CellPress

Trends in Cell Biology

Review

Muscle Stem Cell Quiescence: Controlling Stemness by Staying Asleep

Sara Ancel,[1,2] Pascal Stuelsatz,[1] and Jerome N. Feige[1,2,*]

Muscle stem cells (MuSCs) are tissue-resident stem cells required for growth and repair of skeletal muscle, that are otherwise maintained in a cell-cycle-arrested state called quiescence. While quiescence was originally believed to be a state of cellular inactivity, increasing evidence suggests that quiescence is dynamically regulated and contributes to stemness, the long-term capacity to maintain regenerative functions. Here, we review the current understanding of MuSC quiescence and highlight recently discovered molecular markers, which differentiate depth of quiescence and influence self-renewal capacity. We also discuss how quiescent MuSCs integrate paracrine factors from their niche and dynamically regulate cell signaling, metabolism and proteostasis as they anticipate physiological needs, and how perturbing these cues during aging impairs muscle regeneration.

Impact of Quiescence on MuSC Fate Decisions

Skeletal muscle is a highly plastic tissue with strong potential for tissue expansion and repair. During embryonic and postnatal development, MuSCs contribute to muscle growth and maturation by generating a myogenic progeny that commits to terminal differentiation [1–3]. Some MuSCs escape the muscle building process and anchor to myofibers under the basal lamina, in a prolonged cell-cycle-arrested state of low cellular activity called quiescence [4,5]. Quiescence favors the preservation of stem cell properties by preventing aberrant activation and exhaustion of the stem cell pool while avoiding accumulation of DNA damage and senescence [5]. Following traumatic myofiber injury or chronic regeneration triggered by muscle-wasting diseases, quiescent MuSCs activate and generate myogenic progenitors that drive adult myogenesis for tissue repair (Figure 1 and Box 1). MuSCs also contribute to muscle adaptation to exercise and are necessary for hypertrophy and improved muscle function in response to physical activity [6–8].

The mechanisms governing MuSC activation and fate decisions to control regeneration are well characterized and recently reviewed [9]. In contrast, how the regulation of MuSC quiescence contributes to stemness and long-term regenerative capacity remains more elusive. While quiescence is a cellular state, stemness refers to the molecular processes that maintain the stem cell pool over the long term by instructing efficient cell fate decisions that promote **MuSC self-renewal** (see Glossary) while continuously providing myogenic progenitors. Self-renewal involves both the segregation of cell fate during division and the ability of a daughter stem cell to return to quiescence to maintain the MuSC pool over the long term (Box 2). The link between quiescence and stemness was first highlighted using radioisotope labeling experiments in growing rats, where a fraction of MuSCs that did not get labelled defined a quiescent subpopulation that retained the capacity to generate myogenic progenitors without differentiating, thereby constituting a proliferative reserve for long-term regenerative competency [10]. The hypothesis that maintenance of quiescence is important for MuSC stemness was confirmed more recently using a labeling system that tracks proliferative history *in vivo* where label-retaining cells (LRCs) have not proliferated and mark

Highlights

MuSCs are located on muscle fibers in a reversible cell-cycle-arrested state called quiescence.

Quiescent MuSCs are molecularly and functionally heterogeneous and can be stratified in subpopulations with different dynamics of activation and regenerative potential.

Maintenance and return to quiescence are regulated by interdependent extrinsic and intrinsic cues that coordinate the regenerative capacity of MuSCs and influence their ability to self-renew.

MuSC stemness is the ability to perform efficient cell fate decisions to self-renew and to generate myogenic progenitors for long-term regenerative potential. Quiescence is a way to regulate stemness by preventing MuSC exhaustion.

Aging perturbs cell-autonomous and niche-dependent mechanisms that alter MuSC quiescence and stemness, and impair their long-term regenerative capacity.

[1]Nestlé Institute of Health Sciences, Nestlé Research, EPFL Innovation Park, 1015 Lausanne, Switzerland
[2]School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

*Correspondence:
jerome.feige@rd.nestle.com (J.N. Feige).

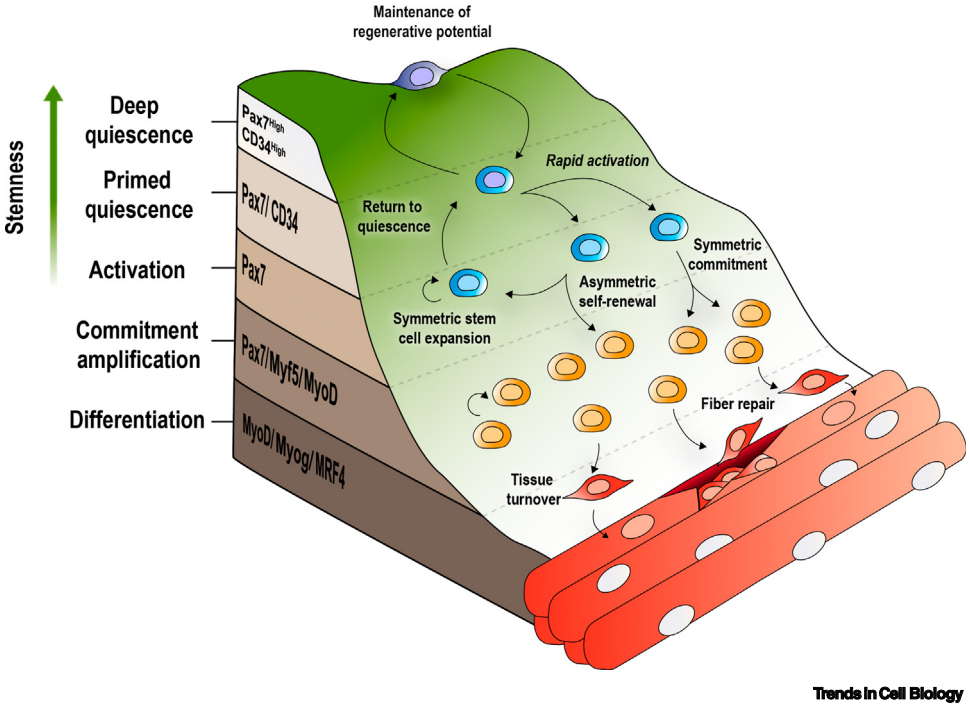

Figure 1. Quiescent MuSCs Control the Regenerative Capacity of Adult Skeletal Muscle and Maintain Long-term Stemness. MuSCs control muscle repair and turnover through different cellular states of quiescence and activation that balance the expansion, differentiation, and fusion of myogenic progenitors to myofibers. Deeply quiescent MuSCs represent a population with higher stemness, while primed quiescent MuSCs activate fast to promote rapid expansion of myogenic progenitors and committed myoblasts lose stemness as they terminally commit to differentiation. Self-renewal and return to quiescence maintain the long-term pool of quiescent MuSCs through asymmetric divisions generating a stem and a committed cell, or symmetric amplification giving rise to two daughter stem cells. Abbreviation: MuSC, muscle stem cell.

quiescence and fast-cycling cells have diluted the label [11]. While non-LRCs are restricted to terminal differentiation, quiescent LRCs transplanted *in vivo* can commit to differentiation and self-renew [11], further supporting the link between quiescence and stemness.

Box 1. From Quiescent MuSCs to Mature Myofibers

Quiescent MuSCs express the paired-box transcription factor Pax7, a canonical myogenic marker that represses genes involved in muscle differentiation and is essential for proper muscle regeneration [9]. Other cell surface markers include syndecans 3 and 4 (Sdc3/4), integrins α7 and β1, CD34, c-Met, neural and vascular cell adhesion molecules (NCAM1 and VCAM1), neural and muscle cadherins (N-CADH and M-CADH), C-X-C motif chemokine receptor 4 (CXCR4), calcitonin receptor (CALCR), and calveolin 1 (CALV1) [86]. These extracellular sensors are unevenly distributed on the surface of quiescent MuSCs to match structural cues from the myofiber and the basal lamina, and integrate different signals from the niche, either through paracrine signaling, cell–cell interaction or extracellular matrix adhesion, which together actively control the quiescent state. To ensure progression through the myogenic program, MuSCs sequentially activate the myogenic regulatory factors, a family of basic helix-loop-helix (bHLH) transcription factors associated with lineage specification and control of muscle regeneration. Members of the MRF family include myogenic factor 5 (Myf5), MyoD, myogenin (Myog) and myogenic regulatory factor 4 (MRF4/Myf6/Herculin). The hierarchical MRF expression patterns drive sequential control of myogenic fate. Whereas Myf5 expression is essentially restricted to undifferentiated myoblasts, MyoD expression extends until early onset of differentiation, and myogenin and MRF4 cover the terminal phases of commitment and differentiation. Even though a small fraction of activated MuSCs will exit the cell cycle and eventually return to quiescence, the majority will progress through differentiation by further downregulating Pax7. Finally, myoblast fusion into multinucleated muscle fibers is governed by myomaker and myomerger, two muscle-specific membrane proteins that are essential in ensuring proper skeletal muscle development and repair [87].

Glossary

5′ AMP-activated protein kinase: heterotrimeric protein complex that acts as an intracellular energy sensor and phosphorylates target proteins in response to AMP levels; master regulator of metabolic homeostasis that stimulates oxidative energy metabolism to increase ATP production.

Autophagy: intracellular process in which large cellular components such as dysfunctional organelles and protein aggregates are engulfed in double-membrane vesicles and targeted to the lysosome for degradation and recycling (see proteostasis).

Cyclin-dependent kinases: family of serine–threonine kinases that bind to regulatory proteins called cyclins. Cyclin–CDK complexes regulate the progression through the cell cycle. Specific combinations of CDKs and cyclins will phosphorylate different cell cycle targets and regulate cell cycle progression.

Cytometry by time-of-flight: immunophenotyping technology that combines flow cytometry and mass spectrometry to detect 40+ proteins simultaneously at the single cell level; relies on the use of isotopically pure metals instead of fluorophores for antibody labelling, extending the multiplexing capability of traditional flow cytometry approaches.

Fibroadipogenic progenitors: tissue-resident mesenchymal cells of the MuSC niche that can differentiate into fibroblasts, adipocytes and possibly osteoblasts/chondrocytes, but also positively support MuSC function through paracrine secretion and cell–cell interactions.

Hes/Hey family: direct target genes of delta-Notch signaling that are transcriptionally activated by the Notch intracellular domain; encode bHLH transcriptional regulators that are involved in maintaining the uncommitted quiescent state, notably by suppressing MyoD expression.

Heterochronic transplantation assay: transplantation of cells (or tissues) from animals of a given condition into recipients of a different condition that is used to differentiate intrinsic cellular changes from extrinsic adaptations in response to the local and systemic environment. These assays have been used in the field to evaluate how aging impacts MuSCs and how paracrine and endocrine signals from a

Box 2. Importance of Division Patterns on MuSC Fate and Return to Quiescence

The ability to regulate fate decisions upon cell division is critical for stemness as MuSCs need to balance differentiation and self-renewal by sensing the needs to generate new progenitors for regeneration while replenishing the stem cell pool and returning to quiescence (see Figure 1 in main text). As they break quiescence, MuSCs can divide asymmetrically to generate one committed and one daughter stem cell, thereby sustaining MuSC self-renewal [86]. In parallel, symmetric modes of division can generate either two other stem cells or two committed progenitors that progress to myogenic differentiation [86], thereby ensuring maintenance of the MuSC pool over the long term. During mitosis, dividing MuSCs are spatially constrained by structural cues that orient proteins involved in the organization of the mitotic spindle, influence cell division, and distribute activators of the myogenic program to the committing cell [88,89]. In addition to its well-documented role in myofiber membrane stabilization, a fundamental role of dystrophin was recently discovered in MuSCs [88]. Dystrophin regulates cell polarity during asymmetric divisions and influences self-renewal by localizing the PAR-complex in the committed cell, where it activates p38α/β MAPK and increases MyoD expression [88]. Similarly, the polarized localization of the epidermal growth factor receptor (EGFR) on the basal surface of quiescent MuSCs orients mitotic centrosomes through Aurora kinase A (Aurka) and supports MuSC self-renewal and return to quiescence through asymmetric divisions [88]. The decision whether a MuSC undergoes a symmetric or asymmetric division is also dictated by the regenerating niche [90]. MuSCs were shown to preferentially undergo symmetric divisions during the early phase of regeneration, consistent with the need for rapid progenitor amplification at this stage [90]. As regeneration progresses, MuSCs switch from symmetric to asymmetric divisions [90], thereby ensuring the generation of uncommitted MuSCs that can return to quiescence to repopulate the stem cell pool and preserve long-term stemness for future regenerative events.

Lately, the standardization of single-cell transcriptomics and proteomics coupled with the development of genetic-lineage-tracing systems for myogenic regulators have provided a fresh perspective on MuSC quiescence by uncovering new molecular and niche factors regulating MuSCs [12]. The field has started to interrogate how these factors crosstalk with each other and how this complex regulation of quiescence dynamically controls the short- and long-term fate of MuSCs. Here, we review the main key regulatory factors that define quiescent MuSC populations, and highlight signaling pathways that jointly govern quiescence and stemness and their impact on MuSC homeostasis and aging. Unless otherwise stated, conclusions are derived from rodent models that facilitate isolation, modeling, and genetic engineering of MuSCs.

Heterogeneity of Quiescent MuSCs as a Cell Fate Determinant

The idea that quiescent MuSCs are functionally heterogenous and comprise various subpopulations with different regenerative potential emerged from studies in rodents [10,13,14], later confirmed in humans [15]. Genetic tools for tracing **myogenic regulatory factors** (MRFs) [16–20] and single-cell profiling technologies [21–26] have recently enabled the characterization of an unforeseen extent of heterogeneity within the quiescent MuSC population, notably at the level of gene expression and cell cycle control.

Heterogeneity Based on Gene Expression

Quiescent MuSC heterogeneity can be defined by differential expression of myogenic markers that also directly influence stemness. Early studies have correlated the absence of myogenic factor (Myf)5 expression with the maintenance of MuSC quiescence [13,16], and Myf5 lineage-tracing studies have further demonstrated the functional heterogeneity of MuSCs [17]. Myf5-lineage-negative MuSCs outperform Myf5-lineage-positive cells in re-establishing the quiescent MuSC pool upon transplantation, suggesting that Myf5-lineage-negative MuSCs retain more stem-like properties [17]. The role of Myf5 in priming MuSC commitment at the expense of stemness has been further confirmed through genetic loss of function as quiescent MuSCs from Myf5 heterozygous mice self-renew and return to quiescence more efficiently after *in vivo* transplantation [18]. **Paired box 7** (Pax7) expression can also define heterogeneity as MuSCs with the highest Pax7 levels have entered a deeply quiescent state characterized by a prolonged time to cell cycle re-entry, lower metabolic activity, and greater stemness upon serial transplantations [19]. Similarly, differential expression of CD34, a canonical MuSC surface marker, delineates stages of MuSC quiescence, with $CD34^{High}$ MuSCs being the

young environment can rescue the defects accumulated in aged cells.
(MuSC) self-renewal: process through which stem cells perpetuate the stem cell pool over the long-term by generating an identical stem cell, either through symmetric (two daughter stem cells) or asymmetric (one stem and one committed daughter cell) divisions.
Myogenic regulatory factors: bHLH transcription factor family composed of Myf5, MyoD, myogenin, and MRF4 that regulates myogenic progression; bind to conserved E-box regions, a motif found in the promoters of many muscle-specific genes.
Paired box 7: transcription factor from the paired-box family; canonical marker of MuSCs and critical for MuSC function.
Proteostasis: processes that dynamically regulate protein homeostasis; regulated by intracellular signaling controlling protein synthesis and ribosomal biogenesis such as mTOR, protein folding chaperones and protein quality control systems and degradation mechanisms through the ubiquitin/proteasome, cathepsin-mediated lysosomal degradation and autophagy.
Pseudotime analysis: bioinformatic methodology used in single-cell transcriptomics (see single-cell RNA sequencing) that infers the progression of an individual cell within a dynamic biological process (development, regeneration, etc.) based on static gene expression snapshots of specific populations.
Quiescence markers: biological markers used to probe quiescent MuSCs. These markers include negative regulators of cell cycle progression (CDK inhibitors), transcriptional regulation (FoxO), and metabolic function (see AMPK and SIRT1), as well as myogenic inhibitory molecules (see Hes/Hey family) and genes involved in cell–cell adhesion (CD34, cadherins, and syndecans).
Single-cell RNA sequencing: sequencing technique used to examine gene expression within individual cells and deconvolute cellular heterogeneity in a mixed population.
Sirtuin 1: NAD^+-dependent deacetylase that acts as low energy-sensor in response to NAD^+ levels; regulates epigenetics and oxidative metabolism by deacetylating histones as well as metabolic enzymes/regulators.

most quiescent population and having better engraftment and self-renewal capabilities several weeks after transplantation [20]. In parallel, single-cell proteomic analysis of MuSC surface markers using **cytometry by time-of-flight** (CyTOF) has discriminated populations of quiescent and activated myogenic progenitors via the expression of either CD9 only or both CD9 and CD104 [21]. While the two populations display similar myogenic capacity *in vitro*, the quiescent, $CD9^{+}/CD104^{-}$ fraction repopulates the MuSC compartment better 5 weeks post-transplantation, suggesting that this population is more prone to self-renewal [21]. Hence, a model emerges where MuSCs sequentially express CD9 and then CD104 as they break quiescence, but whether CD34 regulation overlaps in the same cells and how its relative decline is timed in this process remains to be determined.

Analysis of individual markers has revealed the extent of functional heterogeneity within quiescent MuSCs, but it has remained difficult to assess the overlap between MuSC populations marked by these individual markers. Genome-wide single cell profiling using **single-cell RNA sequencing** (scRNA-seq) has started to uncover how these MuSC populations are interconnected [12]. Based on these results, adult myogenesis can be modeled computationally by ordering myogenic cells along a pseudotemporal regenerative axis that reflects their progression towards differentiation [22,23]. Quiescent MuSCs initiate the trajectory, while genes associated with cell cycle progression, chromatin remodeling, metabolic activity, and translation initiation switch on in activated myoblasts located further down the pseudotemporal axis [22]. In this representation, quiescent MuSCs cluster in two different groups. The first cluster is enriched in **quiescence markers** and includes high Pax7 levels as previously reported using Pax7 reporter mice [19,22], as well as genes related to stress resistance, response to unfolded protein, cell cycle arrest, and circadian rhythm. In contrast, activation of ribosome biogenesis, mRNA processing, translation, and protein stabilization pathways define a second cluster of quiescent MuSCs, which also express (but do not translate) commitment markers such as myogenic differentiation factor (Myo)D and Myf5 [22–24]. While most MuSC clustering analyses have been performed in rodents, heterogeneity within quiescent MuSCs has also been reported in human muscle, where scRNA-seq and **pseudotime analyses** have identified two distinct subpopulations of quiescent MuSCs with similar expression patterns to the rodent populations [25,26]. Collectively, studies on molecular heterogeneity of MuSCs have shown that quiescent MuSCs are diverse and map to a continuum of stages along the quiescence-to-activation trajectory. However, confirmation with genetic models will be key to understand if this clustering is physiologically relevant or could result from changes resulting from the isolation procedure (Box 3).

Box 3. Studying the *in vivo* Quiescent State

Studying cellular quiescence requires MuSCs to be extracted from their environment. Given that MuSCs are poised to rapidly initiate cellular and molecular responses upon changes in their microenvironment, the signature of MuSCs following dissociation differs from the *in vivo* quiescent state [91], and recapitulates markers of *in vivo* activation [94]. Increasing efforts have therefore focused at developing protocols to remove isolation-induced signatures from downstream analyses of MuSC quiescence [80–82]. Freshly isolated MuSCs exhibit gene expression and epigenetic signatures of activation compared to MuSCs whose *in vivo* quiescent transcriptional signature has been preserved through *in situ* muscle fixation [80,81], a method proven compatible with immunofluorescence, RNA sequencing [80,81], and proteome analyses through mass spectrometry [81]. Alternatively, reversible inhibition of *de novo* transcription through treatment with the transcription inhibitor α-amanitin prevents the accumulation of pervasive transcripts and preserves the quiescent transcriptome [82]. In parallel, the broad adoption of single-nucleus RNA sequencing (snRNA-seq) could also facilitate the study of the *in vivo* quiescent state. snRNA-seq bypasses the spurious expression of genes resulting from isolation as tissue preparation for snRNA-seq is short and only considers nuclei, where the absence of ribosomes prevents the translation of mRNAs induced during dissociation [92]. While snRNA-seq has been mostly used for studies on multinucleated muscle fibers [83,84], its potential application to MuSCs could improve our understanding of the endogenous quiescent state. Finally, intravital muscle imaging provided the first snapshots of quiescent MuSC behavior in a physiological environment [93], and could potentially offer a unique *in vivo* perspective on self-renewing events and return to quiescence following injury.

Heterogeneity Based on Cell Cycle Control

Recent evidence suggests that quiescent MuSCs can be heterogeneously arrested in the cell cycle and that these states differ in stemness and regenerative competencies [27–29]. Coculture of quiescent MuSCs with myofibers damaged *ex vivo* triggers G_0-arrested MuSCs to transition into G_1 through myofiber-released factors such as GAPDH [28]. However, these preactivated MuSCs can revert to a G_0 state without undergoing mitosis, suggesting that quiescent MuSCs can reversibly transition between G_0 and G_1 while remaining arrested [28]. Similarly, systemic signals released following muscle injury prime quiescent MuSCs from noninjured muscles into a preactivated state [29]. These distal MuSCs remain quiescent but anticipate cell cycle entry by transitioning from a canonical G_0 to a G_{Alert} state via mTORC1 signaling [29]. In addition to shorter kinetics of cell cycle entry, MuSCs in the G_{Alert} state increase transcriptional and translational activity, activate mitochondrial metabolism, and have enhanced capacity for fiber repair [29]. While this study demonstrates that systemic signals released upon muscle injury regulate the depth of MuSC quiescence remotely, MuSCs in homeostatic conditions can also be stalled at different quiescence depths to respond to injury with different speed and amplitude [19,20].

The evolving understanding of MuSC heterogeneity has revealed that the transition from quiescence to activation does not involve a single event of cell cycle re-entry but rather involves gradual and accumulating changes in cellular metabolism and signaling that fine tune cell fate. This results in heterogenous functionality of quiescent MuSCs where shallow quiescence enables rapid activation and commitment while deep quiescence retains more stem-like properties.

Mechanisms Regulating MuSC Quiescence

Cellular quiescence is dynamically regulated by a cohesive set of extrinsic cues from the niche and by intrinsic molecular mechanisms that work synergistically to regulate cell motility and physiology while repressing cell cycle progression and commitment (Figure 2). Proper regulation of these quiescence-promoting signals is crucial to coordinate the different states and fates of MuSCs to maintain long-term stemness and effective repair.

Regulation of MuSC Quiescence by the Local Niche

The muscle fiber and the extracellular matrix play a critical role in establishing quiescence (Figure 2), in particular by regulating Notch signaling, which was proven essential in this process [30–33]. Quiescent MuSCs express the receptors Notch1–3 that bind to their ligand delta-like 1 (Dll1), located on the muscle fiber. Upon binding to its ligand, the Notch intracellular domain (NICD) translocates to the nucleus where it regulates the expression of quiescence genes, including members of the **Hes/Hey family**. In parallel, Notch also regulates the expression of collagen genes as autocrine production of collagen V by MuSCs is used as a feed-forward mechanism to stabilize the niche and reinforce the quiescent state [30]. Conversely, loss of Notch signaling impairs MuSC self-renewal and triggers stem cell activation, resulting in altered stemness and depletion of the MuSC pool [31–34]. scRNA-seq analyses have shown that a subset of MuSCs critically relies on the Notch2 receptor to re-establish quiescence in self-renewing MuSCs, as blunting the Dll1/Notch2 axis results in failure of MuSCs to retain Pax7 expression and progression towards terminal differentiation [34]. Finally, Notch signaling is further stabilized by FoxO, a transcription factor that is highly expressed in quiescent MuSCs [35]. Constitutive activation of Notch in MuSCs with a perturbed FoxO axis rescues MuSC depletion and re-establishes quiescence in these cells [36]. Overall, these studies have provided critical insights into Notch signaling in MuSCs and established Notch as a master regulator of MuSC quiescence.

Recently, secreted paracrine signals from the niche were also identified as an additional layer of regulation of MuSC quiescence (Figure 2) [37,38]. Under homeostatic conditions, myofibers

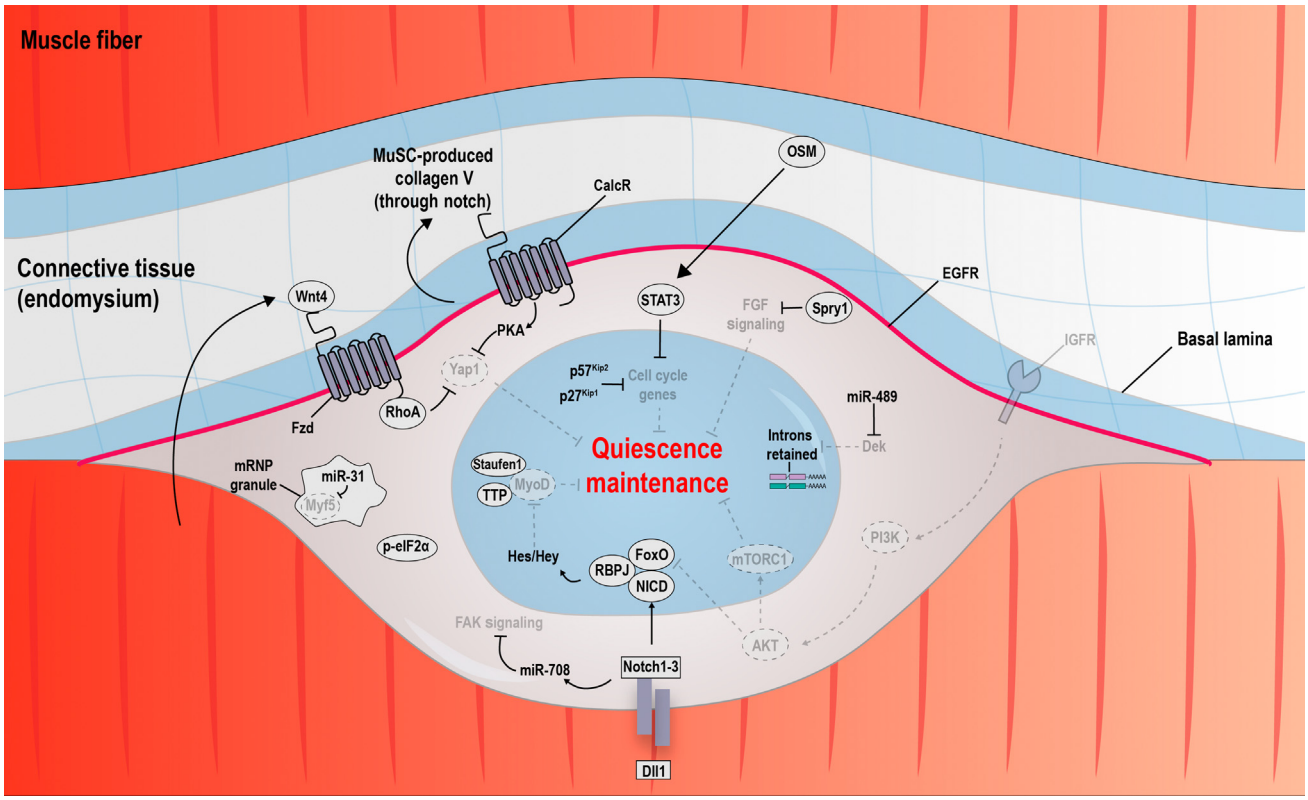

Figure 2. Extrinsic and Intrinsic Mechanisms Regulating MuSC Quiescence. MuSCs consolidate the quiescent state by integrating a set of extrinsic cues from their niche with cell-autonomous signals that jointly maintain the MuSC in a state of reversible cell cycle arrest. Light broken boxes and arrows represent factors and signaling pathways with low activity in the quiescent state. Abbreviations: CalR, calcitonin receptor; CDK, cyclin-dependent kinase; Dll, delta-like ligand; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; FGF, fibroblast growth factor; FoxO, forkhead box O; Fzd, frizzled; IGFR, insulin-like growth factor receptor; mTORC1, mammalian target of rapamycin complex 1; MuSC, muscle stem cell; Myf5, myogenic factor 5; MyoD, myogenic differentiation factor 1; NICD, Notch intracellular domain; OSM, oncostatin M; p-eIF2α, phosphorylated eukaryotic initiation factor 2α subunit; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; RBPJ, recombining binding protein suppressor of hairless; RhoA, ras homolog family member A; Spry1, sprouty1; STAT3, signal transducer and activator of transcription 3; TTP, tristetraprolin; Yap1, yes-associated protein 1.

secrete Wnt4, a soluble Wnt ligand that activates the noncanonical Rho-GTPase in MuSCs to favor quiescence through regulation of the cytoskeleton [37]. Overexpression of Wnt4 in muscle fibers delays cell cycle entry of MuSCs [37], demonstrating that Wnt4 alone is sufficient to reinforce the quiescent state. Conversely, loss of Wnt4 in myofibers results in the upregulation of mTORC1 in MuSCs [37], suggesting that Wnt4 also regulates quiescent MuSC entry into G_{Alert}. Similarly, oncostatin M secretion by muscle fibers induces quiescence in MuSCs and sustains stemness as oncostatin M treatment of MuSCs enhances long-term self-renewal *in vivo* in serial transplantation assays [38].

Beside the myofiber, other cell types in the niche have been implicated in the regulation of quiescence and stemness [39]. Communication with endothelial cells regulates MuSC quiescence as MuSCs secrete the vascular endothelial growth factor (VEGF) to attract endothelial cells to their vicinity, and bind the endothelial-cell-derived Dll4 Notch ligand to maintain quiescence [39]. Vasculature was also shown to control MuSC quiescence and stemness through oxygen availability as hypoxia ameliorates both engraftment and self-renewal [40]. Moreover, a subpopulation of human MuSCs was reported to survive up to 2 weeks after

death while maintaining their regenerative capacity [41]. These results suggest a possible link between quiescence and oxygen availability, where a subpopulation of deeply quiescent MuSCs with low metabolic rates in a hypoxic environment could bear the highest stemness potential.

Finally, **fibroadipogenic progenitors** (FAPs) are niche cells that directly convert into adipocytes in pathological conditions and support MuSC commitment through paracrine signaling after injury [42–44]. Depletion of FAPs *in vivo* results in smaller myofibers along with fewer MuSCs, which points to an underappreciated requirement of FAPs for the long-term maintenance of quiescent MuSCs in adult muscle [45]. Whether this mechanism is regulated through maintenance of MuSC quiescence or via enhanced MuSC self-renewal during tissue turnover remains to be investigated.

Regulation of MuSC Quiescence Through Intracellular Signaling

Most intracellular pathways that govern MuSC quiescence involve molecular inhibitors of cell proliferation (Figure 2). In quiescent MuSCs, mitogenic activity is repressed by **cyclin-dependent kinase** (**CDK**) inhibitors, including $p27^{Kip1}$ and $p57^{Kip2}$ [11,46]. Uninjured muscles from $p27^{Kip1}$-null mice display fewer MuSCs [11], and these MuSCs fail to return to quiescence following two rounds of serial injuries, eventually leading to stem cell exhaustion [11].

Another hallmark of MuSC quiescence is the low activity of the fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) signaling pathways [47], which are intrinsically repressed by negative regulators of their receptors and downstream targets [46]. Genome-wide expression profiling has identified Sprouty1 (Spry1), an inhibitor of FGF signaling, as an essential regulator of MuSC quiescence [48]. Spry1 is specifically expressed in quiescent MuSCs *in vivo* and is essential for MuSC self-renewal [48]. Loss of Spry1 results in fewer quiescent MuSCs after tissue repair, thereby supporting the role of Spry1 in re-establishing quiescence in MuSCs following regeneration and in maintaining long-term MuSC stemness [48]. In parallel, studies on isolated myofibers have shown that pharmacological inhibition of p38α/β MAPKs prevents MuSCs from entering the cell cycle and establishes quiescence in these cells [49]. Moreover, analyzing MuSC division symmetry has also uncovered a role for p38α/β MAPKs in MuSC self-renewal [50]. Upon MuSC division, p38α/β MAPKs asymmetrically localize in the committed cell while the noncommitted MuSC re-enters quiescence [50], highlighting the important role of p38α/β signaling in promoting MuSC commitment and the physiological need to repress p38α/β signaling to return to quiescence during asymmetric self-renewal (Box 2).

Post-transcriptional mechanisms also play an important role in modulating MuSC quiescence (Figure 2). Repression of cell migration and anchoring of MuSCs within their niche by miR-708 consolidates the quiescent state [51]. Moreover, transcriptomic analyses comparing quiescent and activated MuSCs have identified miR-489 as a quiescence-specific miRNA that maintains MuSC quiescence by suppressing Dek [52]. Counteracting Dek prevents the processing and maturation of transcripts regulating commitment, thereby enforcing quiescence [53]. Silencing of commitment genes is also performed through RNA sequestration as Myf5 mRNA can be trapped with miR-31 in messenger ribonucleoprotein (mRNP) granules to prevent Myf5 translation and stabilize the quiescent state [54]. Similarly, MyoD is tightly repressed in quiescent MuSCs by post-transcriptional mechanisms involving tristetraprolin (TTP), an RNA-binding protein that destabilizes the MyoD mRNA [55]. In parallel, Staufen1 also interacts with the 3′-UTR of MyoD mRNA and prevents MyoD translation to maintain quiescence [56]. Taken together, these studies demonstrate the fundamental role of post-transcriptional mechanisms in regulating quiescence.

Regulation of MuSC Quiescence Through Metabolism and Proteostasis

Quiescence is a state of low cellular activity where limited demands for energy are fulfilled by a low metabolic rate. Transcriptomic profiling has demonstrated that quiescent MuSCs rely on slow metabolism through oxidative phosphorylation and fatty acid oxidation [57,58]. However, assessing how metabolism fine tunes quiescence is an experimental challenge as metabolic assays, such as cellular respiration and substrate fluxes, require *in vitro* culture of MuSCs, which breaks quiescence and alters metabolism. Nevertheless, recent studies in which metabolic sensing pathways were genetically modified in mice have started to uncover direct links between energy metabolism and MuSC quiescence [58–60]. In particular, MuSC-specific deletion of the energy sensor **sirtuin 1** (SIRT1) breaks quiescence and induces aberrant MyoD expression and MuSC activation [58]. SIRT1 is an NAD^+ sensor that regulates oxidative metabolism through deacetylation of PGC-1α when the cellular NAD^+/NADH ratio is high [61]. NAD^+ levels are tenfold higher in quiescent than in activated MuSCs [58], and stimulate SIRT1 to deacetylate histone H4K16, thereby repressing genes controlling cell cycle entry and commitment [58]. Thus, the interplay between NAD^+ levels and epigenetic control of gene expression via SIRT1 represents an integrative node through which metabolism can crosstalk with cellular fate.

The energy sensor **AMP-activated protein kinase** (AMPK) is another direct regulator of cellular energy metabolism recently implicated in the regulation of MuSC fate [59,60]. MuSC-specific deletion of AMPKα1 does not affect quiescence in healthy muscle, but increases the ability of MuSCs to return to quiescence after injury by enhancing self-renewal [59]. This cell fate decision favoring quiescence occurs via a metabolic regulation where AMPK directly represses the activity of lactate dehydrogenase (LDH) to control intracellular lactate levels. LDH overexpression in MuSCs mimics the phenotype of AMPKα1 deletion [59], demonstrating that high lactate production is sufficient to promote return to quiescence via self-renewal. Thus, AMPK is required to balance oxidative metabolism and prevent the excessive use of anaerobic glycolysis to regulate cell fate decisions during MuSC specification. Extracellular lactate produced by endothelial cells has also been recently described as a mediator of paracrine communication favoring differentiation and repair in the regenerating MuSC niche [62], but whether this mechanism regulates MuSC self-renewal and return to quiescence remains to be determined. The effects of AMPK on return to quiescence via lactate could either be driven by sensing metabolic changes in the AMP/ATP levels or through post-translational control of AMPK activity through cell signaling. In support of the latter hypothesis, the upstream AMPK activator liver kinase b1 (Lkb1) is required for MuSC quiescence [60]. Lkb1 maintains the quiescent state through AMPK-mediated inhibition of mTOR, which controls MuSC activation [29,60]. Finally, the production of glutamine by macrophages in the regenerating MuSC niche has recently been demonstrated to support MuSC proliferation [63], suggesting that limited bioavailability of glutamine in the intact uninjured niche could be a mechanism preventing metabolic activation and cell cycle entry of quiescent MuSCs.

MuSC quiescence is also regulated through **proteostasis,** the balance between protein synthesis, quality control and degradation [64,65]. MuSCs maintain quiescence through inhibition of the translation initiation factor eIF2α, a master regulator of protein translation [64]. Following mutation of the phosphorylation site on eIF2α, quiescent MuSCs become unable to repress translation initiation, and they break quiescence and activate the myogenic program [64]. While these MuSCs contribute to new myofiber formation, they cannot self-renew *in vivo*, suggesting that robust translational silencing during quiescence is critical, not only to maintain the quiescent state, but also to ensure long-term stemness and regenerative capacity.

Efficient cellular recycling and clearance of protein aggregates through **autophagy** is also required for the maintenance of MuSC quiescence [65]. Disruption of autophagy through MuSC-specific

deletion of Atg7, an essential component of autophagosome formation, results in the loss of quiescent MuSCs in homeostatic muscles [65]. Altered proteostasis through reduced autophagy increases reactive oxygen species (ROS) production and leads to senescence, while pharmacological inhibition of ROS in Atg7-deficient MuSCs prevents senescence and restores self-renewal capacity [65]. These experiments highlight that autophagy is a requirement for both establishment and maintenance of quiescence in MuSCs.

MuSC Quiescence in Aging

Quiescence is efficiently regulated when complex signals perfectly synchronize to control cell fate. Aging is among the best-described conditions in which compromised integrity of diverse cell-autonomous and niche-dependent mechanisms leads to loss of MuSC quiescence through either aberrant activation patterns or irreversible withdrawal from the cell cycle. These defects result in the failure of aged MuSCs to efficiently sustain their long-term regenerative capacity (Figure 3), and further support the link between intrinsic loss of quiescence and stemness.

Aging perturbs the transcriptional and epigenetic landscapes of MuSCs and impairs their functional capacity to properly contribute to tissue repair [66–68]. Aged quiescent MuSCs have a transcriptional activity biased towards activation [66,67], and increased levels of DNA methylation as well as histone H3K27 trimethylation denoting premature activation [67,68]. However,

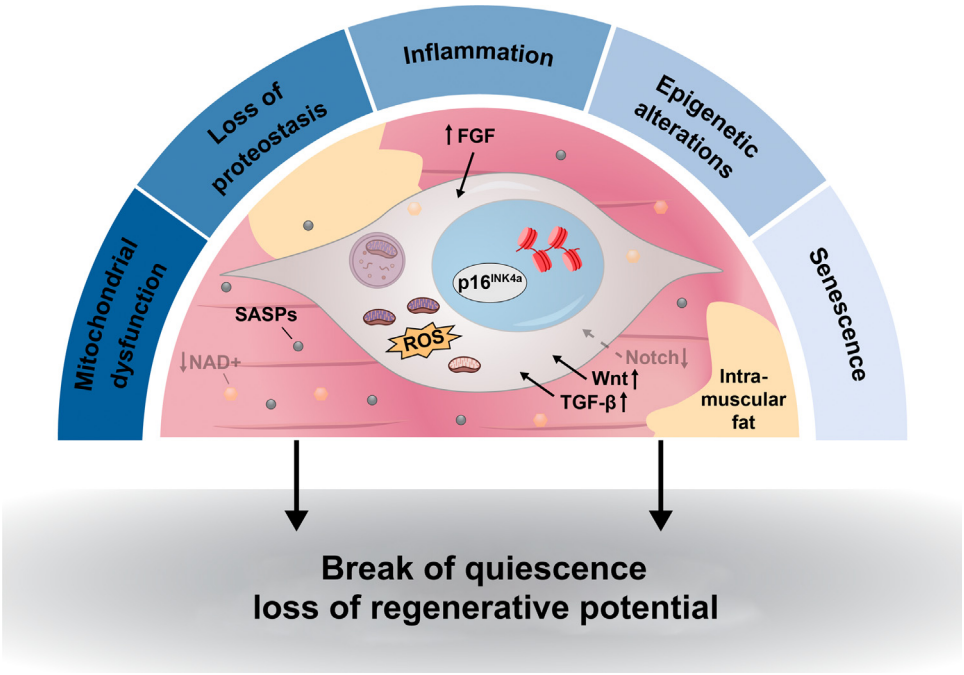

Figure 3. Mechanisms Impairing MuSC Quiescence and Regenerative Potential during Aging. Aging compromises the integrity of diverse cell-autonomous and niche-dependent mechanisms that breaks quiescence, and causes chronic hyperactivation and lack of self-renewal, resulting in MuSC exhaustion and loss of myogenic capacity. These changes include epigenetic changes affecting gene expression, mitochondrial dysfunction and oxidative stress as well as the cell signaling events represented in the figure. In parallel, advanced geriatric age also leads to MuSC exhaustion through cellular senescence, a pathological state of cell cycle arrest where senescent cells negatively influence the stem cell niche through the deleterious cytokines and mediators of the SASP. Abbreviations: FGF, fibroblast growth factor; MuSC, muscle stem cell; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; TGF-β, transforming growth factor β.

studies using *in vivo* multicolor labeling of individual MuSCs have shown that, while aging decreases quiescent MuSC numbers and functional capacity, it does not affect the complexity of the population as a whole as aged MuSCs remain clonally diverse [69].

Aged MuSCs also fail to efficiently self-renew [70–72]. Cycling rates of aged MuSCs in uninjured muscle were investigated using a mouse model with inducible fluorescent labeling of histones where the rapid dilution of the label marks fast-cycling cells [70]. In this model, aged MuSCs cycle more frequently, suggesting that aging alters the quiescent state *in vivo* even in the absence of active tissue repair [70]. Moreover, aged MuSCs fail to repopulate the stem cell compartment and reestablish Pax7 expression following **heterochronic transplantation assays** of aged cells in young muscle [70]. This suggests that the functional defect of aged MuSCs is cell-autonomous and that the inability to preserve quiescence in aging directly contributes to loss of stemness. Mechanistically, the break of MuSC quiescence during aging is governed by the downregulation of the FGF inhibitor Spry1, which deregulates FGF signaling and impairs both quiescence and self-renewal [70]. In parallel, aberrant activation of the p38α/β MAPK signaling in old muscles perturbs the balance between symmetric and asymmetric divisions and reduces MuSC self-renewal [71,72]. This perturbation can be targeted pharmacologically through inhibition of the p38α/β MAPK signaling, which improved self-renewal of aged MuSCs *in vivo* 30 days after transplantation [72].

Loss of stemness in aged MuSCs coincides with the loss of reversible quiescence as they progressively enter senescence [71–74]. In advanced geriatric aging, senescent MuSCs modify the fate of nearby cells by secreting deleterious cytokines collectively referred to as the senescence-associated secretory phenotype (SASP), while irreversibly withdrawing from the cell cycle as they upregulate the CDK inhibitor $p16^{INK4a}$ [71–74]. Accumulation of $p16^{INK4a}$ in aged MuSCs leads to low regenerative function and poor engraftment capacity upon transplantation [73]. $p16^{INK4a}$-silencing in MuSCs, either through genetic knockdown [73] or overexpression of the $p16^{INK4a}$ repressor Slug [74], restores MuSC quiescence and rescues the capacity of MuSCs to engraft and return to quiescence, highlighting a link between the conversion from quiescence to senescence and stemness. Gene expression analyses have shown that the accumulation of $p16^{INK4a}$ is concomitant with the constitutive activation of p38 MAPK signaling in aged MuSCs, which contributes to impaired self-renewal capacity through permanent cell cycle exit [71], while inhibition of p38 MAPK signaling prevents $p16^{INK4a}$ upregulation [71,75]. However, it is currently unclear whether MuSC senescence results from failure to properly reestablish quiescence following proliferation, or if MuSCs progressively degenerate into deeper quiescence and transition to senescence without entering the cell cycle. Further investigation will be required to understand the coexistence and dominance of these senescent transitions as MuSCs age.

Changes in metabolism and proteostasis during aging also affect the ability of MuSCs to maintain quiescence and can be targeted for therapeutic interventions [65,76,77]. NAD^+ levels decline during sarcopenia in mice [78] and humans [79], and treatment with the NAD^+ precursor nicotinamide riboside (NR) in aged mice rescues mitochondrial dysfunction, prevents senescence, and maintains quiescence and activation capacity in MuSCs [76]. Similarly, exercise improves MuSC function and muscle repair during aging by restoring low levels of cyclin D1 to youthful levels in quiescent MuSCs, where it counteracts TGF-β signaling and re-establishes the capacity of MuSCs to reversibly exit the quiescent state in response to injury [77]. Finally, the ability of MuSCs to clear damaged cellular materials through autophagy declines during aging [65]. Reactivating autophagy, either by genetically introducing Atg7 in aged MuSCs or via treatment with the polyamine spermidine or the mTOR inhibitor rapamycin, prevents senescence and rescues the capacity of aged MuSCs to repopulate the stem cell compartment and return to

quiescence following transplantation [65]. Therefore, therapeutic strategies targeting proteostasis and metabolic dysfunction represent a promising avenue to slow down aging of MuSCs and to improve the acute and chronic regenerative capacity of aged skeletal muscle.

Concluding Remarks

Many advances in the understanding of quiescence in MuSCs exemplify its regulatory role in maintaining long-term stemness. Rather than a simple cell cycle-arrested state, MuSC quiescence represents a continuum that requires permanent regulation. Perturbing these quiescent regulatory networks, either genetically or naturally during the aging process, jeopardizes the capacity of MuSCs to meet long-term regenerative needs by impacting stemness and maintenance of the MuSC pool through self-renewal. Beyond the learnings of the past decade on the molecular regulation of quiescence and the recent advances of MuSC heterogeneity at the single cell level, there is a strong need for a better understanding of the endogenous quiescent state. Challenges inherent to the physical location of quiescent MuSCs in the niche have hampered analysis in the native environment without physiological modifications associated with *ex vivo* isolation. Strategies preventing *de novo* transcription during the isolation process have enabled significant progress towards understanding the regulation of the quiescent state *in vivo* [80–84]. Such information will be critical to efficiently recapitulate MuSC function and disease models *in vitro* as the current understanding of stochastic factors that govern MuSC fate precludes maintaining MuSC stemness *in vitro* (see Outstanding Questions). Combined with new organoid or organ-on-chip technologies that can integrate cell–cell interactions and recapitulate complex 3D structures [85], these approaches will represent promising avenues to explore muscle quiescence and find ways to restore stemness and regenerative function in disease models, ultimately leading to new therapeutic opportunities.

Outstanding Questions

Does the maintenance of quiescence directly control stemness or is stemness linked to quiescence indirectly via the ability to self-renew? Do quiescence and stemness share common molecular basis and regulatory mechanisms?

What is the extent of muscle stem cell heterogeneity *in vivo*? Are MuSC subpopulations independent populations with distinct characteristics or do they reflect a continuum of transient states?

How does metabolism regulate MuSC quiescence and fate transitions? Are metabolic changes a cause or a consequence of stem cell transitions?

Does aging differentially affect different quiescent MuSC subpopulations? Can we identify specific signals that influence these subpopulations and specific interventions that drive enhanced therapeutic potential?

How different is the native *in vivo* quiescent state from what can be captured with current experimental approaches?

Can quiescence and stemness be recapitulated *in vitro*? What cell types, growth factors/cytokines, mechanical and structural cues could allow to better maintain stemness *in vitro* and model specificity of different MuSC states such as quiescence, activation, proliferation, and commitment?

Acknowledgments

We thank Florian Bentzinger for critical discussion of the concepts of this review and Anna Weiser for support with artwork.

Declaration of Interests

All authors are employees of Société des Produits Nestlé S.A., Switzerland.

References

1. Bachman, J.F. *et al.* (2018) Prepubertal skeletal muscle growth requires Pax7-expressing satellite cell-derived myonuclear contribution. *Development* 145, dev167197
2. Bachman, J.F. *et al.* (2020) Radiation-induced damage to prepubertal Pax7+ skeletal muscle stem cells drives lifelong deficits in myofiber size and nuclear number. *iScience* 23, 101760
3. Hutcheson, D.A. *et al.* (2009) Embryonic and fetal limb myogenic cells are derived from developmentally distinct progenitors and have different requirements for beta-catenin. *Genes Dev.* 23, 997–1013
4. Gattazzo, F. *et al.* (2020) Distinct phases of postnatal skeletal muscle growth govern the progressive establishment of muscle stem cell quiescence. *Stem Cell Rep.* 15, 597–611
5. Cheung, T.H. and Rando, T.A. (2013) Molecular regulation of stem cell quiescence. *Nat. Rev. Mol. Cell Biol.* 14, 329–340
6. Kadi, F. *et al.* (2004) The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. *J. Physiol.* 558, 1005–1012
7. Fukada, S.I. (2018) The roles of muscle stem cells in muscle injury, atrophy and hypertrophy. *J. Biochem.* 163, 353–358
8. Englund, D.A. *et al.* (2020) Depletion of resident muscle stem cells negatively impacts running volume, physical function, and muscle fiber hypertrophy in response to lifelong physical activity. *Am. J. Physiol. Cell Physiol.* 318, C1178–c1188
9. Dumont, N.A. *et al.* (2015) Satellite cells and skeletal muscle regeneration. *Compr. Physiol.* 5, 1027–1059
10. Schultz, E. (1996) Satellite cell proliferative compartments in growing skeletal muscles. *Dev. Biol.* 175, 84–94
11. Chakkalakal, J.V. *et al.* (2014) Early forming label-retaining muscle stem cells require p27kip1 for maintenance of the primitive state. *Development* 141, 1649–1659
12. Saber, J. *et al.* (2020) Single-cell analyses uncover granularity of muscle stem cells. *F1000Res.* 9, F1000 Faculty Rev-31
13. Yoshida, N. *et al.* (1998) Cell heterogeneity upon myogenic differentiation: down-regulation of MyoD and Myf-5 generates 'reserve cells. *J. Cell Sci.* 111, 769–779
14. Beauchamp, J.R. *et al.* (1999) Dynamics of myoblast transplantation reveal a discrete minority of precursors with stem cell-like properties as the myogenic source. *J. Cell Biol.* 144, 1113–1122
15. Laumonier, T. *et al.* (2017) Human myogenic reserve cells are quiescent stem cells that contribute to muscle regeneration after intramuscular transplantation in immunodeficient mice. *Sci. Rep.* 7, 3462
16. Beauchamp, J.R. *et al.* (2000) Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *J. Cell Biol.* 151, 1221–1234
17. Kuang, S. *et al.* (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell* 129, 999–1010
18. Gayraud-Morel, B. *et al.* (2012) Myf5 haploinsufficiency reveals distinct cell fate potentials for adult skeletal muscle stem cells. *J. Cell Sci.* 125, 1738–1749
19. Rocheteau, P. *et al.* (2012) A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division. *Cell* 148, 112–125

20. García-Prat, L. *et al.* (2020) FoxO maintains a genuine muscle stem-cell quiescent state until geriatric age. *Nat. Cell Biol.* 22, 1307–1318
21. Porpiglia, E. *et al.* (2017) High-resolution myogenic lineage mapping by single-cell mass cytometry. *Nat. Cell Biol.* 19, 558–567
22. Dell'Orso, S. *et al.* (2019) Single cell analysis of adult mouse skeletal muscle stem cells in homeostatic and regenerative conditions. *Development* 146, dev174177
23. De Micheli, A.J. *et al.* (2020) Single-cell analysis of the muscle stem cell hierarchy identifies heterotypic communication signals involved in skeletal muscle regeneration. *Cell Rep.* 30, 3583–3595 e5
24. Cho, D.S. and Doles, J.D. (2017) Single cell transcriptome analysis of muscle satellite cells reveals widespread transcriptional heterogeneity. *Gene* 636, 54–63
25. Barruet, E. *et al.* (2020) Functionally heterogeneous human satellite cells identified by single cell RNA sequencing. *eLife* 9, e51576
26. De Micheli, A.J. *et al.* (2020) A reference single-cell transcriptomic atlas of human skeletal muscle tissue reveals bifurcated muscle stem cell populations. *Skelet. Muscle* 10, 19
27. van Velthoven, C.T.J. and Rando, T.A. (2019) Stem cell quiescence: dynamism, restraint, and cellular idling. *Cell Stem Cell* 24, 213–225
28. Tsuchiya, Y. *et al.* (2020) Damaged myofiber-derived metabolic enzymes act as activators of muscle satellite cells. *Stem Cell Rep.* 15, 926–940
29. Rodgers, J.T. *et al.* (2014) mTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert). *Nature* 510, 393–396
30. Baghdadi, M.B. *et al.* (2018) Reciprocal signalling by Notch-collagen V-CALCR retains muscle stem cells in their niche. *Nature* 557, 714–718
31. Mourikis, P. *et al.* (2012) A critical requirement for notch signaling in maintenance of the quiescent skeletal muscle stem cell state. *Stem Cells* 30, 243–252
32. Bjornson, C.R. *et al.* (2012) Notch signaling is necessary to maintain quiescence in adult muscle stem cells. *Stem Cells* 30, 232–242
33. Fujimaki, S. *et al.* (2018) Notch1 and Notch2 coordinately regulate stem cell function in the quiescent and activated states of muscle satellite cells. *Stem Cells* 36, 278–285
34. Yartseva, V. *et al.* (2020) Heterogeneity of satellite cells implicates DELTA1/NOTCH2 signaling in self-renewal. *Cell Rep.* 30, 1491–1503 e6
35. Gopinath, S.D. *et al.* (2014) FOXO3 promotes quiescence in adult muscle stem cells during the process of self-renewal. *Stem Cell Rep.* 2, 414–426
36. Yue, F. *et al.* (2017) Pten is necessary for the quiescence and maintenance of adult muscle stem cells. *Nat. Commun.* 8, 14328
37. Eliazer, S. *et al.* (2019) Wnt4 from the niche controls the mechano-properties and quiescent state of muscle stem cells. *Cell Stem Cell* 25, 654–665 e4
38. Sampath, S.C. *et al.* (2018) Induction of muscle stem cell quiescence by the secreted niche factor Oncostatin M. *Nat. Commun.* 9, 1531
39. Verma, M. *et al.* (2018) Muscle satellite cell cross-talk with a vascular niche maintains quiescence via VEGF and Notch signaling. *Cell Stem Cell* 23, 530–543 e9
40. Liu, W. *et al.* (2012) Hypoxia promotes satellite cell self-renewal and enhances the efficiency of myoblast transplantation. *Development* 139, 2857–2865
41. Latil, M. *et al.* (2012) Skeletal muscle stem cells adopt a dormant cell state post mortem and retain regenerative capacity. *Nat. Commun.* 3, 903
42. Joe, A.W. *et al.* (2010) Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat. Cell Biol.* 12, 153–163
43. Uezumi, A. *et al.* (2010) Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat. Cell Biol.* 12, 143–152
44. Lukjanenko, L. *et al.* (2019) Aging disrupts muscle stem cell function by impairing matricellular WISP1 secretion from fibro-adipogenic progenitors. *Cell Stem Cell* 24, 433–446 e7
45. Wosczyna, M.N. *et al.* (2019) Mesenchymal stromal cells are required for regeneration and homeostatic maintenance of skeletal muscle. *Cell Rep.* 27, 2029–2035 e5
46. Fukada, S. *et al.* (2007) Molecular signature of quiescent satellite cells in adult skeletal muscle. *Stem Cells* 25, 2448–2459
47. Bentzinger, C.F. *et al.* (2010) Extrinsic regulation of satellite cell specification. *Stem Cell Res Ther* 1, 27
48. Shea, K.L. *et al.* (2010) Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration. *Cell Stem Cell* 6, 117–129
49. Jones, N.C. *et al.* (2005) The p38alpha/beta MAPK functions as a molecular switch to activate the quiescent satellite cell. *J. Cell Biol.* 169, 105–116
50. Troy, A. *et al.* (2012) Coordination of satellite cell activation and self-renewal by Par-complex-dependent asymmetric activation of p38α/β MAPK. *Cell Stem Cell* 11, 541–553
51. Baghdadi, M.B. *et al.* (2018) Notch-induced miR-708 antagonizes satellite cell migration and maintains quiescence. *Cell Stem Cell* 23, 859–868 e5
52. Cheung, T.H. *et al.* (2012) Maintenance of muscle stem-cell quiescence by microRNA-489. *Nature* 482, 524–528
53. Yue, L. *et al.* (2020) Dek modulates global intron retention during muscle stem cells quiescence exit. *Dev. Cell* 53, 661–676 e6
54. Crist, C.G. *et al.* (2012) Muscle satellite cells are primed for myogenesis but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules. *Cell Stem Cell* 11, 118–126
55. Hausburg, M.A. *et al.* (2015) Post-transcriptional regulation of satellite cell quiescence by TTP-mediated mRNA decay. *eLife* 4, e03390
56. de Morrée, A. *et al.* (2017) Staufen1 inhibits MyoD translation to actively maintain muscle stem cell quiescence. *Proc. Natl. Acad. Sci. U. S. A.* 114, E8996–e9005
57. Pala, F. *et al.* (2018) Distinct metabolic states govern skeletal muscle stem cell fates during prenatal and postnatal myogenesis. *J Cell Sci* 131, jcs212977
58. Ryall, J.G. *et al.* (2015) The NAD(+)-dependent SIRT1 deacetylase translates a metabolic switch into regulatory epigenetics in skeletal muscle stem cells. *Cell Stem Cell* 16, 171–183
59. Theret, M. *et al.* (2017) AMPKα1-LDH pathway regulates muscle stem cell self-renewal by controlling metabolic homeostasis. *EMBO J.* 36, 1946–1962
60. Shan, T. *et al.* (2014) Lkb1 is indispensable for skeletal muscle development, regeneration, and satellite cell homeostasis. *Stem Cells* 32, 2893–2907
61. Feige, J.N. *et al.* (2008) Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. *Cell Metab.* 8, 347–358
62. Zhang, J. *et al.* (2020) Endothelial lactate controls muscle regeneration from ischemia by inducing M2-like macrophage polarization. *Cell Metab.* 31, 1136–1153 e7
63. Shang, M. *et al.* (2020) Macrophage-derived glutamine boosts satellite cells and muscle regeneration. *Nature* 587, 626–631
64. Zismanov, V. *et al.* (2016) Phosphorylation of eIF2α is a translational control mechanism regulating muscle stem cell quiescence and self-renewal. *Cell Stem Cell* 18, 79–90
65. García-Prat, L. *et al.* (2016) Autophagy maintains stemness by preventing senescence. *Nature* 529, 37–42
66. Kimmel, J.C. *et al.* (2020) Aging induces aberrant state transition kinetics in murine muscle stem cells. *Development* 147, dev183855
67. Hernando-Herraez, I. *et al.* (2019) Ageing affects DNA methylation drift and transcriptional cell-to-cell variability in mouse muscle stem cells. *Nat. Commun.* 10, 4361
68. Liu, L. *et al.* (2013) Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. *Cell Rep.* 4, 189–204
69. Tierney, M.T. *et al.* (2018) Muscle stem cells exhibit distinct clonal dynamics in response to tissue repair and homeostatic aging. *Cell Stem Cell* 22, 119–127 e3
70. Chakkalakal, J.V. *et al.* (2012) The aged niche disrupts muscle stem cell quiescence. *Nature* 490, 355–360
71. Cosgrove, B.D. *et al.* (2014) Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. *Nat. Med.* 20, 255–264

72. Bernet, J.D. *et al.* (2014) p38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice. *Nat. Med.* 20, 265–271
73. Sousa-Victor, P. *et al.* (2014) Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature* 506, 316–321
74. Zhu, P. *et al.* (2019) The transcription factor Slug represses p16 (Ink4a) and regulates murine muscle stem cell aging. *Nat. Commun.* 10, 2568
75. Papaconstantinou, J. *et al.* (2015) Attenuation of p38α MAPK stress response signaling delays the *in vivo* aging of skeletal muscle myofibers and progenitor cells. *Aging (Albany NY)* 7, 718–733
76. Zhang, H. *et al.* (2016) NAD^+ repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science* 352, 1436–1443
77. Brett, J.O. *et al.* (2020) Exercise rejuvenates quiescent skeletal muscle stem cells in old mice through restoration of Cyclin D1. *Nat. Metab.* 2, 307–317
78. Gomes, A.P. *et al.* (2013) Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell* 155, 1624–1638
79. Migliavacca, E. *et al.* (2019) Mitochondrial oxidative capacity and NAD(+) biosynthesis are reduced in human sarcopenia across ethnicities. *Nat. Commun.* 10, 5808
80. Machado, L. *et al.* (2017) *In situ* fixation redefines quiescence and early activation of skeletal muscle stem cells. *Cell Rep.* 21, 1982–1993
81. Yue, L. and Cheung, T.H. (2020) Protocol for isolation and characterization of *in situ* fixed quiescent muscle stem cells. *STAR Protoc.* 1, 100128
82. van Velthoven, C.T.J. *et al.* (2017) Transcriptional profiling of quiescent muscle stem cells *in vivo*. *Cell Rep.* 21, 1994–2004
83. Petrany, M.J. *et al.* (2020) Single-nucleus RNA-seq identifies transcriptional heterogeneity in multinucleated skeletal myofibers. *Nat. Commun.* 11, 6374
84. Kim, M. *et al.* (2020) Single-nucleus transcriptomics reveals functional compartmentalization in syncytial skeletal muscle cells. *Nat. Commun.* 11, 6375
85. Chal, J. and Pourquié, O. (2017) Making muscle: skeletal myogenesis *in vivo* and *in vitro*. *Development* 144, 2104–2122
86. Feige, P. *et al.* (2018) Orienting muscle stem cells for regeneration in homeostasis, aging, and disease. *Cell Stem Cell* 23, 653–664
87. Millay, D.P. *et al.* (2013) Myomaker is a membrane activator of myoblast fusion and muscle formation. *Nature* 499, 301–305
88. Dumont, N.A. *et al.* (2015) Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. *Nat. Med.* 21, 1455–1463
89. Wang, Y.X. *et al.* (2019) EGFR-Aurka signaling rescues polarity and regeneration defects in dystrophin-deficient muscle stem cells by increasing asymmetric divisions. *Cell Stem Cell* 24, 419–432 e6
90. Evano, B. *et al.* (2020) Dynamics of asymmetric and symmetric divisions of muscle stem cells *in vivo* and on artificial niches. *Cell Rep.* 30, 3195–3206 e7
91. van den Brink, S.C. *et al.* (2017) Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations. *Nat. Methods* 14, 935–936
92. Lacar, B. *et al.* (2016) Nuclear RNA-seq of single neurons reveals molecular signatures of activation. *Nat. Commun.* 7, 11022
93. Webster, M.T. *et al.* (2016) Intravital imaging reveals ghost fibers as architectural units guiding myogenic progenitors during regeneration. *Cell Stem Cell* 18, 243–252
94. Machado, L. *et al.* (2021) Tissue damage induces a conserved stress response that initiates quiescent muscle stem cell activation. *Cell Stem Cell* Published online February 19, 2021. https://doi.org/10.1016/j.stem.2021.01.017