Development of nodes of Ranvier

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The architecture and function of the nodes of Ranvier depend on several specialized cell contacts between the axon and myelinating glial cells. These sites contain highly organized multimolecular complexes of ion channels and cell adhesion molecules, closely connected with the cytoskeleton. Recent findings are beginning to reveal how this organization is achieved during the development of myelinated nerves. The role of membrane proteins involved in axoglial interactions and of associated cytoplasmic molecules is being elucidated, while studies of mutant mice have underlined the importance of glial cells and the specific role of axonal proteins in the organization of axonal domains.

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Abbreviations

Caspr	contactin-associated protein
CNS	central nervous system
Cx29	connexin 29
EBP50	ERM-binding phosphoprotein 50 kDa
ERM	ezrin, radixin, moesin
FERM	four-point-one ERM
GNP	glycophorin C, neurexin IV, paranodin
GPI	glycosylphosphatidylinositol
md	*myelin deficient*
NCP	neurexin IV, Caspr, paranodin
NF	neurofascin
PDZ	PSD95/Discs Large/ZO-1
PNS	peripheral nervous system
PSD95	postsynaptic density protein of 95 kDa
TAG1	transiently expressed axonal glycoprotein 1

Introduction

Many vertebrate axons are surrounded by a myelin sheath allowing rapid and efficient saltatory propagation of action potentials. In myelinated fibres, the contacts between neurons and glial cells display a very high level of spatial and temporal organization, resulting in one of the most elaborate types of cell–cell interaction. The myelinating glial cells — oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS) — are wrapped around the axon, leaving the axolemma relatively uncovered at regularly spaced nodes of Ranvier. The internodal glial membranes are fused to form compact myelin, whereas the cytoplasm-filled paranodal loops of myelinating cells are spirally rolled up around the axon at both sides of the nodes (see [1–3] for recent reviews).

This organization requires a tight developmental control and the formation of a variety of specialized zones of contact between different areas of the myelinating cell membrane (autotypic or reflexive contacts), and between the glial cell and the axon (heterotypic contacts) [4,5]. Each node of Ranvier is flanked by paranodal regions where helicoidally wrapped glial loops are attached to the axonal membrane by a septate-like junction. The segment between nodes of Ranvier is termed the internode. Its outermost part, in contact with paranodes, is referred to as the juxtaparanodal region. Heterotypic axoglial contacts occur at the level of the paranodal junctions, as well as at the nodes, juxtaparanodes and internodes. The nodes are encapsulated by microvilli emanating from the outer aspect of the Schwann cell membrane in the PNS, or by perinodal extensions from astrocytes in the CNS. The underlying axon is organized in distinct functional domains, containing different sets of ion channels, cell adhesion molecules and cytoskeletal linker proteins [1–3]. The two challenges currently facing investigators in this field are: to decipher the molecular organization of the various cell contacts in nodal regions; and to elucidate how this organization is correctly put in place during development. Progress is rapid in these two areas; in this review, we summarize these recent advances. Because the organization of nodal regions in the CNS and PNS appears similar, they are described together; only important differences between oligodendrocytes and myelinating Schwann cells are pointed out.

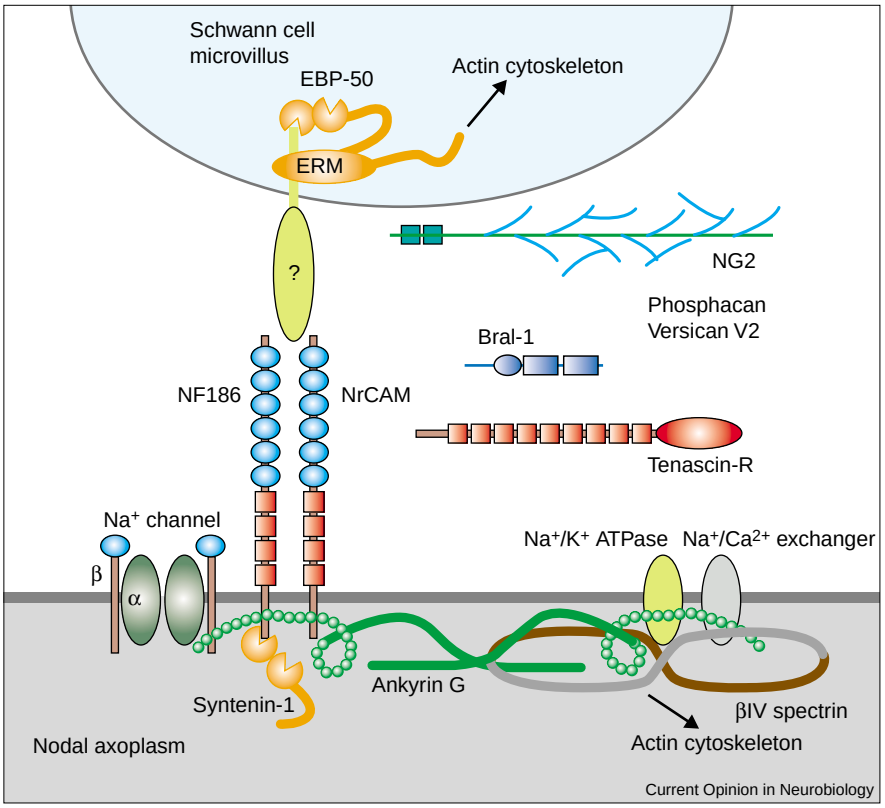
Nodes of Ranvier

The nodes of Ranvier contain Na^+/K^+ ATPases, Na^+/Ca^{2+} exchangers and a high density of voltage-gated Na^+ channels, which allow the generation of the action potential [6]. Na^+ channels are comprised of a pore-forming α subunit and two accessory β subunits, which are related to cell adhesion molecules and anchor the channel to extracellular, as well as intracellular components [7]. The nodes of Ranvier in the adult central and peripheral nervous systems mostly consist of $\alpha Na_V1.6$ [8] and $\beta1$ [9••,10] subunits. The extracellular region of β subunits can associate with itself [11] and with other proteins, including the extracellular matrix protein tenascin R [12] and the cell adhesion molecules neurofascin (186 kDa isoform, NF186) [10] and contactin [13]. Contactin (also termed F3, or F11 in chicken), a glycosylphosphatidylinositol (GPI)-anchored glycoprotein enriched at paranodes (see below) is also present at nodes in the CNS [14]. Interaction with contactin enhances surface expression of Na^+ channels [13], a property reminiscent of its ability to facilitate the addressing of contactin-associated protein (Caspr)/paranodin to the plasma membrane (see below) [15].

Nodal Na^+ channels appear to be part of multimolecular complexes including several intracellular and transmembrane proteins [7] (Figure 1). Within the axoplasm, Na^+

Figure 1

Schematic organization of nodal regions in peripheral nerves. The node is surrounded by Schwann cell microvilli, which contain ERMs and EBP50. These ERMs may provide a connection to actin microfilaments. The nature of the transmembrane protein(s) thought to interact with axonal proteins is not known (?). In the axon, two cell adhesion molecules, NF186 and NrCAM are anchored to ankyrin G, as are the Na^+ channels. βIV spectrin is also associated with ankyrin G. Syntenin-1 can bind to NF186 by a PDZ domain. However, it is not yet known whether it is present at nodes of Ranvier. Additional proteins enriched in nodal axolemma include the Na^+/K^+ ATPase and the Na^+/Ca^{2+} exchanger. Several extracellular matrix proteins are enriched at nodes of Ranvier, including tenascin R, Bral-1, and proteoglycan NG2, as well as phosphacan and versican V2. At CNS nodes, the axonal proteins also include contactin; Schwann cells microvilli are replaced by astrocyte perinodal extensions. Fibronectin type III repeats are shown as red boxes, Ig domains as blue circles, PDZ domains as indented orange circles, and FERM domain as an orange oval.



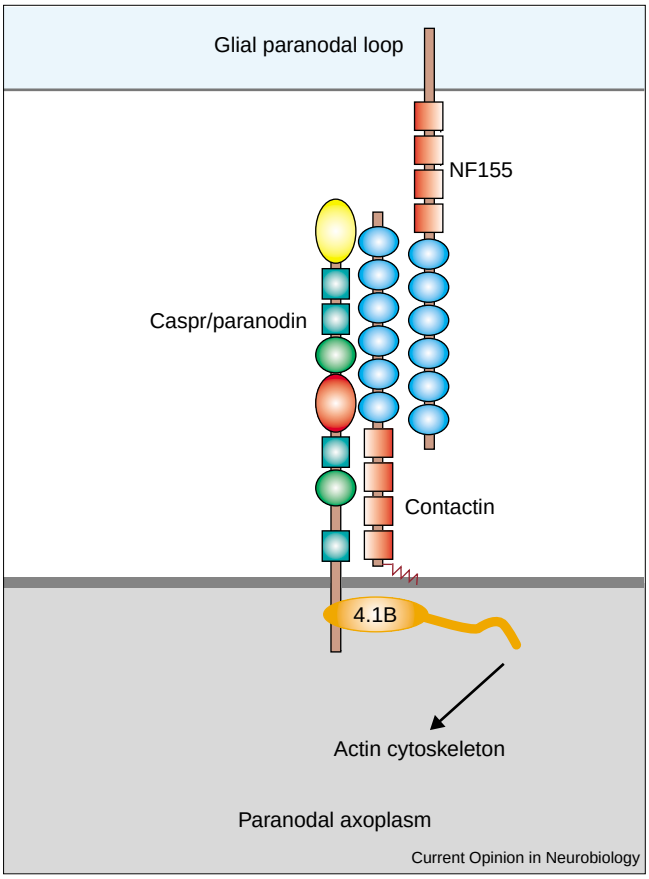
channels are associated with ankyrin G, which belongs to a family of intracellular adaptor proteins involved in targeting membrane proteins to specialized domains. This association is possibly mediated by the β subunit of the Na^+ channel [11] or, as suggested recently, directly by its α subunit [16•]. Ankyrin is also bound to βIV spectrin, a spectrin isoform enriched at nodes of Ranvier and axon initial segments [17,18••]. The intracellular carboxyl (C)-terminal region of NrCAM and NF186, two cell adhesion molecules of the L1 family, highly enriched in nodal regions, is associated with ankyrin G [19]. The C-terminus of NF186 also interacts with syntenin-1, a multifunctional adaptor protein with two PDZ (PSD95/Discs Large/ZO-1) domains [20]. NrCAM is capable of associating with, and clustering specifically NF186 [21•]. Thus, nodal proteins appear to form a meshwork of interacting components, in which transmembrane proteins are associated directly and through intracellular adaptor proteins (Figure 1).

Nodal multimolecular complexes may be further stabilized by binding to extracellular matrix components present at the nodes, including tenascin R [12,22–24], NG2 proteoglycan [25], phosphacan, versican V2, and the brain-specific hyaluronan-binding protein Bral1 [26]. Schwann cell microvilli contain ezrin, radixin, moesin (ERM) proteins and an associated PDZ domain-containing protein, ERM-binding phosphoprotein 50 kDa (EBP50) [27••,28•]. ERM proteins provide a regulated membrane anchoring mechanism for actin microfilaments, which are also enriched in microvilli [29]. It seems likely that these proteins interact with still unidentified transmembrane component(s) at the tip of the microvilli that may bind axonal protein complexes at the nodes.

Paranodal septate-like junction

At the paranodes, the glial loops are tightly attached to the axolemma through a septate-like junction. The two membranes are separated by a narrow (2.5–3 nm) extracellular space interrupted by septa interconnected with the cytoskeleton of glial loops and axons [30]. Freeze fracture analysis revealed that the paranodal junction appears formed by superimposed rows of intramembranous particles, regularly arranged in the glial and axonal membranes [31]. Two proteins are highly enriched in the paranodal axolemma: Caspr/paranodin [32,33] and contactin [14]. Caspr/paranodin belongs to a distinct subgroup of the neurexin superfamily, termed NCP (neurexin IV, Caspr, paranodin), which includes five different Caspr genes in humans (*Caspr1–Caspr5*) [32,34–36], as well as neurexin-IV and axotactin in *Drosophila melanogaster* [37,38]. The association with contactin is necessary for the addressing of Caspr/paranodin to the plasma membrane in transfected cells [15] and its targeting to the axon *in vivo* [14]. Knockout mice lacking Caspr/paranodin or contactin display ataxia, motor deficits and a dramatically reduced nerve conduction velocity [39••,40••]. In these mutants,

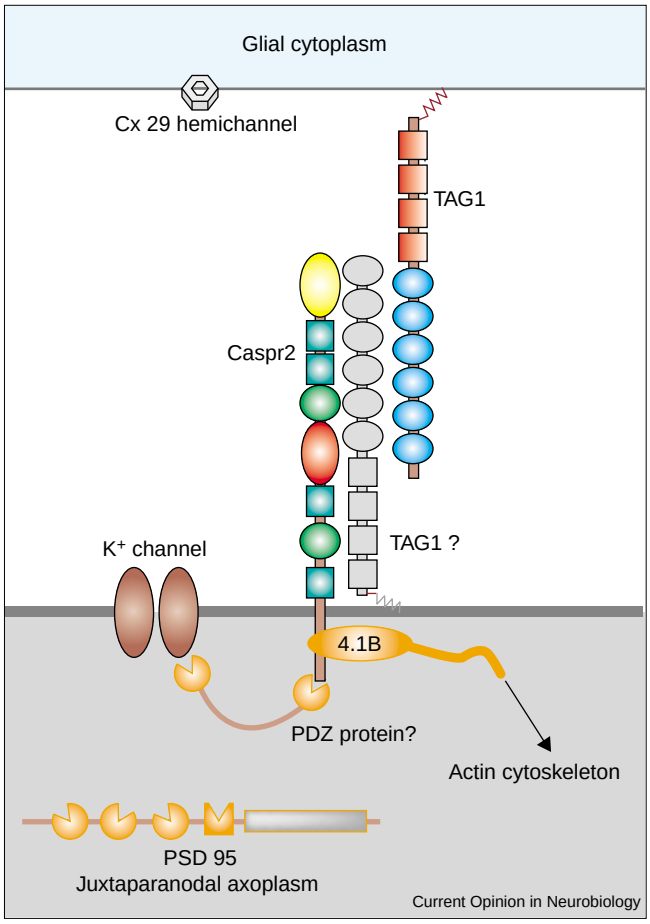
Figure 2



Schematic organization of paranodal regions. The main components of paranodal axolemma are Caspr/paranodin associated with contactin/F3, a GPI-anchored glycoprotein. This complex interacts with a cell adhesion molecule of glial paranodal loops, NF155. The intracellular region of Caspr/paranodin interacts with the FERM domain of protein 4.1B, through a juxtamembrane sequence, the GNP motif. Protein 4.1B provides a potential link with actin microfilaments. Fibronectin type III repeats are shown as red boxes, Ig domains as blue circles, EGF domains as green circles, laminin G domains as blue boxes, fibrinogen domain as a red oval, factor VIII/discoidin domain as a yellow oval, and FERM domain as an orange oval.

the ultrastructure of the paranodes is severely altered: the glial paranodal loops are disorganized, the gap between glial and axonal membranes is increased and the electron-dense material forming the septa in wild-type mice is absent [39••,40••]. The simplest explanation of these findings is that Caspr/paranodin and contactin are essential components of the paranodal macromolecular complexes required for the tight attachment of the two membranes. The paranodal loops of oligodendrocytes and Schwann cells contain the 155 kDa splice isoform of neurofascin (NF155) [41]. Given that the localization of Caspr/paranodin, contactin and NF155 at the paranodes is interdependent [39••,40••,42•] and because NF155 binds to the Caspr–contactin complex [43•], it is very likely that these three proteins form the core of the axoglial cell adhesion apparatus (Figure 2).

Figure 3



Schematic organization of juxtaparanodal regions. The juxtaparanodal axolemma contains Kv1.1 and Kv1.2 K^+ channels and Caspr2, a protein closely related to Caspr/paranodin. These proteins are associated through an as yet unidentified PDZ domain-containing protein (?). PSD95, another PDZ domain-containing protein (or a closely related protein), is enriched at juxtaparanodes but its partners are not known. The Caspr2 intracellular region can associate with protein 4.1B, which provides a link to the actin cytoskeleton. TAG1, a GPI-anchored glycoprotein closely related to contactin/F3, is also enriched at the juxtaparanodes. Although TAG1 is enriched in glial membranes, it is also possibly present on neuronal membranes (as indicated by a gray shading) and could be involved in *cis* or *trans* associations with Caspr2, or in *trans* homophilic interactions with itself. Cx29 is located in the glial membrane where it may form functional hemichannels. Fibronectin type III repeats are shown as red boxes, Ig domains as blue circles, EGF domains as green circles, laminin G domains as blue boxes, fibrinogen domain as a red oval, factor VIII/discoidin domain as a yellow oval, PDZ domains as orange indented circles, SH3 domain as an orange indented box, guanylate kinase domain as an orange rectangle, and FERM domain as an orange oval.

An important feature of paranodal junctions is their tight association with the cytoskeleton in both glial loops and the axoplasm [30]. On the axonal side, the short intracellular domain of Caspr/paranodin provides a site of anchorage for cytoskeleton-associated proteins, through a sequence conserved in glycophrin C, neurexin IV, paranodin (GNP) motif [44]. This motif, now identified in many other

proteins, is a binding site for the four-point-one ERM (FERM) domain of protein 4.1 [32]. Among the isoforms of protein 4.1, encoded by four different genes in mammals, type II, or protein 4.1B, is concentrated at paranodes and juxtaparanodes [42•,45]. Protein 4.1B binds to the GNP motif of Caspr/paranodin *in vitro* and the two proteins coimmunoprecipitate from brain extracts [46••] (N Denisenko, JA Girault, unpublished data). Because protein 4.1B has a conserved actin–spectrin-binding domain, it may associate directly the transmembrane protein complexes to the axonal cortical cytoskeleton (Figure 2). A remarkable feature of paranodal septate-like junctions is their morphological and molecular similarity with invertebrate septate junctions, well characterized in *Drosophila*. In these, neurexin IV, the *Drosophila* homologue of Caspr/paranodin [37], is colocalized with D-contactin (C Faivre-Sarrailh, personal communication) and recruits the protein 4.1 homolog coracle [47].

The juxtaparanodal regions

The Shaker-type K^+ channels, Kv1.1, Kv1.2 and their Kvβ2 subunit are enriched at the juxtaparanodal ends of the internodal axolemma [48]. Caspr2, a protein that displays a 45% amino acid identity with Caspr/paranodin, is enriched in the juxtaparanodal axolemma and is associated with K^+ channels, presumably through a PDZ domain-containing protein [34] (Figure 3). Although such a protein, postsynaptic density protein of 95kDa (PSD95), has been reported to coimmunoprecipitate and colocalize with Kvβ2 in paranodal regions [50•], it does not interact with Caspr2 [36]; Caspr2 is still associated with K^+ channels in PSD95 mutant mice (MN Rasband, personal communication), suggesting that other PDZ-containing protein(s) exist at this site. In addition, similarly to Caspr/paranodin, Caspr2 contains an intracellular GNP motif and directly interacts with 4.1B found at the juxtaparanodes [42•,45,46••]. Transient axonal glycoprotein 1 (TAG1), a GPI-anchored cell adhesion molecule related to contactin, expressed in Schwann cells, oligodendrocytes and neurons, is highly enriched in the juxtaparanodal region [50•]. Finally, the juxtaparanodal glial membrane contains connexin 29 (Cx29), a gap junction protein that may be capable of forming functional hemichannels, possibly involved in K^+ clearance [51•].

Formation of the nodal environs

The differentiation of the nodal regions into the distinct domains seen in the adult nervous system takes place g radually during myelination, and can be grossly divided into distinct coordinated stages: the formation of nodal clusters, which occurs concurrently with, or slightly precedes that of the paranodal junctions, followed by concentration of juxtaparanodal components. Several recent studies using a variety of spontaneous and targeted mutations in mice have demonstrated the essential role of myelinating cells in the formation of distinct axonal domains as detailed below.

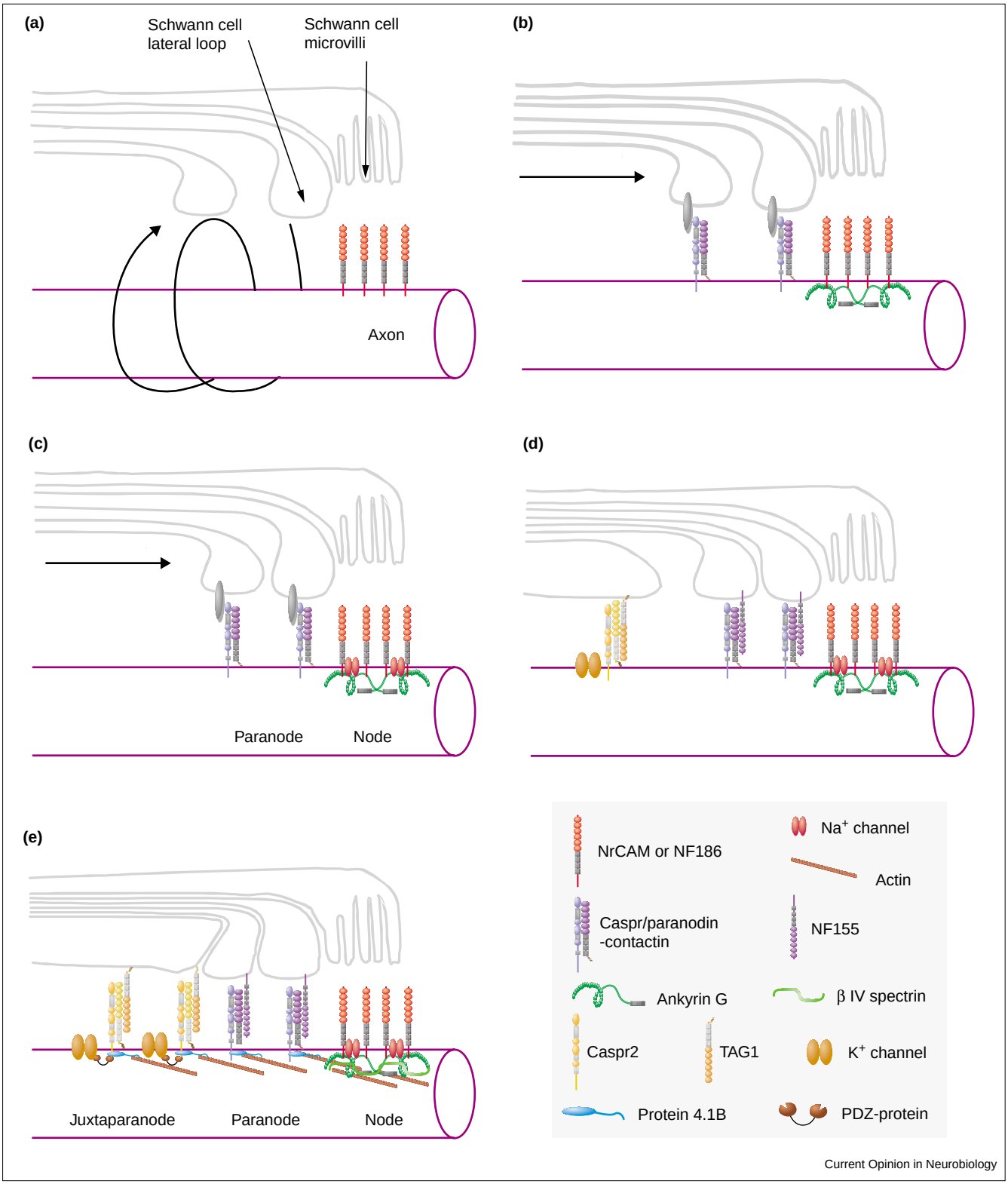
Localization of Na^+ channels at the nodes

Na^+ channels are clustered at early stages during development adjacent to the cellular processes of Schwann cells or oligodendrocytes, suggesting that these clusters are pushed towards the presumptive nodes by the glial paranodal loops (reviewed in [48,52]). However, although the formation of nodal aggregates of ankyrin G and Na^+ channels depends on oligodendrocytes and coincides with the formation of paranodal contacts [53], it occurs independently of the clustering of Caspr/paranodin and does not require the establishment of tight septate-like junctions [39••,40••,54]. This conclusion is also supported by studies of the consequences of spontaneous mutations of the proteolipid (*plp*) gene protein, which trigger a delayed cell death of oligodendrocytes in the CNS of *jimpy* mice and *myelin deficient* (*md*) rats [55•,56,57••]. In these animals, although Caspr/paranodin and NF155 are not detected at paranodes, the nodal aggregates of Na^+ channels and ankyrin G are by and large normally formed [55•,56,57••]. Moreover, the nodal clusters persist even at times when oligodendrocytes have disappeared. However, the requirement of glial cells for Na^+ channel clustering was demonstrated by the early postnatal selective ablation of oligodendrocytes in transgenic mice [58]. In these mice, no clustering of ankyrin G or Na^+ channel was visible, except in contact with the rare spared oligodendrocyte [57••].

The nature of the glial molecule(s) responsible for the clustering of nodal neuronal proteins is not known. Experiments in the PNS suggest that a direct contact between Schwann cells and the axon is required [59], although clustering at a distance from Schwann cells has been observed in nerves of dystrophic mice *in vivo* [60]. During myelination of dorsal root ganglia neurons by Schwann cells *in vitro*, initial clusters of Na^+ channels are detected in association with ERM-positive microvilli processes, suggesting the involvement of a still unknown ERM-binding receptor in channel clustering [27••]. By contrast, oligodendrocytes appear to secrete a soluble factor sufficient to trigger regularly spaced axonal clustering of $Na_V1.2\alpha$ subunits, β2 subunits and ankyrin G in cultured neurons [9••,61]. During normal development, $Na_V1.2$ is first accumulated at immature nodes and later replaced by $Na_V1.6$ as myelination proceeds [62••]. By contrast, $Na_V1.2$ predominates in *shiverer* mice, in which a mutation of myelin basic protein severely impairs the formation of compact myelin, indicating that myelination regulates Na^+ channel switching. Nevertheless, two recent studies demonstrated the presence of $Na_V1.6$ in the nodes of *md* rats and *jimpy* mutant mice [55•,56], suggesting that while inducing the initial $Na_V1.2$ clusters, oligodendrocytes may initiate an intrinsic programme in the axon for later channel switching.

βIV spectrin and, presumably, ankyrin G are essential for the organization of Na^+ channel nodal clusters [18••,63•]. In the PNS, the cell adhesion molecules NrCAM and NF186 cluster first, followed by ankyrin G, and finally Na^+ channels [64]. In the CNS, however, ankyrin G appears at the nodes before clustering of NF186 and Na^+ channels [56]. This temporal succession suggests that adhesion

Figure 4



molecules (NrCAM, NF186, or an as yet unidentified receptor) recruit ankyrin G, which is responsible for the aggregation of Na^+ channels. This model is supported by the inhibition of nodal clustering of Na^+ channels and ankyrin G in myelinating dorsal root ganglia cultures incubated in the presence of NrCAM–Fc fusion protein [21•].

Figure 4 legend

Development of nodes of Ranvier in the PNS. (a–c) The first event appears to be the accumulation of cell adhesion molecules such as NF186 or NrCAM. This event is likely to be triggered by contact with as yet unidentified glial molecules, presumably associated with Schwann cells microvilli (a). The intracellular regions of these cell adhesion molecules interact with ankyrin G, which serves as an anchor for Na^+ channels (b), which may also interact directly with glial molecules through its β-subunit (see Figure 1). Simultaneously, the periaxonal extension of the glial cell wraps around the axon, as shown by the spiraling arrow in (a), giving rise to presumptive paranodal regions, which become progressively packed towards the nodal region, as shown by the straight arrow in (b,c). This lateral movement along the axon contributes significantly to the overall formation of nodes of Ranvier by allowing heminodes formed at the edges of neighboring glial cells to fuse into complete nodes. Although glial cells are essential for the organization of nodal and paranodal axonal proteins, the identity of the first glial molecules interacting with axonal proteins at paranodes is not known with certainty, as indicated by a gray oval in (b,c). (d) Septate-like junctions form progressively at paranodes with the enrichment of NF155 in glial paranodal loops, coincident with the appearance of transverse bands. Following the early differentiation of the nodal and paranodal regions, K^+ channels, Caspr2 and TAG1 accumulate in juxtaparanodal regions. This accumulation, whose mechanism remains to be elucidated, coincides with the formation of compact myelin. In mutants in which paranodal septate-like junctions are altered, Caspr2 and K^+ channels accumulate in paranodal regions, adjacent to nodal clusters. (e) In mature nodal regions, interactions with intracellular proteins appear essential for the stability of all nodal regions. These interactions involve linker and scaffolding proteins that associate transmembrane proteins between themselves and to the cytoskeleton, including actin filaments. These intracellular axonal proteins are essential for the enrichment and/or stability of axonal proteins. In the CNS oligodendrocytes do not possess microvilli, but appear capable to trigger the clustering of some axonal proteins through secreted factor(s). The combined effects of such factors with the subsequent lateral movements generated by the wrapping of oligodendrocyte periaxonal extension could account for the organization of CNS nodes of Ranvier.

The role of ankyrin G appears essential, because its specific deletion in Purkinje cells prevents targeting and accumulation of NrCAM, NF186, and Na^+ channels in axon initial segments [65]. Given the similarities between the axonal components at Ranvier nodes and axonal initial segments, it is likely that ankyrin G is also important in nodal regions. Likewise, the accumulation of Na^+ channels and ankyrin G is dramatically reduced in axon initial segments and nodes of Ranvier of βIV-spectrin-deficient mice [18••], showing the importance of cytoskeletal stabilization for the enrichment of these proteins. Interestingly, although the axonal proteins enriched at axon initial segments and Ranvier nodes are similar, the determinism of their clustering appears different, because initial segments form normally in the absence of oligodendrocytes [57••]. Although extracellular matrix proteins could be good candidates for the regulation of aggregation of axonal proteins, it should be noted that in tenascin R knockout mice, even if the axonal conduction velocities are decreased, the nodal distribution of Na^+ channels appears normal [66].

Development and maintenance of the paranodal junction

The presence of Caspr/paranodin at the paranodes and the juxtamesaxon [32,67], as well as its appearance in a spiral below the overlying turn of the paranodal loops that forms during development [2], strongly suggest that its localization in the axon is regulated by the overlying myelin sheath. Analysis of several myelin mutant animals showed that the continuous presence of normal oligodendrocytes is necessary for the paranodal localization of Caspr/paranodin and contactin [55•,56,57••]. Galactocerebrosides [42•,54] and their sulfated derivative, sulfatide [68,69•], are critical for the correct formation of paranodal axoglial junctions. In mice lacking ceramide galactosyl-transferase, Caspr/paranodin, contactin and NF155 do not accumulate at paranodes, which exhibit morphological abnormalities including the absence of septa [42•,54]. Generation of mice lacking either Caspr/paranodin or contactin demonstrated that both proteins are essential for the formation of the tight paranodal junction, and that their absence results in the disappearance of the transverse bands (intercellular septa), which are the hallmark of this axoglial contact [39••,40••]. Interestingly, accumulation of Caspr/paranodin slightly precedes that of NF155 and the formation of septa [70•], suggesting that NF155 may be responsible for the formation of the septate-like axoglial junction rather than for the initial concentration of Caspr/paranodin at paranodes. Although the intracellular region of Caspr/paranodin is not required for its targeting, it is essential for its stability, as well as that of contactin at the paranodal junction, presumably through its interaction with protein 4.1B [46••]. Thus, Caspr/paranodin may serve as a 'transmembrane scaffold' that stabilizes the adhesion complex at the paranodal junction by connecting it to axonal cytoskeletal components.

Localization of Caspr2/K^+ channel complexes in the juxtaparanodal region

K^+ channel accumulation is detected at relatively late developmental stages, after the node of Ranvier is already formed (reviewed in [48,52]). In the PNS, K^+ channels and Caspr2 are first detected at the nodes and then are relocated to the adjacent juxtaparanodal region [42•,71]. In the CNS, however, K^+ channels are first concentrated in the juxtaparanodal region [53]. These differences suggest that both active exclusion from the paranodes and direct axonal targeting may be involved. Study of mutant mice in which the paranodal enrichment of Caspr/paranodin and contactin is lost, reveals the role of the paranodal junction in providing a barrier that restricts the apparent movement of juxtaparanodal proteins [39••,40••,42•,54,55•,57••]. In these mutants, although the nodal clustering of Na^+ channels is minimally affected, K^+ channels are mislocalized at the paranodes instead of in the juxtaparanodal region. Interestingly, K^+ channels are also mislocalized along myelinated axons of *quivering* mice, which carry a loss-of-function mutation in the βIV spectrin gene [72•]. This is

particularly interesting in light of the observation that βIV spectrin is exclusively localized at the nodes, suggesting that all the nodal subdomains are closely linked through the cortical cytoskeleton.

Is there a glial protein that binds (either directly or indirectly) to K^+ channels causing their lateral movement towards the node? The recent identification of TAG1 at juxtaparanodes makes it a likely candidate for such a function. Its similarity with contactin (48% sequence identity), suggests that TAG1 may associate with Caspr2. Such an association may occur between axonal TAG1 and Caspr2 or between TAG1 present in the glial juxtaparanodal membrane and axonal Caspr2 [50•]. Furthermore, given that TAG1 interacts homophilically [73], its presence in both the axonal and the glial membrane may result in the formation of an adhesion complex consisting of a glial TAG1 molecule and an axonal Caspr2/TAG1 heterodimer.

Conclusions: a working model for the development of nodes of Ranvier

Recent results, together with previous findings and hypotheses [2,3,52] allow the proposal of a simplified scenario for the formation of nodal regions (Figure 4). The first event triggered by myelinating glial cells is the clustering of axonal adhesion proteins, such as NrCAM and NF186, or possibly other molecules. In peripheral nerves, in which Schwann cell extensions cover the nodal region, this appears to require a direct cell–cell contact, whereas in the CNS, where no microvilli abut the nodes, clustering may be triggered in response to a soluble factor. Whatever the nature of extracellular signals, recruitment of ankyrin G, with its multiple protein binding sites, is very probably a major step, allowing the clustering of Na^+ channels with NrCAM and NF186. The presence of βIV spectrin is a critical factor for the stability of these clusters.

In addition, possible repulsive interactions between components of the nodal axolemma and the paranodal glial loops may also exist, helping to prevent paranodal loops from invading the nodal territory. Heminodal clusters can form in contact with one myelinating cell and, as wrapping of the myelinating cell proceeds, be pushed towards the neighboring heminode until they fuse to form a complete node [52,71]. While the periaxonal extension of the myelinating cell rolls up around the axon, and compact myelin is formed, the lateral loops of the glial cells become progressively compacted in the lateral direction to form the paranodal region [2]. At this time Caspr/paranodin–contactin complexes in the axolemma interact with NF155 in the glial membrane to form septate-like junctions. The precise and regular geometric organization of these junctions strongly suggests that additional, presumably intracellular, proteins provide a grid-like meshwork, allowing a regular spacing of intercellular complexes. This intracellular meshwork is likely to include protein 4.1B and other associated proteins and to be connected with axonal cytoskeleton. Thus, paranodal junctions anchor the glial cell membrane to the axolemma, and serve as barriers for stopping the apparent movement of juxtaparanodal components. Although the precise mechanism of the juxtaparanodal accumulation of K^+ channels, Caspr2 and TAG1 remains to be determined, in the PNS these proteins are first detected at nodes, suggesting that they undergo an exclusion mechanism from these regions.

In contrast to the markedly different functional properties of the various nodal domains, their generation and maintenance may involve a limited number of similar molecular mechanisms. Further identification of additional components of these complexes and of their relationships will no doubt shed light on the development of these fascinating structures.

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