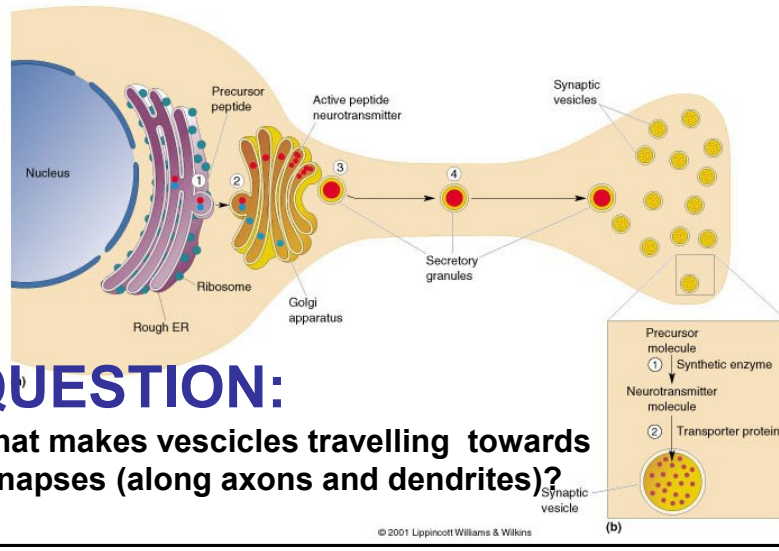
Lesson (10)

Inside the neuron VI:

Cytoskeleton and axonal transport

The classical view of vescicle trafficking in neurons

Figure 5.9
The synthesis and storage of different types of neurotransmitter. (a) Peptides: ① A precursor peptide is synthesized in the rough ER. ② The precursor peptide is cleaved in the Golgi apparatus to yield the active neurotransmitter. ③ Secretory vesicles containing the peptide bud off from the Golgi apparatus. ④ The secretory granules are transported down the axon to the terminal where the peptide is stored. (b) Amine and amino acid neurotransmitters: ① Enzymes convert precursor molecules into neurotransmitter molecules in the cytosol. ② Transporter proteins load the neurotransmitter into synaptic vesicles in the terminal, where they are stored.



QUESTION:

What makes vescicles travelling towards synapses (along axons and dendrites)?

In this lecture we will see:

- **Review of the Cytoskeletal Filaments**
- **Description of the Roles of Filaments in Neurons**
- **Axonal Transport**
- **Microtubule-based Motor Proteins and The Mechanism of Fast Axonal Transport**
- **What is Slow Axonal Transport?**

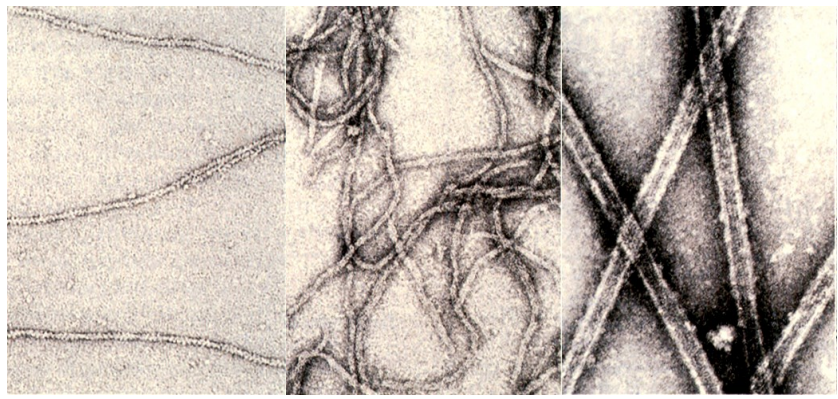
Cytoskeleton

- Functions of Cytoskeleton
  1. Dynamic scaffold
  2. Internal framework
  3. Network of highways
  4. Force generating apparatus – cell movement
  5. m-RNA anchoring
  6. Cell division

Cytoskeleton

- Eukaryotic cell Skeletal System
  - **Microtubules**
    - Rigid tubes
    - *Tubulin*
  - **Microfilaments**
    - Solid / thinner
    - *Actin*
  - **Intermediate filaments**
    - Tough ropelike fibres
    - *Many related proteins*

REVIEW OF CYTOSKELETAL FILAMENTS



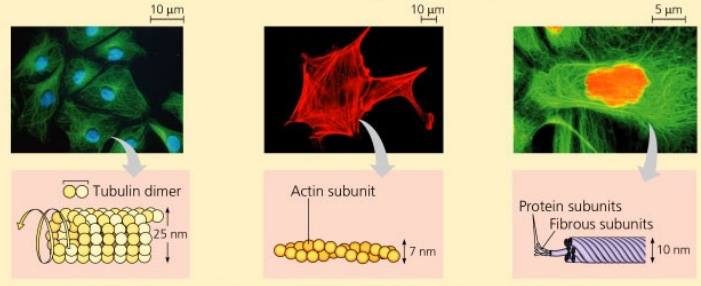
Actin Filament	**IF/ Neurofilaments**	**Microtubules (MT)**
7-9 nm (f-actin)	8-12 nm **(NF)**	24 nm

The Actin and Microtubules Filaments have polarity (different ends) NFs and IFs do not: explanation found in polymerization mechanism.

The three types of cytoskeletal filaments have a differential subcellular distribution

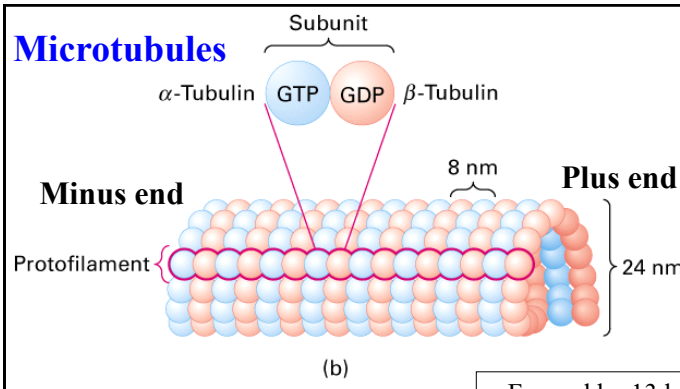
Table 7.2 The Structure and Function of the Cytoskeleton

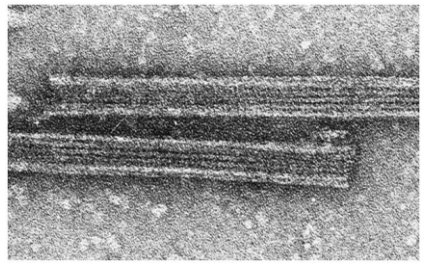
Property	Microtubules	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin	Fibrous proteins supercoiled into thicker cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, consisting of α-tubulin and β-tubulin	Actin	One of several different proteins of the keratin family, depending on cell type
Main functions	Maintenance of cell shape (compression-resisting "girders") Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streaming Cell motility (as in pseudopodia) Cell division (cleavage furrow formation)	Maintenance of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles Formation of nuclear lamina



SOURCE: Adapted from W. M. Becker, L. J. Kleinsmith, and J. Hardin, *The World of the Cell*, 4th ed. (San Francisco, CA: Benjamin Cummings, 2000), p. 753.

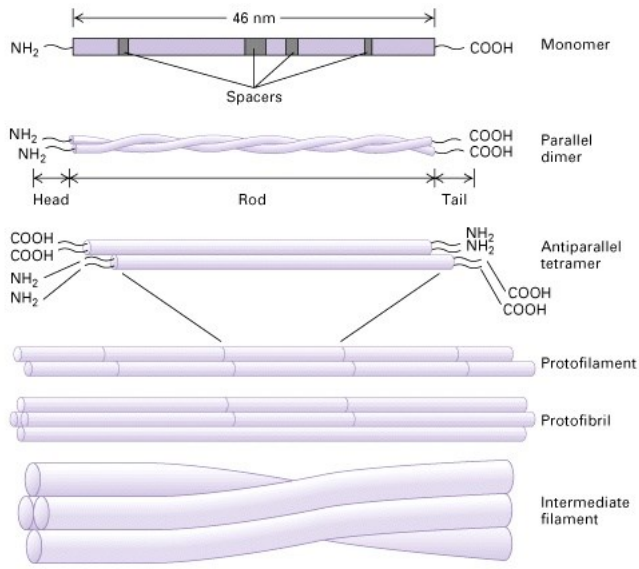
Microtubules





- Formed by 13 longitudinal strands arranged in helical configuration.
- Each strand is composed of aligned globular heterodimers consisting of α- and β-tubulin subunits.
- This leads to polarized assembly with one end having mainly exposed α subunits and the other end having mainly exposed β subunits

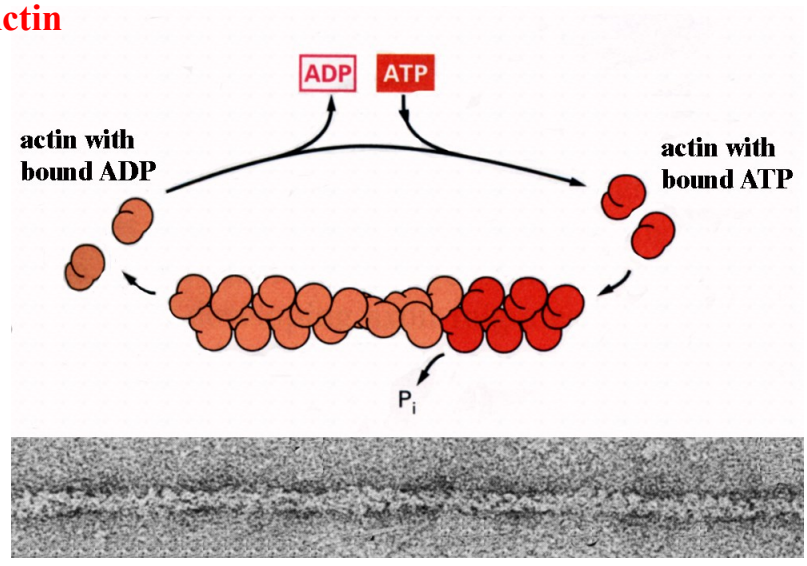
Intermediate Filaments – no polarity



Intermediate Filament types

Class and name	Cell type
Types I and II	
Acidic and basic keratins	Epithelial and endothelial cells
Type III	
Glial fibrillary acidic protein	Astrocytes and nonmyelinating Schwann cells
Vimentin	Neuroblasts, glioblasts, fibroblasts, etc.
Desmin	Smooth muscle
Peripherin	A subset of peripheral and central neurons
Type IV	
NF triplet (NFH, NFM, NFL)	Most neurons, expressed at highest level in large myelinated fibers
α-Internexin	Developing neurons, parallel fibers of cerebellum
Nestin	Early neuroectodermal cells. The most divergent member of this class; some have classified it as a sixth type.
Type V	
Nuclear lamins	Nuclear membranes

Actin



F-actin (actin filament): 2 strands in helix, two distinct ends, Polymerization favored at one (+). Actin "monomer" ~ 40 K Da

Nucleation Is the Rate-limiting Step in the Formation of a Cytoskeletal Polymer

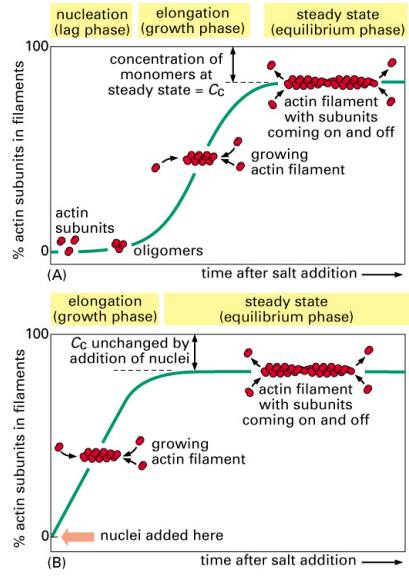


Figure 16–5. Molecular Biology of the Cell, 4th Edition.

The Two Ends of Microtubules and Actin Filaments Are Distinct and Grow at Different Rates

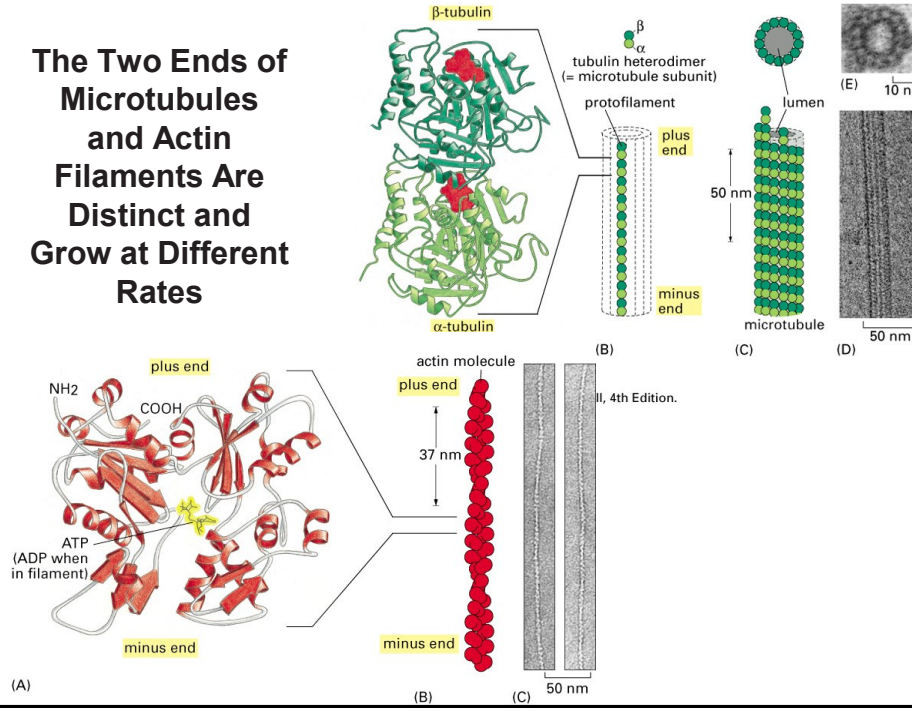


Figure 16–6. Molecular Biology of the Cell, 4th Edition.

Filament Treadmilling and Dynamic Instability Are Consequences of Nucleotide Hydrolysis

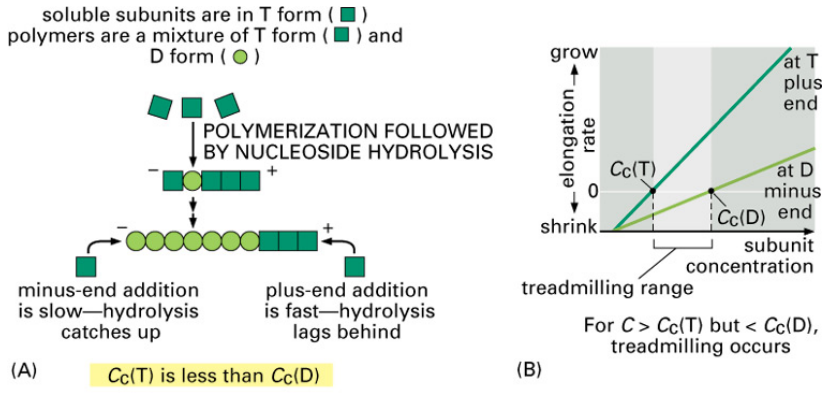
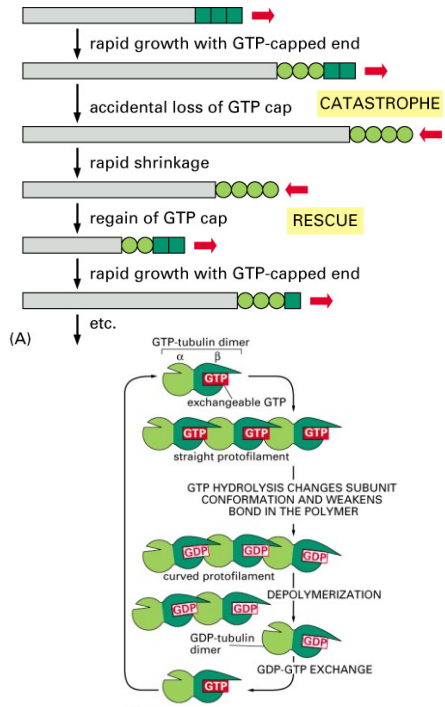


Figure 16–9. Molecular Biology of the Cell, 4th Edition.



Tubulin - dynamic instability

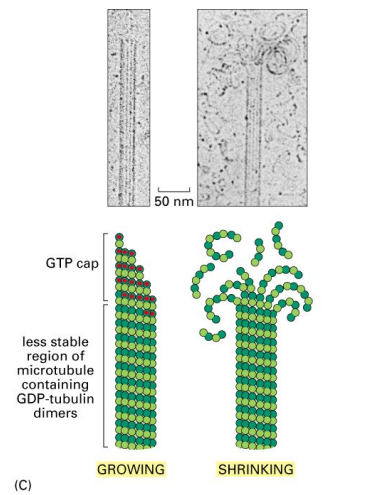


Figure 16–11 part 3 of 3. Molecular Biology of the Cell, 4th Edition.

Regulatory Proteins Bind to Free Subunits, Filaments Sides and Filament Ends

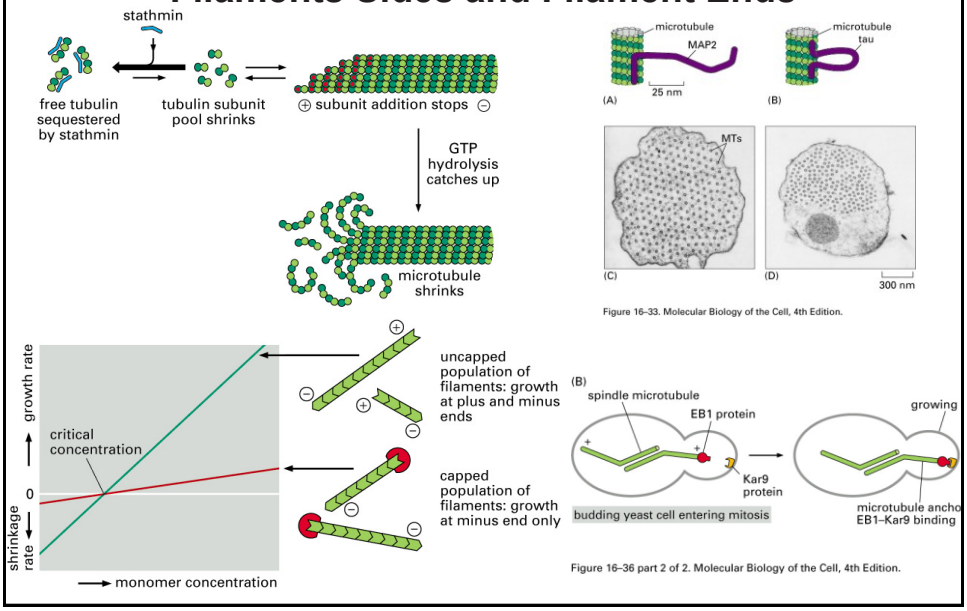


Figure 16–33. Molecular Biology of the Cell, 4th Edition.

Figure 16–36 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Intermediate Filaments Impart Mechanical Stability to Animal Cells

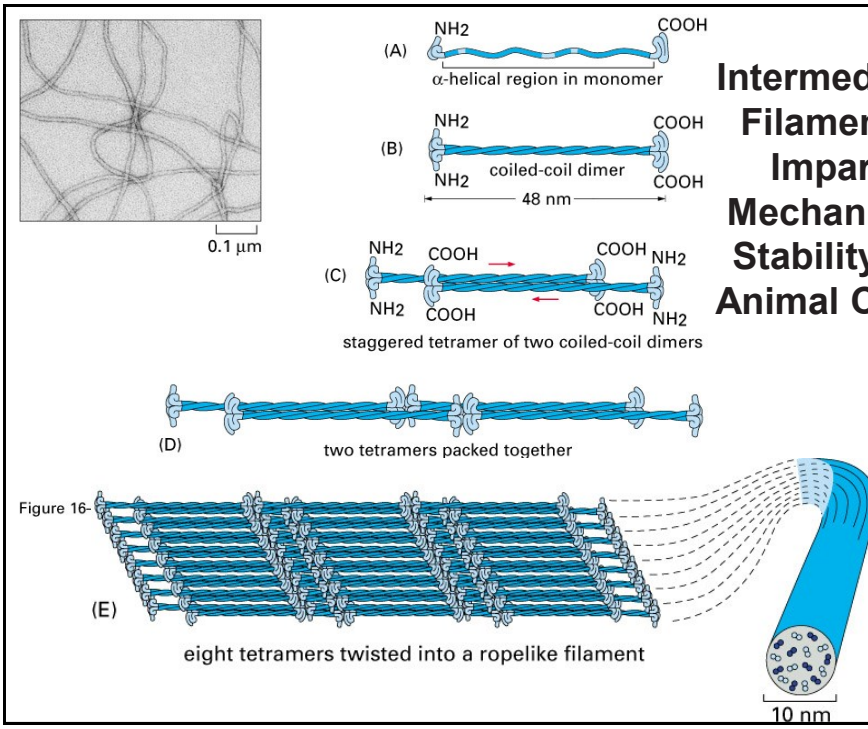
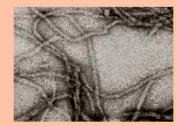
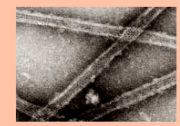
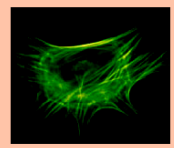
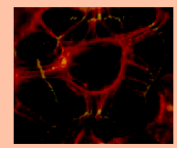
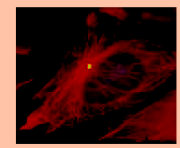


Figure 16-

insight overview

Table 1 **Eukaryotic cytoskeletal polymers**

Polymer	Actin filament	Microtubule	Intermediate filament
Protein subunit	Actin monomer	Tubulin heterodimer	Various proteins with an α-helical coiled-coil
Evolutionary origins	Prokaryotic hexokinase → prokaryotic actin-like proteins	Prokaryotic FtsZ	Early eukaryotic nuclear lamins
Polymerization by nucleation/elongation	Yes	Yes	Probably
Bound nucleotide	ATP	GTP	None
Ageing by nucleotide hydrolysis and phosphate release	Yes, allows binding of proteins that promote disassembly	Yes, destabilizes polymer	No
Flux of subunits through polymer at steady state (treadmilling)	Yes, very slow	Yes, slow	No
Dynamic instability (spontaneous fluctuations in length at steady state)	No	Yes, dramatic	No
Track for motors	Yes, 20 families of myosins	Yes, several dyneins and many families of kinesins	No
Electron micrographs of polymers			
Fluorescence micrographs of cells with polymers			

Micrographs reproduced with permission from ref. 31. The left fluorescence micrograph is from I. Herman, Tufts Medical School; the middle is from E. Smith and E. Fuchs, University of Chicago; and the right is from G. Borisy, University of Wisconsin.

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ROLE OF FILAMENTS IN NEURONS (DESCRIPTIVE)

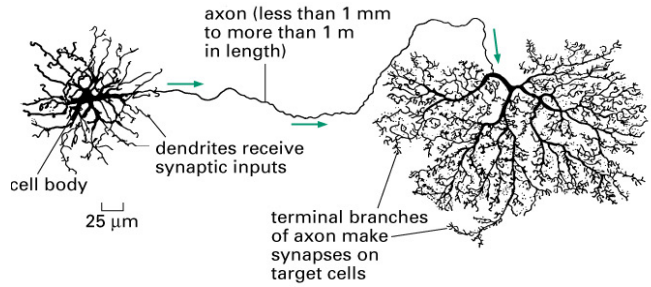
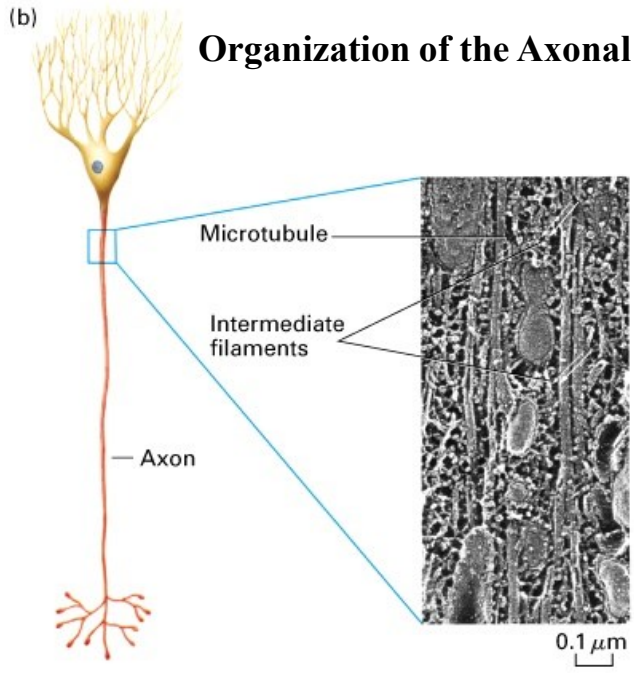


Figure 16–100. Molecular Biology of the Cell, 4th Edition.

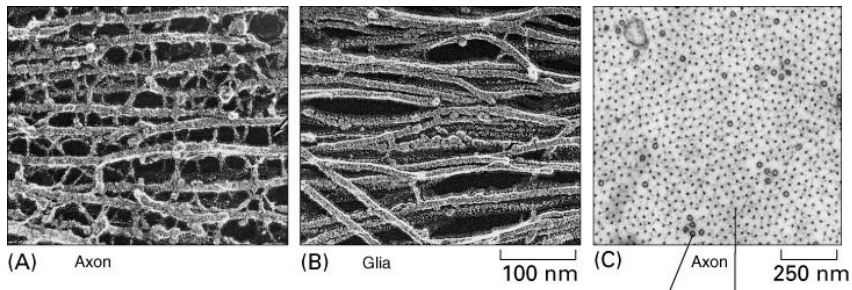
The Cytoskeleton is Essential for the Maintenance of Neuronal Structure

Cell Body & Dendrite, Axon, Synapse, Growth Cone
Many domains/structures all with varied components

Organization of the Axonal Cytoskeleton



IF Network in Neurons and Glia



NF triplet
NFL, NFM, NFH

GFAP

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

ACTIN

Roles:

Cell/ Growth Cone Migration

Synapse Structure

Sensing and Processing

Environmental Cues

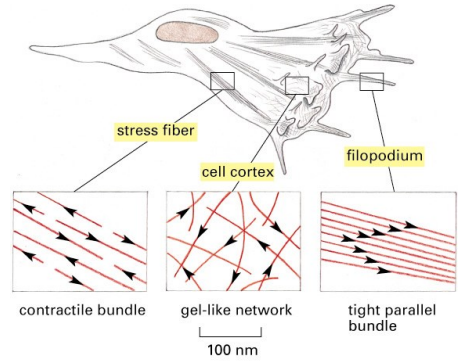
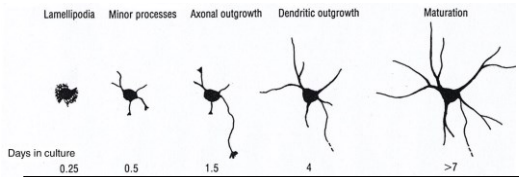
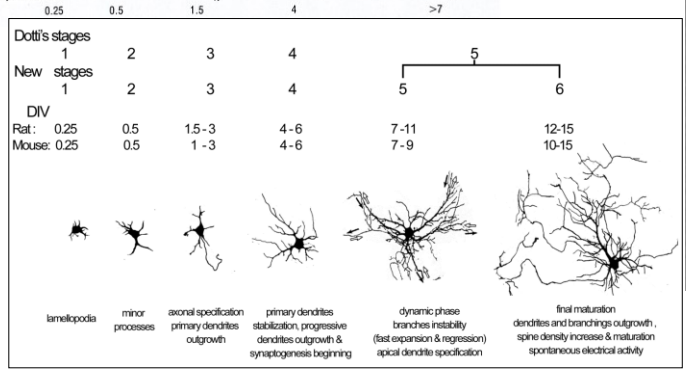


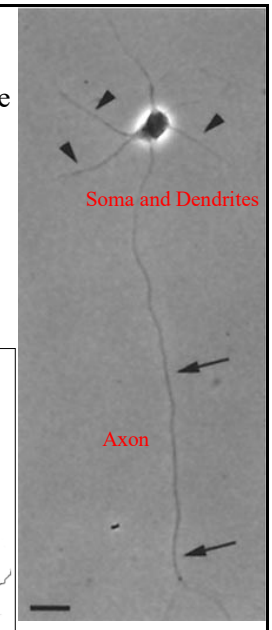
Figure 16–38. Molecular Biology of the Cell, 4th Edition.

Cultured Hippocampal Neurons (Dotti, 1988)

One process becomes the axon and grows dramatically, the other processes become dendrites.

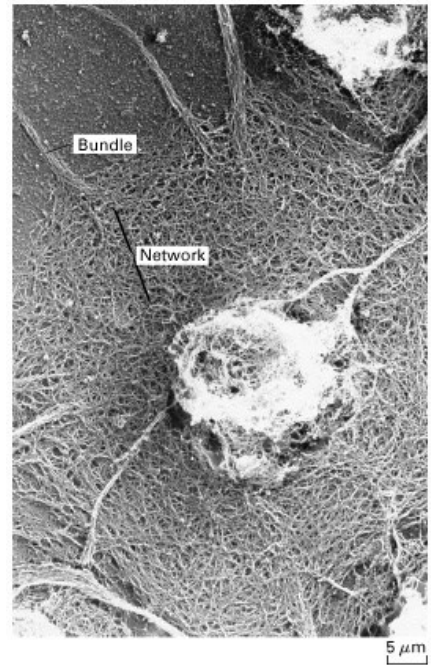






Spreading fibroblast extracted prior to fixation.

Example of filopodial bundles & cortical network



Actin is Enriched in Lamella and Growth Cones

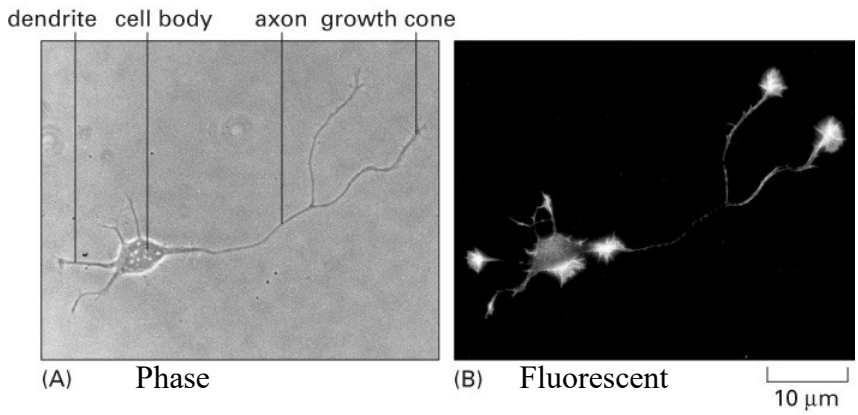
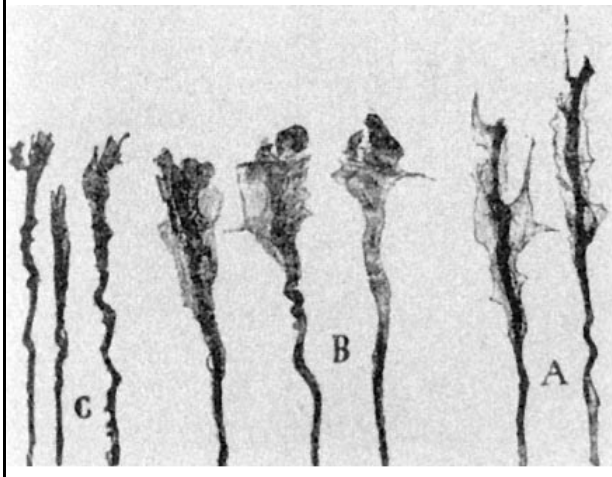


Figure 21–97. Molecular Biology of the Cell, 4th Edition.



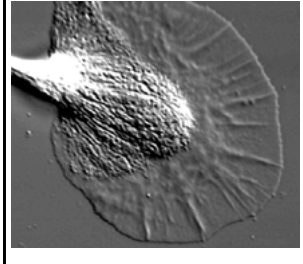
Growth Cones

Mammalian from RyC

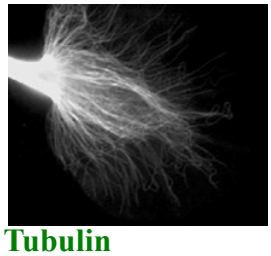
Growth cones sample the environment and respond to signaling cues.

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

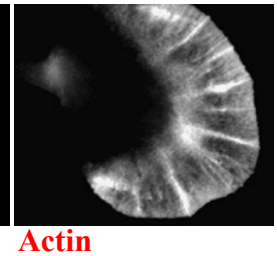
Growth Cone of Aplysia Bag Cell Neuron



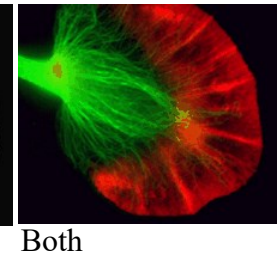
These mollusc neurons are great for growth cone studies. Big and flat, note a peripheral (P) region with actin but relatively empty and the vesicle filled central (C) region (with mts). MTs concentrated in the central region, but some in P region



Tubulin



Actin



Both

Forscher

Model for the Cytoskeleton Organization in Growth Cone

Actin:

- Filopodia with a polarized bundle polymerize at tip
- F-actin network polymerize at membrane
- Actin arcs collection of actin filaments

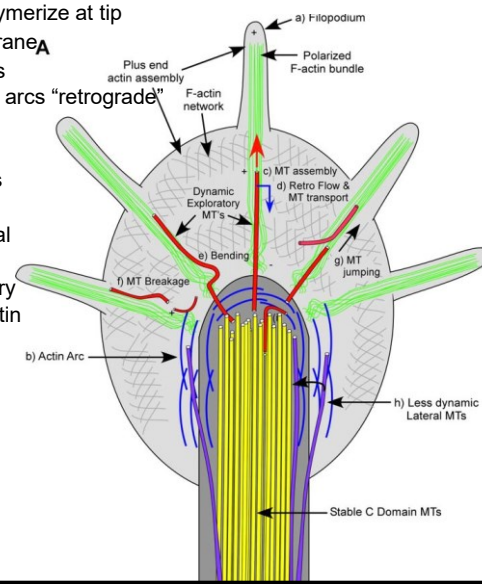
Transport of network from membrane to arcs "retrograde"

MTs:

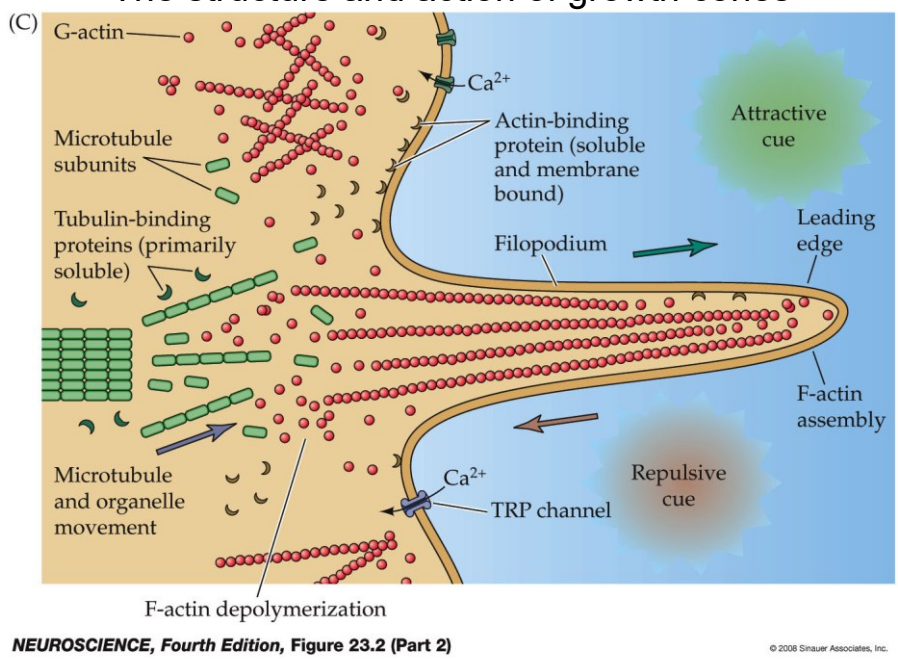
- stable MTs in C domain (bundled?)
- lateral MTs associated with actin arcs
- dynamic unbundled MTs:

-polymerize into the periphery along filopodial F-actin bundles

-are simultaneously cleared from the periphery by catastrophe and coupling to retrograde actin flow



The structure and action of growth cones



NEUROSCIENCE, Fourth Edition, Figure 23.2 (Part 2)

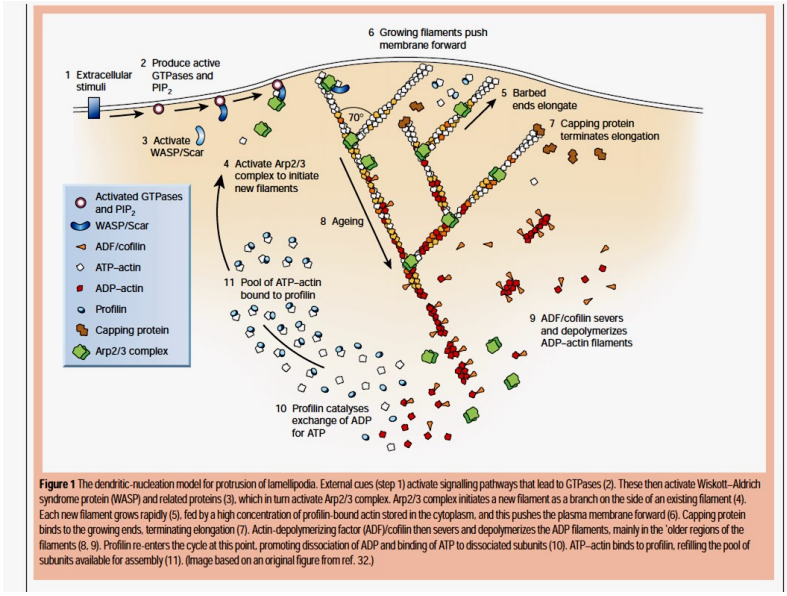


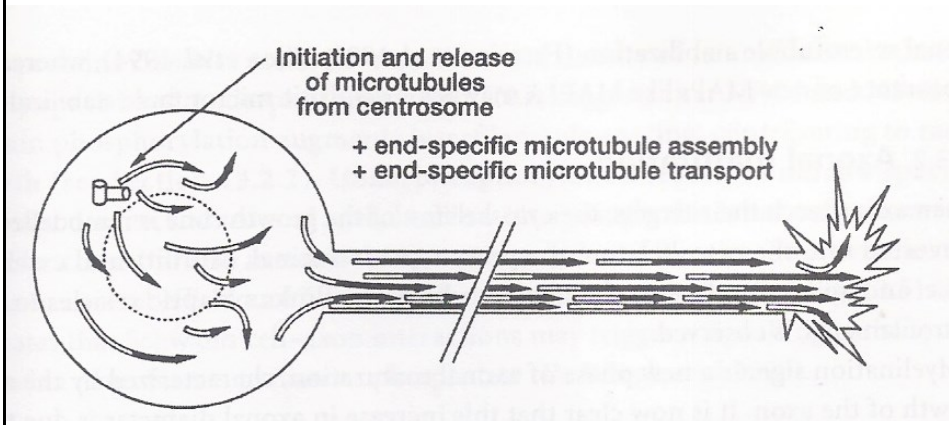
Figure 1 The dendritic-nucleation model for protrusion of lamellipodia. External cues (step 1) activate signalling pathways that lead to GTPases (2). These then activate Wiskott–Aldrich syndrome protein (WASP) and related proteins (3), which in turn activate Arp2/3 complex. Arp2/3 complex initiates a new filament as a branch on the side of an existing filament (4). Each new filament grows rapidly (5), fed by a high concentration of profilin-bound actin stored in the cytoplasm, and this pushes the plasma membrane forward (6). Capping protein binds to the growing ends, terminating elongation (7). Actin-depolymerizing factor (ADF)/cofilin then severs and depolymerizes the ADP filaments, mainly in the 'older regions of the filaments (8, 9). Profilin re-enters the cycle at this point, promoting dissociation of ADP and binding of ATP to dissociated subunits (10). ATP–actin binds to profilin, refilling the pool of subunits available for assembly (11). (Image based on an original figure from ref. 32.)

First, capping protein binds to the growing ends, terminating elongation. Next,a small protein called actin-depolymerizing factor (ADF)/cofilin binds weakly to the side of ADP–Pi actin filaments and promotes dissociation of the g-phosphate. The ADP filaments become a target for higher-affinity binding of ADF/cofilin, leading to their severing and depolymerization. Profilin re-enters the cycle at this point, promoting dissociation of ADP and binding of ATP to dissociated subunits. ATP–actin binds to profilin, refilling the pool of subunits available for assembly.

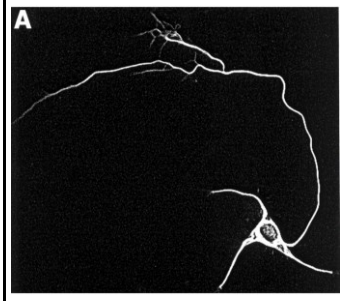
Microtubules

- **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**

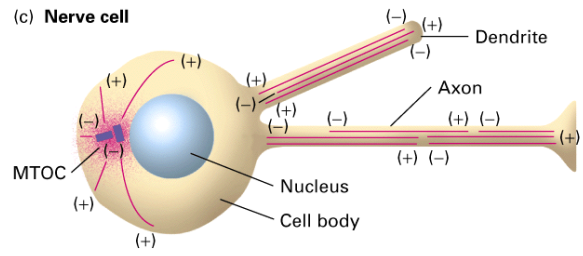
Model showing the Origin of Axonal Microtubules



Axons and Dendrites: Different Microtubule Polarities



Cultured neuron injected with fluorescent tubulin



Axons: uniform MT polarity
all plus ends face the terminal

Dendrites: MTs have mixed polarity

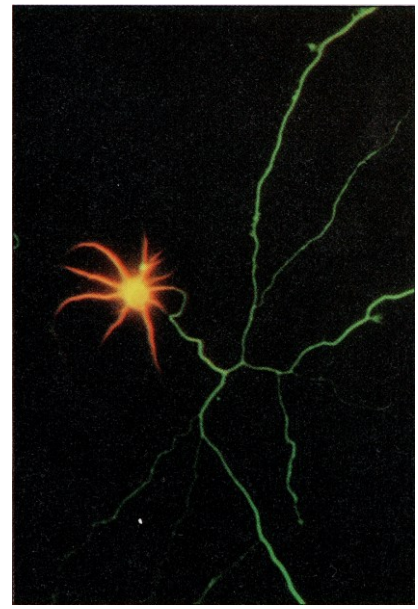
Axons and Dendrites:
Different Microtubule Associated Proteins (MAPs)

Two MAPs (structural):

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

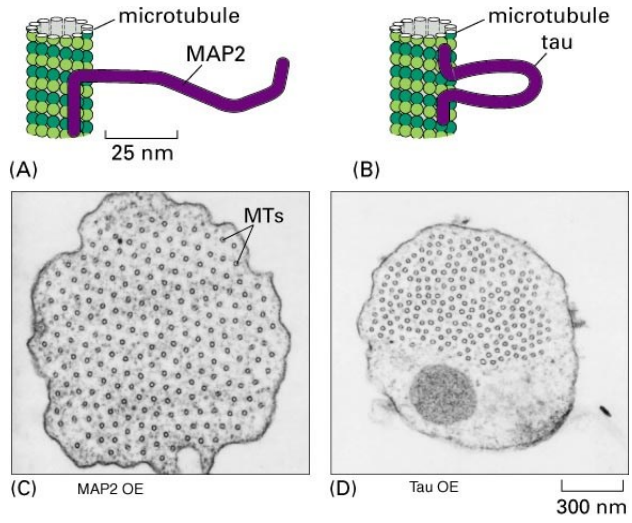
Green: **Tau** (dephosphorylated) in the axon

Cultured hippocampal neuron



MAP2 and tau bind to MTs.

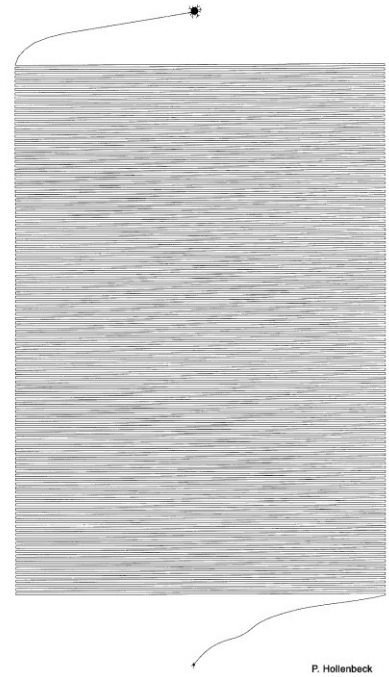
The lengths of their side arms may contribute to spacing of mts.



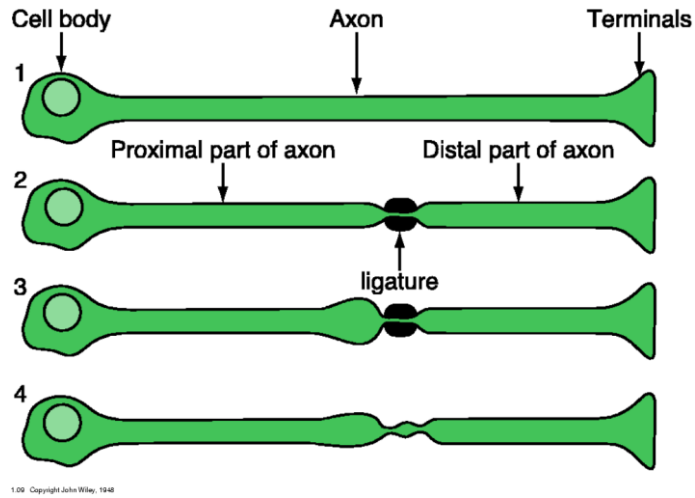
Axonal Transport

Axons can be >95%
of total neuronal volume.

All proteins in the axon are made in cell body and must be transported into and along the axon

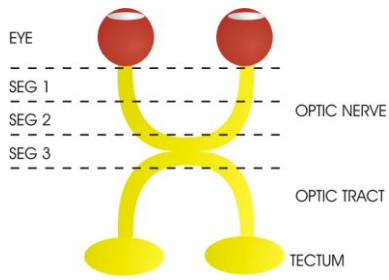


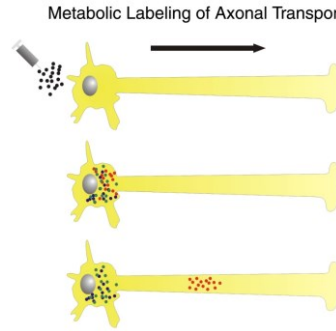
Weiss and Hiscove: Discovery of Axonal Transport



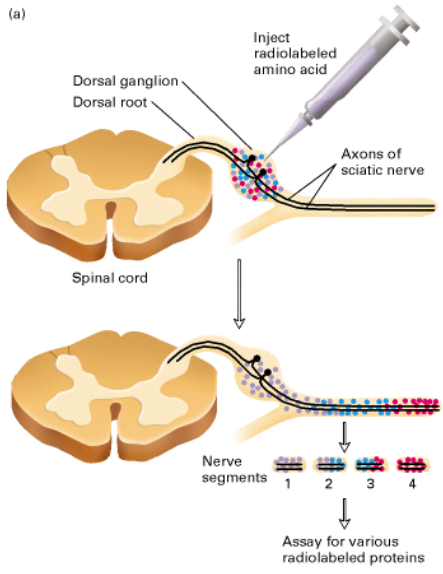
Pulse Chase Analysis of Axonal Transport

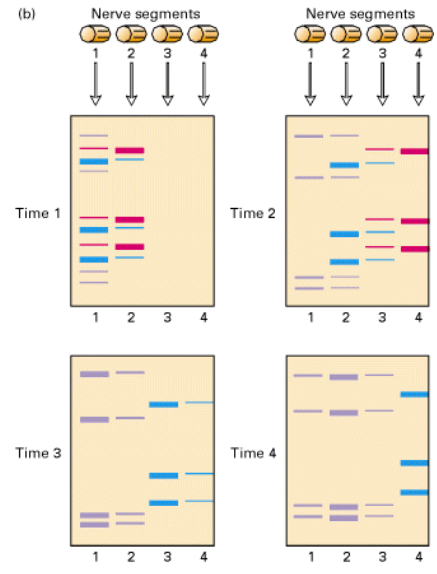
Rat Optic Nerves (sciatic nerves also popular)

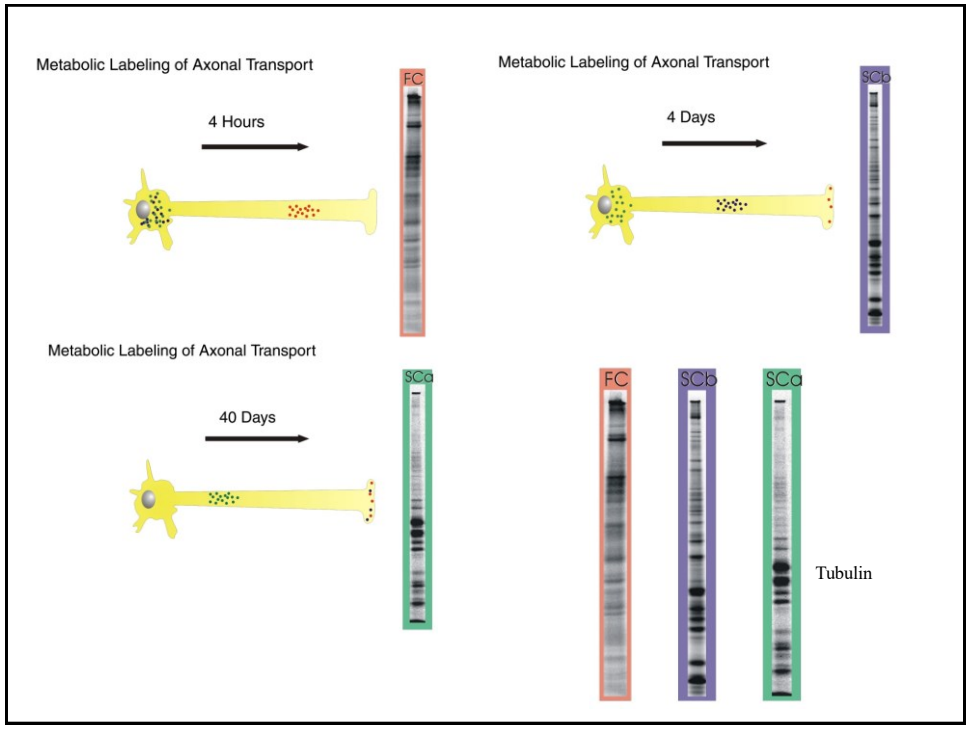




Pioneer experiments on axonal transport: Fast and slow axonal transport







The different transport components

Fast Anterograde Axonal Transport
Membrane-bounded organelles (mbo) 100's of mm/day
(1-2 micron/sec)

Slow Component B Anterograde Axonal Transport
actin and metabolic proteins 2-6 mm/day
(0.02 – 0.07 micron/sec)

Slow Component A Anterograde Axonal Transport
Microtubules, neurofilaments and associated proteins
0.1–1 mm/day (=1000 days to reach the end of a meter-long axon!)
(0.01 – 0.001 micron/sec)

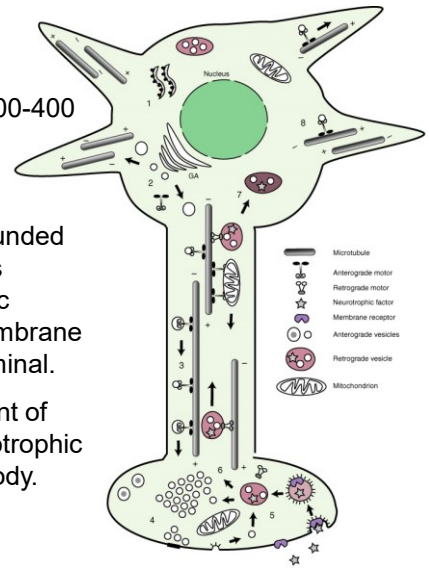
Retrograde Axonal Transport - Fast only
membrane bounded organelles (mbos) 100s of mm/day

Fast Axonal Transport: 100-400 mm/day

Purpose:

Transport membrane-bounded organelles (mbo) such as mitochondria and Sinaptic Vesicles and plasma membrane proteins to the nerve terminal.

Also retrograde movement of vesicles containing neurotrophic factors back to the cell body.



From:
L.Squire et al
Fundamental
Neuroscience
Third edition

FIGURE 2.12 Fast axonal transport represents transport of membrane-associated materials, having both anterograde and retrograde components. For anterograde transport, most polypeptides are synthesized on membrane-bound polysomes, also known as rough endoplasmic reticulum (**1**), and then transferred to the Golgi apparatus for processing and packaging into specific classes of membrane-bound organelles (**2**). Proteins following this pathway include both integral membrane proteins and secretory polypeptides in the lumen of vesicles. Cytoplasmic peripheral membrane proteins like the kinesins are synthesized on the cytoplasmic or free polysomes. Once vesicles have been assembled and the appropriate motors associate with them, they are moved down the axon at a rate of 100–400 mm per day (**3**). Different membrane structures are delivered to different compartments and may be regulated independently. For example, dense core vesicles and synaptic vesicles are both targeted for the presynaptic terminal (**4**), but release of vesicle contents involves distinct pathways. After vesicles merge with the plasma membrane, their protein constituents are taken up by coated pits and vesicles via the receptor-mediated endocytic pathway and delivered to a sorting compartment (**5**). After proper sorting into appropriate compartments, membrane proteins are either committed to retrograde axonal transport or recycled (**6**). Retrograde moving organelles are morphologically and biochemically distinct from anterograde vesicles. These larger vesicles have an average velocity about half that of anterograde transport. The retrograde pathway is an important mechanism for delivery of neurotrophic factors to the cell body. Material delivered by retrograde transport typically fuses with cell body compartments to form mature lysosomes (**7**), where most constituents are recycled. However, neurotrophic factors and neurotrophic viruses can act at the level of the cell body. Although there is evidence that vesicle transport also occurs into dendrites (**8**), less is known about this process. Dendritic vesicle transport is complicated by the fact that dendritic microtubules may have mixed polarity.

Slow Axonal Transport:~0.1-6 mm/day

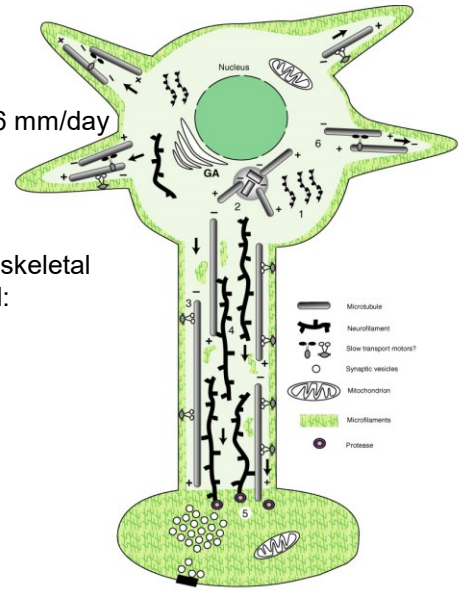
Purpose:

Delivery of cytosolic and cytoskeletal proteins to the nerve terminal:

Microtubules

Neurofilaments

Enzymes



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Fundamental
Neuroscience
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FIGURE 2.11 Slow axonal transport represents the delivery of cytoskeletal and cytoplasmic constituents to the periphery. Cytoplasmic proteins are synthesized on free polysomes and organized for transport as cytoskeletal elements or macromolecular complexes (**1**). The microtubules are formed by nucleation at the microtubule-organizing center near the centriolar complex (**2**) and then released for migration into the axon or dendrites. The molecular mechanisms are not as well understood as those for fast axonal transport, but slow transport appears to be unidirectional with no retrograde component. Recent studies suggest that motors like cytoplasmic dynein may interact with the axonal membrane cytoskeleton to move the microtubules with their plus ends leading (**3**). Neurofilaments do not appear able to move on their own, but hitchhike on the microtubules (**4**). Other cytoplasmic proteins may do the same or they may be moved by other motors. Once cytoplasmic structures reach their destinations, they are degraded by local proteases at a rate that allows either growth (in the case of growth cones) or maintenance of steady-state levels. The different composition and organization of the cytoplasmic elements in dendrites suggest that different pathways may be involved in delivery of cytoskeletal and cytoplasmic materials to the dendrite (**6**). In addition, some mRNAs are transported into the dendrites, but not into axons.

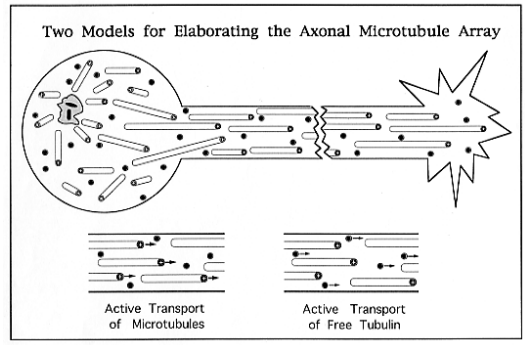
FROM MOLECULES TO NETWORKS John H. Byrne, James L. Roberts

WHAT IS SLOW AXONAL TRANSPORT?

Mechanism(s) of Slow Axonal Transport

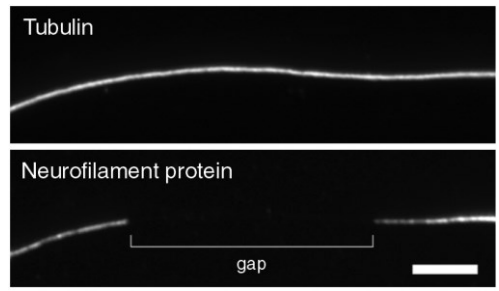
Ancient dispute: polymer or monomer transport; do structures move or monomers (ie f-actin, microtubules, and intermediate filaments, or g-actin, tubulin and NF-H, -M, -L?

Diffusion can not support long axons



There was controversy, but NFs do move as filaments (Brown).

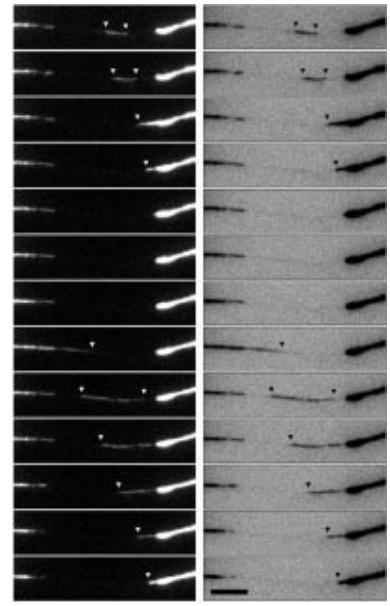
Evidence: Some cultured neurons have natural gaps in their NFs

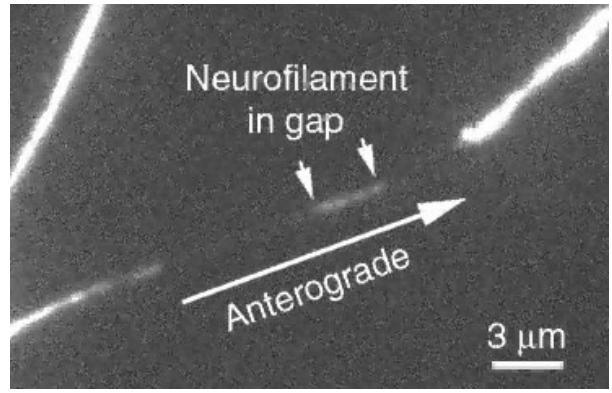


Staining of an axon of cultured neuron
Note tubulin along the whole length
There is a gap in NF length.

With live cell imaging see GFP-NFs move across gap.

BUT!!!!
NFs move in both directions!
&
While moving they move fast!
Seem to move slow because they spend much time not moving.

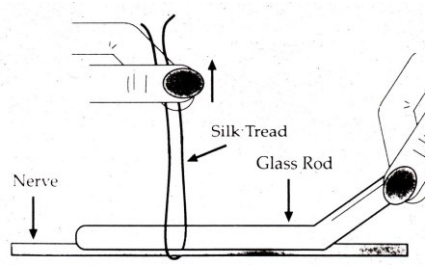




movie

Similar result with NFs when use photobleaching to make gap and with MTs

Ligation Analysis of Fast Axonal Transport Components

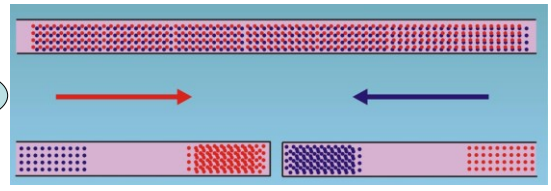


In a single Axon

Control

soma

Ligated



Red: Anterogradely moving organelles
Blue: Retrogradely moving organelles