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Cellular and Molecular Signatures of Muscle Regeneration: Current Concepts and Controversies in Adult Myogenesis

Review

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Adult skeletal muscle generates force in a controlled and directed manner through the contraction of highly specialized, postmitotic, multinucleated myofibers. Lifelong muscle function relies on maintenance and regeneration of myofibers through a highly regulated process beginning with activation of normally quiescent muscle precursor cells and proceeding with formation of proliferating progenitors that fuse to generate differentiated myofibers. In this review, we describe the historical basis and current evidence for the identification of satellite cells as adult muscle stem cells, critically evaluate contributions of other cells to adult myogenesis, and summarize existing data regarding the origins, genetic markers, and molecular regulation of satellite cells in normal, diseased, and aged muscle.

Satellite Cells as Adult Muscle Precursor Cells and Candidate Muscle Stem Cells

Adult skeletal muscle possesses remarkable regenerative capacity, and large numbers of new myotubes normally are formed in only a few days after acute muscle damage (Bintliff and Walker, 1960; LeGros Clark, 1946; Walton and Adams, 1956; Weber, 1863). Early hypotheses proposed that new myofibers were generated via budding of myotubes from existing, injured fibers (LeGros Clark, 1946; Volkmann, 1893); however, further study has demonstrated instead that this rapid repair occurs through the differentiation and subsequent cell fusion of myogenically specified mononuclear precursor cells contained within a population of "satellite cells" positioned between the plasma membrane and the surrounding basal lamina of mature, differentiated muscle fibers (Mauro, 1961; Snow, 1978). Alexander Mauro first proposed in 1961 that satellite cells might represent "dormant myoblasts" left over from embryonic muscle development and capable of recapitulating the developmental program of skeletal myogenesis in response to muscle damage (Mauro, 1961). Subsequent studies, in both the chick (Konigsberg, 1963) and mouse (Yaffe, 1969), demonstrated that multinucleated myotubes could indeed be generated in vitro from single myogenic precursor cells, and that these precursors ultimately derived from muscle satellite cells (Bischoff, 1975). Furthermore, in vivo-labeled (Snow, 1978), and clonally cultured in vitro-labeled (Lipton and Schultz, 1979), satellite cells were shown to participate in the regeneration of damaged muscle when transplanted in vivo, contributing almost exclusively via fusion with pre-existing myofibers. Pulse-chase experiments using a single dose of tritiated thymidine to label dividing cells indicated that DNA synthesis among sublaminar nuclei was limited to satellite cell nuclei, and that true muscle nuclei do not undergo mitosis (Moss and Leblond, 1970). Although these methods labeled satellite cells relatively infrequently, indicating the relative quiescence of satellite cells (Schultz et al., 1978), in some cases DNA label from satellite cells marked during the pulse phase eventually appeared in myonuclei during the chase phase, demonstrating the capacity of at least some satellite cell progeny to incorporate into existing myofibers, even in the absence of acute tissue injury (Moss and Leblond, 1970). Such studies formed the basis for our current view of muscle satellite cells as the primary mediators of postnatal muscle growth and repair. These cells respond to regenerative cues, such as injury or exercise, by proliferating to form myoblasts, which divide a limited number of times before terminally differentiating and fusing to form multinucleated myotubes (reviewed in Morgan and Partridge, 2003; Sloper and Partridge, 1980) (see Figure 1A). The life-long regenerative capacity of satellite cells implies that they can be robustly and perpetually renewed while maintaining the ability to generate differentiated progeny and suggests that they may represent an adult stem cell population for skeletal muscle.

Stem cells are thought to exist in many adult tissues capable of regeneration and are defined by their unique capacity to both self-renew and differentiate. Adult stem cells have been best characterized in the mammalian blood-forming system, where clonogenic, multipotent hematopoietic stem cells (HSC) have been prospectively isolated from bone marrow and demonstrate at the single-cell level the capacity to regenerate the entire hematopoietic system (reviewed in Kondo et al., 2003). While it is clear that satellite cells likewise contain unspecified precursor cells capable of extensive proliferation and differentiation to generate mature myofibers, to formally establish that they indeed function as adult stem cells, it will be necessary to demonstrate that *individual* cells within the satellite cell pool maintain both self-renewal and differentiation potential in vivo. Although this clonal analysis is still lacking, as outlined below, multiple lines of evidence from in vitro and in vivo studies do suggest that muscle stem cells are contained within at least a subset of satellite cells.

First, as noted above, satellite cell number and regenerative capacity normally remain nearly constant through multiple cycles of injury and repair, suggesting satellite cell self-renewal. In addition, studies of isolated single myofibers and primary satellite cells maintained in culture have revealed that some cultured sat-

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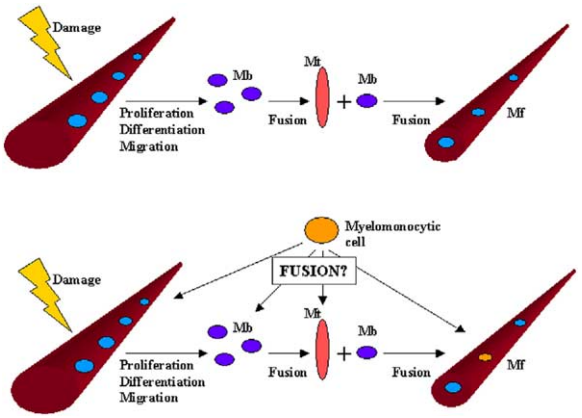

Figure 1. Regeneration of Skeletal Muscle via Cell-Cell Fusion

(A) Repair by endogenous myogenic satellite cells occurs in two phases. First, a subset of differentiated myoblasts (Mb) fuse together to form a nascent myotube (Mt). Next, the Mt attracts and fuses with additional Mb and, with further accretion of nuclei, eventually forms a mature myofiber (Mf).

(B) Contributions from hematopoietic cells. The stage at which heterotypic fusion of myelomonocytic blood-lineage cells with muscle-lineage cells may occur in regenerating muscle has not been defined but could involve interactions with damaged Mf, proliferating Mb, nascent Mt, or newly formed Mf.

ellite cells form clusters that contain both differentiated progeny and cells that regain a phenotype characteristic of quiescent satellite cells (Pax-7$^+$ and MyoD$^-$ or myogenin$^-$) (Olguin and Olwin, 2004; Zammit et al., 2004). Furthermore, populations of neonatal, satellite cell-derived myoblasts have been shown to generate both differentiated myofibers and functional satellite cells in vivo following intramuscular transplantation (Blaveri et al., 1999; Heslop et al., 2001). In ex vivo explant cultures, satellite cells activated by muscle injury give rise to intermediate progenitor cells expressing the myogenic transcription factor Pax-3, which divide asymmetrically and differentiate into Pax-3$^-$, Myf-5^{hi}, desmin^{hi} myoblasts (Conboy and Rando, 2002). This progression along a myogenic lineage in adult muscle resembles embryonic myogenesis, where Pax-3 induces the expression of Myf-5 and MyoD (Maroto et al., 1997), and Pax-3$^+$ pre-myoblast progenitor cells delaminate from somites and migrate into the limb buds to generate fusion-competent myoblasts (Goulding et al., 1994). Finally, the most compelling evidence for the stem cell nature of satellite cells comes from recent studies in which rigorously purified, single, intact myofibers, containing an average of only 7–22 associated satellite cells, were transplanted into the radiation-ablated muscle of immunocompromised, dystrophic (*mdx*-nude) hosts (Collins et al., 2005). The grafted fibers could give rise to over 100 new myofibers, contributing an estimated 25,000–30,000 differentiated myonuclei and, moreover, could regenerate substantially expanded (up to 10-fold) numbers of functional Pax-7$^+$ satellite cells in vivo. These donor-derived satellite cells persisted in the skeletal muscle for at least several weeks and could be reactivated and expanded in response to additional muscle injury (Collins et al., 2005).

Taken together, these data strongly suggest that satellite cells represent self-renewing adult muscle stem cells and alone are sufficient for muscle repair. This conclusion likely will be strengthened by ongoing studies of the capacity for and regulation of satellite cell self-renewal, particularly those testing the continued regenerative potential of single or clonally marked satellite cells in serial transplantations.

Developmental Origins of Skeletal Muscle and Satellite Cells

Myogenic precursors are specified during development by signals emanating from neighboring cells of the notochord, neural tube, and dorsal ectoderm. This specification depends critically on the function of myogenic transcription factors, such as Pax-3 and Pax-7 (Borycki et al., 1999; Cossu et al., 1996a, 1996b; Goulding et al., 1994). Once committed, somite-derived cells migrate to multiple sites of embryonic myogenesis, begin to express the myogenic basic helix-loop-helix transcription factors Myf-5 and MyoD (Birchmeier and Brohmann, 2000), and differentiate into muscle fibers. Somite-derived myogenic progenitors that do not differentiate into myofibers at this time have been suggested instead to be retained into adulthood as muscle satellite cells (Armand et al., 1983; Mauro, 1961; Yablonka-Reuveni et al., 1987). This concept recently has been confirmed in two papers in which myogenic reporter strains and cell-lineage tracing experiments demonstrated that avian (Gros et al., 2005) and mouse (Relaix et al., 2005) embryonic and fetal myogenic progenitor cells arise from the central dermomyotome, following generation of the primary myotome. Importantly, neonatal Pax-7$^+$ progenitor cells found in the satellite cell position were shown to originate also from the avian embryonic dermomyotome, indicating that some of the precursor cells that contribute to embryonic and fetal muscle are retained after birth as satellite cells (Gros et al., 2005). In rapidly growing neonatal muscle, nuclei of satellite cells and myoblasts comprise ~30% of myofiber-associated nuclei, but after cessation of muscle growth, quiescent satellite cells represent only ~5% of adult myofiber nuclei (Cardasis and Cooper, 1975). It remains to be determined, however, whether the quiescent satellite cells in adult muscle have the same developmental origin as embryonic, fetal, and neonatal cells, or, alternatively, if proliferating dermomyotome-derived myofibers-associated progenitors are exhausted during the muscle growth, and subsequent regeneration of the adult muscle invokes a distinct lineage of precursor cells. Additional analysis of myogenic reporter markers (Relaix et al., 2005) in older animals, and after repeated rounds of muscle injury and regeneration, will be particularly informative to address this issue and also may give further insight into the self-renewal potential of embryonically specified dermomyotome-derived satellite cells.

Phenotypic and Functional Heterogeneity of Satellite Cells

Satellite cells are classically defined by their position beneath the basal lamina and by their ability to undergo myogenic differentiation (Beauchamp et al., 2000; Mauro,

1961). However, accumulating evidence suggests that the satellite cell compartment contains cells of distinct ontogeny and function. First, although several markers have been associated with satellite cells, no single marker defines all satellite cells. For example, while most satellite cells express the surface protein CD34 (Beauchamp et al., 2000; Conboy and Rando, 2002; Sherwood et al., 2004a), they can variably express other surface markers (Sherwood et al., 2004a), as well as myogenic transcription factors, such as Pax-7, MyoD, and Myf-5 (Beauchamp et al., 2000; Cornelison and Wold, 1997; Zammit et al., 2004). CD34 and Pax-7 identify quiescent satellite cells, and Pax-7, M-Cadherin, MyoD, and Myf-5 are actually upregulated with differentiation of satellite cells into myoblasts (Morgan and Partridge, 2003; Seale et al., 2000, 2004). This heterogeneity of marker expression may reflect functional differences among satellite cells or distinct stages of myogenic lineage specification or may distinguish myogenic from nonmyogenic cell types within myofiber compartment. In this regard, it is interesting that in vitro studies have shown that some cells emerging from explanted single myofibers can express markers of osteocytes or adipocytes, rather than myogenic markers (Asakura et al., 2001; Csete et al., 2001). Recent studies from our group (Sherwood et al., 2004a) and others (Shefer et al., 2004) have indicated that single cells from within the satellite-cell compartment exhibit mutually exclusive abilities to generate either myogenic or fibroblastic and adipogenic colonies in clonal in vitro assays. Importantly, activated immune or inflammatory cells may also populate the satellite-cell compartment, infiltrating beneath the basal lamina of damaged muscle fibers (Stauber et al., 1988), although such infiltrating hematopoietic cells display no myogenic activity (Sherwood et al., 2004a). Finally, intrinsic differences in proliferation (Beauchamp et al., 1999; Rantanen et al., 1995; Rouger et al., 2004; Yablonka-Reuveni et al., 1987), differentiation (Rantanen et al., 1995; Zammit et al., 2004), and fusogenic capacity (Rouger et al., 2004) among individual satellite cells have been reported. More detailed studies employing prospective isolation and/or clonal marking to further delineate the precise lineage relationships, cellular interaction, and myogenic capacities of these distinct populations of myofiber-associated cells will greatly enhance our understanding of their normal roles and relative importance during muscle regeneration.

Adult Myogenic Cells Distinct from Satellite Cells

Recent reports have suggested that adult skeletal muscle progenitors distinct from satellite cells may function in some models of muscle injury and repair. For example, muscle-resident side population (muSP) cells, defined by their ability to exclude Hoechst 33342 (Asakura et al., 2002), have been shown to contribute to myofibers when injected intramuscularly (McKinney-Freeman et al., 2002) or when cocultured with myoblasts (Asakura et al., 2002), although these cells lack myogenic activity when cultured alone (Asakura et al., 2002). Similarly, although they do not generate myogenic cells when cultured alone, interstitial muscle-resident $CD45^{+}Sca\text{-}1^{+}$ cells reportedly acquire myogenic activity when cocultured with primary myoblasts or in response to muscle injury or Wnt signaling (Polesskaya et al., 2003). Finally, cells with high proliferative potential and surprisingly broad differentiation capacity, and thus termed "muscle-derived stem cells" (MDSC) or "multipotent adult progenitor cells" (MAPC), have been isolated following prolonged culture of cells from muscle (Cao et al., 2003; Jiang et al., 2002; Qu-Petersen et al., 2002).

Descriptions of these distinct subsets of myogenic cells have raised the possibility that multiple mechanisms may support adult skeletal muscle regeneration. Yet, as compared to conventional satellite cells, many of these populations, with the notable exception of cultured MDSC (Qu-Petersen et al., 2002), display vanishingly small myogenic potential. In addition, in all cases, the contribution of these cells to the maintenance or repair of skeletal muscle under physiologic conditions is uncertain, and their therapeutic potential has not been clearly established. Moreover, recent data suggest that satellite cells are alone sufficient to mediate extensive regeneration of damaged adult skeletal muscle in vivo (Collins et al., 2005). Thus, in further assessing the intrinsic myogenic function of these populations, it will be essential to exclude the possibility that their activity derives from "contamination" with a small subset of highly myogenic satellite cells or their progeny or from a change in developmental potential induced by cell culture. The use of lineage-tracing methods (Gros et al., 2005; Relaix et al., 2005) and single myofiber transplantation (Collins et al., 2005) will be a key first step in determining the myogenicity of these populations in relationship to conventional satellite cells.

Bone marrow cells (Ferrari et al., 1998; Fukada et al., 2002; Gussoni et al., 1999; LaBarge and Blau, 2002), and even single hematopoietic stem cells (Camargo et al., 2003; Corbel et al., 2003; Sherwood et al., 2004b), also have been reported to contribute to myofibers when injected directly into injured muscle or intravenously into irradiated injured or dystrophic animals. The frequency with which these unexpected contributions to skeletal muscle have been detected has varied widely and, while generally quite low (<1% of total myofibers; Camargo et al., 2003; Corbel et al., 2003; Ferrari et al., 1998; Gussoni et al., 1999; Sherwood et al., 2004b), has been reported in some cases to reach levels of 5%–12% of differentiated cells (Abedi et al., 2004; LaBarge and Blau, 2002). In this regard, it is worth noting that the method of detection of donor-derived myofibers can significantly influence the estimation of donor contributions, and assay systems employing highly diffusible markers, such as GFP (Jockusch and Voigt, 2003), cannot accurately quantify the numbers of donor myonuclei incorporated due to spread of the marker throughout the myofiber. In any case, such observations initially generated a great deal of excitement, suggesting that bone marrow could represent a reasonably accessible, novel source of regenerative cells for muscle repair; however, as with other nonsatellite cell populations, the physiological significance of bone marrow contributions to skeletal muscle remains uncertain (Ferrari et al., 2001; Gussoni et al., 2002), and accumulating evidence suggests that these events may in fact represent an accidental or even pathological re-

sponse elicited by severe muscle damage and inflammation (see below).

Interestingly, while early efforts aimed at eliciting myogenic activity from bone marrow cells focused largely on the hematopoietic lineages, recent studies have suggested that nonhematopoietic elements isolated from adult bone marrow or from embryonic sites of hematopoietic development might eventually be harnessed for therapeutic skeletal muscle regeneration. For instance, cultured "mesangioblasts" (Minasi et al., 2002), a subset of blood vessel-associated cells originating from the embryonic dorsal aorta region, which have been shown to generate multiple mesodermal cell types following in vivo transplantation, can, when injected intra-arterially into dystrophic α-sarcoglycan knockout mice, contribute to myofiber formation and significantly improve muscle function (Sampaolesi et al., 2003). In addition, clonal, cultured marrow-derived stromal cells, lacking expression of CD34, c-Kit, and CD45, have been shown to participate in myogenesis in vitro and in vivo, regenerating myofibers and sublaminar Pax-7$^+$ satellite cells in immunocompromised dystrophic mice (Dezawa et al., 2005). Significantly, the myogenic contributions from marrow stromal cells observed in this study were much more robust than those typical of BM-derived HSC or hematopoietic progenitor cell subsets; however, it is important to note that the activation of a myogenic program by these stromal cells absolutely required in vitro induction, first by culturing the cells with certain growth factors and then by ectopic expression of constitutively active Notch-1 (Dezawa et al., 2005). Thus, while this work suggests a promising therapeutic potential of marrow stromal cells, these data do not necessarily suggest that analogous populations of marrow cells normally contribute to physiologic muscle regeneration.

In summary, while under particular conditions some cells apparently distinct from satellite cells can contribute to muscle tissue, the preponderance of evidence indicates that the myogenic activity normally responsible for robust adult muscle regeneration is largely restricted to sublaminar CD34$^+$ satellite cells.

Mechanisms of Bone Marrow Contribution to Skeletal Muscle: Cell Fusion or Transdifferentiation?

In most studies in which hematopoietic or bone marrow-derived contributions to skeletal muscle have been detected, significant muscle injury has been necessary, except in particular muscles (e.g., *panniculus carnosus*) (Corbel et al., 2003; Sherwood et al., 2004b). The mechanisms underlying these contributions have been an area of intense research. While initial reports appeared to favor the direct "transdifferentiation" of transplanted BM cells to generate satellite cells (Fukada et al., 2002; LaBarge and Blau, 2002), more recent studies have indicated that donor-marker-expressing myofibers arise via fusion of donor hematopoietic cells with host muscle cells (Camargo et al., 2003; Doyonnas et al., 2004; Sherwood et al., 2004b) (Figure 1B). While the precise cell types involved in these fusion events have not been fully defined, transplantation of BM cells from transgenic mice expressing Cre in a hematopoietic lineage-restricted manner suggests that at least one of the fusion partners is likely to be a committed blood myeloid cell (Camargo et al., 2003; Doyonnas et al., 2004). The stage of differentiation at which muscle-lineage cells participate in heterotypic cell fusions with infiltrating hematopoietic cells remains unknown (Figure 1B). However, when considering the mechanism by which hematopoietic cells fuse with muscle cells, it is important to remember that normally muscle is repaired by fusion of myoblasts with each other, with nascent myotubes, and/or with damaged multinucleated fibers. Thus, macrophages and other inflammatory cells known to infiltrate injured muscle fibers (Stauber et al., 1988) almost certainly are exposed to physiologic fusogenic signals. Significantly, macrophages themselves are known to undergo cell-cell fusion both physiologically, to generate osteoclasts, and pathologically, to generate multinucleated giant cells (Vignery, 2000). Therefore, heterotypic cell fusion between endogenous muscle cells and infiltrating blood cells may occur via mechanisms that normally allow the homotypic fusion of these cells in the context of tissue maintenance and repair. At present the molecular mediators, as well as the overall significance, of these rare events remain unclear. Future studies using coculture or transplantation models will be required to identify the secreted factors, cell-surface receptors, and signaling pathways involved in blood-cell/muscle-cell fusion. Knowledge of these mechanisms ultimately should allow experiments to block its occurrence and thereby test its physiologic importance.

Prospective Isolation of Muscle Precursor Cells

We recently reported a methodology that permits the prospective isolation by fluorescence-activated cell sorting (FACS) of highly myogenic muscle precursor cells from other cell populations contained within the satellite-cell compartment (Sherwood et al., 2004a). Combinatorial analysis of multiple cell-surface markers indicated that autonomously myogenic colony-forming cells (CFC) were highly enriched among the CD45$^-$Sca-1$^-$Mac-1$^-$CXCR4$^+$β1-integrin$^+$ (CSM4B) subset of myofiber-associated satellite cells, and that individual CSM4B cells efficiently form myogenic colonies, which express myosin heavy chain (MyHC) upon induction of myogenic differentiation in vitro. CSM4B cells are contained within a population of cells (CD45$^-$Sca-1$^-$CD34$^+$) that also generates myofibers in vivo upon intramuscular injection and expresses mRNA encoding the myogenic transcription factors MyoD, Myf-5, and Pax-7 (Sherwood et al., 2004a).

The precise relationship of the CSM4B subset of myofiber-associated cells to previously described satellite cells is clearly of interest in synthesizing recent literature and developing a further understanding of skeletal muscle biology and regeneration. These cells coexpress CD34 and c-met, previously reported satellite-cell surface markers (Beauchamp et al., 2000; Cornelison et al., 2001), but are not contained in c-kit$^+$, CD13$^+$, CD71$^+$, Flk-1$^+$, CD105$^+$, CD44$^+$, α1-integrin$^+$, or α6-integrin$^+$ populations of myofiber-associated cells. They also fail to express the pan-hematopoietic marker CD45 and the surface protein Sca-1, in either resting or regenerating (2 days following cardiotoxin injection) muscle; both CD45 and Sca-1 have been reported by others (Polesskaya

et al., 2003) to identify a subset of Wnt-responsive muscle-regenerative cells distinct from satellite cells and likely residing in the muscle interstitium. Finally, CSM4B cells are not derived from transplanted bone marrow cells and are not repopulated from the circulation at detectable levels, indicating that they likely arise from and are maintained by a lineage of cells distinct from bone marrow-derived, hematopoietic, and fibroblastic cells also present in muscle. These data are consistent with previous studies in muscle transplantation systems indicating that muscle repair does not generally involve the recruitment of regenerative cells from a distance (Jockusch and Voigt, 2003; Washabaugh et al., 2004).

Other groups similarly have undertaken phenotypic analysis and prospective isolation of myogenic satellite-cell populations. Using Pax-3/GFP "knockin" mice, in which a GFP marker reports transcriptional activity of the Pax-3 locus, Montarras and colleagues also isolated a highly regenerative population of myogenic precursor cells. Like CSM4B cells, these Pax-3$^+$ cells are largely CD34$^+$, CD45$^-$, and Sca-1$^-$ (Montarras et al., 2005). In these studies, intramuscular delivery of only a few thousand freshly sorted CD34$^+$CD45$^-$Sca-1$^-$ cells yielded substantial regeneration of normal myofibers in irradiated *mdx*-nude recipients; however, strikingly, cell culture prior to transplant dramatically reduced the efficiency of muscle engraftment (by as much as 10-fold).

Biochemical Pathways Regulating Muscle Regeneration

Muscle remodeling involves myogenesis, reinnervation, and revascularization and is regulated by multiple biochemical pathways, including those initiated by inflammatory cytokines, growth factors, and the evolutionarily conserved Notch, Wnt, and Sonic Hedgehog (Shh) signaling pathways (Conboy and Rando, 2002; Husmann et al., 1996; Pola et al., 2003; Polesskaya et al., 2003; Tidball, 2005). Muscle repair coincides with injury-induced inflammation, and some inflammatory cytokines, such as IL-4, LIF, TGF-β, IL-6, and TNF-α regulate myogenic potential (Tidball, 2005). Damaged muscle produces monocyte and macrophage chemoattractants, and blockade of inflammatory cell infiltration impairs muscle regeneration (Chazaud et al., 2003; Jejurikar and Kuzon, 2003; Lescaudron et al., 1999), possibly due to a reduction in macrophage-secreted factors inducing myoblast proliferation (Bondesen et al., 2004; Robertson et al., 1993).

In addition to initiating the inflammatory response, injury promotes the release of growth factors that bind to extracellular matrix (ECM) proteins, such as proteoheparan sulfates (Husmann et al., 1996). This process involves the activity of matrix metalloproteinases, recently shown to play a role in muscle repair (Carmeli et al., 2004). The most studied growth factors participating in muscle maintenance and regeneration are FGFs, HGF, IGF-1, and GDF8/myostatin (Heszele and Price, 2004; Husmann et al., 1996; Lee, 2004; Miller et al., 2000). FGF-2 and HGF promote proliferation of myogenic progenitors and delay their differentiation, in part by inhibiting the expression of myogenic regulatory factors, such as MyoD (Maley et al., 1994; Miller et al., 2000). Both growth factors require heparan sulfate proteoglycans for signaling via their receptors, and syndecan-3 and -4 have been identified as the relevant cell-surface proteoglycans expressed by satellite cells (Cornelison et al., 2001). IGF-1 promotes myogenic differentiation and enhances protein synthesis in differentiated myofibers by activating the translation factor 4E-BP and the ribosomal protein S6 kinase (p70S5K) and by inhibiting muscle-specific E3 ligases that promote protein degradation (reviewed in Heszele and Price, 2004). These mechanisms and the antiapoptotic effects of IGF-1 via the suppression of caspases and activation of Akt (Downward, 2004; Lawlor and Rotwein, 2000) likely explain why IGF-1 attenuates experimentally induced muscle wasting (Shavlakadze et al., 2005). On the other end of the proliferative spectrum, the muscle-specific TGF-β family member, GDF8, inhibits cell-cycle progression in myogenic progenitors during embryonic development and in adult muscle via induction of p21 and suppression of the cyclin-dependent kinase CDK25 (McCroskery et al., 2003; McPherron et al., 1997; Thomas et al., 2000; Zimmers et al., 2002).

The appropriate expansion followed by the timely differentiation of myogenic progenitor cells during muscle repair appear to be regulated by the same conserved mechanisms that orchestrate embryonic organogenesis. Namely, proliferation of satellite cells in response to muscle injury is positively regulated by the Notch pathway, while terminal myogenic differentiation of these cells requires the inhibition of Notch by its intracellular antagonist Numb (Conboy and Rando, 2002). Notch receptor is expressed in quiescent satellite cells, and Notch signal transduction responsible for satellite-cell activation is initiated by the upregulation of Notch ligand, Delta, on the fibers adjacent to the damaged muscle and on the satellite cells themselves (Conboy et al., 2003; Conboy and Rando, 2002). The recent demonstration that BM-derived stromal cells become myogenic after stable expression of constitutively active Notch-1 (Dezawa et al., 2005) may suggest that activation of the Notch pathway generally regulates specification of organ precursor cells toward a myogenic lineage when other myogenic factors, such as FGF-2, are present.

In addition to Notch, Shh mRNA and protein, as well as the Patched receptor, become upregulated in regenerating skeletal muscle (Pola et al., 2003). Moreover, ectopic Shh appears to ameliorate experimentally induced muscle atrophy (Alzghoul et al., 2004), thus demonstrating that yet another classic regulator of embryonic development likely also participates in adult myogenesis. Additionally, Wnt signaling has been reported to be important for the presence of CD45$^+$ cells in regenerating adult muscle, although the physiologic myogenic potential of these cells remains unclear (Polesskaya et al., 2003; Sherwood et al., 2004a). Future experiments that decipher how muscle regulates its own inflammatory response and clarify the temporal and spatial crosstalk between Notch, Shh, and Wnt pathways will be instrumental for providing a better understanding of how cell fate is determined during muscle repair.

Satellite-Cell Activity in Diseased Muscle

In certain pathological states, including congenital myopathies, denervation, and muscle atrophy, satellite-cell numbers and proliferative potential may decrease (Jejurikar and Kuzon, 2003). In muscular dystrophy (MD), repeated cycles of muscle regeneration, brought on by repeated loss of differentiated tissue, may lead to an early loss of the proliferative potential of satellite cells in these patients, and a subsequent failure to maintain muscle homeostasis (Luz et al., 2002). Although the underlying mechanism for this loss of satellite-cell responsiveness in diseased muscle has not been fully elucidated, these findings indicate that under particular circumstances satellite cells may be functionally exhausted. Satellite-cell exhaustion may relate, at least in part, to shortening of telomere ends after repeated rounds of DNA replication (Collins et al., 2003; Di Donna et al., 2003), to recurrent exposure to inflammatory conditions and/or oxidative stress (Renault et al., 2002), to an accumulation of mutations in key satellite-cell regulatory genes, introduced during repeated rounds of proliferation, or to a combination of these and other factors. Nonmyogenic cells in the muscle may also contribute to failed muscle regeneration, as fibroblasts in dystrophic patients have been shown to secrete increased levels of IGF-1 binding proteins, which could sequester this cytokine away from myogenic cells (Melone et al., 2000). A better understanding of the dynamic interplay among distinct populations of cells resident in the muscle and recruited by muscle damage will aid in developing a full picture of the complex cellular networks responsible for myogenesis in healthy and diseased muscle and for designing therapeutic strategies to promote muscle repair.

Age-Related Changes in the Molecular Regulation of Satellite Cell Activity

One characteristic of aging is a decline in the ability of organ stem cells to repair damaged tissues. Adult skeletal muscle is a perfect example of a tissue that robustly regenerates throughout adult life but fails to do so in old age (Grounds, 1998). The reason for this decline in regenerative potential is not completely understood and may involve both intrinsic molecular changes in the stem cells themselves and/or alterations in their aged environment.

As mentioned above, muscle repair relies on Notch activity, which is necessary and sufficient for the activation of satellite cells (Artavanis-Tsakonas et al., 1999; Conboy et al., 2003; Conboy and Rando, 2002). Importantly, Notch receptor continues to be expressed in aged satellite cells, while injury-specific induction of the Notch ligand Delta, and therefore subsequent signal transduction, fail with age, resulting in grossly inefficient regeneration of old muscle tissue (Conboy et al., 2003). Strikingly, productive tissue repair can be restored to old muscle by enforced activation of Notch, while the repair of young muscle is severely perturbed when Notch signaling is inhibited (Conboy et al., 2003). Therefore, it seems that Notch is the key age-related determinant of muscle regenerative potential.

Is the age-related decline in satellite cell regenerative potential intrinsic or dependent on the cell environment? In early muscle transplantation studies, small minced or intact muscle from young or old rodents was transplanted into either young or old muscle beds, and the ability of donor muscle pieces to regenerate in the middle of either young or old host limbs was examined. Amazingly, the efficiency of muscle regeneration was clearly determined in these experiments by the age of the host environment, rather than by the age of the muscle donor (Carlson and Faulkner, 1989; Zacks and Sheff, 1982). In these important studies, the small transplanted muscle was physically isolated from the host satellite cells, revealing the effects of prevalent local and organismal environments on the regenerative potential of the donor satellite cells.

In more recent studies, the age of the systemic environment likewise dominated over the intrinsic age-related regenerative properties of satellite cells when the efficiency of muscle repair was examined in young and old mice with a shared blood circulation (Conboy et al., 2005). Significantly, regeneration-specific Delta-Notch signaling, appropriate activation of satellite cells, and general success in muscle repair were all rejuvenated by the exposure of aged tissue to a young systemic milieu (Conboy et al., 2005). In concert with the above-mentioned pivotal role of satellite cells as muscle stem cells, the aged satellite cells endogenous to the old muscle successfully engaged in tissue repair without any recruitment of young cells from the shared circulation (Conboy et al., 2005). These data strongly suggest that the molecular pathways responsible for muscle repair are regulated by systemic factors and that these factors change with age in ways precluding the activation of satellite cells.

Multiple lines of evidence suggest that many cell-intrinsic changes occur with tissue aging, including the accumulation of oxidative damage, a decline in genome maintenance, and diminished mitochondrial function (Ames, 2004; Golden et al., 2002; Hasty et al., 2003). The rejuvenation of aged stem and progenitor cells by the young extrinsic milieu, even in the presence of these age-related changes, suggests the intriguing possibility that the aging of organ stem cells might be regulated extrinsically and that the molecular changes underlying the loss of the tissue-regenerative potential with age can be reversed or overcome if the stem cell niche is young. Future identification of the relevant extrinsic age-related components will be instrumental for therapies aimed at enhancing the regenerative potential of organ stem cells in aged individuals.

Future Avenues and Perspectives

It is quite clear that endogenous skeletal muscle satellite cells associated with myofibers account for most, if not all, physiologic muscle-regenerative potential and likely represent muscle stem cells. Recent advances have allowed a more refined determination of their origin, position in the myogenic cell lineage, and molecular pathways regulating their function. Other avenues of muscle repair, e.g., by bone marrow-derived cells, may exist; however, unambiguous determination of the precise cell types and specific fusion and reprogramming mechanisms involved in this process will be needed in order to establish whether such cells form muscle tis-

sue under physiologic conditions or can be used therapeutically. Current advances in our understanding of the cellular and molecular mechanisms regulating cell-fate determination and tissue specification during adult muscle repair have indicated a remarkable conservation of developmentally regulated signal transduction pathways, and age-related analysis of these pathways indicates that at least some of them deteriorate in old muscle, causing ineffective tissue repair. The identification of age-related systemic factors that regulate the regenerative capacity of organ stem cells will improve our understanding of aging as a conserved biological process and will help to develop therapies for the enhancement of the regenerative potential often lost in old age or disease.

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References

Abedi, M., Greer, D.A., Colvin, G.A., Demers, D.A., Dooner, M.S., Harpel, J.A., Weier, H.U., Lambert, J.F., and Quesenberry, P.J. (2004). Robust conversion of marrow cells to skeletal muscle with formation of marrow-derived muscle cell colonies: a multifactorial process. Exp. Hematol. *32*, 426–434.

Alzghoul, M.B., Gerrard, D., Watkins, B.A., and Hannon, K. (2004). Ectopic expression of IGF-I and Shh by skeletal muscle inhibits disuse-mediated skeletal muscle atrophy and bone osteopenia in vivo. FASEB J. *18*, 221–223.

Ames, B.N. (2004). Delaying the mitochondrial decay of aging. Ann. N Y Acad. Sci. *1019*, 406–411.

Armand, O., Boutineau, A.M., Mauger, A., Pautou, M.P., and Kieny, M. (1983). Origin of satellite cells in avian skeletal muscles. Arch. Anat. Microsc. Morphol. Exp. *72*, 163–181.

Artavanis-Tsakonas, S., Rand, M.D., and Lake, R.J. (1999). Notch signaling: cell fate control and signal integration in development. Science *284*, 770–776.

Asakura, A., Komaki, M., and Rudnicki, M. (2001). Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation *68*, 245–253.

Asakura, A., Seale, P., Girgis-Gabardo, A., and Rudnicki, M.A. (2002). Myogenic specification of side population cells in skeletal muscle. J. Cell Biol. *159*, 123–134.

Beauchamp, J.R., Morgan, J.E., Pagel, C.N., and Partridge, T.A. (1999). Dynamics of myoblast transplantation reveal a discrete minority of precursors with stem cell-like properties as the myogenic source. J. Cell Biol. *144*, 1113–1122.

Beauchamp, J.R., Heslop, L., Yu, D.S., Tajbakhsh, S., Kelly, R.G., Wernig, A., Buckingham, M.E., Partridge, T.A., and Zammit, P.S. (2000). Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J. Cell Biol. *151*, 1221–1234.

Bintliff, S., and Walker, B.E. (1960). Radioautographic study of skeletal muscle regeneration. Am. J. Anat. *106*, 233.

Birchmeier, C., and Brohmann, H. (2000). Genes that control the development of migrating muscle precursor cells. Curr. Opin. Cell Biol. *12*, 725–730.

Bischoff, R. (1975). Regeneration of single skeletal muscle fibers in vitro. Anat. Rec. *182*, 215–235.

Blaveri, K., Heslop, L., Yu, D.S., Rosenblatt, J.D., Gross, J.G., Partridge, T.A., and Morgan, J.E. (1999). Patterns of repair of dystrophic mouse muscle: studies on isolated fibers. Dev. Dyn. *216*, 244–256.

Bondesen, B.A., Mills, S.T., Kegley, K.M., and Pavlath, G.K. (2004). The COX-2 pathway is essential during early stages of skeletal muscle regeneration. Am. J. Physiol. Cell Physiol. *287*, C475–C483.

Borycki, A.G., Li, J., Jin, F., Emerson, C.P., and Epstein, J.A. (1999). Pax3 functions in cell survival and in pax7 regulation. Development *126*, 1665–1674.

Camargo, F.D., Green, R., Capetenaki, Y., Jackson, K.A., and Goodell, M.A. (2003). Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. Nat. Med. *9*, 1520–1527.

Cao, B., Zheng, B., Jankowski, R.J., Kimura, S., Ikezawa, M., Deasy, B., Cummins, J., Epperly, M., Qu-Petersen, Z., and Huard, J. (2003). Muscle stem cells differentiate into haematopoietic lineages but retain myogenic potential. Nat. Cell Biol. *5*, 640–646.

Cardasis, C.A., and Cooper, G.W. (1975). An analysis of nuclear numbers in individual muscle fibers during differentiation and growth: a satellite cell-muscle fiber growth unit. J. Exp. Zool. *191*, 347–358.

Carlson, B.M., and Faulkner, J.A. (1989). Muscle transplantation between young and old rats: age of host determines recovery. Am. J. Physiol. *256*, C1262–C1266.

Carmeli, E., Moas, M., Reznick, A.Z., and Coleman, R. (2004). Matrix metalloproteinases and skeletal muscle: a brief review. Muscle Nerve *29*, 191–197.

Chazaud, B., Sonnet, C., Lafuste, P., Bassez, G., Rimaniol, A.C., Poron, F., Authier, F.J., Dreyfus, P.A., and Gherardi, R.K. (2003). Satellite cells attract monocytes and use macrophages as a support to escape apoptosis and enhance muscle growth. J. Cell Biol. *163*, 1133–1143.

Collins, C.A., Olsen, I., Zammit, P.S., Heslop, L., Petrie, A., Partridge, T.A., and Morgan, J.E. (2005). Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell *122*, 1–13.

Collins, M., Renault, V., Grobler, L.A., St Clair Gibson, A., Lambert, M.I., Wayne Derman, E., Butler-Browne, G.S., Noakes, T.D., and Mouly, V. (2003). Athletes with exercise-associated fatigue have abnormally short muscle DNA telomeres. Med. Sci. Sports Exerc. *35*, 1524–1528.

Conboy, I.M., and Rando, T.A. (2002). The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Dev. Cell *3*, 397–409.

Conboy, I.M., Conboy, M.J., Smythe, G.M., and Rando, T.A. (2003). Notch-mediated restoration of regenerative potential to aged muscle. Science *302*, 1575–1577.

Conboy, I.M., Conboy, M.J., Wagers, A.J., Girma, E.R., Weissman, I.L., and Rando, T.A. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature *433*, 760–764.

Corbel, S.Y., Lee, A., Yi, L., Duenas, J., Brazelton, T.R., Blau, H.M., and Rossi, F.M. (2003). Contribution of hematopoietic stem cells to skeletal muscle. Nat. Med. *9*, 1528–1532.

Cornelison, D.D., Filla, M.S., Stanley, H.M., Rapraeger, A.C., and Olwin, B.B. (2001). Syndecan-3 and syndecan-4 specifically mark skeletal muscle satellite cells and are implicated in satellite cell maintenance and muscle regeneration. Dev. Biol. *239*, 79–94.

Cornelison, D.D., and Wold, B.J. (1997). Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. Dev. Biol. *191*, 270–283.

Cossu, G., Kelly, R., Tajbakhsh, S., Di Donna, S., Vivarelli, E., and Buckingham, M. (1996a). Activation of different myogenic pathways: myf-5 is induced by the neural tube and MyoD by the dorsal ectoderm in mouse paraxial mesoderm. Development *122*, 429–437.

Cossu, G., Tajbakhsh, S., and Buckingham, M. (1996b). How is myogenesis initiated in the embryo? Trends Genet. *12*, 218–223.

Csete, M., Walikonis, J., Slawny, N., Wei, Y., Korsnes, S., Doyle, J.C., and Wold, B. (2001). Oxygen-mediated regulation of skeletal

muscle satellite cell proliferation and adipogenesis in culture. J. Cell. Physiol. *189*, 189–196.

Dezawa, M., Ishikawa, H., Itokazu, Y., Yoshihara, T., Hoshino, M., Takeda, S., Ide, C., and Nabeshima, Y. (2005). Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science *309*, 314–317.

Di Donna, S., Mamchaoui, K., Cooper, R.N., Seigneurin-Venin, S., Tremblay, J., Butler-Browne, G.S., and Mouly, V. (2003). Telomerase can extend the proliferative capacity of human myoblasts, but does not lead to their immortalization. Mol. Cancer Res. *1*, 643–653.

Downward, J. (2004). PI 3-kinase, Akt and cell survival. Semin. Cell Dev. Biol. *15*, 177–182.

Doyonnas, R., LaBarge, M.A., Sacco, A., Charlton, C., and Blau, H.M. (2004). Hematopoietic contribution to skeletal muscle regeneration by myelomonocytic precursors. Proc. Natl. Acad. Sci. USA *101*, 13507–13512.

Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci, E., Stornaiuolo, A., Cossu, G., and Mavilio, F. (1998). Muscle regeneration by bone marrow-derived myogenic progenitors. Science *279*, 1528–1530.

Ferrari, G., Stornaiuolo, A., and Mavilio, F. (2001). Failure to correct murine muscular dystrophy. Nature *411*, 1014–1015.

Fukada, S., Miyagoe-Suzuki, Y., Tsukihara, H., Yuasa, K., Higuchi, S., Ono, S., Tsujikawa, K., Takeda, S., and Yamamoto, H. (2002). Muscle regeneration by reconstitution with bone marrow or fetal liver cells from green fluorescent protein-gene transgenic mice. J. Cell Sci. *115*, 1285–1293.

Golden, T.R., Hinerfeld, D.A., and Melov, S. (2002). Oxidative stress and aging: beyond correlation. Aging Cell *1*, 117–123.

Goulding, M., Lumsden, A., and Paquette, A.J. (1994). Regulation of Pax-3 expression in the dermomyotome and its role in muscle development. Development *120*, 957–971.

Gros, J., Manceau, M., Thome, V., and Marcelle, C. (2005). A common somitic origin for embryonic muscle progenitors and satellite cells. Nature *435*, 954–958.

Grounds, M.D. (1998). Age-associated changes in the response of skeletal muscle cells to exercise and regeneration. Ann. N Y Acad. Sci. *854*, 78–91.

Gussoni, E., Soneoka, Y., Strickland, C.D., Buzney, E.A., Khan, M.K., Flint, A.F., Kunkel, L.M., and Mulligan, R.C. (1999). Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature *401*, 390–394.

Gussoni, E., Bennett, R.R., Muskiewicz, K.R., Meyerrose, T., Nolta, J.A., Gilgoff, I., Stein, J., Chan, Y.M., Lidov, H.G., Bonnemann, C.G., et al. (2002). Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. J. Clin. Invest. *110*, 807–814.

Hasty, P., Campisi, J., Hoeijmakers, J., van Steeg, H., and Vijg, J. (2003). Aging and genome maintenance: lessons from the mouse? Science *299*, 1355–1359.

Heslop, L., Beauchamp, J.R., Tajbakhsh, S., Buckingham, M.E., Partridge, T.A., and Zammit, P.S. (2001). Transplanted primary neonatal myoblasts can give rise to functional satellite cells as identified using the Myf5nlacZl+ mouse. Gene Ther. *8*, 778–783.

Heszele, M.F., and Price, S.R. (2004). Insulin-like growth factor I: the yin and yang of muscle atrophy. Endocrinology *145*, 4803–4805.

Husmann, I., Soulet, L., Gautron, J., Martelly, I., and Barritault, D. (1996). Growth factors in skeletal muscle regeneration. Cytokine Growth Factor Rev. *7*, 249–258.

Jejurikar, S.S., and Kuzon, W.M., Jr. (2003). Satellite cell depletion in degenerative skeletal muscle. Apoptosis *8*, 573–578.

Jiang, Y., Jahagirdar, B.N., Reinhardt, R.L., Schwartz, R.E., Keene, C.D., Ortiz-Gonzalez, X.R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., et al. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. Nature *418*, 41–49.

Jockusch, H., and Voigt, S. (2003). Migration of adult myogenic precursor cells as revealed by GFP/nLacZ labelling of mouse transplantation chimeras. J. Cell Sci. *116*, 1611–1616.

Kondo, M., Wagers, A.J., Manz, M.G., Prohaska, S.S., Scherer, D.C., Beilhack, G.F., Shizuru, J.A., and Weissman, I.L. (2003). Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu. Rev. Immunol. *21*, 759–806.

Konigsberg, I.R. (1963). Clonal analysis of myogenesis. Science *140*, 1273–1284.

LaBarge, M.A., and Blau, H.M. (2002). Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. Cell *111*, 589–601.

Lawlor, M.A., and Rotwein, P. (2000). Coordinate control of muscle cell survival by distinct insulin-like growth factor activated signaling pathways. J. Cell Biol. *151*, 1131–1140.

Lee, S.J. (2004). Regulation of muscle mass by myostatin. Annu. Rev. Cell Dev. Biol. *20*, 61–86.

LeGros Clark, W.E. (1946). An experimental study of regeneration of mammalian striped muscle. J. Anat. *80*, 24–36.

Lescaudron, L., Peltekian, E., Fontaine-Perus, J., Paulin, D., Zampieri, M., Garcia, L., and Parrish, E. (1999). Blood borne macrophages are essential for the triggering of muscle regeneration following muscle transplant. Neuromuscul. Disord. *9*, 72–80.

Lipton, B.H., and Schultz, E. (1979). Developmental fate of skeletal muscle satellite cells. Science *205*, 1292–1294.

Luz, M.A., Marques, M.J., and Santo Neto, H. (2002). Impaired regeneration of dystrophin-deficient muscle fibers is caused by exhaustion of myogenic cells. Braz. J. Med. Biol. Res. *35*, 691–695.

Maley, M.A., Fan, Y., Beilharz, M.W., and Grounds, M.D. (1994). Intrinsic differences in MyoD and myogenin expression between primary cultures of SJL/J and BALB/C skeletal muscle. Exp. Cell Res. *211*, 99–107.

Maroto, M., Reshef, R., Munsterberg, A.E., Koester, S., Goulding, M., and Lassar, A.B. (1997). Ectopic Pax-3 activates MyoD and Myf-5 expression in embryonic mesoderm and neural tissue. Cell *89*, 139–148.

Mauro, A. (1961). Satellite cells of muscle skeletal fibers. J. Biophys. Biochem. *9*, 493–495.

McCroskery, S., Thomas, M., Maxwell, L., Sharma, M., and Kambadur, R. (2003). Myostatin negatively regulates satellite cell activation and self-renewal. J. Cell Biol. *162*, 1135–1147.

McKinney-Freeman, S.L., Jackson, K.A., Camargo, F.D., Ferrari, G., Mavilio, F., and Goodell, M.A. (2002). Muscle-derived hematopoietic stem cells are hematopoietic in origin. Proc. Natl. Acad. Sci. USA *99*, 1341–1346.

McPherron, A.C., Lawler, A.M., and Lee, S.J. (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature *387*, 83–90.

Melone, M.A., Peluso, G., Galderisi, U., Petillo, O., and Cotrufo, R. (2000). Increased expression of IGF-binding protein-5 in Duchenne muscular dystrophy (DMD) fibroblasts correlates with the fibroblast-induced downregulation of DMD myoblast growth: An in vitro analysis. J. Cell. Physiol. *185*, 143–153.

Miller, K.J., Thaloor, D., Matteson, S., and Pavlath, G.K. (2000). Hepatocyte growth factor affects satellite cell activation and differentiation in regenerating skeletal muscle. Am. J. Physiol. Cell Physiol. *278*, C174–C181.

Minasi, M.G., Riminucci, M., De Angelis, L., Borello, U., Berarducci, B., Innocenzi, A., Caprioli, A., Sirabella, D., Baiocchi, M., De Maria, R., et al. (2002). The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development *129*, 2773–2783.

Montarras, D., Morgan, J., Collins, C., Relaix, F., Zaffran, S., Cumano, A., Partridge, T., and Buckingham, M. (2005). Direct isolation of muscle satellite cells demonstrates their major role in skeletal muscle self renewal. Science. Published online September 1, 2005. 10.1126/science.1114758.

Morgan, J.E., and Partridge, T.A. (2003). Muscle satellite cells. Int. J. Biochem. Cell Biol. *35*, 1151–1156.

Moss, F.P., and Leblond, C.P. (1970). Nature of dividing nuclei in skeletal muscle of growing rats. J. Cell Biol. *44*, 459–462.

Olguin, H.C., and Olwin, B.B. (2004). Pax-7 up-regulation inhibits

myogenesis and cell cycle progression in satellite cells: a potential mechanism for self-renewal. Dev. Biol. *275*, 375–388.

Pola, R., Ling, L.E., Aprahamian, T.R., Barban, E., Bosch-Marce, M., Curry, C., Corbley, M., Kearney, M., Isner, J.M., and Losordo, D.W. (2003). Postnatal recapitulation of embryonic hedgehog pathway in response to skeletal muscle ischemia. Circulation *108*, 479–485.

Polesskaya, A., Seale, P., and Rudnicki, M.A. (2003). Wnt signaling induces the myogenic specification of resident CD45+ adult stem cells during muscle regeneration. Cell *113*, 841–852.

Qu-Petersen, Z., Deasy, B., Jankowski, R., Ikezawa, M., Cummins, J., Pruchnic, R., Mytinger, J., Cao, B., Gates, C., Wernig, A., and Huard, J. (2002). Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. J. Cell Biol. *157*, 851–864.

Rantanen, J., Hurme, T., Lukka, R., Heino, J., and Kalimo, H. (1995). Satellite cell proliferation and the expression of myogenin and desmin in regenerating skeletal muscle: evidence for two different populations of satellite cells. Lab. Invest. *72*, 341–347.

Relaix, F., Rocancourt, D., Mansouri, A., and Buckingham, M. (2005). A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature *435*, 948–953.

Renault, V., Thornell, L.E., Butler-Browne, G., and Mouly, V. (2002). Human skeletal muscle satellite cells: aging, oxidative stress and the mitotic clock. Exp. Gerontol. *37*, 1229–1236.

Robertson, T.A., Maley, M.A., Grounds, M.D., and Papadimitriou, J.M. (1993). The role of macrophages in skeletal muscle regeneration with particular reference to chemotaxis. Exp. Cell Res. *207*, 321–331.

Rouger, K., Brault, M., Daval, N., Leroux, I., Guigand, L., Lesoeur, J., Fernandez, B., and Cherel, Y. (2004). Muscle satellite cell heterogeneity: in vitro and in vivo evidences for populations that fuse differently. Cell Tissue Res. *317*, 319–326.

Sampaolesi, M., Torrente, Y., Innocenzi, A., Tonlorenzi, R., D'Antona, G., Pellegrino, M.A., Barresi, R., Bresolin, N., De Angelis, M.G., Campbell, K.P., et al. (2003). Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science *301*, 487–492.

Shavlakadze, T., White, J.D., Davies, M., Hoh, J.F., and Grounds, M.D. (2005). Insulin-like growth factor I slows the rate of denervation induced skeletal muscle atrophy. Neuromuscul. Disord. *15*, 139–146.

Schultz, E., Gibson, M.C., and Champion, T. (1978). Satellite cells are mitotically quiescent in mature mouse muscle: an EM and radioautographic study. J. Exp. Zool. *206*, 451–456.

Seale, P., Sabourin, L.A., Girgis-Gabardo, A., Mansouri, A., Gruss, P., and Rudnicki, M.A. (2000). Pax7 is required for the specification of myogenic satellite cells. Cell *102*, 777–786.

Seale, P., Ishibashi, J., Holterman, C., and Rudnicki, M.A. (2004). Muscle satellite cell-specific genes identified by genetic profiling of MyoD-deficient myogenic cell. Dev. Biol. *275*, 287–300.

Shefer, G., Wleklinski-Lee, M., and Yablonka-Reuveni, Z. (2004). Skeletal muscle satellite cells can spontaneously enter an alternative mesenchymal pathway. J. Cell Sci. *117*, 5393–5404.

Sherwood, R.I., Christensen, J.L., Conboy, I.M., Conboy, M.J., Rando, T.A., Weissman, I.L., and Wagers, A.J. (2004a). Isolation of adult mouse myogenic progenitors; functional heterogeneity of cells within and engrafting skeletal muscle. Cell *119*, 543–554.

Sherwood, R.I., Christensen, J.L., Weissman, I.L., and Wagers, A.J. (2004b). Determinants of skeletal muscle contribution from circulating cells, bone marrow cells, and hematopoietic stem cells. Stem Cells *22*, 1292–1304.

Sloper, J.C., and Partridge, T.A. (1980). Skeletal muscle: regeneration and transplantation studies. Br. Med. Bull. *36*, 153–158.

Snow, M.H. (1978). An autoradiographic study of satellite cell differentiation into regenerating myotubes following transplantation of muscles in young rats. Cell Tissue Res. *186*, 535–540.

Stauber, W.T., Fritz, V.K., Vogelbach, D.W., and Dahlmann, B. (1988). Characterization of muscles injured by forced lengthening. I. Cellular infiltrates. Med. Sci. Sports Exerc. *20*, 345–353.

Thomas, M., Langley, B., Berry, C., Sharma, M., Kirk, S., Bass, J., and Kambadur, R. (2000). Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. J. Biol. Chem. *275*, 40235–40243.

Tidball, J.G. (2005). Inflammatory processes in muscle injury and repair. Am. J. Physiol. Regul. Integr. Comp. Physiol. *288*, R345–R353.

Vignery, A. (2000). Osteoclasts and giant cells: macrophage-macrophage fusion mechanism. Int. J. Exp. Pathol. *81*, 291–304.

Volkmann, R. (1893). Uber die regeneration des quergestrieften muskelgewebes beim menschen und saugetier. Beitr. Path. Anat. *12*, 233–332.

Walton, J.N., and Adams, R.D. (1956). The response of the normal, the denervated and the dystrophic muscle-cell to injury. J. Pathol. Bacterol. *72*, 273–298.

Washabaugh, C.H., Ontell, M.P., and Ontell, M. (2004). Nonmuscle stem cells fail to significantly contribute to regeneration of normal muscle. Gene Ther. *11*, 1724–1728.

Weber, C.O. (1863). Ueber die regeneration quergestreifter muskelfasern. Zentrabl. Med. Wis. *1*, 529–531.

Yablonka-Reuveni, Z., Quinn, L.S., and Nameroff, M. (1987). Isolation and clonal analysis of satellite cells from chicken pectoralis muscle. Dev. Biol. *119*, 252–259.

Yaffe, D. (1969). Cellular aspects of muscle differentiation in vitro. Curr. Top. Dev. Biol. *4*, 37–77.

Zacks, S.I., and Sheff, M.F. (1982). Age-related impeded regeneration of mouse minced anterior tibial muscle. Muscle Nerve *5*, 152–161.

Zammit, P.S., Golding, J.P., Nagata, Y., Hudon, V., Partridge, T.A., and Beauchamp, J.R. (2004). Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? J. Cell Biol. *166*, 347–357.

Zimmers, T.A., Davies, M.V., Koniaris, L.G., Haynes, P., Esquela, A.F., Tomkinson, K.N., McPherron, A.C., Wolfman, N.M., and Lee, S.J. (2002). Induction of cachexia in mice by systemically administered myostatin. Science *296*, 1486–1488.