Schwann cells as regulators of nerve development

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Abstract

Myelinating and non-myelinating Schwann cells of peripheral nerves are derived from the neural crest via an intermediate cell type, the Schwann cell precursor [K.R. Jessen, A. Brennan, L. Morgan, R. Mirsky, A. Kent, Y. Hashimoto, J. Gavrilovic. The Schwann cell precursor and its fate: a study of cell death and differentiation during gliogenesis in rat embryonic nerves, Neuron 12 (1994) 509–527]. The survival and maturation of Schwann cell precursors is controlled by a neuronally derived signal, β neuregulin. Other factors, in particular endothelins, regulate the timing of precursor maturation and Schwann cell generation. In turn, signals derived from Schwann cell precursors or Schwann cells regulate neuronal numbers during development, and axonal calibre, distribution of ion channels and neurofilament phosphorylation in myelinated axons. Unlike Schwann cell precursors, Schwann cells in older nerves survive in the absence of axons, indicating that a significant change in survival regulation occurs. This is due primarily to the presence of autocrine growth factor loops in Schwann cells, present from embryo day 18 onwards, that are not functional in Schwann cell precursors. The most important components of the autocrine loop are insulin-like growth factors, platelet derived growth factor-BB and neurotrophin 3, which together with laminin support long-term Schwann cell survival. The paracrine dependence of precursors on axons for survival provides a mechanism for matching precursor cell number to axons in embryonic nerves, while the ability of Schwann cells to survive in the absence of axons is an absolute prerequisite for nerve repair following injury. In addition to providing survival factors to neurones and themselves, and signals that determine axonal architecture, Schwann cells also control the formation of peripheral nerve sheaths. This involves Schwann cell-derived Desert Hedgehog, which directs the transition of mesenchymal cells to form the epithelium-like structure of the perineurium. Schwann cells thus signal not only to themselves but also to the other cellular components within the nerve to act as major regulators of nerve development.

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1. Introduction

The work described here illustrates some of the ways in which Schwann cells and their precursors act as a source of a range of developmental signals. These signals influence both cellular differentiation and survival in early nerves. In this way, Schwann cells regulate the development of the three main cell types in nerves: neurones, connective tissue cells, and the Schwann cells themselves by autocrine circuits. In particular, the development of neurones is likely to depend equally on factors from the targets they innervate and on factors from the glia that surround them. Recent progress in identifying the molecules involved in this signalling indicates that developing Schwann cells use a number of different factors to communicate with their neighbours. Further advances in this area, together with the identification of the neurone-derived factors that act on Schwann cells and control their specification, survival and differentiation, will gradually reveal the complex signalling network required to build a peripheral nerve.

2. Schwann cell origins

The majority of Schwann cells in adult nerves originate from the neural crest [1,8,31,39,44]. The crest is also likely to give rise to the other main glial types in the peripheral nervous system, including the teloglia of somatic motor terminals, satellite cells associated with
neuronal cell bodies in sensory, sympathetic and parasympathetic ganglia and the enteric glia in the ganglia of the gut [22,23,53]. The neural crest is probably not the only source of peripheral glia, however, since it appears that some Schwann cells in ventral roots arise from the ventral neural tube and that some satellite and/or Schwann cells or dorsal root ganglia originate from neuroepithelial cells in the spinal cord after migration of the neural crest is complete [10,42,43,57,63].

3. Schwann cell generation

Two intermediate cell types are known to be involved in the generation of Schwann cells from neural crest cells (Fig. 1). The first, the Schwann cell precursor, is found in rat peripheral nerves at embryo day (E) 14 and 15 (mouse E12 and 13). The second, the immature Schwann cell, is present from E17 (mouse E15) to around birth. At this time, the immature cells start to differentiate. Myelinating Schwann cells mature first, the non-myelinating cells appearing later. Therefore, three main transition points are involved in the lineage. These are the transition of crest cells to precursors, of precursors to immature Schwann cells and lastly the formation of the two mature Schwann cell types [32,33,47]. This final step is also axon-dependent and reversible (see later). It is still not clear how the glial lineage originates from the neural crest, a group of cells that also gives rise to other lineages, including neurones and melanocytes. Many crest cells, at least in birds, appear already to have entered distinct lineages at the onset of crest migration [28]. Other studies indicate that instructive signals regulate crest diversification. In a study on clonally derived rat crest cells, β neuregulins (see later) biased crest differentiation towards glia by blocking entry to the neuronal lineage [61]. In related studies, transforming growth factor β (TGFβ) promoted a neuronal phenotype in groups of cells, while it generated smooth muscle cells in sparsely plated cultures. Bone morphogenetic protein 2 (BMP2) stimulated entry to the autonomic neuronal lineage [26,62]. These experiments indicate that β neuregulin may act instructively to promote glial differentiation. They remain, however, to be reconciled with studies on knockout mice. These suggest that β neuregulin is required for neurogenesis from the cephalic neural crest in vivo and that the number of dorsal root ganglion (DRG) neurones is initially normal, rather than excessive, in mice lacking the ErbB3 protein, a major receptor for β neuregulin in crest cells and early glia [46,58].

4. Schwann cell precursors

Schwann cell precursors were first identified as a distinct glial cell type present in peripheral nerves of rat embryos at E14/15 (mouse E12/13) [32]. Like late migrating neural crest cells, Schwann cell precursors in developing nerves express low, basal P0 mRNA levels, providing continuity in molecular phenotype between these two distinctive cell types [40]. They possess other more distinctive molecular and morphological phenotypic properties, which are listed in Table 1. While essentially all the cells in E14 and 15 rat nerves are precursors, by E17 (mouse E15), nearly all the cells are Schwann cells. The generation of Schwann cells from precursors therefore takes place relatively abruptly, although the differences between these two cells extend to a number of diverse and apparently unrelated phenotypes. There is as yet no clear evidence for the persistence of a precursor population in mature nerves, although in view of the existence of adult stem cells in many different tissues it would perhaps be surprising if it did not exist.

A notable feature of the precursor cell is its acute dependence on axonal signals for survival. For details of the experimental methods used to demonstrate this, see [14,21,36]. There is extensive evidence, obtained first in vitro [14,36] and subsequently from transgenic null mice [46,58], that the axonal signal which regulates precursor survival is β neuregulin acting via ErbB3/ErbB2 receptors on the precursors (reviewed in [20,32,48]). β neuregulin
also supports the conversion of isolated precursor cells to Schwann cells [14] and acts as an axon-associated mitogen and survival factor for perinatal Schwann cells [25,49,70,74]. Axonal β neuregulin, signalling via ErbB3 and ErbB2 receptors on glial cells, therefore has a central role in regulating the embryonic development of the Schwann cell lineage.

The precise timing of the Schwann cell precursor to Schwann cell conversion is regulated by two other factors. Fibroblast growth factor [15] positively regulates the rate of the transition while endothelin, acting via endothelin B receptors, negatively regulates the speed of the transition [6].

5. Schwann cells

In rodents, the postnatal formation of myelinating and non-myelinating cells is a slow process, taking several weeks to complete. During the early postnatal period, immature Schwann cells diverge, generating myelinating cells that wrap the large diameter axons and non-myelinating cells that accommodate small diameter axons in shallow troughs along their surface. As mentioned earlier, it is assumed that this process is driven by signalling from axons, although the molecular nature of axon-Schwann cell communication, particularly in myelinating Schwann cells, remains elusive.

Three transcription factors, Sox-10, Oct-6 (SCIP, Tst-1) and Krox-20 (Egr-2), are known to be required in this process [2,3,7,30,37,38,50,66,73]. This topic has been reviewed elsewhere and is outside the scope of this article [7,29,34].

While biochemical and morphological changes occur as both types of Schwann cell differentiate, they are much more extensive in the cells that myelinate [31]. The formation of the myelin sheath requires extensive membrane synthesis, radical changes in gene expression, and cytoskeletal modification to allow membrane spiralling and wrapping. Myelin proteins, which include periaxin, MAG, P0, MBP and PMP22, are strongly up-regulated, while another set of proteins which include N-CAM, p75NGF receptor and GFAP, all of which are expressed by immature Schwann cells and mature non-myelinating cells, are down-regulated [35]. Remarkably, these axon-induced changes are largely reversible. If mature Schwann cells lose contact with axons, for instance following nerve transection, they promptly undergo radical changes in morphology and gene expression leading to developmental regression of individual Schwann cells and myelin breakdown. In nerves, these processes are accompanied by Schwann cell proliferation [17]. The eventual outcome is the generation of a single population of cells that are comparable, although not identical, to immature Schwann cells in neonatal nerves [24,31]. Such cells provide an environment particularly conducive to axonal re-growth, which is probably related to their relatively high expression of neurotrophic factors and cell adhesion molecules [60]. Thus, the dramatic regression response of Schwann cells to loss of axon contact in damaged nerves, together with the autocrine mechanisms that allow Schwann cells to survive in the absence of axonal contact (see later) forms the basis for nerve regeneration and repair in the PNS.

Re-establishment of appropriate axonal contact, as seen in regenerating nerves or in myelinating co-cultures of Schwann cells and neurones, leads to re-differentiation, including myelination (see later and [31,60]). Schwann cells regulate axonal calibre, distribution of ion channels and neurofilament phosphorylation, topics which are outside the subject of this paper, and which are reviewed elsewhere [34].

Table 1
Some of the main differences between precursors and Schwann cells in the sciatic nerve of rat and mousea

<table>
<thead>
<tr>
<th>Precursors</th>
<th>Schwann cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Die by apoptosis when removed from axons and plated in vitro</td>
<td>Full survival under same conditions</td>
</tr>
<tr>
<td>No autocrine loops</td>
<td>Due to autocrine loops</td>
</tr>
<tr>
<td>S100 (cytoplasmic)−b</td>
<td>S100 (cytoplasmic)+</td>
</tr>
<tr>
<td>Do not divide in response to FGFe</td>
<td>Divide in response to FGFs</td>
</tr>
<tr>
<td>Flattened with extensive cell-cell contacts in vitro</td>
<td>Bi- or tri-polar in vitro</td>
</tr>
<tr>
<td>Motility+++</td>
<td>Motility+</td>
</tr>
</tbody>
</table>

a Schwann cell precursors also differ from neural crest cells in a number of characteristics. They express GAP-43 protein, [36] Desert Hedgehog mRNA [55] and brain fatty acid binding binding protein, [7] under conditions where neural crest cells do not.

b Precursors are S100 negative (and Schwann cells are S100 positive) when observed with our routine immunolabelling methods. However, low levels of S100 immunoreactivity are detectable in many precursors in the mouse when the sensitivity of the immunolabelling method is increased significantly.

c This applies to rat only. In the mouse FGF is a mitogen under identical conditions.

d At present this has only been tested thoroughly in the mouse.
5.1. Schwann cells regulate their own survival

Strong evidence that the survival of precursors is acutely dependent on axonal survival signals mediated by β neuregulin has been provided by several laboratories including our own (earlier). The survival of Schwann cells, however, must be regulated in a different way, since transection of adult nerves does not lead to death of Schwann cells [25,74]. In fact, Schwann cells in the distal stump of transected nerves survive for a considerable time in the absence of axons, although their number gradually declines and they lose some responsiveness to extrinsic signals [41]. Likewise, Schwann cells survive well without neurones in vitro under normal culture conditions, i.e. when plated at moderate density in serum-containing or serum-free medium. In contrast, Schwann cell precursors die rapidly in vitro, even when plated at very high density [36]. These observations indicate that Schwann cell development involves a change in survival regulation: the survival of precursors depends on axonal signals, while Schwann cell survival is axon independent.

Schwann cell survival in the absence of axons is critical in the context of nerve regeneration. Schwann cells are left without axons in the nerve segment distal to an injury. For successful repair, axons must enter this segment and grow along it to allow guidance back to their correct targets. Numerous studies have shown that axon re-growth depends on the presence of living Schwann cells in the distal nerve segment, probably because the axons require interactions with Schwann cell associated adhesion molecules such as L1 and N-CAM, with extracellular matrix molecules such as laminin-2 and with trophic factors such as NGF, BDNF and NT3 [17,51]. Nerve regeneration, therefore, depends on a mechanism that allows Schwann cells to survive in the absence of axons.

Work in our laboratory shows that Schwann cells acquire the ability to survive without axons by establishing an autocrine survival loop [45]. Using cell cultures from rat sciatic nerve and brachial plexus, we have demonstrated that these survival loops exist in Schwann cells from E18 and postnatal nerves. They are not, however, functional in Schwann cell precursors from E14 nerves. Work of others on Schwann cell death in transected nerves and nerve terminals from neonatal and older rats, shows that at birth axons still provide detectable survival input (probably mediated by β neuregulins) to the Schwann cell population, including the teloglia of the neuromuscular junction [74]. One to two weeks later, cell death is no longer seen [25,70]. This indicates that the switch from axon-dependent survival to an axon-independent autocrine survival regulation occurs gradually as Schwann cells develop from precursors and mature in early postnatal nerves.

The Schwann cell derived survival signal is not mitogenic for Schwann cells. This observation, plus the fact that the signal does not promote Schwann cell precursor survival, make it unlikely that the signal is β neuregulin, although it does not exclude the possibility that very low levels of β neuregulin might be a component of the signal. The most important components of the autocrine survival include insulin-like growth factor 2 (IGF-2), platelet derived growth factor-BB (PDGF-BB) and NT3. Conditioned medium from densely plated Schwann cell cultures rescues Schwann cells plated at low density from death in a 2-day assay, and these three factors when used in combination prevent Schwann cell death under the same conditions. Furthermore, experiments with blocking antibodies indicate that IGF-2, PDGF-BB and NT3 are present in the Schwann cell conditioned medium, and that they act synergistically to block Schwann cell death [45]. The survival signal is transduced by the mitogen-activated protein kinase pathway. Interestingly, longer term survival requires an additional input signal. This is provided by laminin, which used alone is not a survival factor. The candidate autocrine growth factors and their receptors are expressed in Schwann cells in vitro and in vivo both before and after early postnatal nerve transection, while laminin-2 is a key component of the Schwann cell basal lamina. It is perhaps surprising to find that mRNAs for the growth factors and their receptors are detectable by RT-PCR in E14 sciatic nerve, when the nerve is populated by Schwann cell precursors, despite the fact that these cells do not possess autocrine survival loops. Whether protein expression levels are too low or alternatively some other component of the signalling pathway is not functional is presently unknown. Another factor reported to promote Schwann cell survival in combination with other survival signals is leukemia-inhibitory factor, known to be upregulated following nerve transection [16,68,69,72]. In addition to the autocrine function of these signals, the secretion of these factors by Schwann cells could be of more general significance since they are all known to regulate survival and differentiation of other cells, including neurones.

5.2. Interplay between survival and death

In some situations, cell death in the nervous system is controlled by an interplay between survival factors and negative survival signals that actively induce apoptosis. Based on the evidence described above, it is likely that two sets of signals play a major role in promoting the survival of developing Schwann cells. These are β neuregulins and autocrine Schwann cell signals. β neuregulin is mainly provided by axons, although there are reports of low levels of expression in Schwann cells [11,59,71]. It is probably of paramount importance in embryonic and early postnatal nerves, while the autocrine circuits are active in postnatal cells and likely to be especially significant following injury and consequent loss of axonal
β neuregulin. It is possible that Schwann cell survival is regulated exclusively by these and other positive survival factors, a view that would imply that Schwann cell death is caused by a limited availability of such signals, in line with classical neurotrophic theory. An alternative view suggests that cell death can be caused not only by the absence of survival signals, but also by active cell killing induced by factors that trigger apoptosis [12,56]. NGF is an example of a factor that can act like this, both in retinal development and in Schwann cells [12,18,19,56,65,76].

There is evidence that another growth factor, TGFβ, may also act as a death signal for Schwann cells. TGFβs have a variety of proliferative and phenotypic effects on Schwann cells, and are expressed by Schwann cells in a latent form (for reviews see [47,60]). We have shown that it induces Schwann cell apoptosis under a number of different conditions in vitro. This effect is blocked by the combined presence of β neuregulin and autocrine signals and, in accordance with this, TGFβ kills Schwann cells in the distal stump of cut neonatal nerves but not Schwann cells in normal neonatal nerves. The downstream signalling pathway involves phosphorylation and activation of c-Jun via c-Jun N-terminal kinase (JNK) (L. von Hertzen, R. Mirsky, K.R. Jessen, unpublished) and AP-1 dependent transcription. Expression of dominant negative Jun in Schwann cells reduces TGFβ-induced apoptosis, while over-expression of constitutively active Jun in Schwann cells causes increased cell death when survival signals are removed. A resistance to TGFβ killing develops as Schwann cells differentiate in vivo, and this is related to a failure of TGFβ to activate c-Jun in differentiated cells. Nerve transection leads to elevation of TGFβ1 in the distal stump of neonatal animals, and application of TGFβ to transected neonatal nerves increases cell death in the distal stump [54]. This accords with observations in the adult that suggest that this factor may be involved in events that follow nerve damage, especially in combination with tumour necrosis factor α [60,64] and implicates TGFβ as a negative Schwann cell survival signal in developing and perinatal nerves.

5.3. The control of perineurium formation

We have proposed that mutual signalling systems between neurones and Schwann cells and autocrine signals from Schwann cells to themselves are vital to nerve development and maintenance, but the third cellular component of the nerve must also be considered (Fig. 2). Peripheral nerve fibres (axon-Schwan cell units) are surrounded by a nerve sheath made from collagen fibres and a cellular tube, the perineurium, that also acts as a barrier against unwanted molecules and cellular infiltration. We have shown that a molecule secreted by Schwann cells, Desert Hedgehog, a member of the Hedgehog family of signalling molecules, is involved in the formation of not only the perineurium, but also of the endo- and epi-neurial connective tissue [9,52]. In normal mouse nerves Desert Hedgehog transcripts can be detected by in situ hybridisation in developing nerves as early as E11.5 [4] and the signal is maintained until at least postnatal day 10, being only weakly detectable in adult nerves [55]. mRNA for the Desert Hedgehog receptor Patched [4,67] 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 [55]. In nerve fibroblasts, Desert Hedgehog upregulates patched and hedgehog-interacting-protein mRNA expression. In mice that are null for Desert Hedgehog (dhh), the structure and function of the perineurium are severely abnormal [5]. The perineurium in these mice is abnormally thin, consisting of one to three cell layers, instead of five to eight layers. The perineurial cells have a patchy as opposed to continuous basal lamina, fail to express connexin 43, normally expressed by perineurial cells, and appear wavy, rather than taut as in a normal perineurium. The epi-neurial collagen sheath is also scanty and even absent in some places, whereas there appears to be too many fibroblast 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 cells. They form multiple mini-fascicles within the nerve, resembling the mini-fascicles

![Fig. 2. Diagram of nerve sheath formation. Early in development axons and Schwann cell precursors project towards their target tissues in the absence of mesenchymal ensheathing cells (black, orange and red circles). At around E16 (rat), E14 (mouse) unknown signals recruit mesenchymal cells to surround the nerve and form a loose permeable sheath around it. Desert Hedgehog (Dhh) from Schwann cells, probably acting together with other factors, is needed for the mesenchymo-epithelial transformation that turns this sheath into the multilayered, ordered perineurial sheath which forms a tight barrier between the nerve fibres and the surrounding tissue at around postnatal day 21. Individual cells within the layers are shown. Each cell layer is surrounded by a continuous basal lamina on either side (grey rings). Desert Hedgehog is also involved in the formation of the epineurial collagen sheath which surrounds the perineuria of individual nerve fascicles and binds the nerve together (green ring).](image-url)
that form in regenerating nerves [27]. The nerve-tissue barrier is compromised in terms of permeability to both proteins and migratory cells and the tight-junctions between perineurial cells are ultrastructurally abnormal and immature [55]. Although the movements of the \( dhh^- \) and \( dhh^+ / + \) mice appear indistinguishable when they are running freely [4], nerve conduction velocity in motor fibres, saphenous A fibres and C fibres showed on average a slightly lowered conduction velocity. Desert hedgehog knockout mice are sterile with malformed, hypertrophic testes, with abnormalities in the formation of adult-type Leydig cells, peritubular cells and seminiferous tubules [5,13]. In addition, in adult mice, the Sertoli cell junctions are abnormal (D. Lawson, unpublished observation). A similar phenotype, of partial gonadal dysgenesis with peripheral neuropathy with mini-fascicle formation, has been reported recently in a human patient homozygous for a mutation in the initiation codon of the \( dhh \) open reading frame [75]. This suggests a general requirement for Dhh in testis and nerve formation.

6. Conclusions

The building and maintenance of a peripheral nerve requires interplay between all the cellular components of the nerve. These include Schwann cells, neurones (axons) and the connective tissue cells of the endo-, peri- and epineurium. Here we have provided an overview of some of the signals that are known to be involved, while many others remain to be discovered.

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References


