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How Do the Migratory and Adhesive Properties of the Neural Crest Govern Ganglia Formation in the Avian Peripheral Nervous System?

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The peripheral nervous system derives mainly from the neural crest both in the head and trunk. Using markers such as fibronectin (FN), neural cell-adhesion molecule (NCAM), the nucleolar marker for quail cells in chimaeric embryos, and NC-1, a monoclonal antibody specific to crest cells and their neural derivatives, we have attempted to reconstruct the processes that lead to the formation of peripheral ganglia. Our observations allow us to propose a model of the formation of ganglia based on morphogenetic movements and on variations of crest cell adhesiveness. In most cases, crest cells migrate in morphologically defined and transient pathways that lead them to their final site of arrest; these pathways are always associated with FN, which appears necessary for crest cell attachment and movement in vitro. The directionality of crest cell migration is probably dictated by the cells' motile properties and population pressure in restricted areas suitable for cell movement. The disappearance of the pathways and of the substrate necessary for migration while the population is rapidly dividing may be responsible for the aggregation of crest cells in the case of the sensory ganglia. To the contrary, the aggregation of crest cells into autonomic ganglia (sympathetic, enteric, and ciliary ganglia) does not seem to obey the same rules, no disappearance of the substratum or of the pathways being obvious; rather, their formation seems correlated with the de novo synthesis of adhesive molecules such as NCAM.

Key words: avian embryo, peripheral nervous system, neural crest, cell-adhesion, cell migration

The peripheral nervous system arises entirely from the neural crest in the trunk and has a dual origin in the head (the neural crest and the ectodermal placodes for the sensory ganglia of some cranial nerves). Appropriate cell marking techniques have established very precisely the site of origin along the neural axis of the different ganglia [for an extensive review see 1].

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Let us, for example, consider the trunk level: Crest cells are able to differentiate into sensory and autonomic ganglia and into pigment cells. There is a striking contrast between the uniformity of the premigratory crest cells and the diversity of their differentiated derivatives. In addition, crest cells are able to migrate to widely dispersed areas of the embryo, even though at the onset of migration some of these areas do not yet exist. How can a single embryonic structure generate so many different ganglia in so many different places?

It is clear that the study of the initial and final states cannot solely explain this complex phenomenon. We have to consider carefully each of the intermediate states with special attention to the environment and its modifications. This raises several questions: Why do peripheral ganglia form in particular areas of the embryo? How do they form? What prevents crest cells from migrating in certain territories? Do the surrounding tissues control crest cell migration and, if so, how? What are the morphological cues that direct crest cell migration and arrest? How do the different cell lineages segregate?

Several hypotheses have been proposed to account partially for the precision with which crest cells reach their target sites. These include chemical repulsion from the neural tube or between crest cells themselves [2,3], contact inhibition of movement [4], contact guidance [5,6], haptotaxis [7], chemotaxis [8], and galvanotropism [9].

Here we examine the formation of most of the ganglia of the peripheral nervous system with a particular emphasis on morphogenetic processes and on adhesive properties of crest cells.

FORMATION OF THE SPINAL GANGLIA

The spinal or dorsal root ganglia (DRG) are present at levels posterior to the fifth somite. They lie along the side of the neural tube, among the sclerotomal cells that will differentiate into components of the vertebrae. This position proximate to the dorsal side of the neural tube suggested to early investigators that DRG derive from the neural crest [10]. Previous studies using neural crest markers proposed that crest cells invade the sclerotome but could not simply explain how and why they aggregate into sensory ganglia [7,11,12].

We have investigated this problem using markers of 1) extracellular matrix, 2) cell to cell adhesion, and 3) crest cells themselves. These markers are 1) fibronectin (FN), a glycoprotein that greatly enhances cell spreading, cell adhesion, and cell movement [for a review see 13]; 2) the neural cell-adhesion molecule (NCAM), an integral membrane glycoprotein that mediates the cell to cell adhesion in the nervous system [14]; and 3) the nucleolar marker for quail cells in chimaeric embryos [15] and NC-1, a monoclonal antibody that stains crest cells themselves and most of the neural tissues [16,17]. Using these tools, we have been able to reconstruct step by step the formation of most of the ganglia of the peripheral nervous system.

Soon after their dissociation from the neural epithelium, crest cells encounter an extracellular matrix between the ectoderm and the neural tube (Fig. 1a). This matrix is composed of a meshwork of fibers [4,6] of FN [18–21], collagen types I and III [22], and hyaluronic acid [23] and provides a substratum particularly suitable for migration (see below). Interestingly, this substratum lies between the basement membranes of the epithelia. The part of the basement membrane close to the epithe-

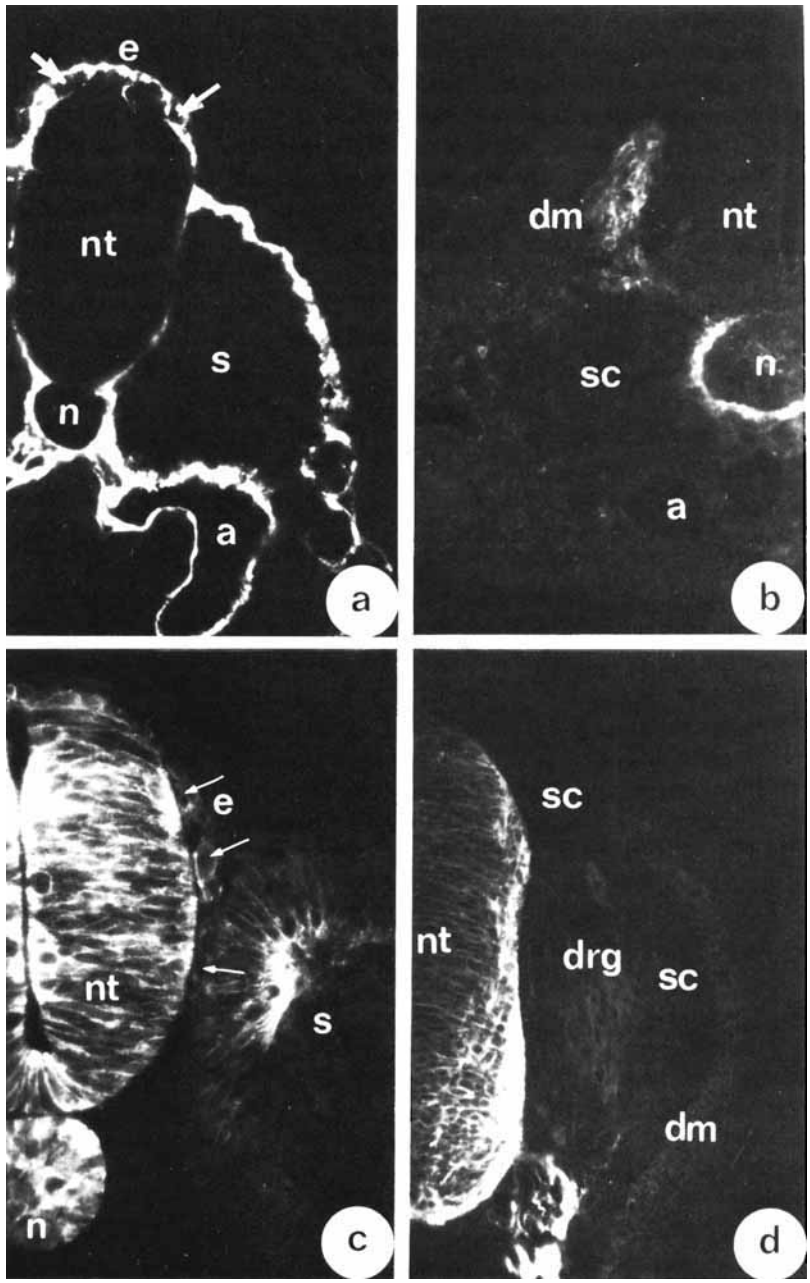

Fig. 1. Formation of the spinal ganglia. a) Immunolabeling for FN of an 18-somite embryo at the fifteenth somite level. FN is present in the basement membranes of the epithelia. Crest cells (arrow) start migrating in an FN-meshwork between the ectoderm and the neural tube. b) Immunolabeling of crest cells with NC-1 of a 32-somite embryo at the fifteenth somite level. Crest cells are only found in a pouch formed by the sclerotome, dermomyotome, and neural tube. c) Immunolabeling for NCAM of a 22-somite embryo at the fifteenth somite level. Though migrating, crest cells (arrows) still express NCAM on their surface. Note, however, that the pioneer crest cells are less stained, suggesting that they gradually lose NCAM. d) Immunolabeling for NCAM of an embryo at stage 23 of Hamburger and Hamilton [58]. The DRG rudiment is hardly stained as compared to the neural tube and motor nerves (compare with the staining of sympathetic ganglia at the same stage, Fig. 2d). a, aorta; dm, dermomyotome; drg, dorsal root ganglion; e, ectoderm; n, notochord; nt, neural tube; s, somite; sc, sclerotome.

lium, the basal lamina, contains laminin [22]. It is noteworthy that, as opposed to the organization of tissues in adults, such a configuration is predominant. Embryonic tissues are mainly organized in epithelial sheets, thus providing many potential pathways for crest cell migration.

After crest cells reach the somite, they begin to migrate as a dense population between the neural tube and the somite. While they progress ventrally, the somite starts to dissociate into the dermomyotome and sclerotome. The latter constitutes a dense mesenchyme that expands either by cell proliferation or by physical pressure [24] towards the notochord and neural tube. As a consequence, the potential pathway along the ventral side of the neural tube becomes obliterated. They accumulate along the lateral border of the neural tube (Fig. 1b), particularly at the level of the anterior part of the somite [our unpublished results], and progressively aggregate into a compact ganglion [see also 25]. Later on, sclerotomal cells will move dorsally and separate the DRG from the neural tube. It should be noted that some crest cells escape and move under the basement membrane of the dermomyotome [16,17]. These crest cells contribute to the Schwann cells of the sensory and motor roots [26, unpublished results].

Concomitant with the formation of the DRG, the extracellular matrix is considerably modified: FN and collagens disappear [21,22] and hyaluronic acid is partly replaced by chondroitin sulfate [23]. It has been proposed that this change in the glycosaminoglycan content [23] or that the disappearance of FN [21] could be responsible for the aggregation of crest cells. However, we cannot determine if the transformation of the matrix is a consequence or a cause of crest cell arrest.

It thus seems that the formation of the spinal ganglion is a consequence of the inability of crest cells to move further forward and backward due to the lack of available space and also of suitable substratum. In this respect, it was interesting to study the distribution of NCAM during the formation of the spinal ganglia. Prior to their migration, presumptive crest cells are associated with the neural epithelium and express NCAM on their surfaces. Surprisingly, the immunofluorescent staining for NCAM does not disappear from crest cells as soon as they detach from the neural tube (Fig. 1c). It should be noted however that the complete disappearance of NCAM from the surface of a cell is not required to get the complete dissociation of the cell from the others—a limited decrease of the number of NCAM molecules may be sufficient to induce a dramatic decrease in cell-cell adhesion [27]. As crest cells continue their migration towards the sclerotome, the staining for NCAM gradually disappears. During the accumulation of crest cells into DRG primordium, NCAM is not expressed significantly; its amount increases again at the time of neuronal differentiation (Fig. 1d), but it remains low compared to the neural tube, motor nerves, and autonomic ganglia (compare with Figs. 2d and 3e). Thus it seems that the compaction of crest cells into spinal ganglia is not rigorously correlated with the appearance of NCAM but rather to the disappearance of pathways of migration.

FORMATION OF THE SYMPATHETIC GANGLIA

The sympathetic ganglia are located in a mesenchyme under the sclerotome near the aorta. The grafting of tritiated thymidine-labeled neural tubes definitively confirmed the crest origin of these ganglia [11]. However, the way crest cells reach the

aorta remained obscure and it was generally accepted that they penetrate through the sclerotome to become located to the ventral side of the embryo [7,11,28]. With this hypothesis, it was necessary to explain the segregation of the sensory and autonomic ganglia as they pass through the sclerotome.

We have investigated the possible presence of crest cells in the extracellular matrix of the intersomitic space. This space is filled partly by the intersomitic arteries derived from the aorta and by an FN-rich matrix between the somites (Fig. 2a). Crest cells rapidly invade this area and within a few hours reach the aorta as a continuous sheet (Fig. 2b,c). Since at this stage the aorta lies dorsal to the endoderm, crest cells cannot migrate further ventrally and spread in a rostral and caudal direction along the aorta. These cells will provide the sympathetic ganglia and the aortic plexuses. Moreover, the aorta is surrounded by an important meshwork of FN, which has been proposed as favoring the adrenergic differentiation of crest cells [29]. The intersomitic pathway is very transient due to the rapid expansion of the sclerotomes and their fusion into the vertebrae. Consequently, sympathetic precursors are precociously separated from the neural tube and the DRG rudiments.

Even though the final localization of crest cells along the aorta can be explained, it is difficult to understand the rapid aggregation of some of them into sympathetic ganglia, because, unlike the situation in the spinal ganglia, crest cells are not locked in a pouch that induces them to aggregate. Furthermore, the behavioral differences between sympathetic precursors aggregating rapidly and plexus precursors remaining for a long period as isolated cells cannot be explained from our data. Nevertheless, we have observed that some crest cells express NCAM very rapidly (Fig. 2d) and form small clusters in contrast to their neighbors, which spread along the aorta and whose NCAM expression is considerably delayed. The disappearance of the extracellular matrix cannot be responsible for the aggregation of crest cells, since the aortic region remains for a long time one of the areas richest in FN in the embryo. It should be noted however that FN and collagens become excluded from the ganglion cell mass as it differentiates [21,22]. Another as yet unrecognized matrix component might possibly influence the motility of crest cells. It seems rather that the aggregation of crest cells into sympathetic ganglia is a secondary event not directly related to their arrest. The de novo expression of NCAM by rapidly dividing cells is likely to be responsible for the formation of the sympathetic ganglia. In this respect, it is interesting to note that in vitro dissociated sympathetic neurons exhibit a strong tendency to move and reaggregate into small ganglia [unpublished results]. One should also remember that crest cells continue to proliferate actively while they already express neuronal markers such as the enzymes of catecholamine metabolism [30].

Finally, it is noteworthy that sympathetic cells undergo at 7 days a second migration, which leads them more laterally [31]. This phenomenon is not yet understood but it probably involved a mechanism different from that of the initial formation of the sympathetic ganglia, since it concerns mature neurons themselves.

The case of the superior cervical ganglia, the most rostral sympathetic ganglion, is much more complex. This ganglion can be considered as a polyganglion: It spreads along several somites and crest cells from the level of the first five somites contribute to its formation [32]. Moreover, regional differences in neural connectivity have been described in the superior cervical ganglion of both the rat [33] and the chick [34].

Neither the formation of this ganglion nor the pathways of migration of the crest cells from which it derives are yet well understood; it seems probable that it is

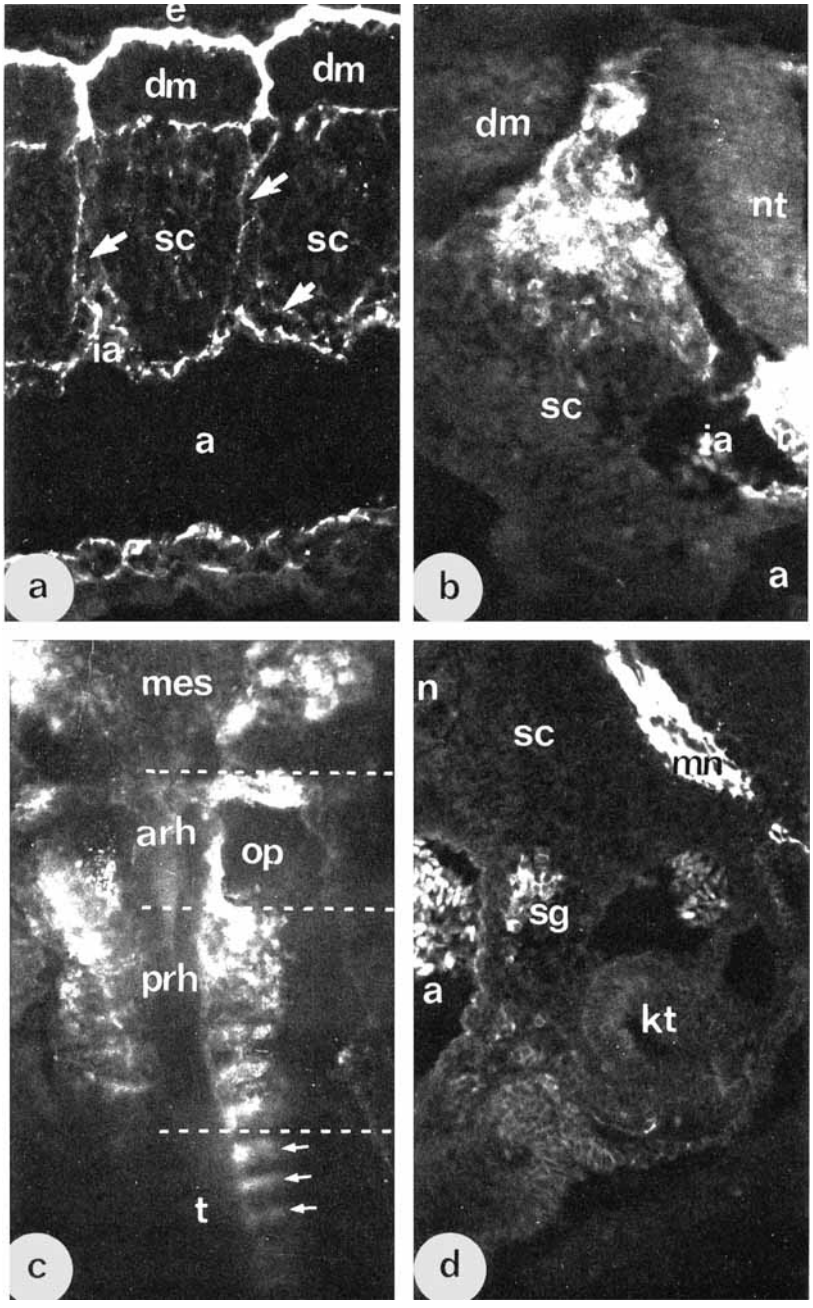

Fig. 2. Formation of the sympathetic ganglia. a) Sagital section of a 28-somite embryo stained for FN and showing crest cells in an FN meshwork along the intersomitic arteries. b) Transverse section of a 28-somite embryo stained with NC-1 at an intersomitic level. Crest cells occupy most of the area along the neural tube from the dorsal aspect of the embryo to the intersomitic arteries (compare with Fig. 1b). c) Whole mount of an 18-somite embryo stained with NC-1. The ectoderm has been peeled off over the head and on the right side of the trunk. At cephalic levels, crest cells migrate as a broad flux under the ectoderm except in the otic placode area. At trunk levels, crest cell fluxes are thin and located in a repeated manner between the somites (arrows). d) Transverse section of a stage 23 embryo stained with NCAM. The sympathetic primordium along the aorta is intensely stained. a, aorta; arh, anterior rhombencephalon; dm, dermomyotome; e, ectoderm; ia, intersomitic artery; kt, kidney tubule; mes, mesencephalon; mn, motor nerve; n, notochord; nt, neural tube; op, otic placode; prh, posterior rhombencephalon; sc, sclerotome; sg, sympathetic ganglion.

composed of initially independent sympathetic ganglia which fuse together during the large and complex morphogenetic movements in the head and neck (especially flexion and curvature). The study of its genesis is further complicated by the presence at this level of numerous ganglia and connective tissues deriving from the neural crest [32,35–38] particularly the enteric precursors. We do not yet know if the superior cervical ganglion shares common precursors with the enteric ganglia (see below) or if it forms by the same processes as the trunk sympathetic ganglia.

FORMATION OF THE PARASYMPATHETIC GANGLIA IN THE GUT

The enteric ganglia are distributed along the entire digestive tract. They are dispersed as a single or double ring of plexuses around the endoderm and among the mesenchymal cells that differentiate mainly into smooth muscles. Although found along the entire gut, the enteric ganglia originate from a precise region of the neural axis: from the vagal region extending from the first to the seventh somite and to a lesser extent from the caudal region [37,39] but not from the trunk region as was formerly thought [40,41]. The precise timing of the gut colonization and neuronal differentiation has been established using chimaeric embryos [39] and grafts of pieces of gut to the chorioallantoic membrane [42]. The colonization of the gut by crest cells is the longest migratory process in the embryo (from day 2 to day 9 of incubation). Here, again, the initial position of the crest cells and the final localization of the enteric ganglia raise questions regarding their formation. Many pathways and mechanisms might be proposed to account for their localization.

The initial steps of crest cell migration at the vagal level take place in an extracellular matrix between the basement membranes of the ectoderm and dermomyotome and between the sclerotome and the dermomyotome [38] leading them to the border of the somatosplanchnopleural epithelium (Fig. 3a). No extensive ventral migration was observed, correlating with the early dissociation of the somite into sclerotome and dermomyotome [21]. At the border of the somatopleure crest cells divide into two streams. One migrates between the ectoderm and the somatopleural epithelium and the other on the side of the splanchnopleural epithelium facing the endoderm. The enteric ganglia derive from the crest cells that take the latter pathway. These crest cells accumulate between the endoderm and the splanchnopleural epithelium. When the dorsal border of the splanchnopleural epithelium starts to delaminate into a thin epithelium and a loose mesenchyme, crest cells penetrate this mesenchyme and migrate caudally, remaining dorsal to the endoderm (Fig. 3b). Later on, crest cells may migrate ventrally as soon as the delayed delamination of the ventral border of the splanchnopleure occurs. During this invasion of the anterior part of the gut, crest cells mostly stay along the splanchnopleural epithelium as a one-cell-thick confluent layer.

In the foregut, the migration of crest cells approximately follows the closure of the gut. As they move, they are delayed by the formation of evaginations of the endoderm (hepatic, biliary, and pancreatic buds). Caudal to the yolk stalk, crest cells encounter the differentiating smooth muscle layer, which they do not seem able to invade. They are found on both sides of the muscle, trapped between the serosa and the circular muscle layer but rather dispersed and far less numerous between the muscle and the endoderm (Fig. 3d). The presence of this muscle layer might account for the formation of two concentric plexuses rather than a secondary migration through muscle cells toward the endoderm.

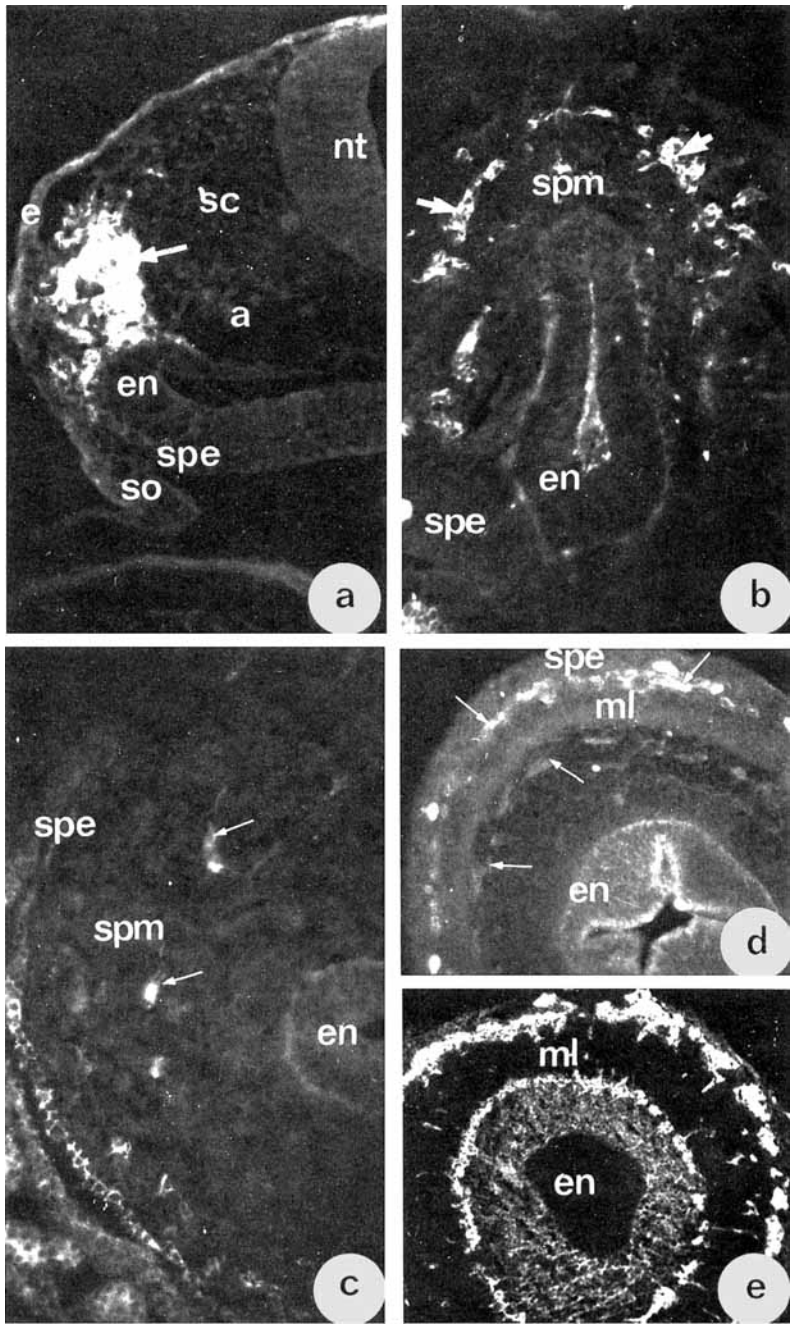

Fig. 3. Formation of the enteric ganglia. a) Migration of crest cells stained with NC-1 at the first somite level of a 22-somite embryo. Crest cells are accumulated on the lateral border of the endoderm. b) Staining of a 36-somite embryo at the level of the developing gizzard reveals neural crest cells (arrows) lining the sinuous border of the splanchnopleural epithelium in its delamination process. c) At 5 days of incubation, crest cells are NCAM positive and start growing axons (arrows) in the proventriculus. d) At 7 days of incubation, enteric precursors (arrows) have passed the umbilicus and the caeca. NC-1 staining shows precursors of the external plexus trapped between the developing longitudinal muscle layer and the well developed circular one. Crest cells belonging to the apparent internal edge of migration are less heavily stained. Note that they are found close to the circular layer and not along the side of the endoderm. e) 11-day-old embryo labelled for NCAM. The two plexuses in the rectum and their interconnecting fibers stain brightly. a, aorta; e, ectoderm; en, endoderm; ml, smooth muscle layer; nt, neural tube; so, somatopleure; spe, splanchnopleural epithelium; spm, splanchnopleural mesoderm.

As assessed by the presence of NCAM on their surface, crest cells aggregate immediately behind the leading edge of migration and they even start growing neurites (Fig. 3c,e). It therefore seems that the front of migration of crest cells divides intensely and is mainly responsible for the progression of crest cells. As was the case for the sympathetic ganglia, there appears no obvious reason for the crest cell arrest since the extracellular matrix does not disappear and no morphological barriers are interposed ahead of them. The appearance of NCAM might be due to the presence of mesenchymal components that induce a rapid differentiation of the crest cells and their arrest. The de novo synthesis of NCAM, concomitant with rapid cell proliferation, might further induce the formation of small ganglia.

We know very little about the pathways and the contribution of caudal crest cells to the innervation of the gut. However, their contribution to the intrinsic innervation of the gut is very limited [42,43]. They participate mostly in the formation of the ganglion of Remak located in the dorsal mesentery.

FORMATION OF THE SENSORY GANGLIA IN THE HEAD

The formation of the ganglia along the cranial nerves involves the neural crest and in some cases the ectodermal placodes. The use of radioisotopic labeling and of the quail chick chimaeras has indicated the relative contribution of the crest and placodes in the complex formation of these ganglia [35,36,44,45]. In all ganglia, the glial cells are derived from the neural crest. In some (geniculate, petrosal, and nodose), the neurons have a placodal origin; in others (jugular and superior), the crest gives rise to both the neuronal and glial populations. Finally, in the trigeminal ganglion both crest and placode cells form neurons.

Let us consider only the migration and aggregation aspect of the formation of these cranial ganglia. The pathways of migration are well documented for those ganglia located close to the neural tube.

The trigeminal ganglion derives from crest cells arising in the anterior rhombencephalon and migrating along the basement membrane of the neural tube. When they encounter the anterior cardinal vein and the cephalic mesenchyme, they accumulate (Fig. 4a) and rapidly aggregate into a ganglion rudiment (Fig. 4b).

The other sensory ganglia that appear in the hindbrain can be divided into two groups according to their final location: the proximal and the distal ones. They both are derived from crest cells migrating out of the posterior rhombencephalon caudal to the otic placodes. Though most of the crest cells at this level migrate laterally under the ectoderm, some of them remain close to the dorsal aspect of the neural tube (Fig. 5a) and differentiate into the proximal ganglia (jugular and superior ganglia). The distal ganglia, ie., the geniculate, petrosal, and nodose ganglia, appear laterally at the site of thickenings of the ectoderm (the ectodermal placodes) and derive from crest cells migrating between the ectoderm and dermomyotome (Fig. 5a). Finally, the outgrowing of the cranial nerves that link the distal ganglia to the proximal ones may utilize crest cells that have migrated under the dermomyotome for their directional cues (Fig. 5b; see also above).

Although the aggregation into the proximal ganglia and also into the tregeminal ganglion can be explained by the same morphological influences as the spinal ganglia (the cephalic mesenchyme and the cardinal vein playing the same role as the sclerotome and NCAM being expressed after the aggregation into ganglion), we do not yet

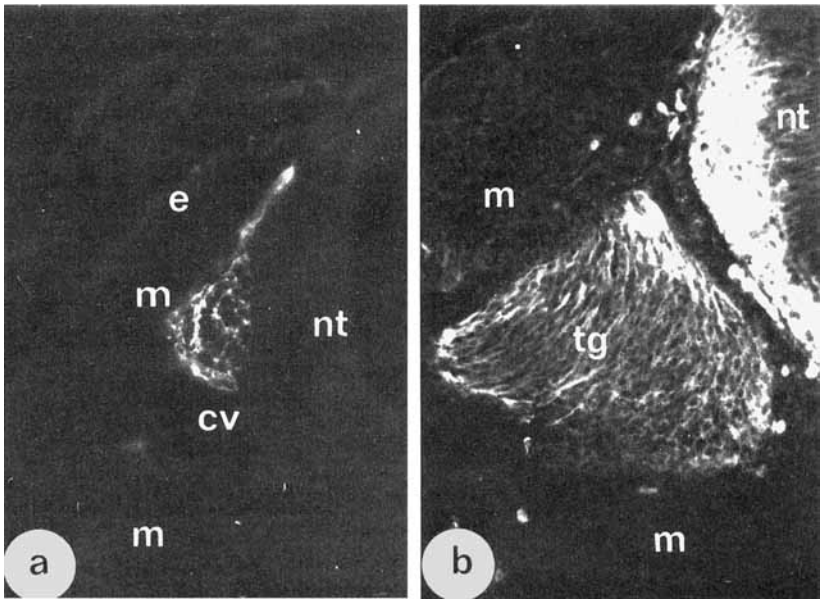

Fig. 4. Formation of the trigeminal ganglion. a) Staining with NC-1 of a 22-somite embryo at the anterior rhombencephalon level. Crest cells are trapped on the border of the anterior cardinal vein, between the neural tube and the cephalic mesenchyme. b) NCAM staining of a stage 21 embryo showing the well developed trigeminal ganglion among the cephalic mesenchyme. cv, cardinal vein; e, ectoderm; m, mesenchyme; nt, neural tube; tg, trigeminal ganglion.

understand how the distal ones form. Indeed the crest cells from which they derive are intermingled with mesenchymal precursors of the pharyngeal pouches, which are able to move further ventrally. Interestingly, the thickening ectodermal placodes express NCAM very early, along with some of the crest cells located very near to them (Fig. 5c,d). One might imagine that a mechanism of specific recognition via NCAM is responsible for the arrest of some crest cells and induces their integration into the placode.

FORMATION OF THE CILIARY GANGLION

Except for numerous autonomic plexuses (the autonomic paraganglia), the ciliary ganglion is the only autonomic ganglion in the head. It is located behind the optic vesicule near the optic nerve and innervates the muscles of the iris and the choroid. The crest cells from which it derives originate from the mesencephalon [46].

The mechanism of formation of this ganglion is unclear; its precursor cells are part of a dense crest population, which also will give rise to connective tissues of the face. During migration, the ciliary precursor cells may be segregated from the

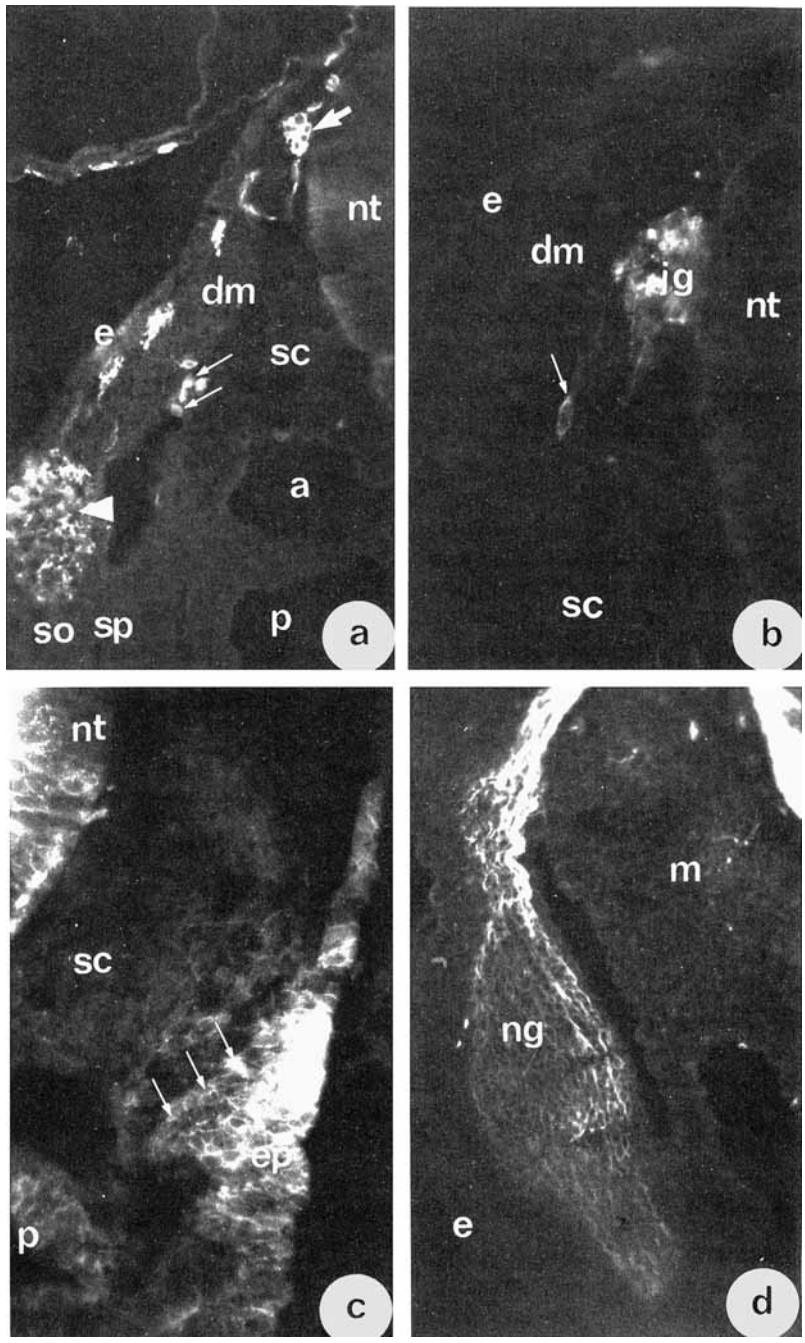

Fig. 5. Formation of the cephalic sensory ganglia. a) Immunolabeling for NC-1 of a 25-somite embryo at the level of the second somite. Crest cells are located mainly in three different places: above the sclerotome, trapped between the dermomyotome and the neural tube (short arrow), dispersed under the dermomyotome (thin arrows), and laterally along side the ectoderm. b) Immunolabeling for NC-1 of a 36-somite embryo at the level of the third somite. The developing jugular ganglion is apparent together with a glial cell accompanying a small neurite that expands from the ganglion. c) In a 28-somite stage embryo, NCAM stains the thickening ectodermal placode and numerous crest cells (arrows) close to it. d) At stage 21, the developed nodose ganglion is heavily stained for NCAM. a, aorta; dm, dermomyotome; e, ectoderm; ep, ectodermal placode; jg, jugular ganglion; m, mesenchyme; ng, nodose ganglion; nt, neural tube; p, pharynx; sc, sclerotome; so, somatopleure; sp, splanchnopleure.

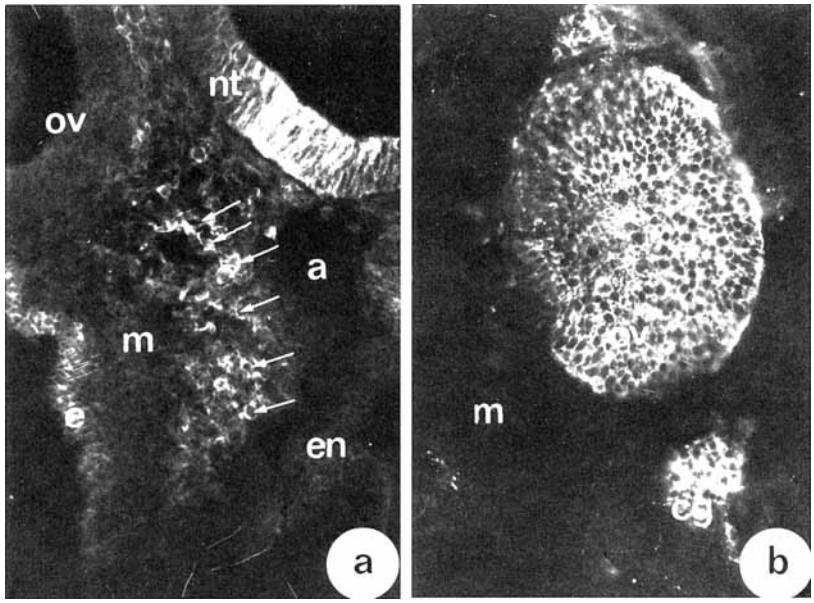

Fig. 6. Formation of the ciliary ganglion. a) A frontal section of a 22-somite embryo stained for NCAM reveals numerous stained cells along the aorta near the optic vesicule. b) NC-1 staining of a 25-somite embryo. Transverse section through the edge of the optic vesicule. The latter reacts strongly with the antibody and so does the developing ciliary ganglion. a, aorta; cg, ciliary ganglion; e, ectoderm; en, endoderm; m, cephalic mesenchyme; nt, neural tube; ov, optic vesicule.

mesectodermal cells both in vivo and in vitro [47–49], but no local segregation of these cell lineages has been detected except for the capacity of pioneer crest cells to produce FN. It has been suggested that crest cells synthesizing FN are the mesectodermal cell precursors that precociously express a mesectodermal property [18], but they may simply reflect a transient physiological property because migrating crest cells encounter a large space filled only with hyaluronic acid [50].

The routes of migration and the exact site of arrest of the ciliary ganglion precursor cells are not known. It has been proposed that these precursors migrate along growing nerves in the head [5]. However, using NCAM immunolabeling, we did not see any staining of growing neurites in the mesencephalon at the stage of crest cell migration. We have, however, detected some NC-1–positive cells exhibiting NCAM, which migrated as a dispersed population along the aorta towards the optic vesicule. These cells could be the ciliary precursor cells exhibiting early a neuronal marker (Fig. 6a,b). The mechanisms of arrest and aggregation of these cells into a ganglion are unknown but the morphology of the eye area suggests that they are similar to those of the other autonomic ganglia.

AN IN VITRO ASSAY FOR STUDYING CREST CELL MIGRATION AND AGGREGATION

We have developed in vitro cultures of crest cells that mimic the in vivo conditions of crest cell migration. When cultured on defined substrates, neural tube explants excised before crest cell emigration produce a halo of cells originating from the dorsal border of the neural tube. In the absence of FN, either in the medium or on the substratum, few crest cells emigrate from the neural tube. Collagen type I and laminin [51] along with hyaluronic acid [28] appear to be poor substrates for migration. Moreover, in vitro binding assays have shown that crest cells do not adhere efficiently to laminin and collagen. On the contrary, FN or FN-containing fibers deposited by fibroblasts are excellent substrates for crest cell adhesion and migration. When confronted with alternating stripes of FN and laminin or glass, crest cells preferentially migrate on FN whereas they aggregate on laminin and do not spread on glass. Finally, crest cells cultured for several days seemed to develop a higher affinity for laminin and progressively decreased their preference for FN.

Using time-lapse cinematography, we have found that the behaviors of crest cells differ according to their position relative to the entire population. Pioneer crest cells at the front of migration exhibit a random migration [2,3]. In contrast, crest cells located within the cell mass maintain a persistent direction of movement. The presence of surrounding cells in a limited environment apparently forces them to move in a straight line.

The role of FN has recently been defined through perturbation experiments. Monovalent antibodies to the cell-binding site of FN or peptides identical to sequence of the cell-binding domain when injected in crest pathways strongly inhibit crest cell displacement. These results demonstrate unequivocally that a direct interaction between the cell-binding site of FN and crest cells is an absolute requirement for their migration [52].

By varying the substrate of migration of crest cells, we obtained the formation of two- and three-dimensional clusters of cells. These clusters quickly exhibit NCAM. An in vitro microaggregation assay revealed that crest cells, according to their dissociation conditions, express either a calcium-dependant or a calcium-independant mechanism of aggregation. Furthermore, anti-NCAM monovalent antibodies strongly inhibit the calcium-independant aggregation of neural crest cells [53].

CONCLUSION

Our study demonstrates that the different ganglia of the peripheral nervous system are derived from crest cells that exhibit distinct patterns of migration and aggregation. However, the initial steps of migration share common properties characterized by several main features: 1) The substratum of migration is acellular and fibrous. Fibronectin, one of its main components, is necessary for transient cell adhesion and movement of crest cells; hyaluronic acid provides space, and collagens maintain the architecture of the tissues. 2) The pathways are organized as channels limited by the basement membranes of the epithelia and are dependent on the morphology of the tissues. 3) Crest cells move in these pathways as a dense cell mass, providing a unidirectional migration.

During the last steps of migration, the behavior of crest cells may vary according to the type of derivatives they give rise to. DRG and proximal ganglion precursors whose duration of migration is short keep a constant pattern of migration. On the contrary, autonomic precursors always begin migrating as a compact population and finish as a dispersed one. This is particularly true for the ciliary and enteric precursors, which migrate even in a cellular environment.

Furthermore, there is no strict correlation between the expression of NCAM and the migratory properties of autonomic precursors: NCAM is expressed by ciliary precursors while they migrate and enteric and sympathetic precursors exhibit NCAM concomitant with their aggregation.

The aggregation follows two types of mechanisms according to the fate of crest cells: 1) The spinal ganglia and the proximal ganglia in the head form as a consequence of the arrest of crest cells. This arrest is due to the presence of morphological barriers followed by the disappearance of substrate and available space for migration. Adhesive molecules are not involved in this process but appear at a low level on the differentiating neurons. 2) In the case of the autonomic ganglia and of the distal ganglia, NCAM appears to be required for crest cell aggregation because no obvious physical barriers are present.

Many aspects of crest migration remain to be elucidated. We cannot yet explain why crest cells sometimes do not utilize some pathways that exhibit, from our point of view, good criteria for cell migration; nor can we understand the mechanisms that trigger crest cell emigration from the neural tube.

Finally, our results, along with previous studies [54–57], suggest that some formerly proposed mechanisms such as chemical repulsion, chemotaxis, and haptotaxis are not likely to be involved in the migratory process of crest cells. However, we cannot exclude a possible local influence of contact inhibition of movement, contact guidance, or electric fields.

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