Spatial and temporal distribution of vinculin and talin in migrating avian neural crest cells and their derivatives

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Summary

Neural crest cells express different adhesion modes at each phase of their development starting with their separation from the neural tube, followed by migration along definite pathways throughout the embryo, and finally to settlement and differentiation in elected embryonic regions. In order to determine possible changes in the cytoskeleton organization and function during these processes, we have studied the in situ distribution of two major cytoskeleton-associated elements involved in the membrane anchorage of actin microfilaments, i.e. vinculin and talin, during the ontogeny of the neural crest and its derivatives in the avian embryo. Prior to emigration, neural crest cells exhibited both vinculin and talin at levels similar to the neighbouring neural epithelial cells, and this expression apparently did not change as cells became endowed with migratory properties. However, vinculin became selectively enhanced in neural crest cells as they further migrated towards their final destination. This increase in vinculin amount was particularly striking in vagal and truncal neural crest cells entering cellular environments, such as the sclerotome and the gut mesenchyme. Talin was also expressed by neural crest cells but, in contrast to vinculin, staining was not conspicuous compared to neighbouring mesenchymal cells. High levels of vinculin persisted throughout embryogenesis in almost all neural derivatives of the neural crest, including the autonomous and sensory ganglia and Schwann cells along the peripheral nerves. In contrast, the non-neural derivatives of the neural crest rapidly lost their prominent vinculin staining after migration. The pattern of talin in the progeny of the neural crest was complex and varied with the cell types: for example, some cranial sensory ganglia expressed high amounts of the molecule whereas autonomic ganglia were nearly devoid of it. Our results suggest that (i) vinculin and talin may follow independent regulatory patterns within the same cell population, (ii) the level of expression of vinculin and talin in neural crest cells may be consistent with the rapid, constant modulations of their adhesive properties, and (iii) the expression patterns of the two molecules may also be correlated with the genesis of the peripheral nervous system.

Key words: neural crest; peripheral nervous system; adherens junctions; vinculin; talin.

Introduction

The neural crest exemplifies the mechanism of rapid modulation of cell adhesion during morphogenesis, and also provides a powerful paradigm for cell migration and cell differentiation (for reviews, see Le Douarin, 1982; Thiery et al. 1985, 1989). The development of the neural crest is a multistep process where cells may be found alternatively in a cohesive or a dispersed state. It starts with the loss of the epithelial arrangement of premigratory crest cells at the apex of the closing neural tube, followed by extensive migration along well characterized pathways through adjacent structures. After reaching their sites of arrest in various areas of the embryo, neural crest cells often coalesce into compact cell collectives and terminate differentiation into a large variety of cell types ranging from neurons and glial cells of the peripheral nervous system to connective tissues in the head and neck.

With the recent advances in the understanding of the mechanism of cell adhesion, it has been possible to determine in greater details how neural crest cells interact with their neighbours or with their immediate environment throughout their ontogeny. Thus, it has been found that surface components involved in cell adhesion are spatially and temporally coordinated in their expression during the various steps of neural crest development. In vivo, neural crest cells lose their intercellular contacts mediated by the cell-adhesion molecule A-CAM (or N-cadherin), and to a certain extent N-CAM, as they emigrate from the neural tube. Conversely, aggregation of neural crest cells into primordia of the peripheral nervous system is correlated with the reappearance of these cell-adhesion molecules.
on the cells' surface (Duband et al. 1985, 1988a; Lallier and Bronner-Fraser, 1988). These modulations in cell-cell interactions are also accompanied by profound changes in the organization and composition of the extracellular matrix in direct contact with neural crest cells (Derby, 1978; Thiery et al. 1982; Krotoski et al. 1986; Rogers et al. 1986; Sternberg and Kimber, 1986; Duband and Thiery, 1987). In particular, fibronectin present in the neural crest environment during their migration is replaced by laminin once they form clusters.

The role of adhesion molecules in the control of neural crest cell behavior has been further investigated in vitro. More specifically, numerous studies have focussed on the mode of interaction of these cells with fibronectin molecules during their migration, and led to the conclusion that, at least in vitro, neural crest cells interact with fibronectin through an RGDS-dependent mechanism involving integrins (for a review, see Duband et al. 1988b).

Yet, while most of these studies have dealt with expression regulation of surface components in connection with changes in the adhesive properties of neural crest cells, little is known about the possible influences of these modulations on the organization and function of the cytoskeleton. Most importantly, adhesion molecules are potential ligands for cytoskeletal components through their cytoplasmic domains, a process necessary for their function (Horwitz et al. 1986; Pollerberg et al. 1986; Volk and Geiger, 1986a,b; Nagafuchi and Takeichi, 1988), indicating that the adhesive behavior of a cell may be controlled not only by surface and extracellular molecules but also by the organization of its cytoskeleton. Because of their specific location at the sites of anchorage of actin microfilaments to the membrane (see for reviews Geiger et al. 1984, 1985; Burnridge, 1986; Burnridge et al. 1988), vinculin and talin are likely to be crucial regulatory elements of the adhesion of cells to their neighbours and to the extracellular matrix. Indeed, purified vinculin has been shown to bind talin which itself binds the cytoplasmic domains of the integrin chains (Burnridge and Mangeat, 1984; Horwitz et al. 1986). Moreover, vinculin injected into live cells is rapidly incorporated into adhesion sites (Burnridge and Feramisco, 1980), and the level of substratum adhesion of cells is directly correlated with the expression of vinculin (Ungar et al. 1986; Bendori et al. 1987).

In the present study, we have examined by in situ immunofluorescence and immunoblotting the distribution patterns of vinculin and talin during development of the neural crest in the avian embryo. It was found that, slightly later after segregation from the neural tube, most neural crest cells exhibited remarkably high levels of vinculin and in a lesser extent of talin compared to the neighbouring tissues. The strong vinculin expression was retained almost permanently in the neural derivatives of the neural crest but not in the mesectodermal tissues and in pigment cells. These observations suggest that vinculin and talin expressions may possibly be connected not only with changes in the adhesiveness of neural crest cells but, most surprisingly, with the process of their differentiation into peripheral ganglia.

**Materials and methods**

**Embryos and immune reagents**

Japanese quail (Coturnix coturnix Japonica) embryos were used throughout the study. Eggs were incubated at 37±1°C and staged according to the number of somite pairs and to the duration of incubation. Rabbit sera directed against chicken gizzard vinculin and talin were kindly provided by Dr B. Geiger (The Weizmann Institute of Science, Rehovot, Israel) and by Dr S. Saga (Nagoya University School of Medicine, Nagoya, Japan), respectively. Vinculin and talin were purified and sera directed against pure fractions of these proteins were prepared and characterized according to Geiger (1979) and Burnridge and Connell (1983). The specificity of the antisera was examined by immunoblot on various embryonic tissues, including quail gizzard, and by immunofluorescence on cultured quail embryo dermal and somitic fibroblasts produced as described in Duband et al. (1986). The staining pattern obtained was in focal contacts which is the typical distribution expected for both molecules (not shown). It should be noted that the antibodies to vinculin are specific for avian vinculin and do not cross-react with vinculin of other species particularly of mammals (see also Bendori et al. 1989). Migrating neural crest cells and their neural derivatives were traced using a mouse monoclonal antibody called NC-1 and characterized previously (Vincent et al. 1983; Vincent and Thiery, 1984, Tucker et al. 1984, 1988).

**Histological sections and immunofluorescence staining**

Embryos were routinely fixed at room temperature in 3.7% formaldehyde in phosphate-buffered saline (PBS) for 1–8 h, depending on the size of the embryos. After several extensive washes in PBS, embryos were embedded in a graduated series of sucrose solutions in PBS (12–18% wt/vol), and frozen in Tissue Tek (Lab-Tek Products) in liquid nitrogen. Sections were cut at 7–12 μm on a cryostat (Bright Instrument Co. Ltd., Huntington, England), and mounted on slides coated with a gelatin solution as in Lohmann et al. (1981). Immunofluorescent staining of sections was performed essentially as described previously (Duband et al. 1986). Generally, for comparison of vinculin and talin stainings, consecutive sections were deposited on two different slides and processed independently for immunofluorescence with either anti-vinculin or anti-talin antibodies. Otherwise, sections and stainings were performed on different embryos at the equivalent developmental stage. Double stainings were performed using as second antibodies tetraethyl rhodamine isothiocyanate- or fluorescein isothiocyanate-conjugated goat antibodies to rabbit immunoglobulins (Nordic, Tilburg, The Netherlands) and biotinylated sheep antibodies to mouse immunoglobulins associated with Texas red- or fluorescein-conjugated streptavidin (Amersham, England). Control stainings were performed using as primary antibodies preimmune sera or non-immune immunoglobulins. No significant staining even in compact tissues was obtained with these control antibodies. Sections were observed with a Leitz Orthoplan epifluorescent microscope.

**Immunoblotting analysis**

Samples of embryonic tissues or cultured cells were briefly homogenized and extracted at 100°C with SDS-sample buffer under reducing conditions. The extracts were clarified by
centrifugation and subjected to SDS-PAGE followed by immunoblotting analysis with antisera to vinculin and talin. PAGE was performed in Laemmli buffer system on slab 7.5% polyacrylamide minigels. Immunoblotting was performed essentially as follows. The protein bands were electrophoretically separated on 7.5% polyacrylamide gels, and then transferred onto a nitrocellulose membrane. The membrane was then incubated with 4% bovine serum albumin solution in PBS for 1 h at 37°C, and then incubated with primary antibody solution for 1 h at 37°C. The membrane was then washed with PBS supplemented with 1% Tween 80 and incubated with 125I-labelled protein A (Amersham) for 1 h at room temperature. After washing, the blot was subjected to autoradiography.

Results

The spatial and temporal distributions of vinculin and talin were examined during the development of the neural crest in the quail embryo using immunofluorescence labeling of cryostat sections as well as using immunoblotting. Particular attention was put on possible variations of the expression of each molecule in correlation with modulation of intercellular interactions and with cell differentiation.

Trunk neural crest and their derivatives

In the avian embryo, trunk neural crest cells start to emigrate from the neural tube a few hours following its complete closure and separation from the ectoderm. At this stage, vinculin exhibited a bright staining throughout the neural tube, including premigratory neural crest cells, whereas talin was found at low levels in neural epithelial cells as compared to the neighbouring tissues (not shown). As neural crest cells emigrated from the dorsal aspect of the neural tube, their vinculin content was not apparently different from that of premigratory cells in contrast to talin which was slightly enhanced. The levels of both molecules were, however, comparable to those in the ectoderm and somite. In contrast, as they approached the area between the neural tube and the dissociating somite, vinculin gradually increased in amount among them whereas talin remained at the same level as before (Fig. 1). Vinculin staining became maximal slightly later when cells migrated in the periphery of the sclerotome in the rostral half of the somite and reached the aortic region. Examination of consecutive sections through the somites revealed coincident patterns of vinculin and NC-1 among neural crest cells, thus permitting this cell population to be discriminated from neighbouring sclerotomal cells using vinculin staining only (Fig. 2). Talin staining was also enhanced in neural crest cells approaching the aortic region, yet not sufficiently as compared to the strongly stained sclerotome to allow their unequivocal distinction (not shown).

After migration, most trunk neural crest cells give rise to neural tissues among which the most prominent are the spinal (or dorsal root) ganglia and the sympathetic ganglia. The former appears as a result of the accumulation of part of the neural crest population along the neural tube in the rostral half of the somite while the latter forms along the aorta (Thiery et al. 1982; Rickmann et al. 1985; Bronner-Fraser, 1986; Loring and Erickson, 1987; Teillet et al. 1987; Lallier and Bronner-Fraser, 1988; see also Fig. 2). Vinculin was abundant in all neural crest cells during the whole process leading to formation of the primordia of both ganglia (Figs 3A; 4A). It should be noted that no change in the level of expression of vinculin could be detected among cells during cessation of migration and aggregation. Again, talin immunoreactivity was clearly detectable among aggregating neural crest cells, yet not more intense than in the neighbouring sclerotome (Figs 3B; 4B). As neurones underwent differentiation and grew neurites, vinculin staining declined slightly in both dorsal root and sympathetic ganglia (Figs 3C; 4C). A more pronounced decrease in staining occurred for talin particularly in the sympathetic ganglia (Figs 3D; 4D). A subpopulation of trunk neural crest cells originating in the lumbosacral region provide the ganglion of Remak which is situated in the dorsal mesentery along the hind-gut (see Le Douarin, 1982). This ganglion exhibited the same vinculin and talin patterns.
Fig. 2. Longitudinal sections through brachial somites of a 35-somite stage embryo showing the coincident stainings for vinculin and NC-1 among the neural crest population. (A) Schematic drawing of a 35-somite stage embryo aimed at showing the levels of sections in B–E. (B, C) A section performed medially along side of the neural axis and (D, E) a section situated more laterally through the somite. Both sections are double stained for vinculin (B, D) and NC-1 (C, E). Note in D the strongly stained smooth muscle cells along the aorta. Arrows point at neural crest cells migrating along the intersomitic arteries in between two adjacent somites. Arrowheads show the boundaries of the somite and A and P indicate the anterior (rostral) and posterior (caudal) aspects of the embryo. a, aorta; dm, dermamyotome; lb, limb bud; ov, omphalomesenteric vessels; s, somite; sc, sclerotome; sg, sympathetic ganglion rudiment.
Fig. 3. Formation of the dorsal root ganglia in the brachial region. (A, B) 40-somite embryo and (C, D) 7-day old embryo stained for vinculin and talin, respectively. Vinculin staining is still intense in cells aggregating into the primordium of the sensory ganglion while talin staining is more discrete and does not stand out compared to the sclerotome. Note that crest cells along the emerging motor nerve also exhibit strong vinculin immunoreactivity. Later on, during ganglion differentiation, while talin is apparently unaltered, vinculin decreases notably in the ganglion, but remains, however, brighter than in the surrounding cartilage. drg, dorsal root ganglion; m, myotome; mn, motor nerve; nt, neural tube; sc, sclerotome; T, talin; V, vinculin.
Fig. 4. Formation of the sympathetic ganglia in the brachial region. (A, B) 40-somite embryo and (C, D) 7-day-old embryo stained for vinculin and talin respectively. Neural crest cells that accumulate along the aorta to provide the sympathetic ganglia can be easily noticed by their bright staining for vinculin but not for talin. This intense staining is retained after differentiation though slightly reduced whereas talin is low. a, aorta; c, cartilage of the vertebrae; sc, sclerotome; sg, sympathetic ganglion; T, talin; V, vinculin.

as the sympathetic ganglia during the time course of its development (not shown).

Part of the neural crest population is known to migrate laterally between the ectoderm and the dermomyotome to differentiate into pigment cells. These cells were originally strongly labelled for vinculin as they entered the lateral pathway, but they rapidly lost their staining as they became mingled among the dermis (not shown).

Vagal neural crest cells and parasympathetic enteric ganglia

The parasympathetic ganglia of the gut derive principally from neural crest cells originating from a region termed vagal region which corresponds to the seven most rostral somites (Le Douarin and Teillet, 1973; Allan and Newgreen, 1980; Tucker et al. 1986). In this region, neural crest cells reach the ventral aspect of the embryo mainly using a lateral route between the
ectoderm and the dermamyotome and along the ventral side of the dermamyotome (Thiery et al. 1982; Tucker et al. 1986). These cells gradually exhibited a strong vinculin staining as they reached the somatopleural and splanchnopleural epithelia (Fig. 5A, B). Talin could also be clearly detected in neural crest cells, but at a level apparently lower than vinculin (Fig. 5C). Thereafter, neural crest cells retained their intense vinculin staining during the whole process of migration and differentiation along the digestive tract (Fig. 5D–H).

Cephalic neural crest cells and their derivatives
As already observed for the neural crest in the trunk and in the neck, premigratory and early migratory neural crest cells in the head were not more intensely labeled for both vinculin and talin than the surrounding tissues (not shown). After migration, a large part of the neural crest population colonize the branchial arches in the ventral side of the head and upper neck where they differentiate into connective tissues in the face and in the lower jaw (Noden, 1978, 1984; Le Lièvre, 1978). At this stage, both vinculin and talin stainings decreased rapidly among neural crest cells (data not shown).

Beside non-neural derivatives, cephalic neural crest cells contribute either entirely or partly to neural structures, including the cranial sensory ganglia and the ciliary ganglia (for reviews, see Le Douarin, 1982; D’Amico-Martel and Noden, 1983). Most cranial sensory ganglia exhibited strong stainings for vinculin and talin during the time course of their differentiation. The case of the trigeminal ganglion is illustrated on Fig. 6A–C. This ganglion arises part from neural crest cells that emigrate from the mid-brain region and part from a local thickening of the ectoderm, termed trigeminal placode (D’Amico-Martel and Noden, 1983). As opposed to neural crest cells that originated from the same region and differentiated into mesodermal tissues, neural crest cells that accumulated along the anterior cardinal vein to contribute to the trigeminal ganglion did not lose their vinculin staining, but rather exhibited an increasingly strong labeling (Fig. 6A). Once the primordium of the ganglion was formed and neuronal differentiation was in process, vinculin staining remained intense in most cells and more particularly in those associated with nerves (Fig. 6B). Interestingly, talin immunoreactivity increased notably at these stages in ganglion cells to levels very similar to those of vinculin (Fig. 6C). The vestibulo-acoustic complex, in contrast, was the only cranial sensory ganglion that did not display any intense vinculin–talin staining, except for a few cell dispersed among the mass of the ganglion and for cells stretched along the emerging nerve (Fig. 6D–F). Finally, the ciliary ganglion exhibited a vinculin–talin pattern different from the cranial sensory ganglia. This autonomous ganglion derives exclusively from neural crest cells originating from the mid-brain (Noden, 1978; Narayan and Narayan, 1978). NC-1-positive cells exhibiting intense vinculin staining were seen precociously as small groups or scattered within the head mesenchyme dorsally to the optic cup (Fig. 6G). In contrast to vinculin, talin was not abundant in those cells (not shown). Later on, as neural crest cells assembled into the ciliary ganglion, stainings for vinculin and talin remained unchanged (Fig. 6H, I).

Vinculin and talin expression in Schwann cells
The neural crest are known to be the source of Schwann cells located along the sensory and motor nerves. It was found that both vinculin and talin were abundant within the cytoplasm of these cells both along cranial sensory nerves as they emerge from the neural epithelium (Fig. 7A, B) and along trunk motor and sensory nerves innervating peripheral structures (Fig. 7C, D). As exemplified on Fig. 7C, D, vinculin (but also talin) was present in high amounts only in Schwann cells but not in nerve fibers.

Discussion
During each step of their development, neural crest cells have to reorganize constantly and rapidly their interactions with either their neighbours and/or the extracellular matrix. For example, during migration, neural crest cells that are spread onto a network of fibronectin and collagen fibrils have to detach locally from these and expand lobopodia in order to form new anchorage sites with other fibers. Likewise, during their segregation from the neural epithelium, they have to destroy preexisting intercellular junctions, lose their epithelial polarity, and modify their association with the extracellular matrix in order to irreversibly separate from contiguous cells. Conversely, during cessation of migration, they have either to establish permanent anchorage sites to the substratum or to develop intercellular contacts with the other cells. At the subcellular level, a prerequisite for the occurrence of these various events is the dynamic organization of the cytoskeleton and of its association with the cellular membrane. Therefore, because of their involvement in the membrane anchorage of actin bundles (Burrige and Fermoisci, 1980; Avnur et al. 1983; Burrige and Mangeat, 1984; Horwitz et al. 1986; Unger et al. 1986; Bendori et al. 1987, 1989), vinculin and talin are good candidate for participating to the control of changes of the architecture of the cytoskeleton in neural crest cells and consequently to the control of the adhesive behavior of these cells.

In the present study, we have examined the spatio-temporal distribution of vinculin and talin during the ontogeny of the neural crest and of its derivatives. Our major observations are (i) vinculin and talin do not follow strictly the same regulatory patterns within the neural crest population; (ii) vinculin is found at strikingly high levels in migrating neural crest cells and in most of their cranial and truncal neural derivatives and...
Fig. 5. Migration of vagal neural crest cells along the digestive tract. Formation of enteric ganglia. (A–C) Transverse sections through the 3rd somite of a 28-somite embryo stained for vinculin (A), NC-1 (B), and talin (C). Vinculin staining becomes markedly strong in neural crest cells when they migrate between the foregut endoderm and the splanchnic mesoderm. Talin is also present in neural crest cells as they enter the foregut environment, but the staining is not sufficiently high to permit the discrimination of cells among the splanchnic mesenchyme. (D–F) Transverse sections through the gizzard of a 38-somite embryo stained for vinculin, NC-1 and talin, respectively; (G, H) Transverse section through the proventriculus of a 7-day-old embryo stained for vinculin and NC-1. At the 38-somite stage, neural crest cells have already colonized the gizzard, and can be easily recognized through their vinculin staining. At 7 days of incubation, neural crest cells are regrouped into ganglion clusters around the circular smooth muscle layer in the proventriculus. Note that both smooth muscle cells and neural crest cells exhibit an intense vinculin staining. Arrows point at neural crest cells. a, aorta; en, endoderm; m, splanchnic mesenchyme; spe, splanchnic epithelium; sml, circular smooth muscle layer; T, talin; V, vinculin.
Fig. 6. Formation of various ganglia in the head. (A–C) Trigeminal ganglion in a 25-somite embryo (A) stained for vinculin and in a 4-day-old embryo (B, C) stained for vinculin and talin. Neural crest cells accumulating along the neural tube and the superior cardinal vein to form part of the trigeminal ganglion exhibit a pronounced vinculin staining. Note that the trigeminal placode is also strongly stained for vinculin. By 4 days of development, the ganglion is brightly stained not only for vinculin but also for talin. (D–F) Vestibulo-acoustic ganglion in a 4-day-old embryo stained for vinculin, NC-1 and talin. Only a few cells dispersed among the mass of the ganglion and around its cortex exhibit a high staining for the three molecules. (G–I) Ciliary ganglion in a 32-somite stage embryo stained for vinculin (G) and at 7 days of incubation stained for vinculin and talin (H, I). Both cells regrouping into clusters at the origin of the ciliary ganglion (arrows in G) and cells present in the differentiating ganglion express high levels of vinculin but not of talin. cg, ciliary ganglion; nt, neural tube; oc, optic cup; tg, trigeminal ganglion; tp, trigeminal placode; vag, vestibulo-acoustic ganglion; T, talin; V, vinculin.
Fig. 7. Vinculin and talin in Schwann cells. (A, B) Transverse section at the mid-brain level of a 25-somite stage embryo showing the ventral aspect of the neural epithelium and the immediate connective tissue. Early emerging cranial sensory nerves are associated with Schwann cells (arrows) that exhibit strong vinculin (A) and talin (B) stainings. (C, D) Distal parts of a truncal motor nerve in a 7-day-old embryo stained for vinculin (C) and NC-1 (D). Note that, as shown by the NC-1 staining, neurites are poorly stained for vinculin (thin arrows). ne, neural epithelium; T, talin; V, vinculin.

Fig. 8. Immunoblot with antibodies to vinculin on extracts of spinal cord (line 1), brain (line 2), retina (line 3), trigeminal ganglia (line 4), ciliary ganglia (line 5), and dorsal root ganglia (line 6) from 8-day old embryos. Lines 1'-6' show the corresponding Coomassie-blue stainings. The approximately same amounts of material were loaded in the different lines. All peripheral ganglia exhibit high amounts of vinculin compared to central nervous tissues. Note that no difference in molecular weight can be detected in the form of vinculin found in the various tissues.

(iii) talin is also expressed by migrating neural crest cells, but at levels apparently less remarkable than vinculin. A few neural derivatives, however, showed notably high levels of talin. These results are summarized on Table 1.

The most striking observation of the present study is certainly the extremely high vinculin content of migrating neural crest cells and their neural derivatives. Our previous in vitro studies on the organization of substratum-contact sites in neural crest cells revealed that vinculin distributes mostly in the cytoplasm of cells and incidentally in focal contacts present at the tip of some but not all lobopodia (Duband et al. 1986). This pattern in migratory cells appeared totally different from that in non-motile cells which exhibited the molecule exclusively in focal contacts. Additionally, fibronectin receptors are diffuse and highly mobile in the membrane of motile cells, a characteristic which is again not shared by immobile cells (Duband et al. 1986, 1988c). From these studies and others (see for a review Duband et al. 1988b), it has been proposed that the particular organization and behavior of fibronectin receptors and cytoskeletal elements in migratory neural crest cells is most suitable to permit locomoting cells to establish transient contacts with their substratum. This model is further confirmed by the recent finding that fibronectin recep-
tors are internalized and recycled in motile cells, possibly allowing the preexisting interactions with the extracellular matrix material to be abolished (Duband et al. 1988b; Bretcher, 1989). Thus, the large cytoplasmic pool of vinculin in migrating neural crest cells would possibly serve as a reservoir of molecules for the rapid establishment of prospective adhesion sites with the substratum.

Since the strong vinculin staining is retained after migration on the neural derivatives of neural crest cells, the function of this molecule may not simply be linked to the particular adhesive behavior of these cells, but also to their differentiation. Indeed, autonomic and sensory ganglia of the peripheral nervous system express high amounts of vinculin and, in some cases, of talin, and this characteristic is specific for neural crest-derived ganglia, as assessed by comparison of various cranial peripheral ganglia of neural crest and placodal origins. In addition, the non-neural derivatives of the neural crest were found to lose their high vinculin content readily during differentiation. It is therefore tempting to suggest that vinculin may be involved in the genesis of the peripheral nervous system, and that expression of vinculin in neural crest cells reflects in part their precocious commitment into neural lineages as they are still in the process migration. In this respect, it should be reminded that a monoclonal antibody, called E/C8 and recognizing an avian-specific neurofilament-associated protein of 73×10^3 Mr, differentially stains subpopulations of the neural crest at the time of migration and also central and peripheral neuronal cells, indicating that this monoclonal antibody identifies an early differentiating subpopulation of the neural crest (Ciment and Weston, 1982, 1985; Ciment et al. 1986). The role of vinculin during neurogenesis, if it exists, thus remains to be determined. Interestingly, it has been recently found that vinculin may carry at least two distinct functional domains involved in the binding to various cytoskeletal molecules, including talin, vinculin itself, and possibly α-actinin (Bendori et al. 1989).

Even though vinculin cannot be called properly a marker for neural crest cells, because it is widely distributed in many tissues, the conspicuous labelling of neural crest cells and their neural derivatives by anti-vinculin antibodies make this molecule attractive to follow their migration and early differentiation in vivo. Indeed, the striking migratory behavior of neural crest cells along with the wide variety of tissues deriving from this structure made it necessary to search for specific markers allowing tracing of these cells during migration and differentiation. A number of monoclonal antibodies have been produced that recognize avian neural crest cells and their derivatives (Ciment and Weston, 1982; Vincent and Thiery, 1984; Tucker et al. 1984; Girdlestone and Weston, 1985; Barbu et al. 1986). However, with the exception of NC-1/HNK-1, which was found to stain very precociously almost all neural crest cells (Vincent and Thiery, 1984; Bronner-Fraser, 1986; Teillet et al. 1987), these monoclonal antibodies stain only part of migrating neural crest cells (e.g. E/C8 and GIN1; Ciment and Weston, 1982; Barbu et al. 1986) and/or appear on neural crest cells at the end or after migration (e.g. E/C8; Ciment and Weston, 1982). In addition, most of these monoclonal antibodies cannot be used appropriately on species other than avians because they exhibit different distribution patterns. For example, the NC-1/HNK-1 epitope was not detectable on migrating neural crest cells and their derivatives in amphibians or mice and was found transiently on the neural crest in rats (Tucker et al. 1988; Erickson et al. 1989). Therefore, so far there is no marker available that would stain all neural crest cells in most species during the whole process of their migration. Since vinculin is not restricted to avians, we propose this molecule as a possible tracer to follow the migration of neural crest cells and the genesis of the peripheral

### Table 1. Expression of vinculin and talin in neural crest cells and their major derivatives.

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nervous system not only in avians but also in amphibians and mammals.

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