SATELLITE CELLS AND THE MUSCLE STEM CELL NICHE

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Yin H, Price F, Rudnicki MA. Satellite Cells and the Muscle Stem Cell Niche. Physiol Rev 93: 23–67, 2013; doi:10.1152/physrev.00043.2011.—Adult skeletal muscle in mammals is a stable tissue under normal circumstances but has remarkable ability to repair after injury. Skeletal muscle regeneration is a highly orchestrated process involving the activation of various cellular and molecular responses. As skeletal muscle stem cells, satellite cells play an indispensable role in this process. The self-renewing proliferation of satellite cells not only maintains the stem cell population but also provides numerous myogenic cells, which proliferate, differentiate, fuse, and lead to new myofiber formation and reconstitution of a functional contractile apparatus. The complex behavior of satellite cells during skeletal muscle regeneration is tightly regulated through the dynamic interplay between intrinsic factors within satellite cells and extrinsic factors constituting the muscle stem cell niche/microenvironment. For the last half century, the advance of molecular biology, cell biology, and genetics has greatly improved our understanding of skeletal muscle biology. Here, we review some recent advances, with focuses on functions of satellite cells and their niche during the process of skeletal muscle regeneration.

I. INTRODUCTION: SATELLITE CELLS AS ADULT STEM CELLS IN MUSCLE

Skeletal muscle is a form of striated muscle tissue, accounting for ~40% of adult human body weight. Skeletal muscle is composed of multinucleated contractile muscle cells (also called myofibers). During development, myofibers are formed by fusion of mesoderm progenitors called myoblasts. In neonatal/juvenile stages, the number of myofibers remains constant, but each myofiber grows in size by fusion of satellite cells, a population of postnatal muscle stem cells. Adult mammalian skeletal muscle is stable under normal conditions, with only sporadic fusion of satellite cells to compensate for muscle turnover caused by daily wear and tear. However, skeletal muscle has a remarkable ability to regenerate after injury. Responding to injury, skeletal muscle undergoes a highly orchestrated degeneration and regenerative process that takes place at the tissue, cellular, and molecular levels. This results in the reformation of innervated, vascularized contractile muscle apparatuses. This regeneration process greatly relies on the dynamic interplay between satellite cells and their environment (stem cell niche). During the last half century, advances in molecular biology, cell biology, and genetics has greatly improved our understanding of skeletal muscle regeneration. In particular, extensive research on satellite cells and their niche has elucidated many cellular and molecular mechanisms that underlie skeletal muscle regeneration. These studies have contributed to the development of therapeutic strategies. These strategies serve to alleviate the physiological and pathological conditions associated with poor muscle regeneration observed in sarcopenia and muscular dystrophy.

Here, we concentrate on the functions of satellite cells and the regulation of their niche during the process of skeletal muscle regeneration. We first describe the current understanding of satellite cells with respect to their characteristics, heterogeneity, and embryonic origin. We then provide an integrated view of the roles played by satellite cells during muscle regeneration and normal postnatal muscle growth. We also discuss the contribution of several nonsatellite cell populations in muscle regeneration and their lineage relationships with satellite cells. Next, we focus on the satellite cell niche with emphasis on the regulatory mechanisms associated with each niche component. We further review the links between malfunction of satellite cells and their niche factors during aging. This review focuses on satellite cells and their niche in mammalian models, paying limited attention to the studies of satellite cell biology in other model organisms.

A. A Brief History of Satellite Cells

Half a century ago, Alexander Mauro observed a group of mononucleated cells at the periphery of adult skeletal muscle myofibers by electron microscopy (329). These cells were named satellite cells due to their sublaminar location...
and intimate association with the plasma membrane of myofibers.

The direct juxtaposition of satellite cells and myofibers immediately raised a hypothesis that these cells may be involved in skeletal muscle growth and regeneration (329). Indeed, experiments by thymidine labeling and electron microscopy demonstrated that satellite cells undergo mitosis, assume a cytoplasm-enriched morphology, and contribute to myofiber nuclei (355, 437). Later on, thymidine tracing experiments indicated that satellite cells are mitotically quiescent in adult muscle but can quickly enter the cell cycle following muscle injury (499). The same study also demonstrated that satellite cells give rise to proliferating myoblasts (myogenic progenitors cells), which were previously shown to form multinucleated myotubes in vitro (276, 499, 574). More definitive evidence came from in vitro cultures of individually dissected myofibers, whereby the behaviors of single myofibers and their resident satellite cells during regeneration can be tracked by phase-contrast microscopy (51, 277). It was observed that myofiber necrosis is accompanied by satellite cell outgrowth, clonal expansion, and later fusion to form functional regenerated myotubes. These experiments support the notion that it is the satellite cell, rather than the myonuclei, that contribute to postnatal muscle growth and repair.

The pivotal function of the satellite cell in muscle regeneration stimulated research to determine their regulatory mechanisms. This started with the finding that quiescent satellite cells on single myofibers were activated to enter the cell cycle by a mitogen originating from crushed skeletal muscles (53). In search of this mitogen, it was found that the proliferation and differentiation of satellite cell-derived myoblasts cultured in vitro respond to various growth factors in a dose-dependent manner (10, 11). Numerous studies further revealed that the proliferation and differentiation of satellite cells during muscle regeneration is profoundly influenced by innervation, the vasculature, hormones, nutrition, and the extent of tissue injury (121, 224, 250, 332, 360, 410). Interestingly, satellite cells in contact with the plasma membrane of intact myofibers were found to have reduced sensitivity to mitogen stimulation (50). All of these findings led to an intriguing notion that satellite cells reside in a special microenvironment in vivo, which profoundly affects their behavior.

By definition, stem cells found in adult tissues can both replicate themselves (self-renew) and give rise to functional progeny (differentiate). Although the ability of satellite cells to differentiate into myofibers was clearly proven, their ability to self-renew was once questioned. The first evidence of satellite cell self-renewal came from a single myofiber transplantation assay. It was found that as few as seven satellite cells, when transplanted into irradiated regeneration-insufficient mice along with their resident single myofiber, can give rise to hundreds of satellite cells and thousands of myonuclei. Most importantly, transplanted satellite cells on a single myofiber were able to support subsequent rounds of muscle regeneration (105). These observations demonstrate that satellite cells are bona fide muscle stem cells.

Asymmetric division is a common manner of stem cell self-renewal. Close examination of satellite cell divisions on single myofibers revealed that satellite cells can undergo both asymmetric division and symmetric division (280). The fashion of symmetric versus asymmetric division largely depends on the relative position of the daughter cells in relation to the myofiber. This finding strongly argues that satellite cell self-renewal is governed by the structure and signaling present in their immediate niche (280). Further investigation has revealed an important role for noncanonical Wnt signaling in the regulation of satellite cell self-renewal (293).

The aforementioned observations, together with those from other studies, formed the basis of our current view of satellite cells as muscle stem cells whose functions are dictated by their surrounding niche.

B. Identification and Isolation of Satellite Cells

Classically, satellite cells were identified based on a unique anatomical location: beneath the surrounding basal lamina and outside the myofiber plasma membrane. This anatomical location gives satellite cells a “wedged” appearance when viewed by electron microscopy (329). This technique also revealed other morphological characteristics of satellite cells: large nuclear-to-cytoplasmic ratio, few organelles, small nucleus, and condensed interphase chromatin. This morphology is in harmony with the notion that most satellite cells, in healthy unstressed muscles, are mitotically quiescent (in G0 phase) and transcriptionally inactive (472). In addition to electron microscopy, satellite cells can be identified by phase-contrast microscopy on single myofiber explants (53). Based on the same principle, the behaviors of satellite cells on single myofiber explants can be recorded by utilizing live cell imaging techniques (490). The identification of satellite cells by fluorescence microscopy relies on specific biomarkers (FIGURE 1). In adult skeletal muscle, all or most of satellite cells express the paired domain transcription factors Pax7 (478) and Pax3 (72), myogenic regulatory factor Myf5 (116), homeobox transcription factor Barx2 (336), cell adhesion protein M-cadherin (244), tyrosine receptor kinase c-Met (13), cell surface attachment receptor a7-integrin (73, 192), cluster of differentiation protein CD34 (37), transmembrane heparan sulfate proteoglycans syndecan-3 and syndecan-4 (113), chemokine receptor CXCR4 (429), caveolae-forming protein caveolin-1 (192, 552), calcitonin receptor (177), and nuclear en-
of these, Pax7 is the canonical biomarker for satellite cells as it is specifically expressed in all quiescent and proliferating satellite cells across multiple species, including human (334), monkey (334), mouse (478), pig (402), chick (216), salamander (353), frog (96), and zebrafish (219). Of note, some of the aforementioned satellite cell markers (e.g., α7-intragem, CD34) are also expressed on other cell types within

**FIGURE 1.** Characteristics of the satellite cell. A: numerous proteins are expressed in satellite cells and have been used as markers to distinguish between surrounding cell types within skeletal muscle. Due to heterogeneity in satellite cell populations, it is unlikely that all of these markers are expressed in a given satellite cell at a specific time. Nevertheless, this panel summarizes the cellular location of markers used to identify satellite cells. B: the satellite cell population is heterogeneous and can be classified in a hierarchical manner based on function and gene expression. Evidence from lineage tracing experiments identified a subpopulation of satellite cells having never expressed the myogenic transcription factor Myf5 (satellite stem cells) are placed hierarchically above satellite cells that have expressed Myf5 at some point during development (satellite myogenic cells). Satellite stem cells, upon asymmetric division (typically in a apical-basal orientation), will give rise to two daughter cells, only one of which has activated Myf5. Functional differences in regenerative potential exist between satellite stem cells and satellite myogenic cells. Following transplantation, satellite stem cells preferentially repopulate the satellite cell niche and contribute to long-term muscle regeneration. In contrast, satellite myogenic cells preferentially differentiate upon transplantation in vivo.
skeletal muscle, and thus should not be utilized alone to unequivocally identify satellite cells. Satellite cells can be identified by fluorescence microscopy via combined immunofluorescence labeling of laminin and M-cadherin, which respectively label the basal lamina and plasma membrane of satellite cells contacting the myofibers (517). In vivo, satellite cell populations can be visualized with the aid of newly developed bioluminescence imaging techniques (454, 558).

Multiple methodologies have been developed to isolate satellite cells from skeletal muscle. The choice of methods largely depends on the isolation scale and the subsequent experiment. In small scale, limited amounts of satellite cells can be released from single myofiber explants by physical trituration (446) or enzymatic digestion (107). In large scale, satellite cells can be isolated from skeletal muscles by fluorescent-activated cell sorting (FACS). In the latter method, single cells are released from muscle tissue chunks by enzymatic digestion, followed by immunofluorescence labeling of satellite cell-specific cell surface markers (positive selection) and definitive cell surface markers for non-satellite cell populations (negative selection). As Pax7 is specifically expressed in satellite cells within skeletal muscle, satellite cells, in both quiescent and proliferating stages, can be FACS-sorted by fluorescent protein expression in tamoxifen-injected animals carrying both a Pax7CreER allele and a fluorescence Cre reporter allele (e.g., R26R-EYFP). In addition, two transgenic mouse strains, which carry fluorescence proteins driven by Nestin- or Pax7-regulatory elements, are also useful for isolating satellite cells by FACS (63, 127).

Protocols have been developed to isolate satellite cells in bulk utilizing their characteristic proliferation and adhesion characteristics under defined culturing conditions (144, 364, 425). It is noteworthy that the satellite cell progeny cultured on regular collagen-coated plastic dishes and under activation conditions, also called satellite cell-derived primary myoblasts, are molecularly and functionally distinct from the satellite cells freshly isolated from muscles (160, 177, 350). In addition, the gene expression profile of these cultured primary myoblasts differs from that of activated satellite cells in vivo (398). These essential differences are possibly due to the lack of a satellite cell niche in in vitro culturing conditions. One challenge in future studies is to understand the components and role of the satellite cell niche to better control and manipulate their quiescence, activation, proliferation, and differentiation.

C. Heterogeneity of Satellite Cell Population

Satellite cells were considered to be a homogeneous population of committed muscle progenitors (52). However, recent evidence demonstrated that the satellite cell population is heterogeneous and that satellite cells differ in their gene expression signatures, myogenic differentiation propensity, stemness, and lineage potential to assume nonmyogenic fates.

With regard to gene expression, it was first observed that only a subset of satellite cells expresses Pax3, a close paralog to Pax7 (350, 431). Although Pax3+ satellite cells tend to be associated with skeletal muscle of certain anatomical locations (e.g., diaphragm and truck muscles), the difference of Pax3 expression does not seem to correlate with their embryonic origins, metabolic fiber types, or types of innervating motor neurons. Examination of the expression of satellite cell markers CD34, M-cadherin, and Myf5 by immunofluorescence staining revealed that a subpopulation of satellite cells do not express these markers (37). Similarly, human satellite cells also manifest heterogeneity in their Pax7, neuronal cell adhesion molecule (NCAM), c-Met, and Dlk1 expression (311). By RT-qPCR, two recent studies showed that satellite cells from head or body muscles are distinct in their molecular signatures (225, 456). Notably, the heterogeneity of gene expression in single satellite cells was investigated in a recent study, wherein the expression of Pax7, Pax3, Myf5, and MyoD was interrogated by RT-qPCR for 40 individually FACS-isolated muscle stem cells (CD45−/CD11b−/CD31−/Sca-1−/α7-intergin+/CD34+) (454). All of these muscle stem cells express Pax7, indicating they are satellite cells. In addition to the varied expression of Pax3, it was also found that 25% of investigated satellite cells also express MyoD, a basic helix-loop-helix transcription factor critical for myogenic commitment and differentiation. A recent study discovered a small subpopulation of satellite cells, characterized by their surface markers Sca-1+/ABCG2+ (as compared with Sca-1−/ABCG2− for most satellite cells), is able to exclude Hoechst 33342 dye and thus belongs to the skeletal muscle side population (satellite-SP cells) (518). After transplantation into damaged muscle, satellite-SP cells can both fuse to regenerating myofibers and return to quiescence in the satellite cell niche. However, by lineage tracing, ABCG2+ cells (including satellite-SP cells) seem to only have minor contribution to myofiber regeneration (146).

Satellite cells are also heterogeneous in their differentiation potential. Satellite cells on single myofibers isolated from various skeletal muscle sources were transplanted into irradiated muscles of mdx/nude mice, which underwent repeated regeneration and were depleted of endogenous satellite cells (105). It was observed that the number of regenerated myofibers contributed by tibialis anterior (TA) originated grafts was significantly less than that derived from either extensor digitorum longus (EDL) or soleus muscle. Although the contribution from donor myofibers cannot be precisely evaluated, this observation strongly suggests inherent differences in proliferation/differentiation potential of satellite cells and/or composition of satellite cell subpopulations from various muscles. In line with this view, continuous BrdU labeling of satellite cells in
vivo revealed two satellite cell populations that are distinct in terms of their mitotic rates (471). It was found that the majority (~80%) of satellite cells readily enter the cell cycle (responsive population), whereas the remaining 20% of satellite cells (reserve population) do this in a much slower manner. It has been proposed that the reserve population maintains in the quiescent state at the beginning of muscle growth/regeneration and only moves into this proliferative state in response to the need for extensive muscle growth/regeneration (470). In line with this view, a recent study traced satellite cell divisions by a fluorescent dye PKH26, which revealed the minority of PKH26<sup>high</sup> slow-dividing satellite cells retained long-term self-renewal ability (388). Notably, a recent study thoroughly compared the gene expression profiles and proliferation/differentiation potential of satellite cells isolated from limb and facial muscles of adult and aged mice (387). This study revealed broad satellite cell heterogeneity at both the population and single-cell levels. Despite their heterogeneous gene expression profiles, satellite cells isolated from limb (EDL) and facial (masseter) muscles are comparable in their ability to repair a limb (TA) muscle injury after transplantation. This finding suggests that although satellite cells from various muscles have distinct gene expression and behaviors in vitro, their regeneration potential in vivo might be largely determined by host stem cell niche and microenvironment.

Most importantly, recent studies revealed satellite cell heterogeneity in terms of their stemness and indicate that only a small percentage of satellite cells are true stem cells (109, 280, 489). By immunofluorescence staining of freshly isolated single myofibers from Myf5<sup>Cre</sup>;R26R-YFP mice, our group demonstrated that 13% of quiescent satellite cells on EDL muscles are LacZ<sup>−</sup>, making them distinct from the LacZ<sup>+</sup> satellite cells (280). As Myf5 is a myogenic regulatory factor, the absence of Myf5 expression suggests a less committed cell fate for those LacZ<sup>−</sup> satellite cells. Moreover, by the Cre-LoxP based lineage tracing technique, our group further discovered that 10% of satellite cells in Myf5<sup>Cre</sup>;R26R-YFP mice do not express YFP (Pax7<sup>+</sup>/YFP<sup>−</sup> satellite cells), indicating that this small percent of satellite cells have never expressed Myf5 as did the majority of satellite cells (Pax7<sup>+</sup>/YFP<sup>+</sup>). Remarkably, these two distinct satellite cell subpopulations also differ in terms of their regenerative potential: the Pax7<sup>+</sup>/YFP<sup>−</sup> satellite cells were able to reconstitute the stem cell niche and repaired muscles in a sustainable manner, whereas the Pax7<sup>+</sup>/YFP<sup>+</sup> satellite cells directly underwent myogenic differentiation when transplanted in regenerating muscles of Pax7<sup>−/−</sup> mice. We further demonstrated that only Pax7<sup>+</sup>/YFP<sup>−</sup> satellite cells could undergo asymmetric cell divisions, giving rise to a Pax7<sup>+</sup>/YFP<sup>−</sup> satellite stem cell and a Pax7<sup>+</sup>/YFP<sup>+</sup> satellite committed progenitor cell. These findings indicate that a hierarchical lineage progression from the Pax7<sup>+</sup>/YFP<sup>−</sup> (satellite stem cell) to the Pax7<sup>+</sup>/YFP<sup>+</sup> (satellite committed progenitors) exists within the total satellite cell population. Consistent with this notion, multiple studies reported that only a subset of satellite cells undergo asymmetric division in vivo or in vitro (108, 109, 489). For example, Numb, an inhibitor of Notch signaling and a cell fate determinant, was found to asymmetrically distribute in some but not all satellite cell divisions (108, 489). By pulse labeling or tandem pulse labeling of growing/regenerating muscles with halogenated thymidine analogs, it was further discovered that all “older” chromosomes (the template chromosome during DNA replication during S phase) cosegregate into the more stem-like daughter cell, whereas “younger” chromosomes are inherited by the more differentiated daughter cell during asymmetric divisions of satellite cells both in vivo and in vitro (109, 489). According to the “immortal DNA strand” hypothesis (75), this preferential retention of “older” chromosomes protects stem cells against the accumulation of mutations introduced during DNA replications (109, 489). Alternatively, it has been also proposed that the nonrandom segregation of sister chromatids, and hence their different epigenetic states, is essential to the gene expression patterns and cellular fates of satellite stem cells and satellite progenitor cells (289). In summary, the aforementioned findings support satellite cell heterogeneity whereby the existence of a hierarchy delineates a small population of true stem cells (satellite stem cells) from a more committed myogenic progenitor population of satellite cells. The satellite stem cells are less committed to the myogenic lineage and tend to retain older template DNA during division. Through asymmetric division, satellite stem cells self-renew to replenish the stem cell pool and produce more committed myogenic progenitors that participate in skeletal muscle growth and regeneration.

Satellite cells also exhibit heterogeneity in respect to their cell fate potential. Our group first revealed that satellite cells have an intrinsic potential to differentiate into multiple mesenchymal lineages (23). When cultured on solubilized basement membrane matrix (matrigel), satellite cells from single myofibers spontaneously differentiate into myocytes, adipocytes, and osteocytes. This finding indicates that satellite cells functionally resemble bone marrow-derived mesenchymal stem cells. This notion is substantiated by another study wherein satellite cells were found to assume the adipocyte lineage, which can be enhanced upon oxygen-rich culture conditions (120). By clonal analysis, it was found that myogenic and adipogenic satellite cells are two separate populations in the satellite cell compartment, although both populations express the myogenic marker Pax7 as well as the adipogenic markers peroxisome proliferator-activated receptors γ (PPARγ) and CCAAT/enhancer binding proteins (C/EBPs) (485). This similar molecular signature may reflect a common developmental origin of satellite cells and adipogenic progenitors in embryonic somites (discussed in sect. IIE). Satellite cell heterogeneity with regard to myogenic and nonmyogenic potential was thoroughly investigated by in vitro differentiation and in vivo transplant assays (486). In this study, myofiber-associated satellite cells were isolated from undamaged skeletal muscle by a two-step enzymatic digestion and sorted by FACS based on
their differential expression of cell surface markers, CD45 and Sca-1. It was found that the vast majority of myogenic satellite cells are within the CD45+/Sca-1− population and exhibited no in vitro differentiation potential into fibroblasts or adipocytes. In contrast, the minor population of CD45+/Sca-1+ satellite cells can differentiate into both fibroblasts and adipocytes in culture, a similarity that is shared with CD45−/Sca-1+ mesenchymal stem cells found in multiple tissues (316, 401, 417, 441, 509, 519). Despite the plasticity of satellite cells in vitro, it is important to note that myogenesis is the predominant fate of satellite cells in vivo as fibrosis or adipose infiltration is not normally observed in young healthy muscle. Furthermore, recent studies indicate that intramuscular adipocytes and fibroblasts can also arise from fibrocyte/adipocyte progenitors (FAPs), which reside in the muscle interstitium (252, 541; and discussed in sect. IIIB). Therefore, recent studies indicate that intramuscular adipocytes and fibroblasts can also arise from fibrocyte/adipocyte progenitors (FAPs), which reside in the muscle interstitium (252, 541; and discussed in sect. IIIB).

Indirect lineage tracing experiments indicate that adipocytes derived from myofibers isolated from MyoD-Cre;R26R-EYFP mice have never transcribed MyoD (507). As the vast majority of adult satellite cells were permanently labeled with EYFP in this experiment, this finding argues that most satellite cells from myofiber cultures do not spontaneously differentiate into adipocytes. Further investigation with more definitive lineage tracing (e.g., Pax7-CreER;R26R-EYFP) methods would clarify the exact contribution of satellite cells to other nonmyogenic lineages both in vitro and in vivo.

As discussed here, multiple lines of evidence demonstrate that satellite cells represent a heterogeneous population. However, our understanding of this heterogeneity is far from complete. First, although several markers can separate the total satellite cell population into functional subpopulations, it is still unknown whether these subpopulations are homogeneous in their function and gene expression. Further studies to identify additional satellite markers will potentially help distinguish the various satellite cell lineages. Moreover, future investigations should attempt to identify the intrinsic differences between satellite cell subpopulations at the molecular and functional levels during muscle regeneration. Such findings would elucidate regulatory mechanisms governing the transition between different satellite cell subpopulations and potentially distinct roles of satellite cell subpopulations during muscle regeneration. In addition, it would be of great importance to understand the dynamics of satellite cell heterogeneity in response to various environmental cues with regard to research in muscle regeneration and disease.

D. Variance of Satellite Cells Number and Location

In addition to the heterogeneity of satellite cells, the quantity of satellite cells differs between muscles, myofiber types, developmental stages, and species. In general, satellite cells account for 30–35% of the sublaminar nuclei on myofibers in early postnatal murine muscles, and this number declines to 2–7% in adult muscles (9, 227, 450, 469). In adults, the percentage of satellite cells in soleus muscle is generally two- to fourfold higher than that in tibialis anterior muscle or EDL muscle (190, 466, 500). Within the same muscle, the number of satellite cells found on slow muscle myofibers (type I) is generally higher than those on fast myofibers (type IIa and type IIb) (190, 324, 383). The biological meaning and the potential regulatory mechanisms underlying these phenomena are poorly understood. However, it is conceivable that these variances may reflect intrinsic heterogeneity of satellite cells on different myofibers and implicate a potential role of myofibers as a niche factor in regulating the homeostasis of their resident satellite cells.

Along a myofiber, the distribution of satellite cells is not random. It has been reported that the density of satellite cells is higher at the ends of the myofibers, where the longitudinal growth of skeletal muscles happens (14). A higher incidence of satellite cells has been observed at perisynaptic regions compared with that at extrasynaptic regions (190, 269, 567). Moreover, satellite cells have been observed in close proximity to capillaries (100, 466). In fact, 88% of satellite cells in adult human muscles were observed to be located within a 21-μm distance of a capillary (100). This tight association with capillaries seemed to be compromised by denervation (130). These observations indicate that homing of satellite cells is influenced by their niche, both by local motor neurons and blood vessels (discussed in sect. IIIIB).

It is noteworthy that some reported variation in satellite cell numbers may be partially due to techniques or statistical analysis employed in satellite cell counting. For example, satellite cell counting based on immunofluorescence labeling of satellite cell specific markers relies on the comparable expression levels of these markers on all satellite cells under investigation. As such, special caution should be taken into account when interpreting data between independent experiments.

E. Origins of Adult Satellite Cells

1. Embryonic origins

By classic techniques of developmental biology, it has long been established that skeletal muscles within both the adult trunk and limbs develop from embryonic somites (462). However, the exact origin of adult satellite cells was obscure until recently.

Early experiments using a quail-chick chimera technique suggested a somitic origin of satellite cells in amniotes (18). Embryonic somites are segments of paraxial mesoderm formed on both sides of the body axis. In this experiment, somites from donor quail embryos were transplanted into host chick embryos. After embryonic development, the contribution of quail cells to the chick satellite cell compart-
ment was determined using Feulgen staining, which distinguishes quail-specific interphase nuclei from those of chick. It was found that donor cells from quail somites integrated into the chick limb and contributed to both terminally differentiated muscle fibers and satellite cells. This finding indicated a common somitic origin for all myogenic cell lineages, including satellite cells. However, the progenitor cell types at the origin and the developmental route remain unknown.

Advances in mouse genetics, particularly the generation of Pax3 and Pax7 knock-in reporter alleles, allows precise tracing of Pax3/Pax7-expressing myogenic progenitor cells during muscle development in a temporal and spatial manner (265, 326, 350, 432, 433). These reporters together with labeling of cells by electroporation (40, 203), retrovirus (464), Cre-LoxP based lineage tracing (263, 300, 464), and traditional quail-chick transplantation (203, 464), jointly shed light on the embryonic origins of adult satellite cells. Accumulating evidence indicates that adult satellite cells originate from the dermomyotome (203, 265, 434, 464), an epithelial structure formed on the dorsal part of the somite. The dermomyotome contains multipotent progenitor cells, which eventually give rise to multiple adult tissues/cell types including dermal fibroblasts, endothelial cells, vascular smooth muscle, brown fat tissue, and all skeletal muscles of the trunk and limbs (71). The cell fate decisions largely depend on the relative position of these multipotent progenitor cells with respect to adjacent tissues such as the notochord, neural tube, dorsal ectoderm, and myotome (71).

Embryonic muscle development takes place in two successive stages. During the first stage, a group of postmitotic mononucleated myocytes, expressing Myf5 and Mrf4, migrate out from the border regions of the dermomyotome and form primitive muscles beneath the dermomyotome (204, 261). These primitive muscles constitute the primary myotome and are the source of fetal and adult trunk muscles. During the second stage of muscle growth, the central portion of the dermomyotome undergoes an epithelial-to-mesenchymal transition (EMT). During EMT, tightly packed epithelial cells tease apart and turn in a loose mesenchymal state prior to assuming different developmental fates: cells in the medial dermomyotome will develop into brown fat, dermis, and trunk muscle, while cells in the lateral dermomyotome will give rise to endothelia and limb muscles. The different cell fates assumed by the same group of progenitor cells are proposed to be due to asymmetric cell division (40, 101). EMT is accompanied by the extensive cell migration (203, 265, 434, 464). With the breakdown of dermomyotome, a group of proliferating progenitor cells, expressing both Pax3 and Pax7, migrate from the central region of the dermomyotome into the previously formed primary myotome. Upon arrival, some progenitor cells continue to proliferate and replenish the progenitor pool. These cells, which were absent from the primary myotome at earlier stages, count for the majority of all proliferating cells in embryonic/fetal trunk muscles (203, 434). Some of the proliferating Pax3+/Pax7+ progenitor cells persist into late fetal development stages and are enveloped beneath the basal lamina of developing myofibers (203, 434). These cells, which reside in the satellite cell compartment, are presumed to subsequently become the postnatal satellite cells in trunk muscles. Besides proliferation, progenitor cells also exit the cell cycle and begin differentiating into embryonic/fetal trunk muscles. Cell cycle withdrawal is concomitant with the expression of the myogenic regulatory factors MyoD and Myf5 (453).

During EMT, another group of proliferating progenitor cells, expressing Pax3 (but not Pax7 in mouse), delaminate from the ventral-lateral border of the dermomyotome and migrate to the limb bud mesenchyme (265, 392, 464). These progenitor cells still maintain their multipotency as they give rise to the limb vascular, lymphatic endothelia, and limb muscles (226, 264). At E11.5 of mouse embryo development, some progenitor cells start to express Pax7 in the anterior limb buds (433). The expression of Pax7 specifies these cells to the myogenic lineage (242). Similar to the myotome-located progenitors, these Pax3+/Pax7+ progenitor cells in limb buds undergo proliferation/differentiation while a portion withdraw from cell cycle and become satellite cells.

All together, these observations indicate that Pax3+/Pax7+ embryonic progenitor cells are the major source of adult satellite cells in trunk and limb muscles; it is, however, noteworthy that the aforementioned observations from lineage tracing and immunofluorescence labeling experiments cannot exclude the possibility that some adult satellite cells may originate from other sources during fetal and postnatal muscle development. For example, embryonic dorsal aorta explants, when cultured and disaggregated in vitro, can efficiently give rise to myogenic precursors (129). These myogenic precursors are similar to satellite cells in their gross morphology and expression of molecular markers. Given that adult satellite cells also express endothelial markers, it was proposed that some adult satellite cells originated from the embryonic dorsal aorta (129). However, recent studies revealed that both skeletal muscles and smooth muscles found in dorsal aorta are derived from the same Pax3+ cell population in the paraxial mesoderm (155). Thus it is also possible that the observed similarities are merely reminiscent of a common embryonic origin before myogenic specification.

Distinct from trunk and limb muscles, head muscles have multiple embryonic origins. The majority of head muscles, including branchiomiect muscles and most extraocular muscles, arise from the cranial paraxial mesoderm (CPM) (223, 540). Posterior neck muscles and tongue develop...
from occipital somites. Similar to progenitor cells migrating to limb buds, the progenitor cells for tongue delaminate from the ventral-lateral border of occipital somites. A small fraction of extraocular muscles also arise from the prechordal mesoderm (PM). On the basis of observations from lineage tracing experiments, it has been found that adult satellite cells of the various head muscles originate from their corresponding embryonic muscles and express distinct combinations of signature genes (225). Unlike trunk and limb progenitors, most progenitor cells for head muscles (except for tongue) express MesP1 rather than Pax3.

2. Alternative origins of adult satellite cells

Accumulating evidence indicates that some adult satellite cells may have alternative origins other than dermomyotome-derived Pax3+/Pax7+ progenitor cells.

First, multiple studies have demonstrated that several types of nonsatellite cells can reconstitute the satellite cell niche and turn into bona fide satellite cells (Pax7-expressing myogenic cells) after transplantation into regenerating skeletal muscles (see sect. IID). It remains, however, largely unknown to what extent these cells contribute to the adult satellite cell pool and muscle development under physiological conditions. Notably, a recent study utilizing TN-APCreERT2 and VE-cadherinCreERT2 alleles showed that alkaline phosphatase (ALPL) expressing pericytes, but not VE-cadherin-expressing endothelial cells, can develop into postnatal satellite cells and participate in normal development of limb muscles (135).

It is noteworthy that some adult satellite cells in mammals may be derived from dedifferentiation of fetal/adult myofibers. It has long been established that the dedifferentiation of “terminally” differentiated multinucleated myofibers occurs in injured skeletal muscle of urodele amphibians, such as the newt (69). Nevertheless, it remains controversial as to whether mammalian myotubes in vitro or myofibers in vivo can undergo a similar dedifferentiation process. Studies utilizing immortalized murine myoblast cell lines (e.g., C2C12 or pmi28 cells) have shown that some myotubes formed by these cells can dedifferentiate into mononucleated myogenic cells in the presence of protein extract from regenerating newt muscles (331), bioactive compounds like myostatin or its derivatives (149, 443), ciliary neurotrophic factor (CNTF) (95), or by genetic manipulations such as overexpression of Msx1 (379), Twist1 (232), Barx2 (335), or knockdown of Rb1 in conjunction with a deficiency for Cdkn2a (p16Ink4a) (395). By a novel fusion-dependent lineage tracing technique, two recent studies reported that differentiated myotubes formed by satellite cell-derived primary myoblasts (397) or muscle-derived cells (MDCs) (359) can dedifferentiate into Pax7-expressing mononucleated myogenic cells in vitro in response to an inhibitor cocktail (a tyrosine phosphatase inhibitor plus an apoptosis inhibitor) or within regenerating muscle in vivo. Although still not experimentally tested on myofibers formed during development, these intriguing observations jointly suggest that some adult satellite cells may be “recycled” from multinucleated myofibers in vivo during postnatal muscle growth or regeneration. Future studies may employ the fusion-dependent lineage tracing technique in chimeric mice to investigate the physiological relevance of this alternative source of satellite cells.

II. FUNCTIONS OF SATELLITE CELLS IN MUSCLE REGENERATION

Skeletal muscles consist of myofibers, neurons, vasculature networks, and connective tissues, of which the structural and functional element of skeletal muscle is the myofiber. Each myofiber is surrounded by the endomysium (also called the basement membrane or basal lamina). Bundles of myofibers are surrounded by the perimysium, while the entire muscle is contained within the epimysium. Each myofiber is anchored at its extremities to tendons or tendon-like fascia at the myotenodinous junctions (MTJs) (531). Myofibers are composed of actin and myosin myofilaments repeated as a sarcomere, which is the basic functional unit of skeletal muscle. Responding to the signals from motor neurons, myofibers depolarize and release calcium from the sarcoplasmic reticulum (SR). This drives the movement of actin and myosin filaments relative to one another and leads to sarcomere shortening and muscle contraction.

Based on their physiological properties, skeletal muscle fibers can be grouped into a slow-contracting/fatigue-resistant type and a fast-contracting/fatigue-susceptible type. Myofibers also vary in terms of their myosin heavy chain (MyHC) isoforms (fast or slow) and metabolism types (oxidative or glycolytic). The choice of myosin gene expression is under the dynamic regulation of thyroid hormone and work load (reviewed in Ref. 28). Recent studies demonstrated that the specification of myosin expression is also regulated by intrinsic microRNAs within MyHC genes (545, 546).

Mammalian skeletal muscle during adulthood is a stable post-mitotic tissue with infrequent turnover of myonuclei (467). Minor lesions inflicted by day-to-day wear and tear can be repaired without causing cell death, inflammatory responses, or histological changes. For instance, local plasma membrane damage caused by spontaneous eccentric muscle contractions can be efficiently repaired by recruiting intracellular vesicles to patch the damaged membrane (30, 508). This repair process involves dysferlin and caveolin-3 (30, 181), and mutations of these genes cause limb girdle muscular dystrophy 2B (LGMD-2B) (36, 314) and 1C (LGMD-1C) (344), respectively. In contrast, severe muscle injuries due to either traumatic lesions (e.g., extensive physical activity such as resistance training, or exposure to myotoxin) or genetic defects (e.g., muscular dystrophies) are accompanied by myofiber necrosis, inflamma-
tory responses, activation of satellite cells, proliferation, and differentiation of satellite cell-derived myoblasts (FIGURE 2). This process, starting from myofiber necrosis and ending with new myofiber formation, is called muscle regeneration. It should be stressed that satellite cells play a pivotal role during muscle regeneration under either physiological conditions (e.g., extensive exercise) (400, 457) or pathological conditions (e.g., myotoxin induced injury) (301, 361, 457). This notion is clearly supported by the findings that ablation of the total satellite cell pool (all Pax7+ cells) in adulthood completely abolished muscle regeneration (301, 361, 457). It has been reported that several types of nonsatellite cells can undergo myogenic differentiation and contribute to muscle regeneration after transplantation into regenerating muscle (24, 210, 287, 345, 413). Nevertheless, the contribution of these cells to adult muscle regeneration seems to be negligible compared with satellite cells, implying the physiological relevance of nonsatellite cell-based myogenesis might depend on Pax7 expression and/or the existence of considerable numbers of satellite cells.

In this section, we examine the extensive cellular and molecular dynamics during muscle regeneration, with emphasis on satellite cell. We also review the potential of nonsatellite cell lineages on muscle regeneration. At the end of this section, we briefly describe the function of satellite cells in normal postnatal muscle development, and compare and contrast this with muscle regeneration in adulthood.

A. An Introduction to Muscle Regeneration

Muscle regeneration occurs in three sequential but overlapping stages: 1) the inflammatory response; 2) the activation, differentiation, and fusion of satellite cells; and 3) the maturation and remodeling of newly formed myofibers.

Muscle degeneration begins with necrosis of damaged muscle fibers. This event is initiated by dissolution of the myofiber sarcolemma, which leads to increased myofiber permeability. Disruption of myofiber integrity is reflected by...
increased plasma levels of muscle proteins and microRNAs, such as creatine kinase (17) and miR-133a (292), which are usually restricted to the myofiber cytosol. Similarly, the compromised sarcolemmal integrity also allows the uptake of low-molecular-weight dyes, such as Evans blue or propidium orange, by the damaged myofiber, which is a reliable indication of sarcolemmal damage associated with extensive exercise and muscle degenerative diseases (218, 328, 396). Moreover, myofiber necrosis is accompanied by increased calcium influx or calcium release from the SR, which in turn activates calcium-dependent proteolysis and drives tissue degeneration (reviewed in Refs. 7, 19, 39). In this process, calpain, a calcium-activated protease, has been shown to cleave myofibrillar and other cytoskeletal proteins (reviewed in Ref. 145). Myofiber necrosis also activates the complement cascade and induces inflammatory responses (389). Subsequent to inflammatory responses, chemotactic recruitment of circulating leukocytes occurs at local sites of damage (reviewed in Ref. 530). Neutrophils are the first inflammatory cells to infiltrate the damaged muscle, with a significant increase in their number being observed as early as 1–6 h after myotoxin- or exercise-induced muscle damage (168). Following neutrophil infiltration, two distinct subpopulations of macrophages sequentially invade the injured muscle and become the predominant inflammatory cells (91). The early invading macrophages, characterized by the surface markers CD68+/CD163+, reach their highest concentration in damaged muscle at ~24 h after the onset of injury and thereafter rapidly decline. These CD68+/CD163+ macrophages secrete proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1, and are responsible for the phagocytosis of cellular debris. A second population of macrophages, characterized by the surface markers CD68-/CD163+, reach their peak at 2–4 days after injury. These macrophages secrete anti-inflammatory cytokines, such as IL-10, and persist in damaged muscle until the termination of inflammation. Notably, the CD68-/CD163+ macrophages also reportedly facilitate the proliferation and differentiation of satellite cells (80, 81, 303, 341, 503).

A highly orchestrated regeneration process follows muscle degeneration. A hallmark of this stage is extensive cell proliferation. Blocking cell proliferation by colchicine treatment (411) or irradiation (423) drastically reduced muscle regenerative capacity. Experiments by [3H]thymidine labeling have clearly demonstrated that the proliferation of satellite cells and their progeny provide a sufficient source of new myonuclei for muscle repair (498, 499, 554; reviewed in Ref. 79 and discussed in sect. II B). It is commonly agreed that following proliferation, myogenic cells differentiate and fuse to existing damaged fibers or fuse with one another to form myofibers de novo. This process, in many but not all respects, recapitulates embryonic myogenesis.

Muscle regeneration can be characterized by a series of morphological characteristics based on histological and immunofluorescence staining. On muscle cross-sections, newly formed myofibers can be readily distinguished by their small caliber and centrally located myonuclei. These myofibers are often basophilic in the beginning of regeneration due to protein synthesis and the expression of embryonic/developmental forms of MyHC (217, 562). On muscle longitudinal sections and on isolated single myofibers, the centrally localized myonuclei were observed in discrete segments of regenerating myofibers or along the entire new myofiber, which suggests that cell fusion during regeneration happens in a focal, rather than diffuse, manner (58). Occasionally, concentrated regenerative processes may appear as local protrusions (also called budding) on myofibers. Muscle regeneration can often lead to architectural changes of the regenerated myofibers, which are presumably due to incomplete fusion of regenerating fibers within the same basal lamina (57, 58, 64, 465). Newly formed myotubes may not fuse to each other, resulting in clusters of small caliber myofibers within the same basal lamina. Alternatively, they may fuse only at one end, leading to the formation of forked (also called branching or splitting) myofibers. Myofiber branching was commonly observed in muscles from patients suffering neuromuscular diseases, in hypertrophied muscles, and in aging muscles, suggesting this phenotype may relate to abnormal muscle regenerative capacity (59, 90). Small regenerating myofibers may also form outside the basal lamina in the interstitium, due to migration of satellite cells or other types of myogenic cells. Finally, the reconstitution of myofiber integrity may be prevented by scar tissue that separates the two regenerative sites, leading to the formation of a new myotendinous junction.

At the end of muscle regeneration, newly formed myofibers increase in size, and myonuclei move to the periphery of the muscle fiber. Under normal conditions, the regenerated muscles are morphologically and functionally indistinguishable from undamaged muscles.

B. Satellite Cell Activation and Differentiation

In intact muscle, satellite cells are sublaminally and mitotically quiescent (G0 phase). Quiescent satellite cells are characterized by their expression of Pax7 but not MyoD or Myogenin (116). Examination of β-galactosidase activity in Myf5-LacZ mice indicated that the Myf5 locus is active in ~90% of quiescent satellite cells, which suggests most satellite cells are committed to the myogenic lineage (37).

Upon exposure to signals from a damaged environment, satellite cells exit their quiescent state and start to proliferate (satellite cell activation). Proliferating satellite cells and their progeny are often referred to as myogenic precursor...
cells (MPC) or adult myoblasts. Satellite cell activation is governed by multiple niche factors and signaling pathways (discussed in detail in sect. IIIA). Satellite cell activation is not only restricted to the site of muscle damage. In fact, localized damage at one end of a muscle fiber leads to the activation of all satellite cells along the same myofiber and migration of these satellite cells to the regeneration site (473). Satellite cell activation is also accompanied by extensive cell mobility/migration. It has been observed that satellite cells can migrate between myofibers and even muscles across barriers of basal lamina and connective tissues during muscle development, growth, and regeneration (241, 251, 557). Recently, sialomucin CD34, whose expression is high on quiescent satellite cells but dramatically reduced during satellite cell activation, was demonstrated to act as an antiadhesive molecule to facilitate migration and promote the proliferation of satellite cells at very early stages of muscle regeneration (8). In addition, dynamic regulation of Eph receptors and ephrin ligands in activated satellite cells and regenerating myofibers have been shown to direct satellite cell migration (506).

Unlike quiescent satellite cells, myogenic precursor cells are characterized by the rapid expression of myogenic transcription factors MyoD (111, 114, 116, 175, 207, 497, 572, 585) and Myf5 (111, 116). Of note, the presence of MyoD, desmin, and Myogenin in satellite cells was observed as early as 12 h after injury, which is before any noticeable sign of satellite cell proliferation (426, 497). This early expression of MyoD is proposed to be associated with a subpopulation of committed satellite cells, which are poised to differentiate without proliferation (426). In contrast, the majority of satellite cells express either MyoD or Myf5 by 24 h following injury (111, 116, 585) and subsequently coexpress both factors by 48 h (111, 116). The ability of satellite cells to upregulate either MyoD or Myf5 suggests these two transcription factors may have different functions in adult myogenesis.

First, MyoD−/− mutant mice display markedly reduced muscle mass (338). This atrophy phenotype is reportedly due to delayed myogenic differentiation (564, 573). Similarly, muscle regeneration is also impaired in MyoD−/− mice, resulting in an increased number of myoblasts within the damaged area (338). These MyoD−/− myoblasts persist for extended periods of time, fail to differentiate, and do not fuse into myotubes. This is consistent with the notion that MyoD−/− myoblasts, when cultured in myogenic differentiation conditions, continue to proliferate and eventually give rise to a decreased number of differentiated mononucleated myocytes (114, 452, 573). Intriguingly, transplanted MyoD−/− myoblasts have been reported to survive and engraft into MyoD+/+ regenerating muscles with improved efficacy (compared with wild-type myoblasts). This phenotype is reportedly due to their increased stem cell characteristics and repressed apoptotic potential (22, 231).

After regeneration, these transplanted MyoD−/− myoblasts not only give rise to myonuclei but also contribute to the satellite cell pool (22). On the other hand, ectopic expression of MyoD in NIH-3T3 and C3H10T1/2 fibroblasts is sufficient to activate the complete myogenic program in these cells (234). Taken together, these observations indicate that expression of MyoD is an important determinant of myogenic differentiation, and in the absence of MyoD, activated myoblasts have a propensity for proliferation and self-renewal (452). In contrast to the MyoD−/− mice, Myf5−/− mutant mice show a myofiber hypertrophy phenotype (187), and the proliferation of Myf5−/− myoblasts is compromised (187, 542). Together, these results implicate a distinct role for Myf5 in adult myoblast proliferation, while MyoD is essential for differentiation. Notably, the disparate functions of Myf5 and MyoD in adult muscle regeneration parallel the proposed roles for these transcription factors throughout the development of distinct myogenic lineages during embryogenesis (188, 213, 256–259; reviewed in Ref. 260). Together, the aforementioned observations suggest a hypothesis that satellite cells enter different myogenic programs depending on whether Myf5 or MyoD expression predominates (450). Predominance of MyoD expression would drive the program toward early differentiation, as exemplified by the behavior of Myf5−/− myoblasts (349). In contrast, predominance of Myf5 expression would direct the program into enhanced proliferation and delayed differentiation, as shown by the behavior of MyoD−/− myoblasts (452). Finally, myoblasts coexpressing both Myf5 and MyoD would exhibit the intermediate growth and differentiation propensities as shown by most of satellite cell-derived myoblasts. This hypothesis is consistent with the observation that MyoD and Myf5 have different expression profiles throughout the cell cycle. MyoD expression peaks in mid G1, whereas Myf5 expression is maximal at the G0 and G2 phases of the cell cycle (273). Therefore, disruptions to the MyoD/Myf5 ratio may determine the choice of myogenic programs. This hypothesis also explains the spectrum of proliferation and differentiation potential observed in different primary myoblast clones cultured in vitro.

Several studies have revealed that MyoD expression in proliferating myoblasts is positively regulated by serum response factor (SRF), which binds to the serum response element (SRE) within the MyoD regulatory region (186, 285). In proliferating myoblasts, SRF only drives low levels of MyoD expression (286), whose activity is inhibited by cyclin D1 induced cyclin dependent kinase 4 (Cdk4) (587). However, the induction of MEF2 expression prior to differentiation enables MEF2 to out-compete SRF for the SRE binding site and leads to high levels of MyoD expression and initiation of differentiation (286). This function of MEF2 is further regulated by a member of the myocardin family of transcription factors, MASTR, whose expression is upregulated in response to muscle injury (348).
Notably, our group recently revealed a pro-proliferation function of MyoD in myoblasts (191). We found that the γ isoform of p38 kinase (p38γ) phosphorylates MyoD, which negates the transcriptional activation potential of MyoD and leads to a repressive MyoD complex occupying the Myogenin promoter (191). This positive effect of p38γ on myoblast proliferation is also supported by the observation that Myogenin is prematurely expressed in p38γ-deficient muscle, which displays markedly reduced myoblast proliferation (191). These results also support the notion that the functional state of MyoD depends on cofactors present in the MyoD transcriptional complex.

Moreover, multiple studies demonstrated that MyoD expression does not always warrant myogenic commitment. Monitoring satellite cell lineage progression revealed that some Pax7+/MyoD+ proliferating myoblasts could retract back to a Pax7+/MyoD- state and eventually return to quiescence (127, 216, 584; discussed in sect. II). In addition, the reciprocal inhibition of Pax7 with MRFs (MyoD and Myogenin) has been revealed in C3H10T1/2 fibroblasts and MM14 myoblasts in vitro (385). It was found that Pax7 decreases MyoD transcription activity and stability, whereas Myogenin represses Pax7 transcription likely via the HMGB1-RAGE axis (385, 438). Based on these observations, it was proposed that the ratio of Pax7 and MyoD activities is critical for satellite cell fate determination (385). A high ratio of Pax7 to MyoD (as seen in quiescent satellite cells) keeps satellite cells in their quiescent state. An intermediate ratio of Pax7 to MyoD allows satellite cells to proliferate, but not differentiate. Satellite cells with a low Pax7-to-MyoD ratio begin to differentiate, and further reduction in Pax7 levels are observed following activation of Myogenin.

After limited rounds of proliferation, the majority of satellite cells enter the myogenic differentiation program and begin to fuse to damaged myofibers or fuse to each other to form new myofibers. The initiation of terminal differentiation starts with the expression of Myogenin and Myf6 (also called Mrf4) (114, 116, 207, 497, 572). The induction of Myogenin expression primarily depends on MyoD and is proposed to enhance expression of a subset of genes previously initiated by MyoD (83). Target genes of MyoD and Myogenin have been revealed by candidate approaches (405), ChIP-on-chip experiments (44, 56, 83), and more recently by ChIP-Seq analysis (84). These investigations jointly reveal a convoluted hierarchical gene expression circuitry centered on MyoD and its immediate downstream targets: Myogenin and Mef2 transcription factors (Mef2s). Based on the temporal expression pattern of MyoD, Myogenin and Mef2s, a feed-forward regulatory circuit is proposed. In this hypothesis, myogenic differentiation is an irreversible procedure and is driven by the sequential expression of key transcription factors (master regulators), which are destined to transduce gene expression signals to their target genes (45, 405). A large portion of target genes induced by MyoD, Myogenin, and Mef2s are muscle-specific structural and contractile genes, such as those encoding actins, myosins, and troponins. The expression of these genes is essential for the proper formation, morphology, and function of skeletal muscle and thus they are regulated by multiple mechanisms (41).

First, the transcriptional activities of MyoD, Myogenin, and Mef2s are regulated by posttranscriptional modifications (reviewed in Ref. 420). The α and β isoforms of p38 kinase (p38-α/β) have an important role in the expression of muscle-specific genes (405) and in muscle terminal differentiation (571). The function of p38-α/β is at least partially responsible for the phosphorylation of Mef2s as inhibition of p38-α/β disrupts the transcriptional activities of Mef2s. In contrast, the expression of constitutively active forms of p38-α/β promotes myogenesis (117, 221, 390, 420, 571, 589). p38-α/β activity stimulates the binding of MyoD and Mef2s to the promoters of muscle-specific genes, leading to the recruitment of chromatin remodeling complexes, and ultimately the RNA polymerase II holoenzyme (405, 424, 491). Similarly, the transcriptional activity of Myogenin is regulated by protein kinase A (PKA) (308) and protein kinase C (PKC) (309). Protein inhibitors of MRFs also control myogenic gene expression. The transcriptional activity of MRFs relies on heterodimerization with E proteins (ITF1, ITF2, E12, E47) (291, 362, 363). This heterodimerization is negatively regulated by a group of inhibition of DNA binding proteins (Ids: Id1, Id2, Id3, and Id4), which are also helix-loop-helix proteins but lack the basic DNA-binding domain (42, 43). Id proteins heterodimerize with E proteins and prevent their association with MRFs, thus abrogating myogenic gene expression (43). Similar results occur when Mist1 directly interacts with MyoD and prevents MyoD from binding E-boxes (298). MyoD activity can be further inhibited by the sequestration of E proteins, by Twist (504). Finally, the transcriptional activity of MyoD is also determined by specific cofactors present on the promoters of myogenic genes (reviewed in Ref. 450). In vitro, MyoD associates with histone acetyltransferases (HATs) p300 and p300/CBP (CREB-binding protein)-associated factor (PCAF) on E-box motifs of its target genes (419). This association is presumed to induce histone acetylation and transcriptional activation (45). MyoD also interacts with histone deactylases (HDACs), which negatively regulate the transcriptional activity of MyoD either directly (325) or in a Mef2-dependent manner (319).

Besides MRFs and their regulators, other factors have been shown to be involved in myogenic differentiation. Micro-RNAs are 20–22nt noncoding small RNAs, which function to repress translation and reduce the stability of their target mRNAs. Recent studies demonstrated that MRFs, such as Myf5, MyoD, and Myogenin, activate the expression of a collection of myogenic microRNAs (e.g., miR-1, miR-133,
miR-206 together called MyomiRs). These myogenic microRNAs, in conjunction with other microRNAs, modulate the expression levels of key myogenic transcription factors and regulators, such as Pax3 (119, 196, 231), Pax7 (94, 139), SRF (93), c-Met (526, 577), and Dek (98) during satellite cell activation/proliferation and differentiation (also reviewed in Refs. 374, 565). Furthermore, recent studies have demonstrated the requirement for caspase-3 and its activation of CAD (caspase-activated DNase) in initiating myoblasts differentiation in vitro (164, 290). Activation of CAD by caspase-3 induces double-stranded DNA breaks (DSBs) in the genome and the association of CAD with various target promoters. One such promoter is the cyclin-dependent kinase (CDK) inhibitor p21(Waf1/Cip1) which following binding by CAD is activated (290). MyoD also induces the expression of p21(Waf1/Cip1) (209, 215) and subsequent permanent cell cycle arrest. p21 is known to dephosphorylate the retinoblastoma protein (Rb) and cause the inactivation of the Rb-associated E2F family of transcription factors known to activate S-phase genes (reviewed in Ref. 559). Thus, through this pathway, MyoD induces permanent cell cycle withdrawal in myoblasts. Consistently, myoblasts in p21−/− mice are defective in cell cycle arrest and myotube formation, leading to increased apoptosis (555, 588). Similarly, Rb−deficient myoblasts cannot complete cell cycle withdrawal and arrest during both S and G2 phases of the cell cycle (378).

After exiting the cell cycle, myogenic cells undergo cell-to-cell fusion to repair damaged myofibers or form nascent multinucleated myofibers. The cellular events in this complex process have been extensively studied (274, 312, 418, 428, 553). Akin to embryonic myogenesis, de novo formation of myofibers during muscle regeneration happens in two stages. In the first stage, individual differentiated myoblasts fuse to one another and generate nascent myotubes with few nuclei. In the second phase, additional myoblasts incorporate into the nascent myotubes, forming a mature myofiber with increased size and expression of contractile proteins. In recent years, studies in myoblast fusion in vitro have identified a cadre of cell surface, extracellular, and intracellular molecules, which are important for these two stages of myogenic cell fusion (reviewed in Ref. 238). For example, cell membrane proteins β1-integrin (474), VLA-4 integrin (445), integrin receptor V-CAM (445), caveolin-3 (180), and transcription factor FKHR (Forkhead in human rhabdomyosarcoma, also called FOXO1a) have been shown to act in myoblast-to-myoblast fusion. Whereas the cytokine IL-4 (238) and calcium and calmodulin activated NFATC2 (nuclear factor of activated T cell isoforms C2) pathway (237) is critical for the fusion of myoblasts with nascent myotubes.

As discussed here, activation of satellite cells following muscle injury results in the expansion of the myogenic cell pool and leads to the initiation of the myogenic program. This program, orchestrated by key transcription factors, dictates the balance between proliferation and differentiation and drives the functional transformation from individual proliferating myogenic cells to a syncytial contractile myofiber. Although numerous studies have shed light on this complex process, there are still many interesting questions that remain to be answered. For example, what are the intrinsic and extrinsic mechanisms that determine the scale of satellite cell proliferation in vivo, which essentially generate sufficient but not excessive numbers of myogenic cells for muscle regeneration? Similarly, what mechanisms regulate the magnitude of muscle regeneration in response to variable levels of damage and eventually prevent muscle atrophy or hypertrophy? With the recent advances in high-throughput sequencing and system biology, it is possible to elucidate a core regulatory network of myogenic gene expression and employ such knowledge on directing myogenic determination and differentiation from multipotent cells? The answers to these questions will improve our understanding of muscle regeneration and facilitate future development of therapeutic approaches to cure muscle diseases.

C. Satellite Cell Self-Renewal

A hallmark of stem cells is the ability to self-renew. Stem cells can divide and self-renew in two fashions: asymmetric cell division and symmetric cell division. In asymmetric cell division, one parental stem cell gives rise to two functionally different daughter cells: one daughter stem cell and another daughter cell destined for differentiation. In symmetric cell division, one parental stem cell divides into two daughter stem cells of equal stemness. In either fashion, the number of stem cells is maintained at a constant level. Stem cell self-renewal by asymmetric cell division is exemplified by neuronal stem cells (590). Stem cell self-renewal by symmetric cell division is frequently observed in hematopoietic stem cells and mammalian male germ line stem cells (spermatogonia) (570).

The self-renewing capability of satellite cells is clearly demonstrated by their remarkable ability to sustain the capacity of muscle to regenerate. For example, in a single myoblast transplantation experiment (105), 7–22 satellite cells together with their intact myofibers were transplanted into irradiated muscles of immunodeficient dystrophic (scid-mdx) mice. It was found that one grafted myoblast can give rise to over 100 new myofibers, which contain ~25,000–30,000 differentiated myonuclei. In addition, the grafted satellite cells can undergo a 10-fold expansion via self-renewal. The expanded satellite cells are functional as they can be activated and support further rounds of muscle regeneration (105). Similarly, the self-renewing capability of satellite cells was further proven by single satellite cell transplantation experiments (454). In this study, single Lin+/α7-integrin+/CD34− cells freshly isolated by FACS were transplanted into irradiated muscles of scid-mdx mice. It was
observed that progeny from these single cells not only generated myofibers, but also migrated to the satellite cell niche and persisted in host muscles (454).

An intriguing question is how satellite cells renew themselves. By using the Cre-LoxP based permanent lineage tracing technique (Myf5Cre;R26R-loxP-stop-loxP-YFP), our group first demonstrated that satellite cells can undergo both asymmetric and symmetric divisions within their natural niche environment in mildly damaged EDL muscle (280). The choice of asymmetric versus symmetric division is largely correlated to the mitotic spindle orientation relative to the longitude axis of the myofiber. Asymmetric divisions are only observed for Pax7+/Myf5− satellite cells, which give rise to one satellite stem cell (Pax7+/Myf5−) and one satellite myogenic (Pax7+/Myf5+) cell. Asymmetric division predominantly happens when the mitotic spindle is perpendicular to the myofiber axis (apical-basal division) with the satellite stem cell (Pax7+/Myf5+) in close contact with the basal lamina (basal) and the Pax7+/Myf5+ satellite myogenic cell adjacent to the myofiber plasma membrane (apical). Occasionally, it was observed that Pax7 expression is dampened in the apical satellite cell, suggesting a progression towards terminal differentiation. Furthermore, the asymmetric nature of this kind of division is also underscored by the observation that the apical Pax7+/Myf5+ cells express higher levels of the Notch ligand Delta-1, whereas the basal Pax7+/Myf5− satellite cells express higher levels of the Notch-3 receptor. Symmetric divisions were observed for both Pax7+/Myf5− satellite cells and Pax7+/Myf5+ satellite cells and in either case the mitotic spindle was frequently parallel to the myofiber axis (planar division) and both daughter cells were in contact with the myofiber plasma membrane and the basal lamina.

When both satellite stem cells and satellite myogenic cells are freshly sorted and transplanted into Pax7+/− mice lacking any functional endogenous satellite cell, both cell types can colonize the host muscle. However, only Pax7+/Myf5− satellite cells can efficiently reconstitute the satellite cell compartment (~7-fold more efficient than Pax7+/Myf5+). These observations indicate that Pax7+/Myf5− satellite cells represent a bona fide stem cell population, which can undergo self-renewal by both asymmetric division and symmetric division (280).

Although asymmetric self-renewal may be sufficient under physiological conditions, satellite stem cells may favor symmetric self-renewal divisions to replenish and expand their population in response to acute need for a large number of satellite cells after injury or disease state (354). Indeed, it was observed that Pax7+/Myf5− satellite stem cells represent ~10% of total satellite cells in intact muscles, whereas this percentage increases to ~30% at 3 wk after injury (280). In search of intrinsic and extrinsic cues regulating Pax7+/Myf5− satellite stem cell self-renewal, our group further revealed that symmetric self-renewal is promoted by a niche factor, Wnt7a, during muscle regeneration (293; discussed in sect. IIIA1A). Therefore, the mode of satellite cell self-renewal is governed by the satellite cell niche.

With regards to activated satellite cells returning to quiescence, observations on the dynamic expression of Pax7, MyoD, and Myogenin in ex vivo cultured single myofibers suggest it is possible (127, 216, 584). After myofiber isolation (the isolation procedure per se is a form of injury to initiate muscle regeneration), satellite cells are activated and rapidly start to express MyoD (572, 584). It has been observed that almost all myoblasts express MyoD after ~24 h of in vitro culture (584). While most of these proliferating Pax7+/MyoD+ myoblasts differentiate into Pax7−/MyoD+/Myogenin+ cells after 72 h of culture, some Pax7+/MyoD− myoblasts (also called reserve cells) nonetheless have been observed to repress MyoD expression and maintain Pax7 expression and eventually withdraw from the cell cycle (216, 584). These Pax7+/MyoD− satellite cells acquired the quiescence marker Nestin, albeit at a much later (3–4 wk in culture) stage (127). These observations suggest that quiescent satellite cells renew by lineage regression from committed myogenic cells at least in vitro, and this renewal may be promoted by Pax7 (584). In addition, the capability of activated satellite cells to generate reserve cells may be an important mechanism to maintain the size of the satellite cell pool, as reduction of such capability was suggested to lead to decreased numbers of satellite cells with age (128). Intriguingly, this lineage regression has also been recently observed in vivo (482). Sprouty-1, a negative regulator of receptor tyrosine kinase (RTK) signaling, plays a critical role in satellite cell renewal (482). Sprouty-1 is expressed in quiescent but not proliferating satellite cells (177, 482). Upon injury, released fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) act through the RTK/ERK pathway to stimulate satellite cell proliferation (482). Most satellite cells entered the cell cycle during regeneration as indicated by BrdU labeling. It was confirmed that some satellite cell-derived myogenic progenitors withdrew from the cell cycle, returned to the satellite cell compartment, and regained Sprouty-1 expression, indicative of satellite cell renewal in vivo. In the absence of Sprouty-1, it was observed that satellite cell-derived myogenic progenitors proliferate and differentiate normally, but markedly reduced numbers of satellite cells exist after regeneration. Further investigations revealed that Sprouty-1 is required only for a distinct subset of myogenic progenitors to return to their quiescence state (482). These results indicate that Sprouty-1 is essential for some satellite cells to regain quiescence following regeneration. The function of Sprouty-1 in this process is most likely due to its inhibitory effect on the ERK pathway and subsequent enhanced cell cycle withdrawal. It should be stressed that this renewal of satellite cells at a population level and the aforementioned self-renewal of satellite cells at a single-cell level are not mutually exclu-
sive but rather represent two different perspectives of the same regeneration process. Future studies are needed to identify the essential differences between Sprouty-1-dependent and Sprouty-1-independent populations and understand whether these differences reflect any other functional distinction. In addition, it would be interesting to investigate the regulatory mechanisms governing Sprouty-1 expression, for example, whether the reappearance of Sprouty-1 is regulated by Pax7 or Wnt signaling.

D. Contributions and Therapeutic Potential of Nonsatellite Cells in Trauma-Induced Muscle Regeneration

1. Bone marrow stem cells

The myogenic potential of bone marrow cells was demonstrated by either intravenous injection of bone marrow cells into subjects following muscle injury or directly via intra muscular injection of whole bone marrow into injured muscles. Both of these methods led to the incorporation of bone marrow-derived cells into newly formed myofibers within regenerating muscles (54, 166, 210). Because of the extremely low efficacy of incorporation, doubts persist regarding the ability of bone marrow-derived cells to reconstitute the satellite cell niche (210). Nevertheless, lineage tracing experiments demonstrated that bone marrow cells, when systematically injected into irradiated mice, were able to reconstitute the satellite cell niche and expressed satellite cell markers, such as Myf5, c-Met, and α7-integrin (287). These bone marrow-derived satellite cells can undergo myogenic differentiation in vitro and give rise to myofibers when injected into damaged muscles (287). Adult bone marrow contains hematopoietic stem cells (HSC) and stromal cells (also called mesenchymal stem cells). Due to a lack of a standardized definition, conflicting results exist regarding the exact bone marrow-derived progenitor cell type(s) that contribute to muscle regeneration. Bone marrow-resident hematopoietic stem cells, isolated by cell surface markers c-Kit+/Sca-1+ or CD45+/Sca-1+, or CD45+/c-Kit+/Sca-1+ are able to incorporate into newly forming myofibers (1, 78, 112, 581), although it was unclear whether the progeny of these cells were able to occupy the satellite cell niche. It was also demonstrated that Mac-1low bone marrow SP cells, particularly those c-Kit+/CD11b- immature cells, were able to generate myofibers (147, 382). On the other hand, mesenchymal stem cells were able to incorporate into myofibers in injured muscles (140, 488) and gave rise to Pax7+ satellite cells, which can support multiple rounds of muscle regeneration (140). Interestingly, the high myofiber turnover rate induced by muscle stress and injury seems to facilitate bone marrow cell engraftment into regenerating muscles (1, 68, 287, 460, 487). These results further emphasize the importance of the microenvironment on satellite cell specification. In accordance with this, stromal cell-derived factor-1 (SDF-1)/chemokine receptor 2 (CXCR4) signaling and chemokine receptor 2 (CCR2) have been implicated in guiding bone marrow cells towards muscle injury sites (429, 510). However, it has also been reported that SDF-1/CXCR4 signaling is dispensable for the homing of CD45+ hematopoietic lineage cells to injured muscle, which are instead responsive to HGF/c-Met signaling (447). Overall, it is noteworthy that bone marrow transplantation has not been reported to robustly or therapeutically ameliorate any muscle disease. Future studies are required to define the subpopulation(s) of bone marrow cells, which have long-term myogenic potential in vivo especially in the context of muscle diseases. Also, it would be interesting to investigate whether bone marrow-derived cells are involved in postnatal muscle growth since satellite cells with similar markers have been identified in adult muscles.

2. Muscle side population cells

Similar to bone marrow, skeletal muscle contains a unique cell population termed muscle side population (SP) cells, which can be isolated based on their ability to efflux Hoechst 33342 dye (210, 245). The majority of muscle SP cells are characterized by CD45−/c-Kit−/Sca-1-1/ABC2-g2+/Pax7-1 Myf5-1/Desmin-1 and reside in the satellite muscle interstitium juxtaposed to blood vessels, which make them distinct from satellite cells and bone marrow-derived SP cells (24, 146, 210, 439, 464). Multiple lines of evidence indicate that muscle SP cells are heterogeneous (24, 464). Initially, a minor population of CD45+ muscle SP cells was identified (24, 464, 478). These CD45+ muscle SP cells persist in Pax7+/− germline null mice and can efficiently differentiate into hematopoietic cells in culture (24, 478). In addition, CD45+ muscle SP cells also have myogenic potential when isolated from both wild-type and Pax7+/− mice. They can be induced to differentiate into myogenic cells following coculture with primary myoblasts or through the forced expression of Pax7 or MyoD (24, 477). In addition, lineage tracing experiments have revealed that only the main population of CD45− muscle SP cells arise from embryonic Pax3+ hypaxial somitic cells, suggesting the minor CD45+ SP cells might have distinct origins, possibly from endothelial, hematopoietic stem, or bone marrow cells (464). This is consistent with results from transcriptome analysis of muscle SP cells in resting and regenerating muscles, which indicate the increased expression of c-Kit and CD45 in the muscle SP population 5 days after injury (337). Furthermore, it was also demonstrated that somite-derived CD45− SP cells have more myogenic potential than CD45+ SP cells when cultured in vitro, suggesting that the developmental origin of SP cells affect their intrinsic myogenic capacity (464). Lastly, a recent study revealed that a subpopulation of satellite cells also have an SP phenotype (518). These SP cells that reside in the satellite cell compartment, termed satellite-SP cells, are characterized by CD45−/Sca-1-1/ABC2-g2−/Pax7-1/Syndecan-4+, which makes them distinct from the majority of CD45− SP cells in muscle. Following direct injection into regenerating muscles, the grafted
Muscle SP cells were found in myofibers and the satellite cell niche, which suggests that these cells can contribute to long-term muscle regeneration (24). Importantly, when introduced intravenously into mdx mice, muscle SP cells from wild-type mice incorporate into host myofibers and restore their dystrophin expression (27, 210), suggesting these cells are able to migrate to regenerating muscles through the bloodstream. Indeed, compared with satellite cells and their derivatives, muscle SP cells are much more efficient at engrafting into mdx hosts after systemic delivery (368). Interestingly, the systemic delivery of wild-type SP cells into mdx mice can also repopulate the bone marrow of an irradiated host, although at lower efficacy compared with bone marrow-derived SP cells (210).

Muscle SP cells constitute a distinct cell population based on their unique properties. However, it is still unclear whether the SP phenotype per se is of any importance to the muscle regeneration process. Although muscle SP cells outperform the main population in muscle regeneration, future studies are needed to carefully compare muscle SP cells with other defined myogenic precursors, such as non-SP satellite cells, based on their myogenic potential.

3. PW1⁺ interstitial cells

It has been reported that some interstitial cells, characterized by their expression of PW1 (also called peg3), might be involved in perinatal skeletal muscle growth (345). Intriguingly, a recent study from the same group reported that adult stem cells/progenitors residing in multiple tissues/organisms can be directly identified and isolated from PW1-reporter mice (46). PW1 is encoded by a ~8.5 kb long, intronless transcript, which is strongly transcribed in skeletal muscle in both embryonic and postnatal stages (435). Within skeletal muscle, PW1 expression was detected in both satellite cells and a group of Sca-1⁻/CD34⁺/Pax7⁻ interstitial cells (PIC) (351, 376, 476). In transgenic mice with a dominant negative form of PW1 (ΔPW1) driven by a Myogenin promoter, embryonic and fetal muscle development is normal but postnatal muscle growth is severely impaired with a profound phenotype, to some extent, reminiscent to that of Pax7⁻/⁻ germ-line mutant mice (376, 478). Compared with wild-type mice, the number of quiescent satellite cells is markedly reduced in postnatal muscle of ΔPW1 mice (376). In contrast, the number of Pax7⁻/PW1⁺ interstitial cells (PIC) is significantly increased within postnatal muscles from Pax7⁻/⁻ mice (345), which is accompanied by progressive loss of Pax7⁺ satellite cells (345, 380, 478). Interestingly, PICs have myogenic potential when cultured in vitro (345). This myogenic potential is enhanced by coculture with satellite cells and is compromised in PICs isolated from Pax7⁻/⁻ mice (345). Importantly, when transplanted into injured muscles, FACS isolated Sca-1⁻/CD34⁺/CD45⁻ PICs give rise to both interstitial cells and Pax7⁺ satellite cells, whereas Sca-1⁻/CD34⁺/CD45⁻ cells (enriched for satellite cells) only derive into Pax7⁺ satellite cells (345).

Although not directly assessed by lineage tracing, these observations together suggest a hypothesis that PICs may contribute to the satellite cell population during postnatal muscle growth and during adult muscle regeneration (345). In addition, Pax7 expression is presumably essential for the myogenic specification of PICs as PICs isolated from Pax7⁻/⁻ mice cannot give rise to myogenic cells in vitro (345). Of note, Pax3 lineage tracing experiments further indicated that PICs do not arise from embryonic Pax3⁺ myogenic progenitor cells, indicating PICs and satellite cells are derived from distinct lineages (345).

These intriguing observations also raised several issues regarding the identity and lineage progression of the Sca-1⁻/CD34⁺/CD45⁻/>50% PW1⁺ interstitial cells. A recent study indicated that FAPs isolated from adult muscle are characterized by similar markers Sca-1⁺/CD34⁺/CD45⁻ (also CD31⁻) but only give rise to adipogenic and fibrogenic lineages in vitro (252 and discussed in sect. III.B1A). Coculture with myogenic cells cannot promote the myogenic potential of FAPs (252). Accordingly, FAPs also do not assume the myogenic lineage in vivo when transplanted into either uninjured or injured muscles. These discrepant observations on PICs and FAPs lead to two possibilities: 1) FAPs are depleted of CD31⁺ cells and thus the myogenic potential resides in these CD31⁺ cells; or more likely 2) PICs and FAPs represent the same cell population but in young and adult stages, respectively. Notably, muscle SP cells (Sca-1⁻/ABCG2⁺) are also CD34⁺ and thus could be originating from the same lineage as FAPs and PICs (226). Future studies may provide direct answers in terms of the lineage origin and hierarchy of these cell types. Future investigations should also focus on the mechanisms that regulate the myogenic potential of these interstitial cells.

4. Muscle-derived stem cells

A series of studies have demonstrated that a distinct cell population, termed muscle-derived stem cells (MDSCs), can be isolated from skeletal muscles based on their weak adhesion characteristics and long-term proliferation behaviors in culture (247, 294, 421, 422). Further characterization of MDSCs indicated that these cells are CD45⁻/M-Cadherin⁻/CD34⁻/Flk-1⁻/Sca-1⁺/Desmin⁺, which makes them distinct from satellite cells and hematopoietic cells but more similar to muscle SP cells (294, 421). MDSCs and satellite cell-derived myoblasts in culture appear to represent two distinct populations as evidenced by the finding that MDSCs can be isolated from Pax7⁻/⁻ germline null mice (318). Like muscle SP cells, the myogenic differentiation of MDSCs depends on Pax7 (318). A unique characteristic of MDSCs is their multipotency (294, 421, 536). It has been reported that MDSCs can differentiate into myogenic, adipogenic, osteogenic, chondrogenic, and hematopoietic lineages (reviewed in Ref. 404). After intra-arterial transplantation, MDSCs contribute to regenerated myofibers, incorporate into the satellite cell niche, and also give...
rise to endothelial and neural cells (421). Interestingly, like muscle SP cells, MDSCs can also repopulate the hematopoietic lineage in irradiated murine hosts, and the reconstituted bone marrow can in turn contribute to muscle regeneration (82). In addition, MDSCs are more efficient than myoblasts at forming dystrophin\(^-\) myofibers when directly transplanted into mdx mice (243). Due to the prolonged isolation procedure of MDSCs and the accompanied dynamics of gene expression, it is difficult to trace the origin of MDSCs in vivo based on cell surface markers. Future investigation by candidate lineage tracing and clonal analysis may help to elucidate the lineage relationship between MDSC and other myogenic cells.

5. Mesoangioblasts

Mesoangioblasts arise from the embryonic dorsal aorta and are characterized by CD34\(^+\)/c-Kit\(^+\)/Flk-1\(^-\)/NKX2.5\(^-\)/Myf5\(^-\)/Oct4\(^-\) expression (129, 343). Mesoangioblasts are highly proliferative when cultured in vitro, and long-term cultured mesoangioblasts are multipotent, being able to give rise to multiple mesodermal lineages, such as bone, cartilage, smooth, cardiac, and skeletal muscle following transplantation (343). Although the contribution of mesoangioblasts to normal muscle development is controversial, multiple studies have demonstrated that mesoangioblasts can be employed to facilitate muscle regeneration, particularly for dystrophic muscles. First, it was found that \(\alpha\)-sarcoglycan\(^-\) mesoangioblasts can be transduced in vitro with \(\alpha\)-sarcoglycan expressing lentiviral vector and robustly generate \(\alpha\)-sarcoglycan\(^+\) myofibers following transplantation into \(\alpha\)-sarcoglycan\(^-\) mice (a model for limb girdle muscular dystrophy) via intra-artery injection (458). The high efficiency of normal myofiber reconstitution observed in this study suggests that intrinsic properties of mesoangioblasts may help them travel to regenerating muscles via the bloodstream. In addition, mesoangioblasts can release immunosuppressive and tolerogenic molecules, which supposedly help mesoangioblasts incorporate into allogeneic dystrophic hosts (211). The myogenic potential of mesoangioblasts isolated from human patient biopsies suffering from dermatomyositis (DM), polymyositis (PM) and inclusion-body myositis (IBM) were compared (352). It was observed that mesoangioblasts from the IBM patients could not differentiate into myofibers when cultured in vitro or xenotransplanted into injured mouse muscles. Interestingly, this defect can be rescued by either transient overexpressing of MyoD or knockdown of a MyoD inhibitor protein, bHLH-B3, which suggests that the myogenic program of mesoangioblasts is MyoD-dependent (352). Several investigations demonstrated that factors involved in normal muscle regeneration also facilitate mesoangioblast-based muscle regeneration. For example, HMGBl, SDF-1, CXCR4, and \(\alpha\)4-integrin have been shown to enhance the migration of mesoangioblasts from blood vessels toward injured muscles (182, 399). Similarly, pretreatment of mesoangioblasts with nitric oxide before transplantation also stimulated \(\alpha\)-sarcoglycan expression in mesoangioblast-derived myofibers (475). Taken together, investigations on mesoangioblasts have revealed great therapeutic potential for the amelioration of human dystrophic diseases. Future studies may focus on identifying the adult counterparts of these cells and characterizing their myogenic potentials.

6. Pericytes

Pericytes (also called Rouget cells or Mural cells) are contractile connective tissue cells residing beneath the microvascular basement membrane. Pericytes originate from the embryonic sclerotome and are believed to regulate the blood flow in capillaries (415, 284, 406). As a multipotent stem cell population, pericytes can differentiate into adipocytes (161), chondrocytes (161), and osteoblasts (142) in vitro. Two recent studies indicated that pericytes, which do not express Pax7, Myf5, or MyoD, can differentiate into skeletal muscles both in vitro and in vivo (135, 136), suggesting pericyte-mediated myogenesis may follow a myogenic differentiation program distinct from that of satellite cells. FACS sorted human pericytes display the following cell surface marker combination: CD45\(^-\)/CD34\(^-\)/CD105\(^-\)/CD146\(^-\)/PDGFR-B\(^1\)/NG2 proteoglycan\(^-\), which makes them distinct from embryonic mesoangioblasts, hematopoietic cells, or satellite cells (136). Intriguingly, cells carrying the same surface marks can be isolated from various tissues, including pancreas, adipose, and placenta and differentiate into skeletal muscle cells when cultured in vitro, irrespective of their origins (118). The fact that pericytes also display cell surface markers characteristic of mesenchymal stem cells (CD10\(^+\)/CD13\(^+\)/CD44\(^+\)/CD73\(^+\)/CD90\(^+\)) raised the hypothesis that pericytes may represent a significant portion of mesenchymal stem cells in the adult (85, 118, 163). Xenographic transplantation of human pericytes into combined immune deficient-X-linked, mouse muscular dystrophy (scid-mdx) mice through the femoral artery gave rise to numerous myofibers expressing human dystrophin (136). Interestingly, a small portion (3–5%) of transplanted pericytes incorporated beneath the basal lamina of myofibers, indicating that pericytes are able to occupy the satellite cell niche in dystrophic muscle. Importantly, pericyte transplantation significantly improved the physiological performance of treated dystrophic muscles, indicating that pericyte-derived myofibers are functional. Similar promising results were achieved in a prototype experiment, wherein pericytes isolated from Duchenne patients were transduced in vitro with a human mini-dystrophin expressing lentiviral vector and transplanted into scid-mdx mice (136). The myogenic potential, together with their abilities to be cultured in vitro and penetrate the blood vessel wall, makes pericytes a promising candidate for future cell-based therapies to treat muscular dystrophy. Future research may investigate whether transplanted pericytes possess sufficient long-term myogenic capability in dystrophic muscles.
7. CD133+ cells

Recent studies demonstrated that a fraction of CD133+ (also called AC133) mononucleated cells in adult peripheral blood have myogenic potential (535). Freshly isolated human CD133+ cells can undergo myogenic differentiation when cocultured with myogenic cells or exposed to Wnt-producing cells in vitro (535). Importantly, when xenotransplanted via intra-arterial or intramuscular injection, these CD133+ cells can fuse with regenerating muscles in scid-mdx mice and improve the force generation of treated muscles. Moreover, donor human CD133+ cells, expressing satellite cell markers M-Cadherin and Myf5, were detected in the satellite cell compartment after transplantation, indicating they can reconstitute the satellite cell niche (535). A small subpopulation of CD133+/CD34+ double positive cells can be identified in the blood and skeletal muscle interstitium (233). Similar to blood-derived CD133+ cells, human muscle-derived CD133+ cells manifested remarkable myogenic differentiation capacity in regenerating muscle (372). As CD133+ cells can be readily isolated from the blood, manipulated in vitro and delivered through the circulation, a couple of studies have explored their application to treating Duchenne muscular dystrophy (DMD). In one of these studies, CD133+ cells isolated from human dystrophic muscles were genetically engineered ex vivo to restore dystrophin expression and subsequently delivered into scid-mdx mice (41). Treated dystrophic muscles were improved in terms of muscle morphology, function, and dystrophin expression, although the long-term effect of this treatment has not been documented so far. The safety of this CD133+ cell therapy (without dystrophin correction) was later confirmed in human DMD patients by autologous transplantation (534). Taken together, CD133+ cells are one of the most promising candidates for cell-based therapy of DMD. Future studies should focus on whether these cells can survive long term and support repeated bouts of regeneration in DMD patients.

8. Embryonic stem cells and induced pluripotent stem cells

Embryonic stem (ES) cells are pluripotent stem cells, which can differentiate into all three germ layers and hence have been extensively studied for cell-based therapies. In an early study, mouse ES cells cocultured with muscle cells were transplanted into irradiated mdx mice by intramuscular injection (47). Donor-derived myofibers were occasionally found on the surface of the host muscles. The low efficacy of engraftment in this study suggested that selective markers might be necessary to enrich myogenic precursors from ES cell cultures. In a later study, a subpopulation of CD73+/N-CAM+ human ES cells, which are enriched for mesenchymal precursors, were injected into regenerating muscle of scid mice (31). It was observed that human donor cells gave rise to myoblasts and established prolonged engraftment; however, the overall efficiency was not compared with other myogenic cells, such as satellite cells. Recently, several strategies have been established to achieve persistent engraftment and avoid teratoma formation. In one study, Pax3 was transiently expressed to induce paraxial mesoderm formation during embryoid body differentiation, and PDGFR-α, a paraxial mesoderm marker, was subsequently used to isolate a homogeneous population of proliferating myogenic progenitors (124). The transplantation of this population into mdx mice resulted in improved muscle function, and teratoma formation was prevented. In another study, PDGFR-α+ cells were directly isolated from ES cells in an earlier stage without Pax3 induction (455). It was found that these early paraxial mesodermal cells, when transplanted into injured muscles, could give rise to bona fide satellite cells that can further differentiate into functional myofibers. Similarly, transient expression of Pax7 seems to facilitate the myogenic specification of ES cells (125). Recently, it was shown that SM/C-2.6+ cells isolated from cultured embryoid bodies can differentiate into skeletal muscle myofibers both in vitro and in vivo (88). When transplanted into injured muscles, these cells can incorporate into the satellite cell compartment and express satellite cell markers, such as Pax7 and M-cadherin. Importantly, these ES-derived satellite cells undergo extensive self-renewal during subsequent muscle injury and can be further transplanted with high engraftment efficiency (88).

Over the last five years, various types of differentiated somatic cells have been successfully induced into pluripotent stem cells (iPS cells) via a process involving transcription factor based reprogramming (371, 384, 515, 516, 582). Given their adult somatic origin and pluripotent nature, iPS cells hold tremendous potential for cell-based therapy. In an attempt to apply iPS cells to muscle regeneration, a recent study demonstrated that SM/C-2.6+ cells isolated from mouse iPS cells have comparable regenerative potential to those from mES cells (346). In addition, conditional expression of Pax7 in human iPS cells was successful in inducing large quantities of myogenic progenitors, which were able to efficiently engraft and produce abundant human-derived dystrophin+ myofibers in dystrophic mouse muscle (123). Notably, a new strategy of iPS cell-based therapy to treat DMD was reported recently (267). In this study, iPS cells were derived from mdx mice or a human DMD patient, and the genetic deficiency of dystrophin in these cells was corrected by transferring a human artificial chromosome (HAC) containing a complete genomic dystrophin sequence via microcell-mediated chromosome transfer (MMCT). These genetically corrected iPS cells can differentiate into muscle-like tissues and can be used to generate chimeric mice, wherein tissue-specific expression of dystrophin was observed (267). These inspiring results warrant future investigations on regulatory mechanisms governing the myogenic specification of ES and iPS cells.
E. Satellite Cells in Perinatal/Juvenile Muscle Growth

In neonatal mouse muscle, satellite cells account for ~30–35% of the sublaminal nuclei on myofibers (9, 227, 471). This proportion of satellite cells decreases while the number of myonuclei increases over postnatal growth (150). Experiments by \(^{3}H\)thymidine labeling indicate that satellite cells are proliferative in growing muscle, give rise to myonuclei, and continue to fuse with myofibers (355, 470, 481). The cell cycle time of juvenile satellite cells in growing rat muscles was determined to be ~32 h, with 14 h within the S phase (471). This high proliferation rate of juvenile satellite cells is likely due to a large requirement for muscle growth at this stage. Like their adult counterparts, juvenile satellite cells are also likely heterogeneous. By continuous BrdU labeling and tandem BrdU/\(^{3}H\)thymidine labeling, two subpopulations of satellite cells in growing muscle have been identified (471). A fast-cycling satellite cell population, accounting for ~80% of the cells, was readily labeled over the first 5 days of continuous BrdU infusion. In contrast, slow-dividing satellite cells incorporated BrdU at a much slower rate, and some of them were not labeled with BrdU even after an additional 9 days. Moreover, only a small portion of the satellite cells labeled with BrdU during the first 5 days could be labeled with \(^{3}H\)thymidine during a second 5-day continuous infusion. This observation suggests that 1) the majority of fast-cycling satellite cells only undergo a limited number of mitotic divisions prior to fusion, and 2) the number of fast-cycling satellite cells in growing muscle are maintained by the slow-cycling satellite cells through asymmetric divisions (471). Interestingly, the functional difference and lineage relationship between these two satellite cell populations during postnatal muscle growth largely resemble that of satellite cells and myoblasts during adult muscle regeneration. This analogy may reflect a common scheme of myogenesis in both postnatal muscle growth and adult muscle regeneration.

Despite their similarities, studies have revealed distinct genetic requirements for both juvenile and adult satellite cell populations. Pax7 is required for the maintenance of a functional myogenic cell population in the perinatal/juvenile stage, as muscle growth and regeneration are severely impaired in Pax7 germline null mutants during this period (279, 393, 478). In contrast, Pax7 seems to be dispensable for adult muscle regeneration in Pax7 conditional knockout mutants, wherein Pax7 expression is ablated only in the adult stage (299). In conditional Pax7 knockout mice, satellite cells, expressing M-cadherin but not Pax7, were observed in the satellite cell compartment in adult muscle after one round of regeneration, and these cells can support a second round of regeneration (299). The regenerative capacity of Pax7− satellite cells was not due to a compensatory mechanism by Pax3, as muscle regeneration is not impaired by simultaneous deletion of both Pax7 and Pax3 in adulthood. It is well known that both juvenile and adult satellite cells express Pax7 (478). The apparent distinct requirement for Pax7 in juvenile but not adult muscle regeneration may suggest that Pax7 is only necessary for the initial establishment of perinatal satellite cells and/or de novo myogenic specification of other nonsatellite cell lineages, which presumably contribute to the total satellite cell pool during postnatal growth. In contrast, the maintenance of satellite cell establishment might be Pax7 independent and the rare occasion of de novo myogenic specification from other nonsatellite cell lineages may be negligible in adulthood. In this sense, the sustained expression of Pax7 in adult satellite cells may be due to sustained interactions with their niche, rather than a necessity for Pax7 function. In support of this hypothesis, genetically marked hematopoietic stem cells (bone marrow derived CD45+/c-Kit+/Sca-1+/Lin− cells) have been shown to express Pax7 once they are present in the satellite cell niche (148, 581).

III. ANATOMIC AND FUNCTIONAL DIMENSIONS OF THE SATELLITE CELL NICHE

Based on the stem cell niche concept, behaviors of tissuespecific stem cells are determined by structural and biochemical cues emanating from the surrounding microenvironment (380). In recent years, adult stem cells and their corresponding niches were identified in numerous tissues including bone marrow (566), brain (322), testis (240), liver (528), intestine (55), heart (302), white fat (441, 519), and skin (174). Similar to these adult stem cells, satellite cells are also present in a highly specified niche, which consists of the extracellular matrix (ECM), vascular and neural networks, different types of surrounding cells, and various diffusible molecules (FIGURE 3). Furthermore, satellite cells, as one of the niche components, also influence each other by means of cell-cell interaction and autocrine or paracrine signals. The dynamic interactions between satellite cells and their niche specifically regulate satellite cell quiescence, self-renewal, proliferation, and differentiation. In this fashion, muscle atrophy or excessive growth is prevented, and the satellite cell pool is maintained during regeneration. Understanding the interactions between satellite cells and their niche is of paramount importance for the development of therapies to treat both age-related skeletal muscle atrophy (sarcopenia) and skeletal muscle diseases. In the following section, we will define the scope of the satellite cell niche and discuss how these niche factors affect satellite cells during muscle regeneration.

A. The Immediate Niche

1. Satellite cell: regulatory signaling pathways

A) WNT SIGNALING. Wnt signaling controls diverse biological processes, such as cell proliferation, cell fate determination,
Signaling within the Immediate Niche

- Satellite cell
- Fibronectin
- bFGF
- Wnt7a
- Basal lamina
- Sarcolemma
- Decorin
- Proteoglycans
- SDF-1
- Laminin
- Entactin
- Collagen
- HGF
- IGF
- Glycoproteins
- Perlecan

Signaling within the Local Milieu

- Fibroblast
- Pericytes
- Motor neurons (axons)
- Perimysium
- Connective tissue
- Capillaries
- Adipocytes

Signaling within the Systemic Milieu

- Immune cells
  - Monocytes
  - Macrophages
  - Neutrophils
- Androgens
- IL-6
  - Myofibers
  - Neutrophils
  - Satellite cells
- Nitric Oxide
  - Epithelial cells
  - Endothelial cells
  - Fibroblasts
  - Macrophages
  - Myofibers
cell adhesion, cell polarity, and morphology. Wnt signaling is activated by binding of extracellular Wnt family glycoproteins with Frizzled receptors and low-density lipoprotein receptor-related protein (LRP5, LRP6) pairs. The Frizzled receptor can initiate two distinct signaling pathways: the Wnt/β-catenin and Wnt/PCP pathways. Wnt/β-catenin pathway is characterized by the regulation of β-catenin stabilization and its entry into the nucleus. The Wnt/PCP pathway is involved in planar cell polarization (PCP) and establishment of polarized cellular structures (183).

In the Wnt/β-catenin pathway, the level and subcellular localization of β-catenin determine the ultimate output. Normal levels of β-catenin dimerize and associate with both transmembrane cadherins and cytoskeleton-bound α-catenin, which promotes cell adhesion and controls cell shape. The normal levels of cytoplasmic β-catenin are maintained by constitutive degradation of excess β-catenin by GSK3-β. In the cytoplasm, GSK3-β and β-catenin complex with Axin and adenomatous polyposis coli (APC), leading to β-catenin phosphorylation by GSK3-β. Phosphorylated β-catenin is bound by the β-TrCP component of the ubiquitin ligase complex and degraded by the proteosome. GSK3-β is constitutively active but can be inhibited by Dshveeled (Dvl), which is activated by Wnts binding to a Frizzled and LRP receptor pair. Thus, when Wnt signaling is active, elevated levels of cytoplasmic β-catenin enter the nucleus and interact with Tcf/Lef transcription factors to regulate gene expression (373).

Accumulating evidence indicates that Wnt/β-catenin signaling is involved in satellite cell function during muscle regeneration, although its defined roles remain controversial. First, Brack et al. (65) suggested that Wnt signaling promotes myogenic commitment and terminal differentiation in adult myogenesis. By examining the effects of exogenous Wnt3a and sFRP3 as activator and inhibitor of Wnt signaling, respectively, it was found that regenerating muscles in adult myogenesis. By examining the effects of exogenous Wnt3a and sFRP3 as activator and inhibitor of Wnt signaling, respectively, it was found that regenerating muscles in vivo and myoblasts derived from cultured single myofibers are responsive to Wnt signaling only at a late stage of differentiation. Activation of Wnt/β-catenin signaling increased both Desmin expression in myogenic cells in vitro and the size of newly formed myofibers in vivo, whereas inhibition of Wnt signaling caused the opposite effects. Interestingly, Notch signaling seems to antagonize the effects of Wnt signaling at early stages of myoblast proliferation. This counteracting effect is reflected by the levels of the active form of GSK3-β, which are high in the early Notch activating stage and low in later Wnt activating stage. It was proposed that low activity of Wnt signaling in early proliferation allows the expansion of enough myoblasts by Notch signaling for later differentiation (65). Notably, although this study reported that the mRNA levels of Wnt ligands and Frizzled receptors within isolated myoblasts increase in late culture, it is still possible that Wnt ligands may originate from other sources in vivo, such as the ECM, and may function through preexisting Frizzled receptors on satellite cells.

Perez-Ruiz et al. (407) proposed that β-catenin promotes self-renewal of satellite cells and prevents them from immediate myogenic differentiation. By retrovirus-based overexpression and RNAi knockdown of proliferating Pax7+/MyoD+ satellite cells on single myofibers, it was found that constitutive expression of stabilized β-catenin, or inhibition of degradation by GSK3β leads to downregulation of MyoD and Myogenin expression. In contrast, when β-catenin was downregulated by RNAi or when its target genes were repressed by the dominant-negative β-catenin-ERD, fewer Pax7+/MyoD− myoblasts were observed. As β-catenin levels determined the observed cellular changes, it was proposed that the Wnt/β-catenin pathway is involved (407). It should be stressed that although MyoD expression can be regulated by directly changing β-catenin levels, the physiological relevance of this phenomenon still largely depends on the availability of Wnt ligands and expression of Frizzled receptors on satellite cells. In addition, a recent study revealed that β-catenin directly interacts with MyoD to enhance MyoD binding to E-box elements to initiate the myogenic program (271). However, in vivo studies are required to further confirm and elaborate on the mechanism whereby β-catenin interacts with MyoD.

Otto et al. (391) proposed that Wnt ligands are important regulators of proliferation for satellite cells during adult muscle regeneration. mRNAs for Wnt ligands (Wnt1, Wnt3a, Wnt5a, Wnt11) were expressed in single myofibers by retrovirus-based overexpression and RNAi knockdown. When exogenous Wnt3a and sFRP3 were expressed in single myofibers (together with satellite cells) upon 2 days of culture in vitro. These data along with reporter assays indicated that Wnt signaling is active at this point. It was found that exogenous

FIGURE 3. The satellite cell niche. A: satellite cells reside between the basal lamina and the sarcolemma of adult skeletal myofibers and as such are influenced by structural and biochemical cues emanating from this microenvironment. A complex set of diffusible molecules (e.g., Wnt, IGF, and FGF) are exchanged between the satellite cell and the myofiber to maintain quiescence or promote activation. In addition, numerous extracellular matrix components and cellular receptors are present either on the surface of the sarcolemma, satellite cell, or contained within the basal lamina. These components comprise the immediate niche of the satellite cell and dictate rapid changes in the satellite cell state. B: the muscle fascicle defines the extremities of the local milieu an environment that is more diverse compared with the immediate niche due to the heterogeneity of cell types and signaling factors. The local milieu surrounding the satellite cell is made up of other myofibers in addition to interstitial cells, capillaries, and neuromuscular junctions. Each of these cell types influences the surrounding environment and thereby affects the state of the satellite cell. C: the systemic milieu contains the greatest diversity with which the satellite cell is influenced. Exposure of satellite cells to the host’s immune system, and circulating hormones along with the skeleton and surrounding skeletal muscles present the broadest environment for the satellite cell. Changes that occur in the systemic milieu affect the satellite cell gradually. Prolonged exposure of signals from the systemic milieu plays an important role in the ability of satellite cells to participate in multiple rounds of regeneration.
Wnt1, Wnt3a, and Wnt5a induced satellite cell proliferation, whereas exogenous Wnt4 and Wnt6, whose expression is absent from regenerating muscle, inhibited the proliferation. This is consistent with the inhibitory effect of ECGC, a potent Wnt signaling inhibitor, on satellite cell proliferation (391). Importantly, the expression and subcellular localization of the active form of β-catenin (Act-β-Cat) was carefully examined by immunofluorescence staining in this study. Nuclear Act-β-Cat was observed only after MyoD expression and was absent in Myogenin cells, which suggests Wnt signaling is inactive either before satellite cell activation or after differentiation determination. This temporal progression of Wnt signaling was further confirmed in mouse regenerating muscles and human dystrophic muscle biopsies. Thus Otto et al. (391) concluded that activating Wnt ligands function to promote satellite cell proliferation during muscle regeneration, whereas inhibitory Wnt ligands antagonize the proliferation by secluding β-catenin to the cell membrane in quiescent satellite cells. Perplexingly, the pro-proliferation function of Wnt3a signaling revealed by this study seems to oppose the pro-differentiation function reported by Brack et al. (65). This might be due to different sources of Wnt ligands, either from cultured Wnt-expressing cells or from recombinant protein, which in turn affect Wnt concentration and effective time.

In addition to β-catenin-dependent gene activation, recent studies indicate that the binding of Wnt ligands with Frizzled/LRP receptors can also lead to a transduction cascade to establish PCP, which is referred to as the Wnt/PCP pathway (reviewed in Ref. 492). In this signaling pathway, signaling from Wnt ligand/receptor binding activates Rho/Rac small GTPase and Jun NH2-terminal kinase (JNK) via Dishevelled. Dishevelled activates Rac, which further activates JNK to assist in the subsequent regulation of cytoskeleton organization and gene expression. The Wnt/PCP signaling pathway plays an important role in the development of anterior-posterior extension of the neural tube and the establishment of the aligned cellular structure in epithelium.

Emerging evidence reveals that satellite cells are under the regulation of Wnt/PCP signaling pathway. Our group demonstrated that quiescent satellite cells can be separated into two functional subpopulations based on their Myf5 expression: Pax7+/Myf5− satellite stem cells and Pax7+/Myf5+ satellite progenitor cells (280). Of interest, we determined that mRNAs of the Frizzled7 receptor are preferentially present in Pax7+/Myf5− quiescent satellite stem cells (293). However, upon injury, Frizzled7 is ubiquitously expressed in all satellite cells and their proliferating progeny. In a frozen injury model, Wnt1, Wnt2, Wnt5b, Wnt8b, Wnt10a, and Wnt16a, Frizzled receptors, and sFRP inhibitors were increased in muscles 3 days postinjury (acute phase of regeneration). In contrast, an increase of Wnt7a and Wnt10a was detected in muscles 6 days postinjury, when satellite cells returned to the sublaminar position. It was determined that Wnt7a acts through the Frizzled7 receptor to specifically activate the Wnt/PCP pathway and induce cell polarity both ex vivo and in vitro. Activation of the Wnt/PCP pathway by recombinant Wnt7a simulated the symmetrical division of satellite cells and hence expanded the satellite stem cell population on single myofiber explants. Knockdown of Wnt/PCP downstream component, Vangl2, by RNAi caused the opposite effects, confirming the involvement of Wnt/PCP pathway in this process. In vivo, Wnt7a injection into regenerating muscles remarkably improved muscle regeneration as evidenced by increased muscle mass and myofiber size. We postulated that the activation of Wnt/PCP pathway promotes the planar alignment of two daughter cells along the myofiber during satellite cell division. This alignment and the induced cell polarity may facilitate the attachment of both daughter cells to the basal lamina and hence maintain their stem cell fate (293). Although it is still unknown, one possible source of endogenous Wnt7a is the regenerative myofiber, given its close proximity to satellite cells and perfect alignment to the basal lamina (65, 413). Intriguingly, a recent study revealed synergetic functions of Wnt/β-catenin and Wnt/PCP pathways in oriented elongation of myocytes during embryonic muscle patterning (205). Thus it is enticing to further speculate that the activated Wnt/β-catenin pathway in regenerating myofibers may induce Wnt7a secretion and activation of the Wnt/PCP pathway in adjacent satellite stem cells, which in turn would direct their symmetric division and replenish the satellite cell pool.

Wnt signaling is also implicated in satellite cell-related transdifferentiation. Our group reported that muscle residing CD45+/Sca1+ cells increased in number and underwent myogenic differentiation in injured muscles but not in intact muscles (413). CD45+/Sca1+ cells express Frizzled receptors and upregulated β-catenin during regeneration, suggesting the Wnt/β-catenin pathway is required for the myogenic commitment of these cells. Indeed, the myogenic commitment of CD45+/Sca1+ cells can be induced by lithium treatment, or coculture with myoblasts or Wnt secreting cells. Daily injections of Wnt signaling inhibitors, sFRPs, severely reduced the myogenic recruitment of CD45+/Sca1+ cells during regeneration. These data further suggest that Wnt signaling is required for muscle regeneration and may increase the myogenic potential of CD45+/Sca1+ cells (413). Similarly, it was reported that β-catenin is required and sufficient for myogenic commitment of P19 embryonic carcinoma cells (408). Of note, a recent study showed that Wnt signaling is important for transdifferentiation of satellite cells into fibrogenic cells (66). Activated satellite cells from old mice have higher Wnt signaling activity and a greater tendency to assume fibrogenic fate in vitro, compared with those from young mice. Exposure of activated satellite cells from old mice to young serum or pairing with young mice in a heterochronic parabiotic system reversed
the Wnt signaling activity and associated fibrogenic tendency, suggesting they are not intrinsic. Conversely, activated satellite cells from young mice exposed to aged serum or linked parabiotically activated Wnt signaling and became fibrogenic. These observations indicated that the fibrogenic transdifferentiation of satellite cells is influenced by an unknown circulating factor (66). As the effect of this factor can be neutralized by a recombinant secreted form of Frizzled, it was believed that an increase in Wnt ligands in old mice may account for the fibrogenic transformation observed (66). Notably, an alternative possibility is that certain Wnt inhibitors are decreased in old mice. This possibility is supported by a recent finding that a Wnt binding protein, Klotho, is a circulating hormone and can affect tissue aging (281, 313). Wnt signaling also controls the balance between myogenic and adipogenic potentials of myoblasts in vitro. First, downregulation of Wnt/β-catenin signaling mediated by Wnt10b was implicated in the increase of adipogenic differentiation of aged myoblasts (527). Similarly, it was observed that in Wnt10b−/− mice, the adipogenic potential of myoblasts increased and excessive lipid accumulated in regenerating myofibers (549). A recent mechanistic study indicated that Wnt10b activated the Wnt/β-catenin pathway and that SREBP-1c-mediated lipogenesis and insulin resistance reciprocally counteract each other and thus determine the myogenic and adipogenic fate of myoblasts (2). The above-mentioned experiments serve to demonstrate the complexities involved in Wnt regulation of myogenesis. Future studies focused on in vivo regulation of a myogenic to adipogenic switch will elaborate on the physiological relevance of the above experimental evidence.

b) NOTCH SIGNALING. Notch signaling regulates cell proliferation, differentiation, and cell fate determination (21). The activation of Notch signaling pathway is initiated by the binding of Delta and Jagged family of ligands to Notch transmembrane receptors, the result of which induces sequential enzymatic cleavage of the Notch receptor and release of an active truncated form of Notch, termed the Notch intracellular domain (NICD). Upon enzymatic cleavage, the NICD translocates from the cytoplasm to the nucleus and activates the CSL (CBF1, Suppressor of Hairless and Lag-1) family of transcription factors, leading to target gene expression.

Notch signaling plays key roles in skeletal muscle regeneration (reviewed in Ref. 320). Upon muscle injury, the Notch ligand Delta is quickly upregulated in both activated satellite cells and in myofibers (108). The upregulation of Delta in satellite cells is accompanied by the appearance of the NICD, indicative of activated Notch signaling. Activation of Notch signaling stimulates the proliferation of satellite cells and their progeny and thus leads to the expansion of proliferating myoblasts. Indeed, inhibition of Notch signaling abolishes satellite cell activation and impairs muscle regeneration (108). Reversely, constitutive Notch activation in satellite cells from Pax7-CreER;ROSA^Notch resulted in increased Pax7^+ satellite cells and impaired muscle regeneration (560). In aged muscles, the expression of Notch in satellite cells remains unchanged (107). However, the induction of Delta in myofibers is no longer responsive to muscle injury, which results in reduced satellite cell proliferation and impaired muscle regeneration (107).

At the onset of myogenic differentiation, however, the inactivation of Notch signaling in myoblasts is required for their fusion (108). Two mechanisms exist to downregulate Notch signaling. The first involves Numb, a Notch signaling inhibitor, which is asymmetrically partitioned during satellite cell/myoblast division (108, 489). The daughter cell inherits Numb and activates the expression of Desmin leading to differentiation (108). Numb inhibits Notch signaling by inducing ubiquitination of the NICD, which abolishes its activity (21). Second, the activation of Wnt signaling in differentiating myoblasts antagonizes Notch signaling and facilitates terminal differentiation (65). This is supported by observations that activation of Notch signaling is associated with phosphorylation of tyrosine 216 of GSK-3b, whereas Notch inhibitor treatment or activation of Wnt signaling in myoblasts coincided with dephosphorylation of this residue (65). Although the precise mechanism remains ambiguous, the crosstalk between Wnt signaling and Notch signaling at Dishevelled and Presenilin has been reported (131). In addition, β-catenin has been reported to associate with MyoD and facilitate the transcription activity of MyoD (271). Thus it would be interesting to investigate whether MyoD/β-catenin can drive the expression of Notch signaling inhibitors, such as Numb, Itch, or the newly reported bHLH transcription factor Stra13 (511).

As both Notch receptors and their ligands are transmembrane proteins, Notch signaling is an important signaling pathway mediating cell-cell communications. Our group recently demonstrated that Megf10, a multiple EGF repeat-containing membrane protein, may function similar to Delta and Jagged in Notch signaling activation (235). Notch-mediated cell-cell communications, presumably conveyed between “bumping” satellite cells or between satellite cells and the myofiber, may serve as a controlling mechanism for satellite cell quiescence and the number of satellite cells on a single myofiber under physiological conditions. In line with this view, double knockout mice of Hey1 and Hey2, two downstream target genes of Notch signaling, showed remarkably reduced number of satellite cells, which is likely due to failure of satellite cells in keeping quiescence in adult muscle (176).

Three Notch receptors are abundantly expressed on satellite cells: Notch1, Notch2, and Notch3. A recent study suggests that Notch3 may act as a Notch1 repressor by activating Nrarlp, a negative-feedback regulator of Notch signaling.
In line with this view, Notch3-deficient mice showed markedly increased numbers of sublaminar quiescent satellite cells and muscle hyperplasia after repetitive muscle injuries, which are phenotypically opposite to those from Notch1 mutants.

In addition to its functions during muscle regeneration, Notch signaling is also involved in the maintenance of cell quiescence in resting health muscle. It was found that genetic abrogation of Rbpj, the main effector for canonical Notch signaling, specifically in satellite cells results in the loss of their quiescent state (356). In Pax7-CT2;Rbpj<sup>fl<sup>ox</sup></sup>-mice, Tamoxifen induction and Rbpj depletion in satellite cells gradually decreased the number of Pax7<sup>+</sup> satellite cells, which is accompanied with spontaneous myogenic differentiation and failure of muscle regeneration upon injury. Interestingly, the spontaneous myogenic differentiation of the majority of mutant satellite cells in this model seems to start with G<sub>0</sub> (quiescent) satellite cells without entry into S phase in vivo.

The above findings support the notion that Notch signaling is critical for satellite cell activation and proliferation. As Notch signaling can be activated by cell-to-cell contact, it is very intriguing to envision that communal satellite cells existing on the same myofiber can signal each other. This community niche effect may serve to determine the number of quiescent satellite cells on a myofiber and/or control the size of the satellite cell pool during regeneration. In this respect, recent studies have revealed a close relationship between Notch signaling and Hippo (Salvador/Warts/Hippo) signaling pathways in determining cell proliferation and organ size in <em>Drosophila</em> (77, 339, 412). It would be interesting to investigate the interactions of these two pathways in satellite cell activation and proliferation.

C) SPHINGOLIPID SIGNALING. Sphingolipids are a large group of naturally occurring glycolipids, characterized by their sphingoid backbone. Although originally recognized as inert precursors and intermediate products in lipid metabolism, sphingolipids, particularly ceramide, ceramide-1-phosphate, sphingosine, and sphingosine-1-phosphate (S1P), have been recently revealed as important bioactive signaling molecules in regulating cell proliferation, migration, death, and senescence (reviewed in Ref. 223). Sphingomyelin is the most abundant component in the sphingolipid pathway and also serves as a precursor of ceramide, sphingosine, and S1P. It was found that sphingomyelin is enriched in plasma membrane of quiescent satellite cells, yet it markedly diminishes after satellite cell activation and reappears in some Pax7<sup>+</sup>/MyoD<sup>−</sup> satellite cells returning to the quiescent state (369). The dynamics of sphingomyelin are connected with the biosynthesis of mitogenic S1P, which promotes satellite cells to enter the cell cycle on ex vivo cultured myofibers and improves muscle regeneration (370, 461). On the other hand, pharmacological inhibition of sphingomyelin-to-S1P processing reduced the number of activated satellite cells and perturbed muscle regeneration. This action of S1P on satellite cell activation and muscle regeneration is in line with the proliferative, proinflammatory (through COX-2) and antiapoptotic (through inhibition of caspase-3 and BAX) functions of S1P-mediated pathways in general. Accumulating evidence indicates that S1P, being one of the more soluble sphingolipids, acts through S1P receptors (S1PRs; a group of high-affinity G protein-coupled receptors) on satellite cells in an autocrine/paracrine fashion (76, 122). In addition, abnormal modulation of S1P activity is also involved in the pathology of muscular dystrophy (as exemplified in <em>mdx</em> mice) (317).

Our current understanding of the functions of bioactive sphingolipids on satellite cells is far from complete. For example, the role of ceramide, another central player of the sphingolipid signaling pathway, on satellite cell activation/proliferation/differentiation during muscle regeneration is vastly unknown. Moreover, whether S1P may bind to other intracellular proteins and exert S1PR-independent actions remains to be investigated.

2. The myofiber niche

The myofibers are the primary component of the satellite cell niche due to their direct contact with satellite cells. The overarching effect provided by the myofiber for satellite cells was revealed through selective killing of myofibers with Marcaine while leaving the basal lamina intact. This resulted in greater numbers of proliferating satellite cells compared with viable myofibers (50). This suggested that the myofiber emanates a “quiescent” signal either by its physical association or by releasing chemical compounds (50, 53). In accordance with this hypothesis, recent studies have revealed numerous satellite cell regulatory factors that are presented on myofibers or secreted by myofibers. First, myofibers secrete SDF-1, which serves as ligand to the cell surface receptor CXCR4 on satellite cells (429, 486). SDF-1/CXCR4 signaling has been shown to stimulate satellite cell migration (429). In addition, it has been found that the transmembrane Notch ligand Delta is upregulated on myofibers after injury, which can activate the Notch signaling cascade in satellite cells and hence induce their proliferation (107). Such an activation signal from myofibers is probably amplified by the expression of both Notch receptors and Delta ligands on satellite cells, functioning in autocrine and juxtacrine fashion (108, 280).

In aged muscle, both the caliber and the number of myofibers decline (202), although the direct effect of these morphological changes is not known. Interestingly, the presence of Delta ligands is not sufficiently expressed on aged myofibers, an indication of diminished Notch signaling possibly compromising satellite cell activation and muscle regeneration (107).
3. ECM and associated factors

The basal lamina of the myofiber is composed of a network of ECM that directly contacts the satellite cell and separates it from the muscle interstitium. The basal lamina of skeletal muscle is composed of type IV collagen, laminin, entactin, fibronectin, perlecan, and decorin glycoproteins along with other proteoglycans (459). These ECM molecules are mainly synthesized and excreted by interstitial fibroblasts but can also be produced and remodeled by myoblasts during muscle development and regeneration (278). Satellite cells reside between the basal lamina, composed primarily of a type IV collagen network, and the apical sarcolemma covered in laminin (568). The basal lamina presents a large number of binding sites for α7β1-integrins; sites that anchor the actin cytoskeleton of satellite cells to the ECM (501; reviewed in Ref. 330). This physical tethering is critical for satellite cell activation as it allows the transduction of extracellular mechanical force into intracellular chemical signals (62; reviewed in Ref. 73). In addition, muscle-specific laminin-2 (α2/β1/γ1 subunits and laminin-4 (α2/β2/γ1) are also associated with myofibers though α7β1-integrins and dystroglycan on the surface of myofibers (208; reviewed in Ref. 459). Proteoglycans reside on the surface of satellite cells and function as receptors to bind a suite of secreted, yet inactive growth factor precursors. These precursors, including HGF (521), basic fibroblast growth factor (bFGF) (141), epidermal growth factor (EGF) (193), insulin-like growth factor isoforms (IGF-I, IGF-II) (323) and various Wnt glycoproteins (65, 293), originate from either satellite cells, myofibers, interstitial cells, or serum. In resting muscle, the sequestering of inactive growth factors exists as a local reservoir, to facilitate a rapid response to muscle injury. These growth factors can be swiftly rendered active by proteolytic enzymes present in serum or interstitium, such as thrombin, serine proteases, and matrix metalloproteinases (MMPs). This highly coordinated process serves to stimulate satellite cell survival, activation, and proliferation upon muscle injury (249, 288, 386, 521, 576).

A) HGF/SCATTER FACTOR. HGF is a heterodimer of its α and β subunits, both of which are processed from a single-chain inactive precursor (pro-HGF). HGF is critical for cell growth, migration, and organ morphogenesis through its mitogen, motogen, and morphogen activities (reviewed in Refs. 381, 586). In skeletal muscle, HGF functions in the early phase of muscle regeneration and is one of the key activators of satellite cells (342, 521). In intact muscle, low levels of HGF mRNA can be detected in satellite cells but not in fibroblasts, suggesting an autocrine function of HGF (13, 484, 521). However, the majority of active and inactive forms of HGF are sequestered by heparan sulfate proteoglycans (HSPGs) within the basal lamina (13, 484, 520, 521). Upon injury, the transcription of HGF is increased in proportion to the degree of injury, and an active form of HGF is released from the ECM without the need of proteolytic cleavage of pro-HGF (484, 520, 521, 525, 569). In vitro and in vivo data have revealed that release of nitric oxide synthase (NOS) from the basal lamina after myofiber stretch or damage leads to the production of nitric oxide (NO), which in turn activates MMPs and release HGF from local HSPGs (522, 523, 576). Alternatively, the observed rapid upregulation of HGF following muscle injury is due to release of HGF from intact organs, such as the spleen (513).

Released HGF and newly synthesized HGF act directly on satellite cells and myoblasts through the cell surface receptor c-Met found on both quiescent and activated satellite cells (13, 116, 179, 521). Multiple studies have demonstrated that the activation of HGF/c-Met pathway stimulates quiescent satellite cells to enter the cell cycle, increases myoblasts proliferation, and inhibits myogenic differentiation (13, 179, 342, 521). Fittingly, HGF isolated from crushed muscle extracts induces quiescent satellite cell activation, and an anti-HGF antibody abolished this activity (521). Furthermore, direct injection of HGF into injured muscle blocked the regeneration process but increased myoblast numbers (342, 521). Forced expression of a constitutive active form of c-Met in C2C12 myoblasts resulted in the inhibition of differentiation (15). HGF inhibits myoblast differentiation by coordinately repressing transcriptional activity of MRF/E-protein complexes, increasing MyoD inhibitor Twist expression, as well as decreasing p27kip1 expression (179, 306). The dual function of HGF in promoting proliferation and inhibiting differentiation involves sustained activation of MAPK/Erk signaling through binding of Grb2 to phosphorylated c-Met (214, 304, 305). Moreover, HGF promotes satellite cell migration to the site of injury as demonstrated by the in vitro chemotactic activity of this factor on satellite cells and C2C12 myoblasts (49, 512, 551). The function of HGF in muscle regeneration is important during the early phase of repair because over time levels of HGF decline and exogenous HGF does not affect muscle regeneration when administrated at later stages (342, 521). Therefore, these results demonstrate that HGF is a satellite cell activator, playing a critical role in the early stage of muscle regeneration. The activation of satellite cells by HGF is due to both transcription activation of HGF in satellite cells and release of HGF from the ECM by MMPs, which coordinately function in an autocrine and paracrine fashion to induce the proliferation of satellite cells.

B) FIBROBLAST GROWTH FACTORS. Fibroblast growth factors (FGFs) are a large family of mitogens, involved in cell growth, survival, migration, and embryonic development. Numerous FGFs have been found in skeletal muscle in vivo and upon culture and differentiation of myoblasts in vitro (12, 199, 222, 266). Inhibition of endogenous FGF-1 by siRNAs led to myogenic differentiation of myoblasts in vitro, while exogenous FGF-1, -2, -4, and -6 robustly stimulate the proliferation of rat primary myoblasts in vitro.
completely abolished in myoblasts derived from and proliferation is impaired and MAPK signaling is compartment (236). In accordance with this, satellite cell activation both FGFs and FGF receptors to promote ligand recognition (115). It is believed that HSPGs interact with implicating separate roles of these two HSPGs in satellite cell function (115). In light of this, mutant mice (115). In contrast, hyperplasia of satellite cells investigations revealed that Although HSPGs are ubiquitously present on most mammalian cells, both quiescent and activated satellite cells specifically express syndecan-3 and syndecan-4 (113). Further investigations revealed that syndecan-3−/− and syndecan-4−/− mutant mice display distinct panels of phenotypes, implicating separate roles of these two HSPGs in satellite cell function (115). It is believed that HSPGs interact with both FGFs and FGF receptors to promote ligand recognition (236). In accordance with this, satellite cell activation and proliferation is impaired and MAPK signaling is completely abolished in myoblasts derived from syndecan-4−/− mutant mice (115). In contrast, hyperplasia of satellite cells and myonuclei and overactivation of MAPK signaling were observed in syndecan-3-mutant mice, which suggests that an inhibitory mechanism is missing (115). In light of this, the downregulation of syndecan-4 mRNA was observed in turkey satellite cells in response to exogenous FGF-2 (548).

FGF signaling is mediated by virtue of four transmembrane tyrosine kinase receptors known as FGFR1–4, which differ in their specificity of ligand binding. Of these, FGFR1 and FGFR4 are the most prominent receptors in rat satellite cells in vitro (172). In vitro, FGF-1 can induce the expression of FGFR1, and this induction effect can be further augmented by HGF (483). Stable overexpression of FGFR1 increases myoblast proliferation and delays their differentiation, whereas overexpression of a dominant negative form of FGFR1 leads to the opposite effects (463). In skeletal muscle, dimerization of FGFs with FGFRs leads to tyrosine phosphorylation of FGFR and activates the downstream Ras/MAPK pathway. This is supported by the finding that constitutively expressed active RAS in myoblasts induces proliferation and blocks differentiation even in the absence of exogenous FGFs (162). In addition, inhibition of mitogen-activated protein kinase kinase (MAPKK) activity completely abolishes the FGF2-dependent inhibition of myogenic differentiation in vitro (561). Therefore, activation of the Ras/MAPK signaling pathway seems to play a central role in FGF/FGFR-mediated pro-proliferation and anti-differentiation effects in skeletal muscle cells.

In addition to the canonical FGF receptors, HSPGs also serve as low-affinity FGF receptors (reviewed in Ref. 427). Although HSPGs are ubiquitously present on most mammalian cells, both quiescent and activated satellite cells specifically express syndecan-3 and syndecan-4 (113). Further investigations revealed that syndecan-3−/− and syndecan-4−/− mutant mice display distinct panels of phenotypes, implicating separate roles of these two HSPGs in satellite cell function (115). It is believed that HSPGs interact with both FGFs and FGF receptors to promote ligand recognition (236). In accordance with this, satellite cell activation and proliferation is impaired and MAPK signaling is completely abolished in myoblasts derived from syndecan-4−/− mutant mice (115). In contrast, hyperplasia of satellite cells and myonuclei and overactivation of MAPK signaling were observed in syndecan-3-mutant mice, which suggests that an inhibitory mechanism is missing (115). In light of this, IGF-I and IGF-II play important roles in the regulation of satellite cell activity (reviewed in Ref. 409). IGF-I has pleiotropic functions including anti-inflammation, cell migration, and stimulation of both proliferation and differentiation in satellite cells. In contrast, IGF-II is believed to only promote myogenic differentiation (11, 103, 151, 169, 170, 547). Elevation of IGF-I signaling within muscle cells in vitro results in muscle hypertrophy with increased DNA and protein content in muscle (6, 34, 87, 103, 365). The hypertrophic effects of IGF-I are attributed to both the activation of satellite cell proliferation, which gives rise to more myonuclei, and protein synthesis, which increases the cytoplasmic-to-DNA volume ratio (32, 35, 366). IGF-II expression and secretion are elevated prior to myoblast differentiation (171), and knock-down of either IGF-I or IGF-II in vitro results in impaired myogenic differentiation (158, 171). Consistently, IGF2−/− mutant mice are ~40% lighter compared with the weight of wild-type mice at birth, and this defect persists postnatally (133).

IGF-I and IGF-II are two growth factors that are involved in the regulation of myoblast proliferation and differentiation. IGF-I binds to the IGF-I receptor (IGF1R), which is a ligand-activated receptor tyrosine kinase. In contrast to IGF-I-induced muscle hypertrophy, IGF1R−/− mutant mice display a dystrophic phenotype and usually die at birth due to weak respiratory muscles (315). Activation of IGF1R in satellite cells induces the expression of MRFs (170) and initiates intracellular signaling cascades involving both mitogenic and myogenic responses (110, 367). Two primary signaling pathways are activated by IGF1R. In the first pathway, activated IGF1R recruits and phosphorylates insulin receptor substrate proteins (IRSs), leading to the activation of phosphatidylinositol 3-kinase (PI3K). Activation of PI3K is involved in multiple cellular processes including an anti-apoptotic effect mediated by activation of the AKT pathway (502), modulation of intracellular calcium levels by the inositol phosphate cascade (156), and promoting protein translation (5). Although evidence supports that IGF-I-dependent satellite cell proliferation is mediated by the AKT/mTOR pathway (220), the activation of PI3K/AKT and p38-MAPK pathways are also involved in myoblast differentiation (197, 539). In the second pathway, IGF1R activates the Ras/Raf/ extracellular response kinases (ERKs) cascade, which in turn activates other protein kinases and several transcription factors (110, 347). The activation of the Ras/Raf/ERK pathway is also required for satellite cell proliferation (110). In addition to the aforementioned pathways, accumulating evidence indicates that the calcium/calcineurin pathway is also involved in IGF-I signaling-dependent muscle differentiation and hypertrophy (137, 173, 366, 479). Specifically, it has been revealed that GATA-2-mediated
myofiber hypertrophy depends on IGF-I stimulation of the calcineurin-mediated signaling pathway (366). Further evidence of the pleiotropic functions of IGF-I involves a role in anti-inflammation demonstrated by a reduction in chronic inflammation upon IGF-I treatment (357).

Activated satellite cells and cultured myoblasts express insulin-like growth factor binding proteins (IGFBPs), which are a family of secreted proteins that specifically bind IGFs and function as carriers during circulation and regulate IGF turnover, transport, and half-life (reviewed in Ref. 253). Of the five IGFBPs, IGFBP-5 plays a critical role in muscle growth and differentiation. IGFBP-5 expression is induced during differentiation and precedes that of IGF-II in cultured myoblasts (246, 436). It was found that IGFBP-5 functions to promote myogenic differentiation by switching on an IGF-II-mediated positive-feedback loop (436). Knockdown of IGFBP-5 impairs myogenesis and suppresses IGF-II expression (436).

Notably, recent studies demonstrated that alternative splicing of IGF-I pre-mRNA gives rise to a unique peptide, termed mechano-growth factor (MGF) (194). MGF promotes myoblast proliferation in vitro (26, 578). However, the existence of MGF and its functions in vivo remain controversial (discussed in Ref. 327).

D) MMPs. Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that can degrade components of the extracellular matrix such as collagens, elastin, fibronectin, laminin, and proteoglycans. In skeletal muscle, MMPs play an important role in satellite cell activation, migration, and differentiation during muscle regeneration (reviewed in Ref. 86). Of more than 20 MMPs, MMP-2, -3, -7, and -9 were found in skeletal muscle. In particular, MMP-2 and MMP-9, which can degrade denatured collagen types (gelatin) and heparan sulfate proteoglycans, have critical functions on ECM remodeling in skeletal muscle regeneration. Upon injury, MMP-2 is secreted by satellite cells and regenerating myofibers, and its activity is elevated in both degenerative and regenerating stages (167, 178, 270). In contrast, MMP-9 is generated by leukocytes and macrophages, and its activity drastically decreases after the degenerative stage (270). In the degeneration stage, activated MMP-2 and MMP-9 degrade collagen IV in the muscle ECM, which allows satellite cells to migrate across the basement membrane to the site of injury (377). In the regeneration stage, it is believed that MMP-2 is involved in remodeling and maintenance of the ECM. Recent studies indicate that NO upregulates enzymatic activity and protein expression levels of MMP-2 (270) and MMP-9, and this activation of MMP-2 is required for the release of HGF from the ECM upon satellite cell activation (575, 576).

E) ECM stiffness. Recent studies reveal a previously unappreciated role for the stiffness of the ECM in regulating satellite cell proliferation and differentiation. Resting healthy skeletal muscles and cultured myotubes possess a similar elastic stiffness (elastic modulus ~12 kPa) (106, 152), whereas aged (184, 444) and dystrophic (152) skeletal muscles are apparently stiffer (elastic modulus ~1418 kPa). These changes of stiffness are presumably due to increased extracellular matrix deposition, in particular collagen deposition by fibroblasts, a result of repeated muscle regeneration. The significance of changes in stiffness was evaluated by assessing the proliferation and differentiation ability of myoblasts on various in vitro culturing surfaces. Primary myoblast proliferation on polyacrylamide gels cross-linked with entactin, type IV collagen, and laminin is optimal at an elastic modulus equal to 21 kPa, while it is impaired on softer (3 kPa) or stiffer (80 kPa) culturing substrates (61). Similarly, C2C12 myoblasts optimally differentiate on collagen-coated polyacrylamide gels that mimic the physiological elasticity of skeletal muscle (12 kPa), whereas either softer (5 kPa) or stiffer (420 kPa) gels greatly compromise their differentiation efficiency (152). Although the stiffness of the ECM surrounding the satellite cell niche has not been directly measured yet, these in vitro data draw attention to a causal relationship between biophysical stimuli and satellite cell function in vivo.

B. The Microenvironment Beyond the Immediate Niche

1. Local milieu

In addition to the immediate niche surrounding satellite cells, local interstitial cells, motor neurons, blood vessels, and their associated secreted factors reside within skeletal muscle and have the potential to regulate satellite cell function and affect muscle regeneration.

A) Interstitial cells. Interstitial cells are the major component of the stromal tissue between the basal lamina and epimysial sheath surrounding skeletal muscle. Fibroblasts comprise a major component of the interstitial stromal cell population (51). Fibroblasts secrete growth factors, such as FGFs, and also contribute to the skeletal muscle ECM by depositing collagen, laminin, fibronectin, HSPGs, tenascin, and NCAM (185). Increased levels of fat and connective tissue (fibrosis), evidence for increased numbers of adipocytes and fibroblasts, respectively, are well documented for several physiological and pathological conditions, such as aging, obesity, and muscular dystrophy (38, 66, 195, 198, 200). It is believed that satellite cells may account for the increase in adipocytes and fibroblasts due to transdifferentiation along alternative mesenchymal lineages. This notion is supported by the observations that myoblasts can undergo adipogenic differentiation in vitro (239, 310, 485, 579) and satellite cells on single myofibers can give rise to...
both adipocytes and fibroblasts in vitro (23, 66). Recently, two studies revealed that these intramuscular adipocytes and fibrocytes can arise from muscle residing fibrocyte/adipocyte progenitors (FAPs) (252, 541).

In one study, a population of SM/C-2.6−/PDGFR-α+ cells is able to differentiate into adipocytes in culture (541). In vivo, these cells localize to the interstitial space between myofibers and muscle bundles. After transplantation into glycerol-injected fatty degenerating muscle, SM/C-2.6−/PDGFR-α− cells can differentiate into adipocytes, a result that is not observed in normal regenerating muscle (541). In another study, a Sca-1+/CD34− or α7-integrin−/CD45−/CD31− cell population was isolated and found to spontaneously form both adipocytes and ER-TR7+ fibroblasts in culture (252). Although the origin of these cells was not directly investigated, it was further demonstrated that these Sca-1+/α7-integrin− cells also express PDGFR-α, and count for the vast majority of PDGFR-α− cells in skeletal muscle (252). This suggests strong similarities exist between the cell populations used in both studies. Consequently, Sca-1+/α7-integrin− cells also only give rise to adipocytes in degenerating muscle in vivo, in contrast to their limited adipogenic potential in intact muscle and in injured regenerating muscle. Moreover, a single Sca-1+/α7-integrin− cell was able to differentiate into both adipocytes and fibroblasts in vitro, indicating that these cells are bipotent and thus termed FAPs (252).

Both studies clearly demonstrate that FAPs do not have myogenic potential either in vitro or in vivo, which makes them distinct from satellite cells and other muscle-residing myogenic cells (252, 541). The distinct adipogenic potentials of FAPs in degenerating and regenerating muscles suggest that the differentiation of FAPs is regulated by their local microenvironment. Indeed, both groups demonstrated that FAPs proliferated and transiently increased in number in regenerating muscle without differentiating into adipocytes or fibroblasts (252, 541). Moreover, when adipogenic FAPs isolated from degenerating muscle were transplanted into regenerating muscle, the regenerative microenvironment was sufficient to inhibit adipogenic differentiation (541). These observations indicate that regenerating muscle provides a specialized microenvironment, which favors myogenic differentiation and maintains FAPs in their undifferentiated state. Importantly, the undifferentiated FAPs in such a microenvironment may facilitate the myogenic differentiation of satellite cells, which is supported by the observation that, in co-culture conditions, FAPs increase the commitment of myoblasts to terminal differentiation (252). IL-6 signaling was implicated in this process as evidenced by induced IL-6 expression in FAPs in response to muscle regeneration (252).

In summary, these intriguing results support the hypothesis that skeletal muscle provides a balanced environment for coexistence of both myogenic precursors (myoblasts) and adipogenic/fibrogenic precursors (FAPs) during muscle regeneration. Regeneration is supported by both satellite cells and FAPs, whereas in degenerating muscle, this balance is disrupted and FAPs differentiate into adipocytes (and presumably also fibroblasts). These findings also raise several interesting questions including: How do satellite cells respond to increased adipocytes and fibroblasts in the aforementioned physiological and pathological conditions? Recent observations regarding the effect of fibrosis on the stiffness of the ECM raise the question as to whether increased depositions from fibroblasts or adipocytes can alter myofiber stiffness and therefore affect the physiological roles attributed to satellite cells (152; discussed in sect. IIIA). Finally, it would be interesting to investigate the embryonic origin and postnatal lineage progression of FAPs, particularly their lineage relationship to the CD45−/Sca-1− mesenchymal stem cells found in multiple tissues (316, 401, 417, 441, 509, 519). Further study is required to understand whether other regulatory factors are also involved in the determination of FAP differentiation and whether these factors change in response to various physiological and pathological conditions.

In addition to FAPs, recent studies identified telocytes (TCs, formerly called interstitial Cajal-like cells or ICLCs) as a new type of cells within muscle interstitium, which are in close vicinity of satellite cells, myofibers, nerve endings, and blood vessels (414). Unlike satellite cells and fibroblasts, skeletal muscle TCs express the cell surface marker c-kit. Transmission electron microscopy (TEM) examination of TCs in muscle and studies on TCs from other tissues suggest TCs transmit intercellular signaling by shedding/intaking microvesicles (also called exosomes), which are enriched for proteins and RNAs (230, 414, 543). Although their exact role in muscle regeneration is still unknown, the finding that these cells secrete VEGF suggests telocytes are an important player in promoting satellite cells self-renewal, facilitating vasculogenesis, and preventing fibrosis (132; further discussed in sect. III B1c).

Another cellular component of skeletal muscle interstitium is the SP, a population of cells characterized by their expression of Abcg2 and hence the ability to efflux Hoechst dye. A recent study indicated that endothelial cells and interstitial cells constitute the major fraction of skeletal muscle SP (146). Ablation of Abcg2 impairs skeletal muscle regeneration. In addition, a small percentage of these SP cells have myogenic potential and may contribute to myogenic regeneration in injured muscle (discussed in sect. IID).

B) MOTOR NEURONS. It is well known that denervation results in progressive skeletal muscle atrophy. During acute denervation of muscle, the percentage of satellite cells increases during the first week, indicating a proliferation phase similar to muscle injury (468). However, long-term denervation
results in a drastic decline in satellite cell numbers (442, 550), which is at least partially due to decreased mitotic capability and increased apoptosis of satellite cells. Experiments illustrate that an absence of PCNA (proliferating cell nuclear antigen)-positive satellite cell can be found on myofibers denervated for more than 1 wk. This implies that long-term denervation may cause satellite cells to lose their capability to enter the mitotic cell cycle (282). Second, satellite cells from muscle denervated for 6 and 10 wk displayed a twofold increase of apoptosis, compared with control cells from normal innervated muscle (248). Although the underlying mechanism of a neural influence on satellite cell behavior is still elusive, neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are involved.

NGF is expressed in developing muscles (563), and its expression is downregulated after birth (153). The expression of NGF reappears in adult muscle under several pathological conditions such as muscular dystrophy (537) and amyotrophic lateral sclerosis (283), wherein expression of NGF was found in regenerating myofibers and connective tissue fibroblasts within the damaged muscles (99, 537), suggesting that it may be involved in muscle regeneration (340). Indeed, ectopic expression of NGF neutralizing antibodies in vivo causes muscle dystrophy (448). Within skeletal muscle, NGF seems to bind to its low-affinity receptor p75NTR (138), which is expressed in satellite cells (358) and human primary myoblasts and myotubes (33). The expression of both NGF and p75NTR is upregulated during primary myoblast differentiation in vitro, and they function to stimulate morphological changes involved in myoblast fusion (138).

Similar to NGF, developing muscles express high levels of BDNF, whereas in adult myofibers it is not detectable (201, 358). BDNF is present in most adult satellite cells, and its expression is correlated with Pax3 expression (358). In cultured primary myoblasts, the expression of BDNF is decreased dramatically during myogenic differentiation; in concordance, a reduction in endogenous BDNF leads to early myogenic differentiation, while addition of exogenous BDNF can reverse this phenotype (358). In vivo, BDNF+/− satellite cells are decreased and hence compromise muscle regeneration (102). These observations suggest that the function of BDNF in adult skeletal muscle is to maintain satellite cells in their quiescent state and prevent their myogenic differentiation (358). This is consistent with the observation that neural electrical activity stimulates the activation of satellite cells (reviewed in Ref. 470) and represses the expression of BDNF (358). Since the TrkB receptor of BDNF was not detected in skeletal muscle, BDNF signaling is proposed to occur via the p75NTR in a similar fashion to NGF.

Taken together, these data indicate that neurotrophic factors function in an autocrine fashion to regulate satellite cell behavior and muscle regeneration. Thus it is conceivable that systematic release of neurotrophic factors after denervation may also exert similar regulatory roles on satellite cell activation and differentiation. In addition, denervation can also cause secondary effects on satellite cells by directly influencing the physiological properties of myofibers (229). Moreover, it has been recently found that satellite cells may control myofiber innervation during muscle regeneration by secreting semaphorin 3A, a well-known factor involved in axon guidance and growth (524). This finding further emphasizes the notion that proper muscle regeneration relies on the dynamic interaction of satellite cells with their niche.

In aged muscle, examination of neuromuscular junctions by EM revealed decreases in nerve terminal area, mitochondria and synaptic vesicles, along with occasional postsynaptic regions (159). These changes, in addition to progressive myofiber atrophy, may potentially disturb signals between myofibers and satellite cells.

**C) VASCULATURE.** Skeletal muscle is nourished by the microvascular network. The importance of this niche component is reflected by the fact that most satellite cells are closely associated with capillaries in intact adult muscle (100, 465). It is also well known that angiogenesis and myogenesis proceed at the same time during muscle regeneration (321). Vascular endothelial growth factor (VEGF) plays an important role in satellite cell function and muscle regeneration (416). It has been reported that overexpression of VEGF by adenovirus can stimulate satellite cell proliferation and improve myofiber regeneration in vivo (20). A recent study revealed that in coculture, endothelial cells promote myoblasts proliferation by secreting a panel of growth factors, such as IGF-I, HGF, FGF, platelet-derived growth factor-BB (PDGF-BB), and VEGF (100). In addition, it was found that periendothelial cells (smooth muscle cells and endomysial fibroblasts) promote a subset of myoblasts (presumably reserve cells) to return to the quiescent state (100). This procedure mimics the self-renewal of satellite cells during muscle regeneration. Indeed, recent studies demonstrated that the Tie-2 receptor is preferentially expressed by quiescent satellite cells and angiopoietin-1/Tie-2 signaling acts through ERK1/2 pathway to promote the reentry to quiescence and thus self-renewal of a subset of satellite cells (3, 100). Based on these observations, it was proposed that during muscle regeneration, while vessels are not stabilized, endothelial cells and myogenic precursors interact with each other to promote both myogenesis and angiogenesis. Once homeostasis of muscle is achieved, the proximity of satellite cells and periendothelial cells permits angiopoietin-1, which is secreted by periendothelial cells, to bind Tie-2 receptors on satellite cells to simultaneously stabilize vessels and promote satellite cell quiescence (4, 100). In addition to endothelial cells, satellite cells and myoblasts, differentiating myofibers also generate VEGF within skle-
tal muscle (70, 92, 189). VEGF can function in an autocrine fashion through VEGF receptors present on various myogenic cells to stimulate migration, prevent apoptosis, and promote differentiation (92, 189). In addition, VEGF stimulation can reportedly activate the Akt pathway and promote myofiber hypertrophy (70, 514).

In aged muscle, it was observed that while myofibers lose their close contact with the microvascular network, there is a corresponding VEGF level decrease (451). In addition, endothelial nitric oxide synthase (eNOS) secreted by endothelial cells is also decreased in aged muscle (580; reviewed in Ref. 67).

Taken together, recent studies have begun to unveil interactions between satellite cells and cells within the microvasculature, experiments that imply a potentially profound functional role necessitating the close proximity of satellite cells to the microvasculature. Future studies are needed to further elucidate these interactions, particularly those of periendothelial cells, during muscle regeneration.

2. Systemic milieu

A) IMMUNE CELLS. Only a small number of immune cells reside within intact skeletal muscle. During the early stages of muscle injury, immune cells are recruited and play important roles in the regeneration process (reviewed in Ref. 532). Upon injury, immune cells rapidly infiltrate the muscle to remove necrotic tissue and secrete soluble factors that serve to activate satellite cells (reviewed in Ref. 530). As such, immune cells constitute a transient local environment for satellite cells. Depletion of macrophages and monocytes impairs subsequent muscle regeneration (206). Satellite cells and immune cells attract one another through chemokines (chemoattraction) (440). Satellite cells have been demonstrated to secrete a panel of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, to facilitate immune cell infiltration and function (reviewed in Ref. 92). In turn, immune cells secrete a wealth of diffusible factors, such as growth factors, IL-6, globular adiponectin, ECM components, and ECM remodeling MMPs. These diffusible factors generate ECM chemoattractive fragments, help satellite cells escape from the basal lamina of myofibers, and promote satellite cell proliferation. In addition, cell-to-cell contact between immune cells and satellite cells protects satellite cells from apoptosis (503).

Aged muscle displays a functional deficit in the immune cells surrounding the satellite cell niche. In vitro studies indicate that the capabilities of free radical generation, phagocytosis, and chemotaxis are decreased in aged immune cells (25). The perturbation between satellite cells and immune cells impedes satellite cell activation and migration.

B) IL-6. IL-6 is a ubiquitous pleiotropic cytokine, which can be produced by many cell types, such as hematopoietic cells, fibroblasts, and vascular endothelial cells. Although IL-6 is primarily produced following an immune response, convincing evidence demonstrates that IL-6 is also involved in satellite cell-mediated muscle hypertrophy and regeneration. Chronic systematic elevation of IL-6 leads to muscle atrophy in aging and disease states (29, 212; reviewed in Ref. 154). Likewise, transgenic mice overexpressing IL-6 display a muscle wasting (cachexia) phenotype (538). It is also well documented that exercise causes an increase in IL-6 levels within muscle (533; reviewed in Ref. 403). During regeneration, satellite cells, myofibers, and neutrophils are the principal sources of IL-6, the expression of which is regulated by calcineurin-NFAT, NF-κB, AP-1, IL-1, and NO signaling pathways (74, 333, 583; reviewed in Ref. 403).

The IL-6 receptor (IL-6Rα) is expressed on the myofiber sarcolemma and is responsive to exercise (268). Muscle-derived IL-6 can act in an autocrine fashion by binding to the IL-6Rα on the sarcolemma and activate the JAK/STAT3 signaling pathway (480; reviewed in Refs. 403, 430). Binding of IL-6 to the receptor IL-6Rα leads to JAK2 phosphorylation and subsequent STAT3 phosphorylation. Phosphorylated STAT3 dimerizes and translocates to the nucleus where it acts as a transcription factor for target gene activation.

It has been shown that IL-6 can also induce satellite cell proliferation (80). Recently, it was demonstrated that IL-6 is involved in satellite cell-mediated muscle hypertrophy (480). Although early studies demonstrated that muscle regeneration in IL-6−/− null mice was comparable to that of wild type (556), a recent study reported a blunted hypertrophic response and less contribution of satellite cell myonuclei to regenerating myofibers (480). Furthermore, it was shown that the proliferation of satellite cells from IL-6−/− mice was impaired both in vivo and in vitro, which is due to the attenuation of STAT3 signaling (480).

C) ANDROGENS. The musculature of men and woman differs in mass, fiber size, and various other physiological properties. These differences are in part due to the anabolic actions of circulating androgens, e.g., testosterone, on muscle size and muscle strength (reviewed in Refs. 97, 228). Androgens bind to androgen receptors (AR) and lead to the dimerization and nuclear translocation of AR. In the nucleus, AR homodimers bind to the DNA motifs or androgen response elements (AREs) and activate transcriptional target genes.

Satellite cells express androgen receptors and are receptive to androgen signaling (143); however, the endogenous androgens acting on satellite cells have yet to be...
determined. Exogenous testosterone increases satellite cell numbers by promoting satellite cell activation and proliferation (255, 494). This function is predicted to involve the induction of Notch signaling in satellite cells (494). Furthermore, androgen administration causes increased myonuclei and muscle hypertrophy in a dose-dependent manner (254, 493, 495). Multiple mechanisms have been implicated in androgen-induced muscle hypertrophy. First, androgen administration has been shown to elevate local IGF-I levels (165, 307) through a postulated induction of IGFR1 expression on satellite cells (529). Androgen administration can also elevate intracellular calcium and inositol 1,4,5-trisphosphate (IP3) levels, which induces phosphorylation of ERK1/2 and contributes to affect the IGF pathway (157). Additionally, androgen may promote muscle hypertrophy by counteracting the catabolic effect of glucocorticoid hormones and promote the myogenic differentiation (48, 496).

During aging, systemic levels of androgens decline, which is concomitant with a loss in muscle mass and a gain in fat mass (228, 544).

**D) NITRIC OXIDE.** NO is a diffusible small molecule, which can freely pass through cell membranes. It can be produced by nitric oxide synthase (NOS) in diverse cell types, including epithelial cells, endothelial cells, fibroblasts, hepatocytes, macrophages, and skeletal muscle cells (60). Currently three NOS isoforms exist: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (394).

NO has profound functions in intact muscle as well as during muscle regeneration (reviewed in Ref. 505). In skeletal muscle, nNOS is constitutively expressed within the sarcolemma of myofibers (275). Loss of nNOS from the sarcolemma (e.g., as a result of a dystrophin deficiency) leads to inflammation and myofiber lysis, implicating a protective function for NO in skeletal muscle (89). iNOS levels are extremely low in intact muscle, yet drastically induced by macrophages and myofibers in response to muscle injury (449; reviewed in Ref. 262). In the degenerating phase, NO, generated by macrophages and myofibers, promotes the lysis of necrotic myofibers by macrophages while reducing other inflammation-induced damage (375; reviewed in Ref. 505). During regeneration, NO induces satellite cell activation, as evidenced by the inhibitory effect of l-arginine methyl ester (l-NAME), a NOS inhibitor, on satellite cell activation (16). NOS inhibition prevents the binding of HGF to the c-Met receptor on satellite cells, whereas treatment with l-arginine, the NOS substrate, resulted in an increase in satellite cell activation. Accordingly, iNOS−/− mice display delayed satellite cell activation following muscle injury, although injured muscles were still able to regenerate (16). The function of NO-dependent satellite cell activation stems from the ability of NO to promote the release of HGF from the ECM (575, 576). MMP-2 was demonstrated to be involved in this process (575). In addition, it was also suggested that NO may regulate satellite cell migration (104) and prevent fibrosis by inhibiting transforming growth factor-β signaling during muscle regeneration (126).

**IV. CONCLUDING REMARKS AND PERSPECTIVES**

Compelling evidence indicates that adult satellite cells represent a heterogeneous population of stem cells and committed myogenic progenitors in skeletal muscle. At the population level, the stem cell characteristics of satellite cells are well represented by their remarkable and sustained ability to support skeletal muscle regeneration. At the individual level, satellite cells vary in their self-renewal, proliferation, and myogenic differentiation potential. These intrinsic differences of satellite cells likely reflect the distinct needs of 1) maintaining a sustainable reservoir of stem cells, 2) producing sufficient numbers of myogenic cells, and 3) generating, during the course of muscle regeneration, functional contractile muscular structures. Throughout regeneration, the hierarchical and collaborative behaviors of satellite cells are influenced by changes in their niche. These changes include diverse molecules, cells, and structures that constitute a dynamic niche continually influencing the multiple tasks set forth for satellite cells.

Satellite cells are an ideal cellular model system to study adult stem cells and tissue regeneration. Extensive studies over the last three decades have accumulated much knowledge and insights. Satellite cells are currently not applicable to regenerative medicine due to difficulties in their isolation and loss of stemness ex vivo. However, satellite cell-based cell therapy would greatly benefit from future studies in lineage progression to better delineate satellite cell hierarchy along with physiologically relevant interactions between satellite cells and other myogenic cells. Moreover, such issues may be resolved following the maturation of techniques involving the generation of iPSC or other types of ES-like multipotent cells. If so, the broad interest of future studies should focus on identification of the intrinsic and extrinsic regulatory mechanisms that govern satellite cell commitment and differentiation throughout the muscle regeneration. Future advances in system biology and bioengineering may hopefully harness these previous achievements towards developing therapeutic approaches for sarcopenia and muscle dystrophic diseases.

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