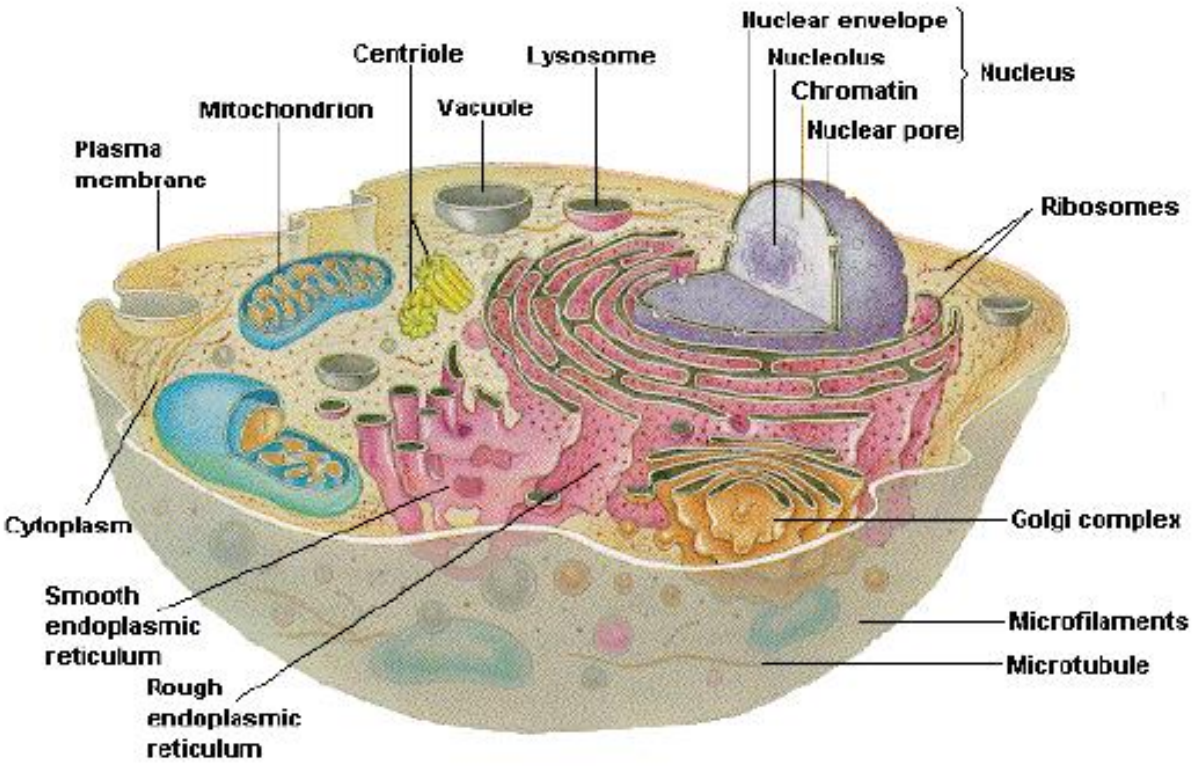
Typical Eukaryotic Cell



ORGANELLES

What is different in a neuron?

- Cytoplasm
- Plasma membrane
- Nucleus
- Mitochondria
- smooth ER
- rough ER
- Golgi
- Membrane Organelles
  - Lysosomes
  - Peroxisomes
  - Endosomes
- Cytoskeleton
  - Microfilament
  - Intermediate filaments
  - Microtubules
- Adherens Junctions
- Centrioles
- Cilia
- Ribosomes
- Proteosomes

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- Ribosomes
- Proteosomes

Neuronal plasma membrane

- Much much larger area than typical cell
  - Long axons and very complex dendrites, axon 20000 x soma size
- Unique electric properties- electrically active
  - many ion channels in neurons
  - K^+, Cl^-, Na^+, Ca^+ channels which determine electrical properties of cell.
- Different local domains with unique properties
  - Nodes of Ranvier, synaptic terminals, axon hillock, dendritic spines.

The neuronal plasma membrane contains several local domains with unique properties

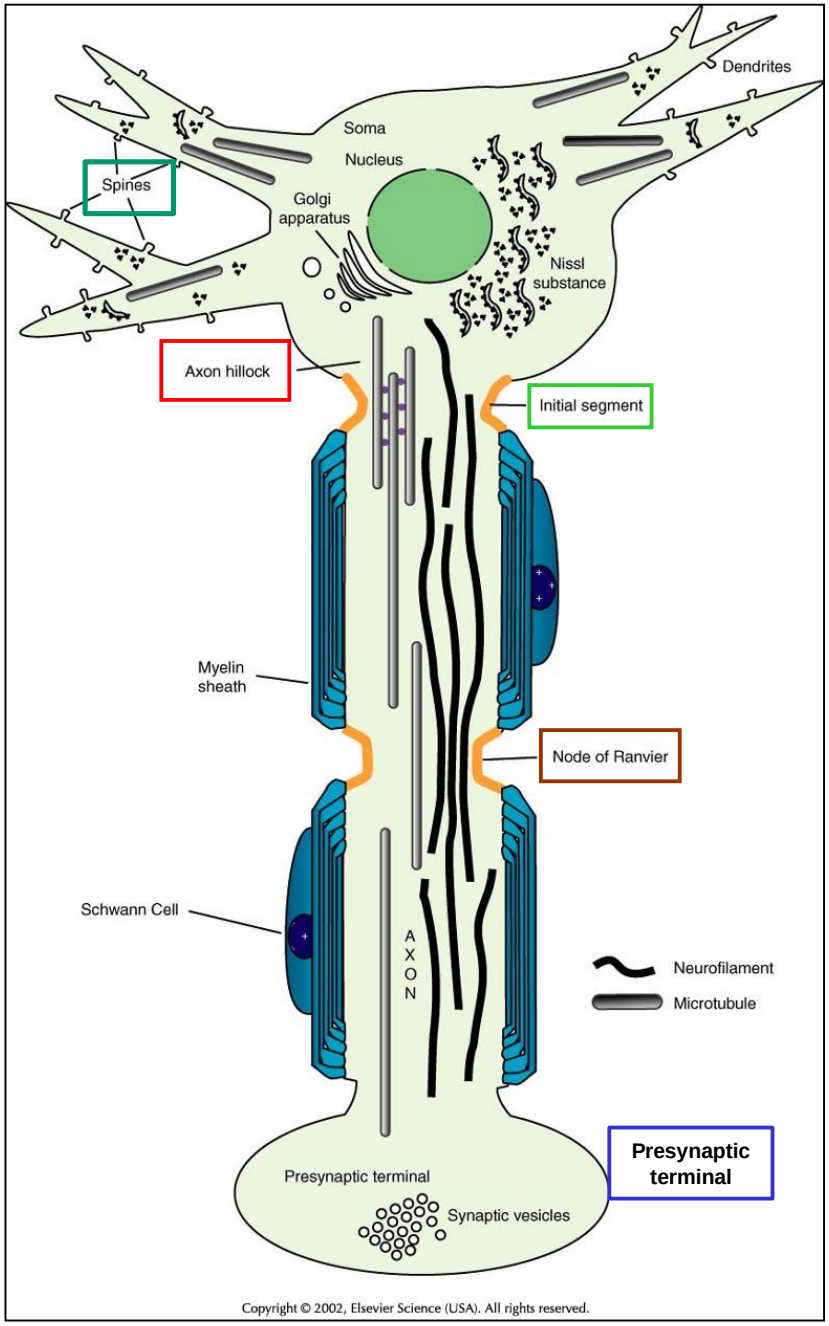
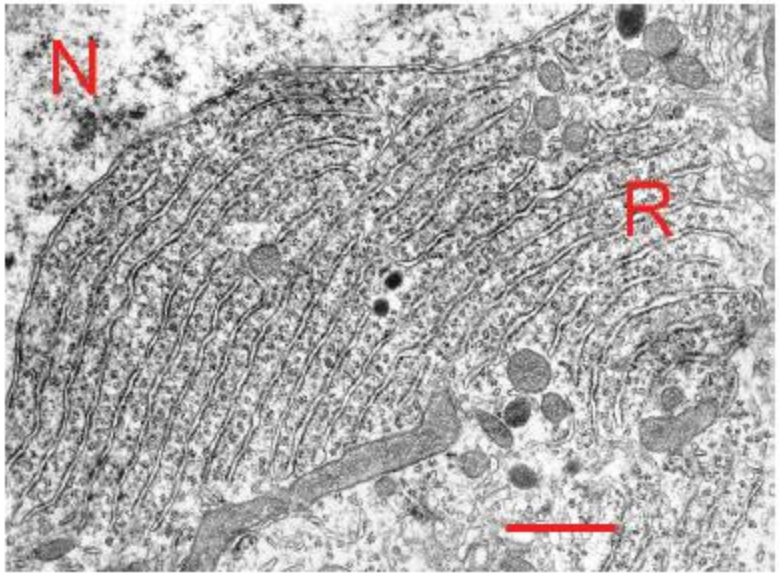
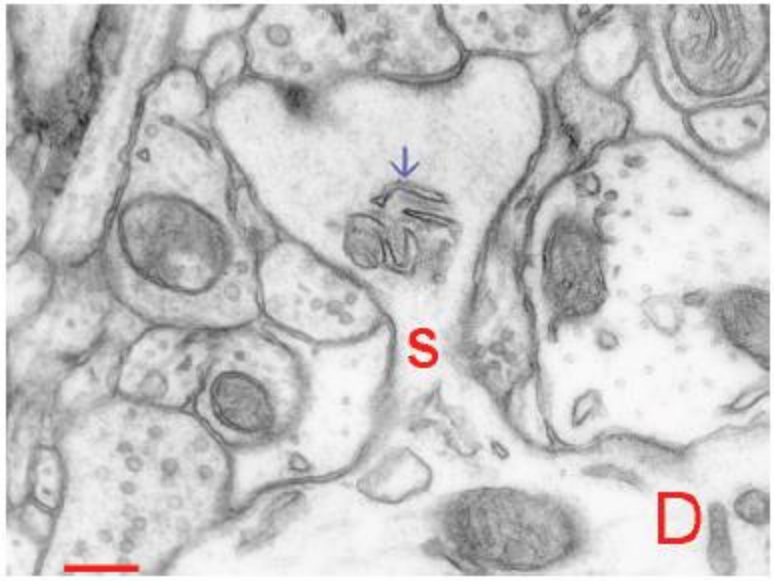


FIGURE 2 Basic elements of neuronal subcellular organization. The neuron consists of a soma, or cell body, in which the nucleus, multiple cytoplasm-filled processes termed dendrites, and the (usually single) axon are placed. The neuron is highly extended in space; a neuron with a cell body of the size shown here could easily maintain an axon several miles in length! The unique shape of each neuron is the result of a cooperative interplay between plasma membrane components (the lipid matrix and associated proteins) and cytoskeletal elements. Most large neurons in vertebrates are myelinated by oligodendrocytes in the CNS and by Schwann cells in the PNS. The compact wraps of myelin encasing the axon distal to the initial segment permit the rapid conduction of the action potential by a process termed "saltatory conduction" (see Chapter 3).

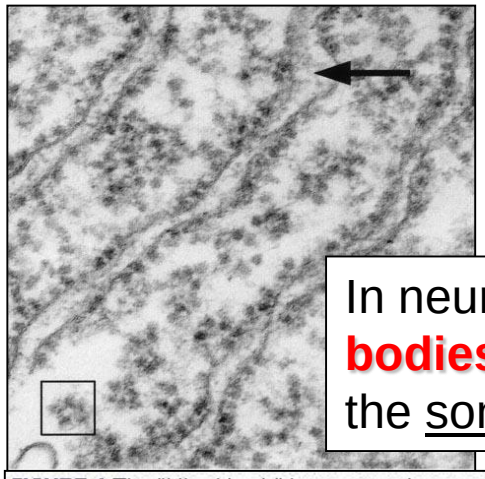
Endoplasmic Reticulum

- important source of calcium
- rER site of translation and insertion of membrane proteins
- rER extensive in many neurons and a major component of Nissl bodies
- Spine apparatus a specialized ER compartment





Where is the protein synthesis machinery in neurons?

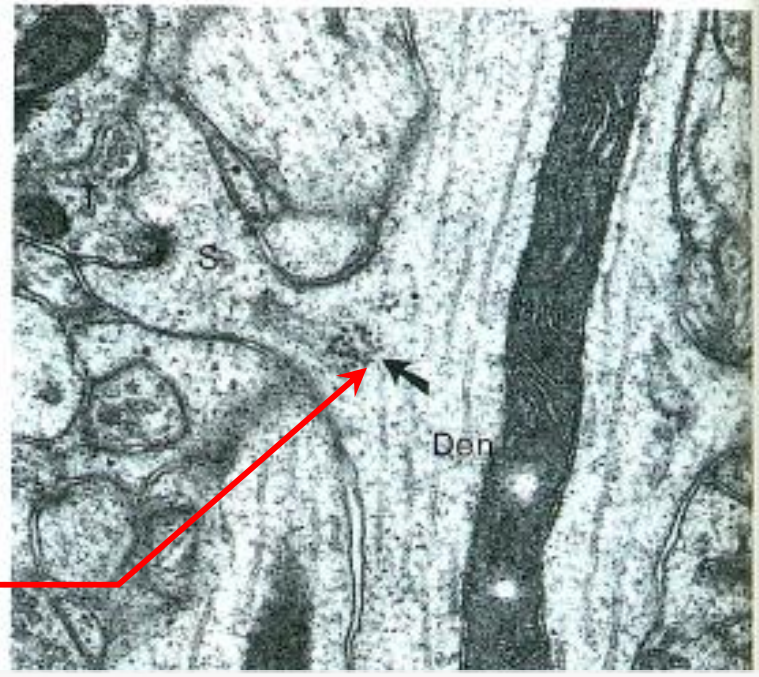


In neurons the **Nissl bodies** are present in the soma and dendrites

FIGURE 1 The "Nissl body" in neurons is an array of cytoplasmic-free polysomal rosettes (boxed) interspersed between rows of rough endoplasmic reticulum (RER) studded with membrane-bound ribosomes. Nascent polypeptide chains emerging from the ribosomal tunnel on the RER are inserted into the lumen (arrow), where they may be processed before transport out of the RER. The relationship between the polypeptide products of these "free" and "bound" polysome populations in the Nissl body, an arrangement that is unique to neurons, is unknown.

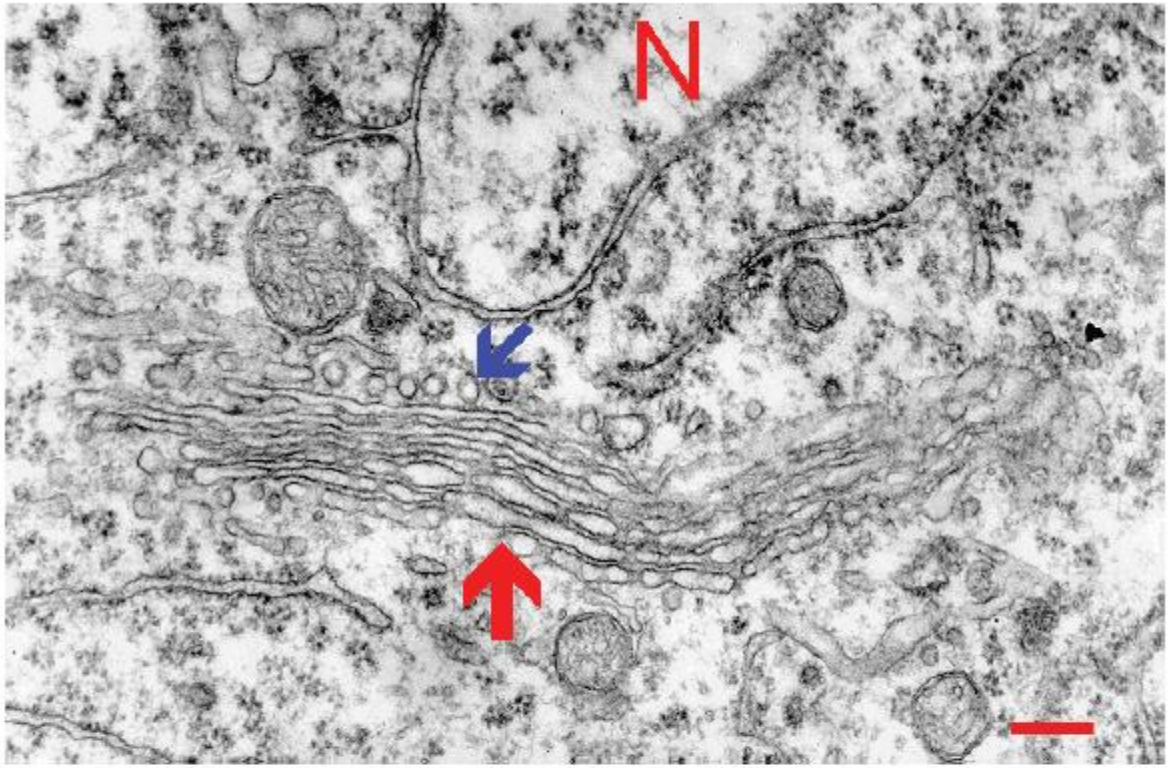
Polyribosomes are located at the basal portion of dendrtitic

Protein targeting in dendritic spines is regulated by local synthesis



B. Polyribosomes in dendrites are selectively located beneath postsynaptic sites. In spine-bearing neurons clusters of polyribosomes are generally found just at the junction of the spine and the main dendritic shaft (arrow). This electron micrograph shows a mushroom-shaped spine synapse in the hippocampal dentate gyrus. (S = spine head; T = presynaptic terminal; Den = main shaft of the dendrite containing a long mitchondrion.) Note the absence of polyribosomes in other parts of the dendritic shaft. × 60,000. (Courtesy of O. Steward, University of Virginia.)

The Golgi



- Sorts, packages, and modifies cargo proteins coming from ER
- Major site of carbohydrate synthesis
- Site of glycosylation and proteoglycan synthesis
- Site of initial aspects of processing of neuropeptides

The Golgi complex is present in the somatodendritic compartment and does not extend into the axon

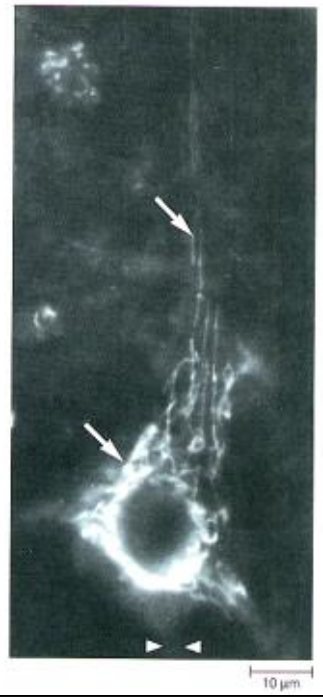
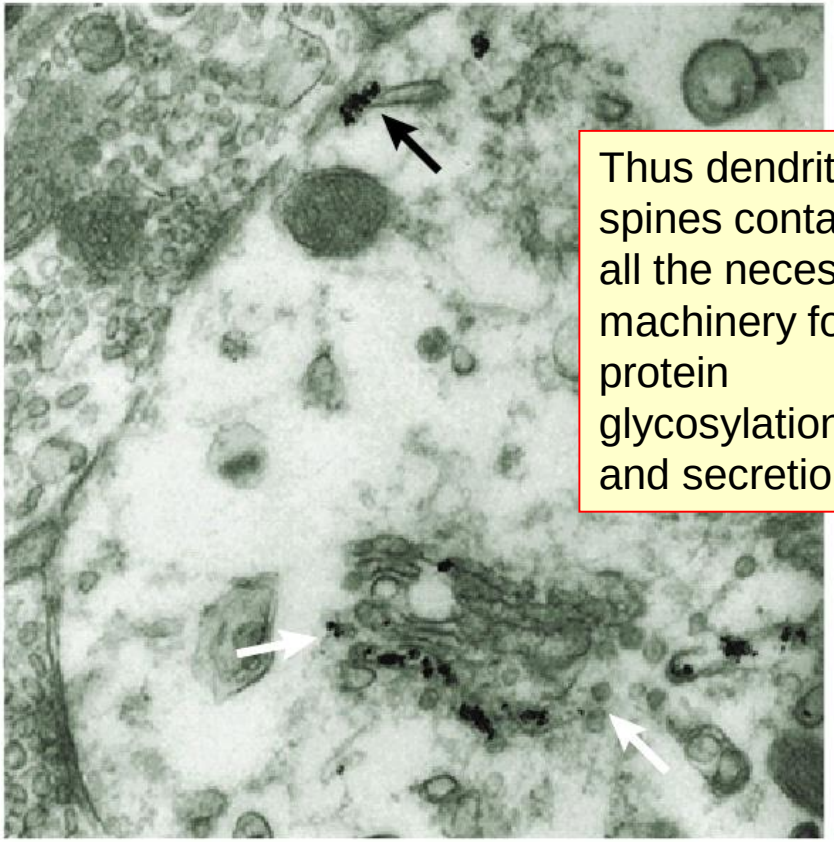


Figure 4-3 Under the light microscope the Golgi complex appears as a network of filaments that extend into dendrites (arrows), but not into the axon. The arrowheads at the bottom indicate the axon hillock. The Golgi complex in this micrograph is in a large neuron of the brain stem immunostained with antibodies specifically directed against this organelle. (From De Camilli et al. 1986.)

Differently from neurons, in most eukaryotic cells the Golgi complex is confined to the perinuclear region



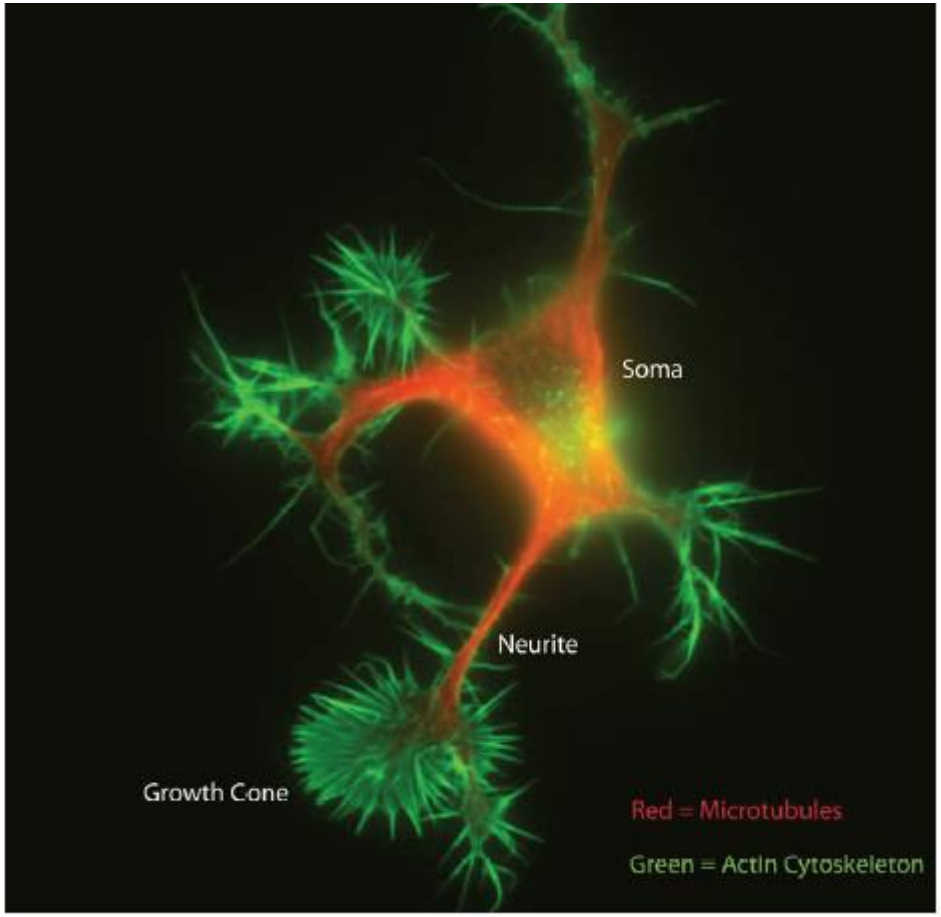
Thus dendritic spines contain all the necessary machinery for protein glycosylation and secretion

Figure 1 | **Electron-microscopic evidence for the existence of Golgi markers in neuronal dendrites.** Using gold-labelled antibodies to trans-Golgi network protein TGN38, this Golgi marker can be visualized on the inside of the dendritic spine. White arrows indicate TGN38 immunoreactivity on dendritic stacks; the black arrow highlights TGN38 immunostaining close to synaptic connections. Reproduced with permission from REF. 12 © 1999 Society for Neuroscience.

Job & Eberwine, 2001
Nature Review Neurosci 2:889-98

Neuronal cytoskeleton

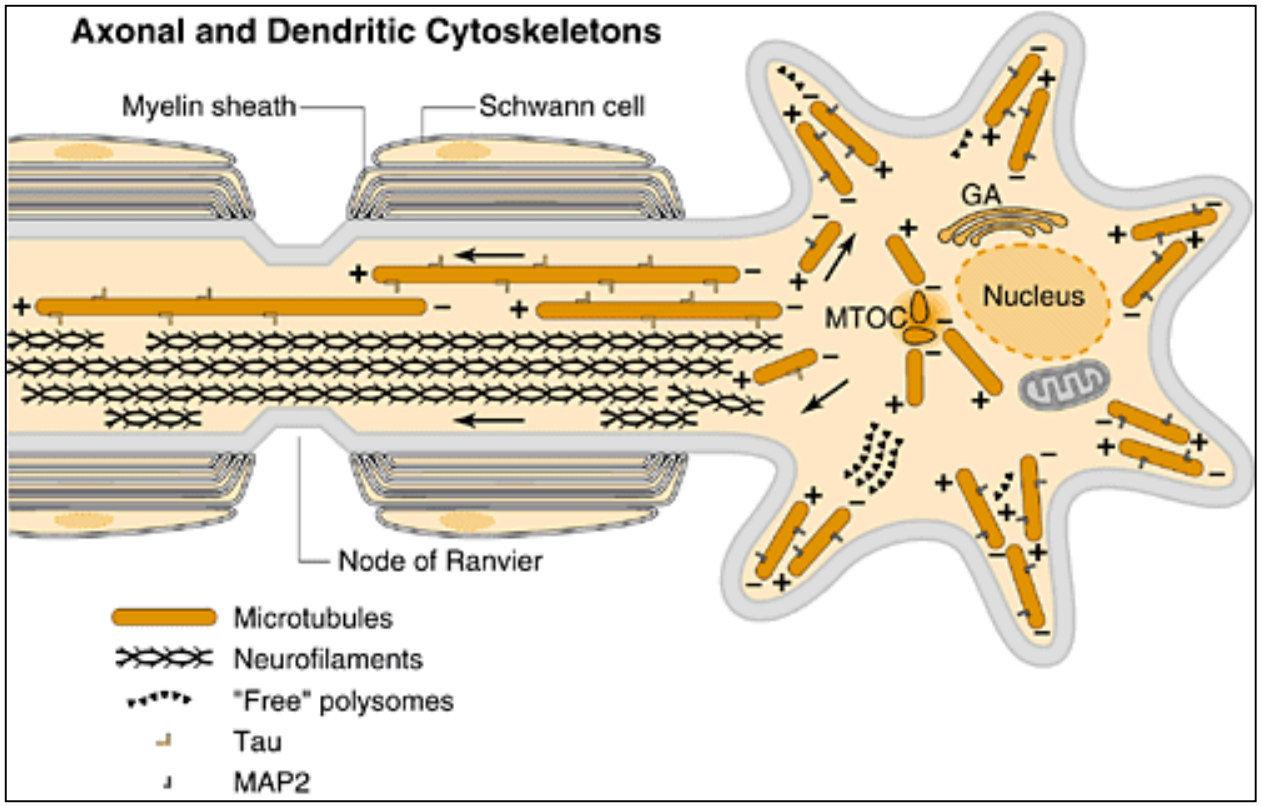
- Microfilaments
- Intermediate Filaments
- Microtubules

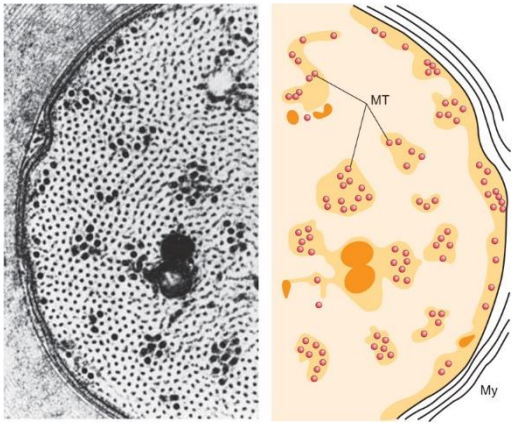


The three types of cytoskeletal filaments have a differential subcellular distribution

Let's have a look to a simple type of polarized cell:
the intestinal epithelial cell

Distrubution of neurofilaments and microtubules in the neuron





Organization of the cytoskeleton in a cross section of an axon.
Left: Electron micrograph of a myelinated toad axon in crosssection. **Right**: Diagram of the same axon. Most of the axonal diameter is taken up by the neurofilaments (*clear area).* The microtubules (*MT)* tend to be found in bundles and are more irregularly spaced. They are surrounded by a fuzzy material that is also visible in the region just below the plasma membrane (*stippled areas,* **right**). These areas are thought to be enriched in actin microfilaments

Neurofilaments are largely excluded from the dendritic compartment and are highly abundant in large axons.

Microtubules in axons and distal dendrites are unipolar, with the + end pointing away from the cell body. However in proximal dendrites are of mixed polarity.

IFs of the nervous system appear as solid, ropelike fibrils 8-12 nm in diameter and can be many micrometers long.

Neurofilaments have an unusual degree of **metabolic stability**, with a special role in stabilizing and maintaining neuronal morphology

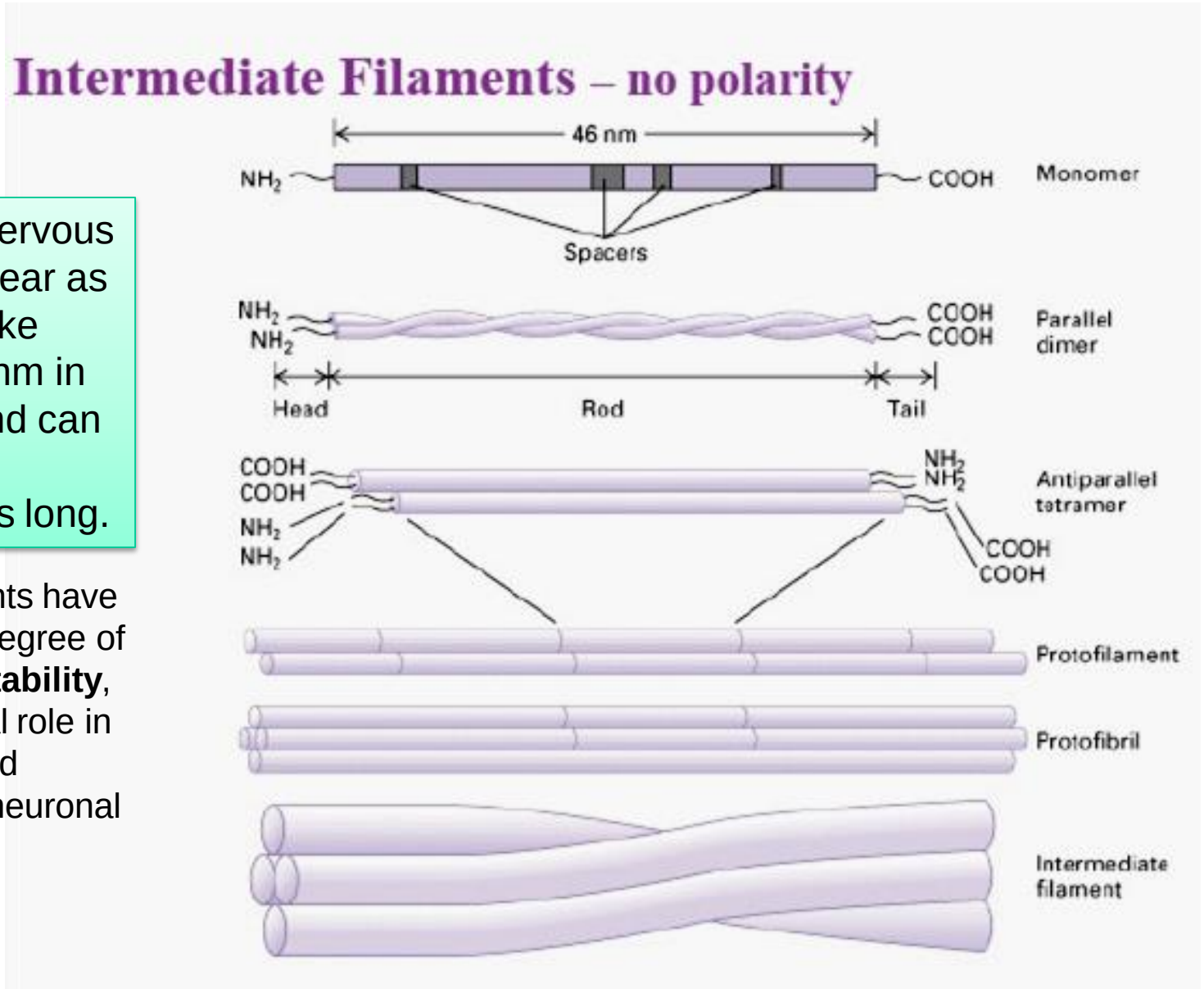
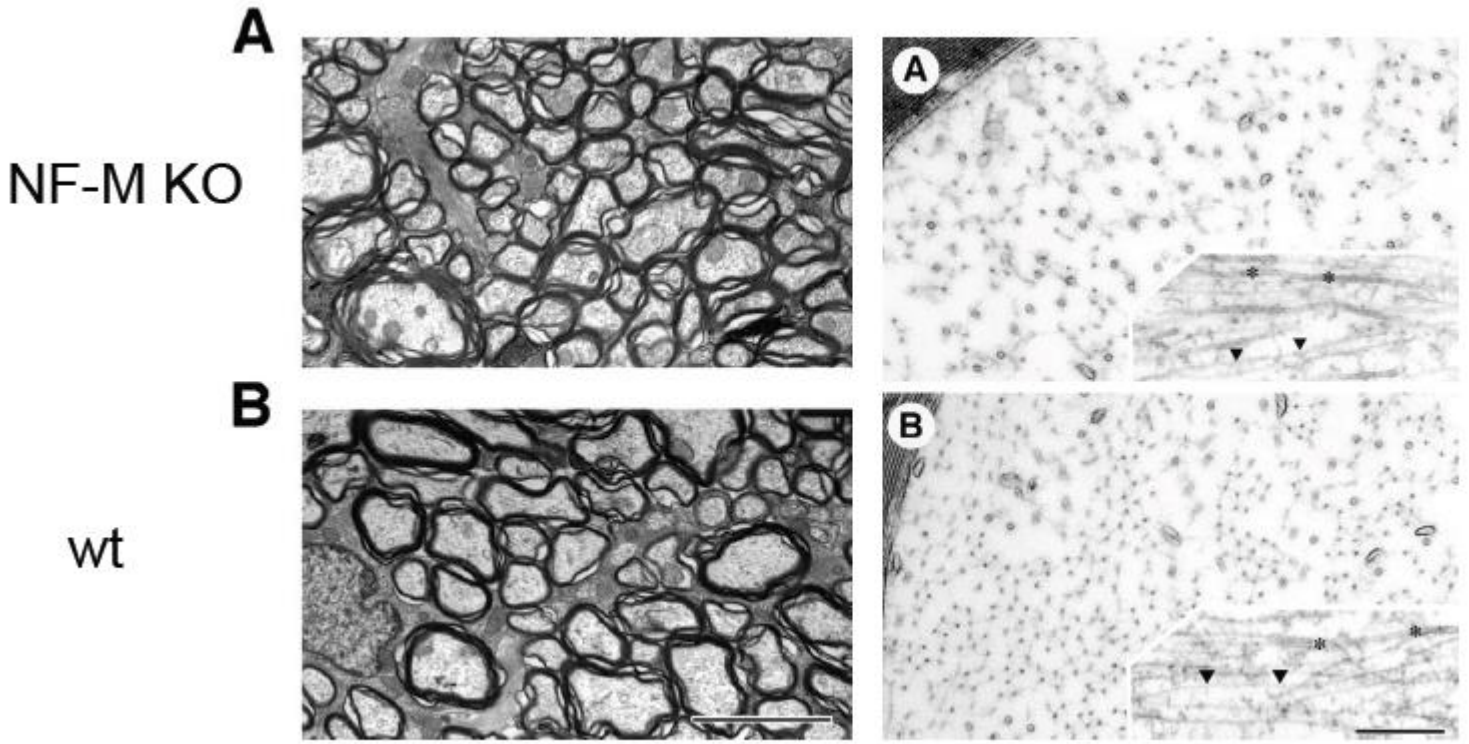


TABLE 6-2 IF Proteins of the Nervous System

IF Type	Subunit	Cell type
Type III	Vimentin	Neural and glial precursors
	GFAP	Astrocytes, some Schwann cells
	Peripherin	Subset of neurons, particularly in PNS, may coassemble with NFH/NFM/NFL
	Desmin	Smooth muscle cells in vasculature
Type IV	NFH	
	NFM	
	NFL	Most neurons, most abundant in large neurons
	α-Internexin	Subset of neurons, particularly parallel fibers in cerebellum, may also coassemble with NFH/NFM/NFL
Type V	Nuclear lamins	Nuclear envelope
Type VI	Nestin	Neuroectodermal precursors in developing brain

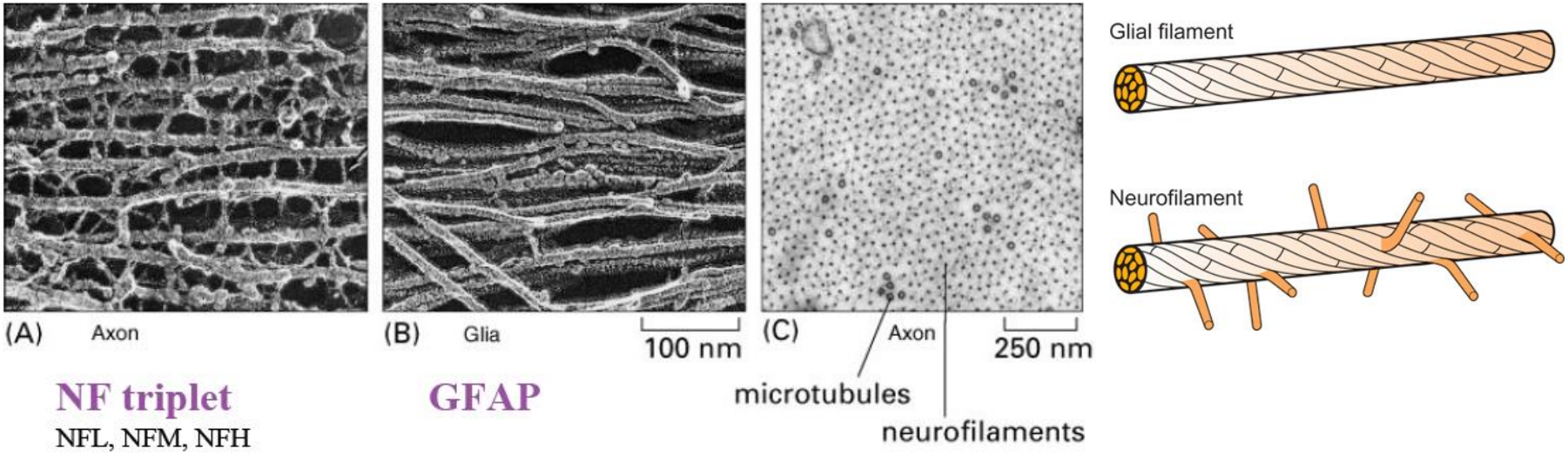
Neurofilaments regulate axonal caliber

- Disruption of NF genes reduces axon diameter >50%



Edgar et al. 1998 J. Cell Biol.141:727-739

Comparison of IF in neurons and glia



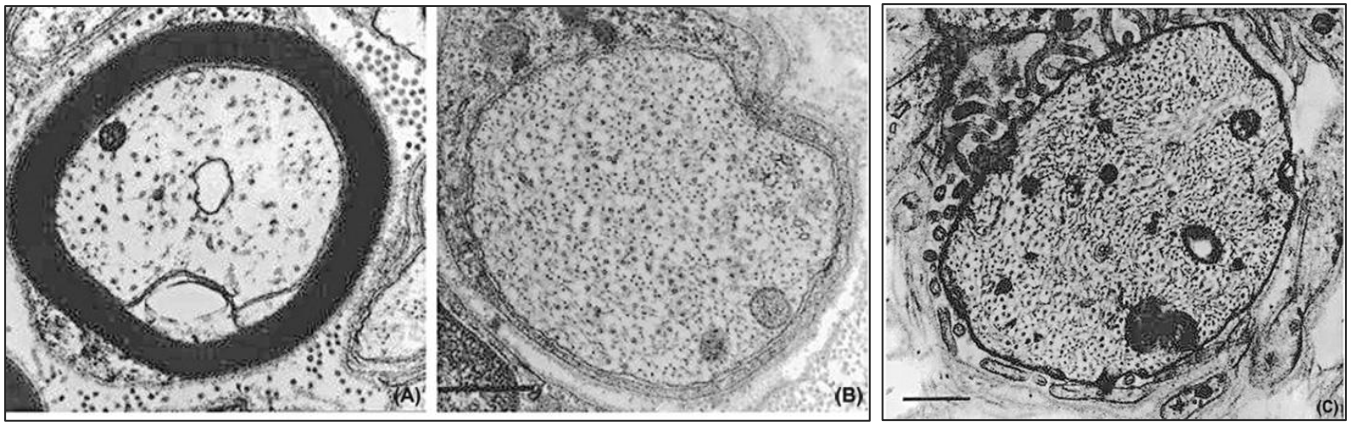
IF in neurons (NFs):

- Represent 13% of total protein in mature neurons
- Neurofilament composed of NF-L, -M, and -H
- NF-L required for all filament assembly
- Side arms of NMF & NFH contribute to wider spacing of NFs relative to glial IFs
- The side arms of NFH & NFM have consensus phosporylation sites. Phosphorylated side arms repel adjacent NFs with similar charge

IF in glia (or other non-neuronal cells):

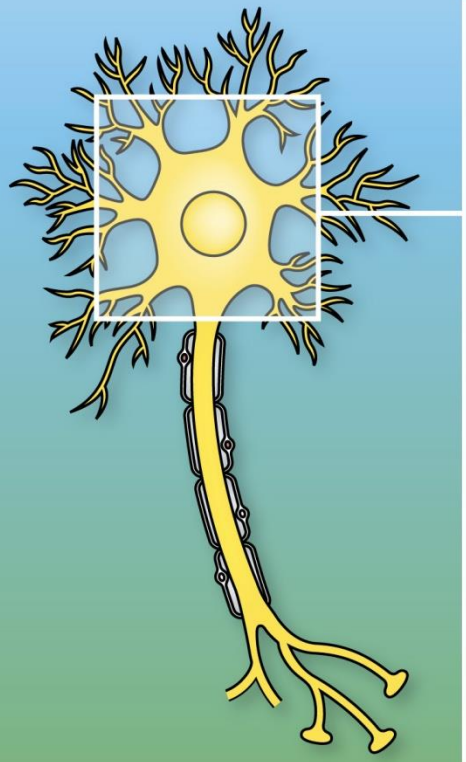
- are shorter and lack side-arms

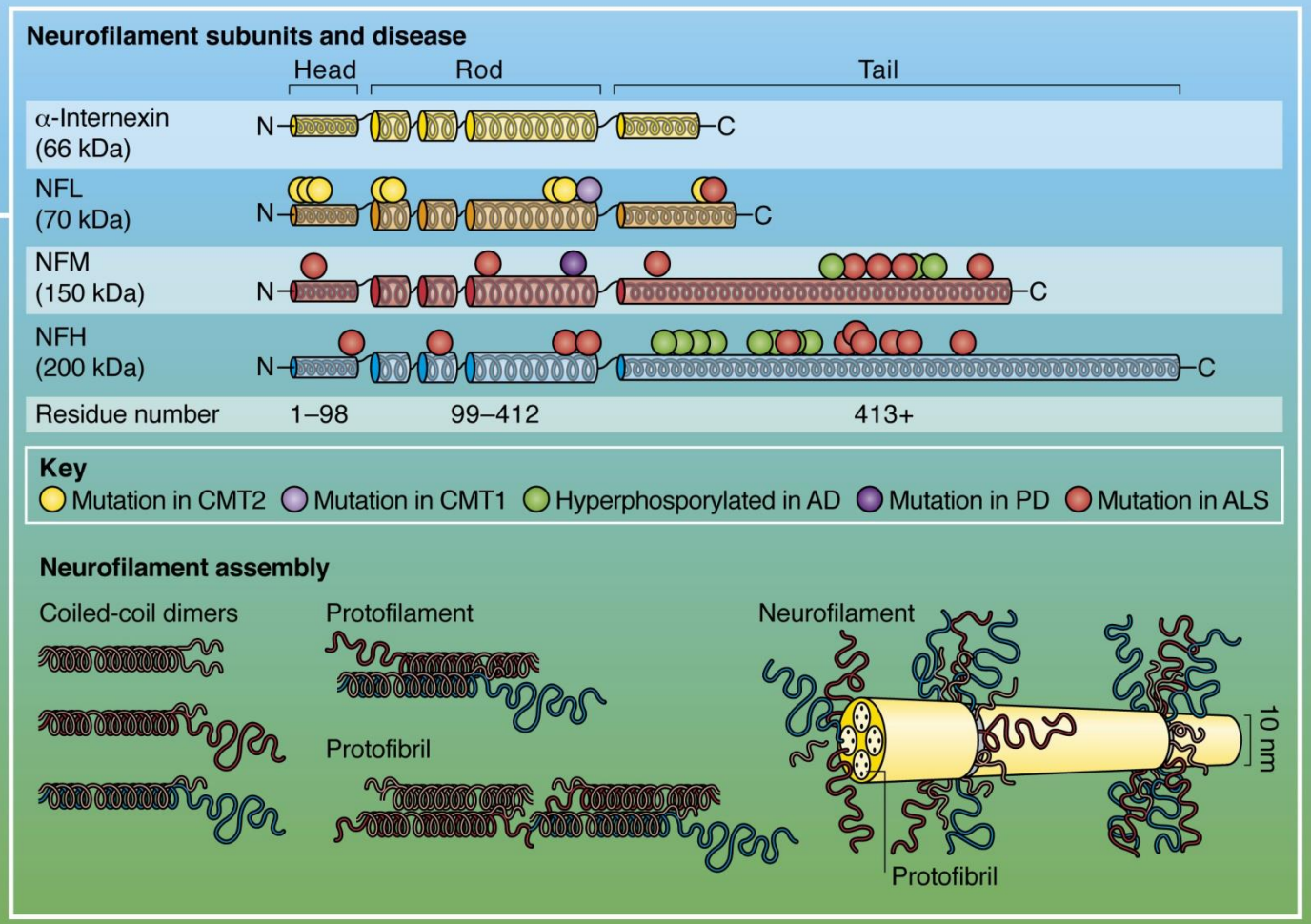
The local environment can alter the organization of the axonal cytoskeleton



(A) In a normal myelinated axon of the sciatic nerve, neurofilaments and microtubules are widely spaced, so they occupy considerable volume. **(B)** In contrast, a comparably sized axon from the sciatic nerve of the **demyelinating Trembler mutant mouse** has a denser cytoplasm, with neurofilaments densely packed. This has been shown to result from a shift in the net **dephosphorylation of neurofilaments produced by demyelination**. This effect on the axonal cytoskeleton is highly localized. **(C)** Similar changes in the organization and phosphorylation of the axonal cytoskeleton occur even over the short gap in the myelin sheath which occurs at the **node of Ranvier**. Such changes illustrate the dynamic nature of the axonal cytoskeleton. Bars represent 0.5 μm with **(A)** and **(B)** at the same magnification. (Micrographs supplied by Sylvie de Waegh and Scott Brady.)

Mutations in neurofilament genes are associated with some neuropathologies





CMT = Charcot Marie-Tooth desease

Tangles or aggregates of NFs are often associated with neurodegenerative diseases

J. Cell Sci. (2012) **125**, 3257–3263

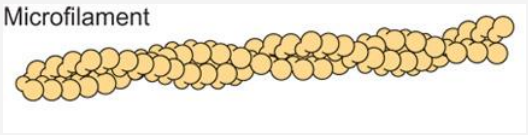
Microfilaments

- Smaller very dynamic structural filaments
- Composed of actin polymers
- Involved in cell motilitv and axon growth via filopodia and lamellipodia
- Involved in endocytosis and intracellular motility
- Structurally component of neuronal spines
- Act with myosins to drive certain intracellular movements
- Synapse structure

ACTIN

Cell/ Growth Cone Migration

Sensing and Processing Environmental Cues



http://cellix.imba.oeaw.ac.at/cytoskeleton

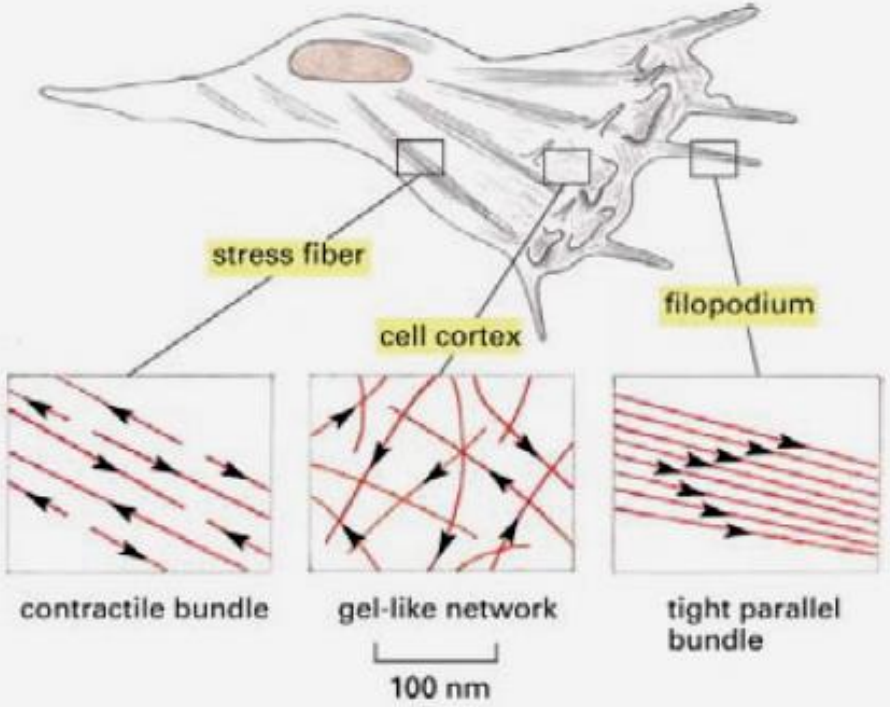


Figure 16–38. Molecular Biology of the Cell, 4th Edition.

Actin is Enriched in Lamella and Growth Cones

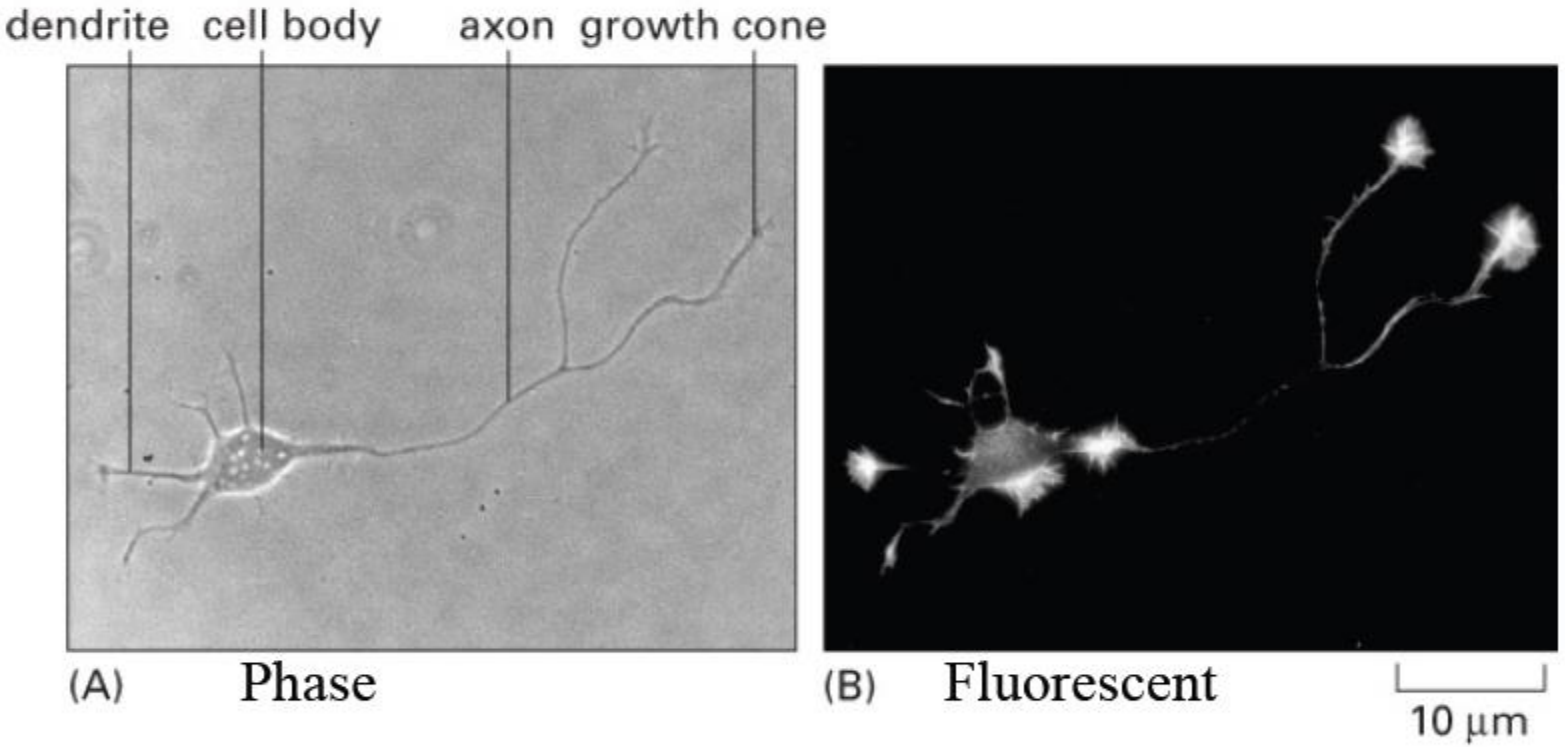
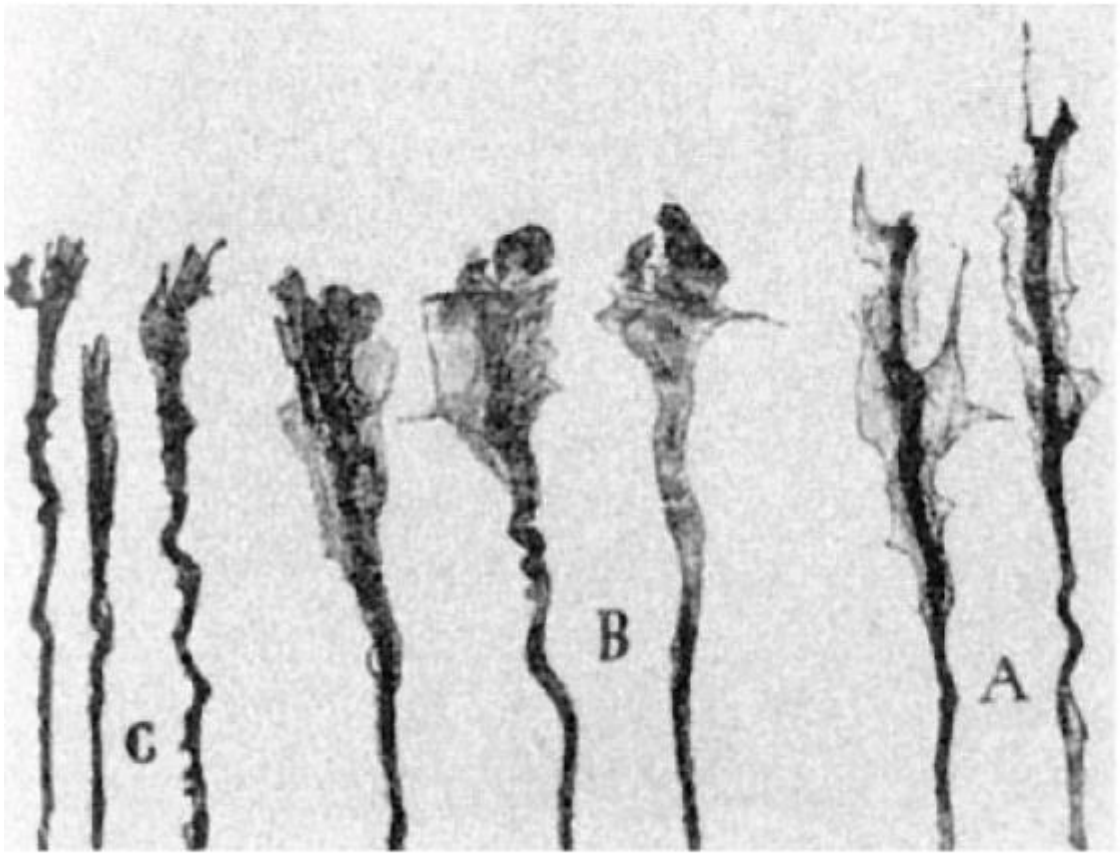


Figure 21–97. Molecular Biology of the Cell, 4th Edition.



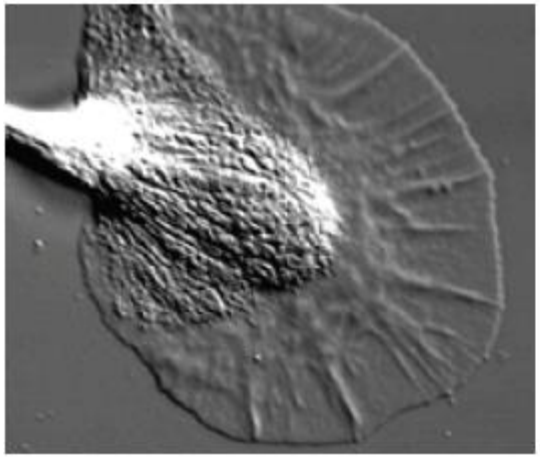
Growth Cones

Mammalian from RyC

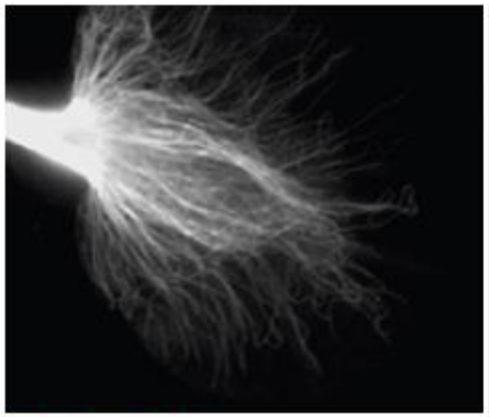
Growth cones sample the environment and respond to signaling cues.

The end results of the signaling pathways are changes in actin and MT cytoskeletal dynamics.

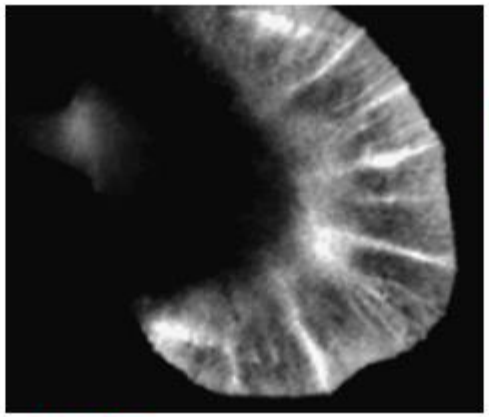
Growth Cone of Aplysia Bag Cell Neuron



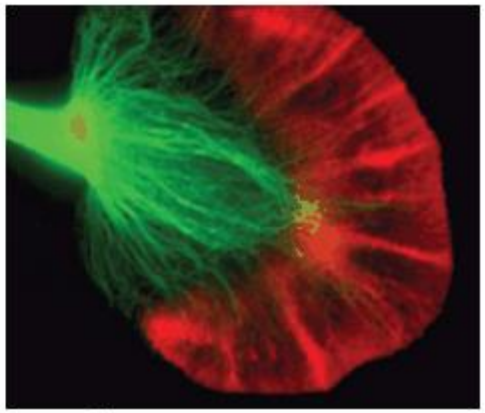
These mollusc neurons are great for growth cone studies. Big and flat, note a peripheral (P) region with actin but relatively empty and the vesicle filled central (C) region (with mts). MTs concentrated in the central region, but some in P region



Tubulin



Actin



Both

TABLE 6-3 **Selected Microfilament-Associated Proteins Expressed in the Nervous System**

Protein	Activity	Cellular location
Actin	Core subunit of MFs	Throughout neurons and glia, enriched in growth cones and in membrane cytoskeleton
Tropomyosin	Stabilize MFs	Co-distributed with most MFs
Spectrin/fodrin	Cross-link MFs in membrane cytoskeleton	Enriched in membrane cytoskeleton
Ankyrin	Links MF/spectrin to membrane proteins	Membrane cytoskeleton, distinct forms in axon, dendrite and nodes of Ranvier
Fimbrin	MF bundling and cross-linking	Growing neurites
Gelsolin	Fragments MFs and nucleates assembly, regulated by Ca^{2+}	Growing neurites, glia, mature neurons
β-thymosin	Binds actin monomers and regulates MF assembly	Growing neurites
Profilin	Binds actin monomers, inhibits MF formation, regulated by selected signal transduction pathways	Growing neurites, glia, mature neurons
Arp2/3	Nucleation of actin MF assembly in cortex and initiation of MF branches	Enriched in cell cortex where MF assembly is active
N-WASP	Interacts with Arp2/3 complex to nucleate branched actin MF assembly	Enriched in cell cortex where MF assembly is active, binds to Cdc42/Rac small GTPases and Arp2/3 complexes
Formin	Nucleates straight actin filaments	Enriched in cell cortex where MF assembly is active, binds to Rho small GTPase

Actin instability is higher in one of neurite growth cones of unpolarized neurons: the future axon

Rac and Cdc42 induce higher activity of actin-severing proteins

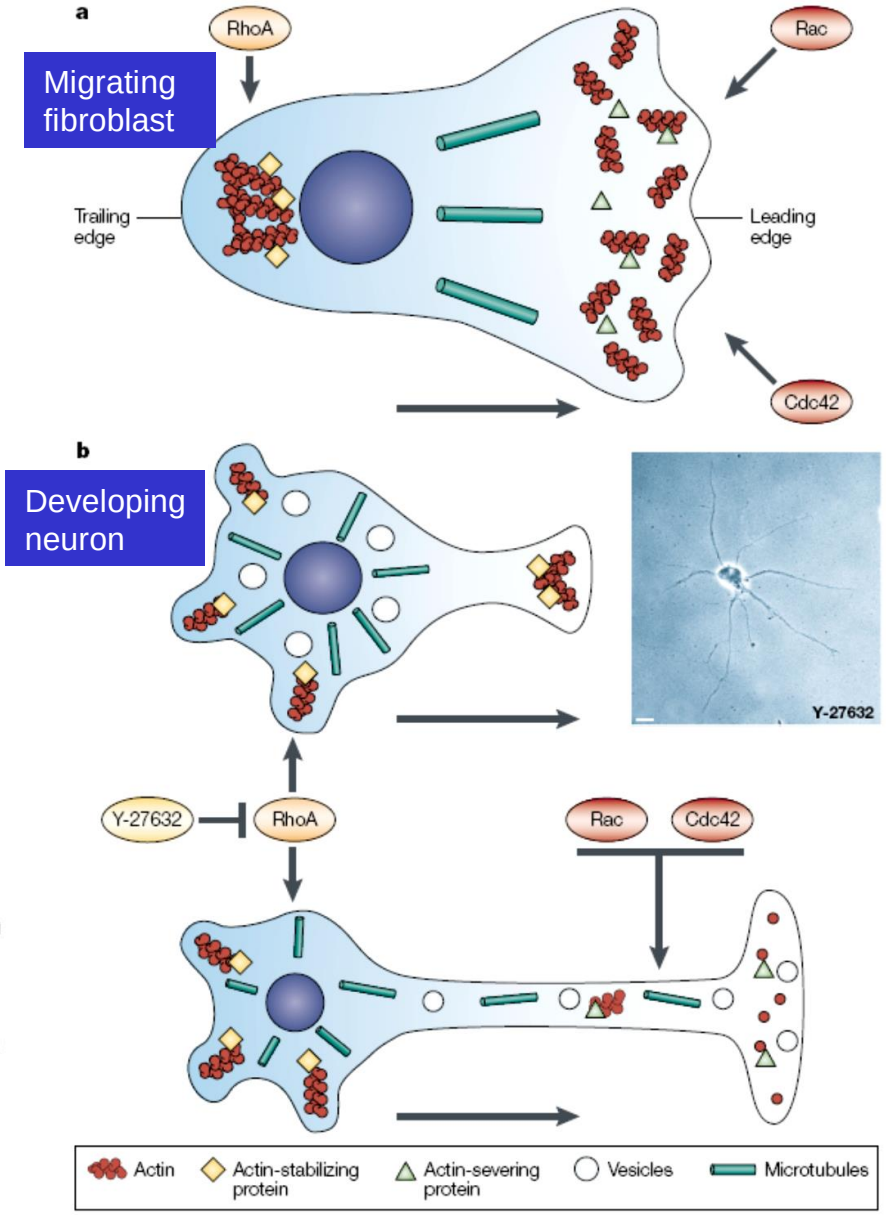


Figure 3 | **Simplified view of the action of Rho proteins in fibroblasts and neurons.** **a** | Diagram depicting the role of the main members of the Rho family in fibroblast migration, illustrating how dominance areas of these molecules affect growth. Rho is more active at the trailing end of the fibroblast, and this induces stress-fibre and focal-adhesion formation through the polymerization of actin, which is thought to stabilize the trailing end of the cell as it migrates towards the external stimulus (the direction of growth is depicted by the black arrow at the bottom of the image). Rac and Cdc42, on the other hand, are dominant at the leading edge and, by depolymerizing actin, they promote the formation of protrusions that underlie the force-mediated movement towards the stimulus. The dynamics of polymerization and depolymerization depend on the balance between proteins that stabilize actin and those that sever microfilaments. The stabilizing actin-binding proteins dominate at the trailing edge, where the RhoA action prevails, whereas actin-severing proteins are more influential at the leading edge, where Rac/Cdc42 exert their effects. **b** | The equivalent diagram in neurons, illustrating how the Rho molecules affect neuritogenesis. As in fibroblasts, the dynamics of filament assembly and disassembly arise from a balance between different types of actin-binding protein that are regulated by Rho GTPases. In this case, quiescent processes, where RhoA is dominant, tend to express molecules that stabilize actin. In a sprouting process, the dominant role of Rac/Cdc42 will tilt the balance in favour of actin-severing proteins. The RhoA downstream kinase ROCK (Rho-associated coiled-coil-containing protein kinase) is specifically inhibited by Y-27632, and treatment of young hippocampal neurons with this inhibitor for 24 h shows how changing the equilibrium between the Rho pathways can lead to an uncontrolled sprouting of new neurites. As observed in the phase-contrast inset image, inhibition of the RhoA pathway induces the sprouting of multiple processes, as RhoA cannot effect its neurite-stabilizing role.

Da Silva and Dotti, 2002
Nature Reviews Neurosci 3:695-704

Microtubules

- **Are the tracks or highways for organelle transport**
- **Maintain elongated (asymmetric) neurite process morphology**
- **Different polarity distribution in axons and dendrites**
- **Microtubule associated proteins (MAPs) contribute to function**
  - **Structural MAPs also different in axons and dendrites**
  - **Motor Proteins are also MAPs**

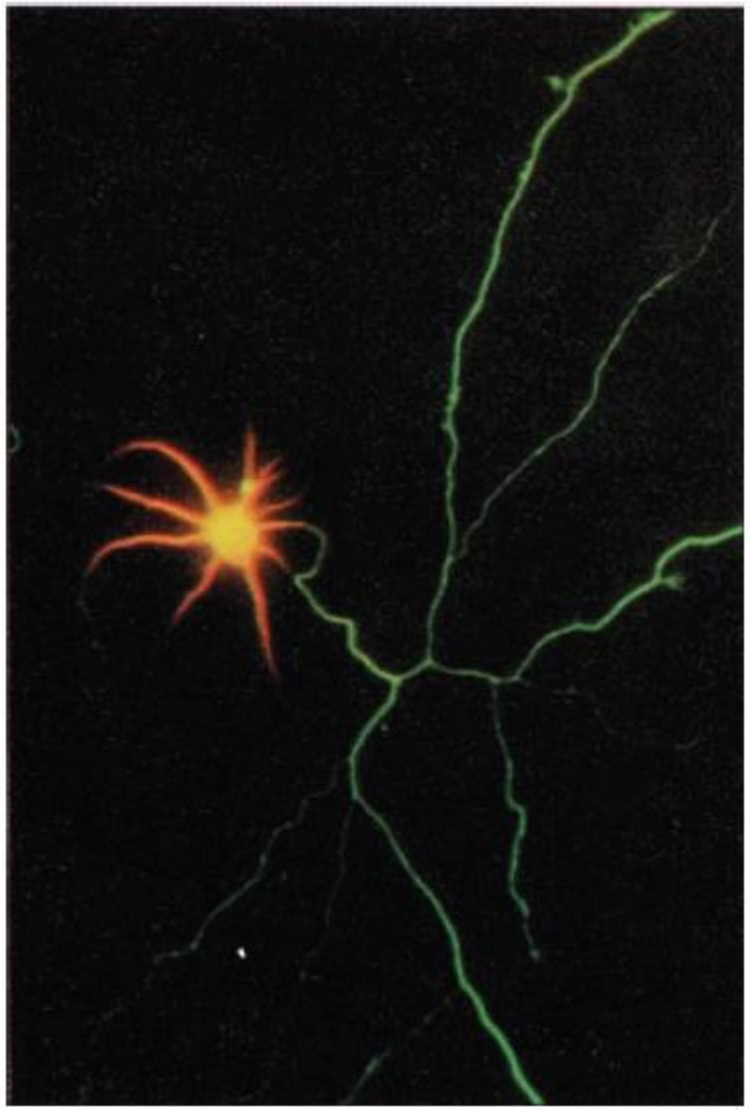
Axons and Dendrites:
Different Microtubule Associated Proteins (MAPs)

Two MAPs (structural):

Red: **MAP2** in the soma and dendrites

Green: **Tau** (dephosphorylated) in the axon

Cultured hippocampal neuron



MAP2 is a marker for neuronal dendrites

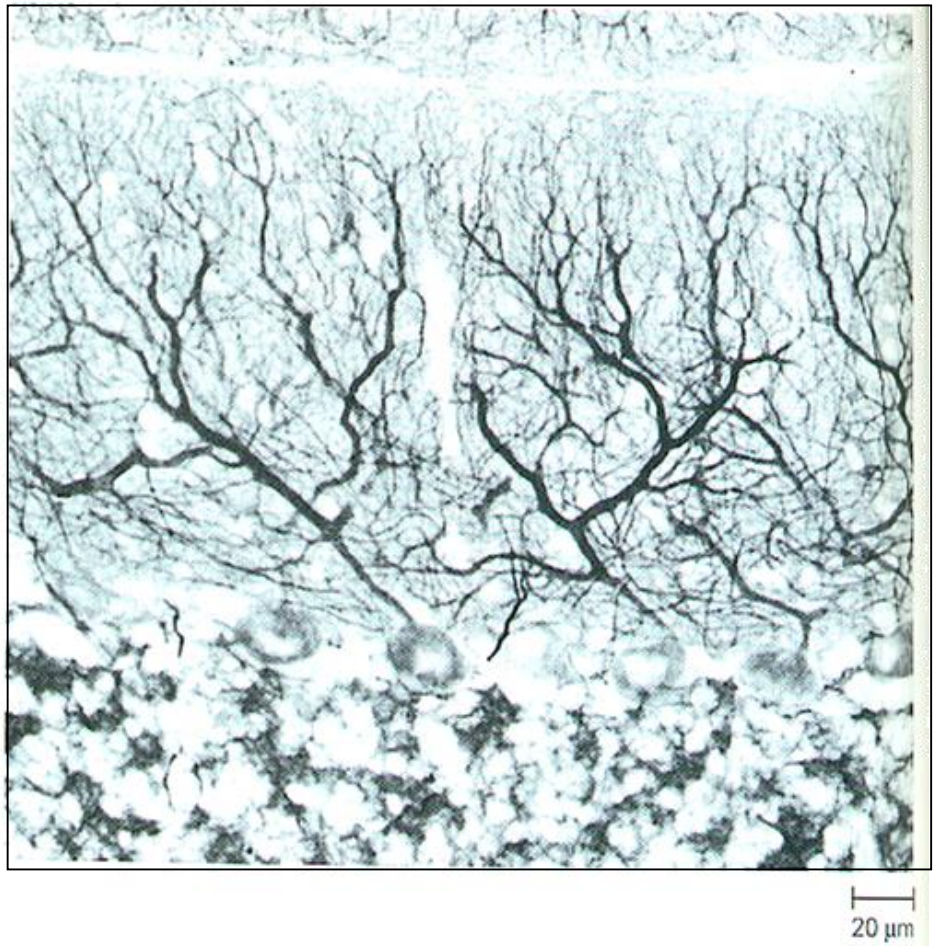


Figure 4-7 The dendritic architecture in the cerebellar cortex is visualized here by immunoperoxidase staining for the microtubule-associated protein MAP2, a dendrite-specific MAP. Dendrites of all classes of neurons are stained. The field is dominated by the dendrites of Purkinje cells. (Courtesy of P. De Camilli.)

Microtubule organization in axons and dendrites

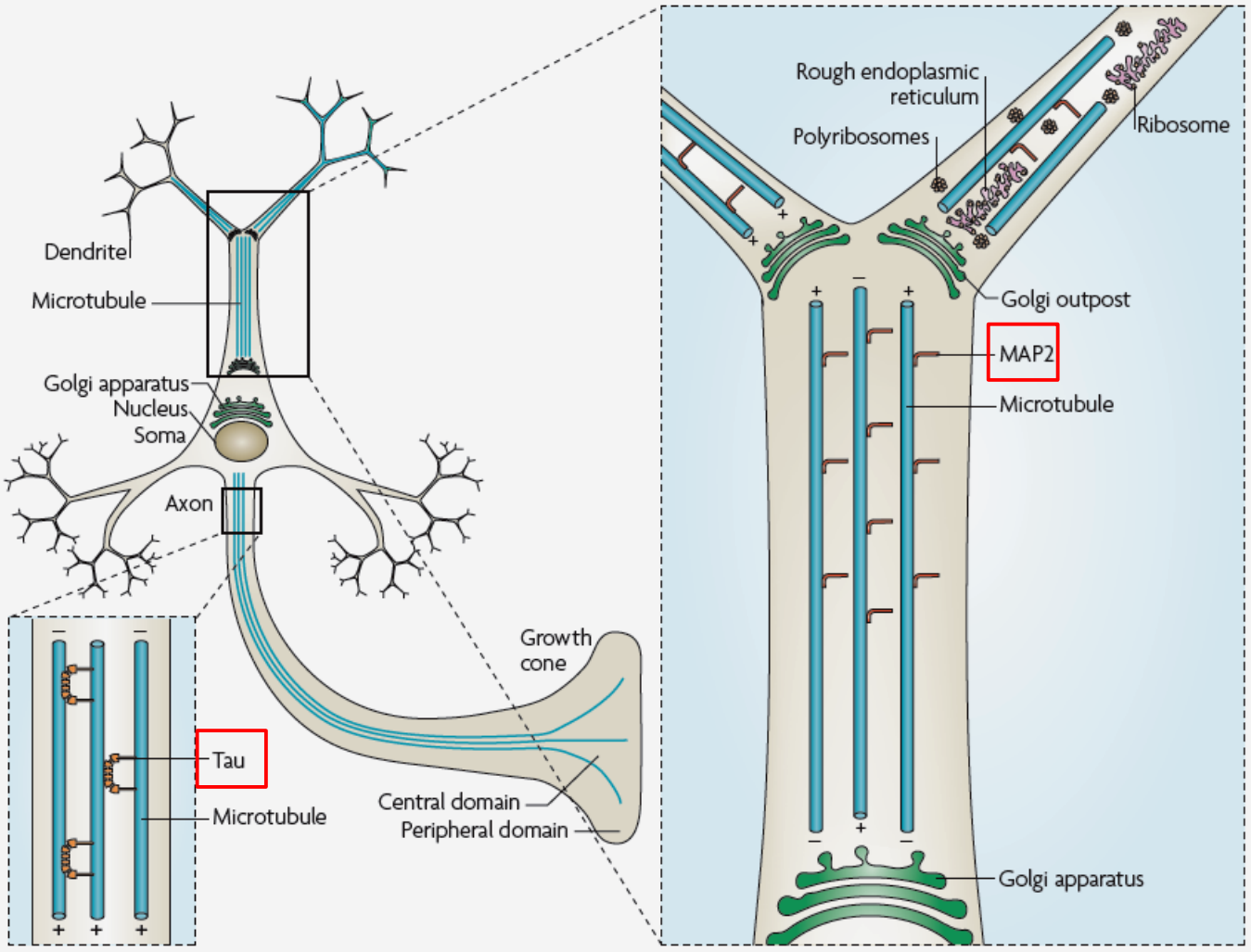


Figure 1 | **Microtubule organization and organelle distribution in axons and dendrites.** Axons have tau-bound microtubules of uniform orientation, whereas dendrites have microtubule-associated protein 2 (MAP2)-bound microtubules of mixed orientation. Dendrites also contain organelles that are not found in axons, such as rough endoplasmic reticulum, polyribosomes and Golgi outposts.

Conde & Caceres, 2009
Nature Reviews Neurosci
doi:10.1038/nrn2631

MAP2 and tau bind to MTs.

The lengths of their side arms may contribute to spacing of mts.

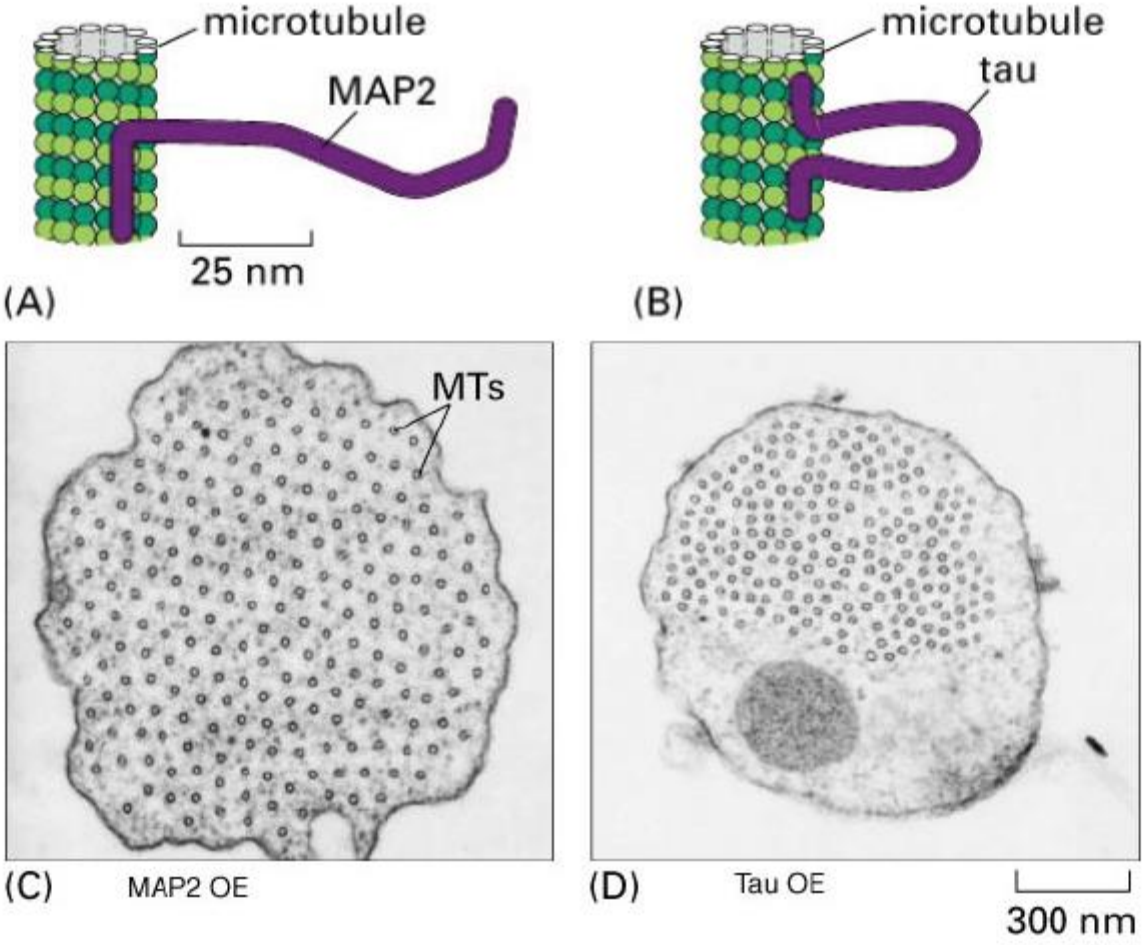


TABLE 8-1. MAJOR MICROTUBULE CYTOSKELETAL PROTEINS OF THE NERVOUS SYSTEM

Protein	Expression pattern and dstribution	Modifications
α-Tubulin (multigene family)	In all cells but some isoforms preferentially expressed in brain	Acetylation, tyrosination
β-Tubulin (multigene family)	In all cells but some isoforms preferentially expressed in brain	Phosphorylation, polyglutamylation
γ-Tubulin	In all cells, pericentriolar region/MTOC	?
MAP1a (multigene family)	Appears late, Wide distribution	Phosphorylation
MAP1b	Appears early, then declines, enriched in axons	Phosphorylation
MAP2a (single gene)	High MW, dendritic in mature neurons	Phosphorylation
MAP2b	High MW, dendritic expressed throughout lifetime	Phosphorylation
MAP 2c	Low MW, dendritic in developing neurons	Phosphorylation
Tau (single gene)		Phosphorylation
High MW	Peripheral axons with distinctive phosphorylation pattern	
Low MW	Enriched in CNS axons with distinctive phosphorylation pattern	Phosphorylation
MAP4	Primarily non-neuronal, multiple forms, widespread distribution	Phosphorylation at mitosis

MAP, microtubule-associated protein; MTOC, microtubule-organizing center; MW, molecular weight

Role of MAPs and other MT regulators

Organize microtubules in neurons

Bundle microtubules (assembly MAPs, MAP1A and MAP1B)

Stabilize microtubules (MAP2, MAP4, Tau, acetylation of MTs)

Control rate of tubulin polymerization
(phosphorylated MAPs cannot bind to MTs)

Microtubule severing proteins (spastin and katanin)

Microtubule-actin linking proteins (spectraplakin)

Microtuble organization in developing axons

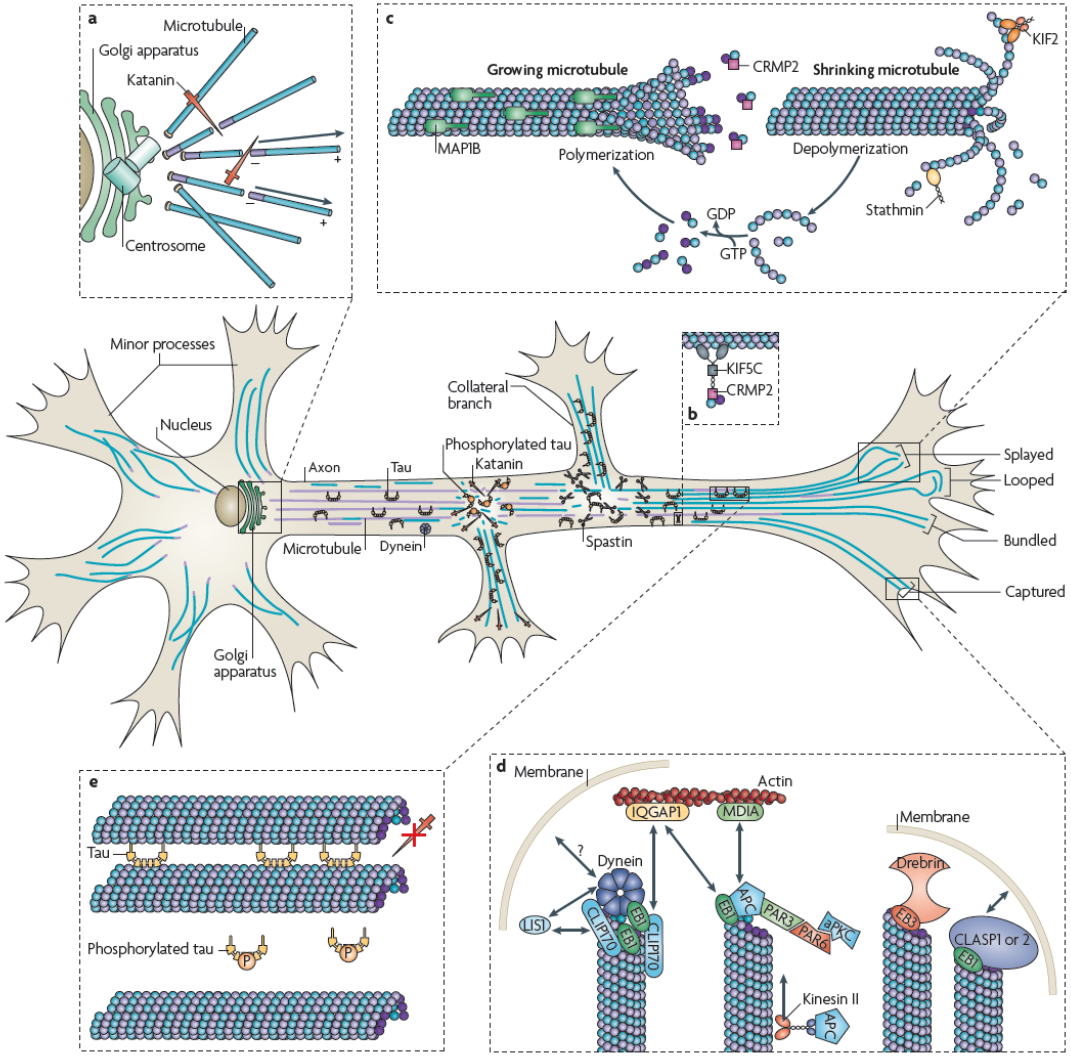


Figure 2 | **Microtubule organization in developing axons.** The organization and regulation of microtubules in a stage 3 hippocampal pyramidal neuron. Dynamic (blue) microtubules predominate in minor processes (short, unbranched neurites) and at the distal end of the axon and collateral branches, whereas stable (purple) microtubules are enriched in the proximal part of the axon. Inset **a** shows katanin-mediated release of microtubules nucleated at the centrosome. Short polymers are transported along microtubules by motors such as dynein. Inset **b** shows the transport of tubulin dimers or oligomers to the growth cone by a complex of kinesin family member 5C (KIF5C) and collpasin response mediator protein 2 (CRMP2; also known as DPYSL2). On entering the axonal growth cone microtubules splay, bend, loop, bundle or get captured at the cell cortex. Inset **c** illustrates the dynamic behaviour of splayed microtubules. Proteins such as CRMP2 promote microtubule assembly, whereas microtubule-associated protein 1B (MAP1B) contributes to the maintenance of microtubule dynamics and KIF2 and stathmin induce microtubule depolymerization. Inset **d** shows the protein machinery that is involved in microtubule capture (see also Supplementary information S1 (table)). Inset **e** shows how tau protects microtubules from katanin-induced severing, thereby contributing to microtubule stabilization and preventing excessive collateral branching. In the axon shaft, the formation of collateral branches is regulated by the action of microtubule severing proteins such as spastin. APC, adenomatous polyposis coli; aPKC, atypical protein kinase C (also known as PRKCI); CLASP, cytoplasmic linker associated protein; CLIP170, CAP-GLY domain containing linker protein 170 (also known as CLIP1); EB, end-binding protein; IQGAP1, IQ motif containing GTPase activating protein 1.

Conde & Caceres, 2009
Nature Reviews Neurosci
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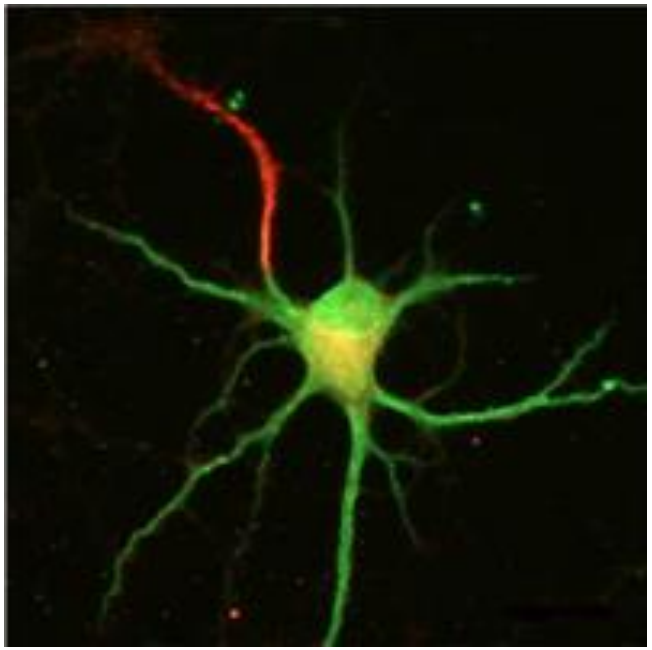
NEURON POLARITY

The unique shape of each neuron is the result of a cooperative interplay between plasma membrane components (the lipid matrix and associated proteins) and cytoskelatal proteins

Although neurons come in many shapes and sizes, they all **polarize** into discrete functional domains



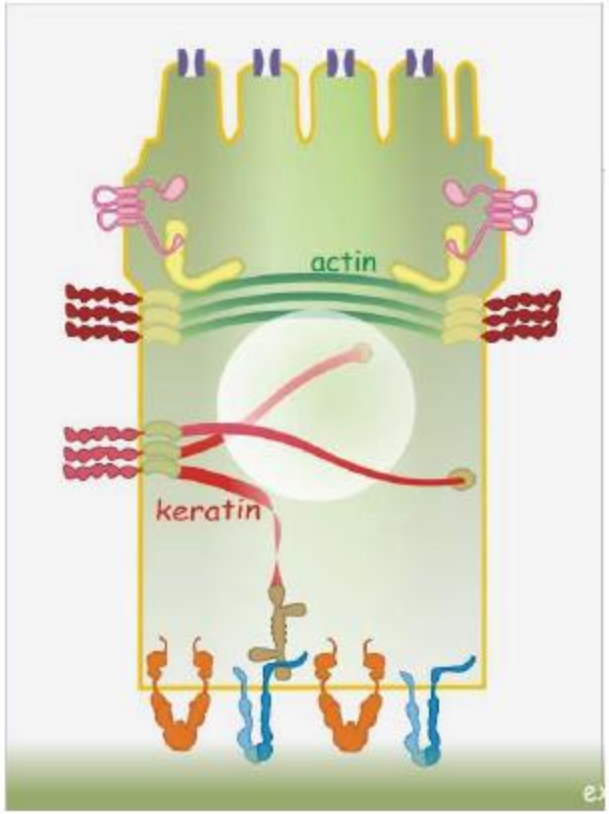
A spinal motor neuron.
Sodium channels (red);
MAP2 (green).

Separating Subcellular Compartments

Polarized cells such as epithelial cells and neurons contain distinct compartments.

Many molecules in one compartment are restricted from entering the other(s).

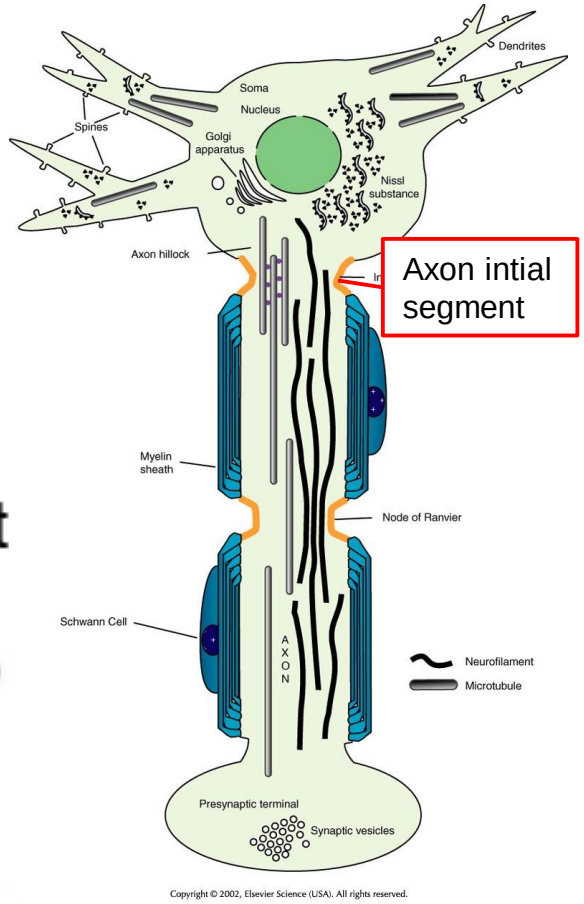
Where is the polarity divison in neurons?



Marking the border between somatodendritic and axonal compartments:

Axon initial segment

- Mature neurons are polarized
- The axon initial segment (AIS) acts as the integrator of subthreshold synaptic events into all or nothing APs.
- Its also a cytoplasmic filter between axon and somatodendritic compartment
- Specific motors carrying axonal cargo can pass through this filter.
- Also barrier to membrane protein diffusion from soma into axon.



How are distinct axonal and somatodendritic domains maintained?

In mature axons diffusion is impeded because of the existence of tight pockets of membrane components anchored to the subadjacent **actin cytoskeleton**.

This not only results in the trapping or immobilization of selected groups of membrane proteins required for electrical transmission, but also prevents the intermixing of freely mobile molecules in the adjacent territories

Dotti & Poo, 2003
Nature Cell Biology 5(7):591-94

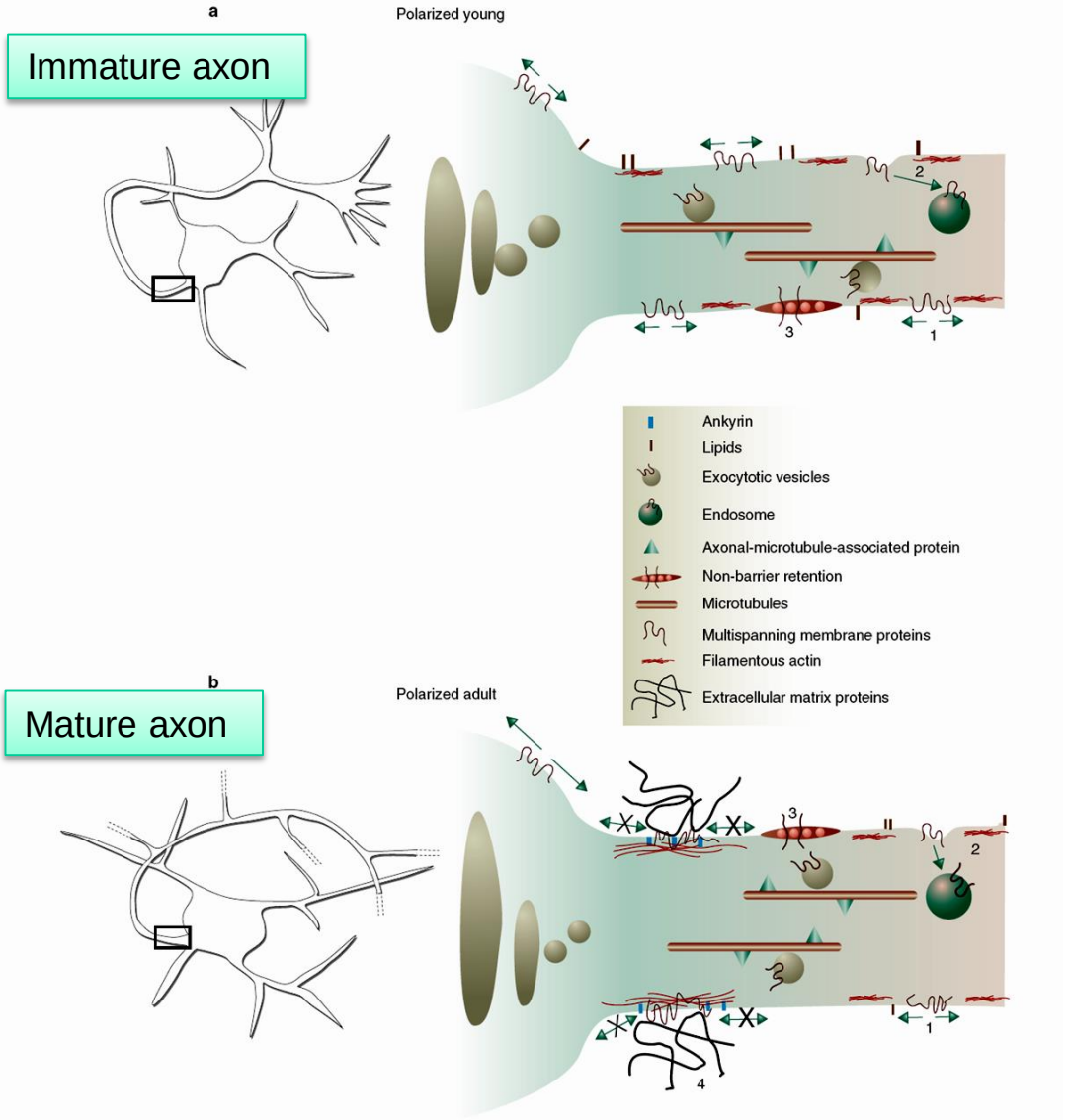


Figure 1 The progression of neuronal polarity. (**a**) During early polarization, membrane carriers containing axonal and dendritic components travel from the cell body to the future axon (framed) and future dendrites along microtubules. On fusion to the membrane, some components diffuse laterally (1), whereas others are retrieved by endocytosis and retrogradely transported (2). These components can thus change distribution from one domain of the neuron (for example, the axon) to the other (the dendrite). Even at these early stages, some components can be specifically retained in the membrane to which they were initially delivered through interactions with the underlying cytoskeleton or in so-called membrane cholesterol-sphyngolipid rich rafts (3). (**b**) Late in development, lateral mixing between axon and dendrites is prevented because a diffusion barrier at the axonal initial segment (framed) has formed (4). This consists of membrane lipid-protein clusters forming a tight connection with the underlying actin cytoskeleton and also with molecules of the extracellular matrix.