

The Skeletal Muscle Satellite Cell: Still Young and Fascinating at 50

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Summary

The skeletal muscle satellite cell was first described and named based on its anatomic location between the myofiber plasma and basement membranes. In 1961, two independent studies by Alexander Mauro and Bernard Katz provided the first electron microscopic descriptions of satellite cells in frog and rat muscles. These cells were soon detected in other vertebrates and acquired candidacy as the source of myogenic cells needed for myofiber growth and repair throughout life. Cultures of isolated myofibers and, subsequently, transplantation of single myofibers demonstrated that satellite cells were myogenic progenitors. More recently, satellite cells were redefined as myogenic stem cells given their ability to self-renew in addition to producing differentiated progeny. Identification of distinctively expressed molecular markers, in particular Pax7, has facilitated detection of satellite cells using light microscopy. Notwithstanding the remarkable progress made since the discovery of satellite cells, researchers have looked for alternative cells with myogenic capacity that can potentially be used for whole body cell-based therapy of skeletal muscle. Yet, new studies show that inducible ablation of satellite cells in adult muscle impairs myofiber regeneration. Thus, on the 50th anniversary since its discovery, the satellite cell's indispensable role in muscle repair has been reaffirmed. (*J Histochem Cytochem* 59:1041–1059, 2011)

Keywords

satellite cell, stem cell, self-renewal, Pax7, nestin-GFP, extrafusal, intrafusal, spindle, myofiber, pericyte, regeneration, aging

The functional units responsible for skeletal muscle contraction are cylindrical, multinucleated muscle fibers (myofibers). These contractile structures are established during embryogenesis, when mononuclear cells known as myoblasts fuse into immature myofibers (myotubes). The myofiber nuclei (myonuclei) are postmitotic and under normal conditions cannot re-enter a proliferative state to contribute additional nuclei. During postnatal life, myofiber growth, homeostasis, and repair rely on satellite cells, myogenic stem cells residing between the myofiber plasmalemma and basal lamina (Fig. 1) (Katz 1961; Mauro 1961; Hawke and Garry 2001; Yablonka-Reuveni and Day 2011). For many years following its discovery in 1961, electron microscopy provided the only definitive method of identification of the skeletal muscle satellite cell. More recently, several molecular markers have been described that can be used to detect satellite cells, making them more accessible for study at the light microscope level. With the extensive use of histological and cytological approaches in the studies of satellite cells, the *Journal of Histochemistry and Cytochemistry* has provided a visible platform for original

publications and reviews on these fascinating cells. Here, we join the celebrations for the satellite cell at 50, discussing selective topics related to satellite cell biology.

Historical Perspective

The capacity of skeletal muscle to regenerate was documented in the 19th century, but it took another century before researchers unveiled the cellular basis of myofiber formation and regeneration (reviewed in Scharner and Zammit 2011). Seminal studies that set the stage for current cell biology of muscle regeneration were published in early 1960s. It was demonstrated that the multi-nucleated myofi-

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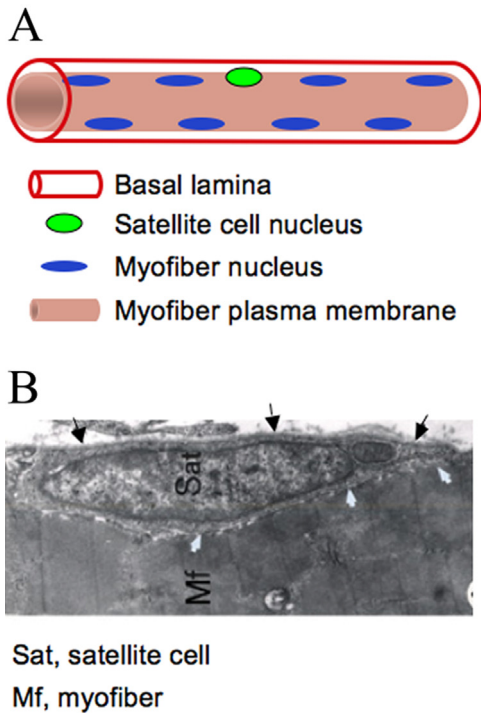


Figure 1. A schematic (A) and electron microscopy image (B) of the satellite cell location. In panel A, nuclei depicted at the myofiber periphery represent the state of healthy adult myofibers; immature myofibers present in regenerating muscles and in muscular dystrophy display centralized myofiber nuclei (not shown) (Yablonka-Reuveni and Day 2011, with kind permission of Springer Science+Business Media). In panel B, black arrows depict the basal lamina, and white arrows depict apposing satellite cell and myofiber membranes; note the sarcomeric organization within the myofiber (Yablonka-Reuveni 1995). The myofiber basement and plasma membranes have been routinely detected by immunostaining with antibodies against laminin and dystrophin, respectively.

ber, the contractile unit of skeletal muscle, is formed by the fusion of mononucleated myoblasts and that single cells, but not myofiber nuclei, are involved in cell proliferation (Bintliff and Walker 1960; Capers 1960; Cooper and Konigsberg 1961; Stockdale and Holtzer 1961). This effectively resolved the “enigmatic” finding reported 44 years earlier (Lewis and Lewis 1917) that myofibers appeared to increase in size and in content of nuclei in the absence of any observable nuclear division within the myofiber. A complementary 1961 discovery consisted of electron microscopic descriptions of an apparently quiescent cell lying on the surface of the myofiber, but beneath its basement membrane, where its peripheral position earned it the name *satellite cell* (Katz 1961; Mauro 1961). Although first detected in frog muscle, the satellite cell presence was immediately confirmed in rat muscle (Mauro 1961), and it was soon shown to occupy a common anatomic position in the majority of vertebrates (reviewed in Grounds and Yablonka-Reuveni 1993).

Upon its discovery, the satellite cell acquired immediate candidacy as the source of myogenic cells for growth and repair of postnatal skeletal muscle. However, the debate about the actual source of myoblasts for muscle regeneration continued as there was no direct evidence that satellite cells were indeed myogenic progenitors (Carlson 1973; Scharner and Zammit 2011). In general, stem/progenitor cells have been identified and characterized in terms of molecular markers, which have then been used to trace them to their anatomic niche within a tissue. In the case of the satellite cell, attribution of a stem cell–like status to an anatomically defined entity made it difficult to devise stringent tests, because its activity during regeneration usually displaces the cell from its position beneath the basal lamina. Thus, the principal defining characteristics of a satellite cell are removed, destroying any formal connection between it and the myoblasts that appear upon injury and eventually form new myofibers. Evidence that satellite cells function as myogenic precursors was initially based on studies of the distribution of labeled thymidine in growing or regenerating muscles (Grounds and Yablonka-Reuveni 1993). Studies using this approach collectively led to the commonly accepted view that satellite cells divide to provide myonuclei to growing myofibers (Moss and Leblond 1971) before becoming mitotically quiescent in normal mature muscle (Schultz et al. 1978).

Conclusive proof that myofibers harbor cells that give rise to myoblasts and multinucleated myotubes was eventually shown with isolated myofibers (Bischoff 1975; Konigsberg et al. 1975). The isolation of viable myofibers was subsequently optimized using collagenase digestion for studies of the myofiber itself (Bekoff and Betz 1977a, 1977b) and for satellite cell studies (Bischoff 1986). This procedure has facilitated effective isolation of intact myofibers with their complete cohort of satellite cells still resident beneath the basal lamina (Bischoff 1986; Yablonka-Reuveni and Rivera 1994; Rosenblatt et al. 1995). Upon myofiber culturing, the satellite cells proliferate, giving rise to satellite cell–derived myoblasts that can differentiate and form multinucleated myotubes. Transplantation of single myofibers into host muscle has provided important evidence that the satellite cells contributed by the donor myofiber indeed act as myogenic stem cells *in vivo*, able to give rise to both new myofibers and, importantly, many new satellite cells (Collins et al. 2005). The satellite cell therefore fulfills the basic definition of a stem cell in that it can give rise to a differentiated cell type and maintain itself by self-renewal.

With the development of contemporary means for genetic labeling of satellite cells, it has become possible to trace their progeny even after muscle tissue has been injured. Lineage-tracing studies relying on inducible activation of Pax7-driven reporter expression in adult muscle have confirmed that satellite cells can repair damaged muscle tissue (Lepper et al. 2009). Furthermore, recent studies using novel genetic models have enabled specific ablation of satellite cells in adult mice and have provided what might

be considered not only strong, but likely the ultimate, evidence for the essential role of satellite cells in muscle regeneration (Lepper et al. 2011; McCarthy et al. 2011; Murphy et al. 2011; Sambasivan et al. 2011).

Functional Satellite Cells Are Required Throughout Life

The distinct anatomic position of the satellite cell on the surface of the myofiber beneath the basal lamina provides immediacy and sensitivity to a postmitotic tissue like skeletal muscle that is critically dependent on mechanical, structural, and functional integrity (Anderson 2006; Ciciliot and Schiaffino 2010). In the juvenile growth phase, when muscles enlarge, satellite cells are proliferative and add nuclei to growing myofibers (Moss and Leblond 1971; Campion 1984; Schultz 1996; Halevy et al. 2004). In most adult muscles, satellite cells are typically quiescent until their activation is invoked by muscle injury (Snow 1978; Grounds and Yablonka-Reuveni 1993; Schultz et al. 1978; Hawke and Garry 2001). Subtle injuries may lead to minimal proliferation of activated satellite cells, whereas major trauma can recruit greater numbers of satellite cells and promote prolonged proliferation prior to differentiation. As small myofiber injuries can occur routinely during daily activity, a mechanism for repair is essential for muscle maintenance throughout life.

Activation of satellite cells is controlled by proximal signals from the muscle niche and microvasculature and through an inflammatory response (Bischoff 1989; Yablonka-Reuveni, Seger et al. 1999; Wozniak et al. 2005; Christov et al. 2007; Carlson et al. 2008; Gopinath and Rando 2008; Shavlakadze et al. 2010). Systemic factors may also regulate satellite cell activation (Conboy et al. 2005; Carlson et al. 2009; Shavlakadze et al. 2010). Among the factors considered to be regulators of satellite cell activation, hepatocyte growth factor (HGF) and neuronal nitric oxide synthase (NOS) have been studied extensively (Tatsumi et al. 1998; Tatsumi et al. 2001; Wozniak et al. 2005; Anderson 2006; Leiter et al. 2011), and fibroblast growth factors (FGFs) appear to play a role in the process as well (Yablonka-Reuveni and Rivera 1994, 1997; Yablonka-Reuveni, Seger et al. 1999; Kastner et al. 2000; Jones et al. 2005; Shefer et al. 2006). We have also detected high-level transcript expression of FGF receptor 1 (FGFR1) and to a lesser extent FGFR4 in freshly isolated satellite cells prepared by FACS sorting from nestin-GFP mice (K. Day and Z. Yablonka-Reuveni, unpublished results). Clearly, additional *in situ* studies within the muscle tissue are needed for further confirmation of the FGF-FGFR role in satellite cell activation.

Following their activation, satellite cells may contribute to repair of damaged myofibers and also generate new myofibers following cell division and fusion of myoblast prog-

eny (Grounds and Yablonka-Reuveni 1993; Collins et al. 2005). Satellite cell behavior is under stringent regulatory control in order to balance various actively maintained states, including quiescence, entry into proliferation and continuity of the cell cycle, and terminal differentiation (Shefer and Yablonka-Reuveni 2008; Day et al. 2009). Furthermore, apart from their ability to fortify myofibers and contribute to muscle regeneration, satellite cells have the capacity to replenish a reserve pool and self-renew, qualifying them as tissue-specific stem cells (Collins et al. 2005; Sacco et al. 2008). It is possible, however, that individual satellite cells differ with regard to their amplification and renewal potential (Kuang et al. 2007; Sacco et al. 2008; Day et al. 2010).

During early postnatal growth, muscle satellite cells can represent about 30% of the nuclei, whereas in the healthy adult satellite cells represent approximately 2%–7% of nuclei within skeletal muscle (Hawke and Garry 2001; Halevy et al. 2004). The number of satellite cells per myofiber or per cross-sectional area may vary immensely between muscles. For example, the fast twitch extensor digitorum longus (EDL) contains fewer satellite cells compared with the slow twitch soleus (Hawke and Garry 2001; Zammit et al. 2002; Shefer et al. 2006). Additionally, as shown in chicken muscle, myofiber ends may have a higher concentration of satellite cells than the rest of the myofiber (Allouh et al. 2008).

There are also reports of an age-associated decline in the number of satellite cells, where the presence and extent of decline may vary by muscle (Shefer et al. 2006; Collins et al. 2007; Day et al. 2010; Shefer et al. 2010). Satellite cell performance may also decline in the aging environment, a possible contributory factor to age-associated muscle deterioration known as sarcopenia (Thompson 2009; Shefer et al. 2010). However, additional studies suggest that initial performance of skeletal muscle progenitors is delayed but not necessarily impaired in aged muscle and that factors beyond satellite cell activity alone may play a role in reducing muscle repair in old age (Carlson and Faulkner 1996; Grounds 1998; Shavlakadze et al. 2010). Indeed, satellite cell activity can be rejuvenated upon exposure of old muscle to a juvenile environment by cross-transplantation or by parabiosis of young and old mice (Carlson and Faulkner 1989; Conboy et al. 2005). Muscle wasting associated with muscular dystrophy is also thought to lead to exhaustion of satellite cells because of the continuous demand for reparative myogenic cells (Blau et al. 1983; Webster and Blau 1990; Aguenouz et al. 2011). However, exercise supports an increase in satellite cells, even in old age when there is a drastic decline in satellite cell numbers at least in some limb muscles (Shefer et al. 2010; Leiter et al. 2011; Smith and Merry 2011). It would be advantageous to gain further insight into the mechanisms involved in this process and whether such an increase in satellite cells is merely an

outcome of muscle activity or an underlying factor in the beneficial effect of exercise on muscle performance. Last, although it is commonly accepted that satellite cells are involved in myofiber growth during the early phase of postnatal life, the role, if any, of satellite cells in myofiber hypertrophy during adult life has been a subject of debate (McCarthy and Esser 2007; O'Connor and Pavlath 2007; O'Connor et al. 2007). A recent study using a genetic mouse model for specific ablation of satellite cells in adult mice concluded that effective myofiber hypertrophy can take place in satellite cell-depleted skeletal muscle, although the typical increase in myofiber nuclei associated with hypertrophy was absent (McCarthy et al. 2011). Also, a more "minor" component of the hypertrophic response, which involves de novo formation of myofibers, was significantly blunted following satellite cell depletion, indicating a distinct requirement for satellite cells in the hypertrophy-associated regenerative process (McCarthy et al. 2011).

Overall, satellite cells are vital to skeletal muscle homeostasis and regeneration throughout life, and understanding the regulation of myogenic stem cells will likely provide valuable insights into muscle wasting in disease and aging.

Detection, Molecular Markers, and Lineage Origin of Satellite Cells

Satellite cells were initially described by their anatomic location on the surface of muscle fibers, between the myofiber plasmalemma and the basal lamina, using electron microscopy (Katz 1961; Mauro 1961; Muir et al. 1965). More recent methods facilitate monitoring these cells by light microscopy based on expression of a range of specific markers that can be detected by immunostaining (reviewed in Biressi and Rando 2010; Boldrin et al. 2010). In particular, specific expression of the paired box transcription factor Pax7 (Seale et al. 2000) and availability of an excellent antibody for immunodetection of this protein provide a consistent means to identify satellite cells in their native position in a range of species including mouse (Seale et al. 2000; Zammit, Golding et al., 2004; Shefer et al. 2006; Day et al. 2007; Day et al. 2010), rat (Shefer et al. 2010), chicken (Halevy et al. 2004; Allouh et al. 2008), and human (Lindstrom and Thornell 2009; Lindstrom et al. 2010).

Genetically manipulated reporter mice that permit direct detection of satellite cells based on specific expression of a fluorophore (e.g., GFP) or β -galactosidase (β -gal) have also become available (Beauchamp et al. 2000; Montarras et al. 2005; Biressi et al. 2007; Day et al. 2007). We have demonstrated that transgenic expression of GFP under the control of nestin regulatory elements (Mignone et al. 2004) allows detection of satellite cells in freshly isolated myofibers (Day et al. 2007). These nestin-GFP mice also facilitate isolation of satellite cells using fluorescent-activated cell sorting (FACS) and subsequent studies of purified populations

(Day et al. 2007). The $Myf5^{nLacZ/+}$ mouse has also provided a means to identify satellite cells in intact muscle and isolated myofibers (Beauchamp et al. 2000; Collins et al. 2005; Zammit et al. 2006; Day et al. 2010; Ono et al. 2010). In this mouse, one of the *Myf5* alleles was modified to direct lacZ expression (Tajbakhsh et al. 1996; Tajbakhsh et al. 1997), resulting in β -gal protein in the nuclei of satellite cells, as originally reported by Beauchamp and colleagues (Beauchamp et al. 2000). We frequently use crosses of nestin-GFP with $Myf5^{nLacZ/+}$ mice, allowing the detection of satellite cells by means of direct fluorescence and X-gal staining (Day et al. 2010). The 3F-nlacZ-E transgenic mice (Kelly et al. 1995) (referred to as MLC3F-nLacZ mice in some studies) are also useful for distinguishing between satellite cells and myofiber nuclei. In these mice, regulatory elements of muscle-specific myosin light chain 3F (MLC3F) drive LacZ expression in myofiber nuclei but not in satellite cells (Beauchamp et al. 2000). A cross of nestin-GFP mice with MLC3F-nLacZ transgenic mice results in a clear distinction between satellite cells (which are GFP⁺ but negative for X-gal) and myofiber nuclei (which are X-gal⁺ but negative for GFP) (Day et al. 2010).

Although studies on the functional role of Pax7 during myogenesis have been published (Olguin et al. 2007; Collins et al. 2009), the role of Pax7 in the development and maintenance of satellite cells remains an enigma. The initial study reporting that satellite cells express Pax7 concluded that this transcription factor is required for specification of satellite cells; the survival of mice in which the Pax7 gene was inactivated was compromised (Seale et al. 2000). Notably, such early lethality in Pax7-null mice could have also been due to failure of Pax7-dependent systems other than muscle, such as the brain. Subsequent studies on Pax7 inactivation in mice showed that it may control survival of satellite cells beyond the neonatal period, because detectable satellite cells exhibited signs of apoptosis and declined rapidly postnatally when Pax7 was absent (Oustanina et al. 2004; Relaix et al. 2006). A more recent study using inducible inactivation of Pax7 indicated that it is only required postnatally up to the juvenile stage to support satellite cells, but not for satellite cell function and maintenance in adult muscle (Lepper et al. 2009). The study suggests a cell-intrinsic difference in Pax7 dependency between the myogenic progenitors supporting neonatal muscle development and adult satellite cells.

The conclusion of the lineage tracing study by Lepper and colleagues seems to reaffirm early studies proposing that satellite cells represent a distinct population of myogenic progenitors that replaces the fetal myogenic population (reviewed in Yablonka-Reuveni 1995). These early studies demonstrated that progeny of myogenic cells isolated from adult muscles displayed various morphological and biochemical features that were distinct from cells derived from myogenic progenitors present in the fetal phase of embryogenesis. These studies further identified the

emergence of “adult type” myogenic progenitors in late fetal phase, corresponding with the development of mature basal lamina around myofibers (Cossu et al. 1987; Cossu and Molinaro 1987; Feldman and Stockdale 1992; Hartley et al. 1991, 1992; Yablonka-Reuveni 1995). In the absence of a direct means to trace the satellite cells themselves, the latter studies established the concept that myogenic progenitors in the adult could be distinct from those present in fetal muscle based on the features of cells cultured from adult muscle. Notably, there is also heterogeneity in the myogenic cells that contribute to muscle development during earlier and later phases of embryogenesis (White et al. 1975; Seed and Hauschka 1988; Stockdale 1992).

Satellite cells share Pax7 expression (as well as Myf5^{nLacZ} and nestin-GFP expression) across all muscles regardless of the embryonic origin of the parent muscle (Day et al. 2007; Harel et al. 2009; Ono et al. 2010). Skeletal muscles of body and limb are derived from somites, but most head muscles originate from cranial mesoderm (Noden and Francis-West 2006; Schienda et al. 2006; Zammit et al. 2006; Harel et al. 2009; Sambasivan et al. 2009; Ono et al. 2010). Lineage tracing studies indicate a common origin for the satellite cells and their parent muscle (Armand et al. 1983; Schienda et al. 2006; Harel et al. 2009; also our unpublished studies with limb, diaphragm, extraocular, and jaw muscles). In limb and trunk muscles, satellite cells are derived from cells that express both Pax7 and its paralog, Pax3, during embryogenesis, whereas progenitors participating in development of many head muscles do not exhibit Pax3 expression (Epstein et al. 1996; Tajbakhsh et al. 1997; Gros et al. 2005; Relaix et al. 2005; Schienda et al. 2006; Harel et al. 2009; Relaix and Marcelle 2009; Sambasivan et al. 2009).

Although Pax7 is commonly expressed in satellite cells, the status of Pax3 in satellite cells is not entirely clear. Even among somite-derived muscles, Pax3 transcripts are expressed at a relatively higher level only by satellite cells in certain muscle groups (e.g., diaphragm), and likewise, Pax3-driven reporter expression is only detected in satellite cells of some adult muscles (Montarras et al. 2005; Relaix et al. 2006; Day et al. 2007). Satellite cell analysis with a well-characterized antibody for Pax3 (a mouse monoclonal antibody that does not cross-react with Pax7) has led researchers to conclude that Pax3 protein is not necessarily expressed by mouse satellite cells, even when they show a relatively high level of Pax3 transcript expression. Indeed, Pax3 protein expression during adult myogenesis appears to be repressed by a specific microRNA, thereby preventing inhibition of differentiation by Pax3 (Crist et al. 2009). However, the detection and specific degradation of Pax3 protein during satellite cell activation have also been described (Boutet et al. 2007). Different from the enigmatic status of Pax3 protein expression in mouse satellite cells, Pax3 protein is readily detected by immunostaining in some satellite cells (Pax7⁺) of chicken muscles, but there is also a

decline with age in Pax3 detection (Kirkpatrick et al. 2009). It is thus possible that Pax3 expression by satellite cells in somite-derived muscles is a residual trait from embryogenesis, where both Pax3 and Pax7 are expressed by myoblasts during development. In contrast, satellite cells in some craniofacial muscles that develop without Pax3 gene activity do not demonstrate Pax3 transcripts or Pax3-driven reporter expression (Harel et al. 2009; Sambasivan et al. 2009; P. Stuelsatz, A. Shearer and Z. Yablonka-Reuveni, unpublished work).

In the original discovery of the satellite cell, it was detected in both intrafusal (Katz 1961) and extrafusal (Mauro 1961) myofibers; in both of these myofiber types satellite cells can be detected by Pax7 expression (Kirkpatrick et al. 2008; Kirkpatrick et al. 2009) (Fig. 2). Intrafusal myofibers are within special structures in skeletal muscles known as spindles, and they function in proprioception (Walro and Kucera 1999; Kirkpatrick et al. 2008; Osterlund et al. 2011). Some intrafusal myofibers extend beyond the spindle capsule (Walro and Kucera 1999), but because of their small diameter and rarity can be overlooked in routine inspection of cross-sections when not inside the capsule. Intrafusal myofibers make up a small subpopulation of the muscle myofibers, whereas the bulk of skeletal muscle myofibers are termed *extrafusal myofibers*. The diameter of intrafusal myofibers remains small from an early postnatal age, and they retain expression of developmental myosins as well as expression of LacZ in myofiber nuclei of Myf5^{nLacZ/+} knock-in mice (Kozeka and Ontell 1981; Walro and Kucera 1999; Zammit, Carvajal et al. 2004; Kirkpatrick et al. 2008; Kirkpatrick et al. 2009; Osterlund et al. 2011). It is unknown whether satellite cells associated with intrafusal versus extrafusal myofibers are functionally unique and/or derived from distinct myogenic populations. A better understanding of the distinctions, if any, between satellite cells in the two types of myofibers is important for regenerative medicine because a complete muscle recovery would require that intrafusal myofiber be present as well for proprioception.

Detection of Satellite Cell Progeny by Temporal Expression Patterns of Myogenic-Related Transcription Factors

At the molecular level, myogenesis of satellite cells is highly orchestrated to ensure that specific genes are regulated in a temporally organized manner according to genetic blueprints, cell cycle requirements, and environmental factors (Yablonka-Reuveni et al. 2008). The resulting pattern of gene expression yields terminally differentiated myoblasts, capable of adding myonuclei to existing myofibers in addition to fusing together to form new myofibers during muscle growth and repair (Charge and Rudnicki 2004; Shefer and Yablonka-Reuveni 2008; Yablonka-Reuveni and Day 2011).

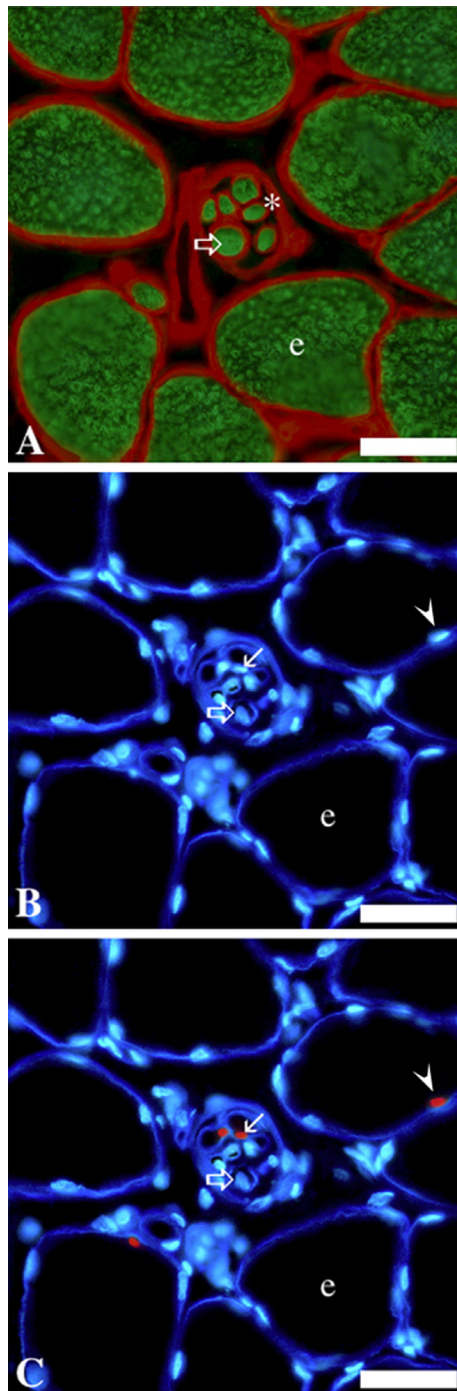


Figure 2. Immunohistochemical labeling of serial cross-sections of anterior latissimus dorsi muscle from a 2-month-old chicken, depicting Pax7⁺ satellite cells in intrafusal and extrafusal myofibers. The small intrafusal fibers (clustered within a spindle capsule) are near the center of each parallel image. A, Myosin (in green) highlights myofiber cross-sectional area; laminin (in red) highlights the myofiber basal lamina and the spindle capsule (identified with an asterisk). B and C, laminin (deep blue) and nuclei (light blue). C, Pax7⁺ nuclei (in red). e, extrafusal fiber; open/large arrow, intrafusal fiber; small arrow, Pax7⁺ cell associated with intrafusal fiber; arrowhead, Pax7⁺ cell associated with an extrafusal fiber. Scale bars = 30 μ m. This figure is adapted from Kirkpatrick et al. (2009) with kind permission from Dr. Benjamin Rosser. Immunostaining reagents and protocols are detailed in Kirkpatrick et al. (2009).

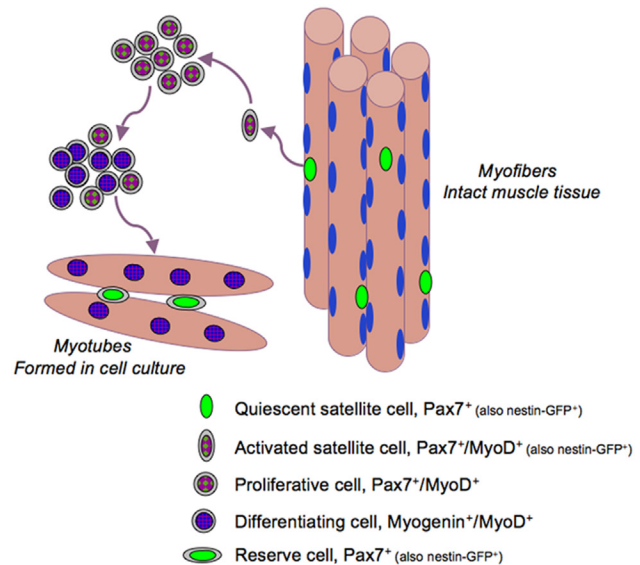


Figure 3. The molecular signature of satellite cells and their progeny upon activation, proliferation, differentiation, and self-renewal. The model is based primarily on cell culture studies (Yablonka-Reuveni and Day 2011, with kind permission of Springer Science+Business Media; edited for nestin-GFP information). Such cell cultures have contributed to the identification of autocrine/paracrine factors that can regulate satellite cell activation and proliferation, demonstrating that hepatocyte growth factors and fibroblast growth factors are crucial growth factors involved in the process (e.g., Shefer et al. 2006; Wozniak et al. 2005; Yablonka-Reuveni and Rivera 1997; Yablonka-Reuveni, Seger et al. 1999; Yamada et al. 2009; Tatsumi et al. 1998; Gal-Levi et al. 1998). The role of many other growth factors and extracellular matrix components has been studied extensively in cell culture as well; for a comprehensive review of these topics that are beyond the scope of this article, see Shefer and Yablonka-Reuveni (2008).

Satellite cell progeny can be distinguished from their quiescent progenitors based on distinctive gene expression patterns (Zammit et al. 2006; Yablonka-Reuveni et al. 2008; Yablonka-Reuveni and Day 2011). In particular, expression of MyoD and myogenin has been used extensively in conjunction with Pax7 (Yablonka-Reuveni and Rivera 1994; Zammit, Carvajal et al. 2004; Shefer et al. 2006; Day et al. 2009). Fig. 3 summarizes Pax7, MyoD, and myogenin expression patterns by satellite cells and their progeny. Proliferating progeny (myoblasts) continue to express Pax7 but, in contrast to their quiescent progenitors, also express MyoD. A decline in Pax7 along with the induction of the muscle-specific transcription factor myogenin marks myoblasts that have entered the differentiation phase and initiated cell cycle withdrawal. Coinciding with or occurring soon after the upregulation of myogenin, differentiating myoblasts initiate expression of various genes encoding structural proteins, such as sarcomeric myosin, and fuse into myotubes (Andres and Walsh 1996; Halevy et al. 2004; Shefer et al. 2006; Yablonka-Reuveni, Rudnicki et al. 1999; reviewed in Yablonka-Reuveni and Day 2011).

Fig. 3 also presents the phases that express the nestin-GFP transgene: that is, quiescent and activated satellite cells and their renewing progeny (Day et al. 2007; Day et al. 2010); additional discussion about satellite cell renewal is detailed in the next section.

Both quiescent and proliferating satellite cells also express the myogenic regulatory factor Myf5 as determined by mRNA analysis (Kastner et al. 2000; Day et al. 2007; Day et al. 2010). Myf5 promoter activity can also be observed through β -gal detection in satellite cells and their proliferating progeny in myogenic cultures from the aforementioned Myf5^{nlacZ/+} mice (Beauchamp et al. 2000; Day et al. 2010). However, detection of the Myf5 protein has not been reported in quiescent satellite cells, although proliferating progenies do express it (Day et al. 2009), albeit at a higher level in myoblasts from mice lacking MyoD compared with Wild-type (Yablonka-Reuveni, Rudnicki et al. 1999). It is therefore possible that whereas the Myf5 promoter is active in quiescent satellite cells, Myf5 protein is not produced until cells begin to proliferate. Ultimately, Myf5 gene expression declines when myoblasts enter differentiation (i.e., withdraw from the cell cycle followed by fusion into myotubes), whereas MyoD expression persists well into the differentiation stage when satellite cells are maintained in our standard culture conditions (Shefer et al. 2006; Day et al. 2009; Yablonka-Reuveni and Day 2011).

The observation that Myf5 expression declines when myoblasts enter differentiation whereas MyoD expression persists suggests that these two myogenic regulatory factors have different roles during myogenesis of satellite cells (reviewed in Day et al. 2009). MyoD null mice contain functional satellite cells that can contribute to normal (albeit slightly delayed) muscle regeneration (Yablonka-Reuveni, Rudnicki et al. 1999; White et al. 2000). However, it was initially reported that MyoD is essential for muscle regeneration (Megency et al. 1996). Indeed, the delayed differentiation observed in primary cell cultures from MyoD null mice (Yablonka-Reuveni, Rudnicki et al. 1999) and the impaired differentiation observed in single myofibers from such mice (Yablonka-Reuveni, Rudnicki et al. 1999; Cornelison et al. 2000) do suggest a cell autonomous defect that might affect the dynamics of muscle repair in vivo. Unpublished studies from our laboratory have also demonstrated that progeny of satellite cells from MyoD null mice differentiated poorly, if at all, when the progenitors were cultured at low or clonal density, but robust differentiation (although delayed) was observed with high-density cultures. Overall, it is possible that absence of MyoD may affect muscle regeneration only in certain types of injury protocols, and this may lead to the different conclusions stated in published in vivo regeneration studies of MyoD null mice (Megency et al. 1996; McIntosh et al. 1998; White et al. 2000).

Early studies that developed several different Myf5 null mice demonstrated neonatal lethality, caused likely by compounded effect of the Myf5 mutations on a neighboring

gene (Olson et al. 1996). However, more recent studies generated Myf5 null mice that survive to adulthood and are fertile, suggesting that Myf5 is not essential for satellite cell function in adult muscle (Gayraud-Morel et al. 2007; Ustanina et al. 2007). Myogenin expression is critical for muscle formation during embryogenesis. However, conditional inactivation of the myogenin gene in the adult muscle does not interfere with myogenesis and raises further questions (and new research directions) about the actual role of myogenin in adult life (Knapp et al. 2006; Meadows et al. 2008). The role of the fourth myogenic regulatory factor, MRF4, remains unknown in myogenesis of satellite cells, but it is the only MRF expressed at a high level in adult myofibers, whereas MyoD, Myf5, and myogenin expression levels are relatively lower (Hinterberger et al. 1991). Notably, MRF4 involvement in downregulating myogenin expression has been reported (Zhang et al. 1995). In two different studies on MRF4 mRNA expression in primary myogenic cultures, this factor was detected before, after, and concurrently with myogenin expression (Smith et al. 1993; Smith et al. 1994). In another study, a reporter gene in a targeted allele of MRF4 was expressed in the differentiated, but not proliferative, state of satellite cell progeny (Gayraud-Morel et al. 2007). Our unpublished work indicates differences between satellite cell cultures from limb and diaphragm muscle in the temporal expression of MRF4; relatively high levels of MRF4 transcripts were detected in limb cultures before the increase in the expression of the differentiation-linked marker myogenin, but in diaphragm cultures MRF4 expression coincided with myogenin expression. Hence, the kinetics and role of MRF4 expression be different depending on the age of the organism and the muscle from which myogenic progenitors are isolated.

Satellite Cell Self-renewal

Routine daily activity leads to subtle muscle injuries and requires nuclear replacement for localized myofiber repair throughout life. Therefore, without its renewal, the satellite cell pool would be depleted. However, very little is known about how the satellite cell pool is retained in vivo. Cell culture studies have demonstrated that coinciding with myoblast differentiation, a subpopulation of mononucleated cells downregulate MyoD expression and exit the cell cycle but maintain Pax7 expression, in contrast with neighboring cells that retain MyoD and upregulate myogenin expression (Halevy et al. 2004; Zammit, Golding et al. 2004; Shefer et al. 2006; Yablonka-Reuveni et al. 2008; Day et al. 2009; Day et al. 2010). BrdU labeling studies suggest that such Pax7⁺/MyoD⁻ cells are derived from Pax7⁺/MyoD⁺ myoblasts that have downregulated MyoD expression and do not continue in the cell cycle or transit into differentiation, thus demonstrating the self-renewing population (Zammit, Golding et al. 2004; Day et al. 2007).

These Pax7⁺/MyoD⁻ nonproliferating cells define a reserve population that presumably reflects satellite cell self-renewal (Fig. 3). Indeed, following transplantation of donor satellite cells into host muscles, satellite cells are detected *in vivo* with donor reporter markers, indicating that at least some of the satellite cells underwent self-renewal (Collins et al. 2005; Sacco et al. 2008). It is sensible to suggest that the donor cells first go through an amplification phase before some of the cells enter the satellite cell niche based on engraftment of single satellite cells that produced both differentiated progeny and renewed satellite cells (Sacco et al. 2008).

There is some evidence that satellite cells self-renew through asymmetric cell divisions controlled by Notch and Wnt signaling pathways (Conboy and Rando 2002; Kuang et al. 2007; Ono et al. 2009; reviewed in Punch et al. 2009; Yablonka-Reuveni and Day 2011). The close association of satellite cells with capillaries residing in the surrounding interstitium also may provide satellite cells with direct signals from pathways traditionally shown to be involved in angiogenesis and vascular integrity (Christov et al. 2007). Interestingly, the angiopoietin-1/Tie2 receptor-signaling pathway may directly control development of reserve cells and entry into quiescence (Abou-Khalil et al. 2009).

As detailed earlier in this article, our laboratory has found that nestin-GFP transgenic expression in mice allows monitoring of satellite cells in isolated myofibers. This transgene also appears to identify the self-renewing, Pax7⁺/MyoD⁻ reserve progeny that develop in cell culture (Day et al. 2007; Day et al. 2010). Satellite cells express nestin-GFP, but this expression is diminished in proliferating and differentiating myoblasts and myotubes. However, cultures with dense myotube networks eventually develop mononuclear, nestin-GFP⁺ cells that do not proliferate (based on BrdU incorporation studies) and are Pax7⁺/MyoD⁻. Examination of satellite cells in clonal cultures showed that nestin-GFP⁺ reserve progeny develop only in dense clones, which accounts for only some of the clones (Day et al. 2007; Day et al. 2010). We also demonstrated an age-linked decline in such clones containing nestin-GFP⁺ reserve cells (Day et al. 2010). This age-linked decline in clones containing reserve cells is concomitant with a decline in satellite cell numbers on myofibers isolated from old mice (Day et al. 2010). Therefore, impairment in satellite cell renewal in old age may lead to the observed decline in satellite cells with age, which also results in a significant number of myofibers without satellite cells, as shown in myofibers from mouse and rat limb muscles (Shefer et al. 2006; Day et al. 2010; Shefer et al. 2010;). It is possible that myofibers lacking satellite cells are more prone to age-associated atrophy, but this issue has not been studied further. In addition to secreted factors, contact with myotubes might provide a signal for self-renewal as also suggested in studies with isolated myofibers maintained in suspension where the satellite cells

remain on the myofiber surface (Zammit, Golding et al. 2004; Kuang et al. 2007; Kuang et al. 2008).

Apart from the aforementioned evidence supporting the hypothesis that the self-renewing population is reserved from the pool of proliferating myoblasts, there is also the demonstration that satellite cells may include a minor subpopulation of stem cells that can self-renew while also providing committed satellite cells within a single proliferative round (Kuang et al. 2007; Kuang et al. 2008; Punch et al. 2009). According to this asymmetric cell division model, most of the satellite cells are “committed,” expressing both Pax7 and Myf5, and only provide committed progeny (i.e., characteristic myogenic cells). The minor “stem cell” population does not display Myf5 gene activity based on Myf5-Cre driven Rosa26-YFP reporter expression, whereas the committed cells do express YFP (Kuang et al. 2007). However, our studies with Myf5^{nLacZ/+} mice have suggested that essentially all satellite cells express the Myf5 reporter, but the cells with weaker expression may only be detected with increased sensitivity of β -gal detection methods (Beauchamp et al. 2000; Day et al. 2010).

Moreover, our unpublished studies with Myf5-Cre-driven reporter expression indicate that a minor population of satellite cells indeed does not show Cre-driven reporter (GFP) expression, and the proliferating/differentiated myoblasts and myotubes developed clonally from such satellite cells also do not express GFP. The animals used in these studies were generated by crossing Myf5-Cre mice (Tallquist et al. 2000; Kuang et al. 2007) with Rosa26-mT/mG mice (Muzumdar et al. 2007). The lack of GFP expression in both the progenitors and their committed progeny introduces a complication to the proposed model of asymmetric cell division when relying on the Myf5-Cre mouse. In contrast with the Myf5-Cre mice, a recent study with the MyoD-Cre/Rosa26-YFP mouse model suggests that all satellite cells are derived from cells that “historically” expressed MyoD and therefore are all committed to myogenesis (Kanisicak et al. 2009). Further studies are required to resolve questions about satellite cell functional heterogeneity and self-renewal based on different strains of reporter mice.

Isolation and Culture Approaches for Satellite Cell Analysis

Much of our understanding of satellite cell biology has arisen from analysis of satellite cells in culture. Satellite cells are routinely isolated from skeletal muscles by enzymatic digestion (Yablonka-Reuveni 2004). Depending on the enzymatic procedure used and the purpose for cell isolation, enrichment for satellite cells beyond the basic isolation protocol is often unnecessary; see for example our studies with Pronase digestion of skeletal muscle (Haley et al. 2004; Shefer et al.

2006; Day et al. 2009; Yablonka-Reuveni 2004; reviewed in Danoviz and Yablonka-Reuveni 2012). Alternatively, satellite cells can be enriched from whole muscle cell suspensions by various approaches that remove myofiber debris present in the initial cell suspension and reduce the presence of fibroblastic cells. Such different enrichment/debris-cleaning approaches include (but are not limited to) (a) initial short-term plating on uncoated tissue culture dishes that results in removal of fast adhering cells followed by culturing of the remaining non-adhering cells (i.e., differential plating) (Richler and Yaffe 1970; Rando and Blau 1994; Qu-Petersen et al. 2002); (b) fractionation on Percoll density gradients (Yablonka-Reuveni and Nameroff 1987; Yablonka-Reuveni et al. 1987; Morgan 1988; Kastner et al. 2000); and (c) cell sorting by forward and side scatter (Yablonka-Reuveni 1988).

In studies where further enrichment of satellite cells is warranted, cells can be isolated by FACS using antibodies that react with satellite cell surface antigens (Sacco et al. 2008; Ieronimakis et al. 2010). First, cells are released from the muscle tissue using collagenase–dispase, an enzyme mixture that preserves cell surface antigens more effectively than Pronase or trypsin digestion methods. Studies from various laboratories (performed mainly with mouse tissue) have established that satellite cells can be isolated based on being negative for CD45, CD31, and Sca1 and positive for CD34 and $\alpha 7$ integrin (Montarras et al. 2005; Sacco et al. 2008; Ieronimakis et al. 2010). Additional cell surface antigens, including CXCR4, $\beta 1$ integrin, and syndecan-4, have also been used for isolation of myogenic progenitors from adult muscle (Cerletti et al. 2008; Tanaka et al. 2009). Fluorescence-based reporter systems in genetically manipulated mouse strains have also permitted reliable isolation and study of satellite cells. For example, we have isolated satellite cells from different muscle groups of transgenic nestin-GFP mice by FACS based on high GFP expression in satellite cells (Day et al. 2007; Day et al. 2010). Pax3- and Pax7-driven reporter expression have also been used to sort mouse satellite cells by FACS (Bosnakovski et al. 2008; Montarras et al. 2005). However, in contrast with the Pax7-driven reporter that is commonly expressed by satellite cells across different muscle groups, Pax3 expression is restricted to select satellite cells (Montarras et al. 2005; Relaix et al. 2006). In addition, mice with a GFP reporter gene targeted into the Myf5 locus have permitted isolation of myogenic cells by FACS (Biressi et al. 2007). However, not all satellite cells in this mouse exhibit detectable GFP expression, so efficiency of satellite cell isolation based on this report is reduced (Christov et al. 2007; Gayraud-Morel et al. 2007). Cre-Lox mouse models offer additional ways for isolating satellite cells based on permanent fluorescent reporter expression in cells derived from myogenic progenitor cells expressing Cre-recombinase driven by genes relevant to the myogenic lineage such as Pax3, Myf5, and

MyoD (Schienda et al. 2006; Kuang et al. 2007; Kanisicak et al. 2009). When working with such Cre-Lox mouse models to sort satellite cells, one should be careful to ensure that the reporter is not expressed in additional cell types during embryogenesis. See for example the report on Myf5-Cre expression at nonmyogenic sites (Gensch et al. 2008).

The ability to isolate and culture single live myofibers (most commonly from rodent models) offers the advantage of following individual resident satellite cell activity in the presence of its parent myofiber (Bischoff 1986; Yablonka-Reuveni and Rivera 1994; Rosenblatt et al. 1995). In adherent myofiber culture models, satellite cells typically emanate from their position on the myofiber and give rise to proliferating myoblasts that differentiate and generate myotubes (Zammit et al. 2002; Shefer et al. 2006). Depending on culture and media conditions, rapid activation, proliferation, and differentiation of satellite cells can take place within the adherent parent myofiber unit (Yablonka-Reuveni and Rivera 1994, 1997; Yablonka-Reuveni, Rudnicki, et al. 1999; Yablonka-Reuveni, Seger, et al. 1999; Kastner et al. 2000; Shefer and Yablonka-Reuveni 2005) (Fig. 4). Alternatively, culture of myofibers in suspension permits the unique ability to observe the behavior of satellite cells as they proceed through multiple rounds of cell division in cell clusters while remaining in contact with the myofiber (Zammit, Golding et al. 2004).

Isolated single myofibers also allow tracing and recording of satellite cell numbers per myofiber based on specific marker expression, with endogenous Pax7 expression becoming a common and direct approach to monitor satellite cells by immunofluorescence (Beauchamp et al. 2000; Zammit et al. 2002; Shefer et al. 2006; Day et al. 2010). Individual progeny of satellite cells can also be analyzed in clonal studies. We have reported on such clonal analyses of satellite cells upon isolation of cells from whole muscles and also from triturated individual myofibers (Shefer et al. 2006; Yablonka-Reuveni et al. 1987; Day et al. 2010). Notably, some researchers have used an approach for satellite cell isolation that is based on bulk preparations of myofiber fragments. It should be noted that myofiber fragments retain “external” connective tissue cells and the preparation requires further enrichments; otherwise, myogenic purity of the resulting cells is drastically reduced.

Studies with myogenic cell lines (including rat L6 and L8 and mouse C2, C2C12, and MM14; Yaffe 1969; Yaffe and Saxel 1977; Clegg et al. 1987; Yablonka-Reuveni et al. 1990; Graves and Yablonka-Reuveni 2000; Jones et al. 2005; Kwiatkowski et al. 2008) have permitted extensive biochemical and molecular analyses, although these models do not always fully adhere to the biology of satellite cells. We have previously published comprehensive summaries of the characteristics of the most commonly used myogenic cell lines (Shefer and Yablonka-Reuveni 2008; Yablonka-Reuveni and Day 2011).

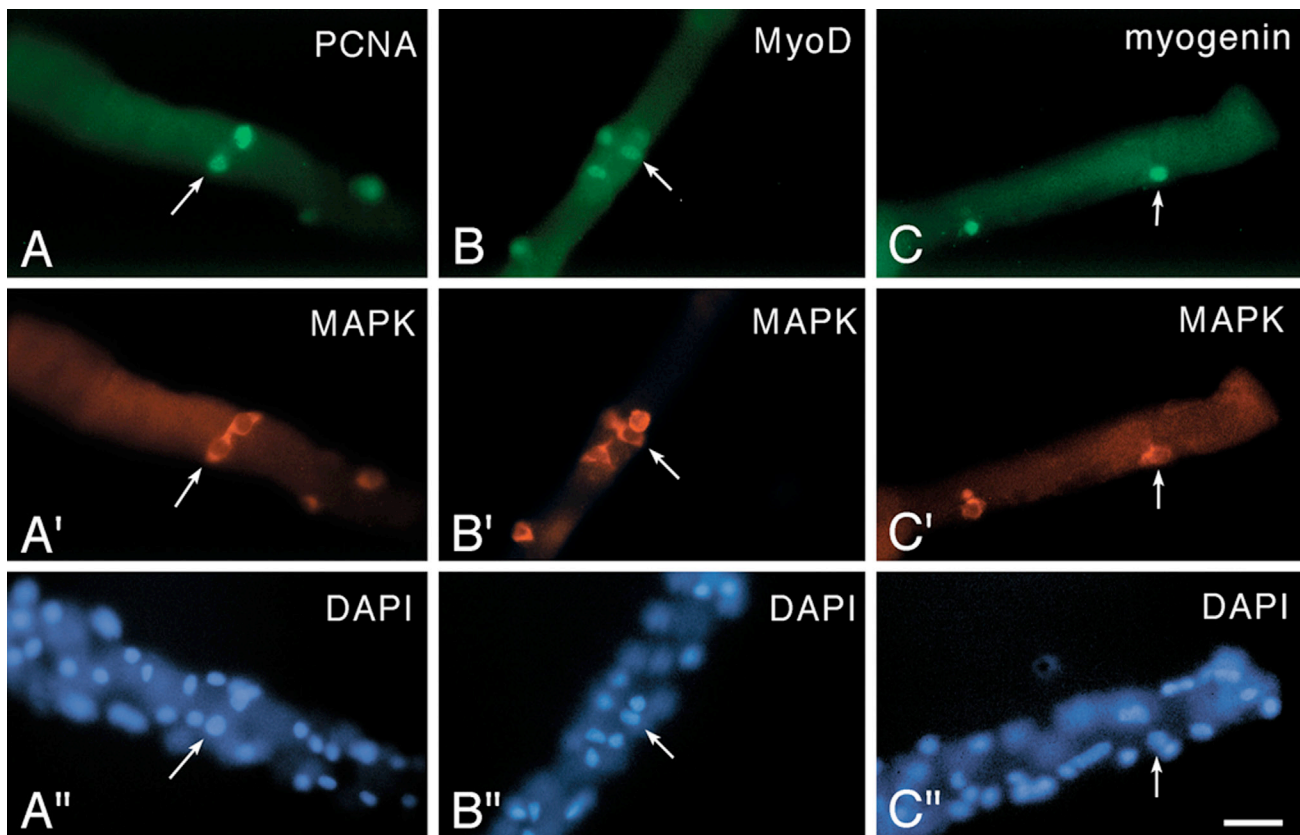


Figure 4. Micrographs of fiber cultures isolated from the flexor digitorum brevis muscle of an 8-week-old rat. Cultures were maintained in basal medium and reacted via double immunofluorescence with a rabbit polyclonal antibody against MAPK (anti-ERK1/2, highlights the cytoplasm of satellite cells as detailed in Yablonka-Reuveni, Seger et al. (1999) and mouse monoclonal antibodies against (A, A') PCNA, which highlights proliferating cells; (B, B') MyoD; and (C, C') myogenin. For each antibody combination, the bottom panel (A''–C'') shows a parallel DAPI stain, which highlights both myofiber nuclei and satellite cell nuclei. Arrows in each set of adjacent panels point to the location of the same cell. Immunostaining with the anti-PCNA/anti-MAPK and the anti-MyoD/anti-MAPK is shown for Day 2 cultures, and immunostaining with the anti-myogenin/anti-MAPK is shown for Day 3 cultures. Not all positive nuclei or cells on the fibers are in the same focal plane. Bar = 34 μ m. This figure was published first in Yablonka-Reuveni, Seger et al. (1999), where additional details regarding immunostaining reagents and protocols are provided. Note that before high-quality antibodies for satellite cell research were available, researchers investigated activation and proliferation of satellite cells on isolated myofibers by tracing tritiated thymidine uptake, which required extra time (even several weeks) to obtain autoradiographic images of the cultures to identify the location of S-phase cells (Bischoff 1989; Schultz 1996; Yablonka-Reuveni and Rivera 1997). This approach provided valuable feedback about response of satellite cells to growth factors, regardless of the tedious method that was used to collect the data (Bischoff 1986).

Satellite Cell Transplantation: Hopes and Hypes for Muscle Regenerative Medicine

Satellite cell behavior has often been investigated by transplantation of cells into host muscles of experimental animals and assessment of donor cell contribution to myofiber formation shown by expression of donor-derived genes (Collins et al. 2005; Montarras et al. 2005; Collins et al. 2007; Cerletti et al. 2008; Sacco et al. 2008). The long-term goal of such studies has been the improvement of muscle quality and performance in cases of severe muscle wasting disorders such as Duchenne muscular dystrophy (Gussoni

et al. 1997; Miller et al. 1997; Tremblay and Skuk 2008; Muir and Chamberlain 2009).

Donor satellite cells contribute myofiber nuclei while also establishing additional satellite cells through self-renewal in experimental mouse models, enabling further understanding of satellite cell biology in the *in vivo* environment (Collins et al. 2007; Sacco et al. 2008). However, limited migration of satellite cells and their progeny, donor–host histocompatibility issues, death of donor cells, and the need to deliver cells to many host muscles at once represent some of the major challenges that have not yet been resolved for cell-based therapies of skeletal muscle wasting disorders (Partridge 2004; Mouly et al. 2005; Lafreniere et al.

2009; Tremblay et al. 2009; Richard et al. 2010; Tedesco et al. 2010). Additionally, some reports have alluded to the failure of satellite cells injected into the circulation to reach target muscles for whole body treatment, although detailed evidence is not available (Sampaolesi et al. 2003; Dellavalle et al. 2007). Myoblasts expanded from satellite cells in culture to multiply the number of donor cells available also cannot be delivered systemically to target muscles and lose regenerative capacity compared with freshly isolated satellite cells upon direct intramuscular delivery (Morgan et al. 1993; Beauchamp et al. 1999; Montarras et al. 2005; Cossu and Sampaolesi 2007). It also appears that muscle injury or irradiation prior to donor cell delivery significantly enhances engraftment, as shown in experimental mouse models (Gross et al. 1999; Collins et al. 2005; Sacco et al. 2008). Therefore, cell-based therapy for skeletal muscle remains a challenge despite the availability and our understanding of satellite cells. Notably, certain cell culture conditions may better preserve the potential of satellite cell progeny to be used as donor cells for muscle repair upon intramuscular transplantation (Gilbert et al. 2010). Expanding human myogenic cell population under such conditions may allow the preparation of sufficient myogenic cells for cell-based muscle therapy of individual muscles or limited muscle groups (e.g., oculopharyngeal muscular dystrophy, Brais 2011; see also Negroni et al. 2011). However, genetic disorders that affect all muscles body-wide (e.g., Duchenne muscular dystrophy) will likely require delivery of donor cells via circulation and possibly combination of cell-based therapies with viral vector or other approaches to deliver therapeutic genes effectively (Muir and Chamberlain 2009).

Are Satellite Cells the Sole Source for Myogenic Progenitors in Adult Muscle?

The discovery of the satellite cell in 1961 established an obvious candidate for the source of new muscle growth and repair, and the satellite cell has remained the uncontested myogenic progenitor of skeletal muscle for many years based on cell culture and *in vivo* studies. More recent studies have demonstrated that new myofibers and new satellite cells can be generated from a few satellite cells resident on a single transplanted myofiber or even from individually transplanted satellite cells (Collins et al. 2005; Sacco et al. 2008). Additionally, lineage-tracing studies relying on inducible activation of Pax7-driven reporter expression in adult muscle have confirmed that satellite cells can repair damaged muscle tissue (Lepper et al. 2009).

However, other cell types isolated from skeletal muscle, such as mesoangioblasts (perivascular cells), myoendothelial cells, side population (SP) cells, interstitial PICs, and

pericytes (i.e., contractile, smooth muscle-like cells engulfing the endothelium at the microvasculature wall), also seem to have some myogenic potency (Montanaro et al. 2004; Dellavalle et al. 2007; Zheng et al. 2007; Mitchell et al. 2010; Tedesco et al. 2010; Negroni et al. 2011). Identification of the aforementioned non-satellite cell myogenic sources has led some to question the classic view that satellite cells are the sole supply of myogenic precursors in postnatal and adult life. However, recent reports using novel genetic mouse models for specific ablation of satellite cells based on Pax7 expression (Lepper et al. 2011; McCarthy et al. 2011; Murphy et al. 2011; Sambasivan et al. 2011) provide once again strong, perhaps ultimate, evidence of the essential role of satellite cells in the regenerative biology of skeletal muscle. Whether the aforementioned non-satellite cell types participate in normal muscle maintenance and repair remains unclear, although these cells might offer alternative avenues for cell-based repair of muscle (especially in muscular dystrophy), because at least some of these cells can reach host muscles upon donor cell delivery via circulation (Gussoni et al. 1999; Dellavalle et al. 2007).

Pericytes (often referred to as “mural cells of the microvasculature”) are a particularly intriguing example of a possible non-satellite cell source of myogenic cells given their mesenchymal plasticity and proximity of the capillary networks to myofibers (Day et al. 2007; Diaz-Flores et al. 2009; Armulik et al. 2011). There is also a degree of resemblance between the pericyte and the satellite cell niche; similar to the satellite cell that is wedged between plasma and basal lamina of the myofiber, the pericyte is situated adjacent to the endothelium, sharing a common basement membrane that surrounds the capillary (Diaz-Flores et al. 1991; Armulik et al. 2005; Diaz-Flores et al. 2009; Armulik et al. 2011). Fig. 5 depicts the proximity of the microvasculature with its associated pericytes to the myofibers. The density of the capillary bed surrounding myofibers ranges widely between muscle groups. For example, extraocular muscles exhibit higher capillary density than the tibialis anterior muscle. We have shown smooth-to-skeletal muscle transition of vascular smooth muscle cell lines, where cells can spontaneously initiate MyoD expression followed by myogenin expression and fusion into myotubes (Graves and Yablonka-Reuveni 2000). In addition, we have shown that retinal derived pericytes can undergo myogenic reprogramming (i.e., express muscle specific genes and transgenes) upon their spontaneous fusion with host myotubes (Kirillova et al. 2007). Published studies on pericyte-like cells being able to contribute to muscle repair have defined cells isolated from skeletal muscle as being pericytes based on features of the cells after propagation in culture (Dellavalle et al. 2007). However, more direct approaches are needed to establish whether pericytes indeed function in routine myofiber maintenance and regeneration. We are presently using pericyte-specific reporter mice to develop means for

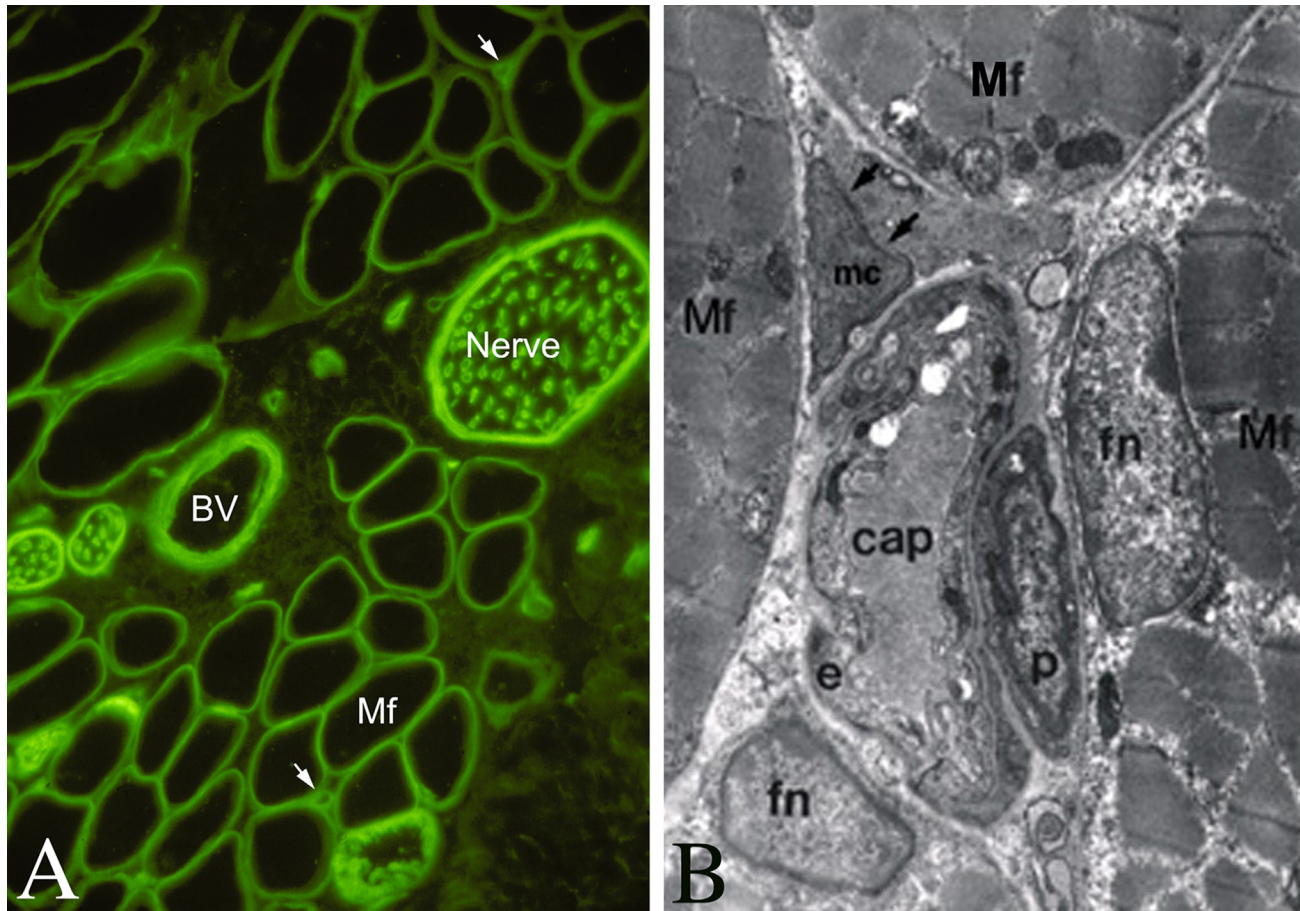


Figure 5. Low- and high-resolution images depicting capillaries of neighboring myofibers. **A**, immunofluorescent image of a cross-section from rat flexor digitorum brevis muscle reacted with an antibody against laminin (green), which highlights the basement membrane of the myofiber (Mf) and other structures in the muscle, including nerve bundles, blood vessels (BV), and capillaries (indicated by arrows). **B**, electron microscopy micrograph of adult chicken muscle (Yablonka-Reuveni 1995) demonstrating the fine details of a capillary surrounded by four myofibers. Mf, myofiber; fn, myofiber nucleus (myonucleus); cap, capillary; e, endothelial cell; p, pericyte; mc, an uncharacterized “mysterious cell,” for which a higher resolution EM revealed a large nucleus and some rER structures in the cytoplasm.

isolating bona fide pericytes and their progenitors with the goal of evaluating the role of pericytes in skeletal myogenesis both in culture and in vivo.

Concluding Remarks

The journey of the satellite cell began with its discovery in 1961. The identification of satellite cell molecular markers has enabled a more efficient analysis of this cell and its progeny both in vivo and in culture, permitting a more refined analysis of cell autonomous and environmental factors affecting satellite cell activation, proliferation, differentiation, and renewal. The development of genetic models for satellite cell analysis has moved satellite cell research to a level never possible before, including the ability to trace satellite cell progeny when the muscle architecture is destroyed upon injury and to study the outcome of satellite

cell ablation. Major emphasis is further placed on developing a means for using satellite cells or alternative populations for muscle repair. Nevertheless, many classic aspects of satellite cell biology await future studies. These include the nature of the satellite cell niche and its role in regulating the quiescent and active states of the satellite cell, mechanisms of satellite cell renewal, the role of environmental and epigenetic factors, and the status of satellite cells in less optimal environments, for example, those caused by muscular dystrophies, aging, obesity, and diabetes. Happy 50th to the skeletal muscle satellite cell! We know you will continue to fascinate us for many more years to come.

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