Morpho-Functional Anatomy

General Organisation of the Peripheral Nerve

The peripheral nerve is the "cable" used by the motor, sensory and vegetative neurons' axons to circulate in the peripheral nervous system. It conveys information between these neurons and their effectors in both directions (sensitive receptors, skeletal muscles and viscera). The afferents towards the periphery correspond to the motor and autonomous functions of the nerve, whilst the efferents, originating from the periphery and in charge of carrying information towards the central nervous system, correspond to the sensory nucleus of the nerve. The information is transmitted as nerve impulses, the properties of which depend on, amongst other things, the intrinsic characteristics of the nerve itself.

In adult state, the nerve fibres, constituted of axons and Schwann cells that are associated to them, are grouped in fascicles, wrapped in the perineurium. The perineurium is constituted of layers of perineurial cells of fibroblastic origin, separated by bundles of collagen and linked together by tight junctions. The nerve fibres are associated to Schwann cells which are the only glial cells of the peripheral nervous system. These have an essential role in axon maintenance, myelination and regeneration processes. The nerve fascicles are contained in an areolar connective tissue known as epineurium which contains fibroblasts, collagen and fat in variable proportions. This sheath participates in the fixation of the nerve inside the surrounding structures. It contains the lymphatic and vascular network (vasa nervorum) which crosses the perineurium to communicate with the network of arterioles and venules in the endoneurium. The epineurium constitutes between 30 and 70% of the total surface of the section of a nerve trunk (Figure 1).

A nerve can be constituted of between one and a hundred or so fascicles, their number and distribution being constantly variable thanks to a great number of exchanges of anastomoses. In addition, to a macroscopic level, anastomoses between different nerves are frequent, for instance, the Martin-Gruber anastomosis between the ulnar and median nerve (Figure 2).

It possesses a resistance to stretching thanks to the double action of the "undulating" architecture of the fascicles and the nerve fibres that it contains (Figure 3), but also thanks to the elasticity of the perineurium. The homeostasis of this micro-environment is obtained and maintained by a complex vascular system and by the active barrier constituted by the perineurium. Like the central nervous system, a real blood-nerve barrier is found, its tightness being linked to the properties of the perineurium and to the presence of tight junctions (zonula occludens) between the capillary endothelial cells that penetrate into the endoneurium and the perineurium cells.

1. Nerve fascicle
2. Vasa nervorum : arteriole
3. Vasa nervorum : venule
4. Epineurium
5. Perineurium
6. Nerve fibre
7. Capillary

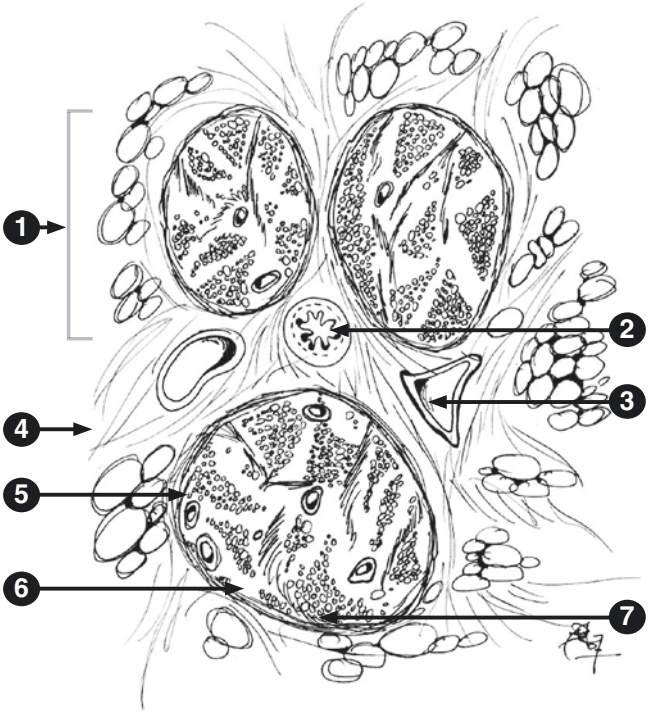

Figure 1. Axial section of a peripheral nerve

Figure 2. Anastomoses of various nerves

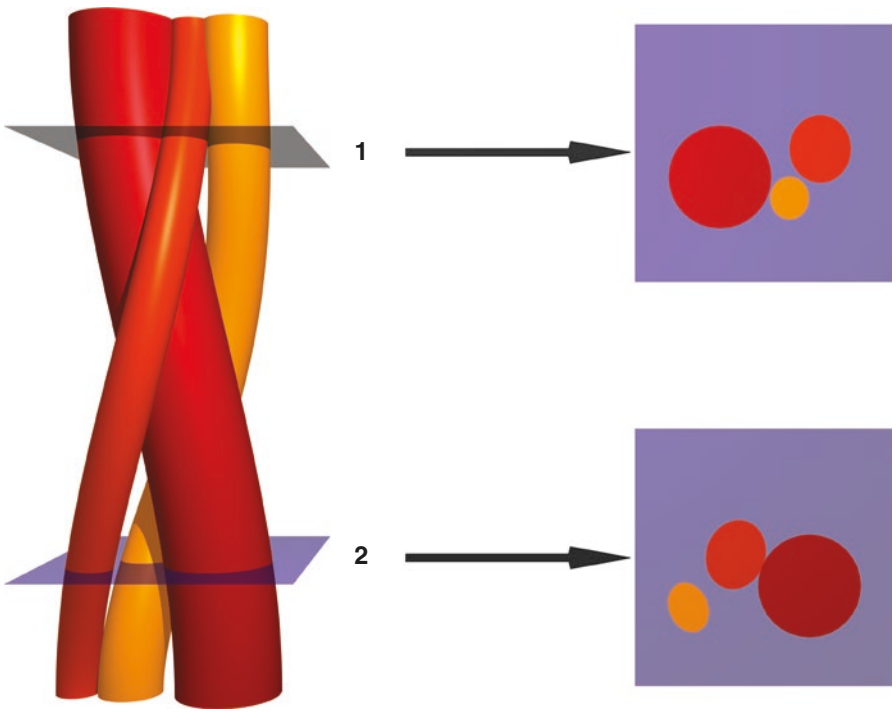

Figure 3. Architecture of fascicles and nerve fibres

The Nerve's Structure and Physiology

Axon

The axon is the cylindrical prolongation of the cytoplasm of the neuron. Its main role is the transmission of nerve impulses. It can only be conceived in the context of a functional unity between the neuron and its target. Its survival is linked to that of the neurons and its targets. Since it does not possess its own capacity of protein biosynthesis, its contents are carried from the core to the periphery by the axonal flow.

Cytoskeleton

The axonal cytoskeleton has a microfibrillar structure composed of three main groups of proteins: the microfilaments, the microtubules and the intermediate filaments including the neurofilaments. These contribute to the maintaining of the shape and growth of the axon. The neurofilaments are constituted of an assembly of three proteins which spread apart during the process of phosphorylation, giving them a fundamental role in the determination of the axonal diameter. This diameter is correlated to myelination, and it is therefore an essential structural parameter. The microfilaments, constituted of an assembly of polymers of globular actin (G-actin), are generally located in areas in motion and at the level of the membrane anchorages which have a significant role in the mobility of the axonal growth cone and in the synaptogenesis. The microtubules, which are heterodimers of alpha and beta tubulin, form hollow tubules on which many other proteins implicated in the processes of assembly and stabilisation as well as the interactions with the rest of the cytoskeleton get fixed on. These microtubules participate to the growth and to the axonal flow.

Axonal Flow

The axonal flow constantly circulates in both anterograde and retrograde directions at variable speeds according to the elements transported and the type of fibres (Table 1). It guarantees a permanent communication between neurons, axon terminations and target cells. It is divided into two fast anterograde and retrograde transports, one slow anterograde transport and one path reserved for mitochondria. On the one hand, the fast anterograde flow transports the vesicular and tubular structures containing the precursors of the neurotransmitters and the membrane proteins, and on the other hand, it transports the mitochondria and membrane lipids. The slow anterograde flow carries the structural proteins of the cytoskeleton and polyproteins. The fast retrograde flow takes back the cellular waste, transports enzymes, growth factors and lysosomal vesicles, and participates in the retro-control of the activity of the soma by the target. This transport is allowed by microtubules thanks to motor proteins (Figure 4): principally kinesin (for the anterograde flow) and dynein (for the retrograde flow).

For the peripheral motor neurons, it is the neuromuscular synapse that corresponds to the extremity of the axon termination relating to its target. At this level, the electric signal is transformed in a chemical signal by mechanisms described hereafter. The arrival of the impulse causes the entrance of calcium by the opening of the voltage-dependent calcium channels, thus triggering a spate of intracellular activation ending with the fusion of the membrane and the synaptic vesicles containing the neurotransmitters, liberated in the synaptic cleft by exocytosis.

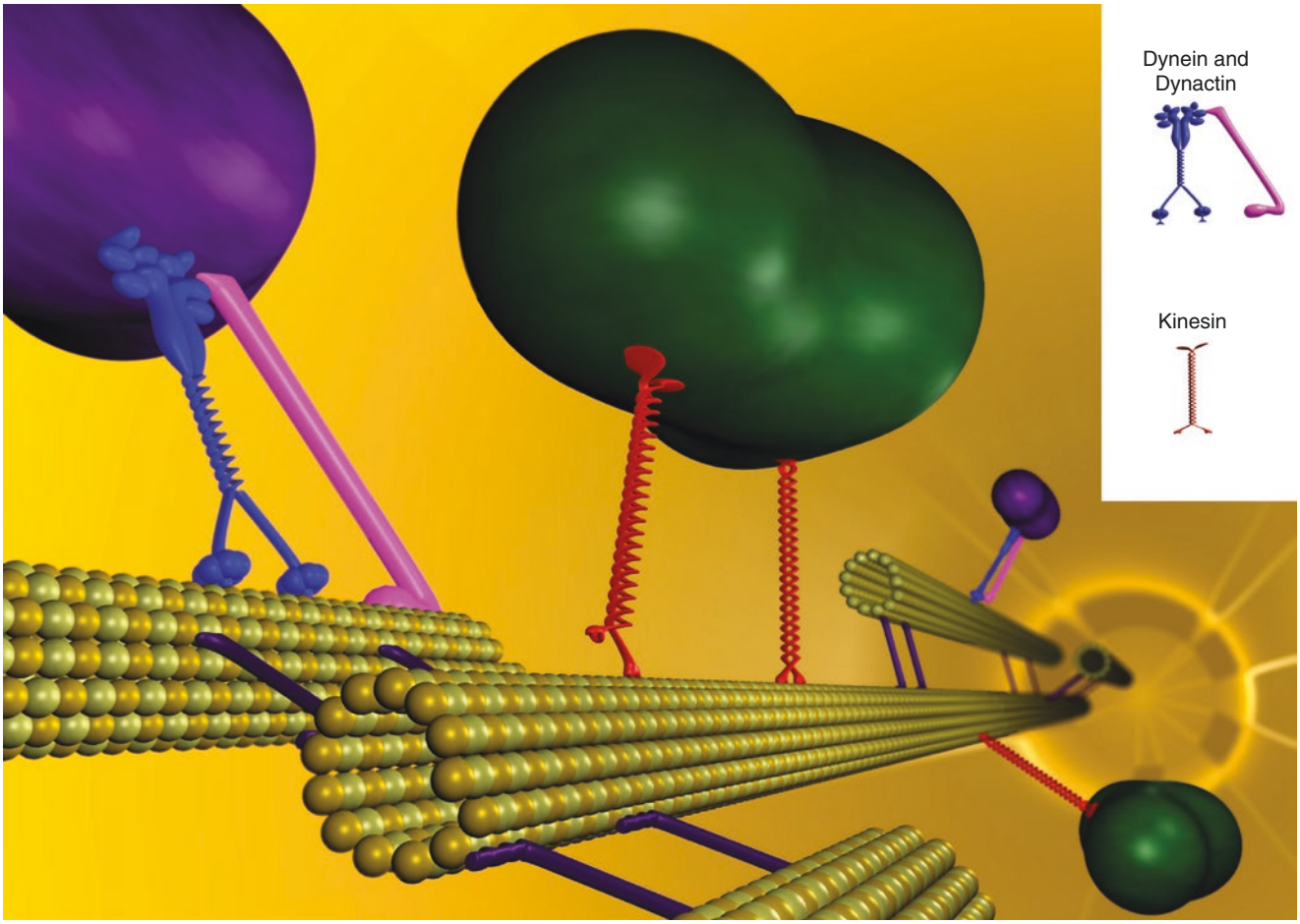

Figure 4. Axonal cytoskeleton

Fibre type		Role	Myelination	Diameter (µm)	Conduction speed (m/s)
Sensory					
Aαβ	Ia	Proprioception: muscle spindles	+	12–20	70–120
	Ib	Golgi tendon organ	+		
	II	Cutaneous sensitivity: touch	+	5–12	30–70
Aδ	III	Cutaneous pressure: temperature	+	2–5	12–30
C	IV	Cutaneous pain: pain	–	0.4–1.2	0.5–2

Table 1. Classification of nerve fibres

Schwann Cell and Myelination

The Schwann cells are the only glial cells represented in the peripheral nervous system (Figure 5). In the mature peripheral nerve, Schwann cells are distributed as longitudinal chains running along the axons. There is a direct relation between the thickness of the myelin sheath and the diameter of the axon and between the diameter of the axon and the internodal distance. The increase of the myelin sheath's thickness and the internodal distance are correlated to that of the diameter of the axon (Figure 6).

Myelination (Figure 7) is observed in the peripheral nervous system (PNS) for axons with a diameter above 1–1.5 µm. The axon's diameter is not the only determining factor of myelination. It follows the histogenesis and happens later, after about 4 months of foetal life. The Schwann cell begins its myelination on a definite segment of the axon. The transitional area separating two myelinated segments is called node of Ranvier. The space separating two nodes of Ranvier is called the internodal space. The myelin sheath ends on each side of a node with a paranodal region.

Myelination speeds up nerve conduction. The conduction of the impulse is continuous (uninterrupted) in the unmyelinated fibres; the maximum obtained speed is limited to 15 m/s. In the myelinated fibres, the excitable membrane is confined to the nodes of Ranvier because the myelin possesses isolating properties. This conduction thus becomes saltatory, from node to node, and can attain speeds up to ten times its original (120 m/s). The number of impulses that can be carried by these fibres is also much greater. Myelination optimises the energetic output of the fibre.

The basal membrane of the Schwann cell directs the axon's growth.

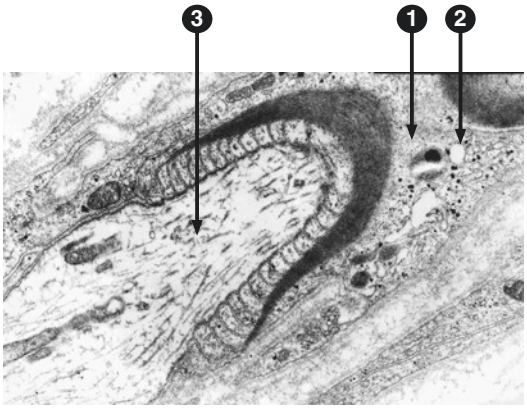

Figure 5. Schwann cell (electron microscopy)

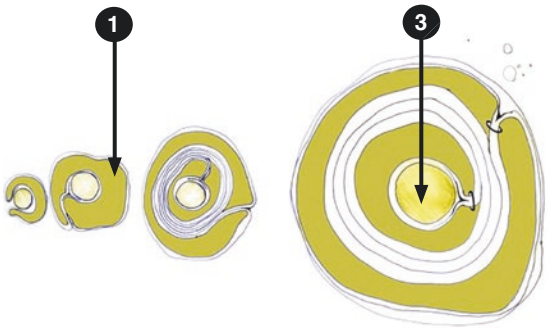

Figure 6. Myelination process (axial section)

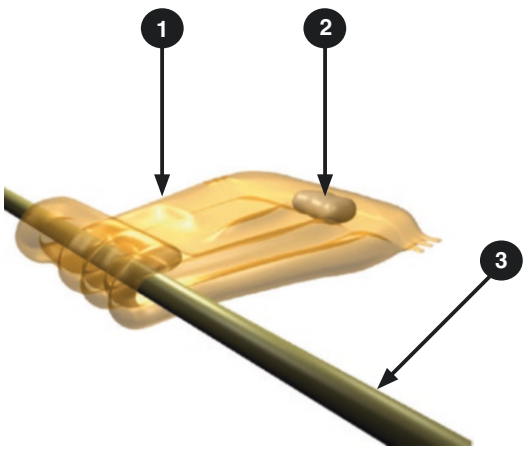

Figure 7. Myelination process

1. Schwann cell
2. Schwann cell nucleus
3. Axon

Mechanical Properties of the Nerves

A peripheral nerve possesses a certain resistance to stretching, thanks to not only the double action of the "undulating" architecture of the fascicles (Figure 3) and the nerve fibres that it contains but also to the elasticity of the perineurium. The tension forces first apply on the fascicle and then on the fibres which, due to this elasticity, keep their normal form for a long time. These forces provoke a shrinking of the fascicle's diameter and an increase of the pressure inside the fascicle that ends up compromising the vascularisation of the nerve if they are applied for too long. A number of factors including the intensity, speed and duration of application of these constraints condition the resistance to stretching. The resistance to these compressing forces varies with the number of fascicles and the girth of the epineurium. The nerves which contain a great number of fascicles and a thin epineurium are weaker against compressing forces (type B fibres compared to type A, in Figure 8), as well as the roots that do not possess a structure corresponding to an epineurium and which have a thinner perineurium.

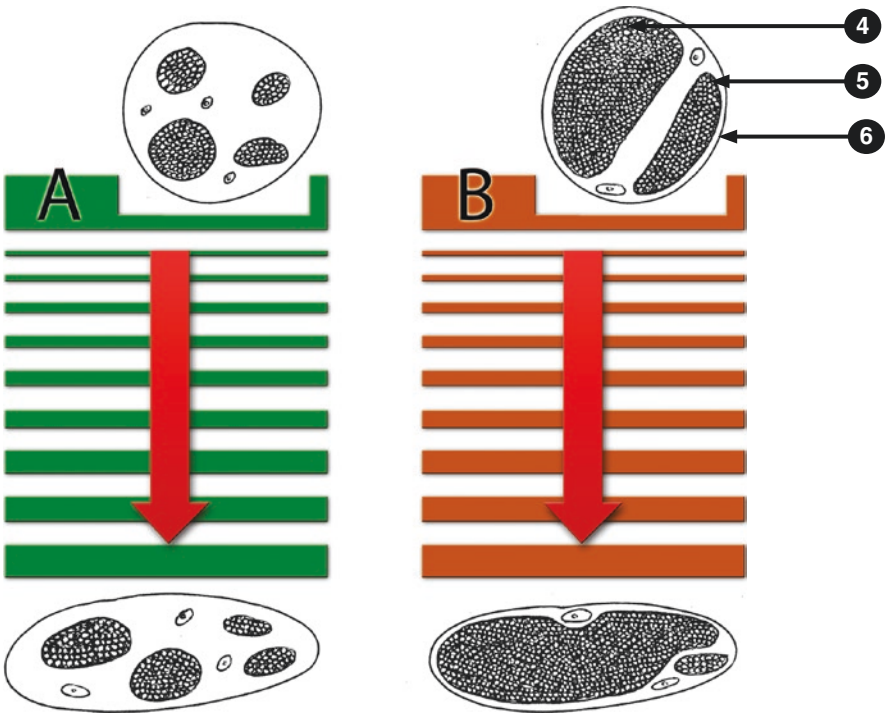

Figure 8. Strength model of a nerve against compression

Vascularisation of the Peripheral Nerves

This vascularisation is special on many fronts. The axon's trophicity is particularly dependent of the endoneurial micro-environment because of the soma's remoteness. The homeostasis of this micro-environment is obtained and maintained by a complex vascular system and by the active barrier constituted by the perineurium. The arterial supply comes from the trunci which are closest to the nerve. Each artery is divided into a descending branch and an ascending branch before splitting into several epineurial branches. There are two distinct systems which are functionally independent but contain a great number of anastomoses: one is extrinsic and constituted of regional feeder vessels and arterio-capillary vessels of the epineurium, and the other is intrinsic and constituted of endoneurial capillaries in a longitudinal distribution (Figure 9). As a result, there is a considerable overlapping between the vascularised areas by the segmental arteries which cross them. The relatively low metabolic needs of the nerve compared to the high basal blood flow and the possibility to function in a situation of anaerobiosis grant the nerve a special resistance to ischemia. However, the central fascicular area remains weaker than the subperineurial area, probably because of a higher density of capillaries and a better penetration of the nutritive substances through the perineurium. There also seems to be a border zone of susceptibility to ischemia between two longitudinal territories. As in the central nervous system, there is a real blood-nerve barrier, its tightness being linked to the properties of the perineurium and to the presence of tight junctions between the endothelial cells of the capillaries penetrating into the endoneurium and the perineurium cells. The epineural and transepineural vasa nervorum are innervated by thin plexuses made of amyelinic vegetative nerve fibres, some being sympathetic (vasoconstricting) and others being parasympathetic (vasodilating). The endoneural capillaries have a smooth, underdeveloped muscular system that suggests a weak autoregulation.

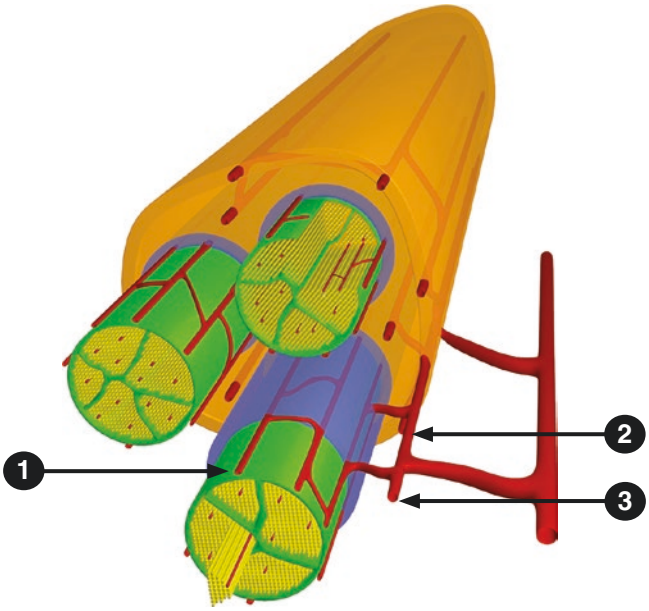

Figure 9a. Longitudinal view of vascularisation

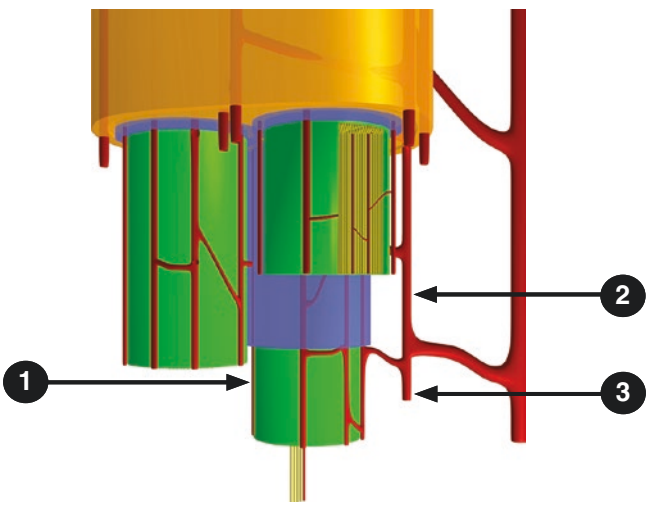

Figure 9b. Side view of vascularisation

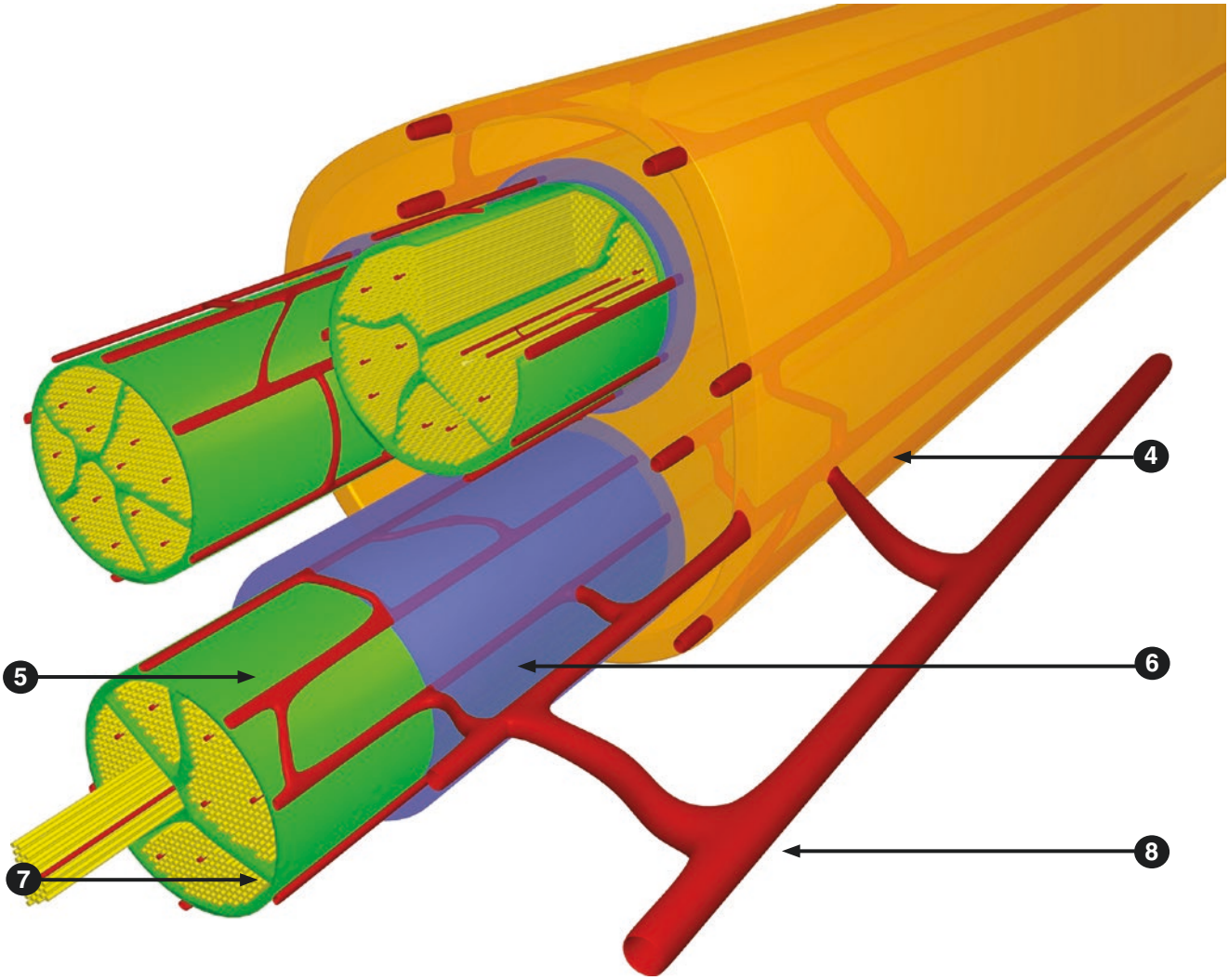

Figure 9c. Microstructure of a peripheral nerve

1. Epineural arterial branch
2. Ascending arterial branch
3. Descending arterial branch
4. Epineurium and connective tissue
5. Endoneurium
6. Perineurium
7. Axon
8. Vasa nervorum

Neuromuscular Junction and Transmission

The musculoskeletal system is the mechanical interface between our nervous system and the external world. The mechanical properties of muscles have been very largely preserved during the phylogenesis of the vertebrates. These have been crucial in the adaptation of the neuronal mechanisms for movement.

A single motor neuron is bombarded by synaptic stimuli, which will result in determining the manner of and intensity at which the target muscle fibre will participate to the realisation of a motor programme. This response of the nerve cell to a stimulus is allowed by a modification of its membrane properties. The neuromuscular synapse is the junction area between the axon of a motor neuron and a muscular cell. In mammalians (with a few exceptions), there is no real contact at the synaptic level. The synaptic gap (between 10 and 40 nm) separating these cells acts as an isolating structure. This neuromuscular junction (NMJ) (Figure 10) is made up of the apposition of highly differentiated domains of three kinds of cells: the nerve termination of the motor neuron, the Schwann cell called "terminal" and the postsynaptic membrane of the muscle fibre. These three elements are surrounded or linked together by a basal lamina, which is a favourable micro-environment for the exchange of molecular signals that control the formation, maturation and sustenance of the NMJ. The NMJ forms a functionally and structurally differentiated complex, the goal of which is to guarantee the synaptic transmission within the neuromuscular apparatus by managing the propagation of the motor neuron's impulse towards the skeletal muscle fibre.

The nerve termination releases a neurotransmitter in the synaptic gap, the acetylcholine (ACh), which connects on specific nicotinic receptors (the receptors of the acetylcholine or AChR), located under the invaginations' cristae or subneural folds of the postsynaptic membrane of the muscle fibre. The activation of these receptors causes a depolarisation of the muscle membrane leading to a chain reaction named excitation-contraction coupling (ECC) inducing the contraction of the adjacent muscle fibre. Several tools have been developed to characterise in a simple way the morphological aspect of the normal NMJ and the abnormalities that ensue from the pathological modifications of these junctions. The advent of molecular biology has allowed the discovery of a great number of synaptic molecules concentrated at the junction and thus favoured the understanding of the physiopathological mechanisms implied in the phenomena of denervation and reinnervation and in neuromuscular pathologies. For example, the congenital myasthenic syndromes, which form a heterogeneous group of affections of genetic origin, lead to a dysfunction of the neuromuscular transmission. Their characterisation relies on bringing to light structural abnormalities in the NMJ, mutations in the genes coding the concentrated proteins at the level of the motor areas, and on the molecular mechanisms by which such mutations induce the illness.

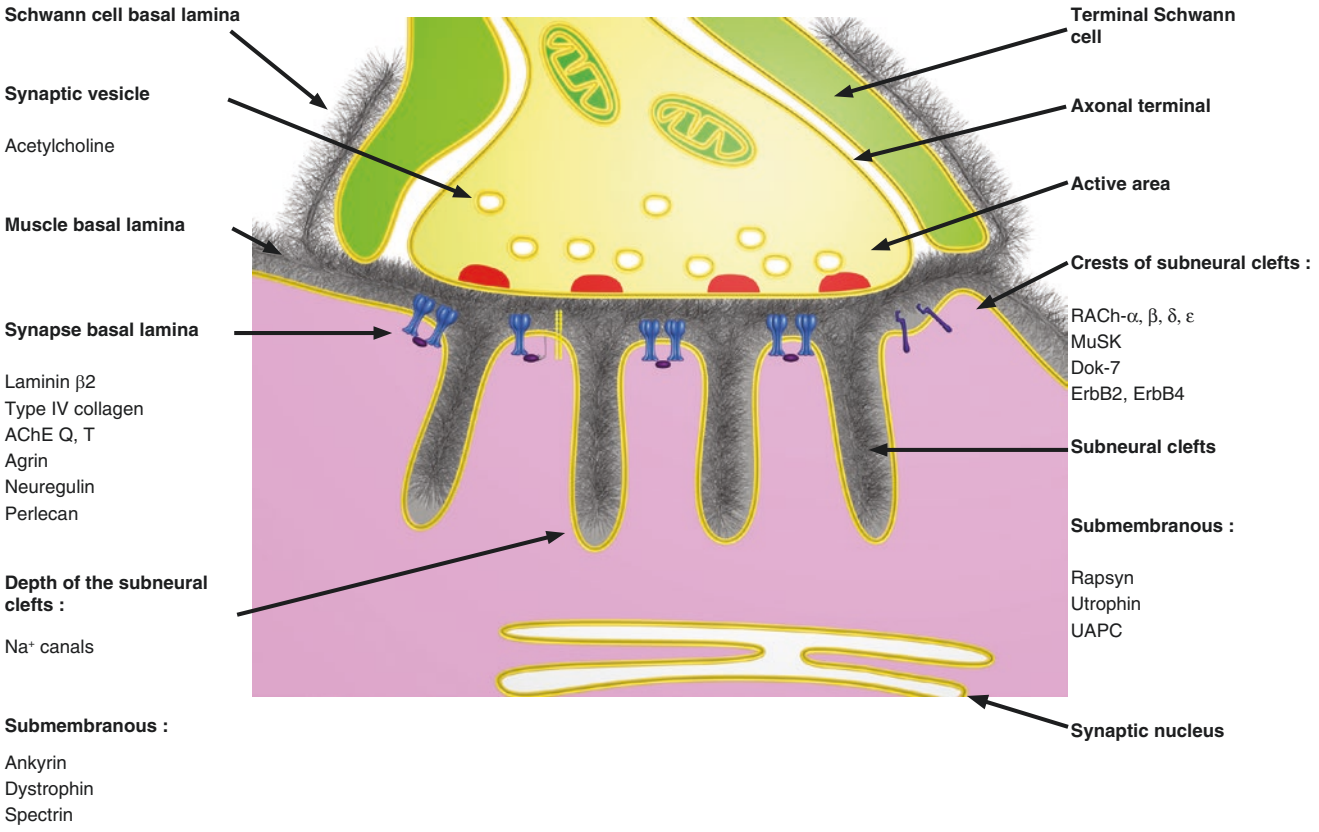

Figure 10. The neuromuscular junction (According to Sanes and Lichtman 1999)

Main Mechanisms of Synaptic Formation

Synaptic formation is a necessary process during neuronal development allowing communication between two neurons. One of the main characteristics of the development of the nervous system is the specificity of its connections. As such, the axons' migration towards their target and the formation of the synapses are selective processes, implicating many recognition molecules, most of which remain unknown.

The synthesis and distribution of the acetylcholine receptors at the level of the postsynaptic membrane of the NMJ indeed seem regulated by anterograde signals originating from the motor neuron. The differentiation of axonal termination is however regulated by retrograde signals. The nerve and muscle have distinct roles in the differentiation of the synaptic compartment. The initial steps of this differentiation and formation of the neuromuscular junction require several postsynaptic molecular agents including receptor tyrosine kinase protein MuSK and rapsyn. The dependency to agrin or motor neuron remains controversial, whilst the following steps of the axonal growth and the sustainment of the postsynaptic apparatus mostly depend on neural agrin and on a specific signal emanating from the nerve fibre, responsible for the dispersion of the remnants of aggregates of ectopic acetylcholine receptors, all this possibly managed by the acetylcholine itself. The neuregulin essentially intervenes in the sustainment of the Schwann cell which guides axonal growth. The synaptic formation of the central nervous system actually presents a high number of similarities with the development of motor innervations. This allows the study of some mechanisms of recovery of the nerve connections after a traumatic or degenerative nerve injury and thus leads to the discovery of new treatments that could favour recovery on a functional point of view.

One can distinguish three fundamental steps in synaptic formation: the creation of a connection between the growing axon and its target cell, the differentiation of the axonal growth cones into a nerve termination and finally the formation of postsynaptic structures in target cells. These steps depend on intercellular interactions mediated by signals, responsible for the recognition by the axon of the appropriate postsynaptic cell, and the coordination of the formation of various pre- and postsynaptic structures at the synapse's level.

As soon as there is contact between the extremity of a growing axon and a myotube, a neurotransmission occurs, even in a rudimentary form, notably by the intermediary of the acetylcholine vesicles. This leads to the creation of the synaptic zone, especially thanks to many retrograde signals, coming from the muscle and going towards the axon. Indeed, the intrinsic properties of the various involved cellular elements are not sufficient. Studies have shown that after a denervation synapses are able to regenerate, especially if there is a preserved postsynaptic membrane. Furthermore, the presynaptic specialisation of the axon starts only after contact with a muscle. It is then obvious that a muscle feedback on the axons exists, but the actual mechanisms are yet to be known. Two types of cell adhesion molecules, the N-CAM and the N-cadherin, situated at the level of the axonal terminations and myotubes, would stabilise the contact between the muscle and nerve.

The synaptic formation completes that of the nervous system by giving it its functionality. It needs a rigorous spatio-temporal organisation: the nerve termination has to reach a specific area of the target cell, and the synaptic membrane needs to be very sensible to the neurotransmitters sent by the corresponding nerve termination. This functional set has to be stable enough to subsist for a whole lifetime, but at the same time adaptable enough to evolve with the learning processes.

Synaptogenesis is a highly specific process as well: even though the pre- and postsynaptic cells are able to synthesise their own components, the exchange of many signals is necessary in order to coordinate their activity at all times. As for the NMJ, in vitro models have initially proved that two molecules, the agrin and the ARIA neuregulin β1, could be responsible for the accumulation, synthesis and maturation of the acetylcholine receptors. Knockout of the genes coding for these two molecules has been used in mice to clarify their role during the junction's development.

The latest concepts have allowed a very clear specification of the role of each of these molecules in the maturation of the NMJ. MuSK remains the hub of postsynaptic differentiation. The accumulation and synthesis of AChR are guided by agrin (aggregation of receptors by way of the interaction of the MuSK/agrin complex with the rapsyn but also with a

characteristic action preventing their separation) and Dok-7 that allows their phosphorylation to MuSK. The maturation of AChR could also result from the interaction between agrin and MuSK via the implication of GTPases (Rac/Cdc42) in the transcriptional regulation of the receptors' subunits (Figure 11). The neuregulin emanating from the nerve would essentially act by its interaction with its receptors situated on the surface of the terminal Schwann cell and is now considered a key molecule in the sustainment of the Schwann cell and so, through these means, of nerve regeneration.

The involvement in the synapses of the CNS of some of these molecular actors illustrates quite well the complexity of the anterograde and retrograde interactions required for the formation, development and sustainment of the NMJ. The scientific interest aroused by the major challenge of public health to try and figure out the mechanisms allowing for neuron plasticity and reparation, especially at the level of the CNS, has led to the discovery of some factors influencing axon regeneration and opened the way to new therapeutic propositions, their aim being to restore function in the event of a nerve injury.

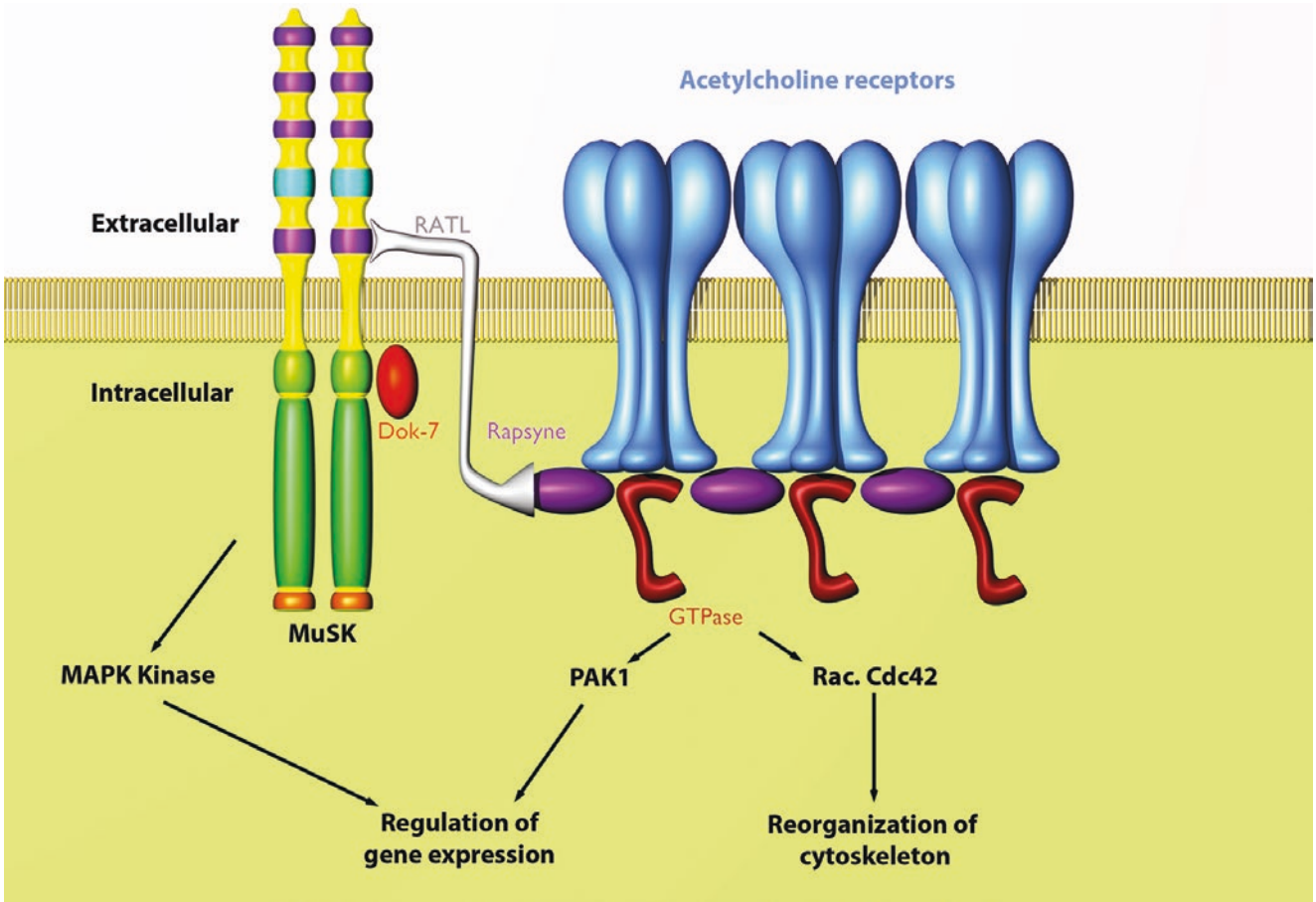

Figure 11. Role of the kinase proteins in the transmission of nerve impulse (According to Valenzuele, 1995, Zhou, 1999)