Electron Tomography of Paranodal Septate-Like Junctions and the Associated Axonal and Glial Cytoskeletons in the Central Nervous System

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The polarized domains of myelinated axons are specifically organized to maximize the efficiency of saltatory conduction. The paranodal region is directly adjacent to the node of Ranvier and contains specialized septate-like junctions that provide adhesion between axons and glial cells and that constitute a lateral diffusion barrier for nodal components. To complement and extend earlier studies on the peripheral nervous system, electron tomography was used to image paranodal regions from the central nervous system (CNS). Our three-dimensional reconstructions revealed short filamentous linkers running directly from the septate-like junctions to neurofilaments, microfilaments, and organelles within the axon. The intercellular spacing between axons and glia was measured to be 7.4 ± 0.6 nm, over twice the value previously reported in the literature (2.5–3.0 nm). Averaging of individual junctions revealed a bifurcated structure in the intercellular space that is consistent with a dimeric complex of cell adhesion molecules composing the septate-like junction. Taken together, these findings provide new insight into the structural organization of CNS paranodes and suggest that, in addition to providing axo-glial adhesion, cytoskeletal linkage to the septate-like junctions may be required to maintain axonal domains and to regulate organelle transport in myelinated axons. © 2010 Wiley-Liss, Inc.

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Efficient propagation of action potentials along nerve fibers relies on axons from the central nervous system (CNS) and peripheral nervous system (PNS) being tightly wrapped with a specialized myelin sheath derived from oligodendrocytes and Schwann cells, respectively (Arroyo and Scherer, 2000). The multiple layers composed of lipid- and protein-rich membranes serve to insulate the axons from the extracellular environment and therefore increase the speed with which electrical impulses travel along the axon. Specifically, the myelin sheath is periodically interrupted at nodes of Ranvier, which produce action potentials that rapidly propagate to the next node by saltatory conduction.

Four distinct regions can be defined along the axonal membrane, each of which harbors a unique set of protein components: node, paranode, juxtaparanode, and internode (Arroyo and Scherer, 2000; Peles and Salzer, 2000; Salzer, 2003). Voltage-gated Na+ channels are restricted to the node and appear to be held there by the cytoskeletal scaffolding proteins ankyrinG and β-IV spectrin (Pan et al., 2006; Yang et al., 2007). In the axonemal, the cell-adhesion molecules (CAMs) neurofascin(NF)186 and NrCAM are also tethered to this ankyrinG/β-IV spectrin scaffold via their cytoplasmic tails and contribute to node formation (Dzhashiashvili et al., 2007).

The paranode flanks both sides of the node and is characterized by septate-like junctions between the axon and the myelinating glial cells (Bhat, 2003). These junctions serve to terminate the myelin sheath and are...
thought to be composed of a complex of three CAMs: Caspr (Paranodin) and contactin from the axolemma and NF155 from the glial cell membrane (Einheber et al., 1997; Menegoz et al., 1997; Charles et al., 2002). In addition to mediating intercellular adhesion and providing a diffusion barrier to ions (Rosenbluth, 2009), the septate-like junctions have recently been implicated in axonal transport and in maintaining the organization of the cytoskeleton. For example, ankyrinB, 4.1B, α-II spectrin, and actin are mislocalized in the Caspr knockout mouse, resulting in a swollen and disorganized cytoskeleton and axonal degeneration (Garcia-Fresco et al., 2006; Ogawa et al., 2006). Also, both these knockout mice and those carrying a β-IV spectrin mutation exhibit an abnormal accumulation of vesicles or mitochondria at the node (Lucas-Gervais et al., 2004; Yang et al., 2004; Einheber et al., 2006; Uemoto et al., 2007).

Ultrastructural studies of peripheral nerves have employed conventional and three-dimensional high-voltage electron microscopy. Early studies showed filaments that appear to attach to the septate-like junctions physically to the paranodal cytoskeletal network (Ichimura and Ellisman, 1991). More recently, electron tomography of peripheral nerves revealed narrow cross-bridges running from the junctions to axoplasmic neurofilaments and glial cytoskeletal filaments (Perkins et al., 2008). We have extended these studies to investigate the structure and cytoskeletal interactions of the septate-like junctions in the CNS. We have used electron tomography to explore the structure of paranodes in thin sections of the corpus callosum of adult mice. This imaging method combines numerous images of a given paranode at a systematic series of tilt angles to generate a three-dimensional volume. The internal details of the volume can be visualized without the superimposition that usually obscures fine cellular components such as cytoskeletal filaments and intercellular junctions (for review see Barcena and Koster, 2009). With such tomograms, we show for the first time that septate-like junctions in paranodes from myelinated, CNS nerve fibers are physically anchored to cytoskeletal elements in the axoplasm (neurofilaments, microfilaments, and microtubules) as well as to vesicles and mitochondria through short, filamentous linkers. We conclude that the association of paranodal septate-like junctions with the axonal cytoskeleton is likely to be important for strengthening the adhesion between axons and glia, for maintaining domain organization at the node of Ranvier, and for regulating organelle trafficking along the axon.

Samples were prepared for electron microscopy by high-pressure freezing and freeze substitution. To start, 1-mm-diameter pieces of the corpus callosum were cut out of the fixed vibratome slices using a tissue punch. This tissue was placed between aluminum specimen carriers, and the 100-μm-thick space was filled with 20% bovine serum albumin. Samples were rapidly frozen with a high-pressure freezer (HPM 101; Bal-tec AG, Liechtenstein) and stored under liquid nitrogen. For freeze substitution, specimen carriers containing frozen tissue were placed into anhydrous acetone containing 2% OsO4 and 0.1% uranyl acetate at –90°C for 24 hr. By using an automated freeze substitution machine (EM AFS; Leica Microsystems), the samples were slowly warmed (5°C/hr) to –60°C and incubated for 12 hr, then to –30°C for an additional 12 hr, to 0°C for 4 hr, and finally to room temperature for 1 hr. Samples were rinsed in acetone and incubated in 70% acetone–30% LX112 epon resin (Ladd Research Industries) for 30 min, followed by 50% acetone–50% epon for 1 hr and 30% acetone–70% epon for 1 hr. They were then incubated twice in 100% epon, first for 2 hr and then overnight before being polymerized at 60°C.

Thin sections (70 nm; Ultruct UCT microtome; Leica Microsystems) of longitudinally oriented myelinated axons were collected on 200 mesh hexagonal copper grids (Ted Pella, Redding, CA) with a collodion support film (Electron Microscopy Sciences, Fort Washington, PA). The sections were counterstained by incubation with 3% uranyl acetate in 30% methanol for 10 min, followed by washing in water, air drying, and incubation with Sato lead stain for 2 min (Sato, 1968). A thin layer of carbon was evaporated on top of the sections to minimize beam-induced specimen shrinkage (Auto306 Vacuum Evaporator; Edwards BOC).

**Collection and Analysis of Tomograms**

Samples were imaged with an electron microscope (CM200; Philips Electron Optics) equipped with a 1,024 × 1,024 CCD camera (MSC 1000; Gatan) at 27,500 nominal magnification (corresponding to a 0.66-nm pixel size) and 1 μm defocus. Dual-axis tilt series were collected with a high-tilt tomography holder (model 916; Gatan) using a tilt range of ±70° and 2° increment (EM-Menu 3.0; TVIPS). To collect the tilt series about the second axis, the sample was removed from the microscope, rotated ~90°, and reinserted. Fiducial-free alignment of projection images and calculation of 3-D volumes was carried out using Protoomo software (Winkler and Taylor, 2006) and creation of dual-axis reconstructions was done in IMOD (Kremer et al., 1996; Mastronarde, 1997). Reconstructions were manually segmented in Amira (Mercury Computer Systems, San Diego, CA) after applying a $3 \times 3 \times 3$ median filter. The intercellular cleft spacing and dimensions of septate-like junctions were measured in ImageJ (National Institutes of Health, Bethesda, Maryland, http://rsb.info.nih.gov/ij/) by drawing a rectangle or square at well-preserved areas of the membrane, integrating the enclosed region, and calculating the average width. Averaging of septate-like junctions was done by selecting 100 individual junctions from eight dual-axis reconstructions. The junctions were selected from reconstructions that origi-
nated from the best-preserved tissue and were harvested from a single animal. The junctions were contained in subvolumes with dimensions of 40 x 40 x 80 pixels and were aligned and averaged using PEET (particle estimation for electron tomography; Nicastro et al., 2006). The subvolumes were subjected to iterative cycles of rotational and translational alignment in three dimensions; the initial reference was selected from the original group of subvolumes and was replaced with the global average of 67 subvolumes with the highest cross-correlation coefficient after each round of refinement. After seven rounds of refinement, 80 subvolumes with the highest cross-correlation values were averaged to produce the final volume.

RESULTS

Axonal and Glial Cytoskeletons of the CNS Associate With the Paranodal Septate-Like Junctions

To investigate the ultrastructure of the paranodal septate-like junctions and their associations with the cytoskeleton, we conducted electron tomography on thin sections (70 nm) of myelinated axons from the corpus callosum of adult wild-type mice. The corpus callosum was selected as the tissue source because coronal vibratome slices of the brain yields a parallel arrangement of myelinated axons that aided in finding paranodal regions cut in longitudinal orientation. To ensure optimal specimen preservation and to minimize the shrinkage and extraction that are associated with conventional preparation methods, we used high-pressure freezing and freeze substitution to preserve the tissue. These procedures resulted in smooth, continuous membranes and dense cytoplasm, indicating excellent preservation and lack of extraction. As expected, longitudinal sections through myelinated axons reveal loops projecting from the myelin sheath that wrap around the paranodal region of the axons (Fig. 1A). In these regions, evenly spaced, transverse bands are seen connecting the glial membrane to the axolemma, which have been previously identified as the septate-like junctions (Laatsch and Cowan, 1966; Fig. 1B,C).

We collected 20 dual-axis tilt series of the paranodal region of myelinated axons from a total of seven thin sections containing comparable features and generated a three-dimensional tomographic reconstruction for each series. Examination of 0.66-nm-thick slices from these reconstructions revealed thin filaments that linked the septate-like junctions to various cytoplasmic components within the axons and glial cells. These thin filamentous linkers were indistinct in projection images because of superimposition of material throughout the 70-nm thickness of the section (e.g., compare Fig. 1C with 2A). In the tomographic sections, these linkers (light blue in Figs. 2, 3) can also be seen attaching to 10-nm filaments (yellow in Fig. 2), to 5-nm filaments (orange in Figs. 2, 3), and to 25-nm filaments (green in Figs. 2, 3), which are consistent with the size and shape of neurofilaments, microfilaments, and microtubules, respectively. Thus, these linkers appear to be part of an extensive, cytoskeletal meshwork that connects neurofilaments, microfilaments, and microtubules to one another as well as to the transverse bands of the septate-like junctions (Fig. 2B,G; Supp. Info. Movies 1 and 2).

Portions of the glial cell cytoskeleton also associate with the transverse bands, although the network of connections is less extensive than in the axoplasm. In the particular cell shown in Figure 2, these glial cell densities appear to link the transverse bands to an invaginated region of the glial membrane (Fig. 2E,H).

Paranodal Septate-Like Junctions Associate With Membranous Organelles in the Axoplasm

We observed various membranous organelles within the paranodal region of the axon. Specifically, organelles shown in Figure 4A,D,G are in close proximity to the axolemma and, based on their appearance, are consistent with transport vesicles, endoplasmic reticulum, vacuoles, and/or prelysosomal organelles. These organelles adopt circular and oval shapes, range in size from 30 to 100 nm in diameter, and are occasionally connected to the septate-like junctions via short filaments (Fig. 4G–I; Supp. Info. Movie 3). In addition, mitochondria with an electron-dense matrix were in close proximity to the axolemma and septate-like junc-

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Electron tomography of the paranode reveals distinct cytoskeletal linkers running from the dense septate-like junctions to the axon and glial cell cytoskeleton. A: A 0.66-nm-thick tomographic slice from a reconstruction of one paranodal loop and the associated axon. Filamentous connections running between the paranodal septate-like junctions and the axon cytoskeleton are evident in the reconstruction (black arrows). Connections between the junctions and glial cytoplasm are also visible (white arrowheads). A neurofilament (NF) and two microtubules (MT) are components of the axoplasm. B: Surface rendering of the reconstruction depicted in A. The glial membrane (red) invaginates into the glial cytoplasm (magenta). Thin and somewhat regularly spaced connections (light blue) bridge the septate-like junctions (purple) to a 10-nm neurofilament (yellow) in the axoplasm. C–H: Close-up views of A,B that show linkages between septate-like junctions and cytoskeletal network in the axon. E,H: Close-up views of A,B that show a linkage between the junctions and an invagination in the glial cell membrane. Scale bars = 100 nm in A,B; 25 nm in C–H. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Architecture and Spatial Arrangement of the Paranodal Septate-Like Junctions**

To characterize the septate-like junctions that connect axons and glial cells at the paranode, we measured the center-to-center spacing and width of the transverse bands as well as the width of the intercellular cleft between the cells (Fig. 5). We found the transverse bands to be regularly arranged with an average center-to-center spacing of 31.0 ± 2.2 nm and an average width of 16.1 ± 3.0 nm. Notably, the average intercellular distance (measured between outer leaflets of the lipid bilayer) was 7.4 ± 0.6 nm.

To evaluate the 3D shape of individual septae, the 3-D volume was viewed from a direction normal to the membrane plane (compare Fig. 6A and B; Supp. Info. Movie 5). These images indicate that individual septae form a continuous belt running around the perimeter of the axon. To illustrate that the continuity of the septae normal to the plane of the section was not an artifact resulting from the limited number of views included in the tomogram (the so-called missing pyramid), Figure 6C shows a comparable view of a vesicle within the axoplasm. The discrete border of this vesicle verifies that the dual-axis data collection scheme is able to resolve discrete features normal to the section plane.
Arrangement of Cell Adhesion Molecules in the Intermembrane Space

To elucidate the arrangement of the adhesion molecules within an individual septum, we calculated an averaged volume by extracting 100 subvolumes from the tomograms (each containing a single transverse band; Fig. 7A) and averaging them together after conducting rotational and translational alignment in three dimensions. As shown in Figure 7B, a central slice through the averaged volume reveals two major densities spanning the intercellular cleft (Supp. Info. Movie 6). Density 1 continues past the plasma membrane and into the cytoplasmic side of the axon, whereas density 2 terminates at the extracellular face of the axolemma. Septate-like junctions (purple), microfilaments (orange), and vesicles (dark blue) are depicted in the surface rendering. D: A 0.66-nm-thick tomographic slice depicting many microtubules running tangentially through the reconstruction. The microtubule closest to the axolemma associates with the septate-like junctions through thin cytoplasmic linkers. E,F: Surface renderings of the volumes depicted in D. Glial and axonal membranes (red), septate-like junctions (purple), microtubules (green), neurofilaments (yellow), and vesicles (dark blue) are depicted in the surface reconstruction. Scale bars = 150 nm in A,B; 75 nm in C,F; 100 nm in D,E. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

Understanding the physical associations between the septate-like adhesive complex at the paranode and the underlying cytoskeletal network continues to be the focus of ongoing research (for review see Susuki and Rasband, 2008). In this study, we have used electron tomography to characterize the structure of paranodes from the CNS for the first time. This technique enhances the visibility of intracellular structures, and we were thus able to show that the paranodal regions of myelinated axons are highly enriched in various cytoskeletal filaments and membranous organelles. Specifically, we observed short linker filaments connecting the regularly spaced septae of the paranodal junctions to microtubules, neurofilaments, microfilaments, and membranous organelles.

Role and Composition of Filamentous Linkers

Our observations generally support previous studies of sciatic nerve and spinal root axons from the PNS (Perkins et al., 2008). However, we observed some interesting differences between the systems. For the first time, we have revealed that, in the CNS, membranous organelles such as mitochondria, transport vesicles, and endoplasmic reticulum are tethered to the septate-like junctions through short filaments. In the PNS, linkers have only been described between cytoskeletal filaments and the junctions and are generally shorter, straighter, and more homogeneous in appearance (Perkins et al., 2008). This suggests that filaments in the CNS are more flexible and that the septate-like junctions could play a role in regulating transport of vesicular cargoes along the
axon. In particular, tethering via the short filaments could serve either to sequester specific membrane-protein components to the paranode or to guide these components through the dense cytoskeletal network within the narrower nerves of the CNS.

Spectrin is a logical candidate for the filamentous linkers observed in this study. Spectrin is a submembranous cytoskeletal scaffolding protein that is essential for membrane stability in many types of cells (for review see Bennett and Baines, 2001). Spectrins are thin, flexible molecules composed of antiparallel α and β chains that laterally associate to form heterodimers, which in turn assemble end-to-end to form tetramers (Bennett and Baines, 2001). There is an abundance of spectrin in the axon, with ~80% located in the central axoplasm, and spectrin has been shown to associate physically with microtubules, neurofilaments, microfilaments, mitochondria, and endoplasmic reticulum (Glenney et al., 1982; Lasek et al., 1984; Zagon et al., 1986). Immunofluorescence has shown colocalization of spectrin with its binding partners ankyrinB and band 4.1 specifically at the paranodal region (Ogawa et al., 2006), and immunoprecipitation suggests formation of a complex between actin, band 4.1, and spectrin (Garcia-Fresco et al., 2006).

A specific association between spectrin and the septate-like junctions is consistent with evidence for 4.1B binding to the cytoplasmic tail of Caspr (Gollan et al., 2002; Denisenko–Nehrbass et al., 2003). Spectrin has distinct binding sites for band 4.1 and for actin (An et al., 2005) and could thus facilitate anchorage of these junctions to the actin cytoskeleton. The importance of 4.1B to the paranode was demonstrated by a recent study of mice lacking the 4.1B binding sequence in the cytoplasmic tail of Caspr, which failed to segregate juxtaparanodal K⁺ channels properly from the paranode. This result supports the idea that linking septate-like junctions to the axonal cytoskeleton is necessary for maintaining the domain organization of myelinated axons (Horresh et al., 2010). The precise supramolecular organization of these elements at the node of Ranvier will require further structural studies at higher resolution.

Fig. 4. Septate-like junctions physically associate with membranous organelles in the axoplasm such as transport vesicles, smooth ER, and mitochondria. A,D,G,J: Gallery of 0.66-nm-thick tomographic slices of various vesicular organelles at the paranode. B,C,E,F,H,I, K,L: Corresponding surface renderings from the tomographic reconstructions. Vesicular structures vary in shape and size ranging from elongated shapes to circular and from 30 to 100 nm in diameter. G-I: Cytoskeletal linkages (light blue) bridge the septate-like junctions (purple) to a vesicle (dark blue; arrows). Glial and axonal membranes, along with any sublemmal cytoplasmic densities, are depicted in red. J-L: An elongated, dense mitochondrion is present, with lipid bilayers of the double membrane and cristae visible (dark blue). Diffuse, axoplasmic material links the mitochondrion to the axolemma (arrow). Scale bars = 120 nm in A,B,D,E,G,H,J,K. 60 nm in C,F,I,L. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Axonal Transport

Anterograde and retrograde transport of organelles along the microtubule network is an important and well-studied phenomenon, which is necessitated by the large distances traversed by axons (Brown et al., 2005). Mitochondria display bidirectional movement with long pauses, thus giving rise to a wide range of average transport rates (Ligon and Steward, 2000). This variable transport rate may reflect targeted delivery of mitochondria to regions of high metabolic demand (Hollenbeck and Saxton, 2005). In keeping with this idea, the electron-dense mitochondrial matrix seen in many of our tomographic volumes may indicate an increased level of respiration near the paranode along with a higher ATP content (Hackenbrock, 1966; Perkins and Ellisman, 2010). The diffuse densities bridging the septate-like junctions to mitochondria may represent the static tethers that have been hypothesized to tether mitochondria at the node (Hollenbeck and Saxton, 2005) and that have been also described in specialized presynaptic region of the calyx of Held (Rowland et al., 2000).

More discrete filamentous linkers were associated with endoplasmic reticulum and transport vesicles, and the idea that they are composed of spectrin is consistent with its transport kinetics. Specifically, spectrin is the only protein transported with both major subcomponents of slow axonal transport (SCa and SCb; Lasek et al., 1984), thus exhibiting transport kinetics similar to microfilaments, neurofilaments, and microtubules (0.2–8.0 mm/day). In addition, a small amount of spectrin...
also associates with vesicular organelles and exhibits the much faster retrograde and anterograde transport kinetics of these cytoplasmic elements (50–100 nm/day; Lasek et al., 1984; Levine and Willard, 1981). Thus, the size, shape, ubiquitous nature, and transport properties of spectrin are all consistent with our observations of filamentous linkers attached to a variety of organelles and cytoskeletal elements at the paranode.

Spatial Arrangement and Architecture of the Septate-Like Junctions

The average center-to-center spacing and width of the septate-like junctions of our samples were 31.0 ± 2.2 nm and 16.1 ± 3.0 nm, respectively. These measurements agree well with previously published values of 30–40 nm (Laatsch and Cowan, 1966) and 25–30 nm (Livingston et al., 1973; Schnapp et al., 1976) for the center-to-center distance and 10–15 nm (Elvín, 1961; Laatsch and Cowan, 1966; Hirano and Demberter, 1967), 14–19 nm (Dermietzel, 1974), and 15 nm (Perkins et al., 2008) for the width. The mechanism for maintaining the regular spacing of these junctions is currently unknown, but it may depend on physical restraints imposed by the cytoskeletal linkers described above. The average intercellular distance between axons and the apposing glial cell was measured to be 7.4 ± 0.6 nm, which is more than twice the value previously reported in the literature (2.5–3.0 nm; Livingston et al., 1973; Dermietzel, 1974; Schnapp et al., 1976). This difference may reflect better sample preservation with the high-pressure freezing and freeze substitution methods used in this study, which generally minimize membrane collapse (McDonald and Auer, 2006). Furthermore, measurements from tomographic slices are less susceptible to artifacts caused by an oblique angle of sectioning, which will reduce the apparent intercellular distance measured in the projection images used for the above-referenced studies. Interestingly, our measurement of the spacing is about half of the intercellular spacing of invertebrate septate junctions (15–17 nm; Lane and Swales, 1982; Tepass et al., 2001).

We observed evenly spaced belts of density when the paranodal loops were viewed from a direction normal to the plane of the membrane (Fig. 6B). This observation is consistent with previous studies showing continuous rows of cell adhesion molecules at the paranode (Livingston et al., 1973; Dermietzel, 1974; Rosenbluth, 1976; Schnapp et al., 1976; Wiley and Ellisman, 1980; Hirano and Demberter, 1982). This organization may be optimal for creating a strong intercellular adhesion as well as a physical barrier for diffusion of membrane-bound components within the axolemma. Averaging of tomographic subvolumes revealed an asymmetric, bifurcated structure for the individual septum that composes the septate-like junction. A prominent sublemmal axoplasmic density may represent cytoplasmic domains.

Fig. 7. Averaged subvolume of the transverse bands reveals bifurcated densities in the intermembrane space. A: Tomographic slice from a septate-like junction used for alignment and averaging. Densities from individual subvolumes have a high noise level. B: Central 0.66-nm slice through the averaged volume of the septate-like junction reveals a strong density 1 that continues into the axoplasm and a weaker density 2 at left. C (left): Projection across the entire averaged tomographic volume. C (right): Isosurface-rendering of the averaged tomographic volume. Scale bars = 100 nm in A, left; 7 nm in A, right, B; 8 nm in C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
of transmembrane proteins or may represent axoplasmic proteins associated with the transverse bands (e.g., 4.1B). It is possible that filamentous linkers attach to this axoplasmic density, limiting the flexibility of one arm of the complex and thus producing a stronger density on one side of the individual septum. The two resolved arms of the septum suggest a dimer of adhesion molecules, which is also consistent with the distribution of densities on the axolemmal side of the membrane in freeze-fracture studies (Wiley and Ellisman, 1980). Plausible elements for this dimer are the Caspr/contactin complex and NF155, although higher resolution or specific labeling of these components will be necessary to confirm these assignments. In the meantime, this tentative assignment suggests that the width of each transverse band is determined by the dimensions of these adhesion molecules and that these complexes associate to form a linear array that runs circumferentially around the axon. The narrow distance between the cell membranes (7.4 nm) suggests that these relatively long, multidomain proteins are folded up in the extracellular space, in contrast to the extended structure adopted by the cadherins in adhesive junctions (He et al., 2003).

Other major classes of adhesive junctions, such as desmosomes and adherens junctions, derive their mechanical strength from coupling the relevant transmembrane proteins to the cytoskeleton via a dense protein scaffold at the junctional level (Getios et al., 2004). Although axons are not required to resist high levels of shear force, it may be desirable to anchor the membrane to the cytoskeletal elements in order to maintain, for example, the width of the node and the distance between nodes along the axon. Indeed, knockout of Caspr or glial ceramide galactosyltransferase leads to dramatic disruption of the cytoskeleton at the node and consequent swelling of the axons in this region (Einheber et al., 2006; Garcia-Fresco et al., 2006).

**CONCLUSIONS**

The observations presented in this report provide new insights into the ultrastructure of neuronal cells from the CNS and suggest that the formation of the septate-like junctions to the cytoskeleton is important for the maintenance of cell–cell adhesion, conduction of organellar transport, and organization of the paranode. These observations may be of particular clinical importance for human demyelinating diseases such as multiple sclerosis (MS); MS is characterized by inflammation of the brain, destruction of myelin and oligodendrocytes, and axonal degeneration (Compston and Coles, 2002), and it is notable that Caspr and NF155 are mislocalized near these lesions (Wolswijk and Balesar, 2003; Howell et al., 2006). Thus, the stability of the membrane-cytoskeletal linkage at the paranode may be an early determinant of axonal integrity by being responsible for the initial organization and segregation of the various domains composing the node of Ranvier.

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