Heterophilic Binding of L1 on Unmyelinated Sensory Axons Mediates Schwann Cell Adhesion and Is Required for Axonal Survival

C.A. Haney,*‡ Z. Sahenk,§ C. Li,‖ V.P. Lemmon,* J. Roder,‖ and B.D. Trapp*‡

*Department of Neuroscience, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106; ‡Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195; §Department of Neurology, Neuromuscular Disease Center, College of Medicine, Ohio State University, Columbus, Ohio 43210; and ‖Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada

Abstract. This study investigated the function of the adhesion molecule L1 in unmyelinated fibers of the peripheral nervous system (PNS) by analysis of L1-deficient mice. We demonstrate that L1 is present on axons and Schwann cells of sensory unmyelinated fibers, but only on Schwann cells of sympathetic unmyelinated fibers. In L1-deficient sensory nerves, Schwann cells formed but failed to retain normal axonal ensheathment. L1-deficient mice had reduced sensory function and loss of unmyelinated axons, while sympathetic unmyelinated axons appeared normal. In nerve transplant studies, loss of axonal-L1, but not Schwann cell-L1, reproduced the L1-deficient phenotype. These data establish that heterophilic axonal-L1 interactions mediate adhesion between unmyelinated sensory axons and Schwann cells, stabilize the polarization of Schwann cell surface membranes, and mediate a trophic effect that assures axonal survival.

Key words: cell adhesion • cell polarity • axonal degeneration • L1 • myelin-associated glycoprotein

Cell–cell adhesion is critical to the development and maintenance of the three dimensional structure of the nervous system. This maintenance is regulated via transmembrane cell adhesion molecules that usually use their intracellular domain to anchor to the cytoskeleton and their extracellular domain to interact with other adhesion or matrix molecules. L1 is a cell adhesion molecule that is expressed on the surface of developing axons, growth cones, and Schwann cells of unmyelinated fibers (Martini and Schachner, 1986). L1 has six immunoglobulin-like domains, five fibronectin type III repeats, a transmembrane region, a cytoplasmic carboxyl tail (Moos et al., 1988), and a molecular mass of ~200 kD. Two alternatively spliced variants have been identified (Miura et al., 1991). Full-length L1 mRNA is found in neurons and the shorter isoform lacking 15 bp in the NH_2-terminal and 12 bp that codes for an RSLE sequence in the cytoplasmic tail is found in nonneuronal cells including Schwann cells and leukocytes. L1 is highly conserved in mammals: human, rat, and mouse L1 share 100% amino acid identity in their cytoplasmic region and between 80–95% identity in the extracellular domains (Hlavin and Lemmon, 1991). Othologous molecules in other species include NgCAM/8D9/G4 (chick) (Lemmon and McLoon, 1986), E587 (goldfish) (Bastmeyer et al., 1995), L1.1 and L1.2 (zebrafish) (Tongiorgi et al., 1995), and neuroglian (*Drosophila*) (Bieber et al., 1989). Such high amino acid conservation during evolution suggests an important functional role for this adhesion molecule.

L1 homophilic and heterophilic interactions can mediate neurite outgrowth (Lagenaur and Lemmon, 1987; Lemmon et al., 1989) or facilitate axon-axon (Stallcup and Beasley, 1985) or axon-Schwann cell adhesion (Seilheimer et al., 1989; Wood et al., 1990). Homophilic L1 interactions on growth cones in vitro activate the FGF receptor which in turn initiates arachidonic acid production, Ca^{2+} influx, cytoskeletal rearrangements, and neurite outgrowth (Williams et al., 1994). The cytoplasmic domain of L1 can bind ankyrin (Davis and Bennett, 1994) and may provide a mechanism for transducing extracellular signals and the dynamic cytoskeletal rearrangements required for cell migration (Burden-Gulley et al., 1997). Homophilic L1 interactions also help bundle axons into discrete fiber tracts (Landmesser et al., 1988). L1 heterophilic interactions with TAG-1/axonin-1, DM1-GRASP, and the $\alpha_v\beta_3$ integrin have also been implicated in neurite outgrowth (Kuhn et al., 1991; DeBernardo and Chang, 1996) or cell migration (Montgomery et al., 1996), however, the downstream mechanisms of these associations are not understood. The

Address correspondence to Bruce D. Trapp, Department of Neurosciences, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195. Tel.: (216) 444-7177. Fax: (216) 444-7927. E-mail: trappb@ccf.org

chondroitan sulfate proteoglycans, neurocan and phosphocan, inhibit L1-mediated neurite outgrowth (Friedlander et al., 1994; Milev et al., 1994). Homophilic interactions between nonneuronal and neuronal L1 promote cell–cell adhesion in vitro (Takeda et al., 1996). Therefore, L1 appears to mediate both migration and adhesion and these differential functions depend upon isoform specificity, membrane location (Takeda et al., 1996; Kamiguchi and Lemmon, 1998), and specificity of homophilic or heterophilic binding partners.

Ensheathment of axons by Schwann cells is essential for normal neuronal function and is mediated by specialized molecules located in the adaxonal Schwann cell membrane and axolemma. Axons require glial trophic support to maintain normal caliber and cytoskeletal architecture, normal axonal transport, and axonal viability (deWaegh et al., 1992; Hsieh et al., 1994; Sanchez et al., 1996; Yin et al., 1998). The myelin-associated glycoprotein (MAG)[1], another member of the immunoglobulin (Ig) superfamily enriched in Schwann cells at the myelin-axon interface (Trapp and Quarles, 1982; Trapp et al., 1989) is essential for normal axon maintenance (Yin et al., 1998). In MAG-deficient mice, PNS axons were myelinated normally (Li et al., 1994; Montag et al., 1994) but showed a progressive axonopathy and axonal degeneration (Yin et al., 1998). Adult unmyelinated axons may be maintained through a similar mechanism involving L1.

The purpose of this study was to investigate the function of L1 in the adult PNS by analysis of L1-deficient and L1/MAG-deficient mice. Myelination proceeded normally in the L1/MAG-deficient animals indicating that neither molecule is essential for axonal ensheathment and spiral wrapping of myelin membranes. In mature L1-deficient mice, Schwann cells fail to maintain ensheathment of unmyelinated sensory axons but not unmyelinated sympathetic axons. Furthermore, our studies established that axonal-L1 maintains Schwann cell ensheathment of adult sensory unmyelinated axons by heterophilic binding mechanisms and that loss of axonal-L1 resulted in axonal degeneration.

Materials and Methods

Generation of Knockout Mice

L1-deficient mice are described in Cohen et al. (1998). In brief, a targeting construct with 5.5 kb of homology with the L1 locus, a thymidine kinase gene and a neomycin resistance gene was used to disrupt the endogenous L1 gene at exon 13 in SV-129 mice. Mice were genotyped by Southern blot analysis of tail DNA. 0.6 kb of L1 cDNA was used as a probe and detected an 11-kb DNA fragment in wild-type and an 8-kb DNA fragment in L1-deficient mice when digested with EcoR1 restriction enzyme.

MAG-deficient mice are described in Li et al. (1994). In brief, a targeting construct with 7.5 kb of homology with the MAG locus, a neomycin resistance gene, and the thymidine kinase gene was used to disrupt the endogenous MAG gene at exon 5 in the 129 × CD1 mouse strain. Southern blot analysis was used to identify the MAG genotype. A 1.6-kb region of the MAG cDNA was used as a probe and detected a 7.5- and a 5.5-kb DNA fragment from wild-type mice and a 5.5- and a 4.5-kb DNA fragment from MAG-deficient mice when digested with BamH1-HindIII restriction enzymes.

Southern Blot Analysis

Genomic DNA was isolated from 1 cm of mouse tail by phenol/chloroform extraction. 20 μg of genomic DNA was digested with restriction enzymes (Boehringer-Mannheim) and run on an 0.8% agarose gel. The DNA was transferred to a Zeta Probe-GT membrane (Bio-Rad Laboratories) and probed with the respective ^{32}P-labeled cDNAs using High Prime (Boehringer-Mannheim). Hybridization was performed at 65°C for 18 h in the presence of 0.5 M $NaPO_4$, 7% SDS, 1% BSA, 1 mM EDTA, 200 μg/ml denatured salmon sperm DNA, and ^{32}P-radiolabeled probe. Blots were washed at 65°C for 1 h with 40 mM $NaPO_4$, 5% SDS, 0.5% BSA, and 1 mM EDTA and exposed to Kodak X-omatAR film with intensifying screens (48 h).

Western Blot Analysis

P60 control, L1-deficient, MAG-deficient, and L1/MAG-deficient mice were killed by cervical dislocation. Brain and sciatic nerves were harvested immediately, placed in 4% SDS, and dissociated with a Polytron homogenizer. 20 μg of CNS and PNS protein were run on 4–20% SDS–polyacrylamide gradient gels (Novex) and transferred to PVDF membrane (Amersham). Membranes were pretreated with 10% nonfat dry milk and 0.1% Tween-20 in PBS for 30 min at 4°C and then transferred to a solution containing primary antibody, 5% nonfat dry milk, 0.1% Tween-20 in PBS, and incubated for 18 h at 4°C. Blots were washed and incubated in appropriate horseradish peroxidase–conjugated secondary antibodies (Amersham) at a dilution of 1:1,000. Labeled protein was detected with an enhanced chemiluminescence kit (ECL; Amersham) according to the manufacturer's directions on Kodak BiomaxMR film. The MAG (Fujita et al., 1990) and L1 (Brittis et al., 1995) antibodies used to assay protein expression are well characterized.

Electron Microscopy

P7 and P60 control, L1-deficient, MAG-deficient, and L1/MAG-deficient mice were perfused intracardially with 4% paraformaldehyde, 2.5% glutaraldehyde, and 0.08 M Sorenson's phosphate buffer. Lumbar dorsal roots 4 and 5 (L4, L5), sciatic nerve, sural nerve, superior cervical ganglia (SCG), and cervical sympathetic trunk (CST) were dissected and post-fixed for 1 h. The tissue was then processed for electron microscopy by standard procedures and embedded in Epon (EMS). Ultrathin sections (80 nm thick) were placed on slot grids (EMS), stained with lead citrate and uranyl acetate, and examined in a Philips 100CX electron microscope. Montages of the entire L4 and L5 dorsal roots were constructed from electron micrographs printed at a final magnification of 4,200×. Total unmyelinated axons were quantified in L4 and in L5 dorsal roots from five control, four L1-deficient, and four L1/MAG-deficient mice. The data was analyzed by the Student's *t* test.

Von Frey Pressure Test

The von Frey test uses Semmes Weinstein Monofilaments (Stoelting Co.) to measure skin sensitivity to an applied pressure. This test is used in clinical neurology to assess light touch and deep pressure cutaneous sensation. Unmyelinated C fibers contribute to these sensations as well as deep burning pain, extreme cold and heat, and crude touch (Omer and Bell-Krotoski, 1998). The monofilament will exert an increasing pressure until it begins to bend. Once bending occurs, a constant force is applied to the region, which allows for a reproducible force level for each filament tested. The filaments give a linear scale of perceived intensity and correlate to a log scale of actual grams of force. To administer the test a mouse is scruffed and turned upside down to allow accessibility to the hind paws. A filament is then used to touch the glabrous region of the paw 10 times in 10 s. A response to any of the 10 monofilament applications (toe curling, paw withdrawal) is scored as a positive sensory response. The data was analyzed by the Student's *t* test.

Immunocytochemistry

P60 wild-type and L1-deficient mice were perfused intracardially with 4% paraformaldehyde. The dorsal roots, dorsal root ganglia (DRG), sciatic nerves and SCG's were removed and cryoprotected in 2.3 M sucrose and 30% polyvinylpyrrolidone. 1-μm cryosections were cut on a Reichart Ul-

1. *Abbreviations used in this paper:* CST, cervical sympathetic trunk; DRG, dorsal root ganglia; L4, lumbar dorsal root 4; L5, lumbar dorsal root 5; MAG, myelin-associated glycoprotein; PNS, peripheral nervous system; SCG, superior sympathetic ganglia.

tracutS (Leica), placed on slides, and incubated in the following solutions: primary antibody overnight at 4°C, biotinylated secondary antibodies (1:500), Avidin/Biotin Complex (1:1,000) (both from Vector Laboratories), 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and 0.4% osmium tetroxide (EMS). Tissue used for teased fiber preparations was postfixed for one hour in 4% paraformaldehyde, separated in 1% Triton X-100 with teasing needles, treated with Triton X-100 overnight at 4°C, incubated in primary antibody for 48 h at 4°C, and stained as described above. Tissue used for free floating sections was postfixed for 1 h after perfusion, cryoprotected in 20% glycerol overnight, and sectioned at a thickness of 20 μm on a Zeiss freezing sliding microtome. Tissue was incubated in primary antibody for 48 h at 4°C and then stained as described above or by immunofluorescent procedures. Sections processed for double-labeling were incubated in both fluorescein-conjugated donkey anti–mouse and Texas red donkey anti–rabbit (Vector Laboratories) secondary antibodies at 1:500 and mounted in Vectashield mounting media (Vector Laboratories).

Antibodies

The polyclonal L1 anti-sera (Brittis et al., 1995) was used at a concentration of 1:2,000 for Western blots and 1:6,000 for immunocytochemistry. MAG polyclonal antisera (Fujita et al., 1990) was used at a concentration of 1:10,000 for both Western blots and immunocytochemistry. The monoclonal CGRP was purchased from Research Biochemicals International and used at a concentration of 1:1,000. The nonphosphorylated neurofilament (SMI-32) was purchased from Sternberger Monoclonals and used at a concentration of 1:15,000.

Sciatic Nerve Transplants

Sciatic nerves segments (4 mm long) were removed from control or L1-deficient mice and sutured into the sciatic nerve of wild-type, L1-deficient, or nude mice as described previously (Sahenk et al., 1999; Sahenk and Chen, 1998). Surgery was performed under sterile conditions and the mice were housed in a sterile environment until they were killed at 60 d after transplantation. No immunosuppression was necessary. For wild-type or L1-deficient recipient mice, cyclosporin A (Sandoz) was injected daily at a dose of 17 mg/kg. This dosage was shown previously to immunosuppress without affecting nerve regeneration (Grochowicz et al., 1985; Yu et al., 1990). Four different transplant paradigms were performed. Control or L1-deficient donor nerves were transplanted into nude mice. Control nerves were transplanted into control or L1-deficient mice (both of the SV-129 strain). At 60 d after surgery, the sciatic nerves were harvested and immersed in 3% glutaraldehyde for 30 min. The transplanted portion of the nerve was identified by the sutures used to secure the end-to-end anastomosis. In reference to the transplanted portions, the regions of the sciatic nerve are designated proximal, transplant, and distal. Each portion of the nerve was cut into an ~2-mm portion, postfixed for 3 h and embedded in Epon for electron microscopic analysis as described above. 1-μm and ultrathin sections were obtained from the middle of each nerve segment (see Fig. 6 D, arrowheads). Unmyelinated axons were analyzed by transmission electron microscopy in all three regions of each transplanted nerve ($n > 3$) and were scored as either greater than one-half ensheathed or less than one-half ensheathed by Schwann cells. In each segment from each transplant paradigm 300 to 1,100 axons were examined and the data was analyzed by the Student's *t* test.

Results

Biochemical Analysis of L1-deficient mice

Mice deficient for both MAG and L1 were generated by initially breeding SV-129 L1 heterozygote females ($-_x/+_x$) with 129 × CD1 MAG-deficient (−/−) males. From these breedings, female L1 heterozygotes ($-_x/+_x$)/MAG heterozygotes (+/−) were identified and bred to MAG-deficient (−/−) males. Female L1 heterozygotes ($-_x/+_x$)/MAG-deficient (−/−) mice were identified and bred with MAG-deficient males to produce L1/MAG-deficient males ($-_x$/ y for the L1 gene and −/− for the MAG gene). MAG-deficient, L1-deficient, and L1/MAG-deficient mice were identified in Southern blots probed with MAG and L1 cDNAs (Fig. 1 A). When probed with the L1 cDNA, wild-type and MAG-deficient DNA (digested with EcoR1) contained an 11-kb band while DNA from L1-deficient and L1/MAG-deficient males contained a band at 8 kb. L1 heterozygote females had bands at both 11 and 8 kb. Wild-type and L1-deficient DNA (digested with BamHI and HindIII) contained two MAG bands (7.5 and 4.5 kb). The MAG-deficient and L1/MAG-deficient mice showed a diagnostic 5.5-kb band as well as the 4.5-kb band. DNA from L1 heterozygote/MAG-deficient females also contained bands at 5.5 and 4.5 kb. The absence of L1 and MAG protein in the respective null mice was confirmed by Western blot analysis of CNS and PNS protein extracts (Fig. 1 B). MAG antibodies detected an appropriate band at 100 kD only in wild-type and L1-deficient mice. L1 antibodies detected a band of ~200 kD only in wild-type and MAG-deficient mice. A large percentage of the L1-deficient and L1/MAG-deficient males were runted from birth and remained smaller than the male littermates until approximately one month of age.

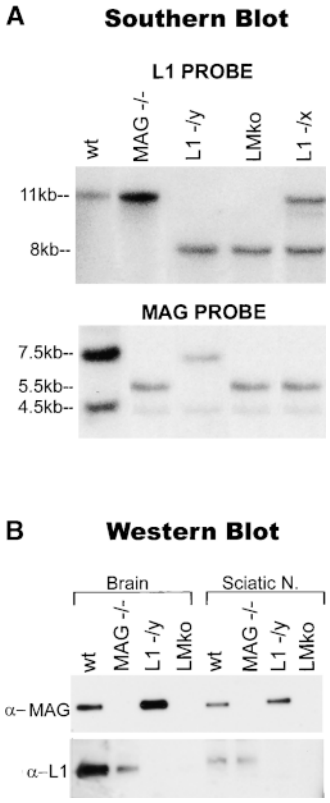

Figure 1. L1 and MAG gene expression in transgenic mice. (A) Southern blot analysis of MAG and L1-deficient mice. The L1 probe (upper panel) detected an 11-kb fragment in wild-type (wt) and MAG-deficient (MAG−/−) mice, and an 8-kb fragment in L1-deficient (L1 −/y) and L1/MAG-deficient (LMko) mice, (EcoR1 cut). The MAG probe (lower panel) detected a 7.5- and a 4.5-kb fragment in wild-type and L1-deficient mice, and a 5.5- and a 4.5-kb fragment in MAG- and L1/MAG-deficient mice (BamHI-HindIII cut). L1 heterozygous (L1 −/x)/MAG-deficient (−/−) females were used as breeders for the L1/MAG-deficient line and showed an 11- and 8-kb band when probed for L1, and a 5.5- and 4.5-kb band when probed for MAG. (B) Western blot analysis of CNS and PNS protein extracts from wild-type, MAG-deficient, L1-deficient, and MAG/L1-deficient mice. MAG antibodies detected a 100-kD band in wild-type and L1-deficient mice. MAG immunoreactivity was not detected in MAG- or L1/MAG-deficient mice. L1 antibodies detected a 200-kD band in wild-type and MAG-deficient mice. L1 immunoreactivity was not detected in L1- or L1/MAG-deficient mice.

L1-Deficient Mice Show Altered Unmyelinated Fibers in Sensory but Not Sympathetic Nerve

The ultrastructural appearance of unmyelinated fibers was

compared in the sciatic nerve and dorsal roots of P60 L1-deficient and wild-type mice. As previously described (Peters et al., 1991), nonmyelinating Schwann cells in wild-type sciatic nerve ensheathed individual axons in separate cytoplasmic troughs (data not shown). In P60 dorsal roots from wild-type mice, many unmyelinated axons still remained in polyaxonal Schwann cell pockets (Fig. 2 A, arrowheads). Unmyelinated fibers in MAG-deficient dorsal roots had a similar ultrastructure as the unmyelinated fibers in wild-type dorsal root (data not shown). In L1-deficient dorsal roots examined at P60, many unmyelinated axons were not surrounded or were partially surrounded by Schwann cell processes. Unensheathed axons often contained fragments of basal lamina on their surface (Fig. 2, B–D, arrows) indicating former Schwann cell ensheathment. Evidence of ongoing axonal degeneration including axonal swelling, dissociation of microtubules and neurofilaments, and clumping of axonal contents (Dyck and Hopkins, 1972) was apparent in the L1-deficient peripheral nerves (Fig. 2 D, asterisk). Small diameter axons without Schwann cells often abutted the basal lamina of myelinated fibers, and nonmyelinating Schwann cells extended processes passing near but not surrounding many naked axons (Fig. 2 D, arrowheads). The basal lamina was often discontinuous on the surface of these L1-deficient nonmyelinating Schwann cell processes. Lateral adhesion between adjacent Schwann cells of unmyelinated fibers was also disrupted (data not shown). Similar phenotypes were also present in sciatic and sural nerves of L1 and L1/MAG-deficient mice. Myelinated fibers in the L1-deficient mouse appeared similar to those in wild-type mice. Myelin membranes were tightly compacted and the periaxonal space was appropriately maintained at 12–14 nm. Myelination proceeded normally in the L1/MAG-deficient mice and fibers analyzed at P60 showed MAG-deficient phenotypes (Yin et al., 1998) in the myelinated fibers and the L1-deficient phenotypes in the unmyelinated fibers.

Unmyelinated fibers in the cervical sympathetic trunk (CST), a predominantly unmyelinated autonomic nerve were also analyzed. Wild-type nonmyelinating Schwann cells of the CST engulf single axons in cytoplasmic troughs. At P60, the ultrastructure of wild-type (Fig. 3 A) and L1-deficient (Fig. 3 B) CST unmyelinated fibers was similar.

Development of Somatic Nerves

Because L1 may function in neural migration and axon guidance, the phenotype associated with the L1-deficient unmyelinated fibers could be related to a developmental abnormality. This possibility was investigated by analysis of young L1-deficient mice. In electron micrographs of

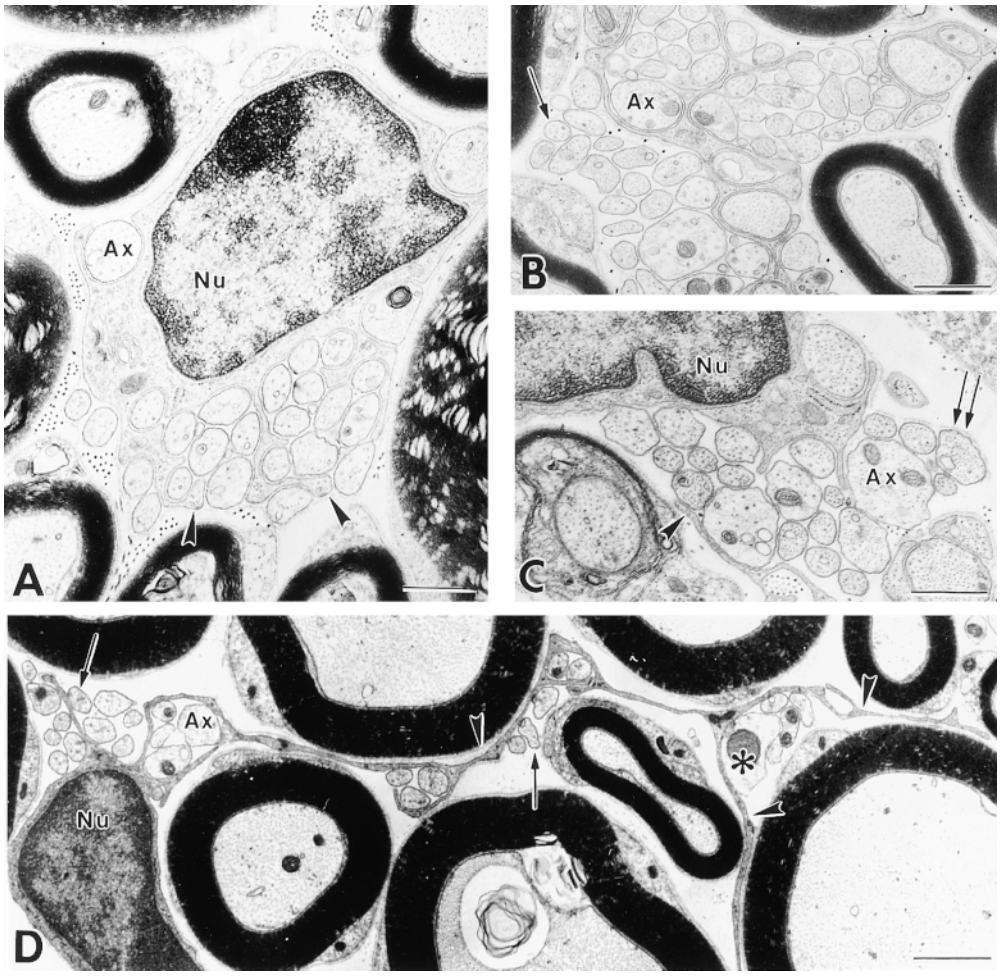

Figure 2. Nonmyelinating Schwann cells fail to maintain ensheathment of P60 L1-deficient sensory axons. In electron micrographs of wild-type (A) dorsal roots, most unmyelinated axons were surrounded by Schwann cells (A, arrowheads). In L1-deficient dorsal roots (B and D) many unmyelinated axons were not surrounded by Schwann cell processes (B and D, arrows), although some were partially surrounded by a basal lamina (C, double arrows). L1-deficient nonmyelinating Schwann cell processes often came into contact but did not ensheathe (D, arrowheads) small diameter axons. Some axons showed signs of degeneration (D, asterisk). Similar abnormalities were present in nonmyelinated fibers in MAG/L1-deficient dorsal root (C). Ax, Axon; Nu, Nucleus. Bars, 1 μm.

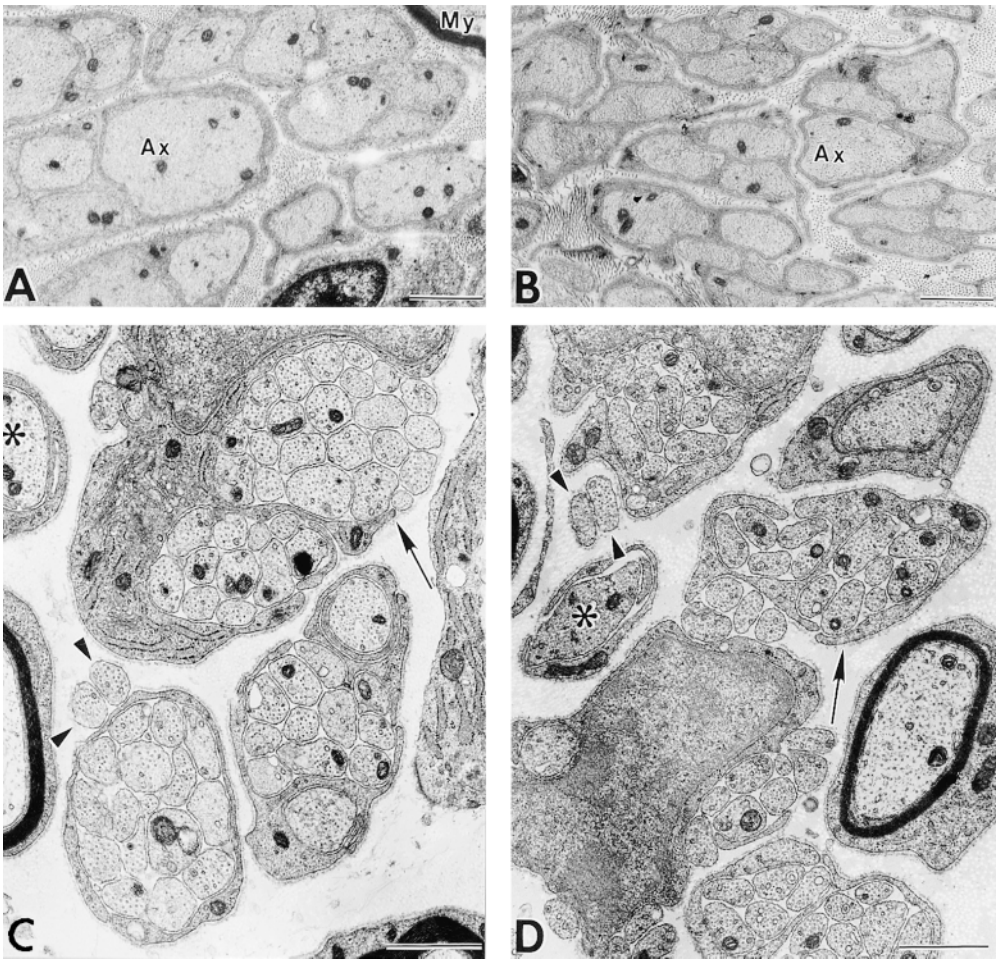

Figure 3. Unmyelinated fibers in adult L1-deficient sympathetic nerves and P7 L1/MAG-deficient sural nerve appear normal. In the cervical sympathetic trunk (CST), an unmyelinated autonomic nerve, from wild-type (A) and L1-deficient mice (B), axons were normally segregated and surrounded by Schwann cell processes. In electron micrographs of sural nerves from P7 wild-type (C) and L1/MAG-deficient mice (D), Schwann cells surround multiple axons (C and D, arrows). Occasional axons are ensheathed by fragments of basal lamina (C and D, arrowheads) and many axons have segregated into one-to-one relationships with Schwann cells (C and D, asterisks). Sural nerves, sciatic nerves, and dorsal roots from P7 L1-deficient mice displayed none of the unmyelinated fiber phenotypes present in adult L1-deficient nerves. Ax, Axon; My, Myelinated fiber. Bars, 1 μm.

sural nerves from P7 mice, the ultrastructural relationship between Schwann cells and axons in wild-type (Fig. 3 C) and L1/MAG-deficient mice (Fig. 3 D) was identical and there was no apparent loss of unmyelinated axons in the L1/MAG-deficient mice. At P14, some small diameter axons were not surrounded by Schwann cell processes and by P28 clusters of small diameter naked axons (data not shown) were present in L1- and L1/MAG-deficient mice.

Decreased Unmyelinated Sensory Axons and Pressure Sensation in L1-deficient Mice

Ultrastructural changes in L1-deficient unmyelinated axons were consistent with ongoing axonal degeneration. To extend this observation, the number of nonmyelinated axons was quantified in electron micrograph montages of the entire fourth and fifth lumbar dorsal roots from five wild-type, four L1-deficient, and four MAG/L1-deficient mice (Fig. 4 A). Lumbar dorsal roots 4 and 5 (L4 and L5) from L1-deficient and L1/MAG-deficient mice showed a 30 and 35% decrease of unmyelinated axons compared with wild-type L4 and L5 dorsal roots. This difference was statistically significant ($P < 0.01$) using the Student's *t* test. Myelinated fiber number was similar in control, L1-deficient, and L1/MAG-deficient dorsal roots (data not shown), indicating that the loss of unmyelinated axons is not a result of an increase in myelinated fibers. In addition, the number of nonmyelinating Schwann cell nuclei per unit area was not significantly different between control and L1-deficient mice (data not shown).

Loss of sensory axons could result from axonal degeneration, neuronal degeneration, or both. Loss of sensory neuronal perikarya is preceded by cytoplasmic vacuolation, followed by cell necrosis, neuronophagia, and proliferation of satellite cells resulting in residual nodules of Nageotte (Thomas et al., 1984). In L1-deficient P60 L4 and L5 dorsal root ganglion, pathological evidence of small sensory neuron degeneration was not detected indicating a primary axonal degeneration. Loss of axons without neuronal degeneration is consistent with early stages of a dying-back axonopathy.

Possible sensory deficiencies due to the loss of unmyelinated axons was investigated by the von Frey Pressure Test (Fig. 4 B) which measures the perception of a predetermined applied force to the skin. The glabrous skin of the hind paws of P60 wild-type ($n = 9$) and L1-deficient mice ($n = 7$) was tested three times each to assess the response to different diameter monofilaments. All wild-type mice responded to 1.02 *g* of force by toe curling and paw withdrawal. The L1-deficient mice responded to monofilament forces between 2.041 and 11.749 grams of pressure. This difference was statistically significant ($P < 0.015$) using the Student's *t* test.

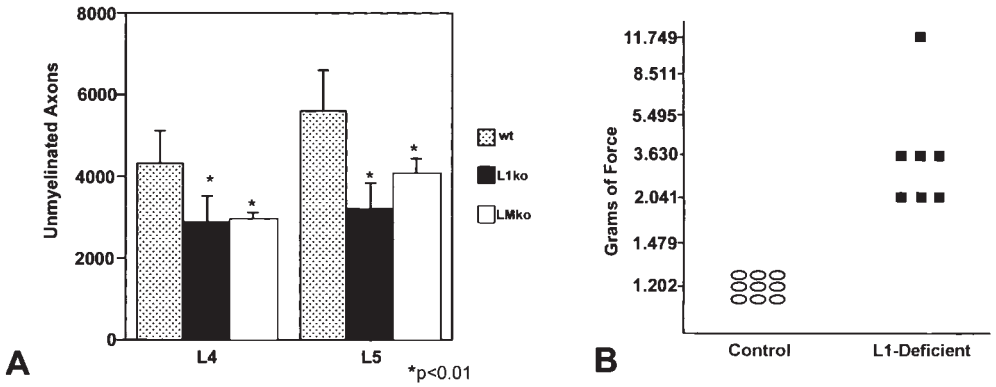

Figure 4. Loss of L1 results in decreased sensory axons and reduced sensory function. (A) All unmyelinated axons in the fourth and fifth lumbar dorsal roots of five control, four L1-deficient, and four L1/MAG-deficient mice were counted in electron micrograph montages (×4200). Compared with control nerves, a 30–35% decrease in the number of unmyelinated axons was detected in L4 and L5 dorsal roots from L1-deficient and L1/MAG-deficient mice. *$P < 0.01$ according to the Student's *t* test. (B) The von Frey pressure test used the Semmes-Weinstein monofilaments to assay sensitivity to deep pressure. All control mice ($n = 9$) responded to the monofilament corresponding to 1.202 grams of force. In contrast, the L1-deficient mice ($n = 7$) responded to monofilament force ranging from 2.041–11.749 grams of pressure. *$P < 0.015$ according to the Student's *t* test.

L1 Distribution in the Adult Peripheral Nervous System

To investigate if the different phenotype in sensory and sympathetic unmyelinated fibers was due to differential expression of L1, the immunocytochemical localization of L1 was compared in dorsal roots and cervical sympathetic trunks from P60 wild-type mice. Unmyelinated fibers in teased fiber preparations from dorsal roots were positive for L1 (Fig. 5 A, arrows). L1 immunoprecipitate was clearly delineated in the nonmyelinating Schwann cells. In transverse one micron thick cryosections from P60 wild-type mouse sciatic nerve L1 antibodies also stained the Schwann cells of unmyelinated fibers (Fig. 5 B, arrows). However, the optical resolution of immunostaining in these preparations was insufficient to determine whether L1 was also present in the small diameter axons. Myelinated fibers (Fig. 5 B, arrowheads) were not labeled with the L1 antibody. Exclusive detection of L1 in unmyelinated fibers in the PNS was consistent with previous reports (Martini and Schachner, 1986).

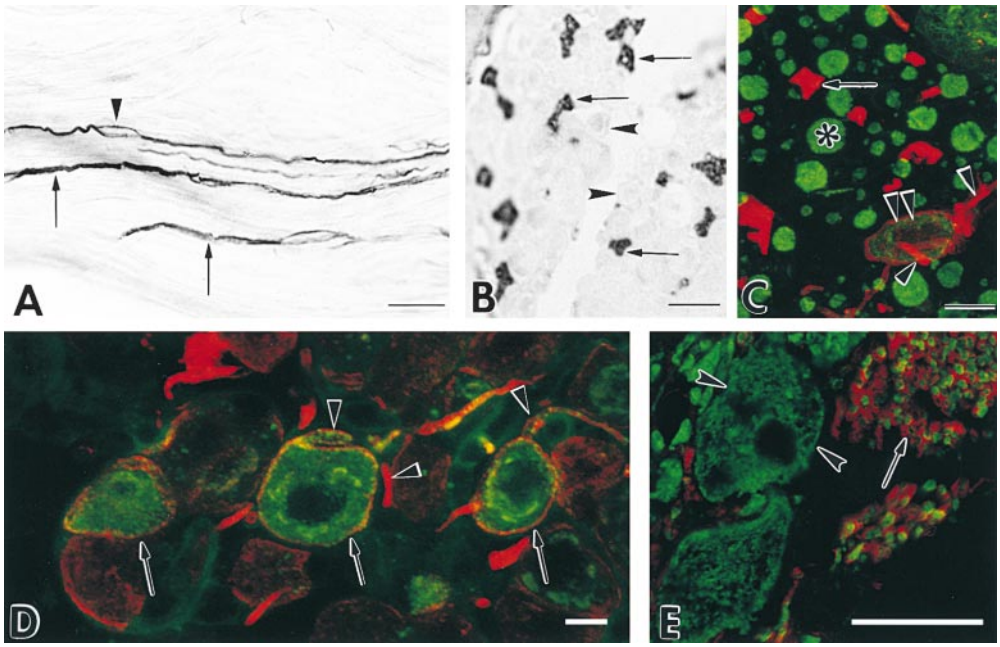

Figure 5. L1 is expressed by adult unmyelinated sensory but not sympathetic axons. In teased nerve fibers from P60 wild-type mouse dorsal root (A), L1 antibodies selectively stained unmyelinated fibers. L1 immunoreactivity surrounds the nucleus of a nonmyelinating Schwann cell (A, arrowhead) and confirms L1 expression by Schwann cells (A, arrows). L1 immunoreactivity in transverse cryosections of P60 wild-type nerve shows typical appearance of nonmyelinated fibers (B, arrows). Myelinated fibers (B, arrowheads) were not labeled with the L1 antibody. Sections (20 μm thick) from dorsal root ganglia (DRG) were double-labeled for L1 (C, red) and nonphosphorylated neurofilament (C, green). The plasma membrane (C, double arrowhead) and axon (C, arrowhead) of the small diameter neurons and the unmyelinated fibers (C, arrow) were L1-positive. Large diameter myelinated axons (C, asterisk) were labeled by neurofilament but not L1 antibodies. In sections (20 μm thick) of P60 DRG, small diameter neurons (D, arrows) were double-labeled for L1 (D, red) and Calcitonin gene-related product (CGRP) (D, green). The axons of these neurons were also L1-positive (D, arrowheads). In sections (20 μm thick) of P60 mouse superior cervical ganglia (SCG), neurons (E, arrowheads) and the axons exiting neuronal cell bodies were positive for neurofilament (green) but not for L1 (red). The nonmyelinating Schwann cells surrounding the SCG axons were L1-positive (E, arrow). Bars: (A) 30 μm; (B–D) 10 μm; (E) 25 μm.

To determine the distribution of L1 on sensory and sympathetic neurons and unmyelinated axons, free floating sections (20 μm thick) of P60 mouse DRG and SCG were double-labeled with L1 and neurofilament antibodies and analyzed by confocal microscopy. Neurofilament antibodies stained large diameter myelinated axons and neuronal perikarya (Fig. 5 C, green). L1 antibodies labeled the surface of small diameter neurons (Fig. 5 C, double arrowhead), the unmyelinated portion of axons as they exit and encircle small dorsal root neurons (Fig. 5 C, single arrowhead), and unmyelinated fibers (Fig. 5 C, arrows). Small diameter axons of the unmyelinated fibers were also stained for neurofilament, but at this magnification they were not resolvable against the intense L1-staining. Consistent with previous results (Faissner et al., 1984), L1 was not detected on the satellite cells surrounding the DRG neuronal cell bodies. L1-positive DRG neuronal perikarya were of small diameter (between 20–25 μm), a characteristic feature of neurons that give rise to unmyelinated axons. To extend this observation, we immunostained DRG sections for L1 and calcitonin gene-related product (CGRP), a neuropeptide in neurons that give rise to unmyelinated axons (Hokfelt et al., 1992). L1 was detected on the surface and axons of CGRP-positive small diameter neurons (Fig. 5 D), but not large diameter CGRP-positive neurons. These data locate L1 on both Schwann cells and axons of unmyelinated sensory fibers. The nonmyelinating Schwann cells that surround SCG axons were also L1-positive (Fig. 5 E). However, L1 was not detected on the surface of SCG neurons (Fig. 5 E) or axons leaving their cell bodies. Therefore, L1 is located on Schwann cells but not axons in unmyelinated sympathetic fibers.

Adult Axonal-L1 Regulates Schwann Cell-Axon Adhesion of Unmyelinated Fibers

The inability of Schwann cells to maintain axonal ensheathment in L1-deficient mice could result from a lack of a homophilic binding between Schwann cell-L1 and axonal-L1, heterophilic binding of Schwann cell-L1, or heterophilic binding of axonal-L1. These possibilities were tested in sciatic nerve transplant paradigms (Aguayo et al., 1977; Sahenk and Chen, 1998; Sahenk et al., 1999) that isolated L1 to either the Schwann cell or the sensory axon. To maintain wild-type nerve transplants into L1-deficient nerves, mice were immunosuppressed with cyclosporin A. To test if cyclosporin A treatment affected axonal regeneration, sciatic nerves from a wild-type SV-129 mice were also transplanted into the sciatic nerves of T cell–deficient nude mice. At 60 d after surgery, axons had regenerated through the graft and donor Schwann cells ensheathed unmyelinated axons and maintained adhesion (Fig. 6 A, arrowheads) in both sets of animals. Four percent or less of the unmyelinated axons in all three regions of the transplanted nerves were less than one-half ensheathed by Schwann cells. As described previously in sciatic nerve transplants (Grochowicz et al., 1985; Yu et al., 1990), these data indicate that axonal regeneration, Schwann cell en-

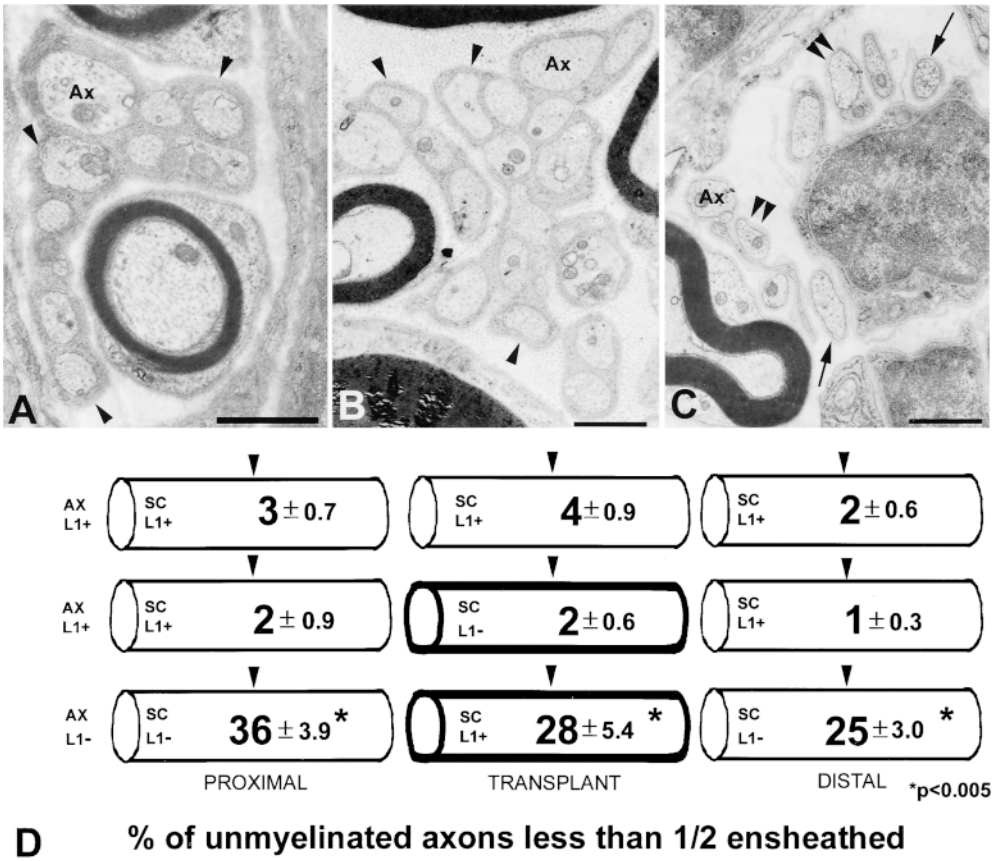

Figure 6. Axonal-L1 mediates adhesion between Schwann cells and unmyelinated sensory axons. A–C are electron micrographs from transplanted region of sciatic nerves. L1-positive Schwann cells normally ensheathe L1-positive axons (A, arrowheads). L1-deficient Schwann cells also normally ensheathe L1-positive axons (B, arrowheads). L1-positive Schwann cells only partially associate with L1-deficient axons (C), and have a morphology similar to that found in somatic nerves of the L1-deficient mouse. Axons were surrounded only by a basal lamina (C, arrows) or only partially in contact with a Schwann cell process (C, double arrowheads). Percentages in D correspond to the number of axons less than half ensheathed by Schwann cells. Arrowheads in D mark the regions of the nerves that were used for sectioning. **P*, 0.005 when compared with same region in other transplant paradigms. Ax, axon. Bars, 1 μm.

sheathment of unmyelinated axons, and myelination (data not shown) occurs in sciatic nerve transplants and is not affected by cyclosporin A treatment.

In transplants where L1-positive axons regenerated through a region of L1-deficient Schwann cells (Fig. 6 D, middle row), unmyelinated axons were normally ensheathed (Fig. 6 B, arrowheads) and could not be distinguished from the proximal or distal region of this control nerve. Two percent of all unmyelinated axons were less than half ensheathed by Schwann cells in all three regions (proximal, transplant, and distal) in this transplant paradigm. In an environment where L1-deficient axons regenerated through a region of L1-positive Schwann cells (Fig. 6 D, third row), 28% of the unmyelinated axons were less than half ensheathed by Schwann cells, indicating that L1-positive Schwann cells are unable to normally maintain ensheathment of L1-deficient axons. In the regions proximal and distal to the transplant where L1 is absent from both the axon and the Schwann cell, 36 and 25% of the unmyelinated axons were less than half ensheathed. The differences in Schwann cell ensheathment of control and L1-deficient axons were statistically significant using the Student's *t* test. These data indicate that loss of axonal-L1 is responsible for the lack of adhesion between Schwann cell and unmyelinated sensory axons.

Discussion

The objective of the present study was to elucidate the function of L1 in unmyelinated fibers of the PNS by analysis of L1-deficient mice. Our data establishes that L1 maintains Schwann cell ensheathment of sensory but not sympathetic unmyelinated axons. This null mutation phenotype was reproduced when normal Schwann cells were transplanted into L1-deficient sciatic nerve, but not when L1-deficient Schwann cells were transplanted into wild-type sciatic nerve. These data demonstrate that heterophilic adhesion of axonal-L1 is essential for maintaining Schwann cell ensheathment of sensory unmyelinated axons (Fig. 7). The L1 null mutation also results in a late onset degeneration of unmyelinated axons and decreased sensory function, consistent with axonal L1 functioning as an adhesion and signaling molecule. Normal ensheathment of unmyelinated sympathetic fibers in L1-deficient mice indicates that the mechanism of adhesion between Schwann cells and unmyelinated axons can be mediated by mechanisms unrelated to L1.

Axonal-L1 Maintains Schwann Cell Adhesion to Sensory Unmyelinated Axons

The present study established that axonal-L1 is essential for maintaining adhesion, ensheathment, and axonal viability of unmyelinated sensory fibers. L1-deficient mice on a C57Black6 background also have altered Schwann cell ensheathment of sensory unmyelinated axons (Dahme et al., 1997). L1-mediated adhesion can occur by both homophilic and heterophilic binding (Grumet and Edelman, 1988; Lemmon et al., 1989; Kuhn et al., 1991; Ruppert et al., 1995; DeBernardo and Chang, 1996; Hortsch, 1996; Montgomery et al., 1996) and a disruption of either may result in the phenotype seen in the L1-deficient mice. These hypotheses were directly tested by sciatic nerve transplant paradigms. Normal ensheathment of sensory axons by transplanted L1-deficient Schwann cells established that adhesion between Schwann cells and sensory unmyelinated axons does not occur by L1 homophilic nor Schwann cell-L1 heterophilic binding. In contrast, when L1 was absent from the axon and present on the transplanted Schwann cells, a phenotype similar to the L1-deficient mouse developed in the grafted nerve. This data isolates axonal-L1 as the molecule responsible for maintenance of sensory axon-Schwann cell ensheathment and indicates a heterophilic mechanism of adhesion between axonal-L1 and a Schwann cell molecule.

Loss of L1 Results in Degeneration of Sensory Unmyelinated Axons

While the number of axons in the corticospinal tract is reduced in L1-deficient mice (Cohen et al., 1998), most CNS and PNS axons reach appropriate targets and form appropriate fiber tracts in developing L1-deficient mice (Cohen et al., 1998; Dahme et al., 1997). Analysis of P7 mice in the present study indicates that unmyelinated fi-

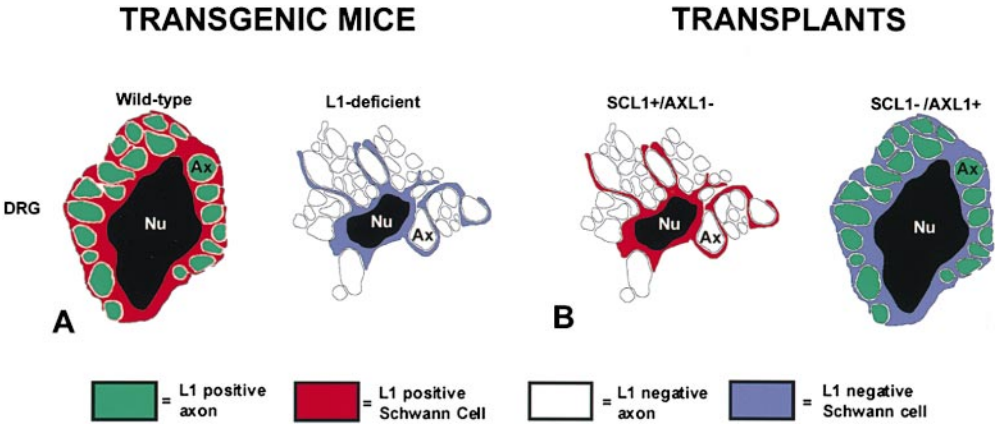

Figure 7. Schematic diagram of phenotypes in L1-deficient mice (A) and sciatic nerve transplants (B). L1 is expressed in Schwann cells (A, red) and unmyelinated axons (A, green) in wild-type mice. In the absence of both axonal and Schwann cell-L1, adhesion between Schwann cells (A, blue) and axons (A, white) is disrupted and axons degenerate. When L1-positive Schwann cells (B, red) are transplanted into L1-deficient nerves (B, white) Schwann cell-axon adhesion is also disrupted. However, Schwann cell-axon adhesion is maintained when L1-deficient Schwann cells (B, blue) are transplanted into L1-positive nerves (B, green).

bers develop normally in the absence of L1. However, by P60, L1-deficient mice have a significant decrease in sensory unmyelinated axons and decreased sensory function. Whereas loss of axonal-L1 is responsible for abnormal axonal ensheathment, several mechanisms could contribute to loss of axons. Schwann cells can provide extrinsic trophic factors or cell surface ligands that regulate the maturation, normal function, and survival of axons.

Adhesion between Schwann cells and sensory axons may be crucial for delivery of Schwann cell trophic factors and the loss of this contact may contribute to axonal degeneration in L1-deficient mice. The majority of mature small diameter DRG neurons express the nerve growth factor (NGF) receptors, p75 and trkA (Mu et al., 1993; DeBernardo and Chang, 1996). A small percentage of these neurons also express the brain-derived neurotropic factor (BDNF)/neurotrophin 4 (NT4) receptor, trkB (McMahon et al., 1999). Schwann cells of unmyelinated fibers secrete NGF (Varon et al., 1981; Bandtlow et al., 1987; Heumann et al., 1987) and before myelination Schwann cells secrete BDNF and CNTF (Friedman et al., 1992, 1996). NT4 mRNA is present at low levels in sciatic nerve (Funakoshi et al., 1993), but it is not known what cell types make and secrete NT4. Lack of ensheathment might disrupt expression or delivery of trophic factors, such as NGF or NT4 and ultimately lead to axonal degeneration.

The Schwann cell periaxonal membrane may contain a ligand that helps stabilize the axonal cytoskeletal architecture and thus assures normal axonal function and survival. In support of this mechanism, myelinated axons in MAG-deficient mice have marked alterations in neurofilament phosphorylation, neurofilament spacing, and axonal diameter that precede a late-onset axonal degeneration (Yin et al., 1998). While our transplantation studies indicate that Schwann cell L1 is not essential for axonal ensheathment, it is possible that Schwann cell-L1 or another molecule functions as a ligand that helps stabilize axonal cytoskeleton and thus assures axonal viability. Chronic disruption of this trophic effect could contribute to axonal degeneration.

The loss of axons in L1-deficient mice may also result from an axonal deficit in processing Schwann cell trophic signals. The correlation between loss of axonal-L1, disruption of Schwann cell ensheathment, and axonal degeneration supports the possibility that axonal-L1 functions as an adhesion and signaling molecule. In the appropriate environment, the cytoplasmic domain of L1 can initiate Ca^{2+} influx during neurite outgrowth (Williams et al., 1994) and Ca^{2+} influx is an early event in Wallerian degeneration (George et al., 1995). It is also possible that the L1 cytoplasmic domain is essential for maintaining the normal architecture of the axonal cytoskeleton by binding to ankyrin (Davis and Bennett, 1994). Ankyrin is enriched beneath the axolemma of unmyelinated sensory axons (Kordeli et al., 1990), and L1 and ankyrin colocalize at regions of cell–cell contact (Burden-Gulley et al., 1997). In the optic nerves of ankyrin-B null mice, L1 was initially expressed by optic nerve axons, but was not detected at P7 when the optic nerves began to degenerate (Scotland et al., 1998). Based upon these correlations, axonal-L1 may assure axonal viability by coordinating signaling events through its cytoplasmic domain or by stabilizing microfilament attachment to the axolemma. The chronic nature and partial penetrance of the axonal degeneration phenotype suggest that the mechanisms responsible for axonal loss are complex and possibly regulated by several interaxonal pathways.

Loss of Axonal-L1 but Not Schwann Cell-L1 Affects Schwann Cell Phenotype

Schwann cells can be considered as polarized epithelial cells with their abaxonal surface attached to the basal lamina, their adaxonal surface attached to axons, and their lateral surface attached to adjoining Schwann cells (Carlsen and Behse, 1980; Trapp et al., 1995). When basal lamina formation is inhibited by vitamin C deprivation, Schwann cells do not myelinate or appropriately ensheathe axons (Eldridge et al., 1987). While initial polarization of unmyelinated Schwann cells occurs in L1-deficient mice, functional polarization is not maintained in adult sensory fibers. The basal lamina is discontinuous, axonal adhesion and lateral adhesion of Schwann cells to neighboring Schwann cells is lost, and Schwann cell processes extend into the endoneurial space. L1-deficient nonmyelinating Schwann cells, therefore, are not able to maintain a mature, nonmigratory, polarized status. However, this effect was not observed when L1-deficient Schwann cells were transplanted into L1-positive axons. Therefore, axonal-L1 appears to stabilize the polarization of Schwann cell surface membranes via heterophilic binding to a Schwann cell molecule.

L1 is Not Essential for Initial Axonal Ensheathment or Myelination

Schwann cells express L1 as they colonize peripheral nerves and sort axons into to polyaxonal pockets or one-to-one relationships (Jessen and Mirsky, 1991). Myelinating Schwann cell membranes that form the initial one and a half spiral wraps around an axon are also L1-positive (Martini and Schachner, 1986). As myelination proceeds, L1 is no longer detected in Schwann cell membranes. L1 antibodies inhibited myelination of DRG axons maintained in vitro (Seilheimer et al., 1989; Wood et al., 1990), suggesting that L1 was essential for axonal ensheathment and initial spiral wrapping of myelin membranes. Normal myelination of two lines of L1 null mice (C57Black6 and SV-129) indicate that L1 is not essential for initial axon ensheathment nor myelination (Dahme et al., 1997; Cohen et al., 1998). Furthermore, it was hypothesized that L1 compensated for loss of MAG during myelination in MAG-deficient mice (Montag et al., 1994). The present study demonstrated normal myelination in the absence of both L1 and MAG. The molecule responsible for spiral wrapping of membranes during myelination is not known. The data presented in this report and normal ensheathment and myelination in MAG/NCAM-deficient mice (Carenini et al., 1997) demonstrate that MAG, L1, and N-CAM do not play an irreplaceable role during spiral wrapping of myelin membranes or initial ensheathment of unmyelinated and myelinated axons.

The authors wish to thank Phil Soriano for providing the L1-deficient mice and for critical reading of the manuscript and Alison Hall for helpful advice on the CGRP studies.

This work was supported by NIH Grant RO1-NS38186 (B.D. Trapp), RO1-NS32770 (V.P. Lemmon), and the Canadian MRC (J. Roder).

Submitted: 8 June 1999
Revised: 23 July 1999
Accepted: 23 July 1999

References

Aguayo, A.J., M. Attiwell, J. Trecarten, S. Perkins, and G.M. Bray. 1977. Abnormal myelination in transplanted trembler mouse Schwann cells. *Nature.* 265:73–75.

Bandtlow, C.E., R. Heumann, M.E. Schwab, and H. Thoenen. 1987. Cellular localization of nerve growth factor synthesis by in-situ hybridization. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:891–899.

Bastmeyer, M., H. Ott, C.A. Leppert, and C.A. Stuermer. 1995. Fish E587 glycoprotein, a member of the L1 family of cell adhesion molecules, participates in axonal fasciculation and the age-related order of ganglion cell axons in the goldfish retina. *J. Cell Biol.* 130:969–976.

Bieber, A.J., P.M. Snow, M. Hortsch, N.H. Patel, J.R. Jacobs, Z.R. Traquina, J. Schilling, and C.S. Goodman. 1989. Drosophila neuroglian: a member of the immunoglobulin superfamily with extensive homology to the vertebrate neural adhesion molecule L1. *Cell.* 59:447–460.

Brittis, P.A., V. Lemmon, U. Rutishauser, and J. Silver. 1995. Unique changes of ganglion cell growth cone behavior following cell adhesion molecule perturbations: a time-lapse study of the living retina. *Mol. Cell. Neurosci.* 6:433–449.

Burden-Gulley, S.M., M. Pendergast, and V. Lemmon. 1997. The role of cell adhesion molecule L1 in axonal extension, growth cone motility, and signal transduction. *Cell Tissue Res.* 290:415–422.

Carenini, S., D. Montag, H. Cremer, M. Schachner, and R. Martini. 1997. Absence of the myelin-associated glycoprotein (MAG) and the neural cell adhesion molecule (N-CAM) interferes with the maintenance, but not with the formation of peripheral myelin. *Cell Tissue Res.* 287:3–9.

Carlsen, F., and F. Behse. 1980. Three dimensional analysis of Schwann cells associated with unmyelinated nerve fibres in human sural nerve. *J. Anat.* 130: 545–557.

Cohen, N.R., J.S.H. Taylor, L.B. Scott, R.W. Guillery, P. Soriano, and A.J.W. Furley. 1998. Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. *Curr. Biol.* 8:26–33.

Dahme, M., U. Bartsch, R. Martini, B. Anliker, M. Schachner, and N. Mantei. 1997. Disruption of the mouse *L1* gene leads to malformations of the nervous system. *Nat. Genet.* 17:346–349.

Davis, J.Q., and V. Bennett. 1994. Ankyrin binding activity shared by the neurofascin/L1/NrCAM family of nervous system cell adhesion molecules. *J. Biol. Chem.* 269:27163–27166.

DeBernardo, A.P., and S. Chang. 1996. Heterophilic interactions of DM-GRASP: GRASP-NgCAM interactions involved in neurite extension. *J. Cell Biol.* 133:657–666.

deWaegh, S.M., V.M.Y. Lee, and S.T. Brady. 1992. Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. *Cell.* 68:451–463.

Dyck, P.J., and A.P. Hopkins. 1972. Electron microscopic observations on degeneration and regeneration of unmyelinated fibers. *Brain.* 95:223–224.

Eldridge, C.F., M.B. Bunge, R.P. Bunge, and P.M. Wood. 1987. Differentiation of axon-related Schwann cells in vitro. I. Ascorbic acid regulates basal lamina assembly and myelin formation. *J. Cell Biol.* 105:1023–1034.

Faissner, A., J. Kruse, J. Nieke, and M. Schachner. 1984. Expression of neural cell adhesion molecule L1 during development, in neurological mutants and in the peripheral nervous system. *Dev. Brain Res.* 15:69–82.

Friedlander, D.R., P. Milev, L. Karthikeyan, R.K. Margolis, R.U. Margolis, and M. Grumet. 1994. The neuronal chondroitin sulfate proteoglycan neurocan binds to the neural cell adhesion molecules Ng-CAM/L1/NILE and N-CAM, and inhibits neuronal adhesion and neurite outgrowth. *J. Cell Biol.* 125:669–680.

Friedman, B., S.S. Scherer, J.S. Rudge, M. Helgren, D. Morrisey, J. McClain, D. Wang, S.J. Wiegand, M.E. Furth, R.M. Lindsay, and N.Y. Ip. 1992. Regulation of ciliary neurotrophic factor expression in myelin-related Schwann cells *in vivo*. *Neuron.* 9:295–305.

Friedman, H.C., T.N. Jelsma, G.M. Bray, and A.J. Aguayo. 1996. A distinct pattern of trophic factor expression in myelin-deficient nerves of *trembler* mice: implications for trophic support by Schwann cells. *J. Neurosci.* 16: 5344–5350.

Fujita, N., S. Sato, H. Ishiguro, T. Inuzuka, H. Baba, T. Kurihara, Y. Takahashi, and T. Miyatake. 1990. The large isoform of myelin-associated glycoprotein is scarcely expressed in the quaking mouse brain. *J. Neurochem.* 55:1056–1059.

Funakoshi, H., J. Frisén, G. Barbany, T. Timmusk, O. Zachrisson, V.M.K. Verge, and H. Persson. 1993. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J. Cell Biol.* 123:455–465.

George, E.B., J.D. Glass, and J.W. Griffin. 1995. Axotomy-induced axonal degeneration is mediated by calcium influx through ion-specific channels. *J. Neurosci.* 15:6452.

Grochowicz, P., M. Schatzle, C. Hammer, W. Olscewski, and W. Brendel. 1985. Revascularization of peripheral nerve transplants. Steromicroangiographic evaluation of revascularization in allogenic transplants, allogenic transplants with cyclosporin A therapy and autologous transplants. *Handchir. Mikrochir. Plast. Chir.* 17(Suppl):17–19.

Grumet, M., and G.M. Edelman. 1988. Neuron-glia cell adhesion molecular interacts with neurons and astroglia via different binding mechanisms. *J. Cell Biol.* 106:487–503.

Heumann, R., S. Korsching, C. Bandtlow, and H. Thoenen. 1987. Changes in nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transaction. *J. Cell Biol.* 104:1623–1631.

Hlavin, M.L., and V. Lemmon. 1991. Molecular structure and functional testing of human L1CAM: an interspecies comparison. *Genomics.* 11:416–423.

Hokfelt, T., U. Arvidsson, S. Ceccatelli, R. Cortes, S. Cullheim, A. Dagerlind, H. Johnson, C. Orazzo, F. Piehl, V. Pieribone, et al. 1992. Calcitonin gene-related peptide in the brain, spinal cord, and some peripheral systems. *Annu. NY Acad. Sci.* 657:119–134.

Hortsch, M. 1996. The L1 family of neural cell adhesion molecules: old proteins performing new tricks. *Neuron.* 17:587–593.

Hsieh, S.-T., G.J. Kidd, T.O. Crawford, Z. Xu, B.D. Trapp, D.W. Cleveland, and J.W. Griffin. 1994. Regional modulation of neurofilament organization by myelination in normal axons. *J. Neurosci.* 14:6392–6401.

Jessen, K.R., and R. Mirsky. 1991. Schwann cell precursors and their development. *Glia.* 4:185–194.

Kamiguchi, H., and V. Lemmon. 1998. A neuronal form of the cell adhesion molecule L1 contains a tyrosine-based signal required for sorting to the axonal growth cone. *J. Neurosci.* 18:3749–3756.

Kordeli, E., J.D. Davis, B.D. Trapp, and V. Bennett. 1990. An isoform of ankyrin is localized at nodes of Ranvier in myelinated axons of central and peripheral nerves. *J. Cell Biol.* 110:1341–1352.

Kuhn, T.B., E.T. Stoeckli, M.A. Condrau, F.G. Rathjen, and P. Sonderegger. 1991. Neurite outgrowth on immobilized axonin-1 is mediated by a heterophilic interaction with L1(G4). *J. Cell Biol.* 115:1113–1126.

Lagenaur, C., and V. Lemmon. 1987. An L1-like molecule, the 8D9 antigen, is a potent substrate for neurite extension. *Proc. Natl. Acad. Sci. USA.* 84:7753–7757.

Landmesser, L., K. Schultz, and U. Rutishauser. 1988. Distinct roles for adhesion molecules during innervation of embryonic chick muscle. *Dev. Biol.* 130:645–670.

Lemmon, V., K.L. Farr, and C. Lagenaur. 1989. L1-mediated axon outgrowth occurs via a homophilic binding mechanism. *Neuron.* 2:1597–1603.

Lemmon, V., and S.C. McLoon. 1986. The appearance of an L1-like molecule in the chick primary visual pathway. *J. Neurosci.* 6:2987–2994.

Li, C., M.B. Tropak, R. Gerial, S. Clapoff, W. Abramow-Newerly, B. Trapp, A. Peterson, and J. Roder. 1994. Myelination in the absence of myelin-associated glycoprotein. *Nature.* 369:747–750.

Martini, R., and M. Schachner. 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. *J. Cell Biol.* 103:2439–2448.

McMahon, S.B., M. Armanini, L.H. Ling, and H.S. Phillips. 1999. Expression and coexpression of trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. *Neuron.* 12:1161–1171.

Milev, P., D.R. Friedlander, T. Sakurai, L. Karthikeyan, M. Flad, R.K. Margolis, M. Grumet, and R.U. Margolis. 1994. Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia, and neural cell adhesion molecules. *J. Cell Biol.* 127:1703–1715.

Miura, M., M. Kobayashi, H. Asou, and K. Uyemura. 1991. Molecular cloning of cDNA encoding the rat neural cell adhesion molecule L1. Two L1 isoforms in the cytoplasmic region are produced by differential splicing. *FEBS Lett.* 289:91–95.

Montag, D., K.P. Giese, U. Bartsch, R. Martini, Y. Land, H. Blüthmann, J. Karthigasan, D.A. Kirschner, E.S. Wintergerst, K.-A. Nave, et al. 1994. Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. *Neuron.* 13:229–246.

Montgomery, A.M.P., J.C. Becker, C.-H. Siu, V.P. Lemmon, D.A. Cheresh, J.D. Pancook, X. Zhao, and R.A. Reisfeld. 1996. Human neural cell adhesion molecule L1 and rat homologue NILE are ligands for integrin a_vb_3. *J. Cell Biol.* 132:475–485.

Moos, M., R. Tacke, H. Scherer, D. Teplow, K. Fruh, and M. Schachner. 1988. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. *Nature.* 334:701–703.

Mu, X., I. Silos-Santiago, S.L. Carroll, and W.D. Snider. 1993. Neurotrophin receptor genes are expressed in distinct patterns in developing dorsal root ganglia. *J. Neurosci.* 13:4029–4041.

Omer, G.E., and J. Bell-Krotoski. 1998. Sensibility testing. *In* Management of Peripheral Nerve Problems. G.E. Omer, M. Spinner, and A.L. Van Beek, editors. W.B. Saunders Company, Philadelphia. 11–28.

Peters, A., S.L. Palay, and H.deF. Webster. 1991. The Fine Structure of the Nervous System: Neurons and their Supporting Cells. Oxford University Press, New York.

Ruppert, M., S. Aigner, M. Hubbe, H. Yagita, and P. Altevogt. 1995. The L1 adhesion molecule is a cellular ligand for VLA-5. *J. Cell Biol.* 131:1881–

1891.
Sahenk, Z., and L. Chen. 1998. Abnormalities in the axonal cytoskeleton induced by a connexin32 mutation in nerve xenografts. *J. Neurosci. Res.* 51: 174–184.
Sahenk, Z., L. Chen, and J.R. Mendell. 1999. Effects of PMP22 duplication and deletions on the axonal cytoskeleton. *Annu. Neurol.* 45:16–24.
Sanchez, I., L. Hassinger, P.A. Paskevich, H.D. Shine, and R.A. Nixon. 1996. Oligodendroglia regulate the regional expansion of axon caliber and local accumulation of neurofilaments during development independently of myelin formation. *J. Neurosci.* 16:5095–5105.
Scotland, P., D. Zhou, H. Benveniste, and V. Bennett. 1998. Nervous system defects of ankyrin$_B$ (−/−) mice suggest functional overlap between the cell adhesion molecule L1 and 440-kD ankyrin$_B$ in premyelinated axons. *J. Cell Biol.* 143:1305–1315.
Seilheimer, B., E. Persohn, and M. Schachner. 1989. Antibodies to the L1 adhesion molecule inhibit Schwann cell ensheathment of neurons *in vitro*. *J. Cell Biol.* 109:3095–3103.
Stallcup, W.B., and L. Beasley. 1985. Involvement of the nerve growth factor-inducible large external glycoprotein (NILE) in neurite fasciculation in primary cultures of rat brain. *Proc. Natl. Acad. Sci. USA*. 82:1276–1280.
Takeda, Y., H. Asou, Y. Murakami, M. Miura, M. Kobayashi, and K. Uyemura. 1996. A nonneuronal isoform of cell adhesion molecule L1: tissue-specific expression and functional analysis. *J. Neurochem*. 66:2338–2349.
Thomas, P.K., D.N. Landon, and R.H.M. King. 1984. Diseases of the peripheral nerves. *In* Greenfield's Neuropathology. J.H. Adams, J.A.N. Corsellis, and L.W. Duchen, editors. J. Wiley & Sons, New York. 807–920.
Tongiorgi, E., R.R. Bernhardt, and M. Schachner. 1995. Zebrafish neurons express two L1-related molecules during early axonogenesis. *J. Neurosci. Res.* 42:547–561.
Trapp, B.D., S.B. Andrews, C. Cootauco, and R.H. Quarles. 1989. The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. *J. Cell Biol.* 109:2417–2426.
Trapp, B.D., G.J. Kidd, P.E. Hauer, E. Mulrenin, C. Haney, and S.B. Andrews. 1995. Polarization of myelinating Schwann cell surface membranes: role of microtubules and the trans-Golgi network. *J. Neurosci.* 15:1797–1807.
Trapp, B.D., and R.H. Quarles. 1982. Presence of the myelin-associated glycoprotein correlates with alterations in the periodicity of peripheral myelin. *J. Cell Biol.* 92:877–882.
Varon, S., S.D. Skaper, and M. Manthorpe. 1981. Trophic activites for dorsal root and sympathetic ganglionic neurons in media conditioned by Schwann and other peripheral cells. *Brain Res.* 227:73–87.
Williams, E.J., J. Furness, F.S. Walsh, and P. Doherty. 1994. Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. *Neuron*. 13:583–594.
Wood, P.M., M. Schachner, and R.P. Bunge. 1990. Inhibition of Schwann cell myelination *in vitro* by antibody to the L1 adhesion molecule. *J. Neurosci.* 10:3635–3645.
Yin, X., T.O. Crawford, J.W. Griffin, P.-H. Tu, V.M.Y. Lee, C. Li, J. Roder, and B.D. Trapp. 1998. Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. *J. Neurosci.* 18:1953–1962.
Yu, L.T., J. England, A. Sumner, D. Larossa, and W.F. Hickey. 1990. Electrophysiologic evaluation of peripheral nerve regeneration through allografts immunosuppressed with cyclosporin. *J. Reconstr. Microsurg.* 6:317–323.