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

Margaret Buckingham

Département de biologie du développement, CNRS–URA 2578, Institut Pasteur, 25, rue du Docteur-Roux, 75724 Paris cedex 15, France

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Presented by Jean-François Bach and Nicole Le Douarin

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

E-mail address: margab@pasteur.fr.

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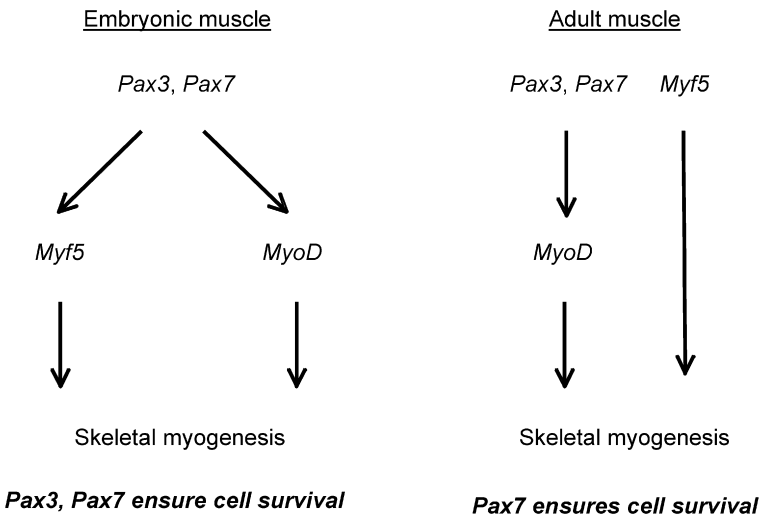

Fig. 1. The role of Pax3 and Pax7 in directing skeletal muscle progenitor cells into the myogenic programme and in ensuring progenitor cell survival. *Myf5* and *MyoD* are myogenic determination genes.

than with previously used crude muscle cell preparations ($\times 100$) or with purified satellite cells that had been expanded in culture prior to grafting ($\geqslant \times 10$). 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 activator [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).