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Myelination and Regional Domain Differentiation of the Axon

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Abstract

During evolution, as organisms increased in complexity and function, the need for the ensheathment and insulation of axons by glia became vital for faster conductance of action potentials in nerves. Myelination, as the process is termed, facilitates the formation of discrete domains within the axolemma that are enriched in ion channels, and macromolecular complexes consisting of cell adhesion molecules and cytoskeletal regulators. While it is known that glia play a substantial role in the coordination and organization of these domains, the mechanisms involved and signals transduced between the axon and glia, as well as the proteins regulating axo–glial junction formation remain elusive. Emerging evidence has shed light on the processes regulating myelination and domain differentiation, and key molecules have been identified that are required for their assembly and maintenance. This review highlights these recent findings, and relates their significance to domain disorganization as seen in several demyelinating disorders and other neuropathies.

1 Introduction

One of the most critical processes of both the central and peripheral nervous systems is myelination, involving the ensheathment and insulation of axons by glial cell membranes. As the glial cells, comprising Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS), contact and continually wrap their membranes around axons, they create polarized domains (Bhat 2003; Salzer 2003). These domains include the node, paranode, juxtaparanode, and internode.

During development, axo–glial interactions mediate the restriction of ion channels into specific membrane domains; that is, potassium channels in the juxtaparanode and sodium channels in the node of Ranvier, which, in turn, allow for the rapid and efficient conduction of the nerve impulse. The exact mechanisms governing the segmentation of ion channels into specific domains is elusive, but evidence has shown that disruption of paranodal axo–glial junctions leads to severe impairments of saltatory conduction, motor coordination, and myelination (Bhat 2003; Salzer 2003; Salzer et al. 2008). These phenotypes are often seen in demyelinating disorders and other neuropathies, which exemplify the importance of axo–glial junctions to the steady state kinetics of the action potential and proper nervous system functioning. Recent findings have identified several critical molecules and signaling pathways mediating the formation of axo–glial junctions and the regional organization of ion channel domains in the axonal membrane. In this review, new advancements in our knowledge of myelination and the differentiation of four domains in myelinated fibers will be highlighted, in addition to

discussing the mechanisms regulating their formation, maintenance during normal functioning, and disease onset and progression.

2 Myelination of Axons

Myelination is a process whereby specialized cells of the nervous system, termed glia, elaborate double membrane wrappings around axons, creating an insulating layer that promotes the fast conduction of nerve impulses. The many wrappings effectively increase total membrane resistance and decrease total membrane capacitance between nodes of Ranvier, which greatly reduces “leakage” of current across the internodal membrane. The “sparing” of axoplasmic current, in combination with very fast internodal electrotonic conduction, rapidly depolarize the downstream nodal membrane to threshold.

While myelination is required in both the PNS and CNS, there are distinct differences between the myelin forming cells with respect to the proteins and the signals required for myelination in these two systems. In the PNS, as Schwann cells differentiate, they will assume one of the two fates: they will either (1) form a 1:1 relationship with an axon and myelinate it or (2) extend multiple processes that will ensheath several axons (Jessen and Mirsky 2005).

Oligodendrocytes, on the other hand, extend multiple processes that will contact and myelinate several axons, up to forty separate axons at a time (Simons and Trotter 2007). While there are several factors that affect glial cell differentiation, such as growth factors and the extracellular matrix (ECM), the most notable is the axon phenotype, which determines its diameter. Those axons greater than 1 μm in diameter will be myelinated; whereas those smaller than 1 μm will be ensheathed. Interestingly, axonal diameter also determines the length of the internode, the segment of myelin between two nodes, as well as the thickness of the myelin layer(s), but the exact mechanisms governing the detection of axonal thickness by Schwann cells and oligodendrocytes remains elusive.

Premyelinating Schwann cells are distinctly bipolar, with processes extending longitudinally along the length of an axon. This extension will ultimately determine the location of the nodes of Ranvier and the internodal length. Once the internode is defined, signals from the axon and the ECM induce Schwann cells to extend their membrane laterally and spiral inwardly around the axon. The continuous wrapping of the Schwann cell membrane facilitates the development of the adaxonal (i.e., adjacent to the axon) and abaxonal (i.e., abutting the ECM) membrane layers. On the abaxonal side, Schwann cells are surrounded by a specialized ECM known as the basal lamina. The basal lamina is unique to the PNS and is formed by the Schwann cells to assist with their maturation and differentiation into a myelinating phenotype (Chernousov and Carey 2000; Court et al. 2006). Another unique feature of peripheral Schwann cells is the formation of nodal microvilli. These structures are small protrusions that extend beyond the distal-most paranodal loop and contact the underlying node. These structures are believed to participate in the formation of the node and mediate communication between the axonal node and the adjacent Schwann cell (Gatto et al. 2003; Ichimura and Ellisman 1991; Melendez-Vasquez et al. 2001).

Oligodendrocytes, unlike Schwann cells, are multipolar cells that have numerous processes extending from their cell bodies. These processes mediate the defasciculation and separation of axons, to which, eventually, the majority of the processes will attach to, and also myelinate several different axons. As mentioned earlier, one oligodendrocyte has the ability to myelinate as many as forty axons (Simons and Trotter 2007). The ensheathment of multiple axons by oligodendrocytes suggest that different signaling mechanisms govern their ability to identify neighboring cells and to distinguish the node and other axonal domains compared to Schwann cells. At this time, little is known about these mechanisms or the molecules involved. Additional distinctions between oligodendrocytes and Schwann cells are the absence of

microvilli overlying the node and a basal lamina, which is absent in the parenchyma of the central nervous system (Hildebrand et al. 1993; Melendez-Vasquez et al. 2001).

Because of the absence of the nodal protrusions, it is unclear how oligodendrocytes mediate intercellular signaling during the formation of the node. There is, however, evidence that oligodendrocytes secrete specific factors that coordinate the clustering of nodal components preceding myelination (Kaplan et al. 2001, 1997). Additionally, the existence of perinodal astrocytes are hypothesized to interact with nodal components, and thus may provide signaling cues to adjacent myelinating oligodendrocytes (Black and Waxman 1988; Hildebrand et al. 1993). The absence of basal lamina from oligodendrocytes suggests that other ECM components or environmental factors may provide the binding sites for anchorage requisite for myelination, but at this time this remains an unresolved issue. Although substantial differences exist between the mechanisms of myelination between PNS Schwann cells and CNS oligodendrocytes, one common feature is the ability of both types of glia to potentiate the development of polarized axonal domains during myelination. The formation of these domains (viz. the node, paranode, juxtaparanode, and internode) is crucial for proper saltatory conduction of the action potential. Key molecules are involved with the formation of these domains and their absence results in grave consequences as discussed below (Fig. 1).

3 Axonal Domains of Myelinated Axons

3.1 The Node of Ranvier

The nodes of Ranvier are short, myelin-free segments of axonal membrane that are distributed at regular intervals along myelinated nerve fibers, in which the action potential is regenerated in a saltatory manner. These regions are enriched in voltage-gated sodium (Nav) ion channels, which occupy a density of approximately $1,500 \mu\text{m}^{-2}$ (Waxman and Ritchie 1993). Nav channels are heterotrimeric complexes comprised of a pore-forming α -subunit that regulates ion flow and one or more transmembrane-spanning β -subunits that mediate both extracellular and intracellular interactions (Isom 2002; Yu and Catterall 2003). During development, a transition between the α -subunits occurs, in which Nav1.2, present in immature nodes, is replaced by Nav1.6 in adult nodes (Boiko et al. 2001). While all mature nodes in the PNS express Nav1.6 exclusively, subsets of adult CNS nodes express Nav1.2 and Nav1.8 (Arroyo et al. 2002). The significance of this exchange in subunits is currently unknown, but may pertain to varying activity of the subunits. What is known is that the Nav channels are essential for the proper conduction of the nerve impulse, because loss of Nav1.6 causes a dramatic decrease in conduction velocities, accompanied with abnormal nodal and paranodal structure (Kearney et al. 2002).

Several proteins expressed in the node are known either to interact with and/or mediate Nav channel function, including the cytoskeletal proteins ankyrin G (AnkG), β IV spectrin, α II spectrin, and the cell adhesion molecules (CAMs) *neurofascin* (NF186), and *NrCAM* (Salzer 2003). AnkG belongs to a family of scaffolding proteins that function to stabilize membrane-associated proteins by linking them to the actin-spectrin cytoskeleton within specialized domains (Bennett and Lambert 1999). It is expressed in both the axon initial segment (AIS) and the nodes of neurons, where it interacts with Nav channels through either their α -, or β -subunit(s) (Bouzidi et al. 2002; Kordeli et al. 1995; Lemaillet et al. 2003; Malhotra et al. 2002). This interaction is essential for the targeting of Nav channels to the AIS, as mice deficient in a cerebellar-specific AnkG show a loss of Nav channel clustering at the AIS of Purkinje neurons and the inability to fire action potentials (Zhou et al. 1998a). Presumably, the loss of AnkG within the nodes may result in a similar loss of Nav channels; however, while the AIS and nodes have many similarities in molecular composition, their functions may be differentially regulated.

AnkG associates with the cytoskeleton through its interaction with b-spectrins; specifically, *bIV spectrin*, which is also localized to the nodes of Ranvier and AISs (Berghs et al. 2000; Komada and Soriano 2002). This interaction is critical for the clustering of AnkG, and in turn, Nav channels to the node inasmuch as loss of *bIV spectrin* in mice results in reduced levels of these proteins in nodes and AISs, increased nodal axonal diameter, and severe tremors and impaired nerve conduction (Komada and Soriano 2002; Lacas-Gervais et al. 2004). Concomitantly, AnkG null Purkinje neurons show loss of β IV spectrin in the AIS, revealing a codependent relationship between AnkG and β IV spectrin and their localization to these critical areas of action potential propagation (Jenkins and Bennett 2001).

Recently, another spectrin, *α II spectrin*, was identified at the nodes, and proposed to have physiological significance to the nodal architecture. Normally, *α II spectrin* is associated with the paranode, but new findings have indicated the presence of *α II spectrin* in immature nodes (Garcia-Fresco et al. 2006; Ogawa et al. 2006). Initially, *α II spectrin* is expressed in both the nodes and the paranodes in developing nerves, but gradually becomes restricted to the paranode as myelination progresses. Although its final site of expression resides at the paranode, *α II spectrin* was proposed to play a role in the assembly of the nodes and the clustering of Nav channels, since loss of its expression in the neurons of zebra fish resulted in abnormal nodal dimensions (Voas et al. 2007). Although electrophysiological data from these mutants could not be assessed, it is probable that the increase in nodal length observed may perturb the propagation of the action potential and slow down nerve conduction. Interestingly, the progressive restriction of *α II spectrin* during myelination may suggest that *β IV spectrin* replaces it in mature nodes. Further analysis of the significance of *α II spectrin* to the node or the paranode may prove to be important to our understanding of how these domains are initially constructed.

Neurofascin (NF186) and *NrCAM* are members of the L1 subfamily of immunoglobulin (Ig) cell adhesion molecules (CAMs) that mediate cell–cell, and cell–matrix interactions (Grumet 1997; Volkmer et al. 1992). Both proteins are expressed in the nodes and AISs and interact with AnkG through a conserved region present in the cytoplasmic domain of each protein (Davis et al. 1996; Lustig et al. 2001; Zhang et al. 1998). The association of AnkG with NF186 is mediated by tyrosine phosphorylation. The unphosphorylated form of NF186 is able to associate with AnkG at the nodes, while phosphorylation perturbs the interaction of NF186 with AnkG (Garver et al. 1997; Zhang et al. 1998). Through their interaction with AnkG, both *NrCAM* and NF186 are thought to coordinate Nav channel clustering and node formation, because accumulation of these CAMs occurs prior to the presence of both AnkG and Nav channels in PNS nodes (Custer et al. 2003; Lambert et al. 1997; Lustig et al. 2001). Additionally, experiments utilizing function-blocking antibodies against the CAMs revealed that both Nav channels and AnkG failed to accumulate at the nodes in *in vitro* cocultures (Lustig et al. 2001). Furthermore, mice deficient in *NrCAM* expression resulted in delayed aggregation of Nav channels and AnkG to the nodes, although they did eventually cluster and the nodes functioned normally (Custer et al. 2003). Conversely, other findings suggest that AnkG is responsible for the initial assembling of Nav channels and CAMs to CNS nodes (Jenkins and Bennett 2002).

At this time, no mutational analysis of NF186 alone has been conducted, but a conventional null mutant lacking both isoforms of neurofascin, NF186, and the glial neurofascin (NF155) expressed in the paranode exhibited complete loss of nodal and paranodal formation (Sherman et al. 2005). These mice died at postnatal day 6 (P6), which prevented further characterization of their functions in axo–glial domain formation and maintenance. However, recent findings by the same group revealed that reexpression of NF186 in the mutant axons resulted in the relocalization of AnkG and Nav channels to the node, suggesting that NF186 coordinates the formation of the node and the clustering of the Nav channels (Zonta et al. 2008). These results

are very compelling and further analysis of a true NF186 knockout would greatly contribute to our future understanding of its role in nodal development and organization.

A unique set of nodal proteins exists that is expressed specifically in the PNS. These proteins, which reside within the Schwann cell microvilli that extend from the outermost paranodal loop of myelin, contact the node and are proposed to function in nodal development and formation. An array of proteins are expressed within these small protrusions, including *gliomedin*, *ERM* (ezrin/radixin/moesin) proteins, *EBP-50* (ezrin binding protein 50), *dystroglycan*, *RhoA-GTPase*, and *syndecans* (Eshed et al. 2005; Gatto et al. 2003; Goutebroze et al. 2003; Melendez-Vasquez et al. 2004, 2001; Saito et al. 2003). Of particular interest is Gliomedin, which was shown to interact with NF186 and NrCAM in the nodal axolemma. This interaction was proposed to coordinate the clustering of these proteins into the PNS node (Eshed et al. 2005). Similarly, ablation of dystroglycan, a laminin receptor, in myelinating Schwann cells resulted in reduced Nav channel clustering at the nodes and disrupted nodal microvilli formation (Saito et al. 2003). These findings indicate that Schwann cells may function to coordinate the initial formation and clustering of nodal components, most likely through their interactions with NF186 and NrCAM, and further exemplify the importance of glial signals to nodal development, particularly in the PNS. While we have yet to discover the exact mechanisms regulating nodal development, it is evident that all the proteins discussed above play significant roles in nodal domain formation, maintenance, and function. Further studies to elucidate their mode of action may provide insight into the mechanisms regulating these processes in disease.

3.2 The Paranode

3.2.1 The Function of the Vertebrate Paranodal Region—The paranode is a region in myelinated nerve fibers where the terminal myelin loops form specialized septate-like junctions with the axolemma. These axo–glial junctions are directly contiguous to the nodes of Ranvier and are thought to act as a barrier or molecular sieve, which impedes free diffusion between the nodal space and juxtaparanodal periaxonal space (Pedraza et al. 2001). As myelination progresses, the internodal myelin layers are compacted. This compaction forces cytoplasm to redistribute outwardly towards the paranode, and results in the formation of the characteristic paranodal loops. These paranodal loops, representing the initial wraps of myelin, also function as an anchorage point to stabilize the glial cell as myelination proceeds. Accordingly, the appearance of transverse bands, or the septate-like junctions, is first observed at the distal-most loop of the preforming paranode. Formation of the bands then progresses inwardly towards the juxtaparanode. The continual wrapping of myelin and the formation of these “septae” serve to cluster the juxtaparanodal potassium (Kv) channels and separate them from the nodal Nav channels. Thus, formation of the paranodal axo–glial junctions is crucial to the demarcation and segmentation of axonal domains in nerve fibers that allow for proper conduction of the nerve impulse (see below).

3.2.2 Functional Relevance of Invertebrate Septate Junctions to Vertebrate Paranodal Axo–Glial Junctions—Septate junctions (SJs) are one of the most widely and diversely expressed junctions in invertebrates. These junctions play critical roles in governing cell polarity, cell adhesion, and the diffusion of molecules between cells (Banerjee et al. 2008, 2006a, b; Baumgartner et al. 1996). During epithelial cell development, SJs form in a region known as the apico-lateral domain, an area just basal to the apical hemiadherens junctions, and zonula adherens. These junctions form in a circumferential pattern and function to maintain epithelial cell polarization and integrity by sustaining a constant distance of 15 nm between adjoining cells. Additionally, SJs were found to act as a diffusion or paracellular barrier to restrict the movement of molecules between the apical and basolateral surfaces of epithelia (Banerjee et al. 2008, 2006a; Carlson et al. 2000; Tepass et al. 2001). Similarly, vertebrate paranodal axo–glial junctions function by providing a periaxonal barrier to ionic

diffusion between regional longitudinal axonal domains of myelinated fibers and thus are considered orthologous to invertebrate SJs (Banerjee and Bhat, 2007; Bhat 2003; Salzer 2003). Perhaps the most relevant example of invertebrate SJs to vertebrate paranodal axo–glial junctions is found in the nervous system of *Drosophila* (Fig. 2). Similar to oligodendrocytes in the mammalian CNS, *Drosophila* glial cells encompass several axons with their membrane, but instead of myelinating the axons, they simply ensheath them (Banerjee and Bhat 2008). A separate layer of perineurial cells develops around the glia, similar to the layer of fibroblasts that form the perineurium in vertebrates, which surrounds myelinated nerve fasciculi in the PNS (Hildebrand et al. 1993; Jessen and Mirsky 2005). To limit the flow of ions from the axons, septate junctions are formed between glial cells in a homotypic fashion and heterotypically between the glial and perineurial cells. As is the case with epithelial cells, these SJs function as a paracellular barrier to regulate or prevent the diffusion of ions and molecules from the hemolymph (Banerjee and Bhat 2007; Tepass et al. 2001). Similarly, vertebrate paranodal axo–glial junctions behave as diffusion barriers between Nav channels in the node and Kv channels in the juxtaparanode. By maintaining the segregation of ion channels into their respective domains, the paranode facilitates proper saltatory conduction, while also ensuring repolarization of the action potential.

The identification of *Drosophila* SJs and the proteins involved in their formation and stabilization has led to the elucidation of several homologues expressed in vertebrate paranodes (Fig. 3). Of note are the *Drosophila* cell adhesion molecules (CAMs) *neurexin IV*, *contactin*, and *neuroglian*, and the cytoskeletal protein, *coracle* (Banerjee et al. 2006b; Faivre-Sarrailh et al. 2004). Loss of expression of these genes has devastating effects on septate junction formation, and the stabilization of the paracellular barrier (Banerjee et al. 2006a; Baumgartner et al. 1996). The vertebrate counterparts of these molecules are *contactin-associated protein* (Caspr), *contactin*, the 155 kDa isoform of neurofascin (NF155), and *protein 4.1B*, respectively (Bhat et al. 2001; Boyle et al. 2001; Peles et al. 1997; Tait et al. 2000). All these proteins localize to the paranode, and through genetic ablation and biochemical analysis we have begun to understand their importance to the formation of the paranode and the maintenance and segregation of axonal domains.

3.2.3 Key Regulators of Paranodal Formation and Stability—Initial studies aimed towards elucidating the proteins involved in the formation of the paranodal axo–glial junctions were focused around the Neurexin/Caspr/Paranodin (NCP) family of cell recognition molecules (Bellen et al. 1998). This superfamily is composed of five vertebrate homologues, that is, Caspr–Caspr5 (Spiegel et al. 2002). Of these isoforms, only Caspr is expressed at the paranodes, where it becomes enriched in the axolemma when myelination arrests (Arroyo et al. 1999; Bhat et al. 2001; Einheber et al. 1997; Menegoz et al. 1997). Caspr (aka Paranodin) is a Type 1 transmembrane protein that is comprised of a large expansive extracellular domain and a short intracellular domain. The extracellular domain of Caspr contains an array of subdomains implicated in cell–cell and cell–matrix interactions, including discoidin, EGF (epidermal growth factor), laminin G, and fibrinogen-like domains (Bellen et al. 1998; Bhat 2003; Denisenko-Nehrbass et al. 2002). While the specific function of each individual domain remains elusive, it is known that the extracellular domain of Caspr is vital for the formation of transverse septae, as evidenced by the absence of these junctions in Caspr-deficient mice (Bhat et al. 2001). The periaxonal space of the Caspr mutants was often invaded by astrocytic process in the CNS, Schwann cell microvilli in the PNS, and extracellular matrix components. The lack of stability resulted in the eversion of the paranodal loops, in which severe ataxia and reduced conduction velocity were also noted in these mice. Additionally, the juxtaparanodal rectifying *Shaker*-like potassium channels, Kv1.1 and 1.2, were frequently mislocalized to the paranodal region, while the nodal Nav channels remained unchanged. This alteration is likely to be mediated by the short intracellular domain of Caspr, which includes proline rich and glycophorin C domains that are known to interact with the actin cytoskeleton and coordinate

it (Denisenko-Nehrbass et al. 2003a, b; Gollan et al. 2002; Menegoz et al. 1997). Thus, these findings exemplified the importance of Caspr to the formation of the axo–glial junctions and to the distribution, segregation, and organization of ion channels within axonal domains.

Support for the role of Caspr in the organization of axonal domains and the formation and stabilization of paranodes came with the discovery of its protein binding partners and regulators. On the axonal side, Caspr associates with contactin, a glycosylphosphatidylinositol (GPI)-anchored protein belonging to the Ig superfamily (Brummendorf and Rathjen 1996; Falk et al. 2002). Contactin is also enriched at the paranodes of myelinated fibers and forms a *cis* interaction with Caspr in the axolemma (Peles et al. 1997; Reid et al. 1994). This interaction is mediated by the extracellular domain of Caspr and the fibronectin III domains of contactin (Bonnon et al. 2003; Faivre-Sarrailh et al. 2000). Genetic ablation of contactin in mice results in an analogous phenotype as Caspr mutants, with the loss of transverse bands and attendant paranodal disorganization (Boyle et al. 2001). In addition, Caspr fails to localize to the plasma membrane, suggesting that contactin may function to transport and/or stabilize Caspr to the paranodal axonal membrane. Indeed, further characterization of the interaction between contactin and Caspr reveals that a mutually exclusive relationship exists between the two proteins. Without contactin, Caspr is retained in the endoplasmic reticulum and fails to traffic to the paranodal axolemma (Bonnon et al. 2003; Faivre-Sarrailh et al. 2000). Concomitantly, contactin cannot stably localize to the paranode in the absence of Caspr, but rather is found in the nodes in the CNS (Bhat et al. 2001; Rios et al. 2000).

Several scaffolding and cytoskeletal components reside at the paranodes, and emerging evidence suggests a critical role for these proteins in the maintenance of axo–glial junctions. Protein 4.1B belongs to the Band 4.1 superfamily of membrane cytoskeletal linking proteins (Hoover and Bryant 2000; Parra et al. 2000; Sun et al. 2002). It is present in the paranodes, and distributed diffusely in the juxtaparanodes of axons; to date, it is the only known protein 4.1 isoform localized to the axo–glial junctions (Ohara et al. 2000). Protein 4.1B contains a conserved FERM (four point one/ezrin/radixin/moesin) domain that mediates its binding with several transmembrane receptors, including Caspr at the paranodes and Caspr2 at the juxtaparanodes (Denisenko-Nehrbass et al. 2003b; Garcia-Fresco et al. 2006; Gollan et al. 2002). The conserved cytoplasmic GNP (glycophorin C/Neurexin IV/paranodin) domain of Caspr mediates its association with protein 4.1B (Gollan et al. 2002; Sousa and Bhat, 2007). Loss of the GNP domain resulted in the internalization of the Caspr–contactin complex from the axonal plasma membrane, suggesting an important role for Protein 4.1B in the stabilization of this complex and axo–glial junctions at the paranode (Gollan et al. 2002). It remains to be seen what effect the loss of protein 4.1B might have on the formation and maintenance of the paranode; however, studies of the *Drosophila* orthologue, coracle, suggest a key role for the FERM domain-containing proteins in the organization and stabilization of septate junctions (Baumgartner et al. 1996; Laval et al. 2008; Ward et al. 1998). Recent work has identified the presence of a macromolecular complex consisting of protein 4.1B, Ankyrin B (AnkB), and the aII and bII spectrins that associates with Caspr and contactin at the paranodal junctions (Garcia-Fresco et al. 2006; Ogawa et al. 2006). Loss of Caspr resulted in the absence of AnkB, or its diffusion out of the paranodes, which reflects the importance of Caspr in orchestrating the assembly of the underlying paranodal cytoskeleton (Garcia-Fresco et al. 2006; Pillai et al. 2007; Sousa and Bhat, 2007). Given that the junctional specialization of the paranode functions as a “fence” in regulating the diffusion of ions associated with functional activity in the axon, it will be interesting to see how future studies may elucidate the roles of these cytoskeletal proteins in segregating the axonal domains.

The interaction(s) between glia and axons is important in regulating myelination and the formation of axonal domains. As oligodendrocytes and Schwann cells associate with the axon, they initiate the clustering of the paranodal components in the axolemma. While several axonal

proteins have been identified at the paranode, only one glial protein is known to localize to the paranodal axo–glial junctions, the 155 kDa isoform of neurofascin, NF155 (Collinson et al. 1998; Moscoso and Sanes 1995; Tait et al. 2000). NF155 is a CAM that belongs to the L1 subgroup of the Ig superfamily (Davis and Bennett 1993; Holm et al. 1996; Volkmer et al. 1992). It differs from the axonally expressed NF186, in that it lacks the mucin-like domain and contains an extra fibronectin type III domain (Davis et al. 1996). The exact mechanisms governing their varied expression pattern is not clear, but over 50 alternatively spliced isoforms exist that appear to be developmentally regulated (Hassel et al. 1997). Initial mutational analysis in mice resulted in the loss of both isoforms of neurofascin, NF186 and NF155 (Sherman et al. 2005). These mice were unable to form either the nodal complex or the paranodal axo–glial junctions and died at postnatal day 6. Caspr and contactin were diffused throughout the axon in these mutants. Interestingly, upon reexpression of NF155 in glia, the paranodes reorganized and Caspr and contactin relocated to the axolemma. This coincided with previous reports that expression of NF155 colocalized with Caspr during the clustering of the paranodal loops during myelination (Tait et al. 2000). Further analysis of the proposed interaction(s) revealed that the extracellular domain of NF155 could associate with the Caspr–contactin complex in vitro (Charles et al. 2002). These results are somewhat controversial, however, as other studies employing similar in vitro techniques find that NF155 binds contactin and that the presence of Caspr perturbs this interaction (Gollan et al. 2003). Although it seems unclear as to how these proteins associate, these results do suggest a role for NF155 in paranodal organization, possibly through interactions with Caspr and/or contactin.

Recent findings utilizing Cre-loxP conditional knockout strategies has provided new insights into the specific role of NF155 in axo–glial formation and maintenance. Using a Cre recombinase driven by the CNPase promoter, which is specific for glia, it was shown that loss of NF155 expression alone results in the loss of septate-like junctions, paranodal disorganization, and the failure to segregate axonal domains (Pillai et al. 2009). Because of the strong correlation of phenotype with those of Caspr and contactin mutants, it was shown that both of these axonally expressed proteins were absent from the paranodal axolemma in NF155 mutant nerves. This suggested that NF155 and Caspr and/or contactin interact with the glial NF155 protein, and that this interaction mediates the formation and stability of the Caspr–contactin complex to the paranodal-domain of the axonal plasma membrane. The ability of NF155 to direct the formation of the paranode and stabilize the complex formation suggests that it may interact with cytoskeletal proteins. Although no protein-binding partners have been identified, it has been shown that the intracellular domain of NF155 contains a FERM domain-binding motif (Gunn-Moore et al. 2006). Given that FERM domain-containing proteins are cytoskeletal linkers, NF155 may coordinate signals originating from the axon that direct changes in the glial cytoskeleton prior to and during myelination; whereby, axonal domains are partitioned.

3.2.4 The Importance of Lipids to Paranodal Formation—As noted previously, myelin is the elaborated glial membrane sheath that circumferentially wraps axons, which becomes compacted to form a multilamellar layer of insulation. The major constituents of myelin are lipids, which make up approximately 70–85% of the dry weight (Morell et al. 1994). Nearly one-third of the lipid mass is comprised of glycosphingolipids, galactosylceramide (GalC), and its sulfated derivative, sulfatide (Coetzee et al. 1996; Norton and Cammer 1984). These lipids were shown to have a significant role in oligodendrocyte development, the initiation of myelination, and the stabilization of the compacted myelin layers (Marcus and Popko 2002). Studies aimed towards elucidating the function of these lipids during myelination resulted in the development of knockout mice deficient in CGT (UDP-galactose:ceramide galactosyltransferase), the enzyme that synthesizes galactosylceramide (Coetzee et al. 1996). Surprisingly, these mice formed myelin, but displayed severe ataxia, tremors, and slowed nerve conduction. Further examination of these mice revealed the

appearance of disorganized and everted paranodal loops and the absence of axo–glial junctions (Dupree et al. 1998).

Similar phenotypes were also observed in CST (cerebroside sulfotransferase) null mice (Honke et al. 2002; Ishibashi et al. 2002). These mice differ from CGT, in that they are unable to synthesize only sulfatide, whereas galactosylceramide is still produced. These findings implied that glial sulphosphingolipids were essential to the formation and/or maintenance of the paranode. The exact mechanisms in which these lipids function to promote axo–glial junction formation are unknown, but it is hypothesized that they are responsible for creating lipid microdomains, where paranodal components may reside and function. In this context, it is noteworthy that studies have indicated that NF155, Caspr, and contactin are present in lipid-rich microdomains (Bonnon et al. 2003; Schafer et al. 2004). Furthermore, NF155, Caspr, and contactin are mislocalized outside of the paranode in CGT mice (Dupree et al. 1999; Hoshi et al. 2007; Poliak et al. 2001). Alternatively, it may be that an unidentified protein(s) may be localized in the domain to interact with these lipids, either in *cis* or *trans* relation, and may thereby direct the localization of Caspr, contactin, and NF155 to the paranode.

3.3 The Juxtaparanodal Region

The juxtaparanode is a potassium channel-rich region that lies underneath the compact myelin sheath, just proximal to the paranodes. It has been proposed that the significance of localizing potassium channels in this domain is concerned with repolarization of the action potential, as well as counteracting instability of excitability, especially in the transition zone between myelinated and unmyelinated portions of distal motor nerve fibers (Chiu et al. 1999; Rasband et al. 1998; Zhou et al. 1998b). Specifically, two rectifying *Shaker*-like potassium channels, Kv1.1 and Kv1.2, occupy a majority of the domain, along with the CAMs, Caspr2, and TAG-1 (Arroyo et al. 1999; Mi and Berkowitz 1995; Rasband et al. 1998; Wang et al. 1993). Caspr2 is expressed strictly in the axon and is functionally related to Caspr, except that it contains a PDZ binding motif in its intracellular domain that Caspr lacks (Poliak et al. 1999). Caspr2 was shown to be critical to the maintenance of Kv channels in the juxtaparanode, as its absence in mice resulted in the diffusion of these channels throughout the internode (Poliak et al. 1999). Subsequently, it was found that the PDZ binding domain links Caspr2 to Kv1.1 and Kv1.2 (Poliak et al. 2003). Initial reports predicted that PSD-95, a PDZ-containing protein found within the juxtaparanode, might be a potential candidate for this interaction, as it has been shown to associate with Kv β 2; however, later studies reported that PSD-95 and Caspr2 do not interact (Baba et al. 1999; Poliak et al. 1999). Furthermore, Kv1.1 and Kv1.2 remained clustered at the juxtaparanodes of PSD-95 deficient mice; therefore, it remains to be elucidated what PDZ protein(s) links Caspr2 and Kv channels in the juxtaparanode (Rasband et al. 2002).

Another critical component of the juxtaparanode is the GPI-anchored adhesion molecule, TAG-1 (Furley et al. 1990; Karagogeos et al. 1991; Traka et al. 2002). TAG-1 belongs to the Ig superfamily and shares 50% sequence homology with contactin; it is expressed in both neurons and myelinating glia (Traka et al. 2002). Like Caspr and contactin in the paranode, TAG-1 and Caspr2 associate and form a *cis* complex within the juxtaparanodal axolemma. This interaction is mediated by the Ig-domains of TAG-1 (Traka et al. 2003; Tzimourakas et al. 2007). Interestingly, the axonal *cis* complex of TAG-1/Caspr2 binds with TAG-1 expressed in glia by homophilic interactions between the TAG-1 molecules (Traka et al. 2003). Mutational analysis in mice revealed that a codependent relationship between TAG-1 and Caspr2 exists, similar to that of Caspr and contactin, where the accumulation of either protein to the juxtaparanode relies on the expression of the other (Poliak et al. 2003; Traka et al. 2003). Deletion of *TAG-1* in mice resulted in the exclusion of Kv1.1 and Kv1.2 channels from the underlying juxtaparanodal axonal membrane concomitant to Caspr2 null mice (Traka et al.

2003). Although the Kv channels were absent from the membrane, no change in conduction velocity was observed in TAG-1 mutant nerves (Traka et al. 2003). Accordingly, Caspr2 mutant mice displayed normal conduction (Poliak et al. 2003). While no electrophysiological abnormalities were observed in TAG-1 or Caspr2 mutant mice, the effects of extended loss of these proteins to action potential propagation and resting potential are unknown. Examining these effects may be of particular interest to disease pathology because Kv1.1 mutant mice display backfiring of the action potential and extended hyperexcitability (Zhou et al. 1999). Additionally, TAG-1 function has been recently implicated in learning and cognition and suggests an important role for these proteins in long-term plasticity (Savvaki et al. 2008).

3.4 The Internodal Region

The internode comprises the area between the juxtaparanodes, and accounts for 99% of the total length of a myelinated nerve segment (Salzer 2003). As previously mentioned, the length of this region is determined by the axonal diameter and represents the most extended region involving axo–glial interactions. Very little is known about the mechanisms regulating the ability of glia to detect the axonal diameter to determine the final internodal length. Additionally, as animals grow, this region lengthens to compensate for the extension of limbs (Abe et al. 2004). A handful of proteins have been found to localize to the adaxonal membrane at the inner-most lip of the myelin membrane. These proteins include the nectin-like proteins, *Nec11* and *Nec14*, the polarity protein *Par-3*, and the *myelin associated glycoprotein* (MAG). The nectin-like proteins (Nec1) are cell adhesion molecules that belong to the Ig superfamily (Takai et al. 2003). They are related to the nectin cell adhesion proteins, but differ by their inability to bind to afadin. Necls are often associated with tight junctions and contain PDZ binding motifs that may facilitate their interaction with cytoskeletal scaffolding proteins. Recent findings have implicated *Nec11* and *Nec14* in myelination. These proteins were expressed in a polarized fashion at the inner mesaxon or at the initial contact of the glial myelin membrane with axons (Maurel et al. 2007). Heterophilic interactions between *Nec11* on axons and *Nec14* on glia mediate the initial attachment and wrapping of the glial membrane (Maurel et al. 2007). Perturbation of *Nec14* by use of RNAi in Schwann cell-DRG neuron cocultures inhibited myelination (Maurel et al. 2007). Similar results were observed in mutant mice deficient in the axonally expressed *Nec11* (Park et al. 2008). These results indicate the importance of these proteins to myelination and axo–glial recognition and adhesion.

Par-3 is a PDZ-containing adaptor protein, which regulates polarity in many cell types (Ohno 2001). The *Drosophila* homolog of *Par-3*, *Bazooka*, functions to establish cell polarity in epithelial cells through its restricted expression at the apical surface (Pinheiro and Montell 2004). Like *Bazooka*, the vertebrate *Par-3* is a polarized protein that is expressed asymmetrically in glia at the junction of the inner mesaxon or the adaxonal layer. It is proposed to facilitate the adhesion of glia to axons (Chan et al. 2006). Disruption of *Par-3* function in Schwann cells resulted in the inability of Schwann cells to adhere to and myelinate axons (Chan et al. 2006). Further analysis determined that *Par-3* is associated with *p75NTR*, a BDNF receptor that promotes myelination through its PDZ1 domain. While other binding partners of *Par-3* are unknown, it is likely that it interacts with many cell adhesion receptors that contain PDZ binding motifs. Of particular interest is the possible association of *Nec14* and *Par-3*. Given their colocalization to the inner mesaxon, the presence of a PDZ binding motif in *Nec14*, and the observation in mice that an association of *Par-3* and nectins occurs, these proteins may interact to coordinate axo–glial adhesion prior to myelination (Takekuni et al. 2003).

4 Mechanisms Regulating Axonal Domain Formation and Maintenance

A key question regarding the assembly of axonal domains remains elusive; namely, what comes first, the node or the paranode? Is formation of the nodes dependent on or independent of paranodal formation? Furthermore, does the long-term maintenance of the node require intact

paranodes? A central theme has emerged from the numerous studies on axo–glial domains, which is that distinct differences exist between the mechanisms regulating the partitioning of axonal domains in the PNS vs. the CNS. This is, to a degree, to be expected as myelination proceeds in different fashions between oligodendrocytes and Schwann cells. Later we attempt to interpret recent findings to shed light on the organization of axonal domains and their dependence on adjacent domains for long-term stability and maintenance.

4.1 Nodal Formation in the PNS and CNS: Extrinsic vs. Intrinsic; Dependent vs. Independent Mechanisms

In the PNS, Schwann cell development and myelination are intimately linked to axonal and ECM stimuli and adhesion. The maturation of Schwann cells into a myelinating phenotype and the segmentation of axonal domains rely on forming this intimate relationship. Studies have shown that PNS domain organization begins with the formation of the node and proceeds inwardly towards the internode (Melendez-Vasquez et al. 2001; Poliak et al. 2001). An extrinsic pattern of nodal assembly is suggested for the PNS as the presence of NF186 and NrCAM precedes that of Nav channels and the cytoskeletal components, AnkG and β IV spectrin (Peles and Salzer 2000; Salzer 2003). Additionally, the appearance of these nodal components is observed before the paranodal proteins, Caspr and contactin, which suggests that PNS nodes form prior to and independently of the paranodes (Melendez-Vasquez et al. 2001).

Schwann cell distal microvilli are believed to coordinate the formation of nodes through their association with NF186 and/or NrCAM. This association may occur through gliomedin, as it is expressed in the nodal microvilli and interacts with both NF186 and NrCAM (Eshed et al. 2005). Disruption of gliomedin by RNAi resulted in the absence of Nav channels at the node and suggests that glia facilitate the initial assembly of the node by mediating NF186 localization. This follows an extrinsic pattern of node formation, in which extracellular signals initiate node formation and also transmit signaling to the axonal cytoskeleton to stabilize the complex once formed. Interestingly, gliomedin can be secreted, resulting in soluble forms that are accumulated and incorporated into the surrounding basal lamina in the perinodal space (Eshed et al. 2007, 2005). In the absence of Schwann cells, axons exposed to the soluble form of gliomedin formed nodes, further supporting a role for glia in the formation of the node extrinsically. Dystroglycan, a laminin receptor expressed in Schwann cell nodal microvilli, may also mediate the extrinsic formation of PNS nodes, as genetic ablation of its gene in mice results in severe conduction blockade, dysmyelination, and the loss of nodal Nav channel clustering (Occhi et al. 2005; Saito et al. 2003). Additionally, these mice form normal axo–glial junctions at the paranodes, suggesting that paranodal formation does not require the formation of the node. It may also indicate that the formation of paranodes alone is not sufficient to cluster the nodal complex, although further studies in these mice revealed that AnkG and NF186 are still clustered at the node in the absence of Na_v channels. This suggests that dystroglycan may simply act to stabilize these ion channels to the node instead of coordinating the organization of the entire node. However, the presence of NF186 at these nodes still suggests that an extrinsic mechanism of assembly may occur albeit through alternative mechanisms.

Nodal formation in the CNS is considered to behave quite differently than that of the PNS. It is hypothesized that instead of an extrinsic glial mediated assembly, a more intrinsic form of coordination occurs, beginning with the cortical axonal cytoskeleton and assembling in association with the axolemma. One of the major factors contributing to this model of an intrinsic assembly is that oligodendrocytes do not extend nodal microvilli (Melendez-Vasquez et al. 2001). Additionally, NF186, NrCAM, and Nav channels were recruited to the node subsequent to the appearance of AnkG in intermediate nodes (Jenkins and Bennett 2002). An attempt to disrupt AnkG resulted in the generation of a cerebellum specific knockout (Zhou et

al. 1998a). Aberrant Nav channel clustering was observed in the AISs of Purkinje neurons, yet AnkG was still present in the nodes of these mutants; therefore, the effects of loss of AnkG on CNS node formation could not be evaluated (Jenkins and Bennett 2001; Zhou et al. 1998a). While it is evident from these findings that AnkG expression is important for Nav channel clustering at AIS, they also present evidence that several isoforms of AnkG may exist that differentially localize to separate areas of Nav channel clustering. Although oligodendrocytes lack nodal microvilli, they still retain the ability to cluster Nav channels through the secretion of soluble factors (Kaplan et al. 1997). This mechanism of clustering is similar to the aggregation of Nav channels to PNS nodes by secreted gliomedin, and suggests that oligodendrocytes may, in fact, assemble nodes without direct contact (Eshed et al. 2007, 2005). Further examination revealed that oligodendrocyte conditioned medium clustered Nav1.2, the immature Nav channel isoform; whereas, Nav1.6 present in mature nodes required myelination (Kaplan et al. 2001). Additionally, the early assembly required an intact axonal cytoskeleton, and suggests that certain intrinsic signals may still be required for CNS nodal formation and maturation. The identification of the soluble factor(s) released by oligodendrocytes will certainly be critical to the future analysis and elucidation of the mechanisms governing CNS node formation.

An alternative method of CNS assembly may involve signaling from perinodal astrocytes that contact the nodal domain. The relationship of these astrocytes to nodal function is not well characterized, but interestingly, these cells were shown to express the cytoskeletal mediating ERM protein *ezrin* (Melendez-Vasquez et al. 2001). As mentioned earlier, ERM proteins are also present in the nodal microvilli of Schwann cells, and their expression at these domains is coordinated with the extrinsic formation of the node in the PNS. Taken together, it is possible that perinodal astrocytes may compensate for the lack of nodal microvilli in oligodendrocytes and provide nodal assembly cues in a similar manner as Schwann cell microvilli. It would be interesting to see if these perinodal astrocytes express gliomedin, or a derivative, as well as dystroglycan.

Yet another possible mechanism of CNS node development is the idea that nodes are formed by the sequestration of nodal components from the axolemma by the formation of the paranodes. In support of this idea is the finding that Caspr expression in the forming paranode preceded the expression of Nav channels in the nodes of optic nerves (Rasband et al. 1999). Since oligodendrocyte myelination does not depend on the demarcation of the internode prior to myelination, as it does with Schwann cells, it is possible that oligodendrocytes begin to wrap their membrane while progressively extending processes along the axon. In this manner, the oligodendrocytes create a barrier that will induce the aggregation of the nodal proteins. In support of this mechanism, it was found in mice that reexpression of NF155 alone, in a complete neurofascin null background, resulted in the clustering of Nav channels (Zonta et al. 2008). These findings implicate a paranodal dependent formation of nodes in the CNS, but these mice also did not live past the complete knockout mice, and no assessment of conduction velocities was performed in these animals. Furthermore, mice deficient in Caspr expression form nodes in the CNS and PNS in the absence of intact axo–glial junctions, suggesting that paranodal formation is not a prerequisite for CNS node formation (Bhat et al. 2001). Concomitantly, NF155-specific knockout mice also form nodes independently of paranodal formation, further supporting the hypothesis that nodes form through separate autonomous mechanisms than paranodes (Pillai et al. 2009). Taken together, it appears that nodal stability may rely on paranodal domains, but the initial assembly of the node forms independently of other axonal domains.

New evidence has emerged regarding the requirement of paranodal axo–glial junctions in the maintenance and long-term stability of the node. Recently, Pillai et al. (2009) tested this hypothesis by knocking out NF155 in adult mice through the use of tamoxifen inducible Cre

recombinase, driven by the proteolipid protein (PLP) promoter (Doerflinger et al. 2003). They allowed the mice to reach adult stage (P23) and subsequently treated the mice with tamoxifen to induce glial-specific genetic ablation of neurofascin (NF155). They found that axo–glial junctions disassembled, and essentially unraveled, from the juxtaparanodal side distally outward towards the node. The progressive unraveling of the paranodes resulted in the invasion of the paranodal space with Kv channels. While the node remained unchanged initially, it was found that with extended time (up to 1 year posttreatment), NF186, Nav channels, and AnkG diffused out of the node (CT and MB, unpublished observation). Upon further examination, these same proteins were diffused out of the AIS, further revealing a progressive loss from the external axonal nodes sequentially upwards towards the AIS at the neuron cell body. This suggests that through development and maturation, the paranodes not only function to sequester ion channels to specific domains, but that they maintain segregation by stabilizing these components. Therefore, even though nodes may form independently of paranodes during development, their long-term integrity may rely upon paranodal axo–glial junctions. As intriguing as these results may be, further experimentation will need to be conducted to elucidate the signaling involved. Additionally, the use of this inducible system may help with future studies to elucidate the effects of progressive myelin loss on axonal domains, much like that seen in multiple sclerosis.

4.2 Disease Manifestation in the Absence of Segmented Axonal Domains

Several human neuropathies and disorders, such as multiple sclerosis and Charcot–Marie Tooth disease, result in the progressive demyelination of nerves, leading to axonal degeneration. There are varied causes to the development of these diseases, be it autoimmune attack or genetic predisposition, but the dissolution of axonal domain organization appears to be a central indicator of the development and progression of these disorders (Berger et al. 2006; Lubetzki et al. 2005; Nave et al. 2007; Oguievetskaia et al. 2005; Shy 2006; Trapp and Nave 2008). While NF155, NF186, Caspr, and contactin have not been directly associated with disease predisposition and onset, they have been shown to be disrupted in disease pathology (Coman et al. 2006; Howell et al. 2006; Mathey et al. 2007; Wolswijk and Balesar 2003). Emerging evidence has revealed that NF155 is targeted in multiple sclerosis (MS) (Howell et al. 2006; Maier et al. 2007, 2005; Mathey et al. 2007). Initially, it was found that NF155 expression was reduced in the paranodes of MS patients, and was considered an early marker for the ensuing demyelination of the nerve tracts (Howell et al. 2006; Maier et al. 2005). Further analysis revealed that NF155 had decreased association with lipid rafts in MS, and therefore, axo–glial junction stability was compromised (Maier et al. 2007). The mislocalization of Kv channels to the paranodes confirmed the dissolution of axo–glial junctions in the diseased lesions (Howell et al. 2006). A recent report revealed the presence of autoantibodies to NF155 and NF186 in patients with MS (Mathey et al. 2007). Interestingly, increasing amounts of autoantibodies were found in patients with chronic progressive MS compared to those with relapsing MS, suggesting that as the disease becomes more severe it targets these critical proteins, preventing the reformation of the paranodes and nodes. The inability of new oligodendrocytes to myelinate the affected lesions and organize axonal domains, due to the lack of both Neurofascins, would promote the axonal degeneration observed in chronic MS individuals. In support of the degeneration of paranodes in MS lesions, it was also found that loss of Caspr expression precedes demyelination in MS patients (Wolswijk and Balesar 2003). Other compelling evidence to support the role of intact axo–glial junctions in the prevention of axonal degeneration is found in Caspr mutants, in which loss of Caspr expression in mice resulted in the presence of axonal swellings and cytoskeletal abnormalities in Purkinje neurons of the cerebellum (Garcia-Fresco et al. 2006). These abnormalities precede the degeneration of axons, as seen in multiple neuropathies (Fabrizi et al. 2007; Lappe-Siefke et al. 2003; Rodriguez and Scheithauer 1994). In addition to their importance to axonal domain organization, the juxtaparanodal proteins, Caspr2 and Tag-1, have been implicated in autism, language impairment, as well as learning and cognition disorders, respectively, suggesting

other roles for these CAMs in nervous system function (Alarcon et al. 2008; Bakkaloglu et al. 2008; Savvaki et al. 2008; Vernes et al. 2008). While genetic ablation of axonal domain proteins has not been identified as the causative agent for disease manifestation, it is clear that these proteins are critical in preventing the progression of demyelinating neuropathies, and serve as possible therapeutic targets for the future treatment of these devastating disorders.

5 Concluding Remarks

To date, significant advancements through the development of mouse models have contributed greatly to our knowledge of the proteins involved in myelination and the segregation of axonal domains. While many of the proteins involved have been identified and characterized, future studies may provide more insight into the signaling mechanisms involved in the formation and stabilization of each domain. The future efforts are likely to be centered on cytoskeletal scaffolding proteins and the signaling mechanisms governing their function, expression, and localization to specific axonal as well as glial domains. Additionally, emerging advances in animal model systems are sure to facilitate research directed towards studying the effects of extended loss of axo–glial junctions. These studies will certainly shed light on the processes, proteins, and functions misregulated during disease, and may reveal new roles for many of the axo–glial junctional proteins in demyelinating disorders, axonopathies, and other neuropathies.

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Fig. 1.

Domain organization in myelinated PNS nerve fibers. Teased sciatic nerve fibers from wild-type ($+/+$; **a**), *Caspr* null (*Caspr*^{-/-}; **b**), and *Neurofascin*^{NF155} (*NF155*) specific null mice (*Cnpcre;Nfasc^{Flox}*; **c**) mice immunostained with antibodies against Kv1.1 (red), Caspr (blue), and Neurofascin 186 (NF186; green). In wild-type nerve fibers, localization of Kv1.1 fluorescence is restricted to the juxta- and paranode. Caspr staining marks the paranode and NF186 is a marker of the nodal region. In *Caspr* null fibers, the lack of paranodal axo-glial junctions results in the diffusion of potassium channels into the paranode, as evident by the presence of Kv1.1 fluorescence adjacent to NF186 staining at the node (**b**). Loss of NF155 expression results in the lack of *Caspr* fluorescence at the paranode and the redistribution of potassium

channels into the paranodal region, similar to *Caspr* mutants (c). In both mutants, the node remains unaltered as indicated by NF186 fluorescence

Fig. 2.

Comparative ultrastructure of *Drosophila* and mouse unmyelinated and myelinated nerve fibers. (a) Cross-sections of peripheral nerve fibers from *Drosophila* show the inner glia (G) ensheathing axons (a). Electron dense, ladder-like structures (arrow) known as septate junctions form between the outer perineurial (P) and the inner ensheathing glial cell (G) membranes (m) of *Drosophila* (arrowheads, D). Electron micrograph cross-section of a Remak bundle in the mouse peripheral nerve (b). Remak bundles consist of several small diameter axons (a) that are ensheathed by a single nonmyelinating Schwann cell. These fibers do not acquire myelination, and are similar in structure to *Drosophila* nerve fibers. Ultrastructure of a single myelinated mouse peripheral nerve fiber in cross-section (c). The continual wrapping of the Schwann cell myelin membrane (my) forms a multilamellar layer that is electron dense. A longitudinal section of the paranodal region of a myelinated axon (a) shows the septate-like junctions that form between the myelin loops (ml) and the underlying axolemma. Scale bars: (a) 2 μ m; (b) 1 μ m; (c) 0.5 μ m; (d, e) 0.2 μ m

Fig. 3.

Major components of septate junctions in *Drosophila* and mouse. The domain structure of *Drosophila* NrX IV, Contactin, Neuroglian, and Coracle, and their vertebrate counterparts in mouse, Caspr, Contactin, NF155, and Protein 4.1B reveals significant homology between these proteins (a). Schematic representation of the proteins involved in the formation of the paranodal axo-glial septate junctions in mouse (b). NF155 is expressed strictly in the myelinating glial within the paranodal loops. The presence of a FERM binding domain within NF155 predicts an interaction with a FERM protein, which may mediate signaling to the glial cytoskeleton. NF155 is hypothesized to bind to either Caspr or Contactin, but the exact mechanisms are yet unknown.