PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Role of Neurotrophins in Development of Neural-Crest Cells that Become Sensory Ganglia

Chaya Kalcheim

Phil. Trans. R. Soc. Lond. B 1996 **351**, 375-381
doi: 10.1098/rstb.1996.0031

The role of neurotrophins in development of neural-crest cells that become sensory ganglia

CHAYA KALCHEIM

Department of Anatomy and Embryology, Hebrew University of Jerusalem, Hadassah Medical School, Jerusalem 91120-P.O.B 12272, Israel

SUMMARY

A fundamental issue of neural-crest ontogeny is understanding how different types of cells are created at the right time and in the correct numbers. Sensory ganglia are among the many derivatives of the vertebrate neural crest. Their proper formation requires the regulation of several processes such as cell fate specification, proliferation, survival, and terminal differentiation. The timescale of the occurrence of processes involved in the regulation of cell number and identity, coincides with key morphogenetic events such as cell migration, homing and gangliogenesis.

To gain insight into these processes, we characterized the cellular basis of metameric migration of neural-crest cells and of consequent ganglion organization, which are imposed by intrinsic differences within rostral and caudal sclerotomal compartments. We also established a transient requirement for neural tube-derived factors in regulating the proliferation, survival and differentiation of prospective DRG cells. Additionally, we showed that cooperation between the mesodermal cells and the neural tube is necessary for modulating cell number in the nascent ganglia. BDNF, NT-3 and basic FGF were found to mediate this environmental signalling. All the above factors display neurogenic activity for a subset of early-committed sensory neuron progenitors. This observation raises the possibility of an early redundancy in the response of individual neural-crest progenitors to distinct factors. This overlap in responsiveness progressively disappears upon the colonization of specific ganglionic sites and the subsequent establishment of selective innervation patterns by post-mitotic sensory neurons.

1. NEURAL CREST MIGRATION AND THE FORMATION OF METAMERIC DORSAL ROOT GANGLIA

Several studies have unravelled the cellular basis underlying the segmental organization of the peripheral nervous system during embryogenesis. Segmentation of the dorsal root ganglia (DRG), the sympathetic ganglia and the peripheral nerves, stems from differences inherent to the paraxial mesoderm, the somites, into which neural-crest cells and motoneuron fibres migrate after leaving the central nervous system (CNS) primordium (Keynes & Stern 1984; Rickmann *et al*. 1985; Kalcheim & Teillet 1989; Goldstein & Kalcheim 1991). Each somite can be subdivided into rostral and caudal domains by their differential ability to support or inhibit, respectively, the migration of neural progenitors and of motoneuron axons (Stern & Keynes 1987; Layer *et al*. 1988; Davies *et al*. 1990). It is now well established that the metameric formation of the DRG and nerves is imposed by this alternation (Keynes & Stern 1984; Lallier & Bronner-Fraser 1988; Kalcheim & Teillet 1989).

In one study we analysed the segmental origin and migratory behaviour of neural-crest cells that give rise to DRG (Teillet *et al*. 1987). We established that each DRG forming in the rostral half of a somite is colonized by crest cells arising from the tube opposite two consecutive segments i.e. the rostral and caudal halves of the corresponding somite and the caudal half of the somite located immediately rostrad along the axis. The convergence of crest cells arising from regions of the axis opposite inhibitory caudal somitic areas is achieved by a short longitudinal migration of progenitors along the neural tube until they reach mesodermal regions with rostral properties. Opposite these permissive regions, the cells can migrate transversally into the sclerotome and contribute to the colonization of the nascent DRG. Moreover, progenitors arising opposite both somitic halves segregate to different locations within the rostrocaudal extent of each DRG, with a pattern that reflects their segmental origin along the neuraxis (Teillet *et al*. 1987).

The importance of the caudal part of each somite in determining final DRG segmentation is best illustrated by the discovery that facing a mesoderm composed of only rostral somitic halves, DRG are continuous and unsegmented but normally located when compared to the control ganglia (Kalcheim & Teillet 1989; Goldstein *et al*. 1990). In contrast, opposite a caudal type of mesoderm, small DRG are formed that remain dorsally located with respect to the somite.

In addition to the differential roles played by rostral and caudal-half somitic cells in neural development, cells within each domain have also diverging fates when considering the formation of the sclerotome-

Phil. Trans. R. Soc. Lond. B (1996) **351**, 375–381
Printed in Great Britain

derived vertebrae. The morphogenetic capabilities of each somitic half differ. Only the caudal half cells are committed to give rise to the vertebral pedicle, and only the rostral half cells are comitted to give rise to the intervertebral disk (Goldstein & Kalcheim 1992).

The various roles in which cells of rostral and caudal somitic domains are involved, suggest that yet unidentified active molecules must be uniquely expressed in either somitic domain (for a growth cone-collapsing activity enriched in caudal-half somites see, for example, Davies *et al.* 1990). Defining the molecular basis underlying somite polarity, and understanding their involvement in segmental patterning of neural-crest cells, will help elucidate the mechanisms that control segmentation of neural and skeletal structures. The distinctive properties of the two somitic domains that determine DRG segmentation also have a profound influence on the regulation of the number of neural-crest cells at migratory and organogenetic stages (see §2).

2. REGULATION OF CELL NUMBER IN NEURAL-CREST CELLS THAT BECOME DORSAL ROOT GANGLIA

(*a*) *BDNF, NT-3 and basic FGF mediate a neural tube effect on neural-crest development*

(i) In vivo *studies*

The influence of the CNS primordium (the source of the neural-crest cells) on the early development of these progenitors into DRG was shown by depriving migrated neural-crest cells of contact with the CNS. The experimental paradigm consisted of implanting an impermeable membrane between neural tube and crest cells at the level of the DRG anlagen (Kalcheim & Le Douarin 1986). Under these conditions, only those neural-crest cells that were separated from the CNS died along the grafted area, and no DRG developed. These results suggested that contact with the neural tube is absolutely needed during migration and early gangliogenesis. However, this requirement diminishes progressively: implantating the mechanical barriers one day later (E4–4.5), when the onset of peripheral target innervation is underway, only partially affected the DRG, resulting in the formation of distally located ganglia whose size was reduced by 30–50 %. These results showed for the first time that, similar to neurons in already differentiated peripheral ganglia, certain neural-crest progenitors may be programmed to die unless provided with specific molecules. It appears that these molecules do not derive from the somites because despite remaining on the somitic side, the neural-crest cells were still unable to survive if separated from the tube.

We then tested for factors able to rescue neural-crest cells experimentally separated from the neural tube. Various embryonic extracts and purified CNS-derived factors were examined using the same paradigm. Pretreatment of the membranes with laminin or collagen followed by a neural tube extract, BDNF, bFGF or NT-3, before grafting, rescued a significant proportion of separated cells. In contrast, treating the membranes with collagen or laminin alone (with a liver extract, or with NGF) was ineffective (Kalcheim & Le Douarin 1986; Kalcheim *et al.* 1987; Kalcheim 1989; G. Brill & C. Kalcheim, unpublished results). Interestingly, the number of cells surviving in the presence of membranes pre-adsorbed with two factors, such as BDNF and bFGF, was not significantly different to the number observed surviving separately with either factor (Kalcheim 1989). These results indicate the existence of an overlapping activity of the above molecules on neural-crest cells that develop into DRG *in vivo*, an observation subsequently confirmed using *in vitro* paradigms.

(ii) *BDNF, NT-3 and bFGF are neurogenic for a subset of cultured neural-crest cells that develops into sensory neurons*

A necessary complement of our *in vivo* studies was the establishment of cell culture systems that would enable us to define more accurately the identity of the responsive progenitors and the type of activities exerted by those factors previously characterized using '*in vivo*' models. To this end, we used a culture system that consisted of incubating neural-crest cells in a serum-free defined medium (Ziller *et al.* 1987). Two main populations of neural-crest cells developed under these conditions. First, HNK-1-immunoreactive cells with flat and polymorphic appearance, a significant proportion of which was mitotically active during the first two days after plating. These progenitors are probably multipotential as they can give rise to adrenergic cells and melanocytes if provided with serum and chick embryo extract (Ziller *et al.* 1987) as well as to yet unidentified nonneuronal cells. Second, a subset of sensory neurons expressing a variety of markers including neurofilament proteins, tetanus toxin binding sites, the HNK-1 and A2B5 epitopes and Substance P (Ziller *et al.* 1983, 1987). As these neurons were shown to appear as early as 15 h after initial plating from a subset of post-mitotic precursors, they may be considered to be at least restricted, if not fully committed, to differentiate along the sensory neuronal lineage. Indeed, *in vivo* and *in vitro* clonal analysis have revealed that different populations of sensory neurons can arise both from early committed progenitors and from late differentiating multipotent precursors (Ziller *et al.* 1987; Baroffio *et al.* 1988; Bronner-Fraser & Fraser 1988; Sieber-Blum 1989).

We find that, similar to the situation in the embryo, several factors can influence the development of avian neural-crest cells that become sensory neurons in culture. For instance, BDNF, NT-3 and bFGF promote the survival and differentiation of a subpopulation of post-mitotic neural-crest cells that develop in defined medium. Under these conditions, however, a significant effect on neurogenesis is only obtained when the neural-crest cells are associated with somites or other mesodermal cells (Kalcheim & Gendreau 1988; Brill *et al.* 1992; Pinco *et al.* 1993), suggesting these may be instrumental in proper presentation of the proteins to the responding cells (see also §2*a*).

Thus the neurotrophins BDNF and NT-3, as well as bFGF, affect neuronal differentiation of a subpopulation of committed crest precursors as well as their survival, both in the embryo and in culture. Furthermore, based on our *in vivo* experiments, it appears that the survival-promoting effect of these

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

factors when provided from the central target, the neural tube, is only transient, and encompasses a time window that spans the migratory and early post-migratory stages at trunk levels of the neuraxis (Kalcheim & Le Douarin 1986). In those stages immediately following, young sensory neuroblasts may require BDNF and NT-3 for maturation, but not for survival (Wright *et al.* 1992). Post-mitotic neurons appear to resume a selective target dependence for survival only after the establishment of stable contacts with the periphery (Barde 1989; Vogel & Davis 1991). These findings illustrate a dynamic type of response to growth factors which changes as a function of maturation toward a differentiated state. Different kinds of response may thus reflect different states of committment of the target cells.

The ability of all three factors to stimulate neurogenesis of the neural-crest progenitors, raises the possibility of a redundancy in the response of individual neural-crest cells to the various factors. Alternatively, each factor might act on non-overlapping subsets of progenitors, all able to give rise to sensory neurons. The possibility that several factors may similarly affect development of immature cells is further illustrated by the finding that both BDNF and NT-3 stimulate maturation of early neurons excised from E4.5 chicken DRG (Wright *et al.* 1992) or from E10–11 mouse trigeminal ganglia (Buchman & Davis 1993). In addition, E6 DRG neurons expressing Substance P-immunoreactivity reveal a complete overlap in their response to both NGF and BDNF. This early redundancy is replaced at later stages by an additive type of effect, suggesting that distinct populations of neurons become responsive to each of the factors (Lindsay *et al.* 1985). Thus some progenitor cells might already express a wide repertoire of functional receptors. It must then be assumed, that one role played by local signals encountered during gangliogenesis, differentiation and target innervation, is to refine this repertoire by stabilizing the expression and function of a given receptor type while causing a down-regulation of 'less relevant' receptor species.

(iii) *Multiple effects of a single factor: The roles of NT-3 in development of neural and somite progenitors*

A central question concerning the mechanisms underlying cell diversification from the early neural-crest, is how do different kinds of neurons and satellite cells arise within an apparently homogeneous micro-environment of a nascent ganglion. The answer to this question is most likely contained in the general notion that cell development depends upon a delicate interplay between environmental factors and progenitor cells displaying progressive restrictions in their developmental potentials. For instance, signals provided during different phases of cell maturation may induce the expression of particular phenotypic traits in multipotent precursors, as well as select for a phenotype to which a progenitor cell is already comitted by stimulating actual cell differentiation.

Indeed the neural-crest cells that colonize a ganglionic anlagen such as the DRG have different states of committment, some of them are able to give rise to sensory and sympathetic neurons or to sensory neurons and satellite cells, whereas others are already restricted to become neurons of the sensory lineage exclusively (Bronner-Fraser & Fraser 1988; Sieber-Blum 1989). We propose that one mechanism accounting for the segregation of cell types within a DRG is the differential responses elicited by a single factor on progenitor cells displaying different degrees of developmental restriction. An example for such a mechanism is provided by the dual activity of NT-3 on neural-crest precursors. NT-3 promotes both neurogenesis of a subset of early-committed, post-mitotic sensory neuron precursors (Pinco *et al.* 1993) and proliferation of another subset of multipotent neural-crest progenitors (Kalcheim *et al.* 1992; Pinco *et al.* 1993). The notion that a single factor may exert multiple activities on cells with different states of committment is further supported by the observation that heterogeneous subsets of neural-crest cells migrating toward the DRG anlagen and colonizing the nascent ganglia transiently express trkC mRNA, the most selective receptor for NT-3 (Kahane & Kalcheim 1994). It is only at later stages, after target innervation, that the expression of the trkC receptor message becomes restricted to defined neuronal populations in the lateroventral part of the ganglia, and also to cell subsets in the mediodorsal region of the DRG (Tesarrollo *et al.* 1993; Kahane & Kalcheim 1994; Zhang *et al.* 1994). The activity of endogenous NT-3 on DRG progenitors was further confirmed by the observation that treatment of young avian embryos with neutralizing antibodies to NT-3 significantly reduced cell number in the sensory ganglia before the onset of programmed cell death (Gaese *et al.* 1994).

In addition to the neurogenic and mitogenic roles of NT-3 on subpopulations of neural-crest cells, this neurotrophin plays several other functions in early phases of development of distinct embryonic precursors (figure 1). NT-3 stimulates the differentiation of neuroepithelial cells into motoneurons expressing the SC-1 and islet-1 epitopes (Averbuch-Heller *et al.* 1994), and promotes primary dermis development from the somite-derived dermatome by stimulating the conversion of the epithelial progenitors into mesenchymal cells of the dermis (Brill *et al.* 1995). Thus the diversity of responses to a single neurotrophin best illustrates the ability of one molecule to play multiple roles on distinct cell types that are segregated in space to different embryonic contexts. This is in addition to the time frame which enables a single factor to display miscellaneous functions during the ontogeny of a given cell type.

(*b*) *Factor-induced development of neural-crest cells into sensory neurons is modulated by the rostral somitic mesoderm*

(i) In vivo *modulation of DRG growth by mesodermal substrates permissive for neural-crest migration*

Manipulating the rostrocaudal composition of the somites which determines ganglion segmentation (see §1) causes not only a change in the metameric

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

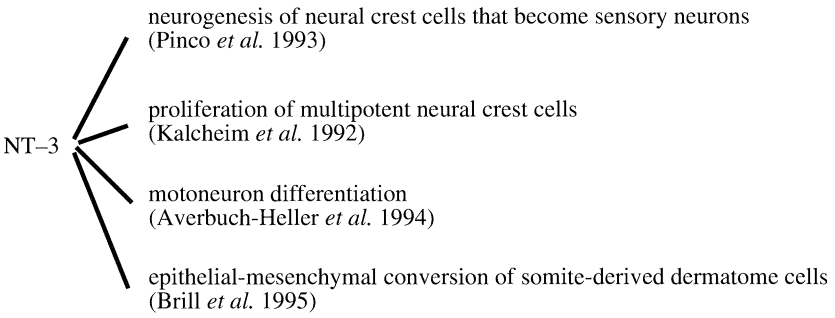

Figure 1. Multiple effects of NT-3 in early development of neural and somite-derived cells. Scheme representing the various effects of NT-3 so far characterized in the avian embryo. NT-3 has at least a dual effect on neural-crest progenitors, it also affects motoneuron differentiation, and is later expressed transiently in subsets of motoneurons different from those expressing BDNF (Kahane *et al.* 1996). NT-3 also affects the early formation of dermis from the epithelial dermatome precursors, both *in vivo* and *in vitro*.

organization of the peripheral ganglia including DRG and sympathetic ganglia but has also profound effects on their size (Goldstein *et al.* 1990; Goldstein & Kalcheim 1991). Grafting multiple rostral half-somites instead of normal segments leads to the formation of unsegmented DRG (polyganglia) whose volume and cell number is significantly greater than the sum of contralateral DRG (Goldstein *et al.* 1990). Using this experimental system we revealed that one mechanism, which accounts for increased ganglion size, is enhanced proliferative activity of the polyganglionic cells (Goldstein *et al.* 1990). In subsequent experiments we showed that the mitogenic effect of rostral half-somite grafts could be mimicked by implanting pieces of lateral plate mesoderm in the place of normal somites (Gvirtzmann *et al.* 1992). A feature common to both tissues is their ability to support migration of neural-crest cells and elongation of axons (Bronner-Fraser & Stern 1991; Gvirtzmann *et al.* 1992). In contrast, grafting of substrates that inhibit the above processes (caudal half-somite, three-dimensional collagen matrices, intermediate plate mesoderm), resulted in the formation of small DRG with reduced proliferative activity (Gvirtzmann *et al.* 1992). Subsequent experiments revealed that the mitogenic effect of the rostral somitic tissue is not due to a rostral somite-derived mitogen as neural-crest cells proliferate to the same extent when co-cultured with either rostral or caudal half-somitic cells (O. Pinco & C. Kalcheim, unpublished observations). Instead, the rostral mesoderm may facilitate the accessibility of CNS-derived molecules to neural-crest cells by permitting their migration or in addition, by producing factors that collaborate with the tube-derived neurotrophins. In support of this notion, we have shown that the mitogenic activity of NT-3 on neural-crest progenitors is enhanced by the presence of mesodermal cells in the cultures (Kalcheim *et al.* 1992).

(ii) *Mesodermal cells modulate the neurogenic effects of BDNF, NT-3 and bFGF on cultured neural-crest progenitors*

We have observed that primary neuronal development from neural-crest progenitors requires a cell-mediated process. Growth factors such as NT-3, BDNF or bFGF have little or no effect on neuronal numbers when added to pure neural-crest cultures (they have, however, significant effects on the nonneuronal cells, see Kalcheim 1989; Brill *et al.* 1992; Kalcheim *et al.* 1992). In contrast, when neural-crest cells are grown on mesodermal cells transfected with the cDNAs coding for these molecules or on naive mesodermal cells to which these factors are exogenously added, neuronal development is greatly stimulated (see Pinco *et al.* 1993 for NT-3, Kalcheim & Gendreau 1988 for BDNF, and Brill *et al.* 1992 for bFGF). Several mechanisms could account for these observations. Mesodermal cells are likely mediators in the presentation of growth factors to responding cells. Thus factors could specifically bind to surface heparan sulfate proteoglycans (Saksela & Rifkin 1990; Brunner *et al.* 1991; Bashkin *et al.* 1992), to the low affinity p75 receptor (Chao 1990; Hempstead *et al.* 1991) present on somitic cells (Heuer *et al.* 1990), or to other membrane or extracellular matrix-associated molecules. These molecules could help generating high affinity binding sites along with the FGF receptor 1 (Heuer *et al.* 1990), with trkB (Klein *et al.* 1990) or trkC (Tesarrollo *et al.* 1993; Kahane & Kalcheim 1994), all receptor types expressed by the target neural-crest cells.

These results support the notion that the neurogenic effect of CNS factors on neural-crest subsets requires a cell-mediated mechanism. *In vivo*, the true substrates for migration of neural-crest progenitors that become DRG are the sclerotomal cells of the rostral somitic domains. It was therefore important to test for their ability to mediate such an effect. Using a culture system of rostral or caudal half-somites separately cocultured with neural-crest cells, we demonstrated the differential ability of rostral somite half-cells to upregulate the neurogenic ability of bFGF (figure 2). In this experiment, rostral and caudal half-epithelial somites were separately cultured and treated with recombinant bFGF, for 1 day. At the end of incubation, excess factor was washed away from the cultures and neural-crest cells were seeded on top of the control and bFGF-treated somites. The extent of neuronal development was measured 20 h later. The level of neuronal development in the absence of added factor was higher on the rostral compared with the caudal somitic substrates (figure, dark bars). This results from

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

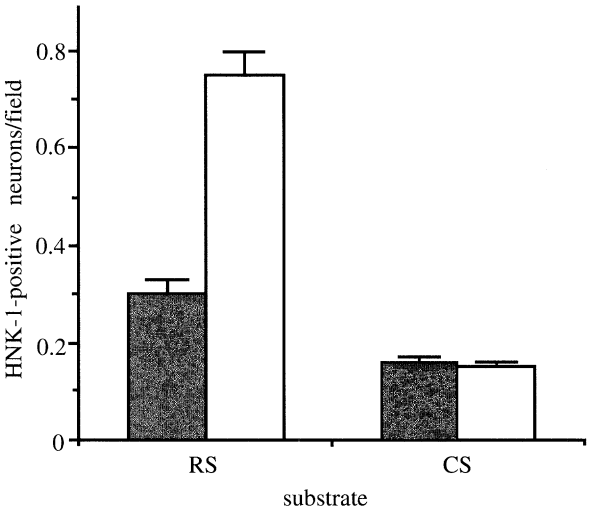

Figure 2. Rostral somitic half-cells mediate the neurogenic effect of bFGF on a subset of neural-crest progenitors. Dark bars represent control; light bars represent bFGF (1 ng ml^{-1}). Rostral somite (RS) and caudal somite (CS) halves were separately cultured in the absence or presence of bFGF (1 ng ml^{-1}) for 20 h. Excess factor was then removed from the somite cultures. Crest cells were dissociated from 45 h clusters and added on top of control and bFGF-treated somites for an additional day. The level of crest-derived bipolar neurons that differentiate from post-mitotic neural-crest cells was monitored by HNK-1 immunostaining. The number of such neurons obtained in control cultures (dark bars) was higher in RS compared to CS. Moreover, only RS cells supported a significant 2.5-fold stimulation in neuronal number in cultures pretreated with the factor (compare light with dark RS bars). No significant effect was obtained either when conditioned media from control or bFGF-treated RS cells was used (not shown). These results suggest that signals associated with RS cell membranes stimulate neuronal differentiation of NC progenitors. Neurons were counted in triplicate cultures. One hundred fields were scored per dish. Results are the average of neurons per field $\pm$ s.d.

a lower extent of adhesion of the progenitor crest cells to the caudal substrates measured 3 h after seeding (not shown). This reduced basal level of neurogenesis observed on the caudal mesoderm does not reflect a selection of specific neuronal precursors, as the development of other crest-derived cell types was equally affected. Lower adhesion to caudal cells may, therefore, be the *in vitro* correlate for the inhibition displayed by cells of the caudal somitic domains on the normal migration of neural-crest progenitors.

As seen in figure 2 (light bars), treatment of rostral half somites, but not of caudal half cells with the factor, resulted in a significantly higher number of neurons developing from the neural-crest progenitors, compared with the respective untreated controls. Thus rostral somitic cells can succesfully modulate bFGF-induced neurogenesis. As depicted schematically in figure 3 and elaborated above, rostral-somite factors can collaborate with CNS molecules to drive crest neurogenesis, or CNS factors can be efficiently presented to the responsive crest cells through initial binding to low affinity sites on the somite cells. Elucidation of the molecular nature of rostral somitic mediation will advance our understanding of the

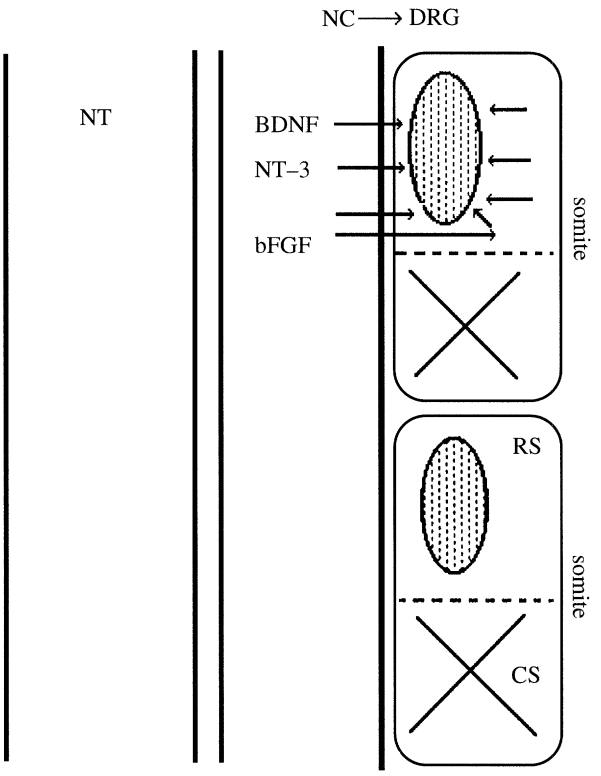

Figure 3. Schematic model illustrating the interactions between neural tube and somites that regulate neural-crest development into DRG. Schematic frontal section illustrating two consecutive somites adjacent to one side of the neural tube (NT). Polarized migration of neural-crest (NC) cells and segmental development of dorsal root ganglia (DRG) within the rostral somitic (RS) domains are driven (at least) by inhibitory properties of the caudal somite halves (CS) on progenitor migration. The NT plays a role in early development of NC cells that become DRG. BDNF, NT-3 and bFGF, all expressed in the NT at the appropriate times (Pinco *et al.* 1993; Kahane *et al.* 1995; Kalcheim & Neufeld 1995), play multiple functions (proliferation, survival, differentiation) on the NC precursors. Their activities are positively modulated by cells of the RS (arrows) via still unknown mechanisms.

mechanisms of early neural-crest differentiation into sensory neurons.

3. CONCLUSIONS AND PERSPECTIVES

Controlled modification of the mesodermal and CNS environments in which the peripheral ganglia arise, has led us to propose a model for DRG development that infers a functional link between mechanisms leading to their metameric organization and the regulation of cell number. The segmentation of developing DRG is regulated by the alternation of permissive and inhibitory properties of distinct domains within the somites. The caudal half of each somite is responsible for ganglion segmentation because of its inhibitory properties on neural crest migration. Initial DRG size is regulated by the neural tube and by the rostral part of each somite (figure 3). The last, being permissive (at least) for neural-crest migration, facilitates DRG growth. Facilitation of ganglion growth may result: (i) from cell surface interactions

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

leading to migration and thus accessibility to the CNS; and (ii) from reciprocal signalling occuring between rostral somite-derived molecules and tube-derived factors.

BDNF, NT-3 and bFGF are all expressed in the early neural tubes of avian embryos and promote neural-crest development both *in vivo* and in culture. Their mechanism of action on the progenitor cells is likely to be complex. Along this line, several issues are worth emphasising. First, a single factor elicits multiple activities on distinct progenitors with varying degrees of committment. Second, several factors have converging activities on a specific subset of progenitor cells that give rise to sensory neurons. Third, the rostral portion of each somite, that supports neural-crest migration also regulates the number of neural-crest cells by modulating the activity of identified growth factors (figure 3).

Future avenues of research should focus on elucidating the molecular heterogeneity between rostral and caudal somitic domains in terms of roles played by differentially expressed genes in the regulation of neural crest segmentation. Along the same line, significant effort should be invested in understanding the nature of the interactions between CNS factors and specific mesodermal genes that control the number of neural-crest cells during migration and condensation to form sensory ganglia.

This work was supported by the Israeli Ministry of Sciences, the Familial Dysautonomia Foundation, and the Council for Tobacco Research.

REFERENCES

Averbuch-Heller, L., Pruginin, M., Kahane, N., Rosenthal, A. & Kalcheim, C. 1994 Neurotrophin-3 stimulates the differentiation of motoneurons from avian neural tube progenitor cells. *Proc. natn. Acad. Sci. U.S.A.* **91**, 3247–3251.

Barde, Y.-A. 1989 Trophic factors and neuronal survival. *Neuron* **2**, 1525–1534.

Baroffio, A., Dupin, E. & Le Douarin, N. M. 1988 Clone-forming ability and differentiation potential of migratory neural crest cells. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5325–5329.

Bashkin, P., Gitay-Goren, H., Neufeld, G. & Vlodavsky, I. 1992 Release of cell surface associated basic fibroblast growth factor by glycosyl phosphatidylinositol specific phospholipase C. *J . cell Physiol.* **151**, 126–131.

Brill, G., Kahane, N., Carmeli, C. von Schack, D., Barde, Y.-A. & Kalcheim, C 1995 Epithelial-mesenchymal conversion of dermatome progenitors requires neural tube-derived signals: Characterization of the role of Neurotrophin-3. *Development* **121**, 2583–2594.

Brill, G., Vaisman, N., Neufeld, G. & Kalcheim, C. 1992 BHK-21-derived cell lines that produce basic fibroblast growth factor, but not parental BHK-21 cells, initiate neuronal differentiation of neural crest progenitors. *Development* **115**, 1059–1069.

Bronner-Fraser, M. & Fraser, S. E. 1988 Cell lineage analysis reveals multipotency of some avian neural crest cells. *Nature, Lond.* **335**, 161–164.

Bronner-Fraser, M. & Stern, C. D. 1991 Effects of mesodermal tissues on avian neural crest migration. *Devl Biol.* **143**, 213–217.

Brunner, G., Gabrilove, J., Rifkin, D. B. & Wilson, E. L. 1991 Phospholipase C release of bFGF from human bone marrow cultures as a biologically active complex with a phosphatidylinositol-anchored heparan sulfate proteoglycan. *J. cell Biol.* **114**, 1275–1283.

Buchman, V. L. & Davis, A. M. 1993 Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. *Development* **118**, 989–1001.

Chao, M. 1992 Neurotrophin receptors: a window into neuronal differentiation. *Neuron* **9**, 583–593.

Davies, J. A., Cook, G. M. W. Stern, C. D. & Keynes, R. J. 1990 Isolation from chick somites of a glycoprotein fraction that causes collapse of dorsal root ganglion growth cones. *Neuron* **2**, 11–20.

Gaese, F., Kolbeck, R. & Barde, Y.-A. 1994 Sensory ganglia require neurotrophin-3 early during development. *Development* **120**, 1613–1619.

Goldstein, R. & Kalcheim C. 1992 Determination of half-somites in skeletal morphogenesis. *Development* **116**, 441–445.

Goldstein, R. & Kalcheim C. 1991 Normal segmentation and size of the primary sympathetic ganglia depend upon the alternation of rostrocaudal properties of the somites. *Development* **112**, 327–334.

Goldstein, R., Teillet, M. A. & Kalcheim, C. 1990 The microenvironment created by grafting multiple rostral half-somites is mitogenic for neural crest cells. *Proc. natn. Acad. Sci. U.S.A.* **87**, 4476–4480.

Gvirtzmann, G., Goldstein, R. & Kalcheim, C. 1992 A positive correlation between permissiveness of mesoderm to neural crest migration and early DRG growth. *J. Neurobiol.* **23**, 205–216.

Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F. & Chao, M. V. 1991 High affinity NGF binding requires coexpression of the trk proto-oncogene and the low affinity receptor. *Nature, Lond.* **350**, 678–683.

Heuer, J. G., von Bartheld, C. S., Kinoshita, Y., Evers, P. & Bothwell, M. 1990 Alternating phases of FGF receptor and NGF receptor expression in the developing chicken nervous system. *Neuron* **5**, 283–296.

Kahane, N. & Kalcheim, C. 1994 Expression of trkC receptor mRNA during development of the avian nervous system. *J. Neurobiol.* **25**, 571–584

Kahane, N., Shelton, D. & Kalcheim, C. 1996 Expression and regulation of BDNF and NT-3 mRNAs in distinct avian motoneuron subsets. *J. Neurobiol.* (In the press.)

Kalcheim, C. 1989 Basic fibroblast growth factor stimulates survival of nonneuronal cells developing from trunk neural crest. *Devl Biol.***134**, 1–10.

Kalcheim, C., Barde, Y. A., Thoenen, H. & Le Douarin, N. M. 1987 In vivo effect of brain-derived neurotrophic factor on the survival of developing dorsal root ganglia. *EMBO J.* **6**, 2871–2873.

Kalcheim, C., Carmeli, C. & Rosenthal, A. 1992 Neurotrophin-3 is a mitogen for cultured neural crest cells. *Proc. natn. Acad. Sci. U.S.A.* **89**, 1661–1665.

Kalcheim, C. & Gendreau, M. 1988 Brain-derived neurotrophic factor enhances survival and differentiation of neuronal precursors in cultured neural crest. *Devl Brain Res.* **41**,79–86.

Kalcheim, C. & Le Douarin, N. M. 1986 Requirement of a neural tube signal for the differentiation of neural crest cells into dorsal root ganglia. *Devl Biol.* **116**, 451–56.

Kalcheim, C. & Neufeld, G. 1990 Expression of basic fibroblast growth factor in the nervous system of early avian embryos. *Development* **109**, 203–215.

Kalcheim, C. & Teillet, M. A. 1989 Consequences of somite manipulation on the pattern of dorsal root ganglion development. *Development* **106**, 85–93.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Keynes, R. J. & Stern, C. D. 1984 Segmentation in the vertebrate nervous system. *Nature, Lond.* **310**, 786–789.

Klein, R., Martin-Zanca, D., Barbacid, M. & Parada L. 1990 Expression of the tyrosine kinase receptor trkB is confined to the murine embryonic and adult nervous system. *Development* **109**, 845–850.

Lallier, T. E. & Bronner-Fraser, M. 1988 A spatial and temporal analysis of dorsal root and sympathetic ganglion formation in the avian embryo. *Devl Biol.* **127**, 99–112.

Layer, P. G. Alber, A. & Rathjen, F. G. 1988 Sequential activation of butyrylcholinesterase in motorneurons and myotomes preceding growth of motor axons. *Development* **102**, 387–396.

Lindsay, R. M., Thoenen, H. & Barde, Y. -A. 1985 Placode and neural crest-derived sensory neurons are responsive at early developmental stages to brain-derived neurotrophic factor. *Devl Biol.* **112**, 319–328.

Pinco, O., Carmeli, C., Rosenthal, A. & Kalcheim, C. 1993 Neurotrophin-3 affects proliferation and differentiation of distinct neural crest cells and is present in the early neural tube of avian embryos. *J. Neurobiol.* **24**, 1626–1641.

Rickmann, M., Fawcett, J. W. & Keynes, R. J. 1985 The migration of neural crest cells and the growth of motor axons through the rostral half of the chick somite. *J. Embryol. exp. Morph.* **90**, 437–455.

Saksela. O. & Rifkin, D. B. 1990 Release of basic fibroblast growth factor-heparan sulfate complexes from endothelial cells by plasminogen activator-mediated proteolytic activity. *J. cell Biol.* **110**, 767–775.

Sieber-Blum, M. 1989 Commitment of neural crest cells to the sensory neuron lineage. *Science, Wash.* **243**, 1608–1611.

Stern, C. D. & Keynes, R. J. 1987 Interactions between somite cells: the formation and maintenance of segment boundaries in the chick embryo. *Development* **99**, 261–272.

Teillet, M. A., Kalcheim, C. & Le Douarin, N. M. 1987 Formation of the dorsal root ganglia in the avian embryo: segmental origin and migratory behavior of neural crest progenitor cells. *Devl Biol.* **120**, 329–347.

Tesarrollo, L., Tsouflas, P., Martin-Zanca, D., Gilbert, D. J., Jenkins, N. A., Copeland, N. G. & Parada, L. 1993 trkC, a receptor for neurotrophin-3, is widely expressed in the developing nervous system and in non-neuronal tissues. *Development* **118**, 463–475.

Vogel, K. S. & Davies, A. M. 1991 The duration of neurotrophic factor independence in early sensory neurons is matched to the time course of target field innervation. *Neuron* **7**, 819–830.

Wright, E. M., Vogel, K. S. & Davies, A. M. 1992 Neurotrophic factors promote the maturation of developing sensory neurons before they become dependent on these factors for survival. *Neuron* **9**, 139–150.

Zhang, D., Yao, L. & Bernd, P. 1994 Expression of trkC and neurotrophin mRNA in dorsal root ganglia and sympathetic ganglia of the queil during development. *J. Neurobiol.* **25**, 1517–1532.

Ziller, C., Fauquet, M., Kalcheim, C., Smith, J. & Le Douarin, N. M. 1987 Cell lineages in peripheral nervous system ontogeny: Medium-induced modulation of neuronal phenotypic expression in neural crest cell cultures. *Devl Biol.* **120**, 101–11.

Ziller, C., Dupin, E., Brazeau, P., Paulin, D. & Le Douarin, N. M. 1983 Early segregation of a neuronal precursor cell line in the neural crest as revealed by culture in a chemically defined medium. *Cell* **32**, 627–638.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES