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Visions & Reflections

Skeletal muscle progenitor cells: from embryo to adult

F. Relaix

Mouse Molecular Genetics Group, INSERM U787, Faculté de Médecine Pitié-Salpétrière, 105 bd de l'Hôpital, 75634 Paris Cedex 13 (France), Fax: +33 1 53 60 08 02, e-mail: frelaix@gmail.com

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Introduction

Skeletal muscle differentiation has long been an appealing model to study the mechanisms of molecular and cellular differentiation. Owing to recent progress and obvious therapeutic arguments, the field of myogenesis is now evolving toward identifying and manipulating muscle progenitor stem cells, with special interest in the mechanisms of cell fate decision and self-renewal. Muscle progenitor cells were first identified in the adult (as satellite cells associated with myofibers) more than 40 years ago by Alexander Mauro [1]. However their origin has remained controversial [2], while their self-renewing activity and regenerative potential has only been recently demonstrated *in vivo* [3, 4]. Reflecting a major evolution in the field, several groups have now published complementary work demonstrating that in mammals and birds there is a new, previously unidentified, population of muscle progenitor cells expressing the paired homeobox transcription factors Pax3 and Pax7, which give rise to nearly all the muscle cells of the embryo, fetus and adult, muscle satellite cells included [5–8].

Classic embryology experiments have demonstrated that most embryonic skeletal muscle cells, with the exception of craniofacial muscles, are derived from the somites, epithelial balls of cells which form from the paraxial mesodem and express Pax3 [see ref. 9]. As a result of inductive interactions from surrounding tissues, soon after their formation, the somites become compartmentalized. The most ventral part undergoes an epithelial to mesenchyme transition, giving rise to the sclerotome (the precursor of vertebrae and ribs). The dorsal part keeps its epithelial character, maintains Pax3 expression and forms the dermomyotome, which will generate the musculature and dermis [reviewed in ref. 9]. Shortly after, a third somitic compartment, the myotome, arises beneath the dermomyotome as the result of delamination of muscle progenitor cells from the dermomyotome [10]. These progenitor cells downregulate Pax3 while entering myogenesis by activation of the genes encoding the myogenic regulatory factors (MRFs) Myf5, Mrf4 and/or MyoD. These factors are essential for the formation of skeletal muscle [11–13]. Soon after the delamination of myogenic cells from the dermomyotomal edges, a myogenic differentiation factor, myogenin [14] will act with Mrf4 and MyoD [15] to drive their terminal differentiation into elongated, mononucleated desmin- and myosin-expressing myocytes in the myotome [10, 16–19].

Following formation of the myotome, successive waves of myogenic cells will use the pre-existing muscle fibers as a scaffold to sustain muscle growth during development. Growth, combined with patterning events, will give rise over time to the complex organization of fetal muscles prefiguring adult organization. In this context, embryonic and fetal myoblasts have been described as giving rise to 1° and 2° fibers, respectively. The developmental origin and relationship of these cells had remained unclear [2].

Initial progress in the field of skeletal myogenesis has been greatly stimulated by earlier studies of muscle differentiation in cell culture models [for reviews, see refs. 20, 21]. Most of these experiments were carried out using stable myogenic cell lines or cultures from either late fetal muscle cells or post-natal satellite cells. These are the major adult stem cell population critical for muscle growth and regeneration [3, 4, 22]. Satellite cells are quiescent myogenic progenitor cells appearing around birth that are

located between the plasma membrane and the surrounding basal lamina of mature, differentiated muscle fibers [1, 23–25]. They are characterized by the expression of Pax7 [26], CD34 and, for the vast majority, an *nLacZ* reporter gene targeted into *Myf5* [27], but do not express detectable levels of Myf5 or MyoD proteins. In the absence of *Pax7*, these adult progenitor cells are lost [26] and regeneration is impaired [28, 29], as a result of compromised proliferation and survival [30]. Despite the fact that *Pax3* is expressed in the satellite cells of a subset of muscles, it does not compensate for the absence of Pax7 in these cells [30]. Upon injury, satellite cells re-enter the cell cycle, proliferate, and then exit the cell cycle to either renew the quiescent satellite cell pool or to differentiate into mature fibers [31].

When isolated on myofibers, or as a pure population, and maintained in high serum conditions, quiescent satellite cells proliferate and activate Myf5 and MyoD, therefore providing a useful cell culture model of muscle regeneration [32]. At high density or upon serum withdrawal they activate *Myogenin*, an event under the control of Myf5 and MyoD [30]. This leads to downregulation of Pax7, cell cycle exit, terminal differentiation and fusion [20, 21] while self-renewing of satellite cells occurs through down-regulation of MyoD in a subset of the cells that maintain Pax7 expression [31, 33–36].

A new population of embryonic and fetal muscle progenitor cells.

These cell culture studies led for many years to the belief that muscle cells originate from a subset of dividing myoblasts located within the muscle masses during development. However, studies in the embryo and fetus have now demonstrated that myogenic cells (defined by the expression of myogenic determination factors) quickly exit the cell cycle to go on to differentiate, implying that the myoblast stage is a transitory step of myogenic progression in the myotome or developping muscle masses [5, 6]. This argued for the presence of an upstream reservoir of muscle progenitor cells that are not expressing myogenic markers [see ref. 37 for discussion].

As *Pax3* expression is a hallmark of early muscle progenitor cells in the somite and limb, while *Pax7* expression characterizes adult muscle satellite cells, expression of these paralog factors was re-investigated throughout myogenic development in the mouse [5, 8] and in the avian embryo [6, 7]. These studies, using specific antibodies [6, 8], in situ hybridization [7], mice with a reporter targeted into the *Pax3* and *Pax7* genes [5] and cell lineage tracing experiments in the chick [6, 7] identified a novel population of cells located within the myotome and maintained within the embryonic and fetal muscle masses. These cells express Pax3 and Pax7, but no myogenic markers. Importantly, this population contributes to muscle growth throughout development and is the major cycling population in the muscle masses [5]. These embryonic and fetal myogenic progenitor cells arise from a Pax3/Pax7 population of cells which delaminate from the central dermomyotome into the early myotome [5–7].

Genetic experiments in the mouse have also now demonstrated that these cells depend upon Pax3 or Pax7 function [5]. *Splotch* embryos [38], in which a mutation disrupts Pax3 function in both *Pax3* alleles [39], have defects in dorsal neural tube closure as well as in neural crest cell delamination and migration in the trunk and heart [38, 40, 41]. In addition, *Pax3* mutant embryos display limb and trunk muscle defects [38, 42–45]. In contrast to *Pax3*-deficient mice, *Pax7* mutant mice present no embryonic muscle defect [5, 46, 47], despite the ability of Pax7 to replace most Pax3 functions [47], reflecting differences in expression rather than functions. Embryos in which both Pax3 and Pax7 have been removed form a primary myotome, but the muscle progenitor cells either die or fail to enter the myogenic program and, as a consequence, no further muscle cells emerge. This points to a key function of these factors in muscle progenitor cell specification and survival [5].

Identification of similar progenitor cell populations and analysis of myogenesis in other vertebrates and in pre-vertebrates with primitive somites such as the cephalochordate *Amphioxus* will be of interest in order to understand from an evolutionary point of view how a second population of myogenic progenitor cells not only evolved but became the major muscle lineage. This likely reflects the requirement in higher vertebrates for massive muscle growth and reorganization during development.

From embryo to adult

While it had been known for two decades that adult satellite cells arose from somite-derived progenitor populations in embryonic and neonatal muscles [48, 49], their precise origin had remained controversial [for reviews and stimulating discussion, see refs. 37, 50]. It has now been demonstrated that most, if not all, satellite cells are derived from the Pax3/Pax7 progenitor cells located in fetal muscles. This has been shown by long-term labeling of the dermomyotome in the chick using both green fluorescent protein electroporation and the quail-chick grafting technique [6], as well as reporter genes targeted into the *Pax3* and *Pax7* loci in the mouse [5]. Although these studies do not exclude other possible origins, these experiments suggest that adult muscle progenitor cells in the trunk and limb derive from a unique structure (the dermomyotome).

Head and limb muscles have distinct origins. Limb muscle also derives from the somites [51, 52]. Recent work using both the chick and mouse lineage tracing techniques has demonstrated that limb muscle satellite cells derive from the population of muscle progenitor cell migrating from the hypaxial somite [53]. Moreover, preliminary data indicate that head muscles also contain a muscle progenitor cell population [F. Relaix and M. Buckingham, unpublished observations], but the link with muscle satellite cells still has to be made. In addition, there are specific mechanisms governing the appearance of these cells that are being investigated.

Several studies in recent years have pointed to possible input from other sources into the myogenic lineage, mainly in the adult (such as bone marrow, the hematopoietic system or the vasculature). These results have been the subject of much discussion and, at least in some cases, remain controversial [50]. A regenerative potential has been demonstrated in the case of the vessel-associated mesoangioblasts [54, 55]. Despite this, it is now generally accepted that the muscle satellite cells account for most physiological muscle regenerative potential [4, 22], and direct access to this population [3] is of considerable interest in the context of muscle diseases. There is little doubt that these findings open important new avenues in regenerative medicine. Regarding possible use of such cell populations for cardiac repair, preliminary experiments did not yield a clear functional benefit [56] and we must await further studies.

Concluding remarks

These analyses have demonstrated that myogenesis in the trunk requires two distinct steps. In the first step, the epithelial borders of the dermomyotome generate a terminally differentiated early myotome [10]. While the myotome is still developing, it is then used as scaffold for migration of a second population of muscle progenitor cells from the central region of the dermomyotome. This second population of cells will then be at the origin of nearly all the embryonic, fetal and adult muscle cells, including the adult muscle progenitor stem cells (satellite cells). These data point to a comprehensive and continuous mode of muscle growth from a progenitor pool that is maintained during development.

However, molecular and functional heterogeneity of muscle progenitor cells may exist. For example, in the adult there is a clear heterogenity of the satellite cells as two populations defined by the expression of either Pax7 or Pax3/Pax7 [30]. This heterogeneity seems to appear at the fetal stage (F. Relaix and M. Buckingham, unpublished observation], but elucidation of its functional significance must await further experiments. Additional heterogeneity may already exist at earlier stages. Despite the fact that primary and secondary fibers derive from the same population of muscle progenitor cells, divergent properties of embryonic and fetal myoblasts [2] may reflect either maturation or heterogeneity within the progenitor pool. Moreover, there might be a functional heterogeneity regarding muscle commitment and/or maintainance of a reserve pool. For example, a subset of the cells might be involved in muscle growth while others might be kept as a reserve population to generate adult muscle progenitor cells.

In conclusion, understanding the signals and molecular regulation ensuring the maintainance of an expanding and maturing pool of mitotic progenitor cells, and how these cells are instructed when to commit to the myogenic lineage is a major challenge for the future.

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