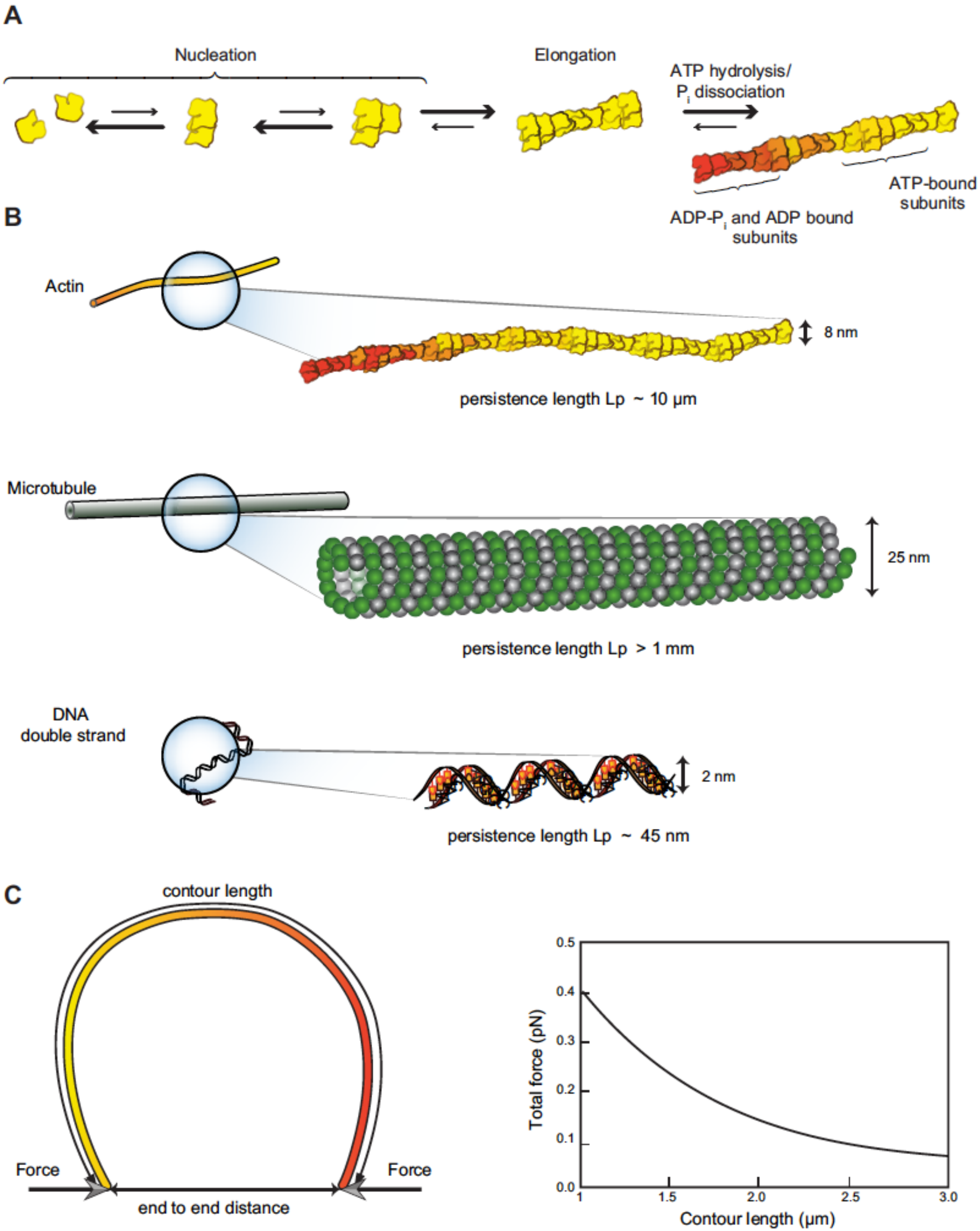


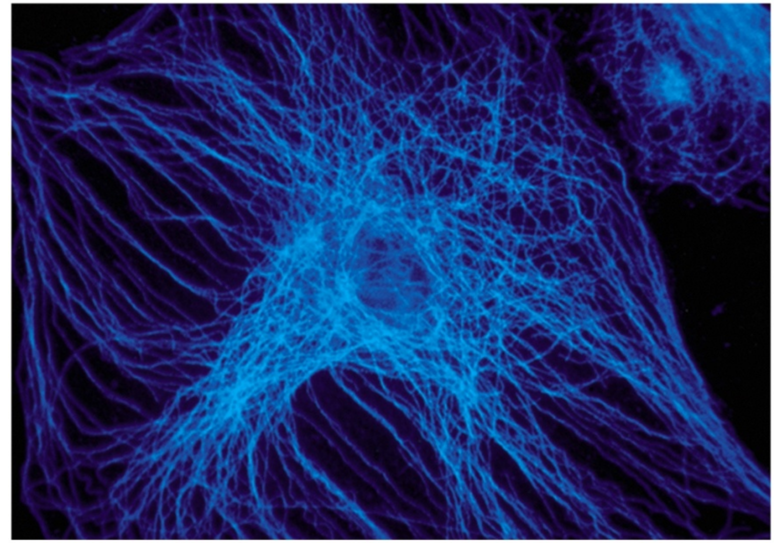
Single filament assembly and mechanics.

A: the kinetics of actin assembly.

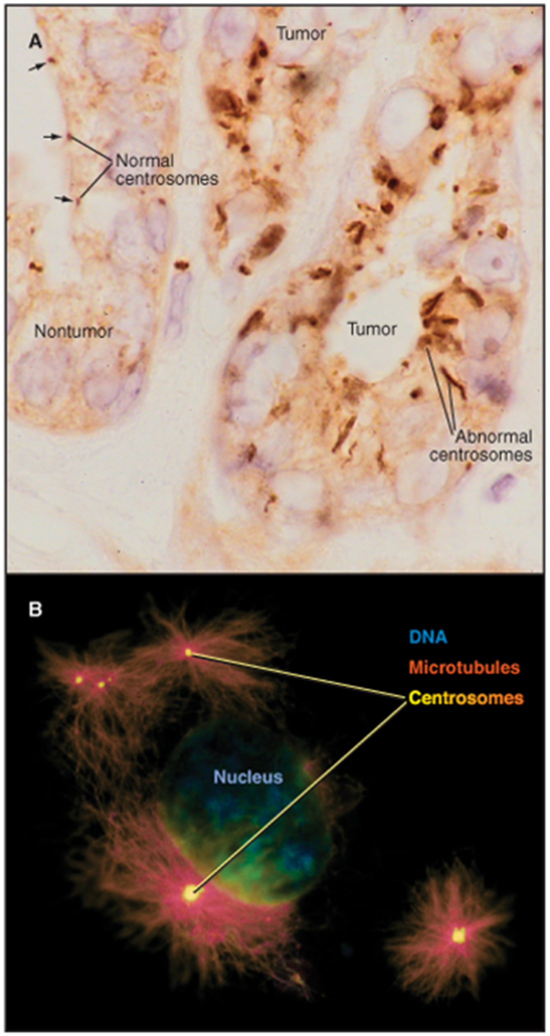
B: persistence lengths of different cytoskeletal elements.

C: at the scale of the cell, actin filaments are almost straight structures, but they can nevertheless buckle under a load.

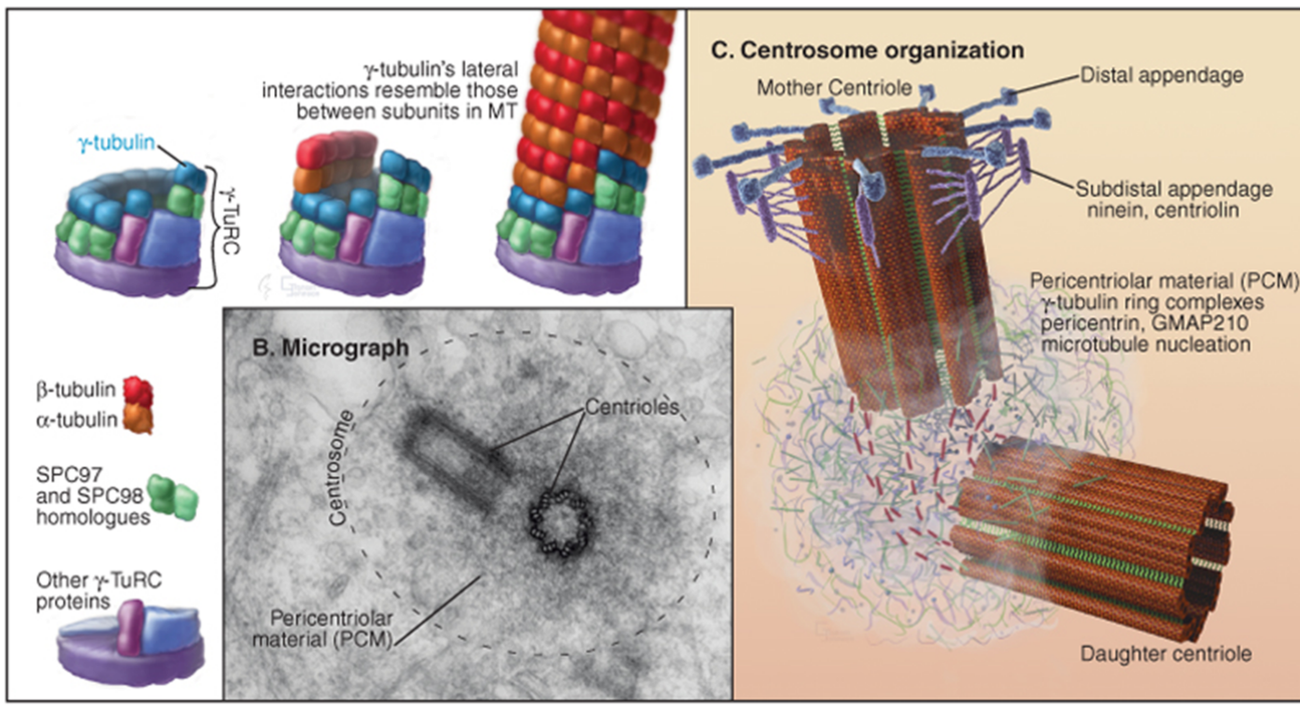
Microtubules

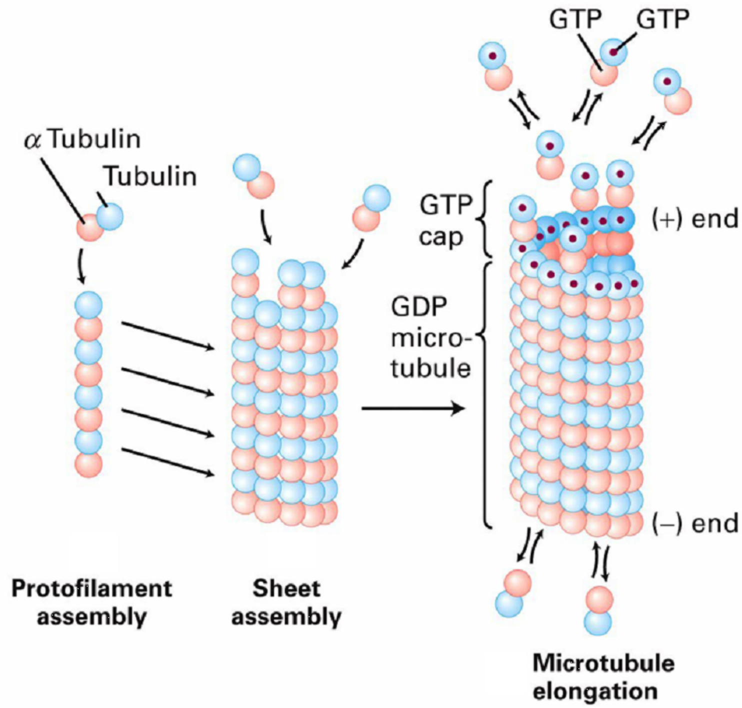


Centrosomes are often abnormal in cancer cells.

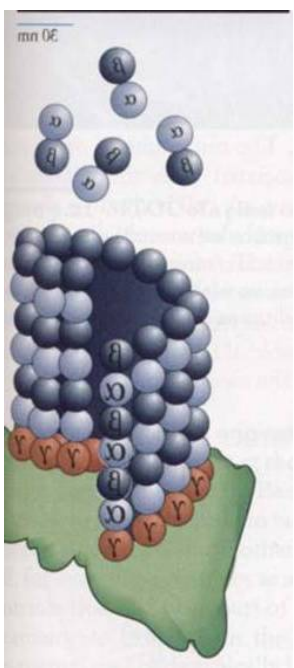


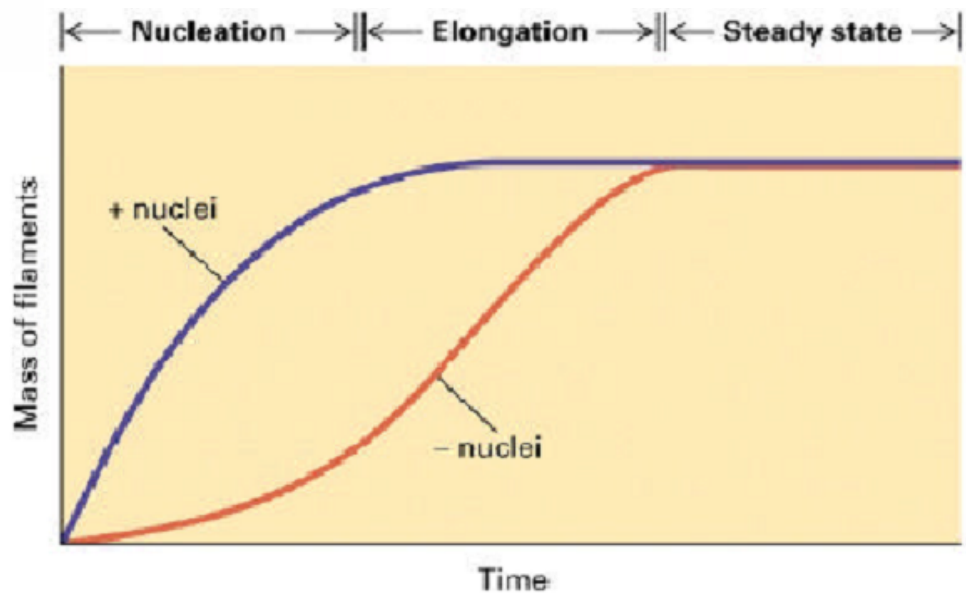
The centrosome is the primary microtubule nucleation site in most cells





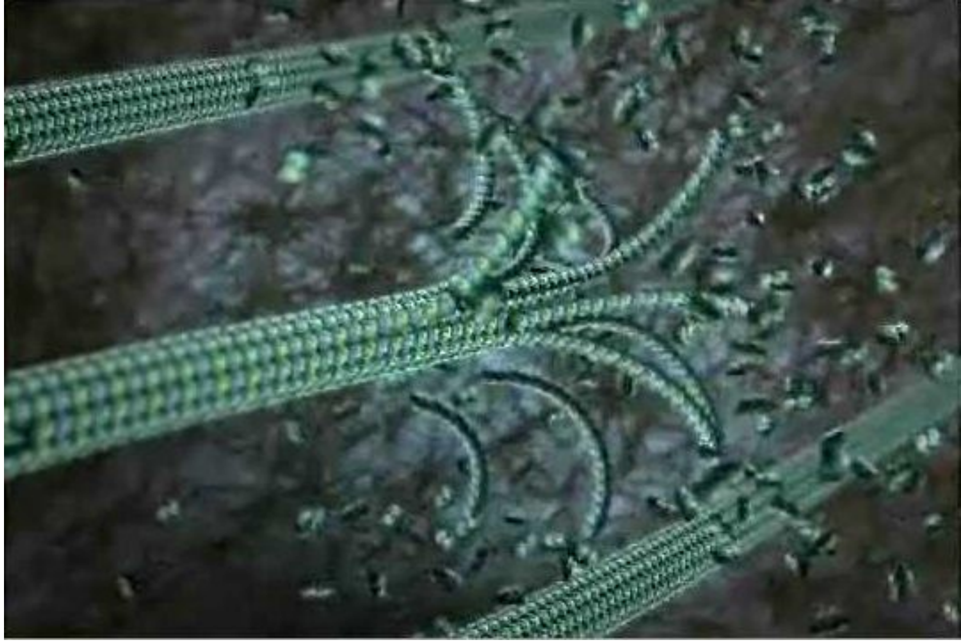
Tubulin assembly





γ-tubulin at initiates synthesis at one end (-) (green).

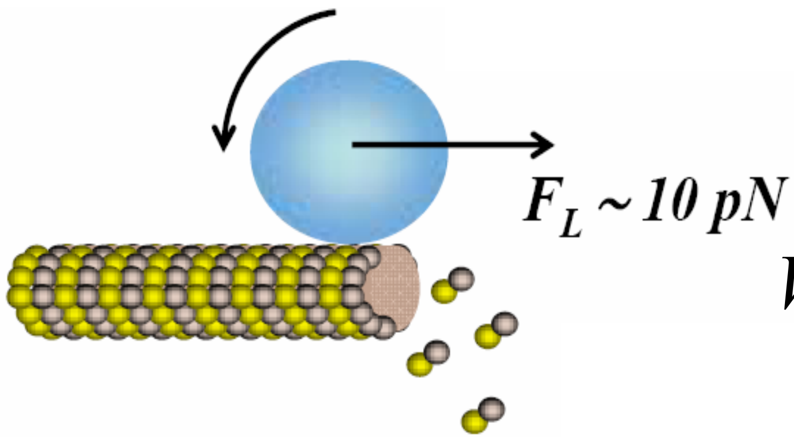
Depolymerization



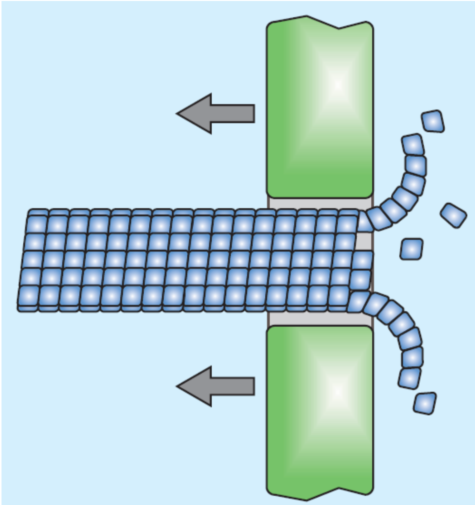
The elasticity of the protofilaments that curve outward at the disassembling plus end drives a sliding collar on the kinetochore toward the minus end (*a power stroke mechanism*).

$$F_L \cdot \delta = k_B T$$

$$\delta = \frac{k_B T}{F_L}$$

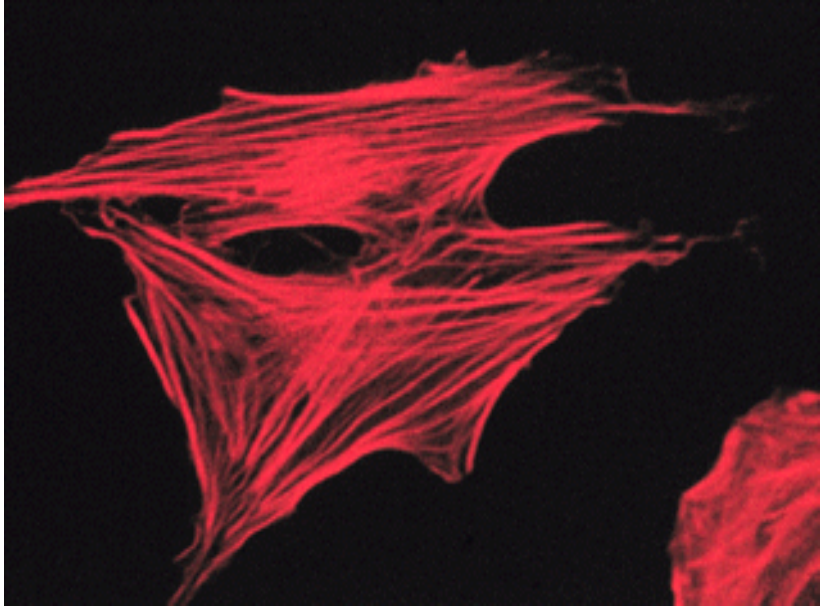


$$V_p = k_{off}\delta = \frac{k_{off} k_B T}{F_L}$$



Tubulin half life is nearly a full day

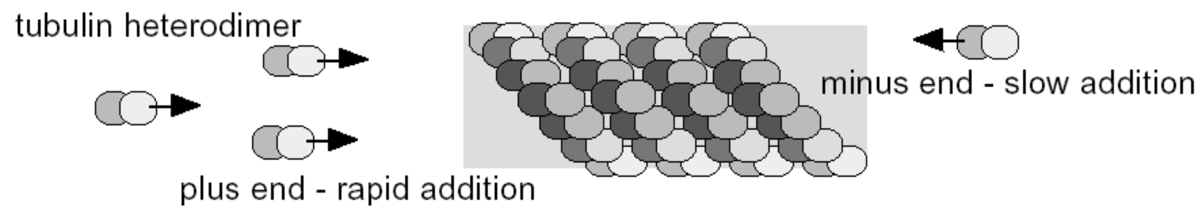
The half life of a given microtubule may be only 10 min.

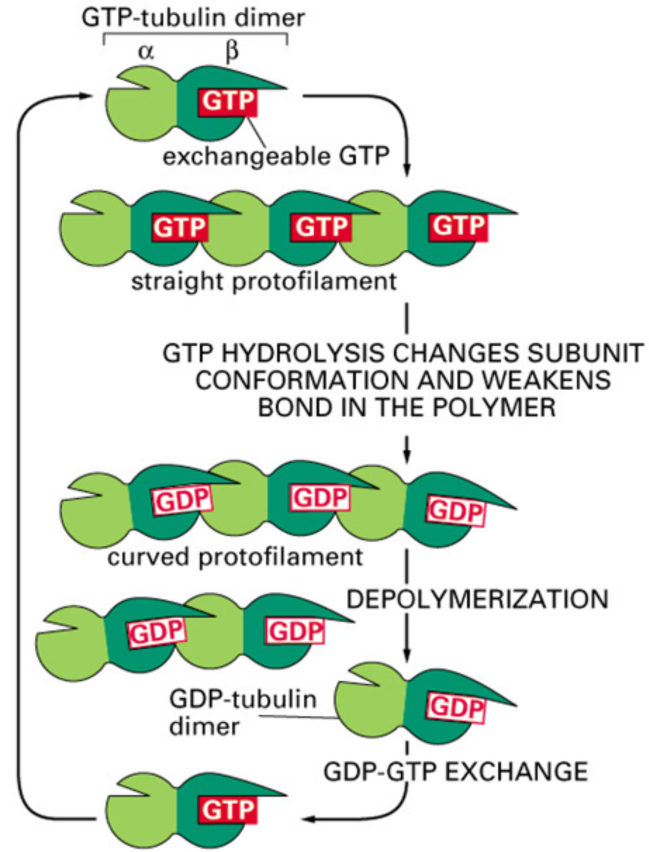


- ***A static microtubule grow in the presence of a non-hydrolysable GTP analogue - a tube has little tensions.***

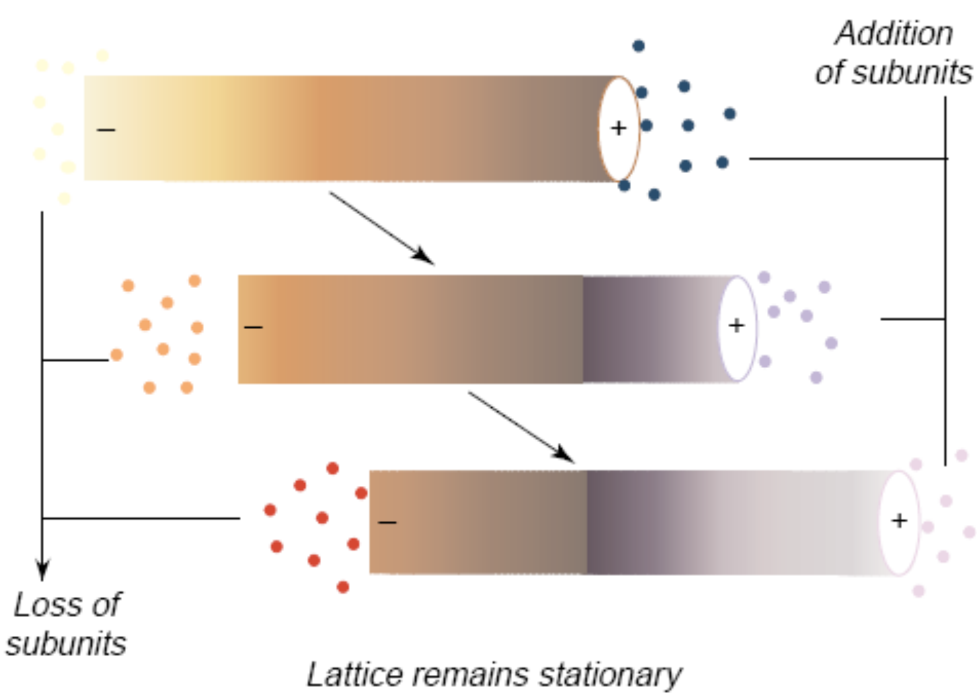
- ***A dynamic microtubule grow in the presence of GTP is a tube ready to crack.***

The tubulin concentration in cells is on the order of 20 μM





- Subunits on the fast end contain GTP.
- The slow end has an GDP containing subunit and depolymerizes.



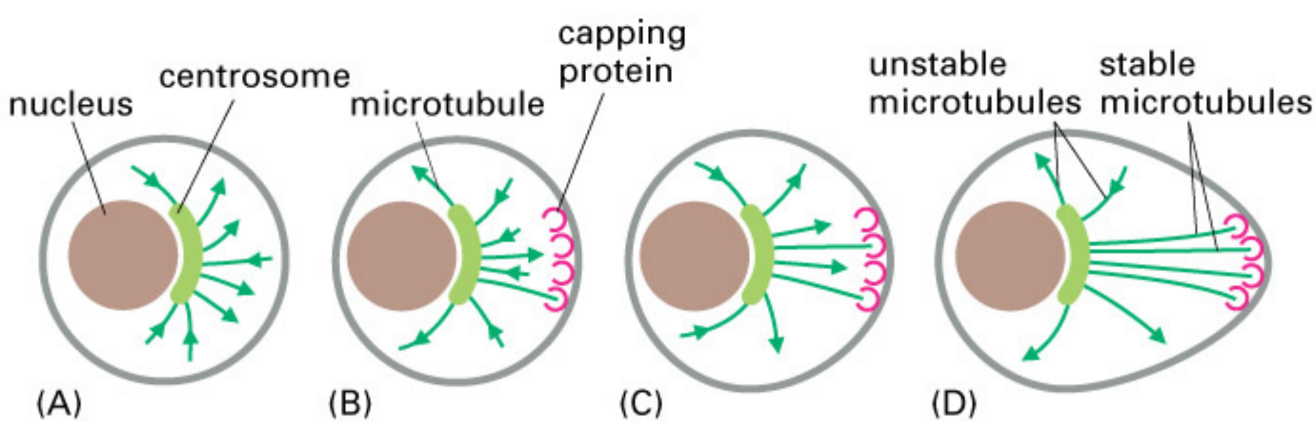
Treadmilling

Treadmilling does not involve actual movement of the microtubule lattice.

Dynamic instability: significance

- Allows 'random' searches
- Allows rapid re-arrangements of MTs
- Makes 'selective stabilization' (at tip) possible
- Can be locally regulated, to drive MTs into or out of an area
- Microtubule dynamics allow the cell to quickly reorganize the network when building a mitotic spindle
- Dynamics also allow microtubules to probe the cytoplasm for specific objects and sites on the plasma membrane - <u>search and capture</u>

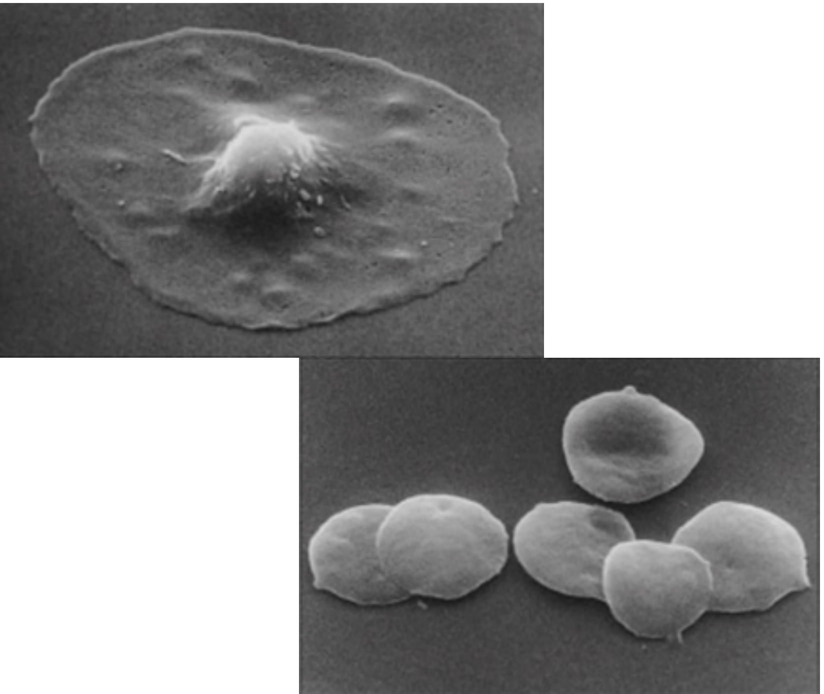
Search & capture during cell polarization

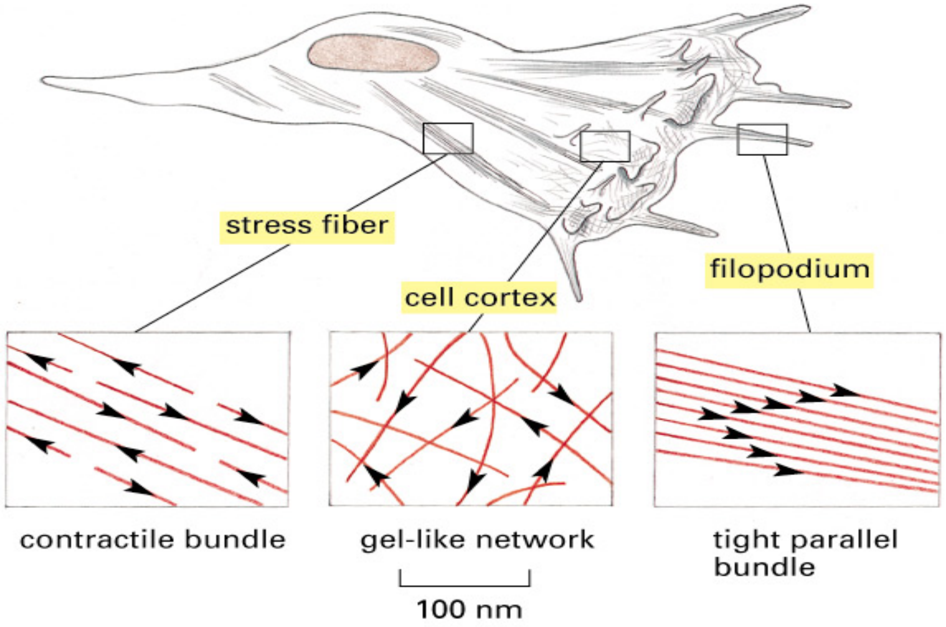


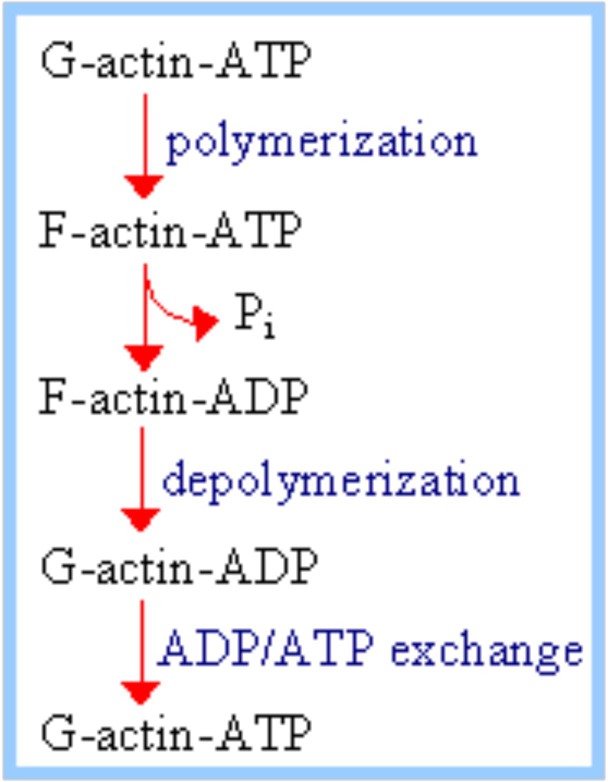
Actin based cell movement

- Wound healing – epidermal cells.
- Immune response – leukocytes – migrate to sites of infection.
- Development – neural crest cells; neuronal process extension.

- Cancer cell metastasis – malignancy determinant.



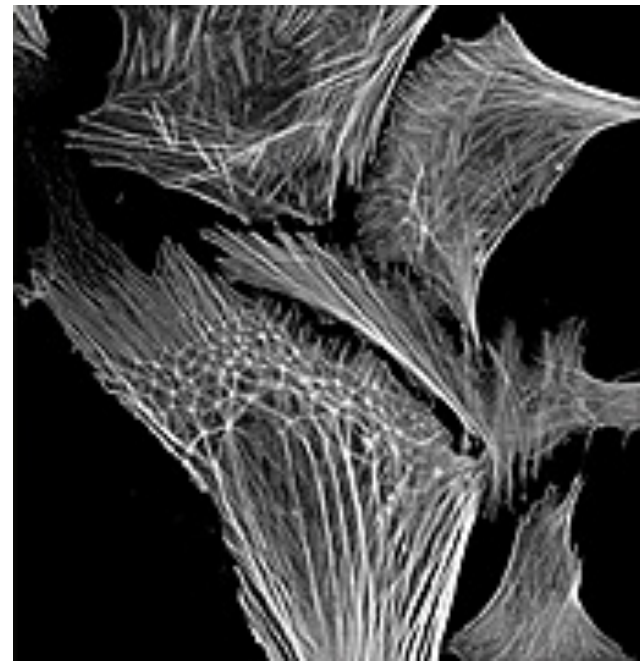


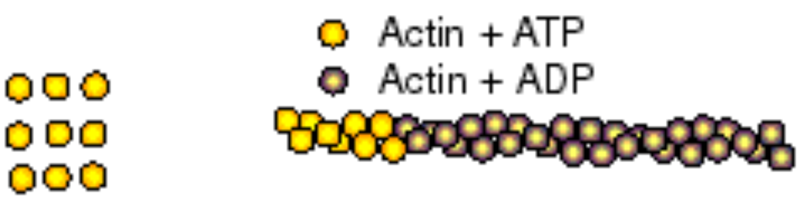


During growth, the energy supplied by ATP/GTP hydrolysis is stored in the lattice as mechanical strain.

This strain powers the fast shortening of disassembling microtubules.

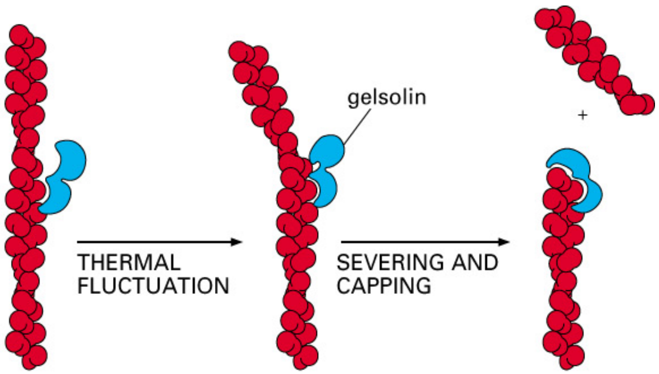
Fibroblasts-actin filaments

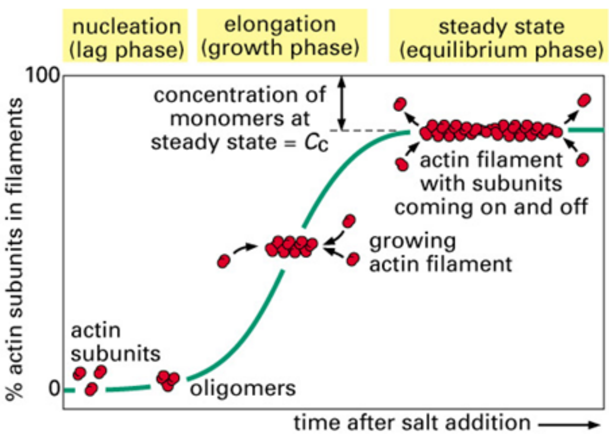


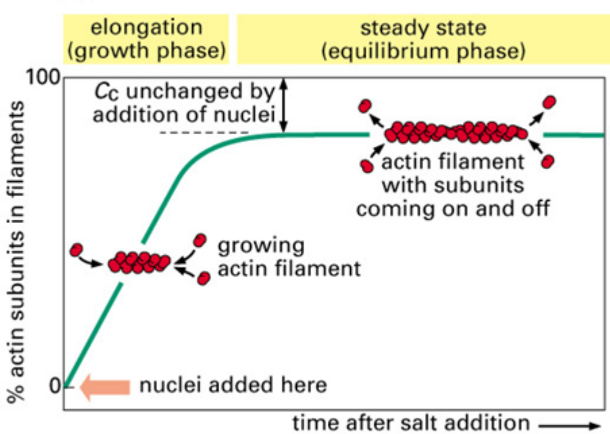


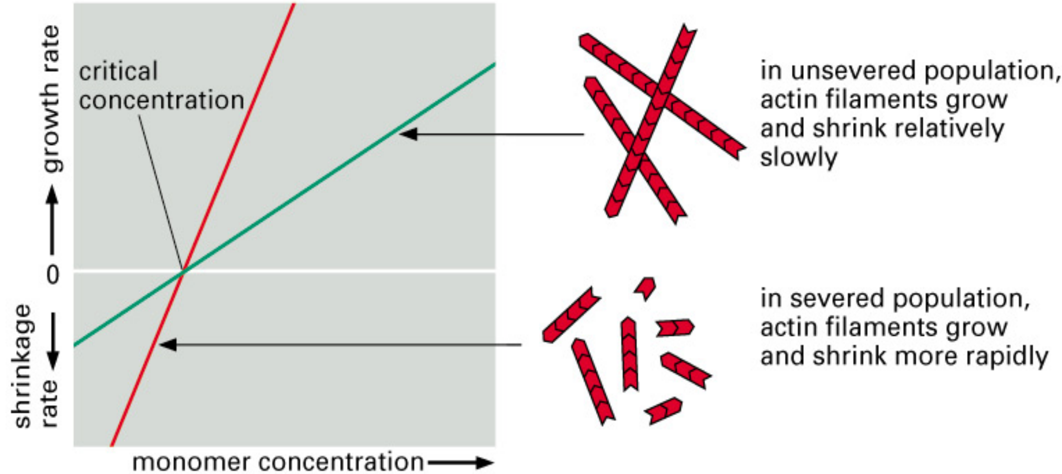
Dynamic instability and treadmilling are phenomena that require energy dissipation, and which could not emerge from a pure self-assembly process.

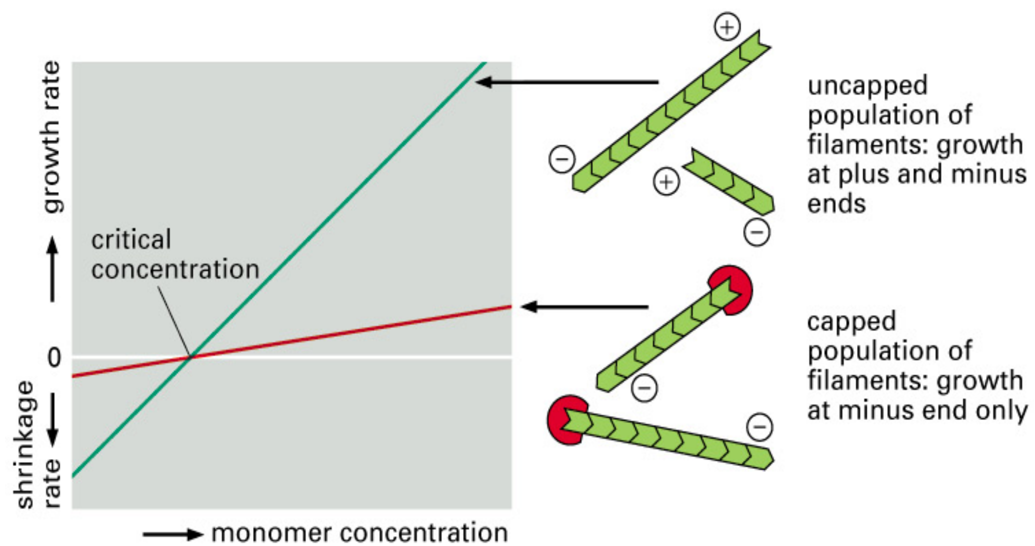
Regulation of polimerization rate



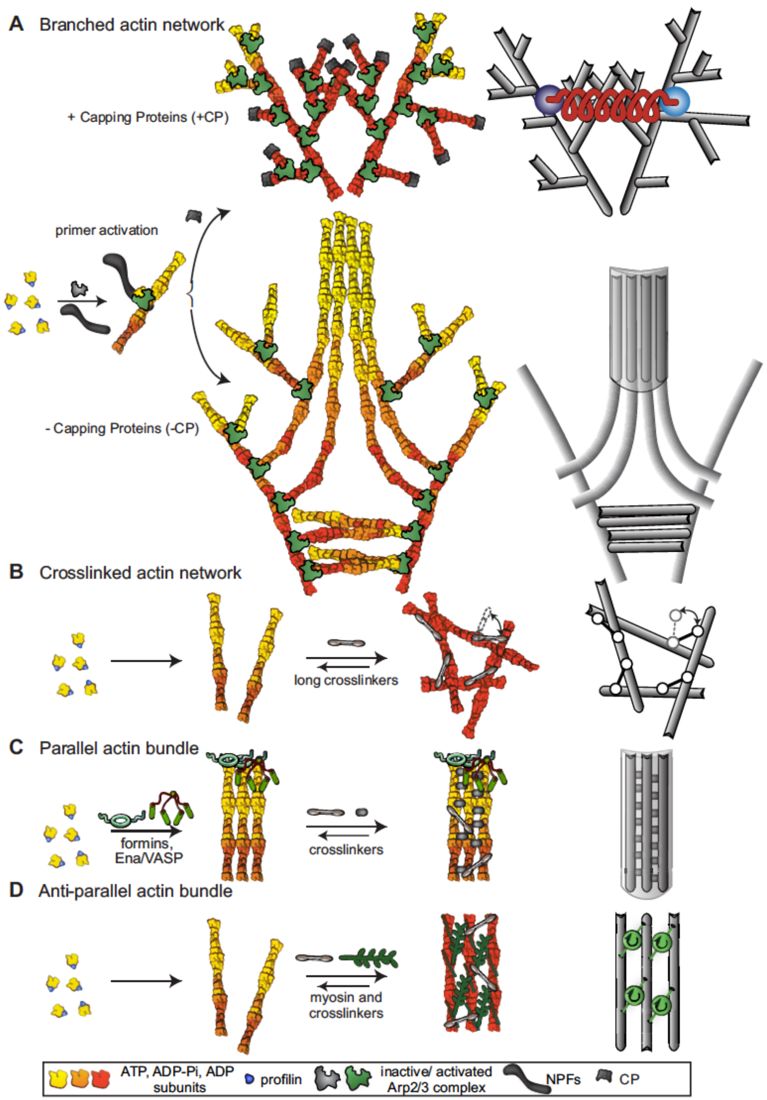


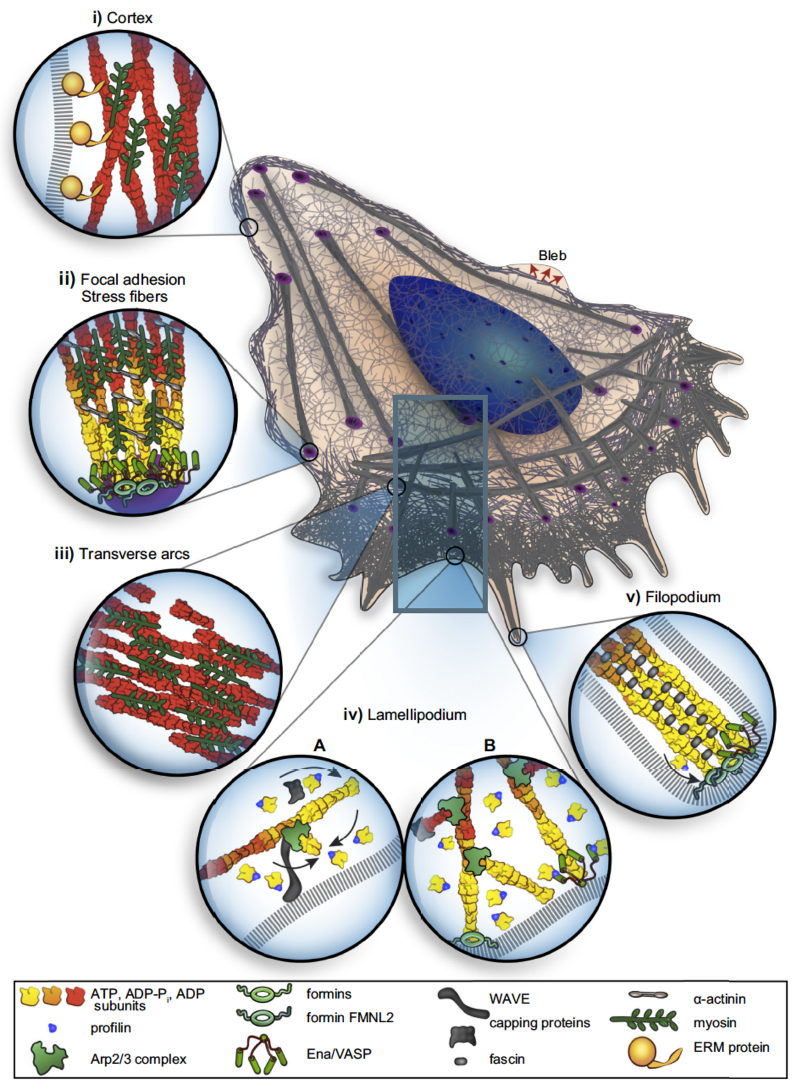




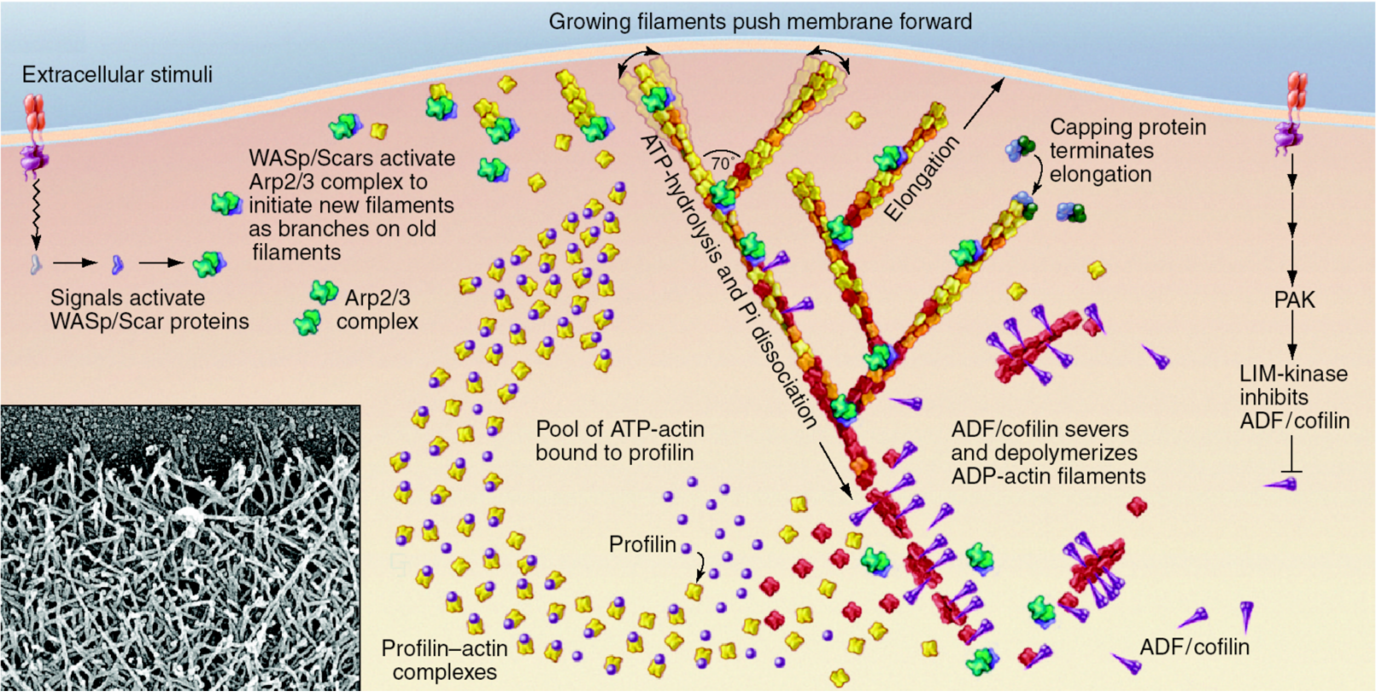


Distinct actin filament organizations and their mechanical description.

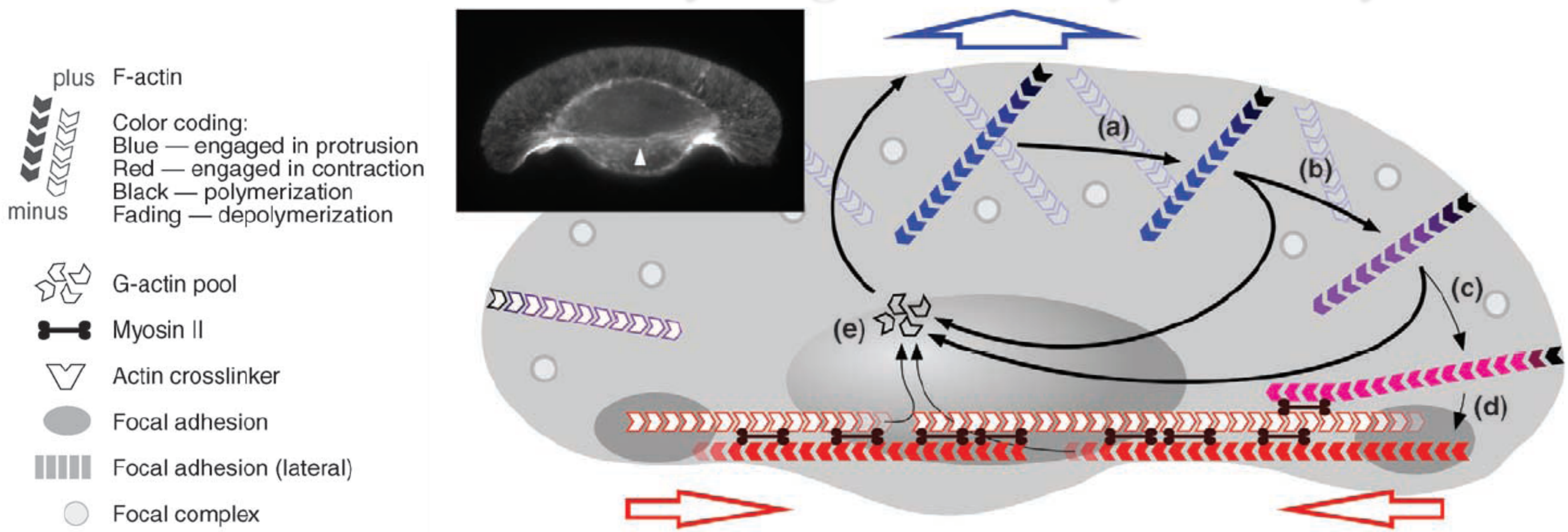




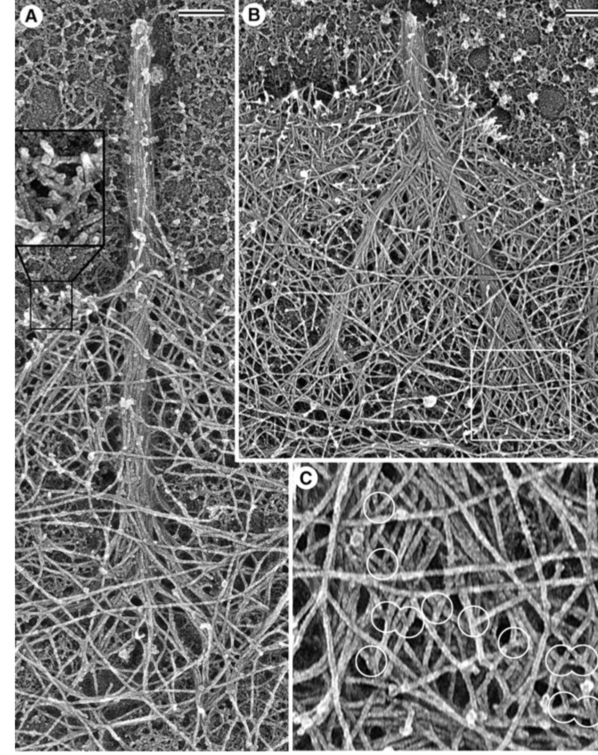
Actin-Binding Proteins Regulate the System



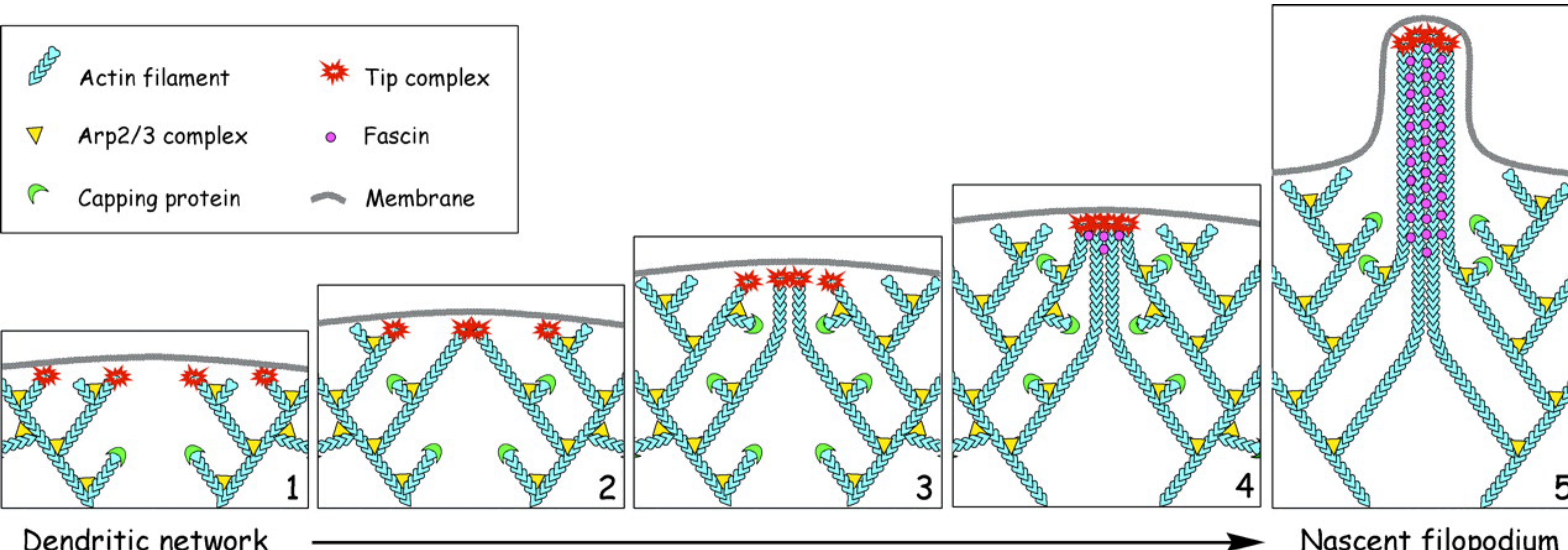
Filament recycling in keratocyte motility.



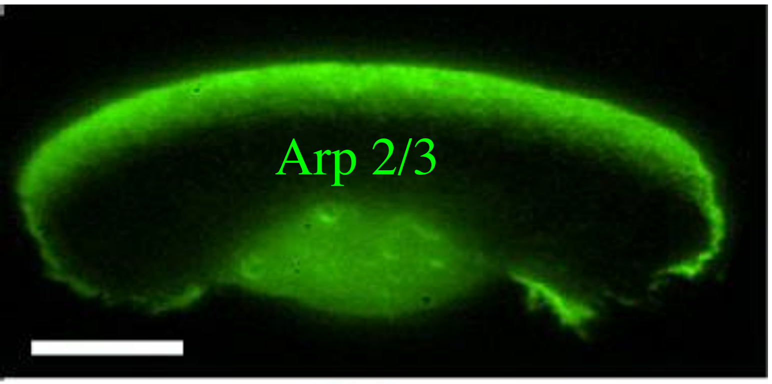
Filopodia are composed of long, unbranched actin filaments

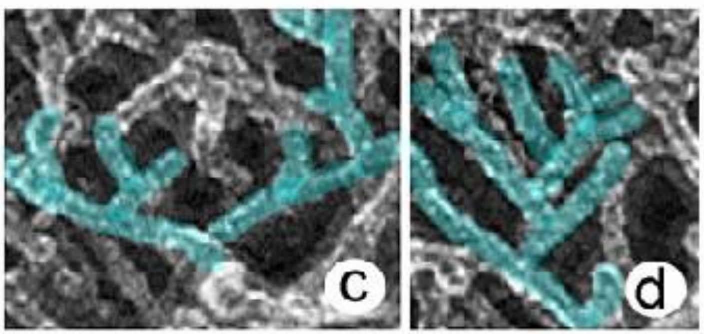


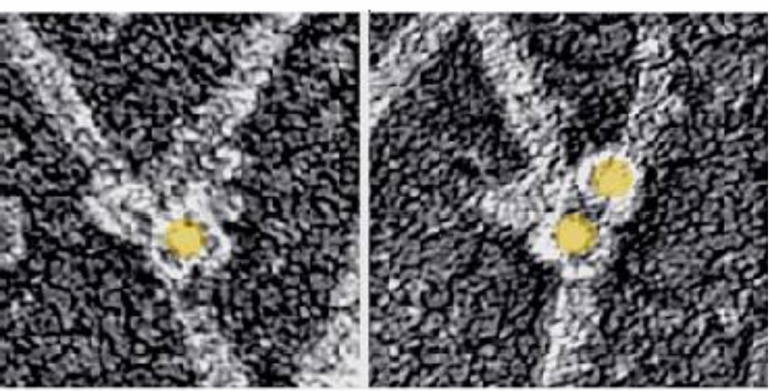
Generating movement
Actin dynamics in filopodia

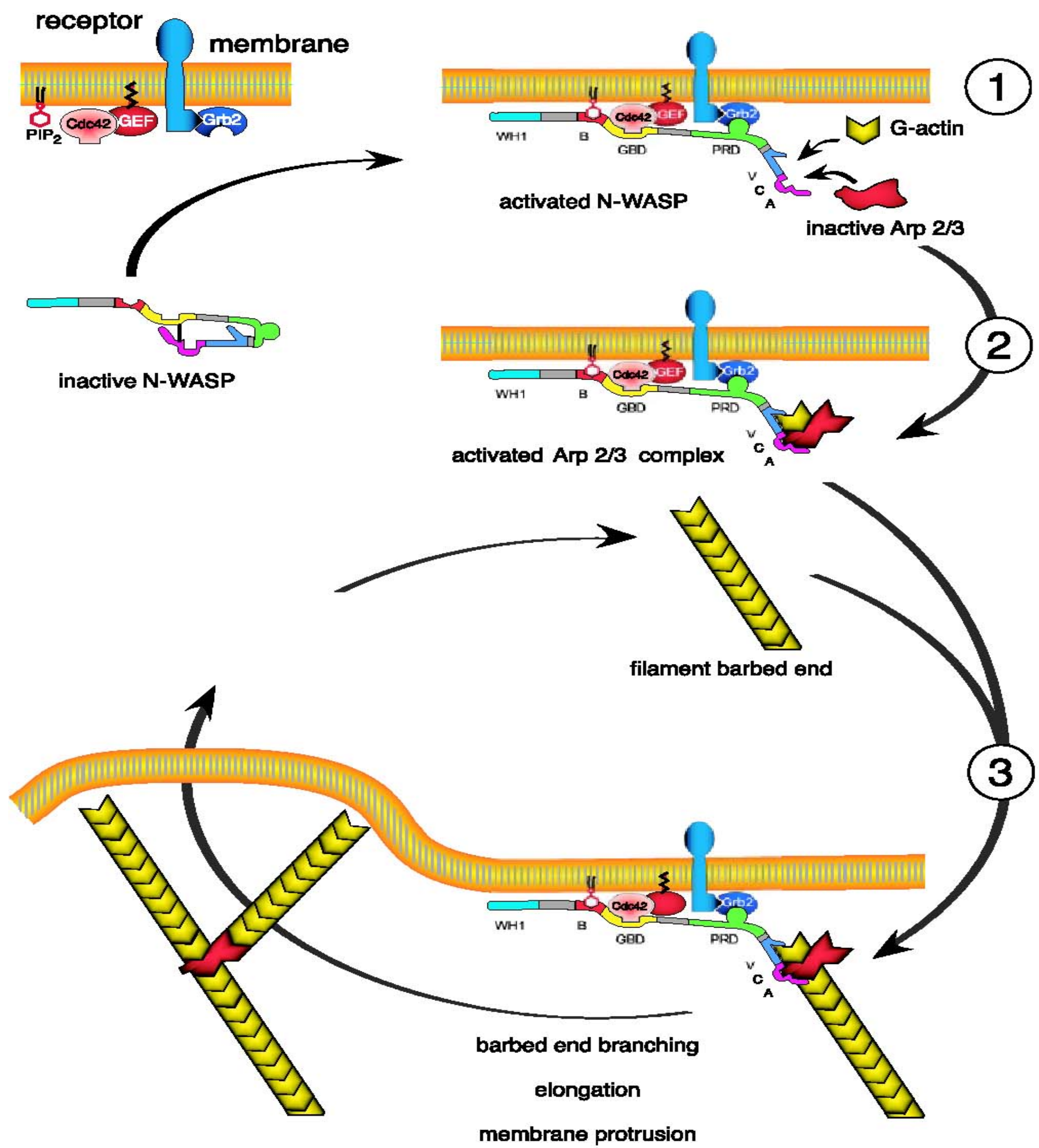


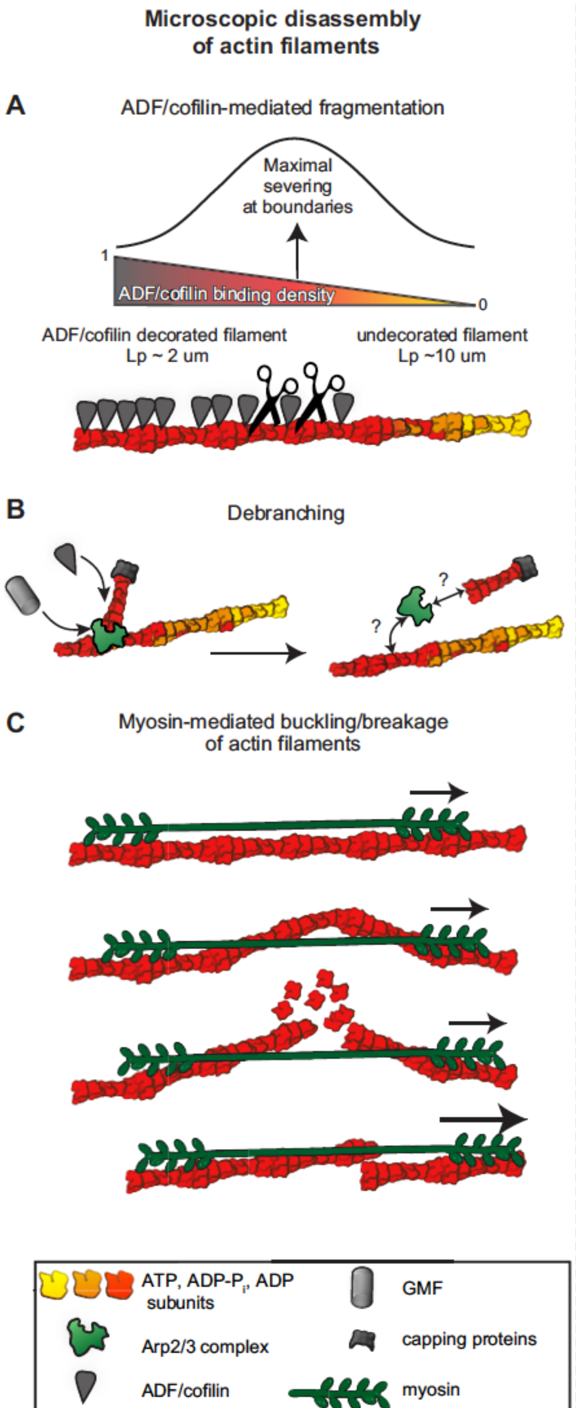
Dynamics of actin polymerization at the leading edge



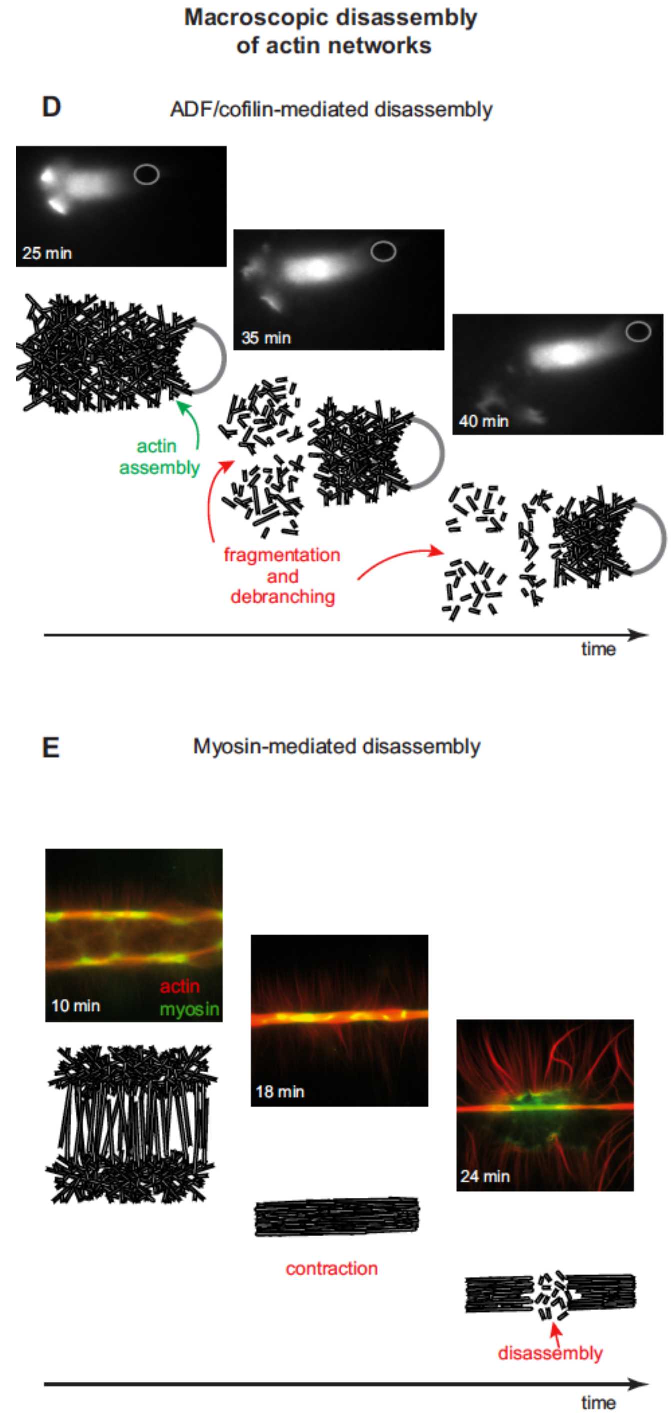




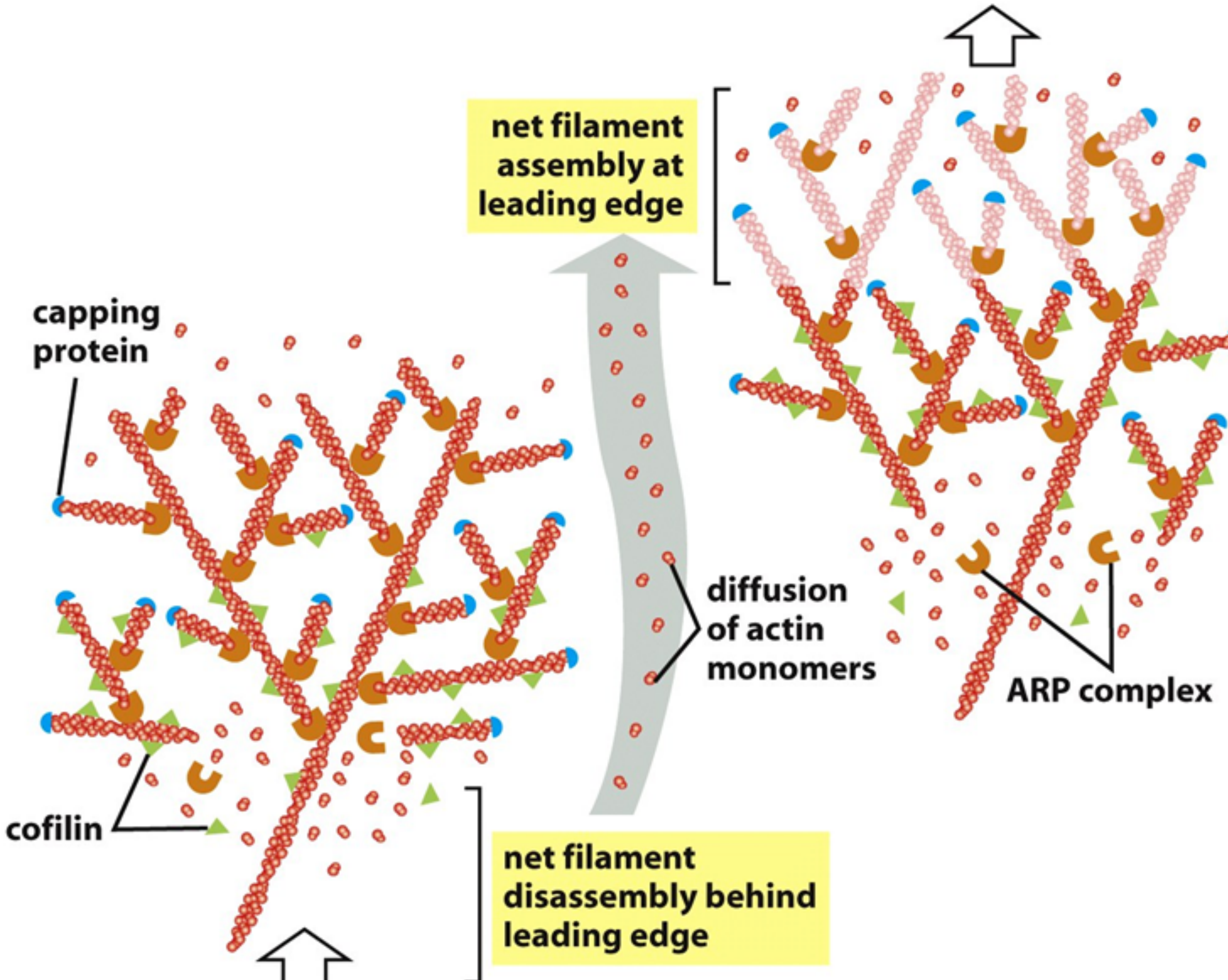




Remodeling and disassembly of dynamic actin structures.

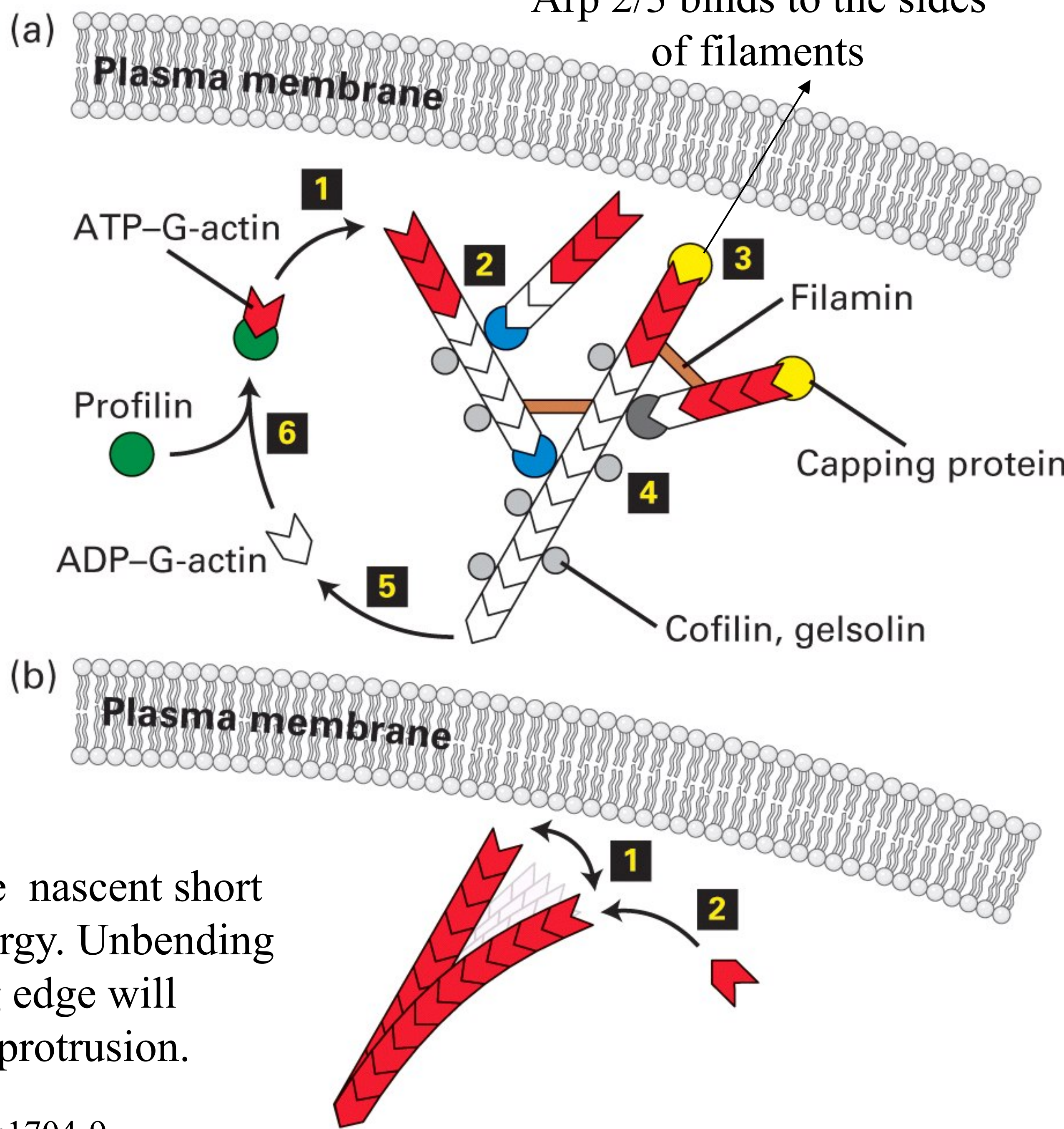


Polymerization based movement



"Elastic Brownian ratchet"

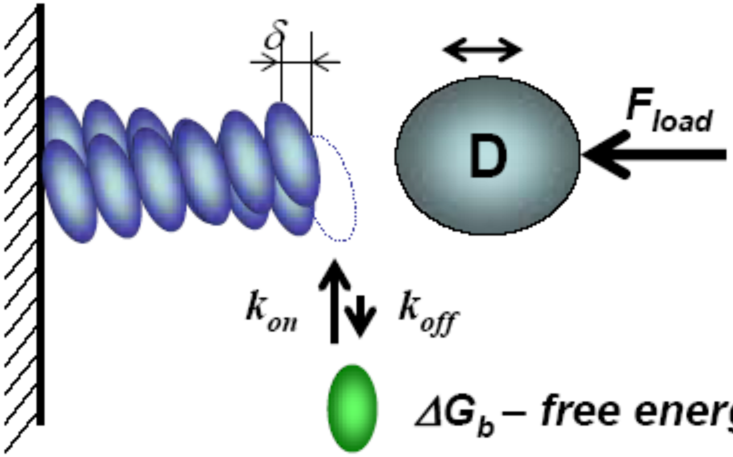
Arp 2/3 binds to the sides of filaments



The thermal energy bends the nascent short filaments, storing elastic energy. Unbending of the end against the leading edge will provide the driving force for protrusion.

Science. 2003 Dec 5;302(5651):1704-9.

Polymerization motors – ratchet mechanism



$k_{on}M$ – *polymer growth*

k_{off} – *polymer disassembly*

ΔG_b – **free energy of monomer binding**

$$\Delta G_{tubulin} = 5\text{–}10\ k_BT$$

The motor does not directly drive the load, but simply rectifies its Brownian diffusion.

$$V_p = \delta\left(k_{on}M^* \exp\left(-\frac{F_L\delta}{k_BT}\right) - k_{off}\right)$$

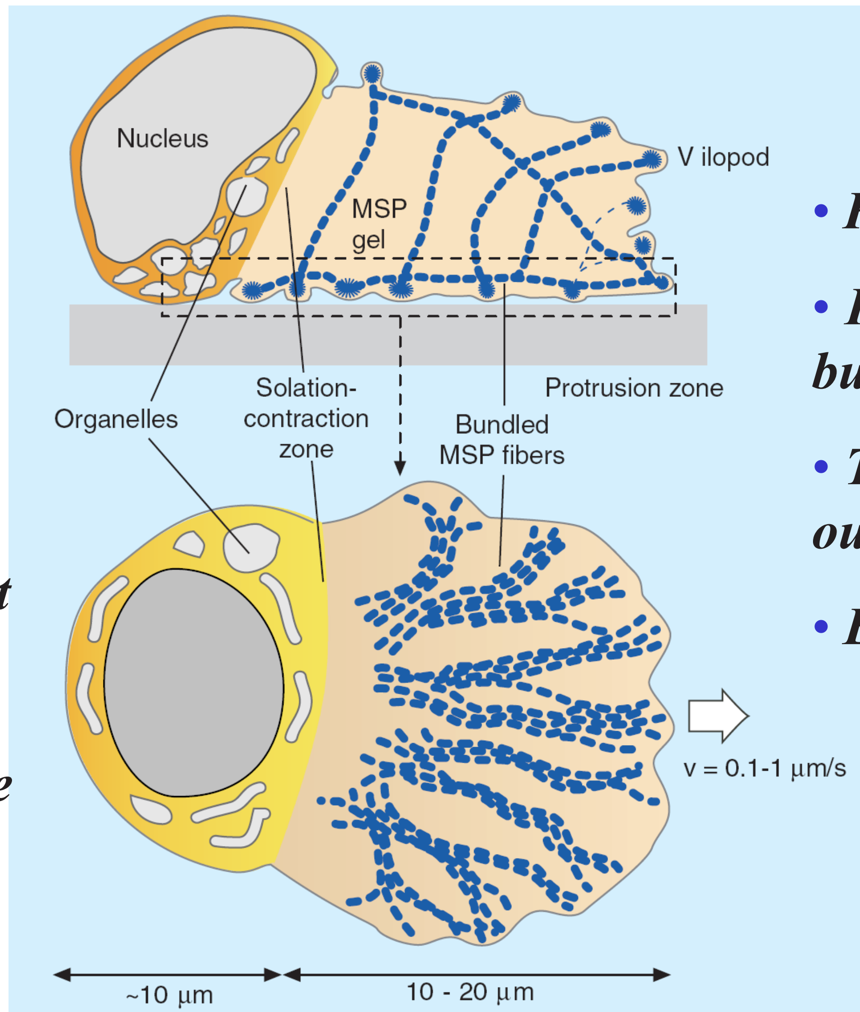
$$F_{stall} = \left(\frac{k_BT}{\delta}\right) * \ln\left(\frac{k_{on}M}{k_{off}}\right)$$

Then, characteristic stall force (V=0) is:

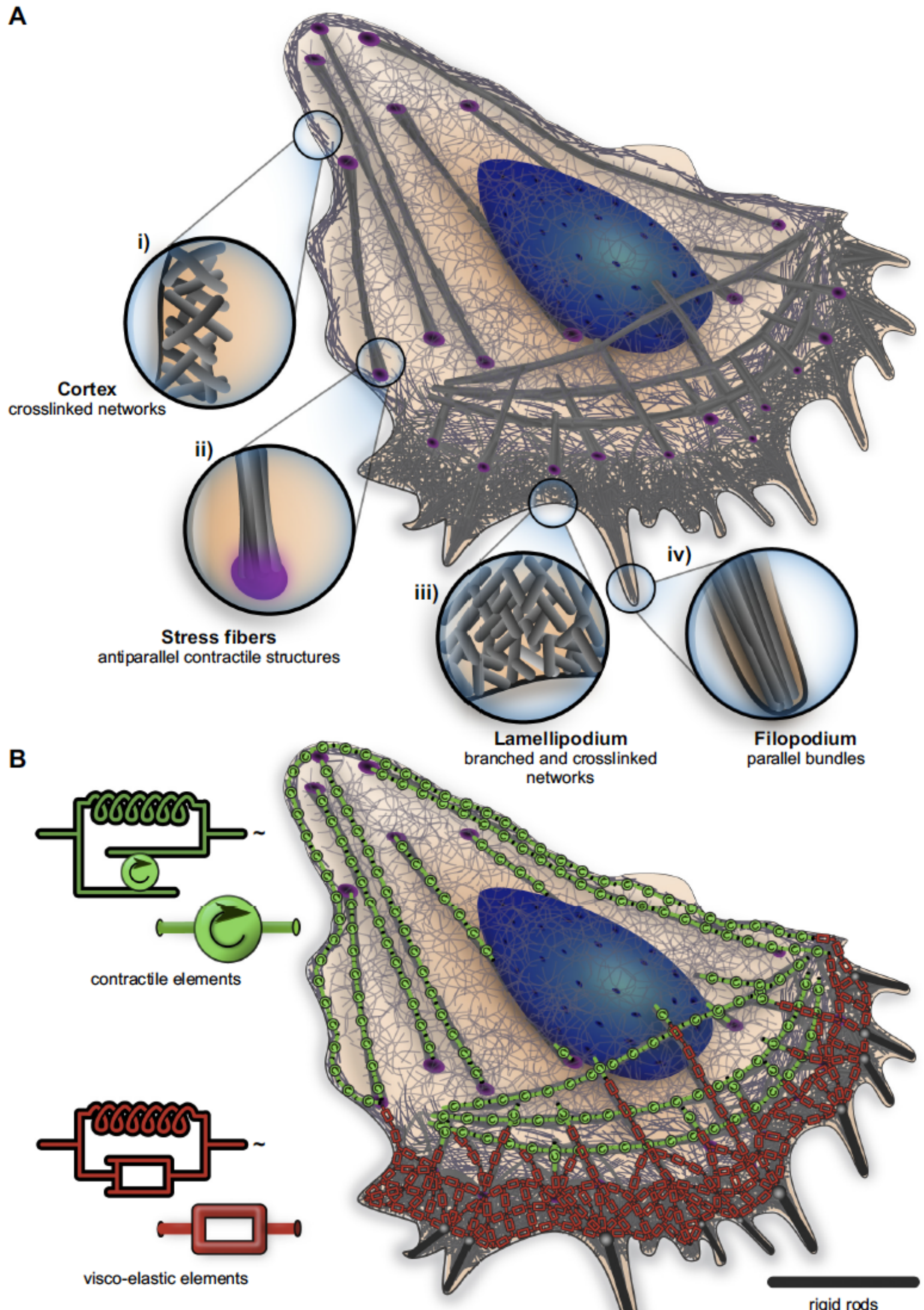
The directional movement

The perinuclear mitochondria generate an anterior–posterior pH gradient which regulates gelation and solation of the gel in the lamellipod.

- ***Low pH.***
- ***The interfilament interactions weaken.***
- ***The filaments unbundle.***
- ***Because the cell front adheres to the substratum, this provides the contractile force to pull the cell body forward.***



- ***High pH.***
- ***Filaments grow and bundle into thick fibers.***
- ***The cell front is pushed out.***
- ***Elastic energy is stored.***



Overlay of actin architecture and mechanics in the moving cell.

A: schematic representation of the cell with the different architectures.

B: overlay of the actin architecture and its mechanical profile.

The red rectangles are the shock absorbers (dashpots) that represent the actin network, while the green circles are active springs due to myosin motor activity.

Listeria utilizes the power of actin polymerization for intracellular movement

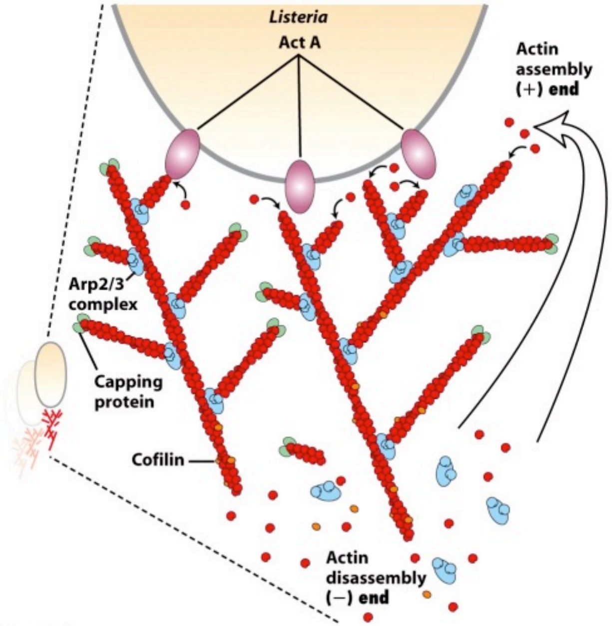


Figure 17-17c
Molecular Cell Biology, Sixth Edition
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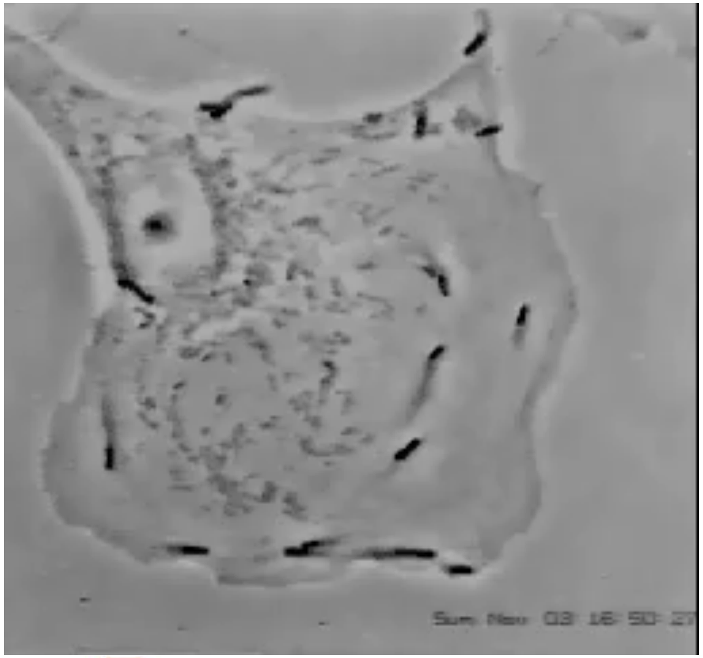

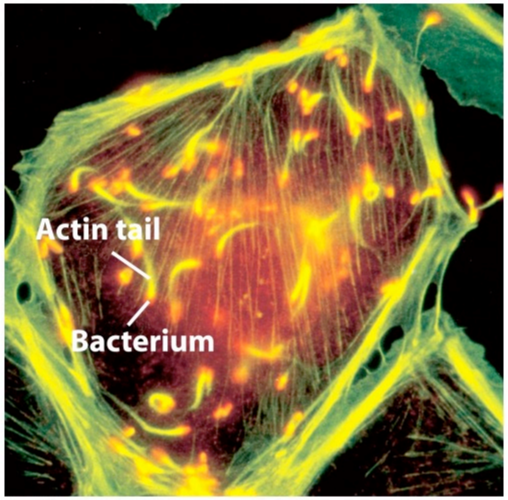

Figure 17-17a
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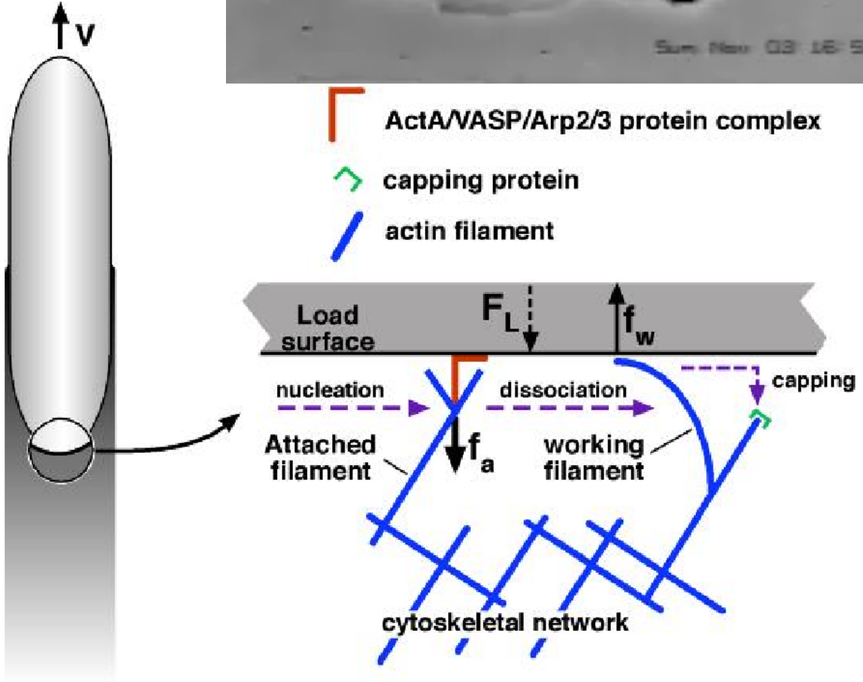
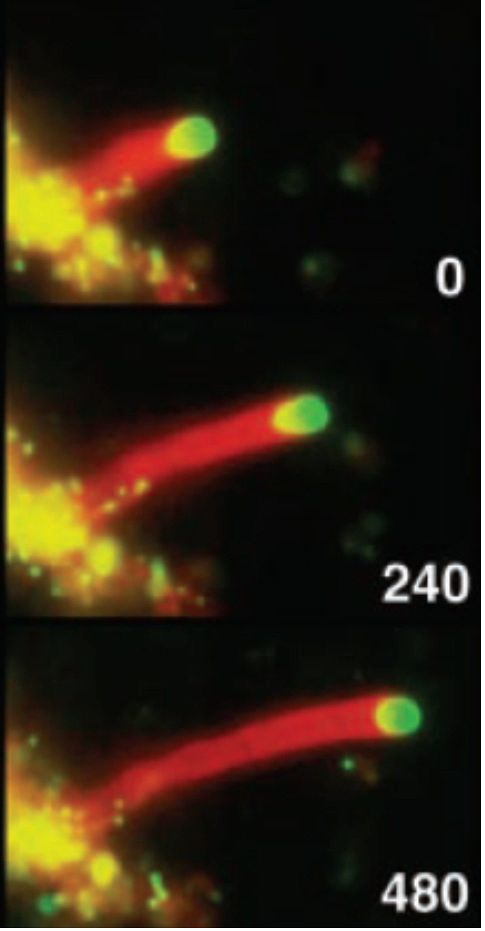
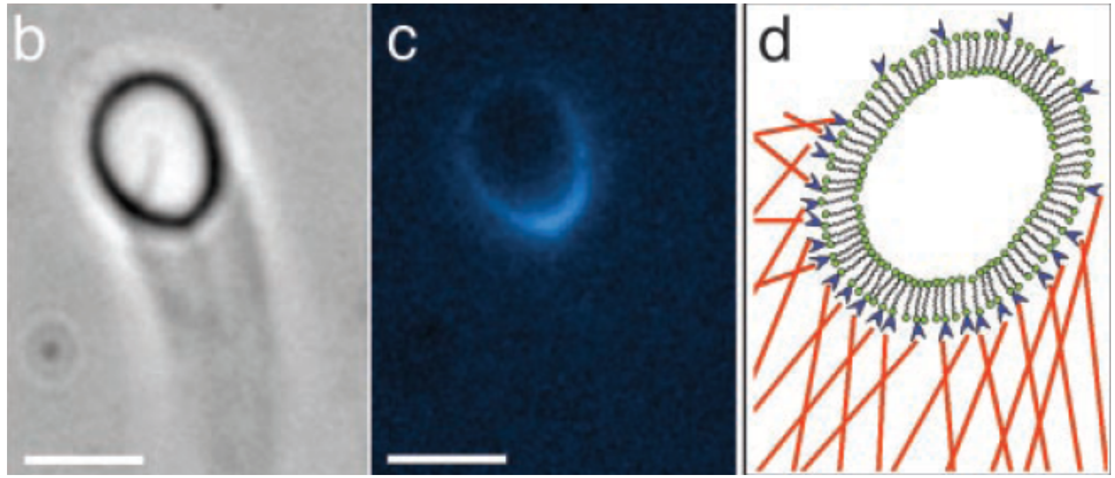
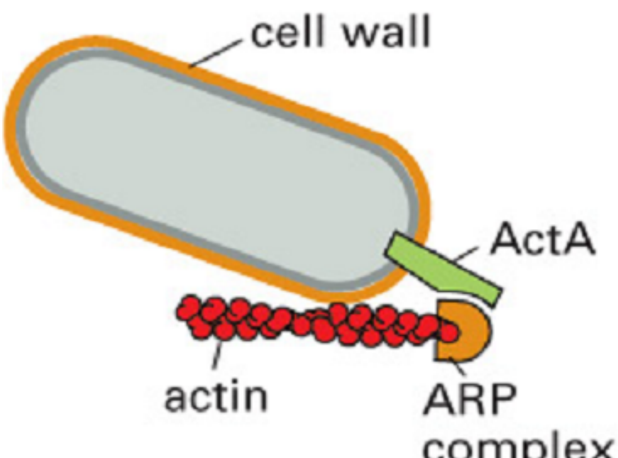


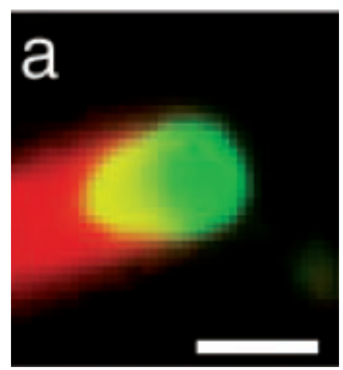


Actin-driven motility of lipid vesicles coated with ActA.

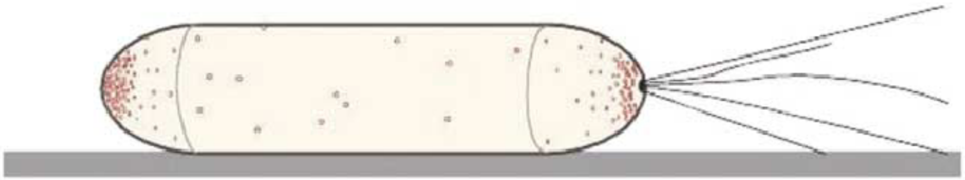
Fluorescently labeled ActA molecules (blue)







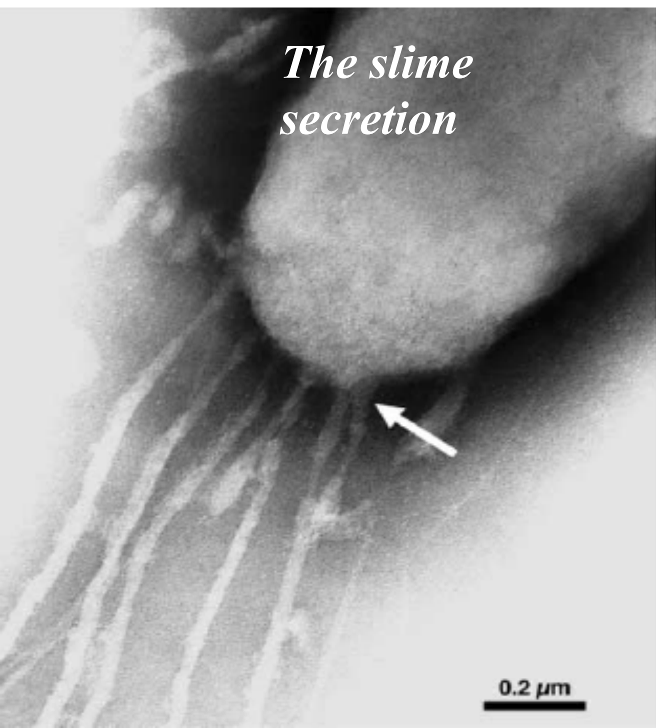
A moving vesicle deformed by actin.

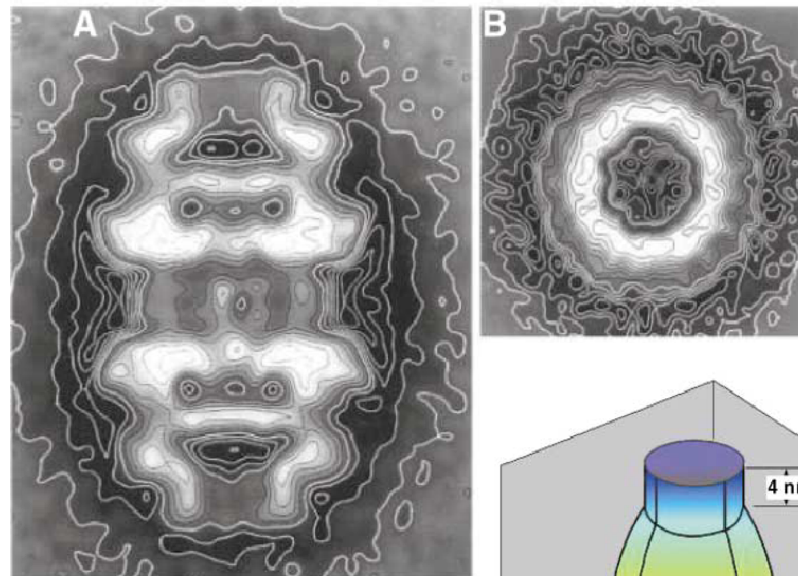


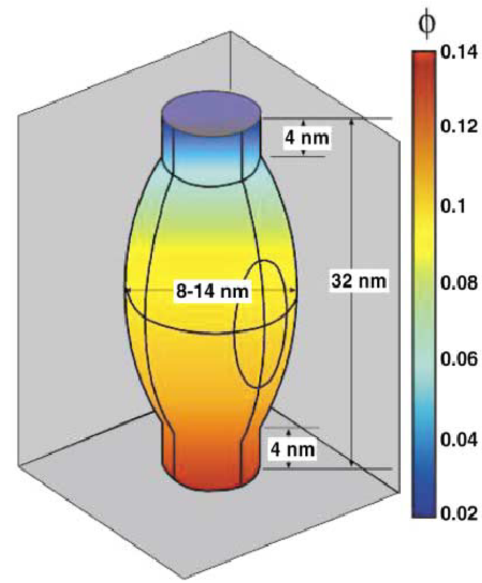
In *M. xanthus* nozzles are clustered at the two cell poles, pili at one pole.

S motility is generated by the pili, which extend, attach to nearby cells, and then retract, pulling the cells together.

A motility is driven by the secretion of mucilage from the nozzles – gliding.



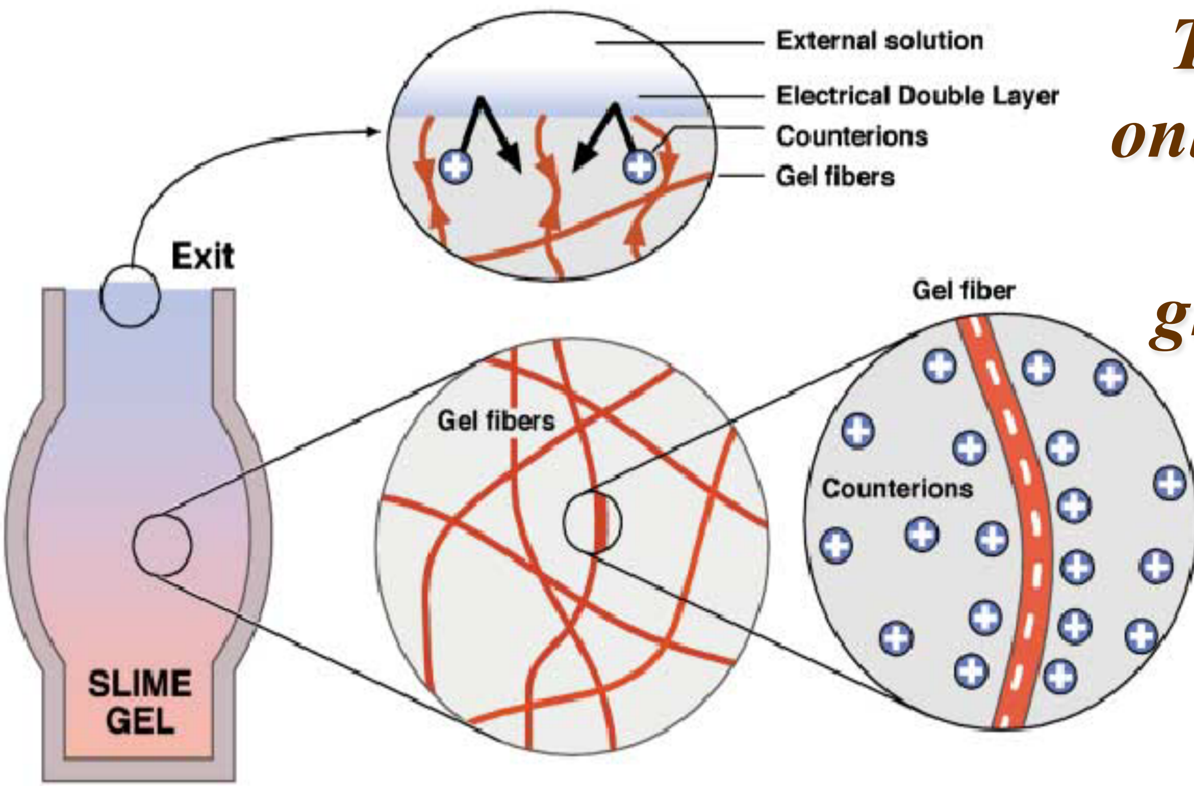




Pore complex in
P. uncinatum

A Model for Nozzle Function

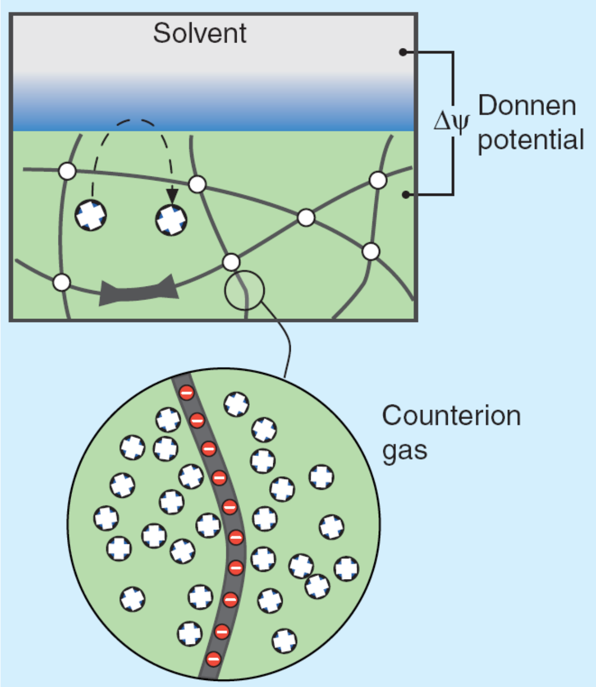
- ***Slime is imported into the proximal end of the nozzle.***
- ***Slime is a creosslinked polyelectrolyte gel (crosslinked fibers).***
- ***A Donnan potential is generated by the mobile counterions.***
- ***The slime is hydrated by water that flows into the nozzle causing the slime to swell.***



The nozzle hydrates only through the nozzle exit – a hydration gradient exists along the nozzle.

Force generation in polyelectrolyte gels

Negatively charged filaments are surrounded by positive counterions that are confined inside gel by the Donnen potential.



The swelling pressure

$$\Pi = \Pi_{Entropic} + \Pi_{Ion} + \Pi_{Elastic} + \Pi_{Interactions}$$

$\Pi_{Entropic}$ – the gel fibers tend to diffuse outward.

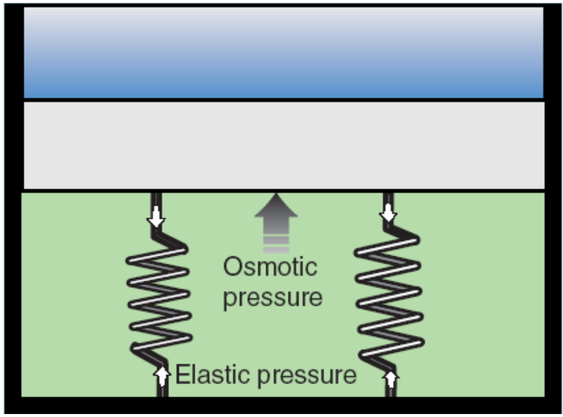
Π_{Ion} – a polyelectrolyte gel contains diffusible counterions. Water will diffuse in.

$\Pi_{Elastic}$ – Gel elasticity tends to resist its tendency to expand outwards.

$\Pi_{Interaction}$ – An attraction between the gel fibers.

$$\Pi_{Entropic} + \Pi_{Interactions} << \Pi_{Ion} + \Pi_{Elastic}$$

$$\Pi_{swell} = \Pi_{osm} - \Pi_{elas}$$



At equilibrium, the elastic tension just counterbalances the osmotic pressure of the gel counterions.

If the cross-links are partially removed – the gel partially ‘solates’ – the elasticity of the gel weakens, allowing the osmotic pressure to expand the gel to a larger volume.

The force of expansion is in the range of hundreds of pN per square micron, and the expansion would take a few tenths of a second for a micron sized ball.