THE ORIGIN AND DEVELOPMENT OF GLIAL CELLS IN PERIPHERAL NERVES

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Abstract | During the development of peripheral nerves, neural crest cells generate myelinating and non-myelinating glial cells in a process that parallels gliogenesis from the germinal layers of the CNS. Unlike central gliogenesis, neural crest development involves a protracted embryonic phase devoted to the generation of, first, the Schwann cell precursor and then the immature Schwann cell, a cell whose fate as a myelinating or non-myelinating cell has yet to be determined. Embryonic nerves therefore offer a particular opportunity to analyse the early steps of gliogenesis from transient multipotent stem cells, and to understand how this process is integrated with organogenesis of peripheral nerves.

Before the onset of gliogenesis in the spinal cord, neural crest cells (BOX 1) have already given rise to the early glial cells that are found among the axons of nascent nerves as they work their way through body tissues to reach distal targets and establish functional links between the CNS and the rest of the body[1,2]. These early embryonic nerves are compact columns built exclusively from axons and tightly associated Schwann cell precursors (SCPs). Notably, they have no reinforcing connective tissue or protective covering, and do not even have their own blood supply. These features arise later, at about the time that nerves reach their targets (REFS 3,4; A. Kumar, R.M. and K.R.J., unpublished observations).

A surprising finding is that although SCPs are intimately associated with the axon bundles of these nerves, they are not actually required for the nerve to grow and reach its final target fields[5–7]. Rather, SCPs have four main functions. Their most obvious role is, of course, as an intermediary precursor stage between neural crest stem cells and Schwann cells and, therefore, as the immediate source of the Schwann cells present in perinatal nerves[6,8,9]. Another major function of these cells is likely to be the provision of essential trophic support for sensory and motor neurons — at limb levels of the spinal cord most of these neurons die in mouse mutants in which SCPs are absent[10]. In addition, SCPs are essential for normal nerve fasciculation[10]. Finally, SCPs might be the source of not only Schwann cells, but also the relatively small population of fibroblasts that is found in peripheral nerves[11].

This last finding accords with previous work showing that SCPs can generate non-glial lineages, including neurons, *in vitro* (see below). In principle, these observations on SCPs parallel the recent observation that radial glia give rise to CNS neurons during development[12,13]. In both cases, cells that are unambiguously glial-like, both in molecular phenotype and also, in the case of SCPs, in showing intimate association with axons, seem to generate unexpected progenies that were previously thought to arise from different lineages. These findings are a useful reminder of the tentative nature of many of the dogmas that shape the field.

Notwithstanding these uncertainties, recent progress in understanding neural crest and Schwann cell development has allowed us to generate a coherent baseline picture of glial development in early peripheral nerves, which we describe in this review. We also discuss the molecules that control glial differentiation and highlight new data that point to unexpected functions, developmental potential and the fate of early glial cells in the PNS.

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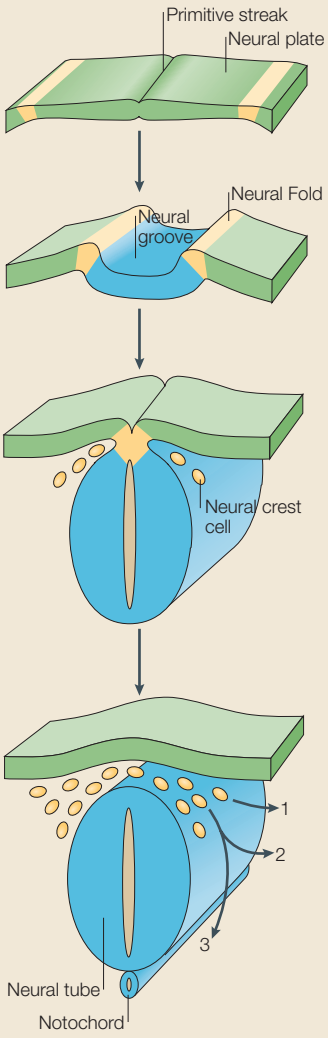
Box 1 | **The neural crest**

In a process known as neurulation, the neural plate, which is found along the dorsal surface of an embryo, gradually folds in on itself to generate the neural groove. As the neural folds fuse to form the neural tube, neural crest cells segregate from the tips of the folds. After taking up an initial position at the dorsal surface of the tube, the crest cells in the trunk region soon migrate along one of two major streams: in a lateral direction (1) to give rise to melanocytes in the skin, and in a ventral direction (2,3) to give rise to neurons in dorsal root sensory ganglia and glia (2), or glia, autonomic neurons and chromaffin cells (3). Neural crest cells in the most anterior part of the trunk, the cardiac crest, also generate fibroblasts and smooth muscle cells, and the cephalic crest in the head region forms the cells of cartilage and bone.

The mechanisms that allow the apparently homogenous population of crest cells to generate such diversity have been intensively studied. It is now considered likely that some neural crest cells are already committed to certain fates, whereas others are multipotent. Although some of these cells may enter lineages in a stochastic and undirected manner, a combination of positive and negative instructive signals probably play an important part in directing neural crest cell differentiation.

How migrating neural crest cells, which initially move through immature connective tissue on each side of the nascent neural tube, end up as Schwann cell precursors in tight association with axons in early embryonic nerves is not clear, either in terms of their detailed migratory route or the signals that cause these cells to adopt an early glial phenotype.



Outline of the Schwann cell lineage

Schwann cells in spinal nerves originate from the neural crest, although the origin of cells in spinal roots is more complex (see below). The end point of Schwann cell development is the formation of myelinating and non-myelinating cells that ensheath large and small diameter axons, respectively, throughout the PNS[4,14–20] (FIG. 1). The other main glial cell types in the PNS are described in BOX 2.

Schwann cell formation is preceded by the generation of two other cell types: SCPs, which are the glial cells of embryonic day (E) 14–15 rat nerves (mouse E12–13), and immature Schwann cells, which are generated from the SCPs from E15 to E17 (mouse E13–15). The latter are the glial cells found in rat nerves from E17–18 to about the time of birth[8,9] (FIG. 2). The postnatal fate of immature Schwann cells is determined by which axons they randomly associate with, with myelination being selectively activated in those cells that happen to envelop single large diameter axons.

These events can be viewed as three main transitions, that is, the transition from migrating neural crest stem cells to SCPs, from SCPs to immature Schwann cells and, lastly, the divergence of this population to form the two mature Schwann cell types. These events are strikingly dependent on survival factors, mitogens and differentiation signals from the axons with which SCPs and Schwann cells continuously associate[4,15]. Another notable feature is plasticity, as much of this developmental sequence is readily reversible: mature myelinating and non-myelinating cells respond to nerve injury by reverting to a phenotype similar to that of immature Schwann cells, and SCPs can be diverted, at least *in vitro*, to other neural crest derivatives[21–25]. Only the middle transition — from SCPs to immature Schwann cells — seems to involve irreversible commitment.

Neural crest cells, SCPs and immature Schwann cells all proliferate rapidly *in vivo*, and the onset of myelination is the only step of differentiation in the entire lineage that is clearly linked to cell cycle exit. Even in this case, cessation of proliferation is reversible, because myelinating cells re-enter the cell cycle as they start to de-differentiate in response to nerve injury[24,26].

Numerous molecules have now been implicated in the regulation of Schwann cell development. It is notable that our knowledge of perinatal events greatly exceeds what we know about the control of early steps in the differentiation of the Schwann cell lineage (FIG. 3).

Markers of lineage progression

A partially overlapping set of molecular differentiation markers can be used to characterize each stage of the Schwann cell lineage. However, each stage can also be identified by additional criteria that relate to signalling responses and changing relationships to other cells and tissues (FIG. 4). Until a few years ago, analysis of lineage progression in this system was limited by a paucity of markers — only S100 calcium-binding protein (S100) and glial fibrillary acidic protein (GFAP) were used, both of which appear late in embryonic nerve development. Now, a number of markers are emerging that offer insights into the intervening developmental steps, allowing new questions to be asked about the mechanisms that control lineage progression.

The markers shown in FIG. 4 fall into 5 main groups: (1) those that are present at all developmental stages, for example, SRY (sex determining region Y) box 10 (SOX10); (2) those expressed by neural crest cells and SCPs, but downregulated by immature Schwann cells, such as activator protein 2α (AP2α); (3) a gene that is expressed only by SCPs — at present cadherin 19 (Cad 19) is the only marker in this category; (4) those present on SCPs and immature Schwann cells but not on migrating neural crest cells, such as brain fatty acid-binding protein (BFABP); and (5) those present on immature Schwann cells but absent or at much lower levels in SCPs, for example, GFAP and S100.

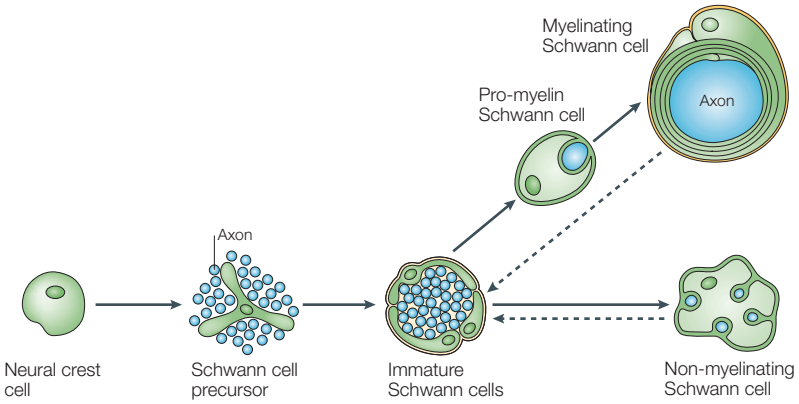


Figure 1 | **The Schwann cell lineage.** Schematic illustration of the main cell types and developmental transitions involved in Schwann cell development. Dashed arrows indicate the reversibility of the final, largely postnatal transition during which mature myelinating and non-myelinating cells are generated. The embryonic phase of Schwann cell development involves three transient cell populations. First, migrating neural crest cells, which are discussed further in BOX 1. Second, Schwann cell precursors (SCPs). These cells express various differentiation markers that are not found in migrating neural crest cells, including brain fatty acid-binding protein (BFABP), protein zero (P0) and desert hedgehog (DHH) (FIG. 4). At any one time, a rapidly developing population of cells — such as the glia of embryonic nerves — will contain some cells that are rather more advanced than others. However, the cells[23] isolated from embryonic day (E) 14 rat nerves by the P07 monoclonal P0 antibody[97] (referred to as 'neural crest stem cells'[23]) are unlikely to be significantly different from the bulk of cells in the nerve, which are referred to here as SCPs (for a detailed discussion, see REFS 98,99). Third, immature Schwann cells. All immature Schwann cells are considered to have the same developmental potential, and their fate is determined by the axons with which they associate. Myelination occurs only in Schwann cells that by chance envelop large diameter axons — Schwann cells that ensheath small diameter axons progress to become mature non-myelinating cells.

Other differences between the three main stages of embryonic Schwann cell development, also shown in FIG. 4, include the following. First, SCPs and immature Schwann cells, but not migrating neural crest cells, are intimately associated with neurons (axons), a characteristic attribute of glial cells (FIGS 2,5). Second, SCPs and migrating neural crest cells show numerous differences in their responses to survival factors[4,15,27]. Third, when compared with migrating neural crest cells, SCPs are also relatively insensitive to the neurogenic action of bone morphogenetic protein 2 (BMP2), and are strongly biased towards Schwann cell generation[28,29].

Additional differences between immature Schwann cells and SCPs include the presence of a basal lamina, which starts to form soon after Schwann cells are generated (A. Kumar, R.M. and K.R.J., unpublished observations). But perhaps the most striking difference between these cells is the ability of Schwann cells to ensure their own survival through the help of autocrine survival circuits[30]. These are missing in SCPs, leaving these cells wholly dependent on survival signals from axons, which are probably mediated to a large extent by β-neuregulin 1 (NRG1; see BOX 3 and below).

Box 2 | Glial cells of the peripheral nervous system

The PNS contains a number of distinct glial cells, each of which is intimately associated with different parts of the neuron or with specific neuronal cell types[94]. Neuronal cell bodies in dorsal root sensory ganglia and in sympathetic and parasympathetic ganglia are covered by flattened sheet-like cells known as satellite cells, whereas axons in nerve trunks are ensheathed by non-myelinating or myelinating Schwann cells, the best known of all peripheral glia. Unlike oligodendrocytes, the myelinating cells of the CNS, each Schwann cell forms myelin around one axon only. For reasons that are not understood, only the larger diameter (>~1 μm) axons of peripheral nerves are myelinated — smaller axons lie in troughs in the surface of non-myelinating Schwann cells. Distinct cells known as olfactory ensheathing cells envelop the axons of the olfactory nerve. Axon terminals at the skeletal neuromuscular junction are tightly covered by terminal glia (teloglia), whereas the terminals of autonomic neurons show only irregular associations with processes of non-myelinating Schwann cells. Many sensory nerve endings in the skin associate with glial cells that form the innermost part of larger structures, for example, the Pacinian corpuscle. Lastly, the complex ganglia of the enteric nervous system harbour glial cells, the enteric glia, that are remarkably similar to astrocytes.

The control of gliogenesis from the neural crest

It has been suggested that in the CNS, glial differentiation represents a 'default pathway' of neural stem cell differentiation[12,13]. We do not yet know whether this is a useful way of thinking about glial cell development in the PNS. However, the functions of the major signalling pathways that have been implicated in the control of gliogenesis from the neural crest — those that involve NRG1, BMP2 and 4, and Notch — are consistent with a default mode. This is because the most obvious functions of these signals in the neural crest are to suppress glial development, or to suppress or activate neuronal development[31–33]. It has been hard to prove that these factors positively initiate glial differentiation from neural crest cells. Similarly, although the transcription factor SOX10 is required for glial development, it is expressed by all neural crest cells and therefore does not seem to be part of a classical inductive signalling cascade for the activation of glial development[34].

A default model would also provide a relatively simple hypothesis explaining how, during gangliogenesis, neurons and glia could form in the same location. It would be sufficient to envisage neurogenic signals acting on neural crest cells, tempered by signals from early neurons (for example, NRG1 or Delta–Notch signalling; see below) that suppress excessive neurogenesis in neighbouring cells.

SOX10. Before investigating the often uncertain function of cell–cell signals in the initiation of glial development, we discuss the transcription factor SOX10, because *Sox10* is the only gene known to be essential for the generation of the glial lineage from trunk crest cells. SOX10 appears to be present in all migrating neural crest cells[34,35]. Expression persists in developing satellite glia in dorsal root sensory ganglia (DRG) and in SCPs in spinal nerves, but is downregulated in early neurons. Matching this pattern, satellite glia and SCPs are missing in mice in which *Sox10* is inactivated, whereas neurons are initially generated in normal numbers[34]. In these mutants, early DRGs contain neural crest-like cells instead of BFABP$^+$ satellite cells (FIG. 4), and nerve trunks also contain a few neural crest-like cells that lack BFABP$^+$. This indicates that in the absence of SOX10 glial specification is blocked, whereas neural crest cells thrive and are able to generate neurons. *In vitro* experiments also support a role for SOX10 in establishing

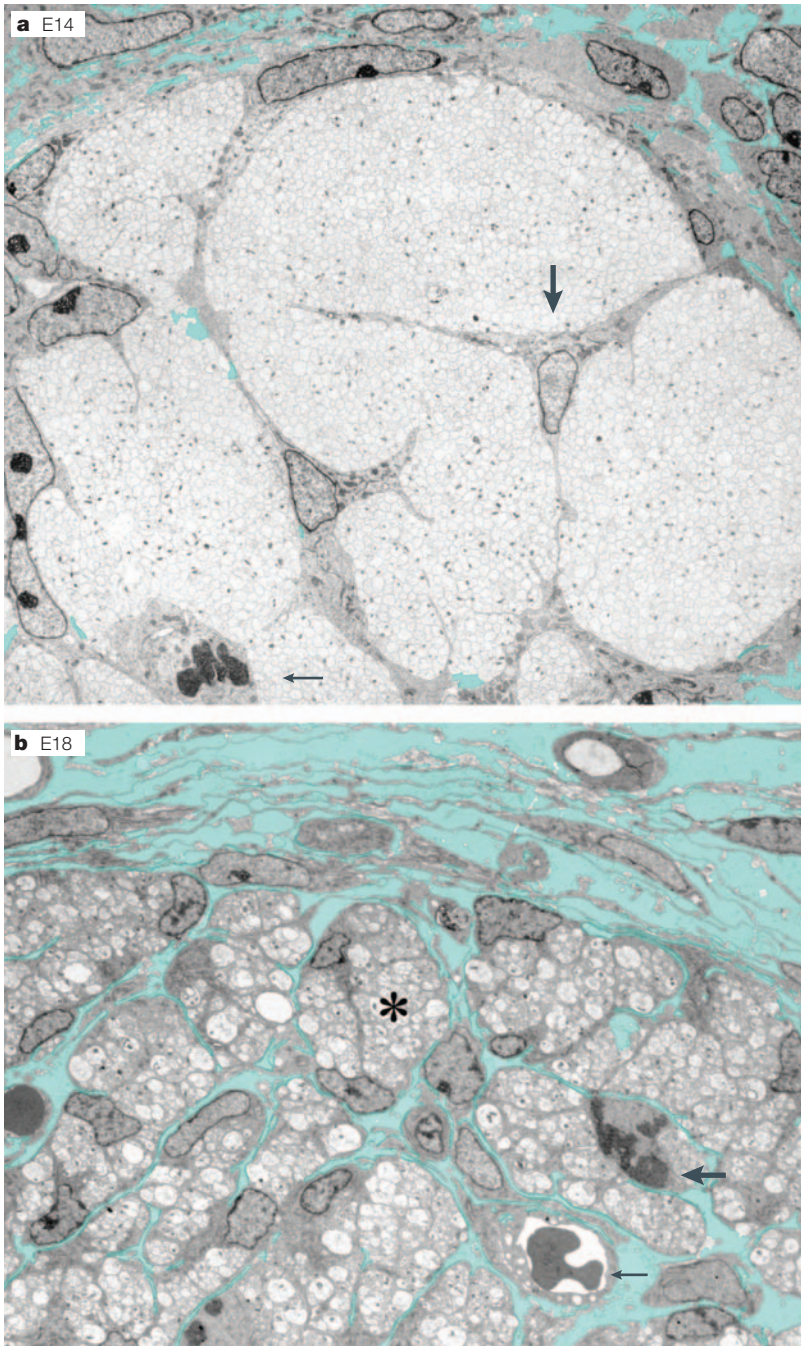


Figure 2 | **The appearance of early cells in the Schwann cell lineage. a** | An electron microscopic image of a transverse section of a nerve in the hindlimb of a rat embryo at embryonic day (E) 14. Schwann cell precursors (SCPs) branch among the axons inside the nerve (big arrow) and are also found in close apposition to axons at the nerve surface. One precursor cell is undergoing mitosis (small arrow). Extracellular connective tissue space (turquoise), which contains mesenchymal cells, surrounds the nerve but is essentially absent from the nerve itself. These nerves are also free of blood vessels and the axons are of smaller and more uniform diameter than those seen in mature nerves. Magnification, ×2000. **b** | Schwann cells in a transverse section of the sciatic nerve of a rat embryo at E18, shown at the same magnification. In marked contrast to the nerve at E14, connective tissue spaces now branch throughout the nerve among compact bundles of immature Schwann cells and their associated axons ('Schwann cell families'[68], for example see asterisk). Blood vessels (small arrow) and fibroblasts (for example, directly above the vessel) have also appeared inside the nerve. One Schwann cell is undergoing mitosis (big arrow). Outside the nerve (in the uppermost part of the picture) connective tissue, which contains flattened fibroblasts of the early developing perineurium and two blood vessels, can be seen.

and maintaining a glial phenotype[35]. One function of SOX10 might be to maintain the ability of early glia to respond to NRG1, as the neuregulin receptor ErbB3 is downregulated in *Sox10* mutants[34].

Neuregulin 1. In neural crest cell cultures, NRG1 inhibits the development of neurons[31], a function that might lead, indirectly, to increased gliogenesis. It is not clear whether NRG1 also restrains neurogenesis *in vivo*, as overproduction of neurons has not been noted in NRG mutants. In similar cultures, GFAP$^+$ Schwann cells appear readily even without the addition of NRG1 (REF. 31), and the same is true if the activation of protein zero (P0) expression, an earlier SCP marker, is used (N. Kazakova, R.M. and K.R.J., unpublished observations). A major population of neural crest-derived glia, the satellite cells of DRGs, is also apparently unaffected in mouse mutants in which NRG1 or the NRG1 receptors ErbB2 or 3 are missing (although SCPs are lost; see below)[10]. Together, these findings indicate that NRG1 signalling is not required for glial differentiation from the neural crest.

The NRG mutants point to two important alternative functions for NRG1 in the development of neural crest derivatives. First, NRG1 is required for the migration of neural crest cells past the location of the DRGs to reach the ventral sites of sympathetic gangliogenesis, as shown by the finding that sympathetic ganglia are hypoplastic in neuregulin mutants[36]. Second, SCPs and, later, Schwann cells are absent or seriously depleted in spinal nerve trunks of the mutant animals[6,7,37]. This probably reflects the role of NRG1 as an axon-derived survival factor and mitogen for SCPs, and failure of migration of precursors from DRGs into spinal nerves might also play a part[9,38]. NRG1 is an essential survival factor for SCPs *in vitro*, and stimulates SCP proliferation. It is also the signal by which DRG neurons rescue SCPs from death *in vitro*[9]. *In vivo*, NRG1 is present at the right time and place to control SCP survival, because it is found in DRG and motor neurons at the stage at which SCPs populate spinal nerves, and it accumulates along axon tracts[39–42]. SCPs depend on axons for survival *in vivo*, and the SCP death that follows nerve injury can be prevented by the application of NRG1 (REF. 43). Comparable results have been obtained with neonatal Schwann cells and terminal glia (known as teloglia)[44,45].

All of these observations indicate that NRG1 is an essential survival factor for SCPs in embryonic nerves. It is likely that the main NRG1 variant responsible for this function is the transmembrane type III isoform, which is the main neuregulin in DRG and motor neurons. In mice that selectively lack this isoform, SCPs initially populate spinal nerves, but their number is severely depleted by E14, which shows that the type III isoform is necessary for the survival of SCPs *in vivo*[38].

NRG1 also promotes Schwann cell survival and proliferation and is likely to be an important component of the axon-associated signal that drives Schwann cell division prior to myelination[46]. There is also evidence that NRG1 accelerates the production of Schwann cells from SCPs (see below).

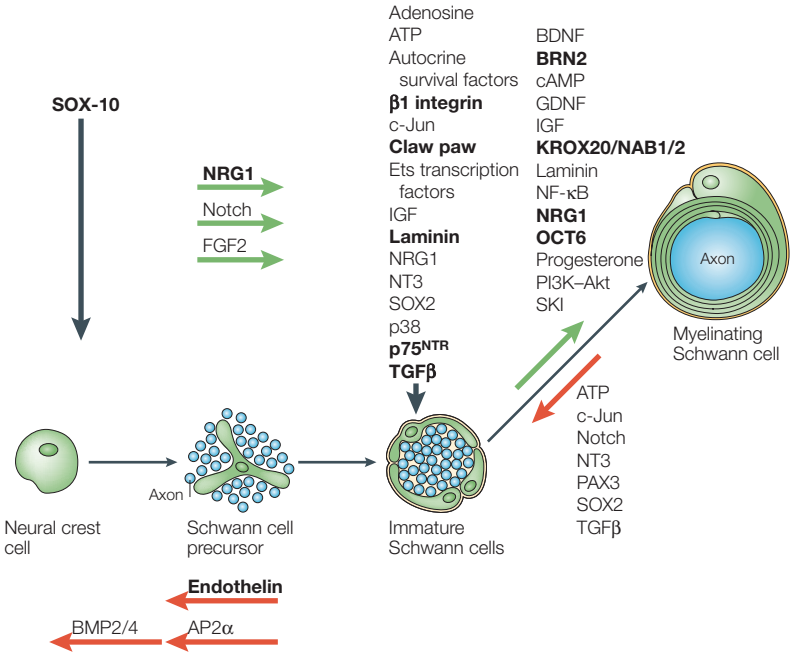


Figure 3 | **Some of the factors that have been implicated in the control of early Schwann cell development and myelination.** Evidence for molecules shown in bold is based on *in vivo* observations in mutant animals. The other molecules have been implicated *in vitro*. In some cases the *in vitro* evidence is substantially more complete than in others. SRY (sex determining region Y) box 10 (SOX10) is required for the generation of all peripheral glia from the neural crest[34], whereas bone morphogenetic proteins (BMPs) inhibit glial differentiation[32]. Axon-derived neuregulin 1 (NRG1), in particular the type III isoform, is necessary for the survival of Schwann cell precursors (SCPs) in embryonic nerves both *in vitro* and *in vivo*[9,10,38]. NRG1, fibroblast growth factor 2 (FGF2) and Notch accelerate the SCP–Schwann cell transition (REFS 33,55,100,101; A. Woodhoo, R.M. and K.R.J., unpublished observations), whereas the transcription factor activator protein 2α (AP2α) and endothelins delay it[55]. In immature Schwann cells, survival is supported by autocrine survival factors, NRG1, Ets transcription factors and laminin[30,44,45,74,102,103], whereas transforming growth factor-β (TGFβ) and the p75 neurotrophin receptor (p75^{NTR}) induce Schwann cell death (REFS 81,82; M. D'Antonio, A. Droggiti, R.M. and K.R.J., unpublished observations). *In vitro* experiments indicate that NRG1 is an axon-associated Schwann cell mitogen, but proliferation is also supported by TGFβ, laminin and various other factors (REFS 46,73,74,104; M. D'Antonio, A. Droggiti, R.M. and K.R.J., unpublished observations). The transcription factors SOX2 and c-Jun support proliferation[83,86], although c-Jun is also required for cell death[83]. ATP and adenosine, however, inhibit Schwann cell division[87]. NRG1, brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), insulin-like growth factors (IGFs) and the p38 pathway function in Schwann cell migration and/or association with axons prior to myelination[75–78]. Radial sorting is impaired in claw paw, laminin and β1 integrin mutants[70–72,74]. Myelination is promoted by the transcription factors early growth response 2 (EGR2, also known as KROX20) with NGFI-A-binding (NAB) proteins, octamer-binding transcription factor 6 (OCT6) and brain 2 class III POU-domain protein (BRN2)[86,91,92], and inhibited by c-Jun, paired box gene 3 (PAX3) and SOX2 (REFS 83,85,86; D. B. Parkinson, R.M. and K.R.J., unpublished observations). Cell-extrinsic signals that promote myelination include glial cell line-derived neurotrophic factor (GDNF), NRG1, IGFs, BDNF, progesterone and laminin[14,42,105–108]. Intracellular phosphatidylinositol 3-kinase (PI3K)–Akt (v-akt murine thymoma viral oncogene homologue) and cyclic AMP (cAMP) activated pathways also promote myelination, whereas it is blocked by Notch activation, NT3 and ATP (REFS 4,87,88,107,109; A. Woodhoo, R.M. and K.R.J., unpublished observations). TGFβ also inhibits myelination, whereas v-ski sarcoma viral oncogene homologue (SKI), which suppresses TGFβ, stimulates it[93,110,111]. NF-κB, nuclear factor-κB.

Therefore, NRG1 acts in a number of different ways to promote the generation and expansion of immature Schwann cells. Furthermore, the inhibitory effect of NRG1 on neurogenesis[31] might act indirectly to increase the production of glial cells, by prolonging the time available for uncommitted neural crest cells to adopt a glial fate. It is likely that the enhancement of Schwann cell generation by NRG1 that has been seen in various *in vitro* situations occurs through a combination of these mechanisms.

Studies on zebrafish indicate that although ErbB2/3 signalling is not needed for Schwann cell generation, it is required for proliferation and migration of Schwann cells along the posterior lateral line[47].

Notch. In the CNS, enforced Notch activation *in vivo* directly or indirectly promotes the generation of glial cells[48]. In the Schwann cell lineage there are intriguing similarities between the actions of Notch and NRG1. Notch activation inhibits the generation of neurons in neural crest cell cultures and increases the number of GFAP$^+$ Schwann cells[29,33,49]. There is also evidence that Notch activation, like NRG1 activation, stimulates the formation of Schwann cells from SCPs (see below) and stimulates Schwann cell proliferation (A. Woodhoo, R.M. and K.R.J., unpublished observations). Cooperative interactions between Notch and NRG1 signalling have been noted previously in the development of astrocytes[50]. It is not yet clear whether Notch acts instructively on neural crest cells to promote gliogenesis. As with NRG1, alternative explanations for the Notch-mediated increase in GFAP$^+$ Schwann cells in neural crest cultures include indirect effects due to inhibition of neurogenesis, stimulation of the SCP–Schwann cell transition, and stimulation of Schwann cell proliferation.

BMP2 and 4. BMPs are important for the generation of sympathetic neurons *in vivo* and stimulate the formation of neurons in neural crest cultures[32,51]. *In vitro*, BMPs are also powerful blockers of glial differentiation from the neural crest[32]. Whether this has a role in normal neural crest development *in vivo* remains to be determined.

The formation of immature Schwann cells

In rats, SCPs convert to immature Schwann cells between E15 and E17 (E13–E15 in mice). This entails a coordinated change in molecular expression, and response to survival signals and mitogens (FIG. 4). At this time connective tissue spaces also open up within the nerves, which become vascularized, and a distinct layer of developing perineurium appears at the nerve surface. The SCP–Schwann cell transition therefore correlates with an important step in the organogenesis of peripheral nerves.

Control of cell survival also changes at this transition, because Schwann cells (but not SCPs) can support their own survival in an autocrine way by secreting a cocktail of survival factors, which, *in vitro*, has been shown to include insulin-like growth factor 2 (IGF2), neurotrophin 3 (NT3), platelet-derived growth factor-β (PDGFB), leukaemia inhibitory factor (LIF) and lysophosphatidic acid (LPA)[30,52,53]. These autocrine survival circuits are probably important in maintaining the survival of Schwann cells in injured nerves even after axons have degenerated. The switch from paracrine dependence on axonal signals to autocrine support of

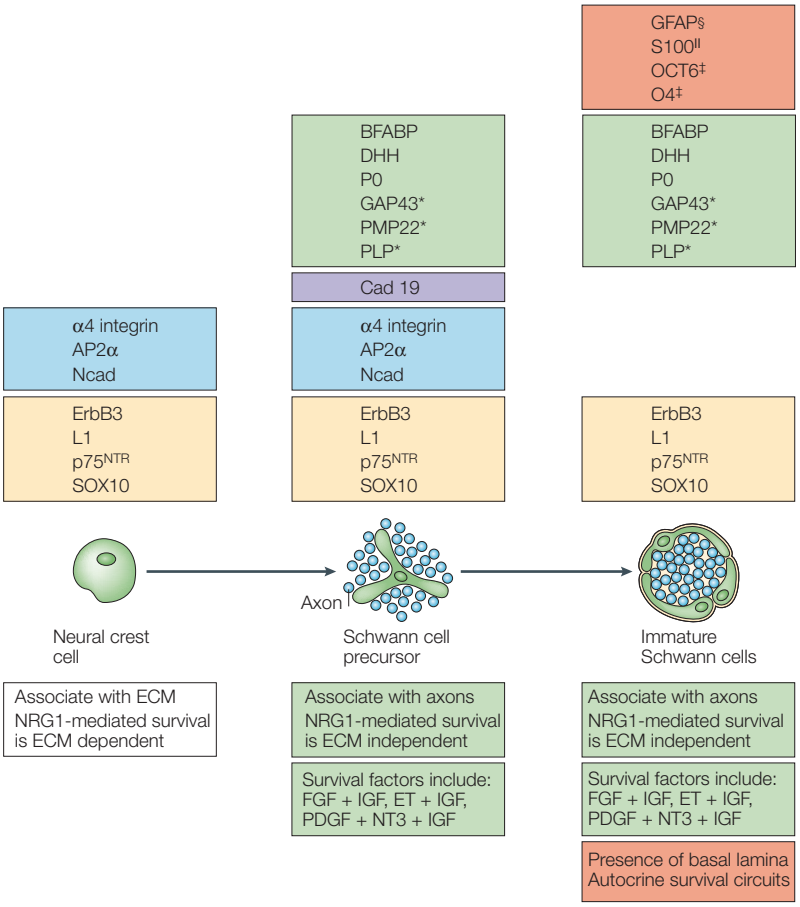


Figure 4 | **Changes in phenotypic profile as cells progress through the embryonic Schwann cell lineage.** Shared profiles are indicated by distinct colours. The boxes above the lineage drawing indicate the changes in gene expression that take place during embryonic Schwann cell development. The gene expression shown here is based on observations of endogenous genes rather than on observations of reporter genes in transgenic animals. Note that Cadherin 19 (Cad 19) is exclusively expressed in Schwann cell precursors (SCPs)[113]. Each developmental stage also involves characteristic relationships with surrounding tissues, and distinctive cell signalling properties (boxes below lineage drawing). For instance, neural crest cells migrate through extracellular matrix. By contrast, SCPs and Schwann cells are embedded among neurons (axons) with minimal extracellular spaces separating them from nerve cell membranes, a characteristic feature of glial cells in the CNS and PNS. Basal lamina is absent from migrating crest cells and SCPs, but appears on Schwann cells. *In vitro*, β-neuregulin 1 (NRG1) only supports neural crest survival in the presence of extracellular matrix (ECM), although this is not required for the NRG1-mediated survival of SCPs and Schwann cells[27]. Migrating neural crest cells also fail to survive in the presence of several factors that support the survival of SCPs and Schwann cells[27], including combinations such as fibroblast growth factor (FGF) plus insulin-like growth factor (IGF), endothelin (ET) plus IGF, and platelet-derved growth factor (PDGF) plus neurotrophin 3 (NT3) and IGF. Schwann cells also have autocrine survival circuits that are absent from SCPs[30]. *Proteins that also appear on neuroblasts/early neurones. ‡Markers that are acutely dependent on axons for expression. §Glial fibrillary acidic protein (GFAP) is a late marker of Schwann cell generation, as significant expression is not seen until about the time of birth. GFAP is reversibly suppressed in myelinating cells. The early expression of GFAP has not yet been carefully examined in mice. ‖SCPs have been shown to be S100 calcium-binding protein (S100)-negative and Schwann cells S100-positive using routine immunohistochemical methods — however, low levels of S100 are detectable in many mouse SCPs when the sensitivity of the assay is significantly increased. α4 integrin[11] (V. Sahni and K.R.J. unpublished observations); AP2-α, activator protein 2α[54]; BFABP, brain fatty acid-binding protein[34]; DHH, desert hedgehog[114–115]; ErbB3, neuregulin receptor[10]; GAP43, growth associated protein 43 (REF. 8); L1, L1 adhesion molecule[4]; N-cad, N-cadherin (I. Wanner and K.R.J., unpublished observations); OCT6, octamer-binding transcription factor 6 (REF. 116); O4, lipid antigen[100]; PLP, proteolipid protein[117]; PMP22, peripheral myelin protein, 22-kDa[22]; P0, protein zero[118]; p75NTR, p75 neurotrophin receptor[4]; SOX10, SRY (sex determining region Y) box 10 (REF. 34).

survival makes biological sense. The former provides a mechanism for matching the numbers of axons and SCPs, whereas the axon-independence of Schwann cell survival ensures that if postnatal nerves are injured, Schwann cells survive to provide essential support for axon regrowth.

Little is known about the transcription factors that control the SCP–Schwann cell transition. It has been suggested that AP2α provides negative regulation, because this factor is strongly downregulated as Schwann cells are generated *in vivo*, and enforced expression in SCPs delays Schwann cell generation *in vitro*[54]. Another negative regulator of Schwann cell generation is endothelin. Endothelins and their receptors are present in embryonic nerves, and in rats with defective endothelin B receptors Schwann cells form prematurely[55]. Evidence for positive regulation of the SCP–Schwann cell transition comes from *in vitro* experiments indicating that NRG1 accelerates the conversion of SCPs to Schwann cells, as well as promoting SCP survival and proliferation[55]. Notch activation also promotes the generation of Schwann cells from SCPs *in vitro* (REF. 33; A. Woodhoo, R.M. and K.R.J., unpublished observations).

Novel origin of Schwann cells in spinal roots

Most of our detailed knowledge about the development of SCPs and immature Schwann cells comes from studies on the spinal nerve trunks that innervate the limbs. How much of it holds true for Schwann cell development in the dorsal and ventral roots of the spinal cord? The observation that S100 appears relatively early in the roots had already indicated that the developmental schedule, at least, differs between root and limb nerves[56]. Now, a study of the fate of boundary cap cells of the spinal cord has revealed a more fundamental difference between these Schwann cell populations[57]. Boundary cap cells originate in the neural crest and take up positions in clusters where dorsal and ventral roots enter and exit the spinal cord. They can be identified by the expression of early growth response 2 (*Egr2*, also known as *Krox20*) long before this gene appears in myelinating Schwann cells. When the fate of these cells is traced *in vivo* using *Krox20*-driven Cre recombinase, it transpires that these cells not only give rise to a small subset of nociceptive neurons and some satellite cells within the DRG, but also give rise to all of the Schwann cells in dorsal roots and many of the Schwann cells in ventral roots. Therefore, boundary cap cells constitute a relatively late reservoir of neural precursors that contribute to the generation of neurons and glia in the PNS. In spinal nerves, few or no boundary cap cell-derived glia were detected. Therefore these findings do not affect the classical notion that the Schwann cells of limb nerves originate in migrating neural crest cells.

The function of Schwann cell precursors

It is generally believed that glial cells provide trophic support for developing neurons. Persuasive *in vivo* evidence for this mechanism comes from investigations

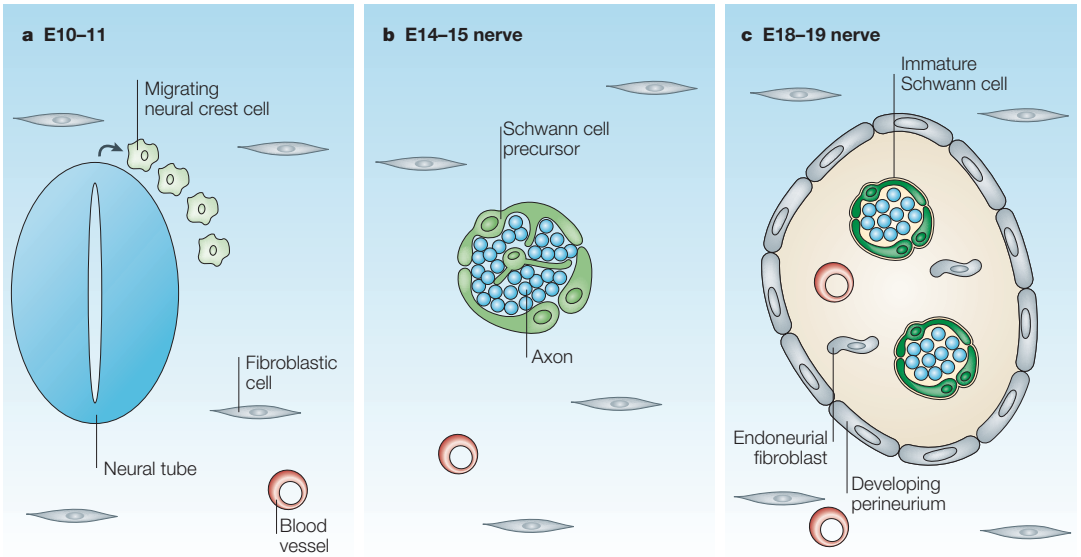


Figure 5 | **Cell and tissue relationships at key stages of Schwann cell development in rodents.** There is a simple relationship between the main stages of embryonic Schwann cell development and organogenesis of spinal nerves. **a** | Migrating crest cells move through immature connective tissue before the time of nerve formation. **b** | Schwann cell precursors (SCPs) are tightly associated with axons and are found in early nerves that are still compact and do not yet contain blood vessels or connective tissue. **c** | Immature Schwann cells are found in nerves that have acquired the basic tissue relationships of adult nerves. By this time, the developing perineurium defines the endoneurial space that now contains, in addition to axon–Schwann cell units, blood vessels, endoneurial fibroblasts and extracellular matrix.

of embryonic nerve development, in which it has been possible to examine what happens when glial cells are deleted from a significant part of the nervous system. This occurs in the major limb nerves of mouse embryos that lack SOX10, isoform III of NRG1, or the NRG1 receptors ErbB2 or ErbB3, owing to the importance of these molecules in gliogenesis and glial survival, as discussed above. Strikingly, in these mutants most of the DRG neurons and motor neurons that project into limb nerves die by E14 and E18 respectively, although these cells are initially generated in normal numbers. This suggests that one of the functions of SCPs and immature Schwann cells is to provide essential survival signals for developing neurons[6,10,34]. Impaired axon–target contacts probably contribute to sensory and motor neuron death in the isoform III neuregulin mutants[38].

Taken together with the finding that axons control the survival of SCPs (see above), these studies identify a discrete phase in early nerve development when neurons and glia depend on each other for survival. An intriguing possibility is the control of neuronal survival by SCPs through back-signalling by the intracellular domain of NRG1 (REF. 58). If this is the case, mutual survival of neurons and glia in embryonic nerves would be ensured by a bidirectional effect of the same molecular interaction, namely the binding of NRG1 to ErbB2 and ErbB3 receptors.

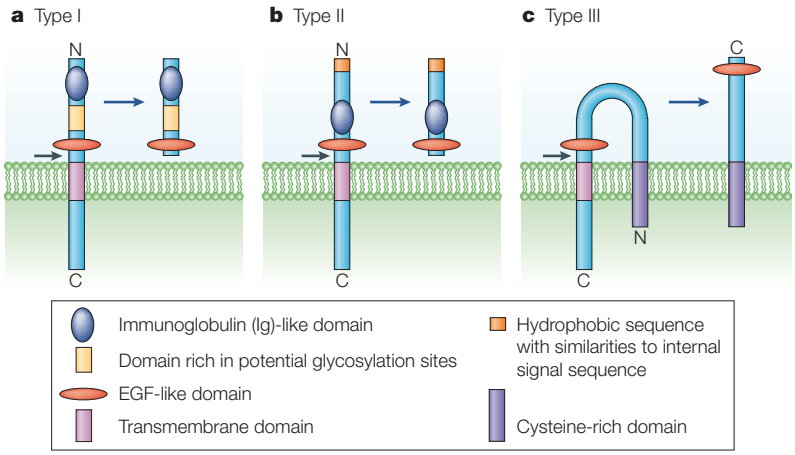
An unexpected morphogenetic role has also been identified for early glia of ventral roots[59]. This function has been seen in a number of mouse lines in which these cells are missing. Such lines include *Sox10* mutants, which fail to generate all glial neural crest derivatives (of which boundary cap cells are a subset), mice in which the boundary cap cells have been selectively killed by diphtheria toxin inserted into the *Krox20* locus, and paired box gene 6 (*Pax6*) mutants, in which failure of neural crest migration leads to the absence of glia in caudal peripheral nerves. In all of these animals the cell bodies of motor neurons are displaced into ventral roots[59]. It seems, therefore, that one of the functions of boundary cap cells, or the SCPs that are derived from them, is to maintain the position of motor neurons in the spinal cord.

Another morphogenetic function of neural crest-derived glia in peripheral nerves is seen in the lateral line of the zebrafish, in which developing glial cells control the formation of secondary neuromasts, organs that are specialized for the detection of water movement[60,61]. SCPs and immature Schwann cells are also important for normal fasciculation of peripheral nerves[7,37].

Recently, a number of classical ideas about developmental potential and lineage restrictions have been challenged by unexpected observations. Probably the most notable example from the nervous system is the finding that neurons can arise from cells that show obvious similarities to astrocytic glial cells, both during development and in the adult[12,62,63].

Another example of a surprising lineage relationship involving glial cells seems to occur in embryonic nerves[11]. In this case, genetic lineage tracing indicates that the relatively small population of fibroblasts found inside nerves (5–10% of the number of Schwann cells at birth) originates from cells in the nerve that are neural crest derived and express desert hedgehog (DHH), and are, therefore, presumably SCPs (FIG. 4). The idea

Box 3 | **Neuregulin 1**



Neuregulin 1 (NRG1) seems to have exceptionally numerous and varied functions in Schwann cell biology. It is involved in neural crest migration, and has been implicated in the lineage specification of neural crest cells and shown to be essential for the survival of Schwann cell precursors (SCPs). It is also involved in Schwann cell generation, proliferation and survival. In postnatal nerves, NRG1 is a positive regulator of myelin sheath thickness, but, paradoxically, also appears to drive the de-differentiation of myelinating cells in injured nerves. No other signalling molecule has been proposed to be so comprehensively involved in the control of Schwann cell development.

There are a surprisingly large number (>15) of NRG1 protein isoforms. The schematic structures of the main isoforms found in the nervous system are shown in the panel. Although splice variants without the transmembrane domain exist for all of these isoforms, transmembrane isoforms (as shown here together with the products of a proteolytic cleavage in the juxta-membrane area) predominate in the nervous system. The epidermal growth factor (EGF) domain is found in all bioactive forms of NRG1 and is sufficient for activation of ErbB receptor-kinase activation. The type III isoform is expressed in axons and is the main regulator of survival of SCPs and myelin sheath thickness. It is thought to have two membrane-spanning domains and to undergo proteolytic cleavage that generates a membrane attached protein carrying the EGF domain[95,96].

NRG1 shows high affinity binding to two receptors, ErbB3 and ErbB4, whereas a related protein, ErbB2, acts as a co-receptor in ErbB3–ErbB2 and ErbB4–ErbB2 complexes. The former is the main NRG1 receptor in peripheral glial cells. The action of axonal NRG1 type III on ErbB3–ErbB2 in developing Schwann cells is probably the best established molecular signalling pathway between neurons and glia in the PNS.

that SCPs generate some fibroblasts in addition to Schwann cells fits well with the observation that both cell types appear in the nerve at the SCP–Schwann cell transition (A. Kumar, R.M. and K.R.J., unpublished observations; also, see above). It is also consistent with the principle, established in previous experiments, that early PNS glia from rodents and birds can generate cells other than Schwann cells. This was first shown in the quail, when P0$^+$ cells from embryonic nerves were found to generate melanocytes *in vitro*[21,64]. More recently, chick SCPs expressing the glial-specific protein SMP (Schwann cell myelin protein) were induced to generate melanocytes by exposure to endothelin, a signal that also seems to act more broadly to promote plasticity of neural crest derivatives[25,65]. The appearance of melanocytes has even been documented in injured nerves of adult mice, particularly in lines that are heterozygous for the neurofibromin 1 gene[66]. In addition, in the rat, P0$^+$ cells from early nerves or DRGs have been shown to have the potential to generate neurons and fibroblasts[22,23].

The generation of fibroblasts from SCPs *in vivo*, if confirmed, would bring the trunk crest lineage in line with the cardiac and cephalic crest lineages, which have long been known to generate connective tissue[67]. But these observations on the developmental potential of SCPs are also in agreement with the emerging concept that early glia can act as multipotent progenitors in the developing nervous system[12,13].

Direct experimental evidence for the more intuitive, if prosaic, idea that SCPs generate Schwann cells includes the following. It is the simplest explanation for the observation that, in spinal nerves of the limbs, SCPs disappear as Schwann cells appear at E15–E17, with substantial numbers of both cell types being present only at E16. Also, in SCP cultures prepared from E14 nerves, the gradual appearance of cells with the phenotype of Schwann cells can be observed directly as SCPs disappear. *In vitro*, the conversion of most of the SCPs to Schwann cells is completed in 3–4 days, which is close to the time course of the SCP–Schwann cell transition *in vivo*[8,9]. Lastly, in mouse mutants that lack SCPs, Schwann cells are not generated[10,34].

A significant narrowing of developmental options clearly takes place at the SCP–Schwann cell transition. Immature Schwann cells have not yet been seen to de-differentiate to SCPs and, *in vitro*, immature Schwann cells are resistant to signals, including BMP2 and fibroblast growth factor 2 (FGF2), that are able to induce the generation of other neural crest derivatives from SCPs[21,23,33]. Their only option seems to be the reversible generation of myelinating or non-myelinating cells, fates that are determined by axon associated signals (see above).

Preparing for myelination

The transition of SCPs to Schwann cells coincides with a major change in the cellular architecture of peripheral nerves. At E14 in rats, limb nerves consist of axons and SCPs. The SCPs are found at the outer margin of the nerves, and inside them as well, connecting to each other through sheet-like processes that communally envelop large numbers of axons. These nerves are compact structures — there are no significant connective tissue spaces, the cellular elements are separated by only minimal gutters and blood vessels are absent (FIG. 2). By E18, the nerves consist of irregular axon–Schwann cell bundles (similar to the 'families' described in newborn nerves[68]) that are surrounded by connective tissue spaces containing endoneurial fibroblasts and blood vessels (A. Kumar, R.M. and K.R.J., unpublished observations). This is the basic relationship between nervous tissue, connective tissue and blood vessels that is seen in adult nerves. We still know little about the signals that control these complex changes and govern the organogenesis of peripheral nerves (FIG. 5).

Around E18 in rats, immature Schwann cells are found communally ensheathing large groups of axons, whereas myelination starts some 3 days later, at birth. This requires radial sorting — a process of radical change in cellular relationships that allows individual Schwann cells to start myelinating single large diameter axons. At the same time, Schwann cell numbers are adjusted by controlling survival and proliferation. While these events take place, premature myelination seems to be prevented by the activity of a number of signalling systems that function as 'myelination brakes'.

Radial sorting. In a process that continues postnatally, the Schwann cell families of late embryonic nerves gradually erode as individual Schwann cells, each associated with a single large diameter axon, segregate from them to form the pro-myelin stage, which is characterized by a 1:1 relationship between axons and Schwann cells[68]. The molecular control of this crucial step is poorly understood. It is impaired in laminin-defective mutants and in the absence of β1 integrin, which is a component of Schwann cell laminin receptors, and also in the claw paw mutant, which has a defect in myelination[69–74].

Various factors affect Schwann cell migration in cell culture and it is possible that these signals govern Schwann cell movements during radial sorting *in vivo*. They include NRG1, IGFs, NT3 and brain-derived neurotrophic factor (BDNF)[75–77]. There is also evidence that activity in the p38 mitogen-activated protein kinase (MAPK) pathway is required prior to myelination, perhaps to attain the correct alignment between axons and Schwann cells[78].

The control of Schwann cell numbers. Another challenge during the late postnatal period is to match the numbers of Schwann cells and axons. Because the period of neuronal death is largely over, this comes down to controlling the rates of Schwann cell survival and proliferation.

Evidence that the axons themselves are major stimulators of Schwann cell proliferation comes from *in vitro* experiments[79], and the idea is supported by the observation that, *in vivo*, Schwann cell proliferation decreases as Schwann cells lose contact with axons in transected nerves in newborn animals[80]. Co-culture studies using Schwann cells and neurons indicate that NRG1 is a major axonal mitogen, but this has not yet been confirmed in mammals *in vivo*[46,47].

Another potential Schwann cell mitogen is transforming growth factor-β (TGFβ). This is found in embryonic nerves, and excision of the type II TGFβ receptor reduces Schwann cell proliferation in E19 mouse sciatic nerves, which shows that TGFβ is directly or indirectly involved in controlling Schwann cell division *in vivo* (M. D'antonio, J. Roes, R.M. and K.R.J., unpublished observations). Interactions with laminin also promote Schwann cell proliferation in developing nerves[73,74].

The survival of immature Schwann cells in late embryonic and perinatal nerves is probably controlled by a balance between factors that support survival and factors that cause death. Survival support comes from axon associated NRG1 and autocrine circuits, as discussed above, and from laminin associated with the basal lamina[30,74]. Two death signals have been identified *in vivo*. One of these acts through the p75 neurotrophin receptor ($p75^{NTR}$), perhaps following activation by binding to nerve growth factor (NGF), and is required for the elevated Schwann cell death that is seen in newborn nerves following injury[81]. The other is likely to be TGFβ, as deletion of type II TGFβ receptors suppresses the normal developmental death in E18 to newborn nerves. The elevated death that follows injury in neonates is also suppressed by injection of TGFβ-blocking antibodies (REF. 82; M. D'antonio, J. Roes, R.M. and K.R.J., unpublished observations) and by deletion of the TGFβ receptor in Schwann cells.

Differentiation brakes. There is now evidence that various signalling pathways that inhibit myelin differentiation are active in immature Schwann cells, and that these pathways are suppressed at the onset of myelination. For instance, the c-Jun-amino (N)-terminal kinase (JNK) pathway is active in Schwann cells of E18 to newborn nerves, where it is required for NRG1 and TGFβ signalling[83]. This pathway is inactivated in individual cells as they start to myelinate by a mechanism that depends on the myelin-associated transcription factor KROX20. If this is prevented, and the JNK pathway remains active, myelination in neuron–Schwann cell co-cultures is blocked and myelin gene expression that would normally result from pro-myelin signals, such as KROX20 or the elevation of cyclic AMP, is inhibited (REF. 84; D. B. Parkinson, A. Bhaskaran, R.M. and K.R.J., unpublished observations). Similarly, Notch signalling promotes proliferation of immature Schwann cells, but is suppressed as cells start to myelinate, and if this is prevented, myelination is blocked (A. Woodhoo, R.M. and K.R.J., unpublished observations). An analogous pattern of action is seen in the transcription factors PAX3 and SOX2: these are expressed before myelination and are involved in proliferation. They are downregulated in myelinating cells and exert a negative effect on myelin differentiation[85,86]. In neuron–Schwann cell co-cultures, axon-derived ATP also delays myelination[87].

These studies are starting to define the signals that determine the immature Schwann cell state. Myelination is activated by inhibition of these pathways together with activation of pro-myelin pathways, which involve the transcription factors KROX20, octamer-binding transcription factor 6 (OCT6) and brain 2 class III POU-domain protein (BRN2), NGFI-A-binding proteins 1 and 2 (NAB1/2), phosphatidylinositol 3-kinase (PI3K) signalling and v-ski sarcoma viral oncogene homologue (SKI)[86,88–93]. The mechanism that initiates these switches remains unclear.

Summary and perspectives

Some 10 years ago, the first potent molecular regulators of glial development from the neural crest were identified, and it was realised that embryonic nerves contain a distinct cell type that is positioned between

neural crest cells and Schwann cells. The first transcription factors that control Schwann cell myelination were also discovered.

Our knowledge of PNS glial development has since been transformed. The main transitions from migrating neural crest cells to myelinating and non-myelinating Schwann cells have been established and each developmental stage can now be unambiguously defined by molecular profile, signalling responses and tissue relationships. Although many important cell-intrinsic, paracrine and autocrine signals have been identified, NRG1, in particular the axon-associated type III isoform, has emerged as a signalling molecule of fundamental importance and considerable versatility, as it is likely to carry out different functions at different stages of the lineage. Understanding how the glial lineage is established from the neural crest, and the role that positive and/or negative inductive signals or default mechanisms have in this key event remain challenging areas. Another important step will be the clarification of the signals that induce myelination.

In many ways our ideas about CNS and PNS glial cells have changed along a similar trajectory in recent years. In both cases, new and unexpected glial functions have been determined and glial cells have been shown to have surprisingly broad developmental potential. It is even possible that they are interchangeable in development, although this has not been tested. Both classes of glia are also increasingly recognized as sources of signals that are essential for the survival and function of neurons and other cells. This key function of glial cells is likely to be more tractable in peripheral nerves because of their relative simplicity. A related issue is the question of to what extent glial cells determine and maintain the higher order organization of the tissue in a specific environment. In peripheral nerves, this issue can be addressed by testing the role of glial-derived signals not only in organogenesis — namely the processes that establish correct tissue relationships during development — but also in the maintenance of tissue function and integrity throughout life.

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Competing interests statement

The authors declare no competing financial interests.

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