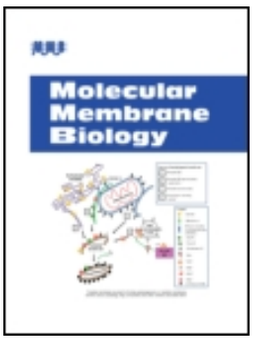

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Cellular junctions of myelinated nerves (Review)

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Summary

Myelinated nerves are specifically designed to allow the efficient and rapid propagation of action potentials. Myelinating glial cells contain several types of cellular junctions that are found between the myelin lamellas themselves in specialized regions of non-compact myelin and between the myelin membrane and the underlying axon. These include most of the junctional specializations found in epithelial cells, including tight, gap and adherens junctions. However, whereas in epithelial cells these junctions are formed between different cells, in myelinating glia these so called autotypic junctions are found between membrane lamellae of the same cell. In addition, myelinating glial cells form a heterotypic septate-like junction with the axon around the nodes of Ranvier and, in the peripheral nerve system, contact the basal lamina, which surrounds myelinating Schwann cells. This short review discusses the structure, molecular composition and function of the junctions present in myelinating cells, concentrating on the axo-glial junction.

Keywords: Myelin, Schwann cells, axo-glial junction, node of Ranvier, Schmidt-Lanterman incisures.

Introduction

Myelin, which is produced by Schwann cells in the peripheral nervous system or by oligodendrocytes in the central nervous system, covers the axon in segments that are separated by the nodes of Ranvier. The myelin sheath reduces the current flow across the axonal membrane by reducing the capacitance and/or increasing the resistance, thereby allowing saltatory movement of the nerve impulse from node-to-node. The myelin membrane is divided into two structurally and biochemically distinct areas: compact myelin, which results from fusion of the outer monolayers of two adjacent layers of the plasma membrane at the internodes, and regions of non-compact myelin (Arroyo and Scherer 2000, Peles and Salzer 2000). In myelinating Schwann cells, regions of non-compact myelin consist of the paranodal loops, Schmidt-Lanterman incisures (SLI), nodal microvilli, and the inner and outer edges of the myelin (figure 1).

At the end of each internodal segment, the compact myelin lamellae open up into a series of cytoplasmic loops called the paranodal loops. These glial loops are being separated from the axon by a narrow gap of 2–3 nm and form with it a series of septate-like junctions. In electron micrographs of longitudinal sections through the paranodal region, these junctions appear as a series of ladder-like densities (i.e. transverse bands) that arise from the axon and contact the glial membranes (Wiley and Ellisman 1980). In addition to generating the axo-glial junction, the paranodal loops are also connected between themselves through adherens, tight and GAP junctions (Fannon *et al*. 1995, Balice-Gordon *et al*. 1998). A second region of non-compact myelin is found at the Schmidt-Lanterman incisures, which are conical compartments of cytoplasm that divide the internodes into small islands of compact myelin. As the myelin lamellae spiral around the axon, the enclosed cytoplasm also follows a helical course, connecting the outer and inner belts of the myelinating glial cell. The incisures provide a relatively short diffusion pathway through the myelin sheath that links the membrane closer to the axon (adaxonal) and the perinuclear region of the Schwann cell (Balice-Gordon *et al*. 1998). An additional function that has been attributed to the incisures is to provide some degree of flexibility, and to protect the peripheral nerve from mechanical stresses during stretching and contraction (Singer and Bryant 1969). While the morphology of the incisures has been described in detail (Robertson 1958, Hall and Williams 1971, Sandri *et al*. 1977, Tetzlaff 1978, 1982), much less is known about the identity of the proteins involved in their development and maintenance. Myelin associated glycoprotein (MAG; Trapp *et al*. 1989) and a 155 kDa isoform of neurofascin (Tait *et al*. 2000), two cell adhesion molecules of the immunoglobulin superfamily, are enriched in the incisures but their function at this site is unknown.

In addition to the paranodal loops and the Schmidt-Lanterman incisures, areas of non-compact myelin are also present in the internal (adaxonal) and external (abaxonal) aspects of the myelin sheath. The adaxonal surface overlays the axon, while the abaxonal surface contacts the basal lamina that surrounds myelinating Schwann cells. At these sites, the edges of the sheath, called the inner and outer mesaxon, respectively, form adherens, and possibly tight junctions with the adjacent membrane layer (figure 1). In the PNS, microvilli are emanating from the outer aspect of the membrane and encapsulate the nodes of Ranvier. In contrast, myelinating oligodendrocytes do not contain such microvilli, and processes of perinodal astrocytes cover nodes in the CNS instead.

Myelinating glial cells are equipped with most of the junctional specializations found in epithelial cells, including, tight, gap and adherens junctions (Mugnaini and Schnapp 1974, Fannon *et al*. 1995, Scherer 1996, Balice-Gordon *et al*. 1998,). However, whereas in epithelial cells these junctions are formed between different cells, in myelinating glia they connect membrane lamellae of the same cell. These have been termed autotypic junctions (Fannon *et al*. 1995). In addition, myelinating glial cells form a heterotypic septate-like junction with the axon around the nodes of Ranvier, and, in the PNS, contact the basal lamina, which surrounds myelinating Schwann cells.

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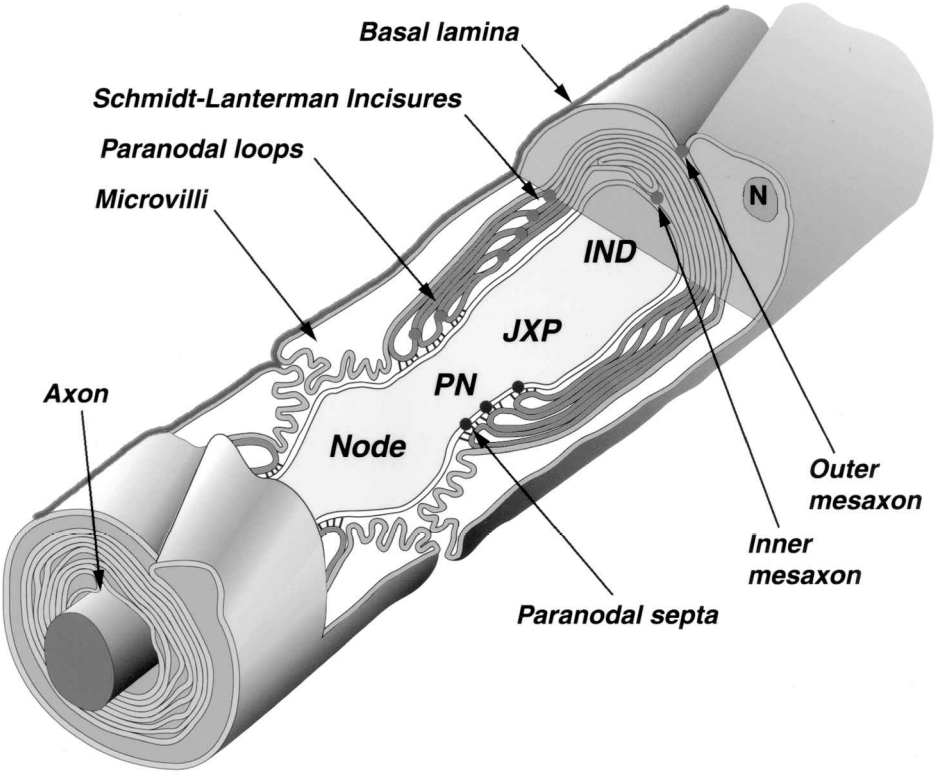

Figure 1. Schematic organization of peripheral myelinated nerve. At the internodes (IND), the axon is surrounded by a compact myelin sheath, which is disrupted by small pockets of cytoplasm at the Schmidt-Lanterman incisures. At the paranodal region (PN), the compact myelin is opened up, generating cytoplasmic loops that form a septate-like junction with the axons adjacent to the node of Ranvier (solid dots, which are coloured blue in the on-line version of this paper). The outer aspect of myelinating Schwann cells extends laterally, generating microvilli that encapsulate the nodes. The entire Schwann cell-axon unit is covered by a basal lamina. Autotypic junctions (shaded dots, which are coloured red in the on-line version of this paper) are found at the inner and outer mesaxon, in the Schmidt-Lanterman incisures and between the glial loops. Myelinated axons are divided into distinct domains to which different proteins are localized. Na^+ channels, neurofascin 186, Nr-CAM, ankyrin G and spectrin βIV are found at the nodes, while Caspr, contactin neurofascin 155 and protein 4.1B are found at the paranodal junction. An additional axonal domain is defined at the juxtaparanodal region (JXP), located at the ends of the internode below the compact myelin, and is enriched in K^+ channels and Caspr2.

Adherens junctions

Autotypic adherens junctions are found at the inner and outer mesaxon, as well as in the incisures and the paranodal loops (Fannon *et al*. 1995). A unique feature of these junctions at the paranodal loops is that the sub-plasmamembrane plaques transverse the entire cytoplasm and are fused to the adjacent plaque (Fannon *et al*. 1995). Similar to other cell types, these junctions contain the calcium-dependent cell adhesion molecule E-cadherin, as well as the cytoplasmic protein β-catenin, which connect it to the actin filaments (Trapp *et al*. 1989, Gumbiner 2000). It was suggested that these junctions function to stabilize newly formed wraps of myelin during development of the nerve (Fannon *et al*. 1995). This is consistent with older observations describing that lowering extracellular calcium, which inhibits cadherin-binding, affecting the integrity of the paranodes (Blank *et al*. 1974). Another proposed function of these junctions is to mediate rapid intracellular signaling, which would bypass a longer course around the spiral turns. In other cell types, adherens junctions contain two adhesion systems, namely the cadherin/catenin and nectin/afadin adhesion systems, which interact directly and cooperate to organize adherens junctions (Tachibana *et al*. 2000). Whether such a cooperation takes place in the generation of autotypic junctions in Schwann cells remains to be determined.

Tight junctions

EM and freeze fracture studies have revealed tight junction strands that are comprised of linear rows of intermembranous particles between adjacent Schwann cell membranes in the circumference of the myelinating cells. They are found in the inner and outer mesaxon, paranodal loops and the Schmidt-Lanterman incisures (Mugnaini and Schnapp 1974, Sandri *et al*. 1977, Tetzlaff 1982). At the incisures, they run spirally following the membrane helix connecting with the marginal junctions which extend the whole length of the internodes at the external and internal mesaxons. Tight junctions are specialized cell–cell contact sites in epithelial and endothelial cells that act as a selective permeability barrier (gate function), and as an intramembranous fence to restrict the movement of lipids and proteins from specific membrane domains (Mitic and Anderson 1998). Their

function in myelinating glia is less clear. They were proposed to link adjacent membranes for increased mechanical strength and to separate the glial outer membrane from the membrane of the compact myelin (Gow *et al*. 1999). Tight junctions are also present at the radial component of myelin, a series of radially arranged intralamellar strands that are found exclusively in CNS myelin (Peters *et al*. 1991). So far, the only tight junction protein found in myelinating glia is Osp/Claudin-11 (Morita *et al*. 1999). In its absence, CNS myelin lacks tight junction strands (Gow *et al*. 1999). However, Osp/claudin-11 cannot be detected in PNS myelin, suggesting that other members of the claudin family may be present in tight junctions in Schwann cells. Two tight junction proteins, including Mupp1 and claudin 5/6 were recently identified in Schmidt-Lanterman incisures in the sciatic nerve (Poliak, Matlis, Sabanay, Tsukita, Scherer and Peles, unpublished data). Mupp1, is a multi-PDZ protein, containing 13 such domains, which bind several proteins, including claudin-1 and junctional adhesion molecule (JAM) (Hamazaki *et al*. 2001). Other tight junction proteins such as occludin and JAM were not found in myelinating Schwann cells (Nagaoka *et al*. 1999, Poliak and Peles, unpublished data).

GAP junctions

GAP junctions flanked between two rows of tight or adherens junctions are found at the incisures and the paranodal loops of Schwann cells (Arroyo and Scherer 2000). Gap junctions result from the alignment and docking of two half channels (connexons) that span the two plasma membranes. Each connexon, in turn, is a multimeric assembly of protein subunits called connexins. Connexins form a multigene family of proteins whose members are distinguished according to their predicted molecular mass. Connexins are the protein subunits of gap junctional intercellular channels, which allow cells to share ions (electrical coupling), small metabolites and second messengers, thus coordinating a wide range of cellular mechanisms. In Schwann cells, gap junction channels are mainly composed of connexin32 (Cx32) (Scherer *et al*. 1995). Dye diffusion studies have demonstrated that these channels provide an intracellular pathway that facilitates the passage of ions and small molecules between the outer and inner cytoplasm (Balice-Gordon *et al*. 1998). Although the same signals may travel through the cytoplasmic spirals, this pathway is most likely several orders of magnitude slower than the putative radial route through intracellular gap junctions. Mutations in Cx32 lead to the development of the X-linked form of Charcot-Marie-Tooth disease (CMTX), demonstrating that Cx32 is essential for myelin homeostasis (Bergoffen *et al*. 1993). However, the precise identity of the signals that need to be exchanged through these channels is presently unknown (Ressot and Bruzzone 2000). To date, more than 150 different mutations in Cx32 were discovered, affecting its folding, targeting and various aspects of its gating properties (Abrams *et al*. 2000, Oh *et al*. 1997). In keeping with the proposed pathogenic role of Cx32, mice lacking Cx32 develop a progressive peripheral neuropathy with features similar to those of CMTX (Anzini *et al*. 1997, Scherer *et al*. 1998). Although much has been learned on the function of gap junctions, less is known about the mechanisms involved in their generation and maintenance. The generation of GAP junctions requires close proximity of the membranes involved and may be connected to the formation of tight and adherens junctions (Musil *et al*. 1990, Guerrier *et al*. 1995, Fujimoto *et al*. 1997, Laird *et al*. 1999). Although it is not clear whether this is the case (Ohsugi *et al*. 1997, Woodward *et al*. 1998), it appears that connexins do interact directly with various components of such junctions, including, ZO-1 and occludin (Giepmans and Moolenaar 1998, Toyofuku *et al*. 1998, Kojima *et al*. 1999, Nusrat *et al*. 2000). Furthermore, it was recently suggested that in addition to its function as a gap junction channel, Cx32 may be regulating the formation of tight junctions (Kojima *et al*. 1999), suggesting that gap and tight junctions in the incisures and the paranodes may be functionally coupled.

Axo-glial junction

The axons of myelinated nerves in the adult nervous system are sub-divided into several distinct functional domains that differ in their molecular composition (Peles and Salzer 2000). This organization is essential for the efficient and rapid propagation of action potentials via saltatory conduction. At the node of Ranvier, the axonal membrane contains high concentration of voltage-gated Na^+ channels that are responsible for the regeneration of the action potential. A second specialized region in the axon is defined at the juxtaparanodal region, located beneath the compact myelin at both sides of each internodal interval. This region is characterized by the presence of delayed rectifier K^+ channels of the Shaker family, Kv1.1, Kv1.2 and their Kvβ2 sub-unit, which may stabilize conduction and help to maintain the internodal resting potential (Rasband and Shrager 2000). At this site, the channels colocalize and create a complex with Caspr2, the second member of a growing family of putative cell recognition molecules (Poliak *et al*. 1999). The juxtaparanodal K^+ channels and Caspr2 are separated from the Na^+ channels at the node of Ranvier by the paranodal junction, a specialized septate-like point of contact that is formed between the axolemma and the paranodal loops of the myelinating cells (figure 2). These specialized junctions appear relatively late during development of myelinated nerves and are being first generated at the node-proximal region by the most outer paranodal loop and, then, continue gradually as additional loops are attached to the axon (Tao-Cheng and Rosenbluth 1983, Rosenbluth 1995, Pedraza *et al*. 2001). As a result, the paranodal junction is composed of a number of rings that represent each turn of the myelin warp and, thus, does not represent a uniform domain. The paranodal axo-glial junction is thought to have several functions: it provides attachment of the myelin sheath to the axon, separates the electrical activity at the node of Ranvier from the internodal region that lies under the compact myelin sheath, and it serves as a boundary that limits the lateral diffusion of membrane components (Rosenbluth 1995, Peles and Salzer 2000). In addition, the paranodal junction may also represent a site for bidirectional signalling between axons and myelinating glial cells.

The axonal membrane at the paranodal junction contains a complex of cell adhesion molecules that includes Caspr

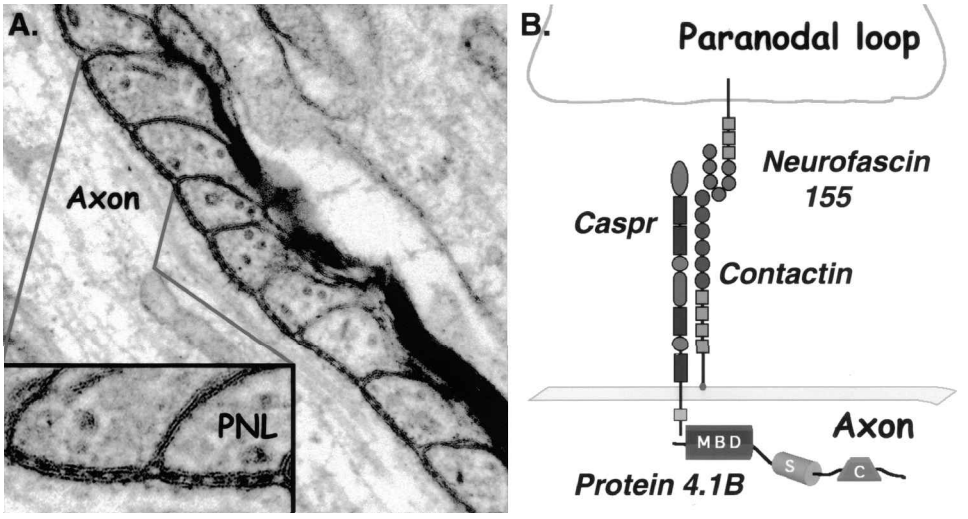

Figure 2. The paranodal axo-glial junction. (*a*) Electron micrograph showing the paranodal junction in longitudinal section of mouse spinal cord. Glial loops (PNL) form close attachment with the axon, separated by a narrow gap of 2–4 nm. This gap is filled with periodic densities, termed 'transverse bands' or 'septate-like' structures. (*b*) Adhesion components of the axo-glial junction include an axonal complex consisting of Caspr and the GPI-linked protein contactin. An isoform of neurofascin (NF155) represents a glial component of the paranodal junction and connects to the Caspr/contactin complex by binding to contactin. The Caspr/contactin complex is connected to the underlying axonal cytoskeleton through the interaction between the cytoplasmic domain of Caspr and protein 4.1B present at paranodes. The EM picture shown in (*a*) was generously provided by Dr Brain Popko, University of Chicago. Panel B was adapted from Peles and Salzer (2000).

(contactin associated protein, also known as paranodin (Einheber *et al*. 1997, Menegoz *et al*. 1997, Peles *et al*. 1997)) and the GPI-linked cell adhesion molecule contactin (Rios *et al*. 2000, Bhat *et al*. 2001, Poliak *et al*. 2001). A glial component of the paranodal junction was recently identified as neurofascin 155 (NF155), a spliced isoform of the cell adhesion molecule neurofascin that is specifically found at the glial loops (Tait *et al*. 2000). In the PNS, these molecules are also found in a single strand opposed to the inner mesaxon of the myelin sheath along the internodes and below the Schmidt-Lanterman incisures (Arroyo *et al*. 1999, Rios *et al*. 2000, Tait *et al*. 2000). Both contactin and NF155 are members of the immunoglobulin superfamily, while Caspr is a transmembrane molecule that shares structural homology with the neurexins, a polymorphic family of proteins involved in cell adhesion and intercellular communication (Bellen *et al*. 1998, Missler and Sudhof 1998). Within the neurexin superfamily, Caspr belongs to a distinct subgroup (named also NCP; Bellen *et al*. 1998), which contains five distinct genes (Caspr-Caspr5 (Peles *et al*. 1997, Poliak *et al*. 1999, Spiegel *et al*. 2002)). All Caspr proteins contain a variety of sub-domains also found in proteins that have been implicated in synaptogenesis, axonal guidance and target recognition. Their extracellular region consists of a mosaic of domains implicated in mediating protein–protein interactions, including discoidin and fibrinogen-like domains, EGF motifs and several regions with homology to the G domain of laminin A, which are thought to mediate cell adhesion. The extracellular region of Caspr binds laterally to contactin when both proteins are expressed in the same cell (i.e. cis interactions; Peles *et al*. 1997), generating a receptor complex that may bind neurofascin (Volkmer *et al*. 1998, Tait *et al*. 2000). The interaction with contactin is required for efficient export of Caspr from the endoplasmic reticulum to the plasma membrane of transfected cells (Faivre-Sarrailh *et al*. 2000), and, accordingly, for its transport from the cell body to the axon (Boyle *et al*. 2001). Furthermore, it appears that the presence of Caspr, contactin and NF155 at the paranodal junction is interdependent, suggesting that they are part of the same adhesion complex at this site (Bhat *et al*. 2001, Boyle *et al*. 2001, Poliak *et al*. 2001). Similarly, disruption of the paranodal junction in galactolipids-deficient mice results in the disappearance of these adhesion components from the paranodes (Dupree *et al*. 1999, Poliak *et al*. 2001). Both Caspr and contactin are essential for the generation of the paranodal junction, and their absence results in the disappearance of the transverse bands, which are the hallmark of this axo-glial contact, and in lower conduction velocity (Bhat *et al*. 2001, Boyle *et al*. 2001). Interestingly, in the peripheral nerves of different paranodal mutant mice, including mice deficient for contactin (Boyle *et al*. 2001), Caspr (Bhat *et al*. 2001) or galactolipids (Dupree *et al*. 1999, Poliak *et al*. 2001), K^+ channels were mainly mislocalized at the paranodal domain instead of the juxtaparanodal region, while clustering of Na^+ channels at the node was minimally affected (some small expansion of the nodal region was detected). These results demonstrate that the generation and maintenance of specialized domains in myelinated fibres may require the presence of barriers that are present at the paranodal junction and restrict the diffusion of axonal proteins. Thus, while structurally distinct, the paranodal junctions may function similarly to tight junctions by providing a fence that restricts the movement of membrane proteins, thereby confining them to specific domains along the axolemma.

The localization of cell adhesion molecules at cell junctions depends on their interaction with cytoplasmic linker proteins, which connect them to the cytoskeleton (Knust 2000). The

cytoplasmic region of NF155, like other forms of neurofascin (i.e. NF186 present at the nodes of Ranvier) contains a short amino acid sequence (FIGQY), which mediates its binding to ankyrin G (Garver *et al*. 1997, Zhang *et al*. 1998). In addition, the carboxy-terminal tail of neurofascin was shown to interact with syntenin-1, a cytoplasmic protein that contains two PDZ domains (Koroll *et al*. 2001). Proteins containing PDZ domains have been implicated in the localization of proteins to specific membrane domains such as cellular junctions, clustering of transmembrane receptors, and recruitment of cytosolic proteins to generate multi-signalling complexes (Scott and Zuker 1998, Gonzalez-Mariscal *et al*. 2000, Sheng and Pak 2000). Nevertheless, it is currently unknown what proteins bind to NF155 in the glial loops as neither syntenin nor ankyrin was localized with NF155 at this site. At the axonal side of the junction, the intracellular domain of Caspr was suggested to bind to members of the protein 4.1 family, which link membrane proteins with the actin/spectrin cytoskeleton (Menegoz *et al*. 1997, Hoover and Bryant 2000, Bennett and Baines 2001). Four different 4.1 proteins are expressed in the nervous system at different sub-cellular locations (Ohara *et al*. 1999, Walensky *et al*. 1999, Parra *et al*. 2000), of which protein 4.1B is concentrated at the axonal paranodes and juxtaparanodal region (Ohara *et al*. 2000, Poliak *et al*. 2001). Caspr interacts with protein 4.1B through a short juxtamembrane sequence found in its cytoplasmic tail, which shows strong similarity to the protein 4.1R-binding site found in erythrocytes glycophorin C (Bennett and Baines 2001). Interestingly, clustering of the Caspr/contactin complex on the cell surface immobilized protein 4.1B into these clusters, suggesting that it is recruited to Caspr-containing sites on the plasma membrane (Gollan *et al*. 2002). Consistent with this notion, protein 4.1B was abnormally distributed along peripheral myelinated axons of the galactolipids-deficient mice, which lack the Caspr/contactin complex in their paranodes (Poliak e*t al*. 2001). It was previously shown that Neurexin-IV, a Drosophila homologue of Caspr and Caspr2, associates and recruits protein 4.1 homologue Coracle to septate junctions (Baumgartner *et al*. 1996). However, in contrast to the complete absence of Coracle from the septate junctions in *neurexin IV* mutants, in *coracle* mutants neurexin IV still reached the lateral membrane but was not subsequently confined at septate junctions (Ward *et al*. 1998). These results indicate that while Coracle does not play a role in the targeting of neurexin IV to the plasma membrane, it is required for its maintenance at the junction. In analogy, a deletion mutant of Caspr that lacks its intracellular domain was targeted to the paranodes, but was not retained properly at the junction, suggesting that maintenance of the Caspr/contactin complex at this site requires its association with protein 4.1B. (Gollan *et al*. 2002).

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