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REVIEW

Expression and functional roles of neural cell surface molecules and extracellular matrix components during development and regeneration of peripheral nerves

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Summary

By combining both immunocytochemical and functional investigations, a hypothetical framework will be developed for the molecular mechanisms underlying neuron-glia interactions during development and regeneration of peripheral nerves. In particular, the immunoglobulin-like molecules L1, N-CAM, MAG and P0, the extracellular matrix molecules laminin and tenascin, and the carbohydrates PSA and L2/HNK-1 will be considered. During early stages of limb bud innervation in embryos, L1 and N-CAM are expressed on axons and Schwann cells and are involved in axonal fasciculation, whereas tenascin is thought to be involved in forming a scaffold around the nerve possibly preventing axons and/or Schwann cells from leaving the nerve. PSA has been shown to be involved in pathway selection at initial stages of limb bud innervation. Later on, when motor axons enter muscles, the carbohydrates determine the branching pattern of the nerves. During myelination, L1 appears to play a pivotal role during the formation of the first Schwann cell loops around the prospective myelin-containing axons. MAG and P0 appear also to be functionally involved at initial stages of myelin formation. Additionally, MAG may contribute to the formation and maintenance of non-compacted myelin and axon-Schwann cell apposition whereas P0 is involved in myelin compaction. Under regenerative conditions, L1, N-CAM, laminin, and tenascin are strongly up-regulated by denervated Schwann cells. *In vitro* observations strongly suggest that these molecules might foster axonal regeneration. The carbohydrate PSA is confined to regrowing axons and is also a candidate to support axonal regrowth. L2/HNK-1, which is found on motor axon-associated Schwann cells, may provide regenerating motor axons with a selective advantage over others resulting in appropriate reinnervation of motor pathways. Since many of the functional studies this review refers to have been performed *in vitro*, some of the conclusions drawn need reexamination *in vivo*. Gene manipulations, such as the generation of null mutants followed by a thorough morphological and immunocytochemical investigation may be a powerful tool to resolve this problem.

Introduction

Axonal growth is dependent upon a variety of extrinsic molecular cues which are provided by the axons' cellular and extracellular environment (see reviews of Aguayo, 1985; Sanes, 1989; Fawcett & Keynes, 1990). The nature of these cues, their location and their mode of regulation are the central questions in developmental neurobiology, since the investigation of such molecular cues may clarify how appropriate connections are made when the nervous system is built up during development or restored after injury.

In the PNS, an important source for such molecular cues is the non-neuronal, supportive cells of peripheral nerve, the Schwann cells. These can either express such cues as intrinsic constituents of their cell membranes or secrete them as extracellular matrix components into the interstitial space (Fawcett & Keynes, 1990; Schachner *et al.*, 1990; Schachner, 1990, for reviews). Whereas axonal growth may depend upon Schwann cell-derived molecular cues, proliferation and differentiation of Schwann cells in turn depends upon axonal contact (Weinberg & Spencer, 1975; Aguayo *et al.*, 1976; Jessen & Mirsky, 1991, 1992). This close functional interrelationship between neurons and glia demonstrates the central role of molecules involved in cell–cell recognition during development and regeneration of the nervous system.

At present, there is a significant body of literature showing that particular neural cell surface and extracellular matrix molecules may be involved in neuron–glia interactions either during development or regeneration or both (see Rutishauser & Jessell, 1988; Lander, 1989; Schachner *et al.*, 1990; Schachner, 1990,

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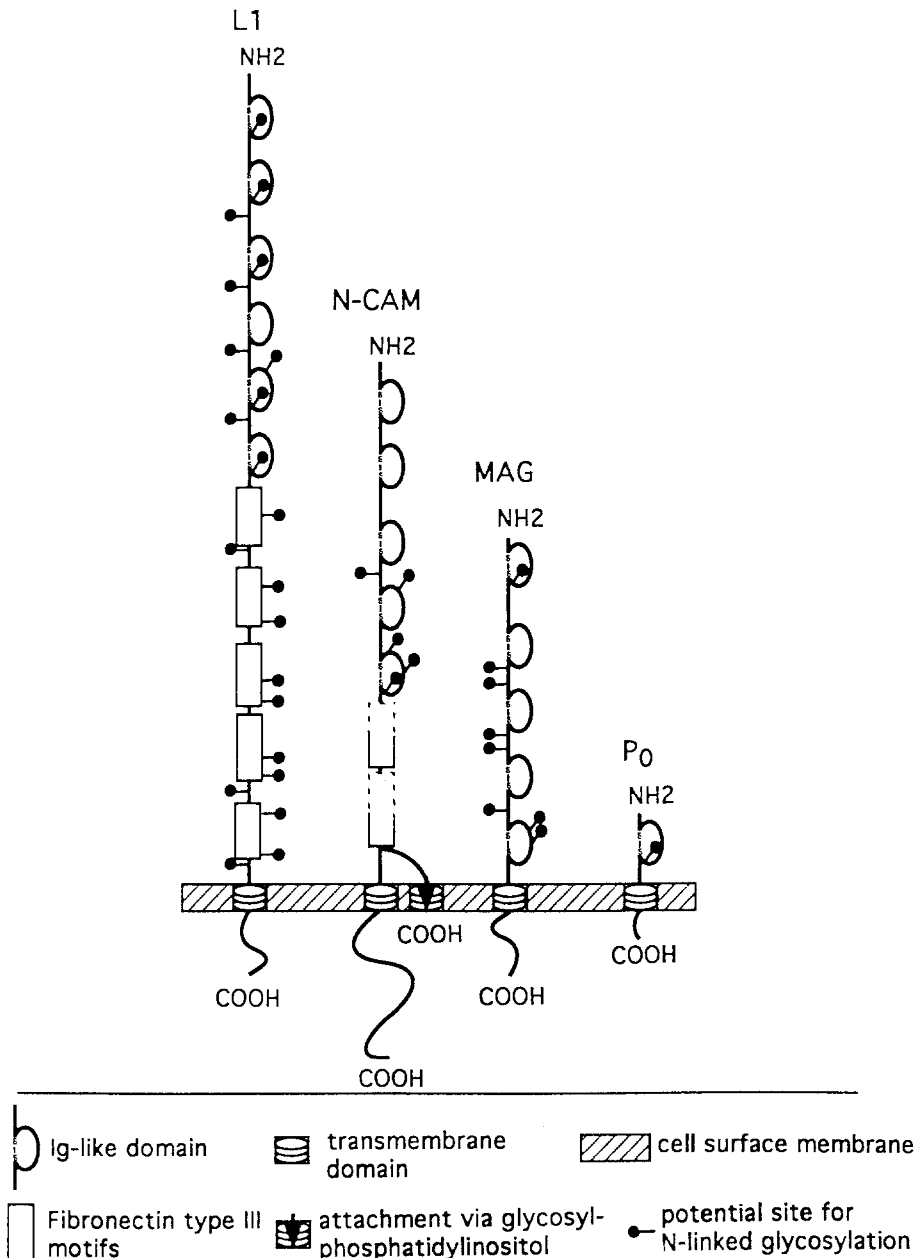


Fig. 1. Schematic presentation of the molecular structure of the cell surface molecules L1, N-CAM, MAG and P0.

for reviews). Except for relatively few *in vivo* studies, most of the functional data are based on *in vitro* observations. Additionally, only a few studies deal with the subcellular localization of neural recognition molecules in the peripheral nerve, although knowledge about their spatio-temporal dynamics is an important prerequisite to understand the molecular mechanisms underlying development and regeneration.

The present review tries to summarize what is known about the distribution of particular recognition molecules in developing and regenerating peripheral nerve in the context of functional studies. The aim is to develop a hypothetical framework for the molecular mechanisms underlying neuron-glia interactions during development and regeneration of peripheral nerves. This framework may also provide insight into the poor regenerative capacity of the CNS of mammals.

CELL SURFACE MOLECULES OF THE IMMUNOGLOBULIN SUPERFAMILY

The adhesive cell surface molecules which have been described can be grouped on the basis of their structure into three major families, the cadherins, the integrins and the immunoglobulin-like molecules (Schachner *et al.*, 1990; Takeichi, 1990; Reichardt & Tomasselli, 1991; Stappert & Kemler, 1993). Func-

tional studies and immunohistochemical investigations on various developmental and regenerative events have been performed mainly for members of the immunoglobulin superfamily (Fig. 1).

L1 is a transmembrane glycoprotein with an apparent molecular weight of 200–230 kDa and an extracellular part consisting of six immunoglobulin-like domains and five fibronectin type III repeats (Rathjen & Schachner, 1984; Moos *et al.*, 1988; Brümmendorf *et al.*, 1989). It is homologous to the NGF-induced large external glycoprotein NILE in rat (Bock *et al.*, 1985) and homologous molecules have also been described in human (Prince *et al.*, 1991; Miura *et al.*, 1991; Hlavin & Lemmon, 1991). In the chicken, three L1-related molecules have been described and termed Ng-CAM (Grumet & Edelman, 1984; Grumet *et al.*, 1984), 8D9 (Lemmon & McLoon, 1986), and G4 (Rathjen *et al.*, 1987). Due to the striking similarities of L1, Ng-CAM, 8D9 and G4 in terms of distribution and function (see Rathjen, 1988, for review), they are treated here as a single group of closely related molecules.

N-CAM is expressed in multiple isoforms (180, 140 and 120 kDa) as a result of alternative splicing (Goridis *et al.*, 1985; Murray *et al.*, 1986; Rutishauser & Goridis, 1986, for review). The extracellular part of all forms consists of five immunoglobulin domains and two fibronectin type III repeats (Barthels *et al.*, 1987; Cunningham *et al.*, 1987; Small *et al.*, 1987; Cunningham & Edelman, 1990, for review). At a specific location on the extracellular domain, the molecule contains a glycosylation site for the unusual carbohydrate structure polysialic acid which can modify the adhesive properties of the molecule (see below).

Myelin-associated glycoprotein (MAG) is an integral plasma membrane glycoprotein containing five immunoglobulin-like domains and shows significant homology to N-CAM (Arquint *et al.*, 1987; Lai *et al.*, 1987a,b; Salzer *et al.*, 1987). In its glycosylated state the molecule has a molecular weight of about 100 kDa (Quarles *et al.*, 1983). In MAG isolated from CNS, two molecular weight forms of 67 and 72 kDa become apparent after deglycosylation (Frail & Brown, 1984). The two molecular forms are the result of alternative splicing (Lai *et al.*, 1987a; Salzer *et al.*, 1987) and show different kinetics of expression during development (Frail *et al.*, 1985; Owens *et al.*, 1990; Inuzuka *et al.*, 1991).

P0 is the major glycoprotein of mammalian PNS myelin. It is an integral membrane molecule with an apparent molecular weight of 28–30 kDa and contains a single immunoglobulin-like domain (Greenfield *et al.*, 1973; Lemke & Axel, 1985; Lemke, 1986; Lemke *et al.*, 1988; Lai *et al.*, 1987a,b; Schneider-Schaulies *et al.*, 1990; Filbin *et al.*, 1990; D'Urso *et al.*, 1990).

EXTRACELLULAR MATRIX MOLECULES

Extracellular matrix components involved in the development and regeneration events of the nervous system include laminin, fibronectin, thrombospondin, various proteoglycans, collagens and tenascin (Fig. 2) (see reviews by Sanes, 1989; Reichardt & Tomaselli, 1991). In this review, predominantly laminin and tenascin will be considered.

Laminin

Laminin is a glycoprotein of cruciform structure comprising three polypeptide chains (A-, B1- and B2-chain), each of which is the product of three different, but related, genes. Each of the three shorter arms of the cruciform molecule contains several EGF-like repeats; along two extended stretches of the single long arm the three polypeptide chains form a triple-coiled rod (Reichardt & Tomaselli, 1991). Recently, three isoforms of particular laminin polypeptide chains have been described (Hunter *et al.*, 1989a,b; Engvall *et al.*, 1990). Merosin M, an A-chain variant of laminin, appears to be the laminin isoform in adult peripheral nerves (Sanes *et al.*, 1990). Another variant of the A chain, named K-laminin, is confined to the dermal–epidermal junction and is not detected in peripheral nerves (Marinkovich *et al.*, 1992). S-laminin, a B1-chain variant, is much more restricted in its distribution than the B1-chain (Hunter *et al.*, 1989a,b). It is found in the basal lamina of the neuromuscular junction and is supposed to provide the stop signal for regenerating motor axons that have arrived at the denervated motor end plates (Hunter *et al.*, 1989a,b; 1991; Sanes *et al.*, 1990).

Tenascin

Tenascin-like molecules have been isolated from various species and tissues by different groups and have thus acquired a variety of different names (see Erickson & Bourdon, 1989, for review). Tenascin is an oligomeric glycoprotein consisting of six disulphide-linked subunits (Erickson & Bourdon, 1989; Chiquet, 1989; Chiquet-Ehrismann, 1990, for reviews). The amino acid sequences of chicken, mouse, human, and amphibian tenascin have been analyzed (Gulcher *et al.*, 1989; Jones *et al.*, 1989; Spring *et al.*, 1989; Onda *et al.*, 1991; Saga *et al.*, 1991; Weller *et al.*, 1991). Each arm of the hexameric molecule contains a globular domain at the amino terminal where the polypeptides are linked together. This globular domain is followed by several EGF-like repeats and various fibronectin type III motifs. The 'outer' terminal of the molecule is represented by a domain similar to fibrinogen. In most of the species investigated tenascin has been shown to occur as polypeptides of various sizes due to alternative splicing (Gulcher *et al.*, 1989; Jones *et al.*, 1989; Spring *et al.*, 1989; Weller *et al.*, 1991).

CARBOHYDRATE STRUCTURES

Cell surface and extracellular matrix molecules are

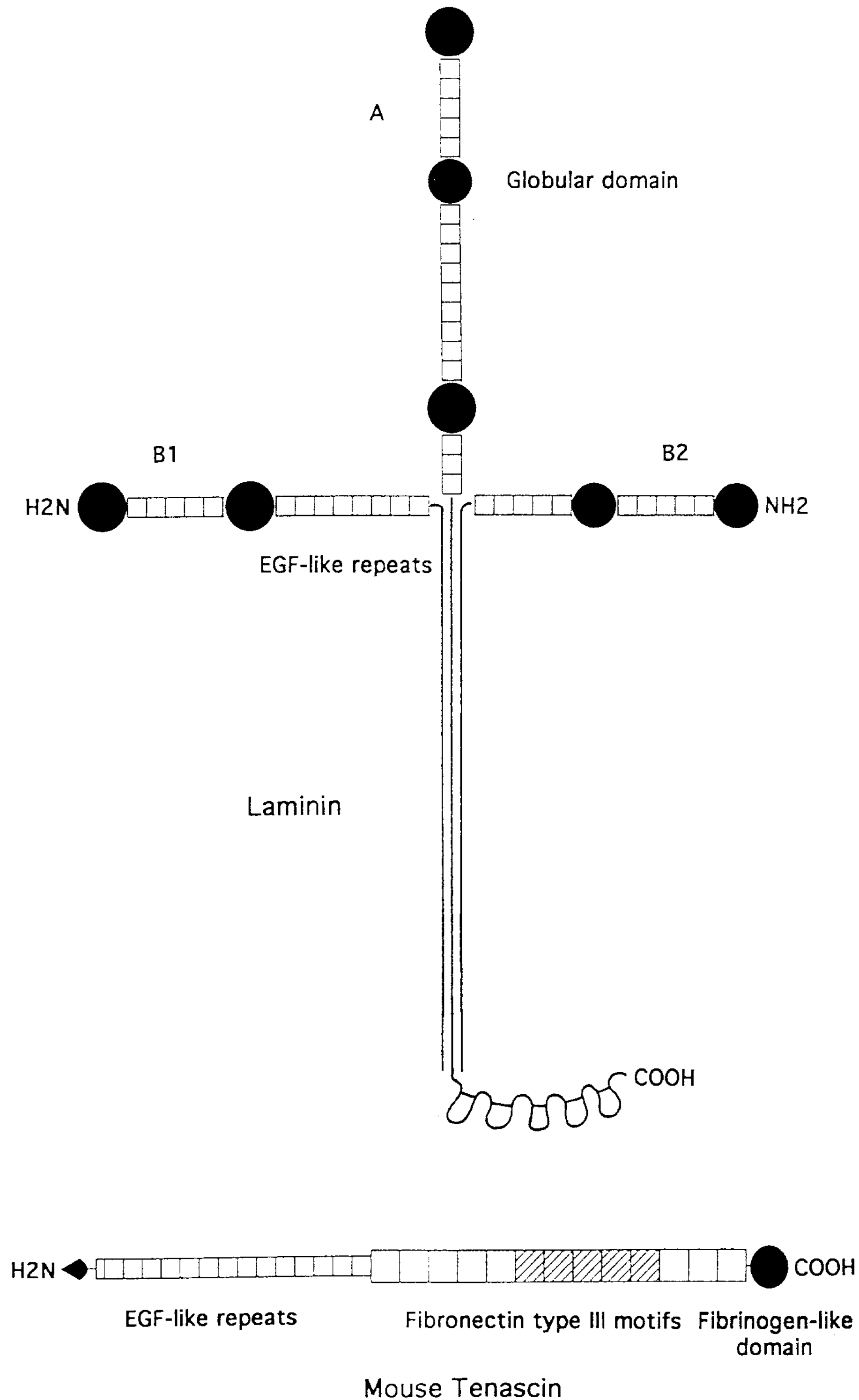


Fig. 2. Schematic presentation of the molecular structure of the extracellular matrix molecule laminin and of one arm of the hexameric extracellular matrix component tenascin.

characterized by particular sets of carbohydrate structures, some of which are thought to modulate the functional capacities of the molecules bearing them (Rutishauser & Jessell, 1988; Jessell *et al.*, 1990, for reviews). There is ample evidence that both polysialic acid and L2/HNK-1 have important functions during development and regeneration of peripheral nerves (see reviews of Rutishauser & Jessell, 1988; Jessell *et al.*, 1990; Schachner *et al.*, 1990, Schachner, 1990).

Polysialic acid (PSA)

This unusual carbohydrate consisting of α2-8-linked N-acetylneuraminic acid units, is predominantly contained in glycans bound to N-CAM. This results in a

reduction in the adhesiveness of the N-CAM because of the high content of negative charges conferred by the PSA groups (Cunningham *et al.*, 1983; Hoffman & Edelman, 1983; Sadoul *et al.*, 1983; Finne, 1990, for review).

L2/HNK-1

This epitope, originally described as a cell surface component of human natural killer cells (Abo & Balch, 1981), forms the basis of a large family of recognition molecules (Schachner *et al.*, 1990). Structural analysis of the carbohydrates was performed on L2/HNK-1-containing glycolipids from human sciatic nerve and embryonic rat brain (Ilyas *et al.*, 1984; Chou *et al.*, 1985, 1986; Noronha *et al.*, 1986; Schwarting *et al.*, 1987). These studies suggest that the L2/HNK-1 epitope is a sulphate-3-glucuronyl moiety (Chou *et al.*, 1986; Ariga *et al.*, 1987).

Expression and possible functions of neural cell surface molecules and extracellular matrix components during development of peripheral nerves

INITIAL INNERVATION OF LIMB BUDS: EVIDENCE THAT BOTH ADHESIVE AND REPULSIVE MOLECULES ARE INVOLVED IN THE FORMATION OF PERIPHERAL NERVES

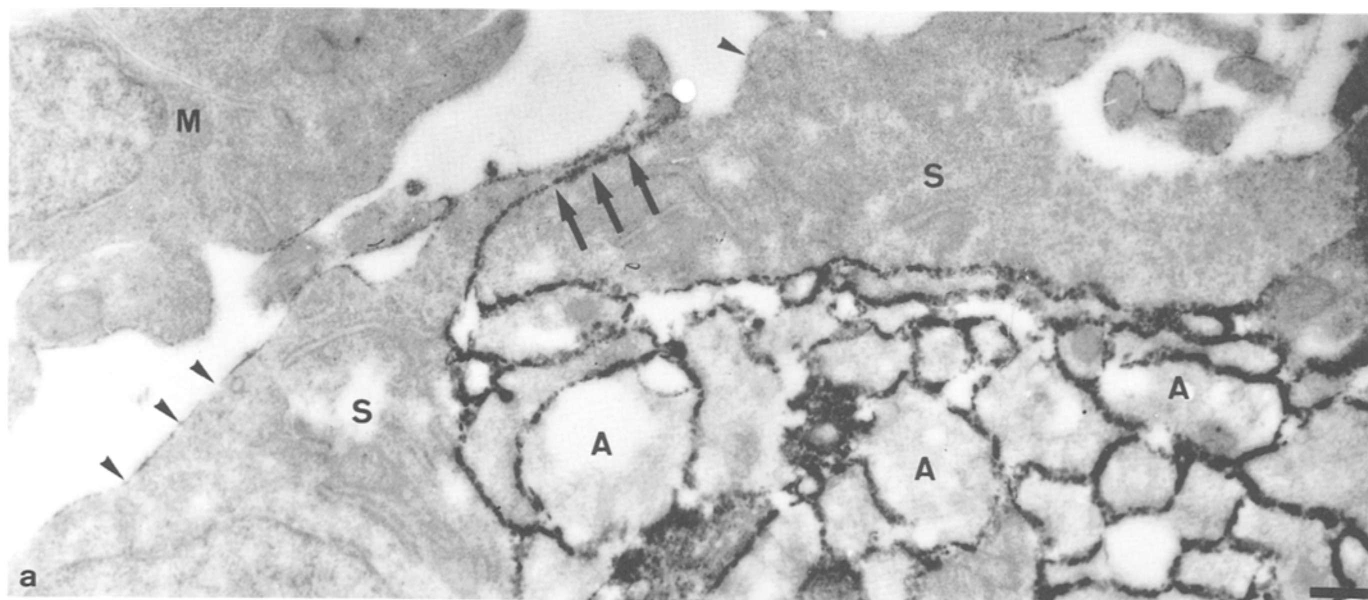
When growing axons enter embryonic limb buds and follow particular pathways, they are already accompanied by Schwann cells (Carpenter & Hollyday, 1992). The distal ends of growing nerves consist of filopodia of the most advanced growth cones and do not contain Schwann cells (Dahm & Landmesser, 1988; Carpenter & Hollyday, 1992). To date, this is the most accepted view of the morphology of growing nerve ends in limb buds although it disagrees with previous reports suggesting that Schwann cells precede the growth cones and guide axons to their peripheral destinations (Noakes & Bennett, 1987; Noakes *et al.*, 1988). More proximal to the nerve tip, the nerve is composed of fasciculated axons surrounded by an epithelial-like sheath of Schwann cells (Peters & Muir, 1959; Cravioto, 1965; Noakes & Bennett, 1987; Ziskind-Conhaim, 1988; Dahm & Landmesser, 1988; Martini & Schachner, 1991).

None of the cell surface molecules, extracellular matrix components or carbohydrate structures analysed so far appears to delineate the exact prospective routes of the nerves through the mesenchymal environment of the limb bud, although there is one report describing an antibody against a not yet completely characterized antigen demarcating mesenchyme-related motor axon pathways (Tanaka *et al.*, 1989).

8D9/G4 and N-CAM are expressed on the surfaces of all fasciculating axons including growth cones and filopodia. Additionally, these antigens are associated with Schwann cells surrounding the embryonic nerves as revealed by immunoelectron microscopy (Martini & Schachner, 1991). Immunolabelling is visible at the interface between axons and Schwann cells and where Schwann cells contact each other. Interestingly, the Schwann cell surfaces bordering the mesenchymal environment are either 8D9/G4- and N-CAM-negative or of very low immunoreactivity (Figs 3a,4). Thus, with respect to their expression of 8D9/G4 and N-CAM, Schwann cells of embryonic peripheral nerves are polarized cells (Martini & Schachner, 1991). With increasing age, however, some Schwann cells come to occupy positions among the axons and lose their polarization.

Among the extracellular matrix components, laminin expression has been recently investigated (Yip & Yip, 1992). The authors found that laminin does not delineate the pathways of growing axons and is, therefore, not a molecule guiding axons to peripheral destinations. Similarly, tenascin expression does not argue in favour of its involvement in axonal guidance, since during innervation of the limb bud, the molecule is restricted to the proximal nerve region at the limb bud base, whereas the nerve tips penetrate tenascin-negative mesenchyme (Fig. 3b,b′). Nerve-related tenascin immunoreactivity is restricted to the 8D9/G4- and N-CAM-negative, abaxonal Schwann cell sites facing the mesenchymal environment (Figs 3c,4). Immunoelectron microscopy shows tenascin in the endoplasmic reticulum of Schwann cells (Fig. 3c; Martini & Schachner, 1991), which suggests that it is produced by Schwann cells, in concordance with recent *in situ* hybridization findings (Wehrle-Haller *et al.*, 1991). Tenascin expression surrounding the nerves is down-regulated at the level where nerves begin to branch (Fig. 3d,d′; Martini & Schachner, 1991). The particular expression pattern of tenascin in the embryonic peripheral nerve suggests that the molecule forms a sort of scaffold around the nerve possibly preventing axons and/or Schwann cells from leaving the nerve. Although there is no evidence from *in vivo* studies to support this hypothesis, a two-choice *in vitro* approach testing the molecule's substrate properties supports the view that tenascin is repulsive for Schwann cells (Martini *et al.*, unpublished).

The proposal that repulsive molecules are functionally involved in the formation of peripheral pathways is not new. Repulsive molecules are supposed to direct growth cones into the anterior parts of somites and repulse them from perinotochordal mesenchyme (Tosney & Oakley, 1990; Oakley & Tosney, 1991; Ranscht & Bronner-Fraser, 1991; Schwab *et al.*, 1993, for review). The most conclusive experiment in this context was provided by Davies and colleagues (1990),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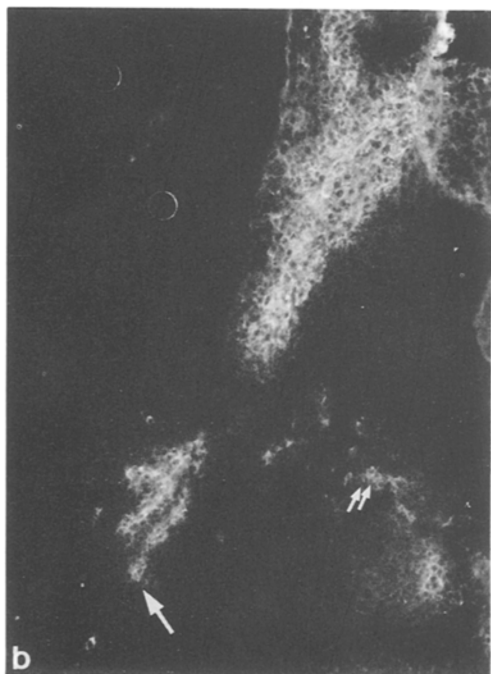
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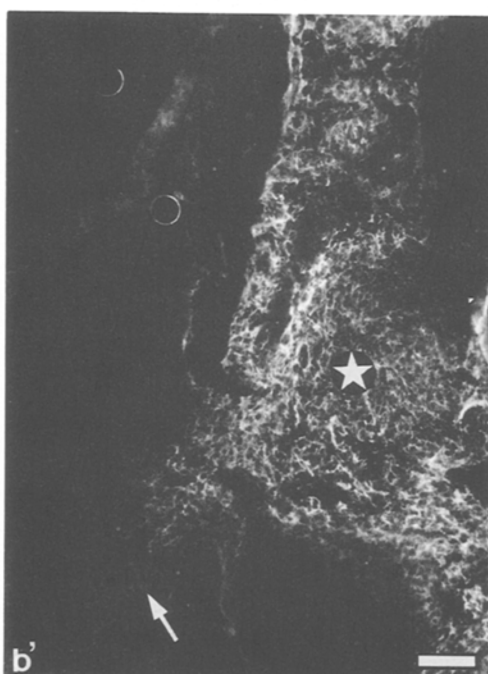
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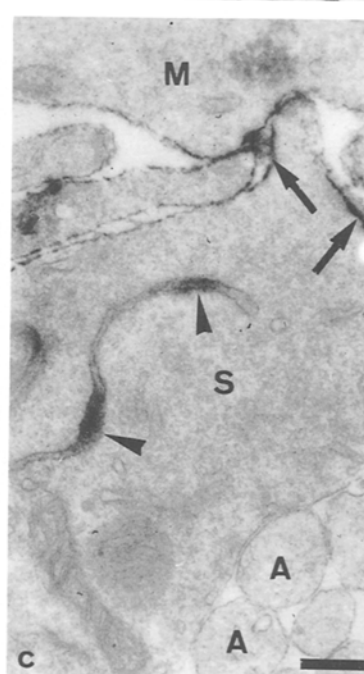
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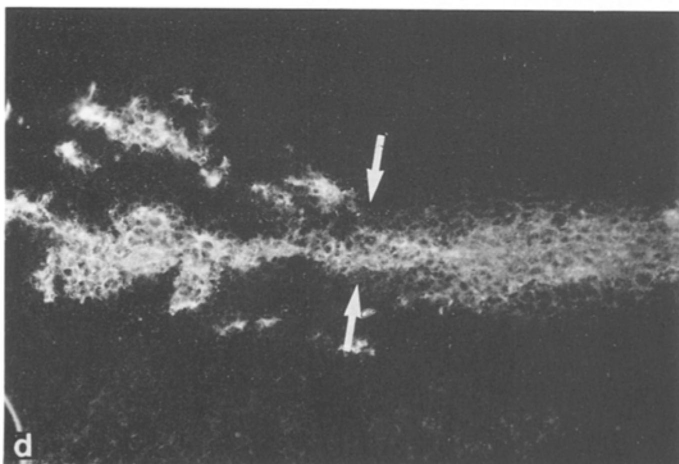
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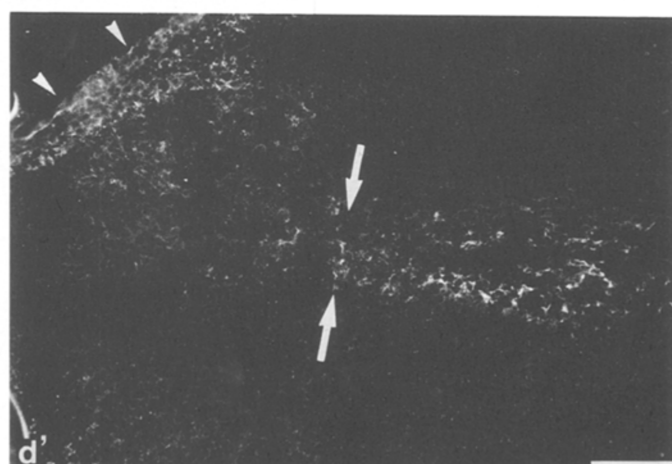
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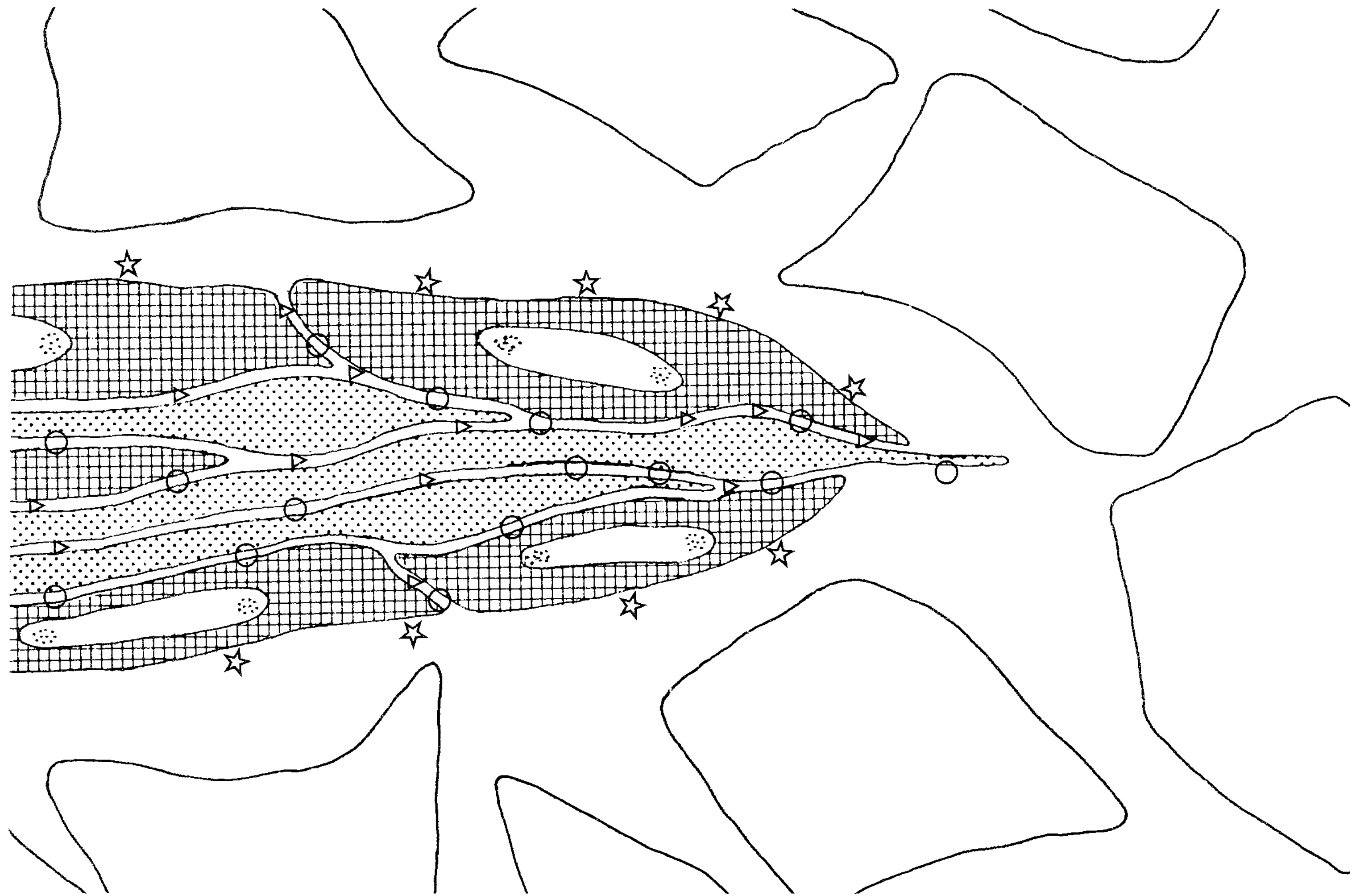


Fig. 4. Schematic representation of 8D9/G4, N-CAM and tenascin expression in a growing, embryonic nerve. Note that 8D9/G4 and N-CAM on the one hand and tenascin on the other spatially exclude each other. Axons are stippled, Schwann cells chequered, mesenchymal cells unshaded. ○ = 8D9/G4; △ = N-CAM; ☆ = tenascin.

Fig. 3. Immunoelectron microscopic localization of G4 (a) and tenascin (c) in the sciatic nerve of a chick hindlimb at embryonic stage 26 and immunohistological localization by double immunofluoresence of L2/HNK-1 (b,d) and tenascin (b′,d′) on longitudinal sections of chick hindlimbs at embryonic stage 24.5 (b,b′) and 28 (d,d′). (a) Cell contacts between axons (A), axons and Schwann cells (S) and between Schwann cells (arrows) are immunoreactive, whereas the Schwann cell surfaces facing the mesenchyme (M) are either G4-negative or very weakly immunoreactive (arrowheads). (b) Monoclonal antibody to L2/HNK-1 was used as a marker for nerves. Arrow marks one of three branchlets of sciatic nerve invading the limb bud mesenchyme. Small double arrow points to autonomic nerves. (b′) Corresponding immunofluorescence to (b) with polyclonal antibodies to tenascin. Tenascin-immunoreactivity is observed in the sclerotome (asterisk) and in a restricted area of the limb bud base. Arrow points to the position of the distal end of the invading nerve as indicated in (b). Note that the distal end of tenascin-immunoreactivity does not extend as deep into the limb bud mesenchyme as the L2/HNK-1-positive nerve. (c) Tenascin-immunoreactivity (arrows) is visible between a Schwann cell (S) and a process of a mesenchymal cell (M). Note immunoreactivity in the endoplasmic reticulum of the Schwann cell (arrowheads). Axons (A) are not in contact with tenascin-immunoreactivity. (d) Monoclonal antibody to L2/HNK-1 was used to label the medial femoral cutaneous nerve, the branchlets of which have just reached the skin. Arrows indicate the point where the nerve begins to arborize. (d′) Tenascin-immunoreactivity is associated with the distal end of the medial femoral cutaneous nerve up to the point where the nerve begins to arborize (arrows; see (d)). Note down-regulated tenascin levels in the region where the nerve is branching and the weak, but distinct, immunoreactivity associated with the innervated skin (arrowheads). Scale bars: (a,c) 0.25 μm; (b′,d′) 50 μm.

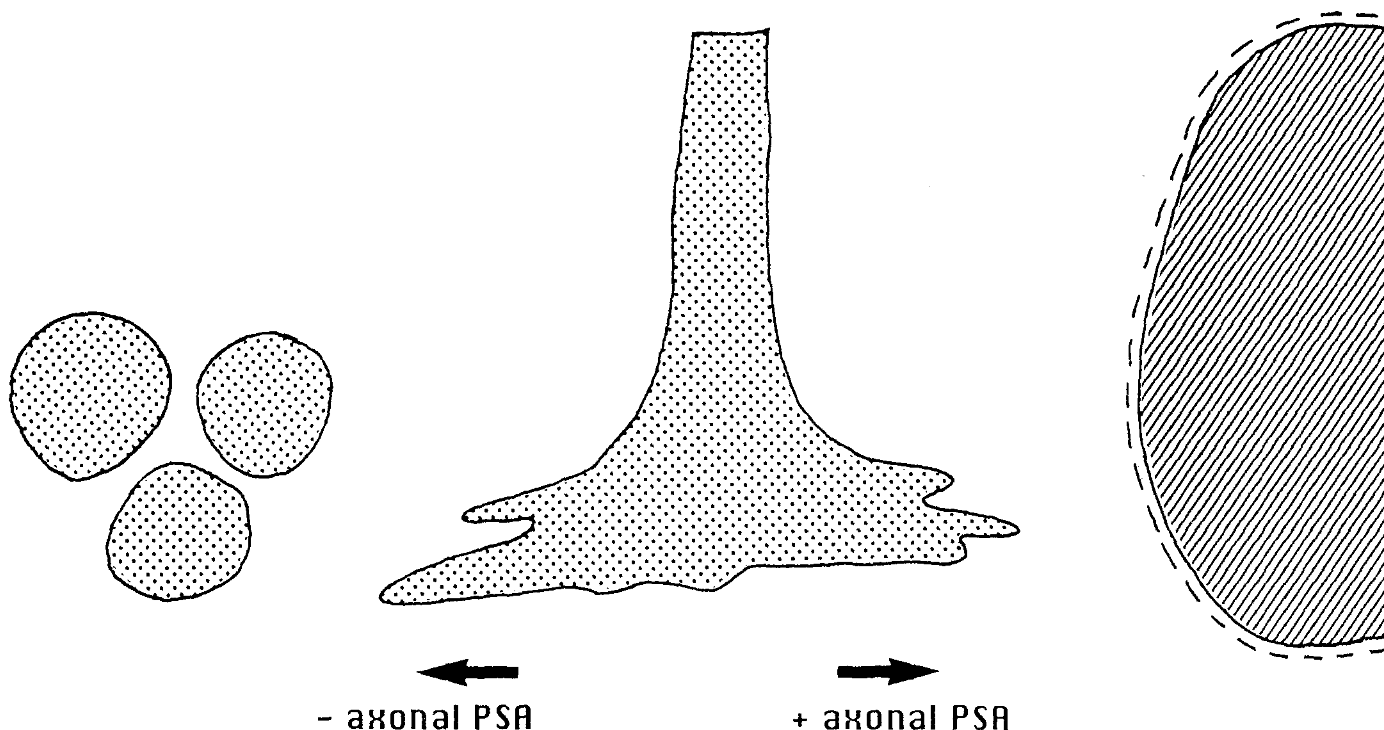


Fig. 5. Schematic representation of how PSA influences the innervation pattern in embryonic muscles. The low adhesive carbohydrate PSA is expressed on axon-related N-CAM molecules but not on N-CAM molecules of myotubes. The fasciculating G4 molecule is confined to axons as well. Myotubes contain the adhesive molecules N-CAM and N-cadherin as well as adhesive extracellular matrix components (laminin, heparasulfate proteoglycan). When PSA is up-regulated on axons (+ axonal PSA), the fasciculating properties of G4 molecules are weakened, resulting in defasciculation and innervation of the myotube. In contrast, low levels of PSA expression or experimental removal of PSA by endoneuraminidase (− axonal PSA) results in a lower degree of muscle nerve branching. Axons are stippled, myotube is hatched (Adapted from Landmesser *et al.*, 1990)

demonstrating that particular peanut agglutinin binding molecules from the anterior aspects of somites cause collapse of growth cones *in vitro*. Growth cone guidance by repulsive extracellular matrix molecules seems also to play a role at later stages of peripheral nerve development, when pelvic girdle precursors and other skeletal primordia are supposed to deviate axons from putative ectopic routes and delineate the borders of the correct pathways (Tosney & Landmesser, 1985; Oakley & Tosney, 1991; Tosney, 1991). The presence of these particular barriers can be correlated with the expression of peanut agglutinin binding sites and chondroitin-6-sulfate immunoreactive molecules which are assumed to form boundaries avoided by the main nerve trunks (Oakley & Tosney, 1991). Interestingly, the down-regulation of peanut-agglutinin-binding sites and chondroitin-6-sulfate immunoreactivity in limb buds that have yet to be innervated coincides temporally with the initial ingrowth of nerves into limb buds (Oakley & Tosney, 1991).

Among the carbohydrates, L2/HNK-1 is rather uniformly expressed in developing peripheral nerves, and all axons and Schwann cells are labelled (Martini & Schachner, 1991). A much more restricted pattern was found when the distribution of PSA was investigated in limb buds. It is predominantly expressed on motor axons projecting into the dorsal muscle masses, but is only scarcely found on motor axons growing into the ventral muscle masses of the limb bud (Tang *et al.*, 1992). This particular expression pattern of the carbohydrate implies a function in pathway selection since misrouted axons are common in embryos injected with endoneuraminidase, an enzyme which removes PSA from N-CAM, (Tang *et al.*, 1992). At later developmental stages, i.e., during innervation of embryonic muscles, the carbohydrate has another crucial function (Fig. 5). At this time, axon-related L1-like molecules (G4) with fasciculating properties compete with defasciculating forces caused by the tendency of G4 and N-CAM expressing motor axons to interact with N-CAM-positive myotubes in an N-CAM-dependent manner (Landmesser *et al.*, 1988). The regulatory element promoting nerve branching within the muscle is the low adhesive carbohydrate PSA expressed on axon-related N-CAM molecules but not on myotubes (Fig. 5). When PSA is up-regulated on axons, the fasciculating function of G4 is weakened, resulting in nerve branching. In contrast, low levels of PSA expression or experimental removal of

Table 1. Expression and possible functions of cell surface and extracellular matrix molecules and carbohydrates during development of peripheral nerves

	Expression	*Function*
L1	On fasciculating axons and SC[a], down-regulation when myelinating SC have made ca 1 1/2 turns around the axon[b], remains expressed on non-myelinating SC and axons[b,c,d,e]	Axonal fasciculation (ivt[1], ivv[2]), initiation of myelination (ivt[3,4])
N-CAM	see L1	Axonal fasciculation (ivt, see review[5]), determination of branching pattern during innervation of muscles (ivv[2])
MAG	On SC after 1 : 1 relationship between SC and axons has established[f] or after SC have made ca 1 1/2 turns around the axon[b], disappears from compact myelin, but remains expressed periaxonally and in non-compacted myelin[b,g]	Functionally involved at initial stages of myelination (ivt[6]), maintenance of axon-SC contact and non-compacted myelin (ivt[7,8,9])
P0	On SC at initial stages of myelination[h], down-regulated in non-compacted myelin[h], but strongly expressed in compacted myelin[g,h]	Functionally involved at initial stages of myelination, compaction of myelin and regulation of other cell surface molecules (ivt[10–12] mu[13])
LAM	Associated with embryonic nerve fascicles[i] (adult nerve: SC basal lamina[j])	Stabilization of embryonic nerves?
TEN	Abaxonal aspect of SC in embryonic nerves[a], (adult nerve: nodes of Ranvier, perineurium[e,k])	Preventing axons and SC from leaving the nerve by repulsive properties (ivt[14])
PSA	Motor axons of dorsal pathways[l]	Pathway selection (ivv[15]), determination of branching pattern (ivv[16])
L2/ HNK-1	On SC and axons[a], down-regulation at early stages of myelination[b], reappearance on motor axon-associated myelinating SC[h]	Sc-axon interaction?

References on the distribution of antigens are indicated by letters, functional investigations by numerals; ivt = *in vitro*, ivv = *in vivo*, mu = PO-deficient mutant

[a] Martini & Schachner, 1991
[b] Martini & Schachner, 1986
[c] Nieke & Schachner, 1985
[d] Mirsky *et al.*, 1986
[e] Rieger *et al.*, 1986
[f] Owens & Bunge, 1989
[g] Trapp *et al.*, 1986
[h] Martini *et al.*, 1988
[i] Yip & Yip, 1992
[j] Kücherer-Eheret *et al.*, 1990
[k] Martini *et al.*, 1990
[l] Tang *et al.*, 1992

[1] Fischer *et al.*, 1986
[2] Landmesser *et al.*, 1988
[3] Seilheimer *et al.*, 1989
[4] Wood *et al.*, 1990
[5] Rutishauser & Jessell, 1988
[6] Owens & Bunge, 1991
[7] Poltorak *et al.*, 1987
[8] Johnson *et al.*, 1989
[9] Sadoul *et al.*, 1990
[10] d'Urso *et al.*, 1990
[11] Filbin *et al.*, 1990
[12] Schneider-Schaulies *et al.*, 1990
[13] Giese *et al.*, 1992
[14] Martini, unpublished
[15] Tang *et al.*, 1992
[16] Landmesser *et al.*, 1990

PSA by endoneuraminidase results in a lower degree of muscle nerve branching (Landmesser *et al.*, 1990; Rutishauser & Landmesser, 1991).

The *in vivo* studies by Landmesser and colleagues are the most conclusive experiments on the functions of particular cell surface molecules during initial innervation of limbs (see Table 1). Nevertheless, some comments concerning the experimental approach used appear to be necessary. Whereas the role of PSA was very elegantly evaluated by removing the carbohydrate by endoneuraminidase followed by investigating the innervation pattern (Landmesser *et al.*, 1990; Rutishauser & Landmesser, 1991; Tang *et al.*, 1992), the functional roles of G4 and N-CAM have been determined by antibody perturbation experiments (Landmesser *et al.*, 1988). Although perturbation experiments have been an important tool in evaluating the functions of proteins, at least two significant shortcomings are inherent in this approach. First, antibodies are large molecules and can, therefore, sterically block functionally important molecules which are located close to the antibody-related antigen (see Künemund *et al.*, 1988). Second, antibodies binding to their corresponding cell surface antigen may activate particular second messenger systems which can modify the behaviour of the cells (Schuch *et al.*, 1989; von Bohlen und Halbach *et al.*, 1992). A more promising experimental approach may be the attenuation of the expression of a particular molecule by introducing antisense RNA into cells by a

retrovirus. By such an approach, Sanes and colleagues were able to block significantly B1 integrin synthesis resulting in the inhibition of neuroblast migration in chicken optic tectum (Galileo *et al.*, 1992; Sanes, 1993).

AXON–SCHWANN CELL RELATIONSHIP IN THE MATURING PERIPHERAL NERVE: SPATIOTEMPORAL REGULATION OF NEURAL CELL SURFACE MOLECULES DURING MYELINATION

During myelination axons larger than about 1 μm become segregated from a bundle of smaller axons and establish a 1:1 relationship with prospective myelinating Schwann cells (Webster *et al.*, 1973; Martin & Webster, 1973; Bunge *et al.*, 1986; Peters *et al.*, 1991). Then, the inner lips of the Schwann cells turn around the axon (Bunge *et al.*, 1989) and after 3–4 loops, myelin compaction occurs in the prospective internodes (Webster *et al.*, 1973; Peters *et al.*, 1991). Axons which are not individually separated remain enclosed by non-myelinating Schwann cells (Peters *et al.*, 1991).

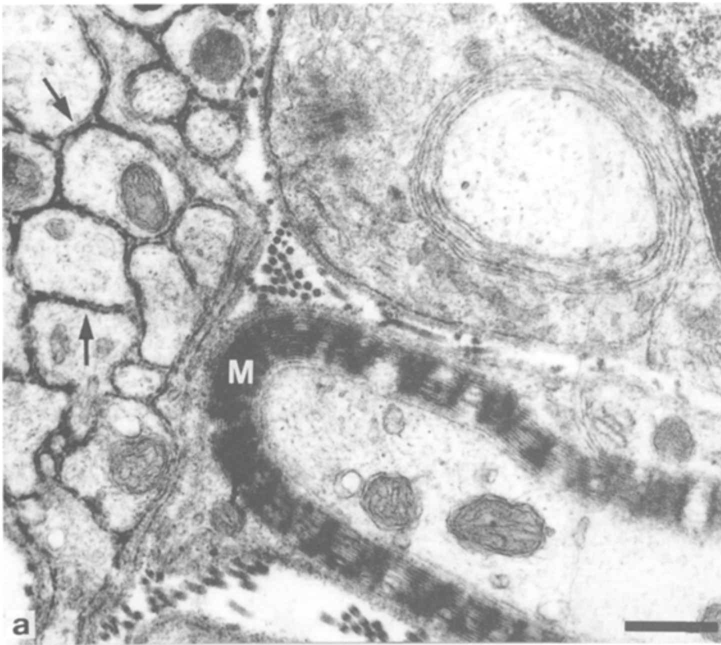
In sciatic nerves of newborn and early postnatal mice, L1 and N-CAM are expressed on fasciculating axons as well as on the Schwann cells that ensheath them (Figs 6a,b, 7; Nieke & Schachner, 1985; Mirsky *et al.*, 1986; Martini & Schachner, 1986). These molecules are also expressed on both Schwann cell–Schwann cell and Schwann cell–axon contacts when axons and Schwann cells are already separated from more immature axonal bundles (Fig. 7). However, when myelination starts, both adhesion molecules are drastically down-regulated, but remain present on non-myelinating Schwann cells and the small calibre axons ensheathed by them (Figs 6a,b, 7; Nieke & Schachner, 1985; Mirsky *et al.*, 1986; Rieger *et al.*, 1986; Martini & Schachner, 1986). It has been shown by both pre- and postembedding immunoelectron microscopy that L1 becomes completely undetectable on both axons and Schwann cells, whereas N-CAM decreases to very low levels and becomes confined to the axon–Schwann cell interface when the myelinating Schwann cell has made one and a half to two turns around the axons (Figs 6a–c, 7; Martini & Schachner, 1986). Similarly, axons in the CNS have been shown to down-regulate L1 to undetectable levels when oligodendrocytes, which do not express the molecule, start to myelinate them (Bartsch *et al.*, 1989).

In the PNS, down-regulation of L1 and N-CAM is accompanied by the loss of the carbohydrate epitope L2/HNK-1. However, L2/HNK-1 remains expressed on a subpopulation of non-myelinating Schwann cells and small calibre axons (Martini & Schachner, 1986). Whereas L1, N-CAM and L2/HNK-1 become down-regulated at the onset of myelination, the adhesive molecules MAG and P0 appear on the cell surfaces of Schwann cells. MAG appears at about the same stage as that at which L1 and N-CAM are down-regulated (Figs 6d,7), whereas P0 is already present at very low levels when the 1:1-relationship between axons and prospective myelinating Schwann cells is established (Fig. 7; Martini & Schachner, 1986; Martini *et al.*, 1988).

The onset of MAG and P0-expression has also been studied *in vitro* using rat sensory neurons and Schwann cells. In this *in vitro* system, MAG is expressed relatively earlier than is the case for the sciatic nerve of the mouse (Owens & Bunge, 1989, 1990). By contrast, P0 has been shown to occur *in vitro* in more mature Schwann cells than in the mouse sciatic nerve (Owens & Bunge, 1989, 1990). These contradictory findings may be explained by experimental variables (sensitivity of the staining procedures; *in vitro* vs *in situ*) or species differences (rat vs mouse). Concerning the latter possibility, in mammals, P0 is not expressed prior to initial stages of myelination (Hahn *et al.*, 1987; Lemke, 1988, Martini *et al.*, 1988, for review), whereas in chicken, Schwann cell precursors express P0 (Bhattacharyya *et al.*, 1991).

In mouse sciatic nerve, the subcellular localization of both MAG and P0 is identical prior to myelin compaction: the turning Schwann cell loops as well as the Schwann cell–axon interface show immunoreactivity (Figs 6d,f, 7; Martini & Schachner, 1986; Martini

Fig. 6. Immunoelectron microscopic localization of L1 (a,b), N-CAM (c), MAG (d,e) and P0 (f) in sciatic nerves of 21- (a–c,e) and 8-day-old (d,f) mice by pre- (a) and post-embedding staining procedures (b–f). In the latter technique, secondary antibodies coupled to 15 nm gold particles were used. The gold particles appear as rounded, electron-dense dots. (a) Note L1-immunoreactivity on fasciculating small calibre axons (arrows), whereas the developing myelin sheath with two turns of the myelinating Schwann cell process and the corresponding large calibre axon are L1-negative. M, compacted myelin. (b) Immunoreactivity is found on cell membranes of both small calibre axons and non-myelinating Schwann cells (S) whereas myelinating Schwann cells and the corresponding axon are L1-negative. M, myelin. (c) N-CAM is expressed periaxonally between axon membrane and compact myelin. Immunoreactivity in compact myelin is rare. M, myelin. (d) MAG expression is found at early stages of myelination in non-compacted Schwann cell loops. Arrows indicate axolemma. (e) When myelin compaction has occurred, MAG is restricted to the periaxonal space and to non-compacted myelin (arrow). M, myelin; N, Schwann cell nucleus. (f) At the onset of myelination, P0 is expressed on the turning loops of the Schwann cell and is thus distributed similarly to MAG at comparable developmental stages (compare left part of the micrograph with (d). When compaction has occurred, P0 is expressed in compact myelin (M, right half of the micrograph). Scale bars: 0.25 μm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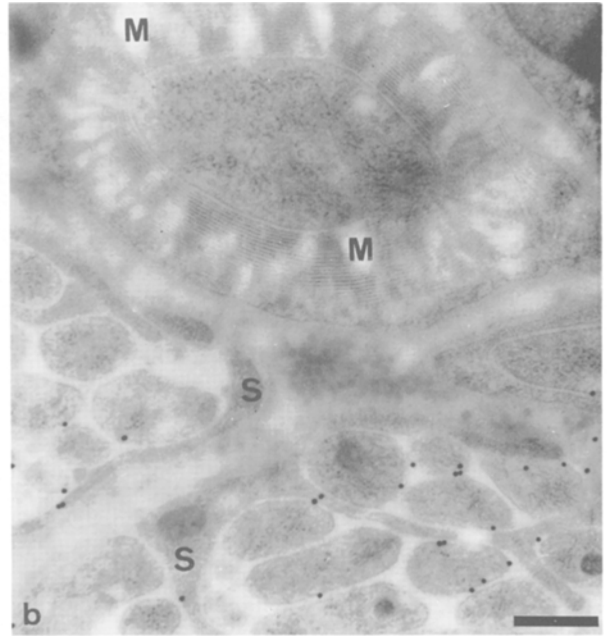
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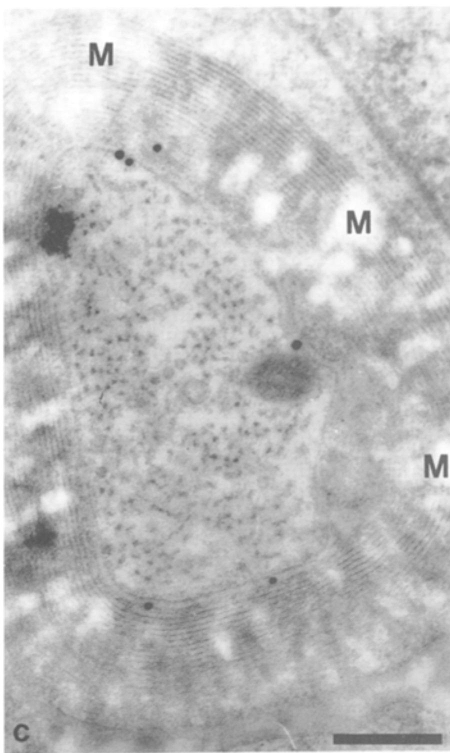
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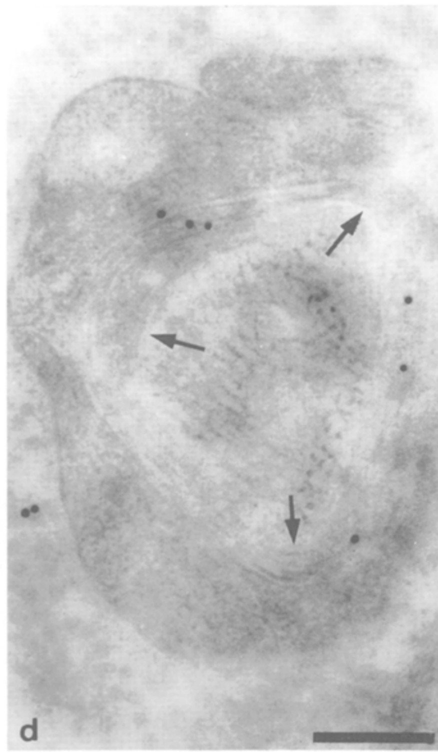
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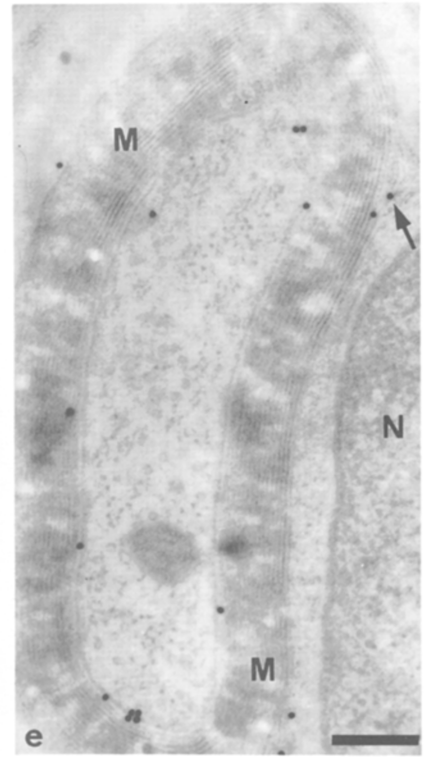
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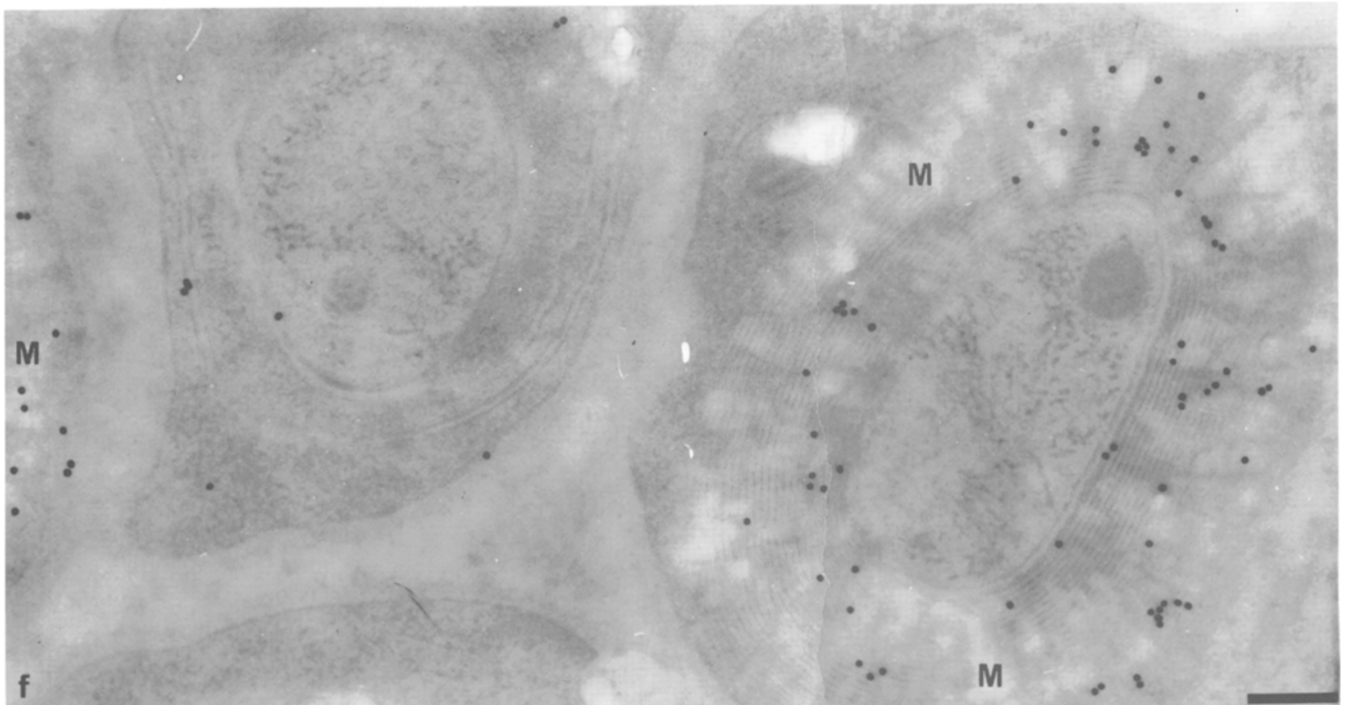
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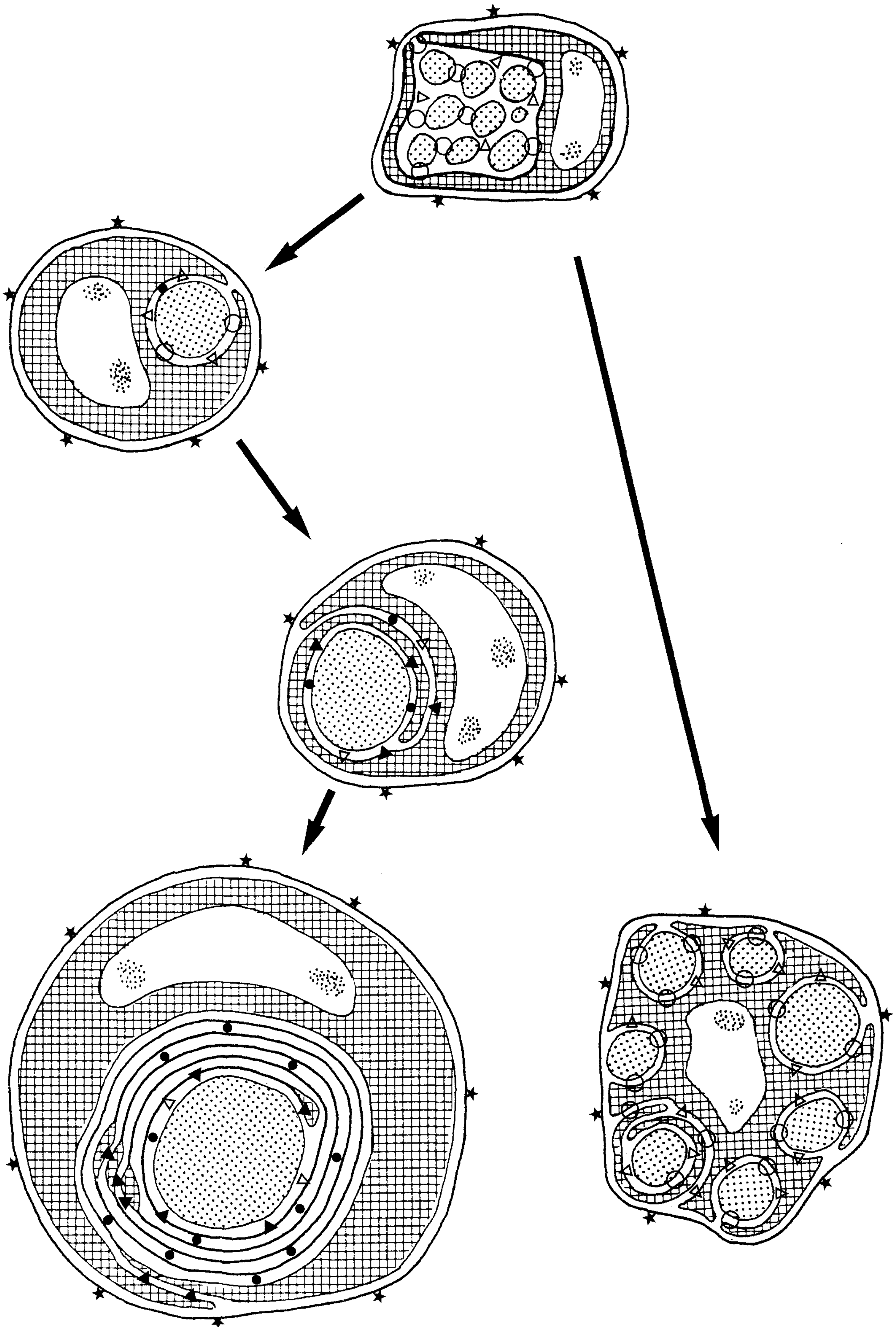


Fig. 7. Schematic representation of L1, N-CAM, MAG, P0 and laminin expression during various stages of Schwann cell maturation. The 'pathway' on the right hand side leads to an unmyelinated fibre, whereas the pathway on the left to a myelinated fibre. Axons are stippled, Schwann cells chequered. ○ = L1; △ = N-CAM; ▲ = MAG; ● = P0, ★ = laminin.

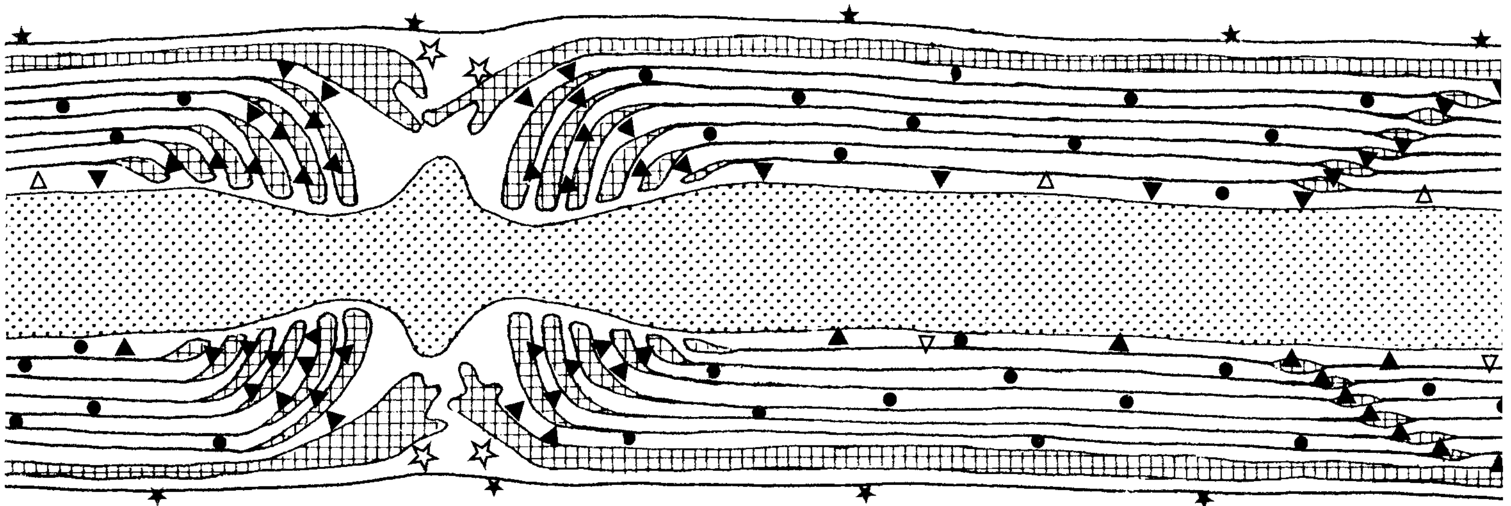


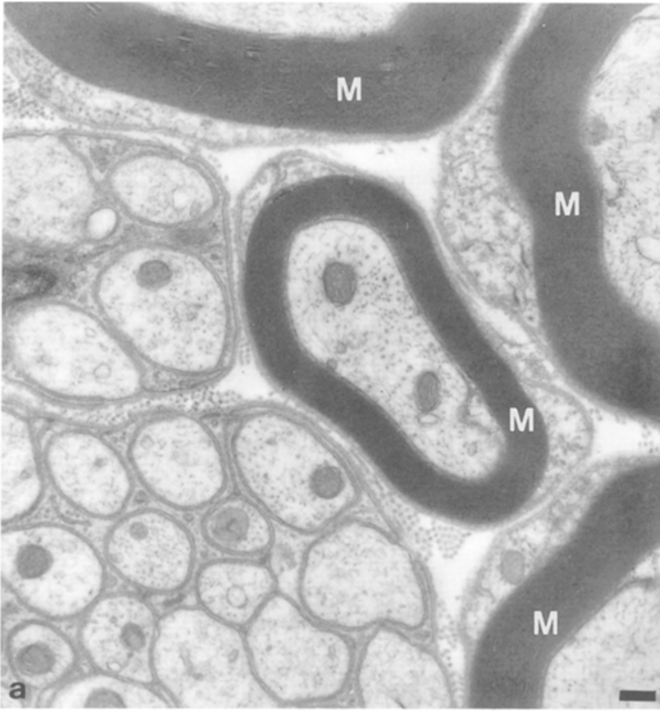
Fig. 8. Schematic representation of N-CAM, MAG, P0 laminin and tenascin expression in a myelinated fibre, including a node of Ranvier (longitudinal section). Note that L1 is not expressed. Axon is stippled, Schwann cells chequered. △ = N-CAM; ▲ = MAG; ● = P0; ★ = laminin; ☆ = tenascin.

et al., 1988). Both molecules, however, differ with respect to their subcellular distribution when compaction of myelin occurs. P0 becomes confined to compacted myelin, whereas MAG is strongly restricted to non-compacted myelin such as the paranodal loops, and the Schmidt-Lanterman incisures (Figs 6e,f, 7,8; Quarles & Trapp, 1984; Trapp & Quarles, 1984; Quarles, 1984; Trapp *et al.*, 1986; Martini & Schachner, 1986). Interestingly, the molecular profile in myelinated fibres continues to change, even after compact myelin has formed, in that the L2/HNK-1 epitope re-appears in the third postnatal week in compact myelin and basal laminae of a subpopulation of Schwann cells which are predominantly associated with motor axons (Martini *et al.*, 1988). In contrast to myelinating Schwann cells, non-myelinating Schwann cells permanently express L1 and N-CAM (Fig. 7). This holds true also for two other cell surface glycoproteins, the low-affinity NGF receptor (Jessen *et al.*, 1990; Stemple & Anderson, 1991) and endopetidase 24.11 (Kioussi *et al.*, 1992).

The different roles of the adhesive molecules during myelination have been predominantly investigated *in vitro*. In co-cultures with dorsal root ganglion neurons and purified Schwann cells, L1 antibodies blocked the extension of processes from Schwann cells between fasciculating neurites (Seilheimer *et al.*, 1989; Wood *et al.*, 1990). This leads to the loss of axonal engulfment and results in a blockade of myelination (Wood *et al.*, 1990). In contrast to L1-specific antibodies, N-CAM-antibodies showed only a partial blockade of Schwann cell engulfment (Seilheimer *et al.*, 1989).

Several *in vitro* studies have shown that MAG is an adhesive molecule and suggest that the molecule mediates adhesion between glial membranes in non-compacted portions of myelin as well as axon-glia-interactions (Poltorak *et al.*, 1987; Johnson *et al.*, 1989; Sadoul *et al.*, 1990). A significant role of MAG during initial stages of myelination has been shown using cultured rat Schwann cells infected with a retrovirus expressing MAG antisense mRNA. Such Schwann cells failed to segregate large calibre axons and to initiate myelination in these cultures (Owens & Bunge, 1991). This implies that MAG may be functionally important even before the 1:1 ratio has been established.

Initial studies on the functional roles of P0 during myelination have also been performed *in vitro*. These studies showed that the molecule is adhesive and may mediate interactions between Schwann cells as well as between Schwann cells and axons (Schneider-Schaulies *et al.*, 1990; Filbin *et al.*, 1990; D'Urso *et al.*, 1990). In order to strengthen the hypothesis that P0 is involved in the formation and maintenance of the myelin sheath of PNS axons, a null-mutation of the P0 gene was introduced into the genome of mice. In such animals, a sub-population of axon–Schwann cell units was found which were obviously arrested at the 1:1 relationship which supports the hypothesis that P0 is involved even at such early stages of myelination (Martini *et al.*, 1988). Furthermore, Schwann cells which succeeded in making some turns around the axon were abnormal, showing predominantly non-compacted myelin-like wrappings (Fig. 9a,b; Giese *et al.*, 1992). However, the disorganization of myelin cannot be regarded as a proof that P0 itself is directly involved in the formation and maintenance of normal myelin sheaths. Myelin-like sheaths of P0-deficient mutants have been shown to be associated with high levels of N-CAM and MAG (Fig. 9c–f), both of which are lacking in compact myelin of wild type mice (Giese *et al.*, 1992). This raises the question whether the



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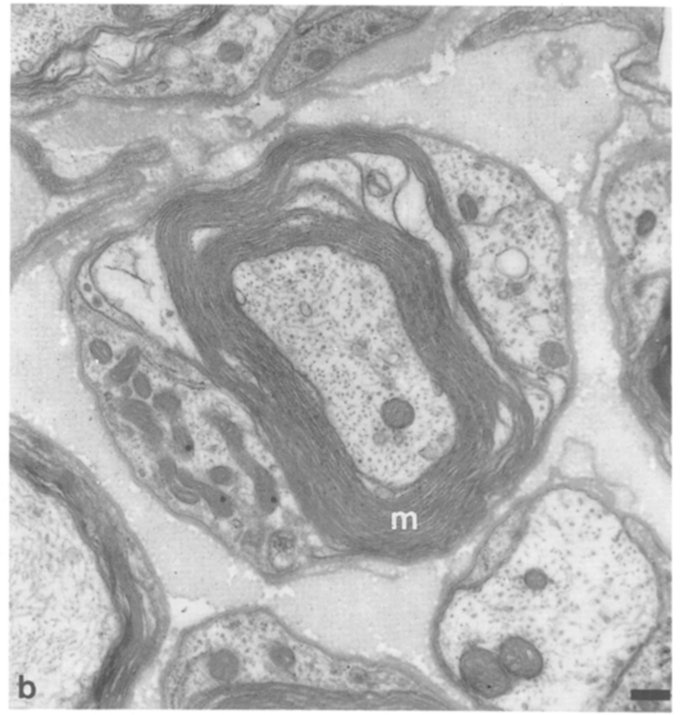

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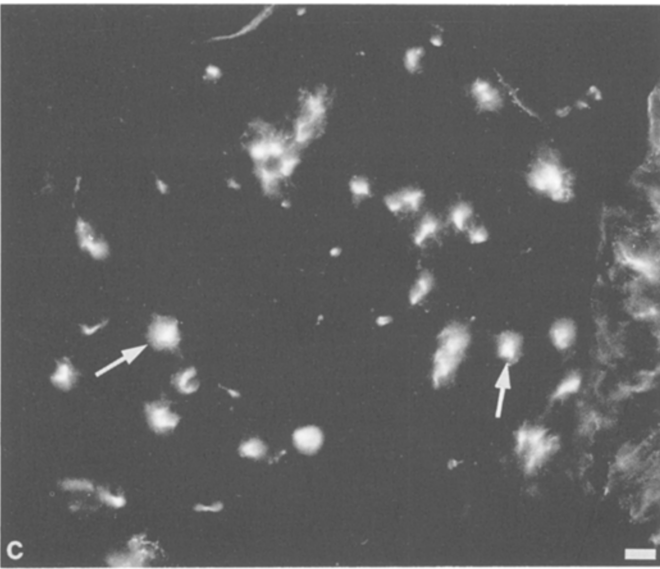

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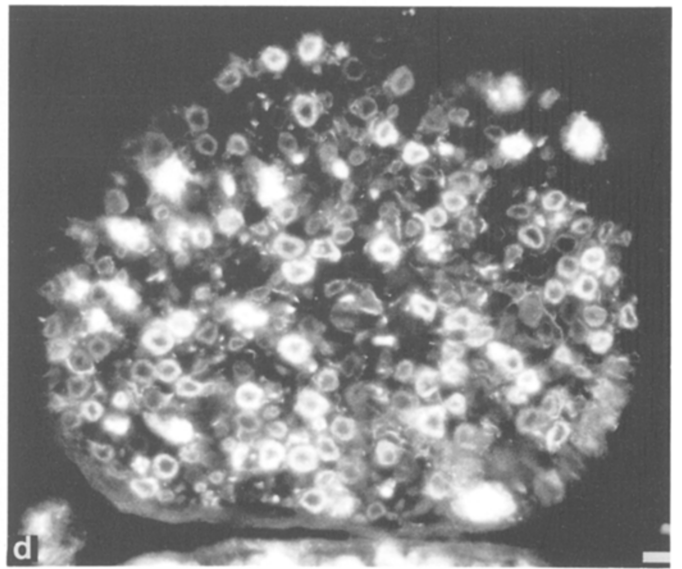

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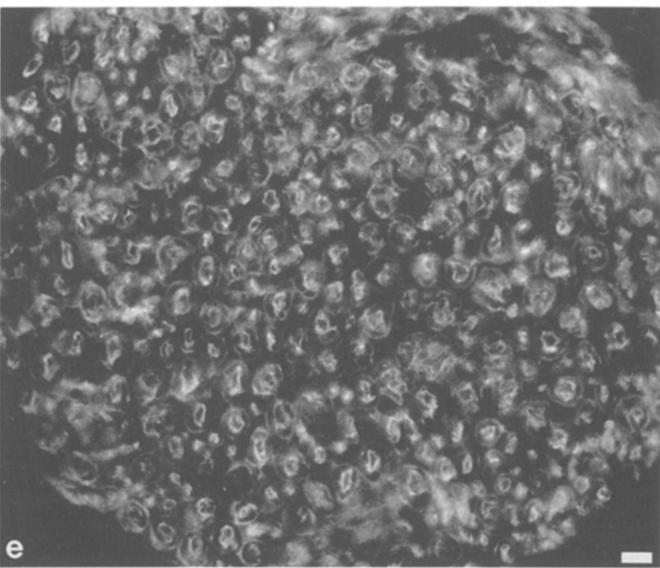

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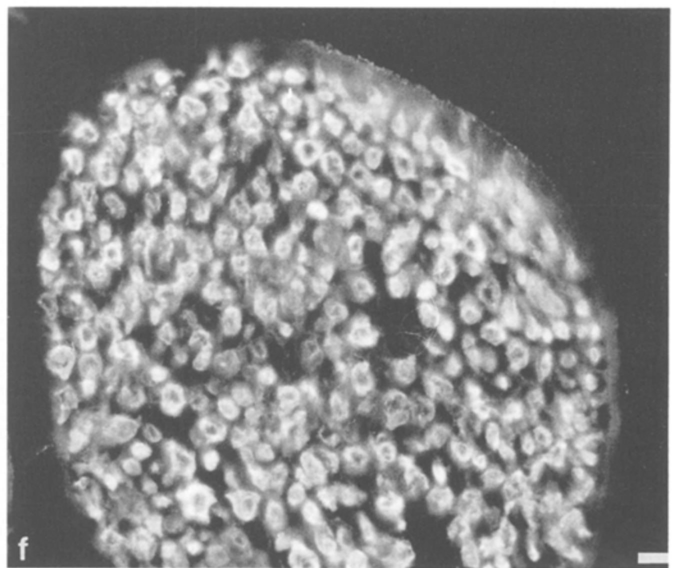

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observed abnormal myelin in P0-deficient mice is caused by the absence of the adhesive properties of P0 or is due to abnormalities in the regulation of other adhesive molecules including N-CAM and MAG.

Expression and possible functions of neural cell surface molecules and extracellular matrix components during regeneration of adult peripheral nerves: evidence for Schwann cell-derived neurite outgrowth-promoting activities

After peripheral nerve interruption, axons in the distal stump degenerate and are removed together with their myelin sheaths (see Hall, 1989; Fawcett & Keynes, 1990, for reviews). These lesion-induced degenerative events, called 'Wallerian degeneration', are accompanied by a rapid proliferation of Schwann cells (Fawcett & Keynes, 1990, for review) and are probably mediated by macrophages which enter the endoneurium after the lesion to remove axonal and myelin debris (Perry & Brown, 1992, for review).

At sites where the nerve is cut, fibroblasts of either endoneurial or perineurial origin form a cellular bridge between the proximal and distal nerve stump (Ramon y Cajal, 1928; Williams *et al.*, 1983; Martini & Schachner, 1988; Martini *et al.*, 1990). This bridge is traversed by regrowing and branching axons and by migratory Schwann cells (Ramon y Cajal, 1928; Williams *et al.*, 1983; Martini *et al.*, 1990). When the regrowing axons have reached the distal stump of the nerve, they grow preferentially along the interface between the inner aspects of the Schwann cell basal laminae and the surfaces of the Schwann cells (Nathaniel & Pease, 1963; Ide *et al.*, 1983; Scherer & Easter, 1984; Kuffler, 1986; Martini & Schachner, 1988). Thus, one would expect that molecules fostering axonal regrowth are particularly expressed at this interface.

In the distal stump the first changes in L1- and N-CAM-expression are seen from postlesion day 4 and 6 onwards when 10–20% of those Schwann cells which have previously formed myelin become L1 and N-CAM positive. Two weeks after the lesion all Schwann cells have become L1- and N-CAM-immunoreactive (Figs. 10a,b, 11; Martini & Schachner, 1988).

Up-regulation of L1- and N-CAM in the distal stump is also supported by immunofluorescence studies of Nieke and Schachner (1985) and Daniloff and colleagues (1986). It is also in concordance with *in vitro* investigations by Jessen and colleagues, although under their cell culture conditions, up-regulation of N-CAM seems to be much more rapid than that observed *in situ* (Jessen *et al.*, 1987). By quantitative Northern blot analysis of distal stumps of rat sciatic nerves, up-regulation of L1- and N-CAM-specific mRNAs appeared after postoperative days 4 and 6, respectively (Tacke & Martini, 1990) thereby paralleling the immunocytochemical data.

The re-appearance of L1 and N-CAM on denervated Schwann cells is possibly an important prerequisite for significant axonal regrowth after nerve injury. Cell culture experiments with mouse dorsal root ganglia neurons and purified Schwann cells from the same species showed that L1 and, to a lesser degree N-CAM, are involved in Schwann cell-mediated neurite extension (Seilheimer & Schachner, 1988). In other *in vitro* studies, Schwann cells from mice were co-cultured with ciliary ganglion neurons from chicken and, again, neurite outgrowth was significantly inhibited by antibodies to L1 (Bixby *et al.*, 1988). Since the antibodies used were generated to the mouse L1 antigen and bind only weakly to related molecules in the chicken (Rathjen & Schachner, 1984), these experiments strongly suggest that the Schwann cell-related molecule is functionally implicated in the neurite outgrowth promoting activities of Schwann cells.

Among the extracellular matrix components, laminin is known to be one of the most effective promotors of neurite extension *in vitro*. As laminin is a component of Schwann cell basal laminae (Figs 8, 11), it has been suggested to be involved in axonal regeneration *in vivo* (Baron-van-Evercooren *et al.*, 1982; Manthorpe *et al.*, 1983; Rogers *et al.*, 1983; see reviews by Sanes, 1989; Reichardt & Tomaselli, 1991). Interestingly, nerve injury leads to an accumulation of laminin at axon-Schwann cell contacts (Kücherer-Ehret *et al.*, 1990).

Fig. 9. Electron microscopy of sciatic nerves (a,b) and immunohistochemical localization of N-CAM (c,d) and MAG (e,f) in muscle branches of femoral nerves of wild type (a,c,e) and P0-deficient mice (b,d,f). (a) Myelinated and non-myelinated axons are visible in this micrograph. Note the compact appearance of myelin sheaths (M). (b) Non-compacted myelin sheath (m) around an axon. Note incomplete Schwann cell ensheathment of the large calibre axon at the lower right hand corner. (c) In the wild type animal, N-CAM-immunoreactivity is confined to non-myelinated axon–Schwann cell units (arrows). The weak periaxonal expression as revealed at the electron microscopic level (Fig. 6c) is not visible by immunofluorescence so that myelinated fibres appear totally negative. (d) In the P0-deficient animal, N-CAM is, in addition to non-myelinated axon–Schwann cell units, highly expressed in myelin-like sheaths. (e) In the wild type animal, MAG immunoreactivity is predominantly found in the periaxonal space (circular structures of high immunoreactivity; compare with Fig. 6e), and to a lower extent, at the outer aspect of the Schwann cell, which most probably represents the Schwann cell basal lamina. (f) In the P0-deficient animal, MAG immunoreactivity occupies the entire extent of the myelin-like sheaths. Scale bars: (a,b) 0.25 μm; (c–f) 10 μm

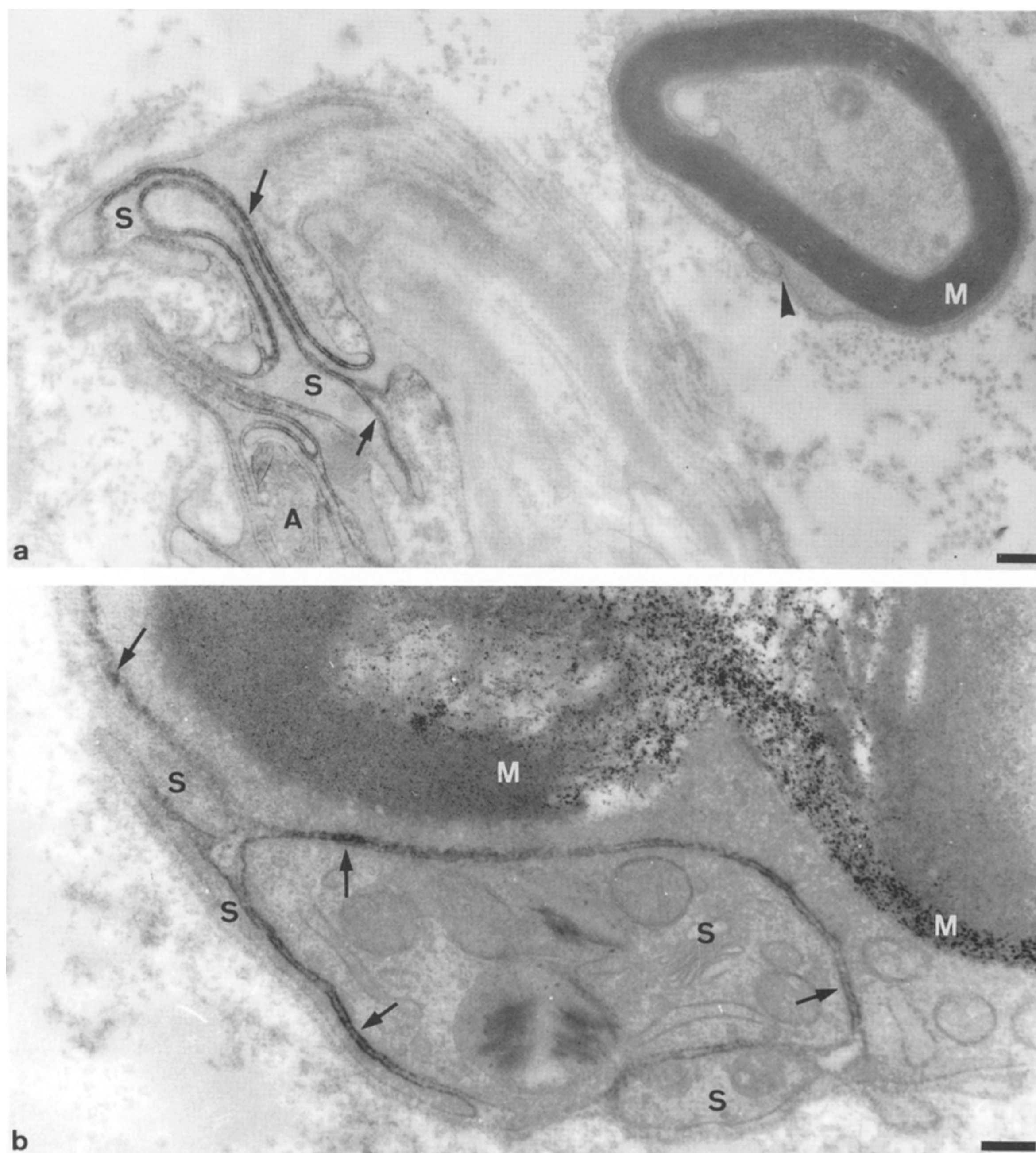

Fig. 10. Immunoelectron microscopic localization of L1 in distal stumps of transected sciatic nerve four (a) and 14 (b) days after the lesion. Sections were taken about 10 mm distal to the transection site. (a) Processes of non-myelinating Schwann cells (S) are L1-positive (arrows), whereas the myelinating Schwann cell does not express detectable levels of L1 (arrowhead). A, L1-negative cell process which is possibly a degenerating axon; M, myelin. (b) Processes of a myelinating Schwann cell (S) are L1-positive where they make contacts with one another (arrows). (L1-immunoreactivity excludes the possibility that the cells are intratubal macrophages, since those have never been observed to express the molecule). M, myelin debris. Scale bars: 0.25 μm

Recent *in vivo* studies using polyclonal antisera against laminin support the view that laminin is involved in promoting the growth of regenerating axons along basal laminae of Schwann cell depleted nerve implants (Wang *et al.*, 1992). In a previous *in vitro* study, Sandrock & Matthew (1987a) showed that a laminin-heparan sulfate complex is involved in neurite extension on fresh-frozen sections of sciatic nerves.

Another extracellular matrix component possibly involved in regeneration is tenascin. In the normal nerve, tenascin expression is confined to the extra-

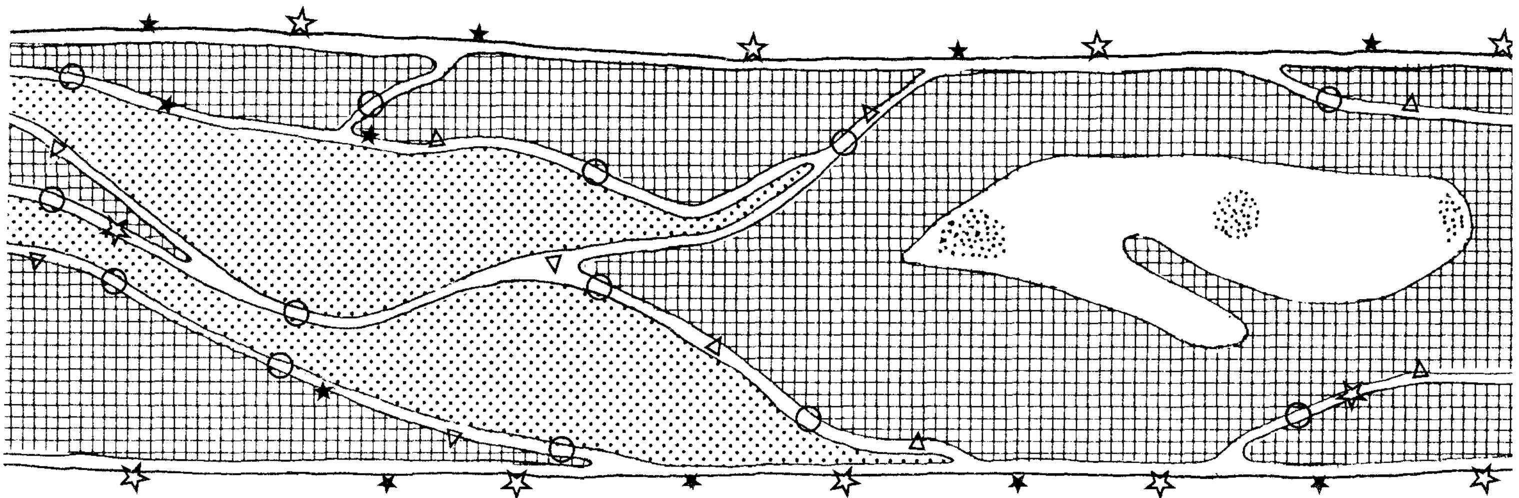

Fig. 11. Schematic representation of L1-, N-CAM-, laminin-, and tenascin-expression in the distal part of a lesioned nerve fibre containing two regrowing axons. Axons and growth cones are stippled, Schwann cells chequered. ○ = L1; △ = N-CAM; ★ = laminin; ☆ = tenascin.

cellular matrix around the nodes of Ranvier and in the perineurium (Fig. 8; Daniloff *et al.*, 1989; Martini *et al.*, 1990). After lesion, the molecule was found in the distal part of the nerve along its entire length, in the distal end of the proximal nerve stump and in the fibroblast-containing bridge connecting both stumps (Figs 11, 12a; Martini *et al.*, 1990).

At the ultrastructural level, tenascin immunoreactivity in denervated nerve stumps is predominantly associated with Schwann cell basal laminae (Martini *et al.*, 1990). Together with the fact that tenascin promotes neurite outgrowth *in vitro* when offered as a uniform substrate (Wehrle & Chiquet, 1990; Lochter *et al.*, 1991; Husmann *et al.*, 1992), the expression of the molecule in association with Schwann cell basal laminae supports the view that tenascin may foster axonal regrowth after a peripheral nerve lesion. This hypothesis is in concordance with the observation that the glial cells of the optic nerve do not significantly up-regulate tenascin after transection (U. Bartsch *et al.*, 1992). In other parts of the CNS, however, tenascin has been found to increase after injury and interpreted as an inhibitory molecule causally related to the low regenerative capacity of the adult CNS (McKeon *et al.*, 1991; Laywell *et al.*, 1992).

The fact that tenascin is associated with Schwann cell basal laminae of denervated nerves does not prove that Schwann cells are the source of this secreted molecule (see Prieto *et al.*, 1990; S. Bartsch *et al.*, 1992). However, by the combination of *in situ* hybridization with a tenascin-specific riboprobe and indirect immunofluorescence using antibodies to L1, Schwann cells were found to be the source of tenascin after denervation (Martini *et al.*, unpublished findings).

The fibroblast-containing territory between the distal end of the proximal stump and the proximal end of the distal stump strongly expressed tenascin in the first and second post-lesion week. It is worthwhile mentioning that the entire mesenchymal bridge was not tenascin-positive; immunoreactivity was confined to the central part of the bridge. Double-immunofluorescence microscopy with antibodies to tenascin and L1 revealed that this region contains regrowing axons and migrating Schwann cells (Fig. 12b,b′; Martini *et al.*, 1990). Moreover, by immunoelectron microscopy, it could be shown that growth cones of regenerating axons directly contacted tenascin-immunoreactive extracellular matrix (Fig. 12c; Martini *et al.*, 1990).

Examination of the expression of carbohydrate structures during nerve regeneration by Western blot analysis suggests that PSA is present in lesioned nerves. However, the exact localization pattern of this carbohydrate has not yet been determined (Daniloff *et al.*, 1986). Recent observations on peripheral nerve grafts implanted into the thalamus show that the carbohydrate is confined to regrowing axons (Fig. 13; Y. Zhang *et al.*, 1993). Together with the findings that removal of PSA inhibits the speed of neurite extension *in vitro* (Boisseau *et al.*, 1991; H. Zhang *et al.*, 1992) and that antibodies to the embryonic form of N-CAM delay functional recovery when applicated into silicon tubes connecting transected nerves (Remsen *et al.*, 1990), these observations suggest that PSA supports axonal regeneration.

L2/HNK-1 is another carbohydrate possibly involved in axonal regeneration. It is detectable in association with Schwann cells in ventral spinal roots and motor axon-related Schwann cells of muscle nerves, but hardly at all in dorsal roots or predominantly sensory, cutaneous nerves (Fig. 14a,b; Brushart, 1988; Martini *et al.*, 1988, 1992, in preparation). Its

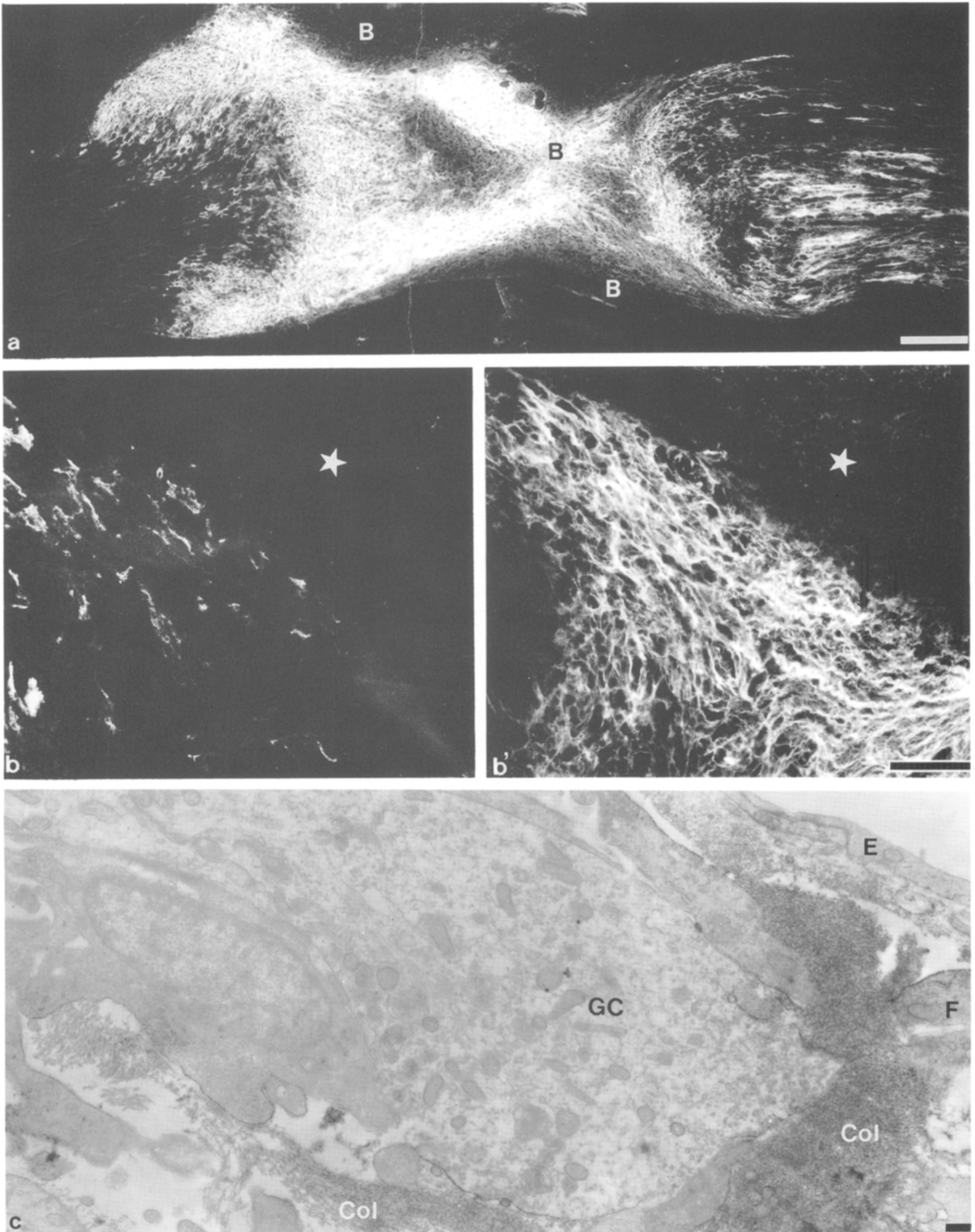

Fig. 12. Single immunofluorescence of tenascin (a), double-immunofluorescence of L1 (b) and tenascin (b'), and immunoelectron microscopy of tenascin (c) in the transected sciatic nerve seven days after the lesion. (a) A cellular bridge (B) links the proximal (left) and the distal (right) nerve stumps. The bridge is predominantly labelled at its inner core, whereas the outer part is negative (see also Figs. b,b'). Note strong immunoreactivity in the endoneurium of the distal nerve stump and weaker immunoreactivity in the endoneurium of the proximal stump. (b,b') Cellular bridge between proximal and distal nerve stumps. Note that L1-positive axons and Schwann cells growing into the bridge (b) are confined to the tenascin-positive region (b') and that tenascin indicates the prospective direction of growth of neural elements from proximal (left) to the distal (right) parts of the bridge. Asterisk in (b) and (b') indicates tenascin-negative region of the bridge. (c) Cellular bridge between proximal and distal nerve stumps. Note direct contact of a regrowing axonal tip (GC) with highly tenascin-immunoreactive collagen fibrils (Col) of the inner core of the bridge. E, endothelial cells; F, fibroblast. Scale bars: (a) 100 μm; (b') 50 μm; (c) 0.5 μm

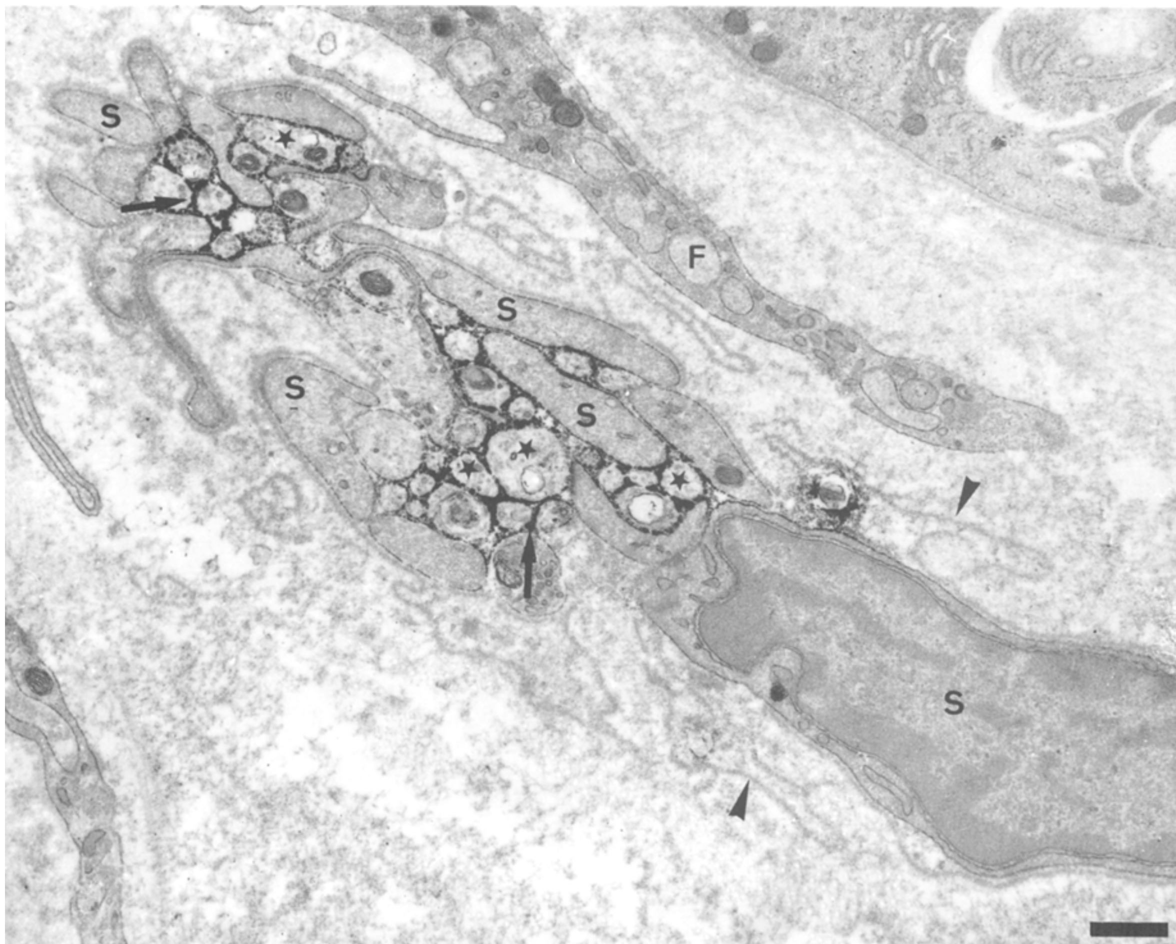

Fig. 13. Immunohistochemical localization of PSA in a peripheral nerve graft 14 days after surgical implantation into the thalamus of an adult rat. Note that strong immunoreactivity (arrows) is confined to regrowing thalamic axons (asterisks), whereas cell contacts between Schwann cell processes (S) are scarcely labelled or PSA-negative. F, endoneurial fibroblast; arrowheads mark Schwann cell basal lamina. Scale bar: 0.5 μm.

characteristic expression pattern and the fact that it is maintained in denervated nerves for 2–3 weeks suggest that the epitope may be involved in pathfinding of motor axons after injury of mixed nerves where motor and sensory axons intermingle (Martini *et al.*, 1988, 1992). The possible functional involvement of L2/HNK-1 carbohydrate-positive Schwann cells in the regeneration of motor axons was tested on cryosections of L2/HNK-1 carbohydrate-positive and negative peripheral nerves. Motor neurons from chicken embryos grew significantly longer (about 30–40%) on muscle nerves and ventral roots than on cutaneous nerve branches and dorsal roots (Martini *et al.*, 1992). This preferential outgrowth of motor neurons on sections of ventral roots could be inhibited by L2/HNK-1-specific antibodies.

The molecular mechanisms by which preferential outgrowth of motor axons may result in an apparently pathway-selective reinnervation of muscle nerves *in vivo* are difficult to imagine at present. In regenerating femoral nerves of rats, motor axons have been reported to explore possible pathways by sending out collateral branches into both appropriate and inappropriate nerve branches. Pathway specificity was only subsequently gained by pruning off those collaterals which had grown into the inappropriate nerve branch (Brushart, 1990, 1993). Combining these and our own observations, it appears plausible that a motor neuron can discriminate between its faster and slower growing axonal collaterals and is able to eliminate the slower ones (Martini *et al.*, 1992). An alternative mechanism could be imagined when one considers that in denervated femoral nerves, motor axons induce L2/HNK-1 almost exclusively in denervated Schwann cells which have been previously associated with motor axons, but not in Schwann cells previously associated with sensory axons (Brushart *et al.*, 1992; Martini *et al.*, in preparation). This preferential

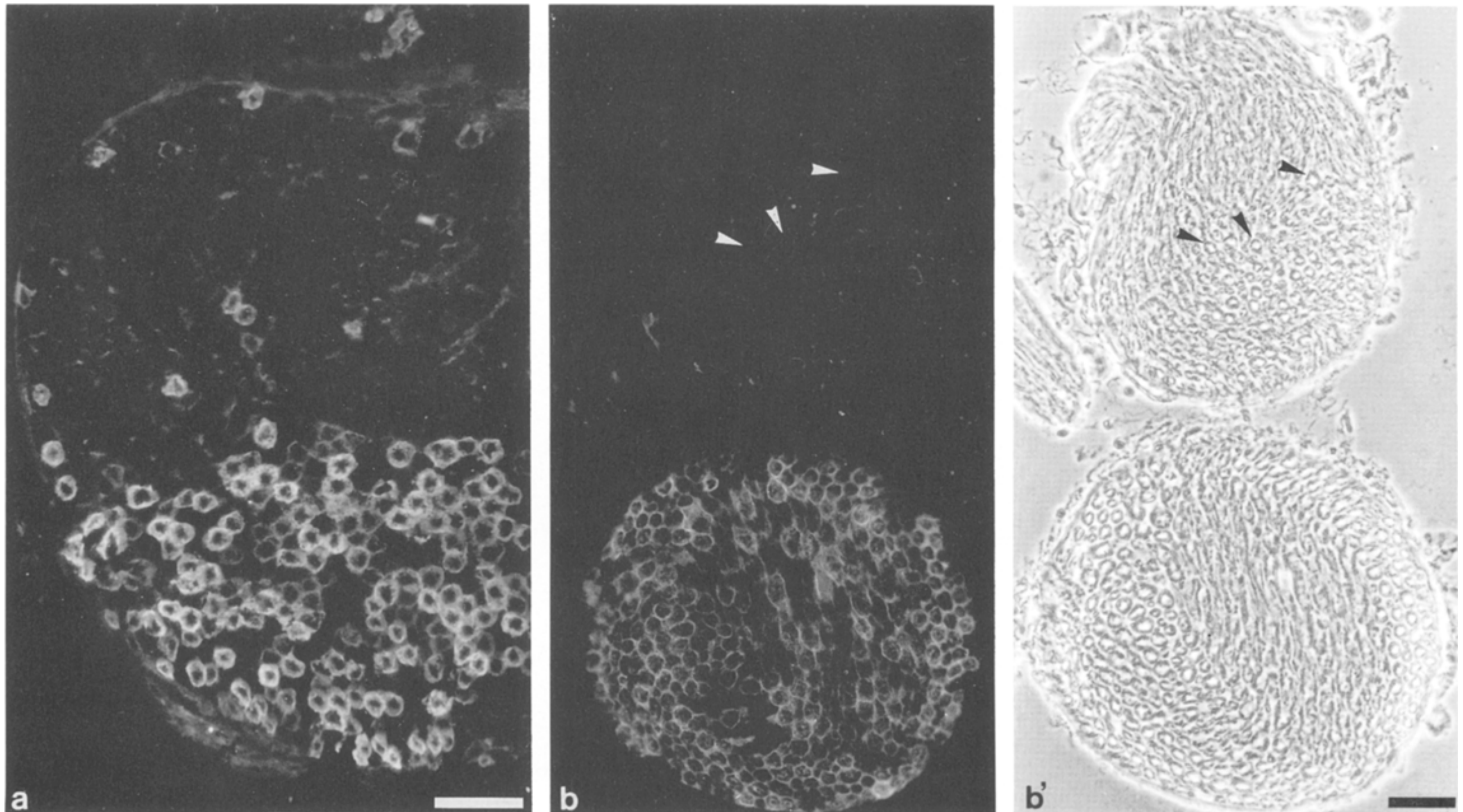

Fig. 14. Immunohistochemical localization of L2/HNK-1 in the spinal nerve (a) of the fourth lumbar segment and in the femoral nerve (b) with corresponding phase contrast micrograph (b'). (a) Myelin sheaths of ventral roots are strongly stained with L2/HNK-1-antibodies (lower part of the micrograph), whereas in the area occupied by the dorsal root ganglion axons only few immunoreactive myelin sheaths are visible (upper part of the micrograph). (b) Myelin sheaths of the muscle branch of the femoral nerve are stained with L2/HNK-1 antibodies (lower part of the micrograph), whereas myelin sheaths of the cutaneous branch are negative (upper part of the micrograph). The location of some of the L2/HNK-1-negative myelin sheaths in the cutaneous branch is indicated by arrowheads. (b') Corresponding phase contrast micrograph to (b). Arrowheads point to myelinating Schwann cells of the cutaneous branch which are indicated as L2/HNK-1-negative myelin sheaths in (b). Scale bars: 50 μm.

bility of previously motor axon-associated Schwann cells to reexpress the epitope may provide regenerating motor axons with a selective advantage over others which prevents them from being pruned (Brushart *et al.*, 1992; Martini *et al.*, in preparation).

Concluding remarks and future perspectives

By the combination of immunocytochemical and functional investigations, a picture emerges of the molecular mechanisms underlying development and regeneration of peripheral nerves (see Tables 1 and 2). According to this picture, particular cell surface and extracellular matrix molecules are involved in the morphogenesis of the peripheral nerve and contribute to the Schwann cells' important ability to mediate axonal growth in the lesioned peripheral nerve. Certainly, this picture may be incomplete and in several respects speculative. The most problematical aspect concerns regeneration, since most experimental evidence is derived from *in vitro* studies (see Table 2) which mimic regenerative events in an unsatisfactory manner. A major shortcoming is that embryonic tissue usually serves as a source for neural cells which may differ from adult cells in many respects. Additionally, in cultures, neuronal outgrowth is limited to a few hundred micrometers, whereas axonal elongation during regeneration *in vivo* can cover several centimeters or even greater distances. Thus, particular efforts appear to be necessary to improve conditions for *in vivo* experiments in adult animals. Such improvements could be the use of smaller sized experimental species (e.g. mouse) in order to avoid drastic dilution of probes (antibodies, antisense RNA). Permanent applications of highly concentrated probe solutions would be another improvement which could be achieved for example by moulding silicon chambers around lesioned nerves (see Remsen *et al.*, 1990). Such

Table 2. Expression and possible functions of cell surface and extracellular matrix molecules and carbohydrates during regeneration of peripheral nerves

	Expression	*Function*
L1	Up-regulation on previously myelinating SC and regrowing large calibre axons[a–c]	Promotion of axonal regrowth (ivt[1,2]), initiation of myelination of regenerated axons (ivt[3,4])
N-CAM	Up-regulation on previously myelinating SC, on regrowing large calibre axons[a–c] and fibroblasts in the bridge connecting proximal and distal stumps[c]	Promotion of axonal regrowth (ivt[2])
MAG	Disappears on previously myelinating SC, re-appears during myelination of regenerated axons[c–f]	Permission or promotion of axonal regrowth along previously myelinating SC at early stages of regeneration (ivt[5]), involved in myelination of regenerated axons as during development
P0	see MAG[d–f]	Permission or promotion of axonal regrowth along previously myelinating SC at early stages of regeneration (ivt[6]), involved in myelination of regenerated axons as during development
LAM	SC basal lamina, SC surface[g]	Promotion of axonal regrowth (ivt, see reviews by[7–9], ivv[10])
TEN	Up-regulation by SC and deposition on their basal laminae[h,i], association with collagen fibrils in the inner core of the bridge between proximal and distal stumps[i]	Promotion of axonal regrowth (ivt[11–13])
PSA	Regenerating axons[j]	Promotion of axonal regrowth (ivt[14,15], ivv[16])
L2/HNK-1	Maintenance of occurrence in motor axon-associated SC for 2–3 weeks[k], re-appearance on motor axon-associated SC[l]	SC mediated preferential reinnervation of muscle nerves by motor axons (ivt[17])

References on the distribution of antigens are indicated by letters, functional investigations by numerals; ivt = *in vitro*, ivv = *in vivo*

[a] Nieke & Schachner, 1985
[b] Daniloff *et al.*, 1986
[c] Martini & Schachner, 1988
[d] Willison *et al.*, 1988
[e] Gupta *et al.*, 1990
[f] Mitchell *et al.*, 1990
[g] Kücherer-Ehret *et al.*, 1990
[h] Daniloff *et al.*, 1989
[i] Martini *et al.*, 1990
[j] Zhang, Y. *et al.*, 1993
[k] Martini *et al.*, 1992
[l] Brushart *et al.*, 1992

[1] Bixby *et al.*, 1988
[2] Seilheimer & Schachner, 1988
[3] Seilheimer *et al.*, 1989
[4] Wood *et al.*, 1990
[5] Johnson *et al.*, 1989
[6] Schneider-Schaulies *et al.*, 1990
[7] Sanes, 1989
[8] Reichardt & Tomaselli, 1991
[9] Sandrock & Matthew, 1987a
[10] Wang *et al.*, 1992
[11] Wehrle & Chiquet, 1990
[12] Lochter *et al.*, 1991
[13] Husmann *et al.*, 1992
[14] Boisseau *et al.*, 1991
[15] Zhang, H. *et al.*, 1992
[16] Remsen *et al.*, 1990
[17] Martini *et al.*, 1992

depots naturally occur in the form of ventricles or eye cups in the CNS (Sandrock & Matthew, 1987b; Schnell & Schwab, 1990). Furthermore, the use of smaller-sized probes (e.g. Fab-fragments) could help to reduce the barrier effects of myelin and the extracellular matrix.

A conceptually very elegant approach circumventing these problems is the generation of null-mutations of particular, functionally important genes and the consequent analysis of the phenotypes resulting from the mutation (Capecchi *et al.*, 1989). Such an approach has been recently reported by Giese and colleagues (1992) in investigations of the functional roles of the P0 protein during myelination (see above). However, the disorganization of the myelin of such mutants could not be interpreted as a strict proof that P0 is directly involved in the formation of normal myelin sheaths, since other cell surface molecules were also abnormally regulated (see Table 1 and Giese *et al.*, 1992). Thus, if it transpires that other null-mutations are also accompanied by the dysregulation of functionally important, non-target genes, the appropriateness of this approach for the evaluation of a particular molecule's function will be limited. In this case, it appears as if one would be forced to go back to conceptually less elegant approaches such as perturbation experiments.

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