# Lecture 4

# Structure and Function of Actin & Myosin

**Outline:**

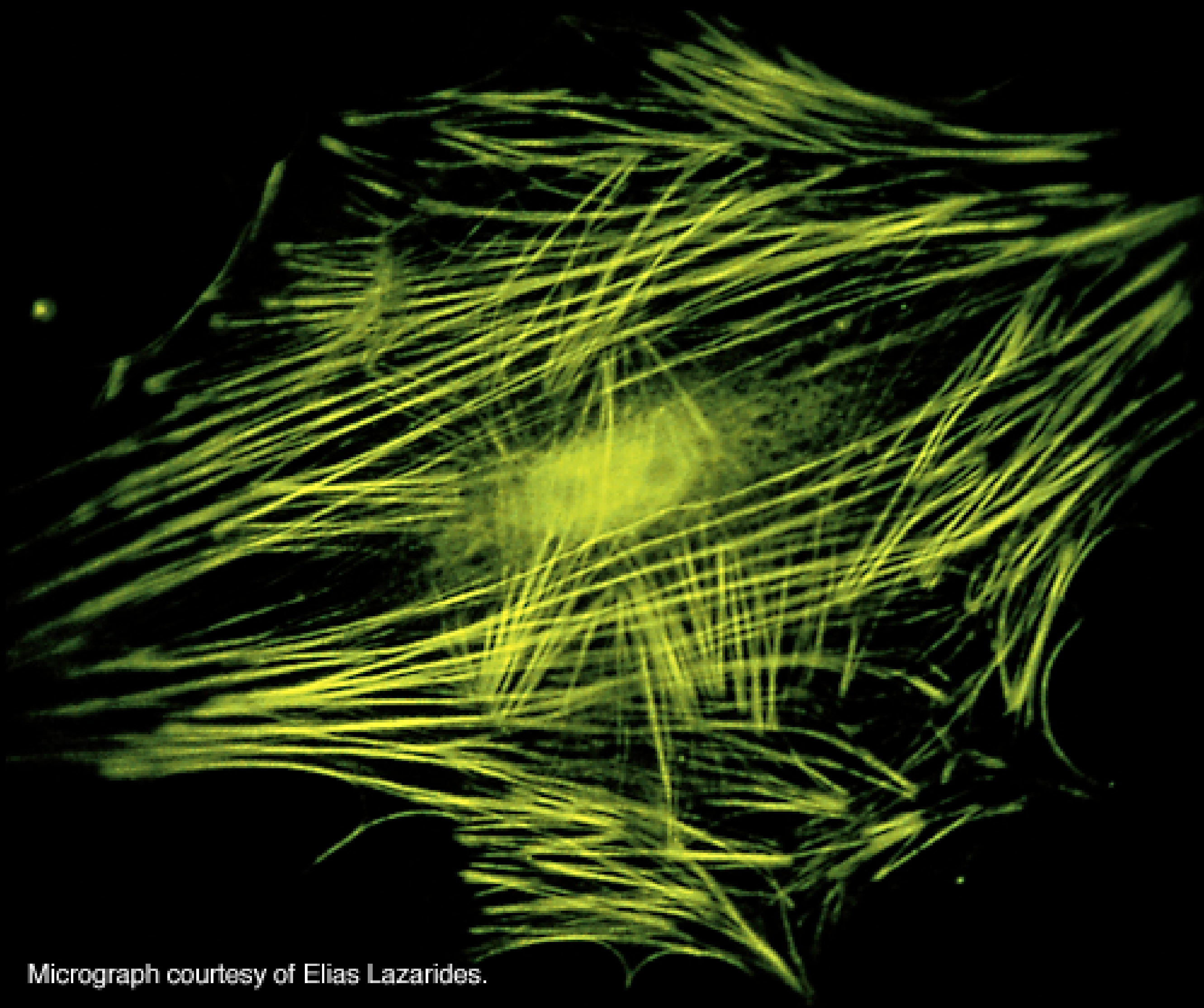
Actin Structure and Regulation

Myosin Structure and Regulation

Functions of Actin and Myosin in Cells

**Paper: Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in Xenopus egg extracts**

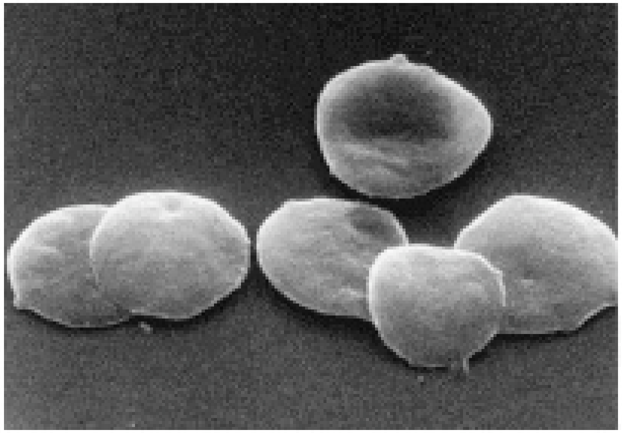
# Stationary cell - stress fibers



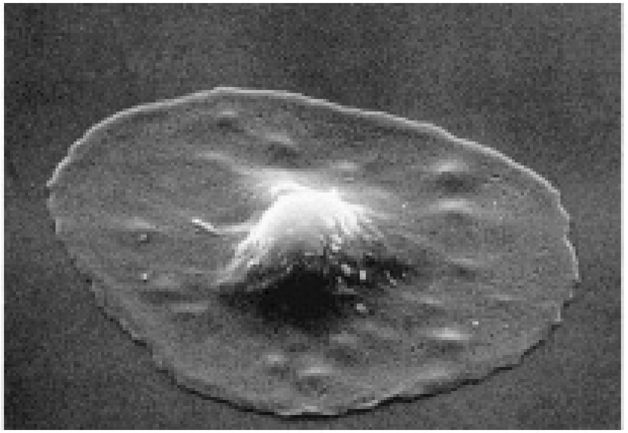
Micrograph courtesy of Elias Lazarides.

# Platelet Dynamics

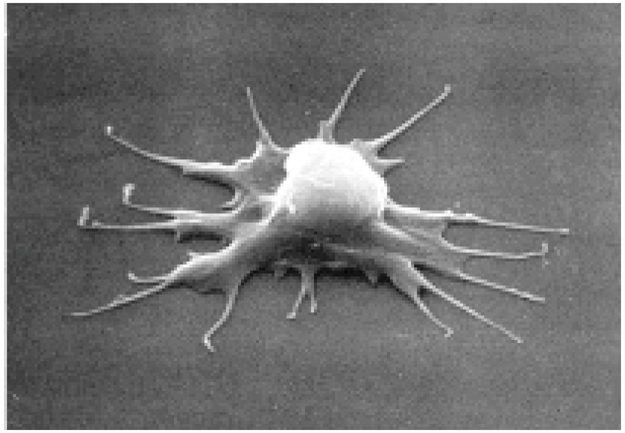
resting



activated



retraction



**Dramatic morphological changes result from reorganization of actin cross-linked to plasma membrane**

# Actin

**highly conserved 375 aa, 43 kD protein**

**the most abundant protein in non-muscle cells : 1-5%**

**roles: cell shape, polarization, locomotion, division; vesicle traffic**

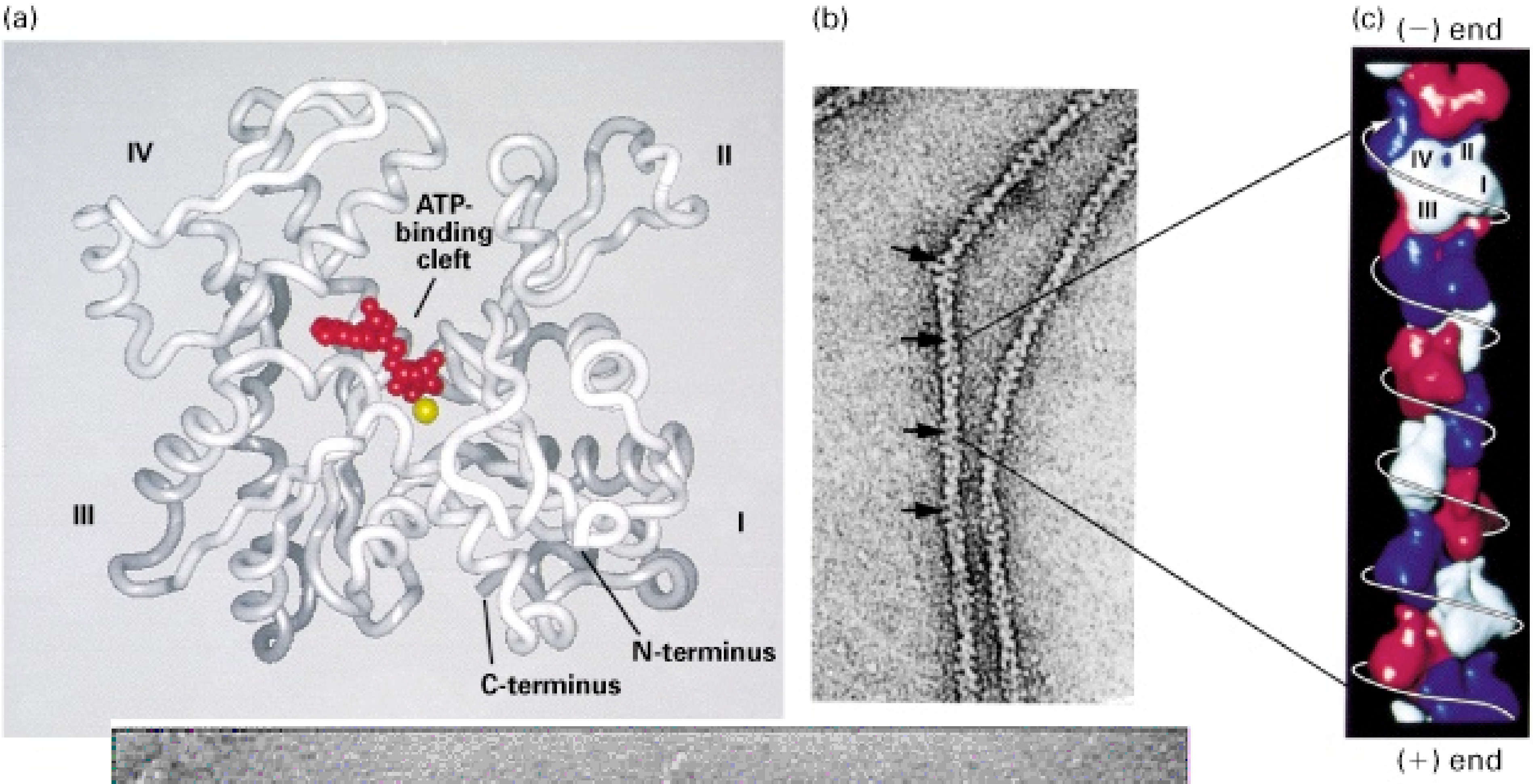
**monomer = G-actin**

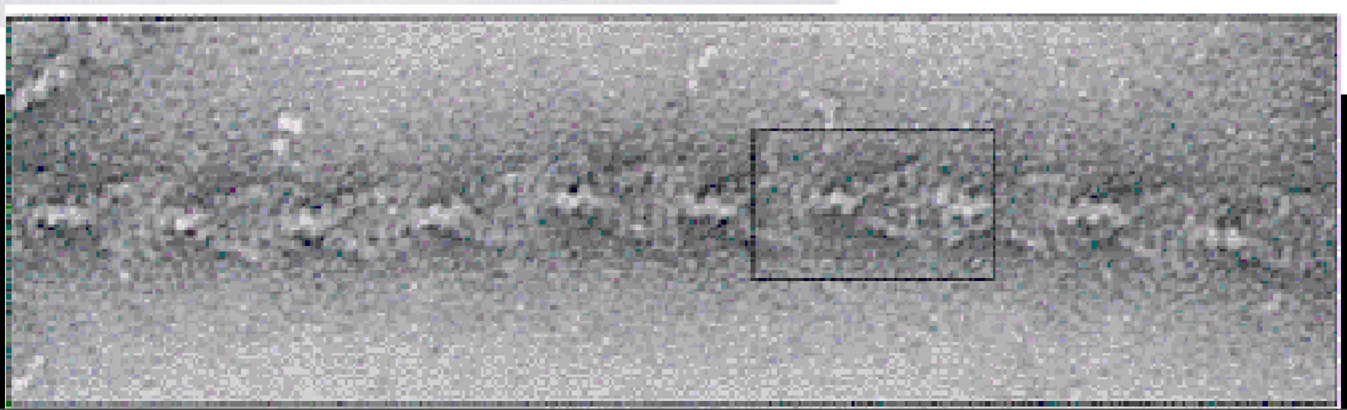
**polymer = F-actin, microfilaments**

**inhibitors:**

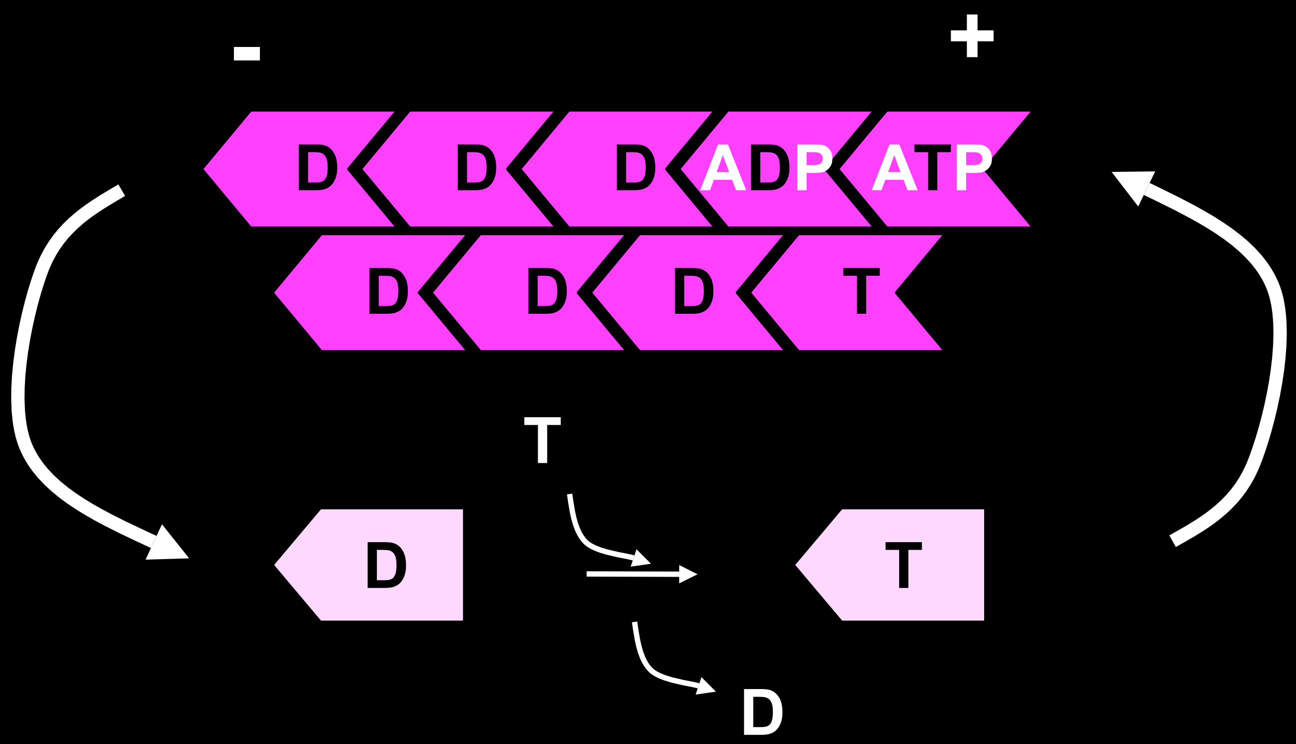
**latrunculin, cytochalasin; phalloidin**

# Actin Structure



# Steady state actin polymer-
# Treadmilling of subunits




Cc(- end) = 0.8 μM > Cc(+ end) = 0.1 μM

filament turnover rate: $t_{1/2}$ = 30 min

rate limiting step = dissociation of ADP actin from minus end

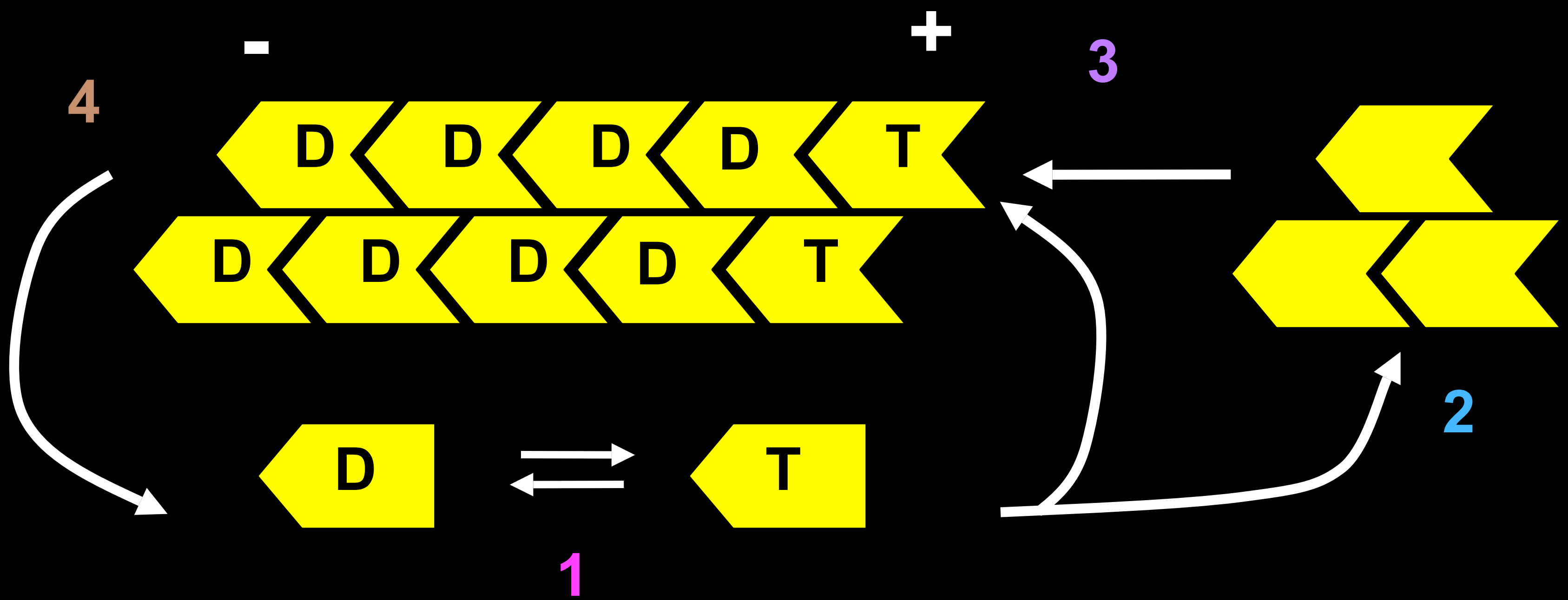
# Actin dynamics in vivo

## Parameters:

1. Spatial and temporal control of polymerization/ depolymerization
2. Turnover
3. Movement of actin filaments - myosins

## Model systems:

1. Fibroblast
2. Keratocyte - epithelial cell
3. Listeria monocytogenes - intracellular bacterial pathogen




1. monomer pool

2. nucleation

3. elongation

4. depolymerization

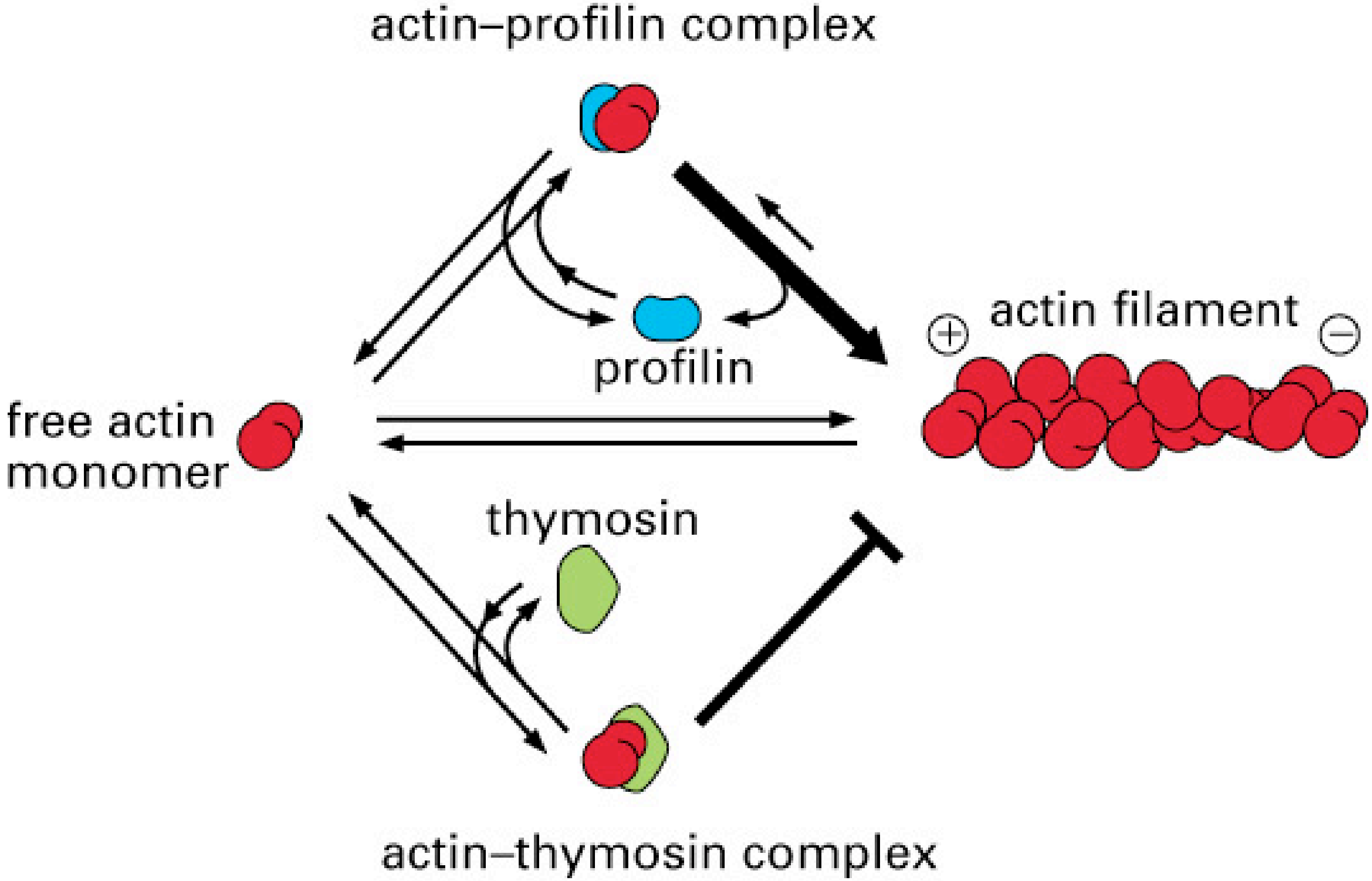
# Regulation of the monomer pool

## Thymosin β4

M.W. 5000
binds 1:1 - enough to buffer all the actin
sequesters actin from polymerizing
localization - diffuse

## Profilin

M.W. 14,000
binds 1:1 - can buffer 20% of actin
promotes nucleototide exchange and polymerization
binds PIP2 and proline-rich sequences
localization - diffuse and leading edge, Listeria surface




PROFILIN COMPETES WITH THYMOSIN
FOR BINDING TO ACTIN MONOMERS
AND PROMOTES ASSEMBLY

# Nucleation

**Arp2/3 complex (Actin Related Protein)**

7 subunits, include actin-related proteins 2 and 3

accelerates actin polymerization

(with activator, eliminates lag phase)

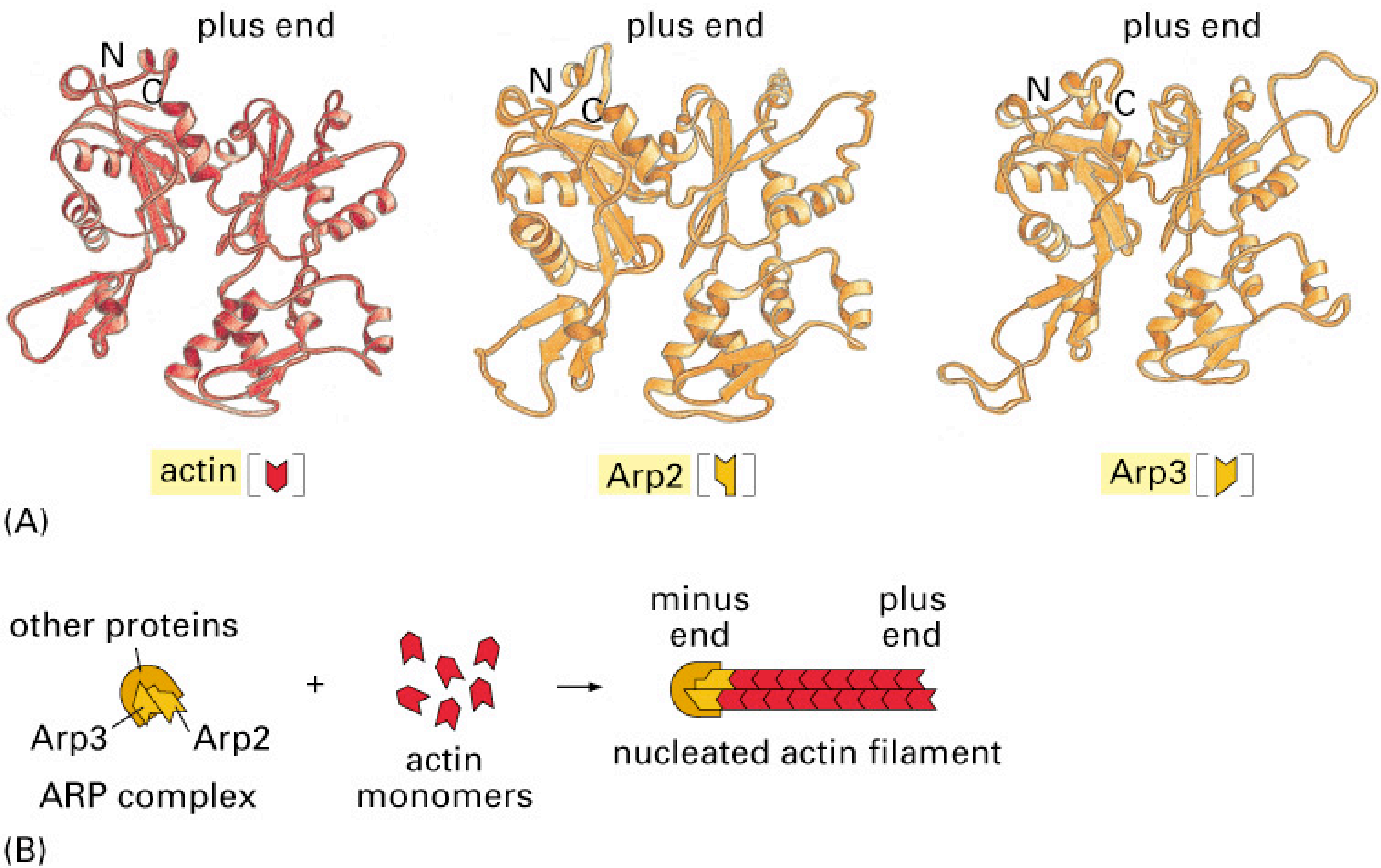
binds (-) ends and filament sides - branching function

promotes actin polymerization at listeria surface

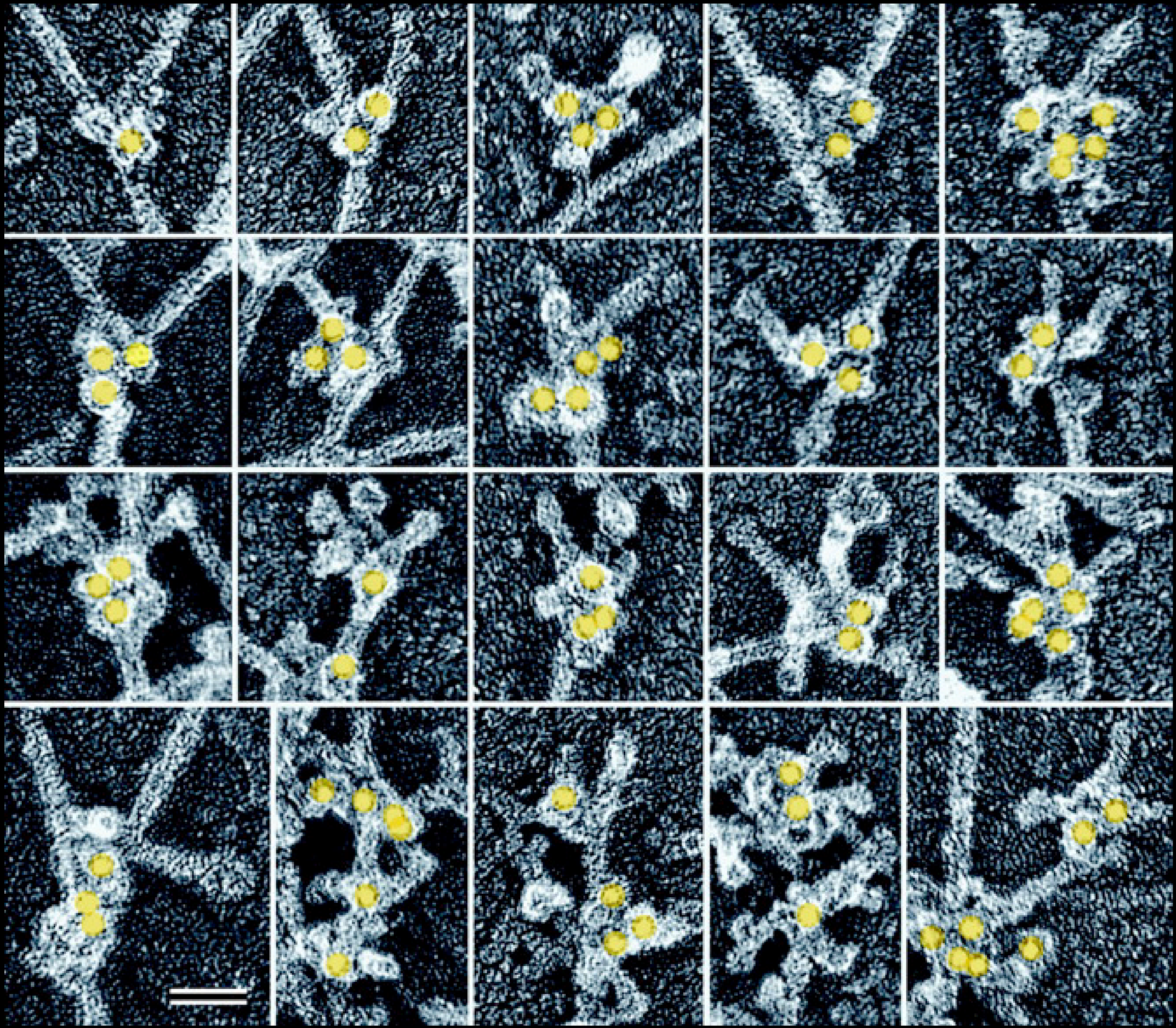
**Activators:**

Listeria: Act A

cells: WASP family proteins


plus end
N
C
plus end
N
C
plus end
N
C
actin
Arp2
Arp3
(A)
other proteins
Arp3
Arp2
ARP complex
+
actin
monomers
minus
end
plus
end
nucleated actin filament
(B)

immuno-EM of Arp2/3 at actin branch points



# Elongation

**Profilin**

**promotes (+) end growth**

**Capping factors:**

**CapZ (Capping protein) - (+) end**

**tropomodulin - (-) end**

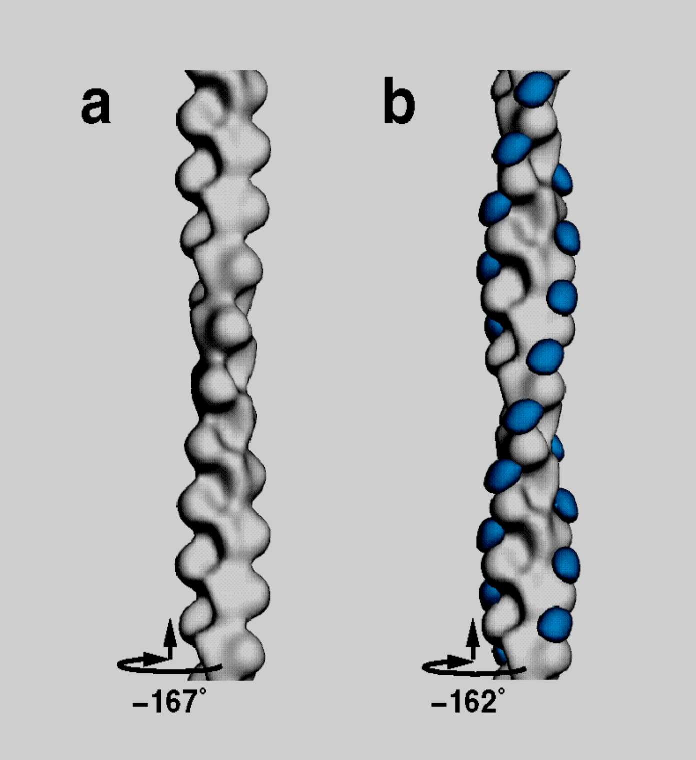
**gelsolin - (+) end**

**can stabilize or destabilize filaments, prevent elongation**

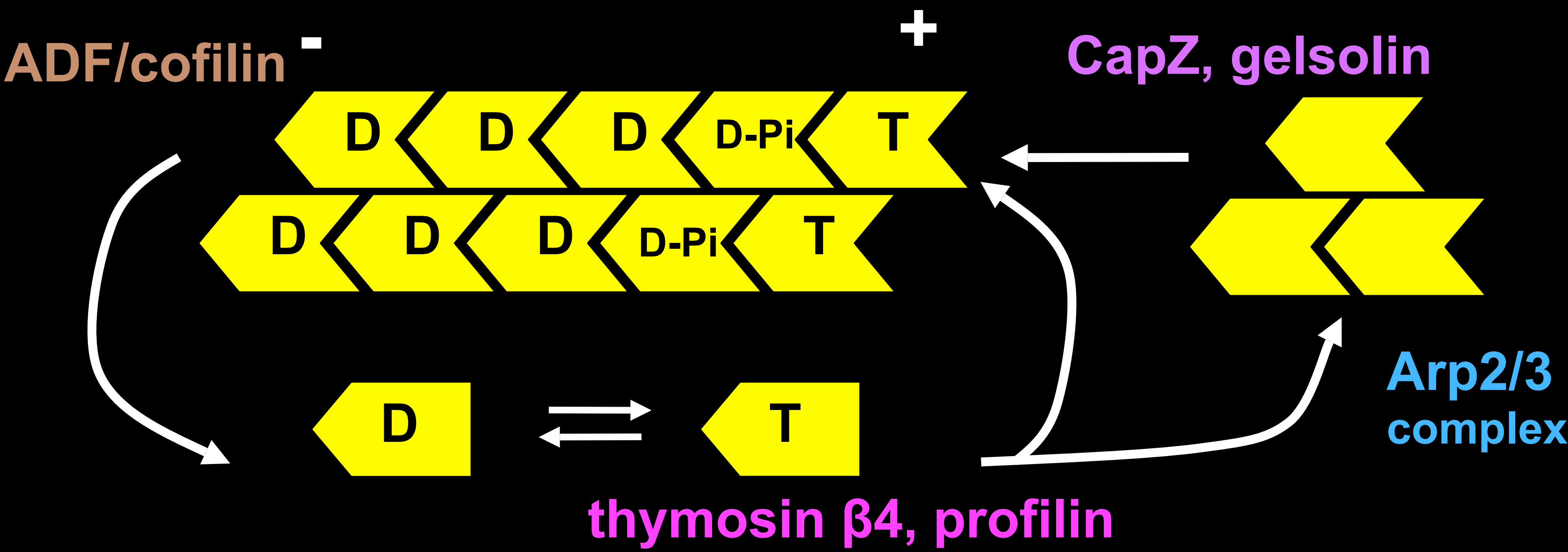
# Depolymerization

**gelsolin**
M.W. 87,000
$Ca^{++}$-dependent severing

**ADF/cofilin**
M.W. 19,000
binds G- and F-actin
accelerates (-) end
depolymerization 25-fold




+ ADF




1. monomer pool
2. nucleation
3. elongation
4. depolymerization

# Myosin - the most studied of all proteins (!?)

large family of myosin-related proteins ~14 in human

common features:

one or two heavy chains and several light chains

**heavy chain:**

1) large globular head:

contains actin-binding and ATPase domains

2) $\alpha$-helical neck region - binds light chains

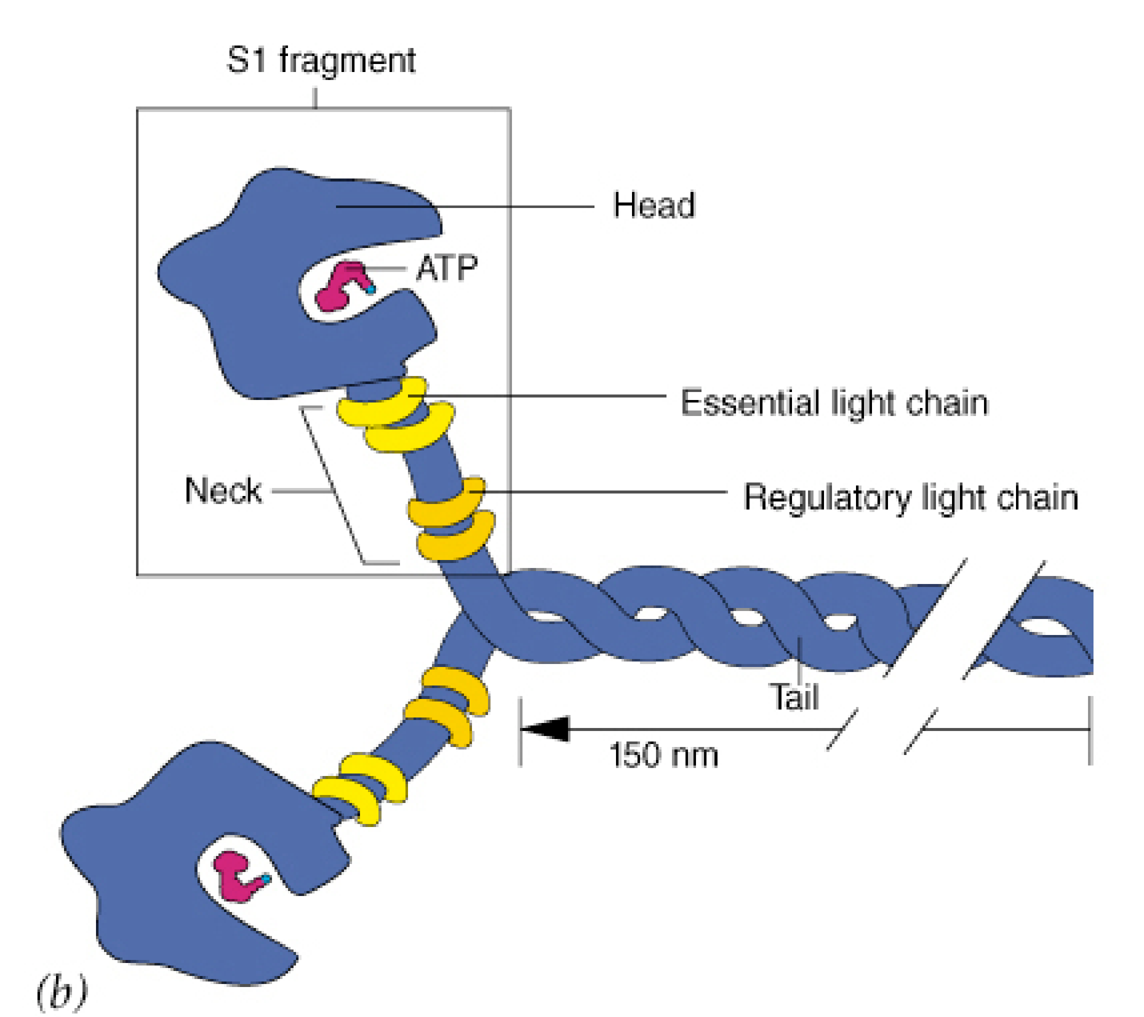
3) tail domain - for oligomerization or cargo binding

**light chains:**

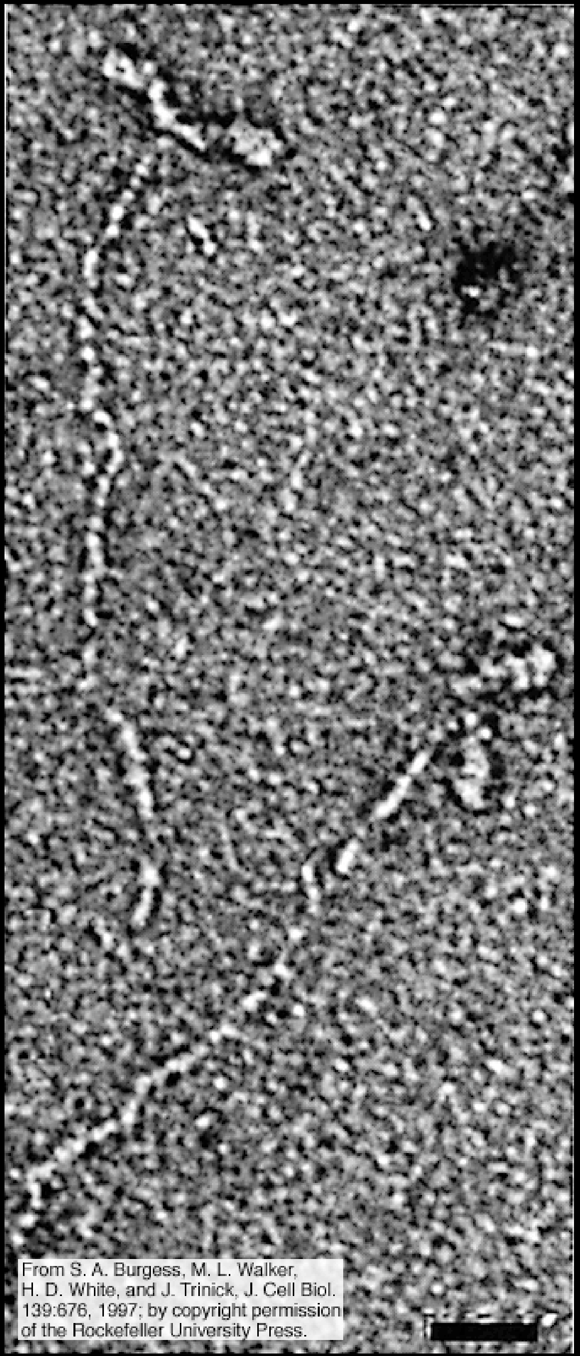
1) calcium-binding proteins, sometimes calmodulin

2) regulate myosin activity

# Myosin II





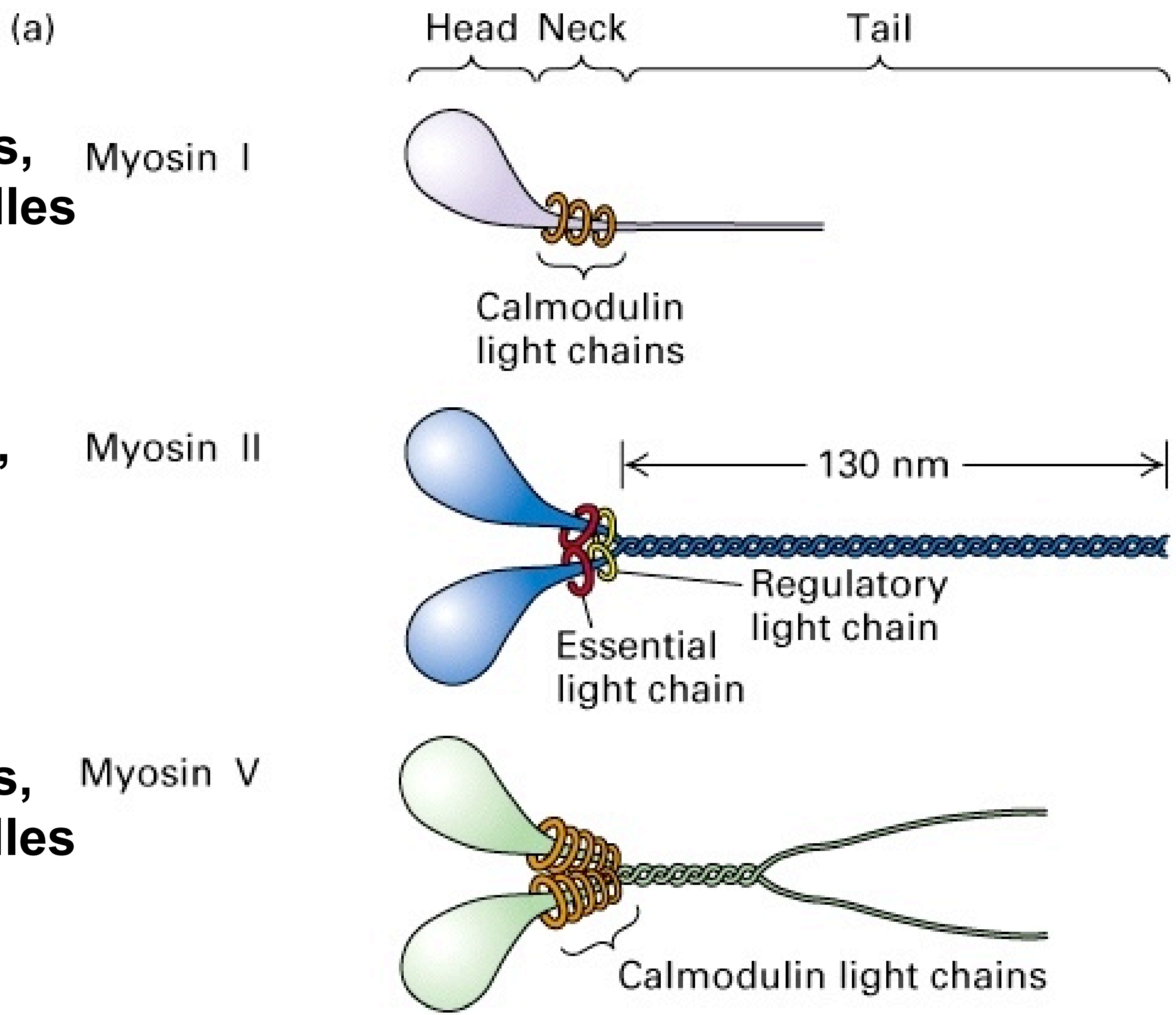



From S. A. Burgess, M. L. Walker, H. D. White, and J. Trinick, J. Cell Biol. 139:676, 1997; by copyright permission of the Rockefeller University Press.

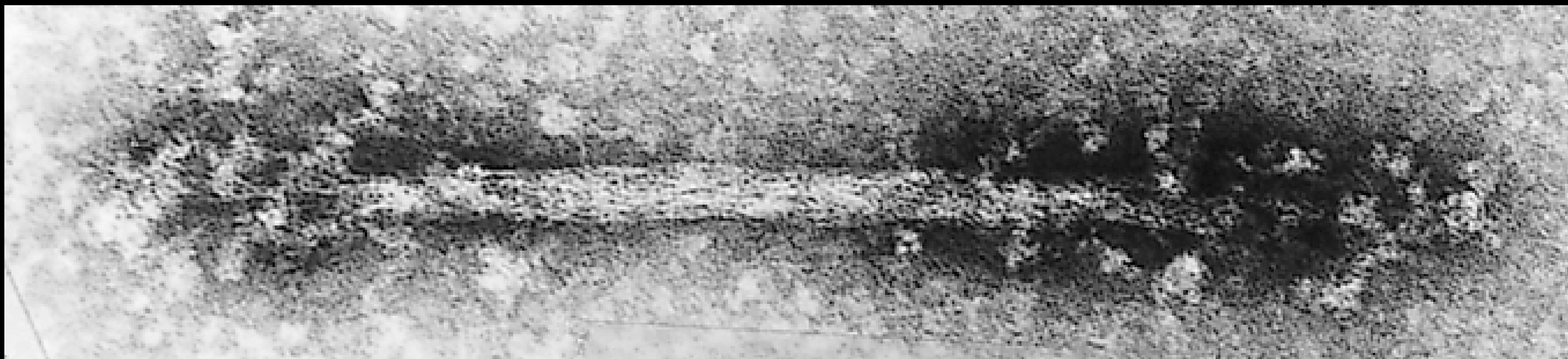
**vesicles, organelles**

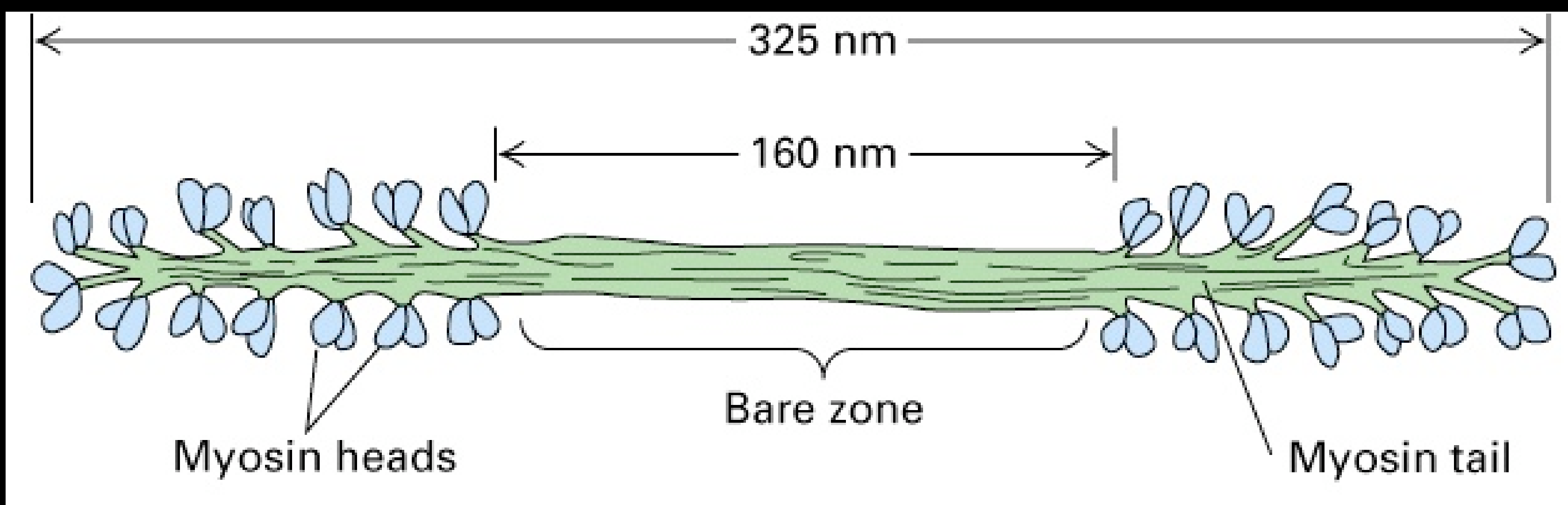
**muscle, stress fibers**

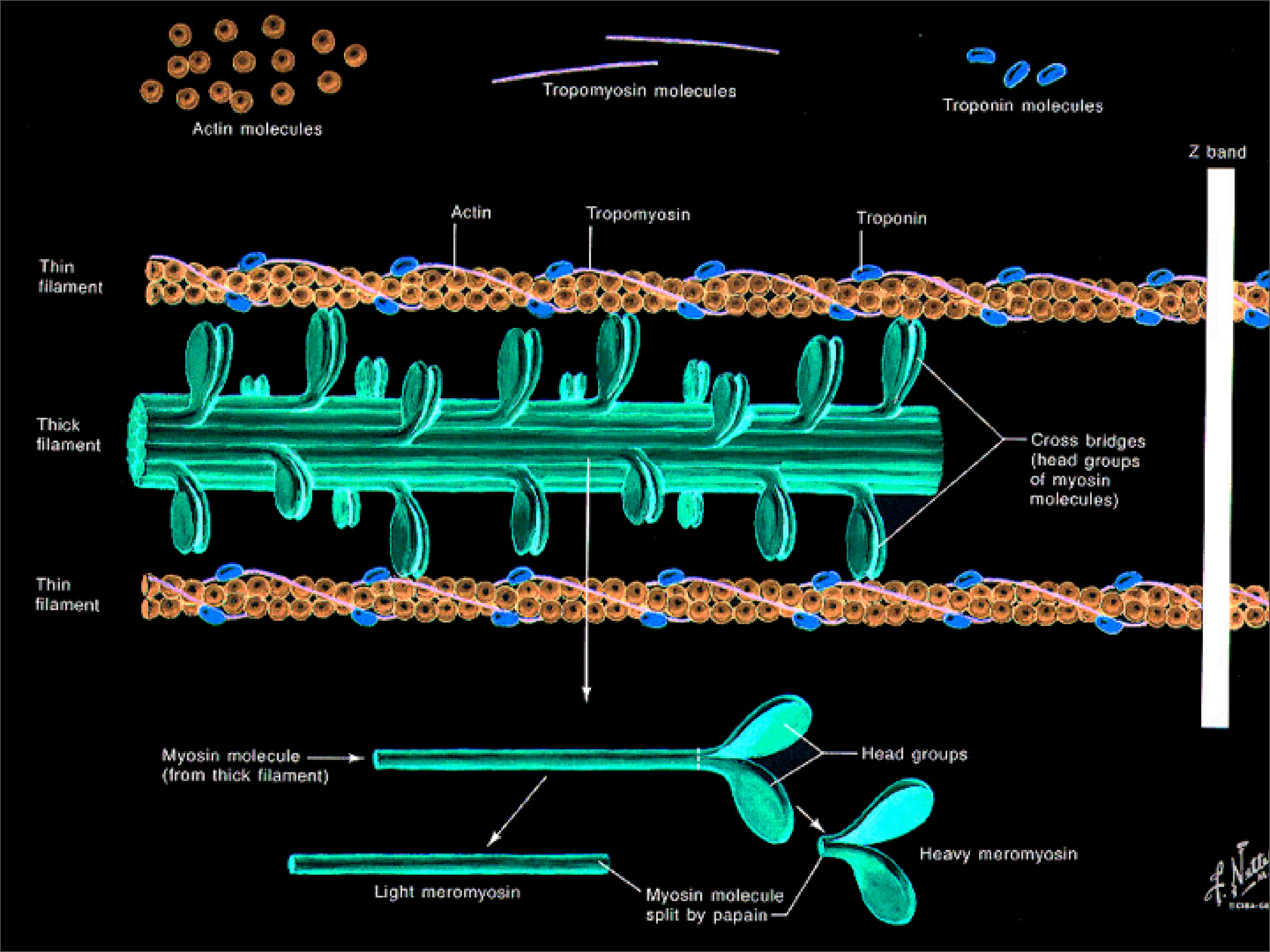
**vesicles, organelles**



# Myosin thick filaments: bipolar





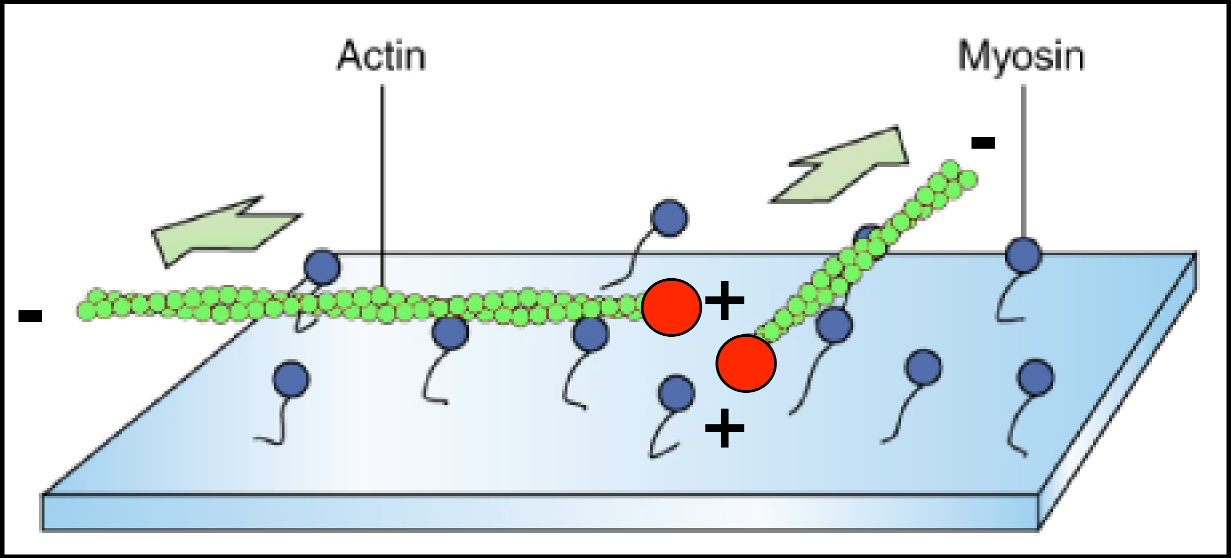


Actin molecules
Tropomyosin molecules
Troponin molecules
Z band
Actin
Tropomyosin
Troponin
Thin filament
Thick filament
Cross bridges (head groups of myosin molecules)
Thin filament
Myosin molecule (from thick filament)
Head groups
Heavy meromyosin
Light meromyosin
Myosin molecule split by papain

# Myosin motility assay

1) Adsorb myosin molecules on glass coverslip in chamber

2) Perfuse in labeled actin filaments and plus ends (and ATP)




3) Observe by fluorescence video microscopy

other myosins can move toward the minus end

muscle myosin

plus end motor

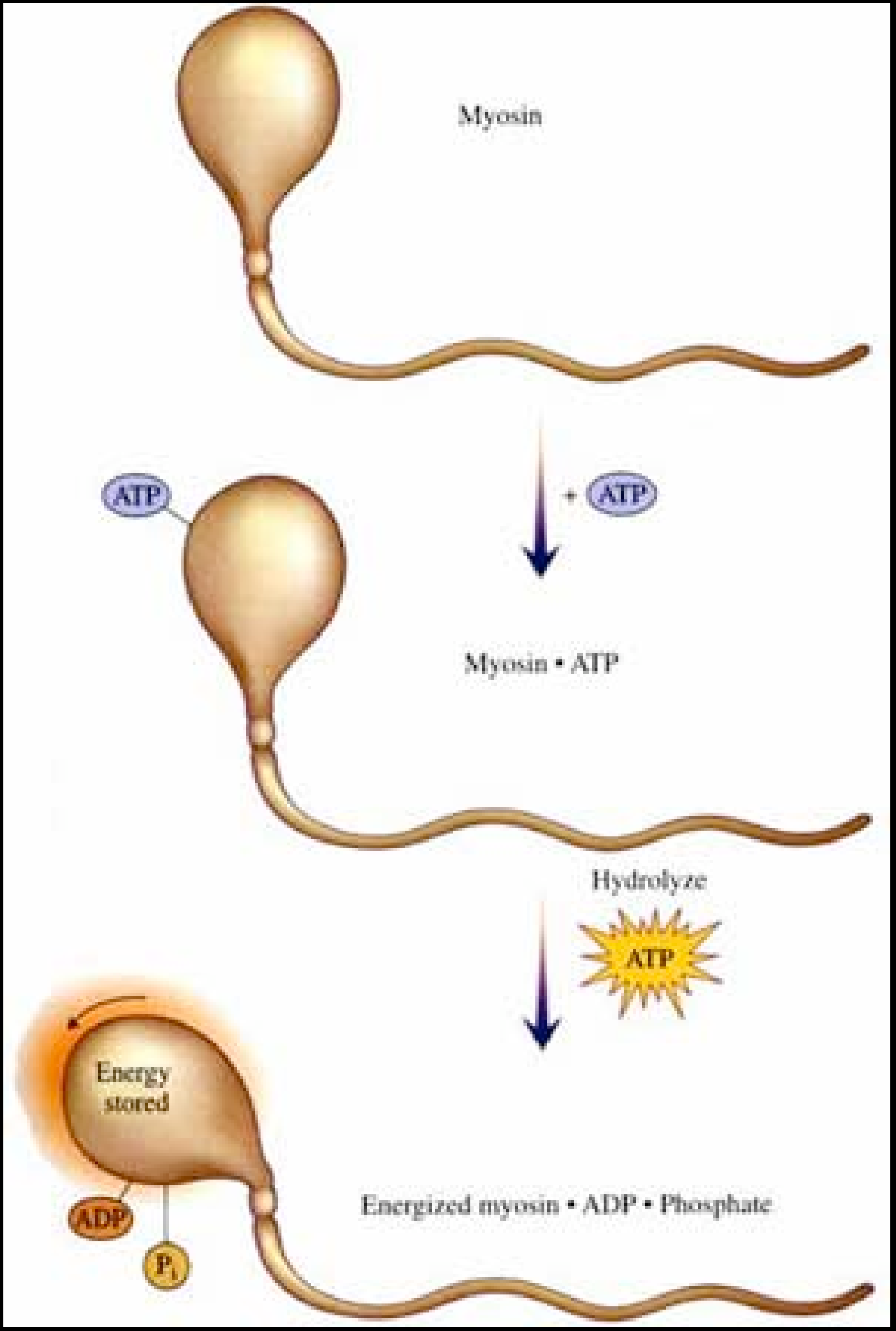
~4.5 μm/sec

# Myosin II mechanism

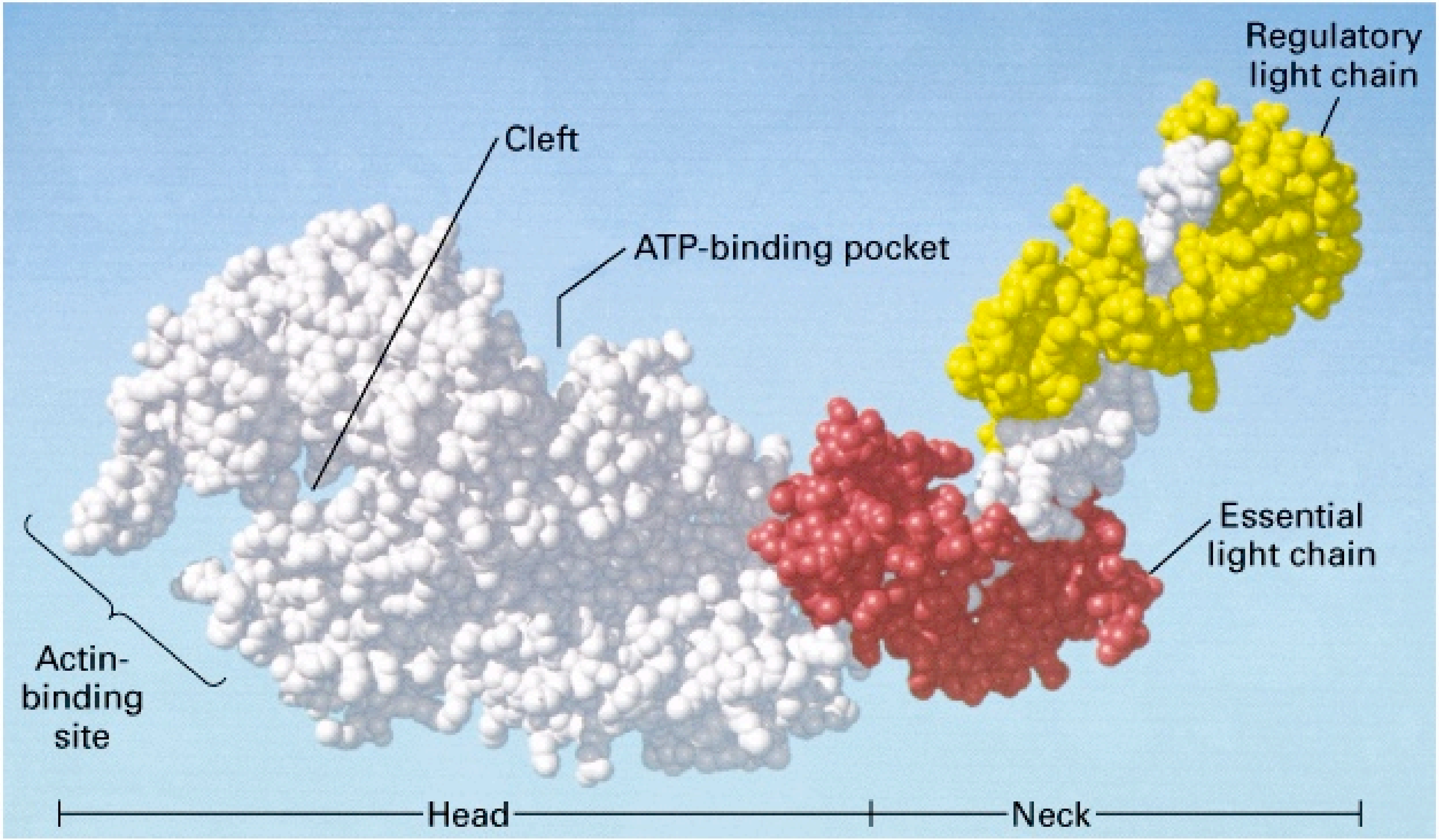

**ATPase activity stimulated by actin:**
**from 4/hour to 20/second**

**ATP binding, hydrolysis and dissociation of ADP-Pi produce a series of allosteric changes in myosin conformation**

**Energy release is coupled to movement**

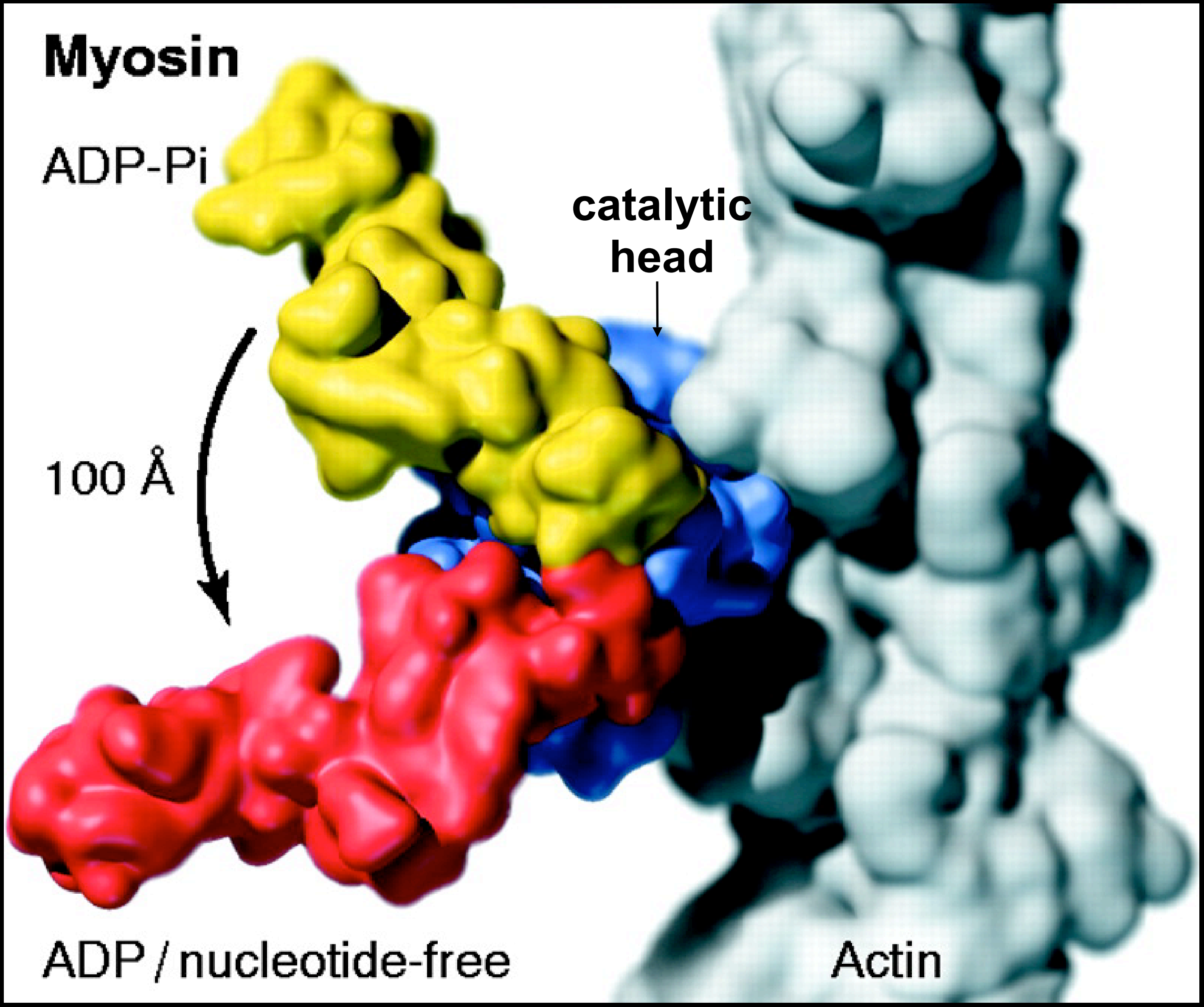

Myosin
ATP
+ ATP
Myosin • ATP
Hydrolyze
ATP
Energy
stored
ADP
$P_i$
Energized myosin • ADP • Phosphate

# Myosin II crystal structure (S1 fragment)

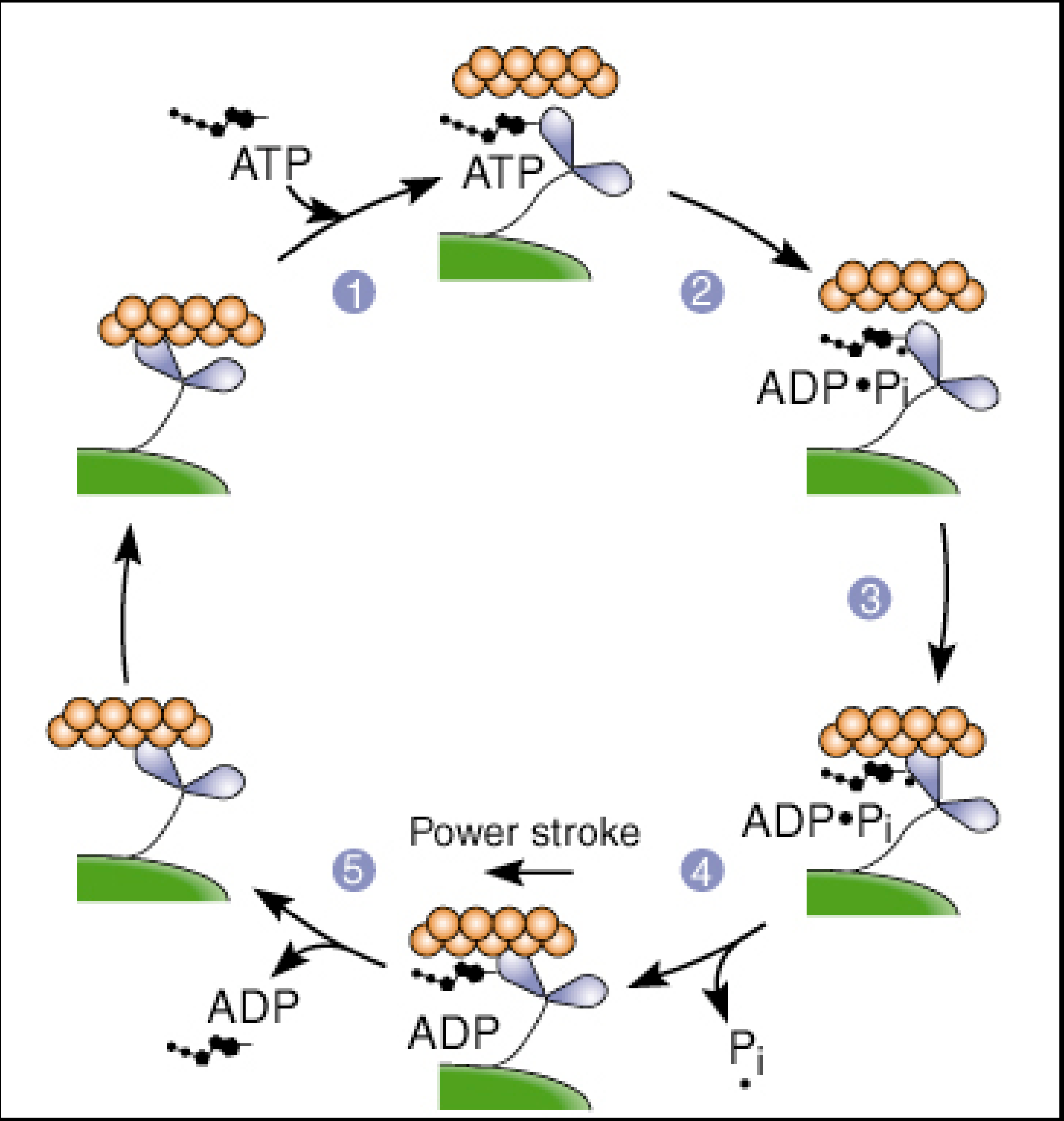


# neck domain = lever arm

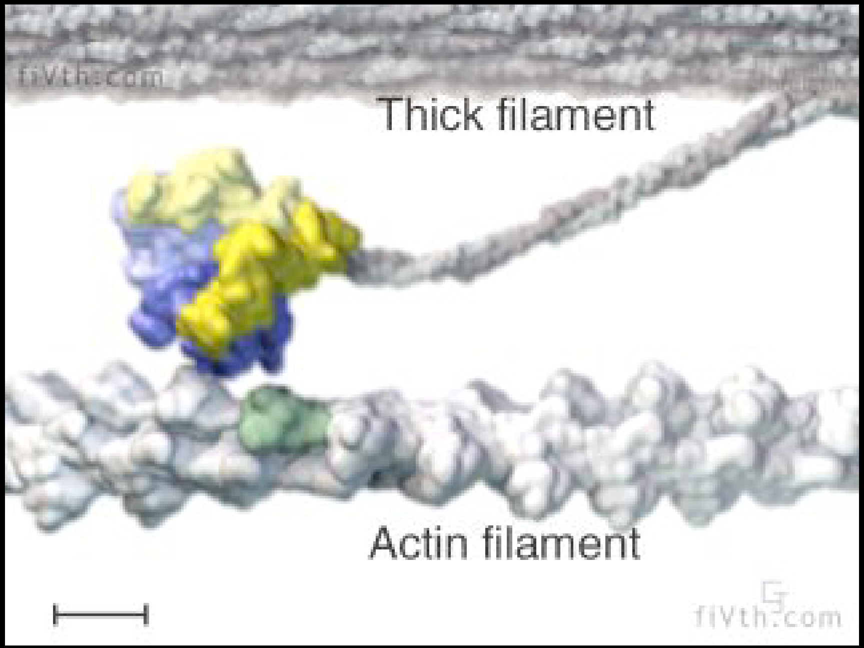
superimpose structures in two different nucleotide states



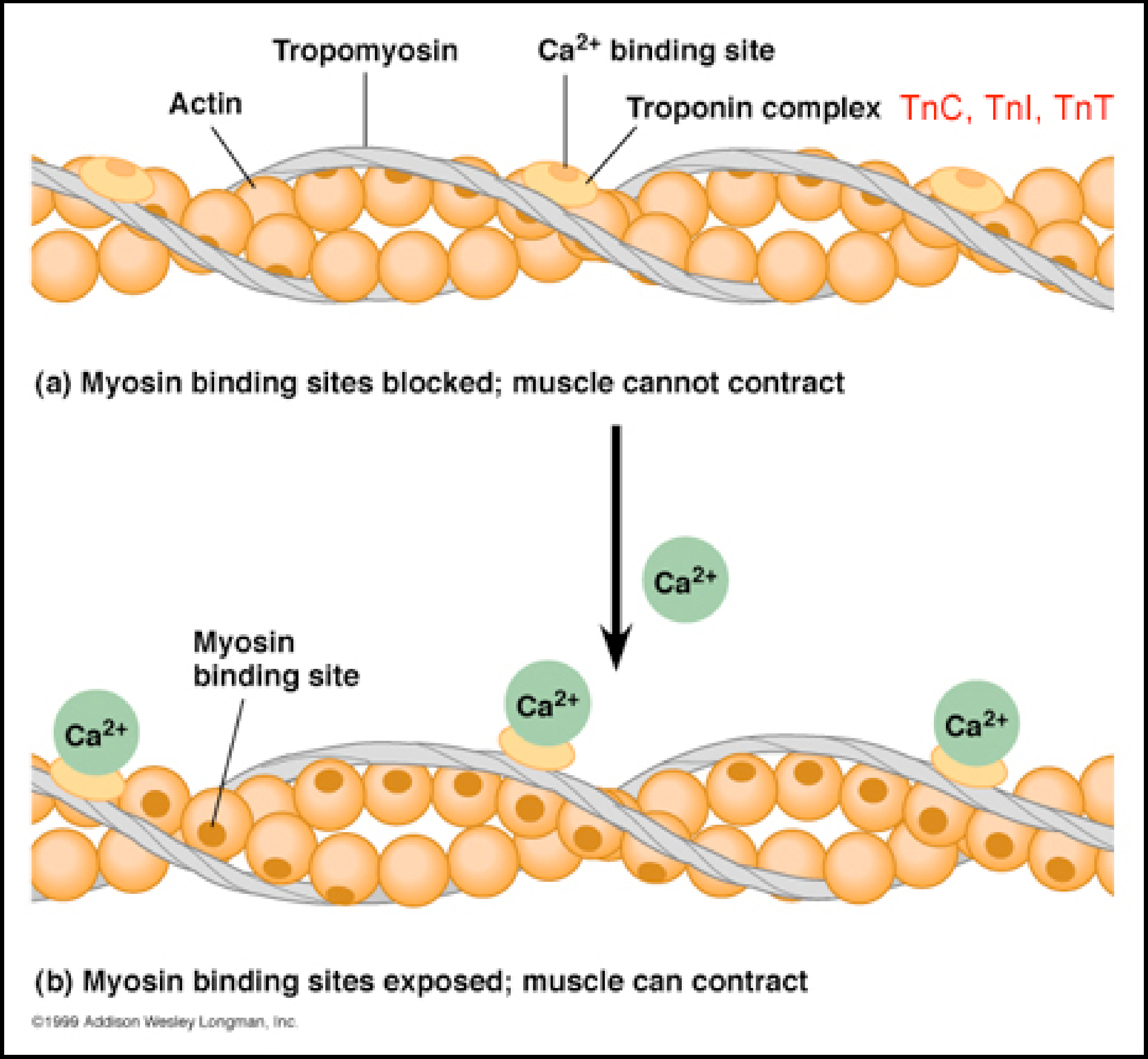
cross bridge cycle



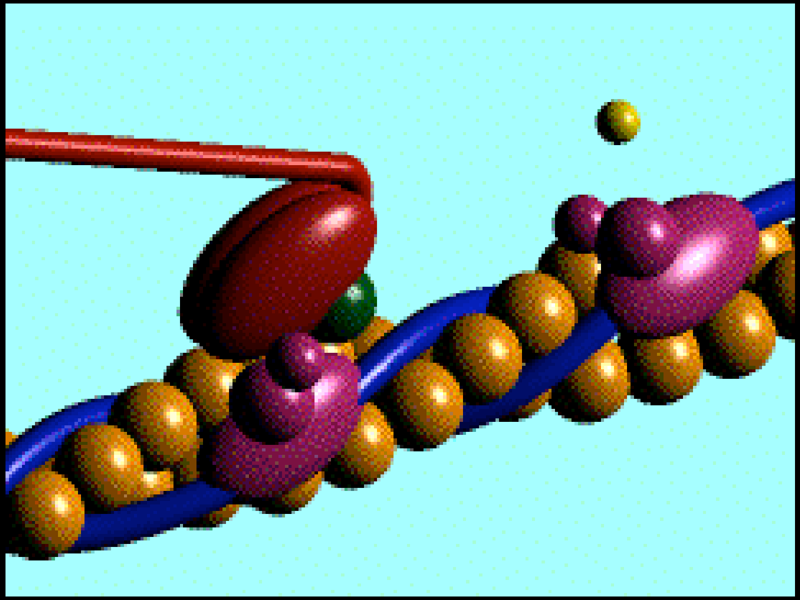
**cross bridge cycle**

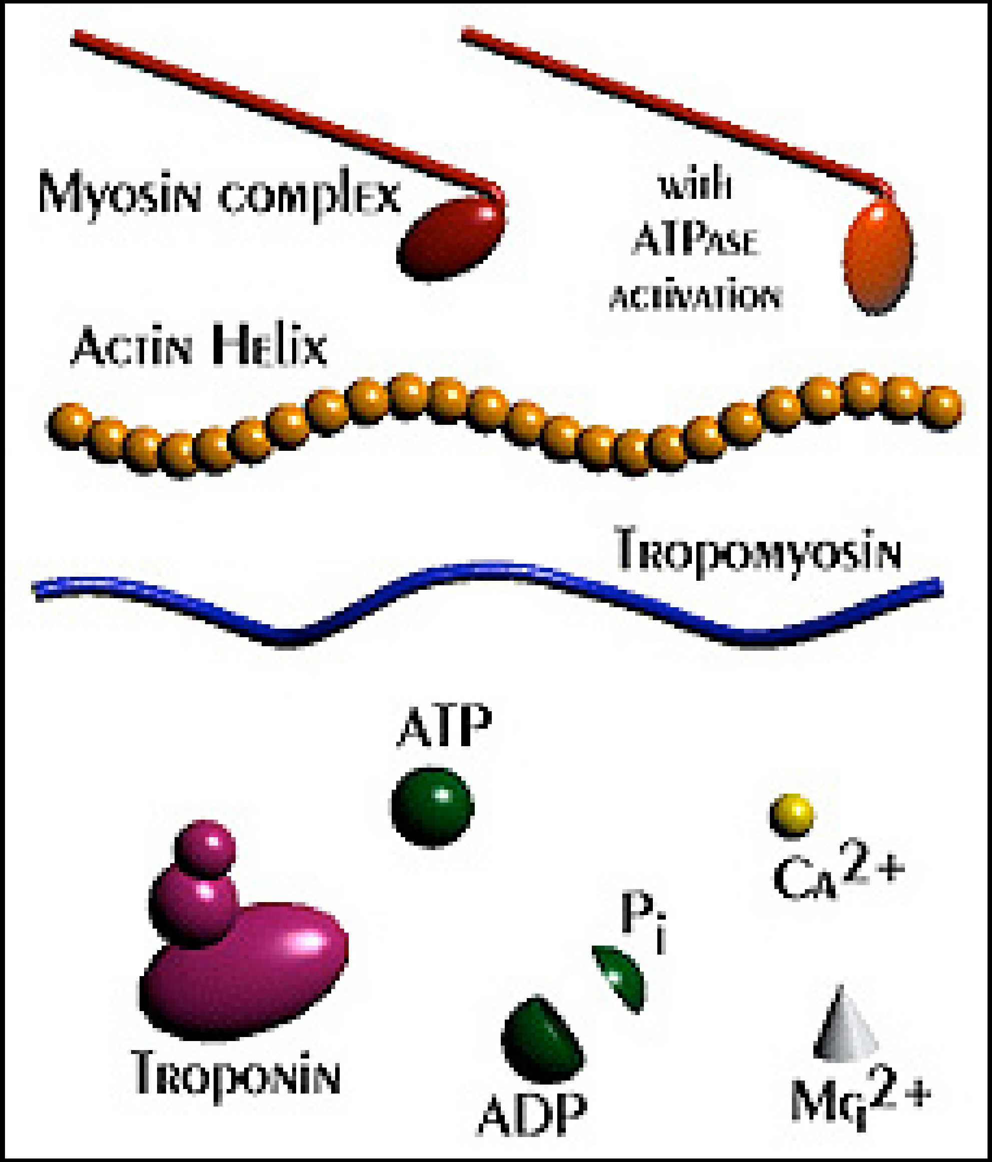


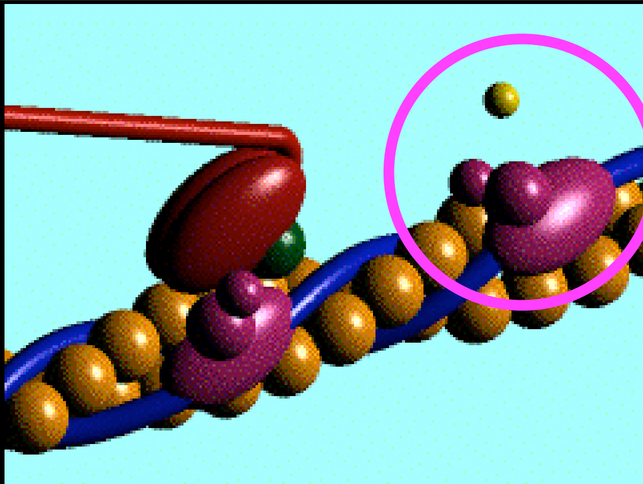
# Myosin mediated movement: in reality more complexly regulated



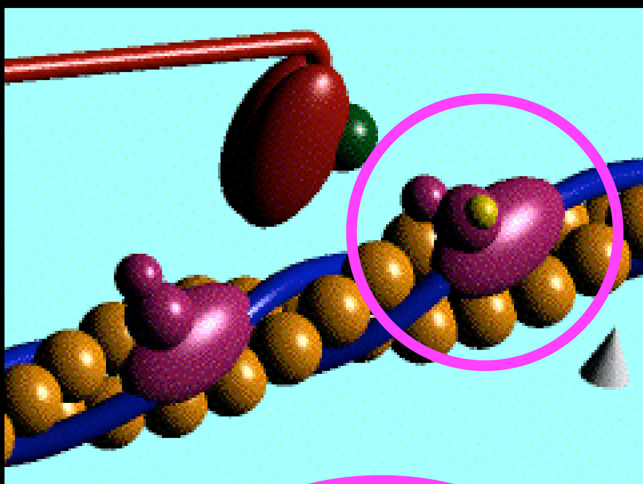

**troponin**
**tropomyosin**



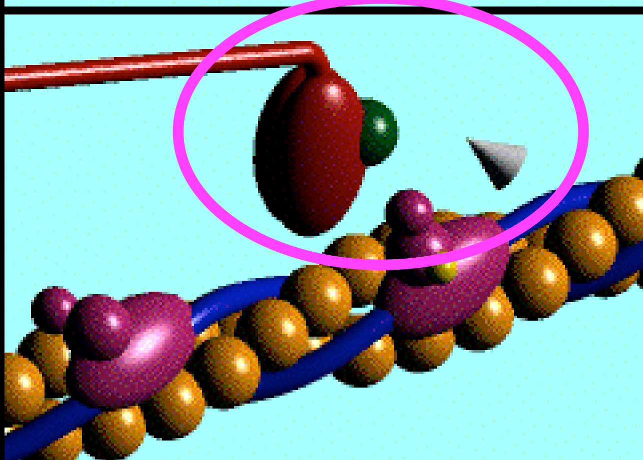

Myosin complex
with
ATPase
activation
Actin Helix
Tropomyosin
ATP
Troponin
ADP
$P_i$
$Ca^{2+}$
$Mg^{2+}$



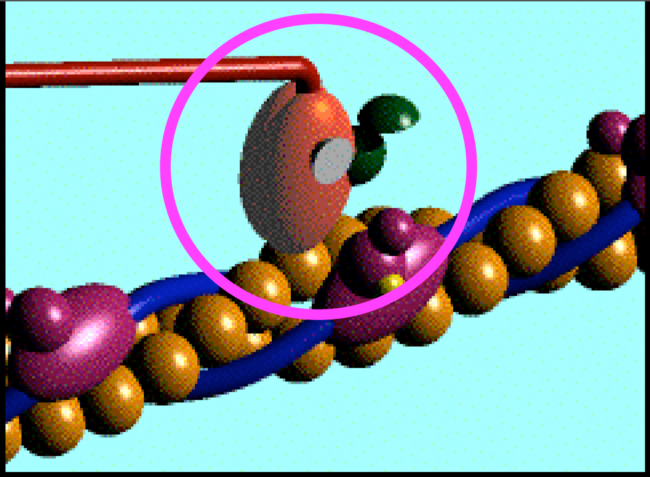
Calcium binds Troponin



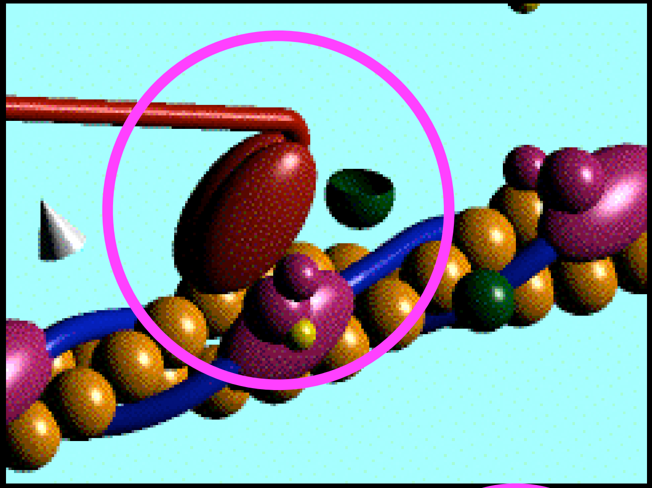
Troponin movement exposes binding site for Myosin head.



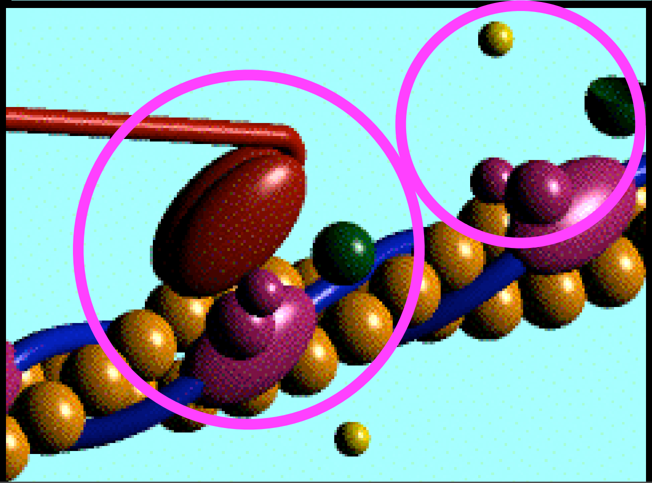
Magnesium ion approaches Myosin head.



Myosin head binds Actin filament.
Magnesium activates Myosin head,
releases Phosphorus from ATP, leaves ADP
causes Myosin head to contract.

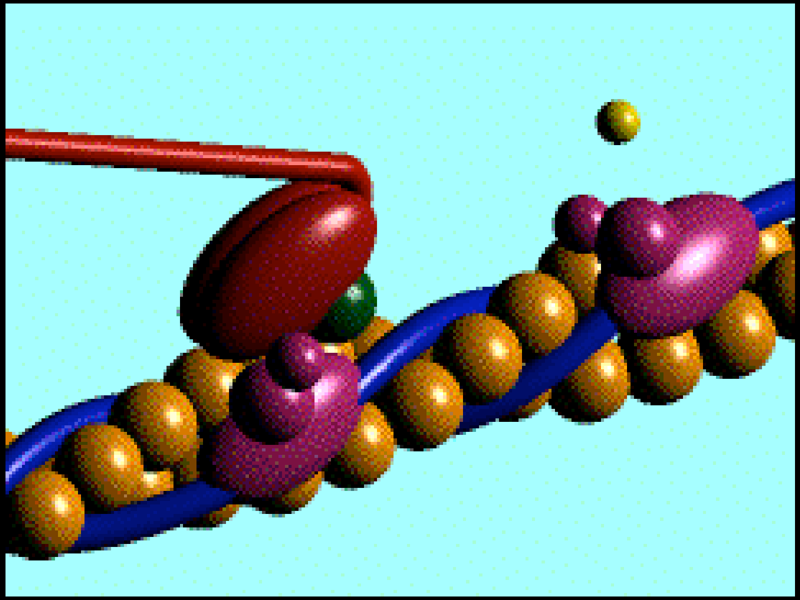


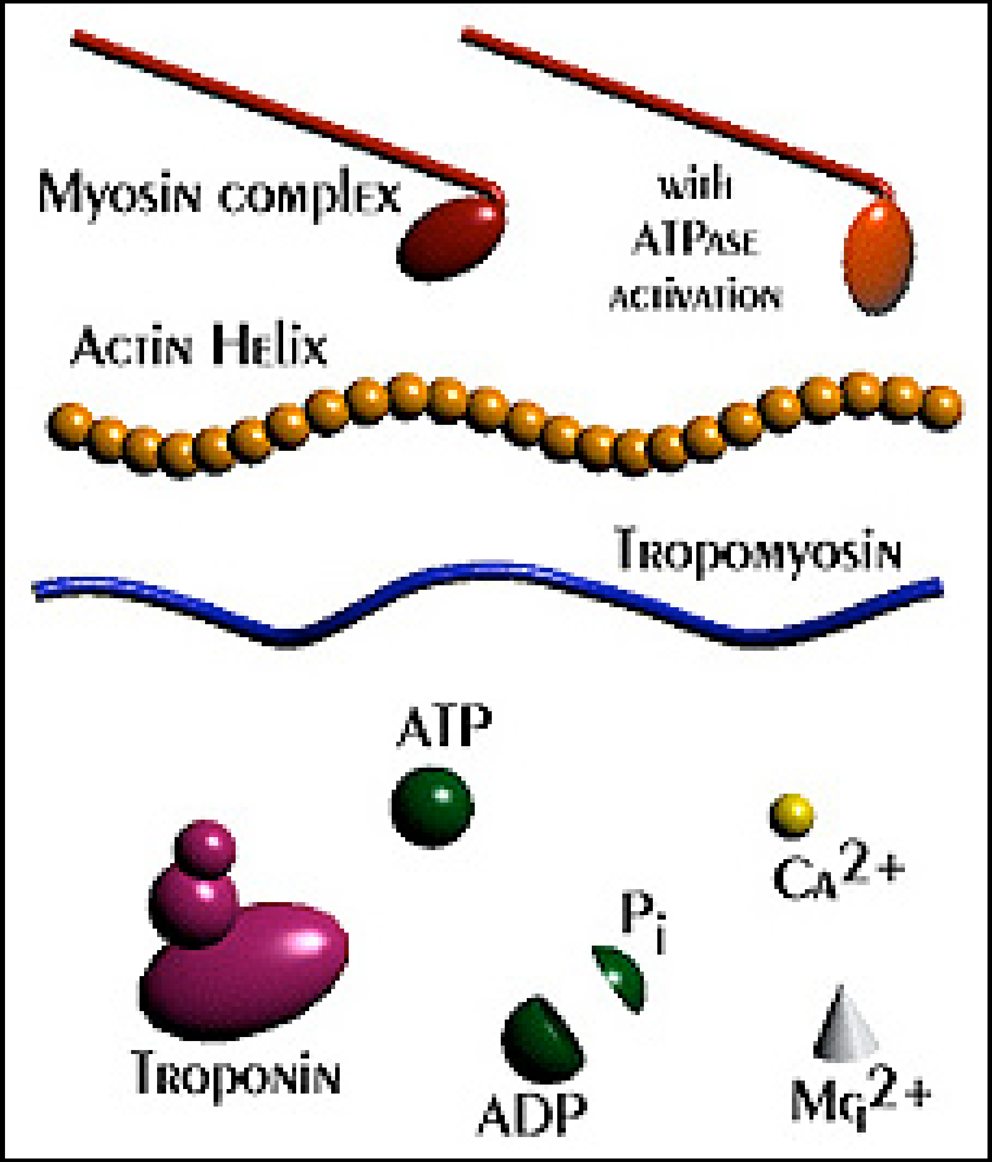
Magnesium and ADP released from Myosin head
ends contraction.

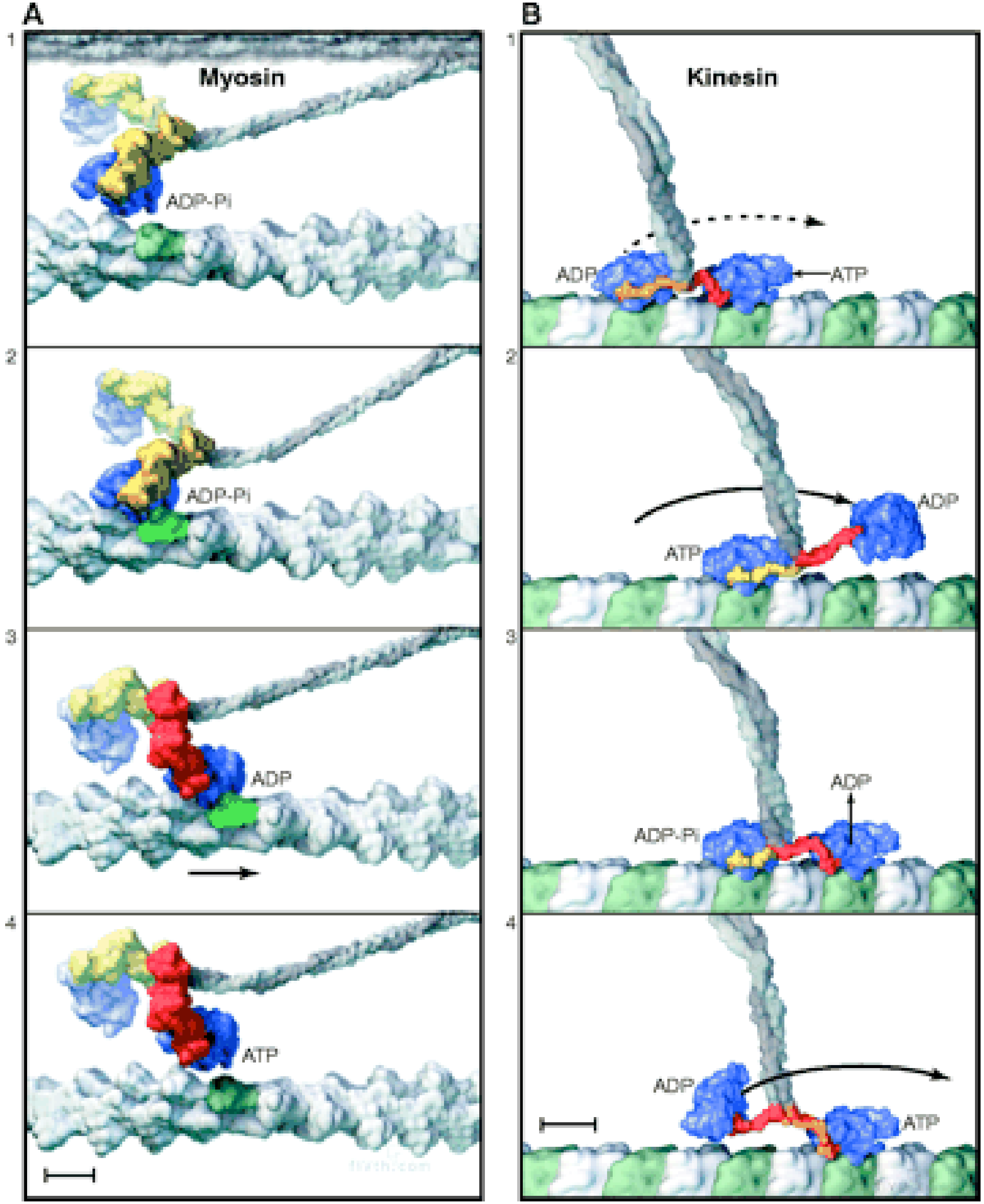


Myosin head releases from Actin filament.
Calcium ion released from Troponin, covers binding site

New calcium ion approaches next Troponin molecule
New ATP molecule approaches Myosin head
beginning the process over again

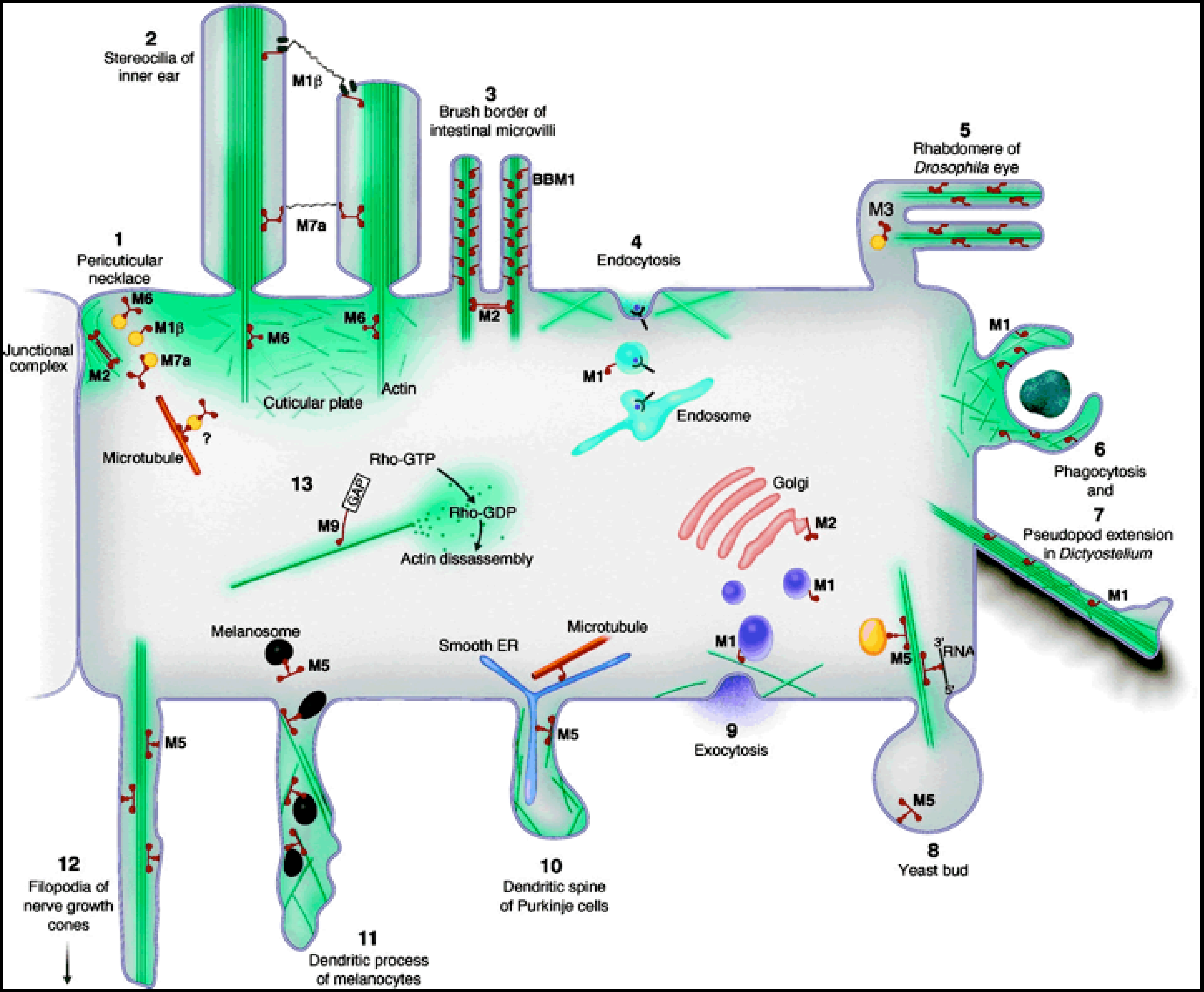



Myosin complex
with
ATPase
activation
Actin Helix
Tropomyosin
ATP
$Ca^{2+}$
$P_i$
Troponin
ADP
$Mg^{2+}$



A
B
1
Myosin
ADP-Pi
2
ADP-Pi
3
ADP
4
ATP
1
Kinesin
ADP
ATP
2
ADP
ATP
3
ADP
ADP-Pi
4
ADP
ATP

# Functions of Actin and Myosin in Cells



# Functions of Actin and Myosin in Cells

Cell Motility

Cell Division

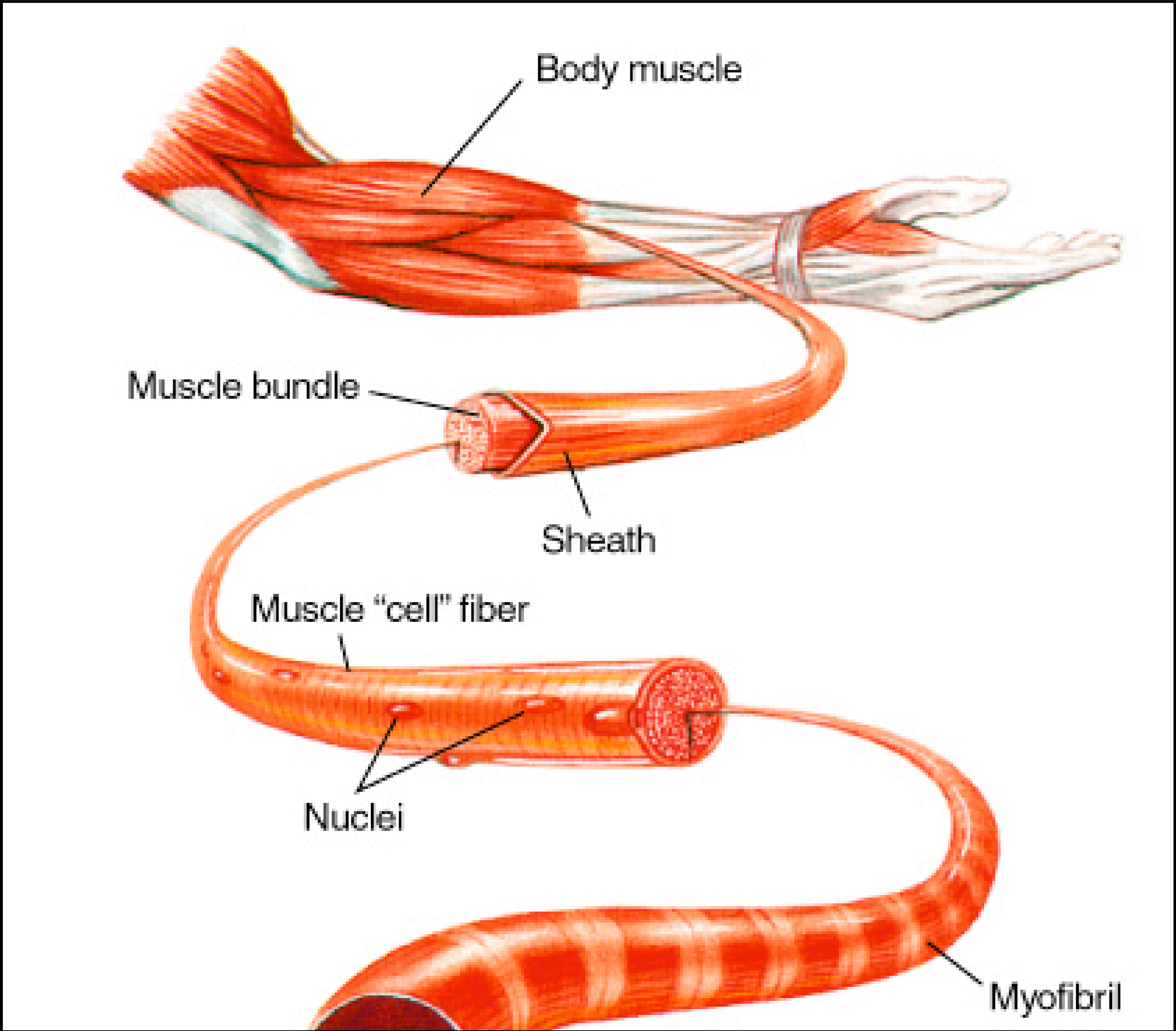
Muscle Contraction

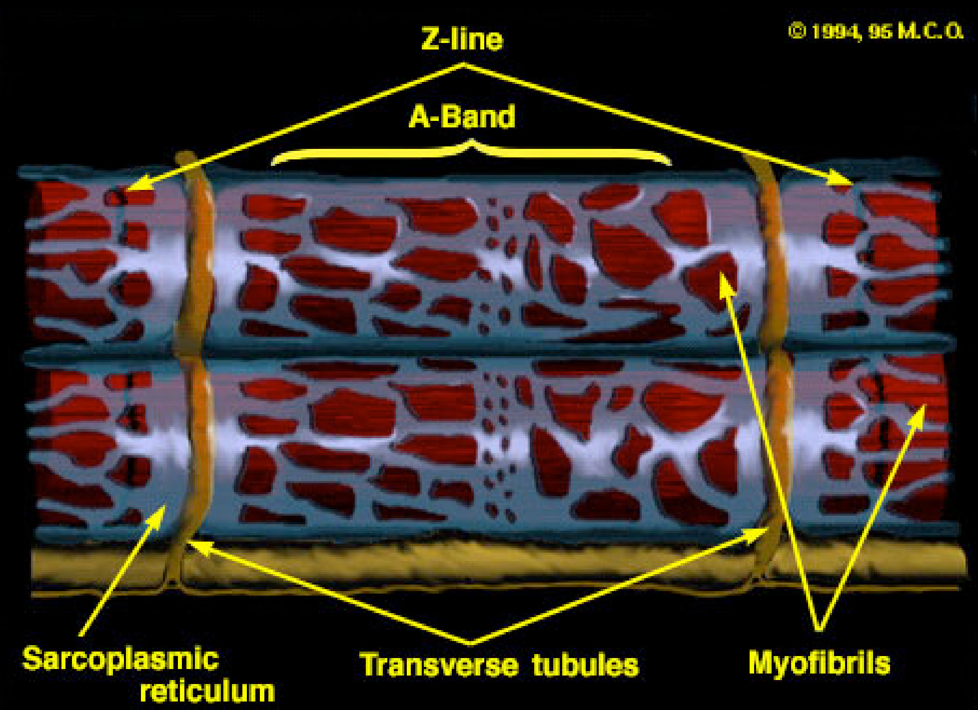
Pathogen Motility and Infection

Cell Protrusions/microvilli

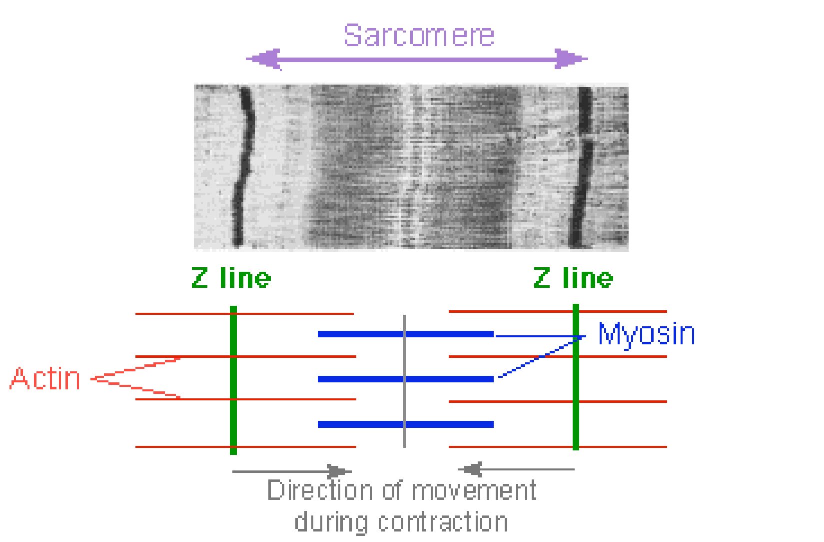
Cell Cortex

Stress Fibers

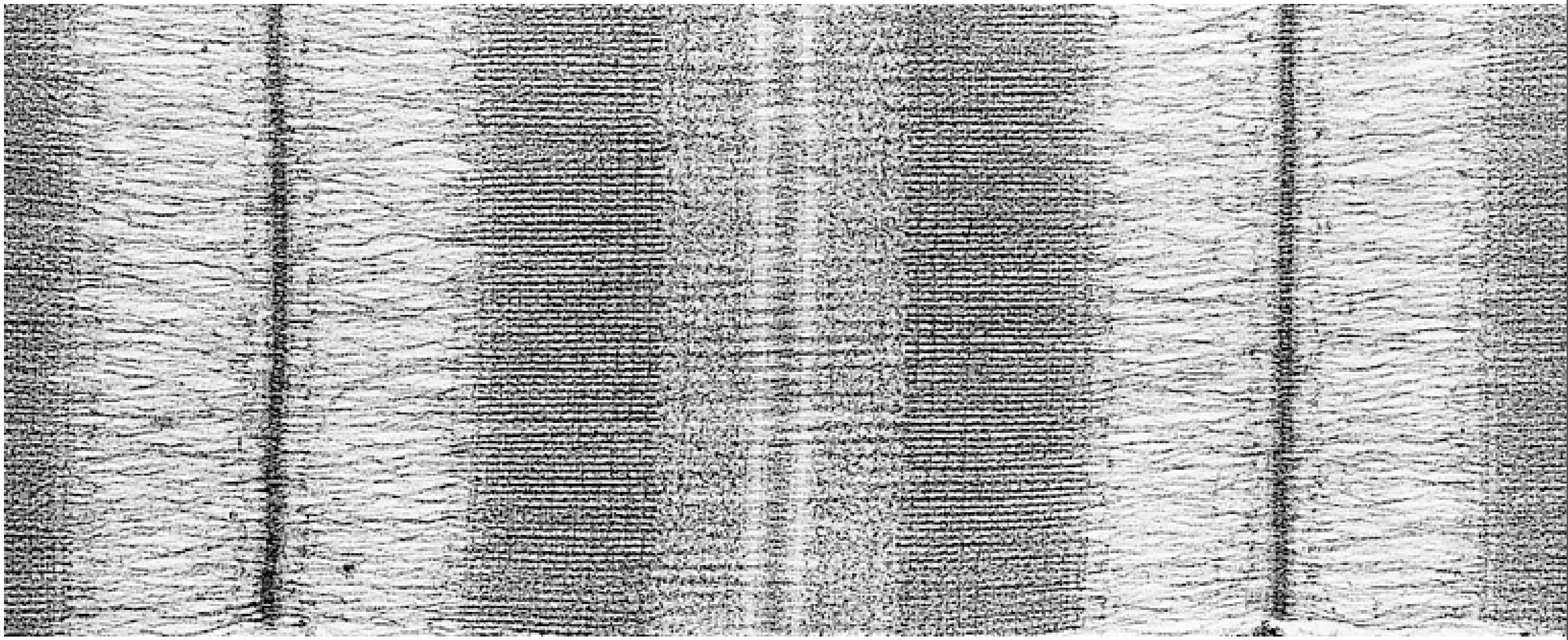

Body muscle
Muscle bundle
Sheath
Muscle “cell” fiber
Nuclei
Myofibril



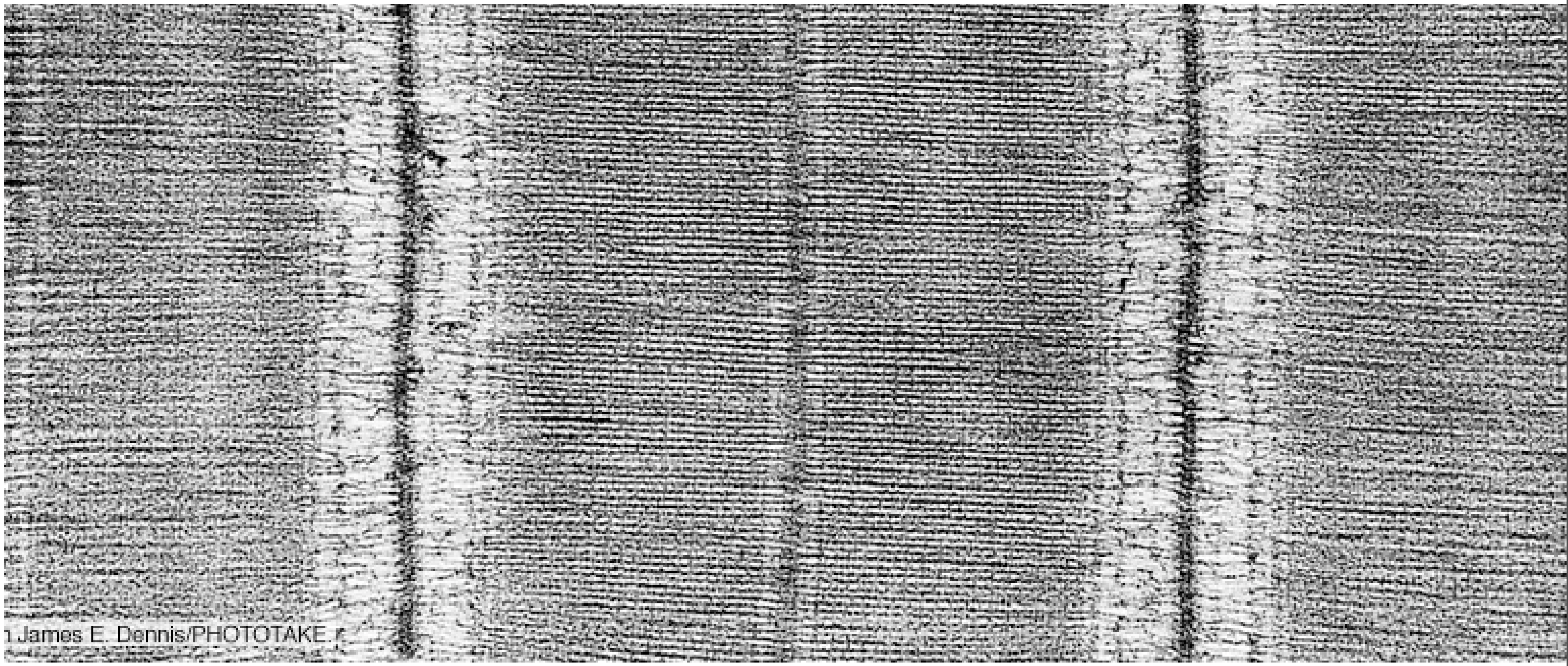

## Sarcomere Cytoarchitecture



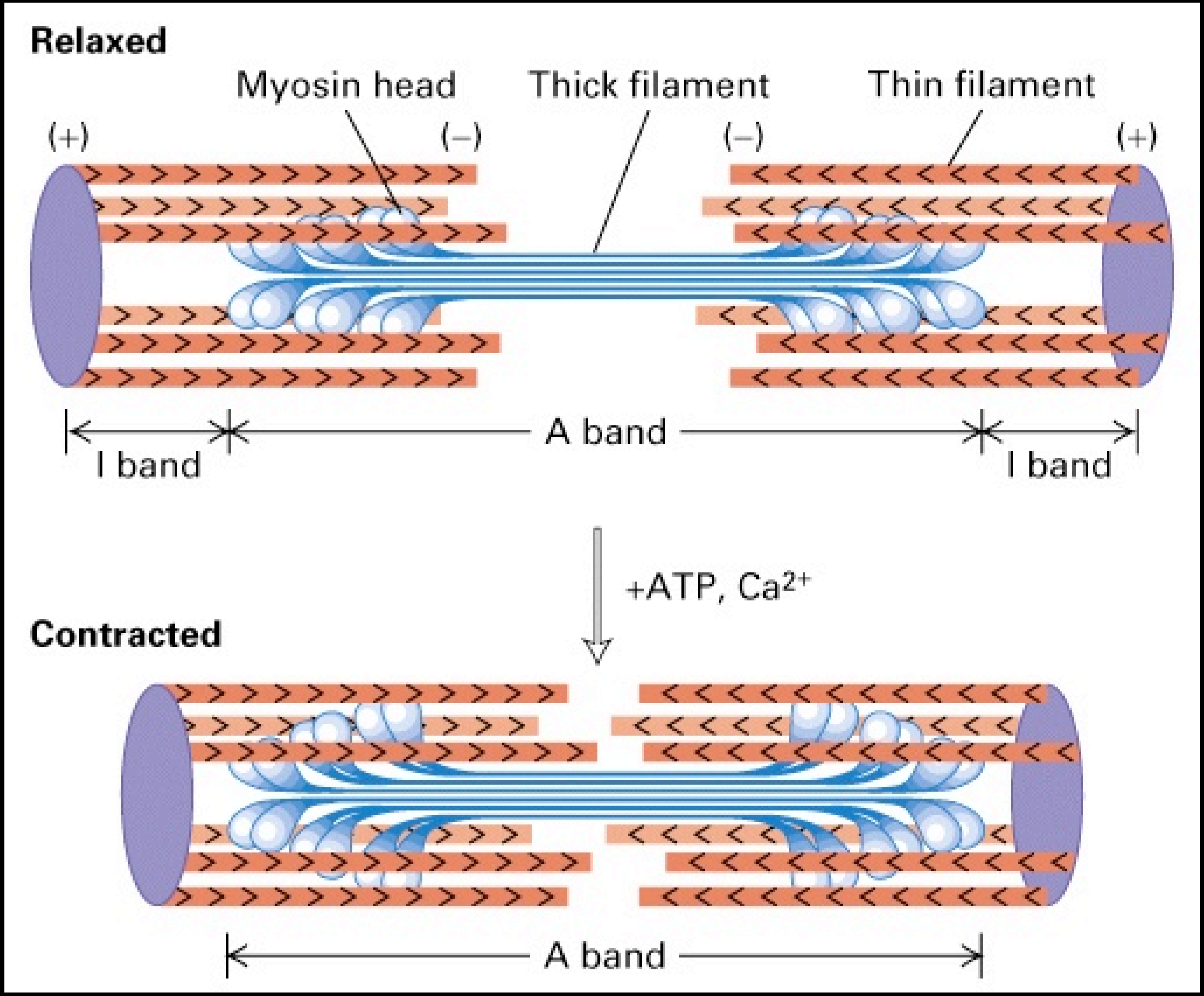
relaxed



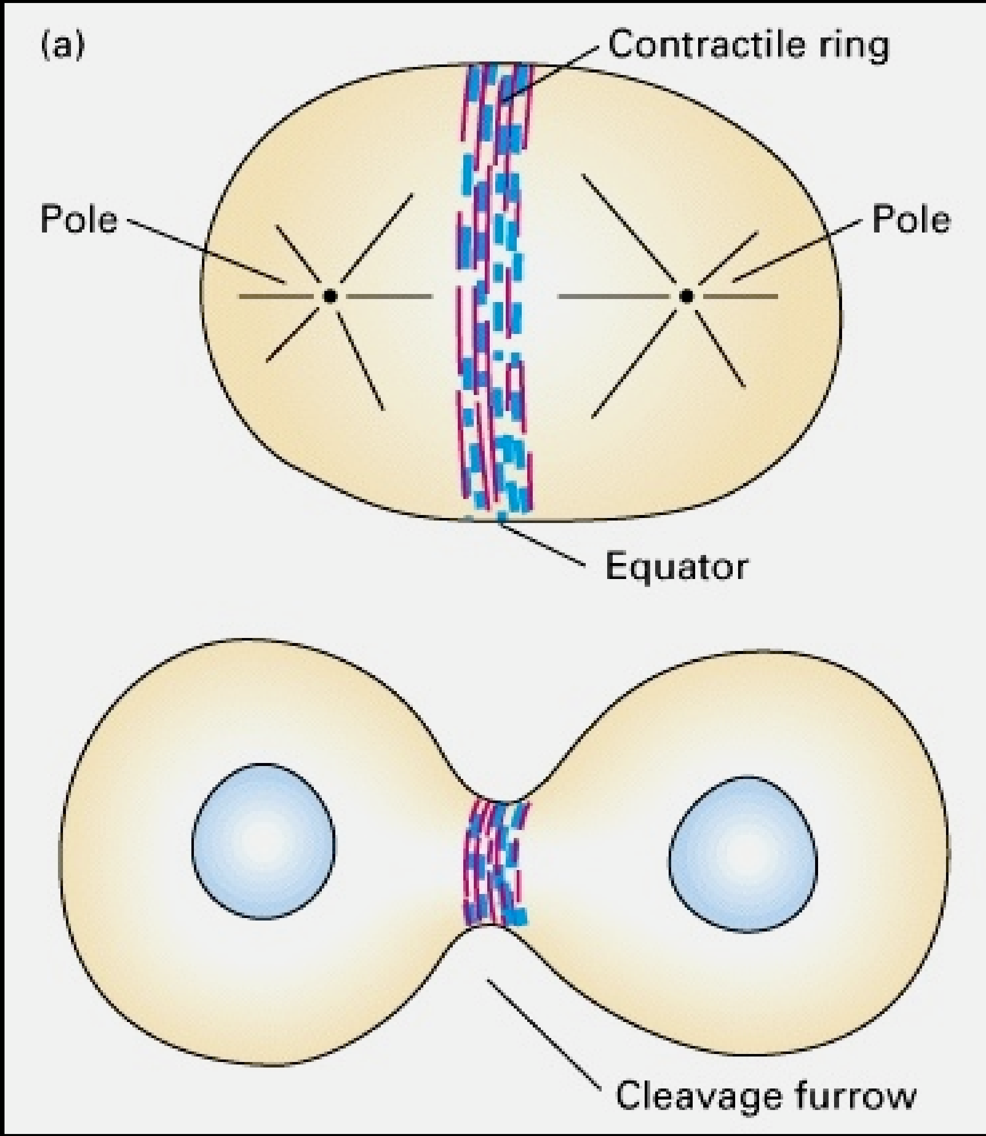
contracted



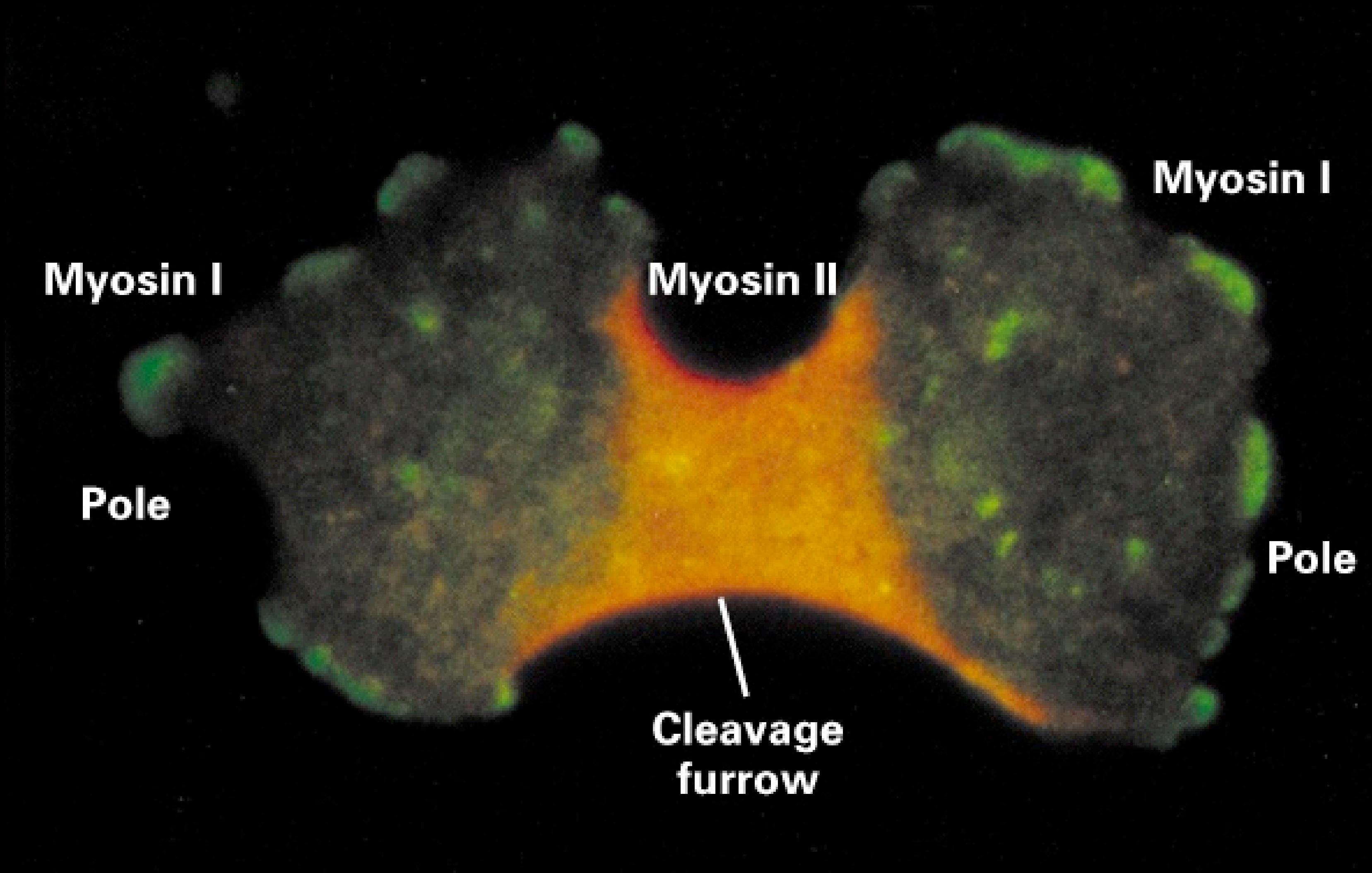
# Muscle Contraction



# Cell Division / Cytokinesis



# *Dictyostelium* amoeba



# Cell Motility

## Swimming

Microtubule-based – cilia, flagella

## Crawling

Actin-based

## purposes:

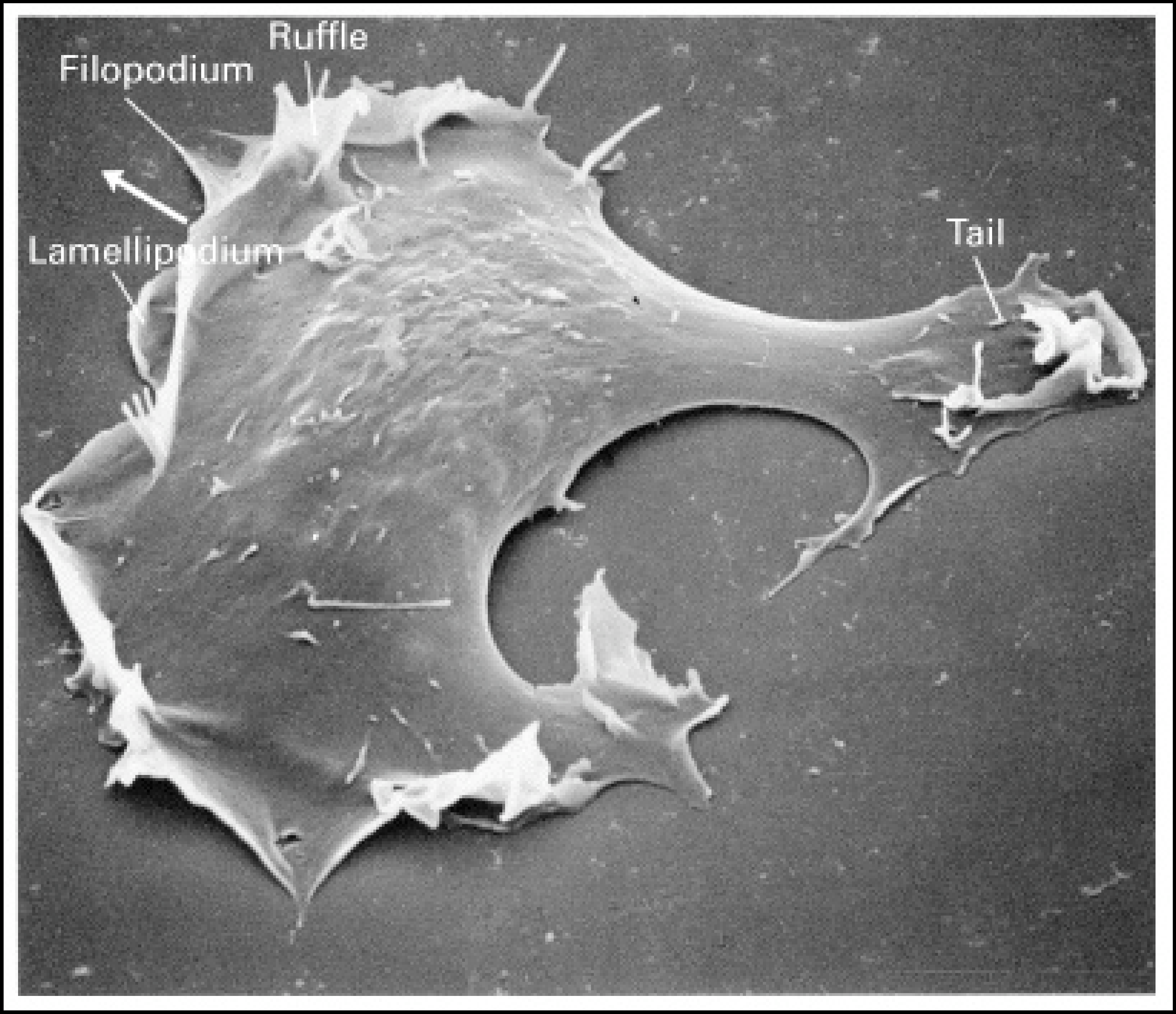
wound healing - epidermal cells

immune response - leukocytes –migrate to sites of infection

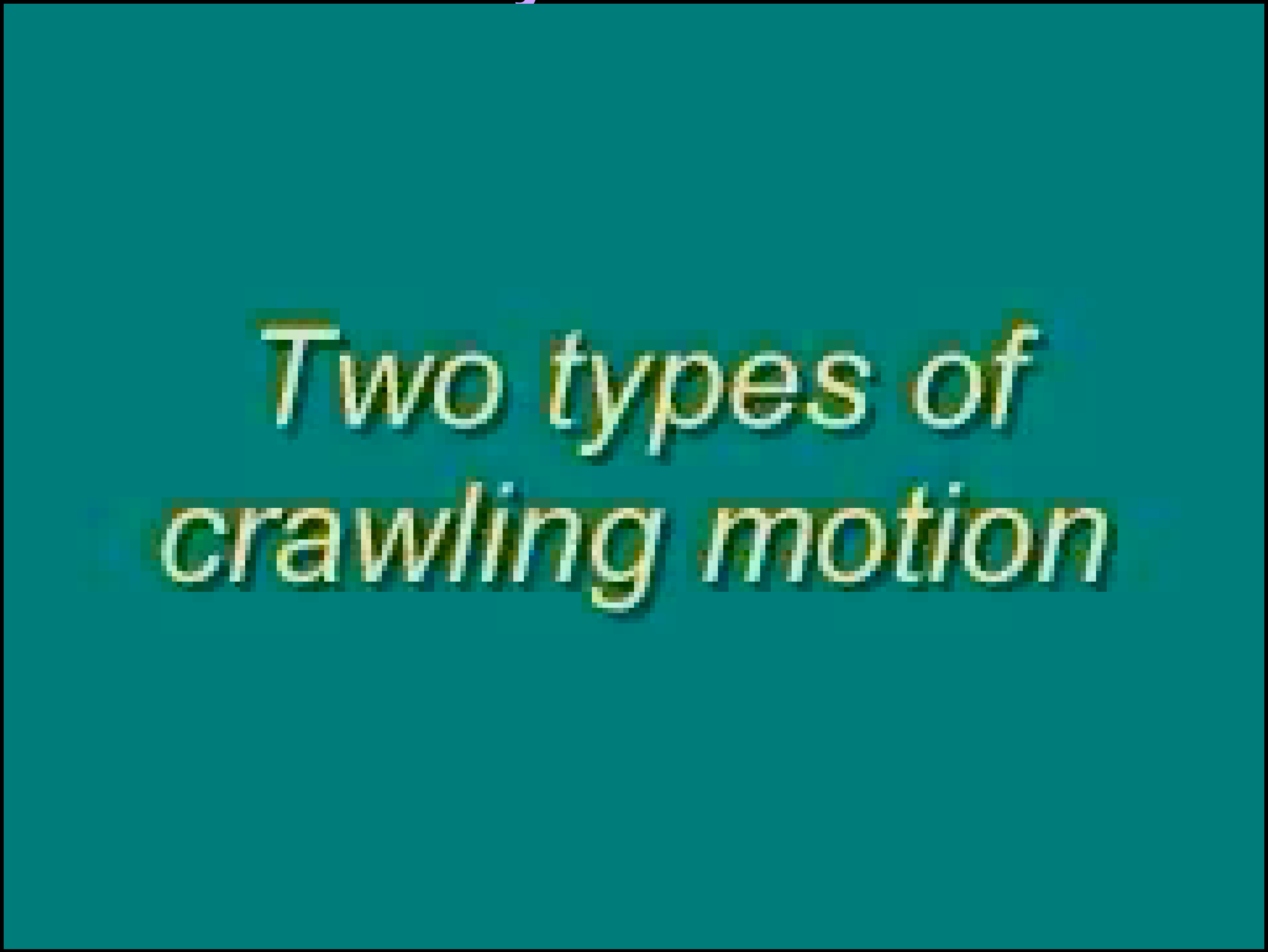
development – neural crest cells; neuronal process extension

cancer cell metastasis –malignancy determinant

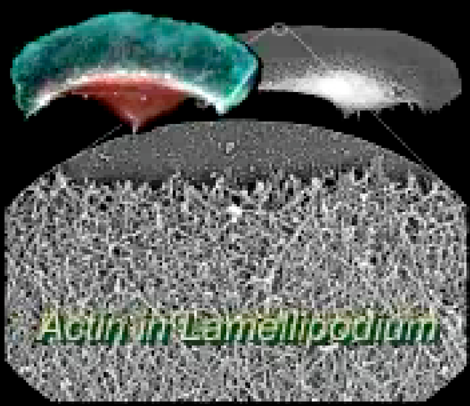
# locomoting cell - filopodia and lamellipodia

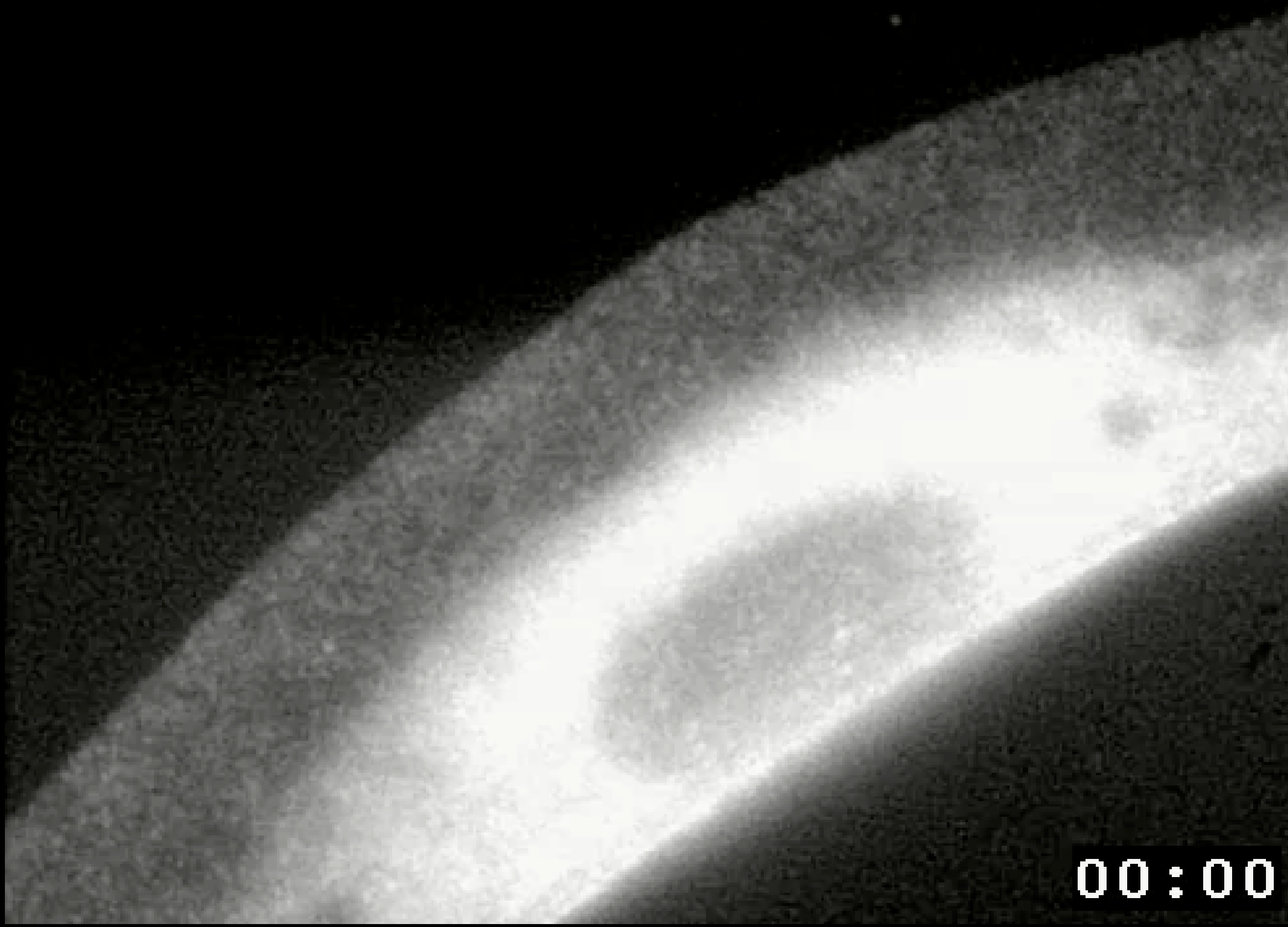


# Fish Keratocytes vs. Fibroblasts



# Actin Dynamics in Moving Keratocytes



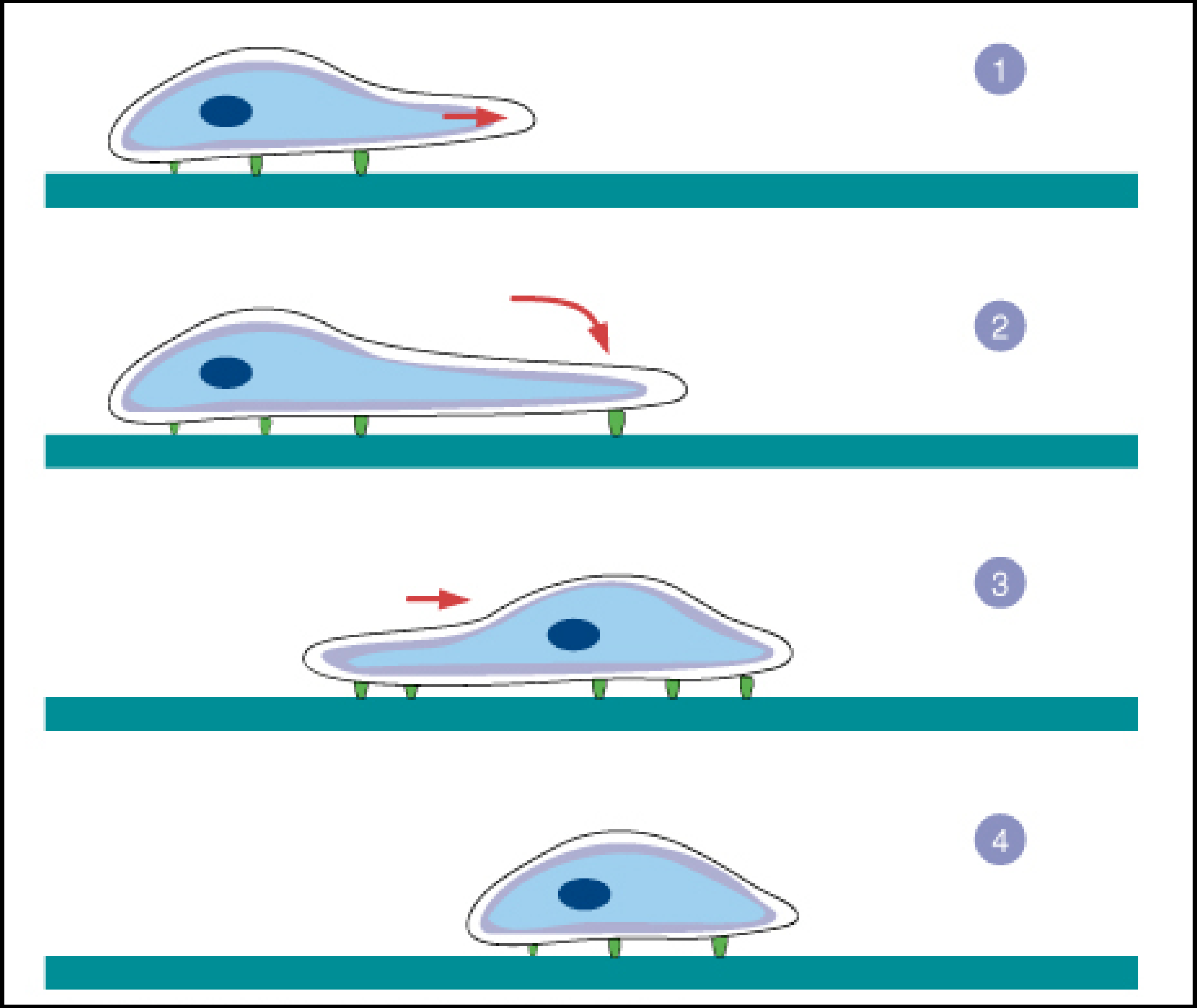




**actin** polymerization at leading edge, treadmill to the rear

# Actin polymerization required for movement

## latrunculin-inhibits actin polymerization


# Crawling: Coordination of 4 processes




protrusion

anchorage

forward movement

tail retraction


protrusion

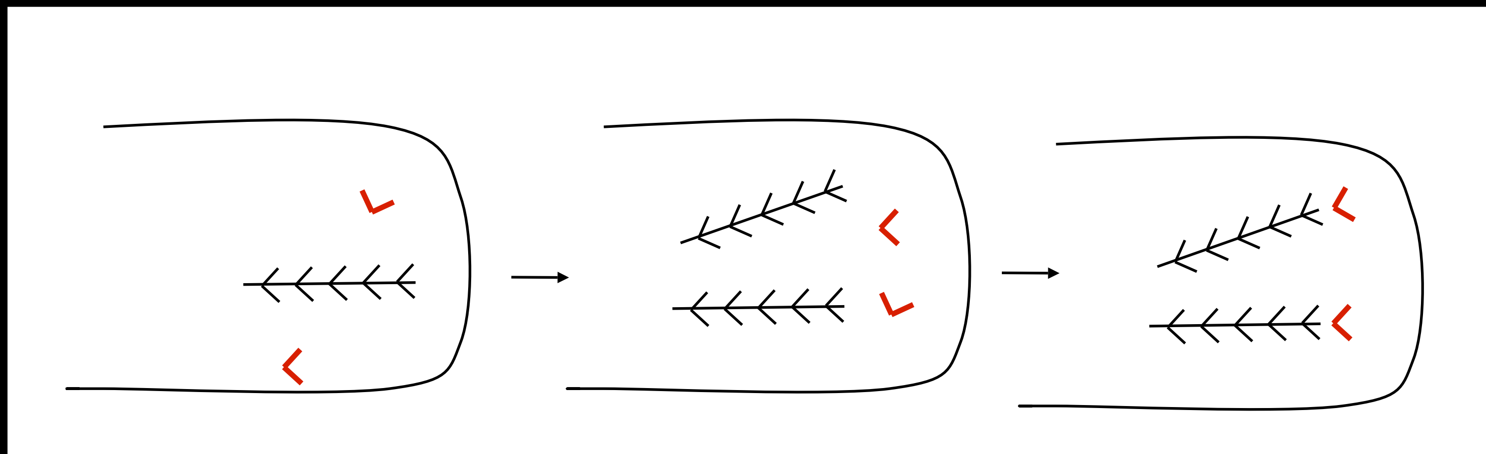
Actin polymerization at leading edge - local force

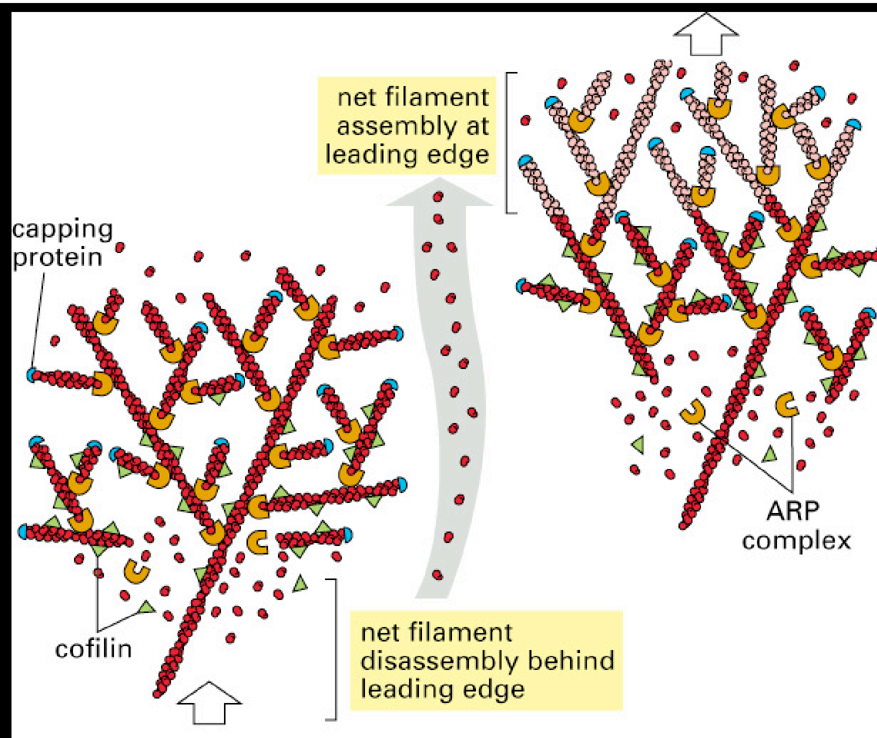
mechanisms:

1) “thermal rachet” - actin polymerization pushes

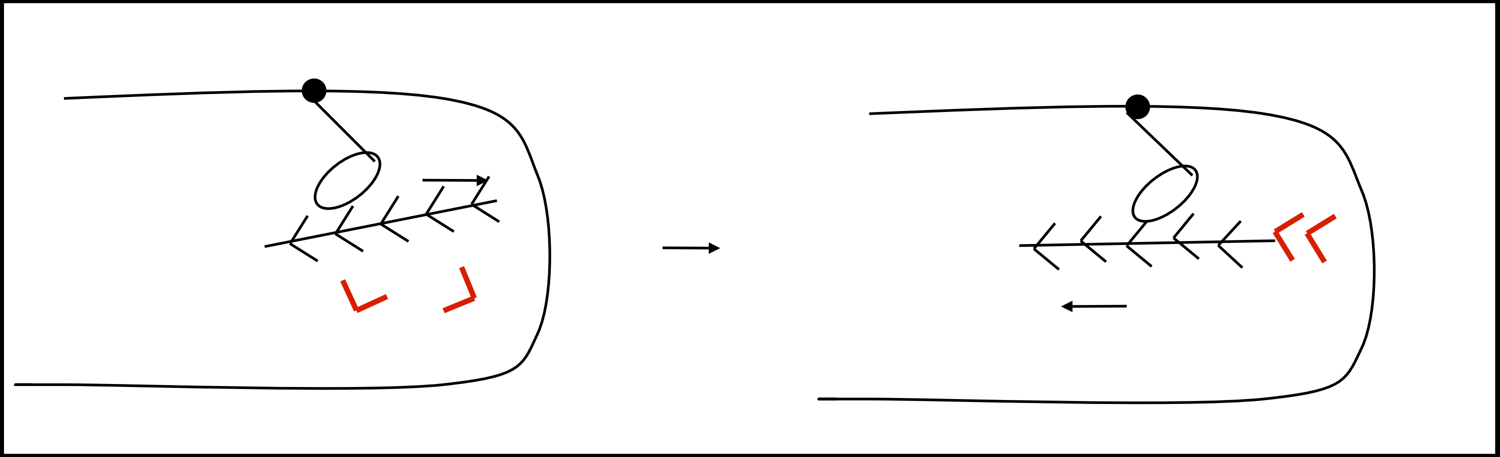
2) myosin I - movement of actin filaments

# 1) thermal rachet - actin polmerization at leading membrane, depolymerization at the rear

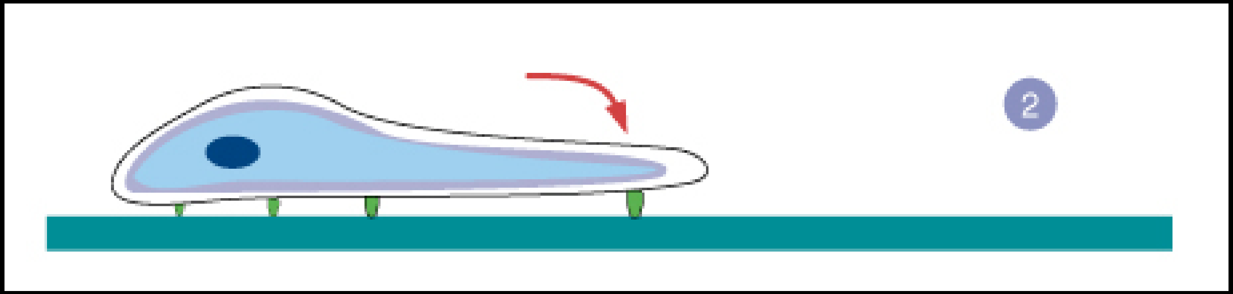




## 2) myosin I - dependent



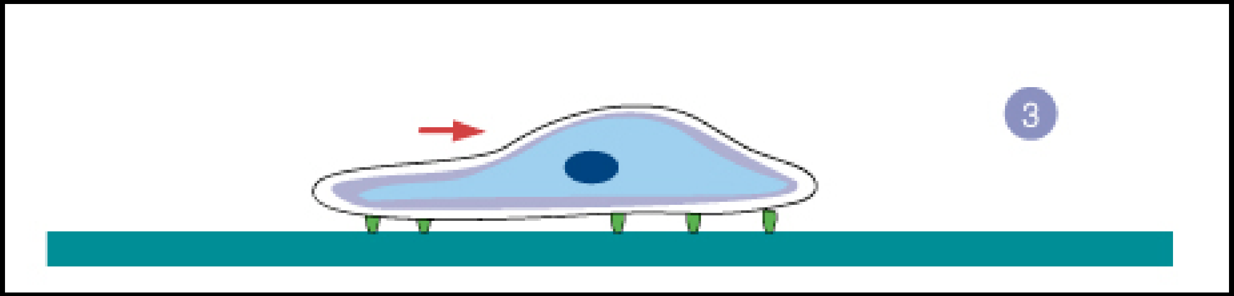
**myosin I could also transport assembly factors to membrane**




anchorage

**Adhesion plaques:**

connect cell to substratum
prevent leading lamella from retracting




forward
movement

**Observations:**

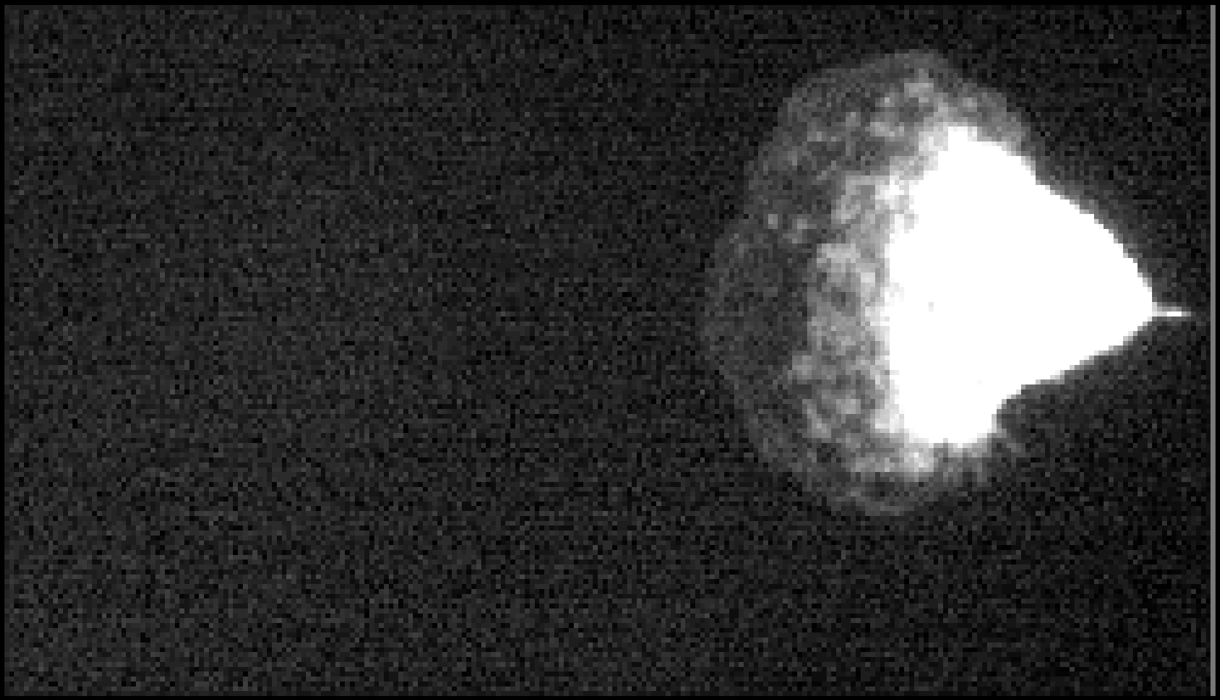
actin networks stationary with respect to substratum
cell body and nucleus rotates
myosin II required

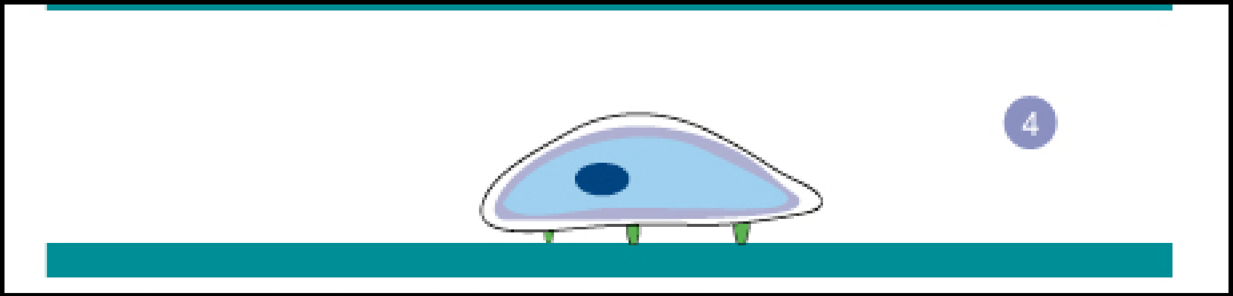
## Proposed mechanisms:

1) sarcomere-like contractions in rear
2) transport along actin arrays

crawling
Dictyostelium
amoeba

# MyosinII (Rhodamine) in Moving Keratocytes

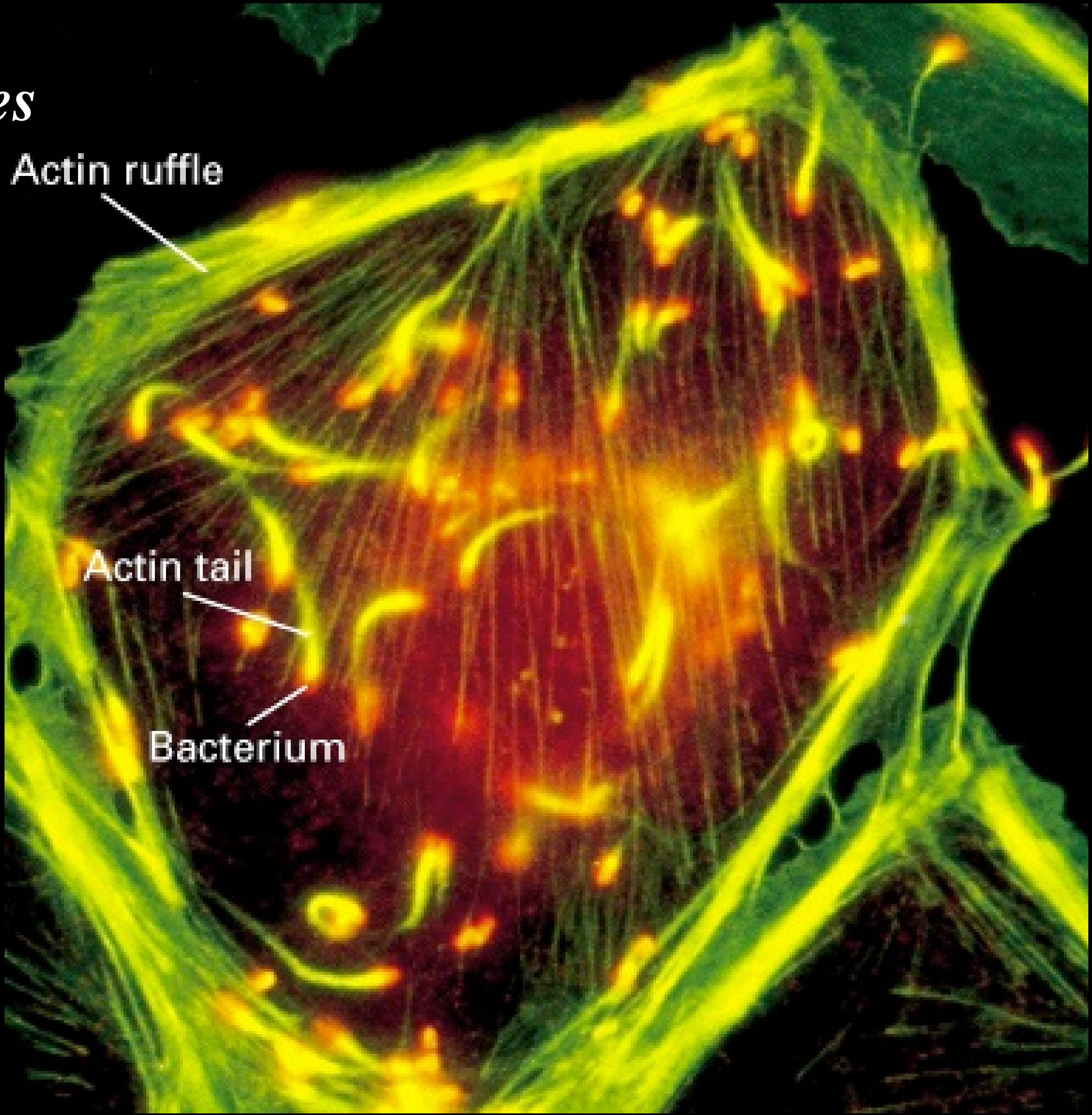





tail retraction

passive - cell snaps loose from adhesion plaques

# *Listeria monocytogenes*

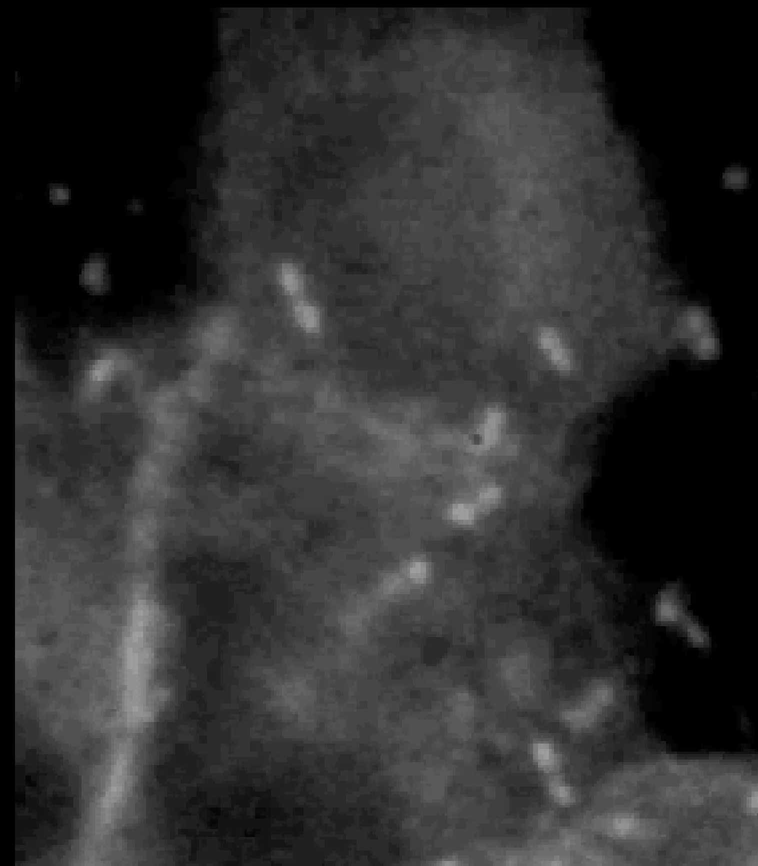



# Co-opts Actin for devious purposes.....

## *Listeria* movement


'comet tails' formed
by actin polymerization

## *Listeria* invasions

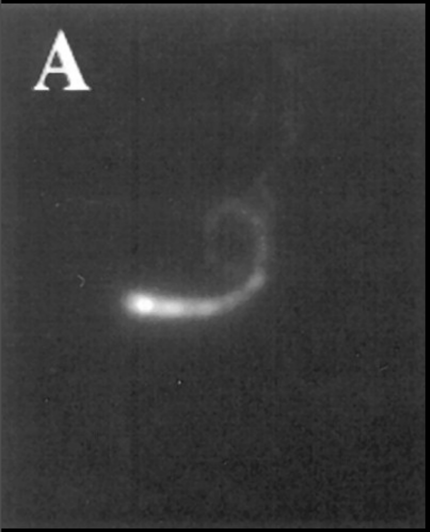


# what controls depolymerization in Listeria tails?

Listeria + cytoplasmic egg extract leads to motility in vitro

immunodeplete gelsolin or ADF/cofilin and observe effects

control



# Important Breakthrough:

## reconstitution of Listeria motility from purified components

**required:**

- Actin and ATP
- Arp2/3 complex
- ADF/cofilin
- Capping protein

**stimulators:**

- VASP -binds ActA, actin, profilin
- Profilin
- α-actinin

**has helped us understand 'normal' cell mechanisms involving actin**

## Next Week:

**Chromosomes and the Cell Cycle**

**Shiv Grewal - 4PM**
**Heterochromatin in Pombe**