BME 42-620 Engineering Molecular Cell Biology

Lecture 08:

Review: Basics of the Diffusion Theory

The Cytoskeleton (I)

Outline

- Background: FRAP & SPT
- Review: microscopic diffusion theory
- Review: macroscopic diffusion theory

- An overview of the cytoskeleton
- Actin and its associated proteins

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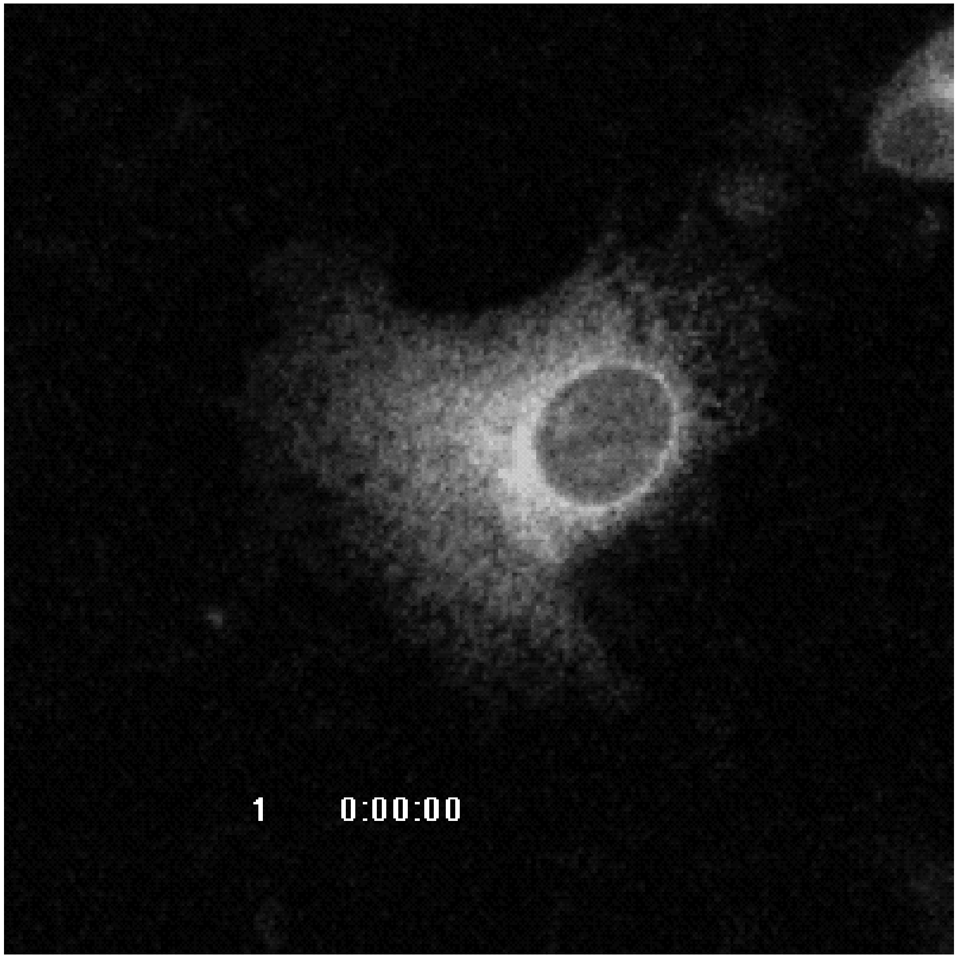
Fluorescence Microscopy of Cell Dynamics



http://lippincottschwartzlab.nichd.nih.gov/video/classic/VSVGrelease.mov

Two Frequently Used Methods to Determine Diffusion Coefficient

- Method 1: Fluorescence recovery after photobleaching
- Method 2: Single particle tracking

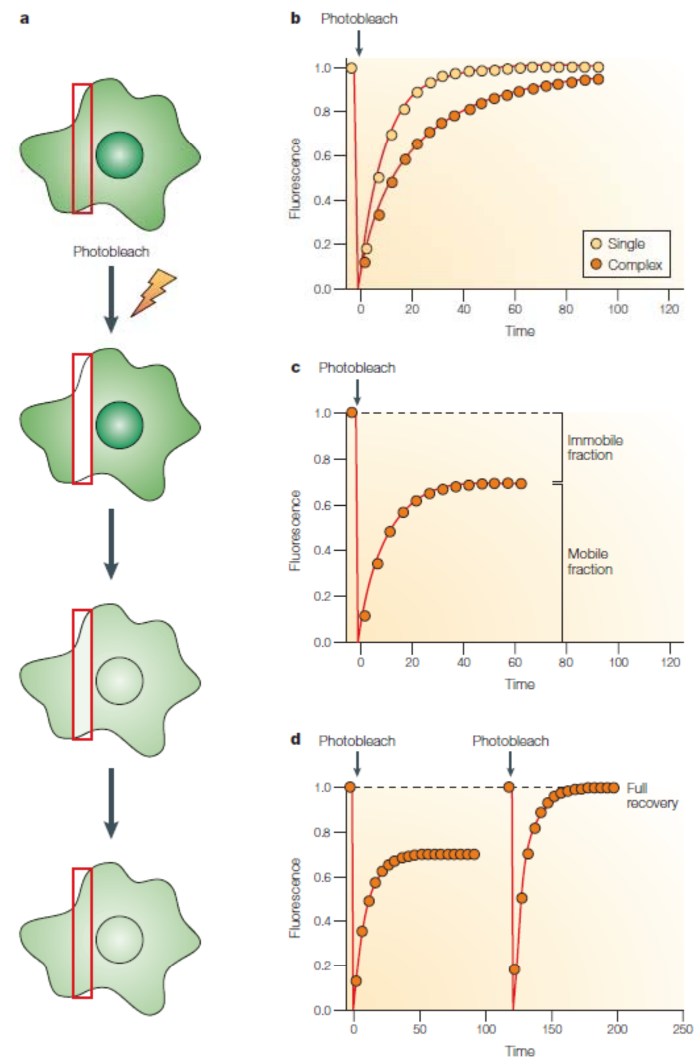
Fluorescence Recovery After Photobleaching (FRAP)

- FRAP provides a convenient approach to visualize diffusion.
- Diffusion coefficient can be estimated from FRAP.

$$D = \frac{w^2}{4t^{1/2}}$$

w: radius of a Gaussian profile bleaching beam

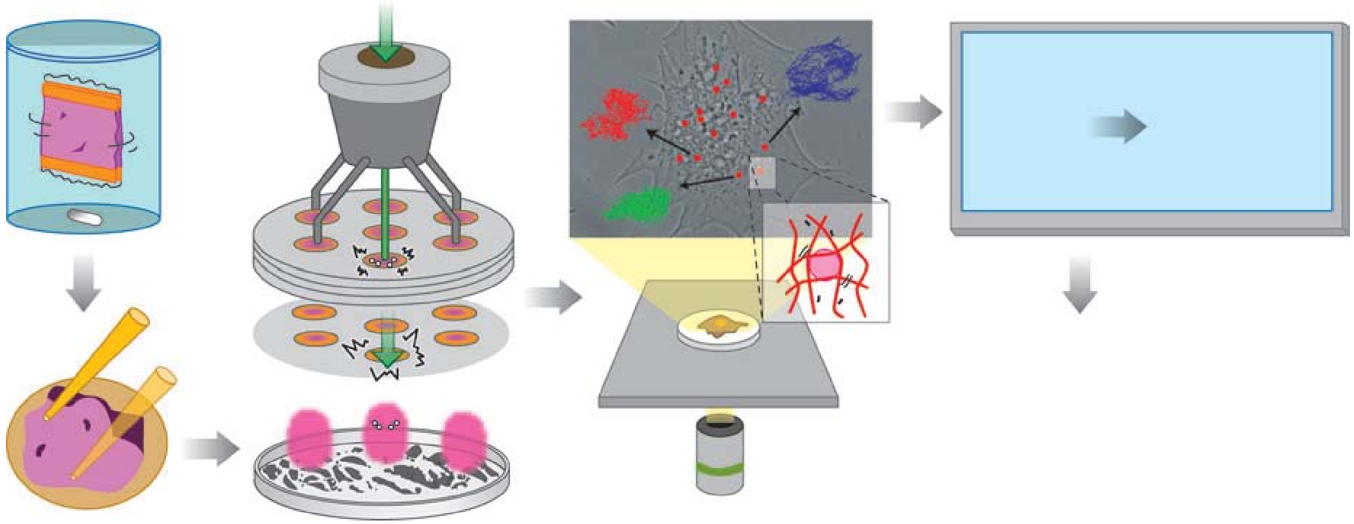
$t^{1/2}$: half time of fluorescence recovery



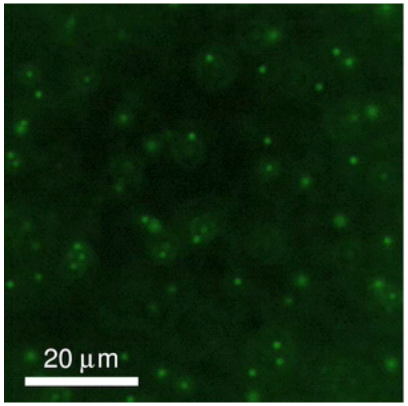
1) D. Axelrod, D.E. Koppel, J. Schlessinger, E. Elson, and W.W. Webb. Mobility Measurement by Analysis of Fluorescence Photobleaching Recovery Kinetics. Biophys. J. 1976; 16(9):1055-1069.

2) J. Lippincott-Schwartz, N. Altan-Bonnet, G. H. Patterson, Photobleaching and photoactivation: following protein dynamics in living cells. Nature Cell Biology, 2003 Sep;Suppl:S7-14.

Single Particle Tracking (SPT)



D. Wirtz, Particle-tracking microrheology of living cells, *Ann. Rev. Biophys*. 38:301-326, 2009.



http://web.mit.edu/savin/Public/.Tutorial_v1.2/Introduction.html

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1D Random Walk in Solution (I)

- Assumptions:

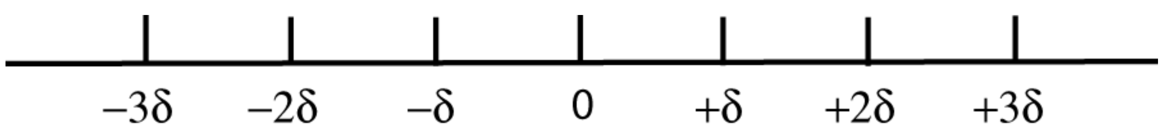
  (1) A particle *i* has equal probabilities to walk to the left and to the right.

  (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 $\delta = v_x \cdot \tau$

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

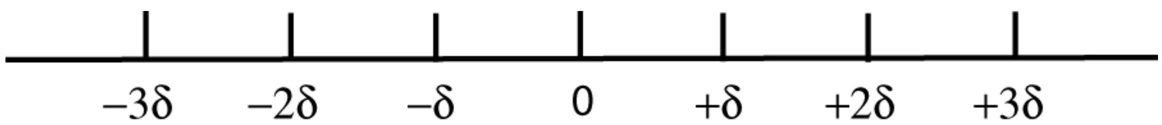


1D Random Walk in Solution (II)

- Property 1: The mean position of an ensemble of particles undergoing random walk remains at the origin.

- The same holds for a single particle over a sufficiently long period of time (ergodicity).

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



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

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

1D Random Walk in Solution (III)

- Property 2: The mean square displacement of an ensemble of particles undergoing random walk increases linearly w.r.t. time.

- Again, the same holds for a single particle.

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

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

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

$$\boxed{D = \frac{\delta^2}{2\tau} = \frac{V_x^2 \tau}{2}}$$

1D Random Walk in Solution (IV)

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

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

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

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

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

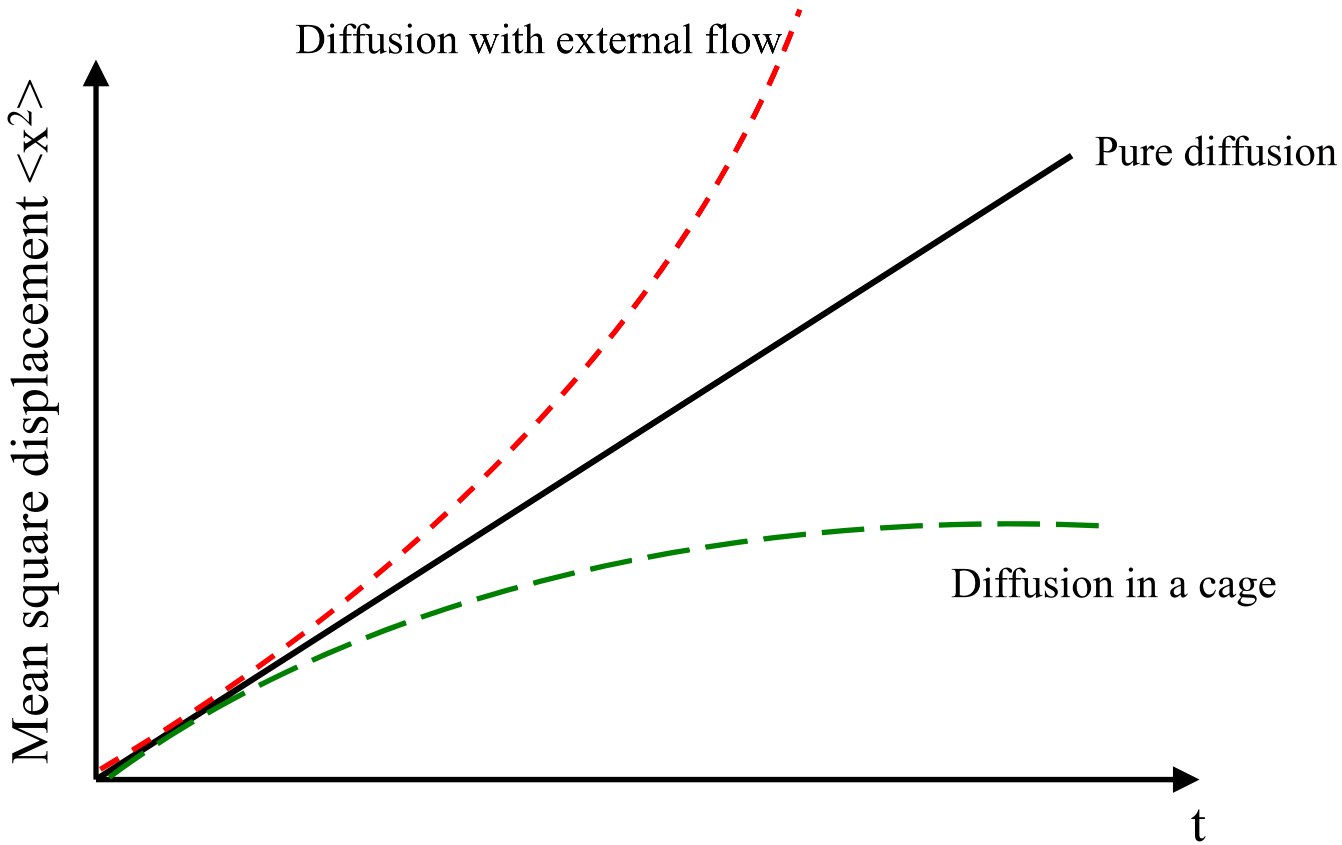
$$p(x) = \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \text{ where } n\delta^2 = 2Dt$$

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 = 1mPa·s^{-1}; T = 25°C; D=2000 µm^2/sec
Protein: Radius = 3nm, viscosity = 0.6915mPa·s^{-1}; T = 37; D = 100 µm^2/sec
Organelle: Radis = 500nm, viscosity = 0.8904mPa·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.

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- Background: FRAP & SPT
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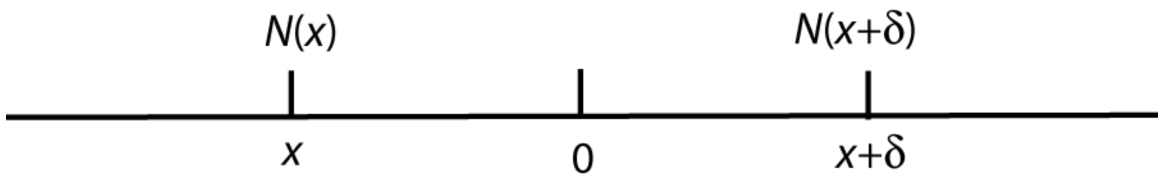
Macroscopic Theory of Diffusion (I)

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



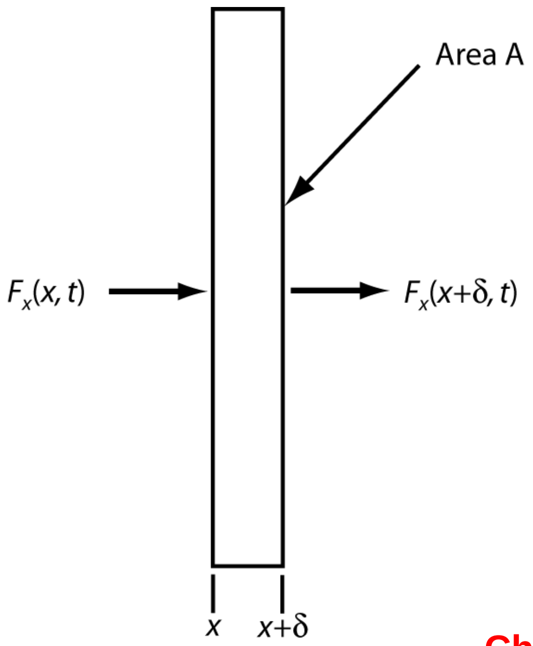
$$-\frac{1}{2}\left[N(x+\delta)-N(x)\right]$$

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

$$\boxed{F_x = -D\frac{\partial C}{\partial x}}$$

Macroscopic Theory of Diffusion (II)

- Fick's second equation



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

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

Change over time $$\frac{\partial C}{\partial t}=-\frac{\partial F_x}{\partial x}=D\frac{\partial^2 C}{\partial x^2}$$ **Change over space**

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

Diffusion Coefficient of a Particle

- Einstein-Smoluchowski Relation

$$v_d = \frac{1}{2} a\tau = \frac{1}{2} \frac{F_x}{m} \tau$$

$$f = \frac{F_x}{v_d} = \frac{2m}{\tau} = \frac{2m \frac{\delta^2}{\tau^2}}{\frac{\delta^2}{\tau}} = \frac{m v_x^2}{D} = \frac{kT}{D}$$

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

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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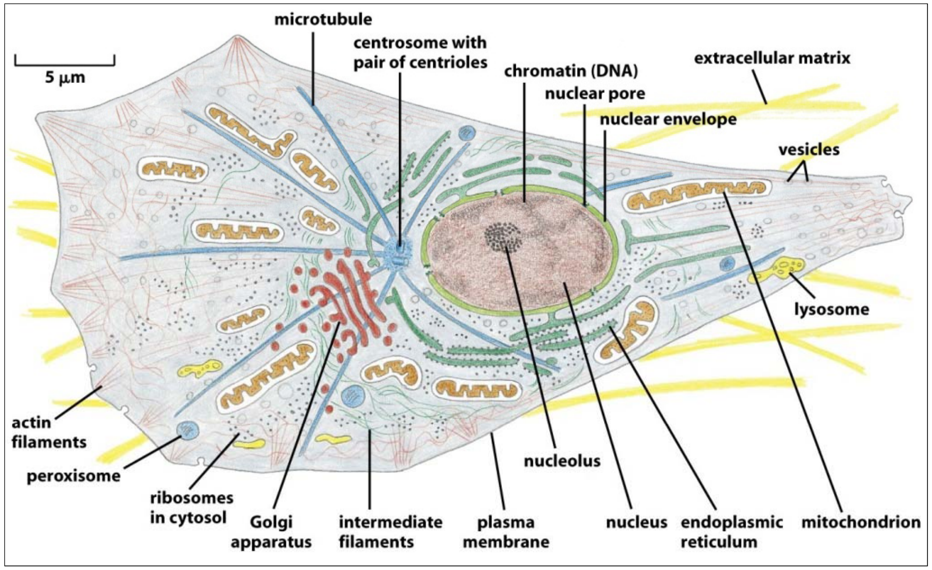
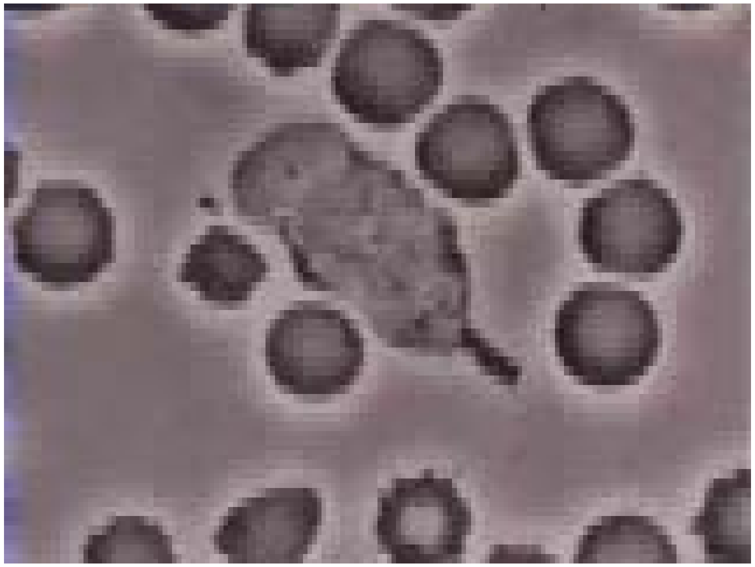
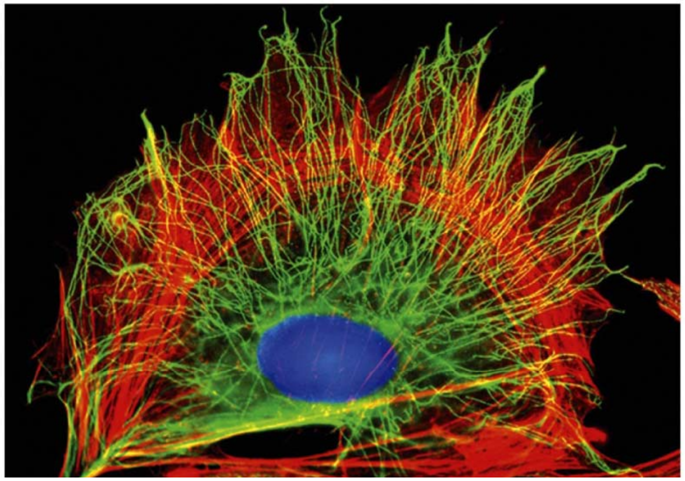
The Cytoskeleton is Highly Dynamic and Regulated

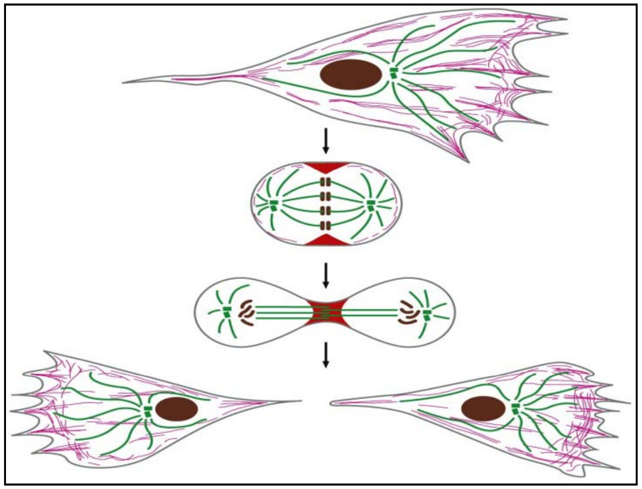




The Cytoskeleton (I)

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





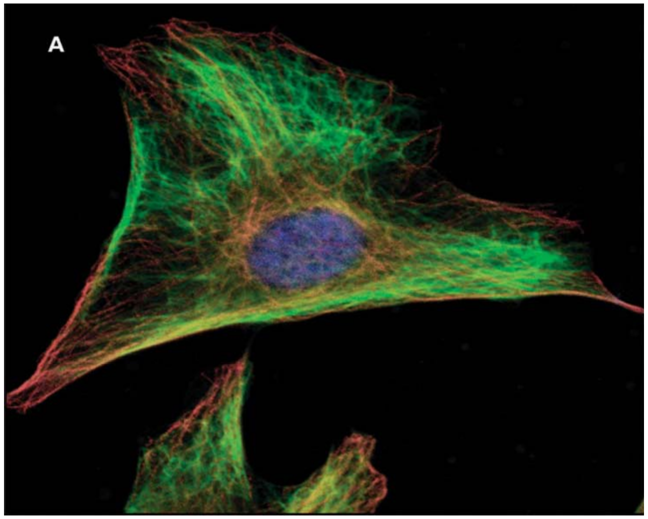
Red: actin
Green: microtubule

The Cytoskeleton (II)

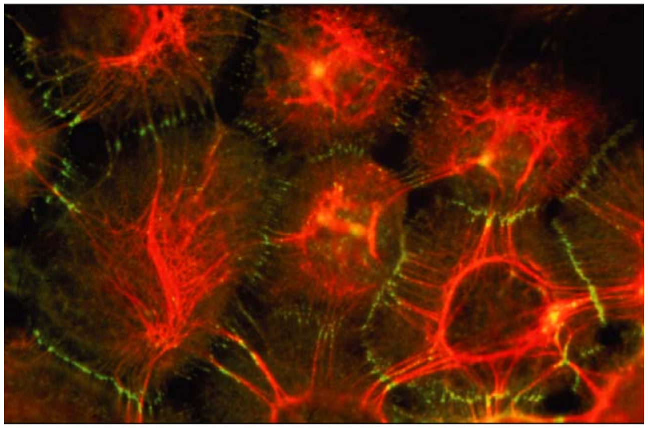
- Intermediate filaments

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

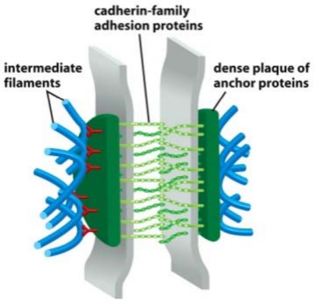
  - cell type
  - cell states (cycle)
  - cell activities



Green: vimentin IF
Red: microtubule

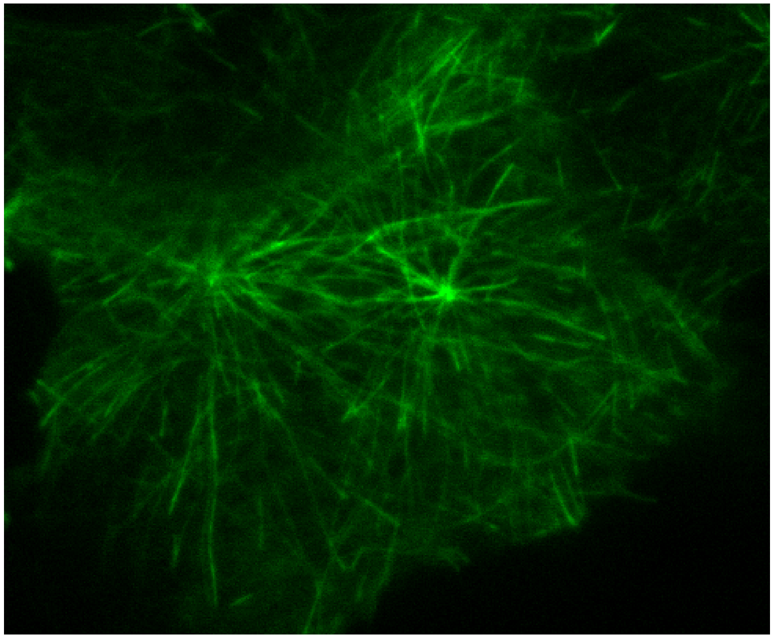


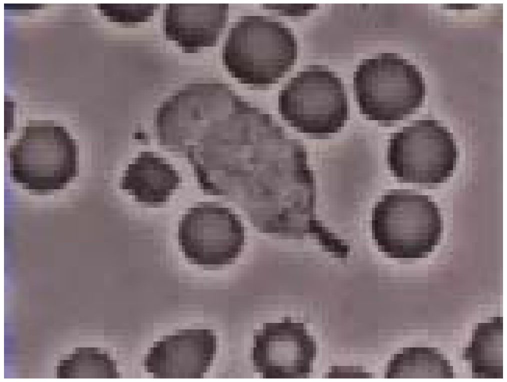
Orange: keratin IF
Green: desmosome

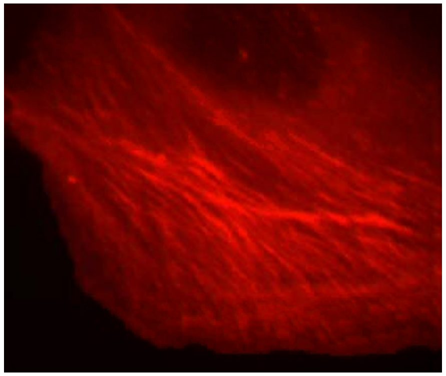


The Cytoskeleton (III)

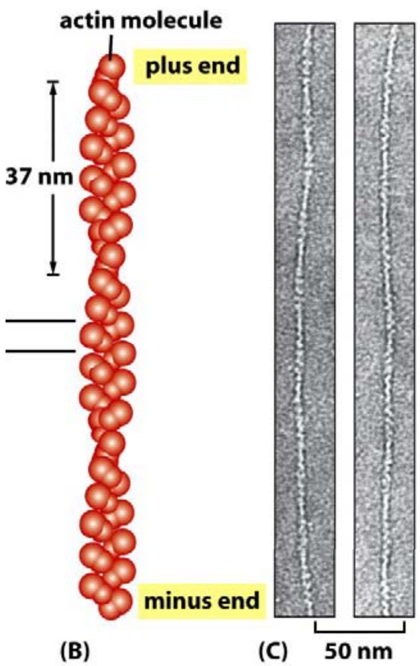
- The cytoskeleton plays a critical role in many basic cellular functions, e.g.
  - structural organization & support
  - shape control
  - intracellular transport
  - force and motion generation
  - signaling integration
- Highly dynamic and adaptive

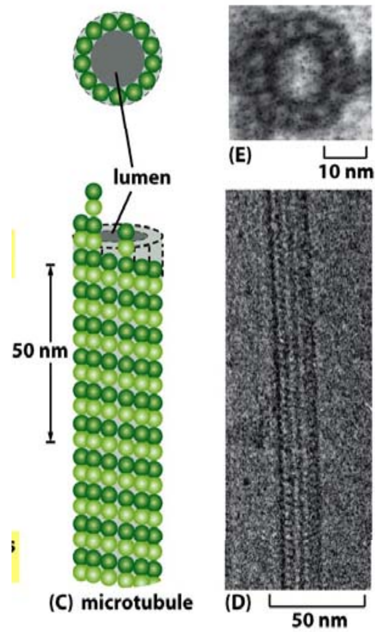


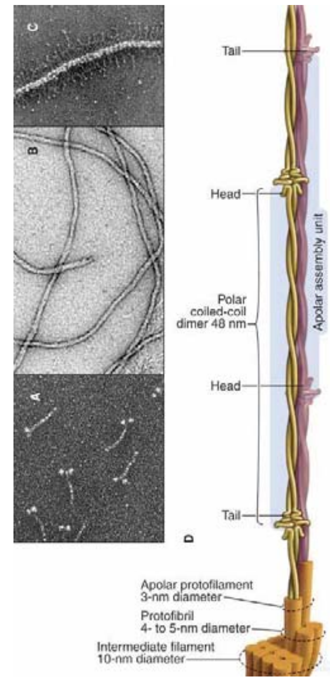




Overview of Cytoskeletal Filaments

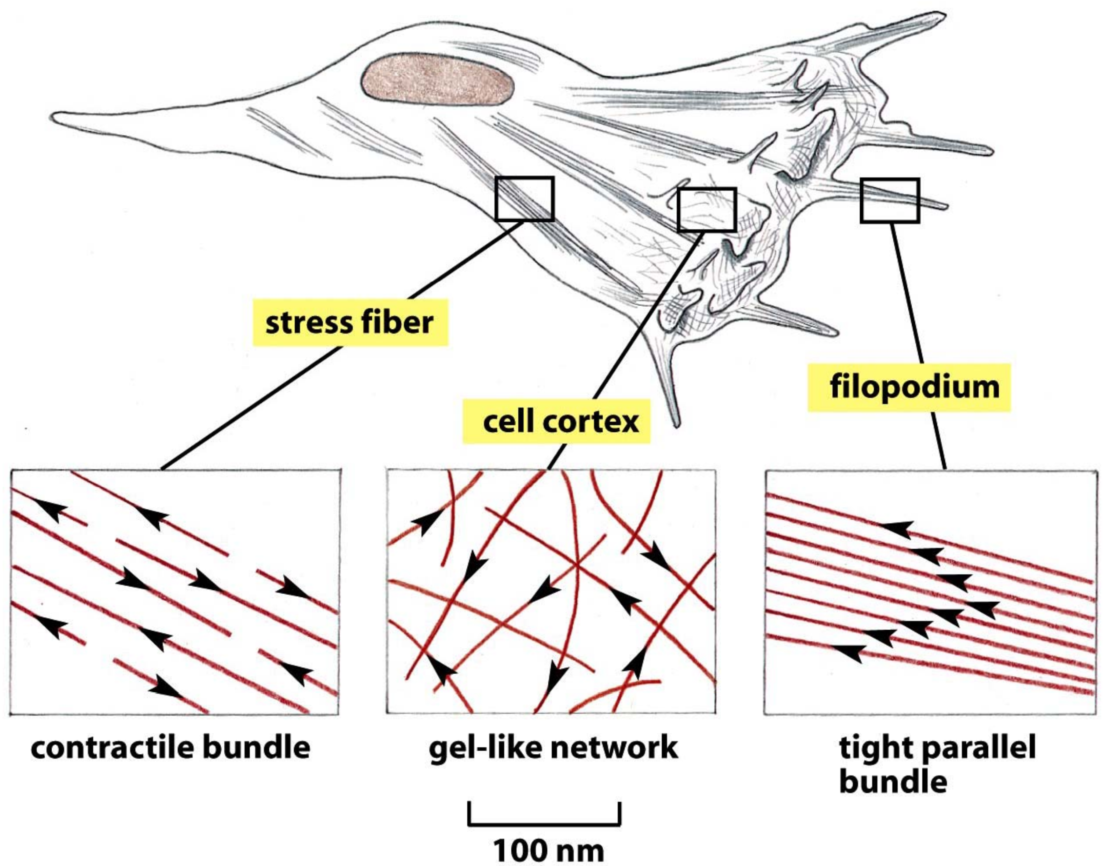






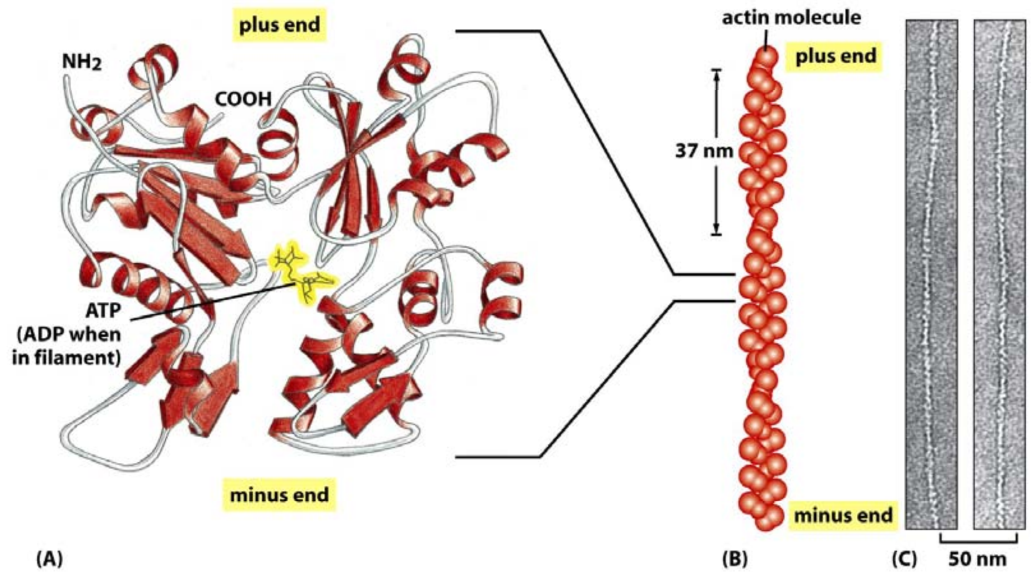
	Shape	Diameter	Subunits	Polarized
actin	cable	~6 nm	actin monomer	yes
microtubule	tube	~25nm	tubulin heterodimer	yes
intermediate filament	rope	~10nm	Various dimers	no

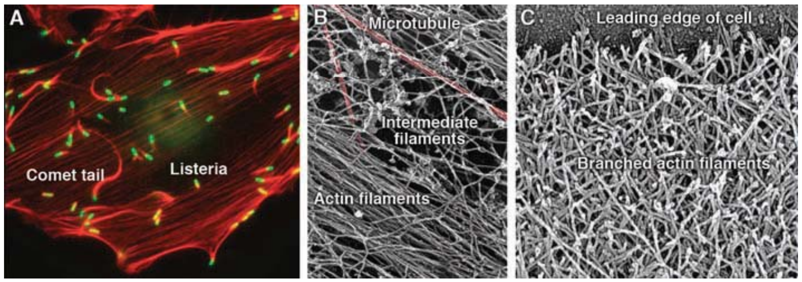
Organization of Actin with a Cell



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





Pollard & Cooper, Science,326-1208, 2009

Basics Terms of Chemical Reaction Kinetics

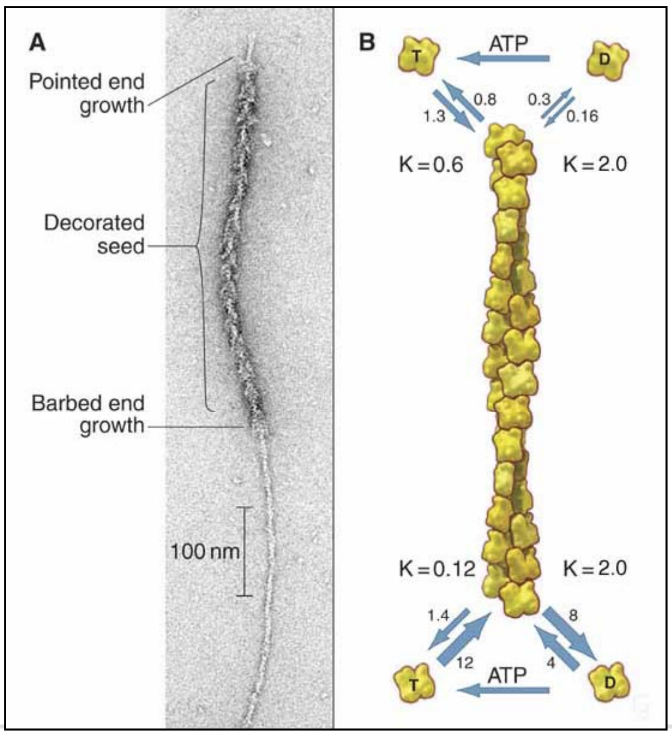
- A reversible bimolecular binding reaction

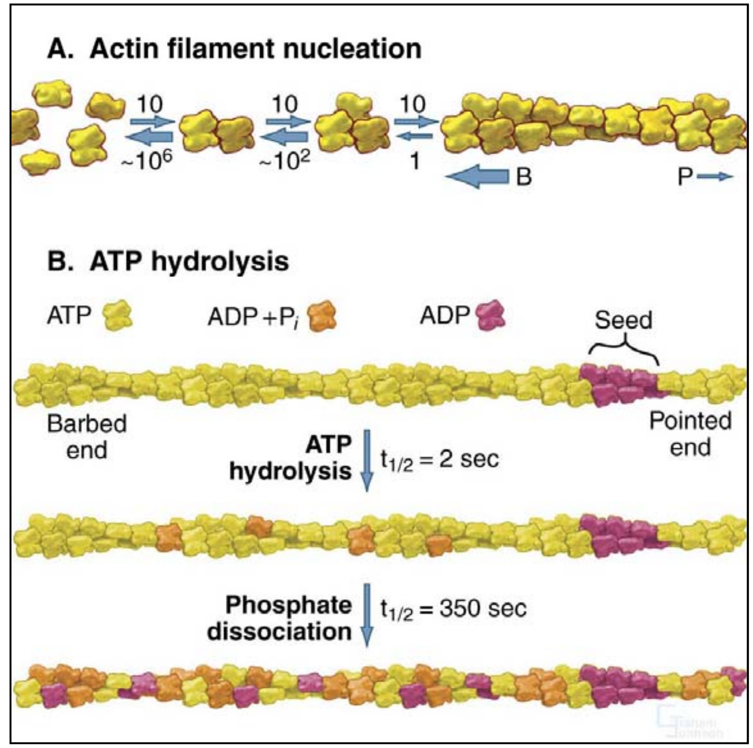
$$A + B \rightleftharpoons AB$$

- 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).





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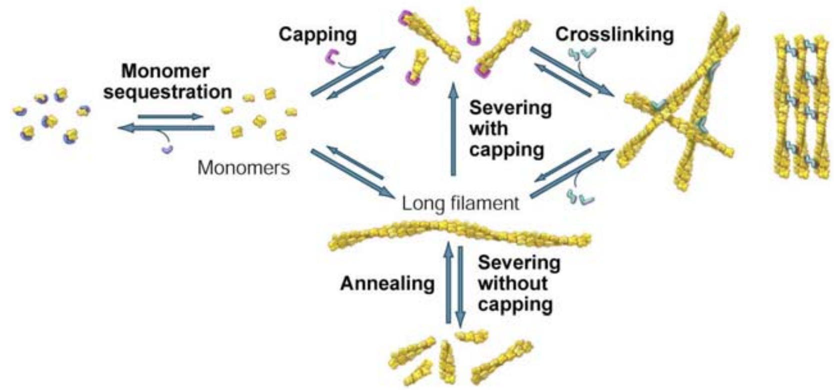
Actin Accessory Proteins (I)

- More than 60 families identified so far.

- Functions



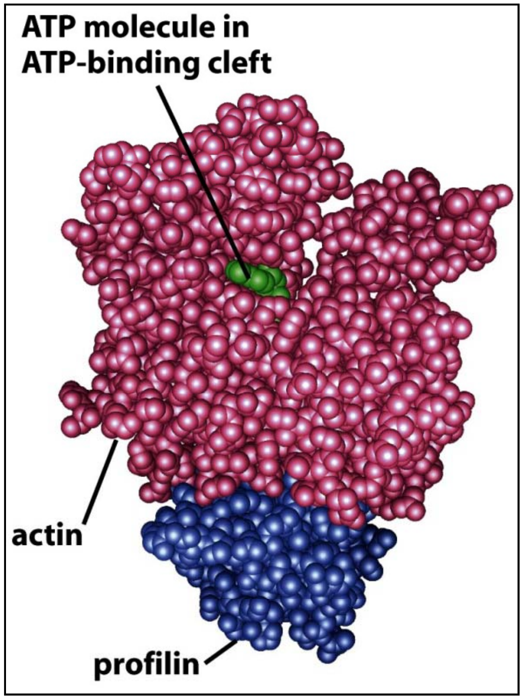
  - Monomer binding
  - Nucleation
  - Filament capping
  - Filament severing
  - Filament side-binding and supporting
  - Filament crosslinking

  - Signaling adapter

- Functional overlap and collaboration between actin-binding proteins

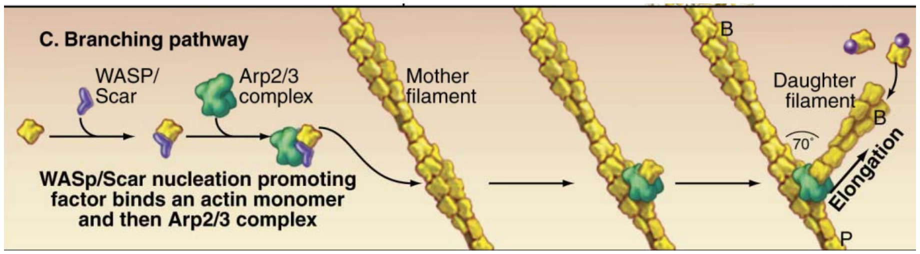
Actin Accessory Proteins (II)

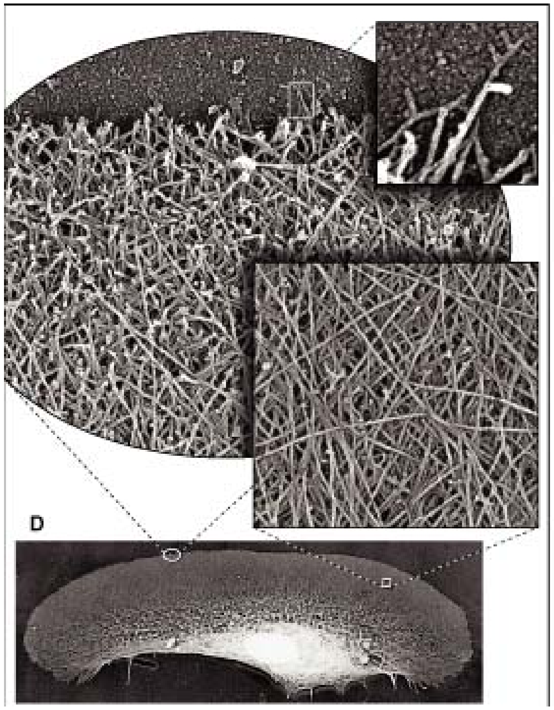
- Monomer binding proteins
  - profilin: to bind actin monomer and accelerate elongation
  - thymosin: to bind and lock actin monomer
  - ADF/cofilin: to bind and destabilize ADP-actin filaments

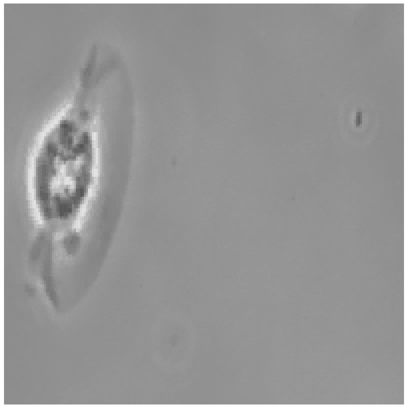


Actin Accessory Proteins (III)

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







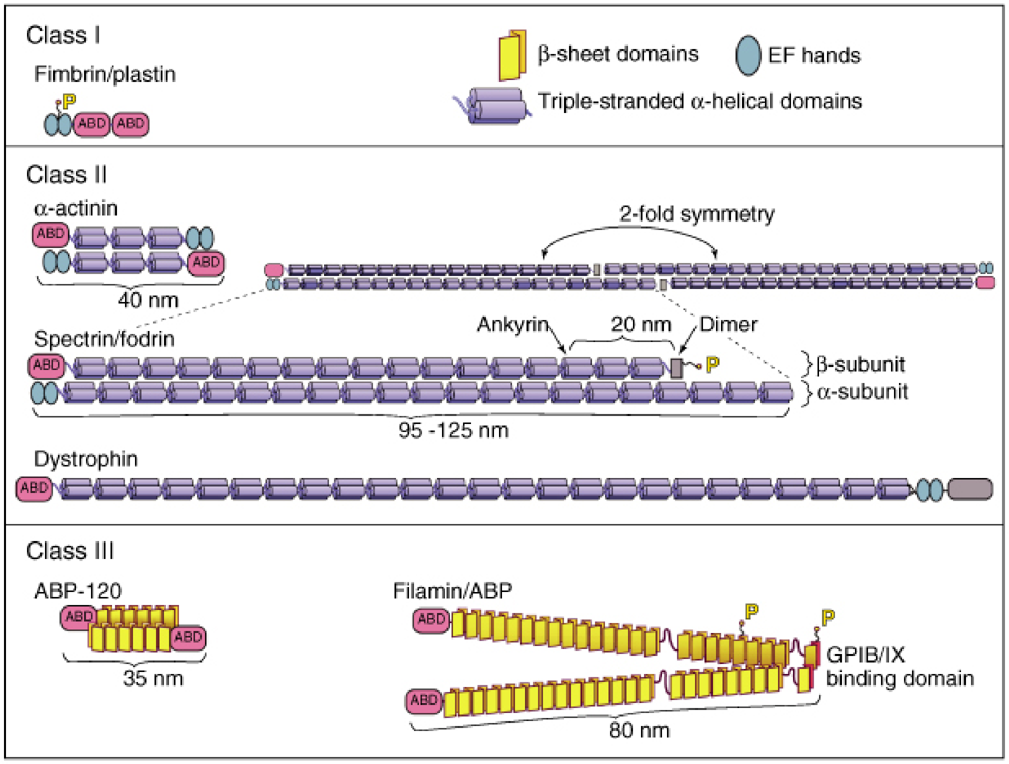
Actin Accessory Proteins (IV)

- 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)

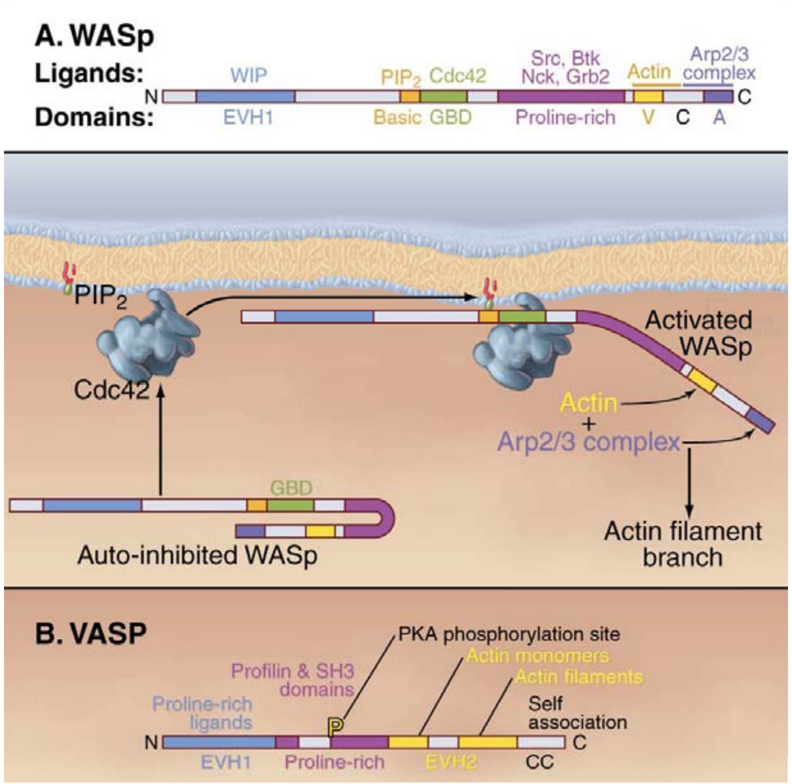
- Actin side-binding proteins
  tropomyosin, nebulin, caldesmon

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



Actin Adapter Protein

- Adaptor proteins such as WASP (a branching mediating factor) & VASP (a polymerization mediating factor) server as connectors between signaling pathways and actin assembly.



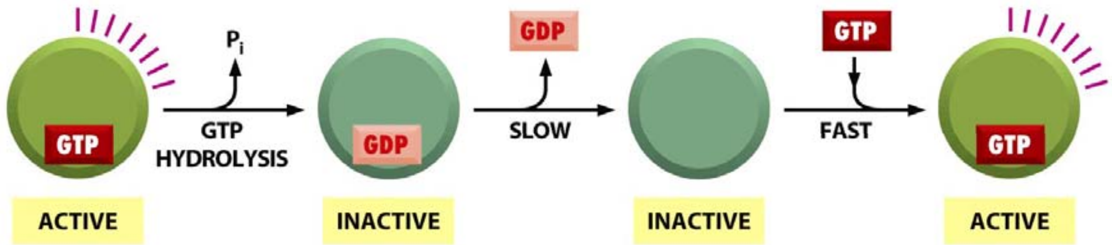
Actin Regulation

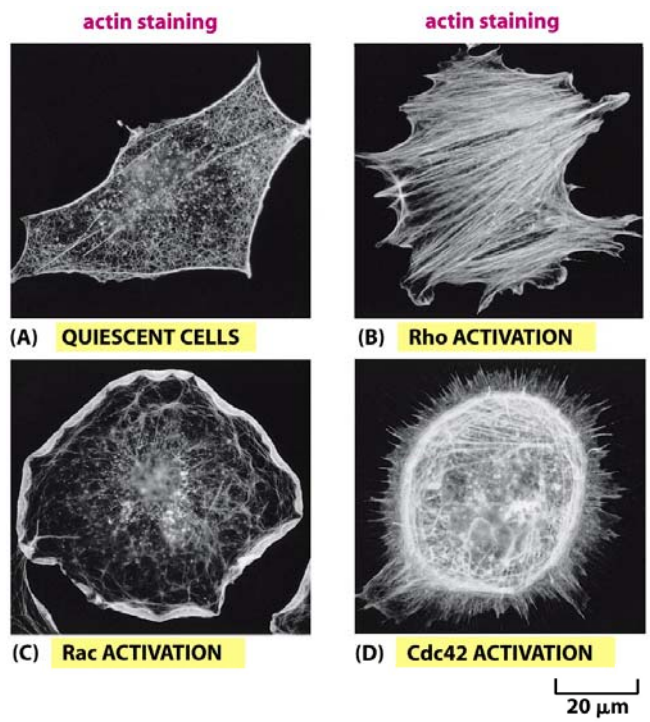
- GTPase: Molecule switch; Family of proteins that are activated by GTP binding and inactivated by GTP hydrolysis and phosphate dissociation.

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

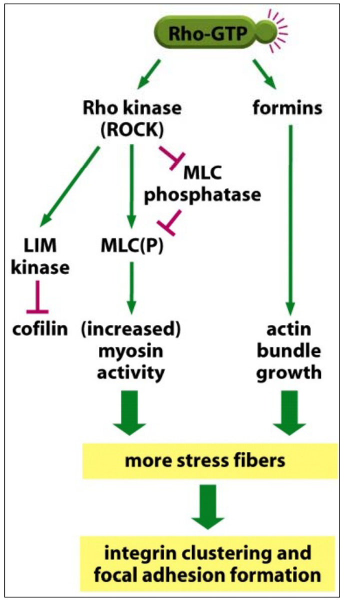
  Rho: its activation promotes actin bundling.

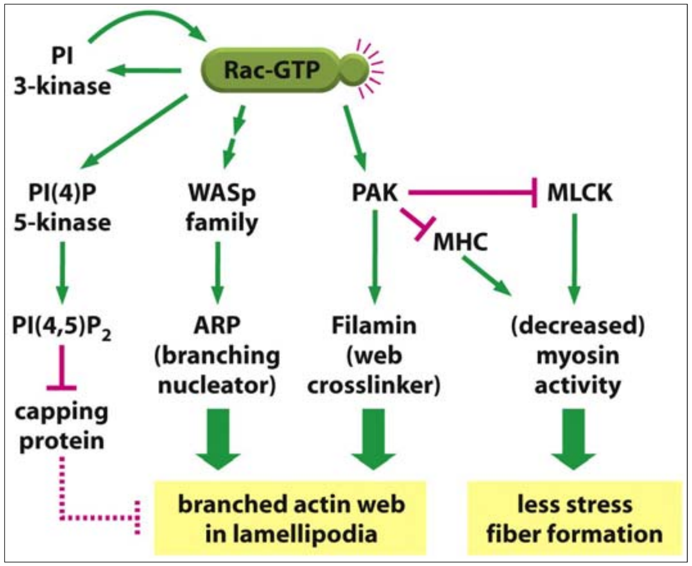
  Rac: its activation promotes polymerization at the cell periphery.

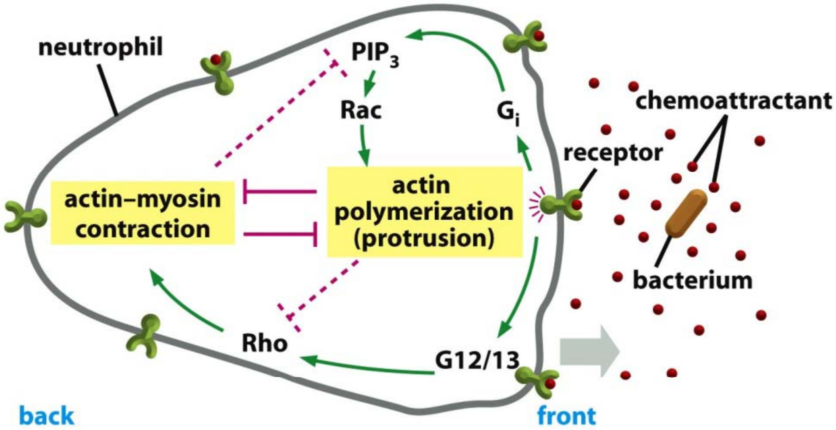


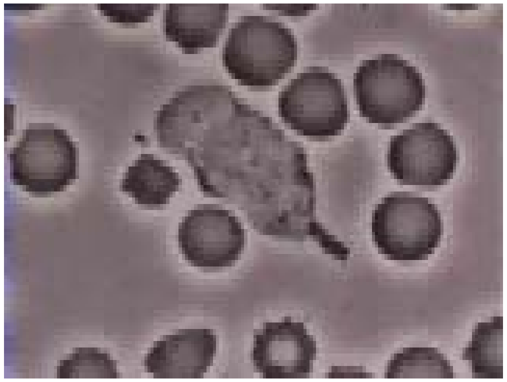


Rac on Actin Organization









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.

Required Reading

- Chapter 16

Questions ?