Biological Frameworks for Engineers

ME 498 / ME 599

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Biological Frameworks for Engineers

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Class Organization

- *Tiny Workhorses* Project

Motor Protein	Grad Student
Actin	Alex
Dynein	Adam
F0F1-ATPase	Brian
Kinesin	Kevin
Myosin II	Grier
Prestin	Evan

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Cell Movement

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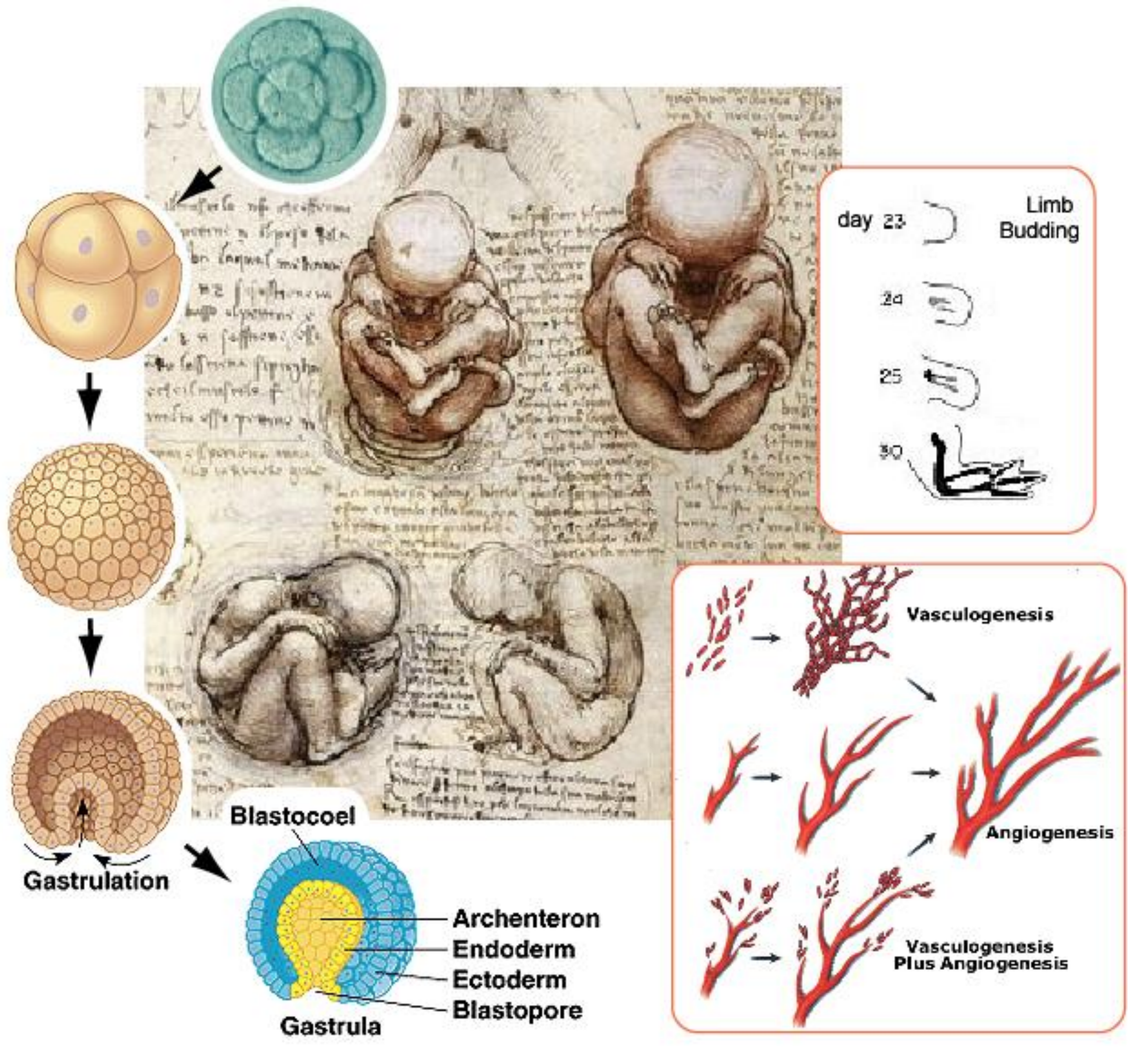
First Movements…



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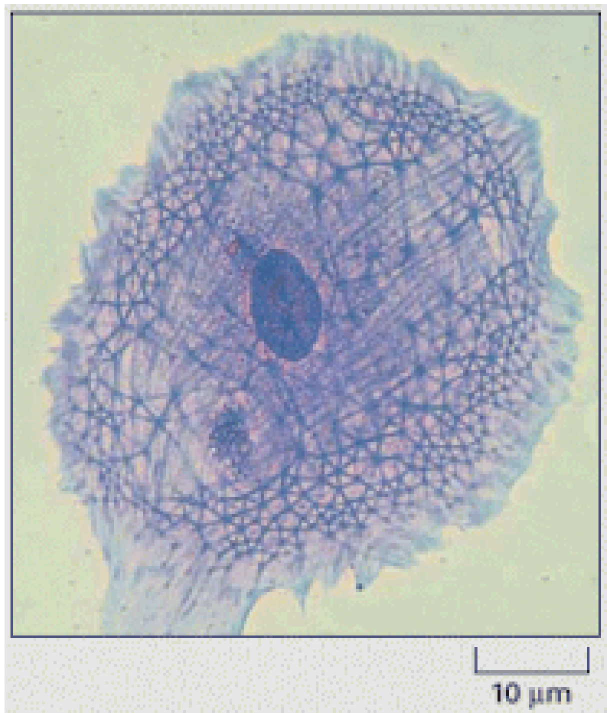
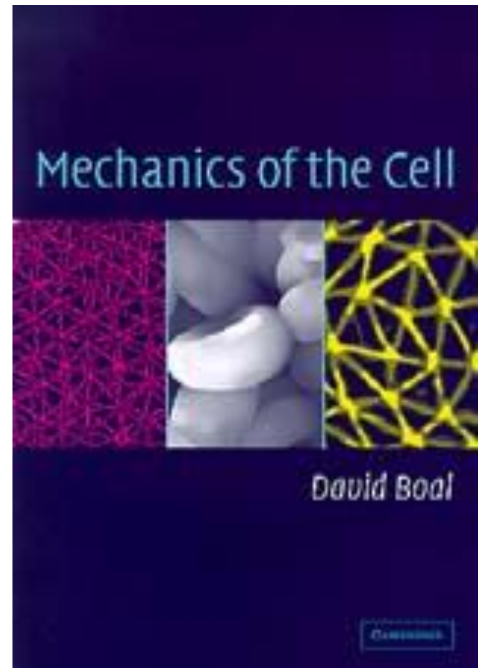
Important Movements

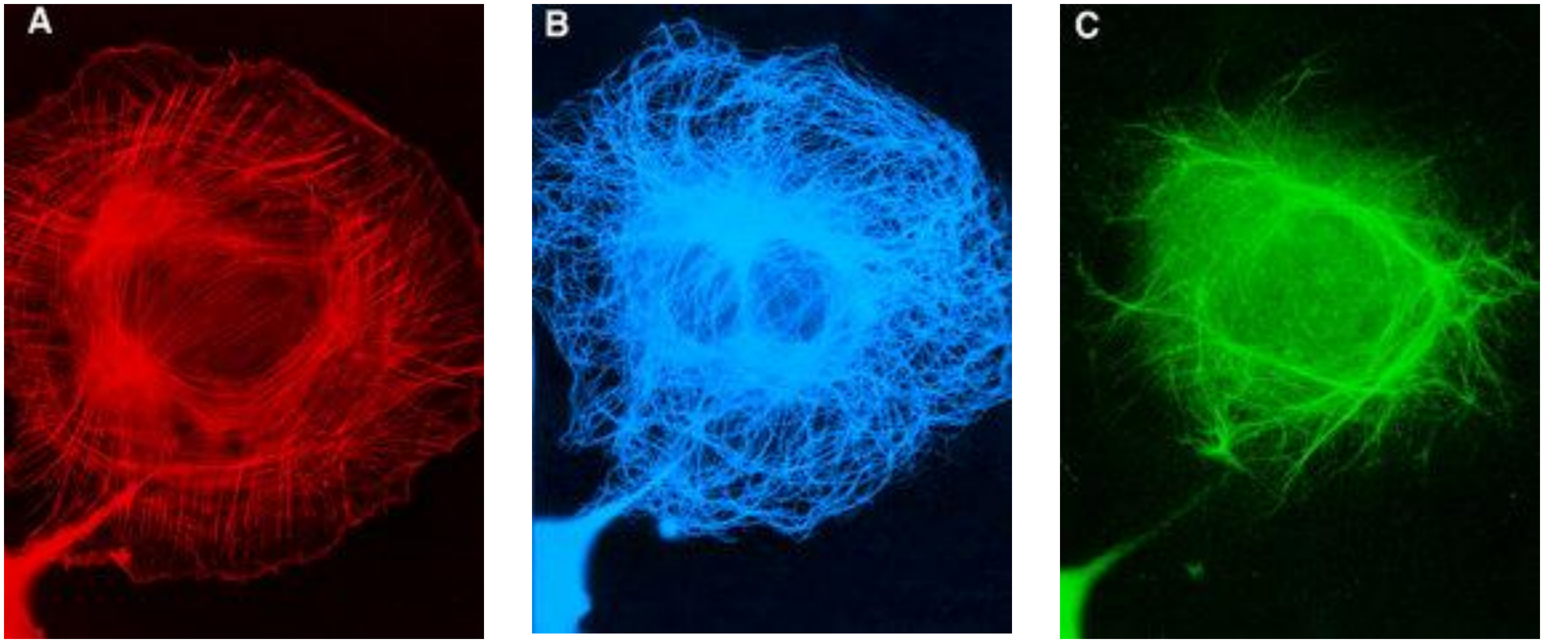
- Development
- Contraction of Cells
- Elongation of Neurons
- Migration of cells
- Flagella and cilia
- Cytokinesis
- Active Intracellular Transport

Cytoskeleton





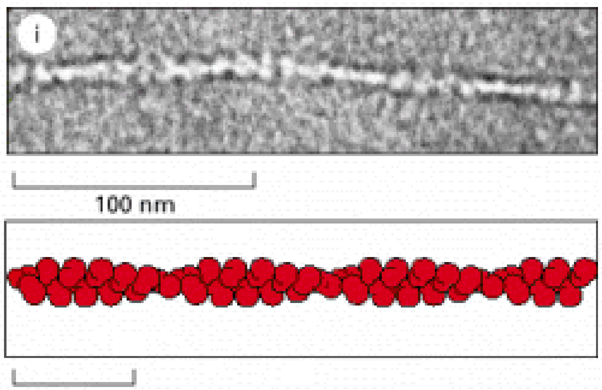
CSK players

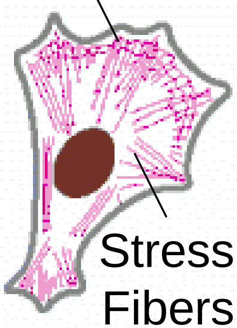


Actin Microtubules (MT) Intermediate Filaments (IF)

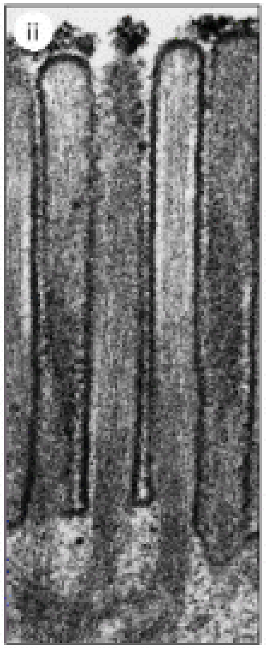
Actin

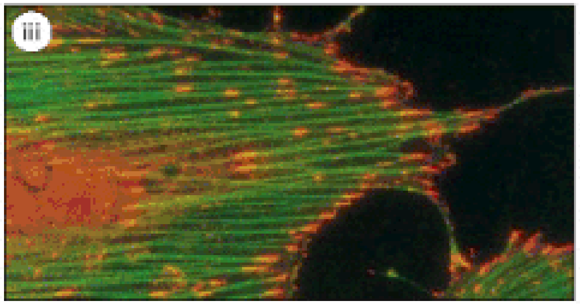
ACTIN FILAMENTS

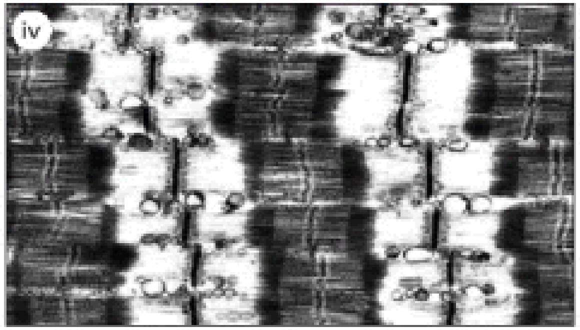




Stress Fibers &
Focal Adhesions







Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

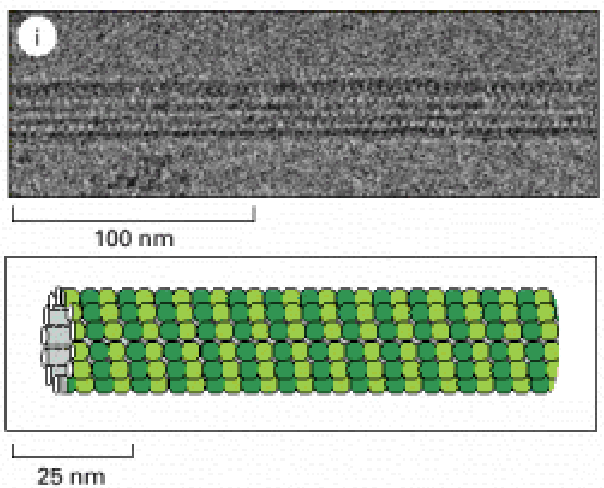
Micrographs courtesy of Roger Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); Keith Burridge (iii).

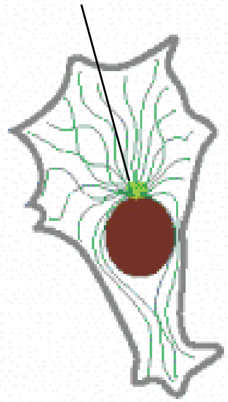
Microvilli

Myofibrils

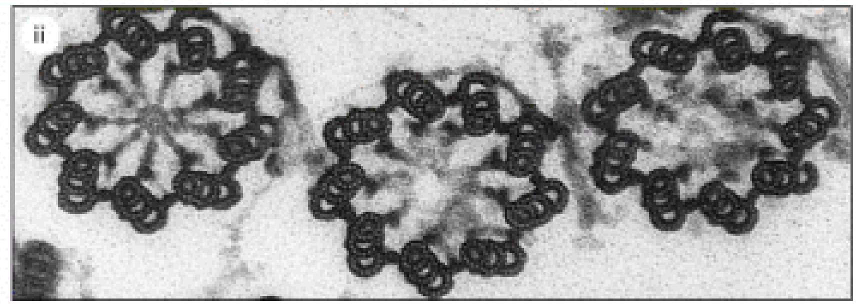
Microtubules

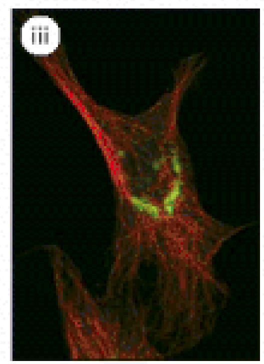
MICROTUBULES

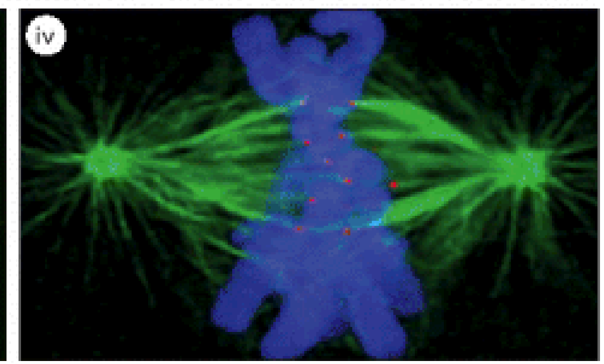




Cilia







Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (iii); A. Desai (iv)

Star-Like

Mitotic Spindle

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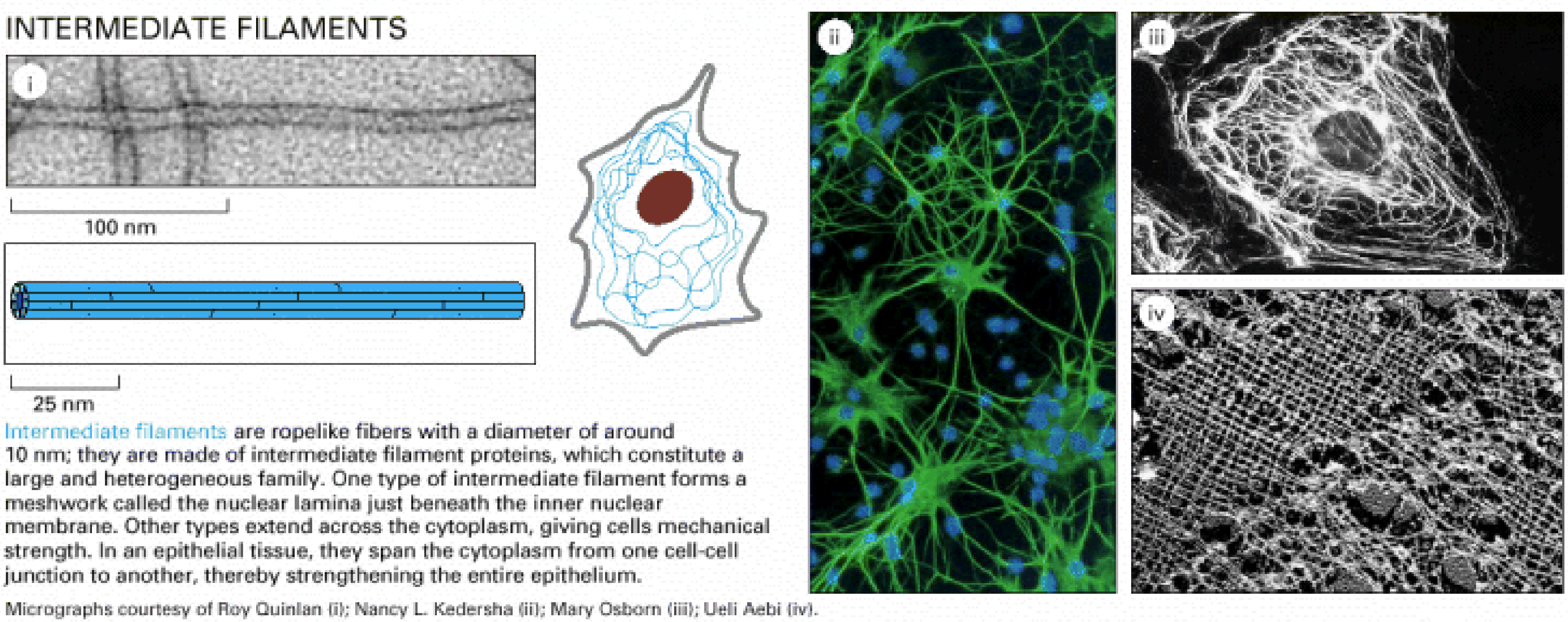
Intermediate Filaments

Strength



Axons Nuclear Lamina

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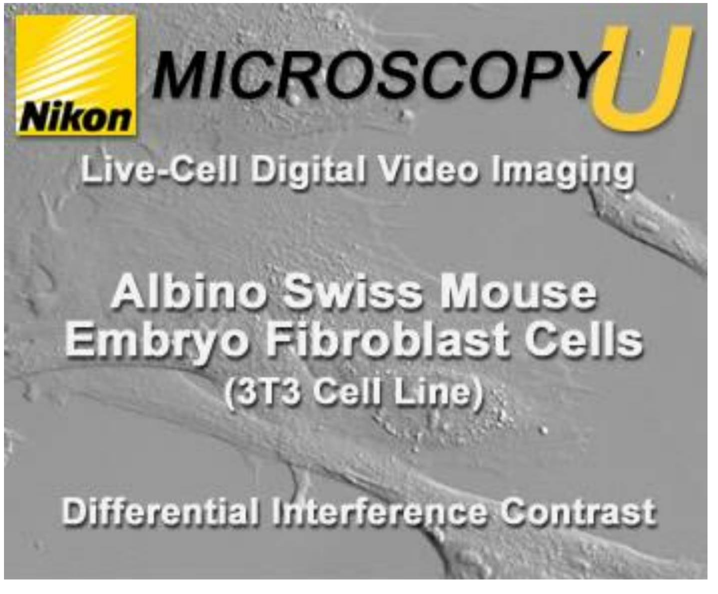
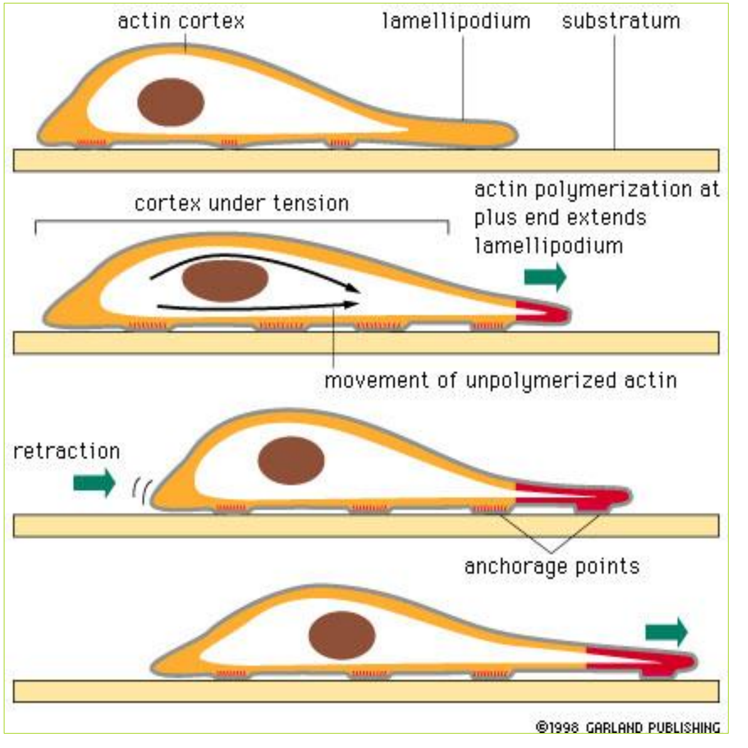
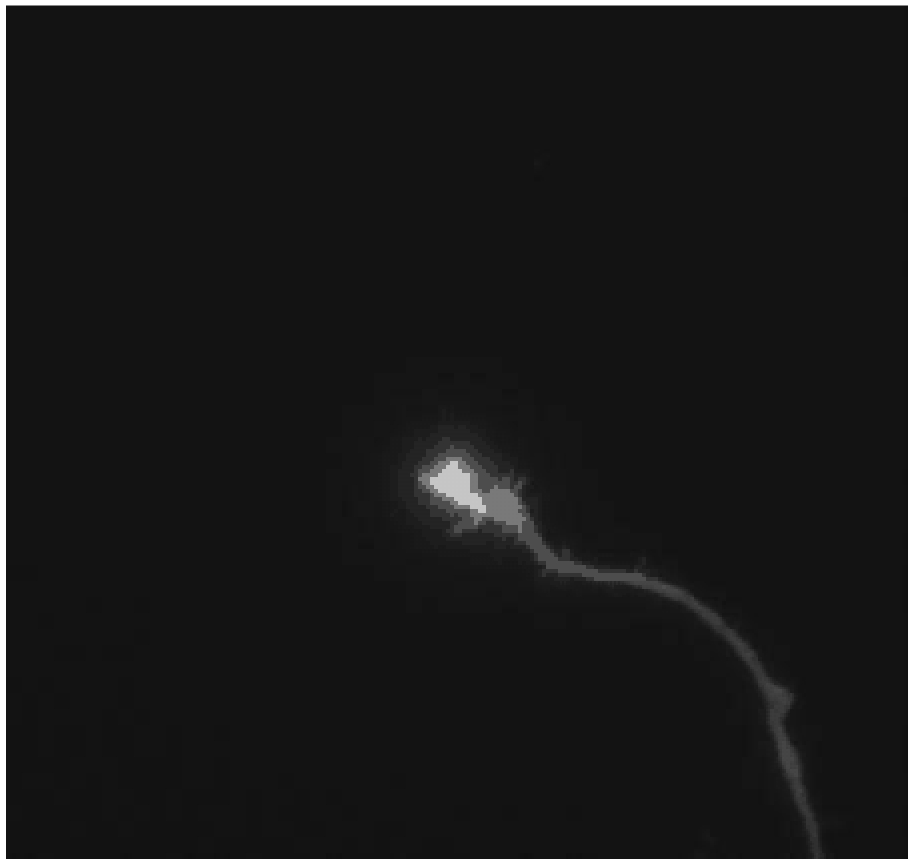
Mechanism of Movement

Polarization… Protrusion… Traction… Retraction…

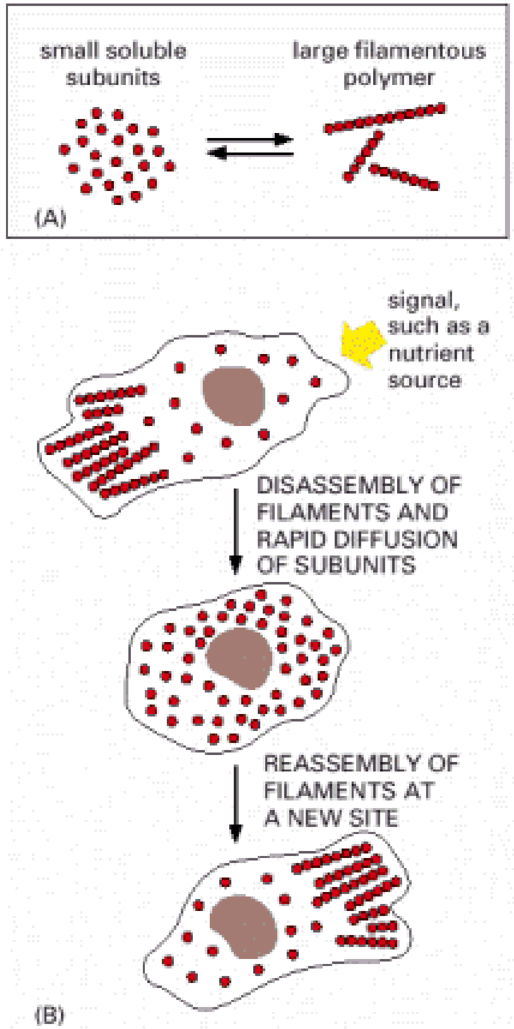




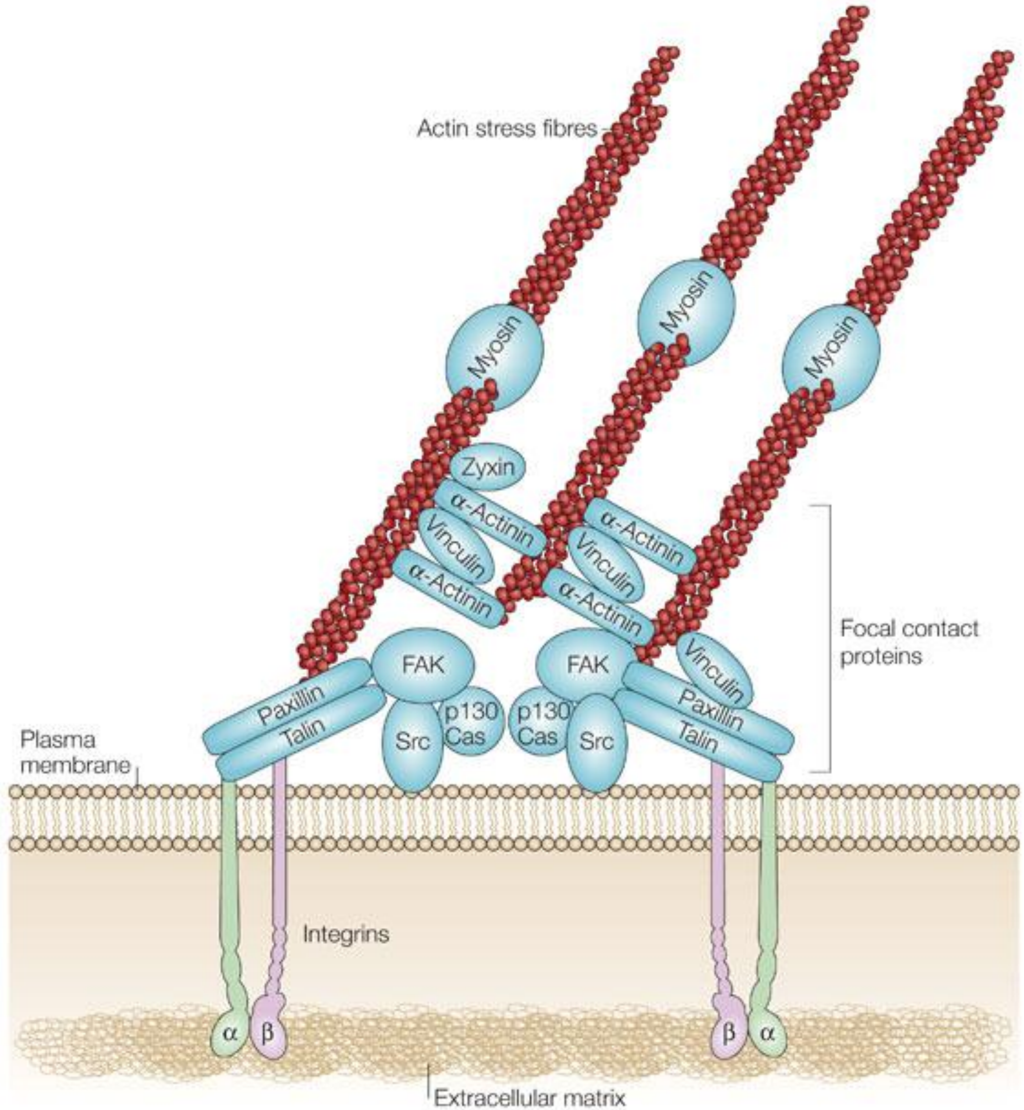
Axon Movement



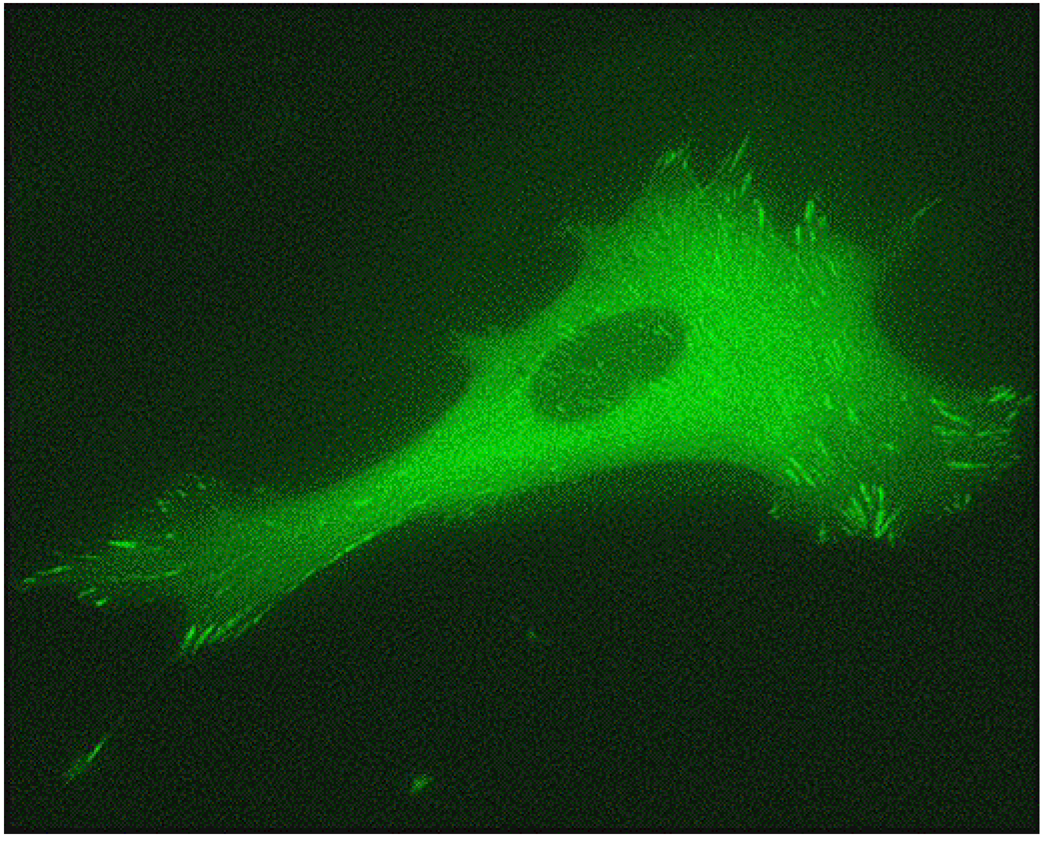
Polarization



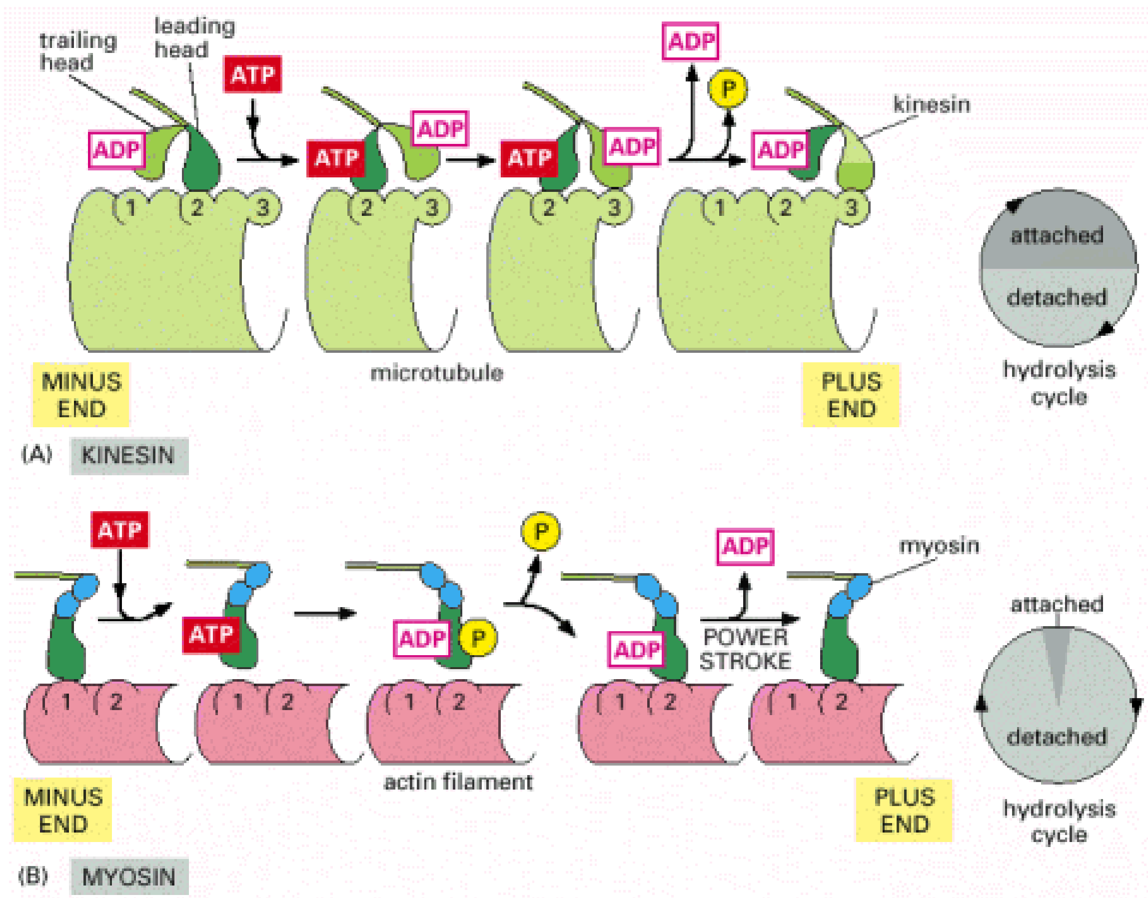
Focal Adhesions



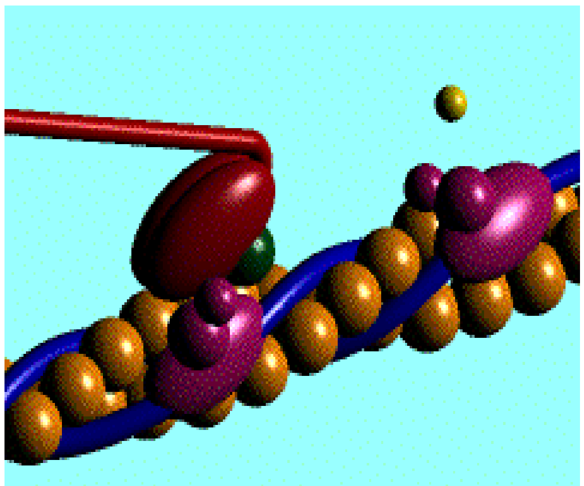
Focal Adhesions

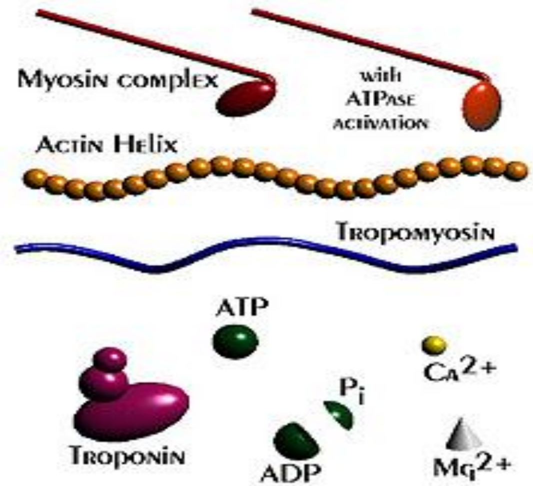


Motor Proteins

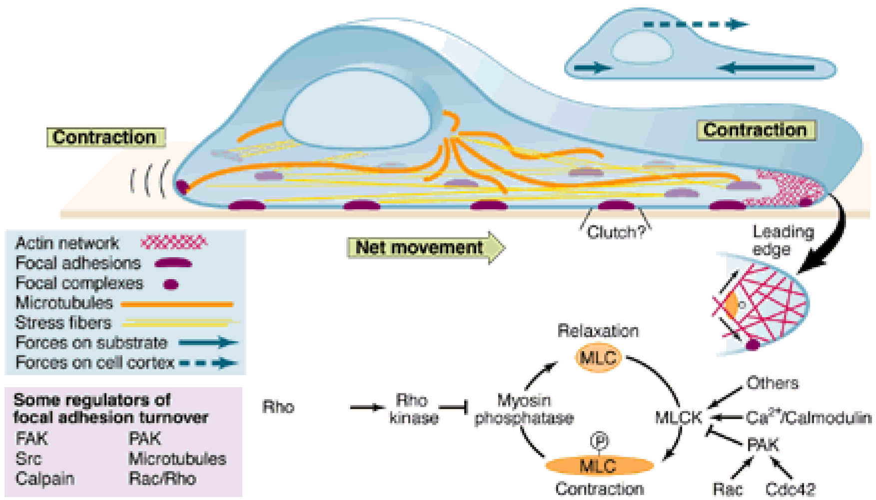


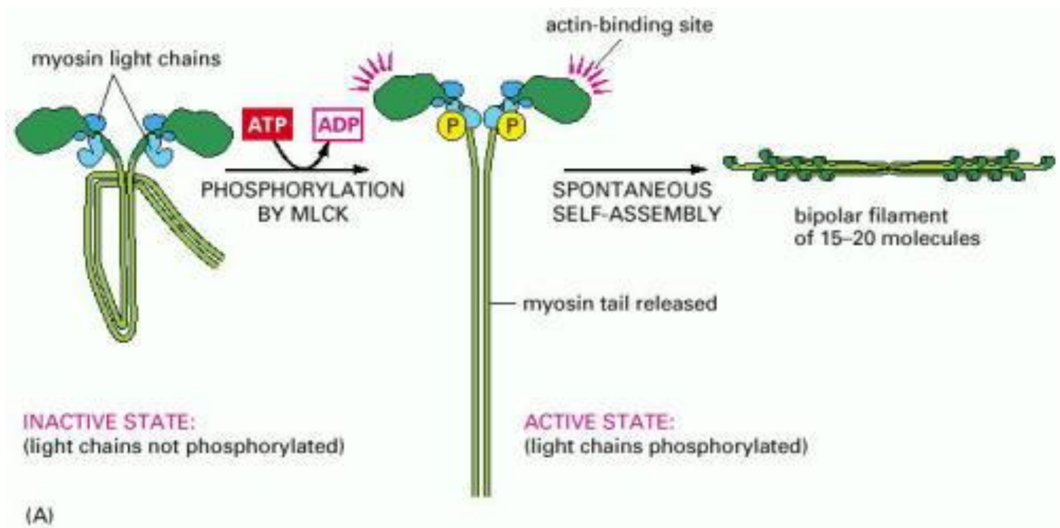
Motor Proteins



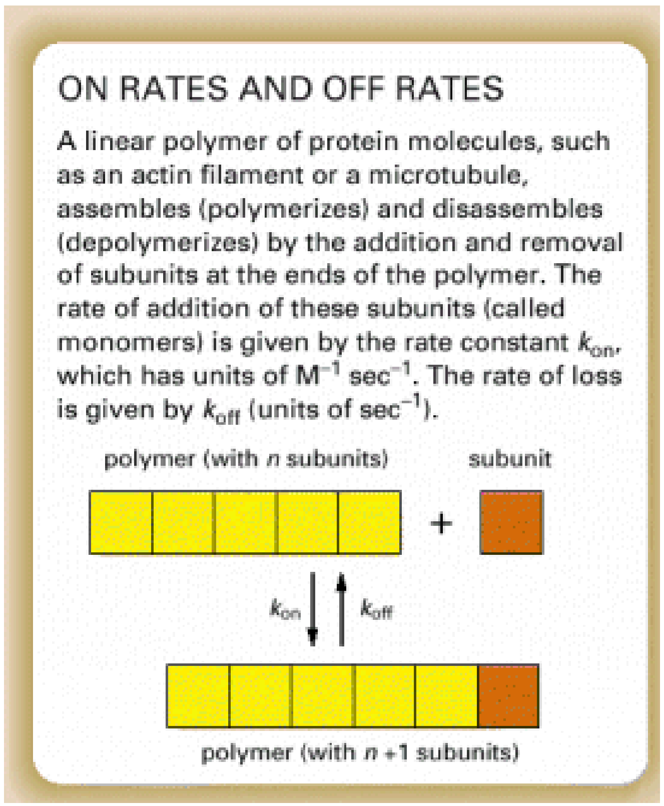


Activation


Contraction
Contraction
Actin network
Focal adhesions
Focal complexes
Microtubules
Stress fibers
Forces on substrate
Forces on cell cortex
Net movement
Clutch?
Leading edge
Some regulators of focal adhesion turnover
FAK
Src
Calpain
PAK
Microtubules
Rac/Rho
Rho
Rho kinase
Myosin phosphatase
Relaxation
MLC
MLC
Contraction
MLCK
Others
Ca2+/Calmodulin
PAK
Rac
Cdc42

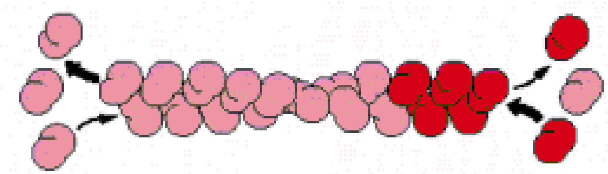

myosin light chains
actin-binding site
ATP
ADP
PHOSPHORYLATION BY MLCK
SPONTANEOUS SELF-ASSEMBLY
bipolar filament of 15–20 molecules
myosin tail released
INACTIVE STATE: (light chains not phosphorylated)
ACTIVE STATE: (light chains phosphorylated)
(A)

Assembly / Disassembly


ON RATES AND OFF RATES

A linear polymer of protein molecules, such as an actin filament or a microtubule, assembles (polymerizes) and disassembles (depolymerizes) by the addition and removal of subunits at the ends of the polymer. The rate of addition of these subunits (called monomers) is given by the rate constant k_{on}, which has units of M^{-1} sec^{-1}. The rate of loss is given by k_{off} (units of sec^{-1}).

M = mol / L



<u>Critical Concentration</u>

C = conc. of free subunits
# Added to filament = k_{on} * C
# Removed to filament = k_{off}

As filament grows, C drops until it reaches a critical value, C_c (critical concentration). At this equilibrium:

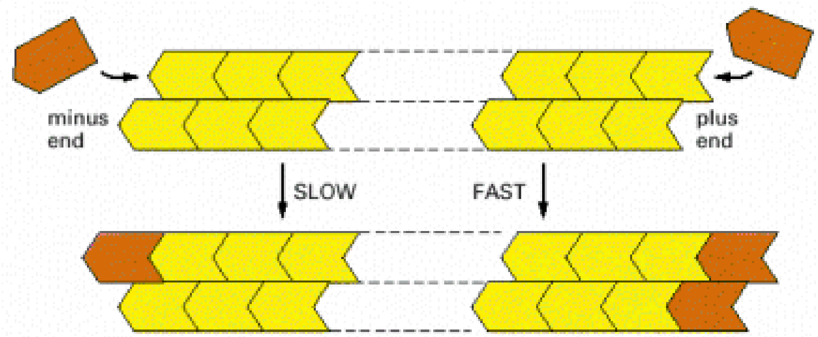
$$k_{on} * C = k_{off}$$

→ $Cc = k_{off} / k_{on}$

Assembly / Disassembly

PLUS AND MINUS ENDS

The two ends of an actin filament or microtubule polymerize at different rates. The fast-growing end is called the plus end, whereas the slow-growing end is called the minus end. The difference in the rates of growth at the two ends is made possible by changes in the conformation of each subunit as it enters the polymer.



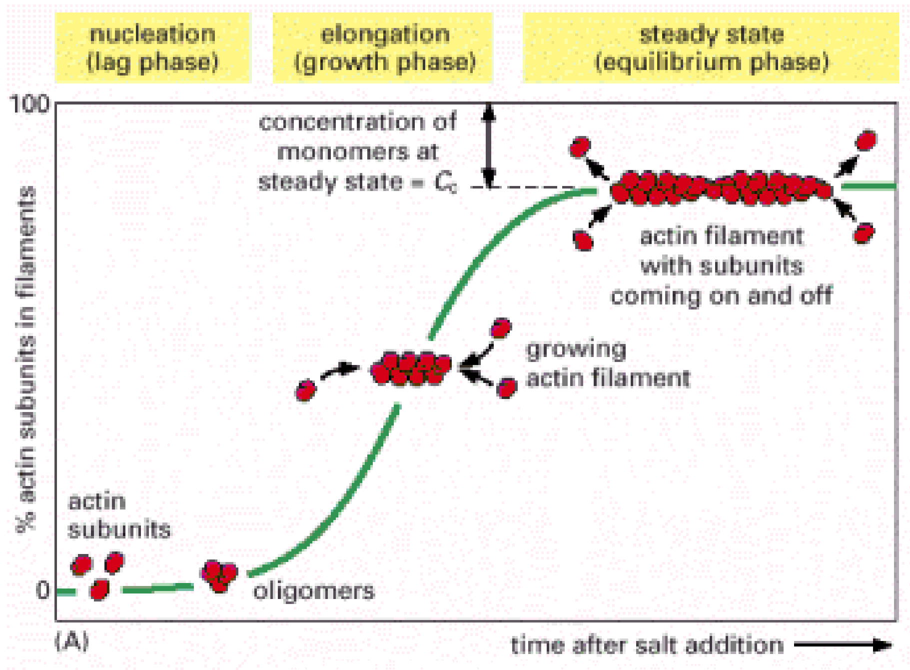
This conformational change affects the rates at which subunits add to the two ends.

Even though k_{on} and k_{off} will have different values for the plus and minus ends of the polymer, their ratio k_{off}/k_{on}—and hence C_c—must be the same at both ends for a simple polymerization reaction (no ATP or GTP hydrolysis). This is because exactly the same subunit interactions are broken when a subunit is lost at either end, and the final state of the subunit after dissociation is identical. Therefore, the ΔG for subunit loss, which determines the equilibrium constant for its association with the end, is identical at both ends: if the plus end grows four times faster than the minus end, it must also shrink four times faster. Thus, for $C > C_c$, both ends grow; for $C < C_c$, both ends shrink.

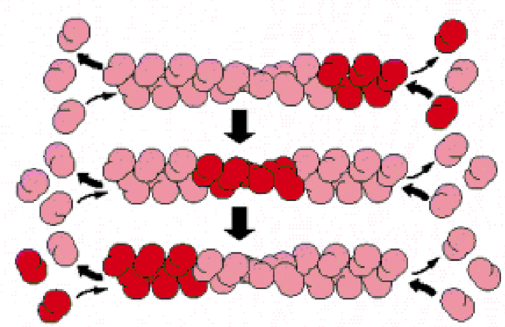
The nucleoside triphosphate hydrolysis that accompanies actin and tubulin polymerization removes this constraint.

Assembly / Disassembly

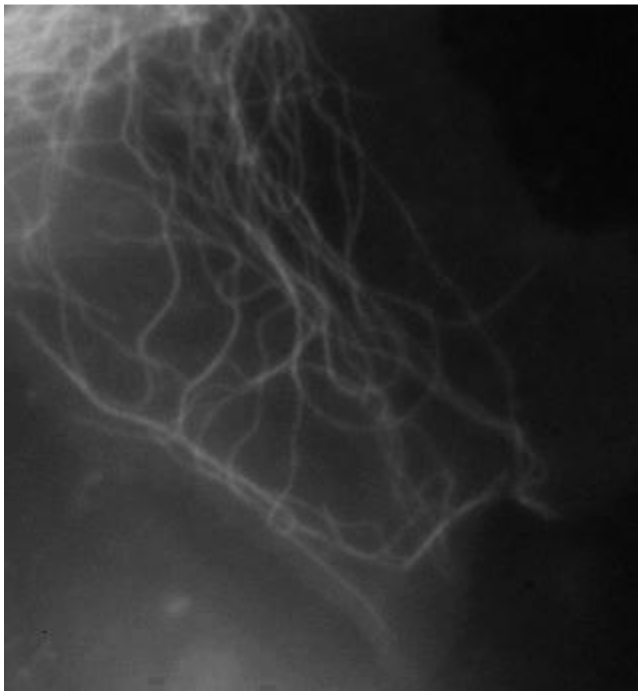
Polymerization

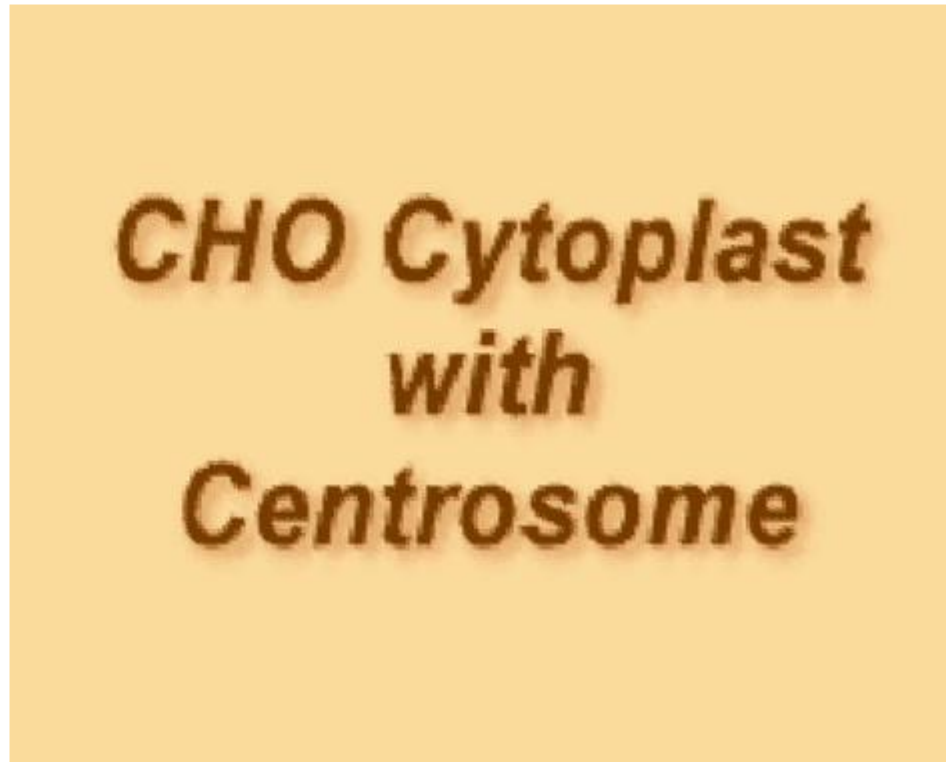


Treadmilling

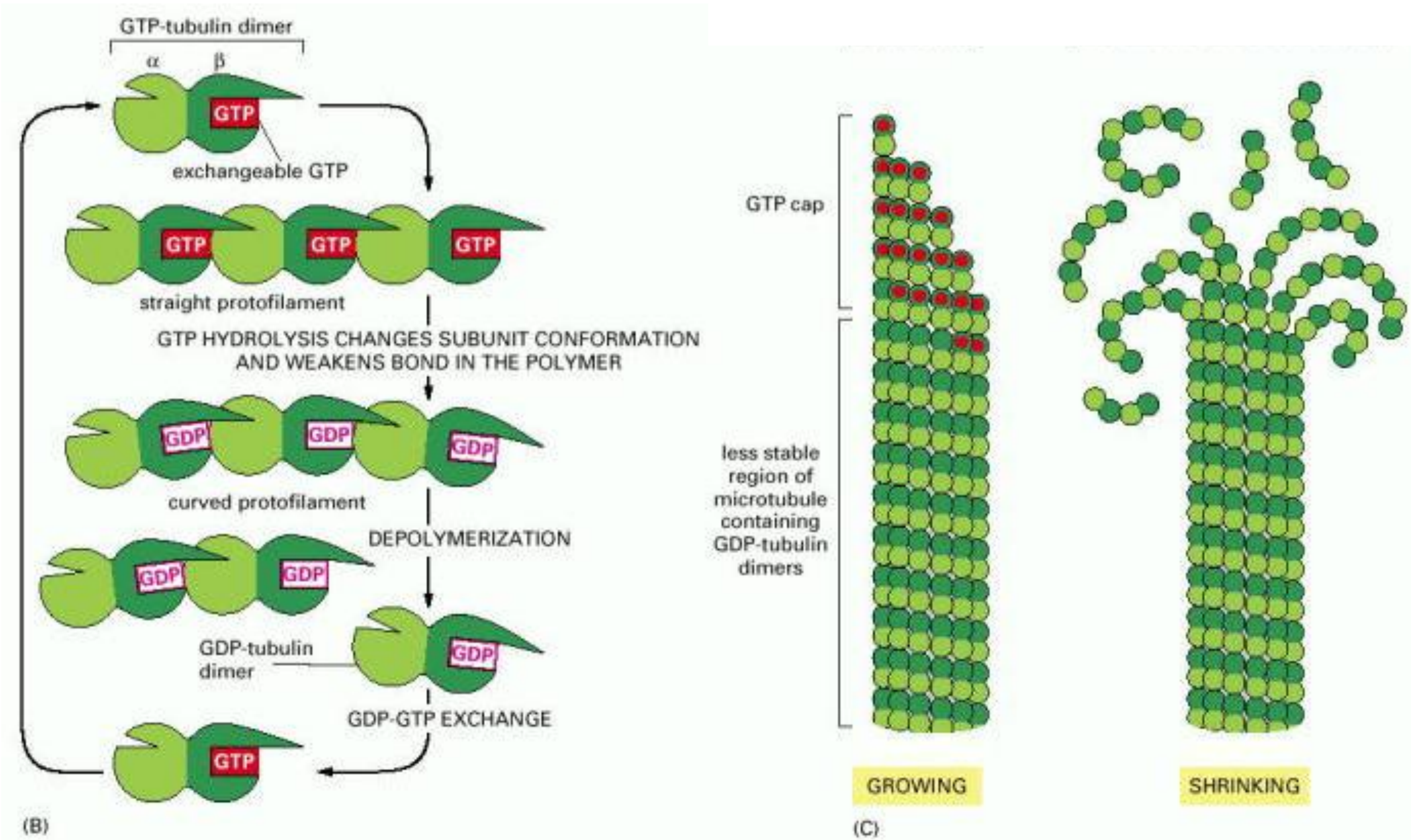


MT Dynamic Instability

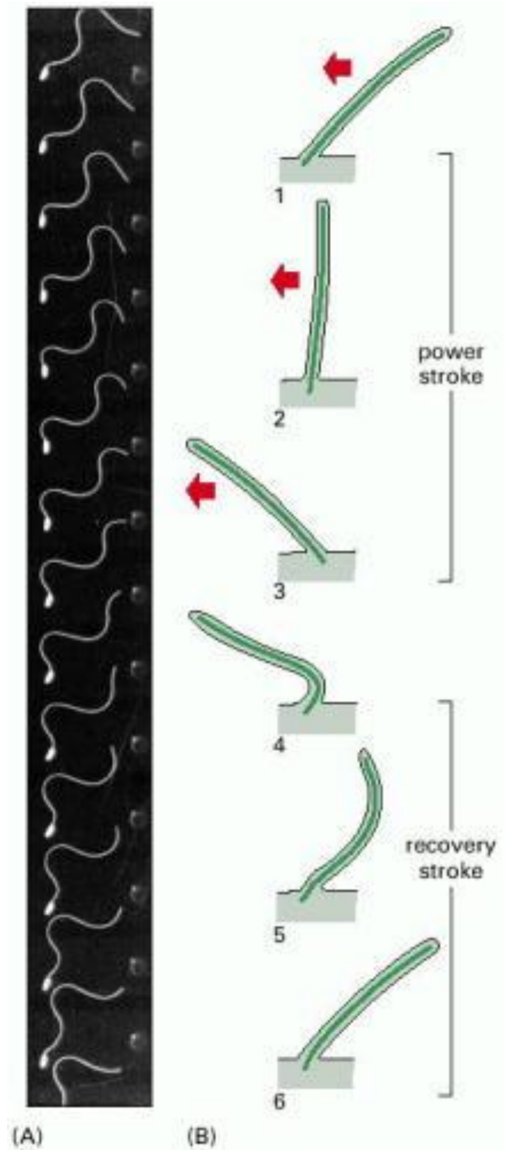




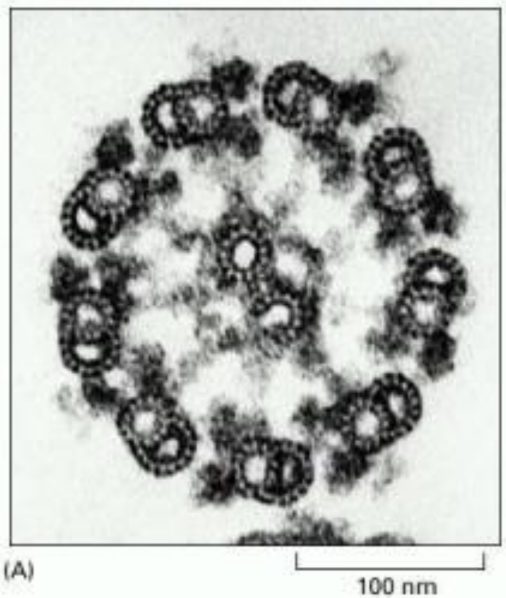
MT Instability

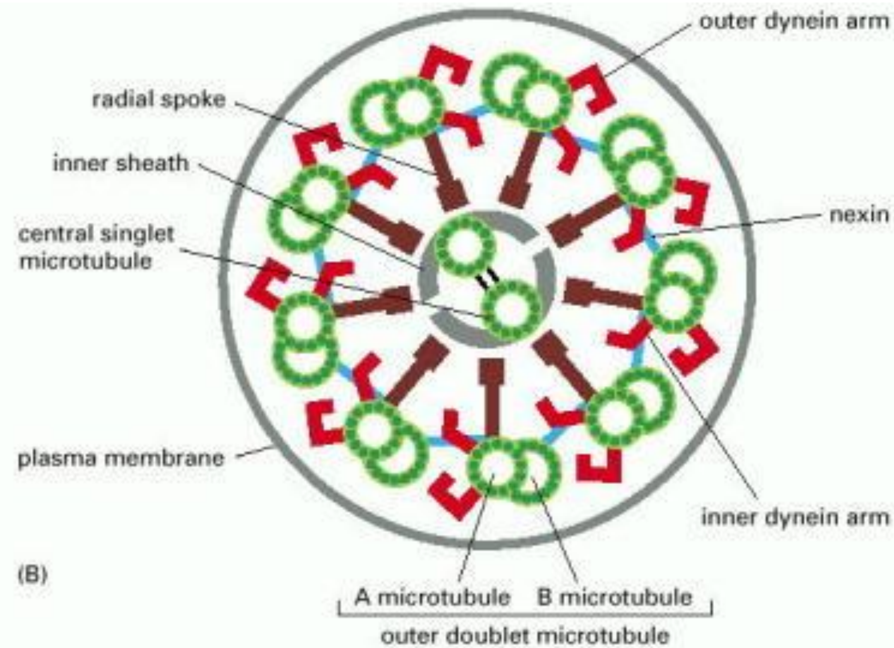


Flagella & Cilia

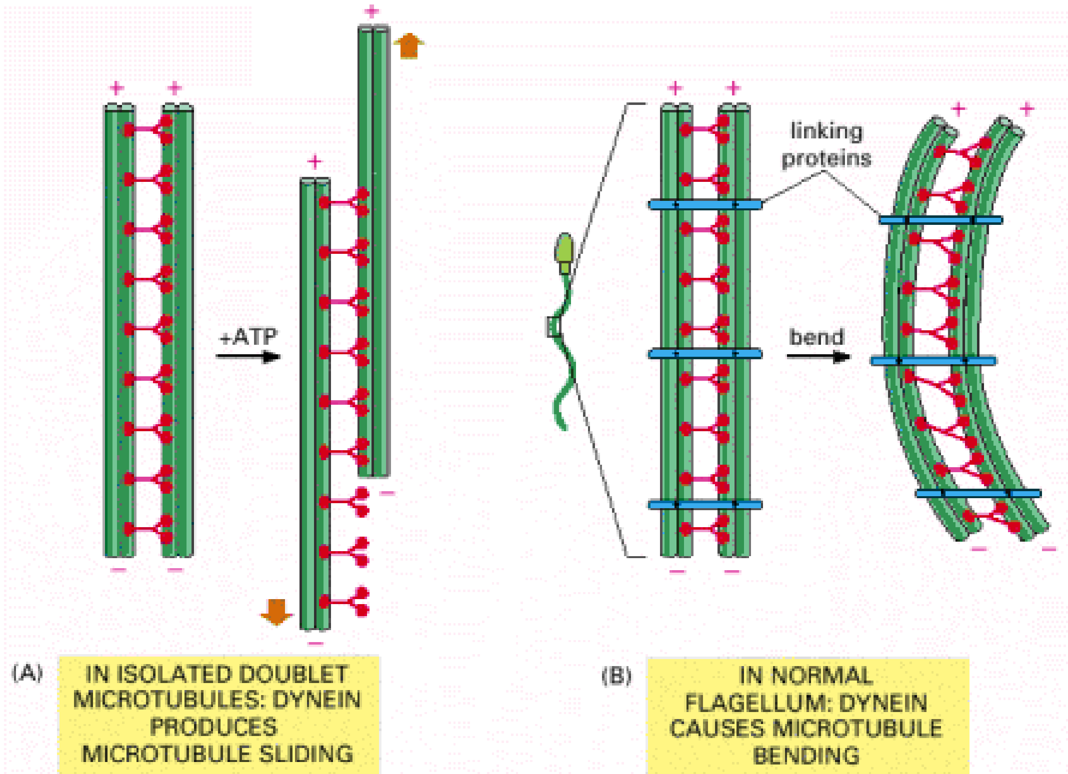


Axoneme

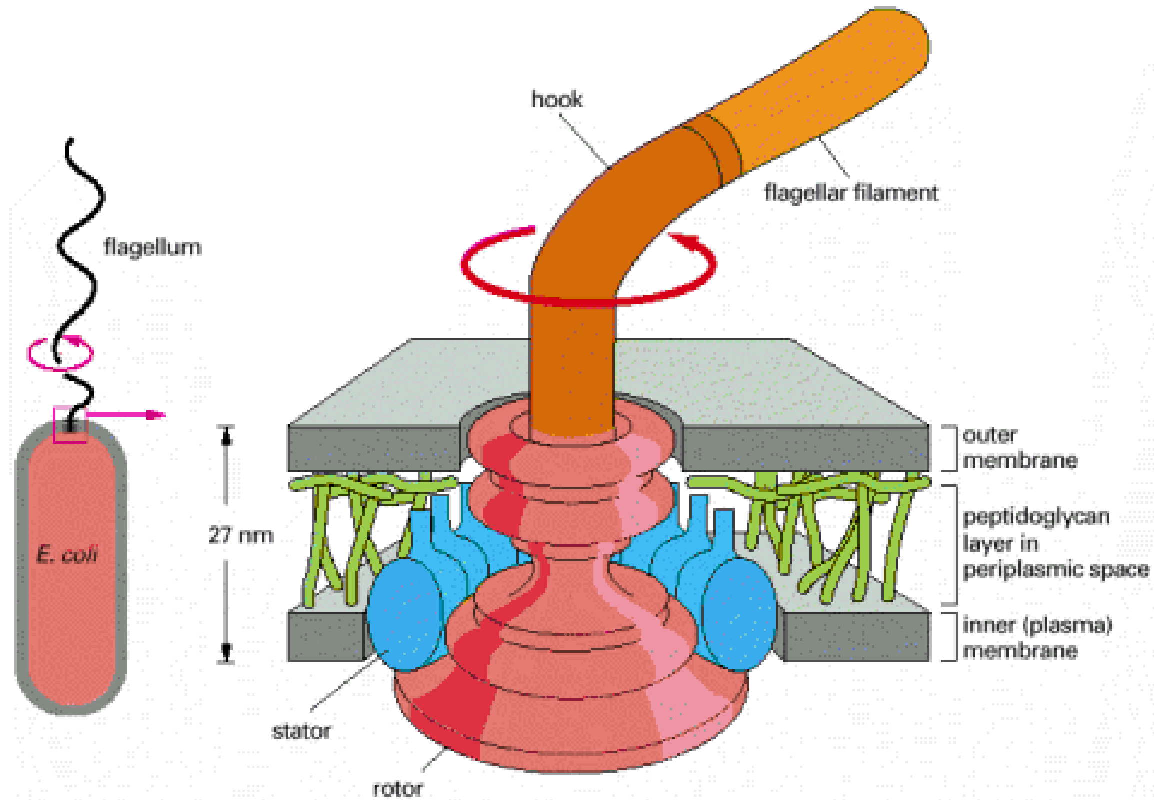

(A)
100 nm


outer dynein arm
radial spoke
inner sheath
central singlet microtubule
nexin
plasma membrane
inner dynein arm
(B)
A microtubule
B microtubule
outer doublet microtubule

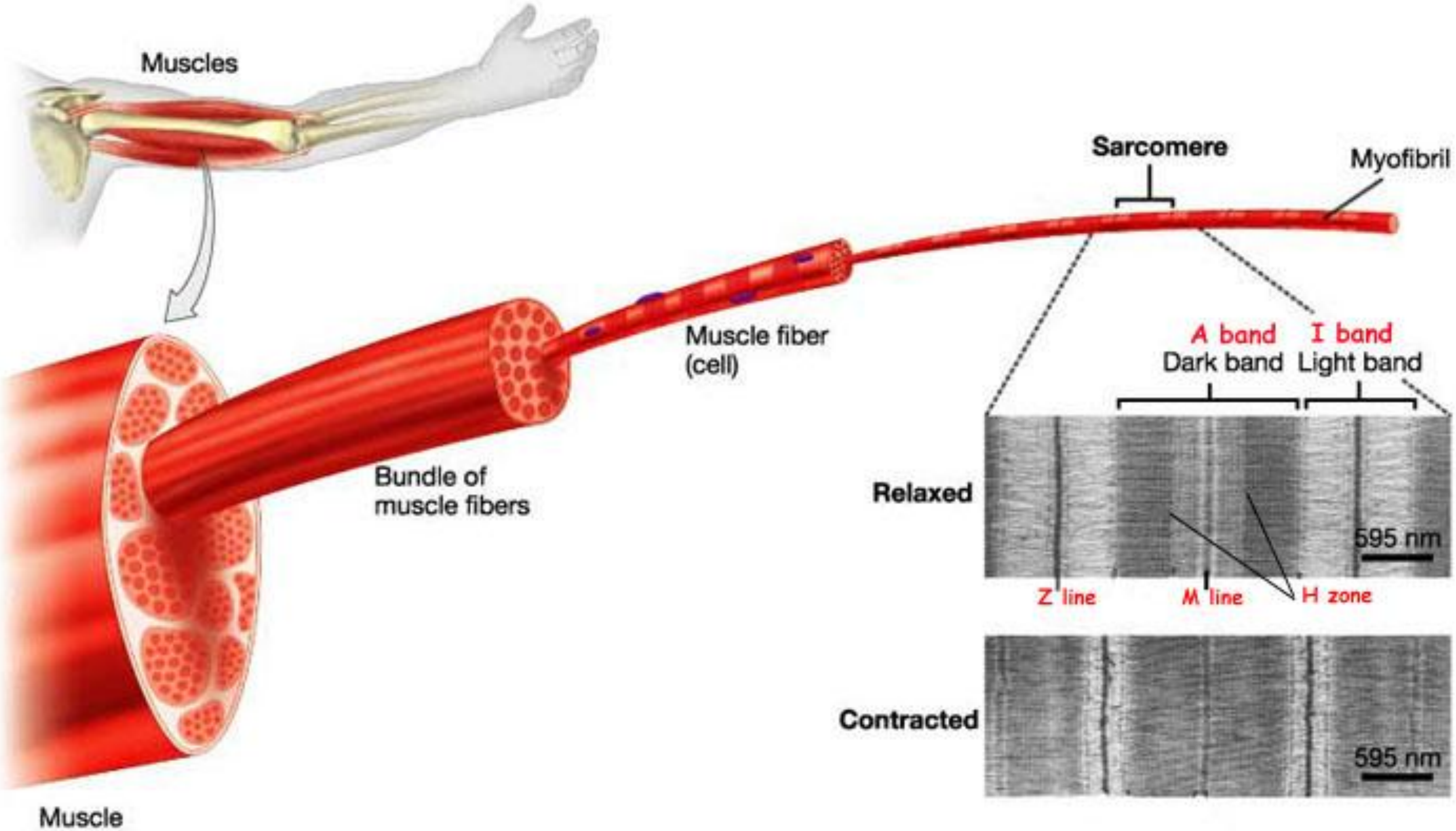
Dynein Bending



Bacteria Flagellar



Muscle Cells



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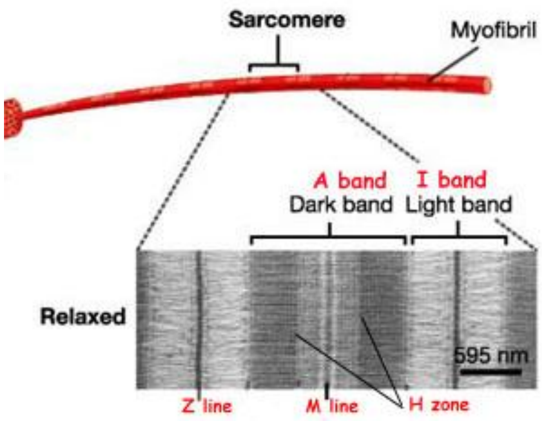
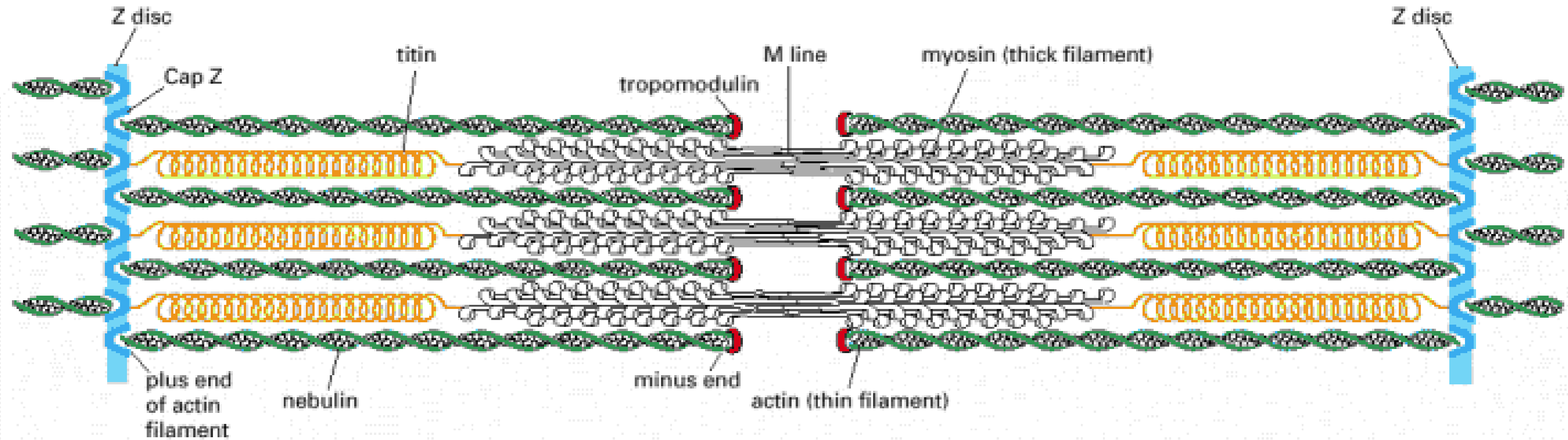
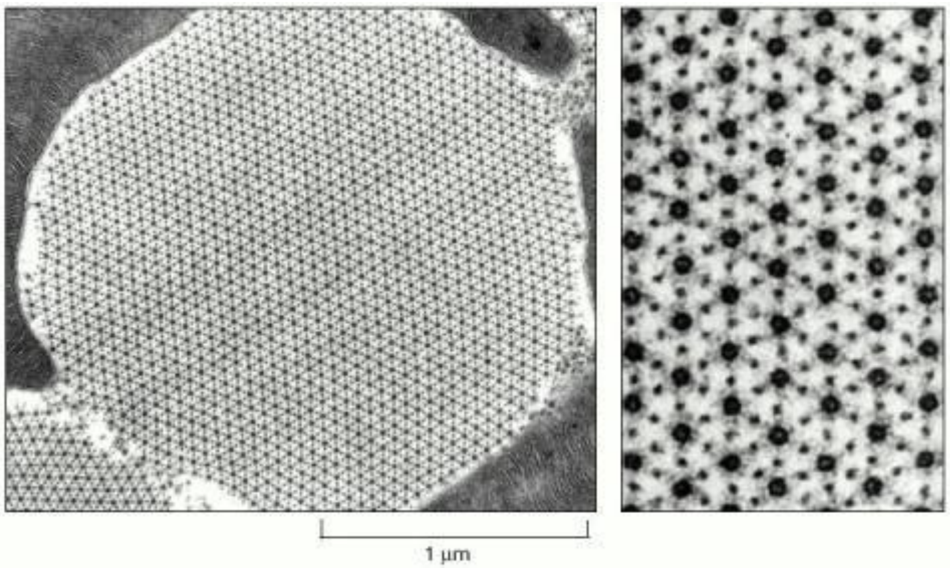
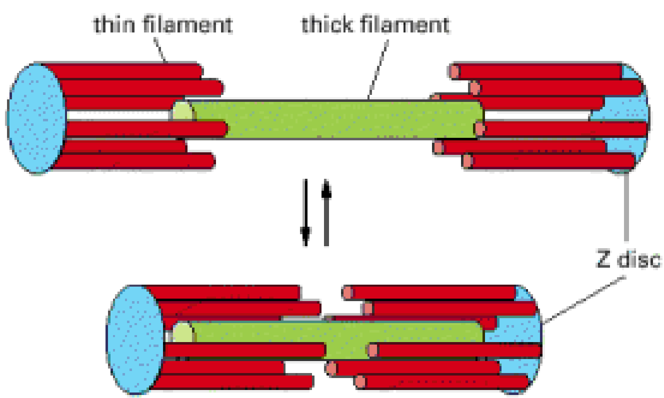
Sarcomere

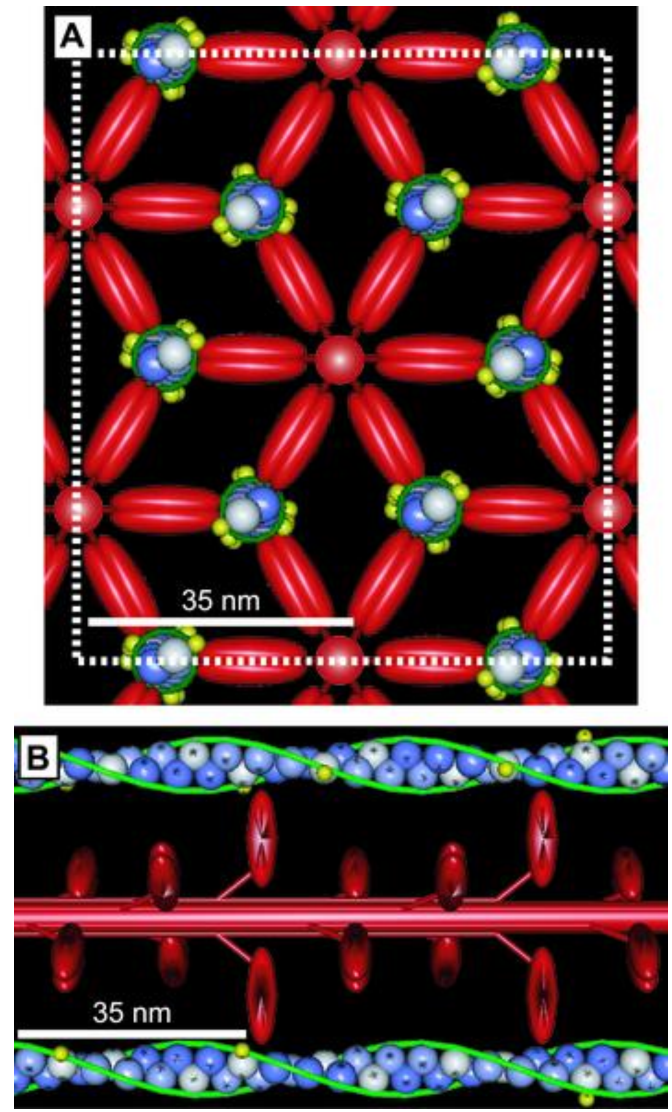

Sarcomere
Myofibril
A band
Dark band
I band
Light band
Relaxed
595 nm
Z line
M line
H zone


Z disc
Cap Z
titin
tropomodulin
M line
myosin (thick filament)
Z disc
plus end
of actin
filament
nebulin
minus end
actin (thin filament)

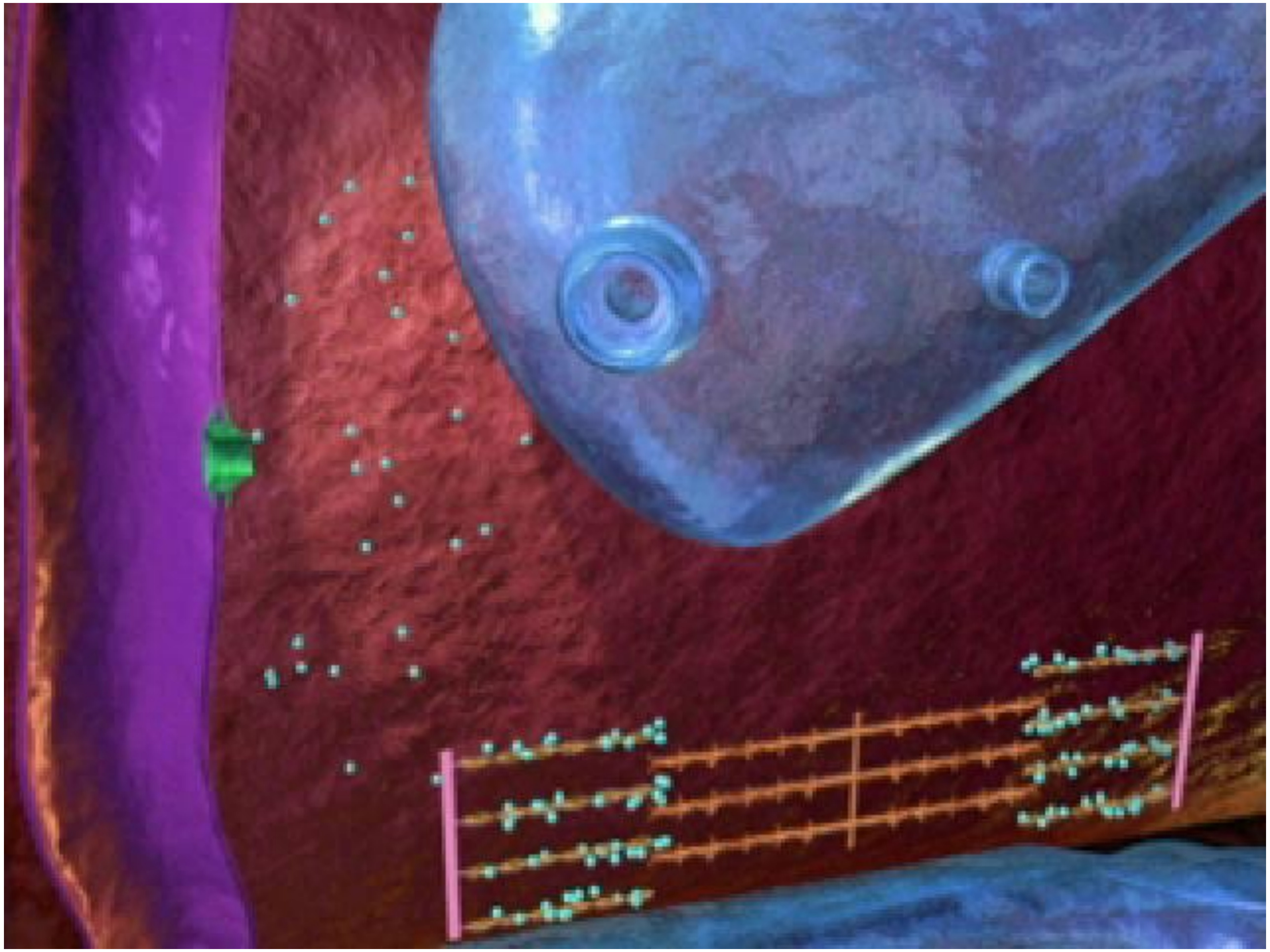
Cross-Section







Contraction



Questions?