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Co-localization of the myelin-associated glycoprotein and the microfilament components, F-actin and spectrin, in Schwann cells of myelinated nerve fibres

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Summary

The myelin-associated glycoprotein (MAG) is an intrinsic membrane protein that is specific for myelinating cells. MAG has been proposed to function in the PNS as an adhesion molecule involved in Schwann cell–axon contact and maintenance of cytoplasmic channels within the myelin sheath. In this report we show that the microfilament components, F-actin and spectrin, co-localize with MAG in periaxonal membranes, Schmidt–Lanterman incisures, paranodal myelin loops, and inner and outer mesaxons of myelinating Schwann cells. F-actin was localized light microscopically by rhodamine-labelled phallicidin binding. Spectrin and MAG were localized by light microscopic and ultrastructural immunocytochemistry. The findings indicate that plasma membrane linkage of F-actin in Schwann cells is likely to occur via spectrin, and raise the possibility that microfilaments interact with the cytoplasmic domain of MAG. An interaction between MAG and microfilaments would be consistent with the proposed function of MAG as an adhesion molecule.

Introduction

Cell shape, motility and adhesion are mediated in part by interactions between actin microfilaments and transmembrane proteins (for reviews, see Geiger, 1983; Satir, 1984; Stossel, 1984). The molecular details of this interaction are best understood in the erythrocyte, where linkage of actin to the cytoplasmic domain of band 3, an integral membrane glycoprotein, occurs via spectrin and ankyrin (Branton *et al.*, 1981; Low, 1986). Characterization of the transmembrane components to which actin is linked in nucleated somatic cells has been elusive. Integral membrane glycoproteins are the most promising candidates and it is likely that individual cellular phenotypes utilize specific transmembrane proteins. The myelin-associated glycoprotein (MAG), a transmembrane protein unique to myelin-forming Schwann cells in the PNS, has been proposed as a candidate for such a role (Trapp & Quarles, 1982, 1984; Quarles, 1983/1984; Trapp *et al.*, 1984a, b; Martini & Schachner, 1986; Poltorak *et al.*, 1987; Salzer *et al.*, 1987; Lai *et al.*, 1988).

This hypothesis predicts the co-localization of actin microfilaments and MAG at the level of plasma membranes. Since the distribution of actin microfilaments within Schwann cells and their myelin sheaths is not known, the present study was designed to determine if two microfilament components, F-actin and spectrin, co-localized with MAG in sections of PNS myelinated fibres.

Immunogold procedures were used to localize MAG in ultrathin cryosections of peripheral nerve. This method avoided many of the inherent limitations of other immunocytochemical approaches and provides additional details of MAG localization in electron micrographs. The localization of actin microfilaments is complicated by their lability to fixation and tissue processing, as well as by the widespread distribution of G-actin in cytoplasm. For these reasons we used a cytochemical stain, rhodamine–phallicidin, that binds specifically to F-actin (Wulf *et al.*, 1979; Barak *et al.*, 1980). The localization of F-actin in cryosections, 1 μm thick, was compared to that of spectrin visualized in 1 μm and ultrathin cryosections by immunofluorescence and immunogold procedures, respectively.

Our results indicate that microfilaments and MAG co-localized in myelinated nerve fibres and represent a

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start in elucidating actin microfilament–Schwann cell plasma membrane interactions. Preliminary results of this study have been published in abstract form (Wong & Griffin, 1982; Trapp *et al.*, 1985, 1986).

Materials and methods

Light microscopy

Frozen sections, 1 μm thick, were obtained according to standard procedures (Tokuyasu, 1980; Griffiths *et al.*, 1983) with minor variations. Adult, 7- and 35-day-old Sprague–Dawley rats were perfused with 4% paraformaldehyde in 0.08 M phosphate buffer. The sciatic nerves were removed, cut into 0.5 cm segments, infiltrated with 1.0 M sucrose containing 4% paraformaldehyde for 12 h, 2.0 M sucrose for 4 h, and 2.3 M sucrose for 4 h. The nerve segments were then mounted on specimen stubs and frozen in liquid nitrogen.

Frozen sections, nominally 1 μm thick, were cut on glass knives in a Reichert ultracryomicrotome maintained at approximately −95° C. The sections were transferred with thawing to polylysine-coated glass coverslips, and washed in phosphate-buffered saline (PBS) (5 × 3 min).

F-actin was localized by floating the coverslips on PBS containing a 1:40 dilution of rhodamine-labelled phallicidin (Molecular Probes, Junction City, OR) for 30 min. The coverslips were then rinsed in PBS (3 × 3 min), mounted on glass slides in 90% glycerol and sealed with nail polish. Co-localization of F-actin and spectrin was performed by sequentially floating the coverslips on drops of PBS containing the following: (i) 3% normal goat serum for 15 min; (ii) rabbit anti-brain spectrin antibody (diluted 1:200) for 60 min; (iii) fluorescein-labelled anti-rabbit IgG (diluted 1:40) and rhodamine-labelled phallicidin (diluted 1:40) for 30 min. The coverslips were rinsed in PBS (3 × 3 min) after steps 2 and 3, and mounted on glass slides as described above. Sections were visualized with fluorescence and phase optics by use of a Zeiss photomicroscope III. The characterization and specificity of the spectrin anti-serum kindly provided by Dr Vann Bennett has been described (Davis & Bennett, 1983).

Electron microscopy

Seven-day-old Sprague-Dawley rats were perfused with a solution containing 2.5% glutaraldehyde, 4.0% paraformaldehyde and 0.08 M phosphate buffer. The L4 and L5 spinal roots were removed, cut into 0.5 cm segments, placed in fixative overnight, then infiltrated with sucrose, frozen and stored in liquid nitrogen as described above. Ultrathin frozen sections (< 120 nm) were cut at approximately −110° C, and transferred to formvar- and carbon-coated, hexagonal-mesh grids. The grids were maintained, sections facing down, on 2% gelatin in PBS at 4° C for 2 h, followed by floating on PBS (5 × 3 min).

The sections were stained, essentially according to published procedures (Tokuyasu, 1980; Griffiths *et al.*, 1983; Slot & Geuze, 1984), by sequentially floating the grids on drops of the following solutions: 10% ovalbulmin and 3% normal goat serum in PBS for 30 min; primary antibodies appropriately diluted in PBS containing 1% ovalbumin and 0.3% normal goat serum (PBS-1) for 1 h; PBS-1 (6 × 5 min); 15-nm colloidal gold-labelled goat anti-rabbit or mouse IgG (Janssen Pharmaceuticals) in PBS-1 for 60 min; and PBS-1 (6 × 5 min). The grids were then rinsed in distilled water (4 × 2 min), stained with neutral uranyl acetate–oxalate (pH 7), washed again in distilled water (4 × 2 min) and embedded in uranyl acetate-containing methyl cellulose (Methocell, Fluka AG, Buchs, Switzerland). Grids were viewed in a JEOL 200-CX electron microscope operated at 80 kV.

The characterization and specificity of the antisera used have been described: MAG (Doberson *et al.*, 1985), spectrin (Davis & Bennett, 1983) P_0 (Trapp *et al.*, 1979, 1981) and neurofilament (Sternberger & Sternberger, 1983).

Results

Distribution of F-actin

The distribution of F-actin was determined by rhodamine-labelled phallicidin binding in 1 μm cryosections of 7-day, 35-day and adult rat sciatic nerve (Figs 1, 2). F-actin was distributed similarly at all ages. In transverse sections, all myelinated fibres contained a periaxonal ring of F-actin staining that always included an intense dot (Figs 1, 2A); as indicated below, these dots correspond to the inner mesaxons. Schmidt–Lanterman incisures, identified by phase-contrast microscopy, were intensely stained (Fig. 2A, B). Longitudinal sections (Fig. 2C) and teased fibres (not shown) confirmed the staining of the incisures, with their characteristic 'arrowhead' appearances. Internodal axoplasm was unstained or very faintly stained (Figs 1, 2A, C, D). The paranodal and nodal regions were prominently stained (Fig. 2D). Much of this

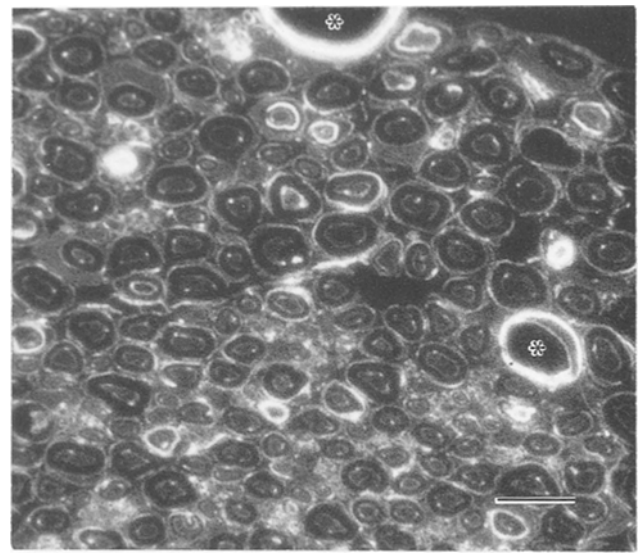

Fig. 1. Rhodamine-labelled phallicidin binding to a 1 μm cryosection of 7-day-old rat sciatic nerve. F-actin was detected in myelinated fibres, unmyelinated fibres and around blood vessels (asterisks). Scale bar: 10 μm.

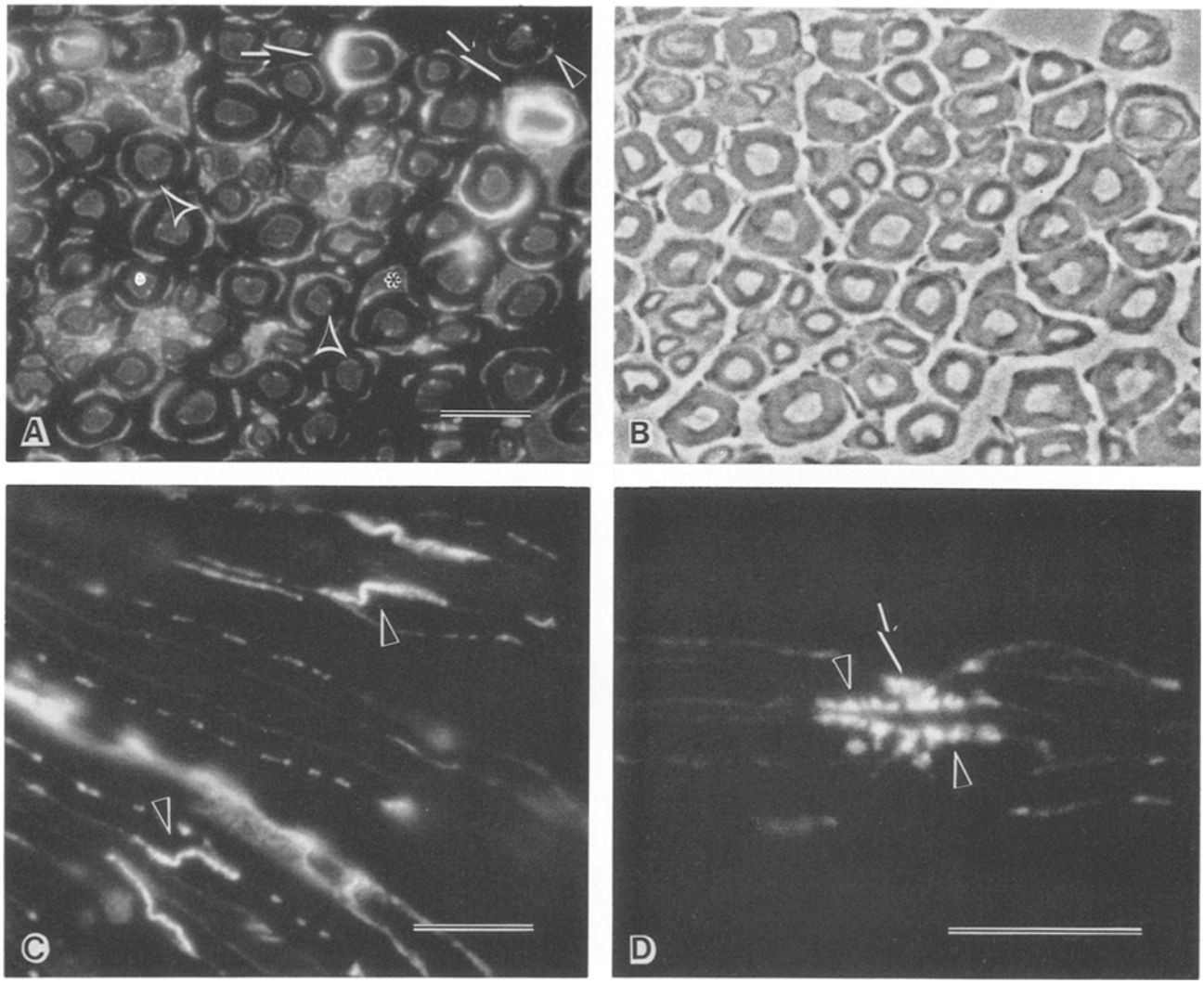

Fig. 2. Rhodamine-labelled phallicidin binding to 1 μm cryosections of sciatic nerves from 35-day-old (A) and adult (C, D) rats. In transversely oriented sections, all myelinated fibres contain a periaxonal ring of F-actin staining that includes an intense dot (A, curved arrowheads). Schmidt–Lanterman incisures are stained intensely (A, arrows) and have typical 'arrowhead' appearances in longitudinal section (C, arrowheads). Paranodal myelin loops (D, arrowheads) and paranodal microvilli (D, arrow) are stained intensely. The outer perimeter of myelinated fibres is partially surrounded by lines of F-actin staining which can include dots or bands of increased staining intensity (A, straight arrowhead). Where the Schwann cell plasmalemma and outer perimeter of compact myelin can be resolved as separate membranes (A, asterisk), both are stained. Compact myelin did not stain. Morphological preservation of A is demonstrated by phase-contrast image (B). Scale bars: 10 μm (A and B are at the same magnification.)

staining was over the paranodal terminal loops of myelin lamellae and Schwann cell microvilli. The resolution was inadequate to determine whether the immediate subaxolemmal portion of the nodal axon might also be stained, but in these regions the centre of the axon was relatively unstained.

The outer perimeter of myelinated fibres also was stained (Figs 1, 2A); typically the staining was interrupted and patchy. The outer perimeter often contained an intense dot or short line of F-actin staining; as indicated below, this probably represents the outer mesaxon. Where Schwann cell cytoplasmic channels could be identified outside the myelin sheath, both the outer margin of compact myelin and the abaxonal Schwann cell plasmalemma were stained (Figs 1, 2A; also see Fig. 3C). Where these cytoplasmic channels were undetectable, F-actin staining was absent or appeared as a single line. At the light microscopic level, we could not determine whether these single lines of staining were associated with the Schwann cell plasmalemma, the outer lamella of myelin, or both. Schwann cell cytoplasm, both in the channels and in

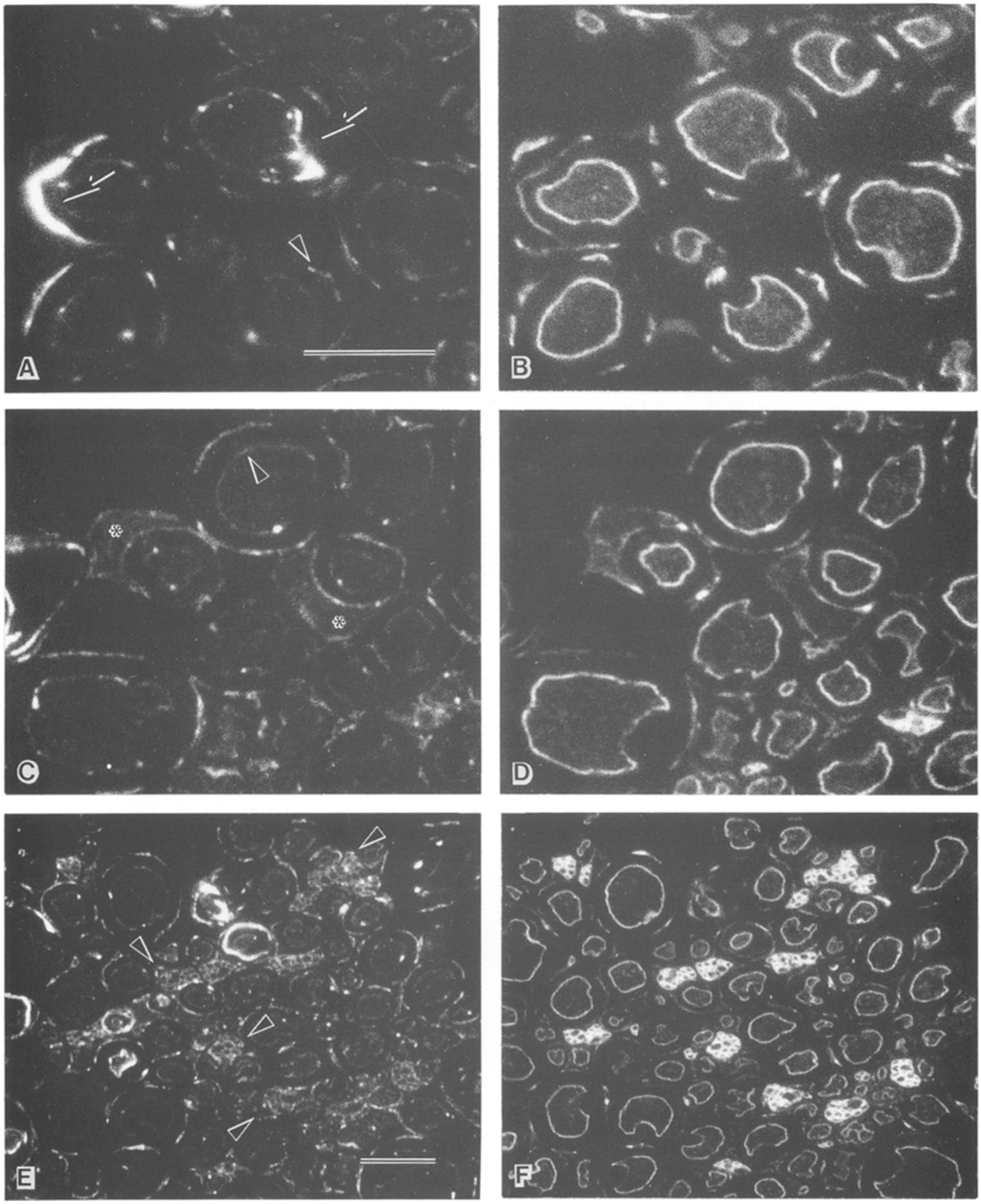

Fig. 3. Comparison of F-actin (A, C, E) and spectrin (B, D, F) localization in 1 μm cryosections of adult rat sciatic nerve as determined by double labelling. F-actin and spectrin co-localized at all locations. However, the width and relative intensity of F-actin staining varied; variations in spectrin staining intensities were less apparent. Note differences at Schmidt–Lanterman incisures (A, arrows), and at the inner and outer perimeter (C and A, arrowheads) of myelin sheaths and unmyelinated fibres (E, arrowheads). Asterisks (C) denote Schwann cell cytoplasmic channels at the outer perimeter of myelinated fibres. Scale bars: 10 μm. (A–D and E, F are at the same magnification.)

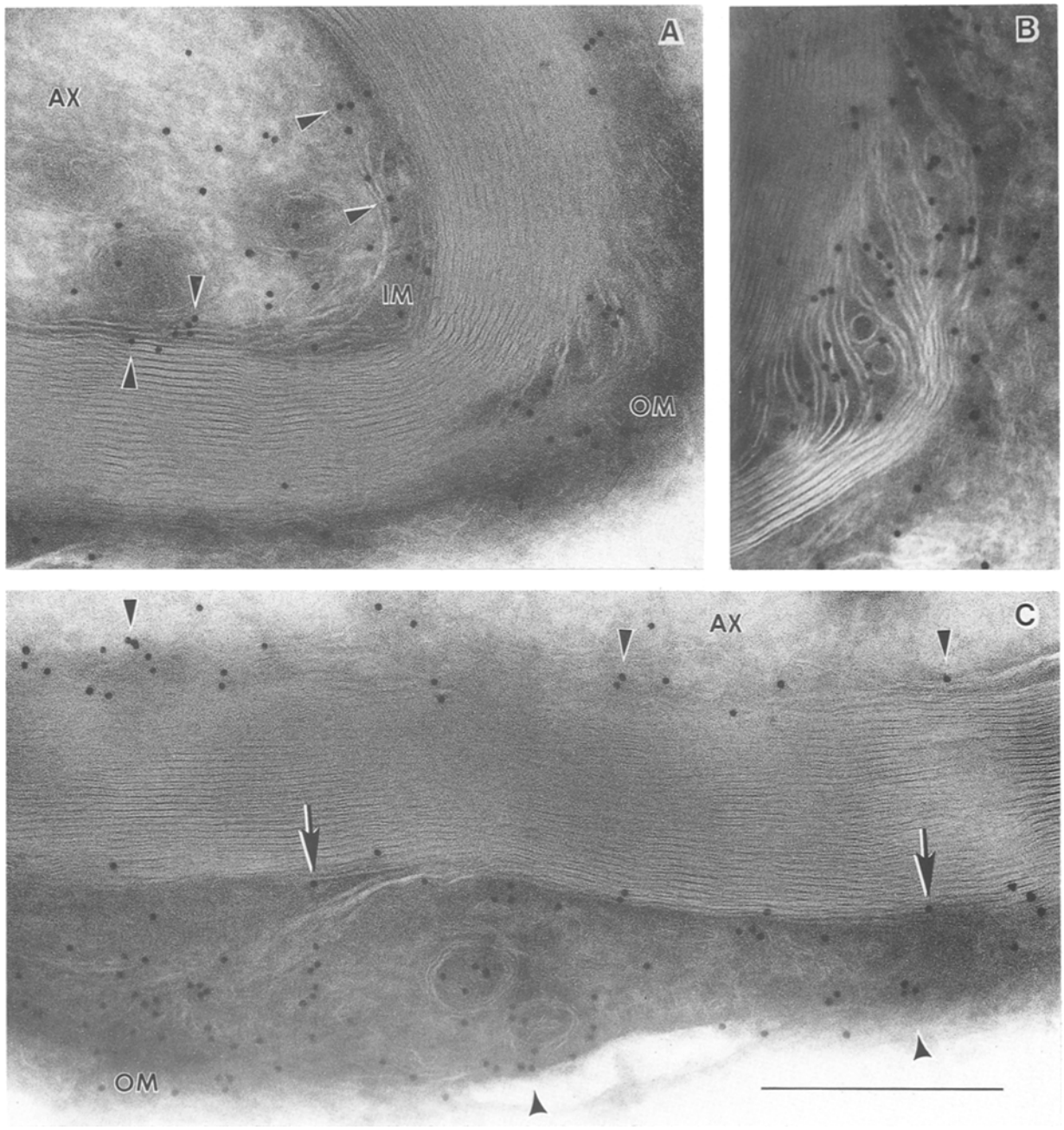

Fig. 4. Electron micrographs of myelinated fibres from 7-day-old rat peripheral nerve immunostained with spectrin antibodies and 15 nm colloidal, gold-labelled secondary antibody. Gold particles are concentrated around periaxonal regions (A and C, straight arrowheads), Schmidt–Lanterman incisures (B), inner mesaxons (A, IM) and outer mesaxons (A and C, OM). Gold particles were rarely found over compact myelin. At the outer perimeter of myelinated fibres, gold particles were associated with the Schwann cell plasma membrane (C, curved arrowheads) and the outer perimeter of compact myelin (C, arrows). Diffuse labelling of axoplasm (AX) and regions of Schwann cell cytoplasm was also present. Scale bar: 0.5 μm.

the perikaryon, was faintly stained. The Schwann cells of unmyelinated fibres were prominently stained (Fig. 3E); within these fibres the axons appeared as dark (unstained) dots.

Distribution of spectrin

F-actin and spectrin were localized in transverse sections of adult sciatic nerve by double labelling (Fig. 3). F-actin and spectrin appeared to co-localize at all locations. The relative width and intensity of labelling with the F-actin probe varied at different locations. For example, F-actin and spectrin co-localized at Schmidt–Lanterman incisures (Fig. 3A, B) but the relative intensity of F-actin staining was greater than that of

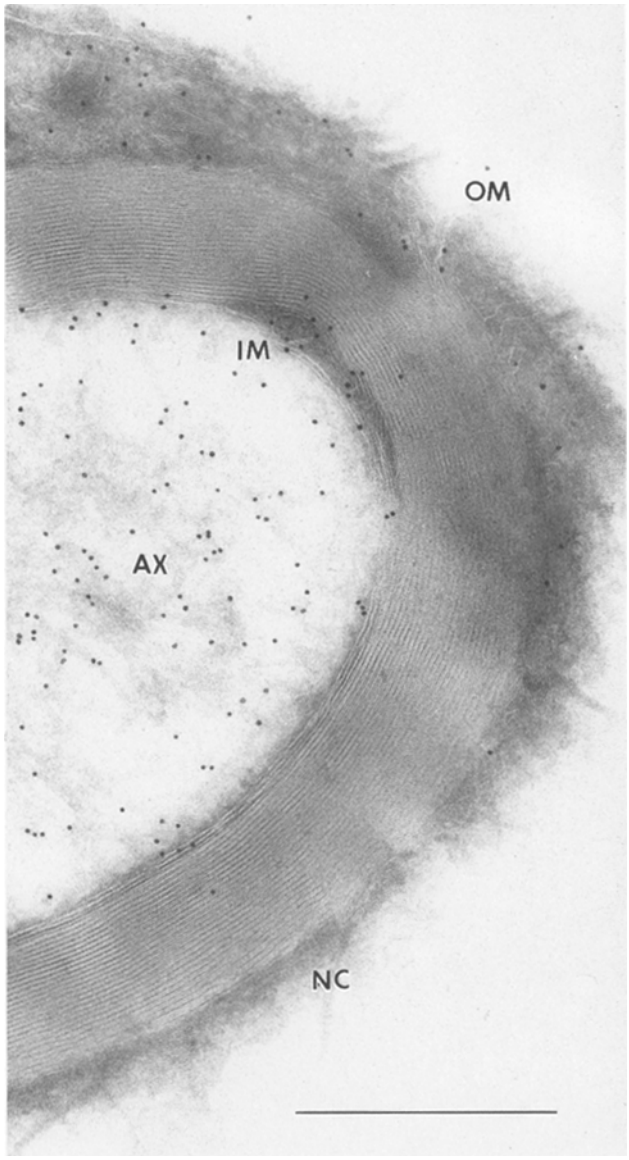

Fig. 5. Electron micrograph of a myelinated axon from a 7-day-old rat peripheral nerve immunostained with spectrin antibodies and 15 nm colloidal, gold-labelled secondary antibody. Gold particles are present at periaxonal regions and at the inner mesaxon (IM). The outer mesaxon (OM) and bordering Schwann cell cytoplasmic channels also contained gold particles. Gold particles were rarely associated with non-channel (NC) regions or compact myelin. Axoplasm (AX) was labelled diffusely. Scale bar: 0.5 μm.

spectrin. The inner mesaxons seen with F-actin staining were not as readily appreciated with anti-spectrin antibodies, although when apparent they co-localized with F-actin. Periaxonal staining of myelinated and unmyelinated fibres (Fig. 3E, F) and staining of the outer perimeter of myelinated fibres (Fig. 3A, B) appeared more prominent for spectrin than for F-actin. Neither F-actin nor spectrin staining was detected in regions of compact myelin. Anti-spectrin antibodies produced weak, diffuse staining of axoplasm and the channels of Schwann cell cytoplasm (Fig. 3D).

Ultrastructural localization of spectrin

Spectrin was localized in ultrathin cryosections of 7-day-old rat peripheral nerve by immunogold procedures (Figs 4–6). At the electron microscopic level, spectrin was enriched in the same regions stained by spectrin antibodies in 1 μm sections. For example, gold particles were concentrated around periaxonal regions (Figs 4A, C, 5), within inner mesaxons (Fig. 4A), Schmidt–Lanterman incisures (Fig. 4B) and outer mesaxons (Fig. 4A, C), but rarely found over compact myelin. The intense dots of outer and inner perimeter staining present in light micrographs are thus likely to represent the inner and outer mesaxon.

Spectrin antibodies also labelled the Schwann cell plasma membrane and the outer compact myelin lamellae where they surround cytoplasmic channels (Figs 4C, 5). However, gold particles were rarely associated with these membranes when they became closely apposed in the non-channel regions (Fig. 5). Schwann cell perinuclear cytoplasm (Fig. 6A) and axoplasm (Figs 4A, 5) were labelled diffusely. A cytoplasmic pool of spectrin was also apparent in the larger cytoplasmic channels located at the outer perimeter of the myelin sheath (Figs 4C, 5).

Ultrastructural localization of P_0 protein and neurofilaments

To establish the specificity of the immunogold procedure, two additional antigens, neurofilament and P_0 protein, were localized. These antigens were chosen because of their known location within axoplasm (neurofilaments) and compact PNS myelin (P_0 protein). Neurofilament antibodies did not label Schwann cell cytoplasm (Fig. 6B) or compact myelin (Fig. 6C). However, as expected, gold particles were diffusely distributed throughout axoplasm (Fig. 6C). Compact PNS myelin was intensely labelled by P_0 antibodies (Fig. 6D).

Ultrastructural localization of MAG

MAG was localized in ultrathin cryosections by immunogold procedures utilizing a monoclonal antibody directed against MAG-polypeptide (Doberson *et al.*, 1985). A consistent finding in all MAG-stained electron micrographs was a paucity of gold particles over compact myelin and axoplasm (Figs 7–9). Gold particles were distributed around the periaxonal membrane (Fig. 7) and the membranes of the outer mesaxon (Fig. 8A), Schmidt–Lanterman incisures (Fig. 8B) and paranodal myelin loops (Fig. 9). These results confirmed previous immunocytochemical studies demonstrating the absence of MAG in compact myelin, and indicated a co-localization of MAG, spectrin and F-actin.

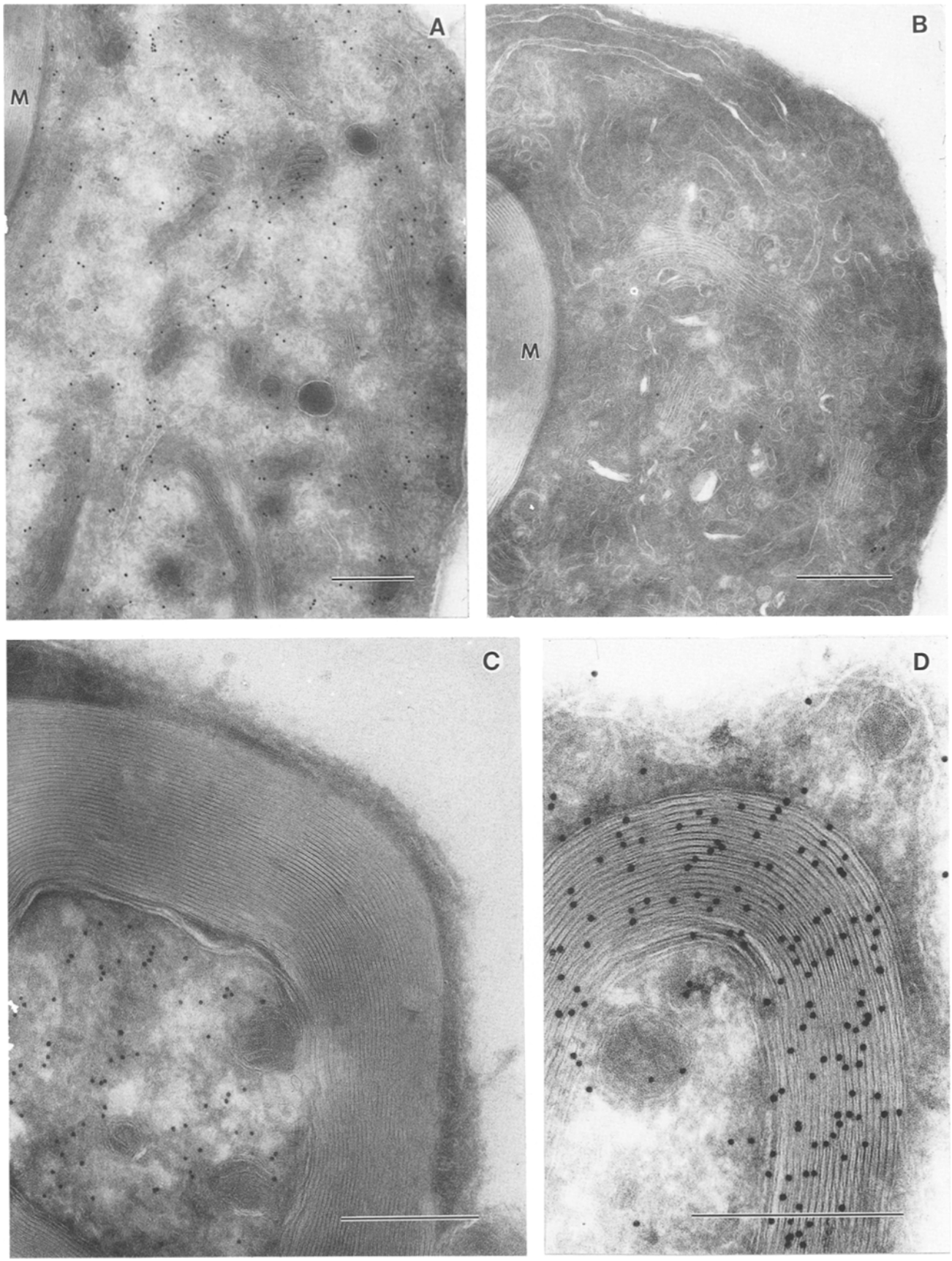

Fig. 6. Electron micrographs of Schwann cell perinuclear regions from a 7-day-old rat peripheral nerve immunostained with spectrin (A), neurofilament (B, C) and P_0 (D) antibodies and 15 nm colloidal, gold-labelled secondary antibody. Schwann cell cytoplasm is diffusely labelled by spectrin antibodies (A), but does not react with neurofilament antibodies (B) that diffusely label axoplasm (C). P_0 antibodies label compact myelin (D). Label M in A and B denotes myelin sheaths. Scale bars: 0.5 μm.

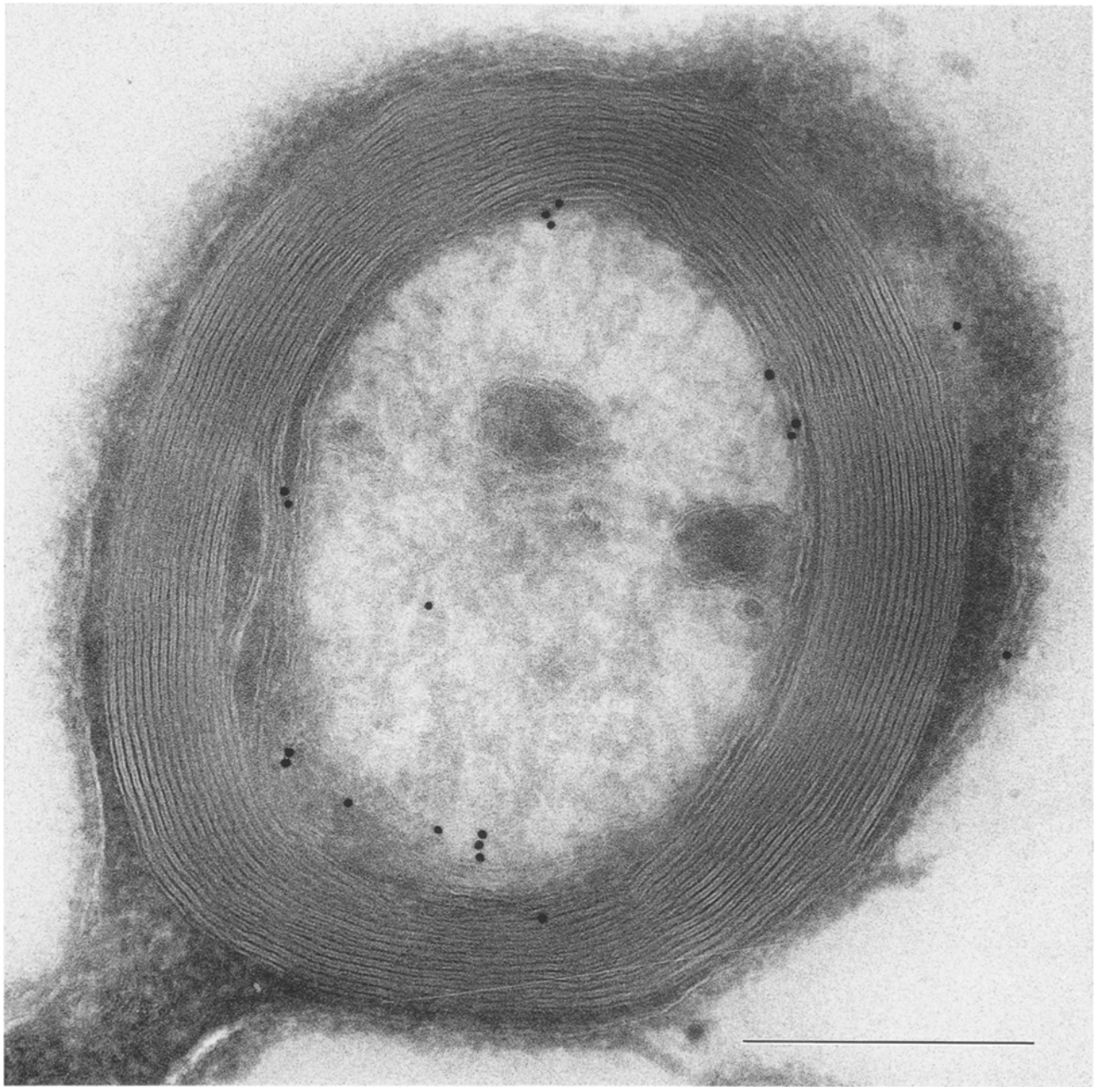

Fig. 7. Electron micrograph of a myelinated fibre from a 7-day-old rat peripheral nerve immunostained with MAG antibodies and 15 nm colloidal, gold-labelled secondary antibodies. Gold particles are concentrated around the periaxonal membrane. Compact myelin and axoplasm are not labelled. Scale bar: 0.5 μm.

Discussion

The major finding of this study was the co-localization of MAG, F-actin and spectrin within discrete regions of the PNS myelin sheath. The precise localization of spectrin and F-actin in Schwann cells and PNS myelin sheaths are described for the first time. These results confirm and extend previous immunocytochemical studies that have localized MAG (Sternberger *et al.*, 1979; Trapp & Quarles 1982, 1984; Trapp *et al.*, 1984a, b; Martini & Schachner, 1986) and spectrin (Levine & Willard, 1981; Repasky *et al.*, 1982; Glenney & Glenney, 1983a, b; Koenig & Repasky, 1985) in peripheral nerve, and raise the possibility that actin microfilaments form stable contacts with the cytoplasmic domain of MAG. Such an interaction would be consistent with the proposed function of MAG as an adhesion molecule (Trapp & Quarles, 1982, 1984;

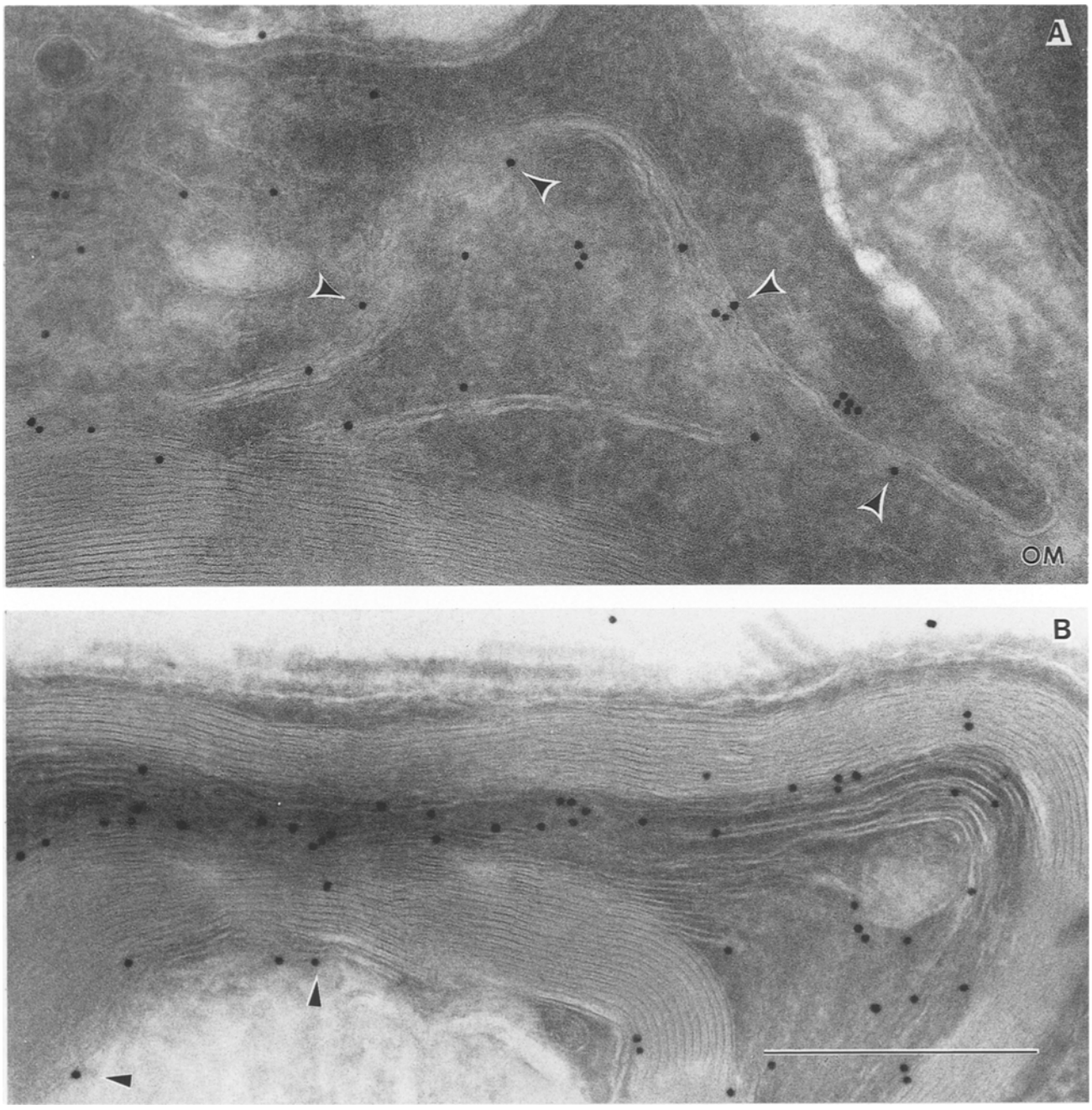

Fig. 8. Electron micrographs of myelinated fibres from a 7-day-old rat peripheral nerve immunostained with MAG antibodies and 15 nm colloidal, gold-labelled secondary antibodies. Gold particles are associated with the membranes (A, arrowheads) of the outer mesaxon (OM) and Schmidt–Lanterman incisures (B). Gold particles are also found at periaxonal regions (B, arrowheads) but are rarely present over axoplasm and compact myelin. Scale bar: 0.5 μm.

Martini & Schachner, 1986; Quarles 1983/1984; Trapp *et al.*, 1984a, b; Poltorak *et al.*, 1987), and suggests a role for MAG–microfilament linkages in a variety of Schwann cell functions.

Methodological considerations

The mushroom toxins, phalloidin and phallicidin, were developed as cytochemical stains (Wulf *et al.*, 1979; Barak *et al.*, 1980) because they selectively bind to F-actin but do not bind to G-actin. This presumably explains the paucity of axonal staining, in that actin is one of the most abundant axonally transported proteins (Black & Lasek, 1979). This indicates that most axoplasmic actin is either in the monomeric form *in vivo*, or assembled into short filament form that is not preserved under the present fixation and prep-

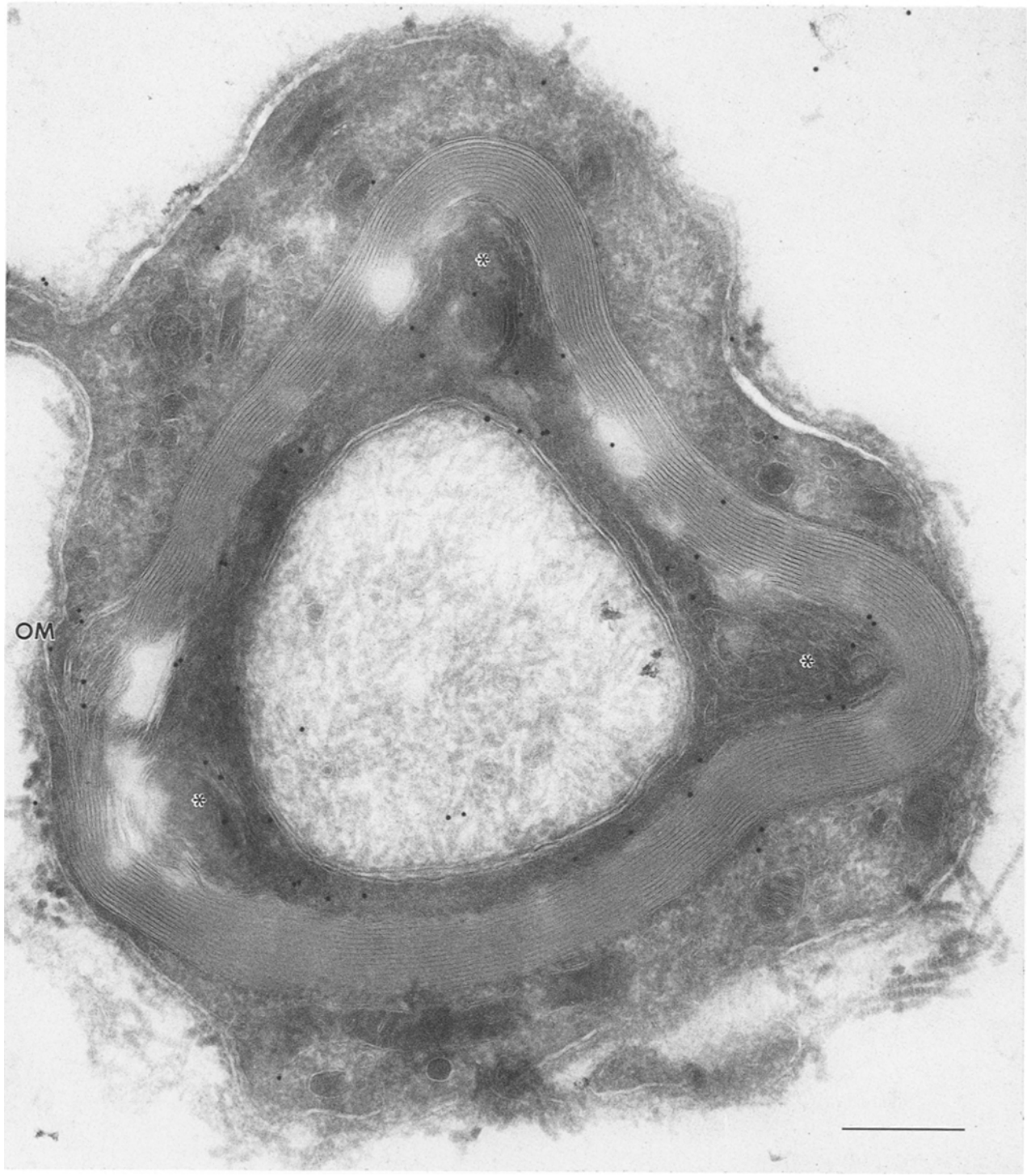

Fig. 9. Electron micrograph of a paranodal region of a myelinated fibre from a 7-day-old rat peripheral nerve immunostained with MAG antibodies and 15 nm colloidal, gold-labelled secondary antibody. Gold particles are associated with the membranes of the paranodal loops (asterisks) and the outer mesaxon (OM). Scale bar: 0.5 μm.

aration procedures (LaFountain *et al.*, 1977; Maupin-Szamier & Pollard, 1978). Spectrin and F-actin were enriched in the same regions of myelinated fibres in light micrographs.

In light micrographs, axoplasm and Schwann cell cytoplasm were also stained by spectrin antibodies. This staining was distributed diffusely and appeared less intense than submembranous staining. These cytoplasmic pools of spectrin were more apparent in immunogold-labelled electron micrographs. This apparent discrepancy between the submembranous enrichment of spectrin in light micrographs and

cytoplasmic enrichment in electron micrographs has been found previously (Levine & Willard, 1981; Repasky *et al.*, 1982; Glenney & Glenney, 1983a, b; Zagon *et al.*, 1986). We suspect that our results are due, in part, to the inability of diffusely scattered fluorescein molecules to produce a significant signal in light micrographs. In contrast, all gold particles are detectable in electron micrographs. It is not clear why spectrin labelling of plasma membranes was not more apparent in our electron micrographs or in those published previously (Zagon *et al.*, 1986). We have found similar results in the distribution of the myelin-associated molecule 2′, 3′-cyclic nucleotide 3′-phosphodiesterase in light and electron micrographs of CNS tissue (Trapp *et al.*, 1988), and we suspect similar findings when localizing other extrinsic membrane proteins that are associated with or enriched beneath plasma membranes. Different fixation conditions were used in the light microscopic and electron microscopic studies presented here. However, the apparent discrepancy between our light and electron microscopic findings cannot be attributed solely to fixation because the number and distribution of gold particles in spectrin-stained, 4% paraformaldehyde-fixed, ultrathin cryosections were comparable to those found in sections fixed with 2.5% glutaraldehyde (data not shown).

Because their distinctive distributions are well established, P_0 (Trapp *et al.*, 1979, 1981) and neurofilament (Sternberger & Sternberger, 1983) staining provided useful controls to evaluate the reliability of the immunolocalization techniques. The results with these antigens show that a variety of cellular antigens can be localized by immunogold procedures in ultrathin cryosections of tissue stringently fixed with glutaraldehyde.

Distribution of microfilaments

F-actin, as identified by rhodamine–phallicidin staining, was concentrated in inner and outer mesaxons, Schmidt–Lanterman incisures, periaxonal membranes, and paranodal terminal loops of non-compacted myelin. Spectrin was identified by light or ultrastructural immunocytochemistry in these same sites. In addition, both of these proteins were present in the channels of Schwann cell cytoplasm outside the myelin sheath; in these channels they appeared to be associated with the outermost myelin lamella and the Schwann cell plasmalemma. In neighbouring regions, in which only scant cytoplasm intervened between the outer myelin lamella and the plasmalemma (Mugnaini *et al.*, 1977), there was little staining.

The co-localization of F-actin and spectrin suggests that Schwann cell spectrin participates in linking F-actin to plasma membranes. Spectrin is a member of a functionally and structurally related family of proteins that are present in most, if not all, mammalian cells (Levine & Willard, 1981; Repasky *et al.*, 1982; Glenney & Glenney 1983a, b; Goodman *et al.*, 1983; Nelson & Lazarides, 1983; Zagon *et al.*, 1986). The affinity purified anti-brain spectrin polyclonal antisera used in the present study reacts with both the α and β subunits of CNS spectrin (Davis & Bennett, 1983) and does not appear to distinguish between various subunit isoforms (V. Bennett, personal communication) that have been identified (Riederer *et al.*, 1986). Brain spectrin binds F-actin, ankyrin and calmodulin (Bennett *et al.*, 1982; Repasky *et al.*, 1982; Nelson & Lazarides, 1983; Davis & Bennett, 1983, 1984; Glenney & Glenney, 1983a, b). The association of brain spectrin with membrane motility (Cheney *et al.*, 1983; Nelson *et al.*, 1983; Koenig & Repasky, 1985) suggests that membrane expansion during myelin formation is mediated by an actin-based motility system.

Localization of MAG

The present study and a recent report (Martini & Schachner, 1986) have provided compelling electron microscopic immunocytochemical evidence confirming enrichment of MAG in discrete regions of the PNS myelin sheath (Trapp & Quarles, 1982). In both studies, the distribution of gold particles in MAG-stained electron micrographs indicated that MAG resides predominantly in Schwann cell membranes demarcating the cytoplasmic channels of the PNS myelin sheath. These results, considered together with the intense labelling of compact PNS myelin by P_0 (Fig. 6D) and myelin basic protein (Martini & Schachner, 1986) antisera, provide no support for the proposal that MAG is present in compact myelin membranes (Webster *et al.*, 1983).

Correlating the immunocytochemical localization of MAG, its chemical composition and biochemical properties, Trapp & Quarles (1982) proposed that MAG functioned as an adhesion molecule, stabilizing the 12–14 nm periaxonal space between myelinating Schwann cells and axons. MAG was also implicated in maintaining the spacing between adjacent Schwann cell membranes in Schmidt–Lanterman incisures, paranodal loops and the inner and outer mesaxons. Recent *in vitro* studies (Poltorak *et al.*, 1987) also supported a role for MAG in cellular adhesion by demonstrating that MAG antibodies in the CNS interfered with neuron–oligodendrocyte adhesion and MAG-containing liposome–neurite adhesion.

A model depicting the possible orientation of MAG within Schwann cell membranes (see Fig. 11 in Trapp *et al.*, 1984b) divided MAG into three domains: (i) a heavily glycosylated extracellular N-terminal domain that mediated membrane adhesion; (ii) a single transmembrane domain; and (iii) a cytoplasmic C-terminal domain, possibly interacting with cytoskeletal components. The orientation of MAG within

membranes, deduced from its amino acid sequence specified by cDNA clones (Arquint *et al.*, 1987; Lai *et al.*, 1987; Salzer *et al.*, 1987), is strikingly similar to the three-domain model discussed above. The N-terminal region of MAG contains the tripeptide sequence Arg-Gly-Asp (RGD) which has been shown to be crucial in mediating interactions between transmembrane and extracellular proteins (Plow *et al.*, 1985; Pytela *et al.*, 1985; Ruoslahti & Pierschbacher, 1986). In some species, MAG shares a carbohydrate determinant (HNK-1) with several other molecules proposed to mediate cell adhesion in the nervous system (McGarry *et al.*, 1983; Kruse *et al.*, 1984).

The N-terminal region of MAG displays significant homology with members of the immunoglobulin gene superfamily (Hood *et al.*, 1985). MAG shows closest homology to N-CAM, a molecule involved in neuronal adhesion and development (Edelman, 1984; Rutishauser, 1984; Hemperly *et al.*, 1986). Since MAG is present in apposing plasma membranes of Schmidt–Lanterman incisures, paranodal loops and mesaxons, adhesion between these membranes may involve homotypic MAG interactions similar to those proposed for N-CAM (Edelman, 1984; Rutishauser, 1984) and P_0 protein (Lemke & Axel, 1985). It is interesting to note that spectrin has been shown to bind an affinity purified isoform of N-CAM (180K) on gel overlays (Pollerberg *et al.*, 1986, 1987). Adhesion between Schwann cell periaxonal membranes and axolemmae may involve heterotypic interactions between MAG and another member of the immunoglobin gene superfamily.

MAG–microfilament interactions

On the basis of their co-localization and the MAG sequence data, we speculate that in myelinating Schwann cells, MAG may be the major integral membrane glycoprotein linked to cortical actin microfilaments. MAG has been deduced to have a cytoplasmic domain of about 100 amino acids containing proteolytic cleavage and phosphorylation sites. The putative cytoplasmic domain of MAG is homologous to a cytoplasmic segment of integrin (Salzer *et al.*, 1987), a transmembrane protein that is linked to actin intracellularly and fibronectin extracellularly (Tamkun *et al.*, 1986). MAG–microfilament linkages would fit well with the postulated role of MAG as an adhesion molecule acting across the periaxonal space (Trapp *et al.*, 1984b) and with a role of maintaining Schwann cell cytoplasmic continuity and preventing fusion of membranes into compact myelin (Trapp & Quarles, 1982). The strict correlation between the presence of MAG in Schwann cell periaxonal membranes and maintenance of Schwann cell periaxonal cytoplasm also supports this hypothesis (Trapp *et al.*, 1984b). In addition, since MAG appears in mesaxon membranes of myelinating Schwann cells even before compact myelin (Trapp *et al.*, 1984b; Trapp & Quarles, 1984; Trapp, 1988), a microfilament-based motility system interacting with MAG could provide force generation for the process of spiral rotation of one mesaxon that is required for formation of the myelin sheath.

Elucidation of molecular aspects of membrane cytoskeleton linkages requires various methodological approaches that all have limitations (Geiger, 1983). The results described in this paper provide the necessary beginning that supports the possible interaction between MAG and actin microfilaments. However, it should be stressed that biochemical and molecular approaches will be required to further substantiate this hypothesis. Because the amino acid sequence of MAG is now known, such studies are now feasible.

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References

ARQUINT, M., RODER, J., CHIA, L.-S., DOWN, J., WILKINSON, O., BAYLEY, H., BRAUN, P. & DUNN, R. (1987) Molecular cloning and primary structure of myelin-associated glycoproteins. *Proceedings of the National Academy of Sciences USA* **84**, 600–4.

BARAK, I. S., YOCUM, R. R., NATHNAGEL, E. A. & WEBB, W. W. (1980) Fluorescence staining of the actin-cytoskeleton in living cells with 7-nitrobenz-2-oxa-13-diazole-phallacidin. *Proceedings of the National Academy of Sciences USA* **77**, 980–5.

BENNETT, V., DAVIS, J. & FOWLER, W. E. (1982) Brain spectrin, a membrane-associated protein related in structure and function to erythrocyte spectrin. *Nature* **229**, 126–31.

BLACK, M. M. & LASEK, R. J. (1979) Axonal transport of actin: Slow component b is the principal source of actin for the axon. *Brain Research* **171**, 401–13.

BRANTON, D., COHEN, C. M. & TYLER, J. (1981) Interaction of cytoskeletal proteins on the human erythrocyte membrane. *Cell* **24**, 24–32.

CHENEY, R., HIROKAWA, N., LEVINE, J. & WILLARD, M. (1983) Intracellular movement of fodrin. *Cell Motility* **3**, 649–55.

DAVIS, J. & BENNETT, V. (1983) Brain spectrin: isolation of subunits and formation of hybrids with erythrocyte spectrin subunits. *Journal of Biological Chemistry* **258**, 7757–66.

DAVIS, J. J. & BENNETT, V. (1984) Brain ankyrin. Purification of a 72,000 Mr spectrin-binding domain. *Journal of Biological Chemistry* **259**, 1874–81.

DOBERSON, M. G., HAMMER, J. K., NORONHA, A. B., MACINTOSH, T. D., TRAPP, B. D., BRADY, R. O. & QUARLES, R. H. (1985) Generation and characterization of mouse monoclonal antibodies to the myelin-associated glycoprotein (MAG). *Neurochemical Research* **10**, 427–37.

EDELMAN, G. M. (1984) Modulation of cell adhesion during induction histogenesis, and perinatal development of the nervous system. *Annual Review of Neuroscience* **7**, 339–77.

GEIGER, B. (1983) Membrane–cytoskeleton interaction. *Biochimica et Biophysica Acta* **737**, 305–41.

GLENNEY, J. R. & GLENNEY, P. (1983a) Spectrin, fodrin and TW260/240: a family of related proteins lining the plasma membrane. *Cell Motility* **3**, 671–82.

GLENNEY, J. R., JR & GLENNEY, P. (1983b) Fodrin is the general spectrin-like protein found in most cells whereas spectrin and the TW protein have a restricted distribution. *Cell* **34**, 503–12.

GOODMAN, S. R., ZAGON, I. S., WHITFIELD, C. F., CASPRIA, L. A., MCLAUGHLIN, P. J. & LASHIEWICZ, T. L. (1983) A spectrin-like protein from mouse brain membranes: immunological and structural correlations with erythrocyte spectrin. *Cell Motility* **3**, 635–47.

GRIFFITHS, G., SIMONS, K., WARREN, G. & TOKUYASU, K. T. (1983) Immunoelectron microscopy using thin frozen sections: Application to studies of intracellular transport of Semliki Forest virus spike glycoproteins. In *Methods of Enzymology* (edited by FLEISHER, S. & FLEISCHER, B.), Vol. 96, pp. 435–50. New York: Academic Press.

HEMPERLY, J. J., MURRAY, B. A., EDELMAN, G. M. & CUNNINGHAM, B. A. (1986) Sequence of cDNA clone encoding the polysialic acid rich and cytoplasmic domains of the neural cell adhesion molecule N-CAM. *Proceedings of the National Academy of Sciences USA* **83**, 3337–41.

HOOD, L., KRONENBERG, M. & HUNKAPILLAR, T. (1985) T Cell antigen receptors and the immunoglobulin supergene family. *Cell* **40**, 225–9.

KOENIG, E. & REPASKY, E. (1985) A regional analysis of β-spectrin in the isolated Mauthner neuron and in isolated axons of the goldfish and rabbit. *Journal of Neuroscience* **5**, 705–14.

KRUSE, J., MAILHAMMER, R., WERNECKE, H., FAISSNER, A., SOMMER, I., GORIDIS, C. & SCHACHNER, M. (1984) Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1. *Nature* **311**, 153–5.

LAFOUNTAIN, J. R., JR, ZOBEL, C. R., THOMAS, H. R. & GALBREATH, C. (1977) Fixation and staining of F-actin and microfilaments using tannic acid. *Journal of Ultrastructure Research* **58**, 78–86.

LAI, C., BROW, M. A., NAVE, K.-A., NORONHA, A. B., QUARLES, R. H., BLOOM, F. E., MILNER, R. J. & SUTCLIFFE, J. G. (1987) Two forms of 1B236/myelin-associated glycoprotein (MAG), a cell adhesion molecule for postnatal neural development, are produced by alternative splicing. *Proceedings of the National Academy of Sciences USA* **84**, 4337–41.

LAI, C., WATSON, J. B., BLOOM, F. L., SUTCLIFFE, J. G. & MILNER, R. J. (1988) Neural protein 1B236/myelin-associated glycoprotein (MAG) defines a subgroup of the immunoglobulin superfamily. *Immunological Reviews* **100**, 127–49.

LEMKE, G. & AXEL, R. (1985) Isolation and sequence of a cDNA encoding the major structural protein of peripheral myelin. *Cell* **40**, 501–8.

LEVINE, J. & WILLARD, M. (1981) Fodrin: axonally transported polypeptides associated with the internal periphery of many cells. *Journal of Cell Biology* **90**, 631–43.

LOW, P. S. (1986) Structure and function of the cytoplasmic domain of band 3: center of erythrocyte membrane–peripheral protein interactions. *Biochimica et Biophysica Acta* **864**, 145–67.

MARTINI, R. & SCHACHNER, M. (1986) Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. *Journal of Cell Biology* **103**, 2439–48.

MAUPIN-SZAMIER, P. & POLLARD, T. D. (1978) Actin filament destruction by osmium tetroxide. *Journal of Cell Biology* **77**, 837–52.

MCGARRY, R. C., HELFAND, S. L., QUARLES, R. H. & RODER, J. C. (1983) Recognition of myelin-associated glycoprotein by the monoclonal antibody HNK-1. *Nature* **306**, 376–8.

MUGNAINI, E., OLSEN, K. K., SCHNAPP, B. & FRIEDRICH, V. L., JR (1977) Distribution of Schwann cell cytoplasm and plasmalemmal vesicles (caveolae) in peripheral myelin sheaths. An electron microscopic study with thin sections and freeze-fracturing. *Journal of Neurocytology* **6**, 647–68.

NELSON, W. J., COLACO, C. A. L. S. & LAZARIDES, E. (1983) Involvement of spectrin in cell-surface receptor capping. *Proceedings of the National Academy of Sciences USA* **80**, 1626–30.

NELSON, W. J. & LAZARIDES, E. (1983) Expression of the β subunit of spectrin in nonerythroid cells. *Proceedings of the National Academy of Sciences USA* **80**, 363–7.

PLOW, E. F., PIERSCHBACHER, M. D., RUOSLAHTI, E., MARGUERIE, G. A. & GINSBERG, M. H. (1985) The effect of Arg-Gly-Asp-containing peptides on fibrinogen and von Willebrand factor binding to platelets. *Proceedings of the National Academy of Sciences USA* **82**, 153–8.

POLLERBERG, G. E., BURRIDGE, K., KREBS, K. E., GOODMAN, S. R. & SCHACHNER, M. (1987) The 180-kD component of the neural cell adhesion molecule N-CAM is involved in cell–cell contacts and cytoskeleton–membrane interactions. *Cell and Tissue Research* **250**, 227–36.

POLLERBERG, G. E., SCHACHNER, M. & DAVOUST, J. (1986) Differentiation state-dependent surface mobilities of two forms of the neural cell adhesion molecule. *Nature* **324**, 462–5.

POLTORAK, M., SADOUL, R., KEILHAUER, G., LANDA, C., FAHRIG, T. & SCHACHNER, M. (1987) Myelin-associated

glycoprotein, a member of the L2/HNK-1 family of neural cell adhesion molecules,. is involved in neuron–oligodendrocyte and oligodendrocyte–oligodendrocyte interaction. *Journal of Cell Biology* **105**, 1893–9.

PYTELA, R., PIERSCHBACHER, M. D. & RUOSLAHTI, E. (1985) A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. *Proceedings of the National Academy of Sciences USA* **82**, 5766–70.

QUARLES, R. H. (1983/1984) Myelin-associated glycoprotein in development and disease. *Developmental Neuroscience* **6**, 285–303.

REPASKY, E. A., GRANGER, B. L. & LAZARIDES, E. (1982) Widespread occurrence of avian spectrin in nonerythroid cells. *Cell* **29**, 821–33.

RIEDERER, B. M., ZAGON, I. S. & GOODMAN, S. R. (1986) Brain spectrin (240/235) and brain spectrin (240/235E): Two distinct spectrin subtypes with different locations within mammalian neural cells. *Journal of Cell Biology* **102**, 2088–97.

RUOSLAHTI, E. & PIERSCHBACHER, M. D. (1986) Arg-Gly-Asp: a versatile cell recognition signal. *Cell* **44**, 517–8.

RUTISHAUSER, U. (1984) Developmental biology of a neural cell adhesion molecule. *Nature* **310**, 549–53.

SALZER, J. L., HOLMES, W. P. & COLMAN, D. R. (1987) The amino acid sequences of the myelin-associated glycoproteins: homology to the immunoglobin gene superfamily. *Journal of Cell Biology* **104**, 957–65.

SATIR, P. (1984) Cytoplasmic matrix: old and new questions. *Journal of Cell Biology* **99**, 225–38.

SLOT, J. W. & GEUZE, H. J. (1984) Gold markers for single and double immunolabeling of ultrathin cryosections. In *Immunolabeling for Electron Microscopy* (edited by POLAK, J. M. & VARNDELL, I. M.), pp. 129–42. Amsterdam: Elsevier.

STERNBERGER, L. A. & STERNBERGER, N. H. (1983) Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments *in situ*. *Proceedings of the National Academy of Sciences USA* **80**, 6126–30.

STERNBERGER, N. H., QUARLES, R. H., ITOYAMA, Y. & WEBSTER, H. DEF. (1979) Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rats. *Proceedings of the National Academy of Sciences USA* **76**, 1510–4.

STOSSEL, T. P. (1984) Contribution of actin to the structure of the cytoplasmic matrix. *Journal of Cell Biology* **99**, 15–21.

TAMKUN, J. W., DESIMONE, D. W., FONDA, D., PATEL, R. S., BUCK, C., HORWITZ, A. F. & HYNES, R. O. (1986) Structure of intergrin, a glycoprotein involved in the transmembrane.linkage between fibronectin and actin. *Cell* **46**, 271–82.

TOKUYASU, K. T. (1980) Immunocytochemistry on ultrathin frozen sections. *Histochemical Journal* **12**, 381–403.

TRAPP, B. D. (1988) Distribution of the myelin-associated glycoprotein during myelin composition in quaking mouse peripheral nerve. *Journal of Cell Biology* **107**, 675–85.

TRAPP, B. D., BERNIER, L., ANDREWS, S. B. & COLMAN, D. R. (1988) Cellular and subcellular distribution of 2′, 3′-cyclic nucleotide 3′-phosphodiesterase and its mRNA in the rat central nervous system. *Journal of Neurochemistry* **51**, 859–68.

TRAPP, B. D., GRIFFIN, J. W., WONG, A., O'CONNELL, M. & ANDREWS, S. B. (1985) Localization of F-actin in one micron frozen sections of myelinated peripheral nerve. *Journal of Cell Biology* **101**, part 2: 33a (abstract).

TRAPP, B. D., ITOYAMA, Y., STERNBERGER, N. H., QUARLES, R. H. & WEBSTER, H. DEF. (1981) Immunocytochemical localization of P_0 protein in Golgi complex membranes and myelin of developing rat Schwann cells. *Journal of Cell Biology* **90**, 1–6.

TRAPP, B. D., MCINTYRE, L. J., QUARLES, R. H., STERNBERGER, N. H. & WEBSTER, H. DEF. (1979) Immunocytochemical localization of rat peripheral nervous system myelin proteins: P_2 protein is not a component of all peripheral nervous system myelin sheaths. *Proceedings of the National Academy of Sciences USA* **76**, 3552–6.

TRAPP, B. D., O'CONNELL, M. F. & ANDREWS, S. B. (1986) Ultrastructural immunolocalization of MAG and P_0 proteins in cryosections of peripheral nerve. *Journal of Cell Biology* **103**, part 2: 228a (abstract).

TRAPP, B. D. & QUARLES, R. H. (1982). Presence of the myelin-associated glycoprotein correlates with alterations in the periodicity of peripheral myelin. *Journal of Cell Biology* **92**, 877–82.

TRAPP, B. D. & QUARLES, R. H. (1984) Immunocytochemical localization of the myelin-associated glycoprotein: fact or artifact? *Journal of Neuroimmunology* **6**, 231–49.

TRAPP, B. D., QUARLES, R. H. & GRIFFIN, J. W. (1984a) Myelin-associated glycoprotein and myelinating Schwann cell–axon interaction in chronic B, B′-iminodipropionitrile neuropathy. *Journal of Cell Biology* **98**, 1272–8.

TRAPP, B. D., QUARLES, R. H. & SUZUKI, K. (1984b) Immunocytochemical studies of quaking mice support a role for the myelin-associated glycoprotein in forming and maintaining the periaxonal space and periaxonal cytoplasmic collar of myelinating Schwann cells. *Journal of Cell Biology* **99**, 594–606.

WEBSTER, H. DEF., PALKOVITS, C. G., STONER, G. L., FAVILLA, J. T., FRAIL, D. E. & BRAUN, P. E. (1983) Myelin-associated glycoprotein: electron microscopic immunocytochemical localization in compact developing and adult central nervous system myelin. *Journal of Neurochemistry* **41**, 1469–79.

WONG, A. & GRIFFIN, J. (1982) Actin localization in Schwann cells of myelinated nerve fibers. *Annals of Neurology* **12**, 106 (abstract).

WULF, E., DEBOBEN, A., BAUTZ, F. A., FAULSTICH, H. & WIELAND, T. (1979) Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proceedings of the National Academy of Sciences USA* **76**, 4498–502.

ZAGON, I. S., HIBEE, R., RIEDERER, B. M. & GOODMAN, S. R. (1986) Spectrin subtypes in mammalian brain: an immunoelectron microscopic study. *Journal of Neuroscience* **6**, 2977–86.