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Neural Crest Cells Prefer the Myotome's Basal Lamina over the Sclerotome as a Substratum

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Anterior sclerotome is presumed to be the only somitic tissue that guides neural crest cells as they migrate ventrally. In contrast, we report here that crest cells prefer the myotome's basal lamina over the sclerotome as a substratum. This conclusion stems from four observations. First, crest cells migrating between the neural tube and somite invade lumbar and thoracic somites only after the myotome has formed a basal lamina, as though they use this basal lamina to penetrate the somite. Second, crest cells alter their trajectories dramatically when they contact this basal lamina. They abruptly turn laterally and align closely with the myotome's basal surface. Third, crest cells invade sclerotome only when they fail to contact this basal lamina. For instance, the lateral half of each myotome is initially devoid of basal lamina. When the first crest cells reach the lateral myotome, they depart from the myotome's basal surface and penetrate lateral sclerotome. Only later, when a higher population density prevents some cells from contacting the basal lamina, do crest cells penetrate medial sclerotome. Conversely, crest cells that migrate between somites do not have access to myotome and fail to turn laterally. Fourth, when we prevent myotome development by surgically removing its precursor (the dermamyotome), crest cells fail to turn laterally within the somite. Instead, they move directly ventrally and colonize medial sclerotome. The preference for myotomal basal lamina implies that anterior sclerotome is a suboptimal environment for neural crest migration. The myotome's basal lamina may facilitate rapid migration through the somite before impediments to ventral migration develop.

INTRODUCTION

After 75 years of studying how neural crest cells and axons navigate through somites, we would appear to know which somitic tissues guide migration. Indeed, researchers in this field generally agree that only the sclerotome imposes patterns on migration within the somite. Sclerotome is thought to pattern migration along both anterior-posterior and dorsal-ventral axes because it is subdivided into domains that inhibit or permit migration. The posterior sclerotome inhibits both crest cells and axons; each population advances only through the permissive anterior sclerotome and thereby forms segmental patterns of ganglia and spinal nerves (Lehmann, 1927; Detwiler, 1937; Weston, 1963; Keynes and Stern, 1984; Rickmann *et al.*, 1985; Bronner-Fraser, 1986; Loring and Erickson, 1987; Teillet *et al.*, 1987; Tosney, 1987, 1988a; Lallier and Bronner-Fraser, 1988; Kalcheim and Teillet, 1989). Likewise, the ventromedial sclerotome and/or notochord inhibit the ventral advance of crest cells and axons and thereby impose patterns along the dorsal-ventral axis (Newgreen *et al.*, 1986; Pettway *et al.*, 1990; Tosney and Oakley, 1990). Indeed, both axons and neural crest cells fail to enter much of the sclerotome during normal development and advance only within a dorsolateral wedge of anterior sclerotome.

These conclusions derive from a strategy that tests whether a tissue provides essential guidance cues by deleting or transplanting the tissue. While powerful, this strategy reveals only essential and irreplaceable cues; it may fail to reveal all the cues that contribute to guidance. Indeed, guidance cues are probably both multiple and redundant. If we delete a tissue that provides one cue, other cues may suffice for relatively normal development. In addition, such studies usually focus on a single consequence of guidance such as segmentation and therefore analyze embryos only after migration is complete, making it difficult to detect subtle alterations in migration itself. A case in point is a study proving that the myotome provides no irreplaceable cues essential for segmentation. When the myotome's precursor is deleted, both ganglia and spinal nerves form in their normal segmental array (Tosney, 1987). However, this study analyzed ganglion formation rather than neural

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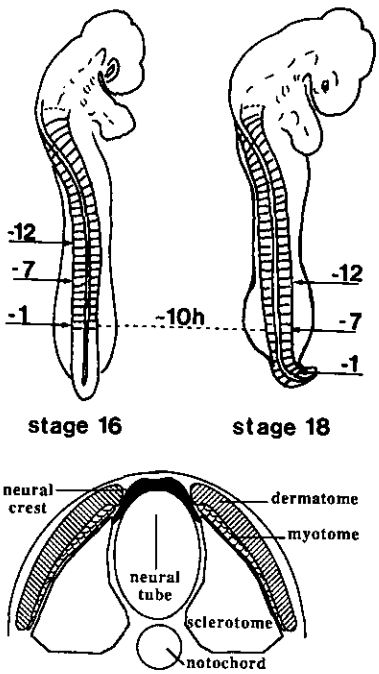


FIG. 1. Schematic diagram (top) illustrates the counting method based on Loring and Erickson (1987) criteria, in which somites are numbered from the posterior, as indicated by minus signs. Unlike the Hamburger and Hamilton (1951) criteria, this method reliably identifies somites of the same degree of maturity in embryos of different stages. At Hamburger-Hamilton stage 16, embryos have 26–28 somites; at stage 18, embryos have 30–36 somites. In our method, the most recently formed pair of somites is labeled "−1" both in a stage 16 embryo (shown with 27 somites) and a stage 18 embryo (shown with 33 somites). As development proceeds, somite pairs are added at about 1.7-hr intervals while somites at each segmental level mature at similar rates (Loring and Erickson, 1987). For example, the dotted line indicates the maturation of somites lying at the 27th segmental level. After about 10 hr of development, this −1 pair of somites will have matured to form the −7 pair of somites in the stage 18 embryo. The schematic cross section below shows the relation between anatomical structures as seen in a −12 somite.

crest cell migration per se and focused on segmentation alone. It does not, therefore, rule out some role for the myotome in guiding neural crest cells.

How can we detect a replaceable or subtle guidance cue? In particular, what evidence would tell us whether the myotome influences neural crest migration? First, spatial and temporal *proximity:* for a tissue to guide, it must be present when and where the cells need guidance. Previous studies have described myotome development, but not in enough detail to show whether it consistently forms before or after crest cells invade the somite (Loring and Erickson, 1987; Kaehn *et al.*, 1988; Layer *et al.*, 1988; Christ *et al.*, 1992). We therefore asked if the myotome forms early enough and close enough to guide crest cells and found that it does. Second, *plausibility:* analysis of normal development must reveal some reproducible association consistent with guidance, such as an alteration of crest cell trajectory on contact, an orientation or alignment related to the tissue, or a close physical association. We found that crest cells' interactions with myotomal basal lamina met all three of these criteria. Crest cells entering the somite unexpectedly fail to move directly into sclerotome but instead turn on contact with the myotome, align along its basal surface, and advance in contact with its basal lamina. Further analysis shows that the detailed trajectories are explicable if crest cells prefer the myotome's basal lamina over the sclerotome as a substratum. Moreover, the dramatic turn crest cells take upon contacting the myotome gave us an assay for our third piece of evidence, an experimental test of *dependence.* We prevented myotome development by excising its precursor, the dermamyotome. Crest cells responded by moving directly into medial sclerotome instead of turning laterally. Our study thus documents a previously unsuspected role for the myotome in guiding neural crest cells.

MATERIALS AND METHODS

HNK-1 and Laminin Immunocytochemistry

Stage 16–19 (Hamburger and Hamilton, 1951) embryos were processed to detect immunoreactivity of either HNK-1 or laminin, or both, by incubating fixed embryos with antibodies before embedding them in plastic as detailed in Loring and Erickson (1987). In brief, embryos were fixed with 4% paraformaldehyde for 20 min, rinsed extensively in phosphate-buffered saline (PBS), and incubated for 3 hr in 3% bovine serum albumin (BSA) in PBS at room temperature. During incubation, embryos were cut into five to seven segment lengths to facilitate antibody penetration. These embryo fragments were incubated overnight in either HNK-1 antibody (ascites fluid diluted 1:1000 in PBS with 3% BSA) or anti-laminin antibody (1:100; generous gift from Hynda Kleinman). Unbound antibody was washed out with a 4-hr incubation in 0.5% BSA in PBS and the specimens were immersed overnight in the secondary antibody (FITC-conjugated goat anti-mouse IgG + IgM + IgA; diluted 1:100 in 0.5% BSA in PBS; Cappell). For double-label, fragments were first processed for HNK-1 immunoreactivity as above and then washed for 20 min in 0.5% BSA, fixed for 10 min with 4% paraformaldehyde to immobilize bound antibodies, washed in four changes of PBS and incubated 15 min in 0.5% BSA in PBS; primary and FITC-conjugated secondary antibodies for laminin immunoreactivity were then applied as above. Labeled fragments were washed extensively in PBS before embedding.

Since plastic embedding better preserves embryonic tissues and provides higher resolution than paraffin or

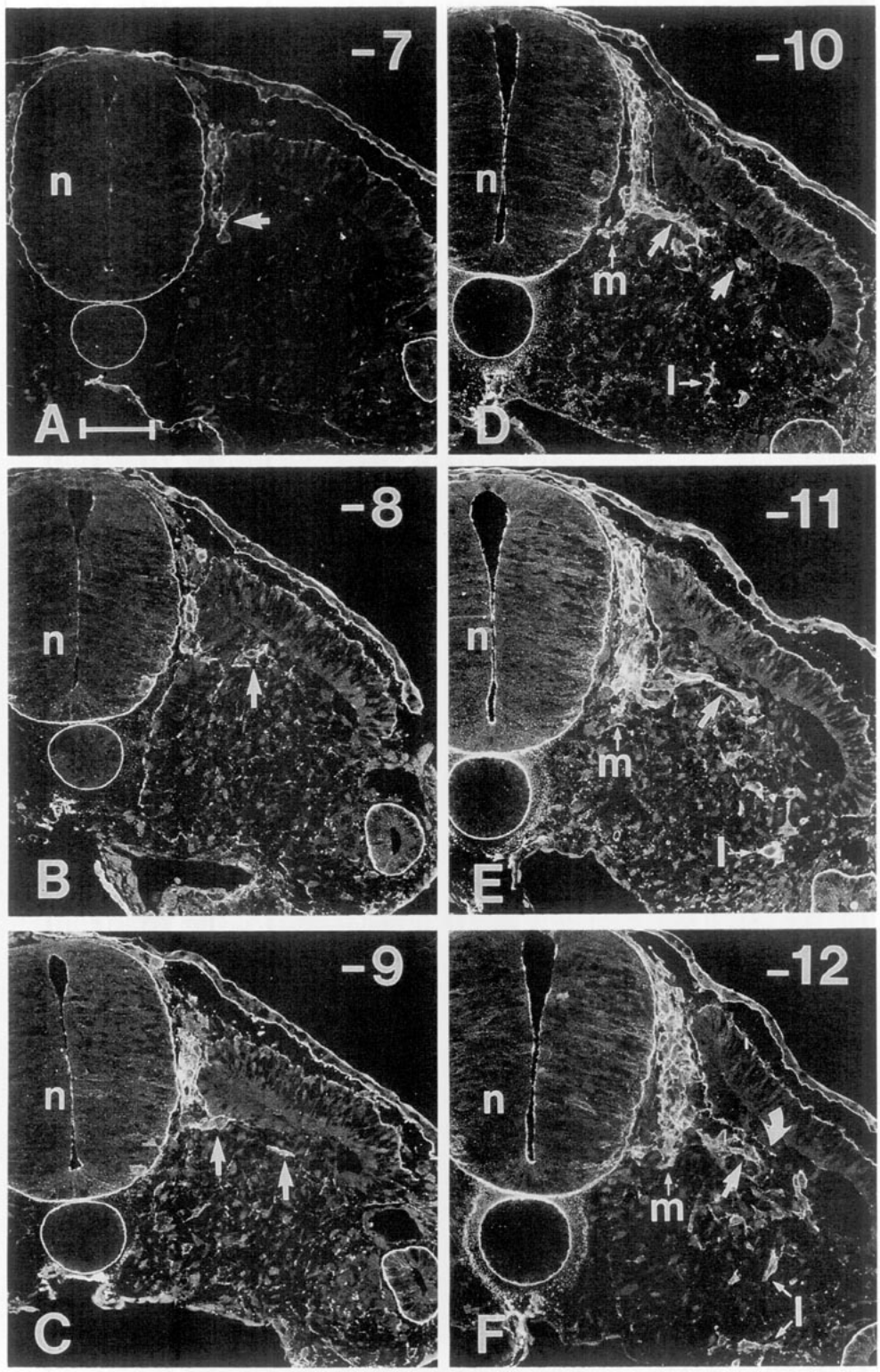
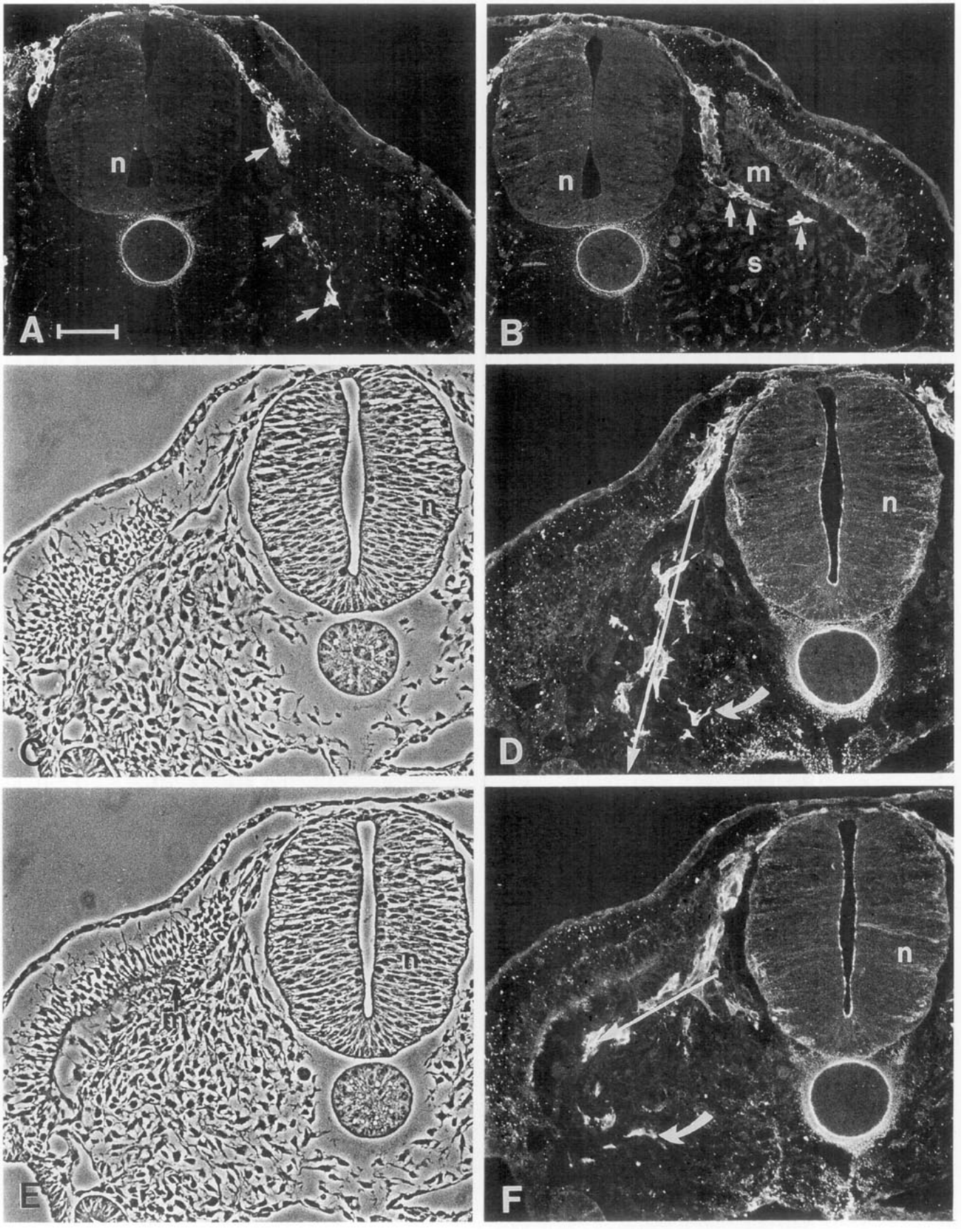


FIG. 2. Stereotyped trajectories of crest cells entering somites −7 through −12 are shown in 2-μm plastic cross sections immunolabeled for both HNK-1 and laminin antigenicity. The extent of local myotome development can be gauged by crossreferences in Fig. 5B. Lateral is to the right. (A) In midanterior somite −7, crest cells have penetrated ventrally to the border between sclerotome and dermatome (arrow). (B) In somite −8, crest cells have penetrated the somite by abruptly turning laterally. They lie at the interface between the myotome and sclerotome (arrow) where they obscure the local, laminin-positive basal lamina. Laminin immunoreactivity is undetectable along the more lateral interface. (C) In somite −9, the most advanced crest cell is spread parallel to the myotome's basal surface and entering crest cells have turned laterally as they encounter the myotome (arrows). (D) In somite −10, the medial myotome is coated by crest cells that have turned to align with it (arrows). A few crest cells have entered medial sclerotome (m) while others lie deep within lateral sclerotome (l). (E) In somite −11, crest cells lie in three streams: a ventrally directed population within medial sclerotome (m), a laterally directed population in contact with medial myotome (arrow), and a ventrally-directed population dispersed within lateral sclerotome (l). Laminin antigenicity is undetectable on lateral myotome (see Fig. 7B). (F) In somite −12, crest cells are more numerous within the somite, but still lie within three streams. Laminin antigenicity is seen on medial myotome (curved arrow) but is patchy or undetectable on lateral myotome. Scale bar, 100 μm; *n*, neural tube.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frozen sections, most labeled preparations were viewed in plastic sections. These sections support high-quality visualization of neural crest cells, the developing myotome, and the laminin-positive basal lamina. After the labeling procedure above, the tissue was postfixed in 2.5% glutaraldehyde, 10% paraformaldehyde, and 2.5% DMSO in PBS at pH 7.4, dehydrated, and embedded in Epon-Araldite. Two-micrometer sections were cut with a diamond histoknife, affixed to glass slides with a drop of water on a warming tray, and mounted in 70% glycerol containing 2% *n*-propyl gallate and 0.1 *M* NaHCO3, pH 7.8.

For ultrastructural observation of crest cell contacts with basal lamina, whole tissues were incubated with HNK-1 antibody as above, incubated with a secondary antibody conjugated to anti-mouse colloidal gold (10 nm, diluted 1:100), washed with PBS, postfixed as above, and embedded in Epon/Araldite. Thin sections were cut using a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Philips 401 transmission electron microscope at 60 kV.

Electron Microscopy

To characterize myotome development ultrastructurally, we first serially sectioned plastic-embedded embryos at 25 μm and then processed selected thick sections for electron microscopy as in Tosney and Landmesser (1986). In brief, embryos were fixed in 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer at pH. 7.2, rinsed in 0.2% cacodylate, postfixed in 0.2% osmium tetroxide in 0.1 *M* cacodylate, dehydrated in graded ethyl alcohols, embedded in Epon-Araldite, and sectioned serially at 25 μm using a rotary microtome with a heated steel knife. Sections were mounted in glycerol. After analysis, selected sections were reembedded, thin-sectioned, mounted on formvar-coated slot grids, stained with uranyl acetate and lead citrate, and examined in a Philips 300 transmission electron microscope at 60 kV.

Additional Morphological Methods

We also examined myotome development with scanning electron microscopy (as in Tosney, 1978 1988b), with frozen sections in which myocytes were identified by immunoreactivity to MAB myo231 (gift of Jean and Bruce Carlson) or to an antibody to desmin (also see Christ *et al.*, 1992) and with paraffin sections in which myocytes were identified by their autofluorescence after glutaraldehyde fixation (see Tosney, 1987). We also examined neural crest migration using DiI labeling (see Serbedzija *et al.*, 1989) and in whole mounts following HNK-1 labeling as above. These results are not shown but they confirm by independent methods the developmental events we describe.

Embryonic Surgeries

To examine experimentally the relation between myotome development and crest cell trajectories, we fully or partially deleted dermamyotomes, the precursors of myotomes and dermatomes, from 14 stage 16–17 embryos using methods detailed previously (Tosney, 1987). In brief, we windowed egg shells and injected black ink below the embryo to enhance visibility. To avoid damaging ectoderm dorsal to operated somites, we slit the ectoderm immediately posterior to the deletion site with a fine tungsten needle and removed one or more dermamyotomes through the slit using a micropipet. Deletions were confined to the five most recently formed somites at lumbar and low thoracic levels (somite numbers 20 to 27, counted from the most anterior somite); at these levels, crest cells had yet to invade somites. After 10–20 hr, embryos were processed to detect HNK-1 antigenicity as above and viewed in serial plastic sections.

RESULTS

To discern a relation between two developmental events, both events must be reproducibly visualized in time and space, even in different embryos. To assure reproducible observations, we identified somites of the same maturity in all embryos studied. Regardless of stage, the relative maturity of each somite is always consistent from embryo to embryo in thoracic and lumbar segments when measured relative to the most re-

FIG. 3. Divergent trajectories within and between somites are shown in 2-μm cross sections labeled for HNK-1 and laminin immunoreactivity. See Fig. 5B for position of sections relative to myotome development. (A) Crest cells (arrows) in an intersegmental space project ventrally, continuing a trajectory tangential to the neural tube. (B) In contrast, within the adjacent, anterior half of somite −9 in the same embryo, crest cells (arrows) abruptly turn laterally and penetrate the somite along the interface between the myotome (m) and sclerotome (s). (C, D) Phase and fluorescent images of the same section through the most anterior edge of somite −11. (C) Myotome is absent, since it extends only between the inner lips of the dermatome rather than penetrating to the outer edges of the somite (see Figs. 5 and 6A). Since the section cuts through the incurled dermatomal lip, many dermatome cells (d) are cut in cross-section and appear rounded. s, sclerotome. (D) In the absence of the myotome, crest cells migrate directly ventrally along a trajectory tangential to the neural tube (long arrow). Some ventral crest cells lie only 50 μm from the notochord (curved arrow). (E, F) Phase and fluorescent images of a section through the midanterior of the same somite shown in C and D. (E) A myotome (m) is distinct. (F) Many crest cells have turned upon contacting the myotome and lie parallel to it (long arrow). Other crest cells are scattered within lateral sclerotome where they lie 100 μm or more from the notochord (curved arrow). Scale bar, 100 μm; n, neural tube.

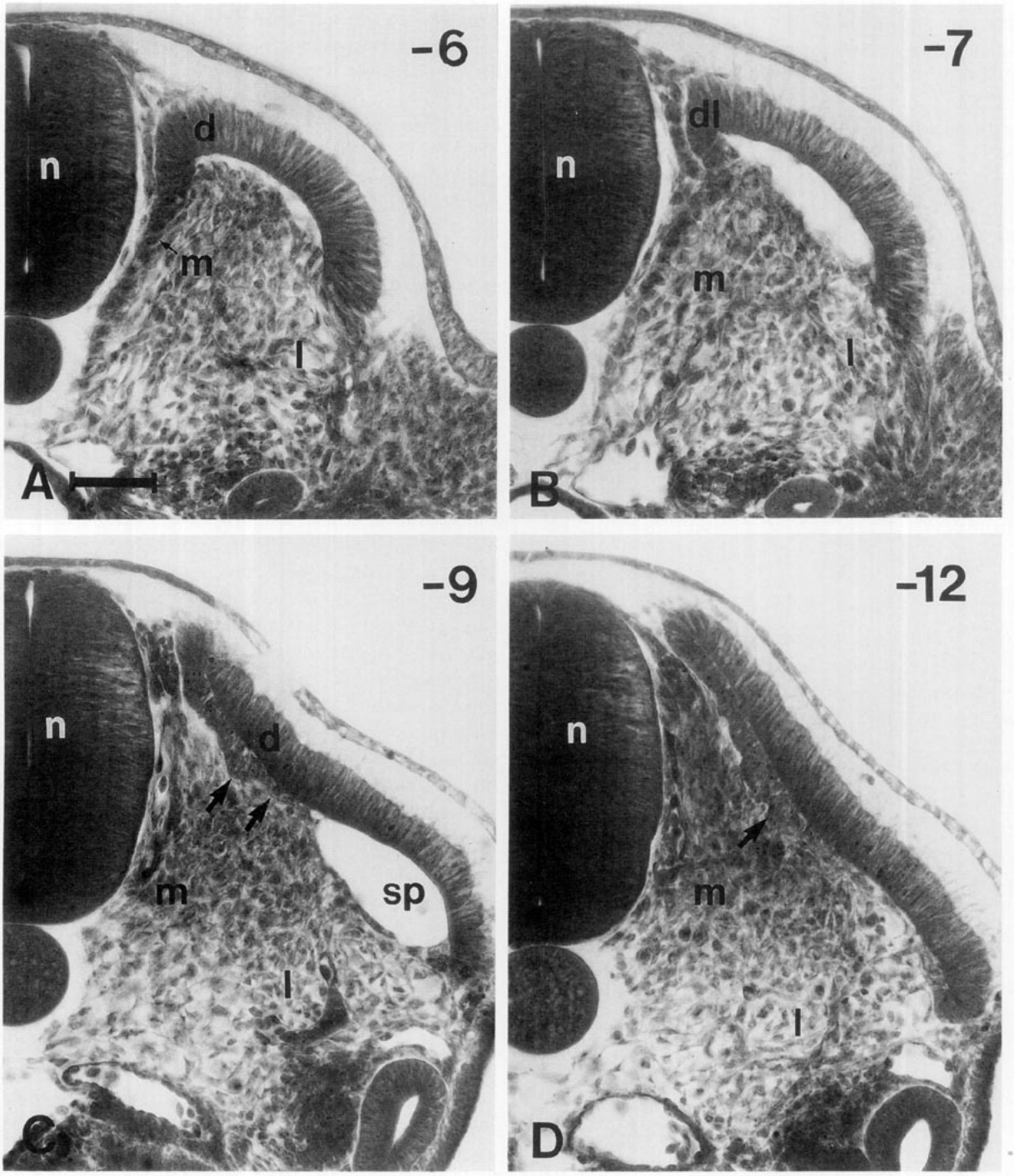


FIG. 4. Medial–lateral differences in somite maturation shown in 25-μm cross sections. Figure 5B shows the position of each section relative to myotome development. m, medial; l, lateral. (A) At somite −6, the lateral sclerotome is a loosely packed mesenchyme while the most medial sclerotome is still an epithelium (arrow) that is contiguous with the dermatome (d). (B) At somite −7, the most medial sclerotome is becoming mesenchymal but the medial half remains more densely packed than the lateral half. The dermatome curves toward the inside of the somite and forms a dermatomal lip (dl) as it separates from sclerotome. (C) By somite −9, a myotome has developed medially (arrows) and lies in contact with the dermatome (d). A space (sp) between dermatome and sclerotome gradually closes as myotome develops. Both medial and lateral sclerotome are fully mesenchymal but medial cells remain closely packed. (D) By somite 12, myocytes underlie the entire dermatome and obliterate the space, but the medial myotome contains more myocytes (arrow). Cells are more dense in medial than in lateral sclerotome. Scale bar, 100 μm; *n*, neural tube.

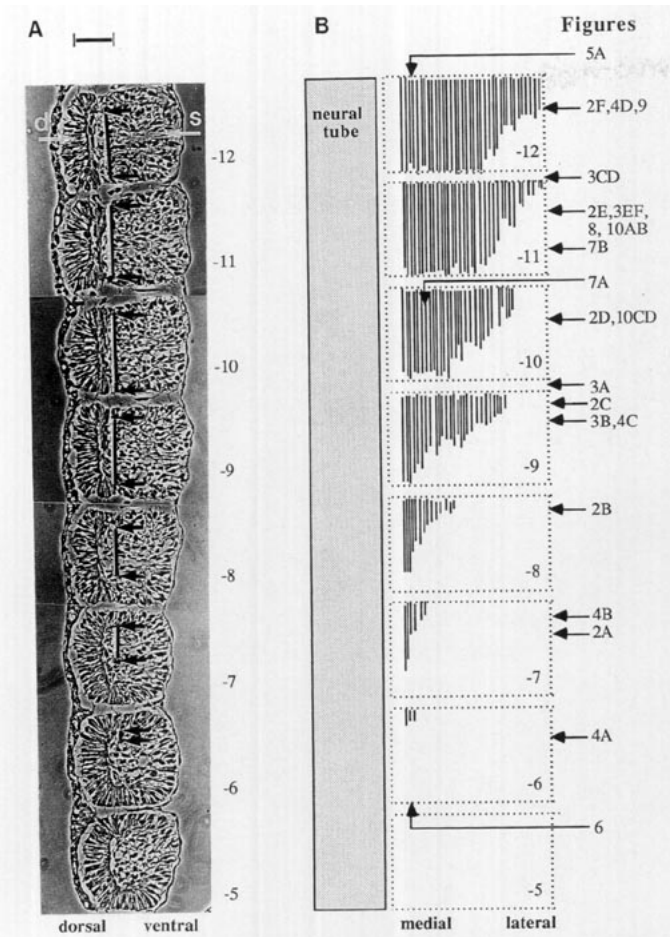


FIG. 5. Myotome development. Anterior is to the top. (A) In plastic sections, developing myotome is readily distinguished from epithelial dermatome (d) and mesenchymal sclerotome (s) by morphology. Myocyte progression within successive somites is shown in this sagittal section taken close to the neural tube. Lines between arrows indicate the anterior to posterior extent of the myotome in each somite. The first myocytes initiate at the anterior edge of somite −6. Myocytes reached the incurved, posterior lip of the dermatome by somite −9. (B) Reconstruction from serial sagittal sections in which lines represent the extent of myotome development as viewed from the dorsal surface. Since myocytes extend progressively across the somite in a medial to lateral wave, the extent of myotome within a cross section will depend on the section's anterior-posterior position within the somite. Therefore, for orientation, notations at right indicate the approximate position of sections shown in other figures. Scale bar, 100 μm.

cently formed somite (Loring and Erickson, 1987). We therefore used an unconventional posterior-to-anterior staging scheme; we counted from the most recently formed pair of somites, which we designated "−1" (Fig. 1). This method also allows us to approximate the duration of events. New somites emerge at intervals of about 1.7 hr (Loring and Erickson, 1987) so that somite −12 is 8 to 9 hr more mature than somite −7. The developmental events described below by somite number are consistent between embryos.

Since the terminology describing morphogenetic processes is sometimes used inconsistently, we first define terms (see Fig. 1 for anatomical relations). The term "dermamyotome" refers to the epithelial precursor of both the myotome and the dermatome. For consistency, we call the dorsal epithelial portion of the somite the dermamyotome in somites −1 to −5, before the first cells emerge to generate the myotome. Thereafter we call it the "dermatome" even though it continues to generate myotome for many hours. We call cells within the developing myotome "myocytes."

Counterintuitive Trajectories within the Somite

Neural crest cells that invade somites display trajectories that are unexpected in three respects (Fig. 2). First, crest cells appear to delay entering the somite. They have early access to the somite because they occupy the space between the somite and the neural tube by somite −5 or −6 (see also Loring and Erickson, 1987; Tosney, 1988b). However, they do not enter the somite until 3-5 hr later, when they penetrate the far anterior of somite −7. Even in somite −7, they have yet to enter the midanterior somite, despite their ventral position next to developing sclerotome (Fig. 2A). Crest cells thus fail to move into sclerotome as soon as they reach it, as though they were awaiting some developmental transformation in the somite. Second, upon entering somites, crest cells turn abruptly. Instead of entering medial sclerotome that lies directly in front of them, they turn and move laterally at the interface between developing myotome and sclerotome (Figs. 2B-2E, somites −8 to −11). Third, once crest cells reach the midpoint of the somite, they again turn and move ventrally. Since they turn ventrally only after traveling dorsal to the medial half of the sclerotome, they colonize lateral sclerotome before they colonize medial sclerotome. For example, by somite −10 crest cells lie far ventrally in the lateral sclerotome but few have colonized the medial sclerotome that lies next to the site where they entered the somite (Fig. 2D). Moreover, none have continued on a lateral trajectory beyond the midpoint of the somite. Crest cells continue to disperse within lateral sclerotome in somites −11 and −12, while they are making little headway within medial sclerotome (Figs. 2E and 2F). Only at more mature somitic levels do crest cells invade medial sclerotome in mass.

This complex trajectory within somites contrasts with the straightforward trajectory between somites where crest cells enter immediately and advance without turning (Fig. 3). Crest cells initially enter the intersegmental space between somite −6 and −7 which lies posterior to the site where crest cells first penetrate somites. Thus, crest cells advance immediately into the

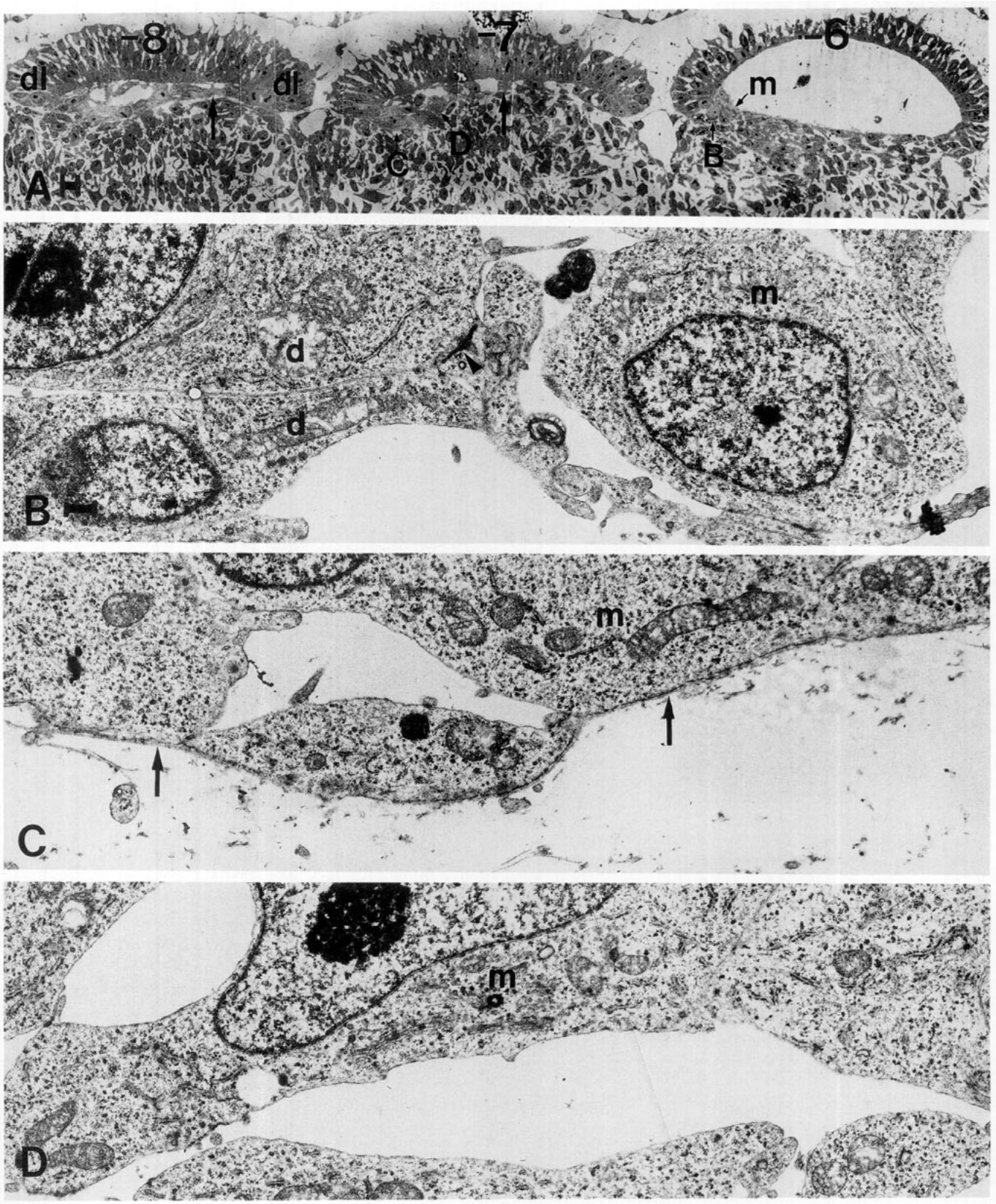


FIG. 6. Early development of myotome and its basal lamina shown in electron micrographs. Note that anterior is to the left, opposite to the orientation in Figs. 5 and 7A. (A) Somites −6, −7, and −8 are shown in a low-magnification sagittal section. The first myocytes (m) are visible at the anterior edge of somite −6. Arrows indicate the most posterior myocytes in somites −7 and −8. Letters indicate sites shown in B–D. dl, dermatomal lips. (B) In somite −6, myocytes (m) have yet to develop a basal lamina. Dermatome cells (d) are more electron-dense and elongated than myocytes and are joined by apical junctions (arrowhead). (C) Myocytes (m) in anterior somite −7, at the site where crest cells first enter, have developed a basal lamina (arrows). (D) Myocytes (m) in midanterior somite −7 lack a basal lamina. Scale bars: A, 10 μm; B–D, 1 μm.

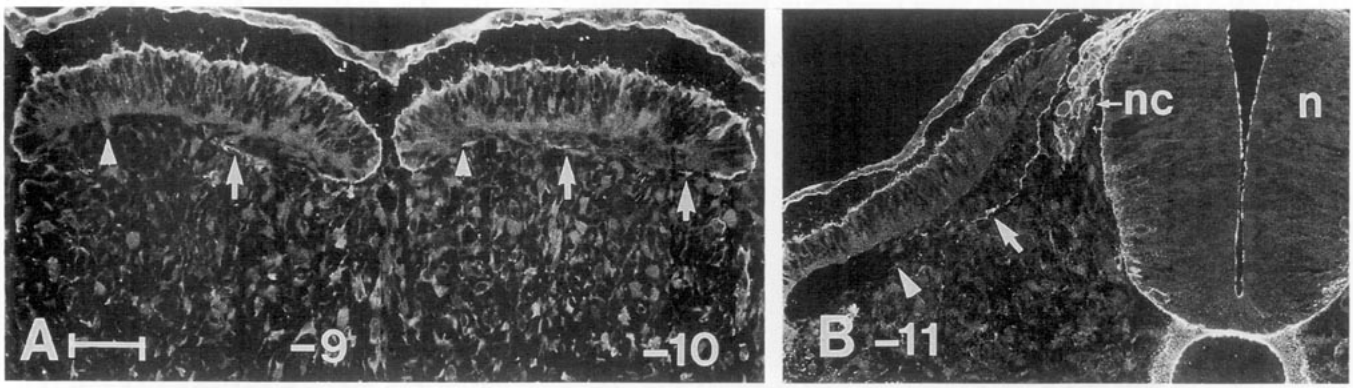


FIG. 7. Basal lamina development on myotomes shown by laminin immunoreactivity in 2-μm sections. (A) Arrowheads indicate the most posterior myocytes detected in this sagittal section of somites −9 and −10. Laminin immunoreactivity (arrows) demarks basal lamina on maturing myotome. Anterior is to the right. (B) Arrow indicates laminin immunoreactivity on medial myotome in posterior somite −11. Arrowhead indicates the most lateral myocyte seen. HNK-1 immunoreactivity indicates neural crest cells (nc). n, neural tube: scale bar, 50 μm.

intersomitic space, but delay entering the somite itself. Moreover, crest cells do not turn abruptly within intersomitic spaces. Instead, they move directly ventrally on a trajectory tangential to the neural tube (compare Figs. 3A and 3B). Trajectories are similarly simple within the most anterior margin of somites, where a direct ventral trajectory contrasts markedly with a lateral trajectory deeper within the same somite (compare Figs. 3C and 3D to 3E and 3F). Moreover, both between somites and at the anterior margin, crest cells colonize more medial sclerotome. The majority occupy midsclerotome about 100 μm from the notochord and a few lie within 50 μm of the notochord (Fig. 3D). In contrast, deeper within the somite, few come within 100 μm of the notochord and the majority lie 100 to 200 μm from the notochord (Fig. 3F).

Somite Maturation

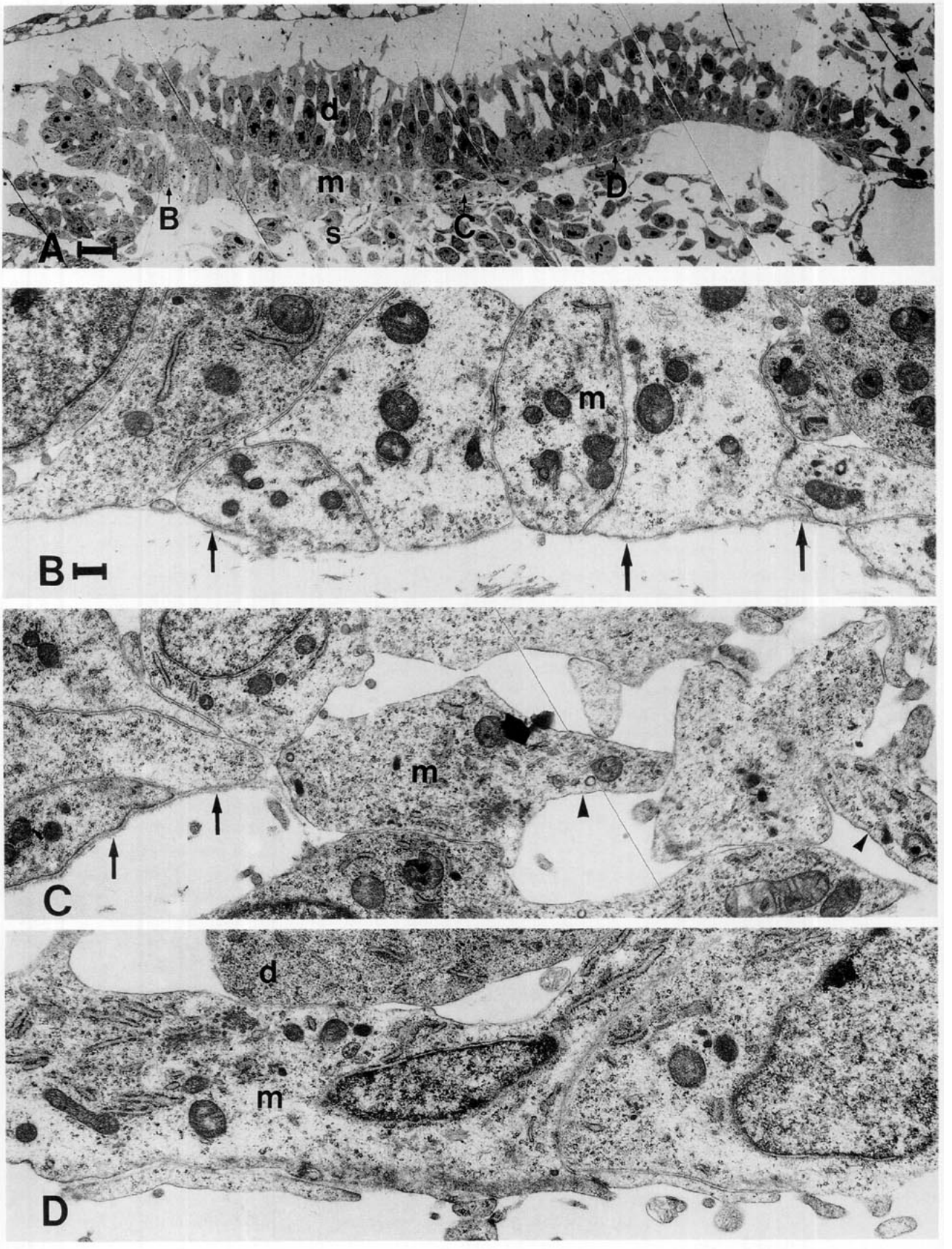
Changes within maturing somites must account for the following three peculiarities of crest cell migration within somites: (1) the time of entry, (2) the abrupt turn and lateral advance within dorsomedial somite, and (3) the ventral turn midway through the somite and advance within lateral rather than medial sclerotome. To identify features that could impose these patterns, we examined somite maturation in detail. We concentrated on identifying alterations at the entrance site and differences along the medial-lateral axis.

The entrance site, the medial aspect of the somite, presents a continuous epithelium as the first crest cells reach prospective sclerotome. The epithelial dermatome has yet to separate from the sclerotome, which is also still epithelial (Fig. 4A). Shortly before crest cells enter, three features at this entrance site alter in concert. The dermatome curves inward toward the center of the somite forming a dermatomal lip and a dent in the somite, myocytes appear medially (see below), and the most medial sclerotome transforms from an epithelium to a mesenchyme (Fig. 4B). Crest cells thereby face a dent in the somite, developing myotome, and mesenchymal sclerotome, any one of which might stimulate entry.

Along the medial-lateral axis, the sclerotome displays a consistent pattern of maturation. Its lateral portion matures first. It forms mesenchyme well before the medial edge (Figs. 4A and 4B). Moreover, lateral cells are less densely packed than are medial cells and remain so through somite −12, the most mature somite examined (Figs. 4C and 4D).

The myotome initiates a medial-to-lateral maturation sequence before crest cells invade the somite. The first myocytes arise from the anterior-medial edge of the dermatome in somite −6, spread proximate to the undersurface of the dermatome, and extend toward the posterior (Fig. 5A). More myocytes progressively arise from the anterior dermatomal lip in a medial to lateral sequence. The pattern produced by this progressive initiation and extension is most obvious when viewed from above (Fig. 5B). In somite −7 (where crest cells have entered the most anterior edge of the somite) the most medial myocytes have extended past the site of entry into the posterior somite. Additional myocytes have initiated lateral to them. By somite −8, a contingent of myocytes occupies the anterior-medial quadrant of the somite (compare to Fig. 2B). By somites −9 and −10, these myocytes have extended to the incurved, posterior lip of the dermatome, a journey taking 5–6 hr.

In addition to the progressive maturation along the medial-lateral axis, each half of the myotome differs distinctly. The lateral half develops more slowly in several respects. First, myocytes in the lateral half initiate less expeditiously and extend more slowly. Those ini-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tiating in lateral somite −9 have yet to extend more than half-way through a segment by somite −12, 5–6 hr later (Fig. 5B). Second, medial myotome is consistently more mature than lateral myotome at any cross-sectional level. For instance, it more rapidly accumulates myocytes. By somite −10, myocytes already lie two to three cells deep in the medial myotome even though myocytes have yet to form in the most lateral regions (Figs. 4C and 8A). In addition, the medial myocytes are more mature morphologically even at somite −12, where myocytes have developed from the medial to the lateral lips of the dermatome (Figs. 4D and 9). In comparison to lateral myocytes, they are more rounded in cross-sectional profile and extend fewer cellular processes. They are separated by less extracellular space and are often less electron-dense. Moreover, they continue to be more numerous, lying three to four cells deep, whereas lateral myocytes lie only one to two cells deep.

The myotome's basal lamina emerges in close concert with crest cell entry into the somite (Fig. 6). Before crest cells enter, myocytes have emerged at the most anterior edge of somite −6, but have yet to develop a basal lamina (Figs. 6A and B). By somite −7, basal lamina has developed but only on the most anterior myotome, where it thus coincides with the site of initial entry (Figs. 6C and 6D). As myocytes assemble in the medial myotome, they quickly develop a basal lamina, as indicated by laminin immunoreactivity (Figs. 7A and 7B). However, basal lamina development always lags behind myocyte extension. Myocytes without basal lamina can always be detected at the expanding edges of the myotome, either with electron microscopy (Figs. 6D, 8D, and 9D) or with laminin immunoreactivity (Figs. 7A and 7B). Immunoelectron microscopy confirms that laminin immunoreactivity viewed with light microscopy accurately delineates this basal lamina (not shown). Moreover, crest cells identified ultrastructurally by their HNK-1 immunoreactivity do contact this basal lamina directly (Fig. 10), a necessary condition for a preferred substratum.

Basal lamina disposition differs in one striking respect along the medial–lateral axis. At the times when crest cells align with the medial myotome's basal surface, only the medial myotome possesses a basal lamina. Both at somites −10 and −12, basal lamina is distinct on medial myotome but abruptly disappears near the myotome's midpoint; it is undetectable on lateral myotome (Figs. 8 and 9). The rather abrupt absence of basal lamina correlates closely with the position where neural crest cells turn away from myotome and move ventrally (compare with Fig. 2). The lateral, naked myocytes appear insufficient to entice crest cells farther laterally. The preferred substratum appears to be basal lamina rather than myocytes themselves.

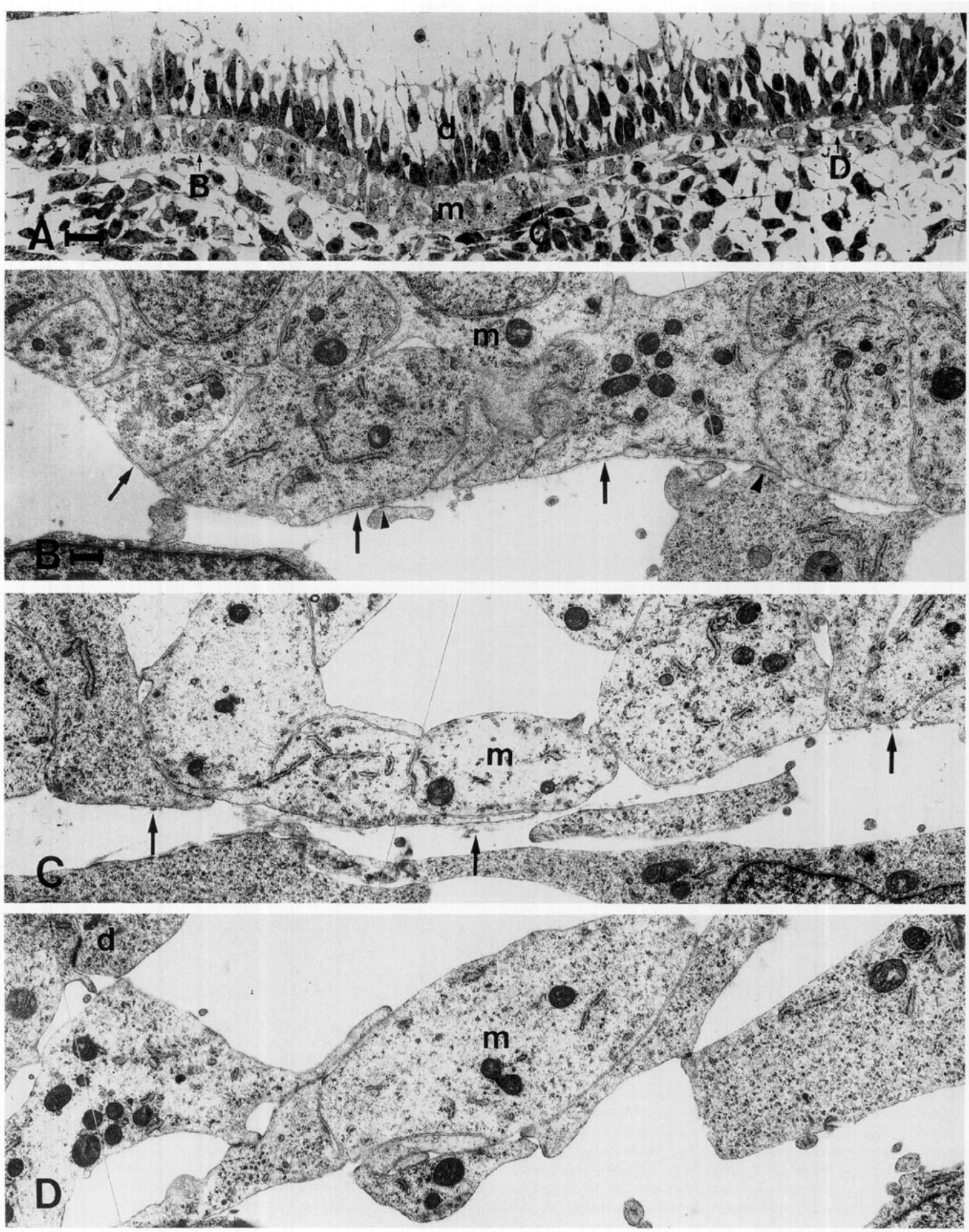
Crest Cells Fail to Turn Laterally When Myotome Is Prevented from Developing

To assess these correlations experimentally, we prevented myotome development by surgically removing its precursor, the dermamyotome. In the myotome's absence, crest cells entered the somite more directly. They consistently failed to turn laterally. Instead they invaded as a ventrally directed group of cells. Their trajectories were similar to those in natural myotome-free regions except that the wedge of invading cells was wider (compare Fig. 11B to Figs. 3A and 3D). The lateral trajectories of crest cells within the somite therefore depend on the myotome.

These surgeries also confirm an earlier report (Tosney, 1987) that the myotome is not *essential* for crest cells to enter the somite. Despite their altered trajectory when the myotome was absent, crest cells entered the sclerotome expeditiously. For instance, in somite −11, crest cells have not obviously delayed their advance in the absence of the myotome (compare Figs. 11B and 2E). In addition, we detected no obvious differences in the numbers of crest cells that entered myotome-free somites although we had the impression that fewer penetrated the ventrolateral sclerotome.

Partial deletions provide further evidence for a contact-mediated affinity between crest cells and the myotome's basal surface. When a small remnant of myotome developed medially, crest cells responded to it. They turned abruptly upon contacting it and aligned with its basal surface (Figs. 11C and 11D). Crest cells heavily coated any medial myotome that developed, as though they preferred to use the myotome's basal surface as a substratum.

FIG. 8. Electron microscopy shows differences in maturity of the myotome and its basal lamina along the medial (left) to lateral axis in somite −10, a level where crest cells have penetrated the somite to its midpoint (compare to Fig. 2D). (A) Even at low magnification, myocytes (m) are distinguishable from dermatome (d) and sclerotome (s) cells by their lower electron density. Medial myocytes are arrayed two to four cells deep, whereas lateral myocytes are sparse and widely spaced. Letters indicate sites shown in B to D. (B) Medially, myocytes (m) closely appose one another, have smooth profiles and few processes, and abut a well-organized basal lamina (arrows). (C) Near its midpoint, the organization of the myotome alters abruptly from one with contiguous myocytes (m) abutting a basal lamina (arrows) to one with widely spaced and irregularly shaped myocytes lacking a basal lamina (arrowheads). (D) Lateral myocytes (m) are distinct from the more electron-dense dermatome cells (d). These myocytes are stellate in profile, often extend processes, and lack a basal lamina. Scale bars: A, 10 μm; B–D, 1 μm.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DISCUSSION

The Myotome's Basal Lamina Is a Preferred but Not Essential Substratum

Patterns of crest cell entry and movement correlate closely with the disposition of basal lamina on the myotome (summarized in Fig. 12). We have provided four lines of evidence that neural crest cells prefer the myotome's basal lamina as a substratum. First, crest cells enter the somite when and where the myotome has developed a basal lamina. Second, crest cells consistently alter their trajectories in accord with myotome development, turning abruptly to align with and progress along its basal surface. Third, crest cells associate with myotomal basal lamina rather than with myocytes. They diverge into sclerotome where this basal lamina is incomplete or when it is rendered inaccessible because it is fully occupied by other crest cells. Fourth, in regions where myotome is normally absent or is deleted, crest cells enter the somite without turning. Neural crest cells, therefore, preferentially use the myotome's basal lamina to invade the somite. This substratum directs them laterally.

Despite their affinity for this basal lamina, crest cells do not require it to enter the somite. They will invade sclerotome from intersegmental spaces where myotome is naturally absent. They will invade after surgeries that prevent myotome from developing. Indeed, even their time of entry appears to be controlled by sclerotome rather than myotome. Crest cells normally enter only after the medial sclerotome has formed mesenchyme (see also Bronner-Fraser and Stern, 1991). In the absence of myotome, crest cells advance without an obvious delay.

The coordinated development of both myotome and sclerotome must produce the stereotyped pattern of neural crest migration within the somite. As the somite matures, the most medial sclerotome becomes mesenchyme that permits entry while the myotome generates basal lamina that provides a preferred substratum for advance. These events need not be linked. For example, at sacral and tail levels, myotome development is delayed relative to sclerotome development. Crest cells enter the somite directly at these levels, without turning (Erickson, unpublished observations).

Must we invoke peculiar properties of the myotomal basal lamina to explain its affinity for crest cells? One observation is consistent with a response specific to myotomal basal lamina. Crest cells fail to associate with the basal laminae of the dermatome or the posterior myotome. However, we know of no molecular features that are expressed in myotomal basal lamina but absent from these other basal laminae. For instance, all are positive for laminin immunoreactivity. Laminin distribution is thus insufficient to explain the affinity, even though laminin could contribute to migration since antibodies to integrins disrupt cranial neural crest migration (Bronner-Fraser, 1985; Lallier *et al.*, 1990; Lallier and Bronner-Fraser, 1991). However, one correlation does distinguish myotomal basal lamina from the basal lamina of the neural tube and dermatome. It fails to express two glucoconjugates that mark inhibitory function in other tissues, peanut agglutinin lectin binding activity or chondroitin-6-sulfate immunoreactivity (see Fig. 2 in Oakley and Tosney, 1991).

Moreover, the basal lamina of the anterior myotome is likely to be the only basal lamina that is accessible for migration, since these other basal laminae lie within inhibitory environments. The dermatomal basal lamina lines the dorsolateral path between the somite and the ectoderm. This path inhibits early crest cell entry (Erickson *et al.*, 1992; Oakley *et al.*, 1994). Likewise, basal lamina on posterior myotome lies adjacent to the inhibitory posterior sclerotome. The permissiveness of adjacent sclerotome may control access to the myotomal basal lamina.

The preference of crest cells for myotomal basal lamina significantly modifies our view of anterior sclerotome as an optimal migration path. Crest cells enter sclerotome as a second choice, preferring the basal lamina as a substratum wherever they can contact it. Similarly, anterior sclerotome is permissive but suboptimal for motor axon advance in culture. These axons advance more effectively on a laminin substratum than on sclerotome populations (Tosney, 1992) and slow their advance upon contacting anterior sclerotome cells (Oakley and Tosney, 1993). The anterior sclerotome is thus not an optimal path, eagerly invaded by migratory neural crest cells and axons. Instead, it appears to be a somewhat hostile environment that permits travel without providing the best possible environment for advance.

Does It Matter?

Does guidance proffered by the myotome contribute to final patterns of crest cell distribution or differentia-

FIG. 9. Electron microscopy shows differences in maturity of the myotome and its basal lamina along the medial (left) to lateral axis in somite −12. (A) Myocytes (m) now underlie the full medial to lateral extent of the dermatome (d) but are more numerous medially. Letters indicate sites shown in B to D. (B) Medial myocytes (m) are generally rounded in profile, closely spaced, and abut a basal lamina (arrows) that is contacted by electron-dense cells and cell processes (arrowheads). (C) Just lateral to the midpoint of the myotome, myocytes (m) abut an immature and discontinuous basal lamina (arrows). (D) Lateral myocytes (m), while still stellate, are more rounded in profile than in somite −10. Basal lamina is absent. d, dermatome cell. Bars: A, 10 μm; B–D, 1 μm.

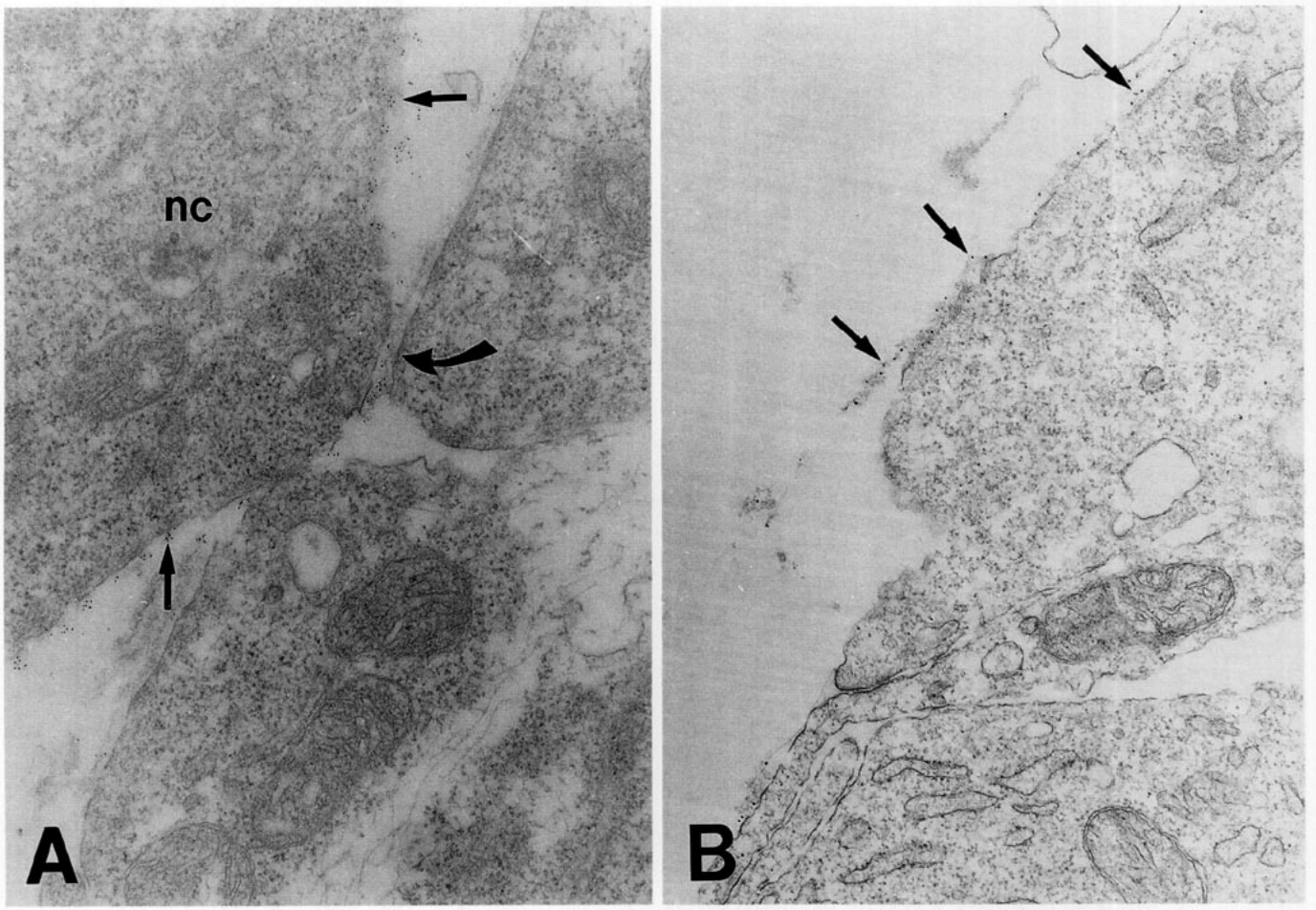

FIG. 10. Immunoelectron microscopy shows that crest cells contact laminin-positive myotomal basal lamina. (A) Colloidal gold (arrows) indicates an HNK-1 labeled crest cell (nc) in contact with the myotomal basal lamina (curved arrow). (B) Colloidal gold (arrows) indicates laminin-immunoreactivity in the myotomal basal lamina. Bars, 1 μm.

tion? On the face of it, the guidance appears subtle, sure to be swamped out by later developmental events. Indeed, the myotome influences only a subpopulation of crest cells, those that contact it. Moreover, it reorients crest cells only within a confined area, the dorsal somite.

One speculative possibility is that trajectories imposed by the myotome's basal lamina assure interactions important to determination. Crest cells responding to this basal lamina could accumulate a unique history of interactions. Those contacting it will interact with its extracellular matrix components, enter more lateral sclerotome and come close to the mesonephros, while those moving between somites will contact intersegmental blood vessels and come close to the perinotochordal mesenchyme. Since the local environment has long been posited to influence neural crest specification (e.g., Weston, 1991), such local effects should be considered seriously.

A second possibility is that this basal lamina helps crest cells reach proper destinations. For instance, it could assure that crest cells penetrate medial sclerotome slowly, so that a ganglion forms in a suitably dorsal position (c.f., Tosney, 1987). Another intriguing possibility is suggested by the rapid ventral migration of crest cells once they enter the lateral sclerotome. By causing crest cells to move laterally, this preferred substratum may assure that crest cells have the earliest possible access to a path that promotes migration ventrally, before impediments develop that prevent ventral migration to a sympathetic ganglion destination. Crest cells do colonize the sympathetic ganglion destination only during a limited period, as though their ventral migration were suddenly curtailed. At the lower thoracic and lumbar levels examined here, only crest cells labeled before stage 18 colonize the sympathetic destination; cells leaving the neural tube at or after stage 18 fail to migrate into this site (Oakley *et al.*, 1994; see Serbed-

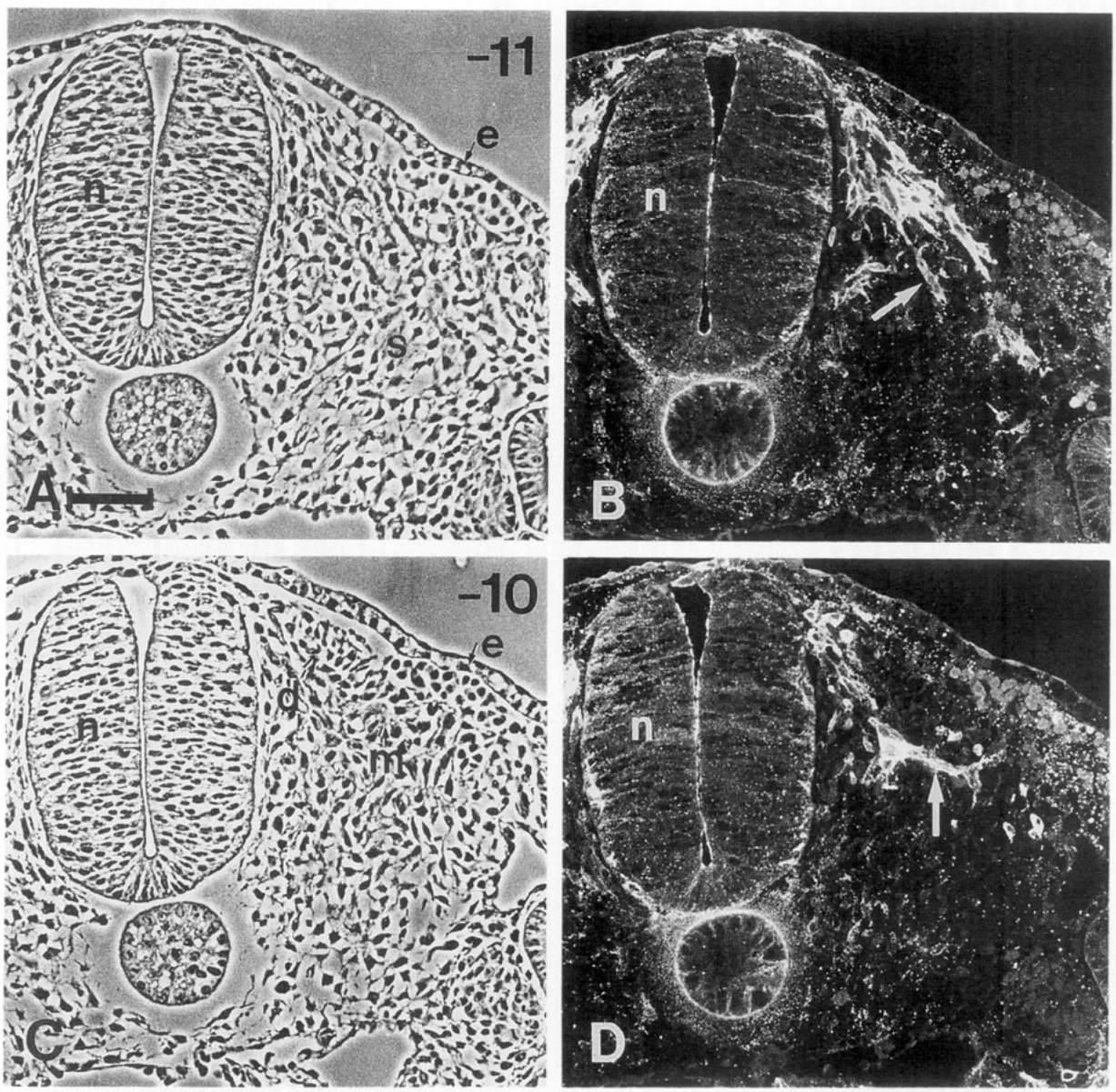

FIG. 11. Ventral migration following dermamyotome deletion. (A, B) Phase and fluorescent images of the same 2-μm cross section through somite −11. (A) Myotome is absent but sclerotome (s) is intact. (B) Rather than turning abruptly on entering sclerotome, crest cells (arrow) migrate ventrally as a wide wedge of cells. (C, D) Phase and fluorescent images of the same 2-μm cross section through somite −10. (C) A remnant of dermatome (d) and myotome (m) lies medially. (D) Crest cells have turned abruptly and conform closely to the undersurface of the myotomal remnant (arrow). Scale bar, 100 μm; n, neural tube. Round cells under the ectoderm (e) are blood cells.

zija *et al.*, 1989 for wing levels). Since crest cells first enter somites at stage 17 at lumbar levels, few may have the opportunity to migrate so far ventrally before an impediment develops.

Two developing structures are likely to impede migration ventrally. First, spinal nerves may preclude ventral migration (Loring and Erickson, 1987). Motor axons eventually extend as a sheet across the entire anterior somite (Tosney and Landmesser, 1985), forming a network that could trap crest cells. This potential impediment develops early. Motor axons extend across sclerotome and reach myotome by stage 18 at low thoracic levels (Tosney, unpublished observations). Experimental manipulations also suggest that motor axons can confine many crest cells to a dorsal location. When deletion of the ventral neural tube prevents motor axons from extending, crest cells migrate farther ventrally (Scott and Erickson, unpublished observations; Battacharyya *et al.*, 1993) and sensory ganglia condense farther ventrally than normal (Tosney and Oakley, 1990). A

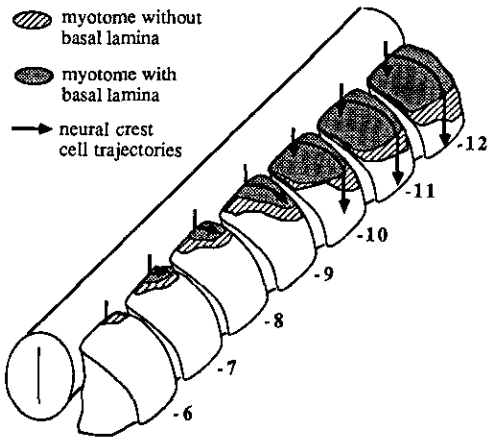

FIG. 12. Drawing summarizes the relation between crest cell trajectories and myotomal basal lamina. Anterior is to the upper right. Dermatome is omitted for clarity. Arrows pointing down indicate ventral migration; curved arrows indicate lateral migration along the myotome's basal surface. Basal lamina first develops on the anterior-medial myotome in somite −7, coincident with crest cell invasion of the somite. Crest cells turn upon contacting this basal lamina and move laterally. By somite −10, crest cells reach the more slowly maturing lateral myotome which lacks basal lamina; here they turn away from the myotome's basal surface and rapidly migrate ventrally through lateral sclerotome. By somite −10, so many crest cells have invaded the somite that the basal lamina is fully occupied. Some crest cells now advance slowly into medial sclerotome. In the intersegmental spaces (not shown), crest cells have no access to myotomal basal lamina and move directly ventrally, without turning.

second impediment to ventral migration is the perinotochordal mesenchyme. This ventromedial portion of the sclerotome develops inhibitory qualities owing to an interaction with notochord (Tosney and Oakley, 1990). The notochord itself appears to inhibit ventral migration, either directly or by altering the ventromedial sclerotome and its extracellular matrix (Newgreen *et al.*, 1986; Pettway *et al.*, 1990). The perinotochordal mesenchyme becomes inhibitory for axons as early as stage 18 at lumbar levels (Tosney and Oakley, 1990; Oakley and Tosney, 1991). Both structures thus form during the period when ventral migration appears to be curtailed.

We require experimental manipulations to determine whether the myotome influences the number of cells migrating ventrally or the differentiation of these cells. An effect on cell numbers is consistent with an enlargement of sensory ganglia after dermamyotome deletion, as though fewer crest cells had penetrated ventrally (Tosney, 1987). However, this evidence is indirect and supports several interpretations. We could directly assess an influence by studying the development of ventral derivatives after dermamyotome deletion. For instance, in the absence of myotome, fewer crest cells may reach the sympathetic ganglion site. Such studies must carefully determine how the manipulation alters actual migration patterns. Before we can do so, we need to know the pattern of ventral migration in more detail than is now available. Such studies are underway.

Myotome and Sclerotome Development

Our study extends previous reports of somite development in two ways. First, we link events in myotome development directly to the sequence of somite maturation by using a staging scheme independent of Hamburger and Hamilton (1951) staging criteria. Since this scheme is easy to apply, our information is more accessible than in previous studies.

Second, our more detailed study reveals developmental differences between medial and lateral somite populations. We show that medial myotome matures more rapidly than lateral myotome. The different rates of maturation extend beyond the earlier initiation of myocytes medially. The medial myocytes extend across the somite more rapidly, they are more mature morphologically, and they more rapidly generate a basal lamina. Conversely, the sclerotome matures in lateral to medial sequence. The lateral sclerotome becomes mesenchyme sooner, remains less dense, and supports more rapid crest cell penetration than medial sclerotome.

These differences are likely to reflect the different origins and fates of medial and lateral somitic populations. The medial and lateral halves of somites originate from spatially distinct populations during gastrulation. Medial halves arise from progenitors in the lateral portion of Hensen's node, while lateral halves arise from progenitors well to the posterior, within the primitive streak (Selleck and Stern, 1991). Medial and lateral populations also differ in fates and modes of development. Medial dermatome generates myocytes *in situ* whereas lateral dermatome generates myocytes by two modes, by contributing myocytes *in situ* to lateral myotomes and by producing cells that migrate into limbs to form myocytes later (Chevallier *et al.*, 1977; Christ *et al.*, 1977; Tosney, 1991; Ordahl and Le Douarin, 1992). The fate of lateral myotomes also varies with axial level. At thoracic levels they form body wall and abdominal muscles, whereas at limb levels they die about the time premyocytes cease their migration into the limb (Tosney, 1991). Moreover, medial and lateral populations require different trophic interactions: only medial myocytes require an early interaction with the neural tube to differentiate (Rong *et al.*, 1992; see also Christ *et al.*, 1992). Although molecular differences between populations within the myotome have yet to be systematically addressed, medial and lateral populations may also differ in their expression of myogenic regulatory genes which are detectable in early myotomes but not in premyocytes migrating into the limb (c.f., de la Brousse and

Emerson, 1990; Ott *et al.*, 1991; Pownall and Emerson, 1992). Similar medial and lateral differences may characterize sclerotome, which has yet to be examined in detail. One consequence of these differences is a stereotyped pattern of basal lamina development, which we show dictates the trajectories of those neural crest cells that contact it.

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