THE LOCAL DIFFERENTIATION OF MYELINATED AXONS AT NODES OF RANVIER

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Efficient and rapid propagation of action potentials in myelinated axons depends on the molecular specialization of the nodes of Ranvier. The nodal region is organized into several distinct domains, each of which contains a unique set of ion channels, cell-adhesion molecules and cytoplasmic adaptor proteins. Voltage-gated Na^+ channels — which are concentrated at the nodes — are separated from K^+ channels — which are clustered at the juxtaparanodal region — by a specialized axoglial contact that is formed between the axon and the myelinating cell at the paranodes. This local differentiation of myelinated axons is tightly regulated by oligodendrocytes and myelinating Schwann cells, and is achieved through complex mechanisms that are used by another specialized cell–cell contact — the synapse.

The evolutionary need for the rapid and efficient conduction of action potentials in vertebrate neurons has resulted in the development of the myelin sheath. Myelin, a multilamellar membrane that is formed by oligodendrocytes in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system (PNS), enwraps the axon in segments that are separated by the nodes of Ranvier (FIG. 1a). The myelin sheath reduces current flow across the axonal membrane by reducing its capacitance and increasing its transverse resistance, thereby allowing the fast, saltatory movement of nerve impulses from node to node[1]. As a consequence, a large number of axons with high conduction velocities could be placed in a limited space, a feature that permitted the development of more complex nervous systems. In addition, saltatory conduction eliminates the need for regenerating the action potential at every point of axonal membrane, therefore reducing the metabolic requirements for neuronal activity.

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Organization and function of the nodal environs

The coordinated differentiation of the axon and its myelinating cell requires a close communication between neurons and glia from early stages of development. Signals provided by the axon regulate the proliferation, survival and differentiation of oligodendrocytes and Schwann cells[2,3], and participate in determining myelin thickness[4]. Reciprocal glial signals affect the axonal cytoskeleton and transport[5], and are required for axonal survival[6,7]. As a result of this reciprocal communication, myelinated fibres acquire structural features that allow them to maximize their conduction velocities. One such feature is the differentiation of the axonal membrane into distinct molecular, structural and functional domains. These domains include the nodes of Ranvier, the paranodal junction, the juxtaparanodes and the internodal region[8,9] (FIGS 1b and 2). We will focus on the molecular mechanisms that underlie the generation and maintenance of these unique axonal domains, which are necessary for normal nerve function.

The node of Ranvier

The nodes of Ranvier are short, periodical interruptions in the myelin sheath, which are spaced at intervals that are about 100 times the axonal diameter. Although PNS and CNS nodes show similar structural characteristics, there are some differences. In peripheral nerves, the

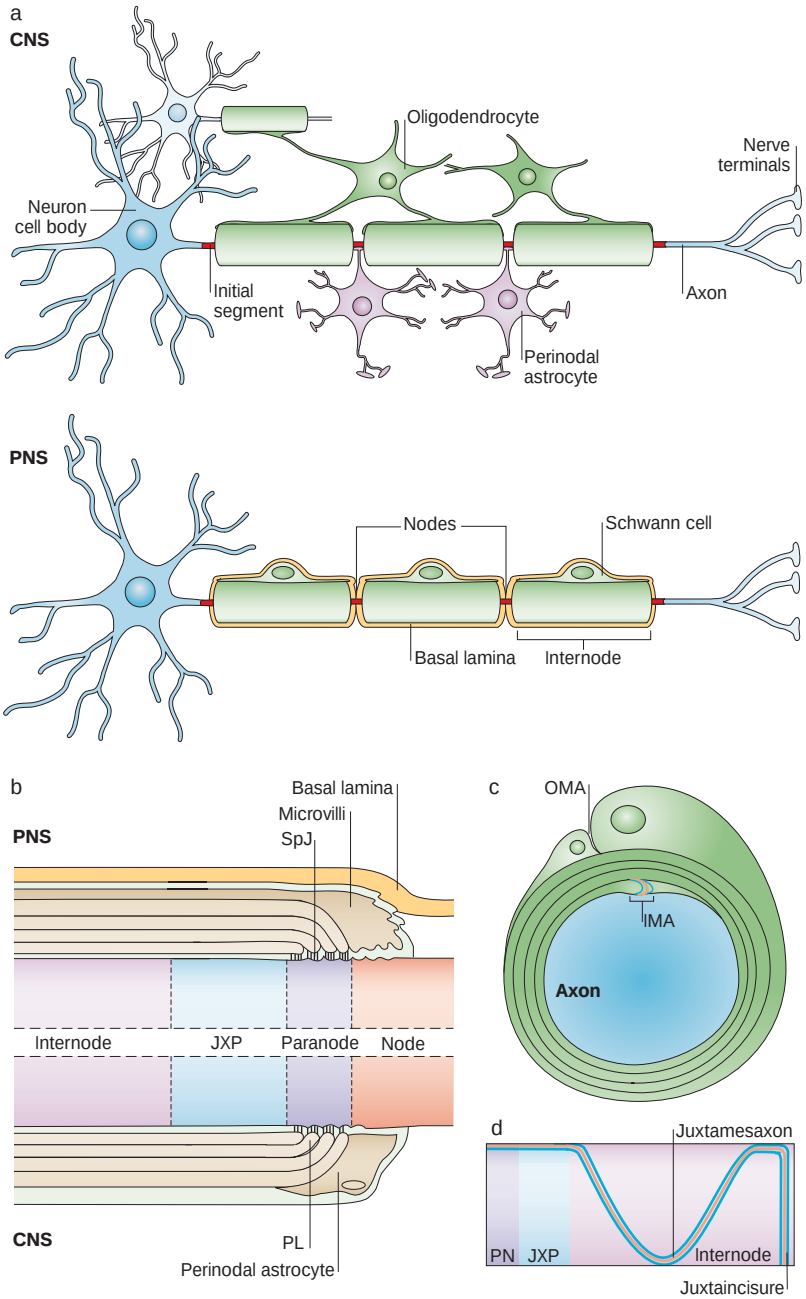


Figure 1 | **Structure of myelinated axons. a** | Myelinating glial cells, oligodendrocytes in the central nervous system (CNS) or Schwann cells in the peripheral nervous system (PNS), form the myelin sheath by enwrapping their membrane several times around the axon. Myelin covers the axon at intervals (internodes), leaving bare gaps — the nodes of Ranvier. Oligodendrocytes can myelinate different axons and several internodes per axon, whereas Schwann cells myelinate a single internode in a single axon. **b** | Schematic longitudinal cut of a myelinated fibre around the node of Ranvier showing a heminode. The node, paranode, juxtaparanode (JXP) and internode are labelled. The node is contacted by Schwann cell microvilli in the PNS or by processes from perinodal astrocytes in the CNS. Myelinated fibres in the PNS are covered by a basal lamina. The paranodal loops form a septate-like junction (SpJ) with the axon. The juxtaparanodal region resides beneath the compact myelin next to the paranode (PN). The internode extends from the juxtaparanodes and lies under the compact myelin. **c** | Schematic cross-section of a myelinated nerve depicting the inner and outer mesaxons (IMA and OMA, respectively). **d** | Drawing of the specializations found along the internodes. A strand composed of paranodal molecules (Caspr, Contactin; red line) flanked by juxtaparanodal proteins (Caspr2, K^+ channels and TAG-1; blue lines) extends along the internodal region (the juxtamesaxon) and below the Schmidt–Lanterman incisures (the juxtaincisure). In addition, Nf155 and ezrin–radixin–moesin proteins, as well as connexins 29 and 32 are found at the glial side, opposite these axonal strands.

entire myelin unit is covered by a basal lamina and the outermost layer (the outer collar) of the Schwann cell extends microvilli that cover the nodes (FIG. 1b). The perinodal space (that is, the space between the axolemma and the basal lamina), which contains the microvilli, is also filled with a filamentous matrix[10]. In the CNS, there is no basal lamina, and the nodes are contacted by perinodal astrocytes[11,12], recently termed synantocytes[13].

The nodal axolemma. The nodes are characterized by a high density (>1200/μm^2) of Na^+ channels that are essential for the generation of the action potential during saltatory conduction[14]. Voltage-gated Na^+ channels are multimeric complexes that consist of a pore-forming α-subunit and one or more auxiliary β-subunits[15] (FIG. 2a). These subunits are encoded by nine α- (*Scn1a–Scn9a*) and four β-subunit genes (*Scn1b–Scn4b*) in mammals[16,17]. Nodes of Ranvier in the adult CNS and PNS mostly contain $Na_v1.6$ (REF. 18). In addition, $Na_v1.2$ and $Na_v1.8$ are found in many CNS nodes[19], whereas $Na_v1.9$ is localized in some nodes in the PNS[20]. During development, both PNS and CNS nodes express $Na_v1.2$, which is later replaced by $Na_v1.6$ (REFS 21,22). The functional significance of this switch is currently unclear, but it might allow neurons to adapt to high-frequency firing[23]. In addition to voltage-gated Na^+ channels, several other transmembrane and cytoskeletal proteins have been identified at the nodal axolemma — the cell-adhesion molecules (CAMs) of the immunoglobulin (Ig) superfamily Nrcam and neurofascin-186 (Nf186)[24], the cytoskeletal adaptor ankyrin G[25,26] and the actin-binding protein spectrin βIV (REF. 27). Recent studies have also disclosed the presence of two K^+ channels at the nodes — $K_v3.1$ (REF. 28) and Kcnq2 (REF. 29). $K_v3.1$ is mainly found in large axons in the CNS and only in few nodes in the PNS, whereas Kcnq2 is located in all PNS nodes and most CNS nodes.

Na^+ channel β-subunits and CAMs. Na^+ channel β-subunits have been shown to modulate channel gating, to facilitate the delivery of Na^+ channels to the cell surface, and to act as CAMs[30]. The extracellular domain of these β-subunits has a single Ig domain[31], which mediates homophilic interactions[32], as well as binding to other nodal components. The β1- and β3-subunits interact in *cis* with Nf186 (REF. 33), and β1 also binds contactin[34], a glycosylphosphatidylinositol (GPI) anchored glycoprotein that is found in all paranodes (see later in text) and in CNS nodes[35]. The interaction with contactin enhances the expression of Na^+ channels on the surface of transfected cells, indicating that this CAM might be important for the expression of Na^+ channels at the node of Ranvier[34,36]. In agreement with this idea, the expression of these channels is markedly reduced in the optic nerve of *contactin*-null mice[37]. The β1- and β2-subunits also interact with the extracellular matrix molecules tenascin-C and tenascin-R[38,39], as well as with phosphacan[40], the secreted form of receptor protein tyrosine phosphatase β (Rptpβ).

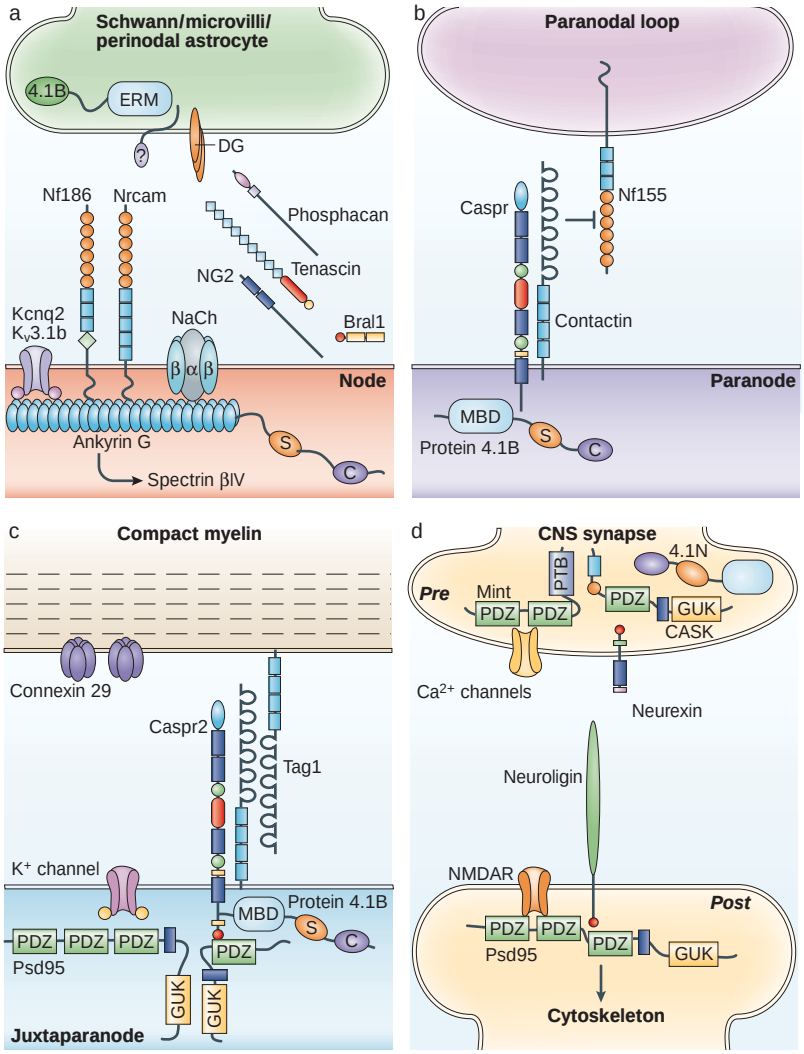


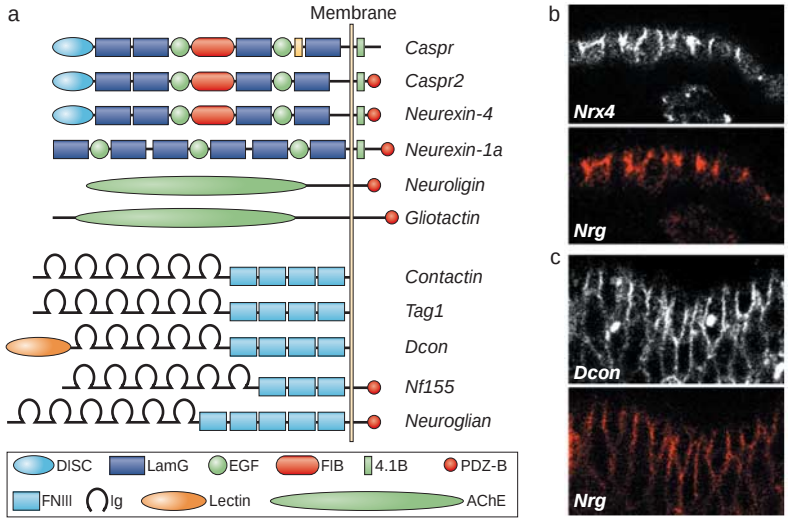
Figure 2 | **Molecular composition of the nodal domains.** The specialized domains around the node of Ranvier are composed of a distinct set of molecules. **a** | At the nodal axolemma, voltage-dependent Na^+ channels are anchored to the cytoskeleton by ankyrin G, which also binds Nf186, Nrcam and $K_v3.1b$. Ankyrin G connects these proteins to the axonal cytoskeleton through spectrin βIV. In the PNS, Schwann cell microvilli express ezrin–radixin–moesin (ERM) proteins and dystroglycan (DG). The nodal gap also contains several extracellular-matrix proteins. NaCh, Na^+ channel; NG2, NG2 proteoglycan. **b** | At the paranodes, a Caspr/contactin complex in the axolemma faces neurofascin 155 (Nf155) at the glial membrane. Whereas contactin alone can bind Nf155, Caspr inhibits this interaction, indicating that the Caspr/contactin complex might bind an unidentified ligand at the glial loops. The cytoplasmic tail of Caspr interacts with protein 4.1B, providing a potential link with the actin cytoskeleton. **c** | At the juxtaparanodal axolemma, voltage-gated K^+ channels are found in a macromolecular complex with Caspr2, protein 4.1B, Psd95 and Tag1. Tag1 is also expressed on the glial membrane and binds the axonal Caspr2/Tag1 complex. Connexin 29, localized at the juxtaparanodal glial membrane, could form functional hemichannels. **d** | At synapses in the central nervous system (CNS), neurexins interact with calmodulin-dependent serine kinase (CASK) and the protein Mint, which in turn can associate with Ca^{2+} channels. CASK could also bind protein 4.1N, further linking the complex to the actin cytoskeleton. The extracellular domain of neurexin binds to neuroligin that is present at the postsynaptic membrane. The cytoplasmic tail of neuroligin interacts with Psd95, which in turn might recruit NMDA (*N*-methyl-D-aspartate) receptors (NMDAR). C, carboxy-terminal ; S, spectrin-binding domain; GUK, guanylate kinase-like domain; MBD, membrane binding domain.

Cytoskeletal proteins. The nodes and the initial segment are enriched in ankyrin G, a membrane–cytoskeleton adaptor that links integral membrane proteins to the spectrin cytoskeleton[25,26]. Ankyrin G interacts with Na^+ channels[41], both with their α- (REF. 42) and β- (REF. 32) subunits, as well as with Nf186, Nrcam[43] and $K_v3.1$ (REF. 28). The β-subunit recruits ankyrin G to the plasma membrane[32] and this interaction is regulated by tyrosine phosphorylation[44]. The binding of ankyrin G to the α-subunit is mediated through a sequence of nine amino acids that is present in all known voltage-gated Na^+ channels[42]. This nine-amino-acid motif is required for the accumulation of the α-subunit in the axon initial segment[45]. Furthermore, this ankyrin-binding site is located within a short sequence that is sufficient to target proteins to the axon initial segment[45]. It remains to be determined whether this short sequence is also necessary for targeting to the nodes of Ranvier. Binding of ankyrin G to the two nodal Ig-CAMs, Nf186 and Nrcam, is mediated by a twelve-amino-acid motif that is found in their cytoplasmic domains[43]. Ankyrin G binds this motif only when it is dephosphorylated[43,46,47], indicating that unidentified tyrosine kinases and phosphatases might regulate this interaction. Tyrosine-phosphorylated neurofascin is located at the glial paranodes[48], but not in the nodes, supporting the idea that nodal neurofascin is closely associated with ankyrin G[49]. Ankyrin G also binds spectrin βIV, a spectrin isoform that is enriched at the nodes of Ranvier and axon initial segments[27], further anchoring the nodal Na^+ channel and Ig-CAMs to the axonal cytoskeleton.

The nodal gap, extracellular matrix and the glial membrane. In the PNS, the nodal gap is filled with Schwann cell microvilli that emanate from the outer aspect of the cell (FIG. 1b). At the proximal region of the microvilli, the membranes of two adjoining Schwann cells are connected by TIGHT JUNCTIONS[50,51]. However, these junctions do not seal the nodal gap, as it was found to be permeable to horseradish peroxidase applied outside the nerve fibres[52]. Three proteins — ezrin, radixin and moesin, as well as the ezrin-binding protein EBP50 and the Rho-A GTPase, are localized at the microvilli[53–55]. These proteins might potentially link the actin-rich microvilli[56] with integral membrane proteins[57]. In addition, several extracellular matrix (ECM) proteins are present in the nodal gap under the basal lamina, including the hyaluronan-binding proteoglycan versican[58], tenascin-C[59,60] and the NG2 proteoglycan[61]. Recently, it was shown that dystroglycan, which is abundantly expressed at the ABAXONAL surface of myelinating Schwann cells[62], is also located at the nodes[63]. Specific ablation of dystroglycan in Schwann cells results in the disorganization of the microvilli, a marked reduction in nodal Na^+ channels and consequently impaired nerve conduction[63].

In contrast to the PNS, processes of perinodal astrocytes contact most of the nodes in the CNS. Here, the nodal gap has been shown to include several proteoglycans and ECM proteins that are produced by oligodendrocytes, including tenascin[64] and phosphacan[65]. The CNS nodal gap also contains the versican-binding protein Bral1, which is produced by neurons[66]. The function of these proteins is presently unclear, although it was suggested that, owing to their high content of acidic disaccharides, they could provide a strong negative environment that serves as an extracellular

Box 1 | Vertebrate axoglial junctions and *Drosophila* septate junctions



In *Drosophila*, a blood–brain barrier is formed by perineural and glial cells, which insulate neurons from the surrounding hemolymph and allow the normal propagation of action potentials. Septate junctions that are present between these cells are necessary for the integrity of this blood–brain barrier[81,166–168]. Septate junctions, which are also found in all invertebrate epithelia, share morphological, functional and molecular similarities with the vertebrate paranodal junction. Both junctions contain regularly spaced electron-dense septa that give them a ladder-like appearance. Disruption of septate junction or paranodal junction integrity results in abnormalities in the propagation of axonal action potentials[81,86,87,90,166]. The basic molecular components of the fly septate junctions seem to be conserved in the vertebrate paranodal junction (a). Paranodal junctions contain a complex of Caspr and contactin[35], whereas neurexin-4 (Nrx4), the fly homologue of Caspr and Caspr2, is found in *Drosophila* septate junctions[81], where it co-localizes and interacts with *Drosophila* contactin (Dcon) (b–c; M. Shelly and E.P., unpublished observations). Both Caspr and Nrx4 associate with 4.1 proteins (4.1B and Coracle, respectively), which stabilize them at the junction[88,101]. *Drosophila* septate junctions also contain the Nf155-homologue neuroglian, which associates with the Nrx4/Coracle complex[167]. The figure shows staining of wild-type *Drosophila* embryos (stage 11–15) with the indicated antibodies; Nrx4, Dcon and neuroglian co-localized at cell junctions of the outer ectoderm. Two other proteins, gliotactin and a Na^+/K^+ ATPase, are localized at septate junctions, and are important for their formation[167,168], but their mammalian homologues have not been reported to reside in the axoglial paranodal junction. Panels b–c show the expression of *Drosophila* homologues of the paranodal junction components in the fly epithelia. AChE, acetylcholinesterase; DISC, discoidin-like domain; EGF, epidermal growth factor; FIB, fibrinogen-like domain; FNIII, fibronectin-III-like domain; Ig, immunoglobulin-like domain; LamG, laminin G; PDZ-B, PDZ-binding domain.

TIGHT JUNCTION
A belt-like region of adhesion between adjacent cells. Tight junctions regulate paracellular flux, and contribute to the maintenance of cell polarity by stopping molecules from diffusing within the plane of the membrane.

ABAXONAL
Term that refers to the outermost layer of the myelin sheath.

TYPE I TRANSMEMBRANE PROTEIN
Molecule with a single transmembrane domain.

Na^+ reservoir in the perinodal space[66]. Both tenascin-C and tenascin-R bind to Na^+ channels[39] and alter their electrophysiological properties[38]. Genetic ablation of tenascin-R resulted in slower nerve conduction, but had no effect on the distribution of Na^+ channels at the nodes, indicating that this interaction might stabilize nodal complexes or regulate channel activity, but is not required for the initial clustering of these channels[67]. Na^+ channels were also reported to bind the cytoplasmic tail and the extracellular domain of Rptpβ[40], a receptor tyrosine phosphatase that has not been reported to be located at the nodal axolemma. Furthermore, the importance of these interactions for the normal physiology of myelinated nerves is not clear, as the distribution of nodal Na^+ channels and the conduction velocity of CNS myelinated axons are normal in Rptpβ-deficient mice[68]. Notably, both tenascin-R and Rptpβ also interact with contactin and Nrcam[69–71], which are present at CNS nodes, indicating the possible existence of large macromolecular complexes at the perinodal space.

The axoglial paranodal junction

Morphology and molecular composition. At both sides of the nodes of Ranvier, the compact myelin membrane opens up and forms cytoplasm-filled glial loops that wind helically around the axon (FIG. 1b). These paranodal loops are connected to the axolemma by a series of ridges (transverse bands) that are reminiscent of invertebrate septate junctions[72] (BOX 1). The axoglial junctions appear relatively late during myelination, being first generated closer to the nodes by the outermost paranodal loop, and continue gradually as additional loops are attached to the axon[73]. As a result, they are composed of a number of rings, each representing a turn of the myelin wrap.

The axonal membrane at the axoglial junction contains a complex of two cell-recognition molecules — contactin-associated protein (Caspr; also known as paranodin)[74,75] and contactin[35] (FIG. 2b). Caspr is a TYPE I TRANSMEMBRANE PROTEIN that belongs to a distinct subgroup of the neurexins, a polymorphic protein family that is involved in cell adhesion and intercellular communication[76,77]. There are five human genes in the Caspr family (*CASPR1–CASPR5* (REFS 78–80)), two in *Drosophila*[81,82] (*nrxIV* and *axo*) and two in *Caenorhabditis elegans* (*itx-1* and *nlr-1*; L. Haklai-Topper and E.P., unpublished observations). These proteins bind several CAMs and should therefore be considered as CAM-associated proteins. Their extracellular region consists of several domains that are implicated in protein–protein interactions, including a discoidin and a fibrinogen-like domain, epidermal growth factor (EGF) motifs, and several regions with homology to the G domain of laminin A (BOX 1). Caspr, but not other members of the Caspr family, forms a complex with contactin only in *cis*[78]. The interaction between Caspr and contactin is required for the efficient export of Caspr from the endoplasmic reticulum to the plasma membrane[83], and regulates the glycosylation and transport of contactin[84]. Caspr and contactin are associated in the endoplasmic reticulum and might be transported through a Golgi-independent pathway to the cell surface[84,85]. In agreement with these *in vitro* findings, Caspr is retained in the neuronal somata and does not reach the axons in *contactin*-deficient mice[86], whereas Caspr is necessary to maintain contactin at the paranodes[84,87,88].

Both Caspr and contactin are essential for the generation of the axoglial junction, and their absence results in the disappearance of septa and a widening of the space between the axon and the paranodal loops[84,86,87]. These results indicate that Caspr and contactin might be part of a paranodal adhesion complex that is required for the tight attachment of the two membranes. This phenotype is similar to those of two other paranodal mutants: the galactolipids-deficient mice, which lack UDP-galactose ceramide galactosyltransferase (Cgt) and do not synthesize galactocerebroside (GalC) and sulfatide,

and cerebroside sulfotransferase (*Cst*)-null mice, which only lack sulfatide[89–93]. In all of these mutants, Caspr and contactin are absent from the paranodes[86,87,93–95]. The way in which the absence of GalC and sulfatide causes paranodal abnormalities is not clear, but it might result from direct binding of sulfatide to the Caspr/contactin complex. Alternatively, given the proposed role of galactolipids in the formation of LIPID RAFTS and the organization of myelin[96,97], their absence might result in a misrouting of junctional glial components to non-compact myelin. The latter possibility is further supported by recent findings, showing that genetic ablation of the myelin and lymphocyte (MAL) protein, a raft-associated molecule that is involved in intracellular trafficking, results in paranodal abnormalities (N. Schaeren-Wiemers and U. Suter, personal communication).

The intracellular regions of Caspr and Caspr2 contain a juxtamembrane sequence that binds protein 4.1B[75,78,88,98], which is present at the paranodes and juxtaparanodes[95,98–100]. Similar to other 4.1 proteins, 4.1B contains a conserved actin–spectrin-binding domain and could therefore immobilize Caspr (and therefore contactin) to the cytoskeleton[88]. Consistent with this idea, protein 4.1B is abnormally distributed along peripheral myelinated axons of mice lacking either contactin or galactolipids, both of which lack paranodal Caspr[88,95]. In these mutants, the position of protein 4.1B correlates strongly with those of Caspr and Caspr2, indicating that they might determine its localization. Furthermore, the cytoplasmic tail of Caspr is required for stabilizing the Caspr/contactin complex at the paranodes, as a Caspr mutant that lacks this domain is not properly maintained at the axoglial junction[88]. So, Caspr seems to serve as a transmembrane scaffold that stabilizes the Caspr/contactin adhesion complex at septate-like junctions by connecting the complex to the axonal cytoskeleton through protein 4.1B. This mechanism closely resembles the function of *Drosophila* neurexin IV, which recruits Coracle (the homologue of protein 4.1) to septate junctions[81,101] (BOX 1). In addition, the cytoplasmic region of Caspr also binds the FERM domain (four-point-one, ezrin–radixin–moesin)-containing protein Schwanomin/merlin[102]. However, the importance of this interaction is less clear, as Schwanomin is not concentrated at the paranodal junction.

The distribution of Caspr and contactin along the internodes[95,103,104] (see later in text), their accumulation at the paranodes as a number of rings that represent each turn of the myelin wrap during development[35,105,106], and the abnormal distribution of Caspr in MULTIPLE SCLEROSIS[107] and in several myelin mutants[19,93–95,105,108] indicate that the myelin sheath dictates the localization of Caspr and contactin in the axolemma. Furthermore, the addition of a soluble Rptpβ, which binds contactin, to myelinating co-cultures perturbs the paranodal accumulation of Caspr, indicating that the localization of the Caspr/contactin complex to this site might be mediated by its interaction with a glial ligand[35]. The most probable candidate to serve as a glial ligand of the Caspr/contactin complex is Nf155, a glial isoform of the CAM neurofascin, which is located across Caspr and contactin at the axoglial junction[48], and is not localized to this site in the absence of Caspr[86,87,95,109]. In agreement with this idea, it was recently reported that a soluble Nf155-Fc chimaera binds to cells that express Caspr and contactin, and precipitates these proteins from rat brain lysates, indicating that Nf155 might indeed serve as a receptor for the Caspr/contactin complex[110]. However, recent studies have challenged this model, showing that, whereas Nf155 binds directly to contactin, Caspr inhibits this interaction. This observation indicates the possible existence of other receptors for the Caspr/contactin complex in myelinating glia[84]. This conclusion agrees with previous observations that show that Nf155 appears much later than Caspr in the paranodes[109].

Function of the paranodal junction. The paranodal junction was proposed to attach the myelin sheath to the axon, to separate the electrical activity at the node of Ranvier from the internodal region under the compact myelin sheath, and to serve as a fence that limits the lateral diffusion of axolemmal proteins[111]. Recent studies using four different paranodal mutant mice — mice lacking *Caspr*, *contactin*, *Cgt* and *Cst*, all of which lack the characteristic septa in their axoglial junction — allowed close examination of these original ideas. In the CNS of these mutants, the paranodal loops are disorganized, with many overlapping and inverted loops that face away from the axon[87,92,112]. In the PNS, the morphological alterations are much milder, possibly due to the presence of the basal lamina; the paranodes are well organized, but there is an increase in the space between the glial membrane and the axon. However, even in the absence of septa, the paranodal loops are still closely attached to the axon in many sites in the PNS and CNS, pointing to the presence of so far unidentified paranodal components that mediate axoglial contact at this site. Together with ultrastructural data showing that the transverse bands are generated late during myelination[73,109], these studies indicate a possible role for the septa in securing the paranodal loops to the axon at the axoglial junction. In agreement with this view, a gradual, age-dependent detachment of the paranodal loops from the axon was observed in the CNS of *Caspr*-null mice[104].

The absence of paranodal septa in all four paranodal mutants results in a reorganization of the axonal membrane[86,87,93–95] (FIG. 3). In these mutants, the *shaker*-type K^+ channels that are normally present in the juxtaparanodal region are mislocalized to the paranodal axon membrane[86,87,93–95]. So, it seems that the paranodal septate junction functions as a barrier that restricts the movement of K^+ channels from under the compact myelin, separating them from the Na^+ channels at the nodes. In contrast to the juxtaparanodal K^+ channels, disruption of the paranodal septa minimally affects the distribution of the nodal Na^+ channels[86,87,94]. There is a small increase in nodal length, accompanied by a reduction in membrane particles at the nodal axolemma, that is detected by FREEZE-FRACTURE electron microscopy, indicating that the paranodal septate junction might not be required for the generation of the nodes[87,104,113].

CIS INTERACTION
Term that refers to the interaction between molecules that are present in the same cell membrane, as opposed to an interaction in trans, in which the interacting molecules are present in opposing membranes.

LIPID RAFTS
Dynamic assemblies of cholesterol and sphingolipids in the plasma membrane.

MULTIPLE SCLEROSIS
A neurodegenerative disorder characterized by demyelination of central nervous system tracts. Symptoms depend on the site of demyelination and include sensory loss, weakness in leg muscles, speech difficulties, loss of coordination and dizziness.

FREEZE FRACTURE
An electron-microscopic method in which rapidly frozen tissue is cracked to produce a fracture plane through the specimen. The surface of the fracture plane is shadowed by a heavy metal, and the specimen is digested away to leave a replica that can be examined under the electron microscope.

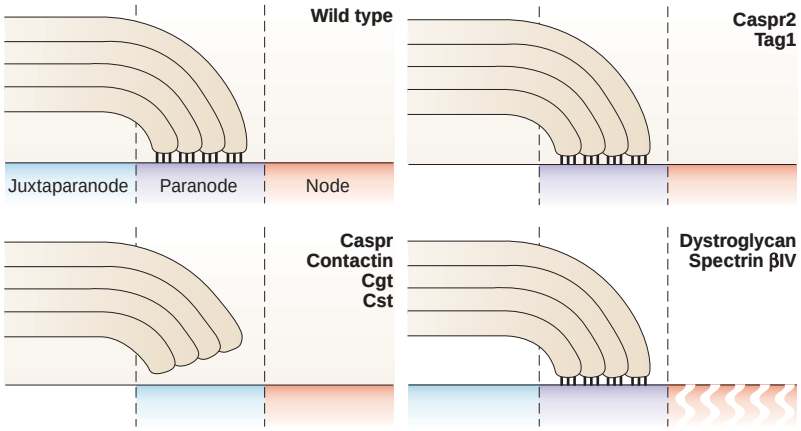


Figure 3 | **Arrangement of the nodal environ in various mutant mice.** A schematic representation of the paranodal loops attaching to the axon. The distribution of nodal (red), paranodal (purple) and juxtaparanodal (blue) proteins is shown in wild-type and the indicated mutant mice. In paranodal mutants (Caspr, contactin, ceramide galactosyltransferase (Cgt) and cerebroside sulfotransferase (Cst), all juxtaparanodal components move to the paranodes; in juxtaparanodal mutants (Caspr2 and Tag1), K^+ channels are dispersed along the internodes; in nodal mutants (dystroglycan and spectrin βIV), Na^+ channel clustering at the nodes is reduced. See TABLES 1 and 2 for a detailed description of these and other mutants.

However, glial attachment at the paranodes in the CNS is required to maintain Na^+ clustering at the nodal axolemma[93,104,113,114].

Juxtaparanodal specialization

The juxtaparanode is located in a short zone just beyond the innermost paranodal junction (FIG. 1b). In freeze-fracture electron microscopy, this region shows randomly distributed particles that are more concentrated near the paranodes and diffuse away towards the internodes[111]. These particles most likely correspond to heteromultimers of the DELAYED RECTIFIER K^+ CHANNELS of the Shaker family, $K_v1.1$, $K_v1.2$ and $K_v\beta2$ (REFS 115,116). At the juxtaparanodal axolemma, these channels co-localize and create a complex with Caspr2, the second member of the Caspr family[79]. In addition, $K_v1.6$ is present at this site, predominantly in small axons[117]. Two other proteins that are found at the juxtaparanodes are transient axonal glycoprotein-1 (Tag1), a GPI-anchored CAM that is related to contactin[118], and connexin 29 (Cx29), which is found at the glial membrane[119,120]. The association of Caspr2 with K^+ channels is mediated by their carboxy-terminal region, most probably through an unidentified PDZ DOMAIN-containing protein. Although one such protein, Psd95, is located at the juxtaparanodes and associates with K^+ channels, it does not mediate the interaction of these channels with Caspr2 or their accumulation at this site[121,122]. Two recent studies showed that Caspr2 and Tag1 form a juxtaparanodal complex, consisting of a glial Tag1 molecule and an axonal Caspr2/Tag1 heterodimer[123,124] (FIG. 2c). This complex is essential for the accumulation of K^+ channels in the juxtaparanodes, as targeted disruption of *Caspr2* or *Tag1* results in a striking reduction in the juxtaparanodal accumulation of these channels in both PNS and CNS axons (FIG. 3). These results indicate that Caspr2 and Tag1 might form a scaffold that enables the positioning of ion channels at specific sites of the plasma membrane, therefore resembling the mechanisms that operate during synapse formation (FIG. 2d).

Role of K^+ channels under the myelin sheath. Juxtaparanodal K^+ channels were proposed to act as an active damper of re-entrant excitation and to help in maintaining the internodal resting potential[125–128]. Although theoretically it is enough to have these channels scattered along the internodes to maintain the resting potential, preventing re-entrant excitation would require a high spatial clustering of K^+ channels near the node. Despite the marked abolishment in juxtaparanodal clustering of $K_v1.1/K_v1.2$ in *Caspr2*- and *Tag1*-knockout mice, there is no change in the excitability of myelinated nerves[123,124]. The observation that the total content of these channels remains constant in both mutants could indicate that the main role for these myelin-concealed K^+ channels is maintaining the internodal resting potential. In addition, a computer model in which both K^+ channel distribution and the axoglial junctional conductance were varied indicated that the clustering of K^+ channels in the juxtaparanode could provide a protective function in axons that might undergo a low degree of demyelination (FIG. 4). A testable implication of this model is that Caspr2 and Tag1 might serve to ensure stability in axons with compromised axoglial junctions.

Another function of the juxtaparanodal K^+ channels might be mediating axoglial communication. In the PNS, these channels are located across from Cx29 hemichannels that are present at the ADAXONAL membrane of myelinating Schwann cells[119,120], which most likely correspond to the rosettes of particles that are seen by freeze-fracture electron microscopy at this site[129]. These hemichannels could provide a direct pathway for K^+ ions from the axon to the overlying glia[119]. This, in turn, would generate an activity-dependent signal into the Schwann cell, reminiscent of electrical synapses formed by GAP JUNCTIONS. In support of this idea, Ca^{2+} transients recorded in Schwann cells upon electrical stimulation of the axon were proposed to be generated by K^+ efflux from the axon that depolarizes the glial membrane[130]. The exchange of information through such an 'axoglial synapse' at the juxtaparanodes could provide an additional mechanism for axon–gliacommunication[131]. Interestingly, the paranodal axoglial junction could also be remodelled by neuronal activity[132], an effect that could be mediated, in part, by controlling the expression of contactin on the axonal surface[133].

Internodal differentiation

Although no junctional specializations are observed between the glia and the axon along the internode, freeze-fracture electron microscopy revealed that the internodal axolemma in the PNS contains longitudinal strands of intramembranous particles that resemble those found in the paranodes and juxtaparanodal region[134,135]. As shown in FIGS 1c,d, Caspr and contactin are located throughout the internodal region in a strand that is flanked by K^+ channels and Caspr2, which apposes the inner mesaxon of the myelin sheath and forms a

DELAYED RECTIFIER K^+ CHANNELS
Slowly activating and very slowly inactivating channels that preferentially pass K^+ out of the cell.

PDZ DOMAIN
A peptide-binding domain that is important for the organization of membrane proteins, particularly at cell–cell junctions, including synapses. It can bind to the carboxyl termini of proteins or can form dimers with other PDZ domains. PDZ domains are named after the proteins in which these sequence motifs were originally identified (PSD95, Discs large, zona occludens 1).

ADAXONAL
Term that refers to the innermost layer of the myelin sheath.

GAP JUNCTIONS
Cellular specializations that allow the non-selective passage of small molecules between the cytoplasm of adjacent cells. They are formed by channels termed connexons — multimeric complexes of proteins known as connexins. Gap junctions are structural elements of electrical synapses.

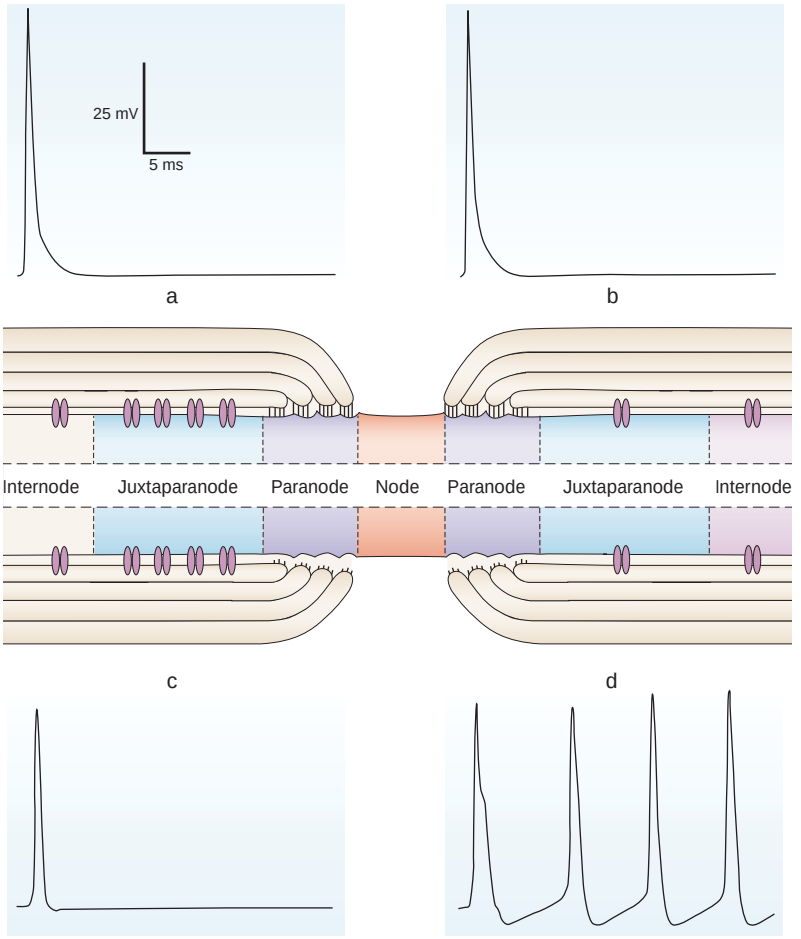


Figure 4 | **A computational model describing a role for juxtaparanodal K^+ channels in myelinated fibres.** Each part of the figure shows a schematic organization of the paranodal junction (black lines) and the distribution of K^+ channels (purple ovals), together with the corresponding action potential recorded after a single stimulus. **a** | The axon has normal properties and responds to a single stimulus with a single action potential. **b** | The paranodal junctions remain normal, but K^+ channel clusters have dissipated into the internode (*Caspr2*$^{-/-}$; *Tag1*$^{-/-}$) and conduction velocity remains normal. **c** | The junctions are loosened to increase conductance to 10% of the value that is observed when they are fully open, but the channels remain clustered. Conduction velocity is slowed by 40%, but is otherwise stable. **d** | The junctions are loosened as in **c**, but the channels are now dispersed as in **b**, and the axon responds to a single stimulus with repetitive action potentials. The model was kindly provided by P. Shrager and used parameters that have been described by Hines and Shrager[169].

circumferential ring just below the inner aspect of the SCHMIDT–LANTERMAN INCISURES[35,95,103]. This line, termed juxtamesaxonal and juxta-incisural[9,136], is a direct continuation of the paranodes/juxtaparanodes. Accordingly, Nf155 (REF. 48), Cx29 (REF. 119) and Tag1 (REF. 118) are localized in a complementary distribution on the adaxonal membrane of myelinating Schwann cells. These findings indicate that the internodal localization of axonal proteins is dictated by the myelin sheath, probably by mechanisms similar to those that operate in the paranode/juxtaparanode. However, recent analysis of *Caspr2*-null mice indicates that different mechanisms might control the localization of K^+ channels in the juxtaparanodes and the juxtamesaxon[124]. The molecular organization of the internodal region is not observed in myelinated nerves in the CNS[48,118,119,137].

SCHMIDT–LANTERMAN INCISURE
A cytoplasmic channel that interconnects the adaxonal and abaxonal layers of the myelin sheath.

LAMINA CRIBROSA
The supporting structure for the optic nerve at the point in which it leaves the eye.

Formation of the nodal environ

The role of myelinating glia. During the development of myelinated nerves in the PNS, the different nodal domains are formed gradually; Na^+ channels are first clustered at the nodes, followed by the generation of the paranodal junction, and later on by the clustering of K^+ channels at the juxtaparanodal region[22,125,138]. In both the CNS and the PNS, Na^+ channels cluster initially at sites that are adjacent to the edges of processes extended by oligodendrocytes[22,105] and myelinating Schwann cells[138,139]. Further longitudinal growth of these processes causes displacement of the clusters until ultimately two neighboring clusters seem to fuse, forming a new node of Ranvier. These results indicate that these Na^+ clusters are positioned by direct glial contact. Accordingly, the distribution of Na^+ channels is diffuse along retinal ganglion cells, but they are clustered at the nodes right after these axons cross the LAMINA CRIBROSA and become myelinated[22]. These channels are not clustered after ablation of oligodendrocytes[140] or Schwann cells[138], and are dispersed during demyelination[141]. Furthermore, nodal Na^+ channels are associated with the edges of myelinating Schwann cells in nerves that display shorter internodes as a result of remyelination[141] or genetic mutation, as seen in the *CLAW PAW* mutant mouse[142]. However, studies using retinal ganglion cells showed that Na^+ clustering could be induced *in vitro* by soluble factors that are secreted by cultured oligodendrocytes[21,143]. Although Schwann cells do not secrete such clustering activity[139], some clustering of Na^+ channels has been detected in the absence of myelinating Schwann cells in dystrophic mice[144]. Recent analyses of dysmyelinating[145,146] or paranodal mutants[104,147], and models of demyelination[114,148] showed that the presence of intact myelinating oligodendrocytes is also required for the developmental switch of Na^+ channel isoform in the nodes. By contrast, $Na_v1.6$ is found in the nodes of two myelin mutants that are associated with oligodendrocyte death and lack normal paranodal junctions — *MYELIN DEFICIENT (MD)* RATS and *JIMPY* mutant mice. This observation indicates that the switch might occur in the absence of normal paranodal contact or myelin[19,108]. Notably, recent analysis of the *SHIVERER* mutant revealed that, whereas axoglial contact is necessary for the expression of $Na_v1.6$ at nodes, it is not required for targeting of this subunit to the axon initial segment, pointing to the existence of multiple targeting mechanisms in myelinated axons[149].

Molecular assembly. During the development of myelinated nerves in the PNS, Nrcam and Nf186 are detected at the nodes first, followed by the appearance of ankyrin G and Na^+ channels[150]. In the CNS, however, ankyrin G is detected at the nodes before the clustering of Nf186 and Na^+ channels[108]. These results indicate that Nrcam, Nf186 or an unidentified ankyrin G-binding protein binds ankyrin G, which in turn recruits Na^+ channels. In support of this model, the addition of a soluble Nrcam to myelinating dorsal root ganglia cultures inhibits Na^+ channel clustering[151]. Moreover, the appearance of ankyrin G and Na^+ channels at the nodes is delayed in *Nrcam*-null mice[152], indicating that this adhesion

Table 1 | Molecular changes at the nodal region in myelin-mutant mice

Mutant/gene	Node	Paranodes	Juxtaparanodes	References
shiverer Mbp mutant (CNS hypomyelination) (slight PNS hypomyelination)	Fewer Na^+ channel clusters; most are atypical. No Na^+ channel isoform switch. Expression of Na^+ channel is elevated. Rare $Na_v1.6$ clusters adjacent to Caspr-labelled zones, but normal clusters in the initial axon segment. No ankyrin G clustering	Axoglial junction abnormalities; aberrant location. Irregular Caspr/Nf155-labelled patches. Caspr next to the few existing nodes	Adult CNS: $K_v1.2$ not clustered; diffusely distributed; present adjacent to few normal nodes. Increased overall expression of $K_v1.2$. PNS: mildly affected. Slight increase in internodal staining and occasional elongated juxtaparanodes	22,48,105, 117,149, 170
trembler Pmp22 mutant (PNS hypomyelination)	Clusters of ankyrin G, Na^+ channels and Nf186; some binary clusters	Terminal loops face outwards in some fibres	$K_v1.1$ is redistributed along the axon	150,179, 171
Plp overexpression (CNS demyelination)	Gradual decrease of Na^+ channel clusters as demyelination progresses; irregular elongated and binary clusters; decrease in $Na_v1.6$ clusters and increase in the total expression of $Na_v1.2$	Marked reduction of paranodal staining of Caspr in optic nerve	K^+ channels decreased markedly with age. Eventually all K^+ channel clusters disappear. Total protein level of K^+ channels is unaltered	114,121, 145
jimpy Plp mutant (oligodendrocyte death) (CNS hypomyelination)	Reduced number of nodes; abnormal shape; binary, broad or dot-like. Clusters of ankyrin G, Na^+ channels and Nf186. Normal Na^+ channel isoform switch	Disrupted. Caspr absent (diffusely distributed). No paranodal ankyrin G is detected during development	Transient clustering of $K_v1.1$ in paranodes, which disappear after 3 weeks	108,121, 140,145
Myelin-deficient (md) rats. Plp mutant (CNS dysmyelination)	Na^+ channel and ankyrin G clusters but many do not surround the full circumference of the axon. Normal Na^+ channels isoform switch. $K_v3.1b$ clusters at nodes	No septate junctions Absence of Caspr, contactin and Nf155; Caspr is diffusely distributed in CNS axons. Total Caspr and contactin protein levels are unaffected	$K_v1.1$ and $K_v1.2$ in paranodes; some nodal staining is detected	19,28, 143
P0 null	Normal Na^+ channels clusters ($Na_v1.6$); some broad and binary clusters in adult; larger nodal gap; aberrant microvilli; shorter internodes. In contrast to WT mice, nodes along the femoral quadriceps motor nerve expresses $Na_v1.8$	Caspr is either asymmetrically present in heminodes (53%), absent (5%) or normal (42%). Normal Caspr is correlated with absence of $Na_v1.8$, representing morphologically normal nodes	Asymmetric distribution of $K_v1.2$ in paranodes; absent in 29% of sites, Caspr2 is shifted or expanded to the paranodes; absent in only 7% of sites	146,172
E-cadherin null *(Schwann-cell specific)*	Normal Na^+ channel distribution	Caspr present at paranodes; normal paranodes.	Normal $K_v1.1$ and $K_v1.2$ clusters	173
Mag null	Na^+ channels clustering is not affected	Caspr and Nf155 staining less defined, diffused along the processes. Partial delay in the formation of septa. More pronounced paranodal loop disorganization in *Mag/Cgt* nulls than in each mutant alone	Caspr2 absent from juxtaparanodes $K_v1.1$ extends to the paranodal region but is normally localized in the adult	109,174
Dystrophic Laminin α2	Short internodal lengths; heminodes. Presence of Na^+ channel and ankyrin G clusters in amyelinated axons; in many cases more extended than WT nodes	No axoglial septa in ventral root. Normal Caspr in sciatic nerve	ND	144,175

CNS, central nervous system; Cgt, ceramide galactosyltransferase; Mag, myelin-associated glycoprotein; Mbp, myelin basic protein; ND, no data; Nf, neurofascin; Plp, myelin proteolipid protein; Pmp, peripheral myelin protein; PNS, peripheral nervous system; WT, wild type.

molecule participates in clustering. The eventual formation of nodes in these animals could be explained by the presence of Nf186, which contains a similar ankyrin G-binding site and could therefore compensate for the absence of Nrcam. The importance of the interaction between ankyrin G and these nodal components was shown in mice lacking the cerebellar isoform of ankyrin G, in which Na^+ channel, Ig-CAMs and spectrin βIV are not clustered in the initial segment of Purkinje cell axons[153,154]. Similarly, spontaneous mutations of spectrin βIV in the *QUIVERING* mice[155], or targeted disruption of this gene[156], results in nodal abnormalities and altered channel distribution. However, ankyrin G is also present at the paranodes during the early development of myelinated axons, indicating that it might not be directly involved in the initial targeting of Na^+ channels to the nodes, but rather be important for their stabilization[105,108]. Furthermore, ankyrin G is normally localized at the nodes in *dystroglycan*-null mice, which display a marked reduction of nodal Na^+ channel clusters[63]. After the initial clustering of nodal components in PNS fibres, Nf155 and the Caspr/Contactin complex accumulate in the paranodal junction[22,35], followed by the arrival of Caspr2 and K^+ channels to the juxtaparanodal region[95,125]. Caspr2, K^+ channels and TAG-1 are first detected at the paranodes, and subsequently relocate

CLAW PAW
Mutant mice in which peripheral myelination is disrupted, but central myelination is unaffected. The responsible gene has not been identified.

MYELIN-DEFICIENT RATS
Strain which the gene for the proteolipid protein is mutated, leading to defective myelination, tremors, ataxia and early death. ataxia, tremor and cerebral atrophy.

Table 2 | Molecular changes in nodal-environ mutants

Mutant/gene	Node	Paranodes	Juxtaparanodes	References
Cgt null (Paranodal)	CNS: Elongated nodes, abnormal shape; heminodes; some clusters do not surround the full axonal circumference. PNS: minor expansion of Na^+ channel clusters and ankyrin G. Age-dependent decrease in the number and intensity of $Na_v1.6$ cluster; increased nodal length	Absence of transverse bands. Caspr and contactin almost completely absent from paranodes; diffused staining of Caspr adjacent to narrow labelling of paranodal K^+ channels; reduced paranodal labelling of Nf155. Reduced accumulation of paranodal 4.1B	K^+ channels, Caspr2 and Tag1 are found in the paranodes in the PNS and are diffused along the internode in the CNS; some paranodal concentration of K^+ channels is observed in spinal cord	94,95,112, 113,118,147
Contactin null (Paranodal)	PNS: normal appearance of Na^+ channels. CNS: fewer and elongated Na^+ channel-labelled nodes	Absence of transverse bands. Absence of Caspr from paranodes (found in soma); reduced paranodal Nf155. 4.1B is diffusely distributed along the axon	PNS: K^+ channels and Caspr2 are found in the paranodes	37,86,88,95
Caspr null (Paranodal)	Elongated nodes (labelled with Na^+ channels, Nrcam and spectrin βIV); CNS nodes progressively disperse; normal ERM positive microvilli. Aberrant Na^+ channel isoform switch in CNS; switch is delayed in the PNS. Increased contactin in CNS nodes	Absence of transverse bands. Absence of contactin and Nf155 from paranodes. Progressive detachment of paranodal loops in CNS	K^+ channels and Caspr2 are found at the paranodes; more diffuse in the CNS than in the PNS. $K_v1.1$ clusters are lost over time in the CNS. Increased juxta-incisural lines along internodes	84,87,104
Cst null (Paranodal)	CNS: elongated nodes, abnormal shape and intensity; binary clusters. Decreased clustering with age (12% at 22 weeks)	Disrupted axoglial junction. Caspr diffusely distributed along the axon	CNS and PNS: diffuse K^+ channels and PSD-95 with some concentration at paranodes. Decreased clustering with age (8% at 22 weeks)	92,93
Caspr2 null (Juxtaparanodal)	Normal appearance	Normal appearance	Reduced K^+ channels clustering. Absence of TAG-1. Intense juxtamesaxonal labelling of K^+ channels	124
Tag1 null (Juxtaparanodal)	Normal appearance	Normal appearance	Reduced K^+ channels clustering. Absence of Caspr2. Small decrease in juxtaparanodal labelling of 4.1B	123,124
Dystroglycan (Nodal)	Reduced Na^+ channel clustering (90%); channels dispersed along a broader region (7%). Normal distribution of ankyrin G, moesin and Nf186. Abnormal microvilli morphology	Normal localization of Nf155	Normal localization of K^+ channels and Caspr2	63
quivering Spectrin βIV (Nodal)	ND	ND	$K_v1.1$ is upregulated and redistributed along the length of the axon	155
Spectrin βIV -genetrap (Nodal)	55% reduction in number of nodes as measured by $Na_v1.6$ immunoreactivity. Reduced intensity of Na^+ channels compared with WT	ND	ND	156
Nrcam null (Nodal)	Delayed Na^+ channel and ankyrin G clustering in PNS	Normal Caspr localization	ND	152
Na^+ channel β2 null (Nodal)	Normal appearance; $Na_v1.6$ appears within a normal time course. Reduced Na^+ current in optic nerve; CAP data consistent with loss of nodal Na^+ channels	Normal Caspr localization	ND	176

CNS, central nervous system; CAP, compound action potential; Cgt, ceramide galactosyltransferase; Cst, cerebroside sulfotransferase; ERM, ezrin/radixin/moesin; ND, no data; Nf, neurofascin; Nrcam, neuronal cell-adhesion molecule; PNS, peripheral nervous system; Tag1, transient axonal glycoprotein-1; WT, wild type.

to the juxtaparanodes as the paranodal junction forms[95,123,125,157]. In the absence of this junction, K^+ channels do not move to the juxtaparanodes and remain adjacent to the nodes[86,87,93–95] (FIG. 4 and, for further information, see TABLES 1 and 2). Further maintenance of K^+ channels at the juxtaparanodal region requires Caspr2 and Tag1, as these channels are redistributed along the internodes in their absence[123,124].

Molecular sieves, pickets and fences. The segregation of proteins to distinct domains in neurons is achieved through specific sorting mechanisms, followed by the anchoring and clustering of these proteins in the plasma membrane. The formation of the nodal environ might involve several distinct molecular mechanisms (FIG. 5). The exclusion of Na^+ channels from the extending edges of myelinating glia during development might be

JIMPY
A mouse strain in which the gene for the proteolipid protein is mutated, leading to defective myelination and oligodendrocyte death.

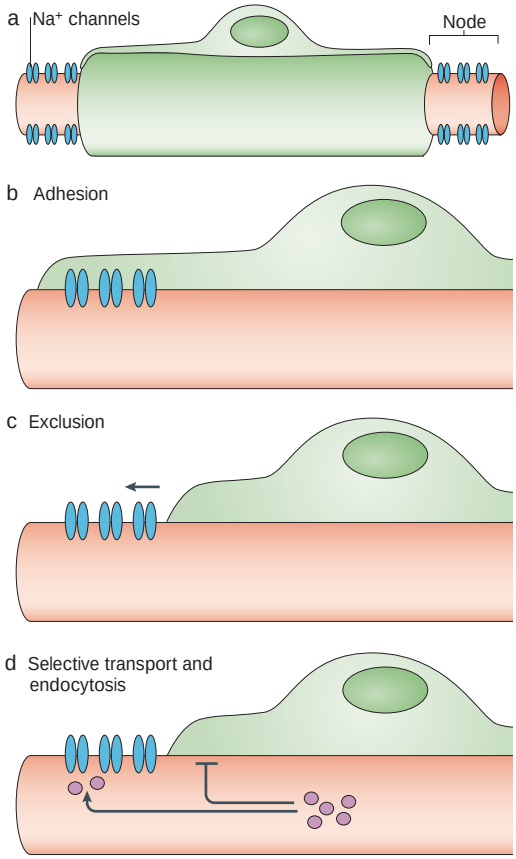


Figure 5 | **Possible mechanisms involved in node formation. a** | Schematic presentation of the appearance of Na^+ channels during development. Contacting processes from myelinating glial cells induce the clustering of molecules at the underlying axonal membrane. This clustering might be mediated by several distinct mechanisms (**b**–**d**), which could operate alone or in concert (see text for details). **b** | Na^+ channels are directed to the nodes by adhesive interactions with Schwann cell microvilli or perinodal astrocytes. **c** | Na^+ channels are excluded from the paranodes either by a molecular sieve or by a repulsive signal that is present at this site. **d** | Clustering of Na^+ channels at the nodes could be achieved by specific transport coupled with selective endocytosis along the internodes.

mediated by a selective molecular filter[111] or sieve[106] that is found at the paranodes (FIG. 5c). It was proposed that such a sieve selectively excludes large protein complexes, including Na^+ channels and Ig-CAMs that are connected to ankyrin G, while allowing the passage of small membrane particles, such as those that correspond to K^+ channels[106,111]. This process requires axoglial contact, but is not mediated by the Caspr/contactin complex, as its absence does not prevent Na^+ channels from clustering at the nodes[86,87]. So, the generation of mature, septa-containing paranodal junctions might not be required for the efficient clustering of Na^+ channels. This conclusion is further supported by freeze-fracture electron microscopic studies, disclosing an early differentiation of the nodes prior to the generation of the paranodal septa[73]. This implies that the initial axoglial contact at the paranodes is required for node formation, independently of the generation of septa. Accordingly, the accumulation of Caspr at the paranodes and the nodal clustering of Na^+ channels occur before the appearance of the septa[109]. It should be noted that gradual detachment of the paranodal loops in the CNS of paranodal mutants is accompanied by the widening of the nodal gap and dispersion of nodal Na^+ channels. This indicates that, although the septa are not required for the initial assembly of Na^+ channels at the nodes, stabilized glial contacts (which depend on septa) at the paranodes might be necessary to maintain these clusters[93,104,113]. Interestingly, clustering of Na^+ channels in the optic nerve of *Caspr*-null mice, which lack the paranodal septa, is associated with adjacent K^+ channel clusters, raising the possibility that Caspr2 and Tag1 compensate at these sites for the absence of Caspr and contactin[104].

In contrast to the clustering of Na^+ channels at the nodes, the formation of septa-containing axoglial junctions is essential for sequestering K^+ channels at the juxtaparanodes[86,87,94,95]. These observations indicate that, once formed, the axoglial septate junction functions as a fence that restricts the movement of these channels and other molecules from beneath the myelin sheath towards the nodes. They also imply that a molecular sieve operating at the paranodes during the formation of the nodes would have to change its properties after the paranodal loops have been secured to the axon by the septate junction. The generation of this fence might be mediated by the attachment of the Caspr/contactin complex to the axonal cytoskeleton[88], binding to a glial ligand, and the assembly of specific lipid microdomains. Although the contribution of the lipid composition of the membrane to the generation of axonal domains is yet to be investigated, it is of interest that contactin[158], Caspr[83] and Tag1 (REF. 159) are associated with rafts.

In addition to the paranodal junction, there might also be a membrane barrier at the nodes. Although, the Caspr2/K^+ channel complex and Tag1 are aberrantly located at the paranodal region in the absence of the paranodal junction, these proteins do not invade the nodes, indicating the existence of an additional barrier at this site[86,87,94]. A nodal barrier might be similar to the diffusion barrier (or a membrane fence) that is found at the axon initial segment, which could be regarded as the first node in most myelinated axons[160]. At the axon initial segment, this fence is formed by a high local concentration of transmembrane proteins that are anchored to the actin cytoskeleton and that serve as pickets, which can block the diffusion of membrane proteins and phospholipids[161]. Interestingly, an intact actin cytoskeleton in retinal ganglion axons is also required for the clustering of Na^+ channels by a soluble factor that is secreted from oligodendrocytes[21].

Two other molecular mechanisms that might operate in the formation of the nodes should be considered. In the PNS, clustering of nodal Na^+ channels during development could also be mediated by contacting glial processes that 'drag' Na^+ channels and Ig-CAMs towards their final position on the axolemma (FIG. 5b). This might be mediated by binding of Na^+ channels to the Schwann cell microvilli, either directly through their β-subunits, or indirectly through Nrcam and Nf186 (REF. 150). During

SHIVERER
A mouse strain in which the gene for myelin basic protein is mutated, leading to a defect in myelination. These animals are characterized by the presence of ataxia, tremor and cerebral atrophy.

QUIVERING
A mouse strain in which the gene for spectrin βIV is mutated, leading to progressive ataxia, tremor, hindlimb paralysis and deafness.

development, the ERM-positive Schwann cell microvilli make early contact with the nodes during their formation[54]. These contact-sites (termed 'caps') contain the phosphorylated adaptor EBP50 and face across axonal ankyrin G[53]. Disruption of microvilli in mice lacking Schwann cell dystroglycan resulted in a striking reduction in clustering of nodal Na^+ channels[63]. It remains to be seen whether dystroglycan binds any of the nodal proteins, thereby mediating this axoglial interaction.

The microvilli also contain other candidate proteins, including L1 (REF. 162) and neurofascin[136], both of which can bind Ig-CAMs present at the axolemma. Finally, it is possible that the clustering of Na^+ channels to the nodes is mediated by downregulation of these channels from beneath the internodes, and by the selective insertion of newly synthesized or recycled molecules to the forming nodal gap (FIG. 5d). Although it is less likely to operate during early development, a specific nodal delivery machinery is anticipated to exist, as indicated by the observations that Na^+ channel isoforms are replaced after the nodes have been formed[22,148], the presence of a high concentration of vesicles at the nodes[163], and the close association of Na^+ channels with microtubules[18]. Similar mechanisms might also operate in the formation of the juxtaparanodes in the CNS where K^+ channels are first detected during development[117,145].

Concluding remarks

The identification of a growing number of molecules that are present at the nodal environ and the generation of mice that lack some of these molecules have provided an initial insight into the mechanisms that are involved in the formation of different axonal domains at and around the nodes of Ranvier. The myelin sheath dictates the localization of molecules in the underlying axon during development and is necessary for their maintenance. Similar to synapses, cytoskeletal scaffolds that link CAMs with ion channels are assembled at the nodes of Ranvier and the juxtaparanodal region. By analogy to the molecular complexity of CNS synapses[164], it is clear that the journey towards the identification of all the proteins that participate in the formation and maintenance of the nodal environ has only begun. The development of specific procedures to isolate myelin and axolemmal proteins[165], coupled with molecular screens to identify protein-interaction networks should disclose many more components in the near future. In addition, the molecular and morphological similarities between the axoglial junction and septate junctions in invertebrates enable us to use *Drosophila* genetics for its study. A key challenge for future studies resides in understanding the role of each molecule during the coordinated differentiation of myelinating glia and their underlying axons.

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Competing interests statement

The authors declare that they have no competing financial interests.

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