Development of the Nodes of Ranvier in Wild Type and Dysmyelinated Mice

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Myelin plays an important role in the saltatory conduction of action potentials in the nervous system. Myelin is composed of primarily lipids (~70%) and proteins (~30%) making it a good insulator for electrical current and the propagation of action potentials (APs) through the axons. The AP is propagated by saltatory conduction created by the influx/efflux of sodium and potassium ions. As the AP propagates along an axon, the strength of current decreases, but if the axon is myelinated the current can travel for a greater distance. In mammals, axons are ensheathed by myelin produced by oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system. Not all axons are myelinated; their myelination depends on their diameter and projection trajectory. In the CNS, myelinated fibers are primarily found in the white matter tracts of the corpus callosum, cerebellum and cranial nerves.

At regular intervals, there is space between the wrappings of oligodendroglial processes, exposing the axon in highly specialized regions known as the nodes of Ranvier. The nodes of Ranvier are divided into three regions: node (~1μm), paranode, juxtaparanode (~5-15μm). In order for AP propagation to occur properly, high densities of voltage-gated sodium (Nav) channels are found in the nodes and high densities of voltage-gated potassium (Kv) channels are found in the juxtaparanodes. The paranode does not contain voltage channels, and acts as a diffusion barrier between the node and juxtaparanode. Aside from Nav and Kv channels, several other molecules have been identified at these sites that affect node of Ranvier formation (Figure 1). In the CNS of the mouse, myelination starts a few days after birth. During postnatal development, nodal molecules appear in an ordered manner following myelination.

Dysmyelination has a drastic effect on the development of nodes of Ranvier. For example, the shiverer and jimpy murine models have been used to look at how genetic dysmyelination affects formation of nodes of Ranvier. Most studies to date used teased fibers of the optic nerve as the primary source due to its accessibility, but some studies have looked at the corpus callosum and cerebellum as well. This review will focus on the development of the nodes of Ranvier in the CNS of mice, and how myelin deficiencies result in their deformation.

Development of the node of Ranvier

Axoglial junction formation and clustering of sodium channels occur in the early stage of myelination.

The formation of axoglial junctions precedes the clustering of sodium channels, suggesting that the initial contact of oligodendrocytes with the axon is necessary for recruitment of nodal proteins. The axoglial junction is formed by the binding of the glial protein neurofascin-155 (nf-155) in a trans-formation to the axonal complex of Contactin-associated protein (Caspr) and Contactin. At post-natal day 6 (P6) in mice, nf-155 is primarily found in the cell body and major branches of the oligodendrocyte, and by P8 it is diffusely distributed along the processes. The formation of the axoglial junction is regulated by the expression of nf-155. When nf-155 is knocked out, Caspr and Contactin no longer localize to the paranode. As nf-155 starts to gather at the paranode, Caspr is detected at the edges of processes on P7 labeled with myelin-associated glycoprotein (MAG), an early myelin marker. The appearance of Caspr precedes Nav channels by about two days and rapidly increases with a rate similar to that of Nav channels. Contactin is found primarily in the paranode with some expression in the node, ~95% and ~5% respectively of total expression. There are two isoforms of Contactin that differ in molecular weight.
One isoform of Contactin is found in the paranode, and the other Contactin is found in the node. Presumably Contactin in the paranode starts to appear at the same time as Caspr as the two molecules are bound together in a cis-formation.

The recruitment of voltage-gated sodium channels to the nodes is preceded by other nodal proteins in addition to the formation of the axoglial junction. Neurofascin-186 is required for the localization of NrCAM, Ankyrin-3/G, and bIV-spectrin. When Neurofascin-186 is deleted, expression of these proteins remains the same, but does not localize to the node. Neurofascin-186 and NrCAM have intracellular binding domains for ankyrin-3/G, forming complexes that anchor Nav channels to the node.

Ankyrin acts as a bridge between membrane proteins, neurofascin-186, Nav channel, NrCAM, and the cytoskeletal protein βIV-Spectrin. Prior to myelination, ankyrin-B is detected diffusely along the entire length of the axon, and is replaced with ankyrin3/G after myelination. After myelination, ankyrin B is restricted to unmyelinated zones. Due to low incidence of neurofascin-186 and ankyrin-3/G detection early in development, it is difficult to determine which protein is present at the clusters first. Ankyrin-3/G is detected at P7, with about two-thirds colocalizing with Nav clusters at P9.

Through interactions in the axon with ankyrin-3/G, neurofascin-186, βIV-spectrin, and NrCAM, sodium channels are stabilized at the nodes. Voltage-gated sodium channels are first detectable in the mouse optic nerve at P9-10. At P9, about two-thirds of ankyrin-3/G clusters colocalized with βIV-spectrin, and the rest did not. The appearance of βIV-spectrin at the same time as Nav channels suggests that the ankyrin-G does not anchor the nodal proteins until the channels are bound. Between P12-P22, there is a rapid increase in the number of channels and clusters formed (Figure 2A). Nav channels are always adjacent to MAG processes, but they do not colocalize with MAG. During the rapid increase in Nav channel number, the nodes can be defined as broad, binary or focal clusters (Figure 3). During the period of increase, the change from broad (immature) to focal (mature) clusters marks the maturation of the node of Ranvier.

The developmentally increasing clustering of Nav channels coincides with the change in the ratio of Nav1.2 to Nav1.6 expression. As the amount of myelin increases, the level of Nav1.6 expression increases, since broad and binary nodes tend to be Nav1.2, and focal nodes are Nav1.6. Nav1.2 is detected on P9-10 at all early nodes with low expression of Nav1.6. Nav1.6 clusters at completion of myelination and whereas Nav1.2 clusters prior to completion of myelination. At P30, the end of myelination of the optic nerve, ~50% of nodes are still positive for Nav1.2, and ~90% are positive for Nav1.6. Figure 2: Quantification of the Replacement of Nav1.2 by Nav1.6 at Nodes of Ranvier in the Myelinating Optic Nerve. (A) The developmental time course of the number of PAN-stained, Nav1.2-positive, and Nav1.6-positive nodes per FOV [Field of View]. Images were taken from both proximal and distal portions of the optic nerve, and 3–5 FOVs were averaged for each developmental stage. Error bars represent ±1 SEM. (B) Proportion of PAN-labeled nodes that were positive for Nav1.2 and Nav1.6 at different ages. (C) Developmental change in the intensity of Nav1.2 and Nav1.6 immunofluorescence relative to the intensity of PAN immunostaining at the same node. Between 12 and 65 sites were measured for each age from Nav1.2/PAN and Nav1.6/PAN double-labeled images [15].
Caspr2 is a member of the neurexin superfamily that binds to and may be involved in the initial recruitment of Kv channels. As the number of axons that have compact myelin increases, myelin forms around P14 just as Kv channels start to localize. But the myelin is still loosely wrapped. Compaction of myelin is needed for the localization of Kv channels. At P10, there is an increase in the number of axons that are myelinated, but the myelin is still loosely wrapped. Compaction of myelin and clustering of potassium channels occurs at the juxtaparanode.18 Kv channels mainly consist of Kv1.1,24,28 Kv1.2 does not localize to the node by itself and is always found with Kv1.1, but Kv1.1 does not require Kv1.2 to be clustered at the juxtaparanode.27 The presence of myelin also has an effect on the clustering of Kv channels. In the unmyelinated region of retinal ganglion cell axons, there is a uniform distribution of Kv channels throughout the axon.25 In the myelinated region there is a discrete distribution to the juxtaparanode.15,18,19 Compact myelin is needed for the localization of Kv channels. At P10, there is an increase in the number of axons that are myelinated, but the myelin is still loosely wrapped. Compaction of the myelin starts around P14 just as Kv channels start to localize. As the number of axons that have compact myelin increases, the number of Kv channel clusters increases.23

Prior to Kv channel clustering, Caspr2 is detected by P12 and may be involved in the initial recruitment of Kv channels. Caspr2 is a member of the neurexin superfamily that binds to TAG-1, stabilizing the juxtaparanode (Figure 4C).24 TAG-1 is located on both the axonal and glial membranes.25 The axonal and glial TAG-1 bind to each other in a trans-formation, while the axonal TAG-1 also binds to Caspr2.26 Traka et al. (2003) and Poliak et al. (2003) showed that the localization of both proteins is dependent on the presence of the other. The Caspr2 knockout resulted in TAG-1 being absent from the juxtaparanode.27 Likewise, a TAG-1 knockout resulted in Caspr2 being redistributed to the internode.26,27

The presence of Caspr2 has a direct effect on the localization of Kv channels. Caspr coimmunoprecipitates with Kv1 channels24, but their interaction can be disrupted with most detergents.28 Poliak et al. (2003) created a Caspr2 knockout mouse demonstrating its effect on Kv channels and development of the juxtaparanode. Kv channels in the absence of Caspr2 are redistributed to the internodal region but do not move toward the paranode. Kv1.2 expression remained similar to that of wild-type expression even though it was not localized directly to the juxtaparanode. Caspr2 is bound to Kv channels through a protein with a PDZ domain.24,28

PSD-95 has three PDZ binding domains that connect it to Kv1 channels. It is not detectable at P14 before Kv channels cluster, but is detectable at some clusters by P17. At P17, there is some colocalization with Kv1.2 and Caspr2, which occurs at most sites by P22.28 Rasband et al. (2002) created a mouse with a truncated PSD-95 mutation that does not affect the expression and localization of Caspr2 or Kv1 channels to the juxtaparanode. Although it appears to have a structural purpose, PSD-95 does not affect the maintenance of Kv1 clusters to the juxtaparanode.

**Figure 4**: Effect of compact myelin on the formation of nodes of Ranvier. In the Corpus Callosum (A,B) and Cerebellum (C,D) of WT mice Caspr (green-A, red-C,D) and Nav1.6 (red-A, B) and Caspr2 (green-C, D) have distinct borders as they form the nodes. Whereas the shiverer has very little Nav1.6 as a result of the aberrant Caspr localization. Caspr2 in the shiverer also has a diffuse distribution along the axons. Scale bars 10 μm.

**Dysmyelinating diseases in the shiverer and jimpys mice result in abnormal formations of the node of Ranvier.**

Compaction of myelin has a major effect on the formation of the node of Ranvier. The *shiverer* is deficient in MBP expression, resulting from a 5 exon deletion in the gene.29 MBP is necessary for the compaction of myelin, forming major dense lines needed for proper axoglial interactions.30-32 Neurofascin-155 is maintained at high levels in the *shiverer*, but tends to be found more in the cell body than the processes.6 Caspr (Figure 4B, 4C) and neurofascin-155 colocalize at ectopic sites along the myelinated regions, but lack distinct boundaries.6,7

Due to the disruption in the axoglial junction in *shiverer*, the distribution of Nav and Kv channels are altered. The major difference in the Nav channel is the severe deficit of Nav1.6 channels. Nav channels in the *shiverer* are diffuse and irregular.7 In the axons of retinal ganglion cells, the sodium channels make the switch from Nav1.2 to Nav1.6, but only in the initial segment.15,35 Nav1.2 expression was increased in hypomyelinated regions, but distinction of broad nodes is difficult to make due to the uniform distribution along the axon.15 Although the *shiverer* is lacking in amount of Nav1.6, it is still found at a few focal sites where Caspr is found to form a paranode (Figure 4B).7 Expression of Kv1.1 and Kv1.2 are increased in cerebellar and subcortical white matter tracts.34 Like the Nav channels, Kv channels have a uniform and aberrant distribution resulting in localization in the node and paranodal regions in addition to the juxtaparanode.18,34 Caspr2 (Figure 4D) has a similar disruption in localization to that of the Kv channels. At sites where Caspr is localized, Kv channels cluster adjacent to each other,2,18 suggesting the formation of...
axoglial junctions may be enough to induce clustering of Kv channels.

Jimpy mutant mice have a mutation in the Plp gene resulting in the death of many of the oligodendrocytes. Due to the lack of oligodendrocytes, the CNS is severely hypomyelinated. Kv channel expression in the jimpy is upregulated with a distribution resembling that of the shiverer. Although a majority of the CNS has aberrant Kv channel distribution, the axons that do have compact myelin form correct channel clusters. Not many studies have used jimpy mice as a dysmyelination model due to their short life span, which is about 25-30 days.

There are many factors that have been identified in the formation of the node of Ranvier, but there are also several unknown factors that may have an additional role. Oligodendrocyte contact with the axon may have an initiating role in node formation as seen in the models of shiverer and jimpy. Although most of the data on the development of the nodes of Ranvier in the CNS comes from teased fibers of the optic nerve it will be important to also look at the developmental time line in other white matter tracts.

There have been several remyelination studies in the PNS, but very few studies of remyelination in the CNS as a way to examine the reformation of the nodes after injury or disease. Our lab is currently using a remyelination model using both fetal and adult human oligodendrocyte progenitor cells (OPCs). The shiverer is used to test the ability of the OPCs to migrate and differentiate into mature oligodendrocytes in the white matter tracts of the corpus callosum and cerebellum (unpublished data). Both adult and fetal cells are successful in maturing to oligodendrocytes after migrating through the tracts and as well as in forming functional axoglial junctions determined by colocalization of MBP and Caspr (unpublished data). We are further examining the ability of the human OPCs to fully reconstitute nodes of Ranvier.

References

27. Inoue, Y., et al., Fine structure of the central myelin sheath in the myelin deficient mutant Shiverer mouse, with special reference to the pattern of

About the Author

Steven Schanz will receive his B.S. in Neuroscience from the University of Rochester in May 2007. After graduation he plans to continue working in the Goldman lab.

My research is focused on using oligodendrocyte progenitor cells as a cell based therapy for treatment of congenital dysmyelinating diseases. We are using both fetal and adult cells from the ventricular zone, an area in the brain that continues to produce stem cells throughout adult life.

How did you become interested in this area of research? What motivated you to do this research?

I have had an interest in neuroscience for a long time before coming to UR. I did an internship at a research lab studying the effect of topography on the growth of neurons, but became more interested in the study of neural stem cells.

How does this research relate to your major/future plans/interests?

As a neuroscience major the research allows me to see the correlation between what we learn in class and how it applies to research currently taking place. It also shows that the glia cells in the brain play a large role in the functioning of the nervous system and it is not all about the neurons.

While doing this research project, what was your biggest obstacle and how did you overcome it?

The biggest obstacle for our project is bridging the gap between mouse and human cells. As in any xenograft, transplants from one species to another, there is a certain level of host rejection. By immunosuppressing or using immunocompromised shiverer mice we have been able to get the human cells to survive longer and prolong the animal’s life significantly.

After completing your project, what do you think was your most fulfilling experience?

In April I will be attending the National Conference on Undergraduate Research to present.

Any advice you can give to fellow undergraduates who would like to do this kind of research (or any other type of research)?

Get involved early on in undergraduate career. By starting early in sophomore year and working for the same lab through out your senior year you will be able to be more involved in the research. Starting early also makes it easier to do a senior thesis if one desires to.