Mechanisms of node of Ranvier assembly

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Abstract | The nodes of Ranvier have clustered Na⁺ and K⁺ channels necessary for rapid and efficient axonal action potential conduction. However, detailed mechanisms of channel clustering have only recently been identified: they include two independent axon–glia interactions that converge on distinct axonal cytoskeletons. Here, we discuss how glial cell adhesion molecules and the extracellular matrix molecules that bind them assemble combinations of ankyrins, spectrins and other cytoskeletal scaffolding proteins, which cluster ion channels. We present a detailed molecular model, incorporating these overlapping mechanisms, to explain how the nodes of Ranvier are assembled in both the peripheral and central nervous systems.

Axolemma
The plasma membrane of the axon.

Extracellular matrix
(ECM). A complex mixture of extracellular macromolecules, including glycoproteins, that surround cells.

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In the early 1800s, engineers dreamed of communication between North America and Europe using a transatlantic telegraph cable. Their vision was realized in 1858 and introduced a new 'Age of Information'[1]. Although this earliest cable only transmitted a few words per hour, it still reduced the time required for the transmission of a message by more than tenfold. Today, modern fibre-optic cable systems have further reduced this time to just milliseconds! However, these technological wonders lag ~400 million years behind the revolution in speed of axonal action potential conduction that occurred with the evolution of myelin and clustered ion channels at the nodes of Ranvier (FIG. 1). In addition to speed, myelin and clustered ion channels also dramatically reduce both the space requirements and metabolic costs of rapid action potential conduction. These evolutionary advances permitted the subsequent development of complex nervous systems[2,3].

The organization of our nervous system is similar to that of our modern transatlantic cable networks. Because signals degrade as they propagate along a cable, repeaters are used to regenerate the signals and increase the transmission range. In myelinated axons, the nodes of Ranvier function as repeaters to regenerate the action potential as it propagates in a saltatory manner along the axon to the nerve terminal, thereby dramatically increasing the speed of action potential propagation[4,5]. A prerequisite for the function of the nodes in saltatory conduction is the high density clustering of Na⁺ channels[6,7] and K⁺ channels[8–12] at the nodal axolemma (FIG. 1b). In addition, the nodes of Ranvier, as well as their flanking paranodal junctions, are comprised of unique cell adhesion molecules (CAMs), cytoskeletal scaffolds and extracellular matrix (ECM) molecules (TABLE 1). As these various molecular components of the nodal environs have come into focus (FIG. 1b,c), the specific molecular

mechanisms for their assembly have been revealed. In this Review, we emphasize and discuss nodal proteins in the context of the multiple overlapping axon–glia interactions that combine and converge on the axonal cytoskeleton to efficiently cluster and maintain high densities of Na⁺ and K⁺ channels at the nodes of Ranvier.
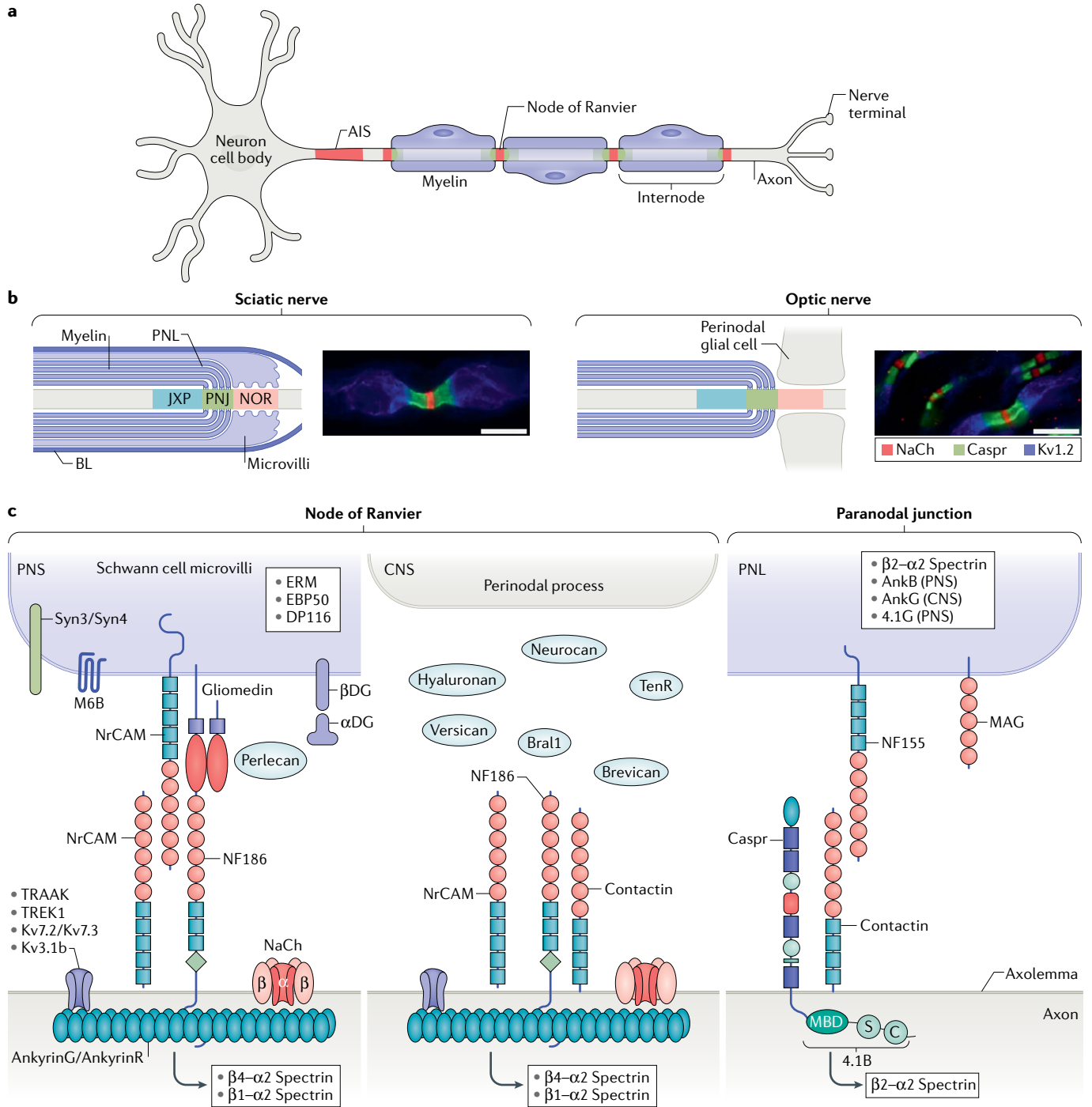
Myelinating glia control node assembly

Myelination is a late developmental process mediated by Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the CNS. For example, in mice, PNS myelination begins at birth and is mostly complete within 2 weeks, although sheath length continues to increase as the animal grows. CNS myelination is more protracted, with significant regional variation. Myelination begins at birth in the spinal cord and at 1 week of age in the optic nerve, being mostly complete by about 1 month of age. Ion channel clustering at the nodes of Ranvier follows and requires myelination, suggesting a causal connection. Together, myelination and the associated clustering of ion channels results in a profound transition in the mechanism of action potential propagation from continuous to saltatory conduction. Because of this dramatic physiological change and its importance for the developing, healthy and diseased brain, it is important to understand how myelinating glia regulate nodal ion channel clustering.

The concept that Schwann cells and oligodendrocytes cluster nodal ion channels was originally proposed by Rosenbluth[13,14] and relied on his detailed descriptions of myelinated axons analysed using freeze-fracture electron microscopy. Later developmental studies using antibodies to detect the earliest clusters of Na⁺ channels showed that they accumulate adjacent to the edges of the forming myelin sheath in both the PNS[15–17] and the CNS[6,18]. Further support that oligodendrocyte and myelinating

a

b

Sciatic nerve

Optic nerve

NaCh   Caspr   Kv1.2

c

Node of Ranvier

Paranodal junction

Schwann cell contact control the assembly of the nodes of Ranvier was obtained from studies demonstrating that channel clustering was largely impaired upon the depletion of oligodendrocytes and myelinating Schwann cells[19,20] or after demyelination[21–23]. While these studies strongly support the notion that axoglial contact clusters Na+ channels, a second model proposed that oligodendrocyte-secreted factors may initiate channel clustering along axons[24]. Recently, two studies[25,26] reported that soluble, oligodendrocyte-derived factors promote the formation of axonal Na+ channel clusters prior to myelination but only in GABAergic neurons; however, the prevalence of these clusters and whether

they eventually become nodes of Ranvier remain unclear[27]. Contact-mediated mechanisms depend on specific axoglial interactions that occur at the forming nodes of Ranvier (that is, 'nodal mechanism') and at the paranodal axoglial junction that border the nodes (that is, 'paranodal mechanism'). The latter is present in both the CNS and the PNS and is formed between the axon and the terminal cytoplasmic loops of oligodendrocytes or myelinating Schwann cells[28]. In contrast to the paranodal junction, axoglial contact at the nodes differs between the PNS and the CNS. In the PNS, the nodal axolemma is contacted by microvilli processes that are formed by the outer aspect of the adjacent myelinating

Microvilli
Small membrane protrusions that increase the surface area of a cell to facilitate adhesion, absorption, or signal transduction.

◄ Fig. 1 | **Organization of the nodal environ. a** | Myelin formed by Schwann cells or oligodendrocytes (not shown) covers the axon in segments (internodes) separated by the nodes of Ranvier. The nodes of Ranvier, which are flanked by the paranodal junction (green) as well as by the axon initial segment (AIS) contain a high density of Na⁺ channels (red). **b** | Schematic organization of a myelinated axon at the node of Ranvier (NOR) in the peripheral nervous system (PNS; sciatic nerve) and the CNS (optic nerve). The NOR, paranodal junction (PNJ; green) and the juxtaparanodal region (JXP; blue) are labelled. The node is contacted by Schwann cell microvilli in the PNS or by processes from perinodal glia in the CNS. The PNJ is formed between the paranodal loops (PNL) of the myelin and the axon. This junction separates the NOR from the JXP, which is enriched in Kv1 channels. In the PNS, myelinated fibres are covered by a basal lamina (BL). Immuno-labelling of sciatic and optic nerves using antibodies to nodal (Na⁺ channels; NaCh), paranodal (Caspr, a paranodal marker protein) and juxtaparanodal (K⁺ channels; Kv1.2) components are shown on the right (scale bars 10 μm). **c** | Molecular composition of nodes and the paranodal junction. The nodal axolemma is enriched in both Na⁺ (REF.[7]) and K⁺ channels (TRAAK, TREK-1, Kv7.2, Kv7.3 and Kv3.1b)[8,9,11,12]. These channels are associated with neurofascin 186 (NF186) and a secreted form of NrCAM as well as with ankyrin G (AnkG), ankyrin R (AnkR), β4, β1 and α2 spectrin (AnkR and β1 spectrin are located at the nodes during node assembly). In the PNS, the nodal gap contains Schwann cell microvilli that are enriched in several transmembrane proteins, including a complex of NrCAM and gliomedin[38], dystroglycan (αDG and βDG)[66], syndecan 3 (Syn3) and Syn4 (REFS[68,69]), and M6B[136]. The microvilli are embedded in heparan sulfate proteoglycan-rich extracellular matrix (that is, the nodal matrix) that also contains perlecan, which regulates the initial clustering of the nodal complex by gliomedin. At the CNS nodal axo-lemma, there is occasionally a direct contact of the perinodal astrocyte or oligodendro-cyte precursor cell processes. Here, the nodal gap contains several chondroitin sulfate proteoglycans and secreted NrCAM[39,71,73]. The paranodal junction in both the PNS and the CNS are formed by the binding of NF155 to an axonal adhesion complex containing contactin and Caspr. The latter binds protein 4.1B, thereby providing a link to β2 and α2 spectrin enriched at this site. On the glial side, NF155 is linked to β2 and α2 spectrin through ankyrin B (AnkB) in Schwann cells and AnkG in oligodendrocytes, both of which are found at paranodes during development and in adults[121]. MBD, membrane binding domain. Part **a** and part **c** adapted from REF.[112], Springer Nature Limited.

Schwann cell[14,29]. In the CNS, oligodendrocytes do not form nodal microvilli but many nodes are contacted by perinodal astrocyte and NG2⁺ (neural/glial antigen 2) glial progenitor cell processes[30–32] (FIG. 1b); although these processes have been proposed to participate in node assembly[33], the evidence to date is inconclusive. As discussed below, these important morphological differences dictate some of the variations in the mechanisms that operate during the assembly and maintenance of the nodes of Ranvier in the PNS and the CNS.

Nodal mechanisms

CAMs mediate axon–glia interactions. What molecular interactions occur at the nodes of Ranvier between myelinating glia and axons? Nodes of Ranvier include the axonal, ankyrin-binding CAMs neurofascin 186 (NF186) and NrCAM[34] as well as the scaffolding protein ankyrin G (AnkG)[35] (FIG. 1c). The discovery of these proteins at the nodes immediately suggested that nodal ion channel clustering is directed by ankyrin-binding CAMs. During early development and before the onset of myelination, NF186 and NrCAM are distributed uniformly along axons[36]. As myelination progresses, these CAMs become enriched at the ends of myelinating glial cells through specific axon–glia interactions (see below)[17,37–39]. At these locations, NF186 then functions as an attachment site to recruit the Na⁺ and K⁺ channel-binding scaffolding protein AnkG.

The importance of NF186 for nodal Na⁺ channel clustering was strongly supported by the observation

Splice variant
variants of a gene product that are produced by alternative splicing of the RNA.

that mice with a disrupted *Nfasc* gene fail to cluster AnkG or Na⁺ channels[40]. However, this conclusion was complicated by the recognition that the two major *Nfasc* gene products are both found at or near the nodes of Ranvier: NF186 in axons and NF155 at paranodes of myelinating Schwann cells and oligodendrocytes. Paranodal NF155 is an obligate component of the par-anodal axoglial junction[41,42]. Thus, using a constitutive *Nfasc*-deficient mouse it was not possible to distinguish the role of axonal NF186 in node formation from that of glial paranodal NF155. Recently, pathogenic human variants of *Nfasc* and its binding partners have been reported, with profound consequences for nervous system function (BOX 1).

To determine the contributions of each *Nfasc* splice variant, Zonta et al.[43] used a transgenic rescue strategy and expressed NF186 or NF155 in neurons or myeli-nating glia of *Nfasc* knockout mice, respectively. The expression of NF186 in neurons alone was sufficient to rescue nodal AnkG and Na⁺ channel clustering. However, transgenic expression of NF155 in myelinat-ing glia also rescued Na⁺ channel and AnkG clustering. Similarly, Na⁺ channels were clustered at the nodes of *Nfasc*-null dorsal root ganglion (DRG) neurons myel-inated in vitro by wild-type Schwann cells[38]; thus, the glial NF155 necessary to form paranodal junctions is sufficient to cluster Na⁺ channels. Together, these results strongly support a model where only one neurofascin isoform is necessary for node formation, although each participates in distinct axon–glia interactions capable of independently clustering ion channels and AnkG at the nodes of Ranvier.

More direct experiments to determine the specific and independent functions of NF155 and NF186 relied on the use of floxed *Nfasc* alleles and *Cre* mouse lines for neurons or glia. For example, the genetic deletion of *Nfasc* only in myelinating glia removed NF155 and disrupted the paranodal junction[42]. Nevertheless, these mice still had clustered nodal Na⁺ channels and AnkG since axonal NF186 was not affected. Thus, an intact paranodal junction is not required for node assembly[44–46]. In contrast to NF155-deficient mice, the specific genetic deletion of neuronal NF186 was reported to block nodal Na⁺ channel clustering[47]. However, a major limitation of these studies was that the *Nefl-Cre* line used to disrupt neuronal *Nfasc* is very inefficient. For example, at post-natal day 11, the majority of nodes (54% in the PNS and 84% in the CNS) still had Na⁺ channels and a significant fraction of nodes lacking NF186 still had Na⁺ channels.

To resolve the question of whether NF186 is required for node assembly, Amor et al.[48] generated two new *Nfasc* floxed alleles and crossed them with highly penetrant *Cre* lines to induce the recombination and deletion of NF186 from PNS and CNS neurons, ret-inal ganglion cells, and spinal cord neurons. In all of these NF186-deficient mice, Na⁺ channels were still clustered at the nodes of Ranvier, while the glial ECM proteins failed to cluster at the nodes. Together, these results show that paranodal mechanisms are sufficient to cluster nodal Na⁺ channels. However, with increas-ing age, there was a gradual reduction in the number of nodes with Na⁺ channels in both the PNS and the CNS,

Table 1 | **Adhesion, ECM and cytoskeletal components of the nodes of Ranvier**

Cell adhesion	CNS	PNS
NF186 (REF.[34])	Membrane, axonal	Membrane, axonal
NrCAM[34,38,39]	Secreted, nodal gap	Secreted, nodal gap and membrane, Schwann microvilli
Dystroglycan[70]	–	Membrane, Schwann microvilli
M6B[136]	–	Membrane, Schwann microvilli
Navβ1,2,4 (REFS[137,138])	Membrane, axonal	Membrane, axonal
Contactin[139]	Source unknown	–
ECM		
Gliomedin[37]	–	Membrane, Schwann microvilli and secreted, nodal gap
Syndecan 3,4 (REF.[69])	–	Membrane, Schwann microvilli
Perlecan[66]	–	Secreted, nodal gap
Collagen XXVII[140]	–	Secreted, nodal gap
Collagen V[68]	–	Secreted, nodal gap
Laminin α2/5β1γ1 (REF.[141])	–	Secreted, nodal gap
Brevican[72]	Secreted, nodal gap	–
Versican V1 (REF.[68])	–	Secreted, nodal gap
Versican V2 (REF.[73])	Secreted, nodal gap	–
Neurocan[77]	Secreted, nodal gap	–
Bral1 (REF.[73])	Secreted, nodal gap	–
Phosphacan[71]	Secreted, nodal gap	–
Tenascin-R[71]	Secreted, nodal gap	–
Hyaluronan[73]	Secreted, nodal gap	–
NG2 (REFS[32,142])	Membrane, glial	Secreted, nodal gap
Cytoskeletal scaffolds		
AnkG[35]	Intracellular, axonal	Intracellular, axonal
AnkR[90]	Intracellular, axonal	Intracellular, axonal
β4 spectrin[143]	Intracellular, axonal	Intracellular, axonal
β2 spectrin[90]	Intracellular, axonal	Intracellular, axonal
α2 spectrin[104]	Intracellular, axonal	Intracellular, axonal
ERM proteins[144,145]	–	Intracellular, Schwann microvilli
EBP50 (REFS[146,147])	–	Intracellular, Schwann microvilli
DP116 (REF.[141])	–	Intracellular, Schwann microvilli

ANKG, ankyrin G; AnkR, ankyrin R; ECM, extracellular matrix; NF186, neurofascin 186; PNS, peripheral nervous system.

a result consistent with the idea that NF186 also helps to maintain and stabilize Na⁺ channels at the nodes[49], most likely through its interaction with ECM proteins. The role of NF186 in stabilizing and maintaining the nodes has important implications for human PNS autoimmune diseases, where NF186 and its glial binding partners can function as autoantigens (BOX 1). These results do not imply that NF186-dependent mechanisms have no role in nodal Na⁺ channel clustering but instead show that NF186 is not essential and that an NF155-dependent mechanism can also cluster nodal Na⁺ channels.

Mice lacking NrCAM have delays in Na⁺ channel clustering[50]. This is rather surprising given the sequence similarity and domain organization shared by these two immunoglobulin superfamily CAMs[51], their precise colocalization along myelinated axons and, in particular, their ability to interact with the same extracellular[37] and intracellular[52] proteins. As shown by Feinberg et al.[38], while NF186 is present at the nodal axolemma as a transmembrane protein, in the PNS, NrCAM is present at both the nodal axolemma (that is, axonodal CAM) and the Schwann cell microvilli (that is, glianodal CAM) that contact the nodes (FIG. 1c). To complicate the setting even further, at peripheral nodes of Ranvier, NrCAM may be found as transmembrane and secreted forms that function in synergy with other glial-derived factors to promote node assembly (discussed below). Nevertheless, the addition of either NrCAM-Fc (a soluble recombinant form of its ectodomain) or NF186-Fc fusion proteins to myelinating DRG–Schwann cell co-cultures inhibits the clustering of Na⁺ channels and AnkG[53,54]. NrCAM-Fc fusion proteins bind NF186, suggesting that NrCAM-Fc inhibits early nodal Na⁺ channel clustering by blocking the normal NF186-dependent Schwann cell–axon interactions. In agreement with a role for NrCAM as a glial ligand rather than as an axonal receptor involved in node assembly, CNS nodes may only contain the secreted but not the transmembrane form of NrCAM[39,43]. In the axon, NrCAM may be involved in the transport of NF186, as the two were recently found to be co-transported anterogradely during myelination[55]. Taken together, these observations show that nodal and paranodal CAMs mediate axon–glia interactions and are central contributors to node assembly.

Glial ECMs organize and maintain the nodes. What mechanisms induce the clustering of NF186 in axons? To address this question in the PNS, Dzhashiashvili et al.[56] constructed chimeric transmembrane proteins containing cytoplasmic or extracellular domains of NF186 and transfected these into DRG neurons in vitro. After inducing myelination by Schwann cells, they found that the extracellular domain of NF186 was required for its clustering at the end of myelinating Schwann cells and for its clearance from beneath the myelin sheath. These results indicate that interactions between NF186 and extracellular glia-derived factors induce the early clustering of NF186 at PNS nodes. In follow-up studies, Zhang et al.[57] further showed that a pre-existing pool of axolemmal NF186 is clustered at forming PNS nodes and that this depends on extracellular interactions. However, after paranodal junctions form, the accumulation of nodal NF186 requires its cytoplasmic ankyrin-binding domain[58]. To address this same question in CNS neurons, Susuki et al.[39] electroporated cortical neurons in utero with GFP-tagged NF186 deletion constructs. They also found that both intracellular and extracellular interactions can independently direct NF186 to CNS nodes. Nodal targeting by the intracellular domain of NF186 required the five-amino acid sequence FIGQY previously shown to mediate its interaction with AnkG[59]. After the nodes have formed and as occurs in the PNS, replenishment and continued accumulation of NF186 in adults requires intact paranodes[58]. Together, these results show that both extrinsic and intrinsic interactions contribute to the targeting of NF186 to the nodes of Ranvier.

Heminodes
The ends of a myelin segment lacking another flanking myelin segment.

Gliomedin clusters PNS Na$^+$ channels. What extrinsic, glia-derived factors cluster and stabilize NF186? The answer for this question is different for the PNS and the CNS and reflects a fundamental difference between these two regarding Na$^+$ channel clustering. In the PNS, the nodal complex (that is, NF186, AnkG, β4 spectrin and Na$^+$ channels) is first clustered at heminodes located at the ends of each developing myelin segment, even before the formation of the paranodal junction[16,17]. In the absence of this heminodal clustering, Na$^+$ channels are concentrated throughout the forming nodal gap[38,48]. In contrast, in the CNS, the nodal complex appears at the nodal gap only after the paranodal junction has formed[18]. Notably, in the developing spinal cord, NF186 and Na$^+$ channels concentrate at the boundaries between the forming nodal gap and the adjacent paranodal junction[60]. This symmetric accumulation of the nodal complexes is distinct from the heminodal clustering detected in the PNS (FIG. 2a) and depends on paranodal cytoskeletal mechanisms (see below).
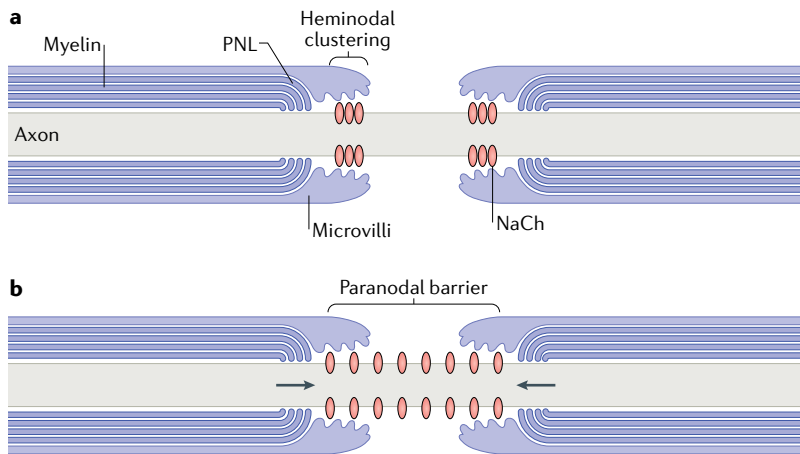
Experiments using mixed DRG neuron and Schwann cell cultures revealed that the extracellular region of neurofascin binds Schwann cells but not neurons[37,54]. This led to the identification of gliomedin, a Schwann cell microvillar protein, as a glial ligand for axonal NF186 (REF.[37]) (FIGS 1c, 3). Gliomedin is a transmembrane protein secreted from the cell surface by a furin protease[61,62]. The binding of gliomedin to NF186 in isolated DRG neurons results in further recruitment and co-clustering of AnkG, β4 spectrin and Na$^+$ channels[37] (FIG. 3). In line with this observation, the clustering of Na$^+$ channels by gliomedin in vitro requires NF186 and is not detected on DRG neurons isolated from *Nfasc*-null mice[38] or from neuron-specific *Nfasc*-conditional knockout mice[48]. Gliomedin contains an olfactomedin domain that mediates its binding to both NF186 and NrCAM[37,63] and a

collagen-like region that enables its oligomerization[61,62]. The presence of these two functional domains within gliomedin form the structural basis for its ability to cluster NF186 on the axolemma[61,64]. During the development of peripheral nerves, gliomedin functions as a glial signal that is required for the initial heminodal clustering of Na$^+$ channels (FIG. 2a). Thus, heminodal Na$^+$ channel clusters do not form after the genetic removal of gliomedin[37,38]. Surprisingly, the clustering of Na$^+$ channels by gliomedin also requires glial but not neuronal expression of NrCAM[38]. Furthermore, the interaction between NrCAM and gliomedin enhances the binding of the latter to DRG neurons[38]. These experiments suggested that transmembrane NrCAM present at the Schwann cell microvilli plays a dual role in Na$^+$ channel clustering by (1) serving as a scaffold that traps gliomedin and (2) enhancing gliomedin's presentation and binding to axonal NF186. Furthermore, the absence of both gliomedin and NrCAM leads to a gradual loss of nodal Na$^+$ channels[48]. Hence, the continuous interaction between gliomedin, NrCAM and NF186 not only mediates the initial clustering of Na$^+$ channels but is also required for their long-term maintenance at the nodes of Ranvier[48,49]. The importance of these axoglial nodal CAMs and gliomedin is also reflected by their involvement in both hereditary and acquired neurological diseases (BOX 1).

Although secreted gliomedin induces clustering of Na$^+$ channels even in the absence of Schwann cells[37], these channels are mainly clustered at the forming nodes of Ranvier, suggesting that the activity of gliomedin is tightly regulated. Such regulation is provided at three levels: (1) by its temporal expression by Schwann cells during development[37], (2) by its proteolytic cleavage[65] and (3) by its binding to NrCAM and heparan sulfate proteoglycans (HSPGs) present at the nodal gap ECM[61,66]. In addition to furin, which is required for the shedding of gliomedin from the plasma membrane, the extracellular domain of gliomedin is cleaved by a bone morphogenetic protein 1 (BMP1)/Tolloid-like (TLD) metalloproteinase[61,62]. This cleavage separates the CAM-binding olfactomedin domain and the N-terminal coiled-coil and collagen domains, which mediate its oligomerization. Recent work by Eshed-Eisenbach et al.[65] revealed that BMP1/TLD-like proteinases confine Na$^+$ channel clusters to the developing nodes of Ranvier by negatively regulating the activity of gliomedin. Eliminating the BMP1/TLD cleavage site in gliomedin or treatment of Schwann cell or DRG neuron co-cultures with a BMP1/TLD inhibitor induced the formation of ectopic axonal Na$^+$ channel clusters. Moreover, Schwann cell deletion of *Bmp1* and *Tll1* genes in mice resulted in ectopic and premature clustering of Na$^+$ channels around early ensheathing Schwann cells before myelination, which altered nerve conduction[65]. Another mode of regulation of gliomedin-dependent Na$^+$ channel clustering in the PNS is provided by HSPGs that are enriched in the nodal gap ECM[61,64]. These nodopathies or paranodopathies are associated with antibodies to nodal gangliosides[170,171], neurofascin, Caspr and contactin[172]. These antibodies are considered pathogenic as they disrupt nodal organization and the attachment of the paranodal loops to the axon[173–175], subsequently leading to abnormal nerve conduction and axon degeneration[176].

Box 1 | Involvement of nodal environ components in human diseases

The importance of the nodes of Ranvier to the normal function of the nervous system is reinforced by the involvement of different nodal as well as paranodal components in neurological diseases. Loss-of-function mutations in gliomedin (*GLDN*) in humans causes lethal congenital contracture syndrome 11 (OMIM#617194) associated with decreased fetal movement in utero, likely due to abnormal nerve conduction[148,149]. Mutations in Caspr (*CNTNAP1*) have also been associated with lethal congenital contracture syndrome type 7 (OMIM#616286)[150,151], with Charcot–Marie–Tooth disease[152] and with congenital hypomyelinating neuropathy (OMIM#18186)[151,153–157]. Hypomyelination in the absence of Caspr likely reflects the recently discovered importance of the intact paranodal junction to CNS myelination[158,159]. In agreement, mutations in neurofascin (*NFASC*), which is critical for the formation of the nodal environ and for myelination[134], cause neurodevelopmental disorder with central and peripheral motor dysfunction (OMIM#609141)[160,161] and peripheral demyelination[162]. In addition to these axoglial cell adhesion molecules, mutations in cytoskeletal components of the nodes and axon initial segments are associated with neurological diseases: mutations in β4 spectrin (*SPTBN4*) in neurodevelopmental disorder with hypotonia, neuropathy, and deafness (OMIM#617519)[106,163,164] and α2 spectrin (*SPTAN1*) in hereditary motor neuropathy and cerebral hypomyelination[165,166]. Finally, autoantibodies to both nodal and paranodal junction components have been identified in patients with chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and Guillain–Barre syndrome[167–169]. These nodopathies or paranodopathies are associated with antibodies to nodal gangliosides[170,171], neurofascin, Caspr and contactin[172]. These antibodies are considered pathogenic as they disrupt nodal organization and the attachment of the paranodal loops to the axon[173–175], subsequently leading to abnormal nerve conduction and axon degeneration[176].

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surrounding the Schwann cell microvilli[66,68]. In cultured Schwann cells, secreted gliomedin is incorporated into the ECM by binding HSPGs, an interaction mediated by its collagen-like domain[61]. Several HSPGs

Fig. 2 | **Two overlapping axoglial adhesion systems assemble the nodes of Ranvier.**
a | The initial clustering of Na^{+} channels occurs at heminodes that flank each of the forming myelin segments and paranodal loops (PNL). This clustering depends on specific axoglial contact made between the forming Schwann cell microvillar processes and the axolemma. **b** | The paranodal junction provides another mechanism for the accumulation of Na^{+} channels by forming a cytoskeletal barrier that can restrict and cluster (arrows) these channels in the nodal gap.

are present at the PNS nodes, including syndecan 3 and 4 (REFS[68,69]), agrin and perlecan[66] (TABLE 1). The latter binds gliomedin and facilitates its Na^{+} channel clustering activity[66]. Perlecan is recruited by dystroglycan, a Schwann cell microvillar protein that is required for the alignment of microvilli processes to the axon as well as for the stabilization of Na^{+} channels at the nodes of Ranvier[70]. Much like mice lacking either gliomedin or NrCAM[38], the specific deletion of dystroglycan in Schwann cells results in reduced Na^{+} channel clustering at the nodes[66]. In summary, assembly of the nodal complex in the PNS is initiated by the binding of Schwann cell-derived gliomedin and NrCAM to axonal NF186. These interactions are further regulated and stabilized by ECM components that are present at the nodal gap. Taken together, these results highlight the central role of Gliomedin and its associated proteins in the earliest clustering of Na^{+} channels in the PNS.

CNS nodal ECM proteins. CNS nodes of Ranvier are also surrounded and enriched with ECM molecules (TABLE 1, FIG. 1), including core, NF186-interacting proteins and peripheral components whose localization depends on the core proteins. More precisely, the chondroitin sulfate proteoglycans (CSPGs) brevican and versican V2, the hyaluronan-binding link protein Bral1, and secreted NrCAM are all found at the nodes and bind directly to nodal NF186 (REFS[39,71–73]) (FIGS 1, 3). Bral1 stabilizes the core CSPGs and loss of Bral1 results in the coincident loss of nodal brevican and versican V2 (REF.[74]); conversely, Bral1 is absent from the nodes after the loss of brevican and versican V2, indicating that these core nodal ECM proteins reciprocally stabilize each other[39]. The peripheral components of the CNS nodal ECM include tenascin-R (TnR), phosphacan and neurocan. Nodal phosphacan requires nodal TnR[75], which in turn requires nodal brevican and versican V2 (REFS[71,76]). The accumulation of nodal neurocan requires Bral1 (REF.[77])

and versican V2 (REF.[39]). Intriguingly, the situation is even more complicated than what is presented above: most nodes have Bral1 and versican V2, but brevican, TnR and phosphacan are preferentially enriched at nodes of Ranvier with a large diameter. The reasons for and how this heterogeneity arises among the nodes is unknown, but it is reminiscent of the diversity of perineuronal nets that also consist of CSPGs and hyaluronan-binding link proteins and that surround the cell bodies of some CNS neurons[78].

In contrast to the nodal CSPGs and Bral1, the clustering of secreted NrCAM does not depend on any component of the nodal proteoglycan-based ECM. Instead, its localization likely depends entirely on NF186 (REF.[48]). Like gliomedin in the PNS, NrCAM in the CNS undergoes multiple proteolytic cleavage events necessary for its shedding. NrCAM is cleaved by furin and at a second site that is likely mediated by an ADAM metalloproteinase; this second site is the major contributor to NrCAM secretion[39]. In contrast to the important role of NrCAM for heminodal clustering of Na^{+} channels in the PNS[38], the function of secreted NrCAM in the CNS remains unknown.

Together, these observations show that a complex network of ECM and secreted proteins is found at CNS nodes. These nodal ECM proteins are sufficient to induce the clustering of NF186 when overexpressed in cultured DRG neurons[39]. Thus, one might be tempted to conclude that the CNS nodal ECM functions much like gliomedin and its associated ECM to initiate Na^{+} channel clustering; however, this would be incorrect. In contrast to gliomedin-NF186 interactions, which are necessary for the earliest heminodal clustering of Na^{+} channels at the ends of elongating myelin sheaths in the PNS[37], the CNS nodal ECM cannot be detected until after the accumulation of NF186 adjacent to well-defined paranodal junctions[39] (FIG. 2). Thus, the CNS nodal ECM is not instructive for CNS nodal protein complex assembly. This indicates that the paranodal mechanism is the primary clustering mechanism in the CNS, with nodal ECM–NF186 interactions functioning as a secondary mechanism. This is consistent with the fact that CNS nodes lacking NF186 and nodal ECM have clustered Na^{+} channels[48]. Conversely, in the absence of paranodal junctions, the nodal ECM stabilizes NF186 in the membrane at nascent nodes between myelin segments, thereby permitting the subsequent (although less efficient) recruitment of AnkG and other components of the nodal protein complex. In support of this model, at postnatal day 18, CNS nodes in mouse spinal cord lacking single or combinations of nodal ECM proteins all have normally clustered Na^{+} channels[39,74]. In contrast, at this same developmental timepoint, only ~70% of nodes have clustered Na^{+} channels in mice lacking paranodal junctions. These results suggest that multiple mechanisms work together in the CNS to form nodes, with the nodal ECM functioning mainly to stabilize the nodal protein complex. Nevertheless, this means that the nodal ECM can also function as a secondary but less efficient mechanism for glia-driven, NF186-dependent assembly of the nodal protein complex. Finally, the nodal ECM may also function as a cation reservoir that serves as an

ion diffusion barrier to facilitate saltatory conduction. For example, Bral1-deficient mice have significantly reduced conduction velocities in the CNS[74].

Nodal scaffolds and cytoskeletons. Nodal NF186, Na+ channels and Kv7.2 and Kv7.3 (*KCNQ2* and *KCNQ3*) K+ channels are all direct binding partners for AnkG. The specific domains and motifs that mediate their interactions have been mapped in detail[10,79–82]. For example, the ankyrin-binding region of Na+ channels is a 22-amino acid motif, located in the channel's II–III linker domain, common to all vertebrate voltage-gated Na+ channels[80,81,83]. The phosphorylation of serine residues in this motif by CK2 increases Na+ channel affinity for ankyrins[84]. This motif is both necessary and sufficient for nodal Na+ channel clustering[83]. Like the nodes of Ranvier, axon initial segments (AIS) have high densities of NF186, Na+ channels, and Kv7.2 and Kv7.3 K+ channels but NF186 does not contribute to AIS AnkG clustering[72,85]. In contrast to the nodes, AIS form spontaneously and do not require myelinating glia for Na+ channel clustering. Because of this difference,

AIS have been used effectively to dissect the intrinsic mechanisms of Na+ channel clustering in axons and the mechanisms discovered there were assumed to apply to the nodes of Ranvier as well. Many in vivo and in vitro studies show that AnkG is required for AIS Na+ channel clustering[56,72,86–88].

One notable exception to this general rule may be the recent identification of the leak K+ channels found at mammalian nodes of Ranvier (FIG. 1). In mammals, the repolarization of the nodal membrane occurs through leak K+ channels rather than voltage-gated K+ channels[89]. These leak K+ channels were recently identified as TREK1 (*KCNK2*) and TRAAK (*KCNK4*)[8,9]. Surprisingly, these leak channels are only found at the nodes of Ranvier and not in AIS, suggesting that they may have novel AnkG-independent mechanisms for their localization.

Nevertheless, the molecular and functional similarities between AIS and the nodes of Ranvier strongly suggested that nodal Na+ channel clustering is mediated through its interactions with AnkG. To directly test this, Ho et al.[90] crossed a floxed *Ank3* (the gene encoding AnkG) allele with *Avil-Cre* mice to disrupt AnkG expression in peripheral sensory neurons. The choice of the *Avil-Cre* line was important since mice lacking AnkG in all neurons die at birth and fail to cluster Na+ channels at AIS[90]. Using *Avil-Cre* mice bypassed perinatal lethality by restricting recombination to DRG sensory neurons and allowing for myelination to occur. Furthermore, since DRG sensory neurons lack AIS[91], by using *Avil-Cre* mice it was possible to distinguish nodal AnkG functions from AIS AnkG functions. In contrast to the expected outcome, mice lacking nodal AnkG in sensory axons had normal behaviour, normal sensory root conduction velocities, and high densities of nodal Na+ channel clusters — even in 2-year-old mice! These mice also lacked nodal β4 spectrin, which links AnkG and the nodal protein complex to the actin cytoskeleton. Thus, AnkG and β4 spectrin are not required for the initial clustering or maintenance of Na+ channels at the nodes of Ranvier.

A hierarchy of nodal scaffolds. How can Na+ channels still be clustered at the nodes of Ranvier in the absence of AnkG and β4 spectrin, when numerous studies show that Na+ channel clustering at the AIS requires AnkG? Ho et al.[90] discovered that a pre-existing pool of axonal ankyrin R (AnkR) and its preferential spectrin binding partner, β1 spectrin, compensate for and replace AnkG and β4 spectrin to rescue nodal Na+ channel clustering (FIG. 3) but not AIS Na+ channel clustering; this is because AnkR lacks the giant exon found in AnkG required for its AIS localization[92,93]. Similarly, the loss of nodal β4 spectrin was accompanied by the loss of nodal AnkG, which was then replaced by AnkR and β1 spectrin[93] (FIG. 3). Thus, the depletion or loss of either AnkG or β4 spectrin is compensated for by an AnkR–β1 spectrin protein complex. The simultaneous deletion of AnkR and AnkG completely blocked nodal Na+ channel clustering, proving the requirement for an ankyrin scaffold to cluster Na+ channels at the nodes of Ranvier. These studies in ankyrin and spectrin-deficient mice were complemented
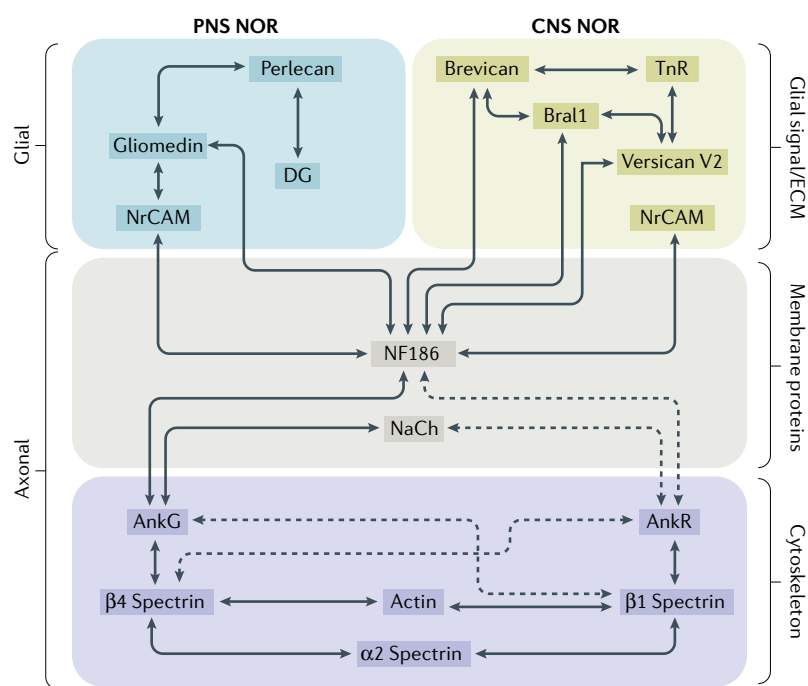
Fig. 3 | **Molecular interactions at the nodes of Ranvier.** Peripheral nervous system (PNS) nodes of the Ranvier (NOR) are surrounded by several interacting and glia-derived proteins. Gliomedin and NrCAM bind to neurofascin 186 (NF186) and function as the glial signal to initiate heminodal clustering of NF186. CNS NOR are also surrounded by interacting extracellular matrix (ECM) proteins and secreted NrCAM. Among these, a set of core ECM proteins binds NF186 and stabilizes it in the axonal membrane. NF186 functions as a binding site for the recruitment of the scaffolding proteins ankyrin G (AnkG) and ankyrin R (AnkR). AnkG is found at mature nodes but AnkR can substitute in mice lacking AnkG; both AnkG and AnkR are found at developing NOR. AnkR is replaced during node maturation because it has a lower affinity for NF186 compared to AnkG (low affinity interactions are indicated by dotted lines). Na+ channels (NaCh) bind directly to AnkG and AnkR, although the interaction with AnkG is stronger. AnkG and AnkR preferentially bind to β4 and β1 spectrin, respectively. Spectrins form a tetramer consisting of two β4 (or β1) spectrins and two α2 spectrin proteins. These spectrin tetramers bind to and link actin rings in a periodic organization along the axon. The spectrin cytoskeleton is required to maintain the nodal protein complex. DG, dystroglycan; TnR, tenascin-R.

by biochemical studies that revealed a hierarchy of preferential binding affinities among nodal proteins: AnkG has a higher affinity for NF186, Na⁺ channels and β4 spectrin than does AnkR, but AnkR has a higher affinity for β1 spectrin than for β4 spectrin. These observations revealed primary and secondary nodal Na⁺ channel clustering mechanisms that depend on the specific affinities of each protein in the network of NF186, Na⁺ channel, ankyrin and spectrin interactions (FIG. 3). During early developmental node formation, AnkR-β1 spectrin may also be found at the nodes. However, with increasing age, these are replaced by AnkG-β4 spectrin due to their higher affinity for NF186 (REF.[90]).

Finally, AnkG may be required for node maturation and maintenance since AnkG-deficient axons degenerate[94]. While it is difficult to determine if the loss of AnkG from nodes or AIS causes axon degeneration or if axon degeneration itself causes the loss of nodal Na⁺ channels, these results are consistent with the idea that AIS AnkG helps preserve axon integrity[86]. This is important since nervous system injuries and diseases can induce the proteolysis and loss of AnkG[95,96].

β4 spectrin links nodal AnkG and Na⁺ channels to the underlying actin cytoskeleton[97,98]. In axons, spectrins and ankyrins form a periodic cytoskeleton with sub-membranous rings of braided actin spaced at 190 nm intervals by spectrin tetramers[99–101]. This cytoskeletal arrangement may help the axon withstand mechanical forces by acting as a tension buffer; modelling suggests that this occurs by reversibly unfolding the repeating spectrin domains to release mechanical stress[102]. Nodal Na⁺ channels, AnkG, β4 spectrin and α2 spectrin are also organized in a periodic manner[103,104]. The loss of nodal β4 spectrin disrupts the nodes of Ranvier, leading to membrane instability and wider nodes[105]. However, these studies did not distinguish between the role of β4 spectrin at AIS and that at the nodes of Ranvier. Since β1 spectrin can compensate for β4 spectrin in AnkG-deficient peripheral sensory axons, the nodes of sensory axons should be normal. Consistent with this idea, human pathogenic variants in β4 spectrin have recently been described (BOX 1) and shown to cause profound intellectual disability and motor axonal neuropathy[106]. Interestingly, these patients have no peripheral sensory abnormalities.

Since β1 and β4 spectrin both function as a tetramer with α2 spectrin, the ablation of α2 spectrin from sensory neurons disrupts the nodes of Ranvier[104]. Although this observation suggests that spectrin cytoskeletons are required for node assembly, this cannot be concluded since paranodes also have a β2 spectrin-dependent cytoskeleton necessary for their proper function[104,107]. Therefore, to determine the specific function of the nodal spectrin cytoskeleton, mice lacking β1 or β4 spectrin, or β1 and β4 spectrin simultaneously in peripheral sensory neurons were recently generated[93]. Mice lacking β1 spectrin alone had no phenotype due to the primary function of β4 spectrin at the nodes. Mice lacking β4 spectrin in peripheral sensory neurons also had normal sensory neuron physiology and Na⁺ channel clustering due to the replacement of nodal β4 spectrin by β1 spectrin. However, mice lacking both β1 and β4 spectrin in sensory neurons had a profound dysfunction of myelinated sensory axons and even showed signs of neuronal injury, including the upregulation of the common stress-responsive transcription factor ATF3. Interestingly, although these mice had normal clustering of Na⁺ channels during early development, over time, they lost nodal Na⁺ channels. Together, these results indicate that nodal β spectrins are not required for the initial clustering of Na⁺ channels but are required to maintain high levels of nodal Na⁺ channels. Furthermore, the axon degeneration and neuronal injury observed in β1–β4 double conditional knockout mice and α2 spectrin conditional knockout mice[93,104] supports the notion that the nodal spectrin cytoskeleton is essential to maintain axon integrity and that the nodes of Ranvier may be particularly vulnerable to mechanical forces, diseases or injuries that can damage axons.

Conceptually, the nodal cytoskeleton may be analogous to a keystone that bears the weight of an arch. As described above, the actin–spectrin cytoskeleton is organized in repeating rings of braided actin spaced by spectrin tetramers[101]. However, these spectrins are thought to be held under constant tension rather than compression[102]. Just as an arch falls without its keystone, the loss of nodal spectrins results in axon injury and degeneration[93,104]. During development, axonal actin–α2–β2 spectrin rings are found throughout the length of the axon. We propose that NF186, located at the ends of elongating myelin sheaths, may act as a 'wedge', breaking the pre-existing actin–α2–β2 spectrin periodic cytoskeleton for the attachment of AnkG and subsequent insertion and nucleation of the nodal actin–α2–β4 spectrin periodic cytoskeleton.

Paranodal mechanisms

The second nodal ion channel clustering mechanism depends on paranodal axoglial junctions. Studies of Na⁺ channel clustering during myelination and remyelination suggested that paranodes function as barriers that 'push' Na⁺ channels already present in the axonal membrane ahead of the myelin sheath as it extends along the axon[15,18,21,108]. These studies were complemented by those showing the sufficiency of paranodal NF155 to cluster Na⁺ channels in NF186-deficient axons[38,43,48]. How do paranodes function as a barrier to induce the clustering of ankyrins and ion channels in an NF186-independent manner? Paranodal junctions are the largest vertebrate intercellular junction and consist of glial NF155 that binds to axonal Caspr and contactin[41,45,109] (FIG. 1c), although the details of these interactions remain controversial[110,111]. Mice lacking any one of these CAMs have disrupted axoglial contact and axonal conduction deficits but they still have clustered Na⁺ channels since the NF186-dependent nodal mechanism remains intact[42,44,45]. Human pathogenic variants of Caspr and NF155 have been reported and these patients have severe nervous system dysfunction (BOX 1).

The most prominent consequence of disrupted paranodal junctions for the organization of axonal ion channels is that Kv1 K⁺ channels, normally excluded from paranodes and confined to juxtaparanodes beneath the myelin sheath, are able to enter the paranodal regions.

The exclusion of these Kv1 channels was assumed to be due to the high density of paranodal CAMs, which were viewed as a membrane protein 'fence' that limited the diffusion of Kv1 channels in the membrane[112,113]. However, the discovery of a specialized paranodal cytoskeleton eventually led to a different mechanistic interpretation.

Paranodal scaffolds and cytoskeletons. The axonal paranodal CAM Caspr has a protein 4.1B-binding domain that was proposed to link the paranodal adhesion complex to the underlying cytoskeleton[114–116]. 4.1B-deficient mice and mutant mice where Caspr is unable to bind to 4.1B still have intact paranodes. However, these same 4.1B-deficient mice have altered domain organization with reduced clustering of Kv1 K$^+$ channels[117–119]. These results suggest that paranodal cytoskeletons contribute to the barrier that excludes Kv1 channels from the paranodes. In addition to protein 4.1B, the biochemical purification of paranodal junctions, followed by mass spectrometry, revealed that the cytoskeletal proteins α2 and β2 spectrin are also enriched at the paranodes[120]; these axonal spectrins co-immunoprecipitate with protein 4.1B. A specific cytoskeleton is also found in glial paranodes (TABLE 1, FIG. 1c) and glial-specific knockouts of AnkB, AnkG and β2 spectrin showed that AnkB–β2 spectrin are found at the paranodes of Schwann cells and that AnkG–β2 spectrin are found at the paranodes of oligodendrocytes, where they associate with NF155 to promote the efficient assembly of the paranodal junction[121,122] (FIG. 1c).

Insight into the cytoskeletal mechanisms controlling axonal AnkG and Na$^+$ channel clustering at the nodes came from studies aimed at determining how AnkG is clustered at AIS. Galiano et al.[123] proposed a model where an axonal α2–β2 spectrin-based cytoskeleton located in the distal axon created an intra-axonal boundary that restricts the location of AnkG to the proximal axon. The extension of this model to myelinated axons suggested that the paranodal α2–β2 spectrin cytoskeleton flanking the nodes might also function as repeating glia-organized intra-axonal boundaries. To test this idea, Zhang et al.[107] generated mice with β2 spectrin-deficient axons; these axons had normal myelin, normal clustering of Na$^+$ channels and intact paranodal junctions, including transverse bands. However, Kv1 K$^+$ channels were no longer restricted to juxtaparanodes and instead were present in paranodes where they overlapped with Caspr. This result demonstrated that the molecular basis of the paranodal barrier is not a membrane 'fence' formed by Caspr–contactin and NF155 but instead depends on the paranodal spectrin cytoskeleton. Brivio et al.[60] provided additional support for the role of the paranodal cytoskeleton in node assembly by constructing transgenic mice expressing a mutant Caspr unable to bind to protein 4.1B on a Caspr-null background. Although paranodal junctions formed normally, CNS node formation was significantly delayed due to the inability to assemble a paranodal cytoskeleton. Thus, paranodal axoglial interactions between NF155, Caspr and contactin assemble a specialized paranodal cytoskeleton comprised of protein 4.1B and α2–β2 spectrin that functions as an intra-axonal boundary to organize axonal membrane domains (FIG. 4a–c).

Although the results described above strongly support a role for the paranodal spectrin cytoskeleton in clustering AnkG and AnkR, it is not clear how this periodic cytoskeleton creates a barrier or intraxonal boundary. Surprisingly, although structurally similar, neither β1 nor β4 spectrin can substitute for paranodal β2 spectrin[93]. Since nodal and paranodal β spectrins both associate with α2 spectrin[104], the specificity for spectrin localization must depend on the β spectrin and its interactions with other cytoskeletal and membrane-associated proteins. In addition, β4 spectrin also undergoes developmental changes in the splice variants found at the nodes. During node formation, a longer β4Σ1 splice variant predominates but is then replaced by a much shorter β4Σ6 splice variant[124]. This shorter variant is interesting because it lacks the canonical actin-binding domain but the spacing of the periodic cytoskeleton does not change. How β4Σ6 interacts with actin remains unknown. We speculate that interactions between the actin–α2–β2–4.1B-based cytoskeleton and paranodal CAMs constitute a very stable sub-membranous cytoskeleton resistant to mixing with actin–α2–β4–AnkG. This paranodal cytoskeleton could then effectively function as a boundary that restricts the location of AnkG–β4-containing or AnkR–β1-containing cytoskeletons to the nodes of Ranvier (FIG. 4c). During normal development, this boundary works in concert with NF186, which has a cytoplasmic binding site for AnkG or AnkR.

Glia-directed node assembly

The existing data support both nodal and paranodal mechanisms that can independently organize axonal membrane domains (FIG. 4). These mechanisms depend on axon–glia interactions that converge on unique nodal and paranodal ankyrin and spectrin cytoskeletons. However, the strongest evidence supporting two glia-dependent mechanisms for nodal Na$^+$ channel clustering comes from analyses of mice where both paranodal and nodal mechanisms are simultaneously disrupted. For example, Feinberg et al.[38] showed that mice lacking both gliomedin and Caspr or NrCAM and Caspr in the PNS have dramatically impaired Na$^+$ channel clustering. The absence of extrinsic nodal ECM proteins necessary to cluster NF186 and paranodal junctions necessary to assemble a paranodal cytoskeleton disrupts nodal Na$^+$ channel clustering. Susuki et al.[39] also generated mice lacking CNS nodal ECM proteins and Caspr; these mice had significantly impaired CNS nodal Na$^+$ channel clustering. However, the most definitive experiment demonstrating that two independent yet overlapping glial mechanisms converge on the cytoskeleton to assemble the nodes of Ranvier came from the analysis of nodes in NF186–β2 spectrin double conditional knockout mice[48]. Whereas single knockouts of axonal NF186 or β2 spectrin still had clustered Na$^+$ channels due to the remaining mechanism, the loss of both NF186 and β2 spectrin blocked nodal clustering of AnkG, β4 spectrin and Na$^+$ channels since both nodal and paranodal mechanisms were disrupted (FIG. 2).

The nodal and paranodal mechanisms for Na$^+$ channel clustering are not employed to the same degree in

Paranodal mechanism: Gial-directed placement of the cytoskeletal barrier
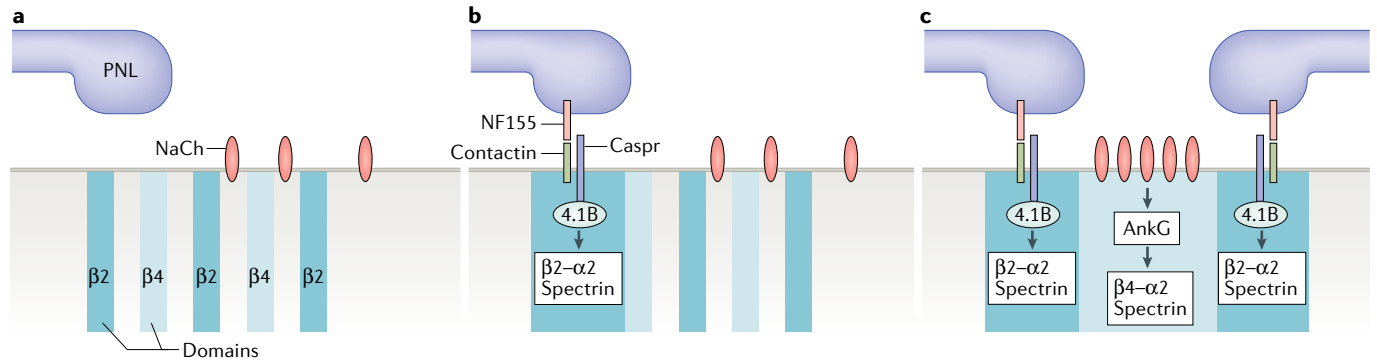
Nodal mechanism: Gial-directed clustering of Na⁺ channels
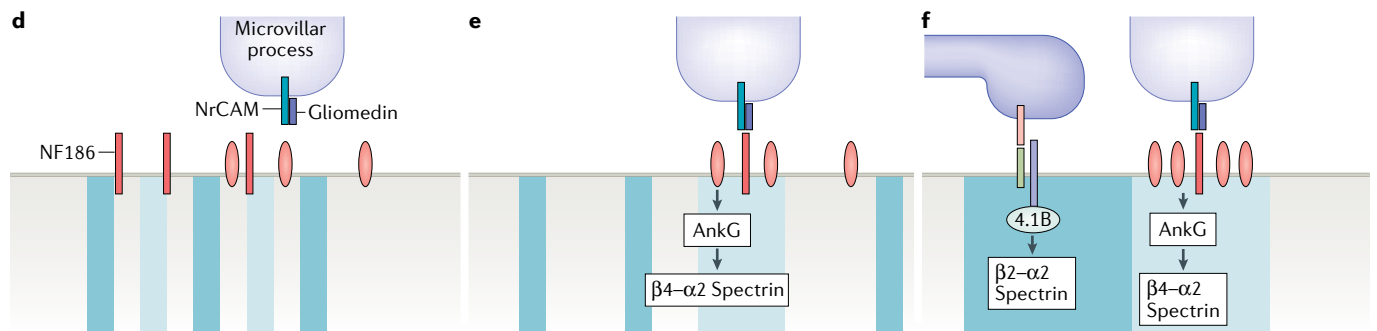
Fig. 4 | **A model for glia-directed placement of axonal cytoskeletal scaffolds and barriers.** Axonal β2 and β4 spectrin occupy mutually exclusive domains (parts **a** and **d**), hence forming the basis for the generation of non-overlapping domains in myelinated axons (parts **c** and **f**). Axonal β2 and β4 spectrin are not clustered in the axon before contact by the paranodal loops (PNL) (part **a**). At the paranodal junction, axoglial contact is mediated by glial neurofascin 155 (NF155) with axonal contactin and Caspr (part **b**). Protein 4.1B binds the cytoplasmic region of Caspr, leading to further recruitment of β2 and α2 spectrin and the formation of a cytoskeletal territory from which β4 spectrin is excluded. The attachment of additional PNLs during the process of myelination results in the expansion of the paranodal junction and the formation of an axonal barrier that restricts Na⁺ channels (NaCh) to the nodal gap (part **c**). Nodal proteins are not clustered prior to contact with the Schwann cell microvilli (part **d**). Initially at heminodes and later on in mature nodes, axoglial contact is mediated by binding of glial gliomedin and NrCAM to axonal NF186 (part **e**). This induces the recruitment of ankyrin G (AnkG) and the clustering of NaCh as well as the recruitment of β4 spectrin and the formation of a cytoskeletal domain that is distinct from the one formed at the flanking paranode. In the PNS, the microvilli-directed nodal mechanism precedes the PNL-directed assembly of the paranodal β2 and α2 spectrin-based cytoskeleton (part **f**).

the PNS and the CNS; in each, one mechanism seems to predominate and play a leading role. For example, in the CNS, paranodal junctions defined by NF155 and Caspr form before the clustering of nodal CSPGs and ECM proteins[39]. Thus, the paranodal mechanism for AnkG and Na⁺ channel clustering precedes the nodal mechanism in the CNS and therefore plays the leading role (FIG. 4a–c). In contrast, in the PNS, gliomedin–NF186 interactions at the edges of the nascent myelin sheath precede the formation of the paranodal junction[37] (FIG. 4d–f). Furthermore, mice lacking Caspr and βIV spectrin have profound node disruption in the CNS compared to the PNS[39]. This difference reflects the leading role of the paranodal mechanism for node formation in the CNS; conversely, a gliomedin–NrCAM–NF186 mechanism is dominant in the PNS.

Conclusion and future directions
In recent years, the importance of glial cells for nervous system function has become clearer as many examples of neuron–glia interactions have been described.

The detailed molecular interactions involved in node of Ranvier assembly represent one of the best examples underlying the profound interdependent morphological, molecular and physiological changes that occur in neurons and their myelinating Schwann cells or oligodendrocytes. Thus, myelinating glia are no longer viewed simply as insulation but rather as active contributors to nervous system development and function.

The mechanisms responsible for node of Ranvier assembly described here represent the synthesis of decades of work from many different laboratories. With the concept and details of two glia-driven and cytoskeleton-dependent mechanisms firmly established, one might be tempted to ask, what more is there to do? Not to worry, many questions remain unanswered. For example, the nodes of Ranvier are frequently viewed as static and unchanging. However, during development, the spacing of nodes changes in concert with the growth of the axon itself. In some CNS circuits, node spacing reflects different but precise demands on conduction velocity[125]. How this is sensed and regulated by

oligodendrocytes remains unknown. Similarly, AIS position and size in some neurons is tuned or even plastic in response to differing levels of activity[126,127]; it will be interesting to determine if the nodes of Ranvier are also dynamic and can be modified by activity. Node plasticity may involve changing the size of the node or the density and composition of nodal ion channels. One recent report suggests that perinodal astrocytes may help tune axonal conduction velocities by regulating the distance between flanking paranodes[128]. Node length can also vary and is predicted to strongly influence action potential propagation[129]. How these morphological properties of nodes are regulated remains unknown. In contrast, in some demyelinated axons, the nodal components and their locations in axons may be extremely stable and may function to constrain the position of the myelin sheath during remyelination[130]; thus, in some circuits, nodes may function as a blueprint that guides remyelination to tune the timing of action potentials or to promote precise metabolic support to the axons within a given circuit.

It is well known that, during CNS node development, the composition of Na$^+$ channels switches from Nav1.2 to Nav1.6 (REF.[6]). Similarly, with increasing age, the nodes of Ranvier in motor axons show a dramatic increase in Nav1.8 (REF.[131]). Furthermore, different types of neurons can have different nodal K$^+$ channels[12] and disease (for example, dysmyelinating neuropathies) can cause aberrant expression of different kinds of nodal ion channels[132]. Thus, node composition may be plastic through currently unknown mechanisms. Another important question that remains unanswered is what mechanisms function to clear membrane proteins (for example, adhesion proteins and ion channels) from beneath the myelin sheath in internodal regions. For instance, the extracellular region of NF186 is required for its clearance from the internodal axolemma; however, the mechanisms remain unknown[39,56].

The use of other vertebrate (that is, myelinated) model organisms besides rodents to elucidate questions of node biology will almost certainly play a major role in answering questions about the dynamic regulation of nodes in both health and disease and the distinct roles that myelinating glia and neurons have in these processes. For example, zebrafish, due to their transparency, amenability to long-term high-resolution live cell imaging in vivo, and the ability to introduce fluorescent tags or reporters, allow for in vivo analysis and investigation of node dynamics and assembly[133,134]. Similarly, the avian nervous system is also providing important insights into how node position along the axon can be locally regulated by oligodendrocytes to influence circuit properties[125].

Although the function of the spectrin cytoskeleton as an intra-axonal boundary or barrier is now recognized, how the spectrin cytoskeleton does this remains obscure. How does the paranodal cytoskeleton restrict the location of α2-β4-AnkG to regions of the axon not covered by myelin? The barrier mechanism does not appear to depend solely on the affinities between the ankyrin and spectrin binding domains[135] but rather additional structural constraints in the spectrin cytoskeleton are conferred by their different subunits; in addition, there may be other as yet unidentified accessory proteins that contribute to the organization of the nodal and paranodal cytoskeleton in ways not yet imagined. Thus, much work remains to be undertaken to understand how the spectrin cytoskeleton functions as a membrane boundary. In conclusion, the elucidation of two independent glia and axonal cytoskeleton-dependent mechanisms that assemble the nodes of Ranvier provides remarkable biological insight into one of the most important evolutionary adaptations that permitted the development of complex vertebrate nervous systems.

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