

Medical sciences / Sciences médicales

Skeletal muscle progenitor cells and the role of *Pax* genes

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Abstract

Satellite cells, which lie under the basal lamina of muscle fibres, are marked by the expression of *Pax7*, and in many muscles of *Pax3* also. A pure population of satellite cells, isolated from a *Pax3^{GFP/+}* mouse line by flow cytometry, contribute very efficiently to skeletal muscle regeneration and also self-renew, thus demonstrating their role as muscle stem cells. *Pax3/7* regulates the entry of these cells into the myogenic programme via the activation of the myogenic determination gene, *MyoD*. *Pax7* is also essential for the survival of satellite cells. This dual role underlines the importance of ensuring that a tissue stem cell that has lost its myogenic instruction should not be left to run amok, with the potential risk of tissue deregulation and cancer. A somite-derived population of *Pax3/Pax7* positive cells is responsible for muscle growth during development and gives rise to the satellite cells of postnatal muscles. In the absence of both *Pax3* and *Pax7*, these cells die or assume other cell fates. *Pax3/7* lies genetically upstream of both *MyoD* and *Myf5*, which determine the skeletal muscle fate of these cells. **To cite this article: M. Buckingham, C. R. Biologies 330 (2007).**

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1. Satellite cells in the repair of skeletal muscle

Skeletal muscle undergoes regeneration in response to injury. Mononucleated cells, known as satellite cells, are located under the basal lamina of the multinucleated muscle fibre. When the fibre is damaged these cells become activated, replicate, and then differentiate to form new fibres, thus permitting muscle repair (Fig. 1) [1]. However, in recent years, the role of muscle-derived satellite cells in this process has been challenged and it has been proposed that cells from other sources such as bone marrow may be contributors of adult muscle

stem cells [2]. It has now become clear that this is not the case [3] and experiments with purified satellite cells have demonstrated their efficiency in muscle repair as well as their capacity to self renew [4,5]. Satellite cells are marked by the expression of *Pax7*, and also in many muscles of its paralogue, *Pax3*. We targeted the mouse *Pax3* gene with a *GFP* reporter sequence, which permitted us to isolate satellite cells by flow cytometry [5]. Clonal analysis demonstrated their myogenicity in culture. Grafting experiments into *mdx nude* mice, that lack dystrophin and undergo continuous regeneration in an attempt to repair the muscle damage which this entails, resulted in the reconstitution of dystrophin positive fibres. Quantitative estimation of grafting efficiency showed that this was much more efficient

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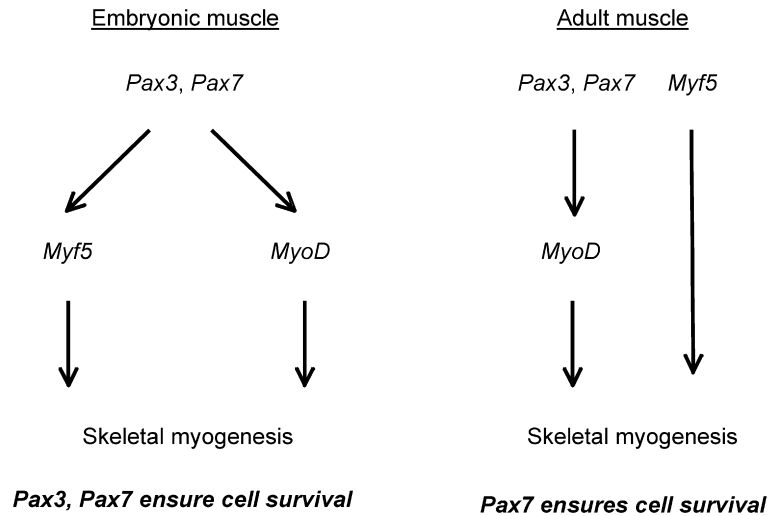


Fig. 1. The role of Pax3 and Pax7 in directing skeletal muscle progenitor cells into the myogenic programme and in ensuring progenitor cell survival. *Myf5* and *MyoD* are myogenic determination genes.

than with previously used crude muscle cell preparations ($\times 100$) or with purified satellite cells that had been expanded in culture prior to grafting ($\geq \times 10$). Furthermore, the presence of GFP positive satellite cells demonstrated the reconstitution of the progenitor cell population.

2. Pax function in satellite cells

In *Pax7* mutant mice, satellite cells are lost and regeneration is compromised. This was first thought to be due to a failure of satellite cell specification [6]. However, some satellite cells are present [7], and we have shown that there is progressive loss of satellite cells after birth, such that at postnatal day two, 80% of the normal number is present whereas by day ten this has fallen to 10% [8]. Postnatal muscle growth is affected, as well as regeneration. Satellite cells undergo apoptosis already at birth, as evidenced by labelling with an antibody to activated caspase 3 on sections of mutant skeletal muscle. Mutant satellite cells in culture also show cell cycle perturbations. The anti-apoptotic role of Pax7 is confirmed by experiments in which a dominant negative form is introduced into normal satellite cells. We had previously shown that Pax3 functions as a transcriptional activator during myogenesis by replacing *Pax3* with a *PAX3-FKHR* allele that encodes the DNA binding domain of Pax3, fused to the strong transcriptional activation domain of FKHR (Foxo1a); this fusion protein saves the myogenic phenotype of the mutant [9]. Furthermore, replacement of *Pax3* by a *Pax7* coding sequence shows that Pax7 also functions as an activa-

tor [10]. We therefore used a sequence encoding a fusion protein with the DNA binding domains of Pax7 or Pax3, followed by the transcriptional repression domain of Engrailed, to produce dominant negative forms of these factors (Pax7-DN, Pax3-DN). These sequences were introduced into adenoviral vectors with a GFP reporter. Infected satellite cells were separated by flow cytometry and tested for propidium iodide uptake as an indicator of cell death. Pax7-DN had a marked apoptotic effect, whereas Pax3-DN did not. This is consistent with the apoptosis of satellite cells in the *Pax7* mutant, where expression of *Pax3* does not save the phenotype [8].

In contrast, Pax3 and Pax7 have similar targets in the context of myogenesis. These factors are key upstream regulators of the myogenic programme (see [11]). Introduction of Pax3-DN or Pax7-DN into satellite cells prevents expression of the myogenic determination gene, *MyoD*. The other key myogenic determination gene, *Myf5*, which is already transcribed at a low level in quiescent satellite cells [12], continues to be expressed. This permits activation of *myogenin* encoding the member of this transcription factor family that controls muscle differentiation, leading to the formation of multinucleated fibres. In satellite cells from *Myf5^{GFP/GFP}* mutant mice [13], myogenesis occurs, but if they are infected with Pax3/7-DN, *myogenin* is not expressed and there is no differentiation, confirming that this requires Myf5 or MyoD. This series of experiments [8] leads to the conclusion that in adult satellite cells, Pax3 and Pax7 regulate myogenesis through *MyoD* (see below).

3. The origin of satellite cells: the formation of skeletal muscle

3.1. The onset of myogenesis in the embryo

The skeletal muscles of the trunk and limbs are derived from somites, segments of paraxial mesoderm that form on either side of the axial structures in the vertebrate embryo (see [14]). As somites mature, the dorsal part retains an epithelial structure, known as the dermomyotome, which is the source of myogenic progenitor cells. Initially these cells delaminate from the edges of the dermomyotome to form the first skeletal muscle, the myotome, immediately under the epithelium, or migrate to more distant sites of myogenesis, such as the developing limbs. The latter process is Pax3 dependent in mammals, while the initial formation of the myotome is orchestrated by Pax3 and the myogenic regulatory genes, *Myf5* and *Mrf4* [13,15]. These are activated by signalling from the surrounding tissues, as exemplified by Wnt and Shh signalling that directly regulate *Myf5* in the epaxial somite [16]. Recently, we have shown that Pax3 directly activates *Myf5* in the hypaxial somite and limb buds [17].

3.2. Pax3/Pax7 positive progenitor cells in developing skeletal muscle

As development proceeds, the epithelial structure of the central dermomyotome disintegrates and cells that express *Pax3* and *Pax7*, already present in this domain, enter the myotome [18,19]. Such Pax positive cells are present in all developing skeletal muscle masses at late embryonic and foetal stages [19–22]. Most of these cells are proliferating and do not express skeletal muscle markers. However, chase experiments show that they

can enter the myogenic programme, with activation of the myogenic determination genes *Myf5* and *MyoD*, followed by their incorporation into skeletal muscle fibres. Lineage tracing experiments, and notably grafts of quail somites into the chick embryo, demonstrate that all these cells in the trunk and limbs derive from somites. Furthermore, as previously suggested [23], the Pax positive satellite cells of postnatal muscle also come from this source [19,22].

In *Pax3/Pax7* double-mutant mouse embryos, which lack both *Pax3* and *Pax7*, there is a major deficit in skeletal muscle; only the early myotome and its derivatives are formed [20]. The *Pax3/Pax7* positive population of cells do not activate *Myf5* or *MyoD* and fail to enter the myogenic programme. They die, or they become incorporated into other tissues. In this major population of skeletal muscle progenitor cells, prior to birth, *Pax3* and *Pax7* regulate both myogenic determination genes, and *Pax3* as well as *Pax7* ensures cell survival.

In the perinatal period, these cells take up a satellite cell position under the basal lamina, which begins to form around muscle fibres. At this stage, many of such quiescent progenitor cells transcribe the *Myf5* gene, suggesting that activated, Pax positive-, cells that had begun to enter the myogenic programme revert to a more progenitor-like state, as quiescent satellite cells (Fig. 2). In these cells, *Myf5* expression is no longer under Pax control. Furthermore, the anti-apoptotic function of *Pax7* compared to *Pax3* is now predominant, again perhaps reflecting the shift in progenitor cell status. At earlier stages, the Pax positive cells either self-renew as stem cells or make muscle. The postnatal satellite cells efficiently repair normal damage, but progressively fail to do so when confronted with the degenerative diseases of skeletal muscle. This may reflect the preponderance of progenitor cells that have lost some

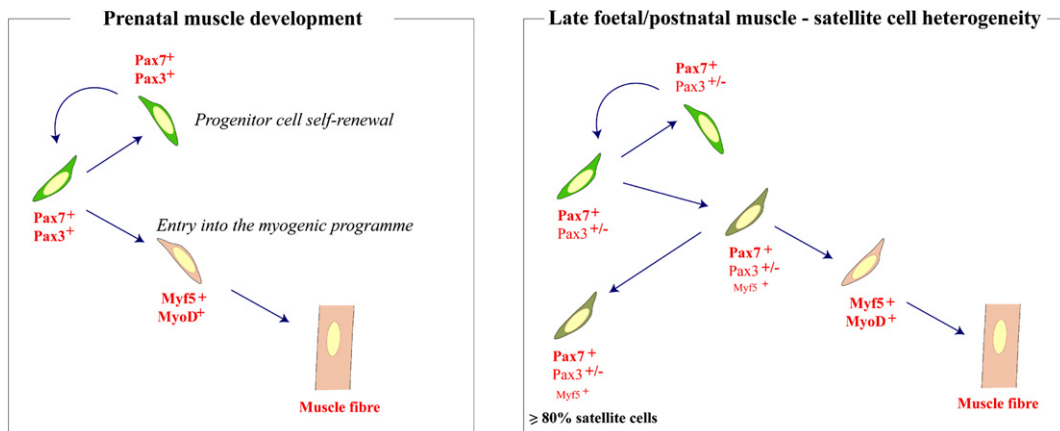


Fig. 2. Schematic representation of progenitor cell self-renewal, and myogenic differentiation in the embryo and the adult.

stem cell like properties, as a result of partial myogenic engagement.

3.3. Regulation of survival and tissue determination in stem cells

Finally, it is important to underline the significance of the regulation of stem cells' survival by the same factors that control the entry of these cells into a tissue differentiation programme. In the absence of such factors, undirected stem cells present a danger for the organism, and this is prevented by their death.

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