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SYMPOSIUM: Peripheral Neuropathies

The Neurobiology of Schwann Cells

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This selective review of Schwann cell biology focuses on questions relating to the origins, development and differentiation of Schwann cells and the signals that control these processes. The importance of neuregulins and their receptors in controlling Schwann cell precursor survival and generation of Schwann cells, and the role of these molecules in Schwann cell biology is addressed. The reciprocal signalling between peripheral glial cells and neurons in development and adult life revealed in recent years is highlighted, and the profound change in survival regulation from neuron-dependent Schwann cell precursors to adult Schwann cells that depend on autocrine survival signals is discussed. Besides providing neuronal and autocrine signals, Schwann cells signal to mesenchymal cells and influence the development of the connective tissue sheaths of peripheral nerves. The importance of Desert Hedgehog in this process is described. The control of gene expression during Schwann cell development and differentiation by transcription factors is reviewed. Knockout of Oct-6 and Krox-20 leads to delay or absence of myelination, and these results are related to morphological or physiological observations on knockout or mutation of myelin-related genes. Finally, the relationship between selected extracellular matrix components, integrins and the cytoskeleton is explored and related to disease.

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Derivation of peripheral glia

Most Schwann cells are derived from the neural crest. The neural crest is a multipotent cell population that arises from dorsal areas of the neural tube. Cells subsequently migrate through distinct pathways in the mesenchyme to form peripheral glia including Schwann cells, enteric glia and satellite cells in addition to neurons, chromaffin cells, and melanocytes. In the head region neural crest cells give rise to a wider range of cell types including cartilage and smooth muscle (reviewed in 3, 4, 70, 81, 92).

One of the questions that has interested us is to consider how early cells become specified Schwann cells. Have some of the migrating crest cells already started to show glial differentiation or does entry to the lineage occur at the time that putative glial cells associate with differentiatied neuronal cell bodies and outgrowing axons? What are the signals that regulate entry to the glial lineage? What are the intermediate stages between lineage entry and the generation of mature myelinating and non-myelinating Schwann cells (Fig 1)?

One of the reasons for our relative ignorance about early glial formation in rodents has been the lack of an early glial differentiation marker. It has now been shown, however, that in both rat and chick some migrating crest cells express a gene that is indicative of a glial phenotype (15, 94, 177). This is the gene for the major peripheral myelin protein P_0. In normal adult nerves expression of this gene is entirely restricted to myelinating Schwann cells, although during development it is expressed, albeit at much lower basal levels, in Schwann cell precursors and premyelinating Schwann cells, regardless of whether they are destined to form myelin or not. Transient expression has also been reported in early postnatal satellite cells in rat DRG (91). Recently, in agreement with earlier findings in the chick, we showed that P_0 mRNA can also be detected in a subpopulation of late migrating crest cells in the trunk region of rats at embryo day (E) 11.5. Clusters of P_0 positive cells

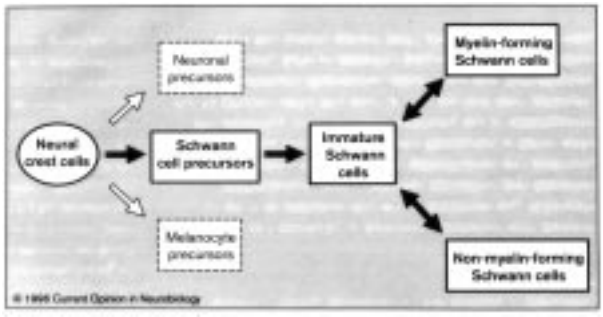


Figure 1. The Schwann cell lineage as characterized in rat and mouse. Reproduced with permission from Mirsky and Jessen (116). For each lineage, neural crest cells generate precursors that in turn give rise to more mature crest derivatives. In the rat and mouse, Schwann cell precursors are a distinct cell type that generates cells with a recognizable Schwann cell phenotype (immature Schwann cells) before birth. This step appears to be irreversible. Further differentiation to form myelinating and non-myelinating cells depends on axon-associated signals and is reversible (indicated by double-headed arrows). Some Schwann cells in spinal roots and dorsal root ganglia originate directly from the neural tube (not shown).

appear to be preferentially located near β3 tubulin positive axons which are projecting from the neural tube and at sites of condensing DRGs. Since there are no published reports of P_0 expression in cells other than Schwann cells or satellite cells it seems likely that initial expression of this gene is a marker of glial differentiation (15, 94). This, however, remains to be proved, and it will be important to examine the relationship between P0 expression in crest cells and expression of early markers of entry to other crest lineages. In quail, Schwann cell myelin protein (SMP), a member of the Ig superfamily, is expressed at E6, somewhat later than P_0, but may also serve as a marker of early Schwann cell lineage cells in birds. Although SMP is not expressed by enteric glia or satellite cells *in vivo*, in culture these cells express the protein (45, 46). The observation that early markers of neuronal differentiation such as β3 tubulin, are generally seen before P_0 expression both *in vivo* and in crest cultures (Lee, Jessen, Mirsky, unpublished) is consistent with the view that early glial differentiation follows rather than precedes early neuronal differentiation (70). It is clear, however, that more needs to be learned about how the appearance of these markers relates to early lineage specific differentiation events before such conclusions can be drawn with certainty.

When we consider extrinsic signals that regulate entry to the glial lineage, very little is known. Experiments on clonally derived rat neural crest cells cultured in a complex medium, show that addition of β neuregulins (see below) can bias crest cell differentiation towards the glial lineage by blocking entry to the neuronal lineage (4, 152, 153). This effect of β neuregulin *in vitro* remains, however, to be reconciled with studies on neuregulin knockout mice which suggest that *in vivo* β neuregulin is required for neurogenesis at least in the cephalic neural crest (112). It may also be relevant in this context that the number of neurons in early DRGs is initially normal in mice lacking one of the major neuregulin receptors erb B3 (139).

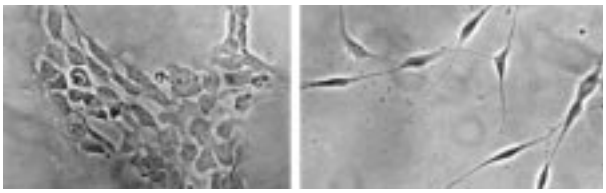
From neural crest to specified Schwann cells

To investigate how neural crest cells turn into Schwann cells, the developing rat sciatic nerve provides a good model system. In this region of the trunk, axons project out into the rat hind limb between E13 and 14, about two days later than the end of neural crest cell migration (138). The glial cells present at this stage are associated with axons that are vigorously projecting towards their target tissues. While these glial cells are a few days older than crest cells at the end of their crest migration, they are at least one week away from myelination, which in the rat starts at around the time of birth. Observation of the E14 nerve at the electron microscope level revealed that the nerve contained glial cells both at the boundary of the nerve and within the nerve. The cells possessed sheet-like processes that contacted one another, encircling large groups of axons, dividing them into territories. To investigate the properties of these cells, nerves were excised from E14 rats. When the cells were dissociated from axons and placed in culture, they underwent programmed cell death within 20 hours, which was surprising since Schwann cells survived well under similar conditions (i.e. at moderate cell density (2-4000 cells per coverslip)). A more extensive comparison of the properties of these cells and Schwann cells revealed that they differed in many other respects (Fig 2). We concluded that the rat sciatic nerve contained at least two distinct cell types in the embryonic period: Schwann cell precursors, present in the nerve at E14 and 15, and Schwann cells, found in the nerve from E16 onwards, and forming the majority of cells in the nerve by E18. The Schwann cell precursor is thus an intermediate cell type in the progression from neural crest cell to Schwann cell (80). Mouse peripheral nerves also contain Schwann cell precursors, but they are present at E12 and 13, two days earlier than in the rat, and the precursor to Schwann cell transition occurs in the developing sciatic nerve between E14 and 15, again two days earlier than in rat. In most respects the properties of the

mouse Schwann cell precursors are similar to those of rat (Dong, Sinanan, Mirsky, Jessen, J. Neurosci. Res., in press).

The role of β neuregulins in the survival of Schwann cell precursors and generation of Schwann cells

The observations that Schwann cell precursors show an intimate morphological relationship with axons, and that Schwann cell precursors did not survive when they were deprived of axonal contact by dissociation, suggested that the survival of Schwann cell precursors in developing nerves was supported by axon-derived signals. In agreement with this idea, conditioned medium from postnatal day 1 DRG neurons supported survival of Schwann cell precursors *in vitro* and allowed them to convert to Schwann cells on schedule (i.e. after 4 days in culture which is equivalent to E18 *in vivo*). Schwann cell precursors also survived when they were placed in close proximity to neurites in sparse cultures of DRG neurons (42), or were exposed to axonal membranes isolated from cultured DRG neurons (Ratner, Mirsky and Jessen, unpublished). The activity associated with axonal membranes and present in the DRG conditioned medium could be blocked by a soluble hybrid protein that contains the extracellular domain of the erb B4 receptor, a high affinity receptor for neuregulin that is highly specific (42). Furthermore, β neuregulins mimicked the effect of the neuron-conditioned medium. They supported Schwann cell precursor survival and allowed the precursors to develop into Schwann cells on schedule *in vitro*. The twin conclusions from this work, i.e. that precursor survival and Schwann cell generation depended on a signal from axons, and that a major component of this signal was β neuregulin, have been confirmed by *in vivo* experiments. Thus, in chick embryos, degeneration of peripheral axons at a stage comparable to rat E14/15 (when the nerves contain precursors), results in precursor death (35). Furthermore, neuregulin mRNA is present at the right time and place to act as a neuron-precursor signal, since strikingly high expression is seen in both DRG and ventral horn motor neurons at E14 and at later ages (13, 102). Lastly, in mice deficient in neuregulin or the major neuregulin receptor, erb B3, there are very few Schwann cell precursors associated with growing embryonic nerves, indicating that neuregulins are required for proper development of these cells (110, 139). It is not at present clear whether the failure of Schwann cell precursors to populate peripheral nerves in the erb B3 deficient mice is exclusively due to death because of a lack of the neuregulin signalling pathway, or whether other factors contribute.



Precursors		Schwann cells
Die by apoptosis when removed from axons and plated in vitro; no autocrine loops	•	Full survival under same conditions; due to autocrine loops
S100 (cytoplasmic)	•	S100 (cytoplasmic)$^{+}$
Do not divide in response to FGFs	•	Divide in response to FGFs
Flattened with extensive cell-cell contacts in vitro	•	Bi- or Tri-polar in vitro
O4^{-}	•	O4^{+}
Krox-20^{-}	•	Krox-20^{+}
Motility^{+++}	•	Motility^{+}

Figure 2. A comparison of the differences in properties of rat and mouse Schwann cell precursors and newborn Schwann cells. S100 (cytopl) + or - refers to the presence of S100 protein in the cytoplasm of the cells, as observed with our routine immunolabelling methods. (Low levels of S100 immunoreactivity are detectable in many precursors in the mouse when the sensitivity of the immunolabelling method is increased significantly). In general, the properties of E12 mouse Schwann cell precursors are similar to those of rat with the exception of the response to FGFs. In the mouse, FGF is a mitogen for Schwann cell precursors.

This could include a failure of neural crest cells/Schwann cell precursors to meet axons at the right time and place and/or a failure to migrate out along the nerves. It has been reported that β neuregulins can promote Schwann cell migration, and we have made similar observations on neural crest cells (101; Calle, Mirsky, Jessen, unpublished). Interestingly, mice that are deficient in β neuregulins containing an Ig domain in the extracellular portion of the molecule (knockout of neuregulin type I and type II) develop normal peripheral nerves, showing that type III neuregulins such as SMDF are sufficient to permit normal Schwann cell development (112).

Whereas neuregulin knockout mice die from heart defects at E11.5, mice lacking the erb B3 receptor survive until birth. The nerves of these animals are essentially devoid of Schwann cells, as expected from the lack of precursors at earlier developmental stages. Nevertheless, axons appear to reach target tissues and

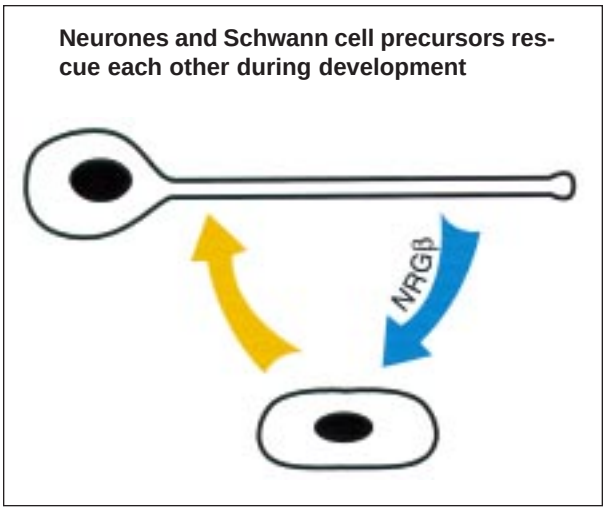


Figure 3. A hypothetical scheme showing how the number of developing neurons and glia may be regulated by reciprocal survival signalling. The molecular identity of the proposed glial derived neuronal survival signal(s) is not clear (see text for details). Schwann cell precursors are supported by b neuregulins (NRGb), and in turn secrete factors that support neuronal survival.

establish normal innervation (110, 139). From this finding, and a previous observation on the Delayed Splotch mutant mouse, it can be concluded that cells of the Schwann cell lineage are not necessary for axons to elongate normally in the embryo, nor are they required for the establishment of appropriate outgrowth patterns (57). However, examination of neuronal survival in the erb B3 knockout mouse points to a different, but equally important, role for Schwann cell precursors and embryonic Schwann cells in the development of peripheral neurons. It was found that although the knockout erb B3 mice initially had normal DRG and motor neuron numbers, they showed an abnormally high incidence of death even at E13, and by E18 about 80% of these neurons had died. This is in excess of the cell death seen in mice lacking individual neurotrophins (97, 168). Since other experiments argue against the possibility that the neuronal death is due to an absence of erb B3 receptors on the neurons themselves, the simplest interpretation of these observations is that signals from the Schwann cell precursors and embryonic Schwann cells are required for the survival of normal numbers of motor and DRG neurons. It is well established that a large number of these neurons die during development in normal animals in the presence of peripheral glia, while in the erb B3 knockout mice an additional number of cells dies, although presumably they have had access to target derived factors. On this basis it could be speculated that most motor and DRG neurons require both target and glial derived factors for survival (39, 40). In view of the regulation of precursor survival discussed above, this also implies that there is a mutual dependence between developing neurons and Schwann cells, in which the survival of Schwann cell precursors depends on neuronal β neuregulins, while the survival of neurons depends on as yet unidentified precursor/Schwann cell-derived survival signals (Fig 3). GDNF, or other newly discovered members of this family including neurturin or persephin, are possible candidate molecules (88, 114). GDNF mRNA is expressed by Schwann cell precursors and developing Schwann cells. It is upregulated in Schwann cells after nerve injury, and acts as a potent survival factor for motor neurons and sensory neurons *in vitro* (23, 67, 69, 117, 157, 174). Recent evidence suggests that Schwann cells, not muscle, are the likely source of GDNF (7). IGFs are another possibility. They have been shown to promote neuronal survival, nerve regeneration or myelination *in vivo*, myelin related differentiation in Schwann cell cultures and are upregulated after nerve transection (31, 83, 84, 131, 134, 160).

Regulation of Schwann cell Phenotype

The differentiation of immature Schwann cells to form the non-myelinating and myelinating cells of mature nerves does not occur if the cells are removed from axons prior to differentiation. In rodents, where this process is essentially postnatal, this can be shown by dissociating cells from nerves and placing them in neuron-free culture, or by transecting neonatal nerves and preventing reinnervation of the distal stump. It has long been concluded from these and related observations, that Schwann cell differentiation is driven by cell-extrinsic signals, most of which come from axons. This signalling must be able to generate two quite distinct Schwann cell variants in appropriate locations, i.e. the non-myelinating cells in association with smaller axons and myelinating cells wrapping the larger axons (Fig 1). These signals orchestrate a large number of biochemical and morphological changes in Schwann cells, a feat that is particularly impressive in the case of myelinating cells, since these cells are extremely specialised and differ radically from the immature cells from which they derive (79, 147, 179). It is a sobering thought that the molecular identity of these differentiation signals is still obscure.

A notable feature of the Schwann cell phenotype is how labile it remains throughout life. If a mature nerve is transected, the myelinating and non-myelinating cells in the distal stump will promptly undergo numerous and

radical alterations in morphology and gene expression. While some of these responses are transient and complex, the eventual outcome is the generation of an apparently single population of cells that show a state of differentiation comparable to that of immature cells prior to the generation of myelinating and non-myelinating cells (116). Similar events can be induced by dissociating cells from adult nerves and plating them in culture without neurons. Both *in vivo* and *in vitro*, this process involves the dedifferentiation or developmental regression of individual Schwann cells and myelin breakdown, while *in vivo* it is accompanied by invasion of macrophages into the denervated stump and a transient phase of Schwann cell proliferation known as Wallerian degeneration. It is likely that signals arising in the distal stump of cut nerves act directly or indirectly to recruit macrophages into the damaged nerve and accelerate the dedifferentiation process (reviewed in 147). Preliminary evidence suggests that two of these signals could be LIF and MCP1 produced by Schwann cells (9, 34, 164; Tofaris, Jessen and Mirsky unpublished). A significant consequence of the Schwann cell response to nerve injury is the generation of an environment that is supportive of axon regrowth. Denervated Schwann cells and endoneurial fibroblasts express higher levels of several potential neurotrophic factors (e.g. NGF, Il-6, LIF, IGF and after several days BDNF and NT4/5) than do their counterparts in normal nerves (19, 38, 58, 71, 100, 109, 113, 134; reviewed in 147). Schwann cells in the distal stump of cut nerves also express adhesion molecules or receptors important for axonal elongation (e.g. L1, N-CAM, N-cadherin, p75NGF receptor) that in mature nerves are restricted to non-myelinating cells (reviewed in 79; 154). Together with the autocrine mechanisms that allow Schwann cells to survive even in the absence of axonal contact (see below), the dramatic response of Schwann cells to nerve injury forms the basis for nerve regeneration and repair in the PNS.

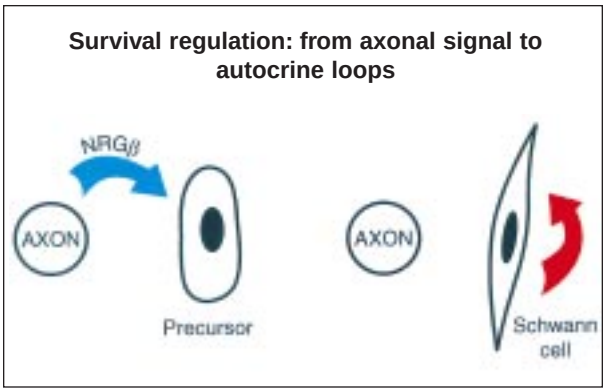


Figure 4. Both axon-derived and autocrine signals regulate survival in the Schwann cell lineage. As development proceeds, there is a shift from an exclusive reliance on axonal signals, b neuregulins (NRGb) to the establishment of autocrine loops as precursors generate Schwann cells. The molecular nature of the autocrine signal is not known.

Regulation of Schwann cell survival

While Schwann cell precursors are obviously dependent on neuronal factors for survival, Schwann cells are not, since adult Schwann cells in the distal stump of transected nerves can survive in the absence of axons, at least for a few months (see below), and cultured neonatal Schwann cells likewise survive well under normal culture conditions i.e. when plated at moderate density in serum-free medium. Schwann cell development therefore involves a change in survival regulation: precursor survival is acutely dependent on axonal signals, while Schwann cell survival is axon-independent, at least in the medium term. This switch in survival mechanisms makes biological sense. The reliance of Schwann cell precursors on close association with axons is likely to help match precursor numbers to axon numbers and should prevent the survival of precursors that have strayed from axons to inappropriate locations. Conversely, the survival of Schwann cells postnatally in the absence of axons ensures that large scale Schwann cell death does not occur in response to axonal damage or loss, which in turn would severely compromise the possibility of axonal regeneration after nerve injury.

The evidence that Schwann cells, unlike precursors, can support their own survival by the help of secreted factors, comes principally from two observations. Firstly, Schwann cell survival is density dependent while precursors die equally readily even at extremely high plating densities. Secondly, Schwann cells in sparse cultures (e.g. 100 cells per coverslip) which die by apoptosis in routine media can be rescued by medium conditioned by dense Schwann cell cultures. Precursors do not respond to this Schwann cell derived survival factor(s) and die equally in Schwann cell conditioned medium (42). Important components of this signal are IGF-2, NT3 and PDFG-BB. It is not mitogenic for Schwann cells and this observation, plus the fact that it does not promote precursor survival (above), render it unlikely that the Schwann cell signal is simply β neuregulin, although it does not exclude the possibility that very low levels of β neuregulin might be a component of the signal. Autocrine survival support can be demonstrated even in early (E18) Schwann cells (Meier

C, Parmantier E, Mirsky R, Jessen KR, J. Neurosci, in press). Nevertheless, the switch from axon-dependent to axon-independent survival is likely to occur gradually as Schwann cells develop from precursors and mature in peripheral nerves. The evidence for this comes from observations on cell death in transected nerves. While a few apoptotic nuclei can be seen in normal sciatic nerves of newborn animals, the number of such nuclei is increased 10 to 20 fold if the cells are deprived of axonal contact by nerve transection. Older nerves, however, contain essentially no apoptotic nuclei and their number does not increase significantly following transection (63, 166). The large majority of cells survive without axons in these experiments, even in newborn nerves. The simplest interpretation of these findings is that at birth axons still provide detectable survival input (probably mediated by β neuregulins) to the Schwann cell population, while this has become insignificant one to two weeks later (Fig 4).

Neuregulins and Schwann cells

β neuregulins undoubtedly have a significant role in the biology of perinatal and mature Schwann cells in addition to their crucial involvement at the early stages of the lineage. It will be interesting to see whether more recently described members of the neuregulin gene family, neuregulin-2 and neuregulin-3, have similar effects, although preliminary reports of their distribution suggest that they may be highly enriched in CNS tissues and heart (26, 27, 30, 72, 176). One of the most important of these functions is likely to be that of acting as an axon-associated Schwann cell mitogen (122), while axon-derived neuregulin is also likely to take some part in promoting the survival of embryonic and early postnatal Schwann cells as mentioned above. The mitogenic effect of β neuregulin is well established. It is seen in several species, including humans (86, 96, 122, 136, 142, 160), and in DRG-Schwann cell co-cultures antibodies to neuregulin and to the neuregulin receptor erb B2 abrogate the axonally induced mitogenic response (122). Freshly isolated adult rat Schwann cells are not initially responsive to β neuregulin when measured by BrDU incorporation, but after culture for 5 days in serum or in b neuregulin they undergo DNA synthesis in response to β neuregulin (43).

β neuregulins also promote Schwann cell survival. Neonatal Schwann cells *in vivo* are susceptible to death after nerve transection (see above). This is true both for Schwann cells at the neuromuscular junction (teloglia) and Schwann cells in neonatal rat sciatic nerves. Both naturally occurring cell death and cell death due to axotomy are only seen during a relatively short period after birth and both can be prevented by injection of exogenous neuregulin (63, 166, 171). It has been reported that neuregulin mRNAs and protein can be detected in cultured Schwann cells and bioactive neuregulin has been detected in conditioned medium from purified Schwann cell cultures (135, 136, 140). Neuregulin mRNA can be detected in adult sciatic nerve by RT-PCR and is up-regulated after nerve transection. α neuregulins appear to be the major isoform detectable in adult nerves after transection, but β forms are also found (28). The function of Schwann cell derived neuregulins is still obscure.

β neuregulins signal via either erb B3 or erb B4 receptors, normally in combination with erb B2 receptors (60). The main receptors in Schwann cells are erbB3 and erb B2, but low amounts of erb B4 are also detectable (28, 63). Schwann cell precursors express erb B3 receptors, but the presence of erb B4 and erb B2 receptors has also been reported (80, 111). During nerve development, both erb B2 and erb B3 receptors are down-regulated as myelination proceeds (28, 37, 63, 166). This is likely to be important, since ongoing expression of these receptors would leave the cells responsive to mitogenic stimulation by axonal β neuregulin, which in turn would prevent myelin differentiation. Nerve transection results in rapid up-regulation of erb B3, erb B2 and also erb B4 (28, 99). β neuregulins induce phosphorylation of both erb B3 and erb B2 receptors (63, 96, 122, 140, 166), and mitogenic signalling via erb B-2 and erb B3 receptors involves activation of ras and the MAP kinase pathway (85, 86). Although β neuregulin does not induce immediate elevation of intracellular cAMP levels in cultured Schwann cells there is a transient elevation of cAMP levels in Schwann cells about 12 hours after addition of β neuregulin (86). One of the signaling pathways used appears to involve the transcription factor CREB (95, 158). β neuregulin induces CREB phosphorylation within 5 minutes of application, probably acting via the MAP kinase pathway. The phosphorylation is sustained for a longer time and is of greater magnitude than that induced by forskolin or cAMP analogues (167).

Trophic factors in adult life

Adult Schwann cells of the distal stump, when kept *in vivo* for 6 months after denervation, down-regulate expression of erb B2 and erb B4 (erb B3 was not examined in this study). When these cells are dissociated and cultured in serum, the receptor down-regulation is reversed, cells express both erb B4 and erb B2 and respond to β neuregulins (99). In adult life, although

Factor	DNA binding domain	*in vivo*	*in vitro*	References
AP2	blZ	+	+	159
CREB	blZ	+	+	86,95,158,167
C/EBPa	blZ	+	+	14,75
E12/47	blZ	+	+	161
c-Jun	blZ	+	+	41,119,158
Jun D	blZ	+/-	+	158
Jun B	blZ	+/-	+	158
Krox-20	Zn finger	+	+	18,123,169,178
Krox-24 (NGF-1A/Egr-1)	Zn finger	+	+	90,106,127,170
NF-κβ		?	+	29
NT3R	Nuclear hormone receptor	+	?	10
Oct-1	Homeodomain	+	+	18
Oct-6	Homeodomain	+	+	12,68,77,119,120,121,175
Pax-3	Paired box domain	+	+	18,87
Sox 10	HMG domain	+	+	89,156
REB	bHLH	+	+	161
Id 1, 2, 3, 4	bHLH	+	+	161

Table 1. Transcription factors associated with Schwann cell precursors and/or Schwann cells.

loss of axonal contact after nerve transection does not lead to immediate Schwann cell death, the Schwann cells in the distal stump eventually die, pointing to the need for neuron-derived or other factors in long term maintenance of Schwann cells. Conversely, the importance of Schwann cell derived CNTF in survival of motor neurons has been shown. Mice deficient in CNTF develop the normal number of motor neurons, but their long term survival is poor, and at several months of age there is significant motor neuron cell loss (105). Despite the fact that knockout of LIF alone produces no significant motor neuron loss, double knockout of LIF and CNTF produces earlier and more severe motor neuron cell death than that seen when CNTF alone is knocked out. This indicates the importance of combinations of growth factors in supporting neuronal survival *in vivo* (150).

Transcription factors in Schwann cell differentiation

Transcription factors that are expressed in Schwann cells or their precursors are summarised in Table 1. Two transcription factors that are important for Schwann cell myelination have been identified. These are the zinc-finger protein Krox-20 and the POU domain protein Oct-6 (Tst-1, SCIP). Knockout of Krox-20 generated by an in-frame insertion of the E.coli lacZ gene leads to arrest of Schwann cell differentiation at the stage just after they have formed a 1:1 relationship with the larger axons, which normally occurs just prior to the onset of myelination. Schwann cells in Krox-20-/- mice can make one and a half spiral turns round the axon but do not form compact myelin. They express pan-Schwann cell markers such as S100 and MAG, which is an early marker of myelin-differentiation. Later gene products such as P_0 and MBP are not expressed at high levels. Krox-20 is sharply upregulated in Schwann cells in normal developing nerve at E15 i.e. at the precursor-Schwann cell transition, and at birth Krox-20 appears to be expressed in most of the Schwann cells in the sciatic nerve, although this is a mixed population with respect to fate, since some of the cells will myelinate while others will give rise to mature non-myelinating Schwann cells. As the nerve develops, Krox-20 expression becomes entirely restricted to those cells that make myelin and these cells continue to make detectable levels of Krox-20 protein throughout life. Following transection of adult nerves, expression of Krox-20 in the distal stump is reduced, a reaction that is in line with that of many other proteins (e.g. myelin proteins) that are positively regulated by neuronal signals and are selectively expressed by myelinating cells (79, 169). Another example of axon-dependent Krox-20 expression is provided by the observation that contact with DRG neurites induces expression from the Krox-20 promoter in Schwann cells that have lost Krox-20 driven expression in culture (123). Interestingly Krox-20 is seen in cells associated with outgrowing axons in the ventral and dorsal roots at E10.5, several days before it is highly expressed in cells of the sciatic nerve. These cells are also S100 positive, suggesting that they may develop a Schwann cell phenotype well ahead of the schedule seen in the sciatic nerve. It is an intriguing feature of Krox-20 expression that it is not normally expressed by satellite cells within

DRGs and is apparently absent even from Schwann cells that are present within the ganglia although many of these cells form myelin. When DRGs are placed in culture Krox-20 expression can be induced in the glial cells by exposure to β neuregulin or a combination of FGF-2 and CNTF (123). Recently it has been found that another zinc-finger factor of the same family, Krox-24 (Egr-1, NGF1A) shows a reciprocal expression to Krox-20 in early nerves and in adult nerves (170). Based on expression of lacZ under the control of the Krox-24 promoter in Krox-24+/- mice, the gene is activated between E11 and 12 in Schwann cell precursors, while expression drops sharply shortly after the precursor-Schwann cell transition, a time when Krox-20 is activated. Surprisingly, Krox-24 reappears on the first day after birth when it is activated for several days in all cells in the nerve; during this period Krox-24 expression overlaps with that of Krox-20. Later on, Krox-24 is downregulated in myelinating Schwann cells, but remains activated in non-myelinating Schwann cells. In the distal stump of cut nerves Krox-24 is strongly activated again in most cells as they dedifferentiate, while Krox-20 falls as discussed above. Many other proteins show a comparable pattern of expression in postnatal nerves to that described here for Krox-24, namely presence in immature cells followed by restriction to non-myelinating cells in the mature nerve; this restriction is lost following loss of axonal contact resulting in re-expression of the protein in all cells in denervated distal stumps or in Schwann cells in neuron-free cultures (79, 116). It will be important to determine whether expression of Krox-24 affects regeneration after cut or crush (170).

Mice deficient in Oct-6 show a similar phenotype to Krox-20 knockout mice, in that myelination is either absent or severely delayed (12, 77). Schwann cells achieve a 1:1 relationship with larger axons by birth, at which stage most mice die of respiratory failure. It is notable that in the mice that survive myelination eventually occurs, resulting in the delayed formation of apparently normal myelin (77). This result could be either because the gene is involved in the timing of the myelination or alternatively because a related but unidentified POU domain gene expressed later in development can compensate for the loss of Oct-6 and enable myelination to proceed, albeit with a delay. Oct-6 mRNA and protein can be detected in Schwann cell precursors, rising to a peak in early postnatal life. Oct-6 protein is clearly detectable in the nuclei of most myelinating Schwann cells during the first week of active myelination (18, 144, 178). Many non-myelinating Schwann cells, particularly in the cervical sympathetic trunk, continue to express Oct-6 at relatively low levels in adult life (18). While the experiments discussed here indicate that Oct-6 function is necessary for myelination to proceed, other studies using a dominant negative construct of Oct-6 (SCIP) under control of the 1.1 kb P_0 promoter have raised the possibility that Oct-6, on the contrary, acts to delay or suppress myelination. Recent studies using this transgenic mouse show that both myelination and axonal regeneration are enhanced (62, 175). This controversy is discussed elsewhere (78, 116). Two different isoforms of the related POU domain transcription factor Oct-1, which is widely expressed in many tissues, can also be detected in Schwann cell precursors and Schwann cells, but it does not appear to be strongly regulated with developmental age (18). It should be noted that there is little evidence that either Krox-20 or Oct-6 are directly involved in the myelination programme. The phenotype of both Krox-20 and Oct-6 knockout mice could therefore be due to a requirement for these genes in Schwann cell maturation prior to myelination.

Recent evidence suggests that a transcription factor of the SRY-like high mobility group (HMG) domain class of transcriptional modulators, Sox 10, may play a role in both PNS and CNS glial development (89, 156). On its own, Sox 10 has no autonomous transcriptional activity in glial cells, but it functions synergistically with Oct-6, and also modulates the activity of Krox-20 and the paired box transcription factor Pax-3 (89). On the basis of indirect evidence, Pax-3 has previously been implicated in the maturation of non-myelinating cells (87), and it can be detected by RT-PCR at low levels in Schwann cells and Schwann cell precursors, peaking at E17 (18). Sox 10 mRNA is expressed in migrating neural crest cells, including melanocyte precursors, and in both Schwann cells and oligodendrocytes later in development (89, 156).

Members of the helix-loop-helix (HLH) class of transcription factors are also expressed in cells of the Schwann cell lineage. In general, three classes of HLH factors exist, ubiquitous A basic(b) HLH factors, cell type specific B bHLH factors and inhibitory factors (Ids) which lack a DNA binding domain and negatively regulate binding to DNA by sequestration of class A proteins. Cell type specific activation is thought to involve heterodimers consisiting of a ubiquitous A class and a specific B class bHLH protein. Class B b HLH factors play crucial roles in both muscle (MyoD family) and nerve development (Mash 1, neurogenin and Neuro D) (4, 82, 93). To date, two members of the ubiquitous A class, REB and E12/47 and all four members of the Id family have been detected in Schwann cell precursors

and Schwann cells. No class B factors have so far been identified (161). However, the promoters of the myelin genes P_0 and MBP, the GAP-43 gene and the low affinity p75NGF receptor all contain the activating E box sequence, to which bHLH dimers bind (32, 33, 161). This fact, together with the fact that Schwann cells express both A class proteins and Ids, makes it likely that B class genes will be identified in Schwann cells.

Other transcription factors that are expressed by Schwann cells include CREB (see above), CEBP/α, members of the Jun family, c-Jun, Jun B and Jun D, and NFκβ, all of which may have roles to play in development or differentiation (reviewed in 159; 81). It is intriguing that NGF, acting through the p75NGF receptor, activates NFκβ, without inducing Schwann cell death although NGF mediated activation of the p75NGF receptor has been shown to induce cell death in other cell types (29).

Myelin proteins and lipids

In the past few years, transgenic and genetic studies have contributed significantly to our knowledge of molecules that are involved in the formation, stability and function of the Schwann cell myelin sheath. These studies are important for an understanding of peripheral nerve pathologies and are reviewed in another chapter in this volume and elsewhere (104, 125, 148, 155, 162). Some of the most important findings are, however, basic to the biology of the Schwann cell and will be highlighted here particularly because some of the phenotypes observed are surprisingly similar to those seen in Krox-20 and Oct-6 knockout mice, suggesting that multiple signals can arrest Schwann cell differentiation at the pro-myelin stage. Knockout of the major peripheral myelin protein P_0, not surprisingly, leads to major disruptions in myelination, since P_0 contributes not only to compaction of the extracellular faces of the myelin membrane by homophilic interaction (47, 56), but also to the compaction of the cytoplasmic faces of the membrane. *in vitro* experiments using epithelial cells transfected with P_0, indicate that it can organise the membrane and cytoskeleton to form desmosomes (44). Importantly, in P_0 knockout mice, development of the myelin sheath is delayed or arrested, with abnormal numbers of axon-Schwann cell units at the 1:1 promyelin stage with a similar morphology to that seen in the Krox-20 and Oct-6 knockout mice (103). Progressive axon loss is also seen, particularly associated with the thickest myelin-like sheaths around the largest axons. The pathology seen in P_0+/- heterozygote mice is also instructive since a crucial role for P_0 in maintenance of myelin integrity is exposed. These mice express half as much P_0 protein as normal mice, and myelin sheaths appear morphologically normal until the 10th postnatal week. By 4 months of age abnormalities in the nerve are apparent, with some myelin sheaths that appear too thin compared with their axon calibre, often surrounded by supernumerary Schwann cells forming onion bulbs, indicative of myelin degeneration and associated Schwann cell proliferation. The results indicate that gene dosage is important, perhaps because there is an insufficient supply of P_0 in the steady state to compensate for normal turnover of P_0 in the membrane (104). Another myelin associated component, MAG, also plays a role in stabilising myelin. In MAG-/- knockout mice, myelin forms normally in the PNS (98, 118), but abnormalities appear in mice that are older than 8 months of age, most probably because MAG has a role in stabilising the interaction between the axon and the inner Schwann cell membrane. Degenerating myelin profiles and supernumerary Schwann cells are seen, particularly associated with the motor axons in the femoral nerve, together with newly formed myelin sheaths of thinner calibre. In mice that express neither MAG nor P_0, myelin formation occurs even later than in mice deficient in P_0 alone, with increased numbers of cells delayed at the pro-myelin stage, suggesting that MAG may play a minor role in this process (104), a result also suggested by *in vitro* experiments using a dominant negative retroviral construct of MAG to inhibit myelination in DRG neuron-Schwann cell co-cultures (132).

Gene dosage effects on myelin structure are also seen in the case of PMP 22, another peripheral myelin protein. This gene is frequently duplicated in Charcot-Marie-Tooth Type 1A peripheral neuropathy and deleted in hereditary neuropathy with liability to pressure palsies (HNPP) (165). Although the precise function of PMP 22 in the myelin membrane is not known, knockout of the gene in mice leads, as in the case of P_0 knockout, to severe delays in myelination, with increased numbers of cells delayed at the pro-myelin stage, focal hypermyelination followed by demyelination, Schwann cell proliferation and cycles of demyelination followed by remyelination, indicating roles in spiralling of the myelin membrane, regulation of myelin thickness and stability (1, 104). Moderate over-expression of mRNA in transgenic rats leads to hypomyelination, demyelination and Schwann cell proliferation, mimicking effects seen in some Charcot-Marie-Tooth Type 1A patients, again pointing to the importance of a need for the prop-

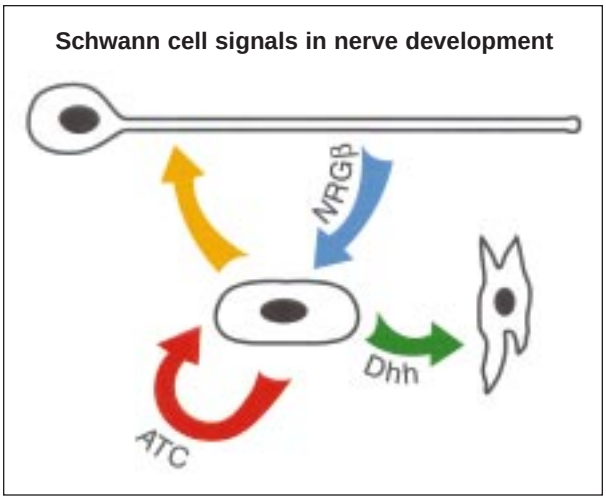


Figure 5. Schwann cells provide autocrine signals (ATC) that sustain their own survival (red arrow), signals that provide support for neurons (yellow arrow) and also, as shown by Desert Hedgehog (Dhh), help to organise the connective tissue sheaths around the nerve (green arrow).

er quantity of protein in the membrane (151). Proteolipid protein, the major myelin CNS protein, and the related protein DM20, are also expressed in Schwann cells. Recent analysis of families with a mild form of Pelizaeus-Merzbacher disease in which there is no expression of either PLP or DM20 protein, indicates that PLP, expressed at low levels in the myelin sheath in normal human nerves, plays a role in stabilising the myelin sheath, since patients with this defect show progressive demyelinating peripheral neuropathy not seen in more severe forms of the disease (59). It is interesting that mutations in the gap junction protein, Connexin 32 cause similar destabilisation of the myelin sheath leading to Charcot-Marie-Tooth Type X neuropathy in humans and destabilisation of the myelin sheath in mice (6, 11, 22). Connexin 32 probably contributes to the gap junctions that connect the cytoplasm of successive paranodal loops and Schmidt-Lantermann incisures (145, 148), and it has been shown that in addition to missense mutations that fail to form functional channels, other mutations lead to dominant negative effects or to functional channels with altered permeability characteristics (22, 128, 130). Thus efficient communication between the Schwann cell nucleus and the adaxonal cytoplasm via gap junctions is vital for the stability of the myelin sheath. In contrast, galactocerebroside or its related molecules sulfatide or galactodiglyceride may contribute to the insulating properties of the myelin sheath. While knockout of the major synthetic enzyme UTP-galactosyl-ceramide transferase has few effects on Schwann cell myelin membrane spiralling, thickness or compaction, perhaps due to the compensating effect of glucocerebroside, it has subtle effects on nerve conduction velocity, which is somewhat slow in mutant mice (21, 36). The results suggest that these lipids maybe involved in the maintenance of an ion permeability barrier in the myelin sheath (162).

Desert Hedgehog and the perineurium

We have described how axonal signals can support Schwann cell precursor and Schwann cell survival, and conversely, have indicated the importance of Schwann cell signals for neuronal survival in development and adult life. The importance of Schwann cells signals for regeneration is well known and will be reviewed elsewhere in this volume. Relatively little attention has been paid to Schwann cell signalling to the mesenchyme. Recently we have obtained evidence that this is important in the formation of the connective tissue sheaths around the nerve, the endo-, peri- and epi-neurium which are of fibroblastic origin (24). These sheaths are important in providing a protective covering around the nerve, and in maintaining the blood-nerve barrier (129). In a mouse in which the Desert Hedgehog gene has been knocked out (17), we have found that the connective tissue sheaths in adult nerves are clearly abnormal (133). In particular the perineurium is abnormally thin, consisting of one to three layers only, instead of 5-8 layers. The perineurial cells have a patchy as opposed to continuous basal lamina, and appear unusually wavy, rather than taut as in a normal perineurium. The epineurial sheath is abnormally thin and absent in some places, whereas there appear to be too many fibroblastic cells within the endoneurium. Unlike normal endoneurial fibroblasts, these cells form junctions with one another and have patchy basal lamina, being indistinguishable at the electron microscope level from the mutant perineurial fibroblasts. They form multiple mini-fascicles within the nerve. Preliminary tests suggest that the blood-nerve barrier is compromised. In normal nerves Desert Hedgehog transcripts can be detected by in situ hybridisation in developing nerves as early as E11.5 (17) and the signal is maintained until at least postnatal day 10, being only weakly detectable in adult nerves (133). mRNA for the Desert Hedgehog receptor Patched (16, 163) can be detected in the mesenchyme immediately around the nerve at E15.5, a stage at which the perineurium is starting to form in the mouse peripheral nervous system, suggesting that Desert Hedgehog molecules secreted by Schwann cells are signalling to the surrounding connective tissue cells to organise the perineurium (133) (Fig 5).

Schwann cell cytoskeleton and interactions with the basal lamina

Signalling from the extracellular matrix, and in particular the basal lamina, plays an important role in polarisation and differentiation of diverse cell types, including Schwann cells. Many studies have indicated the importance of the Schwann cell basal lamina in myelin formation (25). Laminin, a major component of the basal lamina, exists in several different forms and recent studies have highlighted its role in maintaining normal Schwann cell function in peripheral nerves. The major form in the Schwann cell basal lamina is laminin 2 (merosin), (consisting of α2, β1 and γ1 chains), although smaller amounts of laminin 1 are also present (76). It turns out that the skeletal muscle atrophy and complex pathology of peripheral motor nerves seen in the dy/dy mouse and most of the classical forms of human congenital muscular dystrophy are due to a deficiency in the α2 chain of laminin 2 (for review see 20; 107). The most severe Schwann cell abnormalities observed in the dy/dy mouse include areas in the dorsal and ventral spinal roots where naked axons are surrounded by undifferentiated Schwann cells which do not penetrate the axon bundles. More general defects seen not only in the roots but also within peripheral nerve trunks, include inappropriately thin myelin sheaths, excessively wide nodes of Ranvier and a patchy basal lamina surrounding individual nerve fibres (2). Two major receptors for laminin 2 present in the Schwann cell membrane are α-dystroglycan, a member of the dystrophin complex of proteins, and α6β4 integrin (51, 52, 66, 108). Alpha-dystroglycan links to the transmembrane protein β-dystroglycan and thus indirectly to dystrophin, which contains an actin-binding domain and thus is linked to the cytoskeleton (20; see however 143). The other main laminin receptor, α6β4 integrin, is strongly expressed in differentiated Schwann cells including myelinating Schwann cells (49, 53, 54, 126; see 116 for discussion of this point). It is thus likely that interaction of laminin 2 with a-dystroglycan and/or α6β4 integrin is involved in Schwann cell differentiation and in myelin stability. In epithelia, α6β4 is found in hemi-desmosomes where it is linked to keratin filaments, but hemi-desmosomes have not been seen in Schwann cells, and a peripheral nerve phenotype has not been reported in β4 knockout mice (173). Another important interaction of Schwann cell derived laminin 2 is seen in leprosy. This disease, caused by Mycobacterium leprae, is characterised by infiltration and infection of the Schwann cells of sensory nerves of the skin. M leprae binds specifically to the G domain of the laminin α2 chain of laminin 2. It is suggested that in this case the adherence of M leprae via laminin 2 occurs via interaction with a β4 integrin (possibly α6β4) (137).

Several other integrins including α1β1, α2β1, α5β1, α6β1, an unidentified β1 integrin, β8 integrin, αvβ3 and αvβ1 are associated with Schwann cells *in vivo* or in culture (50, 54, 115, 161). Some of their functions are starting to be elucidated. In Schwann cell-neuron cocultures, α6β1 is expressed by Schwann cells prior to myelination and has indirectly been implicated in myelination. Since antibodies to β1 block myelination whereas antibodies to α1β1 do not, this implies that α6β1 (the A variant) is the receptor required for myelination to proceed (54, 73). Integrins are frequently linked to the cytoskeleton and it is interesting to note that drug induced disruption of the actin cytoskeleton, also leads to a block of myelination (55). In migration studies, Schwann cell migration on laminin 1 and 2 in response to neuregulin and forskolin was blocked by antibodies to β1. Antibodies to α6β1 blocked migration on laminin 1 but not laminin 2, while RGD peptides blocked migration on fibronectin, suggesting involvement of αv integrins (115). Thus Schwann cells have a considerable choice of ligands and receptors available for migratory interaction with the extracellular matrix. Possible roles for another integrin, α1β1, are suggested by its distribution *in vivo*. It is not strongly expressed by Schwann cell precursors or embryonic or neonatal Schwann cells in developing nerves but it is expressed by mature nonmyelinating Schwann cells and by all Schwann cells in adult nerve after transection. It might therefore have a role in the interaction of small diameter axons with their surrounding Schwann cells, in the interaction of regenerating axons with Schwann cells or in Schwann cell migration (161). Its expression is upregulated by TGFβs and phorbol esters and down-regulated by neuregulin and forskolin or forskolin alone (50, 161). Prominent β8 immunolabelling has been detected around myelin sheaths in mouse peripheral nerve, suggesting a possible role for this integrin in myelination, but this has not been tested (115). In addition to their association with extracellular matrix components, cytoskeletal components and in other cell systems, growth factors and their receptors, integrins also associate with cell adhesion molecules (74). In a Schwann cell line, the tetraspan membrane protein CD9 is associated with integrins α3, α6 and β1. It appears to be involved in Schwann cell adhesion, proliferation and migration perhaps via its association with integrins (5, 8, 64, 65, 124).

Two other molecules of potential importance in Schwann cells should be mentioned in the context of the

cytoskeleton. The first, periaxin, exists in two alternatively spliced forms. At the N terminus, both forms contain a PDZ domain. This domain is found in proteins that interact with the cytoplasmic tail of plasma membrane proteins and with the cortical cytoskeleton. In myelinating Schwann cells, the large form of periaxin is associated with the plasma membrane, like other proteins containing this motif such as the tight-junction associated protein ZO-1, while the smaller form is in the cytosol (48, 61, 145). At present it is not known what proteins periaxin associates with, either in the membrane or within the myelinating Schwann cell cytoplasm, but its appearance just before the onset of myelination suggests an involvement in myelination. Merlin (Schwannomin), the product of the neurofibromatosis type 2 tumour suppressor gene, is the second molecule of interest. It is related to the radixin/ezrin/moesin family of proteins which link membrane proteins to the actin cytoskeleton in epithelia and other cell types (141, 172). Since mutations in the gene lead to an increased frequency of Schwannomas it is likely to play an important role in the negative control of Schwann cell proliferation. It is localised at the paranodal membranes of myelinating Schwann cells where it co-localizes with the small GTP-binding protein RhoA, associated with actin based movement in other cell types (146). It interacts directly with βII spectrin, present in Schwann cells. Spectrin in turn binds to actin and treatment with antisense nucleotides to merlin causes cells to round up, with concomitant changes to the actin cytoskeleton (149). It will now be important to determine whether merlin interacts with integrins, growth factor receptors or other proteins in the Schwann cell plasma membrane.

Conclusions and future prospects

An understanding of how cells of the Schwann cell lineage develop, from their origins in the neural crest to differentiation into myelinating and non-myelinating Schwann cells is emerging. The use of a combination of more traditional morphological and immunocytochemical methods both *in vivo* and *in vitro* combined with the newer transgenic and molecular biological methods has resulted in significant new insights into Schwann cell neurobiology. In particular the ability to use this wide array of techniques has led to substantial advances in our knowledge of the signals that bias cells towards the lineage, support their survival and progression once they have entered it, and sustain differentiation of the myelin sheath. The demonstration of the importance of the β neuregulin family of growth factors at several stages of the lineage is an outstanding example of this combined approach. Transgenic technology in combination with crystallographic, human genetic and *in vitro* molecular biological methods has contributed to our understanding of factors that contribute to the formation and stability of the myelin sheath and has revealed the role of mutations not only of myelin proteins but also of the gap junction protein connexin 32 in Charcot-Marie Tooth neuropathies. Although we still do not understand the mechanism of their action, two transcription factors involved in myelination, Krox-20 and Oct-6, have been identified, and doubtless other transcription factors that affect not only myelination, but also other stages in the lineage, will be uncovered in the next few years. The linkage between the extracellular matrix, basal lamina, Schwann cell plasma membrane and the cytoplasmic signalling machinery is receiving increased attention, and is implicated in both congenital muscular dystrophy and type 2 neurofibromatosis. Understanding the role of connexin 32 in communication from the abaxonal to the adaxonal Schwann cell cytoplasm represents a challenge that will have important medical implications. Another area that is of current interest is the node of Ranvier. To understand the mechanisms and molecules involved in establishing and maintaining it will be important for our knowledge of both neuronal and glial cell biology. While axonal signals have long been known to control Schwann cell phenotype the identity of the axonal signals that control myelination is still unknown and attempts to identify these will remain at the forefront of research effort. An important role for Schwann cells and their precursors in influencing the survival and differentiation of other cells that make up a peripheral nerve, namely neurons and the mesenchymal cells that contribute to the peri-, epi- and endoneurial sheaths is emerging. The Desert Hedgehog mediated induction of perineurial formation provides an example of Schwann cells taking part in organising nerve development by acting as a source of developmentally important signals. We believe that this illustrates an important role for these cells as active participants in the generation of the PNS and that the near future will see more instances of Schwann cells acting in this context.

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