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Signals that Initiate Myelination in the Developing Mammalian Nervous System

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Abstract

The myelination of axons by oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system is essential for the establishment of saltatory conduction. In the absence or destruction of the myelin sheath, as seen in demyelinating diseases, impulse conduction is impeded resulting in severe sensory and motor deficits. Axon myelination is the culmination of a sequence of events that begins with the differentiation of glial cells and proceeds to the transcription and translation of myelin genes, the elaboration of a myelin sheath, and the recognition and ensheathment of axons. This review examines the regulatory mechanisms for each of these steps and compares and contrasts the role of the axon in initiating myelination in the central and peripheral nervous system.

Index Entries: Oligodendrocyte; Schwann cell; glia; saltatory conduction; myelin; electrical activity; axon caliber; development; differentiation.

Introduction

The speed at which sensory information is conveyed to the brain, and motor information is conveyed to the muscles, is dependent upon two critical features of an axon: its diameter and the presence of a myelin sheath. By increasing the diameter of an axon, a strategy used in the nervous system of both invertebrates and vertebrates, the conduction velocity of a fiber increases as a result of a lower resistance to the flow of current. However, the limits to which the brain can expand in size as a result of this adaptation, and the comparatively larger number of neuronal cells present in the vertebrates, has prompted a second strategy to evolve that increases the speed of action potential propagation with little axon diameter growth (*see* review: Hildebrand et al., 1993). This strategy results from a complex interaction occuring between neurons and oligodendrocytes in the central nervous system (CNS)

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and Schwann cells in the peripheral nervous system (PNS), the two glial cell types capable of synthesizing the membrane structure, myelin. The elaboration of myelin into a sheath by the processes of these cells, and the subsequent wrapping of an axon with this product, changes the way in which electrical signals are transmitted along an axon. Because the ensheathment is not continuous along the length of an axon, but interrupted at regular intervals by areas devoid of myelin and rich in sodium channels (nodes of Ranvier), action potentials propagate along the axon from node to node, i.e., in a saltatory fashion. This spatial arrangement between neurons and their ensheathing cells provides the means by which the nervous system can convey electrical impulses at high speeds. Conversely, any loss of axon myelination, as seen in demyelinating diseases or nervous system trauma, disrupts action potential propagation, resulting in devastating consequences to normal motor and sensory functions (Compston et al., 1991). The myelination of axons in the vertebrate nervous system, therefore, represents a functional adaptation produced by the complex interactions occuring between neurons and glia cells. Ascertaining the regulatory mechanisms involved in this cell–cell interaction for both the CNS and PNS has been the focus of research aimed at restoring function to fiber tracts affected by disease or trauma.

Although the interactions that occur between neurons and either Schwann cells or oligodendrocytes produce the same outcome, that is, an axon ensheathed in myelin, the regulatory mechanisms involved in this event differ for the peripheral and central nervous systems. Myelination in both systems can be regarded as the culmination of a sequence of events that begins with the differentiation of glial cells from mitotically active, migratory precursor cells. Upon becoming postmitotic, these cells proceed to transcribe and translate the genes that encode the myelin proteins. The elaboration of myelin into a sheath by the processes of glial cells and the recognition of target axons are further distinct steps along the pathway leading to myelination. Finally, the initiation of axon ensheathment and the subsequent compaction of the myelin sheath around the axon, completes the stages of myelination. To examine the regulatory mechanisms involved in this whole process, it is therefore necessary to view and define myelination in both the PNS and CNS, as not a single event, but as a compendium of events that may be regulated independently, by glial cell-intrinsic or extrinsic factors.

PNS Myelination

Myelinating and Nonmyelinating Schwann Cells

In the PNS, the myelinating Schwann cells establish a 1:1 relationship with the axons before myelination, with each glial cell producing one internode of myelin. Schwann cells, however, can assume two different phenotypes and differentiate into either myelin-forming or nonmyelin-forming cells depending on the axons they contact (Aguayo et al., 1976; Weinberg and Spencer, 1976; Jessen et al., 1987). Nonmyelin-forming Schwann cells are usually associated with more than one axon and express a specific set of markers, including the low-affinity NGF receptor (NGFR), the growth-associated protein 43 (GAP-43) and the cell-adhesion molecules N-CAM and L1, but do not express myelin-specific proteins (Jessen and Mirsky, 1992). Given the right conditions, both myelin-forming and nonmyelin-forming Schwann cells lose their differentiated phenotype and revert to a cell type similar to immature Schwann cells. This phenotypic regression occurs in vivo during nerve degeneration (for review, *see* Scherer and Salzer, 1996) or in vitro by culturing Schwann cells in serum containing medium in the absence of axonal contact (Mirsky et al., 1980). Upon reestablishing contact with axons, dedifferentiated cells once again adopt a differentiated phenotype (Weinberg and Spencer, 1976; Jessen et al., 1987). Thus, myelin-forming and nonmyelin-forming Schwann cells seem essentially interconvertible with their particular phenotypes being dependent on axonally derived signals.

Extrinsic signals, as exemplified above by the axonal control of terminal differentiation of Schwann cells, play an important role throughout Schwann cell development. During recent years, a variety of factors involved in this process have been identified at the molecular level. Moreover, molecules expressed by the Schwann cell lineage itself, including transcription factors, have been identified and implicated as important players in the developmental process leading to axon myelination in the PNS (Zorick and Lemke, 1996).

Proliferation and Cell Differentiation

Most Schwann cells are derivatives of the neural crest, a group of cells arising along the lateral margins of the neural folds early in development. From their site of origin, neural crest cells migrate throughout the body, proliferate, and give rise to a variety of cell types, including neurons and glial cells of the PNS. Along the developmental pathway from the neural crest cell to the differentiated Schwann cell, several intermediate cell types, each characterized by a distinctive antigenic profile, can be distinguished. Briefly, neural crest cells give rise to Schwann cell precursors that further develop into immature Schwann cells. These latter two stages of the Schwann cell lineage can be distinguished by their expression of the cytoplasmic protein, S-100, i.e., Schwann cell precursors are S-100$^-$, whereas immature Schwann cells and subsequent maturational stages are S-100$^+$ (Jessen et al., 1994). Further maturation of immature Schwann cells leads to the formation of either myelin-forming Schwann cells or nonmyelin-forming Schwann cells (for extensive review, *see* Stewart et al., 1996a). It should be noted that several research groups have further subdivided the immature Schwann cell stage into a premyelinating and promyelinating stage (for review, *see* Zorick and Lemke, 1996).

Several growth factors are involved in the progression of the Schwann cell lineage. Neuregulins (NRGs), a family of proteins that includes glial growth factor (GGF), heregulin, acetylcholine-receptor inducing activity, and neu differentiation factor (NDF), are generated by alternative splicing from a single gene (for review, *see* Lemke, 1996). In vitro experiments suggest that NRGs induce the commitment of neural crest cells to progress along the Schwann cell lineage (Shah et al., 1994). The survival of Schwann cell precursors is dependent on axon influence, and this survival-promoting effect can be mimicked by β isoforms of NRGs (Dong et al., 1995). Further, in the presence of NRGs, surviving precursor cells mature into Schwann cells (S-100$^+$) with a time course similar to the phenotypic switch in the developing nerves in vivo. NRGs are also potent mitogens for Schwann cells in vitro (Minghetti et al., 1996), and may represent part of the mitogenic effect axons exert on these cells (Wood and Bunge, 1975; Morrissey et al., 1995). In addition, it has been suggested that axonally-derived NRGs play a role in matching the number of Schwann cells to the number of nerve fibers by regulating apoptotic cell death of neonatal Schwann cells (Grinspan et al., 1996; Syroid et al., 1996). Finally, at low concentrations neuregulins increase the motility of Schwann cells in vitro, and cause directed migration of these cells when applied focally (Mahanthappa et al., 1996).

The proliferation and differentiation of Schwann cells has been shown to be under the influence of several other growth factors. For example, culturing Schwann cells under conditions that elevate cAMP, together with platelet-derived growth factor (PDGF) or fibroblast growth factor 2 (FGF-2), in serum-free medium, causes the glial cells to proliferate (Stewart et al., 1991). Insulin-like growth factors (IGFs) potentiate the mitogenic effect of PDGF and FGF. On the other hand, in the absence of other growth factors, IGF stimulates the differentiation of Schwann cells induced by cAMP alone, as measured by enhanced expression of the major PNS myelin gene *P0* (Stewart et al., 1996b). Since IGFs in combination with other factors can promote both proliferation and myelin differentiation in Schwann cells in vitro, they may play a role in the transition from proliferating to differentiating Schwann cells in vivo.

Members of the transforming growth factor beta (TGF-β) family antagonize the proliferation-promoting effect of axons on Schwann cells in cocultures (Einheber et al., 1995). TGF-βs also prevent the axon-driven differentiation of Schwann cells towards myelin production (Einheber et al., 1995; Guénard et al., 1995). These results are consistent with findings in cultures of purified Schwann cells, in which low concentrations of TGF-βs suppress P0 induction by cAMP (Morgan et al., 1994). Other phenotypic changes in Schwann cells after TGF-β application include: an increase of NCAM and L1 expression; a decrease in the levels of NGFR; a decrease in the O4 antigen and galactocerebroside, and a reduction of gap-junction coupling (Mews and Meyer, 1993; Chandross et al., 1995; Stewart et al., 1995). Taken together, these results indicate that TGF-βs may be involved in either promoting the nonmyelinating phenotype or maintaining the premyelinating phenotype of Schwann cells in vivo.

Myelin Gene Expression

The expression of myelin-specific proteins (i.e., P0, myelin basic protein [MBP] and so on) at high levels by differentiated, myelin-forming Schwann cells occurs after immature Schwann cells become postmitotic and downregulate markers of the immature stage (e.g., NGFR, N-CAM, and so on). The ensheathment and wrapping of axons with Schwann cell myelin membranes, and the formation of compact myelin, completes the differentiation process. Axons that become myelinated are thought to instruct the associated Schwann cells to initiate and maintain the myelination program (Mirsky and Jessen, 1990). In support of this model, Schwann cells in vivo decrease expression of myelin genes after denervation and re-express these genes only after contact with regenerating axons (Scherer and Salzer, 1996). In vitro, Schwann cells removed from axonal contact rapidly downregulate expression of the myelin genes. In contrast, in cocultures with neurons or in pure cultures with experimentally elevated intracellular levels of cAMP, Schwann cells strongly express myelin-specific genes (Lemke and Chao, 1988; Morgan et al., 1991). Since nerve fibers in vivo and in vitro, and increased cAMP levels in vitro have similar effects on myelin-gene expression, it has been suggested that cAMP may act as a mediator in the signaling process between axons and Schwann cells in vivo. The transcription factor CREB (cAMP responsive element binding protein) that translates an increased intracellular cAMP concentration into an altered expression of appropriate target genes, is present in Schwann cells throughout development (Stewart, 1995). However, since the characterized part of P0-promoter region lacks a target sequence for CREB (Lemke et al., 1988), the induction of P0 expression by cAMP may be indirect, possibly mediated by other gene regulatory proteins. Nevertheless, it remains to be determined if indeed axonal contact causes a significant increase of intracellular cAMP in Schwann cells at all (Meador-Woodruff et al., 1984). The precise role of cAMP in axon-induced myelin gene expression in vivo has yet to be elucidated.

Recently, it was shown that purified rat Schwann cells constitutively express large amounts of P0 and MBP in cultures lacking neurons or agents that increase intracellular cAMP—provided that serum is absent during the culturing process (Cheng and Mudge, 1996). NRGs were found to inhibit this expression of P0. In contrast to the hypothesis that axonal contact is required for myelin gene expression, Cheng and Mudge suggest that constitutive expression of P0 prior to the onset of myelination is blocked by inhibitory signals from the axon. For example, axonally derived NRGs may inhibit myelin-gene expression, and myelination can occur only if this inhibition is relieved or overridden. Regardless of the exact mechanism, however, there is consensus that axons in the peripheral nerves are key players in the control of myelin production by Schwann cells.

Several transcription factors, other than CREB, are reportedly present in Schwann cells. The expression of some of these factors is developmentally regulated (Stewart et al., 1996;

Zorick and Lemke, 1996). Recent experimental work on two transcription factors in particular, Krox-20 and SCIP, has clearly established their important roles in Schwann cell differentiation. Inactivating the zinc finger gene *Krox-20* by targeted, in-frame insertion of the *Escherichia coli lacZ* gene, prevents myelination in the mouse PNS. Schwann cell development in *Krox-20*$^{-/-}$ mutant mice appears to be blocked as cells begin myelin formation (Topilko et al., 1994). In these animals, Schwann cells establish a normal 1:1 association with axons and express the early myelin marker, myelin-associated glycoprotein (MAG), but fail to upregulate the late myelin constituents, such as P0. Using the *lacZ* insertion as a sensitive means to study *Krox-20* expression in *Krox-20*$^{+/-}$ heterozygous mice, it has been shown that the onset of Krox-20 synthesis in vivo coincides with the transition from Schwann cell precursors (S-100^{-}) to immature Schwann cells (S-100^{+}). In agreement with a role for NRGs in this transition (Dong et al., 1995, *see* Proliferation and Cell Differentiation), the expression of Krox-20 by glial cells in cultures of embryonic dorsal root ganglia can be induced by NRGs. Moreover, the expression of Krox-20 by Schwann cells in coculture with neurons, requires continuous axonal contact. These results suggest that Krox-20 is part of the missing link between the signaling process that is believed to occur between axons and Schwann cells (Murphy et al., 1996). It is uncertain, however, if Krox-20 can activate the transcription of myelin genes directly, since target sites for the zinc finger protein in the corresponding promoter regions have not been yet identified.

The POU transcription factor SCIP (also known as Oct-6, Tst-1) has been proposed as a transcriptional repressor of the late myelin genes in vivo. This suggestion was based on the finding that SCIP is able to repress transcription from the MBP and P0 promoters in vitro (Monuki et al., 1990, 1993) and that its axonally induced expression is significantly downregulated in myelinating Schwann cells (Scherer et al., 1994). Mice that express a truncated, dominant negative form of SCIP under the control of the P0 promoter, exhibit premature myelination during postnatal development, hypermyelination in adulthood, and an overall elevated expression of myelin genes (Weinstein et al., 1995). In contrast to these results, peripheral nerves in *SCIP* gene knockout mice are severely hypomyelinated in early postnatal life (Bermingham et al., 1996; Jaegle et al., 1996). Animals that survive to postnatal day 90, however, show normal myelination in the sciatic nerve (Jaegle et al., 1996). These findings suggest that myelin formation in the mutant Schwann cells is delayed as a result of a transient arrest of these cells at a stage prior to axonal wrapping (the promyelinating stage), and that SCIP may be required by Schwann cells in order to proceed to the myelin-forming phenotype.

Initiation of Myelination

The final step in the process leading to the production of a functional myelin sheath is the spiraling of the myelin membrane around the axon and the compaction of the membrane layers. Expression of the major myelin proteins, P0 and MBP, is not a prerequisite for the wrapping of axons, as can be seen in double knockout mice that lack these proteins. In the peripheral nerves of these animals, nerve fibers are wrapped by Schwann cell membranes, however, normal compact myelin sheaths are not formed (Martini et al., 1995). Studies examining the functions of SCIP and Krox-20 (*see* Myelin Gene Expression) further support the view that myelin gene expression and axon myelination may be differentially regulated. In the SCIP-deficient mice, the major myelin-specific genes are expressed around the time of birth, but the Schwann cells are temporarily arrested in the promyelinating stage (Bermingham et al., 1996; Jaegle et al., 1996). In contrast, Schwann cells in mice that lack Krox-20 fail to activate high-level expression of the late myelin genes, but they initiate wrapping and make approximately one-and-a-half turns around the axons (Topilko et al., 1994).

Each axon-Schwann cell element is enclosed with a basal lamina that is produced by the

Schwann cell. The formation of the basal lamina, which requires the presence of neurons in vitro (Bunge et al., 1982), is a prerequisite for myelination. Under conditions that prevent basal lamina deposition in cocultures, Schwann cells still express myelin-specific genes (Owens and Bunge, 1989; Brunden and Brown, 1990), but they fail to ensheath and myelinate the nerve fibers (Bunge and Bunge, 1986). The addition of an exogenous basal lamina or purified laminin to such cultures, however, restores myelin formation by the Schwann cells (Carey et al., 1986; Eldridge et al., 1989). The control of myelination by the basal lamina seems to be mediated by members of the integrin family. This is supported by the finding that Schwann cells express several integrins in vivo and in vitro, (Einheber et al., 1993; Niessen et al., 1994; Feltri et al., 1994), and that antibodies to beta 1 integrin inhibit myelin formation in cocultures (Fernandez-Valle et al., 1994). Beta 1 integrin, therefore, appears to have a critical role in transducing signals that initiate myelination.

It has been suggested that MAG is involved in the initiation of myelin sheath formation. This view is supported by the expression pattern and periaxonal localization of this protein, as well as by its cell-adhesion promoting activity (Martini and Schachner, 1986; Poltorak et al., 1987) Furthermore, myelination is enhanced in cocultures of sensory neurons with transfected Schwann cells that overexpress MAG (Owens et al., 1990), whereas, Schwann cells that express MAG antisense RNA contain reduced levels of MAG and fail to form myelin (Owens and Bunge, 1991). It was, therefore, surprising that knockout mice totally deficient in MAG expression showed near-normal myelination in early adulthood (Li et al., 1994, Montag et al., 1994). Abnormalities were found only in the periaxonal space of myelinated nerve fibers. In animals older than 8 mo, however, axons and myelin degenerated (Fruttiger et al., 1995). These observations suggest that MAG may be involved in maintaining the integrity of myelinated axons rather than in initiating myelin formation. Alternatively, the near-normal myelin formation in MAG knockout mice may be explained by compensation of a crucial MAG function by other molecules. One of the candidate molecules, neural cell adhesion molecule, N-CAM, has recently been tested. Interestingly, mice deficient in both the *MAG* and the *N-CAM* gene still form normal myelin sheaths. Therefore, in MAG knockout mice, N-CAM does not compensate for the potential function of MAG in myelination (Carenini et al., 1997).

The cytoplasmic protein, periaxin, is exclusively found in peripheral myelin. Based on its primary stucture, and its coexpression and colocalization with MAG during early postnatal development, it has been suggested that Periaxin plays a role in axonal ensheathment (Gillespie et al., 1994). The dramatic relocalization of Periaxin from the adaxonal (apposing the axon) to the abaxonal (apposing the basal lamina) side of the Schwann cell membrane after myelin sheath formation suggests another function for this protein in the stabilization of the mature myelin sheath (Scherer et al., 1995). Functional studies have to be performed in order to assess the role of periaxin in myelin formation and/or stabilization.

In contrast to the reported functions of peripheral nerve fibers in Schwann cell proliferation and differentiation, less is known about the role of peripheral axons in controlling the onset of myelination. Early microscopic studies on PNS myelination have found that correlations exist between the diametric size of an axon and the thickness of the myelin sheath as well as its internodal length, i.e., the larger the axon, the thicker the myelin sheath and the longer the internode of myelin (Young, 1945; Friede and Samorajski, 1967; Williams and Wendell-Smith, 1971). From these findings came the view that peripheral axons do exert some influence over myelin sheath formation. Morphological studies have also found a close correlation between the size of an axon and whether or not it is myelinated (Duncan et al., 1934). These observations suggested that a critical axon diameter was required in order to initiate myelination. Although quantitative studies on a variety of peripheral nerves have supported this view (Friede and Samorjski, 1968; Friede, 1972), evi-

dence gathered from other groups do not support this finding and instead show that the onset of myelination occurs over a wide range of axon diameters (Fraher, 1972; Hahn et al., 1987). Experimental evidence, however, has now been obtained for the peripheral nervous system indicating that axon caliber is a crucial determinant of axon myelination (Voyvodic, 1989). It has been found that sympathetic postganglionic axons, a group of fibers normally unmyelinated, will become myelinated when their diameter is increased in response to an experimental expansion of their peripheral target field. Although this study demonstrates the clear influence of axon morphology over the onset of myelination in the PNS, it remains to be seen whether the size of an axon can be related to molecular changes occuring on the surface of the axon membrane, and that it is these changes that initiate myelination.

CNS Myelination

Migration and Cell Differentiation

Our knowledge of the regulatory mechanisms involved in the myelination of fiber tracts in the CNS stems largely from in vitro and in vivo studies that have examined the origin of the oligodendrocyte. This cell has been shown to arise from a progenitor cell, which was originally characterized in vitro by its ability to bind the monoclonal antibody, A2B5, and to form into either an oligodendrocyte or type-2 astrocyte (Raff et al., 1983), thus termed O-2A progenitor cell. Recently, however, it has been suggested that this term only applies to progenitor cells grown in vitro. Indeed, "O-2A progenitor cells" develop exclusively into oligodendrocytes when transplanted into the brain and spinal cord in vivo (Espinosa de los Monteros et al., 1993; Groves et al., 1993) and, in spite of extensive efforts, no conclusive evidence has been found that "type-2 astrocytes" exist in vivo (Fulton et al., 1991, 1992).

An examination of the prenatal rodent brain with probes to platelet-derived growth factor (PDGF) alpha receptor and myelin protein DM20 mRNAs, both of which are expressed by oligodendrocyte progenitor cells, has provided evidence that oligodendrocyte progenitors arise in restricted regions of the CNS (Pringle and Richardson, 1993; Timsit et al., 1995). Their generation, from neuroepithelial cells, has been shown to be dependent on inductive signals from the notochord/floorplate, possibly involving the sonic hedgehog protein (Pringle et al., 1996). From their sites of origin, oligodendrocyte progenitors are thought to migrate through the neuropil to populate the developing fiber tracts. This idea is supported by cell-culture studies that show these cells to be highly migratory (Small et al., 1987; Armstrong et al., 1991; Kiernan and ffrench-Constant, 1993) and capable of directional movement, either along the permissive substrate of cultured radial glial cells (Goldman et al., 1997) or toward distinct molecular gradients (Armstrong et al., 1991). The populations of oligodendrocytes found in the fiber tracts of the adult CNS are, therefore, thought to be derived from migratory pools of oligodendrocyte progenitor cells. The brain obtains its final complement of oligodendrocyte numbers from these original pools during the ensuing period of growth factor-induced progenitor cell proliferation and a much later process of programmed cell death (Barres et al., 1992).

The proliferation of oligodendrocyte progenitor cells from these original cohorts and the timing of their differentiation has been shown in vitro to be regulated by a number of soluble factors (for reviews, *see* Goldman, 1992; McKinnon and Dubois-Dalcq, 1996; McMorris and McKinnon, 1996). One mitogenic factor, PDGF, is secreted by astrocytes, one of the first glial cell types to differentiate in the developing CNS. In the presence of PDGF, progenitor cells will continue dividing until an internal timing mechanism within these cells, which appears to count time or cell division, drives them to terminal differentiation (Raff et al., 1988; Gao et al., 1997). This internal clock, however, can be overridden by the presence of basic fibroblast growth factor (bFGF), which maintains progenitor cells in a proliferative state (McKinnon et al., 1990). In the absence of

further growth factor influence, these cells lose their responsiveness to PDGF and then follow a constitutive pathway towards differentiation into oligodendrocytes. Further factors, such as IGF I can also promote the proliferation and development of progenitor cells into oligodendrocytes in vitro (McMorris and Dubois-Dalcq, 1988). The proliferation and subsequent differentiation of oligodendrocyte progenitor cells is, therefore, regulated by extrinsic factors associated with the cellular environment these cells migrate through. In contrast, further maturation of oligodendrocytes appears to be regulated, not by extrinsic factors, but by an instrinsic program of these cells.

Myelin Gene Expression

The maturation of oligodendrocytes into myelin-forming cells is characterized by the orderly temporal expression of the proteins that make up myelin. Interestingly, the transcription and translation of the myelin-specific genes by oligodendrocytes is much less dependent on axon signaling than the expression of these genes by Schwann cells *(see* Initiation of Myelination). In the absence of neurons, cultured oligodendrocytes, dissociated from developing CNS regions, will mature at the same rate as oligodendrocytes found in the same brain region in vivo (Zeller et al., 1985). That is, the orderly appearance and peak expression of the myelin proteins and their mRNAs, by oligodendrocytes, appears to be independent of continual neuronal influences (Mirsky et al., 1980; Abney et al., 1981; Zeller et al., 1985; Dubois-Dalcq et al., 1986). In vivo studies that examine myelin gene expression in transected fiber tracts, however, have shown that in the absence of axon integrity following optic nerve transection, the expression of myelin genes is reduced from that found in the nerves of age-matched, control animals (Kidd et al., 1990; McPhilemy et al., 1990; Scherer et al., 1992). Although these results may, in part, be explained by the reduction in oligodendrocyte numbers found in nerves after transection (David et al., 1984), they nevertheless, point to some axonal influence on myelin-gene expression. The fact, however, that the oligodendrocytes in these nerves continue to express significant levels of myelin protein mRNAs following loss of axon contact, suggests that transcriptional regulation of the myelin genes is part of the oligodendrocyte's intrinsic program. This is in marked contrast to the pronounced reduction of myelin gene expression seen in denervated Schwann cells *(see* Myelin Gene Expression).

Transcription factors have been identified that are important in the regulation of Schwann cell development *(see* Myelin Gene Expression). However, little is known about transcription factor action in developing oligodendrocytes. Krox-20, for example, is essential for the terminal differentiation of Schwann cells, however, it has not been found to be expressed in the oligodendrocyte cell lineage (Topilko et al., 1994). Accordingly, in *Krox-20* knockout mice, CNS myelination is normal. Although the transcription factor, SCIP, has been found in undifferentiated oligodendrocyte progenitors (Collarini et al., 1992), as well as in developing Schwann cells (Blanchard et al., 1996), it has no effect on CNS myelination, whereas, it has a pronounced effect on PNS myelination (Bermingham et al., 1996; Jaegle et al., 1996). The relevance of SCIP expression in the oligodendrocyte lineage is, therefore, unknown. Further transcription factors of the zinc finger superfamily are expressed in the oligodendrocyte cell lineage, i.e., MyTI, found in oligodendrocyte progenitors (Kim and Hudson, 1992), and rKr1 and rKr2, both found in differentiated oligodendrocytes (Pott et al., 1995; 1996). The function of these factors has yet to be elucidated.

Initiation of Myelination

The elaboration of myelin into a sheath by the processes of oligodendrocytes is a further step that can be achieved in the absence of neuronal influence (Bradel and Prince, 1983; Rome et al., 1986; Szuchet et al., 1986). At this point, the oligodendrocyte must be able to recognize the axon as the target structure for myelin ensheathment. Since the environment of oligo-

dendrocytes contains a variety of cell types (both neuronal and nonneuronal) and their accompanying processes, some type of axon-recognition signal must be present that attracts oligodendrocyte processes. Indeed, in vitro studies using dissociated cultures from embryonic mouse brain have shown that oligodendrocytes will only myelinate the axonal process of a neuron and will not associate with the dendritic processes or, for that matter, any other cell type (Lubetzki et al., 1993). It is likely that oligodendrocytes and/or neurons express cell-surface molecules that could mediate the initial contact and subsequent adhesion of the oligodendrocyte process to the axon. Indeed, oligodendrocytes have been shown to express a full repertoire of cell adhesion molecules prior to and during the initial stage of axon wrapping, i.e., MAG, enriched in the periaxonal membranes of myelinating oligodendrocytes (Trapp et al., 1989); N-cadherin, found on the somata and major processes of oligodendrocytes (Payne et al., 1996); and NCAM, expressed on elaborating myelin membrane (Bhat and Silberberg, 1988). The presence of adhesion molecules (NCAM and N-cadherin), that act via a homophilic binding mechanism, and are found on both neurons and oligodendrocytes (Lander, 1989), could be important in mediating the oligodendrocyte/neuronal contact that presages myelination. Recently, it has been shown that the mediation of this contact is independent of MAG expression. Although antibodies to MAG interfere in vitro with neuron-to-oligodendrocyte adhesion (Poltorak et al., 1987), transgenic mice totally deficient in MAG expression display the same extent of myelination as found in control mice (Li et al., 1994; Montag et al., 1994).

Contact of an oligodendrocyte process with an axon does not, by itself, initiate myelination. Rather, it is thought that both the oligodendrocyte and the axon must "mature" to a point that is conducive for myelination (Morell et al., 1984). For the oligodendrocyte, that point represents a time in maturation when myelin-constituent proteins are expressed and elaborated into a myelin sheath. For an axon, however, this point of maturity is unknown and cannot, therefore, be defined simply as a time concomitant with the expression of a specific protein or the development to a physiological state. Nevertheless, the fact that axons are the sole cellular structure myelinated by an oligodendrocyte (Lubetzki et al., 1993) and that myelination occurs during a precise period of fiber pathway maturation (Morell, 1984) suggests that axons exert an important regulatory function in the initiation of this event.

There are currently two notions on how an axon may initiate myelination. These have arisen from earlier studies examining the development of myelination in a variety of fiber-tract systems. From activity studies it has been suggested that the electrical state of an axon may play a role in triggering myelination (Gyllensten and Malmors, 1963; Tauber et al., 1980; Demerens et al., 1996). Alternatively, measurements of axons at the electron microscopic (EM) level have provided evidence that a minimum diameter of an axon is needed in order to initiate myelination (Duncan, 1934; Peters and Vaughn, 1970; Voyvodic, 1989). The idea that the physiological state of a neuron and its axon plays a role in initiating myelination originates from studies examining myelination in the optic nerves of dark-reared and early eye-opened animals. By examining the optic nerve of mice dark-reared for 3–4 wk, it was found that the number of myelinated fibers was reduced by 12% as compared to age-matched, control mice (Gyllensten and Malmfors, 1963). Similarly, a recent study has shown that intraocular injections of the sodium-channel blocker, tetrodotoxin (TTX), reduces myelin protein expression in the developing optic nerve (Demerens et al., 1996). In a related experiment, it was found that myelin protein expression was elevated above control values in the optic nerves of rabbits whose eyes were artifically opened (Tauber et al., 1980). The general interpretation of these studies, i.e., that electrical activity initiates myelination, has been brought into question because of recent electron microscopic studies on the myelination of fiber tracts silenced by injections of tetrodotoxin. In the absence of electrical

activity in the optic nerves of rats treated with intraocular injections of TTX throughout the first postnatal week, concurrent with the initial period of myelin formation, it was found that both the number of myelinated fibers and the time of myelination onset was the same for both treated and age-matched, control animals (Colello et al., 1995; Crespo et al., 1995). Likewise, treatment of spinal cord explants with TTX fails to block the onset and progression of axon myelination (Shrager and Novakovic, 1995). Additionally, activity blockade by intraocular injection of TTX during optic nerve regeneration in goldfish have no notable affect on the myelination of regenerating fibers (Hayes and Meyer, 1989). Interestingly, two independent groups have found that dark-rearing appears to have no effect on the initiation of myelination in the developing visual pathway (Moore et al., 1976; Fukui et al., 1991).

Although there appear to be contradictory findings in the studies evaluating the influence of electrical activity on the initiation of myelination, the differences might be explained by the known effect of electrical activity on oligodendrocyte progenitor cells. In the presence of electrical activity, progenitor cells are stimulated to proliferate (Barres and Raff, 1993), thereby increasing the number of oligodendrocytes that develop within a fiber tract. Conversely, in the absence of electrical activity during early postnatal development, proliferation of progenitor cells is blocked. Therefore, the total number of oligodendrocytes that differentiate from this progenitor pool is reduced. Since the peak period of progenitor-cell proliferation occurs during the first two postnatal weeks (Small et al., 1987), treatment of newborn animals with either TTX or dark-rearing would reduce the number of available oligodendrocytes to myelinate axons. This effect, which may not be apparent during the first week of myelination, would become more pronounced with time as the reduced oligodendrocyte population reaches its limit on the number of axons it can ensheath. Taken together, the contradictory findings in the studies evaluating the influence of electrical activity on the initiation of myelination can be explained by the pronounced effect of electrical activity on oligodendrocyte-progenitor cell proliferation and not on the initiation of myelination itself. The electrical activity of the axon may not act directly on oligodendrocyte-progenitor cells but rather indirectly by causing astrocytes to produce mitogenic factors (i.e., PDGF) that drive progenitor-cell proliferation (Barres and Raff, 1993).

The second notion of how axons initiate myelination originates from EM studies examining the onset of myelination in the PNS (*see* Initiation of Myelination). These studies have shown that a close correlation exists between the initiation of this event and the caliber of an axon (Matthews and Duncan, 1968; Voyvodic, 1989). This correlation, however, is less apparent for fiber tracts of the CNS, where the range of axon diameters becoming myelinated is larger than that found in the PNS. Nevertheless, EM studies of myelination in the pyramidal tract (Samorajski and Friede, 1968; Reh and Kalil, 1982; Gorgels, 1990), dorsal funiculus (Matthews and Duncan, 1968), corpus callosum (Looney and Elberger, 1986), and optic nerve (Colello et al., 1995) all show that myelination first appears on larger axons and then on progressively smaller ones.

Although it is possible that the diameter of an axon alone can initiate myelination, it is more likely that size reflects a level of maturation of the axon when new factors that act as initiation signals for myelination are being expressed on its surface. Indeed, ultrastructural studies suggest axons undergo membrane remodeling prior to myelination (Hildebrand and Waxman, 1984). Interestingly, Notterpek and Rome (1994) have isolated a monoclonal antibody against an unknown protein(s) on the axolemma, which dramatically blocks myelination in cultures of the cerebellum. Although the antigen has not been fully characterized and its effect on the initiation of myelination has not been tested, their results suggest an involvement of axons in CNS myelination. Alternatively, it is possible that increasing the size of an axon may dilute an already present inhibitory signal for myelination below the threshold for functional activ-

ity. Evidence, however, for this idea has not been reported.

Myelination in the Visual Pathway

Although much of what we know about myelination has been inferred from in vitro studies, each of the events described above that lead to myelination, and the regulatory mechanisms underlying them, have been studied, at one time or another, for the developing visual system. The advantage of using this fiber tract over others for studies on myelination is primarily because it is easily accessable both pre- and postnatally. Also, since the axons of this fiber tract are derived from a single source of neurons within the retina, they can be easily manipulated both physiologically and pharmacologically. From the results of in vivo and in vitro studies on myelination in the optic nerve, we are beginning to identify which of the distinct steps along the pathway to myelination are intrinsically or extrinsically regulated. The emerging model of optic axon myelination is as follows: oligodendrocyte-progenitor cells enter the optic nerve prenatally from the chiasmatic end of the nerve (Small et al., 1987). The factor(s) that influence their directional migration into the nerve are unknown, but they may be related to some property of the recently arriving optic axons. Indeed, rat retinal ganglion cells are physiologically active at the time of progenitor-cell migration (Galli and Maffei, 1988). Activity-induced growth factor release by already present type-I astrocytes (Barres et al., 1993) could establish a molecular gradient for progenitor-cell migration. Alternatively, the presence of favorable substrate proteins found on the surface of early axons (Hynes and Lander, 1992) could act to guide progenitor cells into the nerve. Since oligodendrocyte-progenitor cells are found throughout the length of the rat nerve by E17 (Small et al., 1987), and the first to differentiate are found nearest the chiasm around P3 (Colello et al., 1995), this would suggest that the optic nerve population is set up by a "wave" of mitotic cells moving towards the orbit, with post-mitotic cells (differentiated oligodendrocytes) taking up positions in a chiasm-to-eye arrangement. Upon differentiation, oligodendrocytes begin to transcribe myelin protein genes at a time similar to that observed for oligodendrocytes grown in vitro in the absence of neurons (Zeller et al., 1985; Dubois-Dalcq et al., 1986). This suggests the presence of an intrinsic program for myelin-gene expression that runs independent of neuronal influences. The subsequent appearance of myelin proteins occurs with the same time delay for oligodendrocytes located in any part of the nerve (Colello et al., 1995), suggesting that the event of translation does not involve additional regulatory mechanisms (*see* also Mirsky et al., 1980). However, the event of myelin ensheathment appears to be regulated by an extrinsic signal and is not part of this intrinsic oligodendrocyte program. This is evident by the difference in time delay between myelin-protein production and myelin ensheathment for oligodendrocytes located on opposite sides of the nerve (eye vs chiasm). Myelin ensheathment begins at approx P6–7 in a clear eye-to-chiasm gradient (Skoff et al., 1980; Colello et al., 1995). This suggests the presence of a myelin initiation signal, which is graded along the nerve and strongest near the eye. Although the signal(s) that initiates axon myelination is not known, blocking electrical activity in the optic nerve does not alter the onset of optic axon myelination (Colello et al., 1995; Crespo et al., 1995). These results argue that optic axon myelination is initiated by an activity-independent mechanism. Nevertheless, the fact that oligodendrocytes solely ensheath axons and no other cellular structure (Lubetzki et al., 1993) suggests that the signal must be axon-derived. The ensuing myelination of optic axons is accompanied by further diametric growth (Colello et al., 1994), culminating in the attainment of full physiological maturity by the axon population.

Myelination of CNS Axons by Schwann Cells and PNS Axons by Oligodendrocytes

Much of our understanding of the events involved in myelination has been obtained from

observing myelination under normal conditions, that is, oligodendrocytes interacting with CNS fibers and Schwann cells interacting with PNS fibers. Several experimental and pathological conditions exist, however, that provide a unique opportunity to observe Schwann cells interacting with CNS axons, and oligodendrocytes interacting with peripheral axons. These studies, described below, have provided evidence that a common neuronal mechanism may initiate myelination by both Schwann cells and oligodendrocytes.

Schwann cells are prevented from entering the CNS by the glial-limiting membrane, a structure formed by astrocytes at the transition zone between the CNS and PNS (Fraher, 1992). Under experimental as well as pathological conditons, however, this barrier can break down, allowing Schwann cells to migrate into the CNS and myelinate CNS axons (Gilmore and Sims, 1986; Duncan and Hoffman, 1996; for review, *see* Franklin and Blakemore, 1993). In addition, transplanted Schwann cells can remyelinate focal lesions in the CNS that are devoid of myelin (for review, *see* Duncan, 1996), leading to the restoration of near-normal conduction properties to these axons (Felts and Smith, 1992). Furthermore, in vitro, Schwann cells have the ability to myelinate CNS axons just as readily as PNS axons (Bahr et al., 1991). A corollary to these studies is that oligodendrocytes will myelinate peripheral axons. Indeed, in graft studies in which regenerating PNS axons encounter oligodendrocytes, it has been demonstrated that peripheral axons can induce oligodendrocytes to myelinate (Aguayo et al., 1978; Weinberg and Spencer, 1979). Taken together, these results suggest that the axon signal(s) that lead to the myelination of nerve fibers may be similar in both the PNS and CNS, and that Schwann cells and oligodendrocytes have the capacity to interpret these signals in both systems.

Summary

Recent cell and molecular biological studies have provided insight into the mechanisms underlying the development of Schwann cell and oligodendrocyte precursors into fully differentiated, myelin-forming cells. It is now becoming clear that this maturational proccess is comprised of a number of distinct stages, each independently regulated by cell intrinsic and/or extrinsic factors. Although many of these stages and factors have helped to define the differences between Schwann cells and oligodendrocytes, there is an increasing amount of evidence that the development of these cells is often under comparable regulatory control. In vitro studies have demonstrated that the proliferation and differentiation of both Schwann cells and oligodendrocytes requires growth factors, some of which, e.g., FGF, IGF, PDGF, have similar effects on the two cell types. Culture studies have further shown that oligodendrocytes and, to a limited extent, Schwann cells are capable of expressing the myelin genes in the absence of any neuronal influence. Finally, the myelination of axons by both these cells appears to require an axon signal which, in some way, is related to the caliber of the axon. This myelination signal may be similar or even identical for both the CNS and PNS, as suggested by the ability of both Schwann cells and oligodendrocytes to myelinate fibers derived from either system. It is likely, therefore, that future myelination studies will continue to reveal fundamental similarities between the oligodendrocyte and the Schwann cell.

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