Skeletal muscle formation in vertebrates

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Research in the past year has added to our understanding of the signalling systems that specify myogenic identity in the embryo and of the regulation and roles of MyoD family members. New insights into the movement of muscle precursor cells include the demonstration that Lbx1 is essential for their migration from the somite to some but not all sites of muscle formation elsewhere. Later in development, ras as well as calcineurin signalling is now implicated in the definition of slow versus fast fibre types. The myogenic identity of precursor cells in the adult depends on Pax7, the orthologue of Pax3 which is required for early myogenesis; this finding is of major importance for muscle regeneration and the active field of stem cell research.

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Abbreviations

cdk	cyclin-dependent kinase
IGF	insulin growth factor
FGF	fibroblast growth factor
MRF	myogenic regulatory factor
PDGF	platelet-derived growth factor
TGF-β	transforming growth factor β
Shh	Sonic hedgehog

Introduction

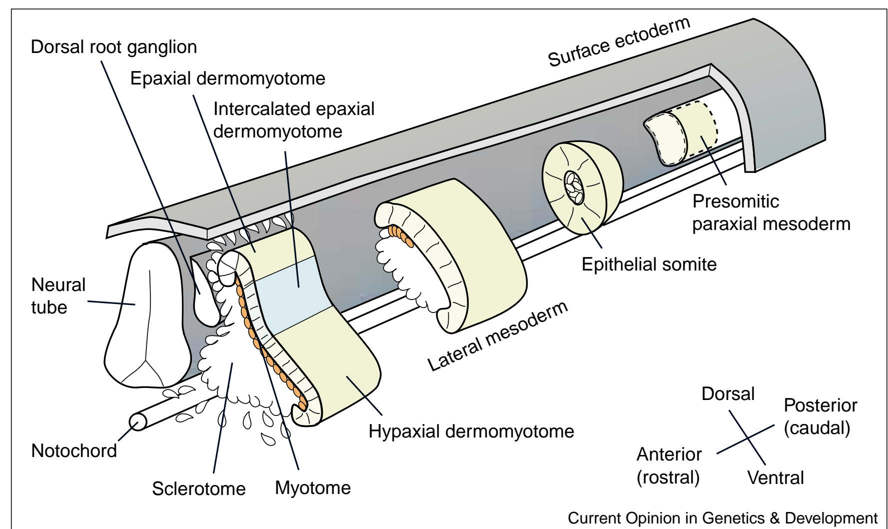
Skeletal myogenesis is initiated in the embryo as a result of signalling molecules from surrounding tissues that specify myogenic cell fate. Further identification of such signalling systems, together with the expression of their intracellular components and inhibitors, continues to be an active area of research. Such studies tend to concentrate on one system, although the interaction between pathways is beginning to be explored. The integration of positive and negative signals at the level of the target genes is an important facet of this. Molecular data are not yet available, but transgenic analysis of DNA sequences which regulate *Myf5* reveals the complexity of elements required to direct the full spatio-temporal expression of this myogenic determination factor. Myf5 and MyoD are required for the acquisition of myogenic identity. Their role in chromatin remodeling is being explored and it has been shown that MyoD, and also MEF2 involved in the transcriptional activation of muscle genes, interact with enzymes which modify chromatin structure. The relation between myogenic factors such as Myf5 and MyoD, expressed in dividing myoblasts, and cell cycle regulators is another focus of interest. It is not clear how these factors influence decisions to either divide, differentiate or enter a quiescent state, but their interaction with cell cycle components and their degradation in a specific phase of the cycle suggest that this may be the case. In this context Myf5 differs from MyoD. These observations, made with cultured cells, are not yet fully integrated into the *in vivo* picture, but further analysis in the mouse of mutants and mutant combinations for this family of myogenic regulators continues to reveal distinct functions in muscle differentiation. An unexpected phenotype associated with some of these mutations is rib malformation. Recent papers have addressed the origin of rib precursors. In addition to possible cellular effects of perturbations in myogenesis on these cells, there is now evidence that interference with sequences within the *Myf5* locus probably affects another gene implicated in rib formation.

Muscle progenitor cells migrate from the somite, where they originate, to found muscle masses in the body and limbs. Comparison of fin muscle formation between different types of fish provides insight into the evolution of appendicular muscle formation. In higher vertebrates, a number of genes have been implicated in this process; interestingly mutations in some of them in the mouse affect specific limb muscles leaving others intact. The molecular targets of the homeobox proteins that show such differential effects is not yet known. In the last few years, the Six family of homeodomain proteins, which are also probably important for the muscle progenitor cell population, have been identified as major players, with MEF2 and MyoD family members, in the transcriptional activation of muscle genes in differentiating muscle cells. Recently, progress has been made in understanding how fibre type specific muscle gene expression is regulated. This later aspect of muscle development had long remained obscure. Intracellular signalling pathways and potential transcriptional effectors are now being characterized. Lastly, research on another aspect of skeletal muscle formation, that of regeneration in the adult, is advancing rapidly. More markers for muscle precursor cells are becoming available and the topical issue of a stem cell contribution has been the subject of a number of recent publications. The analogy with myogenesis in the embryo is underlined by the recent demonstration that a transcription factor of the Pax family, closely related to Pax3, an important regulator of early muscle formation, is a key determinant for adult muscle cells.

Specification of myogenic identity by signalling molecules

Most skeletal muscle in vertebrates forms from progenitor cells present in somites which arise by segmentation of paraxial mesoderm on either side of the neural tube and notochord. Craniofacial muscles derive from prechordal and presomitic as well as somitic paraxial mesoderm (see [1]). Signals from the surrounding tissues lead to the specification of myogenic and dermal progenitors in the dorsal somite, which initially retains an epithelial structure, the

Figure 1



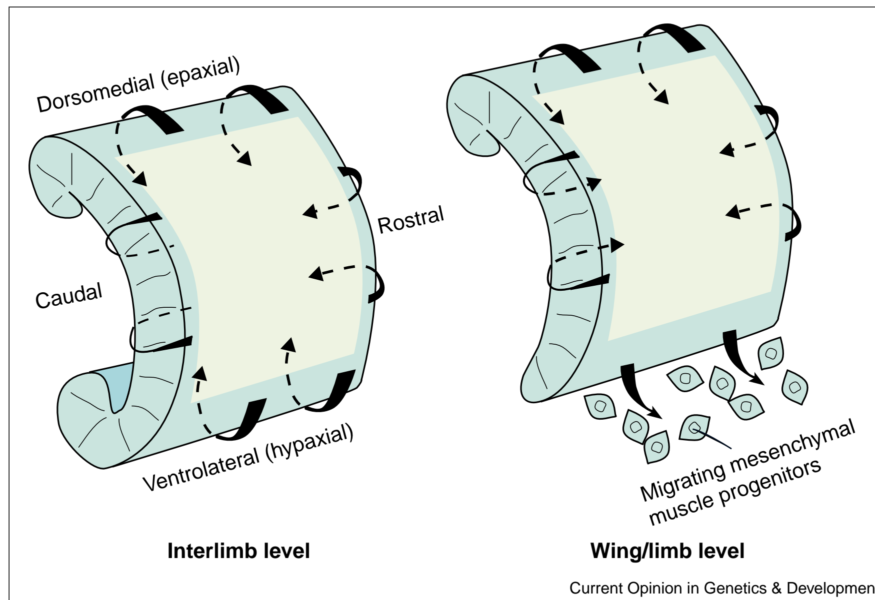
Schematic representation of vertebrate somitogenesis as it occurs in the mouse embryo. Somites are formed and mature following a rostrocaudal gradient on either side of the axial structures.

dermomyotome. In the ventral somite, signalling specifies the mesenchymal cells of the sclerotome which will contribute the axial skeleton (see Figure 1). In the past decade many candidate signalling molecules implicated in myogenic cell specification have been described. Myogenic identity is acquired as a result of activation of the myogenic determination genes, notably those encoding the bHLH proteins Myf5 and/or Myod. Wnts produced by the dorsal neural tube and surface ectoderm and Sonic hedgehog (Shh) from the notochord and floor plate of the neural tube have been identified as positive effectors of myogenesis (see [2]).

Ectopic expression of Wnts 1, 3a, and 4 overrides ventralising signals in the chick somite leading to the activation of genes encoding dorsal markers such as Pax3, Paraxis and Myod [3]. The Wnt receptor, Frizzled1, as well as intracellular effectors of Wnt signalling, β-catenin and the transcription factor TCF, are already present in the presomitic mesoderm, but are upregulated in the dorsal somite prior to *Myod* activation. Later TCF and β-catenin become restricted to the myotome [4•]. The adjacent tissues regulate their expression, as do the candidate signalling molecules which they produce; *β-catenin* expression in the myotome depends on Wnt1 or 3a, acting with Shh, suggesting that these Wnts, produced by the neural tube, activate myogenesis through the TCF/β-catenin pathway. This is in contrast to Wnts 5a and 7a, which, together with Wnt11, act through another Ca^{2+}-dependent intracellular pathway (see [5]). This class of Wnts alter cell movement, reducing cell adhesion; in the zebrafish, Wnt11 is necessary for cell movement at gastrulation [6]. In Amphioxus, *Wnt11* is expressed in a pattern complementary to *Wnt8* in the medial edge of the somite where myotome forms in this chordate [7]. Interestingly, *Wnt11* is expressed in avian somites in the epaxial lip of the dermomyotome from which muscle progenitor cells will migrate to the myotome [8]. Secreted forms of the Wnt receptors, known as Frzb and/or Sfrp, bind Wnts and potentially inhibit their function. Both *Frzb* and *Sfrp2* genes are expressed in the myotome in chick embryos where they may titrate Wnt signalling [9]. Sfrp2, present at lower levels in the presomitic mesoderm and dorsal somite, is expressed at a high level in the sclerotome of mouse embryos. In explant experiments, it specifically inhibits the action of Wnts 1 and 4. *Sfrp2* expression is upregulated by Shh, thus providing a mechanism for antagonising the dorsaling effect of Wnt1 and Wnt4 signalling on sclerotome development [10•].

Reciprocally, Wnts regulate effectors of the Shh signalling pathway — the Gli zinc finger transcription factors [11•]. Initially *Gli1*, *2* and *3* are expressed throughout the avian epithelial somite, but subsequently *Gli1* transcripts acquire a more ventral localisation, whereas Gli2 and 3 become restricted to the dermomyotome and myotome. Surface ectoderm initially represses *Gli3* expression in presomitic paraxial mesoderm, while its presence is subsequently necessary for expression of *Gli2* and *3* as the somite forms. Wnts 1 and 4 can mimic these effects, through a β-catenin dependent pathway. As these Wnts are mainly produced by the neural tube, this would suggest that their effects may be additional to those of surface ectoderm. *Gli1* expression is regulated by Shh, which also plays a role in the dorsalisation of *Gli2* and *Gli3* expression in the somite. In the *Shh* null mouse, *Myf5* expression and myogenesis are specifically compromised in the epaxial domain of the somite [12], although some expression is still detectable in this region of the myotome adjacent to the neural tube/notochord [13], suggesting that in this domain other signalling systems such as that of the Wnts act independently of Shh, which may be required only for activation of the epaxial *Myf5* enhancer in the dermomyotome (see [14•]). Hypaxial myogenesis in this mutant is not compromised, although subsequent development of limb muscle masses is severely affected. Experiments with limb cultures suggest that Shh may be necessary to maintain the

Figure 2



Schematic representation of proposed muscle progenitor cell migration from the edges of the dermomyotome to the underlying myotome and from the hypaxial dermomyotome to more distant sites of muscle formation.

expression of myogenic regulatory factors and hence on-going myogenesis [13].

If negative regulators of positive signals, such as the Frzb/Sfrp Wnt antagonists expressed in the ventral somite, may modulate the effects of these pathways, myogenesis is also negatively regulated by BMP signalling, which has been shown to be antagonized in the dorsal somite by noggin (see [2]). Another candidate negative regulator of myogenesis is Notch signalling. Indeed it has been proposed that this may antagonise Wnt signalling (see [15]), either by binding of Wnt to the Notch receptor, or more probably at the level of Dishevelled, potentially involved in the intracellular signalling of both pathways. A further level of interaction has now emerged with the demonstration that Presenilin1, involved in the processing of Notch, also binds to β-catenin, negatively regulating β-catenin/TCF dependent signalling and hence antagonizing the action of the class of Wnts which act through this pathway [16]. Notch receptors and ligands are present at sites of myogenesis and therefore potentially implicated in this later process as well as playing a key role in the segmentation of paraxial mesoderm where Notch signalling orchestrates the somitic clock (see [17]). The presence of several isoforms may account for the lack of an obvious myogenic phenotype in mouse mutants where somitogenesis is perturbed. In addition to possible indirect effects, activation of the Notch pathway has been shown to inhibit *Myod* expression and muscle differentiation in cultured myogenic cells, and recently a similar effect has been demonstrated *in vivo* in the somite [18•] and limb [19•] of chick embryos. Retroviral mediated over-expression of Delta1 leads to down-regulation of *Myod* in postmitotic cells and prevents muscle cell differentiation. There is no effect on *Pax3* or *Myf5* expression. This would suggest that, in contrast to neurogenesis, where cell fate decisions and cell cycle exit are affected, activation of Notch signalling acts downstream of these events during myogenesis in the avian embryo.

In addition to regulation of myogenesis through effects on the expression of *Myf5* or *Myod*, signalling molecules like the Wnts and Shh, also potentially act on the proliferation and survival of myogenic cells. Other factors implicated in myogenesis can also act at this level. Insulin and IGFII, produced by the neural tube and by somites, act synergistically with Shh, bFGF and TGF-β to promote myogenesis in somite cultures [20•]. They also stimulate somite cell proliferation and influence apoptosis. *FGF4* expression in the myotome is directly regulated by Shh, as well as by myogenic regulatory factors (MRFs) [21]. Another regulatory influence on the onset of myogenesis in the embryo is the community effect. In an assay designed to test candidate community factors, it was found that eFGF can mimic the interaction between nearby precursor cells necessary for the maintenance of tissue specific gene expression and differentiation [22]. In the presence of eFGF, a single muscle precursor cell will differentiate. Furthermore eFGF is expressed in muscle precursor cells of the *Xenopus* embryo at the appropriate time to mediate the community effect.

Cell movement from the dermomyotome to the myotome

Prior to muscle cell differentiation, muscle progenitor cells are located in the dermomyotome. These cells receive dorsal signals and enter the myogenic programme, with expression of myogenic determination genes already in the epaxial (and hypaxial) dermomyotome (see [2]). Indeed, the dorso/ventral axis of the somite is determined very rapidly after formation of the epithelial somite, with acquisition of myogenic cell fate preceding that of the sclerotome,

ventrally (see [23]). The way in which myogenic precursor cells move from the dermomyotome to form the myotome has been a subject of controversy (Figure 2). On the basis of cell labelling experiments on inter-limb level chick somites, Denetclaw and Ordahl [24•] show that cells delaminate from the length of the dorsomedial and ventrolateral lips of the dermomyotome and then translocate to form the differentiated fibres of the myotome which have a rostral/caudal orientation. Kalcheim and co-workers [25•] also identify the dorsomedial and ventrolateral lips as major sites of delamination, followed by longitudinal migration to the rostral/caudal edges of the dermomyotome where the cells then differentiate. This group has stressed that these edges of the dermomyotome also give rise to delaminating muscle precursors. It is notable that in the *Myf5* mouse mutant, muscle precursor cells which already transcribe the gene, accumulate along all four edges of the dermomyotome (see [2]). Cells in the sublip region of the chick somite are Myod and Myf5 positive, but do not yet express the myoblast marker desmin. They are still dividing and express the FGF-4 receptor (FREK) [25•]. This is in contrast to the first wave of myogenic cells, described as pioneers [26], which arise from the medial part of the early epithelial somite as post-mitotic Myod-positive cells. As development proceeds, the dermomyotome elongates dorsomedially as well as ventrolaterally [24•], giving rise to the epaxial and hypaxial somitic buds, respectively (Spörle, 2001). At this stage in amniotes, an intercalated epaxial domain can be distinguished on the basis of specific gene expression patterns (e.g. *En1*). Early differentiating myogenic cells underlie this domain, which leads to an interesting comparison with the adaxial domain of more primitive vertebrates such as zebrafish [27•].

Myf5 gene regulation

Either directly or indirectly, the signalling pathways that influence the onset of myogenesis lead to activation of the myogenic determination genes *Myf5* and *Myod*. *Myod* regulation depends on proximal and distal enhancer sequences, necessary for later fœtal and embryonic expression, respectively (see [2]). It has been shown recently that *Myf5* regulatory sequences extend over at least 96 kbp upstream of the mouse gene [14•]. More proximal elements are involved in branchial arch and early somite expression; an epaxial enhancer drives the earliest *Myf5* expression in the epaxial dermomyotome [28•]. An enhancer element located between –58/–48 kbp from *Myf5* directs expression to the myotome, to sites in the brain where the gene is transcribed (but the protein does not accumulate [29]), and to the limbs [14•]. Sequences further upstream are necessary for the expression of the gene at later stages in head and some trunk muscles. These and previous papers on the regulation of *Myf5* (see [2]) point to the integration of multiple signals necessary for the correct spatiotemporal expression of this key myogenic determination gene. The gene encoding another MRF, Mrf4, probably involved, like Myogenin, in myogenic differentiation rather than determination, lies immediately upstream of *Myf5* and it is not always clear at present which sequences in, and upstream of, the locus regulate which gene. It is also not yet known which sequence motifs and transcription factors translate the signalling information essential for expression. The latter is also true for *Myod*.

Given the complexity of regulatory sequences for the mouse *Myf5* gene, it is surprising that the expression pattern of the equivalent gene in the zebrafish embryo can be reproduced with a green fluorescent protein transgene under the control of only 82 bp of upstream sequence [30]. It is also interesting to note significant expression of zebrafish *Myf5* in presomitic mesoderm as well as in somites.

Myogenic regulatory factors: molecular aspects

In the context of their upstream role as myogenic determination factors, expressed prior to muscle cell differentiation, Myf5 and Myod are potentially involved with cell-cycle regulation and chromatin remodelling.

In the muscle cell lines where cell-cycle regulation has been studied, both genes are expressed by dividing myoblasts. There is considerable recent literature (see [31]) on the interaction between these myogenic factors and the cell cycle. Myf5 and Myod are specifically degraded at mitosis and G_1/S, respectively, and this is mediated by phosphorylation via cyclin-dependent kinases (cdks). A high level of Myf5 at mitosis perturbs cell cycling [32]; Myf5, but not Myod is high in G_0 quiescent myoblasts (see [31]). Stabilisation of Myod, due to inhibition of G_1/S cdks and also to direct interaction with p57 (a cdk inhibitor) [33] or pRb (retinoblastoma protein; an E2F inhibitor) is associated with cell cycle arrest, leading to differentiation [34]. In the absence of Myf5 or Myod, myoblast cell growth is compromised [35]. There is little information on the regulation of the cell cycle/onset of myogenesis *in vivo*, although severe defects in skeletal muscle development are seen in mice lacking both p21 and p57 (cdk inhibitors) [36]. This important aspect, with the potential implication also of asymmetric cell division in the acquisition of myogenic cell fate, documented in *Drosophila* (see Baylies and Michelson, this issue [pp 431–439]) remains to be explored in the vertebrate embryo.

In a classic paper, Gerber *et al.* [37] showed that myogenic regulatory factors, especially Myf5 and Myod, can re-model chromatin through protein domains which are distinct from those involved in transcriptional activation *per se*. Myod has been shown to interact with the BRG1 and BRM (SWI/SNF) subunits of the chromatin-remodelling ATPase. This promotes myogenic conversion of fibroblasts, altering the chromatin structure of muscle genes such as *MCPK* [38•]. In the past few years, a number of papers have shown interactions between Myod and the histone transacetylases pCAF and CBP/p300 which function as transcriptional co-activators. Furthermore, acetylation of Myod by pCAF [39•] or p300 [40•] is necessary for myogenic conversion and increases its affinity for muscle specific promoters, hence

promoting myogenic differentiation. In contrast, histone deacetylation inhibits gene activity; interaction between histone deacetylase HDACI and Myod prevents premature activation of the myogenic programme in dividing myoblasts [41•]. Similarly, interaction of MEF2 transcription factors, which are regulated by MRFs such as Myod, with HDACIV and V, suppresses myogenic differentiation [42•,43•]. Repression can be overcome by calcium–calmodulin-dependent kinase signalling which induces nuclear export of these HDACs, thus releasing MEF2 and the block on muscle cell differentiation [43•,44].

Myogenic regulatory factors: specific roles in muscle differentiation

Studies on cultured cells and mutant mouse embryos have demonstrated that Myf5 and Myod are involved in determining skeletal muscle cell fate with a potential role in remodeling chromatin and regulating the crucial balance between cell proliferation and differentiation. This is also the case in a rare example of transdifferentiation from smooth to skeletal muscle in the developing oesophagus; a change in cell fate that is Myf5-dependent [45]. Myogenin, Mrf4 and also Myod are implicated in the subsequent activation of muscle-specific genes during myogenic differentiation. Compound mutants have given further information on the extent to which each factor has a specific role. Compensation between family members depends, of course, on their levels and patterns of expression as well as their function. Levels of the differentiation factor Myogenin may be critical but differences in function are revealed by an assay with *Myogenin* null ES cells where large fully differentiated muscle fibres are recovered by over-expression of *Myogenin* but not *Myod* [46]. In the absence of Myogenin, Myod and Mrf4, muscle differentiation does not occur either *in vivo* or in cell culture, demonstrating that Myf5 alone cannot activate differentiation [47]. Interestingly desmin staining, which marks myoblasts, is absent, indicating that the muscle precursor cells present in the myotome and elsewhere do not progress to this point. Valdez *et al.* [47] argue that the muscle phenotype is not simply caused by insufficient MRF levels on the basis of observations on heterozygote/homozygote mutant combinations.

Myogenesis and distal rib formation

Mutation of *Myf5* prevents the formation of the early myotome but also has an unexpected effect on distal rib formation. This is also seen with mutations in the adjacent *Mrf4* gene, possibly as a result of perturbations which these mutations produce in *Myf5* transcription [48]. Removal of the *PGKneo* selection cassette from two *Myf5* mutant alleles restored correct rib formation, leading to the suggestion that the cassette perturbs the transcription of other genes in the locus necessary for rib formation [49•]. In further *Myf5* null alleles, however, the insertion of other coding sequences, irrespective of either the presence or absence of the *neo* cassette, results in varying degrees of rib abnormality [50]. The exact site of insertion in the locus would appear to be critical in this respect. In addition to perturbation of the *Mrf4–Myf5* locus, mutation of the *Myogenin* gene also leads to a mild rib phenotype, which becomes more severe in double or triple mutants with *Mrf4* or *MyoD*. Early muscle defects are notable, particularly in the hypaxial myotome, and, later, in intercostal muscle morphology [51].

In attempting to explain the cause of the rib phenotype, it is important to establish the embryological origin of the ribs. Manipulation of chick/quail chimaeras establishes that the distal ribs derive from the lateral half of the somite [52]. Classically, somitically derived cartilage and bone were believed to originate from the sclerotome where *MRFs* are not normally expressed but it has been proposed that the distal ribs arise from the hypaxial dermomyotome [53], although this has been challenged [54]. If distal rib precursors, like those of skeletal muscle, originate from a common pool of dermomyotomal progenitors it is understandable that in the absence of a muscle determination factor such as Myf5 their fate may be perturbed. Indeed it is notable that embryos lacking Pax3, necessary for the maintenance of the hypaxial dermomyotome, also have a rib phenotype (see [55]). However, Pax3 is also required for the formation of some hypaxial musculature, so that an indirect effect of *Pax3* and *MRF* mutations on the sclerotomal cells underlying the hypaxial myotome, via a defect in the initial formation of this muscle, is probable. This may be as a result of a lack of growth factor production, such as FGFs (but see [49•]) or PDGFA [50]. Later muscle perturbations may also affect rib outgrowth. Clarification of this phenomenon will be facilitated when 'clean' mutations are generated which affect only *Mrf4* or *Myf5*.

The hypaxial dermomyotome and muscle cell migration

The hypaxial dermomyotome in amniotes gives rise to muscle in two distinct ways (Figure 2). Ventrolateral extension contributes the hypaxial myotome from which ventral body wall muscles, for example, are derived at the interlimb level. Alternatively, migratory mesenchymal precursor cells leave the dermomyotome to found more distant muscle masses such as those of the limbs. Neyt *et al.* [56•] in a paper which addresses the evolution of appendicular musculature, show that in a teleost fish, the zebrafish, fin muscle is formed in this way whereas in the chondrichthyan dogfish, the more primitive mechanism of myotome elongation underlies the formation of some if not all fin muscles (see [27•]).

A number of homeobox genes are implicated in the survival, delamination and migration of muscle progenitor cells from the hypaxial dermomyotome to sites of muscle formation elsewhere in the body and limbs (see Table 1). These include *Lbx1*, expressed in such migrating cells and in the hypaxial myotome of somites which give rise to them. In three recent papers [57••–59••], *Lbx1* null mice were found to lack most hindlimb muscles and to have abnormalities in forelimb musculature, as well as milder abnormalities in hypoglossal chord derivatives such as

some tongue muscles. In these embryos, cells delaminate from an apparently normal hypaxial dermomyotome. There is no premature muscle cell differentiation, but cells fail to migrate correctly, as evidenced by accumulation beside the somite at the hindlimb level, mislocation of ventral muscle cells to dorsal muscles at the forelimb level, and retarded migration/differentiation in the hypoglossal chord. This is in contrast to *Mox2* mutants, which have defects in limb muscle formation but where muscle progenitor cells appear to migrate normally [60]. The c-Met receptor tyrosine kinase and its ligand SF/HGF are essential for the delamination of cells from the dermomyotome at all axial levels and are potentially important also for guiding migration. In their absence migrating muscle progenitors and the muscles they form are absent (see [61]). Recently it was shown that in the absence of GabI, a docking protein that interacts with c-Met, the efficiency of delamination is reduced and muscle progenitor cell migration is impaired with consequences for the formation of muscle masses derived from these cells in the limbs and diaphragm [62]. Interestingly as in the case of *Lbx1*, *Mox2* and c-*Met–Grb2* [63] mutants, specific and distinct muscles are affected.

Another homeobox gene, *En2*, is expressed specifically in migratory muscle cells of the first branchial arch and the jaw muscles derived from them. Although there are no gross defects in these muscles in mutant embryos, En2 has some effect on fibre size and metabolic state [64].

Pax3 is an important regulator of myogenesis. It is necessary for *Myod* activation in the absence of Myf5 and plays a key role in the establishment/survival of cells in the hypaxial dermomyotome, and in the delamination and migration of myogenic progenitors (see [2]). In its absence, no limb or diaphragm muscles form and other hypaxial muscles are affected. In the absence of Pax3, *Lbx1* and c-*Met* expression in the somite is severely compromised (see [61]). In an *in vivo* notochord challenge experiment, cells expressing *Pax3*, prior to *MRF* gene activation, were found to be already restricted to the muscle lineage [65]. In p19 embryonal carcinoma cells, Pax3 induces expression of *Six1* and its co-factor *Eya2* as well as *Mox1*, prior to *Myod* and *Myogenin* expression, whereas in the absence of functional Pax3 skeletal myogenesis is abolished [66]. The homeobox protein Six1, together with Six4, is expressed in the dermomyotome and in migrating myogenic progenitor cells; ectopic expression experiments in the chick embryo show that they can induce *Myod* expression. These transcription factors also play a role in muscle differentiation, acting through *MEF3* motifs essential for the activation of *Myogenin* and other muscle genes (see [67]). Compound mutations will probably be necessary in order to define their precise place in the *Pax3*/*MRF* genetic hierarchy. Mutation of mouse *Six5*, potentially involved in Steinhert's myotonic dystrophy and expressed in muscle, as well as other tissues in the embryo, does not give a muscle phenotype, although the mutants have other features of the disease [68,69].

Table 1

Homeobox proteins implicated in the formation of limb muscles, prior to the onset of myogenesis.

Gene	Mutant phenotype/Potential function
Pax3−/−	Disintegration of the hypaxial dermomyotome, loss of progenitor muscle cells, no determination, no migration, no limb muscles.
c-Met−/−/*HGF*−/−	Hypaxial dermomyotome intact, muscle progenitor cells fail to delaminate – no limb muscles.
Gab1−/−	Delamination impaired, migration impaired – specific muscles affected.
Lbx1−/−	Muscle progenitor cells delaminate but fail to migrate/locate correctly – specific muscles affected in the forelimb, reduction or loss of hindlimb muscles.
Mox2−/−	Migration apparently normal – specific muscle defects with reduction in size in the forelimb, reduction or loss of hindlimb muscles.
Msx1	Potentially involved in the proliferation versus differentiation of muscle progenitor cells in the forelimb (see [80]).
Six1,4(5)	Expressed in the hypaxial dermomyotome and migrating muscle progenitor cells as well as limb muscles. Potentially implicated in muscle cell specification as well as later differentiation.

Later muscle development: regulation of muscle fibre type

The onset of muscle formation in the amniote embryo corresponds to so-called 'primary' myogenesis, followed, from about embryonic day 14 in the mouse, by a second wave of 'secondary' muscle fibre formation, as a result of proliferation and differentiation of myoblasts which had remained quiescent. This correlates with the onset of innervation, leading subsequently to the acquisition of mature fibre type identity (see [70]). The molecular mechanisms regulating these later stages of muscle development remain relatively unexplored; however, recently the signalling pathways and transcriptional factors implicated in controlling slow versus fast fibre phenotypes have begun to be elucidated. Slow fibres will revert to a fast phenotype on denervation. It has been proposed that a calcineurin-activated Ca^{2+}-dependent pathway acting through the NFAT transcription factor plays an important role in controlling gene expression in slow fibres (see [71]). Slow muscle specific regulatory sequences do not necessarily have critical NFAT sites [72], however, and MEF2 now emerges as another mediator of calcineurin signalling and of other Ca^{2+}-dependent signalling pathways potentially involved in transducing the signal from the nerve (see [71]). In addition, slow fibre type specificity has been shown to depend on an Ebox motif [73,74], which in the case of the slow *TroponinI* gene binds a TFII-1 like factor [73]. Ras signalling through a MAPK/ERK pathway is another important mediator of slow muscle specific gene activation, mimicking the effect of slow motor neurons on myosin gene expression [75••]. There may be several convergent pathways that regulate fibre type specific gene expression.

Regeneration: satellite cells and stem cells

Satellite cells which lie between the basal lamina and the muscle fibre constitute a reservoir of undifferentiated muscle precursor cells that are activated in response to muscle damage, leading to regeneration of adult skeletal muscle (see [76]). They also play an important role in postnatal growth. Although many of the regulatory genes implicated in muscle formation in the embryo are also important in muscle formation by satellite cells, the source of these cells may be quite different from that of embryonic muscle. It has been suggested that they may have an endothelial cell origin [77] and, indeed, adult satellite cells express endothelial, as well as myogenic, markers. Quiescent satellite cells, visualised on isolated muscle fibres, express an isoform of CD34, a marker of haematopoietic stem cells, as well as Myf5, detected as β-galactosidase labelling in a *Myf5–nlacZ* heterozygote mouse [78], consistent with Myf5 accumulation in quiescent G_o myoblasts (see [31]). *Myf5* transcripts, however, were not detected in a PCR-based analysis of isolated satellite cells [79] where, interestingly, mRNA for myostatin, a TGF-β like factor implicated in limiting muscle growth, and Msx1 a homeobox protein implicated in the negative regulation of myogenesis (see [80]), marked quiescent cells. Once satellite cells are activated, cell-cycle markers and *Myod* as well as *Myf5* transcripts are now detectable. Subsequent satellite cell differentiation is marked by the appearance of *Myogenin*, but not *Mrf4* transcripts. Myod is known to be important for muscle regeneration (see [76]) and in this analysis most satellite cells from *Myod* mutant mice failed to differentiate; those that did were Myf5-positive. c-*Met* transcripts mark satellite cells but *Pax3* transcripts have not been detected. However, the orthologue of Pax3, Pax7 is present and recently it has been shown to play an essential role in the specification of these cells; in *Pax7* null mice, satellite cells are absent [81••].

In recent years, the presence of multipotent cells, which are capable of contributing to skeletal muscle, has been demonstrated, first in bone marrow and then in muscle itself. In *Pax7* mutants, the haematopoietic potential of these cells is increased, suggesting that they can no longer engage in a muscle programme [81••]. The location of such cells is not known, although the presence of occasional β-galactosidase (*Myf5–nlacZ*) negative, CD34 negative cells on isolated fibres may indicate that they have a similar localisation to satellite cells [78]. They are characterised by the expression of the early haematopoietic stem cell marker Sca1; a putative stem cell clone isolated from adult muscle also expresses another marker of this type, Flk1. This clone is capable of undergoing both myogenic and osteogenic differentiation, and contributes to regeneration of these tissues *in vivo* [82]. Some mesodermal cell lines, such as 10T1/2 are multipotent and indeed even the C_2 muscle cell line can give rise to other mesodermal cell types. The nuclei of C_2 myotubes can be forced back into the cell cycle and this is now demonstrated by ectopic expression of *Msx1*. However, in this case not only were proliferating myogenic cells derived from the myotube nuclei but 10% of clones were multipotent, re-differentiating as osteoblasts, chondroblasts, adipocytes and myogenic cells [83]. This situation resembles that of regeneration in urodeles; amputation of a newt limb leads to major regeneration, with reactivation of myotube nuclei at the site of the blastema [84]. It will be interesting to see if this phenomenon is mediated by Msx1.

In conclusion, the regulatory genes necessary for the acquisition of myogenic identity and subsequent muscle regeneration are similar to those deployed in the embryo. It is not yet clear which environmental signalling molecules elicit their expression (see [76]), nor to what extent the myogenic strategy is strictly equivalent. As in the embryo, the important regulatory question of proliferative versus myogenic responses remains open, both at the stem cell and the satellite cell level.

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