BME 42-620 Engineering Molecular Cell Biology

Lecture 07:

Basics of the Diffusion Theory

The Cytoskeleton (I)

Outline

- Diffusion: microscopic theory
- Diffusion: macroscopic theory
- A method to determine the diffusion coefficient

- An overview of the cytoskeleton

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Introduction

Table 2–2 The Approximate Chemical Composition of a Bacterial Cell

	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	~300
Macromolecules (proteins, nucleic acids, and	26	~3000

- Cellular molecules are subject to thermal force due to collisions with water and other molecules.

- The resulting motion and energy are called thermal motion and thermal energy.

Movement of a Free Molecule (I)

- The average kinetic energy of a particle of mass *m* and velocity *v* is

$$\left\langle \frac{1}{2} m v_x^2 \right\rangle = \frac{kT}{2}$$

Boltzmann constant=1.381×10^{-23} J/K

$t_K = t_C + 273.15$

1 Joule = 1 N·m = 1 kg·m^2 / s^2

where k is Boltzmann's constant and T is absolute temperature (Einstein 1905).

- Principle of equipartition of energy

$$\left\langle \frac{1}{2} m v^2 \right\rangle = \frac{3 \cdot kT}{2}$$

Movement of a Free Molecule (II)

- Molecular mass of GFP is 27 kDa. One atomic mass unit (Da) is 1.6606×10^{-24}g. So the mass of one GFP molecule is 4.48×10^{-20}g.

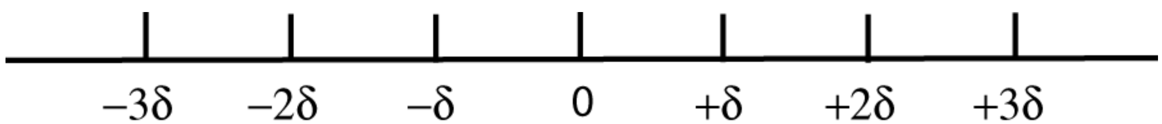
  At 27 degree C, kT is $4.14 \times 10^{-14} \text{g} \cdot \text{cm}^2/\text{sec}^2$.

$$\sqrt{\langle v_x^2 \rangle} = \sqrt{\frac{kT}{m}} = 961.51 \text{ cm/sec}$$

1D Random Walk in Solution (I)

- Assumptions:
  (1) A particle *i* has equal probabilities to walk to the left and to the right.
  (2) Particle movement at consecutive time points are independent.
  (3) Movement of different particles are independent.
  (4) Each particle moves at a average step size of $\delta = v_x \cdot \tau$

$$x_i(n) = x_i(n-1) \pm \delta$$

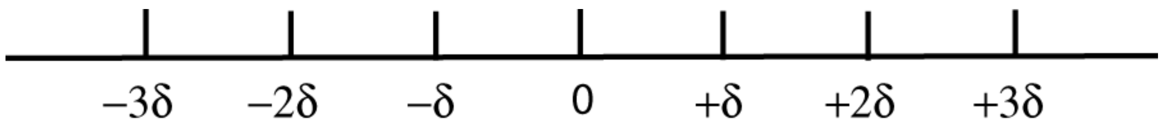


$$\langle x(n) \rangle = \frac{1}{N}\sum_{i=1}^{N} x_i(n) = \frac{1}{N}\sum_{i=1}^{N}\left[x_i(n-1) \pm \delta\right]$$
$$= \frac{1}{N}\sum_{i=1}^{N} x_i(n-1) = \langle x(n-1) \rangle$$

1D Random Walk in Solution (II)

- Property 1: The mean position of a particle (or an ensemble of particles) undergoing random walk remains at the origin.

$$x_i(n) = x_i(n-1) \pm \delta$$



$$\langle x(n) \rangle = \frac{1}{N}\sum_{i=1}^{N} x_i(n) = \frac{1}{N}\sum_{i=1}^{N}\left[x_i(n-1) \pm \delta\right]$$

$$= \frac{1}{N}\sum_{i=1}^{N} x_i(n-1) = \langle x(n-1) \rangle$$

1D Random Walk in Solution (III)

- Property 2: The mean square displacement of a particle undergoing random walk increases linearly w.r.t. time.

$$\langle x^2(n)\rangle = \frac{1}{N}\sum_{i=1}^{N} x_i^2(n) = \frac{1}{N}\sum_{i=1}^{N}\left[x_i^2(n-1) \pm 2\delta x_i(n-1) + \delta^2\right]$$
$$= \langle x^2(n-1)\rangle + \delta^2$$

$$\langle x^2(n)\rangle = n\delta^2 = \frac{t}{\tau}\delta^2 = 2Dt \qquad \langle r^2(n)\rangle = \langle x^2(n) + y^2(n)\rangle = 4Dt$$

$$\langle r^2(n)\rangle = \langle x^2(n) + y^2(n) + z^2(n)\rangle = 6Dt$$

1D Random Walk in Solution (IV)

- Property 3: The displacement of a particle follows a normal distribution.

$$p(k;n) = \frac{n!}{k!(n-k)!}\frac{1}{2^k}\frac{1}{2^{n-k}}$$

$$p(k) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(k-\mu)^2}{2\sigma^2}} \text{ where } \sigma^2 = \frac{n}{4} \text{ and } \mu = \frac{n}{2}$$

$$x(n) = [k - (n-k)]\delta = (2k - n)\delta \qquad \langle x(n) \rangle = (2\langle k \rangle - n)\delta = 0$$

$$\langle x^2(n) \rangle = (4\langle k^2 \rangle - 4\langle k \rangle n + n^2)\delta^2 = (n^2 + n - 2n^2 + n^2)\delta^2 = n\delta^2$$

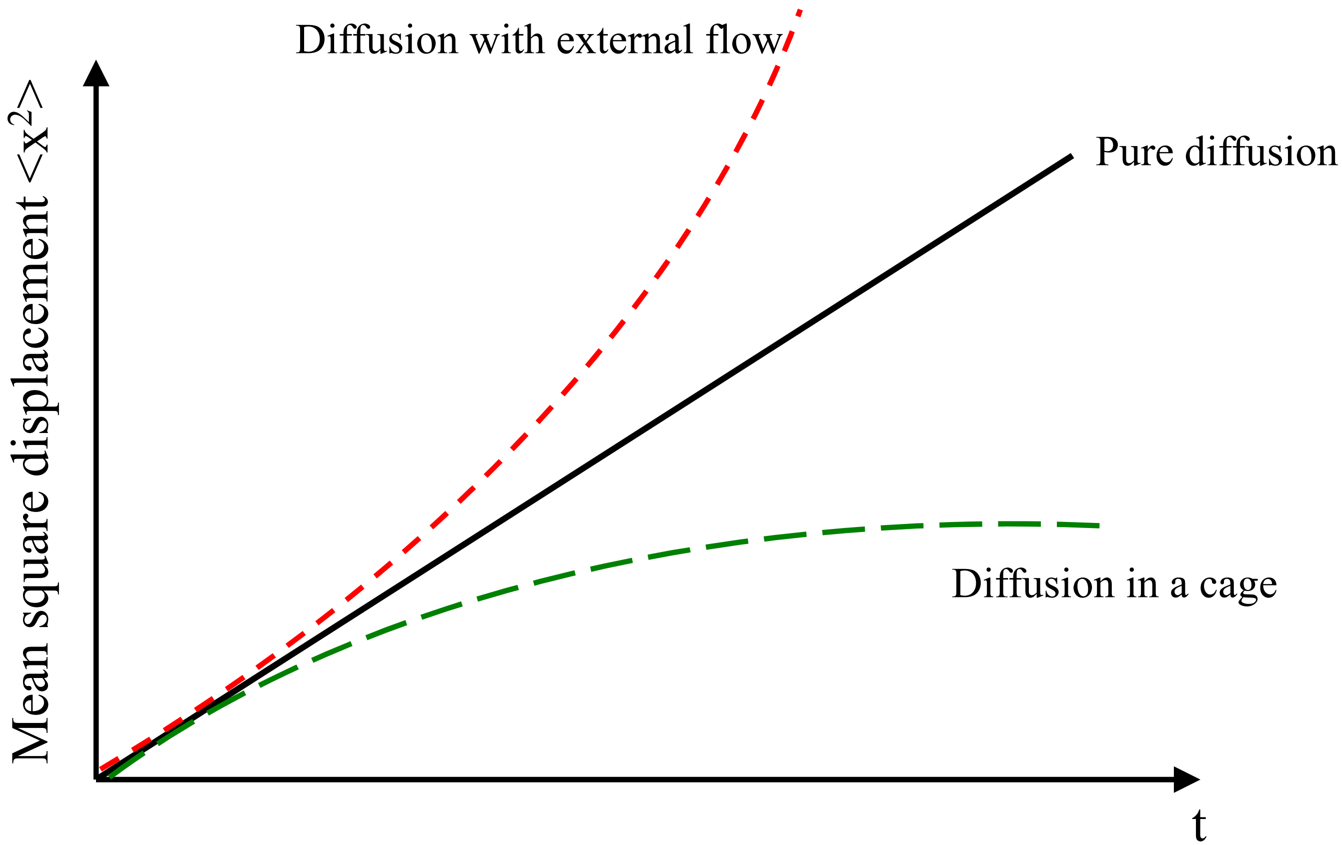
$$p(x) = \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \text{ where } n\delta^2 = 2Dt$$

Application of the Microscopic Theory (I)

Object	Distance diffused			
	1 μm	**100 μm**	**1 mm**	**1 m**
K^+	0.25ms	2.5s	2.5×10^4s (7 hrs)	2.5×10^8s (8 yrs)
Protein	5ms	50s	5.0×10^5s (6 days)	5.0×10^9s (150 yrs)
Organelle	1s	10^4s (3 hrs)	10^8s (3 yrs)	10^{12}s (31710 yers)

K+: Radius = 0.1nm, viscosity = $1\text{mPa}\cdot\text{s}^{-1}$; T = 25°C; D=2000 μm^2/sec
Protein: Radius = 3nm, viscosity = $0.6915\text{mPa}\cdot\text{s}^{-1}$; T = 37; D = 100 μm^2/sec
Organelle: Radis = 500nm, viscosity = $0.8904\text{mPa}\cdot\text{s}^{-1}$; T = 25°C; D = 0.5 μm^2/sec

Application of the Microscopic Theory (II)



H. Qian, M. P. Sheetz, E. L. Elson, *Single particle tracking: analysis of diffusion and flow in two-dimensional systems*, Biophysical Journal, 60(4):910-921, 1991.

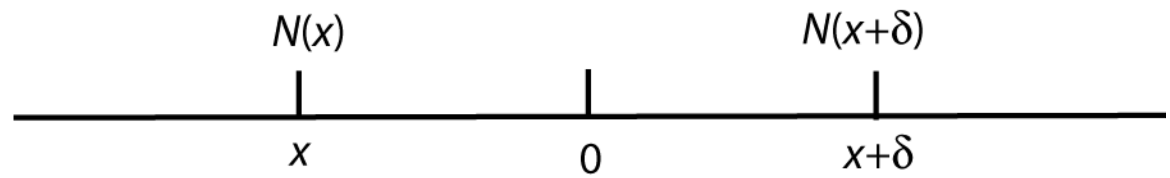
Outline

- Diffusion: microscopic theory
- Diffusion: macroscopic theory
- A method to determine the diffusion coefficient

- An overview of the cytoskeleton

Macroscopic Theory of Diffusion (I)

- Fick's first equation: net flux is proportional to the spatial gradient of the concentration function.

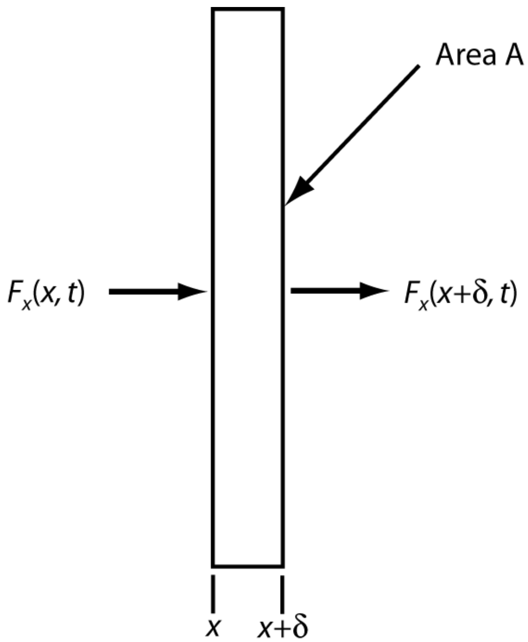


$$-\frac{1}{2}\left[N(x+\delta)-N(x)\right]$$

$$\begin{aligned}
F_x &= \lim_{\delta\to 0} -\frac{1}{2}\left[N(x+\delta)-N(x)\right]/A\tau \\
&= \lim_{\delta\to 0} -\frac{\delta^2}{2\tau}\frac{1}{\delta}\left[\frac{N(x+\delta)}{A\delta}-\frac{N(x)}{A\delta}\right] \\
&= \lim_{\delta\to 0} -D\frac{1}{\delta}\left[C(x+\delta)-C(x)\right] \\
&= -D\frac{\partial C}{\partial x}
\end{aligned}$$

Macroscopic Theory of Diffusion (II)

- Fick's second equation



$$\left[C(t+\tau)-C(t)\right] = -\frac{1}{A\delta}\left[F_x(x+\delta)-F_x(x)\right]A\tau$$

$$\frac{1}{\tau}\left[C(t+\tau)-C(t)\right] = -\frac{1}{\tau}\frac{1}{A\delta}\left[F_x(x+\delta)-F_x(x)\right]A\tau$$
$$= -\frac{1}{\delta}\left[F_x(x+\delta)-F_x(x)\right]$$

$$\frac{\partial C}{\partial t} = -\frac{\partial F_x}{\partial x} = D\frac{\partial^2 C}{\partial x^2}$$

The time rate of change in concentration is proportional to the curvature of the concentration function.

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A Method to Measure Diffusion Coefficient

- Einstein-Smoluchowski Relation

$$v_d = \frac{1}{2} a\tau = \frac{1}{2}\frac{F_x}{m}\tau \qquad f = \frac{F_x}{v_d} = \frac{2m}{\tau} = \frac{2m\frac{\delta^2}{\tau^2}}{\frac{\delta^2}{\tau}} = \frac{mv_x^2}{D} = \frac{kT}{D}$$

$$D = \frac{kT}{f}$$

f: viscous drag coefficient

- Stokes' relation: the viscous drag coefficient of a sphere moving in an unbounded fluid

$$f = 6\pi\eta r$$

η: viscousity
r: radius

An example of D calculation

- Calculation of diffusion coefficient

$$D = \frac{kT}{6\pi\eta r}$$

- $k=1.381\times10^{-23}$J/k$=1.381\times10^{-17}$ N·μm/k
- T = 273.15 + 25
- $\eta=0.8904$mPa·s$=0.8904\times10^{-3}\times10^{-12}$N·μm^{-2}·s
- r= 500nm=0.5μm
- D=0.5 μm^2/s

References

- Howard Berg, *Random Walks in Biology*, Princeton University Press, 1993.
- Jonathon Howard, *Mechanics of Motor Proteins and the Cytoskeleton*, Sinauer Associated, 2001.

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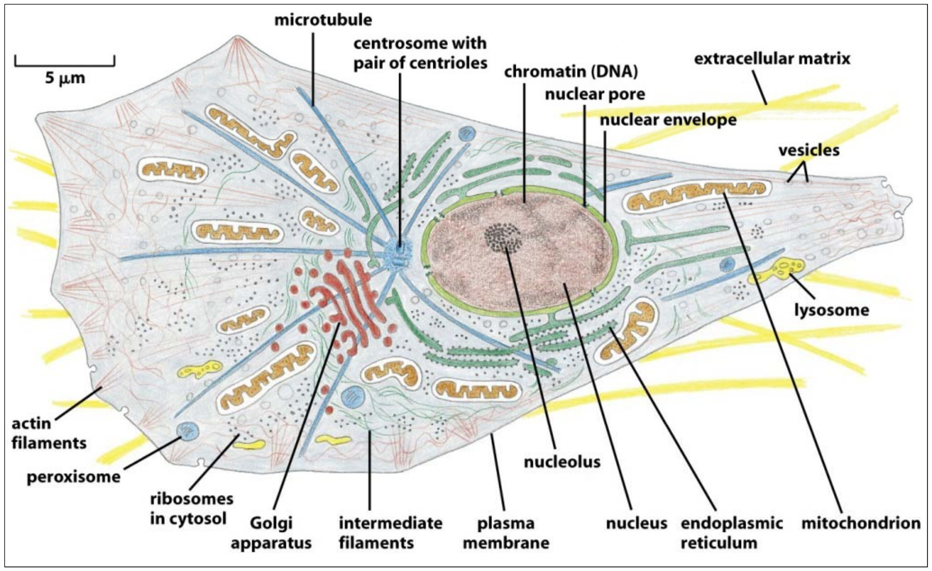
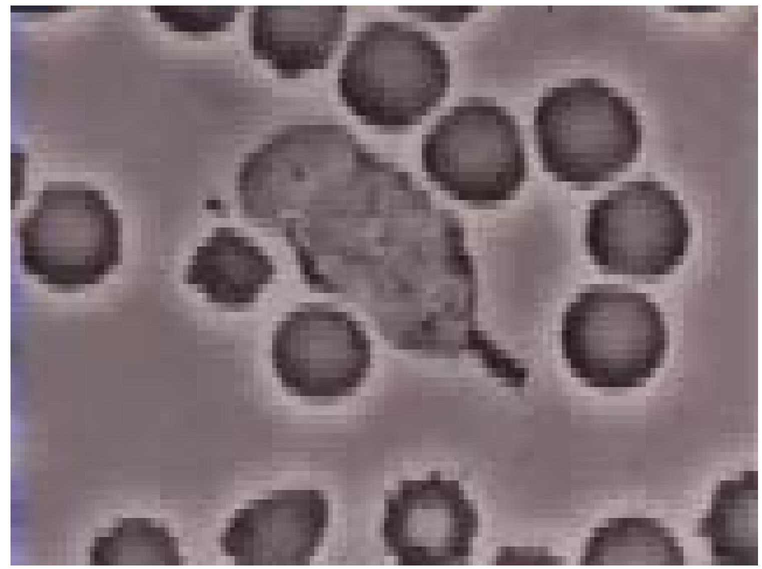
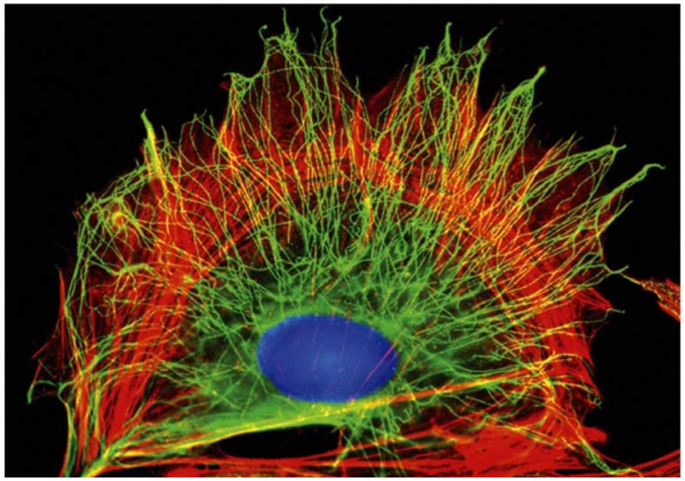
The Cytoskeleton is Highly Dynamic

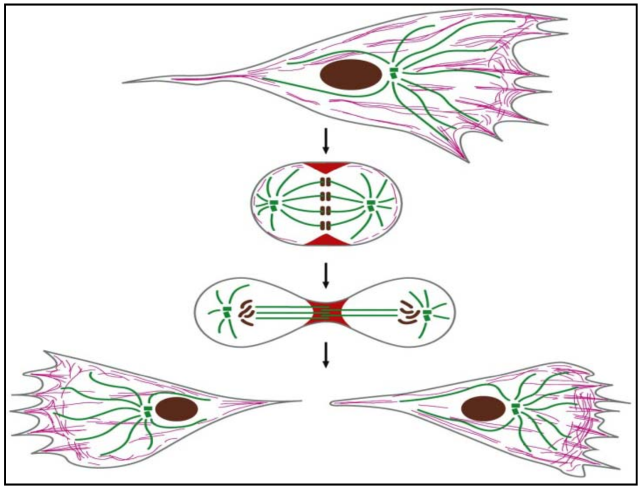




The Cytoskeleton (I)

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





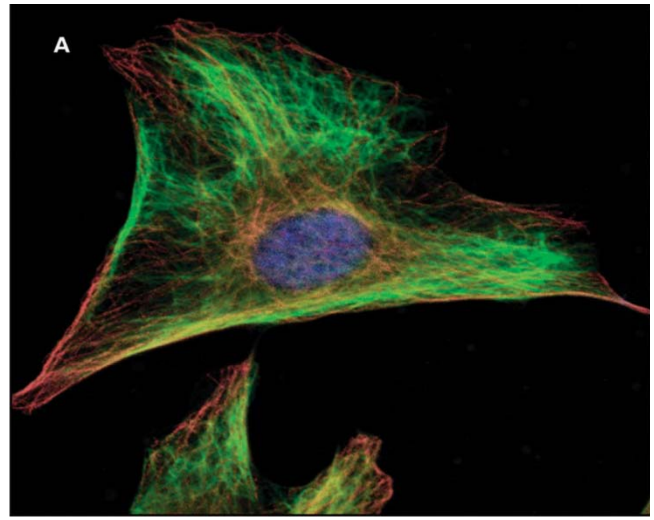
Red: actin
Green: microtubule

The Cytoskeleton (II)

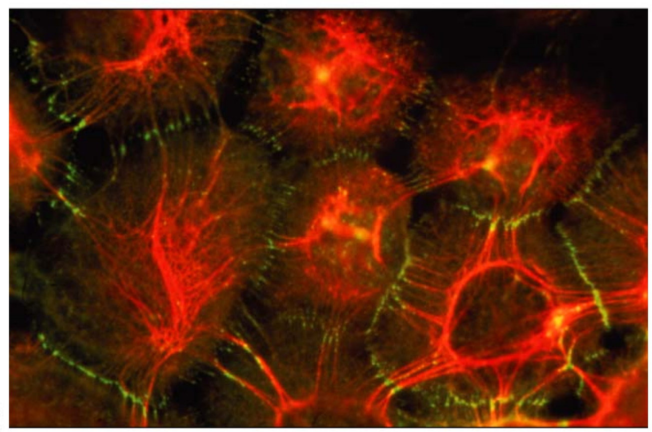
- Intermediate filaments

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

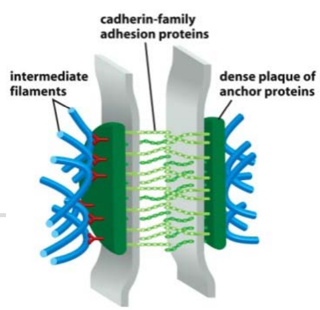
  - cell type
  - cell states (cycle)
  - cell activities



Green: vimentin IF
Red: microtubule

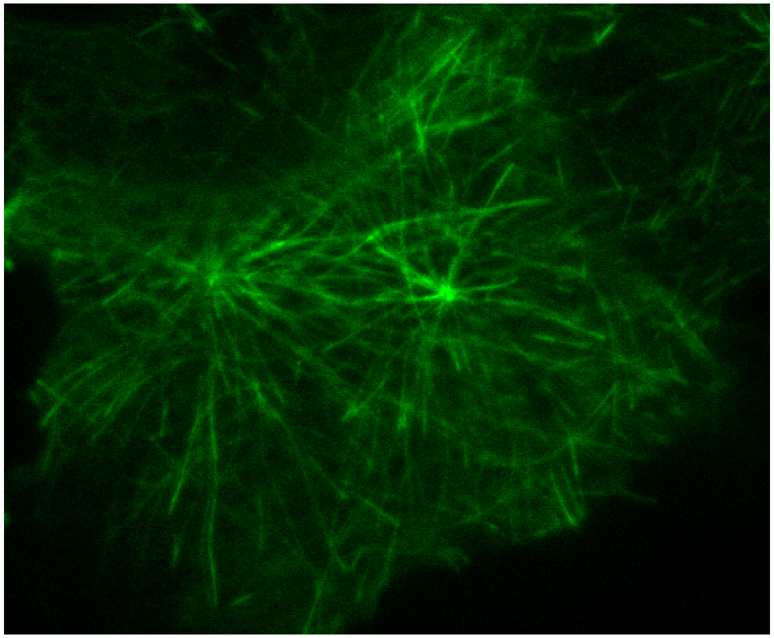


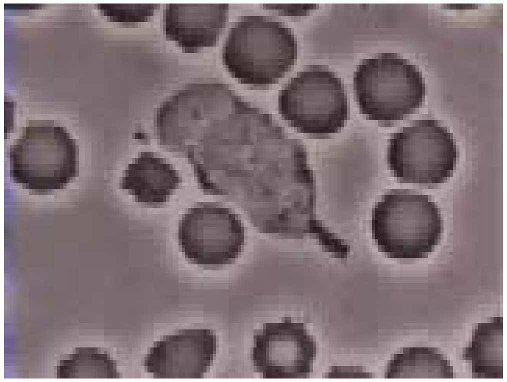
Orange: keratin IF
Green: desmosome

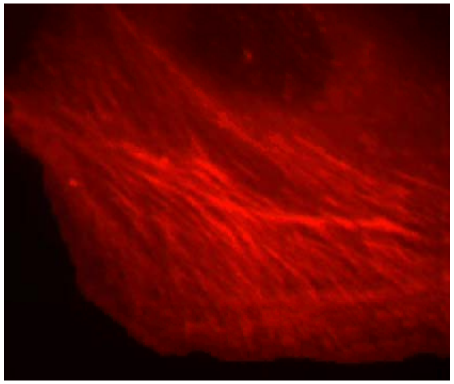


The Cytoskeleton (III)

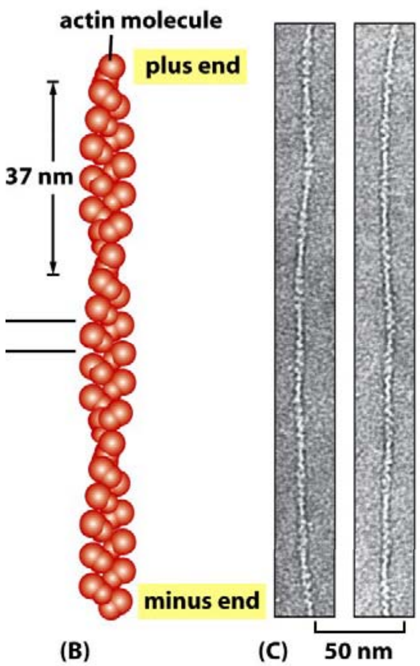
- The cytoskeleton plays a critical role in many basic cellular functions, e.g.
  - structural organization & support
  - shape control
  - intracellular transport
  - force and motion generation
  - signaling integration
- Highly dynamic and adaptive

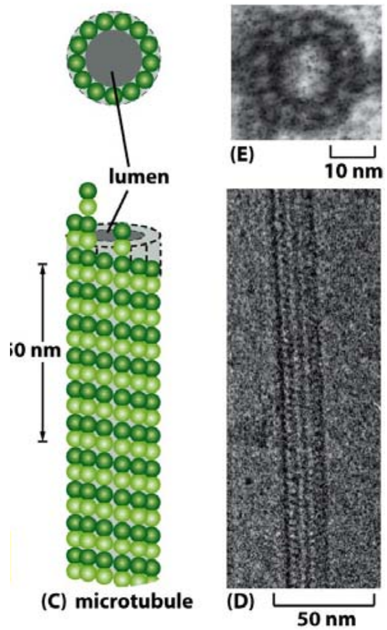


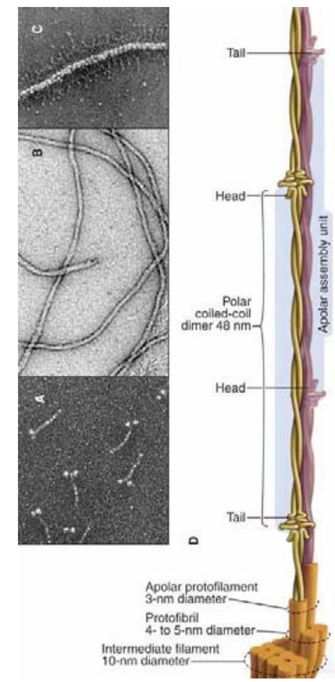




Overview of Cytoskeletal Filaments

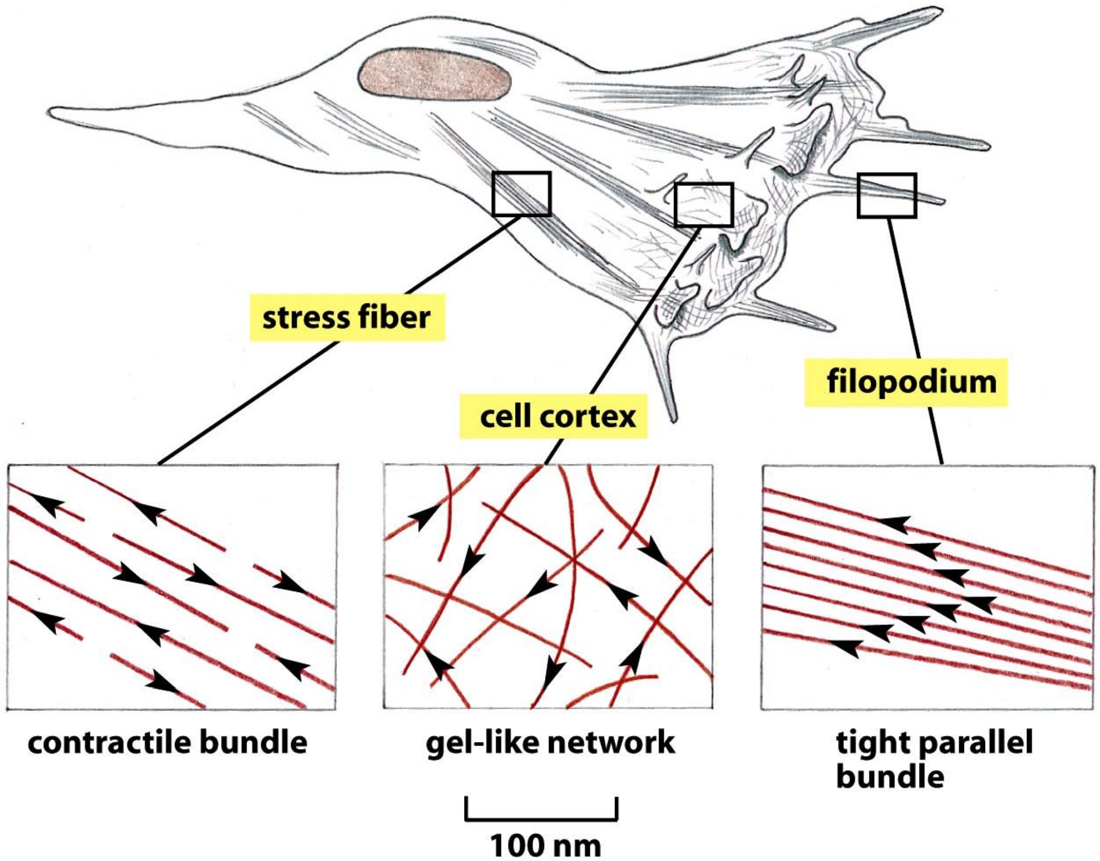






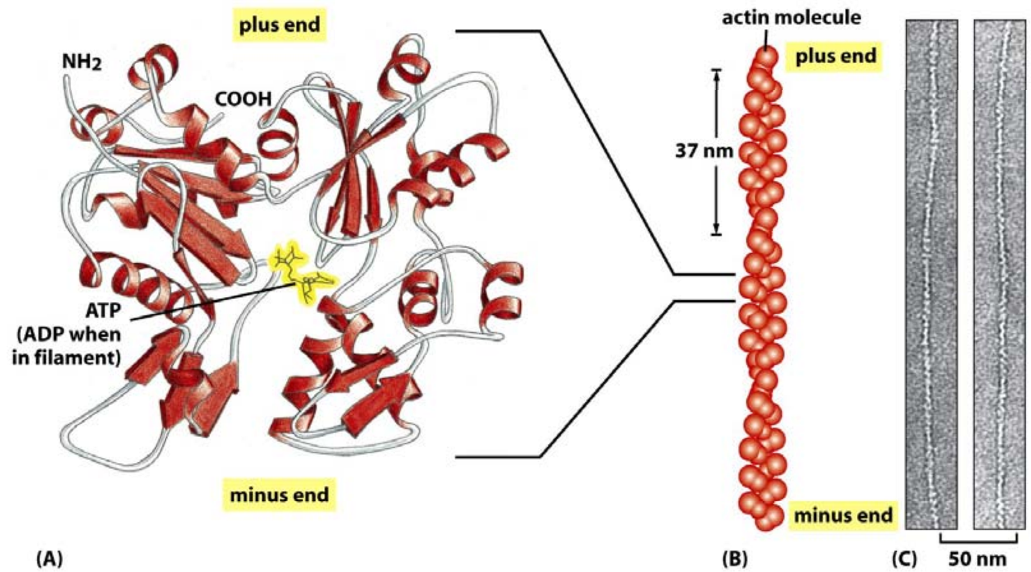
	Shape	Diameter	Subunits	Polarized
actin	cable	~6 nm	actin monomer	yes
microtubule	tube	~25nm	tubulin heterodimer	yes
intermediate filament	rope	~10nm	Various dimers	no

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

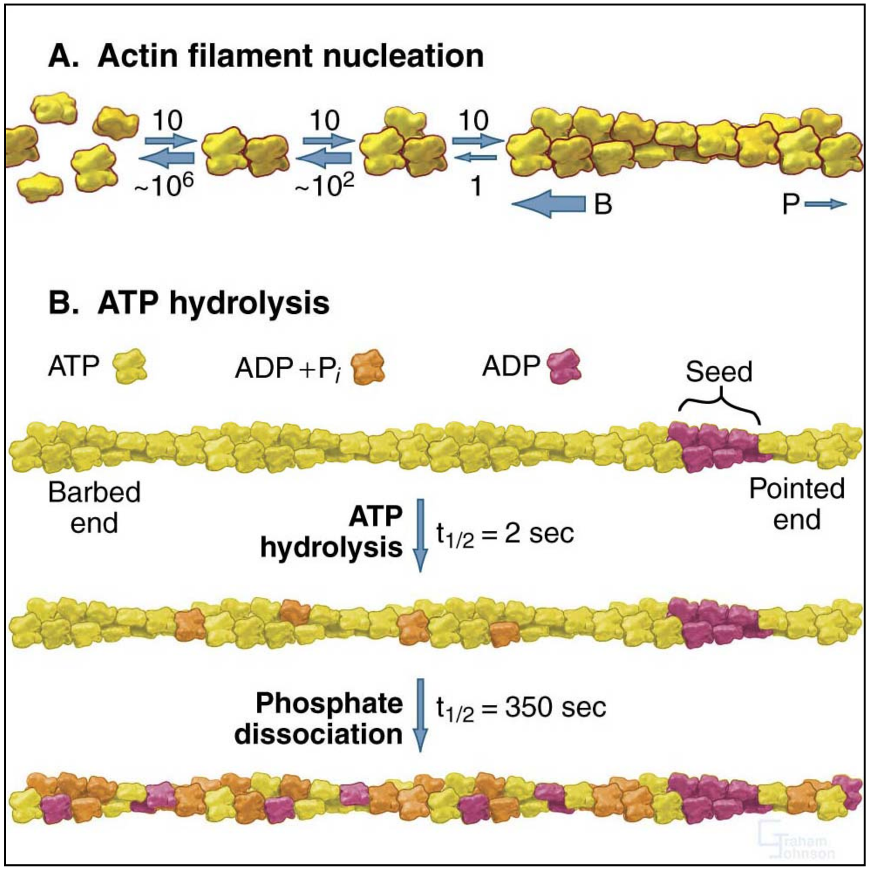
- A reversible bimolecular binding reaction

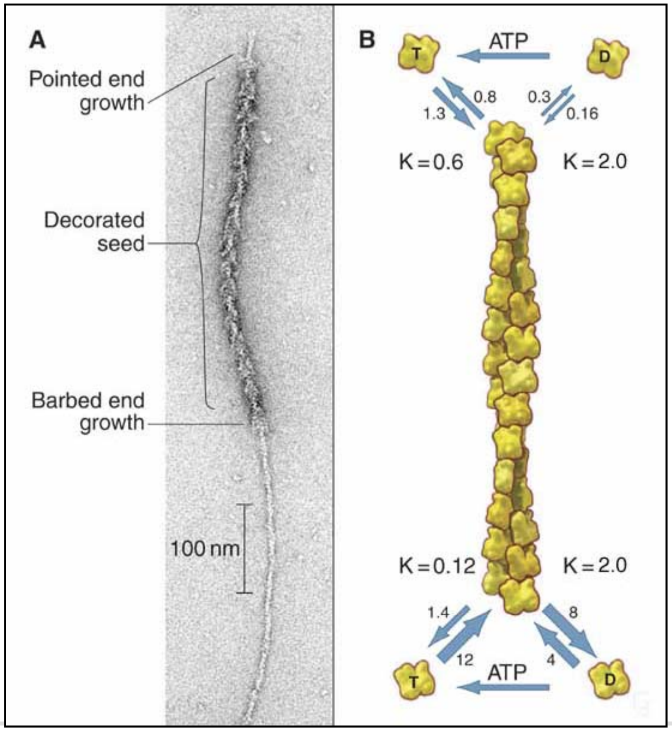
$$A + B \rightleftharpoons AB$$

- 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

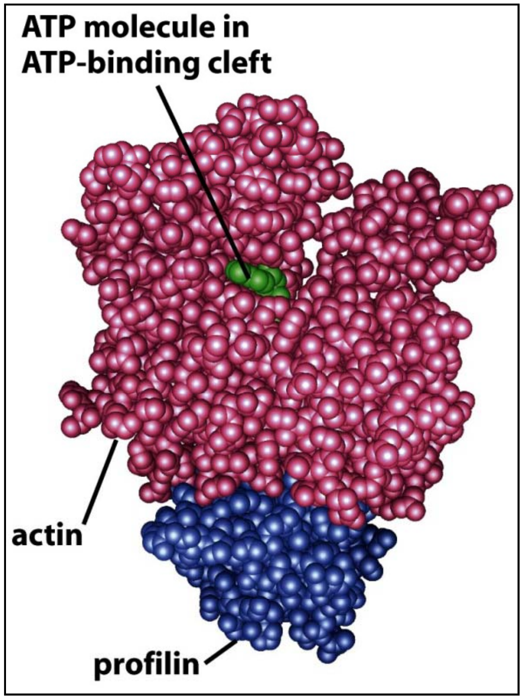
  - Signaling adapter

- Functional overlap and collaboration between actin-binding proteins

Questions ?

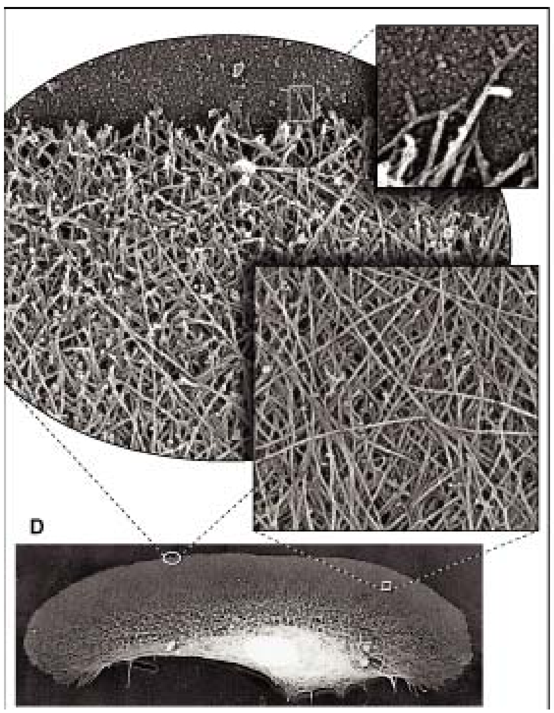
Actin Accessory Proteins (II)

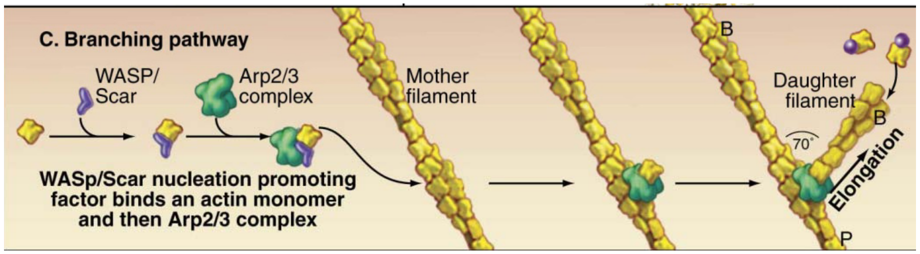
- Monomer binding proteins
  - profilin: to bind actin monomer and accelerate elongation
  - thymosin: to bind and lock actin monomer
  - ADF/cofilin: to bind and destabilize ADP-actin filaments

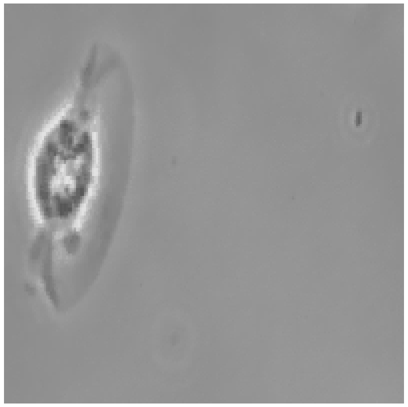


Actin Accessory Proteins (III)

- 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

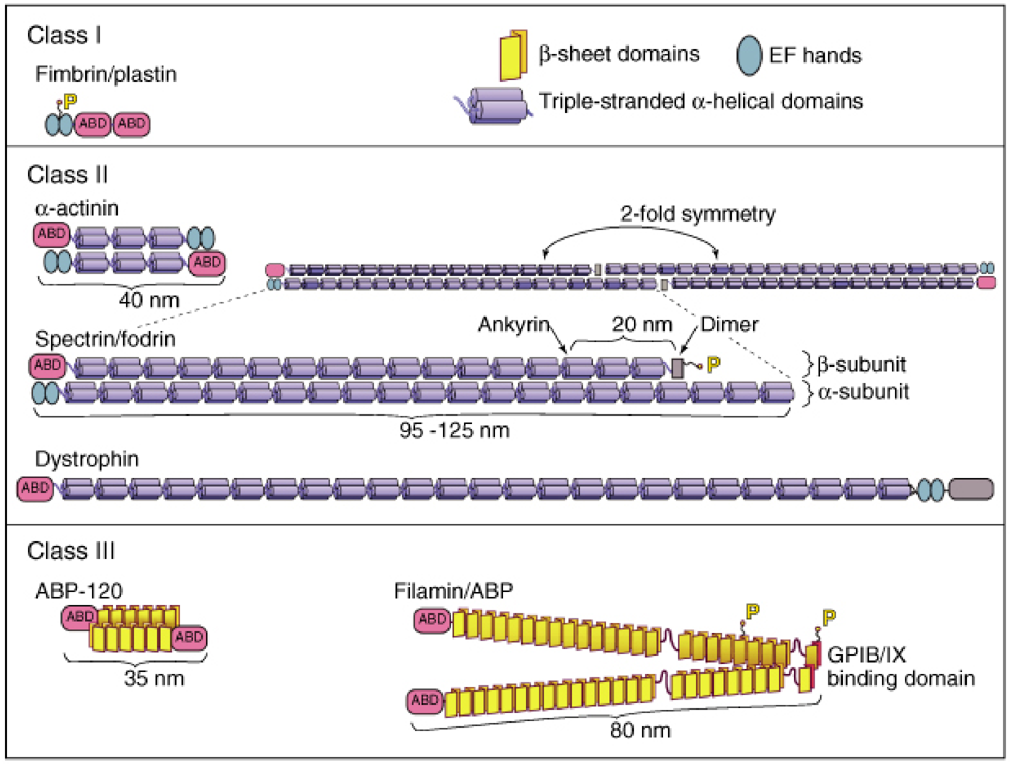
- Actin severing protein

- Three families of proteins perform both functions
  - Gelsolin
  - Fragmin-severin
  - ADF/cofilin

Actin Accessory Proteins (V)

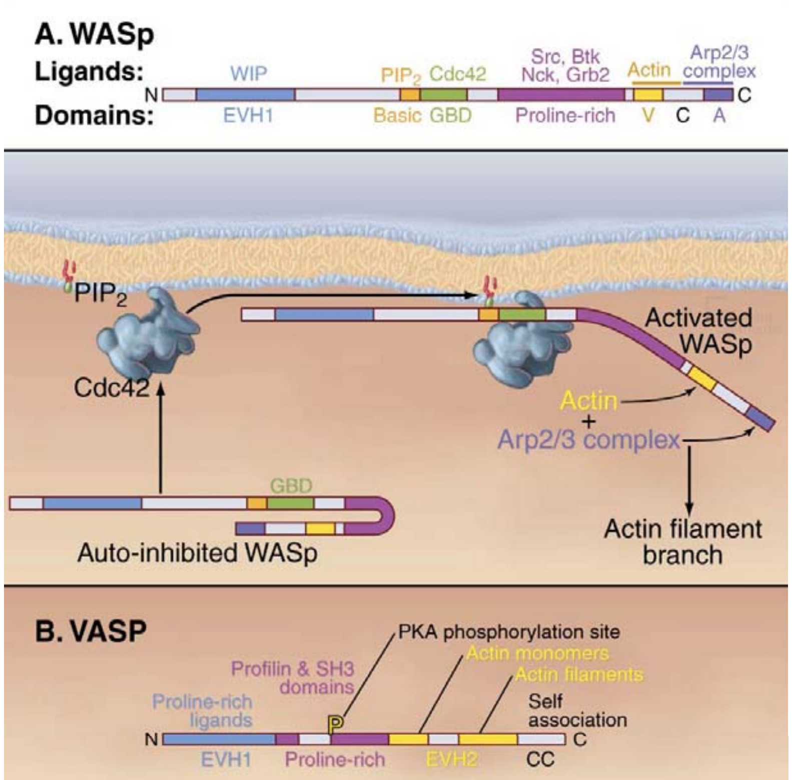
- Actin side-binding proteins
  tropomyosin, nebulin, caldesmon

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



Actin Adapter Protein

- WASP & VASP



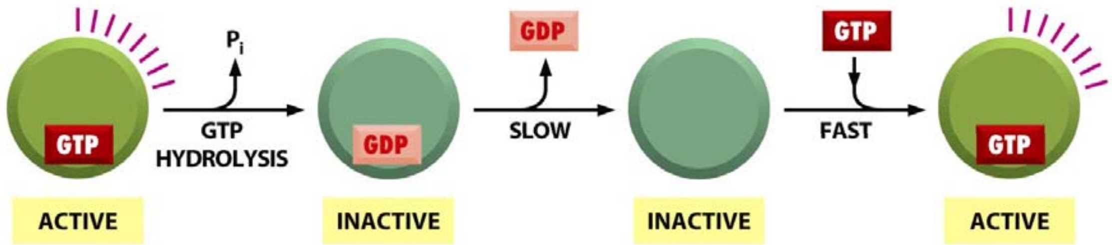
Actin Regulation

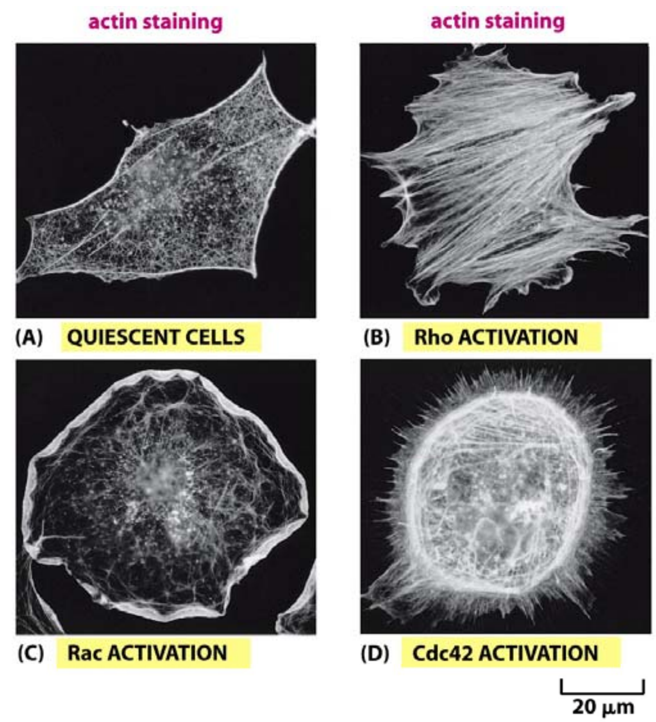
- GTPase: Molecule switch; Family of proteins that are activated by GTP binding and inactivated by GTP hydrolysis and phosphate dissociation.

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

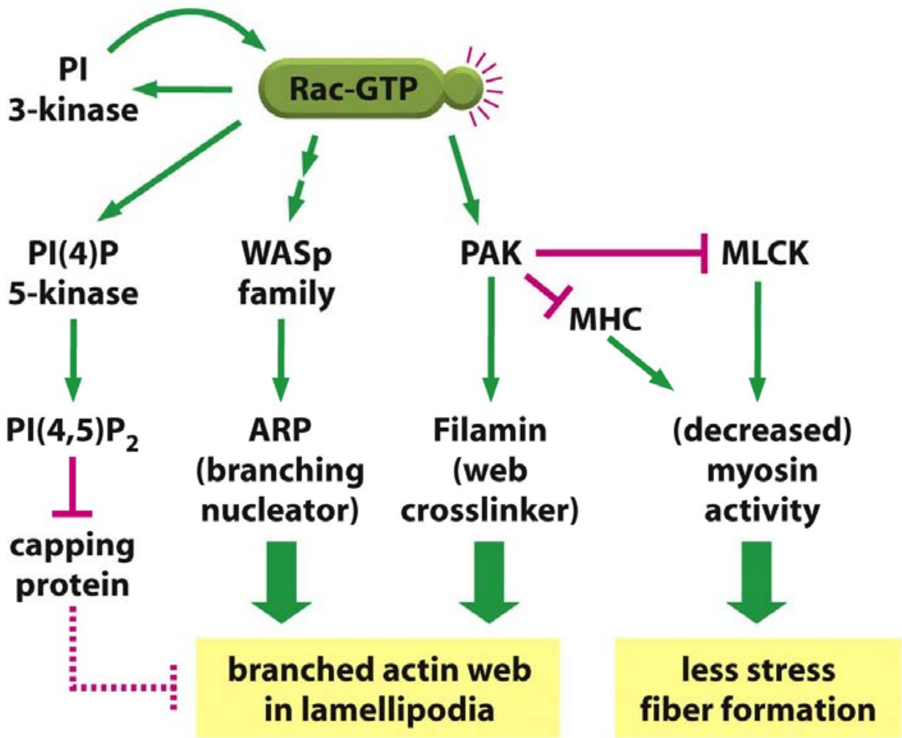
  Rho: its activation promotes actin bundling.

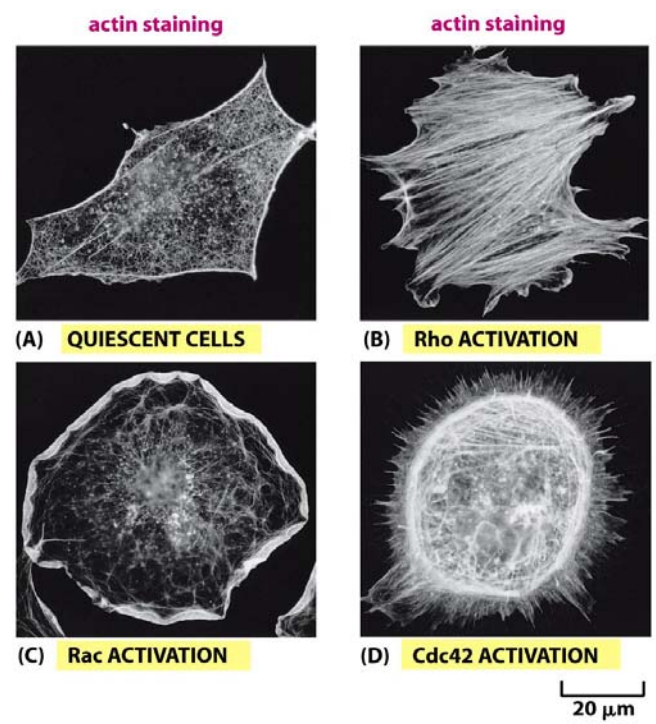
  Rac: its activation promotes polymerization at the cell periphery.





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.