MECHANISMS OF AXON ENSHEATHMENT AND MYELIN GROWTH

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Abstract | The evolution of complex nervous systems in vertebrates has been accompanied by, and probably dependent on, the acquisition of the myelin sheath. Although there has been substantial progress in our understanding of the factors that determine glial cell fate, much less is known about the cellular mechanisms that determine how the myelin sheath is extended and stabilized around axons. This review highlights four crucial stages of myelination, namely, the selection of axons and initiation of cell–cell interactions between them and glial cells, the establishment of stable intercellular contact and assembly of the nodes of Ranvier, regulation of myelin thickness and, finally, longitudinal extension of myelin segments in response to the lengthening of axons during postnatal growth.

Functional integration of the vertebrate nervous system's byzantine cytoarchitecture requires rapid nerve impulse conduction. During vertebrate evolution this has been achieved through the development of myelin-forming glial cells — oligodendrocytes in the CNS, and Schwann cells in the PNS. These cells wrap around axons so that the molecular machinery responsible for propagating action potentials is concentrated at regular, discontinuous sites along the axon. These are known as nodes of Ranvier. The presence of myelin as an internodal insulator ensures that membrane depolarization can only occur at the nodes. The result is rapid, saltatory (from the Latin *saltare*, to jump, or to dance) nerve conduction.

The myelin sheath is one of the best studied mammalian membranes, not least because of its vital function, and also owing to its abundance and the ease of isolation of enriched myelin fractions. Consequently, there is a vast literature on the biochemical and biophysical properties of this membrane in health and disease, and considerable detail has been amassed about the biosynthesis of its constituent lipids and proteins (for a review, see REF. 1). Furthermore, and reflecting our growing understanding of how nervous systems develop in general, the embryonic origins and cell lineages of oligodendrocytes and Schwann cells and their precursors have been more clearly defined over the past few years (for reviews, see REFS. 2,3). These discoveries have revealed the involvement of a steadily increasing number of receptor signalling pathways and transcription factors in the differentiation of glial cells. Therefore, there has been, and continues to be, steady progress in our understanding of where myelin-forming glia come from and which molecules regulate their specification.

In spite of this burgeoning knowledge, until recently surprisingly little was known about the molecular basis and dynamics of the cell–cell interactions that determine how the myelin sheath is extended and stabilized around axons in the first place. Progress has also been slow in understanding the mechanisms that allow nerves to continue growing in the postnatal animal. These are key questions for understanding nervous system function, as they relate directly to the role of myelin as an insulator of nerve fibres and to the way that myelin-forming glia participate in the assembly of nodes of Ranvier. These issues are of more than academic interest, as progress in revealing the mechanisms of repair and the essential role of myelin-forming glia

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in providing trophic support for axons will inevitably affect the development of therapies to arrest or even reverse the neurodegeneration that accompanies demyelinating diseases[4]. The purpose of this review is to discuss recent progress in understanding the molecular bases of these crucial stages of development in myelinated nerves. We use examples from the CNS and the PNS where appropriate, and draw attention to persisting gaps in our knowledge.

Selection of axons and initiation of contact

The reasons why some axons are myelinated and others are not are still baffling. In general, a minimum calibre is required (~1 μm) before an axon can be myelinated. The physiological rationale for this threshold is that the saltatory mode of conduction would probably not confer significantly enhanced rates of nerve impulse transmission on small calibre fibres, but how axons of a minimum calibre are selected for myelination is still not understood. This is of particular relevance in the context of demyelinating diseases such as MULTIPLE SCLEROSIS, for which it has been proposed that changes in the expression of proteins on the axonal surface might make axons less susceptible to remyelination[5].

Certain cell adhesion molecules, such as L1 and polysialylated NCAM (neural cell adhesion molecule), are known to be expressed on unmyelinated axons and to be downregulated during axonal myelination[5,6]. However, whether or not the disappearance of these molecules is mechanistically connected to axon ensheathment is not clear. The L1-knockout mouse shows no obvious derangements in myelination, such as ensheathment of inappropriate axons[7]. Other candidates for sensing axonally presented molecules on oligodendrocytes and Schwann cells include the integrins and neurofascin. Integrins seem to be most important for survival mechanisms in early oligodendrocytes (for reviews, see REFS 8,9) and in Schwann cells their best-characterized role is in the radial sorting of unmyelinated axons[10]. Laminins, the ligands for some integrins, also have an important role in the defasciculation of axons[11]. Dystroglycan, the other laminin receptor on Schwann cells, is not vital for the early stages of myelination, and seems to be more important for the organization of the nodal microvilli[12]. The neurofascin gene and its products do play an important part in later stages of myelination (see below), however, they do not seem to be necessary for initial ensheathment (D.L.S. *et al.*, unpublished observations).

The role of neurotrophins in the earliest stages of axon engagement has attracted renewed interest. The prototypical neurotrophin is nerve growth factor (NGF), and studies on the ability of NGF to promote neuronal survival have a long history[13,14]. It has recently been shown that NGF might also have a role in regulating myelination[15]. NGF stimulates myelination by Schwann cells but inhibits oligodendrocyte-mediated myelination. Antibody-blocking experiments showed that NGF exerts its effect through binding to tyrosine kinase TrkA receptors, which was later confirmed by the use of reagents that independently activate these receptors. Interestingly, the effects of NGF on the cell biology of myelin-forming glia seem to be indirect, that is, the signals that affect myelination arise from axons in response to the binding of NGF to axonal TrkA receptors. TrkA receptor involvement in mediating the action of NGF was shown by the fact that myelination of a sub-population of dorsal root ganglion neurons that were dependent on bone-derived neurotrophic factor (BDNF) for survival was unaffected by NGF. It is possible that NGF could affect myelination by influencing the diameter of responsive axons, but this still leaves open the question of how myelin-forming glia can recognize differences in axonal size. Nevertheless, these observations have confirmed the importance of the properties of the axon itself as a determinant of myelination. Even in axons that express TrkA, it seems that responsiveness to NGF cannot be the whole story, as the unmyelinated sensory C-fibres of the PNS express TrkA receptors but are not myelinated.

The fact that NGF seems to act through axonal but not glial TrkA receptors begs the question as to the nature of the axonal signals that are produced in response to the binding of NGF and that influence the cell biology of oligodendrocytes and Schwann cells. Ligand binding to Trk receptors causes autophosphorylation, which is followed by binding of various adaptor proteins, including phospholipase C-γ1 (PLCγ1), Src homology 2 domain-containing transforming protein (SHC), phosphatidyl-inositol 3–kinase (PI3K) and extracellular signal-regulated kinase 1 (ERK1). Each of these has been implicated in signalling pathways that converge on the nucleus. It is therefore likely that receptor activation influences the transcription of neuronal genes that can modulate the ability of oligodendrocytes and Schwann cells to myelinate. Possible candidates for the neuronal effector molecules that influence glia to myelinate include secreted molecules such as NEUREGULINS and the small molecules known to act at glial purinergic receptors[16].

Myelin process extension around target axons

The function of myelin in the CNS and PNS appears to be identical, but there are differences in the cell biology of myelination when oligodendrocytes are compared with Schwann cells. For example, the Schwann cell has an intimate association with the axon that it myelinates, and lines up along the axon to define a single internode, whereas oligodendrocytes extend several processes, each of which myelinates distinct internodes, often on different axons. Nevertheless, in both cases a myelinating process that is continuous with the plasma membrane must be assembled and extended. Current concepts of LIPID RAFTS, which propose the existence of microdomains in membranes, might help to explain how proteins and lipids are delivered to the growing membrane (for an alternative perspective on rafts, see REF. 17). However, such concepts might be less useful for understanding how the myelin macrodomain, with its distinct protein and lipid content, is stably segregated from the plasma membrane of the myelin-forming glial cell.

MULTIPLE SCLEROSIS
A disease of the CNS that is characterized by focal areas in which myelin is lost from axons, leading to axon degeneration.

NEUREGULINS
A family of receptor tyrosine kinases related to epidermal growth factor (EGF). The receptors for neuregulins are the ErbB family of tyrosine kinase transmembrane receptors.

LIPID RAFTS
Domains within plasma membranes believed to be enriched in cholesterol and glycolipids that are proposed to aid the delivery of subsets of membrane proteins to the plasma membrane and serve as sites for concentrating signalling molecules.

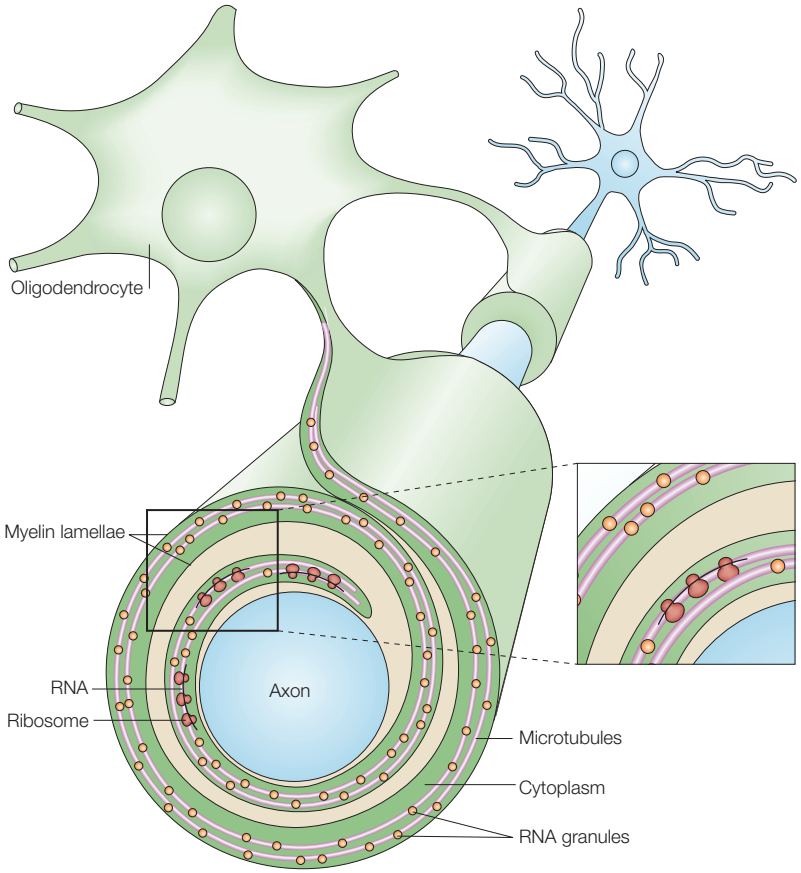

Figure 1 | **Ensheathment of an axon by oligodendrocytes.** An oligodendrocyte extends and surrounds neighbouring axons and the myelinating process develops a distinct protein and lipid composition. The synthesis of some myelin proteins — and presumably the assembly of new membrane — occurs at sites distal to the cell body as a result of microtubule-based transport of RNA granules (orange circles) that contain mRNA (blue lines) and ribosomes (red circles) from the nucleus to the paranodes.

The myelin membrane contains a high percentage of lipids compared with the plasma membranes of most eukaryotic cells. Cholesterol is a major constituent, and mice with oligodendrocytes that lack the ability to synthesize cholesterol show a delay in myelination that seems to be at least partially compensated for by cholesterol uptake[18]. There has been particular interest in determining the significance of the high galactolipid content of myelin. Mice with a disruption in the gene that encodes the biosynthetic enzyme UDP-galactose:ceramide galactosyl transferase (CGT) cannot synthesize galactolipids, but seem to compensate by replacing these lipids with their gluco-analogues[19–21]. Surprisingly, only the myelin of the CNS shows any resulting structural and functional defects, including disrupted PARANODAL AXO–GLIAL JUNCTIONS and reduced nerve conduction velocities. In order to discriminate between the effects of the absence of galactolipids and their sulphated derivatives, mice that lack cerebroside sulphotransferase were generated. These mice show a two- to threefold enhancement in the number of terminally differentiated oligodendrocytes, both in culture and *in vivo*, and also have disrupted paranodes like their CGT-null counterparts[22,23]. It would seem, therefore, that the effects on myelin domain structure seen in mice lacking CGT might result primarily from the absence of sulphatide. The precise function of both lipids remains to be determined.

PARANODAL AXO–GLIAL JUNCTIONS
Major sites of physical interaction between myelin-forming glial cells and the axon that lie on either side of the nodes of Ranvier and are characterized by septate-like junctions.

Lipids are probably targeted to the growing process as a consequence of their interactions with particular proteins (for an example, see REF. 24). If this is so, then the question remains as to how proteins are segregated into nascent myelin. It seems likely that this occurs as a result of a combination of factors, such as the specific targeting of proteins during their biosynthesis, and the consolidation of *cis* associations between proteins within the myelin domain by *trans* adhesive associations during compaction and axon–glia interaction.

The discovery that myelin basic protein (MBP) is synthesized in the growing myelin process[25] was a major step forwards in our understanding of how proteins might be delivered to the myelin membrane. This was one of the first demonstrations of localized mRNA translation in a eukaryotic cell, and indicated that MBP is incorporated into the growing myelinating process at sites that are quite distant from the oligodendrocyte cell body (FIG. 1). This concentration of MBP mRNA at distal sites has also been observed in Schwann cells, which indicates a common mechanism for process assembly in the CNS and PNS[26,27]. Surprisingly, few other mRNAs have been shown to be similarly localized in either oligodendrocytes or Schwann cells, and those that have been identified are of unknown function[28].

How might the mRNAs for myelin proteins be translocated to the growing process for local translation? A series of studies from the laboratory of E. Barbarese and J. H. Carson has firmly implicated the microtubule system in oligodendrocytes. Translocated mRNAs, such as MBP mRNA, contain an A2RE sequence element that binds the heteronuclear ribonucleoprotein (hnRNP) A2 protein[29]. Localization of this protein in oligodendrocytes is microtubule-dependent, and recent data show that a microtubule-associated protein, TOG2, is probably responsible for the association of hnRNP A2-positive mRNA granules with microtubules during transport and/or localization[30]. It seems likely that a similar system operates in Schwann cells, as transport of MBP mRNA from the nucleus to distal sites at the paranodal regions also requires intact microtubules[26].

There is a growing appreciation that the transport of mRNAs in ribonucleoprotein granules from the nucleus to distal sites in polarized cells (such as glia and neurons) might add another layer of regulation to both the assembly and the local function of membrane domains. Recent work has indicated that the targeting and local translation of neuronal mRNAs might also require the association of short (~20 nucleotides long) microRNAs (miRNAs) with both mRNAs and polyribosomes in RNA granules. Base-pairing of these miRNAs to complementary sequences in target RNAs is believed to suppress translation until the granules reach their target sites — for example, dendritic synapses[31,32]. How translation arrest is released is not yet clear. However, the fact that the complement of miRNAs changes as neurons mature indicates that these regulatory mechanisms might operate

during development as well as in response to local synaptic activity. It will be of great interest to determine whether similar mechanisms operate in myelin-forming glia during both myelination and remyelination.

In addition to microtubules, other components of the glial cytoskeleton, such as the microfilaments, are likely to have a role during process extension. An important step in determining these was the recent identification of Rho kinase (ROCK) as a key regulator of myelinating process morphology and extension in Schwann cells[33]. ROCK regulates the actin–myosin mechanical transduction system by phosphorylating myosin light chains. ROCK itself is an effector of Rho, a well-known regulator of actin cytoskeleton dynamics in various cell types. In Schwann cells, ROCK inhibition does not influence proliferation or differentiation. However, in the absence of ROCK activity the single myelinating process of the Schwann cell splits to form many smaller internodes, each with their appropriate paranodes and nodes. As we discuss below when considering the mechanisms of Schwann cell elongation and the functional consequences of short internodes, splitting of the myelinated segments would be expected to have deleterious consequences for nerve conduction velocities. Nevertheless, these experiments do point to a role for actomyosin in ensuring that the internode comprises a single Schwann cell.

A role for ROCK signalling has also been demonstrated in oligodendrocytes[34]. Sphingosine 1-phosphate receptor 5 (S1P5, also known as EDG8) is a high-affinity sphingosine 1-phosphate receptor that is restricted to oligodendrocytes in the CNS. Stimulation of oligodendrocytes with sphingosine 1-phosphate can affect two distinct signalling pathways — one of which mediates myelin process retraction, the other cell survival. Process retraction occurs through a Rho kinase–collapsin response-mediated protein signalling pathway, whereas the sphingosine 1-phosphate-induced survival of mature oligodendrocytes is mediated through a pertussis toxin-sensitive, Akt (v-akt murine thymoma viral oncogene homologue)-dependent pathway. This has parallels with experiments discussed below, in which oligodendrocyte-conditioned medium promoted the survival of neurons through similar signalling pathways[35]. Interestingly, activated Rho has also been shown to have a role in process retraction in response to signalling by the Src (v-sarcoma viral oncogene homologue) family kinase Fyn[36]. By contrast, activation of Cdc42 (derived from cell division cycle 42)–Rac by Fyn caused process extension.

Independent evidence for the role of the actin filament system in myelination has been provided by studies in which the actin cytoskeleton was disrupted using cytochalasin D[37]. In myelinating Schwann cell–neuron co-cultures, low concentrations of cytochalasin D prevented spiralling of the myelin process around the axon. Indeed, in the absence of intact microfilaments the expression of genes encoding myelin proteins was inhibited so that the myelinating process itself was no longer extended as the inner mesaxon: it is this inner mesaxon that is believed to spiral around the axon to form the multilamellar myelin sheath[38]. Evidently, the microfilament system is at, or close to, the nexus that links the pattern of gene expression in myelin-forming glia to their response to axonal signalling.

Rho, Cdc42 AND Rac
Rho GTPases are small (20–30 kDa) GTP-binding proteins of the Ras superfamily and are divided into three main subgroups, Rho, Cdc42 and Rac, which have distinct effects on microfilament dynamics.

Axo–glial contact and formation of the node

An early event during myelination is the formation of an axo–glial junction between the distal, uncompacted loops of myelin and the axolemma, which will define the paranodal domain and separate the node from the juxtaparanode. The molecular constituents of these three domains and current ideas about how they might be assembled have recently been reviewed elsewhere (see REFS 39,40). In summary, the paranodal junction comprises the axonal proteins Caspr (contactin-associated protein, also known as paranodin) and contactin, and their probable glial partner neurofascin 155 (Nfasc155)[5,41–44]. At about the same time that the first axo–glial junctional components are detectable, the first constituents of the node are also detectable; these include Nfasc186 (a neuronal isoform of neurofascin), ankyrin G, neural–glial-related cell adhesion molecule (NrCAM) and βIV-spectrin. These are followed by the voltage-gated sodium channels that are required for propagating the action potential.

Ensheathment by myelin-forming glia and node formation are almost certainly inextricably linked (FIG. 2a). Nevertheless, the precise mechanistic relationship between the two events is unclear. An attractive idea was that the paranodal septate junctions might act like a snow-plough, pushing the nodal components together and assembling nodal complexes. However, mice lacking either Caspr or contactin, and the myelin-deficient (*md*) rat all have disrupted axo–glial junctions, and are still able to cluster sodium channels adjacent to myelinating processes, although these are slightly less concentrated than normal[45–47]. As studies of the *md* rat have revealed, myelination *per se* is not essential for sodium channel clustering. There seem to be some differences between the CNS and PNS; for example, it has been reported that diffusible factors from oligodendrocytes are sufficient to cause sodium channel clustering in retinal ganglion axons[48]. However, cell–cell contact seems to be essential in the PNS. At present, NrCAM and Nfasc186 seem to be the best candidates for 'nucleating' the assembly of nodal components, although mice that lack NrCAM can still cluster sodium channels at nodes of Ranvier[49,50]. The weight of evidence indicates that an intact paranodal axo–glial junction is not essential for nodal formation, although it probably has a role in helping to maintain the tight clustering of nodal components in the mature nerve.

A similar repertoire of proteins to those found at the node of Ranvier also assembles at axon initial segments (AIS) (FIG. 2b). In the cerebellum, the axons of basket cells make synaptic contacts with the AIS of Purkinje cells, and abnormal distribution of Nfasc186 in the AIS of mice that lack ankyrin G disrupts this synaptic connection[51]. Loss of βIV-spectrin disrupts ankyrin G and sodium channel localization at the AIS, and would presumably also result in the disruption of Nfasc186 (REF. 52).

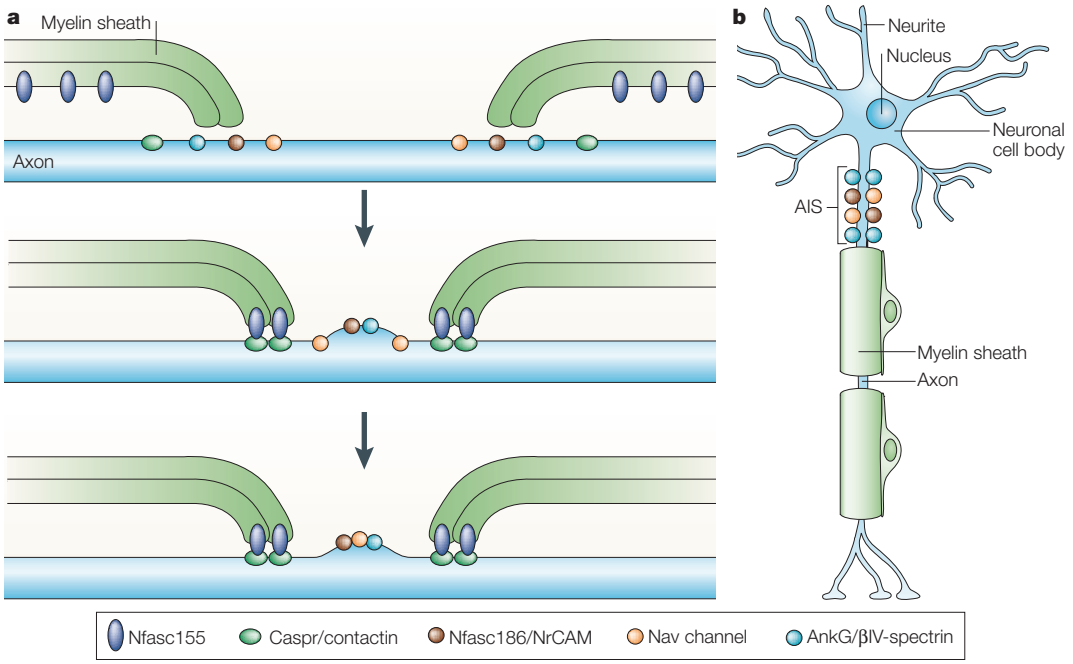

Figure 2 | **Myelination causes clustering of the sodium channel complex at nodes of Ranvier and axon initial segments. a** | Initially the adhesion molecules of the future paranodal region and the components of the node are distributed diffusely along the axon and myelinating process. Establishment of paranodal axo–glial junctions between glial neurofascin 155 (Nfasc155) and axonal contactin-associated protein (Caspr) and contactin coincides with the clustering of some of the nodal components, such as Nfasc186, NrCAM, ankyrin G (AnkG) and βIV-spectrin, which is followed by the clustering of voltage-gated sodium (Nav) channels. **b** | The axon initial segment (AIS) is the site at which action potentials are initiated as a result of various synaptic inputs arriving at the neuron. The composition of the AIS is similar to that of the node, which indicates that there could be similar mechanisms for the assembly of the sodium channel complex.

Whether these effects on sodium channel clustering are the direct result of a loss of Nfasc186 remains to be seen. It is also not certain that the mechanisms for the assembly of the AIS are identical to those that operate at the node, despite their similar protein compositions. The sodium channel Nav1.2 subunit, for example, is specifically targeted to the AIS of hippocampal neurons by means of its ankyrin G binding domain[53].

Regulation of myelin thickness

The g-ratio is the ratio of the axonal diameter divided by the diameter of the axon and its myelin sheath. Most myelinated axons in any given animal have the same g-ratio and this value is usually between 0.6 and 0.7. This means that the thickness of the myelin sheath varies according to the diameter of the axon: bigger axons have thicker myelin, and vice versa.

It has been known for some time that neuregulin signalling is important for Schwann cell development. The neuregulin–ErbB receptor system can also have a considerable influence on the thickness of the myelin sheath. Mice lacking the ErbB3 receptor are completely deficient in Schwann cells[54], whereas in the absence of the ErbB2 receptor myelinating Schwann cells are produced, but the myelin sheaths are thinner than usual[55]. Mice haploinsufficient for the neuregulin 1 (*Nrg1*) gene also have thinner peripheral myelin sheaths than wild-type mice, which has brought a clearer focus to bear on both the neuregulins that are produced by this gene and their cognate receptor, ErbB2 (REF. 56). Interestingly, ErbB2 receptor heterozygous null mice have normal myelin, which indicates that the ligand, an *Nrg1* gene product, might be the limiting factor in this system. The *Nrg1* gene can produce up to 16 protein isoforms through a combination of alternative splicing and promoter selection, so it was naturally of interest to know which isoform(s) were responsible. Transgenic overexpression in neurons of the cDNA for the NRG1 type III protein driven by the promoter of the gene that encodes the Thy-1 antigen (a murine immunoglobulin expressed at high levels in neurons and lymphocytes) showed that it was active in regulating myelin thickness. Internodal lengths appear to be unaffected by the neuregulin–ErbB system.

The exception to the constant g-ratio rule is seen in axons that have been remyelinated — for example, after peripheral damage and regeneration — in which the sheaths are typically thinner than expected. This might reflect an uncoupling between the delivery of appropriate amounts of neuregulin and normal myelin assembly. It could also reflect reduced efficacy of the ErbB2 receptor. If the amount of neuregulin regulates myelin thickness *in vivo*, the question remains as to how axonal diameter is linked to neuregulin expression. In order to further evaluate the role of the neuregulin–ErbB system in regulating myelin thickness and its relationship to axon diameter, it would interesting to assess the functional consequences of transgenic overexpression of NRG1 type III on regeneration.

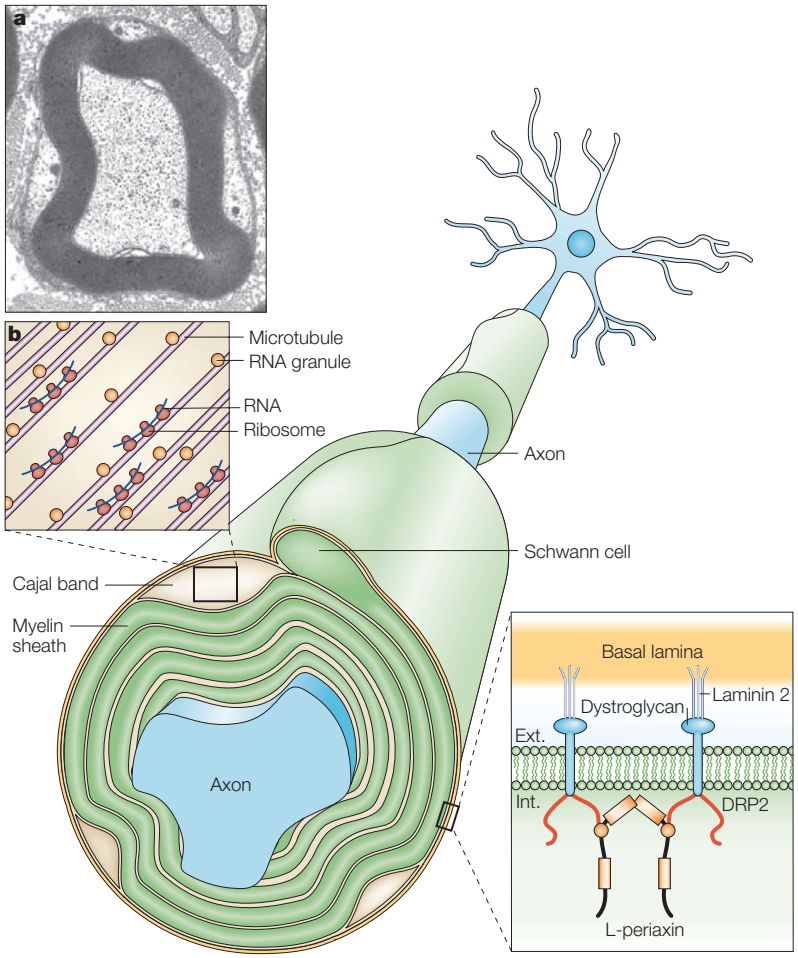

Figure 3 | **Cajal bands form channels for mRNA transport in Schwann cells. a** | Electron micrograph of a transverse section of the quadriceps nerve. **b** | Cajal bands are cytoplasm-filled channels that lie underneath the plasma membrane of the Schwann cell. The cytoplasm is squeezed between appositions that form between the outer surface of the myelin sheath and the cytoplasmic face of the Schwann cell plasma membrane, and contains the periaxin–dystrophin-related protein 2 (DRP2–dystroglycan complex; see right inset). Cajal bands contain microtubules that participate in the delivery of mRNA from the nucleus to distal sites at which it is translated, as occurs in oligodendrocytes (see FIG. 1). Importantly, intact Cajal bands seem to be vital for the transport function of microtubules in Schwann cells. Disruption of the periaxin–DRP2–dystroglycan complex that is responsible for forming the Cajal bands also prevents microtubule-based mRNA transport. Panel **a** modified, with permission, from REF. 25 © (2004) Macmillan Magazines Ltd.

There is also the possibility that other signalling systems might participate in determining the extent of myelin synthesis. Recently, BDNF and the neurotrophin p75 receptor have also been implicated in regulating later stages of myelination, including myelin thickness[57]. Interestingly, although transgenic overexpression of BDNF increases both axonal diameter and myelin thickness, the effect is greater on the latter, which indicates that the effects of BDNF cannot be simply the result of enhanced axonal calibre. Therefore, neuregulins might not be the only factors that are important for regulating myelin thickness.

Growth of nerves and myelinated segments

There has been a wealth of new data about the molecular basis of axon pathfinding and synaptogenesis in recent years. However, we know less about how nerves grow during postnatal development in mammals. For example, some peripheral nerves will have to increase in length by more than four times after myelination has already begun, which naturally raises the question of what happens to the internodal segments of myelin as the axons elongate. This could have implications for nerve function as, according to some models, the properties of myelinated axons predict that internodal length might influence the rate of nerve impulse transmission, although this is controversial[58–60]. Recent work on mice that lack the Schwann cell-specific protein PERIAXIN has provided new insights into these issues.

The Schwann cell–axon unit is surrounded by a basement membrane that is crucial for Schwann cell differentiation and myelination. Dystroglycan complexes are important receptors for the basement membrane protein laminin, and periaxin is a component of one such receptor complex in the Schwann cell plasma membrane[61,62]. Dystroglycan is linked in this complex to the protein L-periaxin by dystrophin-related protein 2 (DRP2), a fourth member of the dystrophin family (FIG. 3). The L-periaxin–DRP2–dystroglycan (PDG) complex is clustered at sites between the abaxonal surface of the myelin sheath and the Schwann cell plasma membrane, and forms cytoplasmic channels in a pattern that accurately delineates what the great Spanish neuroanatomist Santiago Ramon y Cajal described as the "longitudinal bands embedded in a meshwork of protoplasmic trabeculae"[63]. Ramon y Cajal's 'longitudinal bands' have now been renamed CAJAL BANDS[26]. Disruption of the PDG complex also disrupts the Cajal bands, and these mutant Schwann cells are impaired in their ability to elongate during nerve growth[26,62]. This strongly indicates that Cajal bands have an important role in regulating the ability of Schwann cells to increase in length.

Microtubules play an important part in myelin assembly, so it is perhaps not surprising that these cytoskeletal structures are much less organized in the absence of Cajal bands[26]. In association with these derangements, the concentration of MBP mRNA at the distal regions of the Schwann cell was abolished, which indicates that the function of the microtubules in RNA translocation was seriously impaired (FIG. 3). Interestingly, at these early stages of nerve growth, disruption of mRNA translocation did not affect myelin thickness. This indicates that elongation and myelin thickness are regulated independently, as suggested by the absence of an effect of varying neuregulin expression on internodal length[56].

At early stages of development the only aspect of Schwann cell function that seems to be affected by the absence of Cajal bands is their internodal length. This provided an excellent opportunity to determine experimentally whether internodal length influences the rate of nerve conduction. Decreased internodal lengths did reduce rates of conduction in accordance with predictions from a recent, rather sophisticated mathematical model[26,64]. Furthermore, in chimaeric nerves that contain approximately equal numbers of mutant and wild-type Schwann cells, the conduction rates were halfway between the two extremes. The mutant Schwann cells in the chimaeric nerves maintained their short

PERIAXIN
A protein of myelinating Schwann cells that is concentrated at zones of adhesion between the abaxonal (outermost) myelin lamella and the Schwann cell plasma membrane in a complex with DRP2 and dystroglycan.

CAJAL BANDS
Cytoplasmic channels that lie beneath the surface of the Schwann cell plasma membrane and that are separated from each other by the appositions formed by the periaxin–DRP2–dystroglycan complexes.

CHARCOT-MARIE-TOOTH DISEASE
An inherited degenerative neuropathy of the PNS that is caused by more than 30 different mutations. Although most forms are primarily demyelinating, loss of proper glial contact generally results in axonal degeneration.

phenotype, which indicates that the ability to elongate was a cell-autonomous property of the Schwann cell.

Myelinating glia promote axonal survival

This is perhaps the most important issue if we wish to apply what we learn about axon–glia interactions to treating human demyelinating diseases. The disastrous consequences of demyelination on axon degeneration explains the serious long-term disabilities that patients experience in the CNS disease multiple sclerosis and in demyelinating diseases of the PNS such as CHARCOT-MARIE-TOOTH DISEASE[4,65]. Myelin itself might not be a crucial factor, as in mice with oligodendrocytes that lacked a functional cyclic nucleotide phosphodiesterase 1 (*Cnp1*) gene, axonal degeneration could occur even after mild derangements to glial cells without any obvious effect on myelination[66].

The fact that ensheathment of axons by myelin-forming glia has profound effects on the axon is clear from the fact that myelination establishes distinct domains in the axonal membrane where proteins associate in specific macromolecular assemblies, such as the sodium channel complex at the node of Ranvier. However, the influence of glial contact is not restricted to the axonal membrane. The work of Brady and colleagues showed that myelinating Schwann cells not only affect the extent of neurofilament phosphorylation but also reduce the rates of slow axonal transport[67]. Axonal calibre was also reduced by the absence of glial contact, presumably as a consequence of the reduced neurofilament phosphorylation. Similar effects of demyelination have been observed in the CNS, where reduced rates of fast axonal transport were also observed[68]. Aberrant axonal transport can certainly lead to axonal degeneration, as has been shown in both animal models and human diseases in which mutations that affect the microtubule-based motors kinesin KIF5A, KIF1B and the dynein–dynactin complex cause axonal degeneration[69–72].

Apart from the secretory product of oligodendrocytes that causes the clustering of sodium channels in axons[48], there is recent evidence that oligodendrocytes secrete factors that promote neuronal survival in culture[35]. Embryonic rat neurons exposed to oligodendrocyte-conditioned medium show increased survival through a PI3K–Akt signalling pathway. Interestingly, axonal length was also increased and this seems to occur through a glial cell line-derived neurotrophic factor (GDNF)–mitogen-activated protein kinase (MAPK)-mediated system.

At present, links between aberrant ensheathment and altered axonal transport seem to be one of the most interesting avenues for obtaining mechanistic insights into why axonal degeneration occurs after demyelination. This is obviously proving to be quite a challenge as it is still not clear how demyelination influences the activities of the presumed kinases that are responsible for neurofilament phosphorylation. Nevertheless, the prospects are good because various other kinases that influence axonal transport and targeting, including cyclin-dependent kinase 5 and glycogen synthase kinase 3, have recently been identified[73].

Concluding remarks and future perspectives

The association between myelin-forming glia and the axons that they ensheath is a remarkable example of cell–cell interaction, not least because the paranodal axo–glial junction is by far the largest intercellular adhesion complex found in vertebrate biology. Glia influence the establishment of distinct axonal domains and the subsequent growth of both myelin and myelinated nerves. These processes are the result of a fascinating combination of reciprocal intercellular signalling and cell autonomous mechanisms. Although we now have a much clearer idea of the constituents of the paranodal and nodal domains, the key molecules that nucleate their assembly and how these molecules are targeted to these domains are still not clear. With respect to the node of Ranvier, the current assumption is that one or a few proteins in the axon pioneer the assembly of the node in response to either diffusible or membrane-bound proteins that originate from the ensheathing glial cells. Once these nodal proteins arrive at the nascent node they might act as anchors for the clustering of other proteins, including sodium channels themselves. Although the snow-plough model, by which the extending glial sheath pushes these pioneer axonal proteins to the nascent node, is intuitively attractive, it seems unlikely to be correct in a simple mechanical sense because physical interaction at the paranode between axonal and glial cell is not essential for sodium channel clustering, at least in the CNS. Clearly, identifying the signalling pathways that link glia to the trafficking of membrane proteins in neurons will be a key area for further study.

Progress in these areas and in understanding how axons are selected for myelination will no doubt accelerate as the involvement of more candidate proteins is revealed, in addition to the neuregulins and neurotrophic factors. An important challenge will be to understand the molecular basis for the longer term effects of glial ensheathment on the cell biology of the axon. These include the molecular mechanisms through which glia promote axon survival and the factors (and reasons) that underlie the relatively late switch in sodium channel type at the nodes of Ranvier[70,71,74,75].

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