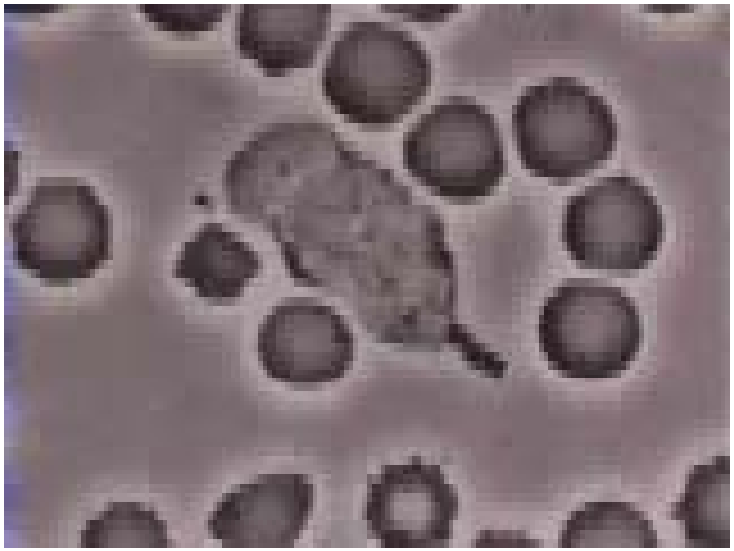
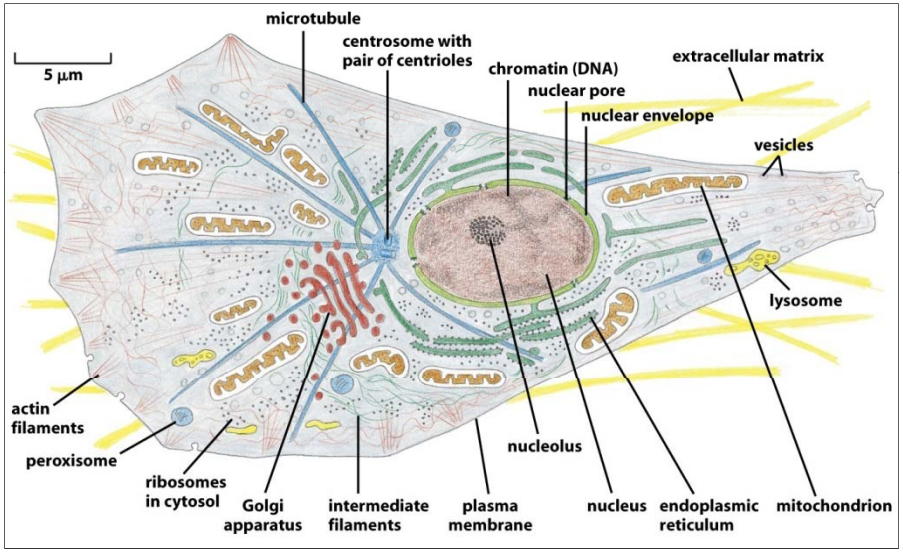
Engineering Molecular Cell Biology
Lecture 8, Fall 2010

The Cytoskeleton (I)

Chapters 33, 34, 35



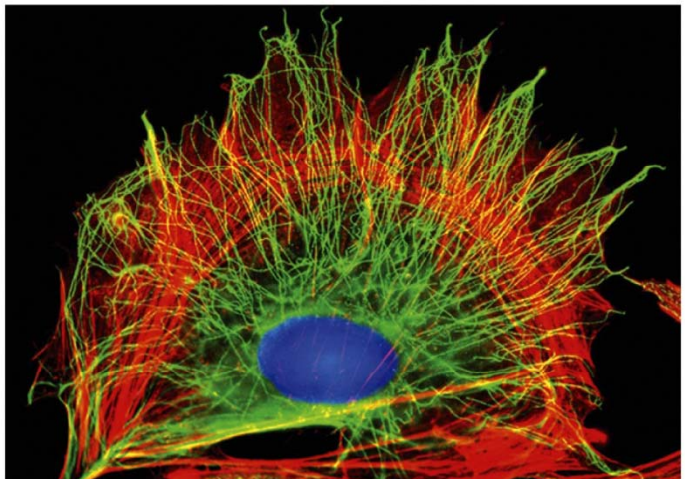

microtubule
centrosome with pair of centrioles
5 μm
chromatin (DNA)
nuclear pore
nuclear envelope
extracellular matrix
vesicles
lysosome
actin filaments
peroxisome
ribosomes in cytosol
Golgi apparatus
intermediate filaments
plasma membrane
nucleolus
nucleus
endoplasmic reticulum
mitochondrion

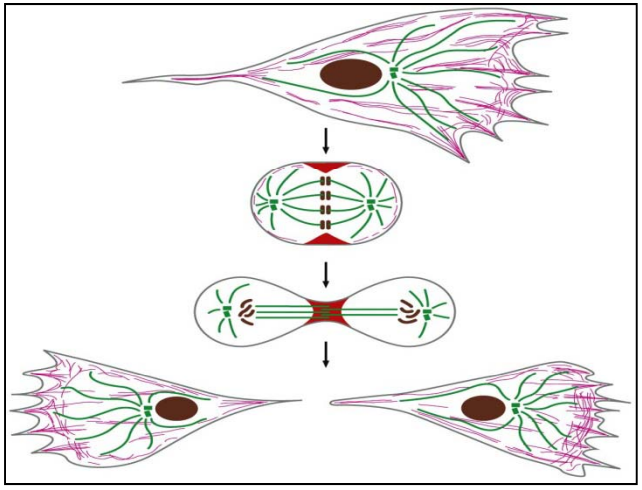
Outline

- Overview
- Actin and its accessory proteins
- Microtubule and its accessory proteins
- Centrosomes
- Intermediate filaments and its accessory proteins

The Cytoskeleton (I)

- Three classes of filaments
  - actin: stress fiber; cell cortex; filopodium
  - microtubule: centrosome
  - intermediate filaments





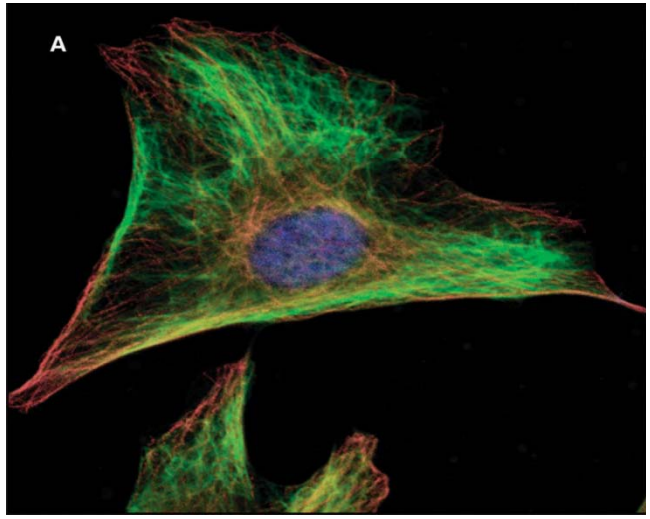
Red: **actin**
Green: **microtubule**

The Cytoskeleton (II)

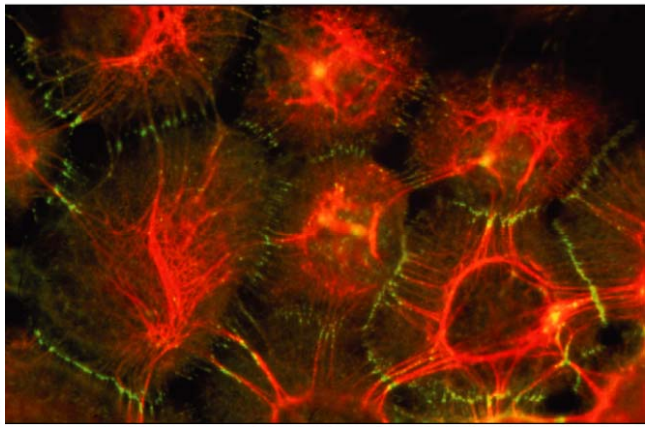
- Intermediate filaments

- Spatial organization of cytoskeletal filaments is dependent on many factors, e.g.

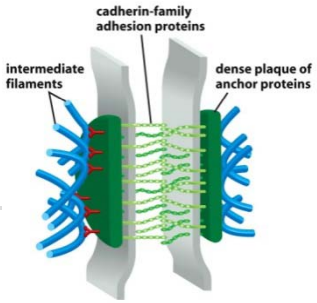
  - cell type
  - cell states (cycle)
  - cell activities



Green: vimentin IF
Red: microtubule

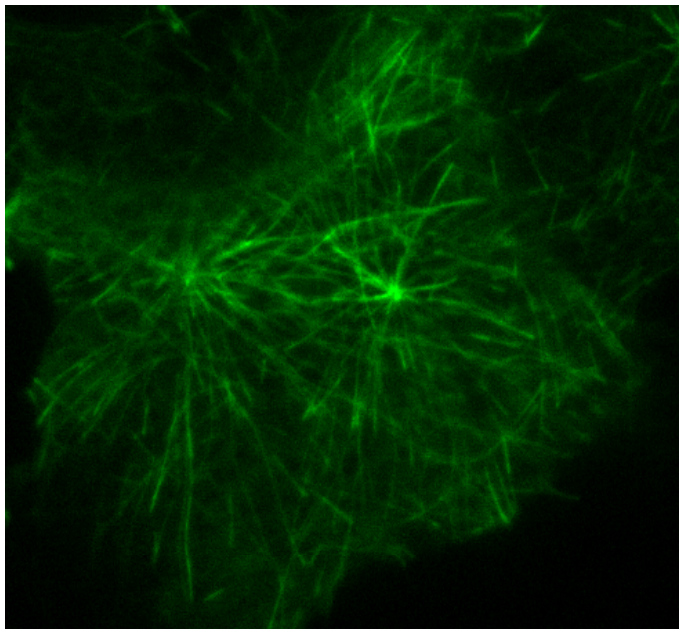


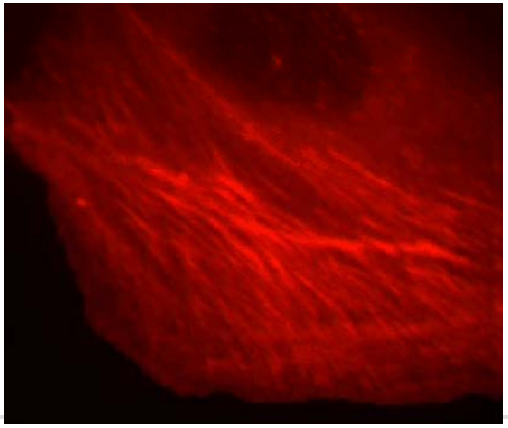
Orange: keratin IF
Green: desmosome

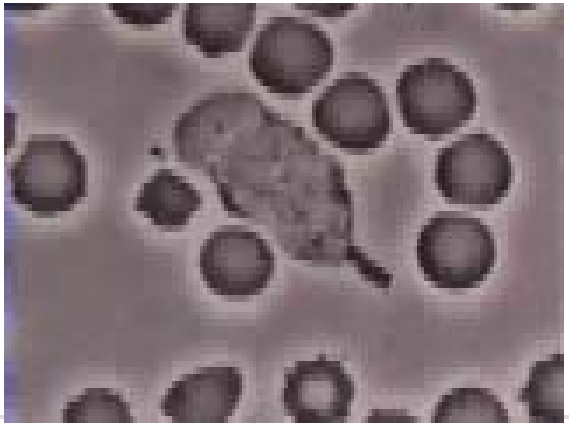


The Cytoskeleton (III)

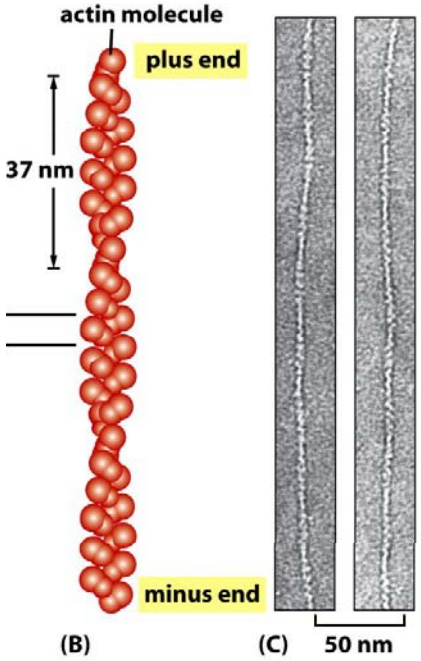
- The cytoskeleton plays a critical role in many basic cellular functions, e.g.
  - structural organization & support
  - shape control
  - intracellular transport
  - force and motion generation
  - signaling integration
- Highly dynamic and adaptive

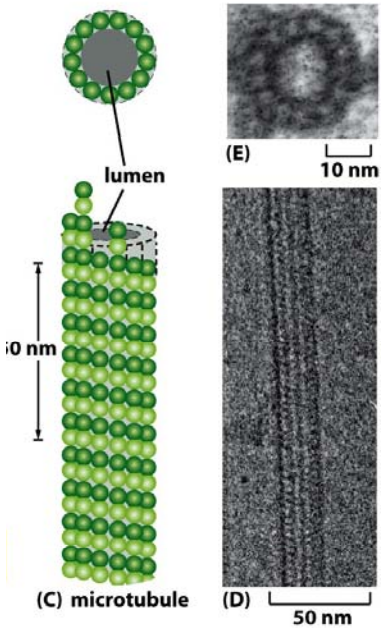


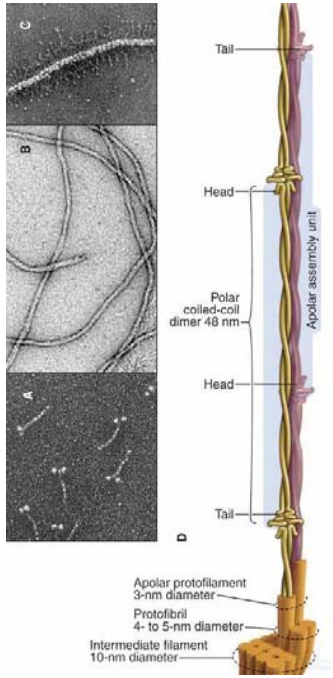




Overview of Cytoskeletal Filaments

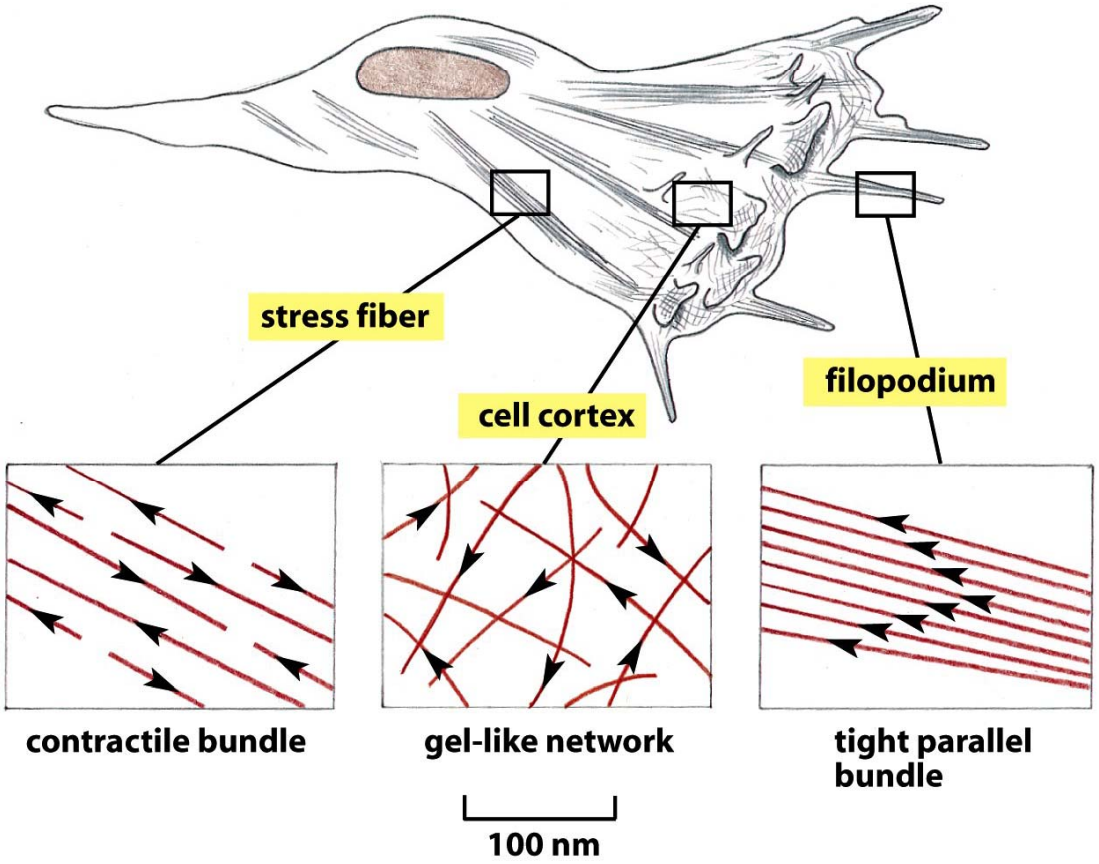






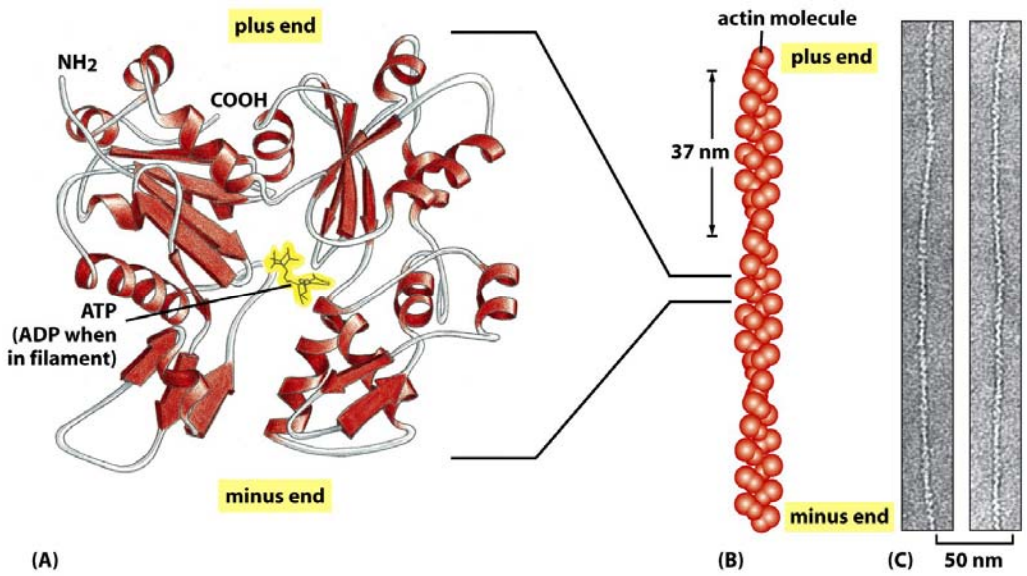
	Shape	Diameter	Subunits	Polarized
actin	cable	~6 nm	actin monomer	yes
microtubule	tube	~25nm	tubulin heterodimer	yes
intermediate filament	rope	~10nm	Various dimers	no

Organization of Actin with a Cell



Actin Structure and Function

- Each actin subunit is a globular monomer.
- One ATP binding site per monomer.
- Functions
  - Cell migration
  - Cell shape
  - Used as tracks for myosin for short distance transport



Basics Terms of Chemical Reaction Kinetics

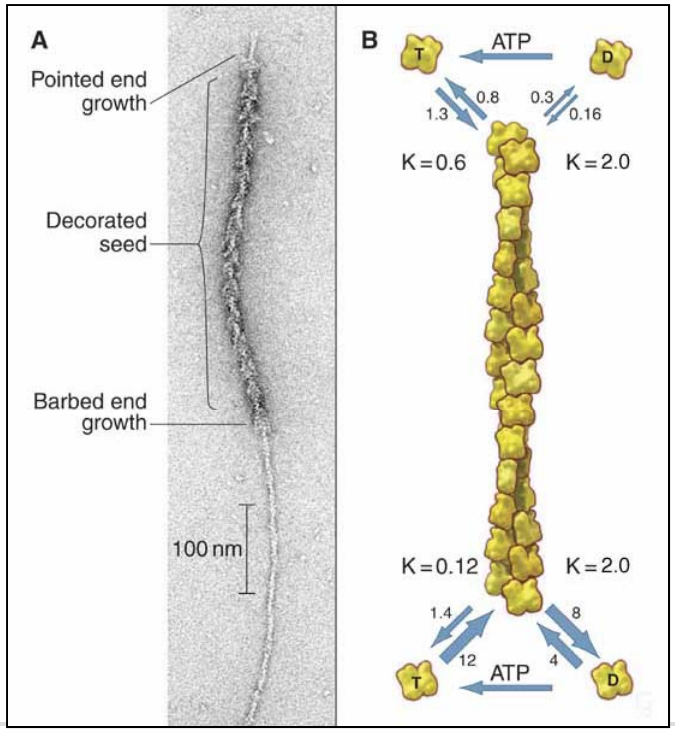
- A reversible bimolecular binding reaction

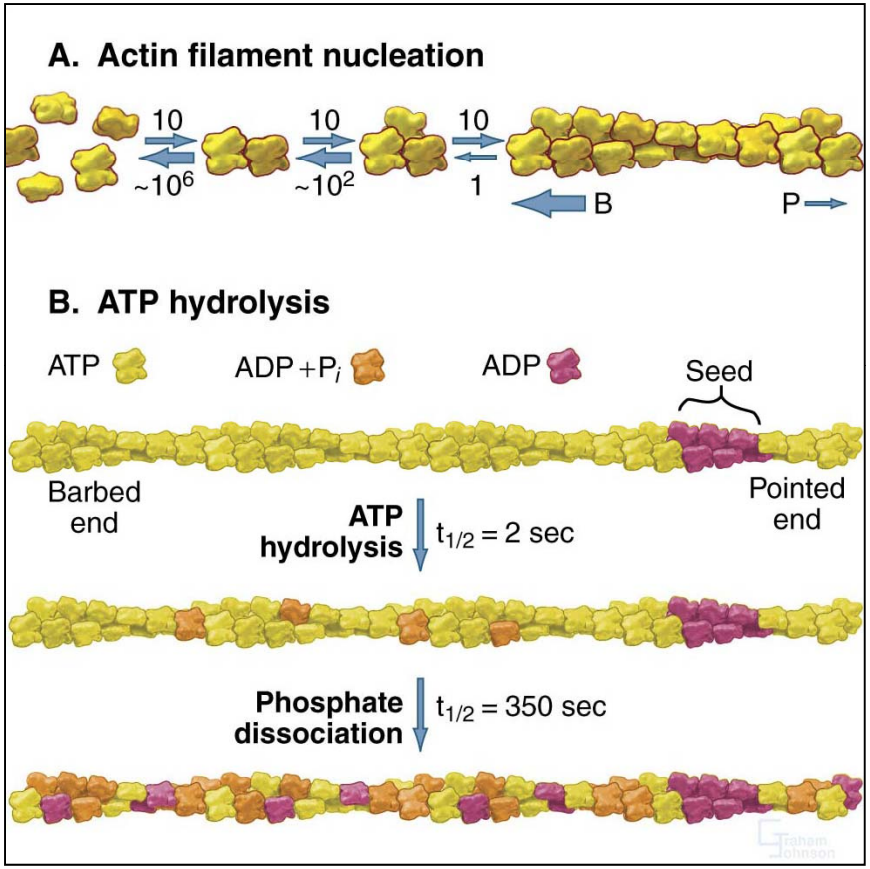
$$A + B \rightleftharpoons AB$$

- Rate of association = $k_+[A][B]$
- Rate of disassociation = $k_-[AB]$
- At equilibrium $k_+[A][B] = k_-[AB]$

Actin Nucleation and Nucleotide Hydrolysis

- Actin polymerizes and depolymerizes substantially faster at the plus end (barbed end) than at the minus end (pointed end).





Actin Accessory Proteins (I)

- More than 60 families identified so far.

- Functions

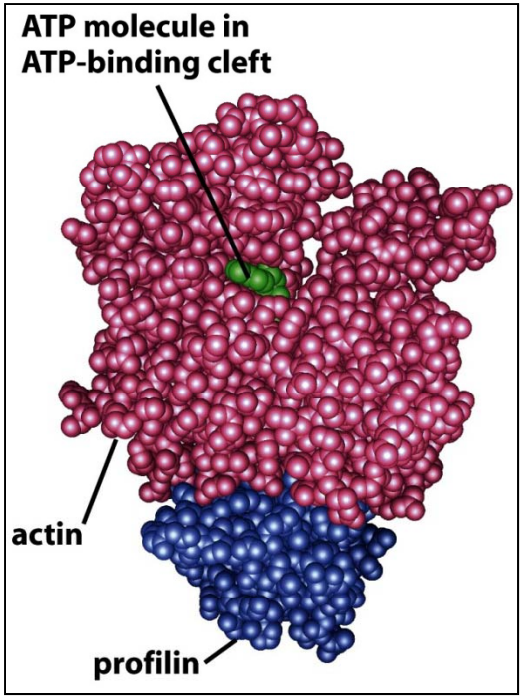
  - Monomer binding
  - Nucleation
  - Filament capping
  - Filament severing
  - Filament side-binding and supporting
  - Filament crosslinking

  - Signaling adapter

- Functional overlap and collaboration between actin-binding proteins

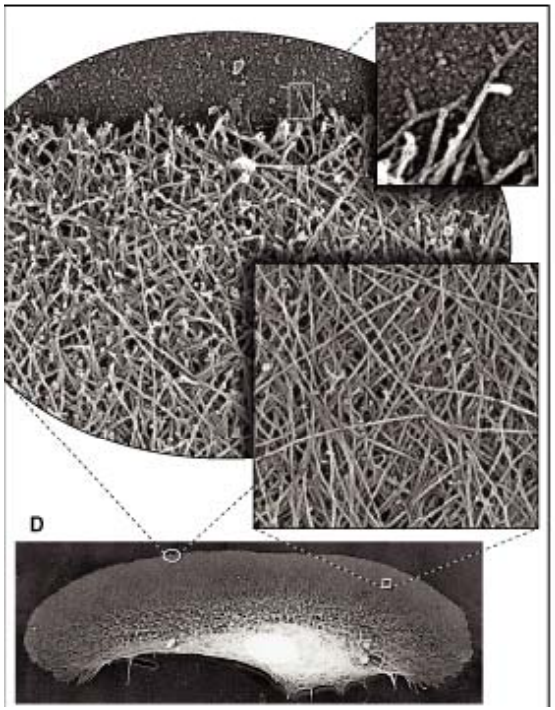
Actin Accessory Proteins (II)

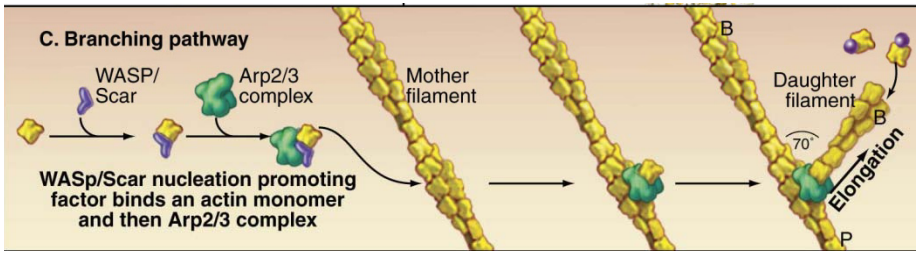
- Monomer binding proteins
  - profilin: to bind actin monomer and accelerate elongation
  - thymosin: to bind and lock actin monomer
  - ADF/cofilin: to bind and destabilize ADP-actin filaments

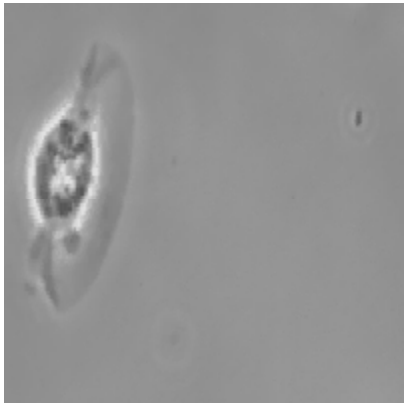


Actin Accessory Proteins (III)

- Actin nucleation
  - Formins: to initiate unbranched actin filaments
  - Arp2/3: to bind the side of actin and initiate branching







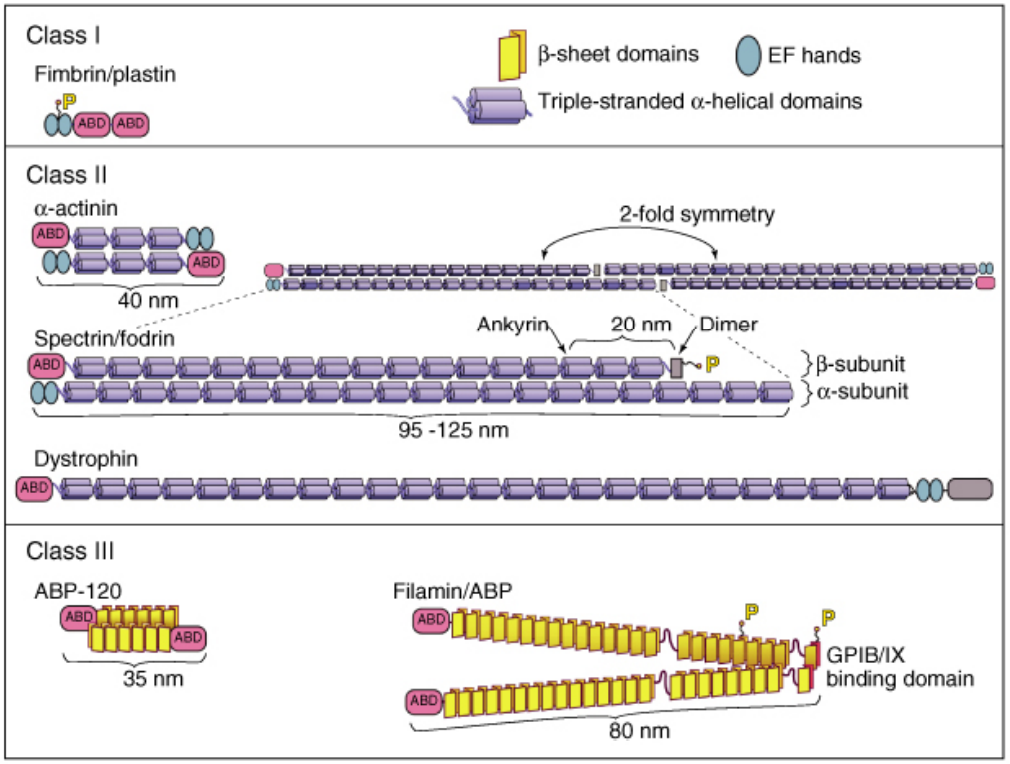
Actin Accessory Proteins (IV)

- Actin capping protein
  - Blocks subunit addition and disassociation
- Actin severing protein
- Three families of proteins perform both functions
  - Gelsolin
  - Fragmin-severin
  - ADF/cofilin

Actin Accessory Proteins (V)

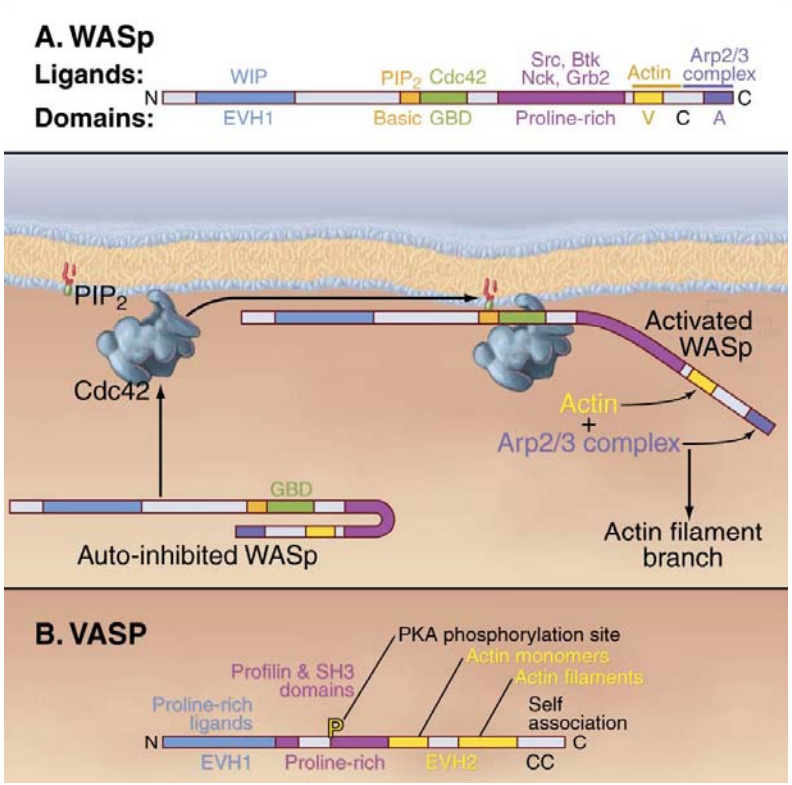
- Actin side-binding proteins
  tropomyosin, nebulin, caldesmon

- Actin crosslinking
  - α-actinin
  - filamin
  - spectrin
  - ERM



Actin Adapter Protein

- WASP & VASP



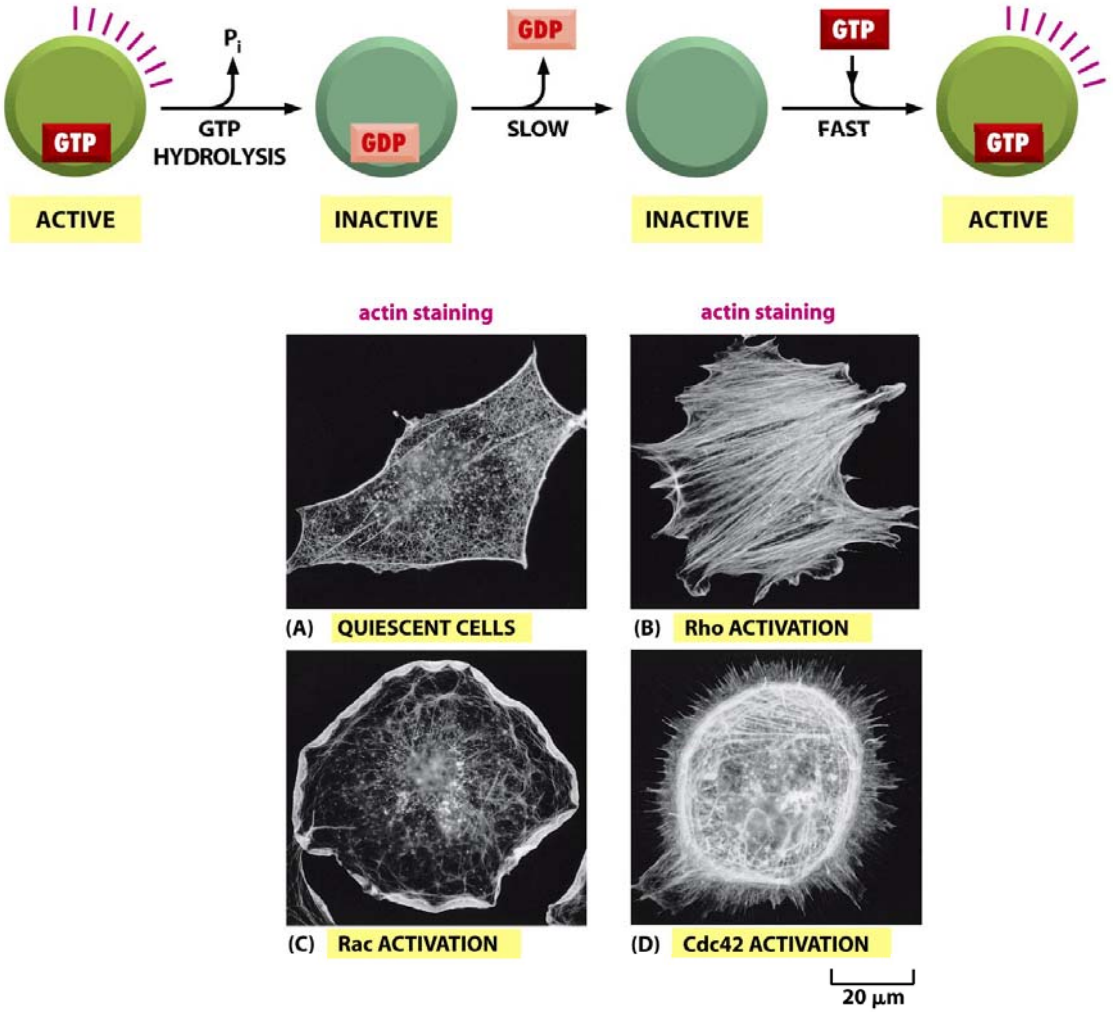
Actin Regulation

- GTPase: Molecule switch; Family of proteins that are activated by GTP binding and inactivated by GTP hydrolysis and phosphate dissociation.

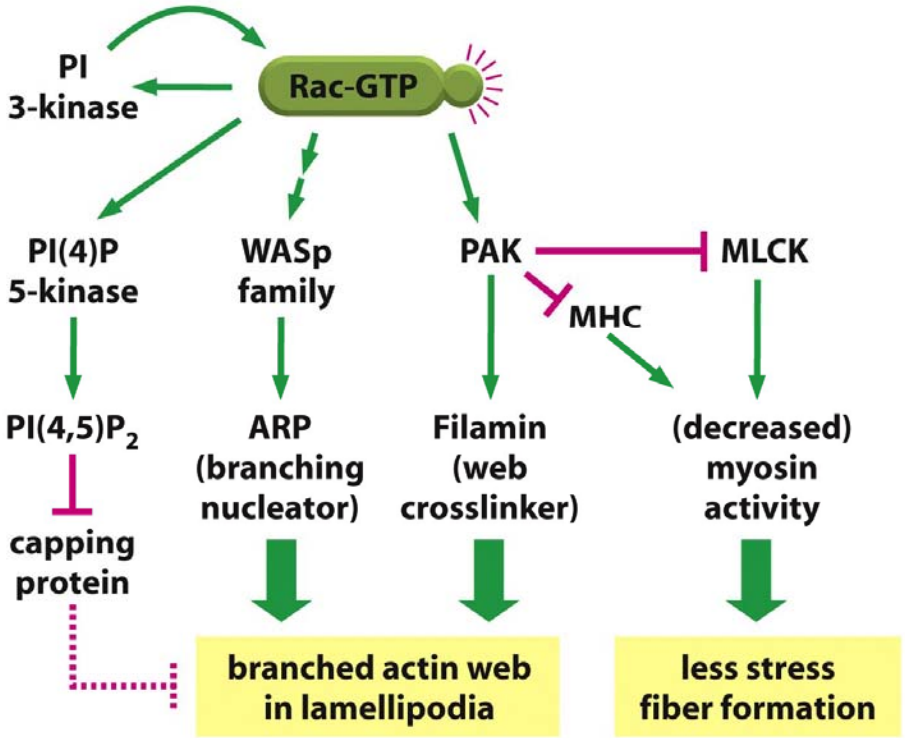
- Rho GTPase:
  cdc42: its activation triggers actin polymerization and bundling at filopodia.

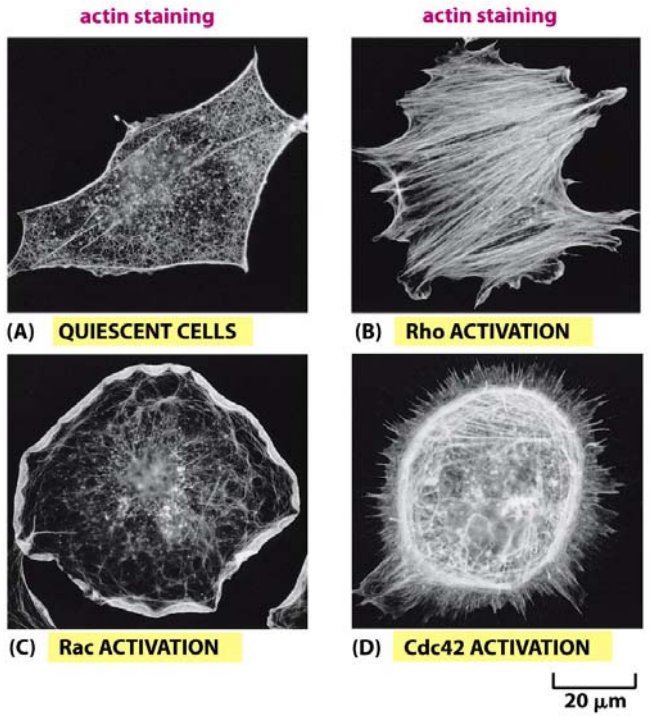
  Rho: its activation promotes actin bundling.

  Rac: its activation promotes polymerization at the cell periphery.



Rac on Actin Organization





Summary: actin

- Relatively soft (quantification in following lectures).
- Often form bundles; mechanical strength comes mostly from bundling and crosslinking.
- Mostly function to withstand tension rather than compression.
- Relatively stable and easy to work with (biochemically).

Summary: actin accessory proteins

- Different proteins have distinct functions.
- Proteins with multiple functional domains can have multiple functions.
- Some of them are essential.
- Most of the proteins have functional overlap.

Questions?