Review

Cell PRESS

Stem cells in the hood: the skeletal muscle niche

Alice Pannérec, Giovanna Marazzi and David Sassoon

Myology Group, UMR S 787 INSERM, Université Pierre et Marie Curie Paris VI, Paris, 75634, France

It is generally accepted that the principal resident progenitor underlying regenerative capacity in skeletal muscle is the satellite cell. Satellite cells are present throughout life even though regenerative capacity declines with age and disease. Recently, other stem cell populations have been identified that can participate in muscle growth and regeneration. These cells may provide therapeutically useful sources of muscle stem cells as an alternative to satellite cells; however, the roles of these nonsatellite cell populations during muscle homeostasis, regeneration, and aging are unclear. Here, we discuss how the stem cell neighborhood influences satellite cell behavior and bring together recent discoveries pertaining to a wide variety of adult stem cells, including muscle stem cells and their niche.

Diversity of muscle progenitors

Adult skeletal muscle is composed primarily of multinucleate myofibers, each surrounded by a basal lamina. A population of single cells, referred to as satellite cells, is found underneath the muscle fiber basal lamina and closely juxtaposed to the muscle fiber plasma membrane [1] (Box 1 and Figure 1). Under normal physiological conditions in adult muscle, satellite cells are quiescent and can be identified by the expression of a number of genes including Pax7 and α7-integrin [2,3]. Upon injury, satellite cells activate expression of the myogenic regulatory factors Myf5 and MyoD, re-enter the cell cycle, differentiate, and ultimately fuse to form new fibers ([1,4,5] and for a review see [6]). Although the majority of satellite cells participate in muscle repair, a small proportion of cells exit the cell cycle and replace the satellite cell population [7,8], providing a mechanism to support multiple rounds of injury. Although substantial evidence points to satellite cells as the major skeletal muscle progenitor/stem cell, there are many reports of nonsatellite cell populations with myogenic capacity following engraftment into muscle tissue (Table 1).

Identification of one of the first nonsatellite cell populations with myogenic capacity came from experiments in which bone-marrow-derived cells were transplanted into lethally irradiated mice. Following muscle injury, these bone-marrow-derived cells participated directly in skeletal muscle regeneration [9,10]. Further analyses of the hematopoietic population revealed a novel population of cells, referred to as side population (SP) cells based on their ability to exclude Hoechst dye [11]. Following engraftment, SP cells reconstitute the hematopoietic lineage as well as give rise to new satellite cells and myofibers, albeit at extremely low levels [12,13]. Transplantation of SP cells into *mdx* mice (a model for Duchenne muscular dystrophy) restored dystrophin expression and improved muscle function [9]. In humans, a subpopulation of hematopoietic cells expressing the cell surface antigen, CD133, also displays myogenic capacity following co-culture with myoblasts [14]. Intramuscular and intra-arterial injection of these cells into *scid/mdx* mice resulted in significant recovery of muscle morphology, function, and dystrophin expression [15], and these cells are presently being tested in the clinic [16]. More recently, a muscle-resident population with myogenic potential located within the interstitial space and characterized by PW1/paternally expressed gene 3 (Peg3) expression, referred to as PICs ($PW1^+$ interstitial cells), has been identified [17]. PICs isolated from early postnatal muscle can generate new fibers, contribute to the satellite cell pool following engraftment into damaged muscle, and give rise to more PICs (the latter property not shared by satellite cells). Lineage tracing experiments have demonstrated that PICs and satellite cells do not share the same embryonic origin (Pax3 dermamyotome somite). Coupled with the observation that PICs also give rise to nonskeletal muscle lineages, it has been suggested that PICs constitute an upstream stem population and play a role during postnatal growth [17], which corresponds to a period of rapid muscle mass and nuclear accumulation [18]. In this context, it will be important to determine the potential of PICs in the adult. A recent study defined another interstitial population of adult muscle stem cells characterized by β4-integrin expression that can participate in muscle repair following engraftment into *mdx* mice [19]. Whether these $\beta4\text{-integrin}^+$ cells are the same population as PICs remains to be determined.

An additional progenitor population with high therapeutic potential are the mesoangioblasts (Mabs), which can be isolated from either embryonic [20,21] or postnatal muscle vasculature [22]. Mabs robustly participate in muscle repair following engraftment or arterial delivery in both mice [23] and dogs [24], and are presently being tested for therapeutic capabilities in a clinical trial. Although the anatomical location of adult Mabs is unclear, compelling data suggest that they are derived from pericytes that also show robust myogenic capacity *in vitro* and *in vivo* [25]. Pericytes can be distinguished from endothelial cells by alkaline phosphatase (AP) expression and do not express myogenic or endothelial markers. Using lineage tracing, it has been shown

Corresponding author: Sassoon, D. (david.a.sassoon@gmail.com).
Keywords: satellite cell; regeneration; stem cell; aging; skeletal muscle; nonsatellite stem cell; adult stem cells.

  http://dx.doi.org/10.1016/j.molmed.2012.07.004 Trends in Molecular Medicine xx (2012) 1–8

Box 1. The satellite cell

Alexander Mauro first identified satellite cells based upon their anatomical location between the basal lamina and the myofiber plasma membrane. Based on this location 'satellite' to the myofiber, he proposed that these cells were resident skeletal muscle progenitors [1]. Since their discovery, extensive effort has been made to understand the origin and role of satellite cells. Satellite cells originate from Pax3-expressing progenitors in the somites of the embryo that migrate to the limb bud where they subsequently upregulate Pax7 and other myogenic regulatory factors [82,83]. Although the majority of these progenitors undergo differentiation and form primitive nascent myofibers, constituting the basis for the formation of additional muscle during postnatal growth, a small subset of these cells adopt a satellite cell position [5]. In adult muscle, satellite cells express Pax7 and remain quiescent under normal physiological conditions. Following muscle damage, satellite cells re-enter the cell cycle and generate myoblasts that eventually fuse together or with damaged fibers [84]. During this process, satellite cells are replaced by self-renewal through asymmetric division of a small fraction of the satellite cell pool [85]. The satellite cell was a focus for therapeutic applications for muscle diseases and led to several clinical trials in the 1990s; however, their poor survival, migration, and inability to undergo sufficient self-renewal following engraftment has led the field to seek alternative approaches (reviewed in [86]).

that pericytes contribute to postnatal muscle growth and regeneration *in situ*, demonstrating a *bona fide* contribution to normal muscle growth by a nonsatellite cell population during postnatal development [26]. The studies outlined above clearly establish that nonsatellite cell progenitors are competent to contribute to muscle repair and raise the possibility that multiple cell types support adult skeletal muscle regeneration and postnatal muscle growth. However, with the exception of pericytes, direct contribution of these progenitors to postnatal myogenesis and muscle repair has not been firmly demonstrated, and in the case of pericytes this contribution is still relatively minor.

Recently, two laboratories have created murine models in which satellite cells can be conditionally depleted in order to test whether muscle regeneration is possible in the absence of satellite cells [27,28]. In one case, the human diphtheria toxin receptor was expressed under the control of the murine *Pax7* locus so that injection of diphtheria toxin killed cells expressing Pax7 [28]; in the other study, mice expressing tamoxifen-inducible conditional recombinase (CRE) under the control of Pax7 were crossed with mice expressing an inducible diphtheria toxin, leading to

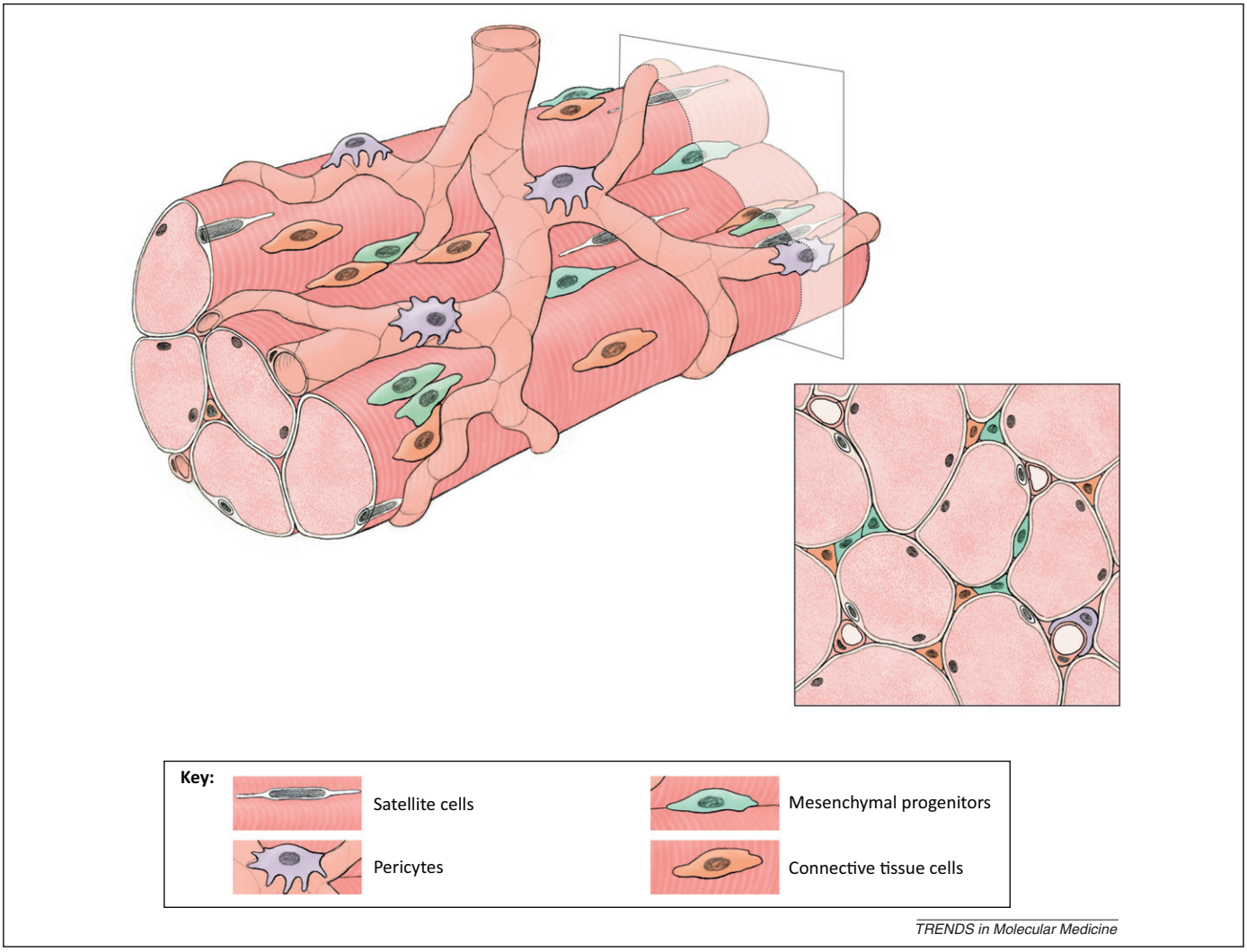

Figure 1. The muscle neighborhood. Skeletal muscle is composed of myofibers, containing myonuclei, and satellite cells (white) that reside beneath the basal lamina and constitute the major muscle stem cell population (Box 1). Blood vessels, composed of endothelial cells, permeate the interstitial space of the muscle fibers, and in addition to providing a blood supply the endothelial cells promote satellite cell proliferation through secretion of growth factors and delivery of circulating inflammatory cells. Pericytes (purple) actively contribute to postnatal muscle growth and regeneration. The interstitial space is occupied by mesenchymal progenitors (green) as well as connective tissue cells (orange).

Table 1. Comparative capacities of myogenic progenitor cells

Cell type	Markers	Proliferation *in vitro*	Systemic delivery	Repopulation of the satellite cell niche	Transplantation efficiency (number or % of positive myofibers, TA)[a]	Improvement of muscle disease	Refs
Satellite cells	Pax7, CD34, c-met	High	No	Yes	>500 fibers (i.m)	*mdx* mice	[4,88]
SP	Hoechst exclusion, CD45, Sca1	Low	Yes	Yes	<50 (8%) (i.a.)	*mdx* mice	[9]
CD133+ cells	CD133, CD34, Thy1, CD45	Low	Yes	Yes	50–100 fibers (i.a)	*mdx* mice	[15]
Mabs	CD34, c-kit, Flk1	High	Yes	??	>20% (i.a)	α-sarco mice, dystrophic dogs	[23,24]
Pericytes	AP, NG-2 proteoglycan	High	Yes	Yes	300–500 i.a, 20 i.m	*mdx* mice	[25]
PICs	PW1	Low	Not tested	Yes	60% (i.m)	Not tested	[17]
β4 integrin+ cells	β4 integrin	High	Not tested	??	50–100 (5–10%) (i.m)	*mdx* mice	[19]

[a]Abbreviations: i.a., intra arterial; i.m., intramuscular.

the induction of diphtheria toxin expression in $Pax7^+$ cells and subsequent loss of satellite cells [27]. Both studies demonstrated that eliminating the satellite cells in adult muscle severely impairs the regenerative response following injury. These studies led to the conclusion that satellite cells are indispensable for muscle repair. It was further demonstrated that vasculature and innervation were not affected by diphtheria toxin, and that other progenitors such as PICs and, presumably, pericytes were still present following satellite cell depletion based on PW1 expression in interstitial cells [28]. Based on these results, it would appear that nonsatellite myogenic populations cannot give rise to new muscle fibers under normal physiological conditions. However, several caveats must be considered in the interpretation of these experiments. First, it is not clear whether the depletion of satellite cells is a neutral event. Specifically, depletion of satellite cells leads to a decrease in muscle mass in the absence of any apparent injury, thus it is possible that some stress or inflammatory processes disturb the muscle environment prior to injury. Second, it is possible that the presence of satellite cells is necessary to recruit nonsatellite cell progenitors into the myogenic lineage through physical and/or molecular paracrine interactions. The impact of the removal of any single population on the stem cell niche may be paramount for understanding these results.

The cellular neighborhood

Satellite cells grown *in vitro* are capable of proliferating and forming multinucleate myotubes, and it is this inherent and stable myogenic property first observed in the 1960s [29–31] that is primarily responsible for the significant advances made in the field of muscle stem cell biology. Although primary satellite cells display pronounced myogenic capacity, even in defined media, their behavior can be modulated dramatically by growth factors, neighboring cells, and the surrounding cellular matrix. It has been shown that even brief periods of satellite cell growth *in vitro* leads to a marked reduction in their myogenic and self-renewal capacity when re-engrafted into damaged muscle, as compared with freshly isolated satellite cells [4]. Blau and colleagues demonstrated that a more elastic cell substrate (in contrast to 'hard' plastic) increased primary myoblast self-renewal and regenerative capacity when grafted into injured muscle [32]. In addition to the physical properties of the satellite cell environment, cytokines and other diffusible molecules produced by surrounding cells are important. Christov *et al.* [33] have observed that satellite cells are invariably located near small vessels and that endothelial cells promote satellite cell proliferation through insulin-like growth factor (IGF)-1, fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) secretion. In turn, differentiating myoblasts promote angiogenesis. They have proposed that positive feedback between these two cell types promotes faster and properly patterned tissue repair. Following muscle damage, proinflammatory monocytes accumulate at the injury site and clear away cellular debris. This step is followed by a conversion of monocytes into anti-inflammatory macrophages that stimulate myogenesis and fiber growth [34]. Completely depleting inflammatory cells blocks muscle regeneration, demonstrating their crucial role in muscle repair [34]. In addition, macrophages exert an antiapoptotic effect on myoblasts through cell adhesion molecules and recruit Mabs through high mobility group box 1 (HMGB1) and matrix metalloproteinase (MMP)9 secretion [35,36]. Taken together, these data demonstrate vascular-mediated paracrine effects on the stem cell niche that play a key role in coordinating stem cell responses [37,38]. In addition to the presence of vascular cells, other interstitial cells support muscle regeneration. First, a population of connective tissue fibroblasts that are identified by transcription factor 4 (Tcf4) expression proliferate in close proximity to satellite cells following injury [39]. Conditional ablation of $Tcf4^+$ cells prior to muscle damage leads to smaller regenerated fibers and a decreased population of satellite cells due to premature differentiation, suggesting that fibroblasts provide critical signals during regeneration [39]. This has been confirmed by *in vitro* experiments in which myoblasts cultured in the presence of $Tcf4^+$ fibroblasts form larger myotubes containing more nuclei [40,41]. In turn, the authors have shown that satellite cell ablation not only leads to a complete loss of muscle regeneration, as observed by others, but also to a defect in fibroblast recruitment at the onset of regeneration followed by connective tissue expansion [39]. Taken together, these results demonstrate that satellite cells and fibroblasts reciprocally regulate the expansion of the other to ensure efficient muscle repair. Furthermore, it is proposed that fibroblasts and atypical myogenic stem cells are recruited to the site of injury by factors secreted by satellite cells [42].

In addition to the myogenic and fibroblast populations, two resident adipogenic populations have recently been

identified in skeletal muscle. Mesenchymal progenitors are characterized by platelet-derived growth factor receptor (PDGFR)α expression [43], and fibro/adipogenic progenitors (FAPs) have been isolated based upon stem cell antigen 1 (Sca1) expression [44]. These cells display a strong adipogenic potential *in vitro* and differentiate into fat when engrafted into pathological muscle, which is not the case when engrafted into healthy muscle. In addition, FAPs are activated upon injury and promote myoblasts differentiation through cell–cell signaling [44], whereas adipogenesis of PDGFRα$^+$ cells is strongly inhibited by myotubes [43]. It is tempting to speculate that mesenchymal PDGFRα$^+$ cells and FAPs are overlapping populations; nonetheless, these adipogenic progenitors share the properties of adopting different fates depending on the surrounding environment, as well as promoting differentiation of neighboring myogenic progenitors [44]. The authors propose that a balance between satellite-cell-dependent myogenesis and PDGFRα$^+$ cell/FAP-dependent adipogenesis regulates muscle homeostasis and regeneration. Taken together, these data clearly demonstrate interactions between multiple resident cell populations that promote muscle progenitor activation. The deregulation of any single cell population in muscle tissue is therefore likely to have a strong impact on all the resident populations and, therefore, must be taken into account when proposing a 'central' role for any single cell type during the regenerative process (Figure 1). Similarly, changes in this local environment may contribute to muscle pathologies and age-related loss of muscle stem cell competence (Figure 2).

Age-related loss of regenerative capacity and the stem cell neighborhood

A progressive loss of stem cell competence occurs with age and is associated with chronic diseases in mammals (Box 2). In diseased and aged muscle, myofibers are replaced by fat and fibrous tissue and the remaining fibers decrease in mass. The number of satellite cells in muscle declines soon after birth from 30% of myonuclei in the neonate to 4% in the adult, followed by a small decrease to 2% in the old mouse [45]. It has been demonstrated that satellite cells undergo a decrease in levels of Pax7 expression, leading to a loss of myogenicity accompanied by increased levels of apoptosis [46]. However, not all satellite cells appear to undergo this decrease and retain myogenic and self-renewal capacities comparable to those of young

Box 2. Muscle diseases

Muscle diseases are primarily congenital, including most notably the muscular dystrophies (Duchenne, Emery–Dreyfus and Limb–Girdle), centronuclear myopathies, and neuromuscular diseases (amyotrophic lateral sclerosis and spinal muscular atrophy). Muscular dystrophies are characterized by progressive muscle weakness due to mutations in sarcolemmal proteins (dystrophin, sarcoglycans and dysferlin), nuclear proteins (emerin and lamin A/C), or extracellular proteins (collagen-6 and α-2 laminin). Although clinical symptoms are well described, no treatment has yet been fully successful.

Most muscular dystrophies involve an eventual exhaustion of the stem cell pool or a change in their fate, in which the muscle tissue becomes infiltrated with fibrotic and fat tissue. This evolution is similar to sarcopenia that occurs during physiological aging, involving a gradual loss of muscle mass and a decline in regenerative capacity.

In the *mdx* mouse model, the satellite cell population is reduced, as compared to healthy mice, and becomes depleted following repeated cycles of degeneration/regeneration. These repeated cycles of stem cell activation and proliferation lead to telomere shortening [87] and have been proposed to lead to the accumulation of mutations in key regulatory genes required for proper self-renewal and myogenic competence. Therefore, although Duchenne muscular dystrophy (DMD) disease progression is driven by dystrophin deficiency, the pathology exacerbates as a result of stem cell dysfunction. In this context, therapies designed to promote stem cell competence may ultimately slow disease progression.

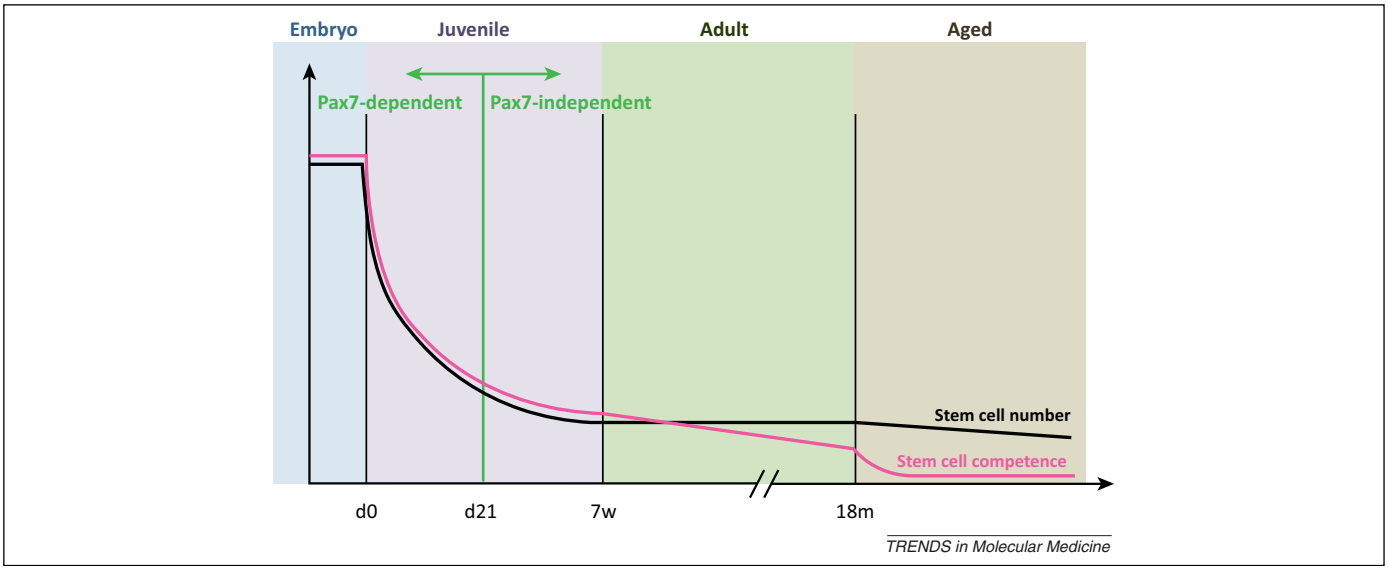

Figure 2. Dynamics of stem cell competence with age. At birth, high numbers of stem cells are followed by a dramatic decrease within the first days and/or weeks of life, depending on the tissue. In skeletal muscle, this juvenile period is characterized by rapid postnatal growth and the presence of a variety of stem cells that are highly plastic and able to contribute to multiple tissue lineages [12,17,43]. In the mouse, Pax7 is required for both postnatal muscle growth and regeneration during the first 3 weeks of life [2,67,68]; after 3 weeks, regeneration can occur in the absence of Pax7 [68]. During adult life, stem cell number is maintained, however, their capacity to repair muscle (stem cell competence) continues to decrease due to an increase in inhibitory factors in their environment until about 18 months, at which point, regenerative capacity is compromised. An additional decline in stem cell competence occurs in aged muscle. Taken together, these results reveal three distinct genetic and cellular phases of postnatal skeletal muscle stem cell behavior: a juvenile phase (0–3 weeks); adult phase (3 weeks to ~18 months); and an aged phase (18 months to end of life).

satellite cells, suggesting that other factors contribute to age-related changes in muscle regenerative capacity [45–47]. An aged-related delay in the early inflammatory response following muscle injury was reported in mice, although this did not impair the regeneration process [48]. *In vivo*, proliferative and regenerative capacities of satellite cells are restored in old mice when exposed to circulating factors from young mice [49]. By contrast, serum obtained from old mice provokes an old age muscle regenerative phenotype when injected into young mice. This old-age-inducing effect has been attributed to elevated circulating Wnt molecules, activating downstream targets such as Axin2 and β-catenin in aged satellite cells, and leading to conversion to a fibrogenic lineage [50]. Conversely, canonical Wnt signaling inhibition by Frizzled-related protein 3 or Dickkopf-related protein 1 (DKK1) in old mice restores regenerative potential [50]. Wnt molecules may be secreted by tissue-resident endothelial precursors in old muscle. Specifically, endothelial precursors are activated during neoangiogenesis following muscle injury and inhibit myoblast proliferation through Wnt3a production [51,52]. In addition, impaired muscle regeneration in aged animals has been linked to a decline in Notch signaling [53]. Insufficient upregulation of the Notch ligand, Delta-1, in satellite cells following injury in old animals leads to a decrease in their myogenic capacities, which can be restored by forced activation of Notch. In turn, *in vivo* inhibition of Notch signaling reduces muscle regeneration in young mice and results in a phenotype similar to that of old muscle [53]. In addition, Notch signaling has recently been shown to be necessary for satellite cell maintenance, such that satellite cells undergo accelerated terminal differentiation without self-renewal in the absence of Notch, resulting in satellite cell depletion [54]. Loss of Notch activation in aged muscle has been associated with high levels of transforming growth factor (TGF)β-1 and cyclin-dependent kinase (cdk) inhibitors in satellite cells [55]. Abnormally high levels of pSmad-3 in aged satellite cells impair regenerative capacity, whereas regenerative competence can be restored *in vivo* by pSmad-3 shRNA blockade. Activation of Notch blocks the pSmad-3-mediated upregulation of the cdks p15, p16, p21 and p27, whereas inhibition of Notch induces these cdks [55,56]. Therefore, endogenous Notch and pSmad-3 antagonize each other in the control of satellite cell proliferation, and deregulation of this balance in old animals is proposed to lead to a decrease in regenerative potential. These data reveal that the progressive decrease in regenerative capacity during muscle aging is due to a progressive switch from Notch to Wnt/TGF-β molecules secretion in the stem cell environment, rather than intrinsic loss of myogenic cell competence.

Can we gain insight from lower vertebrate regeneration?

Fish and amphibians retain the capacity to regenerate tissues fully throughout life, whereas progressive loss of regenerative capacity is common in mammals. Specifically, the adult zebrafish heart can regenerate following ventricular resection [57], whereas mammals lose this capacity soon after birth [58]. A striking demonstration of stem cell capacity in lower vertebrates was recently reported in newt lens subjected to 15 rounds of injury over 16 years without any decline in regenerative capacity [59]. Finally, in addition to cell and organ replacement, amphibians can form a blastema following limb amputation, consisting of dedifferentiated mesenchymal cells that will repattern and redifferentiate to generate the entire appendage. Although limb amputation in amphibians activates resident $Pax7^+$ satellite cells [60], a concomitant dedifferentiation of mature fibers is also observed, which give rise to mononucleated cells, accounting for approximately 30% of the blastema [61]. Although dedifferentiation is an extremely rare event in mammals, one recent report suggests that a similar process occurs in skeletal muscle upon injury, as witnessed with a Cre-Lox-βgal system based on the expression of muscle creatine kinase to tag specifically differentiated multinuclear myofibers [62]. In this study, mononuclear βgal^+ cells were detected, among which some were also $Pax7^+$ and capable of redifferentiating into myotubes *in vitro* after injury [62]. Although the contribution of dedifferentiated myofibers during muscle regeneration remains a subject of scrutiny, this study suggests that myofibers are an additional source of myogenic progenitors for muscle repair.

Understanding the mechanisms underlying dedifferentiation promises tremendous therapeutic applications. Blau and colleagues recently demonstrated that committed myoblasts are reprogrammed by inactivating the tumor suppressors retinoblastoma protein (Rb) and ARF [63]. Upon Rb and p16/19 inactivation, myonuclei re-entered the cell cycle and myotubes dedifferentiated in mononucleated cells. These cells expand under growth conditions and redifferentiate into mature myotubes. Following engraftment, these cells are incorporated into pre-existing fibers [63]. Similarly, Conboy and colleagues have shown that inhibitors of tyrosine phosphatases and apoptosis provoke the dedifferentiation of myotubes into myogenic competent mononucleated cells. They obtained 'reprogrammed' muscle progenitors expressing Pax7 and MyoD capable of differentiating into skeletal muscle *in vitro* and *in vivo* [64]. Even if dedifferentiation does not normally occur during mammalian tissue regeneration, the underlying cellular machinery has not been lost during mammalian radiation and can be artificially reactivated. Presumably this loss of cellular plasticity and stem cell competence arose during evolution, implying that the loss of this regenerative capacity carries some unique advantage. Moreover, the observation that genes involved in tumor suppression need to be deactivated, at least transiently, in order to provoke this process in mammals suggests that there may have been an evolutionary trade-off between mechanisms preventing cancer and mechanisms supporting robust regenerative capacities.

Adolescence–a critical period for stem cells?

A preprogrammed adult body size, and by consequence, organ size, is a hallmark of mammals, whereas many lower vertebrates display large variations in adult body size and, in some cases, postnatal growth is maintained throughout life (reviewed in [65,66]). The continuous growth of lower vertebrates is presumably linked to their enhanced

regenerative capacities, and recent studies have demonstrated that mammals possess an enhanced regenerative capacity during the initial days or weeks after birth. In the case of the heart, which can regenerate in fish but not in mice, it has recently been shown that partial ventricle resection of the mouse heart performed within the first postnatal day provokes complete regeneration through induction of heart cardiomyocyte proliferation; this capacity is lost by 1 week [58]. In skeletal muscle, genetic evidence highlights the roles of Pax7 in postnatal skeletal muscle growth. Mice constitutively lacking Pax7 display a normal pool of satellite cells upon birth but their number rapidly declines by 2–3 weeks after birth concomitant with poor postnatal growth and survival [2]. Furthermore, these mice show very poor muscle regenerative capacity, which led initially to the proposal that Pax7 is required for satellite cell maintenance and self-renewal [2,67]. However, in the context of a conditional Pax7 mutant, it was found that Pax7 is dispensable for satellite cell function when inactivated specifically in the adult [68]. By contrast, when Pax7 is inactivated between birth and 3 weeks of age, postnatal muscle development and regenerative capacity is abrogated, with the most severe phenotypes corresponding to ablation of Pax7 expression during the first week of life [68]. These results demonstrate that there is a critical period near 3 weeks of age when the muscle stem cell niche undergoes a critical change from juvenile to adult. Whether this change is due to progenitors entering a more stable quiescent state or some other unidentified event, including changes in the stem cell neighborhood, remains to be determined.

Genomic imprinting – a mammalian feature of stem cells

Recent studies have implicated parentally imprinted genes in the regulation of postnatal growth and adult somatic stem cells. Parental imprinting is an epigenetic control specific to mammals that involves silencing one allele, and it occurs on $<1\%$ of the human and mouse genome (reviewed in [69,70]). Several parentally imprinted genes regulate embryonic development [71,72]. As a group, they are expressed at high levels in fetal and newborn tissues and decline within the first few weeks after birth in mice [73,74]. Our laboratory identified the parentally imprinted gene PW1/Peg3 in a screen designed to isolate early stem cell markers in the skeletal muscle lineage [75]. Subsequent analyses have revealed PW1/Peg3 expression is restricted to stem cells in all adult tissues examined, including the skin, blood, bone, testes, and central nervous system (CNS) [76]. The expression of a universal adult stem cell marker lends support to the idea that common regulatory mechanisms underlie adult stem cell function regardless of tissue origin; a notion that has been proposed for many years but has previously lacked much supporting data [77]. Our findings were followed by the discovery of Goodell and colleagues that a group of parentally imprinted genes including p57, H19, mesoderm-specific transcript (Mest), Peg3/PW1, Dlk1, and insulin-like growth factor 2 (IGF2) are expressed in adult somatic stem cells but not in their differentiated progeny [74]. In addition, two groups have recently demonstrated that p57, a well-known regulator of the cell cycle, plays a role in maintaining the hematopoietic stem cell pool [78,79]. Similarly, Dlk1 has been shown to be important for skeletal muscle growth and differentiation [80] and neurogenic progression [81]. Moreover, these authors have demonstrated that neural stem cells selectively undergo a 'relaxation of imprinting' during postnatal development that is required for proper neurogenesis [81]. This finding raises the interesting possibility that biallelic expression of imprinted genes may constitute an important regulatory event during stem cell progression and differentiation. As most but not all parentally imprinted genes are shared among all vertebrates, it will be of interest to determine whether the same set of genes are expressed in lower vertebrate stem cells or whether this set of imprinted genes is specific to mammalian stem cells.

Concluding remarks and future perspectives

Muscle growth and maintenance during the lifetime of an animal reveals at least three distinct stages after birth – juvenile, adult, and old age – during which stem cells change in behavior and competence. The juvenile period in mice (0–3 weeks) is characterized by pronounced growth and a marked increase in muscle mass and size. In this context, stem cells are required to build the muscle in young mice. As stated above, the switch from a Pax7-dependent to a Pax7-independent state at 3 weeks old, coinciding with a decline in the rate of postnatal growth, provides direct genetic evidence that the stem cells undergo a change in their genetic circuitry during this transition, likely reflecting a change in their role. This change may also reflect the requirement for Pax7 in the myogenicity of PICs during postnatal growth [17]. In the context of muscular dystrophies, this raises the question as to whether it is possible to develop therapeutics that can induce or maintain juvenile stem cells that may represent a more competent and plastic stem cell population, as compared with adult stem cells. Whether imprinted gene regulation or tumor suppressor genes underlie the 'more limited' potentials of mammalian stem cells as opposed to those in lower vertebrates may also offer potential therapeutic solutions. Why mammals and lower vertebrates differ in their regenerative potential can now be addressed with the advent of high-throughput 'omics' approaches. Determining how the niche changes, which signals are present during each of the postnatal stages, and what role other cells in the niche play will provide targets for therapeutic development that promise to be more tractable than direct stem cell engraftment. As such, the field of adult stem cells is entering a larger evolutionary context. Should we seek to induce amphibian-like characteristics in mammalian stem cells, we will need to establish a clearer understanding of what distinguishes juvenile postnatal tissue from adult and old tissue as well as fully appreciate why mammals have evolutionarily acquired this more limited potential and what Faustian bargain may have been made along the way.

Acknowledgments

We gratefully thank Drs V. Besson and K. Bismuth for fruitful discussions and critical reading of the manuscript. This work was supported by the French Ministry of Research 'Chaire d'Excellence' and the Muscular Dystrophy Association of America to D.S. and the European Community Seventh Framework Program projects OPTISTEM (Optimization of stem cell therapy for degenerative epithelial and muscle diseases contract

number Health-F5-2009-223098) and ENDOSTEM (Activation of vasculature associated stem cells and muscle stem cells for the repair and maintenance of muscle tissue-agreement number 241440). The Myology Group is the beneficiary of a Strategic Plan Support from the Association Francaise contre les Myopathies (AFM) and is affiliated with the Institute of Myology. A.P. was supported by the French Ministry of Research and the Fondation pour la Recherche Medicale (Programme 'Espoirs de la Recherche', number FDT20110922527). The authors have no conflict of interest.

References

1 Mauro, A. (1961) Satellite cell of skeletal muscle fibers. *J. Biophys. Biochem. Cytol.* 9, 493–495
2 Seale, P. *et al.* (2000) Pax7 is required for the specification of myogenic satellite cells. *Cell* 102, 777–786
3 Burkin, D.J. and Kaufman, S.J. (1999) The alpha7beta1 integrin in muscle development and disease. *Cell Tissue Res.* 296, 183–190
4 Montarras, D. *et al.* (2005) Direct isolation of satellite cells for skeletal muscle regeneration. *Science* 309, 2064–2067
5 Relaix, F. *et al.* (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. *Nature* 435, 948–953
6 Dhawan, J. and Rando, T.A. (2005) Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment. *Trends Cell Biol.* 15, 666–673
7 Zammit, P.S. *et al.* (2004) Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? *J. Cell Biol.* 166, 347–357
8 Olguin, H.C. and Olwin, B.B. (2004) Pax-7 up-regulation inhibits myogenesis and cell cycle progression in satellite cells: a potential mechanism for self-renewal. *Dev. Biol.* 275, 375–388
9 Gussoni, E. *et al.* (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* 401, 390–394
10 Ferrari, G. *et al.* (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279, 1528–1530
11 Goodell, M.A. *et al.* (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J. Exp. Med.* 183, 1797–1806
12 Jackson, K.A. *et al.* (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A.* 96, 14482–14486
13 Asakura, A. *et al.* (2002) Myogenic specification of side population cells in skeletal muscle. *J. Cell Biol.* 159, 123–134
14 Torrente, Y. (2004) Human circulating AC133+ stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J. Clin. Investig.* 114, 182–195
15 Benchaouir, R. *et al.* (2007) Restoration of human dystrophin following transplantation of exon-skipping-engineered DMD patient stem cells into dystrophic mice. *Cell Stem Cell* 1, 646–657
16 Torrente, Y. *et al.* (2007) Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. *Cell Transplant.* 16, 563–577
17 Mitchell, K.J. *et al.* (2010) Identification and characterization of a non-satellite cell muscle resident progenitor during postnatal development. *Nat. Cell Biol.* 12, 257–266
18 Young, V.R. (1974) Regulation of protein synthesis and skeletal muscle growth. *J. Anim. Sci.* 38, 1054–1070
19 Liadaki, K. *et al.* (2012) beta4 integrin marks interstitial myogenic progenitor cells in adult murine skeletal muscle. *J. Histochem. Cytochem.* 60, 31–44
20 De Angelis, L. *et al.* (1999) Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration. *J. Cell Biol.* 147, 869–878
21 Minasi, M.G. *et al.* (2002) The mesoangioblast a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. *Development* 129, 2773–2783
22 Tonlorenzi, R. *et al.* (2007) Isolation and characterization of mesoangioblasts from mouse, dog, and human tissues. *Curr. Protoc. Stem Cell Biol.* Chapter 2, Unit 2B 1
23 Sampaolesi, M. *et al.* (2003) Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science* 301, 487–492
24 Sampaolesi, M. *et al.* (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature* 444, 574–579
25 Dellavalle, A. *et al.* (2007) Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nat. Cell Biol.* 9, 255–267
26 Dellavalle, A. *et al.* (2011) Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. *Nat. Commun.* 2, 499
27 Lepper, C. *et al.* (2011) An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. *Development* 138, 3639–3646
28 Sambasivan, R. *et al.* (2011) Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development* 138, 3647–3656
29 Konigsberg, I.R. (1961) Cellular differentiation in colonies derived from single cells platings of freshly isolated chick embryo muscle cells. *Proc. Natl. Acad. Sci. U.S.A.* 47, 1868–1872
30 Konigsberg, I.R. (1961) Some aspects of myogenesis *in vitro*. *Circulation* 24, 447–457
31 Bischoff, R. (1975) Regeneration of single skeletal muscle fibers *in vitro*. *Anat. Rec.* 182, 215–235
32 Gilbert, P.M. *et al.* (2010) Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* 329, 1078–1081
33 Christov, C. *et al.* (2007) Muscle satellite cells and endothelial cells: close neighbors and privileged partners. *Mol. Biol. Cell* 18, 1397–1409
34 Arnold, L. *et al.* (2007) Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J. Exp. Med.* 204, 1057–1069
35 Sonnet, C. *et al.* (2006) Human macrophages rescue myoblasts and myotubes from apoptosis through a set of adhesion molecular systems. *J. Cell Sci.* 119, 2497–2507
36 Lolmede, K. *et al.* (2009) Inflammatory and alternatively activated human macrophages attract vessel-associated stem cells, relying on separate HMGB1- and MMP-9-dependent pathways. *J. Leukoc. Biol.* 85, 779–787
37 Abou-Khalil, R. *et al.* (2009) Autocrine and paracrine angiopoietin 1/Tie-2 signaling promotes muscle satellite cell self-renewal. *Cell Stem Cell* 5, 298–309
38 Mounier, R. *et al.* (2011) Blood vessels and the satellite cell niche. *Curr. Top. Dev. Biol.* 96, 121–138
39 Murphy, M.M. *et al.* (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development* 138, 3625–3637
40 Mathew, S.J. *et al.* (2011) Connective tissue fibroblasts and Tcf4 regulate myogenesis. *Development* 138, 371–384
41 Cooper, S.T. *et al.* (2004) C2C12 co-culture on a fibroblast substratum enables sustained survival of contractile, highly differentiated myotubes with peripheral nuclei and adult fast myosin expression. *Cell Motil. Cytoskeleton* 58, 200–211
42 Wang, Y.X. and Rudnicki, M.A. (2012) Satellite cells, the engines of muscle repair. *Nat. Rev. Mol. Cell Biol.* 13, 127–133
43 Uezumi, A. *et al.* (2010) Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat. Cell Biol.* 12, 143–152
44 Joe, A.W. *et al.* (2010) Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat. Cell Biol.* 12, 153–163
45 Shefer, G. *et al.* (2006) Satellite-cell pool size does matter: defining the myogenic potency of aging skeletal muscle. *Dev. Biol.* 294, 50–66
46 Collins, C.A. *et al.* (2007) A population of myogenic stem cells that survives skeletal muscle aging. *Stem Cells* 25, 885–894
47 Carlson, M.E. and Conboy, I.M. (2007) Loss of stem cell regenerative capacity within aged niches. *Aging Cell* 6, 371–382
48 Smythe, G.M. *et al.* (2008) Age influences the early events of skeletal muscle regeneration: studies of whole muscle grafts transplanted between young (8 weeks) and old (13-21 months) mice. *Exp. Gerontol.* 43, 550–562
49 Conboy, I.M. *et al.* (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433, 760–764
50 Brack, A.S. *et al.* (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* 317, 807–810
51 Grenier, G. *et al.* (2007) Resident endothelial precursors in muscle, adipose, and dermis contribute to postnatal vasculogenesis. *Stem Cells* 25, 3101–3110

52 Trensz, F. *et al.* (2010) A muscle resident cell population promotes fibrosis in hindlimb skeletal muscles of mdx mice through the Wnt canonical pathway. *Am. J. Physiol. Cell Physiol.* 299, C939–C947
53 Conboy, I.M. *et al.* (2003) Notch-mediated restoration of regenerative potential to aged muscle. *Science* 302, 1575–1577
54 Bjornson, C.R. *et al.* (2012) Notch signaling is necessary to maintain quiescence in adult muscle stem cells. *Stem Cells* 30, 232–242
55 Carlson, M.E. *et al.* (2009) Relative roles of TGF-beta1 and Wnt in the systemic regulation and aging of satellite cell responses. *Aging Cell* 8, 676–689
56 Carlson, M.E. *et al.* (2008) Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. *Nature* 454, 528–532
57 Poss, K.D. *et al.* (2002) Heart regeneration in zebrafish. *Science* 298, 2188–2190
58 Porrello, E.R. *et al.* (2011) Transient regenerative potential of the neonatal mouse heart. *Science* 331, 1078–1080
59 Eguchi, G. *et al.* (2011) Regenerative capacity in newts is not altered by repeated regeneration and ageing. *Nat. Commun.* 2, 384
60 Morrison, J.I. *et al.* (2006) Salamander limb regeneration involves the activation of a multipotent skeletal muscle satellite cell population. *J. Cell Biol.* 172, 433–440
61 Echeverri, K. *et al.* (2001) *In vivo* imaging indicates muscle fiber dedifferentiation is a major contributor to the regenerating tail blastema. *Dev. Biol.* 236, 151–164
62 Mu, X. *et al.* (2011) Study of muscle cell dedifferentiation after skeletal muscle injury of mice with a Cre-Lox system. *PLoS ONE* 6, e16699
63 Pajcini, K.V. *et al.* (2010) Transient inactivation of Rb and ARF yields regenerative cells from postmitotic mammalian muscle. *Cell Stem Cell* 7, 198–213
64 Paliwal, P. and Conboy, I.M. (2011) Inhibitors of tyrosine phosphatases and apoptosis reprogram lineage-marked differentiated muscle to myogenic progenitor cells. *Chem. Biol.* 18, 1153–1166
65 Lui, J.C. and Baron, J. (2011) Mechanisms limiting body growth in mammals. *Endocr. Rev.* 32, 422–440
66 Johnston, I.A. *et al.* (2011) Growth and the regulation of myotomal muscle mass in teleost fish. *J. Exp. Biol.* 214, 1617–1628
67 Oustanina, S. *et al.* (2004) Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification. *EMBO J.* 23, 3430–3439
68 Lepper, C. *et al.* (2009) Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. *Nature* 460, 627–631
69 Bartolomei, M.S. and Ferguson-Smith, A.C. (2011) Mammalian genomic imprinting. *Cold Spring Harb. Perspect. Biol.* 3, http://dx.doi.org/10.1101/cshperspect.a002592
70 Kaneda, M. (2011) Genomic imprinting in mammals-epigenetic parental memories. *Differentiation* 82, 51–56
71 Ono, R. *et al.* (2006) Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. *Nat. Genet.* 38, 101–106
72 Sekita, Y. *et al.* (2008) Role of retrotransposon-derived imprinted gene. *Nat. Genet.* 40, 243–248
73 Lui, J.C. *et al.* (2008) An imprinted gene network that controls mammalian somatic growth is down-regulated during postnatal growth deceleration in multiple organs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R189–R196
74 Berg, J.S. *et al.* (2011) Imprinted genes that regulate early mammalian growth are coexpressed in somatic stem cells. *PLoS ONE* 6, e26410
75 Relaix, F. *et al.* (1996) Pw1, a novel zinc finger gene implicated in the myogenic and neuronal lineages. *Dev. Biol.* 177, 383–396
76 Besson, V. *et al.* (2011) PW1 gene/paternally expressed gene 3 (PW1/Peg3) identifies multiple adult stem and progenitor cell populations. *Proc. Natl. Acad. Sci. U.S.A.* 108, 11470–11475
77 Varrault, A. *et al.* (2006) Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. *Dev. Cell* 11, 711–722
78 Matsumoto, A. *et al.* (2011) p57 is required for quiescence and maintenance of adult hematopoietic stem cells. *Cell Stem Cell* 9, 262–271
79 Zou, P. *et al.* (2011) p57(Kip2) and p27(Kip1) cooperate to maintain hematopoietic stem cell quiescence through interactions with Hsc70. *Cell Stem Cell* 9, 247–261
80 Waddell, J.N. *et al.* (2010) Dlk1 is necessary for proper skeletal muscle development and regeneration. *PLoS ONE* 5, e15055
81 Ferron, S.R. *et al.* (2011) Postnatal loss of Dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis. *Nature* 475, 381–385
82 Kassar-Duchossoy, L. *et al.* (2005) Pax3/Pax7 mark a novel population of primitive myogenic cells during development. *Genes Dev.* 19, 1426–1431
83 Gros, J. *et al.* (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. *Nature* 435, 954–958
84 Reznik, M. (1969) Thymidine-3H uptake by satellite cells of regenerating skeletal muscle. *J. Cell Biol.* 40, 568–571
85 Kuang, S. *et al.* (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell* 129, 999–1010
86 Menasche, P. (2007) Skeletal myoblasts as a therapeutic agent. *Prog. Cardiovasc. Dis.* 50, 7–17
87 Sacco, A. *et al.* (2010) Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice. *Cell* 143, 1059–1071
88 Partridge, T.A. *et al.* (1989) Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. *Nature* 337, 176–179