The EMBO Journal Vol.18 No.24 pp.6867–6872, 1999

NEW EMBO MEMBER'S REVIEW

Wnt signaling and the activation of myogenesis in mammals

Giulio Cossu[1] and Ugo Borello

Istituto Pasteur-Fondazione Cenci Bolognetti, Dipartimento di Istologia ed Embriologia Medica, Universitá di Roma 'La Sapienza', Via A.Scarpa 14, 00161 Roma, Italy

[1]Corresponding author
e-mail: cossu@axrma.uniroma1.it

In the amniote embryos, specification of skeletal myoblasts occurs in the paraxial mesoderm in response to a number of signaling molecules produced by neighboring tissues such as neural tube, notochord and dorsal ectoderm. Candidate molecules for this complex signaling activity include Sonic hedgehog, Wnts and Noggin as positive activators and BMP4 as a possible inhibitor. Recently, the receptors and the post-receptor pathways for Sonic hedgehog and Wnts have been characterized, and this has opened up the possibility of linking these signaling events to the activation of myogenic regulatory factor genes such as *Myf5* and *MyoD* and functionally related genes such as *Pax3*. Here we focus on the role of Wnts, their putative receptors Frizzled and the soluble antagonist Frzb1 in regulating mammalian myogenesis. Although it is becoming evident that the signaling downstream of Frizzled receptors is much more complex than anticipated, it is conceivable that it may lead to transcriptional activation of *Myf5* and *MyoD* and to initiation of myogenesis. However, the fact that both Wnts and Sonic hedgehog have a strong effect on cell proliferation and survival suggests that they may contribute to the overall process of myogenesis by a combination of these different biological activities.

Keywords: Frizzled/Frzb1/myogenesis/Shh/somites/Wnt

Neighboring tissues influence somite differentiation

All skeletal muscles of the vertebrate body, with the exception of most head muscles, are derived from somites, epithelial structures which form in the paraxial mesoderm in a cranio–caudal developmental sequence over an extended period of embryogenesis (Brand-Saberi and Christ, 1999). Muscle progenitor cells become specified within the dermomyotome, which is located dorsally under the ectoderm. Cells located in the ventral sclerotome, adjacent to the notochord, will contribute to cartilage and bone. At the onset of somitogenesis, cell fate is not yet determined since rotation of the epithelial somite in a dorsal/ventral or medial/lateral direction does not perturb subsequent differentiation that takes place according to the final orientation of the somite. This strongly suggests that signals from the environment specify the identity of cells within the newly formed somite.

Work carried out in the 1980s (reviewed in Cossu *et al.*, 1996a) established that in explants of paraxial mesoderm, axial structures (the neural tube–notochord complex) are required to promote myogenesis, but only in pre-somitic mesoderm and newly formed somites: more mature somites do not need the presence of neighboring tissues. Further experiments showed that only precursors of epaxial (back) muscles, located in the dorso-medial domain of newly formed somites, are dependent upon signals from axial structures; in contrast, precursors of hypaxial (limb and body wall) muscles, located in the lateral half of the paraxial mesoderm, do not need the neural tube–notochord complex but rather require a signal from dorsal ectoderm for myogenic commitment (reviewed in Cossu *et al.*, 1996a). It was later shown that explants of murine paraxial mesoderm, when cultured in the presence of axial structures, activate *Myf5*. In contrast, they will activate *MyoD* when cultured with their own dorsal ectoderm (Cossu *et al.*, 1996b). This suggests that in mammals, axial structures activate myogenesis through a *Myf5*-dependent pathway, while dorsal ectoderm acts through a *MyoD*-dependent pathway. It is important to stress here that this does not mean that medial progenitor cells express *Myf5* and lateral cells *MyoD*. The situation is more complex: medial cells express *Myf5* and are indeed absent in *Myf5* null embryos (although only at an early stage; Braun *et al.*, 1992). In lateral cells, *Myf5* may activate *MyoD* and myogenesis (see arrow in Figure 2), but this is not the only possibility since dorsal ectoderm can still activate *MyoD* in the absence of *Myf5* (possibly through *Pax3*; Tajbakhsh *et al.*, 1997). Soon after these initial inductive events, the great majority of myogenic cells express both MyoD and Myf5 proteins, although at different levels. This correlates with observations on older heterozygote *Myf5/nlacZ* mice where different types of muscle fibers all express β-galactosidase, as well as MyoD. It also explains the phenotype of the single *MyoD* or *Myf5* knockout mice where *Myf5* null embryos initially have epaxial muscle defects whereas *MyoD* null embryos have predominantly hypaxial muscle defects (Kablar *et al.*, 1997; S.Tajbakhsh and M.Buckingham, unpublished results) but the remaining gene is sufficient to support almost normal skeletal muscle development throughout the body.

In the lateral myogenic progenitor cells, *MyoD* expression and subsequent terminal differentiation are transiently repressed in order to allow migration to the limb and body wall where muscle formation will take place. Although irreversibly committed (as shown by classic transplantation experiments), these myogenic progenitors do not express any member of the *MyoD* family either in the somite or during migration (Tajbakhsh and Buckingham, 1994), when they can be identified by the expression of the

transcription factor Pax3 (Bober *et al.*, 1994) and the receptor tyrosine kinase c-Met (Bladt *et al.*, 1995). It is therefore likely that their differentiation is repressed by signals derived from adjacent tissues. Mechanical separation of the lateral plate mesoderm from the paraxial mesoderm in the chick embryo induced expression of *MyoD* in the lateral half of somites where it is not observed normally. Furthermore, it was demonstrated that cells expressing BMP4 (Pourquié *et al.*, 1996) could replace this inhibitory activity.

Signaling molecules replace the activity of neighboring tissues

A ventralizing signal emanating from the notochord and subsequently from the floor plate of the neural tube is required to activate *Pax1* and specify a sclerotomal fate: Sonic hedgehog (Shh), normally produced by the notochord and the floor plate, can mimic its action (Fan and Tessier-Lavigne, 1994). However, it has also been shown that Shh is required to promote myogenesis, and indeed in *Shh* null embryos epaxial myogenesis is absent whereas progenitors of hypaxial myogenesis, initially located in the dorso-lateral region of newly formed somites and thus more distant from the notochord, are specified normally (Boricky *et al.*, 1999). In addition, members of the Wnt family were also shown to activate myogenesis in the dorsal part of the somite (Münsterberg *et al.*, 1995; Stern *et al.*, 1995).

Because the onset of *MyoD* and *Myf5* expression is spatially and temporally regulated in mouse embryos, it was important to know whether the differential activation of *Myf5* and *MyoD* is promoted by different members of the Wnt family that are expressed in neural tube or in the dorsal ectoderm (Parr *et al.*, 1993). Indeed, we observed that the *Myf5*-inducing activity of the neural tube can be replaced by cells expressing *Wnt1* and, to a lesser extent, *Wnt4*, while *MyoD* activation by dorsal ectoderm can be replaced by *Wnt7a*-expressing cells (Tajbakhsh *et al.*, 1998). *Wnt7a* is expressed in the correct spatio-temporal pattern to be a candidate molecule for this activity. Sonic hedgehog synergizes with both Wnt1 and Wnt7a in explants from paraxial mesoderm of early E8.5 but not older E9.5 embryos.

With current information at hand, a parsimonious model of signaling activity can be proposed, as shown in Figure 1. Shh, produced by notochord and floor plate, activates *Pax1* in the future sclerotome and, in conjunction with BMP4 (Murtaugh *et al.*, 1999), initiates chondrogenesis in this anlagen. Shh, in conjunction with Wnt1 (and possibly other Wnts), activates myogenesis in the future dermomyotome, via a *Myf5*-dependent pathway. Different Wnts such as Wnt7a activate myogenesis in the lateral domain, probably through a *MyoD*-dependent pathway. This activity is inhibited by BMP4 to prevent premature differentiation; the negative action of BMP4 is counteracted, probably through direct protein–protein interaction, by Noggin which is produced by the dorsal neural tube in a Wnt-dependent manner (Hirsinger *et al.*, 1997; Marcelle *et al.*, 1997).

Although the model illustrated in Figure 1 accommodates our present understanding, the rapid increase in the number of known molecules potentially involved in signaling during embryogenesis suggests that the model we now have will become more elaborate, and thus formal experimental evidence will be required. This will probably include *in situ* inhibition by specific antibodies (when they become available) together with detailed analysis of mutant and possibly compound mutant embryos.

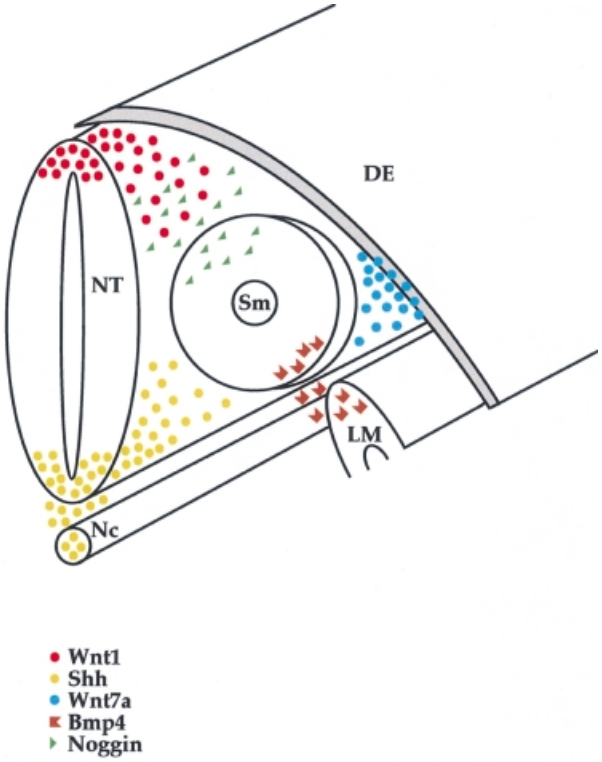

Fig. 1. A simplified scheme of signaling molecules in newly formed epithelial somite. Shh (ochre dots), produced by notochord (Nc) and floor plate, acts on the ventral domain of newly formed epithelial somites, inducing sclerotome, and also on the dorso-medial domain, inducing medial dermomyotome. Wnt1 (red dots), produced by dorsal neural tube (NT), acts (with Shh) on the dorso-medial domain of newly formed somites (Sm), where *Myf5* expression is observed soon after and epaxial progenitors are specified. Wnt7a (blue dots), produced by dorsal ectoderm (DE), acts on the dorso-lateral domain, where hypaxial progenitors are specified. BMP4 (brown polygons), produced by lateral mesoderm (LM), prevents *MyoD* activation and early differentiation in the lateral domain of somites. Its action is counteracted by direct binding of Noggin (green triangles) produced by dorsal neural tube.

From signaling molecules to downstream genes

If Shh and Wnts are the main 'instructive' molecules required to activate myogenesis, as current evidence suggests, then it becomes important to identify and understand their receptors and the downstream post-receptorial pathways that lead to activation of *Myf5* and *MyoD*. Also the relative role and mechanism of cooperation between the two different molecules need to be understood. At present, most studies are based on gene expression analysis, explant cultures and analysis of mutant phenotypes.

Recent work by Boricky *et al.* (1998) showed that genes of the Shh signal response pathway (Murone *et al.*, 1999), specifically *Patched*, the Shh receptor, and *Gli*, zinc finger transcription factors, are activated in coordination with somite formation. They also showed

that the expression of *Patched*, *Gli* and *Gli2/4* is patterned differentially in the somite. This may explain how the same signaling molecule can specify two mutually exclusive fates such as cartilage and skeletal muscle. This provides an alternative explanation to a model in which a concentration gradient of Shh discriminates between sclerotomal and myotomal induction, as supported by notochord transplantation studies (Pourquié *et al.*, 1993). More recently, the same authors (Boricky *et al.*, 1999) showed that in *Shh* null embryos, epaxial myogenesis is abolished, while development of hypaxial musculature proceeds quite normally. This is in keeping with a crucial role for Shh in initiating epaxial myogenesis, before or together with the action of Wnts (epaxial muscle cells are the first to differentiate in the myotome). However, it is puzzling that in mouse explant cultures Shh can also cooperate with Wnt7a to activate *MyoD* (lateral pathway), even though neither Shh nor other members of the family have been detected in dorsal ectoderm. It remains to be verified whether hypaxial (lateral) myogenesis is independent of this signaling molecule *in vivo* and requires other, as yet unknown molecules that cooperate with Wnts.

Vertebrate homologs of the products *Drosophila Frizzled* are putative Wnt receptors (Bhanot *et al.*, 1996). Several members of this family have been cloned in different organisms (Wodarz and Nusse, 1998; Dierick and Bejsovec, 1999). In order to correlate the Wnt-dependent activation of myogenesis with the expression of specific *Frizzled* candidate receptors, we studied the expression of eight murine *Frizzled* (*1* and *3–9*) genes during mouse somitogenesis. All the *Frizzled* genes studied have a complex and partially overlapping pattern of expression in different regions of the embryo, and many of them (*Fz1*, *3*, *7*, *8* and *9*) have specific expression in the epithelial somites as well as in the newly formed myotomes (Borello *et al.*, 1999b). Since different domains of somites are defined by differential and overlapping expression of the various basic helix–loop–helix (bHLH) transcription factors (Patapoutian *et al.*, 1995), it is tempting to speculate a possible correlation between the subsets of bHLH and Frizzled expressed. However, this analysis awaits development of specific antibodies that may distinguish among different *Frizzled* genes at a single cell level. In newly formed somites, *Fz1* is expressed along the medial border, consistent with a possible preferential interaction with Wnt1 which is produced from the adjacent dorsal neural tube. On the other hand, *Fz7* is expressed in a pattern complementary to *Fz1*, i.e. along the lateral and caudal edge of newly formed somites, consistent with the possibility of a preferential interaction with Wnt7a.

Wnt1 acts through the classic *Dishevelled*→ *GSK3*→ β-*catenin*→ *Tcf* pathway (Wodarz and Nusse, 1998; Eastman and Grosschedl, 1999); in contrast, *Wnt7a* appears to act via a β-catenin-independent pathway (Kengaku *et al.*, 1998) and leads to activation of *MyoD* rather than *Myf5* (Tajbakhsh *et al.*, 1998). It is thus tempting to speculate that Fz1 and Fz7 may mediate the differential response to Wnt1 and Wnt7a and activate different intracellular pathways (via either β-catenin or G proteins/C kinase; Sheldahl *et al.*, 1999) leading to differential activation of *Myf5* or *MyoD* in epaxial or hypaxial progenitors (Figure 2). In the last few months, understanding of the *Wnt/Fz* signaling pathway has grown dramatically in

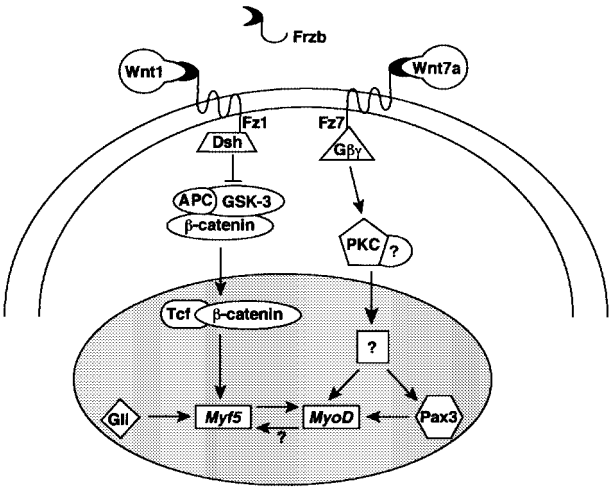

Fig. 2. A model of Wnt signaling in somites. Wnt1 binds to Fz1 and, through a β-catenin pathway, leads to activation of *Myf5*. *Gli* also converges on *Myf5* activation. In contrast, Wnt7a binds to Fz7 and, through a β-catenin independent pathway (G protein/C kinase?), leads to activation of *MyoD*. *Pax3* also converges on *MyoD* activation, but whatever is downstream of the signaling is not known. Furthermore, *Myf5* can activate *MyoD*, while the reverse is uncertain. Frzb1 can interfere with this process by sequestering Wnts in the extracellular space. Note that no experimental evidence for direct Wnt1–Fz1 or Wnt7a–Fz7 preferential binding is available so far.

complexity, with new proteins appearing continuously. Some of these proteins may act as co-receptors (proteoglycans and LDL receptor homologs) while others interact with the axin–GSK3–β-catenin complex (kinases, phosphatases, etc.), suggesting the existence of several possible steps of regulation as well as branching of the pathway (Peifer, 1999; Peters *et al.,* 1999). This suggests that, in addition or alternatively to differential expression of *Wnt* receptors, other downstream molecules may be responsible for differential activation of *Myf5* and *MyoD*. In any case, *in vitro* functional experiments with dominant-negative molecules and specific inhibitors as well as analysis of appropriate null embryos will be needed to test whether Wnt1/Fz1 and Wnt7a/Fz7 (for once at least the numbers coincide) are key activators of *Myf5* and *MyoD*, respectively, and to begin to unravel the specificity of each transduction pathway.

Another possible level of regulation of Wnt signaling may be exerted by the sFRPs (soluble Frizzled-related proteins), a new class of genes recently identified in several laboratories (Leyns *et al.*, 1997; Wang *et al.*, 1997). These are secreted molecules with a strong homology to the Frizzled extracellular domain. Among the genes examined, only *Frzb1* was found to be expressed in the pre-somitic mesoderm and newly formed somites and thus its possible role in regulating myogenesis was investigated in detail. Frzb1 totally inhibits myogenesis in cultures of pre-somitic mesoderm or newly formed somites, but has no effect on more mature somites or on myogenic cell lines, and thus appears to act differently from intracellular myogenic inhibitors such as Id or Twist. In order to examine the effect of *Frzb1* overexpression *in vivo*, we developed a method based on transient transfection of cells with a *Frzb1* expression vector and injection of transfected cells into the placenta of pregnant females before the onset of materno-fetal circulation. Frzb1,

secreted by transfected cells, accumulated in the embryo and caused a marked reduction of caudal structures. Myogenesis was strongly reduced and, in the most severe cases, abolished. Genes downstream of the *Wnt* signaling pathway such as *En1*, *Noggin* and *Myf5* were down-regulated but *Pax3* and *Mox1* were not, thereby excluding a generalized toxic effect (Borello *et al.*, 1999a). The results obtained with this new method are in keeping with the *Wnt1/Wnt3a* double knockout and corroborate the idea that Wnt signals may act by regulating both myogenic commitment and expansion of committed cells in the mouse mesoderm. Indeed, in mouse embryos lacking both *Wnt1* and *Wnt3a*, the medial compartment of the dermomyotome is not formed and the expression of *Myf5* is severely reduced (Ikeya and Takada, 1998) but not abolished, probably because partial activation by Shh has occurred.

From the data discussed above, it appears that, at least medially, Shh and Wnts cooperate to activate myogenesis; they may also instruct diversification between epaxial and hypaxial myogenesis. How this can be achieved in molecular terms is still far from clear. Gli zinc fingers, activated by Shh, may bind directly to regulatory regions of *Myf5* and *MyoD* promoters, whose complexity has made these studies difficult. Similarly, Wnt1-activated Tcf–β-catenin complex may directly activate transcription of target genes (among which are possibly *Myf5* and *MyoD*) but also contribute to changes in chromatin configuration at these loci, making them more easily accessible to other transcription factors. It should be remembered, however, that Shh has been reported to be an important survival factor for paraxial mesoderm (Teillet *et al.*, 1998; Cann *et al.*, 1999) and it also has mitogenic activity on myoblasts (Duprez *et al.*, 1998). Several Wnts, including Wnt1 and Wnt7a, have strong mitogenic and often transforming activity and most probably also act as survival factors (Wodarz and Nusse, 1999). Thus both molecules have the potential both to activate, directly or indirectly, transcription of *Myf5* and *MyoD*, and to promote survival and expansion of the committed population. In other words, if we assume that a myotome is formed of four differentiated cells, these may in one extreme case derive from immediate differentiation of four progenitors responding to an inducing molecule. In the opposite situation, only one cell is induced that divides twice and generates four differentiated cells (Figure 3). In reality, combined action of Shh/Wnt on transcriptional activation, proliferation and survival must account for the final number of differentiated cells in a given structure such as the myotome. This is also relevant to the last issue discussed in this review, namely how different fates are chosen within contiguous and probably equivalent cells of the epithelial dermomyotome (Tajbakhsh and Cossu, 1997).

The generation of myoblast diversity

It is important to recognize that the signals from neighboring tissues do not lead directly to terminal myogenic differentiation, at least in the majority of somitic cells exposed to the signaling molecules. Only a fraction of the cells exposed to Shh and Wnts signals are induced to differentiate terminally into myotomal muscle, while the remaining cells are probably kept in a committed but

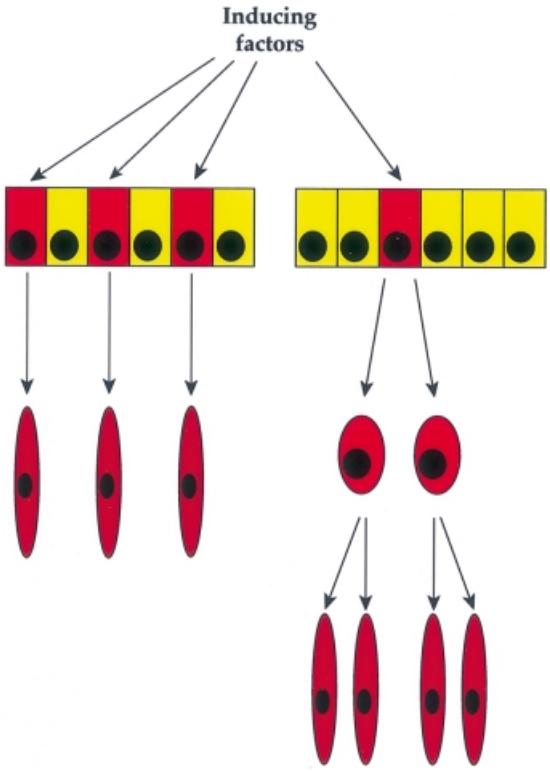

Fig. 3. Alternative mechanisms for the generation of a myotome. In response to inducing factors, several committed cells may differentiate directly without cell division (left). Alternatively, fewer committed cells need to proliferate in order to attain the final number of differentiated myotomal cells (right). In both cases, inhibition of differentiation of surrounding dermomyotomal cells is required.

undifferentiated state. In this state, cells will divide, proliferate and sometimes die until the correct number of myoblasts is attained in the right place and at the right time to produce primary and secondary fibers and satellite cells during later development (Cossu *et al.*, 1996a).

Although not proved definitively (see Bianco and Cossu, 1999; Miller *et al.*, 1999), it is widely accepted that commitment of all myogenic progenitors occurs in the epithelial dermomyotome. It is thus necessary to postulate a mechanism by which one committed cell may prevent neighboring cells from adopting the same fate. In *Drosophila*, lateral inhibition through *Notch* and *Delta* has been shown as the probable mechanism by which adult myogenic progenitors are selected in response to *Wg* signaling (Baylies *et al.*, 1998). It thus appears likely that a similar mechanism may operate in the mammalian somite. Indeed, several *Delta* and *Notch* genes are expressed in the somites (McGrew and Pourquié, 1998) and Notch inhibits myogenesis, probably through different intracellular mechanisms (Nofziger *et al.*, 1999; Wilson-Rawls *et al.*, 1999). However direct evidence for a role for *Notch* in diversifying cell fate in mammalian somites is still missing.

Receptors for growth factors may be pertinent targets for *Notch* signaling. It has been proposed that the dorsal portion of the neural tube produces growth factors such as fibroblast growth factors (FGFs) that prevent differentiation of committed progenitors (Buffinger and Stockdale, 1995). If this is the case, only progenitors that will form the myotome may express few or no receptors (possibly

as a consequence of *Notch* expression) and thus undergo terminal differentiation. From this point of view, it is interesting to note that the neural tube produces various FGFs, and the first cells which form the myotome are the only myogenic cells which do not express the FGF receptor FREK (Marcelle *et al.*, 1995). While a causal relationship between lack of growth factor receptors and early differentiation remains to be demonstrated, this mechanism may be operating at different phases of muscle histogenesis (Cusella De Angelis *et al.*, 1994) to explain the occurrence of different developmental choices within an apparently homogeneous population of committed myogenic progenitors.

Acknowledgements

We thank M.Buckingham, S.Tajbakhsh and B.Wold for helpful discussions and critical reading of the manuscript. Work in the authors' laboratory was supported by grants from Telethon, Fondazione Istituto Pasteur-Cenci Bolognetti, EC, HFSP and MURST.

References

Baylies,M.K., Bate,M. and Ruiz Gomez,M. (1998) Myogenesis: a view from *Drosophila*. *Cell*, **93**, 921–927.

Bhanot,P., Brink,M., Samos,C.H., Hsieh,J.C., Wang,Y., Macke,J.P., Andrew,D., Nathans,J. and Nusse,R. (1996) A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature*, **382**, 225–230.

Bianco,P. and Cossu,G. (1999) *Uno, nessuno e centomila*. Searching for the identity of mesodermal progenitors. *Exp. Cell Res*., **251**, 257–263.

Bladt,F., Riethmacher,D., Isenmann,S., Aguzzi,A. and Birchmeier,C. (1995) Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature*, **376**, 768–771.

Bober,E., Franz,T., Arnold,H.H., Gruss,P. and Tremblay,P. (1994) Pax-3 is required for the development of limb muscles: a possible role for the migration of dermomyotomal muscle progenitor cells. *Development*, **120**, 603–612.

Borello,U., Coletta,M., Tajbakhsh,S., Leyns,L., De Robertis,E.M., Buckingham,M. and Cossu,G. (1999a) Trans-placental delivery of the Wnt antagonist Frzb1 inhibits development of caudal paraxial mesoderm and skeletal myogenesis in mouse embryos. *Development*, **126**, 4247–4255.

Borello,U., Buffa,V., Sonnino,C., Melchionna,R., Vivarelli,E. and Cossu,G. (1999b) Differential expression of the Wnt putative receptors *Frizzled* during mouse somitogenesis. *Mech. Dev.*, in press.

Borycki,A.G., Mendham,L. and Emerson,C.P.,Jr (1998) Control of somite patterning by Sonic hedgehog and its downstream signal response genes. *Development*, **125**, 777–790.

Borycki,A.G., Brunk,B., Tajbakhsh,S., Buckingham,M., Chiang,C. and Emerson, C.P.,Jr (1999) Sonic hedgehog controls epaxial muscle determination through Myf5 activation. *Development*, **126**, 4053–4063.

Brand-Saberi,B. and Christ,B. (1999) Genetic and epigenetic control of muscle development in vertebrates. *Cell Tissue Res.*, **296**, 199–212.

Braun,T., Rudnicki,M.A., Arnold,H.H. and Jaenisch,R. (1992) Targeted inactivation of the muscle regulatory gene *Myf-5* results in abnormal rib development and perinatal death. *Cell*, **71**, 369–382.

Buffinger,N. and Stockdale,F.E. (1995) Myogenic specification of somites is mediated by diffusible factors. *Dev. Biol*., **169**, 96–108.

Cann,G.M., Lee,J.W. and Stockdale,F.E. (1999) Sonic hedgehog enhances somite cell viability and formation of primary slow muscle fibers in avian segmented mesoderm. *Anat. Embryol.*, **200**, 3, 239–252.

Cossu,G., Tajbakhsh,S. and Buckingham,M. (1996a) How is myogenesis initiated in the embryo? *Trends Genet.*, **12**, 218–223.

Cossu,G., Kelly,R., Tajbakhsh,S., Di Donna,S., Vivarelli,E. and Buckingham,M. (1996b) Activation of different myogenic pathways: *Myf5* is induced by the neural tube and *MyoD* by the dorsal ectoderm in mouse paraxial mesoderm. *Development*, **122**, 429–437.

Cusella-DeAngelis,M.G., Molinari,S., Ledonne,A., Coletta,M., Vivarelli,E., Bouché,M., Molinaro,M., Ferrari,S. and Cossu,G. (1994) Differential response of embryonic and fetal myoblasts to TGFβ: a possible regulatory mechanism of skeletal muscle histogenesis. *Development*, **120**, 925–933.

Dierick,H. and Bejsovec,A. (1999) Cellular mechanisms of wingless/Wnt signal transduction. *Curr. Top. Dev. Biol*., **43**, 153–190.

Duprez,D., Fournier-Thibault,C. and Le Douarin,N. (1998) Sonic Hedgehog induces proliferation of committed skeletal muscle cells in the chick limb. *Development*, **125**, 495–505.

Eastman,Q. and Grosschedl,R. (1999) Regulation of LEF-1/TCF transcription factors by Wnt and other signals. *Curr. Opin. Cell Biol*., **11**, 233–240.

Fan,C. and Tessier-Lavigne,M. (1994) Patterning of mammalian somites by dorsal ectoderm and notochord: evidence for sclerotome induction by a hedgehog homolog. *Cell*, **79**, 1175–1186.

Hirsinger,E., Duprez,D., Jouve,C., Malapert,P., Cooke,J. and Pourquié,O. (1997) Noggin acts downstream of Wnt and Sonic Hedgehog to antagonize BMP4 in avian somite patterning. *Development*, **124**, 4605–4614.

Ikeya,M. and Takada,S. (1998) Wnt signaling from the dorsal neural tube is required for the formation of the medial dermomyotome. *Development*, **125**, 4969–4976.

Kablar,B., Krastel,K., Ying,C., Asakura,A., Tapscott,S.J. and Rudnicki,M.A. (1997) *MyoD* and *Myf5* differentially regulate the development of limb versus trunk skeletal muscle. *Development*, **124**, 4729–4738.

Kengaku,M., Capdevila,J., Rodriguez-Esteban,C., De La Peña,J., Johnson,R.L., Izpisúa Belmonte,J.C., Clifford,J. and Tabin,C.J. (1998) WNT3a regulates AER formation and utilizes an intracellular signaling pathway distinct from the dorsoventral signal WNT7a during chick limb morphogenesis. *Science*, **280**, 1274–1277.

Leyns,L., Bouwmeester,T., Kim,S.-H., Piccolo,S. and De Robertis,E.M. (1997) Frzb1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell*, **88**, 747–756.

Marcelle,C., Wolf,J. and Bronner-Fraser,M. (1995) The *in vivo* expression of the FGF receptor FREK mRNA in avian myoblasts suggests a role in muscle growth and differentiation. *Dev. Biol.*, **172**, 100–114.

Marcelle,C., Stark,M.R. and Bronner-Fraser,M. (1997) Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite. *Development*, **124**, 3955–3963.

McGrew,M.J. and Pourquié,O. (1998) Somitogenesis: segmenting a vertebrate. *Curr. Opin. Genet. Dev.*, **8**, 487–493.

Miller,J.B., Schaefer,L. and Dominov,J.A. (1999) Seeking muscle stem cells. *Curr. Top. Dev. Biol.*, **43**, 191–219.

Münsterberg,A.E., Kitajewski,J., Bumcroft,D.A., McMahon,A.P. and Lassar,A.B. (1995) Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. *Genes Dev.*, **9**, 2911–2922.

Murone,M., Rosenthal,A. and de Sauvage,F.J. (1999) Sonic hedgehog signaling by the patched–smoothened receptor complex. *Curr. Biol.*, **9**, 76–84.

Murtaugh,L.C., Chyung,J.H. and Lassar,A.B. (1999) Sonic hedgehog promotes somitic chondrogenesis by altering the cellular response to BMP signaling. *Genes Dev.*, **13**, 225–237.

Nofziger,D., Miyamoto,A., Lyons,K.M. and Weinmaster,G. (1999) Notch signaling imposes two distinct blocks in the differentiation of C2C12 myoblasts. *Development*, **126**, 1689–1702.

Parr,B.A. and McMahon,A.P. (1995) Dorsalization by Wnt7a of DV and AP axes of mouse limb. *Nature*, **374**, 350–353.

Parr,B.A., Shea,M.J., Vassileva,G. and McMahon,A.P. (1993) Mouse *Wnt* genes exhibit discrete domains of expression in early embryonic CNS and limb buds. *Development*, **119**, 247–261.

Patapoutian,A., Yoon,J.K., Miner,J.H., Wang,S., Stark,K. and Wold,B. (1995) Disruption of the mouse *MRF4* gene identifies multiple waves of myogenesis in the myotome *Development*, **121**, 3347–3358.

Peifer, M. (1999) Signal transduction. Neither straight nor narrow. *Nature*, **400**, 213–215.

Peters,J.M., McKay,R.M., McKay,J.P. and Graff,J.M. (1999) Casein kinase I transduces Wnt signals. *Nature*, **401**, 345–350.

Pourquié,O., Coltey,M., Teillet,M.A., Ordahl,C. and Le Douarin,N.M. (1993) Control of dorsoventral patterning of somitic derivatives by notochord and floor plate. *Proc. Natl Acad. Sci. USA,* **90**, 5242–5246.

Pourquié,O. *et al*. (1996) Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell*, **84**, 461–471.

Sheldahl,L.C., Park,M., Malbon,C.C. and Moon,R.T. (1999) Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr. Biol.*, **9**, 695–698.

Stern,H.M., Brown,A.M. and Hauschka,S.D. (1995) Myogenesis in paraxial mesoderm: preferential induction by dorsal neural tube and by cells expressing Wnt-1. *Development*, **121**, 3675–3686.

Tajbakhsh,S. and Buckingham,M. (1994) Mouse limb muscle is

determined in the absence of the earliest myogenic factor *Myf5*. *Proc. Natl Acad. Sci. USA*, **9**, 747–751.

Tajbakhsh,S. and Cossu,G. (1997) Establishing myogenic identity during somitogenesis. *Curr. Opin. Genet. Dev.*, **7**, 634–641.

Tajbakhsh,S., Rocancourt,D., Cossu,G. and Buckingham,M. (1997) Redefining the genetic hierarchies controlling skeletal myogenesis: *Pax-3* and *Myf5* act upstream of *MyoD*. *Cell*, **89**, 127–138.

Tajbakhsh,S., Borello,U., Vivarelli,E., Kelly,R., Papkoff,J., Duprez,D., Buckingham,M. and Cossu,G. (1998) 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*, **125**, 4155–4162.

Teillet,M.-A., Watanabe,Y., Jeffs,P., Duprez,D., Lapointe,F. and Le Douarin,N.M. (1998) Sonic hedgehog is required for survival of both myogenic and chondrogenic somitic lineages. *Development*, **125**, 2019–2030.

Wang,S., Krinks,M., Lin,K., Luyten,F.P. and Moos,M.,Jr (1997) Frzb a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell*, **88**, 757–766.

Wilson-Rawls,J., Molkentin,J.D., Black,B.L. and Olson,E.N. (1999) Activated Notch inhibits myogenic activity of the MADS-box transcription factor myocyte enhancer factor 2C. *Mol. Cell. Biol.*, **19**, 2853–2862.

Wodarz,A. and Nusse,R. (1998) Mechanisms of Wnt signaling in development. *Annu. Rev. Cell. Dev. Biol*., **14**, 59–88.

Received August 12, 1999; revised October 15, 1999; accepted October 22, 1999