

# Developmental regulation of somite derivatives: muscle, cartilage and tendon

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Recent research has broadened significantly our understanding of how the somite, a specialized mesodermal structure found in vertebrate embryos, gives rise to the cartilage, muscle and tendon cell lineages. The specification of somite derivatives involves the action of patterning signals secreted from adjacent tissue combined with the activation, in particular somitic compartments, of genes promoting cell lineage specification.

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## Abbreviations

<b>BMP</b>	bone morphogenetic protein
<b>DML</b>	dorsomedial lip
<b>Gas1</b>	Growth-arrest specific gene 1
<b>ES</b>	epaxial somite
<b>FGF</b>	fibroblast growth factor
<b>Hh</b>	Hedgehog
<b>HSPG</b>	heparan sulfate proteoglycan
<b>MEF2</b>	Myocyte enhancer 2
<b>MRF</b>	myogenic regulatory factor
<b>PSM</b>	presomitic mesoderm
<b>Sfrp2</b>	Secreted frizzled-related protein 2
<b>Shh</b>	Sonic hedgehog
<b>Smo</b>	Smoothed
<b>VLL</b>	ventrolateral lip

## Introduction

In the vertebrate embryo, the axial skeleton, skeletal muscle and dorsal dermis arise from somites (reviewed in [1]). These transient, segmentally organized mesodermal structures bud off as epithelial spheres from the cranial end of the unsegmented presomitic mesoderm (PSM) that lies on either side of the neural tube. Once formed, the epithelial somite is patterned rapidly into distinct compartments that subsequently give rise to distinct cell lineages. In response to signals from surrounding tissues, the ventral portion of the epithelial somite de-epithelializes to form the mesenchymal sclerotome, whereas the dorsal portion, or dermomyotome, remains an epithelial sheet. As the somite matures, cells delaminate from the dermomyotome edges and migrate underneath to form a third compartment, the myotome, which is located between the dermomyotome and sclerotome. The sclerotome is the source of the axial skeleton, the myotome contains skeletal muscle precursors, and the dermomyotome contributes to the dorsal dermis and skeletal muscle (see [1] and Figure 1).

Fate maps reveal that the PSM, epithelial somite and somitic compartments can be subdivided further into distinct medial

and lateral components [2–4]. The ventromedial sclerotome gives rise to the vertebral bodies, intervertebral discs, neural arches and proximal ribs; the dorsomedial sclerotome gives rise to the spinous process; and the ventrolateral sclerotome gives rise to the distal portion of the ribs — with possible distal rib contribution from the lateral dermomyotome [3,5,6].

All epaxial and hypaxial skeletal muscle (which is located, respectively, dorsal and ventral to the notochord and defined by their source of innervation) is derived from the dermomyotome [2,7]. At the dorsomedial edge or lip (DML) of the dermomyotome, cells move underneath to form the epaxial myotome [8], which then differentiates rapidly into the back muscles. Central dermomyotome cells de-epithelialize to form the dorsal dermis [3]. The ventrolateral dermomyotome's behavior depends on axial level. At limb bud levels, ventrolateral edge dermomyotome cells delaminate and migrate into the lateral plate mesoderm where they differentiate to form limb and limb girdle muscle [9]. At interlimb levels, cells at the ventrolateral edge or lip (VLL) of the dermomyotome translocate underneath to produce the hypaxial myotome [10,11]. The ventrolateral dermomyotome and myotome invade the lateral plate mesoderm together as a somitic bud, which gives rise to the body wall and abdominal muscle [12] (Figure 1).

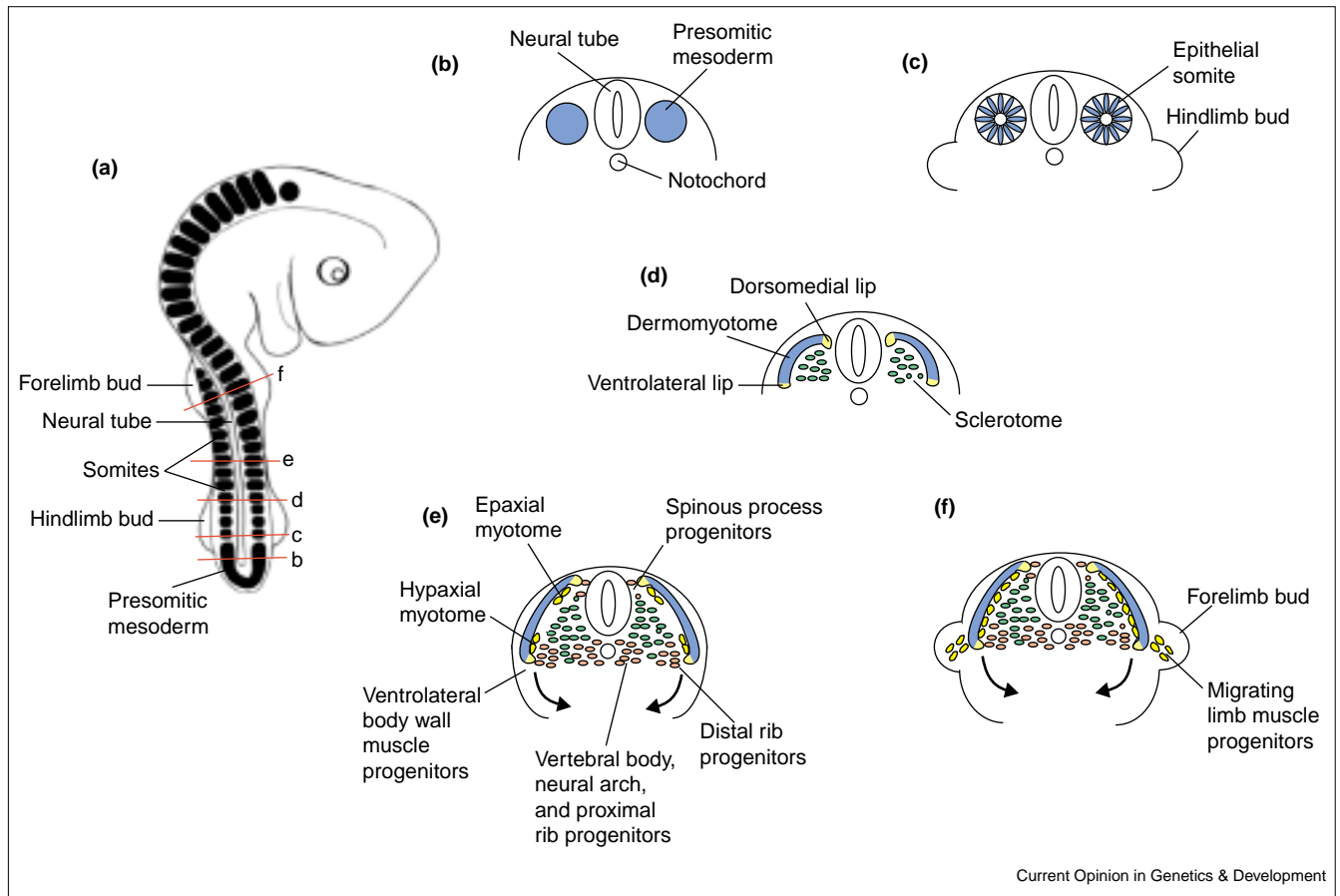
Here we review advances made in the past year in somite patterning and compartmentalization, and in the regulation of somitic derivatives.

## Myotome formation

Although the dermomyotomal origin of the myotome is well established (reviewed in [1]), the mechanism by which dermomyotomal cells generate the myotome remains the subject of much debate. Two principal models have emerged from experiments with avian embryos; although the differences between them have not been resolved as yet, in the past year much evidence has been published in support of each.

Ordahl and colleagues propose that DML [8] and VLL cells [10] translocate directly underneath the dermomyotome to form the subjacent myotome layer. Once in the myotome, myofibers elongate below the dermomyotome rostrally and caudally, and myotome expansion proceeds mediolaterally; thus, older myofibers are displaced laterally as newer fibers translocate into the myotome medially. Surgical ablation and dye-labeling studies show that the DML is both necessary and sufficient for the formation and growth of the epaxial myotome and overlying dermomyotome epithelium, and that DML cells translocate directly into the myotome without prior translational movement [13,14].

Figure 1



Overview of somite formation and compartmentalization in a 3-day (28-somite) chick embryo. (a) Somites (black) bud off from the cranial end of the presomitic mesoderm (PSM), which is located on either side of the neural tube. Somite formation and maturation occur in a craniocaudal gradient. (b–f) Cross-sections through the embryo at the indicated axial levels. (b) Section at the level of the PSM (blue). Surrounding tissues, such as the neural tube and notochord, are involved in patterning the newly segmented somite. (c) Section through a just-formed epithelial somite (blue) at the hindlimb level. (d) In response to signals from surrounding tissues, the somite divides into two compartments: the ventral mesenchymal sclerotome (green), and the dorsal epithelial dermomyotome (blue). The sclerotome will give rise to the axial skeleton, and the dermomyotome to the dorsal dermis and skeletal muscle. The dorsomedial and ventrolateral lips (DML and VLL) of the dermomyotome (yellow) are the initial delamination sites for cells that will form a third compartment, the myotome. (e) As the somite matures, cells delaminate from the dermomyotome edges and migrate

underneath to form the myotome (yellow), which contains the skeletal muscle progenitors. The epaxial myotome, which is derived from the DML, differentiates rapidly to form the back muscles. At thoracic levels, VLL dermomyotome cells give rise to the hypaxial myotome, which, together with the lateral dermomyotome, forms a somitic bud that invades the lateral plate mesoderm and gives rise to the body wall and abdominal muscle (indicated by arrows). The cells at the corners of the sclerotome (pink) begin to be specified into different components of the axial skeleton. Ventromedial sclerotome will give rise to the vertebral bodies, neural arches and proximal ribs; dorsomedial sclerotome will give rise to the spinous process of the vertebrae; and ventrolateral sclerotome, which invades the lateral plate mesoderm, will give rise to the distal portion of the ribs, with a possible contribution from the lateral dermomyotome. (f) At the level of the limb bud, cells from the lateral edge of the dermomyotome (yellow) delaminate and migrate into the lateral plate mesoderm, where they differentiate to form limb and limb girdle muscle.

In the second model, Kalchauer and colleagues [15,16] propose that there are distinct waves of myotome growth. In the first wave, the dorsomedial somite is the initial source of epaxial myotome progenitors. But instead of translocating directly, muscle progenitors first migrate rostrally before integrating into the incipient myotome. Subsequent growth occurs when a second wave of myogenic precursors translocate directly into the myotome from the rostral and caudal dermomyotome lips, and then intercalate with pre-existing myofibers. If any DML or

VLL contribution to myotome growth does occur, it is thought to be preceded by translational movement to the rostral and caudal dermomyotome lips [11].

Dye-lineage analysis shows that the DML and VLL muscle precursors first delaminate into the 'sub-lip domain', an intermediate layer located underneath the DML and VLL [17], and then migrate to the rostral and caudal lips before translocating into the myotome. Although the sub-lip domain cells do not express the

DML/VLL marker, Pax3, or desmin, a marker of differentiated myofibers, they do express the myogenic progenitor markers, MyoD and Myf5, as well as the fibroblast growth factor (FGF) receptor, FREK, a marker of proliferating cells. Thus, sub-lip domain cells are both mitotically active and specified to become myofibers, which suggests that they contribute to myotome growth.

A later, third wave of myotome growth occurs when another population of mitotically active cells enter the myotome from the rostral and caudal dermomyotome edges [18<sup>•</sup>]. Unlike the muscle progenitors that contributed to the first and second waves, these cells do not express MyoD and Myf5; however, they do express FREK and they respond to FGF4 expression in the differentiated myotome by proliferating and moving toward the center of the myotome. This identification of a later appearing, mitotically competent population of muscle progenitors provides a mechanism for growth of the postmitotic myotome.

While most studies on myotome formation employ the easily manipulable avian embryo, Eloy-Trinquet and Nicolas [19<sup>•</sup>] have used lineage analysis of LacZ clones in the mouse [20] to observe the separation of the myotome into epaxial and hypaxial domains. Analysis of LacZ clones shows that medial and lateral myotome precursors are regionalized before somite segmentation and separate clonally once segmentation has occurred. On the basis of the mediolateral location of the LacZ clones, Eloy-Trinquet and Nicolas [19<sup>•</sup>] propose a regionalized model for myotome formation in the mouse embryo in which the site of dermomyotomal myogenic precursors corresponds to that of their descendents in the subjacent myotome. Thus, the dorsomedial dermomyotome, central dermomyotome and ventrolateral dermomyotome would be the respective sources of the dorsalmost epaxial myotome, central myotome and hypaxial myotome. Although the regionalized model is not consistent with the proposed mediolateral direction of myotome growth [21], analysis of somitic gene expression domains does suggest that the medial and lateral dermomyotome and myotome are subdivided into a dorsal-most area including the DML, a centrally located intercalated region and a ventral-most area including the VLL [22<sup>•</sup>].

### Lateral sclerotome and distal rib formation

Shortly before sclerotome formation, Pax1 is activated in the ventral somite and continues to be expressed in the sclerotome after de-epithelialization. During vertebral morphogenesis, Pax1 remains a good marker of the ventromedial sclerotome but is downregulated in the dorsomedial and ventrolateral sclerotome. Although it is thought that the distal rib primordia arise from the lateral portion of the somite [3], there has been some debate over whether the lateral dermomyotome or lateral sclerotome is the source [5,6]. Absence of a ventrolateral sclerotome marker heretofore made it difficult to analyze the behavior of these cells.

Sudo *et al.* [23<sup>•</sup>] have now shown, however, that in chick the Mesenchymal forkhead-1 gene (Mfh-1) is highly expressed in the sclerotome, including the ventrolateral sclerotome component of the somitic bud that invades the lateral plate mesoderm at thoracic levels, and also seems to be expressed in the prospective cartilaginous tissue during rib formation. A comparison of Mfh-1 expression with that of Pax3, which marks the dermomyotomal somitic bud that gives rise to the body wall and abdominal muscles, shows that the two are closely associated; this suggests that rib and body wall muscle primordia invade the lateral plate mesoderm as a unit, and that interactions might occur between the incipient ribs and intercostal muscles during their migration and development. Such interactions might explain why manipulations that cause defects in dermomyotome development, such as removing the surface ectoderm, also cause distal rib defects [5,23<sup>•</sup>,24,25] and why several gene mutations that alter dermomyotome and myotome formation in the mouse, such as Pax3, Myogenic regulatory factor 4 (MRF4) and Myogenin, also result in distal rib defects [26,27].

Bone morphogenetic proteins (BMPs) that are expressed in the lateral plate mesoderm seem to be involved in penetration of the somatopleure by both the dorsal and ventral somitic bud: inhibition of BMP signaling by Noggin results in impaired somitic bud invasion and defective distal rib formation [23<sup>•</sup>].

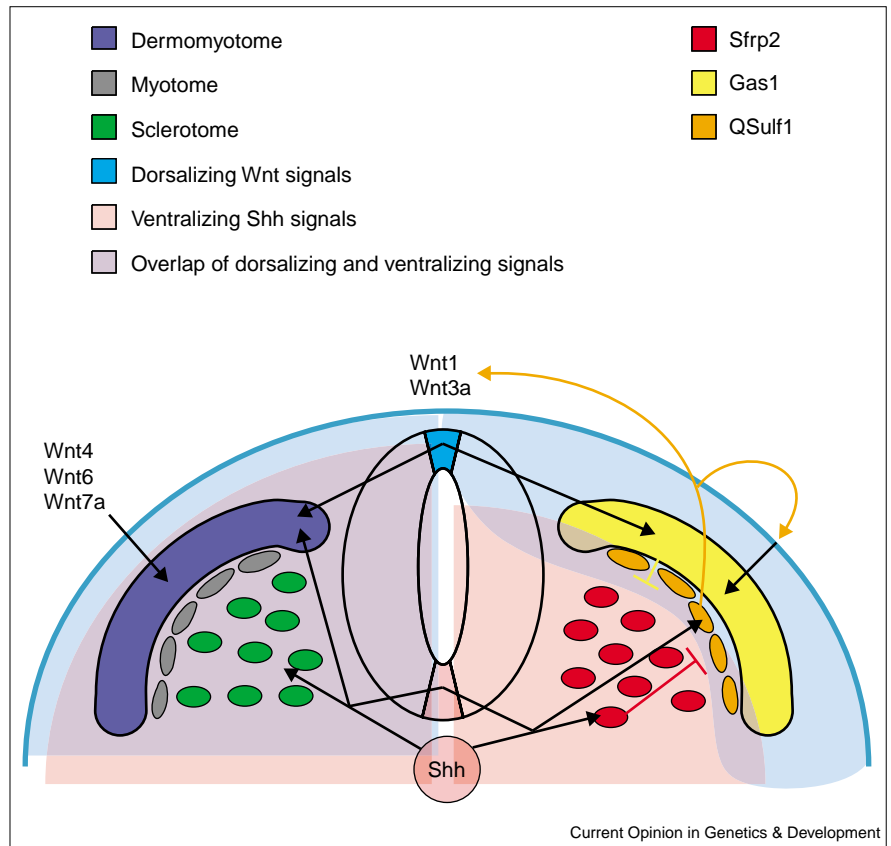
### Localization of signaling during dorsoventral patterning of the somite

Grafting and rotation experiments in the avian embryo show that cells in the newly formed somite are not yet restricted in their fate [2,28,29]. But the mediolateral and dorsoventral axes become patterned in response to extrinsic signals from surrounding tissues, after which the various somitic compartments become determined [30,31]. Some of the tissue interactions and signals that pattern the somitic compartments have been identified through ablation, grafting and coculture experiments in chick and through mutation analysis in mice.

Ventral midline expression of Sonic hedgehog (Shh) and Noggin in the notochord and floorplate are required for inducing and maintaining the sclerotome [24,32–42]. Medial dermomyotome formation depends on the expression of Wnt1 and Wnt3a in the dorsal neural tube [24,35,37,38,43–49], and lateral dermomyotome induction requires contact-mediated signaling by Wnt4, Wnt6 and Wnt7a in the surface ectoderm [24,35,45]. Myotome formation is dependent both on ventral midline Shh signaling for inducing expression of the earliest MRFs, MyoD and Myf5 [24,37,38,40,50,51], and on Wnt signaling from the dorsal neural tube and surface ectoderm for maintaining MRF expression [24,25,37,38,48,52,53]. Because Shh and Wnts are secreted molecules that can act at a distance greater than the length of a somite *in vitro* [35,45,54], it is likely that somitic dorsoventral patterning results from a

Figure 2

A model for the localization of signaling during dorsoventral patterning. A cross-section through a compartmentalized somite. Shortly after formation, the dorsoventral axis is patterned rapidly in response to extrinsic signals from surrounding tissues (left). Dorsoventral patterning of the somite results from a balance between gradients of dorsalizing and ventralizing signals. At high concentrations ventralizing signals, namely Shh, which is expressed in the notochord and floorplate (pink), induce sclerotome formation (green) and dorsalizing signals, namely Wnts, which are expressed in the dorsal neural tube and surface ectoderm (light blue), induce dermomyotome formation (dark blue). When ventralizing and dorsalizing signals are present at intermediate levels, the myotome is induced (gray). As Shh and Wnts are secreted molecules with long-range signaling activities that can act at a distance of more than one somite's length *in vitro* (indicated by opposing and overlapping pink and light blue gradients; overlap is purple), how are dorsalizing signals prevented from dorsalizing the ventral somite and vice versa? As shown on the right, molecules that inhibit long-range signaling activities or that act to localize signal responses have been identified. Sfrp2 (red), a Wnt antagonist, is activated in the ventral somite by Shh, where it functions to block (red lines) Wnts from dorsalizing the sclerotome. Reciprocally, Gas1 (yellow), a Shh antagonist, is activated in the dorsal somite by Wnts, where it functions to block (yellow lines) Shh from ventralizing the dermomyotome. Last, QSulf1 (orange), an extracellular sulfatase, is activated in the epaxial myogenic progenitors of the myotome



in response to Shh signaling. QSulf1 potentiates Wnt signaling (orange arrows) by enhancing the ability of the QSulf1-expressing cells to respond to Wnts. In this model, the long-range activities of dorsalizing and

ventralizing signals are restricted (indicated by localized pink and light blue gradients) by competitive inhibitors and by signal-response localization, which results in proper dorsoventral patterning of the somite.

balance between gradients of dorsalizing and ventralizing signals. Thus, at high concentrations ventralizing signals induce sclerotome and dorsalizing signals induce dermomyotome, whereas at intermediate concentrations both signals induce myotome [24,37,38] (Figure 2).

How, then, are dorsalizing signals prevented from dorsalizing the ventral somite and ventralizing signals prevented from ventralizing the dorsal somite — given that both sets of signals have long-range activity? One possibility is that competitive inhibitors limit the distance at which the signaling molecules can act. The Wnt antagonist Secreted frizzled-related protein 2 (Sfrp2) is expressed in the sclerotome and upregulated in PSM explants in response to Shh signaling, and can block the dermomyotome-inducing activity of Wnt1 and Wnt4 in PSM explants [55]. Expression of a Shh-inducible Wnt antagonist in the ventral somite suggests that Shh itself blocks the long-range dorsalizing activity of Wnts in the sclerotome.

Similarly, there is evidence for a Wnt-inducible antagonist of Shh signaling that functions during somite patterning.

Identified in a screen for genes whose products interact with Shh, Growth-arrest specific gene 1 (Gas1), a glycosylphosphatidylinositol-linked membrane glycoprotein, is expressed in the dorsal somite and induced by Wnts in PSM explant cultures [56\*]. Gas1 can bind recombinant Shh protein and seems to diminish Shh activity in explant cultures, as assayed by transcription of the Shh-responsive genes *Pax1* and *Patched*. So like Sfrp2, Gas1 might function in the dorsal somite as a Wnt-inducible Shh antagonist that binds Shh and prevents long-range Shh signaling from ventralizing the dorsal somite (Figure 2).

Long-range signaling activity can also be restricted through the localization of signal responses. Heparan sulfate proteoglycans (HSPGs) are cell-surface localized molecules that influence the activity of various developmental signals, such as FGFs and Wnts, by restricting their availability for receptor binding. The ability of HSPGs to regulate cell signaling depends on their sulfation state. QSulf1, a member of a family of evolutionarily conserved heparan-specific *N*-acetyl glucosamine sulfatases that function in the desulfation of HSPGs, was identified in a

screen for Shh response genes that are activated in quail embryos during somite formation [57\*\*].

Q<sub>Sulf1</sub> is coexpressed with MyoD and Myf5 in epaxial muscle progenitors — a population of cells dependent on ventral midline Shh signaling [24,36,40,42,50,51,58–60]. Using antisense oligonucleotides, Dhoot *et al.* [57\*\*] demonstrated that Q<sub>Sulf1</sub> activation in the epaxial somite requires Shh signaling. Loss of Q<sub>Sulf1</sub> results in loss of MyoD expression, while Myf5 and Pax1, two targets of Shh signaling, remain unaffected. Since MyoD is induced by Wnts [61,62], it is possible that Shh-induced Q<sub>Sulf1</sub> acts to modulate Wnt signaling.

Q<sub>Sulf1</sub> is localized at the cell surface, where it functions as a sulfatase, and can activate Wnt signaling 17-fold in C2C12 muscle progenitor cells cocultured with Wnt1-expressing cells. Thus, it is likely that HSPGs in the extracellular matrix normally bind secreted Wnts and prevent them from binding their receptors. In cells with extracellular Q<sub>Sulf1</sub>, however, HSPGs are desulfated and bound Wnts are released for receptor binding and activation of the Wnt pathway. The induction of Q<sub>Sulf1</sub> by Shh in epaxial muscle progenitors provides a rationale for how Wnt signaling is activated in this particular dorsal cell population and is restricted from influencing the ventral compartments (Figure 2).

### Short-range Shh signaling and axial cartilage development

It is well established that secreted signals from ventral midline tissues are required for formation of the sclerotome and axial skeleton [24,32–34,58]. In molecular terms, short-range ventral midline Shh signaling induces sclerotome formation during somitic dorsoventral patterning [35,36,39,40,54,63,64], and induction of Pax1 and Pax9 by Shh in the ventral somite is necessary for vertebral and rib development [63,65–68]. Although studies in quail embryo indicate that Shh is both necessary and sufficient for inducing Pax1 in the ventral somite [40], analyses of Shh mutant mice have been less conclusive.

In mice with targeted disruption of Shh, most sclerotomal derivatives, including the whole vertebral column and part of the ribs, are missing [39]. Weak Pax1 expression is induced in the ventral somite but subsequently lost when Pax1-expressing cells undergo apoptosis, which suggests that, unlike in the avian embryo, Shh is required in the mouse for maintaining but not inducing Pax1. Analysis of Pax1 expression in mice with mutations in Smoothened (Smo) has resolved this discrepancy [69\*]. Smo, a multipass membrane protein, is involved in signaling by all three vertebrate Hh homologs, Shh, Indian Hh, and Desert Hh; thus, a Smo mutation results in loss of all Hh signaling. Unlike in Shh mutants, Pax1 is not activated in the sclerotome in Smo mutants, which suggests that induction of Pax1 in Shh mutants is probably dependent on another Hh signal, possibly Indian Hh, that is expressed in the

developing embryonic gut or yolk sac endoderm [69\*]. Thus, in the mouse embryo, as in the chick embryo, there is an absolute requirement for Hh signaling in Pax1 induction.

Although Shh drives sclerotome induction, differentiation into some axial cartilage elements may not require the maintenance of Shh signaling — particularly for sclerotome cells, which migrate dorsally during vertebral development. Dorsomedial sclerotome downregulates the expression of Pax1, which suggests that Shh acts only transiently on these cells. Although Shh signaling has a role in development of the ventral vertebral structures that arise from the ventromedial sclerotome, which expresses Pax1 and migrates ventrally, BMP4 functions in the development of dorsal vertebral structures that arise from the dorsomedial sclerotome, which shuts off Pax1 and migrates dorsally [63,70,71].

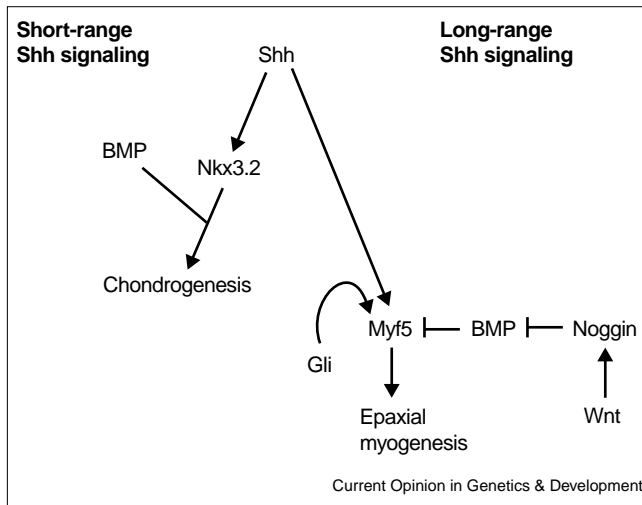
In a PSM explant model for BMP-dependent chondrogenesis, BMPs promoted chondrogenesis only after sustained or transient exposure to Shh [64], which suggests that Shh may render sclerotomal cells competent to respond to cartilage-inducing BMP signals. These results imply the existence of a chondrogenic-promoting factor that is initiated by transient Shh signals and maintained by BMPs after cessation of Shh signaling. Murtaugh *et al.* [72\*\*] have now identified such a factor.

Bapx1/Nkx3.2, a homeodomain transcriptional repressor, is initially expressed in a domain similar to that of Pax1 in the mouse [73] and the chick [72\*\*], which suggests that Nkx3.2 might, like Pax1, be induced by Shh. But unlike Pax1 expression, Nkx3.2 expression is maintained during chondrogenesis. In PSM explants, Nkx3.2 is induced by transient exposure of Shh and maintained by BMP signals. Misexpression of Nkx3.2 in somitic tissue renders the cells responsive to cartilage-inducing BMP signals even when previous Shh signaling is absent. Furthermore, misexpression of Nkx3.2 *in vivo* leads to expansion of the axial skeleton and, when Nkx3.2 is converted to a transcriptional activator, axial chondrogenesis is inhibited. Thus, Nkx3.2 functions in somitic tissue downstream of short-range Shh signaling to make the sclerotome competent to form cartilage, possibly by repressing transcription of factors that inhibit the chondrogenic-promoting activity of BMPs (Figure 3).

### Long-range Shh signaling and muscle development

Although it is clear that Shh is required for the development of epaxial myogenic progenitors [37,38,40,42,50,57\*\*,58,60], there is debate over whether Shh drives the survival and proliferation of specific cell types or specifies cell lineage. Studies have shown that Shh is necessary for both the survival of myogenic and chondrogenic precursors in the somites [74] and the survival and proliferation of hypaxial limb muscles [75,76]. Analysis of Shh mutant mice has shown both that Shh is needed for activating Myf5 and MyoD in the epaxial (but not the hypaxial) somite and that, although Shh functions in the survival of sclerotomal

Figure 3



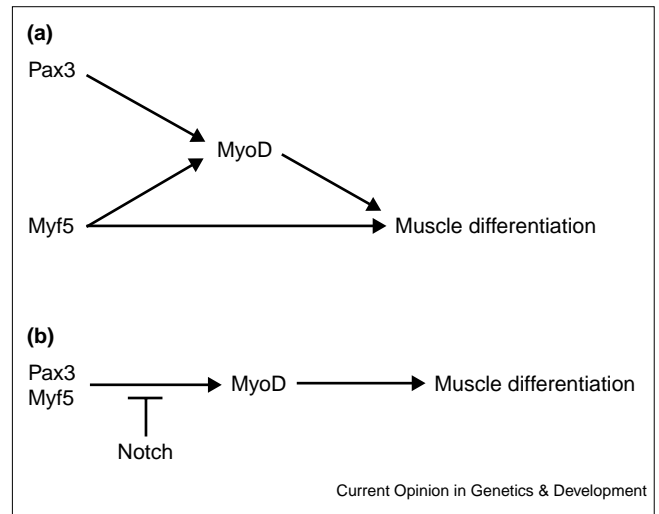
Short- and long-range Shh signaling and the formation of somite derivatives. Short-range Shh signals are essential for the differentiation of sclerotome into cartilage. Transient Shh signaling makes somitic cells competent to respond to the chondrogenic-promoting activity of bone morphogenetic proteins (BMPs). The role of Shh in BMP-dependent chondrogenesis is mediated by the transcriptional repressor Nkx3.2, which is activated in the sclerotome in response to transient Shh signaling and acts downstream of Shh to promote chondrogenesis in the presence of BMPs. Long-range Shh signals are essential for inducing epaxial myogenic precursors. Mediated by a Gli transcription factor binding site in the epaxial somite (ES) enhancer of Myf5, Shh directly activates Myf5 in the DML. A model [77••] for the localization of long-range Shh signaling to the epaxial somite is shown. Expression from the Myf5 ES enhancer is inhibited by BMPs, but this repression is relieved selectively in Myf5-expressing myogenic precursors through the activity of the BMP antagonist Noggin, which is induced in the DML in response to Wnts secreted from the dorsal neural tube.

cells in the ventral somite, loss of MRF expression in epaxial myogenic progenitors is not due to changes in cell survival or proliferation [59].

It has now been shown that Shh functions directly in the specification of epaxial muscle. Gustafsson *et al.* [77••] demonstrated the presence of an essential binding site for Gli transcription factors (the effectors of Shh signaling that act in responding cells) in the Myf5 epaxial somite (ES) enhancer that drives Myf5 expression in epaxial muscle progenitors during somite development [77••,78,79]. Because the ES enhancer is not activated either when the Gli-binding site is mutated or in Shh mutant mice, it is evident that Shh signaling directly activates Myf5 expression in epaxial muscle progenitors. This study provides the first demonstration that Shh signaling is required for the specification of a specific cell lineage; furthermore, as was suspected from *in vivo* and *in vitro* work, Shh signaling can act over long distances in the somite, as has been shown in the limb after amino-terminal processing and cholesterol modification of Shh [80•,81•] (Figure 3).

The Gli transcription factors responsible for activating the Myf5 ES enhancer are not yet clear. In avian embryos, in

Figure 4



Trunk myogenic pathways in mouse and chick. (a) In the mouse, analysis of mutants suggests the existence of two parallel myogenic pathways in which either Myf5 or MyoD is sufficient for muscle development; however, MyoD is activated by Myf5 in one pathway and by Pax3 in the other. (b) In the chick, by contrast, it seems that there is a single myogenic pathway in which Pax3 and Myf5 are expressed in proliferating myogenic progenitors. MyoD is activated in these cells, through inhibition of Notch signaling, once they are postmitotic, which leads to subsequent muscle differentiation. Thus, muscle differentiation cannot occur without prior activation of MyoD.

response to Wnt signals from the dorsal neural tube and surface ectoderm, Gli2 and Gli3 are localized to epaxial muscle progenitors at the site of Myf5 activation [40,60]. It will be interesting to determine whether similar regulation and localization of Glis occur in the mouse somite.

The Myf5 ES enhancer is required for Myf5 activation in the DML epaxial muscle progenitors, but not in the hypaxial muscle progenitors nor in the DML progenitors once they have moved into the myotome or medial dermomyotome [77••]. Thus, there must be some mechanism that restricts Shh-induced activation of the ES enhancer to the DML cells and that prevents activation in other somitic regions and shuts off activation once the DML progenitors have migrated. Gustafsson *et al.* [77••] propose that lateralizing BMP signals from the lateral plate mesoderm may repress activation of the ES enhancer in the lateral somite, while Noggin expression in the DML blocks the lateralizing activity of BMP4, thereby permitting Shh signals to activate the ES enhancer in the DML (Figure 3).

### MRFs and muscle differentiation

The basic helix-loop-helix MRFs, Myf5, MyoD, Myogenin and MRF4, are transcriptional activators of skeletal muscle genes that are each capable of activating the skeletal muscle differentiation program when expressed in nonmuscle cell types (reviewed in [82]). In mouse embryogenesis, Myf5 and MyoD are activated at different times and in different

domains, with *Myf5* expressed first in DML epaxial myogenic precursors before myotome formation and then in the myotome itself. *MyoD* expression is initiated slightly later: first in the somitic dorsomedial quadrant and then in the incipient myotome. Despite these differences in expression induction, *Myf5* and *MyoD* seem to compensate for one another because single *Myf5* or *MyoD* mutants develop normal skeletal muscle, but double mutants do not form muscle.

The transcription factor *Pax3*, which is localized to the developing dermomyotome during somite compartmentalization and then upregulated at the DML and VLL, is also important in muscle development. Because no *MyoD* expression or trunk muscle formation is seen in mice with mutations in both *Pax3* and *Myf5*, it has been proposed that mice possess two parallel trunk myogenic pathways in which either *Myf5* or *MyoD* is sufficient for muscle development; however, *MyoD* is turned on by *Myf5* in one pathway and by *Pax3* in the other [83] (Figure 4).

Recent work on the role of Notch signaling in myogenesis suggests that a different hierarchy might operate among *Pax3*, *Myf5* and *MyoD* in chick [84•]. As in the mouse, *Myf5* is the first MRF activated [84•,85•] and is initially co-expressed with *Pax3* in the DML, where proliferating cells are thought to reside [84•]. *MyoD*, which is expressed later, is predominantly restricted to the postmitotic myotome. On the basis of the localization of MRF genes in the DML and myotome, it seems that proliferating myogenic cells express *Pax3* and *Myf5* in the DML and then activate *MyoD* when postmitotic. Notch signaling pathway components, including the Notch1 receptor and the *Delta1* and *Serrate2* Notch ligands, are also expressed in subsets of differentiating myogenic cells, which suggests that the Notch pathway has a role in the progression from proliferating myogenic precursor to differentiated myofiber [84•].

Activation of Notch signaling through forced expression of *Delta1* in the developing somite leads to strong down-regulation of *MyoD* and complete inhibition of muscle differentiation, but has no effect on *Pax3* or *Myf5* expression, myotome formation, or cell-cycle exit [84•]. It thus seems that Notch signaling acts in postmitotic myogenic progenitors to activate *MyoD*, which suggests that in chick there may be a trunk myogenic pathway — different from the two identified in mouse — in which *Pax3* and *Myf5* are expressed in proliferating myogenic progenitors and *MyoD* is activated in *Myf5*-expressing cells once they are postmitotic, which leads to subsequent muscle differentiation. In this pathway, then, muscle differentiation cannot occur without prior activation of *MyoD* (Figure 4). Similar results have been obtained for chick limb myogenesis [86].

The differences between the *Pax3/Myf5/MyoD* hierarchies in the mouse and the chick might merely reflect differences in experimental model or, more intriguingly, they might represent actual species-specific programs of muscle differentiation.

### **Cis regulation of muscle development**

The *cis*-regulatory elements that are involved in MRF expression have been studied extensively to determine how MRFs control muscle differentiation. *Myf5 cis* regulation is of particular interest, owing to its early and essential role in muscle development. The *Myf5* locus shows a complex arrangement of discrete enhancers that are required to drive *Myf5* expression in the epaxial and hypaxial somites, individual branchial arches, individual hypaxial muscle progenitor populations and central nervous system [78,79,87•]. As has been pointed out [79,87•], the modular arrangement of *Myf5* enhancers might have evolved in response to a need for *Myf5* activation in distinct muscle progenitor populations surrounded by different signaling environments that induce *Myf5* expression through different pathways. In addition, the identification of particular enhancers for individual hypaxial muscle progenitor populations suggests a mechanism whereby new hypaxial muscle groups, such as limb muscle, might have emerged during vertebrate evolution [78,79,87•].

*Myf5* and *MRF4* are tightly linked on the same chromosome in teleost fish, birds and mammals [88], which suggests that there must be selective pressure on the locus to maintain this arrangement. Carvajal *et al.* [87•] provide evidence for this possibility. Using double-reporter constructs, they identified elements in the *Myf5/MRF4* locus that are required for the expression of each gene. Analysis of *MRF4* reporter constructs showed that in addition to being expressed in the central myotome, *MRF4* is also expressed in the ventral myotome at a slightly later stage. Notably, the element driving *MRF4* expression in this ventral domain overlaps with an element that drives *Myf5* expression in a similar ventral domain, which suggests that a single element might control the expression of both genes in the ventral myotome. A shared regulatory element might explain the persistence of the *Myf5/MRF4* linkage throughout vertebrate evolution.

Development of skeletal muscle is controlled by both the myocyte enhancer 2 (MEF2) family of MADS transcription factors and MRFs. MEF2s and MRFs interact to promote differentiation and to autoregulate and crossregulate each other [89]. Forced expression of MRFs in nonmuscle cell lines activates expression of MEF2 and, although the reverse is not true, promoters of two MRFs, *Myogenin* and *MRF4*, contain binding sites for both MEF2s and MRFs (see references in [90•]). To better understand the interaction between MRFs and MEF2s, Wang *et al.* [90•] identified the *cis*-regulatory elements driving expression of *Mef2c* in skeletal muscle. Interestingly, these elements contain an MRF-binding site as well as an adjacent MEF2-binding site. Mutational analysis showed that the MRF-binding site is essential for inducing expression of *Mef2c* during skeletal muscle development, and that the MEF2-binding site is required for *Mef2c* maintenance. This analysis of the *cis*-regulatory elements of *Mef2c*, combined with the identification of MEF2-binding sites in MRF promoters, demonstrates the essential reciprocal

interactions that occur between MRFs and MEF2s during the specification and stabilization of the skeletal muscle lineage.

### A somitic compartment of tendon progenitors

A functional musculoskeletal system depends completely on the coordinated development of muscle, cartilage and tendon, and in particular on the elaborate tendon attachments transmitting force from muscle to bone. But whereas the somitic lineages of the muscular and skeletal systems are well understood, the origin of trunk tendons is not — mainly because there have been no markers of somitic tendon progenitors until recently.

Schweitzer *et al.* [91\*] have now shown, however, that the basic helix–loop–helix transcription factor Scleraxis is expressed both in the mature tendons and ligaments of the limbs and trunk and in their progenitor populations, including a population of cells in the developing somite. Using this marker, it will be possible to begin looking at tendon morphogenesis as well as the signals required for the specification and development of tendon progenitors. The origin and regulation of this fourth somitic cell lineage is an exciting area for study and the focus of current research in our laboratory.

### Conclusions

How the somite — a ball of cells that are initially equivalent in potential — becomes patterned and compartmentalized to form distinct lineages has been an important field of research for over a century (reviewed in [1]). Current advances are being made in our understanding on a molecular level of how somite compartments form and interact, and what signals are required to induce their differentiation into muscle, cartilage and tendon.

Analysis of the developmental regulation of somite derivatives at the level of transcription factors and *cis* regulation will eventually uncover the vital connections between patterning by extrinsic signaling molecules, expression of cell fate regulators and cell lineage specification.

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### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Brand-Saberi B, Christ B: **Evolution and development of distinct cell lineages derived from somites.** *Curr Top Dev Biol* 2000, **48**:1-42.
2. Ordahl CP, Le Douarin NM: **Two myogenic lineages within the developing somite.** *Development* 1992, **114**:339-353.
3. Olivera-Martinez I, Coltey M, Dhouailly D, Pourquie O: **Mediolateral somitic origin of ribs and dermis determined by quail-chick chimeras.** *Development* 2000, **127**:4611-4617.
4. Freitas C, Rodrigues S, Charrier JB, Teillet MA, Palmeirim I: **Evidence for medial/lateral specification and positional information within the presomitic mesoderm.** *Development* 2001, **128**:5139-5147.
5. Huang R, Zhi Q, Schmidt C, Wiltling J, Brand-Saberi B, Christ B: **Sclerotomal origin of the ribs.** *Development* 2000, **127**:527-532.
6. Kato N, Aoyama H: **Dermomyotomal origin of the ribs as revealed by extirpation and transplantation experiments in chick and quail embryos.** *Development* 1998, **125**:3437-3443.
7. Huang R, Christ B: **Origin of the epaxial and hypaxial myotome in avian embryos.** *Anat Embryol* 2000, **202**:369-374.
8. Denetclaw WF Jr, Christ B, Ordahl CP: **Location and growth of epaxial myotome precursor cells.** *Development* 1997, **124**:1601-1610.
9. Chevallier A, Kiény M, Mauger A: **Limb-somite relationship: origin of the limb musculature.** *J Embryol Exp Morphol* 1977, **41**:245-258.
10. Denetclaw WF, Ordahl CP: **The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos.** *Development* 2000, **127**:893-905.
11. Cinnamon Y, Kahane N, Kalcheim C: **Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome.** *Development* 1999, **126**:4305-4315.
12. Christ B, Jacob M, Jacob HJ: **On the origin and development of the ventrolateral abdominal muscles in the avian embryo. An experimental and ultrastructural study.** *Anat Embryol* 1983, **166**:87-101.
13. Denetclaw WF Jr, Berdougo E, Venters SJ, Ordahl CP: **Morphogenetic cell movements in the middle region of the dermomyotome dorsomedial lip associated with patterning and growth of the primary epaxial myotome.** *Development* 2001, **128**:1745-1755. See annotation [14\*].
14. Ordahl CP, Berdougo E, Venters SJ, Denetclaw WF Jr: **The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium.** *Development* 2001, **128**:1731-1744. These two papers [13\*,14\*] use a combination of embryo surgery and dye-lineage analysis to investigate the role of the DML in myotomal growth and patterning. In contrast to Cinnamon *et al.* [17\*], the authors identify direct translocation of muscle progenitors from the DML into the myotome as the primary source of cells driving myotomal and dermomyotomal growth.
15. Kahane N, Cinnamon Y, Kalcheim C: **The origin and fate of pioneer myotomal cells in the avian embryo.** *Mech Dev* 1998, **74**:59-73.
16. Kahane N, Cinnamon Y, Kalcheim C: **The cellular mechanism by which the dermomyotome contributes to the second wave of myotome development.** *Development* 1998, **125**:4259-4271.
17. Cinnamon Y, Kahane N, Bachelet I, Kalcheim C: **The sub-lip domain — a distinct pathway for myotome precursors that demonstrate rostral–caudal migration.** *Development* 2001, **128**:341-351. The 'sub-lip domain' is identified as the site of rostral–caudal migration of myogenic precursors after their translocation from the DML and VLL. In contrast to Ordahl *et al.* [13\*,14\*], the authors refute the notion that DML and VLL muscle precursors translocate directly into the myotome and instead suggest that migration in the 'sub-lip domain' must first occur before these precursors can contribute to myotome formation.
18. Kahane N, Cinnamon Y, Bachelet I, Kalcheim C: **The third wave of myotome colonization by mitotically competent progenitors: regulating the balance between differentiation and proliferation during muscle development.** *Development* 2001, **128**:2187-2198. The authors describe a population of late-appearing, mitotically active muscle progenitors that express the FREK receptor, proliferate in response to FGF4 signals from the differentiated myotome, and subsequently contribute to the third wave of myotome growth.
19. Eloy-Trinquet S, Nicolas JF: **Clonal separation and regionalisation during formation of the medial and lateral myotomes in the mouse embryo.** *Development* 2002, **129**:111-122. Using the LaacZ method of lineage analysis, the authors show clonal separation of medial and lateral myotomes in the mouse and suggest a regionalized model of myotome formation in which discrete dermomyotome domains give rise to sub-jacent myotome.
20. Eloy-Trinquet S, Mathis L, Nicolas JF: **Retrospective tracing of the developmental lineage of the mouse myotome.** *Curr Top Dev Biol* 2000, **47**:33-80.
21. Venters SJ, Thorsteinsdottir S, Duxson MJ: **Early development of the myotome in the mouse.** *Dev Dyn* 1999, **216**:219-232.
22. Sporle R: **Epaxial–adaxial–hypaxial regionalisation of the vertebrate somite: evidence for a somitic organiser and a mirror-image duplication.** *Dev Genes Evol* 2001, **211**:198-217. Analysis of gene expression domains in the mouse somite suggests that in amniotes the epaxial dermomyotome and myotome is subdivided into dorsal-most

and intercalated subregions. The authors propose that the epaxial somitic bud is a mirror image of the hypaxial somitic bud and that the intercalated domain may be related to the adaxial domain seen in more primitive vertebrates.

23. Sudo H, Takahashi Y, Tonegawa A, Arase Y, Aoyama H, Mizutani K, Koseki Y, Moriya H, Wilting J, Christ B, Koseki H: **Inductive signals from the somatopleure mediated by bone morphogenetic proteins are essential for the formation of the sternal component of avian ribs.** *Dev Biol* 2001, **232**:284-300.
- cMfh-1 is expressed in the sclerotome and marks the site of the ventral somitic bud that invades the lateral plate mesoderm and forms the sternal rib primordia. The authors also show that expression of bone morphogenetic protein in the lateral plate is involved in somitic bud migration and distal rib formation, as these processes are disrupted in the presence of Noggin.
24. Dietrich S, Schubert FR, Lumsden A: **Control of dorsoventral pattern in the chick paraxial mesoderm.** *Development* 1997, **124**:3895-3908.
25. Dietrich S, Schubert FR, Healy C, Sharpe PT, Lumsden A: **Specification of the hypaxial musculature.** *Development* 1998, **125**:2235-2249.
26. Henderson DJ, Conway SJ, Copp AJ: **Rib truncations and fusions in the Sp2H mouse reveal a role for Pax3 in specification of the ventro-lateral and posterior parts of the somite.** *Dev Biol* 1999, **209**:143-158.
27. Vivian JL, Olson EN, Klein WH: **Thoracic skeletal defects in myogenin- and MRF4-deficient mice correlate with early defects in myotome and intercostal musculature.** *Dev Biol* 2000, **224**:29-41.
28. Aoyama H, Asamoto K: **Determination of somite cells: independence of cell differentiation and morphogenesis.** *Development* 1988, **104**:15-28.
29. Dockter JL, Ordahl C: **Dorsoventral axis determination in the somite: a re-examination.** *Development* 2000, **127**:2201-2205.
30. Dockter JL, Ordahl CP: **Determination of sclerotome to the cartilage fate.** *Development* 1998, **125**:2113-2124.
31. Williams BA, Ordahl CP: **Emergence of determined myotome precursor cells in the somite.** *Development* 1997, **124**:4983-4997.
32. Watterson R, Fowler I, Fowler B: **The role of the neural tube and notochord in development of the axial skeleton of the chick.** *Am J Anat* 1954, **95**:337-399.
33. Pourquie O, Coltey M, Teillet MA, Ordahl C, Le Douarin NM: **Control of dorsoventral patterning of somitic derivatives by notochord and floor plate.** *Proc Natl Acad Sci USA* 1993, **90**:5242-5246.
34. Brand-Saberi B, Ebensperger C, Wilting J, Balling R, Christ B: **The ventralizing effect of the notochord on somite differentiation in chick embryos.** *Anat Embryol* 1993, **188**:239-245.
35. Fan CM, Tessier-Lavigne M: **Patterning of mammalian somites by surface ectoderm and notochord: evidence for sclerotome induction by a hedgehog homolog.** *Cell* 1994, **79**:1175-1186.
36. Johnson RL, Laufer E, Riddle RD, Tabin C: **Ectopic expression of Sonic hedgehog alters dorsal-ventral patterning of somites.** *Cell* 1994, **79**:1165-1173.
37. Munsterberg AE, Lassar AB: **Combinatorial signals from the neural tube, floor plate and notochord induce myogenic bHLH gene expression in the somite.** *Development* 1995, **121**:651-660.
38. Munsterberg AE, Kitajewski J, Bumcrot DA, McMahon AP, Lassar AB: **Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite.** *Genes Dev* 1995, **9**:2911-2922.
39. Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA: **Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function.** *Nature* 1996, **383**:407-413.
40. Borycki AG, Mendham L, Emerson CP Jr: **Control of somite patterning by Sonic hedgehog and its downstream signal response genes.** *Development* 1998, **125**:777-790.
41. McMahon JA, Takada S, Zimmerman LB, Fan CM, Harland RM, McMahon AP: **Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite.** *Genes Dev* 1998, **12**:1438-1452.
42. Dietrich S, Schubert FR, Gruss P, Lumsden A: **The role of the notochord for epaxial myotome formation in the mouse.** *Cell Mol Biol (Noisy-le-grand)* 1999, **45**:601-616.
43. Christ B, Brand-Saberi B, Grim M, Wilting J: **Local signalling in dermomyotomal cell type specification.** *Anat Embryol* 1992, **186**:505-510.
44. Spence MS, Yip J, Erickson CA: **The dorsal neural tube organizes the dermomyotome and induces axial myocytes in the avian embryo.** *Development* 1996, **122**:231-241.
45. Fan CM, Lee CS, Tessier-Lavigne M: **A role for WNT proteins in induction of dermomyotome.** *Dev Biol* 1997, **191**:160-165.
46. Capdevila J, Tabin C, Johnson RL: **Control of dorsoventral somite patterning by Wnt-1 and  $\beta$ -catenin.** *Dev Biol* 1998, **193**:182-194.
47. Ikeya M, Takada S: **Wnt signaling from the dorsal neural tube is required for the formation of the medial dermomyotome.** *Development* 1998, **125**:4969-4976.
48. Wagner J, Schmidt C, Nikowits W Jr, Christ B: **Compartmentalization of the somite and myogenesis in chick embryos are influenced by wnt expression.** *Dev Biol* 2000, **228**:86-94.
49. Olivera-Martinez I, Thelu J, Teillet MA, Dhouailly D: **Dorsal dermis development depends on a signal from the dorsal neural tube, which can be substituted by Wnt-1.** *Mech Dev* 2001, **100**:233-244.
50. Rong PM, Teillet MA, Ziller C, Le Douarin NM: **The neural tube/notochord complex is necessary for vertebral but not limb and body wall striated muscle differentiation.** *Development* 1992, **115**:657-672.
51. Pownall ME, Strunk KE, Emerson CP Jr: **Notochord signals control the transcriptional cascade of myogenic bHLH genes in somites of quail embryos.** *Development* 1996, **122**:1475-1488.
52. Buffinger N, Stockdale FE: **Myogenic specification in somites: induction by axial structures.** *Development* 1994, **120**:1443-1452.
53. Cossu G, Kelly R, Tajbakhsh S, Di Donna S, Vivarelli E, Buckingham M: **Activation of different myogenic pathways: Myf-5 is induced by the neural tube and MyoD by the dorsal ectoderm in mouse paraxial mesoderm.** *Development* 1996, **122**:429-437.
54. Fan CM, Porter JA, Chiang C, Chang DT, Beachy PA, Tessier-Lavigne M: **Long-range sclerotome induction by sonic hedgehog: direct role of the amino-terminal cleavage product and modulation by the cyclic AMP signaling pathway.** *Cell* 1995, **81**:457-465.
55. Lee CS, Buttitta LA, May NR, Kispert A, Fan CM: **SHH-N upregulates *Sfrp2* to mediate its competitive interaction with WNT1 and WNT4 in the somitic mesoderm.** *Development* 2000, **127**:109-118.
56. Lee CS, Buttitta L, Fan CM: **Evidence that the WNT-inducible growth arrest-specific gene 1 encodes an antagonist of sonic hedgehog signaling in the somite.** *Proc Natl Acad Sci USA* 2001, **98**:11347-11352.
- Gas1, a Wnt-inducible antagonist of Shh signaling, is shown to be activated in the dorsal somite as a possible prevention against the ventralizing effect of long-range Shh signals on the dorsal somite.
57. Dhoot GK, Gustafsson MK, Ai X, Sun W, Standiford DM, Emerson CP Jr: **Regulation of Wnt signaling and embryo patterning by an extracellular sulfatase.** *Science* 2001, **293**:1663-1666.
- QSulf1, an extracellular sulfatase induced in epaxial muscle progenitors by Shh, is required for expression of MyoD in these cells. QSulf1 potentiates Wnt signaling, probably through the desulfation of heparan sulfate proteoglycans, which suggests a mechanism whereby a ventralizing signal (Shh) functions to localize the activity of dorsalizing signals (Wnts) to appropriate cells in the dorsal somite.
58. Borycki AG, Strunk KE, Savary R, Emerson CP Jr: **Distinct signal/response mechanisms regulate pax1 and QmyoD activation in sclerotomal and myotomal lineages of quail somites.** *Dev Biol* 1997, **185**:185-200.
59. Borycki AG, Brunk B, Tajbakhsh S, Buckingham M, Chiang C, Emerson CP Jr: **Sonic hedgehog controls epaxial muscle determination through Myf5 activation.** *Development* 1999, **126**:4053-4063.
60. Borycki A, Brown AM, Emerson CP Jr: **Shh and Wnt signaling pathways converge to control *Gli* gene activation in avian somites.** *Development* 2000, **127**:2075-2087.
61. Hirsinger E, Duprez D, Jouve C, Malapert P, Cooke J, Pourquie O: **Noggin acts downstream of Wnt and Sonic Hedgehog to antagonize BMP4 in avian somite patterning.** *Development* 1997, **124**:4605-4614.
62. 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.** *Development* 1998, **125**:4155-4162.

63. Watanabe Y, Duprez D, Monsoro-Burq AH, Vincent C, Le Douarin NM: **Two domains in vertebral development: antagonistic regulation by SHH and BMP4 proteins.** *Development* 1998, **125**:2631-2639.
64. Murtaugh LC, Chyung JH, Lassar AB: **Sonic hedgehog promotes somitic chondrogenesis by altering the cellular response to BMP signaling.** *Genes Dev* 1999, **13**:225-237.
65. Koseki H, Wallin J, Wilting J, Mizutani Y, Kispert A, Ebensperger C, Herrmann BG, Christ B, Balling R: **A role for Pax-1 as a mediator of notochordal signals during the dorsoventral specification of vertebrae.** *Development* 1993, **119**:649-660.
66. Wallin J, Wilting J, Koseki H, Fritsch R, Christ B, Balling R: **The role of Pax-1 in axial skeleton development.** *Development* 1994, **120**:1109-1121.
67. Ebensperger C, Wilting J, Brand-Saberi B, Mizutani Y, Christ B, Balling R, Koseki H: **Pax-1, a regulator of sclerotome development is induced by notochord and floor plate signals in avian embryos.** *Anat Embryol* 1995, **191**:297-310.
68. Peters H, Wilm B, Sakai N, Imai K, Maas R, Balling R: **Pax1 and Pax9 synergistically regulate vertebral column development.** *Development* 1999, **126**:5399-5408.
69. Zhang XM, Ramalho-Santos M, McMahon AP: **Smoothed mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R asymmetry by the mouse node.** *Cell* 2001, **105**:781-792.
- Analysis of Smoothed mutants, in which all Hedgehog signaling is blocked, shows an absolute requirement for Hedgehog proteins in activating sclerotomal expression of Pax1 in embryos at the 10-somite stage.
70. Monsoro-Burq AH, Bontoux M, Teillet MA, Le Douarin NM: **Heterogeneity in the development of the vertebra.** *Proc Natl Acad Sci USA* 1994, **91**:10435-10439.
71. Monsoro-Burq AH, Duprez D, Watanabe Y, Bontoux M, Vincent C, Brickell P, Le Douarin N: **The role of bone morphogenetic proteins in vertebral development.** *Development* 1996, **122**:3607-3616.
72. Murtaugh LC, Zeng L, Chyung JH, Lassar AB: **The chick transcriptional repressor Nkx3.2 acts downstream of Shh to promote BMP-dependent axial chondrogenesis.** *Dev Cell* 2001, **1**:411-422.
- Nkx3.2 is identified as a transcriptional repressor that is activated by transient Shh signaling in presomitic mesoderm explants and that promotes chondrogenesis in the presence of BMPs. Overexpression of Nkx3.2 activates the chondrogenic program both in explants and after *in vivo* misexpression, which suggests that the normal function of Nkx3.2 is to repress transcription of factors that inhibit the chondrogenic-promoting activity of BMPs.
73. Tribioli C, Lufkin T: **The murine Bapx1 homeobox gene plays a critical role in embryonic development of the axial skeleton and spleen.** *Development* 1999, **126**:5699-5711.
74. Teillet M, Watanabe Y, Jeffs P, Duprez D, Lapointe F, Le Douarin NM: **Sonic hedgehog is required for survival of both myogenic and chondrogenic somitic lineages.** *Development* 1998, **125**:2019-2030.
75. Duprez D, Fournier-Thibault C, Le Douarin N: **Sonic Hedgehog induces proliferation of committed skeletal muscle cells in the chick limb.** *Development* 1998, **125**:495-505.
76. Kruger M, Mennerich D, Fees S, Schafer R, Mundlos S, Braun T: **Sonic hedgehog is a survival factor for hypaxial muscles during mouse development.** *Development* 2001, **128**:743-752.
77. Gustafsson MK, Pan H, Pinney DF, Liu Y, Lewandowski A, Epstein DJ, Emerson CP Jr: **Myf5 is a direct target of long-range Shh signaling and Gli regulation for muscle specification.** *Genes Dev* 2002, **16**:114-126.
- The authors identify an essential Gli transcription factor binding site in the Myf5 epaxial somite enhancer that is required for Shh-mediated induction of epaxial myogenic precursors. A direct role for Shh is implicated in the specification of muscle lineage, and the importance of long-range Shh signaling during somite development is highlighted.
78. Hadchouel J, Tajbakhsh S, Primig M, Chang TH, Daubas P, Rocancourt D, Buckingham M: **Modular long-range regulation of Myf5 reveals unexpected heterogeneity between skeletal muscles in the mouse embryo.** *Development* 2000, **127**:4455-4467.
79. Summerbell D, Ashby PR, Coutelle O, Cox D, Yee S, Rigby PW: **The expression of Myf5 in the developing mouse embryo is controlled by discrete and dispersed enhancers specific for particular populations of skeletal muscle precursors.** *Development* 2000, **127**:3745-3757.
80. Lewis PM, Dunn MP, McMahon JA, Logan M, Martin JF, St-Jacques B, McMahon AP: **Cholesterol modification of sonic hedgehog is required for long-range signaling activity and effective modulation of signaling by Ptc1.** *Cell* 2001, **105**:599-612.
- See annotation [81\*].
81. Zeng X, Goetz JA, Suber LM, Scott WJ Jr, Schreiner CM, Robbins DJ: **A freely diffusible form of Sonic hedgehog mediates long-range signalling.** *Nature* 2001, **411**:716-720.
- These two papers [80\*,81\*] show that amino-terminal processing and cholesterol modification of Shh produce an active signal that participates in long-range signaling both *in vitro* and in the vertebrate limb.
82. Weintraub H, Davis R, Tapscott S, Thayer M, Krause M, Benezra R, Blackwell TK, Turner D, Rupp R, Hollenberg S *et al.*: **The myoD gene family: nodal point during specification of the muscle cell lineage.** *Science* 1991, **251**:761-766.
83. 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.
84. Hirsinger E, Malapert P, Dubrulle J, Delfini MC, Duprez D, Henrique D, Ish-Horowitz D, Pourquie O: **Notch signalling acts in postmitotic avian myogenic cells to control MyoD activation.** *Development* 2001, **128**:107-116.
- The Notch signaling pathway is shown to function in postmitotic myogenic precursors to prevent these cells from activating MyoD and undergoing muscle differentiation.
85. Kiefer JC, Hauschka SD: **Myf-5 is transiently expressed in nonmuscle mesoderm and exhibits dynamic regional changes within the presegmented mesoderm and somites I-IV.** *Dev Biol* 2001, **232**:77-90.
- Together with [84\*], this paper shows that Myf5 is the first MRF to be activated in somitic muscle progenitors in the chick, as it is in the mouse.
86. Delfini M, Hirsinger E, Pourquie O, Duprez D: **Delta 1-activated notch inhibits muscle differentiation without affecting Myf5 and Pax3 expression in chick limb myogenesis.** *Development* 2000, **127**:5213-5224.
87. Carvajal JJ, Cox D, Summerbell D, Rigby PW: **A BAC transgenic analysis of the Mrf4/Myf5 locus reveals interdigitated elements that control activation and maintenance of gene expression during muscle development.** *Development* 2001, **128**:1857-1868.
- Bacterial artificial chromosome (BAC) transgenic analysis of the *cis*-regulatory elements that control expression of Myf5 and MRF4 is described. Both genes are shown to be controlled by complex modular elements that drive expression in specific muscle progenitor populations. In addition, the finding that activation of Myf5 and MRF4 in the ventral somite might be controlled by a common enhancer provides one explanation for the linkage of these genes throughout vertebrate evolution.
88. Olson EN, Arnold HH, Rigby PW, Wold BJ: **Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene MRF4.** *Cell* 1996, **85**:1-4.
89. Molkenin JD, Black BL, Martin JF, Olson EN: **Cooperative activation of muscle gene expression by MEF2 and myogenic bHLH proteins.** *Cell* 1995, **83**:1125-1136.
90. Wang DZ, Valdez MR, McAnally J, Richardson J, Olson EN: **The Mef2c gene is a direct transcriptional target of myogenic bHLH and MEF2 proteins during skeletal muscle development.** *Development* 2001, **128**:4623-4633.
- The *cis*-regulatory elements that drive expression of Mef2c in skeletal muscle are identified and shown to contain a MRF-binding site necessary for inducing expression of Mef2c and a MEF2-binding site necessary for maintaining expression of Mef2c. The interaction between MRFs and MEF2s are thus shown to be essential for specification and stabilization of the skeletal muscle lineage.
91. Schweitzer R, Chyung JH, Murtaugh LC, Brent AE, Rosen V, Olson EN, Lassar A, Tabin CJ: **Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments.** *Development* 2001, **128**:3855-3866.
- Expression of the basic helix-loop-helix protein Scleraxis in tendon progenitors and mature trunk and limb tendons is described, and regulation of Scleraxis expression in the limb is analyzed. The authors identify a compartment of somitic Scleraxis-expressing tendon progenitors, which paves the way for future studies on the origin and regulation of somitic tendons.