Development 101, 247–254 (1987)
Printed in Great Britain © The Company of Biologists Limited 1987

The neural tube origin of ventral root sheath cells in the chick embryo

E. R. LUNN[1], J. SCOURFIELD[1], R. J. KEYNES[1] and C. D. STERN[2]

[1]*Department of Anatomy, Downing Street, Cambridge CB2 3DY, UK*
[2]*Department of Human Anatomy, South Parks Road, Oxford OX1 3QX, UK*

Summary

The embryonic origin of peripheral nerve Schwann/sheath cells is still uncertain. Although the neural crest is known to be an important source, it is not clear whether the ventral neural tube also contributes a progenitor population for motor axons. We have used the techniques of immunohistochemistry, electron microscopy and quail–chick grafting to examine this problem. Immunohistochemistry with monoclonal antibody HNK-1 identified a cluster of immunoreactive cells in the sclerotome, at the site of the future ventral root. With the electron microscope, nucleated cells could be seen breaching the basal lamina of the neural tube, exclusively in the region of the ventral root and preceding axon outgrowth. After grafting a length of crest-ablated quail neural tube in place of host chick neural tube, a population of quail cells was found localized to the ventral root exit zone, associated with the ventral root axons. Taken together, these observations support the possibility of a neural tube origin for ventral root sheath cells, although we found no evidence for a more extensive migration of these cells. The ventral root cells share certain phenotypic traits, such as HNK-1 immunoreactivity, with neural-crest-derived Schwann cells, but are not necessarily identical to them. We argue that while they may help motor axons to exit the neural tube at the correct position, they are unlikely to guide axons beyond the immediate vicinity of the neural tube.

Key words: ventral root, sheath cells, Schwann cells, chick embryo, neural tube.

Introduction

The embryonic origin of Schwann or sheath cells was the subject of much debate in the early part of this century, when three cell populations, the neural tube, the neural crest and the mesoderm, were considered possible sources. A mesodermal origin was rejected at an early stage, after Harrison (1904, 1906) showed that removal of the neural crest in frog embryos led to motor nerves devoid of sheath cells. Subsequent experiments produced conflicting results. Kuntz (1922), for example, claimed that removal of the neural crest and dorsal neural tube in frog and chick embryos did not cause loss of motor nerve Schwann cells, and proposed a ventral neural tube origin instead. This was supported by Raven (1937), using xenoplastic transplantation in amphibian embryos. Detwiler (1937), staining the amphibian neural crest with vital dyes, once again proposed a predominantly neural crest origin, but suggested (as had Harrison, 1924) that the ventral neural tube may make an additional contribution later in development. Jones (1939), who studied sections, stained with haematoxylin and eosin, of both normal and crest-ablated chick embryos, decided that dorsal root ganglion Schwann cells have a neural crest origin whereas ventral root Schwann cells emigrate from the neural tube.

In his book 'The Neural Crest', Hörstadius (1950) summarized all this by remarking that 'evidently the problem of the origin of the sheath cells of Schwann is not solved yet'. With the advent of autoradiography, Weston (1963) again noted the possibility of a dual origin of Schwann cells and pointed out that they could yet turn out to be solely of neural tube origin. The debate then appears to have been abandoned without any definite conclusion having been drawn. With the exception of one brief report (Wachtler, 1985), recent accounts nevertheless state that Schwann/sheath cells are all of neural crest origin and do not consider the possibility that motor axon Schwann/sheath cells could have a neural tube origin. The issue is not a trivial one. For example, if the lineage of all motor axon sheath cells is different from

that of sensory axon sheath cells, we might expect them to differ in other respects, such as the production of molecules with guidance or trophic functions.

In a study using monoclonal antibodies that recognize both neural crest cells and Schwann/sheath cells, we observed a population of immunoreactive cells located at the ventral root in crest-ablated chick embryos (Rickmann, Fawcett & Keynes, 1985). We therefore decided to re-examine the origin of sheath cells, using immunohistochemistry, electron microscopy and the quail–chick grafting method (Le Douarin, 1973) in the hope that these comparatively recent techniques might resolve some of the old uncertainties.

Materials and methods

Immunohistochemistry

Transverse sections of ten normal embryos, stage 17 (Hamburger & Hamilton, 1951), were prepared and processed for indirect immunoperoxidase staining with monoclonal antibody HNK-1 (Becton Dickinson anti Leu-7), according to the protocol of Rickmann *et al.* (1985). Briefly, embryos were aldehyde/immersion fixed and embedded in 20% bovine serum albumin (hardened in aldehyde fixative), after which 50–100 μm sections were cut on a freezing microtome. These were then stained by an avidin–biotin–peroxidase procedure using HNK-1, intensified with osmium tetroxide and embedded in Spurr's resin, after which semithin transverse sections were cut with an ultramicrotome.

Electron microscopy

Five stage-17 embryos were fixed for 4 h in 2% glutaraldehyde and 2% formaldehyde (in 0·1 M-Pipes buffer containing 1·5% sucrose, pH 7·2, at 4°C). Specimens were washed for 24 h in Pipes buffer and then postfixed in 1% osmium tetroxide. Block staining was performed in a saturated solution of uranyl acetate in maleate buffer (160 mOs), followed by dehydration through alcohols. TAAB resin was used for embedding, after which thin sections (40–60 nm) were cut on a Huxley MK1 ultramicrotome, mounted on copper grids and double stained with uranyl acetate and lead citrate. Sections were viewed in a Philips EM 300 at 80 kV.

Chick–quail chimaeras

Fertile hens' and quails' eggs were incubated at 38°C to stages 12–13. Since motor axons first emerge from the neural tube opposite the wing bud between stages 16 and 17 (Keynes & Stern, 1984), no ventral roots had formed in donor or host embryos prior to operation. In the trunk region, neural crest migration is first observed three somites cranial to the most recently formed somite (Rickmann *et al.* 1985); neural tube in donor and host embryos was removed opposite the most caudal somites and cranial segmental plate, before the beginning of crest emigration. The length of tissue removed was equivalent to four somites.

The host hens' eggs were prepared as follows: a window was cut with a scalpel blade and the embryo floated up to the level of the shell by adding calcium- and magnesium-free Tyrode's solution (CMF), to which had been added a solution of 10000 i.u. ml^{-1} penicillin and 1 mg ml^{-1} Streptomycin in 0·9% saline (Sigma) to a final dilution of 1:100. 0·1 ml of ink solution (Pelikan Fount India, diluted 1 in 10 with CMF) was injected into the sub-blastodermic space so that the embryo could be seen against a dark background. A rim of silicone grease was then placed around the edges of the window and a drop of CMF made to cover the embryo. Visibility was significantly enhanced with tangential fibre-optic illumination (Hara, 1970). The vitelline membrane was peeled back over the most caudal somites and cranial segmental plate, and the neural tube and notochord were excised using a Weck microsurgical knife. 0·1% trypsin (Difco, in CMF) was sometimes used to help separate the notochord from the endoderm.

The donor quail grafts were prepared from embryos of the same developmental stage as the chick hosts. Each embryo was pinned out in a Sylgard dish with its ventral side uppermost, immersed in 0·1% trypsin in CMF and the endoderm peeled off. Neural tube and notochord (of the same craniocaudal level and length as that excised from the host) were easily separated from the somites and segmental plate. The trypsin was replaced by CMF and the dorsal half of the neural tube was then cut off with a knife to remove the neural crest. The notochord was used as a marker for the ventral side of the grafted neural tube. It was essential to remove the host notochord, for it has been shown that an additional notochord can lead to abnormalities of ventral root emergence (van Straaten *et al.* 1985). The graft was transferred to the host with a micropipette, placed in position in its normal orientation and 1·5 ml of albumen was withdrawn to bring the embryo down into the egg once again. The egg was then sealed with PVC tape and incubated at 38°C for a further 2–4 days.

Chimaeric embryos were fixed in Zenker's solution, dehydrated through alcohols and wax-embedded. The blocks were sectioned transversely at 7 μm, stained by Feulgen's method (Le Douarin, 1973) and mounted in Permount (Fisher).

Results

Immunohistochemistry

During the earliest stages of neural crest cell migration in the chick embryo, HNK-1 can be used to distinguish crest cells from the surrounding somite-derived cells, by virtue of its selective binding to neural crest cells (Tucker *et al.* 1984; Rickmann *et al.* 1985). By this means, it has been shown that crest cells are confined to the cranial half-sclerotome as they pass through the segmental mesoderm (Rickmann *et al.* 1985). Motor axons are also restricted to this part of the sclerotome as they grow out from the ventral neural tube. They leave the neural tube in a punctuated manner, growing first from cell bodies sited opposite cranial half-sclerotome, and later from

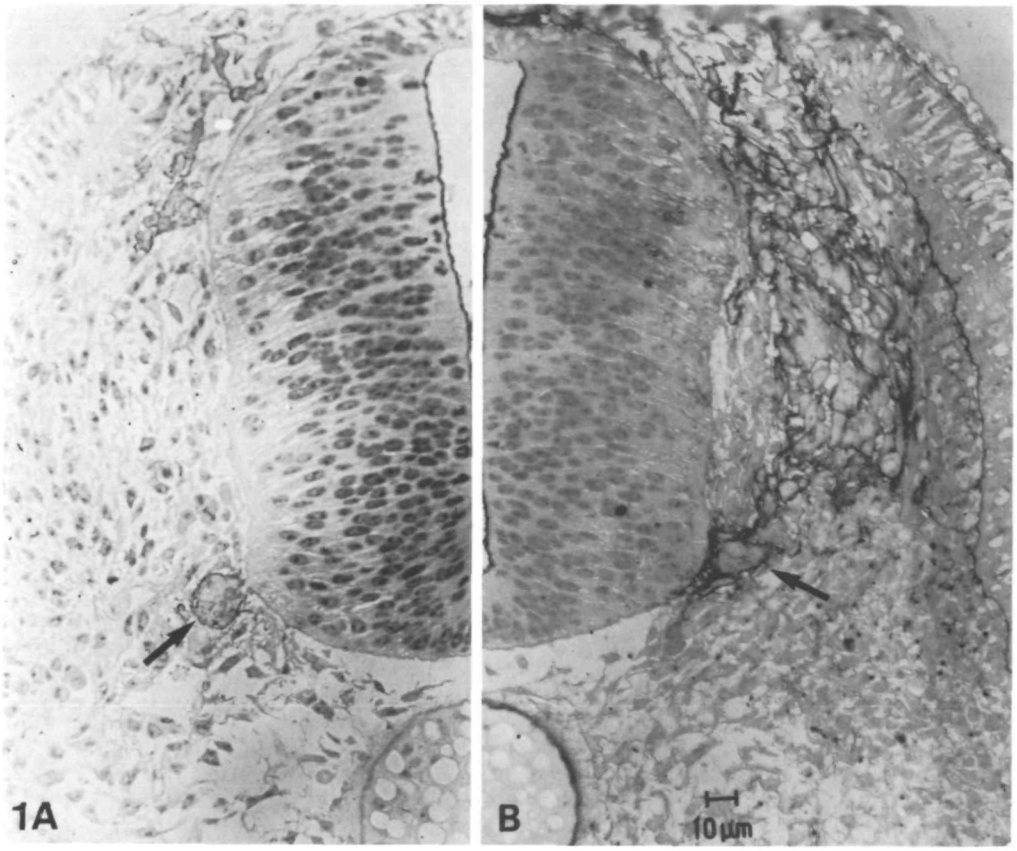

Fig. 1. A composite of transverse semithin sections through the caudal (A) and cranial (B) half-sclerotomes of a wing segment in a stage-17 embryo, stained with HNK-1. (A) In the caudal half-sclerotome, immunoreactive cells are restricted to two areas: immediately dorsal to the sclerotome, adjacent to dorsal neural tube, and as a distinct cluster associated with the site of the future ventral root (arrow). Note the absence of immunoreactive cells within the neural tube. (B) In the cranial half-sclerotome, immunoreactive neural crest cells are now widespread, ventral to the dermomyotome. A cluster of HNK-1-positive cells can again be seen in association with the ventral root region of the neural tube (arrow).

those opposite caudal half-sclerotome (Keynes & Stern, 1984). It was of interest to see whether HNK-1-positive cells were associated with motor axons at the point of axon emergence from the neural tube. In transverse sections through the caudal halves of wing somites of stage-17 embryos, where the neural crest is unable to migrate, a distinct cluster of HNK-1-positive cells was seen adjacent to the neural tube, at the point of future axon emergence (Fig. 1A). In sections through the cranial halves of these same somites, HNK-1-positive cells were widespread in the sclerotome (cf. Rickmann *et al.* 1985). As in the caudal halves, they were clustered around the ventral root zone of the neural tube (Fig. 1B). All the cells **within** the neural tube in this region were themselves HNK-1 negative (Fig. 1A,B).

Electron microscopy

Further evidence for a neural tube origin of these cell clusters was sought by electron microscopy of transverse sections through the wing region of normal stage-17 embryos. In the caudal half-sclerotome, nucleated cells were seen breaching the basal lamina of the neural tube, again exclusively in the region of the future ventral root (Fig. 2A). They were sometimes associated with filopodial processes presumed to be derived from motoneurones. Sections through the cranial half-sclerotome showed the same phenomenon, with, as expected, the additional appearance of fully emergent axon profiles (Fig. 2B).

Chick–quail chimaeras

While the observations described above suggested that the ventral neural tube does contribute a population of axon-associated cells, it remained possible that the ventral cell clusters were nevertheless derived from the neural crest. Chick–quail chimaeras were therefore constructed to see whether quail cells emigrated from a grafted, crest-ablated neural tube along with motor axons. Fifteen grafted embryos survived to stages 20–26, when they were assessed by Feulgen staining, the quail cells being distinguishable

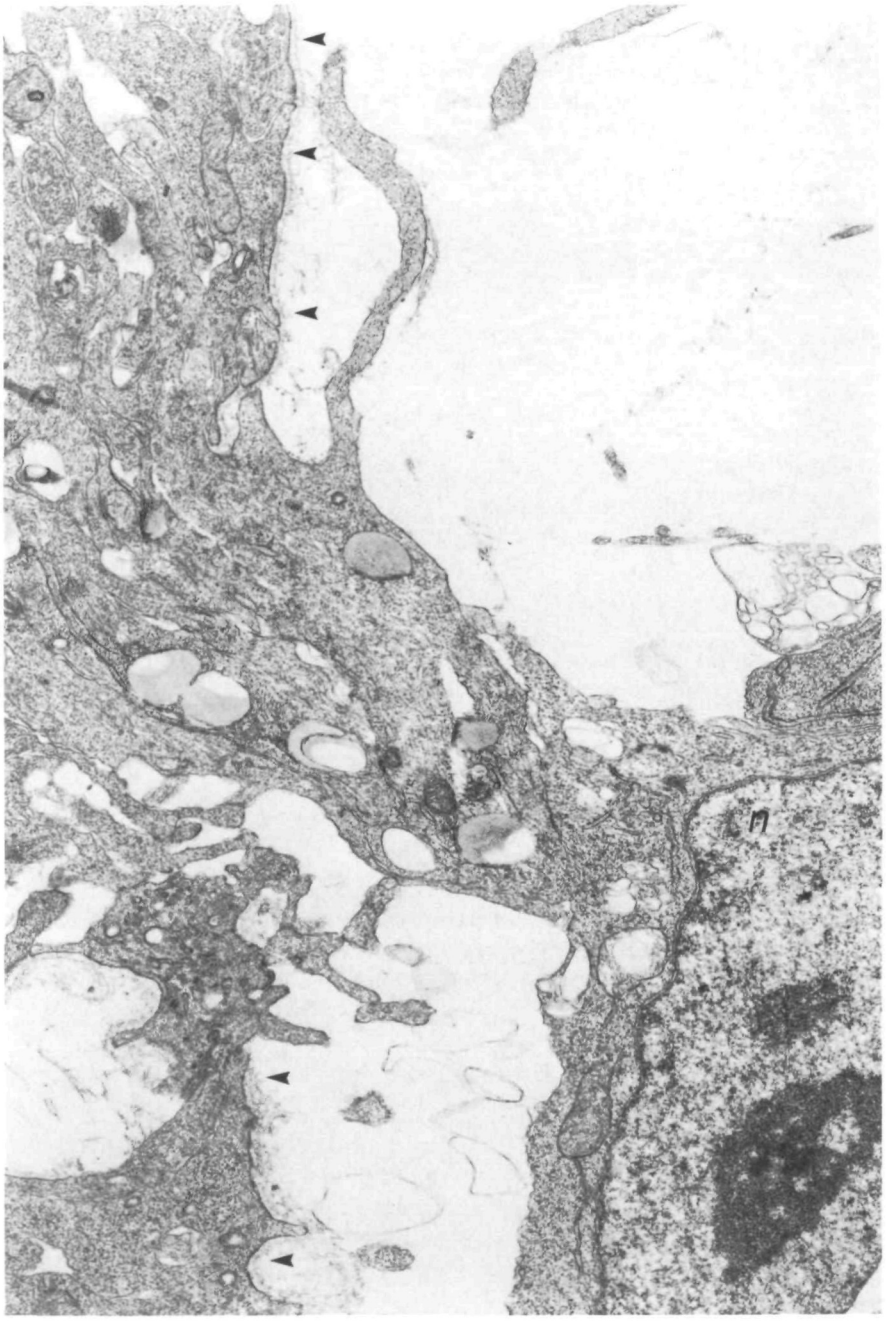

Fig. 2. (A) Electron micrograph of a transverse section through the ventral root region of the neural tube (left) and adjacent caudal half-sclerotome (right) of the wing region in a stage-17 chick embryo. Dorsal is uppermost. The basal lamina on the surface of the neural tube is arrowed, and is breached by a nucleated cell (*n*), whose cytoplasm extends within the confines of the neural tube. ×18750.

by their prominent nucleolar staining (Le Douarin, 1973). Although ventral root cells were first detectable at stage 17 (see above), they were assessed in the chimaeras at later stages so that their migration distance could be estimated simultaneously. Eight embryos were subsequently excluded from the analysis, because only a few quail cells were found to be present, or the graft was malpositioned or severely kinked. In the seven remaining grafts, the spinal cord was usually incompletely formed, since only the ventral portion of the neural tube had been grafted. In some cases, however, the host neural tube and crest had restored the missing dorsal part of the graft or had displaced part of the graft.

Contamination of the grafts by donor neural crest or somite cells, due to incomplete removal of the crest or imperfect dissection of the donor neural tube, was also anticipated. The presence or otherwise of quail neural crest could be determined by studying the dorsal root ganglia; if these contained quail cells it was assumed that quail crest was present in that area. Donor somite cell contamination was assessed by the presence or absence of quail cells isolated within the host sclerotome. Using these criteria, the average length of each graft that was free of contaminating cells was 74·0 % of the total graft length (Table 1).

In contamination-free sections, quail cells were frequently associated with emerging ventral root

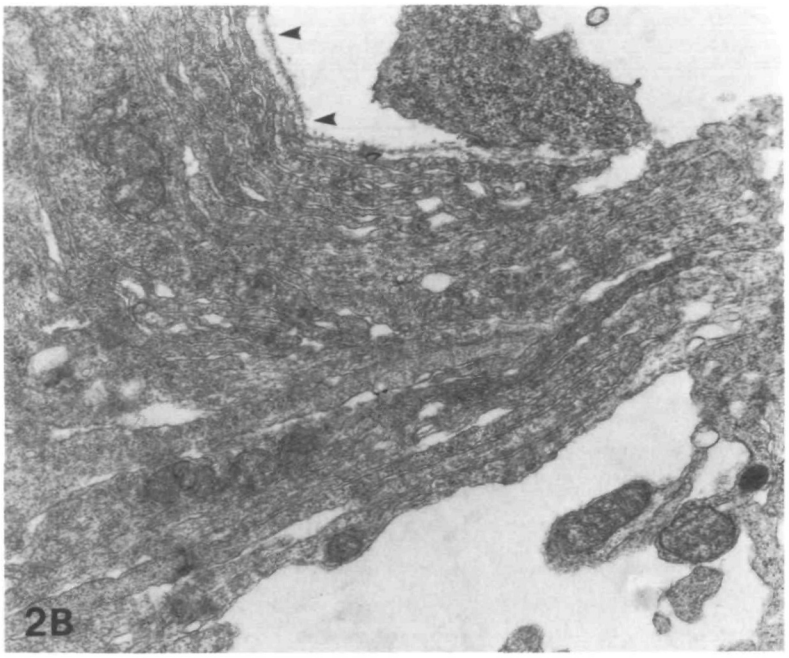

Fig. 2. (B) Ventral root exit zone opposite cranial half-sclerotome of a stage-17 chick embryo, wing region. Same orientation as Fig. 2A. The basal lamina of the neural tube (arrowed) is breached by numerous parallel axon profiles. ×21 000.

axons in the sclerotome immediately external to the neural tube; cells were seen, on average, in 62·0 % of these sections (Table 1). At all the stages examined, the quail cells extended, at most, about 150 μm into the sclerotome (Fig. 3). The ventral root axons could be identified clearly with phase-contrast optics and no quail cells were associated with their more distal regions.

Where regeneration by the host neural tube had occurred, cells of host origin were associated much more extensively with the motor axons derived from the graft. The ventral roots were also thicker in these areas, presumably reflecting a greater degree of axon outgrowth under these conditions.

Discussion

Since Harrison's (1904, 1906) original experiments there has been little doubt that Schwann/sheath cells can arise from the neural crest. A recent demonstration of this has come from the experiments of Le Lievre, Schweizer, Ziller & Le Douarin (1980), who grafted fragments of quail neural crest into chick embryos, between the somites and the neural tube, and showed that quail cells subsequently came to line the nerve fibres of the spinal roots. Over the years, however, there has been disagreement as to whether the ventral neural tube also makes a contribution, leading Weston (1963) to point out that sheath cells could be entirely of neural tube origin.

Table 1. *Results of the quail–chick chimaeras*

A Graft	B Stage examined	C Graft length (μm)	D % Sections free of contaminating cells	E % Contamination-free sections with quail cells at ventral root
1	20	497	88·7	65·0
2	23	644	74·2	30·4
3	24	210	76·7	82·6
4	26	490	91·4	56·3
5	26	700	57·0	91·2
6	26	413	69·5	85·4
7	26	777	60·8	23·7
			$\bar{x} = 74{\cdot}0\,\% \pm 4{\cdot}5$	$\bar{x} = 62{\cdot}0\,\% \pm 9{\cdot}4$

Column B shows the stage at which each grafted embryo was fixed, sectioned and stained; grafts were transplanted at stages 12–13 (see Materials and methods). Column C shows the final length of each graft, calculated as the product of the number of sections containing grafted quail neural tube cells and the section thickness (7 μm). Column D shows the percentage of sections found to be free of contaminating cells, as determined by the absence of quail cells in the dorsal root ganglia and the absence of isolated clusters of quail cells in the host sclerotome. Column E shows the percentage of contamination-free sections that contained quail cells localized to the ventral root. For columns D and E, the mean percentage ± S.E.M. ($\bar{x}$) is also given

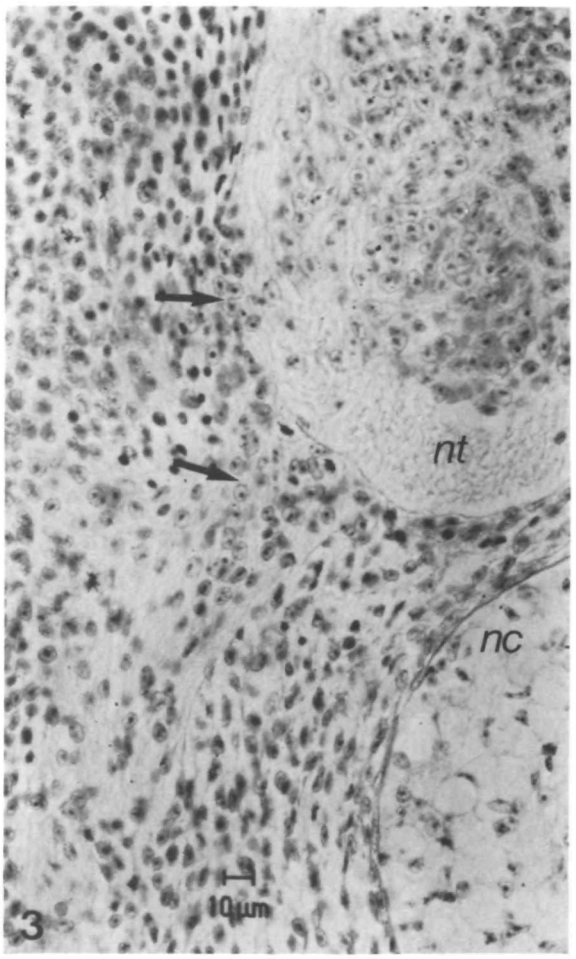

Fig. 3. Transverse section through a grafted chick embryo, showing the ventral portion of the neural tube (*nt*), and notochord (*nc*); both are composed of cells with prominent nucleoli, denoting their quail origin. Clusters of quail cells (arrows) can be seen associated with the proximal 150 μm of the ventral root in the adjacent host sclerotome.

We should first briefly discuss the likely reasons for the previous uncertainties. The early studies (see Introduction) established the ectodermal origin of sheath cells, but were far from unanimous as to their exact provenance. the traditional approach of neural crest ablation could always be criticized because of the difficulty in determining accurately the extent of extirpation *in vivo*; furthermore, a convincing analysis of the results depended upon adequate recognition of the cells, for example in distinguishing them from sclerotome cells, which was not always possible with the routine histological stains used.

Another approach was to detect and label neural crest cells before their migration and differentiation. With vital staining (e.g. Detwiler, 1937), diffusion of dye into neighbouring cells reduced precision. Nuclear size markers (Raven, 1937) had the attendant problem of size overlap between graft and host cells. Tritium labelling was introduced by Weston (1963) in an attempt to overcome these deficiencies. Using labelled crestless neural tube grafts, he again observed that sheath cells emigrate from the neural tube with the motor axons, but could not determine the full extent of this emigration because of the transitory nature of the marker.

Using the stable quail marker (Le Douarin, 1973), our results confirm the long-standing suspicion of a dual origin of sheath cells. We find, however, that the contribution of the ventral neural tube is not as extensive as some authors have previously claimed. Jones (1939), for example, suggested that in chick embryos all the ventral root sheath cells come from the neural tube; Raven (1937) also proposed an important role for neural tube-derived cells in amphibian embryos. Unless it is argued that in removing the dorsal half of the neural tube we have also removed sheath cell progenitors destined to exit through the ventral root, or that there are large differences between birds and amphibia, we do not agree. The limited ventral emigration seen in chick/quail chimaeras is consistent with the limited emigration noted by Harrison in both teleost (Harrison, 1901) and amphibian (Harrison, 1924) embryos. A number of descriptive studies of cyclostome and elasmobranch embryos have suggested a more extensive ventral emigration in these vertebrate classes (e.g. Neal, 1914; see Harrison, 1924), but this needs experimental confirmation. Finally, it seems reasonable to suggest that the cells that emigrate from the neural tube in the embryo might correspond, in the adult, to the well-described 'dome' of glial cells projecting beyond the surface of the spinal cord (see Gamble, 1976).

The question arises as to the phenotypic identity of these ventral cells – whether, in other words, they are identical to neural-crest-derived sheath/Schwann cells or whether they resemble more closely the glia of the central nervous system. So far, we can draw only limited conclusions. Like crest cells, they are immunoreactive with HNK-1 antibody; the HNK-1 epitope is present on a family of related molecules, among them myelin-associated glycoprotein, N-CAM and L-1 (Kruse *et al.* 1984) and J-1. (Kruse *et al.* 1985). Hockfield & McKay (1985) have raised a monoclonal antibody, Rat-401, which also recognizes cells in the ventral root with characteristics of the HNK-1-positive cells. Rat-401 further recognizes neural crest and radial glial cells, and mature Schwann cells (Friedman & Hockfield, 1985). Assuming that the same ventral root cell population is positive for HNK-1 and Rat-401, then presumably it shares characteristics with peripheral glial cells and at least some central glial cells. In an EM study, Fraher & Rossiter (1983) also noted clusters of cells at the ventral roots of E-13 rat

embryos, with characteristics 'resembling those of astrocytes in the adjacent CNS–PNS transition zone'.

Cell processes emerging at the chick embryo ventral root are immunoreactive for both N-CAM (Tosney, Watanabe, Landmesser & Rutishauser, 1986) and Ng-CAM (Thiery, Delouvée, Grumet & Edelman, 1985). While these processes may belong exclusively to motoneurones, the possibility does arise that the ventral root sheath cells are also positive for N-CAM and Ng-CAM.

The final question concerns the function of these cells during neural development. With electron microscopy, nucleated cells were seen breaching the basal lamina of the neural tube even prior to axon outgrowth, opposite caudal half-sclerotome and localized to the site of the future ventral root. In an EM study of amphibian embryos, Nordlander, Singer, Beck & Singer (1981) also noted the presence of nucleated cells breaching the neural tube basal lamina at the ventral root. Nordlander *et al.* (1981) and Hockfield & McKay (1985) have suggested that non-neuronal cells emerging from the ventral root might guide motor axons to the periphery. Certainly it is possible that the ventral root cells direct motor axons to exit from the neural tube at the correct position. Since we cannot tell whether the first cell processes to breach the basal lamina surrounding the neural tube are these sheath cells or, alternatively, growth cone filopodia, it is difficult to be definite on this point. However, our observations do suggest that if these cells guide motor axons, they are unlikely to do so beyond the immediate vicinity of the neural tube, in view of their limited range of migration. It seems equally plausible that their primary role is one of local trophic support and myelination for motor axons.

We thank Marie Watkins and Jeremy Skepper for expert technical assistance.

References

Detwiler, S. R. (1937). Application of vital dyes to the study of sheath cell origin. *Proc. Soc. exp. Biol. Med.* **37**, 380–382.

Fraher, J. P. & Rossiter, J. P. (1983). Cell clusters on fetal rat ventral roots: prenatal development. *J. Anat.* **136**, 111–128.

Friedman, B. & Hockfield, S. J. (1985). Monoclonal antibody Rat-401 identifies developing Schwann cells. *Soc. Neurosci. Abstr.* **11**, 291·10.

Gamble, H. J. (1976). In *The Peripheral Nerve* (ed. D. N. Landon), pp. 330–354. London: Chapman & Hall.

Hamburger, V. & Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49–92.

Hara, K. (1970). "Dark-field" illumination for microsurgical operations on chick blastoderms *in vitro*. *Mikroskopie* **26**, 61–63.

Harrison, R. G. (1901). Über die histogenese des peripheren nervensystems bei Salmo salar. *Archiv f. mikr. Anat.* **57**, 354–444.

Harrison, R. G. (1904). Neue Versuche und Beobachtungen uber die Entwicklung der peripheren Nerven der Wirbeltiere. *Sitzungsber. Niederrh. Ges. Natur. u. Heilkunde zu Bonn* **S** 55–62.

Harrison, R. G. (1906). Further experiments on the development of peripheral nerves. *Am. J. Anat.* **5**, 121–131.

Harrison, R. G. (1924). Neuroblast versus sheath cell in the development of peripheral nerves. *J. comp. Neurol.* **37**, 123–206.

Hockfield, S. & McKay, D. G. (1985). Identification of major cell classes in the developing mammalian nervous system. *J. Neurosci.* **5**, 3310–3328.

Hörstadius, S. (1950). *The Neural Crest.* Oxford University Press.

Jones, D. S. (1939). Studies on the origin of sheath cells and sympathetic ganglia in the chick. *Anat. Rec.* **73**, 343–359.

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

Kruse, J., Keilhauer, G., Faissner, A., Timpl, R. & Schachner, M. (1985). The J1 glycoprotein – a novel nervous system cell adhesion molecule of the L2/HNK1 family. *Nature, Lond.* **316**, 146–148.

Kruse, J., Maihammer, R., Wernicke, H., Faissner, A., Sommer, I., Goridis, C. & Schachner, M. (1984). Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognised by monoclonal antibodies L2 and HNK-1. *Nature, Lond.* **311**, 153–155.

Kuntz, A. (1922). Experimental studies on the histogenesis of the sympathetic nervous system. *J. comp. Neurol.* **34**, 1–26.

Le Douarin, N. M. (1973). A Feulgen-positive nucleolus. *Expl Cell Res.* **77**, 459–468.

Le Lievre, C. S., Schweizer, G. G., Ziller, C. M. & Le Douarin, N. M. (1980). Restrictions of developmental capabilities in neural crest derivatives as tested by in vivo transplantation experiments. *Devl Biol.* **77**, 362–378.

Neal, H. V. (1914). The morphology of the eye muscle nerves. *J. Morph.* **25**, 1–187.

Nordlander, R. H., Singer, J. F., Beck, R. & Singer, M. (1981). An ultrastructural examination of early ventral root formation in Amphibia. *J. comp. Neurol.* **199**, 535–551.

Raven, C. P. (1937). Experiments on the origin of sheath cells and sympathetic neuroblasts in amphibia. *J. comp. Neurol.* **67**, 221–280.

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.

THIERY, J-P., DELOUVÉE, A., GRUMET, M. & EDELMAN, G. M. (1985). Initial appearance and regional distribution of the neuron–glia cell adhesion molecule in the chick embryo. *J. Cell Biol.* **100**, 422–456.

TOSNEY, K. W., WATANABE, M., LANDMESSER, L. & RUTISHAUSER, U. (1986). The distribution of NCAM in the chick hindlimb during axon outgrowth and synaptogenesis. *Devl Biol.* **114**, 437–452.

TUCKER, G. C., AOYAMA, H., LIPINSKI, M., TURSZ, T. & THIERY, J.-P. (1984). Identical reactivity of monoclonal antibodies HNK-1 and NC-1: conservation in vertebrates on cells derived from the neural primordium and on some leukocytes. *Cell Differentiation* **14**, 223–230.

VAN STRAATEN, H. W. M., THORS, F., WIERTZ-HOESSELS, L., HEKKING, J. & DRUKKER, J. (1985). Effect of notochordal implant on the early morphogenesis of the neural tube and neuroblasts; histometrical and histological results. *Devl Biol.* **110**, 247–254.

WACHTLER, F. (1985). Über den möglichen doppelten Ursprung von Schwannschen Zellen. *Verh. Anat. Ges.* **79**, 603–604.

WESTON, J. A. (1963). A radioautographic analysis of the migration and localisation of trunk neural crest cells in the chick. *Devl Biol.* **6**, 279–310.

(*Accepted 8 June 1987*)