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Control of somite patterning by signals from the lateral plate

(*MyoD/myf5/Pax-3*/myogenesis)

OLIVIER POURQUIÉ*, MONIQUE COLTEY, CHRISTIANE BRÉANT, AND NICOLE M. LE DOUARIN

Institut d'Embryologie Cellulaire et Moléculaire du Centre National de la Recherche Scientifique et du Collège de France, 49 bis Avenue de la Belle Gabrielle, 94736 Nogent sur Marne Cedex, France

Contributed by Nicole M. Le Douarin, December 30, 1994

ABSTRACT **The body musculature of higher vertebrates is composed of the epaxial muscles, associated with the vertebral column, and of the hypaxial muscles of the limbs and ventro-lateral body wall. Both sets of muscles arise from different cell populations within the dermomyotomal component of the somite. Myogenesis first occurs in the medial somitic cells that will form the epaxial muscles and starts with a significant delay in cells derived from the lateral somitic moiety that migrate to yield the hypaxial muscles. The newly formed somite is mostly composed of unspecified cells, and the determination of somitic compartments toward specific lineages is controlled by environmental cues. In this report, we show that determinant signals for lateral somite specification are provided by the lateral plate. They result in a blockade of the myogenic program, which maintains the lateral somitic cells as undifferentiated muscle progenitors expressing the *Pax-3* gene, and represses the activation of the *MyoD* family genes. *In vivo*, this mechanism could account for the delay observed in the onset of myogenesis between muscles of the epaxial and hypaxial domains.**

The somites are transient metameric structures characteristic of the chordates. In vertebrates, they appear as pairs of epithelial spheres that bud off from the unsegmented paraxial mesoderm in a cranio-caudal direction. Somites subsequently become polarized into a ventral mesenchymal compartment, the sclerotome, which yields the axial skeleton, and a dorsal epithelial component, the dermomyotome, from which arise all striated muscles of the adult except those of the head (1, 2). Cell lineage analysis and surgical experiments in birds have demonstrated that the epithelial somite can be subdivided into a medial and a lateral compartment. Both somitic moieties originate from different regions of Hensen's node (3) and contribute to the formation of different sets of muscles (4). The medial somitic half gives rise to the paraxial muscles of the vertebral column (epaxial domain), whereas the lateral somitic cells migrate to form the musculature of the limbs and body wall (hypaxial domain).

Several genes controlling the early steps of somitic cell commitment toward the muscle lineage have now been identified. Their expression pattern has been well characterized in chicken and mouse embryos. The earliest of these genes is the *Pax-3* transcription factor (5–7). It is expressed in the early epithelial somite and becomes restricted to the dermomyotome. Later on, a higher expression level is retained by muscle progenitors of the lateral somitic half. It has been suggested that somitic cells must down-regulate the expression of *Pax-3* in order to proceed in their differentiation toward the muscle lineage (5). In the mouse mutant Splotch (6, 7), mutation of *Pax-3* leads to the absence of limb muscles altogether.

The next genes to be activated are the myogenic factors *MyoD* and *myf5*. These genes are transcription factors, first identified by their ability to initiate the myogenic program when introduced into fibroblasts (8). In the chicken embryo, the *MyoD* and *myf5* genes are expressed first in the dorsomedial region of the somite and then in the myotomal cells (9). Cells derived from the lateral somitic moiety show a different schedule of expression of these genes. They retain strong *Pax-3* expression as they migrate toward their differentiation site (5–7). Activation of the *MyoD* family genes in cells derived

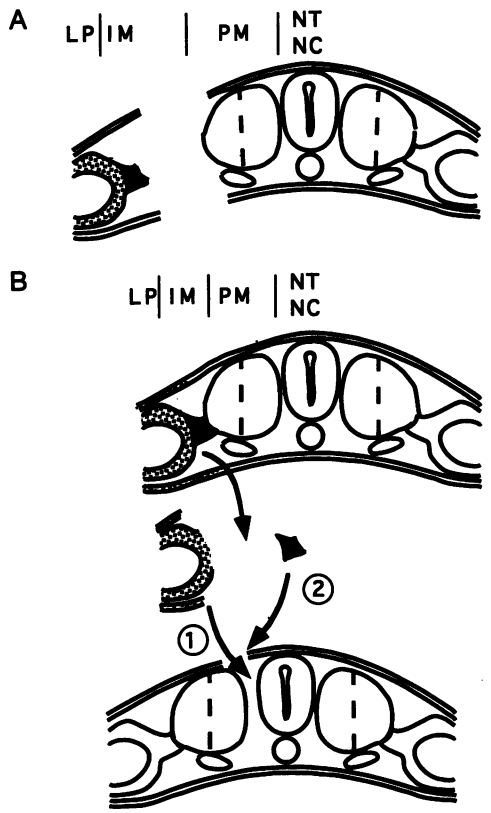

FIG. 1. Schematic transverse sections of 2-day quail or chicken embryos at the level of the unsegmented paraxial mesoderm showing the microsurgical operations. (*A*) Separation of the paraxial mesoderm from the intermediate and lateral plate mesoderm. A slit was made through all three germ layers using a microscalpel, so that both parts differentiated independently. The operation was performed at the level of the unsegmented paraxial mesoderm and of the four last-formed somites. (*B*) Graft of the lateral plate (1) or of the intermediate mesoderm (2). Either the intermediate mesoderm (in black) or a strip of lateral plate (stippled area) was removed from a stage-matched donor embryo using a microscalpel. The graft was implanted into a slit between the neural tube and the unsegmented paraxial mesoderm as described (19). IM, intermediate mesoderm; LP, lateral plate; NC, notochord; NT, neural tube; PM, paraxial mesoderm. The hatched line in the paraxial mesoderm symbolizes the demarcation between the medial and lateral domains.

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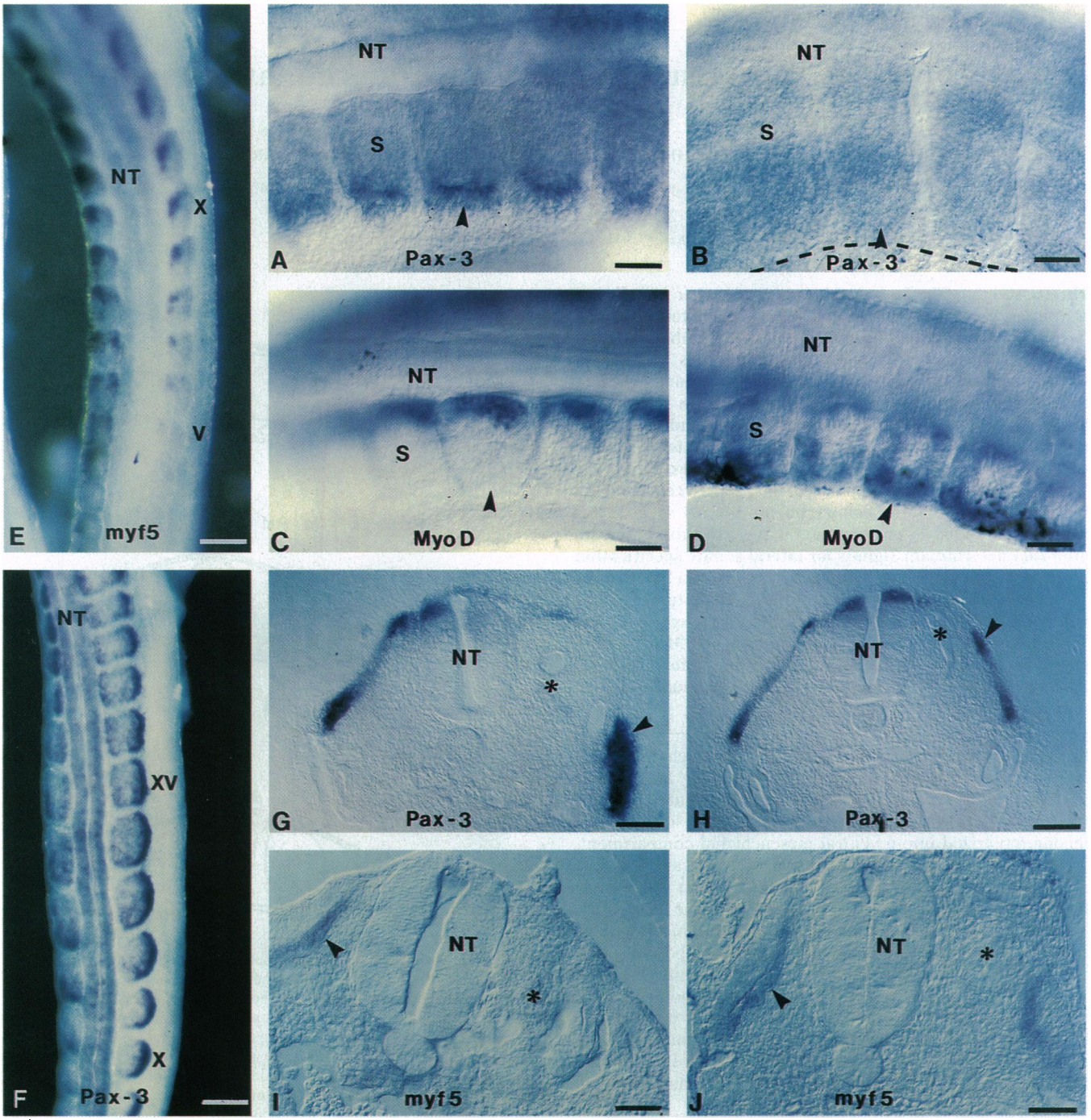

FIG. 2. (*A–D*) Separation of the paraxial mesoderm from its lateral environment leads to *Pax-3* down-regulation and *MyoD* up-regulation in cells of the lateral somitic half. Lateral views of the upper thoracic region of 3-day embryos. Dorsal is up. (Bar = 50 μm.) (*A*) Somites X–XV from a normal 30-somite chicken embryo hybridized with the *Pax-3* probe. The strong expression domain (arrowhead) corresponds to the lateral somitic cells that give rise to the precursors of the limbs and body wall muscles. Anterior is to the right. (*B*) Somites X–XV from a 30-somite chicken embryo operated at the 18-somite stage hybridized with the *Pax-3* probe. The slit is indicated by broken lines. The lateral domain of *Pax-3* expression is absent (arrowhead). Anterior is to the left. (*C*) Somites VI–X from a normal 30-somite quail embryo hybridized with the *MyoD* probe. The positive cells are early myotome cells located in the dorso-medial domain of the somite. *MyoD* message is not detected in the lateral domain (arrowhead). Anterior is to the right. (*D*) Somites VI–X from a 30-somite quail embryo operated at the 16-somite stage. *MyoD* is maintained in the medial cells but becomes expressed elsewhere in the somite. The expression level is particularly intense in cells of the lateral somitic half (arrowhead). Anterior is to the left. (*E*) Low-power dorsal view of a 28-somite quail embryo operated at the 15-somite stage, hybridized with the *myf5* probe. Somite numbers are indicated in roman numerals. The slit is on the left side of the embryo and anterior is up. Clear up-regulation of *myf5* is observed in the lateral somitic half on the operated side. (Bar = 150 μm.) (*F*) Low-power dorsal view of a 26-somite chicken embryo operated at the 13-somite stage hybridized with the *Pax-3* probe. *Pax-3* is down-regulated in the lateral domain. Somite numbers are indicated in roman numerals. (Bar = 150 μm.) (*G*) Transverse section at the level of somites X–XII from a 30-somite chicken embryo operated at the 15-somite stage hybridized in whole mount with the *Pax-3* probe. The graft of lateral plate (asterisk) between the neural tube and the paraxial mesoderm induces a strong expression of *Pax-3* message in the medial somitic cells (arrowhead) when compared with its distribution in the control side. (Bar = 100 μm.) (*H*) Transverse section at the level of somites X–XII from a 32-somite chicken embryo operated at the 16-somite stage. The graft of intermediate mesoderm (asterisk) has no effect on *Pax-3* expression in the medial somitic half (arrowhead). (Bar = 100 μm.) (*I* and *J*) The lateral plate (*I*) but not the

(*Legend continues on the following page.*)

from the lateral somitic half starts only when they have settled in their definitive location—that is, 2 days later than in cells of the medial half (9, 10).

Several lines of evidence have shown that in the newly formed somite, cells are not definitively committed to their lineage, thus implying that their differentiation is largely controlled by environmental cues (4, 11). Muscle differentiation from the medial somitic moiety depends upon signals arising from the axial organs—i.e., neural tube and notochord (12–17)—although the instructive or permissive nature of these signals is not clear yet. Cells of the lateral somitic half, which by their position would not be subjected to such signals, activate the myogenic program later on, when their new environment provides them with the appropriate factors. The problem we addressed in this study was to know why and under what influence the myogenic program is delayed in cells of the lateral somitic moiety. We show that the lateral plate delivers signals to the nearby paraxial mesoderm that block the onset of myogenesis in cells of the lateral somitic half by maintaining them in a *Pax-3* undifferentiated state.

MATERIALS AND METHODS

Eggs and Embryos. Chicken (JA57 from Institut de Sélection Animale, Lyon, France) and quail embryos were obtained from commercial sources. The eggs were incubated in a humidified atmosphere at 38°C and the embryos were staged by the number of somite pairs formed or according to the developmental tables of Hamburger and Hamilton (HH) (18).

Separation of the Paraxial Mesoderm from the Intermediate Mesoderm and the Lateral Plate. A slit through all three germ layers was made laterally to the somites and the unsegmented paraxial mesoderm using a microscalpel on 13- to 22-somite chicken and quail embryos (Fig. 1*A*). The slit was performed over the length of the unsegmented paraxial mesoderm and the last 4 segmented somites. Embryos were reincubated for periods of time ranging from 6 to 24 hr. They were fixed in 4% paraformaldehyde and processed for whole mount *in situ* hybridization with the *Pax-3*, *myf5*, and *MyoD* probes.

Graft of the Intermediate Mesoderm and Lateral Plate Between the Neural Tube and the Unsegmented Paraxial Mesoderm. Embryos were operated at the 12- to 22-somite stage. In one series of experiments, a strip of lateral plate was cut at the level of the most recently formed somites using a microscalpel. The strip was grafted into a groove made between the neural tube and the unsegmented paraxial mesoderm (Fig. 1*B*). In the second series of experiments, the Wolffian duct, associated with intermediate mesoderm, was isolated from stage-matched embryos after treatment of the embryo with 4× pancreatin (GIBCO) and grafted as described above. As a control, a fragment of egg shell membrane was grafted between the neural tube and the paraxial mesoderm. In all series, the graft was placed over a length of 2–6 presumptive somites at the level of the unsegmented paraxial mesoderm. The embryos were allowed to develop for 16–24 hr and then fixed in 4% paraformaldehyde. They were then processed for whole mount *in situ* hybridization with the *Pax-3*, *MyoD*, and *myf5* probes.

Whole Mount *in Situ* Hybridization. Probes derived from the quail *MyoD* and *myf5* genes were a generous gift of Charles Emerson (University of Pennsylvania, Philadelphia) and are described in ref. 9. They were found to give a stronger signal in quail embryos and were therefore used only in this species. The *Pax-3* probe is a generous gift from Peter Gruss (Max-Planck-Institute for Biochemistry, Göttingen, Germany). It was produced from a 660-bp *Eco*RV fragment derived from the chicken Pax-3 cDNA, cloned in Bluescript KS. The plasmid was linearized with *Xba* I and the cRNA was synthesized using the T3 polymerase. This probe was used with chicken embryos. Operated embryos ranging from stage 16 HH to stage 21 HH were fixed 24 hr in fresh 4% paraformaldehyde in phosphate-buffered saline (PBS), washed once in PBT (PBS/0.25% Tween 20), then dehydrated through a series of 5-min washes in 25%, 50%, 75%, and 100% methanol, and stored at −20°C. After rehydration, hybridization was performed as described (20). Embryos were then photographed as whole mounts in PBT using a Wild stereomicroscope or a Nikon Microphot FXA. Some were embedded in 7.5% gelatin/15% sucrose in PBS and serially sectioned at 30 μm using a cryostat (Bright). Sections were photographed using a Nikon Microphot FXA with Nomarski optics.

RESULTS

Separation of the Paraxial Mesoderm from Its Lateral Environment. The newly formed somite is flanked laterally by the intermediate mesoderm, which includes the Wolffian duct. As soon as the somite differentiates into a dermomyotome and a sclerotome, the intermediate mesoderm takes a ventral position and the lateral dermomyotome, which yields the muscle progenitors of the hypaxial domain, comes in contact with the lateral plate mesoderm. To see if the lateral plate could influence gene activity in the lateral dermomyotome, we have microsurgically separated the paraxial mesoderm from its lateral environment by making a slit through the three germ layers (Fig. 1*A*). The onset of myogenesis in the lateral somitic cells was monitored by examining the expression of the *Pax-3*, *MyoD*, and *myf5* genes in chicken and quail embryos. The *Pax-3* probe is derived from the chicken *Pax-3* cDNA and was used with chicken embryos, whereas the *MyoD* and *myf5* probes are of quail origin and were used with quail embryos. The same experiments were carried out in the two species in which both sets of genes exhibit the same expression schedule (refs. 9 and 21; unpublished observations). Embryos were examined 16–24 hr after the operation. During this period, between 8 and 15 somites have formed in the paraxial mesoderm, without contact with the lateral environment. In this report, we have used the somite staging defined in ref. 22—i.e., the last somite formed is designated as somite I, and the roman numeral corresponds to the number of the somite considered starting from the last segmented somite. In addition, the total somite number of the embryo is added after the roman numeral. In normal embryos, *Pax-3* was found to be expressed homogeneously in the dermomyotome of somites I/25–35 to VIII/25–35 and then to be progressively more strongly expressed in the lateral domain from somite VIII/25–35. A fainter medial domain of expression corresponding to the dorso-medial lip of the dermomyotome persisted at this level. *MyoD* was detected from the level of somite I/25–35 and was found to be expressed in a narrow medial domain close to the neural tube in older somites. *myf5* was not detected in somites I–III/25–35 but exhibited the same expression domain as *MyoD* in somites IV–X/25–35. From somite XI/25–35, it expanded laterally. By comparing the operated side with the unoperated one, we observed a strong down-regulation of the *Pax-3* gene ($n = 9$), in the lateral domain of somites facing the slit down to somite I/25–35 (Fig. 2 *A*, *B*, and *F*). This down-regulation was

intermediate mesoderm (*J*) inhibits *myf5* expression in the medial somitic half. Transverse sections at the level of somites IX–XII of 30-somite quail embryos operated at the 14-somite stage hybridized as whole mounts with the *myf5* probe. *myf5* expression in the myotome is shown by arrowheads. (Bar = 50 μm.) (*I*) No *myf5* staining is detected in the dermomyotome (arrowhead) facing the lateral plate graft (asterisk). (*J*) Implantation of the intermediate mesoderm (asterisk) has no effect on *myf5* expression in the medial somitic half. NT, neural tube; S, somite.

particularly obvious at the level of somites VIII–X/25–35 and rostrally, where the strong lateral domain of *Pax-3* expression corresponding to the hypaxial muscle progenitors had disappeared. Conversely, a strong up-regulation of the myogenic factors *MyoD* ($n = 7$) and *myf5* ($n = 14$) in the lateral somitic cells was observed in these operated embryos. The *MyoD* message was detected in the lateral somitic half over the whole length of the slit down to somites I–II/25–35 (Fig. 2 *C* and *D*). *myf5* was also found to be strongly expressed in the lateral somitic half of somites III/25–35 to XV/25–35 (Fig. 2*E*). In somites rostral to XV/25–35, *myf5* expression domain had expanded into the lateral somitic domain; thus it was not possible to distinguish between a lateral activation and the normal expression pattern. This down-regulation of *Pax-3* and activation of the myogenic factors in the lateral somitic half were observed even when the somites were already formed at the time of the operation. These results demonstrate that in the absence of contact with the lateral mesoderm, cells of the lateral somitic moiety are able to initiate the myogenic program and behave, in this respect, like cells of the medial somitic half.

Graft of Components of the Lateral Somitic Environment. Either the intermediate mesoderm or a strip of lateral plate including the ectoderm, the endoderm, and the somatic and splanchnic mesoderm was grafted into a slit made between the neural tube and the paraxial mesoderm of embryos at the 12- to 22-somite stage (Fig. 1*B*). In this setting, the medial somitic cells are subjected to a lateral environment during their maturation. Grafted embryos were analyzed 16–24 hr later. In the grafted embryos, the size of the somites was found to be reduced on the operated side. This effect was proportional to the size of the grafted tissue and is probably due to the separation of the paraxial mesoderm from the neural tube and notochord, which are known to provide a trophic factor(s) for the medial somitic cells (12, 13). When embryos grafted with the strip of lateral plate tissue were analyzed 16 hr later, the graft was found at the level of somites VII–X/25–30, where *Pax-3* expression is uniform in the dermomyotome. In the somites in contact with the graft, a strong up-regulation of *Pax-3* was observed in the medial somitic half ($n = 3$). When the embryos were analyzed 24 hr after the operation, the graft was found at the level of somites X–XV/30–35, at a level where *Pax-3* is more strongly expressed in the lateral somitic half. In these animals, the somites adjacent to the graft exhibited a strong up-regulation of the *Pax-3* gene in the medial domain ($n = 3$) (Fig. 2*G*). In all embryos examined, the myogenic factors *MyoD* and *myf5* were down-regulated or completely absent ($n = 6$) (Fig. 2*I*). In this respect, these medial cells behave like lateral somitic cells. When the embryos were grafted under the same conditions with the intermediate mesoderm ($n = 8$) (Fig. 2 *H* and *J*) or with a piece of egg shell membrane ($n = 4$) (data not shown), *Pax-3* expression was not modified and *MyoD* and *myf5* were still expressed. These experiments show that the lateral somitic environment, but not the intermediate mesoderm, is able to produce a signal up-regulating the expression of *Pax-3* in medial cells and to inhibit normal myogenesis.

DISCUSSION

Our experiments demonstrate that separation of the paraxial mesoderm from its lateral environment leads to a down-regulation of the early myogenic marker *Pax-3* and the activation of the *MyoD* and *myf5* genes in the lateral somitic cells. In normal conditions, these cells retain expression of *Pax-3* and do not start expressing genes of the *MyoD* family before they have reached their target site about 2 days later. Thus, the lateral environment must provide the lateral somitic cells with a previously undescribed signal repressing the onset of myogenesis. This signal must be present at the transitional phase between the unsegmented and segmented state of the paraxial mesoderm—i.e., when the onset of the *MyoD* family genes is first detected in the medial cells. It is likely to be maintained in the lateral environment until the hypaxial muscle progenitors have reached their target site and activated the *MyoD* family genes.

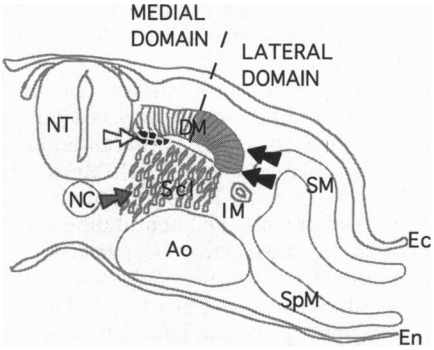

FIG. 3. Summary of local influences on the developing paraxial mesoderm. Schematic transverse section of a differentiated somite and its environment in a 2-day chicken embryo at the trunk level. The black cells in the medial somitic half correspond to the early myotome cells expressing *MyoD* and *myf5* genes. The grey domain of the dermomyotome corresponds to the lateral somitic cells, which strongly express *Pax-3* and yield the progenitors of the hypaxial muscles. The axial organs provide at least two signals acting on the differentiation of the medial somitic domain. One is a survival factor provided by the notochord (NC) and the neural tube (NT) (12, 13). It is necessary for ventral and dorsal cells to differentiate into sclerotome (Scl) and dermomyotome (DM), respectively. The second signal (grey arrow) is a ventral inducer produced first by the notochord and later by the floor plate. This signal recruits nearby cells of the paraxial mesoderm toward a ventral phenotype (sclerotome). Cells escaping this signal would differentiate by default in dorsal lineages (muscle and dermis) (19). An alternative hypothesis resides in the existence of a third signal (white arrow), produced by the neural tube and the notochord, which promotes the onset of myogenesis in the medial somitic half (13–17). The lateral signal identified in this work arises from the lateral plate (black arrows) and transiently represses the onset of myogenesis in the lateral part of the somite. Ao, aorta; Ec, ectoderm; En, endoderm; IM, intermediate mesoderm; SM, somatic mesoderm; SpM, splanchnic mesoderm.

It should be noted that, as previously established (4), surgical replacement of the lateral by the medial half of the newly formed somite leads to a normal muscle pattern. In line with these observations, we find here that the graft of lateral plate facing the medial somitic half alters the normal behavior of these cells. In the grafted embryos, medial cells maintain the expression of the *Pax-3* gene and do not express the myogenic factors *MyoD* and *myf5*, thus behaving as lateral somitic cells. This indicates that the medial somitic half is able to respond to signals provided by the lateral environment.

Most of the current models for myogenic induction from the somite are solely based on signals produced by the axial organs, the neural tube and the notochord. Several *in vitro* studies indicate that the neural tube and notochord promote muscle differentiation from the paraxial mesoderm (14–17). *In vivo*, the role of these structures on *MyoD* activation remains controversial (17, 23). Ablation experiments in the chicken embryo have shown that medial somitic cells require the presence of axial organs for their survival (12, 13). Nonetheless, in the ablated embryos, differentiation of cells of the lateral somitic half proceeds normally, leading to the development of normal limb and body wall muscles, indicating that differentiation of this lineage is independent of the axial organs at that stage (13). These experiments, together with the present results, indicate that the delay of the activation of the myogenic program observed in lateral somitic cells can be

accounted for by the inhibition arising from the lateral plate, rather than from a lack of influence by the axial organs.

Several growth factors such as basic fibroblast growth factor (bFGF) or transforming growth factor β (TGFβ) are known to inhibit myogenesis in already committed cells, by maintaining them in a proliferative state (24). An attractive hypothesis is that the lateral plate is able to produce such a factor, which could diffuse laterally and reach the lateral somitic half. However, none of the FGFs or the TGFs examined so far displays a restricted distribution to the lateral plate (25–27). Since both factors belong to multigene families, it is still possible that a yet unidentified member might exhibit such a distribution. Alternatively, they might act in a paracrine fashion within the somite, their production being controlled by another factor produced by the lateral plate. These hypotheses imply a fairly long-range diffusion and do not explain how the medial cells escape such a signal. One possibility is that the neural tube produces a short-range signal that counteracts the effect of this factor in the nearby cells. On the other hand, the lateral somite is known to be connected to the lateral plate by intercellular bridges, thus raising the possibility of a contact-mediated signal between these two cell populations (22). In this context, the signal would be restricted to the lateral part of the somite.

In a previous report, we analyzed the role of notochord and floor plate in the dorso-ventral polarization of the somite (19). We have shown that the graft of an ectopic notochord, facing the dorso-medial paraxial mesoderm, prevents the formation of epaxial muscles and dermis and converts the entire medial somite into cartilage. From these results, we inferred that the notochord and floor plate provide the nearby paraxial mesodermal cells with a signal recruiting them toward a ventral—i.e., sclerotomal—differentiation pathway. Differentiation of the dorsal somitic lineages (i.e., muscle and dermis) could then occur as a default pathway for the cells escaping the influence of the notochord and floor plate (Fig. 3). Our present results are in agreement with this idea, since the lateral plate does not appear to play any instructive role for the cells of the lateral somitic half but only modulates the onset of myogenesis in these cells.

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1. Wachtler, F. & Christ, B. (1992) *Semin. Dev. Biol.* **3,** 217–227.
2. Couly, G. F., Coltey, P. M. & Le Douarin, N. M. (1992) *Development (Cambridge, U.K.)* **114,** 1–15.
3. Selleck, M. A. & Stern, C. D. (1991) *Development (Cambridge, U.K.)* **112,** 615–626.
4. Ordahl, C. P. & Le Douarin, N. M. (1992) *Development (Cambridge, U.K.)* **114,** 339–353.
5. Williams, B. A. & Ordahl, C. P. (1994) *Development (Cambridge, U.K.)* **120,** 785–796.
6. Goulding, M., Lumsden, A. & Paquette, A. J. (1994) *Development (Cambridge, U.K.)* **120,** 957–971.
7. Bober, E., Franz, T., Arnold, H. H., Gruss, P. & Tremblay, P. (1994) *Development (Cambridge, U.K.)* **120,** 603–612.
8. Weintraub, H., Davis, R., Tapscott, S., Thayer, M., Krause, M., Benezra, R., Blackwell, T. K., Turner, D., Rupp, R., Hollenberg, S., Zhuang, Y. & Lassar, A. (1991) *Science* **251,** 761–766.
9. Pownall, M. E. & Emerson, C. P. (1992) *Dev. Biol.* **151,** 67–79.
10. Buckingham, M. (1992) *Trends Genet.* **8,** 144–148.
11. Christ, B., Brand-Saberi, B., Grim, M. & Wilting, J. (1992) *Anat. Embryol.* **186,** 505–510.
12. Teillet, M. A. & Le Douarin, N. M. (1983) *Dev. Biol.* **98,** 192–211.
13. Rong, P. M., Teillet, M. A., Ziller, C. & Le Douarin, N. M. (1992) *Development (Cambridge, U.K.)* **115,** 657–672.
14. Vivarelli, E. & Cossu, G. (1986) *Dev. Biol.* **117,** 319–325.
15. Kenny-Mobs, T. & Thorogood, P. (1987) *Development (Cambridge, U.K.)* **100,** 449–462.
16. Buffinger, N. & Stockdale, F. E. (1994) *Development (Cambridge, U.K.)* **120,** 1443–1452.
17. Emerson, C. P., Jr. (1993) *Curr. Opin. Cell Biol.* **5,** 1057–1064.
18. Hamburger, V. & Hamilton, H. L. (1951) *J. Morphol.* **88,** 49–92.
19. Pourquié, O., Coltey, M., Teillet, M. A., Ordahl, C. & Le Douarin, N. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 5242–5246.
20. Chedotal, A., Pourquié, O. & Sotelo, C. (1995) *Eur. J. Neurosci.* **7,** 198–212.
21. Piette, J., Huchet, M., Duclert, A., Fujisawa-Sehara, A. & Changeux, J. P. (1992) *Mech. Dev.* **37,** 95–106.
22. Christ, B. & Ordahl, C. P. (1995) *Anat. Embryol.*, in press.
23. Bober, E., Brand-Saberi, B., Ebensperger, C., Wilting, J., Balling, R., Paterson, B., Arnold, H. H. & Christ, B. (1994) *Development (Cambridge, U.K.)* **120,** 3073–3082.
24. Olson, E. N. (1992) *Dev. Biol.* **154,** 261–272.
25. Kalcheim, C. & Neufeld, G. (1990) *Development (Cambridge, U.K.)* **109,** 203–215.
26. Pelton, R. W., Saxena, B., Jones, M., Moses, H. L. & Gold, L. I. (1991) *J. Cell Biol.* **115,** 1091–1105.
27. Millan, F. A., Dehnez, F., Kondaiah, P. & Akhurst, R. (1991) *Development (Cambridge, U.K.)* **111,** 131–144.