sup>69,72</sup>. In addition, MuSCs, NSCs, and HFSCs undergoing activation increase autophagy to process large biomolecules into building blocks<sup>136–140</sup>. HSCs show signs of a mitochondrial checkpoint, in which mitochondrial biosynthesis can cause stress, which in turn pushes the cell towards quiescence<sup>141–143</sup>. This process suggests the existence of quiescence checkpoints analogous



to the cell cycle checkpoints, in which the cell can evaluate whether or not to progress with activation.

Stem cells are activated by growth factors (Figure 2b). MuSCs, NSCs, HSCs, and HFSCs undergoing activation trigger p38 MAP kinase signaling, and inhibition of p38 reduces activation<sup>130,144–147</sup>. Activation of HSCs, MuSCs, and HFSCs also upregulate PI3K-AKT (PKB) signaling and interfering with this pathway limits activation<sup>130,148,149</sup>. Activation of stem cells increases mTOR signaling<sup>73,150–153</sup>. In response to injuries in a different tissue, MuSCs and HSCs increase mTOR signaling to enter an “alert” state<sup>73,74</sup> (Figure 3). MuSCs without the mTORC1 subunit regulatory-associated protein of mTOR (RAPTOR) can no longer enter the “Alert” state and show delayed activation<sup>73</sup>.

Quiescent stem cells are heterogeneous in their gene-expression levels and ability to undergo activation, which results in cells with varying degrees of “quiescence depth”<sup>154</sup>. For example, subpopulations of HSCs with low levels of CDK6 or the receptor tyrosine kinase KIT take longer to become active than subpopulations with higher levels of CDK6 or KIT<sup>134,155</sup>. MuSCs with high levels of the transcription factor PAX7 take longer to become active than MuSCs with low levels of PAX7 (Ref.<sup>156</sup>). NSCs with high levels of tyrosine-protein kinase ABL1 take longer to become active than NSCs with low levels of ABL1 (Ref.<sup>157</sup>). Quiescent stem cells can adjust quiescence depth in response to external cues. For example, distant tissue injuries can induce the more shallow Alert state in quiescent MuSCs and HSCs<sup>73,74</sup>, whereas fasting and ketosis can induce a deeper quiescent state in MuSCs, marked by delayed activation<sup>158</sup>, and norepinephrine can increase quiescence depth in HFSCs by limiting the expression of FGF18 (Ref.<sup>159</sup>). A deeper quiescent state can confer protection against certain stresses such as irradiation and chemotherapy agents<sup>2,158,160</sup>. However, it also hampers the cell’s ability to execute its function and generally correlates with impaired regeneration and impaired homeostasis during aging (BOX 1)<sup>158</sup>.

### Re-entry into the quiescent state

Activated stem cells will at some point replenish the stem cell pool by undergoing re-quiescence, which involves exiting the cell cycle and reverting to the phenotype of a quiescent stem cell<sup>161–164</sup> (Figure 1b). Generally, stem cells enter the quiescent state from the G1 phase of the cell cycle, although there is evidence of NSCs entering quiescence in G2 (Ref.<sup>165</sup>). Re-quiescence occurs in concordance with a loss of growth signals and the reconstruction of the stem cell niche<sup>163,166</sup> (Figure 2c). Not surprisingly, deletion of the genes encoding receptors that convey niche signals for the maintenance of quiescence results in depletion of the stem cell pool<sup>19,20,167–170</sup>. Whether this is due to a defect in quiescence entry, or confounded by defects in other processes, is unclear. Conditional deletion of the gene encoding the Notch ligand Delta-like 1 (DLL-1) considerably reduces the ability of activated MuSCs to re-enter the quiescent state following muscle injury, resulting in a loss of MuSCs<sup>171</sup>.

Stem cells that enter the quiescent state must also repress the growth signaling pathways that drive activation. NSCs expressing tumor necrosis factor- $\alpha$  receptor 1 (TNFR1; also known as TNFRSF1A) will re-enter the quiescent state following stimulation with lipopolysaccharides, which triggers a systemic immune response<sup>172</sup>. This response depends



on TNFR1-dependent phosphorylation of p38 (Figure 2b,c), and p38 inhibitors can partially abrogate re-quiescence. This function of p38 is consistent with observations in proliferating human and mouse MuSCs, in which inhibition of p38 enhances cell expansion<sup>173,174</sup>. In quiescent MuSCs, inhibition of p38 prevents cell cycle entry<sup>144</sup>, highlighting the role of cellular context on signaling pathways. Deletion of the gene encoding SPRY1, an inhibitor of ERK, abolishes MuSC re-quiescence due to increased ERK signaling<sup>175</sup>. Deletion of *Mek1* or *Pten*, both negative regulators of PI3K, results in gradual depletion of the quiescent stem cell pool<sup>98,151,176–181</sup>. The AKT inhibitor Inositol(1,4,5)trisphosphate 3-kinase b (ITPKB) is key for HSC re-quiescence<sup>182</sup>. HSCs without ITPKB activate prematurely and fail to reestablish a population of quiescent HSCs, resulting in hematopoietic failure and anemia. This phenotype suggests that inhibition of AKT signaling is key for re-quiescence<sup>182</sup> (Figure 2c).

### Self-renewal

Self-renewal is a key process for maintaining the populations of all stem cells. Self-renewal is not equated with quiescence entry since, for example, proliferating stem cells in the epithelium of the gut undergo continuous self-renewal without passing through a quiescent state<sup>183</sup>. However, to self-renew, quiescent stem cells must go through one cell division and therefore require cell cycle entry and exit. Activated stem cells must decide whether to continue with proliferation, whether to exit the cell cycle and differentiate, or whether to re-enter the quiescent state.

### Post-transcriptional quiescence control

There has been a strong interest in understanding the mechanisms that regulate stem cell quiescence. Transcriptional gene regulation has key roles in controlling the quiescent state<sup>184–188</sup>; however, differential expression of transcripts only partially determines protein abundance. In fact, in somatic cells, mRNA abundance alone often cannot explain observed protein levels<sup>189</sup>, and RNA and protein abundance often do not correlate in quiescent stem cells<sup>26,34,38,190–195</sup>. We therefore focus in this Review on regulation of stem cell quiescence by post-transcriptional mechanisms, and examine their roles in the control of quiescence maintenance, exit and entry.

### RNA regulation in the nucleus

Following transcription, the pre-mRNA undergoes a series of processing steps, which can be regulated to control the amount of protein that is produced. These mechanisms include alternative polyadenylation (APA), alternative splicing, and RNA modifications such as adenosine and cytosine methylation and adenosine-to-inosine (A-I) editing. The mature mRNA can be degraded, stored, or translated. In the following sections, we discuss how these post-transcriptional mechanisms regulate processes related to stem cell quiescence (Table 1).

**Alternative polyadenylation.**—One of the best studied post-transcriptional regulatory mechanisms is APA, in which cells create mRNA isoforms usually with different 3' untranslated regions (3'UTRs)<sup>196</sup>. Transcript isoforms with different 3'UTRs can be

detected in multiple mouse quiescent stem cell populations, including HSCs, MuSCs, HFSCs, and NSCs<sup>195,197–200</sup>. HSCs exiting quiescence exhibit a global switch to shorter transcript isoforms<sup>197</sup> (Figure 4). Known regulators of APA are dynamically regulated in HSCs and MuSCs<sup>195,197</sup>.

APA of specific genes can profoundly affect quiescence. APA of PAX3 transcripts alters the propensity of MuSCs to exit quiescence<sup>195</sup>. Polyadenylation sites (PASs) in the proximal part of the PAX3 3'UTR are blocked by U1 small nuclear RNA (snRNA), leading to the production of long isoforms that are subject to translation repression by microRNAs (miRNAs) (Figure 4a). Functional knockdown of U1 snRNA results in the expression of shorter PAX3 mRNAs, increased PAX3 protein levels, and MuSC activation, whereas U1 snRNA overexpression prevents MuSC activation<sup>195</sup>. Quiescent FAPs express mRNA isoforms of platelet-derived growth factor receptor alpha (PDGFR $\alpha$ ) that terminate at either of two PASs in the 3'UTR, or at an intronic PAS<sup>201</sup>. Transcripts generated from the intronic PAS generate a truncated protein lacking the intracellular kinase domain. Blocking the intronic PAS with antisense oligonucleotides results in increased levels of full-length mRNA and protein and in increased exit from quiescence. By contrast, blocking distal PASs leads to increased intronic-PAS choice, production of truncated mRNA and protein, blunted PDGFR $\alpha$  signaling, and reduced activation<sup>201</sup>. These findings show that APA affects protein levels in quiescent stem cells. This effect differs from what has been observed in somatic non-stem cells, where APA affects mRNA stability or localization<sup>202,203</sup>, and thus may be a specific feature of quiescent stem cells.

APA also regulates quiescence exit (Figure 4b). Depletion of NUDT21 (also known as cleavage and polyadenylation specificity factor subunit 5) prevents HSC activation and subsequent differentiation. During normal HSC activation, NUDT21 induces a switch towards shorter glutaminase mRNA isoforms, leading to increased glutaminase protein levels, and inhibiting glutaminase activity can similarly block HSC activation<sup>197</sup>. Diaphragm MuSCs express higher levels of PAX3 compared to MuSCs in lower hindlimb muscles, leading to increased activation rates<sup>195</sup>. Loss of PAX3 or forced lengthening of PAX3 mRNAs reduces MuSC activation<sup>195</sup>. Higher levels of PAX3 also confer stress resistance to activated MuSCs<sup>95,204</sup>. In response to stress, PAX3-expressing MuSCs have lower ROS levels, increased cell size, and higher mTORC1 signaling, and display increased activation kinetics, all suggesting these cells are in an “Alert” state<sup>95,204</sup>. FAP activation affects APA of PDGFR $\alpha$ <sup>201</sup>. Increasing intronic PAS choice leads to reduced proliferation and reduced fibrosis, showing that APA is key for quiescence exit. By contrast, the role of APA in quiescence entry is much less clear (Figure 4c). Depletion of the APA regulator polyadenylate-binding nuclear protein 1 (PABPN1; also known as PABP-2) in HSCs results in a global switch towards shorter mRNA isoforms and reduced serial engraftment potential, but has no effect on HSC survival<sup>197</sup>.

**RNA editing.**—In addition to changing mRNA 3' ends, cells can modify individual nucleotides in the transcript. One established mechanism for nucleotide modification is RNA editing, in which adenosine is deaminated to inosine (A-I editing)<sup>205</sup>. A-I editing has been reported in quiescent HSCs and NSCs<sup>206,207</sup>. The enzymes catalysing RNA editing — ADAR (ADAR1), ADAR2 and AIMP2 — are widely expressed and their

mRNAs can be found in quiescent MuSCs, NSCs and HSCs in cell atlas transcriptomics data<sup>208–210</sup>. Moreover, at least in HSCs, ADAR1 expression is dynamically regulated and increases in progenitor cells compared to HSCs<sup>211</sup>. In HSCs, overexpression of ADAR, but not of a mutant lacking enzymatic activity, results in activation into the cell cycle (Figure 4b)<sup>206</sup>. ADAR overexpression results in editing of the primary miRNA pri-miR-26a, preventing its maturation, and of a miRNA binding sites in the 3'UTR of MDM2, leading to mRNA stabilization<sup>206</sup>. By contrast, deletion of *Adar* leads to quiescence maintenance and increased levels of the cell cycle inhibitor CDKN1A<sup>206</sup>. Although these experiments show that RNA editing is a key mechanism controlling HSC quiescence, it remains an open question whether ADAR also acts in a non-enzymatic manner<sup>212</sup>.

**RNA methylation.**—RNA can undergo extensive methylation in cells<sup>213</sup>, and this modification is often mutually exclusive with RNA editing<sup>214</sup>. In quiescent HSCs, mRNAs of an estimated 9% of the expressed genes contain *N*<sup>6</sup>-methyladenosine (m<sup>6</sup>A), and this fraction decreases with activation<sup>215–217</sup>. The m<sup>6</sup>A writer METTL3 is expressed in HSCs, and methylation enzymes can also be detected in other quiescent stem cell populations in cell atlas data<sup>208–210,215,216</sup>. Conditional deletion of *Mettl3* from HSCs leads to quiescence exit and a gradual increase in HSC numbers<sup>215,218,219</sup> (Figure 4a). One target of METTL3 is MYC mRNA, which has increased stability when methylated<sup>215,218</sup>. Similarly, conditional loss of METTL3 in MuSCs causes an initial increase in MuSC numbers immediately after injury, suggesting enhanced activation<sup>220</sup>. Myc, well-known for its role in cell cycle progression and cell expansion, is important for quiescence maintenance in HSCs and NSCs<sup>221,222</sup>. This coincides with a loss in NOTCH mRNA methylation and decreased protein levels, while mRNA levels remain stable (suggesting decreased translation of the mRNA; see below)<sup>220</sup>. Deletion of *Mettl3* or *Mettl14* leads to reduced m<sup>6</sup>A-mRNA levels and delayed cell-cycle exit in embryonic NSCs, resulting in a failure to establish the quiescent NSC pool<sup>223</sup> (Figure 4c). In HFSCs, the MYC-induced SUN2 catalyzes the formation of 5-methylcytosine (m<sup>5</sup>C) in tRNA (Figure 4b). Expression of SUN2 in HFSCs increases in anagen and its deletion prolongs quiescence maintenance, resulting in a delayed hair cycle<sup>224</sup>.

**Alternative splicing.**—Alternatively spliced mRNA isoforms are common in all cells<sup>210,225,226</sup>, but the observation that known splicing factors are dynamically regulated during MuSC and HSC activation indicates that alternative splicing could be an important regulatory mechanism in quiescent stem cells<sup>227–229</sup>. However, surprisingly little is known about the regulation and function of alternatively spliced isoforms in quiescent stem cells. The RNA binding protein quaking (QKI) regulates alternative splicing of cell polarity genes in MuSCs. Conditional deletion of *Qki* results in alternative splicing of ITGA7 and Numb, and reduced activation and assymetrical division<sup>230</sup>. Protein arginine N-methyltransferase 5 (PRMT5) controls mRNA splicing in quiescent HSCs<sup>231</sup>. Following conditional deletion of *Prmt5*, HSCs show a global changes in alternative splicing, increased protein synthesis and increased HSC numbers, all indicating quiescence exit<sup>231</sup>. Thrombopoietin signaling through its receptor MPL can promote either quiescence or proliferation of HSCs in a concentration-dependent manner. Sensitivity of HSCs to thrombopoietin depends on alternative splicing of the receptor MPL, which is expressed as a full-length or as

a truncated, dominant-negative isoform; the RNA-binding protein OTT1 (also known as RBM15) determines the relative abundance of the two isoforms through alternative splicing<sup>232</sup>. Accordingly, by regulating the mRNA splicing of a key gene, HSCs can fine-tune their readiness to exit the quiescent state.

Splicing can also be repressed, leading to intron retention<sup>233</sup>. Transcript isoforms that retain introns accumulate in quiescent MuSCs and HSCs<sup>231,234,235</sup>. In MuSCs, overexpression of the oncogene and proliferation regulator DEK results in intron excision of intron-retaining mRNAs, which is followed by MuSC activation<sup>234</sup> (Figure 4). Depletion of DEK blocks intron excision and prevents cell cycle entry<sup>234</sup>, indicating that intron retention is key for quiescence maintenance. Loss of DEK triggers HSC activation and diminished self-renewal<sup>236</sup>, but whether this involves altered intron retention is unknown.

**Nuclear export.**—Mature mRNAs are exported to the cytoplasm for translation<sup>237</sup>. Unsurprisingly, deletion of the gene encoding the mRNA export protein THO complex subunit 5 (THOC5) homolog leads to a dramatic loss of HSCs<sup>238</sup>, likely due to general toxicity of the loss of RNA export. Cells can regulate the export of specific RNAs. Quiescent NSCs in zebrafish express miR-9, which forms a complex with Argonaute (AGO) proteins, a component of the RNA-induced silencing complex, and accumulates in the nucleus<sup>239</sup>. Nuclear accumulation of miR-9 leads to quiescence maintenance, whereas cytoplasmic retention of miR-9 leads quiescence exit, although the downstream mRNA targets of miR-9, and whether any target mRNA is co-transported with it, remain unclear<sup>239</sup>.

### RNA regulation in the cytoplasm

Following export into the cytoplasm, the mature mRNA can be subjected to several regulatory processes that control when and where a protein is produced. These mechanisms include RNA degradation by small RNAs, as well as RNA degradation by RNA-binding proteins. Transcripts can also be stored in mRNA granules. In the following sections, we discuss how these post-transcriptional mechanisms regulate processes related to stem cell quiescence (Figure 5).

**RNA degradation by microRNAs.**—Mammalian cells can degrade mRNA through several classes of small RNAs, chiefly among them miRNAs. miRNAs are processed from precursors by Dicer and other ribonucleases and target mRNAs through sequence complementarity (mostly) to 3'UTRs, in association with Argonaute proteins. miRNAs are well known to affect the quiescent state<sup>240</sup> (Figure 5). Conditional deletion of *Dicer* results in activation and apoptosis in MuSCs and in apoptosis and a self-renewal defect in HSCs<sup>241,242</sup> (Figure 5a,c). These phenotypes are at least partly due to disrupted miRNA-mediated mRNA degradation. MuSCs maintain quiescence by expressing miR-489 and miR-708, which downregulate DEK and tensin 3 (TNS3) mRNAs, respectively<sup>242,243</sup>. Overexpression of miR-489 or miR-708 prolongs quiescence, whereas their depletion triggers quiescence exit. Finally, quiescent MuSCs use miR-195 and miR-497 to destabilize mRNAs encoding the cell cycle factors CDC25A (also known as M-phase inducer phosphatase 1), CDC25B (also known as M-phase inducer phosphatase 2) and CCND2 (also known as G1/S-specific cyclin D2), thereby supporting quiescence maintenance<sup>244</sup>. In

HSCs, miR-21 decreases PDCD4 expression and function in the NF $\kappa$ B pathway to maintain HSC quiescence. Loss of miR-21 results in activation, whereas its overexpression prevents activation<sup>245</sup> (Figure 5a).

In contrast to MuSCs and HSCs, deletion of *Dicer* has no apparent effect on quiescence maintenance in NSCs or HFSCs, but leads to a loss of activated progenitors and subsequent defects in neurogenesis or hair cycle progression, respectively<sup>246–248</sup>. Nevertheless, individual miRNAs are important for quiescence in both NSCs and HFSCs. In NSCs, miR-204 controls quiescence by enabling the degradation of the mRNAs of the pro-activation factors MEIS2 and SOX11 (Ref.<sup>249</sup>). However, quiescent NSCs acquire miR-204 from the choroid plexus and do not depend on *Dicer* for its synthesis<sup>249</sup>. In HFSCs, miR-29a and/or miR-29b1 destabilize cyclin mRNAs and components of the WNT signaling pathway (a potent morphogen pathway that controls cell expansion in mouse and human HFSCs, as well as in mouse MuSCs, whereas it maintains quiescence in mouse HSCs<sup>250–254</sup>), and loss of miR-29a and miR-29b1 results in activation<sup>250</sup> (Figure 5a).

As quiescent stem cells become active, the miRNAs that prevent activation are downregulated. MuSCs undergoing activation decrease the expression of miR-195 and miR-497, resulting in increased stability of CDC25A, CDC25B and CCND2 (Ref.<sup>244</sup>). In parallel, the stem cells induce the expression of new miRNAs network that destabilizes mRNAs involved in quiescence maintenance. HSCs lacking miR-132 and miR-212 have stable FOXO3 mRNA levels and are more quiescent, show better engraftment and less apoptosis, and undergo slower activation following stimulation by lipopolysaccharides (Figure 5b)<sup>255</sup>. The transcription factor FOXO3 plays a role in maintaining and establishing the quiescent state<sup>256,257</sup>. Conversely, to establish the quiescence program, the cell needs to suppress the ongoing activation program. This may involve the degradation of specific transcripts. However, to the best of our knowledge, no studies yet have directly investigated the role of RNA degradation in the process of quiescence entry. Nevertheless, many studies of miRNAs report on decreased stem cell numbers following regeneration, hinting at a change in self-renewal and/or quiescence entry<sup>243,258,259</sup>. For example, long term depletion of miR-708 leads to apoptosis-independent reduction in MuSC numbers<sup>243</sup> (Figure 5c).

**small-RNA-independent RNA degradation.**—In addition to miRNAs, quiescent stem cells use small-RNA-independent mechanisms to destabilize specific transcripts. Quiescent MuSCs express the mRNA decay activator TTP (also known as ZFP36), which degrades MyoD mRNA<sup>190</sup> (Figure 5a). TTP loss results in MyoD mRNA accumulation and MuSC activation<sup>190</sup>. By contrast, *Tip* deletion in HSCs does not affect quiescence<sup>260</sup>, possibly due to redundancy with its family members. MuSCs also express the RNA-binding protein (RBP) AUF1 (also known as HNRNPD), which targets for degradation transcripts containing AU-rich elements. MuSCs lacking AUF1 accumulate matrix metalloproteinase 9 (MMP9) mRNA, resulting in increased matrix remodeling and reduced quiescence entry<sup>261</sup>. In HSCs, deletion of the gene encoding the RBP and m<sup>6</sup>A reader YTHDF2 leads to stabilization of methylated mRNAs that encode transcription factors such as TAL1, which in turn increases the numbers of quiescent HSCs<sup>216</sup> (Figure 5a). RBPs can also stabilize mRNAs<sup>262</sup>, for example as shown by the function of ELAV proteins on the MSII mRNA in NSCs<sup>263,264</sup>.

**RNA storage.**—Storage of mRNA is an intriguing possible mechanism of cellular memory of the activated state and priming of activation<sup>265</sup>. Processing bodies [G] (P-bodies) and RNA granules [G] store RNA and are key for stem cell differentiation and lymphocyte activation due to their regulation of differentiation and cell cycle factors<sup>266,267</sup>, yet they are poorly studied in the context of stem cell quiescence. The RNA granule protein FMRP is highly expressed in quiescent MuSCs and NSCs and its loss causes aberrant activation<sup>268–270</sup>. In quiescent MuSCs, miR-31 ensures the sequestration of its target mRNA myogenic factor 5 (MYF5; a myogenesis factor) in RNA granules containing the RNA helicase DDX6 (Figure 5a). Antagomirs against miR-31 result in loss of RNA granules and in MuSC activation<sup>194</sup>. Moreover, blocking RNA granules disassembly by inhibition of phosphatase 2A activity prevents MYF5 protein accumulation<sup>194</sup>. When stem cells undergo activation, they dissolve the RNA granules, and forced maintenance of RNA granules prolongs quiescence in MuSCs<sup>194</sup>. It is unclear whether MuSCs accumulate the MYF5 RNA in the quiescent state or during quiescence entry. In oocytes (see below), DDX6 is essential for establishing quiescence<sup>271</sup>. In the absence of DDX6, oocytes do not form RNA granules, fail to repress PI3K–AKT signaling and continuously proliferate<sup>271</sup> (Figure 5c).

### Protein synthesis and degradation

Quiescent stem cells show low levels of mRNA translation, even though the core components of the translation machinery are expressed<sup>26,75,98,272–275</sup>. Quiescent HSCs express similar levels of core rRNAs and ribosomal proteins compared to their activated counterparts<sup>98,275</sup>. Nevertheless, quiescent stem cells increase protein translation during activation<sup>26,75,98,272–274</sup>. The core translation machinery, including rRNA and ribosomal proteins, is dynamically regulated in both quiescent and activating stem cells<sup>26,276,277</sup>.

**RNA folding.**—To be translated, mRNAs need to be (un)folded such that the ribosome can access the start codon. 5'UTRs of mRNAs can contain regulatory elements that affect mRNA folding and accessibility, and that can affect activation of quiescent stem cells. Expression of DHX36, a helicase that unwinds RNA G-quadruplex (rG4) structures, increases with MuSC and HSC activation and its loss resulted in an activation defect<sup>278,279</sup>. In MuSCs, DHX36 specifically regulates the translation of GNAI2 mRNA by unwinding its 5' UTR rG4 structures<sup>278</sup>.

**Translation initiation.**—HSCs not expressing the ribosome assembly factor Notchless protein homolog 1 (NLE1) accumulate immature ribosomal RNAs and exit quiescence<sup>280</sup> (Figure 5a). Deletion of the transcription factor RUNX1 in HSCs similarly leads to reduced ribosome biosynthesis and protein translation and reduced HSC activation<sup>281</sup>. Among the direct targets of RUNX1 are ribosomal genes and rRNAs<sup>281</sup>. In MuSCs, eukaryotic translation initiation factor 2 alpha (eIF2 $\alpha$ ) is phosphorylated to inhibit translation<sup>273</sup>. Following the replacement of the eIF2A gene in MuSCs by a mutant gene that renders the protein resistant to phosphorylation, MuSCs undergo activation and fail to self-renew<sup>273</sup> (Figure 5a,c).

Although phosphorylated eIF2 $\alpha$  generally inhibits translation, certain mRNAs escape this inhibition. The 5' UTR of the TACC3 mRNA (encoding a mitotic spindle stability factor)



contains translation-inhibiting upstream open reading frames (uORFs); in stress conditions, translation of this mRNA is enabled by phospho-eIF2 $\alpha$ -mediated readthrough of these uORFs<sup>282</sup>. Loss of TACC3 results in a MuSC activation defect, likely due to defective spindle formation<sup>282</sup>. Cytoplasmic polyadenylation element binding protein 1 (CPEB1) promotes MyoD1 protein synthesis by binding to the cytoplasmic polyadenylation elements within its 3' UTRs to regulate MuSC activation and muscle regeneration<sup>283</sup>. In contrast to HSCs, in MuSCs, NLE1 increases early during activation, and conditional deletion of *Nle1* results in accumulation of unprocessed rRNAs and in prolonged quiescence<sup>277</sup> (Figure 5b).

The fact that manipulation of ribosome biosynthesis has immediate effects on stem-cell quiescence exit is somewhat surprising, since, at least in HSCs, there is no shortage in functional ribosomes<sup>98</sup>. A possible mechanism for these immediate effects could be the biogenesis and activity of specific, specialised ribosome subunits.

**Translation repression.**—Cells can repress the translation of specific mRNAs through RBPs or miRNAs<sup>284</sup>. Quiescent HFSCs express the Sonic Hedgehog (SHH) mRNA, but repress its translation through the RBP Musashi to maintain the quiescent state<sup>36</sup>. Loss of Musashi leads to SHH signaling and HFSC activation, whereas induced overexpression of Musashi prolongs telogen<sup>36</sup> (Figure 5a). Similarly, quiescent MuSCs express the mRNA of the activation factor MyoD, but repress its translation through the RBP Staufin-1<sup>34</sup>. Loss of Staufin-1 leads to MyoD translation and MuSC activation<sup>34</sup>. Staufin-1–RNA immunoprecipitation experiments revealed that its main targets are mRNA of cell cycle regulators<sup>34</sup>. Quiescent NSCs express the mRNA of the centromere protein Ninein<sup>285</sup>. The exon-junction complex [G] binds the Ninein mRNA and prevents its translation, while enabling its transport to centrosomes for localized translation<sup>285</sup>. Finally, miRNAs can repress translation by targeting the AGO2-containing RNA-induced silencing complex to mRNAs. AGO2 enables translation repression and loss of AGO2 limits HSC activation<sup>286,287</sup>. Quiescent MuSCs in hind limb muscles maintain the quiescent state through miR-206-mediated translational repression of PAX3 mRNA; loss of miR-206 results in PAX3 translation and MuSC activation<sup>195</sup>.

Translation depends on mTORC1 activity (Figure 5b). Quiescent NSCs maintain low mTOR signaling by expressing MFGE8 (also known as lactadherin)<sup>288</sup>. Loss of MFGE8 induces mTOR signaling and subsequent NSC activation, which can be rescued with the mTOR inhibitor rapamycin<sup>288</sup>. Loss of mTOR limits activation in HSCs, whereas mTOR signalling gain-of-function induces activation and limits self-renewal<sup>152</sup>. MuSCs, FAPs and HSCs can enter an alert state in response injury-induced systemic signals, in which they remain quiescent, but show active mTOR signalling and can enter the cell cycle more rapidly<sup>73,74</sup>. Deletion of *Rptor* (which encodes RAPTOR) or *Met* prevents the alert response<sup>73</sup>. Restraining the activity of the mTOR–eIF4EBP1 and mTOR–S6K1 (also known as RPS6KB1) axes is crucial for self-renewal<sup>150,152,289</sup>. Finally, tRNA processing modulates translation; processing of tRNA-derived stress-induced small RNAs (tiRNAs) by the RNase angiogenin was shown to maintain HSC quiescence<sup>290</sup>. Transfection of one specific tRNA precursor, tiRNA-Gly-GCC, can block translation in HSCs, which leads to increased self-renewal<sup>290</sup>.



**Protein degradation.**—Cells can regulate protein abundance through targeted protein degradation. E3 ubiquitin ligases covalently link ubiquitin moieties to proteins to target them for proteasome-mediated degradation (see more on protein modifications below). HSCs and MuSCs undergoing activation show increased ubiquitin expression, global protein ubiquitylation and proteasome activity<sup>291,292</sup> (Figure 6). Nevertheless, the expression of some E3 ubiquitin ligases in quiescent HSCs and NSCs declines with activation<sup>35,293–295</sup>, indicating that in addition to roles in quiescence exit, protein degradation has roles in quiescence maintenance. Deletion of the genes encoding the E3 ubiquitin ligases FBW7, HUWE1 or MAEA results in HSC activation<sup>293–295</sup>. FBW7 prevents the accumulation of MYC<sup>294</sup>, whereas MAEA targets cytokine receptors for degradation through ubiquitin-dependent internalization and autophagy<sup>294,295</sup> (Figure 6a). In contrast to HSCs, loss of HUWE1 has no apparent effect on quiescent NSCs<sup>35</sup>.

Quiescent NSCs express the mRNA, but not the protein of the transcription factor ASCL1 due to immediate degradation of the newly translated protein<sup>296</sup>. The ASCL1 protein can be stabilized through binding the transcription factor E47. However, in quiescent NSCs, E47 is sequestered by ID4, rendering it unable to bind ASCL1. Loss of ID4 prevents this sequestration, causing ASCL1 accumulation and NSC activation<sup>296</sup>. Quiescent NSCs degrade epidermal growth factor receptor (EGFR) through LRIG1-dependent ubiquitylation followed by lysosomal degradation. In the absence of LRIG1, NSCs show increased EGFR signaling and activation<sup>16,297</sup> (Figure 6a). Overexpression of constitutively active EGFR can force quiescent NSCs to undergo activation<sup>298</sup>. Finally, deletion of the gene encoding the endoplasmic reticulum associated degradation [G] protein SEL1L results in HSC activation<sup>299</sup>. SEL1L induces degradation of the mTOR activator RHEB and loss of RHEB can rescue the SEL1L phenotype<sup>299</sup>.

In HFSCs undergoing activation, SIRT7 deacetylates the transcription factor NFATC1, which leads to its proteasomal degradation<sup>300</sup>. In the absence of SIRT7, NFATC1 is not degraded and the cells stay quiescent<sup>300</sup> (Figure 6b). In MuSCs, deletion of the essential proteasome component RPT3 (also known as 26S proteasome regulatory subunit 6B) results in reduced proteolytic activity and proliferation and in apoptosis after injury<sup>292</sup>. Chaperone-mediated autophagy is essential for HSC and MuSC activation<sup>138,301</sup>. As discussed above, NSCs undergoing activation upregulate EGFR expression and signaling and downregulate of ID4 in order to stabilize ASCL1 (Ref.<sup>296,298</sup>). In activated MuSCs and HSCs, the cells that express high levels of ID1 show slow cycling and increased self-renewal<sup>302,303</sup>, suggesting that E47 sequestration by ID proteins could be important for quiescence exit in other stem cell populations as well.

In addition to clearing proteins involved in regulation of the quiescent state, stem cells must also clear protein aggregates. NSCs undergoing activation use the intermediate filament vimentin to localize proteasomes to protein aggregates and facilitate protein turnover, and this clearance of aggregates allows the NSCs to become active<sup>304</sup>. Loss of vimentin causes a decline in protein degradation and a delay in NSC activation<sup>304</sup>.

Stem cells entering the quiescent state must dispose of the proteins that drive activation and the cell cycle. Unsurprisingly, targeted protein degradation is therefore an important

mechanism for quiescence entry. In NSCs, HUWE1 ubiquitylates the ASCL1 to induce its degradation and block transcription of cyclin D. This is key for entry into the quiescent state, as NSCs without HUWE1 fail to enter the quiescent state, resulting in NSC depletion and loss of neurogenesis<sup>35</sup> (Figure 6c). HUWE1 enables re-quiescence also in HSCs, where it induces the degradation of N-MYC<sup>293</sup>. HSCs lacking the E3 ligase MAEA fail to reconstitute a quiescent stem cell population, suggesting a defect in quiescence entry<sup>295</sup>. NSCs without LRIG1 have more surface EGFR and are less responsive to quiescence-inducing BMPs<sup>16</sup>.

### Post-translational modifications

All cells regulate protein function through post-translational modifications (PTMs), of which some of the best characterized are the histone PTMs, which have epigenetic roles. However, few studies actually examine the role of a specific modification of a specific protein in the regulation of stem cell quiescence. Loss of the phosphatase PTEN results in activation of HSCs, NSCs, HFSCs and MuSCs and gradual depletion of the stem cell pool<sup>98,177–179,305</sup> (Figure 2c). Genetic interference with kinase signaling, such as the PI3K or MAPK pathways, affects quiescence<sup>130,144–149,176</sup>. Loss of glycosylation enzymes leads to activation of HSCs, MuSCs, and NSCs, possibly due to changes in the abundance of glycosylated Notch proteins<sup>306–308</sup>. However, none of these studies evaluate the effect of the modification on protein abundance and the quiescent state.

Histones bear many PTMs to regulate chromatin accessibility. Histone acetylation, methylation, phosphorylation and ubiquitylation have all been described in quiescent stem cells<sup>2,309</sup>. As discussed above, the gene encoding eIF2 $\alpha$  was replaced in MuSCs with a mutant gene that encodes eIF2 $\alpha$  that cannot be phosphorylated at Ser51, leading to MuSC activation, thereby directly demonstrating the importance of this particular PTM in stem cell quiescence<sup>273</sup>. MuSCs bearing a mutation in the gene encoding the transcription factor PAX7 rendering the protein insensitive to Lys193 acetylation show decreased quiescence entry after injury, due to changes in the protein's DNA binding capacity<sup>310</sup>. Similarly, HFSCs bearing a mutation in the gene encoding the transcription factor NFATC1 rendering the protein insensitive to Lys612 acetylation, show decreased NFATC1 stability and quiescence exit<sup>300</sup>. Other known PTMs such as sumoylation, crotonylation, citrullination and glycosylation are yet to be studied in quiescent stem cells.

### Post-transcriptional regulation of germ line quiescence

One unique stem cell model that is well-known for post-transcriptional regulation of the quiescent state is the oocyte<sup>311–315</sup>. Oocytes are adult-derived germ line stem cells that can maintain a quiescent state for decades until they are activated in response to luteinizing hormone. Upon their activation, oocytes either proceed to ovulation or die, but they never self-renew. In contrast to somatic stem cells, oocytes undergo chromatin condensation and are arrested in the prophase of the first meiosis stage. Although low levels of transcription can occur at the condensed chromatin, oocytes largely depend on post-transcriptional regulation of pre-synthesized mRNAs to regulate the quiescent state. Oocyte quiescence maintenance and exit depend on a set of key genes similar to somatic stem cell quiescence.

Oocytes express high levels of CDK inhibitors and low levels of cyclins. Mice lacking p27 are infertile and have multi-oocyte follicles, consistent with a loss of quiescence<sup>316</sup>. Rb is unphosphorylated and active in oocytes and its conditional deletion results in activation and formation of teratomas<sup>317</sup>. Conditional loss of *Cdk1* results in oocytes that cannot exit quiescence<sup>318</sup>.

To enter quiescence, oocytes express ZFP36L2, which degrades the mRNAs of transcription regulators to ensure transcriptional gene silencing<sup>315</sup>. Like somatic stem cells, oocytes express mRNA isoforms with different UTRs, which results in different levels of translation. However, these mRNAs are not yet polyadenylated. Instead, translation is regulated by cytoplasmic polyadenylation by CPEB1 upon activation<sup>319</sup>, which enables mRNA loading into ribosomes<sup>320</sup>. Activated oocytes undergo a shift towards isoforms with longer 3' UTRs<sup>321</sup>. However, in contrast to somatic stem cells, this shift is caused by differences in mRNA degradation rather than by alternative cleavage and polyadenylation. Surprisingly, miRNAs, though present, are unable to repress their target genes due to lack of expression of Argonaute proteins, which explains the stability of the long 3' UTR isoforms<sup>321</sup>. Activated oocytes do degrade 20% of their transcriptome through the activity of the m<sup>6</sup>A reader YTHDF2. Loss of YTHDF2 causes infertility due to apoptosis of activated oocytes after completion of meiosis<sup>322,323</sup>. Finally, recent work has shown that quiescent oocytes store maternal mRNAs encoding such RNA degradation factors in specialized mitochondria-associated structures marked by Zygote Arrest 1 (ZAR1)<sup>324</sup>. Although these regulatory mechanisms might be unique to the oocyte, they can serve as examples of potential new mechanisms waiting to be uncovered in the regulation of somatic stem cell quiescence.

## Conclusions and future directions

Quiescent stem cells are essential for tissue homeostasis and regeneration and their dysfunction contributes to aging. Genetic loss-of-function studies revealed that stem cells actively maintain the quiescent state and recent work has begun to uncover layers of regulatory control that act post-transcriptionally to fine-tune the amount of protein that is produced. These studies reveal a dramatic and dynamic complexity beyond transcriptional profiles that underscores how stem cell quiescence and its related processes are heavily regulated, biologically complex phenomena. Moreover, it highlights the difficulties of extrapolating transcriptome data to protein levels.

Adult stem cells offer the potential to develop new cell-based therapies. The post-transcriptional regulatory mechanisms offer potential footholds to improve stem cell function in vivo. Antisense oligonucleotides can affect quiescent stem cell processes in vivo by targeting alternative polyadenylation, miRNA-dependent regulation, or translation<sup>34,195,201,239,325</sup>. This opens the door to therapeutics to enhance quiescent stem cell function, analogous to the antisense oligonucleotide technologies that are currently in clinic. Moreover, improved understanding of the post-transcriptional mechanisms that enable quiescence entry could enable interventions to improve stem cell potency following ex vivo stem cell expansion by pushing the expanded stem cells into the quiescent state for improved autologous transplantation. Allowing the cells to expand before a return to quiescence would also circumvent the barrier to gene therapies posed by the lack of homologous

recombination in the quiescent state. Finally, cancer stem cells [G] can adopt a quiescent state, which renders them resistant to therapy, and these quiescent cancer stem cells are thought to contribute to post-treatment relapse<sup>326,327</sup>. Many of the signaling pathways that are important for stem cell quiescence (e.g. Notch, mTORC, p38 MAPK, and CDKN1B), also play a role in cancer stem cell quiescence<sup>326</sup>. Therefore, a better understanding of the mechanisms that regulate stem cell quiescence could help pave the way toward therapeutics that specifically target the quiescent cancer stem cell, either by activating those cells to render them sensitive to other therapies, or by prolonging quiescence to prevent activation and relapse.

While the studies discussed in this review offer a glimpse of the regulatory mechanisms of quiescence maintenance, exit, and entry, ultimately, the field needs better tools to follow cells as they undergo the different processes. The identification of specific cell surface markers for quiescent stem cells will facilitate the prospective isolation and study of these cells. The field needs better lineage tracers to track cells as they enter the quiescent state. One challenge is that under certain conditions, the first division may be asymmetric, resulting in a daughter cell that immediately enters the quiescent state. This can be followed with DNA nucleotide analogs, but the time frame may be too short to allow for genomic recombination to activate lineage tracers. One exciting area of development is DNA barcoding, which has allowed for the fate mapping and simultaneous transcriptional profiling of single HSCs after in vivo or ex vivo activation of a barcode library<sup>27,328</sup>. A further challenge in the study of stem cell quiescence is that isolation of quiescent stem cells from their native environment tends to mimic injury and leads to cell cycle entry and changes in gene expression<sup>14,43,329</sup>. Accordingly, there is a need for improved models for the study, and manipulation, of ex vivo stem cell quiescence<sup>14,18,330,331</sup>. For example, BMP4 can induce in vitro quiescence in NSCs, and the HDAC6-inhibitor Tubastatin A can induce in vitro quiescence in MuSCs<sup>21,96,186,232,239,307,332–334</sup>. Finally, the majority of studies of stem cell quiescence have been done using mice. The field needs a better understanding of stem cell quiescence in other model organisms and would benefit from a non-human primate model to enable translational research. In summary, the post-transcriptional regulation of stem cell quiescence is an exciting and open field with the potential to impact regenerative medicine and our understanding of stem cell biology.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## GLOSSARY

### *Differentiate:*

Differentiation is the process by which a stem cell withdraws from the cell cycle and adopts specialized tissue functions, while losing stem cell functions such as the ability to self-renew

### *Embryonic stem cells:*

Cells derived from the blastocyst of the embryo and able to form all tissue lineages

### *Germline stem cells:*

Cells that can generate the haploid gametes

***Induced pluripotent stem cells:***

Cells created from somatic cells through the overexpression of specific transcription factors, rendering them immortal and able to form all tissue lineages

***Adult stem cells:***

Rare populations of cells that are found in the body throughout the majority of postnatal life and give rise to a limited number of mature cell types that build the tissue in which they reside

***Autologous stem cell transplantation therapies:***

Procedures in which stem cells are isolated from a person and placed back following expansion and/or modification such as gene correction

***Stem cell activation:***

The process by which a quiescent stem cell exits the quiescent state and enters the cell cycle

***Trithorax group:***

family of proteins that modify or remodel histones to activate genes and keep them active

***Non-homologous end-joining:***

A mechanism of DNA double-strand breaks repair without the need for a homologous template

***ATR:***

A serine/threonine-protein kinase that senses persistent single-stranded DNA and activates a DNA damage checkpoint, leading to cell cycle arrest

***Processing bodies:***

Cytoplasmic ribonucleoprotein (RNP) granules primarily composed of translationally repressed mRNAs

***RNA granules:***

Non-membrane-bound organelles composed of RNA and protein

***Exon-junction complex:***

Protein complex that forms on a pre-messenger RNA strand at the junction of two exons immediately after splicing

***Endoplasmic reticulum associated degradation (ERAD):***

A pathway that targets misfolded proteins in the endoplasmic reticulum for ubiquitylation and proteasomal degradation

***Cancer stem cells:***

Cancer cells with stem cell properties such as self-renewal; some cancer stem cells can adopt a quiescent state

**Bulge:**

Morphologically distinct area in the hair follicle in between the opening of sebaceous gland and the attachment site of the arrector pili muscle that functions as the HFSC niche

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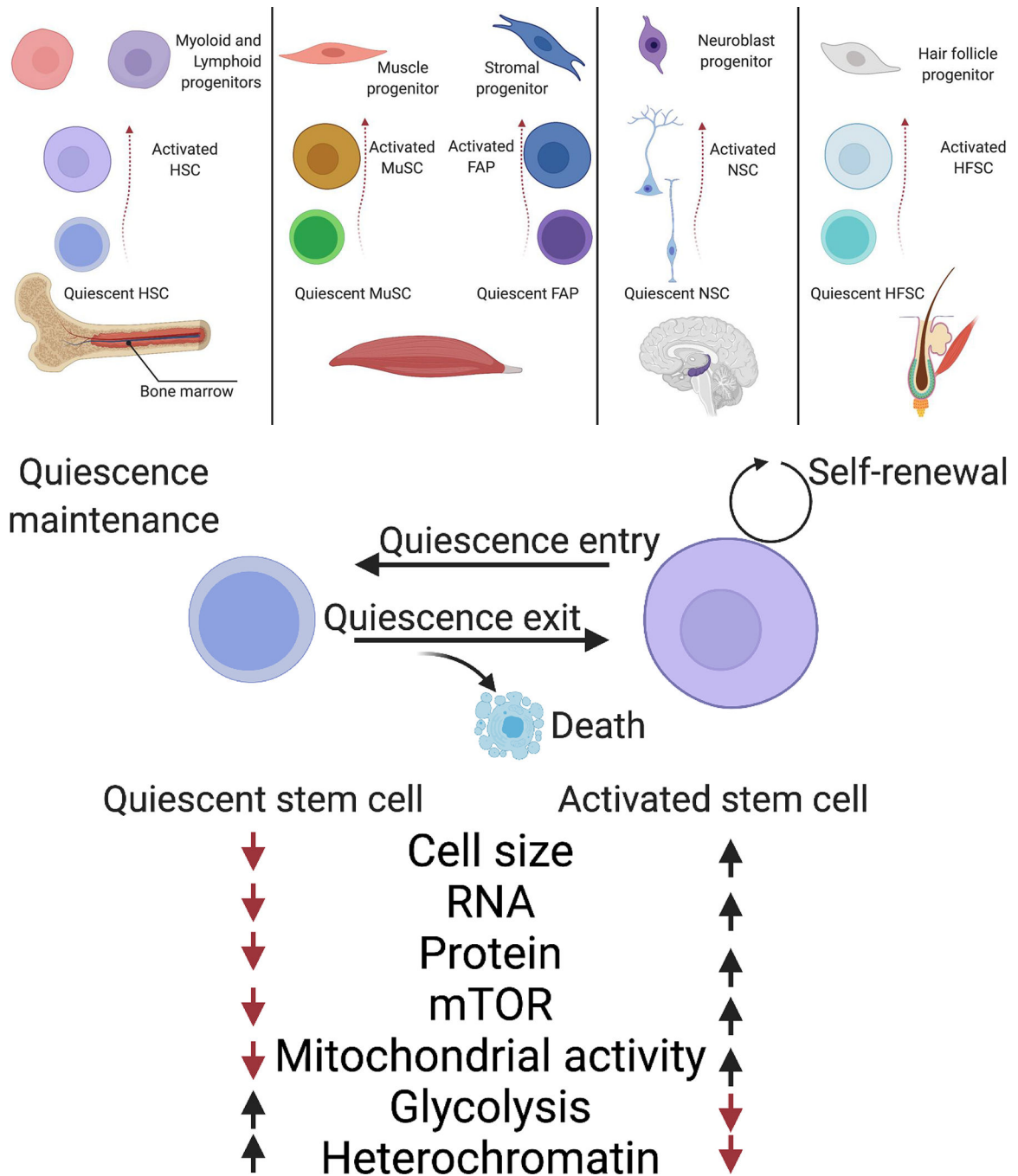
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**BOX 1:****Changes or loss of stem cell quiescence during aging.**

Aging disrupts many aspects of stem cell functionality and impairs tissue homeostasis and regeneration<sup>353,354</sup>. Aged quiescent stem cells (stem cells obtained from aged mice) show a functional decline, which is linked to changes in the quiescent state<sup>355–358</sup>. Strikingly, despite this cell-intrinsic dysfunction, the transcriptomes of aged neural stem cells (NSCs) and skeletal-muscle stem cells (MuSCs) are remarkably similar to the transcriptomes of cells obtained from young mice<sup>40,54,359</sup>. This finding indicates that post-transcriptional gene regulation mechanisms have a role in age-related quiescent stem cell dysfunction.

A number of observations support this idea. Over the course of aging, there is a global switch to expression of longer mRNA isoforms in populations of quiescent HSCs<sup>360</sup>, hinting to dynamic regulation of alternative cleavage and polyadenylation of mRNA and/or of transcript stability. Aged MuSCs accumulate the alternative polyadenylation factor U1 snRNA, and as a result express longer isoforms of CD47, and show reduced proliferation<sup>361</sup>. Aged NSCs accumulate ubiquitinated proteins and protein aggregates, suggesting changes in proteostasis<sup>139,362, 343</sup>. The E3 ubiquitin ligase FBXW7, which ensures degradation of MYC and NOTCH1 during activation out of the quiescent state, is downregulated in aged hair follicle stem cells (HFSCs)<sup>363,364</sup>. However, few studies have directly investigated the role of post-transcriptional regulation in aged quiescent stem cells or how it contributes to aging phenotypes of the quiescent stem cell. In one example, aged HFSC were shown to produce the protease ELANE, which degrades the collagen COL17A1, resulting in premature activation and depletion of the HFSC pool<sup>363</sup>. Induction of COL17A1 expression could rescue the loss of quiescent HFSCs<sup>363</sup>. However, a better understanding of the RNA regulation in aged quiescent stem cells is needed.

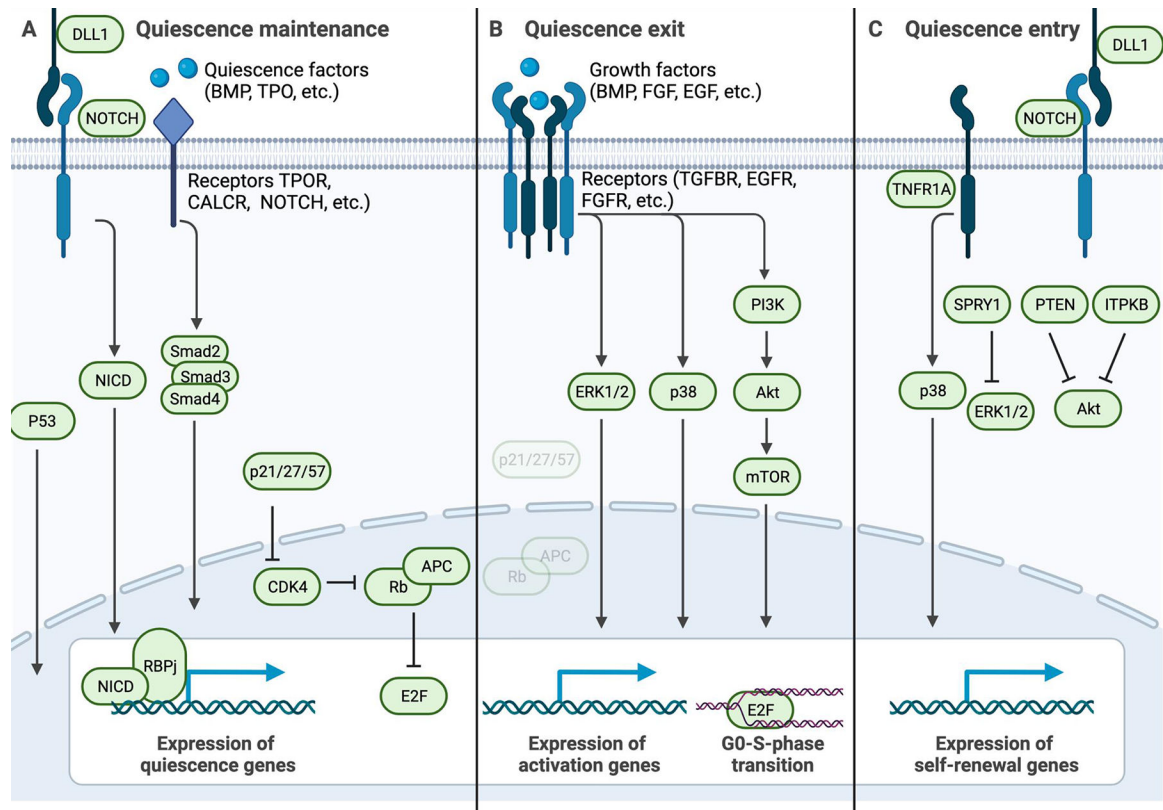


**Figure 1: Types of quiescent stem cells and their features.**

A) Populations of quiescent stem cells have been identified in several tissues. The bone marrow harbors populations of quiescent hematopoietic stem cells (HSCs)<sup>337</sup>, which are established in mice up to six weeks postnatally<sup>60</sup>. HSCs are required to generate the progenitors that sustain the daily production of billions of blood cells and can regenerate the complete blood-cell system following transplantation or injury<sup>46</sup>. Around 6% of mouse HSCs enter the cell cycle each day, though distinct subsets display different activation parameters<sup>46,338,339</sup>. Skeletal muscle contains a population of quiescent stem cells known

as skeletal-muscle stem cells (MuSCs). MuSCs are established around two months after birth in mice<sup>340</sup>. MuSCs are essential to generate the progenitors that regenerate skeletal muscles<sup>341–343</sup>. Around 0.3–1.0% of mouse MuSCs enter the cell cycle each day, depending on the muscle, and their progeny fuse with mature myofibers, though their impact on muscle homeostasis is limited<sup>195,344,345</sup>. A quiescent population of mesenchymal stem cells also called fibro-adipogenic progenitors (FAPs) supports muscle regeneration and homeostasis<sup>346,347</sup>. Around 1% of mouse FAPs enters the cell cycle daily<sup>348</sup>. The adult brain hosts populations of quiescent neural stem cells (NSCs) in the subventricular zone lining the lateral ventricles and in the subgranular zone of the dentate gyrus in the hippocampus<sup>349</sup>. NSCs are established in mice by the second week after birth<sup>161</sup>. They are required to generate the progenitors that sustain continued formation of new neurons, astrocytes and oligodendrocytes. Around 12% of mouse NSCs enter the cell cycle each day<sup>350</sup>. The hair follicles in the skin harbor populations of quiescent hair follicle stem cells (HFSCs). Hair follicles undergo cyclic rounds of growth (anagen), degeneration (catagen) and rest (telogen), termed the “hair cycle”<sup>351</sup>. Quiescent HFSCs are established by 3 weeks after birth at the end of the first hair cycle in mice<sup>36</sup>. During anagen, HFSCs are essential to generate the progenitors that sustain hair growth. They generate the progeny that will start a new hair cycle. Around 0.5% of mouse HFSCs in the bulge [G] enter the cell cycle each day during telogen<sup>352</sup>. Overview of the tissue, time of establishment and spontaneous activation rates of five common adult quiescent stem cell types: HSCs<sup>46,60,337–339</sup>, MuSCs<sup>195,340–345</sup>, FAPs<sup>346–348</sup>, NSCs<sup>161,349,350</sup>, and HFSCs<sup>36,351,352</sup>.

**B)** Quiescent stem cells display a number of distinct features compared to their activated counterparts and undergo different processes, including maintenance of the quiescent state, exit from the quiescent state (activation), and re-entry into the quiescent state (re-quiescence, or self-renewal). At any point in time, quiescent stem cells must ensure survival and may undergo apoptosis. Quiescent stem cells possess several features distinct from their activated counterparts, including small cell size, low RNA and protein content, low mTOR signaling and mitochondrial activity, high use of glycolysis and more heterochromatin, as well as stem cell type specific expression of transcription factors PAX7 (Paired Box 7, MuSCs), TCF3 and TCF4 (Transcription Factor 3 and 4, HFSCs), HOXB5 and TCF15 (Homeobox B5 and Transcription Factor 15, HSCs), and ID3 (Inhibitor of DNA Binding 3, NSCs).



**Figure 2: Key pathways active in quiescent stem cell processes.**

Schematic highlighting key signaling pathways that are active during quiescence maintenance, exit, or entry. **A**) Signals from the niche to the quiescent stem cell that maintain the quiescent state, such as ligands for Notch receptors including DLL1 (delta-like protein 1), and quiescence factors including BMP (bone morphogenic protein), TPO (thrombopoietin), calcitonin, and COLV (collagen V) to their respective receptors NOTCH, BMPRII (bone morphogenic protein receptor 2), TPOR (thrombopoietin receptor), and CALCR (calcitonin receptor). Such receptor-mediated signaling induces gene expression patterns that maintain the quiescent state, via signalling intermediates such as NICD-RBPj, and SMAD2–4. The expression of cell-cycle inhibitors such as p21 similarly invokes quiescence maintenance via inhibition of CDK4 or CDK6, which ensures that APC/C (anaphase promoting complex/cyclosome) and Rb (retinoblastoma) block E2F and S-phase entry. **B**) In response to growth factors, combined with the loss of signals listed in panel (A), phosphorylation cascades such as the PI3K-AKT pathway and the p38 MAP kinase pathway induce quiescence exit and cell cycle entry. **C**) To exit the cell cycle and enter the quiescent state, stem cells interact with NOTCH ligands and suppress the phosphorylation cascades listed in panel (B). In activated NSCs, TNFR1 (tumor necrosis factor 1 receptor) signaling through p38 MAP kinase is key for re-entry into the quiescent state. PTEN (phosphatase and tensin homolog), MEK1 (MAPK/ERK kinase 1), and ITPKB (inositoltrisphosphate 3-kinase b) inhibit AKT, while Sprouty1 (SPRY1) suppresses ERK signaling to stop mitogenic signaling.



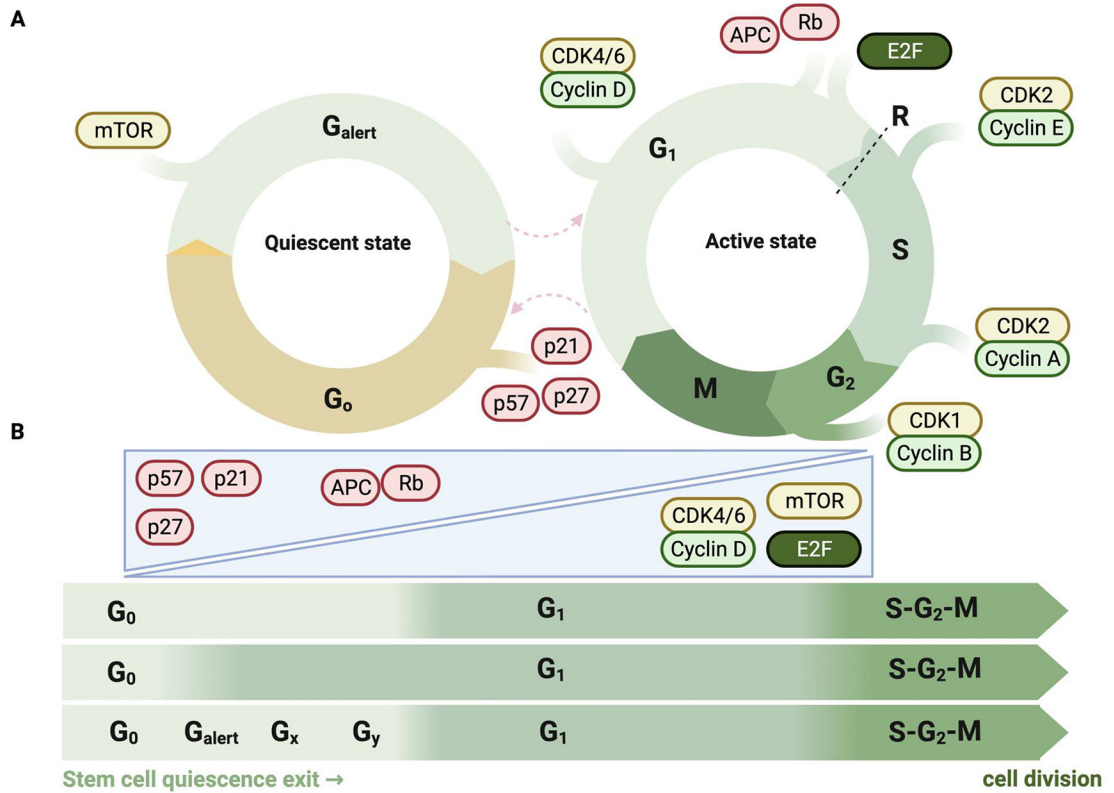
NICD, notch intracellular domain; RBPj, recombination signal binding protein for immunoglobulin kappa j region; CDK4/6, cyclin dependent kinase 4/6; PI3K, phosphatidylinositol-3 kinase.

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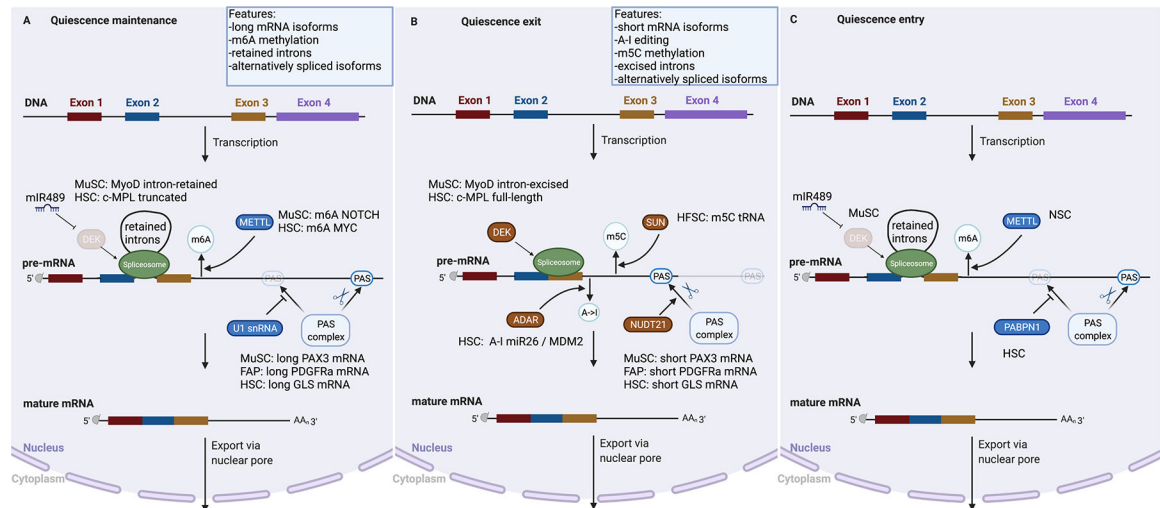
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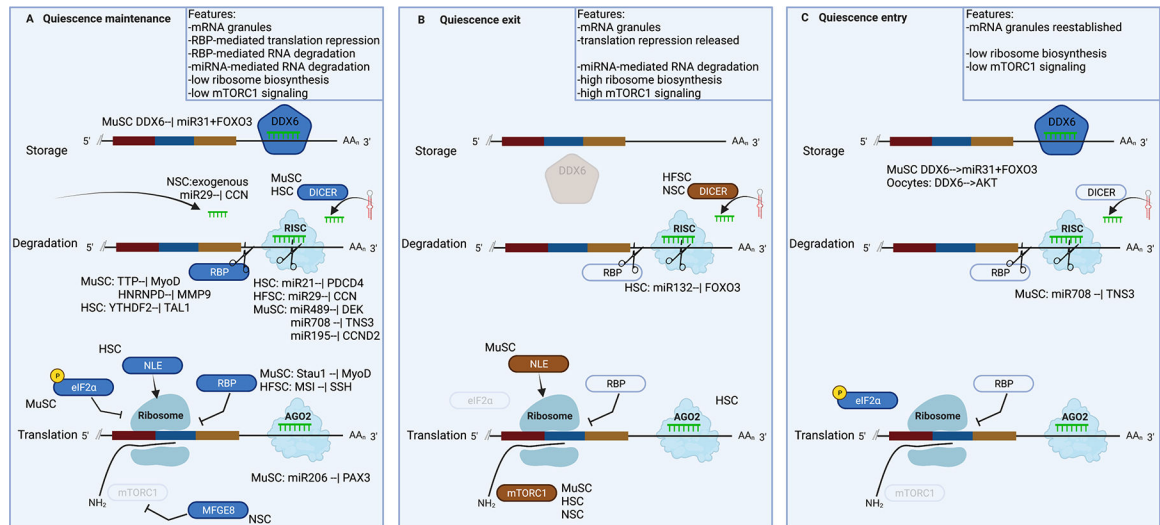
**Figure 3: Quiescence and the cell cycle.**

**A)** Progression of somatic (stem) cells through the G<sub>1</sub>, S, G<sub>2</sub> and M phases of the cell cycle involves intricate regulation by cyclins, cyclin-dependent kinases (CDKs), and their inhibitors. Central to cell-cycle entry and exit is the phosphorylation status of the retinoblastoma (Rb) proteins. Unphosphorylated Rb is chromatin-bound and blocks E2F transcription factors from inducing key proliferation genes. In pro-growth conditions, cyclin D activates CDK4 and CDK6, which phosphorylate (P) Rb and enable transcription of E2F target genes. Somatic stem cells enter the quiescent state early in G<sub>1</sub>, as a result of the downregulation of G<sub>1</sub> cyclins — cyclin D and cyclin E (not shown) — and the upregulation of the CDK interacting protein (CIP) and kinase inhibitory protein (KIP) family of inhibitors, including p21, p27 and p57, which inhibit CDK4 and CDK6 (not shown). Quiescent stem cells can enter an ‘Alert’ state characterized by mTOR signalling and a shorter time to cell-cycle entry. **B)** The first division of the activation of quiescent stem cell is considerably longer than subsequent cell divisions. It is currently unclear whether that first division consists of a long G<sub>0</sub> phase (top), a long G<sub>1</sub> phase (middle), or several G<sub>0</sub> sub-phases following each other in manner similar to the conventional cell cycle phases (bottom).



**Figure 4: Post-transcriptional regulatory mechanisms in the nucleus that control stem cell quiescence.**

Quiescent stem cells regulate mRNA and protein levels through a variety of post-transcriptional mechanisms. Selection of a cleavage and polyadenylation site (PAS) at the pre-mRNA can be blocked by cleavage and polyadenylation (CPA) factors. For example, polyadenylate-binding nuclear protein 1 (PABPN1) and U1 small nuclear RNA (U1 snRNA) block the use of a proximal PAS and thus favour the selection of a distal PAS and production of a mRNA isoform with a longer 3' untranslated region (3'UTR); by contrast, NUDT21 (Nudix Hydrolase 21, also known as Cleavage and Polyadenylation Specificity Factor 25 KDa Subunit) favours the selection of a proximal PAS and production of a mRNA isoform with a shorter 3'UTR. The sequence of the pre-mRNA can be modified by ADAR (adenosine deaminase RNA specific) proteins, which deaminate adenosine nucleotides into inosine (A-I editing); by METTL methyltransferases that produce  $N^6$ -methyladenosine ( $m^6A$ ), and by the SUN2 methyltransferase, which produces 5-methylcytosine ( $m^5C$ ). In addition, the mRNA sequence can be modified by alternative splicing; for example, the protein DEK (DEK Proto-Oncogene) can temporarily delay intron removal. The mature capped, spliced and polyadenylated mRNA is subsequently transported to the cytoplasm. METTL3,14, N6-adenosine-methyltransferase complex catalytic subunit 3,14;  $m^6A$ , N6-Methyladenosine;  $m^5C$ , 5-Methylcytosine; c-MPL, myeloproliferative leukemia protein, also known as thrombopoietin receptor; SUN2, sad1 and UNC84 domain containing 2; PAX3, paired-box 3; PDGFRA, platelet-derived growth factor receptor alpha; GLS, glutamine synthase; MDM2, mouse double minute 2 proto-oncogene; ADAR, A-I, Adenosine-to-Inosine RNA editing; NOTCH, Notch receptor; MYC, m proto-oncogene; miR, microRNA; MYOD, myogenic differentiation 1; THOC5, THO complex 5



**Figure 5: Post-transcriptional regulatory mechanisms in the cytoplasm that control stem cell quiescence.**

The mature mRNA can be degraded by miRNA-containing RISC complexes or RNA binding proteins (RBPs) with RNase activity like TTP, stored in granules like DDX6-containing p-bodies, or translated by ribosomes. Translation can be regulated by RNA binding proteins like STAU1 (Staufen 1) or by mTORC signaling, and by phosphorylation of eIF2a.

DDX6, DEAD-box helicase 6; MYF5, myogenic factor 5; miR, microRNA; 3'UTR, 3' untranslated region; DICER, double-stranded RNA-specific endoribonuclease; RISC, RNA-induced silencing complex; RBP, RNA-binding protein; MyoD, myogenic differentiation 1; HNRNPD, heterogeneous nuclear ribonucleoprotein D; MMP9, matrix metalloproteinase 9; YTHDF2, YTH N6-methyladenosine RNA binding protein 2; TAL1, T-cell acute lymphocytic leukemia protein 1; PDCD4, programmed cell death 4; DEK, DEK proto-oncogene; TNS3, tensin 3; CCND2, cyclin d2; CDC25A,B, cell division cycle 25a,b; NLE1, notchless homolog 1; eIF2a, eukaryotic translation initiation factor 2A; MSI, musashi RNA binding protein 1; SSH, slingshot protein phosphatase; PAX3, paired box 3; AGO2, argonaute 2; mTOR1, mammalian target of rapamycin 1; MFGE8, milk fat globule EGF and factor V/VIII domain containing; FOXO3, forkhead box o3; AKT, AKT serine/threonine kinase, also known as protein kinase b.



**Table 1:**

Overview of post-transcriptional mechanisms that regulate different processes in quiescent stem cells.

Mechanism	Stem cell model	Effector	Target mRNA	Molecular effect	Effect on stem cell quiescence	Refs
Alternative polyadenylation	MuSCs	U1 snRNA high	PAX3	Shorter 3'UTRs	exit	195
	MuSCs	U1 snRNA low	PAX3	Longer 3'UTRs	maintenance	195
	FAPs	NA	PDGFRA	Intronic 3'UTRs	maintenance	201
	FAPs	NA	PDGFRA	Longer 3'UTRs	exit	201
	HSCs	NUDT21 high	Glutaminase	Shorter 3'UTRs	exit	197
	HSCs	NUDT21 low	Glutaminase	Longer 3'UTRs	maintenance	197
	HSCs	PABPN1	NA	longer 3'UTRs	entry	197
RNA methylation (m <sup>6</sup> A and m <sup>5</sup> C)	MuSCs	METTL3	NOTCH2 receptor	Stabilization	maintenance	220
	HSCs	METTL3	MYC	Stabilization	maintenance	215,218,219
	HSCs	IGF2BP2	BMI1	Stabilization	maintenance	217
	NSCs	METTL3 and METTL14	NA	Global methylation	entry	223
	NSCs	FTO	SOCS5	Destabilization	entry	335,336
	HFSCs	SUN2	tRNA	Stabilization	exit	224
RNA editing (A-I)	HSCs	ADAR proteins	MDM2	Stabilization	exit	206
	HSCs	ADAR proteins	pre-miR-26	Stabilization	exit	206
Intron retention	MuSCs	DEK	MyoD	Intron excision	exit	234
	HSCs	DEK	NA	NA	maintenance	236
Alternative splicing	HSCs	OTT1 high	c-MPL	truncated protein	maintenance	232
	HSCs	OTT1 low	c-MPL	Full-length protein	exit	232
	HSCs	PRMT5	NA	Global isoforms	maintenance	231
	MuSCs	QKI	ITGA7	Alternative isoforms	exit	230
Nuclear export or import	NSCs	THOC5	NA	NA	maintenance	238
	NSCs	miR9	NA	nuclear accumulation	maintenance	239
RNA storage	MuSCs	DDX6	miR31, MYF5	Storage	maintenance	194
	Oocytes	DDX6	PI3K-AKT pathway	Storage	entry	271
RNA degradation	MuSCs	miR489	DEK	Degradation	maintenance	242
	MuSCs	miR708	TNS3	Degradation	maintenance	243
	MuSCs	miR195/497	CCND2	Degradation	maintenance	244
	HSCs	miR21	PDCD4	Degradation	maintenance	245
	NSCs	miR24	SOX11, MEIS2	Degradation	maintenance	249
	HFSCs	miR29	WNT, BMP pathways	Degradation	maintenance	250



Mechanism	Stem cell model	Effector	Target mRNA	Molecular effect	Effect on stem cell quiescence	Refs
	MuSCs, HSCs	DICER	NA	miRNA maturation	maintenance	241,242
	NSCs, HFSCs	DICER	NA	miRNA maturation	exit	246-248
	HSCs	miR132/212	FOXO3	Degradation	exit	255
	MuSCs	miR708	NA	NA	entry	243
	MuSCs	TTP	MyoD	Degradation	maintenance	190
	MuSCs	HNRNPD1	MMP9	Degradation	maintenance	261
	HSCs	YTHDF2	TAL1	Degradation	maintenance	216
RNA folding	MuSCs	DHX36	GNAI2	Translation	exit	278
	HSCs	DHX36	NA	Translation	exit	279
Translation repression	MuSCs	STAU1	MyoD	Repression	maintenance	34
	HFSCs	MSI	SHH	Repression	maintenance	36
	MuSCs	miR206	PAX3	Repression	maintenance	195
	NSC	MFGE8	mTORC1	Global translation	maintenance	288
	MuSCs, HSCs, FAPs	RAPTOR or cMET	NA	Global translation	exit	73,74
Translation initiation	MuSCs	eIF2a	NA	Global translation	maintenance	273
	HSCs	NLE	rRNA	Ribosome biosynthesis	maintenance	280
	MuSCs	NLE	rRNA	Ribosome biosynthesis	exit	277
	HSCs	RUNX1	ribosomal genes	Ribosome biosynthesis	maintenance	281
Proteasomal degradation	HFSCs	SIRT7	NFATC1	deacetylation and degradation	exit	300
	HSCs	FBW7	MYC	Proteasomal degradation	maintenance	294
	NSCs	HUWE1	ASCL1	Proteasomal degradation	entry	35
	HSCs	HUWE1	MYC	Proteasomal degradation	entry	293
	HSCs	SEL1	RHEB	Repression of mTORC1	maintenance	299
	NSCs	VIM	NA	localized proteasomes	exit	304
Lysosomal degradation	NSCs	LRIG	Surface EGFR	Lysosomal degradation	maintenance, entry	16,297
	HSCs	MAEA	Surface cytokine receptors	Lysosomal degradation	maintenance	294,295
Protein phosphorylation	MuSCs	PERK	eIF2a	translation repression	maintenance, entry	273
	MuSCs, HSCs, NSCs, HFSCs	PTEN	NA	NA	maintenance, entry	98,177-179,305

Mechanism	Stem cell model	Effector	Target mRNA	Molecular effect	Effect on stem cell quiescence	Refs
	MuSCs, NSCs, HSCs	PI3K-AKT	NA	NA	maintenance, entry	130,148,149
	MuSCs, NSCs, HSCs	MAPK	NA	NA	maintenance, entry	130,144-147
Protein acetylation	HFSCs	SIRT7	NFATC1	deacetylation and degradation	exit	300
	HFSCs	SIRT7	NFATC1 Lys612	degradation	exit	300
	MuSCs	MIST1 and SIRT2	PAX7 Lys193	DNA binding	entry	310
Protein glycosylation	MuSCs, HSCs, NSCs	Glycosylation enzymes	NA	Global glycosylation	maintenance	306-308
	MuSCs, NSCs, HSCs	NA	NOTCH receptors	Stability	maintenance	306-308

FAPs, fibro-adipogenic progenitors; HFSCs, hair follicle stem cells; HSCs, hematopoietic stem cells; MuSCs, skeletal-muscle stem cells; NA, not available; NSCs, neural stem cells; UTR, untranslated region.