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# Molecular Constituents of the Node of Ranvier

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## Abstract

The interaction between neurons and glial cells that results in myelin formation represents one of the most remarkable intercellular events in development. This is especially evident at the primary functional site within this structure, the node of Ranvier. Recent experiments have revealed a surprising level of complexity within this zone, with several components, including ion channels, sequestered with a very high degree of precision and sharply demarcated borders. We discuss the current state of knowledge of the cellular and molecular mechanisms responsible for the formation and maintenance of the node. In normal axons, $Na^+$ channels are present at high density within the nodal gap, and voltage-dependent $K^+$ channels are sequestered on the internodal side of the paranode—a region known as the juxtaparanode. Modifying the expression of certain surface adhesion molecules that have been recently identified, markedly alters this pattern. There is a special emphasis on contactin, a protein with multiple roles in the nervous system. In central nervous system (CNS) myelinated fibers, contactin is localized within both the nodal gap and paranodes, and appears to have unique functions in each zone. New experiments on contactin-null mutant mice help to define these mechanisms.

**Index Entries:** Node of Ranvier; axon; myelin; $Na^+$ channel; $K^+$ channel; paranode; contactin.

## Introduction

The node of Ranvier has evolved as a highly efficient and reliable signal generator in the nervous system. Propagating action potentials in the human peripheral nervous system may pass through more than 1000 nodes in a single axon to reach their target, and the failure rate at each site must therefore be vanishingly small. Very early experiments defined the essential elements of the physiology of myelinated axons. Over 50 yr ago Tasaki and Takeuchi *(1)* and Huxley and Staempfli *(2)* demonstrated that, during conduction, inward current was uniquely recorded at nodes of

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Ranvier, defining the concept of 'saltatory conduction'. The first experiments to examine single nodes under voltage clamp were done on amphibian axons. The responses to depolarizing pulses consisted of a transient inward $Na^+$ current, and a delayed steady-state outward $K^+$ current—records not much unlike the classical sweeps from unmyelinated invertebrate axons. Because voltage-dependent channels under the myelin would in all likelihood not be activated during any of these experiments, it could not be determined if the channels responsible for these "active" currents were specifically sequestered within the nodal gap or, rather, were more uniformly distributed. There was thus little reason to suspect any unusual axolemmal architecture. A series of experiments in the late 1970s and early 1980s significantly altered that view. Waxman and his colleagues *(3)* discovered that ferric ion–ferrocyanide stained the cytoplasmic surface preferentially at nodes of Ranvier, suggesting a biochemical difference in the cytoskeleton of nodes vs internodes. Ultrastructural analysis revealed a distinct pattern of intramembranous particles in freeze–fracture replicas. High densities of large particles were seen in both the node and juxtaparanode, and much lower densities in the axoglial junction region of the paranode and in the remainder of the internode *(4,5).* Saxitoxin (STX) is a highly specific blocker of axonal $Na^+$ channels, and the binding of $^3$H-STX has been quantitated and used to estimate $Na^+$-channel density. Ritchie and Rogart *(6)* found no significant difference in binding in intact and homogenized nerve, and concluded that virtually all axonal $Na^+$ channels were localized at nodes, where they would be accessible to the toxin in intact fibers. The Ritchie laboratory later showed that Schwann cells can also express $Na^+$ channels, and that this is likely to have led to a considerable overestimation of the nodal density of $Na^+$ channels in the earlier work *(7,8).* Patch-clamp recording from demyelinated axons demonstrated a sharp gradient in $Na^+$-channel density at nodes of Ranvier, and measured an internodal concentration about 4% of that at nodes *(9-11).* These internodal channels constituting the majority of axonal channels due to the 1000-fold difference in areas of node and internode, serve as a reservoir that can be tapped into to populate new nodes during remyelination *(12).* They also play important roles in conduction of demyelinated fibers *(10,13).* Measurements of $Na^+$ channel density at nodes by electrophysiological methods have been summarized by Hille *(14),* and lead to a best estimate of 1000–1500/$\mu m^2$. This agrees with the density of intramembraneous particles in freeze–fracture replicas (approx 1300/$\mu m^2$) *(4,15).*

The distribution and role of $K^+$ channels in axons is more complicated. Chiu et al. *(16)* successfully placed mammalian nodes of Ranvier under voltage clamp and showed that, unlike their amphibian counterparts, mammalian nodes had a high density of $Na^+$ channels, but lacked voltage-dependent $K^+$ channels. Chiu and Ritchie *(17)* later demonstrated that $K^+$ currents could be elicited following paranodal demyelination, and it was subsequently shown through immunocytochemistry that Shaker-type $K^+$ channels Kv1.1 and Kv1.2, and their associated Kvβ2 subunit were clustered in juxtaparanodes—internodal zones just beyond the paranodal terminal loops (*18-20*). Therefore it became evident that the myelinated axon, and the node of Ranvier in particular, were structurally quite complex, with $Na^+$ and $K^+$ channels sequestered in different compartments, and with functional consequences attending pathological alterations.

Over the past several years, there has been much progress in identifying the cellular mechanisms and molecular constituents involved in the neuron–glial interactions that initiate and maintain the structure of myelinated axons, and the node in particular. The sketch in Fig. 1 illustrates many of the components that have been characterized to date. Voltage-dependent $Na^+$ channels all include a large (approx 260 kDa) α-subunit that contains the pore and much of the gating machinery. In mammalian cells, several auxiliary $Na^+$ channel β-subunits that modulate expression and

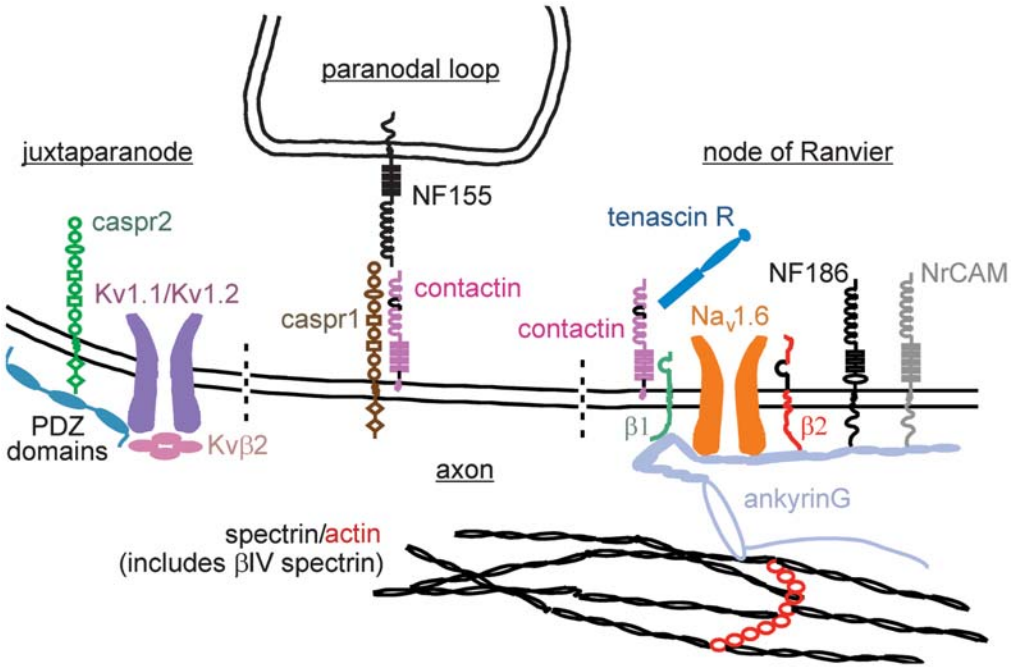


Fig. 1. Diagram showing the structure and location of many of the constituents thought to play a role in the expression and function of ion channels within the region of the node of Ranvier in the CNS. The sketch emphasizes the domain structure of these proteins and is thus not to scale. Only one paranodal loop is illustrated, and it appears rather distant from the axolemma. Dashed lines separate node from paranode, and paranode from juxtaparanode. Although single ankyrin G and PDZ domain molecules are shown for simplicity, several may be involved in anchoring and linking their respective binding proteins. The ion channel α-subunits have multiple membrane-spanning segments. Contactin isoforms at the node and paranode differ in glycosylation *(24).* The adherens and gap junctions that link paranodal loops, and are essential for myelin integrity are not shown *(94–96).*

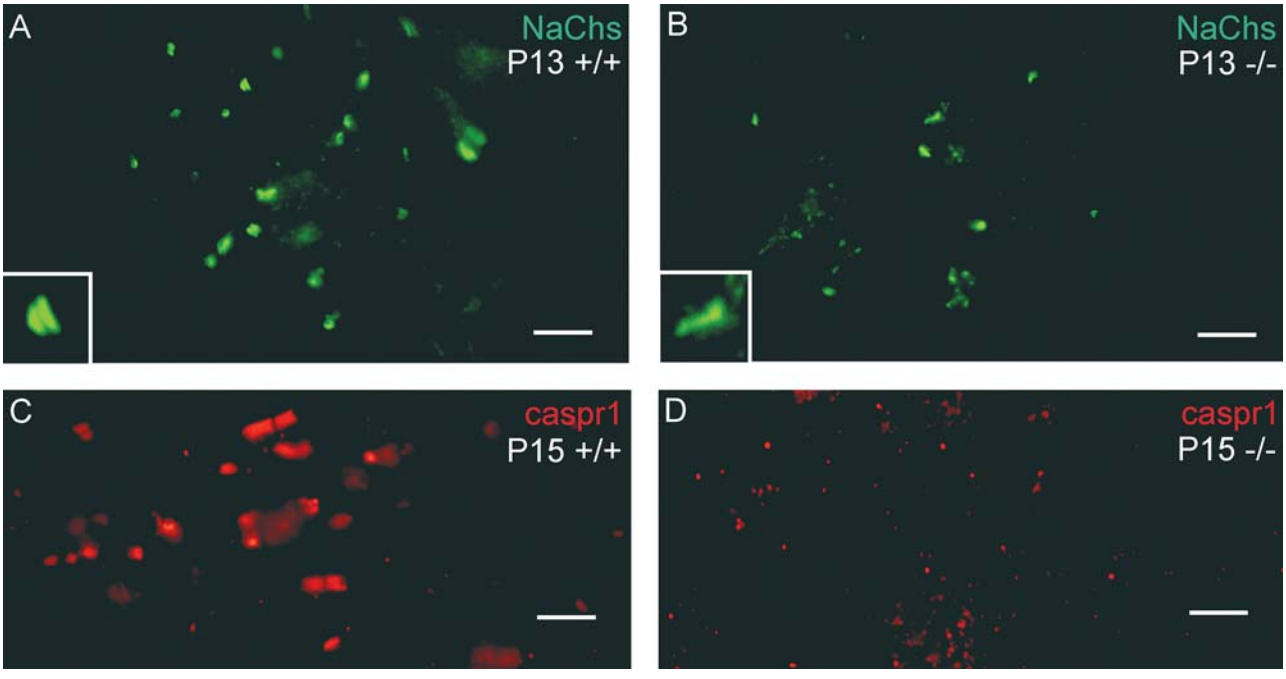


Fig. 2. Immunofluorescence labeling of Na+ channels (NaChs; green; **A**,**B**) and caspr1 (red; **C**,**D**) in optic nerves of wild-type (+/+; **A**,**C**) and contactin null mutant (–/–; **B**,**D**) mice. Nerves were prepared and labeled as described *(25,46).* Results were similar at P13 and P15 (Na+ channel data at P15 is plotted in Fig. 3). The insets show enlarged views of sites within the panel. Scale bars 10 μm.

gating have been identified. Two are shown in the diagram, and will be discussed in more detail. Within the nodal gap, $Na^+$ channels are linked to the spectrin/actin cytoskeleton by ankyrin G *(21,22).* Either through direct interaction, or via common association with ankyrin G, $Na^+$ channels are thought to form a complex with several membrane proteins and extracellular matrix components that colocalize at the node. These include contactin, NrCAM, the 186 kDa isoform of neurofascin (NF186), and tenascin R *(23-30).* A number of distinct proteins (e.g., caspr1, NF155, and an isoform of contactin) have been identified within the paranodal zone, some forming the axo-glial junctions that stabilize myelin structure and provide electrical isolation for the node *(24,27,31).* Furthermore, nodal $Na^+$channel α-subunits are developmentally regulated. $Na_v1.2$ is first clustered at immature nodes, and then replaced by $Na_v1.6$ *(32,33).* In unmyelinated axons, or in axons in which myelination is disrupted, this isoform switch does not take place *(33,34).* $Na_v1.6$ (previously known as NaCh6 and Scn8a), has a resurgent current during repolarization of action potentials that alters the frequency of action potentials in neuronal cell bodies *(35).* The possible influence of this unusual gating on conduction in myelinated axons is not clear, but may contribute to instabilities when $K^+$ channels are blocked or missing *(36-38).* Finally, there has been progress in characterizing molecules with a specific localization within the juxtaparanodal region populated by voltage-dependent $K^+$ channels, including caspr2—another member of the neurexin family *(39).* Details about all of the components illustrated in Fig. 1 are discussed in appropriate sections below.

## Axo-Glial Interactions

Of significant importance in our understanding of axonal development is the issue of exactly how the ion channels that underlie excitability are localized in their respective compartments. Several key questions have emerged, and most of these remain key topics of debate. An early theory for $Na^+$ channel clustering at the node suggests that the axo-glial junctions forming at paranodes serve as barriers to confine the channels after they have accumulated in the nodal gap, or even to play a role in the initial clustering *(4,5).* Several later experimental findings are supportive of this hypothesis. Studying the formation of new nodes of Ranvier during remyelination, Dugandzija-Novakovic et al. *(40)* found that clusters of axonal $Na^+$ channels first formed at the edges of Schwann cell processes. As these processes grew longitudinally, the clusters appeared to move with them, since they remained always at their tip. Ultimately, clusters associated with neighboring Schwann cells were seen to fuse, forming a node. A similar mechanism was subsequently found active during development, and it was further shown that Schwann cells had to reach the stage of commitment to myelination, as defined by expression of myelin-associated glycoprotein (MAG, *[41]*) before they could induce $Na^+$-channel clustering *(42).* At the ultrastructural level, $Na^+$ channels appeared to be excluded from regions of close axon–Schwann cell contact, since clusters were typically just beyond the edge of a Schwann cell process, rather than under it *(43).* Studies were greatly accelerated by the discovery of caspr/paranodin, a neurexin-family membrane protein which is present at high density at paranodes *(44,45),* and which has been shown to form part of the axo-glial junctions at that site (*31*). This protein will be called caspr1 here as there are now several known isoforms. Rasband et al. *(46)* studied node-of-Ranvier formation in the developing optic nerve, and showed that caspr1 accumulation preceded $Na^+$-channel clustering by about 2 d.

More recent evidence, suggests that mature axo-glial junctions (visible as transverse bands in electron micrographs) are not obligatory for $Na^+$-channel accumulation. Coetzee et al. *(47)* produced mice with the enzyme UDP-galactose:ceramide galactosyl transferase (CGT) genetically deleted, resulting in an inability to

synthesize galactocerebroside (GalC) and sulfatide. While the peripheral nervous system (PNS) is mostly structurally normal in these animals, paranodes in the CNS are markedly disrupted. The transverse bands characteristic of mature junctions are absent, and caspr1 is diffusely distributed. $Na^+$ channels are present at high density at nodes, but the clusters are longer than normal *(48,49).* $K^+$ channels are displaced from the juxtaparanode to the paranode, as is caspr2 *(49,50).* A very similar ion channel distribution was seen in the caspr1-null mutant *(51).* In the CNS, paranodal loops were displaced and transverse bands were absent. $Na^+$ channel clusters were present at nodes, but were more diffuse than in wild-type. $K^+$ channels were paranodal. Nodal $Na^+$ channel clusters are also abnormal in the *jimpy* mouse, which similarly has a loss of axo-glial junctions *(52,53).* However, results are complicated because the point mutation in proteolipid protein in *jimpy* causes oligodendroglial cell death. From all of this evidence, it seems apparent that axo-glial junctions are not the sole driving force behind $Na^+$-channel clustering at nodes in the CNS. The initial stages in clustering that place a high density of $Na^+$-channels at the node, may rather be a result of an exclusion of these channels from other regions of close contact between axons and glial cells *(54);* or in a very different hypothesis, be driven by a soluble factor secreted by oligodendrocytes independent of direct contact *(34,55).* On the other hand, these junctions do appear to play an important role in determining ion channel distributions. In each of the mutant animals in which the paranodes are disrupted, $Na^+$-channel clusters are significantly distorted in shape and size. This finding, together with the distinct spatial relationship showing the junctional zones as borders surrounding islands of $Na^+$-channels in *Shiverer*—seen by both freeze–fracture *(56,57)* and immunocytochemistry *(46)*—provides a compelling argument for the involvement of these junctions in $Na^+$-channel localization. There is evidence from freeze–fracture studies that the initial steps in channel clustering may require only early immature paranodal axon–glial specializations *(58).* It seems clear that this issue is best attacked in studies that combine immunocytochemistry for definitive channel identification, and ultrastructural analysis for high resolution morphology. Finally, all cases in which the junctions are disrupted, Shaker-type $K^+$ channels are no longer confined to juxtaparanodes, and are found adjacent to the paranodal loops.

## Surface vs Secreted Proteins

One rather fundamental question that was alluded to above remains: do glia influence $Na^+$-channel clustering at nodes of Ranvier via direct contact, or through the secretion of soluble factors? It should first be recognized that not all $Na^+$-channel sequestration requires glial involvement. $Na^+$ channel clusters form at axonal initial segments of spinal motor neurons that are grown in highly purified cultures that include virtually no glial cells *(59).* However, it is generally accepted that clusters at sites thought destined to become nodes of Ranvier require, or are strongly enhanced by, glia. In the PNS, almost all evidence points to a contact-dependent mechanism. Early experiments on remyelinating and developing axons were described above. Schwann cells induced $Na^+$-channel clustering only after initiating myelination. Recent in vitro studies demonstrated that clustering in dorsal root ganglion (DRG) neurons occurred only under contact with myelinating Schwann cells *(60).* If Schwann cells were included, but myelination was prevented, or if conditioned medium from myelinating co-cultures was added to purified neuronal cultures, clustering failed to occur. Furthermore, Melendez-Vasquez et al. *(61)* studied the ezrin-radixin-moesin (ERM) family of proteins that link the actin cytoskeleton to the membrane. It was shown that during development of the PNS, the ERM proteins are expressed at the tips of Schwann cell processes, likely in advance of the clustering of ankyrin G in the underlying axolemma. The ERM pro-

teins later are present in the microvilli that overlie nodes of Ranvier. The idea that these proteins may cluster nodal constituents by attraction, constitutes an alternative hypothesis to the idea that $Na^+$ channels are clustered by exclusion from regions of Schwann cell adherence *(43,54),* but is still a contact-dependent mechanism. On the other hand, in cultures of retinal ganglion cells (RGCs), $Na^+$-channel clustering is dependent on a soluble factor released by oligodendrocytes *(34,55).* Oligodendrocyte conditioned medium increased the number of clusters seen in RGCs in a dose-dependent manner. An important limitation of this work is that in contrast to the PNS co-cultures, the RGCs were never myelinated in vitro. Thus, while the authors consider these clusters to be "nodal" (i.e., destined to be nodes of Ranvier), there is no direct supporting evidence, and some results even appear contradictory. Clusters formed close to the soma, but in vivo nodes are not seen on the proximal side of the lamina cribrosa. Only $Na_v1.2$ channels were detected in these clusters: $Na_v1.6$ is seen only in myelinating axons in vivo. The sites of high-channel density seen appeared to be in small swellings of the axons, not clearly indicative of surface expression, and were at very short spacing. Similar sites have been observed in mouse PNS axons at postnatal d 1, but they are short-lived, disappearing within 1–2 d, and never forming nodes of Ranvier *(62).* Nonetheless, many authors find that the soluble-factor hypothesis is consistent with their results; it remains intriguing that mechanisms of $Na^+$-channel clustering in the CNS may be markedly different from those in the periphery. Induction by a soluble factor implies that the axon determines the site of nodal clustering, while the contact hypothesis suggests that it is the growth of glial processes that moves channels to their ultimate destinations. Because axons from DRGs and spinal motor neurons (SMNs) traverse both CNS and PNS and are myelinated by oligodendrocytes and Schwann cells, respectively, in these regions, in order for the two mechanisms to coexist, individual axons would have to use them both. Further-more, the axon-determined nodes would be close to the neuronal soma in SMNs, but distant in DRGs. It may be that only initial steps in clustering in the CNS are induced by soluble glial factors, and that the ultimate positioning of nodes is determined later by myelinating oligodendroglia, which also induce the subtype switch to $Na_v1.6$ *(34).*

As noted above, mammalian voltage-dependent $Na^+$ channels are heteromultimeric, and include one of at least 10 known α-subunits in association with auxiliary β-subunits. Three β-subunits and one splice variant have been identified to date. β1 is non-covalently associated with the α-subunit, while β2 is linked to α by disulfide bonds *(63).* β3 has high homology to β1 *(64).* β1A is a higher-molecular-weight splice-variant of β1 and may be the preferential form expressed by certain neurons, while β1 is expressed by oligodendroglia *(34,65).* Each of these β-subunits has an extracellular region containing a single Ig-type domain, a single transmembrane segment, and an intracellular zone. The β-subunits appear to play important roles in many aspects of $Na^+$ channel expression and function. They modulate, for example, the voltage dependence of gating *(63,66).* The β-subunits bear homology to, or associate with, several key proteins in myelinated axons. Homophilic binding of these proteins promotes cell adhesion and recruits ankyrin to points of cell–cell contact in transfected cell lines *(67).* The Ig-fold of β1 is homologous to that of P0, a major PNS protein that mediates homophilic interactions in compact myelin *(68,69).*

## Contactin

As a result of both homologous sequence and colocalization with $Na^+$ channels, contactin is of special interest for its possible involvement in $Na^+$-channel expression. Contactin, known also as F3 or F11 in various species, is a glycosyl-phosphatidylinositol (GPI) anchored protein whose extracellular region contains four fibronectin type III

domains, and six Ig-like domains *(70-73).* Contactin is expressed by both neurons and oligodendroglia, and is involved in axon and dendritic guidance in the cerebellum *(74).* As shown in Fig. 1, in CNS axons contactin is expressed in both the nodal gap and the paranodal region, although the isoforms present in these zones differ in glycosylation *(24).* Within the paranode, contactin is associated with caspr1 in the axolemma, and this complex binds the 155 kDa form of neurofascin that is expressed by glia at the base of the paranodal loops *(27,75).* The third Ig-domain of contactin has sequence homology to the Ig-domain of β2, and the juxtamembrane regions of these two proteins are also homologous (indicated by the black zones in Fig. 1) *(76,77).*

The distribution of contactin and its homology to β2 has stimulated a number of recent studies aimed at exploring its possible role in $Na^+$-channel expression in myelinated axons. Kazarinova-Noyes et al. *(25)* showed that contactin and $Na^+$ channels could be reciprocally co-immunoprecipitated from solubilized rat-brain membranes. Chinese-hamster-lung (CHL) cells were stably transfected with various combinations of the $Na_v1.2$ α-subunit, β1, β2, and contactin. Peak inward $Na^+$ currents were measured under whole-cell patch clamp. Cells transfected with $Na_v1.2$, β1, and contactin had four to five times higher $Na^+$ currents than cells with $Na_v1.2$ alone, or in combination with contactin and/or β2. Tritiated saxitoxin ($^3$H-STX) binding was likewise 4–5 times higher in the $Na_v1.2$/β1/contactin cells. Thus, substituting contactin for its homolog β2 increased the functional surface density of $Na^+$ channels in these cells, and this increase was dependent on the β1-subunit *(25).* CHL cells transfected with contactin plus just one $Na^+$-channel subunit were immunoprecipitated with antibodies to the subunit, and blots were probed with anti-contactin antibodies. Antibodies to neither $Na_v1.2$ α-nor β2-subunits immunoprecipitated contactin from the respective cells. However, anti-β1 antibodies did bring down contactin from β1/contactin cells. Results of this biochemical analysis were entirely consistent with the physiological measurements, and point to a specific interaction between contactin and β1 to modulate $Na^+$-channel expression. These authors also examined the immunocytochemical localization of contactin in myelinated axons in both the PNS and CNS. Contactin was only minimally detected within the nodal gap of both developing and adult axons in sciatic nerves, and was primarily confined to paranodes. During remyelination that followed lysolecithin-initiated demyelination, contactin transiently colocalized with $Na^+$ channels at new nodes, but was largely gone within 2 w. In contrast, strong contactin immunofluorescence was found at nodes in both optic nerve and brain, in the adult as well as during early development. Within the CNS there is thus, both morphological and biochemical evidence for an interaction between $Na^+$ channels and contactin. Liu et al. *(78)* have recently shown that the expression of $Na_v1.9$/NaN is also enhanced by contactin, and they provide evidence for colocalization in sensory neurons in the PNS. Biochemically, however, the interaction with contactin (GPI-anchored) is shown to be at the C-terminus of $Na_v1.9$ (intracellular) and therefore is difficult to envision for more than a very brief period.

The possibility that contactin may play a role in establishing the high density of $Na^+$ channels at CNS nodes of Ranvier has recently been further tested in our laboratory through experiments on contactin-null mutant mice *(74)* obtained from Dr. B. Ranscht. Homozygous mutants are ataxic and die by P18. Cryosections of optic nerves from contactin –/– mice at P13–P15 and wild–type littermates were labeled with antibodies to pan voltage-dependent $Na^+$ channels and to caspr1. Figure 2 shows representative fields of view. In +/+ axons, $Na^+$-channel clusters are seen at numerous sites, with most identifiable as nodes of Ranvier based on length (<2 μm) and double-line structure indicative of surface label (*See* Fig. 2A, inset). In the contactin –/– optic nerve, there were fewer $Na^+$-channel clusters, and many appeared to be misshapen, lacking a

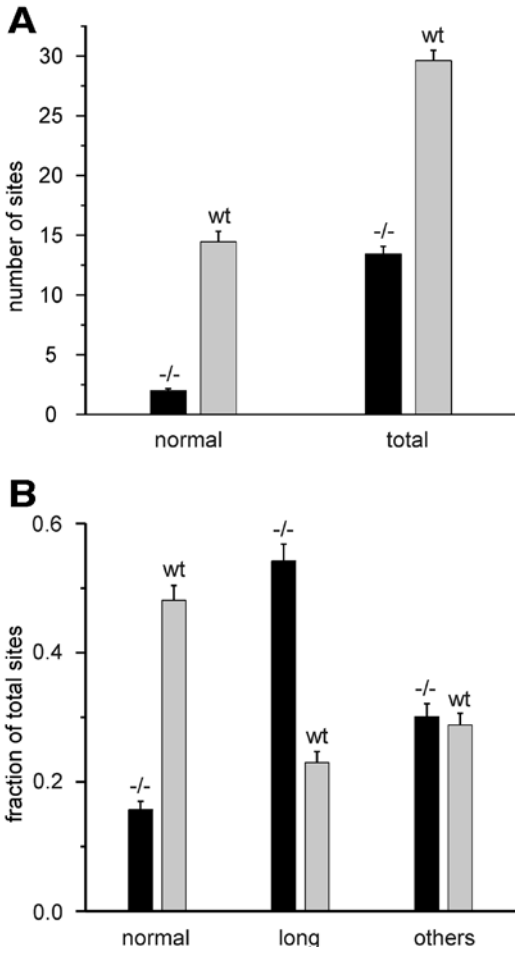


Fig. 3. Quantitation of nodal $Na^+$ channel clusters in wild type and contactin –/– mice at P15. (**A**) Number of sites per field of view in optic nerve cryosections. Sites categorized as normal were similar to the inset in Fig. 2A. (**B**) Immunocytochemistry data plotted as fraction of total sites. Bars show means +/– SEM. Between wild-type and –/–, $p < 0.001$ (2-tailed t-test) in all cases, except "others."

clear double line, and extending for >2 μm (*See* Fig. 2B, inset). Caspr immunofluorescence is present at paranodes in the wild-type nerve (*See* Fig. 2C), but no significant label was seen in the contactin-null mutant (*See* Fig. 2D). $Na^+$-channel clusters were quantitated by categorizing and counting sites in multiple fields of view in cryosections. In Fig. 3A we plot the number of sites per field of view, both as normal nodal clusters and as the total of normal plus abnormal zones. The number of normal nodes is dramatically reduced in the contactin-null mutant, and there are about one-half the number of total $Na^+$-channel clusters. In Fig. 3B the fraction of total sites in each category is plotted. Whereas 48% of clusters appeared as normal-looking nodes in +/+ axons, only 16% of –/– sites were normal. The fraction of long clusters in null mutants was about double that of wild-type mice. In all animals approx 30% of sites were short but misshapen, possibly due to distortions during sectioning.

Compound action potentials were elicited from optic nerves using suction electrodes for stimulation and recording. A total of eight nerves were tested, four from –/– mice and four from wild-type animals, all between P13–P15. Examples at P15 are shown in Fig. 4A. There was a consistent difference in the signals across all cases. Wild-type responses consisted of two clearly distinguishable peaks, while null mutant nerves had only one—with at most a very small shoulder at shorter times. Conduction was uniformly faster at 37°C (right column) than at room temperature (25 +/– 1°C). The amplitudes are somewhat arbitrary in these external records, and the primary information is in the shape and timing of the signals. Conduction velocities were calculated from the length of the nerve between the electrodes and the time-to-peak of the action potentials. These are plotted for the P15 nerves in Fig. 4B. The single peak in –/– nerves (black bars) clearly corresponded to the slower of the two peaks from wild-type littermates (gray bars). The fast component was virtually absent in the null mutant.

Thus, in the contactin-null mutant there are: (A) fewer nodes; (B) distorted morphology of the nodal $Na^+$-channel clusters that do exist; (C) absence of caspr1 clusters; and (D) slower conduction due to a loss of the faster peak in the compound action potential. Earlier studies demonstrated that contactin could enhance $Na^+$-channel surface expression through an association with the β1-subunit *(25).* Loss of

this mechanism in the contactin –/– animal could result in fewer nodes and slower conduction (A and D). Thus, the results on the null mutant support the idea that contactin plays a role in determining levels of expression of axonal $Na^+$ channels. Alternatively, could a defect in myelination that results in (B) and (C) above induce a reduction in number of nodes and slower velocities independent of the role of contactin in controlling $Na^+$-channel expression? The CGT and caspr1 –/– mice have distorted $Na^+$-channel cluster shape and loss of clustered caspr1. They also have a lowered conduction velocity, but there is no evidence for a reduction in frequency of nodes *(47–49,51).* Thus, disruption of paranodal myelin alone might be responsible for the alteration in nodal size and shape, but is not likely to also account for the significant reduction in numbers of nodes seen in the contactin –/– optic nerve.

Caspr1 is not localized at paranodes in the contactin –/– optic nerve, although it is synthesized in the CNS of these mice *(79).* Faivre-Sarrailh et al. *(80)* showed that contactin is required for caspr1 expression in the surface membrane in cell lines, and the absence of caspr1 clusters in the contactin-null mutant optic nerve is thus in vivo confirmation of this interaction. In the PNS of contactin-null mutants, caspr1 was likewise absent from paranodes, the transverse bands were missing, and the gap between paranodal loops and the axolemma was wider *(79).* As noted earlier, the CAP pattern (*see* Fig. 4A) showed not only a decreased conduction velocity, but more importantly a loss of the faster of two components, suggesting that a specific population of fast-conducting axons was not developing normally. While this could reflect defects in myelination, there is another possibility. Retinal ganglion cells have been classified in groups W, X, and Y by functional criteria, but they also tend to differ in size and axonal-conduction velocity. W cells are smallest and slowest, Y cells largest and fastest. In adult rat and mouse optic nerves, three components can be distinguished in the CAP *(46).* It is thus possible that

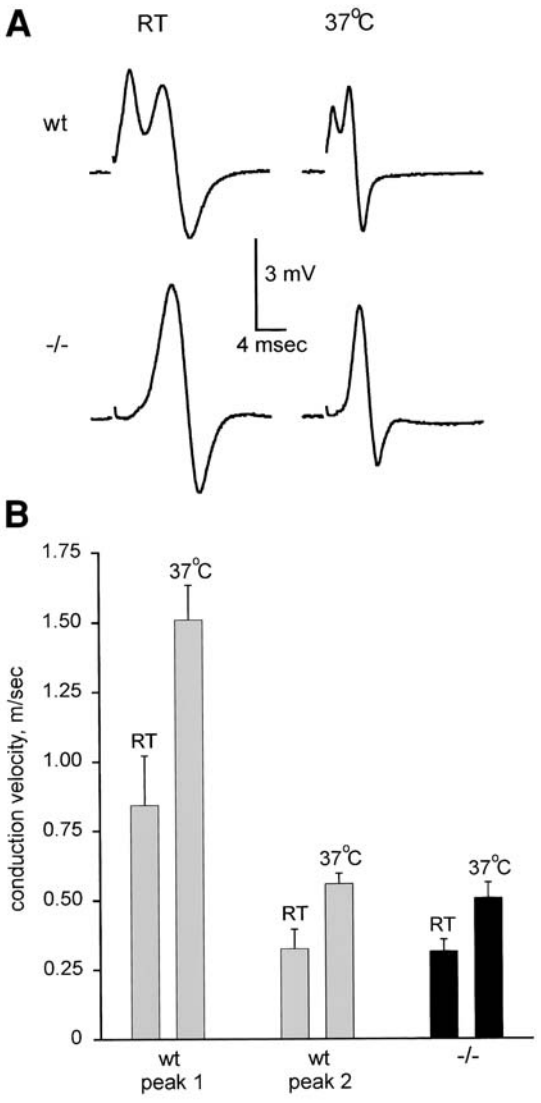


Fig. 4. Conduction in contactin-null mutant optic nerves. Compound action potentials (CAPs) and conduction velocities were measured by drawing ends of optic nerves into suction electrodes for stimulation, and recording as described *(46).* (**A**) CAPs in wild type (top) and contactin –/– (bottom) mice at P15, at both room temperature (RT, 24–26°C) and 37°C. (**B**) Conduction velocities at P15. Bars show means +/– SEM. Between –/– and peak 1 of wild type, $p<0.05$.

the absence of a fast component in the CAP reflects deficiencies in ganglion cell development. The reduction in number of nodes in contactin –/– optic nerves is also consistent

with this idea. Finally, since contactin is expressed by oligodendrocytes *(81)*, their number may be reduced in the contactin-null mutant also; this has not yet been investigated. Thus, while the reduced clustering of $Na^+$ channels that follows genetic deletion of contactin could reflect the role of this protein in directly modulating channel surface expression, it is not possible to rule out other, more indirect mechanisms.

## Cell Adhesion Molecules

Other cell adhesion molecules (CAMs) or CAM-binding partners in the nodal region also play important roles in axon development. Lambert et al. *(26)* studied developing myelinated axons at postnatal d 2 and found that NrCAM and neurofascin clustered in the nodal gap prior to ankyrin G, suggesting a role in initiating $Na^+$-channel clustering. This idea has gained support through experiments using an NrCAM-Fc fusion protein as a competitive blocking compound in the DRG-Schwann cell co-culture system *(82)*. This protein did not block myelination, but it more than doubled the number of nodal sites (tips of myelin basic protein positive processes) that lacked clusters of $Na^+$ channels. The authors suggest that NrCAM-Fc may act by blocking *cis* interactions of neurofascin with NrCAM or $Na^+$ channel β-subunits on the axon surface. Tenascin R, an extracellular matrix protein that accumulates at CNS nodes of Ranvier, binds to contactin *(63,83–85)*, and alters $Na^+$-channel function, but not expression. The conduction velocity of tenascin R null mutant mouse optic nerves is reduced to about 50% of the wild-type value, but a quantitative analysis of $Na^+$-channel immunofluorescence showed no difference in clustering at nodes *(30)*. Application of a recombinant amino-terminal domain of tenascin-R to *Xenopus* oocytes expressing $Na^+$ channels increased peak $Na^+$ current with no change in kinetics (*86*). Tenascin R may therefore interact with $Na^+$ channels to relieve resting inactivation, or to remove an endogenous blocking particle. Receptor protein tyrosine phosphatase-β (RPTPβ) is expressed by glia and binds tenascin, NrCAM, and contactin *(87–89)*. RPTPβ associates with and modulates $Na^+$ channels through control of phosphorylation *(90)*. RPTPβ is not included in Fig. 1 because its localization in the nodal region has not yet been determined. Node formation, $Na^+$-channel clustering, and axonal-conduction were all completely normal in an RPTPβ null mutant mouse *(91)*.

## Potassium Channels

While the role of nodal $Na^+$ channels in conduction is clear, the function of the juxtaparanodal $K^+$ channels has been more elusive. In normal adult PNS axons, the $K^+$-channel blocker 4-aminopyridine (4-AP) has virtually no effect on conduction. Since 4-AP is permeant, it is likely that it has access to the juxtaparanodal channels. Genetic deletion of Kv1.1 was initially found to have only modest effects on axonal conduction *(37)*, but a more thorough analysis revealed that close to the nerve terminal of motor neurons, this channel prevents backfiring *(36)*. As noted earlier, the $Na_v1.6$ channel is susceptible to re-entry excitation, and the juxtaparanodal $K^+$ channels may serve to damp this instability. At most nodes, the combination of passive cable and active ion-channel properties is sufficient to produce stable electrical properties independently of Kv1.1. However, as analyzed by Chiu et al. *(92)*, when the cable geometry changes abruptly (as happens close to the nerve terminal or at a branch point), the juxtaparanodal channels play a more crucial role. There are also two situations in which the Shaker-type $K^+$ channels are transiently present in the nodal gap of PNS axons. Early in development Kv1.1/Kv1.2 are nodal, and application of 4-AP induces repetitive firing, as measured in single fibers in the sciatic nerve *(38)*. Thus, these channels appear to stabilize conduction while the paranodal axo-glial junctions are

immature. During demyelination, the juxtaparanodal $K^+$ channels are dispersed, and early in remyelination they reappear first within the gap of new nodes. These axons are also highly sensitive to 4-AP *(93).* Interestingly, the situation is quite different in the CNS. Normal adult optic nerve compound action potentials are significantly widened by 4-AP. The Kv1.1 null mutant mice develop epileptic seizures, suggesting an important role in the brain, but not necessarily in myelinated axons *(37).*

There has been some progress in identifying molecular association in the juxtaparanode. Caspr2 colocalizes with $K^+$ channels in the juxtaparanode of optic nerve axons, both during development and in the adult *(39).* Kv1.2 or Kvβ2 reciprocally coimmunoprecipitate caspr2 from rat brain lysates, but this interaction is likely to be indirect since stable complexes could not be demonstrated in transfected cell lines. The C-terminal region of Kv1.1, Kv1.2, and caspr2 all have PDZ domain-binding sites, and these sites are important in the association of these proteins *(39).* Thus, it is likely that a PDZ domain-containing protein links $K^+$ channels and caspr2 in a complex at the juxtaparanode. In the CGT and contactin-null mutants, with axo-glial junctions absent, both caspr2 and $K^+$ channels are mislocated to the paranode *(50).* Following demyelination, nodal $Na^+$ channels remain clustered for at least 1 wk, while $K^+$ channels are rapidly dispersed *(40,93).* Thus, linkage of an ion channel to the cytoskeleton via ankyrin confers a much higher level of stability than does the PDZ domain-mediated complex.

## Summary and Conclusions

On a cellular level, there has been significant progress in understanding the events leading to the formation of nodes of Ranvier, including the establishment of a high density of $Na^+$ channels. There is strong evidence that within the PNS, the clustering of these channels is controlled by adherent Schwann cells following the differentiation of these cells to a myelinating phenotype. While early events in CNS axons may be initiated by as yet uncharacterized secreted factors, recent work now points to contact-dependent mechanisms for controlling the expression of the correct $Na^+$ channel subunits, and perhaps also the location of nodes along the axon. The key task now is to elucidate the molecular means of glial–axonal communication that is responsible for the formation and maintenance of nodal architecture. When glial cells become committed to myelination, their pattern of protein expression changes radically, with numerous constituents down-regulated, and many others synthesized at high levels, and trafficked to specific sites along glial processes. The timing and spatial organization of many of these latter components are suggestive of an important role in ion-channel organization, but there remains little in the way of direct evidence for this involvement. Progress has been more rapid on the axonal side. Many key surface and intracellular proteins have been identified, biochemical associations characterized, and functions tested by ligand competition or genetic deletion. The multiplicity of effects that is often seen on complete elimination of a candidate protein has rendered interpretation difficult in many cases. Better approaches may reside in more difficult genetic techniques involving control of temporal or regional expression patterns. The reward will be an understanding of the gateway through which much of the information in the nervous system must pass.

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