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REVIEW

A New Look at the Origin, Function, and "Stem-Cell" Status of Muscle Satellite Cells

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Muscle satellite cells have long been considered a distinct myogenic lineage responsible for postnatal growth, repair, and maintenance of skeletal muscle. Recent studies in mice, however, have revealed the potential for highly purified hematopoietic stem cells from bone marrow to participate in muscle regeneration. Perhaps more significantly, a population of putative stem cells isolated directly from skeletal muscle efficiently reconstitutes the hematopoietic compartment and participates in muscle regeneration following intravenous injection in mice. The plasticity of muscle stem cells has raised important questions regarding the relationship between the muscle-derived stem cells and the skeletal muscle satellite cells. Furthermore, the ability of hematopoietic cells to undergo myogenesis has prompted new investigations into the embryonic origin of satellite cells. Recent developmental studies suggest that a population of satellite cells is derived from progenitors in the embryonic vasculature. Taken together, these studies provide the first evidence that pluripotential stem cells are present within adult skeletal muscle. Tissue-specific stem cells, including satellite cells, may share a common embryonic origin and possess the capacity to activate diverse genetic programs in response to environmental stimuli. Manipulation of such tissue-specific stem cells may eventually revolutionize therapies for degenerative diseases, including muscular dystrophy. © 2000 Academic Press

Key Words: **satellite cells; myogenic stem cells; muscle regeneration; Myf5; MyoD.**

INTRODUCTION

Skeletal muscle satellite cells were first described in frog muscle by Mauro (1961) based on their morphology and position relative to mature myofibers and were later identified in adult avian and mammalian muscle (Armand *et al.*, 1983; Schultz, 1976; reviewed by Bischoff, 1994). Satellite cells adhere to the surface of myotubes prior to the formation of the basal lamina, such that the basal lamina surrounding the myofiber and satellite cells is continuous (Armand *et al.*, 1983; Bischoff, 1990, 1994). Satellite cells mediate the postnatal growth of muscle and are the primary means by which the mass of adult muscle is formed (Schultz, 1989, 1996). The overall population of satellite cells decreases with increasing age in rodents (Gibson and Schultz, 1983; Grounds, 1998). At birth satellite cells account for about 32% of muscle nuclei followed by a drop to less than 5% in the adult (2 months for mice) (Bischoff, 1994). This decline in satellite cell nuclei as the postnatal muscle develops is a direct reflection of satellite cell fusion into new or preexisting myofibers.

Satellite cells in adult skeletal muscle are normally mitotically quiescent but are activated (i.e., initiate multiple rounds of proliferation) in response to stress induced by weight-bearing exercise or trauma (Appell *et al.*, 1988; Rosenblatt *et al.*, 1994; Schultz *et al.*, 1985; reviewed by Bischoff, 1994; Grounds, 1998). The descendants of activated satellite cells, called myogenic precursor cells (mpcs),[2] undergo multiple rounds of division prior to fusing with existing or new myofibers (Appell *et al.*, 1988; Bischoff, 1994; Grounds and Yablonka-Reuveni, 1993). Satellite cells appear to form a population of stem cells that are distinct from their daughter mpcs as defined by biological and biochemical criteria (Grounds and Yablonka-Reuveni,

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[2] Abbreviations used: HSC, hematopoietic stem cell; NSC, neural stem cell; mpc, myogenic precursor cell; PCNA, proliferating cell nuclear antigen; MRF, myogenic regulatory factor; MSC, muscle stem cell; SP, side population (exclude Hoechst dye).

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1993; Bischoff, 1994). The number of quiescent satellite cells in adult muscle remains relatively constant over multiple cycles of degeneration and regeneration, demonstrating an inherent capacity for self-renewal (Gibson and Schultz, 1983; Schultz and Jaryszak, 1985). However, the numbers and doubling potential of satellite cells become severely reduced in muscle diseases such as Duchenne muscular dystrophy presumably due to high levels of ongoing regeneration (Bulfield *et al.*, 1984; Emery, 1998; Schultz and Jaryszak, 1985; Webster and Blau, 1990).

The essential role of satellite cells in muscle regeneration, muscle hypertrophy, and postnatal muscle growth is well documented (Rosenblatt *et al.*, 1994; Schultz, 1996; Darr and Schultz, 1987; Grounds and Yablonka-Reuveni, 1993; Grounds, 1998). However, an understanding of the molecular mechanisms that regulate the activation and function of muscle stem cells has remained elusive. Here we review the molecular events which have been implicated in muscle regeneration, including the pivotal role played by the myogenic regulatory factors (MRFs) in the activation and differentiation of satellite cells. In addition, we discuss the embryonic origin of satellite cells and consider whether these cells represent tissue-specific pluripotent stem cells.

MYOGENIC REGULATORY FACTORS IN SATELLITE CELL ACTIVATION AND DIFFERENTIATION

The MyoD family of bHLH transcription factors is required for the commitment and differentiation of embryonic myoblasts during development. The primary MRFs, Myf5 and MyoD, are required for the determination of myoblasts, whereas the secondary MRFs, myogenin and MRF4, function to regulate terminal differentiation (reviewed by Megeney and Rudnicki, 1995; Arnold and Winter, 1998).

The MRF expression program during satellite cell activation, proliferation, and differentiation is analogous to the program manifested during the embryonic development of skeletal muscle. Quiescent satellite cells express no detectable levels of MRFs. MyoD is rapidly up-regulated within 12 h of experimentally induced muscle injury prior to expression of proliferating cell nuclear antigen (PCNA), a marker for cell proliferation. Myogenin is expressed last during the time associated with fusion and differentiation (Smith *et al.*, 1994; Yablonka-Reuveni and Rivera, 1994). Cornelison and Wold (1997) performed an elegant experiment in which gene expression was assessed by RT-PCR of individual satellite cells following their activation in intact mouse muscle fibers. Quiescent satellite cells express no detectable MRFs but do express the *c-Met* receptor tyrosine kinase, the receptor for hepatocyte growth factor (HGF/SF). Activated satellite cells (satellite cells entering the cell cycle) first express either *Myf5* or *MyoD* followed soon after by coexpression of *Myf5* and *MyoD.* Following proliferation, *myogenin* and *MRF4* are expressed in cells beginning their differentiation program. The absence of MRF mRNA in satellite cells prior to activation is consistent with the hypothesis that satellite cells represent a stem cell with an identity distinct from that of myoblasts. It is interesting therefore to speculate that *de novo* activation of *Myf5* and *MyoD* transcription occurs in response to inductive signals analogous to those that occur during the specification of the myogenic lineage during embryonic development.

Analysis of muscle regeneration in mice lacking MyoD has revealed an essential role for MyoD in regulating satellite cell function. To investigate the role of MyoD in satellite cell function, *MyoD−/−* mice (Rudnicki *et al.*, 1992) were interbred with *mdx* mice. The *mdx* mice carry a loss-of-function point mutation in the X-linked *dystrophin* gene and thus represent an animal model for human Duchenne and Becker muscular dystrophy (Bulfield *et al.*, 1984; Sicinski *et al.*, 1989). The compound mutant mice exhibit markedly increased penetrance of the *mdx* phenotype characterized by muscle atrophy and increased myopathy leading to premature death (Megeney *et al.*, 1996). By 3 to 5 months of age, *mdx:MyoD−/−* mice develop a profound dorsal–ventral curvature of the spine, similar to the lordosis and kyphosis of patients with Duchenne muscular dystrophy, and an abnormal waddling gait characterized by weight bearing on the hocks. The animals become progressively less active, with concomitant weight loss prior to premature death around 12 months of age.

Skeletal muscle from *MyoD−/−* mice displays a strikingly reduced capacity for regeneration following injury (Megeney *et al.*, 1996). Electron microscopic examination of *MyoD*-deficient muscle reveals morphologically normal satellite cells whose numbers are increased 1.8-fold in *MyoD−/−* muscle and 13-fold in *mdx:MyoD−/−* muscle. However, cellular proliferation during regeneration of *mdx: MyoD−/−* muscle is not detectable by [^{3}H]thymidine incorporation or immunohistochemistry with antibody reactive to PCNA. These data suggest a model in which up-regulation of *MyoD* is required for satellite cells to enter the mpc proliferative phase that precedes terminal differentiation. In the absence of MyoD, myogenic progenitors undergo an apparent increase in numbers as a consequence of an increased propensity for self-renewal rather than progression through their developmental program. Taken together, these experiments strongly support the hypothesis that satellite cells form a stem cell compartment that is the source of mpcs (Megeney *et al.*, 1996).

To gain insight into the regeneration deficit of *MyoD−/−* muscle, satellite cell-derived primary cultures were generated from adult *MyoD−/−* hindlimb muscle for analysis of their proliferative and differentiation potential. Low-passage *MyoD−/−* myogenic cells exhibit a stellate flattened morphology distinct from the compact rounded morphology of wild-type myoblasts (Sabourin *et al.*, 1999). Myogenic cells lacking *MyoD* express c-Met, but do not express desmin, an intermediate filament protein typically expressed in cultured myoblasts *in vitro* and mpcs *in vivo.* Under conditions that normally induce differentiation of

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wild-type myoblasts, *MyoD−/−* cells continue to proliferate and only after several days yield reduced numbers of predominantly mononuclear myocytes (Sabourin *et al.*, 1999; Yablonka-Reuveni *et al.*, 1999). Interestingly, expression of *IGF1* (insulin-like growth factor-1) is markedly increased in *MyoD−/−* myogenic cells cultured under low-mitogen conditions, suggesting that MyoD normally negatively regulates *IGF1* expression in primary myoblasts. Therefore, IGF1 may promote proliferation and inhibit differentiation in *MyoD−/−* myoblasts via an autocrine loop. In addition, expression of *M-cadherin* is notably decreased in *MyoD−/−* myogenic cells and a requirement for M-cadherin has been reported for cell-cycle withdrawal and myoblast fusion (Irintchev *et al.*, 1994; Zeschnigk *et al.*, 1995). Taken together, these data suggest that *MyoD−/−* myogenic cells represent an intermediate stage between a satellite cell and a mpc (Sabourin *et al.*, 1999). A definitive role for Myf5 in satellite cell activation has yet to be established since it has not been possible to analyze muscle regeneration in *Myf5−/−* mice, which die perinatally (Braun *et al.*, 1992).

DEVELOPMENTAL ORIGIN OF SATELLITE CELLS

Satellite cells are believed to constitute a myogenic cell lineage distinct from the embryonic lineages and first appear in the limbs of mouse embryos at about 17.5 days postcoitum (Cossu *et al.*, 1985; Feldman and Stockdale, 1992; Hartley *et al.*, 1992; DeAngelis *et al.*, 1999). Adult satellite cells may also be further divided into subclasses based on the fiber type in which they take up residence in the mature muscle. It is clear from several studies that satellite cells form fibers genetically similar to the muscle from which they originate (Rosenblatt *et al.*, 1996; Feldman and Stockdale, 1991; Hoh and Hughes, 1991; Matsuda *et al.*, 1983).

Quail–chick chimera experiments revealed the presence of satellite cell nuclei derived from implanted quail somite associated with host chick myofibers (Armand *et al.*, 1983). However, the somitic domain responsible for the generation of satellite cells and the developmental progression of satellite cell progenitors was not fully explored. Recent work has revealed that clonal skeletal myogenic cells that closely resemble satellite-cell-derived myogenic precursors are readily isolated from the embryonic dorsal aorta of mouse embryos (DeAngelis *et al.*, 1999). Moreover, analogous myogenic precursors are found in the limbs of later stage *c-Met−/−* and *Pax3−/−* mutant embryos that do not contain migratory myoblasts from the somite. Myogenic cells isolated from forelimbs of these mutant mice may arise from endothelial cells within the developing limb bud. This recent study predicts that differentiation of multipotential precursors associated with the embryonic vasculature occurs as a function of the tissue which is perfused, e.g., vessels which perfuse skeletal muscle contain progenitors with the capacity to become satellite cells in this environment. Consistent with this hypothesis, aorta-derived myogenic cells express myogenic and endothelial markers that are also expressed in adult satellite cells (DeAngelis *et al.*, 1999).

The dorsal aorta is colonized by migratory populations of somitic angioblasts derived from the paraxial mesoderm (in somite) which differentiate from the mesoderm as solitary cells and eventually fuse to form blood vessels (Dzierzak, 1999; Pardanaud and Dieterlen-Lievre, 1999; Pardanaud *et al.*, 1996). This raises the possibility that a proportion of satellite cells are derived from progenitors in the paraxial mesoderm, which also give rise to endothelial cells. Alternatively satellite cells may be derived from multipotent endothelial cell precursors during development. Therefore the presence of myogenic cells in dorsal aortic explants does not preclude the possibility of an indirect somitic origin for satellite cells. Collectively these results support a model for satellite cell development, which occurs independent of embryonic myogenesis (Bianco and Cossu, 1999; DeAngelis *et al.*, 1999).

Quiescent satellite cells adjacent to mature fibers express c-Met and M-cadherin proteins but do not express markers of committed myoblasts, such as *Myf5, MyoD,* or *desmin* (Cornelison and Wold, 1997; Irintchev *et al.*, 1994; Yablonka-Reuveni and Rivera, 1994). This observation is consistent with the possibility that satellite cells represent a pluripotential stem cell population. Committed myoblasts would therefore be generated as a function of their microenvironment and available growth factors. Such a model is also consistent with the observed ability of adult muscle-derived stem cells to repopulate the hematopoietic compartment as well as to give rise to skeletal myocytes following intravenous injection into irradiated mice (Bittner *et al.*, 1999; Ferrari *et al.*, 1998; Gussoni *et al.*, 1999).

The pluripotentiality of muscle-derived stem cells has important implications for the role of the MRFs in the ontogeny of satellite cells. Muscle satellite cells are commonly considered a distinct, yet committed population of myogenic cells. Accordingly, the immediate progenitors to satellite cells would down-regulate MRF expression prior to entering quiescence. The pluripotentiality of muscle-derived stem cells, however, raises the possibility of a MRF-independent mechanism for satellite cell development.

SATELLITE CELL SELF-RENEWAL

An outstanding issue that is poorly understood is the mechanism by which satellite cells undergo self-renewal in adult skeletal muscle. If satellite cells are identical to muscle-derived stem cells isolated by Gussoni and colleagues (1999), self-renewal of the stem cell compartment may occur prior to and completely independent of MRF expression. For example, muscle stem cells may undergo asymmetric cell division to generate two daughter cells: a committed myogenic precursor and a pluripotent "self" (Fig. 1A).

Alternatively, expression of Myf5 alone in myogenic cells

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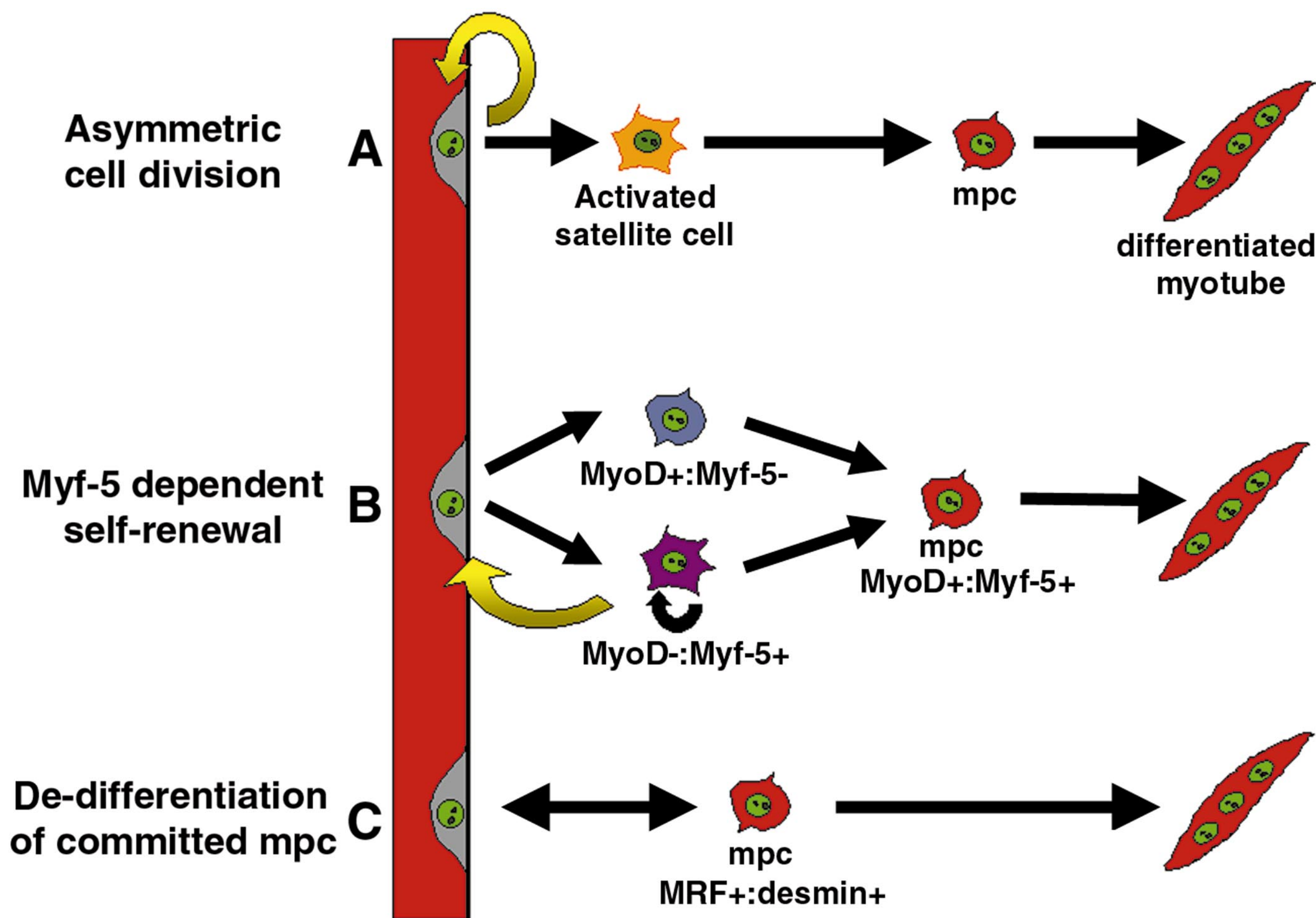

FIG. 1. Models postulated for self-renewal of muscle satellite cells. (A) Asymmetric stem cell division may generate two daughter cells: a committed myogenic precursor and an uncommitted pluripotent "self." (B) Self-renewal of the satellite cell compartment may be Myf5 dependent. In this model, initial activation of Myf5 without MyoD expression defines a developmental stage of satellite cell activation in which the stem cells undergo self-renewal. (C) Alternatively dedifferentiation of committed mpcs, expressing MyoD, Myf5, and other myoblast markers, may give rise to quiescent satellite cells.

may facilitate stem cell self-renewal within the context of skeletal muscle (Fig. 1B). The phenotype of primary *MyoD*−/− myogenic cells is consistent with the notion that *MyoD*−/− myogenic cells represent an intermediate stage between a quiescent satellite cell and a myogenic precursor cell (Sabourin *et al.*, 1999; Yablonka-Reuveni *et al.*, 1999). Interestingly, RT-PCR analysis reveals that activated satellite cells first express either *Myf5* alone or *MyoD* alone, prior to coexpressing *Myf5* and *MyoD* and subsequently progressing through the myogenic program (Cornelison and Wold, 1997). Together these data suggest that expression of *Myf5* alone may define a developmental stage during which satellite cells undergo self-renewal (Fig. 1B).

Another possibility which requires consideration is that "dedifferentiation" of previously committed mpcs could give rise to quiescent satellite cells (Fig. 1C). The existence of mechanisms for homing of satellite cells to discrete locations or grooves along muscle fibers, possibly involving cell adhesion molecules such as M-cadherin (Irintchev *et al.*, 1994; Kaufmann *et al.*, 1999; Zeschnigk *et al.*, 1995), supports the notion that the immediate progenitors of satellite cells express tissue-specific molecules normally expressed in committed mpcs. Importantly, none of the above models are mutually exclusive and all could coexist to maintain the steady-state numbers of satellite cells.

ACTIVATION OF SATELLITE CELLS DURING MUSCLE REGENERATION

Satellite cells are activated in response to diverse stimuli, for example injury, denervation, exercise, or stretching (Appell *et al.*, 1988; Darr and Schultz, 1987; Schultz *et al.*, 1985; Grounds and Yablonka-Reuveni, 1993; reviewed by Grounds, 1998). However, the molecular mechanisms that regulate the activation of satellite cells leading to their

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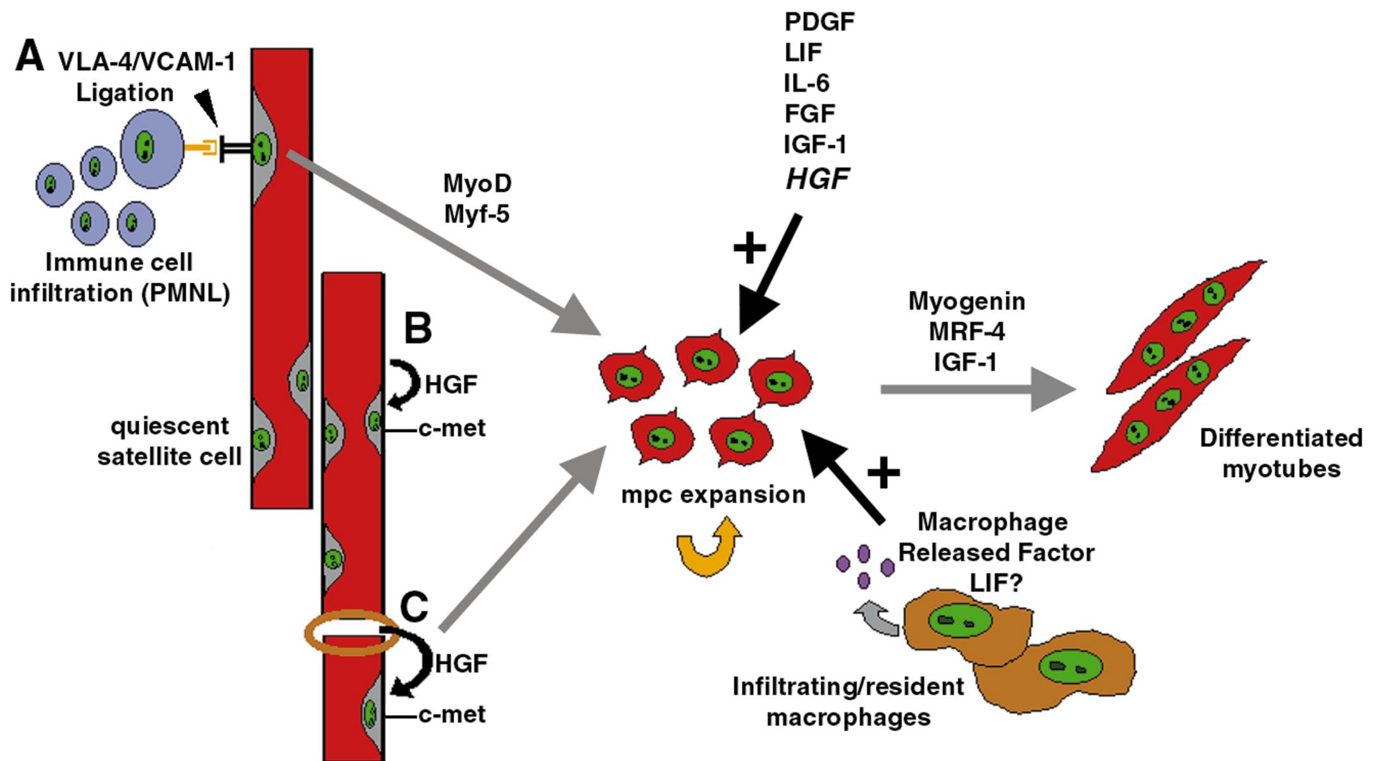

FIG. 2. A summary of molecular events implicated in satellite cell activation, during skeletal muscle regeneration. (A) Satellite cell activation may result from the ligation of integrin molecules, namely VLA-4 on infiltrating PMNL (polymorphonuclear leukocytes) and VCAM-1 on resident satellite cells. (B and C) HGF is also postulated to activate satellite cells through its cognate receptor c-Met, expressed in quiescent satellite cells. (B) HGF may be produced by undamaged myofibers in response to physiological stimuli. (C) Alternatively, damage to the basal lamina and extracellular matrix of myofibers may release HGF, which is normally sequestered here. Activation of quiescent satellite cells results in expression of MyoD and/or Myf5 and the generation of daughter mpcs. Several growth factors have been implicated in expansion of the mpc compartment, including HGF, PDGF, LIF, IL-6, FGF, and IGF1. Also a growth factor secreted from infiltrating macrophages (possibly LIF) is essential for mpc proliferation. Following a proliferative burst, mpcs undergo terminal differentiation in the formation of myocytes, which requires the expression of myogenin and MRF4 before fusing with existing or new fibers. IGF1 has also been postulated to induce muscle hypertrophy in fibers, independent of its role in mpc proliferation.

entry into the cell cycle remain to be elucidated. Nevertheless, a number of studies have implicated diverse mechanisms involved in the activation of satellite cells including the inflammatory response and the release of critical growth factors (Fig. 2).

A role for leukocytes in satellite cell activation has been proposed based on the observation that quiescent satellite cells express vascular cell adhesion molecule-1 (VCAM1) (a cell surface integrin molecule), whereas infiltrating leukocytes express the specific coreceptor VLA-4 (integrin $\alpha4\beta1$) (Fig. 2A) (Jesse *et al.*, 1998; Rosen *et al.*, 1992; Yang *et al.*, 1996). A model has been suggested in which cell–cell interactions mediated by VCAM1/VLA-4 ligation may initiate genetic responses within satellite cells and immune cells to promote regeneration (Jesse *et al.*, 1998).

Polymorphonuclear lymphocytes and macrophages (activated monocytes) migrate to sites of tissue damage within a few hours after trauma to muscle. Macrophages, however, are the dominant immune cells present within regenerating muscle at 48 h postinjury (Orimo *et al.*, 1991; Tidball, 1995). The role of macrophages in muscle regeneration is twofold in that macrophages phagocytose necrotic cell debris as well as secrete a soluble growth factor (yet to be characterized), which exerts a specific mitogenic effect on myoblasts (Fig. 2) (Cantini and Carraro, 1995; Cantini *et al.*, 1994; Merly *et al.*, 1999). A vital role for macrophages in muscle regeneration is supported by the observation that myogenesis is markedly impaired in the absence of macrophage infiltration (Lescaudron *et al.*, 1999).

The cytokines IL-6 (interleukin-6) and LIF (leukemia inhibitory factor) stimulate the proliferation of mpcs in culture (Austin *et al.*, 1992; Kurek *et al.*, 1996). Interestingly, LIF expression is markedly increased 3 h after muscle injury (Kurek *et al.*, 1996), suggesting that damaged muscle secretes LIF prior to infiltration of immune cells. *In situ* mRNA analysis of LIF in regenerating diaphragm of *mdx* mice reveals that *LIF* mRNA is produced by muscle and resident nonmuscle cells (Kurek *et al.*, 1996). In contrast,

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the source of IL-6 is from infiltrating immune cells which appear in regenerating muscle between 12 and 24 h following injury (Kurek *et al.*, 1996). LIF and IL-6 may represent the putative growth factors secreted by macrophages in regenerating muscle that stimulate mpc proliferation (Cantini *et al.*, 1994).

A role for IGF1 is supported by experiments in which direct infusion of IGF1 into the tibialis anterior muscles of adult rats led to increased total muscle protein and DNA content, demonstrating skeletal muscle hypertrophy concomitant with satellite cell activation (Adams and Haddad, 1996; Adams and McCue, 1998; Edwall *et al.*, 1989; Rosenblatt *et al.*, 1994). Recently, IGF1 has been demonstrated to induce the calcineurin–nuclear factor of activated T cells signaling pathway leading to activation of GATA-2, a transcription factor whose up-regulation is associated with myofiber hypertrophy (increase in protein content and size of myofibers) (Musaro *et al.*, 1999; Shibasaki *et al.*, 1996). Therefore, IGF1 likely stimulates both mpc proliferation and muscle hypertrophy during muscle regeneration (Fig. 2). Whether IGF1 directly stimulates satellite cell activation remains to be determined. In addition, a role for fibroblast growth factor-6 (FGF-6) in regulating the proliferation of mpcs has been proposed based on the muscle regeneration deficit in mice carrying a targeted null mutation in *FGF-6* (Floss *et al.*, 1997).

The actual physiological stimulus for satellite cell activation *in vivo* has yet to be defined; however, HGF/SF has emerged as a strong candidate for such a role. HGF is a potent mitogen and chemotactic agent for satellite cells both *in vitro* and *in vivo* (Allen *et al.*, 1995; Sheehan and Allen, 1999; Bischoff, 1997; reviewed by Birchmeier and Gherardi, 1998). Quiescent satellite cells express the *c-Met* proto-oncogene, which is the receptor for HGF (Cornelison and Wold, 1997). Therefore, as HGF is highly expressed in regenerating muscle, it may be responsible for satellite cell activation and/or expansion of the mpc pool prior to the formation of new myofibers (Figs. 2B and 2C). Moreover, injection of recombinant HGF directly into muscle has been shown to activate resident satellite cells (Tatsumi *et al.*, 1998). In support of a role for HGF/c-Met signaling in mpc proliferation, forced expression of an activated form of the c-Met receptor in C2C12 mouse myoblasts (cell line derived from satellite cells) results in their morphological transformation and subsequent inhibition of differentiation (Anastasi *et al.*, 1997). These results imply that activation of c-Met is required for the proliferation of mpcs, but down-regulation of the signaling pathway is necessary to allow terminal differentiation and fusion to form multinucleated myofibers.

The source of HGF in regenerating muscle remains to be identified. A report by Gal-Levi *et al.* (1998) demonstrates reciprocal expression of *c-Met* and *HGF* in growing myoblasts and myotubes, i.e., myoblasts express *c-Met* alone, whereas newly formed myotubes express *HGF* alone. This result implies that secretion of HGF by intact myotubes promotes mpc proliferation in regenerating muscle (Fig. 2B). Interestingly, HGF exerts a potent chemotactic effect on cultured myoblasts (Bischoff, 1997), demonstrating a possible role for HGF in promoting the migration of activated satellite cells to distal sites of injury (Watt *et al.*, 1987; Schultz *et al.*, 1986, 1988; Hughes and Blau, 1990). However, Anastasi and colleagues (1997) have demonstrated expression of both *c-Met* and *HGF* in proliferating myoblasts, thus establishing an autocrine loop for myoblast expansion. HGF may also be released from the extracellular matrix through damage to the basal lamina (Fig. 2C). Although a mechanism for secretion of HGF in damaged muscle is not established, there is strong evidence to suggest that HGF/c-Met signal transduction plays a pivotal role in muscle regeneration.

TISSUE-SPECIFIC STEM CELLS: MONOPOTENTIAL OR PLURIPOTENTIAL?

Satellite cells have long been considered monopotential, with the potential to give rise only to cells of the myogenic lineage (Bischoff, 1994). However, recent experiments have raised important questions regarding the developmental potential of stem cells derived from diverse tissues, including muscle, bone marrow, and brain.

Recent studies have demonstrated the ability of bone marrow cells to give rise to myogenic cells that can participate in muscle regeneration. Ferrari and colleagues (1998) performed a study in which bone marrow from mice carrying a transgene, in which β-galactosidase is under the control of a muscle-specific promoter, were transplanted into immunodeficient mice. Following chemically induced muscle damage, small numbers of lacZ-stained nuclei were detected in regenerating muscle fibers. In support of this work, Bittner *et al.* (1999) similarly demonstrated recruitment of donor-derived bone marrow cells to skeletal and cardiac muscles of *mdx* mice. Donor male nuclei (detected by Y-chromosome-specific fluorescence *in situ* hybridization) were also detected in endothelial cells. Collectively these studies indicate the capacity of bone-marrow-derived stem cells to give rise to cells of the myogenic and other lineages. However, there was no indication that the grafted cells made any contribution to the satellite cell compartment, suggesting that donor myogenic cells can only undergo terminal differentiation.

Recently, Gussoni *et al.* (1999) demonstrated that highly purified hematopoietic stem cells (HSCs) readily contribute nuclei to regenerating muscle fibers following intravenous injection into irradiated *mdx* mouse recipients. The HSCs were purified by fluorescence-activated cell sorting (FACS) of cells that exclude Hoechst dye (Fig. 3A). The FACS method of isolating HSCs (also called side population or SP cells) is dependent upon the principal that marrow-derived pluripotent stem cells stain poorly with Hoechst 33342 due to high expression of multidrug resistant-like proteins (Goodell *et al.*, 1996, 1997; reviewed by Jackson *et al.*, 1999). Importantly, following intravenous injection of 2000–5000 HSCs into lethally irradiated *mdx* mice, dystro-

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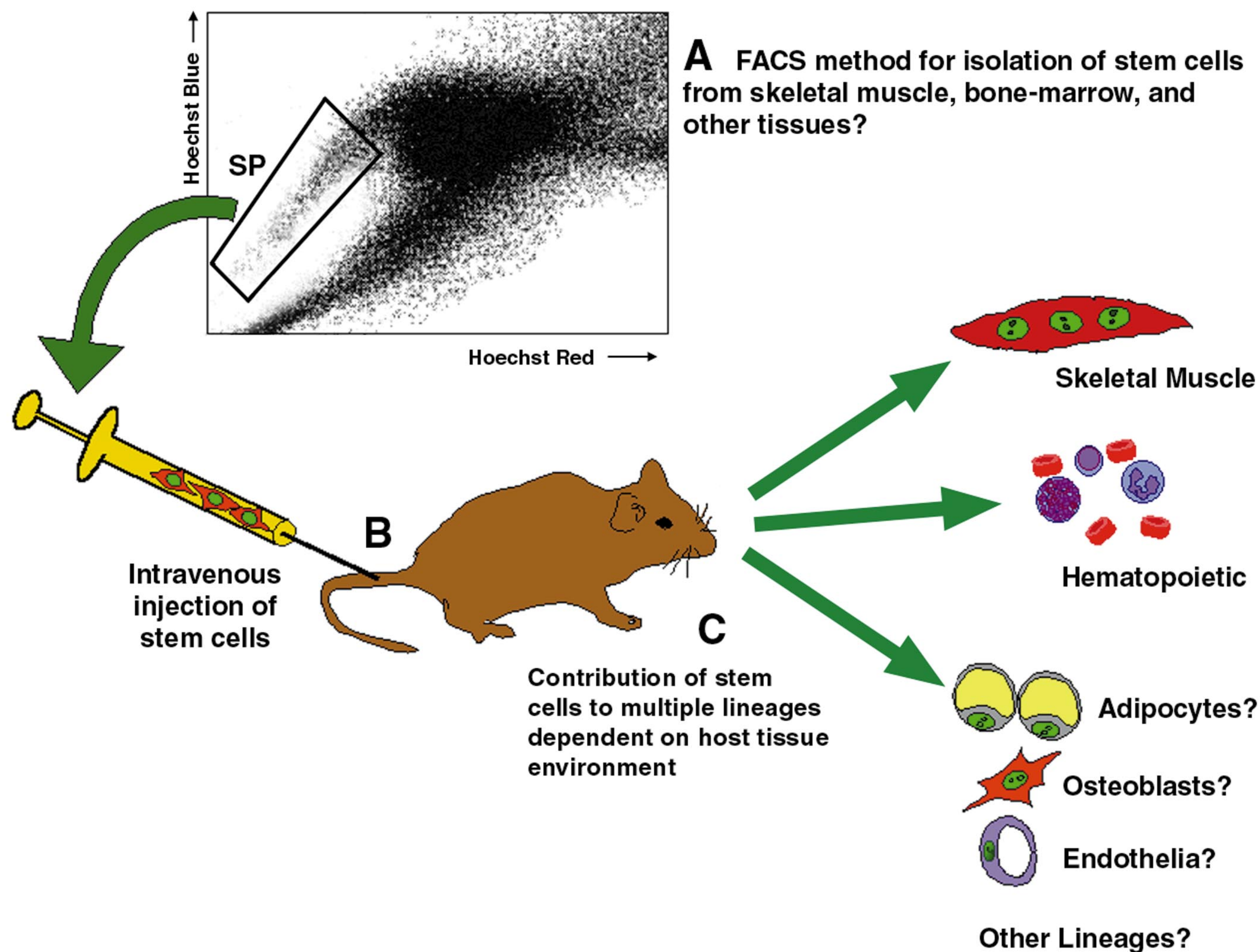

FIG. 3. Pluripotentiality of adult tissue-specific stem cells. (A) Highly purified stem cells are isolated from adult tissue, including bone marrow and skeletal muscle, based on exclusion of Hoechst dye. FACS (fluorescence-activated cell sorting) is used to isolate the side population (SP) of Hoechst-excluding cells. (B and C) Purified stem cells give rise to skeletal muscle cells and hematopoietic cells following intravenous injections in mice. It is possible that such cells could be isolated from many tissues and contribute to multiple lineages following intravenous injection. It is increasingly evident that many (if not all) differentiated tissues contain pluripotential stem cells capable of responding to environmental cues.

phin expression was restored in about 4% of the muscle fibers by 12 weeks after engraftment. Similar to the results of Ferrari *et al.* (1998) and Bittner *et al.* (1999), no donor-derived male nuclei were observed to segregate into the satellite cell compartment.

Gussoni and colleagues then investigated whether the Hoechst/FACS method could be used to purify stem cells directly from muscle tissue. Indeed, muscle stem cells (MSCs) (also called muscle SP cells) were readily isolated from muscle and these differentiated into desmin-expressing myoblasts in cell culture (Fig. 3C). Following intravenous injection, MSCs efficiently contributed to regenerating myofibers, and donor-derived nuclei were detected in up to 9% of muscle fibers in recipients. Importantly, in contrast to marrow-derived HSCs, MSCs apparently gave rise to satellite cells in recently regenerated host fibers following transplantation. Further experiments demonstrating colocalization of satellite cell-specific markers and donor nuclei are required to directly address this question. Strikingly, MSCs efficiently reconstituted the complete repertoire of the hematopoietic system when injected into lethally irradiated recipients. A study by Jackson *et al.* (1999) has further demonstrated that MSCs contribute to all major blood lineages 3 months following injection. Taken together, these experiments unequivocally demonstrate that muscle tissue contains a population of

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pluripotent stem cells that can be highly purified based on Hoechst exclusion (Gussoni *et al.*, 1999; Jackson *et al.*, 1999).

An analogous study revealed that neural stem cells (NSCs) can similarly repopulate the bone marrow/blood system in lethally irradiated mouse recipients (Bjornson *et al.*, 1999). These NSCs were previously thought to be limited in potential to the development of neurons, oligodendrocytes, and astrocytes. Moreover, marrow-derived cells differentiate into astroglia and microglia following injection into brain (Eglitis and Mezey, 1997). Taken together, these results suggest that many or all tissues contain a population of pluripotent stem cells. Therefore, these studies challenge the widely held view that tissue-specific stem cells are predetermined, i.e., monopotential or able to give rise only to a particular cell type. In fact these stem cells appear to be pluripotential or even totipotent, possessing the ability to activate various genetic programs when exposed to the appropriate environment (Fig. 3C). Thus, such pluripotential stem cells must differentiate as a function of the growth factors and signals provided by their host tissue.

CONCLUSIONS AND PROSPECTS

The study of the muscle satellite cell as a stem cell and its role in skeletal muscle regeneration is still in its infancy. Exciting new experiments revealing the pluripotential nature of muscle-derived stem cells have raised several intriguing questions. Are the muscle-derived pluripotent MSCs described by Gussoni *et al.* (1999) identical to satellite cells? Although, it is certainly possible that satellite cells are indeed the MSCs, other interpretations cannot be dismissed. MSCs may represent a developmental stage immediately upstream of the satellite cell, i.e., MSCs may be the direct progenitors of muscle satellite cells. Alternatively, MSCs may represent a transient cell population of recently activated satellite cells prior to the expression of markers typical of committed myoblasts. Moreover, it remains possible that MSCs represent an independent stem cell population, separate from satellite cells. A biochemical and molecular analysis of MSCs should resolve this question.

The capacity for HSCs to participate in myogenesis and in turn the ability of MSCs and NSCs to repopulate the hematopoietic system are suggestive of common developmental progenitors for these tissue-specific stem cells. The ability of embryonic vasculature to give rise to satellite cells certainly supports this idea (DeAngelis *et al.*, 1999). While a common embryonic origin for tissue-specific stem cells cannot be discounted, it is also possible that stem cells, including HSCs, NSCs, and MSCs, express a common array of genes reflecting their primitive status. The ability of tissue-specific cells to activate diverse genetic programs in response to environmental cues is striking. It will now be important to characterize the influence of growth factors and components of the extracellular matrix responsible for activating genetic responses within stem cells.

The pluripotent nature of adult stem cells isolated from diverse tissues raises the possibility of stem cell therapy for a variety of degenerative diseases, including muscular dystrophy. For example, the potential to deliver myogenic stem cells in a disseminated manner through the circulation has opened a new vista of possibilities. Clearly, gaining a more complete understanding of the molecular mechanisms that regulate the development and function of muscle-derived stem cells will have a profound impact on the way we view and exploit adult stem cells.

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