

Neurofilaments: Organization and Function in Neurons

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Neurofilaments in Neuronal Function

Introduction to Neurofilaments

A neuron is composed of the neuronal cell body (perikaryon) and several processes (dendrites and axon), of which the axon is usually the longest one. The axon is comparable to an electrical wire that the neuronal cell body extends to other neurons or muscles to transmit its signal. Assembled neurofilaments are an essential part of the cytoarchitecture of mature axons and are particularly important in establishing the diameter of the largest axons, where they are the most abundant structural component. The first description of this fibrous axonal network dates back to the late nineteenth century, when newly developed silver staining methods allowed anatomists to visualize so-called neurofibrils within axons. Neurofilaments form a very dense network of discontinuous, cablelike filaments (oriented along the axon) which are interconnected by small extensions. This reversibly cross-linked meshwork of structural elements provides mechanical stability to the axon (Figure 1).

Structure of the Neurofilament Proteins

The subunits of neurofilaments belong to one of five classes of proteins and assemble into long protein polymers named intermediate filaments. There are more than 50 different kinds of these proteins in humans. Intermediate filaments are involved in cell architecture of most eukaryotic cells. The name 'intermediate' derives from the diameter of the assembled filaments (8–10 nm), which distinguishes them from the two other major structural polymers of the cytoskeleton, the actin microfilaments (6 nm) and the microtubules (24 nm). In a large myelinated axon, the neurofilaments make up the bulk of the intra-axonal volume, with long polymers interlinked to each other and to the thicker and structurally more rigid microtubules, which are outnumbered (by 10- to 30-fold) by neurofilaments. The actin microfilaments form a spiderweb-like structure underneath the axonal membrane. Both neurofilaments and microtubules are aligned in the same orientation along the axon, and all three cytoskeletal structures (actin microfilaments, neurofilaments, and microtubules) are interconnected by a separate family of proteins,

the plakins, to produce a reversibly cross-linked filament meshwork that proves a flexible, deformable array that provides mechanical strength.

Neurofilaments are obligate heteropolymers requiring their smallest subunit, neurofilament light (NF-L; 60 kDa), and the two large subunits, neurofilament medium (NF-M; 90 kDa) and neurofilament heavy (NF-H; 115 kDa). Like all other intermediate filament subunits, the molecular structure of each of the three neurofilament subunits contains a central helical rod domain of approximately 310 amino acids and a subtype-specific globular domain at the head. The rod domain is highly hydrophobic and can form stable coiled-coil dimers with two protein subunits wrapped around each other within the rod domain, forming an initial assembly unit around 50 nm in length. The actual neurofilament is built up by assembling the coiled-coil dimers and then aligning both longitudinally and laterally in a staggered fashion, ultimately to form a ropelike structure 10 nm in diameter and of indeterminate length. NF-M and NF-H are unique among the intermediate filament subunits in that each contains an extended tail domain (about 400 and 600 amino acids long, respectively) which protrudes from the axis of the assembled filament. The NF-M and NF-H tail domains protrude up to 80 nm from the assembled filaments and interact with neighboring neurofilaments and microtubules (Figure 1). The stoichiometry of the neurofilament subunit composition varies but is approximately 5:3:1 for NF-L:NF-M:NF-H.

Peripherin and α -internexin represent two additional components of neuronal intermediate filaments present in a subset of neurons. They share the structural features of intermediate filament subunits, and both can co-assemble with neurofilaments. In the mature nervous system, peripherin is expressed predominantly in small-caliber sensory neurons of the peripheral nervous system and also to some extent in motor neurons of the central nervous system (CNS). α -Internexin is a component of certain types of CNS neurons, mainly the cerebellar granule cells.

Function of Neurofilaments

In the human nervous system, the largest neurons and the ones with the longest axons are the lumbar spinal cord motor neurons, which mainly innervate the leg muscles. There are thousands of these motor neurons, each with a cell body around 50 μ m in diameter and each extending a single axon to its target muscle. These are the most asymmetric cells in nature, with axons in humans up to a meter in length (the full length of the leg), but even the largest have a maximal

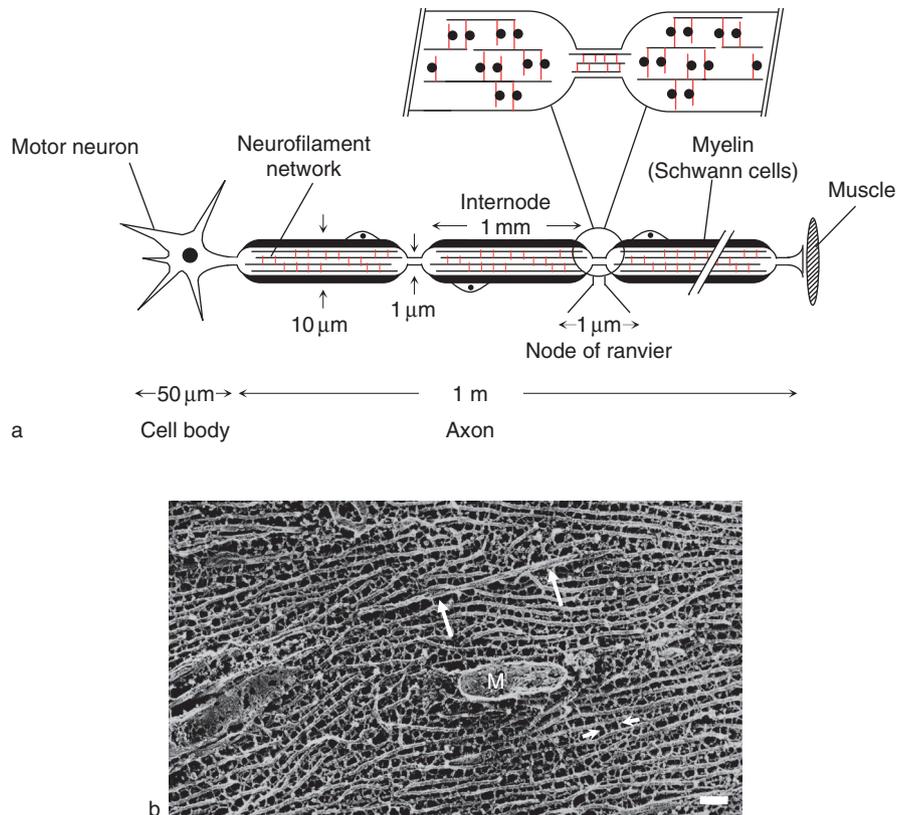


Figure 1 Neurofilaments are the major component of the cytoskeleton of large myelinated axons. (a) Schematic representation of the structural features of a human spinal cord motor neuron innervating muscle with the myelinating Schwann cells along the axon. The neurofilament network is represented by black lines, with the cross-linking neurofilament tail domains in red. The enlargement shows that the tail domains of the neurofilaments are heavily phosphorylated (black circles) in the internodes (large axonal caliber), whereas at the Node of Ranvier they are not (small axonal caliber). (b) Neurofilaments in the axon. Quick-freeze deep-etch view of the axonal cytoskeleton from a large myelinated axon, showing the neurofilament network. Arrowheads point to cross-bridges between the 10-nm-diameter neurofilaments, and arrows point to the single microtubule in the field. M, mitochondria. Scale bar = 100 nm. Reproduced from Hirokawa N (1982) Cross-linker system between neurofilaments, microtubules, and membranous organelles in frog axons revealed by the quick-freeze, deep-etching method. *Journal of Cell Biology* 94(1): 129–142, by copyright permission of The Rockefeller University Press.

axonal diameter ($14\mu\text{m}$ in humans) that is only $1/70\,000$ th of their length. A real-life example of these cell dimensions is a soccer ball (with a diameter of 22 cm) as the cell body and a tube 6 cm in diameter extending over the length of 44 soccer fields, each 100 m long, as its axon. A large motor axon is more than 2000 times the volume of its cell body.

Neurofilaments are central determinants in generating normal axonal diameters. Mature axonal caliber is of importance for normal neuronal function because the speed of transmission of the electrical signal along the axon (the nerve conduction velocity) is directly proportional to the diameter (the bigger the diameter, the faster the speed). Complete loss of neurofilaments caused by loss of the major neurofilament subunit NF-L (either by gene deletion or by mutation that precludes synthesis of NF-L) blocks growth in axonal diameter that normally is contemporaneous with myelination. In mice, loss of neurofilaments is

asymptomatic, but in larger animals (as seen in the quiver quail), absence of neurofilaments and reduced conduction velocity are accompanied by constant quivering and behavioral abnormalities.

The extensive, multiphosphorylated tail domains of NF-M and NF-H subunits (in human with 43 or 44 sites on NF-H and at least seven sites on NF-M) have, for a long time, been proposed to mediate neurofilament-dependent growth of axonal diameter that takes place within myelinated axonal segments. Although it has been proposed that space-filling properties of neurofilaments are achieved by electrostatic repulsion between these highly charged domains on adjacent neurofilaments, this is not the case. Deletion of the entire NF-H tail domain has little effect on radial growth, with organization of the remaining neurofilament array largely unaffected. Removal of the NF-M tail, on the other hand, dramatically limits acquisition of correct axonal diameter, producing a

50% reduction of caliber (fourfold reduction in overall axonal volume) and concomitant lower nerve conduction velocities. Replacement by transplantation of normal Schwann cells with mutant ones that are unable to produce myelin blocks this growth in diameter, but only within the unmyelinated segment, precluding neurofilament tail phosphorylation in the transplanted axonal region, whereas in the neighboring nerve regions with normal myelination, caliber and phosphorylation state remain normal. Thus, growth in diameter is limited to myelinated portions of the axon, evidence supporting the current model that correct diameter is achieved through an 'outside-in' signal generated by the enwrapping myelinating Schwann cells (Figure 1).

The Involvement of Neurofilaments in Neurodegenerative Diseases

Abnormal assembly and/or accumulations of neurofilaments are thought to be involved in the pathology of several human neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), infantile spinal muscular atrophy, and hereditary sensory-motor neuropathy. This is especially so in ALS (known familiarly in the United States as Lou Gehrig's disease), the most prominent human adult motor neuron disease. ALS is typically fatal, with onset between 50 and 60 years of age and complete paralysis within 1–5 years after onset, mainly resulting from premature death of upper (brain) and lower (spinal cord) motor neurons, especially the subset with the largest axons and the highest neurofilament contents. Aberrant neurofilament accumulations both in the neuronal cell bodies and in axons are a major pathological hallmark in both sporadic and familial ALS cases (Figure 2).

Disorganized Neurofilament Arrays as a Cause for Motor Neuron Disease

Altered neurofilament organization can be a primary cause of motor neuron disease. Expression of a mutant NF-L subunit whose incorporation into neurofilaments disrupts their continued assembly provokes an aggressive, fatal, early onset motor neuron disease producing paralysis caused by massive neurodegeneration and death of motor neurons. Even increased synthesis of normal NF-L or NF-H subunits has been shown to produce abnormal accumulations of neurofilaments in motor neuron cell bodies and proximal axons, accompanied by motor neuron dysfunction and atrophy of the target muscles, very similar to the pathological signs of ALS.



Figure 2 Accumulations of neurofilaments in motor neurons in the spinal cord of a patient with amyotrophic lateral sclerosis. Most of the perinuclear area is occupied by abnormal aggregates of 10 nm neurofilaments. Scale bar = 40 μ m. Reproduced with permission from Hirano A (1988) *Color Atlas of Pathology of the Nervous System*, 2nd edn. Tokyo: Igaku-Shoin Medical Press, with permission from Igaku-Shoin Ltd.

Mutations in Neurofilament Genes Linked to Human Motor Neuron Diseases

Genetic evidence in humans has suggested mutations in neurofilament genes as contributors or direct causes for human motor neuron diseases. The most compelling evidence comes from missense mutations in the *NF-L* gene that are strongly associated with human Charcot-Marie-Tooth Disease Type 2E (CMT2E). CMT2E is a milder motor neuron disease than ALS, with an earlier onset and a slower progression and typified by muscle weakness and (depending on the severity) partial paralysis. With respect to ALS, several mutations have been found in the tail domain of the *NF-H* gene in a small group of sporadic ALS patients but not in control individuals. A large-scale sequencing of DNAs from ALS and control individuals has demonstrated that neurofilament mutations are not a significant primary cause of ALS, albeit they may be risk factors for ALS. These findings suggest, but fall short of proving, linkage of neurofilament sequence variants as contributors to human disease.

Peripherin is another intermediate filament subunit of mature motor neurons, and an abnormal version of it has also been suggested to be involved in ALS. Peripherin is normally encoded by two differentially spliced mRNAs transcribed from the same gene and encoding proteins with slightly different molecular weights (58 and 56 kDa). An aberrantly spliced form that generates a 61 kDa protein and that is both assembly incompetent and toxic *in vitro* in neuronal cells has been detected in motor neurons of a few human ALS patients and in ALS mouse models, suggesting a linkage of aberrantly spliced peripherin and human motor neuron disease.

Axonal Neurofilaments as Determinants of Neurodegeneration in ALS

Most incidences of ALS are sporadic, that is, they are without evidence for a genetic origin. Approximately 10% of ALS is inherited in an autosomal dominant manner, and a proportion of those are caused by missense mutations in the gene encoding the ubiquitously expressed Cu/Zn-superoxide dismutase 1 (SOD1). Mice that accumulate such mutations develop a fatal-progressive motor neuron disease comparable to ALS in humans, accompanied by massive motor neuron death and paralysis. As in ALS in humans, it is specifically the largest motor neurons with the biggest axons and highest neurofilament content that are most affected.

Neurofilament accumulations in spinal motor neurons are prominent hallmarks of all ALS disease forms, including mutant SOD1-induced ALS, both in humans and in transgenic mouse models. Elimination of assembled neurofilaments from axons (by deleting NF-L) or trapping most neurofilaments in motor neuron cell bodies (by overexpressing NF-H) both greatly slow ALS-like disease. Although the disease-modifying effect of altering neurofilament content could be achieved through increased accumulation of neurofilaments in the neuronal cell bodies or their removal from axons, removal of the NF-M and NF-H tail domains (by gene replacement in mice) provides a comparable benefit without altering axonal neurofilament content. This outcome eliminates the possibility that the phosphorylation site-rich tail domains of NF-M and NF-H can serve as phosphorylation sinks for buffering a detrimental mutant SOD1-induced hyperactivation of kinases, including cyclin-dependent kinase 5. Thus, neurofilaments are a modifier of ALS caused by mutant SOD1, probably through their influence on slowing transport of other cargoes transported along the axon.

Conclusion

Neurofilaments are essential for establishing a flexible, deformable, reversibly cross-linked array that supports growth in axonal diameter. Absence of neurofilaments reduces conduction velocity, resulting in behavioral abnormalities. Changes or dysregulations

of the neuronal neurofilament network can induce pathological signs of neurodegeneration and even neuronal death. Certain human neurodegenerative diseases, such as CMT2E, are directly caused by mutations in a neurofilament gene. Other human neurodegenerative diseases, such as the motor neuron disease ALS, show pathological neurofilament accumulations, although mutations in any of the three neurofilament genes represent disease modifiers rather than direct causes. Changes in the neurofilament network can strongly influence the ALS disease course.

See also: Amyotrophic Lateral Sclerosis (ALS); Axonal Transport and ALS; Axonal Transport and Neurodegenerative Diseases; Intermediate Filaments.

Further Reading

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