INTRODUCTION

Myelin is a multi-lamellar structure that surrounds axons and increases axonal conduction velocity. It is formed by the spiral wrapping of the cell membrane of Schwann cells (in the PNS) and oligodendrocytes (in the CNS). Compact myelin is chiefly composed of lipids, but also contains a unique set of proteins; in the PNS, these are P0, myelin basic protein (MBP), and peripheral myelin protein-22 kD (PMP-22) (Lemke, 1992; 1993). Other membrane proteins are excluded from compact myelin even though their expression is characteristic of myelinating Schwann cells. Their discrete localization emphasizes that myelinating Schwann cells appear to be polarized epithelial cells, whose plasma membrane contains several distinct domains, each of which has its own distinct repertoire of proteins. Thus, myelin-associated glycoprotein (MAG), the gap junction protein connexin32, oligodendrocyte-myelin glycoprotein and E-cadherin are all found in the perinodal regions and the Schmidt-Lanterman incisures (Trapp and Quares, 1982; Bergoffen et al., 1993; Apostolski et al., 1994; Fannon et al., 1995; Scherer et al., 1995) In contrast, the integrins α6 and β4 are localized to the (abaxonal) surface of myelinating Schwann cells, which apposes their basal laminae (Einheber et al., 1993; Feltri et al., 1994). The adaxonal surface and its associated periaxonal rim of cytoplasm, which appose the axon, are characterized by the expression of MAG, spectrin and F-actin (Trapp et al., 1989). This complex organization of the Schwann cell-axon unit is presumed to contribute to the structural basis required for the formation and stabilization of the myelin sheath.

In addition to myelin-forming Schwann cells, mature peripheral nerve also contains non-myelinating Schwann cells. They ensheathe one or more axons without forming a myelin sheath around any of them (Peters et al., 1991). In addition to these morphological distinctions, non-myelinating and myelinating Schwann cells have different molecular phenotypes. Non-myelinating Schwann cells express glial fibrillary acid protein (GFAP), p75/low-affinity nerve growth factor receptor (NGFR), growth-associated protein 43 kD (GAP-43), neural cell adhesion molecule (N-CAM), and the cell adhesion molecule L1, but not P0, MBP, PMP-22 and MAG (Mirsky and Jessen, 1990; Snipes et al., 1992; Curtis et al., 1992; Scherer et al., 1994b). Conversely, myelinating Schwann cells do not express GFAP, NGFR, GAP-43, N-CAM and L1. In spite of these differences, the phenotype of Schwann cells depends on their association with axons, and can be experimentally altered.

SUMMARY

Periaxin is a newly described protein that is expressed exclusively by myelinating Schwann cells. In developing nerves, periaxin is first detected as Schwann cells ensheathe axons, prior to the appearance of the proteins that characterize the myelin sheath. Periaxin is initially concentrated in the adaxonal membrane (apposing the axon) but, during development, as myelin sheaths mature, periaxin becomes predominately localized at the abaxonal Schwann cell membrane (apposing the basal lamina). In permanently axotomized adult nerves, periaxin is lost from the abaxonal and adaxonal membranes, becomes associated with degenerating myelin sheaths and is phagocytosed by macrophages. In crushed nerves, in which axons regenerate and are remyelinated, periaxin is first detected in the adaxonal membrane as Schwann cells ensheathe regenerating axons, but again prior to the appearance of other myelin proteins. Periaxin mRNA and protein levels change in parallel with those of other myelin-related genes after permanent axotomy and crush. These data demonstrate that periaxin is expressed by myelinating Schwann cells in a dynamic, developmentally regulated manner. The shift in localization of periaxin in the Schwann cell after completion of the spirialization phase of myelination suggests that periaxin participates in membrane-protein interactions that are required to stabilize the mature myelin sheath.

Key words: Wallerian degeneration, peripheral nerve, axotomy, nerve growth factor receptor, myelin, Schwann cell, rat
so that non-myelinating Schwann cells can become myelinating Schwann cells and vice versa. Thus, previously non-myelinating Schwann cells can form myelin sheaths if they are transplanted into a nerve that normally contains many myelinating Schwann cells (Langley and Anderson, 1903; Simpson and Young, 1945; Aguayo et al., 1976b; Weinberg and Spencer, 1976). This finding demonstrates that axons, and not the Schwann cells themselves, determine whether a Schwann cell has a myelinating or a non-myelinating phenotype.

The maintenance of the myelinating phenotype requires continuous axon-Schwann cell interactions, as illustrated by the sequence of events that follow axotomy, which causes axons to degenerate distal to the lesion. Within a few days of axotomy, the axons and myelin sheaths begin to degenerate, but the Schwann cells survive and even proliferate. Myelinating Schwann cells cease expressing high levels of myelin-related proteins and their mRNAs, and begin expressing ones that are more characteristic of non-myelinating Schwann cells, such as the NGFR and GAP-43 (Taniuchi et al., 1988; Curtis et al., 1992; Plantinga et al., 1993; Scherer et al., 1994b). However, if axons regenerate and are remyelinated, the pattern of gene expression reverses: the expression of myelin-related genes increases and the expression of NGFR and GAP-43 falls (Taniuchi et al., 1988; Plantinga et al., 1993; Scherer et al., 1994b). The coordinate increase in myelin-related genes occurs in a manner that appears quite similar to that seen during the onset of myelination in developing nerves. The levels of myelin-related proteins and mRNAs increase, probably because the rate of their transcription increases (Wood and Engel, 1976; Uyemura et al., 1979; Stahl et al., 1990; Wiktorowicz and Roach, 1991). Thus, there is a coordinate program of myelin gene expression, regulated at the level of transcription, which accompanies the synthesis of myelin during both development and regeneration.

In this paper, we have investigated the expression and localization of a newly described protein, periaxin (Gillespie et al., 1994), which is probably the same protein previously identified as P170 and SAG (Shuman et al., 1986; Dieperink et al., 1992). In adult nerve, we find that periaxin is expressed exclusively by myelinating Schwann cells and is predominately localized to their abaxonal surface. In developing and regenerating nerves, however, periaxin is predominately localized to the adaxonal surface of Schwann cells as they begin to form compact myelin. Periaxin immunoreactivity is seen before those of MAG, MBP or P0, which is consistent with the electron microscopic evidence that it is expressed by ensheathing Schwann cells (Gillespie et al., 1994). Periaxin mRNA levels change in parallel with those of other myelin-related genes in permanently axotomized and regenerating nerves. These data show that periaxin is a myelin-related protein, and the dynamic changes in its localization during ensheathment and myelination suggest that it may play an important role in these processes.

MATERIALS AND METHODS

Sciatic nerve transection and crush

Using aseptic technique, the sciatic nerves of anesthetized (50 mg/kg pentobarbital i.p.), adult (10-13 week old) Sprague-Dawley rats were exposed at the sciatic notch. Some nerves were doubly ligated, trans-
Fig. 2. Immunohistochemical analysis of periaxin expression in Wallerian degeneration. These are longitudinal sections of the distal nerve stump of an adult rat sciatic nerve at 8 (E), 12 (A,B), and 24 (C,D) days post-transection. Periaxin immunoreactivity is green (fluorescein); the rhodamine is either S-100β (B), MBP (D) or ED-1 (E). Pairs of photomicrographs (A,B and C,D) were taken from the same field; (E) is a confocal image. (A,B) Periaxin-immunoreactive material (p) is found in both S-100β-positive (Schwann cells) and S-100β-negative cells (macrophages). (C,D) Periaxin-immunoreactive material (p) is associated with MBP-positive myelin ovoids. (E) Periaxin-immunoreactive material (p) is found within both ED-1-positive (macrophages) and ED-1-negative cells (Schwann cells). Scale bar: 10 μm.

Fig. 3. Immunohistochemical analysis of periaxin in regenerating adult rat sciatic nerve. Longitudinal sections from the distal nerve stump at 12 days (A-D) and transverse sections at 24 days (E,F) postcrush were double-labeled for periaxin (A,C,E; fluorescein) and either P0 (B) or MAG (D,F). The periaxin-positive Schwann cells that are associated with regenerating axons form long strands that run between clumps of degenerating periaxin-positive debris (A,C). Two periaxin-positive, MAG-negative Schwann cells are indicated by arrowheads (C,D), whereas most of the periaxin-positive cells are P0-negative (A,B). In transverse sections, many myelinating Schwann cells have a double ring of periaxin labeling: an inner (adaxonal) ring that superimposes with MAG immunoreactivity (arrows), and an outer ring that marks the abaxonal surface (arrowheads). Scale bars: 50 μm (A-D); 10 μm (E,F).
sected with iridectomy scissors, and the two nerve stumps were sutured at least 1 cm apart; this technique prevents axonal regeneration to the distal nerve stump for at least 2 months. Nerve crush was produced by tightly compressing the sciatic nerve at the sciatic notch with flattened forceps twice, each time for 10 seconds; this technique causes all of the axons to degenerate, but allows axonal regeneration. At varying times after nerve injury, the animals were killed by CO2 inhalation, the distal nerve stumps were removed, and the most proximal 2-3 mm were trimmed off. For transected nerves, the entire distal nerve stump was taken from just below the lesion to the ankle (about 4 cm long). For crushed nerves, the distal nerve stump was divided into two equal segments, termed the proximal and distal segments, each about 2 cm long. The nerves were immediately frozen in liquid nitrogen and stored at −80°C. Unlesioned sciatic nerves were obtained from animals of varying ages, from P1 (the day after birth) to P90. All animal protocols were approved by the Institutional Animal Care and Use Committee of The University of Pennsylvania.

Northern blotting
RNA was isolated from rat sciatic nerves and Schwann cells by CsCl gradient centrifugation (Chirgwin et al., 1979). Equal samples (10 µg) of total RNA were electrophoresed in 1% agarose, 2.2 M formaldehyde gels, transferred to nylon membranes (Duralon, Stratagene) in 6x SSC, and u.v. cross-linked (0.12 joules). Blots were prehybridized, hybridized and washed using standard techniques; the final stringency of the wash was 0.2x SSC at 65°C for 30 minutes (Sambrook et al., 1989). The following cDNAs were utilized as probes, a 1.1 kb fragment of rat periaxin (Gillespie et al., 1994), a full-length cDNA of rat P0 (Lemke and Axel, 1985), a 0.7 kb BamHI fragment of rat NGF (Radeke et al., 1987) and a full-length cDNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH; (Fort et al., 1985). Plasmid inserts were isolated after restriction endonuclease digestion, separated by agarose gel electrophoresis and purified by electrolution.

Electron microscopy
P28 Wistar rats were anesthetized and perfused with 4% formaldehyde (freshly prepared from paraformaldehyde) in 0.01 M periodate, 0.075 M lysine, 0.1 M phosphate buffer at pH 7.4 (McLean and Nakane, 1974). The sciatic and trigeminal nerves were removed and fixed for another 2-3 hours at room temperature. Sciatic nerves were removed and fixed by immersion in the same fixative for 2-3 hours at room temperature. The tissues were processed according to the method of Berryman et al. (1992). Briefly, tissues were washed several times with 0.1 M phosphate buffer containing 3.5% sucrose and stained with 0.2% tannic acid in the same buffer for 1 hour at 4°C (subsequent steps were performed at 4°C). After several washes, aldehydes were quenched in 50 mM NH4Cl in the same buffer. The tissues were then washed four times in 0.1 M maleate buffer (pH 6.2) containing 4% sucrose, followed by 1% uranyl acetate in maleate-sucrose buffer for 1 hour. The tissues were dehydrated to 90% ethanol (from 70% ethanol onward all steps were at −20°C), absolute ethanol for 15 minutes then infiltrated with a 1:1 ratio of LR Gold (Agar Scientific Ltd., Stanstead, Essex) and ethanol, followed by a 7:3 ratio of LR Gold and ethanol, and two changes of LR Gold for 1 hour and overnight. The tissues were infiltrated in two changes of LR Gold containing 0.5% benzoin methyl ether, for 1 hour and then overnight, and embedded in gelatin capsules. Polymerization was by u.v. irradiation at a wavelength of 365 nm for 48 hours at −20°C.

RESULTS

Periaxin is expressed by myelinating Schwann cells
Since periaxin has only been found in myelinating Schwann cells (Gillespie et al., 1994), we compared its localization to those of other Schwann cell markers in the adult rat PNS. As shown in Fig. 1A, periaxin immunoreactivity stopped abruptly at the interface of the PNS and CNS, demonstrating that it is not expressed by oligodendrocytes. P0 and PMP-22 immunoreactivities were also confined to myelinating Schwann cells (data not shown), whereas MAG (Fig. 1B), MBP (data not shown), and S-100β immunoreactivities (data not shown) were found in both the PNS and the CNS. In both the nerve roots and the sciatic nerve of adult rats, periaxin was predominately localized to the axonal surface of myelinating Schwann cells, which apposes their basal laminae (Fig. 1A,C,E). There was also distinct staining of the adaxonal surface, which apposes the axon, as well as of the incisures and paranodes (Fig. 1A,C,E), but compact myelin was not labeled. We compared the localization of periaxin with that of MAG (Fig. 1B,D), which was localized at the adaxonal membrane of...
Schwann cells and oligodendrocytes (Sternberger et al., 1979), and even more prominently in the incisures and paranodes (Trapp and Quarles, 1982). To determine whether periaxin was expressed by non-myelinating Schwann cells, we examined the cervical sympathetic trunk, which is mostly composed of unmyelinated axons and their (non-myelinating) Schwann cells (Aguayo et al., 1976a). Periaxin immunoreactivity was found only around the thinly myelinated axons (Fig. 1F), which was confirmed by double-labeling for P0 and MBP (data not shown). Thus, in the mature PNS, periaxin is exclusively found in myelinating Schwann cells, predominantly at the abaxonal surface.

Periaxin has a similar mobility by SDS-polyacrylamide gel electrophoresis to two proteins previously isolated from peripheral nerve myelin, P170 and SAG (Shuman et al., 1986; Dieperink et al., 1992). When sections of adult spinal cord were labeled with two rabbit and three guinea pig antisera against P170 (kindly provided by Dr David Pleasure), all stained myelinating Schwann cells in an identical pattern to that of the periaxin antiserum. As this pattern of staining is essentially the same as described for SAG (Dieperink et al., 1992), periaxin, P170 and SAG are probably the same protein.

**Periaxin expression in degenerating and regenerating nerves**

It was of interest to learn what happens to periaxin after nerve injury, since most myelin-related proteins disappear after axotomy, as the distal nerve stump undergoes Wallerian degeneration (Mirsky and Jessen, 1990; Scherer and Salzer, 1995). Thus, we examined the distal nerve stumps after both permanent axotomy (transsection) and nerve crush, which also causes Wallerian degeneration, but allows axonal regeneration. By 4 days postlesion, the smooth rim of abaxonal periaxin immunoreactivity was largely gone; focal aggregates of periaxin were seen in the Schwann cell cytoplasm, but separate from the degenerating myelin sheaths. After 4 days, most of periaxin immunoreactivity was found in large aggregates, often associated with the degenerating myelin sheaths, the so-called myelin ovoids, which contained the myelin proteins P0, MBP and MAG (Fig. 2C,D). The amount of periaxin-positive material, as well as myelin debris, fell progressively after 8 days postinjury, but was still found here and there even at 58 days postlesion. The periaxin aggregates were initially found mostly in Schwann cells and not in macrophages, but by 58 days postlesion, most of the periaxin-immunoreactive material was found in macrophages (Fig. 2A,B,E). Thus, even though periaxin is not present in compact myelin, it is degraded along with the components of the myelin sheath by Schwann cells and macrophages (Stoll et al., 1989).

Since regenerating axons are remyelinated after nerve crush (Ramon y Cajal, 1928), we could evaluate further how axon-Schwan cell interactions regulate periaxin expression. In crushed nerves, the periaxin that was previously associated with myelinating Schwann cells appeared to be degraded as in transected nerves but, in addition, periaxin was re-expressed as Schwann cells ensheathed and myelinated regenerating axons (Fig. 3). Schwann cells with periaxin-positive sheaths ensheathing regenerating axons, which were labeled by a rat monoclonal antibody against neurofilaments, were seen in the proximal segment of distal nerve stumps at 8 days. By 12 days, these Schwann cells were much more numerous and extended much further into the distal nerve stump and, by 24 and 58 days postcrush, they were found throughout the full extent of the distal nerve stump. As the temporal and spatial gradient of periaxin-expressing Schwann cells is consistent with the idea that Schwann cells re-express periaxin as they remyelinate axons, we performed double labeling for periaxin and MAG, P0 or MBP to determine whether these proteins were co-expressed. At 8 days (data not shown) and 12 days postcrush (Fig. 3C,D), most periaxin-positive cells were also MAG-positive, although a few were MAG-negative. In contrast, at these early stages of regeneration, many periaxin-positive (and MAG-positive) cells were P0- (Fig. 3A,B) and MBP-negative (not shown). At 24 (Fig. 3E,F) and 58 days (not shown) most of the periaxin-positive Schwann cells also expressed MAG, P0 and MBP. These data demonstrate that periaxin and MAG are re-expressed by Schwann cells as they ensheath and remyelinate axons, and prior to the onset of P0 and MBP expression.

We also noted a change in the distribution of periaxin immunoreactivity during remyelination. Between 12 and 24 days postcrush, it was difficult to determine whether the periaxin immunoreactivity was abaxonal or adaxonal, owing to the thinness of the myelin sheath. At the level of the Schwann cell nucleus, where the nucleus and perinuclear cytoplasm separated the abaxonal and adaxonal surfaces, it was possible to determine that there was both abaxonal and adaxonal periaxin immunoreactivity, and that the latter typically predominated (Fig. 3E,F). By 58 days postcrush, most of the periaxin immunoreactivity was found abaxonally (data not shown), as in normal adult nerve. Thus, the localization of periaxin changes dynamically as Schwann cells ensheath and remyelinate axons.

To confirm that the amount of periaxin protein changed as predicted by the above immunohistochemistry, we performed western blot analysis using the rabbit polyclonal serum against rat periaxin fusion protein. After transection, the amount of periaxin protein decreased to a low level by 24 days and remained at this level until at least 60 days post-transection (data not shown). In crushed nerves, the amount of periaxin decreased to a nadir at 28 days, then returned to nearly normal levels at 60 days postcrush (data not shown). Thus, the level of periaxin protein changed in parallel with the immunohistochemical changes in periaxin expression in both transected and crushed nerves. These results show that the expression of periaxin, like other myelin-related proteins, depends on axon-Schwan cell interactions (Mirsky and Jessen, 1990; Scherer and Salzer, 1995).

**Periaxin expression in developing peripheral nerve**

The shift in the subcellular localization of periaxin during remyelination probably reconciles the contrasting immunohistochemical data from adult and neonatal nerves. In adult nerves, periaxin is predominately abaxonally localized (Fig. 1A), whereas we previously reported that periaxin was mostly adaxonal, which was based on our observations of neonatal nerves (Gillespie et al., 1994). To confirm that periaxin is redistributed during development, we analyzed P1, P6 and P15 sciatic nerves, spanning the most active period of myelination (Webster and Favilla, 1984). As expected, the number of periaxin-positive, as well as MAG-, MBP- and P0-positive Schwann cells increased dramatically during this period (Hahn et al., 1987). While there were numerous Schwann cells that
had robust periaxin staining both adaxonally and abaxonally in P1 and P6 nerves, by P15 abaxonal staining predominated in most myelinating Schwann cells. To determine whether periaxin was expressed prior to MAG, MBP and P0, we performed double-labeling. At P1, most of the periaxin-positive Schwann cells were also MAG-positive, but there were many periaxin-positive and MBP- or P0-negative cells (Fig. 4C-F). These data suggest that periaxin expression in developing nerves is similar to that in regenerating nerves: the expression of periaxin immunoreactivity preceded that of MAG, P0 and MBP, and that the predominant localization of periaxin changes from adaxonal to abaxonal.

This shift in localization during development was confirmed by immunoelectron microscopy. In P3 sciatic nerve, periaxin was found to be localized both adaxonally and abaxonally (Fig. 5A). In P28 trigeminal nerve, however, the amount of adaxonal labeling was considerably diminished, whereas the abaxonal surface remained heavily labeled (Fig. 5B). Although adult trigeminal nerve is illustrated, the localization of periaxin was identical in adult sciatic nerve (data not shown).

### Periaxin mRNA levels depend on axon-Schwann cell interactions

The above data, taken together, show that periaxin is expressed in concert with the other myelin-related genes in Schwann cells. Since the mRNA levels of these genes are exquisitely sensitive to the disruption of axon-Schwann cell interactions (Scherer and Salzer, 1995), we analyzed the levels of periaxin mRNA after permanent axotomy (transection) and nerve crush by northern blot analysis. To better illustrate the changes in gene expression in crushed nerves, we divided the distal nerve stumps into a proximal and a distal segment, as axons regenerate in a proximal-to-distal manner.

As shown in Fig. 6A, the steady state level of periaxin mRNA fell dramatically by 4 days posttransection and did not return even by 58 days. After nerve crush, the level of periaxin mRNA also fell over a similar time course, but began to return by 12 days in the proximal segment and by 24 days in the distal nerve segment (Fig. 6B). The level of periaxin mRNA in both segments continued to increase until at least 58 days postcrush. We compared the changes in periaxin mRNA with that of P0, NGFR and GAPDH, by reprobing the same blots. The changes in the levels of periaxin and P0 mRNA were strikingly similar in both transected and crushed nerves. The level of NGFR mRNA, in contrast, was reciprocally related to those of periaxin and P0, both in transected and in crushed nerves. The level of GAPDH mRNA did not change significantly after transection or crush.

These data indicate that axon-Schwann cell interactions are necessary to maintain the high level of periaxin mRNA, and that the increase in the expression of myelin-related genes, including periaxin, in regenerating nerves is mediated by axon-Schwann cell interactions. To demonstrate directly that regenerating axons were required for the return of periaxin mRNA in crushed nerves, we transected regenerating nerves at 24 days postcrush, when periaxin levels had begun to return. As shown in Fig. 6C, 2 days after transection of the regenerating nerve, the level of periaxin mRNA fell in the distal nerve stump. The blot was reprobed for P0 and NGFR mRNA, thereby demonstrating a fall in the expression of other myelin-related mRNAs, and an increase in the expression of genes expressed by denervated Schwann cells, respectively (Gupta et al., 1993; Scherer et al., 1994a).

### DISCUSSION

Periaxin is a protein whose deduced amino acid sequence predicts a cytoplasmic protein (Gillespie et al., 1994). We have shown that periaxin is expressed by myelinating Schwann cells during development and regenerative nerve growth.
cells, and that its localization changes dynamically during ensheathment and myelination. Since P170 and SAG have the same molecular weight and insolubility in Triton-X as periaxin, as well as the same immunohistochemical localization, all three are probably the same protein (Shuman et al., 1986; Dieperink et al., 1992; Gillespie et al., 1994). Furthermore, like other myelin-related proteins, the expression periaxin mRNA and protein is tightly regulated by axon-Schwann cell interactions (Scherer and Salzer, 1995). These data suggest that periaxin has a specific function in myelinating Schwann cells.

**Periaxin is expressed in ensheathing Schwann cells**

In both developing and regenerating nerves, we found that periaxin is initially found predominately in the adaxonal Schwann cell membrane. At the ultrastructural level, periaxin is expressed as soon as Schwann cells ensheathe axons in a 1:1 relationship, which corresponds to the onset of MAG expression (Gillespie et al., 1994; Martini and Schachner, 1986, 1988). The onset of P0 expression is slightly later, as it is first expressed after a few lamellae of compact myelin are formed (Hahn et al., 1987; Lamperth et al., 1990; Martini et al., 1988). Although periaxin, MAG, and P0 all appear to be expressed at roughly the same time, we found a few Schwann cells were periaxin-positive and MAG-negative, and many that were periaxin-positive and P0- and MBP-negative (see also Owens and Bunge, 1989), indicating that periaxin, MAG and P0 are expressed in that order in individual, myelinating Schwann cells. The earlier onset of periaxin and MAG immunoreactivities, however, do not necessarily mean that their corresponding mRNAs are expressed earlier than those of P0 and MBP. In oligodendrocytes, the onset of proteolipid protein (PLP) expression is delayed by as much as 3 days relative to other MBP, whereas the onset of PLP and MBP is delayed by as much as 3 days relative to other MBP, whereas the onset of PLP and MBP

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**Fig. 5.** Comparison of periaxin localization in longitudinal sections of P3 sciatic nerve (A) and P28 (B) rat trigeminal nerve by immunoelectron microscopy. A basal lamina (between arrowheads) surrounds compact myelin (m), which in turn, surrounds the axon (a). The axolemma is indicated by arrows. Gold particles (10 nm) are abundantly detected in at both the adaxonal and abaxonal myelin membranes at P3 (A), whereas by P28 the shift to a predominantly abaxonal localization has become apparent. Scale bar: 200 nm.

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**Fig. 6.** Northern blot analysis of normal and lesioned adult rat sciatic nerves. Each lane contains an equal amount (10 μg) of total RNA isolated from the distal stumps of sciatic nerves that had been transected (A), crushed (B) or transected 24 days after nerve crush and allowed to degenerate for an additional 2 days (C). The number of days after each of these lesions in indicated; the ‘0’ time point is from unlesioned nerves. In crushed nerves (B), the distal nerve stumps were divided into proximal (P) and distal (D) segments of equal lengths. The blots were successively hybridized with a radiolabeled cDNA probe for periaxin (A), NGFR (C), GAPDH (D) and P0 (B). The films were exposed for the following times: periaxin, 2 weeks; P0, 2 hours; NGFR, 1 day; GAPDH, 3 days. The periaxin signal is composed of two transcripts that are similar in size (data not shown); these two bands are difficult to discern on these blots.

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The identity of the band recognized by the periaxin probe in the 24 day transected sample is not known, but is probably due to cross-hybridization with a plasmid that contaminated this sample of RNA.
mRNA expression is the same (Dubois-Dalcq et al., 1987; Grinspan et al., 1993).

The localization of periaxin is developmentally regulated

We found that periaxin was predominately localized to the adaxonal Schwann membrane during ensheathment and early myelination, then predominately to the abaxonal membrane as myelination continued. These data reconcile previous reports on the localization of periaxin, SAG and P170 in peripheral nerve. Like the ultrastructural study of Gillespie et al. (1994), we find that periaxin is abundant in the adaxonal Schwann cell membrane in early postnatal rat sciatic sciatic nerve. SAG was localized to the adaxonal and abaxonal Schwann cell membranes of myelinated axons in human neurofibromas (Dieperink et al., 1992), which agrees with our localization in adult nerves. Finally, although P170 was initially reported to be in compact myelin (Shuman et al., 1986), we have repeated this experiment using the same antibodies and find that it is predominantly localized to the abaxonal membrane in adult nerve.

The developmental regulation of periaxin localization may reflect the reorganization of the Schwann cell membrane during myelination. There is a growing body of evidence that, as Schwann cells ensheathe axons, they become polarized. In axon-Schwann cell co-cultures, Schwann cells need to interact with axons in order to assemble a basal lamina (Clark and Bunge, 1989), and Schwann cells need a basal lamina in order to properly ensheath and myelinate axons and to express MAG and P0 (Eldridge et al., 1987, 1989; Fernandez-Valle et al., 1993). The redistribution of periaxin from the adaxonal to the abaxonal Schwann cell membrane may mark the polarization of myelinating Schwann cells. Periaxin is not the only protein to be redistributed, since MAG becomes restricted to the adaxonal surface as Schwann cells ensheath axons (Martini and Schachner, 1986, 1988). Finally, the integrin subunits α6 and β4 are found only on the abaxonal surface (Einheber et al., 1993; Feltri et al., 1994), even as they first appear in the rat sciatic nerve (about P6, Scherer, unpublished observations). Thus, periaxin, MAG and α6β4 integrin are all restricted with respect to the adaxonal and abaxonal Schwann cell membrane. The spatial restriction of these molecules is presumably important for their function in myelinating Schwann cells, although to date this has been shown directly only for MAG (Owens and Bunge, 1989; Li et al., 1994; Montag et al., 1994). Analysis of the molecular basis by which the polarization of periaxin distribution is accomplished during the maturation of the myelin sheath is likely to foster a better understanding of the cell biology of myelination in the peripheral nerve.

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