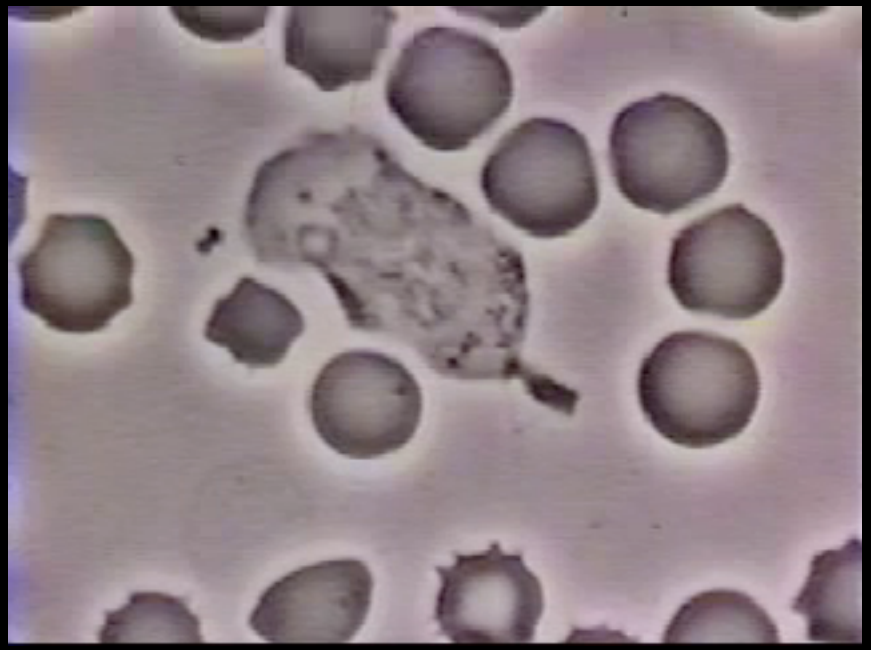
Microtubules, Motors and Movement: Cell Motility and the Cytoskeleton



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Outline

I. Overview

II. Microtubules and Actin Filaments
- Structure and polymerization
- Dynamics
- Nucleation centers
- Associated proteins and assemblies

III. Motor Proteins
- Actin - Myosins
- Microtubules - Kinesins and Dynein

IV. Cell Machines based on Actin and Microtubules

V. Intermediate Filaments

Reading in Textbooks

- Alberts et al., Molecular Biology of the Cell, 6th ed., 2014, Garland.
  - Chap. 16 - The Cytoskeleton.
  - Available as eBook, https://bit.ly/2AR19DR.

- Lodish et al., Molecular Cell Biology, 8th ed., 2016, Macmillan.
  - Chaps. 17 & 18 - Microfilaments, Microtubules and Intermediate Filaments.
  - Available as eBook and hard copy, https://bit.ly/2Qy9Hog.

- Pollard et al., Cell Biology, 3rd ed., 2016, Elsevier.
  - Section IX: Cytoskeleton and Cell Motility. Chaps 33-39.
  - Available as eBook and hard copy, https://amzn.to/2OwoAWC.

Movies and Videos Online

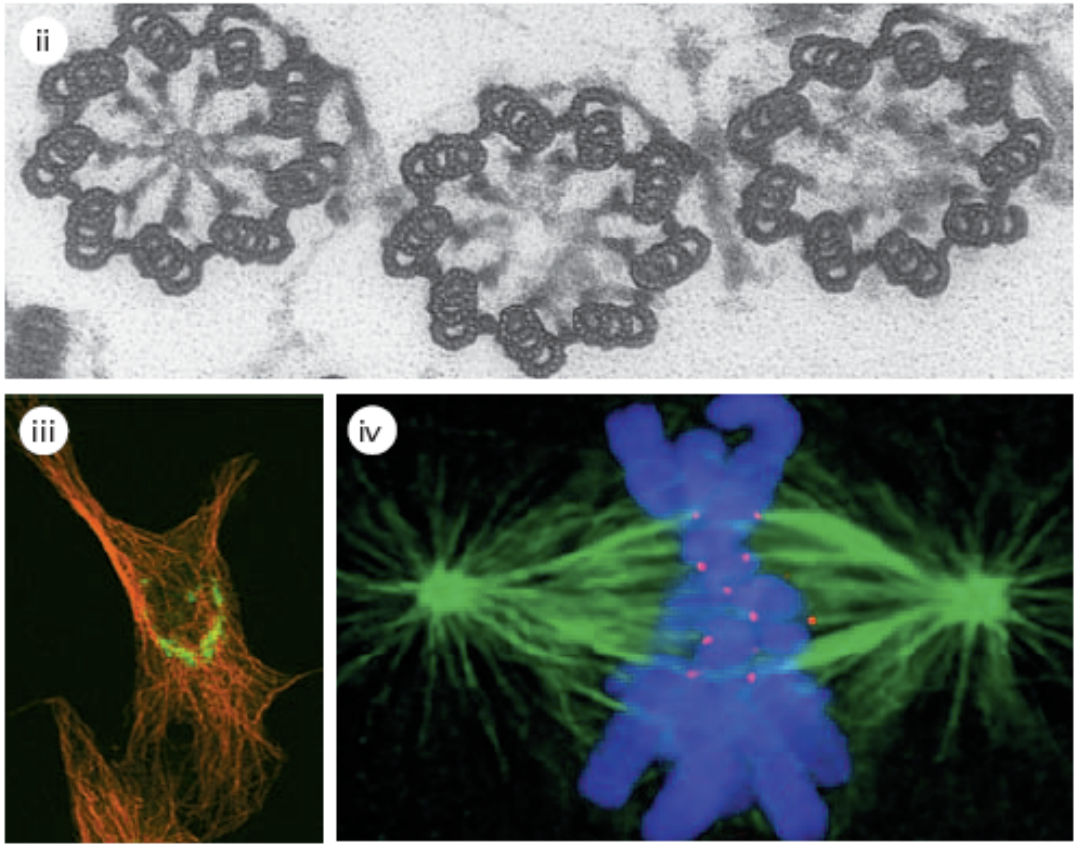
- Nikon Microscope Company
  - http://www.microscopyu.com/galleries/
    - Swept Field Confocal Digital Video Gallery
    - Live-Cell Imaging Cell Motility
- Ted Salmon Lab at Chapel Hill
  - http://labs.bio.unc.edu/Salmon/salmonlabmovies.html
- Am Soc for Cell Biology
  - Videos - News and Education, http://www.ibiology.org/
  - Webinars on Imaging - http://www.ascb.org/webinars/

Related Course Work

- First-Year Lectures
  - Histology
    - Cell-cell Junctions in Epithelial Cell Biology
    - Muscle Cell Biology
  - Physiology
    - Muscle
  - MFM
    - Membrane Receptors and Signaling
  - Neuroscience
    - Axonal Transport and Neurofilaments
- Second-Year Lectures
  - Hematology, Dermatology, Cardiology, Neurology

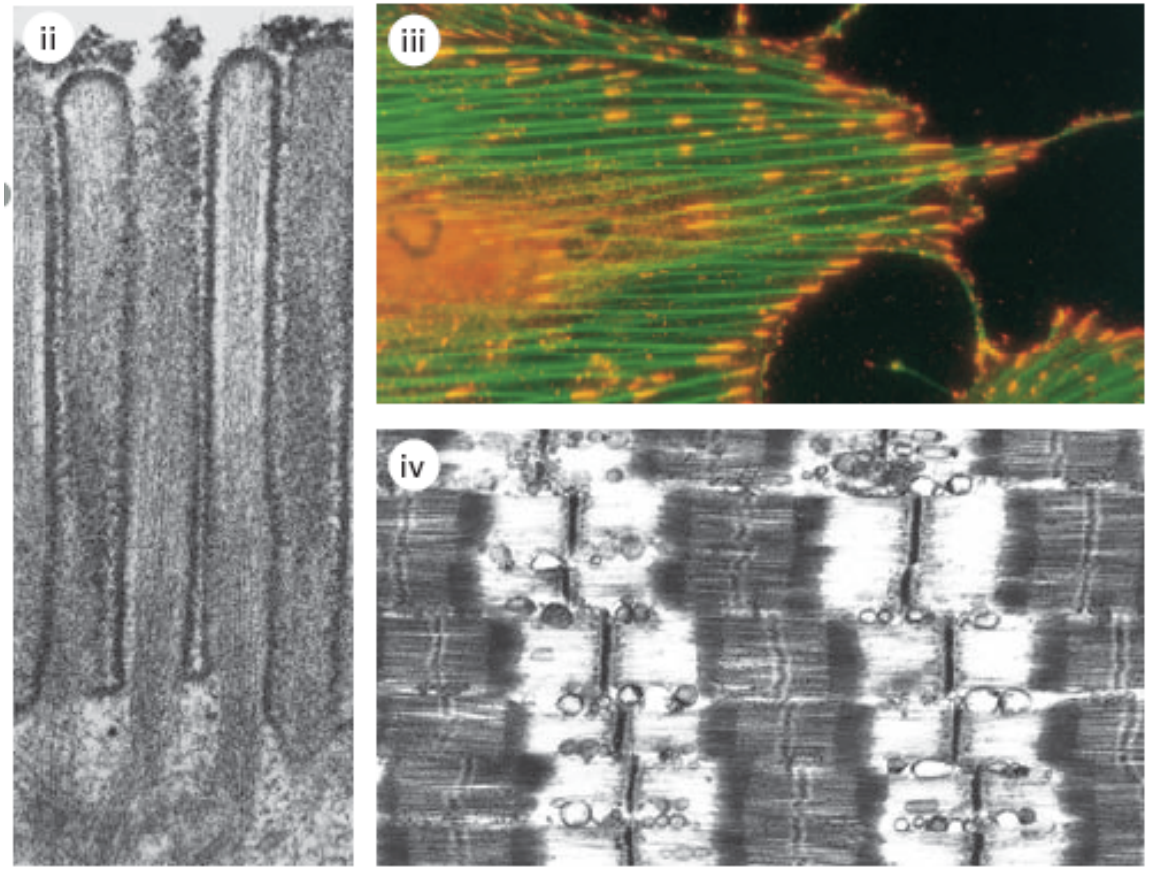
Microtubules

- Rigid hollow cylinders of 25 nm diameter
- In all eukaryotic cells (FtsZ in bacteria)
- Involved in stable and dynamic structures



Actin

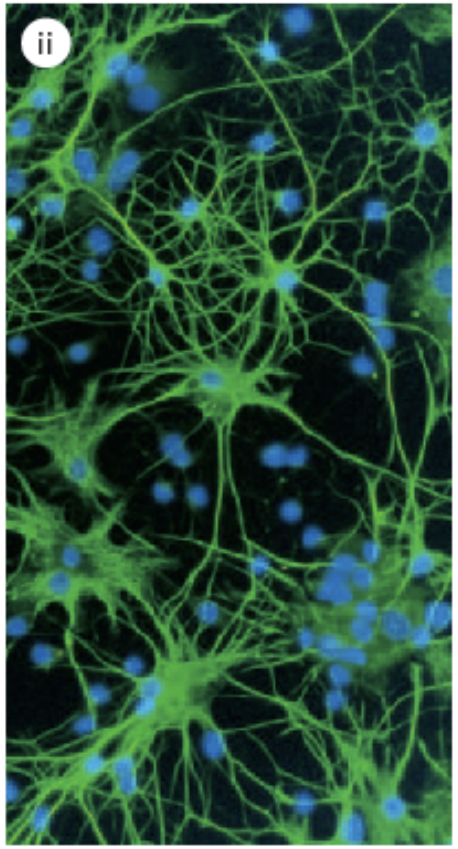
- Flexible microfilaments of 5-10nm diameter
- In all eukaryotic cells (MreB in bacteria)
- Involved in stable and dynamic structures

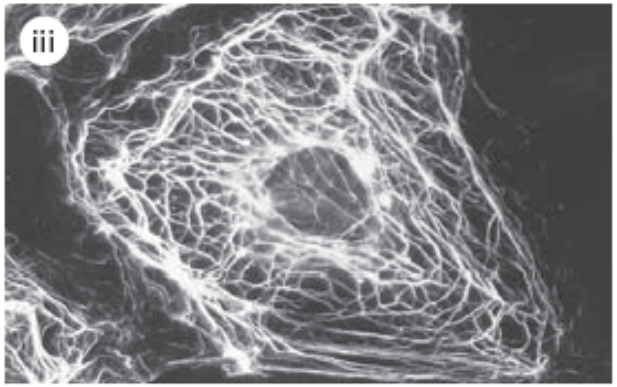


Intermediate Filaments (IF)

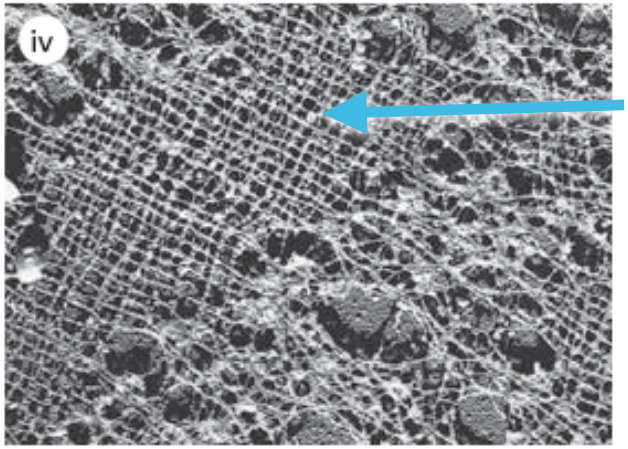
- Rope-like
- Expressed in most metazoans

Neurofilaments





Vimentin



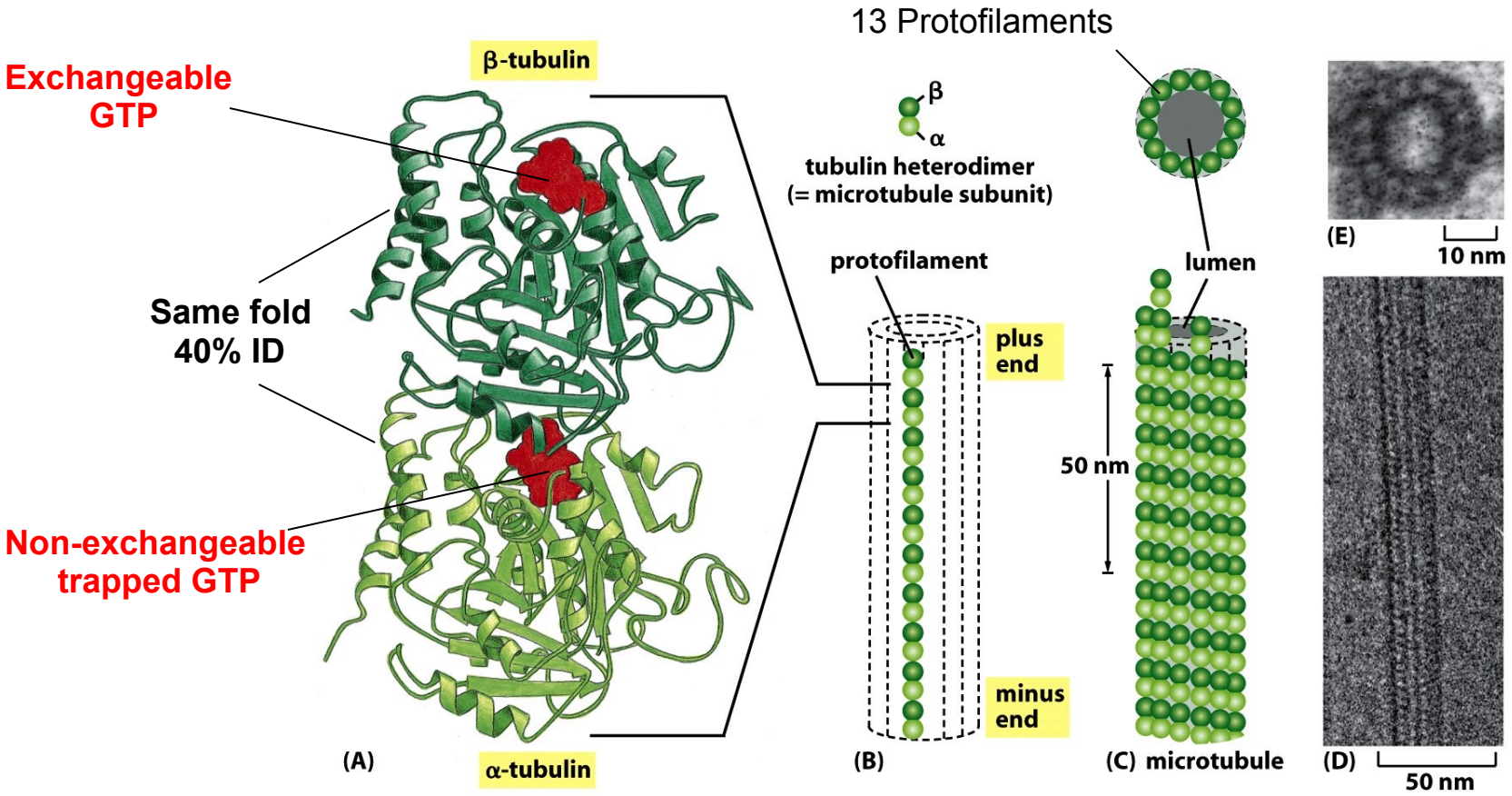
Nuclear lamins

II. Microtubules and Actin Filaments

- Structure and polymerization
- Dynamics
- Nucleation centers
- Associated proteins and assemblies

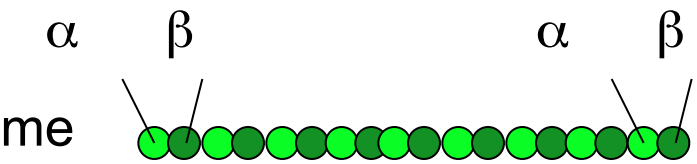
Microtubule Structure

Building block = heterodimers of α and β-tubulin



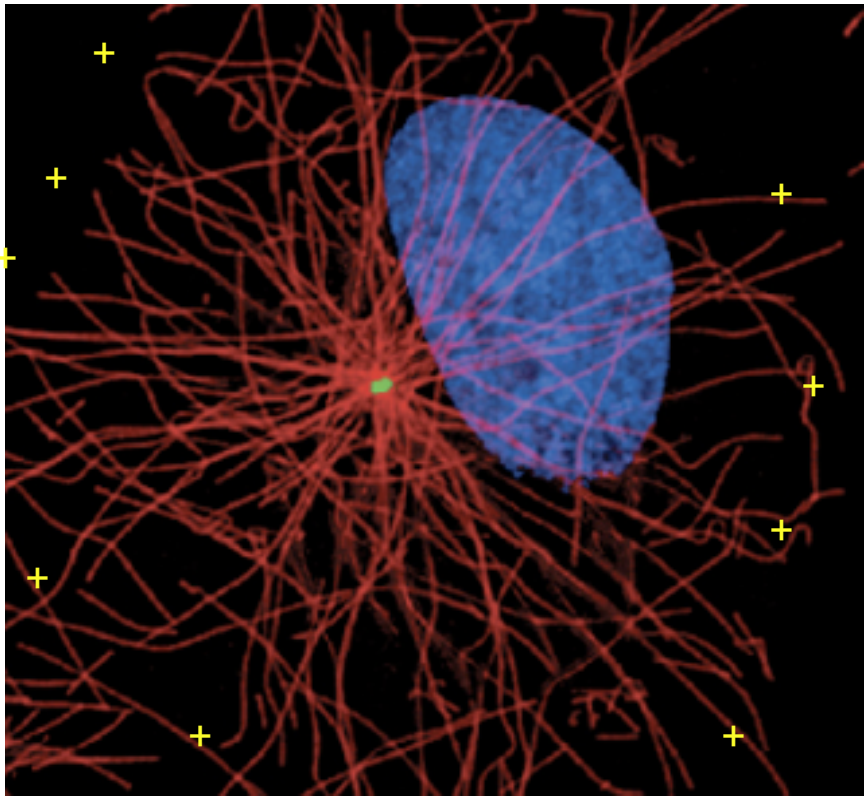
Microtubule Structure

Filament polarity conferred by alpha/beta heterodimer

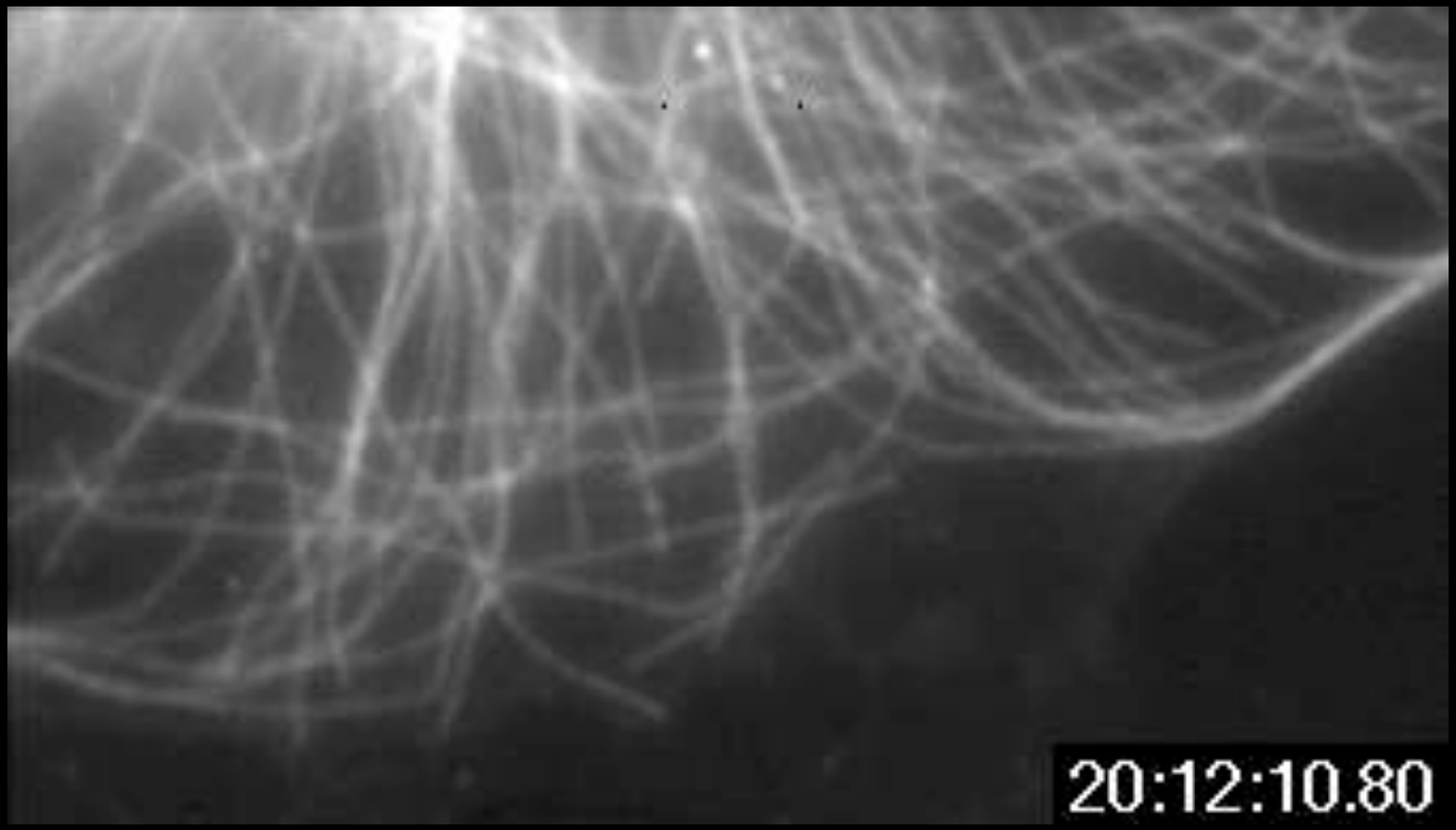


Centrosome
(Minus End)

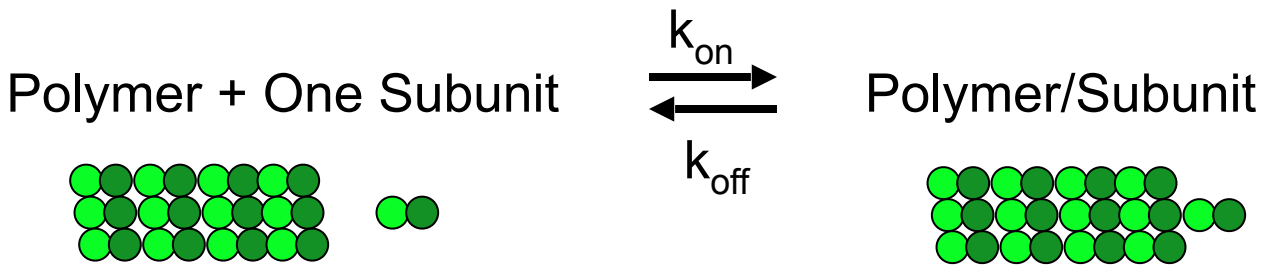
Plasma Membrane
(Plus End)



Microtubules: In vivo dynamics



Microtubules: Polymerization Dynamics



Rate of subunit addition = k_{on} x [S]

Rate of subunit loss = k_{off}

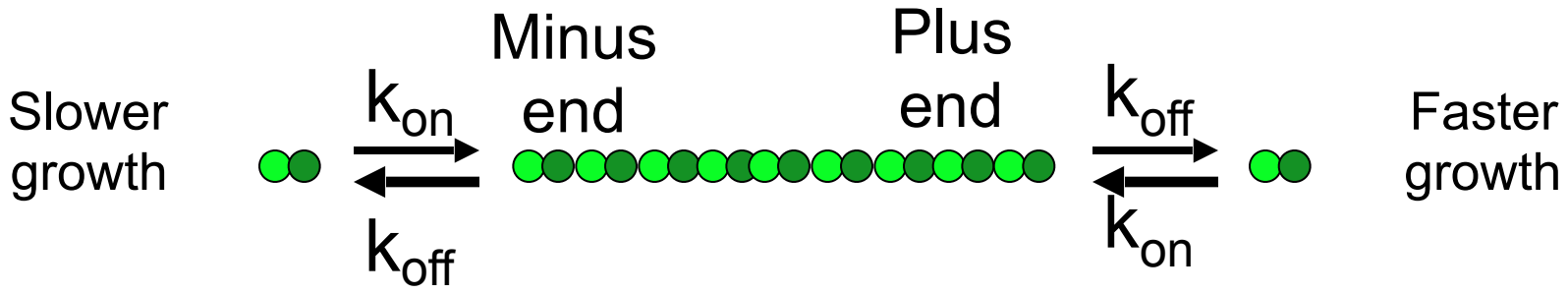
As the polymer grows, subunit concentration [S] diminishes until it reaches a so-called "critical concentration" [Cc] for which …

Rate of subunit addition = Rate of subunit loss

$$k_{on} \times [Cc] = k_{off}$$

$$[Cc] = k_{off} / k_{on}$$

Microtubules: Polymerization Dynamics



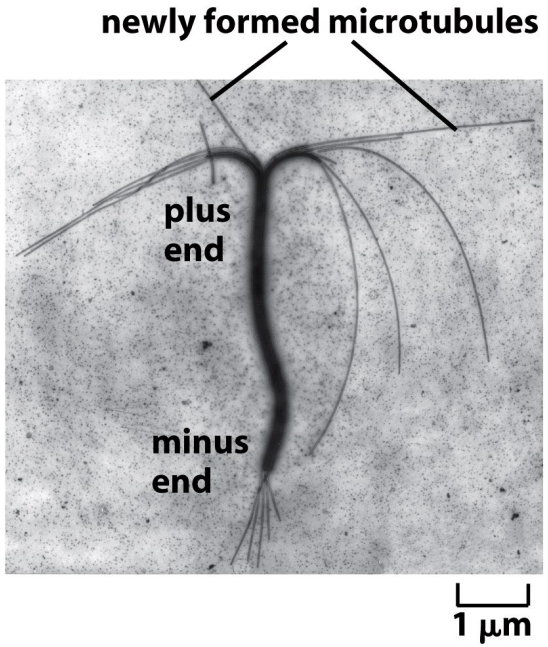
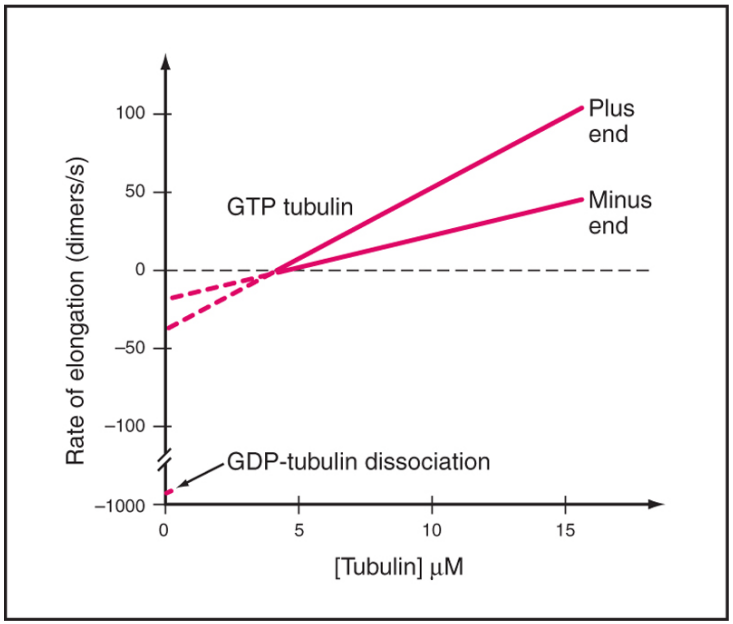
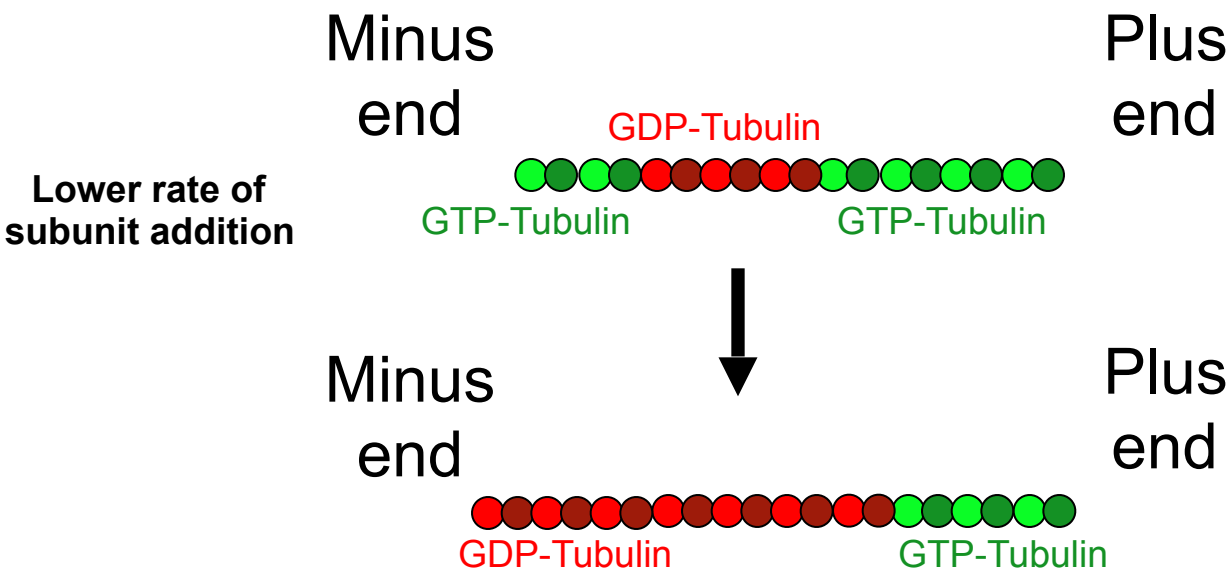


Figure 16-13 Molecular Biology of the Cell 5/e (© Garland Science 2008)



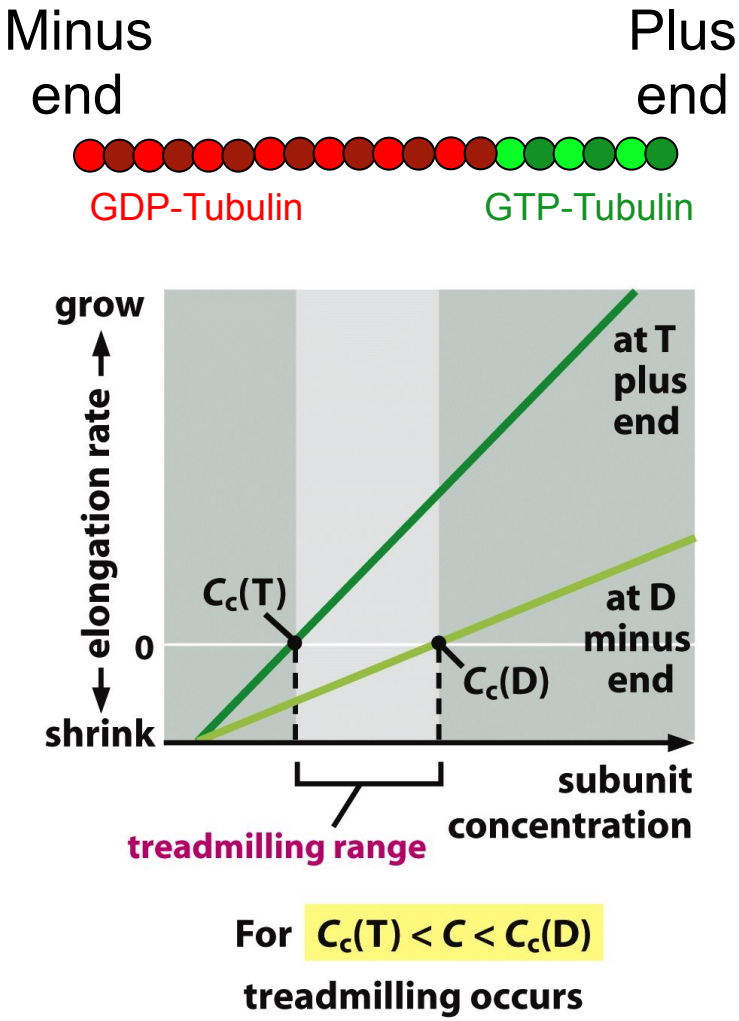
Microtubules: Dynamics

Enzymatic activity of tubulin catalyzes hydrolysis of bound GTP to GDP



Hydrolysis catches up with the minus end

Microtubules: Dynamics - “Treadmilling”



GDP tubulin leans toward disassembly

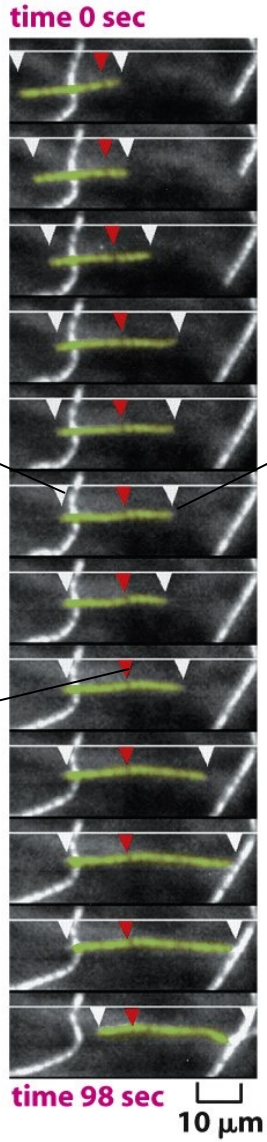
k_{off} GDP tubulin **>>** k_{off} GTP tubulin

Cc(D)**>>**Cc(T)

For certain concentrations of subunits, minus end will shrink while plus end will elongate

=> “Treadmilling”

Microtubules: Dynamics - “Treadmilling”



Shrinking at Minus end

Elongation at Plus end

“Fixed point”

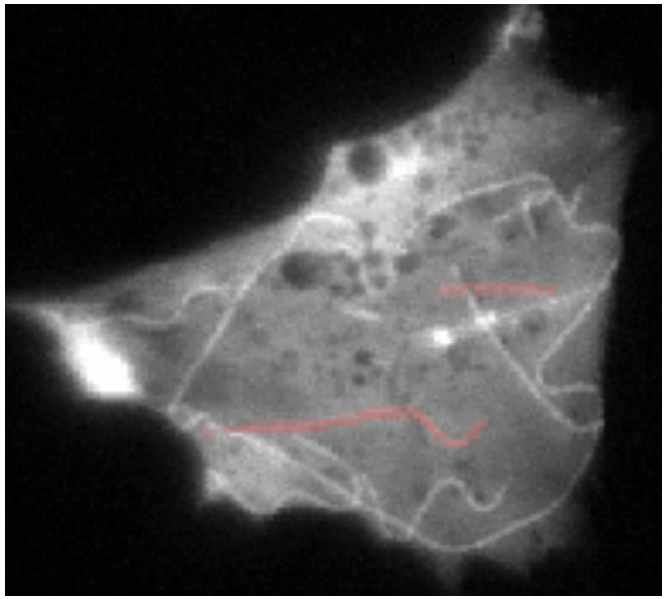


Figure 16-15 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Microtubules: Dynamics

"Dynamic Instability" = Growth and Shrinkage Alternate with Stochastic Transitions, termed "Catastrophe" and "Rescue"

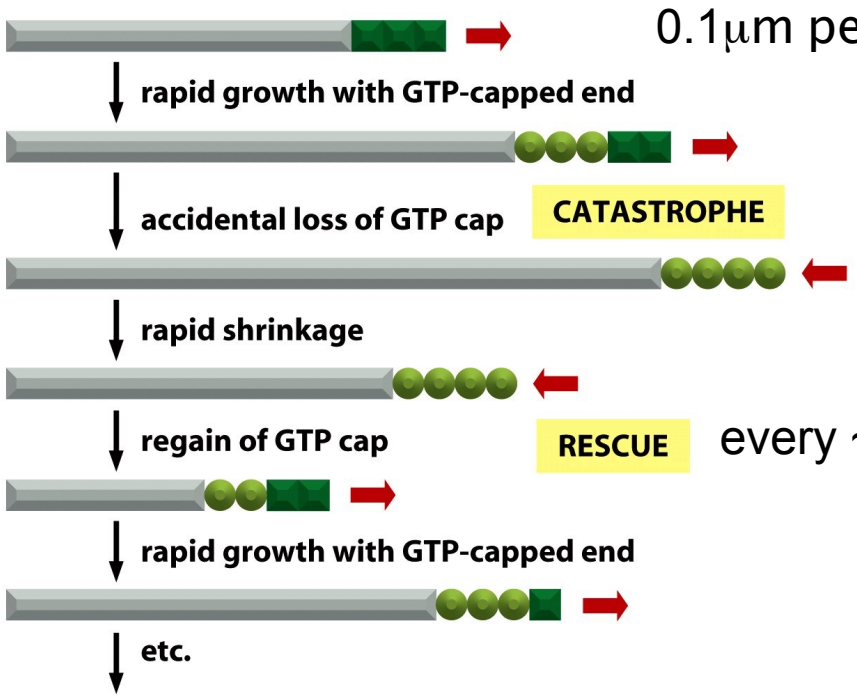


Figure 16-16a Molecular Biology of the Cell 5/e (© Garland Science 2008)

0.1μm per second

1000 dimers or 0.5 μm per second
every ~70 seconds

every ~20 seconds

Microtubules: Dynamic Instability

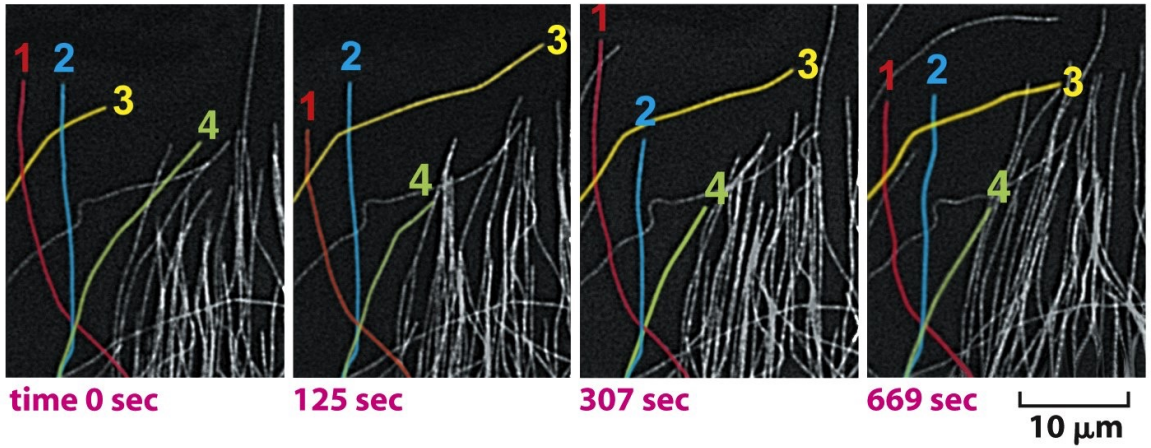
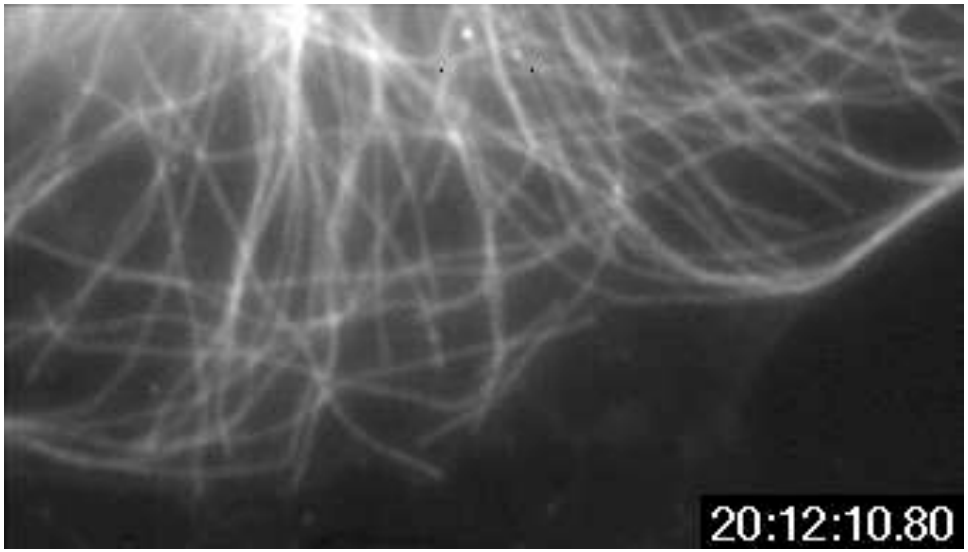
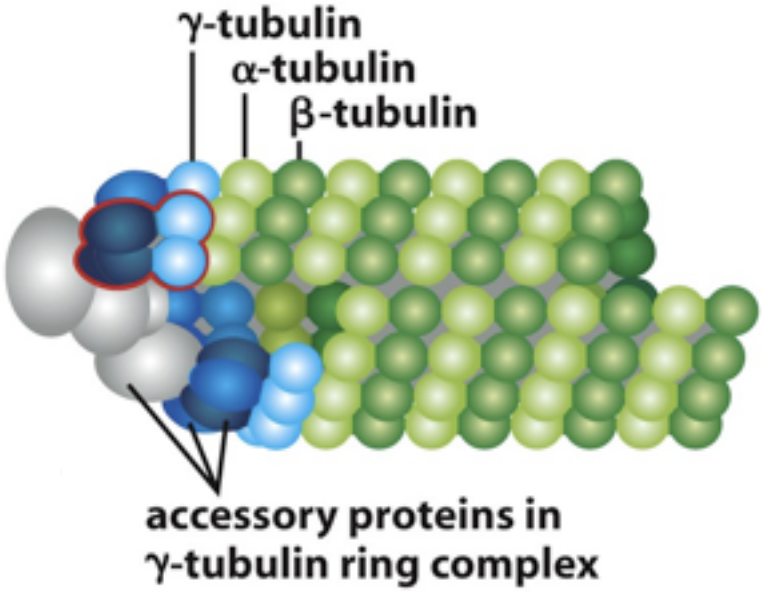


Figure 16-17 Molecular Biology of the Cell 5/e (© Garland Science 2008)



Microtubules: Nucleation by Centrosome



Individual microtubules minus ends created by Gamma-Tubulin Ring Complex, "γ-TuRC"

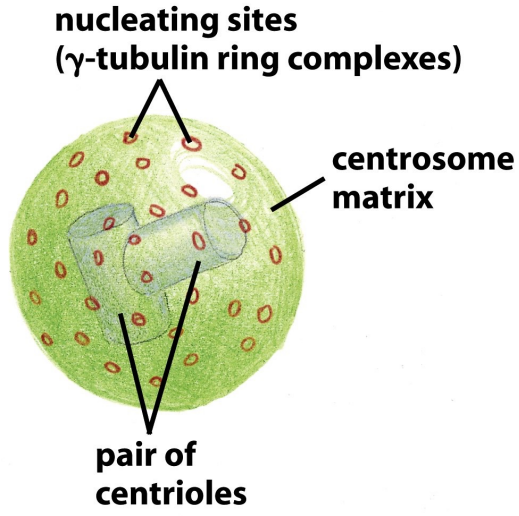


Figure 16-30a Molecular Biology of the Cell 5/e (© Garland Science 2008)

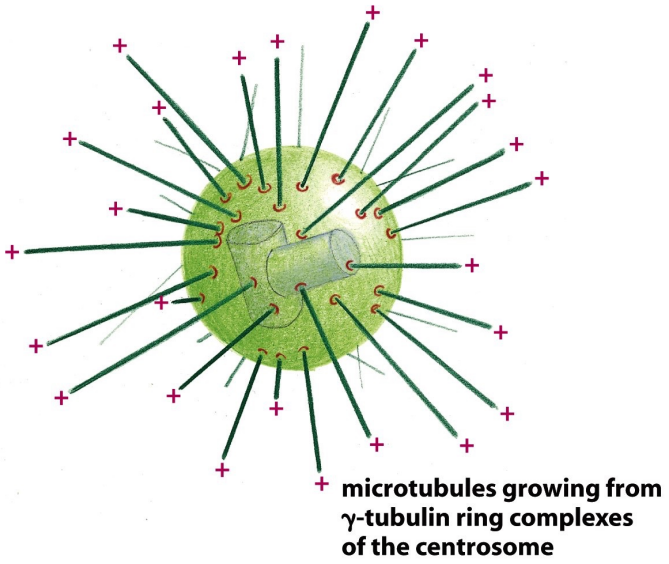
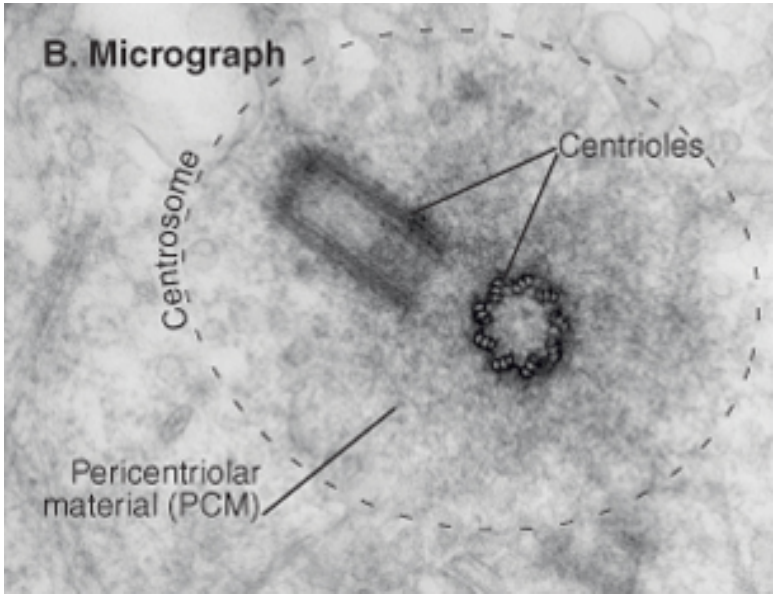
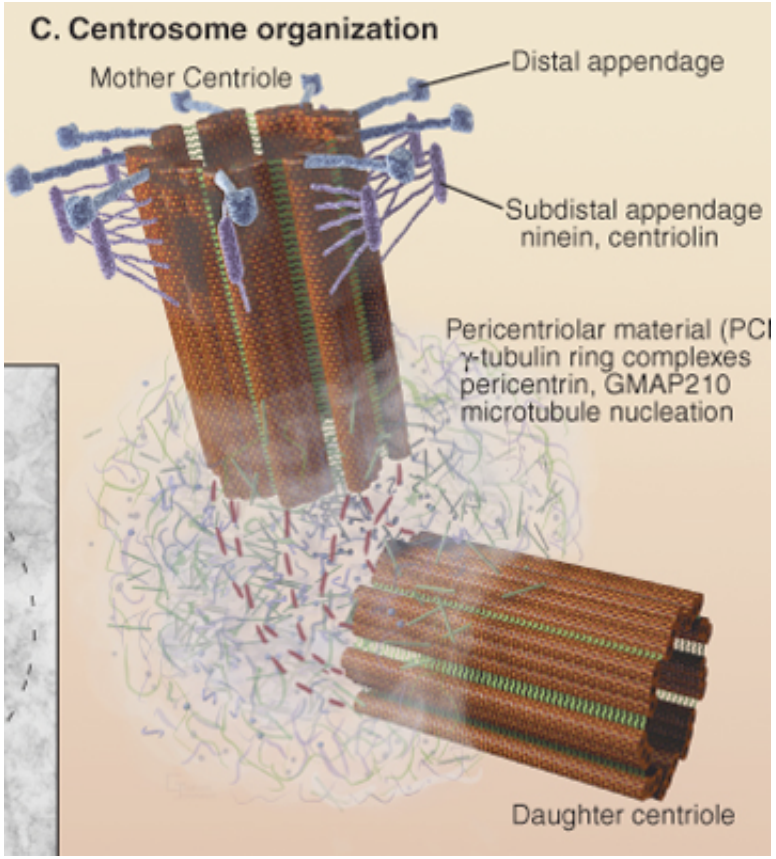


Figure 16-30b Molecular Biology of the Cell 5/e (© Garland Science 2008)

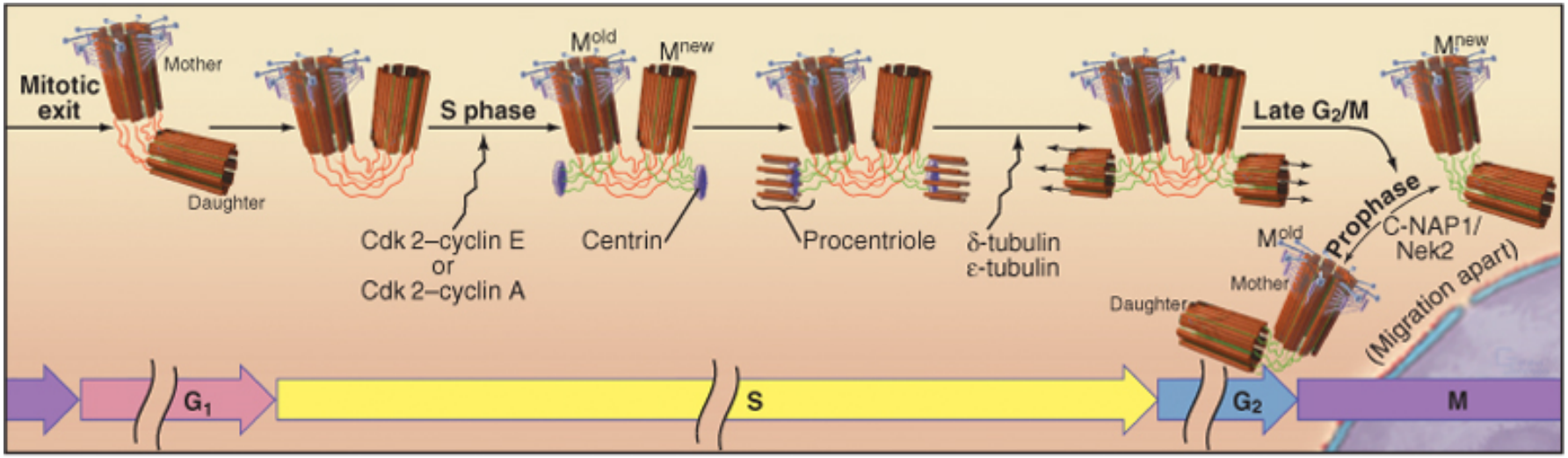
Microtubules: Centrosome

- 2 perpendicular centrioles (Microtubules + other proteins)
- Pericentriolar amorphous material





Microtubules: Centrosome & Centriole Duplication



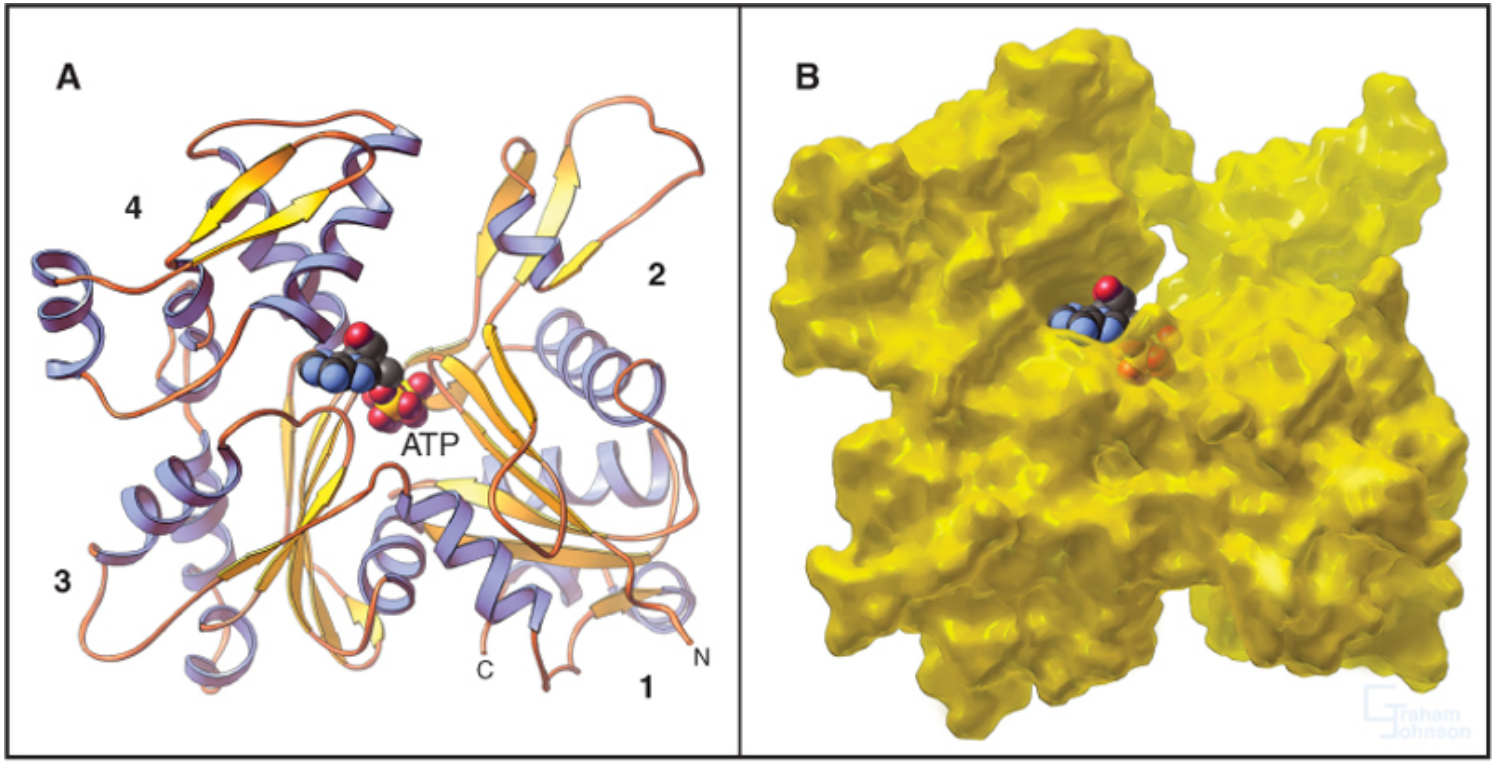
Microtubules: Drugs that affect dynamics

- Taxol
  - Bark of the Pacific yew tree “Taxus brevifolia”
  - Breast cancer and other solid tumors
  - Mechanism uncertain
- Colchicine
  - Autumn crocus
  - Gout (from 1500 BC), other inflammatory disease
- Vincristine / vinblastine
  - Vinca plants
  - Childhood leukemia
  - Inhibits mitosis

Actin

Subunit is a ***monomer*** that binds ***ATP***

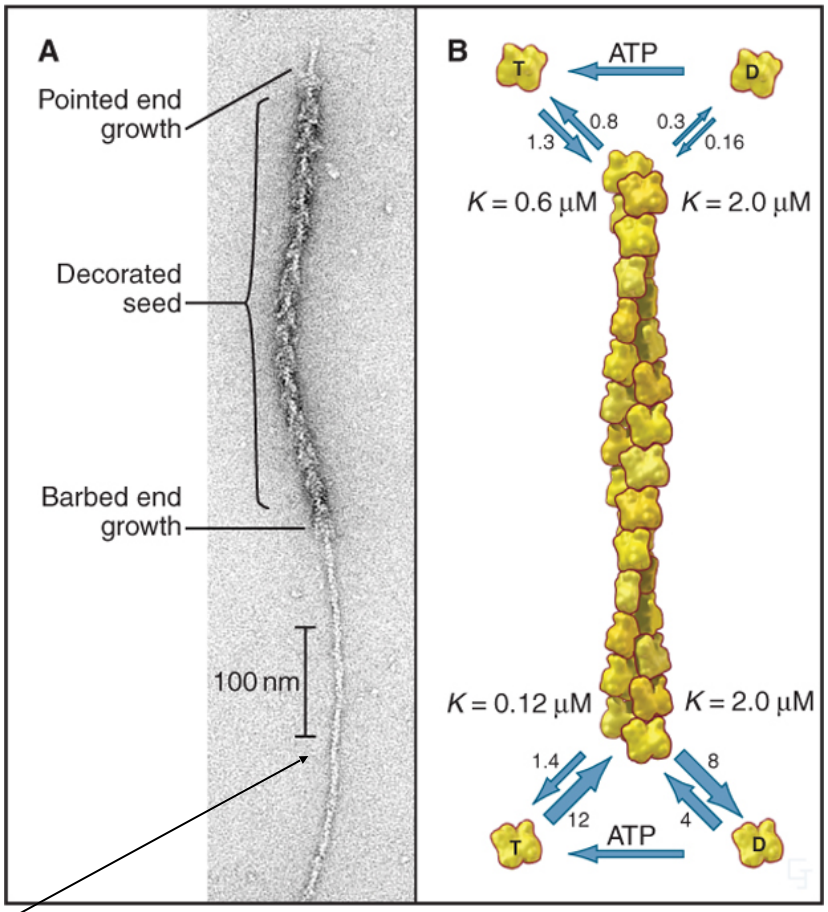
ATP-binding cleft towards the Pointed / Minus (-) end



Actin

Flexible filament composed of two protofilaments

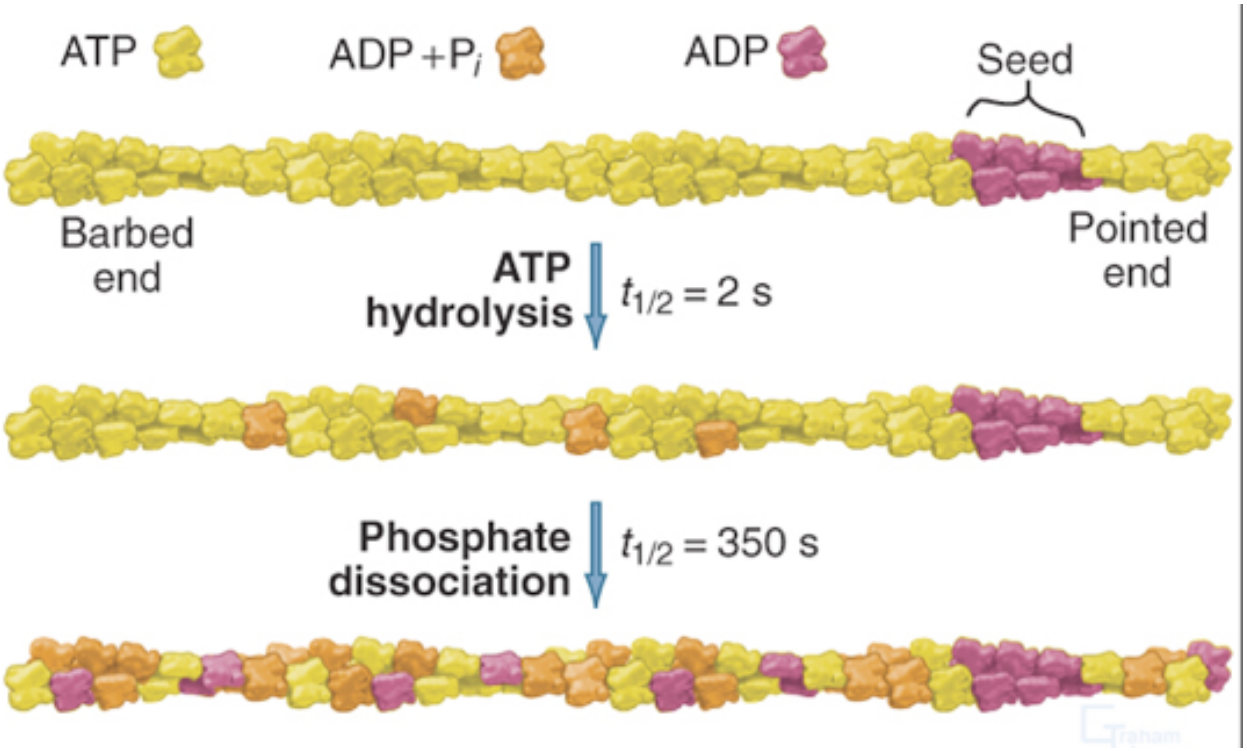
Pointed end = Minus end



Barbed end = Plus end

Faster Growth at Barbed (plus) End

Actin: Dynamics



ATP hydrolysis occurs at random time (first-order decay)

Actin: Nucleation

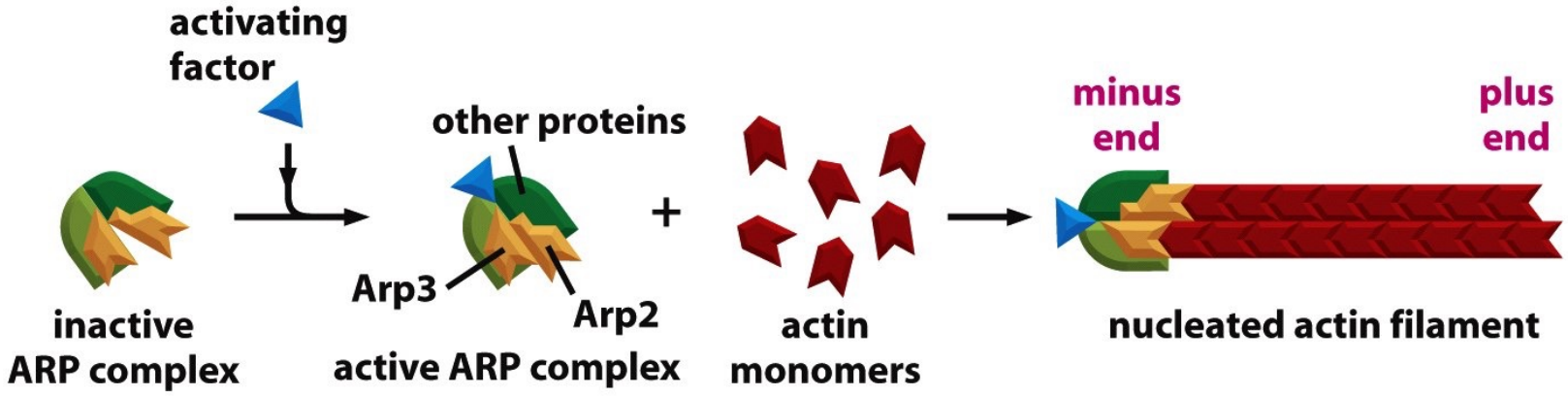


Figure 16-34b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Actin filaments often nucleated near membranes

Actin-related-protein (Arp) 2/3 complex is one major nucleator

Actin: Branching Network of Filaments created by Arp2/3 Complex

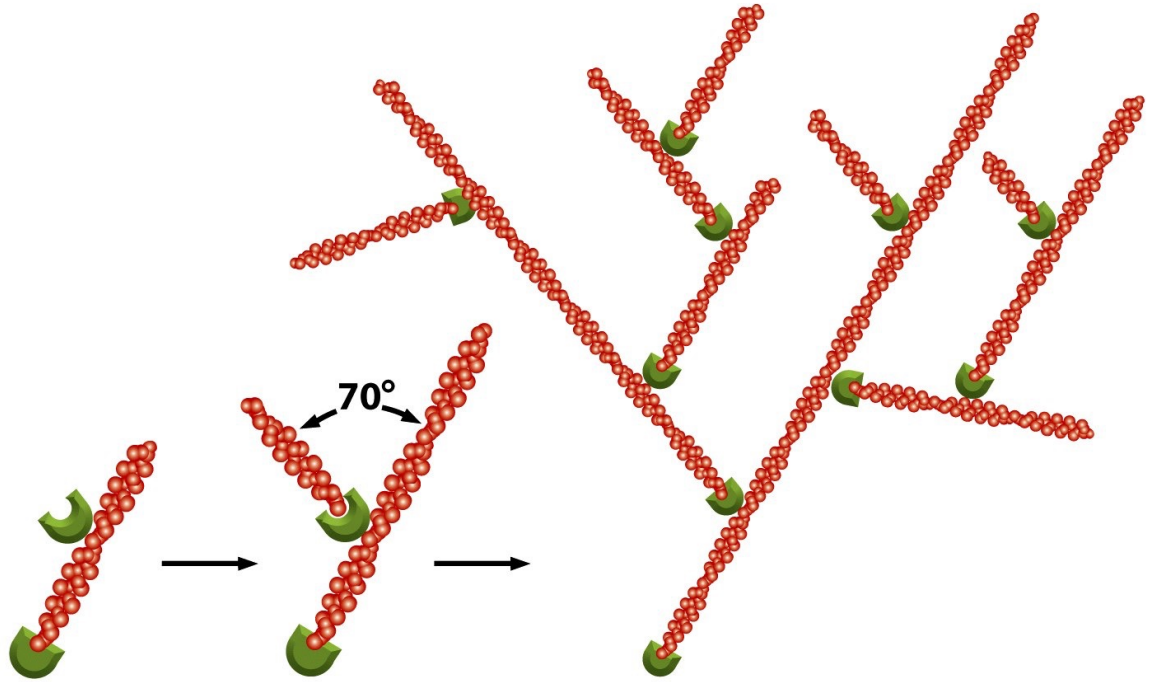
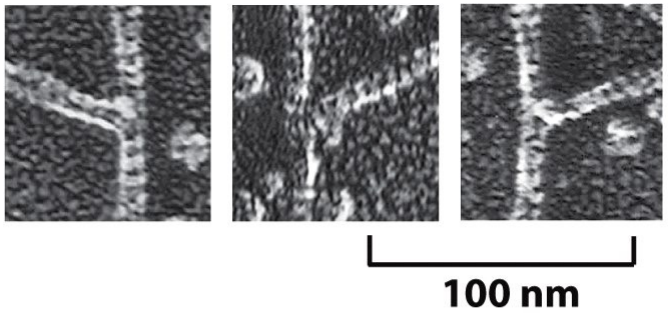
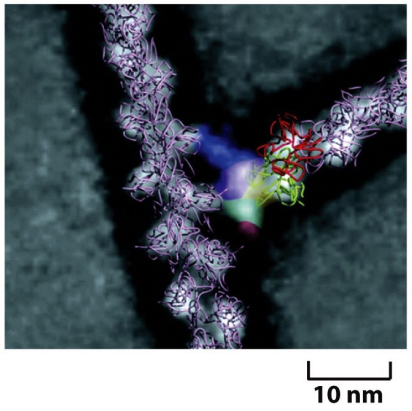
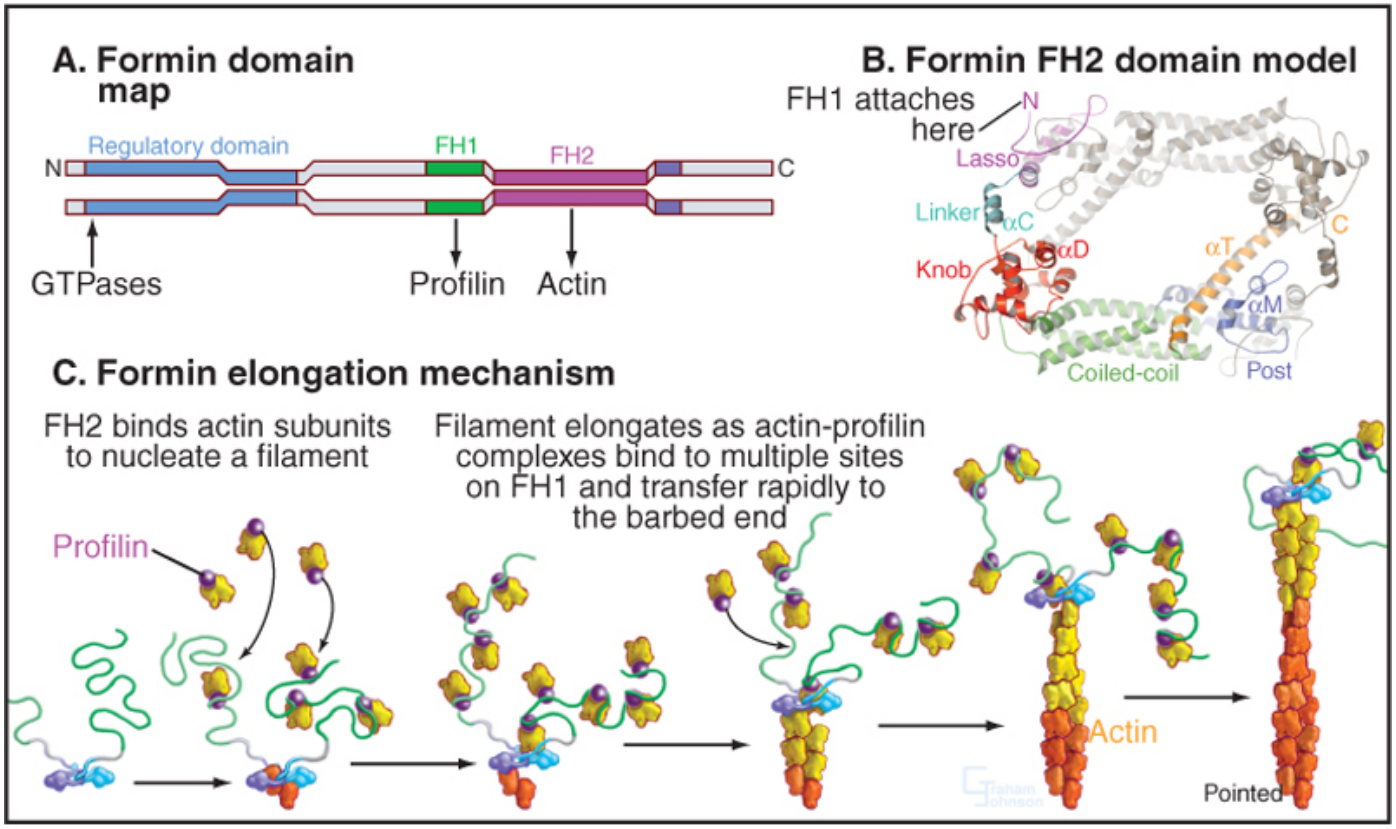


Figure 16-34c Molecular Biology of the Cell 5/e (© Garland Science 2008)





Actin: Nucleation and Elongation by Formins



Formin dimer nucleates - but NO branch between actin filaments

In cells, multiple formins -> different structures with different functions

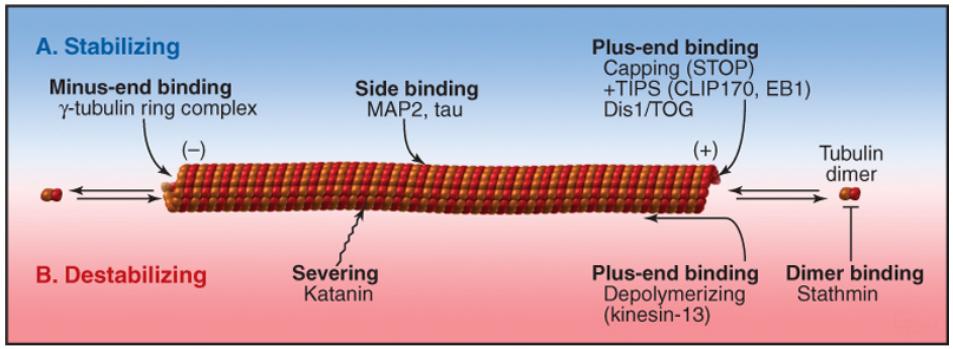
Actin: Nucleation and Elongation by Formins

Julian Eskin

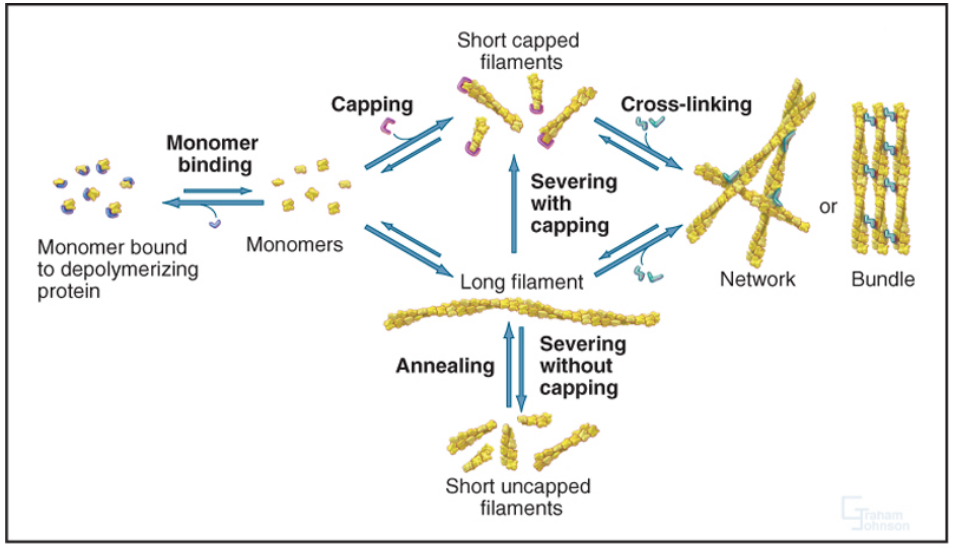
Microtubule- and Actin-associated proteins

- Monomer binding proteins (kinetics modification)
- Polymer tip-associated proteins (steric modification)
- Polymer lateral binding (stability and mechanics)
- Polymer severing

Microtubules



Actin

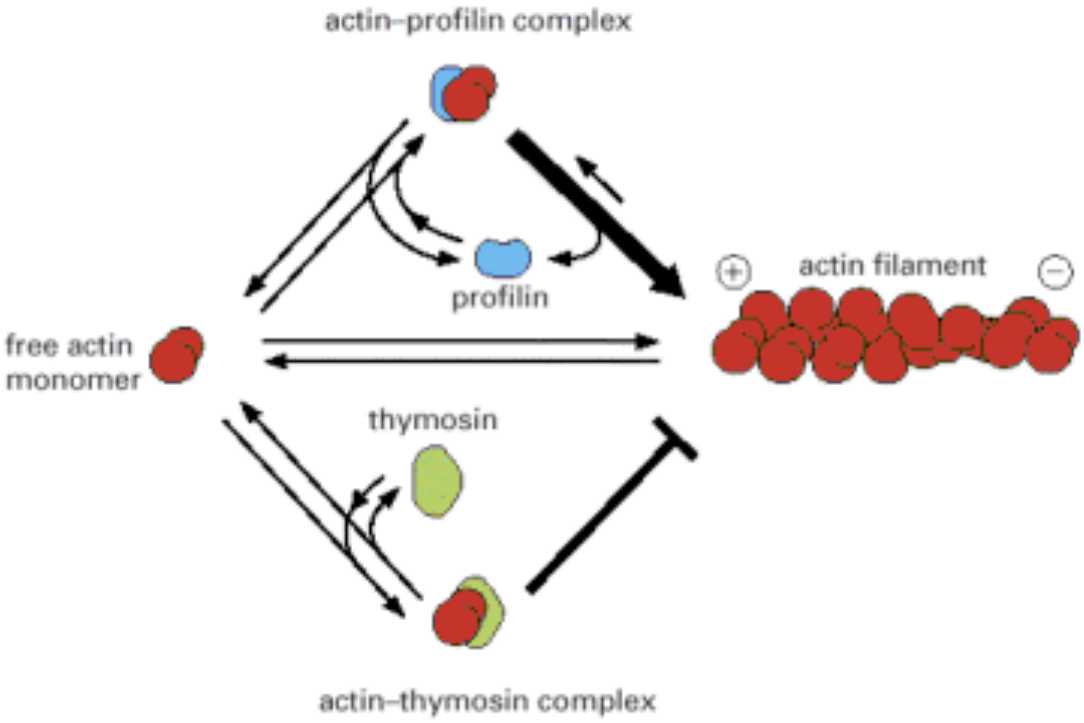


Monomer-binding Proteins

Actin: Thymosin/Profilin

In cells, ~50% of actin in filament, 50% (50-200 μM) unpolymerized, but Cc ATP-actin <1 μM). Other proteins bind and sequester actin monomers.

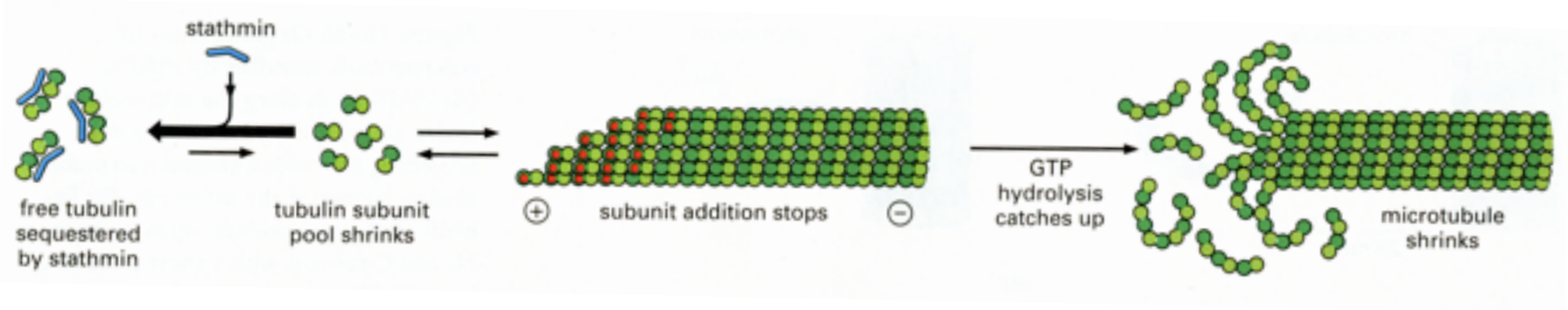
Thymosin: blocks polymerization of G-actin at either + or - end
Profilin: Binds + end of G-actin and promote polymerization at + end only



Monomer-binding Proteins

Microtubules: **Stathmin**

Same principle as thymosin for actin. Binds two tubulin dimers and slows down polymerization rate. Leads to increased frequency of catastrophe because GTP hydrolysis “catches up” with end.

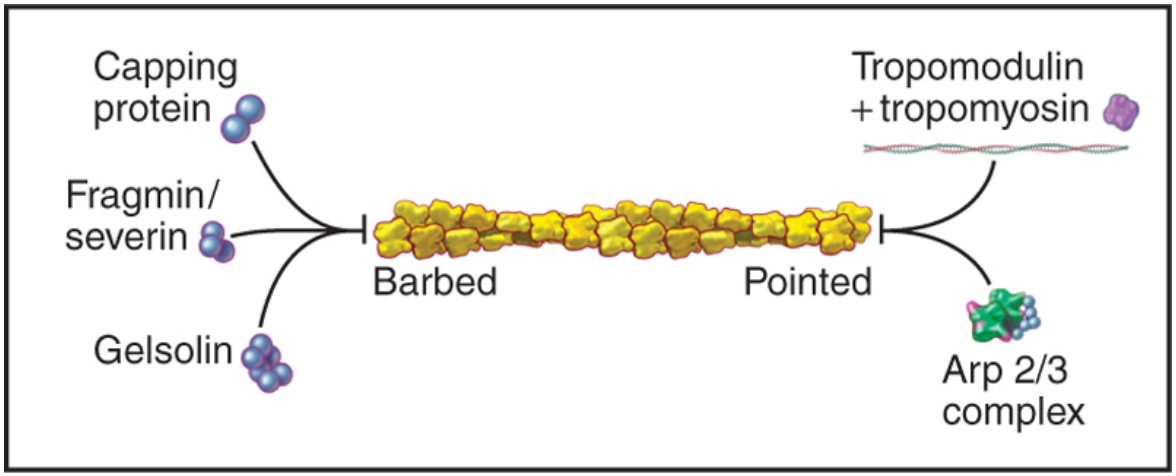


End-binding Proteins - Actin

Gelsolins
-bind to side and barbed (+) end of filaments
-block association or dissociation of actin subunit

Heterodimeric capping proteins (CapZ):
-Two subunits bind to barbed (+) end
-Also present on barbed (+) end of actin filaments in Z disk

Tropomodulin:
-caps pointed (-) end of stable actin filaments in cooperation with tropomyosin



End-binding Proteins - Microtubules

MAPs: microtubule-associated proteins
Kinesin-13: promotes catastrophe

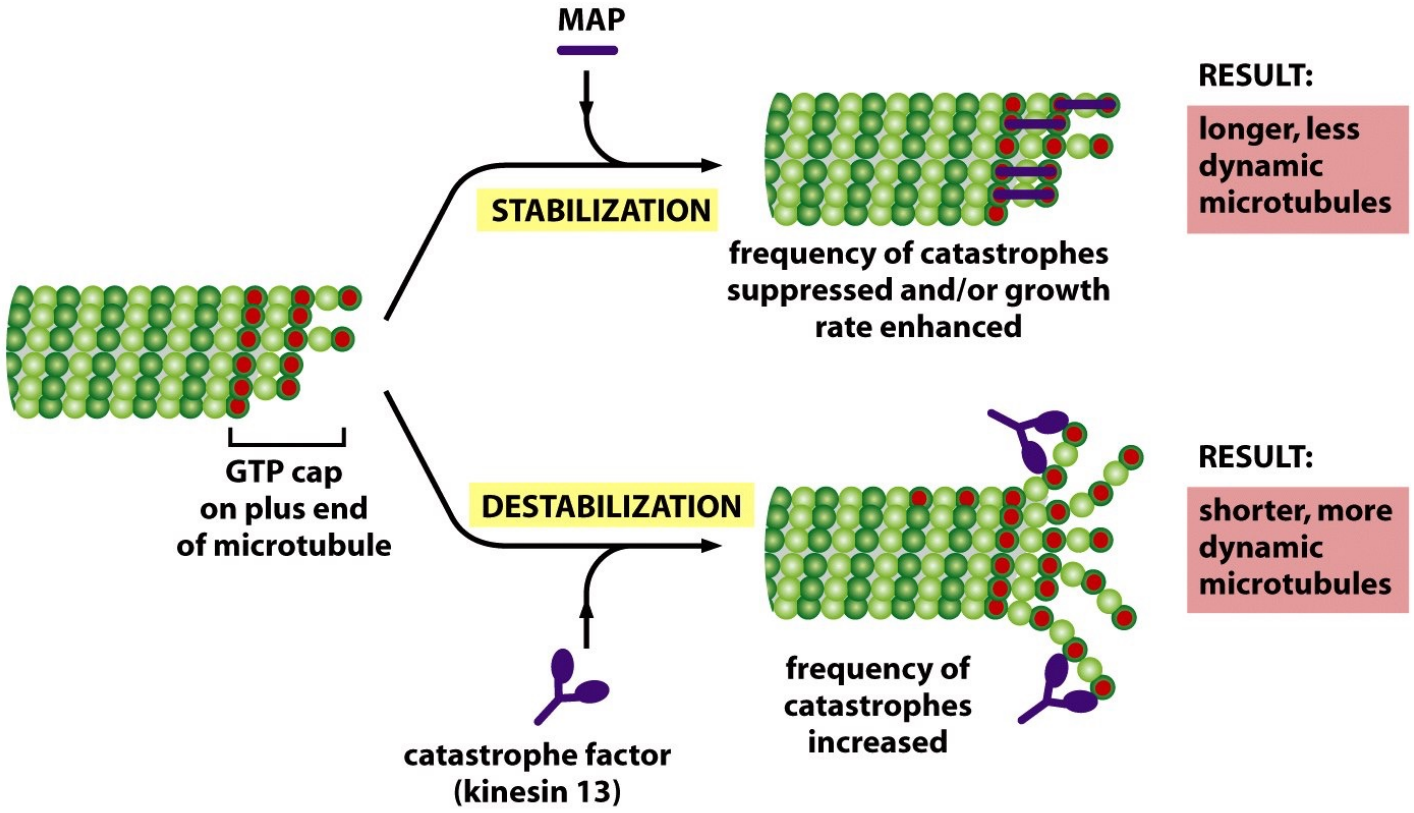
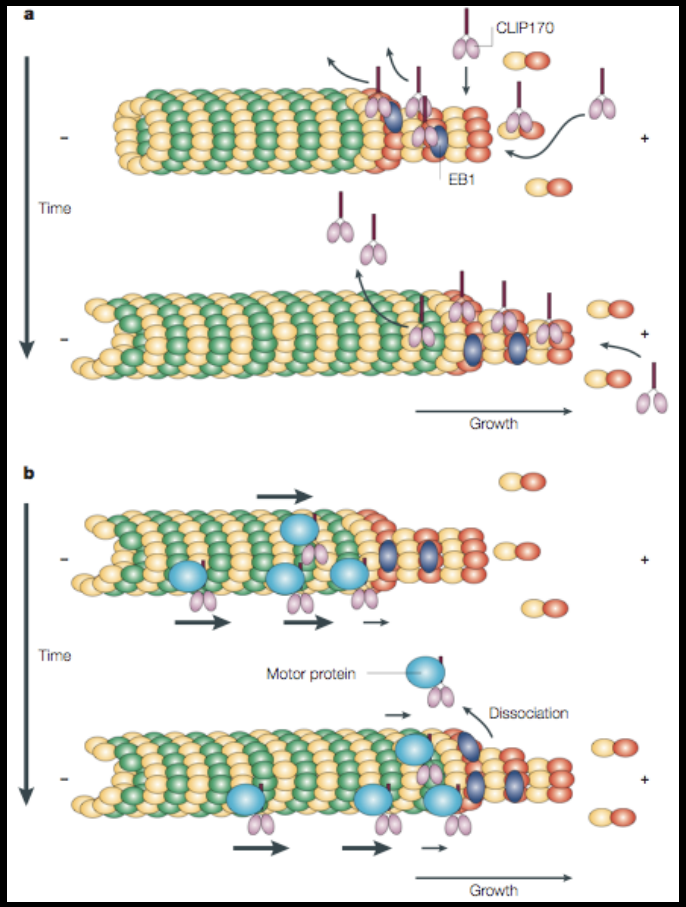


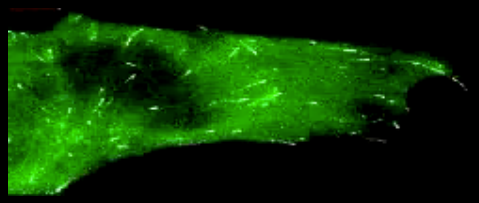
Figure 16-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

End-binding Proteins - Microtubules

Plus-end tracking proteins (+TIPS): CLIP170 and EB1

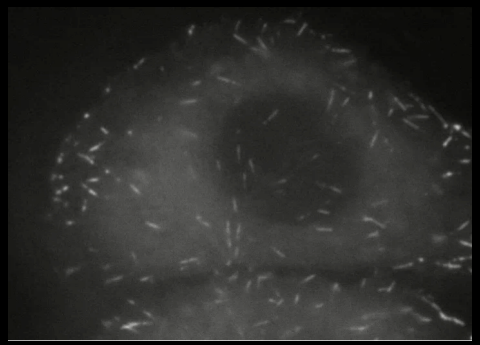
Deposition by Treadmilling





EB1 - GFP

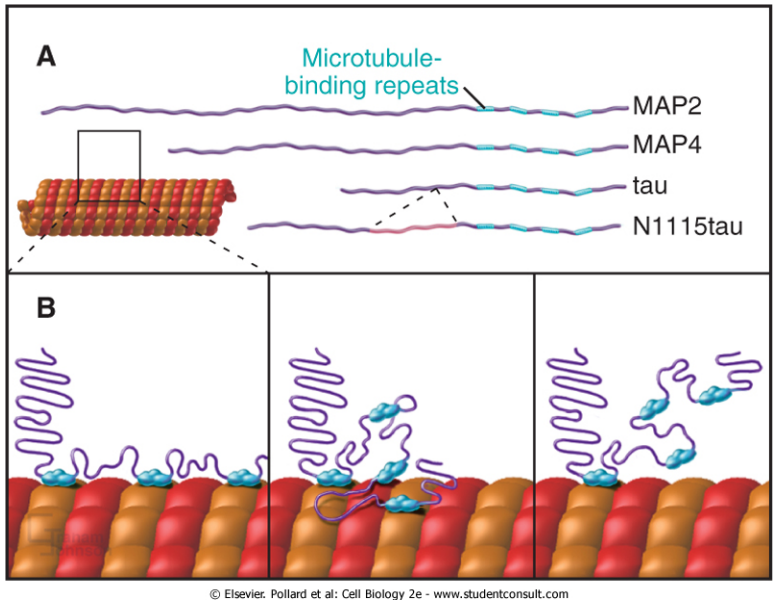
Deposition by motor proteins

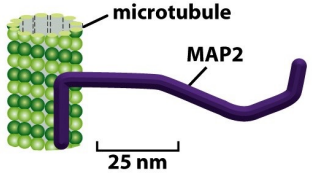


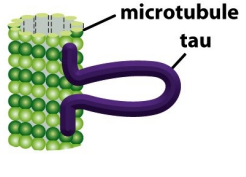
CLIP170 - GFP

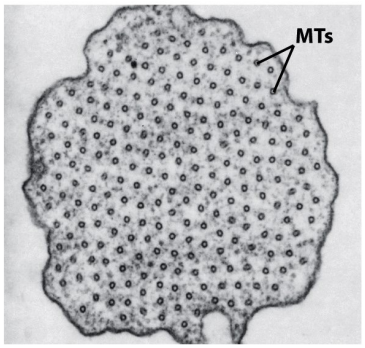
Side-binding Proteins - Microtubules

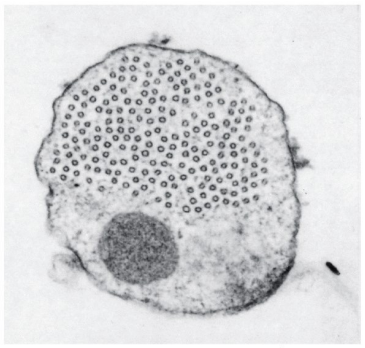
Tau and MAP2 (neurons, brain)
XMAP (ubiquitous)

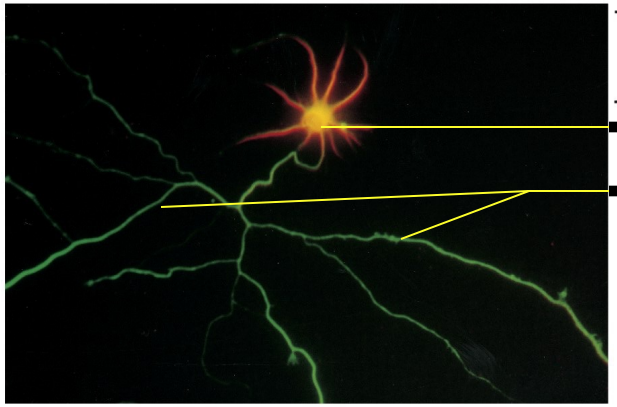












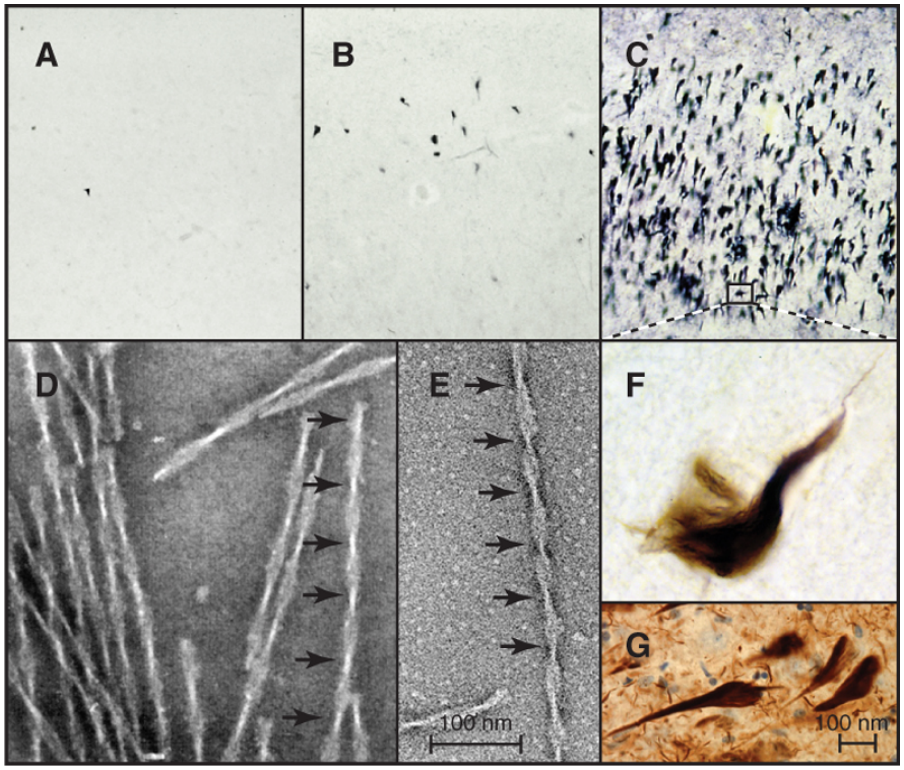
MAP2

Tau

Tau and Alzheimer Disease (AD)

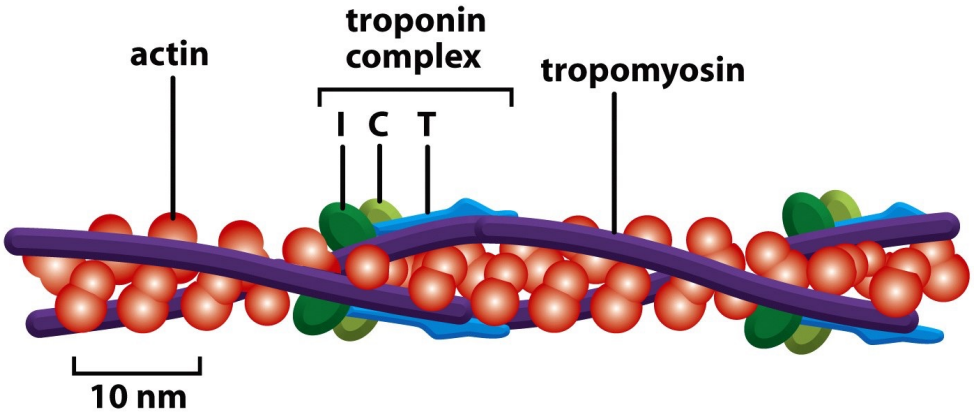
Tau forms intracellular paired helical filaments. Aggregate into neurofibrillary “tangles.” Tangle number correlates with disease severity.

Tau mutations: associated with rare cases of dementia.
Tau phosphorylation dissociates tau from Microtubules. Hyperphosphorylated tau then proteolytically fragmented and aggregates.

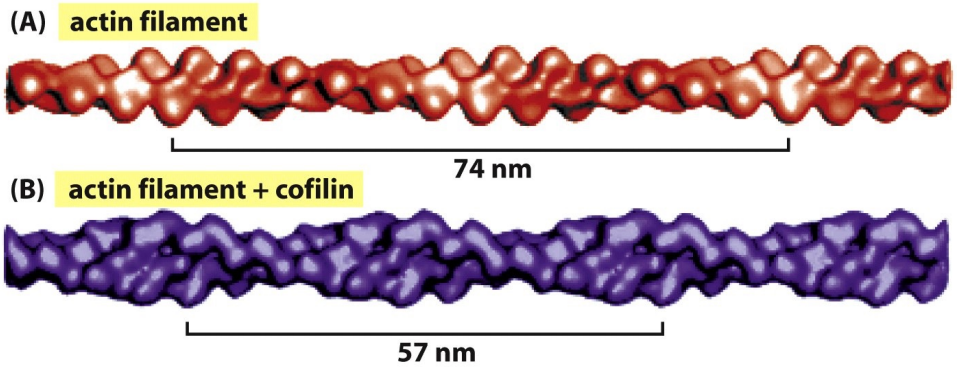


Side-binding Proteins for Actin Filaments

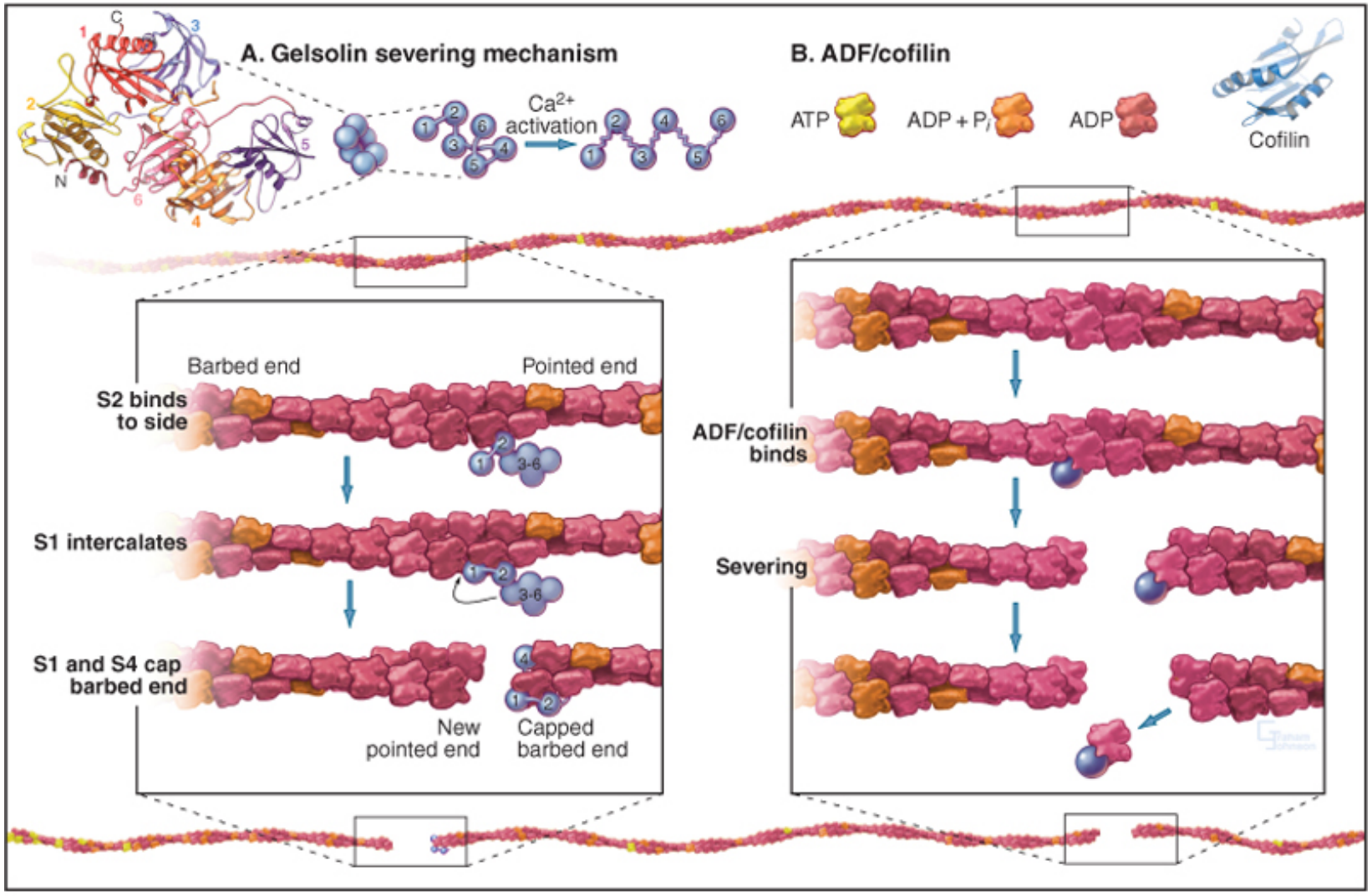
Tropomyosin (muscle and non-muscle)
Increase tensile strength of actin - stronger filaments



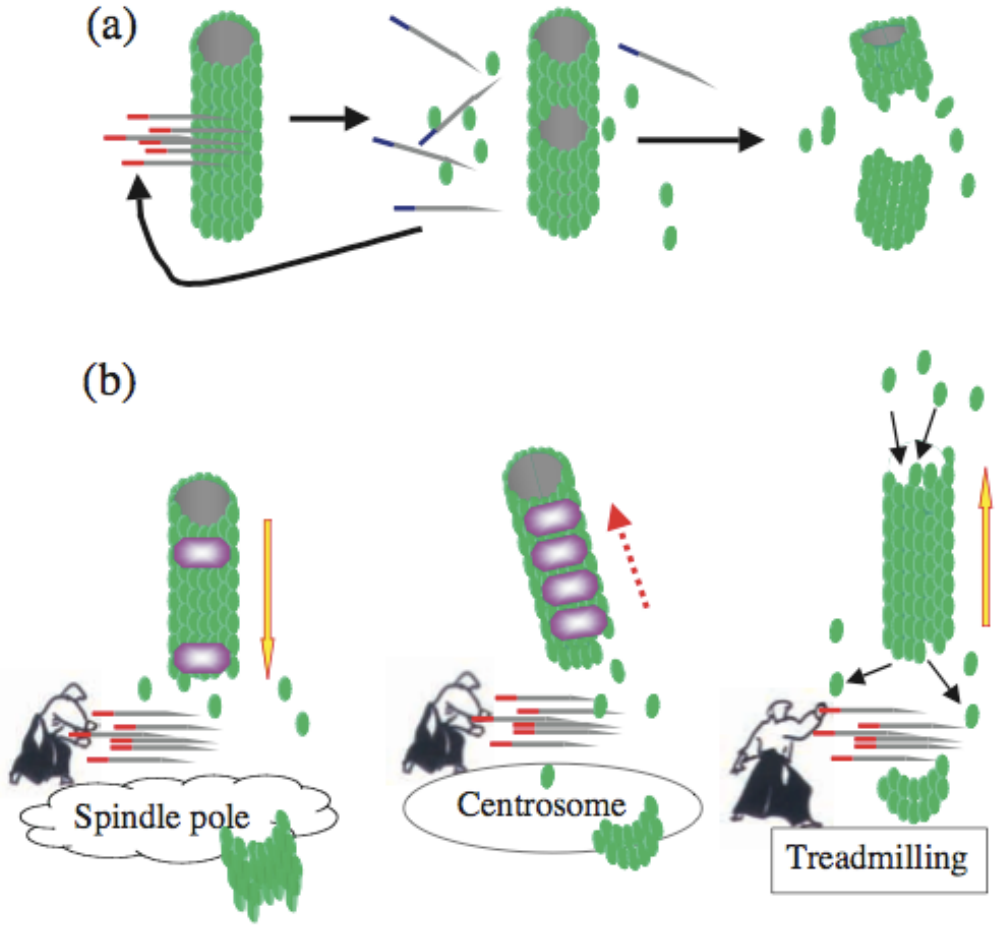
Cofilin
Increase twisting of protofilaments - promotes severing



Actin Filament Severing Proteins: Gelsolin and Cofilin



Microtubule Severing by Katanin (AAA ATPase)

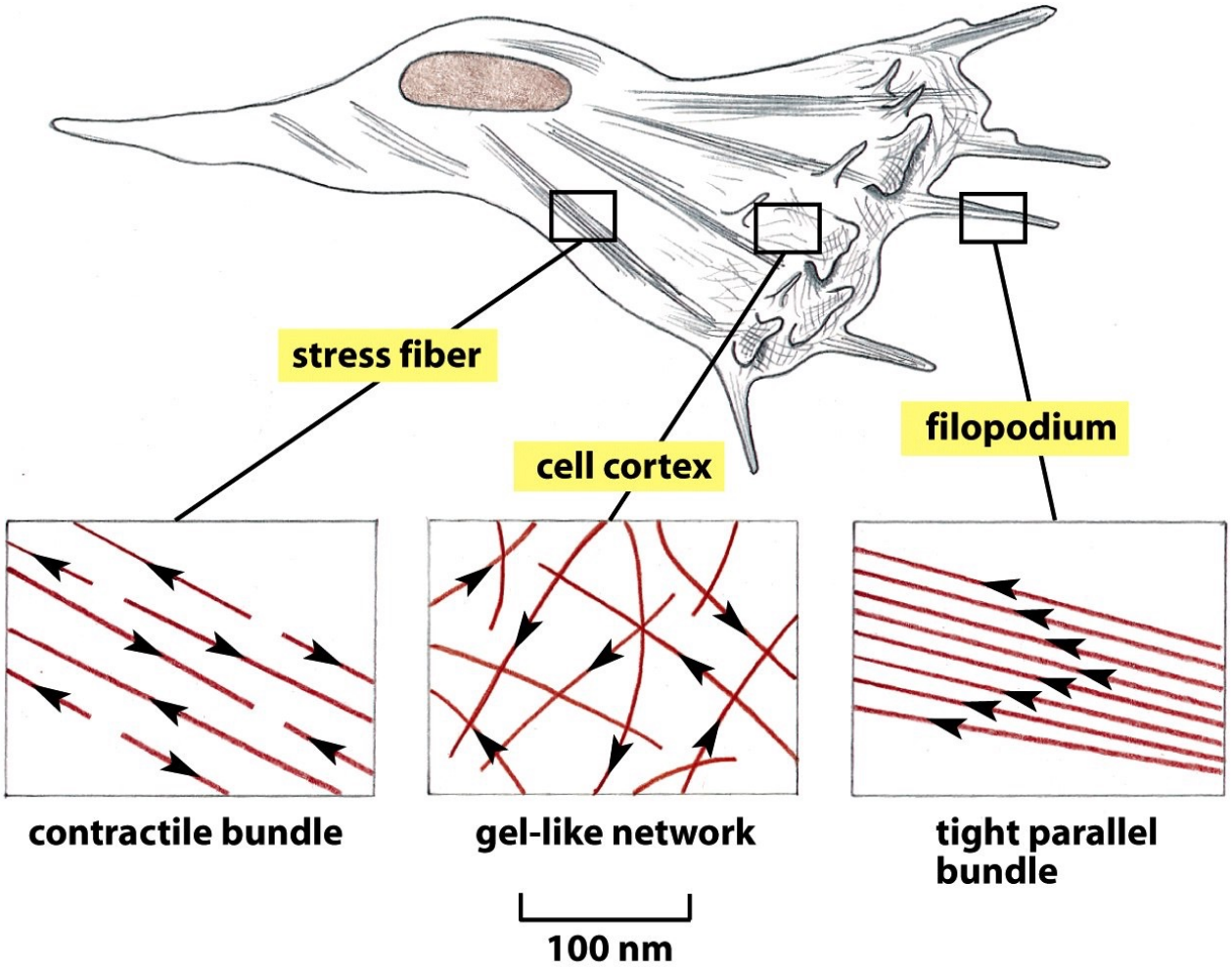


Higher-order Ultrastructural Organization

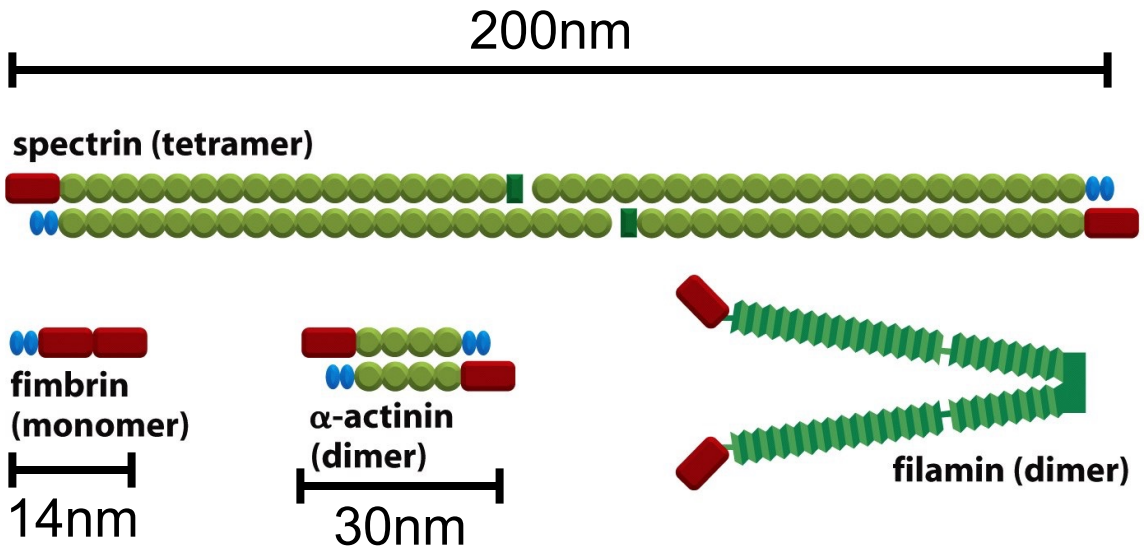
How do cells organize microtubules and actin filaments into large structures and nano-machines?

Actin: Bundles or Gel-like Networks

Cross-linking by specific proteins



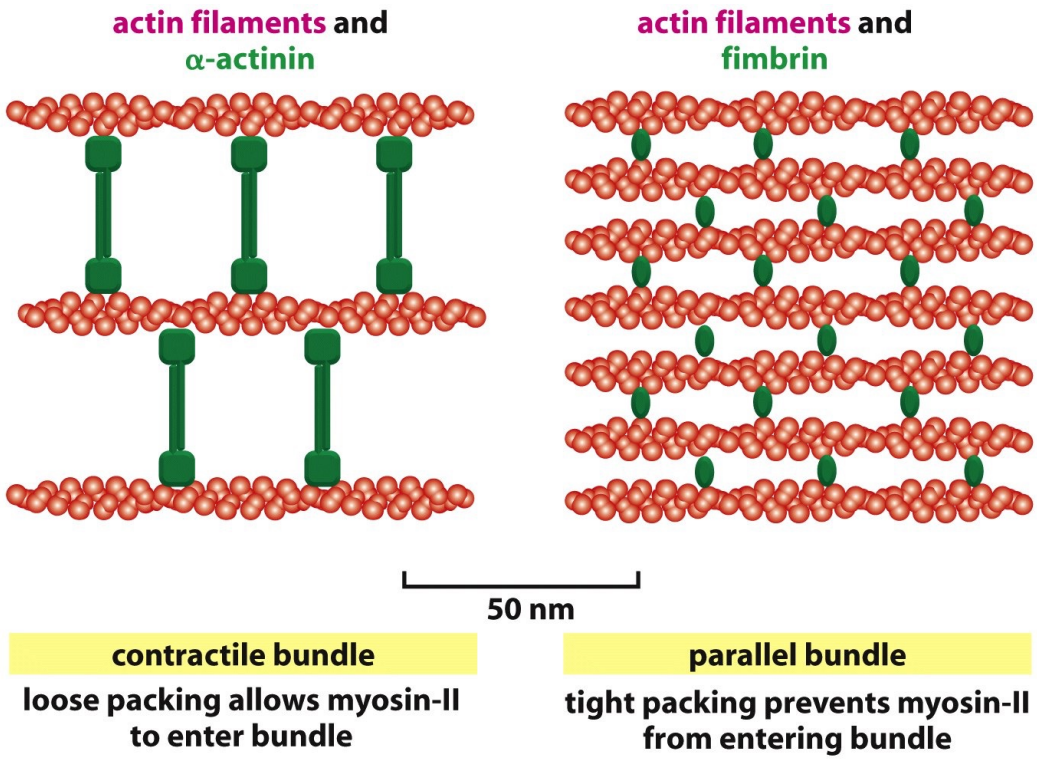
Actin: Filament-Bundling Proteins



Actin: Bundles of Filaments in Cells

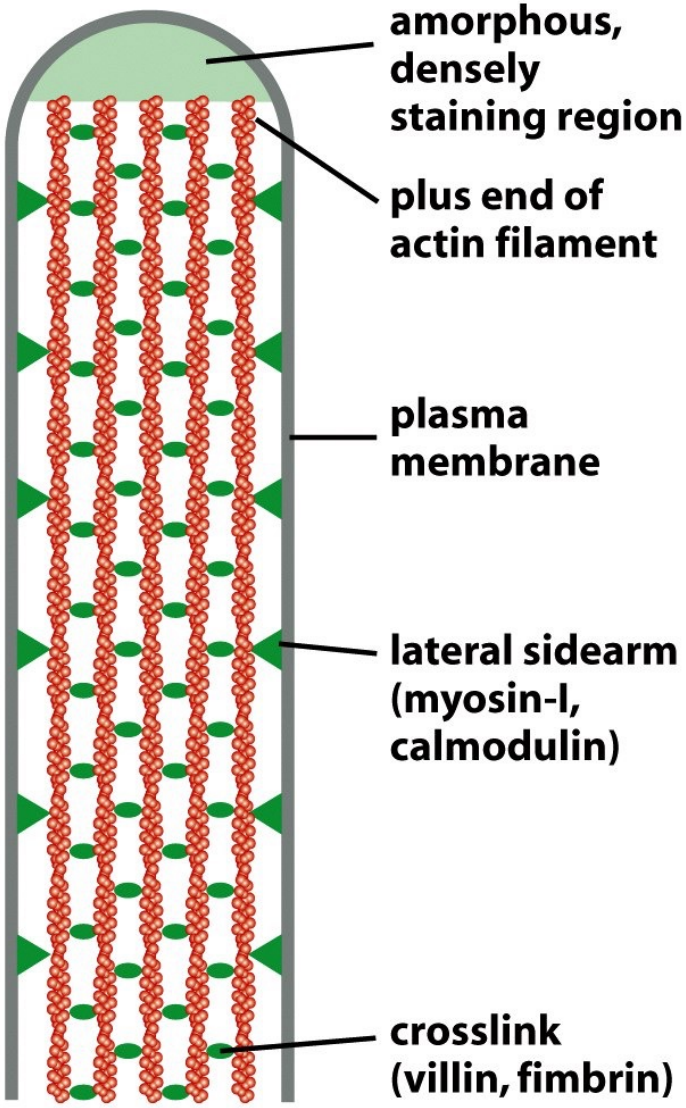
Contractile Bundles
"stress fibers" of non-muscle cells

Parallel bundles
microvilli and filopodia



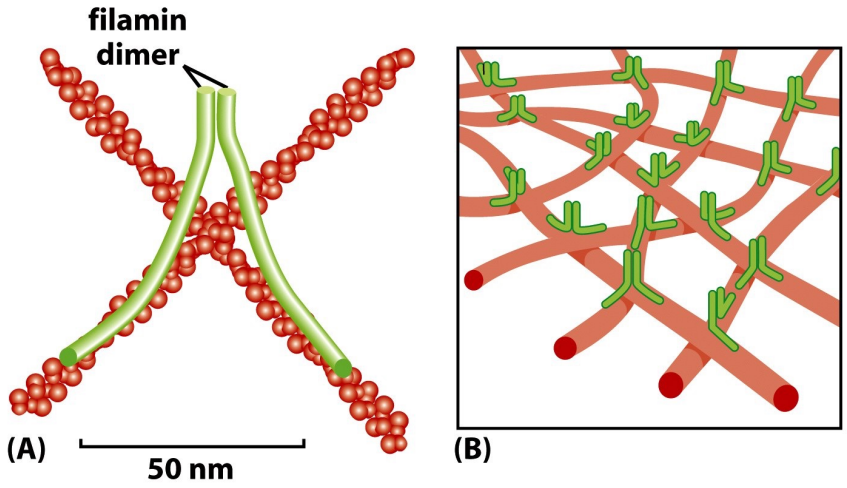
Actin: Bundles of Filaments in Cells

Microvilli



Actin: Gel-like Network of Filaments in Cells

Lamellipodia

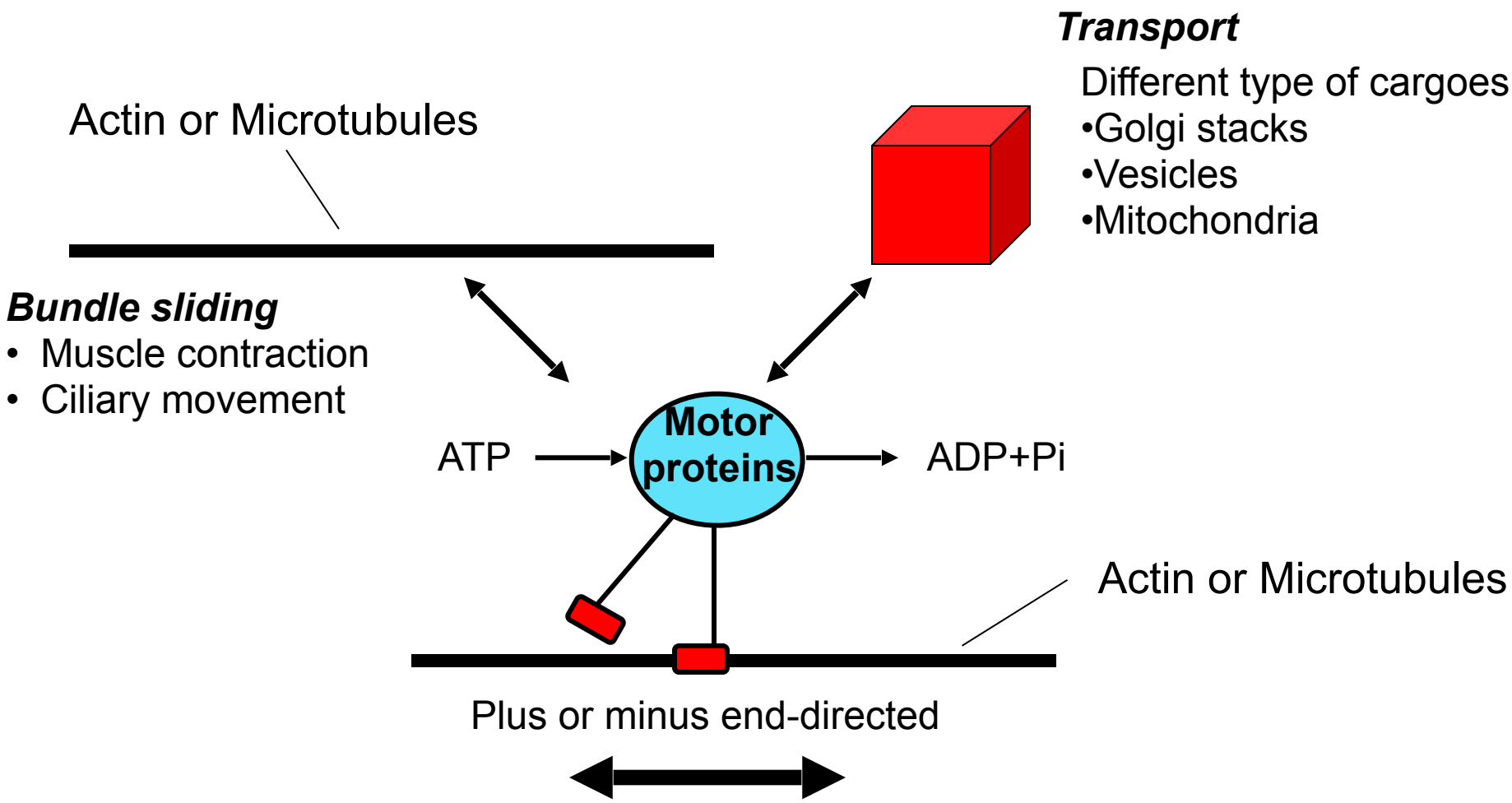


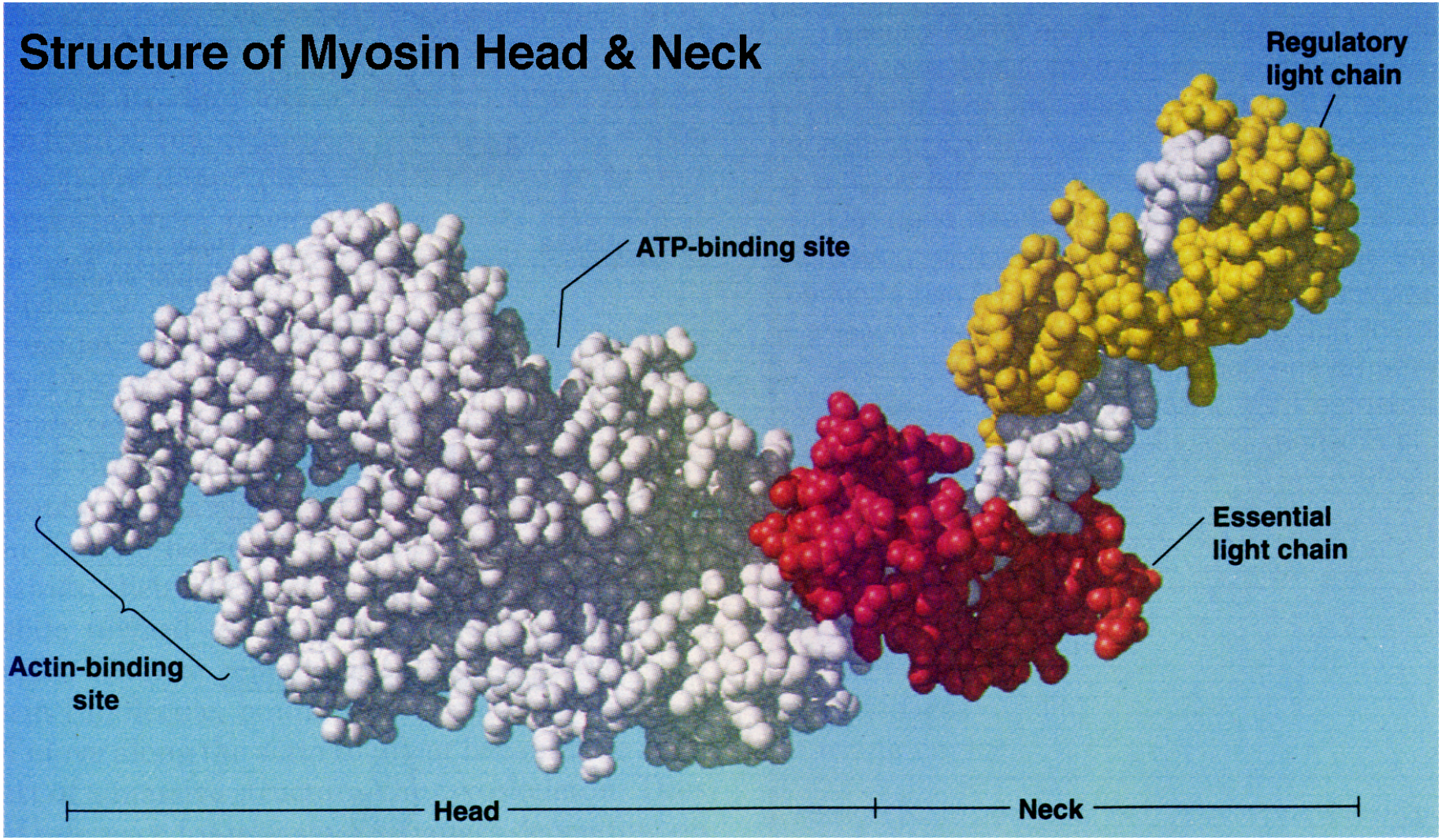
III. Motor Proteins

Actin - Myosins

Microtubules - Kinesins and Dynein

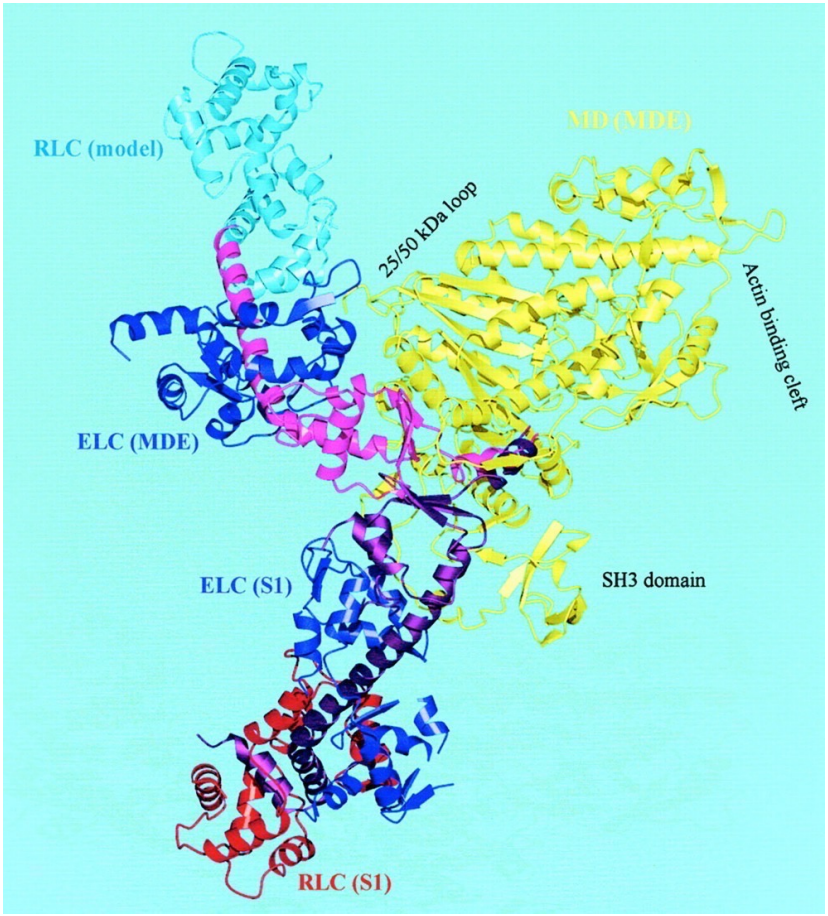
Motor Proteins




Structure of Myosin Head & Neck
Regulatory light chain
ATP-binding site
Essential light chain
Actin-binding site
Head
Neck

Twisting Head Model

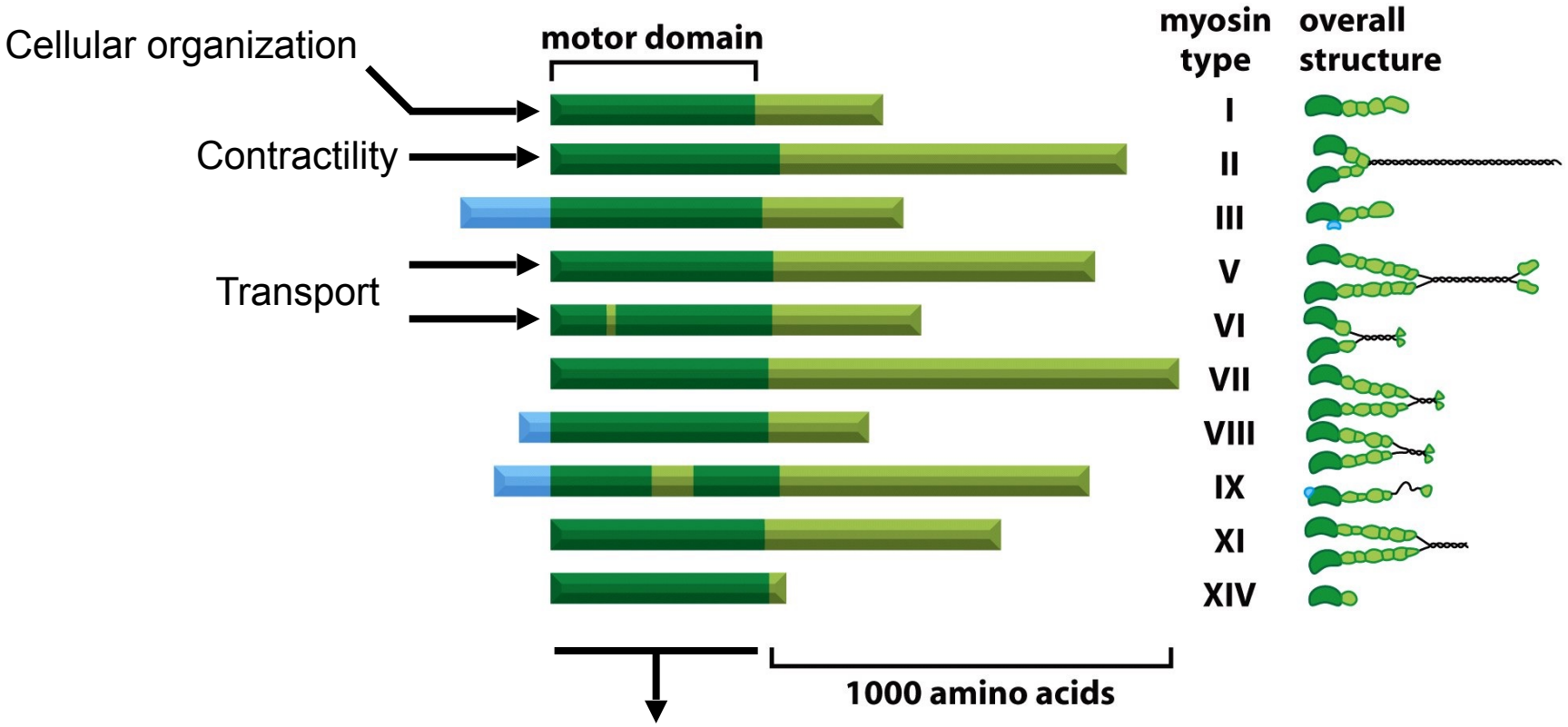
Myosin Lever Arm Orientations



Actin-based motors: Myosin superfamily

Humans have ~40 myosin genes

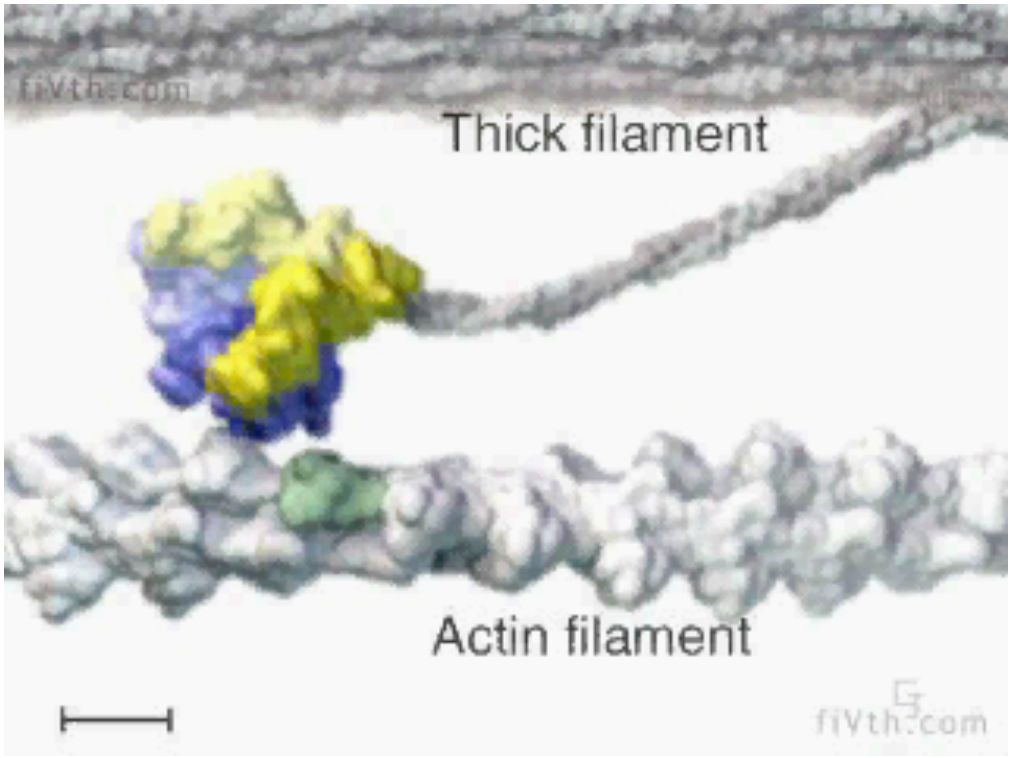
Walk to Barbed (+) end of Actin Filament (except Myosin VI)



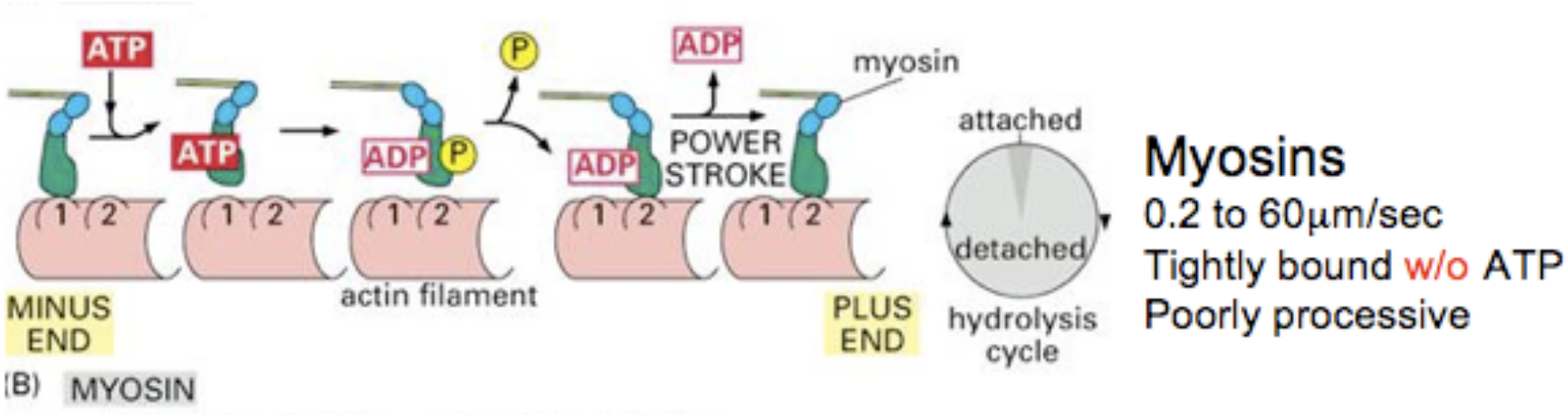
Only the motor domain is conserved

ATPase and Actin binding Sites: Linked Functions

Myosin-II Dimer Moving on Actin Filament



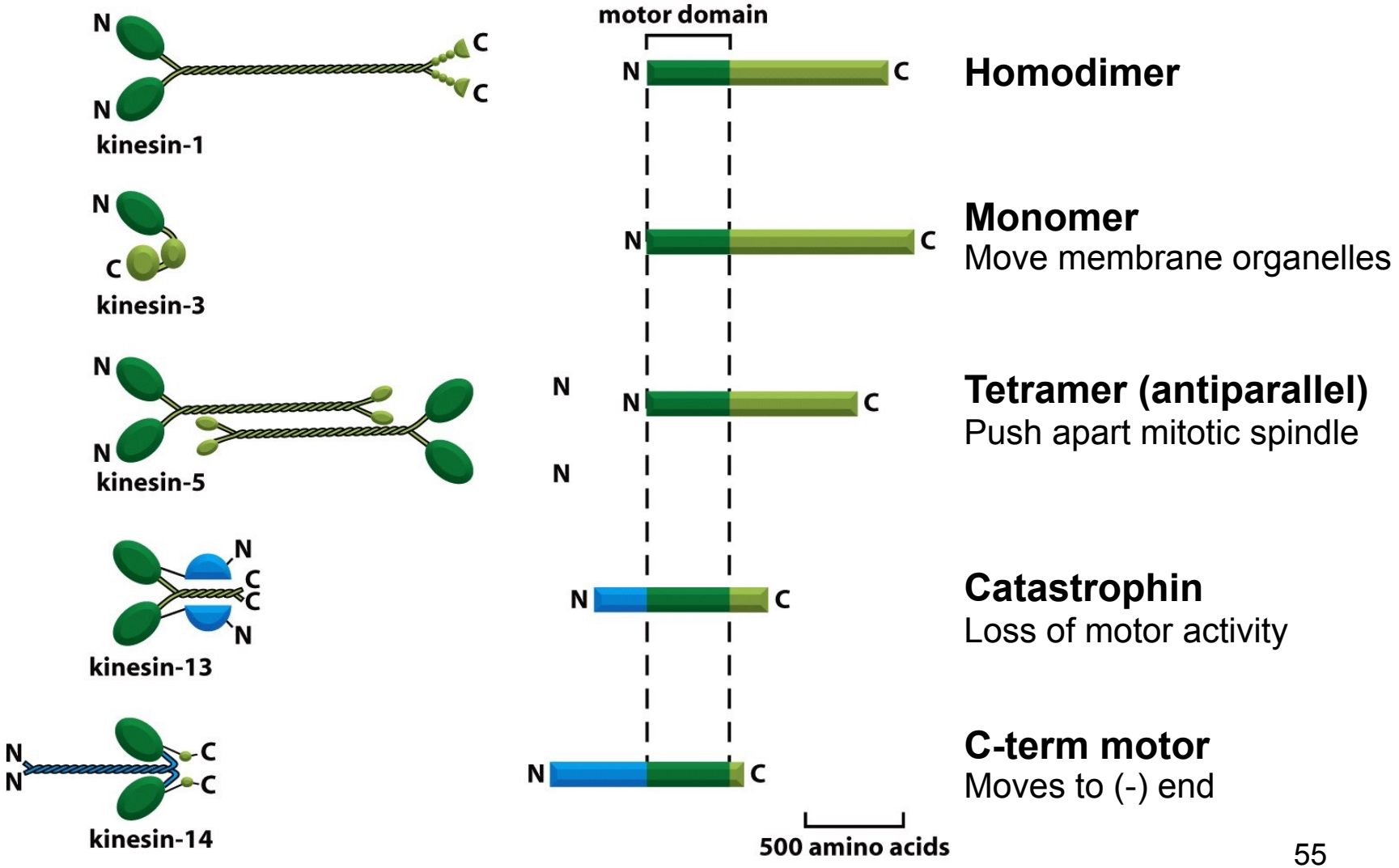
Myosin Motor: Motion from Chemical Energy



Binding of ATP -> decreased affinity for actin (myosin head detaches)
Weak binding of myosin to another (next) actin -> release of Pi -> tight binding
Closing of ATP-binding cleft -> large conformational change (5 nm displacement)
ADP released during power stroke

Microtubule-based motors: Kinesin superfamily

Humans: 40 kinesin genes. Generally Plus (+) end directed.



Homodimer

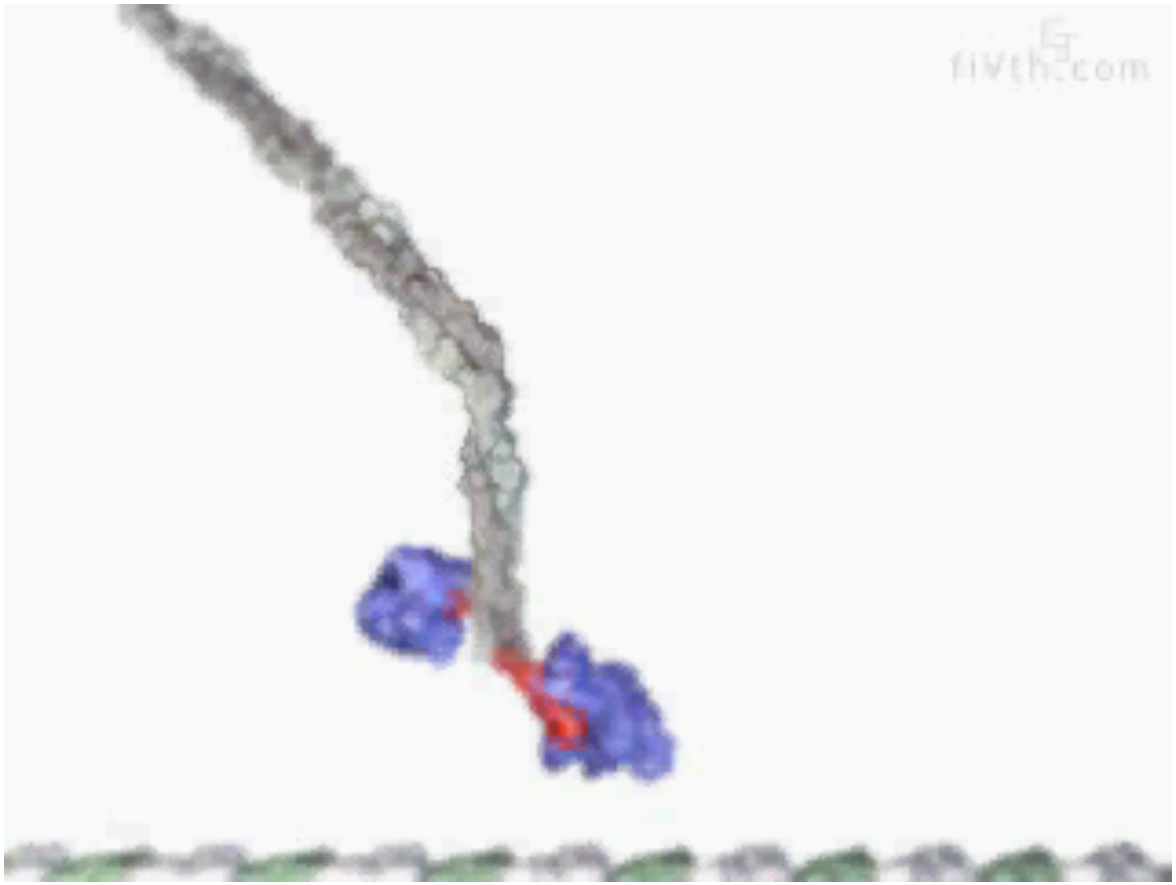
Monomer
Move membrane organelles

Tetramer (antiparallel)
Push apart mitotic spindle

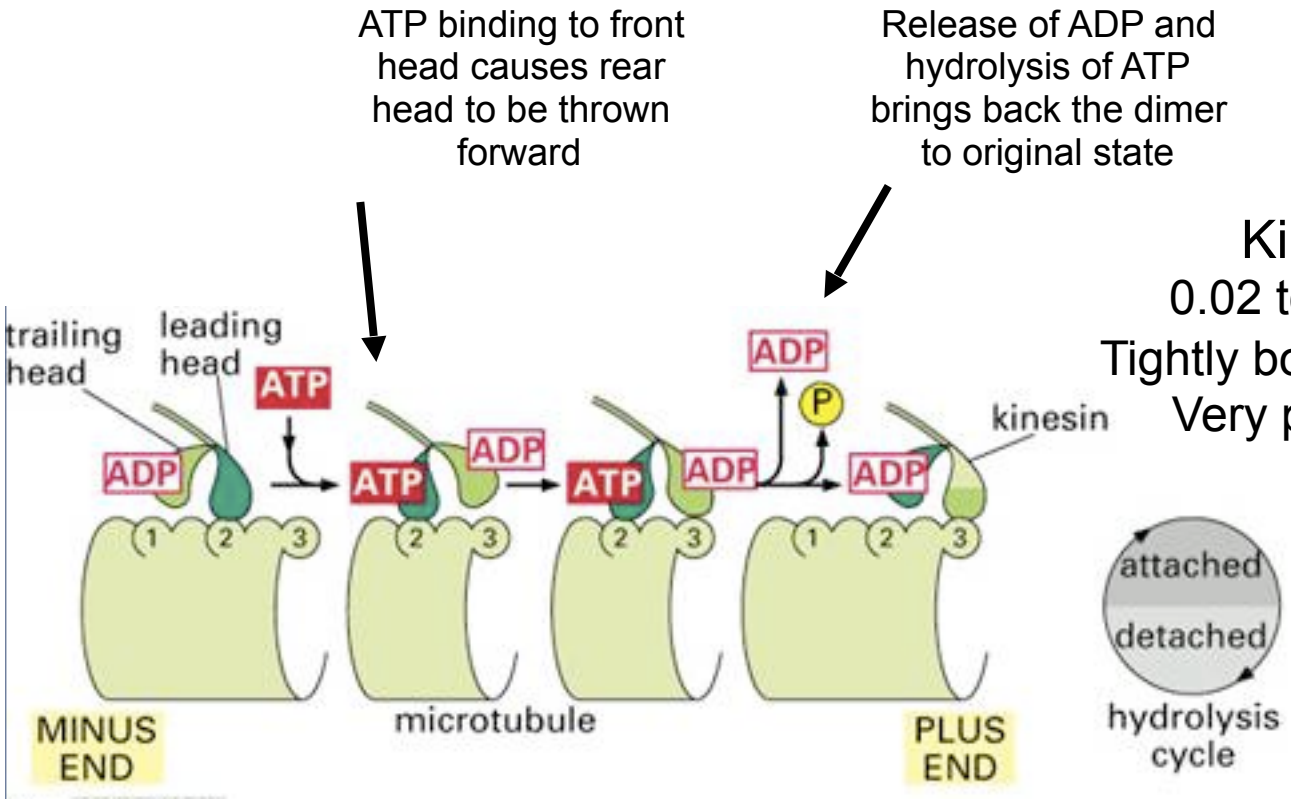
Catastrophin
Loss of motor activity

C-term motor
Moves to (-) end

Kinesin-1 Dimer Moving on Microtubule



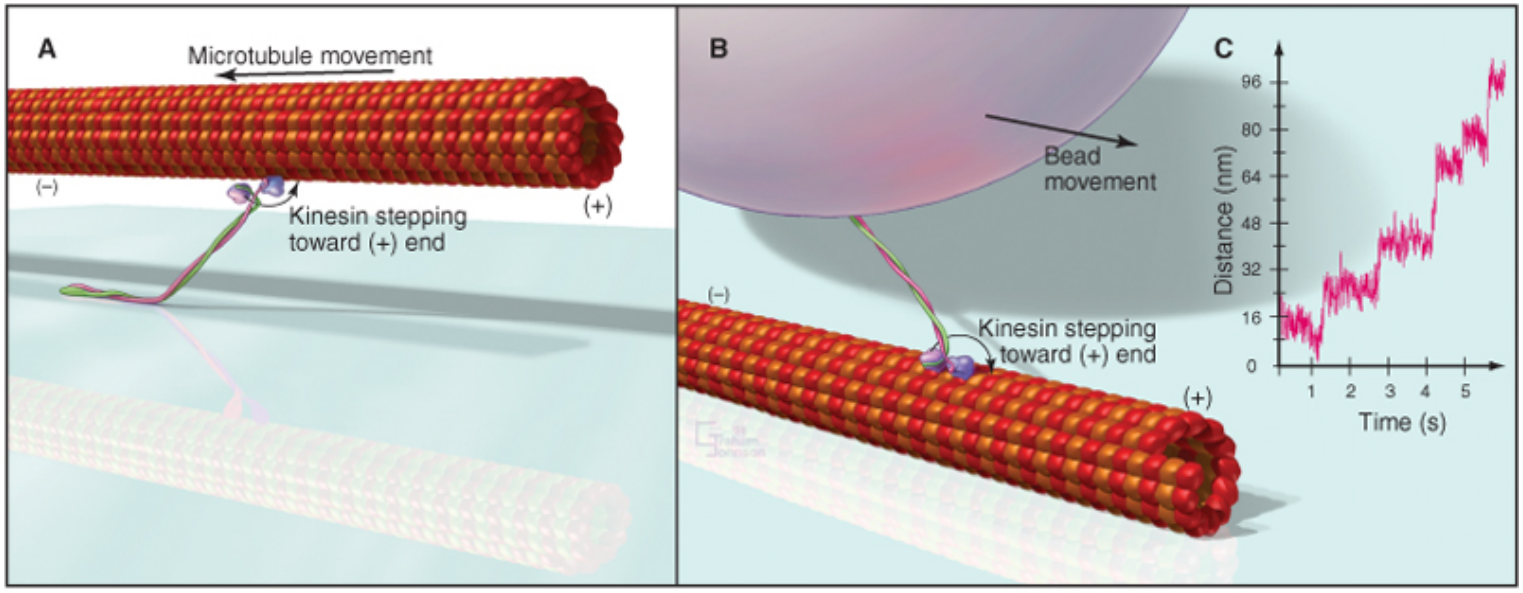
Kinesin Motor: Motion from Chemical Energy

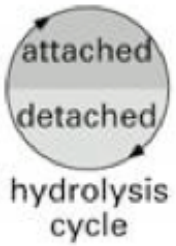


Kinesins
0.02 to 2μm/sec
Tightly bound with ATP
Very processive

Microtubule-based Motors

Kinesin-1

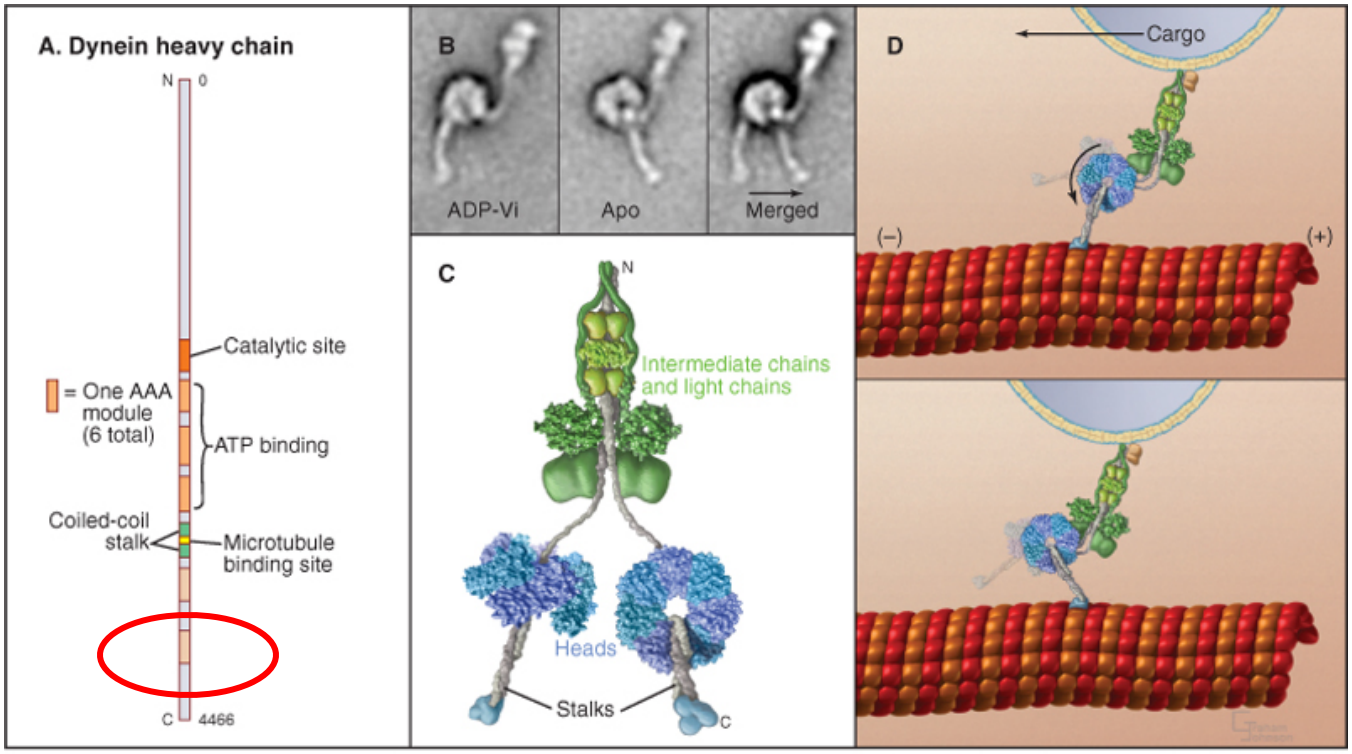




→ Avoids loss of cargo

Microtubule-based Motors: Dyneins

- Different evolutionary origin from Myosins and Kinesins
- AAA proteins (ATPases Associated with diverse Activities)
- Cytoplasmic dynein - vesicle transport and position
- Motor component of cilia and flagella

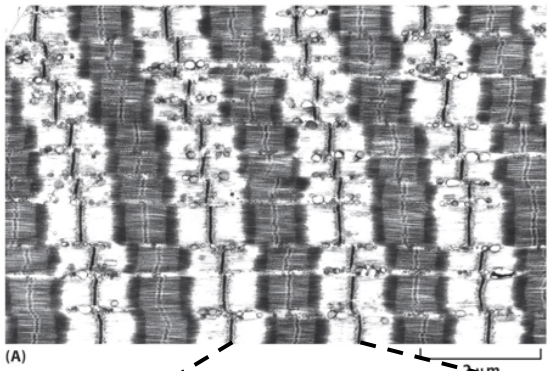


IV. Cellular Machines Based on Microtubules and Actin

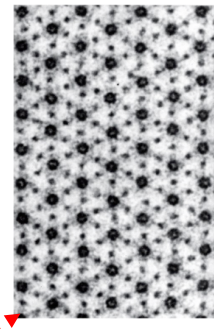
- Skeletal muscle (Actin with myosin-II)
- Cilia, flagella (Microtubules with dynein)
- Mitotic spindle (Microtubules with kinesin & dynein)

Striated (Skeletal & Cardiac) Muscle

Filaments, Sarcomeres, Myofibrils

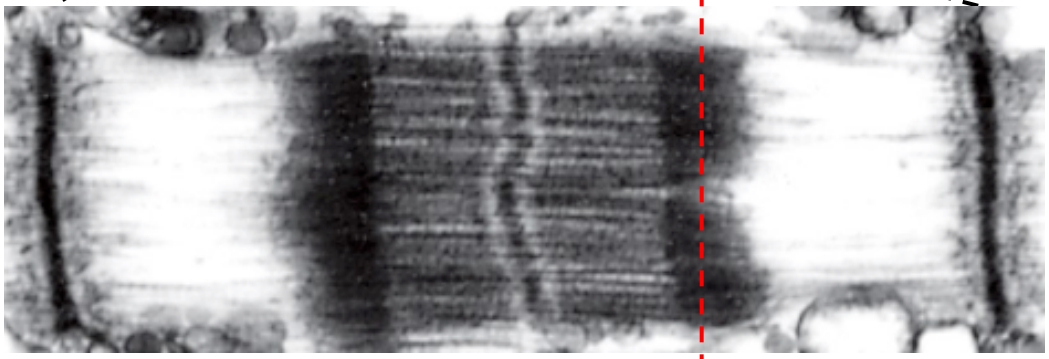


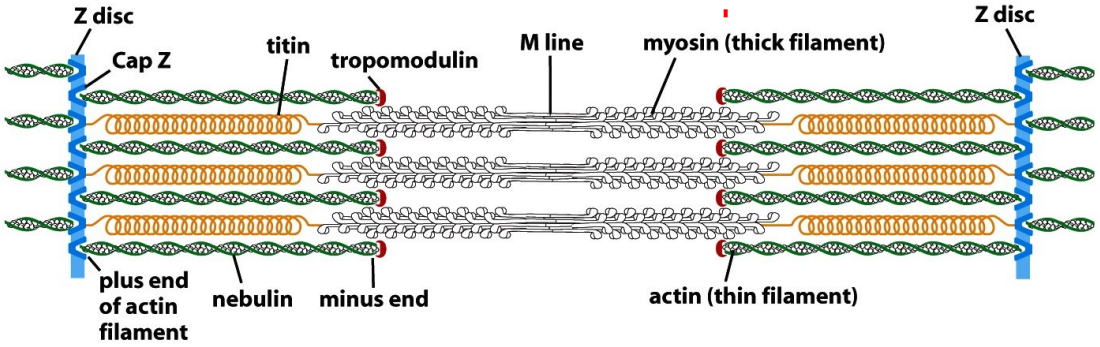
Cross-Section



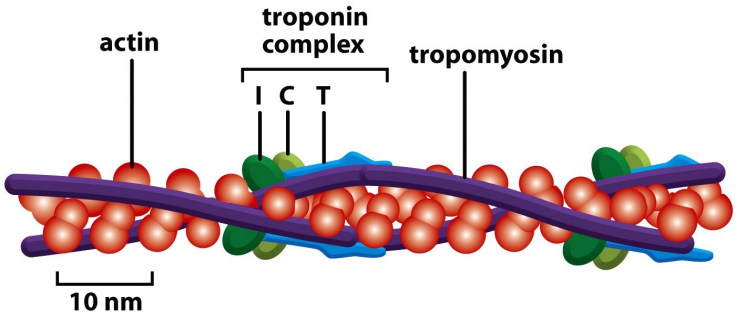
← Myosin-II (Thick)

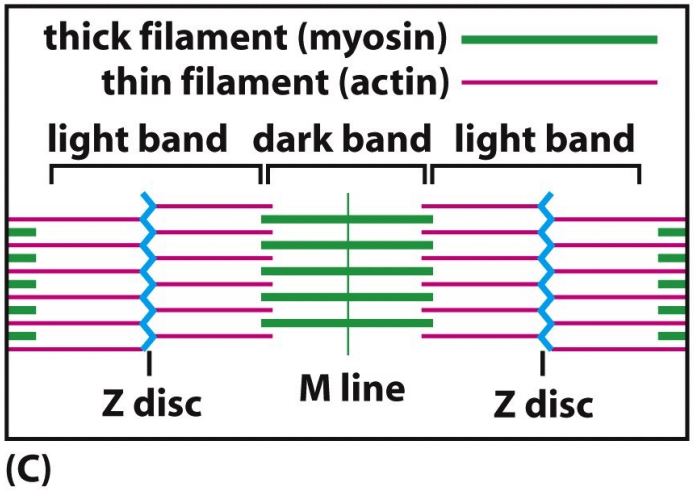
← Actin Filament (Thin)



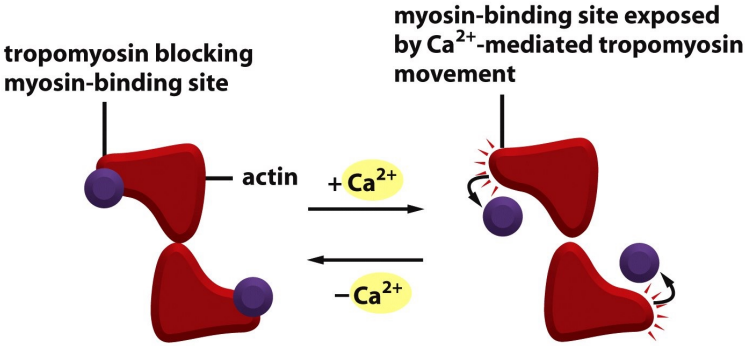


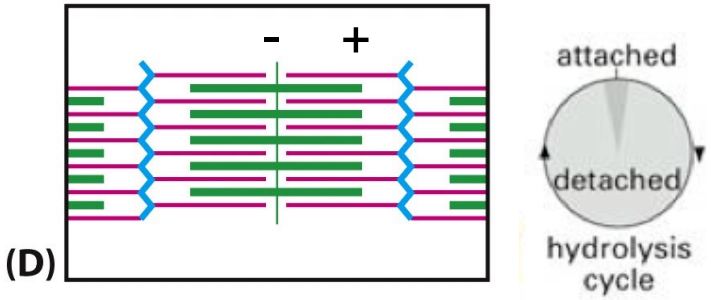
Ca2+-Based Regulation of Contraction





(C)





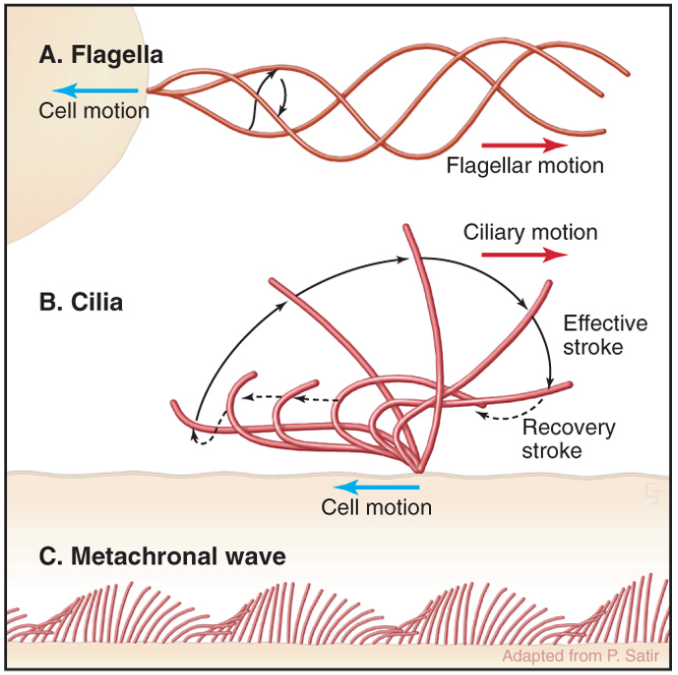
Familial Hypertrophic Cardiomyopathy

- Inherited human disease
  - Heart dysfunction - sudden death
- Autosomal dominant
- Myosin heavy chain gene first
- Other sarcomere components
- Subtle mutations
  - Mild effects on biochemical function

Cilia and Flagella

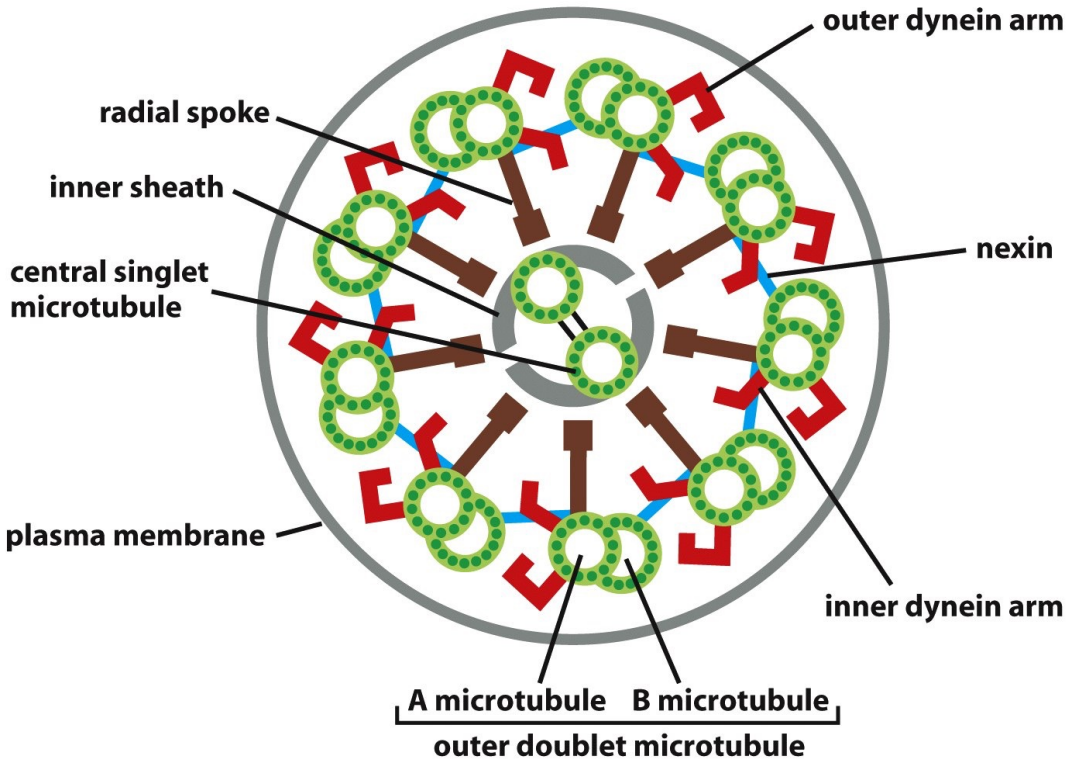
Flagella
-helical motion
-sperm and many protozoa

Cilia
-whip-like motion (swimming breast stroke)
-move cells in fluids or fluids over cells



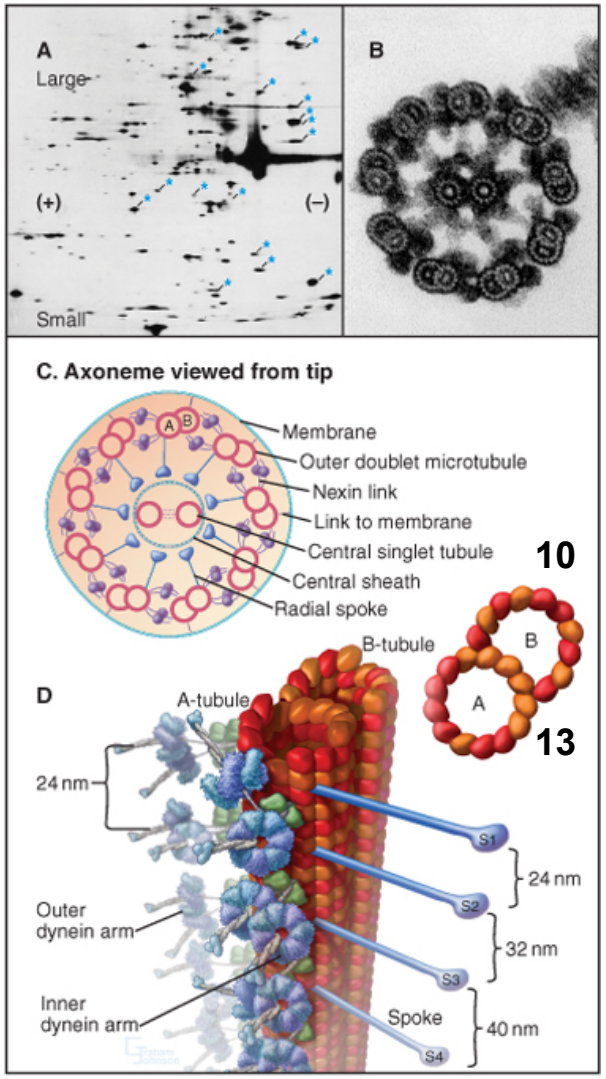
Axoneme: Motor Core of Cilia and Flagella

"9+2"

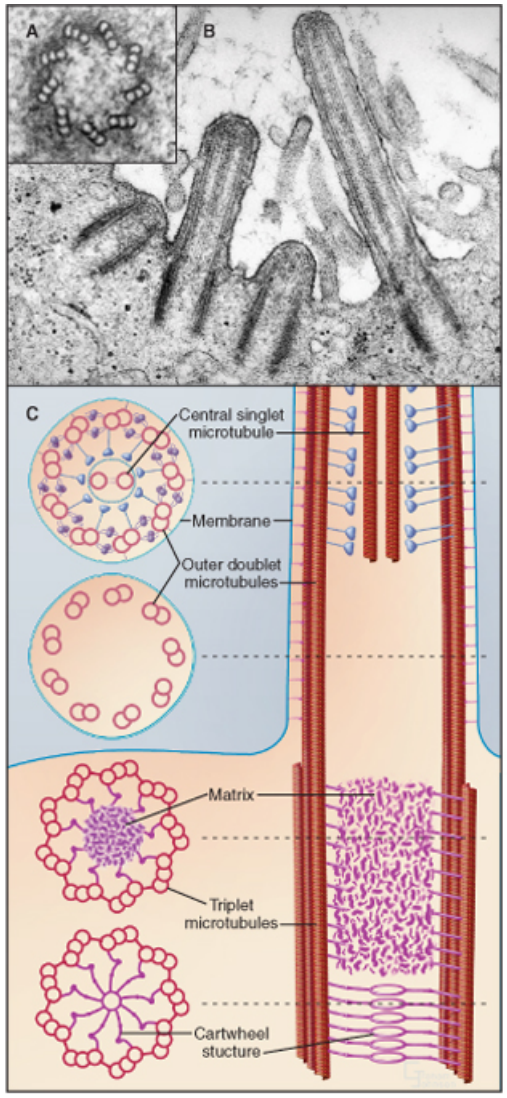


How Motion is Generated

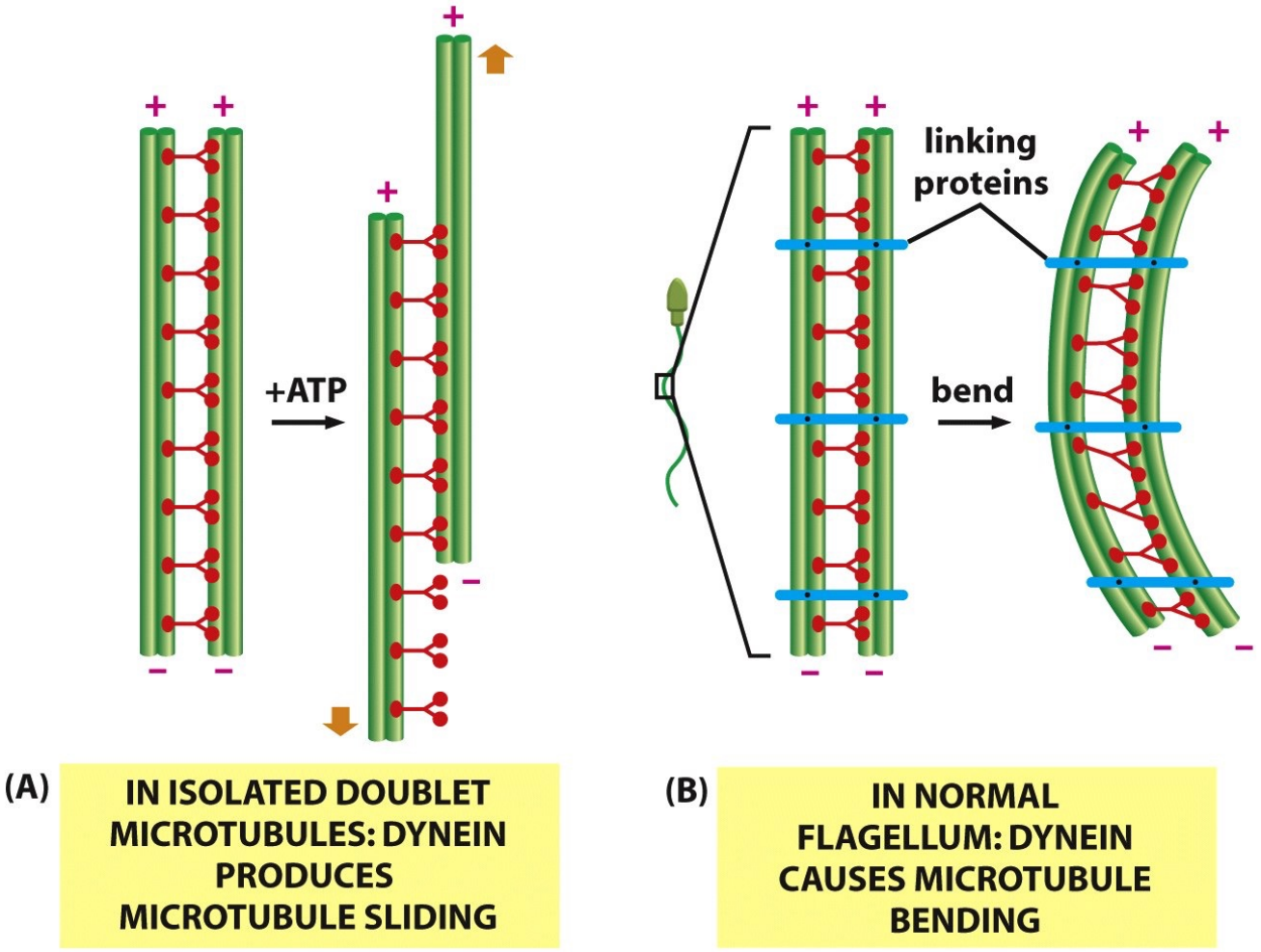
Dynein Arms Connect the Microtubule Pairs



Microtubule Pairs Anchored at Base



How Motion is Generated



Mitotic Spindle: Movement of Chromosomes

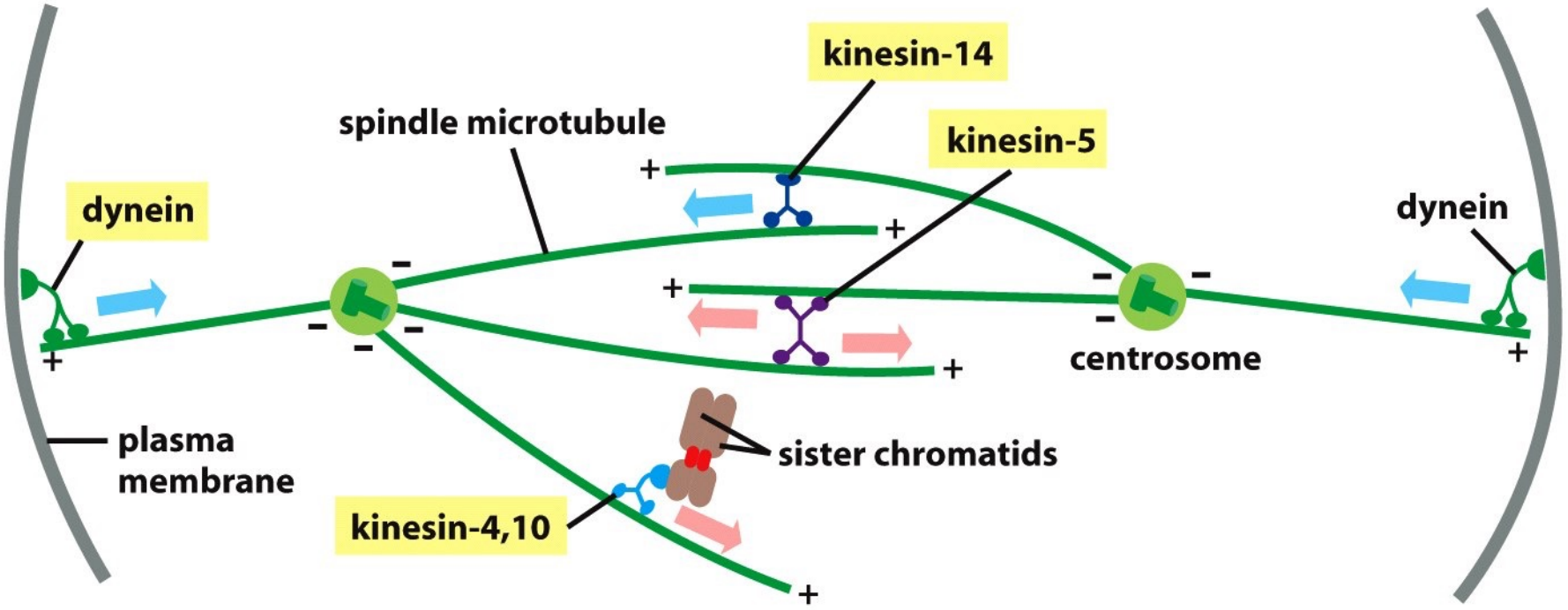


Figure 17-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Intermediate Filaments

- Filaments 10 nm wide => “intermediate”
- Present in Metazoa / Animals
  - i.e. not Plants or Unicellular Organisms
- Complex Gene Superfamily
  - 70 in Human Genome
- Specific Expression at Different Times and Places

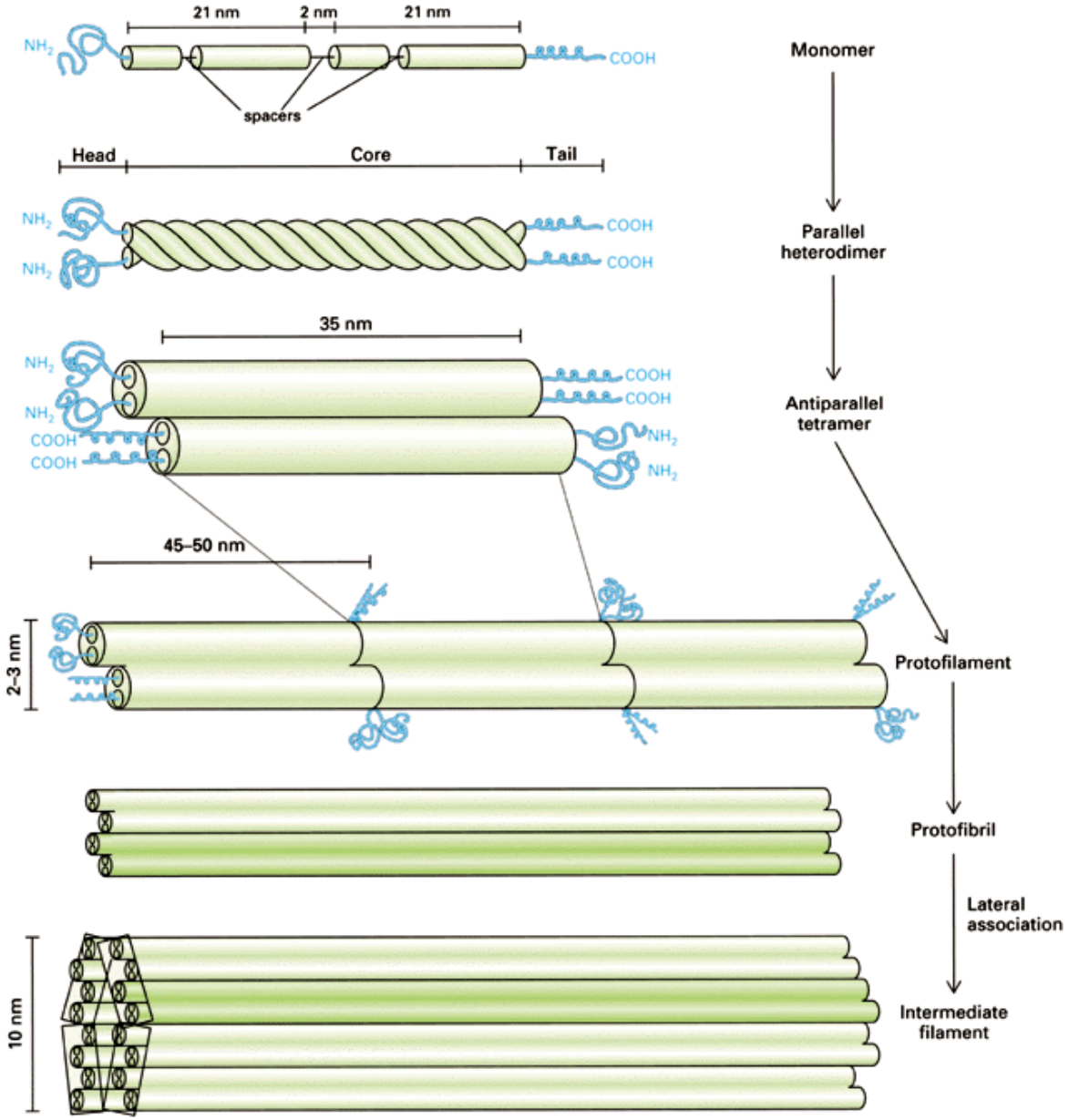
Biochemical Properties In Vitro

- Very stable. Little subunit exchange.
- Very strong. Filaments do not break.
  - MT's strong but brittle
  - Actin weak

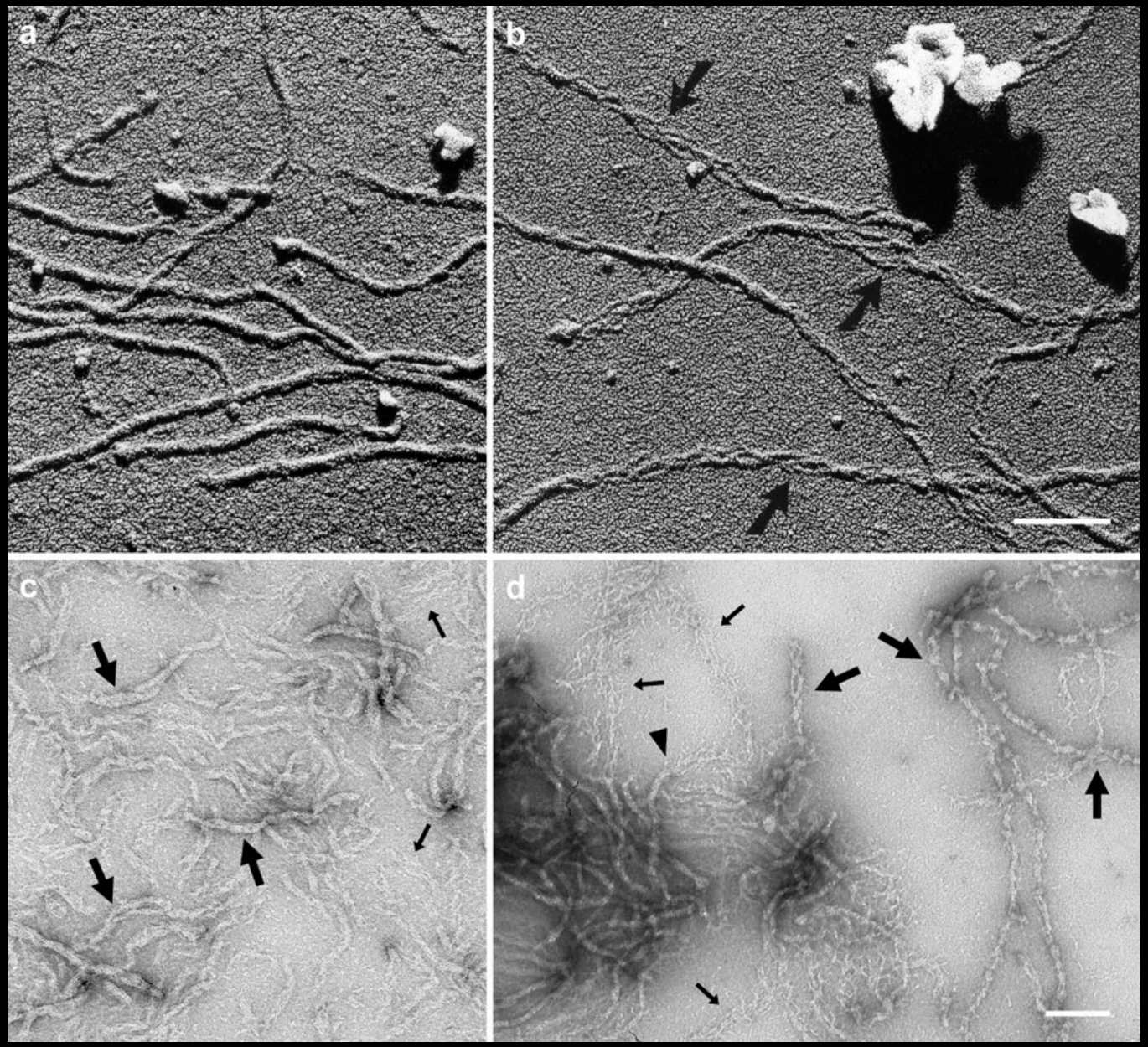
Potential Functions In Vivo

- Mechanical Strength of Cytoplasm
- Help a Layer of Epithelial Cells Resist Shear Stress - Filaments Connect to Cell-cell Junctions
- Hold Nucleus in Center of Cell

Structure & Assembly



Filament Unraveling: EM



Classes of IF Proteins

Class	*Name*	*Cells*	*Number of Isoforms*	*Size (kD)*	*Polymers*
I	Acidic Keratin	Epithelia	~15	40-60	Obligate Heteropolymers
II	Basic Keratin	Epithelia	~15	50-70	One acidic + one basic
III	Vimentin	Mesenchymal	1	53	
III	Desmin	Muscle	1	52	Homopolymers (single
III	Glial Fibrillary Acidic Protein (GFAP)	Glia	1	51	type of subunit) or co-polymers w/ each
III	Peripherin	Neurons	>1	58	other at varied ratios
IV	Neurofilament H	Neurons	1	135-150	
IV	Neurofilament M	Neurons	1	105-110	H & M each require
IV	Neurofilament L	Neurons	1	60-70	L for polymer
IV	Nestin	Glial scars, Early neurons & muscle	1	240	
V	Lamin A	All	1	60-75	Homopolymers or
V	Lamin B	All	1	60-75	Heteropolymer

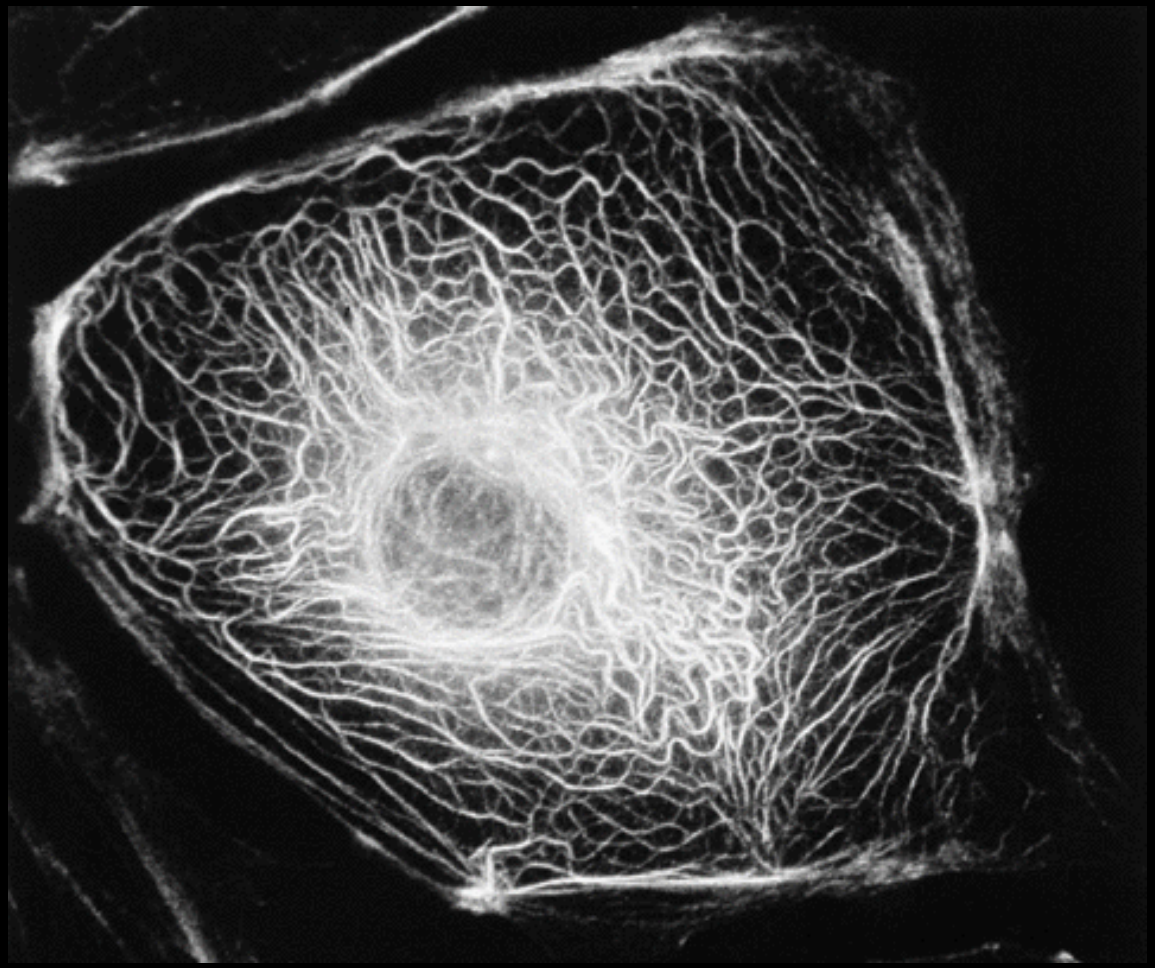
Regulation of IF Assembly

- Notoriously Stable
  - No Nucleotide
- Filaments Move Little
  - Precursors Move More
- Disassemble Somewhat during Mitosis
  - Phosphorylation by Cyclin-dependent Kinase (CDK)

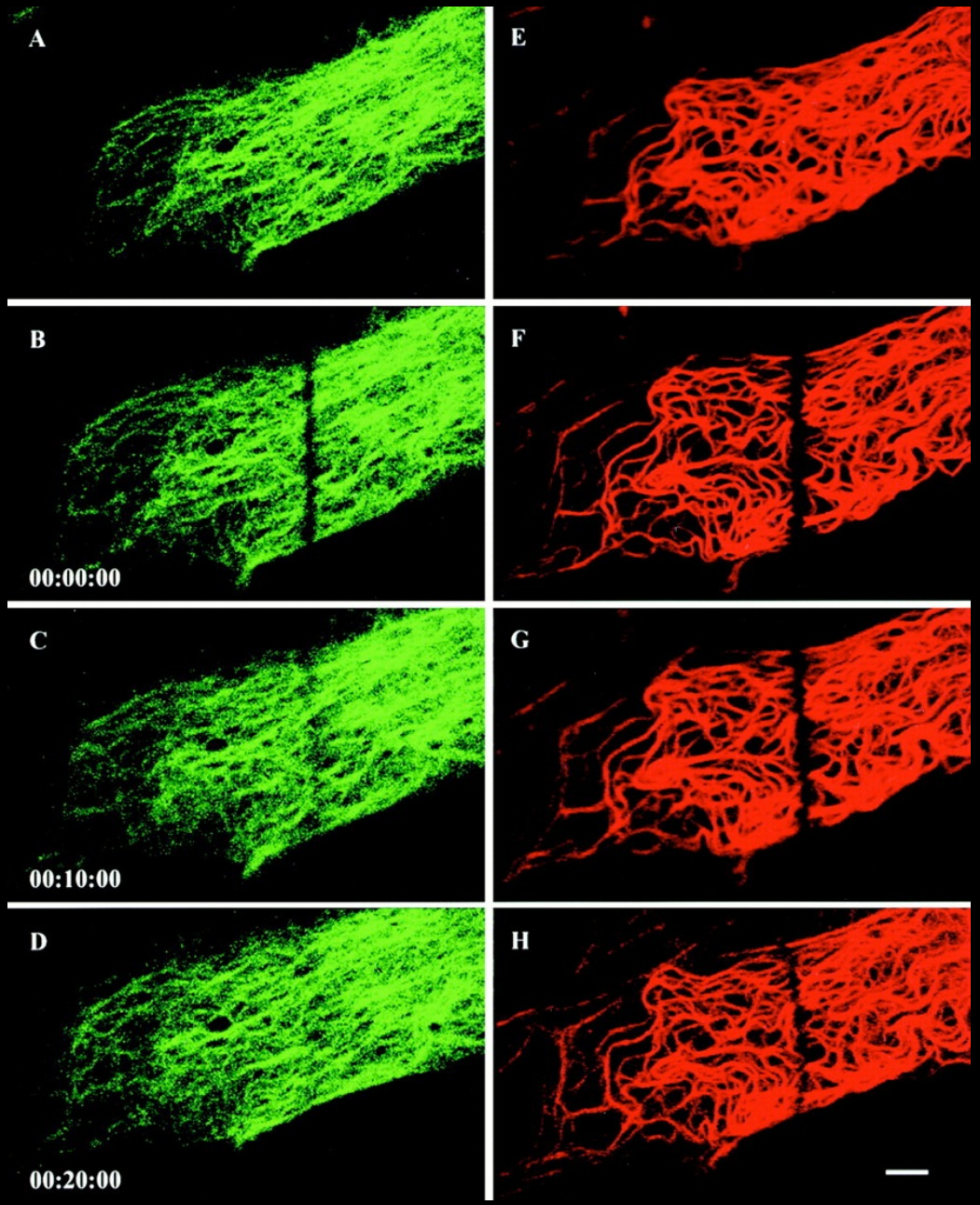
Vimentin

- All Cells in Early Development
- Cage Around Nucleus
- Interacts with Microtubules
- Vimentin Knockout Mouse
  - Initially normal at gross inspection
  - Cultured cells have altered properties of uncertain significance

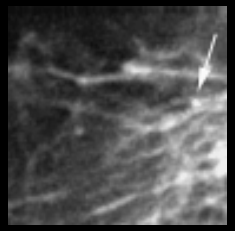
Vimentin: Cage around Nucleus



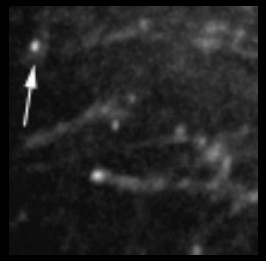
IFs are Dynamic but Relatively Stable: FRAP of Vimentin vs. Keratin in One Cell



Dynamics of IF Particles in Cell Periphery



11 micrometers
over 10 minutes



18 micrometers
over 10 minutes

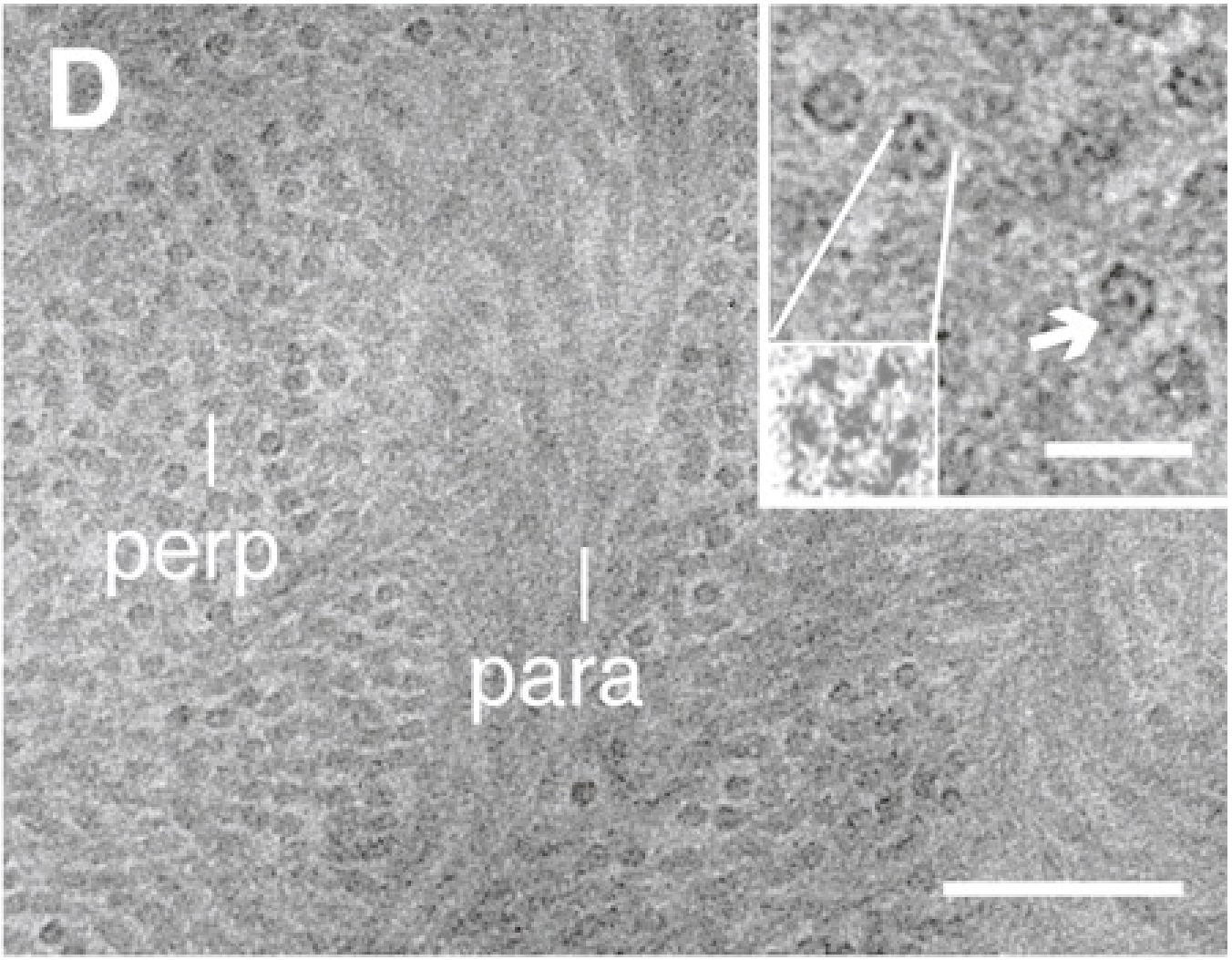
Desmin

- Expressed in Muscle
- Elastic Elements to Prevent Over-stretching
- Connects / Aligns Z lines
- Knockout Mouse - Deranged Myofibril Architecture

Keratins

- Expressed in Epithelia
- Keratin Filaments Connect to Desmosome and Hemidesmosomes
- Differentiation of Epidermis includes Production of Massive Amounts of Keratin
- Provides Outer Protection of Skin
- Composes Hair, Nails, Feathers, etc.

Density of Keratin Filaments in Outer Epidermis Layers

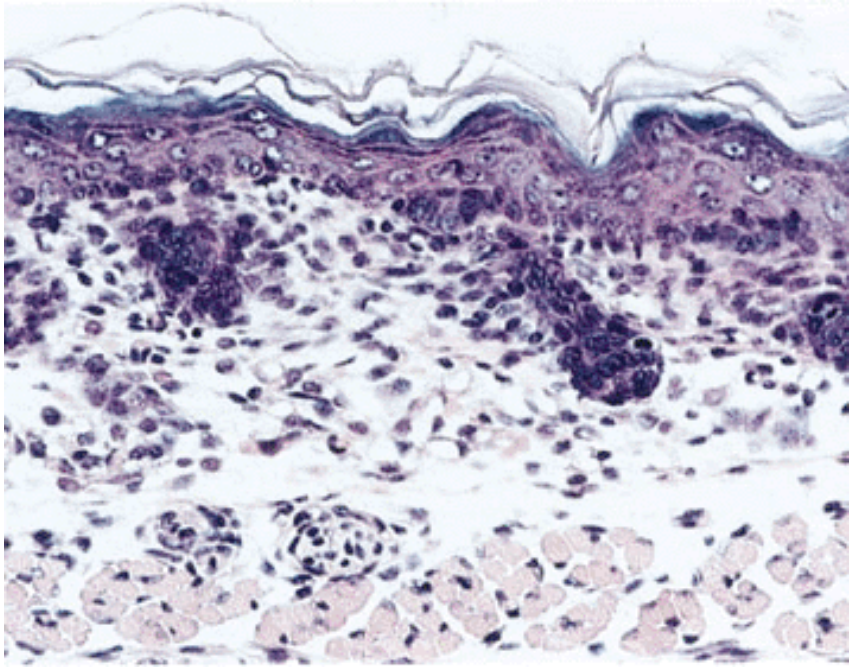


Keratin Mutations are Basis for Human Epidermal Diseases

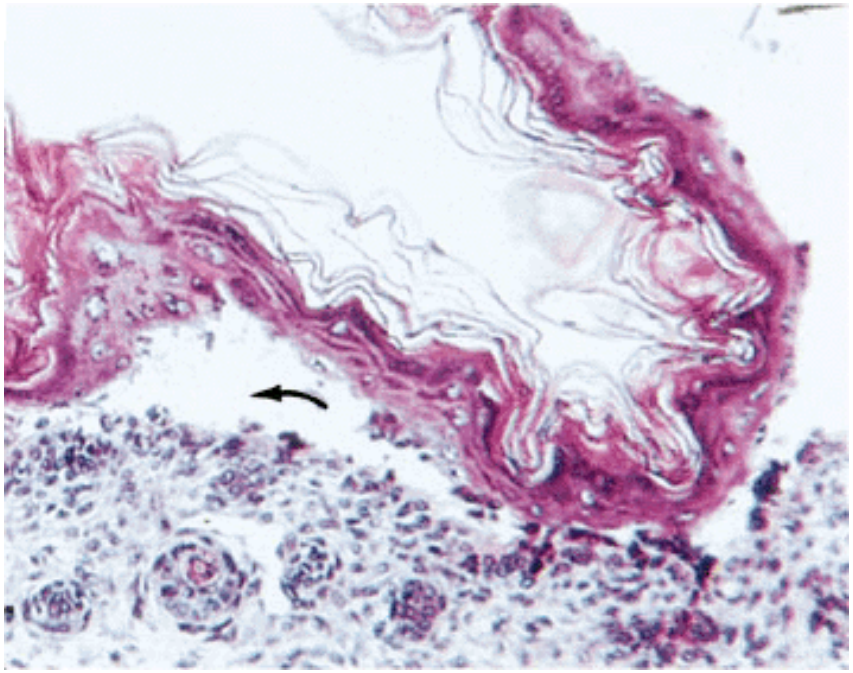
- Structure/Function Analysis of Keratin Assembly
- Point Mutation in Terminal Domain Fails to Assemble
- Mutant is Dominant, even in Low Amounts, in Cultured Cells and Mice

Epidermolysis Bullosa Simplex

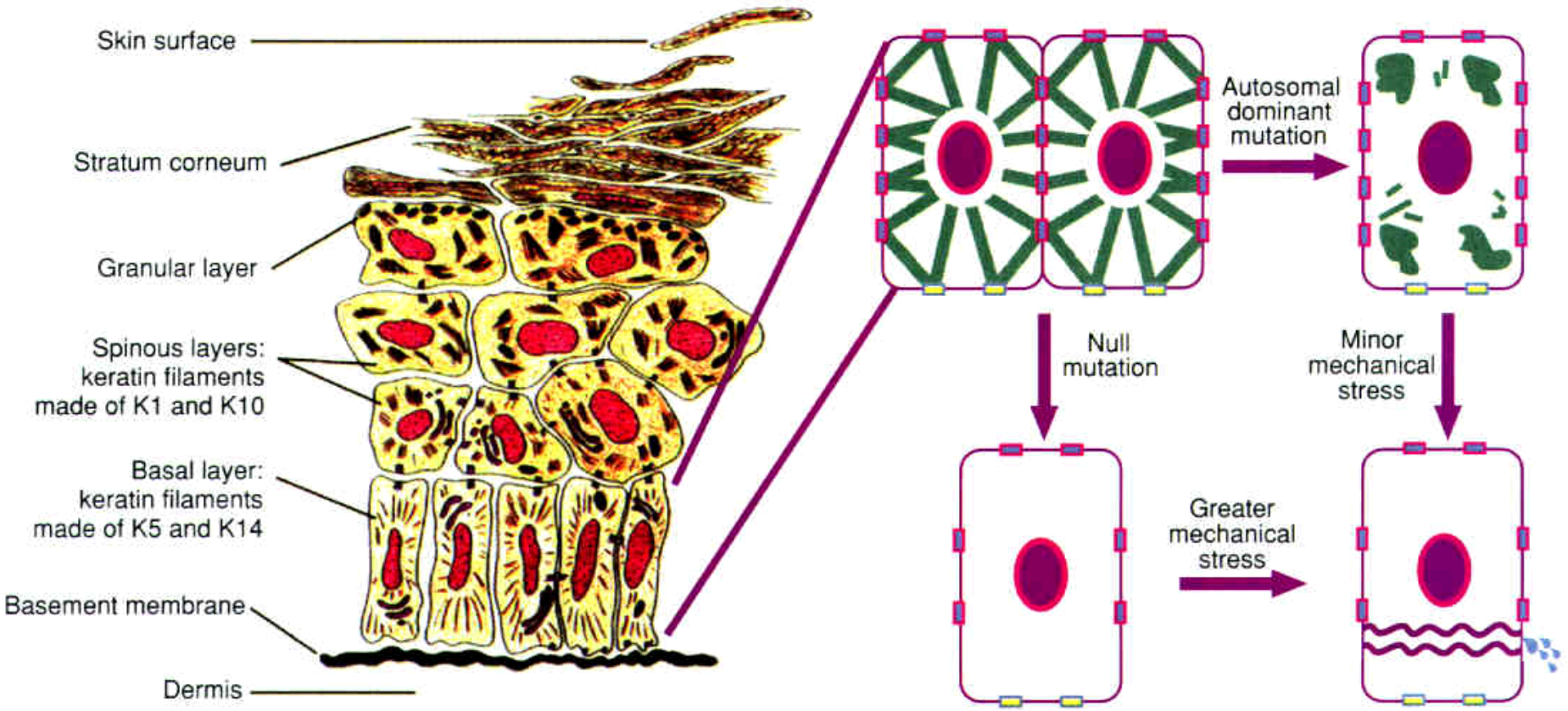
Wild-type



Mutant

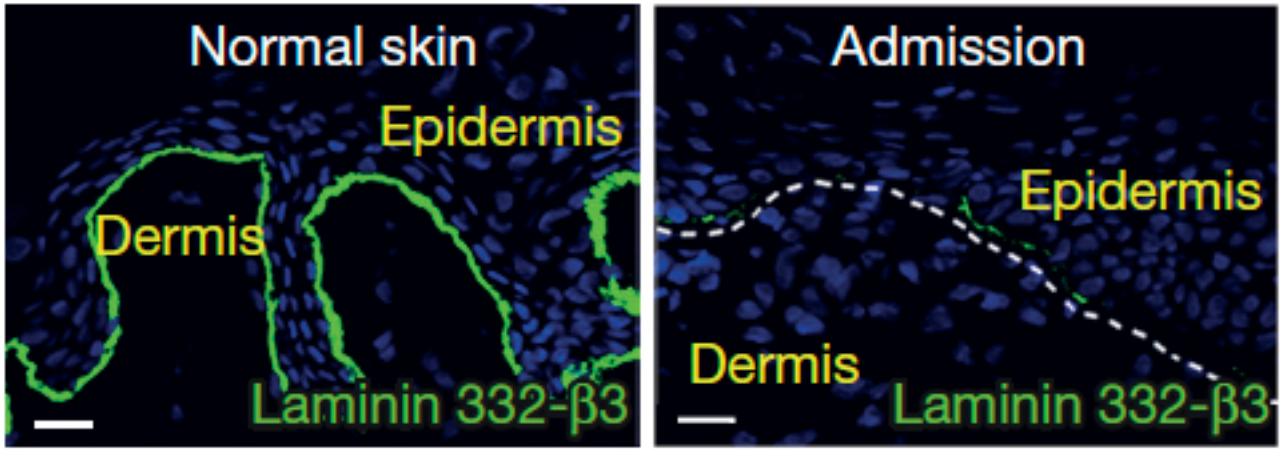


Keratins and EBS



Gene Therapy for Epidermolysis Bullosa

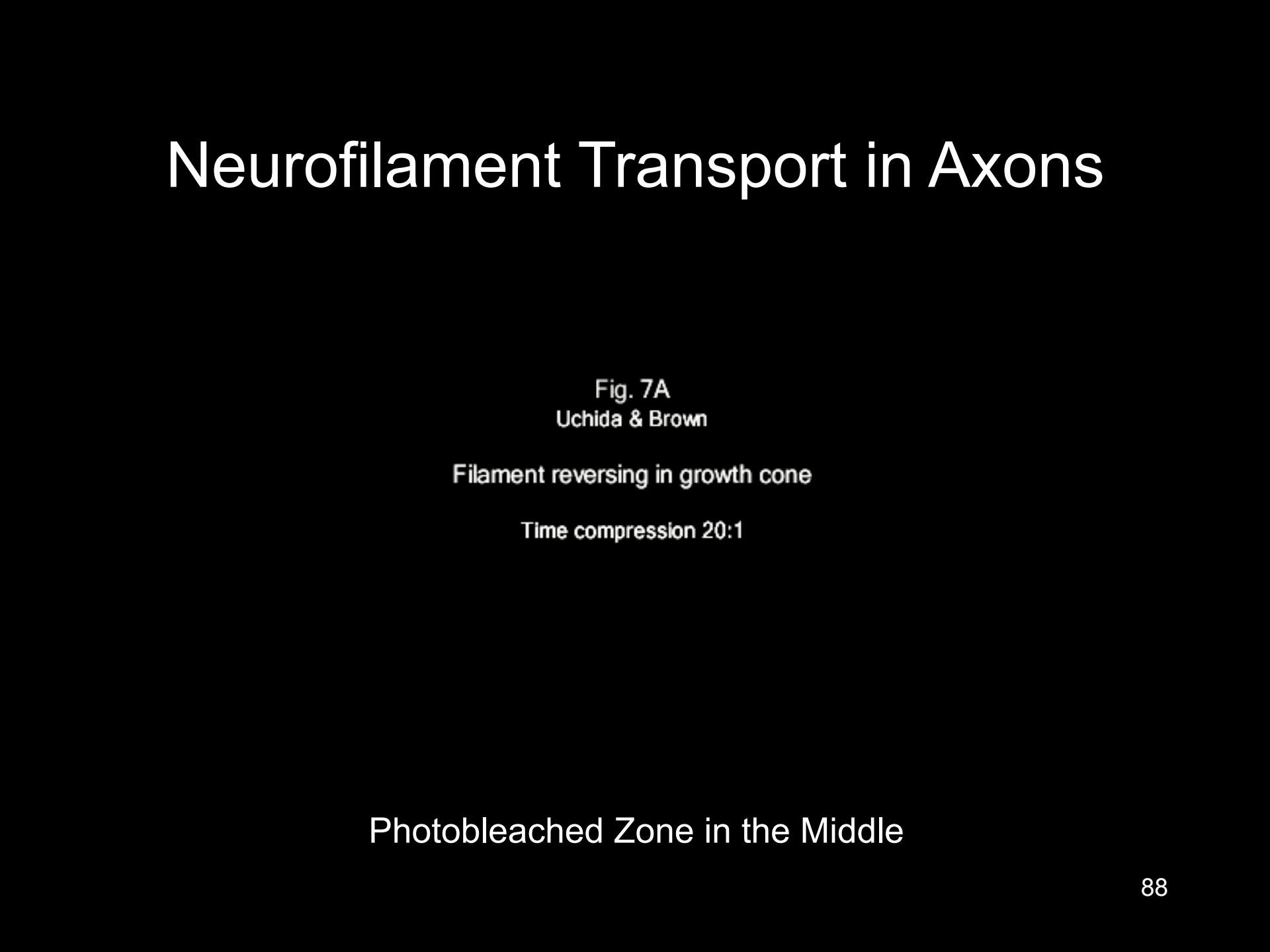
- "Gene Therapy Creates Replacement Skin to Save a Dying Boy." NY Times, 2017 Nov 8.
- Anchors for epidermis to dermis. Recessive mutations: Add wild-type protein.
- Laminins.
  - "Regeneration of the entire human epidermis using transgenic stem cells." www.nature.com/articles/nature24487
- Collagen Type VII.
  - "Safety and Wound Outcomes Following Genetically Corrected Autologous Epidermal Grafts in Patients With Recessive Dystrophic Epidermolysis Bullosa." JAMA. 2016. 316:1808.



Neurons

- Neurofilament H, M, L Copolymer
- Prevent Axon Breakage
- Diseases with Clumps of Neurofilaments
  - Superoxide dismutase model for ALS
  - Clumps are secondary, not causative

Neurofilament Transport in Axons



Photobleached Zone in the Middle

Lamins

- Square Lattice on Inner Surface of Nuclear Membrane
- Present in Metazoans (Animals, not Plants or unicellular organisms)
- Mitosis Breakdown
  - Phosphorylation of A & C by Cyclin-dependent Kinase
  - B remains with Membrane
- Mutations Cause Accelerated Aging Diseases
  - Progerias - Dominant Mutations

Nuclear Lamina

Nuclear Pores

Square Latticework by EM