Organization of Ion Channels in the Myelinated Nerve Fiber

Stephen G. Waxman and J. Murdoch Ritchie

Summary. The functional organization of the mammalian myelinated nerve fiber is complex and elegant. In contrast to nonmyelinated axons, whose membranes have a relatively uniform structure, the mammalian myelinated axon exhibits a high degree of regional specialization that extends to the location of voltage-dependent ion channels within the axon membrane. Sodium and potassium channels are segregated into complementary membrane domains, with a distribution reflecting that of the overlying Schwann or glial cells. This complexity of organization has important implications for physiology and pathophysiology, particularly with respect to the development of myelinated fibers.

Despite the crucial role of the myelinated nerve fiber the nervous system, its functional organization is only now being delineated. Nonmyelinated axons have been regarded as having a uniform membrane structure that underlies a continuous mode of impulse conduction. In contrast, it has become clear that the myelinated fiber has a complex structure, in which there is concomitant differentiation of the myelin-forming cell, the myelin sheath, and the axon itself. In this article, we review recent data on the distribution of the voltage-sensitive sodium channel, and of the voltage-sensitive potassium channel, within mammalian myelinated fibers. These two types of channels, in fact, exhibit complementary distributions within the membrane of the myelinated fiber in mammals. The organization of the mammalian myelinated fiber has important implications not only for normal neural function, but also for the pathophysiology of demyelinating diseases and other disorders of myelinated fibers.

The myelin sheath, which originates from the Schwann cell in the peripheral nervous system (PNS) (*1*) and the oligodendrocyte in the central nervous system (CNS) (*2*), consists of a compacted spiral of glial membrane surrounding the axon. This anatomical arrangement provides for a high transverse resistance and low capacitance (*3, 4*). Myelinated fibers are periodically punctuated by nodes of Ranvier, sites at which the myelin is interrupted. The nodes usually extend for approximately 1 μm along the fiber axis. The axon membrane itself (also termed the axolemma) is highly differentiated at the nodes of Ranvier, exhibiting different properties in that region compared to other sites along the fiber.

Tasaki (*5*) and Huxley and Stämpfli (*6*) provided early physiological demonstrations of the saltatory nature of conduction in myelinated fibers and showed that excitability was localized to the region of nodes of Ranvier. Observations in the internode (the region between consecutive nodes of Ranvier) indicated that the membrane currents there were passive, with the internode acting as a set of resistances and capacitances in parallel. On the basis of these and subsequent findings, computer simulations of the action potential in myelinated fibers can now be carried out; these simulations compare well with data obtained under various experimental conditions (*7*). While there is less evidence concerning the mode of conduction in central myelinated fibers, its saltatory nature has been demonstrated (*4, 8*).

Structure of the Axon Membrane

The saltatory mode of conduction in myelinated fibers raises an important question with respect to the structure of the axon itself; namely, the distribution of ion channels along the myelinated axon. The demonstration of saltatory conduction, while indicating that the nodal axon membrane must exhibit the capability for action potential electrogenesis, provides little information about the intrinsic properties of the internodal axon membrane under the myelin sheath. This internodal axolemma could, in principle, contain voltage-sensitive sodium channels, which are not activated during impulse conduction because the transmembrane potential is attenuated by the overlying myelin. Alternatively, the internodal membrane could lack sodium channels. In the case of normally myelinated fibers, this would not result in inexcitability, since most of the action current is shunted from one active node to the next with relatively little capacitative loss through the myelinated internode.

Voltage-Sensitive Sodium Channels

Early experiments to examine the functional organization of the internodal axon membrane depended on a modification of the longitudinal current analysis technique (*6*) and examined conduction in single ventral root fibers demyelinated with diphtheria toxin (*9*). Although the resolution of the technique was not sufficient in this early study to determine unequivocally whether the internode exhibited excitability, these experiments were important since they focused attention on the axon membrane.

Morphological and physiological studies have shown that the axon membrane in myelinated fibers exhibits a complex organization with respect to ion channels. Cytochemical investigations reveal distinct structural differences between nodal and internodal axolemma (*10*). The axon membrane at the node of Ranvier is structurally similar to the membrane of the axon initial segment, which is a site of high sodium channel density (*11, 12*). Studies in model systems such as the electrocyte axons in the gymnotid *Sternarchus*, which contain both excitable and inexcitable nodes (*13*), demonstrate a correlation between cytochemical properties and the capability for normal nodal action potential electrogenesis (*12*). The results suggest a high density of sodium channels at the nodal membrane. Staining with monoclonal antibodies to sodium channels also suggests a high channel density at the node (*14*).

Studies with tritium-labeled saxitoxin have provided important information about the localization of sodium chan-

Stephen G. Waxman is professor and associate chairman of the Department of Neurology at Stanford University School of Medicine, and director of the Neurological Unit, Veterans Administration Medical Center, Palo Alto, California 94304. J. Murdoch Ritchie is Eugene Higgins Professor of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510.

nels in nodal and internodal membrane. This approach, developed by Ritchie and his colleagues (*15, 16*), suggests a sodium channel density of about 10^4 per square micrometer in the axon membrane at the nodes of Ranvier. In contrast, these studies indicate a sodium channel density of $<25/\mu m^2$ in the internodal axon membrane under the myelin sheath in normal fibers; this density is so low that the membrane is essentially inexcitable (*16*). Similar studies on mammalian nonmyelinated axons (C fibers) (*15, 17*) suggest that if the channel distribution is uniform, the sodium channel density is 100 to $200/\mu m^2$—a value close to that predicted to maximize conduction velocity in nonmyelinated axons (*18*).

The freeze-fracture technique provides a quantitative morphological probe of membrane structure and demonstrates structural differences between nodal axon membrane and paranodal or internodal membrane (*19–21*). In axons examined by freeze-fracture, the external membrane leaflet (E face) at the node of Ranvier differs from that of most other biological membranes. The nodal E face contains a high density ($\sim 1300/\mu m^2$) of intramembranous particles, which are larger than those located in other parts of the axolemma (Fig. 1). It has been suggested that the nodal E-face particles may be related to sodium channels (*19, 20, 21*), although a one-to-one correlation between E-face particles and sodium channels has not yet been made. The internodal-paranodal membrane contains, in most regions, many fewer (100 to 200 μm^2) particles than found in the nodal membrane. Some small collections of large E-face particles, bounded by adjacent terminating glial loops, are present in the paranode; these particles may represent "ectopic" sodium channels (*19*). If such ectopic channels are present, their discharge through the paranodal capacitance and resistance could account for delayed depolarization and repetitive firing at some nodes (*23–25*). Voltage clamp studies have not, however, demonstrated a paranodal contribution to inward sodium currents after sudden loosening of the myelin (*23*).

Voltage clamp studies (*23, 24*) also differentiate between nodal and internodal membrane, with sodium currents being limited to the former (Fig. 2, A and B). Gating current measurements (*26, 27*) in rabbit nodes suggest a maximum of 82,000 sodium channels per node (corresponding to a channel density of $\sim 1500/\mu m^2$). Current fluctuation analysis demonstrates a means of 21,000 sodium channels at nodes in rat sciatic nerve (*28*). While these methods yield values that are not quantitatively identical, it is clear that sodium channel density in the nodal membrane is at least $10^3/\mu m^2$, a value is much higher than for most other excitable membranes (*24*). This conclusion is consistent with the morphological results. The picture that emerges is one of a highly nonuniform distribution of sodium channels in normal myelinated fibers, with a high density of channels at the nodes of Ranvier (where they are required for saltatory conduction), and with a very low density (probably too low to support action potential electrogenesis) in the internodal axon membrane under the myelin sheath.

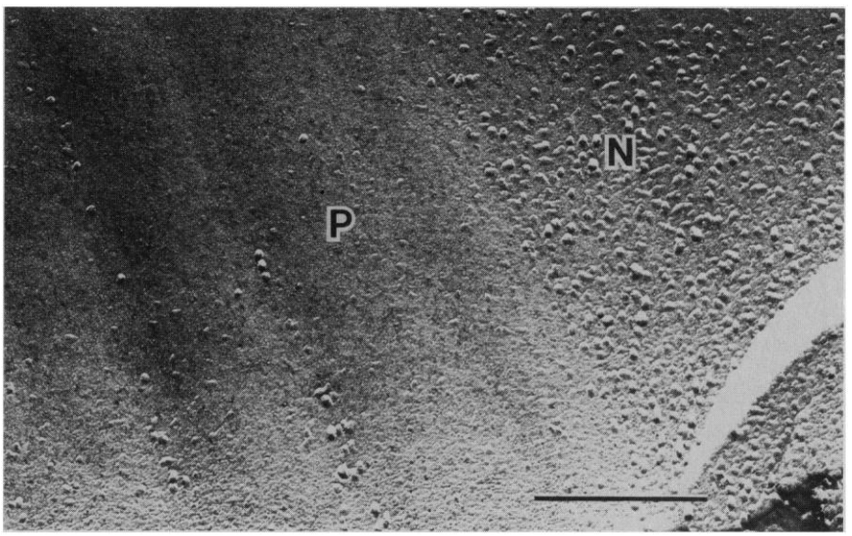

Fig. 1. Freeze-fracture electron micrograph showing the external leaflet (E-face) of the axolemma from a myelinated fiber in rat optic nerve. The high density of intramembranous particles in the axolemma at the node (N) can be compared to that at the paranode (P) (scale, 0.25 μm).

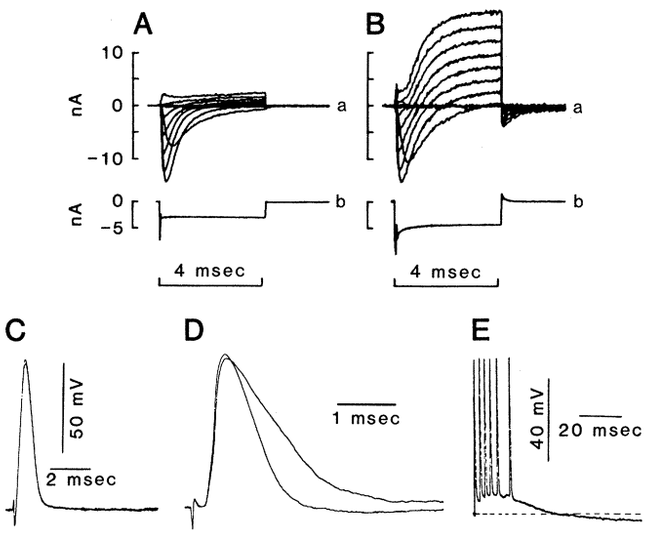

Fig. 2. Ionic currents in a single rabbit node of Ranvier. The upper records (a) show the ionic currents (with capacity transient and leakage currents subtracted) in response to a series of depolarizing pulses in the normal node (A), and after acute treatment to produce paranodal demyelination (B). The lower records (b) show the capacity transient and leakage currents in response to a small hyperpolarizing pulse. In (B) the appearance of the large outward current is associated with a large increase in the area under the capacity transient (that is, with a large increase in electrical capacity) but no, or relatively little, increase in the sodium or leakage currents. The "off" capacity transient is distorted because of a change in sampling rate at the end of the pulse. [Modified from Chiu and Ritchie (*38*)] (C to E) Intraaxonal recordings from normally myelinated (C), premyelinated (D), and myelinated regenerated (E) rat sciatic nerve fibers, showing the effects of blockade of potassium conductance on action potential electrogenesis. (C) Superimposed intraaxonal recordings from normal myelinated fiber, before and after treatment with 4-aminopyridine (4-AP) at 0.5 m*M*. There is no alteration of action potential waveform or duration after blockade of potassium conductance. (D) Intraaxonal recordings from regenerating fiber studied prior to myelination. The action potential is prolonged after blockage of potassium conductance with 4-AP at 0.5 m*M*. Voltage calibration in (C) pertains to both C and D. [C and D are modified from Kocsis *et al.* (*37*)] (E) Intraaxonal recording from myelinated axon in regenerated (1 year after a crushing injury) sciatic nerve fiber of rat. A burst of action potentials, superimposed on a delayed membrane depolarization, is elicited by a single stimulus. [Modified from Kocsis and Waxman (*69*)]

Voltage-Sensitive Potassium Channels

The spatial heterogeneity of the axon membrane also extends to voltage-sensitive potassium channels, which in mammalian (but interestingly not amphibian) myelinated axons may exhibit a distribution complementary to that of sodium channels. Several lines of evidence indicate that the classical model of nerve membrane excitation (whereby repolarization involves potassium channel activation) does not strictly apply to myelinated fibers in mammals. A voltage-dependent potassium conductance (g_K) mediates repolarization of the action potential in mammalian nonmyelinated fibers (*29–32*). In contrast, voltage clamp studies on mammalian myelinated PNS fibers indicate that voltage-dependent potassium conductance is normally attenuated or lacking at the nodal membrane (Fig. 2A), with repolarization occurring by rapid sodium inactivation and by an outward leakage current (*33, 34*). This leakage current is presumably carried by potassium ions flowing outward through voltage-independent channels that accept several cations (*35*). Similar results have been obtained by intraaxonal recording in myelinated axons in situ in mammalian PNS and CNS (Fig. 2C) (*36, 37*).

Potassium channels, although rare in the mammalian nodal membrane, are nevertheless normally present in the paranodal and internodal axon membrane under the myelin sheath. Voltage clamp studies have demonstrated the appearance of voltage-dependent potassium conductance in otherwise normal mammalian nerve fibers after sudden disruption of the myelin (Fig. 2B) (*23, 27, 38*). Similarly, voltage clamp (*39*) and longitudinal current analysis studies (*40*) on chronically demyelinated fibers indicate the presence of potassium channels in the denuded internodal axon membrane. Finally, studies of developing tissues show that, while potassium channels mediate action potential repolarization in premyelinated fibers, the role of potassium channels in repolarization is attenuated as they are covered by myelin during development (*12, 31*). The function of the internodal potassium channels is, at present, not fully understood; nor is it clear whether there are several types of potassium channels, as has been shown at frog nodes of Ranvier (*41*). A role in stabilizing the axon so as to prevent repetitive firing in response to single stimuli has been suggested (*24, 37, 42*). It is also possible that these channels contribute to the resting potential of the myelinated fiber (*43*).

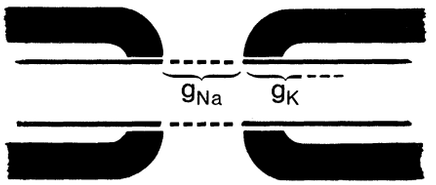

Fig. 3. Working model of the myelinated fiber. A node of Ranvier bounded on either side by the paranodal portion of the myelin sheath is shown schematically in longitudinal section. Sodium channels (g_{Na}) are clustered in high concentration in the axon membrane at the node but are present in low density or are absent in the internodal axon membrane. Potassium channels (g_K) are located in the axon membrane beneath the myelin sheath in paranodal and internode regions.

A model of the above considerations (Fig. 3) emphasizes the segregation of sodium and potassium channels in nodal and internodal regions of the mammalian axolemma. Although there are probably differences in the detailed organization of fibers of different sizes or types (*42, 44*), the concept that nodal and internodal domains of the axon membrane are structurally and functionally distinct, with excitability normally confined to the nodal region, or its vicinity, is now well established.

Axonal Plasticity and Development

Cytochemical (*12*), freeze-fracture (*45–47*), and conventional thin-section (*48*) studies in PNS and CNS indicate that patches of nodal membrane begin to differentiate prior to the formation of myelin or paranodal axoglial junctions. Smith and his colleagues (*49*), using longitudinal current analysis, have also noted the development of "phi-nodes" (foci of inward current, corresponding to collections of sodium channels) at the putative sites of developing nodes of Ranvier, in ventral root axons demyelinated with lysophosphatidyl choline (lysolecithin) several days prior to the formation of new myelin sheaths. Thus, it appears that sodium channels can cluster at the site of developing nodes of Ranvier, prior to the formation of mature myelin. This observation is not unexpected, since a considerable amount of inward current is required at the newly formed nodes if saltatory conduction is to take place.

An important issue in the development of the neuron concerns the signal (or signals) for myelination. There is now convincing evidence that myelination is a specific process (*12, 50*). The possibility of a cell surface–associated signal has been suggested (*51*). As was noted above, patches of nodal membrane differentiate prior to formation of compact myelin sheaths. It has been suggested that the differentiation of nodal and internodal membrane may provide a signal demarcating regions to be covered by myelin sheaths (*12, 45, 46*).

The contribution of potassium channels to action potential electrogenesis changes during the development of myelinated fibers. In premyelinated fibers before the formation of compact sheaths, repolarization is mediated in part by voltage-sensitive potassium channels. Thus, in premyelinated PNS (*31*) and CNS (*12*) fibers, blockade of potassium channels with 4-aminopyridine (4-AP) leads to delayed repolarization and a consequent broadening of the action potential. Maturation of the fiber is not complete, however, with the formation of compact myelin. In immature myelinated fibers, blockade of potassium channels leads not only to a broadening of the action potentially (*12, 31*) but also to burst activity (that is, brief periods of repetitive firing) in response to single stimuli; such stimulus-evoked bursting is not observed in mature myelinated fibers (*25, 42*). With maturation there is a decrease in the extent to which fibers respond to potassium channel blockade by bursting and action potential broadening (*25, 31*). It is not clear whether these changes in firing pattern are due to (i) an alteration in channel properties, (ii) regional redistribution of potassium channels (such as movement of potassium channels away from nodes), (iii) an alteration in the accessibility of the periaxonal submyelinic compartment due to maturation of the paranodal seal, (iv) changes in paranodal resistance, or (v) changes in capacitance with maturation (or a combination of the above). Freeze-fracture studies have demonstrated quantitative differences in nodal and internodal membrane structure of early myelinated, compared to mature myelinated, axons in the optic nerve (*21*). There is also some evidence for remodeling of the compact myelin sheath during development (*12, 52*).

Demyelination and Remyelination

The model shown in Fig. 3 has important implications for the pathophysiology of demyelinated fibers. After the acute loss of myelin, the density of sodium channels in the demyelinated area is probably too low to support impulse conduction. In addition, the presence of potassium channels in the denuded axon membrane tends to hold the demyelinat-

ed axon membrane close to the potassium equilibrium potential, and thus to interfere further with conduction (*24, 53*). This would provide a structural basis for the block of conduction that follows acute demyelination.

However, this conduction block need not be permanent. It is now established that remyelination can restore conduction in previously demyelinated fibers (*54*). In addition, several modes of recovery of conduction through chronically demyelinated axon regions have been described. Bostock and Sears (*55*) described continuous conduction through ventral root axon regions after demyelination with diphtheria toxin. Nonuniform conduction involving "phi-nodes," which probably represent aggregations of sodium channels, has been described as occurring prior to remyelination in ventral root axons demyelinated with lysophosphatidyl choline (*49*). Cytochemical studies, in fact, have demonstrated the spread of nodal membrane characteristics through demyelinated axon regions (*56*). It is not yet clear, in these cases of conduction through demyelinated fibers, whether the acquisition of excitability in demyelinated regions reflects redistribution of preexisting sodium channels, or production of new channels and their insertion into demyelinated membrane. Conduction block due to increased threshold, or impedance mismatch as a result of inadequate current density at the junction between normally myelinated and demyelinated axon regions, must be overcome in order for impulses to invade the demyelinated region. However, small changes in fiber geometry (*57, 58*) or membrane properties (*59*) may subserve this function. If impedance mismatch is overcome, demyelinated axon regions containing a lower-than-nodal density of sodium channels can sustain continuous conduction (*57*).

Remyelinated fibers conduct impulses at velocities close to normal despite their reduced internode distances (*60*). Rasminsky suggested that this reflects the development of near normal membrane properties at the newly formed nodes (*61*). Studies in the spinal cord indeed indicate that nodes formed along remyelinated fibers recapitulate normal cytochemical properties (*62*). There is an increase in saxitoxin binding in remyelinated peripheral nerve, which is proportional to the increase in nodal area imposed by the shorter spacing between nodes (*63*). This finding suggests that newly formed (remyelinated) nodes exhibit a near normal number of sodium channels, reflecting synthesis of new channels. In at least some remyelinated fibers, repolarization of the action potential continues to involve a voltage-dependent potassium conductance for as long as 6 months after remyelination (*63*).

Modification of the kinetics of sodium and potassium channels may provide increased current density in demyelinated fibers, and thus promote conduction across demyelinated axon regions (*64, 65*). Decreases in temperature, for example, lead to increased safety factor in some demyelinated fibers, as a result of the increased time integral of current (*66*). This can lead to transient clinical improvement, despite the concomitant decrease in conduction velocity. Reduction in serum-ionized calcium, which increases axonal excitability, facilitates conduction in demyelinated fibers, leading to transient improvements in the functional status of patients with multiple sclerosis (*67*). The effects of low calcium treatment are, however, transitory, lasting minutes to hours and thus are not of practical clinical value. Nevertheless, these results indicate that the pharmacological manipulation of membrane excitability may provide a rationale for development of symptomatic therapies in demyelinating diseases. Reversible abolition of conduction block in demyelinated fibers is produced by application of *Leiurus quinquestriatus* toxin, which blocks sodium inactivation and thereby increases the duration of the action current (*29*). Reduction in conduction block in demyelinated fibers is also produced by application of 4-AP, an agent that blocks potassium channels (*53*). In ligature-demyelinated peripheral nerve, application of 4-AP leads to increased excitability (*68*). Since agents such as 4-AP also affect normal elements in the white matter and neuropil, in addition to altering the conduction properties of demyelinated fibers (*30, 65*), it is important to develop methods that selectively increase the safety factor of conduction in demyelinated fibers.

Regeneration After Axonal Injury

In premyelinated regenerating fibers in the rat (*37*), as in premyelinated developing fibers (*12*), blockade of g_K leads to broadening of the action potential. This result suggests a role of potassium channels in repolarization of the action potential in premyelinated regenerating axons. After myelination of the regenerating fibers, potassium channel blockade continues to affect the nerve impulse, producing broadening of the action potential (*31*) or burst activity in response to single stimuli (*37*). This sensitivity to potassium channel blocking agents is lost in the normal course of myelinated axon maturation (*12, 25, 31*). However, in the rat this sensitivity is retained in long-term (2-year) regenerated axons, in which conduction velocity has recovered to nearly normal and functional recovery is, in many cases, nearly complete (*69*). Thus, the functional organization of some long-term regenerated mammalian axons is similar to that of remyelinated fibers (*63*) and immature myelinated fibers. It is interesting, in this regard, that internode distances in regenerated and remyelinated fibers remain relatively short (*70*) and do not exhibit the degree of lengthening that occurs during normal development. Morphological studies suggest that there is continued remodeling of the myelin even in long-term regenerated fibers (*71*).

Extraneuronal Sodium and Potassium Channels

A growing body of evidence suggests that voltage-dependent sodium and potassium channels are not necessarily restricted to muscle and nerve fibers. Thus, veratridine depolarizes Schwann cells enveloping the squid giant axon; this effect is reversed or abolished by tetrodotoxin (*72*). Furthermore, veratridine increases sodium influx into cultured fibroblast and glial cells (*73*), an action that is also blocked by tetrodotoxin.

Stronger evidence for the presence of nonneuronal voltage-dependent channels is the fact that in the rabbit (but not in the rat) the Schwann cells that proliferate after peripheral nerve section and Wallerian degeneration, bind two to three times as much saxitoxin (per unit weight) as does the original nerve trunk (*74*). This finding suggests that some mammalian Schwann cells express plasmalemmal voltage-dependent sodium channels. More recently, Ritchie *et al.* (*75*) showed that rabbit cultured Schwann cells bind saxitoxin while rat Schwann cells do so only to a very limited extent; moreover, patch-clamp studies have shown that rabbit cultured Schwann cell membranes contain voltage-dependent sodium and potassium channels whose kinetic properties are virtually the same as those in nodal membrane (*75*). Recent studies have also demonstrated voltage-sensitive potassium channels in fibroblasts, and sodium and potassium channels in astrocytes (*76*). The function of these extraneuronal channels remains unclear at present.

They are unlikely to be active normally since at the low resting potential of Schwann cells and astrocytes, the sodium channels are virtually completely inactivated. It is possible that they are involved in channel turnover or maintenance in the axon.

Neuroglial Relationships and Ion Channel Distribution

The mechanisms by which sodium channels are clustered in high density and potassium channels are excluded at the nodal region are not fully understood. It has been suggested that the paranodal axoglial junction serves as a barrier to diffusion of ion channels within the plane of the membrane (*19*). Alternatively, ion channels at the node may be cross-linked to one another, to other membrane structures, or to nonmembranous elements (either outside the membrane, or in the axoplasm subjacent to it) (*77*); in this respect, the cytoskeleton may play a role in axon membrane organization (*78*). During normal development, ion channels can cluster at the node prior to the formation of myelin or paranodal axoglial junctions (*12, 45–47*). A recent study (*79*) on the density of sodium channels in nervous tissue of the myelin-deficient *md* rat indicates that sodium channel insertion and maintenance within the axolemma during development is at least partially independent of myelination. Moreover, freeze-fracture studies show that some aspects of the structural development of premyelinated axolemma continue even in a glial cell–deficient environment (*80*).

Nevertheless, glial cell interactions may play a role in the development and maintenance of the normal axon membrane; studies on abnormally myelinated axons in the *dy/dy* mouse suggest that failure of this interaction may lead to abnormalities of ion channel distribution (*81*). A possible morphological basis for such interaction is found in the network of Schwann cell processes that surround (and closely approach) the axon membrane at normal peripheral nodes (*62*). Similar astrocytic processes come into close proximity with the axon at central nodes (*48, 83*). Astrocytic processes are also seen at developing central nodes of Ranvier (even in glial cell–deprived tracts where axoglial associations are less likely to occur on a random basis), suggesting a role of the astrocyte in the development of the node (*84*). The demonstration of voltage-sensitive sodium channels in Schwann cells (*74, 75*) and astrocytes (*76*) suggests the possibility of extra-axonal synthesis of ion channels, and their subsequent insertion into the axon membrane. Although membrane-to-membrane transfer has not been directly demonstrated, transport of materials as large as proteins between the Schwann cell and axon has been shown to occur (*85*).

Membrane patches with increased E-face particle density (presumably corresponding to "hot spots" of increased sodium channel density) are present along nonmyelinated axons in the retinal nerve fiber layer (*86*). There is a close spatial relation between these membrane particles and surrounding astroglial processes. Axoglial junctions of the paranodal type are not present in these regions, and these astrocytic processes do not circumferentially surround the axon. This axoglial interaction, which includes a close spatial relationship between glial cell contact and the differentiation of the axon surface, probably involves an interface of a type different from the paranodal junction (*87*). Regardless of the underlying mechanism for axoglial communication, it is likely that node formation involves a mutual differentiation of the axon and nearby Schwann or glial cells. The situation may be similar to that at the neuromuscular junction, where clustering of acetylcholine receptors in the membrane of the postsynaptic muscle cell reflects contact with a second cell type (in the case of the neuromuscular junction, the presynaptic terminal) (*88*).

Conclusion

The ionic basis of conduction in myelinated nerve fibers was initially considered as being the same as that established more than 30 years ago for nonmyelinated fibers by the work of Hodgkin and Huxley (*89*). However, it has become increasingly clear that the myelinated axon is not simply a scaled-down version of the squid giant axon, which happens to be covered along most of its length by an insulating myelin sheath, except at the nodes of Ranvier where the axolemma is exposed. As shown in this article, the mammalian myelinated fiber exhibits a structural differentiation which extends to the location of the ion channels in the axon membrane, where the complementary distribution of sodium and potassium channels reflects the location of the overlying myelin sheath. In functional terms, the differentiation of the myelin fiber is well matched to functional requirements. This mode of organization is also highly relevant to the pathophysiology of demyelinating disease such as multiple sclerosis. Finally, the mammalian myelinated fiber may hold a number of important lessons with respect to the developmental mechanisms by which cell membranes are organized and by which different cell types recognize and interact with each other and differentiate in a coordinated manner.

References and Notes

1. B. B. Geren, *Exp. Cell. Res.* **7**, 558 (1954); J. D. Robertson, *J. Physiol. (London)* **135**, 56 (1957).
2. A. Peters, *J. Biophys. Biochem. Cytol.* **7**, 121 (1960); M. B. Bunge, R. P. Bunge, G. D. Pappas, *J. Cell Biol.* **12**, 448 (1962); S. G. Waxman and T. J. Sims, *Brain Res.* **292**, 179 (1984).
3. J. M. Ritchie, in *Myelin*, P. Morell, Ed. (Plenum, New York, 1984), p. 117; P. G. Funch and D. S. Faber, *Science* **225**, 538 (1984).
4. I. Tasaki, *Nervous Transmission* (Thomas, Springfield, Ill., 1953).
5. ———, *Am. J. Physiol.* **127**, 211 (1939).
6. A. F. Huxley and R. Stämpfli, *J. Physiol. (London)* **108**, 315 (1949).
7. Z. Koles and M. Rasminsky, *ibid.* **227**, 351 (1972); C. Schauf and F. Davis, *J. Neurol. Neurosurg. Psychiatry* **37**, 152 (1974); S. Waxman and M. Brill, *ibid.* **41**, 408 (1978); J. Moore *et al.*, *Biophys. J.* **21**, 147 (1978); S. Waxman and S. Wood, *Brain Res.* **294**, 111 (1984).
8. S. Bement and J. B. Ranck, *Exp. Neurol.* **24**, 147 (1969); J. D. Kocsis and S. G. Waxman, *Adv. Neurol.* **31**, 299 (1981); P. G. Funch and D. S. Faber, *J. Neurophysiol.* **47**, 1214 (1982).
9. M. Rasminsky and T. A. Sears, *J. Physiol. (London)* **227**, 323 (1972).
10. D. C. Quick and S. G. Waxman, *J. Neurol. Sci.* **31**, 1 (1977); S. G. Waxman and D. C. Quick, *Brain Res.* **144**, 1 (1978).
11. F. A. Dodge and J. Cooley, *IBM J. Res. Dev.* **17**, 219 (1973); S. G. Waxman and D. C. Quick in *Physiology and Pathobiology of Axons*, S. G. Waxman, Ed. (Raven, New York, 1978), pp. 125–131; W. A. Catterall, *J. Neurosci.* **1**, 777 (1981).
12. S. G. Waxman and R. E. Foster, *Brain Res. Rev.* **2**, 205 (1980).
13. M. V. L. Bennett, *Annu. Rev. Physiol.* **32**, 471 (1970); S. G. Waxman, G. D. Pappas, M. V. L. Bennett, *J. Cell Biol.* **53**, 210 (1972).
14. M. H. Ellisman and S. R. Levinson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6707 (1982); B. Haimovich *et al.*, *J. Neurosci.* **4**, 2259 (1984); H. Meiri *et al.*, *Brain Res.* **310**, 168 (1984).
15. J. M. Ritchie, R. B. Rogart, G. Strichartz, *J. Physiol. (London)* **261**, 477 (1976).
16. J. M. Ritchie and R. B. Rogart, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 211 (1977).
17. R. G. Pellegrino, P. S. Spencer, J. M. Ritchie, *Brain Res.* **305**, 307 (1984).
18. A. L. Hodgkin, *Philos. Trans. R. Soc. London Ser. B.* **270**, 297 (1975).
19. J. Rosenbluth, *J. Neurocytol.* **5**, 731 (1976).
20. C. Kristol, C. Sandri, K. Akert, *Brain Res.* **142**, 391 (1978).
21. J. A. Black, R. E. Foster, S. G. Waxman, *ibid.* **250**, 1 (1982).
22. J. Rosenbluth, *Adv. Neurol.* **31**, 391 (1981).
23. S. Y. Chiu and J. M. Ritchie, *J. Physiol. (London)* **304**, 123 (1981).
24. J. M. Ritchie and S. Y. Chiu, *Adv. Neurol.* **31**, 329 (1981).
25. J. D. Kocsis, J. A. Ruiz, S. G. Waxman, *J. Neurophysiol.* **50**, 449 (1983).
26. S. Y. Chiu, *J. Physiol. (London)* **309**, 499 (1980).
27. ——— and J. M. Ritchie, *Adv. Neurol.* **31**, 313 (1981).
28. B. Neumcke and R. Stämpfli, *J. Physiol. (London)* **309**, 163 (1982).
29. H. Bostock, R. M. Sherratt, T. A. Sears, *Nature (London)* **274**, 385 (1978).
30. J. D. Kocsis, R. C. Malenka, S. G. Waxman, *Brain Res.* **207**, 321 (1981); *J. Physiol. (London)* **334**, 225 (1983).
31. J. M. Ritchie, *Proc. R. Soc. London Ser. B.* **215**, 273 (1982).
32. R. J. Preston, S. G. Waxman, J. D. Kocsis, *Exp. Neurol.* **79**, 808 (1983).
33. S. Y. Chiu, J. M. Ritchie, R. B. Rogart, D. Stagg, *J. Physiol. (London)* **292**, 149 (1979).
34. T. Brismar, *ibid.* **298**, 171 (1980).

35. B. Hille, *J. Gen. Physiol.* **61**, 669 (1973).
36. J. D. Kocsis and S. G. Waxman, *Nature (London)* **287**, 348 (1980); *Adv. Neurol.* **31**, 299 (1981).
37. _____, C. Hildebrand, J. Ruiz, *Proc. R. Soc. London Ser. B.* **217**, 77 (1982).
38. S. Y. Chiu and J. M. Ritchie, *Nature (London)* **284**, 170 (1980).
39. T. Brismar, *Acta Physiol. Scand.* **113**, 161, 167 (1981).
40. H. Bostock, T. A. Sears, M. Sherratt, *J. Physiol. (London)* **313**, 301 (1981).
41. J. M. Dubois, *ibid.* **318**, 297 (1981).
42. C. Bowe, J. D. Kocsis, S. G. Waxman, *Soc. Neurosci. Abstr.* **257**, 15 (1984); *Proc. R. Soc. London Ser. B* **224**, 355 (1985).
43. S. Y. Chiu and J. M. Ritchie, *Proc. R Soc. London Ser. B.* **220**, 415 (1984).
44. S. G. Waxman, *Ann. Intern. Med.* **52**, 297 (1980); K. J. Smith and C. Schauf, *Nature (London)* **293**, 297 (1981); C. Raine, *J. Neurocytol.* **11**, 935 (1982).
45. C. A. Wiley-Livingston and M. H. Ellisman, *Dev. Biol.* **79**, 334 (1980).
46. S. G. Waxman, J. A. Black, R. E. Foster, *Neurology* **32**, 418 (1982).
47. J. H. Tao-Cheng and J. Rosenbluth, *Dev. Brain Res.* **3**, 577 (1982); *ibid.* **9**, 251 (1983).
48. C. Hildebrand and S. G. Waxman, *J. Comp. Neurol.* **224**, 25 (1984).
49. H. Bostock, S. M. Hall, K. J. Smith, *J. Physiol. (London)* **308**, 21 (1980); K. J. Smith, H. Bostock, S. M. Hall, *J. Neurol. Sci.* **54**, 13 (1982).
50. S. G. Waxman, *Brain Res.* **47**, 269 (1972).
51. R. O. Brady and R. H. Quarles, *Mol. Cell. Biochem.* **2**, 23 (1973); P. Spencer and H. J. Weinberg, in *Physiology and Pathobiology of Axons*, S. G. Waxman, Ed. (Raven, New York, 1978), p. 389; S. G. Waxman and R. E. Foster, *Proc. R. Soc. London Ser. B* **209**, 441 (1980).
52. C.-H. Berthold and S. Skoglund, *Acta Soc. Med. Ups.* **73**, 127 (1968); K. Friede and C. Hildebrand, *J. Anat.* **134**, 517 (1982).
53. R. M. Sherratt, H. Bostock, T. A. Sears, *Nature (London)* **283**, 570 (1980); E. Targ and J. D. Kocsis, *Brain Res.*, in press; J. D. Kocsis and S. G. Waxman, in *Handbook of Neurology. Demyelinating Diseases*, J. Koetsier, Ed. (Elsevier, Amsterdam, in press).
54. K. Smith, W. Blakemore, W. McDonald, *Nature (London)* **280**, 395 (1979); *Brain* **104**, 383 (1981).
55. H. Bostock and T. Sears, *Nature (London)* **263**, 786 (1976); *J. Physiol. (London)* **280**, 273 (1978).
56. R. E. Foster, C. C. Whalen, S. G. Waxman, *Science* **210**, 661 (1980); F. Coria, M. Berciano, J. Berciano, M. LaFarga, *Brain Res.* **291**, 369 (1984).
57. S. G. Waxman and M. H. Brill, *J. Neurol. Neurosurg. Psychiatry* **41**, 408 (1978).
58. T. A. Sears, H. Bostock, M. Sherratt, *Neurology* **28** (No. 2), 21 (1978).
59. S. G. Waxman and S. L. Wood, *Brain Res.* **294**, 111 (1984).
60. B. G. Cragg and P. K. Thomas, *J. Physiol. (London)* **171**, 164 (1964).
61. M. Rasminsky, in *Physiology and Pathobiology of Axons*, S. G. Waxman, Ed. (Raven, New York, 1978), pp. 361–376.
62. L. P. Weiner, S. G. Waxman, S. Stohlman, A. Kwan, *Ann. Neurol.* **8**, 580 (1980).
63. J. M. Ritchie, H. P. Rang, R. Pellegrino, *Nature (London)* **294**, 257 (1981).
64. C. L. Schauf and F. A. Davis, *J. Neurol. Neurosurg. Psychiatry* **37**, 152 (1974); F. A. Davis and C. L. Schauf, *Adv. Neurol.* **31**, 505 (1981).
65. T. A. Sears and H. Bostock, *Adv. Neurol.* **31**, 357 (1981); R. E. Jones *et al.*, *J. Neurol. Sci.* **60**, 353 (1983).
66. F. A. Davis and S. Jacobson, *J. Neurol. Neurosurg. Psychiatry* **34**, 551 (1971); M. Rasminsky, *Arch. Neurol.* **28**, 287 (1973).
67. F. A. Davis *et al.*, *J. Neurol. Neurosurg. Psychiatry* **13**, 723 (1970).
68. S. G. Waxman, J. D. Kocsis, D. L. Eng, *Muscle Nerve* **8**, 85 (1985).
69. J. D. Kocsis and S. G. Waxman, *Nature (London)* **304**, 640 (1983).
70. F. Sanders and D. Whitteridge, *J. Physiol. (London)* **105**, 152 (1946); B. Cragg and P. K. Thomas, *ibid.* **171**, 164 (1964); W. I. McDonald, *Br. Med. Bull.* **30**, 186 (1974).
71. C. Hildebrand, J. D. Kocsis, S. Berglund, S. G. Waxman, *Brain Res.*, in press.
72. J. Villegas, C. Sevcik, F. Barnola, R. Villegas, *J. Gen. Physiol.* **67**, 369 (1976).
73. R. Munson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6425 (1979); G. Romey *et al.*, *Biochim. Biophys. Acta* **556**, 344 (1979); J. Pouyssegur *et al.*, *Nature (London)* **286**, 162 (1980); G. Reiser *et al.*, *Brain Res.* **261**, 335 (1983).
74. J. M. Ritchie and H. P. Rang, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2803 (1983).
75. S. Y. Chiu, P. Shrager, J. M. Ritchie, *Nature (London)* **311**, 156 (1984); P. Shrager, S. Y. Chiu, J. M. Ritchie, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 948 (1985).
76. S. Bevan, S. Y. Chiu, P. Gray, J. M. Ritchie, *J. Physiol. (London)* **361**, 18 (1985).
77. S. G. Waxman and D. C. Quick, *Brain Res.* **144**, 1 (1978); M. H. Ellisman, *J. Neurocytol.* **8**, 719 (1979); J. Fukuda *et al.*, *Nature (London)* **294**, 82 (1981).
78. M. H. Ellisman, J. D. Lindsey, C. Wiley-Livingston, S. R. Levinson, in *Structure and Function in Excitable Cells*, D. Chang *et al.*, Eds. (Plenum, New York, 1983), pp. 3–24; N. Hirokawa, *ibid.*, pp. 113–143.
79. A. L. Oaklander, R. Pellegrino, J. M. Ritchie, *Brain Res.* **307**, 393 (1984).
80. J. A. Black, T. J. Sims, S. G. Waxman, S. A. Gilmore, *Soc. Neurosci. Abstr.* **340**, 13 (1984); *J. Neurocytol.* **14**, 79 (1985).
81. H. Bostock and M. Rasminsky, *J. Physiol. (London)* **340**, 145 (1983).
82. D. Landon and S. Hall, in *Peripheral Nerve*, D. Landon, Ed. (Chapman & Hall, London, 1976), pp. 1–105; M. Rydmark and C. Berthold, *J. Neurocytol.* **12**, 537 (1983).
83. C. Hildebrand, *Acta Physiol. Scand. Suppl.* **364**, 81 (1971); _____ and S. G. Waxman, *J. Comp. Neurol.* **224**, 25 (1984); C. Raine, *J. Neurocytol.* **13**, 21 (1984).
84. T. J. Sims, S. G. Waxman, J. A. Black, S. A. Gilmore, *Brain Res.*, in press.
85. J. S. Kriegler, N. Krishnan, M. Singer, *Adv. Neurol.* **31**, 479 (1981); H. Gainer, I. Tasaki, R. J. Lasek, *J. Cell Biol.* **74**, 524 (1977).
86. C. Hildebrand and S. G. Waxman, *Brain Res.* **258**, 23 (1983); J. A. Black, S. G. Waxman, C. Hildebrand, *J. Neurocytol.* **13**, 417 (1984).
87. One possibility is that an extracellular matrix substance is produced by some astrocytes [P. Liesi, D. Dahl, M. Vaheri, *J. Cell Biol.* **96**, 920 (1983); J. Bernstein *et al.*, *Brain Res.*, in press]. There is evidence that some membrane receptors (acetylcholine receptors) are clustered by contact with the extracellular basal lamina [L. Rubin and U. J. McMahan, in *Diseases of the Motor Unit*, D. Schotland, Ed. (Wiley, New York, 1982), pp. 187–197].
88. A. J. Harris, *Annu. Rev. Physiol.* **36**, 251 (1974); H. Fertuck and M. M. Salpeter, *J. Cell Biol.* **69**, 144 (1976); D. Fambrough, *Physiol. Rev.* **59**, 165 (1979).
89. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **117**, 500 (1952).
90. Supported in part by grants from the U.S. Public Health Service (NS 15320, NS 12327, and NS 08304), from the National Multiple Sclerosis Society (RG 1231 and RG 1162), by the Medical Research Service of the Veterans Administration, by the Folger Foundation, and by the Allen Charitable Trust.