CHAPTER

2

Neuron–glial cell cooperation

There are roughly twice as many glial cells as there are neurons in the central nervous system. They occupy the space between neurons and neuronal processes and separate neurons from blood vessels. As a result, the extracellular space between the plasma membranes of different cells is narrow, of the order of 15–20 nm.

Virchow (1846) was the first to propose the existence of non-neuronal tissue in the central nervous system. He named it 'nevroglie' (nerve glue), because it appeared to stick the neurons together. Following this, Deiters (1865) and Golgi (1885) identified glial cells as making up the nevroglie and distinguished them from neurons.

There are several categories of glial cells. Depending on their anatomical position they are classed as follows:

- *Central glia* are found in the central nervous system, and comprise four cell types: astrocytes, oligodendrocytes, microglia (these three types are also known as interstitial glia, because they are found in interneuronal spaces) and ependymal cells which form the epithelial surface covering the walls of the cerebral ventricles and of the central canal of the spinal cord.
- *Peripheral glia* comprise a single type: Schwann cells. These cells ensheath the axons and encapsulate the cell bodies of neurons. In the latter case, they are also called satellite cells.

Glial cells, excluding microglia, have an ectodermal origin. Those of the central nervous system derive from the germinal neural epithelium (neural tube), while peripheral glia (Schwann cells) are derived from the neural crest. Microglia, in contrast, have a mesodermal origin.

Glial cells have morphological as well as functional and metabolic characteristics that distinguish them from neurons:

- They do not generate or conduct action potentials. Thus, although they extend processes, these are only of one type and are neither dendrites nor axons.
- They do not establish chemical synapses between themselves, with neurons, or any other cell type.
- Unlike most neurons in humans, glial cells are capable of division for at least several years postnatally.

Nervous tissue is made compact by glial cells and for this reason they are often ascribed the role of supporting tissue. However, as we will see in this chapter, they have additional functions. We will explain in this chapter the roles of astrocytes, oligodendrocytes and Schwann cells, only.

2.1 ASTROCYTES FORM A VAST CELLULAR NETWORK OR SYNCYTIUM BETWEEN NEURONS, BLOOD VESSELS AND THE SURFACE OF THE BRAIN

2.1.1 Astrocytes are star-shaped cells characterized by the presence of glial filaments in their cytoplasm

Astrocytes are small star-shaped cells with numerous fine, tortuous, ramified processes covered with varicosities (**Figure 2.1**). The cell body is typically 9–10 μm in diameter and the processes extend radially over 40–50 μm. These often have enlarged terminals in

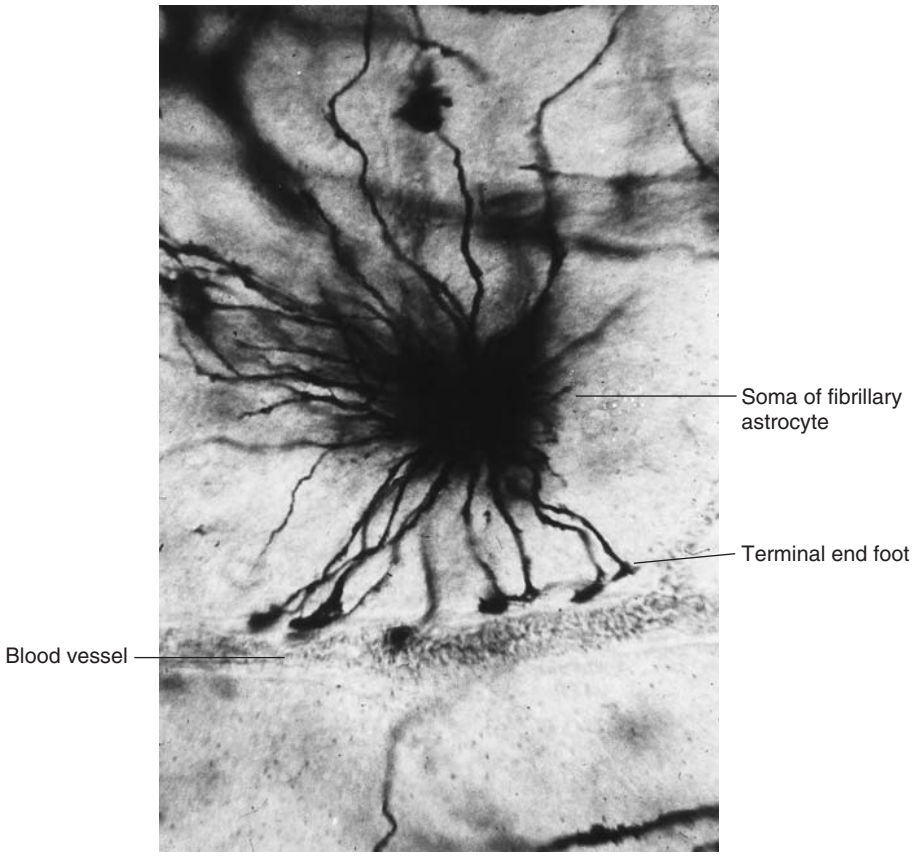

FIGURE 2.1 Fibrillary astrocyte.
Micrograph of a fibrillary astrocyte stained with a Golgi stain observed through an optical microscope. The processes of this astrocyte make contact with a blood vessel: these are the terminal end feet. Photograph by Olivier Robain.

contact with neurons or non-neuronal tissue (like the walls of blood vessels).

Two kinds of astrocytes are recognized. Some astrocytes contain in their cytoplasm numerous glial filaments: these are fibrillary astrocytes, principally located in the white matter (**Figure 2.1**). They have numerous, radial processes which are infrequently branched and covered with 'expansions en brindilles'. Other astrocytes contain few, if any, glial filaments: these are protoplasmic astrocytes, found normally in the grey matter. They have more delicate processes, some of which are velate (veil-like). Both types of astrocytes send out processes that end on the walls of blood vessels or beneath the pial surface of the brain and spinal cord.

The principal ultrastructural characteristics of astrocytes are the glial filaments and glycogen granules present in the cytoplasm of their somata and processes. The filaments are 'intermediate filaments' with an average diameter of 8–10 μm. They are composed of a protein specific to astrocytes, glial fibrillary acidic protein (GFAP), consisting of a single type of subunit with a molecular weight of 50 kD, different from that of neurofilaments. This characteristic has been exploited as a method of identifying astrocytes. By using an antiserum to glial fibrillary acidic protein (anti-GFAP) linked to fluorescein, one can stain astrocytes, *in situ* or in culture, without marking either neurons or other types of glial cells.

Astrocytes, like all glial cells, do not form chemical synapses. They do, however, mutually form junctional complexes. Two types of junctions have been demonstrated: communicating junctions (or gap junctions) and desmosomes (puncta adhaerentia). Coupled to each other by numerous junctional complexes, astrocytes therefore constitute a vast cellular network, or syncytium, extending from neurons to blood vessels and the external surface of the brain.

2.1.2 Astrocytes maintain the blood–brain barrier in the adult brain

The essential characteristic of astrocytic processes is their termination on the walls of blood vessels in astrocytic end feet (**Figure 2.2**). Here the end feet are joined

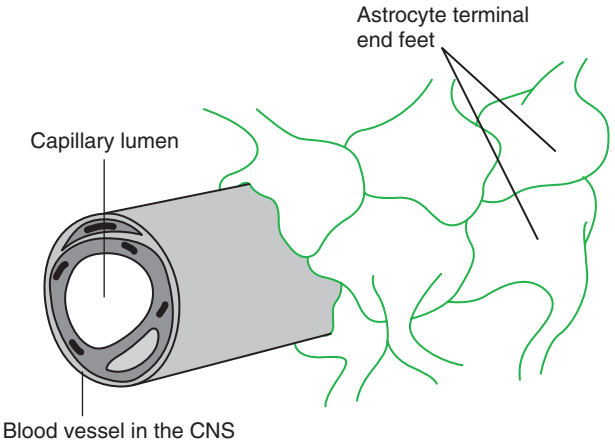

FIGURE 2.2 Diagram of the covering formed by astrocyte end feet around a capillary in the central nervous system (CNS). From Goldstein G and Betz L (1986) La barrière qui protège le cerveau. *Pour la Science*, November, 84–94, with permission.

by gap junctions and desmosomes, forming a 'palisade' between neurons and vascular endothelial cells. The space between the layer of astrocyte end feet and the endothelial cells is about 40–100 nm and is occupied by a basal lamina. Astrocytes also send processes to the external surface of the central nervous system where the astrocyte end feet, together with the basal lamina that they produce, form the 'glia limitans externa', which separates the pia mater from the nervous tissue. Astrocytes therefore constitute a barrier between neurons and the external medium (blood), preventing access of substances foreign to the central nervous system. They thus protect neurons. This barrier is not, however, totally impermeable and astrocytes are involved in selective exchange processes.

Astrocyte end feet are not the blood–brain barrier. This is formed, in most regions of the central nervous system, by vascular endothelial cells joined together by tight junctions. Even though the astrocyte end feet do not form the blood–brain barrier, they have an important role in its development and maintenance. Thus, if the layer of astrocyte end feet in the adult is destroyed, by a tumour or by allergic illnesses, for example, the capillary endothelial cells immediately take on the characteristics normally observed in capillaries outside the central nervous system: they are no longer bound by tight junctions and become 'fenestrated'. In such capillaries the blood–brain barrier no longer exists.

2.1.3 Astrocytes regulate the ionic composition of the extracellular fluid

We have seen that astrocyte end feet are involved in the formation and maintenance of the blood–brain barrier, and that astrocytes thus contribute to regulation of the brain extracellular fluid. However, astrocytes have other important roles in controlling the composition of the extracellular fluid. We shall consider as an example the regulation of the extracellular potassium concentration.

The extracellular potassium concentration needs to be tightly regulated: if potassium increased it would depolarize neurons. This would first increase neuronal excitability and then inactivate action potential propagation. Regulation of the extracellular potassium concentration must occur in the face of large fluxes of potassium ions into the extracellular space during neuronal activity, when potassium ions leave neurons through voltage-activated potassium channels (see Sections 4.3 and 5.3). Astrocytes are thought to regulate extracellular potassium by the mechanism of 'spatial buffering'. This means that astrocytes take up potassium ions in regions where the concentration rises and eventually release through their end feet an equivalent amount of potassium ions into the vicinity of blood vessels or across the *glia limitans externa*. The details of the process are complicated, but potassium ions are thought to enter astrocytes via channels or the sodium pump and to exit at the end feet through channels. This potassium buffering role of astrocytes is likely to be of particular importance at the nodes of Ranvier, where marked accumulation of potassium ions in the restricted extracellular space can occur, due to the conduction of action potentials.

2.1.4 Astrocytes take part in the neurotransmitter cycle

After neurotransmitters are released during synaptic transmission, they need to be removed from the extracellular space to prevent the extracellular neurotransmitter concentration from rising. Steady high concentrations of transmitter would interfere with synaptic transmission, and long-lasting activation of receptors (particularly glutamate receptors) can damage neurons. Most transmitters are removed from the extracellular space by reuptake into cells (but acetylcholine is hydrolyzed; see **Figure 6.12**). Transmitters are taken up by specialized carrier molecules in the cell membrane. Although both neurons and glia express such carrier proteins, it seems that uptake into astrocytes is of particular importance. This is especially clear for the case of glutamate: astrocytes have an enormous capacity to take up this transmitter, presumably reflecting the abundance of this transmitter and the toxicity to neurons of high glutamate concentrations.

Besides their role in transmitter clearance from the synaptic cleft (by recapture), astrocytes play a role in the

synthesis of transmitters and particularly glutamate and GABA. For example, thanks to the presence of glutamine synthetase in astrocytes (see **Figure 10.13**), glutamine is formed from glutamate. Glutamine is then uptaken by neurons and transformed back in glutamate.

2.2 OLIGODENDROCYTES FORM THE MYELIN SHEATHS OF AXONS IN THE CENTRAL NERVOUS SYSTEM AND ALLOW THE CLUSTERING OF NA^+ CHANNELS AT NODES OF RANVIER

Two types of oligodendrocyte are recognized: interfascicular or myelinizing oligodendrocytes, found in the white matter where they make the sheaths of myelinated axons; and satellite oligodendrocytes which surround neuronal somata in the grey matter. We will deal with the former type in detail. Their major role is, by forming the myelin sheath, to electrically isolate segments of axons, induce the formation of clusters of Na^+ channels at nodes of Ranvier and therefore to allow the fast propagation of Na^+ action potentials (see Section 4.4).

2.2.1 Processes of interfascicular oligodendrocytes electrically isolate segments of central axons by forming the lipid-rich myelin sheath

The cell bodies of interfascicular oligodendrocytes are situated between bundles of axons

Interfascicular, or myelinizing, oligodendrocytes have small spherical or polyhedral cell bodies of diameter 6–8 μm and few processes. They are called interfascicular because their cell bodies are aligned between bundles (fascicles) of axons. They are distinguished from astrocytes by the sites of termination of their processes: oligodendrocyte processes enwrap axons and make no contact with blood vessels.

Observed by electron microscopy, the nucleus and perikaryon of oligodendrocytes appear dark (**Figure 2.3**), there are no glial filaments, and there are many microtubules in the somatic and dendritic cytoplasm. Because of this, oligodendrocyte processes may be confused with fine dendrites, and it is by the absence of chemical synapses that the glial processes are identified.

Oligodendrocytes can be identified by immunohistochemistry. This is done using an antigalactoceramide immune serum (anti-gal-C), galactoceramide being a glycolipid found exclusively in the membrane of processes of myelinizing oligodendrocytes.

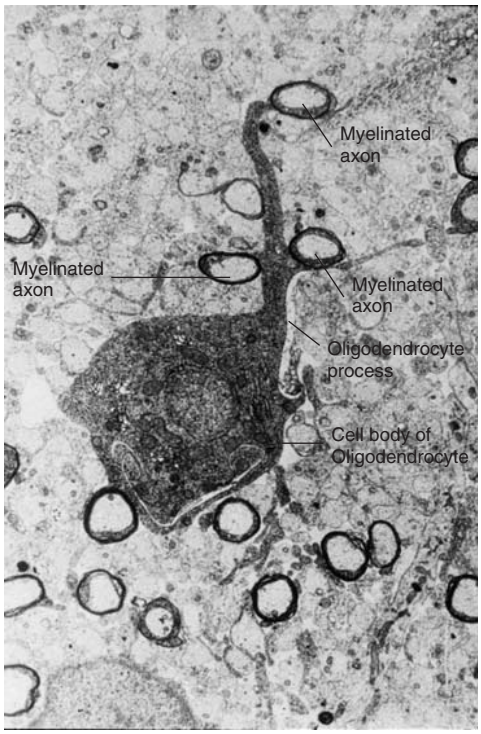

FIGURE 2.3 Myelinating oligodendrocyte.
Electron micrograph of an oligodendrocyte. The cell body and one of its processes enwrapping several axons can be seen. Section taken through the spinal cord. Photograph by Olivier Robain.

The myelin sheath is a compact roll of the plasmalemma of an oligodendrocyte process: this glial membrane is rich in lipids

Myelinated axons are surrounded by a succession of myelin segments, each about 1 mm long. The covered regions of axons alternate with short exposed lengths where the axonal membrane (axolemma) is not covered. These unmyelinated regions (of the order of a micron) are called nodes of Ranvier (**Figures 2.4** and **2.5a**).

A myelinated segment comprises the length of axon covered by an oligodendrocyte. One oligodendrocyte can form 20–70 myelin segments around different axons (**Figure 2.4**). Thus the degeneration or dysfunction of a single oligodendrocyte leads to the disappearance of myelin segments on several different axons.

Formation and ultrastructure of a myelin segment

Myelinization represents a crucial stage in the ontogenesis of the nervous system. In the human at birth, myelinization is only just beginning, and in some regions is not complete even by the end of the second year of life. The first step in the process is migration of oligodendrocytes into the bundles of axons, then the myelinization of some, but not all, axons. Once contact

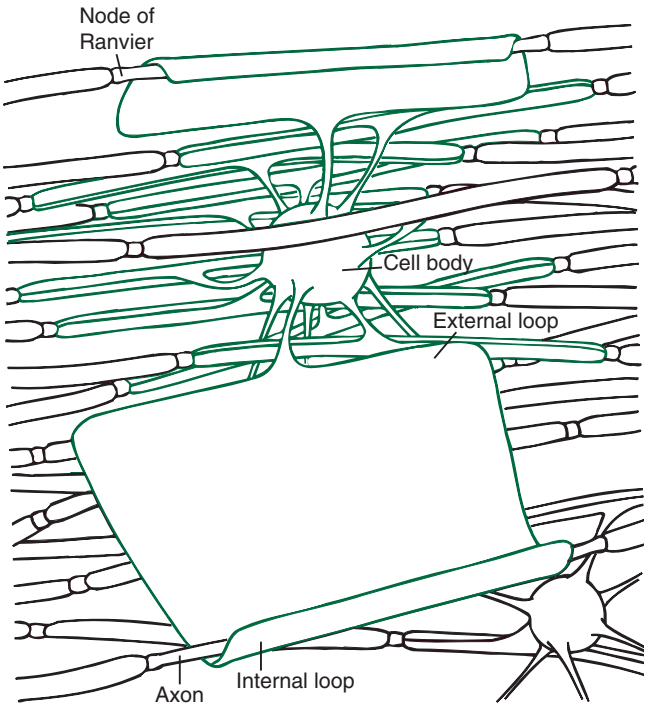

FIGURE 2.4 Diagram of a myelinating oligodendrocyte and its numerous processes.
Each form a segment of myelin around a different axon in the central nervous system. Two myelin segments are represented, one partially unrolled, the other completely unrolled. Drawing by Tom Prentiss. In Morell P and Norton W (1980) La myéline et la sclérose en plaques. *Pour la Science* **33**, with permission.

has been made between the oligodendrocyte and axon, the initial turn of myelin around the axon is rapidly formed. Myelin is then slowly deposited over a period which in humans can reach several months. Myelinization is responsible for a large part of the increase in weight of the central nervous system following the end of neurogenesis.

In order to form the compact spiral of myelin membrane, the oligodendrocyte process must roll itself around the axon many times (up to 40 turns) (**Figure 2.5**). It is the terminal portion of the process, called the inner loop, situated at the interior of the roll, which progressively spirals around the axon. This movement necessitates the sliding of myelin sheets which are not firmly attached. During this period, the oligodendrocyte synthesizes several times its own weight of myelin membrane each day.

Within the spiral the cytoplasm disappears entirely (except at the internal and external loops). The internal leaflets of the plasma membranes can thus adhere to each other. This adhesion is so intimate that the internal leaflets virtually fuse, forming the period, or major, dense line of thickness of 3 nm (**Figure 2.5b**). The extracellular space between the different turns of membrane also disappears, and the external leaflets also stick to each other. This apposition is, however, less close and a small space remains between the external leaflets. The apposed external leaflets form the minor, or interperiod, dense line (**Figure 2.5b**).

Thus, a cross-section of a myelinated axon observed by electron microscopy shows alternating dark and light lines forming a spiral around the axon. The major dense line terminates where the internal leaflets separate to enclose the cytoplasm within the external loop. The interperiod dense line disappears at the surface of the sheath at the end of the spiral (**Figure 2.5b**).

In the central nervous system there is no basal lamina around myelin segments, so myelin segments of adjacent axons may adhere to each other forming an interperiod dense line.

Myelin

Myelin consists of a compact spiral (without intracellular or extracellular space) of glial plasma membrane of a very particular composition. Lipids make up about 70% of the dry weight of myelin and proteins only 30%. Compared with the membranes of other cells, this represents an inversion of the lipid:protein ratio (**Figure 2.6**).

This lipid-rich membrane is highly enriched in glycosphingolipids and cholesterol. The major glycosphingolipids in myelin are galactosylceramide and its sulfated derivative sulfatide (20% of lipid dry weight). There is also an unusually high proportion of ethanolamine phosphoglycerides in the plasmalogen form, which accounts for one-third of the phospholipids.

In myelin, a number of structural classes of proteins are present. Some proteins are extremely hydrophobic membrane-embedded polypeptides, some integral membrane proteins have a single transmembrane domain and clearly define extra- and intracellular domains, and some of the myelin proteins are cytosolic; however, they are often intimately associated with the myelin membrane.

Myelin basic protein (MBP) and the proteolipid proteins (PLP/DM20) are the two major myelin proteins in the CNS. Myelin basic proteins are found on the cytoplasmic side and play a role in the adhesion of the internal leaflets of the specialized oligodendroglial plasma membrane. Proteolipid proteins are integral membrane proteins. Though they are in high abundance (they represent around 50% of the total myelin protein in the central nervous system) their exact biological role has not yet been elucidated.

We have seen that myelin has an inverted lipid:protein ratio, while the cell body membrane of the oligodendrocyte has a ratio comparable to that of other cell

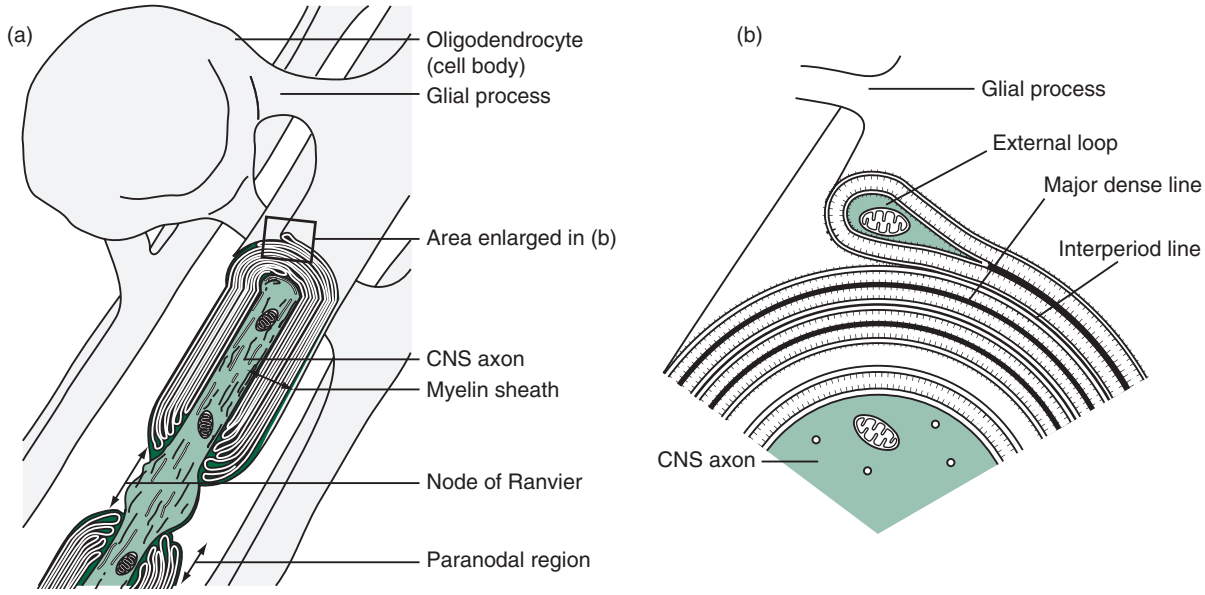

FIGURE 2.5 Myelin sheath of central axons.
(a) Three-dimensional diagram of the myelin sheath of an axon in the central nervous system (CNS). The sheath is formed by a succession of compact rolls of glial processes from different oligodendrocytes. **(b)** Cross-section through a myelin sheath. The dark lines, or major dense lines, and clear bands (in the middle of which are found the interperiod lines) visible with electron microscopy are accounted for by the manner in which the myelin membrane surrounds the axon, and by the composition of the membrane. The dark lines represent the adhesion of the internal leaflets of the myelin membrane while the interperiod lines represent the adhesion of the external leaflets. The lines are formed by membrane proteins while the clear bands are formed by the lipid bilayer. Drawing (a) from Bunge MB, Bunge RP, Ris H (1961) Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord. *J. Biophys. Biochem. Cytol.* **10**, 67–94, with permission of Rockerfeller University Press. Drawing (b) by Tom Prentiss. In Morell P and Norton W (1980) La myéline et la sclérose en plaques. *Pour la Science* **33**, with permission.

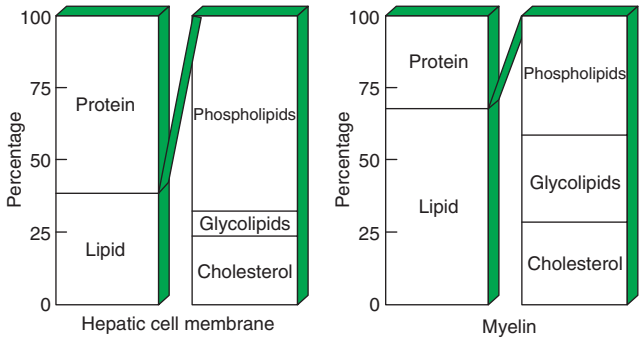

FIGURE 2.6 Comparison of the lipid content of plasma membrane and myelin.
The protein:lipid ratio is inverted between the two membranes. The proportions of the three groups of lipids are also different.

membranes. As the myelin of the oligodendrocyte process is in continuity with the plasma membrane of the cell body, it is necessary to postulate gradients in the composition of lipids and proteins (in opposite directions to each other) between the cell body and the various processes. During the active phase of myelination, each oligodendrocyte must produce as much as 5–50,000 μm^2 of myelin membrane surface area per day.

Nodes of Ranvier

In the central nervous system the nodes of Ranvier, regions between myelin segments, are relatively long (several microns) compared with those in the peripheral nervous system. Here the axolemma is exposed and an accumulation of dense material is seen on the cytoplasmic side. The myelin sheath does not terminate abruptly. Successive layers of myelin membrane terminate at regularly spaced intervals along the axon, the internal layers (close to the axon) terminating first. This staggered termination of the different layers of myelin constitutes the paranodal region (**Figure 2.5a**).

2.2.2 Myelination enables rapid conduction of action potentials for two reasons

Isolation of internode axonal segments

The high lipid content and compact structure of the myelin sheath help make it impermeable to hydrophilic substances such as ions. It prevents transmembrane ion fluxes and acts as a good electrical insulator between the intracellular (i.e. intra-axonal) and extracellular

media. Between the nodes of Ranvier the axon therefore behaves as an insulated cable. This permits rapid, saltatory conduction of action potentials along the axon (see Section 5.4).

Formation of Ranvier nodes with a high density of Na^+ channels

Na^+ channels are clustered in very high density within the nodal gap whereas voltage-dependent K^+ channels are segregated in juxta-paranodal regions, beneath overlying myelin (see **Figure 2.5a**). To test whether oligodendrocyte contact with axon influences Na^+ channel distribution, nodes of Ranvier in the brain of hypomyelinating mouse *Shiverer* are examined. *Shiverer* mice have oligodendrocytes that ensheath axons but do not form compact myelin and axoglial junctions. In these mutant mice, there are far fewer Na^+ channel clusters than in control littermates and aberrant locations of Na^+ channels are observed. If Na^+ channel clustering depends only on the presence of oligodendrocytes and is independent of myelin and oligodendroglial contact, one would expect to find normal Na^+ channel distribution along axons.

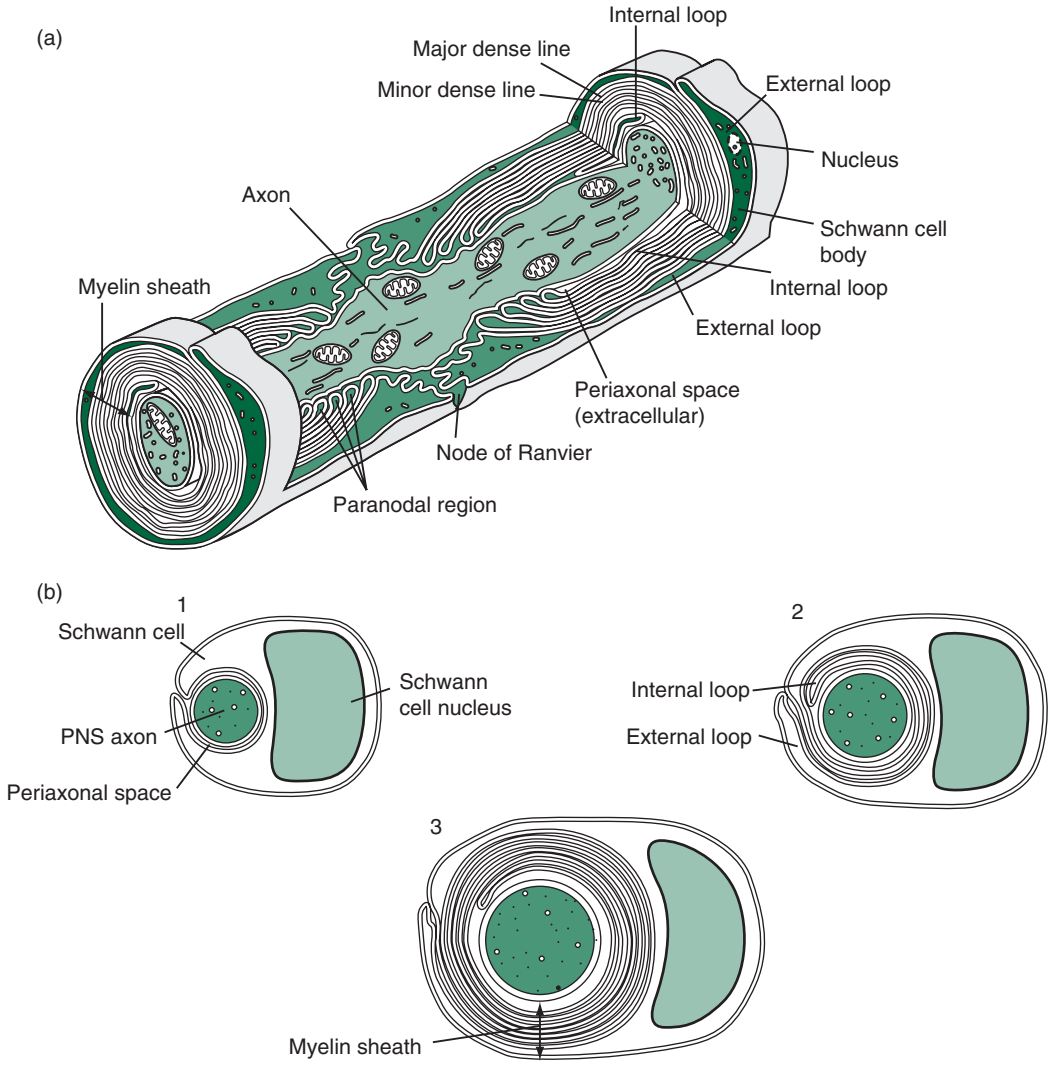

FIGURE 2.7 Myelin sheath of a peripheral axon.
(a) Three-dimensional diagram of the myelin sheath of an axon of the peripheral nervous system (PNS). The sheath is formed by successive rolled Schwann cells. **(b)** Process of myelinization. The internal loop wraps around the axon several times. During this process the axon grows and the myelin becomes compact. Contact between the Schwann cell and axon occurs only at the paranodal and nodal regions. Elsewhere an extracellular, or periaxonal, space always remains. Drawing (a) adapted from Maillet M (1977) *Le Tissu Nerveux*, Paris: Vigot, with permission. Drawing (b) by Tom Prentiss. In Morell P and Norton W (1980) La myéline et la sclérose en plaques. *Pour la Science* **33**, with permission.

2.3 SCHWANN CELLS ARE THE GLIAL CELLS OF THE PERIPHERAL NERVOUS SYSTEM; THEY FORM THE MYELIN SHEATH OF AXONS OR ENCAPSULATE NEURONS

There are three types of Schwann cell:

- those forming the myelin sheath of peripheral myelinated axons (myelinating Schwann cells);
- those encapsulating non-myelinated peripheral axons (non-myelinating Schwann cells); those that encapsulate the bodies of ganglion cells (non-myelinating Schwann cells or satellite cells).

2.3.1 Myelinating Schwann cells make the myelin sheath of peripheral axons

Along an axon, several Schwann cells form successive segments of the myelin sheath. In contrast to oligodendrocytes, it is not a process that enwraps the peripheral axon to form the segment of myelin, but the whole Schwann cell (**Figure 2.7**). Each Schwann cell therefore forms only one myelin segment.

The composition of peripheral myelin differs from that of central myelin only in the proteins it contains. The principal protein constituents of peripheral myelin are: peripheral myelin protein 2 (P2), protein zero (P0) and myelin basic proteins (MBPs). The first two proteins are specific to peripheral myelin. MBP comprises a major part of the cytosolic protein of myelin and is present both in the CNS and PNS. Protein zero is a glycoprotein that has adhesive properties and is located in the interperiod line. It functions, in part, as a homotypic adhesion molecule throughout the full thickness of the myelin sheath. It is a good marker for myelinating Schwann cells as it represents over 50% of total PNS myelin protein.

2.3.2 Non-myelinating Schwann cells encapsulate the axons and cell bodies of peripheral neurons

Non-myelinated axons are not uncovered in the peripheral nervous system as they are in the central nervous system; they are encapsulated. A single non-myelinating Schwann cell surrounds several axons (about 5–20) for a distance of 200–500 μm in man.

In addition, spinal and cranial ganglia contain a large number of Schwann cells that do not produce myelin. These Schwann cells cover the somata of the ganglionic cells, leaving an extracellular space of about 20 nm between themselves and the surface of the covered neuron.

The lipid and protein composition of the plasma membrane of non-myelinating Schwann cells is the same as that of other eukaryotic cells (30% lipid, 70% protein).

Apart from their role in the saltatory conduction of action potentials (myelinating Schwann cells), Schwann cells also play a role in the regeneration of peripheral nerve cells. It has long been known that cut peripheral nerves can, within certain limits, regrow and reinnervate deafferented regions while central axons are not capable of this. This property of regeneration is due in large part to an enabling effect of Schwann cells on axon regrowth.

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