MOLECULAR MECHANISMS AND INTERACTIONS AT THE NODE OF RANVIER: A STRUCTURAL PERSPECTIVE

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Abstract

Action potentials are conducted along axons in a saltatory fashion, being reinforced periodically at the nodes of Ranvier (NOR), where there is a gap in the myelin sheath that covers the axon. The NOR is organized into multiple compartments. The electrical signal is boosted via ion channels at the nodal gap, which in turn are supported by adhesion proteins and the cytoskeleton. The septate-like paranodal junctions, formed by a protein complex that mediates the axon-glia contact, separates the components of the nodal gap from those of the juxtaparanode, where other adhesion proteins and ion channels are located underneath the myelin. Disruption of the NOR may cause a wide range of neurological diseases. While the molecular mechanisms and interactions at the NOR have been studied extensively in cell biology research, the structural biology perspective has only recently gained more attention with the publication of structures of ion channels and adhesion and cytoskeletal proteins. Here, I review the molecular mechanisms and interactions at the NOR in light of available structural data on its molecular components, particularly voltage and mechanosensitivity of ion channels. Future study of the molecular structures, especially *in situ*, will further increase our understanding of the (dys)function of the NOR.

Introduction

Signal transmission over long distances in the nervous system is achieved by conduction of electrical signals, or action potentials, along the axons of neurons, which in humans can be up to a meter long. The axon is wrapped in a layer of myelin, produced by glial cells; oligodendrocytes in the central nervous system (CNS) or Schwann cells in the peripheral nervous system (PNS). The myelin sheath is not continuous, as there are periodic 'gaps' (~1 µm) where the axon is exposed (Fig 1A); these gaps are termed the nodes of Ranvier (NOR). The action potential is conducted in a saltatory fashion, and is reinforced at the NORs, thus greatly accelerating the signal transmission [1] [2].

The NOR is organized into three main compartments, each with a specialized assembly of molecular components (Fig 1B). At the nodal gap, Na^+ and K^+ ion channels are present to propagate the action potential, and they are stabilized by adhesion proteins and the cytoskeleton. An important distinction between the CNS and the PNS is that in the PNS, the microvilli of the myelinating Schwann cells contact the axon at the nodal gap via the adhesion proteins. This is not the case in the CNS, where the axon is exposed to the extracellular matrix and perinodal glial cells instead. The nodal gap is flanked by the paranodes, which form septate-like junctions between the axon and the glial cells. The paranodal junction is an important barrier that separates the nodal gap from the juxtaparanode. At the juxtaparanode, there is also axon-glia contact facilitated by adhesion proteins, as well as K^+ ion channels. Like at the nodal gap, the molecular components of the paranode and juxtaparanode are connected to a specialized cytoskeleton [2] [3].

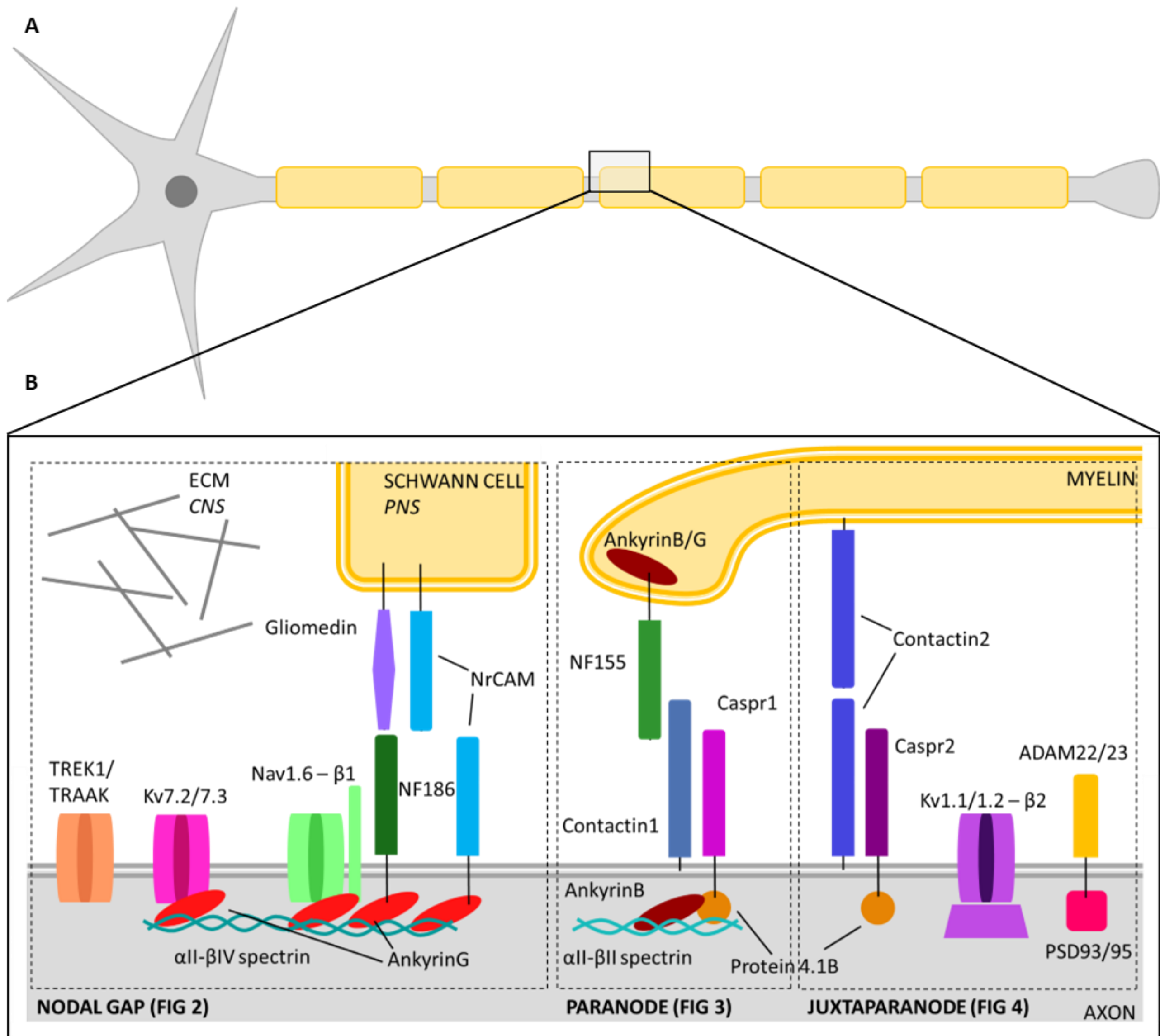


Figure 1: Overview of the node of Ranvier. *(A) Schematic drawing of an axon (gray) wrapped in myelin (gold). One node of Ranvier is indicated by a square. (B) Cross-section of the node of Ranvier, with the molecular components as cartoons. Each segment (nodal gap, paranode and juxtaparanode) is indicated.*

Given its importance in signal conduction, it is perhaps not surprising that the NOR and its components have been implicated in a number of neurological disorders. Mutations in ion channels and adhesion proteins are associated with, among others, epilepsy and autism. Moreover, disruption of axon-glia contacts seems to underlie demyelination in autoimmune diseases such as multiple sclerosis, and proteins at the NOR can be the target of autoantibodies. To better understand the causes of these diseases and to design treatments, it is crucial that the structure and function of the molecular components of the NOR are studied in detail [2] [3].

The molecular mechanisms and interactions at the NOR have been investigated in the field of cell biology for about three decades. The mechanisms underlying the assembly and (dys)function of the NOR are still being elucidated, but considerable progress has been made. On the other hand, structural data on the ion channels, adhesion proteins and cytoskeleton has been sparse. However, in recent years more and more structures are being published (Table 1), providing new insights into their function, particularly for voltage and mechanosensitive ion channels. While many excellent reviews on the molecular composition of the NOR and its role in disease have been written [1] [2] [3], these generally

do not include the structural biology research. In this review, I will provide an overview of the molecular mechanisms and interactions in each compartment of the node of Ranvier, and discuss the new insights that have come from the recently resolved structures of its components.

Table 1: Overview of available structures of node of Ranvier protein components.

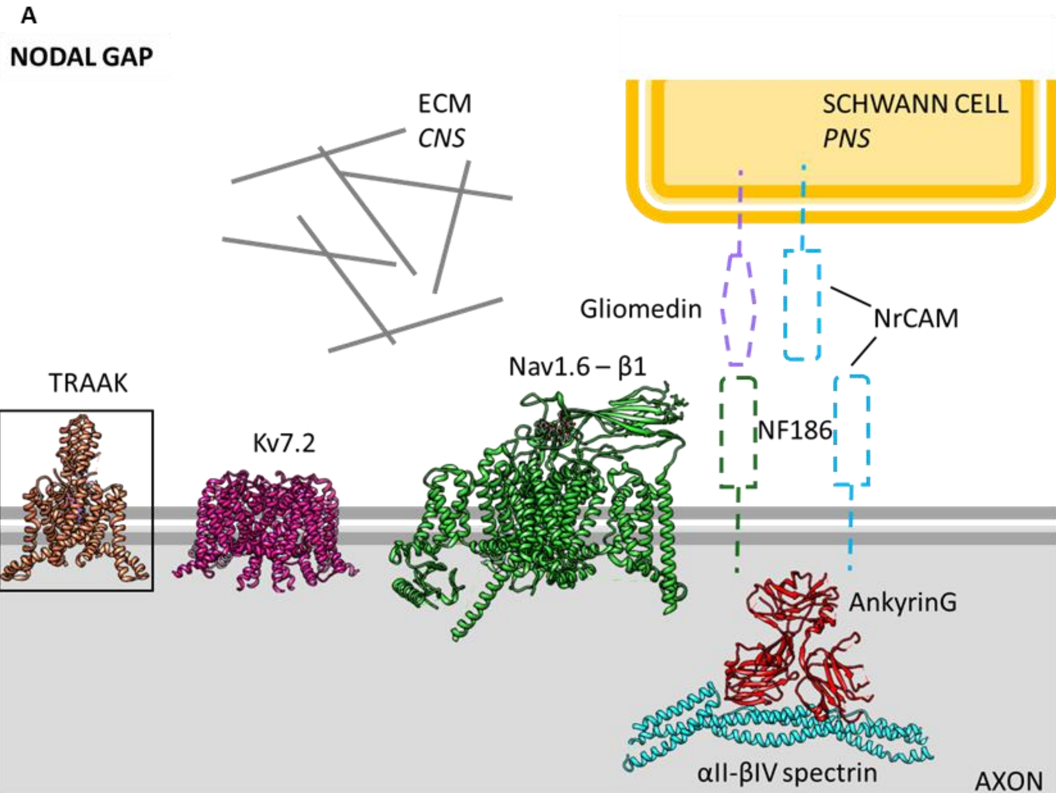
Compartment	Protein(s)	Resolution (Å)	PDB ID/reference
Nodal gap	Nav1.6 with β1 subunit	3.1	8FHD; Fan et al, 2023
	Nav1.6 with β1/2 subunits	3.4	8GZ1; Li et al, 2023
	Kv7.2	3.1	7CR0; Li et al, 2021
	TRAAK	2.5	4WFE/4WFF; Brohawn et al, 2014
	TREK1	3.1	6CQ6; Lolicato et al, 2020
	NF186 (Ig1-4) homodimer	2.6/3.2	3P3Y/3P40; Liu et al, 2011
	AnkyrinG with NF186 fragment	2.5	7XCE; He et al, 2022
	AnkyrinG (ZZU) with βIV-spectrin	4.3	6M3R; Li et al, 2020
Paranode	Contactin1 (Ig1-6) with NF155 (Ig1-6)	4.8	7OL4; Chataigner et al, 2022
	NF155 (Ig1-6) homodimer	3.0	7OK5; Chataigner et al, 2022
	Contactin1 (Ig1-6) homodimer	3.9	7OL2; Chataigner et al, 2022
	Contactin1 (FNIII1-3)	2.5	5E53; Nikolaienko et al, 2016
Paranode/ Juxtaparanode	4.1B with TSLC1 peptide	2.3	3BIN; Busam et al, 2011
Juxtaparanode	Contactin2 (Ig1-6) homodimer	3.5	8A0Y; Chataigner et al, 2023
	Contactin2 (Ig1-4) homodimer	3.1	2OM5; Mörtl et al, 2007
	Contactin2 (FNIII1-3)	2.0	5E7L; Nikolaienko et al, 2016
	Caspr2 (discoidin domain)	1.3	5Y4M; Liang et al, 2019
	Kv1.2 with β subunit	2.9	2A79; Long et al, 2005

Nodal gap

Voltage-gated Na^+ channels associate with β subunits and adhesion proteins for their function

For signal propagation at the NOR, voltage-gated Na^+ channels are clustered at the nodal gap (Fig 2A). While Nav1.2 is clustered at immature nodes, during development it is replaced by Nav1.6 [4]. The Nav channels consist of an α subunit with four repeat domains of six transmembrane segments each, including a voltage-sensing domain [5] [6]. The α subunit by itself forms a functional channel, but in the brain it is associated with one or more β subunits, which have regulatory functions. Four β subunits exist (β1-4), all containing an immunoglobulin (Ig) domain [7]. Aside from their interaction with the Nav α subunit, β subunits bind a multitude of adhesion and cytoskeletal proteins, both in *cis* and in *trans*. The β1 subunit, for instance, has been found to associate with other β subunits, AnkyrinB and G, Contactin1, Neurofascin155 and 186 and NrCAM [7]. Notably, loss of β1 in mice results in disruption of the NOR and also causes a neurological phenotype, with symptoms including ataxia and seizures [8].

Two structures of the human Nav1.6 channel were recently reported. The first [5] shows the typical four-domain architecture of the α subunit, and its association with β1. It was observed that the extracellular loop of the first domain, which is part of the selectivity filter, is stabilized by both the Ig domain of the β1 subunit and glycosylation of a conserved asparagine residue. The voltage-sensing domains were all in the inactivated conformation, and the pore was closed. As Nav1.6 appears to be



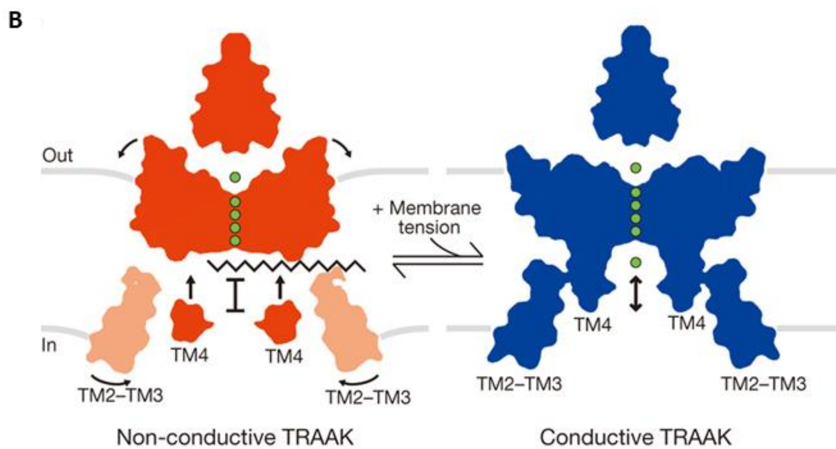


Figure 2: Overview of the nodal gap. *(A) Experimental structures of the TRAAK K2P channel (4WFF), Kv7.2 channel (7CR0), Nav1.6 with β1 subunit (8FHD) and the complex of the AnkyrinG exZZU-tandem and βIV-spectrin repeats (6M3R). (B) Mechanism of mechanosensitivity of the TRAAK channel. Membrane tension-dependent movement of transmembrane helices can close the lateral cavity, relieving the channel blockage by a lipid. Adapted from Brohawn et al, 2014.*

related to neurological disorders including epilepsy, the authors also mapped disease-related mutations to the structure. They found that many of these affect the voltage-sensing domains, suggesting that aberrant channel (in)activation may be an underlying mechanism of epilepsy. In the other structure [6], a truncated but still functional Nav1.6 construct was resolved together with both the β1 and β2 subunits. This revealed a similar overall architecture, which showed that the Ig domain of β2 is on the extracellular side of the channel too, but opposite from β1. In addition to mutation mapping (which also highlighted the voltage-sensing domains), three potential Na^{+} binding sites were identified in the extracellular selectivity filter. Furthermore, the structure of Nav1.6 bound to an

inhibitor (4,9-ah-TTX) revealed a similar binding pocket as the well-known inhibitor TTX, occupying the Na^+ binding site in the selectivity filter.

In the PNS, the clustering of Na^+ channels is mediated by the microvilli of the Schwann cells. These express gliomedin, which then recruits Neurofascin186 on the axonal side [9] [10]. This interaction is enhanced by the glial NrCAM that is found on the Schwann cell microvilli, where it seems to trap gliomedin [9]. Next, the Na^+ channels are recruited, as well as the cytoskeletal scaffolds AnkyrinG and βIV-spectrin [10] [11]. Notably, the Ankyrin binding domain of Nav1.6 has been found to be necessary and sufficient for its targeting to the NOR [12], and it has been suggested that Ankyrin and Nav1.6 are co-transported to the NOR [13]. The mechanism of Na^+ channel clustering in the CNS is less clear. It was reported that this involves three distinct mechanisms, namely the recruitment of Neurofascin186 by the extracellular matrix, separation of the nodal components by the paranodal junctions, and stabilization of the Na^+ channels by the axonal cytoskeleton. These mechanisms seem to be complementary, as loss of one mechanism does not affect the NOR but loss of multiple mechanisms does [14] [15]. Interestingly, a recent swine model of concussion [16] showed loss of Nav1.6 from the nodal gap. Furthermore, the NOR architecture was disrupted, as the NG components Neurofascin186, AnkyrinG and βIV-spectrin diffused into the paranodal region. This may represent an underlying mechanism of brain dysfunction after concussion.

Structures show mechanisms of voltage-sensitive and leak K^+ channels

At the NOR, Na^+ channels are not the only ion channels present. There are K^+ channels as well, both voltage-sensitive and leak channels. The voltage-gated Kv7.2/7.3 channels (also known as KCNQ2/3) seem to play a role in the stabilization of the resting potential, and mutations of these channels can cause epilepsy [17] [18]. Kv7.2/7.3 channels are homo- or heteromeric assemblies of four subunits with six transmembrane helices each, and the structure of the (truncated but functional) Kv7.2 homotetramer was recently reported [18]. This revealed a 'domain-swapping' conformation, where the S1-4 helices of each subunit constitute the voltage-sensing domains, and the ion pore is formed by the S5-6 helices. Although the voltage-sensing domains are in an activated state, the pore is closed, which is likely due to lack of regulation by $PI(4,5)P_2$, which would be present in the lipid membrane of a cell. Furthermore, structures of Kv7.2 in complex with the small-molecule activators ztz240 (an analog of ICA-27243) and RTG (retigabine) showed that these bind to the voltage-sensing domains and the pore domain, respectively. While ztz240 appears to hold the voltage-sensing domain in an activated conformation by acting as a wedge, the activation mechanism of RTG seems to be allosteric. The Kv7.2/7.3 channels are also regulated by calmodulin, which in the structure was observed to bind to the cytosolic domain of Kv7.2, confirmed by mass spectrometry. Since Kv7.2/7.3 contain an Ankyrin binding motif similar to that of Nav1.6 channels, it is not unlikely that they are targeted to the NOR by similar mechanisms [19].

The leak K^+ channels TRAAK and TREK1 have been found at the NOR of afferent nerves in mice [20]. They are part of the K2P family, which are dimeric channels that are distinct from the voltage-gated K^+ channels. It was observed that TRAAK and TREK1 display thermal and mechanical sensitivity, and were required for high-frequency firing of the neurons by ensuring repolarization of the action potential. From structural data, a model (Fig 2B) has been established for the mechanosensitivity of TRAAK [21]. This involves a lateral intramembrane cavity that allows a lipid acyl chain to enter and block the ion pore. However, the membrane tension-dependent movement of a transmembrane helix can seal the lateral opening, ensuring that the ion pore is conductive. For TREK1, it has been shown how the K^+ concentration-dependent conductance is regulated by the selectivity filter, so-called C-type gating [22]. Changes in the K^+ concentration cause conformational changes in the selectivity filter, which are important for the stability of the ion binding sites in the selectivity filter. There is no structural

information on the mechanisms of K2P thermosensitivity, but it has been observed that the intracellular C-terminal domain of TREK1 is responsible for heat-sensing [23].

Neurofascin186 and NrCAM are adhesion proteins at the nodal gap

The nodal adhesion molecules Neurofascin and NrCAM are both members of the L1 subfamily of the IgCAMs. Neurofascin has two major splice variants, namely the axonal Neurofascin186 and the glial Neurofascin155, the latter of which functions at the paranode (see below). Like many other IgCAMs, Neurofascin consists of six Ig domains (Ig1-6), three to five FnIII domains, as well as a transmembrane helix and a short cytoplasmic domain that contains an Ankyrin binding motif. The main differences between Neurofascin186 and 155 are a short loop that is inserted between Ig2-3 of Neurofascin155 only, and the presence of a PAT domain between two FnIII domains in Neurofascin186 [24]. Structural study of the Ig1-4 domains of Neurofascin186 [25] shows the formation of a characteristic horseshoe-like fold, where the Ig1-2 segment folds onto the Ig3-4 module, with interactions between Ig1-4 and Ig2-3. In this report, it was also observed that Neurofascin186 has the potential to form homophilic dimers, through interaction of the Ig1-2 domains. This interaction is mediated by the formation of an intermolecular 'super β-sheet', as well as a hydrophobic cluster, which are both highly conserved in the L1 family. The structure suggests that the Neurofascin186 dimerization represents a *trans* homophilic interaction, but since Neurofascin186 is only expressed by axons, it is unclear whether this is physiologically relevant at the NOR. Neurofascin186 has been found to directly interact with its fellow L1 family member NrCAM [26].

NrCAM has a similar domain architecture as the other L1 family members and is expressed by both glial cells and neurons [27]. No structural studies of NrCAM have been published so far. As discussed above, glial NrCAM seems to play a role in Na^+ channel clustering by trapping gliomedin at the Schwann cell microvilli [9]. The function of axonal NrCAM is less clear, but it may be involved in interactions with the cytoskeleton, since it contains an Ankyrin binding motif [27]. Furthermore, it was observed that NrCAM is co-transported with Neurofascin186 [28].

Organization and function of the cytoskeleton at the nodal gap

The arrangement of the molecular components of the NOR is stabilized by the cytoskeleton. Many adhesion proteins and ion channels, such as Neurofascin186 [29], Nav1.6 [12] and Kv7.2/7.3 [19] contain sequences that mediate their binding to Ankyrins. The structure of the Ankyrin binding motif of Neurofascin186 bound to the Ankyrin repeat domain of AnkyrinG (R8-14 of 24 Ankyrin repeats in total) was recently resolved [29]. This revealed the canonical Ankyrin repeat domain fold, with the Neurofascin peptide forming hydrogen bonds and hydrophobic interactions with the inner groove of the Ankyrin repeats. The Ankyrin binding motif of Neurofascin is highly conserved among the other L1 family members, suggesting that their interaction with AnkyrinG would be similar. Notably, in the same study, mutations linked to L1 syndrome, which is characterized by symptoms including mental retardation, were found to be involved in binding of the L1 family member L1CAM to AnkyrinG.

Ankyrins, in turn, act as scaffolds linking the membrane proteins to the cytoskeleton via spectrin, which forms tetramers consisting of two α and two β subunits. At the nodal gap, the Ankyrin and spectrin isoforms are AnkyrinG and βIV-spectrin and αII-spectrin, respectively [30] [31]. However, it seems that AnkyrinG and βIV-spectrin are dispensable, as their absence can be compensated for by AnkyrinR and βI-spectrin [30]. Structural studies of the binding of Ankyrins to β-spectrins have been performed [32]. The spectrin binding domain of Ankyrin is formed by a $ZU5_N$–$ZU5_C$–UPA tandem, but spectrin binding is strongly increased by extension of the domain to include a conserved sequence N-terminal to the ZZU tandem (referred to as exZZU). From the structure of the AnkyrinG exZZU tandem bound to βIV-spectrin repeats R13-15, it was observed that the exZZU tandem has a cloverleaf-like architecture, with

the main interface involving Ankyrin $ZU5_N$ and UPA and spectrin R14. Moreover, the structures of AnkyrinG bound to βII-spectrin and AnkyrinB bound to βIV-spectrin were determined as well, and these were very similar to the AnkyrinG/βIV-spectrin complex. Thus, the specificity of the pairwise interactions between Ankyrins and β-spectrins is likely regulated by additional mechanisms.

Using stochastic optical reconstruction microscopy (STORM), a superresolution fluorescence imaging method, the organization of the axonal cytoskeleton was revealed [33]. The ubiquitous cytoskeletal protein actin forms ring-like structures around the axon circumference, which are spaced ~190 nm apart, comparable to the length of a spectrin tetramer. Alternating with the actin rings, spectrin formed periodic structures in the longitudinal direction. This arrangement was confirmed by results from stimulated emission depletion (STED) microscopy [34], which also showed that Ankyrins have a similar periodicity, both at the nodal gap and at the paranodes and juxtaparanodes. Furthermore, the ion channels (with the notable exception of juxtaparanodal Kv1.2) and adhesion proteins of the NOR were found to exhibit ~190 nm spacing as well. However, the molecular details of this organization remain to be resolved.

Paranode

Axon-glia interactions mediated by IgCAM adhesion proteins

At the paranode (Fig 3), a septate-like junction is formed by a ternary complex consisting of Contactin1 (also known as Contactin or F3) and Caspr1 (Contactin-associated protein 1, or paranodin) on the axon, and Neurofascin155 on the glial side, as observed by both cell-adhesion and biochemical experiments [35]. These proteins are involved in a range of diseases, including demyelinating disorders such as multiple sclerosis (MS) and chronic inflammatory demyelinating polyneuropathy (CIDP), and may be a target of autoantibodies [2].

Recently, structural studies of the interaction between Contactin1 and Neurofascin155 [36] have provided insights into the adhesion mechanisms. As mentioned above, Neurofascin155 contains six Ig-domains (Ig1-6) with a loop between Ig2-3, followed by four FnIII domains, a transmembrane helix and an intracellular domain. The architecture of the Contactins (another IgCAM subfamily) is very similar, with the difference being that they lack transmembrane and cytoplasmic regions, and are attached to the membrane by a GPI-anchor. The structures of the Ig1-6 domains of both Neurofascin155 and Contactin1 show the horseshoe fold of Ig1-4, like that of Neurofascin186 [25]. The Ig5-6 segment is connected to the horseshoe by a (flexible) V-turn, which is sharper in Neurofascin155 than in Contactin1. Interaction between the two proteins again occurs via the conserved Ig1-2 interfaces with the formation of an intermolecular 'super β-sheet', and is modulated by glycosylation of aspargines on both binding partners. The authors also observed the formation of Neurofascin155 homodimers, in which the interaction interface overlaps with that of the heterodimer with Contactin1, but which have a lower affinity. It is unclear what the relevance of this *trans* homophilic adhesion could be at the paranode, since Neurofascin155 is only expressed in glial cells. Furthermore, Contactin1 had a weak propensity in solution for the formation of zipper-like oligomers, mediated by Ig3-6. Interestingly, this *cis* interaction did not obscure the interface for binding of Contactin1 to Neurofascin155, and thus it could be involved in the formation of the septate-like junctions. While the structure of the full ectodomain of Contactin1 is yet to be resolved, it has been determined that the FnIII1-3 domains of the Contactin family proteins adopt an extended but bent conformation [37]. Integration of these and other structural data has allowed for modelling of the Contactin1 ectodomain, which shows an extended, S-shaped architecture ~20 nm in length, which matches quite well with results from SAXS experiments [36].

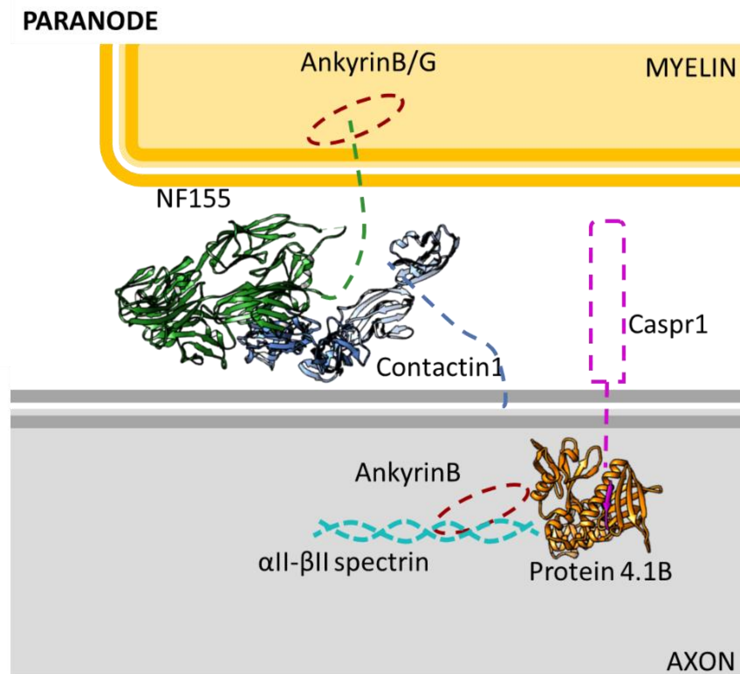


Figure 3: Overview of the paranode. *Experimental structures of the Contactin1 (Ig1-6)/Neurofascin155 (Ig1-6) complex (7OL4) and the TSLC1 peptide bound to protein 4.1B FERM domain (3BIN).*

Connection of adhesion proteins to the cytoskeleton is essential for barrier formation

It has been reported that Contactin1 is required for the formation of the paranodal junction and for the targeting of Caspr1 to the paranode. From coimmunoprecipitation experiments it was observed that the interaction is mediated by the Ig domain of Contactin1, although the FnIII domain was also necessary for proper Caspr1 targeting [38]. No structural data for Caspr1, either alone or in complex with other proteins, has been published so far. The function of Caspr1 seems to be to act as a transmembrane scaffold for the Caspr1/Contactin1 complex. This is achieved by interaction of the cytoplasmic domain of Caspr1, which contains a glycophorin C-like sequence, with the FERM domain of protein 4.1B (also known as DAL1), which links it to the cytoskeleton [39] [40]. For TSLC1, a non-neuronal adhesion protein that also interacts with protein 4.1B through a glycophorin C-like motif, the structure of this peptide bound to the protein 4.1B FERM domain has been resolved [41]. This shows a three-lobed fold of protein 4.1B, with the peptide bound in a mostly hydrophobic pocket in the C-terminal lobe. Thus, this may provide a model for the interaction of Caspr1 with protein 4.1B.

The scaffolding and cytoskeletal proteins at the paranode are AnkyrinB and βII- and αII-spectrin, which form a macromolecular complex with protein 4.1B that cofractionates with Caspr1, Contactin1 and Neurofascin155 [42]. Notably, loss of Caspr1 disrupted the localization of AnkyrinB at the paranode, but AnkyrinB localization was not affected by Caspr2 (juxtaparanodal) or βIV-spectrin (nodal gap), indicating specificity of the paranodal cytoskeleton. The structure of the complex between protein 4.1B and the cytoskeletal components is not known, but binding of AnkyrinB to βII-spectrin would likely resemble the interaction between AnkyrinG and βIV-spectrin [32], as discussed above. Meanwhile, on the glial side, Neurofascin155 has been observed to interact with AnkyrinB (in PNS Schwann cells) or AnkyrinG (in CNS oligodendrocytes) [43], probably in a mode similar to that of Neurofascin186 and AnkyrinG in the axon at the nodal gap [29]. Interestingly, AnkyrinG but not AnkyrinB was required for

formation of the paranodal junctions, while targeting of Ankyrins was not dependent on Neurofascin155.

The function of the paranode as a barrier restricting the ion channels to the nodal gap and the juxtaparanode is clear, as loss of the adhesion proteins disrupts the separation [44] [45]. Therefore, it was hypothesized that the Contactin1/Caspr1/Neurofascin155 complex itself formed the boundary. However, the molecular mechanism that underlies the segregation has been found to rely on the cytoskeleton, since loss of βII-spectrin causes diffusion of juxtaparanodal Kv1.2 channels into the paranode [46]. However, this does not affect the formation of the septate-like junctions, indicating that the axon-glia contact established by Caspr1, Contactin1 and Neurofascin155 is not dependent on the underlying cytoskeleton, nor is it directly responsible for the formation of the barrier. In line with this, it has been shown that protein 4.1B is not required for the stability of Caspr1 at the paranode [47]. Taken together with the observation that Caspr1 is needed for recruitment of AnkyrinB [42], it seems that the organization of the paranodal cytoskeleton depends on the paranodal junction adhesion proteins, but not vice versa.

Juxtaparanode

Structural features and function of the juxtaparanodal adhesion complex proteins

At the juxtaparanode (Fig 4A), there is also a ternary complex that mediates contact between the axon and the glial cells, namely Contactin2 (also known as TAG1 or axonin1) on both the axonal and glial side, and Caspr2 on the axon. Similarly to the paranode, presence of Contactin2 is required for targeting of Caspr2, and also for localization of Kv1.1/1.2 channels (discussed further below) [48]. Structures of fragments of Contactin2 have been resolved, again revealing a horseshoe fold of the Ig1-4 domains, which homodimerizes through Ig1-2 [49], and a bent conformation of FnIII1-3 [37]. A recent investigation of Contactin2 Ig1-6 [50] observed two potential modes of homodimerization; one involving the 'classic' Ig1-2 interface, which resembles but is more extensive than the previously reported dimer, and one mediated by a different, likewise conserved Ig3-6 interface, which could be relevant for the formation of larger assemblies. The dimerization appears to be regulated by asparagine glycosylation, similar to the Contactin1/Neurofascin155 complex [36]. The authors modelled the full ectodomain of Contactin2 based on experimental data combined with *in silico* models, resulting in an S-shaped architecture that resembles the model for Contactin1 [36]. Calculated projections of the Contactin2 model corresponded well to negative stain electron micrographs, which also show the flexibility of the ectodomain.

The direct interaction of Contactin2 with Caspr2 and Kv1.1/1.2 appears to be mediated by the Contactin2 Ig domain, as this domain is necessary and sufficient for their coimmunoprecipitation [51]. Curiously, it has been reported [52] that the paranodal Contactin1 is able to bind to Caspr2, although it is not clear what the physiological role of this interaction is. In the same experiment, association of Contactin2 with Caspr2 was not observed, possibly due to a lower affinity. Like its paranodal counterpart Caspr1, Caspr2 interacts with protein 4.1B as well [39], which is likely the link to the cytoskeleton at the juxtaparanode. Immunofluorescence microscopy experiments also show colocalization of Caspr2 with Kv1.2, and to a lesser extent, protein 4.1B [47]. Moreover, loss of protein 4.1B disrupted the localization of Caspr2 (again, like Caspr1) and Kv1.2 at the juxtaparanodes.

Biochemical characterization of the Caspr2 ectodomain [52] revealed that it is glycosylated at 12 sites, and that it exists as a monomer in solution. Furthermore, a homology model suggested that Caspr2 adopts a compact cloverleaf-like fold, and this was supported by data from SAXS and negative stain EM.

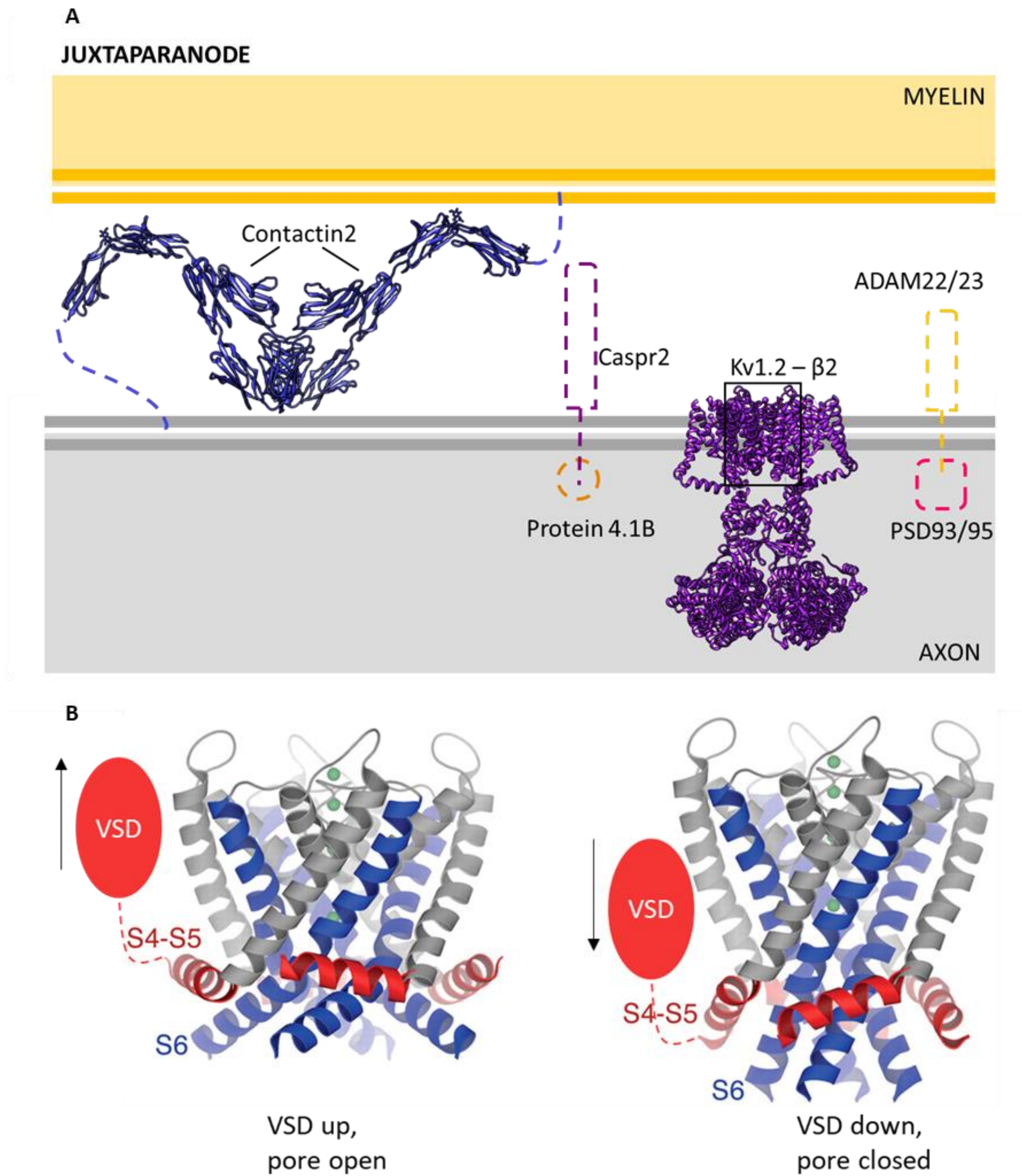


Figure 4: Overview of the juxtaparanode. *(A) Experimental structures of the Contactin2 (Ig1-6) homodimer (8A0Y) and the Kv1.2 channel with β2 subunit (2A79). (B) Mechanism of voltage sensing of the Kv1.2 channel. Membrane potential-dependent movement of the voltage sensing domains is coupled to the pore domain via linker helices. Adapted from Long et al, 2005b.*

The structure of the N-terminal discoidin domain of Caspr2 has been determined experimentally [53], showing a barrel-like conformation (matching the homology model) made up of β-sheets. Notably, the loop region on one side of the barrel was predicted to contain epitopes for autoantibodies, which was confirmed by ELISA (enzyme-linked immunosorbent assay) experiments with antibodies from patients with limbic encephalitis, an autoimmune disorder.

Targeting and function of juxtaparanodal K^+ channels

The juxtaparanodal Kv1.2 channel was the first mammalian voltage-gated K^+ channel to have its structure experimentally resolved [54]. This showed a tetrameric architecture, with a central ion pore

flanked by voltage-sensing domains. Below this transmembrane segment, on the cytoplasmic side, a T1 domain is found, associated with a likewise tetrameric β2 subunit (not one of the β subunits that associate with Nav channels), which seems to have a regulatory function. The structure also revealed the mechanism of ion channel voltage sensing [55]; the voltage-sensing domains detect the membrane potential through positively charged arginine residues, and in response move in the membrane. When the membrane is depolarized (i.e. positive inside), the voltage sensors move up, and conversely move down in a hyperpolarized membrane. The sensors are coupled to the ion pore through linker helices, and thus the pore can open or close in a voltage-dependent manner (Fig 4B).

Besides the Contactin2/Caspr2 complex and the K^+ channels, ADAM22/23 (part of the disintegrin and metalloproteinase family, but lacking proteinase activity) and PSD93/95 (membrane-associated guanylate kinases, or MAGUKs) are found at the juxtaparanodes as well. ADAM22 coimmunoprecipitates and colocalizes with Kv1.1/1.2, PSD93, PSD95 and the extracellular ligand LGI1. However, neither ADAM22 nor PSD93/95 influence the electrophysiological properties of Kv1.2, and ADAM22 is not required for Kv1.2 or Caspr2 targeting to the juxtaparanode. On the other hand, the clustering of PSD93/95 is dependent on ADAM22 [56]. As for ADAM23, a recent study [57] found that it also colocalizes with Kv1.1/1.2 at the juxtaparanodes, and is necessary for Kv1.1/1.2 and Caspr2 accumulation and stability, but its function depends on the presence of the ligand LGI3. Interestingly, results from the same report suggest that the role of the juxtaparanodal Kv1.1/1.2 channels is to regulate the refractory period for high frequency firing, as this is prolonged upon loss of Kv1.1/1.2 function.

Future perspectives

Our understanding of the molecular mechanisms and interactions at the node of Ranvier is ever-increasing, especially as more and more structures of the molecular components are resolved. Mainly, the mechanisms of voltage and mechanosensitivity of the ion channels are now much clearer, which will hopefully contribute to the treatment of diseases characterized by aberrant channel function, such as epilepsy. Nevertheless, there still remains a lot of work to be done, as there are still proteins, such as NrCAM and Caspr1/2, for which there is little to no structural data available. Moreover, for many adhesion proteins (Neurofascin, Contactins) only fragments have been structurally characterized, and it is not clear what the conformation of the full protein looks like. The molecular details of the formation of intermolecular complexes, which is critical for the function of the NOR, also remain largely unknown, although recent progress has been made here.

It should be noted that the structures discussed in this review were generally determined with the proteins or protein fragments in solution, and are thus disconnected from their native cellular environment. However, for a complete picture of the molecular mechanisms and interactions in a more physiologically relevant context, they should be studied *in situ*. Recent advances in cryo-electron tomography [58] have enabled the structural characterization of biomacromolecules in the cell. For example, this technique has been used to study the structure of the cytoskeleton in the neural growth cone, revealing the actin bundles and microtubules in molecular detail [59]. In the future, cryo-electron tomography could be applied to the NOR to resolve, for instance, the molecular details of the macromolecular complexes that link the membrane proteins and the cytoskeleton together. These and other experiments will be instrumental in bridging the gap between cell biology and structural biology research. Overall, knowledge of the molecular mechanisms and interactions at the NOR is critical for the elucidation of the cause and potential treatment of neurological disorders, including epilepsy and demyelinating autoimmune diseases.

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Lay summary

To allow us to think, move and feel, the cells in our nervous system (neurons), transport information as electrical signals. These are conducted along the axons, which are long extensions of neurons that can be up to a meter long in humans. The axon is wrapped in a layer of a fatty substance called myelin, which can be compared to the plastic insulation layer around electric cables. The sheath of myelin not only protects the axon, but also helps to prevent the electrical signal from weakening. Importantly, the myelin layer is not continuous, meaning that there are small gaps where the axon is uncovered. These are called the nodes of Ranvier (NOR). At the NOR, the electrical signal is reinforced, meaning that it basically 'jumps' forward along the axon from one NOR to the next. Thus, it is conducted with high speed, which is necessary to, for example, control our movements.

For the boosting of the electrical signal at the NOR, charged particles (ions), need to be able to enter or exit the axon. They are transported in or out of the axon through special proteins that form channels in the axon's membrane. To keep these ion channels in place, they interact with other proteins in the membrane, the so-called adhesion proteins. At the edges of the gap, other adhesion proteins make contacts between the axon and the myelin layer, and ensure they stay together. Also, the ion channels and adhesion proteins are connected to the cytoskeleton of the neuron, a large network of proteins that gives it its structure.

Because the NOR is so important in the conduction of signals by neurons, there can be serious consequences when something goes wrong with the molecules there. For example, mutations in the ion channels can interfere with the signal propagation, which may cause the neuron to 'fire' too much, leading to disorders like epilepsy or autism. Furthermore, in some autoimmune diseases our body will make antibodies against the proteins at the NOR and attack it. This can damage the myelin layer, and thereby make signal conduction much slower, which is the case in multiple sclerosis (MS).

To understand and treat such diseases, we need to know how the proteins at the NOR work and interact with each other. This has been studied extensively over the past three decades, and much progress has been made. However, until recently we did not know much about what the proteins and protein complexes look like, which is very important in the design of drugs, for example. In this article, I have reviewed the recent advances in the so-called structural biology of the NOR. The structures of the NOR proteins have given us new insights into how they work, but they are often determined when the proteins are on their own, which is obviously different from their environment in the neuron. In the future, technological advances should allow us to study them in a more 'natural' context, further increasing our understanding of their (dys)function.

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