INTRODUCTION

The intracellular mechanisms that lead to skeletal myogenesis are now quite well understood, mainly because of extensive work on the MyoD family of basic helix-loop-helix factors (Weintraub et al., 1991) and, to a minor extent, other transcription factors that activate transcription of muscle genes. In contrast, the extracellular signals that activate myogenesis are still unknown. Studies, mainly conducted in the avian system, leave us with a complex picture (Emerson, 1993). Transplantation of defined regions of Hensen’s node from quail into the wing bud or coelomic cavity of chick suggested that skeletal muscle cells become determined after gastrulation (Krenn et al., 1988). A large body of evidence points to a key role of the neural tube in promoting myogenesis in vivo (Packard and Jacobson, 1976; Vivarelli and Cossu, 1986; Kenny-Mobbs and Thorogood, 1987; Rong et al., 1992; Christ et al., 1992; Buffinger and Stockdale, 1994, 1995; Stern and Hauschka, 1995; Munstenberg and Lassar, 1995), although there is evidence suggesting that the neural tube promotes survival of already committed cells rather than directly inducing myogenic determination (Teillet and Le Douarin, 1983). It is also clear that the effect of the neural tube is limited to that population of myogenic precursors destined to form dorsal (epaxial)
can be recognized by a simple and sensitive histo-chemical stain such that presomite mesoderm from these mice can be used as tissue explants in reconstitution experiments with nontransgenic tissues from the same species.

**MATERIALS AND METHODS**

**Mouse lines**

The MLC3F-nlacZ construct contains 2 kb of the mouse fast myosin light chain 3 (MLC3F) promoter together with 1 kb of 3′ flanking DNA, which includes a muscle-specific enhancer, fused to an nlacZ-SV40 poly(A) sequence. In two independent transgenic lines, the nlacZ reporter gene is strongly expressed in skeletal muscle from day 9 of embryonic development (Kelly et al., 1995). Heterozygous or homozygous transgenic males were crossed with CD1 outbred female mice.

A positive/negative selection vector containing 5.5 kb and 1.1 kb of the 5′ and 3′ flanking homology regions was used to introduce the nlacZ and neomycin-resistance genes into the myf-5 locus. The nlacZ was introduced in frame into the first exon of the myf-5 gene such that the expression of this reporter gene is under the transcriptional and translational control of the endogenous myf-5 locus. Independent ES

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**Fig. 1.** Scheme of a caudal transverse section of a 20- to 25-somite mouse embryo (9.5 dpc) showing the microdissections (broken lines) performed to isolate the paraxial mesoderm with or without various adjacent tissues. (A) The whole paraxial and dorsolateral structures are cultured together without axial structures. (B) The whole paraxial mesoderm is cultured together with axial structures but without dorsolateral tissues. (C) Isolated paraxial mesoderm. (D) Isolated paraxial mesoderm cultured with its own adjacent dorsal ectoderm. (E) Paraxial and lateral mesoderm cultured without axial structures and dorsal ectoderm. Isolated paraxial mesoderm was also recombined in vitro with axial structures (F), dorsal aorta (G), dorsal ectoderm (H) and lateral mesoderm (I). DA, dorsal aorta; DE, dorsal ectoderm; LM, lateral mesoderm; NT, neural tube; PM, paraxial mesoderm.
Fig. 2. Organ culture of UPM (22-somite embryo) from MLC3FlacZ transgenic mice, showing the number of β-galactosidase-positive cells developed after 3 days in vitro. (A) UMP cultured with the adjacent axial structures (neural tube/notochord). (B) UPM cultured in isolation; (C) UPM cultured with the adjacent dorsolateral structures. Bar, 40 µm.

Fig. 3. The effect of surrounding tissues on myogenic differentiation of paraxial mesoderm. The unsegmented paraxial mesoderm (UPM), the last-formed somite (Som I) or the last three formed somites (Som I-III) were dissected and cultured either in isolation (−), or with the adjacent axial structures (AS, neural tube and notochord), dorsolateral structures (DLS, dorsal ectoderm, lateral mesoderm and dorsal aorta), lateral structures (LS, without dorsal ectoderm), dorsal ectoderm (DE). Alternatively, the same paraxial structures were isolated and then re-aggregated with axial structures (+AS), dorsolateral structures (+DLS), lateral structures (+LS), dorsal ectoderm (+DE) and dorsal aorta (+DA). After 3 days in culture the number of β-galactosidase-positive cells was recorded. Each point is the average ± s.e. of at least five separate experiments, each performed in triplicate.

Fig. 4. Organ culture of somite I (22-somite embryo) from MLC3FlacZ transgenic mice, cultured with the adjacent dorsal ectoderm showing a large number of β-galactosidase-positive cells developed after 3 days in vitro (A). The presence of the ectoderm is confirmed after staining with an anti-keratin monoclonal antibody (C). This antibody only recognizes the ectoderm (arrow) among the dorsal structures of a 22-somite embryo at the level of the unsegmented paraxial mesoderm (D). I, intestine; NT, neural tube; PM, paraxial mesoderm. Phase contrast shown in B.
clones containing the mutated myf-5 allele were used to generate heterozygous mice (Tajbaksh and Buckingham, 1995).

**Organ and cell cultures**

Embryos were dated taking day 0.5 postcoitus (p.c.) as the day of the vaginal plug. For most experiments, embryos ranging in age from 20 to 25 somites (9.5 days p.c.) were isolated in PBS. The heart of the MLC3F-nlacZ embryos, where the transgene is also expressed (Kelly et al., 1995), and the cranial myotomes of the myf-5-nlacZ embryos, were stained for β-galactosidase activity (β-gal) and β-gal-positive and -negative embryos were pooled separately. The tissues were then digested with 0.25% pancreatin-0.1% trypsin for 5 minutes at 4°C. After the enzymatic digestion, the various tissues were mechanically separated according to the experimental scheme and then cultured in complete medium (see below). In experiments where re-aggregation of different tissues was required, the tissue fragments were seeded together (under the dissecting microscope) on a layer of 10T1/2 cells, to which they adhered within 10 minutes, and the dishes were then carefully transferred to the incubator. Preliminary experiments had shown that 10T1/2 cells did not alter the extent of myogenic differentiation.

In other experiments, dissected halves of UPM (with or without axial structures or dorsal ectoderm) were mechanically dissociated by gently pipetting through the yellow tip of a Gilson pipette to produce a suspension containing single cells and clusters ranging up to approximately 100-200 cells. This suspension was plated in complete medium on collagen-coated dishes. Alternatively, the cell suspensions from the medial and lateral halves of enzymatically dissected UPM were mixed with a similar suspension obtained from axial structures of sibling embryos.

All cultures were grown in RPMI medium (GIBCO) supplemented with 10% fetal calf serum (Flow), 300 mM β-mercaptoethanol and 50 μg/ml gentamycin. At the times indicated, cultures were fixed and stained for β-galactosidase activity and/or incubated with different antibodies.

**Immunocytochemistry**

Immunocytochemistry on tissue sections and cultured cells was carried out as described (Cusella-De Angelis et al., 1994; Tajbaksh et al., 1994) using the following antibodies:

1. anti-β-galactosidase monoclonal antibody (from Sigma);
2. FDS anti-myogenin monoclonal antibody (Cusella De Angelis et al., 1992), donated by W. Wright.
3. MF20 anti-sarcomeric myosin monoclonal antibody (Bader et al., 1982), donated by D. Fischman.
4. a rabbit polyclonal anti-MyoD antibody (Koishi et al. 1995), donated by J. Harris.
5. a rabbit polyclonal anti-β-galactosidase antibody (Tajbaksh et al., 1994), donated by O. Puijalon.
6. an anti-keratin rat monoclonal antibody, donated by D. Paulin.
7. a rabbit polyclonal anti-sarcomeric myosin antibody (Cusella De Angelis et al., 1994), produced in our laboratory.
8. an anti-PECAM rat monoclonal antibody, which recognizes dorsal aorta, donated by E. Dejana.

Polyclonal antibodies and the anti-β-galactosidase monoclonal were diluted 1:100 before use; other monoclonal antibodies were used undiluted supernatant.

**RESULTS**

Myogenic differentiation of mesoderm depends on dorsolateral structures as well as axial structures

In order to investigate how axial structures and, possibly, a corresponding dorsolateral structure may influence terminal differentiation in newly formed somites as well as in unsegmented paraxial mesoderm (UPM), we performed three types of experiments, illustrated in Fig. 1. In the first type of experiment, single somites or streaks of newly formed somites, or UPM (from MLC3F-nlacZ, 22- to 24-somite embryos) were isolated from axial structures (neural tube and notochord) but not from dorsolateral structures of the body (dorsal ectoderm, lateral mesoderm and dorsal aorta); also the same structures from the paraxial mesoderm were isolated from dorsolateral but not from axial structures (A and B in Fig. 1). Finally, UPM or somites were isolated from all surrounding tissues (C in Fig. 1). The explants were cultured for 3 days and the number of β-galactosidase-positive nuclei was then recorded after staining for the enzymatic activity. Preliminary experiments (not shown) had demonstrated that β-gal-positive nuclei invariably localized within myosin-positive cells and that cells expressing neurofilaments or glial fibrillar acidic protein were present only in cultures that included the neural tube (immuno-cytochemical specific markers for dorsolateral mesoderm are not available and therefore this control was not possible).

Fig. 2 shows an example of these cultures: UPM cultured with adjacent axial structures gave rise to a large number of β-gal-positive (i.e. differentiated) cells; in contrast, very few β-gal-positive cells were generated from UPM cultured in isolation. However, a good extent of differentiation occurred when UPM was cultured with adjacent lateral structures, in the absence of the neural tube. A quantitative analysis, summarizing more than 20 experiments is reported in Fig. 3. It is clear from the data that the axial structures are required for optimal differentiation of UPM or newly formed somite(s), but that differentiation can also be induced by dorsolateral structures. Occasionally, we noticed more differentiation in isolated somites or UPM, possibly due to incomplete removal of surrounding tissues (see below).

**The source of the dorsolateral signals**

In the second series of experiments, we attempted to determine which among the various dorsolateral structures can induce myogenesis in explants of paraxial mesoderm. For this purpose, somites or UPM were isolated from all surrounding tissues and then reaggregated in vitro with either axial or lateral structures isolated from non-transgenic siblings. Specifically, the UPM (or Som I or Som I-III) were recombined with either the dorsal aorta, recognized by an anti-PECAM antibody (G in Fig. 1), or with dorsal ectoderm, recognized by an anti-keratin antibody (H in Fig. 1), or with adjacent lateral mesoderm, including mesonephron, aorta and coelomic epithelia but not dorsal ectoderm (I in Fig. 1). Reconstitution experiments with axial structures were also carried out as positive controls (F in Fig. 1). However, under the conditions tested, only axial structures reproducibly induced a high extent of differentiation (Fig. 3). In the case of dorsal ectoderm, the experiments were technically difficult, as often the pancreatin-digested ectoderm would roll on itself, making it difficult to recombine it with the mesoderm. Because of the reported observations that dorsal ectoderm will induce dorsal markers only if left in contact with the mesoderm (Kuratani et al., 1994; Fan and Tessier-Lavigne, 1994), we performed a third series of experiments, by culturing UPM or newly formed somites either in isolation or with their own dorsal ectoderm or with their own lateral mesoderm (C, D and E in Fig. 1). Fig. 4A and C shows a very
high extent of differentiation in somite I cultured with its own dorsal ectoderm, revealed by an anti-keratin antibody, while somites cultured in isolation or only with lateral mesoderm showed little or no differentiation (data not shown). Morphological analysis of the unsegmented mesoderm and newly formed somites from mouse embryos at a 22-somite stage, revealed close contact between paraxial mesoderm and the dorsal ectoderm, revealed by the anti-keratin antibody (Fig. 4B,D). In order to demonstrate the existence of both positive and negative signals emanating from dorsolateral structures, we isolated the UPM of 22-somite embryos with or without dorsal ectoderm and with or without lateral mesoderm. The explants were grown in culture and the number of differentiated cells under each condition was monitored daily for 5 days. All cultures were then stained with anti-keratin antibodies to confirm the presence or the absence of ectoderm. In three separate experiments (Fig. 5 shows a representative experiment), no differentiation occurred in cultures of UPM alone or of UPM with lateral mesoderm only. In contrast, rapid differentiation occurred in cultures of UPM also containing dorsal ectoderm. Cultures containing both dorsal ectoderm and lateral mesoderm showed a delayed appearance of differentiated cells which, after longer periods in culture, were more numerous than in the corresponding cultures with dorsal ectoderm only. The simplest interpretation of these data is the release of an inhibitory signal from the lateral mesoderm which delayed differentiation of myogenic cells. If this signal is a growth factor, then the cells would be prevented from differentiating by being forced to divide, thus explaining the final increase in total number.

**Medial signals activate myf5 while dorsolateral signals activate MyoD**

We next investigated whether medial and dorsolateral signaling tissues, i.e. neural tube/notochord complex and the dorsal ectoderm, activate the myogenic program in the precursor populations through the same or different members of the MyoD family of basic-HLH transcription factors. Evidence already exists showing earlier dorsal expression of myf-5 and later ventral expression of MyoD in newly formed myotomes (Ott et al., 1991; Smith et al., 1994; Goldhamer et al., 1995). However, to address this point in detail, it is necessary to identify the expression of both genes at a single cell level. We took advantage of mice that carry nlacZ targeted to the myf-5 gene, so that all the cells that express the gene can be identified by staining for β-galactosidase activity or with antibodies against β-galactosidase. In control double-labeling experiments, anti-myf-5 polyclonal (Smith et al., 1994) and anti-β-galactosidase monoclonal antibodies labeled the same nuclei in both cryostat sections and cultured cells. Most of the experiments described here were carried out using a polyclonal antibody against MyoD and a monoclonal anti-β-galactosidase antibody to localize the presence of one or both proteins inside the nucleus of myogenic cells both in vivo and in culture. Fig. 6 shows a transverse section (at the level of the forelimb) of a 10.5 dpc heterozygous myf5/nLacZ embryo, double stained with antibodies against MyoD and β-galactosidase. It is clear from the figure that there is a large overlapping area of expression of the two genes, localized to the same nucleus in about half of the cells (double arrowheads in Fig. 6C,D); it also appears that there is a ventrolateral area of the somite muscle where MyoD but not myf-5 is expressed at detectable levels (arrows in Fig. 6A,B), while myf-5 appears to be predominantly expressed in the dorsomedial area. Because MyoD has a later onset of expression in vivo and can be clearly detected only when the myotome is formed (Buckingham, 1992), we performed experiments aimed to clarify the onset of expression of the two genes in the median and lateral halves of the paraxial mesoderm, by physically separating the median half of UPM with adjacent inducing tissues (neural tube, notochord) from the lateral half (with its own ectoderm), and growing the two separate halves as high density cell cultures. The tissues were mechanically dissociated to obtain a suspension of small clusters of cells so that inductive interactions among different cells types would not be disrupted, while allowing double immunofluorescence to be performed at different times on the resulting multilayer culture. Fig. 7 shows that after 1 day in vitro, several cells derived from the medial half (roughly from 50 to 100 cells per UPM) would express myf-5 but not MyoD, with rare cells expressing both myf-5 and MyoD. In contrast the cultures derived from the lateral half contained a lower number of positive cells and these cells expressed MyoD but not myf-5. By the next day in vitro, cultures of both medial and lateral halves contained several hundreds of cells that coexpressed both proteins although with variable relative intensity, making quantification very difficult (Fig. 8). A minority of cells expressed predominantly either myf-5 or MyoD, but cells expressing only one of the two gene products at a detectable level were rare, at least in vitro.

We next investigated whether myogenic precursor cells in the dorsal portion of the UPM are already determined in their capacity for activating one or the other myogenic program (through myf-5 or MyoD) or whether they are instructed by different signals derived from axial structures and dorsal ectoderm. For this purpose, the medial and the lateral halves of the UPM were dissected free of any adjacent tissues and...
mixed with a similar cellular suspension derived from axial structures. After 1 day in culture, several cells derived from both the medial and the lateral halves of the UPM expressed myf-5 but not MyoD, thus showing that axial structures can activate myf-5 in myogenic precursor cells independently from their previous location in the paraxial mesoderm (data not shown). The complementary experiment could not be performed, since dorsal ectoderm will not induce myogenesis once separated from the underlying mesoderm.

**DISCUSSION**

The data reported here show that both dorsal ectoderm and axial structures will induce cells in the paraxial mesoderm to undergo myogenesis. Furthermore, we show that signal(s) emanating from the axial structures, which induce myogenesis in the medial half of paraxial mesoderm, lead to activation of myf-5, while signals derived from the dorsal ectoderm lead to activation of MyoD laterally; in this case, however, differentiation is probably repressed by signals originating from lateral mesoderm. This information is summarized in a model of myogenic induction in mammals, shown in Fig. 9.

**Dorsal ectoderm induces myogenesis in the paraxial mesoderm**

In addition to a well-established signal emanating from axial structures (neural tube and notochord), a dorsolateral signal exists that induces myogenesis in the lateral half of the paraxial mesoderm. This signal is derived from the dorsal ectoderm and only acts if a close contact between the two tissues is preserved. Our data showing that newly formed somites or UPM will differentiate if dorsal ectoderm is not removed, are in good agreement with the observation that expression of Pax-3, a marker of dorsal somitic mesoderm, temporally and spatially preceding expression of myogenic markers, takes place in explants of somites in close contact with dorsal ectoderm (Fan and Tessier-Lavigne, 1994). The requirement for a close contact between the basal side of the ectoderm and the paraxial mesoderm would explain why, in different assays where ectoderm is cultured in proximity or across a filter (Stern and Hauschka, 1995; Buffinger and Stockdale, 1995), differentiation is not induced. In our own experience, ectoderm did not induce myogenesis in reconstitution experiments where it did not remain adherent to the mesoderm. In a previous study, Kenny-Mobbs and Thorogood (1987) reported differentiation of isolated brachial somites when reconstituted with adjacent epithelium. Here again, the variability reported might have been due to the more or less intimate reconstitution of the two tissues and to the side of the epithelium facing the mesoderm.

It could be argued that neural crest cells leave the neural tube and migrate between the dorsal ectoderm and the somites just at the onset of somitogenesis and may therefore be responsible for the inductive effect (Bonner-Fraser, 1993). However, if the neural tube is removed at the level of the unsegmented mesoderm as in the experiments described here, then few if any neural crest cells should have already left the neuroepithelium (Serbedzija et al., 1990), making it unlikely that these cells contribute to the inductive effect.

**Differentiation of myogenic cells in the lateral half of the paraxial mesoderm is delayed by the lateral mesoderm**

Dorsal ectoderm is adjacent to the dorsolateral portion of the segmental plate and newly formed somites. This region

![Fig. 6](image-url) Double immunofluorescence of a transverse section at the forelimb bud level of a 45-somite (10.5 dpc) myf-5/nlacZ heterozygous embryo, stained with an anti-β-galactosidase monoclonal antibody (A,C) and with anti-MyoD polyclonal antibody (B,D). Staining for β-galactosidase (identifying myf-5-expressing cells) is confined to the dorsal portion of the myotome (D), whereas staining for MyoD extends more ventrally (shown by an arrow in A and B), although overlapping with the majority of the myf-5-positive area. Higher magnification of a dorsal area, shown in C and D, reveals nuclei expressing myf-5 but not MyoD (arrowhead), nuclei expressing MyoD but not myf-5 (arrow) and nuclei expressing both proteins (double arrowheads). Bar, 40 μm.
harbors the myogenic precursors of the hypaxial muscle, which will leave the dermomyotome and migrate to the limb and the body wall. These cells do not express any member of the MyoD family either in the somite or during migration (Tajbakhsh and Buckingham, 1994), and yet they are committed irreversibly to myogenesis as shown by classic transplantation experiments (Chevallier et al., 1977; Jacob et al., 1977). It is therefore possible that differentiation of these cells is repressed by signals derived from adjacent tissues. In the explant cultures described here, differentiation of lateral myogenic cells is delayed by the lateral mesoderm which may produce growth factors or factors stimulating cell movement.

Fig. 7. Double immunofluorescence of a 1-day-old culture derived from the medial half of the UPM with the adjacent neural tube (A,B) and from the lateral half of the UPM with the adjacent dorsolateral structures (C,D) isolated from a myf-5/nlslacZ 22-somite heterozygous embryo, stained with an anti-β-galactosidase monoclonal antibody (A,C) and with an anti-MyoD polyclonal antibody (B,D). Most cells in culture from the medial half express β-galactosidase but not MyoD (shown by arrowhead); the opposite is true for cultures from the lateral half (arrows). Rare double-labeled cells are shown by double arrowheads. Fluorescence in the bottom-left corner in C and D is due to a clump of undissociated tissue and is not specific (i.e. nuclear). Bar, 10 µm.

Fig. 8. Double immunofluorescence of a 2-day-old culture derived from the medial half of the UPM with the adjacent neural tube isolated from a myf-5/nlslacZ 22-somite heterozygous embryo, stained with an anti-β-galactosidase monoclonal antibody (A) and with an anti-MyoD polyclonal antibody (B). Most cells express both antigens although to a variable extent: One cell expressing high levels of β-galactosidase and low levels of MyoD is shown by an arrowhead; cells expressing high levels of MyoD and low levels of β-galactosidase are shown by arrows, double-labeled cells are shown by double arrowheads. Bar, 15 µm.
Myo D is activated before myf-5 in the lateral half of the segmental plate, whereas myf-5 is activated first in the medial half

In dissociated high-density cultures obtained from the lateral half of the segmental plate, a certain number of cells, which probably receive signals from overlying ectodermal cells, begin to express one member of the family by the first day in culture: this is invariably MyoD, strongly suggesting that dorsal ectoderm activates myogenesis through a MyoD-dependent pathway. By the second day in culture, the great majority of positive cells express both MyoD and myf-5, although with variable intensity. The very large increase in the total number of positive cells makes it impossible to determine whether MyoD activates myf-5 in cultures from the lateral half. This does not occur in myogenically converted muscle cell lines (Montarras et al., 1991). Either this activation takes place in vivo or, alternatively, the majority of newly differentiating cells now express both genes simultaneously. At this time, myogenin and myosin also begin to be expressed in the myogenic population (unpublished observations). Myf-5 is the only member of the MyoD family initially expressed in the large majority of myogenic cells cultured from the medio-lateral half of the mouse segmental plate. Here again, by the second day in vitro, most cells express both genes, and this is probably due to myf-5 activation of MyoD given that this occurs under a variety of in vitro conditions (Montarras et al., 1991). Culturing cells from the lateral half of the UPM with cells derived from axial structures resulted in activation of myf-5, thus suggesting that cells in the segmental plate are not already committed to a medial (via myf-5) or to a lateral (via MyoD) fate. This is in agreement with the notion that replacing the medial half of a newly formed somite with the corresponding lateral half does not perturb development (Ordahl and Le Douarin, 1991) indicating persistent plasticity of myogenic precursors at this stage of development.

Myf-5 is the factor expressed in MyoD-negative, primordial myoblasts (Cusella De Angelis et al., 1992), which appear to be insensitive to inhibitory signals (growth factors) and initiate myotome formation. However, since the myotome is initially composed of a relatively small number of post-mitotic myocytes, other myogenic precursor cells must continue to proliferate before differentiating subsequently to form the remaining epaxial musculature. In this context, it has been proposed that the dorsal portion of the neural tube inhibits terminal myogenic differentiation through production of growth factors (Buffinger and Stockdale, 1995).

Two signals and two pathways for myogenic induction?

MyoD and myf-5 are the two members of the family of bHLH myogenic factors present in dividing myoblasts in culture (Buckingham, 1994) and mutations in both genes result in a dramatic impairment of the myoblast cell population in vivo (Rudnicki et al., 1993). During mouse embryogenesis, myf-5 is expressed first, in the medial lip of the dermatomyotome before overt muscle differentiation (Ott et al., 1991), in response, as shown here, to signals from axial structures. We now show that MyoD is activated in cells cultured from the lateral part of the paraxial mesoderm in response to signals from the dorsal ectoderm, thus suggesting that in vivo the activation of myogenesis in the lateral part of paraxial mesoderm occurs through a MyoD-dependent pathway, even if its expression is repressed by the lateral mesoderm. Subsequently, many muscle cells coexpress MyoD and myf-5 in vitro and in vivo (this manuscript and also Ott et al., 1991). Independent activation of myf-5 and MyoD accounts for the formation of most skeletal muscle in mice in which only the myf-5 or MyoD gene is mutated (Rudnicki et al., 1992; Braun et al., 1992). This suggests that, although initially precursor cells responding to environmental signals will activate one or the other gene, subsequently the majority of myogenic cells can use either and normally use both factors.

It is tempting to speculate that myf-5 and MyoD may have duplicated from a common ancestor with diversification of dorsal and ventral muscles, possibly with the appearance of primitive vertebrates. Whether the myf-5 and MyoD proteins have intrinsically different properties remains to be established and, indeed, the fact that in birds the two genes seem to have exchanged roles (Pownall and Emerson, 1992) in terms of which is activated first might argue against this. However, it is clear that during the diversification of this gene family, the two genes acquired different regulatory sequences that determine their response to signals from axial and dorsolateral structures. The molecules that regulate the expression of myf-5 and MyoD remain to be determined.
REFERENCES


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