Inhibition of noggin expression in the dorsal neural tube by somitogenesis: a mechanism for coordinating the timing of neural crest emigration

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SUMMARY

We have previously shown that axial-dependent delamination of specified neural crest cells is triggered by BMP4 and negatively regulated by noggin. Increasing activity of BMP4 towards the rostral part of the axis is achieved by graded expression of noggin in the dorsal neural tube, the latter being high opposite unsegmented mesoderm, and progressively downregulated facing epithelial and dissociating somites, coinciding in time and axial level with initial delamination of neural crest cells (Sela-Donenfeld, D. and Kalcheim, C. (1999) Development 126, 4749-4762). Here we report that this gradient-like expression of noggin in the neuroepithelium is controlled by the paraxial mesoderm. Deletion of epithelial somites prevented normal downregulation of noggin in the neural tube. Furthermore, partial ablation of either the dorsal half or only the dorsomedial portion of epithelial somites was sufficient to maintain high noggin expression. In contrast, deletion of the segmental plate had no effect. These data suggest that the dorsomedial region of developing somites produces an inhibitor of noggin transcription in the dorsal neural tube. Consistent with this notion, grafting dissociating somites in the place of the unsegmented mesoderm precociously downregulated the expression of noggin and triggered premature emigration of neural crest progenitors from the caudal neural tube. Thus, opposite the unsegmented mesoderm, where noggin expression is high in the neural tube, BMP4 is inactive and neural crest cells fail to delaminate. Upon somitogenesis and further dissociation, the dorsomedial portion of the somite inhibits noggin transcription. Progressive loss of noggin activity releases BMP4 from inhibition, resulting in crest cell emigration. We propose that this inhibitory crosstalk between paraxial mesoderm and neural primordium controls the timing of neural crest delamination to match the development of a suitable mesodermal substrate for subsequent crest migration.

Key words: Avian embryo, BMP, Cadherin, Cell delamination, Dermomyotome, Epithelial-mesenchymal conversion, RhoB, Slug, TGFβ.

INTRODUCTION

The neural crest (NC) arises in the lateral tips of the neural folds that become the dorsal midline upon formation of the neural tube. Its importance stems from the immense variety of derivatives that this discrete group of cells yields during ontogeny, such as all the glia and most neurons of the peripheral nervous system. NC cells also develop into distinct endocrine and paraendocrine cells, and chromaffin cells of the adrenal medulla. Notably, all the melanocytes of the body, except for the retinal pigment, also arise from the NC. Most strikingly, at cranial levels of the axis, the ectodermal NC gives rise to most of the skeleton and connective tissue of the head, face and neck, structures that elsewhere in the body are exclusively of mesodermal origin. Thus, the ontogeny of the NC embodies the most fundamental questions of development, i.e. the understanding of differential specification of cell lineages, cell migration and phenotypic differentiation (Le Douarin and Kalcheim, 1999).

Recent studies have shown that NC cells are generated by interactions that take place between the prospective epidermis and the neuroectoderm (Dickinson et al., 1995; Liem et al., 1995; Mancilla and Mayor, 1996; Mouri and Jacobson, 1989, 1990; Rollhauser-ter-Horst, 1979). Although being initially an integral part of the neuroepithelium, specified NC cells separate from the dorsal neural tube by a process of epitheliomesenchymal transition and become a motile cell population (reviewed by Erickson (1993) and Kalcheim (2000)). These mesenchymal cells then migrate through stereotypic pathways, characteristic of embryonic stage and axial level. In the trunk, NC cells migrate between adjacent somites and through individual somites along well-defined pathways (Bronner-Fraser, 1986; Loring and Erickson, 1987; Rickmann et al., 1985; Teillet et al., 1987). Moreover, domain-specific differences within somites have been shown to control the segmental distribution of the migrating cells (Debby-Brafman et al., 1999; Eikholt et al., 1999; Kalcheim and Teillet, 1989; Krull et al., 1997; Wang and Anderson, 1997), as well as their differential localization along the dorsoventral extent of the somite, to give rise to distinct ganglia (Debby-Brafman et al., 1999; Goldstein and Kalcheim, 1991). Observation of normal embryos also reveals that the onset of NC emigration
from the neural tube is in phase with somite development. In general (with slight differences depending on age), opposite the unsegmented paraxial mesoderm, NC cells are still confined to the dorsal neural tube. NC delamination begins facing epithelial somites and continues following somite dissociation into sclerotome and dermomyotome, when migration into the somitic mesoderm is already underway (Sela-Donenfeld and Kalcheim, 1999; Teillet et al., 1987). Thus, while the relationship between NC migration and somitic properties is well established, the possible role of the somites in regulating the onset of NC migration remains unexplored.

We have recently shown that delamination of NC cells in the trunk is triggered by a balance between the activities of BMP4 and noggin from the dorsal neural tube (Sela-Donenfeld and Kalcheim, 1999), a mechanism similar to, but separable both in time and space from that of NC specification induced by ectodermal BMPs (Liem et al., 1995, 1997; Liu and Jessell, 1998; Marchant et al., 1998). The above balance is likely to be established by axial-level differences in the expression of noggin, which are consistent throughout a variety of embryonic stages. Whereas the expression of Bmp4 mRNA is homogeneous along the dorsal neural tube, that of noggin is progressively downregulated caudorostrally (Hirsinger et al., 1997; Marcelle et al., 1997; Reshef et al., 1998; Tonegawa and Takahashi, 1998). Importantly, this gradient-like distribution of noggin transcripts coincides both with initial delamination of NC cells and with somite development. Noggin expression is high opposite the unsegmented mesoderm, where NC cells are still premigratory, and is downregulated progressively facing epithelial and dissociating somites where NC emigration begins (Sela-Donenfeld and Kalcheim, 1999). Since we have found that perturbation of the BMP4/noggin balance by overexpression of noggin inhibits NC delamination (Sela-Donenfeld and Kalcheim, 1999), a central question for understanding the regulation of NC cell emigration by noggin is elucidating what factors establish the gradient-like expression of noggin along the dorsal neural tube.

In the present study we have tested the hypothesis that somitogenesis controls emigration of NC cells by modifying the levels of noggin transcription in the dorsal neural tube. To this end, we performed somite manipulations in ovo followed by examination of the expression of noggin mRNA and NC emigration. We find that deletion of either whole, dorsal halves or only dorsomedial quadrants of epithelial somites prevented normal downregulation of noggin in the neural tube thus maintaining high expression of the gene, which is otherwise restricted to the caudal neural tube. In contrast, unilateral or bilateral deletions of the segmental plate did not reduce the normal high levels of noggin transcription present in the neural tube. These data suggest that the dorsomedial region of developing somites produces an inhibitor of noggin transcription in the dorsal neural tube. In agreement with this notion, grafting dissociating somites in the place of the unsegmented mesoderm precociously downregulated the expression of noggin and triggered premature emigration of Dil-labeled NC progenitors from the caudal neural tube.

We conclude that although NC delamination is topographically restricted to the dorsal midline of the neural tube where it is regulated by local signals (BMP4/noggin), the overall process is modulated by dynamic changes in the adjacent somites. The latter, acting in a stage- and domain-specific manner, inhibit the transcription of the noggin gene, a possible upstream factor in a cascade leading to the physical separation of NC progenitors from the neuroepithelium.

**MATERIALS AND METHODS**

**Embryos**

Fertilized chick (Gallus gallus) and quail (Coturnix coturnix Japonica) eggs obtained from commercial sources were used for this study.

**In ovo microsurgery**

Ablation of segmental plate mesoderm or epithelial somites

Chick embryos aged 16-18 somite pairs were windowed and their vitelline membrane was removed. Following a short exposure to 25% pancreatin in phosphate buffered saline (PBS, pH 7.4; Kalcheim and Teillet, 1989), a fragment of presomitic mesoderm with a length corresponding to four to five prospective somites was removed either unilaterally or bilaterally at a level corresponding to the midsegmental plate (see Fig. 2A). In a separate series of embryos, the caudal three to five epithelial somites were unilaterally removed either totally or partially (see Fig. 3). Partial ablations consisted of removing only the dorsal somite halves, or the dorsomedial region. Embryos were further incubated for 5 or 10 hours, and then fixed with 4% formaldehyde for in situ hybridization.

**Grafts of somites in the place of presomitic mesoderm and vice-versa**

Donor quail embryos aged 25-27 somites were pinned ventral side down on Sylgard-coated dishes. The fourth to the ninth last somites, corresponding to the onset of dissociation into dermomyotome and sclerotome, were excised in one piece, along with a narrow strip of intermediate and lateral plate mesoderm. Resections were performed from the right side of embryos in the presence of 25% pancreatin diluted in PBS, which also assisted in separation from the ectoderm and endoderm. Donor somites were then grafted in the gap left after removal of the unsegmented mesoderm of younger chick hosts (see above). Special care was taken in keeping the correct dorsoventral and mediolateral orientations. In some cases the grafts were tilted clockwise with respect to the midaxial orientation so that the dermomyotome localizes far from the dorsal neural tube. In another set of experiments, a fragment of caudal segmental plate mesoderm of 16-20 somite-stage donors was grafted in place of the four recently formed epithelial somites of age-matched recipients. Embryos were incubated for additional 5 or 10 hours and then fixed with 4% formaldehyde for in situ hybridization or detection of migrating neural crest cells (see below).

**CM-Dil injections**

CM-Dil (C-7000, Molecular Probes) was dissolved in absolute ethanol to a concentration of 1 mg/ml. Just before starting the injections, it was further diluted to a final concentration of 0.1 mg/ml in 10% sucrose in water. Glass micropipettes with a tip opening of approx. 10 μm were backfilled with the CM-Dil solution, which was then microinjected into the lumen of the neural tube of host chick embryos aged 16-18 somites. Following neural tube labeling, unilateral removal of the segmental plate and somite grafting were performed as described above. Operated embryos were then incubated for additional 10 hours, fixed with 4% formaldehyde, embedded in paraffin wax and serially sectioned at 7 μm.

**In situ hybridization**

Whole-mount in situ hybridization was performed as described in
Kahane et al. (1998) with chick-specific probes to noggin (Reshef et al., 1998), Bmp4 (Francis-West et al., 1994), Pax6 (see Pituello et al., 1999) or Gli1 (see Borycki et al., 2000), followed by paraffin embedding and serial sectioning at 10 μm.

RESULTS

The relationship between expression of noggin, onset of neural crest migration and somitogenesis

In 15-35 somite-stage embryos, noggin mRNA was dynamically distributed along the rostrocaudal axis of chick embryos (Fig. 1 and data not shown). In all ages considered, expression in the dorsal domain of the neural tube was high opposite the segmental plate mesoderm (Fig. 1A,B), a region containing premigratory NC cells and showing no apparent emigration (Sela-Donenfeld and Kalcheim, 1999; Teillet et al., 1989 and see Fig. 6A). In 15-25 somite-old embryos, levels of noggin mRNA were comparatively lower adjacent to the newly formed epithelial somites (Fig. 1C,D). Among these, the signal was stronger opposite the youngest two pairs (Fig. 1C) and somewhat weaker towards the third and fourth pairs (Fig. 1D) in line with the onset of NC delamination (see Fig. 6B,C). Interestingly, in older embryos (more than 25 somite pairs), noggin expression facing all epithelial somites was already undetectable (data not shown). This observation correlates well with the age-dependent delamination of NC cells, which begins at slightly more caudal areas of the neuraxis in progressively older embryos (not shown). At the level of dissociated somites, noggin signal was absent at all ages examined (Fig. 1E) and both NC emigration and migration was well under way (Sela-Donenfeld and Kalcheim, 1999, see also Fig. 6D). Notably, a dynamic pattern of expression of noggin was also observed in the somitic mesoderm as a function of development (details in legend to Fig. 1, and see also Hirsinger et al., 1997; Marcelle et al., 1997; Reshef et al., 1998).

Effect of paraxial mesoderm ablations on the expression of noggin mRNA in the dorsal neural tube

The inverse relationship between noggin expression in the neural tube and the acquisition of migratory properties by NC cells, both in register with somite development, raises the hypothesis that somitogenesis regulates the gradient-like expression of noggin along the neuraxis and consequently, the delamination of NC progenitors. To begin addressing whether there is a functional relation between somitogenesis and noggin expression, two possibilities were examined. The high expression of noggin in the dorsal neural tube facing the unsegmented mesoderm could result from a maintenance/inductive effect by this tissue. Alternatively, the progressive downregulation of the transcription of the noggin gene towards more rostral areas of the dorsal neuroepithelium could be explained by an inhibitory activity emitted by developing somites.

To test the first possibility, unilateral ablations of the midsegmental plate were performed as shown in Fig. 2A,B. In all embryos examined 5 hours after the operation (n=10), a stage corresponding to the transition between the unsegmented mesoderm and young epithelial somites, the noggin signal on the operated side was strong and indistinguishable from that on the contralateral unoperated side (Fig. 2B). To overcome a possible compensation by the contralateral mesoderm, bilateral removal of the segmental plate was performed. In the three cases examined the expression of noggin in the dorsal neural tube remained strong (Fig. 2C), similar to the intensity observed in the same embryos in regions limiting the excision as well as opposite segmental plates of intact embryos (Fig. 1B and data not shown). These results suggest that the segmental plate mesoderm has no effect on maintenance of high levels of noggin expression in the caudal portion of the dorsal neural tube.

Complete unilateral resections of epithelial somites were then performed as shown in Fig. 3A. 5 hours after surgery, the somites on the contralateral intact sides fully dissociated into sclerotome and dermomyotome and noggin signal in the dorsal neural tube was not apparent any longer (Fig. 3B), similar to the situation in normal embryos (Fig. 1E). In striking contrast, opposite the gap left after somite removal, noggin expression remained high in the dorsal hemi-neural tube (Fig. 3B) in 12 out of 14 experimental embryos. In addition, removal of the unsegmented mesoderm followed by a longer incubation of 10-12 hours to a stage corresponding to dissociated somites had a similar effect as epithelial somite removal followed by short incubations (n=4 out of 4 embryos, not shown). Maintenance of noggin beyond the normal timing in the absence of mesoderm indicated that the developing somites (from epithelial stage onward) emitted an inhibitor of noggin transcription in the neural tube. To localize more precisely the topographical source of such activity, deletions of either the dorsal (Fig. 3C) or the dorsomedial quadrant (Fig. 3D) of forming somites were performed. In both cases, partial ablation of the mesoderm was sufficient to maintain high noggin expression in the experimental hemi-neural tubes of all embryos (n=5 and 7, respectively). To control for possible effects of the ectoderm on regulation of noggin expression in the tube, ectoderm resections were performed unilaterally from over the segmental plate mesoderm or the epithelial somites with no apparent effect when compared with the contralateral sides (n=9/9 and 5/5, respectively, data not shown). Thus, the dorsomedial quadrant of the developing somite that is in contact with the dorsal domain of the neural tube, but not the segmental plate mesoderm or overlying ectoderm, appears to be the source of an activity that inhibits the transcription of noggin in the neuroepithelium.

To further control for the specificity of the ablations on transcription of the noggin gene, somite deletions were followed by examination 5-8 hours later of the patterns of expression of several genes. BMP4 is expressed in the dorsalmost neural tube (Fig. 4A,B, and see also Sela-Donenfeld and Kalcheim, 1999), PAX6 is expressed in the dorsal half of the tube (Fig. 4C,D, Goulding et al., 1993) and GLI1 is distributed in the tube in a ventral to dorsal gradient of intensity except for the floor plate and in the somites it reveals a stronger expression intensity in their ventromedial aspect (Fig. 4E,F, see also Borycki et al., 2000). As seen in Fig. 4, unilateral deletions of epithelial somites had no effect on the expression pattern or intensity of either of the above genes (n=6, 5 and 3, respectively). These results suggest that whereas somite manipulations altered the normal gradient of noggin expression (Fig. 3), they had no general effect on neural tube patterning.
Grafts of dissociating somites in the place of unsegmented mesoderm prematurely downregulate expression of noggin and trigger precocious emigration of NC cells

To further examine the possibility that the downregulation of noggin expression along the neural tube results from an inhibitory activity produced by the developing somites, stripes of dissociating somites were grafted in the place of the unsegmented mesoderm opposite the caudal neural tube where levels of noggin transcription are high (see Fig. 1). Because results of the deletions suggested that the dorsomedial portion of the somites was sufficient to elicit a change in normal expression of the gene, we chose to backgraft slightly older, dissociating somites rather than young epithelial somites, as only the former are already specified in their dorsoventral and mediolateral axes (Christ and Ordahl, 1995). In 11 of 13 operations in which the normal dorsoventral and mediolateral positioning of the implants was kept, a total unilateral downregulation of noggin transcription was observed opposite the grafts 5 hours following surgery, whereas high expression was apparent in the contralateral control side of the tubes (Fig. 5B-D). In contrast, when the grafts were slightly tilted in a clockwise orientation, such that the dorsomedial portion of the dermomyotomes remained far from the axis, no downregulation was obtained in the dorsal neural tube facing the grafts in any of the five embryos operated (Fig. 5E).

Together with the deletion experiments, these results strengthen the notion that developing somites produce an inhibitor of noggin synthesis in the tube, and further suggest that the source of this inhibitory activity specifically originates in their dorsomedial region.

Fig. 1. The relationship between expression of noggin in the dorsal neural tube and somitogenesis. (A) Whole-mount in situ hybridization of a 20 somite-stage chick embryo with a noggin probe. Note the caudorostral gradient of noggin expression along the neural tube (rostral is towards the right). (B-E) Transverse sections through broken lines in A. (B) Section through the segmental plate showing strong signal in the dorsal domain of the neural tube. (C,D) Opposite young epithelial somites, noggin transcription is still high in the dorsal neural tube (C) and becomes progressively weaker towards more rostral epithelial somites (D). (E) Facing dissociated somites, noggin signal has totally disappeared from the neural tube. A dynamic expression pattern is also observed in the developing mesoderm. Noggin mRNA is expressed in the lateral part of the segmental plate (B), then in the corresponding region of the youngest epithelial somites (C). The signal progressively spreads towards dorsomedial regions of the paraxial mesoderm (D) until becoming restricted to the dorsomedial lip of the dermomyotome (E). DM, dermomyotome; ES, epithelial somite; NO, notochord; NT, neural tube; Scl, sclerotome; SP, segmental plate. Scale bar: 50 µm in B-E.

Fig. 2. Deletions of the segmental plate mesoderm have no short-term effect on the expression of noggin in the dorsal neural tube. (A) Unilateral deletion of the mid-segmental plate (arrows) in a 18 somite-stage embryo. (B) Transverse section through an embryo operated on as shown in A and further incubated for 5 hours. Note that in the control side, a young epithelial somite has formed opposite which noggin expression is still high in the corresponding side of the neural tube. Facing the gap left after removal of the segmental plate, noggin expression is similar to the contralateral side. (C) Following bilateral removal of the segmental plate mesoderm, noggin mRNA remains high in the neural tube 5 hours after the operation. ES, epithelial somite; LPM, lateral plate mesoderm; NO, notochord; NT, neural tube; SP, segmental plate. Scale bar: 60 µm in B,C.
**Fig. 3.** Complete or partial deletions of epithelial somites prevent the normal downregulation of *noggin* mRNA in the dorsal neural tube. (A) Unilateral removal of the last-formed epithelial somites (arrows) in a 18 somite stage chick embryo. (B) Transverse section through an embryo operated on as shown in A. Note that in the control side, the somite has dissociated into sclerotome (Scl) and dermomyotome (DM), and *noggin* signal in the neural tube of the ipsilateral side is not present any longer. In contrast, removal of the whole somite resulted in maintenance of *noggin* in the corresponding hemi-dorsal tube. (C) Removal of the dorsal half of epithelial somites also prevented the normal downregulation in *noggin* mRNA observed in the normal contralateral side. (D) Whole-mount in situ hybridization of an embryo operated at the 25 somite-stage, in which the dorsomedial quarter of the rostralmost part of the segmental plate was removed. Five hours later, *noggin* signal was maintained in the dorsal hemi-tube along the ablated region (arrow), while it was already downregulated in the control side. Note in the operated side that the lateral aspect of the mesoderm, which also expresses *noggin* is apposed to the midline, owing to removal of the dorsomedial portion of the mesoderm (arrow). Note as well that in embryos older than 25 somites, *noggin* mRNA becomes downregulated opposite newly forming somites (see text). In A and D, rostral is towards the right. ES, epithelial somite; LPM, lateral plate mesoderm; NO, notochord; NT, neural tube; SP, segmental plate. Scale bar: 60 μm in B,C.

**Fig. 4.** Somite deletions have no effect on expression of BMP4, PAX6 or GLI1 mRNAs. Whole-mount in situ hybridizations (A,C,E) and transverse sections (B,D,F) of embryos hybridized with avian-specific probes for BMP4, PAX6 and GLI1, respectively. Areas between arrows in the right side of each image represent the unilateral deletions. In the transverse sections the intermediate mesoderm (IM) is now adjacent to the midline in the operated sides (right). Note that expression intensities have not changed for any of the tested genes. LPM, lateral plate mesoderm; NO, notochord; NT, neural tube; S, somite. Scale bar: 60 μm.
To examine whether the downregulation in noggin mRNA caused by ectopic somite grafting is associated with premature delamination of NC cells, neural tubes of host embryos were labeled with the cell tracker CM-DiI followed by back-transplantation of dissociating somites opposite caudal neural tube regions. 10 out of 11 operated embryos fixed 10 hours following the procedure, revealed a premature, unilateral emigration of DiI-positive NC cells from the caudal neural tube into the grafted tissue, whereas the normal contralateral sides showed either no apparent emigration or just a beginning of NC delamination, depending on the exact axial level considered (Fig. 6E-G). In contrast, mere deletion of epithelial somites (n=9) or similar deletions followed by grafting of caudal segmental plates (n=12), two procedures that resulted in maintenance of high levels of noggin in the dorsal tube, whereas the normal contralateral sides showed either no apparent emigration or just a beginning of NC delamination, depending on the exact axial level considered (Fig. 6E-G). In contrast, mere deletion of epithelial somites (n=9) or similar deletions followed by grafting of caudal segmental plates (n=12), two procedures that resulted in maintenance of high levels of noggin in the dorsal tube (Fig. 3 and data not shown), prevented in all cases the emigration of neural crest cells at a time when they normally emerge from the control contralateral sides (Fig. 7). In contrast to all the experimental embryos described above, normal embryos labeled with CM-DiI always showed bilateral symmetry in the behavior of emigrating NC cells (Fig. 6A-D).

Thus, both the regulation of noggin transcription in the dorsal neural tube and the onset of NC emigration are controlled by somite properties. Moreover, since NC delamination is controlled by noggin-dependent BMP4 activity (Sela-Donenfeld and Kalcheim, 1999), we conclude that all above processes are part of a regulatory cascade that modulates, at least, the timely emigration of NC cells.

**Discussion**

In a previous study we have shown that BMP4, expressed in the dorsal domain of the neural tube, triggers delamination of specified NC cells from the neural primordium. Increasing concentrations of BMP4 are likely to be reached by progressive downregulation of the expression of its specific inhibitor noggin, which occurs in a caudorostral direction along the same area of the neural tube. Based on these data, we suggested that BMP4 and noggin act as a regulatory unit to affect the epitheliomesenchymal conversion of pre-migratory progenitors (Sela-Donenfeld and Kalcheim, 1999). In the present study we have further analyzed the regulation of NC emigration by asking what factors contribute to the establishment of the gradient-like expression of noggin along the rostrocaudal axis of the neural tube. We report that this pattern of noggin transcription is established by signal(s) emanating from the adjacent somites. Deletion of epithelial somites either in their totality or partial ablation of their dorsal or only dorsomedial quadrant, resulted in failure of otherwise normal downregulation of noggin mRNA signal in the dorsal tube. Conversely, back-transplantation of developing somites into the segmental plate mesoderm caused a premature disappearance of neural tube-specific noggin opposite the grafts. In contrast, the high levels of noggin expression in caudal areas of the neuraxis do not seem to be regulated by mesodermal signals, as deletion of the segmental plate had no effect on the normal expression pattern. In addition, we show...
Fig. 6. Precocious emigration of NC cells opposite grafts of dissociating somites in the place of the segmental plate mesoderm. (A-D) Transverse sections through normal embryos that were microinjected with CM-DiI into the lumen of the neural tube at the age of 16-18 somites and further incubated for 10 hours. Note in A that no emigration occurs facing the segmental plate. (B) A beginning of delamination in the midline is apparent opposite epithelial somites (arrowhead); (C) more cells delaminate and advance dorsoventrally (arrowheads) facing the somite at the beginning of dissociation (intermediate stage, Teillet et al., 1987), and migration is underway (arrowheads) opposite the dissociated somites (D). In all cases the behavior of NC cells is bilaterally symmetrical. (E-G) Transverse sections through three embryos that were microinjected as depicted above. Following neural tube labeling, unilateral removal of the segmental plate and somite grafting were performed as described in Materials and Methods. NC cells emerge unilaterally from the caudal neural tube (arrowheads) facing the grafts. DM, dermomyotome; ES, epithelial somite; NT, neural tube; Scl, sclerotome; SP, segmental plate. Scale bar: 110 μm in A-G.

Fig. 7. Deletion of epithelial somites or deletion followed by segmental plate grafts prevent emigration of neural crest cells. Images represent transverse sections through embryos that were microinjected with CM-DiI into the lumen of the neural tube (as described in Materials and Methods) and then subjected to somite deletions (A-D) or a similar ablation followed by grafting a fragment of caudal segmental plate (E,F). Neural crest cells emerge opposite the normal sides in each case (arrowheads) either facing the dissociated somites (A,B,E,F) or the region approaching an intersomite (C,D). In contrast, no emigration is apparent facing the deleted somites (A-D) or the grafted segmental plate (E,F). (B,D) Note that following somite deletions and further incubation for 10 hours, the somatopleural layer of the lateral plate mesoderm approaches the neural tube. DM, dermomyotome, E, ectoderm, IEC, intra-embryonic coelom, LPM, lateral plate mesoderm, NO, notochord, NT, neural tube, Scl, sclerotome. Scale bar: 40 μm.
that the premature downregulation of noggin transcription in caudal neural tubes opposite grafts of dissociating somites is accompanied by precocious emigration of NC cells from the neural primordium. Taken together, results of this and our previous study strongly suggest that the developing somites modulate expression of noggin in the dorsal midline of the neural tube, which in turn modifies levels of BMP4 activity that trigger delamination of NC cells.

Two features of such a putative somite-derived activity are worth discussing. The first concerns its source. Deletion and ablation/grafting experiments suggest that the origin of this activity is the dorsomedial quadrant of the developing somite. Analysis of the pattern of noggin expression in embryos at different stages would indicate that in young embryos (approx. 15-20 somite pairs), such an activity develops preferentially upon somite dissociation, whereas in older embryos, it is already present at the level of epithelial somites. The dorsomedial region of epithelial somites is likely to be already specified as it contains a subset of epithelial post-mitotic progenitors expressing MyoD that give rise to the primary myotome (Kahane et al., 1998). Furthermore, upon somite dissociation and formation of the dorsomedial lip (DML) of the dermomyotome, the dermomyotome and the DML region in particular express a variety of factors and receptors such as noggin itself (Hirsinger et al., 1997; Marcelle et al., 1997; Reshef et al., 1998; Sela-Donenfeld and Kalcheim, 1999; Tonegawa and Takahashi, 1998 and see Fig. 1), WNT11 (Marcelle et al., 1997) and PAX3 (Goulding et al., 1994; Williams and Ordahl, 1994), which are implicated in patterning the dermomyotome and the myotome; the neurotrophin-3 receptor TrkC, which has been found to mediate the transition between the epithelial dermomyotome and the mesenchymal dermis (Brill et al., 1995); follistatin and the follistatin-like gene Flik (Amthor et al., 1996), etc. Thus, the dorsomedial area of the developing somites may be the source of a variety of factors acting either locally on somite development and/or upon the synthesis of factors produced in the adjacent dorsal neural tube. Clearly, the activity here defined must operate over a short range as all changes detected in the dorsal tube concerned the side opposite the operations exclusively; bilateral effects were never detected. Moreover, responsiveness to this factor(s) should be restricted to the dorsal neural tube, as transcription of noggin in the DML itself is not affected.

The second issue concerns the nature of the somite-derived factor, which we find to act by inhibiting the transcription of the noggin gene, itself an inhibitor of the BMP signaling pathway that acts to positively regulate NC delamination. Although the identity of this activity remains unknown, as does the mechanism by which it operates to modulate noggin transcription, the present finding suggests that inhibitory signals arising upon somitogenesis mediate interactions between somites and neural tube. Positive signal(s) emanating from developing somites and acting upon the cervical neural tube to activate expression of PAX6 have been documented (Pituello et al., 1998). Furthermore, there is increasing evidence that the paraxial mesoderm plays a role in patterning rostrocaudal properties in the early neuroepithelium at defined times of development (Bang et al., 1997, 1999; Itasaki et al., 1996; Grapin-Botton et al., 1997; Muhr et al., 1997). In addition, axial-level differences between thoracic and brachial somites were found to initiate specification of motoneuron subtype identities (Ensini et al., 1998).

Signaling in the opposite direction from the neuroepithelium towards the somites to affect patterning and later differentiation has been extensively documented as well (see for example Brill et al., 1995; Hirsinger et al., 1997; Ikeya and Tanaka, 1998; Marcelle et al., 1997; Spence et al., 1996). The possibility exists that the inhibitor of noggin transcription is produced upon somitogenesis independent of signals from axial structures. Alternatively, the dorsal neural tube would be a suitable candidate to trigger the production of such a factor in the dorsomedial mesoderm. Regardless of its identity, the initial expression of such a factor must precede the onset of noggin downregulation in the dorsal neural tube. Because this happens at slightly different levels in younger versus older embryos, we favor at this point the explanation that the production of such a somitic factor is a regulated rather than a somite-autonomous process. If this is the case, we would predict that a continuous functional loop transmits information between the neural tube and developing paraxial mesoderm (and vice-versa) that regulates progressive morphogenetic

Fig. 8. Initial emigration of NC cells is modulated by somitogenesis-induced inhibition of the transcription of noggin in the dorsal neural tube and consequent release of BMP4 activity. At caudal levels of the neuraxis opposite the segmental plate mesoderm, noggin expression is high in the dorsal domain of the neural tube, BMP4 is inactive and NC cells fail to delaminate. Upon somitogenesis and further somite dissociation, the dorsomedial portion of the somite produces an activity that inhibits noggin transcription in the neural tube. Progressive loss of noggin activity releases BMP4 from inhibition, resulting in NC cell emigration. This inhibitory crosstalk between paraxial mesoderm and neural primordium is likely to control the appropriate timing of NC delamination to correspond to the elaboration of a suitable mesodermal substrate that supports subsequent cell migration.
events in both tissues. Preliminary results in fact suggest that somite-dependent downregulation of noggin that enables BMP4 activity in the dorsal neural tube is necessary for consequent induction of noggin expression in the DML (Sela-Donenfeld and Kalcheim, unpublished).

In contrast to the regulation of noggin production opposite somites, the high levels of noggin expression in caudal areas of the neuraxis did not seem to be regulated by mesodermal signals, as deletion of the segmental plate had no effect on the normal expression pattern. Furthermore, caudal segmental plates implanted in the place of epithelial somites resulted in high noggin expression in the corresponding hemi-neural tubes, a phenotype most likely due to the mere removal of the epithelial somites, which we showed to be sufficient to prevent downregulation of noggin transcription. Consistent with the lack of noggin disappearance, NC cells failed to leave the neural tube facing the ectopic unsegmented mesoderm, whereas they exited the tube opposite the control side, which already contained dissociated somites by the time of fixation. These results further strengthen the functional link between somitogenesis, noggin expression and NC emigration.

This and our previous study highlight the importance of the gradient of noggin production along the dorsal neural tube as an upstream event in triggering BMP4 activity and consequent NC delamination. It is important to establish whether species other than avians use the same molecular interactions. In Xenopus and mouse, noggin is expressed in the dorsal part of the neural tube (Smith and Harland, 1992; McMahon et al., 1998); however, it is unclear whether its pattern is homogeneous or whether it decreases gradually along the axis in correlation with emigration of NC progenitors. Additional inhibitors of BMP activity have been described (Glinka et al., 1998; Hsu et al., 1998; Joseph and Melton, 1997; Mariani and Harland, 1998; Pearce et al., 1999; Stanley et al., 1998) and their possible expression in the dorsal tube and physiological relevance to NC delamination remain an unanswered question. Similarly, the mouse dorsal neural tube expresses GDF7, BMP6 and BMP7 (Lee et al., 1998). Thus, the possibility exists that different members of the TGFβ superfamily, as well as different BMP inhibitors, might account for the regulation of NC cell delamination in different species. Of interest is the observation that FRZB1, a specific inhibitor of the WNT signaling pathway, which is also operative in the neural primordium, is expressed in the dorsal domain of the tube and displays a caudal to rostral gradient of transcription (Baranski et al., 2000; Duprez et al., 1999; Ladher et al., 2000), suggesting the possibility of complex regulatory mechanisms within the neuroepithelium.

The migration of NC cells at trunk regions of the axis is known to be regulated by properties inherent to the somitic mesoderm. Although the pathways of migration have been fully defined and the elucidation of mechanisms regulating patterned migration through the somites has significantly progressed during the past few years (Kalcheim, 2000 and see Introduction), the factors that control delamination of NC cells from the neural tube have remained elusive. In a previous paper, we reported that a balance between BMP4 and noggin in the dorsal neural tube was responsible for triggering changes in expression of cadherin 6B and rhoB that, among other proteins, are likely to cause intercellular detachment, cytoskeletal changes and generate NC motility (Sela-Donenfeld and Kalcheim, 1999, see also Liu and Jessell, 1998). Here we provide further evidence that the balance between the relevant signals within the neural tube depends upon somitogenesis (Fig. 8). Based on the pivotal role of the somites in controlling the progression of NC migration, we propose that this early interaction between somites and dorsal neural tube, mediated by inhibition of noggin production, serves to signal the exact timing for NC emigration to match the establishment of an appropriate somitic substrate that supports further cell movement.

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