Biophysics Course

Quantitative Aspects of Cell Function
(Cells as Sophisticated Machines)

Cell Biology Section
Michael Sheetz
e-mail: ms2001@columbia.edu
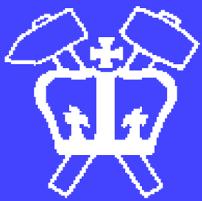
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Definition of a Cell:

Our working definition of a biological cell is a self-contained unit that is capable of duplicating itself given the proper nutrients and environment. In general, cells are enclosed by a lipid bilayer and contain the genetic material needed to direct the continued propagation of the cell.
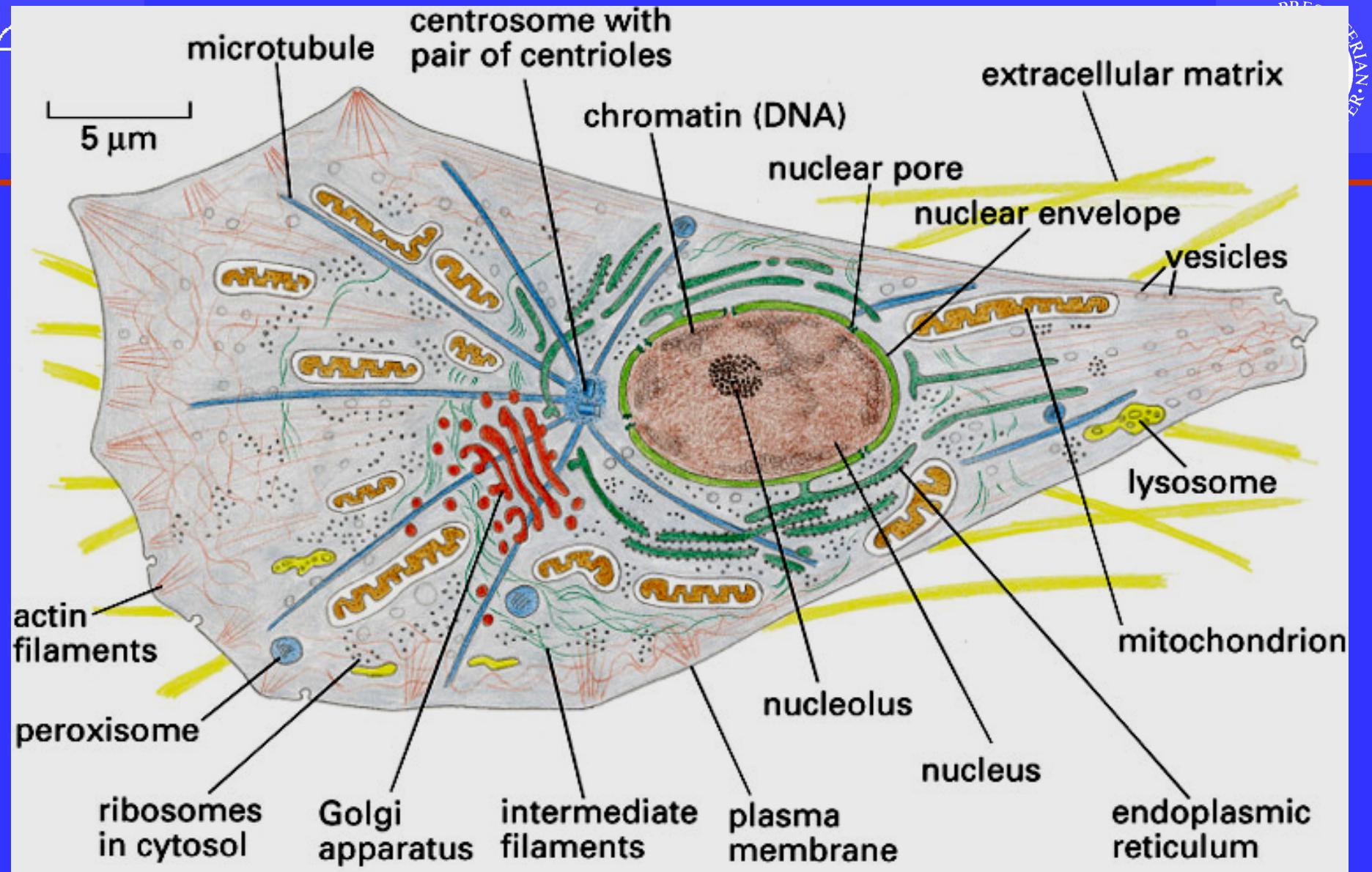
Figure 1–31. Molecular Biology of the Cell, 4th Edition.

The following labels appear in the figure:

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

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ANIMAL CELL — thin section of a generalized animal cell
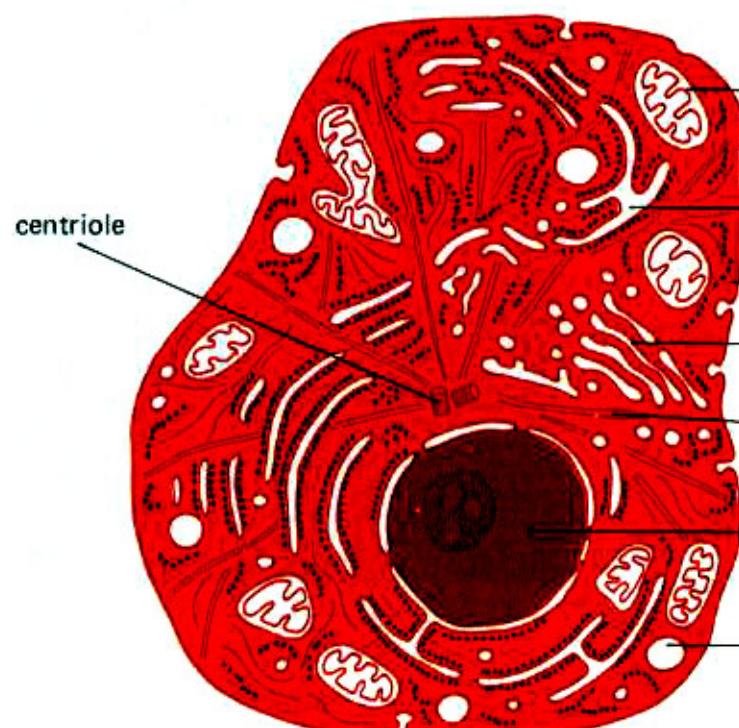
PLANT CELL — thin section of a generalized cell from a higher plant

centriole

mitochondria
plasma membrane
endoplasmic reticulum
cytosol
Golgi apparatus
filamentous cytoskeleton
nucleus
lysosomes
peroxisomes

cell wall
chloroplast
vacuole

10–30 μm

10–100 μm
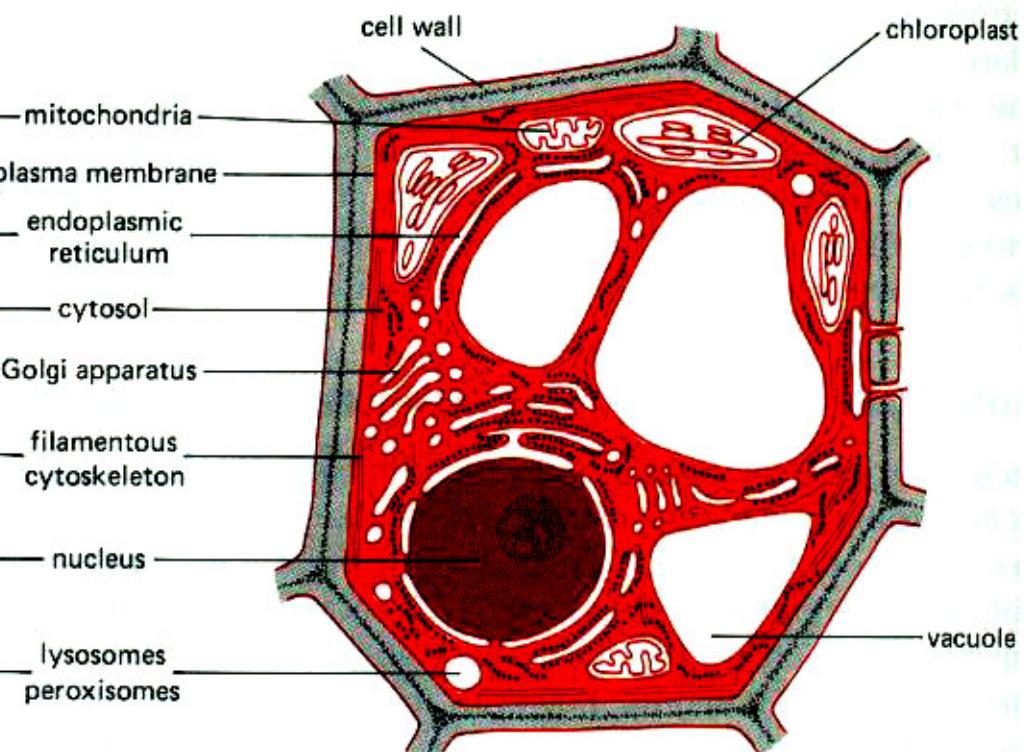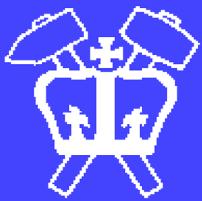
Engineering Cells for Robustness

Compartmentalization of Functions: It is much easier to perform a complex function if the local environment can be customized

Modularity in Functions: If we consider highly engineered items, they typically have modules that contain the machinery to complete a given function.

Term limits: If a cell continues down a given path for a long period, there

Figure 1–52. Molecular Biology of the Cell, 4th Edition.
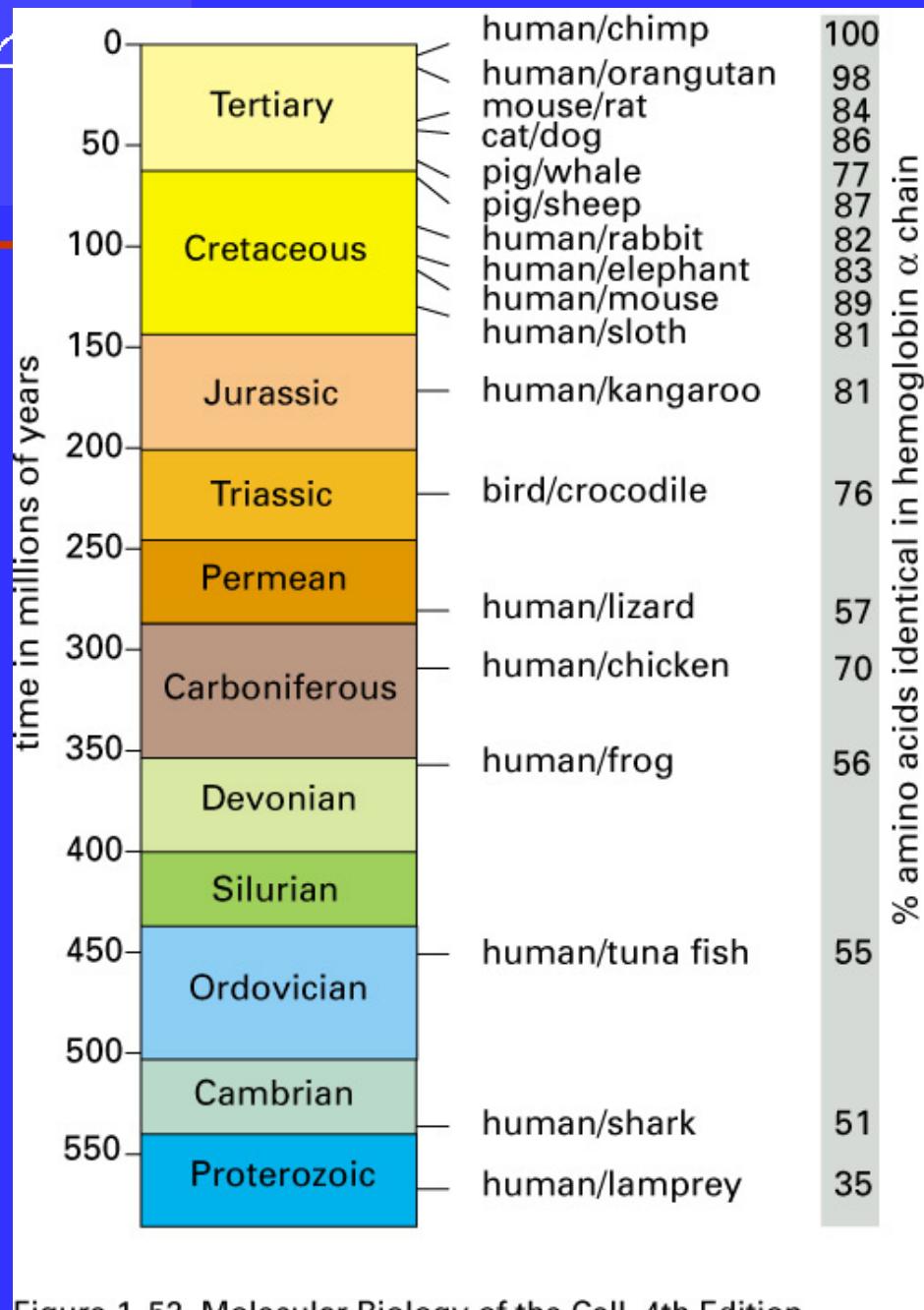
This is a comparison of the % identity between sequences of human hemoglobin and other species as a function of the time since they diverged in evolution.

Note: the sequences of human and tuna hemoglobin are 55% identical because they evolved independently for 450 million years.

•Cell Size and Number of Molecules

•Volume of a 3T3 cell of 15 μm in diameter ($4/3\pi r^3$ = 2000 μm^3 or 2 x 10^{-9} cm^3) vs. bacterium two microns in length and 0.8 micron in diameter (volume lπr^2 = 1 μm^3 or 1x10^{-12} cm^3)

•Protein Concentration in Cytoplasm   ~180 mg/ml (average protein is 50 kDa, the 3.2 mM protein or 2 x10^{18} molecules per ml)

•No. Proteins/Cell is 4 x 10^9 molecules per eukaryotic cell or 2 x 10^6 molecules per prokaryotic cell (if 10,000 different proteins for the eukaryotic and 2,000 for the prokaryotic, then about 10^5 molecules of a protein per cell)

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Cells have been around for about 2 billion years.

Just imagine the sophistication of PDAs in 2 billion years
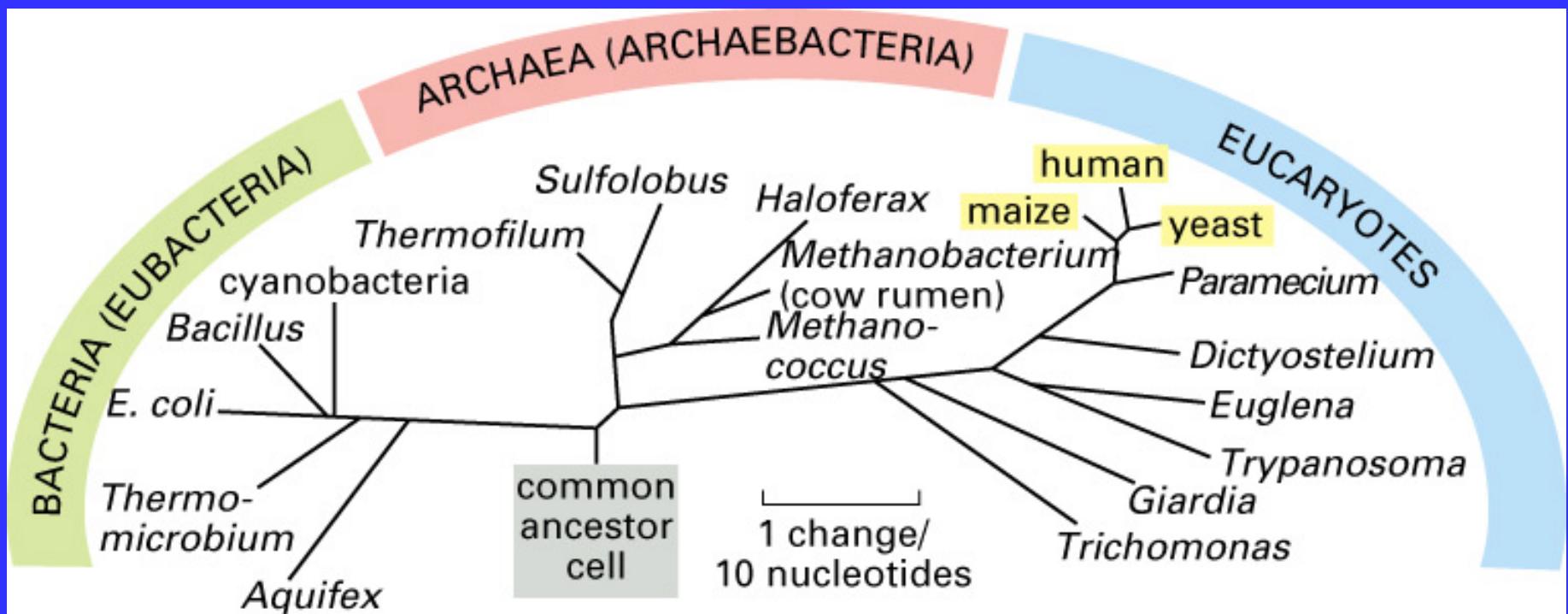
Figure 1–21. Molecular Biology of the Cell, 4th Edition.
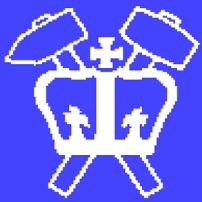
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•Cell Functions: Systems Bioengineering

- •Cells optimize functions for efficiency and robustness, similar to optimization of industrial production
- •Functions can be dissected into steps performed by modules
- •Modules contain many proteins and communicate with other modules
- •Quantitative measures of function are important: death vs. life is far from full story
- •Control theory approaches are useful
- •Compartmentalization and term limits correlate robustness
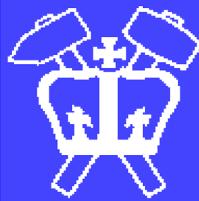
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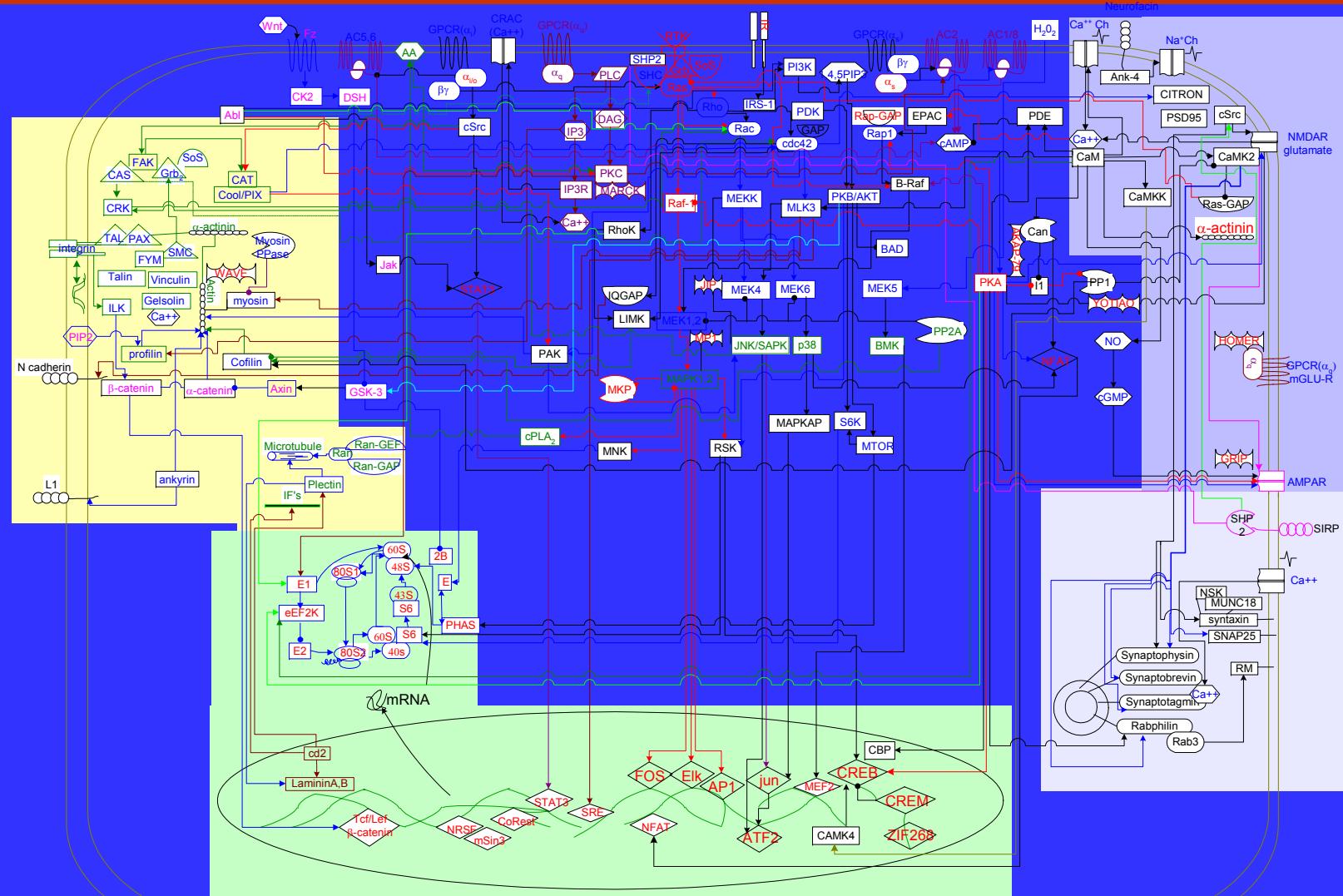
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Systems Engineering and Cells

Systems Engineering in Chemical Engineering involves the optimization of a process for the production of a chemical from raw material

Cell Selection The process of selection of cells for survival with limited resources or changing environmental conditions results in a similar optimization.

Computer or Automobile Evolution Many commercial products have evolved similarly from a basic functioning unit to a highly sophisticated system with many engineered features.
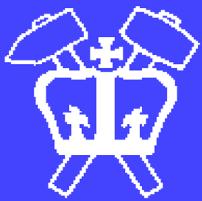
Cell Optimization and Robustness

Robustness in the context of cellular functions means that the important task can be completed even as conditions vary. Some of the obvious variables for cells are listed below:

1. number of proteins per cell

2. salinity and pH

3. temperature

4. nutrient level

5. environmental factors.

Cells Have Different Phases for Different Functions

Specialized Functions Require Phase Change: Many functions of cells are so complex that the phase of the cell must be altered through changes in state of many proteins or expression.

Phase Changes Are Discontinuous: Cells appear most often to undergo a rapid switch from one phase to another with the appropriate signal.

Populations of Clonal Cells Can Be in Many Phases: Even though a group of cells originated from the same cell and are grown in the same medium, they can be in different phases.

Is all DNA replicated?

Is environment favorable?

G2 CHECKPOINT

Are all chromosomes attached to the spindle?

METAPHASE CHECKPOINT

ENTER M

EXIT M

M

G2

CONTROLLER

G1

S

ENTER S

G1 CHECKPOINT

Is environment favorable?

Figure 17–14. Molecular Biology of the Cell, 4th Edition.
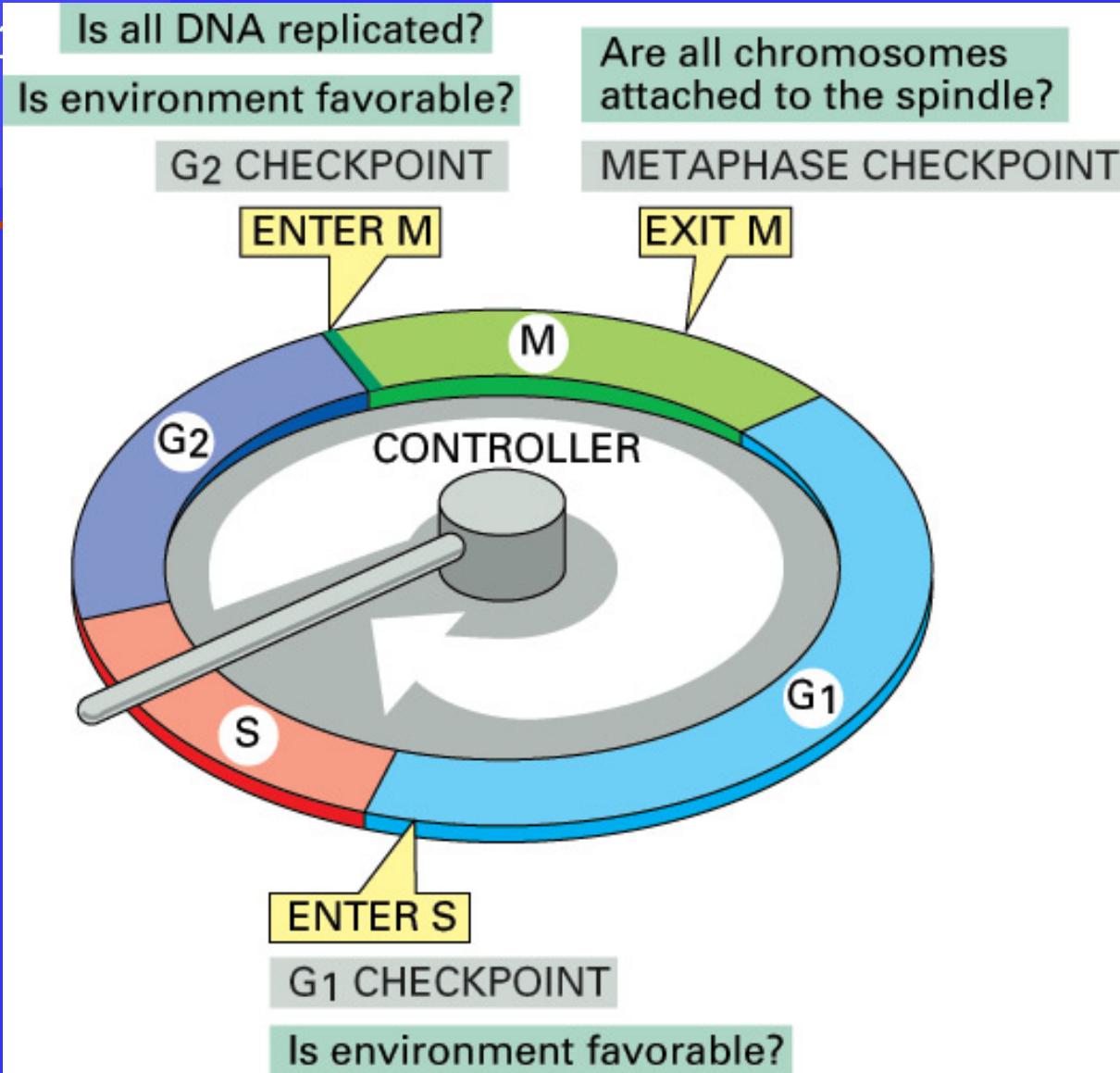
Phases of the cell cycle are bounded by definite checkpoints that aid in making the transitions abrupt.

The Critical Function of Cells is to Propagate DNA

DNA Encodes Plan for the Organism: Survival of the organism means propagation of the DNA.

Information Exchange is Critical for Survival: When one organism in a population has a mutation that enables it to survive a challenge, others will potentially benefit by sharing that information.

•**Cell Size and Number of Molecules**

•**Volume of a 3T3 cell of 15 μm in diameter ($4/3\pi r^3 = 2000$ μm^3 or 2×10^{-9} cm^3) vs. bacterium two microns in length and 0.8 micron in diameter (volume l$\pi r^2 = 1$ μm^3 or 1×10^{-12} cm^3)**

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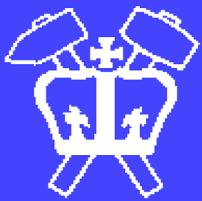
Life at Low Reynolds Number (diffusion and transport)

- Reynold's number $\qquad R = vL\rho/\eta$

- Example: fish vs. bacterium
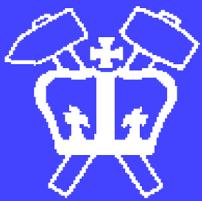
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- Reynold's number $R = vL\rho/\eta$

- fish of density approximately that of water ($\rho = 1$ gm/cc), length of 10 cm (L), moving at a velocity of 100 cm/sec (v) in water ($\eta = 0.01$ g/cm sec), we calculate R to be about 10^5.

- bacterium of the same density, length of 1 micron (L = 10^{-4} cm), moving at a velocity of 10^{-3} cm/sec through water, we calculate R to be 10^{-5}.
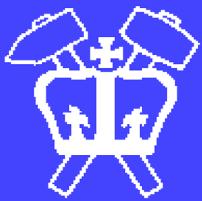
Viscous Drag on Particles

- Einstein-Smoluchowski relation

- $$v_d \ \phi_d \ = F_x$$

- The drift velocity of the particle (v_d) is related to the external force (F_x) by a constant called the frictional drag coefficient (ϕ_d)
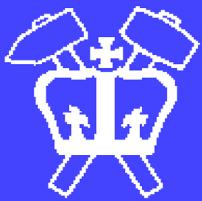
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- Because the drag is the same for diffusion as for externally applied forces, the diffusion coefficient can be derived

$$D = kT/ \phi_d$$

- For the special case of a spherical particle, Stokes' law gives the relationship between force and velocity.

$$f = 6\pi\eta r \, v$$
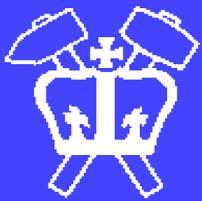
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- For a sphere we know from Stokes' law that $\phi_d = 6\pi\eta r$, which enables us now to calculate D directly

$$D_{sphere} = kT/6\pi\eta r$$

- For a one micron sphere in water $\phi_d = 9.5 \times 10^{-6}$ g/sec and $D_{sphere} = 4.4 \times 10^{-9}$ cm^2/sec
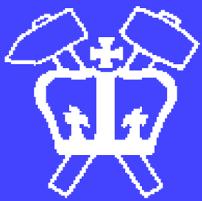
One-dimensional Diffusion

Assumptions:

1. Steps of r length occur at regular intervals (τ)

2. The direction of each step is equally likely to be + or – independent of previous steps.

3. Each object moves independent of other particles.
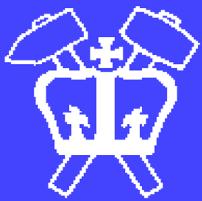
Root-mean-square displacement

• Single particle tracking of gold particles or single fluorescent molecules enables diffusion measurements at the single molecule level.

• $$2D_1t = <\Delta X^2>$$
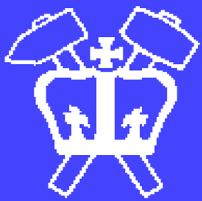
Gaussian Distribution of Diffusing Particles

- If all of the particles are at the origin originally, the distribution after many elemental steps follows a Gaussian

- $P(x)dx = (1/(4\pi Dt)^{1/2}) \, e^{-x^2/4Dt} \, dx$

- For a normal curve the fraction of the area within one standard deviation ($s = (2Dt)^{1/2}$) is approximately 68% of the total area
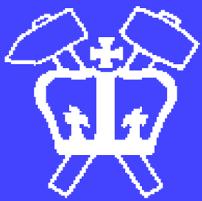
- •Practical Implications of the Diffusion Equation

- •For a cell ($v = 3000$ μm^3 or a cylinder 2 μm high and 44 μm in diameter), diffusion of typical proteins would take ~40 sec to travel about 20 microns ($D = 10^{-7}$ cm^2/sec)

- •For an axon one meter in length, typical proteins would require 10^{11} seconds or about 3,000 years
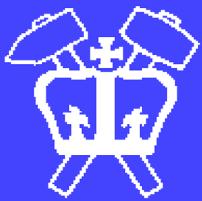
•<u>Non-ideal Diffusive Processes</u>

•Recent analyses of single particle tracking of diffusing proteins, vesicles, etc in cytoplasm have found many MSD versus time plots are non-linear

•Two different types of non-linearity are observed often in cells; confined diffusion and flow plus diffusion

•Confined Diffusion

•Many objects in cells have limited access to different regions of cytoplasm.

•Endoplasm, MT

•Ectoplasm, cortex
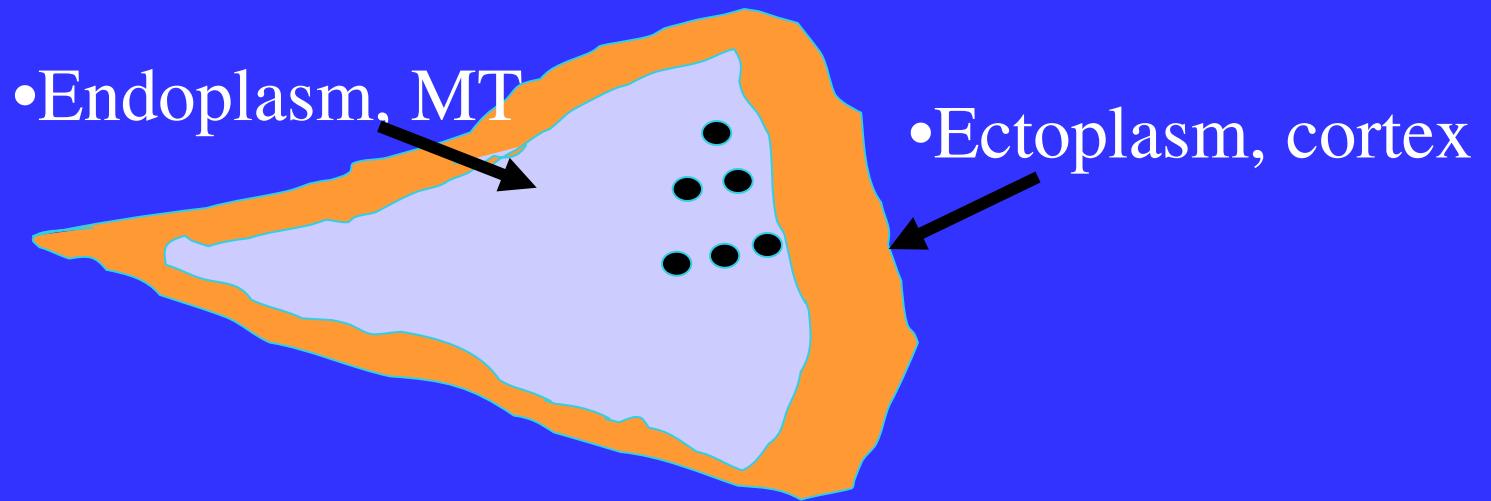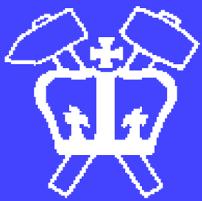
• Diffusion in a Flowing Medium

•If a particle is diffusing within a medium that is moving or if the particle has a drift generated by a constant force (e.g. magnetic), then MSD versus time will show a positive deviation (quadratic).

• $\quad <\Delta X^2> = 2D_1 t + (vt)^2$
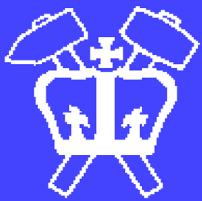
•Diffusive Transport

•We will consider a simple case of synthesis and assembly in cytoplasm. Site A is where a protein is being translated and folded properly. Site B is where the protein is assembled into a working complex. Proteins need to get from A to B for assembly. How can we describe the process?
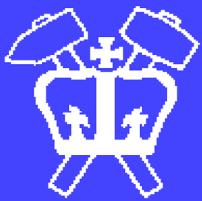
•One-dimensional Diffusive Transport

•One way to understand diffusive transport is to go back to the diffusing drunks and to talk about 2 bars at closing. Assume that the bars are one step from each other and that 200 are in one vs. 100 in the other bar. At early times will there be a net transport?

Fick's Law.

$$J_x = -D \, dC/dx$$

where Jx is the flux in the x direction, D is the diffusion coefficient, dC/dx is the concentration gradient in the x direction.

Implications of Low Concentration of Sites in Genome

1. To specify 2 sites in 6 billion base pairs, you need 16 base pairs (4 possibilities for each base, which means 4,294,967,296 possible 16mer sequences). The bases do not have to be sequential and are likely to be spaced around the activation site.

2. Binding is governed by normal binding kinetics

   Binding constant $K_A = k_a/k_d$

   Dissociation constant $K_D = k_d/k_a$

For a protein of concentration [x], the rate of binding to immobile sites, y, is given by the equation

   $dy/dt = k_a [x]$ and dissociation by $dxy/dt = k_d$

Diffusion-Limited Binding Defines the Lifetime of Complexes

The lifetime of protein complexes controls the dynamics of functions such as transcription. In other words, if a protein-protein interaction does not fall apart, i.e. binding is very strong and the dissociation constant (k_d) is very slow, transcription may not turn off.

Because diffusion of proteins to sites determines the rates of most protein-protein interactions, they have a $k_a = 2 \times 10^6 \, M^{-1} \, s^{-1}$

If $K_D = k_d/k_b$, then $k_d = k_b \times K_D = 2 \times 10^6 \, M^{-1} \, s^{-1} \, K_D$

For $K_D = 10^{-6} \, M$, then $k_d = 2 \, s^{-1}$ then

For $K_D = 10^{-9} \, M$, then $k_d = 2 \times 10^{-3} \, s^{-1}$ or a lifetime of 500 s.

Biophysical Analysis of Membrane Functions by Laser Tweezers

- **Plasma Membrane Functions (Control by PIP2 Levels in Plasma Membrane)**
  - Cell Motility
  - Endocytosis (Cell Volume regulation)
  - Membrane resealing
- **Modified Model of Membrane Structure**

Evidence for the Bilayer– Couple hypothesis from membrane mechanics
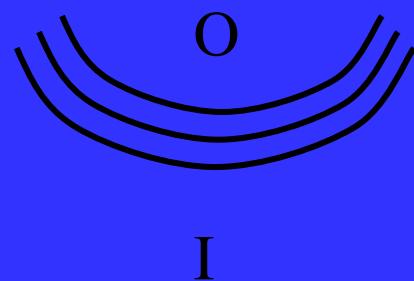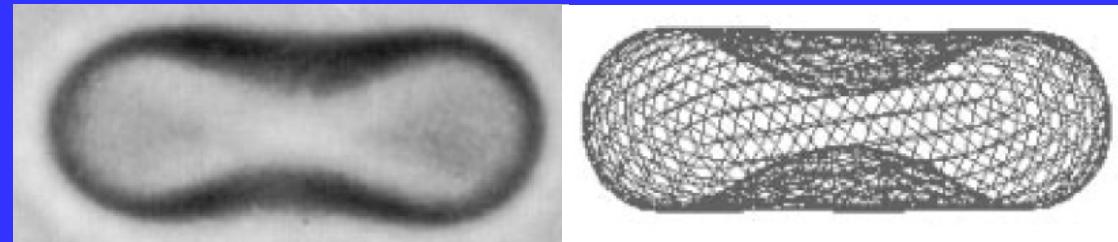
$$\frac{\text{Inner Mono Expansion}}{\text{Outer Mono Expansion}}$$
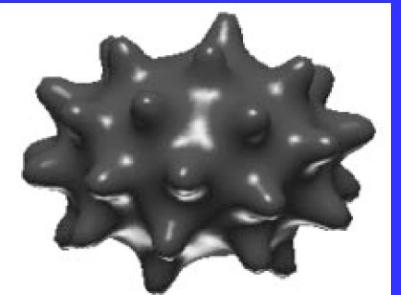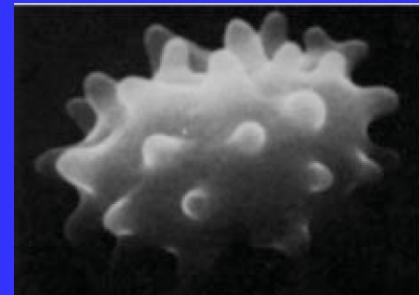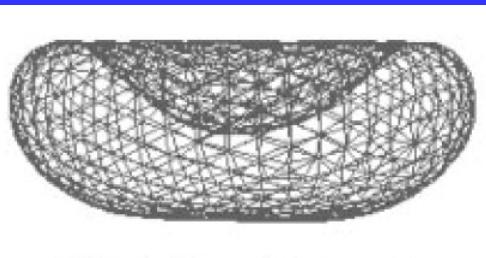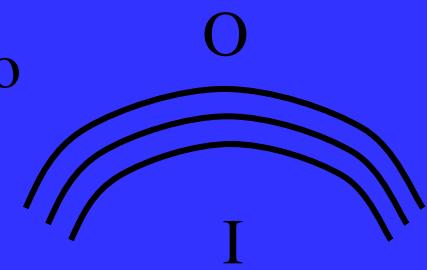
Lim et al., (2003) PNAS. 99:16766-9
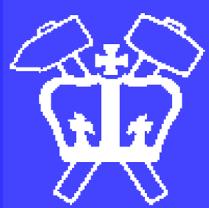
Tether Formation on Fibroblasts
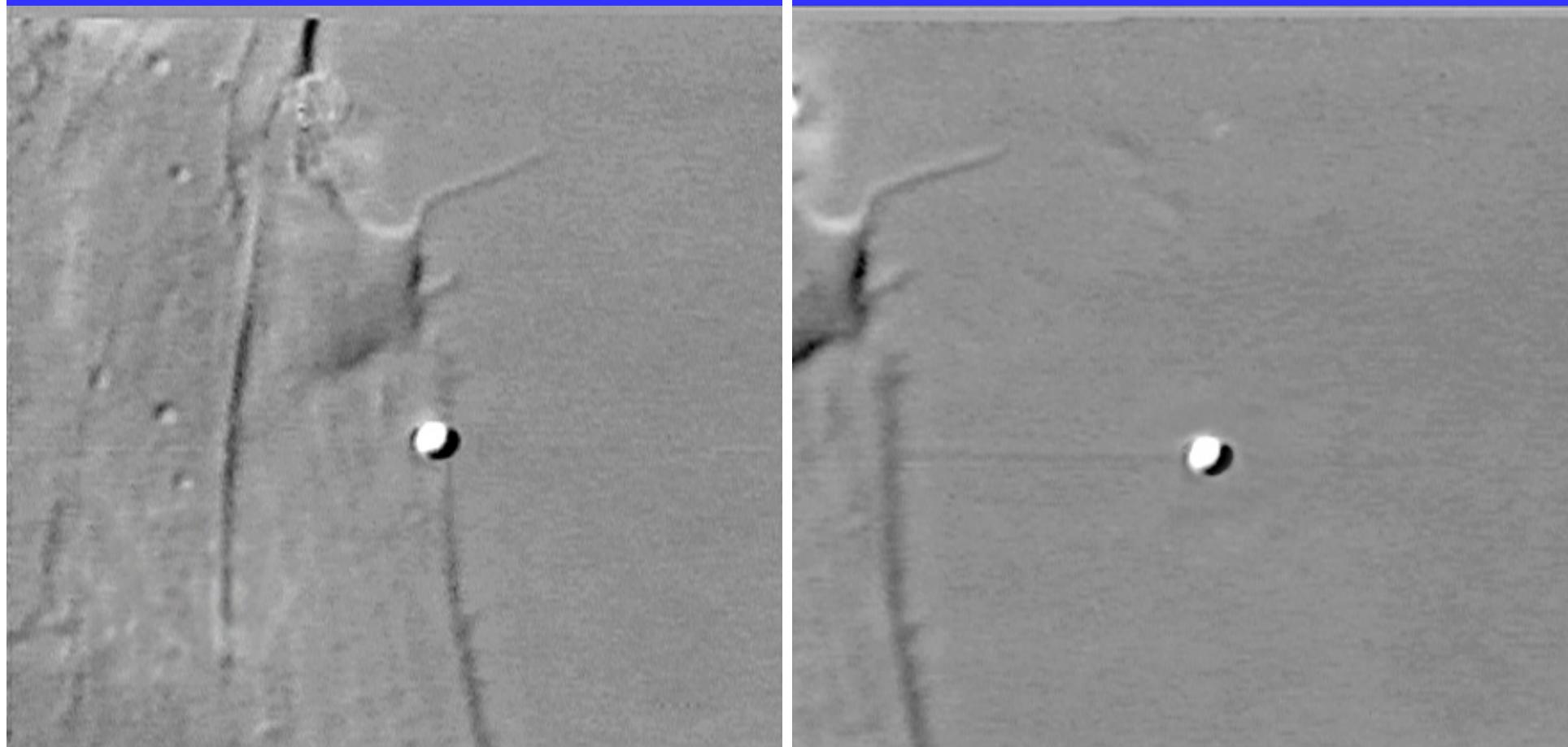
A
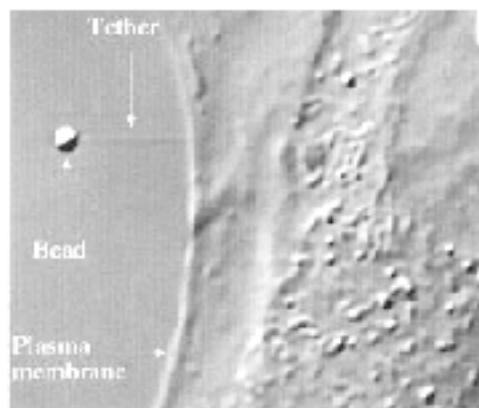
B
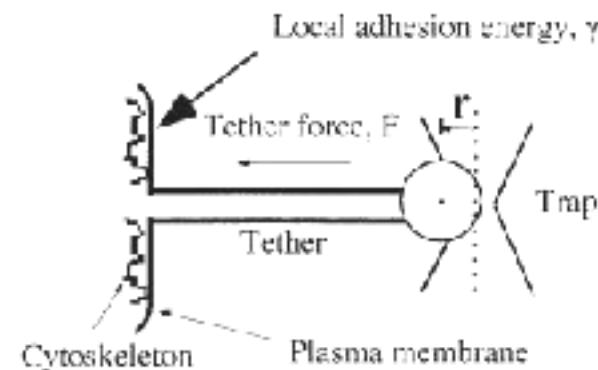
C
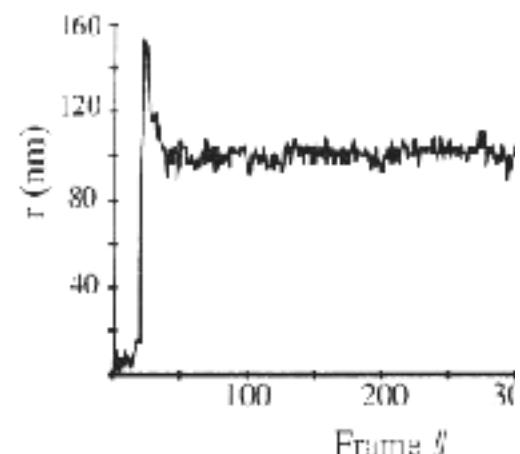
D


E

Apparent Membrane Tension

γ

B
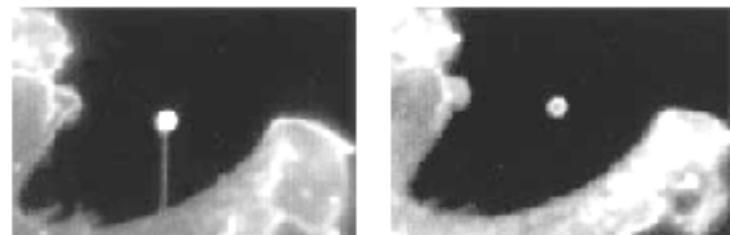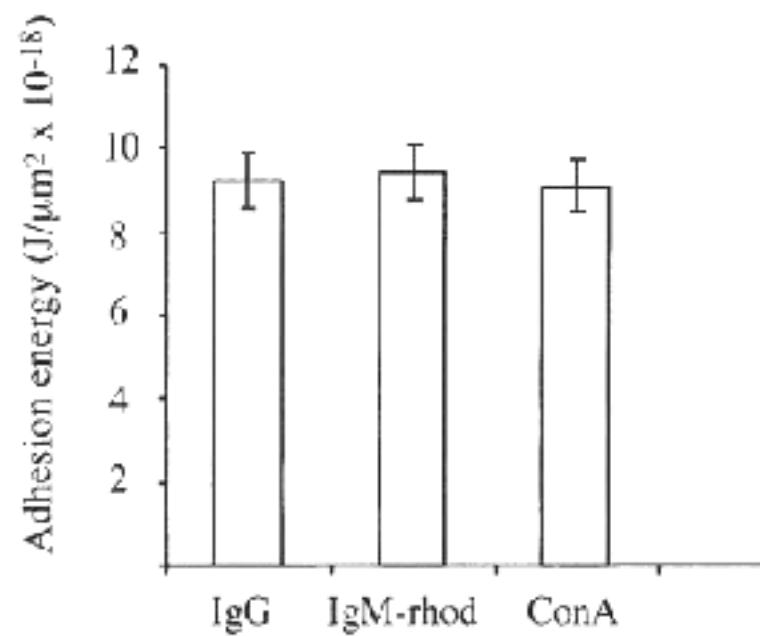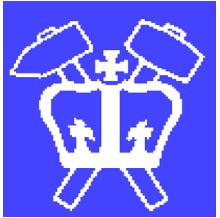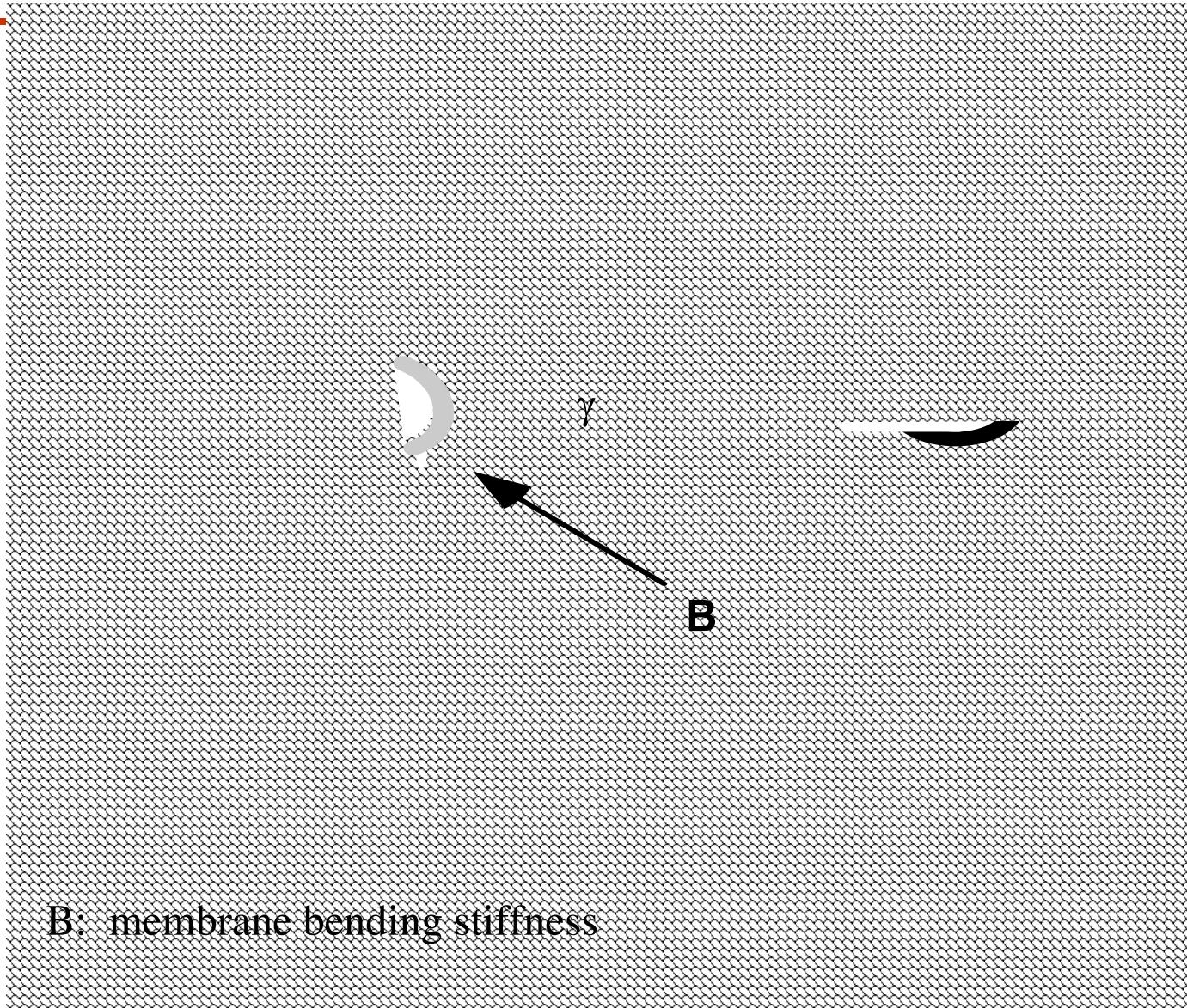
B: membrane bending stiffness

# Tether Force vs. Length Indicates Membrane Reservoir is Present
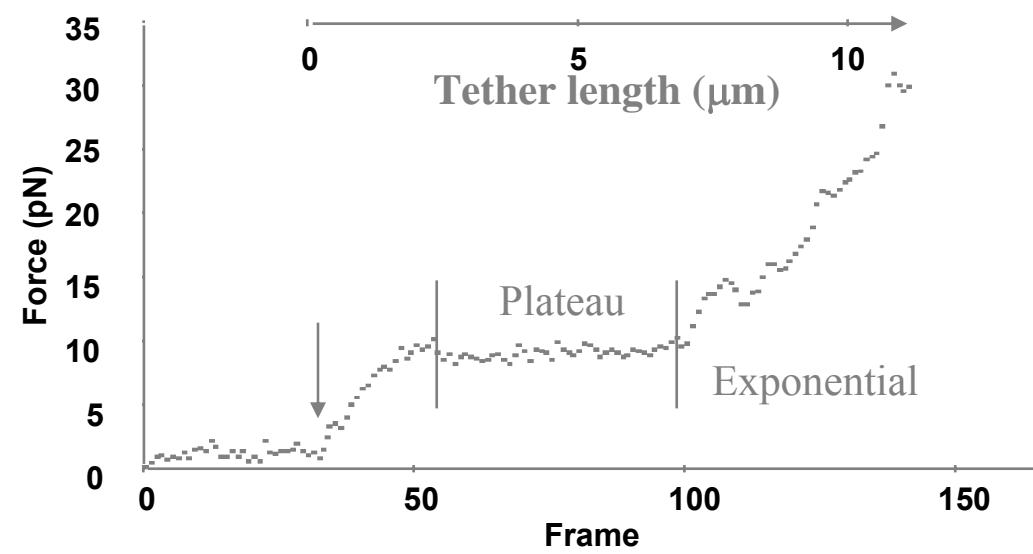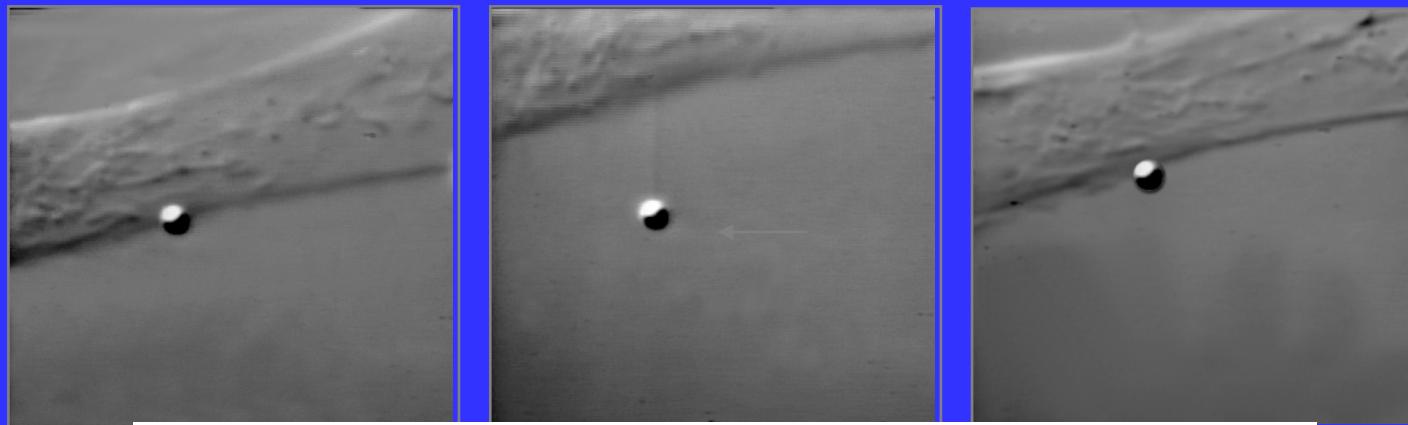
Raucher and Sheetz, Biophys J 77:1992 (1999).

Reservoir Increases With Each Tether Pull
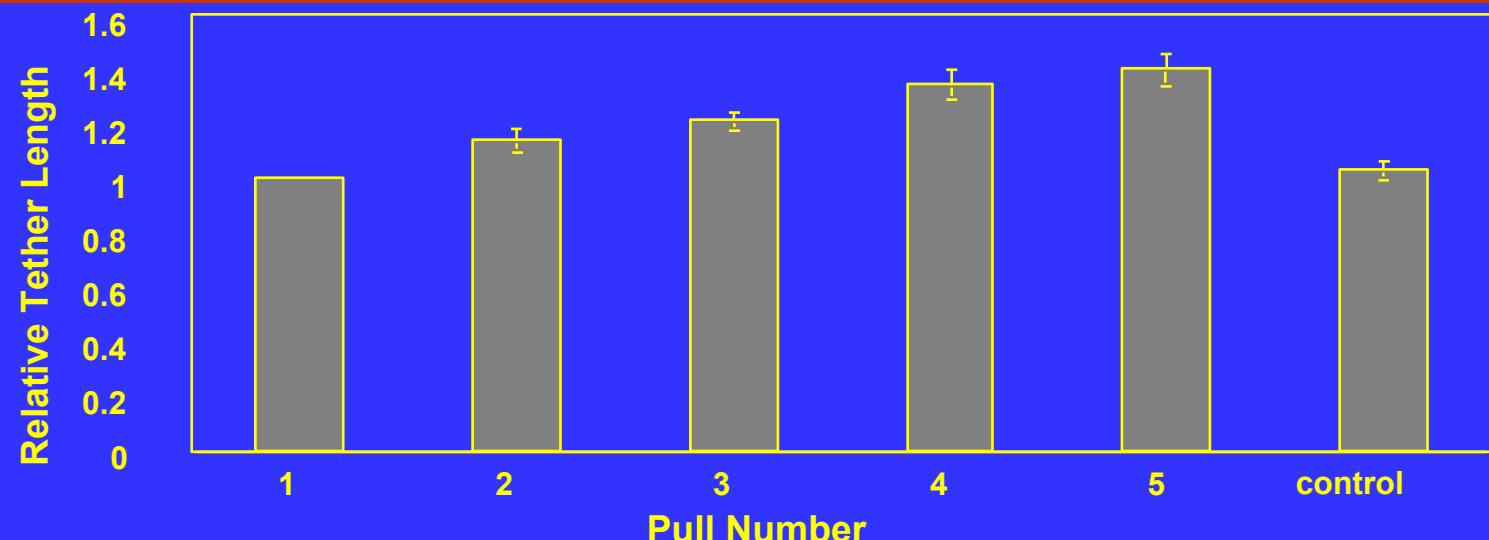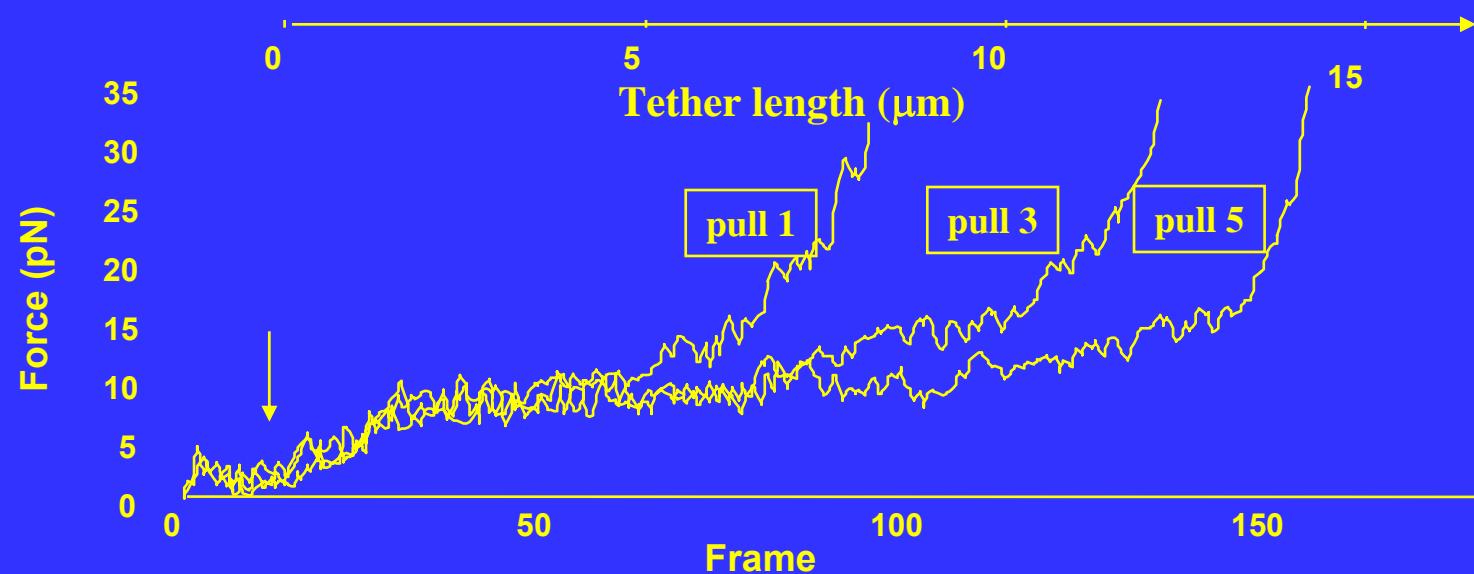
Reservoir Increase Is Sensed Over Whole Cell
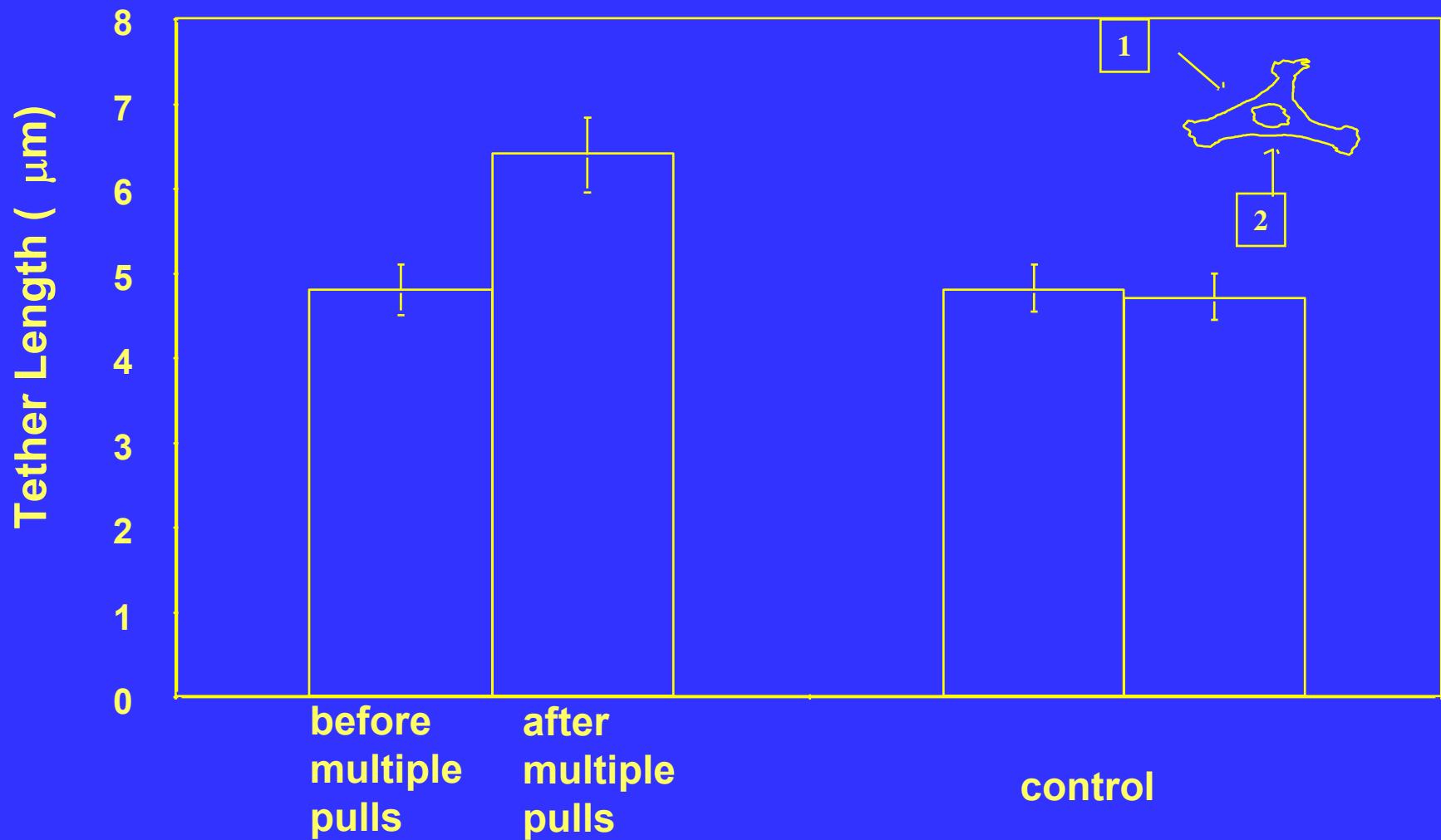
Raucher & Sheetz (1999) Biophys. J. 77:1992.
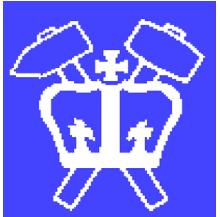
Summary

• In-plane tension is small and is continuous over the whole cell surface

• Membrane-cytoskeleton interaction is the major component of the apparent membrane tension

Substrate Rigidity Can Direct Movement
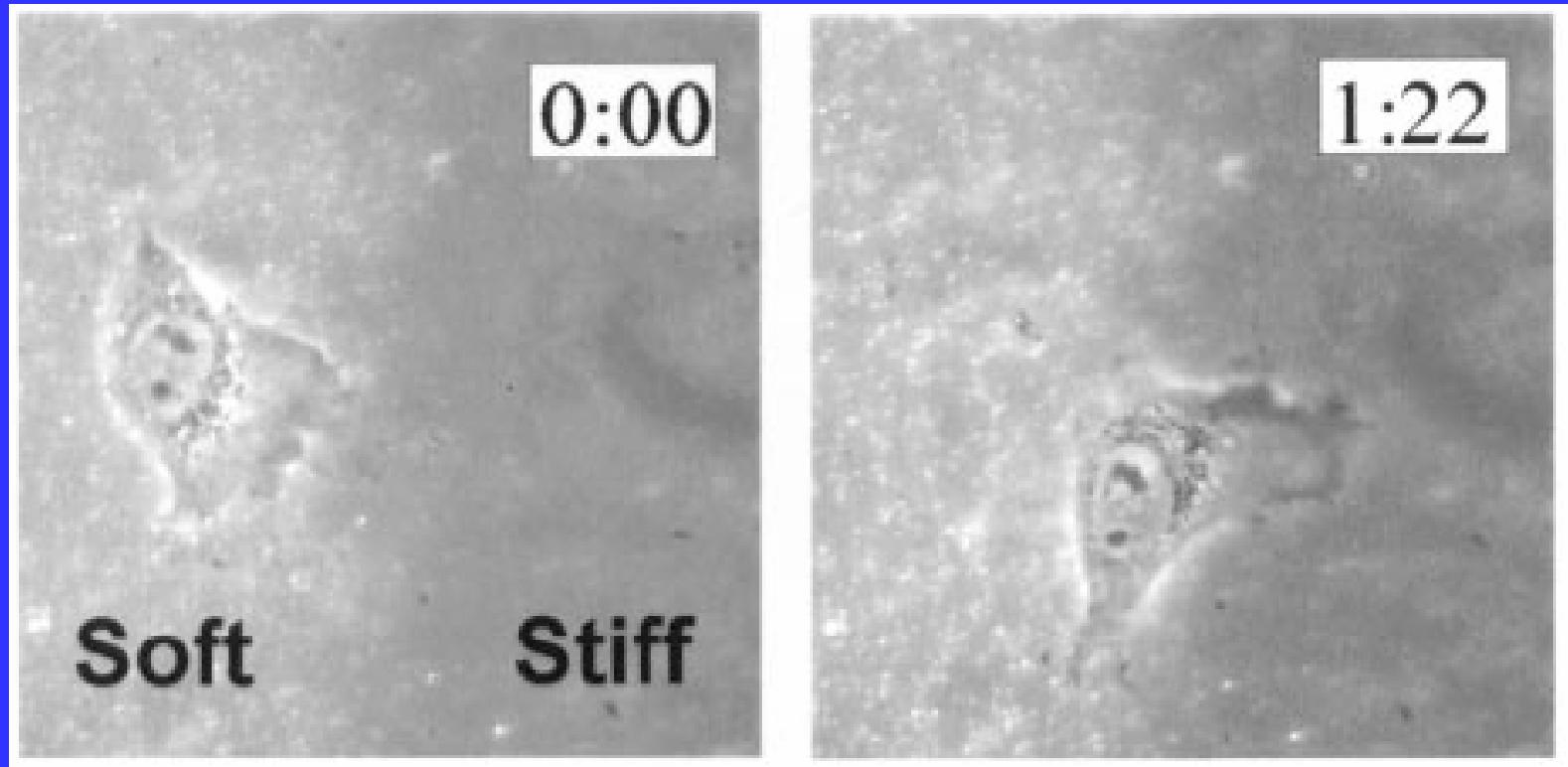
- Lo et al., 2000. Biophys. J. 79:144-152

Cell Biophysics: Analysis of Physical Basis of Cell Functions

- **Physical and chemical analysis of cell functions is critical**

- **Tension in Membrane is Critical for Membrane Functions (Global Control)**

- **Cell Forces Generated and Sensed by Cytoskeleton**
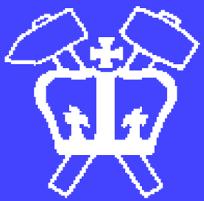
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Papers for Discussion:

- Wednesday (Mangon and Alon. 2003. Proc. Natl Acad. Sci USA. 100:11980-4. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14530388 ;

- Thursday (Pollard. 1986. J. Cell Biol. 103:2747. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3793756)

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