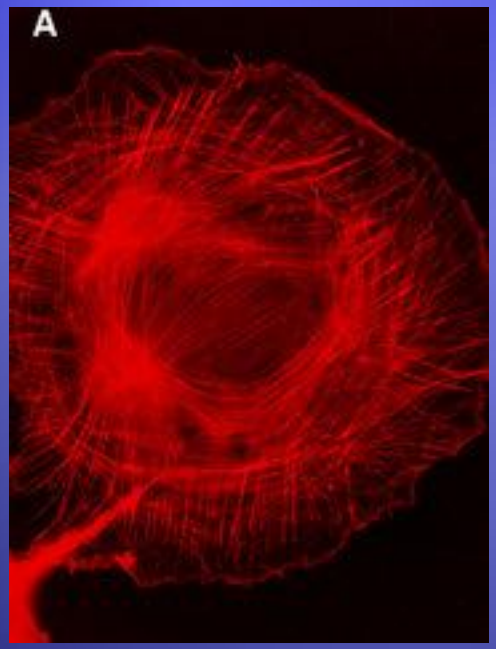
Session 4

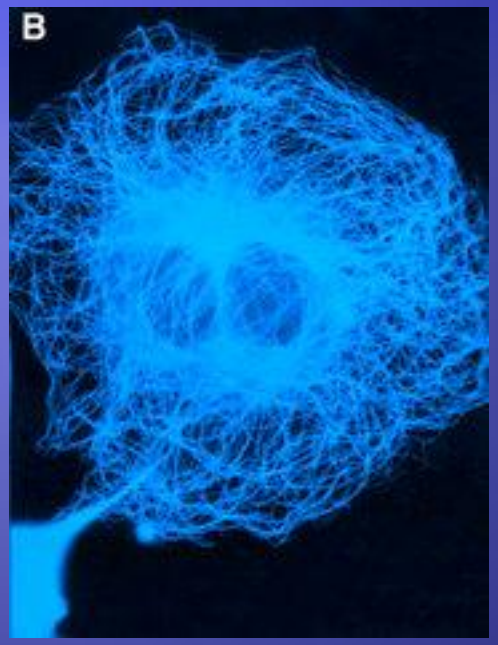
CYTOSKELETON

Cytoskeleton

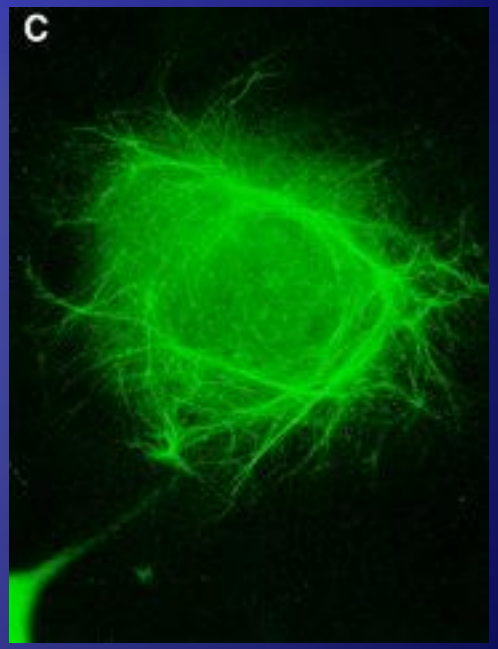
- Three fundamental cytoskeletal filaments



Actin



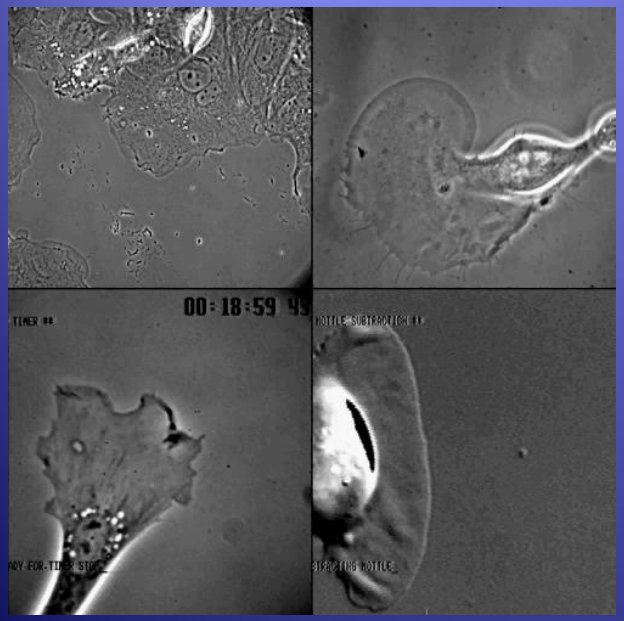
Microtubules (MT)

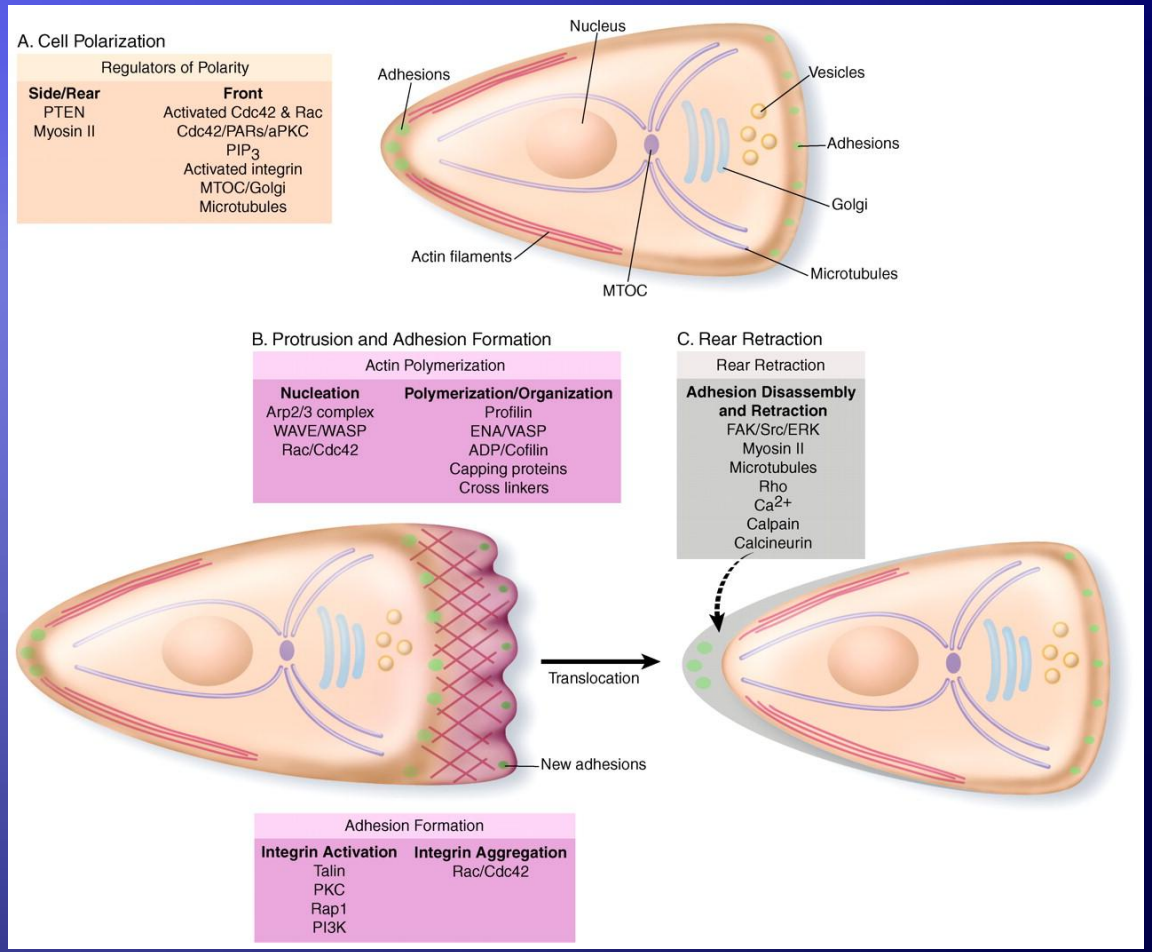


Intermediate Filaments (IF)

Dynamic Structure

- Cell Crawling
- Phagocytosis
- Cytokinesis
- Muscle contraction
- Gastrulation

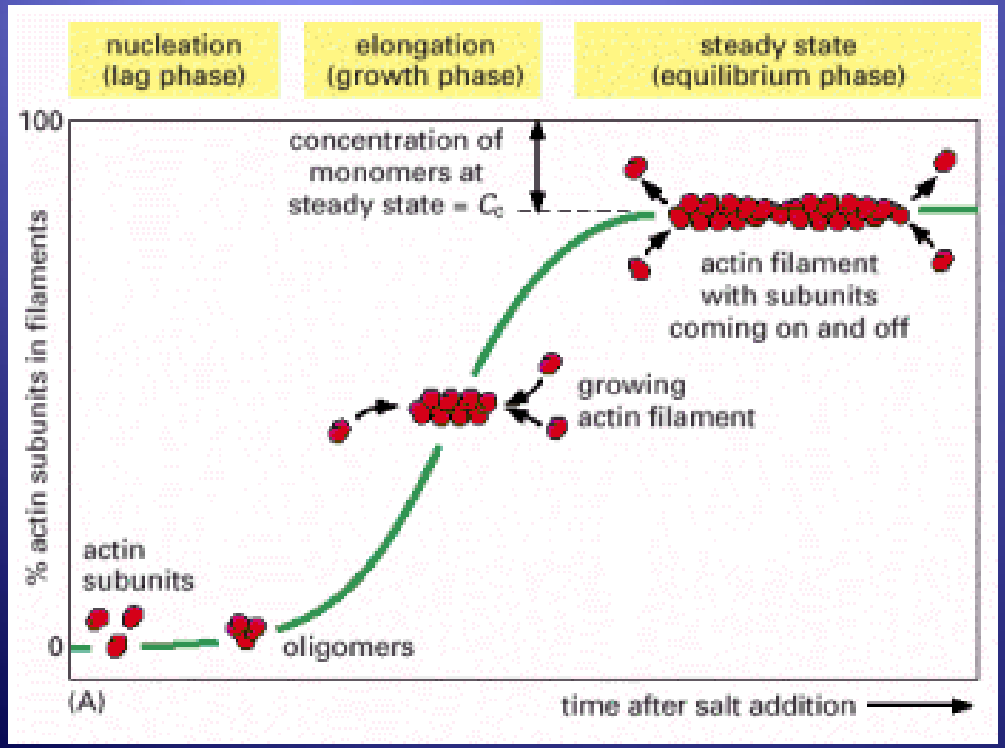


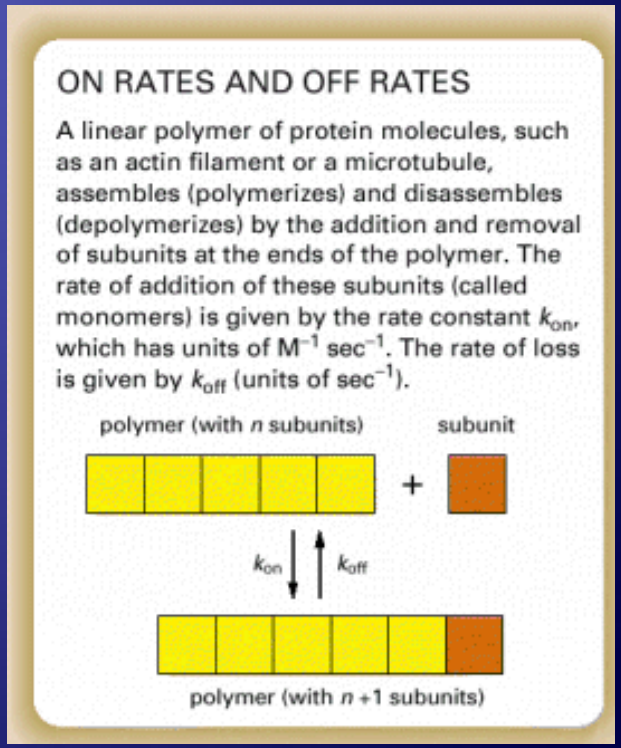


Top left: mouse fibroblasts in scratch wound assay (3h). Top right: mouse melanoma cell (20min) Bottom left: a chick fibroblasts (2h). Bottom right: trout epidermal keratocyte (4min).

Polymerization of Subunits

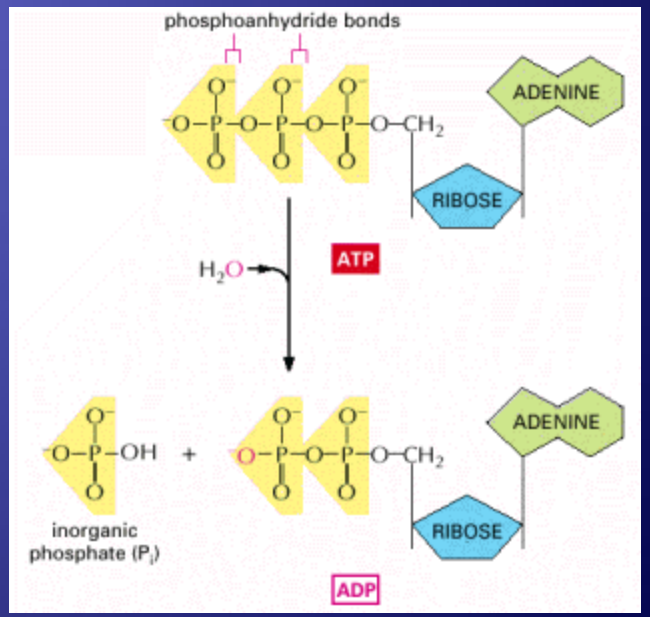
- Assembly/Disassembly of Monomers Subunits
- Treadmilling of monomers keeps a flux of subunits but a steady-stead length.

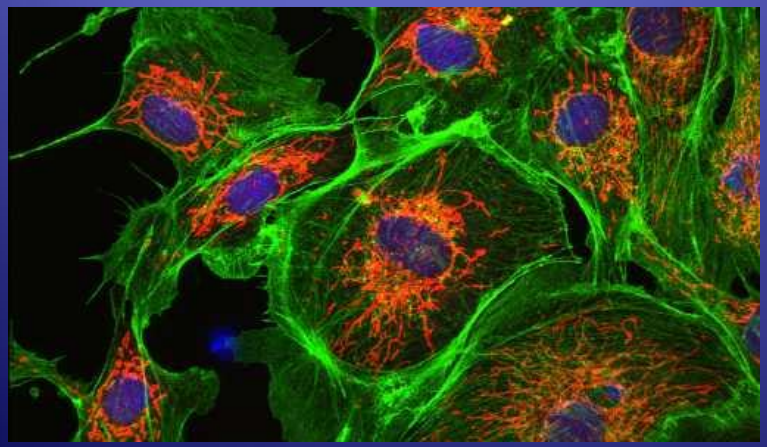




Adenosine & Guanosine Triphosphate

- Hydrolysis of ATP or GTP is coupled to cytoskeletal assembly
  - $ATP + H_2O \rightarrow ADP + Pi$
  - $GTP + H_2O \rightarrow GDP + Pi$
- ATP, GTP synthesized in mitochondrial



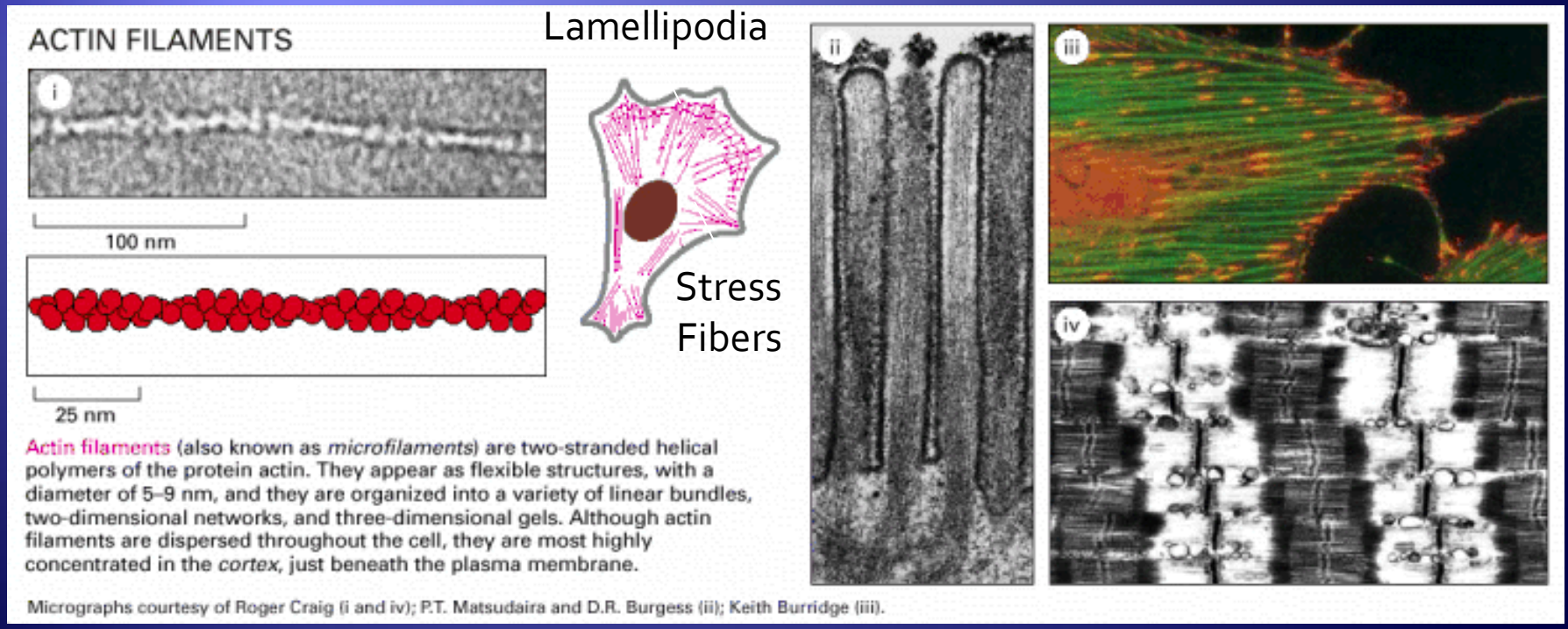


DAPI, Phalloidin, Mitotracker

Actin

- Most versatile and abundant protein of cells
- G-actin: 375 amino acids, 42 kDa

Stress Fibers & Focal Adhesions

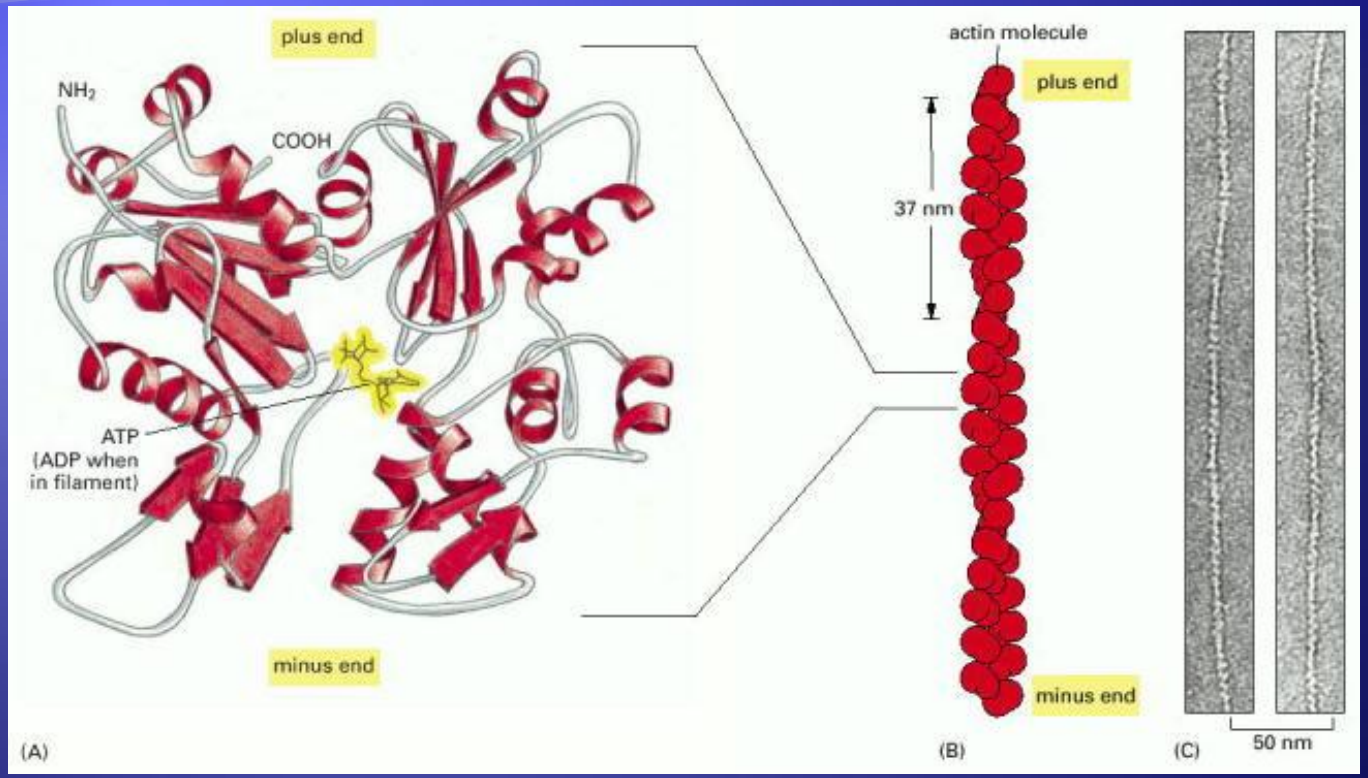


Microvilli

Myofibrils

Actin

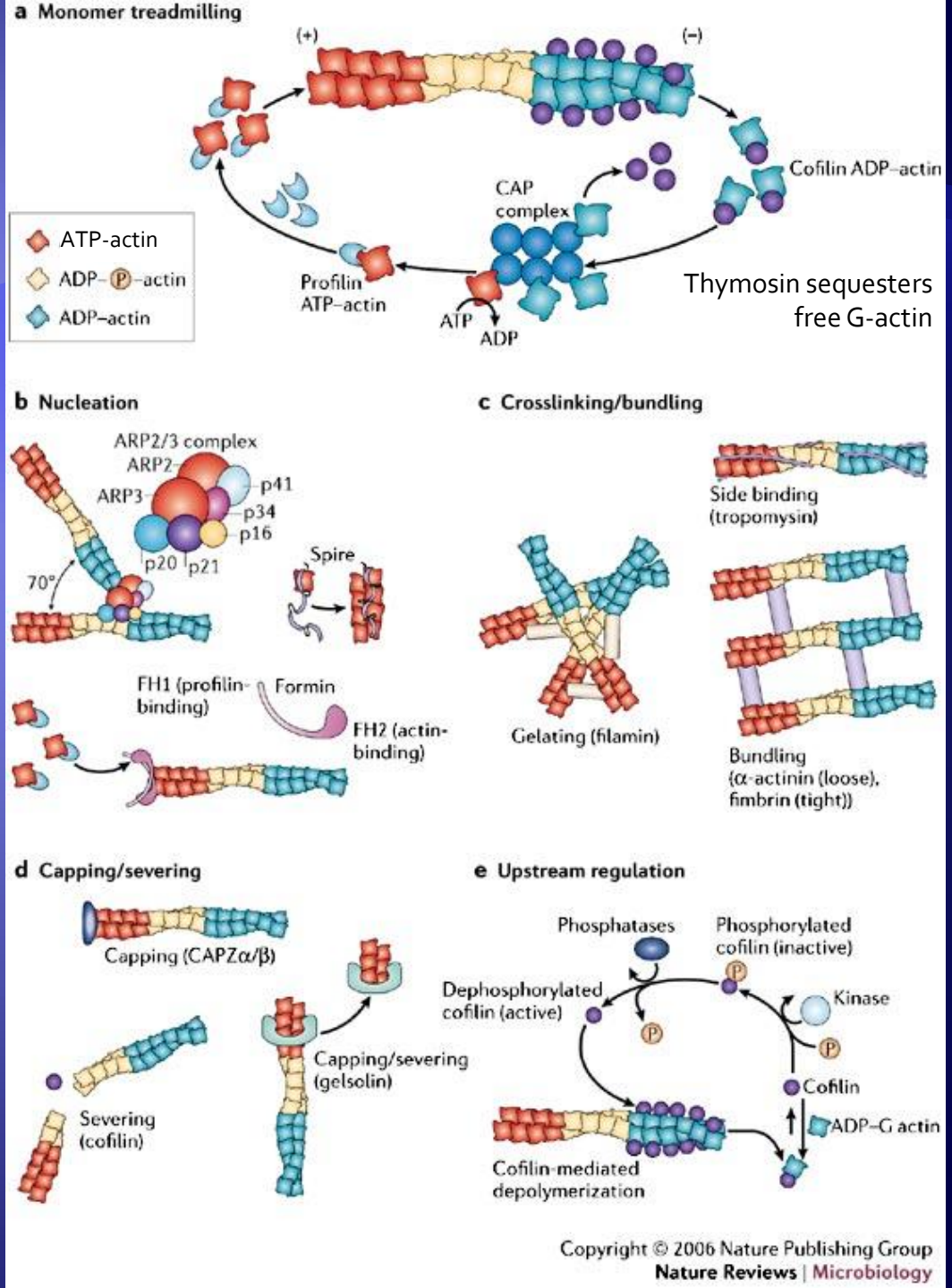
- Assembly is regulated by ATP binding



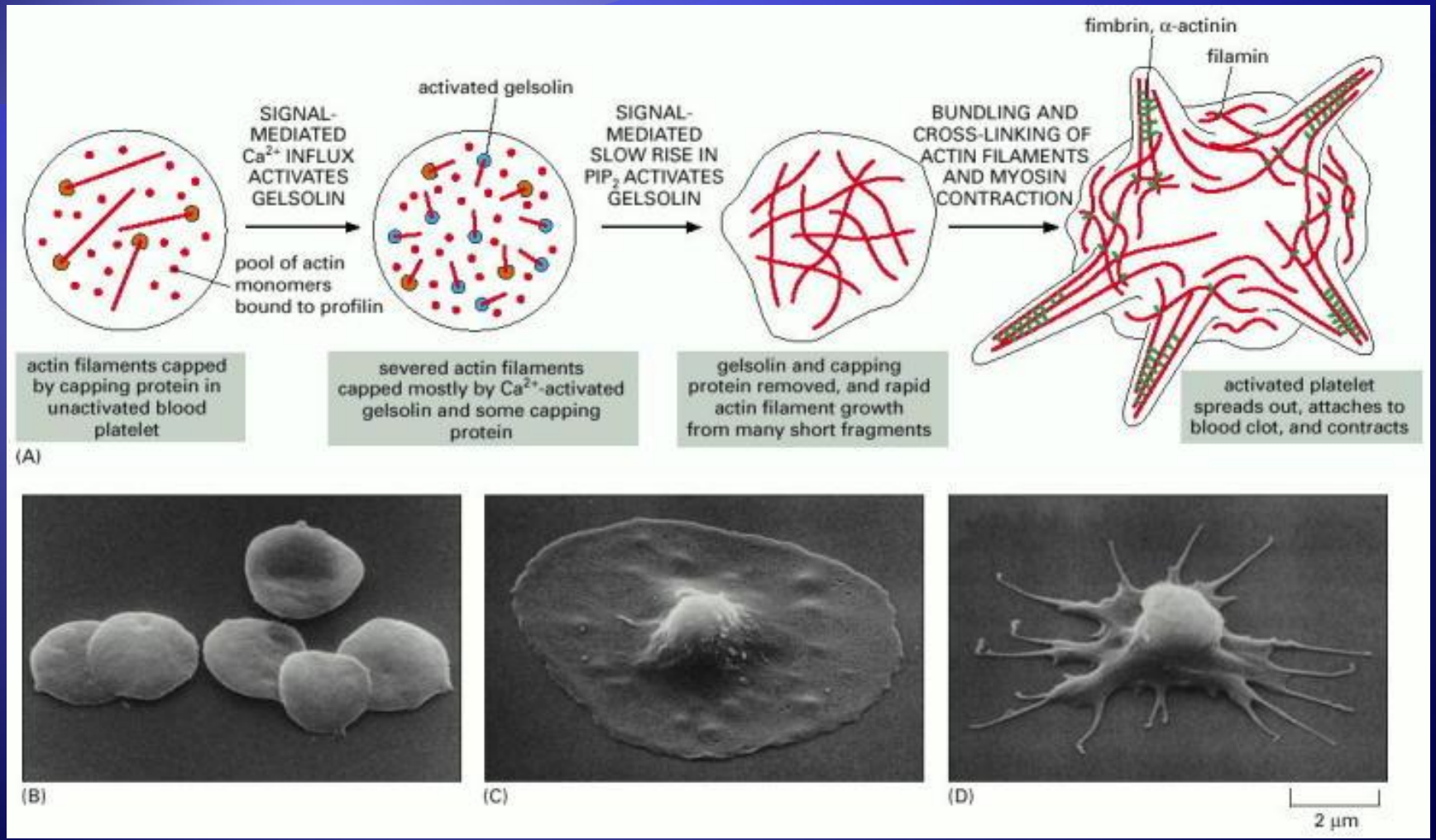
- Six major isoforms of actin found in different tissue that differ by their N-terminal sequence

Actin Binding Proteins

A) Treadmilling
B) Nucleation
C) Crosslinking
D) Disruption
E) Regulation



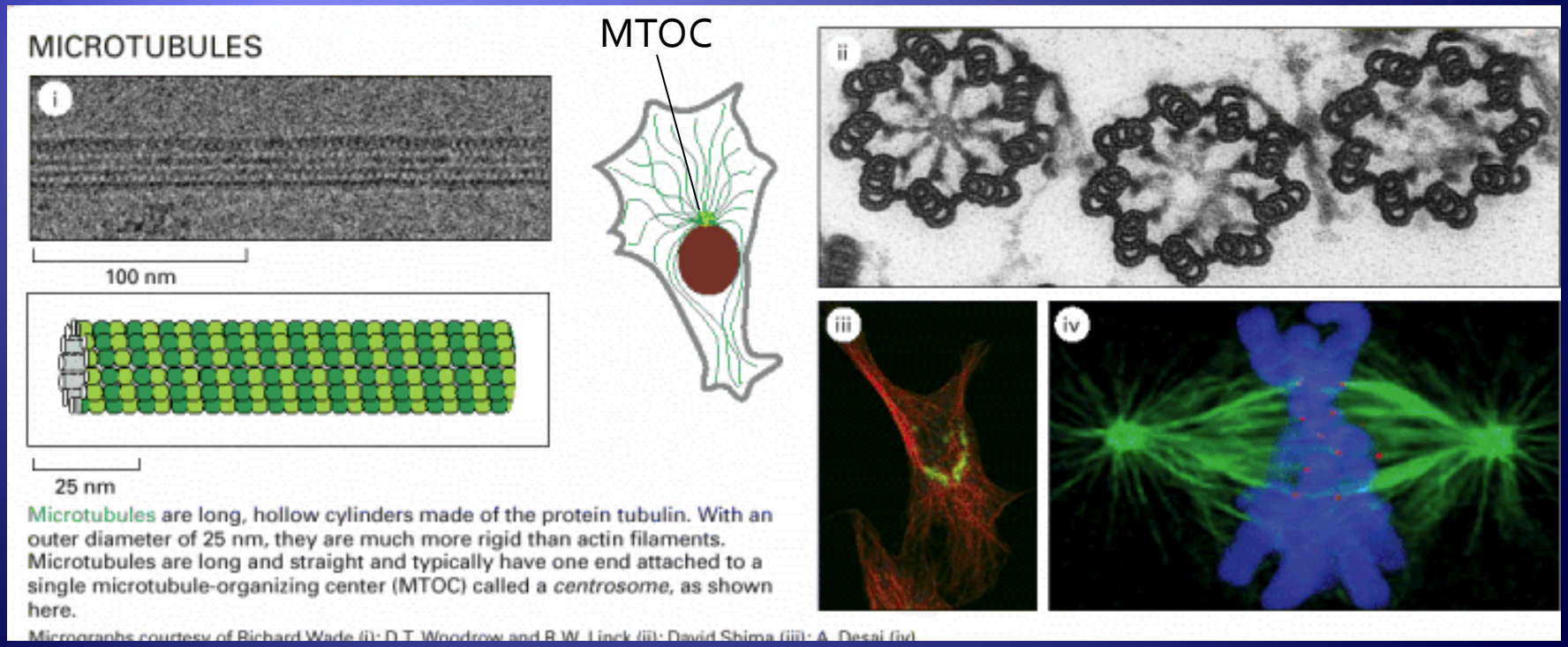
Rapid response of actin



Microtubules

- Hollow, stiff filaments that direct traffic
- Tubulin: 50 kDa

Cilia (axoneme)

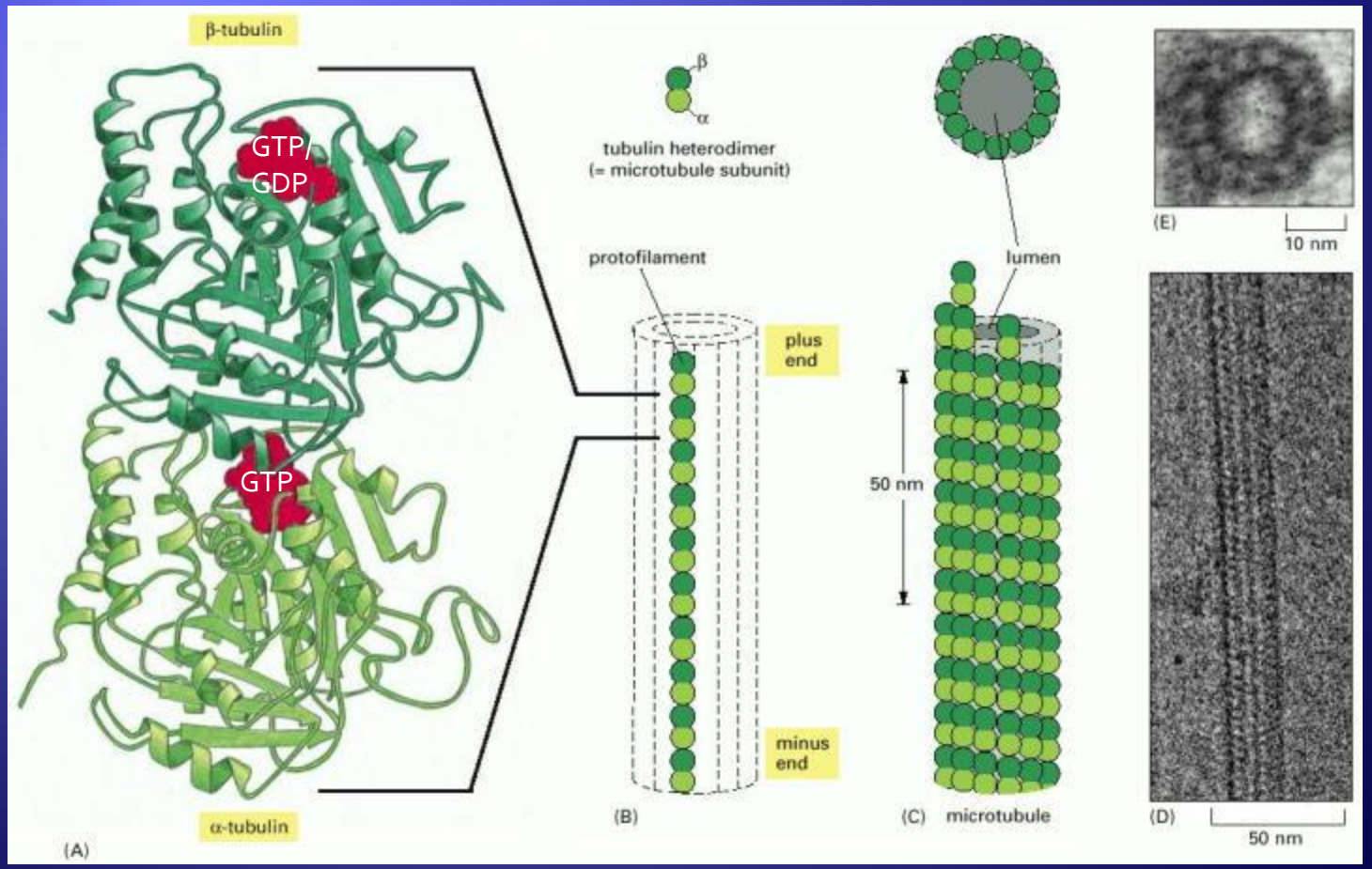


Star-Like

Mitotic Spindle

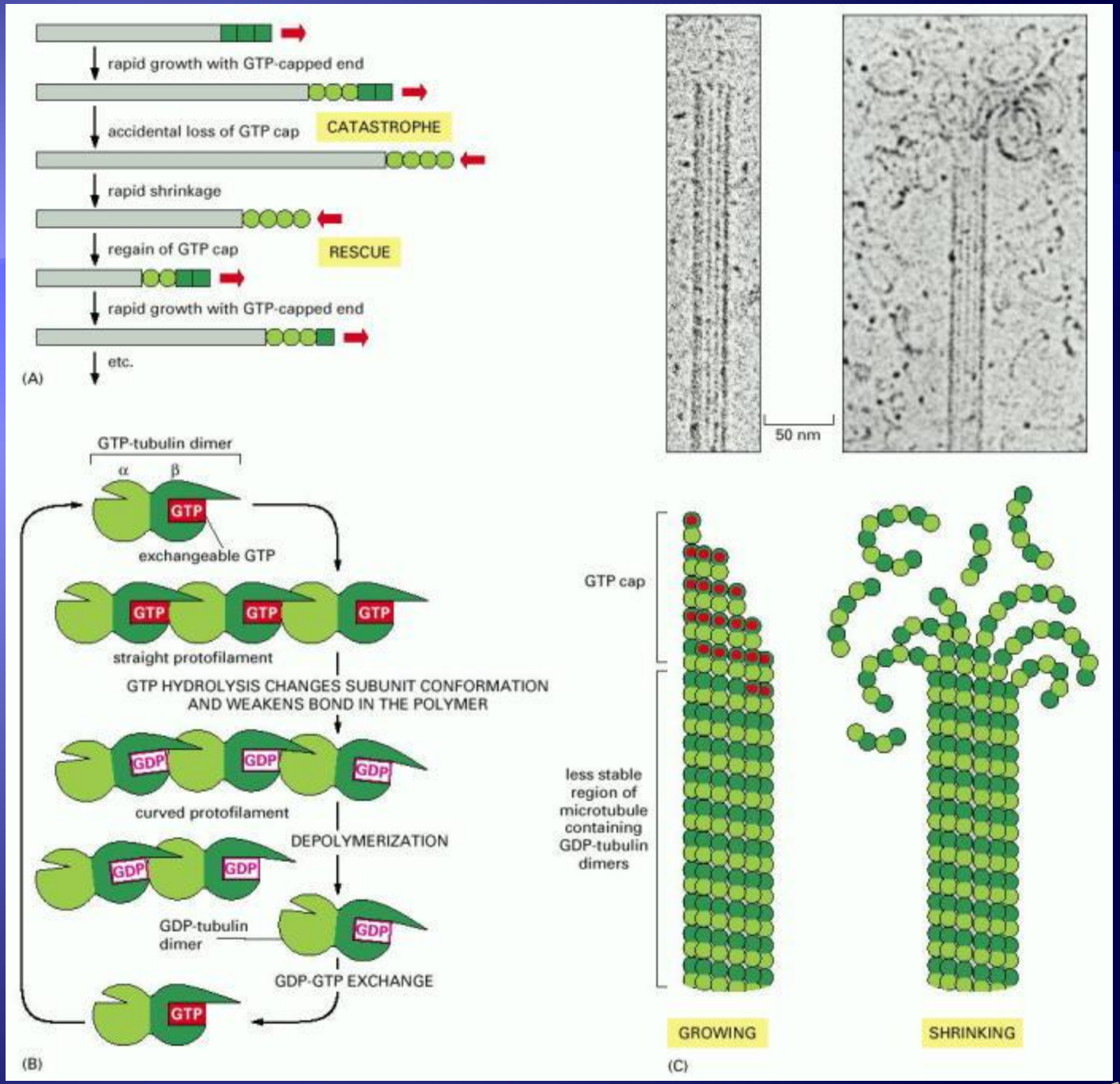
Microtubule Assembly

- Heterodimers of α-tubulin and β-tubulin



Dynamic Instability

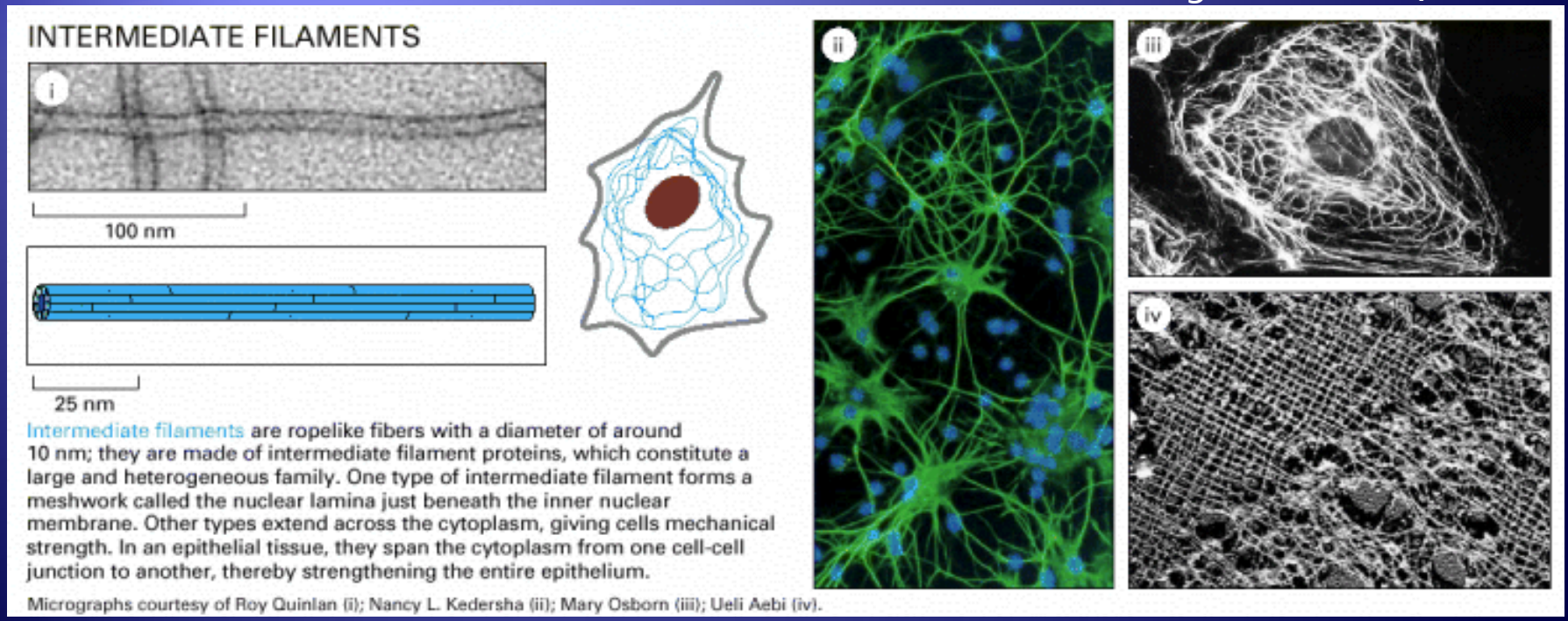
- Free ends abruptly alternate between assembly & catastrophe phases.



Intermediate Filaments

- Defines cell shape and mechanical properties
- Major types: lamins, vimentin, desmin, keratins, neurofilaments.

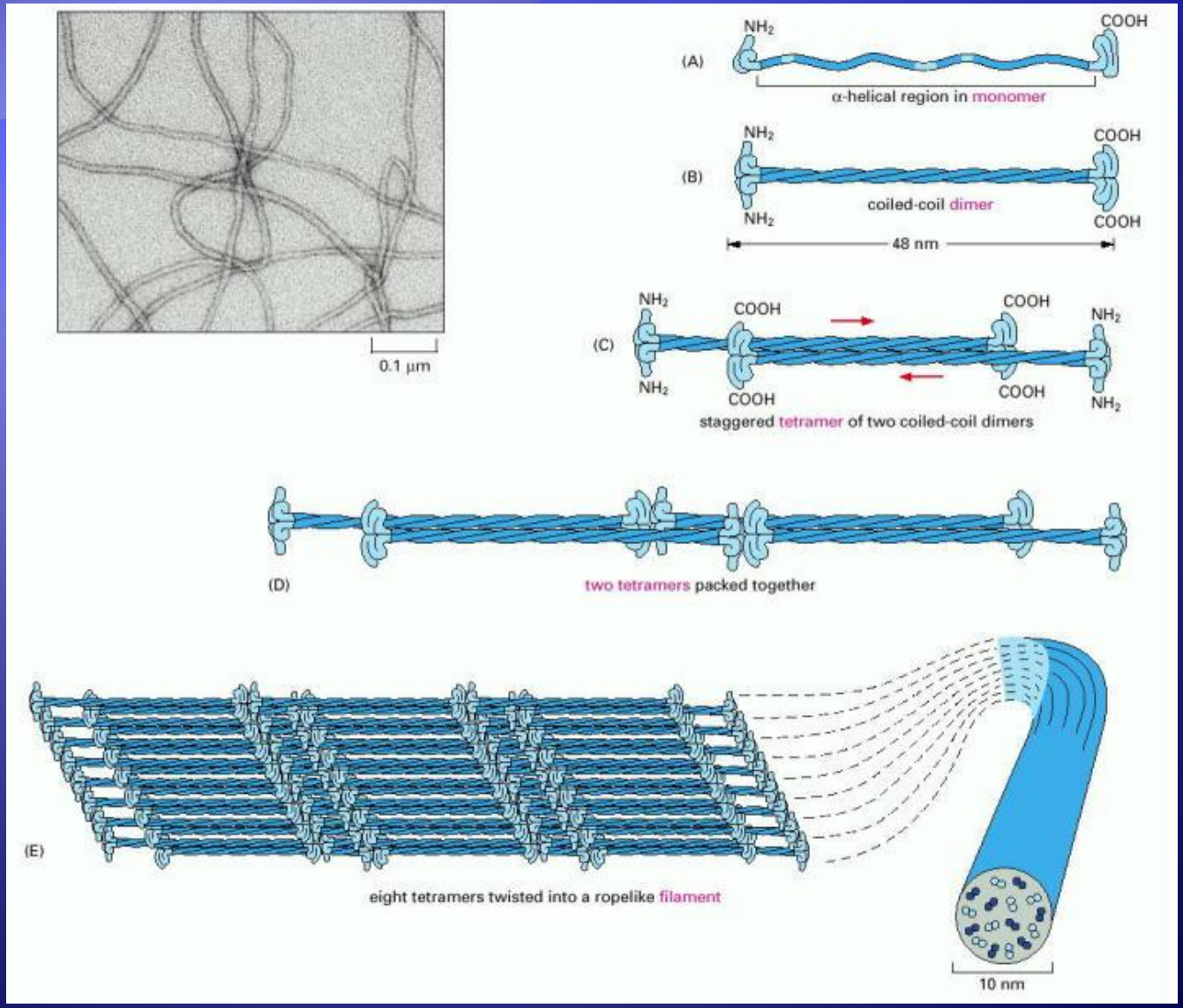
Strength (vimentin, desmin)



Axons (neurofilaments)

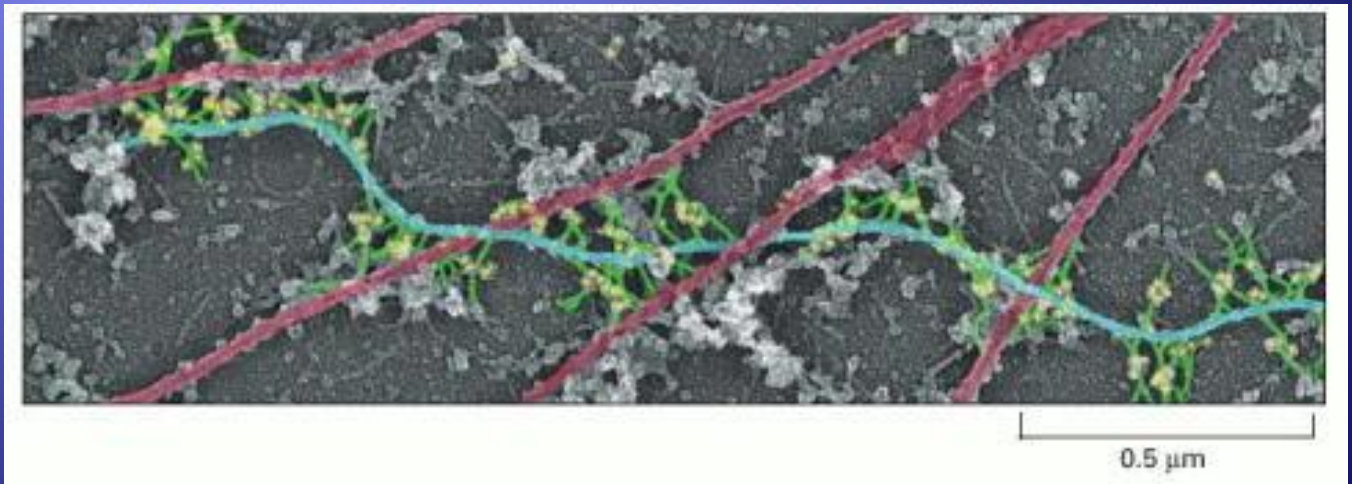
Nuclear Lamina

Coiled-Coil Dimer



CSK Cross-linking

- Intermediate filaments (blue), plectin (green), Microtubules (red), anti-plectin labelled gold particles (yellow)

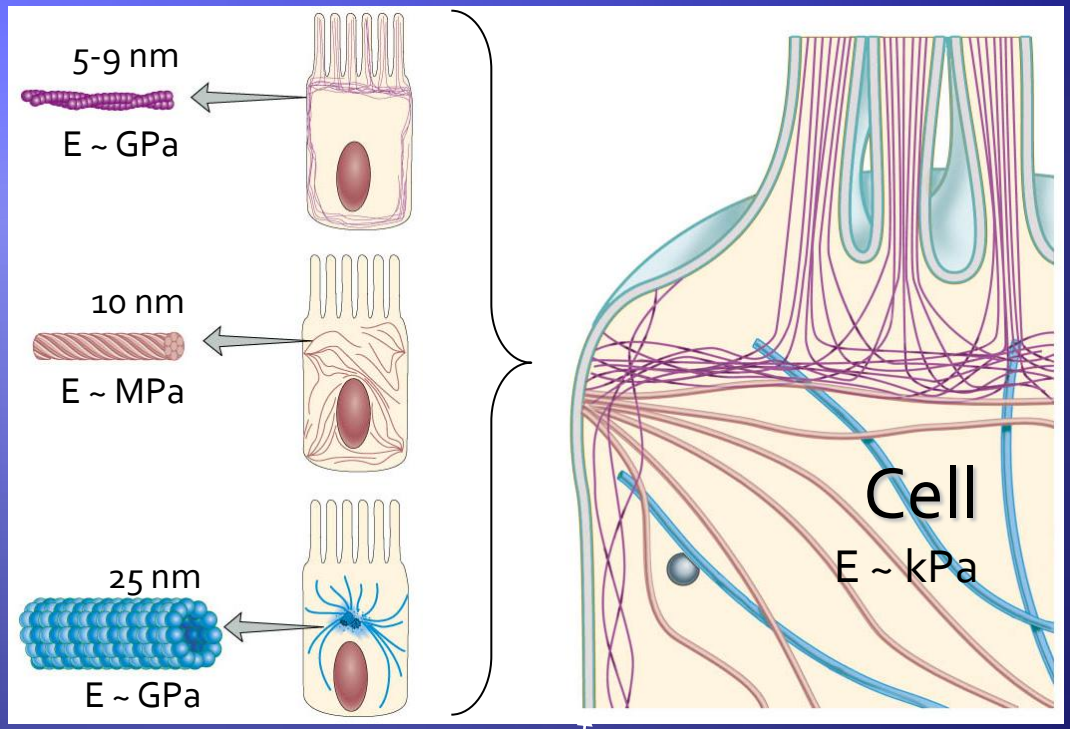


Cytoskeletal disruption drugs

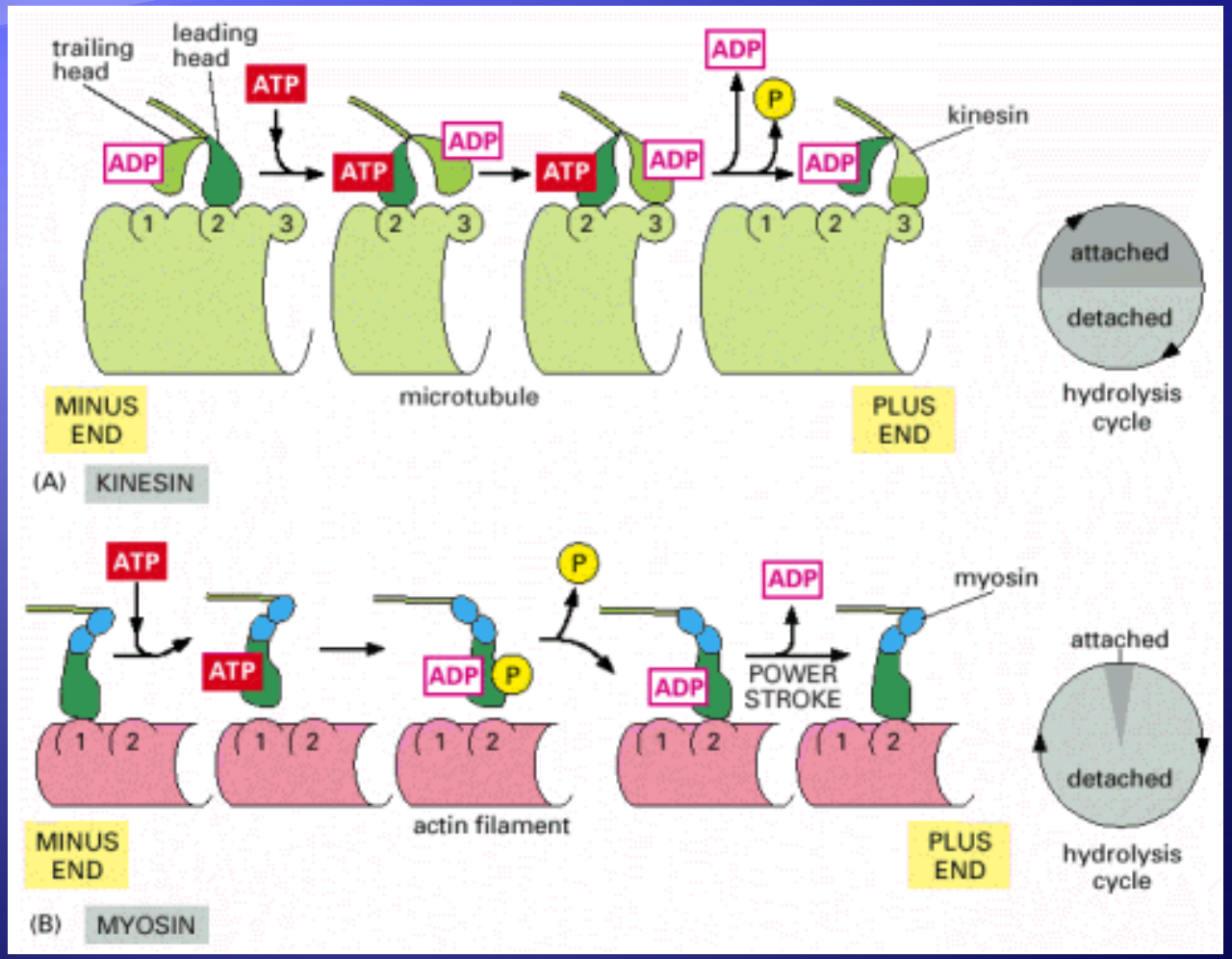
- ACTIN-SPECIFIC DRUGS
  - Phalloidin binds and stabilizes filaments
  - Cytochalasin caps filament plus ends
  - Latrunculin binds subunits and prevents their polymerization
- MICROTUBULE-SPECIFIC DRUGS
  - Taxol binds and stabilizes microtubules
  - Colchicine binds subunits and prevents polymerization
  - Nocodazole binds subunits and prevents polymerization

Simple cellular biomechanics

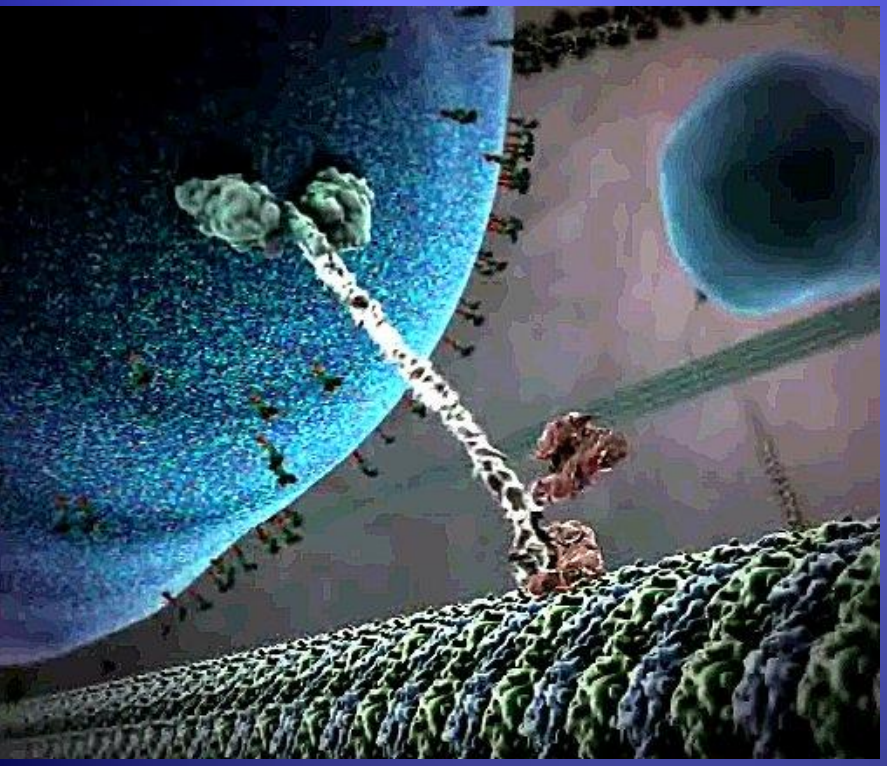
- Stiff polymer filaments form a soft cell

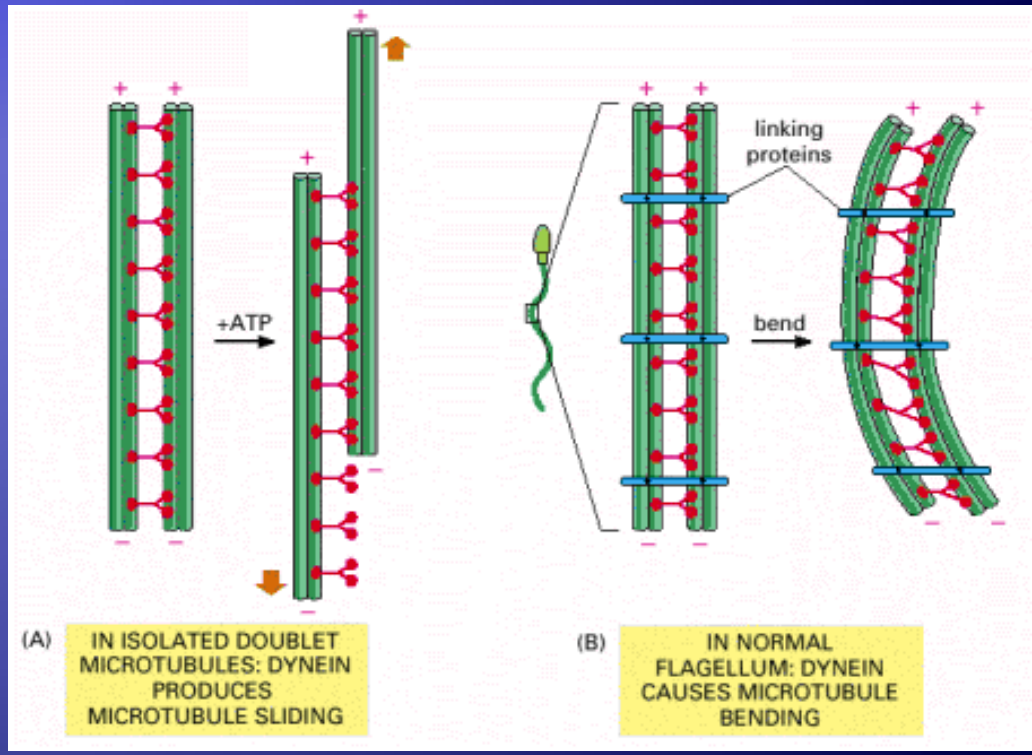


Molecular Motors

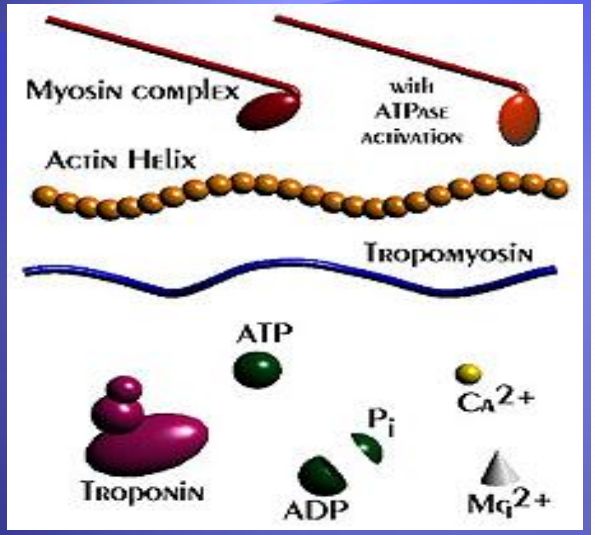


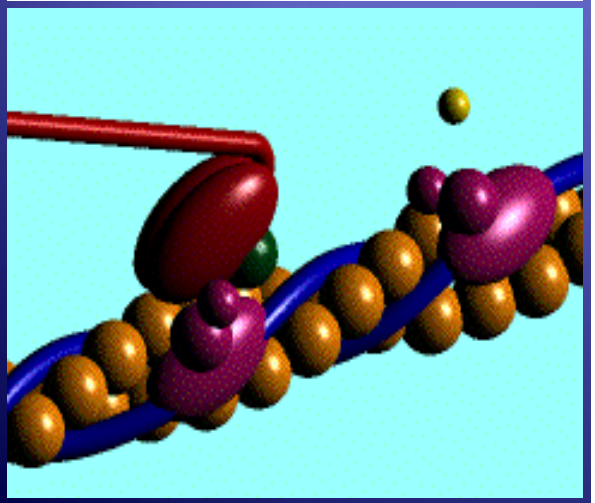
Kinesin & Dynein

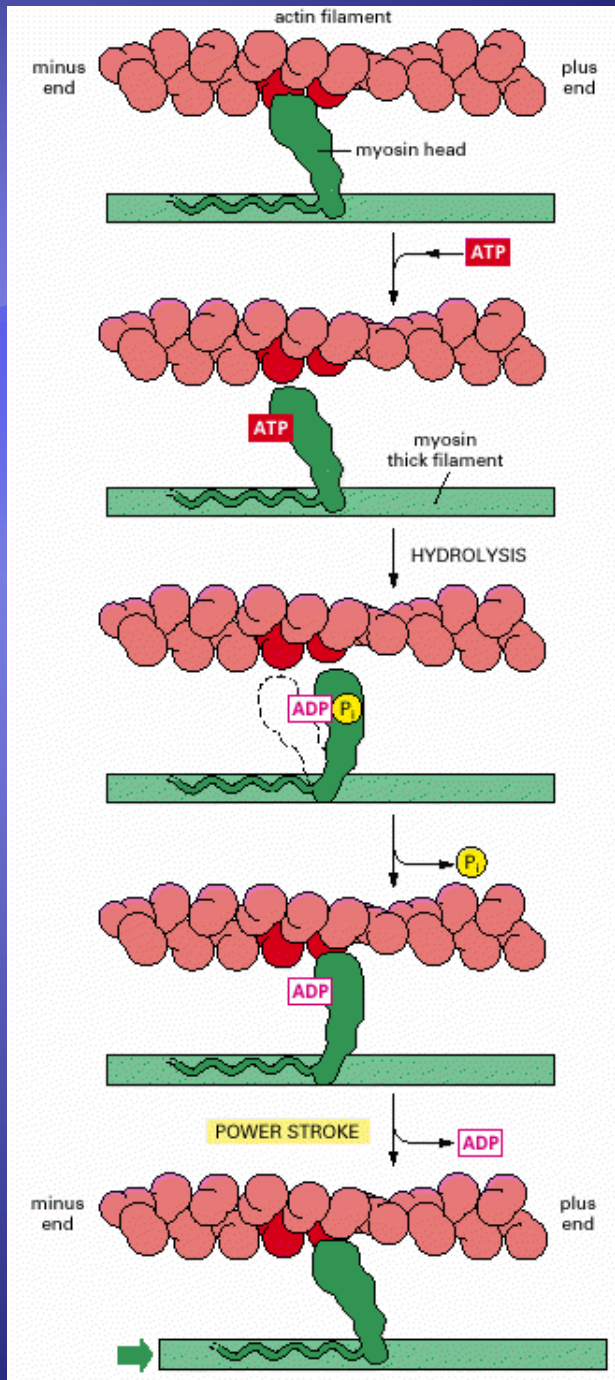




Myosin







ATTACHED At the start of the cycle shown in this figure, a myosin head lacking a bound nucleotide is locked tightly onto an actin filament in a *rigor* configuration (so named because it is responsible for *rigor mortis*, the rigidity of death). In an actively contracting muscle, this state is very short-lived, being rapidly terminated by the binding of a molecule of ATP.

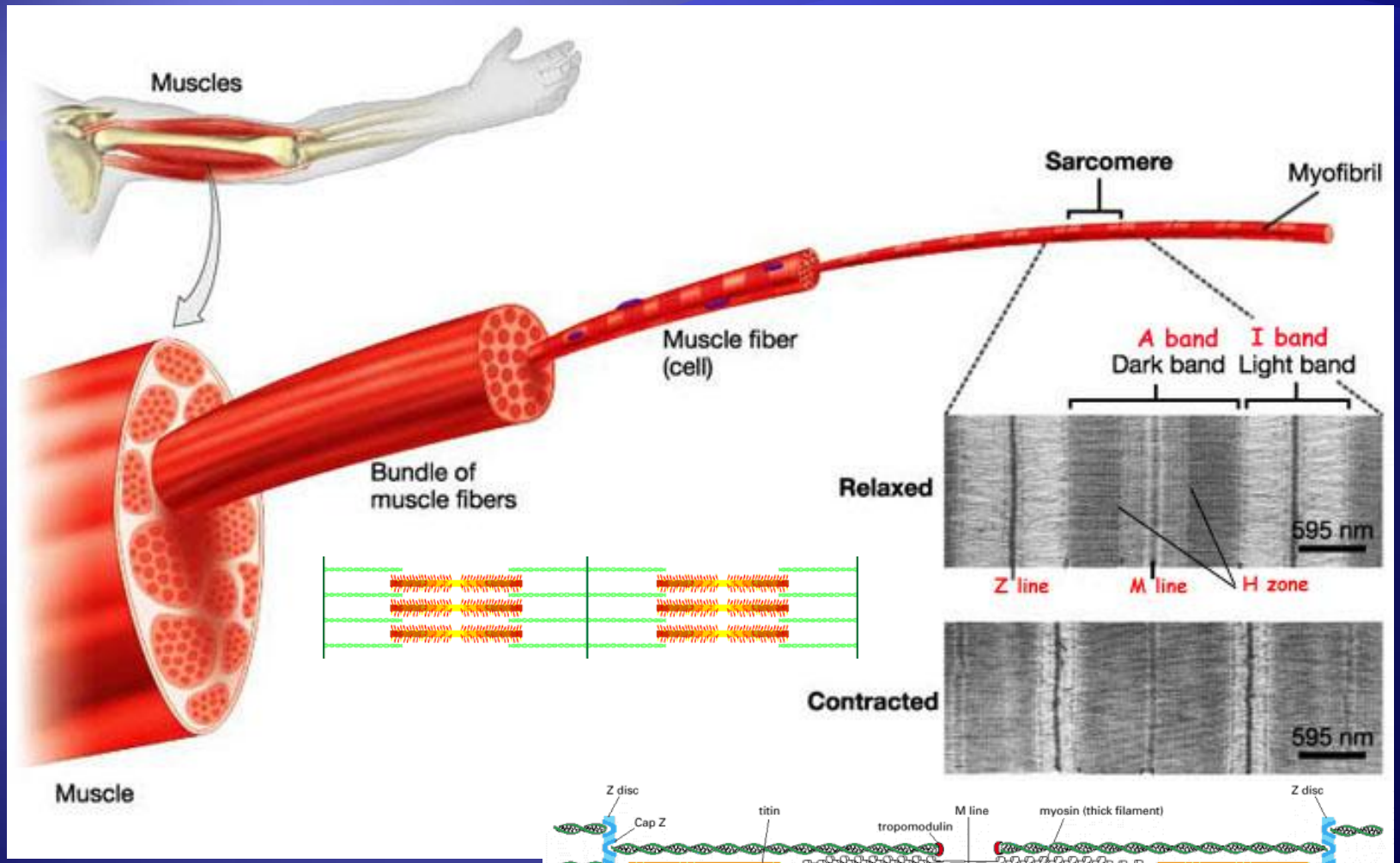
RELEASED A molecule of ATP binds to the large cleft on the "back" of the head (that is, on the side furthest from the actin filament) and immediately causes a slight change in the conformation of the domains that make up the actin-binding site. This reduces the affinity of the head for actin and allows it to move along the filament. (The space drawn here between the head and actin emphasizes this change, although in reality the head probably remains very close to the actin.)

COCKED The cleft closes like a clam shell around the ATP molecule, triggering a large shape change that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (P_i) produced remain tightly bound to the protein.

FORCE-GENERATING A weak binding of the myosin head to a new site on the actin filament causes release of the inorganic phosphate produced by ATP hydrolysis, concomitantly with the tight binding of the head to actin. This release triggers the power stroke—the force-generating change in shape during which the head regains its original conformation. In the course of the power stroke, the head loses its bound ADP, thereby returning to the start of a new cycle.

ATTACHED At the end of the cycle, the myosin head is again locked tightly to the actin filament in a rigor configuration. Note that the head has moved to a new position on the actin filament.

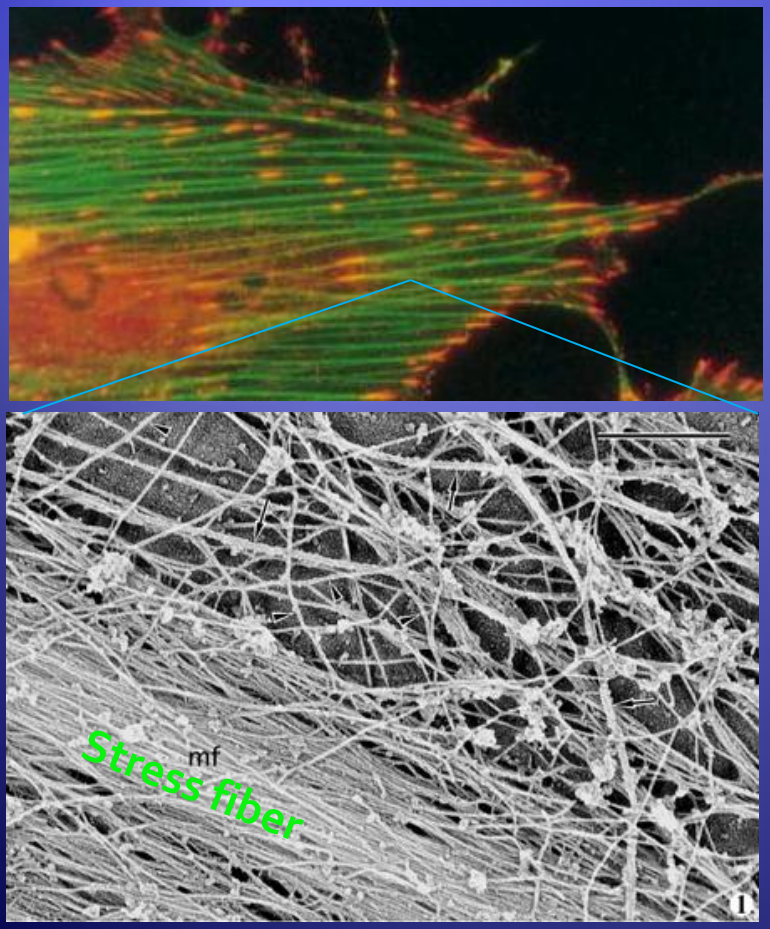
Sarcomeres

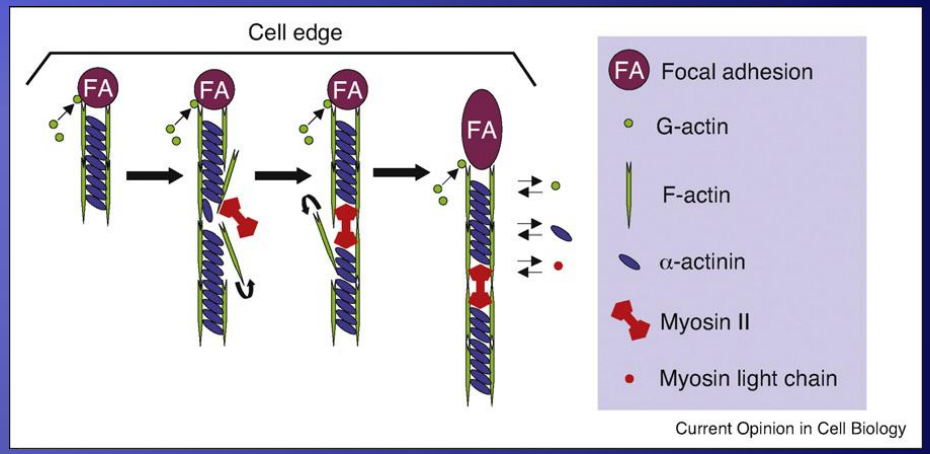


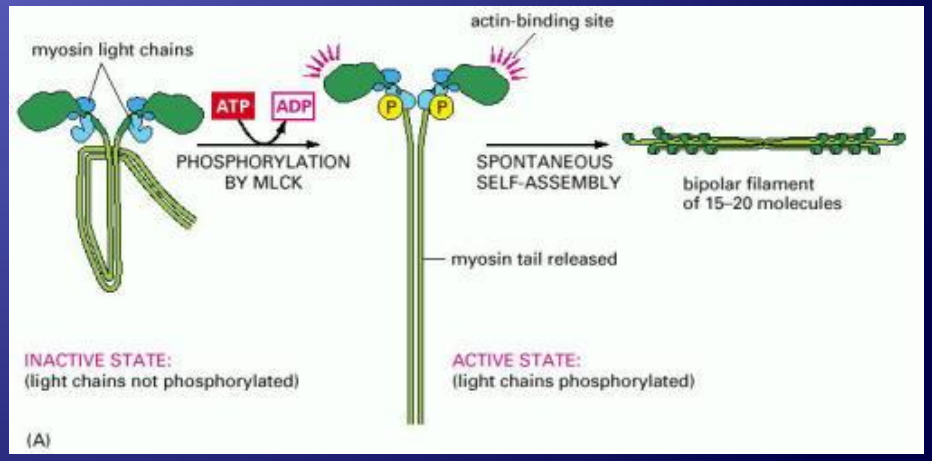
http://www.youtube.com/watch?v=ren_IQPOhJc

Stress Fibers

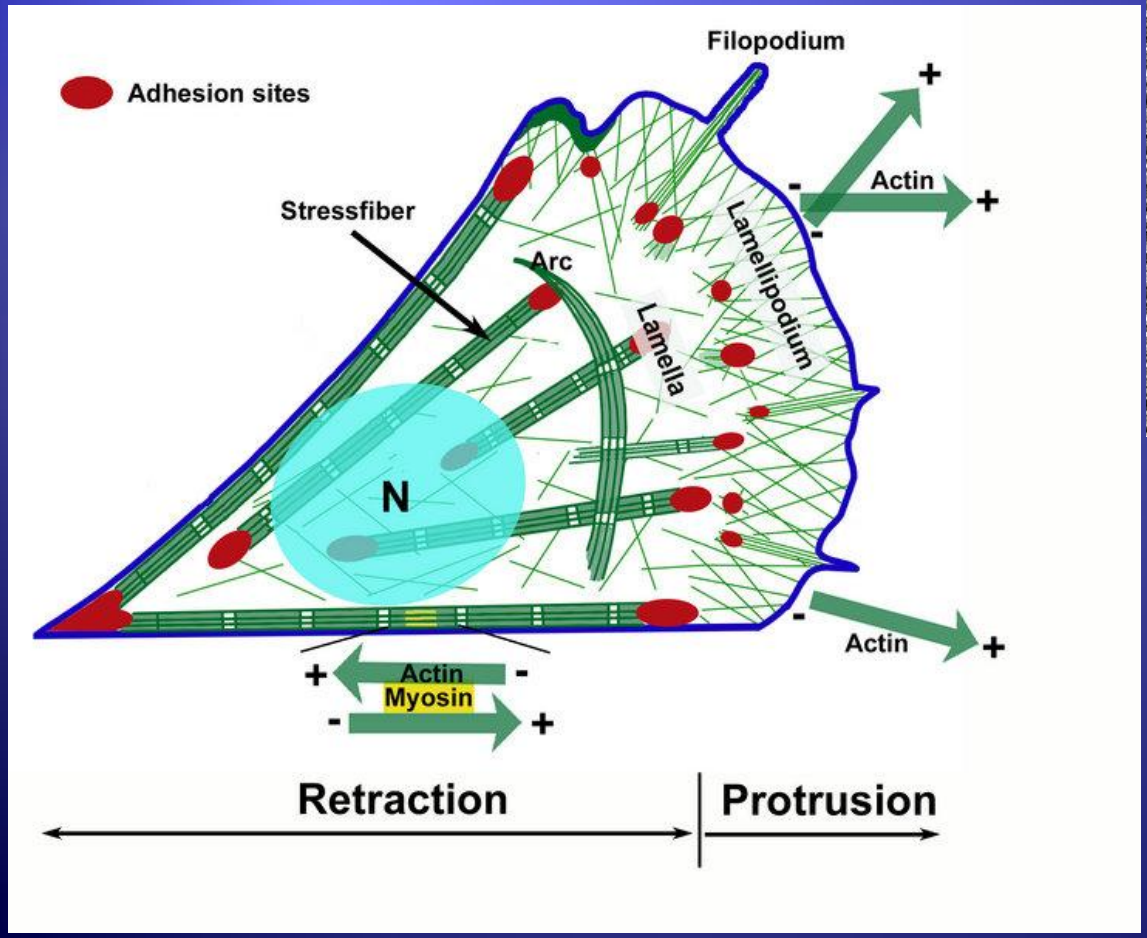
- Transient bundled structures

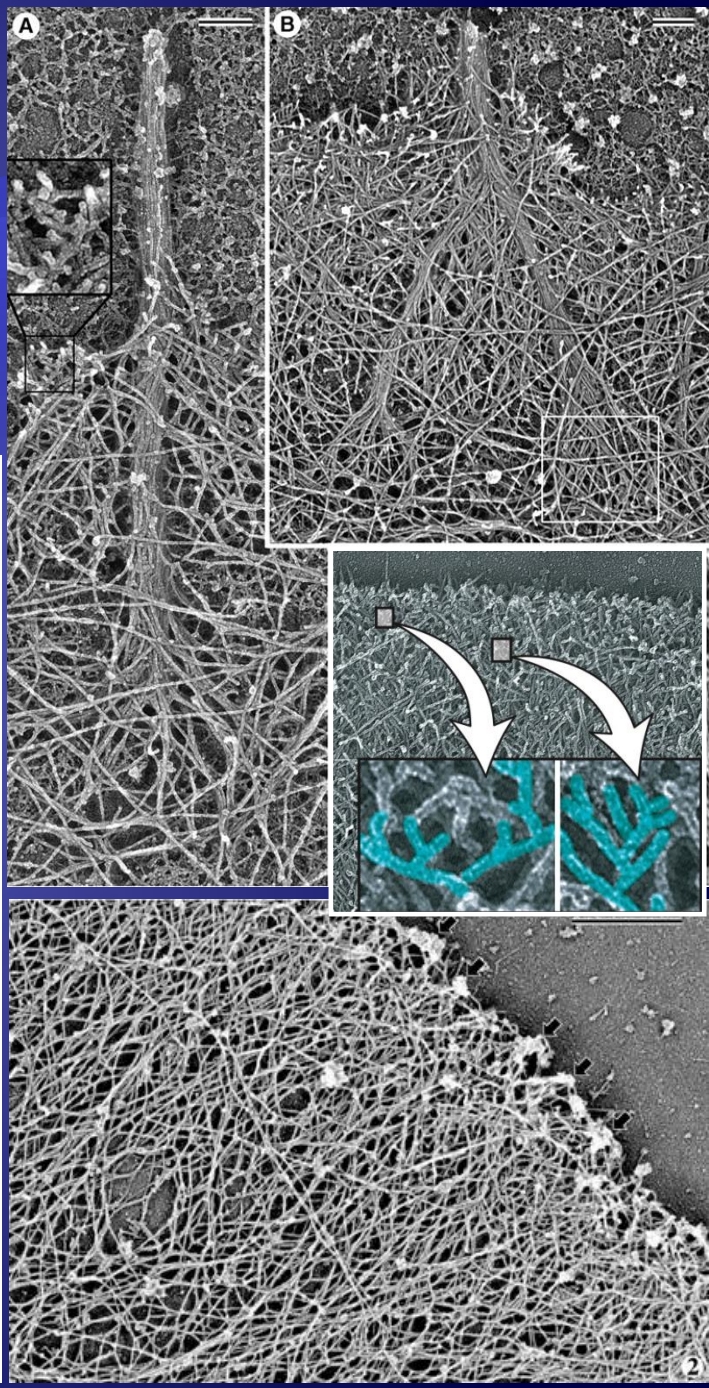






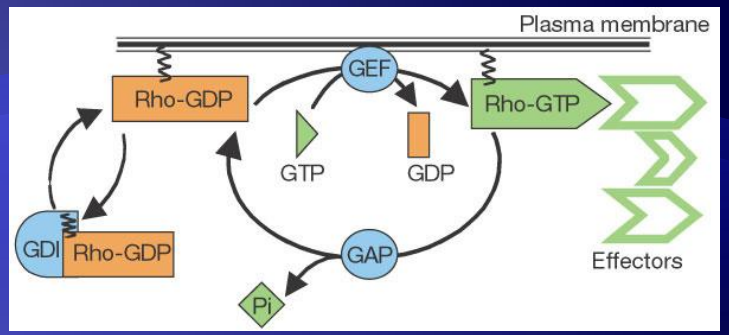
Lamellapodia & Filopodia

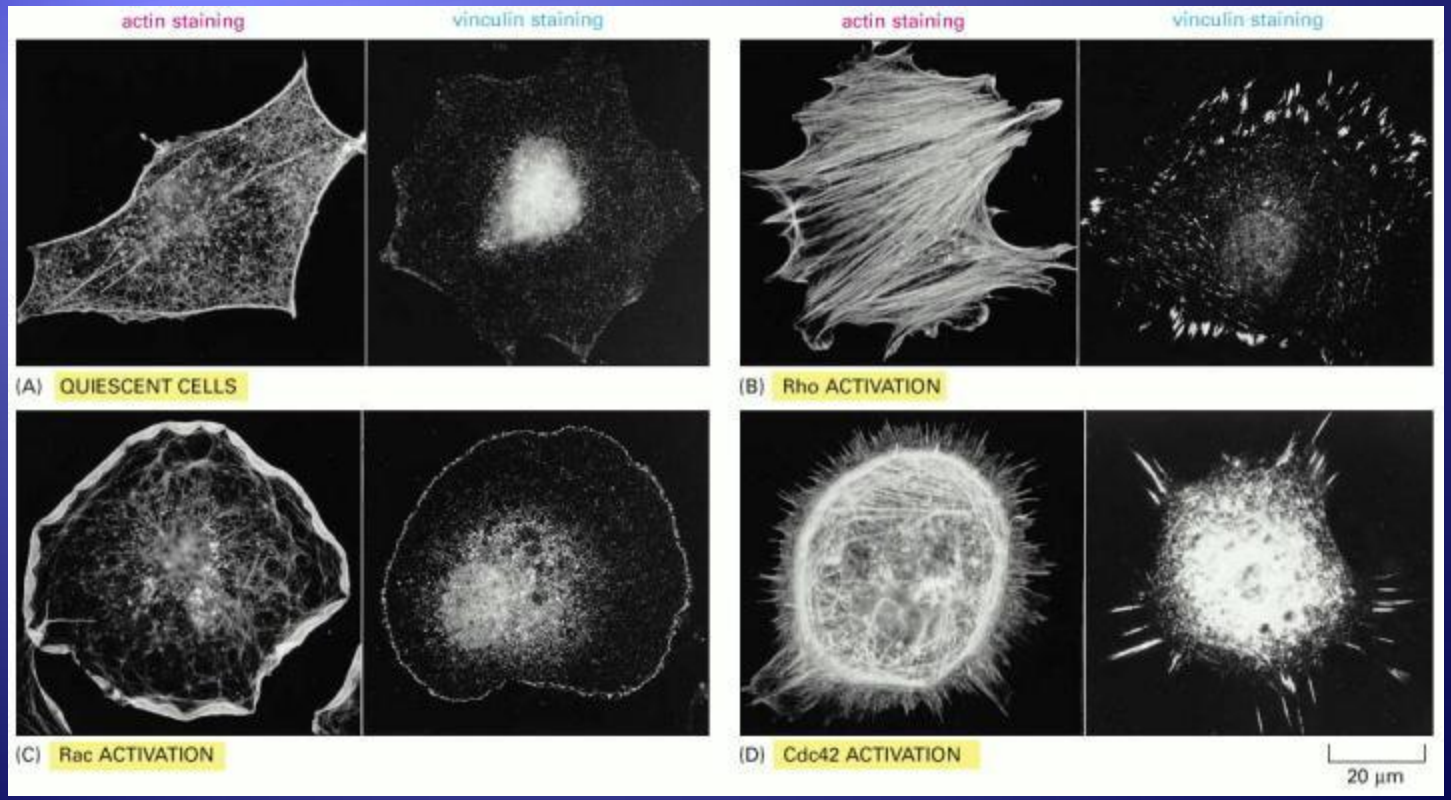




Rho Family GTPases

- An interesting discovery by Anne Ridley and Alan Hall while studying the oncogene *ras*…





Rho and Rac Effectors

- Rho promotes CSK tension and Rac helps migration and cell elongation