Myogenic satellite cells: physiology to molecular biology

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Hawke, Thomas J., and Daniel J. Garry. Myogenic satellite cells: physiology to molecular biology. J Appl Physiol 91: 534–551, 2001.—Adult skeletal muscle has a remarkable ability to regenerate following myotrauma. Because adult myofibers are terminally differentiated, the regeneration of skeletal muscle is largely dependent on a small population of resident cells termed satellite cells. Although this population of cells was identified 40 years ago, little is known regarding the molecular phenotype or regulation of the satellite cell. The use of cell culture techniques and transgenic animal models has improved our understanding of this unique cell population; however, the capacity and potential of these cells remain ill-defined. This review will highlight the origin and unique markers of the satellite cell population, the regulation by growth factors, and the response to physiological and pathological stimuli. We conclude by highlighting the potential therapeutic uses of satellite cells and identifying future research goals for the study of satellite cell biology.

skeletal muscle; stem cells; regeneration; aging; transgenic models

SKELETAL MUSCLES OF ADULT mammalian species exhibit a remarkable capacity to adapt to physiological demands such as growth, training, and injury. The processes by which these adaptations occur are largely attributed to a small population of cells that are resident in adult skeletal muscle and are referred to as satellite cells. After their initial identification in 1961 (98), Mauro (117) described a cell closely associated with the periphery of the frog myofiber and termed it a satellite cell based on its location. Quiescent satellite cells are physically distinct from the adult myofiber as they reside in indentations between the sarcolemma and the basal lamina (130). Adult skeletal muscle fibers are terminally differentiated such that muscle growth and regeneration are accomplished by satellite cells. In the unperturbed state, these cells remain in a nonproliferative, quiescent state. However, in response to stimuli such as myotrauma, satellite cells become activated, proliferate, and express myogenic markers (satellite cells expressing myogenic markers are also termed myoblasts). Ultimately, these cells fuse to existing muscle fibers or fuse together to form new myofibers during regeneration of damaged skeletal muscle (12, 167).

Since the original description of the myogenic satellite cell, considerable interest and research efforts have focused on myogenic satellite cell biology. These research efforts have enhanced our understanding of muscle growth, remodeling, and regeneration. In addition, new paradigms have been proposed regarding the regenerative capacity and the plasticity of the myogenic satellite cell population (108, 119, 139, 168, 169). These paradigms suggest that the satellite cell population not only has a remarkable capacity for muscle regeneration but may also contribute to alternative muscle and non-muscle lineages and may have clinical applications in the treatment of devastating and deadly diseases such as muscular dystrophy.

The current review attempts to integrate the anatomic, physiological, biochemical, and molecular properties that regulate the myogenic satellite cell population. We begin with a brief overview of vertebrate
myogenesis, highlighting the populations of myoblast precursor cells that contribute to muscle development, and outline a well-described genetic hierarchy that is important in muscle specification. We describe the limited gene expression profile and the distinguishing morphological characteristics of the satellite cell population. We describe the inductive signals that regulate the satellite cell in vitro and in well-described physiological models. Finally, we will review the stem cell-like features of the satellite cells with emphasis on the novel strategies that may be pursued in the future for the treatment of debilitating myopathies.

Importantly, myogenic satellite cell biology remains an emerging field of scientific inquiry, such that satisfying and complete answers to the most fundamental questions are currently unavailable. In this review, we will provide a summary of the current knowledge in this area and highlight fertile areas for future research.

**BUILDING OF MUSCLE WITH WAVES OF PRECURSOR CELLS**

Anatomic and molecular mechanisms during muscle regeneration have been postulated to recapitulate muscle development. Although current evidence suggests the regenerative process may be more complex, an understanding of muscle development is important to appreciate the anatomic and molecular network associated with muscle regeneration.

During embryogenesis, the head, trunk, and limb skeletal muscles develop as separate lineages. Of the three germ layers in the early embryo, the paraxial mesoderm gives rise to the somite (Fig. 1A). The somite is subdivided into the dorsomedial (epaxial) domain, which generates the muscles of the back, and the ventrolateral (hypaxial) domain, which gives rise to the abdominal, intercostal, and limb musculature (see Refs. 123 and 140 for review).

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**Fig. 1. Derivation of muscle precursor cells during mouse embryogenesis.**

A: precursor cells from the epaxial region of the somite migrate to form the back musculature. Precursor cells from the hypaxial region of the somite migrate to the newly formed limb buds. Note the migrating limb precursor cells form dorsal and ventral masses, which will later become the extensor and flexor muscle groups of the limb. Surrounding structures such as the neural tube, notochord, dorsal aorta, and overlying ectoderm potentially provide signals regulating precursor cell movements and fate. B: members of the MyoD family play an integral role in skeletal muscle myogenesis. MyoD and myf5 expression is involved in determination of precursor cells to a myogenic fate, whereas myogenin and MRF4 expression is associated with terminal differentiation.
During somitogenesis, cross talk involving growth factors (Wnt proteins, Sonic hedgehog, bone morphogenetic proteins, and so forth) and transcription factors (myf5, MyoD, Pax-3, and so forth) occurs between the developing somite and the anatomically adjacent structures, including the overlying ectoderm, the ventromedial neural tube and notochord, and the vascular structures including the aorta (38, 113, 123, 131, 145). The constellation of these positional cues (i.e., growth and transcription factors) results in the specification of muscle through the regulation of a distinct molecular (hierarchical) cascade.

Since the discovery of MyoD in 1987, the role of the myogenic basic helix-loop-helix (bHLH) transcription factors in skeletal myogenesis has been defined in elegant detail by several groups (19, 126, 137, 147, 148, 192, 200, 207). This subset of the bHLH family includes MyoD, myf5, myogenin, and MRF4. Each of these myogenic bHLH proteins forms heterodimeric DNA binding complexes that include other bHLH proteins of the E2 gene family (E12 and E47) and bind a canonical DNA sequence, CANNTG (E-box), within enhancer elements of genes that encode terminal differentiation markers of the skeletal muscle lineage (41, 103). MyoD family members share the ability to activate skeletal muscle differentiation when expressed ectopically in nonmuscle cells (115, 193). The essential role played by bHLH proteins in skeletal myogenesis has been demonstrated unambiguously by gene disruption experiments (78, 138, 142, 147, 148, 157, 185). The results of these gene knockout experiments support a role for myf5 and MyoD in the determination of the myogenic cell fate and the formation of myoblasts during embryogenesis (Fig. 1B). Myogenin and MRF4 appear to function in activation of muscle differentiation (149, 175).

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MUSCLE PRECURSOR CELLS AND LIMB DEVELOPMENT

Whereas the cells associated with the epaxial region of the somite contribute to the primary myocytes of the myotome, the hypaxial region of the somite (at the level of the limb) contributes to the migratory limb muscle precursor cells (141). Specification, migration, and differentiation of the myogenic precursor cells to their distal targets are complex processes involving intrinsic and extrinsic cues of which little is known. Recent studies have shown that these myogenic precursor cells express the paired domain transcription factor Pax-3 (181), the tyrosine kinase receptor c-Met (205), the homeodomain transcriptional repressor msx1 (11, 189), and the homeodomain transcription factor Lbx 1 (17, 71) but lack expression of the myogenic regulatory factors of the MyoD family. After migration to the developing limb, these precursor cells coalesce into the dorsal and ventral premuscle masses, which will be the future flexor and extensor compartments of the forelimb and now express members of the MyoD family (19, 137, 141).

Targeted gene disruption studies (i.e., knockout mouse models) have begun to provide insight into the molecular regulation of limb development. In mice lacking either Pax-3 (16, 60) or c-Met (50), limb precursor cells fail to migrate into the limb, resulting in the complete loss of limb muscles. Combinatorial knockout experiments crossing the Pax-3 mutant mouse (Splotch) into the myf5 null background result in a further perturbation of myogenesis and an absence of both limb and body wall muscle (181). Additional studies support a genetic hierarchy where Pax-3 mediates the activation of other myogenic regulatory factors (myf5 and MyoD) and functions as a key regulator of somitic myogenesis (113, 181). In addition, Pax-3 functions in the specification of the limb precursor cells and is upstream of both c-Met and Lbx 1 (17, 50, 60, 181). Inactivation of the Lbx 1 locus by homologous recombination results in an extensive loss of limb muscles, although residual muscle groups are still present. This finding suggests that Lbx 1 is not required for the specification of limb muscles but may function in the determination of which migratory highway the precursors should pursue (17, 71).

Proliferating limb myoblasts coalesce into the ventral and dorsal premuscle masses, withdraw from the cell cycle, and form multinucleated primary myofibers at approximately embryonic day 13 postcoitum (E13) in the mouse embryo. In a process that is less clearly defined, secondary myofibers form parallel to the primary fibers and constitute the predominant multinucleated myofibers during the latter stages of embryogenesis (E15–E16 in the mouse) and in postnatal skeletal muscle (56, 123). Studies suggest that two distinct lineages generate primary myofibers (i.e., embryonic myoblasts) and the secondary myofibers (i.e., fetal myoblasts). Furthermore, primary myofibers differ from secondary myofibers in their temporal devel-
The discovery of satellite cells in the early 1960s demonstrated the existence of yet an additional population of proliferative cells that contributed to postnatal growth, the maintenance of adult skeletal muscle, and the repair of damaged myofibers. Myogenic satellite cells are present in the limbs of midgestational mouse embryos after E15 (35). After birth, the satellite cell population accounts for ~30% of sublaminar muscle nuclei in neonatal hindlimb skeletal muscle (12). These neonatal satellite cells fuse to growing myofibers to contribute additional nuclei during postnatal growth of skeletal muscle.

SOMATIC VS. NONSOMATIC ORIGIN OF SATELLITE CELLS

Previous studies support the hypothesis that muscle precursor cells, including the myogenic satellite cell population, originate from the multipotential mesodermal cells of the somite (58, 141, 167). The support for this hypothesis is primarily derived from chimeric or interspecies grafting experiments that have been performed in avian models. These fate-mapping studies involved the transplantation (or exchange) of embryonic somites from donor quail embryos into host chick embryos (27, 105). The transplanted quail cells have distinguishing morphological characteristics and were observed to migrate from the somite and contribute to both the limb muscles and the satellite cell population in postnatal chick skeletal muscle. Satellite cells have been isolated from the fetal skeletal muscle from an E15 or older mouse embryo, suggesting that satellite cells populate the developing limb during the latter stages of embryogenesis (34, 35, 36). Whether the satellite cells migrate from the somite as a distinct lineage or whether they originate from a preexisting lineage (i.e., embryonic or fetal myoblasts) in the developing limb is unclear. Nevertheless, the underlying concept was that each of the myoblast precursor cells (i.e., embryonic myoblasts, fetal myoblasts, and satellite cells) was a derivative of the somite.

This paradigm has recently been challenged, as studies have suggested that multipotential cells of nonsomatic origin may be the precursors of the satellite cell (45, 139). De Angelis et al. (45) reported that cells isolated from the embryonic dorsal aorta had a similar morphological appearance and a similar profile of gene expression to that of satellite cells. Furthermore, transplantation of the aorta-derived myogenic cells into newborn mice revealed that this cell population participated in postnatal muscle growth, regeneration, and fusion with resident satellite cells. The authors proposed that satellite cells may be derived from endothelial cells or a precursor common to both the satellite cell and the endothelial cell.

Derivation of the satellite cell from the somite or a nonsomatic source need not be mutually exclusive. Conceivably, both lineages may contribute under physiological or pathological states to the satellite cell population. Additional fate-mapping strategies will be needed to further define and dissect the lineage derivation of all the satellite cells that reside in adult skeletal muscle. These studies will be important in defining whether there is a common lineage source for the entire satellite cell population and a common lineage source for cells that have regenerative capacities in both muscle and nonmuscle tissues.

SATellite CELL IDENTIFICATION

Anatomic identification. Resident within adult skeletal muscle is a pool of undifferentiated mononuclear cells termed satellite cells because of their anatomic location at the periphery of the mature, multinucleated myotube. The defining characteristic of the satellite cell is that the basal lamina that surrounds the satellite cell and the associated myofiber is continuous (167). As shown in Fig. 2, the identification of this cell population has historically utilized ultrastructural techniques (66, 161). Other distinguishing morphological features of the satellite cell population include a relatively high nuclear-to-cytoplasmic ratio with few organelles, a smaller nuclear size compared with the adjacent nucleus of the myotube, and an increase in the amount of nuclear heterochromatin compared with that of the myonucleus (167). These morphological features are consistent with the finding that satellite cells are relatively quiescent and transcriptionally less active. These distinguishing features are absent following activation or proliferation of the satellite cells in response to growth, remodeling, or muscle injury. After activation, the satellite cells are more easily identified as they appear as a swelling on the myofiber with cytoplasmic processes that extend from one or both poles of the cell (Fig. 2; Ref. 167). Associated with the increase in mitotic activity, there is a reduction in heterochromatin, an increase in cytoplasmic-to-nuclear ratio, and an increase in the number of intracellular organelles (167).

Satellite cell markers. The profile of gene expression of the quiescent satellite cell as well as their activated and proliferating progeny is largely unknown. The quiescent satellite cells do not express myogenic regulatory factors of the MyoD or MEF2 families or other known markers of terminal differentiation (33, 119, 201). Through the identification of satellite cell markers, biologists will be able to address issues related to the developmental origin of the satellite cell, the cell cycle control, and the molecular regulation of this unique cell population during growth and regeneration. Several satellite cell markers have been identified and are restricted to either the quiescent, activated, or proliferative state or are expressed more broadly (Table 1).

We have previously determined that myocyte nuclear factor (MNF or Foxk1), a member of the winged helix transcription factor family, is localized to the quiescent satellite cell in adult skeletal muscle (64). We have identified two alternatively spliced isoforms for MNF and termed them MNF-α and MNF-β (64,
203, 204). These two alternatively spliced isoforms are reciprocally expressed during myogenesis and during muscle regeneration, suggesting that the two isoforms of MNF may exert opposing effects on target genes at discrete steps during muscle regeneration or in renewal of the satellite cell population (63). Using a RT-PCR assay, we have shown that MNF-β is the principal form expressed in quiescent satellite cells, whereas MNF-α predominates in proliferating satellite cells following muscle injury. Disruption of the MNF locus, mutating both isoforms, resulted in a severe growth deficit, a marked impairment in muscle regeneration (63), and a decreased number of satellite cells in adult MNF mutant skeletal muscle (Hawke and Garry, unpublished observations). Additional winged helix family members have also been identified in stem cells or regenerating cells including Genesis (Foxd3; Ref. 83), which is expressed selectively in embryonic stem cells, and a protein related to hepatocyte nuclear factor-3 (HNF3/forkhead homolog 11), which has been identified in regenerating hepatocytes (206).

Utilizing single cell RT-PCR analysis, Cornelison and Wold (33) characterized the satellite cells as a heterogeneous population based on their profile of gene expression. Additionally, they identified c-Met, the receptor for hepatocyte growth factor (HGF), as a marker of quiescent satellite cells. HGF is a potent mitogen for satellite cells and has been shown to be important in the migration of the myogenic precursor cells from the somite to the developing limb (5, 14). Moreover, c-Met deficient embryos fail to form limb skeletal muscle due to a lack of myogenic precursor cells (14, 112).

Irintchev and colleagues (87) identified M-cadherin, a calcium-dependent cell adhesion molecule, as a unique marker of the satellite cell pool. M-cadherin is only expressed in a subpopulation of the quiescent cell pool; however, its expression is increased when the satellite cells become activated in response to a stimulus (9, 33). Recent studies suggest that other cell adhesion molecules, neural cell adhesion molecule (NCAM) and vascular adhesion molecule-1 (VCAM-1), are also potential markers of quiescent satellite cells (39, 89). The role of these adhesion molecules is unclear but collectively (NCAM, VCAM-1, and M-cadherin) may function in the adhesion of the satellite cell to the basal lamina of the myofiber and may participate in the migratory capacity of this cell population in response to stimuli. NCAM is expressed in both myofibers and satellite cells, whereas VCAM-1 is broadly expressed in adult skeletal muscle. In the uninjured muscle fiber, the satellite cell is quiescent and rests in an indentation in the adult muscle fiber. The satellite cells can be distinguished from the myonuclei by a surrounding basal lamina and more abundant heterochromatin. When the fiber becomes injured, the satellite cells become activated and increase their cytoplasmic content. The cytoplasmic processes allow for chemotaxis of the satellite cell along the myofiber. Bar = 1 μm.

Table 1. Expression patterns of satellite cell markers in adult skeletal muscle

<table>
<thead>
<tr>
<th>Molecular Marker</th>
<th>Expression Observed in the Adult</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNF</td>
<td>Quiescent, activated, and proliferating satellite cells</td>
<td>64</td>
</tr>
<tr>
<td>Pax7</td>
<td>Quiescent, activated, and proliferating satellite cells</td>
<td>169</td>
</tr>
<tr>
<td>c-Met</td>
<td>Quiescent, activated, and proliferating satellite cells</td>
<td>33</td>
</tr>
<tr>
<td>M-cadherin</td>
<td>Quiescent, activated, and proliferating satellite cells</td>
<td>33</td>
</tr>
<tr>
<td>NCAM</td>
<td>Quiescent, activated, and proliferating satellite cells; synaptic junctions in adult myofibers</td>
<td>39</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Quiescent, activated, and proliferating satellite cells</td>
<td>89</td>
</tr>
<tr>
<td>Desmin</td>
<td>Activated and proliferating satellite cells</td>
<td>15</td>
</tr>
<tr>
<td>myf5</td>
<td>Activated and proliferating satellite cells</td>
<td>33</td>
</tr>
<tr>
<td>MyoD</td>
<td>Activated and proliferating satellite cells</td>
<td>33, 124</td>
</tr>
<tr>
<td>BrdU</td>
<td>Proliferating cells</td>
<td>163</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cells</td>
<td>90</td>
</tr>
<tr>
<td>[3H]thymidine</td>
<td>Proliferating cells</td>
<td>128</td>
</tr>
</tbody>
</table>

Expression of selected molecular markers used to identify the satellite cell population in adult skeletal muscle is outlined. MNF, myocyte nuclear factor; NCAM and VCAM, neural cell and vascular adhesion molecule; BrdU, bromodeoxyuridine; PCNA, proliferating cell nuclear antigen.
expressed during embryogenesis but limited to satellite cells in adult muscle (39). Furthermore, VCAM-1 has been shown to mediate satellite cell interaction with leukocytes following injury (89).

Recent work from the Rudnicki laboratory (169) identified the paired box transcription factor, Pax7, expressed selectively in quiescent and proliferating satellite cells. Analysis of the Pax7 mutant skeletal muscle revealed a complete absence of satellite cells. This novel finding supports the hypothesis that Pax7 is essential for the specification of the satellite cell population. Future studies will be important in the definition of Pax7 downstream target genes in the satellite cell population and may provide insight into the regulation of this cellular pool.

Identification of other satellite cell markers is the focus of current research efforts. Emerging candidates include the cell surface antigen Sca-1 (stem cell antigen-1; Refs. 88, 177), the glycoprotein Leu-19 (94, 162), the anti-apoptotic factor Bcl-2 (106, 123), CD34 (9), and interferon regulatory factor-2, which is a transcription factor that mediates VCAM-1 expression in skeletal muscle (89).

SATELLITE CELL QUANTITATION AND DISTRIBUTION

Quantitation of the satellite cell population in adult skeletal muscle has been possible primarily through the use of ultrastructural techniques. More recently, immunohistochemical techniques have been utilized for the identification of the satellite cell pool. Although a limited number of markers for the quiescent satellite cell population exist, proliferating satellite cells, as measured by [3H]thymidine or bromodeoxyuridine (BrdU) incorporation, can be identified immunohistochemically for the coexpression of the MyoD family members or the intermediate filament protein, desmin (15, 104, 123). MyoD expression occurs early during the activation of the satellite cell population (within 6 h following muscle injury) (63, 74, 100, 119, 134). In addition, several nonselective markers of cellular proliferation have been used to characterize the proliferating satellite cell pool. These markers of cellular proliferation include proliferating cell nuclear antigen (90), BrdU (163), and [3H]thymidine (128).

Satellite cell number is dependent on the species, age, and muscle fiber type (Table 2; see Ref. 167 for review). Satellite cells constitute ~30% of the muscle nuclei in the neonate and decrease with age to ~4% in the adult and ~2% in the senile (29–30 mo) mouse (176). The decrease in the percentage of satellite cells with aging (i.e., senile rodent) is the result of an increase in myonuclei (oxidative and glycolytic myofibers) and a decrease in total number of satellite cells (glycolytic myofibers) (12, 66, 167).

The satellite cell distribution between muscle groups is a result of the heterogeneity in satellite cell content.
between muscle fiber types. An increase in satellite cell density has been demonstrated in association with the proximity of capillaries (161), myonuclei (18, 161), and motoneuron junctions (199). The proximity of satellite cells to these anatomic structures suggests a permissive role in the regulation of the cellular pool. In support of this hypothesis, oxidative fibers (characterized by increased capillary and motoneuron density compared with glycolytic myofibers) demonstrate a five to six times greater satellite cell content (65, 161).

GROWTH FACTORS AS REGULATORS OF THE SATELLITE CELL POPULATION

The process of muscle regeneration requires the influence of growth factors and a sequence of cellular events, which results in the regulation of the satellite cell population (Table 3, Fig. 3; Refs. 73, 168). Many of the studies that have examined the effect of growth factors on satellite cell biology have utilized satellite cell cultures. These studies have defined the effect of growth factors alone or in combination and have provided valuable insight into the regulation of the satellite cell. Admittedly, in vitro studies are limited due to the lack of permissive and repressive factors that are present in vivo and may influence cellular activity. Currently, most satellite cell cultures are derived from neonatal skeletal muscle due to the abundance of satellite cells in these tissues compared with older animals (>30% in young animals vs. ~5% in older animals; Ref. 143). The population and age of the satellite cell is an important consideration in cell culture preparations as the response of aged, quiescent satellite cells to growth factor stimulation differs compared with young, proliferating satellite cells (182). This section will provide a brief introduction of growth factors that are important in the regulation of satellite cell proliferation, differentiation, and motility (for review, see Table 3).

Insulin-like growth factors. Skeletal muscle secretes insulin-like growth factors I and II (IGF-I and IGF-II), which are known to be important in the regulation of insulin metabolism (3, 109, 186). In addition, these growth factors are important in the regulation of skeletal muscle regeneration. IGF-I and IGF-II increase satellite cell proliferation and differentiation in vitro (Table 3). The importance of these growth factors was demonstrated with the intramuscular administration of IGF-I into older, injured animals. In this study, IGF-I administration (using an osmotic minipump) resulted in enhanced satellite cell proliferation and increased muscle mass (26). Moreover, skeletal muscle overload or eccentric exercise results in elevated IGF-I levels, increased DNA content (suggesting an increase in satellite cell proliferation), and a compensatory hypertrophy of skeletal muscle (1, 202).

IGF-I appears to utilize multiple signaling pathways in the regulation of the satellite cell pool. The calcineurin/NFAT, mitogen-activated protein (MAP) kinase, and phosphatidylinositol-3-OH kinase (PI-3K) pathways have all been implicated in satellite cell proliferation (25, 32, 170). IGF-I-stimulated satellite cell differentiation appears to be mediated through the PI-3K pathway (32). Additional studies, utilizing genetic mouse models (i.e., transgenic overexpression or knockout models), may further define the regulation and signaling pathways of the IGFs and satellite cell biology (8, 25, 132).

Hepatocyte growth factor. Hepatocyte growth factor (HGF) is a multifunctional cytokine initially described as a mitogen in mature hepatocytes (122). Recently, HGF and its receptor c-Met have been localized to satellite cells and adjacent myofibers but are absent in the adjacent fibroblasts. In addition, HGF expression is proportional to the degree of muscle injury (33, 174,

Table 3. Factors affecting satellite cell activity

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Chemotactic Ability</th>
<th>Proliferation</th>
<th>Differentiation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF</td>
<td>↑</td>
<td>↓</td>
<td>79</td>
<td>3, 54</td>
</tr>
<tr>
<td>HGF</td>
<td>↑</td>
<td>↑</td>
<td>13</td>
<td>152, 162</td>
</tr>
<tr>
<td>IGF-I</td>
<td>↑</td>
<td>↑</td>
<td>13</td>
<td>4, 124, 182</td>
</tr>
<tr>
<td>IGF-II</td>
<td>↑</td>
<td>↑</td>
<td>79</td>
<td>54, 152, 208</td>
</tr>
<tr>
<td>TGF-β</td>
<td>↑</td>
<td>↑</td>
<td>3</td>
<td>13, 208</td>
</tr>
<tr>
<td>Macrophages</td>
<td>↑</td>
<td>↑</td>
<td>121</td>
<td>128, 86(t)</td>
</tr>
<tr>
<td>Crushed muscle/platelet-derived extract</td>
<td>↑</td>
<td>↓</td>
<td>79</td>
<td>13, 152</td>
</tr>
<tr>
<td>LIF</td>
<td>↑</td>
<td>NE</td>
<td>7</td>
<td>152, 7</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑</td>
<td>↑</td>
<td>7</td>
<td>152, 121</td>
</tr>
<tr>
<td>PDGFAB</td>
<td>NE</td>
<td>NE</td>
<td>79</td>
<td>152, 54</td>
</tr>
<tr>
<td>PDGFB</td>
<td>NE</td>
<td>↑</td>
<td>152</td>
<td>121, 13</td>
</tr>
<tr>
<td>EGF</td>
<td>↑</td>
<td>↑</td>
<td>13</td>
<td>54, 26(t)</td>
</tr>
</tbody>
</table>

A number of factors affect satellite cell proliferation, differentiation, and chemotaxis. All studies were performed using cell culture techniques except those denoted with a (t), which were performed using skeletal muscle tissue. NE, no effect. FBF, HGF, IGF, TGF, PDGF, and EGF, fibroblast, hepatocyte, insulin-like, transforming, platelet-derived, and endothelial growth factor, respectively. LIF, leukemia-inhibitory factor. *Stimulation of satellite cell proliferation in the presence of serum but no effect in serum-free medium. ↑ and ↓. Increase or decrease in effect, respectively.
Multiple roles for HGF have been proposed for the regulation of the satellite cell, including a role as a potent chemotactic factor, an activator of the satellite cell, and an inhibitor of myoblast differentiation (Table 3). HGF is capable of activating and selectively promoting satellite cell proliferation (4). Furthermore, HGF administration attenuates satellite cell differentiation through the transcriptional inhibition of the myogenic regulatory factors (i.e., MyoD and myogenin) (62).

Fibroblast growth factors. Fibroblast growth factor (FGF) has nine different isoforms (FGF-1 to FGF-9). Although many of the FGF isoforms are broadly expressed, FGF-6 is restricted to skeletal muscle (59). Sheehan and Allen (173) investigated in detail the role of the FGF family on satellite cell proliferation in culture. In these studies, it was demonstrated that FGF-1, -2, -4, -6, and -9 stimulated cellular proliferation, whereas FGF-5, -7, and -8 had no mitogenic activity. The investigators further observed that addition of HGF to either FGF-2, -4, -6, or -9 resulted in a synergistic increase in satellite cell proliferation. In addition to an increase in satellite cell proliferation, the FGF family has also been observed to attenuate satellite cell differentiation to myofibers (30, 91, 173, 178).

Floss et al. (59) reported that mice deficient for FGF-6 (i.e., knockout mice at the FGF-6 locus) have impaired satellite cell proliferation and a subsequent defect in muscle regeneration in response to a crush injury. In contrast, Fiore et al. (57) pursued a similar targeting strategy to mutate the FGF-6 locus and observed an absence of defects in response to either a crush injury or chemically induced injury (notexin). Consequently, the functional role(s) of FGF-6 during muscle regeneration remains unclear. Nevertheless, these studies underscore the ability of redundant family members to compensate for one another and result in preserved function under pathological conditions (i.e., mouse knockout models).

The release of FGF-2 from the damaged myofibers, like HGF, is proportional to the degree of injury (29). FGF levels are coordinated with FGF receptor expression. When receptor expression is increased, satellite cells propagated in culture demonstrate an increased proliferation and decreased differentiation (160). Conversely, when receptor expression is diminished, proliferation is decreased and there is a concomitant increase in satellite cell differentiation. Interestingly, during the period of satellite cell activation and proliferation (0–48 h after injury), FGF receptor (FGF-R1) mRNA is increased fivefold, and this increase is further enhanced in the presence of HGF (173).

The signaling pathway(s) that transduces the FGF signal has recently been investigated with the use of both transgenic techniques and pharmacological inhibitors (92). These studies revealed that the MAP kinase pathway is important in transducing the FGF-induced increase in satellite cell proliferation; however, the...
MAP kinase signaling pathway did not mediate the FGF-mediated repression of satellite cell differentiation.

**Transforming growth factors.** Transforming growth factor-β (TGF-β) is the prototypical family member of cytokines that includes bone morphogenic protein and growth-differentiation factors. The TGF-β family of cytokines transduces their signal through the SMAD factor-b (TGF-β interaction. FGF-mediated repression of satellite cell differentiation by TGF-β, like FGF, persists even in nonmuscle cell lines engineered to ectopically express members of the MyoD family (97, 115, 184). The combination of IGF-I or FGF with TGF-β was unable to alter the TGF-β-induced attenuation of satellite cell differentiation; however, TGF-β action had little effect on IGF-I- or FGF-mediated increases in proliferation (70). During muscle regeneration, TGF-β receptor levels (TGF-β RII) and TGF ligand are reciprocally expressed, resulting in the initial promotion of cellular proliferation followed by enhanced muscle differentiation (159).

**Interleukin-6 cytokines.** Leukemia inhibitory factor (LIF) and interleukin-6 (IL-6) are members of the IL-6 family of cytokines produced by many different cells, including myoblasts and macrophages. These cytokines share a common receptor component, and their actions are mediated through the same signaling pathways (81, 144). Skeletal muscle regeneration after injury in LIF mutant mice is attenuated, whereas exogenous administration of LIF increased the regenerative process and produced enlarged myofibers (101). The permissive effect of LIF was associated only with the muscle lineage and had no effect on nonmuscle cells in skeletal muscle (101). IL-6 promotes the degradation of necrotic tissue, synchronizes the cell cycle of satellite cells, and induces apoptosis of macrophages following muscle injury (22). Unlike LIF, however, IL-6 expression in injured muscle does not increase satellite cell proliferation (96). Collectively, this family of growth factors appears to play an integral role in skeletal muscle regeneration.

Many other factors may be involved in the regulation of the satellite cell in adult skeletal muscle. Nitric oxide, platelet-derived growth factor, endothelial-derived growth factor, and testosterone have been shown to mediate satellite cell activity (6, 93, 133). Obviously, the regulation of satellite cells is orchestrated by numerous factors in a temporal and concentration-dependent fashion during regeneration.

Few animal studies have examined the effect of growth factors in vivo. Chakravarthy et al. (26) observed that local IGF-I administration to atrophied muscle increased satellite cell proliferation and muscle mass within 2 wk. Unlike the observations with IGF-I, the intramuscular injection of HGF, at specified intervals following skeletal muscle injury, increased satellite cell proliferation and either had no effect or impaired the rate of regeneration (124). Similarly, administration of FGF at timed intervals and selected dosages did not appreciably affect muscle regeneration (125). Future studies that combine cell culture methodologies and overexpression or loss of function models using molecular technologies will be helpful in the definition of the role of growth factors in satellite cell biology.

**FUNCTIONAL RESPONSES OF SATTELITE CELLS TO PHYSIOLOGICAL STIMULI**

**Hypertrophic stimuli.** Load-induced hypertrophy (chronic stretch, agonist ablation, tenotomy) and resistance training are physiological challenges that promote a hypertrophic response in both human and animal models (154–156, 197). Hypertrophic growth of skeletal muscle is stimulated by short bursts of muscle activity against high resistance. Resistance training induces muscle hypertrophy through a process of satellite cell activation, proliferation, chemotaxis, and fusion to existing myofibers to contribute to muscle growth (Fig. 4; Ref. 167). The migratory capacity (chemotaxis) of satellite cells is dependent on the integrity of the basal lamina. After the rupture or interruption of the basal lamina in response to myotrauma, satellite cells may migrate to adjacent myofibers utilizing tissue bridges (164, 191). In response to limited myotrauma, where no rupture of the basal lamina occurs, satellite cells migrate from the proximal intact portion of the myofiber, under the basal lamina, to the site of injury to participate in the repair process (165, 167).

Exercise-induced myotrauma initiates an immune response, resulting in the influx of macrophages into the damaged region. After the acute insult, macrophage infiltration peaks within 48 h (186). Initially, the role of these blood-borne macrophages was believed to be limited to phagocytosis and the digestion of myonecrotic fibers. However, additional roles for macrophages during the early stages of muscle repair are emerging. Macrophages are essential in the orchestration of the repair process as they secrete a collection of cytokine factors that regulate the satellite cell pool (133). Importantly, in the absence of a macrophage response, muscle regeneration is absent; in the presence of an enhanced macrophage response, there is an increase in satellite cell proliferation and differentiation (110).

In response to resistance training, myotrauma results in the release of growth factors that will, in part, regulate the satellite cell population during regeneration (Fig. 3 and Table 3). For example, IGF-I is upregulated in response to hypertrophic signals in skeletal muscle and promotes proliferation and fusion of the satellite cell pool (1, 26, 186). As outlined in the previous section, additional growth factors and/or cytokines including LIF and members of the TGF-β family may play a role in the signaling or commissioning of the satellite cells to participate in the hypertrophic remodeling response. Although a number of questions remain regarding the role of the satellite cell in muscle remodeling, the primary physiological consequence of the
hypertrophic response is to produce a muscle with a greater capacity for peak force generation.

**Atrophic stimuli.** Atrophy of skeletal muscle results in a reduction in myonuclei number and can be induced by numerous factors including denervation, hindlimb suspension, and malnutrition (73). Atrophy and remodeling that result from muscle disuse can be produced in laboratory rodents physiologically by tail/hindlimb suspension or immobilization of specific muscle groups in plaster casts or pathologically through denervation. The response of the satellite cells appears to be pleiotropic and dependent on the atrophic stimulus.

In adolescent rats, hindlimb suspension results in an irreversible remodeling process, including decreased satellite cell content and an impaired proliferative capacity within 3 days of unloading in both the oxidative slow-twitch soleus and the fast-twitch extensor digitorum longus (EDL) muscles (44, 129). Thus the atrophic stimulus in the adolescent animal may irreversibly alter the developmental program for myofibers to accrue nuclei even with the resumption of weight bearing. A similar reduction in the satellite cell population was observed in adult rat hindlimbs using an immobilization model as an alternative atrophic stimulus (188). In contrast to adolescent animals, remobilization of the hindlimb was accompanied by increased myofiber regeneration, supporting the hypothesis that, following completion of the developmental program, adult satellite cells are capable of activation and proliferation to repopulate atrophied muscle (129, 188).

Unlike the other forms of atrophy, denervation is a pathological rather than physiological stimulus. Denervation produces a form of disuse atrophy that includes myofiber degeneration and is accompanied by distinctive changes in the myonuclei and quiescent satellite cells (102, 111, 153). In the unperturbed condition, there are an increased number of satellite cells associated with the neuromuscular junction (199). It is conceivable that neurotrophic factors are important in satellite cell homeostasis. A number of laboratories have reported that the percentage of satellite cells increase (from 3 to 9%) during the initial period following denervation (118, 187). However, a prolonged period of denervation results in a significant decrease in the percentage of satellite cells (3 to 1% following an 18-mo period of denervation). Viguie et al. (187) hypothesize that the progressive decline in the satellite cell pool may be the result of satellite cell apoptosis. Alternatively, denervation may result in a lack of neurotrophic input (including growth factors) that negatively impacts satellite cell function and content.

Long-term denervation has considerable clinical implications. Denervation for periods of 6–18 mo results in the inability of skeletal muscle to reestablish a preinjury functional capacity even if neuronal sprouting and regeneration occurs (180). The mechanisms for this phenomenon are unclear, but considerable data support the conclusion that the intact neuromuscular junction and the denervation model mediate positive and repressive influences, respectively, on the satellite cell pool (187).

**Aging.** Recent advances have allowed biologists to interrogate the proliferative history of a cellular population. With each cell replication, there is ~100 bp lost from the ends of eukaryotic chromosomes (47, 48, 151, 172). The ends of the chromosomes contain TTAGGG repeats termed telomeres. The length of these telomeres reflects the number of replications of a particular cell and its proliferative capacity. Using this technology, investigators now have the ability to analyze the proliferative history and future capacity of the satellite cell, providing valuable insight into the effects of aging and disease in this cell population.
As age progresses, there is an impairment of skeletal muscle regeneration following injury (see Ref. 72 for review). A decrease in satellite cell number and/or proliferative capacity has been used to explain this phenomena. Support for this hypothesis is observed in rodent models, as increasing age is associated with a decrease in satellite cell number and a reduced proliferative capacity (52, 66, 166). In contrast to the rodent model, a decrease in human satellite cell population and proliferative ability is observed only during the childhood years. For example, neonatal (5-day-old) and infant (5-mo-old) satellite cells are capable of ~60 and 45 replications, respectively, whereas 9-yr-old and ≥60-yr-old humans are both capable of 20–30 replications (151). However, as aging progresses, satellite cells (>60-yr-old skeletal muscle) fuse to form thinner, more fragile myotubes (151). Thus, despite a normal ability to proliferate, the satellite cells of older humans have a reduced capacity to repopulate the myofiber population.

The impaired regenerative response that is observed with aging in humans thus appears to be much more complex than satellite cell senescence alone. Results from cross-transplantation experiments suggest that the host environment is a critical factor in the ability of older skeletal muscle to regenerate (23, 24). Cross transplantation of EDL muscles between 4-mo-old and 24-mo-old rats demonstrated that mature skeletal muscle was as capable as young skeletal muscle in recovering from transplant and from toxin-induced injury. However, young (4-mo-old) nerve-muscle autografts functioned significantly better than old (24-mo-old) nerve-muscle autografts, as determined by the measurement of mass and maximum isometric force, suggesting that the ability for neuronal regeneration may be a critical factor in activating the satellite cell response and, ultimately, regenerating the damaged muscle (23, 24).

Other factors within the host environment affect the efficiency of skeletal muscle regeneration as aging progresses. A thickening of the basal lamina (176), increased fibrosis within skeletal muscle (114), and reduced capillary density (31) may also contribute to impaired regeneration. Inflammatory factors (macrophages and associated cytokines) are essential for the normal satellite cell response to injury. Aging negatively impacts the immune response, resulting in a decrease of the inflammatory factors and macrophages (43). In association with an impaired immune response, there are reduced serum levels of growth factors including IGF-I in aged rats and humans (150, 183). After multiple cycles of atrophy, there is no restoration of muscle mass or satellite cell proliferation in aged rats even after 9 wk of recovery. However, local IGF-I administration to the atrophied muscle resulted in significant increases in mass and satellite cell proliferation within 2 wk (26).

An unresolved question with regard to satellite cells and the aging process is whether repeated exhaustive and/or resistance training exercise programs have a negative impact on the long-term satellite cell content? This question is applicable to the young population as well. If satellite cells have a limited proliferative capacity (~60 doublings), does a lifetime of intense exercise have a negative influence on their ability to regenerate skeletal muscle as aging progresses?

FUNCTIONAL RESPONSES TO DISEASE STATES

Most myopathies have a molecular mutation that affects the structural or cytoskeletal proteins in skeletal muscle. Duchenne muscular dystrophy (DMD) is the most common and the most devastating of the muscular dystrophies (20, 55, 76, 82, 127). Disease progression and death are ultimately due to a failure of the myogenic satellite cells to maintain muscle regeneration (36, 80). DMD is a recessive X-linked disease that results in a null mutation at the dystrophin locus (20, 82, 127). The absence of this cytoskeletal protein renders the muscle fiber extremely fragile. In response to mechanical stress associated with repeated contraction, there is widespread degeneration. The satellite cells respond to the injury by repopulating the injured skeletal muscle with defective myofibers lacking dystrophin. This process results in continuous degeneration-regeneration cycles and ultimately exhausts the satellite cell pool (36, 80).

Clinical symptoms are apparent by 4–5 yr of age in boys with DMD (10, 20, 127). DMD patients (4–5 yr of age) have been shown to undergo more skeletal muscle regeneration than that measured in a total of six normal patients over 60 yr of age (46). These results were confirmed by Renault et al. (151), who demonstrated that the proliferative life span of satellite cells derived from a 9-yr-old DMD patient was approximately one-third of an age-matched control. Proliferative fatigue or senescence of the satellite cell population and the milieu of the DMD skeletal muscle may collectively impair the proliferative or regenerative capacity of this cell population. In the DMD patient, increased levels of IGF binding proteins (IGFBP) are released by fibroblasts. The elevated IGFBP sequesters IGF-I, limiting its bioavailability for satellite cells and ultimately resulting in increased skeletal muscle fibrosis (120). The evidence to date demonstrates the tremendous strain and ultimate failure of the satellite cell population to adequately compensate for the persistent degeneration-regeneration process that is occurring in the DMD skeletal muscle.

GENETIC MOUSE MODELS OF MYOPATHY

A number of gene knockout mouse models and experimental methods are available for studies of muscle regeneration and satellite cell biology. Although the mdx mouse, which lacks dystrophin, has provided important insights into the pathophysiology of DMD, the myopathy in these animals does not represent the myopathic process of DMD in humans (75). The mdx mice have a normal life span, a temporally restricted myofiber degeneration, and adapt to muscle degeneration with an expansion of the satellite cell pool and muscle hypertrophy, thereby avoiding the compro-
mised muscle function that afflicts humans who lack dystrophin (51, 69, 116, 179). In addition to the spontaneous dystrophin mutation in the \textit{mdx} mouse, myopathic mouse models include genetically engineered mouse strains with knockouts of utrophin (49, 69), MyoD (119), or MNF (63) crossed into the \textit{mdx} background. Each of these double mutant mouse models exhibit features that more closely resemble DMD in humans, including a severe myopathy, an impaired regenerative capacity, and a decreased life span.

**MUSCLE REGENERATION MODELS**

An alternative strategy that has been successfully used for the study of satellite cell activation, proliferation, regeneration, and self-renewal is to experimentally produce a controlled skeletal muscle injury. Strategies including crush (101), freeze (40), or chemically induced injury (42, 63) have all been successfully used to study satellite cell biology. Perhaps the most extensive and reproducible muscle injury is the delivery of cardiotoxin (purified from the venom of the \textit{Naja nigricollis} snake) into the hindlimb skeletal muscle of the mouse (53, 63, 134). The intramuscular injection of 100 \(\mu\)l of 10 \(\mu\)M cardiotoxin into the gastrocnemius muscle results in 80–90\% muscle degeneration (Fig. 5). After cardiotoxin-induced injury, satellite cells become activated within 6 h of injury (Garry, unpublished observations). In response to locally released growth factors from injured myotubes and macrophages, the satellite cells proliferate extensively within 2–3 days of injury (63, 64). Approximately 5 days after injury, the satellite cells withdraw from the cell cycle and either self-renew or form differentiated myotubes that contain a central nucleus (63, 64). With the use of this cardiotoxin-induced injury protocol, the architecture of the injured muscle is largely restored within 10 days after injury.

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**Fig. 5. Skeletal muscle response to toxin-induced injury.** A: transverse section of the adult gastrocnemius muscle stained with hematoxylin and eosin at defined intervals following cardiotoxin-induced injury. After cardiotoxin delivery, there is evidence of extensive myonecrosis and edema of the myofibers (12 h; denoted by \(a\)). A hypercellular response (proliferating satellite cells and inflammatory cells) is observed within 2 days of injury. Muscle regeneration is evident within 5 days of injury. At this time, newly regenerated myofibers are evident as small, basophilic, centrally-nucleated myofibers (denoted by \(b\)). The architecture of the muscle is largely restored within \(\sim\)10 days following injury. The newly regenerated myofiber displays numerous centrally aligned nuclei, demonstrating the fusion of many satellite cells to form a single myofiber (hyperplasia) as denoted by the myofiber designated \(c\). B: schematic diagram emphasizing the temporal pattern of satellite cell proliferation and muscle differentiation following a chemically induced injury of adult mouse skeletal muscle. Bar = 100 \(\mu\)m.
injury (Fig. 5). The complete profile of intrinsic and extrinsic cues that regulate the satellite cell population during muscle regeneration remains unclear. Therefore, the use of reproducible experimental injuries such as cardiotoxin-induced injury will be important to evaluate and define the regulation of the satellite cell population in molecular and physiological myopathic models. Additional myonecrotic agents such as notoxin have been used with similar success (107), resulting in a well-characterized regenerative response in skeletal muscle.

**SATELLITE CELLS AS MUSCLE PRECURSOR CELLS OR STEM CELLS**

Myogenic satellite cells have a tremendous proliferative capacity and are capable of self-renewal. These tissue-specific progenitor cells or satellite cells are important in the maintenance and regeneration of skeletal muscle. Progenitor cells that are resident in other adult tissues have stem cell characteristics, as they are capable of self-renewal and multilineage differentiation along a specified molecular pathway (61, 194, 195). For example, Clarke et al. (28) revealed that neural stem cells isolated from the adult mouse brain had a very broad developmental capacity and could contribute to virtually all tissues when delivered into the developing embryo. These results suggest that adult stem cells or progenitor cells are capable of dedetermination and/or transdifferentiation to adopt alternative lineages in a permissive environment. Unlike the neural stem cell population, the potential of the satellite cell pool in adult skeletal muscle remains incompletely defined. Further definition of the gene expression profile of the satellite cell population and an enhanced understanding of the molecular events responsible for satellite cell development, activation, proliferation, and self-renewal will define the stem cell characteristics of the satellite cell.

**SIDE POPULATION CELLS**

Recent studies have identified a population of pluripotent stem cells from adult skeletal muscle termed side population (SP) cells. Skeletal muscle- and bone marrow-derived SP cells are isolated using a DNA-binding dye (Hoechst 33342) and dual-wavelength flow cytometric analysis (67, 68, 77). This method, which relies on the differential ability of the SP cells to efflux the Hoechst dye, defines a small and homogeneous population of cells that can adopt alternative fates in permissive environments (77, 88). For example, Guszoni et al. (77) demonstrated that SP cells isolated from adult bone marrow were able to reconstitute the irradiated *mdx* mouse bone marrow, and later these cells were recruited from the bone marrow to participate in muscle repair. Furthermore, Jackson et al. (88) utilized the same protocol to isolate muscle SP cells from adult mice and observed that as few as 100 SP cells could reconstitute the entire bone marrow of a lethally irradiated mouse. These results demonstrate that adult skeletal muscle contains a stem cell population that can be purified based on the exclusion of Hoechst dye and can adopt alternative fates. Further studies are necessary to determine whether the SP cells are satellite cell progenitors, a subpopulation of the satellite cells, or an independent progenitor cell population that are resident in skeletal muscle.

Alternative sources of proliferating myoblasts have recently been described. Odellberg et al. (136) recently reported that terminally differentiated myoblasts are capable of dedifferentiation to form myoblasts when exposed to the homeobox-containing transcriptional repressor, *msx1*. Furthermore, in response to appropriate cues (i.e., conditioned media), these dedifferentiated myoblasts have the capacity to adopt alternative phenotypes. Collectively, these studies are challenging the established developmental paradigms regarding satellite cell biology and the plasticity or the potential of the terminally differentiated myotube for dedifferentiation. Further studies are necessary to define the molecular regulation of this dedifferentiation process but clearly have applications for the treatment of myopathies including DMD.

**FUTURE PROSPECTS**

Since the initial description of the satellite cell approximately 40 years ago, a number of anatomic and physiological studies have established the importance of this cellular pool in the growth, remodeling, and regeneration of adult skeletal muscle. The profile of gene expression that regulates the cell cycle progression of the satellite cell from a quiescent to an activated and proliferating state remains ill-defined. Despite its complexity, future challenges for this field will include the definition of the development, maintenance, self-renewal, and potentiality of the satellite cell population. These challenges will require an integration of each of the biological disciplines, including the use of increasingly powerful molecular biological tools.

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**REFERENCES**


