All muscle satellite cells are equal, but are some more equal than others?

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Summary

Skeletal muscle is an accessible adult stem-cell model in which differentiated myofibres are maintained and repaired by a self-renewing stem-cell compartment. These resident stem cells, which are known as satellite cells, lie on the surface of the muscle fibre, between the plasmalemma and overlying basal lamina. Although they are normally mitotically quiescent in adult muscle, satellite cells can be activated when needed to generate myoblasts, which eventually differentiate to provide new myonuclei for the homeostasis, hypertrophy and repair of muscle fibres, or fuse together to form new myofibres for regeneration. Satellite cells also self-renew in order to maintain a viable stem-cell pool that is able to respond to repeated demand. The study of the control of self-renewal has led to the idea that the satellite-cell pool might be heterogeneous: that is it might contain both self-renewing satellite 'stem' cells and myogenic precursors with limited replicative potential in the same anatomical location. The regulatory circuits that control satellite-cell self-renewal are beginning to be deciphered, with Pax7, and Notch and Wnt signalling being clearly implicated. This Commentary seeks to integrate these interesting new findings into the wider context of satellite-cell biology, and to highlight some of the many outstanding questions.

Introduction

The remarkable regenerative capacity of skeletal muscle was elegantly demonstrated by Studitsky, who removed and minced an entire muscle, then showed that it functionally regenerated when replaced in situ (Studitsky, 1964). The repair and regeneration of syncytial muscle fibres (myofibres) is performed by muscle satellite cells. These resident stem cells were named on the basis of their anatomical location: they lie on the surface of the myofibre, between the plasmalemma and the overlying basal lamina, in both extrafusal myofibres (Mauro, 1961) and the intrafusal myofibres of the muscle spindle (Katz, 1961). Satellite cells generate muscle precursor cells (myoblasts) that then proliferate before they either fuse into an existing myofibre to become post-mitotic nuclei (myonuclei), or fuse together to form myotubes (immature myofibres) (Bischoff, 1975; Konigsberg et al., 1975; Lipton and Schultz, 1979). The main role of the satellite cell during the early postnatal period is to provide myonuclei for skeletal muscle growth. In adult muscle, its role changes to one of providing myonuclei for homeostasis and hypertrophy, or in response to the more sporadic demands for myofibre repair and regeneration (Zammit et al., 2006).

This Commentary focuses on two fundamental questions in satellite-cell biology. First, are all satellite cells capable of behaving as stem cells or is there a dedicated satellite 'stem' cell lineage within the satellite-cell compartment? Second, how is satellite cell self-renewal regulated? Emerging evidence clearly implicates Notch and Wnt signalling in control of satellite-cell fate.

The developmental origin of satellite cells

The musculature of the trunk, the limbs and a part of the head arises from stem cells in the mesoderm-derived somites, which are transitory embryonic structures that form in pairs, one on either side of the neural tube (Stockdale et al., 2000; Sambasivan and Tajbakhsh, 2007). Recently, myogenic stem cells have been directly identified in the mouse somite by their molecular profile: they express the paired box (Pax) transcription factor Pax3 and, later, Pax7, but not muscle-specific proteins, such as the members of the myogenic-regulatory-factor family (which comprises Myf5, MyoD, Mrf4 and myogenin; Fig. 1). During development, these cells continually generate embryonic and foetal myoblasts, which are identified by the expression of Myf5 (the earliest marker of the myogenic lineage) and/or MyoD (Gros et al., 2005; Kassar-Duchossoy et al., 2005; Relaix et al., 2005).

Transplantation assays and lineage-tracing indicate that most satellite cells in the late foetal period are also derived from somites, and it has been argued that these myogenic stem cells are the source (Armand et al., 1983; Gros et al., 2005; Schienda et al., 2006) (Fig. 1). Satellite cells currently lack a unique molecular signature that can be used to distinguish them from other myoblasts during development. Functional characteristics, which include the myosin-heavy-chain isoforms that are expressed after differentiation and the timing of acetylcholine-receptor expression, suggest that satellite cells first emerge during the foetal period in rodents and birds (Cossu and Molinaro, 1987; Feldman et al., 1993; Hartley et al., 1991; Yablonka-Reuveni, 1995). However, only when the basal lamina forms around myotubes towards the end of the foetal period can cells first be classified as satellite cells by using anatomical criteria (Kelly and Zacks, 1969; Ontell and Kozeka, 1984).

Satellite cells in growing postnatal muscle

Satellite cells account for ~30-35% of the sublaminal nuclei of myofibres in early postnatal mouse muscle, but this proportion falls over time and, by adulthood, only 1-4% of nuclei belong to satellite cells (Allbrook et al., 1971; Hellmuth and Allbrook, 1971; Schultz, 1974). By contrast, the number of myonuclei in muscle increases during postnatal growth (Enesco and Puddy, 1964). Satellite cells proliferate in growing muscle (Shafiq et al., 1968), and tracking

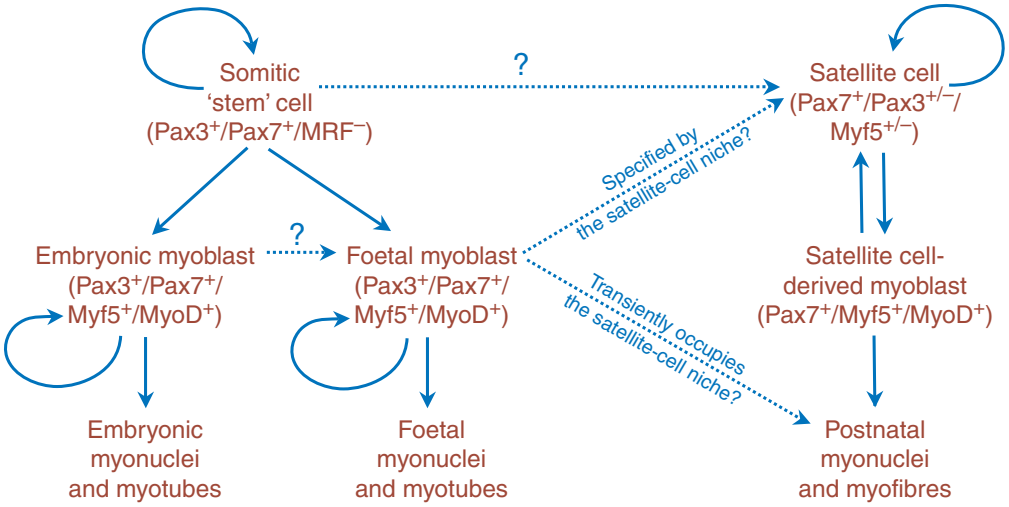

Fig. 1. Possible origins of satellite cells. Self-renewing somitic stem cells can be identified by the expression of Pax3 and Pax7, but lack expression of myogenic regulatory factors (MRFs), such as Myf5 and MyoD. These stem cells generate embryonic and foetal myoblasts, which proliferate before differentiating and fusing to produce myotubes. Somitic stem cells may become satellite cells directly if they are partitioned beneath the basal lamina as it forms. The satellite-cell niche might also be transiently occupied by foetal myoblasts, which differentiate to contribute myonuclei for the initial phases of post-natal growth. Such foetal myoblasts might also be specified by the niche to become satellite cells. Satellite cells can self-renew, and produce satellite-cell-derived myoblasts that proliferate and differentiate to contribute myonuclei to growing muscle.

DNA replication during S phase by using tritiated thymidine suggested that labelled satellite cells give rise to myonuclei following cell division (Moss and Leblond, 1970); this was later confirmed by many other studies. Importantly, the tracking of DNA replication also indicated that satellite-cell divisions could be asymmetric in growing muscle and give rise to both myonuclei and satellite cells, which suggests that satellite cells self-renew (Moss and Leblond, 1971). The satellite-cell population in growing muscle can be separated into two groups: a fast-dividing population that undergoes limited replication before differentiating, and a slow-dividing population that might return to G0 between cycles and give rise to the fast-dividing population (Schultz, 1996).

Together, these data suggest that, during the early postnatal period, fast-dividing satellite cells initially undergo asymmetric divisions to produce both myonuclei for muscle growth and satellite cells, but later undergo symmetric divisions, so that few of the fast-dividing cells remain as satellite cells in adult muscle. The bulk of the satellite cells that exist in adults, therefore, presumably derives from the slow-dividing population. This raises the question of whether most satellite cells are initially merely 'trapped' foetal myoblasts that had been adjacent to a myotube upon formation of the basal lamina, and are destined for effecting muscle growth. If so, do a proportion then become specified as satellite cells by the satellite-cell niche (see below)? The imposition of a stem-cell fate on a more mature cell type is not unprecedented [for example, this occurs in the ovaries of adult *Drosophila melanogaster* (Kai and Spradling, 2004)]. Alternatively, the niche might initially be occupied by a mixture of foetal myoblasts together with a dedicated satellite-cell lineage, cells of which are destined to become satellite cells in the adult.

The satellite-cell niche

The identity and function of stem cells, including satellite cells, is supported by the local microenvironment, which forms the basis of the stem-cell niche. The importance of the stem-cell niche is evident in several well-defined systems, including haematopoietic and intestinal-crypt stem cells (e.g. Scadden, 2006). The niche is dynamic: it maintains stem-cell quiescence, but also contributes to the activation of stem cells when required. In satellite-cell function, the niche is clearly important; dramatically large numbers of new myonuclei and satellite cells can be produced by transplanting relatively few (<40) satellite cells that have been retained in their niche on an isolated myofibre (Collins et al., 2005). The satellite-cell niche is polarised, and comprises the underlying myofibre and the overlying basal lamina (Mauro, 1961); microvasculature is often located nearby (Christov et al., 2007). Recently, it has been suggested that the orientation of satellite-cell division within the niche influences asymmetric cell division. On myofibres isolated from regenerating muscle, apical-basal division (perpendicular to the myofibre) resulted in asymmetric divisions more often than did planar division (in the plane of the myofibre) (Kuang et al., 2007).

Satellite cells communicate with the underlying myofibre via cell-adhesion proteins that include N-cadherin and M-cadherin (Irintchev et al., 1994). The myofibre component of the niche presumably responds to local and systemic cues by presenting Notch ligands that are necessary for Notch signalling, which is involved in the activation of satellite cells (Conboy et al., 2003). Satellite cells also communicate directly with the overlying basal lamina via integrins, particularly $\alpha 7\beta 1$ integrin (LaBarge and Blau, 2002). In addition, satellite cells in their niche are exposed to diffusible factors. Some of these factors – such as hepatocyte growth factor, which is required for activation – are stored in an inert form in the basal lamina (Tatsumi and Allen, 2004), whereas others might emanate from various sources including the myofibre, the vasculature, the immune system and interstitial cells. Satellite cells might also be regulated by mechanical, chemical and electrical activity (Tatsumi et al., 2001). Indeed, changes in the muscle environment and satellite-cell niche, rather than modification of the satellite cells themselves, appear to be the main factor that is responsible for the declining regenerative response of 'old' muscle (Collins et al., 2007; Conboy

and Rando, 2005; Shefer et al., 2006). As this brief description demonstrates, however, much remains to be discovered regarding how the niche regulates satellite-cell function (reviewed in Gopinath and Rando, 2008).

Satellite cells in adult muscle

Mature skeletal muscle is relatively stable tissue (Schmalbruch and Lewis, 2000; Spalding et al., 2005), so the homeostatic demand on satellite cells is low and the vast majority become mitotically quiescent (Schultz et al., 1978). However, satellite cells in adult muscle remain able to be activated when necessary and generate myoblasts for the production of myonuclei for homeostasis, hypertrophy and repair, or entire myofibres for muscle regeneration (Bischoff, 1975; Konigsberg et al., 1975; Lipton and Schultz, 1979).

Satellite cells self-renew in adult muscle

Satellite cells activate and proliferate efficiently: satellite cells that are resident on an isolated myofibre (which comprise only 3-5.5% of all myofibre nuclei) can produce enough myoblasts in vitro to replace all the myofibre myonuclei within 4-5 days (Zammit et al., 2002). Furthermore, the satellite-cell pool continues to respond efficiently even when the muscle is subjected to repeated severe damage (Luz et al., 2002; Sadeh et al., 1985). In the study by Sadeh and colleagues (Sadeh et al., 1985), rats were given weekly injections of bupivacaine for 6 months, and it has been estimated that this would elicit at least 20 cycles of extensive muscle degeneration and regeneration, which would require an estimated minimum of 80 doubling events per satellite cell (Bischoff and Franzini-Armstrong, 2004).

How is such a robust regenerative potential maintained? Haematopoietic stem-cell function has been tested by transplanting cell populations into hosts whose own stem-cell compartment has been destroyed by irradiation. Similar assays that use transplantation into skeletal muscle (with or without local irradiation) have been developed to analyse the fate and function of myogenic precursor cells. On the basis of such transplantation models, it has long been known that grafted myoblasts not only generate myonuclei (Lipton and Schultz, 1979) but also produce viable myogenic precursors (Cousins et al., 2004; Gross and Morgan, 1999; Heslop et al., 2001; Morgan et al., 1994; Yao and Kurachi, 1993). This assay was later refined by grafting a single myofibre, which has the advantage of transplanting only a limited number of satellite cells; these are also retained in their niche between the plasmalemma and basal lamina (Collins et al., 2005). Large numbers of new myonuclei and functional satellite cells can result from such transplantation: for example, an extensor digitorum longus myofibre, which was associated with approximately seven satellite cells, was estimated to have produced ~11 times as many new satellite cells, in addition to many myonuclei (Collins et al., 2005). This formally showed that at least some satellite cells were capable of self-renewal. Moreover, the loss of regenerative ability that is caused by destroying satellite cells (and any other resident stem cells) by local irradiation can be partially restored by grafting just one myofibre (Collins et al., 2005).

How do satellite cells self-renew?

Viable myofibres can be isolated and maintained in culture, which allows the associated satellite cells to activate and proliferate while being retained in their niche (Zammit et al., 2004). This technique has made it possible to study individual satellite cells during lineage progression. Quiescent satellite cells express several markers, which include Pax7, M-cadherin, CD34 and – in *Myf5*$^{nlacZ/+}$ mice – β-galactosidase from the targeted *Myf5* allele (Beauchamp et al., 2000; Irintchev et al., 1994; Seale et al., 2000). MyoD is rapidly induced during activation in satellite cells in vivo and, in vitro, virtually all satellite cells express MyoD after ~24 hours of culture (Grounds et al., 1992; Yablonka-Reuveni et al., 2008; Yablonka-Reuveni and Rivera, 1994; Zammit et al., 2004). After proliferating as Pax7-positive and MyoD-positive satellite-cell-derived myoblasts, most cells then downregulate Pax7, maintain MyoD expression and commit to myogenic differentiation. Others, however, downregulate MyoD but maintain Pax7 expression and eventually withdraw from the cell cycle (Zammit et al., 2004); they also exhibit an increase in the levels of sphingomyelin (Nagata et al., 2006a) and the re-expression of a nestin transgene (Day et al., 2007), which are characteristics of quiescence. Similar observations have been made in growing chicken (Halevy et al., 2004) and rat muscle (Schultz et al., 2006).

The observations above led us to propose the model of satellite-cell self-renewal that is depicted in Fig. 2A, in which satellite cells initially activate MyoD before MyoD is lost from some cells as a cell-fate decision is made (or imposed) to self-renew, rather than to differentiate (Zammit et al., 2004). The expression of MyoD is normally associated with the initiation of a transcriptional cascade that culminates in myogenic differentiation, even in non-muscle cells (Weintraub et al., 1991). The activity of MyoD can, however, be controlled by its post-translational modification, by its association with repressor proteins or by inhibiting its interaction with DNA (Berkes and Tapscott, 2005), all of which allow it to be expressed in proliferating myoblasts without necessarily causing immediate differentiation. MyoD can be downregulated in satellite cells after division, and cell pairs in which MyoD remains expressed in only one cell have been observed (Zammit et al., 2004); and low or absent MyoD is associated with enhanced proliferation and delayed or perturbed myogenic differentiation (Asakura et al., 2007; White et al., 2000; Yablonka-Reuveni et al., 1999). The model shown in Fig. 2A is based largely on observations made in vitro under culture conditions containing high levels of serum and chick-embryo extract, and the determination of culture conditions in which stem-cell characteristics are retained can be challenging. Thus, the generation of new tools, such as targeted alleles of *Pax7* and *MyoD*, is required to rigorously examine this model in vivo.

First among equals – is the satellite-cell population homogeneous?

The model of satellite cell self-renewal proposed in Fig. 2A also implies that satellite cells are a relatively homogeneous population, in which cells activate and express MyoD before the decision to self-renew or differentiate is made. Thus, most satellite cells are capable of self-renewal and the decision is likely to be controlled by signals from the myofibre, from differentiating satellite-cell progeny or from the changing regenerating environment (Day et al., 2007; Zammit et al., 2004). Over time, however, the satellite-cell population might evolve into a continuum of cells with more (or fewer) stem-cell characteristics, perhaps because some cells have been activated less frequently, or have undergone fewer divisions [as has recently been proposed for skin stem cells (Clayton et al., 2007)]. Alternatively, the satellite-cell population might be composed of both lineage-based satellite 'stem' cells and myogenic precursors in the same anatomical location. However the satellite-cell pool is organised, the expression of MyoD might remain a common step in the activation of all types of satellite cells. Not all

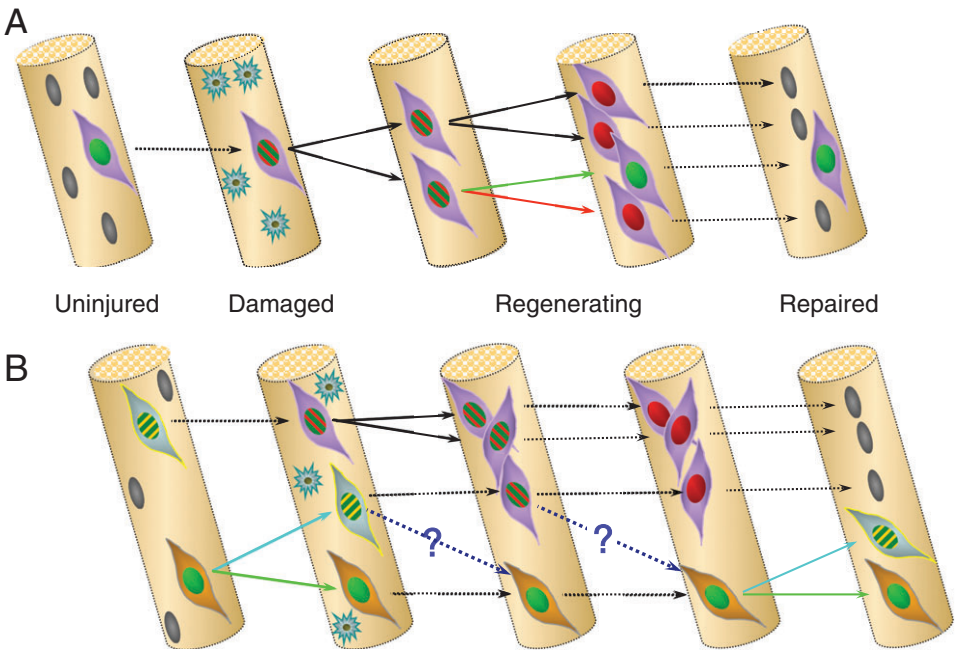

Fig. 2. Two possible modes of satellite-cell self-renewal. (A) Quiescent Pax7-positive satellite cells (green nucleus) co-express MyoD (green and red nucleus) when they are activated following muscle damage. After proliferating as Pax7-positive and MyoD-positive satellite-cell-derived myoblasts, most cells downregulate Pax7, maintain MyoD expression (red) and differentiate to replace lost myonuclei (black). In other satellite-cell progeny, however, MyoD expression is lost and Pax7 expression is maintained (green nucleus) as the cell self-renews. (B) There are two types of Pax7-positive satellite cell, those that have expressed Myf5 (green and yellow nucleus) and those that have not (green nucleus). The Pax7-positive and Myf5-positive satellite cells (green and yellow nucleus) are essentially transit-amplifying cells that are able to give rise to Pax7-positive and MyoD-positive myoblasts (green and red nucleus) that undergo limited proliferation before downregulating Pax7 expression (red nucleus) and differentiating. By contrast, the Pax7-positive, Myf5-negative satellite 'stem' cell (green nucleus) can divide asymmetrically, giving rise both to more satellite-stem-cell progeny for the maintenance of the stem-cell pool, and to Pax7-positive and Myf5-positive satellite cells (green and yellow nucleus) to replace myonuclei.

satellite cells express markers such as *Myf5*-driven expression of β-galactosidase (*Myf5*$^{nlacZ/+}$ mouse), and levels of *Pax3*-driven expression of eGFP (*Pax3*$^{eGFP/+}$ mouse) vary between populations, although such phenotypic heterogeneity might simply reflect a dynamic state of protein expression (Beauchamp et al., 2000; Day et al., 2007; Kuang et al., 2007; Relaix et al., 2006). The constant emergence of new satellite-cell markers, such as lysenin (Nagata et al., 2006b), caveolin 1 (Volonte et al., 2005) and the calcitonin receptor (Fukada et al., 2007), might help further to identify prospective sub-populations. On the functional level, a proportion of satellite-cell-derived myoblasts express myogenin within 8 hours of muscle injury, which shows that they commit to differentiation with little or no proliferation; by contrast, the remaining cells do not divide within the first 24 hours (Rantanen et al., 1995). In vitro, satellite cells exhibit heterogeneity in both their proliferation rate and their clonogenic capacity (Molnar et al., 1996; Schultz and Lipton, 1982).

Are some satellite cells unequal? Is there a satellite 'stem' cell?

A recent study by Rudnicki and colleagues has sought to address the question of satellite-cell heterogeneity directly (Kuang et al., 2007). The authors have shown that ~90% of satellite cells on myofibres of adult *Myf5*$^{cre/+}$ mice had had a 'myogenic experience' and expressed *Myf5* at some point (as shown by the presence of YFP from the recombined targeted *ROSA* locus). The remaining ~10% of satellite cells were YFP-negative, and were able to produce further YFP-negative and YFP-positive cells both in vitro and in vivo. When grafted into *Pax7*-null mice, these YFP-negative cells gave rise to approximately three times more Pax7-positive satellite cells than the YFP-positive cells, and a quarter of these remained YFP-negative. It was proposed by the authors that these YFP-negative cells correspond to a dedicated subset of satellite cells that have more stem-cell-like characteristics (satellite 'stem' cells), and that the YFP-positive cells are their transit-amplifying progeny that can undergo limited symmetric proliferation to generate myonuclei (Kuang et al., 2007). This model of satellite-cell self-renewal is depicted in Fig. 2B. Satellite 'stem' cells are defined by the absence of recombination at the *ROSA-YFP* locus, which is attributed to a lack of expression of *Myf5*-driven *Cre*; this phenotype could, however, result from too little (or too brief) an expression of *Myf5*-driven *Cre* for efficient recombination, or from an inability of the recombined *ROSA* locus to drive *YFP* expression in all quiescent satellite cells. Furthermore, the YFP-positive population might well contain – and, indeed, generate – cells in which *Myf5* is no longer expressed (YFP-positive Myf5-Cre negative cells), which would have a phenotype that is equivalent to that of the YFP-negative population. Importantly, YFP-positive cells do give rise to satellite cells when grafted, albeit fewer than YFP-negative cells. Does this mean that YFP-positive cells are also capable of self-renewal, similar to the YFP-negative cells? Or does this occur because of the presence of YFP-positive Myf5-Cre negative cells in the YFP-positive population? A positive marker of YFP-negative cells [similar to one that has recently been described for crypt stem cells of the small intestine (Barker et al., 2007)] and the use of other targeted

alleles (especially *MyoD*) to drive the expression of *Cre* would help to advance these important observations.

The presence of satellite 'stem' cells has also been examined by pulsing regenerating muscle with halogenated thymidine analogues. A proportion of satellite-cell divisions in vivo and in vitro have been observed to be asymmetric, with the labelled DNA being transferred to the daughter cell that has the self-renewal phenotype (Conboy et al., 2007; Shinin et al., 2006). It has been proposed that this label retention identified satellite 'stem' cells, because the cells contained non-equivalent genomic DNA strands of which the older 'template' strand was protected from DNA replication errors according to Cairn's 'immortal DNA' hypothesis for stem cells (Cairns, 1975). However, label retention is not a universal characteristic of stem cells in all tissues (Waghmare et al., 2008), and it has recently been shown that even crypt stem cells of the small intestine may not retain label (Barker et al., 2007). It is crucial to determine how these label-retaining cells respond to further bouts of muscle injury: if they are satellite 'stem' cells, they should remain at a relatively constant level as they would retain the label by dividing asymmetrically to generate BrdU-negative myonuclei. A caveat is that BrdU is not simply a passive lineage marker, but can repress *MyoD* expression (Ogino et al., 2002) and inhibit myogenic differentiation (Bischoff and Holtzer, 1970). Rather than the prevention of replication errors, the main purpose of non-random segregation of chromosomes might instead be to enable differential gene expression and, therefore, different cell fates of the two progenies – the 'silent sister' hypothesis (Lansdorp, 2007).

What role does Pax7 have in satellite-cell function?

The absence of Pax7 uncouples developmental and postnatal myogenesis, such that muscle development in utero is largely unperturbed but muscle growth postnatally is compromised (Kuang et al., 2006; Oustanina et al., 2004; Seale et al., 2000). Satellite cells are present in *Pax7*-null mice, but exist in reduced numbers that fall further during postnatal development (Kuang et al., 2006; Oustanina et al., 2004; Relaix et al., 2006). Pax7 can activate transcription in quiescent satellite cells and those that adopt a self-renewal phenotype (Zammit et al., 2006) and might influence satellite-cell fate by controlling the expression of the myogenic regulatory factors: Pax7 can modulate the expression of *Myf5* (McKinnell et al., 2008) and *MyoD* (Zammit et al., 2006), and Pax7 transcriptional activity is required to maintain *MyoD* expression (Relaix et al., 2006). Pax7 is rarely co-expressed with myogenin (Halevy et al., 2004; Olguin and Olwin, 2004; Zammit et al., 2004) and constitutive expression of Pax7 delays the induction of myogenin expression in satellite cells (Zammit et al., 2006). In C3H10T1/2 cells that have been converted to a myogenic phenotype by MyoD expression, the overexpression of Pax7 prevents the induction of myogenin expression, whereas the overexpression of myogenin results in the downregulation of Pax7 – it is not known, however, whether such interactions occur in myoblasts (Olguin et al., 2007). Recently, it has been found that Pax7 also interacts with Wdr5 and Ash2L, which are core proteins of the histone methyltransferase complex; this suggests that Pax7 also functions through epigenetic modification (McKinnell et al., 2008). Defects in proliferation and differentiation are apparent in *Pax7*-null myoblasts in culture (Kuang et al., 2006; Oustanina et al., 2004), and a dominant-negative Pax7 mutant elicits myoblast death (Relaix et al., 2006). Therefore, Pax7 certainly seems to have a role in modulating the expression of myogenic regulatory factors, maintaining proliferation and preventing precocious myogenic differentiation; but whether it actively promotes self-renewal, as proposed by Olwin and colleagues (Olguin and Olwin, 2004), is in debate.

Notch signalling influences satellite-cell specification and fate choice

In mammals, there are four Notch receptors (Notch1 to Notch4), which are activated through interaction with their ligands delta-like 1 (Dll1), delta-like 3 (Dll3) and delta-like 4 (Dll4), and jagged 1 and jagged 2 (Jag1 and Jag2, respectively). Ligand binding to one of the Notch receptors results in the proteolytic cleavage of the Notch intracellular domain (NICD) by γ-secretase. The NICD then translocates to the nucleus, where it interacts directly with the transcription factor RBPJ (also known as CBF1) to displace co-repressors and recruit coactivators to activate target genes. The conditional knockout of *RBPJ,* in somitic cells and those cells that migrate to 'seed' the muscle fields of the limbs, diaphragm and tongue, results in their uncontrolled myogenic differentiation (Vasyutina et al., 2007). Similarly, reduced Notch signalling in hypomorphic *Dll1*-mutant mice results in a similar phenotype (Schuster-Gossler et al., 2007). Notch signalling is, therefore, key to preventing the precocious differentiation of myogenic stem and progenitor cells during development. Importantly, the satellite-cell niche is unoccupied in the muscle of mice in which *RBPJ* is conditionally knocked out; this indicates that depletion of these myogenic stem and progenitor cells prevents the appearance of satellite cells during the foetal period.

Satellite cells in adult express *Notch1*, *Notch2* and *Notch3*, together with Notch ligands *Dll1* and *Jag1* (Conboy and Rando, 2002; Fukada et al., 2007). Notch signalling inhibits differentiation in C2 cells (Kopan et al., 1994; Nofziger et al., 1999), and satellite-cell activation in mouse is accompanied by activation of Notch1, which leads to proliferation and, if maintained, prevents satellite-cell differentiation (Conboy and Rando, 2002). Similarly, maintaining Notch activity by targeted disruption of the transcriptional repressor *Stra 13* results in perturbed satellite-cell differentiation and compromised muscle regeneration (Sun et al., 2007). That Notch signalling prevents differentiation does not necessarily imply that it promotes self-renewal; however, inhibiting the Notch pathway with the γ-secretase-inhibitor DAPT does cause a shift to a Pax7-negative and MyoD-positive pro-differentiation phenotype (Kuang et al., 2007). Some satellite-cell divisions result in the asymmetric distribution of Numb, which inhibits Notch signalling by binding to the NICD and preventing its nuclear translocation. Presumably, therefore, asymmetry in Numb distribution leads to different transcriptional programs in each cell progeny, although it is unclear whether self-renewal or differentiation is promoted by the presence of Numb (Conboy and Rando, 2002; Shinin et al., 2006). Finally, it has recently been reported that Notch signalling in satellite cells is antagonised by Wnt3a to promote differentiation (Brack et al., 2008).

Wnt signalling in controlling satellite-cell function

The Wnt proteins belong to a large family of secreted signalling molecules that act through distinct canonical and non-canonical pathways. The canonical pathway involves the stabilisation of β-catenin, which then translocates to the nucleus to control transcription by means of the T-cell factor (TCF)/lymphocyte enhancement factor (LEF) family of transcription factors (Willert and Jones, 2006). β-catenin is expressed by adult rat myogenic cells (Ishido et al., 2006; Wrobel et al., 2007) and by mouse C2 cells

(Goichberg et al., 2001). Constitutive expression of β-catenin or inhibition of endogenous protein degradation both result in a greater proportion of Pax7-positive MyoD-negative satellite cells, and a decreased proportion of differentiating cells. Conversely, silencing β-catenin by RNA interference, or by using a dominant-negative β-catenin mutant to repress its transcriptional targets, reduces self-renewal and promotes differentiation (Perez-Ruiz et al., 2008). This suggests a role for β-catenin in controlling the transcription of target genes in proliferating satellite cells to promote self-renewal. It has recently been shown that β-catenin can directly interact with MyoD in C2 cells, and can increase its transcriptional activity, which shows that β-catenin might also act independently of TCF/LEF (Kim et al., 2008); this activity is in addition to its more established role in the formation of complexes with cadherin family members within adherens junctions at the cell surface, as part of the myoblast fusion process (Goichberg et al., 2001). As the manipulation of β-catenin levels has been reported to act both to inhibit (Gavard et al., 2004; Goichberg et al., 2001; Perez-Ruiz et al., 2008) and promote (Brack et al., 2008; Kim et al., 2008) myogenic differentiation, β-catenin might have roles in multiple signalling cascades and must therefore be tightly regulated. It remains to be determined whether Wnt proteins control the actions of β-catenin in order to influence cell fate in satellite cells, but the recent finding that Wnt signalling helps to maintain quiescence in haematopoetic stem cells indicates that it is a possible mechanism (Fleming et al., 2008).

Conclusions and perspectives

Satellite cells provide us with a model system for the study of adult stem cells, in which a defined and representative stem-cell population can be readily obtained and identified. In contrast to the haematopoietic system, for example, muscle is a stable tissue with a low rate of turnover, which makes the control of quiescence and activation amenable to study. A central question concerns the composition of the satellite-cell pool. Is it a homogeneous population in which any cell can self-renew in response to environmental cues? Or does it exhibit a lineage-based heterogeneity, in which satellite 'stem' cells maintain the pool of satellite cells, the bulk of which merely perform a transit-amplifying role? Are different modes of self-renewal employed or are different populations of satellite cells recruited for muscle growth when compared with the maintenance of adult muscle? Even in mature muscle, satellite-cell responses might differ according to the situation: at one extreme is homeostasis, which requires the occasional replacement of a few myonuclei – at the other is muscle regeneration, which requires the synchronous activation and rapid expansion of the whole pool to generate many thousands of myonuclei. New molecular markers of satellite cells are emerging and, together with new model animals (that use conditional alleles of *Pax7* and *MyoD*, for example), these will hopefully help to resolve this question. Progress towards understanding the control of satellite-cell-fate choice is being made – Pax, and Notch and Wnt signalling are implicated in this regulation, but there is clearly much more work to be done.

In addition to the inherent interest of satellite cells as an adult stem-cell paradigm, satellite-cell biology also has major implications for muscle disease. In muscular dystrophies, for example, the common features of the ~34 clinical disorders are chronic skeletal-muscle wasting and degeneration, which leads to muscle weakness, and even to complete loss of function of most muscles in conditions such as Duchenne muscular dystrophy (Lovering et al., 2005). Therefore, investigating the regulation of satellite cells contributes to our understanding of why satellite cells initially maintain muscle function, but then gradually fail to regenerate dystrophic muscle efficiently. Moreover, there is emerging evidence that in some dystrophic conditions, such as Emery-Dreifuss muscular dystrophy, the primary mutation not only elicits muscle wasting but might also directly compromise satellite-cell function, which could actively contribute to the progression of the disease (Bakay et al., 2006; Gnocchi et al., 2008). Manipulation of the satellite-cell pool could both augment and prolong muscle function, which would be of obvious benefit to patients. It would also have the additional benefit of maintaining – for longer – a muscle environment that can still respond to other forms of treatment, which would extend the window of opportunity for therapeutic intervention.

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