Published in final edited form as:

Curr Opin Clin Nutr Metab Care. 2010 May ; 13(3): 243–248. doi:10.1097/MCO.0b013e328336ea98.

Muscle stem cells in developmental and regenerative myogenesis

Jong-Sun Kang¹ and Robert S. Krauss²

¹ Samsung Biomedical Research Institute, SungKyunKwan University School of Medicine, Suwon 440-746, South Korea

² Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY 10029, USA

Abstract

Purpose of review—Skeletal muscle development serves as a paradigm for cell lineage specification and cell differentiation. Adult skeletal muscle has high regenerative capacity, with satellite cells the primary source of this capability. This review describes recent findings on developmental and adult myogenesis with emphasis on emerging distinctions between various muscle groups and stages of myogenesis.

Recent findings—Muscle progenitors of the body are derived from multipotent cells of the dermomyotome and express the transcription factors Pax3 and Pax7. These cells self-renew or induce expression of muscle regulatory factors (MRFs) and differentiate. The roles of Pax3⁺, Pax7⁺ and specific MRF⁺ progenitor populations in trunk and limb myogenesis have been identified through cell ablation in the mouse. Various head muscles and associated satellite cells have differing developmental origins, and rely on distinct combinations of transcriptional regulators, than trunk and limb muscles. Several genetic and sorting protocols demonstrate that satellite cells are heterogeneous with some possessing stem cell properties; the relative roles of lineage and niche in these properties are being explored. While cellular mechanisms of developmental, post-natal and adult regenerative myogenesis are thought to be similar, recent studies reveal distinct genetic requirements for embryonic, fetal, post-natal and adult regenerative myogenesis.

Summary—Genetic determinants of formation or repair of various muscles during different stages myogenesis are unexpectedly diverse. Future studies should illuminate these differences, as well as mechanisms that underlie stem cell properties of satellite cells.

Keywords

myogenesis; muscle stem cell; satellite cell; muscle cell lineage; muscle regeneration; Pax3; Pax7; myogenic regulatory factor

Introduction

Skeletal muscle fibers form throughout the body and during the entire lifespan of vertebrates. Recent work indicates that diverse populations of muscle stem and progenitor cells with distinct developmental programs and/or genetic requirements are important for production and/or regeneration of various muscles. In this review we discuss recent

publications on muscle stem and progenitor cells in developmental and regenerative myogenesis. Recent reviews on these and related subjects are also recommended [1–3].

Embryonic origins of trunk and limb muscles

Skeletal muscle, the most abundant tissue in the vertebrate body, develops in sequential but overlapping stages [4]. Muscles of the trunk and limbs arise from somites, segmented structures that form from paraxial mesoderm in an anterior-to-posterior manner. Muscle progenitor cells are derived from the dorsal epithelial region of the maturing somite, the dermomyotome [4,5]. The dermomyotome also give rises to cells of the endothelial, vascular smooth muscle, dermal and brown fat lineages, and some dermomyotomal cells are multipotent, their fates being influenced by specific signaling pathways and interactions between transcription factors [1]. For example, BMP signaling stimulates production of endothelial cells, while Notch signaling promotes the smooth muscle fate over the skeletal muscle fate [6*]. The transcriptional regulator PRDM16 interacts with C/EBP β to steer Myf5⁺ myogenic cells into the brown fat lineage; brown adipose tissue from *Prdm16* mutant mice fails to acquire a complete differentiation program and expresses skeletal muscle-specific genes [7*,8*].

In response to signals from the adjacent notochord, neural tube and surface ectoderm, some dermomyotomal progenitors become committed to the skeletal muscle lineage and form the myotome, a set of differentiated muscle cells that underlies the dermomyotome and which elongate parallel to the axis of the embryo over the entire somite length [5]. While the myotome represents the first differentiated muscle in the embryo, few dermomyotomal muscle progenitors actually differentiate into myotomal fibers. The majority of body musculature is derived from a highly proliferative population of Pax3⁺/Pax7⁺ cells that emerge from the central dermomyotome [9–11]. The timing of the emergence of this population is regulated by myotome-derived FGF8, which stimulates expression of the transcription factor *Snail1* in dermomyotomal cells and a consequent epithelial-to-mesenchymal transition [12]. Resultant Pax3⁺/Pax7⁺ progenitor cells are capable of both proliferation/self-renewal and differentiation along the skeletal muscle lineage, defining them as stem cells.

Several signaling pathways contribute to regulation of the balance between self-renewal and differentiation of these cells so as to produce skeletal muscles of appropriate size at the appropriate time. Notch signaling inhibits differentiation, and mice lacking the Notch ligand Delta1 or the pathway-responsive transcription factor RBP-J display precocious differentiation with consequent depletion of the progenitor pool and diminished musculature [13,14]. Overexpression of the asymmetric cell fate determinant Numb, an inhibitor of Notch signaling, in the avian dermomyotome drives cells into a myotomal fate [6*]. However, mice expressing a *Numb* transgene in a similar compartment led to an expanded pool of Pax3⁺/Pax7⁺ progenitor cells and increased numbers of cells committed to both dermal and myotomal fates [15]. Numb may act by more than one mechanism [16], and relative timing of overexpression plus potential loss of asymmetric distribution may produce distinct outcomes with these different model systems. Numb (and its paralog Numbl like) is likely to be a key factor in generation of appropriate cell numbers for dermomyotome-derived lineages. Mice lacking both Numb and Numbl like die at ~E9.5 [17]; future conditional mutagenesis approaches should be very informative.

Sprouty1, a negative regulator of receptor tyrosine kinase signaling, also stimulates self-renewal of Pax3⁺/Pax7⁺ progenitor cells [18*]. Conversely, Pax3 directly activates expression of *Fgfr4* (encoding the receptor tyrosine kinase fibroblast growth factor receptor 4), which promotes entry into the differentiation program [18*]. A second signaling factor

that drives entry into the muscle-specific lineage is myostatin, a TGF β superfamily member that induces expression of p21 and MyoD, driving cell cycle exit and muscle-specific transcription [19]. It is noteworthy that major signaling pathways such as FGF and Notch are used reiteratively in the progression from multipotent dermomyotomal progenitor to determined myogenic precursor. Consistent with this notion, myostatin also plays a distinct, later role in regulation of muscle mass in the adult mouse [20].

Pax3⁺ and Pax3⁺/Pax7⁺ progenitor cells are specified to become muscle lineage-committed myoblasts through the action of the myogenic regulatory factors (MRFs) Myf5, MRF4 and MyoD, while differentiation of myoblasts is regulated by myogenin, MyoD and MRF4. Pax3 and Pax7 expression are down-regulated in most muscles during this process, with Pax7 expression retained by satellite cells [9–11]. Genetic cell lineage tracing and cell ablation studies (the latter through use of mice carrying locus-specific *Cre* knock-in and conditional *Rosa26-DTA* [diphtheria toxin A] alleles) have been used to investigate fates and functions of Pax3⁺, Pax7⁺ and individual MRF-expressing cells. Pax3⁺ cells give rise to embryonic muscle and to Pax7⁺ cells, and ablation of Pax3⁺ cells results in failure of embryonic myogenesis [21*]. This is in contrast to Pax3^{-/-} mice, in which only limb muscles are lost [22], revealing that Pax3 itself is required only for limb musculature but Pax3-expressing progenitors are essential to produce both trunk and limb musculature [21*]. It was not possible to study later stages of muscle development in Pax3⁺ cell-ablated mice due to embryonic lethality, but ablation of Pax7⁺ cells results in defective fetal myogenesis in the trunk and limbs [21*]. This is in contrast to Pax7^{-/-} mice, which develop muscle normally but have defects in maintenance of adult satellite cells [23,24], and is consistent with the notion that Pax3⁺/Pax7⁺ progenitors give rise to the vast majority of the somatic musculature [9–11].

Similar approaches have been taken to ablate cells that express *Myf5*, *Myogenin* or *Mrf4*. A majority of muscle precursor cells express both *Myf5* and *MyoD*, with *Myf5* expression beginning much earlier than *MyoD* expression [5]. Myogenic cell determination in the mouse occurs in the absence of either gene singly, but simultaneous removal of both leads to a failure of fetal myogenesis and animals that lack skeletal muscle at birth [25,26]. Myf5⁺ cells contribute to muscle masses throughout the trunk, limbs and head, though the distribution is variable within and between muscles [27*,28*]. Ablation of Myf5⁺ cells results in aberrant early, somitic myogenesis but such mice did not display significant loss of musculature at later stages [27*,28*]. Therefore a cell lineage in which *Myf5* expression is insufficiently robust to drive Cre-mediated recombination (and which expresses and is presumably dependent on *MyoD*) exists and is sufficient to produce normal musculature, including fast and slow fibers. In contrast, ablation of myogenin⁺ and MRF4⁺ cells resulted in loss of myofibers, consistent with their later expression and roles in differentiation [27*, 28*]. It will be interesting to use the *MyoD-cre* allele [29] for similar experiments.

Embryonic origins of head muscles

Recent studies also reveal that specific groups of head muscles rely on combinations of transcriptional regulators distinct from those used by body muscles during their development. In somite-derived muscles, Pax3 works with *Myf5/Mrf4* and lies upstream of *MyoD*, and Pax3 is sufficient to rescue *MyoD* expression in the absence of *Myf5/Mrf4* [25,30]. In branchial arch muscles, *Tbx1* plays a similar role to Pax3 in body muscles, and Pax3 is dispensable. In contrast, extraocular muscles appear to lack this type of pathway: *MyoD* is not expressed, and these muscles do not develop, in the absence of *Myf5/Mrf4* [31*]. Therefore, unlike body and branchial arch muscle progenitors, extraocular muscle progenitors lack a *Myf5/Mrf4*-independent pathway to activate *MyoD*. Extraocular muscles do not form in mice null for *Pitx2* but *Pitx2* is also involved in body and branchial arch

muscle development, and *Pitx2*'s role in extraocular muscles is not analogous to that of *Pax3* or *Tbx1* [31*].

These differing genetic requirements are reflected by the distinct developmental origins of these muscle groups. Body muscles are mainly derived from Pax3⁺/Pax7⁺ progenitors that originate in somites; recent lineage tracing studies indicate that branchial arch muscles arise from MesP1⁺/Isl1⁺/Nkx2.5⁺ progenitors that originate in cranial paraxial and splanchnic mesoderm, and extraocular muscles arise from Mesp1⁺ progenitors that originate in cranial paraxial and prechordal mesoderm [32*]. The functional roles of MesP1, Isl1 and Nkx2.5 in head muscle development are unclear as these factors are involved in cardiogenesis, and mouse mutants die at ~E10.5. The links between heart and head muscle morphogenesis are intriguing from developmental and evolutionary viewpoints [33*].

Developmental origins of satellite cells

Satellite cells are quiescent muscle progenitors located between myofibers and their surrounding basal lamina. Adult skeletal muscle displays high regenerative ability, even after repetitive injury, and satellite cells are the primary source of this capability [34,35]. All satellite cells express Pax7, which is required for their survival [23,24], but satellite cells of different muscles have distinct developmental origins. Satellite cells of the trunk and limbs originate from the same dermomyotome-derived Pax3⁺/Pax7⁺ muscle progenitor population that gives rise to the body musculature [9–11]. Similar to the developmental origins of extraocular and branchial arch muscles, satellite cells of these muscles are derived from head mesoderm [31*,32*]. The satellite cells of these diverse muscle groups express markers that reflect their distinct origins, an observation with significant implications for myopathies/dystrophies that target specific muscle groups; these signatures are not, however, maintained when the cells are transplanted to other muscles, suggesting both developmental history and niche are important [31*,32*,36].

Satellite cell heterogeneity

Upon muscle injury, quiescent satellite cells are activated to proliferate, differentiate and fuse with each other or preexisting myofibers to regenerate the muscle [34,35]. Inherent in the notion that at least some satellite cells are stem cells is that, following injury, some fraction self-renews to reconstitute the quiescent satellite cell pool [34,35]. Several studies have shown that freshly isolated or fiber-associated satellite cells participate in muscle repair upon engraftment and also reenter the satellite cell niche [37–41*]. This is true even for single transplanted cells, formally demonstrating self-renewal and differentiation capability [41*].

Numerous studies indicate that satellite cells are a heterogeneous population, with some, rather than all, satellite cells having stem cell properties [34,35]. Efforts to identify this fraction are ongoing. Kuang et al. used mice carrying *Myf5-cre* and conditional *Rosa26-YFP* alleles and found that about 10% of Pax7⁺ cells in the satellite cell niche are YFP⁺ (i.e., had never expressed Myf5) [39]. The capacity of these cells to engraft injured muscle and repopulate the satellite cell compartment is superior to Pax7⁺/YFP⁺ cells, and they produce both Pax7⁺/YFP⁺ and Pax7⁺/YFP[−] progeny through asymmetric division [39]. Satellite cells that never expressed *Myf5* in their history therefore behave as stem cells. Furthermore, Pax7⁺/YFP[−], but not Pax7⁺/YFP⁺, cells express the Wnt receptor Fzd7, and Wnt7a ligand induced during muscle injury stimulates symmetric division of these cells via the planar cell polarity (non-canonical) pathway, expanding the stem cell pool [42*].

It might be predicted that satellite cells which never expressed *Myf5* would also never have expressed *MyoD*. However, 98–100% of the quiescent satellite cells in young, uninjured

mice that carry *MyoD-cre* and *ROSA26* reporter alleles expressed *MyoD* in their history [29]. This fits observations on satellite cells from single-fiber cultures, where some Pax7⁺/MyoD⁺ cells divide to produce one Pax7⁺/MyoD⁺ cell and one Pax7⁺/MyoD⁻ cell [43]. A possible scenario consistent with all these results is that stem cells identified as Pax7⁺/YFP⁻ might arise from the Myf5-independent embryonic muscle progenitors identified in the Myf5⁺ cell ablation studies discussed above [27*,28*].

Sorting satellite cells for specific surface markers (e.g., CD34) strongly enriches for cells that engraft into myofibers and occupy the satellite cell niche [37,40,41*]. Tanaka et al. sorted for both satellite cell markers and markers of side population (SP) cells (cells found in many tissues with potential stem-like properties) to isolate so-called satellite-SP cells [44*]. Less than 10% of SP cells expressed satellite cell markers and <10% of satellite cells expressed SP cell markers; cells that expressed both represented ~0.25% of mononuclear cells in muscle and were myogenic in vitro and highly efficient at engrafting the satellite cell niche when transplanted into injured muscle [44*]. It will be interesting to determine the relationship between these cells and the Pax7⁺/YFP⁻ cells described above. It is also important to decipher the extent to which stem cell properties of specific satellite cells are determined by their lineage versus maintained by a specific niche. With regard to the latter possibility, satellite cells express the receptor tyrosine kinase Tie2, and smooth muscle cells and fibroblasts, both found in proximity to satellite cells in muscle [45], express its ligand angiopoietin1. Angiopoietin1 signals via Tie2 and ERK1/2 MAP kinase to promote satellite cell quiescence and may therefore be involved in regulating self-renewal [46].

Satellite cell activation and differentiation

Notch signaling plays an important role in proliferation of satellite cells, blocking differentiation until there is sufficient expansion of progenitor cells for repair [39,47]. There is a transition from Notch signaling to canonical Wnt signaling at the onset of differentiation [48]. The temporal balance between these pathways is important as premature Wnt signaling results in premature differentiation, and blockade of Wnt signaling impairs differentiation [48]. Cross-talk between the two pathways occurs via GSK3 β , an inhibitor of canonical Wnt signaling. Notch activates GSK3 β , and reduction of GSK3 β activity at the transition contributes to Wnt-mediated accumulation of β -catenin and differentiation [48]. In addition, BCL9, a co-regulator of β -catenin and TCF/LEF-mediated transcription, is induced during regenerative myogenesis and promotes Wnt-dependent differentiation of adult muscle progenitors [49].

In contrast, studies with satellite cells associated with isolated myofibers revealed a different role for β -catenin. Nuclear β -catenin levels decrease during differentiation, overexpression of β -catenin or inhibition GSK3 β activity results in more Pax7⁺/MyoD⁻ cells, and depletion of β -catenin by RNAi enhances differentiation [50]. β -catenin therefore promotes satellite cell self-renewal at the expense of differentiation. Exposure of such cultures to canonical Wnts also stimulates satellite cell proliferation [51]. Each of these studies was performed with different protocols, making them hard to compare directly. Furthermore, β -catenin is a multifunctional protein and may have multiple roles with overlapping time frames within muscles that regenerate somewhat asynchronously [35].

Distinct genetic requirements at different stages of myogenesis

The cellular mechanisms of developmental, post-natal and adult regenerative myogenesis are thought to be similar. However, recent studies have revealed some distinct genetic requirements for embryonic, fetal, post-natal and adult regenerative myogenesis. *Myogenin*-null mice die at birth due to a severe deficiency of myoblast differentiation [52], and myogenin is the only MRF without a compensatory factor during developmental

myogenesis. In contrast, mice in which *myogenin* is conditionally deleted 24 hr after birth display normal post-natal muscle development (during which muscle mass increases tremendously), indicating a major difference in requirement for *myogenin* function in embryonic/fetal and post-natal myogenesis [53]. Satellite cells in which *myogenin* is deleted in vitro form myotubes normally, but have an altered pattern of gene expression that is mirrored in conditionally-deleted muscle in vivo. It will be important to assess the ability of adult *myogenin*-null mice to repair muscle injury.

Pax7 is expressed in all adult satellite cells and is required for their maintenance in the post-natal period. Adult *Pax7*^{-/-} animals (i.e., *Pax7*-null from conception) have a severe defect in regenerative myogenesis [23,24]. Surprisingly, Lepper et al. found that *Pax7* is not required for adult limb muscle regeneration if genetically removed after three weeks of age [54*]. *M-cadherin*⁺ (another satellite cell marker) cells are found in the satellite cell compartment after injury to such mice, and they can repair a second round of muscle injury, suggesting an ability to replenish adult muscle progenitor cells. The regenerative capability of *Pax7*-null satellite cells was not due to compensation by *Pax3*, as simultaneous deletion of *Pax7* and *Pax3* in adulthood also does not hamper muscle injury repair.

Conclusion

The study of muscle development and regeneration has been a source of valuable insight into the processes of cell lineage specification, cell differentiation and tissue repair after injury. Sophisticated genetic and embryological analyses have provided information on the diverse genetic determinants required for these processes. This is expected to continue, particularly in the identification of stem cells within the overall satellite cell population and the relative roles of developmental history and niche in governing the behavior of these cells. The unexpected findings that the roles of key regulators are restricted to specific temporal windows (e.g., *myogenin* in myogenic differentiation and *Pax7* in satellite cell maintenance) will surely spur much additional work on age-dependent differences in myogenesis and satellite and muscle stem cells.

Acknowledgments

Work in the authors' laboratories on this topic is funded by the National Institutes of Health (RSK) and the Korea Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A084126) (JSK).

References

1. Buckingham M, Vincent SD. Distinct and dynamic myogenic populations in the vertebrate embryo. *Curr Opin Genet Dev.* 2009; 19:444–453. [PubMed: 19762225]
2. Relaix F, Marcelle C. Muscle stem cells. *Curr Opin Cell Biol.* 2009; 21:748–753. [PubMed: 19932015]
3. Tajbakhsh S. Skeletal muscle stem cells in developmental versus regenerative myogenesis. *J Intern Med.* 2009; 266:372–389. [PubMed: 19765181]
4. Biressi S, Molinaro M, Cossu G. Cellular heterogeneity during vertebrate skeletal muscle development. *Dev Biol.* 2007; 308:281–293. [PubMed: 17612520]
5. Tajbakhsh S, Buckingham M. The birth of muscle progenitor cells in the mouse: spatiotemporal considerations. *Curr Top Dev Biol.* 2000; 48:225–268. [PubMed: 10635461]
- 6*. Ben-Yair R, Kalcheim C. Notch and bone morphogenetic protein differentially act on dermomyotome cells to generate endothelium, smooth, and striated muscle. *J Cell Biol.* 2008; 180:607–618. Studies with the chick embryo demonstrate the influence of Notch and BMP signaling on various cell lineage fates of multipotent dermomyotomal cells. [PubMed: 18268106]

- 7*. Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gyi SP, Spiegelman BM. Initiation of myoblast to brown fat switch by a PRDM16-C/EBP- β transcriptional complex. *Nature*. 2009; 460:1154–1158. [PubMed: 19641492]
- 8*. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. 2008; 454:961–967. This study, along with [7*] by the same group, shows that brown adipose tissue and skeletal muscle share a common, Myf5-expressing progenitor. The brown fat fate is driven by a transcriptional complex that includes Prdm16 and C/EBP- β . [PubMed: 18719582]
9. Gros J, Manceau M, Thomé V, Marcelle C. A common somitic origin for embryonic muscle progenitors and satellite cells. *Nature*. 2005; 435:954–958. [PubMed: 15843802]
10. Kassam-Duchossoy L, Giaccone E, Gayraud-Morel B, Jory A, Gomès D, Tajbakhsh S. Pax3/Pax7 mark a novel population of primitive myogenic cells during development. *Genes Dev*. 2005; 19:1426–1431. [PubMed: 15964993]
11. Relaix F, Rocancourt D, Mansouri A, Buckingham M. A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. *Nature*. 2005; 435:948–953. [PubMed: 15843801]
12. Delfini MC, De La Celle M, Gros J, Serralbo O, Marics I, Seux M, Scaal M, Marcelle C. The timing of emergence of muscle progenitors is controlled by an FGF/ERK/SNAIL1 pathway. *Dev Biol*. 2009; 333:229–237. [PubMed: 19445915]
13. 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 (USA)*. 2007; 104:537–542. [PubMed: 17194759]
14. Vasyutina E, Lenhard DC, Wende H, Erdmann B, Epstein JA, Birchmeier C. RBP-J (Rbpsi) is essential to maintain muscle progenitor cells and to generate satellite cells. *Proc Natl Acad Sci (USA)*. 2007; 104:4443–4448. [PubMed: 17360543]
15. Jory A, Le Roux, Gayraud-Morel B, Rocheteau P, Cohen-Tannoudji M, Cumano A, Tajbakhsh S. Numb promotes an increase in skeletal muscle progenitor cells in the embryonic somite. *Stem Cells*. 2009; 27:2769–2780. [PubMed: 19785007]
16. Gulino A, Di Marcotullio L, Screpanti I. The multiple functions of Numb. *Exp Cell Res*. 2009 Epub ahead of print. 10.1016/j.yexcr.2009.11.017
17. Petersen PH, Zou K, Krauss S, Zhong W. Continuing role for mouse Numb and Numbl in maintaining progenitor cells during cortical neurogenesis. *Nat Neurosci*. 2004; 7:803–811. [PubMed: 15273690]
- 18*. Lagha M, Kormish JD, Rocancourt D, Manceau M, 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; 22:1828–1837. This study showed that *Fgfr4* is a direct target of Pax3 that promotes myogenic differentiation. *Sprouty1*, a negative regulator of receptor tyrosine kinase signaling, promotes self-renewal of progenitors, and its expression is also controlled by Pax3. [PubMed: 18593883]
19. Manceau M, Gros J, Savage K, Thomé V, McPherron A, Paterson B, Marcelle C. Myostatin promotes the terminal differentiation of embryonic muscle progenitors. *Genes Dev*. 2008; 22:668–681. [PubMed: 18316481]
20. Amthor H, Otto A, Vulin A, Rochat A, Dumonceaux J, Garcia L, Mouisel E, Hourdé C, Macharia R, Friedrichs M, et al. Muscle hypertrophy driven by myostatin blockade does not require stem/precursor-cell activity. *Proc Natl Acad Sci (USA)*. 2009; 106:7479–7484. [PubMed: 19383783]
- 21*. 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; 23:997–1013. This study used conditional mutagenesis approaches in the mouse to trace Pax3⁺ and Pax7⁺ lineages and their contributions to trunk and limb muscle development. [PubMed: 19346403]
22. Bober E, Franz T, Arnold HH, Gruss P, Tremblay P. Pax-3 is required for the development of limb muscles: a possible role for the migration of dermomyotomal muscle progenitor cells. *Development*. 1994; 120:603–612. [PubMed: 8162858]
23. Oustanina S, Hause G, Braun T. Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification. *EMBO J*. 2004; 23:3430–3439. [PubMed: 15282552]

24. Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA. Pax7 is required for the specification of myogenic satellite cells. *Cell*. 2000; 102:777–786. [PubMed: 11030621]
25. Kassar-Duchossoy L, Gayraud-Morel B, Gomes D, Rocancourt D, Buckingham M, Shinin V, Tajbakhsh S. Mrf4 determines skeletal muscle identity in Myf5:Myod double-mutant mice. *Nature*. 2004; 431:466–471. [PubMed: 15386014]
26. Rudnicki MA, Schneglesberg PNJ, Stead RH, Braun T, Arnold H-H, Jaenisch R. MyoD or Myf-5 is required for the formation of skeletal muscle. *Cell*. 1993; 75:1351–1359. [PubMed: 8269513]
- 27*. Gensch N, Borchardt T, Schneider A, Riethmacher D, Braun T. Different autonomous myogenic cell populations revealed by ablation of Myf5-expressing cells during mouse embryogenesis. *Development*. 2008; 135:1597–1604. [PubMed: 18367555]
- 28*. Haldar M, Karan G, Tvrdik P, Capecchi MR. Two cell lineages, myf5 and myf5-independent, participate in mouse skeletal myogenesis. *Dev Cell*. 2008; 14:437–445. This study and [27*] used conditional mutagenesis in the mouse to identify a *Myf5*-independent population of muscle precursors that expresses *MyoD* and is sufficient to produce normal body musculature. Ablation of cells that express *MRF4* or *myogenin* [27*] cannot be similarly compensated. [PubMed: 18331721]
29. Kanisicak O, Mendez JJ, Yamamoto S, Yamamoto M, Goldhamer DJ. Progenitors of skeletal muscle satellite cells express the muscle determination gene, *MyoD*. *Dev Biol*. 2009; 332:131–141. [PubMed: 19464281]
30. 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; 89:127–138. [PubMed: 9094721]
- 31*. Sambasivan R, Gayraud-Morel B, Dumas G, Cimper C, Paisant S, Kelly RG, Tajbakhsh S. Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates. *Dev Cell*. 2009; 16:810–821. This study identified the distinct genetic determinants required for formation of various head muscles and how these differ from those of trunk and limb muscles. [PubMed: 19531352]
- 32*. Harel I, Nathan E, Tirosh-Finkel L, Zigdon H, Guimarães-Camboa N, Evans SM, Tzahor E. Distinct origins and genetic programs of head muscle satellite cells. *Dev Cell*. 2009; 16:822–832. This study used lineage tracing in the chick and mouse to identify the developmental origins of various craniofacial muscles and their associated satellite cells. [PubMed: 19531353]
- 33*. Tzahor E. Heart and craniofacial muscle development: a new developmental theme of distinct myogenic fields. *Dev Biol*. 2009; 327:273–279. A timely review on the similarities between development of cardiac and head skeletal muscles. [PubMed: 19162003]
34. Kuang S, Gillespie MA, Rudnicki MA. Niche regulation of muscle satellite cell self-renewal and differentiation. *Cell Stem Cell*. 2008; 2:22–31. [PubMed: 18371418]
35. Zammit PS. All muscle satellite cells are equal, but are some more equal than others? *J Cell Sci*. 2008; 121:2975–2982. [PubMed: 18768931]
36. Ono Y, Boldrin L, Knopp P, Morgan JE, Zammit PS. Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomeric muscles. *Dev Biol*. 2010; 337:29–41. [PubMed: 19835858]
37. Cerletti M, Jurga S, Witzak CA, Hirshman MF, Shadrach JL, Goodyear LJ, Wagers AJ. Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. *Cell*. 2008; 134:37–47. [PubMed: 18614009]
38. Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell*. 2005; 122:289–301. [PubMed: 16051152]
39. Kuang S, Kuroda K, Le Grand F, Rudnicki MA. Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell*. 2007; 129:999–1010. [PubMed: 17540178]
40. Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, Partridge T, Buckingham M. Direct isolation of satellite cells for skeletal muscle regeneration. *Science*. 2005; 309:2064–2067. [PubMed: 16141372]
- 41*. Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM. Self-renewal and expansion of single transplanted muscle stem cells. *Nature*. 2008; 456:502–506. This study used sensitive

bioluminescence imaging to show that single cells sorted for satellite cell markers could both self-renew and differentiate after transplantation, formally demonstrating stem cell properties at the single cell level. [PubMed: 18806774]

- 42*. Le Grand F, Jones AE, Seale V, Scimè A, Rudnicki MA. Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. *Cell Stem Cell*. 2009; 4:535–547. Satellite cells that never expressed *Myf5* display stem cell properties, including asymmetric cell division [39]. This study showed that these cells express the Wnt receptor Fzd7, which signals via the planar cell polarity pathway to promote symmetric divisions that expand the stem cell pool. [PubMed: 19497282]
43. Zammit PS, Golding JP, Nagata Y, Hudon V, Partridge TA, Beauchamp JR. Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? *J Cell Biol*. 2004; 166:347–357. [PubMed: 15277541]
- 44*. Tanaka KK, Hall JK, Troy AA, Cornelison DD, Majka SM, Olwin BB. Syndecan-4-expressing muscle progenitor cells in the SP engraft as satellite cells during muscle regeneration. *Cell Stem Cell*. 2009; 4:217–215. This study sorted mononuclear muscle cells for both satellite and SP cell markers to identify a rare population of cells that engraft the satellite cell niche with high efficiency after injury. [PubMed: 19265661]
45. Christov C, Chrétien F, Abou-Khalil R, Bassez G, Vallet G, Authier FJ, Bassaglia Y, Shinin V, Tajbakhsh S, Chazaud B, et al. Muscle satellite cells and endothelial cells: close neighbors and privileged partners. *Mol Biol Cell*. 2007; 18:1397–1409. [PubMed: 17287398]
46. Abou-Khalil R, Le Grand F, Pallafacchina G, Valable S, Authier FJ, Rudnicki MA, Gherardi RK, Germain S, Chretien F, Sotiropoulos A, et al. Autocrine and paracrine angiopoietin 1/Tie-2 signaling promotes muscle satellite cell self-renewal. *Cell Stem Cell*. 2009; 4:298–309. [PubMed: 19733541]
47. Conboy IM, Rando TA. The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. *Dev Cell*. 2002; 3:397–409. [PubMed: 12361602]
48. Brack AS, Conboy IM, Conboy MJ, Shen J, Rando TA. A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. *Cell Stem Cell*. 2008; 2:50–59. [PubMed: 18371421]
49. Brack AS, Murphy-Seiler F, Hanifi J, Deka J, Eyckerman S, Keller C, Aguet M, Rando TA. BCL9 is an essential component of canonical Wnt signaling that mediates the differentiation of myogenic progenitors during muscle regeneration. *Dev Biol*. 2009; 335:93–105. [PubMed: 19699733]
50. Perez-Ruiz A, Ono Y, Gnocchi VF, Zammit PS. β -catenin promotes self-renewal of skeletal-muscle satellite cells. *J Cell Sci*. 2008; 121:1373–1382. [PubMed: 18397993]
51. Otto A, Schmidt C, Luke G, Allen S, Valasek P, Muntoni F, Lawrence-Watt D, Patel K. Canonical Wnt signalling induces satellite-cell proliferation during adult skeletal muscle regeneration. *J Cell Sci*. 2008; 121:2939–2950. [PubMed: 18697834]
52. 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; 364:501–506. [PubMed: 8393145]
53. Meadows E, Cho JH, Flynn JM, Klein WH. Myogenin regulates a distinct genetic program in adult muscle stem cells. *Dev Biol*. 2008; 322:406–414. [PubMed: 18721801]
- 54*. Lepper C, Conway SJ, Fan CM. Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. *Nature*. 2009; 460:627–631. This study shows that Pax7 is not required for adult regenerative myogenesis after three weeks of age even in response to repetitive injury. Pax3 does not act as a compensatory factor. [PubMed: 19554048]