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REVIEW ARTICLE

Role of Integrins in Peripheral Nerves and Hereditary Neuropathies

Caterina Berti, Alessandro Nodari, Lawrence Wrabetz, and Maria Laura Feltri*

San Raffaele Scientific Institute, DIBIT, Via Olgettina 58, 20132 Milano, Italy

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Abstract

Interactions between Schwann cells and extracellular matrix on one surface, and axons on the other, are required for correct myelination in the developing peripheral nervous system. Integrins are transmembrane proteins that mediate the former in association with other surface receptors. This review focuses on the role that integrins play in the development of the peripheral nervous system, and in inherited human peripheral neuropathies. Here we describe recent findings on integrin signaling to different intracellular pathways, focusing on cell adhesion, migration, and polarization. Then we use information derived from recent experiments of targeted mutagenesis in mice to show that, consistent with temporally regulated expression, different integrins serve multiple roles in developing nerve.

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Index Entries: Laminin; integrins; Schwann cell; MDC1A; conditional mutagenesis.

Introduction

Schwann cells are the glial cells of the peripheral nervous system (PNS). They originate from neural crest cells (NCC) during development and migrate along growing axons (longitudinal migration). Beginning at E15.5 in the mouse, immature Schwann cells ensheath and sequentially sort bundles of axons, to achieve a one-to-one relationship with axons destined to be myelinated (radial sorting). During postnatal development Schwann cells enwrap and myelinate single axons. The myelin sheath defines axonal domains and provides low-capacitance ensheathment for the axon, allowing rapid conduction of action potentials (Waxman and Bangalore, 2004). Significant acquired damage to the myelin sheath disrupts the propagation of nerve signals, but also affects survival, maintenance, and differentiation of axons (Trapp and Kidd, 2004). The process of myelination is also affected in several demyelinating inherited neuropathies in which mutations in genes encoding myelin proteins, like myelin protein

*Author to whom all correspondence and reprint requests should be addressed. E-mail: feltri.laura@hsr.it

zero (P0) and peripheral myelin protein 22 (PMP22), or other Schwann cell genes, cause disorders of the PNS (reviewed in Wrabetz et al., 2004).

Extensive data also indicates that adhesion of Schwann cell to extracellular matrix (ECM) is crucial for nerve fiber morphogenesis and myelination. Anchorage to ECM is mediated via different cell surface receptors, such as those of the integrin superfamily, that transduce signals necessary for cellular processes such as migration, proliferation, differentiation, and polarization (reviewed in Bokel and Brown, 2002). In the PNS, integrins on Schwann cells that bind laminins in the basal lamina (BL) and various cytoskeleton components inside the cell, are responsible for correct myelination. In fact, spontaneous mutations in laminin genes cause a peripheral neuropathy in *dystrophic* (d*y*/d*y or* dy^{2J}/dy^{2J}) mice and in merosin-deficient congenital muscular dystrophy type 1A [MDC1A] patients. Targeted mutagenesis in mice has revealed the function of various laminin isoforms and their receptors in nerve development (Feltri et al., 2002; Chen and Strickland, 2003; Yang et al., 2005; Yu et al., 2005). Although no integrin genes have been found mutated in inherited neuropathies, perhaps because it would cause a lethal phenotype, it is possible that integrin interactors and effectors will be discovered as Charcot-Marie-Tooth (CMT) genes. Indeed, recent findings already suggest that integrins may interact with CMT proteins such as PMP22 (Amici and Notterpek, 2005) and periaxin (PRX)/dystroglycan (Sherman et al., 2001).

Laminins in PNS and Schwann Cell BL

Laminins are trimers formed by an α-, β-, and γ-subunit, each encoded by a different gene. To date, 15 isoforms, made up of five α-chains, four β-chains, and three γ-chains have been found (reviewed in Yin et al., 2003). The most abundant isoforms expressed during early development by Schwann cells are laminin-2 ($\alpha_2\beta_1\gamma_1$) and -8 ($\alpha_4\beta_1\gamma_1$) (Lentz et al., 1997; Wallquist et al., 2002). In mature peripheral nerves, laminins are differentially expressed: laminin-2 and -8 in the BL surrounding Schwann cells and laminin-9 ($\alpha_4\beta_2\gamma_1$) and -11 ($\alpha_5\beta_2\gamma_1$) in the BL of perineurial cells (Sanes et al., 1990; Patton et al., 1997; Wallquist et al., 2002). Recently, laminin-α_5 has been detected in Schwann cell BL only over the node of Ranvier (Vagnerova, 2003; Occhi et al., 2005).

In the endoneurium, each Schwann cell is surrounded by a BL. BL is a thin sheet of organized ECM visible by electron microscopy as a three-layered structure. Lamina fibroreticularis is proximal to the cell membrane, whereas *lamina lucida* and *lamina densa* are the most external layers. BL is a common feature of polarized cells (such as epithelial cells) and it is present at the epithelial–mesenchymal interface of most tissues (Miosge, 2001). Schwann cell BL is made up of collagens, proteoglycans, and noncollagenous glycoproteins including laminin, fibronectin, and entactin/nidogen (Bunge, 1993; Chernousov and Carey, 2000). Seminal studies by the group of Mary Bunge first indicated that assembly of BL was required for myelination (reviewed in Bunge, 1993). Recent studies in vitro (Podratz et al., 1998, 2004) and in vivo (Nakagawa et al., 2001; Yang et al., 2005) have demonstrated that not BL integrity *per se*, but mainly signals from laminins are required for a correct myelination.

The first evidence for the importance of laminin in myelination was derived from in vitro experiments on Schwann cell/neuron cocultures (Fernandez-Valle et al., 1994; Podratz et al., 2001), and characterization in vivo of laminin-α_2 mutations in both human (Helbling-Leclerc et al., 1995) and mice (Xu et al., 1994; Sunada et al., 1995). Loss or impairment of laminin-2, owing to a mutation of the *Lama2* gene, in the dystrophic mouse (Xu et al., 1994; Sunada et al., 1995) and human patients (Helbling-Leclerc et al., 1995) causes a congenital muscular dystrophy and a dysmyelinating neuropathy (MDC1A), characterized by the presence of Schwann cells not able to enwrap and myelinate axons (sorting defects), more severe in proximal (roots) than in distal PNS. More recently, further evidence for the essential role of laminins in myelination came from studies of targeted mutagenesis of various laminin isoforms in mice. These studies emphasize that laminin-related nerve defects are the consequence of altered nerve development, and begin to dissect the role of the different laminin isoforms. Inactivation of the α_4-laminin chain gene in mice results in loss of laminin-8 isoform and causes mild defects in axonal sorting in both roots and distal nerves (Wallquist et al., 2002; Yang et al., 2005). More severe defects in radial sorting are observed in transgenic mice with double deletion of the α_2- and α_4-laminin genes (loss of laminin-2 and -8): in this case all proximal and distal axons remained unsorted

(Yang et al., 2005). Similarly, mice subjected to selective inactivation of the laminin-γ_1 chain in Schwann cells using the Cre/LoxP system, and therefore lacking all of the laminin isoforms, present a deficit in axonal sorting in both roots and distal nerves (Chen and Strickland, 2003; Yu et al., 2005). In all these mutants, defects begin in late embryonic development and are readily apparent at birth.

An interesting observation is that the topography of sorting abnormalities in proximal (roots) as compared with distal (e.g., sciatic nerves) PNS varies among different mutant mice. This could be explained by the presence of molecules with similar function (redundancy), or upregulation of others laminins (compensation) in roots and in the distal nerves. The hypothesis of redundancy is supported by the more evenly distributed defect obtained by eliminating both α_4- and α_8-laminins (Yang et al., 2005; Yu et al., 2005). The hypothesis of compensation is supported by the finding that α_1-laminin is upregulated in distal nerves of dy^{2J}/dy^{2J} mice (Previtali et al., 2003b); and by the fact that ectopic α_5-laminin expression rescues the sorting defects in dy/α_4 null mice (Yang et al., 2005).

In addition to axonal sorting, laminins in Schwann cells activate intracellular signaling pathways that are necessary for many other developmental steps, such as proliferation, survival, differentiation (Chen and Strickland, 2003; Yang et al., 2005; Yu et al., 2005), and organization of the node of Ranvier (Bradley et al., 1977; Jaros and Bradley, 1979; Occhi et al., 2005). Mice lacking laminin-γ_1 in Schwann cells have less Schwann cells, resulting from increased apoptosis and decreased proliferation (Yu et al., 2005), whereas mice lacking both α_2- and α_4-laminin show a decrease in Schwann cell proliferation (Yang et al., 2005). It remains to be elucidated whether this effect is a direct consequence of an impaired laminin signal, or since mutant Schwann cells do not associate properly with axons, if it is due to the absence of axonal mitogenic signals. Moreover, these mutants have abnormalities suggesting an important role for laminins in the architecture of myelin internodes, such as a shorter internodal length and abnormally wide nodes of Ranvier (Bradley et al., 1977; Jaros and Bradley, 1979; Occhi et al., 2005). Recently, it has been shown that laminin defects also affect the proper clustering of sodium channels at nodes and this is also found in nerves from MDC1A patients (Occhi et al., 2005).

Thus, multiple laminin isoforms subserve multiple roles in developing nerves. An important factor in mediating these different roles could be the interaction between specific laminins and specific receptors. In other tissues it has been demonstrated that these interactions play a key role in laminin polymerization and assembly, and induce a matrix–receptor–cytoskeleton network (Colognato et al., 1999; Colognato and Yurchenco, 2000; Henry et al., 2001; ffrench-Constant and Colognato, 2004) that is important for tissue morphogenesis (reviewed in Miner and Yurchenco, 2004).

Integrins and Their Functions

Integrins are a large family of cell surface receptors involved in different cellular processes such as cell adhesion, migration, proliferation, differentiation, and polarity (reviewed in van der Flier and Sonnenberg, 2001). They are transmembrane dimeric proteins made up of α- and β-subunits associated by noncovalent bonds. Different combinations of 18-α- and 8-β-subunits in mammals result in multiple integrin heterodimers (Giancotti, 2000; Hynes and Zhao, 2000), with various and specific affinity for ligands in the ECM, such as laminin, fibronectin, vitronectin, and various collagens. In addition, most integrin heterodimers bind several ECM components. So integrins are characterized by two different versions of redundancy: the first is in forming heterodimers and the second is in their ligand binding. Despite this redundancy and the possibility of compensation, a crucial role for many integrin has been demonstrated by studying knockout mice lacking different integrins (reviewed in ffrench-Constant and Colognato, 2004). All integrin subunits have a large extracellular domain, a single transmembrane domain, and a short cytoplasmic domain, with the exception of vertebrate β_4-subunit, which is characterized by a large cytoplasmic tail (Hynes, 1992). Intracellular integrins bind different cytoskeleton components, such as cytoskeleton-associated proteins (α-actinin, talin, and filamin), signaling molecules (integrin-linked kinase [ILK] and focal adhesion kinase [FAK]), and calcium-binding proteins (reviewed in van der Flier and Sonnenberg, 2001), acting not only as anchoring proteins, but also as signaling receptors. In fact, interaction between integrins and their ligands (*in-trans* binding) or cell surface and cytoskeleton proteins

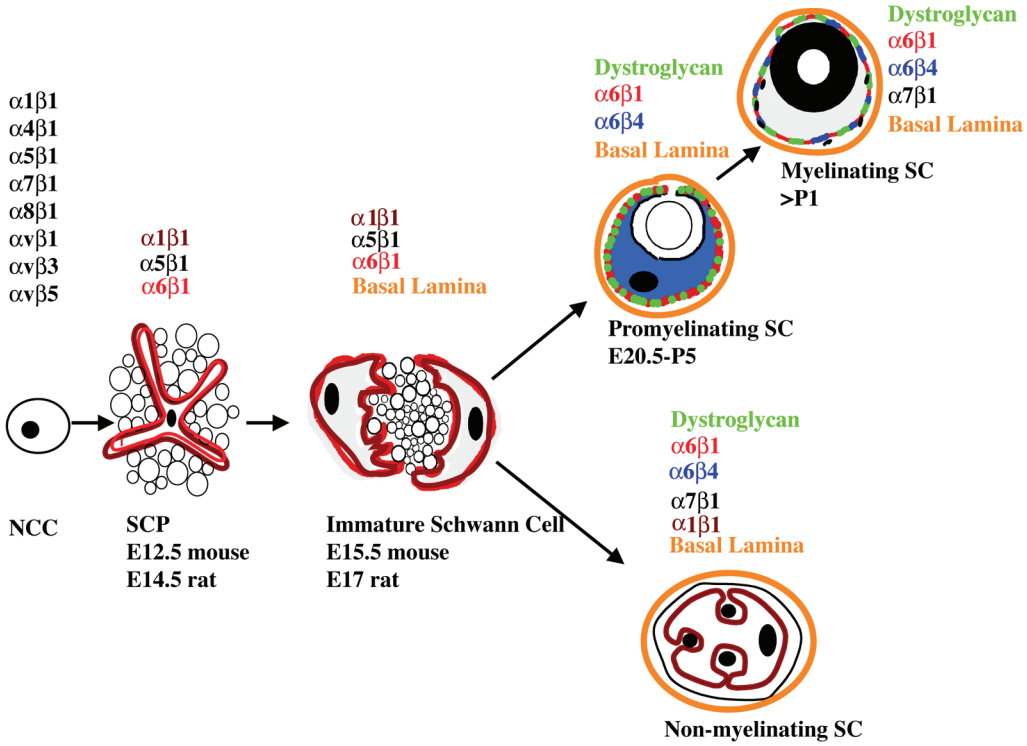

Fig. 1. Differential distribution of ECM receptors during SC development. NCC express several integrin receptors, but in the following steps of development the distribution of these receptors becomes more restricted. $\alpha_6\beta_1$ appears in SCP and in immature SCs, and remains expressed throughout the whole of the SC lineage. $\alpha_6\beta_4$ starts to be expressed by promyelinating SCs, first in a diffuse fashion, and then polarized only after myelination. $\alpha_7\beta_1$ is expressed after the first postnatal week. Whereas there is no integrin expressed only in myelin-forming SCs, $\alpha_1\beta_1$-integrin is specific for nonmyelin-forming SC. SC, Schwann cell; NCC, Neural crest cells; SCP, Schwann cell precursor.

(*in-cis* binding), is responsible for the activation of both an outside-in and an inside-out signaling pathway (Geiger et al., 2001; ffrench-Constant and Colognato, 2004). Through the outside-in signaling the cell receives information from the ECM and responds by changing conformation and linking several adaptors and intracellular signaling molecules (ffrench-Constant and Colognato, 2004; Lee and Juliano, 2004). The inside-out signaling induces a change in integrin status, such as modulation of ligand-binding affinity (activation or inactivation) (Geiger et al., 2001; ffrench-Constant and Colognato, 2004).

Integrins in the Schwann Cell Lineage

In the PNS, integrins are present in both neurons and glia, except for the heterodimer $\alpha_6\beta_4$, only expressed by Schwann cells (Clegg et al., 2003; Feltri and Wrabetz, 2005). Like laminins, integrins manifest differential timing of expression in developing nerve and have differential location in the adult nerve, suggesting distinct roles (Previtali et al., 2003b) (Fig. 1). Mouse Schwann cell precursors (SCP) migrate from neural crest along neurites (embryonic day E 12–13) and differentiate into immature Schwann cells (E 15–16). Before birth, those Schwann cells destined to myelinate axons extend cytoplasmic processes to separate axon bundles and achieve a one-to-one relationship with larger diameter axons, becoming a promyelinating Schwann cell. After birth they enwrap these axons with up to 50–100 turns of membrane and form myelin. Those Schwann cells not destined to myelinate axons, called nonmyelinating Schwann cells, differentiate later at postnatal day P15 and unsheathe multiple small caliber axons.

In the following sections of this review, we will describe different integrins expressed during development in the PNS, from neural crest to mature Schwann cells, and the signaling pathways that they activate. Although Schwann cells express several integrins that bind different ECM components, such as laminins, fibronectin, vitronectin, and some collagen isoforms, more focus will be devoted to integrins that serve as laminin receptors, namely $\alpha_6\beta_1$, $\alpha_6\beta_4$, and $\alpha_7\beta_1$, because their role has been characterized in vivo and they mediate laminin-signaling pathways deficient in hereditary neuropathies. Other laminin receptors, not reviewed in this article, are dystroglycan (Ervasti and Campbell, 1993) and some members of collagen superfamily, such as collagen XIII (Franzke et al., 2003). Dystroglycan, in particular, has been shown to have a key role in myelination (Saito et al., 2003).

Neural Crest Cells

NCCs are a transient group of cells that delaminate from the dorsal part of the neural tube during embryonic development. From the trunk region, NCCs migrate along defined pathways of ECM proteins in order to reach different areas of the embryo, in which they undergo terminal differentiation into sensory neurons, peripheral glia, melanocytes, and craniofacial structures (reviewed in Le Douarin and Ziller, 1993). During migration, NCCs traverse regions rich in ECM components such as fibronectin, collagens, and laminins (Erickson and Perris, 1993; Le Douarin and Kalcheim, 1999). The role of different ECM components to NCC migration is not completely understood because of several layers of complexity including functional redundancy; balance between permissive, nonpermissive, and inhibitory components; differences between species and between cranial and trunk NCC (reviewed in Le Douarin and Kalcheim, 1999, Perris and Perissinotto, 2000). Avian NCCs adhere and migrate onto fibronectin, laminin1, vitronectin and collagens (Rovasio et al., 1983; Perris et al., 1989; Desban and Duband, 1997). In vitro and in vivo experiments suggest that fibronectin and type-I collagen are necessary for NCC migration, with possibly a cooperation between them and other ECM molecules (Rovasio et al., 1983; Duband and Thiery, 1987; Bilozur and Hay, 1988; Perris et al., 1989; McCarthy and Hay, 1991). Furthemore, in vitro and expression studies suggest that laminin is necessary for NCC delamination from the neural tube (Duband and Thiery, 1987; Bilozur and Hay, 1988), where it affects NCC migration in a dose-dependent manner (Perris et al., 1989) and depending on NCC origin, along the sagittal plane (Strachan and Condic, 2003, 2004). Gene deletion studies in mice so far have confirmed a role for fibronectin and γ_1-containing laminins in promoting NCC migration (reviewed in Perris and Perissinotto, 2000). In contrast, a role in vivo has not been confirmed for other ECM components such as vitronectin, tenascin, and several collagen types (reviewed in Perris and Perissinotto, 2000).

NCC expresses in vitro a large repertoire of integrins: $\alpha_1\beta_1$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_8\beta_1$, $\alpha v\beta_1$, $\alpha v\beta_3$, $\alpha v\beta_5$, and $\alpha v\beta_8$ (Kil et al., 1996; Desban and Duband, 1997; Testaz et al., 1999). Similar to the situation for ECM components, many studies suggested a role for integrins in NCC migration. For example, interfering with integrins forming fibronectin or laminin receptors *in-ovo* inhibit NCC migration (reviewed in Bronner-Fraser, 1993). In vitro experiments have demonstrated a role for $\alpha_1\beta_1$, $\alpha v\beta_1$, and $\alpha_8\beta_1$ in cell spreading, and a role for $\alpha_3\beta_1$-, $\alpha v\beta_3$-, and $\alpha_8\beta_1$-integrins in NCC migration (Testaz et al., 1999). Other experiments suggest instead a major role in NCC migration for $\alpha_1\beta_1$ (Lallier and Bronner-Fraser, 1993, among others) and a secondary role for $\alpha_3\beta_1$-integrin (Desban and Duband, 1997). Surprisingly, a generation of mice lacking integrin subunits showed no or minor effects in NCC development (reviewed in Perris and Perissinotto, 2000), although a transient effect on migration of NCC derivatives after ablation of β_1-integrin was reported recently (Pietri et al., 2004). Generation of knockout mice for α_4- and α_5-integrins demonstrated that both of these integrins control glial cell number by affecting NCC survival and proliferation respectively, but not migration and differentiation (Haack and Hynes, 2001).

Signals Involved

Although signaling pathways activated by several factors, such as wnt, bone morphogenetic proteins or fibroblast growth factor, are important for NCC development, no direct links with integrins have been described (reviewed in Gammill and Bronner-Fraser, 2003). Liu and Jessel described a role

for Rho B in the early delamination of NCC from the neural tube, but without excluding a role for other Rho proteins in later events (Liu and Jessell, 1998). Furthermore, it has been recently shown that wnt11 plays a role in NCC migration on fibronectin through the noncanonical wnt signaling pathway (De Calisto et al., 2005). In principle, these two pathways could cooperate with integrins in NCC migration. In addition, involvement of cytoskeletal protein paxillin in NCC migration can be postulated. Paxillin is an adaptor protein that integrates adhesion and growth factor-dependent signals with changes in actin organization and gene expression (Turner, 2000). Paxillin is expressed in migrating NCC and their derivatives (sensory neurons and glial cells) and isolated paxillin-null cells manifest impaired cell migration and spreading, which suggest a role for paxillin in NCC migration and differentiation (Hagel et al., 2002). This question will require conditional mutagenesis, as paxillin knockout mice show a phenotype similar to fibronectin knockout mice: appearance of defects at E8.5 and death at E9.5 (Hagel et al., 2002). Finally, it could be hypothesized that some of these signaling pathways may act on the transcription factor Krox20/Egr2, as it is involved in a second-wave migration of some NCC derivatives: Krox 20 expression is transiently restricted to the NCC-derived boundary cap cells at E10 and E15.5 in the trunk, before being induced in immature Schwann cells (Maro et al., 2004). It has been recently shown that boundary cap cells give rise to a second wave of migration that contributes to neurons and glia in dorsal root ganglia and dorsal roots, and that Krox-20 is important in this process (Maro et al., 2004).

SCPs and Immature Schwann Cells

Expression and Role of Integrins

SCPs appear at E12.5 in the mouse (E14.5 in the rat), Fig. 1. Their differentiation from NCC is associated with a rearrangement of the set of ECM receptors and associated molecules on the surface of the cells. SCPs migrate within mature nerve branches and they possess extensive sheet-like processes that contact and form junctions with processes from neighboring SCPs, thereby surrounding large groups of axons with which they are in close apposition (reviewed in Jessen and Mirsky, 2004). SCPs are destined to become immature Schwann cells, a transition that occurs by E15.5 in the mouse (E17 in the rat). Both SCPs and immature Schwann cells are actively proliferating cells that migrate along bundles of axons.

Immature Schwann cells associate with bundles of axons and insert cellular processes into them in order to segregate axons larger than 1 μm. One of the characteristics of the commitment to immature Schwann cell fate is the assembly of a BL at their abaxonal surface. Immature rat Schwann cells in vivo express $\alpha_1\beta_1$-, $\alpha_5\beta_2$-, and $\alpha_6\beta_1$-integrins, and low levels of $\alpha_2\beta_1$ - and $\alpha_3\beta_1$-integrin (Fernandez-Valle et al., 1994; Stewart et al., 1997; Previtali et al., 2003b). In vitro work on postnatal rat Schwann cells described the presence of $\alpha_6\beta_1$, $\alpha v\beta_8$ as a fibronectin receptor, an low levels of $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, and $\alpha v\beta_3$-integrins (Milner et al., 1997). Given that Schwann cells dedifferentiate in culture in the absence of axons, this expression may represent that of premyelinating Schwann cells.

Generation of conditional null mice for β_1-integrin with two different Cre mice produces severe sorting defects in peripheral nerves, demonstrating that a receptor containing β_1-integrin is the major candidate to function in radial axonal sorting (Feltri et al., 2002; Pietri et al., 2004). β_1-integrin null Schwann cells in vivo show morphological evidence suggesting a defect in cytoplasmic processes formation and maintenance, indicating a major role for β_1-integrin in cytoskeletal/actin rearrangements during axonal sorting (Feltri et al., 2002). Interestingly, only minor sorting defects were found in spinal roots or in trigeminal nerves (Feltri et al., 2002). However, the sorting defect was not caused by impaired longitudinal migration, because β_1-integrin-null Schwann cells manifest only slightly delayed longitudinal migration along axons (Pietri et al., 2004) and Schwann cell number in β_1-integrin-null sciatic nerves is not decreased (Feltri, Nodari and Messing, unpublished data). These results may suggest that β_1-integrins have a role in radial sorting only in specific districts of the PNS, as has been seen for different laminins (Yang et al., 2005). In vitro experiment on rat Schwann cells with blocking antibodies has revealed that an $\alpha\beta_1$-integrin receptor is necessary for migration on laminin. Because $\alpha_6\beta_1$-integrin is the major laminin receptor expressed by immature Schwann cells, this should be the receptor involved. However,

antibody-blocking experiments in vitro showed that blockage of α_6-integrin subunit does not impair Schwann cell migration on laminin (Milner et al., 1997), whereas block of $\alpha v\beta_8$-integrin impairs migration on fibronectin (Milner et al., 1997). Assuming that longitudinal migration of Schwann cells occurs on fibronectin and radial sorting depends on laminins, it is possible that $\alpha v\beta_8$-integrin is the integrin receptor responsible for longitudinal migration, whereas β_1-integrins are necessary for radial sorting (at least in sciatic nerves).

In addition to cytoskeletal rearrangements associated with radial sorting, the process of axonal fasciculation involves also the matching of Schwann cell and axon numbers. The matching is achieved by both an increase in proliferation and, as they are dependent on axonal contact for survival, an increase in apoptosis for supernumerary Schwann cells (reviewed in Jessen and Mirsky, 1997; Mirsky and Jessen, 1999). The absence of α_2- and α_4-laminin chains causes an impairment in Schwann cell proliferation (Yang et al., 2005), whereas absence of γ_1-laminins causes both reduced proliferation and survival, more evident postnatally, but also present before birth (Yu et al., 2005). Interestingly, in the absence of β_1-integrin, Schwann cell proliferation and survival are normal (Feltri et al., 2002). Because all laminin receptors detected before birth contain β_1-integrin (Previtali et al., 2003b), and no other laminin receptor is newly expressed in the absence of β_1-integrin (Feltri et al., 2002), perhaps an unidentified receptor mediates proliferation/survival signals from laminins.

Signals Involved

SCPS migrate longitudinally along axons, and when they become immature Schwann cells their number increases owing to proliferation and survival. Thus, most of the studies of SCPs and immature Schwann cells have focused on the expression of growth factors, their receptors, the signaling cascade that they activate, and transcription factors (reviewed in Jessen and Mirsky, 2004). In immature Schwann cells, proliferation is promoted by neuregulin-1, which induces dimerization and phosphorylation of the neuregulin receptors ErbB2 and ErbB3 (reviewed in Jessen and Mirsky, 2004). It has been proposed that this pathway may cooperate with a pathway activated by $\alpha_6\beta_1$-integrin to control cell proliferation: an in vitro model by Fernandez-Valle and colleagues proposes that in subconfluent Schwann cells a protein complex, similar to that forming at focal adhesions, made up of paxillin, schwannomin/merlin, FAK, β_1-integrin, and ErbB2/ErbB3 is localized at the membrane and promotes motility and cell growth. When Schwann cells reach confluency, glial growth factor receptor is internalized in a merlin-dependent manner and the complex is disrupted, promoting cell cycle arrest (Fernandez-Valle et al., 2002). Interestingly, this pathway may link $\alpha_6\beta_1$-integrins to the development of tumors in the hereditary disorder neurofibromatosis type 2 (NF2), as NF2 is caused by mutations in schwannomin/merlin that lead to the formation of vestibular schwannomas (Rouleau et al., 1993; Trofatter et al., 1993). Mutations in NF2 often fall in the exon 2 of merlin, which encodes a domain important for the interaction with paxillin and may impair this regulation (Fernandez-Valle et al., 2002).

So far, little is known about the signals involved in radial sorting. The β_1-integrin complex present in Schwann cells and including FAK, paxillin, and merlin (Obremski et al., 1998; Chen et al., 2000) could be important during axonal sorting. Other possible players are ezrin-radixin-moesin (ERM) proteins, activated by the GTPase RhoA, similarly to the member of the same protein superfamily, merlin (Shaw et al., 2001). ERM proteins are localized to early Schwann cell processes opposed to forming nodes of Ranvier, and at microvilli tips in immature nerves (Shaw et al., 1998; Gatto et al., 2003). It is possible that during axonal sorting, Schwann cells activate a signaling cascade through Rho GTPases and ERM proteins downstream of different receptors in the different districts of the PNS.

If longitudinal migration of immature Schwann cells and radial sorting of promyelinating Schwann cells are processes regulated by different ECM molecules and integrin receptors, it is also possible that downstream signaling is different in the two processes, even if the final effect of actin cytoskeleton polymerization, process elongation, and cellular advancement is similar. Indeed disruption of actin polymerization in dorsal root ganglia–Schwann cell cultures interfere with axonal association and segregation and with the transcriptional induction of myelin genes (Fernandez-Valle et al., 1997).

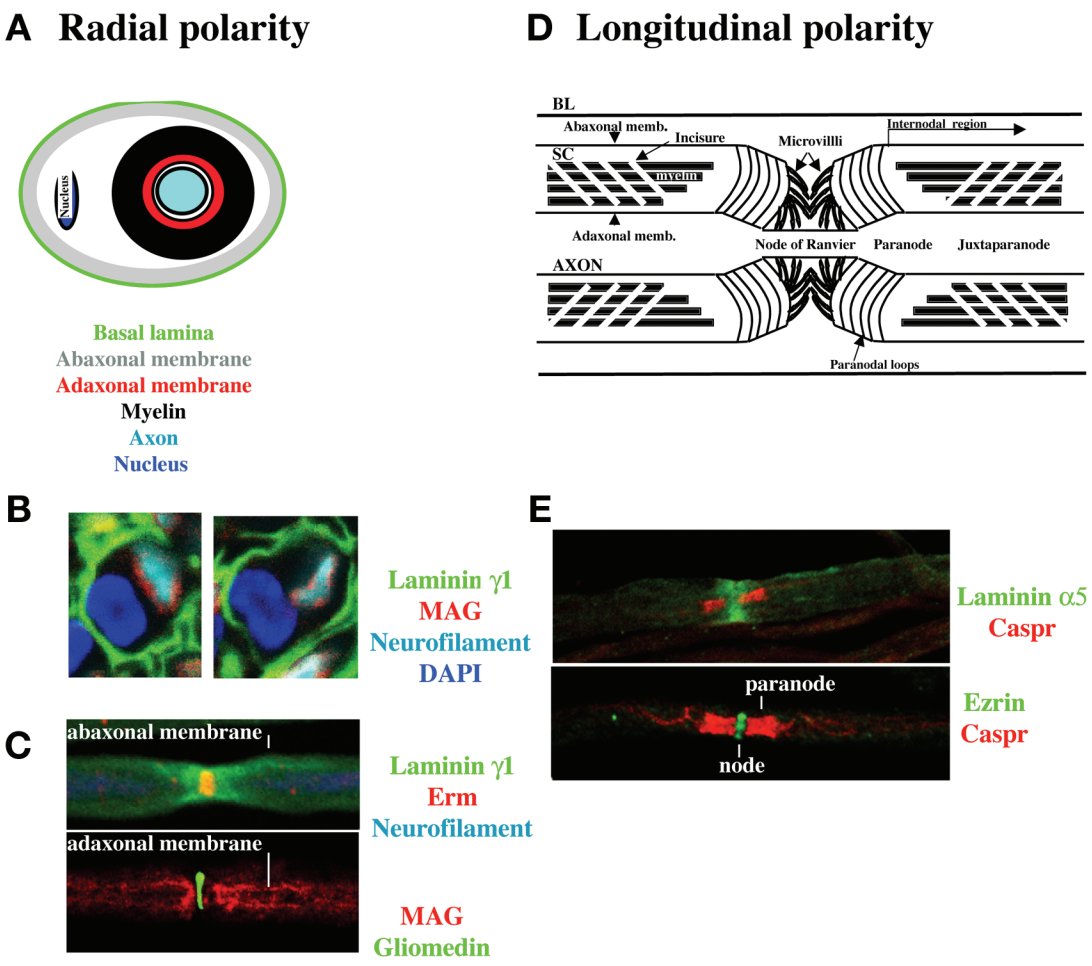

Fig. 2. Schwann cell polarity. Radial (**A,B,** and **C**) and longitudinal (**D,E**) polarity. (A) Scheme, and (B) confocal image of a transverse section of a myelinated axon. Blue denotes neurofilaments staining; red MAG staining, an adaxonal marker; black, compact myelin; and green γ_1-laminin, an extracellular component. (C) A confocal image of teased fibers representing radial polarity in a longitudinal perspective. In green, staining for laminin-γ_1 showing the abaxonal surface. In red, staining for MAG, labels the adaxonal surface. The node of Ranvier is indicated by staining for two different markers: ERM in red (upper panel C) and gliomedin in green (lower panel C). (D,E) Longitudinal polarity: organization of a myelinated fiber represented by (D) a schematic view and (E) a confocal image of a teased fiber. Some components of this organized structure manifest a very restricted expression: (upper panel E) Laminin-α_5 in the BL is detected only over the node and the paranode. (Lower panel E) ERM proteins are present exclusively in the microvilli (green signal). The paranode region is labeled with antibodies against Caspr (red signal). BL, basal lamina; MAG, myelin-associated protein; ERM, ezrin-radixin-moesin.

Promyelinating and Myelinating Schwann Cells

Expression of Integrins

Differentiation of immature Schwann cells into promyelinating Schwann cells occurs around birth and coincides with the achievement of a one-to-one relationship with a larger caliber axon. In the process, promyelinating Schwann cells must reorganize a BL around themselves. When Schwann cells achieve the promyelinating state, they start to express the non-integrin laminin receptor dystroglycan. Myelinating Schwann cells express $\alpha_6\beta_4$- and $\alpha_7\beta_1$-integrin receptors and still maintain $\alpha_6\beta_1$ at the abaxonal surface (Fig. 2). Immunofluorescence experiments on

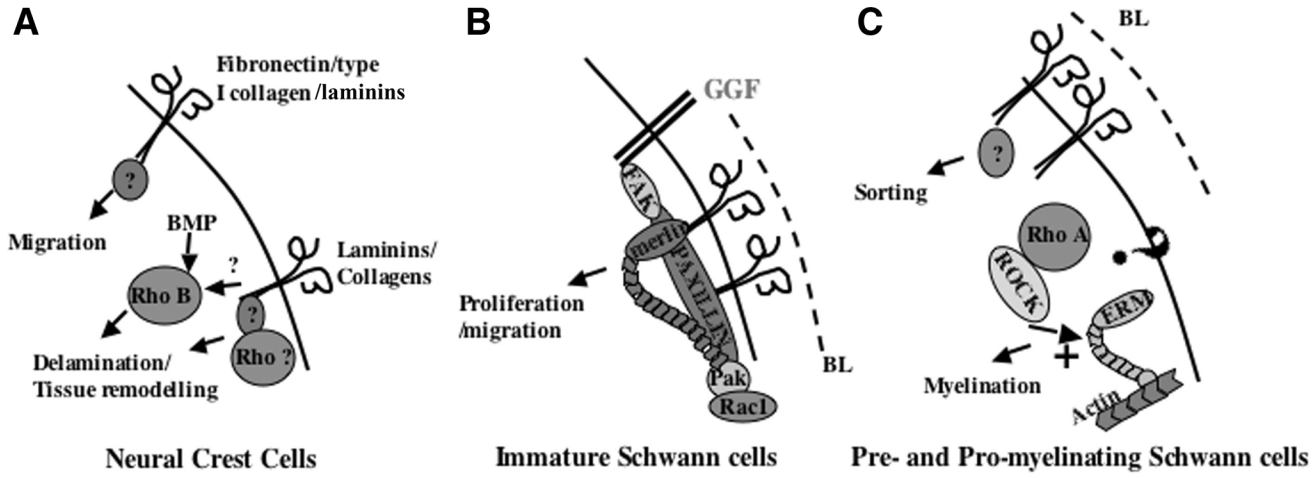

Fig. 3. Known signaling pathways engaged on integrin activation in the Schwann cell lineage. **(A)** NCC migrate prevalently on fibronectin and type-I collagen through $\alpha_3\beta_1$-, $\alpha v\beta_3$-, and $\alpha_8\beta_1$-integrins. Early delamination of NCCs from the neural tube is dependent on Rho B, which is activated by BMP and probably by laminins via an unknown pathway. Later stages of delamination, instead, are Rho B-independent and laminin-dependent probably via other Rho proteins. **(B)** Immature Schwann cells are actively proliferating and migrating cells. It has been proposed that a protein complex containing merlin/schwannomin and paxillin senses cell density and controls proliferation and migration on fibronectin/laminin (Fernandez-Valle et al., 2002). **(C)** Premyelinating Schwann cells interact with laminins in order to radially extend processes through bundles of axons. Signaling is probably mediated by Rho small GTPases. GTPases have a role in myelination: in fact, Rho A is implicated in internode formation via ROCK. ROCK also activates ERM proteins that may be involved in the very first stages of myelination. NCC, neural crest cells; BMP, bone morphogenic factor; GGF, glial growth factor; BL, basal lamina; Rock, Rho-associated kinases; ERM, ezrin-radixin-moesin.

spinal root and sciatic nerve sections of E17.5 mice suggested that $\alpha_6\beta_4$-integrin starts to be expressed in a nonpolarized, diffuse pattern just before the onset of myelination (Previtali et al., 2003b). When Schwann cells start to form myelin, $\alpha_6\beta_4$-integrin appears in a polarized pattern of expression at the abaxonal surface (Feltri et al., 1994; Quattrini et al., 1996; Previtali et al., 2003b). $\alpha_7\beta_1$-integrin starts to be expressed in the first week after birth at the abaxonal surface of myelinating Schwann cells (Previtali et al., 2003a). Constitutive ablation of α_7-subunit does not cause any impairment in myelinating Schwann cells (Previtali et al., 2003a), whereas analysis of nerve development in a β_4-integrin-conditional null mouse is in progress.

Signals Involved

Little is known about the signaling downstream of integrins in promyelinating and myelinating Schwann cells. As for other small Rho GTPases, RhoA may be downstream of integrins. In particular there is evidence that RhoA signaling may have a role in myelination, after Schwann cells have achieved the promyelinating state. In fact, inhibition of an effector of RhoA, Rho-associated kinase (ROCK) in Schwann cell–neuron cocultures affects ERM phosphorylation and results in aberrant myelination with shorter internodes and multiple myelin domains (Fig. 3C). ROCK inhibition does not alter Schwann cell proliferation and apoptosis (Melendez-Vasquez et al., 2004), at least at the stage examined.

Nonmyelinating Schwann Cells

Nonmyelinating forming Schwann cells express specifically $\alpha_1\beta_1$-integrin, a dual laminin–collagen receptor (Toyota et al., 1990; Fernandez-Valle et al., 1994; Stewart et al., 1997). Integrin-$\alpha_1\beta_1$ is also upregulated in dedifferentiating Schwann cells after injury, and it is downregulated in myelin-forming Schwann cells after axonal contact. Generation of α_1-integrin-null mice (Gardner et al., 1996) did not

produce any obvious nerve phenotype, but non-myelinating Schwann cells were not specifically investigated. It is also plausible that α_1-integrin is required for nerve regeneration in a restricted window of time after injury.

Schwann Cell Polarity

Polarity is a common feature of a broad variety of cell types. In prokaryotes it is restricted to cell division, whereas in eukaryotes it is associated with various cellular functions, such as asymmetric division in the embryo, epithelial morphogenesis, cell migration, and signaling. Mammalian epithelia are a geometrically simple model system for polarity: they are polarized along their apical–basal axis (A/B polarity) and they polarize in distinct steps: establishment of cell–cell contact, formation of specific cellular junctions and segregation of apico–basolateral membrane domains, all steps necessary for the maintenance of cell polarity in all systems (reviewed in Drubin and Nelson, 1996). Similar to epithelia, neurons are also highly polarized cells with two distinct types of processes that extend from the cell body: multiple dendrites from one side and a single axon from the opposite side, very early regulatory mechanisms control this specification (Li, 2005).

Schwann cells also develop a precise polarity resulting from reciprocal interactions with axons that permits organization of a myelinated fiber (Salzer, 2003). The geometric organization of a myelinated fiber is more complicated than that of stratified epithelia, as the Schwann cell must achieve dual polarities, both radial and longitudinal (Fig. 2). Similar to all other polarized cells, this corresponds to precise compartmentalization of different molecular components. Radial polarity in a myelin forming Schwann cell includes an abaxonal surface, away from the axon and adjacent to the BL. This domain contains mainly ECM receptors including integrins (Fig. 2A–C). Crossing the compact myelin sheath, there is an adaxonal surface next to the axon, highly enriched in adhesion molecules that may mediate interactions with the axon, such as myelin-associated glycoprotein (MAG) (Fig. 2A–C). The longitudinal polarity instead is defined by nodes of Ranvier (Fig. 2C–E). The myelin segment, which extends from one node to another, corresponds to the elongation of a single Schwann cell and is called internode. The region closest to the node is called paranode and the juxtaparanode separates it from the internode (Fig. 2). At the level of the node, Schwann cells form cytoplasmic extensions that connect nodal axolemma, called microvilli. Longitudinal polarity is characterized by a precise distribution of specific proteins as well. For example, microvilli contain F-actin, ERM proteins (Fig. 2C–E), gliomedin (Fig. 2C, lower panel) (Eshed et al., 2005) and syndecans (Goutebroze et al., 2003). In analogy with epithelia, Schwann cells form specific cellular junctions that separate regions of compact and non-compact myelin. Compact myelin consists of alternating apposed layers known as the intraperiod and major dense line and contains myelin proteins such as P0, PMP22, and myelin basic protein. Noncompact myelin contains specialized junctional proteins forming adherens, tight, and gap junctions. These junctions are present at the level of the paranode, in which they connect different wraps of myelin sheath (autotypic junctions) or seal axonal and Schwann cells membranes (axo-glial junctions), and in Schmidt-Lanterman incisure of Schwann cells, in which they serve as a connection between inner and outer cytoplasmic portions of the same Schwann cells. The peculiarity of Schwann cell junctions is that they may be present between two different membranes of the same Schwannn cell (autotypic junctions).

Preliminary evidence suggests that laminins and integrins may be important in regulating Schwann cell polarity. For example, the expression of integrins in Schwann cells is polarized and this is developmentally regulated. The $\alpha_6\beta_4$-receptor is diffusely distributed in promyelinating Schwann cells, and become restricted to the abaxonal region in myelinating Schwann cells (Fig. 1) (Feltri et al., 1994; Previtali et al., 2003b). Moreover, laminin isoforms are differentially distributed along the BL over Schwann cells during development and in adult tissue (reviewed in Feltri and Wrabetz, 2005). For example, it was recently shown that laminin-α_5 specifically localized over node and paranode (Vagnerova, 2003; Occhi et al., 2005) suggesting a possible role of this laminin in that domain, related to longitudinal polarity (Fig. 2E).

Conclusions

Although laminin genes are mutated in human diseases that include peripheral neuropathies, it is

unlikely that we will find mutations in integrin genes in hereditary neuropathies, owing to the fact that they would lead to a lethal phenotype, or due to the presence or compensation/redundancy. Despite this, in vivo studies have demonstrated that integrins are important for several steps in Schwann cell development: Schwann cell-specific disruption of β_1-integrin causes a severe neuropathy (Feltri et al., 2002) with defects in radial sorting, and integrins are probably also involved in formation and maintenance of a normal myelin sheaths and polarized fibers. This, taken together with the multiple nerve phenotypes of laminin mutants, supports the hypothesis that differential temporal and topographic expression of laminins and their receptors subserve multiple roles in nerve development.

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