Skeletal Muscle Development: From Stem Cells to Body Movement

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Contents

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G. Rodrigues, B. A. J. Roelen (eds.), *Concepts and Applications of Stem Cell Biology*, Learning Materials in Biosciences, https://doi.org/10.1007/978-3-030-43939-2_9

What Will You Learn in This Chapter?

- In this chapter, the example of skeletal muscle development will be used to learn the following:
- How and where muscle stem cells (MuSCs) arise.
- How myogenesis starts in the embryo.
- How MuSCs interact with each other and with their neighbours, be it differentiated muscles cells or other cell types, and how they respond to the cues around them.
- How some MuSCs can stay undifferentiated and proliferative while others differentiate and construct the muscle tissue.
- How skeletal muscle is constructed progressively and in separate phases.
- How MuSC characteristics change over developmental time in harmony with their changing environment.
- How some MuSCs stay undifferentiated, even in the formed tissue after birth, and are set aside as quiescent MuSCs in the adult.
- How skeletal muscle becomes a highly regenerative tissue that responds to exercise or damage by activating its quiescent MuSCs which repair the muscle.
- How certain muscle diseases, such as muscular dystrophies, seriously affect muscle construction and function.
- How many outstanding questions regarding the development and plasticity of skeletal muscle remain and why they need to be addressed to fully understand this amazing tissue and to develop therapeutic strategies for muscle-related diseases.

9.1 Anatomy of Skeletal Muscle

In vertebrates, there are three types of muscles: (1) smooth muscles which are found in internal organs and around blood vessels, (2) cardiac muscle of the heart, responsible for the pumping of our blood, and (3) skeletal muscles which are attached to our bones and enable us to breathe, move, maintain our posture and produce heat. While smooth and cardiac muscles are tuned by the autonomous nervous system, the contraction of skeletal muscles is controlled by our will, through the action of motor neurons. Thus, skeletal muscles enable us to walk, run, jump as well as dance and smile: in essence, express who we are.

Skeletal muscle cells are large multinucleated cells surrounded by a specialised extracellular matrix called basement membrane (Fig. 9.1a). They are called muscle fibres because they have a long cylindrical shape, lie parallel to each other within a muscle and generally stretch from tendon to tendon (Fig. 9.1a). Connective tissue organises the superstructure of the muscle: it provides routes for blood vessels and nerve fibres and organises muscle fibres in different subunits. Considering the connective tissue layers from internal to external: individual muscle fibres are wrapped by the endomysium, the perimysium organises them into bundles (or fascicles) and the epimysium surrounds the whole muscle (Fig. 9.1a). These three sheaths are continuous with each other and extend to attach directly to the bone or to join the tendon which anchors to the bone or cartilage. They can, therefore, transmit forces generated by the contraction of the fibres.

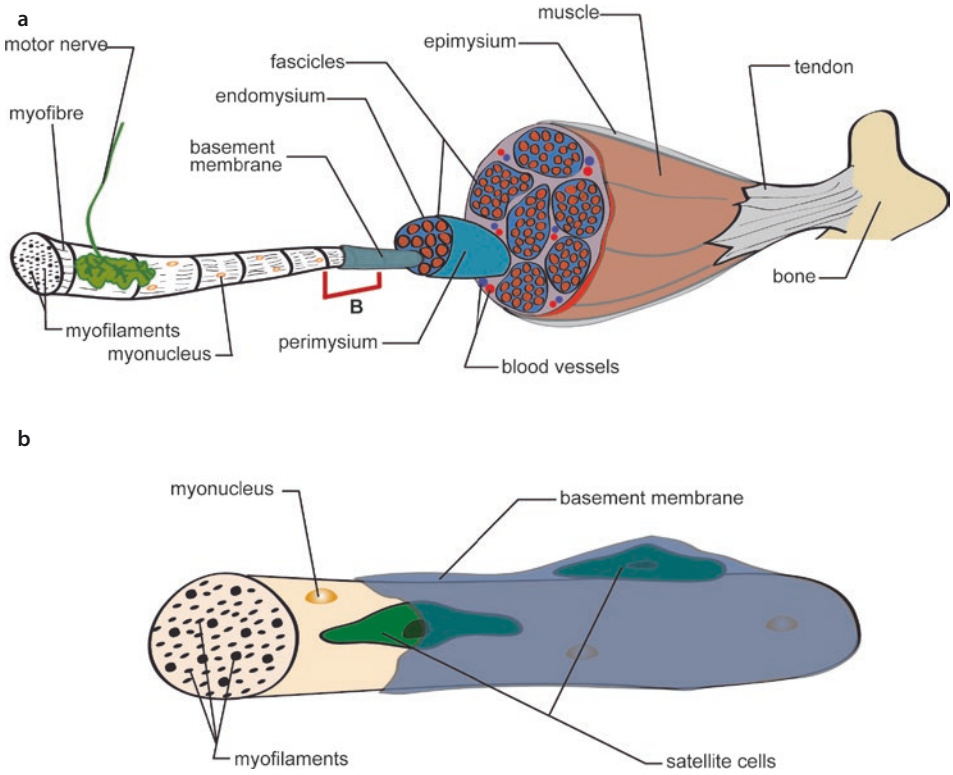


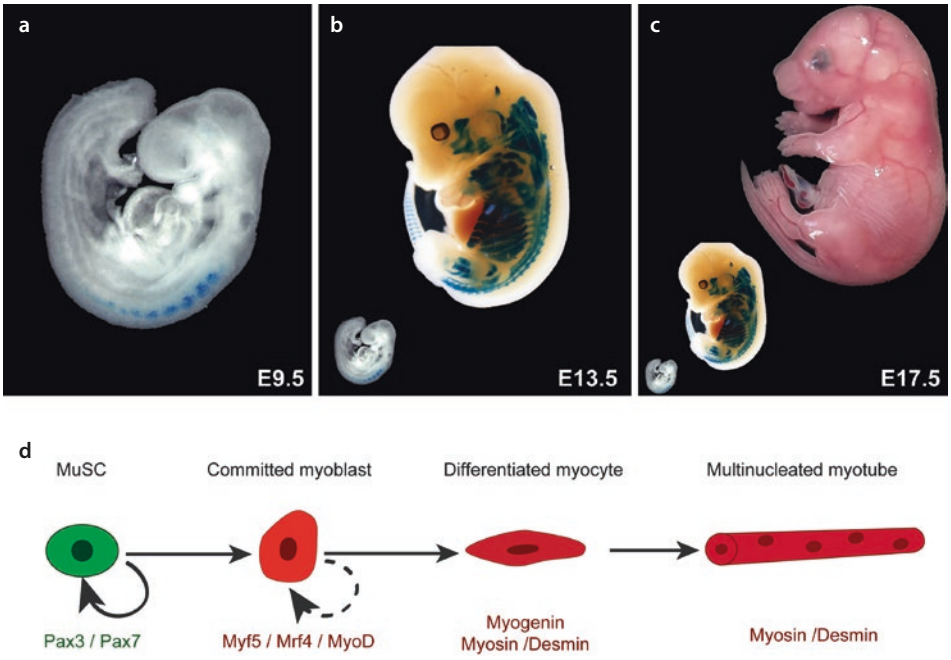
Fig. 9.1 Muscle anatomy. **a** Adult skeletal muscles are part of the musculo-skeletal system which is composed of bone, tendon attaching the bone to the muscle, nerves which enable inputs to order contraction, the skeletal muscle and the blood vessels. The whole muscle is highly organised by its connective tissues (endomysium, perimysium and epimysium) wrapping muscle structures. The muscle cell itself, called myofibre or muscle fibre, is a multinucleated cell containing myofilaments which enable its contraction. It is wrapped by its basement membrane. **b** Muscle stem cells (MuSCs; green) lie underneath the basement membrane of the myofibre, scattered along the fibre as satellites (hence their designation as satellite cells). Myonucleus refers to the nuclei of the muscle fibre

Muscle fibres are highly specialised cells with their many nuclei situated in the periphery of the fibres. The sarcoplasm (cytoplasm of the muscle) is packed with contractile units called the sarcomeres. Within the sarcomeres, thick and thin filaments (containing, respectively, the myosin and the actin filaments) alternate and make the striation of the muscle visible by microscopy. This particular organisation of the filaments is essential for the contraction of the cell. Skeletal muscle contraction is triggered and controlled by motor nerves (Fig. 9.1a). When a signal is sent by some specific motor nerves, the muscle fibres within a certain muscle contract in synchrony, enabled by some specific superstructure of the muscle, and produce the movement.

Close examination of a muscle fibre reveals the presence of small elongated cells lying between the muscle fibre and its basement membrane (Fig. 9.1b). These are adult muscle stem cells (MuSCs), also called satellite cells [1] because of their position near each muscle fibre. In healthy adult muscles, these MuSCs remain quiescent. However, if the muscle is injured, they are activated and proliferate. Subsequently, some of them differentiate and repair the muscle, while others return to a quiescent state under the basement membrane.

9.2 Skeletal Muscle Development at a Glance

Skeletal muscles occupy a huge volume in our bodies and a great number of cells are needed to construct them. Differentiation of the first skeletal muscle cells starts very early in embryonic development (blue cells in ◘ Fig. 9.2a) and this process proceeds at a steady pace thereafter [2, 3]. This progressive build-up of skeletal muscle is achieved through a tightly regulated balance between proliferation and differentiation of MuSCs. In essence, at each stage of development, some MuSCs differentiate, while others keep proliferating, generating enough cells to maintain skeletal muscle differentiation and growth until adulthood. Towards the end of embryonic development and before foetal development starts, all muscle groups have formed (blue cells in ◘ Fig. 9.2b), have been innervated and connected to the skeleton via tendons.



◘ **Fig. 9.2** Myogenesis goes through distinct steps. **a** Skeletal muscle development starts early in development (E8.5-E9.0 in the mouse). Myogenesis is first triggered in the dorso-medial lip of the dermomyotome and cells that activate *Myf5* expression (blue; seen through X-gal staining of heterozygous *Myf5*^{nLacZ} mice; courtesy of S. Tajbakhsh) enter the myotome as committed myoblasts and differentiate into myotomal myocytes. **b** Towards the end of primary myogenesis (here shown for E13.5 in the mouse), the basic muscle pattern has formed. By E14.5, these 'miniature muscles' have been innervated and connected to tendons. Muscles (blue) are visualised with X-gal staining in mice with the myosin light chain type 3F (*Mlc3f*) transgene; (courtesy of M. Buckingham). Inset shows the relative size of the E13.5 compared to the E9.5 embryos. **c** Mouse foetus at E17.5, showing the increase in size between E9.5 (first inset), E13.5 (second inset) and E17.5. **d** Myogenesis goes through distinct steps: a Pax3- and/or Pax7-expressing MuSC turns on Myf5, Mrf4 and/or MyoD, designated myogenic regulatory factors (MRFs), which turns them into committed myoblasts. These myoblasts can divide a few times, but after they upregulate the MRF Myogenin, a differentiation factor, they exit the cell cycle and start expressing muscle structural proteins such as myosins and desmin. These differentiated myocytes then fuse with each other forming multinucleated myotubes, which mature into muscle fibres. During foetal and perinatal stages, myocytes can also fuse with existing myofibres, increasing their size

During foetal development, each muscle grows tremendously through the addition of differentiated cells to the muscle pattern established during embryonic development, and in tune with the growth of the foetus (Fig. 9.2c). Growth then continues after birth, until adulthood.

MuSCs are multipotent stem cells, which in the trunk and limbs are characterised by the expression of Pax3 and/or Pax7 (Fig. 9.2d; [3, 4]). MuSCs enter the myogenic differentiation programme when they start expressing a specific set of transcription factors called the myogenic regulatory factors (MRFs). MRFs are members of the MyoD family of myogenic basic helix–loop–helix (bHLH) transcription factors and their expression leads to myogenic determination and differentiation [5, 6]. In vertebrates, there are four MRFs named Myf5, Mrf4 (also known as Myf6), MyoD and Myogenin [7]. When a MuSC turns on the expression of Myf5, MyoD or Mrf4, it becomes committed to myogenesis and is termed a myoblast. Committed myoblasts can divide a few times (Fig. 9.2d). However, after entering the committed state, myoblasts upregulate the fourth MRF, Myogenin, which leads to their exit from the cell cycle, the synthesis of muscle structural proteins such as desmin and myosins and their terminal differentiation (Fig. 9.2d). Finally, terminally differentiated myocytes fuse with each other or to existing muscle fibres (Fig. 9.2d), thus contributing to muscle growth.

9.3 Muscle Stem Cells: Where Do They Come From?

MuSCs of the trunk and limbs are derived from transient embryonic structures called somites. Somites are formed progressively and in pairs early in development, one somite on each side of the neural tube and the notochord [8]. They form from the unsegmented presomitic mesoderm, budding off at regular intervals as metameric spheres of epithelial cells encompassing a central cavity, named somitocoel (Fig. 9.3a; [9]).

Soon after epithelial somites form, they give rise to different compartments. The ventral portion of each somite undergoes an epithelium to mesenchyme transition to form the sclerotome, a compartment containing the precursors of the vertebrae and ribs (Fig. 9.3b; [10]). Later, the dorsal-most part of the sclerotome, which is called the syndetome [11], is specified into tendon precursors which will form the tendons attaching the axial muscles to the vertebrae and ribs.

Cells in the dorsal somite remain epithelial and form a compartment designated the dermomyotome (Fig. 9.3b). All MuSCs (except those of the head) are derived from the dermomyotomes present along the rostro-caudal axis from neck to tail. Dermomyotomal cells are multipotent and, although their major derivative is MuSCs, certain regions or cells of each dermomyotome give rise to other cell types, such as precursors of the dorsal dermis, smooth muscle, endothelia and brown fat [12–15].

The dermomyotome is an epithelial sheet whose four extremities curl into four contiguous lips - defined as dorso-medial, ventro-lateral, rostral and caudal lips - surrounding what is termed the central dermomyotome. Dermomyotomal cells express the transcription factors Pax3 and/or Pax7, which mark their myogenic potential [16–19]. The dermomyotomal epithelium is lined dorsally by a basement membrane which prevents precocious myogenic differentiation in the dermomyotome

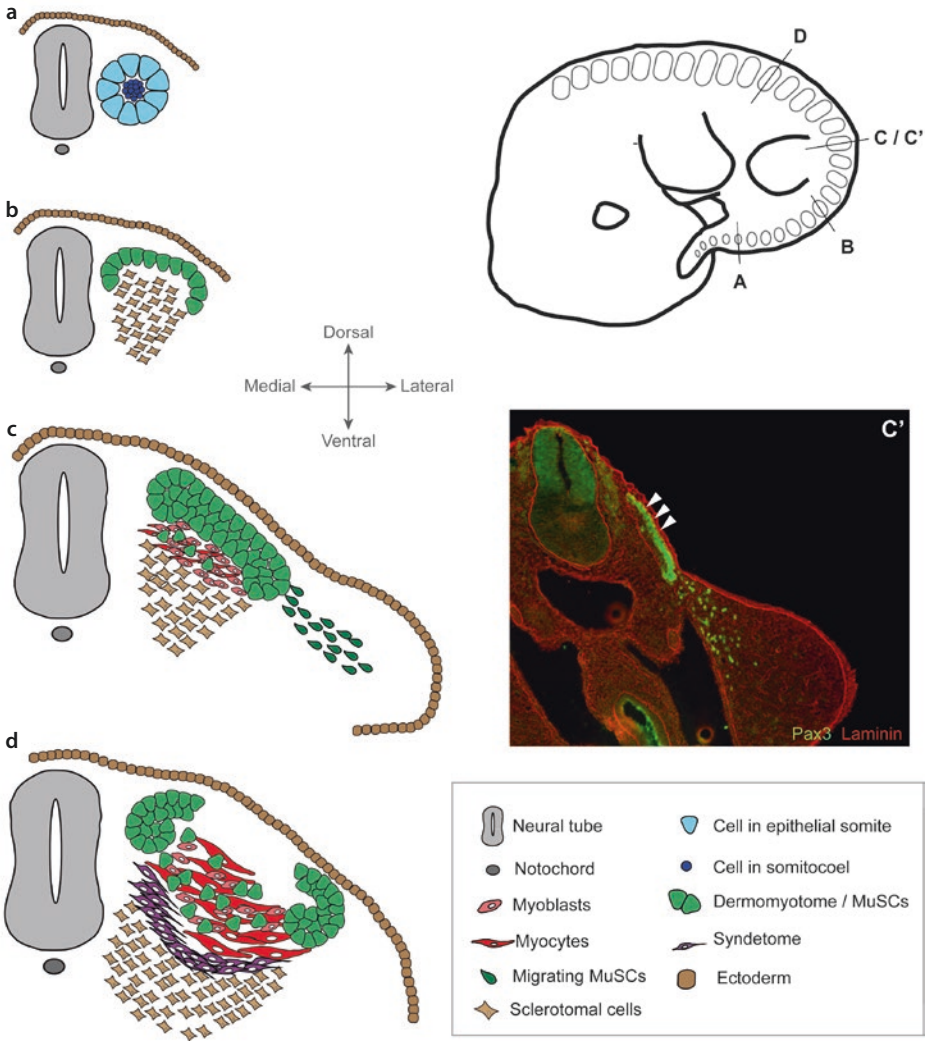


Fig. 9.3 Embryonic origin of skeletal muscle. Schematic illustration of somite development along the caudal to the rostral axis at four different levels of an E11.5 mouse embryo (see drawing, top right). Since embryos at this stage are more developed in their rostral part than their caudal region, this analysis is also a temporal one. **a** Epithelial somites are rosettes of epithelioid cells (blue) with their apical side turned towards a central somitocoel which contains a few mesenchymal cells (dark blue). **b** The ventral portion of the somite de-epithelializes to form the sclerotome (brown), while the dorsal portion remains epithelial and is designated the dermomyotome (green). **c** The dermomyotome originates MuSCs from its edges, and these MuSCs enter the area ventral to the dermomyotome, where they differentiate, first into myoblasts (pink cells) and later into myotomal myocytes (red cells). At limb level, the ventro-lateral lip of the dermomyotome originates Pax3-positive MuSCs (green cells) that migrate to the limb bud. **c'** Transverse section of a mouse embryo stained by immunofluorescence showing Pax3-positive (green) cells in the neural tube and in the dermomyotome which includes MuSCs and Pax3-positive MuSCs migrating from the dermomyotome into the limb bud. Laminin (red) lines the dermomyotomal epithelium (arrowheads), contributing to maintaining its non-differentiated state. Laminin also stains all other basement membranes present at this stage (e.g. ectoderm, neural tube). **d** The central dermomyotome dissociates and many MuSCs (green) colonise the myotome. Some remain proliferative to compose the resident pool of stem cells, while others differentiate giving rise to skeletal muscles. Communication between the myotome and the dorsal sclerotome induces the syndetome (purple)

(Fig. 9.3c'; [20]). As the dermomyotome grows, MuSCs delaminate in synchronous waves from the four dermomyotomal lips and colonise the area underneath to form the myotome, where myogenic differentiation starts (Fig. 9.3c). These are the dermomyotome lip-derived MuSCs (Table 9.1). At limb levels, MuSCs from the ventro-lateral dermomyotomal lip develop in a different way because they delaminate and migrate towards the limb bud (Fig. 9.3c') and only differentiate upon arrival to their target sites [21]. Dermomyotome-derived MuSCs also migrate to form the diaphragm and tongue [22, 23]. The dermomyotome eventually de-epithelializes, releasing proliferative MuSCs into the myotome (Fig. 9.3d). The MuSCs that migrate to the limbs, diaphragm and tongue and the MuSCs derived from the central dermomyotome are designated embryonic MuSCs (Table 9.1).

Once in their muscle mass (myotome, limb muscle or other) MuSCs have two possible fates: either they activate the myogenic differentiation programme and differentiate, or they remain proliferative to maintain the MuSC pool within the muscle mass. Some of these proliferative MuSCs come to differentiate later during development while others are put aside as quiescent adult MuSCs [16–19].

9.4 Onset of Skeletal Muscle Development

9.4.1 Triggers of Myogenic Differentiation

The segmentally organised myotomes are the first skeletal muscles to form in the vertebrate embryo and how they form has been the subject of intensive study. Early studies showed that extrinsic signals coming from the neighbouring tissues play key roles in activating MRF expression and consequently triggering myogenic differentiation (Fig. 9.4a; [14, 24, 25]). Here, we will focus on the dorso-medial lip of the dermomyotome, which is where myogenesis starts in amniote embryos, and we will see how multiple signals converge to trigger its onset.

Recent studies in the chick embryo have shown that neural crest cells migrating from the dorsal neural tube and passing the dorso-medial lip of the dermomyotome contribute to trigger myogenesis. Migrating neural crest cells were shown to express Delta-like ligand 1 (Dll1) which binds to Notch receptors on cells in the dorso-medial lip, leading to a transient activation of Notch which culminates in Myf5 activation in these cells (Fig. 9.4b; [26, 27]). Migrating neural crest cells also express a transmembrane heparan sulphate proteoglycan, named glypican 4 (GLP4), on their cell surface, which carries Wnts from the dorsal neural tube to the dorso-medial dermomyotomal lip (Fig. 9.4b; [28]). Studies in both chick and mouse have shown that Wnt signalling in the dorso-medial lip synergizes with Sonic hedgehog (Shh) signalling to activate Myf5 expression (Fig. 9.4c; [29]). Shh is secreted from the notochord and neural tube floor plate, travels through the sclerotome and activates Gli2 and Gli3 in the cells of the dorso-medial lip (Fig. 9.4c; [30, 31]). Shh is, however, not able to trigger the myogenic differentiation programme by itself. Rather, it seems that Wnts (Wnt1, 3a, 4) from the dorsal neural tube and ectoderm act through β-catenin which synergises with Shh signalling to activate Myf5 in the dorso-medial lip (Fig. 9.4c; [29]). β-catenin also induces the expression of Noggin, a bone mor-

Table 9.1 MuSC terminology

MuSC type (/alternative name)	Pax gene expression	Stage (in mouse)	Location	Arises from	Differentiates into
MuSCs from dermomyotome lips / founder MuSCs[a]	First none[a], then Pax3	E8.5/9.0–E11.0	Trunk	Dorso-medial, rostral, caudal and ventro-lateral dermomyotome lips (in trunk)	Myotome
Embryonic MuSCs (trunk) / founder MuSCs[b]	Pax3/ Pax7	E11.0–E14.5	Trunk	Central dermomyotome	Primary (embryonic) myofibres in trunk
Embryonic MuSCs (limbs) / founder MuSCs[3]	Pax3	E10.5–E14.5	Limb-levels	Ventro-lateral dermomyotome lip (limb levels)	Primary (embryonic) myofibres in limbs
Foetal MuSCs	Pax7	E14.5–birth	Trunk and limbs	Embryonic MuSCs[b]	Secondary (foetal) myofibres. Also contribute to the growth of all myofibres.
Perinatal MuSCs / juvenile satellite cells	Pax7	Birth–P21	Trunk and limbs	Foetal MuSCs[b]	Contribute to the growth of all myofibres. Generate some new myofibres.
Adult MuSCs / Satellite cells	Pax7	P21 onwards	Trunk and limbs	Perinatal MuSCs[b]	Enter quiescence. Activated upon growth, exercise or injury and contribute to muscle repair. After repair, some of them reenter quiescence.

Simplified terminology of the different types of dermomyotome-derived MuSCs (head MuSCs are not included). Six major MuSCs types have been defined, but it is important to appreciate that each MuSC type is heterogeneous and that the significance of this heterogeneity is presently unclear (based on [2, 3, 90, 93])

[a]The first cells to differentiate from the dermomyotome lips do not express Pax3. They may not be true muscle stem cells

[b]Although the current view is that one MuSC type develops into the next type, the possibility that different subpopulations within the dermomyotome originate the different MuSC types cannot be excluded

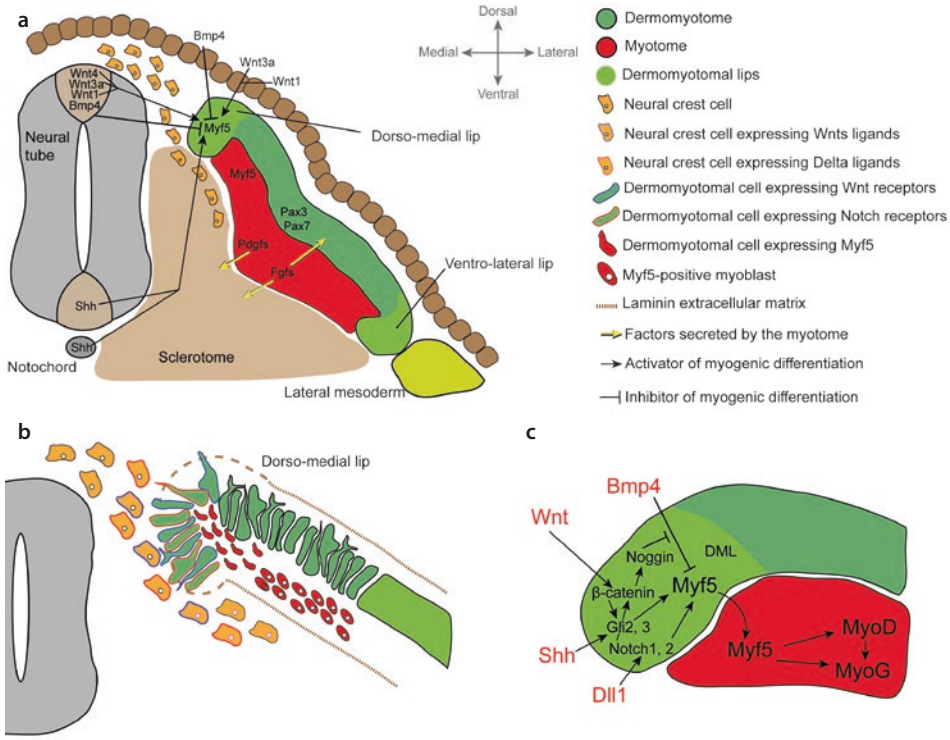


Fig. 9.4 Induction of myotome formation by neighbouring tissues. **a** Global overview of the extrinsic signals that trigger myogenesis in the dorso-medial lip of the dermomyotome. A combination of factors is thought to lead to Myf5 activation in this region. Neural crest cells migrate from the dorsal neural tube and bring the Notch ligand Dll1 and Wnts on their cell surface to the cells of the dorso-medial lip as they migrate past this region (see B for more details). In parallel, Shh secreted from the notochord and the floor plate of the neural tube acts together with Wnt and Notch signalling to upregulate Myf5 in this region. In contrast, Bmp4 secreted by the neural tube and ectoderm acts as a repressor of myogenesis. As myogenesis progresses, cells in the myotome (red) produce Pdgfs that regulate sclerotome differentiation and Fgfs, which induce syndetome formation and the dissociation of the central dermomyotome. **b** Migratory neural crest cells expressing Delta and carrying Wnt ligands from the dorsal neural tube briefly bind to filopodia extended by cells in the dorso-medial lip which have Notch and Frizzled receptors. These two signals, as well as Shh coming from the notochord and floor plate (see A), induce Myf5 expression and dermomyotomal cells delaminate as committed myoblasts (red cells) into the myotomal area. The brown dashed lines represent the dermomyotomal and myotomal basement membranes, which are discontinuous. Notably, the holes in the basement membrane appear to enable cell–cell interactions between dermomyotomal cells and the neural crest cells. **c** Schematic summary of the interplay between the different signalling pathways involved in the induction of myogenesis in the dorso-medial lip (bright green). Dll1 induces Notch activity which brings β-catenin from the membrane to the cytoplasm in the cells of the dorso-medial lip, Wnt signalling acts through β-catenin to induce Noggin and promotes Gli2 and Gli3 expression and Shh signalling in turn activates Gli2 and Gli3. The result is that attenuated Bmp4 signalling lifts the block on Myf5 transcription, while Notch, Wnt and Shh signalling appear to cooperate to activate Myf5. The Myf5-expressing myoblasts then enter the myotome (red) where they express additional MRFs and differentiate into myotomal myocytes

phogenetic protein (Bmp) antagonist [32]. Bmp4 secreted from the dorsal ectoderm and neural tube normally represses myogenesis [33]. However, through the activation of Noggin in the dorso-medial lip, Bmp signalling is specifically blocked in this region and allows for Myf5 activation (◘ Fig. 9.4c). In summary, Notch, Wnt and Shh signalling converge in cells of the dorso-medial dermomyotomal lip where they appear to collaborate to counteract myogenic repressors and activate myogenesis (◘ Fig. 9.4c).

Myogenesis continues in the dorso-medial lip and meanwhile also starts in the ventro-lateral, rostral and caudal dermomyotomal lips [34–36]. As new myoblasts enter the myotome and differentiate, the myotome grows in both the dorsal–ventral and medial–lateral direction. The myotome itself also starts influencing its neighbours, for example by producing platelet-derived growth factors (Pdgfs) and fibroblast growth factors (Fgfs) [37–39]. Pdgfs influence differentiation in the sclerotome (◘ Fig. 9.4a; [40]), while Fgfs induce syndetome specification [39] and act on the central dermomyotome, promoting its subsequent de-epithelialization (◘ Fig. 9.4a; [41]). This de-epithelialization brings the proliferative Pax3- and/or Pax7-positive embryonic MuSCs into the myotome [16–19].

The extrinsic signals that trigger myogenesis in other sites of the embryo are not exactly the same [14, 42]. Nevertheless, even if the details differ, the Wnt, Shh, Fgf, Bmp and Notch signalling pathways are consistently involved in both trunk and limb myogenesis. An interesting difference is that during limb muscle development differentiated myocytes fuse into myotubes faster than those of the myotome [43], most likely because they are evolutionary more recent and are not restrained by the developmental programme specific to the myotome [14, 44].

Head muscle development is very different. Head mesoderm, from which head MuSCs derive, does not express Pax3 [45] and Pax7 is only upregulated after developing muscle masses have formed [18, 19, 46]. Nevertheless, as in trunk and limbs, myogenic differentiation goes through the MRFs, but their specification involves the transcription factors, Tbx1 and/or Pitx2, suggesting that myogenesis in the head took a different evolutionary route from trunk (and limb) myogenesis [23].

9.4.2 Keeping the Balance Between Differentiation and Self-renewal of MuSCs in Space and Time

Skeletal muscle development from here on proceeds through different steps which take place at different time points and do not have the same role [47]. First, embryonic MuSCs undergo primary myogenesis which sets the basic muscle pattern (between the E11.0 and E14.5 in the mouse). During secondary myogenesis, this basic pattern is used as a scaffold for tremendous muscle growth (from E14.5 to birth in the mouse) [48, 49]. Finally, muscle growth after birth is driven by perinatal MuSCs (also called juvenile satellite cells; ◘ Table 9.1) which are not yet quiescent. We will look at these different steps in more detail in the next section.

However, one obvious question that arises when studying any step of myogenesis is how some MuSCs are kept in an undifferentiated state within the developing muscles while others are induced to enter myogenesis. In other words, how do some MuSCs avoid differentiation in an environment that promotes entry into the myogenic programme? This balance between proliferation and differentiation is regulated

by the Notch signalling pathway [50, 51]. During early stages of myogenesis in the trunk and limbs, differentiating myoblasts express Notch ligands which can bind to Notch receptors expressed on MuSCs and maintain their undifferentiated and proliferative state [52–54]. At later stages of myogenesis, not only myoblasts, but also the forming myofibres express Notch ligands [55]. Indeed, when the Notch intracellular domain (NICD), the part of the Notch receptor that enters the nucleus to activate target genes is overexpressed in MuSCs, they remain undifferentiated and proliferative until late foetal development and no skeletal muscles form [56]. Importantly, this Notch signalling is not transient (which leads to Myf5 activation in the dorso-medial lip in the chick; [26]), but sustained. Sustained Notch signalling is thus the single most important pathway in maintaining the pool of MuSCs throughout myogenic development.

9.5 Primary Myogenesis: Construction of the Skeletal Muscle Pattern

9.5.1 Primary Myogenesis

Primary myogenesis is essential to organise the basic muscle pattern and set the connection between muscles and their tendons and nerves. In the trunk, primary myogenesis starts after myotome development, when MuSCs from the dissociating dermomyotome enter the myotome (◘ Fig. 9.5a; [57, 58]; ◘ Table 9.1). In the limbs, and other regions receiving migrating dermomyotome-derived MuSCs, it starts after the arrival of the MuSCs to their target sites (◘ Fig. 9.5a; [59]; ◘ Table 9.1).

It is interesting to note that the environment of primary myogenesis is different from that of the later myogenesis steps. Obviously, as myogenesis advances, the tissues surrounding the developing muscles also advance in their development and, therefore, change their repertoire of secreted factors. Thus, MuSC identity changes over time and these environmental cues appear to play a major role in this change (◘ Fig. 9.5b; [50, 60]; ◘ Table 9.1).

In the limb, primary myoblasts (also called embryonic myoblasts) differentiate from MuSCs expressing Pax3 ([61]; ◘ Table 9.1). In the trunk, the same presumably holds true, although since Pax7 is expressed earlier in the trunk (i.e. in the central dermomyotome), primary myoblasts are probably also derived from cells co-expressing Pax3 and Pax7 ([19], ◘ Table 9.1). Curiously, embryonic MuSCs and myoblasts express Hox genes, which later stage myogenic cells do not [62]. It is interesting to speculate that these Hox genes are important to construct the muscle pattern during primary myogenesis. Multiple fusion events between differentiating myoblasts and/or the existing myocytes quickly generate the first primary myotubes (◘ Fig. 9.5a). These primary myotubes extend from tendon to tendon, are fully differentiated and can contract. However, they are few in number and have a small cross-sectional area (◘ Fig. 9.5c; [63]). Although the primary myotubes are multinucleated, their nuclei are centred in the cell.

One other striking difference between primary myogenesis and later stages of myogenesis is that there is a total absence of laminin matrix around the primary

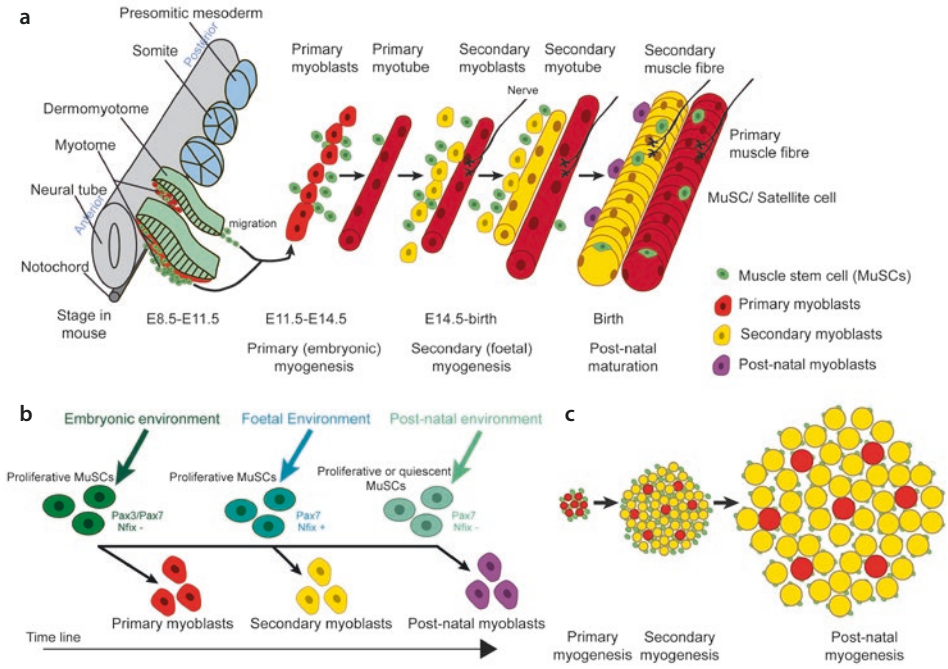


Fig. 9.5 Summary of skeletal muscle development. **a** All the muscles of the vertebrate body (except those of the head) are derived from MuSCs originating from the dermomyotomes of the somites. MuSCs (green) either undergo long-range migration and differentiate after reaching their target site or translocate underneath the dermomyotome and differentiate there to form the segmented myotomes which will later transform into axial muscles. MuSCs either stay proliferative or differentiate into primary (red) then secondary (yellow) myoblasts and finally into post-natal (perinatal or adult) myoblasts, depending on when during development they enter the differentiation programme. Primary myoblasts fuse with each other forming multinucleated primary myotubes (red), then secondary myoblasts first fuse with each other forming secondary myotubes (yellow) and then with all the existing myotubes, increasing their size. During post-natal stages, myotubes mature into myofibres and MuSCs present at those stages come to enter quiescence and occupy a position as satellite cells along the fibres. **b** MuSCs develop different stage-specific identities. Tissue complexity increases over time, which leads to a constantly changing environment. For example, in developing skeletal muscle, other cell types such as in-growing blood vessels and nerves send different cues to their muscle neighbours at different developmental stages. MuSCs are sensitive to their surrounding environment and their identity changes as development proceeds. **c** Transverse view of skeletal muscle during the three steps of muscle growth during myogenesis: During primary myogenesis, primary myofibres (red) develop to make the muscle pattern of the musculo-skeletal system. During secondary myogenesis, the number of fibres increases drastically as secondary myoblasts form secondary myotubes (yellow) around the primary myotubes. This phase defines the number of fibres of each muscle of the adult. In late foetal perinatal stages, growth is normally only by cell-mediated hypertrophy where each fibre grows by addition (fusion) of myoblasts but the number of fibres stays the same

muscle fibres [64]. Indeed, primary myotubes not only lack a basement membrane but they do not express any laminin receptors [65]. The developmental significance of this fact is presently not known, but it is possible that since primary myotubes are contacted by nerves and surrounded by mesenchymal cells, it is important that no extracellular matrix barrier prevents this communication.

9.5.2 Innervation

As soon as primary myogenesis is underway, nerves invade the presumptive muscle masses [66]. Sensory neurons derive from the neural crest cells which migrate to form the dorsal root ganglia, located in pairs on each side of the neural tube. The body of the neurons is in the dorsal root ganglia and they extend their axons and dendrites from there. Motor neurons exit the ventral part of the neural tube and mix with the sensory neurons to form the spinal nerves and they migrate together towards their targets [67]. The axons of motor neurons migrate to their target through precise paths. Their growth cones sense the different cell surface-bound molecules along the path and are attracted or repulsed by them, allowing these 'seeker heads' to find the way [68]. As nerves grow, neural crest cell precursors of Schwann cells migrate along just after them [69]. Once at their target sites, they differentiate into Schwann cells. The whole peripheral nervous system is thus in place to innervate the muscles.

When motor neurons contact muscle fibres, an intricate communication between the two cells takes place to form the specialised area called the synapse (◘ Fig. 9.6a–c; [70–72]). In the synapse region, the nerve terminal and the muscle endplate become specialised areas within both cells, so that neural signals come to command the contraction of the muscle cell (◘ Fig. 9.6c).

9.5.3 Myogenesis and the Development of Muscle Connective Tissue Including Tendons

Muscle anchors to tendons at each end of the muscle fibre and muscle membrane receptors such as integrin α7β1 [73–75], or dystroglycan [76] play a role in organising these connections. Muscle connective tissue including tendons and muscle cells derive from different lineages, but both tissues are essential for each other's development [77–79]. Indeed, the communication between muscle and tendon progenitors has been reported early in development. For example, fibroblast growth factor 4 (Fgf4) secreted by the myotome is necessary for the induction of *Scleraxis* expression (a marker of tendinocyte specification) in the axial system [80]. In the limb, Fgf4 and Fgf8 secreted by the tips of muscle fibres allow the specification of tendon cells [81, 82]. In reverse, if tendinocytes are absent, or if they do not express the transcription factor Tcf4, muscle development is impaired [77, 79]. Therefore, muscle connective tissue, tendon and muscle tissue grow and organise themselves together [11, 77]. Communication between tendon cells and muscle cells ensures their synchronous development, and at the end of primary myogenesis, both are well organised (◘ Fig. 9.7).

In conclusion, through primary myogenesis, primary muscle fibres form, are innervated and come to stretch from tendon to tendon, where they attach to the developing cartilage. They can be considered 'miniature muscles' which serve as a template for the construction of the definitive muscle. It is on top of this template that secondary myogenesis will take place (◘ Fig. 9.5a, c).

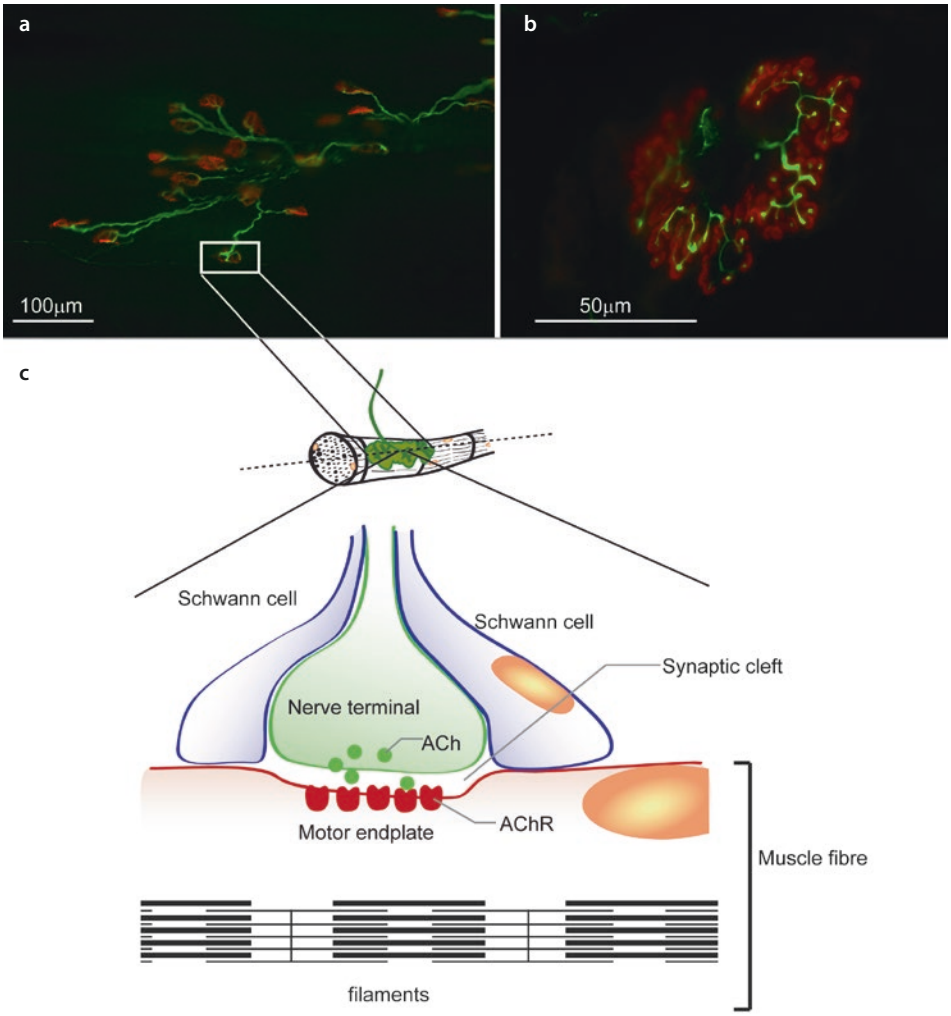


Fig. 9.6 The synapse. **a** Whole mount preparation of the diaphragm of an adult mouse. Motor neurons – stained by immunohistochemistry with antibodies against neurofilament and synaptophysin (both green) – contact the muscle cells at very specific areas called the endplates – stained with α-bungarotoxin (red) which binds to acetylcholine receptors. **b** Section of the extensor digitorum longus muscle of an adult mouse stained by immunohistochemistry (as in a.) to show a motor endplate and its nerve terminal. **c** The synapse is the specialised area where the motor nerve axon contacts the muscle fibre to send signals and control its contraction. The nerve terminal releases neurotransmitters (acetylcholine (ACh)) in the synaptic cleft, which binds to ACh receptors (AChR), located in the motor endplate of the muscle fibre

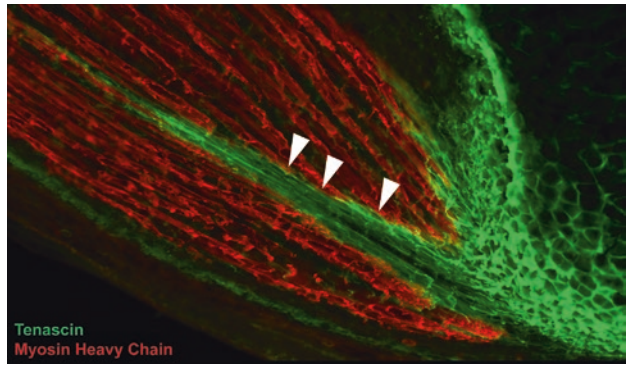


Fig. 9.7 Muscle fibres and tendons. At the end of primary myogenesis (E14.5 in the mouse embryo), muscles have connected to the bone via tendons. Here, a longitudinal section of a forelimb muscle and its tendon are stained by immunohistochemistry with antibodies against tenascin (green) an extracellular matrix component present in tendons and against myosin heavy chain (red) which stains the muscle fibres. Note how the tendon matrix is continuous with the muscle connective tissue (arrowheads)

9.6 Secondary Myogenesis: The Growth of the Embryonic Muscle Pattern

9.6.1 Secondary Myogenesis

During secondary myogenesis, MuSCs which did not differentiate during primary myogenesis proliferate enormously. Some of them become committed myoblasts which differentiate and fuse to form secondary myotubes (Fig. 9.5a) while others stay proliferative. Interestingly, differentiation and fusion of secondary myoblasts start at the innervation point of the primary myotubes, which is located near their centre [63]. Secondary myotubes are initially smaller than primary myotubes and form all around them, using them as a scaffold (Fig. 9.5c). They then extend along the primary myotubes in both directions, to finally run the whole length of the muscle and insert into the tendons [63, 83, 84]. Secondary myotubes are numerous and make most of the adult muscle fibres (Fig. 9.5a, c). Towards the end of foetal development, the formation of new secondary myofibres slows down, as differentiated myoblasts start to preferentially fuse with all existing myofibres and increase their size. This pattern of growth continues after birth.

During secondary myogenesis, the myofibre basement membrane is progressively assembled. At first, this matrix is discontinuous [64] but at the end of foetal development it forms a thin sheet wrapped around the fibre (Fig. 9.8a). This basement membrane interacts with the muscle fibre through an adhesion complex which bridges the actin cytoskeleton of the myofibre with laminin, the major constituent of the basement membrane (Fig. 9.8b). Moreover, MuSCs become wrapped within this basement membrane which, together with the myofibre, constitutes their niche. Mutations in the proteins composing this adhesion complex linking muscle cells to the basement membrane lead to diseases called muscular dystrophies.

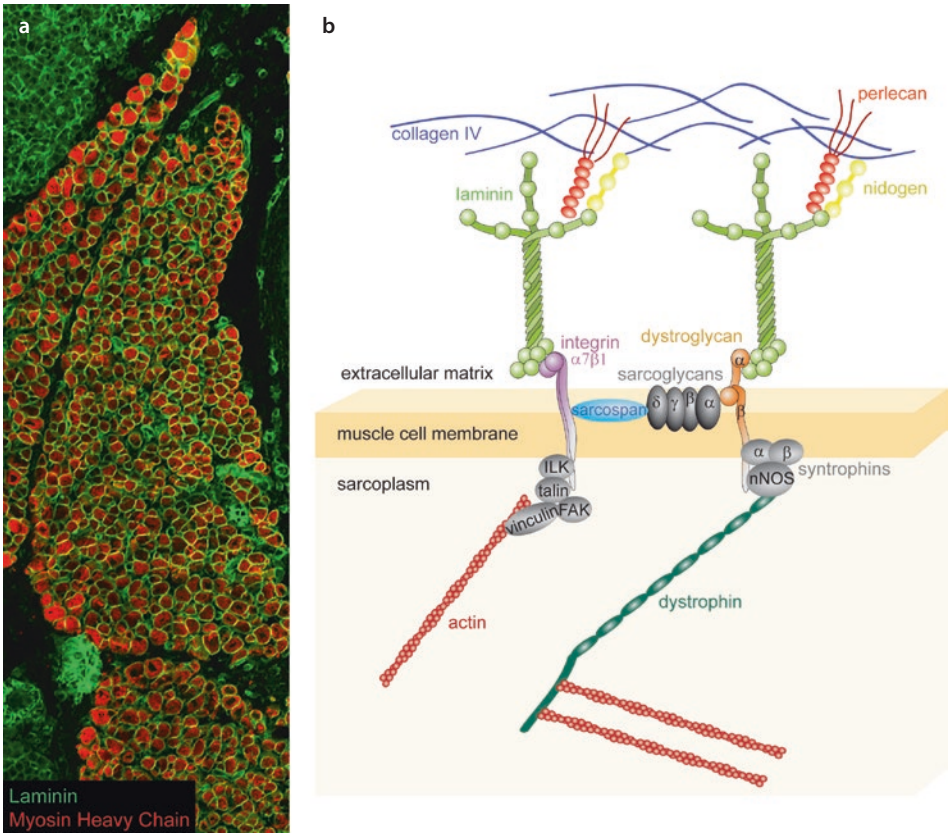


Fig. 9.8 Connection between the muscle fibre cytoskeleton and its basement membrane. **a** Transverse section of an E17.5 mouse foetus showing the deep back muscles stained with an antibody against myosin heavy chain (red) marking the muscle fibres and with an antibody recognising all muscle laminins (green). At this stage, a continuous laminin matrix surrounds each myofibre. **b** Schematic drawing showing the major proteins involved in the connection between the muscle cell cytoskeleton and its surrounding basement membrane [105, 109]. In adult muscle, the predominant laminin isoform is laminin 211

9.6.2 Change in MuSC Identity

As mentioned earlier, the profile of MuSCs producing secondary (or foetal) myoblasts is different from that of the profile of MuSCs producing primary (or embryonic) myoblasts (Fig. 9.5b). In fact, foetal MuSCs express Pax7 and no longer express Pax3 (Table 9.1) and they also express the transcription factor Nuclear factor one X (Nfix) (Fig. 9.5b), which acts as a switch between embryonic and foetal MuSC identity [60]. In the limb, Pax7-positive, Pax3-negative cells arise later than the earlier Pax3-positive MuSCs [43] and first become detectable near the point where nerves enter the muscle masses [66]. Moreover, denervation affects secondary myogenesis more than primary myogenesis [85]. These two facts taken together raise

the interesting possibility that nerves may be important to convert embryonic MuSCs into foetal MuSCs, which will later originate secondary myofibres.

If embryonic and foetal MuSCs show some differences in their profile, embryonic and foetal myoblasts show drastic differences. They respond differently to hormones and growth factors [86, 87] and their gene expression profile is very different [62]. For example, integrin-α7 and Pax7 are more expressed in foetal myoblasts than in embryonic myoblasts, whereas Pax3 and Paraxis are more expressed in embryonic myoblasts.

9.7 Muscle Development and Regeneration After Birth

9.7.1 Perinatal Muscle Growth

Skeletal muscle development continues after birth with an intense growth of muscle mass for the first 3 weeks in the mouse. Most secondary myofibres form before birth. Thus, muscle growth late in foetal development and early post-natal development primarily involves the proliferation of MuSCs and the differentiation of some of them into myoblasts which fuse with the existing myofibres, increasing their size (designated cell-mediated hypertrophy; [88]). Again, the identity of MuSCs changes from the foetal to the perinatal stage. Foetal MuSCs have a higher resistance to differentiation and a higher self-renewing potential than perinatal MuSCs [2, 89]. This makes sense as one considers that foetal MuSCs not only generate the myoblasts that form all the secondary myofibres within the foetal muscles but at the same time also build up a MuSC population that will support enormous muscle growth perinatally. However, as the environment switches from foetal to perinatal, MuSCs become progressively more prone to differentiation [90]. Perinatal MuSCs are located under the myofibre basement membrane and tend to divide asymmetrically ([88]; see Fig. 9.9a, b). This type of division produces a MuSC and a committed myoblast which differentiates and then fuses with the myofibre, contributing to its growth [88, 91].

9.7.2 The Setting Aside of Satellite Cells

Perinatal MuSC number remains relatively constant from birth until P14, indicating that asymmetric MuSC divisions are the norm in this period [88]. However, between P14 and P21, the number of perinatal MuSCs is progressively reduced, which correlates with slower proliferation rates and, by P21, MuSCs have entered quiescence [88]. The perinatal period thus starts with the growth of myofibres by cell-mediated hypertrophy and ends at P21 after which quiescent adult MuSCs or satellite cells (Table 9.1) have been set aside. Importantly, myofibre growth after P21 does not involve the addition of new cells to the fibre; rather, adult myofibres are thought to grow exclusively by protein synthesis [88, 92]. However, as we will see in the next section, if the muscle is injured, the quiescent satellite cells are activated and, through a process called muscle regeneration, can restore the structure and function of the damaged area.

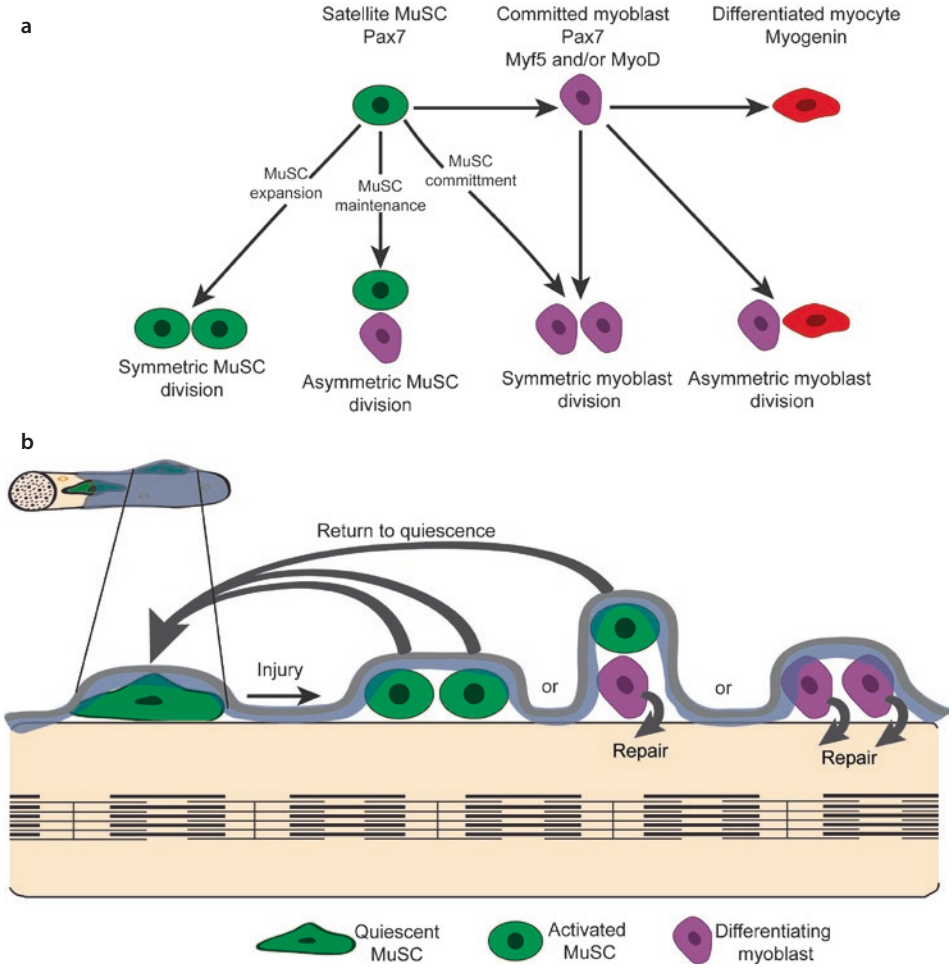


Fig. 9.9 Muscle regeneration. **a** Proposed modes of MuSC division and/or differentiation. MuSC can divide symmetrically into two MuSCs (MuSC expansion), they can divide asymmetrically into one MuSC and one myoblast (MuSC maintenance) or they can commit to myogenesis and divide into two myoblasts (MuSC commitment). MuSCs can also differentiate into myoblasts and give rise to a myocyte without dividing. Myoblasts can divide into either two myoblasts or one myoblast and one myocyte. Myoblasts can also differentiate without dividing. **b** Skeletal muscle is capable of regeneration. When the muscle experiences assault due to exercise, injury or disease, quiescent MuSCs – also called satellite cells – are activated and proliferate. These cells can either divide (1) symmetrically, giving rise to two MuSCs, which in turn can proliferate more, (2) asymmetrically, originating one MuSC and one committed myoblast, or (3) they initiate the differentiation programme and then divide symmetrically into two committed myoblasts. Committed myoblasts either differentiate without dividing or undergo a limited number of divisions before they undergo terminal differentiation and fuse with the existing myofibres. Importantly, MuSCs, which stay undifferentiated during the regeneration process, can return to a quiescent state, thus maintaining the pool of quiescent MuSCs ready for the next assault on the muscle

9.7.3 Skeletal Muscle Regeneration

Adult skeletal muscle has a remarkable ability to regenerate, even in mammals, and this property is due to the setting aside of quiescent adult MuSCs (the so-called satellite cells) during development. Quiescent satellite cells maintain a low metabolic state, are resistant to DNA damage and can retain their stem cell properties for a lifetime [93]. Upon injury, the damaged skeletal muscle releases factors, such as fibroblast and insulin-like growth factors, which induce satellite cell proliferation, while matrix metalloproteases degrade the extracellular matrix, releasing the satellite cells as well as extracellular matrix-bound mitotic factors [91, 93]. These activated adult MuSCs then rapidly migrate bidirectionally along the muscle basement membrane of the damaged fibres (designated 'ghost fibres'; [94]). There, they divide symmetrically (◘ Fig. 9.9a, b) generating cells which become evenly distributed along the longitudinal axis of the ghost fibres [94]. Signals involving fibronectin - a component of the interstitial matrix which gets exposed upon injury - and Wnt7a promote these symmetric cell divisions that expand the MuSC pool [95, 96]. After an initial boost of self-renewing symmetric cell divisions, MuSCs start undergoing asymmetric divisions, where one cell stays in contact with the basement membrane and remains a MuSC, while the other upregulates MyoD and gets committed to myogenesis (◘ Fig. 9.9a, b). These asymmetric divisions involve mechanisms that enhance the segregation of the two cell types. For example, the committed myoblast expresses Delta, which binds to Notch receptors on the MuSC, reinforcing its stem cell identity [97]. It is unclear what leads to this switch in types of divisions. Probably many factors play a role in regulating the balance between symmetric and asymmetric divisions. One such factor may be the size of the injury. A small injury may not 'need' a symmetric expansion of MuSCs, since asymmetric divisions may suffice to repair the damaged myofibre and replenish the MuSC pool, while a large injury may 'require' expansive MuSC divisions before myoblast differentiation sets in. Another alternative is that the MuSC pool that gets activated upon injury is heterogeneous and different subsets of MuSCs undergo symmetric versus asymmetric divisions. If this is the case, different signals would stimulate these two subtypes differently. It is known that adult MuSCs are heterogeneous [3, 90, 98], but it is presently not clear how this heterogeneity plays out in an injury setting.

Other modes of MuSC divisions have also been proposed [91, 98]. MuSC may start differentiating before dividing and thus give rise to two committed myoblasts (◘ Fig. 9.9a, b), a situation which leads to a net loss of MuSCs. Furthermore, one of the committed myoblasts may differentiate faster than the other one, and thus not have time to divide, leading to fewer numbers of fusion-competent myoblasts available for muscle repair (◘ Fig. 9.9a). These two latter modes of MuSC divisions appear to occur more often in aged skeletal muscles, possibly reflecting age-related changes in the MuSCs themselves and/or of the surrounding environment that stops being able to support the types of divisions that maintain the MuSC pool [91, 99].

After muscle regeneration has been completed in a healthy muscle, some MuSCs return to quiescence, thus replenishing the pool. Several mechanisms are involved in promoting this transition. For example, Delta on the muscle fibre binds to Notch receptors on the MuSCs and Notch signalling increases Pax7 expression, promoting

the quiescent state [100–102]. These MuSCs entering quiescence also express Sprouty which counteracts Fgfs [103]. Interestingly, Notch signalling has recently been shown to also activate collagen V production by quiescent MuSCs which in turn binds to the calcitonin receptor on these cells, generating an autocrine loop that further reinforces the quiescent state [104].

9.8 How Does the Study of Muscle Stem Cells in Development Contribute to the Study of Skeletal Muscle Diseases?

Muscular dystrophies are inherited diseases that lead to muscle weakness and tissue degeneration [105, 106]. There are more than 30 different muscular dystrophies and they vary in severity. However, several muscular dystrophies are devastating diseases because of the tissue degeneration involved. Patients growing with these dystrophies gradually lose the ability to walk, will have difficulty breathing and eating, which may eventually lead to premature death [105, 106]. Many (but not all) of the muscular dystrophies are due to mutations in the proteins that connect the actin cytoskeleton of muscle fibres (and its associated MuSCs) to the extracellular matrix [107]. For example, to name only a few, mutations in the intracellular protein dystrophin (◘ Fig. 9.8b) lead to either Duchenne muscular dystrophy (DMD) or the milder Becker's muscular dystrophy (BMD). Mutations in the transmembrane sarcoglycans, which bind to dystroglycan (◘ Fig. 9.8b), or in enzymes which regulate the glycosylation of α-dystroglycan, lead to certain types of limb–girdle muscular dystrophies (LGMD), affecting primarily the pelvic and shoulder girdles [108]. Finally, mutations in the laminin α2 chain of laminin 211 (◘ Fig. 9.8b) lead to Merosin-deficient congenital muscular dystrophy 1A (MDC1A), which is characterized by severe muscle weakness from birth and usually leads to premature death [109].

There are presently no specific treatments for muscular dystrophies and therapy is limited to palliative care. Given the debilitating nature of these diseases, there is an urgent need to find ways to improve muscle function and halt tissue degeneration. A huge effort has gone into addressing how the proteins mutated in muscular dystrophies contribute to normal skeletal muscle development and function. One important conclusion from this work is that these so-called 'structural' proteins not only have structural functions but are also members of integrated signalling networks [105]. Another important conclusion is that several muscular dystrophies lead to changes in the pool of MuSCs. There is evidence for defects in the development of MuSCs in mouse models of DMD and MDC1A [64, 110], as well as perturbations in the regenerative response of these cells upon muscle injury [111, 112].

The study of normal skeletal muscle development and regeneration is important to be able to design therapies that address the underlying processes that go wrong in muscle diseases. The better we understand how the communication between cells occurs, what signalling pathways are used and how MuSCs adapt their response to the need of the tissue at each point of development and regeneration, the more likely we are to be able to design strategies to improve muscle function in a disease setting. This applies not only to the muscular dystrophies discussed

Take-Home Message

- MuSCs arise early in development, generate all the myonuclei of skeletal muscles while simultaneously maintaining a MuSC population which enters quiescence in adult muscles.
  - MuSCs change over developmental time, acquiring characteristics which are appropriate for each stage of myogenesis, but how these changes are regulated is not well understood.
  - Myogenesis is triggered by extrinsic factors, which act on MuSCs and activate transcription factors, the myogenic regulatory factors (the MRFs), leading to myoblast differentiation.
  - Differentiated myocytes fuse to generate multinucleated myotubes which mature into muscle fibres.
- The construction of skeletal muscle occurs in different phases over time, each phase playing a specific role in the construction of the tissue:
  - Primary myogenesis generates the muscle pattern, i.e. 'miniature muscles' connected via muscle connective tissue including tendons to the skeleton and innervated by the peripheral nervous system.
  - Secondary myogenesis builds on the pattern generated through primary myogenesis, leading to a several-fold increase in the number of muscle fibres within each muscle.
  - During perinatal myogenesis, muscle fibres increase in size through the differentiation of myoblasts that fuse with the existing fibres.
  - At the end of perinatal myogenesis, MuSCs enter quiescence and are called satellite cells.
- Skeletal muscle has an amazingly high regenerative potential, which is due to the presence of adult, quiescent MuSCs, which activate, proliferate and repair the tissue upon injury.
- Skeletal muscle diseases, such as mutations in the linkage between the muscle cell cytoskeleton and the surrounding extracellular matrix, lead to muscle weakness and can be extremely debilitating.
- Increasing our knowledge on how MuSCs interact with their complex environment to construct a healthy tissue and successfully repair it will hopefully bring us closer to efficient treatments of muscle diseases.

briefly above, but also to other disease states that impact muscle function, such as cancer-induced cachexia and age-related sarcopenia.

Acknowledgements We thank the members of our group, particularly Gabriela Rodrigues, Luís Marques and Inês Antunes, for their contributions to this chapter, and multiple generations of students of the MSc in Evolutionary and Developmental Biology (► http://bed.campus.ciencias.ulisboa.pt/) for their interest in this topic. The MF20 and Pax3 antibodies were developed by DA Fischman and CP Ordahl, respectively, and were obtained from the Developmental Studies Hybridoma Bank, developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biology, Iowa City, IA52242, USA. The original data shown in figures in this chapter were obtained within projects financed by Fundação para a Ciência e a Tecnologia (FCT), Portugal (PTDC/SAU-BID/120130/2010) and Association Française

contre les Myopathies (AFM) – Téléthon, France (project n° 19959). MD and ABG were supported by fellowships SFRH/BPD/65370/2009 and SFRH/BD/90827/2012 from FCT. ABG is an alumnus of the MSc in Evolutionary and Developmental Biology.

References

1. Mauro A. Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol. 1961;9:493–5.
2. Biressi S, Molinaro M, Cossu G. Cellular heterogeneity during vertebrate skeletal muscle development. Dev Biol. 2007;308(2):281–93.
3. Tajbakhsh S. Skeletal muscle stem cells in developmental versus regenerative myogenesis. J Intern Med. 2009;266(4):372–89.
4. Relaix F, Marcelle C. Muscle stem cells. Curr Opin Cell Biol. 2009;21(6):748–53.
5. Hollway G, Currie P. Vertebrate myotome development. Birth Defects Res C Embryo Today. 2005;75(3):172–9.
6. Emerson CP Jr. Embryonic signals for skeletal myogenesis: arriving at the beginning. Curr Opin Cell Biol. 1993;5(6):1057–64.
7. Pownall ME, Emerson CP Jr. Sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos. Dev Biol. 1992;151(1):67–79.
8. Ordahl CP, Le Douarin NM. Two myogenic lineages within the developing somite. Development. 1992;114:339–53.
9. Christ B, Ordahl CP. Early stages of chick somite development. Anat Embryol (Berl). 1995;191(5):381–96.
10. Monsoro-Burq AH. Sclerotome development and morphogenesis: when experimental embryology meets genetics. Int J Dev Biol. 2005;49(2–3):301–8.
11. Brent AE, Schweitzer R, Tabin CJ. A somitic compartment of tendon progenitors. Cell. 2003;113(2):235–48.
12. Buckingham M. Myogenic progenitor cells and skeletal myogenesis in vertebrates. Curr Opin Genet Dev. 2006;16(5):525–32.
13. Christ B, Huang R, Scaal M. Amniote somite derivatives. Dev Dyn. 2007;236(9):2382–96.
14. Deries M, Thorsteinsdóttir S. Axial and limb muscle development: dialogue with the neighbourhood. Cell Mol Life Sci. 2016;73(23):4415–31.
15. Thorsteinsdóttir S, Deries M, Cachaço AS, Bajanca F. The extracellular matrix dimension of skeletal muscle development. Dev Biol. 2011;354(2):191–207.
16. Ben-Yair R, Kalcheim C. Lineage analysis of the avian dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. Development. 2005;132(4):689–701.
17. Gros J, Manceau M, Thomé V, Marcelle C. A common somitic origin for embryonic muscle progenitors and satellite cells. Nature. 2005;435(7044):954–8.
18. Kassar-Duchossoy L, Giacone E, Gayraud-Morel B, Jory A, Gomes D, Tajbakhsh S. Pax3/Pax7 mark a novel population of primitive myogenic cells during development. Genes Dev. 2005;19(12):1426–31.
19. Relaix F, Rocancourt D, Mansouri A, Buckingham M. A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature. 2005;435(7044):948–53.
20. Bajanca F, Luz M, Raymond K, Martins GG, Sonnenberg A, Tajbakhsh S, et al. Integrin α6β1-laminin interactions regulate early myotome formation in the mouse embryo. Development. 2006;133(9):1635–44.
21. Buckingham M. How the community effect orchestrates muscle differentiation. BioEssays. 2003;25(1):13–6.
22. Babiuk RP, Zhang W, Clugston R, Allan DW, Greer JJ. Embryological origins and development of the rat diaphragm. J Comp Neurol. 2003;455(4):477–87.
23. Sambasivan R, Kuratani S, Tajbakhsh S. An eye on the head: the development and evolution of craniofacial muscles. Development. 2011;138(12):2401–15.
24. Munsterberg AE, Lassar AB. Combinatorial signals from the neural tube, floor plate and notochord induce myogenic bHLH gene expression in the somite. Development. 1995;121(3):651–60.
25. Chang CN, Kioussi C. Location, location, location: signals in muscle specification. J Dev Biol. 2018;18:6(2).

26. Rios AC, Serralbo O, Salgado D, Marcelle C. Neural crest regulates myogenesis through the transient activation of NOTCH. Nature. 2011;473(7348):532–5.
27. Sieiro D, Rios AC, Hirst CE, Marcelle C. Cytoplasmic NOTCH and membrane-derived beta-catenin link cell fate choice to epithelial-mesenchymal transition during myogenesis. elife. 2016;24:5.
28. Serralbo O, Marcelle C. Migrating cells mediate long-range WNT signaling. Development. 2014;141(10):2057–63.
29. Borello U, Berarducci B, Murphy P, Bajard L, Buffa V, Piccolo S, et al. The Wnt/β-catenin pathway regulates Gli-mediated Myf5 expression during somitogenesis. Development. 2006;133(18):3723–32.
30. Borycki AG, Brunk B, Tajbakhsh S, Buckingham M, Chiang C, Emerson CP Jr. Sonic hedgehog controls epaxial muscle determination through Myf5 activation. Development. 1999;126(18):4053–63.
31. Gustafsson MK, Pan H, Pinney DF, Liu Y, Lewandowski A, Epstein DJ, et al. Myf5 is a direct target of long-range Shh signaling and Gli regulation for muscle specification. Genes Dev. 2002;16(1):114–26.
32. Marcelle C, Stark MR, Bronner-Fraser M. Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite. Development. 1997;124(20):3955–63.
33. Kablar B, Rudnicki MA. Skeletal muscle development in the mouse embryo. Histol Histopathol. 2000;15(2):649–56.
34. Venters SJ, Thorsteinsdóttir S, Duxson MJ. Early development of the myotome in the mouse. Dev Dyn. 1999;216(3):219–32.
35. Gros J, Scaal M, Marcelle C. A two-step mechanism for myotome formation in chick. Dev Cell. 2004;6(6):875–82.
36. Kalcheim C, Ben-Yair R. Cell rearrangements during development of the somite and its derivatives. Curr Opin Genet Dev. 2005;15(4):371–80.
37. deLapeyrière O, Ollendorff V, Planche J, Ott MO, Pizette S, Coulier F, et al. Expression of the Fgf6 Gene is restricted to developing skeletal muscle in the mouse embryo. Development. 1993;118(2):601–11.
38. Han JK, Martin GR. Embryonic expression of Fgf-6 is restricted to the skeletal muscle lineage. Dev Biol. 1993;158(2):549–54.
39. Brent AE, Braun T, Tabin CJ. Genetic analysis of interactions between the somitic muscle, cartilage and tendon cell lineages during mouse development. Development. 2005;132(3):515–28.
40. Vinagre T, Moncaut N, Carapuco M, Novoa A, Bom J, Mallo M. Evidence for a myotomal Hox/Myf cascade governing nonautonomous control of rib specification within global vertebral domains. Dev Cell. 2010;18(4):655–61.
41. Delfini MC, De La Celle M, Gros J, Serralbo O, Marics I, Seux M, et al. The timing of emergence of muscle progenitors is controlled by an FGF/ERK/SNAIL1 pathway. Dev Biol. 2009;333(2):229–37.
42. Francis-West PH, Antoni L, Anakwe K. Regulation of myogenic differentiation in the developing limb bud. J Anat. 2003;202(1):69–81.
43. Lee AS, Harris J, Bate M, Vijayraghavan K, Fisher L, Tajbakhsh S, et al. Initiation of primary myogenesis in amniote limb muscles. Dev Dyn. 2013;242(9):1043–55.
44. Deries M, Collins JJ, Duxson MJ. The mammalian myotome: a muscle with no innervation. Evol Dev. 2008;10(6):746–55.
45. Tajbakhsh S, Rocancourt D, Cossu G, Buckingham M. Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. Cell. 1997;89(1):127–38.
46. Nogueira JM, Hawrot K, Sharpe C, Noble A, Wood WM, Jorge EC, et al. The emergence of Pax7-expressing muscle stem cells during vertebrate head muscle development. Front Aging Neurosci. 2015;7:62.
47. Kelly AM, Zacks SI. The histogenesis of rat intercostal muscle. J Cell Biol. 1969;42:135–53.
48. Ross JJ, Duxson MJ, Harris AJ. Formation of primary and secondary myotubes in rat lumbrical muscles. Development. 1987;100(3):383–94.
49. Ontell M, Hughes D, Bourke D. Secondary myogenesis or normal muscle produces abnormal myotubes. Anat Rec. 1982;204:199–207.
50. Mourikis P, Sambasivan R, Castel D, Rocheteau P, Bizzarro V, Tajbakhsh S. A critical requirement for notch signaling in maintenance of the quiescent skeletal muscle stem cell state. Stem Cells. 2012;30(2):243–52.

51. Vasyutina E, Lenhard DC, Birchmeier C. Notch function in myogenesis. Cell Cycle. 2007;6(12):1451–4.
52. Hirsinger E, Malapert P, Dubrulle J, Delfini MC, Duprez D, Henrique D, et al. Notch signalling acts in postmitotic avian myogenic cells to control MyoD activation. Development. 2001;128(1):107–16.
53. Delfini MC, Hirsinger E, Pourquié O, Duprez D. Delta 1-activated notch inhibits muscle differentiation without affecting Myf5 and Pax3 expression in chick limb myogenesis. Development. 2000; 127(23):5213–24.
54. Schuster-Gossler K, Cordes R, Gossler A. Premature myogenic differentiation and depletion of progenitor cells cause severe muscle hypotrophy in Delta1 mutants. Proc Natl Acad Sci U S A. 2007;104(2):537–42.
55. Beckers J, Clark A, Wunsch K, Hrabe De Angelis M, Gossler A. Expression of the mouse Delta1 gene during organogenesis and fetal development. Mech Dev. 1999;84(1–2):165–8.
56. Mourikis P, Gopalakrishnan S, Sambasivan R, Tajbakhsh S. Cell-autonomous Notch activity maintains the temporal specification potential of skeletal muscle stem cells. Development. 2012;139(24): 4536–48.
57. Deries M, Gonçalves AB, Vaz R, Martins GG, Rodrigues G, Thorsteinsdóttir S. Extracellular matrix remodeling accompanies axial muscle development and morphogenesis in the mouse. Dev Dyn. 2012;241(2):350–64.
58. Deries M, Schweitzer R, Duxson MJ. Developmental fate of the mammalian myotome. Dev Dyn. 2010;239(11):2898–910.
59. Goulding M, Lumsden A, Paquette AJ. Regulation of Pax-3 Expression in the dermomyotome and its role in muscle development. Development. 1994;120(4):957–71.
60. Messina G, Biressi S, Monteverde S, Magli A, Cassano M, Perani L, et al. Nfix regulates fetal-specific transcription in developing skeletal muscle. Cell. 2010;140(4):554–66.
61. Hutcheson DA, Zhao J, Merrell A, Haldar M, Kardon G. Embryonic and fetal limb myogenic cells are derived from developmentally distinct progenitors and have different requirements for beta-catenin. Genes Dev. 2009;23(8):997–1013.
62. Biressi S, Tagliafico E, Lamorte G, Monteverde S, Tenedini E, Roncaglia E, et al. Intrinsic phenotypic diversity of embryonic and fetal myoblasts is revealed by genome-wide gene expression analysis on purified cells. Dev Biol. 2007;304(2):633–51.
63. Duxson MJ, Usson Y. Cellular insertion of primary and secondary myotubes in embryonic rat muscles. Development. 1989;107:243–51.
64. Nunes AM, Wuebbles RD, Sarathy A, Fontelonga TM, Deries M, Burkin DJ, et al. Impaired fetal muscle development and JAK-STAT activation mark disease onset and progression in a mouse model for merosin-deficient congenital muscular dystrophy. Hum Mol Genet. 2017;26(11):2018–33.
65. Cachaço AS, Pereira CS, Pardal RG, Bajanca F, Thorsteinsdóttir S. Integrin repertoire on myogenic cells changes during the course of primary myogenesis in the mouse. Dev Dyn. 2005;232(4):1069–78.
66. Hurren B, Collins JJ, Duxson MJ, Deries M. First neuromuscular contact correlates with onset of primary myogenesis in rat and mouse limb muscles. PLoS One. 2015;10(7):e0133811.
67. Marmigère F, Ernfors P. Specification and connectivity of neuronal subtypes in the sensory lineage. Nat Rev Neurosci. 2007;8(2):114–27.
68. Bonanomi D, Pfaff SL. Motor axon pathfinding. Cold Spring Harb Perspect Biol. 2010;2(3):a001735.
69. Jessen KR, Mirsky R, Lloyd AC. Schwann cells: development and role in nerve repair. Cold Spring Harb Perspect Biol. 2015;7(7):a020487.
70. Weatherbee SD, Anderson KV, Niswander LA. LDL-receptor-related protein 4 is crucial for formation of the neuromuscular junction. Development. 2006;133(24):4993–5000.
71. Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH, et al. Lrp4 is a receptor for Agrin and forms a complex with MuSK. Cell. 2008;135(2):334–42.
72. Burden SJ, Yumoto N, Zhang W. The role of MuSK in synapse formation and neuromuscular disease. Cold Spring Harb Perspect Biol. 2013;5(5):a009167.
73. Bao ZZ, Lakonishok M, Kaufman S, Horwitz AF. α7β1 integrin is a component of the myotendinous junction on skeletal muscle. J Cell Sci. 1993;106(Part 2):579–90.
74. Velling T, Collo G, Sorokin L, Durbeej M, Zhang H, Gullberg D. Distinct α7Aβ1 and α7Bβ1 integrin expression patterns during mouse development: α7A is restricted to skeletal muscle but

α7B is expressed in striated muscle, vasculature, and nervous system. Dev Dyn. 1996;207(4):355–71.
75. van der Flier A, Gaspar AC, Thorsteinsdóttir S, Baudoin C, Groeneveld E, Mummery CL, et al. Spatial and temporal expression of the β1D integrin during mouse development. Dev Dyn. 1997;210(4):472–86.
76. Nawrotzki R, Willem M, Miosge N, Brinkmeier H, Mayer U. Defective integrin switch and matrix composition at alpha 7-deficient myotendinous junctions precede the onset of muscular dystrophy in mice. Hum Mol Genet. 2003;12(5):483–95.
77. Kardon G. Muscle and tendon morphogenesis in the avian hind limb. Development. 1998;125(20):4019–32.
78. Chevallier A, Kieny M. On the role of the connective tissue in the patterning of the chick limb musculature. Wilhelm Roux Arch Dev Biol. 1982;191:277–80.
79. Mathew SJ, Hansen JM, Merrell AJ, Murphy MM, Lawson JA, Hutcheson DA, et al. Connective tissue fibroblasts and Tcf4 regulate myogenesis. Development. 2011;138(2):371–84.
80. Brent AE, Tabin CJ. FGF acts directly on the somitic tendon progenitors through the Ets transcription factors Pea3 and Erm to regulate scleraxis expression. Development. 2004;131(16):3885–96.
81. Edom-Vovard F, Schuler B, Bonnin MA, Teillet MA, Duprez D. Fgf4 positively regulates scleraxis and tenascin expression in chick limb tendons. Dev Biol. 2002;247(2):351–66.
82. Eloy-Trinquet S, Wang H, Edom-Vovard F, Duprez D. Fgf signaling components are associated with muscles and tendons during limb development. Dev Dyn. 2009;238(5):1195–206.
83. Ontell M, Hughes D, Bourke D. Morphometric analysis of the developing mouse soleus muscle. Am J Anat. 1988;181:279–88.
84. Duxson MJ, Usson Y, Harris AJ. The origin of secondary myotubes in mammalian skeletal muscles: ultrastructural studies. Development. 1989;107:743–50.
85. Harris AJ. Embryonic growth and innervation of rat skeletal muscles. I. Neural regulation of muscle fibre numbers. Philos Trans R Soc Lond Ser B Biol Sci. 1981;293(1065):257–77.
86. Cossu G, Ranaldi G, Senni MI, Molinaro M, Vivarelli E. 'Early' mammalian myoblasts are resistant to phorbol ester-induced block of differentiation. Development. 1988;102(1):65–9.
87. Cusella-De Angelis MG, Molinari S, Le Donne A, Coletta M, Vivarelli E, Bouche M, et al. Differential response of embryonic and fetal myoblasts to TGFβ: a possible regulatory mechanism of skeletal muscle histogenesis. Development. 1994;120(4):925–33.
88. White RB, Bierinx AS, Gnocchi VF, Zammit PS. Dynamics of muscle fibre growth during postnatal mouse development. BMC Dev Biol. 2010;10:21.
89. Tierney MT, Gromova A, Sesillo FB, Sala D, Spenle C, Orend G, et al. Autonomous extracellular matrix remodeling controls a progressive adaptation in muscle stem cell regenerative capacity during development. Cell Rep. 2016;14(8):1940–52.
90. Tierney MT, Sacco A. Satellite cell heterogeneity in skeletal muscle homeostasis. Trends Cell Biol. 2016;26(6):434–44.
91. Dumont NA, Bentzinger CF, Sincennes MC, Rudnicki MA. Satellite cells and skeletal muscle regeneration. Compr Physiol. 2015;5(3):1027–59.
92. Ontell M, Kozeka K. Organogenesis of the mouse extensor digitorum logus muscle: a quantitative study. Am J Anat. 1984;171(2):149–61.
93. Wang YX, Dumont NA, Rudnicki MA. Muscle stem cells at a glance. J Cell Sci. 2014;127(21):4543–8.
94. Webster MT, Manor U, Lippincott-Schwartz J, Fan CM. Intravital imaging reveals ghost fibers as architectural units guiding myogenic progenitors during regeneration. Cell Stem Cell. 2016;18(2):243–52.
95. Bentzinger CF, Wang YX, von Maltzahn J, Rudnicki MA. The emerging biology of muscle stem cells: implications for cell-based therapies. BioEssays. 2013;35(3):231–41.
96. Le Grand F, Jones AE, Seale V, Scime A, Rudnicki MA. Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. Cell Stem Cell. 2009;4(6):535–47.
97. Kuang S, Kuroda K, Le Grand F, Rudnicki MA. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell. 2007;129(5):999–1010.
98. Kuang S, Gillespie MA, Rudnicki MA. Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell. 2008;2(1):22–31.
99. Blau HM, Cosgrove BD, Ho AT. The central role of muscle stem cells in regenerative failure with aging. Nat Med. 2015;21(8):854–62.

100. Fukada S, Yamaguchi M, Kokubo H, Ogawa R, Uezumi A, Yoneda T, et al. Hesr1 and Hesr3 are essential to generate undifferentiated quiescent satellite cells and to maintain satellite cell numbers. Development. 2011;138(21):4609–19.
101. Wen Y, Bi P, Liu W, Asakura A, Keller C, Kuang S. Constitutive Notch activation upregulates Pax7 and promotes the self-renewal of skeletal muscle satellite cells. Mol Cell Biol. 2012;32(12):2300–11.
102. Bjornson CR, Cheung TH, Liu L, Tripathi PV, Steeper KM, Rando TA. Notch signaling is necessary to maintain quiescence in adult muscle stem cells. Stem Cells. 2012;30(2):232–42.
103. Shea KL, Xiang W, LaPorta VS, Licht JD, Keller C, Basson MA, et al. Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration. Cell Stem Cell. 2010;6(2):117–29.
104. Baghdadi MB, Castel D, Machado L, Fukada SI, Birk DE, Relaix F, et al. Reciprocal signalling by Notch-Collagen V-CALCR retains muscle stem cells in their niche. Nature. 2018;557(7707):714–8.
105. Davies KE, Nowak KJ. Molecular mechanisms of muscular dystrophies: old and new players. Nat Rev Mol Cell Biol. 2006;7(10):762–73.
106. Mendell JR, Clark KR. Challenges for gene therapy for muscular dystrophy. Curr Neurol Neurosci Rep. 2006;6(1):47–56.
107. Van Ry PM, Fontelonga TM, Barraza-Flores P, Sarathy A, Nunes AM, Burkin DJ. ECM-related myopathies and muscular dystrophies: pros and cons of protein therapies. Compr Physiol. 2017;7(4):1519–36.
108. Laval SH, Bushby KM. Limb-girdle muscular dystrophies--from genetics to molecular pathology. Neuropathol Appl Neurobiol. 2004;30(2):91–105.
109. Gawlik KI, Durbeej M. Skeletal muscle laminin and MDC1A: pathogenesis and treatment strategies. Skelet Muscle. 2011;1(1):9.
110. Merrick D, Stadler LK, Larner D, Smith J. Muscular dystrophy begins early in embryonic development deriving from stem cell loss and disrupted skeletal muscle formation. Dis Model Mech. 2009;2(7–8):374–88.
111. Dumont NA, Wang YX, von Maltzahn J, Pasut A, Bentzinger CF, Brun CE, et al. Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. Nat Med. 2015;21(12):1455–63.
112. Van Ry PM, Minogue P, Hodges BL, Burkin DJ. Laminin-111 improves muscle repair in a mouse model of merosin-deficient congenital muscular dystrophy. Hum Mol Genet. 2014;23(2):383–96.