Master regulators of skeletal muscle lineage development and pluripotent stem cells differentiation

Joana Esteves de Lima and Frédéric Relaix*

Abstract
In vertebrates, the skeletal muscles of the body and their associated stem cells originate from muscle progenitor cells, during development. The specification of the muscles of the trunk, head and limbs, relies on the activity of distinct genetic hierarchies. The major regulators of trunk and limb muscle specification are the paired-homeobox transcription factors PAX3 and PAX7. Distinct gene regulatory networks drive the formation of the different muscles of the head. Despite the redeployment of diverse upstream regulators of muscle progenitor differentiation, the commitment towards the myogenic fate requires the expression of the early myogenic regulatory factors MYF5, MRF4, MYOD and the late differentiation marker MYOG. The expression of these genes is activated by muscle progenitors throughout development, in several waves of myogenic differentiation, constituting the embryonic, fetal and postnatal phases of muscle growth. In order to achieve myogenic cell commitment while maintaining an undifferentiated pool of muscle progenitors, several signaling pathways regulate the switch between proliferation and differentiation of myoblasts. The identification of the gene regulatory networks operating during myogenesis is crucial for the development of in vitro protocols to differentiate pluripotent stem cells into myoblasts required for regenerative medicine.

Keywords: Myogenesis, Muscle progenitor, PAX3, PAX7, MRF, MYF5, MYOD, MYOG, hiPSC, ESC

Background
Skeletal muscles constitute the most abundant tissue in the vertebrate body. The voluntary contraction of the muscles requires electrical stimuli provided by motoneurons to the fibers. Then, the mechanical forces generated by muscle fiber contraction are transmitted to the bones (or connective tissues) by the tendons. The contractibility of the fibers relies on the structural organization of the actin and myosin proteins that constitute the sarcomeres.

In tetrapods, skeletal muscles from the trunk and limbs derive from the somites, while head muscles originate from the cranial and prechordal mesoderm. During development, paraxial mesoderm in the trunk undergoes segmentation, forming the somites, which are transient mesodermal structures that subsequently mature into distinct layers, the dermomyotome, the myotome, the syndetome and the sclerotome (Fig. 1A) (Buckingham and Rigby, 2014). Dorsally, the dermomyotome gives rise to the muscles of the trunk and limbs, the dermis of the back, and contributes to the formation of vascular and brown adipocyte tissue cells. Ventrally, the syndetome and the sclerotome form the tendons and bones of the trunk, respectively. The cells from the epaxial (medial) and hypaxial (lateral) edges of the dermomyotome undergo epithelial-to-mesenchymal transition (EMT) and delaminate, migrate ventrally and give rise to the myotome, where the first myoblast fusion events take place (Fig. 1A) (Buckingham and Relaix, 2007). In a second wave of colonization of the myotome, the progenitor cells present in the central dermomyotome delaminate and migrate ventrally, intercalating within the primary fibers of the myotome. Within the myotome, these cells...
can either differentiate into myoblasts or self-renew, maintaining an undifferentiated progenitor pool. Subsequently, the myotome expands ventrally, and complex patterning events will give rise to trunk muscles. Laterally, the delaminating cells migrate to distant locations and provide the muscles of the limbs, diaphragm and the hypoglossal cord (Buckingham and Rigby, 2014). The skeletal muscles of the head derive mostly from the cranial mesoderm, while the extraocular muscles originate from the most anterior mesoderm, the prechordal mesoderm (Comai et al., 2020; Noden and Francis-West, 2006). The formation of most of the head muscles relies on the branchial arches which, like the somites, are transient mesodermal structures that align ventrally, along the pharynx. Although the master regulators of skeletal muscle commitment are shared between head and trunk muscles, muscle specification in these regions relies on distinct upstream transcription factors.

**Main Text**

**PAX transcription factors and myogenic specification**

Skeletal muscle development of the trunk and limbs relies on the presence of progenitor cells that express the paired-homeobox transcription factors PAX3 and PAX7. These genes are major upstream regulators of myogenesis but their expression is not muscle-specific, being also present in neural crest cells and neuro-ectoderm, in the dorsal part of the neural tube and in the central nervous system, where they play essential roles in tissue specification and organ development (Buckingham and Relaix, 2007). PAX3 is expressed in the paraxial mesoderm and in the early epithelial somite but its expression becomes restricted to the dermomyotome in the mature somite. The expression of PAX7 however, is induced later in the central dermomyotome, where it co-localizes with PAX3. Studies performed in chick and mouse embryos identified this group of cells co-expressing PAX3 and PAX7 and demonstrated that it contributes to the formation of all the muscles of the trunk and limbs and to the associated muscle stem cells (satellite cells) (Gros et al., 2005; Kassar-Duchossoy, 2005; Relaix et al., 2005; Schienda et al., 2006). PAX3 and PAX7 share conserved DNA binding domains, and display partial functional overlap in myogenesis (Relaix et al., 2004, 2006). In Pax3-deficient embryos, trunk muscle formation is impaired, cells of the hypaxial domain of the dermomyotome undergo cell death and consequently limb muscles and other muscles of migratory origin are absent (Table 1) (Bober et al., 1994; Epstein et al., 1996; Goulding et al., 1994; Relaix et al., 2003; Tremblay et al., 1998). The severe phenotype associated with the hypaxial dermomyotome in Pax3-mutant embryos is consistent with higher PAX3 expression in the hypaxial domain (Kassar-Duchossoy, 2005; Relaix et al., 2005). In contrast, deleting PAX7 does not affect embryonic development (Table 1) (Mansouri et al., 1996; Relaix et al., 2006; Seale et al., 2000). However, Pax7-deficient mice progressively lose the satellite cell pool, which compromises muscle homeostasis and regeneration (Table 1). This is consistent with PAX7 being expressed in all adult muscle stem cells and required for their self-renewal, survival, propagation and maintenance (Addicks et al., 2019; Kuang et al., 2006; Lepper et al., 2009; Oustanina et al., 2004; Relaix et al., 2006; Seale et al., 2000; Sincennes et al., 2021). Furthermore, mouse embryos lacking PAX3 and PAX7 display a complete loss of trunk and limb muscles with only the primary myotome being formed (Table 1).
Therefore, PAX3 and PAX7 cannot compensate for one another during development or in satellite cell maintenance, which could be associated with the presence of distinct downstream gene regulatory networks.

Until recently, very few PAX3/PAX7 targets have been identified (Bajard et al., 2006; Epstein et al., 1996; Esteves de Lima et al., 2021a; Lagha et al., 2008; Sato et al., 2010). A chromatin immunoprecipitation followed by sequencing (ChIP-seq) performed in myoblasts ectopically expressing a tagged version of PAX3 and PAX7 identified a large set of target genes (Soleimani et al., 2012). Moreover, this analysis highlighted that in adult myoblasts, PAX7 shares similar binding sites as those of PAX3 but also recognizes newly identified regions in the loci of myogenic genes (Soleimani et al., 2012). In addition, while PAX3 displays more affinity for paired-box motifs on DNA, PAX7 mainly binds homeobox sites, which may contribute to the functional differences observed for each of these genes (Soleimani et al., 2012). A specific antibody recognizing the fusion protein PAX3-FOXO1 was used to identify PAX3-FOXO1 direct target genes (Cao et al., 2010). The PAX3-FOXO1 fusion protein contains the PAX3 DNA binding domains and the FOXO1 transactivation domain contributing to the majority of rhabdomyosarcoma cases via impairment of myogenic differentiation (Calhabeu et al., 2013). The vast majority of PAX3-FOXO1 binding sites were identified in introns and in distal intergenic regions conserved in vertebrates (Cao et al., 2010). In addition, a binding motif analysis revealed that there is a selective enrichment of PAX3-FOXO1 in genomic regions containing PAX3 and E-box motifs (Cao et al., 2010).

A list of the phenotypes associated with PAX3, PAX7 and MRF mutations is provided in Table 1.

### Table 1

| Mutated genes | Phenotype | Lethality | References |
|---------------|-----------|-----------|------------|
| Pax3          | Disorganized myotome | Embryonic lethal (E15.5-E18.5) | (Bober et al., 1994; Goulding et al., 1994; Epstein et al., 1996; Tremblay et al., 1998; Relaix et al., 2003) |
|               | Impaired trunk myogenesis |  | |
|               | No migratory muscles formed |  | |
| Pax7          | Normal embryogenesis | Postnatal lethal (2 to 3 weeks after birth) | (Mansouri et al., 1996; Seale et al., 2000; Oustanina et al., 2004; Kuang et al., 2006; Relaix et al., 2006) |
|               | Reduced muscle masses |  | |
|               | Satellite cell loss after birth |  | |
| Pax3:Pax7     | No trunk muscles | Embryonic lethal | (Relaix et al., 2005) |
|               | Normal embryogenesis |  | |
| Pax3:Myf5     | No trunk muscles | Embryonic lethal | (Tajbakhsh et al., 1997) |
|               | No muscles of migratory origin |  | |
| Myf5          | Delayed myotome formation | Postnatal lethal (abnormal rib formation) | (Braun et al., 1992; Braun et al., 1994; Kablar et al., 1997; Tajbakhsh et al., 1998) |
|               | Delayed epaxial development |  | |
|               | Normal musculature |  | |
| Mrf4          | Upregulation of Myog expression | Postnatal lethal (abnormal rib formation) | (Zhang et al., 1995; Patapoutian et al., 1995) |
|               | Normal musculature |  | |
| Myod          | Upregulation of Myf5 expression | Viable and fertile | (Rudnicki et al., 1992; Kablar et al., 1997) |
|               | Delayed hypaxial development |  | |
|               | Normal musculature |  | |
| Myog          | No myoblast differentiation | Postnatal lethal | (Hasty et al., 1993; Nabeshima et al., 1993) |
|               | Severely reduced musculature |  | |
| Myod:Myf5:Mrf4| No embryonic myogenesis | Postnatal lethal | (Rudnicki et al., 1993) |
|               | Lack of trunk and limb muscles |  | |
| Myf5:Myod     | MRF4-dependent embryonic myogenesis only | Postnatal lethal | (Kassar-Duchossoy et al., 2004) |
|               | Defective fetal myogenesis |  | |
|               | Lack of trunk and limb muscles |  | |
While the muscles of the trunk and limbs rely on PAX3 and PAX7 for skeletal muscle lineage specification, Pax3 and Pax7 are not expressed in the developing head muscles and in the Pax3:\Myf5 double mutant embryos the head muscles are spared (Tajbakhsh et al., 1997). The specification of skeletal muscle in the head relies on distinct genetic hierarchies, including the transcription factors Capsulin and MYOR, the T-box transcription factor 1 (TBX1) and the paired-like homeodomain transcription factor 2 (PITX2), that act genetically upstream of Myf5, Mrf4 and Myod expression in separate head muscle groups. Mutations in any of these genes leads to distinct head muscle defects (Dong et al., 2006; Kelly et al., 2004; Lu et al., 2002, 1999; Noden and Francis-West, 2006).

Master regulators of skeletal muscle determination

Distinct gene regulatory networks operate in different body regions (head, trunk, limbs) to activate the expression of the Myogenic Regulatory Factors (MRF) in muscle progenitors. The MRFs are a family of basic-Helix-Loop-Helix (bHLH) transcription factors that recognize the E-box consensus motif CANNTG in DNA. In order to bind DNA, MRF proteins need to heterodimerize with the ubiquitous bHLH E-proteins (Lassar et al., 1991). Expression of the MRF family of transcription factors is sufficient to drive a myogenic fate and the family includes MYF5, MYOD, MRF4 and MYOG. The first identified MRF was MYOD, which was able to convert fibroblasts into myoblasts, a landmark discovery for the understanding of myogenic determination while MYOD is a terminal differentiation driver of myogenesis (Hasty et al., 1993; Nabeshima et al., 1993). Different MRFs contain similar and conserved protein domains and show some degree of functional overlap. The constitutive ablation of Myod in mouse embryos does not lead to major muscle defects and Myf5 expression is maintained and/or upregulated, indicating a compensatory mechanism (Table 1) (Kablar et al., 1997; Rudnicki et al., 1992). Conversely, in Myf5-null embryos skeletal muscle develops normally despite delayed Myod expression and primary myotome and epaxial-derived muscle formation (Table 1) (Braun et al., 1992, 1994; Kablar et al., 1997). The delayed expression of Myod in Myf5 mutants indicate that MYOD have a more specific role in governing myogenesis epaxially. Analysis of the Pax3:Myf5 double mutant embryos placed Pax3 and Myf5 hierarchically upstream of MYOD, since in these compound mutant embryos no trunk muscles are formed (Table 1) (Tajbakhsh et al., 1997). However, it remains unclear if this phenotype is linked to direct genetic interactions and/or combined specific cellular defects of Pax3- (loss of hypaxial and impaired epaxial dermomyotome) and Myf5-mutants (delayed myotome formation with progenitor cells stalled at the edges of the dermomyotome). Regarding MRFs compensation, combined ablations usually lead to severe skeletal muscle development impairment (Table 1), such as in the double knockout Myf5:Myod mutant (Kassar-Duchossoy et al., 2004) or when Myod;Myf5(Mrf4) are deleted (Rudnicki et al., 1993). Conversely, early myogenic specification and determination is not affected in Myog-null embryos, but differentiation of the myoblasts and myofiber formation is abolished in vivo (Table 1) (Hasty et al., 1993; Nabeshima et al., 1993).

The first cells to express MRFs are located at the epaxial lip of the dermomyotome that activate Myf5 when exposed to WNT signals from the neural tube and ectoderm, and SHH signals from the notochord (Fig. 1B) (Buckingham and Rigby, 2014; Ott et al., 1991; Tajbakhsh et al., 1998). The effector proteins of the WNT and SHH signaling pathways, GLI and TCF, respectively, recognize their binding sites on the early epaxial enhancer (EEE) of Myf5 (~5.5 kb), activating its expression in these cells (Borello et al., 2006; Gustafsson et al., 2002). PAX3 also regulates Myf5 expression epaxially via an indirect mechanism by which it activates the transcription of Dmrt2 that in turn binds to the EEE of Myf5 and positively regulates its expression (Sato et al., 2010). The cells committed to the myogenic lineage delaminate and migrate under the dermomyotome initiating myotome formation. While Myf5 expression in the epaxial domain of the dermomyotome can take place independently of Pax3, in the hypaxial domain, Pax3 binds directly to the ~57.5 kb regulatory region of the Myf5 gene required for appropriate Myf5 expression (Fig. 1B) (Bajard et al., 2006). The expression of Pax3 is essential not only for activating myogenic specification but also for cell survival (Relaix et al., 2004, 2005). Members of the Sine oculis homeobox (SIX) and the Eyes absent homologue (EYA) protein families, including SIX1, SIX4, EYA1 and EYA2 are co-expressed with Pax3 in the dermomyotome and regulate its expression (Fig. 1B) (Grifone et al., 2005, 2007). Overexpression of the SIX family member SIX1 activates Pax3 in chick embryos (Heanne et al., 1999). In compound mouse mutants SIX1;SIX4 and Eya1;Eya2, Pax3 expression is lost and the embryos show a similar phenotype to Pax3-deficient embryos: a loss of the hypaxial lip domain and lack of limb muscles, showing that SIX and EYA transcription factors also lie genetically upstream of Pax3 (Grifone et al., 2007).
SIX proteins also regulate myogenesis epaxially where SIX1 and SIX4 directly bind to regulatory sequences of MRF4 (Fig. 1B) (Grifone et al., 2005). In the Six1;Six4 double knockout embryos, MRF4 expression is lost and consequently that of MyoD is downregulated (Grifone et al., 2005; Relaix et al., 2013). In addition to the regulation of MyoD expression by MRF4 epaxially; MRF4 and MYF5 can regulate MYOD expression hypaxially (Fig. 1B) (Kassar-Duchossoy et al., 2004).

In addition to the gene regulatory networks underlying skeletal muscle specification and commitment, recent studies on chromatin organization and architecture uncovered further players regulating myogenic gene expression. The ubiquitous expressed protein LIM domain binding protein 1 (LDB1) has been identified as a PAX3 interacting protein that is required for myogenic specification (Magli et al., 2019b). LDB1 regulates the formation of specific topologically associated domains (TAD) by mediating looping interactions at the Pax3 locus and allowing Pax3 myogenic activity (Magli et al., 2019b). In addition, Pax3 and Pax7 are able to remodel chromatin accessibility in myogenic-associated loci in PSCs with induced Pax3 or Pax7 expression (Lilja et al., 2017; Magli et al., 2019a). In the case of Pax3, analysis of mesodermal cells derived from Pax3-overexpressing mouse ESCs showed that Pax3 increases chromatin accessibility in regions containing its binding sites and that this occurs in cooperation with SIX4 and TEA domain family member 2 (TEAD2) for robust myogenic commitment (Magli et al., 2019a). In mouse PSCs, overexpression of Pax7 leads to an increase in chromatin accessibility and acquisition of active histone modifications in muscle progenitors and committed myoblasts (Lilja et al., 2017). While some gene clusters, associated with myogenic determination, maintained these chromatin features if Pax7 activity was removed, other clusters required the permanent presence of Pax7 to sustain the histone modifications and accessibility (Lilja et al., 2017). Moreover, Pax7 binds to a majority of super-enhancers that contribute to the assembly of TADs associated with myogenic differentiation (Zhang et al., 2020). In addition to Pax3/7 proteins, MYOD can also regulate chromatin architecture. Regulatory regions of myogenic differentiation genes rely on MYOD interaction with the ubiquitous protein chromodomain helicase DNA binding 2 (CHD2) to modulate histone H3.3 variant incorporation into nucleosomes, which regulates downstream myogenic transcription (Harada et al., 2012). The incorporation of the histone variant H3.3 by the histone chaperone HIRA in regulatory regions of myogenic genes, such as Pax7, was further shown to be required to maintain myogenic cell identity (Esteves de Lima et al., 2021b). Furthermore, active chromatin marks on the regulatory regions of Pax7 and Myf5 that rely on the methyltransferase MLL1, regulate their expression and satellite cell function in vivo (Addicks et al., 2019).

**Muscles of migratory origin**

The migratory progenitors that contribute to the formation of muscles in distinct body regions are under distinct molecular regulation to maintain an undifferentiated state during migration and correctly reach the final location. This is the case of muscles like the diaphragm, the hypoglossal cord-derived tongue muscle and the limb muscles, with the former being the most studied and genetically understood. The skeletal muscles of the limbs are formed from the migration of progenitors that delaminate from the hypaxial dermomyotome of somites facing the limb buds, while the hypoglossal cord muscles derive from the occipital and cervical somites (Fig. 1A) (Chevallier et al., 1977; Christ et al., 1974; Relaix et al., 2003). In Pax3-deficient embryos migratory myogenesis is abolished (Bober et al., 1994; Relaix et al., 2003, 2004). Several gene regulatory networks operate downstream of Pax3 in the hypaxial dermomyotome to regulate muscle progenitor migration. Pax3 is phosphorylated by the serine-threonine kinase B-RAF and this is required for Pax3 gene target activation and progenitor migration (Shin et al., 2016). Pax3 controls the EMT of progenitor cells by directly regulating the expression of the tyrosine kinase receptor C-MET in the hypaxial progenitor cells required for migratory myogenesis (Bladt et al., 1995; Daston et al., 1996; Dietrich et al., 1999; Epstein et al., 1996; Yang et al., 1996). The controlled migration of the muscle progenitors towards the limb bud relies on the expression of the C-MET ligand, hepatocyte growth factor (HGF) in the limb bud mesoderm (Bladt et al., 1995; Brand-Saberi et al., 1996). In the c-Met- and in the Hgf-null embryos, delamination and migration of muscle progenitors do not occur, and migratory muscles are not formed, while axial muscles show no defects (Bladt et al., 1995; Dietrich et al., 1999). The homeodomain transcription factor ladybird 1 (Lbx1) is expressed in the long-range migratory progenitors of the hypaxial dermomyotomes of the limbs, occipital and cervical somites and its expression is regulated by Pax3 (Jagla et al., 1995; Mennerich et al., 1998). In Lbx1-mutant embryos, migration of the muscles progenitors towards the limbs is impaired (Brohmann et al., 2000; Gross et al., 2000; Schäfer and Braun, 1999). In particular, progenitor cells that colonize the dorsal forelimb buds are absent, while ventrally, the progenitor cells are present but abnormally distributed, highlighting a cell-specific response to cues that guide migration and maintain the migratory potential of these cells (Brohmann et al., 2000). In addition,
LBX1-regulated migration of the muscle progenitors towards the limb bud relies on LBX1 phosphorylation by FGFR8 and the ERK pathway in chick embryos (Mas selink et al., 2017). Another signaling pathway operating during muscle progenitor migration relies on the chemokine receptor CXCR4, whose expression is regulated by LBX1, and its ligand stromal cell-derived factor 1 (SDF1), expressed in the limb bud mesenchyme. CXCR4 is expressed in muscle progenitors that have already delaminated from the hypaxial dermomyotome and during migration towards the limb its expression co-localizes with LBX1 and PAX3 (Vasyutina et al., 2005).

Embryonic and fetal myogenesis

Myogenesis occurs in successive and overlapping phases with two major waves of muscle formation, embryonic and fetal myogenesis. Embryonic skeletal muscle development starts at E10.5 in the mouse and relies on the PAX3-positive muscle progenitors (Biressi et al., 2007a). Upon differentiation, PAX3/PAX7-positive progenitors located in the myotome start to express MRFs, differentiate and fuse, giving rise to the first embryonic myofibers. Later, fetal myogenesis is initiated around E14.5 in the mouse, with muscle progenitors expressing PAX7 and differentiating into myoblasts that fuse between themselves and with the pre-existing embryonic fibers, allowing muscle growth (Biressi et al., 2007a). The presence of distinct progenitors that co-exist in the muscles but undergo differentiation at different developmental stages is regulated by intrinsic and extrinsic factors. The proliferation of PAX7-positive cells occurs preferentially at the muscle tips, which is specifically enriched in signals such as FGF and BMP (Edom-Vovard et al., 2001; Esteves de Lima et al., 2014; Gros et al., 2005; Kassar-Duchossoy, 2005; Ontell and Kozeka, 1984; Picard and Marcelle, 2013; Relaix et al., 2005). This specific positioning of the progenitor cells under the basal lamina is regulated by the SIX1 and SIX4 transcription factors: in the Six1;Six4 null embryos PAX7-positive cells are impaired and do not adopt a homing position in the trunk muscles (Wurmser et al., 2020). In addition, NOTCH activity in progenitor cells is required to stimulate the production of their own basal lamina environment and to adhere to the fibers (Baghdadi et al., 2018; Bröhl et al., 2012).

The specific development of distinct muscle progenitor cell populations may be linked to differential response to external stimuli. For instance, exposing cultured myoblasts to ligands from the transforming growth factor (TGFβ) family (TGFβ1, BMP4) or to the phorbol ester TPA, leads to inhibition of fetal myoblast differentiation, while embryonic myoblasts are not affected (Biressi et al., 2007a). Genome-wide transcriptomic analysis performed in embryonic and fetal muscle progenitors isolated from mouse embryonic muscles identified groups of genes that were differentially expressed between these two populations (Biressi et al., 2007b). Embryonic myoblasts express high levels of the TGF and BMP inhibitors SMAD6 and SMAD7, which could explain the lack of effect of these molecules in culture (Biressi et al., 2007b). In addition, fetal myoblasts express high levels of PAX7 and genes associated with a more mature muscle phenotype including muscle creatine kinase (MCK), integrin α7 (ITGA7) and several laminins (Biressi et al., 2007b). Moreover, embryonic myoblasts that undergo differentiation express the slow myosin heavy chain (MYHC) isofrom (MYH7) that is repressed in fetal myoblasts through a mechanism involving the fetal-specific transcription factor nuclear factor I X (NFIX) (Taglietti et al., 2016). NFIX is highly expressed in fetal but not embryonic myoblasts and it has been identified as a master regulator of the genetic switch between embryonic and fetal myogenesis (Biressi et al., 2007b; Messina et al., 2010; Taglietti et al., 2016). NFIX expression is directly activated by PAX7 binding to its promoter. Although PAX7 is sufficient to induce NFIX expression, NFIX is only mildly affected in Pax7-null embryos, showing that PAX7 is not required for NFIX expression, suggesting a possible overlapping activity (Messina et al., 2010). The small GTPase RHOA has been identified as a regulator of NFIX, by repressing the ERK signaling pathway, which positively regulates NFIX expression during fetal myogenesis (Taglietti et al., 2018).

Major signaling pathways modulating myogenesis

During development, the balance between proliferation and differentiation of myogenic progenitors is tightly regulated to allow muscle growth while maintaining a pool of undifferentiated progenitors. Several signaling pathways play a role in the switch between proliferation and differentiation of skeletal muscle progenitor cells during development. The NOTCH signaling pathway is a major regulator of the muscle progenitor pool and inhibits myoblast differentiation in several model organisms (Bjornson et al., 2012; Bröhl et al., 2012; Delfini et al., 2000; Esteves de Lima et al., 2016; Hirsinger et al., 2001; Lahmann et al., 2019; Mourikis et al., 2012a, 2012b; Pascoal et al., 2013; Schuster-Gossler et al., 2007; Vasyutina et al., 2007). In the chick, overexpression of the NOTCH ligand DELTA1 inhibits myogenic differentiation in the somites and developing embryonic and fetal limb muscles (Delfini et al., 2000; Esteves de Lima et al., 2016; Hirsinger et al., 2001). Consistently, ablation of
RBPJ, the NOTCH transcriptional co-effector, in muscle progenitors of mouse embryos leads to a premature shift towards differentiation and a progressive loss of the embryonic pool of PAX3/PAX7 progenitor cells (Vasyutina et al., 2007). Importantly, the number of muscle progenitors expressing MYOD is increased in these mutant embryos, and deleting Myod in the Rbpj conditional knockout rescues the phenotype and preserves the progenitor pool (Bröhl et al., 2012; Vasyutina et al., 2007). Moreover, in chick fetal development, signaling from NOTCH ligands expressed in the developing fibers are required to maintain the muscle progenitor cells in a NOTCH ON state and to avoid premature differentiation (Esteves de Lima et al., 2016). Conversely, expression of a constitutively active form of NOTCH (NOTCH intracellular domain, NICD) in myoblasts (MYF5-positive cells) leads to impaired muscle differentiation but maintains the progenitor pool (Mourikis et al., 2012a). In addition, mutating DELTA1 in mouse embryos leads to decreased muscle differentiation associated with the loss of progenitor cells (Schuster-Gossler et al., 2007). This is consistent with the concept that NOTCH signaling is very high in muscle progenitors and declines throughout differentiation (Mourikis et al., 2012a). The NOTCH downstream target hairy and enhancer of split-1 (HES1) is expressed in an oscillatory manner in developing and adult muscle stem cells (Lahmann et al., 2019). Cycling HES1 expression regulates the downstream target MYOD which consequently also oscillates; allowing the maintenance of the undifferentiated and proliferative state of the stem cells (Lahmann et al., 2019).

Other signaling pathways are also involved in maintaining muscle progenitors in a proliferative state, such as BMP signaling (Borok et al., 2020). During limb development, BMP signals from the ectoderm sustain the proliferation of PAX3-positive cells present in the muscle masses of chick embryos (Amthor et al., 1998). BMP gain- and loss-of-function experiments in chick fetal limbs demonstrated that increased BMP signaling leads to higher PAX7-positive cell number and increased muscle mass, while blocking BMP impairs muscle growth, suggesting a positive role of BMP in myogenic progenitor cell proliferation (Wang et al., 2010). In the mouse, BMP regulates post-natal growth and adult muscle homeostasis via regulation of satellite cell proliferation, while inhibiting BMP signaling impairs growth (Sartori et al., 2013; Stantzou et al., 2017). In addition, the BMP target gene inhibitor of DNA-binding protein 1 (ID1) is required to maintain satellite cells in a proliferative and undifferentiated state (Friedrichs et al., 2011; Ono et al., 2010).

The requirement for WNT signaling during myogenesis is distinct between embryonic and fetal phases (Hutcheson et al., 2009). Embryonic myoblasts in mouse developing limbs do not require WNT for correct progenitor and fiber formation, while fetal progenitor expression of a constitutively active form of β-catenin, an effector of the WNT pathway, increases PAX7-positive cell number (Hutcheson et al., 2009). In addition, WNT7a positively regulates the symmetric expansion of satellite cells during regeneration contributing to the maintenance of the stem cell pool (Le Grand et al., 2009). Muscle stem cell renewal is also regulated by SIX1 during muscle regeneration (Le Grand et al., 2012). More recently, post-translational modifications of PAX7 were also shown to regulate its activity and stem cell renewal. PAX7 acetylation by the acetyltransferase MYST1 regulates the satellite cell pool and the number of asymmetric cell divisions (Sincennes et al., 2021). Moreover, methylation of PAX7 by the arginine methyltransferase CARM1 is required for MLL1 recruitment to the Myf5 locus to activate its expression following asymmetric cell division (Addicks et al., 2019; Kawabe et al., 2012).

Recapitulating development in vitro

Research on muscle development has shifted to a regenerative medicine phase, where scientists modify the expression of genes essential for making muscle in vivo in order to recapitulate myogenesis in a petri dish. In vitro myogenesis protocols are now used both to grow functional muscles in the laboratory for tissue replacement, and to model muscle-associated diseases in vitro for cell therapy or drug discovery.

The conditional induction of PAX3 or PAX7 expression during mouse ESCs differentiation into embryoid bodies (EB), combined with cell sorting to enrich for paraxial mesoderm (PDGFRα-positive, FLK1-negative), efficiently produces a myogenic progenitor population (Table 2) (Darabi et al., 2011). Similarly, PAX7-conditional induction in mouse ESCs and PSCs robustly initiates the muscle program (Darabi et al., 2011; Kim et al., 2021; Lilja et al., 2017). Importantly, the muscle progenitors derived from PAX3 or PAX7-induced ESCs can contribute to muscle regeneration when grafted into the muscles of the Duchenne dystrophy mouse model (mdx) that lacks the dystrophin protein. When engrafted, these cells are capable of generating healthy fibers that express dystrophin and replenish the satellite cells which once again adopt a position under the basal lamina (Darabi et al., 2008, 2011; Filareto et al., 2013; Kim et al., 2021). These are important findings for using these techniques for regenerative medicine and ex vivo gene therapy approaches.

Ectopic expression of MYOD is also able to drive human induced PSCs or mouse ESCs into myogenic differentiation (Table 2) (Albini et al., 2013; Dekel et al., 1992; Goudenege et al., 2012; Tanaka et al., 2013; Warren...
This method allows one to bypass the EB and mesodermal differentiation steps required in other protocols, leading to direct and efficient generation of myoblasts. The ectopic expression of BAF60C, a chromatin remodeler from the SWI/SNF family and MYOD cofactor, in induced PSCs allows chromatin remodelling at target myogenic genes and activation of their expression (Albini et al., 2013). However, while MYOD-expressing PSCs are able to engraft and give rise to muscle fibers, the capacity of MYOD-positive cells to contribute to the stem cell pool has not been addressed (Goudenege et al., 2012). Concerns regarding the use of transgene methods in disease therapy are associated with the innate response to viral particles when using viruses to deliver the genetic material and the risk of genomic recombination when delivering DNA. In order to bypass innate anti-viral responses, the use of synthetic and modified MYOD1 messenger RNA combined with interferon inhibitors efficiently directed hiPSC (human induced pluripotent stem cells) differentiation into myoblasts (Warren et al., 2010). To overcome concerns associated with random genomic integration of transgenes, a promising strategy uses specific targeting to genomic safe harbors sites. The targeting of PAX7 to such genomic regions provided myoblasts displaying high efficiency in vivo engraftment, an alternative approach to generate PSC-derived myogenic progenitors that could potentially be of therapeutic use (Kim et al., 2021).

One common drawback of most iPSC-differentiation protocols is the inability to produce mature cell types in vitro. This is also the case for myogenic differentiation protocols that do not yield fully differentiated myotubes in vitro and prevents a deeper analysis of the late myogenic differentiation phases. In fact, the myotubes present in these cultures usually have an embryonic or early fetal phenotype and lack the capacity to mature into late fetal or adult myofibers. Transcriptomic analysis of human PSC-derived myogenic progenitors and human fetal myoblasts highlighted differences in the

| Table 2 | Overview of the different established protocols to differentiate myoblasts from pluripotent stem cells. Arrows represent the bypass of the associated cell population on each protocol |
|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Method                          | Treatment            | Cell population                                      | Advantages                              | Disadvantages              |
| Stem cells (mESC, hiPSC)        |                       | Paraxial mesoderm — Myogenic progenitors — Myoblasts | - Fast                                  | - Ectopic gene expression   |
| Transgene                        | PAX3 induction       | —                                                   | - Highly pure                            | - Cell sorting             |
|                                  | PAX7 induction       | —                                                   | - High number of myogenic progenitors   |                          |
|                                  |                       | —                                                   | - Contribute to fibers and satellite cells |                          |
| MYOD induction                   | —                   | —                                                   | - Fast                                  | - Ectopic gene expression   |
|                                  | —                   | —                                                   | - Highly pure                            | - No myogenic progenitors  |
|                                  | —                   | —                                                   | - High number of myoblasts               |                          |
|                                  | —                   | —                                                   | - Contribute to fibers                   |                          |
| Transgene-free                   | WNT activator       | —                                                   | - No exogenous DNA                       | - Long                    |
|                                  | BMP inhibitor        | —                                                   | - No cell sorting                        | - Heterogeneous population |
|                                  | Growth factors      | —                                                   | - Contribute to fibers and satellite cells |                          |
expression of signaling pathways components between these two populations (Hicks et al., 2018). Human fetal myoblasts downregulate the expression of TGFβ signaling activators while human PSC-derived myogenic progenitors express high levels of these growth factors. When culturing human PSC-derived myogenic progenitors with a TGFβ inhibitor, the myotubes obtained are thicker, have increased levels of expression of fetal and adult MYHC and present a higher level of sarcomeric organization (Hicks et al., 2018). Moreover, the combination of prednisolone, with the aim of recapitulating the glucocorticoid signaling burst operating during human fetal development, and TGFβ inhibitor in the differentiation culture medium significantly improved the morphology of hiPSC-derived fibers towards a more mature phenotype (Al Tanoury et al., 2021). Gene expression profiling from hiPSC-derived MYF5+ cells (Myf5-tdTomato reporter line) revealed that late-stage cultured MYF5+ cells presented a fetal myogenic stem cell phenotype and engrafted with higher efficiency than early-stage cultured MYF5+ cells (Zhao et al., 2020). In addition to the transcriptomic differences, the inability to achieve mature myotubes in vitro can also be associated with the lack of positional information cues that operate in vivo to regulate muscle specification and the metabolic complexity of adult muscle fibers. In fact, single-nuclei RNA-seq analysis of adult muscle fibers revealed the presence of distinct myonuclear domains, highlighting the complexity of the transcriptome of the myonuclei along the fiber (Cramer et al., 2020; Dos Santos et al., 2020; Kim et al., 2020). The question of whether the regionalization of the nuclei within the myotube can be modulated in vitro remains a question and a challenge for the field.

The optimization of protocols to differentiate human PSCs into myoblasts for use in therapeutic approaches also relies on the understanding of the molecular networks operating during human myogenesis that may differ from those of the mouse. Therefore, the development of databases and resources to increase the knowledge of human myogenesis is of major importance. Recently, a scRNA-seq experiment performed in embryonic, fetal and postnatal human skeletal muscles allowed the mapping of myogenic differentiation trajectories of human myoblasts (Xi et al., 2020). These data can be compared to those of the PSC-derived myoblast populations generated in vitro and will contribute to the optimization of protocols that aim to produce myogenic cells from PSCs (Xi et al., 2020).

**Conclusions**

The development of skeletal muscle progenitors is tightly regulated and relies on the hierarchical expression of genes that coordinate their specification and commitment. The role of the PAX genes as well as that of the MRF transcription factors regulating myogenesis is well established. In addition, several genome-wide analyses for PAX3 and PAX7 binding sites have been performed, revealing many potential PAX3/PAX7 target genes. However, only recently has the muscle field started to appreciate the role of these factors as tissue-specific chromatin remodeling factors that rely on interactions with ubiquitous chromatin architecture regulators. Furthermore, the identification of upstream regulators of PAX3 and PAX7 as well as their regulatory regions remains to be done. The answers to these questions could lead to the understanding of how these genes act and regulate downstream targets by inter-playing with chromatin remodelers and also how they are regulated in different developmental contexts.

**Abbreviations**

EMT: Epithelial-to-mesenchymal transition; PSCs: Pluripotent stem cells; MRF: Myogenic regulatory factors; bHLH: Basic helix-loop-helix; EEE: Early epaxial enhancer; ESCs: Embryonic stem cells; ChIP-seq: Chromatin immunoprecipitation-sequencing; TAD: Topologically associated domains; MYHC: Myosin heavy chain; EB: Embryoid body; hiPSCs: Human induced pluripotent stem cells; scRNA-seq: Single cell RNA-sequencing.

**Acknowledgements**

We thank Matthew Borok, Valentina Taglietti and Despoina Mademtzoglou for reading and commenting on the MS. We thank all lab members for constructive scientific discussion.

**Authors’ contributions**

JEdL and FR searched the literature and wrote the manuscript. The authors read and approved the final manuscript.

**Funding**

FR laboratory is supported by funding from Association Française contre les Myopathies (AFM) via TRANSLAMUSCLE (PROJECT 19507 and 22946); Agence Nationale pour la Recherche (ANR) grant Epimuscle (ANR 11 BSV2 017 02), RHU CARMMA (ANR-15-RHUS-0003).

**Availability of data and materials**

Not applicable.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no conflict of interest.

**Received:** 3 June 2021   **Accepted:** 24 August 2021

**Published online:** 01 October 2021

**References**

Addicks GC, Brun CE, Sincennes MC, Saber J, Porter CJ, Francis Stewart A, Ernst P, Rudnicki MA. MLL1 is required for PAX7 expression and satellite cell self-renewal in mice. Nat Commun. 2019;10:4256.
Esteves de Lima J, Blavet C, Bonnin M-A, Hirsinger E, Comai G, Yvernogeau L, Delfini M-C, Bellenger L, Mella S, Nassari S, et al. Unexpected contribution of fibroblasts to muscle lineage as a mechanism for limb muscle patterning. Nat Commun. 2021;12:3851.

Filareto A, Parker S, Darabi R, Borges L, Iacovino M, Schaaf T, Mayerhofer T, Chamberlain JS, Erasti JM, McVor RS, et al. An ex vivo gene therapy approach to treat muscular dystrophy using inducible pluripotent stem cells. Nat Commun. 2013;4:1549.

Friedrichs M, Winsdorfer F, Flohé SB, Schneider S, Wueellng M, Vorkamp A. BMP signaling balances proliferation and differentiation of muscle satellite cell descendents. BMC Cell Biol. 2011;12:26.

Goudenege S, Lebel C, Huot NB, Dufour C, Fujii I, Gekas J, Rousseau J, Tremblay MJ. Myoblasts Derived From Normal hESCs and Dystrophic hiPSCs Efficiently Fuse With Existing Muscle Fibers Following Transplantation. Mol Ther. 2012;20:2153–67.

Goulding M, Lumsden A, Paquette AJ. Regulation of Pax-3 expression in the dermomyotome and its role in muscle development. Dev Camb Engl. 1994;120:567–71.

Grifone R, Demignon J, Houbron C, Souil E, Niro C, Seller MJ, Hamard G, Gros J, Manceau R, Thomé V, Marcelle C. A common somitic origin for embryonic muscle progenitors and satellite cells. Nature. 2005;435:954–8.

Grifone R, Demignon J, Giordani J, Niro C, Souil E, Bertin F, Laclet C, Xu P-X, Maire P, Eyea1 and Eyea2 proteins are required for hypaxial somitic myogenesis in the mouse embryo. Dev Camb Engl. 2005;132:2325–49.

Grifone R, Demignon J, Giordani J, Niro C, Souil E, Bertin F, Laclet C, Xu P-X, Maire P, Eyea1 and Eyea2 proteins are required for hypaxial somitic myogenesis in the mouse embryo. Dev Biol. 2007;302:602–16.

Gross J, Manceau M, Thomé V, Marcelle C. A common somitic origin for embryonic muscle progenitors and satellite cells. Nature. 2005;435:954–8.

Hirsinger E, Malapert P, Dubrulle J, Delfini M-C, Duprez D, Henrique D, Hasty P, Bradley A, Morris JH, Edmondson DG, Venuti JM, Olson EN, Klein WH. Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. Nature. 1993;364:501–6.

Heaume TA, Reshef R, Davis RJ, Mardon G, Oliver G, Tomarev S, Lassar AB, Tabin CJ. Synergistic regulation of vertebrate muscle development by Dach2, Eyea2, and Six1, homologs of genes required for Drosophila eye forma-
tion. Genes Dev. 1999;13:3231–43.

Hicks MR, Hiserodt J, Paras K, Fujiwara W, Eskin A, Jan M, Young CS, Eavesen D, Nelson SF, et al. ERBB3 and NGRFR mark a distinct skeletal muscle progenitor cell in human development and hPSCs. Nat Cell Biol. 2018;20:204–57.

Hirsinger E, Malapert P, Dubrulle J, Delfini M-C, Duprez D, Henrique D, Ish-Horowicz D, Pourquie O. Notch signalling acts in postmitotic avian myogenic cells to control MyoD activation. Development. 2001;128:107–16.

Hutchesson DA, Zhao J, Merrell A, Haldar M, Kardon G. Embryonic and fetal muscle cells are derived from developmentally distinct progenitors and have different requirements for -catenin. Genes Dev. 2009;23:997–1013.

Jagla K, Döll P, Mattei MG, Jagla T, Schuhbaur B, Dretzen G, Bellard F, Bellard M. Mouse Lbx1 and human Lbx1 define a novel mammalian homeobox gene family related to the Drosophila lady bird genes. Mech Dev. 1995;53:345–56.

Kelly RG, Majewsksa LA, Papaioannou VE. The del22q11.2 candidate gene Tbx1 regulates branchiomyogenesis. Hum Mol Genet. 2004;13:2829–40.

Kim M, Franke V, Brandt B, Lowenstein ED, Schiowel V, Spuler S, Akalin A, Birchmeier C. Single-nucleus transcriptomics reveals functional compartmentalization in syncytial skeletal muscle cells. Nat Commun. 2020;11:6375.

Kim H, Selvaraj S, Kiley J, Azzag K, Garay BI, Perlengero RCR. Genomic Safe Harbor Expression of Pax7 for the Generation of Engraftable Myogenic Progenitors. Stem Cell Rep. 2021;10:16–10.

Kouang S, Chargé SB, Seale P, Huh M, Rudnicki MA. Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. J Cell Biol. 2006;172:103–13.

Lagha M, Kornish JD, Rocancourt D, Manceau R, Epstein JA, Zaret KS, Relaix F, Buckingham ME. Pax3 regulation of FGF signaling affects the progression of embryonic progenitor cells into the myogenic program. Genes Dev. 2008;22:1828–37.

Lahmann I, Bohl D, Zylaniou T, Isomura A, Czajkowski MT, Kapoor V, Grieger J, Ruffault P-L, Madernzegoulo D, Zammitt PS, et al. Oscillations of MyoD and Hes1 proteins regulate the maintenance of activated muscle stem cells. Genes Dev. 2019;33:524–35.

Lassar AB, Davis RL, Wright WE, Kadesch T, Murre C, Voronova A, Baltimore D, Weintraub H. Functional activity of myogenic HLH proteins requires hetero-oligomerization with E12/E47-like proteins in vivo. Cell. 1991;66:305–15.

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;4:535–47.

Le Grand F, Grifone R, Mourikis P, Houbron C, Gigaud C, Pujol J, Mallet M, Pages G, Rudnicki M, Tajbakhsh S, et al. Six1 regulates stem cell repair potential and self-renewal during skeletal muscle regeneration. J Cell Biol. 2012;198:815–32.

Lepper C, Conway SJ, Fan CM. Adult satellite cells and embryonic muscle pro-
genitors have distinct genetic requirements. Nature. 2009;460:627–31.

Lilja KC, Zhang N, Magli A, Gunduz V, Bowman CJ, Arpke RW, Darabi R, Kyba M, Perlengero R, Dynlacht BD. Pax7 remodels the chratamin landscape in skeletal muscle stem cells. PLoS ONE. 2017;12:e0171690.

Lu MF, Pressman C, Dyer R, Johnson RL, Martin JF. Function of Riger syndrome gene in left-right asymmetry and craniofacial development. Nature. 1999;401:276–8.

Lu J-R, Basset-Duby R, Hawkins A, Chang P, Valdez R, Wu H, Gan L, Shelton JM, Richardson JA, Olson EN. Control of fetal muscle development by MyoR and capulin. Science. 2002;298:2378–81.

Magli A, Baik J, Mills L, Kwak IY, Dillon BS, Mondragon Gonzalez R, Stafford DA, Swanson SA, Stewart R, Thomson JA, et al. Time-dependent Pax3-
mediated chratamin remodeling and cooperation with Six4 and Tead2 specify the skeletal myogenic lineage in developing mesoderms. PLoS Biol. 2019;17:e300153.

Mageni A, Baik J, Pota P, Cordenho CO, Kwak IY, Gaurin DJ, Love PE, Dynlacht BD, Perlengero RCR. Pax3 cooperates with Ldb1 to direct local chromosome architecture during myogenic lineage specification. Nat Commun. 2019;10:2316.

Mansouri A, Stoykova A, Torres M, Gruss P. Dysgenesis of cephalic neural crest derivatives in Pax7−/− mutant mice. Development. 1996;122:831–8.

Masselink W, Masaki M, Siesco D, Marcelle C, Currie PD. Phosphorylation of Lbx1 controls lateral myoblast migration into the limb. Dev Biol. 2017;430:302–9.

Mennerich D, Schafer K, Braun T. Pax-3 is necessary but not sufficient for Lbx1 expression in myogenic precursor cells of the limb. Mech Dev. 1998;73:147–58.

Messinga G, Briosi S, Monteverde S, Magli A, Cassano M, Perani L, Roncaglia E, Tagliafico E, Starnes L, Campbell CE. Six4 regulates fetal-specific transcrip-
tion in developing skeletal muscle. Cell. 2010;140:554–66.

Mourikis P, Gopalakrishnan S, Sambasivan R, Tajbakhsh S. Cell-autonomous MyoR and capsulin. Science. 2002;298:2378–81.

Mourikis P, Sambasivan R, Castel D, Rocheteau P, Bizzarro V, Tajbakhsh S. Myf5 is a direct target of long-range Shh signaling and Gli regulation for muscle specification. Genes Dev. 2002;16:114–26.

Niu J, Mineo R, Itoh H, Tazawa K, Fujita R, Weintraub H. Functional activity of myogenic HLH proteins requires hetero-oligomerization with E12/E47-like proteins in vivo. Cell. 1991;66:305–15.

Nizrist M, Fricke K, Darabi R, Kong W, Biressi S, Magli A, Rudnicki MA. CARM1 regulates Pax7 transcriptional activity through ML1/2 recruitment during asymmetric stem cell divisions. Cell Stem Cell. 2012;11:33–45.

Nizrist M, Fricke K, Darabi R, Kong W, Biressi S, Magli A, Rudnicki MA. CARM1 regulates Pax7 transcriptional activity through ML1/2 recruitment during asymmetric stem cell divisions. Cell Stem Cell. 2012;11:33–45.
Nallandian M, Zhao M, Sasaki-Honda M, Jonouchi T, Lucena-Cacace A, Mizusawa T, Yasuda M, Yoshida Y, Hotta A, Sakurai H. Characterization of hiPSC-derived muscle progenitors reveals distinctive markers for myogenic cell purification toward cell therapy. Stem Cell Rep. 2021;16:883–98.

Noden DM, Francis-West P. The differentiation and morphogenesis of craniofacial muscles. Dev Dyn. 2006;235:1194–218.

Ono Y, Calhabeu F, Morgan JE, Katagiri T, Amthor H, Zammitt PS. BMP signalling permits population expansion by preventing premature myogenic differentiation in muscle satellite cells. Cell Death Differ. 2010;18:222–34.

Ostell M, Kozek K. The organogenesis of murine striated muscle: a cytoarchitectural study. Am J Anat. 1984;171:133–48.

Ott MO, Bober E, Lyons G, Arnold H, Buckingham M. Early expression of the myogenic regulatory gene, myf-5, in precursor cells of skeletal muscle in the mouse embryo. Dev Camb Engl. 1991;111:1097–107.

Oustanina S, Hause G, Braun T. Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification. EMBO J. 2004;23:9430–9.

Pascoal S, Esteves de Lima J, Leslie JD, Hughes SM, Saleé L. Notch signalling is required for the formation of structurally stable muscle fibres in zebrafish. PLoS One. 2013;8.

Patapoutian A, Yoon JK, Miner JH, Wang S, Stark K, Wold B. Disruption of the mouse MRF4 gene identifies multiple waves of myogenesis in the myotome. Dev Camb Engl. 1995;121:3347–58.

Picard CA, Marcelle C. Two distinct muscle progenitor populations coexist throughout amniote development. Dev Biol. 2013;373:141–8.

Relaix F, Polimeni M, Rocancourt D, Poncetto C, Schafer BW, Buckingham M. The transcriptional activator PAX3-FKHR rescues the defects of Pax3 mutants mice but induces a myogenic gain-of-function phenotype with ligand-independent activation of Mitf signaling in vivo. Genes Dev. 2003;17:2950–65.

Relaix F, Rocancourt D, Mansouri A, Buckingham M. Divergent functions of murine Pax3 and Pax7 in limb muscle development. Genes Dev. 2004;18:1088–105.

Relaix F, Rocancourt D, Mansouri A, Buckingham M. A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature. 2005;435:948–53.

Relaix F, Montarras D, Zaffran S, Gayraud-Morel B, Rocancourt D, Tajbakhsh S, Mansouri A, Cumano A, Buckingham M. Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor cells. J Cell Biol. 2006;172:91–102.

Relaix F, Demignon J, Laclef C, Pujol J, Santolini M, Niro C, Lagha M, Rocancourt D, Buckingham M. The transcriptional activator PAX3-FKHR rescues the defects of Pax3 mutant mice but induces a myogenic gain-of-function phenotype with ligand-independent activation of Mitf signaling in vivo. Genes Dev. 2003;17:2950–65.

Relaix F, Demignon J, Laclef C, Pujol J, Santolini M, Niro C, Lagha M, Rocancourt D, Buckingham M. The transcriptional activator PAX3-FKHR rescues the defects of Pax3 mutant mice but induces a myogenic gain-of-function phenotype with ligand-independent activation of Mitf signaling in vivo. Genes Dev. 2003;17:2950–65.

Santori R, Schirwis E, Blaauw B, Bortolanza S, Zhao J, Enzo E, Stantzou A, Moui R, Rudnicki MA. Acetylation of PAX7 controls muscle stem cell self-renewal and differentiation potential in mice. Nat Commun. 2021;12:3253.

Soleimani VD, Punch VG, Kawabe Y, Jones AE, Palidwor GA, Porter CJ, Cross JW, Carvalaj JJ, Kockx CEM, van Uckcn, W.F.J., et al. Transcriptional dominance of Pax7 in adult myogenesis is due to high-affinity recognition of homeodomain motifs. Dev Cell. 2012;22:1208–20.

Stantzou A, Schirwis E, Swist S, Alonso-Martín S, Polydorou I, Zarrouki F, Mouiel S, Beley C, Julien A, Le Grand F, et al. BMP signalling regulates satellite cell-dependent postnatal muscle growth. Dev Camb Engl. 2017;14:2737–47.

Taglietti V, Maroli G, Cermeniatti S, Monteversedi S, Ferrante A, Rossi G, Cossu G, Beltrame M, Messina G. Nfix induces a switch in Sox6 transcriptional activity to regulate MyHC-I expression in fetal muscle. Cell Rep. 2016;17:2354–66.

Taglietti V, Angelini G, Mura G, Bonfanti C, Caruso E, Monteversedi S, Le Carrou G, Tajbakhsh S, Relax S, Messina G. Rhoa and ERK signalling regulate the expression of the transcription factor Nfix in myogenic cells. Development. 2018;145(21):dev163956.

Tajbakhsh S, Borello U, Vivarelli E, Kelly R, Papkoff J, Duprez D, Buckingham M, Cossu G. Differential activation of Myf5 and MyoD by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of Myf5. Dev Camb Engl. 1998;125:4155–62.

Tanaka A, Wolten K, Miyake K, Hotta A, Ikeya M, Yamamoto T, Nishino T, Shiogi E, Sehara-Fujisawa A, Manabe Y, et al. Efficient and reproducible myogenic differentiation from human iPSC cells: prospects for modeling Miyoshi Myopathy in vitro. PLoS ONE. 2013;8:e65140.

Tremblay P, Dietrich S, Mercikray M, Schubert FR, Li Z, Paulin D. A crucial role for Pax3 in the development of the hypaxial musculature and the long-range migration of muscle precursors. Dev Biol. 1998;203:46–61.

Vasyutina E, Stebler J, Brand-Saberi B, Schulz S, Raz E, Birchmeier C. CXCR4 and Gab1 cooperate to control the development of migrating myogenic progenitor cells. Genes Dev. 2005;19:2187–98.

Vasyutina E, Lenhard DC, Wende H, Erdmann B, Epstein JA, Birchmeier C. RBP-J (Rbpsuh) is essential to maintain muscle progenitor cells and to generate satellite cells. Proc Natl Acad Sci. 2007;104:4443–8.

Wang H, Noulet F, Edom-Vovard F, Le Grand F, Duprez D, Buckingham M. Bmp signalling at the tips of skeletal muscles regulates the number of fetal muscle progenitors and satellite cells during development. Dev Cell. 2010;18:643–54.

Warren L, Manos PD, Ahfeldt T, Loh Y-H, Li H, Lau F, Elina W, Mandal PK, Smith ZD, Messner A, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010;7:618–20.

Weintraub H, Dvarak VJ, Verma I, Davis R, Hollenberg S, Snider L, Lasser A, Tascott SJ. Muscle-specific transcriptional activation by MyoD. Genes Dev. 1991;5:1377–86.

Wurmsmer M, Chaverot N, Madani R, Sakai H, Negroni E, Demignon J, Sainte-Bure P, Mouly V, Amthor H, Tascott S, et al. (2020). SIX1 and SIX4 homeoproteins regulate Pax7+ progenitor cell properties during fetal epaxial myogenesis. Development 147 (19): dev185975.

Xi H, Langerman J, Sabri S, Chien P, Young CS, Younesi S, Hicks M, Gonzalez K, Fujivara W, Marzi J, et al. A human skeletal muscle atlas identifies the trajectories of stem and progenitor cells across development and from human pluripotent stem cells. Cell Stem Cell. 2020;27:158-176 e10.

Yang XM, Vogan K, Gross P, Park M. Expression of the met receptor tyrosine kinase in muscle progenitor cells in somites and limbs is absent in Splotch mice. Dev Camb Engl. 1996;122:2163–71.

Zhang W, Behringer RR, and Olson EN. Inactivation of the myogenic bHLH gene MRF4 results in upregulation of myogenin and rib abnormalities. Genes Dev. 1995;9:1388–99.
Zhao M, Tazumi A, Takayama S, Takenaka-Ninagawa N, Nalbandian M, Nagai M, Nakamura Y, Nakasa M, Watanabe A, Ikeya M, et al. Induced fetal human muscle stem cells with high therapeutic potential in a mouse muscular dystrophy model. Stem Cell Rep. 2020;15:80–94.

**Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.