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The muscle stem cell niche at a glance

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ABSTRACT

Skeletal muscle stem cells (MuSCs, also called satellite cells) are the source of the robust regenerative capability of this tissue. The hallmark property of MuSCs at homeostasis is quiescence, a reversible state of cell cycle arrest required for long-term preservation of the stem cell population. MuSCs reside between an individual myofiber and an enwrapping basal lamina, defining the immediate MuSC niche. Additional cell types outside the basal lamina, in the interstitial space, also contribute to niche function. Quiescence is actively maintained by multiple niche-derived signals, including adhesion molecules presented from the myofiber surface and basal lamina, as well as soluble signaling factors produced by myofibers and interstitial cell types. In this Cell Science at a Glance article and accompanying poster, we present the most recent information on how niche signals promote MuSC quiescence and provide perspectives for further research.

KEY WORDS: Muscle, Muscle stem cell, Stem cell niche, Quiescence, Cell adhesion, Cell signaling

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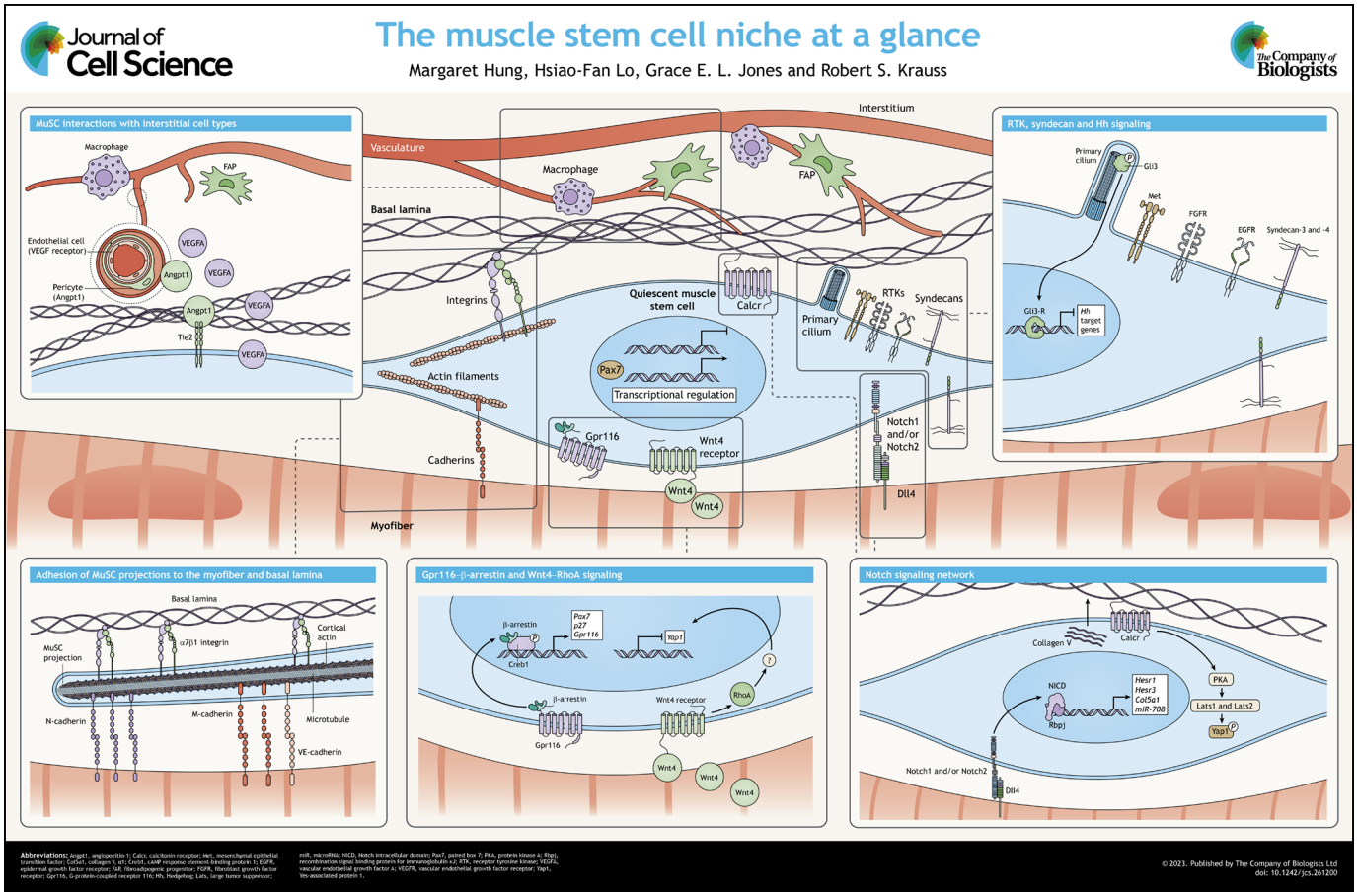
Introduction

Stem cells reside in a specific microenvironment, or niche, which provides regulatory signals that sustain their stem cell properties (Scadden, 2014). Niche-derived signals arise from direct contact between stem cells and niche cells, as well as from soluble factors and extracellular matrix molecules secreted by multiple cell types that contribute to niche function. Muscle stem cells (MuSCs, also called satellite cells) are the source of the remarkable regenerative capability of skeletal muscle (Relaix et al., 2021; Sousa-Victor et al.,



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2022). MuSCs reside in a well-defined anatomical location, sandwiched between skeletal muscle fibers (myofibers) and a basal lamina that ensheathes each myofiber (Evano and Tajbakhsh, 2018; Mashinchian et al., 2018) (see poster). The myofiber and the basal lamina define the immediate MuSC niche, and MuSCs express adhesion molecules and signaling receptors that interact with factors provided by them. Additional cell types reside outside the basal lamina, in the interstitial space, and some can function as niche components that, in contrast to myofibers and the basal lamina, do not physically contact MuSCs (Evano and Tajbakhsh, 2018; Mashinchian et al., 2018).

A hallmark property of MuSCs at homeostasis is quiescence, a state of reversible cell cycle arrest. Quiescence is actively maintained by a combination of cell-autonomous factors and niche-derived signals (Ancel et al., 2021). Among the former are specific transcriptional regulators, including the transcription factor Pax7, which is expressed by all MuSCs and required for their quiescence, while the latter are the topic of this article. Following injury, MuSCs break quiescence in a poorly understood process called activation (see Box 1). Activated MuSCs enter the cell cycle, proliferate and differentiate to regenerate muscle; additionally, some MuSCs self-renew to replenish the stem cell compartment (Hardy et al., 2016; Schmidt et al., 2019). The MuSC niche undergoes substantial changes during muscle regeneration, with cellular composition changing over time after muscle injury (Evano and Tajbakhsh, 2018; Fuchs and Blau, 2020; Kann et al., 2021; Mashinchian et al., 2018; Wosczyna and Rando, 2018). For example, circulating neutrophils and macrophages infiltrate the damaged muscle area within hours of injury (Evano and Tajbakhsh, 2018; Fuchs and Blau, 2020; Kann et al., 2021; Mashinchian et al., 2018; Wosczyna and Rando, 2018). By the time regeneration is complete, some MuSCs have self-renewed, repopulated a restored homeostatic niche and returned to quiescence, but the timing of these steps is unresolved (Cutler et al., 2022; Evano et al., 2020; Kuang et al., 2007). During injury repair, MuSCs and other muscle-resident and infiltrating cells undergo multiple transient and complex interactions with each other. The MuSC niche also undergoes changes during muscle disease and aging, but discussion of these aspects is beyond the scope of this article. Readers are directed to other reviews on the topic of the cellular environment encountered by MuSCs during these processes (Evano and Tajbakhsh, 2018; Fuchs and Blau, 2020; Kann et al., 2021; Mashinchian et al., 2018; Wosczyna and Rando, 2018). In this Cell Science at a Glance article, we discuss the mechanisms by which the MuSC niche promotes homeostatic maintenance of stem cell quiescence. Most work on quiescent MuSCs has been performed with mice, and the data referred to in the article come from studies in mice.

Box 1. Monitoring MuSC quiescence and activation

MuSCs in a healthy adult mammal are quiescent until the muscle is injured. In response to muscle injury, MuSCs become activated and proliferate to produce muscle progenitors (myoblasts) to repair the damage. Agents of injury used in experimental settings include physical injury and lethal myofiber depolarization with $BaCl_2$ or snake venom toxins (Hardy et al., 2016). Very vigorous muscle exercise can also activate MuSCs (Fukada and Nakamura, 2021). Mice have been the animal model of choice to study this process, in part due to the availability of good markers of quiescence and the various stages of muscle regeneration (Schmidt et al., 2019). All quiescent MuSCs express the transcription factor Pax7, which serves as a marker of MuSCs in uninjured animals and is also the basis for Cre drivers that allow specific conditional mutation of genes in these cells (Lepper and Fan, 2010; Murphy et al., 2011). Additional markers of quiescent MuSCs include α7 integrin, VCAM1, CD34 and M-cadherin; these can be used to identify MuSCs on muscle sections and some can be used for purification of MuSCs by fluorescence-activated cell sorting (Liu et al., 2015). As MuSCs enter the activation process, changes in morphology and gene expression occur in distinct stages. The first *de novo* change to gene expression is induction of immediate early genes, such as *Fos* (Almada et al., 2021; Machado et al., 2017). Expression of the myogenic determinant proteins Myf5 and MyoD (also known as MyoD1) follows, as does expression of markers of cell proliferation such as Ki67 (also known as Mki67). Each of these factors have been used as markers of MuSC activation. These cells proliferate as transit-amplifying myoblasts. When sufficient myoblast progeny are produced, the muscle differentiation-promoting transcription factor myogenin is induced and Pax7 is fully downregulated; these cells differentiate and fuse to form new myofibers or repair damaged ones, which express markers of mature muscle, such as myofiber-specific myosin isoforms.

Adhesion of MuSCs to components of the immediate niche

MuSCs are polarized cells, due in part to differential adhesion to myofibers and the basal lamina at their apical and basal surfaces, respectively (see poster). MuSCs and myofibers each express several different cadherins, including N-, M- and VE-cadherins, which bind in a homophilic fashion, bringing the myofiber sarcolemma and MuSC apical plasma membrane into close proximity (Goel et al., 2017; Kann and Krauss, 2019). Genetic removal of M-cadherin has little effect on MuSCs, whereas removal of N-cadherin renders the cells prone to breaking quiescence in the absence of injury (Goel et al., 2017). Despite this propensity, MuSCs lacking N-cadherin remain polarized and under the basal lamina, successfully participate in muscle regeneration and are proficient at self-renewal (Goel et al., 2017). These capabilities are due to partial compensation by other cadherins, as complete loss of cadherin-based adhesion results in loss of MuSC polarity, exit from the niche and MuSC attrition (Hung et al., 2023 preprint).

Quiescent MuSCs have long heterogeneous projections that are proposed to act as sensors of niche signals that differentially regulate quiescence versus activation (Kann et al., 2022; Krauss and Kann, 2023; Ma et al., 2022). Different cadherins display distinct localizations. M-cadherin, which is dispensable for MuSC quiescence, is found at high levels around the body of the MuSC, as well as on projections (see poster). N-cadherin, which is required for stable quiescence, is often enriched on projections, including at their tips; furthermore, loss of N-cadherin leads to loss of projections (Kann et al., 2022). Therefore, maintenance of MuSC projections correlates with a maintenance of quiescence. MuSC projections have a core of microtubules that is surrounded by a ring of cortical F-actin. The catenin proteins that link cadherins to the cytoskeleton are also localized at the cortex of the MuSC apical membrane (Goel et al., 2017; Hung et al., 2023 preprint; Kann et al., 2022). We speculate that cadherin-based junctions link to cytoskeletal F-actin and microtubules to promote a quiescent MuSC structure and function, including projection outgrowth and/or maintenance.

On the basal membrane of MuSCs are receptors for laminin, notably α7β1 integrin (Sacco et al., 2008). Genetic removal of β1 integrin from MuSCs leads to loss of apical-basal polarity, a break in quiescence, cell differentiation and fusion with the adjacent myofiber (Rozo et al., 2016). β1 integrin also heterodimerizes with other α-integrin subunits to form receptors for fibronectin and several types of collagen that are found in either the basal lamina or interstitial extracellular matrix (ECM) (Schüler et al., 2022). However, quiescent MuSCs do not express high levels of such

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heterodimers (Schüler et al., 2022), so loss of laminin receptor function is the most likely driver of the β1 integrin mutant phenotype. Quiescent MuSCs also express a second laminin receptor, dystroglycan, a key component of the dystrophin glycoprotein complex (DGC), mutations of which produce various forms of muscular dystrophy (Dumont et al., 2015). These diseases appear to arise from combined loss of DGC functions in myofibers and MuSCs (Dumont and Rudnicki, 2016). Both integrins and DGC link the cell surface to the intracellular F-actin cytoskeleton, promoting adhesion-mediated cell polarity and structural integrity. In addition to their cytoskeletal linkages, cadherins and integrins regulate signaling pathways, both directly and indirectly (Parsons et al., 2010; Priya and Yap, 2015), but there is little information on pathways downstream of these adhesion molecules in quiescent MuSCs.

The interstitial ECM is a complex mixture of collagens and proteoglycans that interact to provide a structural network of appropriate tissue pliancy [see Schüler et al. (2022) for a detailed review of MuSCs and the ECM]. This interaction is likely to be very important for homeostatic MuSC regulation, but could occur indirectly, with local and overall tissue stiffness sensed by MuSCs via mechanisms that have not yet been identified.

Signaling to MuSCs from the immediate niche

Maintenance of quiescence is critical for long-term MuSC function, and a deregulation of MuSC quiescence can result in premature differentiation or cell death, in turn impairing regeneration after injury. Quiescence is actively maintained by multiple signaling pathways, with niche-derived ligands presented to receptors expressed by MuSCs (see poster). Myofibers express the Notch ligand Dll4, which activates Notch receptors on quiescent MuSCs (Eliazer et al., 2020; Kann and Krauss, 2019). This leads to proteolytic cleavage and cytoplasmic release of the Notch intracellular domain (NICD), which interacts with the transcription factor Rbpj to drive expression of Notch pathway target genes. Conditional mutation of Rbpj in adult mouse MuSCs leads to a break in quiescence, rapid differentiation and fusion with myofibers (usually without completing a full cell cycle), and eventual loss of more than 95% of MuSCs (Bjornson et al., 2012; Mourikis et al., 2012). Conditional mutation of Dll4 in myofibers leads to a similar, if somewhat weaker, phenotype and a strong reduction of Notch-dependent gene expression in MuSCs (Eliazer et al., 2020). These results argue that Dll4 is the major Notch ligand and myofibers the major source for Notch activity in MuSCs at homeostasis. The weaker phenotype, relative to MuSC-specific loss of Rbpj, might be due to Dll4 provided by endothelial cells, ligand-independent Notch signaling or Notch-independent Rbpj functions (Tao et al., 2023; Verma et al., 2018). These phenomena have been observed *in vitro* but require further experimentation to assess their *in vivo* contribution. Quiescent MuSCs express multiple Notch isoforms (Gioftsidi et al., 2022), with Notch1 and Notch2 acting redundantly to maintain the quiescent MuSC pool (Fujimaki et al., 2018).

Notch–Rbpj signaling induces expression of transcriptional regulators of the Hes/Hey family in all Notch-responsive cells (Weber et al., 2014), including *Hesr1* (also known as *Hey1*) and *Hesr3* (also known as *Heyl*) in MuSCs (Mourikis et al., 2012). Combined germline mutation of *Hesr1* and *Hesr3* leads to postnatal loss of MuSCs via premature differentiation (Fukada et al., 2011). However, additional direct Rbpj target genes in MuSCs are critical for maintenance of quiescence, including those encoding collagen V (ColV) (Baghdadi et al., 2018a). ColV secreted by MuSCs acts via an autocrine mechanism by binding to calcitonin receptor (Calcr), a G protein-coupled receptor (GPCR) long known to be a marker of quiescent MuSCs (Baghdadi et al., 2018a; Fukada et al., 2007). Conditional mutation of *Col5a1* or *Calcr* in MuSCs results in a phenotype similar to that of loss-of-function mutations of the Notch pathway (Baghdadi et al., 2018a; Yamaguchi et al., 2015). Calcr signals to maintain quiescence via generation of cAMP, which activates protein kinase A. Protein kinase A in turn stimulates activity of the Lats1 and Lats2 protein kinases, which phosphorylate the transcriptional regulator Yap1, preventing its translocation to the nucleus, where it promotes MuSC activation (Zhang et al., 2019). Notch signaling also drives expression of miR-708, a microRNA that targets tensin 3, a focal adhesion protein that inhibits migration of MuSCs and stabilizes their retention in the immediate niche (Baghdadi et al., 2018b).

Wnt4 is another myofiber-derived quiescence-promoting factor. Myofibers secrete Wnt4, which stimulates a non-canonical Wnt signaling pathway in MuSCs that involves activation of the small GTPase RhoA, ultimately repressing expression of Yap1 by mechanisms that are not yet clear (Eliazer et al., 2019). The Wnt4–RhoA signaling axis is also important for retention of MuSCs within the immediate niche, as a high percentage of MuSCs deprived of Wnt4 or RhoA are found in the interstitial space outside the basal lamina and in between myofibers. Accordingly, Wnt4–RhoA signaling might impact MuSC adhesion within the niche. Consistent with this, MuSCs deprived of myofiber-derived Wnt4 have reduced levels of phosphorylated focal adhesion kinase, a target of integrin signaling, which is critical for basal lamina adhesion (Eliazer et al., 2019).

The cell surface receptor Gpr116 (also known as Adgrf5) has recently been reported to promote MuSC quiescence (Sénéchal et al., 2022). Gpr116 is a member of the adhesion GPCR subfamily; these are seven-transmembrane receptors with very long N-terminal ectodomains that can bind ECM proteins (Vizurraga et al., 2020). The N-terminal region is also subject to autoproteolysis, exposing a short peptide sequence, the so-called Stachel peptide, that acts as a signaling ligand for the receptor (Vizurraga et al., 2020). Genetic removal of Gpr116 from adult MuSCs leads to a break in quiescence and entry into the cell cycle, resulting in slow attrition of MuSCs; here, loss of *Gpr116*-null MuSCs occurs over 6 to 12 months, as opposed to just a few weeks observed for various Notch pathway mutants (Bjornson et al., 2012; Eliazer et al., 2020; Mourikis et al., 2012; Sénéchal et al., 2022; Yamaguchi et al., 2015). Treatment of MuSCs with Gpr116 Stachel peptide stimulates a signaling pathway whereby the GPCR regulator and signal transducer β-arrestin translocates to the nucleus and associates with the transcription factor Creb1 to promote expression of quiescence-associated genes, including *Pax7*, the cell cycle inhibitor *p27* (also known as *Cdkn1b*) and *Gpr116* itself (Sénéchal et al., 2022). Many adhesion GPCRs are regulated by binding to components of the ECM (Vizurraga et al., 2020), and identifying whether this plays a role in Gpr116 action in MuSCs, and if so, which ECM factors are involved, will be important.

Quiescent MuSCs express multiple receptor tyrosine kinases (RTKs), including the hepatocyte growth factor receptor (Met; also known as hepatocyte growth factor receptor), fibroblast growth factor receptors (FGFRs) and epidermal growth factor receptor (EGFR) (Wang et al., 2019; Webster and Fan, 2013; Yablonka-Reuveni et al., 2015). It appears that these receptors have only a minor role in homeostatic maintenance of quiescence, but they are important for timely activation, proliferation and migration of MuSCs following injury. A similar situation appears to be true for Hedgehog (Hh) pathway signaling (Brun et al., 2022; Cruz-Migoni et al., 2019 preprint; Jaafar Marican et al., 2016; Palla et al., 2022).

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Quiescent MuSCs express components of the Hh signaling machinery, and they possess a primary cilium, an organelle required for both the repressive state of the pathway in the absence of Hh ligand and the activated state in the presence of ligand (Gigante and Caspary, 2020). In quiescent MuSCs, Gli3 is phosphorylated and processed into a repressor form at the primary cilium, leading to repression of Hh pathway target genes and maintenance of the quiescent state (Brun et al., 2022). In contrast, activation of Hh signaling might be important during regeneration. Interestingly, aberrant regulation of RTK and Hh pathways underlies some of the reduction in stem cell activity observed in aged mice of ∼2 years of age (Bernet et al., 2014; Chakkalakal et al., 2012; Palla et al., 2022). Finally, quiescent MuSCs express syndecan-3 and syndecan-4, transmembrane cell surface proteins that serve as coreceptors and regulators of Notch, RTKs, Wnt and additional signaling pathways (Bentzinger et al., 2013; Cornelison et al., 2001; Pisconti et al., 2012). Syndecan-3 and syndecan-4 provide non-redundant and complex functions to MuSCs *in vivo*, including regulation of quiescence, activation and regeneration, but the relative importance of the various pathways syndecans regulate during these events is not clear (Cornelison et al., 2004; Pisconti et al., 2016).

Other niche cells and factors

Multiple cell types reside near MuSCs, but outside the basal lamina in the interstitium between myofibers; these include fibroadipogenic progenitors (FAPs), macrophages and cells associated with the vasculature (Relaix et al., 2021) (see poster). Capillaries are found in close proximity to most MuSCs, and several studies indicate that MuSCs communicate with both endothelial cells (ECs) and pericytes during muscle development and regeneration (Abou-Khalil et al., 2009; Christov et al., 2007; Kostallari et al., 2015). MuSCs express vascular endothelial growth factor A (VEGFA), whereas ECs express VEGF receptors (Verma et al., 2018). MuSC-specific genetic removal of VEGFA reduces the proximity between MuSCs and blood vessels at homeostasis (Verma et al., 2018). A population of MuSCs that appear to have the most quiescent stem cell-like character were found to be in the closest proximity to blood vessels (Verma et al., 2018). These results suggest that MuSCs help sculpt their own niche by attracting and retaining local microvasculature, which in turn contributes to maintenance of MuSC quiescence. The identities of the vasculature-derived factors that promote MuSC quiescence are unknown. It has been proposed that Notch ligands might be provided to MuSCs by ECs (Verma et al., 2018), but, as discussed above, the major source of these ligands appears to be the myofiber (Eliazer et al., 2020; Verma et al., 2018).

In mice, the adult compliment of quiescent MuSCs is established in the early postnatal period, up to 8 weeks after birth (Gattazzo et al., 2020; White et al., 2010). During this time, a subset of pericytes (cells embedded in the capillary basal lamina) secrete angiopoietin-1 (Angpt1), whereas MuSC progenitor cells express the Angpt1 receptor Tie2 (also known as Tek) (Kostallari et al., 2015). Pericyte-derived Angpt1 promotes entry of MuSC progenitors into quiescence, thereby participating in establishment of the adult MuSC population (Kostallari et al., 2015). It is not known whether sustained Angpt1–Tie2 signaling is required for maintenance of quiescence of adult MuSCs, but if this is the case, it could reflect a mechanism through which the microvasculature acts in the MuSC niche to promote homeostatic stem cell properties.

In addition to cells associated with blood vessels, the muscle interstitium also harbors multiple types of connective tissue cells, including FAPs (Theret et al., 2021). It is well established that FAPs communicate with MuSCs during muscle regeneration, and mice in which FAPs have been selectively killed, display inefficient regeneration (Murphy et al., 2011; Wosczyna et al., 2019). In the absence of injury, loss of FAPs has little effect on muscle or MuSC numbers in the short term (2 weeks), but by nine months results in muscle atrophy (Wosczyna et al., 2019). This is accompanied by a reduction in MuSC number, but it is not known whether this is based on direct effects of FAPs on MuSCs or on muscle tissue more generally.

In summary, the preceding discussion offers a view of the complex relationship between MuSCs and the niche that sustains their hallmark property at homeostasis – quiescence. Multiple distinct niche cues are required for maintenance of quiescence, including adhesion molecules and secreted factors derived from several cell types.

Perspectives

As discussed here, during muscle homeostasis, the MuSC niche provides polarized adhesion surfaces and multiple signaling factors that are essential for the maintenance of MuSC quiescence and long-term preservation of the stem cell compartment. These observations also raise several questions. First, why are so many different pathways employed in the maintenance of quiescence, and yet why is quiescence broken when individual pathways are genetically removed? This points to only limited compensation between these pathways, but it seems likely that the identified niche signals work in an integrated fashion to maintain quiescence. If so, it is important to probe how this is accomplished. Second, how many more niche-derived signaling pathways that maintain quiescence remain to be discovered, in addition to the many major adhesion and signaling pathways already identified; how close are we to a complete list? It is highly likely that biomechanical cues derived from the niche will be important regulators of MuSC behavior, but little is known of how this occurs (Krauss and Kann, 2023). Another important issue is how quiescence-promoting pathways are downregulated or overridden during injury-stimulated MuSC activation. Thus far, mouse genetic approaches have been critical for the identification of MuSC niche factors and the mechanisms by which they exert their effects. More recently, *ex vivo* preparations that accurately replicate critical features of MuSC quiescence are being developed (Jacques et al., 2022; Quarta et al., 2016), which should provide more efficient means to address the questions posed here. A complete understanding of how the niche maintains homeostatic MuSC behavior is relevant to the potential use of MuSCs for cell therapies in diseases such as Duchenne muscular dystrophy. In mice, freshly isolated MuSCs retain aspects of the quiescent phenotype and engraft efficiently into injured muscles, whereas their cultured myoblast progeny only do so inefficiently (Montarras et al., 2005; Sacco et al., 2008). Understanding how to maintain the features of quiescence that permit engraftment, especially those from niche-derived signals, could therefore pay dividends in translational settings.

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Competing interests

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High-resolution poster and poster panels

A high-resolution version of the poster and individual poster panels are available for downloading at https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.261200#supplementary-data.

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