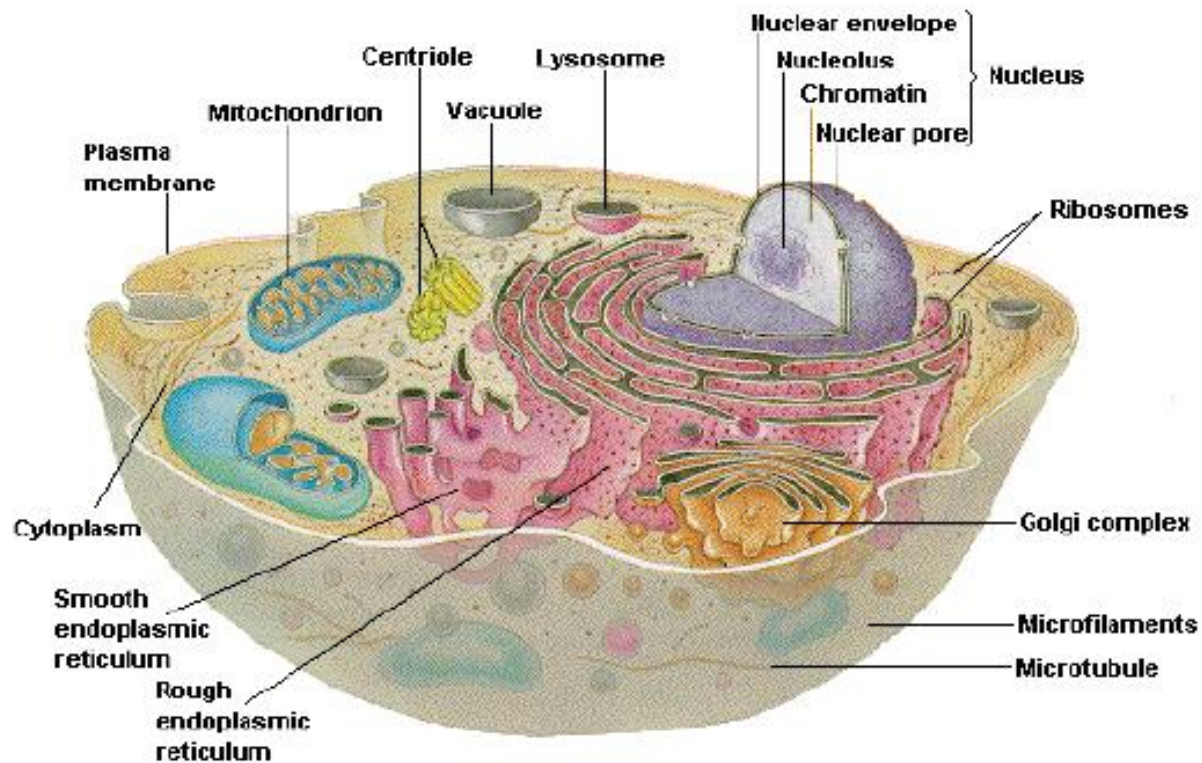


# Typical Eukaryotic Cell



# 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

# ORGANELLES

## What is different in a neuron?

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Cilia

Ribosomes

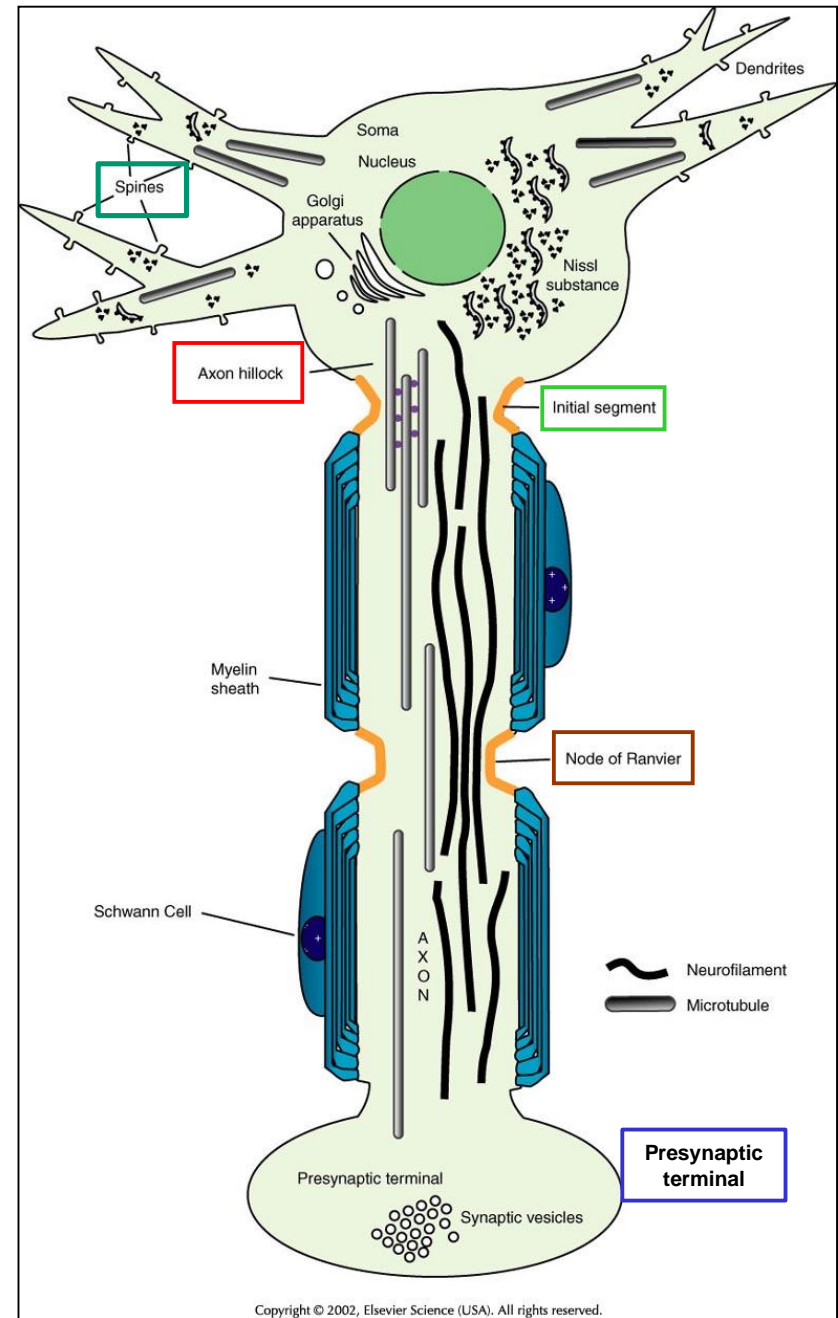
Proteosomes

# 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

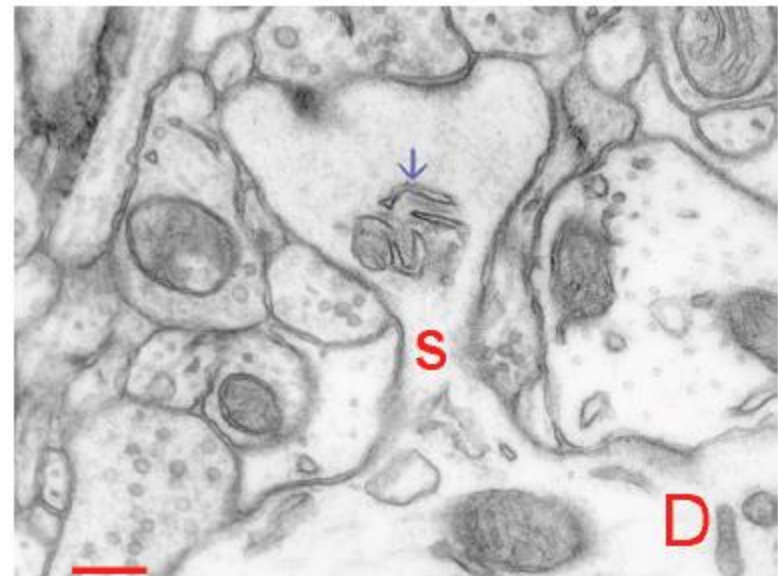
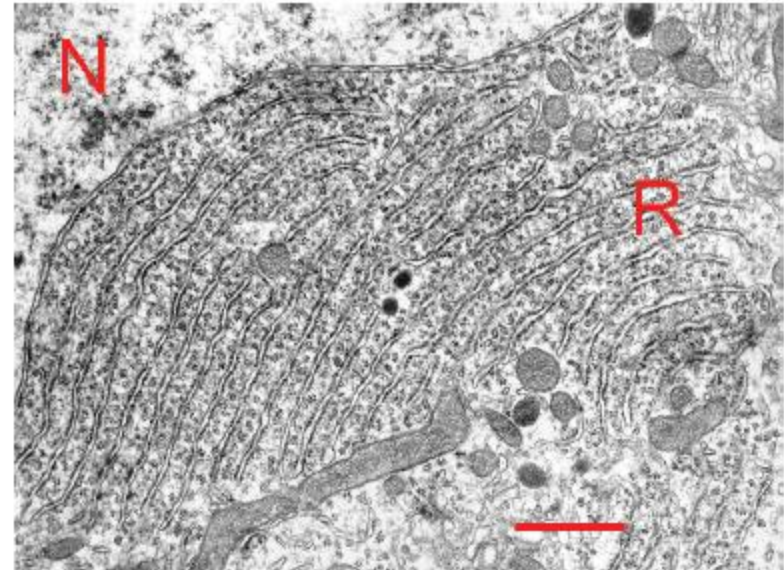
**FIGURE 2** Basic elements of neuronal subcellular organization. The neuron consists of a soma, or cell body, in which the nucleus, multiple cytoplasm-filled processes termed dendrites, and the (usually single) axon are placed. The neuron is highly extended in space; a neuron with a cell body of the 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



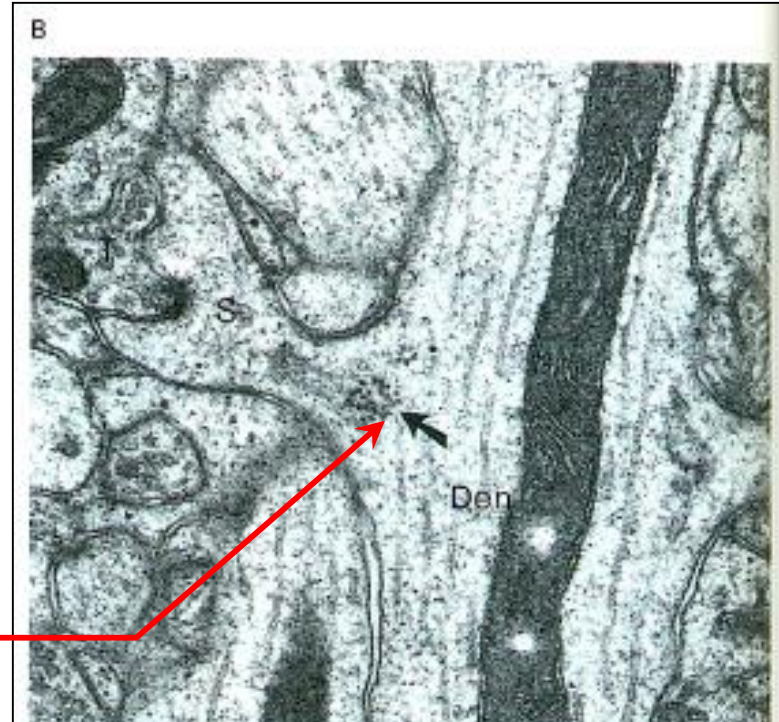
# Where is the protein synthesis machinery in neurons?

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 dendritic

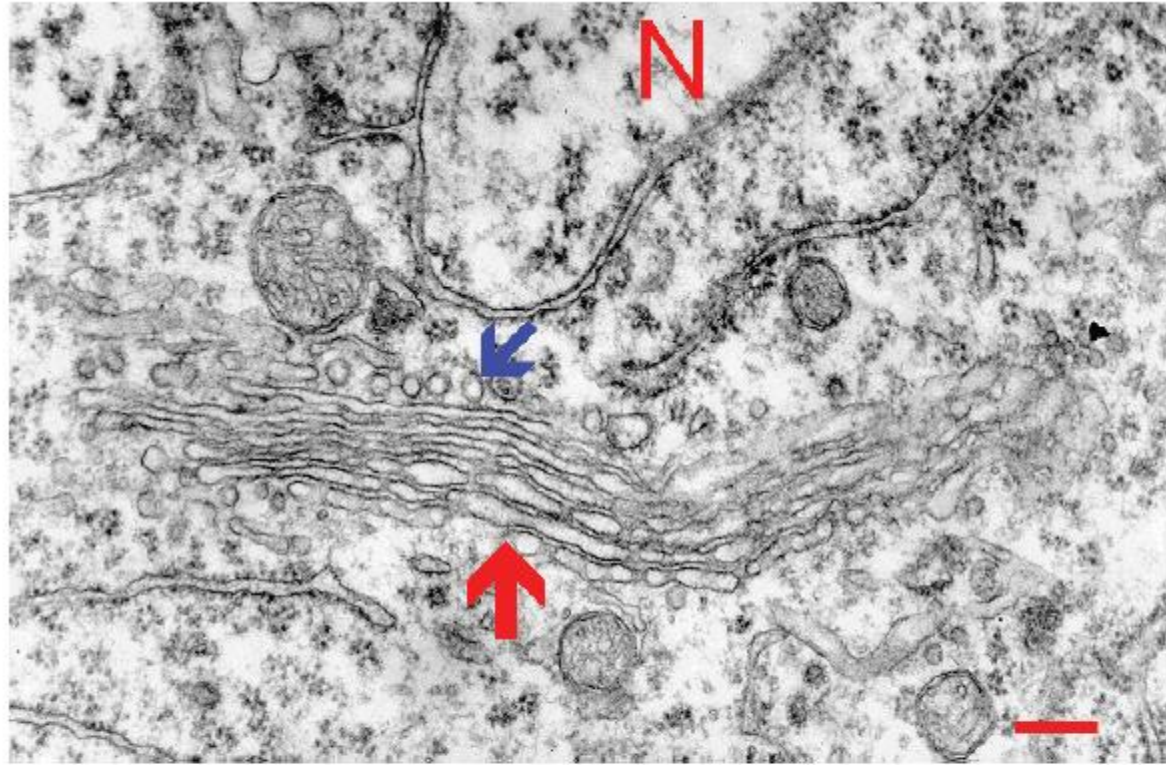
Protein targeting in dendritic spines is regulated by local synthesis



**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 mitochondrion.) Note the absence of polyribosomes in other parts of the dendritic shaft.  $\times 60,000$ . (Courtesy of O. Steward, University of Virginia.)



# The Golgi

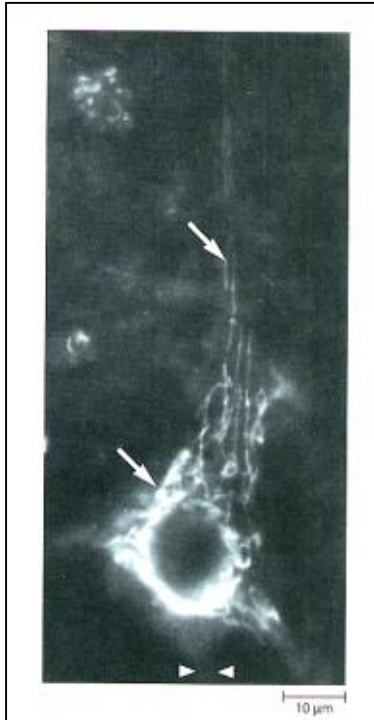


- Sorts, packages, and modifies cargo proteins coming from ER
- Major site of carbohydrate synthesis
- Site of glycosylation and proteoglycan synthesis
- Site of initial aspects of processing of neuropeptides

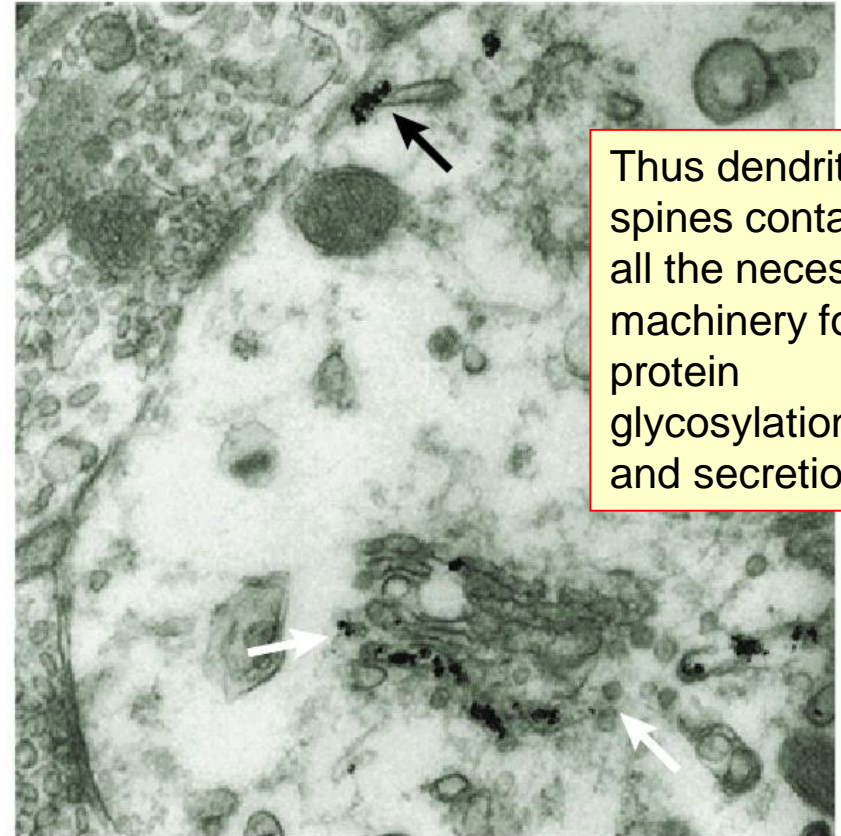


**The Golgi complex** is present in the somatodendritic compartment and does not extend into the axon

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



**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.)



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

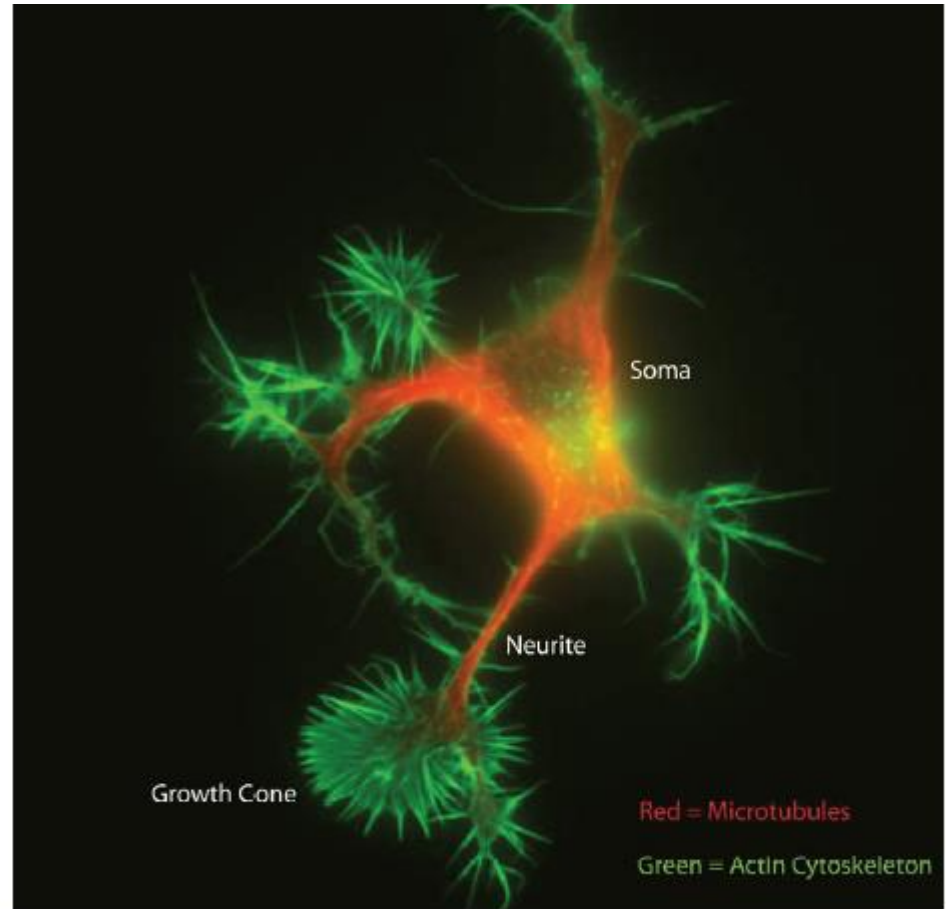
**Figure 1 | Electron-microscopic evidence for the existence of Golgi markers in neuronal dendrites.** Using gold-labelled antibodies to trans-Golgi network protein TGN38, this Golgi marker can be visualized on the inside of the dendritic spine. White arrows indicate TGN38 immunoreactivity on dendritic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

# Neuronal cytoskeleton

Microfilaments

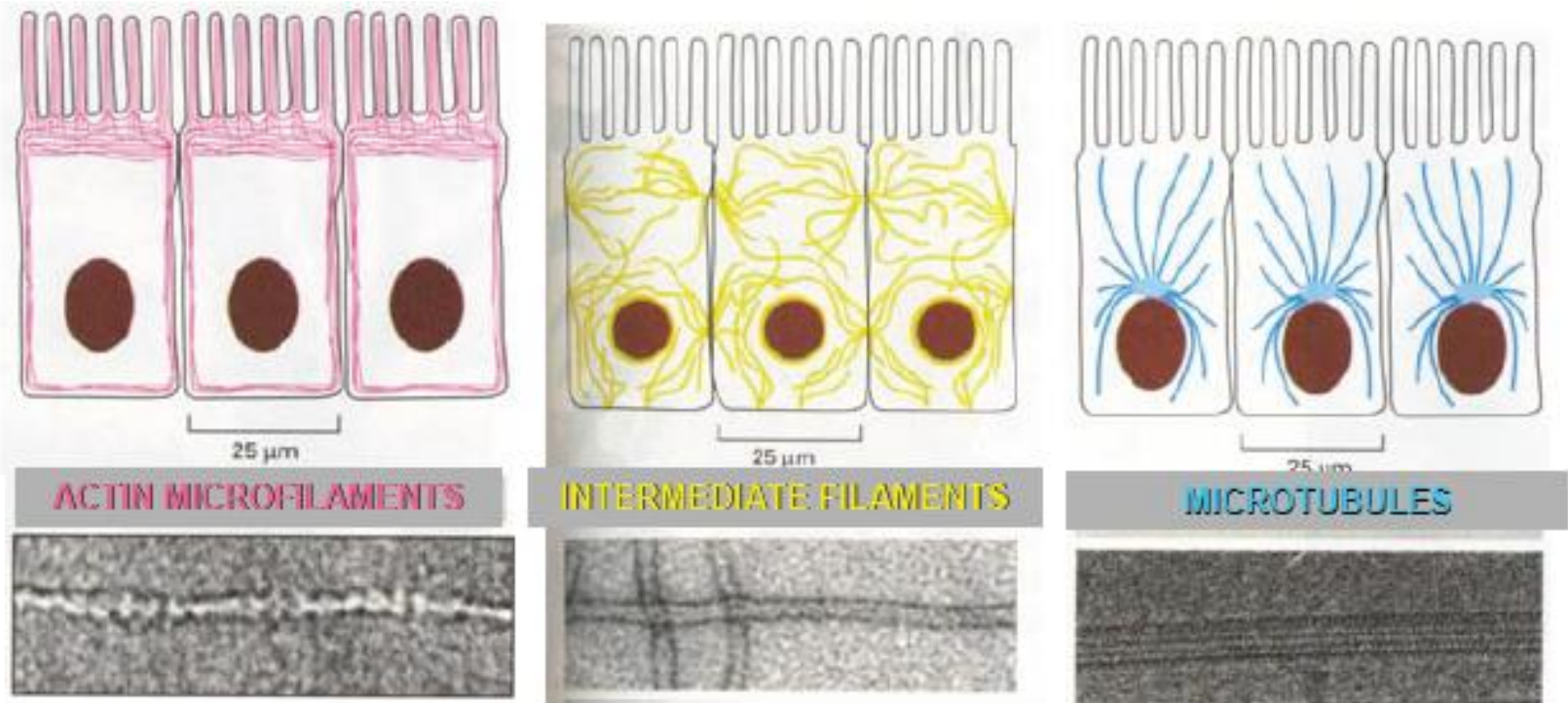
Intermediate  
Filaments

Microtubules



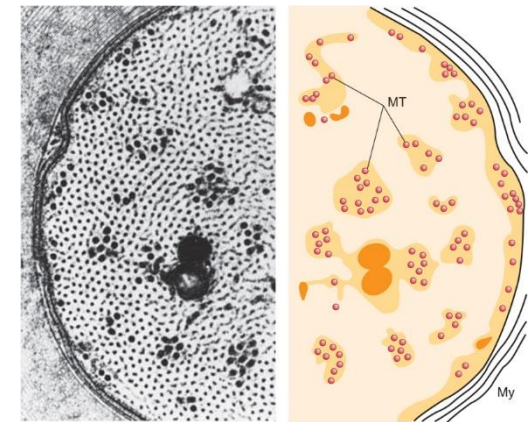
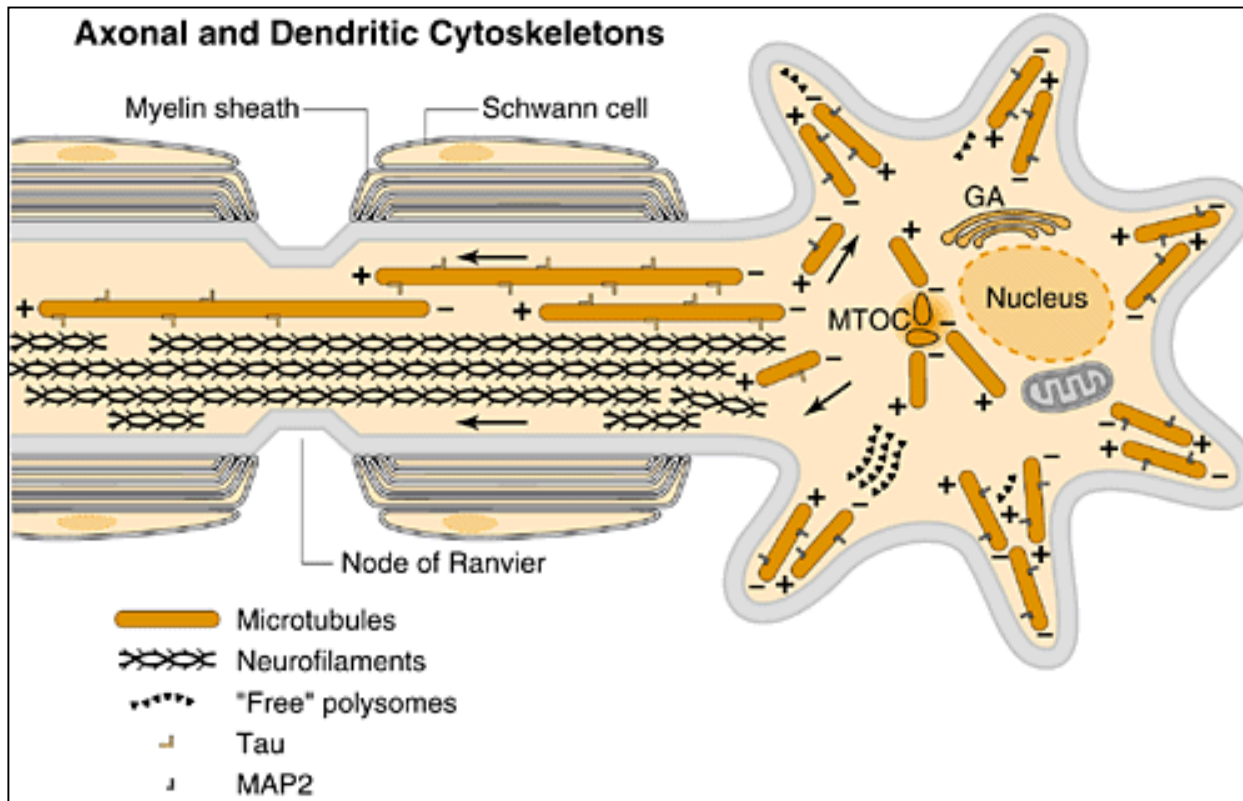
# The three types of cytoskeletal filaments have a differential subcellular distribution

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





# Distribution of neurofilaments and microtubules in the neuron



Cross section of an axon

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

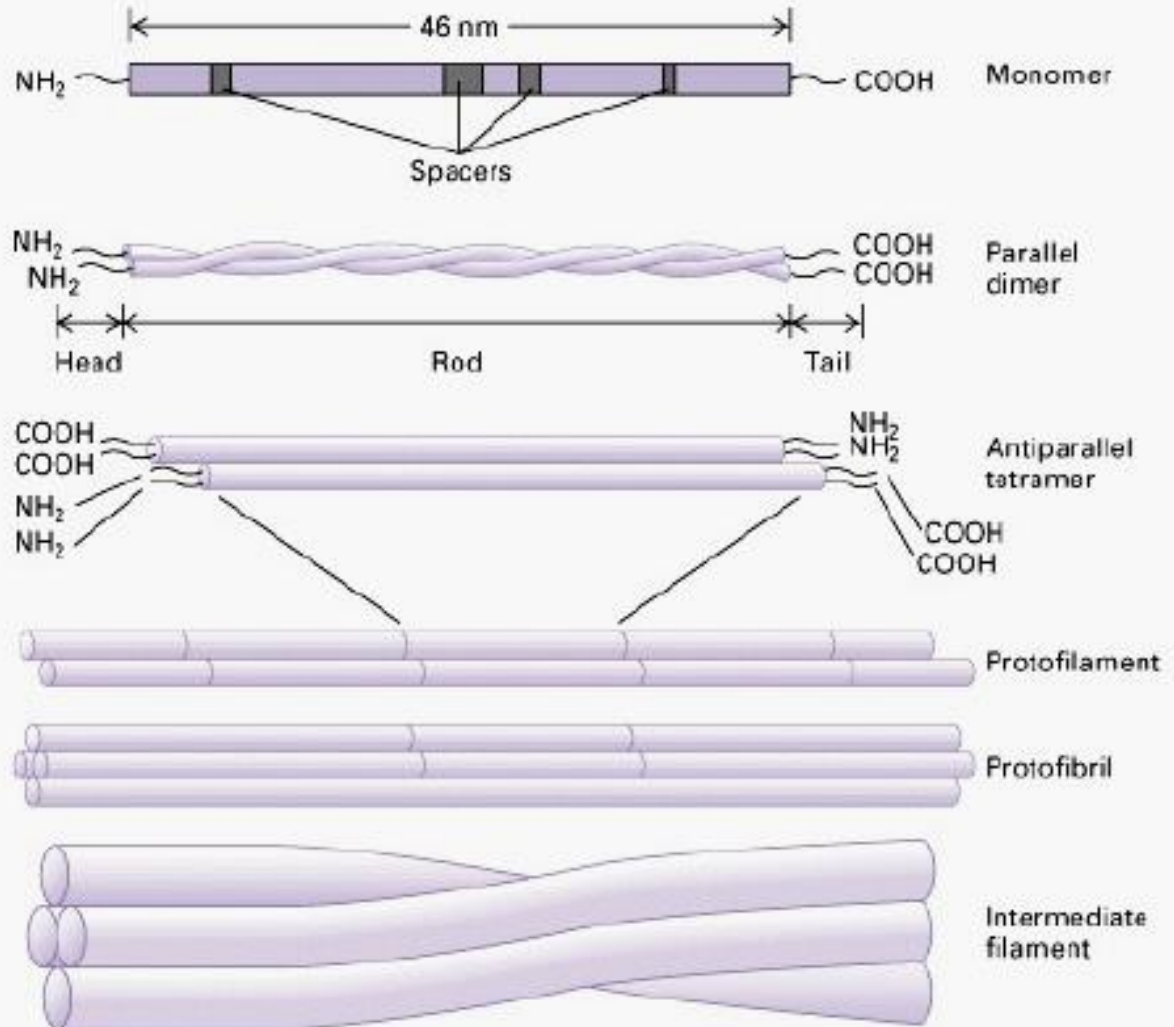
**Microtubules** in axons and distal dendrites are unipolar, with the + end pointing away from the cell body. However in proximal dendrites are of mixed polarity.



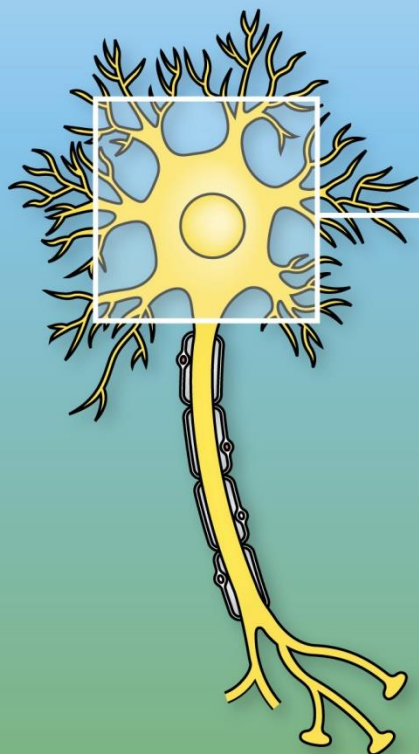
## Intermediate Filaments – no polarity

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

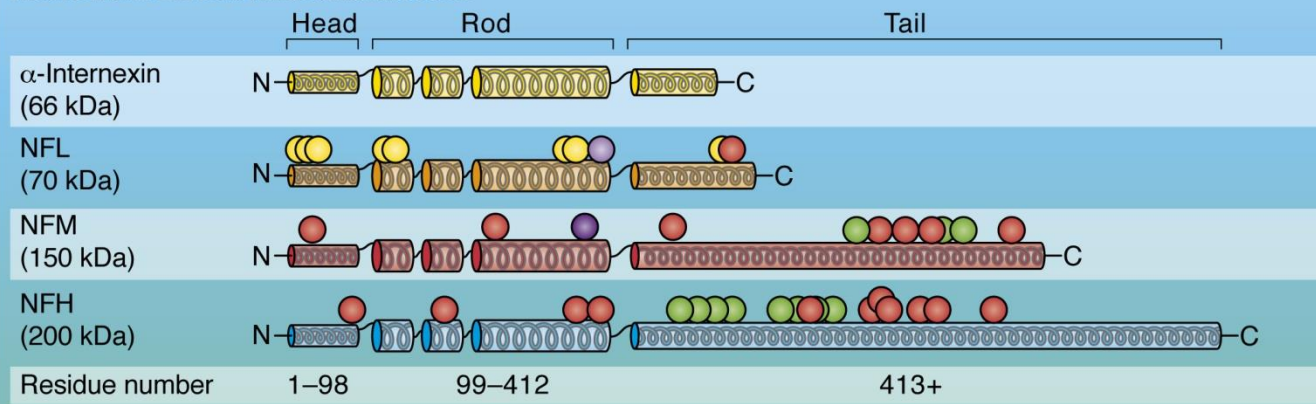
Neurofilaments have an unusual degree of **metabolic stability**, with a special role in stabilizing and maintaining neuronal morphology



# Neurofilament structure



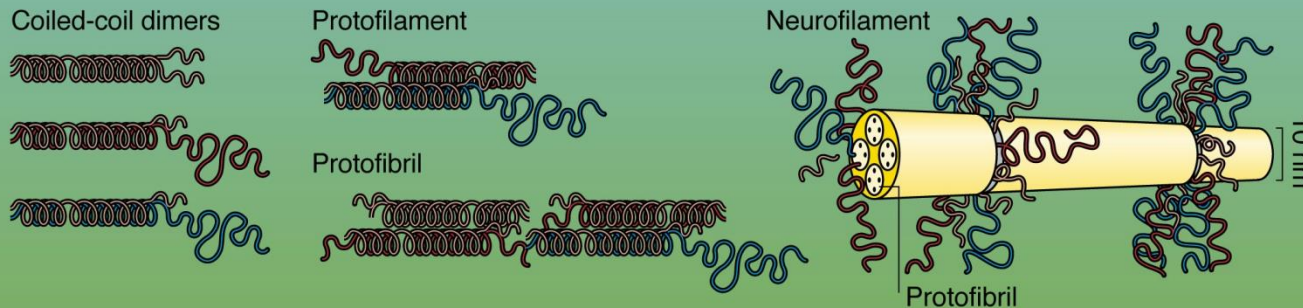
## Neurofilament subunits and disease



## Key

● Mutation in CMT2   
 ● Mutation in CMT1   
 ● Hyperphosphorylated in AD   
 ● Mutation in PD   
 ● Mutation in ALS

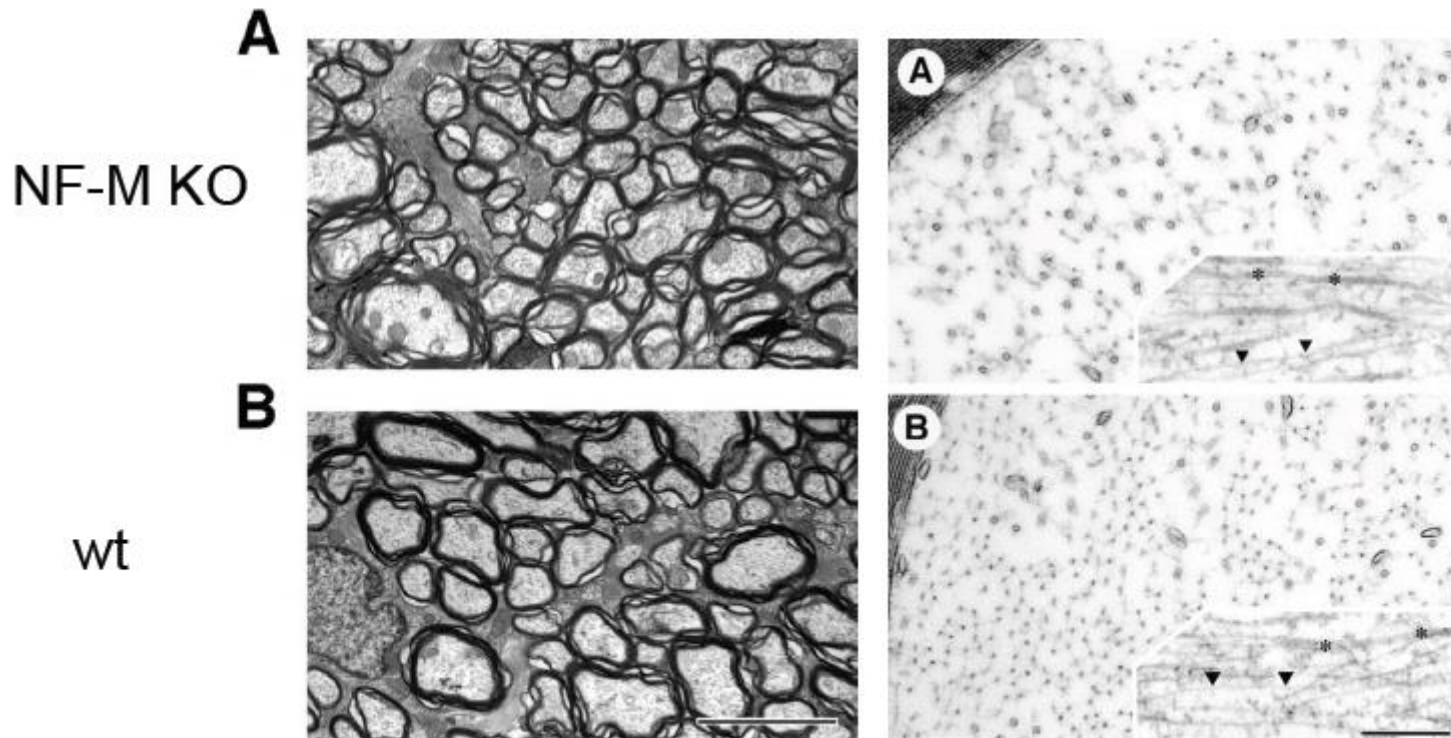
## Neurofilament assembly



CMT = Charcot Marie-Tooth disease

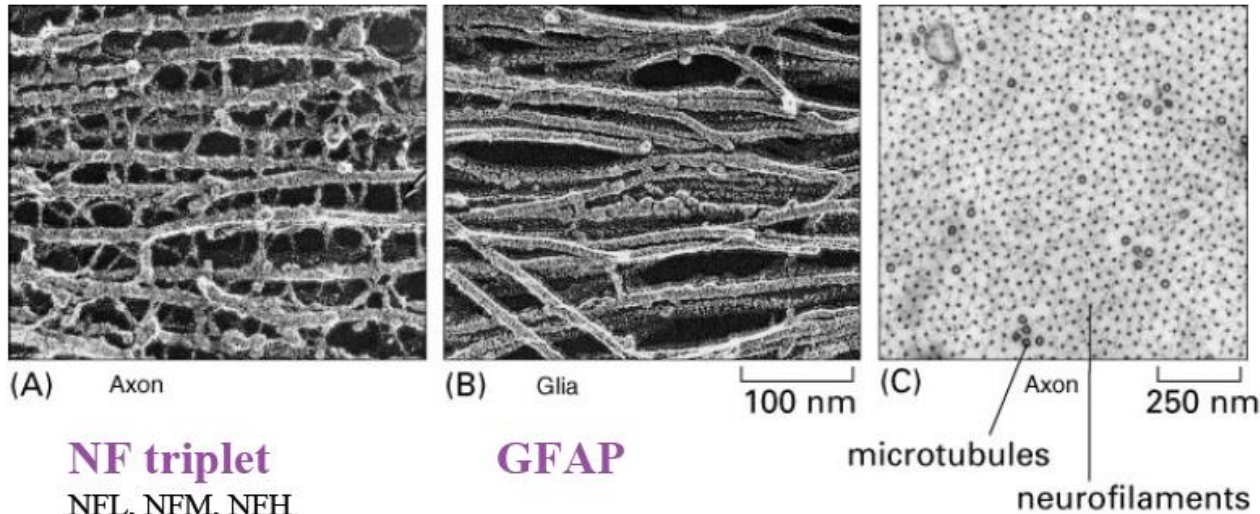
# Neurofilaments regulate axon caliber

- Represent 13% of total protein in mature neurons
- Neurofilament composed of NF-L, -M, and -H
- NF-L required for all filament assembly
- Disruption of NF genes reduces axon diameter >50%





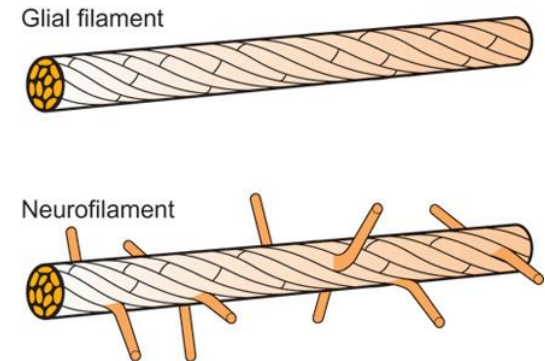
# IF Network in Neurons and Glia



## Side arms of NFM & NFH:

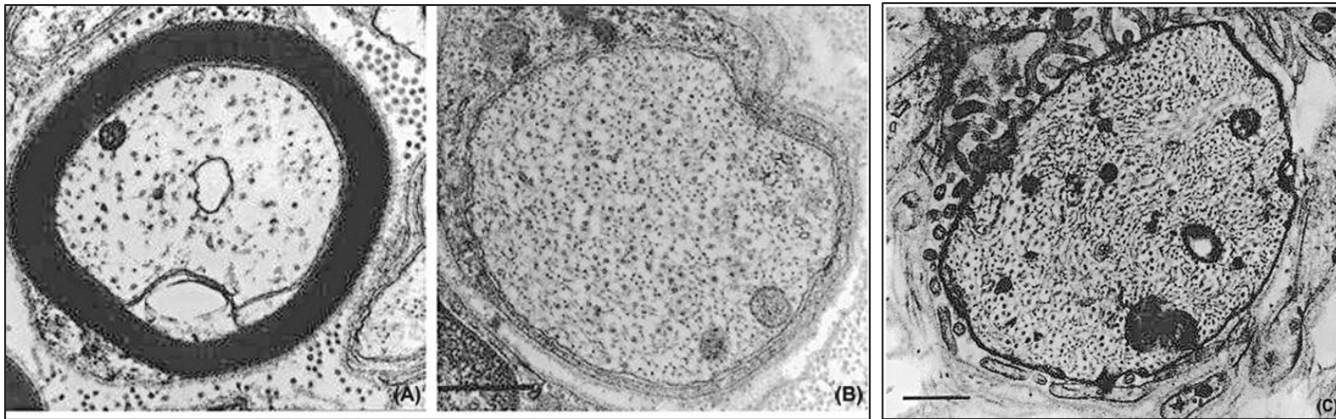
Contribute to wider spacing of NFs relative to glial IFs  
a role in determining axonal caliber – role of phosphorylation

**Tangles or aggregates of NFs are often associated with neurodegenerative diseases**





# 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  $\mu\text{m}$  with **(A)** and **(B)** at the same magnification. (Micrographs supplied by Sylvie de Waegh and Scott Brady.)

# Microfilaments

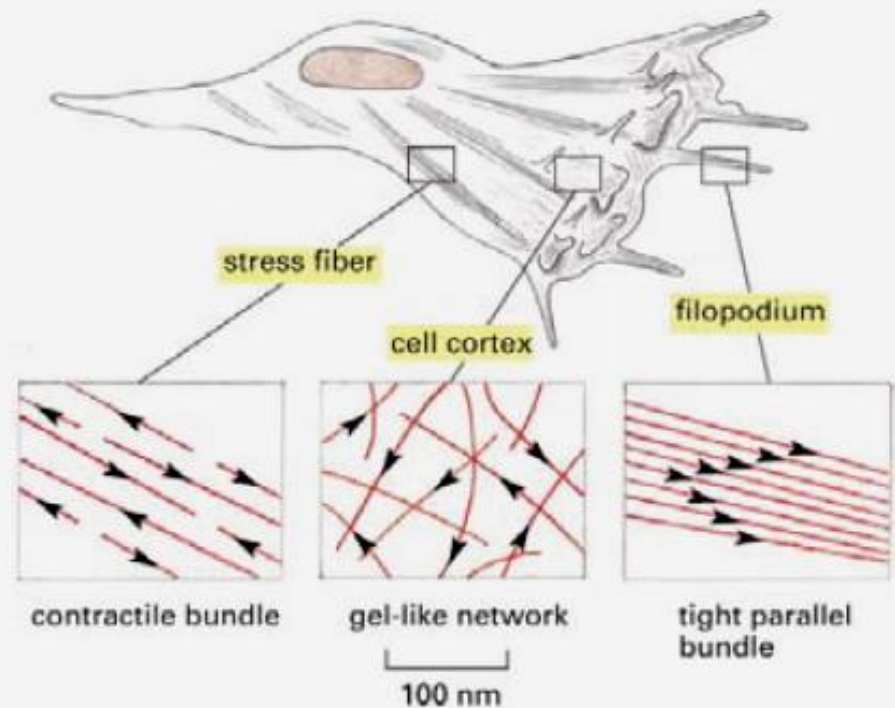
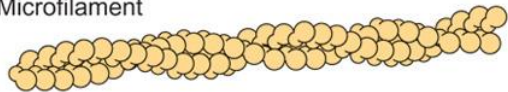
- Smaller very dynamic structural filaments
- Composed of actin polymers
- Involved in cell motility and axon growth via filopodia and lamellipodia
- Involved in endocytosis and intracellular motility
- Structurally component of neuronal spines
- Act with myosins to drive certain intracellular movements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

# ACTIN

## Cell/ Growth Cone Migration

## Sensing and Processing Environmental Cues

Microfilament



<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

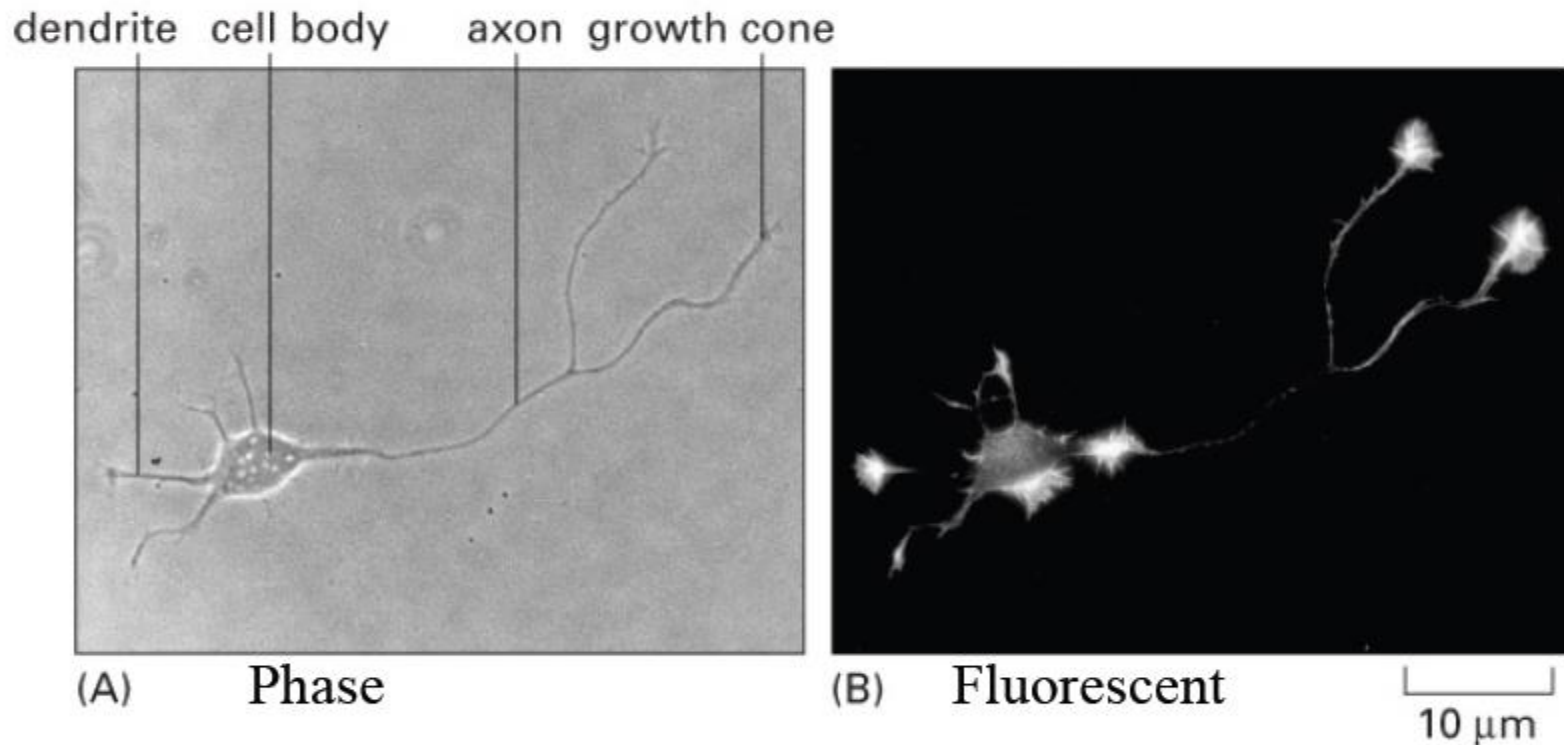
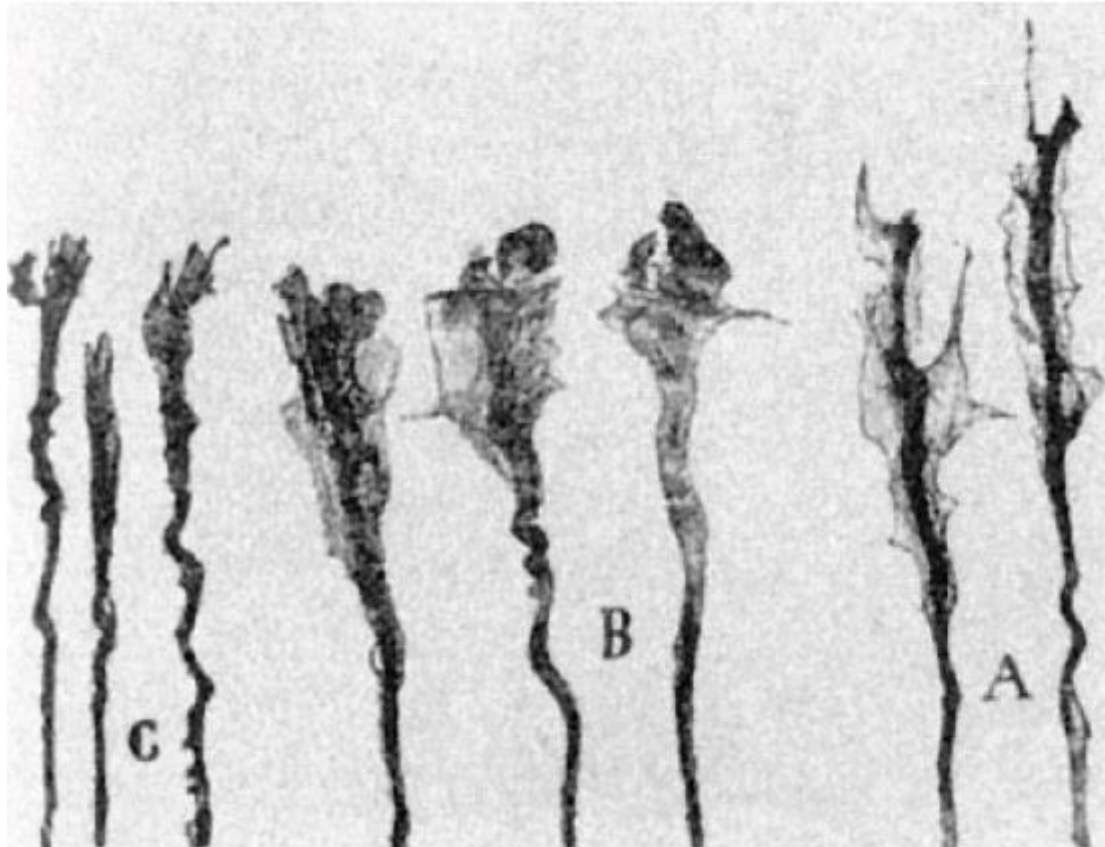


Figure 21-97. Molecular Biology of the Cell, 4th Edition.





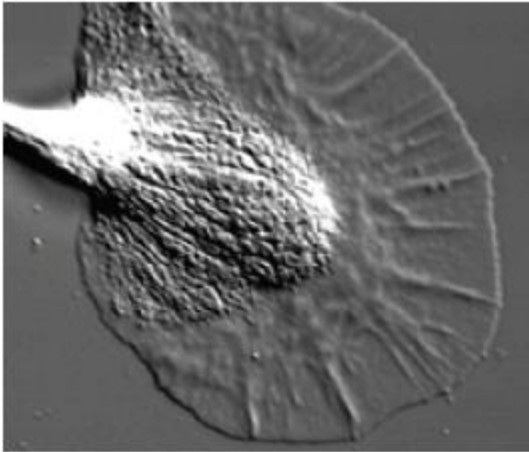
## Growth Cones

Mammalian from RyC

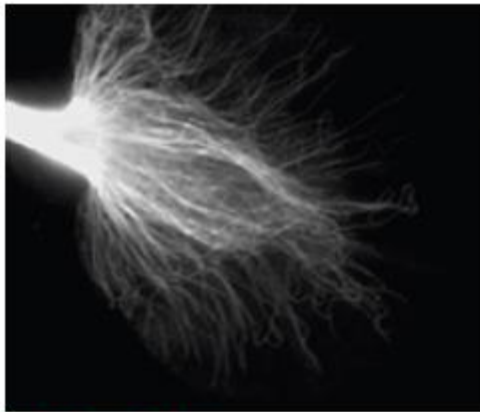
Growth cones sample the environment and respond to signaling cues.

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

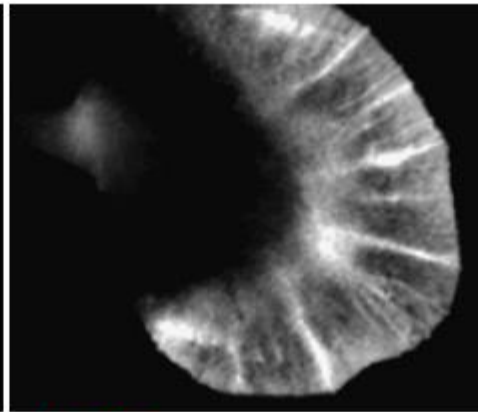
## Growth Cone of Aplysia Bag Cell Neuron



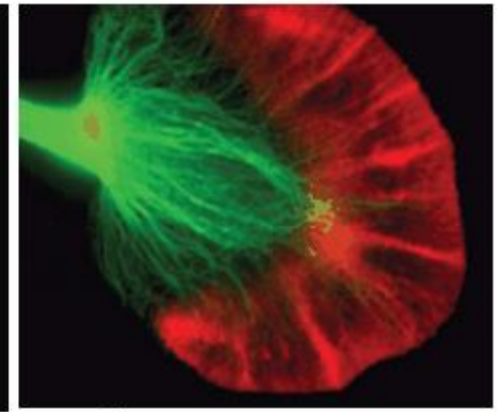
These mollusc neurons are great for growth cone studies. Big and flat, note a peripheral (P) region with actin but relatively empty and the vesicle filled central (C) region (with mts). MTs concentrated in the central region, but some in P region



**Tubulin**



**Actin**



**Both**



# 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**



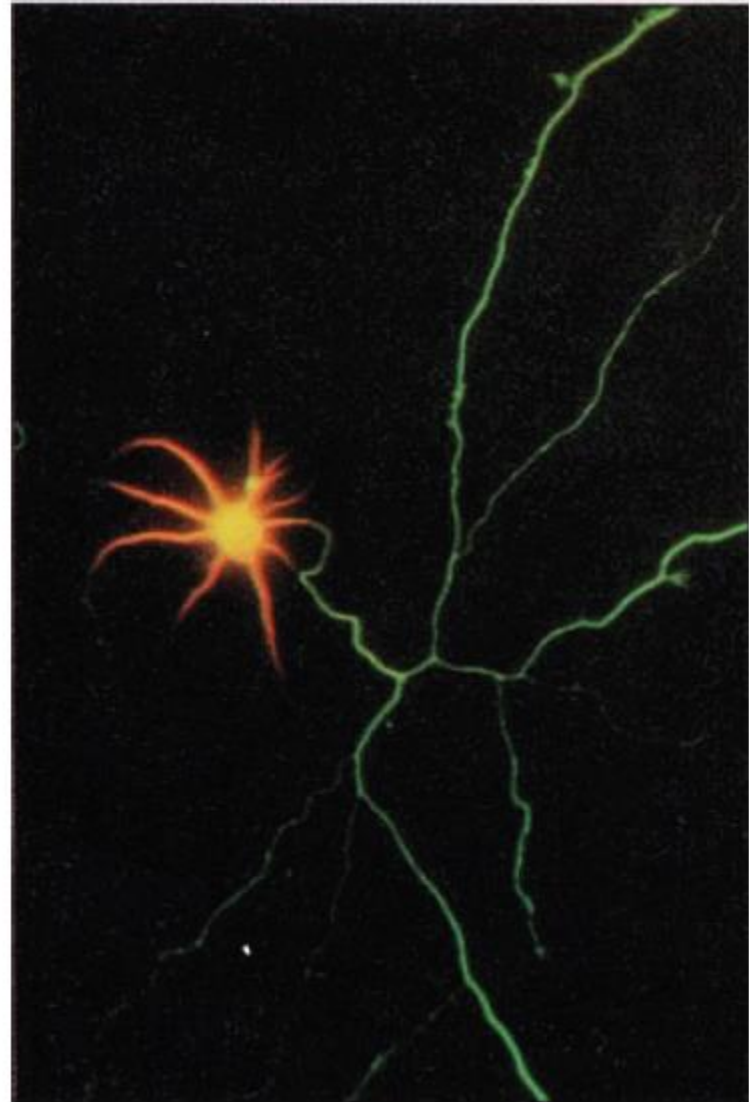
## **Axons and Dendrites: Different Microtubule Associated Proteins (MAPs)**

Two MAPs (structural):

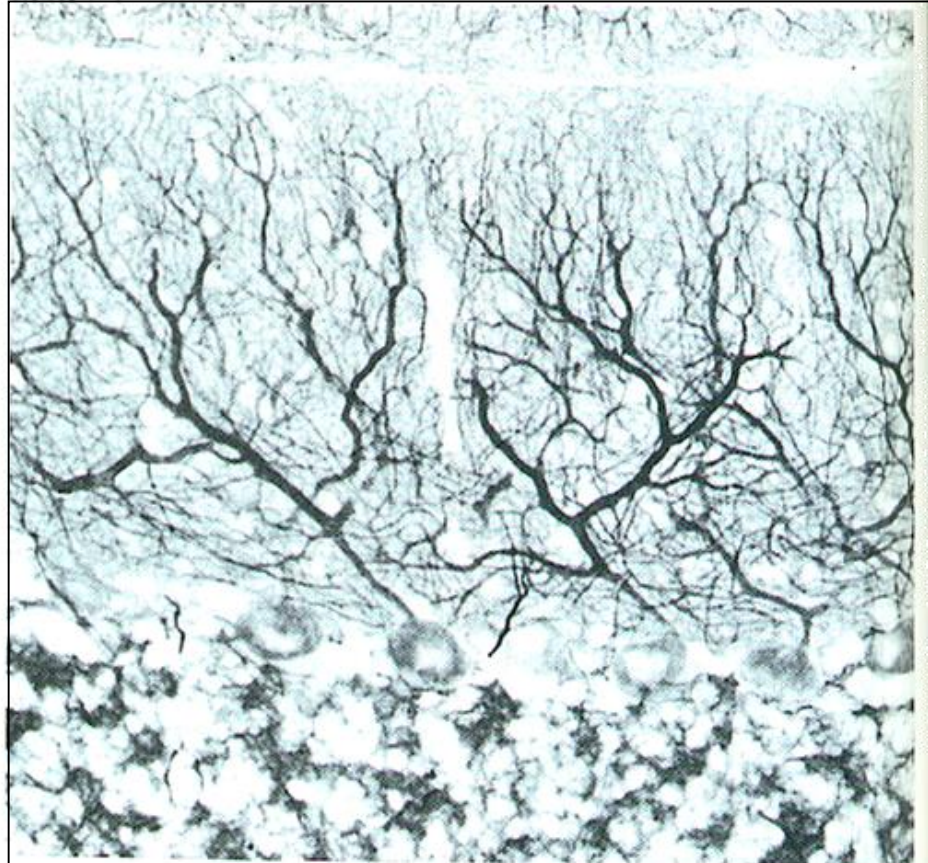
**Red: MAP2** in the soma and dendrites

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

Cultured hippocampal neuron



MAP2 is a marker  
for neuronal  
dendrites



20 μm

**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

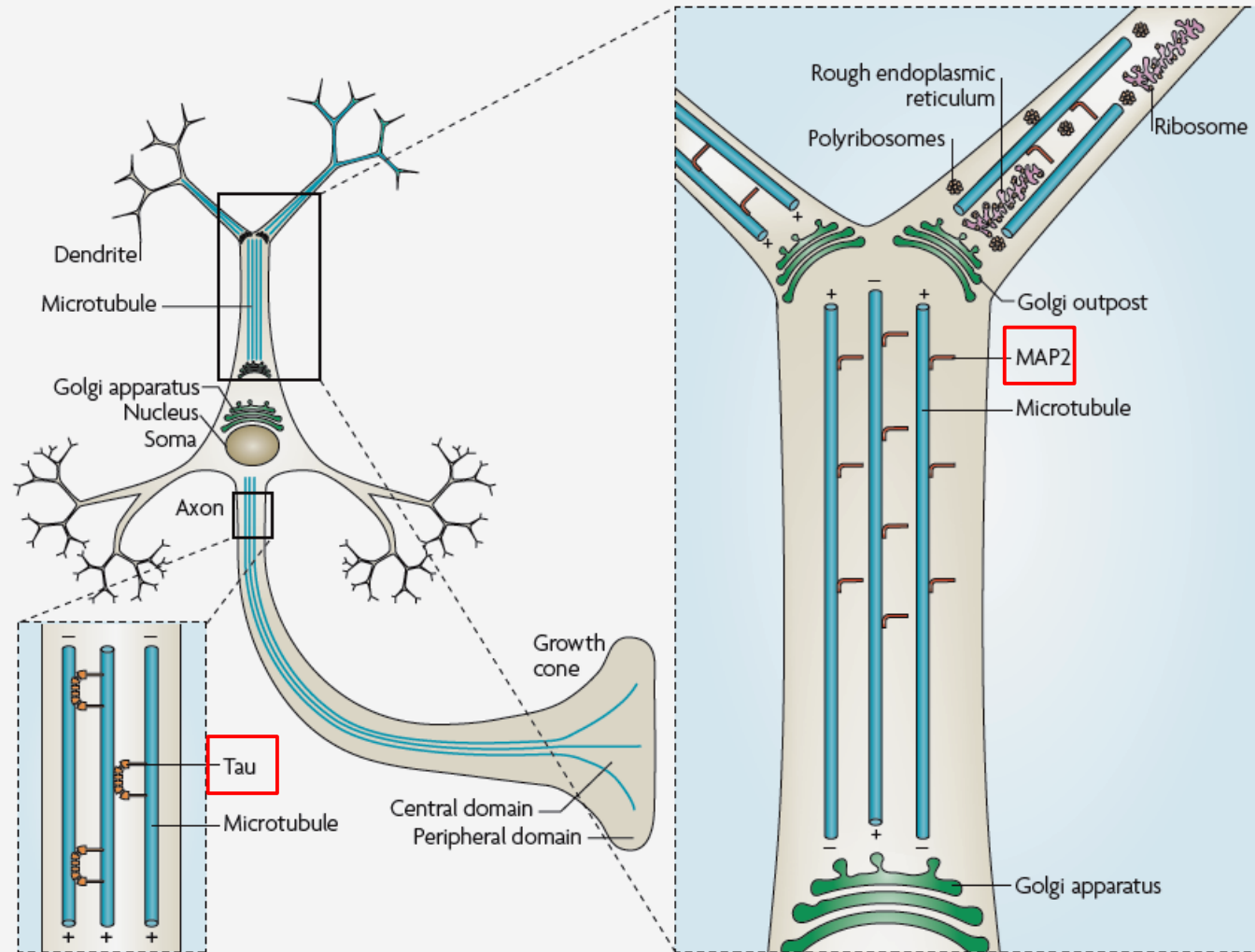
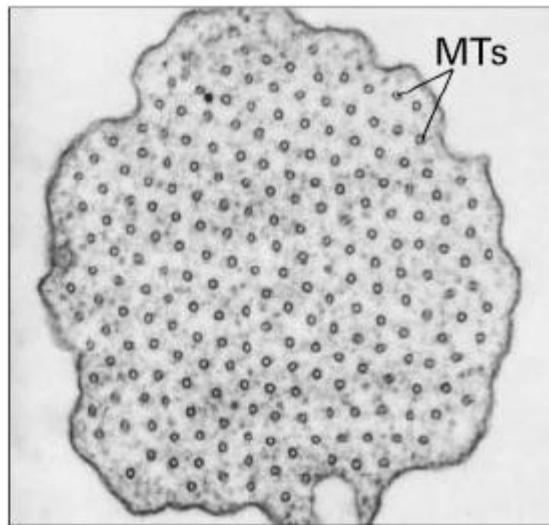
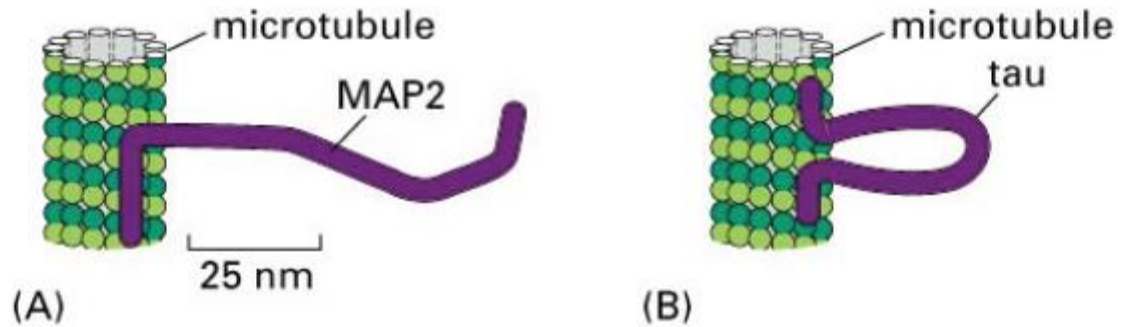


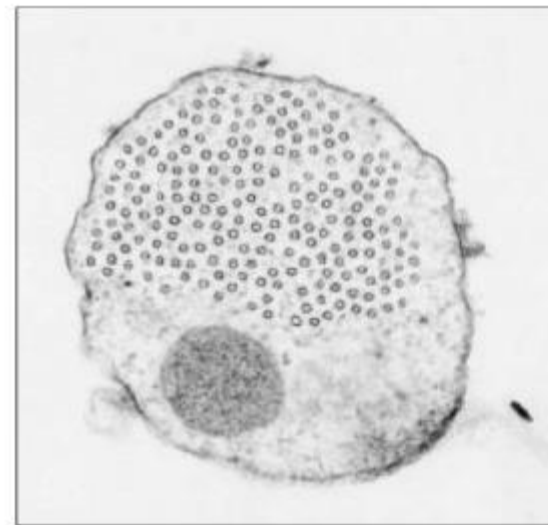
Figure 1 | **Microtubule organization and organelle distribution in axons and dendrites.** Axons have tau-bound microtubules of uniform orientation, whereas dendrites have microtubule-associated protein 2 (MAP2)-bound microtubules of mixed orientation. Dendrites also contain organelles that are not found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

# MAP2 and tau bind to MTs.

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



(C) MAP2 OE



(D) Tau OE  
300 nm



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

<b>Protein</b>	<b>Expression pattern and distribution</b>	<b>Modifications</b>
<b><math>\alpha</math>-Tubulin</b> (multigene family)	In all cells but some isoforms preferentially expressed in brain	Acetylation, tyrosination
<b><math>\beta</math>-Tubulin</b> (multigene family)	In all cells but some isoforms preferentially expressed in brain	Phosphorylation, polyglutamylation
<b><math>\gamma</math>-Tubulin</b>	In all cells, pericentriolar region/MTOC	?
<b>MAP1a</b> (multigene family)	Appears late, Wide distribution	Phosphorylation
<b>MAP1b</b>	Appears early, then declines, enriched in <u>axons</u>	Phosphorylation
<b>MAP2a</b> (single gene)	High MW, <u>dendritic in mature neurons</u>	Phosphorylation
<b>MAP2b</b>	High MW, <u>dendritic expressed throughout lifetime</u>	Phosphorylation
<b>MAP 2c</b>	Low MW, <u>dendritic in developing neurons</u>	Phosphorylation
<b>Tau</b> (single gene)		Phosphorylation
<b>High MW</b>	<u>Peripheral axons</u> with distinctive phosphorylation pattern	
<b>Low MW</b>	Enriched in <u>CNS axons</u> with distinctive phosphorylation pattern	Phosphorylation
<b>MAP4</b>	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)

# Microtubule organization in developing axons

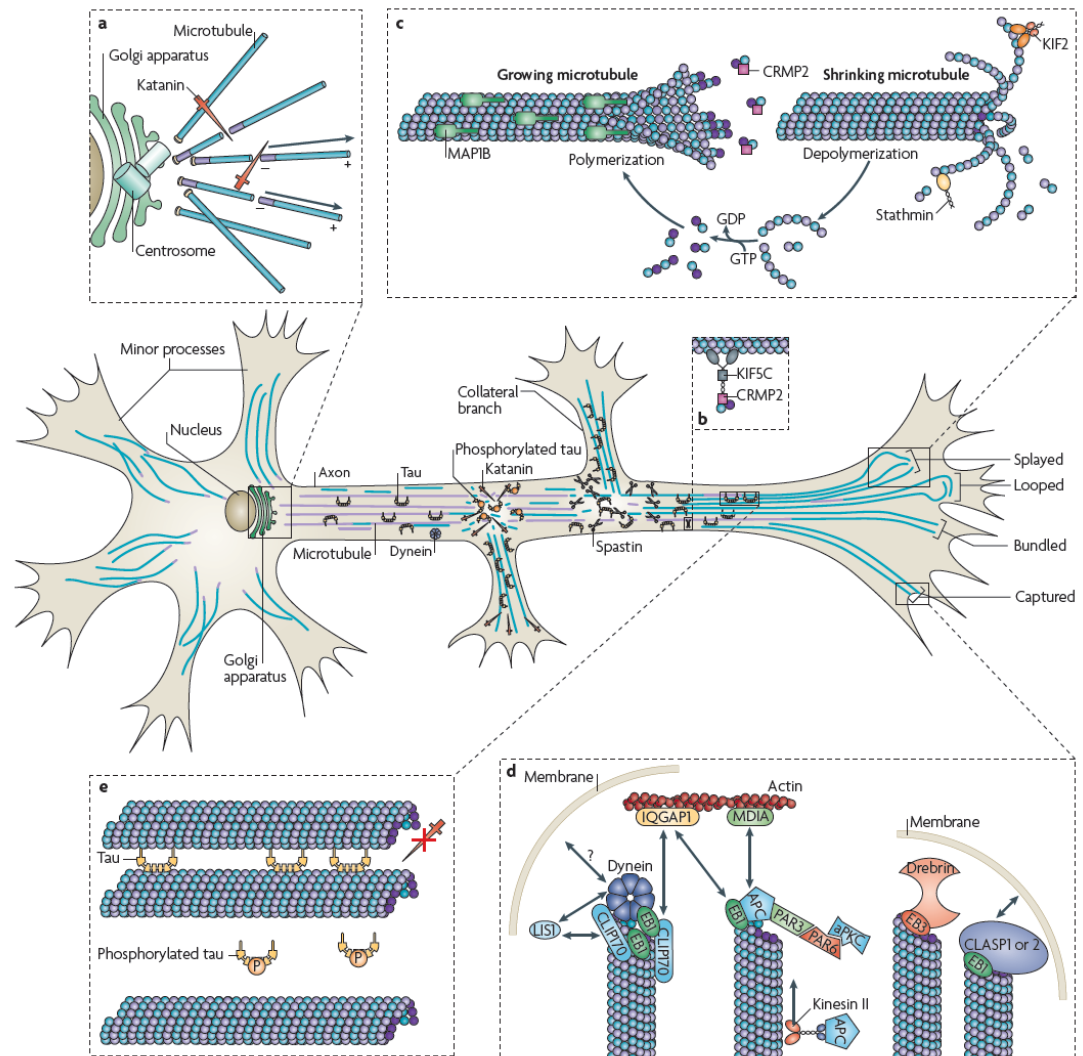
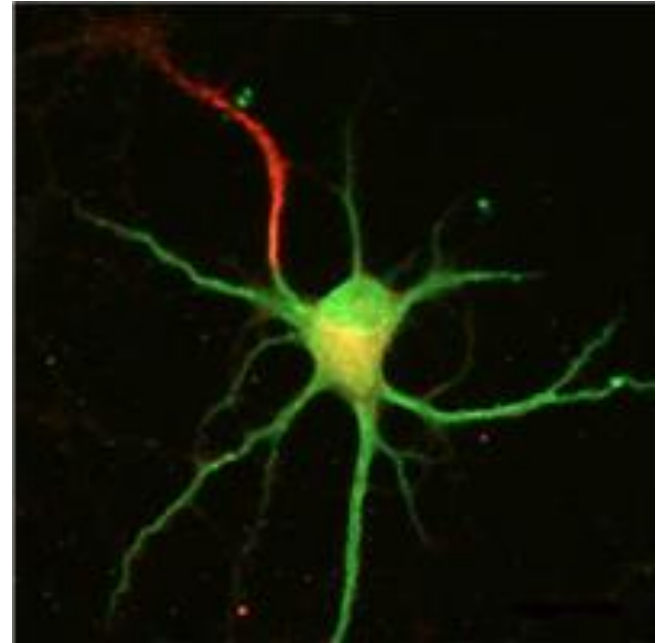


Figure 2 | **Microtubule organization in developing axons.** The organization and regulation of microtubules in a stage 3 hippocampal pyramidal neuron. Dynamic (blue) microtubules predominate in minor processes (short, unbranched neurites) and at the distal end of the axon and collateral branches, whereas 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 collapsin 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 PRKC); 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.

# NEURON POLARITY

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

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 proteins



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

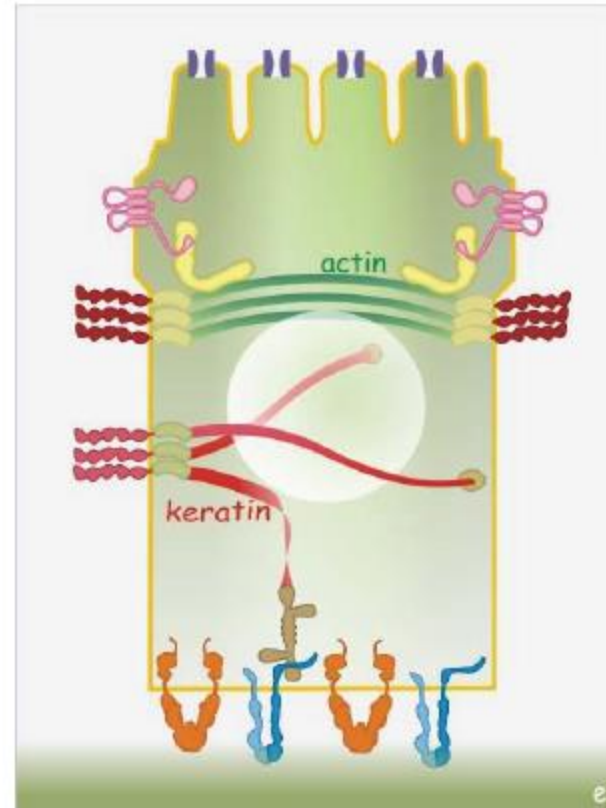


# Separating Subcellular Compartments

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

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

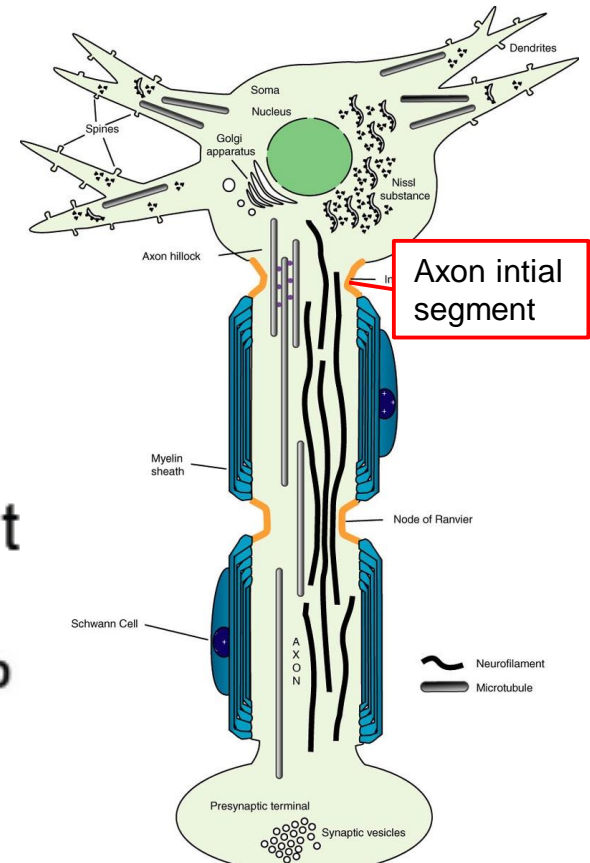
Where is the polarity division in neurons?



## Marking the border between somatodendritic and axonal compartments:

# Axon initial segment

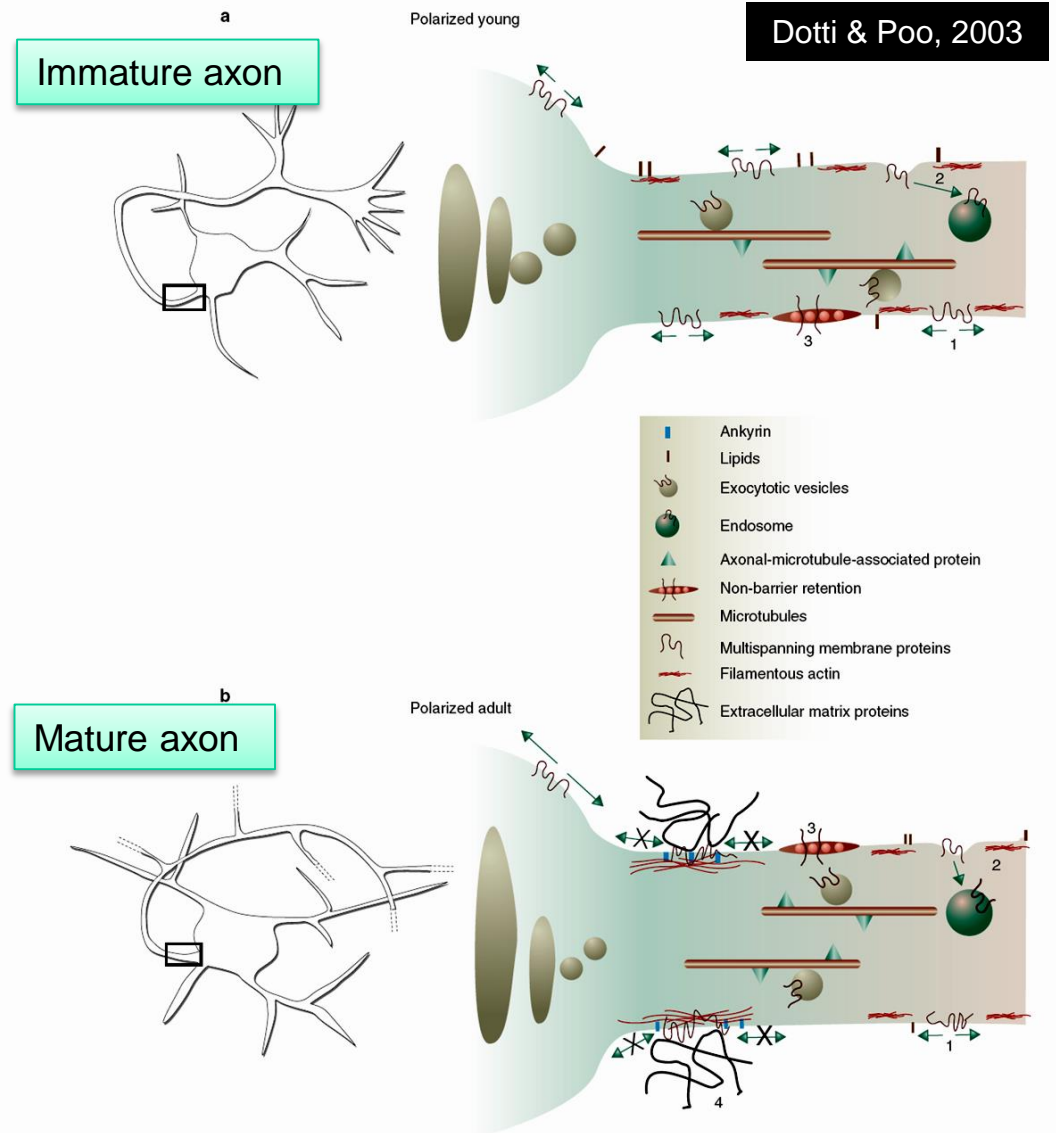
- Mature neurons are polarized
- The axon initial segment (AIS) acts as the integrator of subthreshold synaptic events into all or nothing APs.
- Its also a cytoplasmic filter between axon and somatodendritic compartment
- Specific motors carrying axonal cargo can pass through this filter.
- Also barrier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



**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-sphingolipid 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.