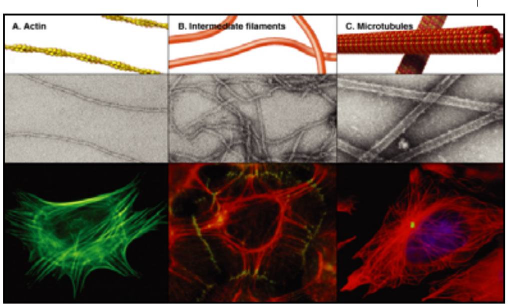
Actin Cytoskeleton

Cytoskeleton

- Cytoplasmic array of fibers
- Maintains cell structure and disperses tensile forces
- Needed for movement both within the cell and of the entire cell
  - Organelle transport
  - Mitosis
  - Phagocytosis
  - Wound healing
  - Morphogenesis

Three types of cytoskeletal filaments



Cytoskeleton

- Three types of cytosolic filaments
  - Microfilaments – actin filaments
  - Intermediate filaments
  - Microtubules
- All are protein polymers
- Dynamic structures with filaments able to grow and shrink rapidly

Actin filaments (a.k.a microfilaments)

- Are one of the three protein filament systems that comprise the cytoskeleton
- Major system for cell motility
- Also form tracks for myosin directed movements

Actin

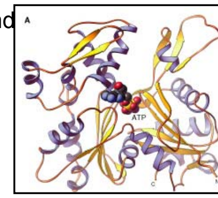
- Humans (6 actin genes)
- α actins - four of them
  - in muscle cells
  - associated with contractile structures
- β and γ actins
  - non muscle cells
  - localized at the front of moving cell

Actin

- Most abundant protein in eukaryotic cells - 10% mass
- Encoded by highly conserved gene family
- Exists as a globular monomer - G actin or linear polymer - F actin
  - G actin monomers polymerize to form strings of F actin

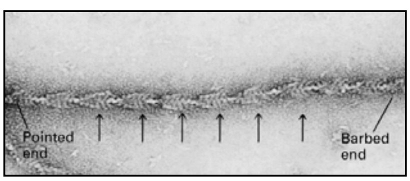
Structure of actin

- Two domains
- Each molecule has Mg^{2+} and ATP or ADP in the ATP fold
  - stabilizes the molecule
- Behaves like a hinge
  - Barbed end
  - Pointed end



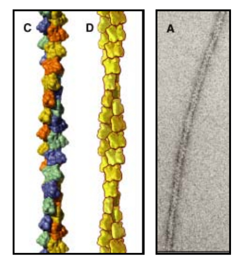
Actin has structural and functional polarity

- Actins fit inside each other
- In a filament all subunits have the same polarity



F actin or actin polymer

- Linear chain of G actins
- Twisted strings of beads on a helical structure

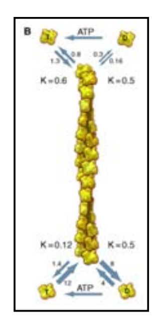


Polymerization of actin

- Actin **self-assembles** by means of molecular interactions
- Actin grows and shrinks by addition and loss of subunits **at both ends**
- Process is reversible
- No need for accessory proteins
  - Addition of salts (Mg^{2+}, Na^{+}, K^{+}) can polymerize actin *in vitro*

Kinetics of actin polymerization

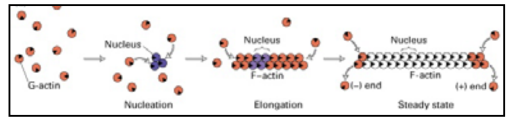
- Polymerization is accompanied by hydrolysis of ATP
  - But hydrolysis is not necessary for polymerization
- Barbed end elongates 5-10x faster than a pointed end



Actin polymerization in vitro proceeds in three steps

- **Nucleation** - aggregation of actin molecules into short oligomeres
  - Seed or nucleus - a 3-4 subunit aggregate
  - Dimer is very kinetically unfavorable
  - Slow (lag period)
- **Elongation** - addition of actin subunits
- **Steady state** – dynamic, addition and loss

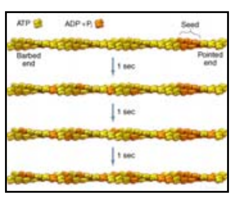
Polymerization of actin filaments



- Critical concentration – a concentration of actin monomers when addition and loss are the same – it looks as actin was stable

ATP hydrolysis

- Free actin is preferably bound to ATP
- After incorporation into filament ATP is irreversibly hydrolyzed to ADP
- ATP hydrolysis modifies the behavior of actin filaments
- Determines the affinity of actin for actin binding proteins

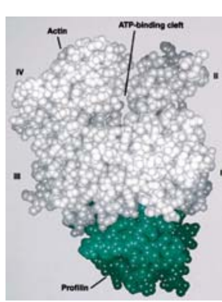


Actin monomer binding proteins

- Control pool of unpolymerized actin
- Help regenerate the nucleotide bound to actin
  - When actin loses the subunit it is bound to ADP and has to be changed to ATP
- Two proteins
  - Profilin
  - Thymosin $\beta 4$

Profilin

- Cytosolic protein
  - Functions like a buffer
  - Binds to ATP-G actin in 1:1 stable complex
  - Buffers only 20% (concentration of profilin = only 20% concentration of actin)



Profilin

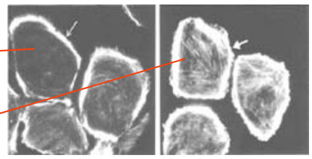
- Inhibits addition of monomers to pointed (slow-growing) end effectively making the barbed end to grow faster
  - Interacts with second messengers - place for controlling of actin cytoskeleton by membrane receptors
- After addition profilin dissociates from the complex
- Helps in conversion of ADP actin back to ATP bound form

Thymosin β4

- Abundant in cytosol - 2 x concentration of actin
- Binds to ATP-G-actin in 1:1 complex preventing polymerization

Thymosin β4 inhibits polymerization of actin

control

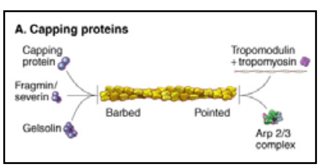


Actin capping proteins

- Preferably bind to polymerized actin
- Permit growth only at the opposite end
- Stabilize the filament
  - If a filament is capped at both ends it is effectively stabilized
- Controlled by second messenger pathways

Actin capping proteins

- Some of them cap barbed end – slow the growth
- Some of them cap pointed end – speed up the growth because there is no loss



Actin severing proteins

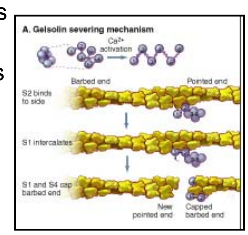
- Control of actin filaments lengths
- **Break filaments into shorter fragments**
- Change the conformation of the subunits causing strain and breakage of the bonds
- Increase the turnover of the filament

Actin-severing proteins

- Gelsolin and cofilin
- Regulated by several second messenger pathways
- Process is calcium dependent

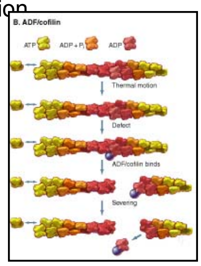
Gelsolin

- Binds to the side and breaks the filament
- After breaking gelsolin stays attached to barbed end and prevents addition of actin subunits (capping)
- Pointed ends are uncapped and shortened



Cofilin

- Bind to "defective" conformation of actin
- Destabilizes and breaks the filament



Toxins can disrupt the monomer-polymer equilibrium

- Cytochalasin D
  - A fungal alkaloid
  - Binds to the barbed end of F actin
  - Blocks addition of new G actins
- Latrunculin
  - Toxin from sponges
  - Binds G actin
  - Inhibits actin from adding to a filament

Both toxins shift equilibrium toward depolymerization

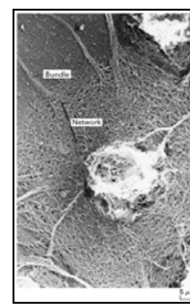
Toxins can disrupt the monomer-polymer equilibrium

- Phalloidin
  - fungal toxin
  - binds at the interface between subunits in F actin
  - locks adjacent subunits together
  - fluorescent labeled phalloidin is used in microscopy to visualize F actin

Prevents depolymerization actin – actin stays polymerized

Organization of actin cytoskeleton

- Organized into bundles and networks of filaments
- Bundles and networks are held together by actin cross-linking proteins



Organization of actin cytoskeleton

bundles

- closely packed parallel arrays
- usually connected by fascin or similar actin cross-linking proteins

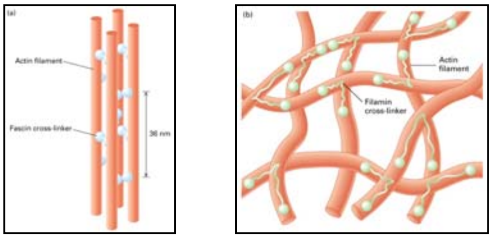
networks

- loosely packed criss-crossed fibers
- usually connected by filamin or similar actin cross-linking proteins

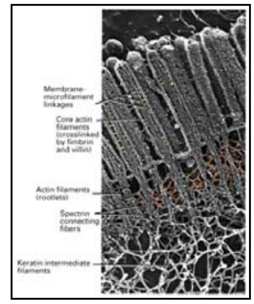
Actin cross-linking proteins

- Hold together actin filaments
- Have two binding sites - one for each filament
- Short cross-linking proteins support bundles
- Long cross-linking proteins support networks

Actin cross-linking proteins bridge actin filaments

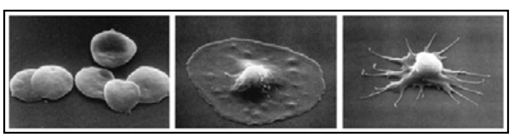


Actin bundles support projections of cell membrane



Change of shape of platelets during blood clotting

- During blood clotting, platelets change shape due to changes in the actin cytoskeleton

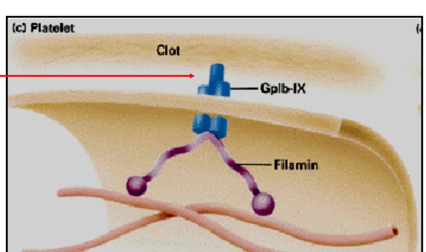


Platelet cytoskeleton

- A network of actin filaments is attached to the integral membrane glycoprotein complex Gp1b-IX by filamin (actin cross-linking protein)
- Gp1b-IX is also a receptor for two blood clotting proteins

Platelet cytoskeleton

Gp1b-IX is also a receptor for two blood clotting proteins



Muscle cell cytoskeleton

- Dystrophin (actin cross-linking protein) attaches actin filaments to the membrane
- Duchenne muscular dystrophy - lack of functional dystrophin – membrane is not properly supported and easily damaged