Schwann cells and their precursors emerge as major regulators of nerve development

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It is becoming ever clearer that Schwann cells and Schwann-cell precursors are an important source of developmental signals in embryonic and neonatal nerves. This article reviews experiments showing that these signals regulate the survival and differentiation of other cells in early nerves. The evidence indicates that glial-derived signals are necessary for neuronal survival at crucial periods of development, that they regulate the molecular and functional specialization of axons and that they control the maturation of the perineurial sheath that protects nerves from inflammation and unwanted macro-molecules produced in the surrounding tissues. Furthermore, an autocrine survival circuit enables Schwann cells in postnatal nerves to survive in the absence of axons, a vital requirement for successful nerve regeneration following injury. The molecular identity of these signals and their receptors is currently being determined.


**SCHWANN CELLS** are renowned for their plasticity and their reliance on neuron-derived signals during development and in maturity. In developing nerves, axonal signals determine the survival, proliferation and differentiation of these cells. Even mature myelinating and non-myelinating cells revert to an immature phenotype if the axons they contact are made to degenerate by nerve transaction. Consequently, cells of the Schwann-cell lineage are often viewed as comparatively passive targets of extrinsic signals that drive differentiation, seen in its most complex form in the myelinating Schwann cell. It is becoming clear, however, that this is only one side of a more-involved tale. In this review, evidence from published and ongoing work is brought together building a different story: that Schwann cells and their distinct precursor cells are active regulators of nerve development. Evidence will be discussed that Schwann-cell signals crucially influence the development of all main components of peripheral nerves, namely the neurons, connective-tissue cells and even the Schwann cells themselves, via autocrine loops.

**An overview of Schwann-cell development**

The Schwann-cell lineage and its origin in the neural crest

The basics of the Schwann-cell lineage have now been elucidated (Fig. 1). Most Schwann cells develop from the neural crest. They appear in mature nerves as two quite different cells, the non-myelinating and myelinating Schwann cells, which are probably present in comparable numbers throughout the PNS. The generation of these cells from neural-crest cells involves the formation of two main intermediates, the Schwann-cell precursor, which is typically found in rat nerves at embryonic-day 14 (E14) and E15 (mouse E12 and E13; Fig. 2) and the immature Schwann cell, present from E17 (mouse E15) to the time of birth (Fig. 3). At this time, the cells start to differentiate, first along the myelin pathway, with mature non-myelinating cells appearing later. The lineage, therefore, involves three main transition points, that is, the transition of crest cells to precursors, of precursors to immature Schwann cells and finally the ultimate, and largely reversible, formation of the two mature Schwann-cell types (Fig. 4).
It is still unclear how the glial lineage originates from the neural crest, a group of cells that also gives rise to other lineages, including neurons and melanocytes. Many crest cells, at least in birds, appear already to have entered distinct lineages at the onset of crest migration. Other studies indicate that instructive signals regulate crest diversification. In a study on clonally derived rat crest cells, β-neuregulin (see below) biased crest differentiation towards glia by blocking entry to the neuronal lineage. In comparable studies, transforming growth factor β (TGFβ) promoted the generation of smooth-muscle cells and bone morphogenetic protein 2 (BMP2) stimulated entry to the autonomic neuronal lineage.

Thus experiments indicate that β-neuregulin might act instructively to promote glial differentiation. However, they remain to be reconciled with studies on knockout mice, which suggest that β-neuregulin is required for neurogenesis from the cephalic neural crest in vivo and which show that the number of dorsal root ganglion (DRG) neurones is initially normal, rather than excessive, in mice lacking EBR83 (avian erythroblastosis oncogene B3), a major receptor for β-neuregulin in crest cells and early glia.

One of the reasons for our ignorance about early glial development has been the lack of an early glial differentiation marker. However, it has now been shown that, in rat and chick, some migrating crest cells express a gene that is indicative of a glial phenotype. Perhaps surprisingly, this is the gene for the major peripheral myelin protein, P0. 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 immature Schwann cells, regardless of whether they are destined to form myelin or not. The striking increase in P0 synthesis that is induced by axons selectively in those cells that form myelin, therefore, represents an increase of pre-existing basal levels of this protein rather than novel gene expression. This is mirrored by axonally signalled suppression of even basal P0 levels in those cells that do not form myelin. This might be due to axonal survival signals. There is, as yet, no clear evidence for the persistence of a precursor population in mature nerves.

One of the most notable features of the precursor cell is its acute dependence on axonal survival signals. There is more extensive evidence, obtained first in vitro and subsequently from knockout animals, that the axonal signal regulating precursor-cell survival is β-neuregulin (reviewed in Refs 1,2). β-neuregulin also supports the conversion of isolated precursor cells to Schwann cells in vitro with a time course that is similar to that of Schwann-cell generation from precursors in vivo (see above). It also acts as an axon-associated mitogen and survival factor for perinatal Schwann cells. Signalling that involves axonal β-neuregulin binding to EBR83 on glial cells is, therefore, of paramount importance.

Schwann-cell precursors and the generation of Schwann cells

Schwann-cell precursors are the distinct glial cell type present in peripheral nerves of rat embryos at embryonic-day 15 (E15). The nuclei of three of the precursors are indicated (P1, P2 and P3). These cells display low, basal P0 levels and, as argued above, it is likely that this provides the continuity that allows us to trace the lineage from immature Schwann cells in perinatal nerves right back to its origin in the neural crest. Some of the distinctive phenotypic properties of the Schwann-cell precursors are listed in Table 1. While essentially all the cells in E14 and E15 rat nerves are precursors, by E17 (mouse E15), nearly all the cells are Schwann cells. Therefore, the generation of Schwann cells from precursors takes place abruptly, although the differences between these two cell types 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.
**REVIEW** K.R. Jessen and R. Mirsky – Schwann-cell signalling

Importance for regulating the embryonic development of the Schwann-cell lineage.

In a slow process, which in rodents starts around birth and takes several weeks to complete, the immature Schwann cells diverge, generating the myelinating cells that wrap the large-diameter axons and the non-myelinating cells that accommodate small diameter axons in shallow troughs along their surface. It is a central dogma in the field that this process is driven by signalling from axons, although the molecular nature of this axon–Schwann-cell communication has remained elusive. While biochemical and morphological changes occur in both types of Schwann-cell differentiation, they are much more extensive in the cells that myelinate. These cells carry out the large amount of membrane synthesis and wrapping needed to form the myelin sheath, and undergo extensive changes in gene expression and protein synthesis. The myelin proteins, which include P0, myelin basic protein (MBP) and peripheral myelin protein (PMP22), are upregulated. This is followed by downregulation of another group of proteins, exemplified by neural cell-adhesion molecule (NCAM), the neurotrophin receptor p75, and glial fibrillary acidic protein (GFAP), that are synthesized by immature Schwann cells and mature non-myelinating cells. Remarkably, these axon-induced changes are largely reversible. If mature Schwann cells lose contact with axons, for example, following nerve transection, they promptly undergo radical changes in morphology and gene expression that lead to developmental regression of individual Schwann cells and myelin breakdown. These processes are accompanied by Schwann-cell proliferation. 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. Presumably, owing to relatively high levels of neurotrophic factors and cell-adhesion molecules in these cells, they form an environment that is particularly conducive to axonal re-growth. Thus, the dramatic regression response of Schwann cells to loss of axonal contact in damaged nerves, together with the autocrine mechanisms that allow Schwann cells to survive in the absence of axonal contact (see below) forms the basis for nerve regeneration and repair in the PNS.

Transcription factors in Schwann-cell differentiation

When compared with our knowledge of transcription factors in insect glia and some other vertebrate cells, including neurones, our knowledge of transcription factors in vertebrate glial development is less advanced. Two of the most promising forays into this area have been made in studies on Schwann cells and concern the POU-domain factors OCT6 (also known as POU3F1, SCIP and TST1) and the zinc-finger protein, EGR2 (also known as Krox-20). Strikingly, the knockout of either...
of these factors in transgenic mice results in an arrest of Schwann-cell myelination17–19. Further exploration of the role of OCT6 has continued to produce intriguing results. In an alternative approach to inactivation of OCT6, the gene for S100A, a dominant-negative protein with respect to OCT6, was expressed under the promoter of \( p_{\pi} \) in transgenic mice. A major aim of this work was to achieve selective inactivation of S106 in cells that had been induced to myelinate, a significant consideration in view of the fact that OCT6 is expressed, albeit at lower levels, in Schwann-cell precursors and immature Schwann cells well before myelination20. However, rather than blocking myelination, an expectation based on the phenotype of S106−/− mice, myelination in those animals was both premature and excessive21. Remarkably, Schwann cells from these mice appear to promote axon outgrowth strongly when they are implanted into regenerating nerves of normal animals22. In order to explain some of these findings, it has been suggested that in these mice, \( \Delta S C E P \) might block OCT6 function, at least partially, or, alternatively, that it might act as a gene activator23,24. Finally, it should be noted that the myelination arrest in OCT6−/− mice is only temporary, which perhaps suggests the existence of other POU-domain factors in Schwann cells that can replace OCT6 during myelination25. Clearly we are some way from understanding the function of POU-domain proteins in Schwann-cell development.

Inactivation of OCT6 or Er81 in mice blocks Schwann-cell development at the pro-myelination stage. At this point, Schwann cells that are due to myelinate envelop large-diameter axons but do not wrap around them. In addition, the strong myelination-associated upregulation of \( p_{\pi} \) and MBP has not yet taken place. There is some evidence that a number of other unrelated ways of interfering with Schwann-cell development also block myelination at a similar stage. Pro-myelina arrest is, for example, seen in neurons–Schwann-cell co-cultures where myelination is prevented by using antibodies to galactocerebroside26. Similarly, mice in which the gene encoding \( p_{\pi} \) is mildly overexpressed or knocked out, show reduced myelination, with many cells appearing to stall at the pro-myelina stage27. (L. Wraits, pers. comm.)

<table>
<thead>
<tr>
<th>Precursors</th>
<th>Schwann cells</th>
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<tr>
<td>Died by apoptosis when removed from axons and plated in vitro because they have no autocrine loops</td>
<td>Full survival when removed from axons and plated in vitro owing to autocrine loops</td>
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<tr>
<td>S106(zygoplastic) negative</td>
<td>S106(zygoplastic) positive</td>
</tr>
<tr>
<td>Do not divide in response to fibroblast growth factor</td>
<td>Divides in response to fibroblast growth factor</td>
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<tr>
<td>Flattened, with extensive cell–cell contacts in vitro</td>
<td>Bi- or tri-polar in vitro</td>
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<td>04 antigen negative</td>
<td>04 antigen positive</td>
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<tr>
<td>High motility</td>
<td>Low motility</td>
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*In this system include the zinc-finger protein EGR1 (also known as Krox-24) and SOX10, a member of the SRY-like high mobility group (HMG) domain class of transcription factors. It will also be interesting to see whether the ongoing search for cell-type-specific B-class basic helix–loop–helix (bHLH) factors in Schwann cells proves successful. The promoters of the genes for P0, MBP and \( \rho_{\pi} \) all contain the activating E-box sequence to which bHLH dimers bind. This, together with the fact that Schwann cells have recently been shown to synthesise two groups of proteins, A-class bHLH proteins and Id factors, which are thought to regulate specific B-class bHLH activity, makes it likely that important B-class genes will be identified in Schwann cells.*

Schwann cells and their precursors are active regulators of nerve development

The control of neuronal survival

Perhaps the most striking proposal for a regulatory role for PNS glia has come from observations on the \( E \) mice mentioned earlier. These experiments have raised the possibility that the survival of major neuronal populations, including DRG neurons and motoneurons, depends on trophic support from Schwann-cell precursors and early Schwann cells. In this context, the key observation was that the \( E \) mice, which lack Schwann-cell precursors and Schwann cells, also lost most of their sensory and motoneurons during the second half of embryonic development. Examination of neurone numbers in these animals showed that although the number of DRG neurones was normal at E12, the number of neurones was reduced by about 80% by E14. The motoneurones died somewhat later: their numbers were still normal at E16 but by E18 about 80% were lost, judging from counts of motor axons. The death of the DRG neurones is probably unrelated to interactions between neurones and their peripheral targets, as it takes place before most axons have reached their target fields. Experiments on chimeric animals also argue against the possibility that the DRG or motoneurones have died owing to absence of EBR3 on the neurones themselves. Therefore, the simplest explanation for the loss of DRG neurones is that signals from Schwann-cell precursors (the cells missing from the nerves of these animals during the time of great DRG neuron death between E12 and E14) are normally required for the survival of these cells. This suggests a sequential model in which survival of DRG neurones is regulated, initially, by glial-derived signals as the axons elongate towards their targets and, subsequently, by
target-derived signals once the axons have reached their target fields, as described in the classical neurotrophic theory40,41. This would agree with previous work showing that sensory neurones require growth-factor support early in development and prior to target innervation42–44. Motoneurone death in the Erbb3 knockout mouse occurs relatively late, and after most neurones have reached and interacted with their target, that is, skeletal muscle. This indicates that for motoneurones, glial- and target-derived survival signals are likely to act at the same time, rather than sequentially as proposed above for DRG neurones, and also that the glial source of the motoneurone survival signal is the immature Schwann cell rather than the Schwann-cell precursor.

The search is now on for neuronal survival factors that are derived from developing Schwann cells. Although ciliary neurotrophic factor (CNTF) in conjunction with leukemia inhibitory factor take part in regulating motoneurone death in the adult, this combination is unlikely to be involved in controlling motoneurone survival in the embryo, as CNTF is only present in Schwann cells postnatally45,46. A better candidate, at present, is glial cell-line-derived neurotrophic factor (GDNF) and its relatives, which include neurturin. GDNF mRNA is found in Schwann-cell precursors and immature Schwann cells in embryonic nerves. It is a potent survival factor for motoneurones in vitro and mice that lack GDNF lose about 20% of their motoneurones by birth47,48. On the basis of experiments with a Schwann-cell line that secretes GDNF, it has been proposed that Schwann-cell-derived GDNF acts synergistically with muscle-derived neurotrophin 1 to control motoneurone death49. The fact that DRG and motoneurone loss in mice that lack developing Schwann cells is more extensive than that generally seen in mice lacking single neurotrophic factors or their receptors, could mean that these cells support neuronal survival using a cocktail of factors. It is also likely that the Schwann-cell-derived trophic factors that support motoneurones differ from the precursor-derived trophic factors required by DRG neurones. GDNF, for example, is unlikely to be employed by Schwann-cell precursors to support survival of DRG neurones between E12 and E14, the period of DRG neurone death in the Erbb3 knockout mouse, given that the Ret GDNF receptor is only found in a few DRG neurones at this stage48. The Ret receptor is, however, found in early motoneurones and in DRG neurones later in development. This could account for the 23% loss of DRG neurones in GDNF knockout mice at birth48. While the identity of any trophic signals derived from Schwann-cell precursors is unknown, a number of observations point to neurotrophin 3 (NT3) as a possible candidate. It is produced by Schwann cells at birth and by tissues associated with embryonic ganglia. Unambiguous evidence for its presence in precursors is, however, not yet available49. In vitro, NT3 promotes survival and differentiation of early sensory neurones. Embryos into which neutralizing NT3 antibodies have been injected, and NO knockout animals have a reduced number of DRG neurones early in embryonic development50,51. All of these observations indicate that this neurotrophin has a role in supporting DRG neurones before target innervation. Nevertheless, it remains to be seen whether NT3 is a component of a signal by which Schwann-cell precursors support the survival of early DRG neurones.

The node of Ranvier

Another event in neuronal development that is regulated by Schwann cells is the formation of the nodes of Ranvier. In this case, it is likely that signals from developing Schwann cells have a crucial role in inducing the complex molecular specialization of the nodal membrane, in particular the striking clustering of Na+ channels that is vital for the function of myelinated axons. The Ranvier node is a remarkably intricate structure morphologically and in molecular composition52–54 (Fig. 5). While the structure has been known for some time, the corresponding molecular organization is still emerging. It appears that each structural compartment of the node is characterized by a distinct group of proteins. The paranodal Schwann-cell membranes possess voltage-gated K+ channels, which are likely to be involved in K+ buffering at the node, connexin 32, a component of the gap junctions between the paranodal loops, and the adhesion molecules E-cadherin and myelin-associated glycoprotein (MAG)55–58. The axonal membrane at the paranode contains contactin-associated glycoprotein (known as caspr or paranodin), a transmembrane protein with a large extracellular domain. It is likely that this glycoprotein interacts with the axonal cytoskeleton, being bound to L1 protein, while its extracellular domain might be involved in forming the intimate junctions between the axon and the paranodal Schwann-cell loops seen using electron microscopy59.60. Two distinct protein variants, 480 and 270 kD, derived from the axon, gene are major components of the axonal cytoskeleton that underlies the node itself. This region also contains neuronal NgCAM-related cell-adhesion molecule (NeCAM) and a distinct isoform of neurofascin, both of which contain aspartyl-binding domains. It has been proposed that an axon–neurofascin–NeCAM complex forms a transmembrane structure in the nodal membrane that corresponds to the transmembrane filaments previously seen with electron microscopy59. It is likely that another major function for axonin at the nodal membrane is the anchoring of voltage-gated Na+ channels. Functionally, these channels are the key molecules at the node. They are highly concentrated in...
the nodal membrane and represent its defining molecular feature. Thus, most of the recent work on how Schwann cells regulate nodal development has relied on examining the levels of Na\(^+\) channels. These experiments, which were carried out on many different systems, have shown a strong correlation between the presence of Schwann cells (although not necessarily in direct contact with axons—see below) and the appearance of focal aggregates of Na\(^+\) channels in axonal membranes.\(^{10,11,56,57}\) This leaves little doubt that the production of Na\(^+\) channel clusters depends on factors released from Schwann cells. A feature of nodal development that has become clear from these studies is the striking spatial relationship between Na\(^+\) channel clusters and Schwann cells. This is seen in a number of situations: during nerve development, during repair following chemical myelination, and when Schwann cells are added to axons in vitro. In all these cases, Na\(^+\) channels are almost always restricted to the axon membrane at the edge of a Schwann cell or its process. A similar correlation is found between the location of Schwann cells and the distribution of intramembranous particles characteristically found at Ranvier nodes.\(^{26}\) In remyelinating nerves the distance between Na\(^+\) channel clusters increases as Schwann cells elongate along the axons. The channel clusters remain associated with the edge of individual Schwann cells as they move and they appear to fuse when two cells come into close proximity with each other. This indicates an intriguing dynamic relationship between clusters of Na\(^+\) channels and the position of Schwann cells along axons. This observation together with those that correlate the location of clusters with the edges of Schwann cells, are open to different interpretations. In particular, they do not tell us unambiguously whether the Schwann cells determine channel clustering, or whether the clusters determine the location of Schwann cells. While these and related questions have caused lively controversy in the past, three recent papers could point to how some of the differing views might be reconciled. Experiments by Kaplan et al.\(^{58}\) on the effects of oligodendrocytes on Na\(^+\) channel formation in vitro, indicate that a protein secreted by oligodendrocytes is required for these cells to produce immunohistochemically detectable clusters of Na\(^+\) channels. Furthermore, in the presence of this signal, but in the absence of direct contact with oligodendrocytes, the channel clusters show a regular spacing along the axons. The observations of Deenick et al.\(^{59}\) on the dystrophic mouse 129/Leda\(^{60}\), provide another example of Na\(^+\) channel clusters apparently forming in the absence of direct glial contact. In the spinal roots of these mice, there are abnormal areas that contain bundles of closely packed, large-diameter unmyleinated axons. Remarkably, these naked axons exhibit discrete focal concentrations of Na\(^+\) channels. Schwann cells appear to be entirely absent from the axons in these regions, although they are present and myelinate surrounding axons. In this context, the observations of Hinson et al.\(^{61}\) are particularly pertinent because they suggest that Schwann cells, like oligodendrocytes, can promote the formation of Na\(^+\) channel clusters at a distance. In these in vitro experiments, addition of Schwann cells to Schwann-cell-depleted neuronal cultures selectively increased the levels of Na\(^+\) channel mRNA corresponding to selected Na\(^+\) channel subunits, including NaG, Na6, SNS and NaL2. Of particular relevance is the finding that exposing the neurones to Schwann-cell-conditioned medium increased the levels of Na\(^+\) channel protein significantly, as judged by immunohistochemistry. Similar results were obtained by adding Schwann cells. Although much remains unclear, these and older data can be reconciled by suggesting two separable interactions between axons and Schwann cells during the early stages of nodal development. First, axons respond to a soluble Schwann-cell signal by increasing production of Na\(^+\) channels and are capable of organizing the channels to form focal clusters in the membrane, at least in certain circumstances. Second, interactions between these clusters, or molecules associated with them, and Schwann-cell surface molecules restrict the distribution of clusters in the axonal membrane. In all these situations it is known, it seems clear that Schwann-cell signals that are capable of both inducing and positioning Na\(^+\) cluster clusters in neurones are a part of the developmental dialogue that exists between these two cell types. Regulation of axonal structure and neurofilament phosphorylation by myelinating Schwann cells Several studies point to the existence of other Schwann-cell–axon signals that are separate from those involved in the organization of Na\(^+\) channels at the node of Ranvier. The trembler mouse mutant has been an important model for revealing these interactions. These animals suffer from a defect in a myelin protein, PMP22, and show severe hypomyelination of axons in peripheral nerves. In an attempt to restore normal myelination, the loss of myelin, the trembler axons have reduced diameter, increased neurofilament density and reduced neurofilament phosphorylation. The rate of slow axonal transport is also reduced. Grafting of trembler Schwann cells onto normal nerves shows that these changes exhibit remarkable spatial restriction: they are only seen in hypomyelinated axonal segments while adjacent, myelinated segments of the same axon are normal.\(^{70}\) Myelinating Schwann cell and neurofilament phosphorylation has been seen in normal axons.\(^{71}\) This study shows that the neurofilaments directly underlying the node of Ranvier are markedly less phosphorylated than the filaments in the immediate vicinity that lie underneath the myelin sheath of the internode. Examination of normal sensory neurones reveals a picture that is consistent with results from the trembler mouse studies, as the unmethylated stem process of sensory axons has a small diameter, a high neurofilament density and low levels of filament phosphorylation when compared with a myelinated part of the same axon nearby.\(^{72}\) In an important extension of the work on the trembler mouse described above, it has been shown that genetically engineered hypomyelination produces effects on axonal structure and phosphorylation that are similar to those seen in trembler mice, and vary in severity according to the degree of hypomyelination.\(^{73}\) Oligodendrocytes appear to exert a control over axonal phenotype in the same way as Schwann cells (for example, see Ref. 74). Together, these experiments suggest that signals from myelinating Schwann cells regulate, in concert, axonal structure and function, both directly and locally. In particular, it has been suggested that Schwann-cell signals regulate a kinase-phosphatase cycle that is
Fig. 6. A diagrammatic transverse section of a nerve showing the organization of the nerve sheath that protects peripheral axons from chemical and mechanical assault. The sheath has three components: the outermost part is the epineurium, which is condensed connective tissue that contains large amounts of collagen fibres. It lies immediately outside a cellular tube, the perineurium, which surrounds the in–Schwann-cell unit of the nerve. The wall of the perineurium consists of several layers of flattened cells that are covered by a basal lamina and form tight, impermeable junctions with each other. Inside the perineurium, individual axon–Schwann-cell units lie embedded in the collagen-rich matrix of the endoneurium.

REVIEW

From axonal signals to autocrine loops: the proposed changes in survival regulation during Schwann-cell development. The survival of E14 Schwann-cell precursors (P) is regulated in a paracrine manner by β-neuregulin (NRGβ) derived from axons (A, Aβ). During development, Schwann cells (S) gradually establish autocrine circuits, which, in neuronal nerves, act in parallel with the axonal signal (not shown). In older nerves (right), the autocrine signal supports Schwann-cell survival in the absence of axons. Experiments indicate that in Schwann cells from seven-day-old nerves, insulin-like growth factor 2 (IGF2), neuregulin 1 (NRG1) and platelet-derived growth factor BB (PDGF-BB) are major components of this autocrine signal (right), although it is likely that other components are also involved.

Schwann cells regulate their own survival

Several groups have provided strong evidence that the survival of precursors is acutely dependent on axonal survival signals produced by β-neuregulin (see above). The survival of Schwann cells, however, must be restricted in a different way because the transaction of adult nerves does not lead to death of Schwann cells. In fact, Schwann cells in the distal stump of transected nerves are able to survive for a considerable length of time in the absence of axons, although their number gradually declines and they lose some of their responsivity to extrinsic signals. Likewise, Schwann cells survive well without neurons in vitro under normal culture conditions, that is, when plated at moderate density in serum-containing or serum-free media. In contrast, Schwann-cell precursors die rapidly in vitro, even when plated at very high density. These observations show that Schwann-cell development involves a change in survival regulation: the survival of Schwann-cell precursors depends on axonal signals, whereas Schwann-cell survival is axon independent.

How is Schwann-cell survival ensured in the absence of axons? Answering this question is crucial in the context of nerve regeneration. Schwann cells are left without axons in the distal nerve segment following injury. For repair to be successful, the axons have to enter this segment and grow along it, as this will guide them towards correct targets. Numerous studies have shown that this axon re-growth depends on the presence of living Schwann cells in the distal nerve segment, probably because the axons require interactions with the collagen-rich matrix of the endoneurium. Of interest, the collagen-rich matrix of the endoneurium, the perineurium, individual axon–Schwann-cell units lie embedded in lamina and form tight, impermeable junctions with each other. Inside the perineurium, which surrounds the in–Schwann-cell unit of the nerve, the wall of the perineurium consists of several layers of flattened cells that are covered by a basal lamina and form tight, impermeable junctions with each other. Inside the perineurium, individual axon–Schwann-cell units lie embedded in the collagen-rich matrix of the endoneurium.

The control of perineurium formation

Recent work on the development of the perineurium has shown that Schwann-cell signalling is not restricted to maintaining a dialogue with neurones. The perineurial sheath surrounds peripheral nerves and acts as a protective diffusion barrier (Fig. 6). Recent evidence has been obtained 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 the endo- and epi-neurial connective tissue. In normal mouse nerves, Desert Hedgehog transcripts can be detected by in situ hybridization in developing nerves as early as E11.5 (Ref. 78), and the signal is maintained until at least postnatal-day 10, being only weakly detectable in adult nerves. The mRNA for Patched, a Desert Hedgehog receptor, can be detected in the mesenchyme immediately surrounding the nerve at E15.5, the stage at which the perineurium starts to form in the mouse PNS, suggesting that Desert Hedgehog molecules secreted by Schwann cells are signalling to the surrounding connective tissue cells to organize the perineurium. This was confirmed subsequently when the structure and function of the perineurium in a Desert Hedgehog knockout mouse was examined. 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 and appear to be ‘wavy’, rather than taut as in a normal perineurium. The epineural collagen sheath is also scantly and even absent in some places, whereas there appear to be many perineurial 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 around the nerve. They form multiple mini-fascicles within the nerve, which resemble the minifascicles that form in regenerating nerves. 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. Thus, not only do Schwann cells and their precursors signal to neurones, but they are also involved in fashioning the connective-tissue sheaths of the nerves.

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Schwann-cell-associated adhesion molecules and trophic factors\(^{14,15}\). Nerve regeneration, therefore, depends on the mechanism that allows Schwann cells, unlike their precursors, to survive in the absence of axons.

Recent work has indicated that Schwann cells acquire the ability to survive without axons by establishing an autocrine survival loop. Using cell cultures, the existence of such survival loops has been demonstrated in Schwann cells taken from E18 and postnatal nerves, but these loops are absent in Schwann-cell precursors in E14 precursors, to survive in the absence of axons.

The molecular identity of the Schwann-cell-derived survival signal has been investigated. It is not mitogenic for Schwann cells and is unable to promote precursor survival, which means the Schwann-cell signal is unlikely to be simply β-nerve growth factor-2 (β-NGF)- or platelet-derived growth factor-β (PDGF-βB)-like. It is clear that Schwann-cell survival requires a signal from axons, which can be mimicked in vitro by a cocktail of growth factors, which include insulin-like growth factor 2 (IGF2), platelet-derived growth factor-BB (PDGF-BB) and NGF, myelinated-associated glycoprotein, N-myc, NT3, neurotrophin 3, PDGF-BB, platelet-derived growth factor-BB, 5′-nucleotidase or Schwann-cell precursors, which secrete these factors by an autocrine circuit that does not depend on factors from the target tissue cells and, via autocrine circuits, the Schwann cells themselves. In particular, the development of neurones is likely to depend 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 of research, together with the identification of the neurone-derived factors that act on Schwann cells and control their specification, survival and differentiation, should gradually resolve the complex signalling network required to build a peripheral nerve.

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**Selected references**


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**Fig. 8. A summary of developmental signaling from Schwann cells.** Signals from developing Schwann cells (S) and their precursors regulate numerous events in nerve development. Molecules that have been implicated in these signaling pathways are indicated. Abbreviations: DNH, Desert Hedgehog; F, fibroblast; GDNF, glial-derived neurotrophic factor; IGF2, insulin-like growth factor 2; MAG, myelin-associated glycoprotein; N, neurite; NT3, neurotrophin 3; PDGF-BB, platelet-derived growth factor BB; S, Schwann cell or Schwann-cell precursor; T, unknown signal.
Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives

Rainer Rupprecht and Florian Holsboer

Steroids influence neuronal function by binding to intracellular receptors that can act as transcription factors and regulate gene expression. In addition, some so-called 'neuroactive steroids' are potent modulators of an array of ligand-gated ion channels and of distinct G-protein coupled receptors via nongenomic mechanisms, and they can influence sleep and memory. This article describes how these neuroactive steroids modulate neurotransmitter receptors and addresses the neuropsychopharmacological potential that arises from the intracellular crosstalk between genomic and nongenomic steroid effects. Neuroactive steroids could also have a role in the response to stress and the treatment of psychiatric disorders, such as depression, and, as they affect a broad spectrum of behavioral functions through their unique molecular properties, they could constitute a yet unexploited class of drugs.


STEROID-HORMONE action involves binding of steroids to their respective intracellular receptors. These receptors change their conformation subsequently to dissociate from chaperone molecules, for example, the heat-shock proteins, and translocate to the nucleus where they bind as homodimers or heterodimers to the respective response elements that are located in the regulatory regions of target promoters. Alternatively, these ligand-activated receptors can influence transactivation through protein–protein interaction with other transcription factors. Thus, steroid-hormone receptors act as transcription factors in the regulation of gene expression. In the past decade, considerable evidence has emerged that some steroids might alter neuronal excitability via the cell surface through interaction with specific neurotransmitter receptors. The term 'neuroactive steroids' has been adopted for steroids with these particular properties. While the action of steroids at the genome requires a time period that lasts from minutes to hours and is limited by the rate of protein biosynthesis, the modulatory effects of neuroactive steroids occur during a millisecond to second time window. Thus, the genomic and nongenomic effects of steroids within the CNS provide the molecular basis for a broad spectrum of steroid action on neuronal function and plasticity.

Are naturally occurring steroids specific for distinct intracellular receptors or distinct neurotransmitter receptors? Do they act via the genomic or the nongenomic pathway? It has long been believed that steroid hormones act exclusively through the classical genomic pathway, whereas some neuroactive steroids that do not bind to either known steroid receptors [for example, 3α-reduced metabolites of progesterone and deoxy-corticosterone, such as 3α,5α-tetrahydroprogesterone (also known as 3α,5α-DHOP), 3α-hydroxy-5α-pregnane-20-one and allopregnanolone and 3α,5α-tetrahydroxy-3α-pregnan20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnan-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydroxy-3α-pregnane-20-one and allotetrahydrox-