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Control of dorsoventral patterning of somitic derivatives by notochord and floor plate

(BEN glycoprotein/muscle differentiation/cartilage/myotome/sclerotome)

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ABSTRACT **We have examined the effect of implantation of a supernumerary notochord or floor plate on dorsoventral somitic organization. We show that notochord and floor plate are able to inhibit the differentiation of the dorsal somitic derivatives—i.e., axial muscles and dermis—thus converting the entire somite into cartilage, which normally arises only from its ventral part. We infer from these results that the dorsoventral patterning of somitic derivatives is controlled by signals provided by ventral axial structures.**

In the vertebrate embryo, one manifestation of anteroposterior polarity is the segmentation of the paraxial mesoderm into somites. The somites are formed by the organization of mesenchymal cells in epithelial balls which are progressively generated according to a craniocaudal gradient on both sides of the neural tube. They become secondarily polarized along the dorsoventral axis by their segregation into a dorsal and a ventral component, designated dermomyotome and sclerotome, respectively. The dermomyotome arises from the dorsal part of the somite (1) and differentiates into striated muscles (for the myotome) and dermis (for the dermatome). The sclerotome yields the axial skeleton—i.e., the vertebrae, intervertebral disks, and ribs (2). The early epithelial somites can also be divided into a lateral and medial moiety which differ by their origin during gastrulation and by their subsequent fate (3, 4).

We are interested in the role of notochord and neural tube in the development of somites and that of neural crest derivatives, which are intimately associated (5, 6). The notochord is an axial structure of mesodermal origin which plays a critical role in establishing the dorsoventral polarity of the neural tube (7). It induces the ventral midline of the neuroepithelium to differentiate into a specialized group of cells, the floor plate (8, 9), which in turn acquires inductive properties that promote differentiation of motoneurons in the ventral horns (7). Notochord and floor plate are able, when grafted dorsally or laterally to the neural tube in 2-day chicken embryos, to induce ectopically ventral-like structures—i.e., floor plate and motoneurons (7, 9). Although the role of neural tube and notochord in the development of the axial skeleton and in cartilage induction has been thoroughly studied (10), their role in the patterning of the mesoderm is poorly understood. In view of the close developmental relationships between the axial structures (neural tube and notochord) and the paraxial mesoderm in the vertebrate embryo, we decided to examine how the notochord and the neural tube could act in the segregation of the different cell lineages arising from the somites. For this purpose, we have grafted the notochord or different portions of the neural tube between the paraxial mesoderm and the neural tube and examined the grafts' effects on somitic cell differentiation. Our results indicate that the notochord and floor plate are able to "ventralize" the somitic mesoderm, as was shown by others to occur for the neural tube. This suggests a central role for the notochord not only in the dorsoventral organization of the neural tube but also in that of somitic mesoderm.

MATERIALS AND METHODS

Chicken embryos (JA 57 from Institut de Sélection Animale, Lyon, France) and quail embryos were obtained from commercial sources. Microsurgery was performed *in ovo* at stages ranging from 8 to 25 somites. Three series of operations were performed. In the first series (Fig. 1, arrows a and b), either the notochord ($n > 30$) or various parts of the neural tube ($n > 20$) were grafted into a groove made with a microscalpel between the neural tube and the paraxial mesoderm. The notochord was removed from the trunk of embryos ranging from 8 to 20 somites after incubation for 5 min with 20% pancreatin (GIBCO) in Ca^{2+}- and Mg^{2+}-free Tyrode's solution. For neural tube grafts, the truncal region of the neural tube from embryos at stages 15–22 of Hamburger and Hamilton (11) was enzymatically dissociated and the ventral zone, including the endogenous floor plate, as well as dorsal and lateral portions were cut out using Pascheff scissors or microscalpels. Grafts were usually performed over a length corresponding to about 10 somites at the level of the last somites formed and of the unsegmented plate. After the operation, the embryos were incubated for 1–8 days. For sham operations ($n = 8$), a cat hair or a baby hair was grafted by the same protocol. No perturbation of development was observed in these cases. In a second series of experiments (Fig. 1, arrow c), embryos which received an ectopic graft of notochord were used as donors of neural tube. The neural tube was dissociated and the region of the neuroepithelium facing the grafted notochord, including the induced floor plate, was isolated and grafted as in the first experimental series. As a control, the ventral region of the neural tube, including the endogenous floor plate, was grafted according to the same procedure. In the third series of experiments (Fig. 1, arrow d), 15- to 16-somite chicken embryos were deprived of the notochord in the unsegmented region as described (5). One day later, the operated embryos [stages 18–19 of Hamburger and Hamilton (11)] were used as donors for grafts of the ventral part of the neural tube. Some embryos deprived of notochord were fixed at different stages between embryonic day 3 (E3) and E7 as controls.

The embryos were fixed in Carnoy's fixative, embedded in paraffin, and serially sectioned. They were analyzed by immunocytochemistry using anti-BEN antibody to identify

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Abbreviation: E*n*, embryonic day *n*.

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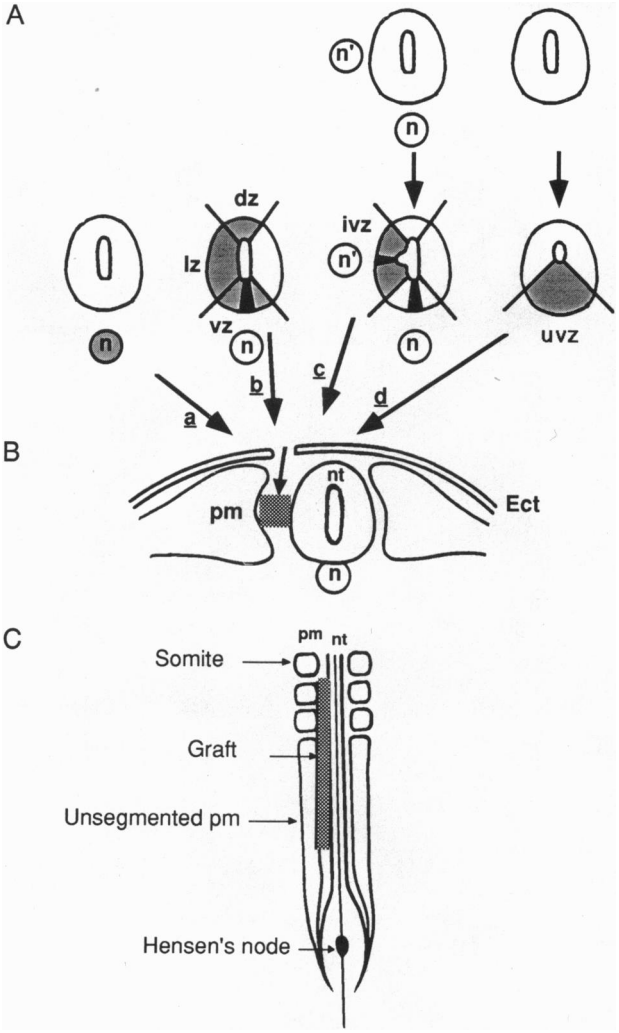

FIG. 1. Schematic drawing of the grafting procedure. (*A*) Representation of the different tissues used as implants (shaded) as indicated by arrows: a, notochord (n); b, ventral zone (vz) of the neural tube, including the floor plate (black area), or lateral zone (lz), or dorsal zone (dz), including the roof plate from normal embryo; c, induced ectopic ventral zone (ivz) including the secondary floor plate from an embryo having received a graft of notochord (n'); d, uninduced ventral zone (uvz) lacking the floor plate, from an embryo deprived of notochord. (*B*) Schematic transverse section of an embryo at grafting time. The piece of tissue (shaded) was implanted into a slit made between the neural tube (nt) and the paraxial mesoderm (pm). Ect, ectoderm. (*C*) Representation of the longitudinal extent of the graft (shaded) along the rostrocaudal axis. To analyze the effect of the implant with respect to the degree of differentiation of the mesoderm at the time of implantation, most of the operations were performed in the region of the junction of the somites and the unsegmented plate.

the floor plate and the motoneurons (12), together with monoclonal antibody 13F4 to identify muscle cells (13). The effects of such operations on the differentiation of somitic derivatives were analyzed from E2.5 to E10.

RESULTS

Graft of a Notochord Between the Neural Tube and the Paraxial Mesoderm. The effect of grafting an additional notochord laterally to the neural tube varied according to the somitic level and the developmental stage of the embryo at grafting time: the older the embryo, the less the intensity of the effect observed. As described earlier (7, 9), the notochord induced a supernumerary floor plate in the lateral wall of the spinal cord and the differentiation of extra motoneurons in the dorsolateral half of the neural tube. This induction of ventral structures in the neural tube was evidenced by using an anti-BEN monoclonal antibody. BEN is a glycoprotein of the immunoglobulin superfamily whose expression in the neural tube is restricted to floor-plate cells and motoneurons (12, 14). Although in many cases the induced floor plate did not express the BEN epitope, a characteristic wedging of the neural tube was observed (15). The influence of the graft on the unsegmented mesoderm was already detectable at E2.5 and E3. At the level of the graft, somite segmentation proceeded normally, but the dermomyotome was greatly reduced or completely absent (Fig. 2*A*). This resulted later on in the absence or extreme reduction of the myotome and dermis. In embryos examined from E7 onward, it was obvious that the sclerotomal derivatives of the somites were expanded in the vicinity of the implanted notochord. Cartilage was much more abundant on the operated than on the contralateral side, whereas the dorsal derivatives—i.e., paravertebral muscles and dermis—were absent (Fig. 2*D*).

When the notochord was implanted at the level of the somites that were already formed, the result was merely a disturbance of the spatial relationships between myotome and sclerotome. The myotome appeared reduced and located more laterally and ventrally than in the normal situation. In the region at the border between segmented and unsegmented mesoderm, a transition between the total absence of the dermomyotome and its displacement to a lateroventral position was observed. In most cases, the development of limb, girdle, and body wall muscles was normal. Therefore, cells derived from the lateral part of the somite were not affected by the graft.

Grafts of Different Portions of the Neural Tube. Implantation of an additional notochord laterally to the neural tube at E2 is thus able to inhibit the differentiation of dorsal muscles and dermis while increasing the size of ventral somitic derivatives—i.e., cartilage. This result extends the ventralizing activity of the notochord on the neural tube to somitic derivatives. It cannot be excluded that the latter is indirectly mediated through the neural tube. To determine whether the ventralizing properties of the notochord on the mesoderm were shared by the floor plate, we applied the same experimental paradigm to different portions of the neural tube.

When the floor plate was included in the graft placed laterally to the neural tube, the effect on somite differentiation was similar to that produced by the notochord. In all cases, the grafted floor plate could be easily identified by its anti-BEN reactivity and its epithelial structure. In embryos up to E4, an ectopic floor plate, which could be either BEN-positive or BEN-negative, was induced by floor plate grafts. Moreover, the dermomyotome was extremely reduced and often completely absent in the area where the mesoderm facing the implant was unsegmented at grafting time (Fig. 2*B*). In embryos older than E6, more cartilage developed on the operated side than on the control side. Moreover, dorsal muscles and dermis were absent at the level of the graft (Fig. 2*E*). When lateral or dorsal neural tube was grafted as a control, only a mechanical effect on the development of the dorsal structures of the embryo was observed (Fig. 2*C*).

Graft of an Ectopic Floor Plate Induced by a Notochord. An ectopically induced floor plate has functional properties similar to the endogenous one, at least in terms of production of a chemoattractant for commissural neurons (16). To determine whether the inductive properties on the mesoderm were also present in the induced floor plate, embryos received a notochord graft as described above, and 1 day later (at the 36-somite stage) the region of the induced floor plate was in turn grafted in the unsegmented region of a chicken embryo host ($n = 2$). In both embryos, sacrificed at E5 and

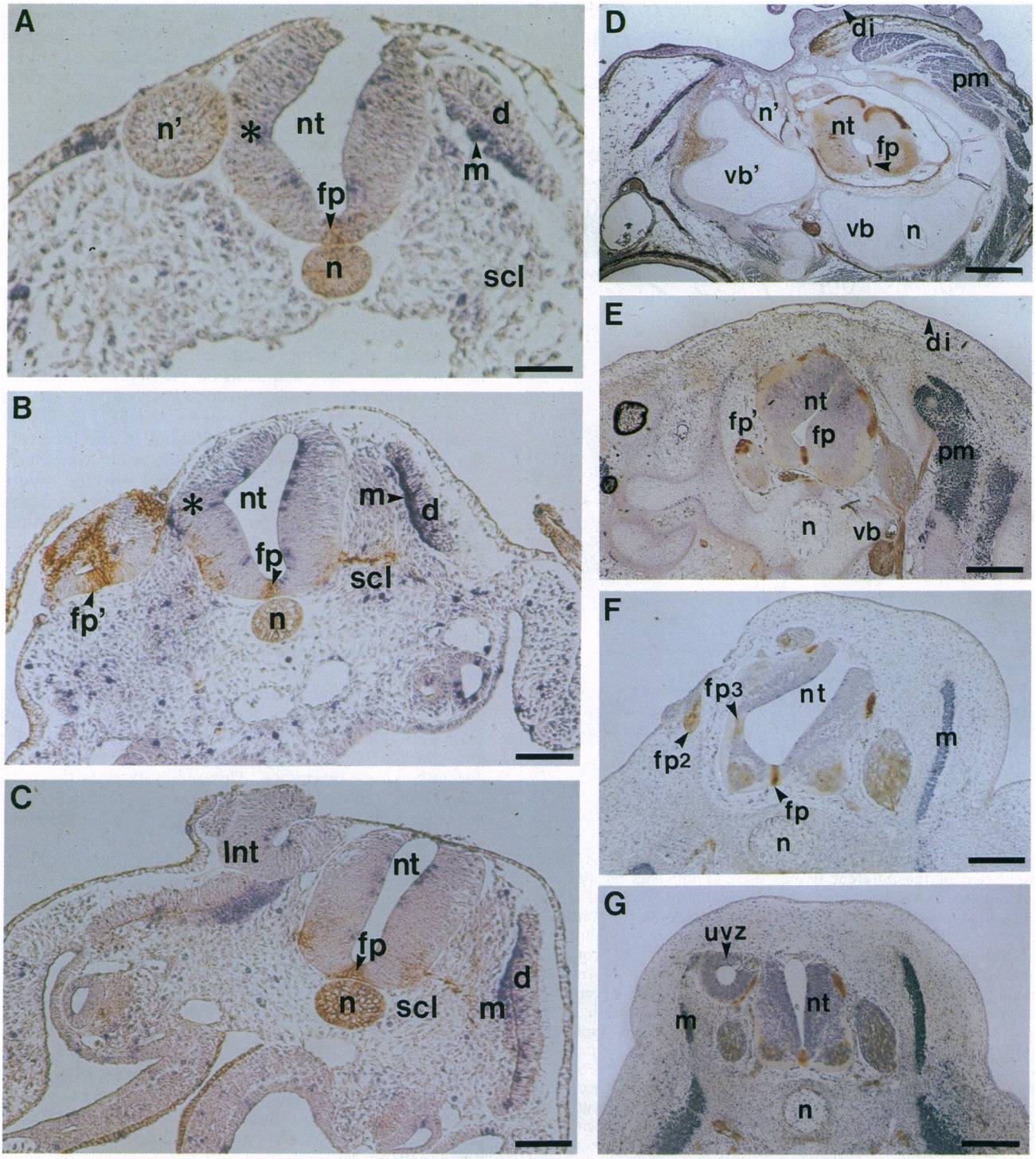

FIG. 2. (*A–E*) Effect of notochord, floor plate, or lateral neural tube graft on the differentiation of dorsal somitic derivatives. Shown are transverse sections of grafted chicken embryos double-stained with anti-BEN monoclonal antibody, recognizing the ventral structures of the neural tube [revealed with the peroxidase reaction (brown)] and monoclonal antibody 13F4, recognizing the muscle lineage [revealed with the alkaline phosphatase reaction (blue)]. (*A*) E2.5 embryo. A notochord (n') was grafted into the unsegmented region of a 10-somite embryo (see Fig. 1, arrow a). The inductive effect of the notochord on the neural tube (nt) is evidenced by the wedging of the tube (asterisk), although no BEN immunoreactivity is observed. The dermomyotome is completely absent on the operated side. (Bar = 40 μm.) (*B*) E2.5 embryo. A ventral portion of the neural tube including the floor plate (fp') and part of the motoneuron pools (as evidenced by anti-BEN reactivity in the implant) from a stage-20 chicken embryo was implanted in the unsegmented region of a 19-somite embryo (see Fig. 1, arrow b). The effect is identical to that of the notochord. On the neural tube, the implant produces a wedging characteristic of floor-plate induction (asterisk) and no dermomyotome is seen on the operated side. (Bar = 70 μm.) (*C*) E2.5 embryo. A fragment of lateral neural tube (lnt) from a stage-20 chicken embryo was grafted into the unsegmented region of a 22-somite embryo (see Fig. 1, arrow b). No effect is observed on the neural tube, and the dermomyotome appears unaffected by the presence of the graft. (Bar = 70 μm.) (*D*) E8 embryo. A notochord was implanted in the unsegmented region of a 14-somite embryo. On the operated side, the dorsal derivatives—paravertebral muscles (pm) and dermis (di)—have disappeared, whereas a large mass of cartilage has formed near the supernumerary notochord (n') appearing as an extra vertebral body (vb'). (Bar = 300 μm.) (*E*) E7 embryo. A floor plate (fp') was implanted in the unsegmented region at the 10-somite stage. An effect identical to that of notochord was produced on somitic derivatives. Dorsal muscles and dermis have disappeared from the region above the graft and extra

E6, the graft which exhibited anti-BEN immunoreactivity induced a tertiary floor plate in the lateral wall of the host's neural tube. In one embryo this floor plate was BEN-positive (Fig. 2*F*); in the other, although this floor plate was morphologically well characterized, it was BEN negative. In both embryos, its effect on the somitic mesoderm was identical to the effect of the endogeneous floor plate. The dorsal structures derived from the somites were completely absent.

Graft of the Ventral Part of a Neural Tube Deprived of Notochord. As known from previous studies (17) and controlled in embryos sacrificed at E3–E4, the excision of the notochord in the unsegmented region profoundly affects the development of the neural tube and the somites. Staining with anti-BEN antibody showed that a portion of the neural tube developing in the absence of notochord lacked the floor plate and motoneurons. Moreover, pairs of somites fused on the midline and produced a single mass of muscle, positive for the muscle-specific monoclonal antibody 13F4, located underneath the neural tube (data not shown). The ventral portion of such a neural tube devoid of floor plate was grafted as before into the unsegmented region of E2 embryos (n = 5). No perturbation of the host neural tube was observed (Fig. 2*G*), and dermomyotomes were displaced only by a mechanical effect as is observed in grafts of lateral or dorsal portions of the neural tube.

DISCUSSION

Our results indicate that it is possible to profoundly modify the fate of the somites when an implant of notochord or floor plate is inserted between the neural tube and the unsegmented paraxial mesoderm (Fig. 3). We have shown that there is a temporal window during which notochord and floor plate are able to prevent the development of the dermis and axial muscles. Somitic derivatives formed in these circumstances are mostly cartilage and mesenchyme. Such a drastic effect is observed only when the extra notochord or floor plate acts on nonsegmented paraxial mesoderm. Therefore, dermomyotome and sclerotome determination should take place around the time of somite formation. Embryonic manipulations on the last segmented somites, such as 180° rotation of the somitic block along the dorsoventral axis (18), or grafting of the ventral part of the somite in place of the dorsal part (19), do not profoundly affect the formation and further development of the dermomyotome and sclerotome, thus showing that a certain plasticity still exists at the level of the last somites. This means that cells of the newly formed somite are still able to interpret positional information directing axial mesoderm differentiation. However, soon after segmentation, the compartments of the somites become irreversibly committed to their respective lineages. This notion is in agreement with the expression of the earliest myogenic control genes, which is detected soon after somite segmentation (20). Moreover, single-cell labeling experiments have shown that cells of the rostral two-thirds of the segmental plate are committed to a somitic fate, but not to a particular lineage (dermatome, myotome, and sclerotome) (21).

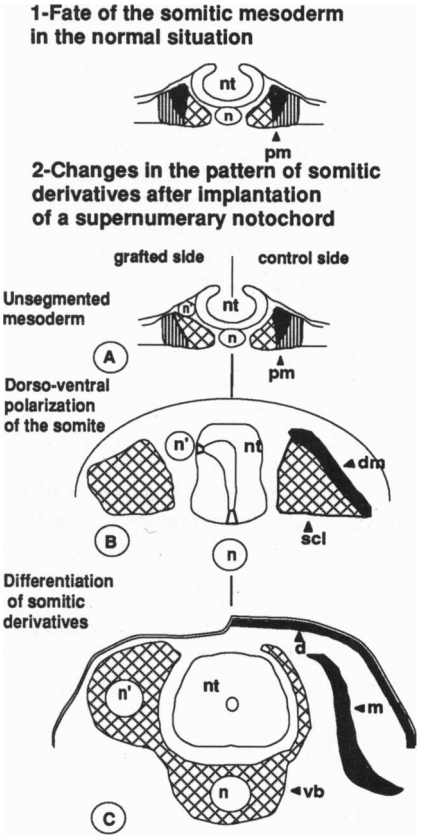

FIG. 3. Part 1. Schematic representation of the normal presumptive cell fate in the unsegmented paraxial mesoderm. Vertical bars, lateral somitic half destined to migrate into the limbs and lateral body wall, where it will differentiate into muscle cells; black area, dorsal area of the medial somitic half (presumptive dermomyotome); cross-hatched area, ventral area of the medial somitic half (presumptive sclerotome). Part 2. Increasing stages (A–C) of somitic maturation showing the influence of the ventral axial organs (e.g., notochord) on the dorsoventral organization of the somitic derivatives. We propose that implantation of the graft into the unsegmented region (stage A) produces a ventralizing effect on cells normally destined to a dorsal fate. This results in the absence of dermomyotome (stage B) and in the increased volume of the sclerotome which, later on, can develop an extra vertebral body around the supernumerary notochord (stage C). d, Dermis; dm, dermomyotome; m, muscle; n, notochord; n', implanted notochord; nt, neural tube; pm, paraxial mesoderm; scl, sclerotome; vb, vertebral body.

We have found that notochord and floor plate can produce *in vivo* factors that induce somitic cells to differentiate into cartilage [in agreement with early experiments describing this effect mostly *in vitro* (10)] but that also prevent the somitic cells from taking the dermomyotomal differentiation pathway. Two possibilities can be proposed at this point. First, the presumptive sclerotomal and dermomyotomal precursor cells may be scattered in the paraxial mesoderm, and the ventral axial organs may act by rescuing the former from cell death and inducing the latter to die. This possibility seems

cartilage has been produced. (Bar = 270 μm.) (*F*) E5 embryo with graft of an induced floor plate (see Fig. 1, arrow c). A notochord was grafted in the unsegmented region of 20-somite embryo. The ectopic ventral zone induced in the lateral part of the neural tube was removed at the 36-somite stage at the level of somites 25–28 and grafted into the unsegmented region of a E2 host. The grafted induced floor plate (fp2) is BEN-positive and the tertiary floor plate (fp3) induced in the host's neural tube is also BEN-positive. The effect on the somitic mesoderm is identical to that of the graft of a primary floor plate (see *B* and *E*). (Bar = 230 μm.) (*G*) E5 embryo with graft of a ventral zone lacking the floor plate (see Fig. 1, arrow d). An embryo was deprived of the notochord at the 18-somite stage. The ventral zone of the neural tube was removed at the 32-somite stage in the region of somites 28–31. It was then grafted into the unsegmented region of a 20-somite embryo. No perturbation of the somitic derivatives was observed. (Bar = 220 μm.) d, Dermatome; fp, floor plate; m, myotome; n, notochord; scl, sclerotome; uvz, uninduced ventral zone; vb, vertebral body.

unlikely due to the small amount of cell death detectable in the somites at that stage, which, moreover, seems most likely to concern the neural crest cells (22). The second possibility is that dermomyotomal differentiation constitutes a default pathway that takes place in the absence of the cartilage-inducing factors originating from the ventral axial structures (i.e., notochord and floor plate). This would be in agreement with the fact that cells from the lateral somitic half, which migrate away at an early developmental stage and thus escape the influence of the ventral axial structures, become muscle cells (4). This is also in line with the results of notochord ablation experiments where differentiation of the myotome was not hampered (17) when the neural tube lacked a floor plate and acquired a dorsal phenotype ventrally (7). Notochord ablation experiments have been performed in various species by using different kinds of techniques such as LiCl treatment (23, 24), γ irradiation of Hensen's node (25), or surgical removal of the notochord (26, 27). In all the studies reported so far, if the notochord was removed early enough, no floor plate differentiation occurred and the myotomes fused and differentiated ventrally below the neural tube (23–25). In the absence of notochord, sclerotome and, therefore, cartilage differentiation does not seem to occur (23).

A possible scenario concerning the temporal sequence of secondary inductions in the dorsal position of the vertebrate embryo is as follows. The first inductive signal is likely to come from the notochord and to occur before segmentation of the paraxial mesoderm. It is responsible for the ventralization of the neural tube, where a new inductive center (the floor plate) differentiates. It is also responsible for the ventralization of the somites and induction of the sclerotome in the epithelial somites. The second step of induction arises from the floor plate. This leads to the induction of ventral structures in the neural tube (i.e., motoneurons) and contributes with the notochord to the differentiation of the vertebrae from the somites. A gradient of morphogen arising from the notochord and the floor plate may act by inducing cells of the nearby neural tube and paraxial mesoderm to differentiate into ventral derivatives. In the absence of this morphogen, the default differentiation pathway would be of the dorsal type. Such a model explains the conflicting results observed in notochord ablation experiments. If the ablation is performed after the first induction has occurred (e.g., by removing the notochord in the segmented region), roughly normal development of the somitic and neural derivatives is observed (5, 26, 27). In contrast, if ablation is performed before this first induction has occurred (at the level of the unsegmented plate), then differentiation of the neural tube and the somite are of the dorsal type (17, 23–25).

The results presented here show a striking parallel between the dorsoventral polarizing activity of notochord and floor plate on the neural tube and paraxial mesoderm. Whether the molecular nature of the two inductive signals is the same in the two systems remains to be determined.

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1. Christ, B. & Wilting, J. (1992) *Ann. Anat.* **174,** 23–32.
2. Gumpel-Pinot, M. (1984) *Chimeras in Developmental Biology*, eds. Le Douarin, N. M. & McLaren, A. (Academic, New York), pp. 281–308.
3. Selleck, M. A. J. & Stern, C. D. (1991) *Development* **112,** 615–626.
4. Ordahl, C. P. & Le Douarin, N. M. (1992) *Development* **114,** 339–353.
5. Teillet, M. A. & Le Douarin, N. M. (1983) *Dev. Biol.* **98,** 192–211.
6. Rong, P. M., Teillet, M. A., Ziller, C. & Le Douarin, N. M. (1992) *Development* **115,** 657–672.
7. Yamada, T., Placzeck, M., Tanaka, H., Dodd, J. & Jessell, T. M. (1991) *Cell* **64,** 635–647.
8. Watterson, R. L., Goodheart, C. R. & Lindberg, G. (1955) *Anat. Rec.* **122,** 539–559.
9. Van Straaten, H. W. M., Hekking, J. W. M., Thors, F., Wierz, E. L. J. M. & Drukker, J. (1985) *Acta Morphol. Neerl. Scand.* **23,** 91–97.
10. Hall, B. K. (1977) *Adv. Anat. Embryol. Cell Biol.* **53,** 1–49.
11. Hamburger, V. & Hamilton, H. L. (1951) *J. Morphol.* **88,** 49–92.
12. Pourquié, O., Coltey, M., Thomas, J. L. & Le Douarin, N. M. (1990) *Development* **109,** 743–752.
13. Rong, P. M., Ziller, C., Pena-Melian, A. & Le Douarin, N. M. (1987) *Dev. Biol.* **122,** 338–353.
14. Pourquié, O., Corbel, C., Le Caer, J.-P., Rossier, J. & Le Douarin, N. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5261–5265.
15. Smith, J. L. & Schoenwolf, G. C. (1989) *J. Exp. Zool.* **250,** 49–62.
16. Placzeck, M., Yamada, T., Teissier-Lavigne, M., Jessell, T. & Dodd, J. (1991) *Development Suppl.* **2,** 105–122.
17. Van Straaten, H. W. M. & Hekking, J. W. M. (1992) *Anat. Embryol.* **184,** 55–63.
18. Aoyama, H. & Asamoto, K. (1988) *Development* **104,** 15–28.
19. Christ, B., Brand-Saberi, B., Grim, M. & Wilting, J. (1992) *Anat. Embryol.* **186,** 505–510.
20. Pownall, M. E. & Emerson, C. P., Jr. (1992) *Dev. Biol.* **151,** 67–79.
21. Stern, C. D., Fraser, S. E., Keynes, R. J. & Primmett, D. R. N. (1988) *Development* **104,** Suppl., 231–244.
22. Jeffs, P. & Osmond, M. (1992) *Anat. Embryol.* **185,** 589–598.
23. Lehmann, F. E. (1935) *Rev. Suisse Zool.* **42,** 405–415.
24. Cohen, A. (1938) *J. Exp. Zool.* **79,** 461–473.
25. Wolff, E. (1936) Doctoral thesis (University of Strasbourg).
26. Kitchin, I. C. (1949) *J. Exp. Zool.* **112,** 393–415.
27. Strudel, G. (1955) *Arch. Anat. Microsc. Morphol. Exp.* **44,** 209–235.