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## Skeletal muscle satellite cells and adult myogenesis

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### Abstract

Research focusing on the canonical adult myogenic progenitor, the skeletal muscle satellite cell, is still an ever-growing field 46 years from their initial description. Recent publications revealed numerous new aspects of satellite cell biology, starting from their developmental life to their role as the principal self-renewing myogenic stem cell in the adult skeletal muscle and finally their loss during aging. The myogenic potential of satellite cells is under the molecular control of specific Paired-box and bHLH transcription factors which tightly orchestrated balance during adult skeletal muscle regeneration account for their effectiveness. New reports also demonstrated satellite cells' continuous relationships with blood vessels and the high myogenic potential of new stem cell subsets related to both lineages.

### Introduction

In this review, we discuss recent achievements in the studies of adult myogenesis and, specifically, on publications that carried major advances in our understanding of the cellular and molecular regulation of the muscle fibers' satellite cells biology. We focused on the major mechanisms responsible for the maintenance and homeostasis of satellite cell during the whole life of a mammal, from the birth of their precursors in the embryonic paraxial mesoderm, through their implication in senescence of the muscle tissue. The last years provided a first overview of the hierarchy within this lineage and numerous research teams are currently trying to find which muscle-derived cell population harbors the most potent regenerative and self-renewal potential and, in parallel, which signals direct the self-renewal of satellite cells. In addition, recent advances clarified the intrinsic and extrinsic signals leading the quiescent satellite cells to become activated and subsequent timing of activation and repression of muscle-specific transcription factors enabling the progeny of activated satellite cells to efficiently regenerate damaged skeletal muscle fibers.

### Developmental origin of satellite cells

All the skeletal muscles of the body and the limbs derive from the somites, segmental derivatives of the paraxial mesoderm. As the somite matures, myogenic progenitor cells become confined to the dorso-lateral part of the somite: the dermomyotome. Skeletal myogenesis is then initiated in myogenic cells originating from the dermomyotome lips that differentiate to form primary muscle fibers (see [1] for review). Subsequently, a progenitor population that expresses Pax3 and Pax7 arise from the central portion of the dermomyotome and is maintained throughout embryogenesis within the developing skeletal muscles [2–4].

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Late in fetal development, the resident progenitor population generates cells in a satellite position around myofibers, which are marked by the expression of Pax7 [2–4]. Lineage tracing experiments using the Cre/LoxP recombination system further demonstrated that limb muscle satellite cells arise from hypaxial cells expressing Pax3. In parallel, it is an interesting fact that a significant number of limb muscle Side Population stem cells are also derived from the hypaxial somite and perhaps share a common ancestor with satellite cells [5].

Still little is known about the molecular signals that regulate the resident progenitor cells embryonic life, but recent reports highlighted the importance of Notch signaling (one the most recurrent signaling pathway directing stem cells expansion and fate determination) in satellite cell ontogenesis. Manipulation of either the Notch ligand Delta1 (Dll1) or the Notch downstream transcription factor RBP-J (Rbpsi) in mice embryo demonstrated an essential role for Notch in the survival of the Pax3/7+ve cells during embryogenesis. In the context of mice heteroallelic for a null mutation and an hypomorphic Dll1 allele [6\*], or when floxed RBP-J alleles are conditionally recombined under the control of myogenic genes [7\*], resident progenitor cells are formed initially, but they undergo an uncontrolled myogenic differentiation. This precocious differentiation leads to a progressive depletion of the progenitor pool and a subsequent absence of progenitor cells and/or satellite cells at the beginning of fetal life [6\*,7\*]. The recent discovery of satellite cell embryonic origin brought a lot of interesting questions to the field, and studies concerning the implication of well-known signaling pathways previously described as major regulators of embryonic myogenesis, such as Wnt signals or Myostatin/Follistatin antagonism in the regulation of satellite cell development will, without any doubts, be of critical interests in the next few years.

## Activation from quiescence

In the adult, satellite cells are mitotically quiescent and reside in a niche between the basal lamina and the sarcolemma of their associated muscle fibers. In that particular state, they exhibit limited gene expression and protein synthesis but they can become activated in response to stress that is induced by weight bearing or by trauma, such as injury or in the context of a myo-degenerative disease (see [8] for review). What control the transition between quiescence and proliferation, the “activation trigger”, remains largely unknown. A signal intrinsic to the cell itself is the production of sphingosine-1-phosphate from the inner leaflet of the plasma membrane. Sphingosine-1-phosphate is required for satellite cell entry in the cell cycle and inhibition of its synthesis dramatically abrogates muscle regeneration [9\*].

Extrinsic mechanical stretch to the fiber also triggers numerous intracellular signals, including nitric oxide synthesis, which results in hepatocyte growth factor (HGF) release and satellite cell activation, this suggesting that the HGF receptor c-Met may be an early gene in satellite cell activation [10]. Nitric oxide also induces expression of Follistatin [11], a fusigenic secreted molecule, known to antagonize Myostatin, a negative regulator of myogenesis expressed by quiescent satellite cells thus maybe contributing to satellite cells exit from quiescence.

Microenvironment-secreted growth factors are third stimulus for satellite cell activation. As a matter of fact, fibroblasts growth factors (FGF) are known to induce pro-myogenic MAPK signaling cascades and the p38 $\alpha$ / $\beta$  MAPK is required for satellite cell activation and regulates the quiescent state of satellite cells [12]. Analysis of p38 $\alpha$  mutant showed that p38 $\alpha$  abrogation induces delayed cell-cycle exit and altered expression of cell-cycle regulators in cultured myoblasts. As a result of continuous proliferation, and lack of growth arrest feed-back, p38 $\alpha$  mutants showed increased myoblast proliferation in the neonatal period [13]. An important question that now remains to be elucidated is whether adult satellite cells that have been activated during regeneration can return to quiescence in p38 $\alpha$  mutant muscles. An alternative way p38 $\alpha$  / $\beta$  can be activated during myogenesis is through the surface protein Cdo via

interaction with the scaffold protein JLP [14], this predicting a still unknown helper cell to satellite cell activation mechanism.

## Self-renewal and stem cell potential

Following activation, satellite cell will leave their niche and move outside of the basal lamina, start to cycle and coexpress Pax7 and MyoD. The descendants of activated satellite cells, the skeletal myoblasts, undergo multiple rounds of division and most of them will downregulate Pax7, express myogenin and differentiate to fuse and form multinucleated myofibers (Figure 1).

A fraction of these myoblasts will maintain Pax7, loose expression of myogenic markers and eventually leave the cell cycle [15,16]. These {Pax7+ve, MyoD-ve} cells have thus been postulated to represent a self-renewing fraction and further transplantation of freshly isolated satellite cells or intact myofibers with their associated satellite cells in irradiated hosts proved that satellite cells can self-renew, replenish a depleted niche, and that their progeny is capable of efficient regeneration [17,18].

In the last couple years, researchers aimed to discover which signals direct the self-renewal of satellite cells, and if it is a potential that all satellite cell possess or only a minority of them. A first insight came from lineage tracing experiments using Myf5-reporter mice, which showed that muscle satellite cells are a heterogeneous mixture of stem cells and committed progenitors. In fact, satellite “stem” cells which never expressed Myf5 can asymmetrically divide, and give rise to a Myf5+ve daughter committed to myogenesis as well as a self-renewing Myf5-ve daughter (Figure 2). Prospective isolations and transplantations of both cell sub-types then demonstrated that Myf5-ve stem cells can extensively contribute to the satellite cell reservoir [19\*\*].

More than 30 years ago, the Cairns hypothesis of the “immortal strand” proposed that stem cell that divide asymmetrically can retain the oldest DNA strand in the less committed cell, thus limiting mutations in the self-renewing pool [20]. That hypothesis was recently tested in the muscle system, and using pulse-chase labeling of stem cells with halogenated thymidine analogs, researchers were able to demonstrate co-segregation of template DNA strands in dividing skeletal myoblasts [21\*\*,22\*]. Further to that point, the label-retaining cells were shown to co-segregate with the immature cell determinants Numb and/or Sca1, while the non-retaining cells are proposed to follow a myogenic pathway. However, the Cairns hypothesis does not appear to be commonly accepted as a common stem cell feature, since only a few cell types show asymmetric strand segregation. Hence, haematopoietic stem cells were recently reported as to not asymmetrically segregate chromosomes or retain BrdU [23]. Another interesting explanation is that asymmetric cell divisions and cell fate are codirected by epigenetic differences between sister chromatids and that the DNA strands bearing the more “active” stem cell genes are co-segregated for the maintenance of self-renewal properties [24].

## Molecular regulation of satellite cell proliferation and differentiation

Satellite cell myogenic potential mostly relies on the expression of Pax genes and Myogenic Regulatory Factors (MRFs; MyoD, Myf5, myogenin, MRF4), sequential activation and repression of Pax3/7 and MRFs is required for the progression of skeletal myoblasts through myogenesis. Pax7 is essential for the post-natal maintenance and self-renewal of satellite cells [25,26\*]. At birth, satellite cell numbers are close to normal in Pax7 mutant mice, but they are progressively lost, mainly because of cell death in the absence of Pax7. Remnant satellite cells can be detected in the absence of Pax7, but they are defective, and unable to support efficient regeneration [26\*]. Pax3 is also expressed in a subset of quiescent satellite cells and can be

detected after activation in most skeletal myoblasts [27\*]. Its roles in muscle regeneration are not well defined, since Pax3-null mice dies in utero and still no adult conditional mutation of Pax3 has been described. Pax3 and Pax7 play similar roles in the activation of myogenic genes, such as MyoD, but only Pax7 possess a survival and anti-apoptotic function [27\*].

Recent works demonstrated the importance of Pax proteins regulation during adult myogenesis. Pax3 is regulated by monoubiquitination and proteasomal degradation during satellite cell activation. Sustained expression of stable mutant form Pax3 inhibited myogenic differentiation, demonstrating that control of Pax3 degradation is an essential step for the progression of myogenic program beyond commitment [28\*]. Pax7 induces myoblast proliferation and delay their differentiation, thus not by blocking myogenin expression [29] but by regulation of MyoD [30], in parallel, myogenin directly affects Pax7 protein expression and is postulated as being a regulator of Pax7 down-regulation during differentiation [30].

In contrast with the Pax3/7 genes, MyoD and Myf5 possess clearly defined specific roles in satellite cell biology. MyoD is required for the differentiation potential of skeletal myoblasts [31,32] whereas Myf5 regulates their proliferation rate and homeostasis [33\*,34\*]. Interestingly, while both Myf5 and MyoD can each compensate for the loss of the other during embryogenesis [35], they cannot efficiently compensate for each other in an adult context. When MyoD-null and Myf5-null mice were crossed with *mdx* mutants both compound showed constant regeneration and aggravation of the dystrophic phenotype [33\*,34\*,36]. Myf5 deficiency leading to a lack of myoblast amplification and loss of MyoD induced an increased propensity for self-renewal rather than progression through the myogenic differentiation.

The differentiation factors myogenin and MRF4 are not involved in satellite cell development or maintenance [33] but induction of myogenin is necessary and sufficient for the formation of myotubes and fibers. As a proof of the latter, mice with a conditional post-natal recombination of myogenin show a reduced muscle mass, thus suggesting that the absence of myogenin prevented the skeletal myoblasts from contributing to post-natal muscle growth [37].

## Satellite cells depletion and inhibition during aging

Sarcopenia, the degenerative loss of skeletal muscle mass and strength, occurs during senescence and results in a reduction in the performance of muscles in old age (see [38] for review). The regenerative potential of skeletal muscle itself diminishes with aging, this related to a decline in satellite cells activity. Impairment in satellite cell function as a source of new myonuclei can be related to a diminution of the satellite cell pool, in fact, the abundance of resident satellite cells declines with age in myofibers from both fast- and slow- twitch muscles, but the down-sized population is still able to support effective myogenesis in vitro [39]. To this extend, transplantation experiments showed that satellite cells associated with aged fibers, can mediate extensive regeneration, and provide new satellite cell to the host muscle pool, thus demonstrating that the self-renewal and myogenic potential of young and old satellite cells is similar [40\*]. The changes in the extrinsic microenvironment inhibit satellite cells competences and increase in tissue fibrosis. As a demonstration of the latter, regenerative capacity and proliferation of satellite cells within old skeletal muscle were restored when exposed to a young systemic environment by parabiotic pairings of old and young animals [41]. Further lineage tracing experiments showed that cells that once were Pax7+ve satellite cells can loose Pax7 expression during muscle aging in mice, and differentiate into fibroblasts in old muscle, hence contributing to fibrosis. This loss of stem cell phenotype, results from an elevated canonical Wnt signalling induced by component of the old serum [42\*\*].

## Alternative source of regeneration; myogenesis from blood vessels

Myogenic and angiogenic cells are closely related during embryonic development. To that extend, a recent study demonstrated that cells of the dorsal aorta and of the myotome derive from a common precursor during early embryogenesis [43]. In fact, developing blood vessels and myogenic precursors are in close vicinity during embryonic development and interact with each others [44]. It has also been demonstrated that fetal myogenic progenitors and muscle fiber-associated endothelial cells share myogenic potential [45]. In post-natal life, satellite cells and endothelial cells are close neighbors and interact with each others during physiological processes and muscle regeneration [46]. In parallel, the mesoangioblast, an adult vessel-derived stem cell, possess high myogenic potential and is highly effective vector for muscle reconstruction in the context of muscular dystrophy [47]. Recent experiments carried out from human muscle tissue have maybe shaken the position of satellite cells as the major effectors of muscle regeneration and homeostasis. Indeed, pericytes associated with microvasculature walls are myogenic precursor [48\*\*] as well as multipotent myogenic cells related to the endothelial cell lineage [49\*] can efficiently contribute to myogenic regeneration. It is still unknown, at the moment, if human mesoangioblasts/pericytes and myo-endothelial cells are actually part of the normal homeostasis and regeneration during normal life. These cells possess a high myogenic potential *in vitro* and after transplantation in a dystrophic host, but they represent a very smaller progenitor population compared to satellite cells [48\*\*,49\*].

## Conclusion

Skeletal muscle satellite cells are a heterogeneous population of stem and progenitor cells which are necessary for skeletal muscle embryonic development, repair, homeostasis and senescence. Satellite cells are the main source for myofibers repair in the adult, and once woken-up from quiescence, they can generate differentiated cells and self-renewing stem cells by asymmetric division. Other vessel-related progenitors can also contribute to myofibers reconstruction, but our current opinion, without impeding any of the considerable impact for cell-mediated therapy of the latest studies, is that muscle satellite cells represent the major source of myogenic cells in the adult, and that vessel-related progenitor cells account for a secondary source of myogenesis as well as being able to contribute to neoangiogenesis in human muscle. From discussing with the researchers in our field, we can say that the study of satellite cells, their niche and regenerative capacities, is accelerating, and clearly, there will numerous breakthrough advances in the next few years in both developmental and molecular biology as well as medicine.

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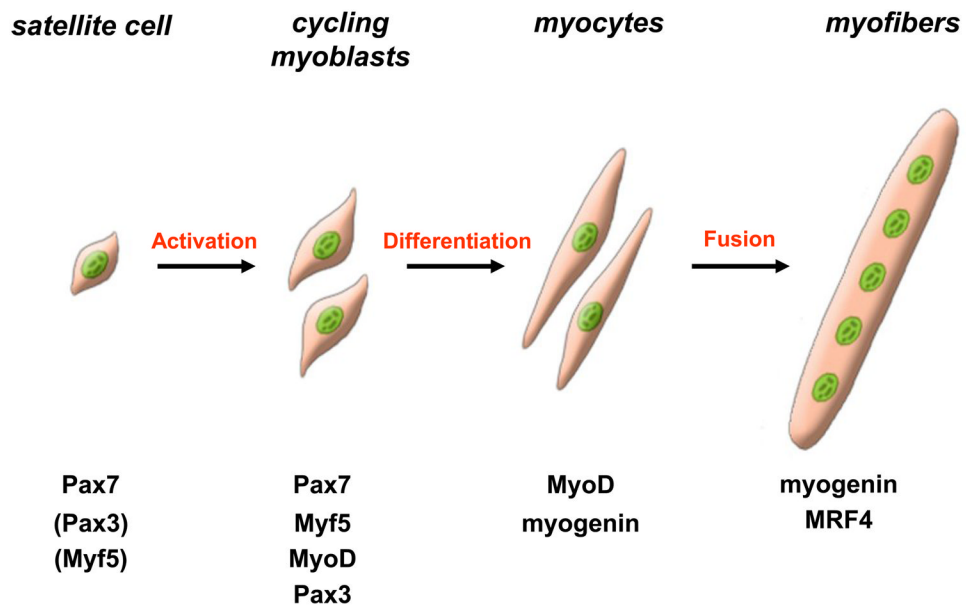


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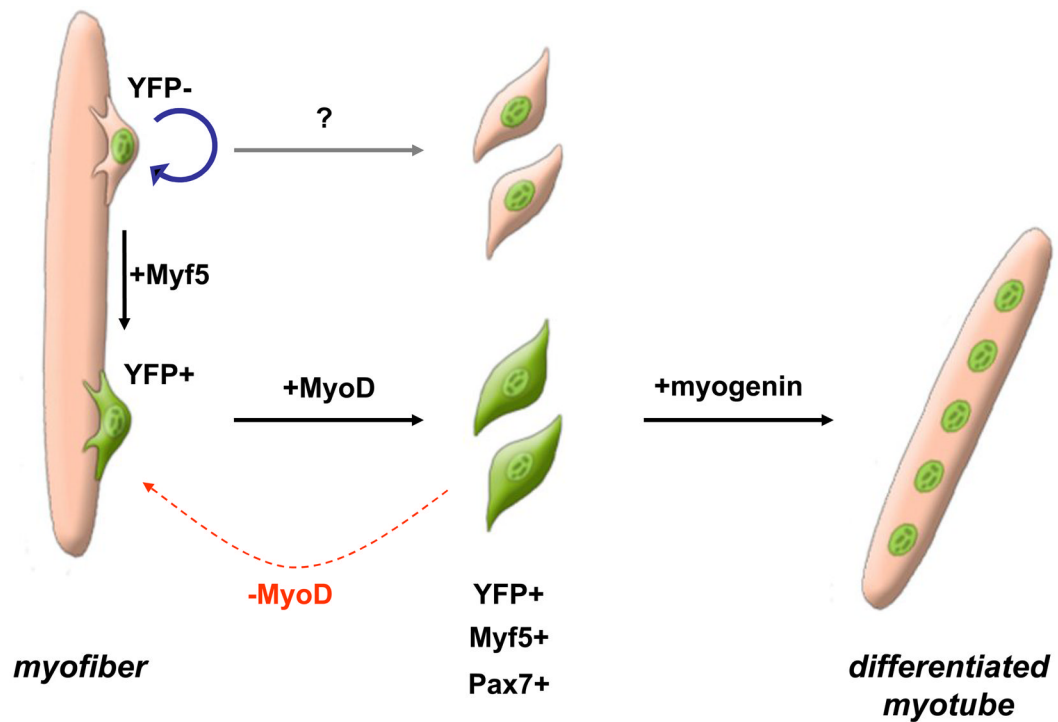
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**Figure 1. Schematic representation of adult myogenesis**

Quiescent skeletal muscle satellite cell can become activated following stimuli originating from their associated fiber or from the micro-environment. Their proliferating progeny, the skeletal myoblasts, express the paired-box transcription factors Pax7 and Pax3, as well as the myogenic regulatory factors Myf5 and MyoD. Once committed to differentiation, myoblasts stop cycling and lose expression of Pax7, Pax3 and Myf5. Differentiating myogenin+ve myocytes will then align and fuse to form multinucleated myofibers. MRF4 is further required for hypertrophy of the new fibers.



**Figure 2. Satellite cell hierarchy and self-renewal**

During regeneration, satellite stem (Myf5YFP-ve) cells can asymmetrically divide and give rise to a self-renewing daughter (blue arrow) and a committed (Myf5YFP+ve) daughter. Committed cells become activated, leave the sub-laminar niche and proliferate. Myf5+ve cycling myoblasts mainly differentiate and contribute to new fiber formation. Some Myf5+ve myoblasts can also downregulate MyoD and return to quiescence (red arrow), thus replenishing the niche. Myf5-ve stem cells are also postulated as being able to give rise to uncommitted progenitors which will participate in regeneration of the tissue (grey arrow).