### Abstract

Immature Schwann cells found in perinatal rodent nerves are generated from Schwann cell precursors (SCPs) that originate from the neural crest. Immature Schwann cells generate the myelinating and non-myelinating Schwann cells of adult nerves. When axons degenerate following injury, Schwann cells demyelinate, proliferate and dedifferentiate to assume a molecular phenotype similar to that of immature cells, a process essential for successful nerve regeneration. Increasing evidence indicates that Schwann cell dedifferentiation involves activation of specific receptors, intracellular signalling pathways and transcription factors in a manner analogous to myelination. We have investigated the roles of Notch and the transcription factor c-Jun in development and after nerve transection. In vivo, Notch signalling regulates the transition from SCP to Schwann cell, times Schwann cell generation, controls Schwann cell proliferation and acts as a brake on myelination. Notch is elevated in injured nerves where it accelerates the rate of dedifferentiation. Likewise, the transcription factor c-Jun is required for Schwann cell proliferation and death and is down-regulated by Krox-20 on myelination. Forced expression of c-Jun in Schwann cells prevents myelination, and in injured nerves, c-Jun is required for appropriate dedifferentiation, the re-emergence of the immature Schwann cell state and nerve regeneration. Thus, both Notch and c-Jun are negative regulators of myelination. The growing realisation that myelination is subject to negative as well as positive controls and progress in molecular identification of negative regulators is likely to impact on our understanding of demyelinating disease and mechanisms that control nerve repair.

**Key words:** demyelination, neuregulin, PNS, regeneration, Schwann cell lineage

### Introduction

The two main cells that reside in adult peripheral nerves are myelinating Schwann cells, which surround large axons, and non-myelinating Schwann cells, which enclose smaller axons in invaginations of their surfaces. One of the most striking features of these cells is their plasticity. This property is seen most obviously in the dedifferentiation of both myelinating and non-myelinating Schwann cells that occurs after nerve cut or crush injury but is also seen when myelinating Schwann cells dedifferentiate in neuron-free cultures. The dedifferentiated Schwann cells possess a molecular phenotype that is in many ways similar to that of immature Schwann cells during development. On dedifferentiation, myelinating cells down-regulate myelin-associated molecules, re-express cell adhesion molecules such as neural cell adhesion molecule (NCAM) and L1 and express growth factors and cytokines, which are conducive to axonal regeneration (Jessen and Mirsky, 2005). An important question that we are interested in addressing is whether the...
demineralization process simply results from reduced activity in pro-myelin signalling pathways, which is the conventional view, or whether, in a mirror image of myelination, it requires activation of specific cell-intrinsic dedifferentiation signalling machinery (Fig. 1).

An Outline of Schwann Cell Development

To set the scene, it is appropriate to outline the process of Schwann cell development. Myelinating and non-myelinating Schwann cells of adult spinal nerves are derived from the neural crest (for reviews, see Jessen and Mirsky, 1999; 2005; Lobsiger et al., 2002) (Fig. 2). Schwann cells in dorsal and ventral roots and satellite glial cells within the ganglion also originate in part from other early cell pools such as boundary cap cells (Marto et al., 2004). There are three main developmental transitions involved. First, Schwann cell precursors (SCPs), which occupy nerve trunks at embryonic day (E) 12/13 in the mouse, are generated from migrating neural crest cells. Next, SCPs transform into immature Schwann cells, which occupy nerves from E15/E16. Finally around birth, these cells start to generate first the myelinating Schwann cells and subsequently the non-myelinating Schwann cells found in adult nerves. All these transition points involve a choice of fate because in vivo neural crest cells give rise to several other cell types besides glia, SCPs give rise to Schwann cells and endoneurial fibroblasts, and immature Schwann cells will become either myelinating or non-myelinating cells depending on the axons with which they associate (reviewed in Jessen and Mirsky, 2005).

The extracellular signals that control fate choice in the Schwann cell lineage are not well understood. It is unclear what signals or mechanisms enable or direct crest cells to enter the glial lineage in vivo, and the signalling systems that promote myelination in Schwann cells are only starting to be revealed (see below). Despite this, three cell-cell signals that regulate Schwann cell development have been identified by a combination of in vitro and in vivo experiments. These are neuregulin-1, which carries out a number of major functions in developing and myelinating nerves, endothelin, which is involved in the SCP/Schwann cell transition, and Notch signalling (Brennan et al., 2000; Garratt et al., 2000b; Wakamatsu et al., 2000; Kubu et al., 2002; Nave and Salzer, 2006) (see below).

In addition to these signals, important roles have been revealed for extracellular matrix molecules, their receptors and downstream intracellular effectors in Schwann cell proliferation and the radial sorting of axons that occurs prior to myelination and in myelination itself (reviewed in Feltri and Wrabetz, 2005; Court et al., 2006; Chen et al., 2007). This includes molecules such as LG14 and ADAM 22 (Agee et al., 2005; Bermingham et al., 2006), laminins and their receptors, β1 integrins and dystroglycans and the small GTPases, cdc42 and Rac1 (Chen and Strickland, 2003; Court et al., 2006; Benninger et al., 2007; Nodari et al., 2007). A detailed discussion of these mechanisms is outside the scope of this review.

Two transcription factors are known to be indispensable for Schwann cell development. The first, Sox10, is essential for the generation of the earliest cells in the Schwann cell lineage and has recently been shown to be required for myelination (Britsch et al., 2001; Schreiner et al., 2007; reviewed in Jessen and Mirsky, 2005), while the second, Krox-20 (Egr-2), is essential for myelination (Topilko et al., 1994; Topilko and Meijer, 2001). In Krox-20 null mice, Schwann cells adopt a 1:1 relationship with axons but fail to myelinate, indicating the importance of this transcription factor in regulating the myelination programme (Topilko et al., 1994). It is also required for long-term maintenance of myelin because tamoxifen-induced excision of Krox-20 in adult nerves results in demyelination and dedifferentiation (Decker et al., 2006). Furthermore, transfection or infection of Krox-20 into dedifferentiated cultured mouse or rat Schwann cells from neonatal sciatic nerves is sufficient to induce a wide range of myelin genes and proteins (Nagarajan et al., 2001; Parkinson et al., 2004). It also, as we have shown, inactivates the proliferative response of cultured Schwann cells to neuregulin-1 and the death response to transforming growth factor (TGFβ), both of which are characteristics of the immature Schwann cell phenotype (Zorick et al., 1999; Parkinson et al., 2001; 2004).

Other transcription factors Oct-6 (SCIP, Tst-1, and POU5f1) and, to a lesser extent, Brn-2 are required for the correct timing of myelination, while in vitro evidence suggests that the transcription factor NFκB is involved in the control of Oct-6 expression and is thus

![Figure 1. Regulators of myelination. The myelination status of Schwann cells can be viewed as being determined by the balance between opposing signalling systems. Positive regulators (green) dominate in normal nerves, while the balance shifts to negative regulators (orange) in injured and pathological nerves. During development, negative regulators may also take part in timing the onset and rate of myelination.](image-url)
also likely to be involved in the timing of myelination (Topilko and Meier, 2001; Jaegle et al., 2003; Nickols et al., 2003).

In vivo, SCPs and immature Schwann cells proliferate rapidly with a peak of DNA incorporation at the immature Schwann cell stage (Stewart et al., 1993; Yu et al., 2005). Therefore, cell division is compatible with the differentiation processes that transform the phenotype of crest cells, first, to that of SCPs and then to that of immature Schwann cells. Exit from the cell cycle occurs only at the final transition as immature Schwann cells differentiate into myelinating and non-myelinating cells.

A remarkable feature of the Schwann cell lineage is the rapid reversibility of this last step of Schwann cell development (Fig. 2). Removal of Schwann cells from contact with axons, which initiates the reversal, can be achieved either by injuring nerves in vivo or by dissociating cells from post-natal nerves and placing them in culture without neurons. Both in vivo and in vitro, the process entails the developmental regression and dedifferentiation of individual Schwann cells and myelin breakdown. In vivo, Schwann cells start to digest their own myelin, aided at later stages in the process by macrophages (Hirata and Kawabuchi, 2002). In this way, it is likely that myelin-related inhibitors of axon regeneration are removed. As the characteristic molecular markers and structural features of myelinating and non-myelinating cells are lost, the cells start to proliferate and re-express markers of immature Schwann cells prior to myelination, including molecules associated with the promotion of axonal growth such as L1 and NCAM (Fig. 3). They also up-regulate expression of trophic factors and cytokines including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 (MCP-1) and extracellular matrix molecules including laminin-2 and 8 (for reviews of this topic, see Scherer and Salzer, 2001; Chen et al., 2007). The phenotype of these denervated Schwann cells is very related to the phenotype of immature Schwann cells prior to myelination, and they form a favourable substrate for regrowing axons. On re-association with axons, these cells redifferentiate and myelinate readily during nerve regeneration (Fawcett and Keynes, 1990; Scherer and Salzer, 2001).

Recent evidence from our own and other laboratories suggests that the dedifferentiation process depends on the activation of particular intracellular signalling pathways and transcription factors. These signals, which oppose positive regulators of myelination that include Krox-20, cAMP activation and axonal myelination signals such as neuregulin-1, may collectively be referred to as negative regulators of myelination (Fig. 1). The identification of negative regulators of myelination, i.e., specific signalling pathways and transcription factors that inhibit or reverse myelination, makes it possible to view the myelination status of Schwann cells as determined by the balance between opposing signalling systems.

Some of the other transitions in the lineage may also be reversible. Although reversal of the phenotype...
of immature Schwann cells leading to the reformation of SCPs has not been observed, analogy with other tissues suggests that it may well be possible. SCPs have a unique, glial-associated molecular and cytoarchitectural phenotype that differentiates them unambiguously from neural crest and other crest derivatives (Figs. 4 and 5) (Jessen and Mirsky, 2005; Wanner et al., 2006a). In their normal signalling environment in developing nerve trunks, evidence suggests that although the majority become Schwann cells, a minority become endoneurial fibroblasts, while in culture, they can also give rise to neurons and smooth muscle cells (Morrison et al., 1999; Joseph et al., 2004). It is also possible that some SCP-like cells might be present in perinatal nerves (Bixby et al., 2002) and even adult nerves may contain cells with an early phenotype corresponding to SCPs or immature Schwann cells (Rizvi et al., 2002). Many other tissues, including the central nervous system (CNS), the enteric nervous system, muscle and the haematopoietic system, retain cell populations with a phenotype and developmental potential reminiscent of early developing cells in their respective lineages.

**Lineage Analysis**

The use of a set of well-defined molecular markers has greatly facilitated analysis of the different stages of the Schwann cell lineage (Figs. 3 and 4). The transition from the neural crest to the SCP stage is marked by the appearance of brain fatty acid–binding protein (BFABP) (mouse not rat), myelin protein zero (P₀) mRNA, desert...
hedgehog, connexin 29 and cadherin 19 (Jessen and Mirsky, 2005; Li et al., 2007). Cadherin 19 is interesting because it is down-regulated again as SCPs generate Schwann cells and is therefore the only well-characterised marker that is restricted to the SCP stage, at least in vivo (Takahashi and Osumi, 2005).

Figure 4. The phenotypic characteristics of each of the key stages in embryonic development. The boxes above the lineage drawing show the molecular profile of each step. Yellow boxes show molecules expressed by all three cell types, while the other boxes show stage-restricted molecules. Note that cadherin 19 (Cad 19) is exclusively expressed by Schwann cell precursors (SCPs). Each developmental stage involves characteristic relationships with surrounding tissues and distinctive cell signalling properties (boxes below lineage drawing). For instance, neural crest cells associate with and migrate through the extracellular matrix (ECM). In 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 peripheral nervous system and central nervous system. Basal lamina is absent from migrating crest cells and SCPs but appears on Schwann cells. In vitro, neuregulin-1 (NRG1) only supports crest cell survival in the presence of ECM, although this is not required for neuregulin-1-supported survival of SCPs and Schwann cells. Migrating neural crest cells also fail to survive in the presence of several factors that support the survival of SCPs and Schwann cells, including combinations such as fibroblast growth factor (FGF) plus insulin-like growth factor (IGF), endothelin (ET) plus IGF and platelet-derived growth factor (PDGF) plus neurotrophin 3 (NT3) and IGF. Schwann cells also have autocrine survival circuits that are absent from SCPs. *Proteins that also appear on neuroblasts/early neurons. †Markers that are acutely dependent on axons for expression. ‡Gliarial fibillary acidic protein (GFAP) is a late marker of Schwann cell generation because 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 negative and Schwann cells to be S100 positive using routine immunohistochemical methods – low levels of S100 are, however, detectable in many mouse SCPs when the sensitivity of the assay is increased. ‡‡Integrin α4 (V. Sahni and K.R.J, University College London, London, unpublished observations), AP2α, activator protein 2α; BFA, brain fatty acid–binding protein; DHH, desert hedgehog; ErbB3, neuregulin receptor; GAP43, growth-associated protein 43; L1, L1 cell adhesion molecule; Ncad, N-cadherin; Oct6, octamer-binding transcription factor 6; O4, oligodendrocyte; PLP, proteolipid protein; PMP22, peripheral myelin protein 22 kDa; P0, protein zero; P75NTR, p75 neurotrophin receptor; Sox10, SRY (sex-determining region Y) box 10. Reproduced from Nature Reviews Neuroscience with modifications (Jessen and Mirsky, 2005).
Immature Schwann cells express S100β, GFAP and the glycolipid antigen O4, molecules that are not expressed or expressed only at very low levels by SCPs. At the transition between SCPs and Schwann cells, the transcription factor AP2α is down-regulated in addition to the down-regulation of cadherin 19 mentioned earlier (Jessen and Mirsky, 2005).

At the onset of myelination, myelin-associated molecules including Krox-20, myelin-associated glycoprotein, periaxin, P0, myelin basic protein, peripheral myelin protein 22, plasmolipin and galactocerebroside are all strongly up-regulated. Myelination is also accompanied by equally extensive down-regulation of a set of molecules that characterise the immature Schwann cell stage such as L1, NCAM and the p75 neurotrophin receptor. Most of these are still expressed by mature non-myelinating Schwann cells. Additionally, these cells express at least two molecules, α1β1 integrin and galactocerebroside, which are not expressed by immature Schwann cells (Jessen and Mirsky, 2005) (Fig. 3).

As mentioned earlier, the reversion of the molecular phenotype of myelinating cells to one similar to that of immature Schwann cells is seen after nerve injury or when myelinating cells are dissociated from their accompanying axons (Fig. 2). Recently, the use of genescreen arrays has revealed many other significant differences in mRNA expression between the myelinated and the immature state, but at present, few of these have been definitively validated as markers of transitions in the lineage (Araki et al., 2001; Nagarajan et al., 2001; 2002; Bermingham et al., 2002; Verheijen et al., 2003; Buchstaller et al., 2004; Le et al., 2005a; 2005b; Bosse et al., 2006; D’Antonio et al., 2006a).

**Schwann Cell Precursors**

The SCP defines the first stage of glial differentiation in peripheral nerve trunks (Jessen et al., 1994; Dong et al., 1995; 1999; Jessen and Mirsky, 2005; 2007). Most or all of the cells in the limb nerves of E14/15 rats (E12/E13 mice) are SCPs, although it is possible that a few neural crest-like cells remain in these nerves (see above). The boundary cap cells found in dorsal and ventral roots at this stage are distinct from SCPs. In vivo boundary cap cells give rise to almost all the Schwann cells in the dorsal roots, and they also generate a small population of sensory neurons (Maro et al., 2004). Unlike Schwann cells, they transiently express Krox-20 at an early developmental stage (around E10) and have important functions in maintaining the CNS/peripheral nervous system (PNS) boundary (Maro et al., 2004; Bron et al., 2007).
Schwann Cell Lineage

The Importance of Neuregulin-1 in the Schwann Cell Lineage

As should already be evident, the importance of neuregulin-1 signalling at all stages of Schwann cell development from the neural crest stage to fully...
Differentiated Schwann cells cannot be overemphasized (Garratt et al., 2000b; Lemke, 2006; Nave and Salzer, 2006). In an impure form, neuregulin-1 was initially isolated as one of the first mitogens for cultured rat Schwann cells, being later identified as the type II isoform of neuregulin-1 (Marchionni et al., 1993). Neuregulin-1 is considered to be the major axonally derived Schwann cell mitogen. As mentioned earlier, mice lacking either the neuregulin-1 or the neuregulin receptors ErbB2 or ErbB3 have embryonic nerve trunks that are essentially devoid of SCPs, although, interestingly, development of satellite glia within DRGs appears to be normal, as is the initial development of DRG neurons (Meyer and Birchmeier, 1995; Riethmacher et al., 1997; Morris et al., 1999; Woldeyesus et al., 1999; Garratt et al., 2000b). Sympathetic ganglia, however, fail to develop normally in these animals (Britsch et al., 1998). These striking phenotypes have provided essential clues for understanding the role of neuregulin signaling in embryonic development of the PNS.

Because satellite glia within ganglia and Schwann cells along nerve trunks both develop from neural crest cells, the normal occurrence of DRG satellite cells in neuregulin mutants suggests that neuregulin-1 signaling is not obligatory for glial differentiation from the neural crest in vivo. This is in line with the observation that GFAP-positive glia develop readily from cultured neural crest cells with or without added neuregulin (Shah et al., 1994). Although neuregulin-1 strongly represses the formation of neurons from cultured crest cells, the neuregulin mutants do not provide any obvious indication, such as overproduction of neurons, to support the idea that neuregulin-1 constrains neurogenesis in vivo. The most important reason for the remarkable reduction or absence of SCPs from nerve trunks in neuregulin mutants is likely to be connected to the role of neuregulin-1 as a survival factor for SCPs and early Schwann cells (Dong et al., 1995; Leimeroth et al., 2002; Jessen and Mirsky, 2005). Unlike Schwann cells, SCPs cannot support their own survival by autocrine mechanisms and instead depend entirely on juxtacrine signals from axons (see above). In developing nerves, they are closely apposed to axons and die rapidly when they are removed from axonal contact either by culture in the absence of axons or by nerve transection in vivo (Jessen et al., 1994; Dong et al., 1995; Ciutat et al., 1996; Winseck and Oppenheim, 2006).

Neuregulin-1 isoforms are found in vivo in embryonic DRG and motor neurons and accumulate along axonal tracts (Marchionni et al., 1993; Loeb et al., 1999). Furthermore, cultured DRG neurons are a potent source of both axon-associated and axon-secreted survival signals that have been identified as neuregulin-1 isoforms (Dong et al., 1995; Taveggia et al., 2005). Neuregulin-1 is therefore available at the right time and place to enable the survival of precursors in embryonic spinal nerves. Mutants lacking isoform III of neuregulin-1, the major isoform in peripheral nerves, provide further support for these observations (Wolpowitz et al., 2000). In these mice, early SCPs start populating nerves at E11, but by E14, a time when precursors are converting rapidly to Schwann cells, the number of cells in the nerves is severely depleted. This argues against the idea that defective migration of precursors into nerve trunks is the major reason for absence of precursors in the nerves of neuregulin mutants (below). Neuregulin-1 also supports the survival of early glia in embryonic chick nerves (Winseck and Oppenheim, 2006). Taken together, these observations suggest that a major function of neuregulin-1 in embryonic nerves is to ensure the survival of SCPs.

Defective migration is unlikely to be the major explanation of the lack of SCPs in developing nerves in neuregulin signaling mutants. On the other hand, the failure of these mutants to develop sympathetic ganglia is likely to be caused by impairment in the migration of neural crest cells along the ventromedial pathway to the sympathetic ganglia (Britsch et al., 1998). From studies on mutant mice, it has been concluded that isoform I neu-differentiation factor (NDF), acetylated choline receptor inducing activity (ARIA), isoform II glial growth factor (GGF) and, in particular, isoform III (SMDF, cysteine-rich domain-neuregulin-1) are the most important neuregulin-1 isoforms for PNS and Schwann cell development (Garratt et al., 2000b; Nave and Salzer, 2006). Animals with inactivation of the neuregulin-1 gene, and therefore of all three isoforms, or of the neuregulin receptors ErbB2 or ErbB3, have fewest SCPs in their peripheral nerve trunks. Mice with selective inactivation of neuregulin-1 isoform III initially show a milder phenotype, although at later stages, SCP numbers are severely depleted and Schwann cells are essentially absent from the distal portion of the nerves (above) (Wolpowitz et al., 2000). In contrast, in mice lacking neuregulin-1 isoforms I and II, glia develop normally, although muscle spindle development is deficient (Meyer et al., 1997; Hippenmeyer et al., 2002). Therefore, isoform III is most crucial for Schwann cell development. Isoforms I and II contain an immunoglobulin domain, bind heparin and are cleaved from the membrane by metalloproteases, forming soluble proteins that associate with cell surfaces (Adikofer and Lai, 2000; Nave and Salzer, 2006), whereas isoform III is mostly expressed as a transmembrane protein (Wang et al., 2001). Membrane-bound, but not soluble, isoform III induces S100 and Oct-6 expression in P0-positive cultured non-neuronal cells from embryonic rat DRGs (Leimeroth et al., 2002). This suggests, first, that membrane-bound forms may have functions not shared by soluble forms and, second, that one of the isoforms has a unique function that is provided only in a membrane-bound form.
functions of neuregulin-1 is to accelerate the SCP/Schwann cell transition (see also Brennan et al., 2000).

An important new function for neuregulin-1 has been revealed by experiments using mice in which either erbB2 in Schwann cells or neuregulin-1 type III in neurons has been conditionally excised or overexpressed. In these mice, excision of erbB2, or haploinsufficiency of neuregulin-1 type III, results in hypomyelination of peripheral nerves, while overexpression of neuregulin-1 type III in neurons results in hypermyelination (Garratt et al., 2000a; Michailov et al., 2004). Experiments in tissue culture further reveal that lack of neuregulin-1 type III in DRG neurons results in defective axonal ensheathment and lack of myelination, while overexpression of the same isoform in sympathetic neurons, which in culture are normally poorly ensheathed and do not myelinate, results in ensheathment and myelination (Taveggia et al., 2005). Taken together, these results indicate that neuregulin-1 type III regulates the thickness of the myelin sheath, is important in axonal ensheathment and is likely to be part of the signalling mechanism from axons that controls myelination in the PNS (Nave and Salzer, 2006). Part of the programme by which neuregulin-1 controls both CNS and PNS myelination is likely to involve neuregulin processing. This involves cleavage by beta-site amyloid precursor protein-cleaving enzyme 1 because knockout of this enzyme results in a delay both in myelination and in hypomyelination (Hu et al., 2006; Willem et al., 2006).

As mentioned above, it is commonly assumed that axonal neuregulin-1 type III is a major mitogen for Schwann cells during development, although in vivo evidence for this plausible notion in mammals is missing (Nave and Salzer, 2006). In zebrafish, ErbB2 receptor blockers reduce proliferation in vivo (Lyons et al., 2005). The only growth factor implicated in driving Schwann cell proliferation in mammalian Schwann cells during development in vivo is TGFβ (D’Antonio et al., 2006b) (see below).

The possibility that axonal neuregulin-1 is a Schwann cell mitogen in vivo raises, however, an important question. Namely, how does the signalling environment within the Schwann cell change as it prepares to myelinate so that neuregulin-1 type III stops driving cell division and instead promotes myelination?

TGFβ and the Schwann Cell Lineage

In vitro, TGFβ has long been known to stimulate Schwann cell proliferation or death, depending on the culture conditions used (reviewed in Jessen and Mirsky, 2003). More recently, this has been investigated using mice with conditional inactivation of TGFβ type II receptor, which results in ablation of TGFβ signalling specifically in Schwann cells. Schwann cell proliferation in perinatal nerves of these mice is substantially reduced. Schwann cell death, however, is also decreased, with the result that Schwann cell numbers are not substantially altered in the absence of TGFβ signals. It has been suggested that the biological significance of TGFβ signalling in perinatal nerves is to amplify the proliferation of cells with tight axonal contact while promoting the death of supernumerary cells with less effective axonal affiliation. Lack of TGFβ signalling in vivo does not affect myelination in contrast to expectations raised by results obtained previously using cell culture systems (D’Antonio et al., 2006b).

Multiple Roles of Notch in Controlling Schwann Cell Development and Dedifferentiation

As mentioned previously, the role of Notch in controlling fate determination in the neural crest is complex and in many aspects incompletely understood. We have recently been investigating the role of Notch at specific well-defined stages of the Schwann cell lineage. We find that the effect of Notch is highly dependent on the cell type in the lineage in which it is acting. Our results indicate that Notch signalling is unlikely to have an instructive role in directing the fate of neural crest cells toward the Schwann cell lineage (Jessen and Mirsky, 2005; Woodhoo et al., 2007, University College London, London) (unpublished observations). This is in agreement with earlier results on the role of Notch in the chick neural crest (Wakamatsu et al., 2000). Rather, during development, Notch acts as a maturation signal for SCPS, as a mitogen for immature Schwann cells and as an inhibitor of myelination. In injured adult nerves, Notch signalling accelerates dedifferentiation (Woodhoo et al., 2004; 2007, University College London, London, unpublished observations).

Interestingly, Krox-20, a transcription factor with a major role in promoting myelination, as mentioned earlier, represses Notch, and this repression is necessary for the timely onset of myelination in perinatal nerves. Because Notch is re-expressed in injured nerves, where it drives dedifferentiation of myelinating cells, the common effect of Notch in developing and injured adult nerves is to promote or maintain the immature or dedifferentiated Schwann cell state (Woodhoo et al., 2007, University College London, London) (unpublished observations).

C-Jun Is a Negative Regulator of Myelination That Drives Schwann Cell Dedifferentiation In Vivo

C-Jun is a leucine-zipper zinc-finger transcription factor, a key component of the AP-1 transcription
complex, and the terminal component of the Jun-N-terminal kinase (JNK) pathway (e.g., Mechta-Grigoriou et al., 2001). It is strongly expressed in immature Schwann cells at E17 prior to the onset of myelination, in cultured Schwann cells and in Schwann cells in the distal stump of transected nerves (De Felice and Hunt, 1994; Stewart, 1995; Shy et al., 1996; Parkinson et al., 2004). We have shown that in vitro c-Jun is required for Schwann cell proliferation in response to neuregulin-1 (Parkinson et al., 2004), and is also required for TGFβ-induced cell death, an event that occurs in vivo both during normal development and after neonatal nerve transection (Parkinson et al., 2001; D’Antonio et al., 2006b). In contrast to the high c-Jun levels prior to myelination and in injured nerves, c-Jun expression levels in myelinating cells are low (Parkinson et al., 2004; Bhaskaran et al., 2007). The expression profile suggests that c-Jun may play a role in immature Schwann cells and dedifferentiating Schwann cells.

As mentioned above, Krox-20 is the single most important transcription factor in the control of myelination (Topilko et al., 1994; Zorick et al., 1999; Nagarajan et al., 2001; Parkinson et al., 2004; Decker et al., 2006). Krox-20 also inactivates the proliferative response of cultured Schwann cells to neuregulin-1 and the death response to TGFβ, both of which are characteristics of the immature Schwann cell phenotype (Parkinson et al., 2001; 2004). Significantly, Krox-20 suppresses c-Jun, in line with the low c-Jun levels in myelinating cells. This is seen both in vitro and in vivo, where c-Jun levels remain high in post-natal nerves of Krox-20 null mice, although they are suppressed in wild-type nerves. The suppression of c-Jun is therefore likely to be part of the mechanism by which Krox-20 prevents proliferation and death in response to neuregulin-1 and TGFβ, respectively (Parkinson et al., 2004).

We have also found that c-Jun is an important regulator of the differentiation state of Schwann cells. c-Jun acts as a potent suppressor of the myelin phenotype, a function that appears to be independent of its role in proliferation and death. c-Jun blocks myelination in neuron-Schwann cell co-cultures, and when c-Jun is expressed in Krox-20-expressing cells in culture, it prevents the up-regulation of P0 and periaxin that normally occurs in response to Krox-20 (Parkinson et al., 2008).

We have recently examined early post-natal nerves in mice with selective inactivation of c-Jun in Schwann cells. Perhaps surprisingly, myelination is not obviously accelerated or otherwise disturbed in these nerves, as might have been expected in the absence of a protein that potentially opposes myelination signals (as shown in the cell culture experiments mentioned above). This is likely to reflect the fact that even in normal nerves, c-Jun is effectively suppressed by Krox-20-related signalling at the onset of myelination. Enforced c-Jun expression also inhibits the expression of Krox-20, P0 and periaxin seen in response to elevation of cAMP levels.

It was examination of the opposite process of dedifferentiation that has revealed a striking role for c-Jun, namely that of driving myelinating cells back toward the immature state. When nerves are transected, c-Jun is strongly up-regulated as myelinating cells dedifferentiate during Wallerian degeneration. We found that this process is markedly delayed in nerves with selective inactivation of c-Jun in Schwann cells, a finding that we have backed up by observations in c-Jun null cells in vitro and by the use of JNK inhibitors. It seems clear therefore that the negative influence of c-Jun on myelin differentiation that can be demonstrated in a number of in vitro tests is, in vivo, more important for pushing the dedifferentiation programme in injured or pathological nerves than for regulation of myelination during normal development. An important consequence of the delayed dedifferentiation seen in the absence of c-Jun in Schwann cells is a striking absence of functional recovery after nerve injury (Arthur-Farraj et al., 2007; Parkinson et al., 2007; 2008).

**Negative Regulators of Myelination: Multiple Mechanisms**

Apart from the in vivo observations on Notch and c-Jun signalling outlined above, the idea that demyelination requires the engagement of distinct intracellular signalling pathways and transcription factors has been raised elsewhere in the context of extracellular-related kinase (ERK)1/2 activation and the transcription factor Sox2 in cell culture studies, and both ERK1,2 and Sox2 are up-regulated after injury (Sheu et al., 2000; Harrisingh et al., 2004; Ogata et al., 2004; Le et al., 2005a; Agthong et al., 2006). Other in vitro experiments also show that p38 mitogen-activated protein kinase, which is activated in injured nerves (Myers et al., 2003), is important for pushing dedifferentiation (Fragoso et al., 2003; Bhaskaran et al., 2007). Thus, it is already evident that many distinct mechanisms, when activated in injured or pathological nerves, have the potential to drive the dedifferentiation of myelinating cells. In addition, delayed myelination is seen in mice with genetic inactivation of toll-like receptors, nitric oxide synthase, phospholipase A2 and matrix metalloprotease 9, although in these cases, it is not entirely clear whether Schwann cells or macrophages are playing the major role (Levy et al., 2001; De et al., 2003; Shubayev et al., 2006; Boivin et al., 2007).

In future work, it will be important to investigate the relationship between signalling pathways within Schwann cells that negatively regulate myelination and...
cell-extrinsic signals that are injury related and arise as a result of nerve damage. It will also be of interest to determine the relative contribution of these systems and haematogenous macrophages in transforming injured nerves into an environment that supports axon growth and nerve repair. An equally important question is whether inappropriate activation of negative myelin regulators contributes to the demyelination that occurs in inherited and acquired peripheral neuropathies.

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