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Specificity of Sympathetic Preganglionic Projections in the Chick Is Influenced by the Somitic Mesoderm

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It has been shown that the development of segmentally specific sympathetic preganglionic projections in the chick is influenced by the tissue environment along the pathway of the preganglionic axons. The cellular origin of this influence, however, is not known. In the present study, transplantation of quail somites into chick hosts showed that the cells in the local environment of the sympathetic trunk are derived from the somite. Surgical manipulations of chick somites then were performed to investigate whether somites play a role in the establishment of preganglionic projection patterns. When cervical somites were transplanted to the thoracic region, preganglionic neurons adjacent to the transplanted somites projected aberrantly. In addition, when somites were removed, the pattern of preganglionic axonal projections to their target ganglia was altered. These results indicate that the specificity of sympathetic preganglionic projections is influenced by the somitic mesoderm.

Key words: axon guidance; somite removal; somite transplantation; chick embryo; preganglionic axon; somitic mesoderm

The projection of sympathetic preganglionic axons in the sympathetic trunk of the chick is specific. It has been suggested that the tissue environment surrounding the preganglionic axon pathway influences the timing and direction of preganglionic projections (Yip, 1987, 1990). A previous study using neural crest removal has demonstrated that neural crest derivatives do not play a major role in directing preganglionic axon outgrowth (Yip, 1987). This study examines whether the somitic mesoderm is involved in the guidance of preganglionic axons.

The somitic mesoderm has been shown to be important in the development of the peripheral nervous system (Tosney, 1988a). First, somite removals (Lehmann, 1927; Tosney, 1988b) and additions (Detwiler, 1934) disrupt the normal segmentation of peripheral nerves and ganglia. In addition, neural crest migration and outgrowth of sensory and motor axons are restricted to the anterior halves of somites (Keynes and Stern, 1984). The preference of nerve cells for the anterior half of the somite may be attributed to the presence of molecules in the anterior half of the somite that promote neuronal growth and/or molecules in the posterior half of the somite that inhibit neuronal growth. Indeed, a glycoprotein isolated from the posterior half of the somite has been shown to cause growth cone collapse (Davies et al., 1990). The somitic mesoderm also has been implicated in the guidance of motor axons in the zebrafish (Eisen and Pike, 1991). Whether factors in the somitic mesoderm also determine the pattern of sympathetic preganglionic projections is not known. Given the location of the sympathetic trunk along each side of the vertebral column (a somite-derived structure), it is conceivable that the cues that guide sympathetic preganglionic axons originate in the somite.

In the normal embryo, the outgrowth of sympathetic preganglionic axons follows a highly precise spatiotemporal pattern (Yip, 1990). First, preganglionic projections are segmentally specific. For example, preganglionic neurons arising from the T1 spinal cord segment project predominantly in the rostral direction, whereas those arising from the T4 spinal cord segment project predominantly in the caudal direction. In addition, the outgrowth of preganglionic axons proceeds in a rostral-to-caudal sequence. Moreover, each ganglion receives projections from a characteristic set of preganglionic neurons arising from several contiguous spinal cord segments (Yip, 1986a, 1990). In the present study, quail somite transplantation shows that somitic derivatives surround the developing sympathetic trunk at the time of preganglionic axon outgrowth. Somite translocation experiments were performed to examine whether the specific location of somites along the rostrocaudal axis can influence the direction of preganglionic projections. Additionally, somite ablations were used to evaluate whether the absence of somites affects the segmentally specific projection patterns of preganglionic axons to sympathetic ganglia. Results from these experiments show that cues guiding preganglionic axonal projections can be localized to the somitic mesoderm.

MATERIALS AND METHODS

White leghorn eggs (Penn State University, University Park, PA) and Japanese quail eggs (Karasoulas Quail Farm, Lake Elinsore, CA) were used in the present study. All embryos were incubated at 37°C in 70% humidity in a forced-draft incubator. For embryo manipulation, a window was opened in the shell of the egg. Embryos were lightly stained with 0.1% neutral red in saline and staged according to Hamburger and Hamilton (1951). Segmental levels were determined by counting somites (Levi-Montalcini, 1950). After surgical manipulation, the window was sealed with a glass coverslip and paraffin wax and the embryo was returned to the incubator until it was killed.

Quail–chick somite transplantation. Quail cells can be distinguished from chick cells by immunostaining and histochemical staining (Le-Douarin and Teillet, 1974; Yip, 1986b; Sharma et al., 1995). Therefore, they have been used as natural cell markers for cell migration in chick hosts. Quail–chick somite transplantation was used here to determine whether tissues in the region of preganglionic growth were derived from the somite. For somite transplantation, a strip of segmental plate, together with the last two to three newly formed somites, was removed with a tungsten needle from one side of a stage 14 quail donor and transplanted homotopically into a similarly staged chick host (Fig. 1*A*). To

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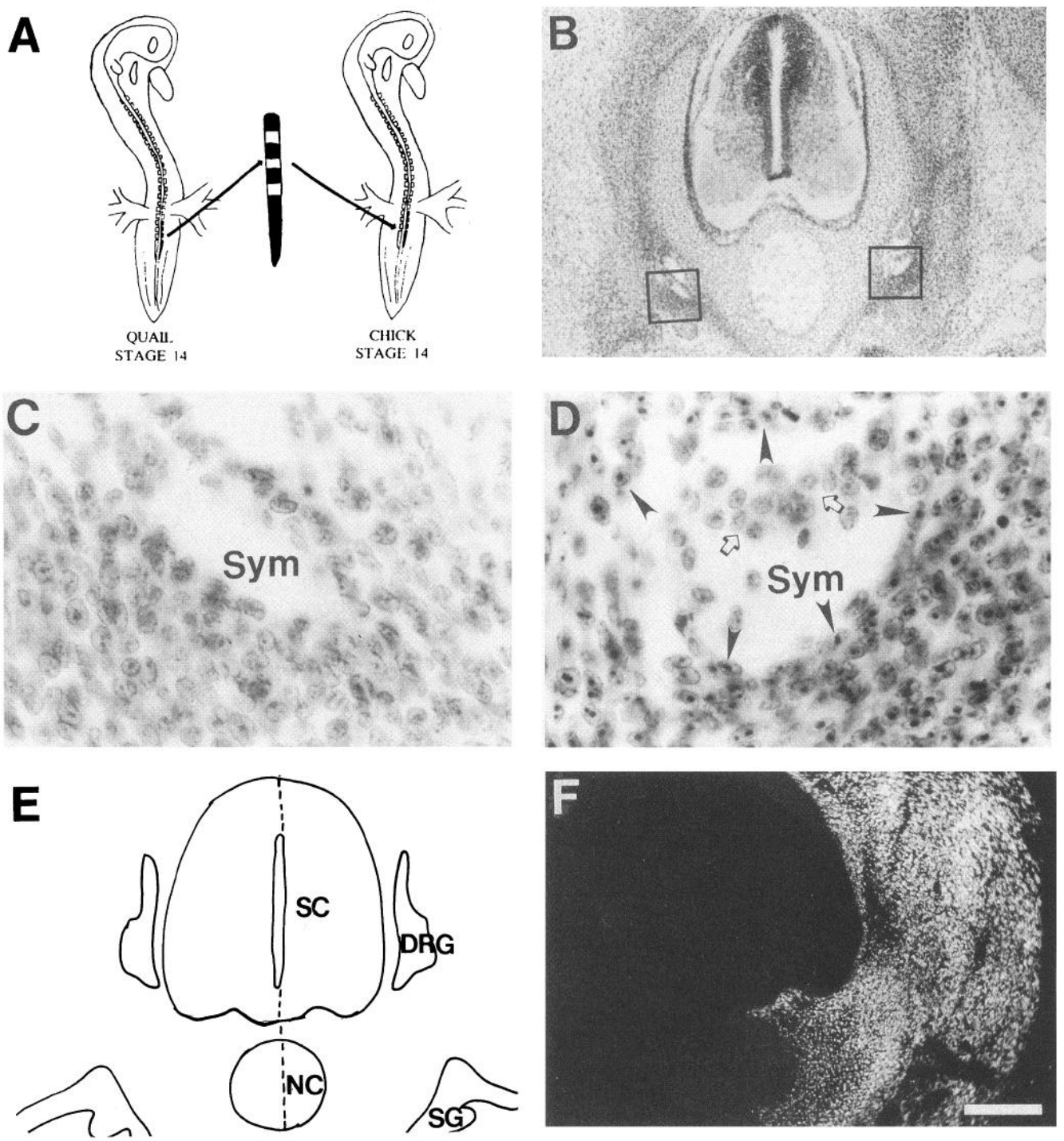

Figure 1. Quail–chick chimera shows that the cells immediately surrounding the sympathetic trunk are derived from the somitic mesoderm. *A*, The last two to three newly formed somites, together with a strip of segmental plate, were removed unilaterally from a stage 14 quail donor and transplanted homotopically into a similarly staged chick host. *B*, Feulgen DNA staining of a transverse section from a resultant embryo at stage 29. The transplant was on the right side of the embryo. *Boxed areas* show sympathetic trunk regions. *C*, High-power micrograph of boxed area on the control side (*left box* in *B*) shows chick cells surrounding the sympathetic trunk (*Sym*). *D*, High-power micrograph of boxed area on the experimental side (*right box* in *B*) shows that cells immediately surrounding the sympathetic trunk (*Sym*) are of quail origin, indicating that they are derived from the transplanted quail somite. Note that quail cells (*solid arrows*) are distinguished from chick cells (*open arrows*) by their condensed heterochromatin. Some ganglion cells are found in the sympathetic trunk of the experimental side; these are of chick origin. *E*, Schematic drawing of immunostained section in *F* shows the spinal cord (*SC*), dorsal root ganglia (*DRG*), sympathetic ganglia (*SG*), and the notochord (*NC*). *F*, Transverse section of a stage 28 chick embryo that had its somite on the right replaced by that of a quail and immunostained with an antibody specific for quail cells (QCPN). Note that quail cells are restricted to the *right* side of the section. Cells immediately surrounding the sympathetic ganglion are of quail origin. The spinal cord, dorsal root ganglia, sympathetic ganglia, and the notochord are devoid of quail cells. Scale bar: *B*, 150 μm; *C*, *D*, 20 μm; *F*, 150 μm.

examine the cellular origin of the tissue surrounding the sympathetic trunk, chimeric embryos were killed between stages 20 and 35. They were processed in one of two ways: (1) in Carnoy solution, embedded in paraffin, and stained for DNA (Feulgen, 1924); or (2) in 4% *p*-formaldehyde, sectioned with a cryostat, and immunostained for quail cells. The quail-specific antibody QCPN (Developmental Hybridoma Bank, University of Iowa, Iowa City, IA) was used to identify quail cells in chimeric embryos. No cells in chick embryos are QCPN-positive (information from Developmental Hybridoma Bank).

Cervical–thoracic somite transplantation. Somites from cervical levels C7–C11 of stage 14 donors were removed unilaterally and transplanted into stage 14 hosts that had an equivalent length of the T1 somite and adjoining thoracic segmental plate removed (Fig. 2*A*). Host embryos were returned to the incubator until they were killed at stages 29–31. For all embryos, the direction of T1 preganglionic projections on both control and experimental sides was determined by orthograde horseradish peroxidase (HRP)-labeling techniques. Additional sham-operated controls were provided by embryos that had the T1 somite and adjoining thoracic segmental plate removed and reimplanted into the same embryos. In normal embryos, thoracic somitic mesoderm gives rise to ribs, whereas cervical somitic mesoderm does not. Thus, the absence of ribs at thoracic levels after cervical–thoracic somite transplantation implies complete and accurate manipulations.

Somite removal. The newly formed somites and segmental plate extending from the lower cervical to upper thoracic levels (C14–T3) were removed on one side of stage 14 embryos. The contralateral side served as the control. In one group of embryos, the somites simply were removed (Fig. 3*A*). In another group, the somites were removed similarly but replaced with a strip of lateral plate to minimize the migration and filling in of cells by the adjacent somitic mesoderm (Fig. 4*A*). Operated embryos were returned to the incubator until stages 29–32. Preganglionic neurons projecting to the C15 or C16 ganglion on each side of the sympathetic chain were labeled retrogradely with HRP. To ensure that somite removal did not interfere with the development of the preganglionic cell column, six stage 30–32 somite-removed embryos were frozen with liquid nitrogen and sectioned in the horizontal or transverse plane at 20 μm. Sections then were immunostained with monoclonal antibody E/C8 (a generous gift from Dr. G. Ciment, Oregon Health Science University) to show the presence of preganglionic neurons in the spinal cord and with monoclonal antibody M1 (a generous gift from Dr. M. Chiquet, Biozentrum; Basel, Switzerland) against tenascin to reveal the vertebrae. Detailed immunostaining procedures have been described in a previous publication (Yip et al., 1995).

Axonal tracing with HRP. Embryos were eviscerated and maintained in oxygenated culture medium (minimum essential medium, Gibco, Gaithersburg, MD). A dorsal laminectomy was performed to expose the spinal cord for oxygenation. For orthograde labeling of T1 preganglionic axons, ~0.2 μl of 30% HRP/1% lysolecithin solution (Frank et al., 1980) was pressure-injected with a micropipette (20 μm tip diameter) into the T1 spinal cord segment. For retrograde labeling of preganglionic neurons in somite-deleted embryos, a similar volume of HRP was injected into the C15 or C16 ganglia on the control side. Because the sympathetic ganglia on the experimental side of these embryos usually were less defined in their size and location, a somewhat larger volume of HRP was injected into the C15 or C16 ganglia and/or paravertebral area; this ensures that preganglionic axons projecting through this area are labeled. After HRP injections, the preparation was maintained in oxygenated culture medium at 34°C for 5–7 hr to allow for the transport of HRP (Landmesser, 1978). Thereafter, the embryos were fixed for 2 hr with a phosphate-buffered fixative consisting of a mixture of 1% *p*-formaldehyde, 2.5% glutaraldehyde, and 4% sucrose, equilibrated in 30% phosphate-buffered sucrose, and serially sectioned with a cryostat at 30 μm. For orthograde HRP-labeling studies, sections were cut in the sagittal plane; for retrograde HRP-labeling studies, sections were cut in the horizontal plane. Sections were mounted on gelatin-coated slides and reacted for the presence of HRP using diaminobenzidine as the chromagen (Adams, 1978). Final preganglionic projection patterns were determined by serial reconstructions using camera lucida drawings.

RESULTS

The tissue immediately surrounding the sympathetic trunk is derived from the somite

As a first step toward determining whether the cues that guide sympathetic preganglionic axons could reside in the somitic mesoderm, the tissue immediately surrounding the sympathetic trunk was examined to determine whether it is derived from the somite. In all chick embryos that had their somites replaced by those of the quail (n = 16), both immunostaining (n = 6) and DNA histochemical staining (n = 10) showed that in the region of the transplant, the cells surrounding the sympathetic trunk of the chick host were of quail origin (Fig. 1). Examination of chimeric embryos during early axon outgrowth revealed that before nerve arrival, the mesenchyme through which nerves grow was made up entirely of quail cells. However, after formation of nerve tracts, quail cells were found only on the outside of the nerve tract. All peripheral nerves, as well as sensory and sympathetic ganglia, were devoid of quail cells. As expected for chimeric embryos, derivatives of the somite including the dermis, epaxial muscle, and vertebrae on the experimental side were made up of quail cells. The same structures on the contralateral control side were free of quail cells. These results indicate that the pathway along which sympathetic preganglionic axons grow is derived from the somitic mesoderm.

Cervical–thoracic somite transplantation alters the direction of sympathetic preganglionic projections

A more direct way to assess whether the cues that guide sympathetic preganglionic axons reside in the somitic mesoderm is to examine whether somite manipulation alters the direction of preganglionic projections. In normal embryos, T1 preganglionic neurons project predominantly in the rostral direction. Their axons extend into the sympathetic trunk at stage 27, turn rostrally, and reach three to six segments by stages 28–32. T4 preganglionic neurons, in contrast, project predominantly in the caudal direction. Because preganglionic neurons in various positions along the rostrocaudal axis project in specific directions, it is possible that they respond to guidance cues that are peculiar to the somitic mesoderm of their segmental level. To ascertain whether cervical somitic mesoderm differs from thoracic somitic mesoderm in its ability to influence T1 preganglionic projections, the C7–C11 somites on one side of stage 14 embryos were removed and transplanted to upper thoracic levels (starting from T1) of similarly staged hosts (Fig. 2*A*).

The resultant embryos showed that T1 preganglionic projections on the experimental side differed significantly from the contralateral control side. Figure 2*B* shows the T1 preganglionic projections of all 33 cervical–thoracic somite-transplanted embryos (stages 29–31). Projections on the experimental side differed from those on the contralateral control side mainly in caudal projection (Fig. 2*C,D*). In 29 (87%) embryos, T1 preganglionic axons on the experimental side extended more than one segment caudally, with axons from 27 (81%) embryos extending between two and four segments caudally. In contrast, only one embryo had T1 preganglionic axons on the control side extending more than one segment caudally. There was no significant difference in rostral projection between T1 preganglionic neurons on the control and experimental sides. There also was no difference between T1 preganglionic projections on the control side of cervical–thoracic somite-transplanted embryos and T1 preganglionic projections from sham-operated control embryos (n = 14; Fig. 2*E*). The finding that cervical–thoracic somite transplantation alters the direction of preganglionic projections suggests that preganglionic axons respond to cues in the somitic mesoderm.

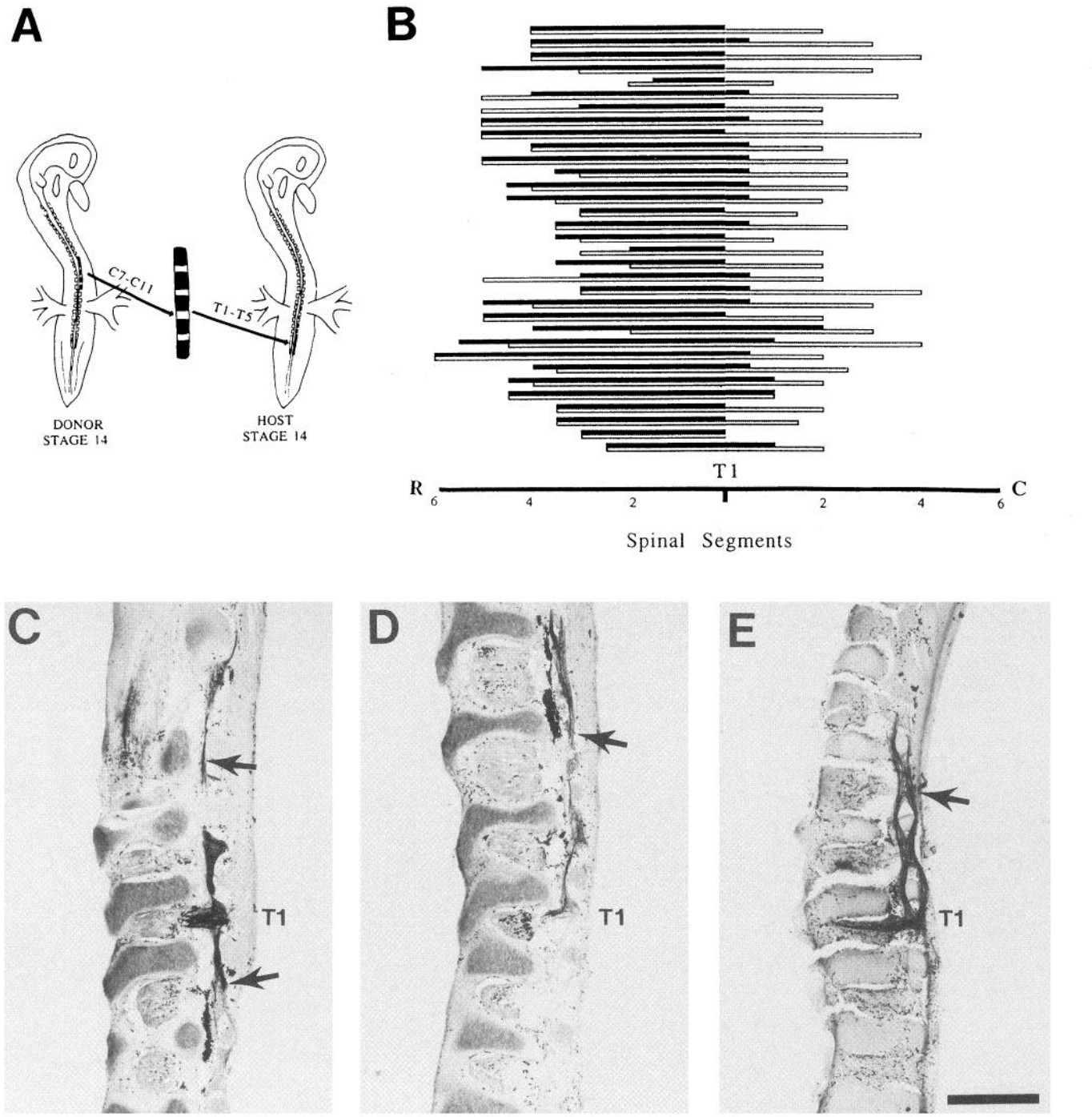

Figure 2. Cervical–thoracic somite transplantation altered the direction of T1 preganglionic projections. *A*, The C7–C11 somites on one side of a stage 14 embryo were removed and transplanted to the presumptive T1–T5 level of a similarly staged host. *B*, T1 preganglionic projections in the resultant embryos (stages 29–31, $n = 33$). Each set of *bars* represents one embryo; the *solid bar* shows projections on the control side, and the *open bar* shows projections on the experimental side. Taking T1 as the midpoint, the number of spinal segments projected rostrally (*R*) is indicated to the *left*, and the number of spinal segments projected caudally (*C*) is indicated to the *right*. T1 preganglionic projections on the control side were predominantly rostral, extending two to six spinal segments at these stages. In only one case were axons extended more than one segment caudally. T1 preganglionic neurons on the experimental side projected bidirectionally, with 29 embryos projecting more than one segment caudally. *C*, Sagittal section from the experimental side of a stage 30 embryo showing that T1 preganglionic neurons project in both rostral and caudal directions (*arrows*). *D*, Sagittal section from the control side of the same embryo showing that T1 preganglionic neurons project in the rostral direction (*arrow*). *E*, Sagittal section from a stage 30 sham-operated embryo showing that T1 preganglionic neurons also project in the rostral direction (*arrow*). Note that no significant caudal projection can be seen in *D* or *E*. Scale bar, 500 μm.

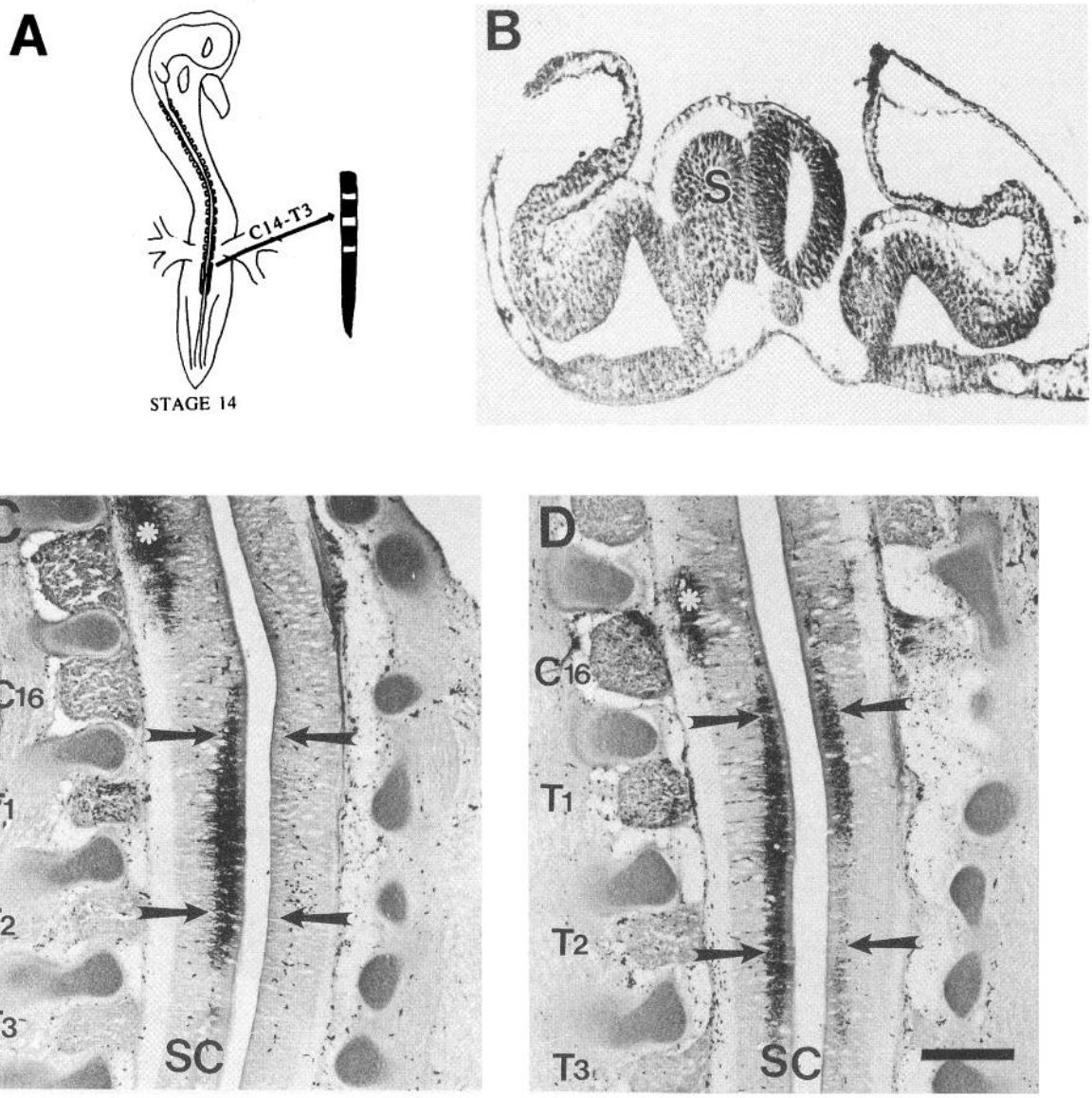

Figure 3. Somite removal disrupts the pattern of preganglionic projections. *A*, The C14–T3 somite/segmental plate was removed on one side of a stage 14 embryo. *B*, Transverse section of an embryo that had its somite removed 3 hr earlier. Note that the somite (*S*) clearly is absent on the operated side (*right*). *C*, Horizontal section from a stage 32 somite-removed embryo showing the distribution of preganglionic neurons retrogradely labeled by injecting HRP into the C15 ganglia. *Arrows* point to the location of the preganglionic cell column in the spinal cord (*SC*). On the control side (*left*), preganglionic neurons from the C16–T3 spinal cord segments projected to the C15 ganglion. On the experimental side (*right*) of this embryo few, if any, preganglionic neurons projected to the C15 ganglion. *D*, In another stage 32 somite-removed embryo, preganglionic neurons from the C16–T3 spinal cord (*SC*) segments on the control side (*left*) projected to the C16 ganglion. However, on the experimental side (*right*), a majority of the T2 and T3 preganglionic neurons failed to project to the C16 ganglion. Labeled motoneurons (*) result from HRP leakage at the injection site. Scale bar: *B*, 100 μm; *C*, *D*, 350 μm.

Somite removal disrupts the pattern of preganglionic projections to their target ganglia

In normal embryos, the C15 and C16 ganglia receive projections from preganglionic neurons arising from the C16–T3 spinal cord segments. The number of preganglionic neurons projecting to these ganglia is greatest from the T1 spinal segment and decreases with distance in more rostral and caudal spinal cord segments (Yip, 1986b, 1990). Therefore, another way to determine whether cues that guide sympathetic preganglionic axons reside in the somitic mesoderm is to examine whether somite removal disrupts the pattern of preganglionic projections to their target ganglia. For this purpose, two series of somite-deleted embryos were prepared. In one series of embryos, several segments of the newly formed somites in lower cervical levels and the adjoining segmental plate in upper thoracic levels were removed unilaterally (Fig. 3). In another series, the somites were removed unilaterally but replaced with a strip of lateral plate mesoderm (Fig. 4). Retrograde HRP labeling was used to assess the preganglionic neurons projecting to the C15 or C16 ganglia on both the control and experimental sides of each embryo.

The resultant somite-deleted embryos show various degrees of loss of labeled preganglionic neurons on the experimental side (Table 1). Of 13 embryos that had their somites removed but not replaced by the lateral plate mesoderm, 3 (23%) embryos showed

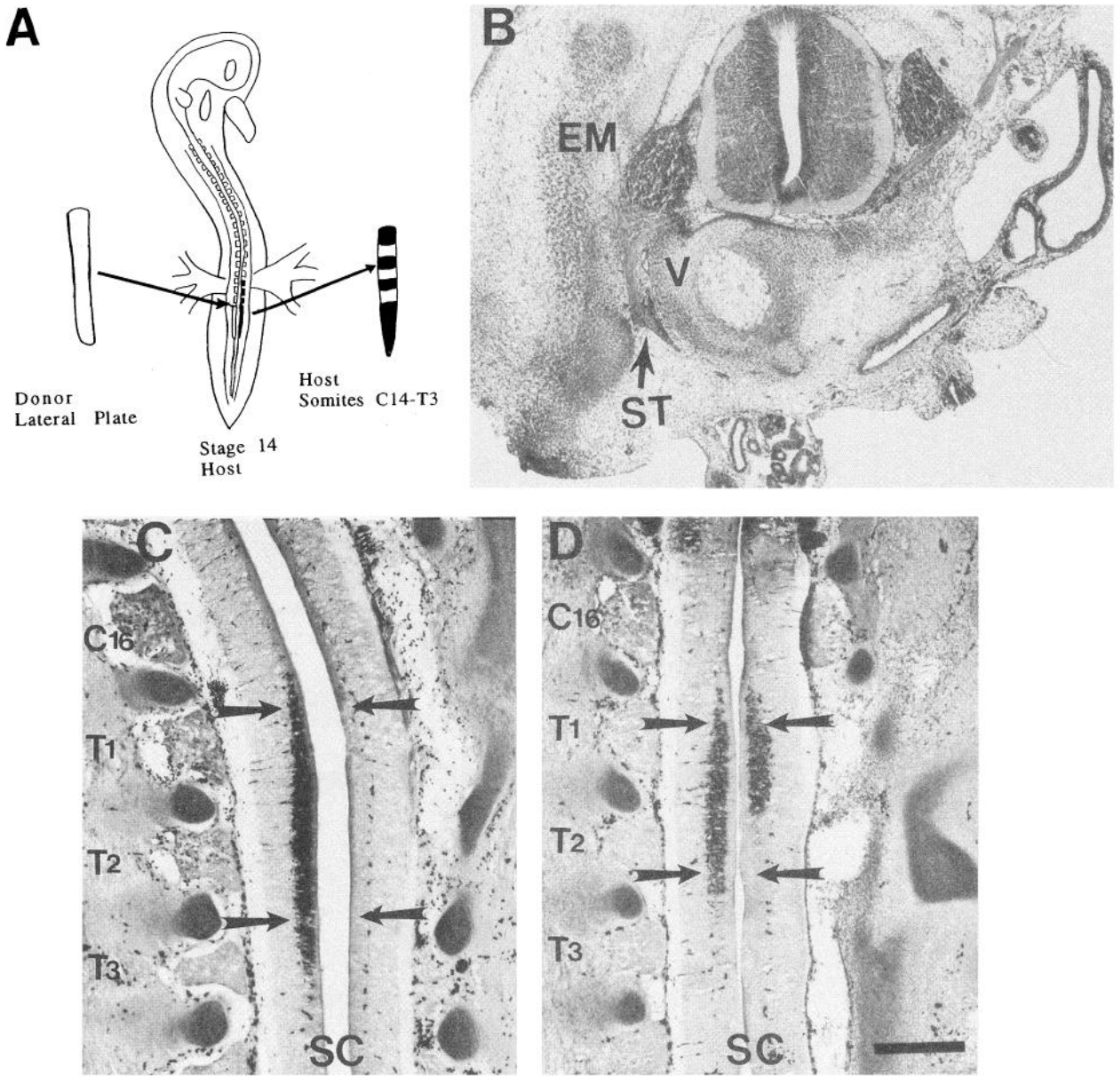

Figure 4. Replacement of somite by lateral plate mesoderm also disrupts the pattern of preganglionic projections. *A*, The C14–T3 somite/segmental plate was removed on one side of a stage 14 embryo and replaced with a strip of lateral plate from the same level of a similarly staged donor. *B*, Transverse section from a resultant embryo at stage 30 showing the absence of the epaxial muscle (*EM*) and severe depletion of the vertebra (*V*) on the experimental (*right*) side. There also is no identifiable sympathetic trunk (*ST*) on the experimental side. *C*, Horizontal section from a stage 32 embryo shows that injection of HRP into the C15 ganglia labeled a column of preganglionic neurons extending from the C16–T3 spinal cord (*SC*) segments on the control (*left*) side but labeled few, if any, preganglionic neurons on the experimental (*right*) side. *Arrows* point to the location of the preganglionic cell column. *D*, In another embryo, HRP injected into the C15 ganglia labeled a normal complement of preganglionic neurons on the control (*left*) side but only a truncated column of neurons on the experimental (*right*) side. These results indicate that many preganglionic neurons failed to project to their target ganglia when the somitic mesoderm was replaced by the lateral plate mesoderm. Scale bar: *B*, 250 μm; *C*, *D*, 350 μm.

no labeled preganglionic neurons in the spinal cord of the experimental side, indicating that few, if any, neurons projected to the C15 or C16 ganglia (Fig. 3*C*). In addition, 5 (38%) embryos showed a truncated column of labeled neurons (Fig. 3*D*) or a drastic reduction in labeled neurons throughout the normal preganglionic cell column, indicating that many preganglionic neurons had not found their way to their target ganglia. Five (38%) embryos showed no apparent difference in the extent of labeled neurons between the control and experimental sides. In 12 embryos that had their somites replaced by the lateral plate mesoderm, 3 (25%) embryos had no labeled preganglionic neurons on the experimental side (Fig. 4*C*). Six (50%) embryos showed either a truncated column of labeled neurons or a drastic reduction in labeled neurons (Fig. 4*D*). Three (25%) embryos showed no obvious difference between the control and experimental sides. The absence or loss of labeled preganglionic neurons in somite-deleted embryos suggests that preganglionic neurons either have failed to project into the sympathetic trunk or have projected to regions other than, or in addition to, their normal target ganglia. Indeed, when orthograde HRP labeling was used

Table 1. Number of somite-deleted embryos showing complete loss, partial loss, or no obvious loss of retrogradely labeled preganglionic neurons on the experimental side

	Complete Loss	Partial Loss	No Loss
Somite removal $n = 13$	3 (23%)	5 (38%)	5 (38%)
Somite replacement with lateral plate mesoderm $n = 12$	3 (25%)	6 (50%)	3 (25%)

to trace T1 preganglionic axons in four additional embryos that had their somites replaced unilaterally by lateral plate mesoderm, two embryos did not show any labeled axons in the sympathetic trunk, and two embryos showed labeled axons projecting in both rostral and caudal directions in the sympathetic trunk. T1 preganglionic neurons on the control side projected as in normal embryos—four segments in the rostral direction (data not shown).

Various structural deficits also were found in somite-deleted embryos that showed complete absence or obvious reduction of labeled neurons on the experimental side. The vertebrae, ribs, and epaxial muscle (all somite derivatives) were either absent or severely depleted. Segmentation of peripheral nerves and ganglia also was generally lost. Consistent with the finding that many preganglionic axons failed to project to their target ganglia, the sympathetic trunk on the experimental side was unidentifiable or greatly reduced in size (Fig. 4*B*). In contrast, embryos with no obvious reduction in labeled preganglionic neurons showed no obvious structural deficits on the operated side, suggesting incomplete somite removal or regulation by the adjacent intact somites.

An alternative explanation for the absence of labeled cells is that the preganglionic cell column fails to develop in the absence of the somite. However, this is not the case. Immunostaining of six embryos that had their thoracic somites removed showed that, in all cases, the formation of the preganglionic cell column continues unimpaired (Fig. 5). Taken together, these results indicate that in the absence of the somite, preganglionic neurons fail to project to their proper target, suggesting that the somitic mesoderm somehow is involved in the establishment of the segmentally specific pattern of sympathetic preganglionic projections.

DISCUSSION

A major issue in the specificity of preganglionic projections is which cells or cell products provide the cues for the guidance of preganglionic axons. To address this issue, one needs to know what cells are in the pathway of preganglionic growth. Results from this study indicate that a majority of the cells in the pathway of preganglionic growth are derived from the somitic mesoderm. Other cells present, however, are sympathetic ganglion cells and Schwann cells, both of which are derived from the neural crest. Yet when the neural crest is removed, sympathetic preganglionic axons still project in the proper direction and to the regions in which the ganglia would have been formed (Yip, 1987). Therefore, sympathetic ganglion cells are not required for the guidance of their preganglionic axons. Schwann cells, likewise, do not seem to be required for preganglionic axon guidance, because they too are removed during neural crest extirpation. The absence of Schwann cells in neural crest-removed embryos has been confirmed recently using a monoclonal antibody described by Bhattacharyya et al. (1991) as a marker (our unpublished observations). Therefore, it is possible that the somitic mesoderm or its products provide the directional cues for preganglionic projections.

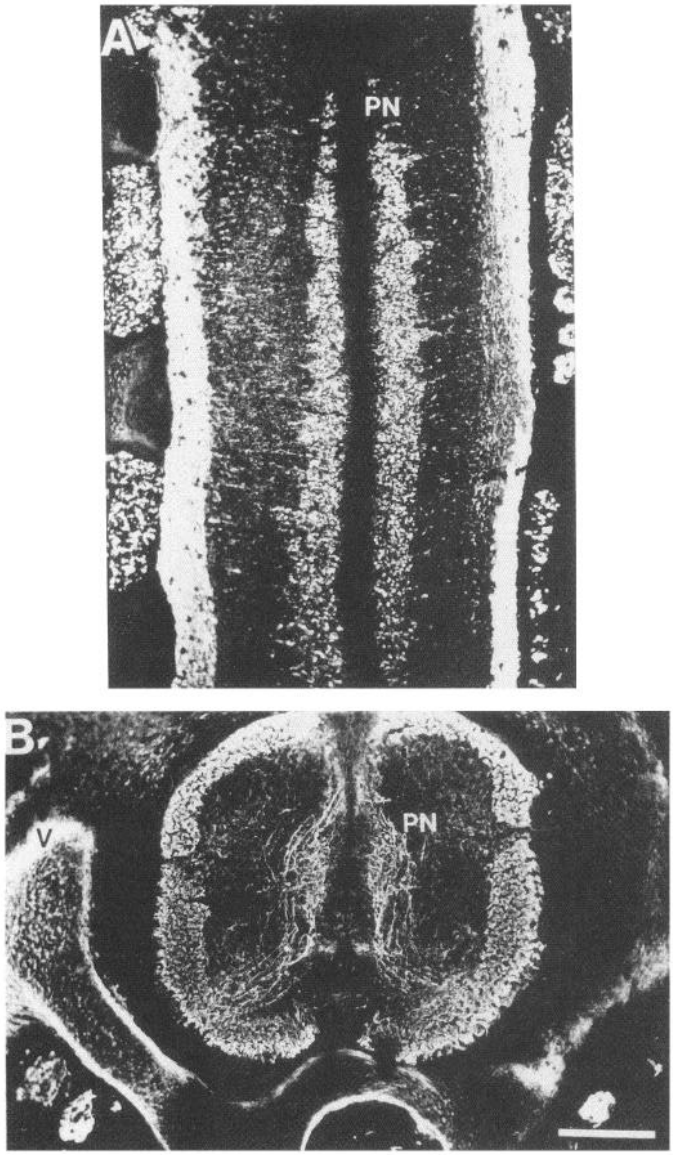

Figure 5. Somite removal does not impair the formation of the preganglionic cell column. *A*, Micrograph shows a horizontal section of a stage 32 somite-removed embryo. *B*, Micrograph shows a transverse section of a stage 30 somite-removed embryo. In both embryos, the somites on the *right* were removed. Sections were immunostained with a neurofilament antibody (E/C8) to reveal the preganglionic neurons (*PN*) and with tenascin antibody (M1) to reveal the vertebrae (*V*), a derivative of the somite. Micrographs were double-exposed to show both structures. No significant difference in the PN column was found between the experimental (*right*) and control (*left*) sides. However, as expected for somite-removed embryos, the vertebrae on the experimental side are missing. Scale bar, 200 μm.

Indeed, when cervical somites were transplanted to the thoracic region, the direction of T1 preganglionic projections was changed. In addition, somite removal disrupted the projection of preganglionic axons to their target ganglia. These results clearly show that the somitic mesoderm somehow is involved in the segmental specificity of preganglionic projections. What aspect of the somite is responsible, then, for the guidance of preganglionic axons? The fact that the direction of T1 preganglionic axons was altered when they were confronted with cervical somitic mesoderm raises the possibility that preganglionic axon guidance is determined by an

intrinsic identity of the somite based on its position along the rostrocaudal axis. Thus, in the normal embryo, preganglionic axons that are confronted with T1 somitic mesoderm project rostrally, and preganglionic axons that are confronted with T4 somitic mesoderm project caudally. Consistent with this idea is that when the T1–T4 spinal cord segment is rotated 180° along the rostrocaudal axis, translocated preganglionic neurons do not retrace their original pathways; instead, these neurons project in directions that are appropriate to their new segmental location (Yip, 1990).

There is evidence that somites along different levels of the rostrocaudal axis possess some kind of intrinsic identity. For example, somites in the thoracic region normally give rise to ribs, whereas somites in the cervical region do not (Kieny et al., 1972). Moreover, when thoracic somites are transplanted to the cervical region, ribs are produced by the transplanted somites. Whether the intrinsic segmental identity of the somitic mesoderm influences the direction of preganglionic projections is not known. However, it has been suggested that synapse formation of preganglionic neurons can be influenced by the positional identity of intercostal muscle, a somite derivative. When intercostal muscles from more rostral segmental levels were transplanted to the region occupied by the excised superior cervical ganglion and allowed to be reinnervated by preganglionic axons of the cervical sympathetic trunk, they tended to be reinnervated by neurons of more rostral segmental origins. Conversely, intercostal muscles transplanted from more caudal segmental levels tended to be reinnervated by preganglionic neurons arising from caudal spinal cord segments (Wigston and Sanes, 1985). The basis for the positional identity of somites is not clear. However, it probably involves *hox* genes, because *hox* gene expression has been shown to correspond to somite boundaries along the rostrocaudal axis (Kessel and Gruss, 1991; Hunt and Krumlauf, 1992).

Results from cervical–thoracic somite transplantation experiments also raise the possibility that preganglionic axon outgrowth is influenced by the maturation state of the somitic mesoderm, which renders the somitic mesoderm permissive to axon growth. In the developing nervous system, it is common that axons do not extend into their target area until the appearance of some signals at the target area. For example, in the innervation of the chick limbs, motor axons undergo a waiting period at the plexus region until cleavage of primary muscle masses (Hollyday, 1983; Tosney and Landmesser, 1985). In the developing cat visual system, axons of lateral geniculate nucleus neurons wait at the subplate region for weeks before their target neurons reach their positions in the cortex (Luskin and Shatz, 1985). Sympathetic preganglionic neurons also undergo a waiting period before projecting into the sympathetic trunk (Tamarkin and Yip, 1994). Preganglionic neurons are born from stage 18 to stage 24 (Prasad and Hollyday, 1991) but do not extend axons into the sympathetic trunk until stage 27 (Yip, 1990). Additionally, when the T1–T4 neural tube of an older donor was transplanted homotopically into a younger host, the transplanted preganglionic neurons projected according to the schedule of their host (Yip, 1990). This suggests that the maturation state of the somitic mesoderm influences the outgrowth of preganglionic axons. Because of the rostrocaudal gradient of development, this state of maturation also is attained by the somitic mesoderm in a rostrocaudal sequence. Thus, in the normal embryo, T1 preganglionic axons may encounter first a permissive pathway in the rostral–cervical somitic mesoderm and project predominantly in the rostral direction. When cervical somites are transplanted to the thoracic region, T1 preganglionic axons are confronted not only with a normal permissive environment rostrally, but also with a permissive environment in the caudal transplanted cervical somitic mesoderm. Therefore, these axons respond by projecting in both rostral and caudal directions.

In summary, the present study has identified the somitic mesoderm as a source of guidance cues for preganglionic axons. Although the nature of those cues is presently unknown, results from this and previous studies are consistent with the view that preganglionic projections can be influenced by the intrinsic identity and/or maturation state of the somitic mesoderm. Future studies will be directed at determining the contribution of each of these factors on the specificity of preganglionic projections.

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