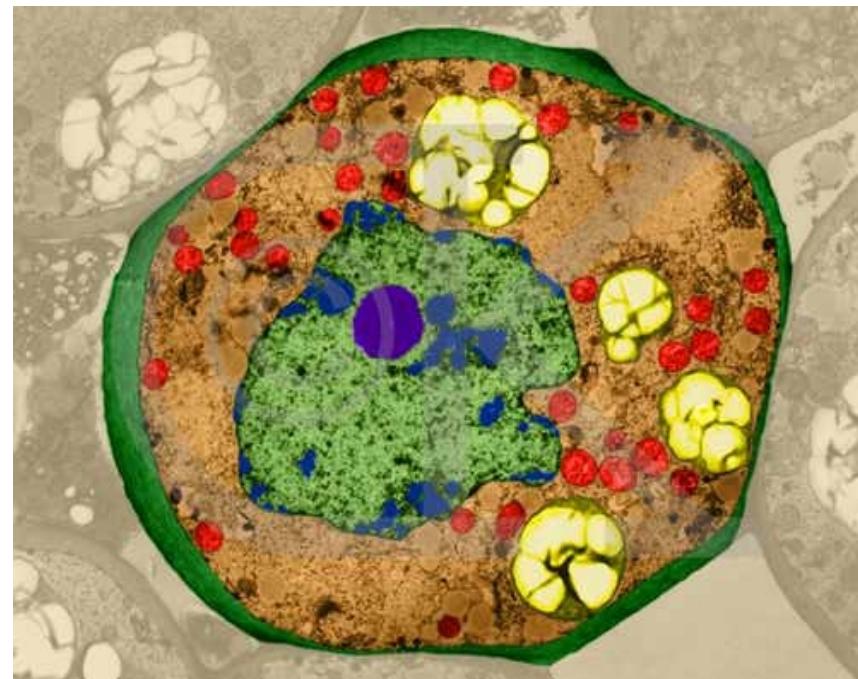


Introduction to Molecular and Cellular Biology

LECTURES 10-11:

Cell organization III



LECTURES 10-11: CELL ORGANIZATION III

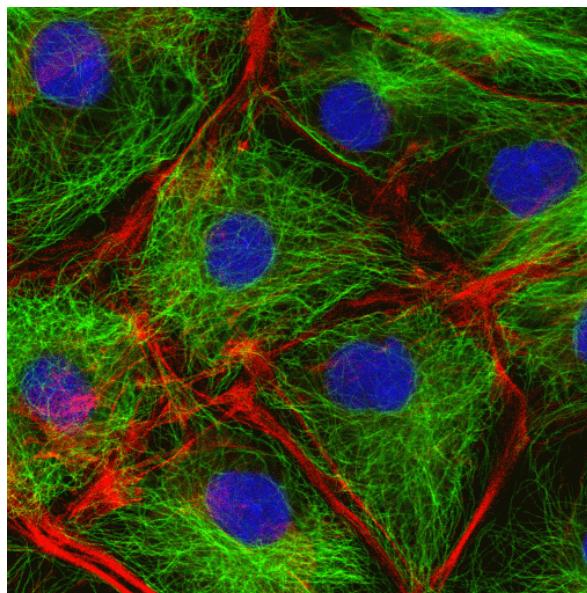
- Introduction to cytoskeleton
- Intermediate filaments
- Microtubules
- Actin filaments
- Muscle contraction
- Regulation of cytoskeleton biogenesis
- Polarity of the cell and cytoskeleton:
 - cell crawling (adhesion and traction)
 - chemotaxis
 - yeast budding
 - neuron specialization



CYTOSKELETON: INTRODUCTION

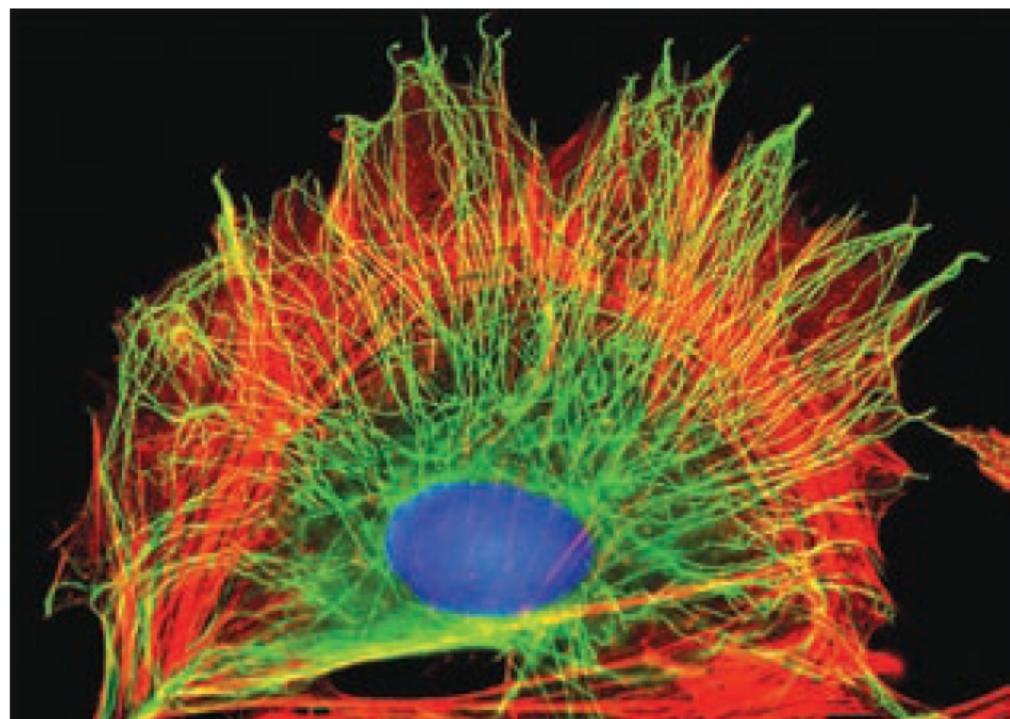
The cytoskeleton is a system of intracellular filaments and tubules in the cytoplasm crucial for cell shape, division and function

- 1903. Nikolai Koltsov: the shape of the cell is determined by a network of tubules => cytoskeleton.
- 1929. Rudolph Peters: protein structures dynamically coordinate the biochemistry in the cell.
- 1931. Paul Wintrebert introduced the term “cytosquelette”.



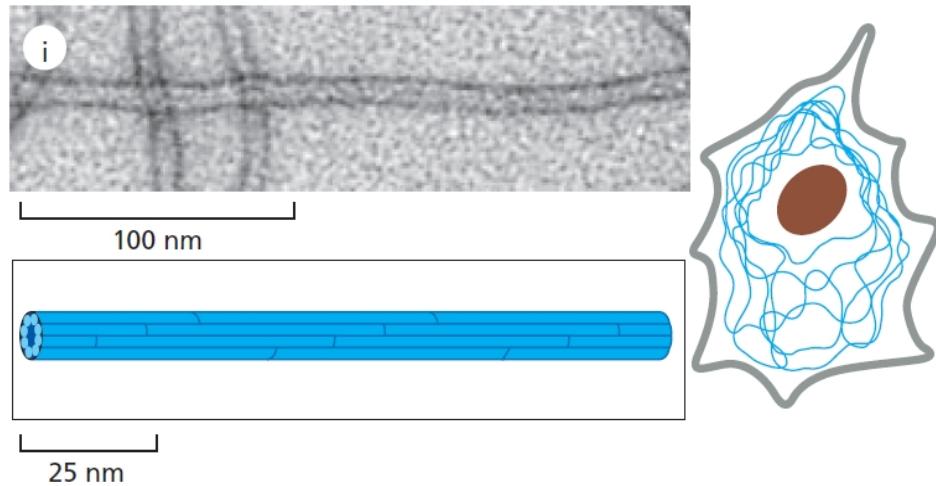
CYTOSKELETON: INTRODUCTION

- Volume support: plants (cell wall) and animals (no cell wall)
- Prokaryotes and eukaryotes have homologous cytoskeletal proteins
- Highly dynamic structure
- Not only “bones” but also “muscles” and “motors” of the cell
- Contributes to the understanding of the “crowding effect” for both organelles and macromolecules
- Components:
 - intermediate filaments
 - microtubules
 - actin filaments

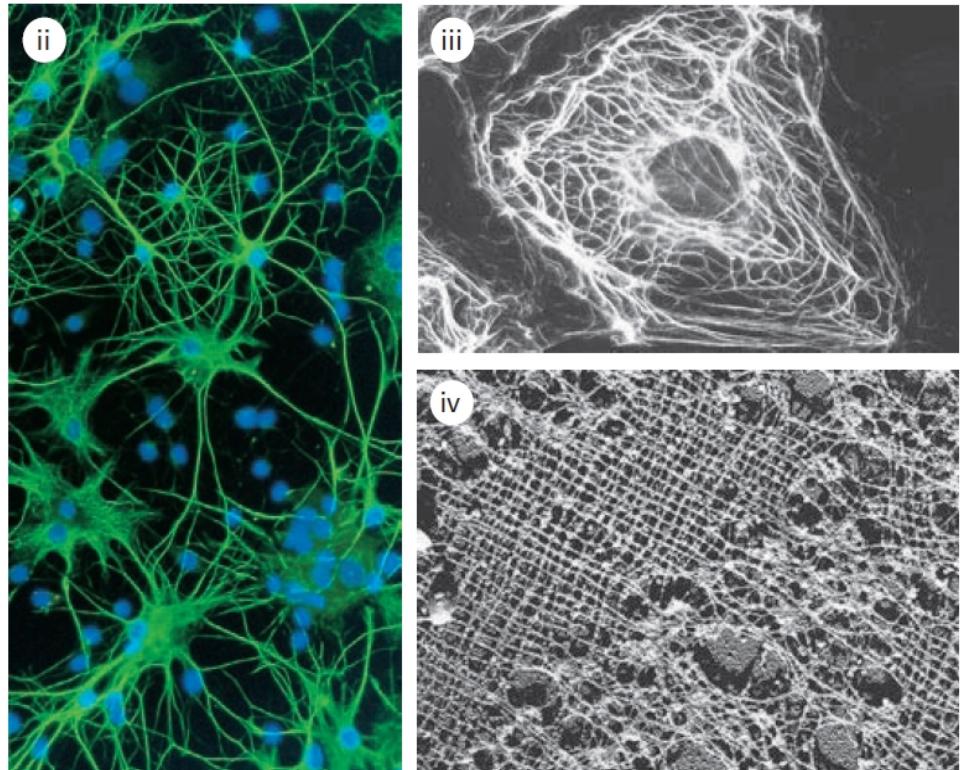


10 μm

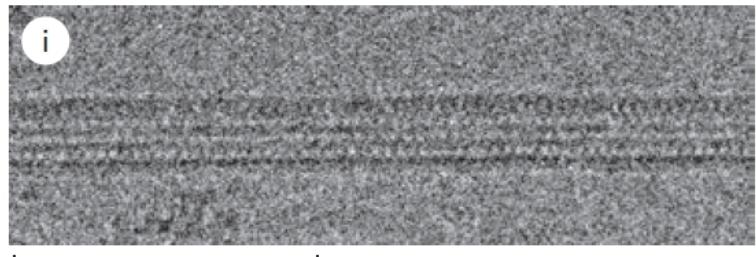
CYTOSKELETON TYPES OVERVIEW: INTERMEDIATE FILAMENTS



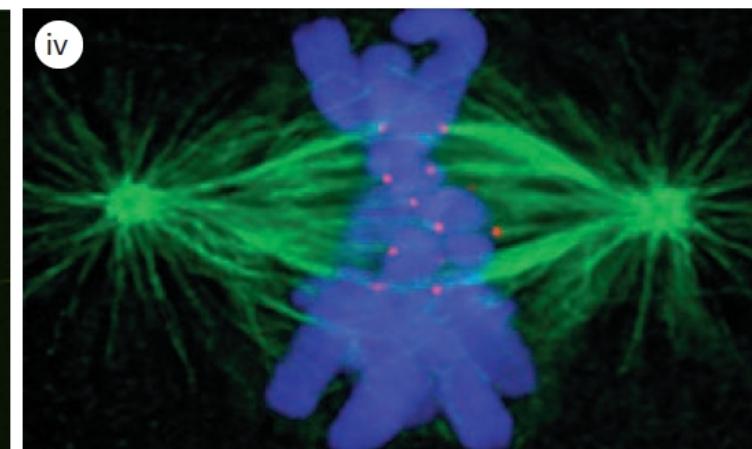
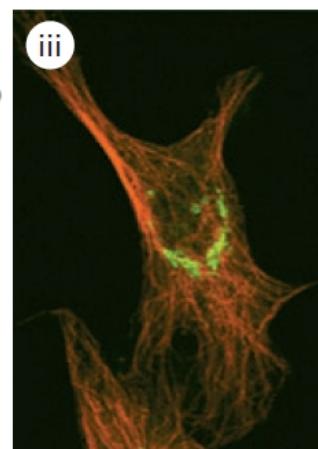
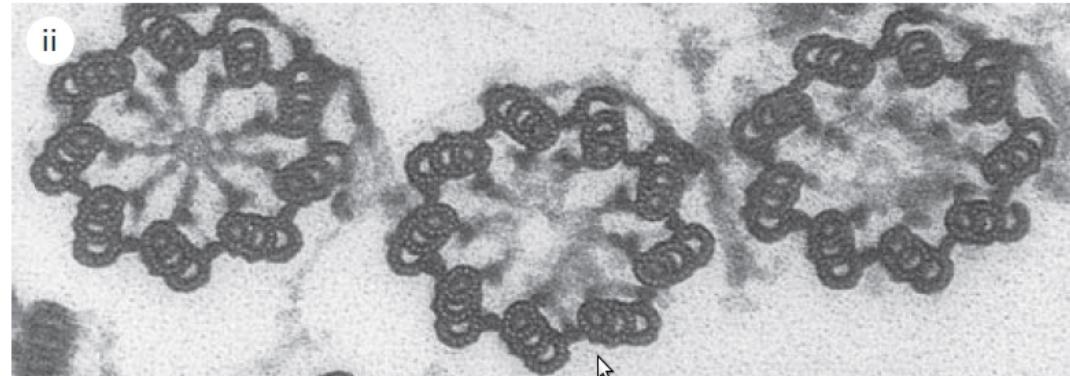
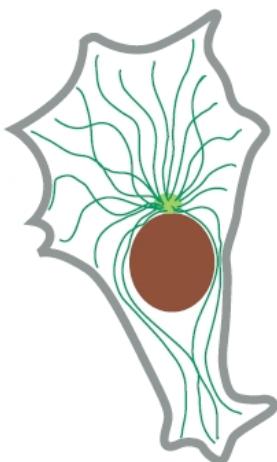
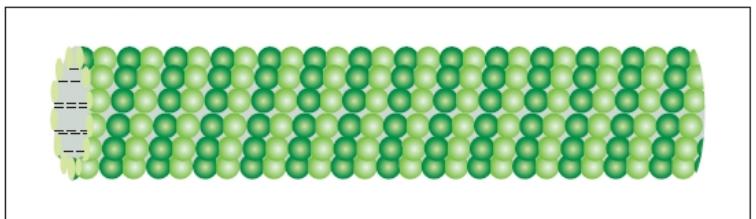
Diameter = 10 nm



CYTOSKELETON TYPES OVERVIEW: MICROTUBULES

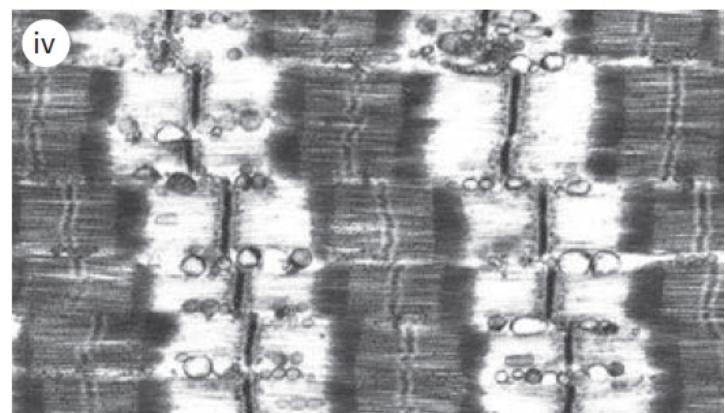
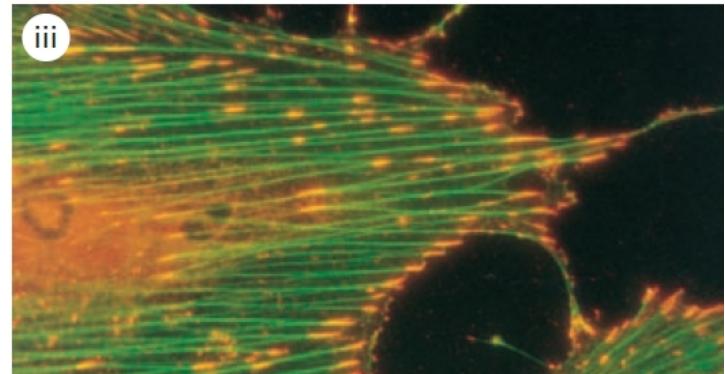
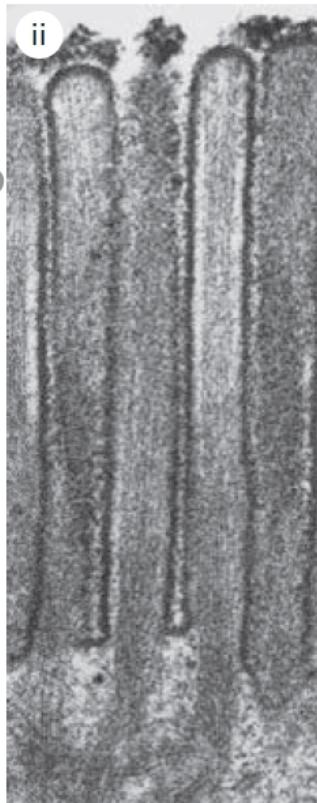
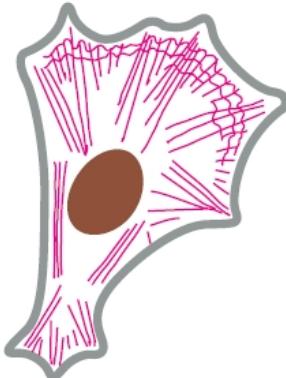
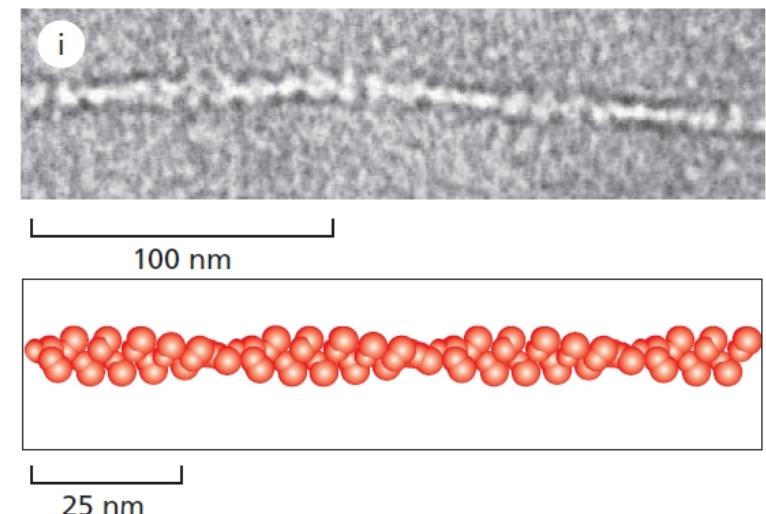


100 nm



Diameter = 25 nm

CYTOSKELETON TYPES OVERVIEW: ACTIN FILAMENTS

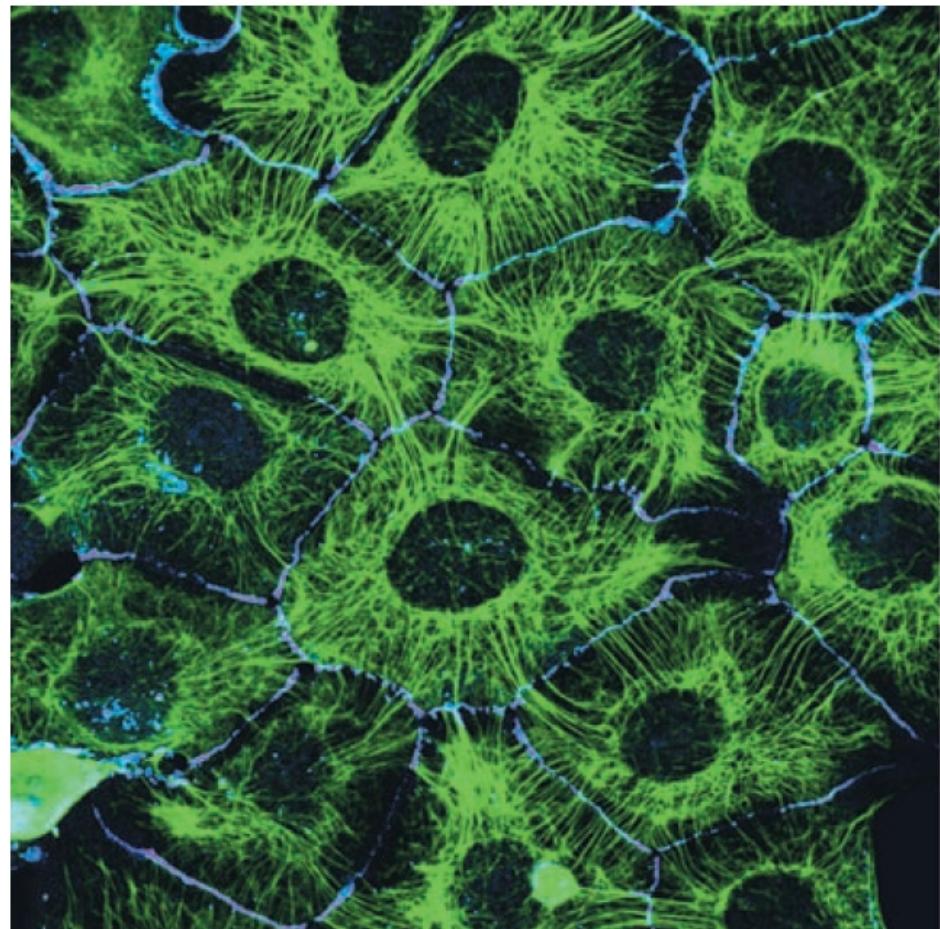


Diameter = 5-9 nm

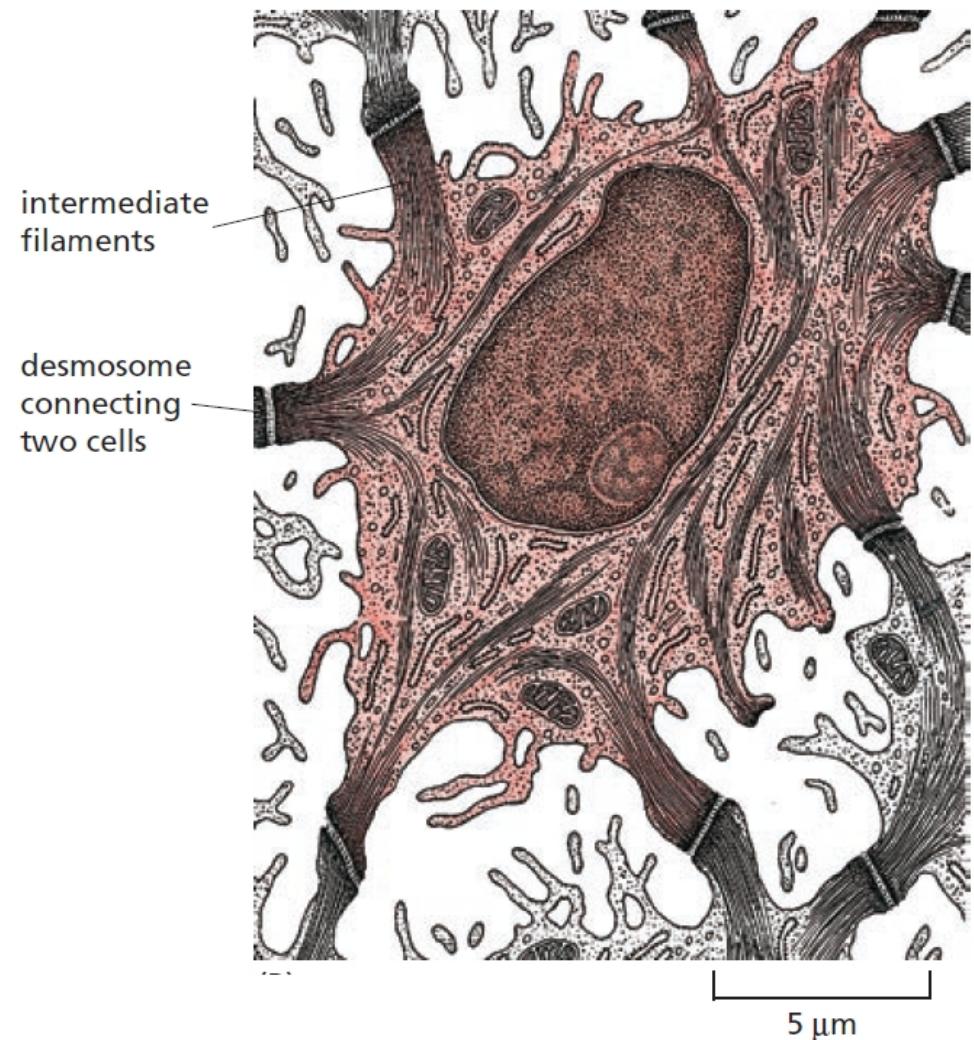
INTERMEDIATE FILAMENTS

- “Intermediate”: diameter (10 nm) is between actin-containing filaments and myosin filaments (found in smooth muscles)
- The most durable of all CS components
- Structure remains intact under strong salt solution/non-ionic detergents
- In most animal cells
- Anchored to plasma membrane by desmosomes (cell junctions, L25-26)
- Localization:
 - cytoplasmic
 - nuclear (nuclear lamina)
- Function:
 - strengthening cells
 - protection from the mechanical stress

INTERMEDIATE FILAMENTS



Keratin filaments in epithelial cells

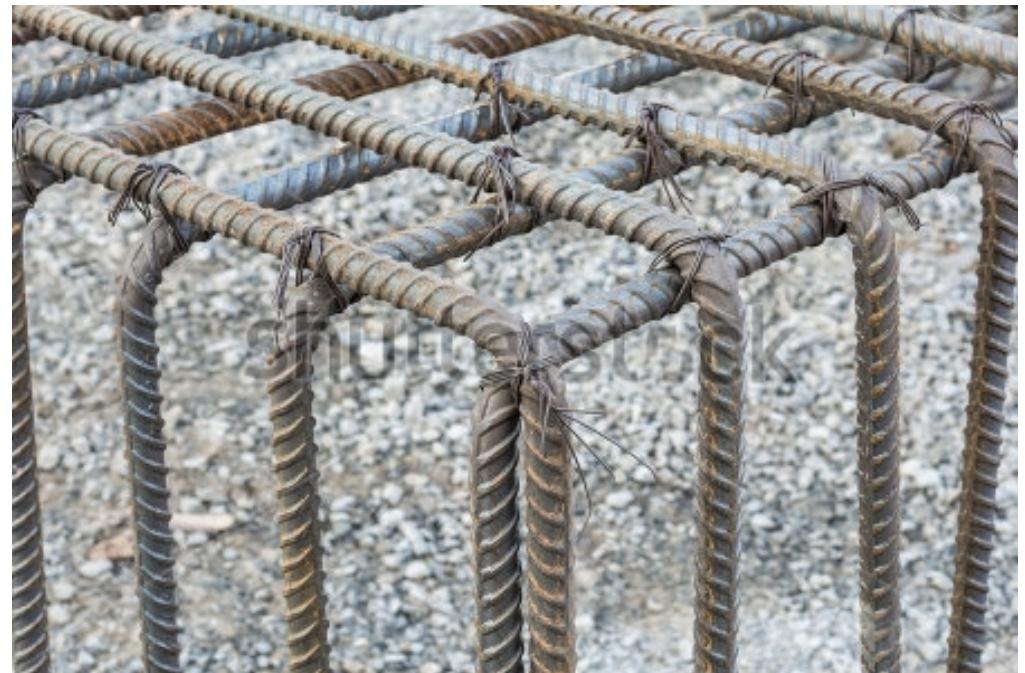


5 μm

MECHANICAL ANALOGY TO INTERMEDIATE FILAMENTS

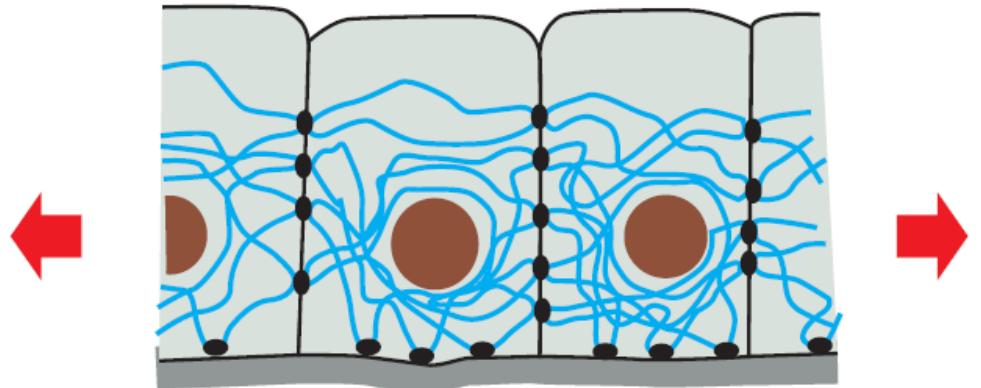


Fibreglass: carbon fibers

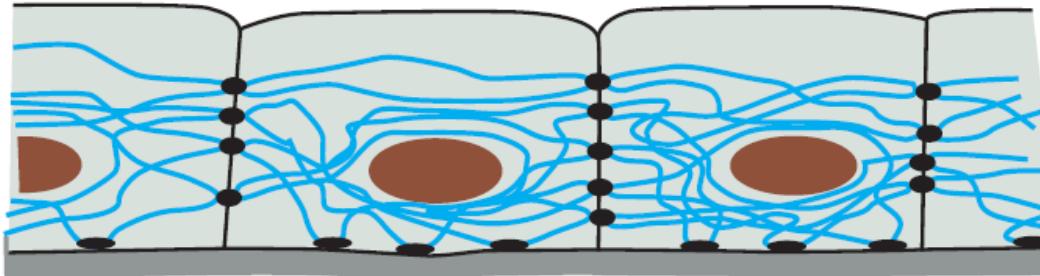


Concrete: steel bars

INTERMEDIATE FILAMENTS IN CELL UNDER MECHANICAL STRESS

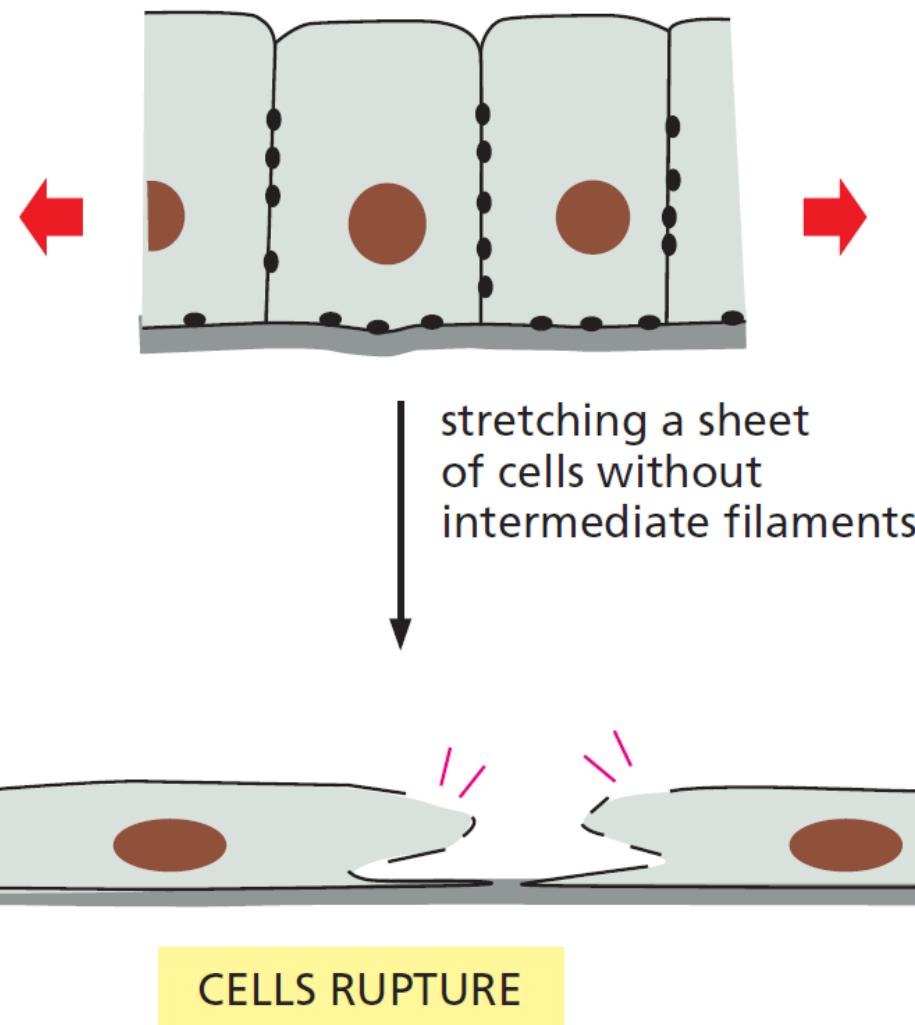


stretching a sheet
of cells with
intermediate filaments



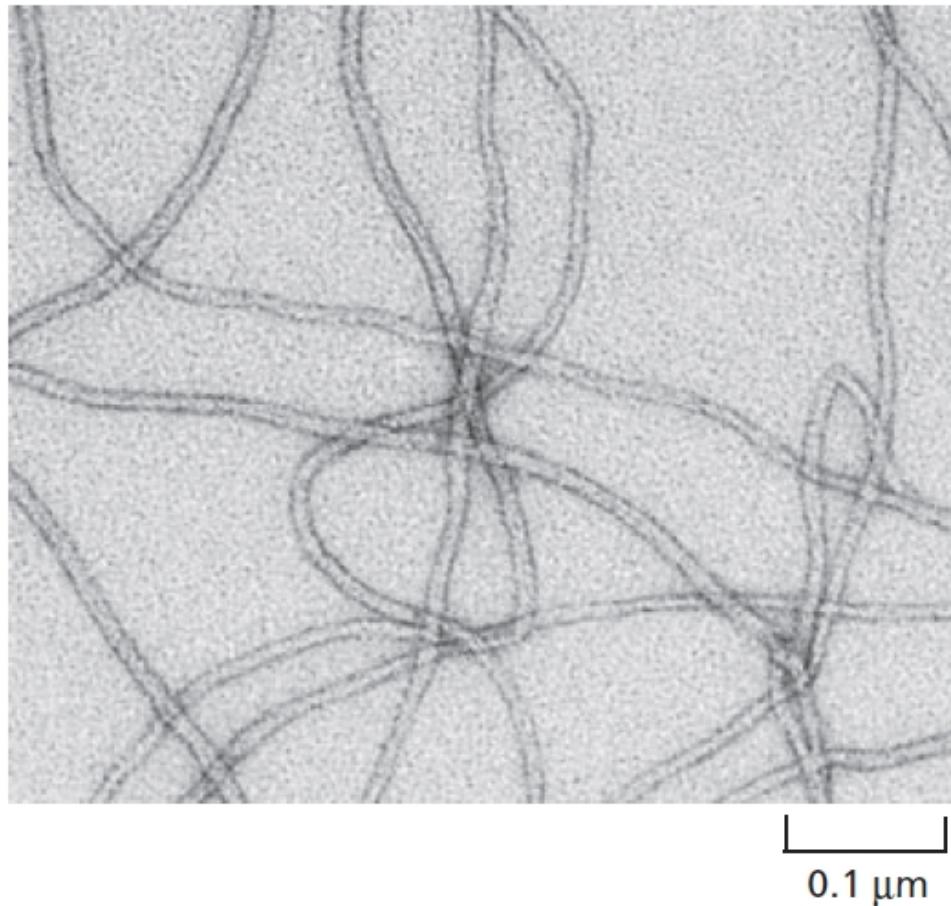
CELLS REMAIN INTACT AND TOGETHER

INTERMEDIATE FILAMENTS IN CELL UNDER MECHANICAL STRESS

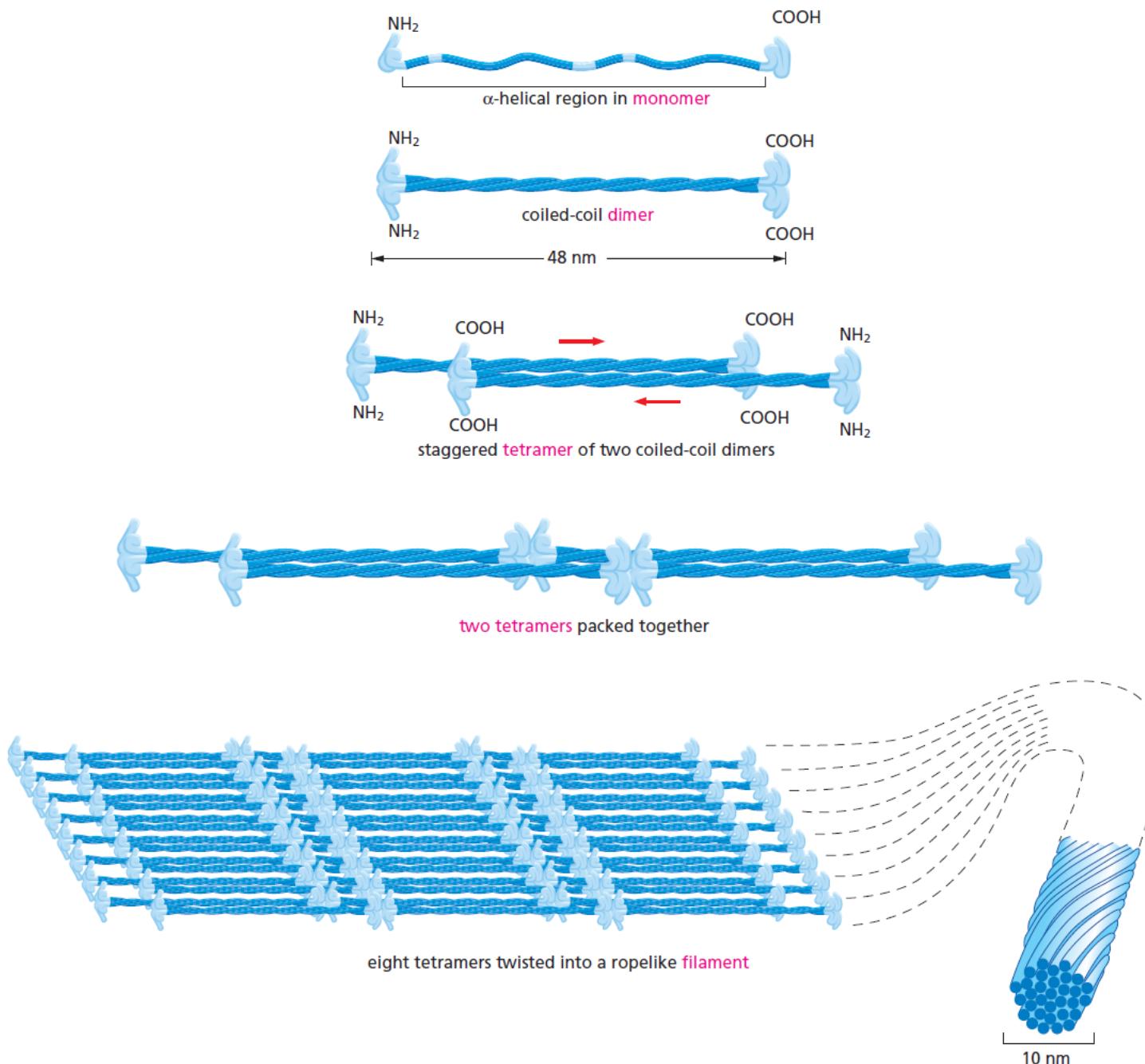


STRUCTURE OF INTERMEDIATE FILAMENTS

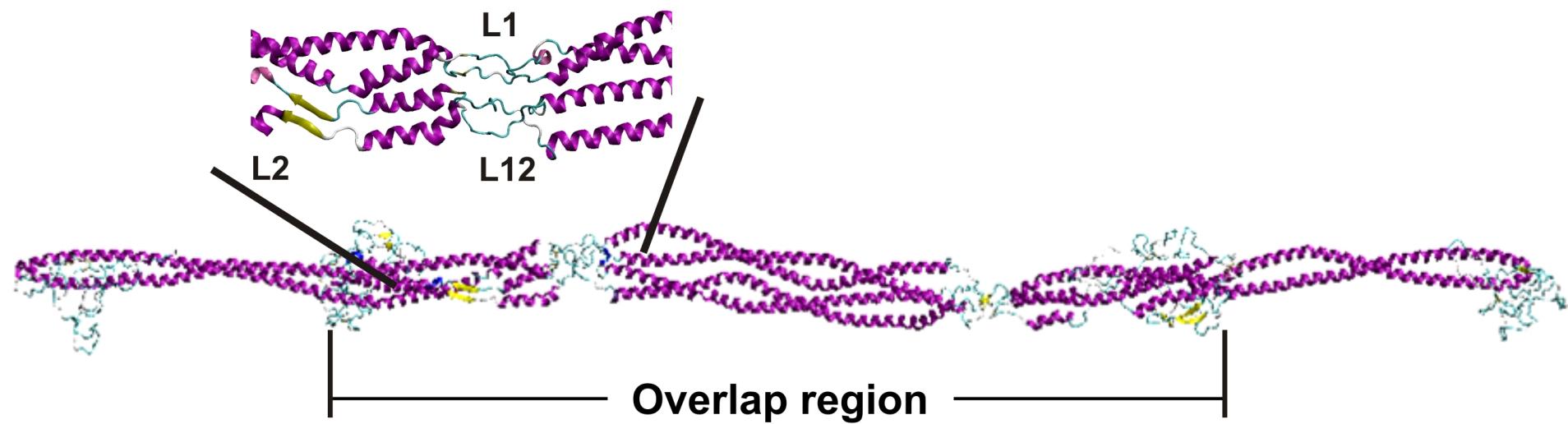
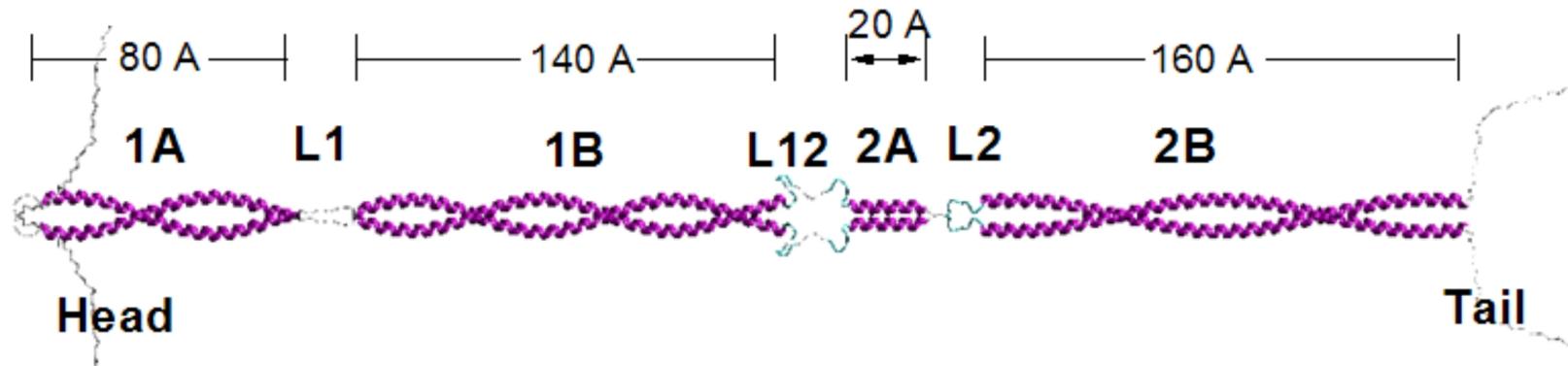
- Shape: long twisted ropes
- Oligomeric associations



STRUCTURE OF INTERMEDIATE FILAMENTS



STRUCTURE OF INTERMEDIATE FILAMENTS



Vimentin intermediate filament

STRUCTURE OF INTERMEDIATE FILAMENTS

➤ Central elongated α -helical domain:

4 α -helical regions (1A, 1B, 2A, 2B) separated by 3 linkers (β -turns)

➤ N- and C-terminal globular domain

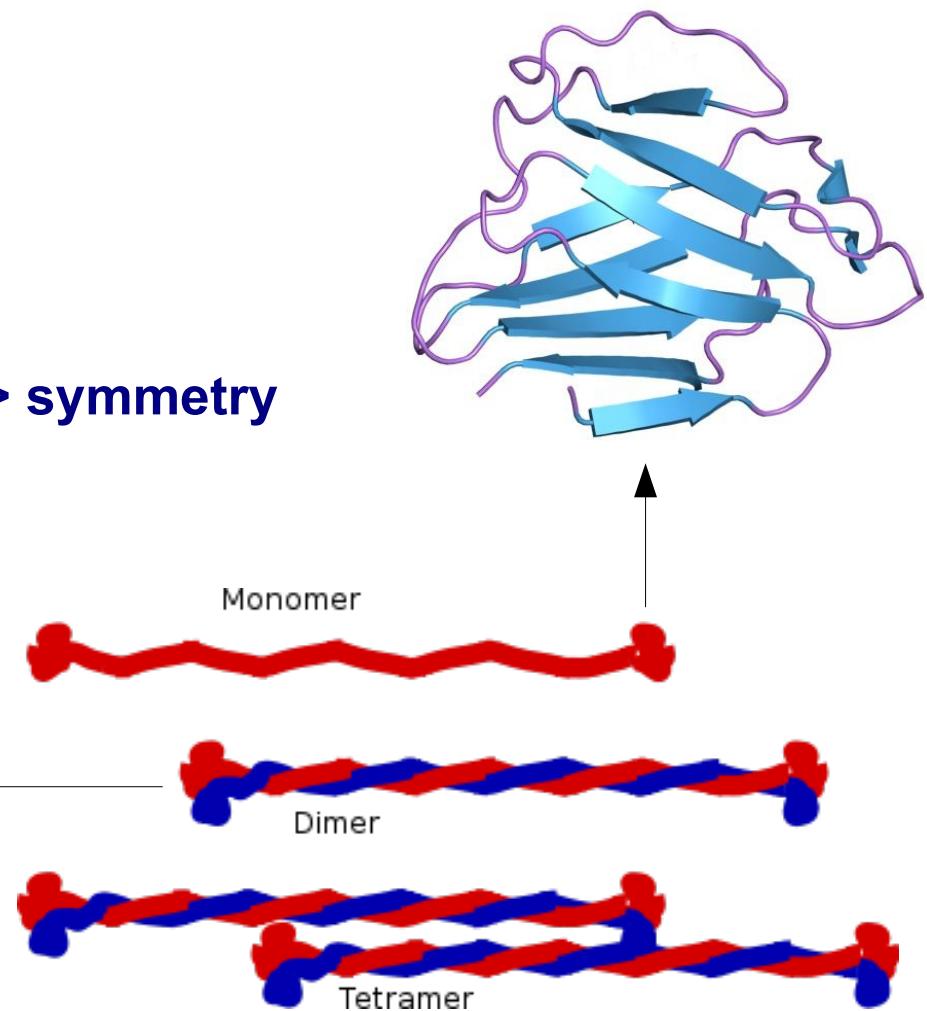
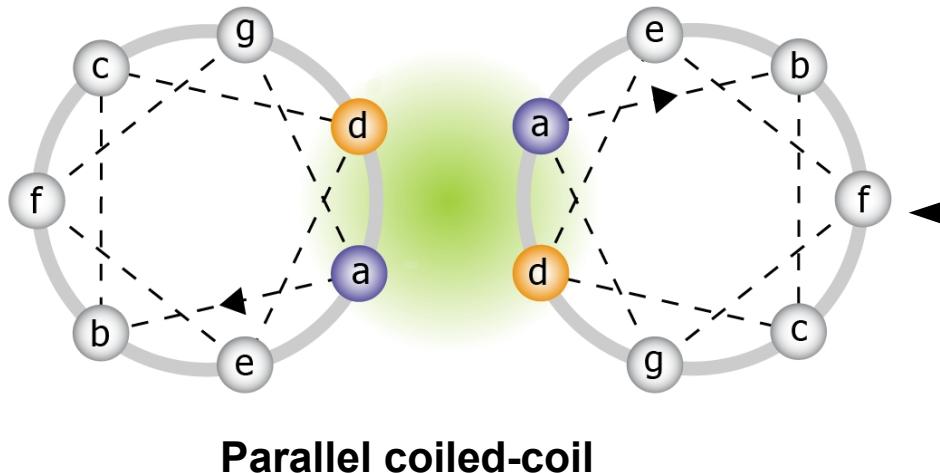
- N- terminal domain is DNA-binding

- C-terminal is very variable

➤ Monomers in dimer are parallel

➤ Dimers in tetramers are antiparallel => symmetry

➤ No NTP binding site



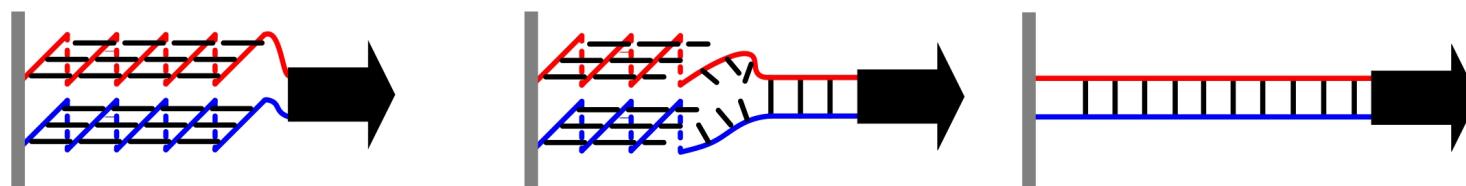
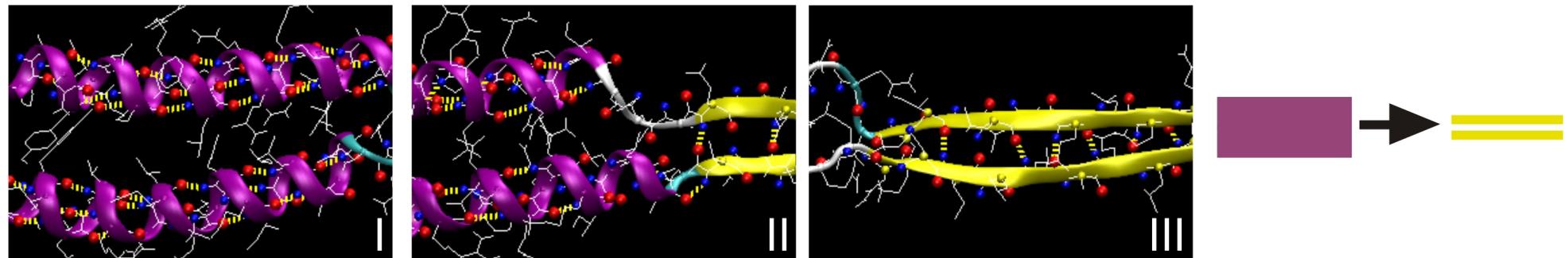
STRUCTURAL TRANSITION UNDER STRESS

α -helical \Rightarrow β -sheet under the stress

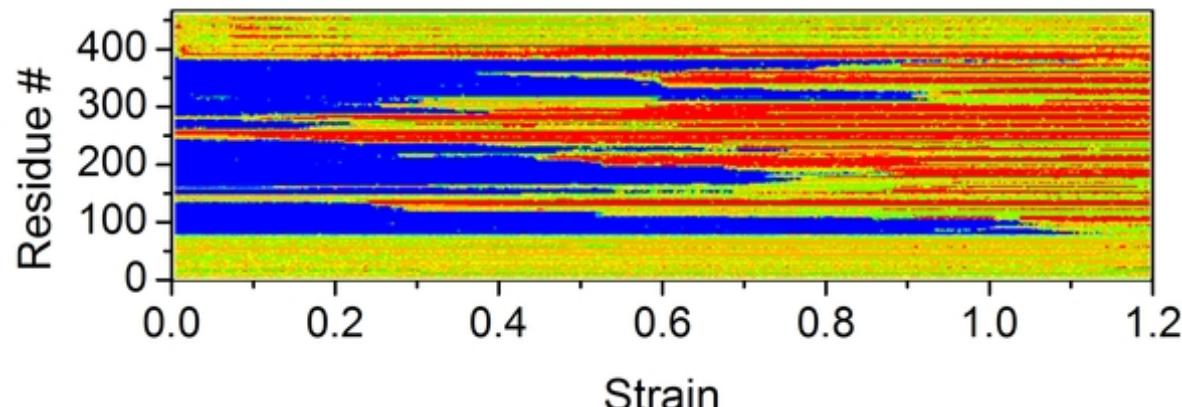


STRUCTURAL TRANSITION UNDER STRESS

α -helical \Rightarrow β -sheet under the stress

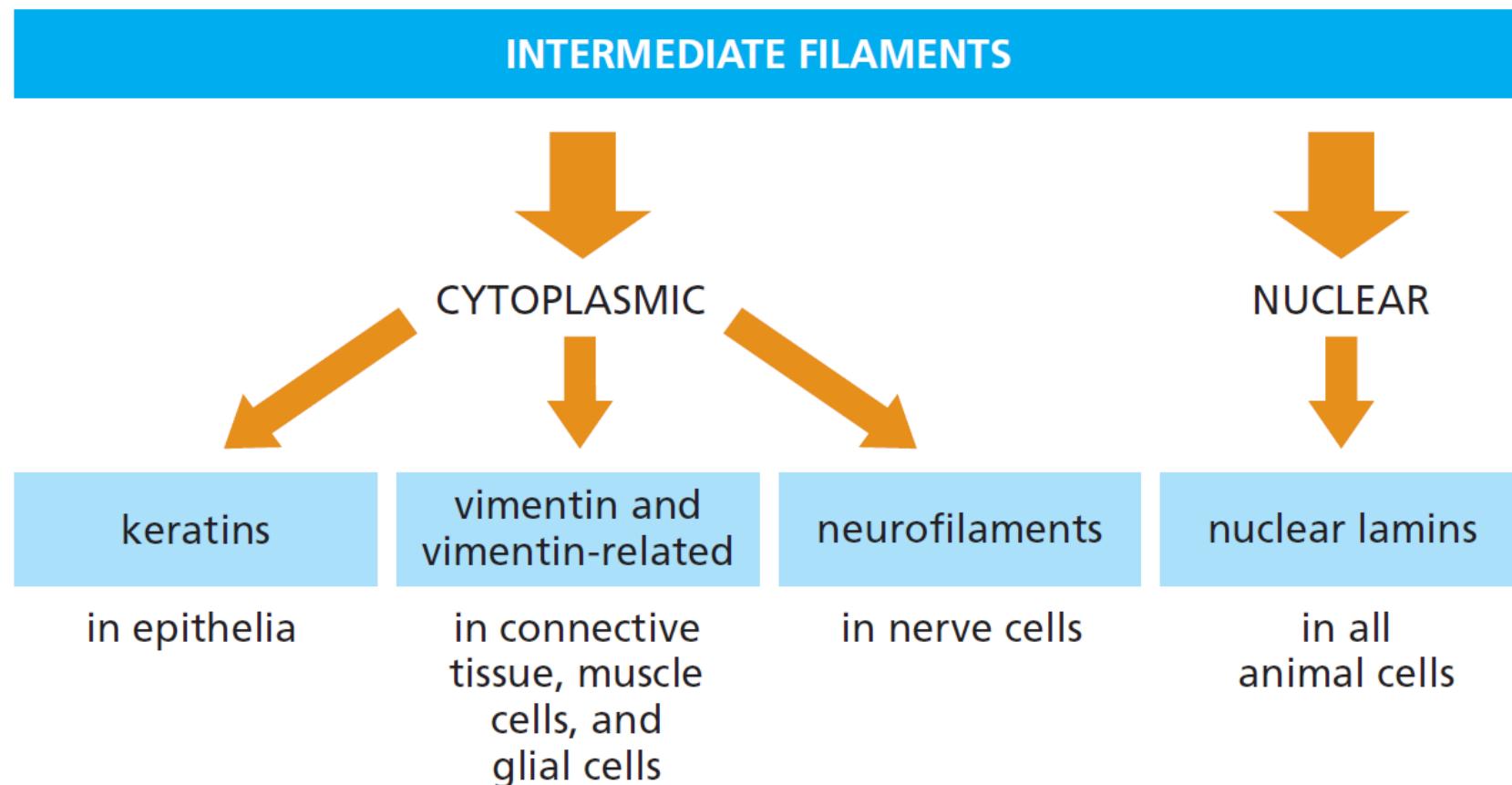


AH3HPH IB C T BS



INTERMEDIATE FILAMENTS CLASSES

- Keratin filaments (epithelium)
- Vimentin and vimentin-related (connective, muscle, glia tissue)
- Neurofilaments (neural cells)
- Nuclear lamins (all animal nuclei)



INTERMEDIATE FILAMENTS PROTEINS

➤ ~ 70 different genes encoding IF proteins

➤ 6 types:

➤ Type I and II: Acidic and Basic Keratins

- epithelial keratins: ~20

- trichocytic keratins (hair, nails, horns): ~10

- 50-90% sequence identity, 30% globular (~100 aa), 70%- helical (~300 aa)

- helical regions: 7-residues repeats

- globular domains: V1, V2 (H1, H2): Gly- and Ser-rich, insoluble

➤ Type III:

- desmin (sarcomers in muscles)

- glial fibrillary acidic protein (glia)

- peripherin (peripheral neurons)

- vimentin (fibroblasts, leukocytes, endothelial cells): organelles, nucleus signal



Epidermolysis bullosa

INTERMEDIATE FILAMENTS PROTEINS

➤ Type IV:

- **α -internexin (neuroblasts)**
- **neurofilaments (axons of vertebrate neurons)**
- **synemin (ECM muscles): binds desmin**
- **syncolin (ECM muscles): binds desmin**

➤ Type V:

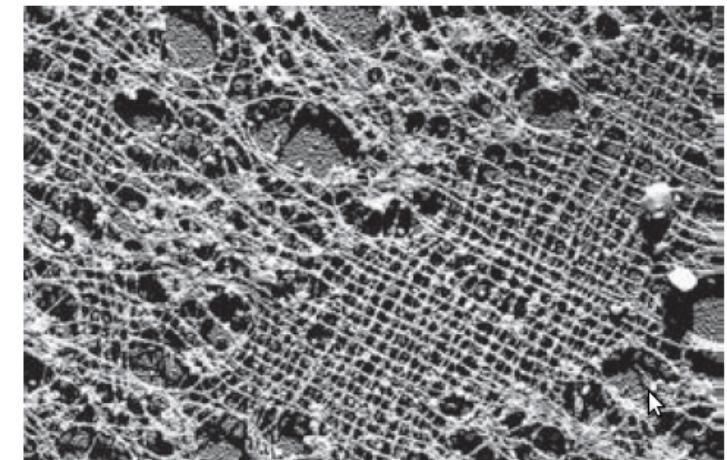
- **lamins (nuclei): A- everywhere, B- gastrulation; differ in pl, length**

➤ Type VI:

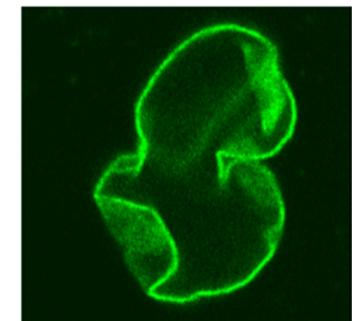
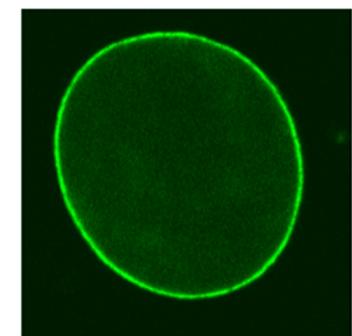
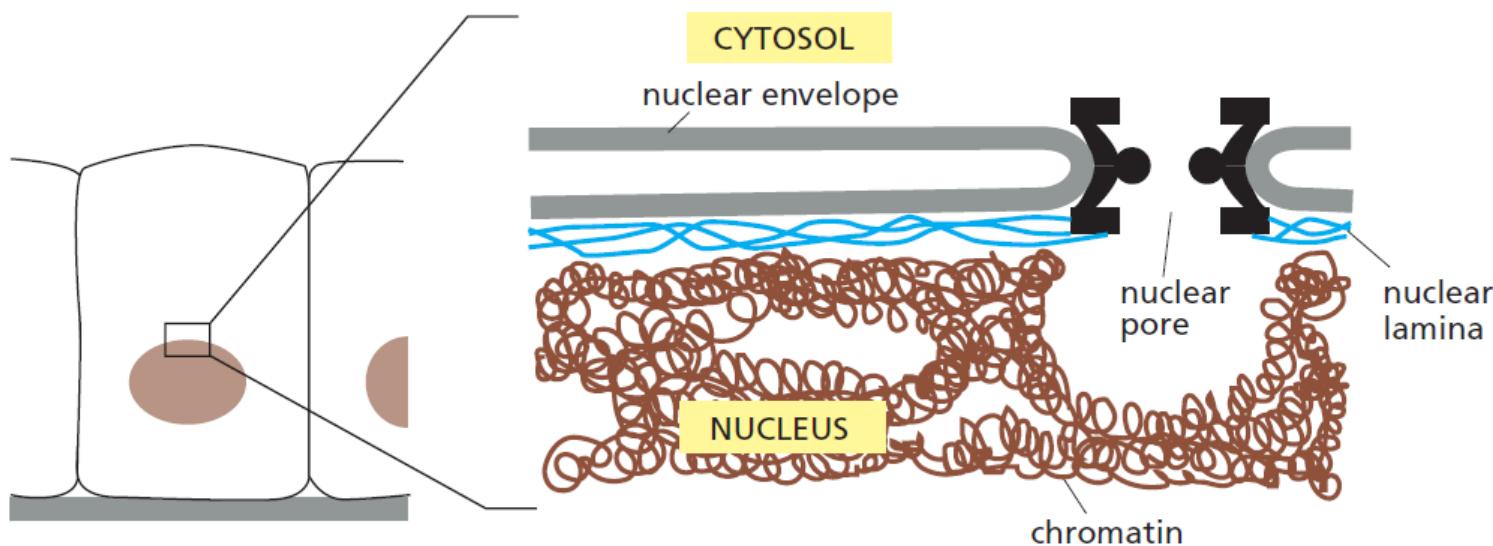
- **nestin (axons): marker for the neural stem cells**

LAMINS

- Reassemble/assemble after each cell-division
- Phosphorylation/dephosphorylation regulation
- Involved in cell division mechanism
- Disorder: progeria



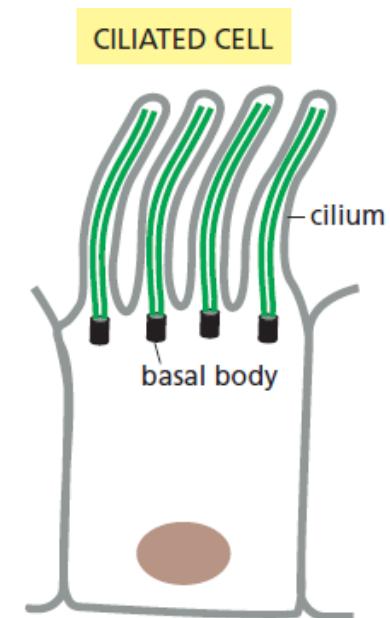
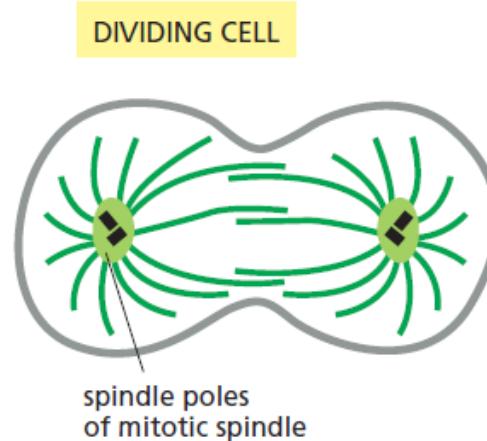
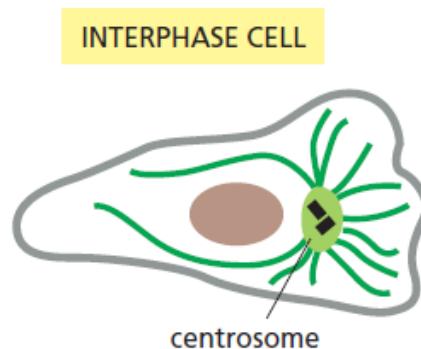
1 μm



Nucleus:
healthy vs. progeria

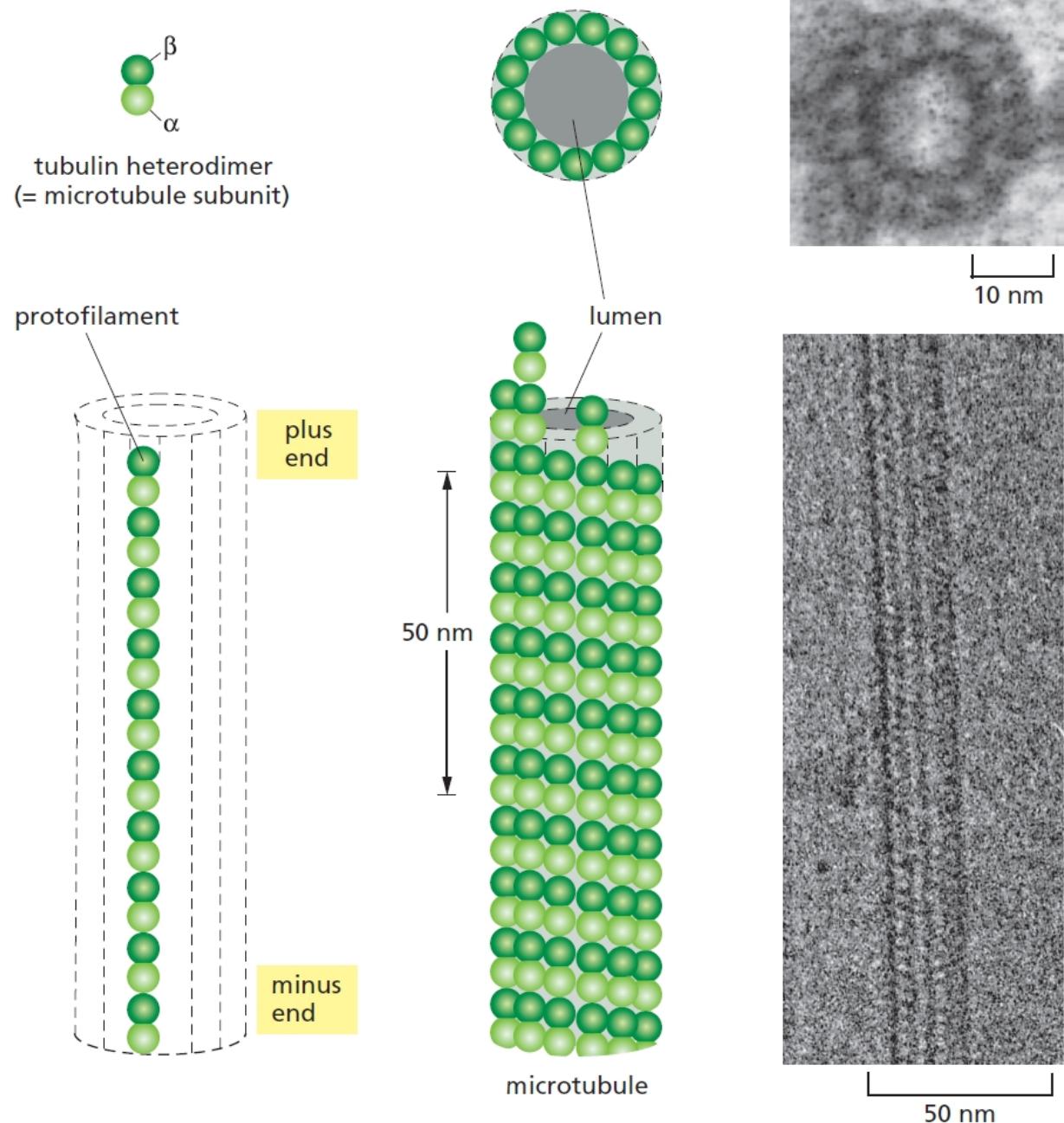
MICROTUBULES

- $d = 25 \text{ nm}$
- Long, hollow and stiff
- Assemble and disassemble
- Functions: cell migration, mitosis, cilia/flagella, signaling via transport



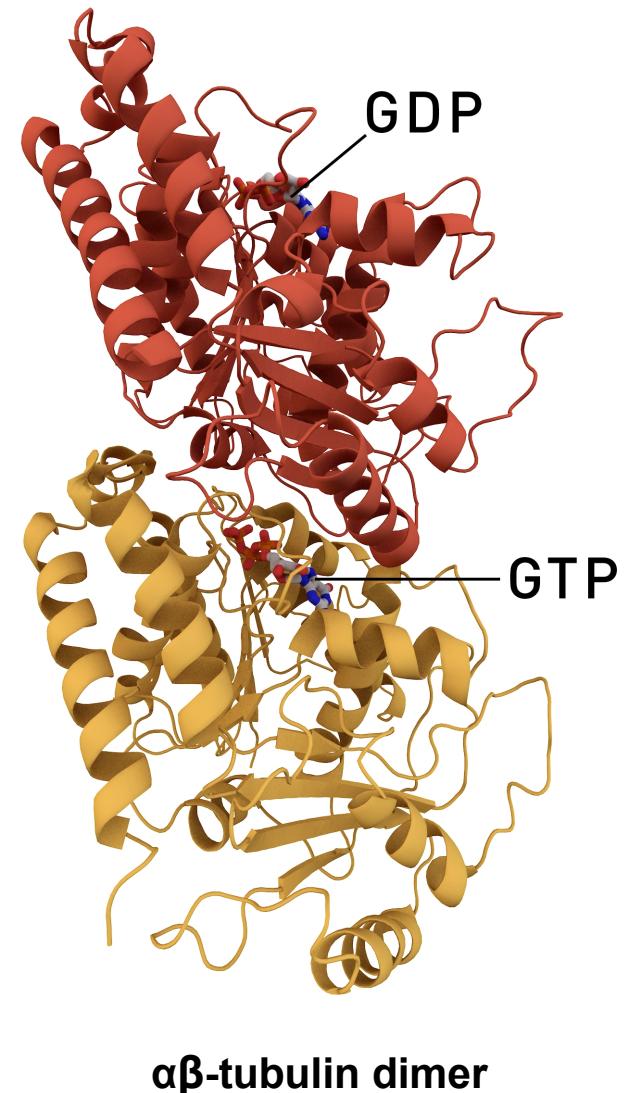
MICROTUBULES STRUCTURE

- **α - and β -tubulins are non-covalently bound => protofilaments**
- **13 protofilaments => tubelike structure**
- **Polarity (α - and β -tubulins at different ends: – and +)**
- **Different rate of addition new units**



TUBULINS

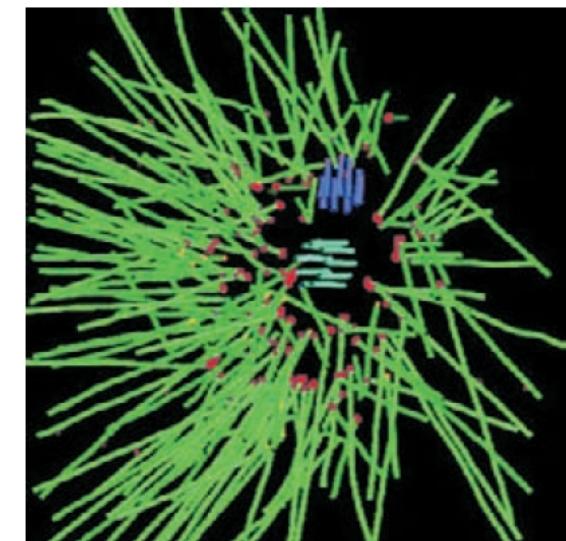
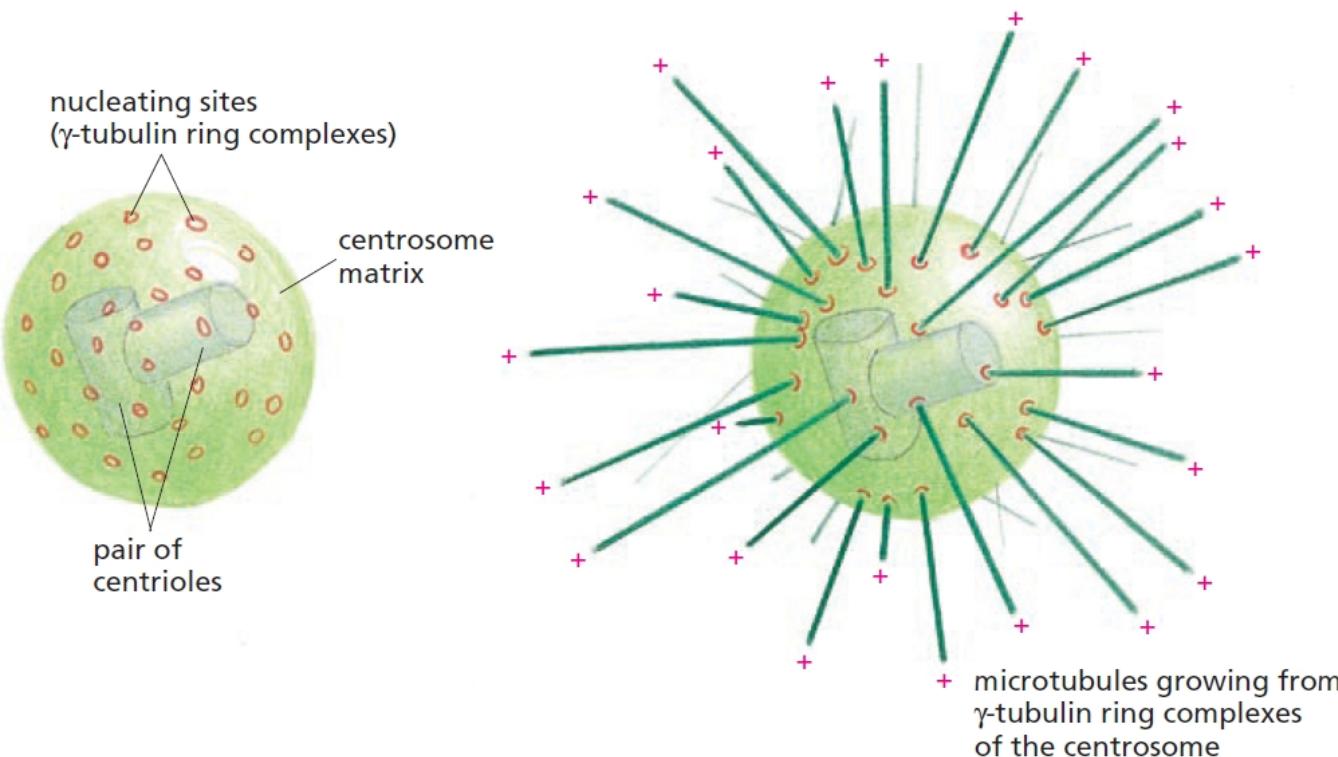
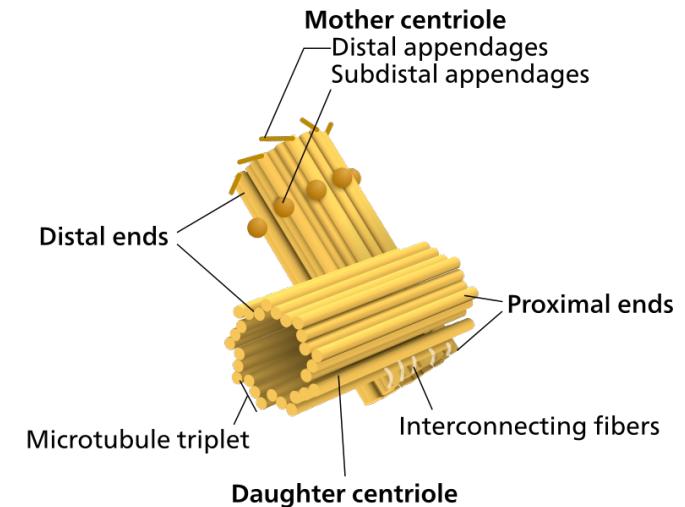
- Tubulin/FtsZ family, GTP-ase protein domain
- Always bound to GDP or GTP
- GTP hydrolysis => change of the conformation
- Types (eukaryotes):
 - α -tubulin, 8 subtypes
 - β -tubulin, 9 subtypes
 - γ -tubulin (centrosomes, spindle poles), 7 subtypes
 - δ -, ϵ -tubulin (centriole, mitotic spindle)
 - ζ -tubulin (in protozoa)
- Types (prokaryotes): BtubA/B, FtsZ, TubZ, CetZ



CENTROSOME

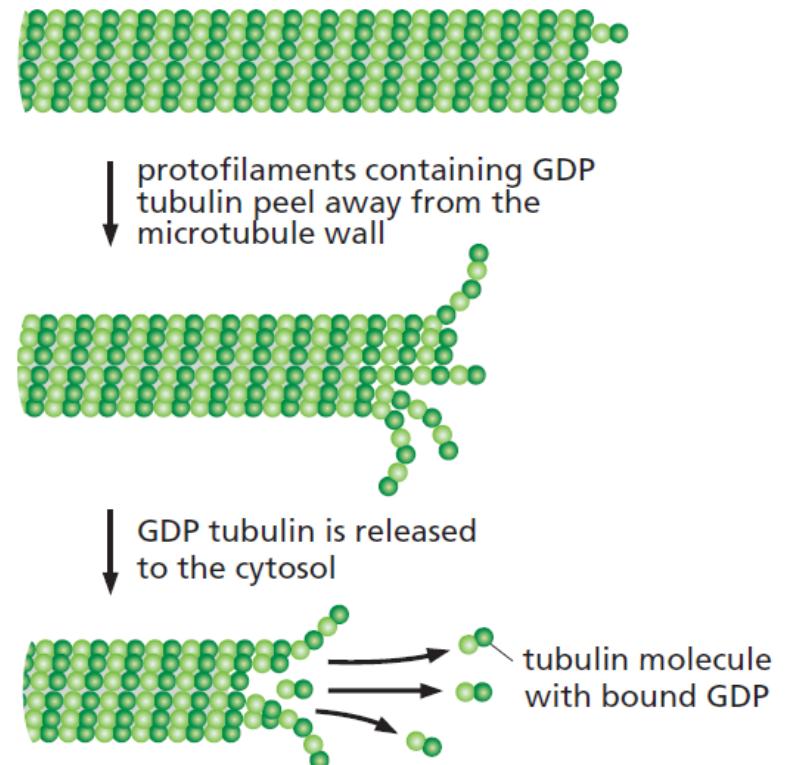
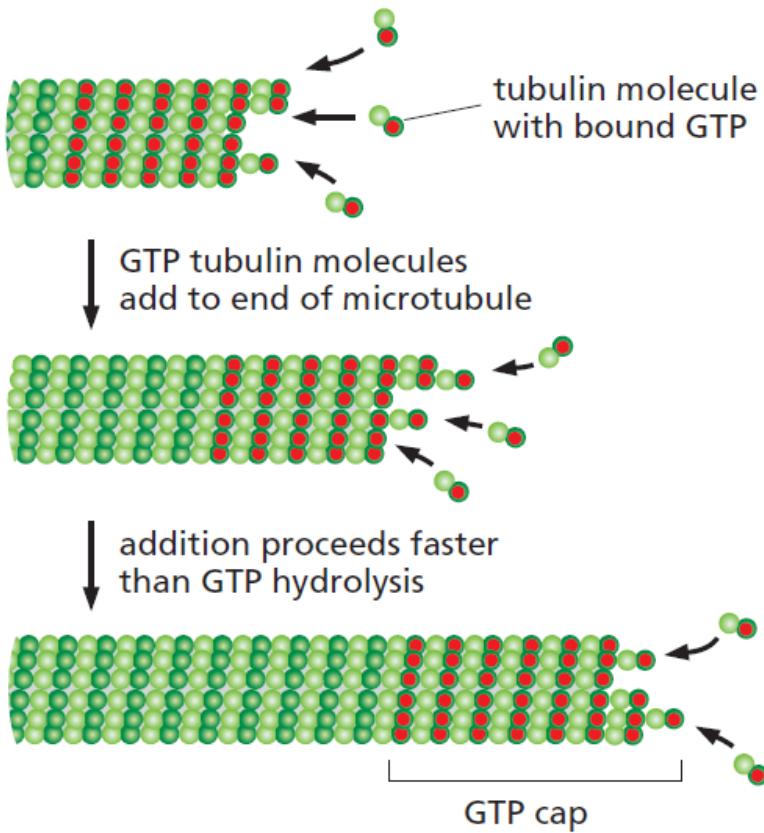
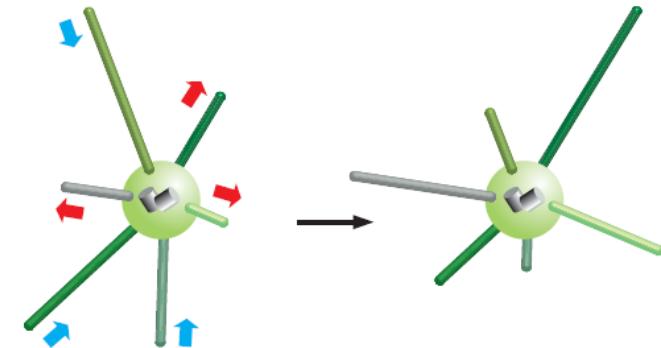
Centrosome: organizing and center for microtubules in cytoplasm

- γ -tubulin form rings (nucleating sites)
- $\alpha\beta$ -unit is attached to the ring
- Growth in “plus” direction
- Centrioles
- *In vitro* vs. *in vivo* assembly



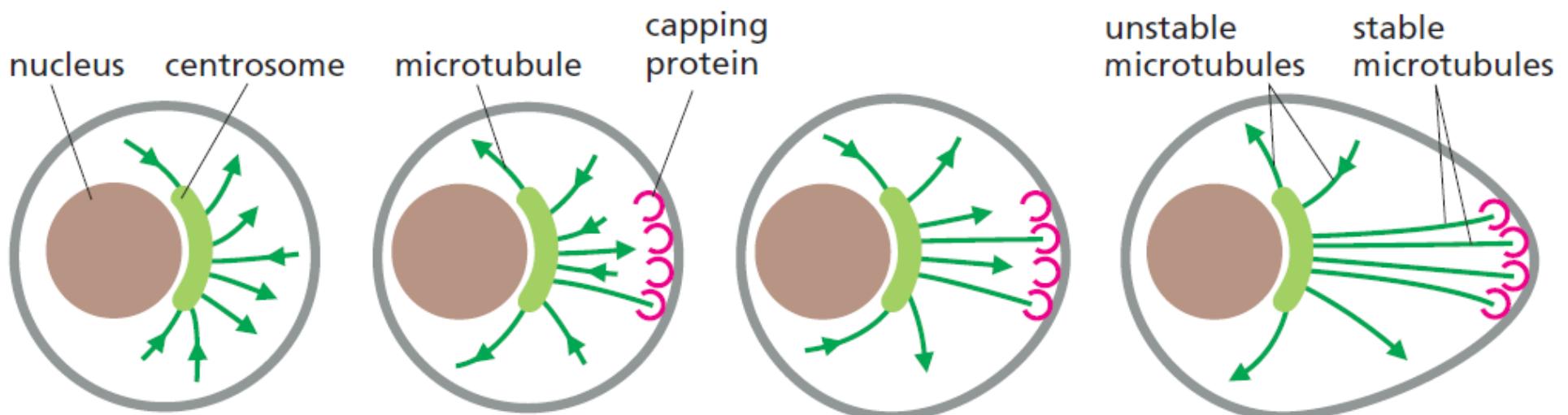
MICROTUBULES GROWTH

- Each one has an independent rate/behaviour
- Random growth/shrinking => dynamic instability
- GDP/GTP binding defines growth/shrinking



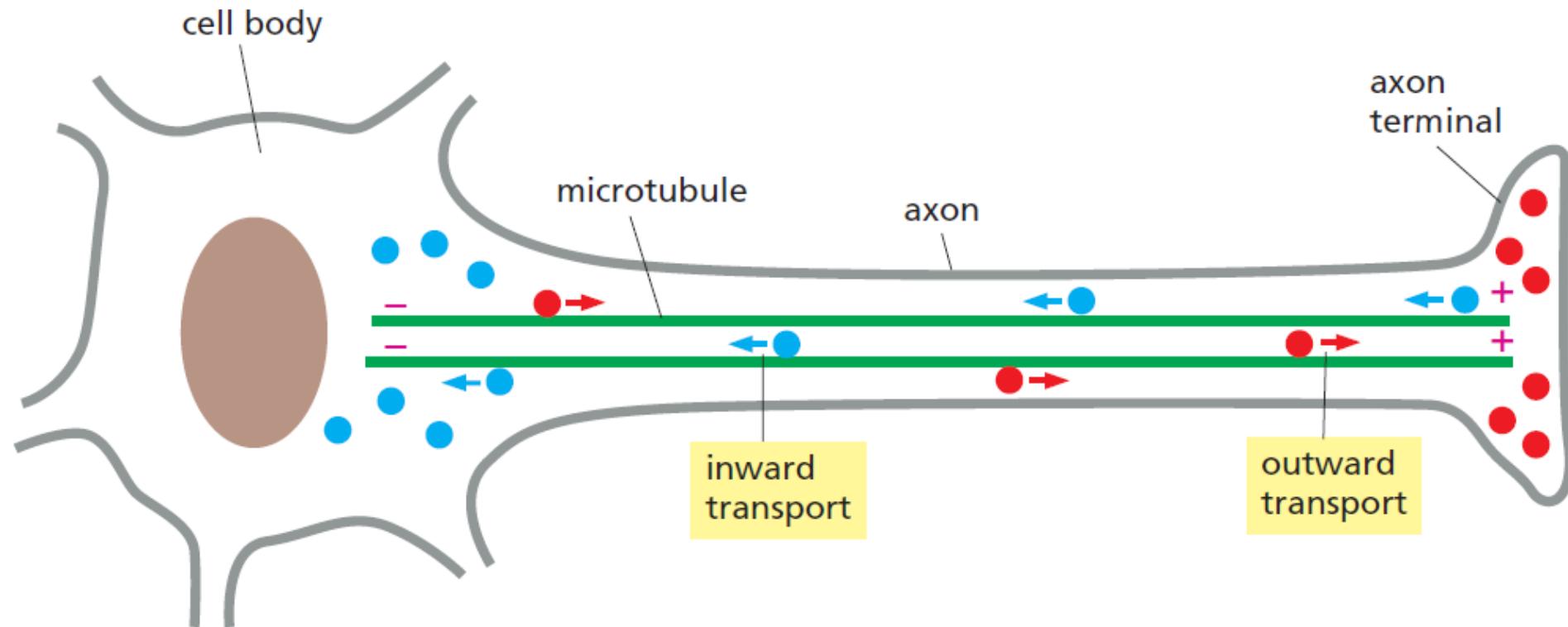
MICROTUBULES GROWTH

- Growth/shrinking can be stabilized
 - Colchicine: prevents polymerization
 - Taxol: stabilized microtubules
- } Severe effects in mitosis



MICROTUBULES IN CELL ORGANIZATION

- Growth/shrinking rates changes are utilized in mitosis
- Cells polarization involvement
- Transport: high effectivity in comparison to free diffusion
- Cargo: vesicles, organelles, macromolecular complexes



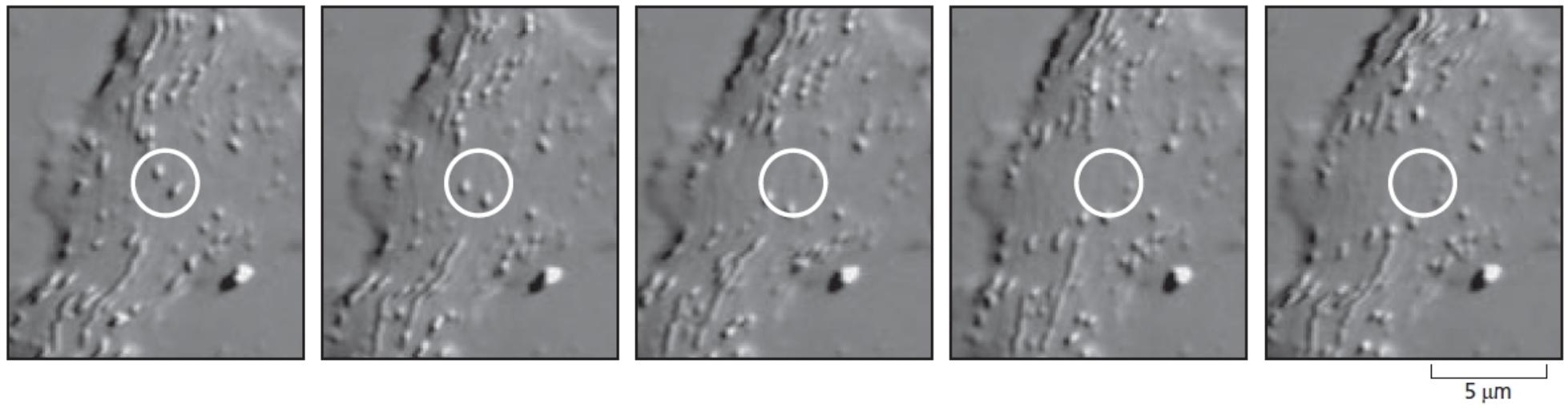
MICROTUBULES IN TRANSPORT

➤ Movements in the cytoplasm:

- diffusion
- directed, many of them are saltatory

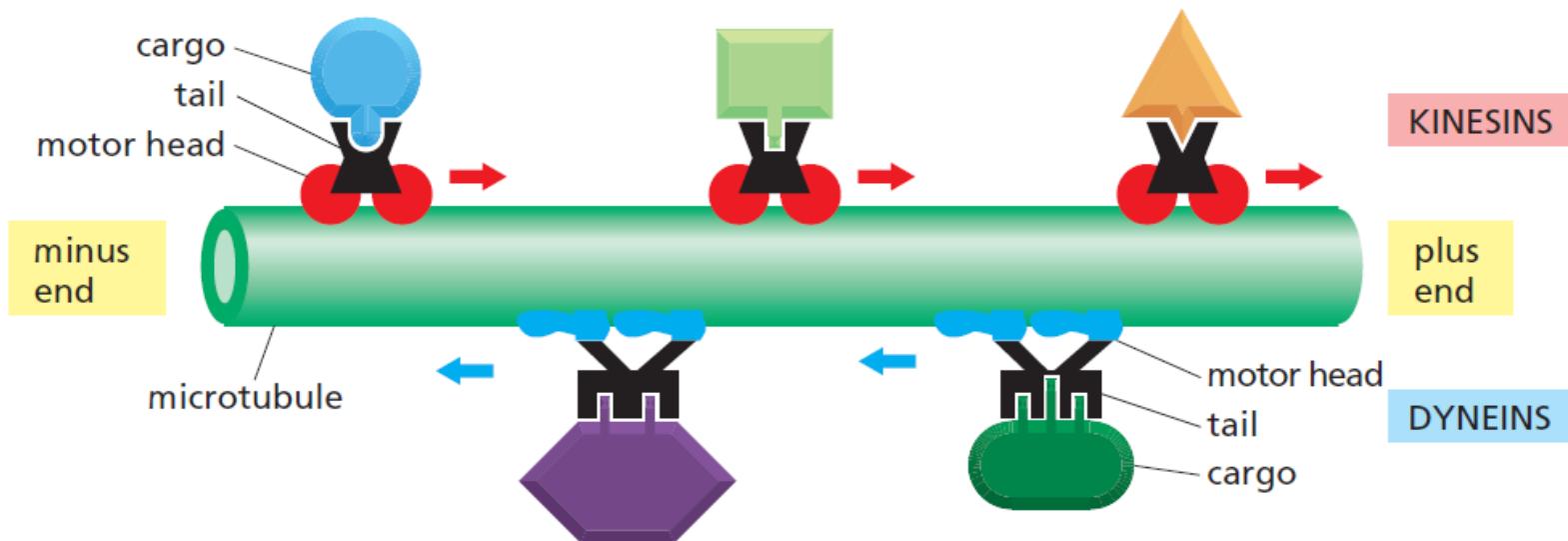
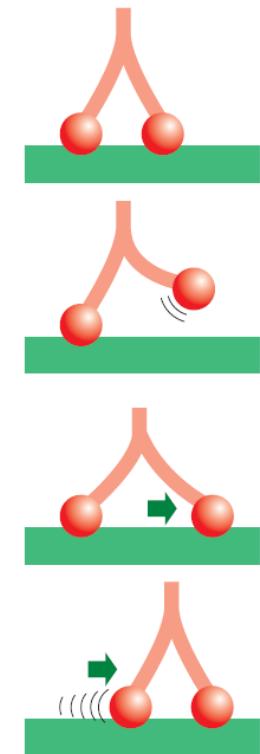
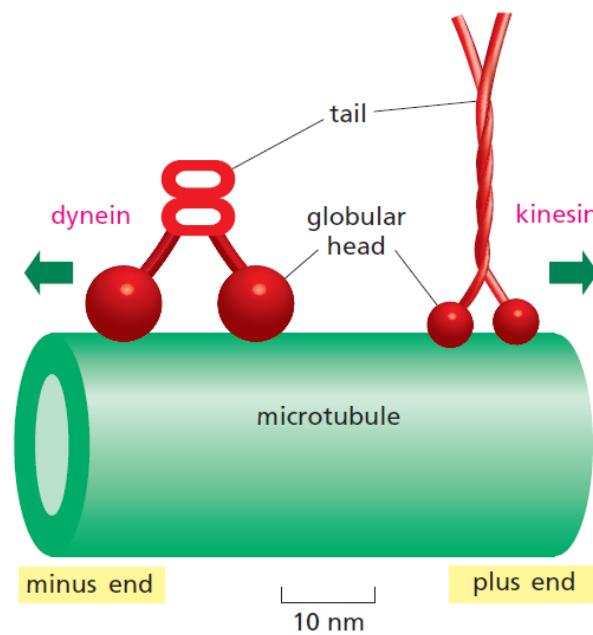
➤ Motor proteins: ATP energy is used for the movements:

- kinesin “-” => “+”
- dynein “+” => “-”



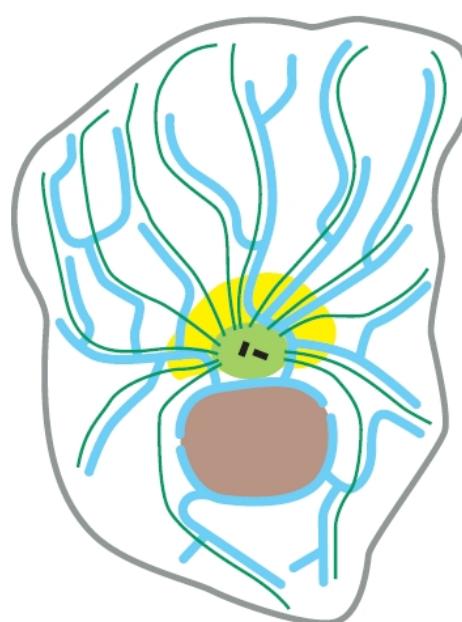
$t = 400\text{ms}$

MOTOR PROTEINS: PRINCIPLE

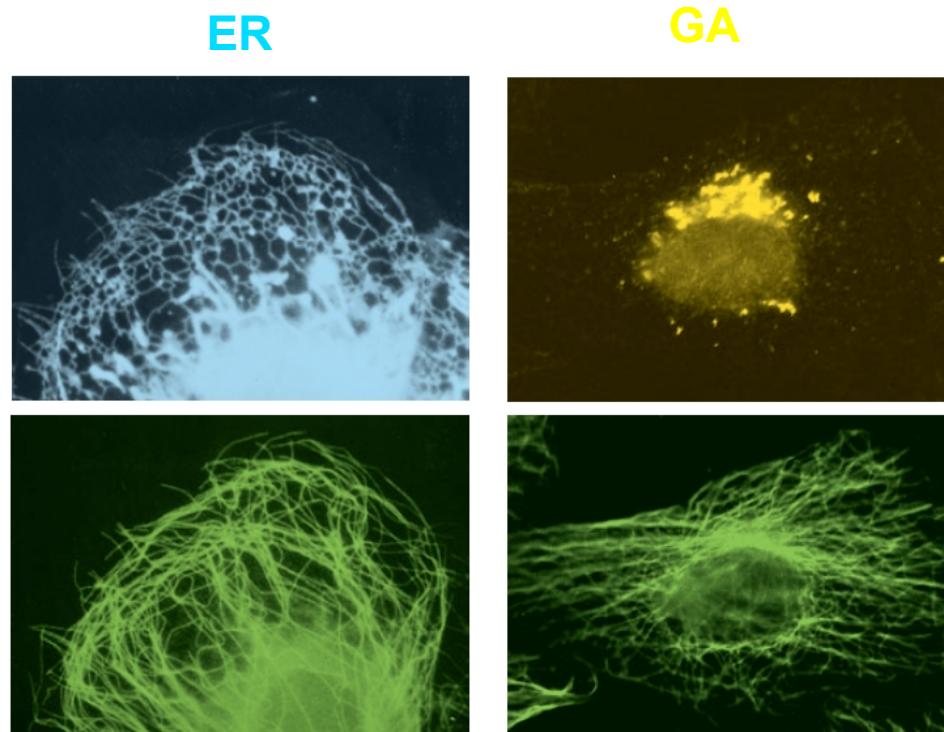


MICROTUBULES ARRANGE ORGANELLES IN CELL

- ER growth in the direction of microtubules
- GA is located near the centrosome
- Kinesins are attached to ER: pulling outward
- Dyneins are attached to GA: shaping near centrosome



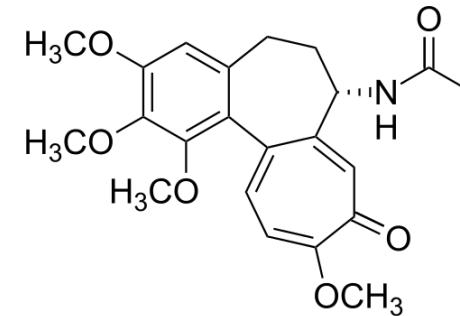
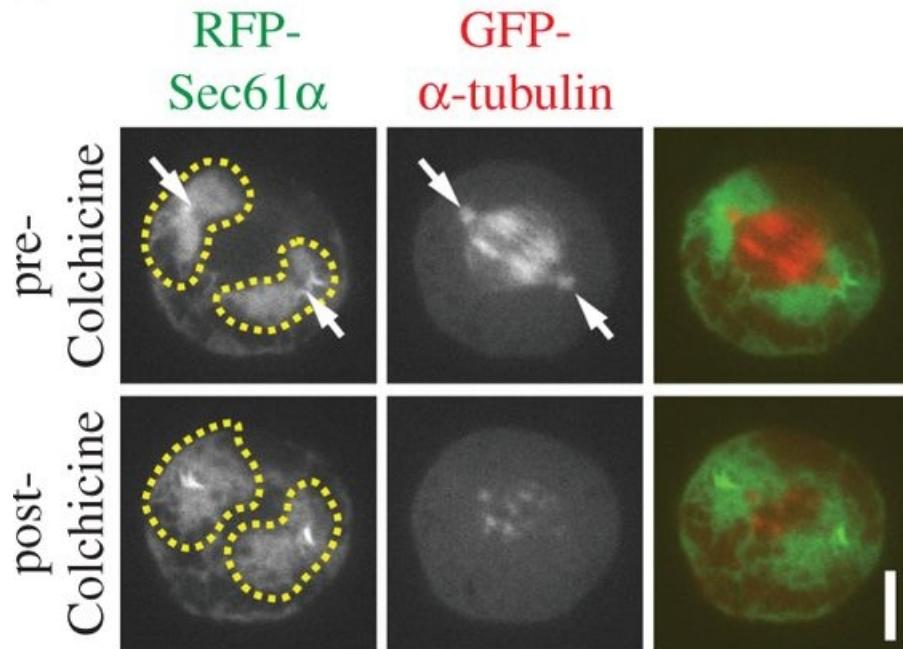
Microtubules
ER



Microtubules

DISRUPTION OF MICROTUBULES

Colchicine affects the geometry of ER

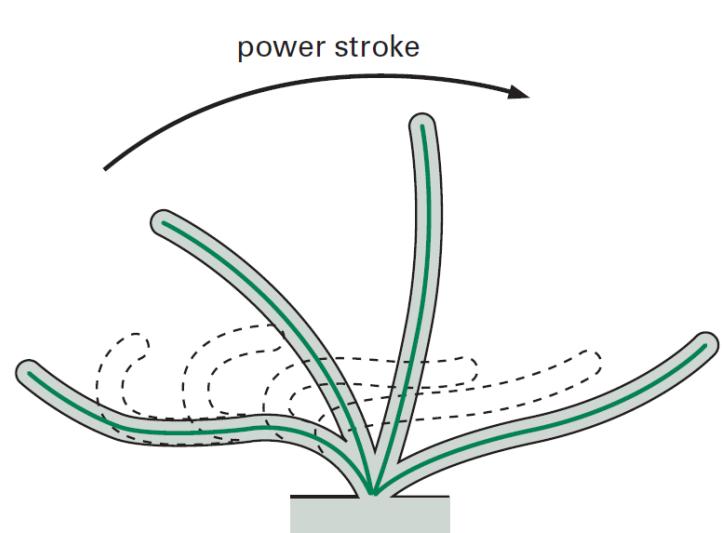
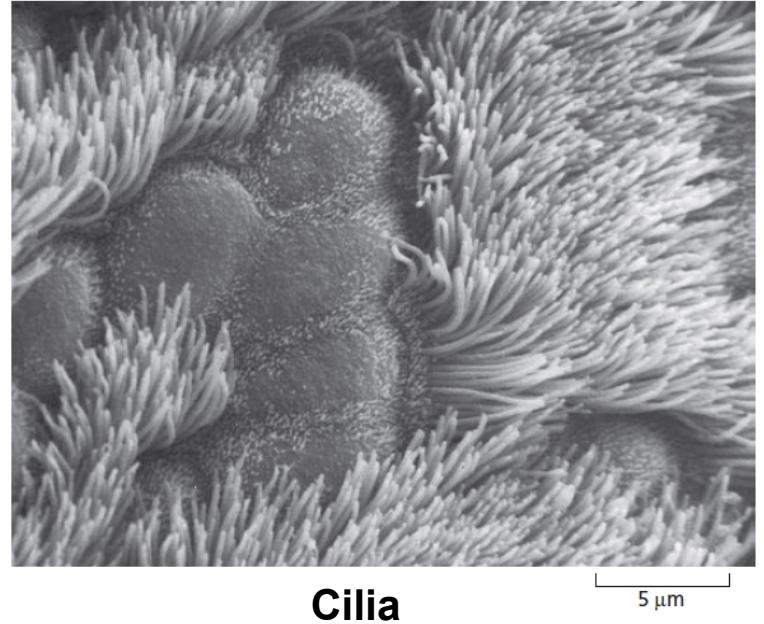


Colchicine

- Experiment:
 - biomarker
 - fluorescence

CILIA

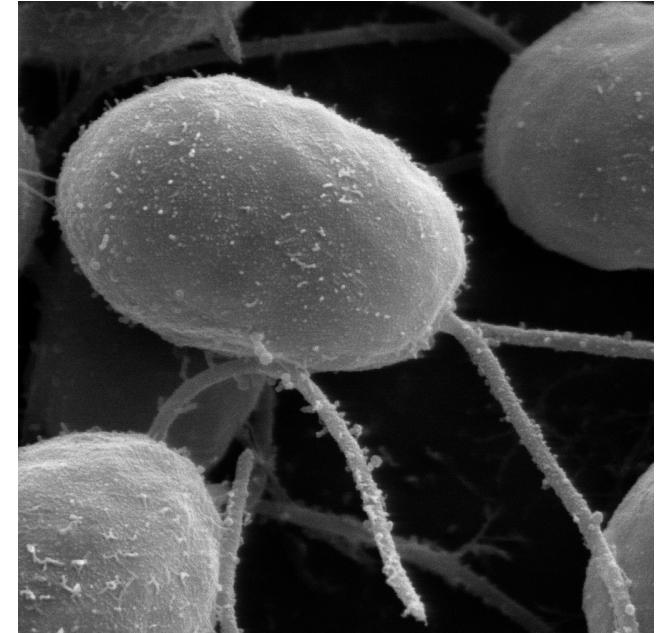
- 250 nm long hair-like organelle
- Organized in bundle of microtubules
- Grows from basal body
- Function:
 - moving and sweeping food
 - sensoric
- 9 double filaments + 2 from the ring
- Ciliary dynein
- Kartagener's syndrome:
 - men's infertility
 - decreased bronchial defence



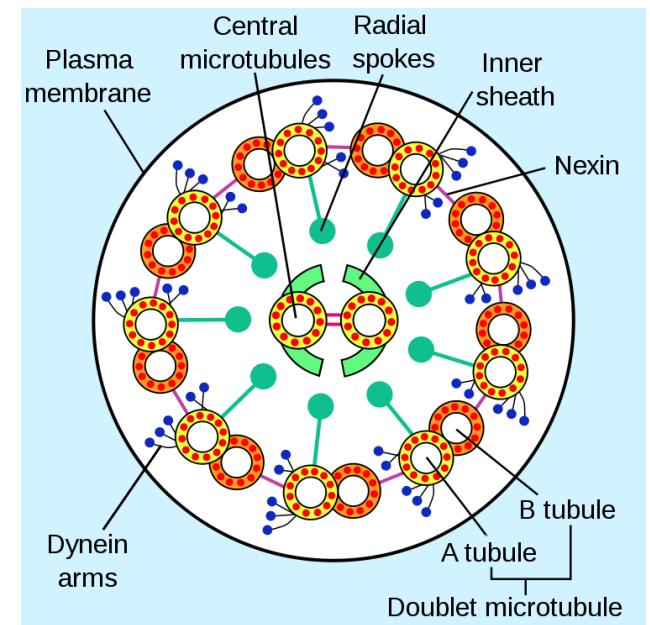
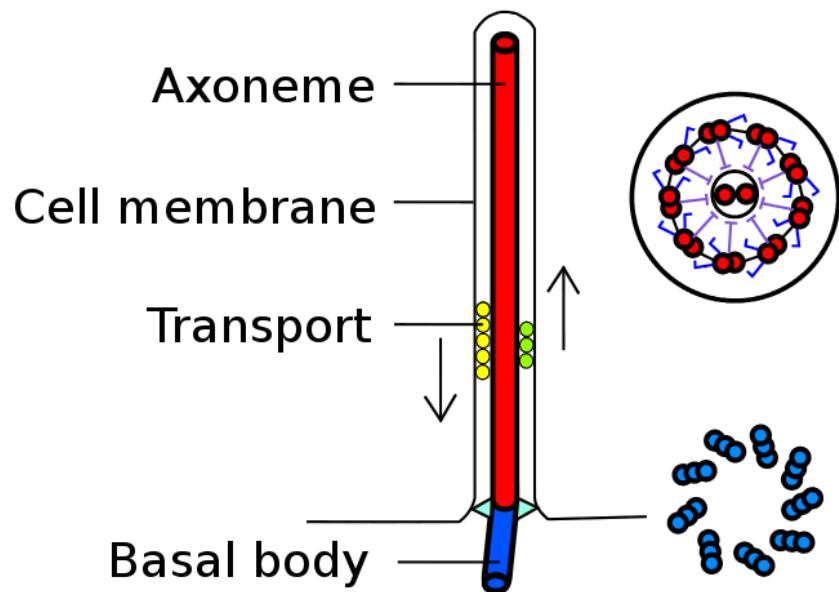
$t \sim 0.1 \text{ s}$

FLAGELLA

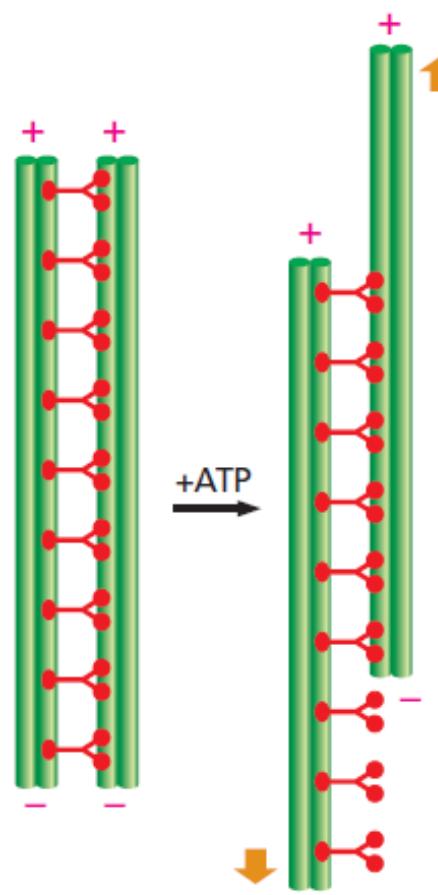
- ~ 2000 nm long
- Function: moving and sweeping food
- 9 double filaments + 2 from the ring
- Eukaryotic (ATP) and prokaryotic (H^+)
- Transport (intraflagellar): TM proteins
- Linking proteins



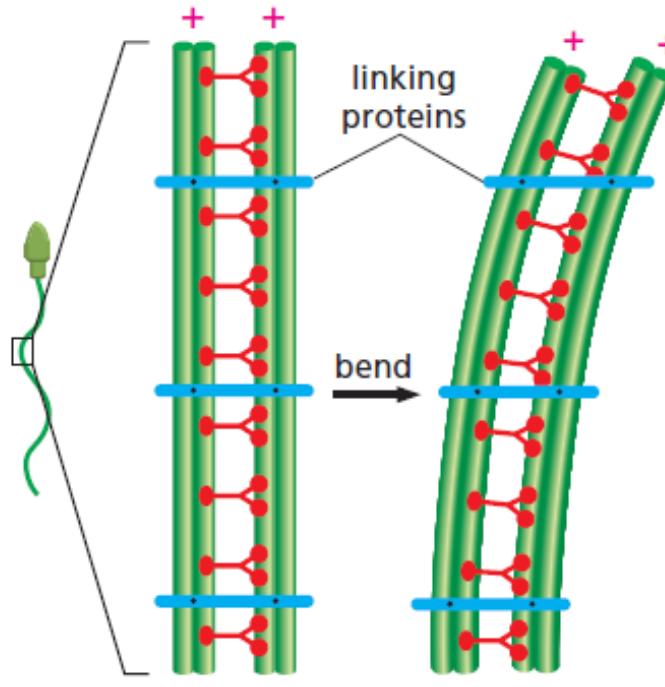
Flagella



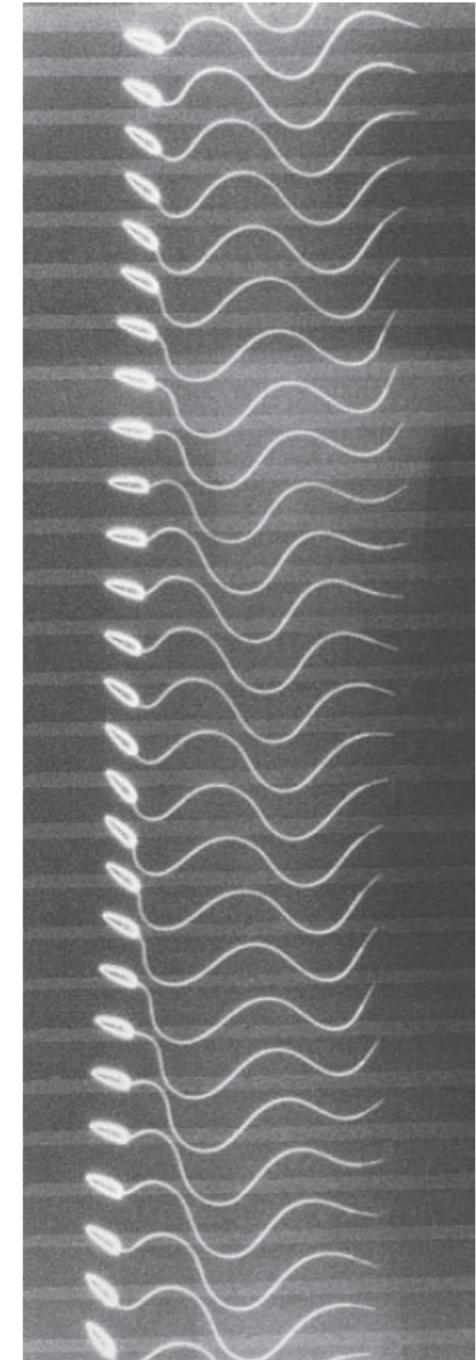
FLAGELLA



IN ISOLATED DOUBLET
MICROTUBULES: DYNEIN
PRODUCES
MICROTUBULE SLIDING

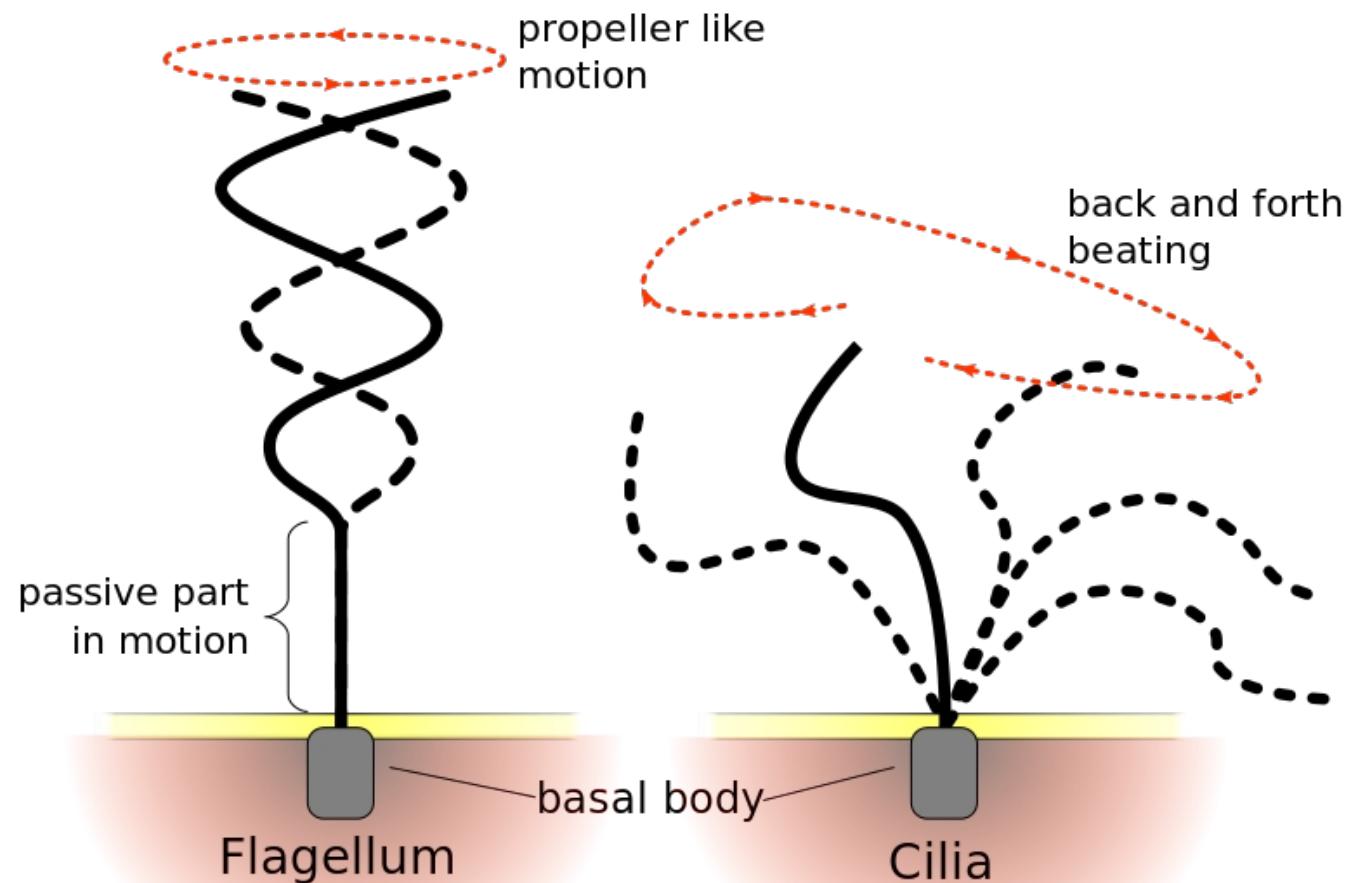


IN NORMAL
FLAGELLUM: DYNEIN
CAUSES MICROTUBULE
BENDING



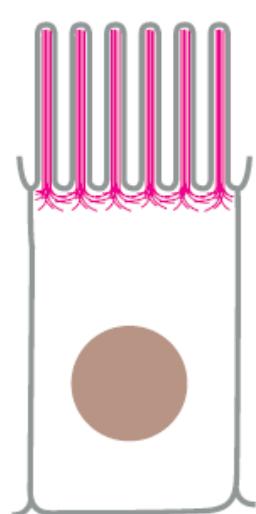
$t \sim 1\text{ s}$

CILIA vs. FLAGELLA

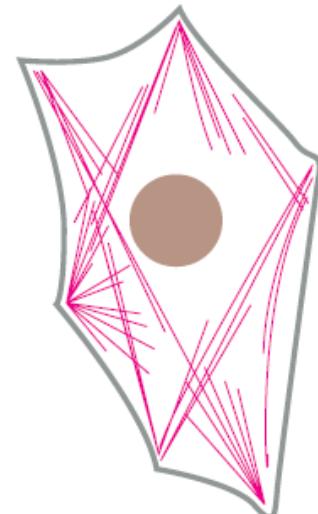


ACTIN FILAMENTS

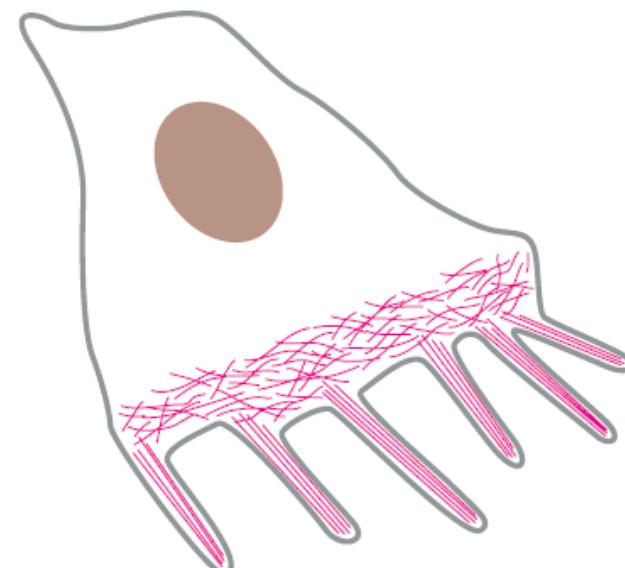
- = Microfilaments
- D = 5-9 nm
- Thin and very flexible
- Composition: actin + actin-binding proteins
- Functions: movements on the surface (crawling, phagocytosis, division), support of the shape
- Structures: microvilli, contractive bundles, contractive ring
- Polarity



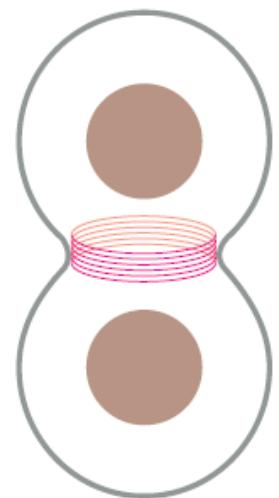
microvilli



contractive bundles



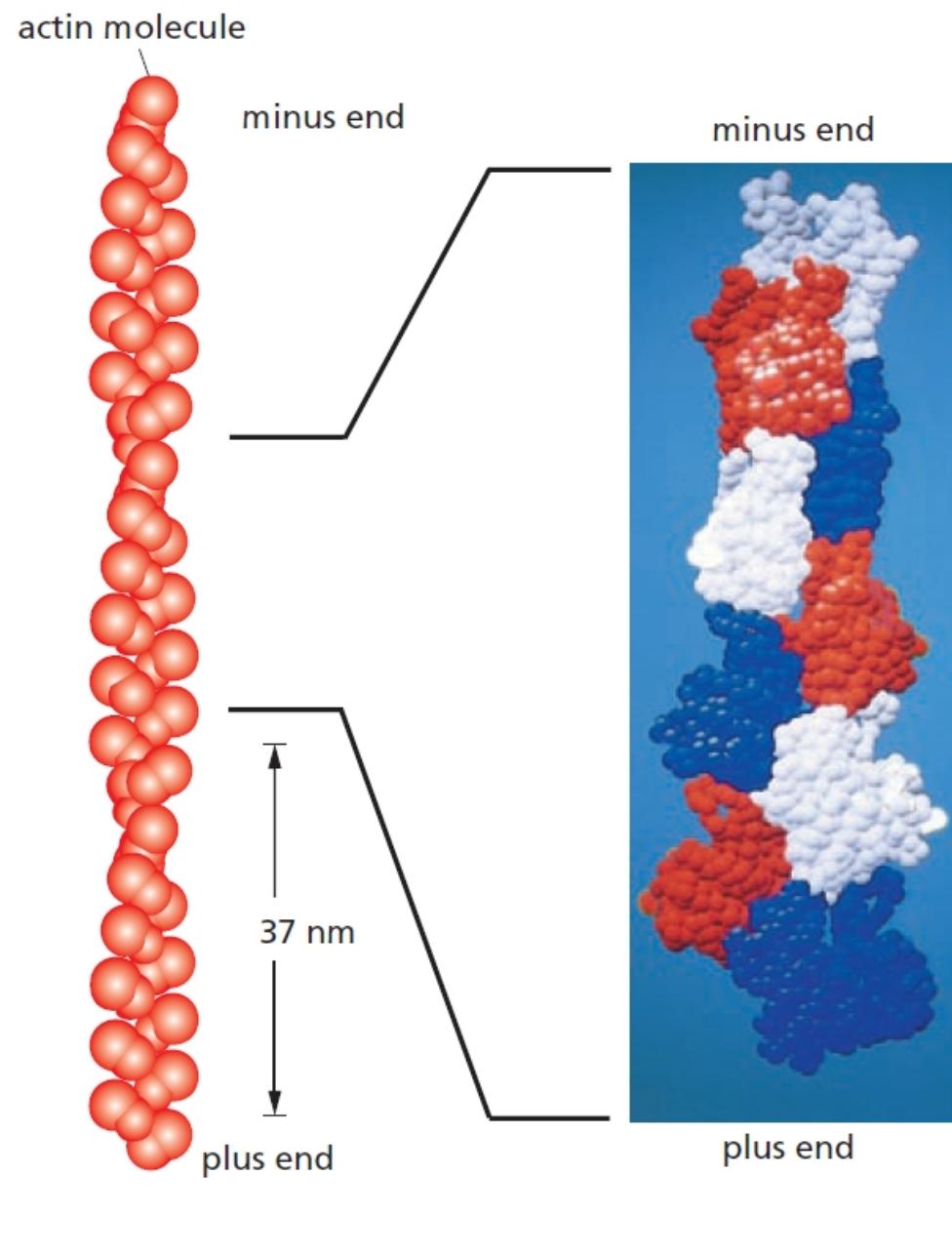
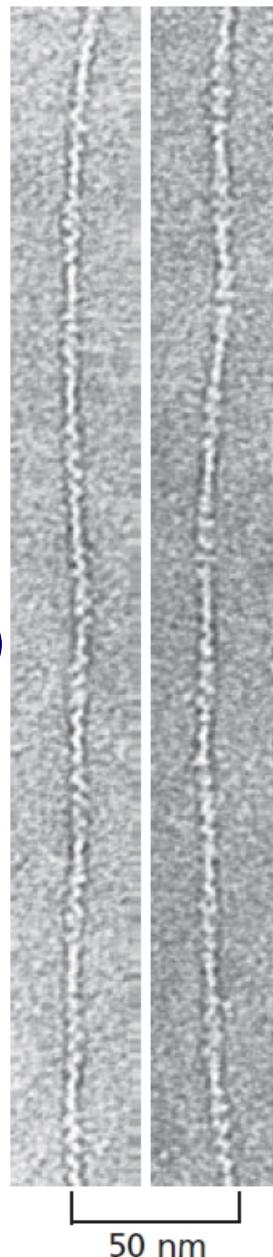
lamellipodia, filopodia



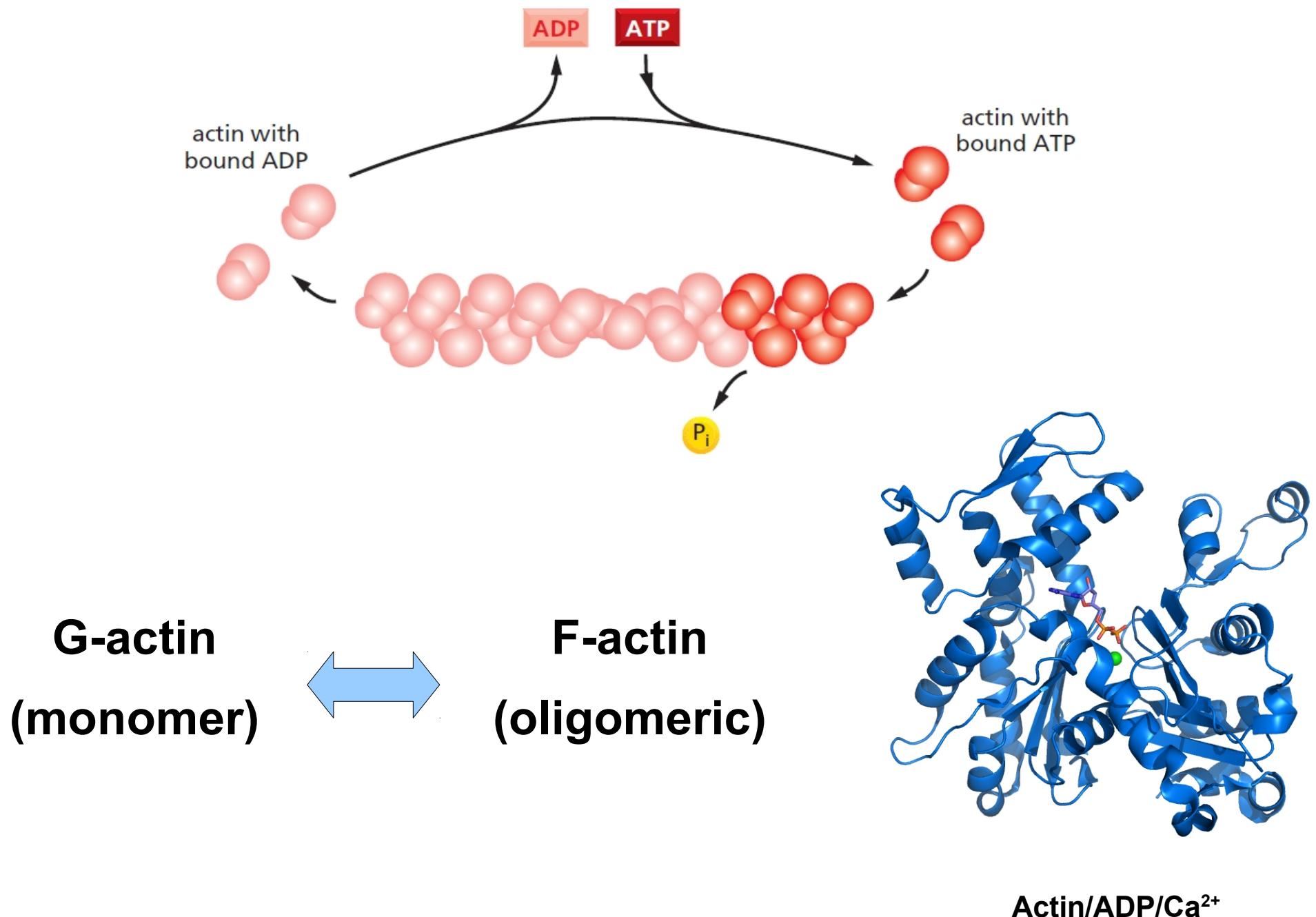
contractive rings

ACTIN FILAMENTS STRUCTURE

- Repeat: 37 nm, 2.7 SU/turn
- Each actin =>
4 interacting partners
- ADP/ATP binding
- Unstable alone
- Toxins:
 - cytochalasin (degradation)
 - phalloidin (assembly)

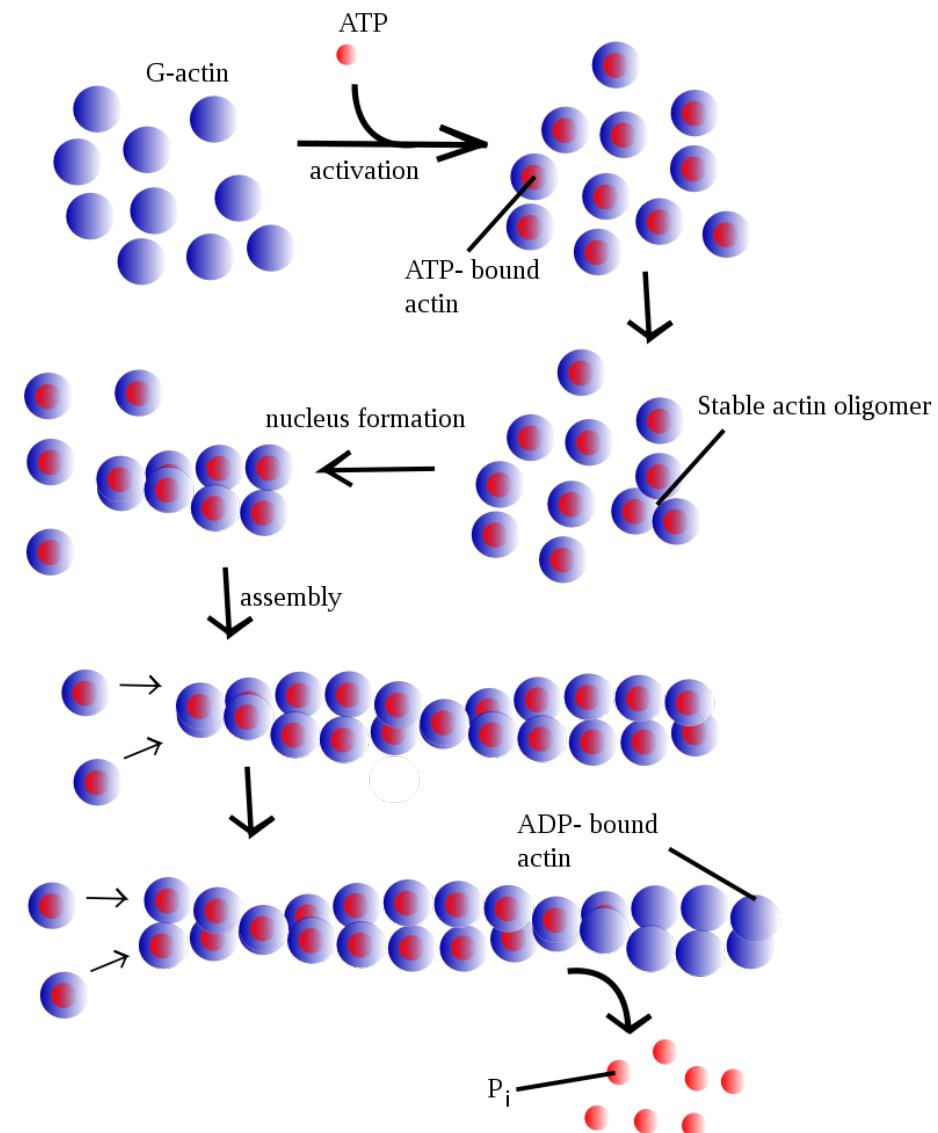


ACTIN FILAMENTS GROWTH: SCHEME



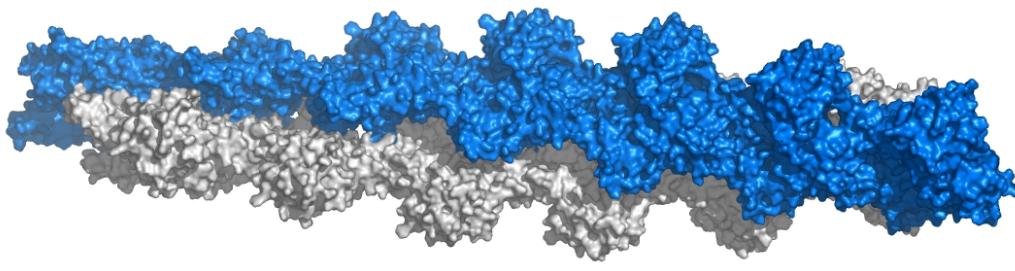
ACTIN FILAMENTS GROWTH: NUCLEATION

- Nucleation factor: Arp2/3
- Exchange ADP/ATP: thymosin, profilin
- Steps:
 - activation phase (divalent ions)
 - **G*** conformational changes
 - unstable dimers/trimers
 - “stationary equilibrium”
- Critical concentration (0.1 μM)
- ATP hydrolysis:
 - stochastic/vectorial
 - ADP/ATP bound concentration defines the way of growth/disassembly

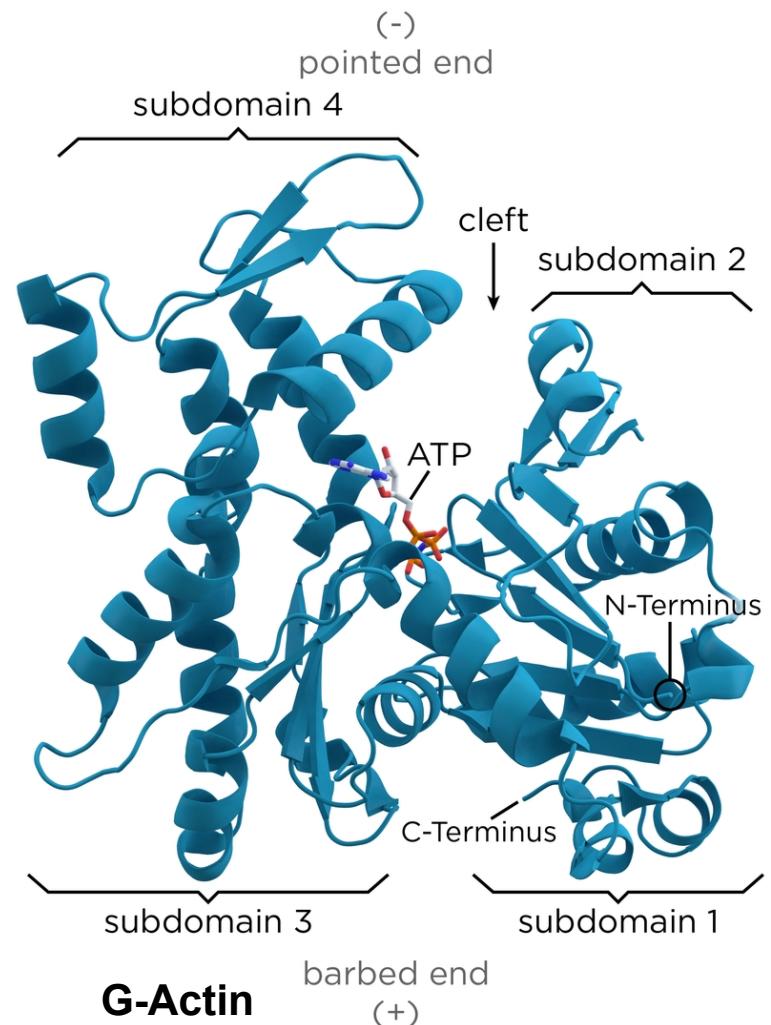


ACTIN

- ATP-ase fold
- 5% of total protein is actin, half is in actin filaments
- Discovered as an activator for myosin; ATP addition => increased viscosity
- One of the most conserved in evolution
- Other centers than catalytic for divalent ions



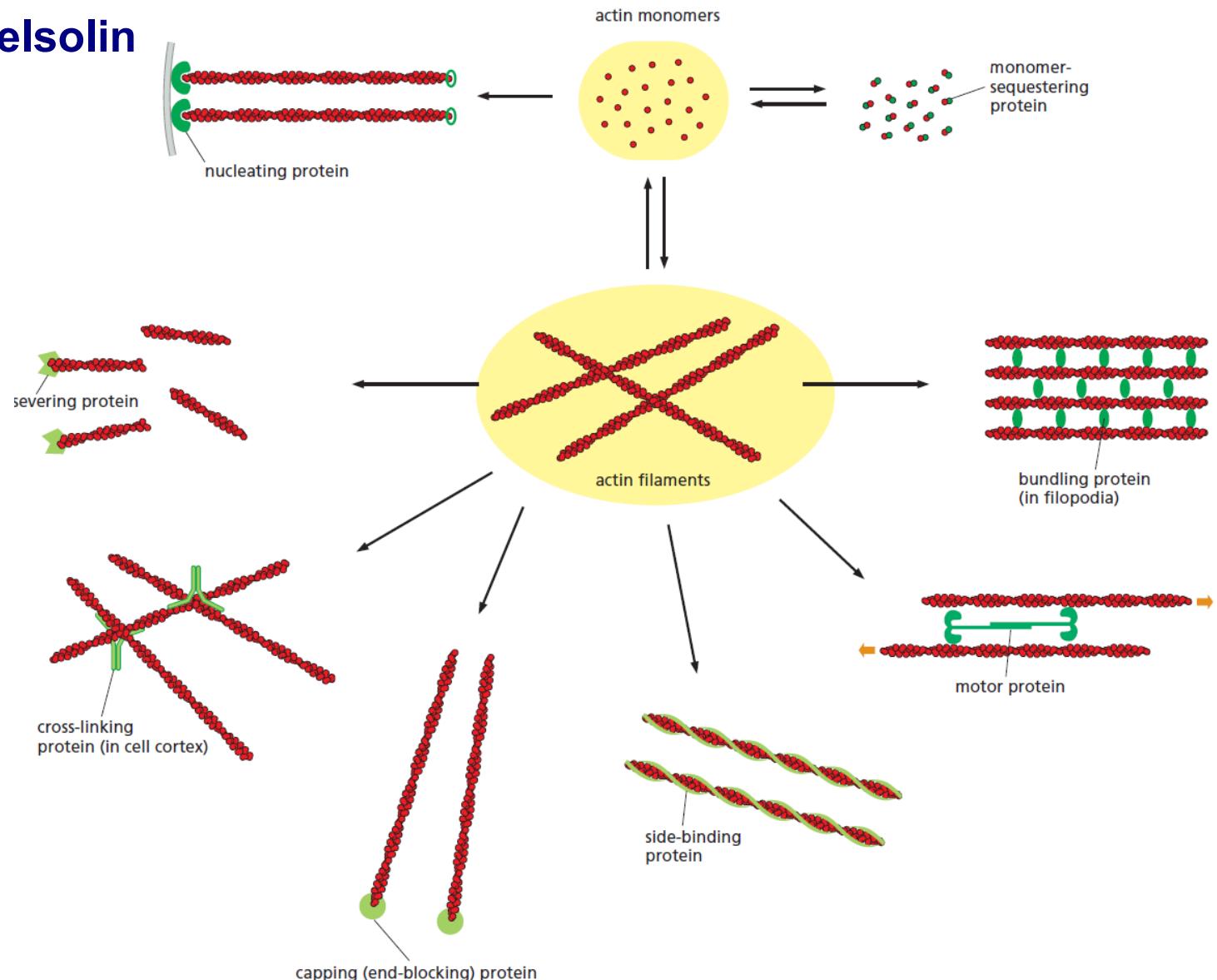
F-Actin



G-Actin

ACTIN-BINDING PROTEINS

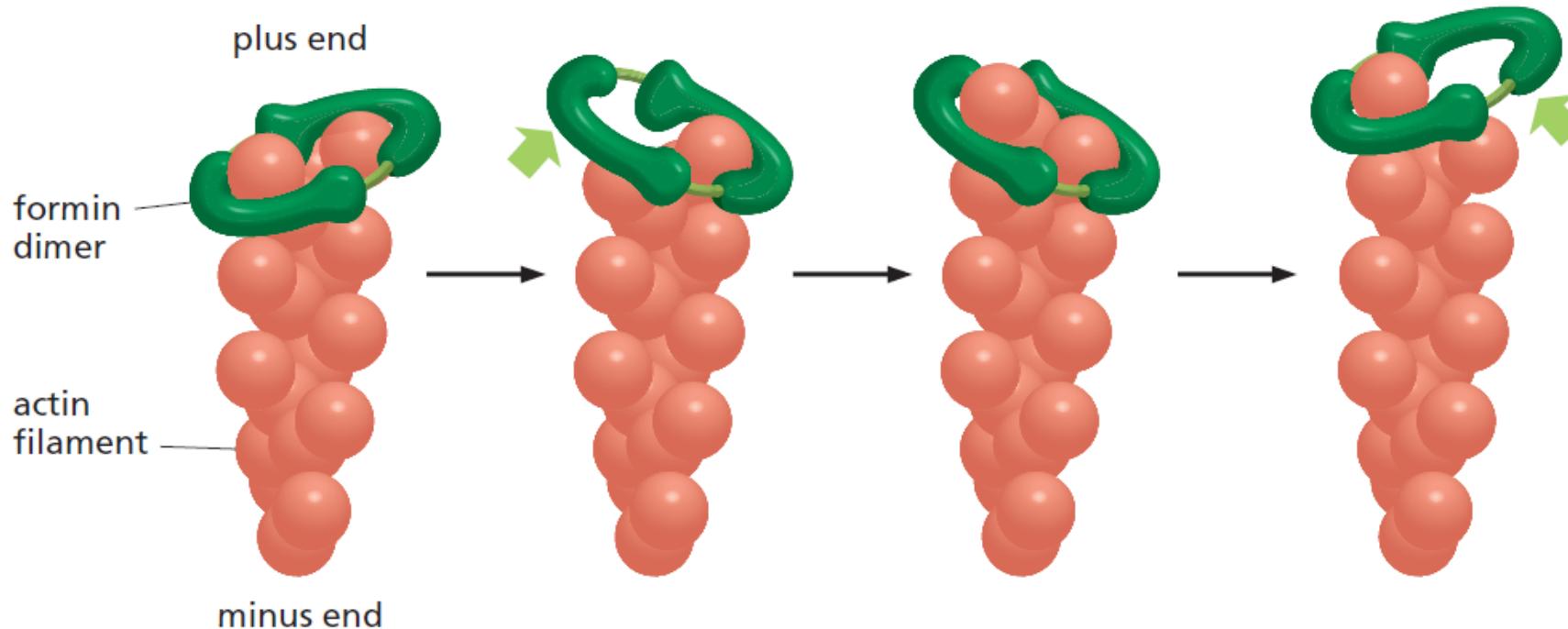
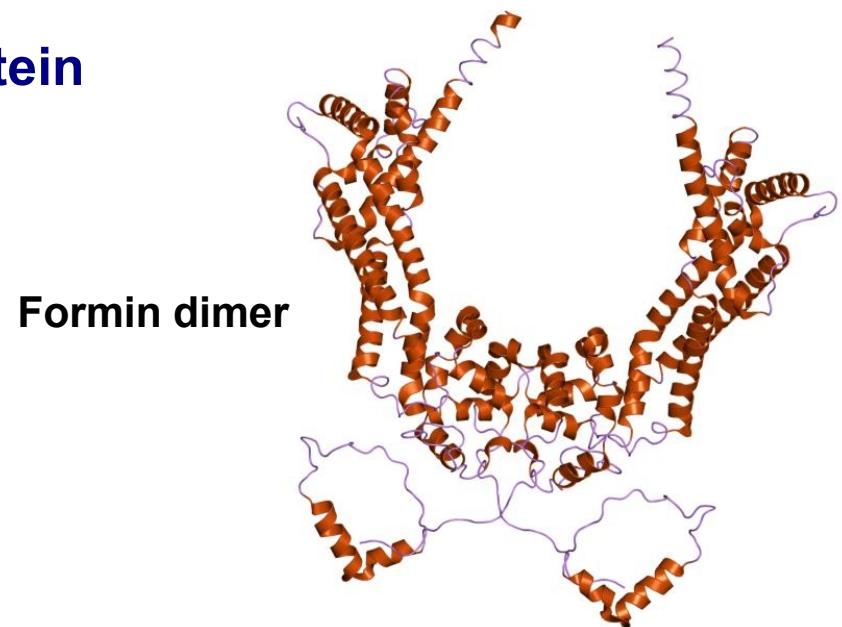
- End-capping proteins: thymosin, profilin
- Control assembly: formins, actin-related proteins
- Filament-severing: gelsolin
- Motor proteins
- Bundling proteins
- Cross-linking



FORMIN'S MECHANISM

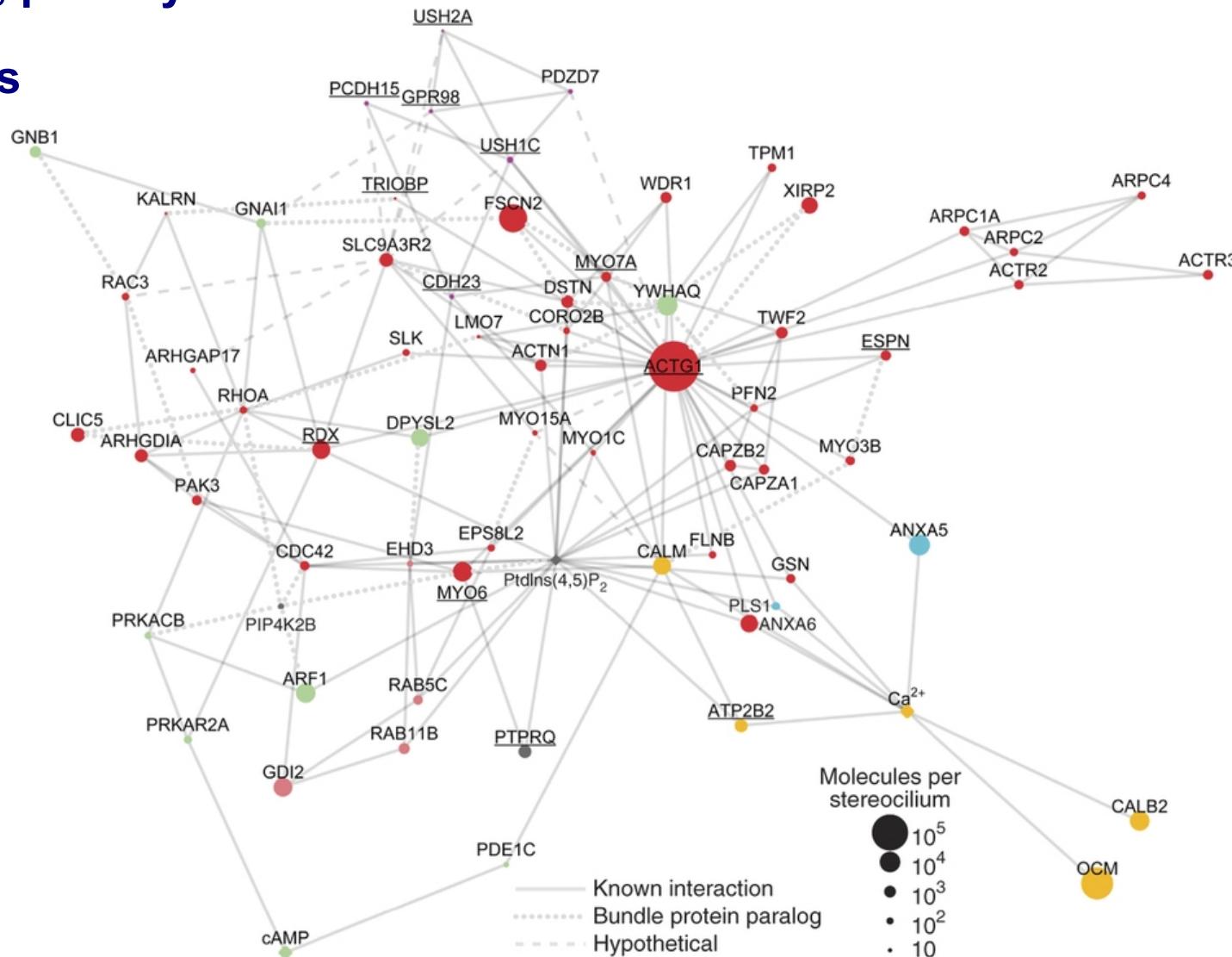
Rho-GTPase effector protein

- Holds a dimeric unit at the plus-end
- Assists binding of the next monomer



ACTIN-BINDING PROTEINS

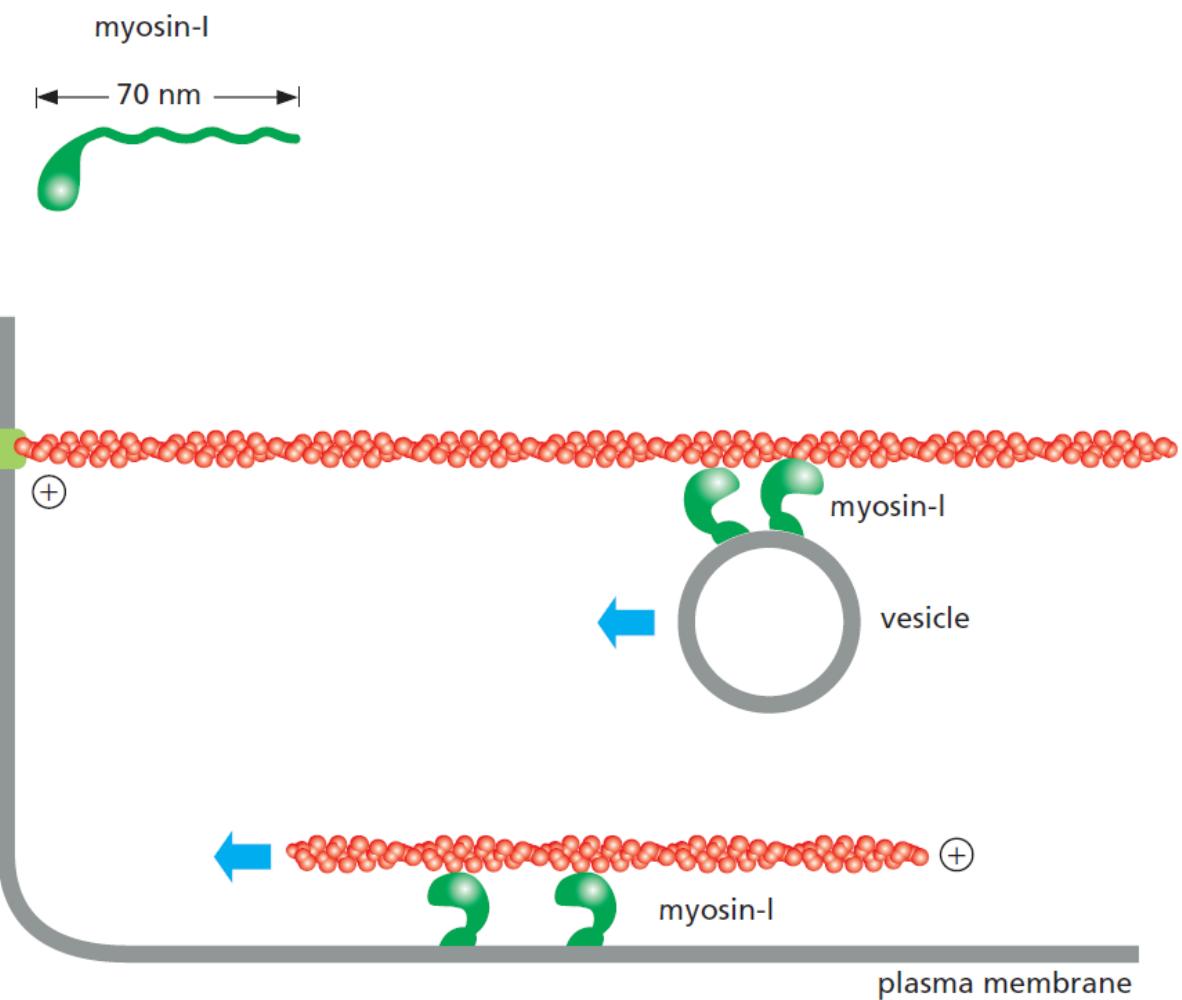
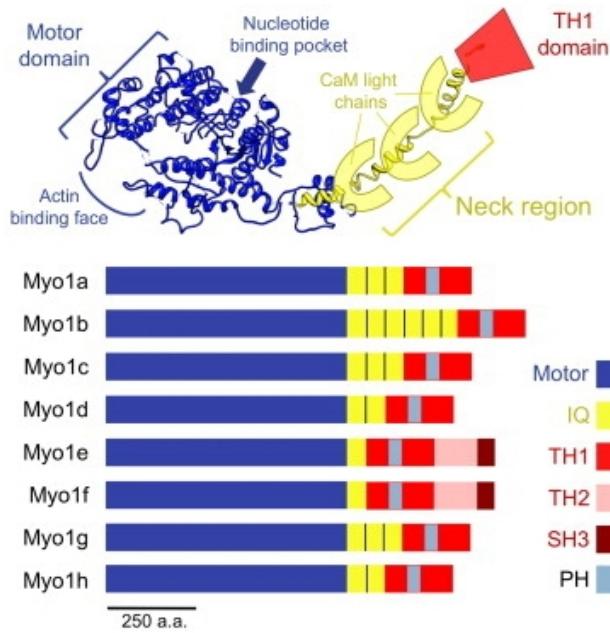
- Myosins
- Cell division, budding, polarity
- Secretion, endocytosis
- Lipid synthesis
- Filament dynamics
- ...



ACTIN-ASSOCIATED MOTOR PROTEINS

Myosins:

- Myosin I (everywhere)
 - head interacts with actin
 - tail is variable for cargo
- Myosin II (muscle)

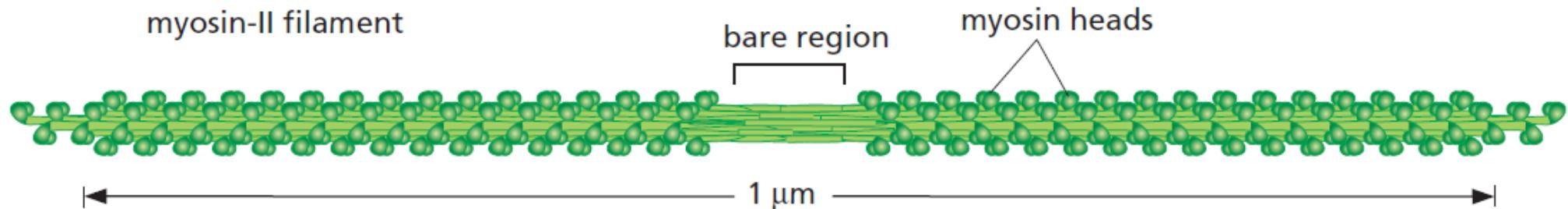
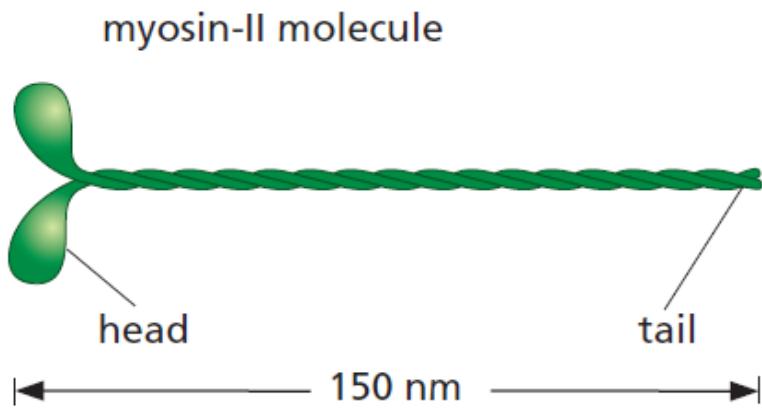


MUSCLE CONTRACTION

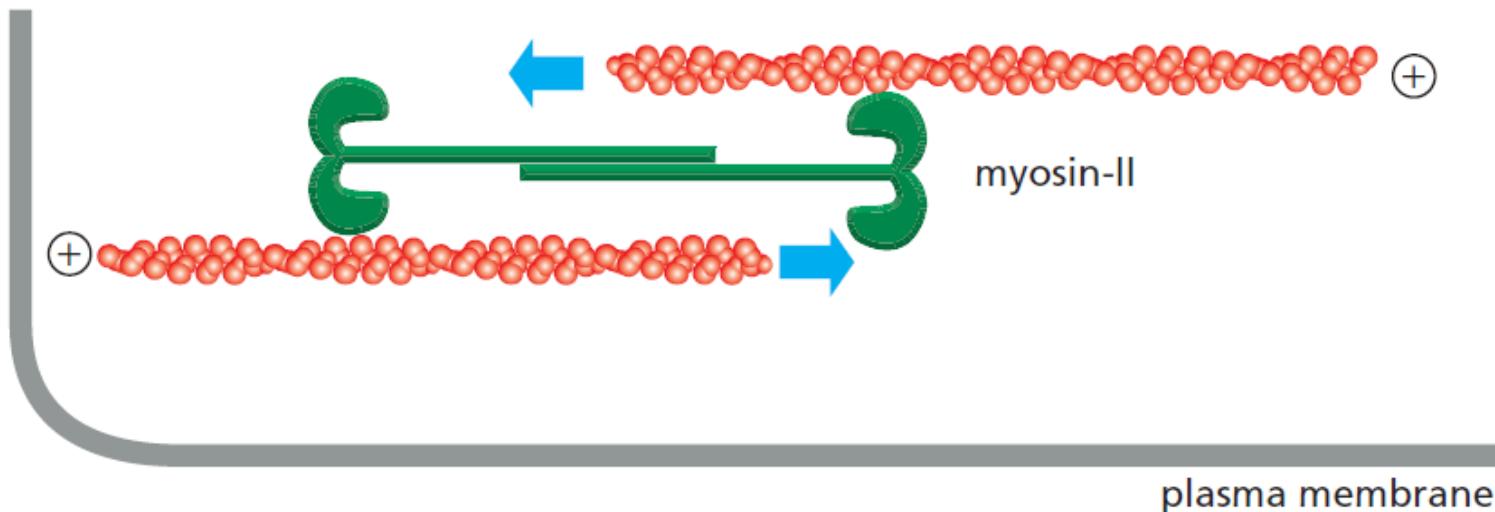
Actin and myosin play a key role in the work of muscles

➤ Myosin II:

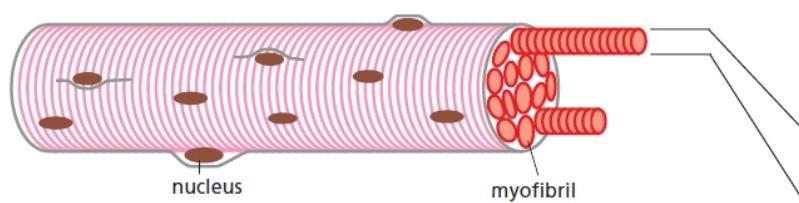
- 2 ATPase head + a long rod-tail
- contractile bundles



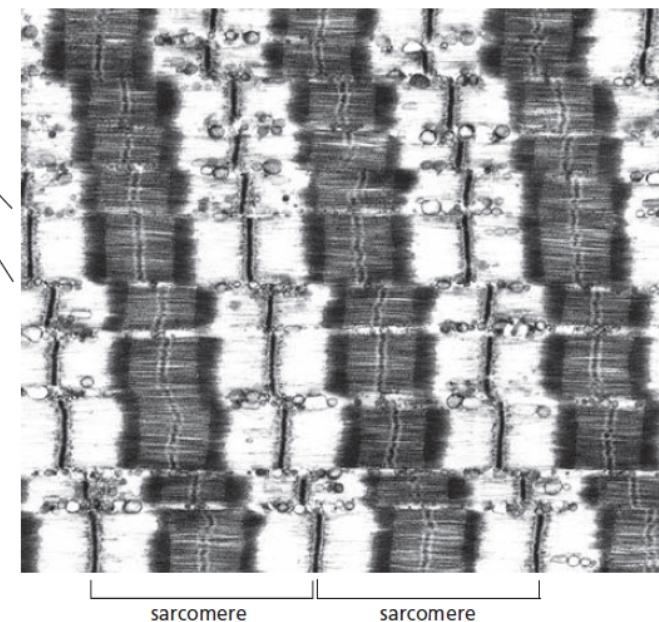
MUSCLE CONTRACTION



Sarcomers: contractile units

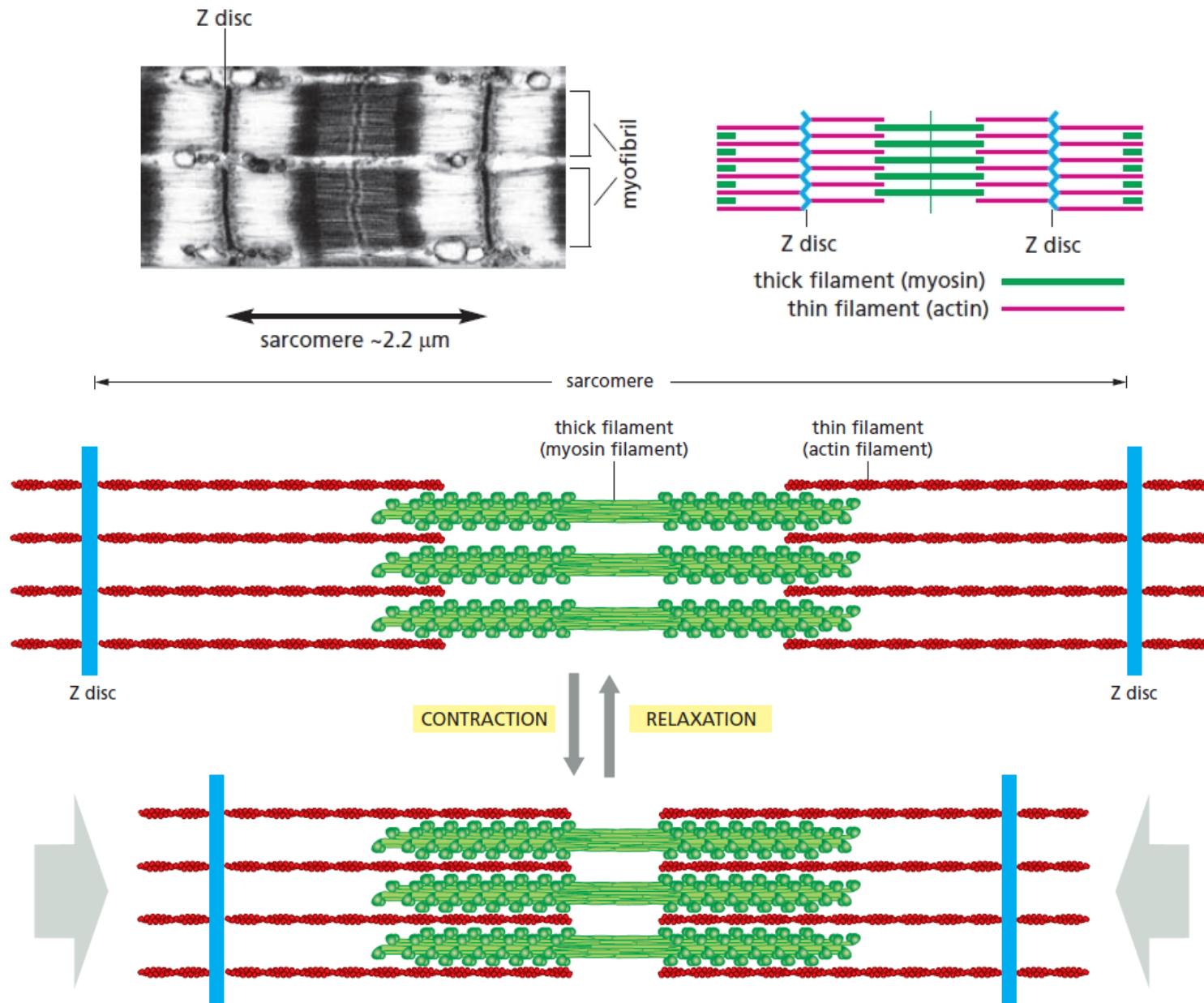


Multinuclear skeletal muscle cell



MUSCLE CONTRACTION

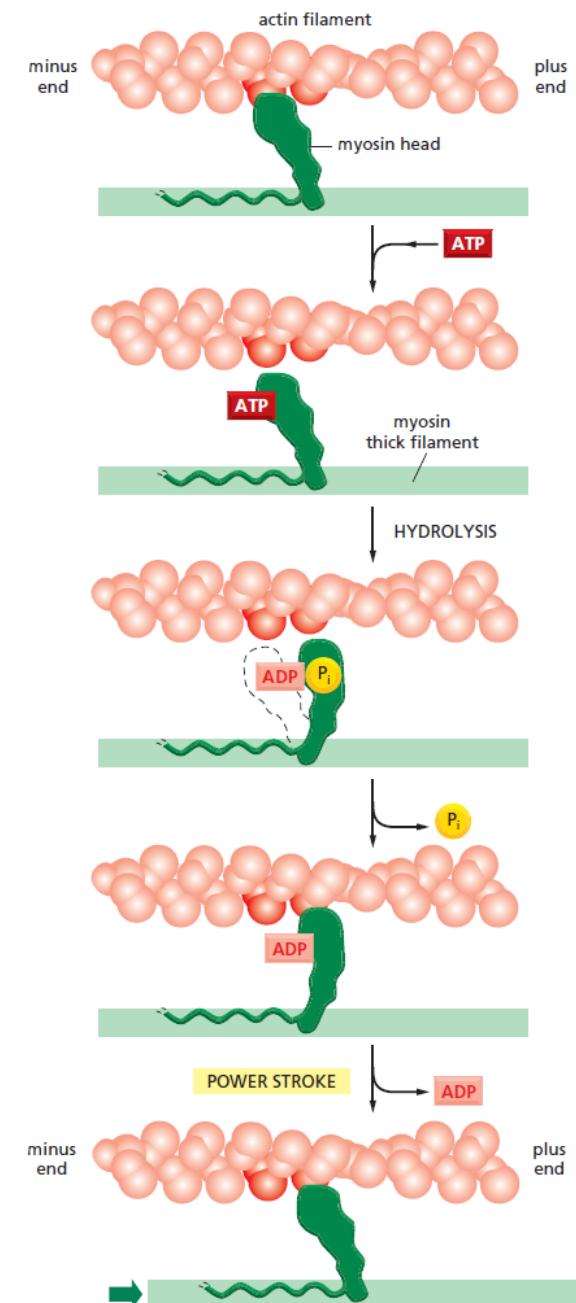
Z discs: anchoring points of actin at their plus-ends



Contraction: simultaneous shortening of all sarcomers

MUSCLE CONTRACTION: MOLECULAR MOVEMENT

➤ Attached: ATP-unbound rigor configuration



➤ Released: ATP is bound, allosteric conformational change => myosin starts to slide along actin

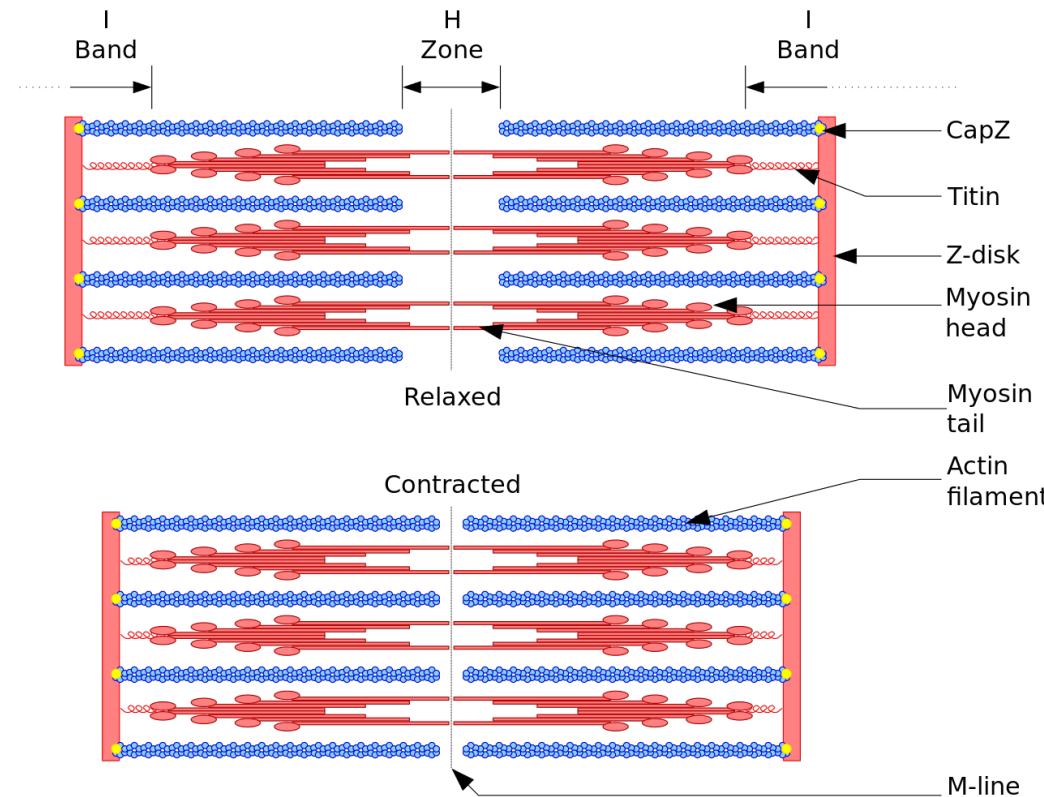
➤ Hydrolysis: $\text{ATP} \Rightarrow \text{ADP} + \text{P}_i$

➤ Force generating: tight binding again

➤ Attached again: ADP leaves

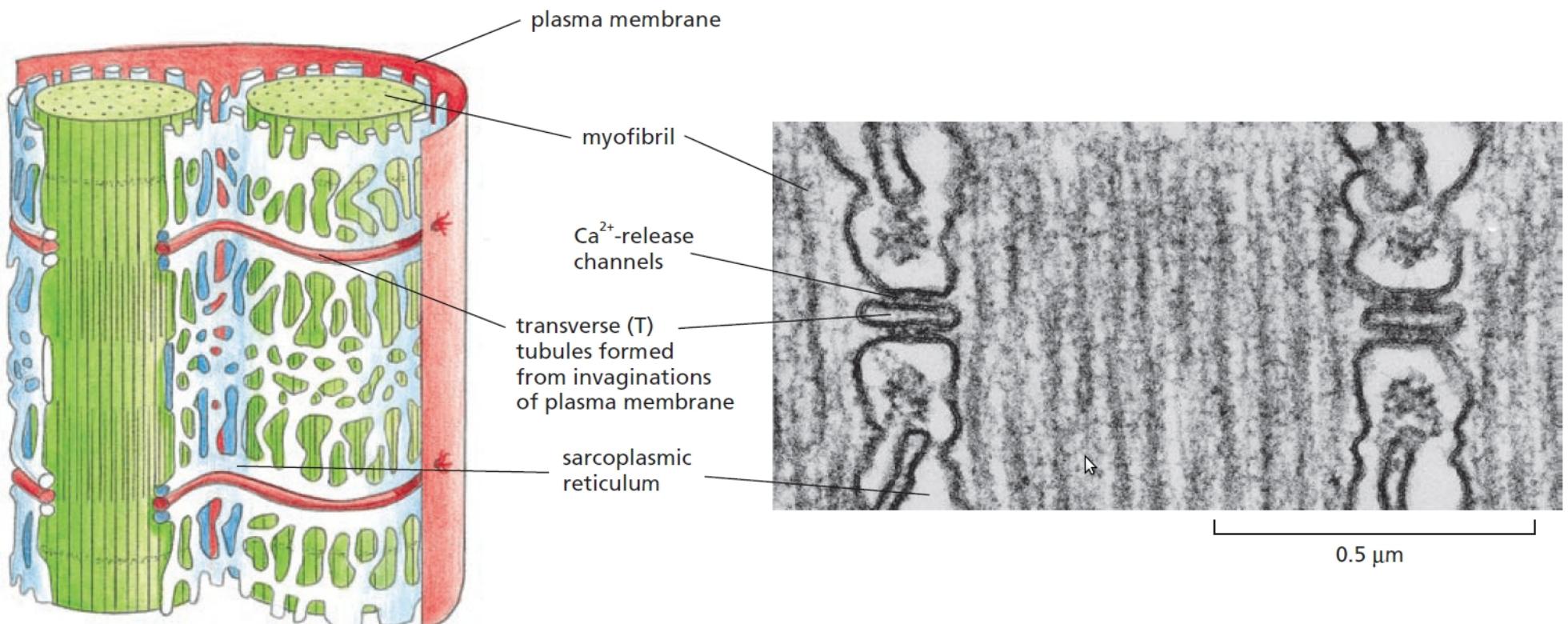
MUSCLE CONTRACTION: SOME NUMBERS

- Movement (sliding) $\sim 5 \text{ nm} / 1 \text{ ATP}$
- Speed of sliding $15 \mu\text{m/s}$
- Myosin filament: ~ 300 myosin heads
- Attachment/detachment $\sim 0.2 \text{ s}$
- Fully extended ($3 \mu\text{m}$) => contracted ($2 \mu\text{m}$) $\sim 0.1 \text{ s}$
- Signal $\sim \text{ms}$



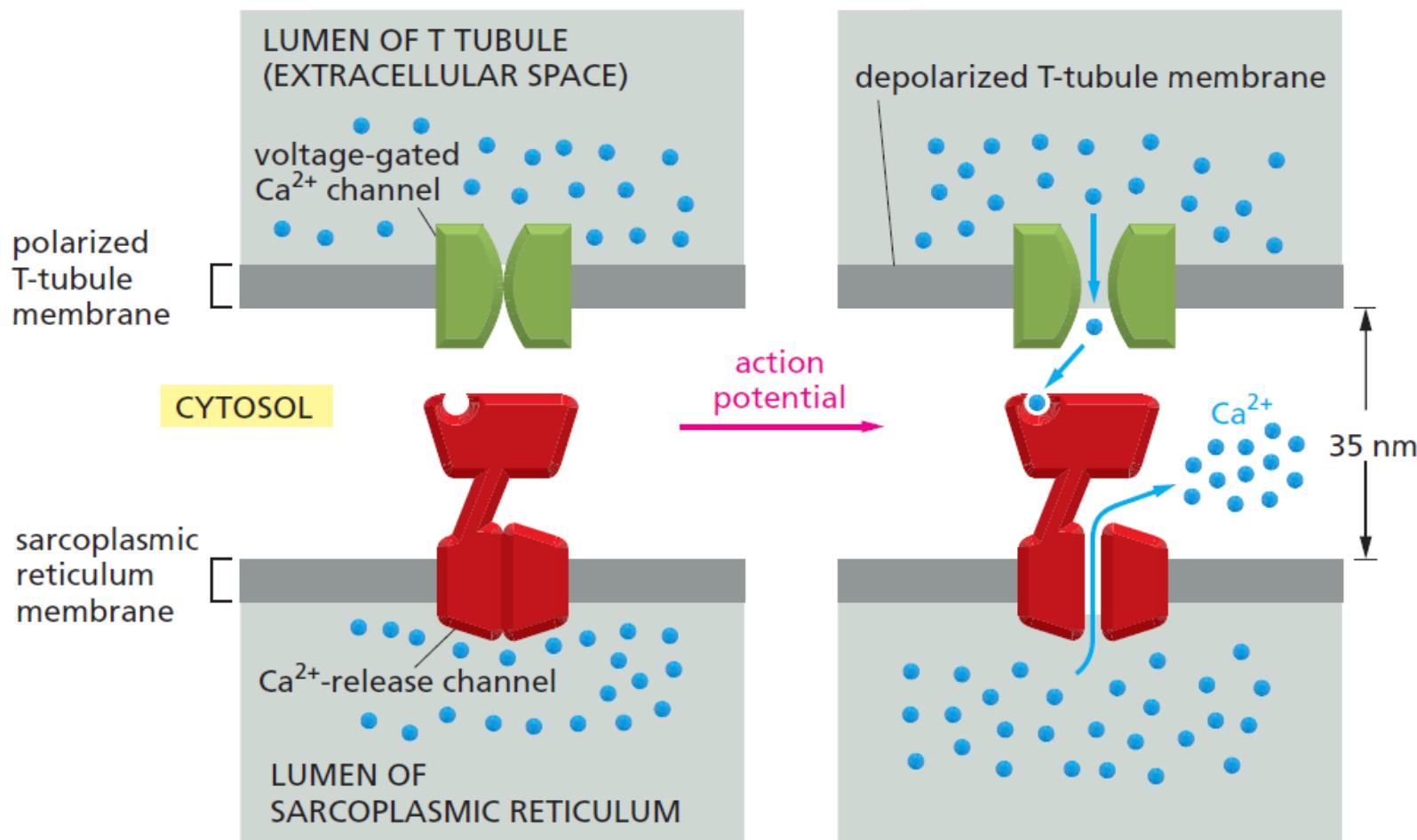
MUSCLE CONTRACTION: CNS SIGNAL

- Signal is required for contractions
- Action potential through traverse tubules => sarcoplasmic reticulum



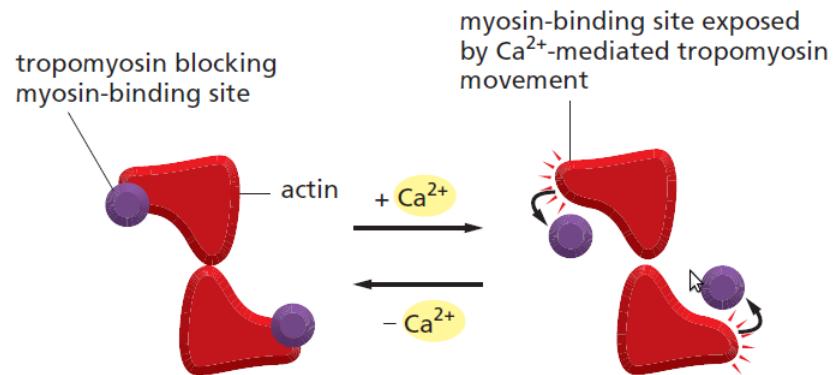
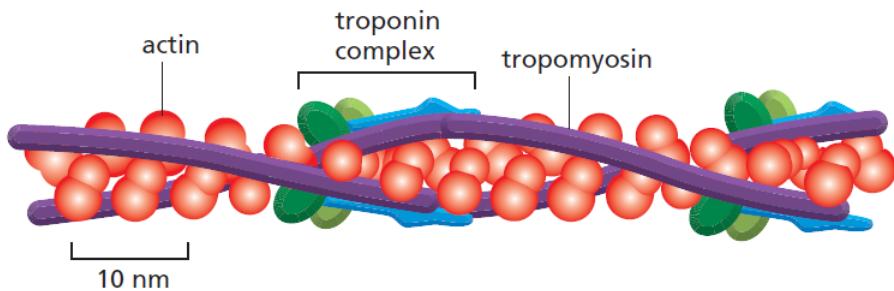
MUSCLE CONTRACTION: CNS SIGNAL

- Ca^{2+} is released from sarcoplasmic reticulum
- => Ca^{2+} -sensoric proteins



MUSCLE CONTRACTION: CNS SIGNAL

- Tropomyosin sterically blocks the access of myosin to actin filament
- Troponin is bound to tropomyosin and changes its conformation by sensing Ca^{2+} : myosin head accesses actin filament



- Ca^{2+} is pumped back
- Phosphorylation is an another way of regulation contraction events:
 - slower
 - more universal reaction to many other signals

REGULATION OF CYTOSKELETON BIOGENESIS

➤ Nucleation of filament assembly:

- γ -tubulin (MT)
- ARP-complex (AF)

➤ Binding to the free subunits:

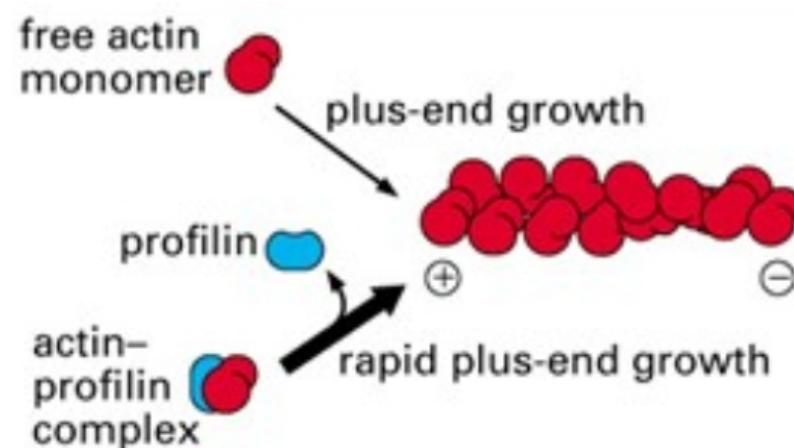
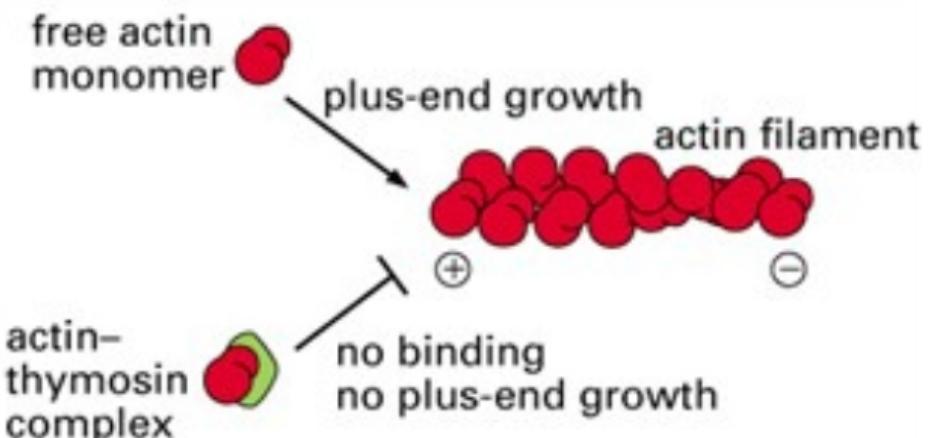
- thymosin/profilin (AF) => ATP site
- stathmin (MT) <= phosphorylation

➤ Binding along the sides:

- MAPs, tau (MT-associated protein)
- tropomyosin (AF),
- cofilin- depolymerizing factor (AF)

➤ Binding to the ends:

- CapZ (AF in “plus”-end) <= PIP₂
- tropomodulin (AF in “minus”-end)
- catastrophin (MT in “plus”-end): pry protofilaments away from each other
- γ -tubulin (MT in “minus”-end)



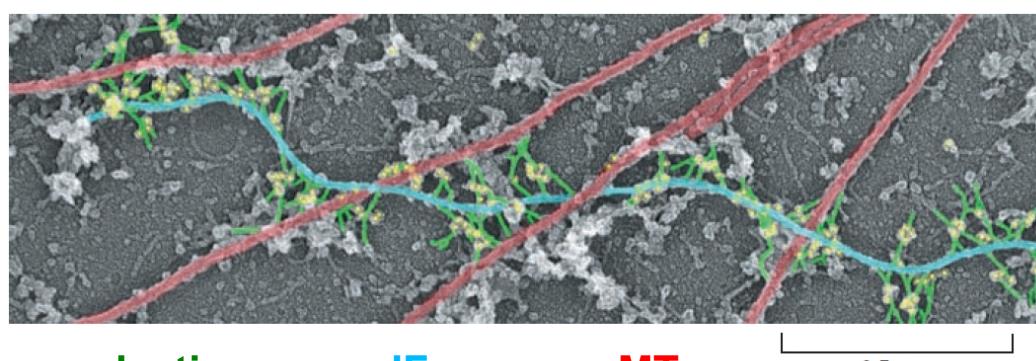
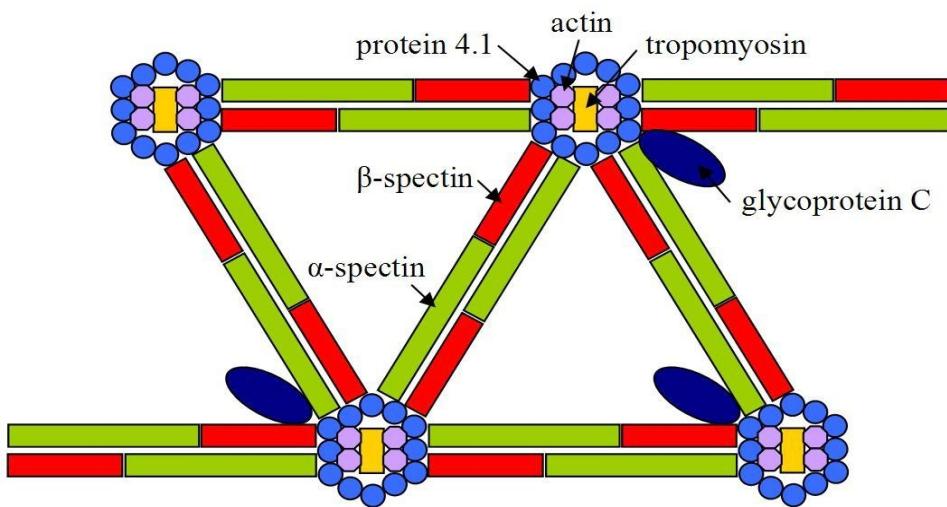
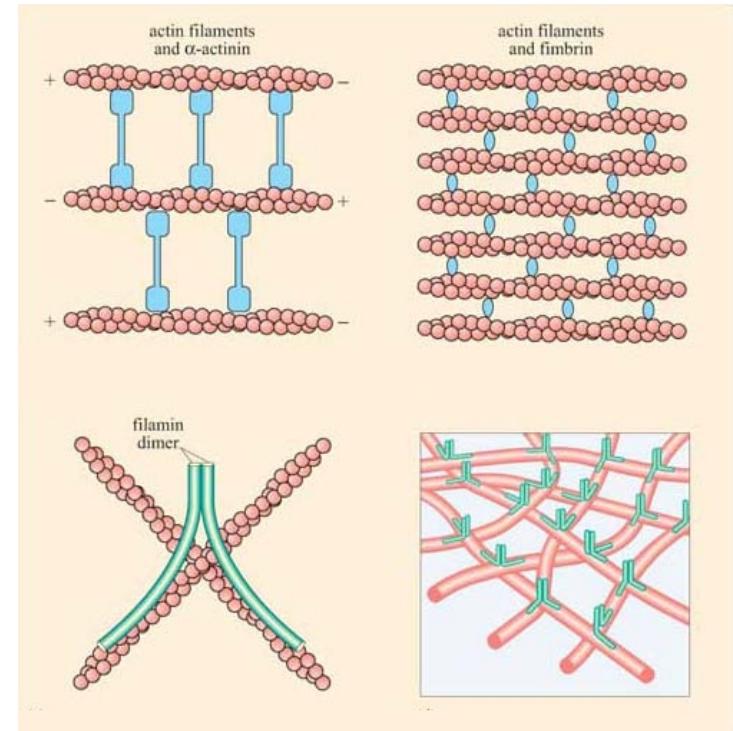
REGULATION OF CYTOSKELETON BIOGENESIS

➤ High-order structures organization:

- centrosome (MT)

➤ Cross-linking:

- NF-M, FN-H (IF): via C-termini
- flaggrin (IF)
- plectin (IF-MT-myosin)
- bundling proteins (AF): fimbrin, α -actinin, villin, spectrin
- gel-forming proteins (AF): filamin



plectin

IF

MT

0.5 μm

REGULATION OF CYTOSKELETON BIOGENESIS

➤ Severing proteins => breaking into many small ones/rates change:

- katanin (MT): ATP-dependent

- gelsolin (AF): Ca^{2+} -dependent

- * platelet activation (AF): PIP_2 -dependent

➤ Binding to membrane proteins:

- spectrin, villin (AF)

- ERM proteins (AF): bind actin and CD44, PIP_2 -dependent

➤ Strong attachments to membrane:

- focal contacts (AF): integrins to ECM,

- FAK (focal adhesion kinase)

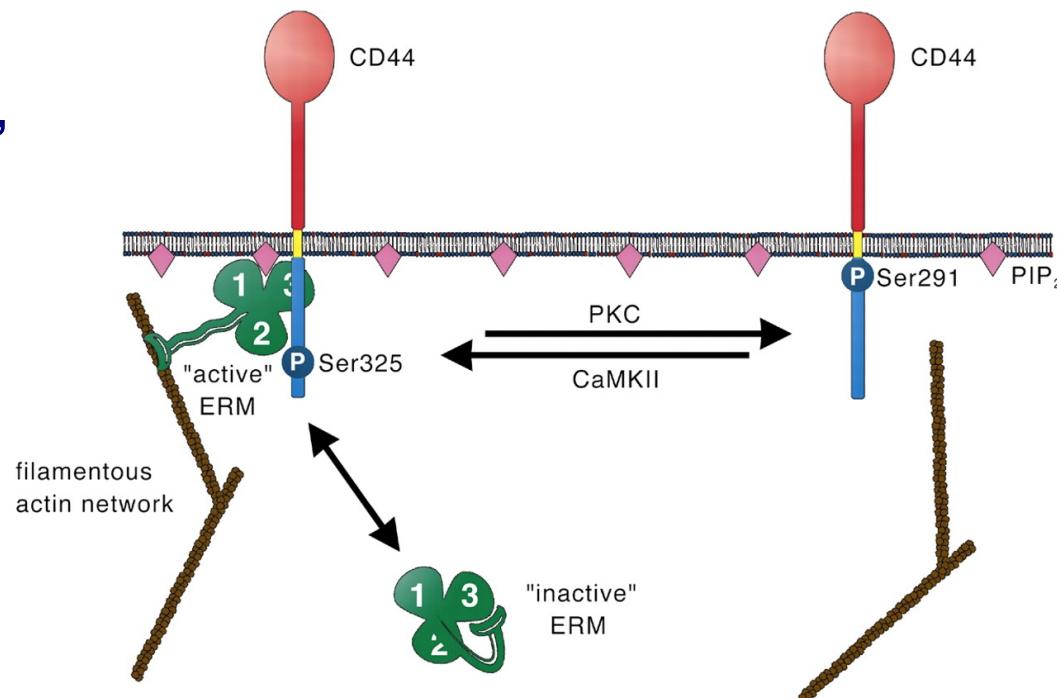
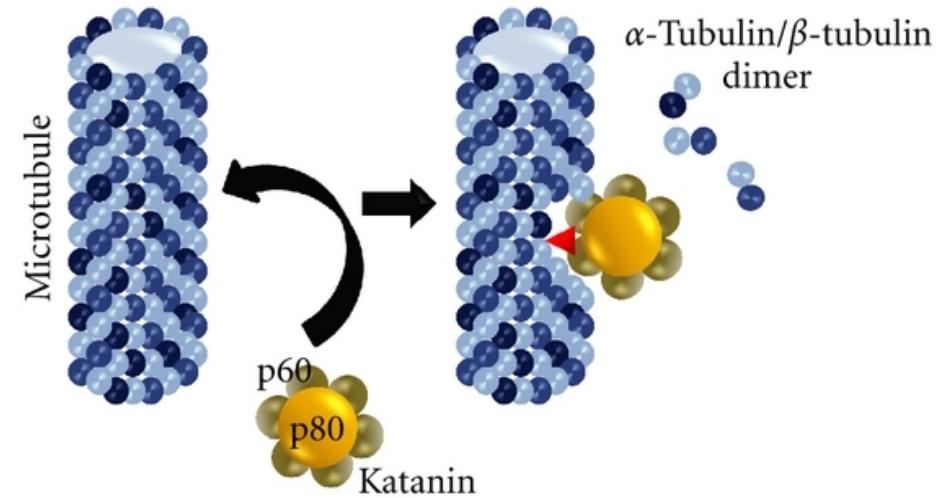
- adhesion belts (AF): cadherin

- desmosomes (IF)

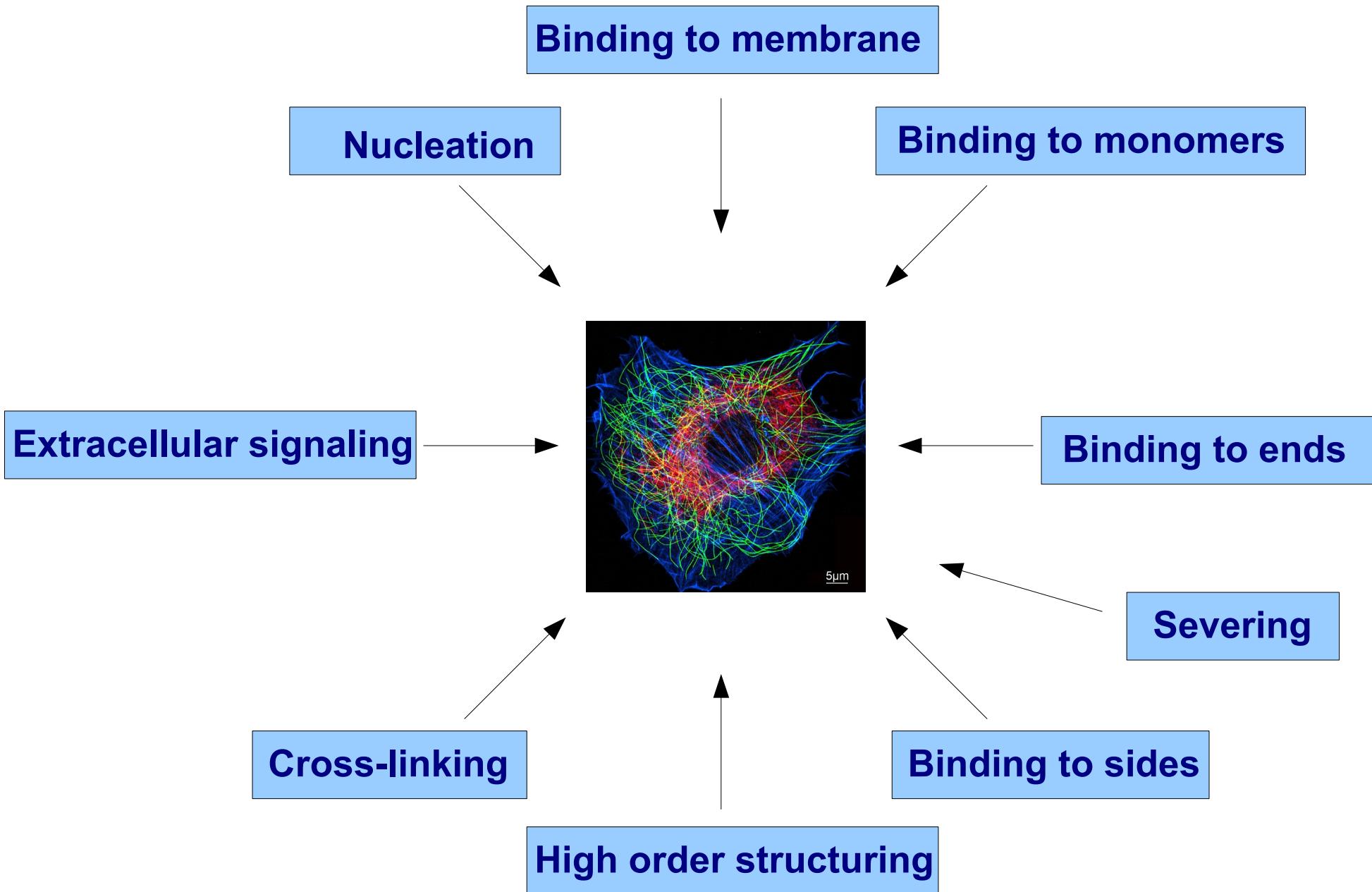
➤ Extracellular signaling:

- Rho protein family (GTPases)

- WASp proteins



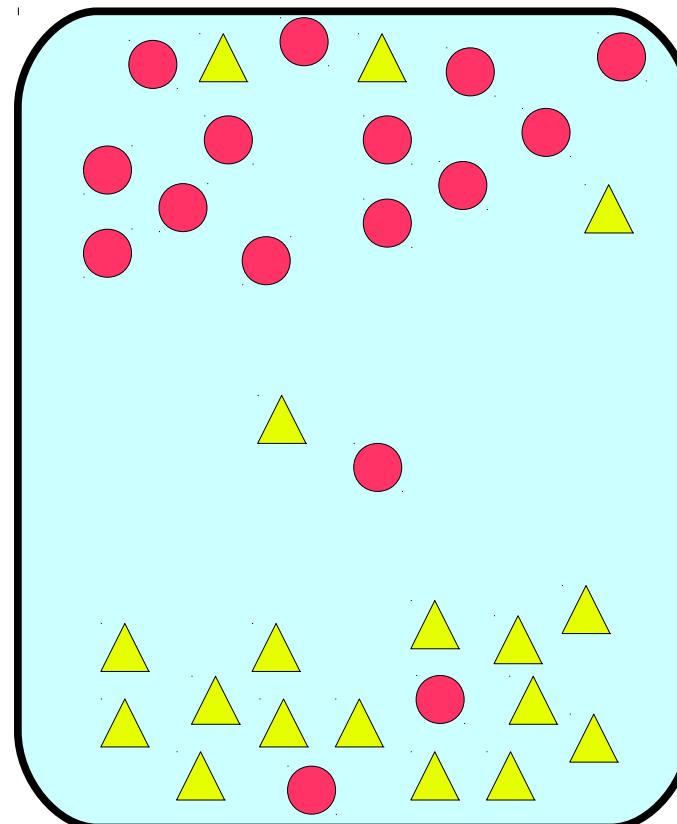
REGULATION OF CYTOSKELETON BIOGENESIS: SUMMARY



CYTOSKELETON AND POLARITY

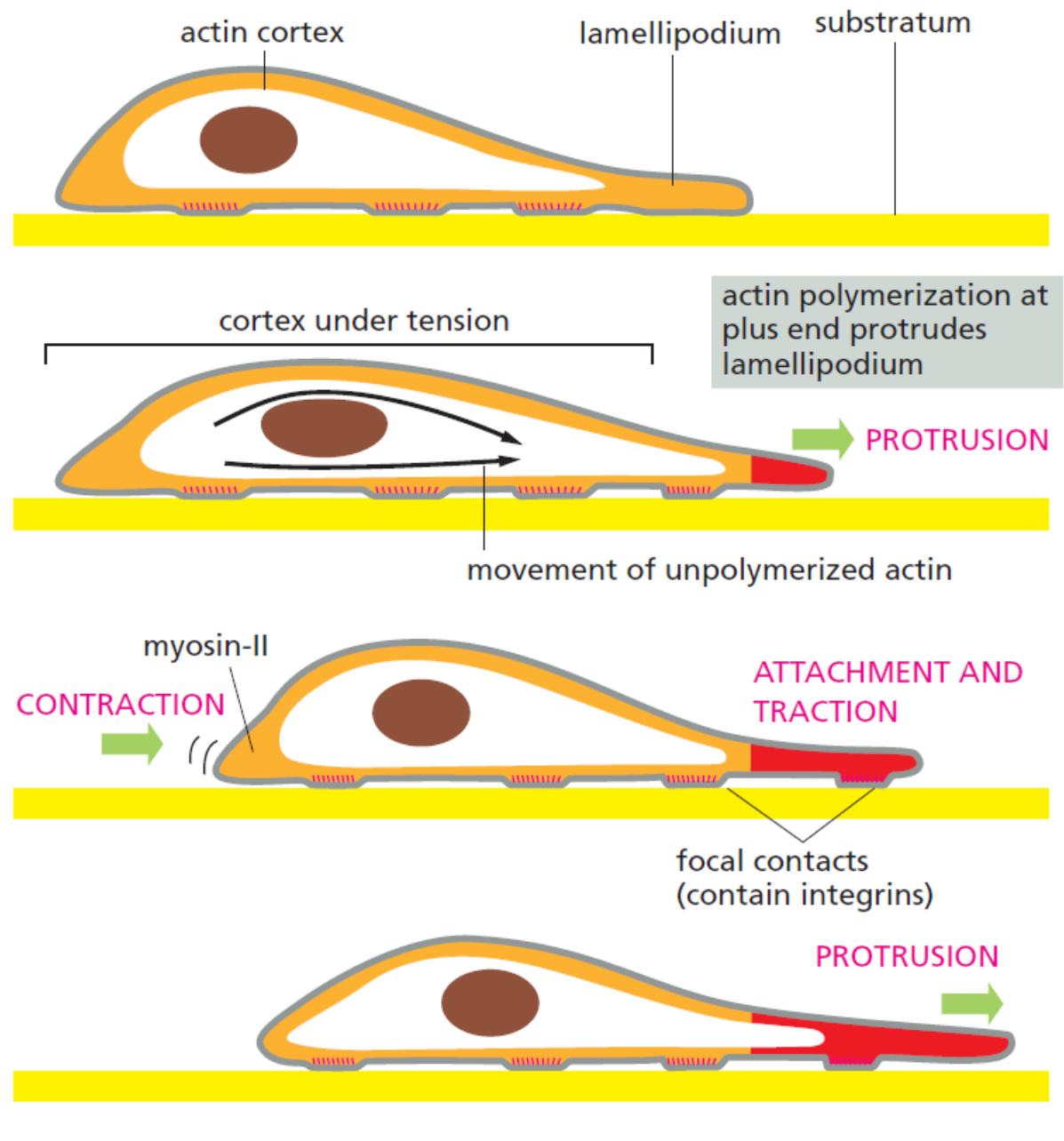
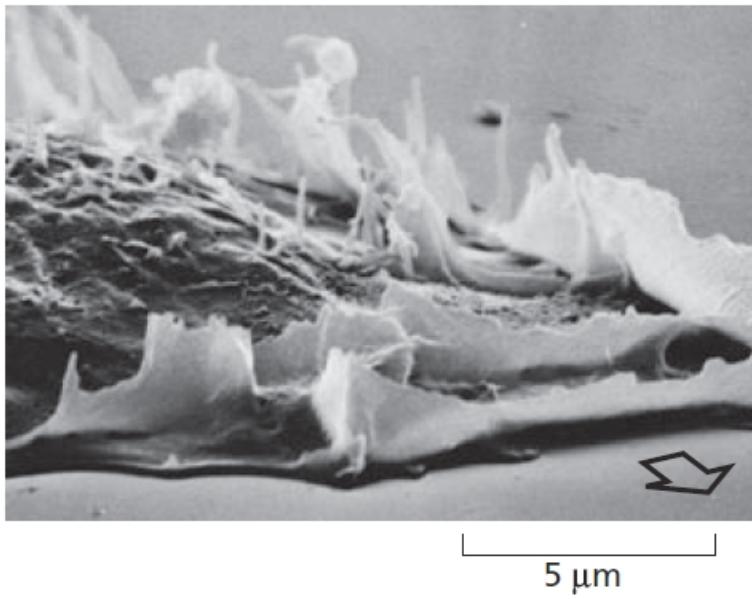
Cell polarity and related mechanism of cell behaviour are defined by cytoskeleton

- Cell crawling (adhesion and traction)
- Chemotaxis
- Yeast budding
- Neuron specialization

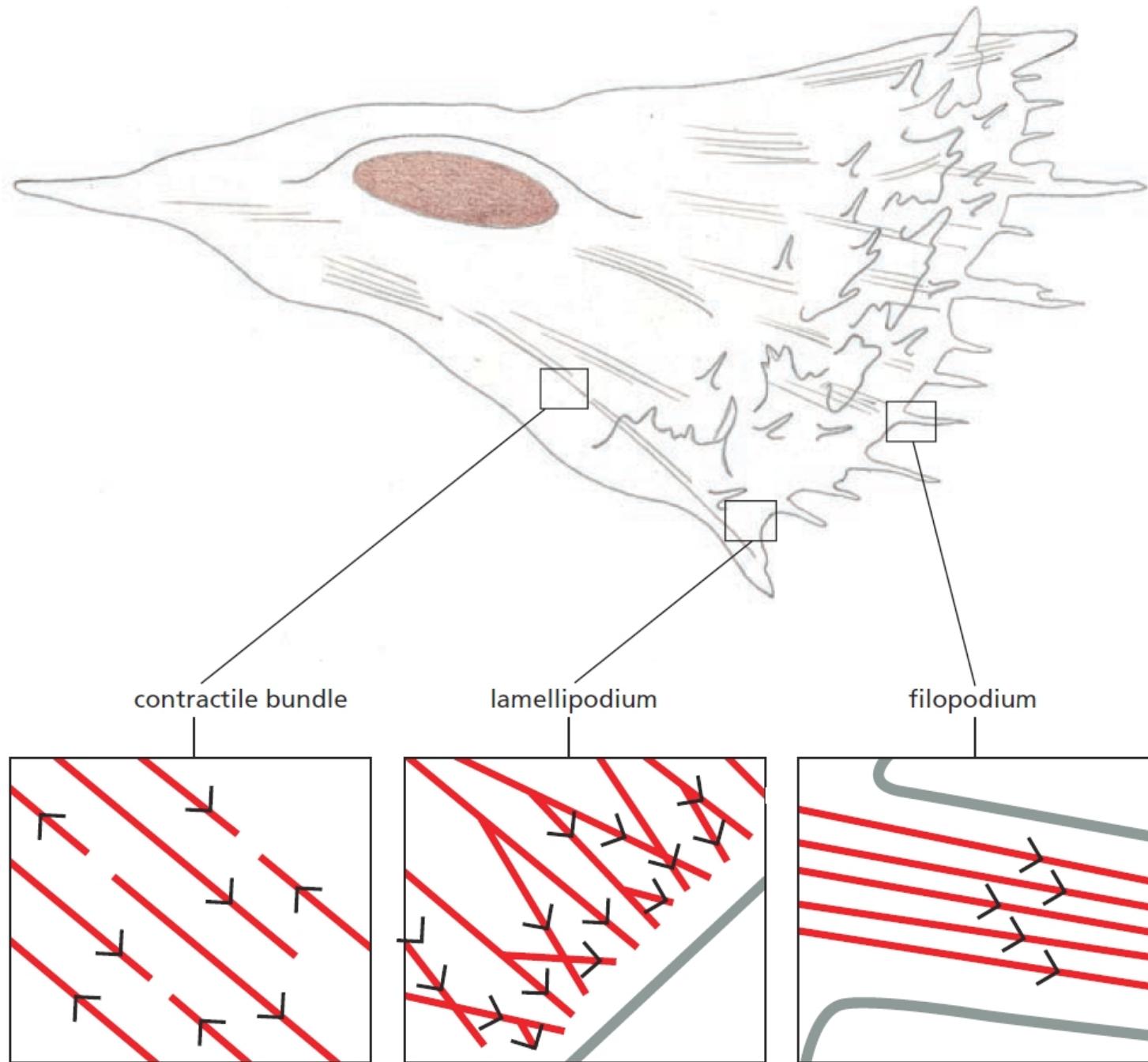


ACTIN FILAMENTS IN CELL CRAWLING

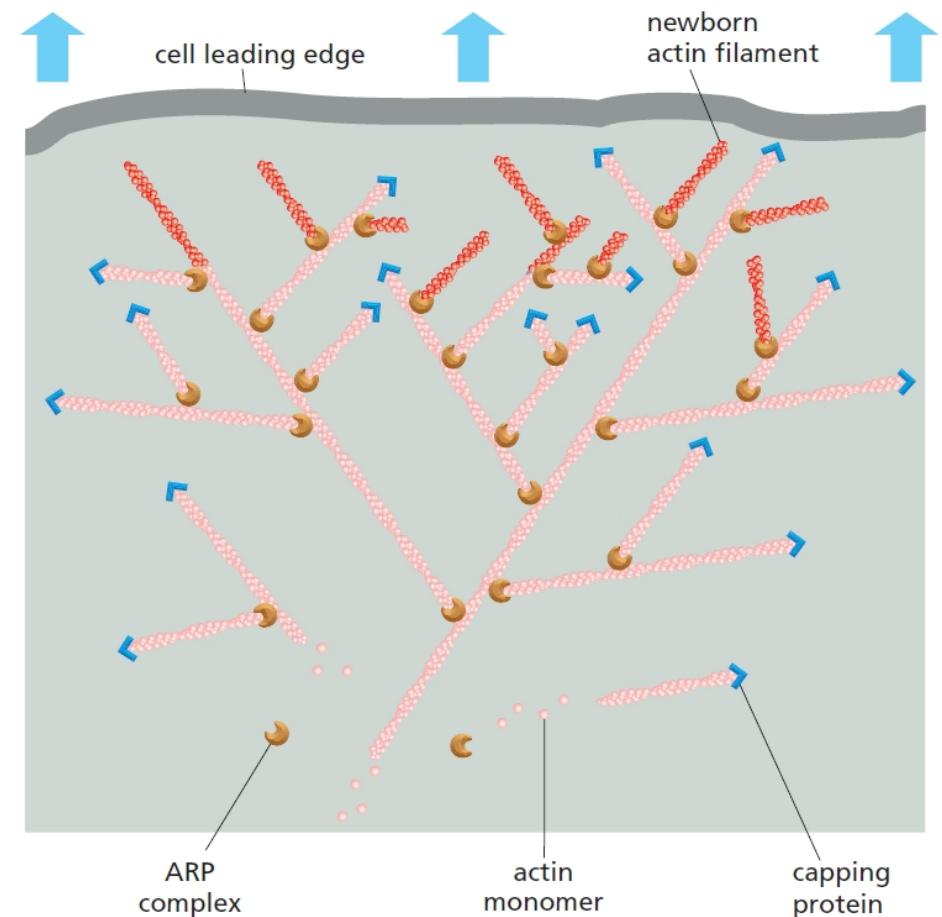
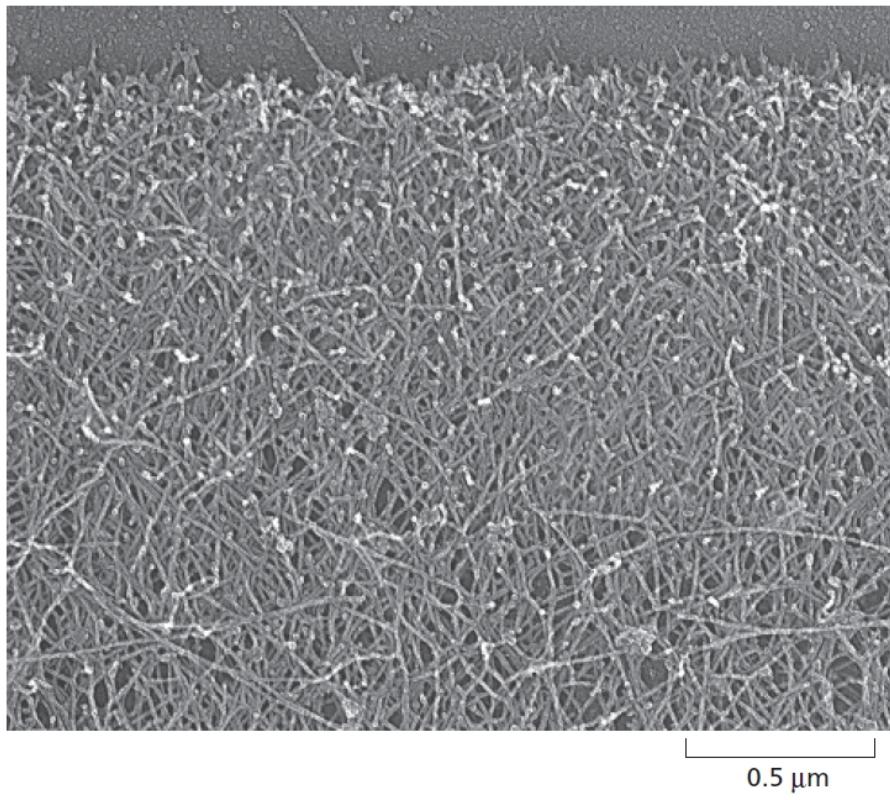
- Protrusion appears
- Adhesion to the surface
- The rest of the cell moves
- Integrins can fix protrusions to surrounding cells



ACTIN FILAMENTS IN CELL CRAWLING



FILAPODIA GROWTH IS ASSOCIATED WITH ACTIN-BINDING PROTEINS



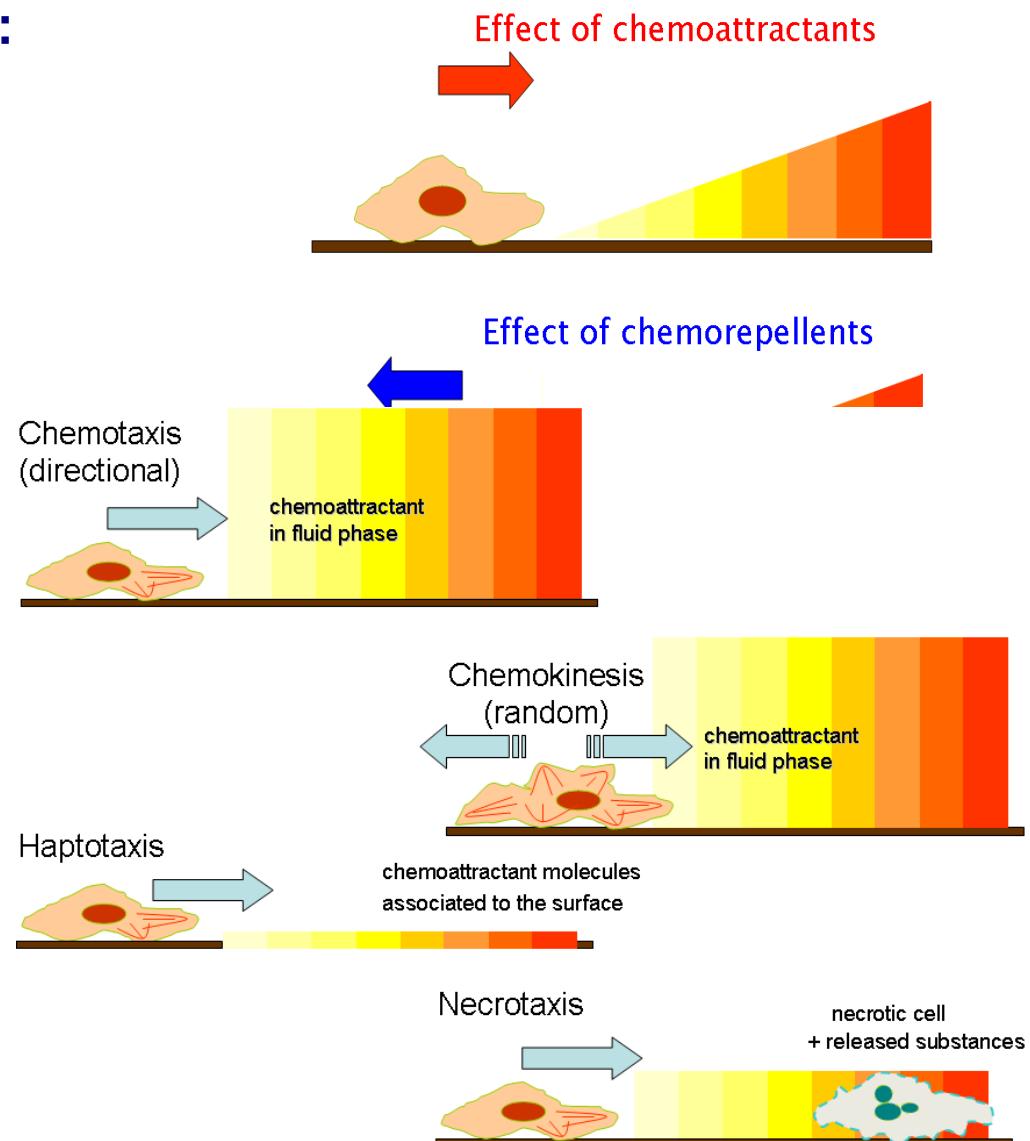
CHEMOTAXIS

- Cell polarization is tightly connected to the environment
- Chemotaxis: movement controlled by a gradient of a diffusible chemical
- Chemoattractants/chemorepellents:

- inorganic salts
- aminoacids
- chemokines

- Classification for eukaryotes:

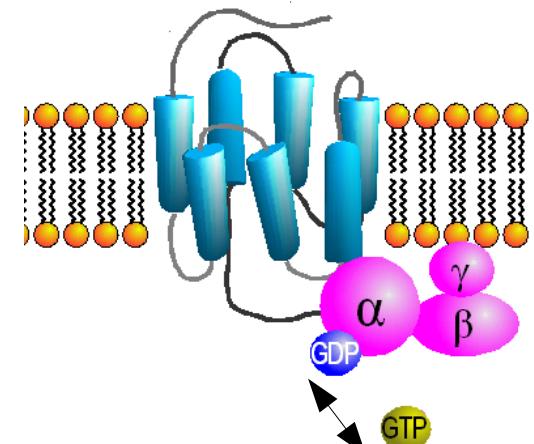
- chemokinesis
- haptotaxis
- necrotaxis



MOLECULAR BASIS OF EUKARYOTIC CHEMOTAXIS

➤ Receptors:

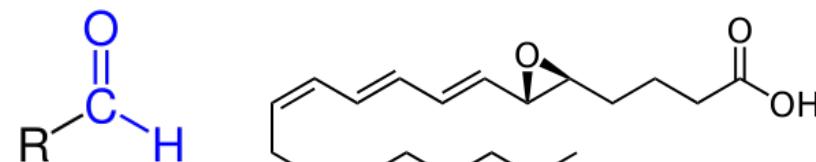
- GPCR (G-protein coupled receptors)
- formyl-peptide receptors
- chemokine receptors
- leukotriene receptors



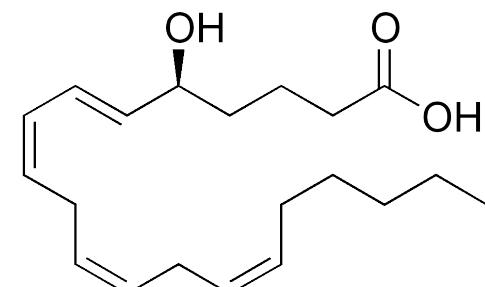
G-protein + GPCR

➤ Ligands:

- formyl-peptides
- complement 3a and 5a proteins
- chemokines
- leukotrienes
- 5-Hydroxyicosatetraenoic acid



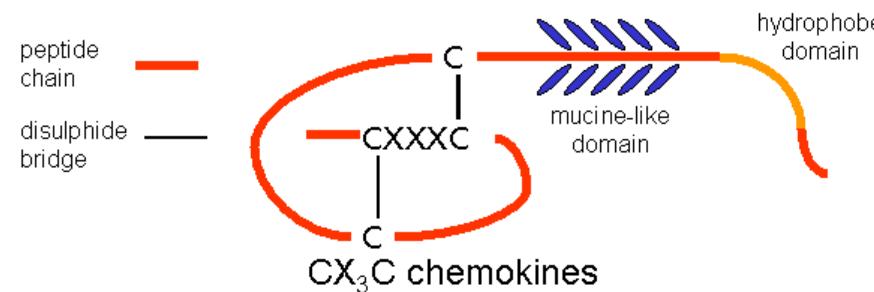
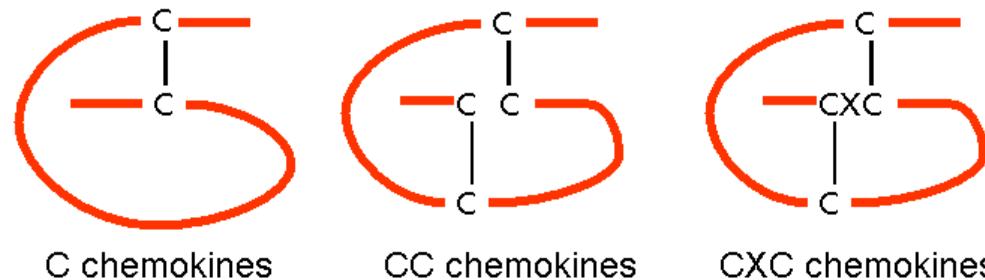
Leukotriene



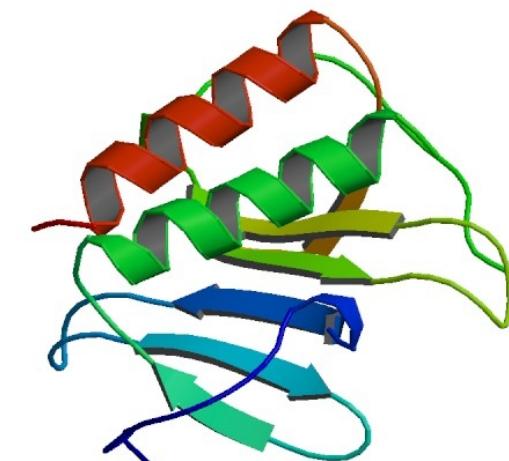
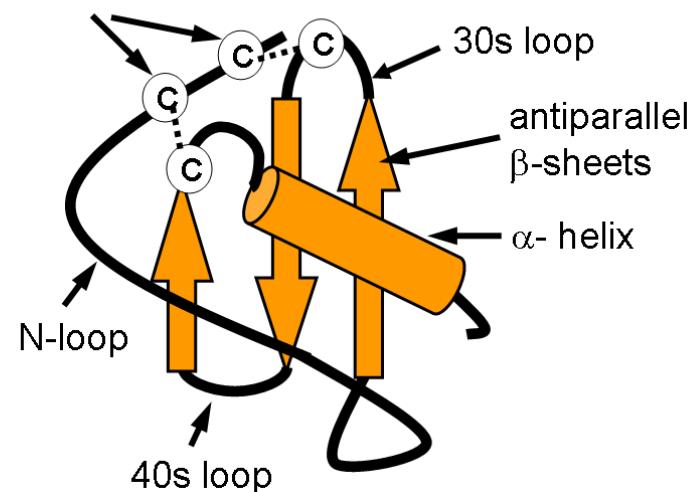
5-Hydroxyicosatetraenoic acid

CHEMOKINES

Chemokines: class of small cytokines



disulphide bridges of Cys-Cys



IL-8

CHEMOTAXIS OF NEUTROPHILS

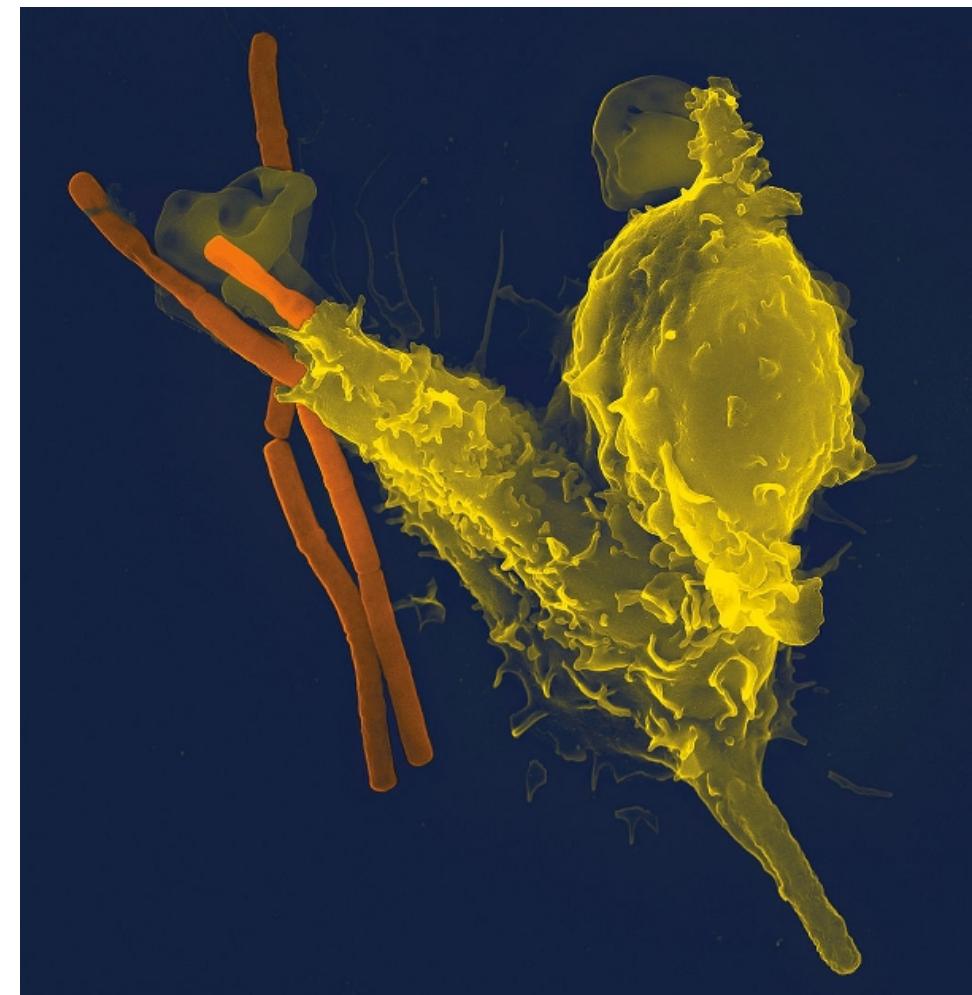
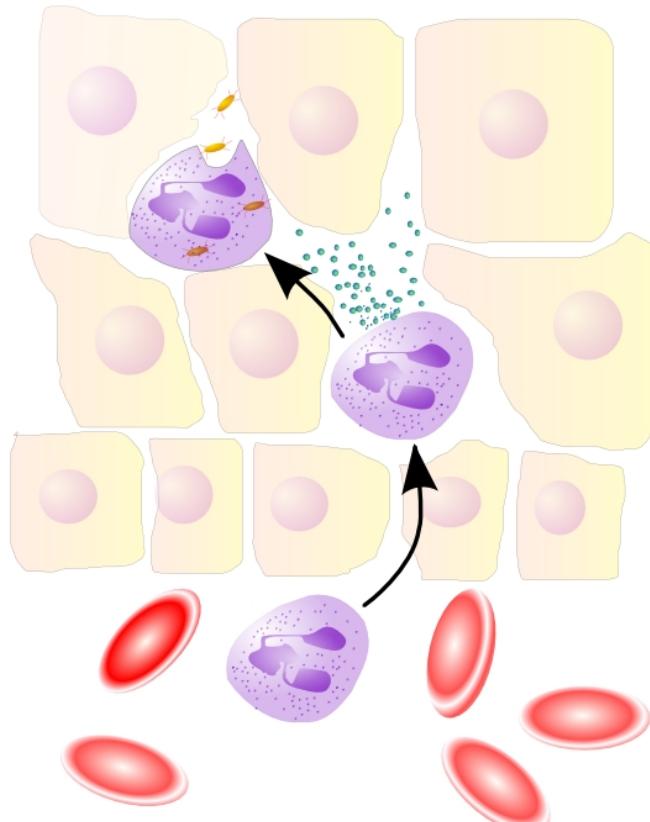
➤ Neutrophils => phagocytosis

- sensitive receptors to N-formylated peptide

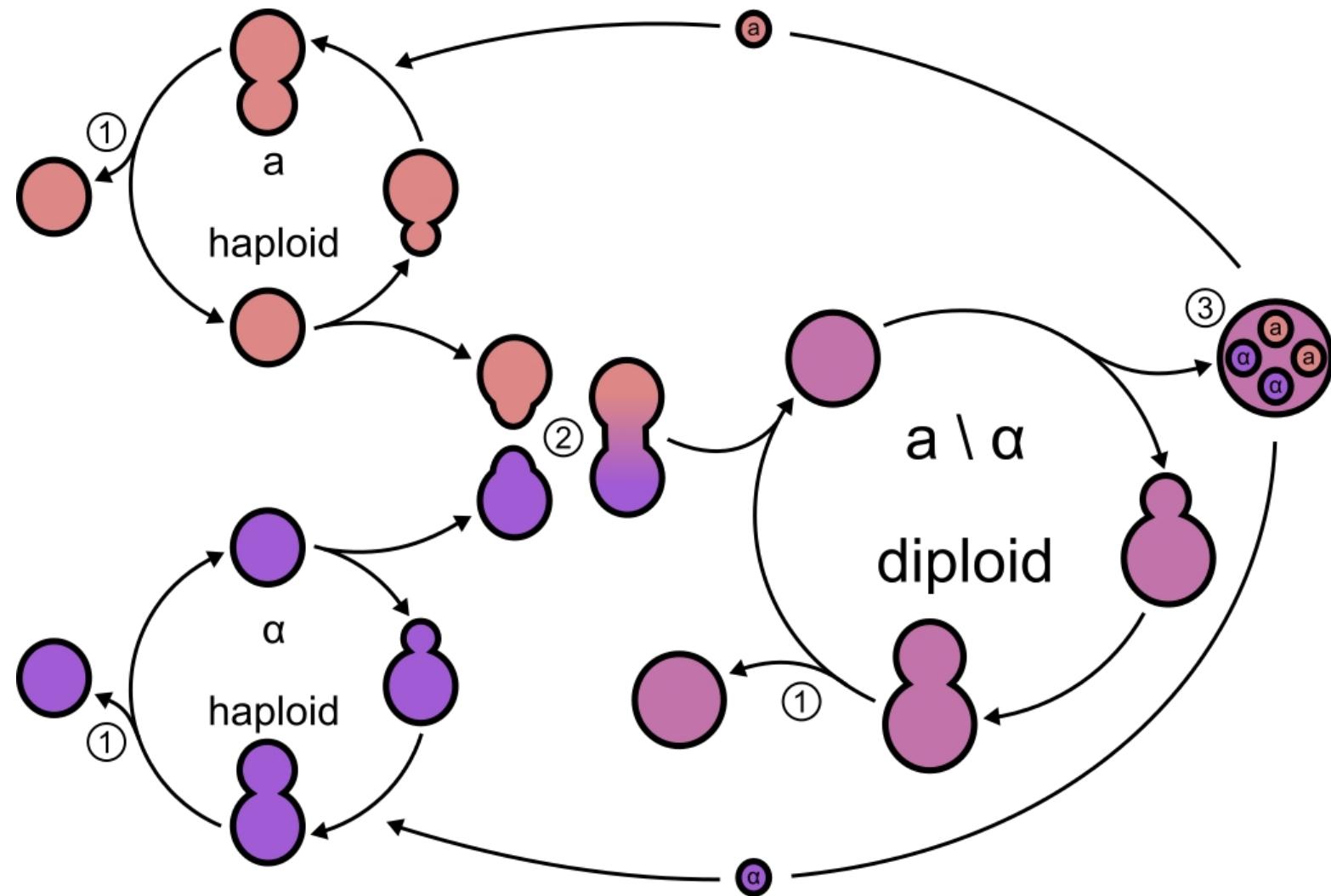
- turning on the actin assembly (Rho GTPases)

- microtubules growth, shifting centrosomes

- adhesion involvement

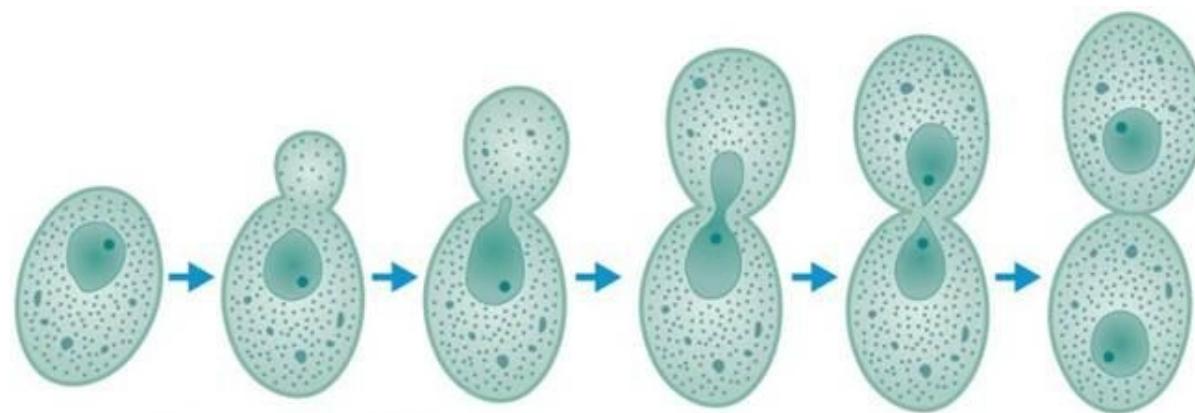
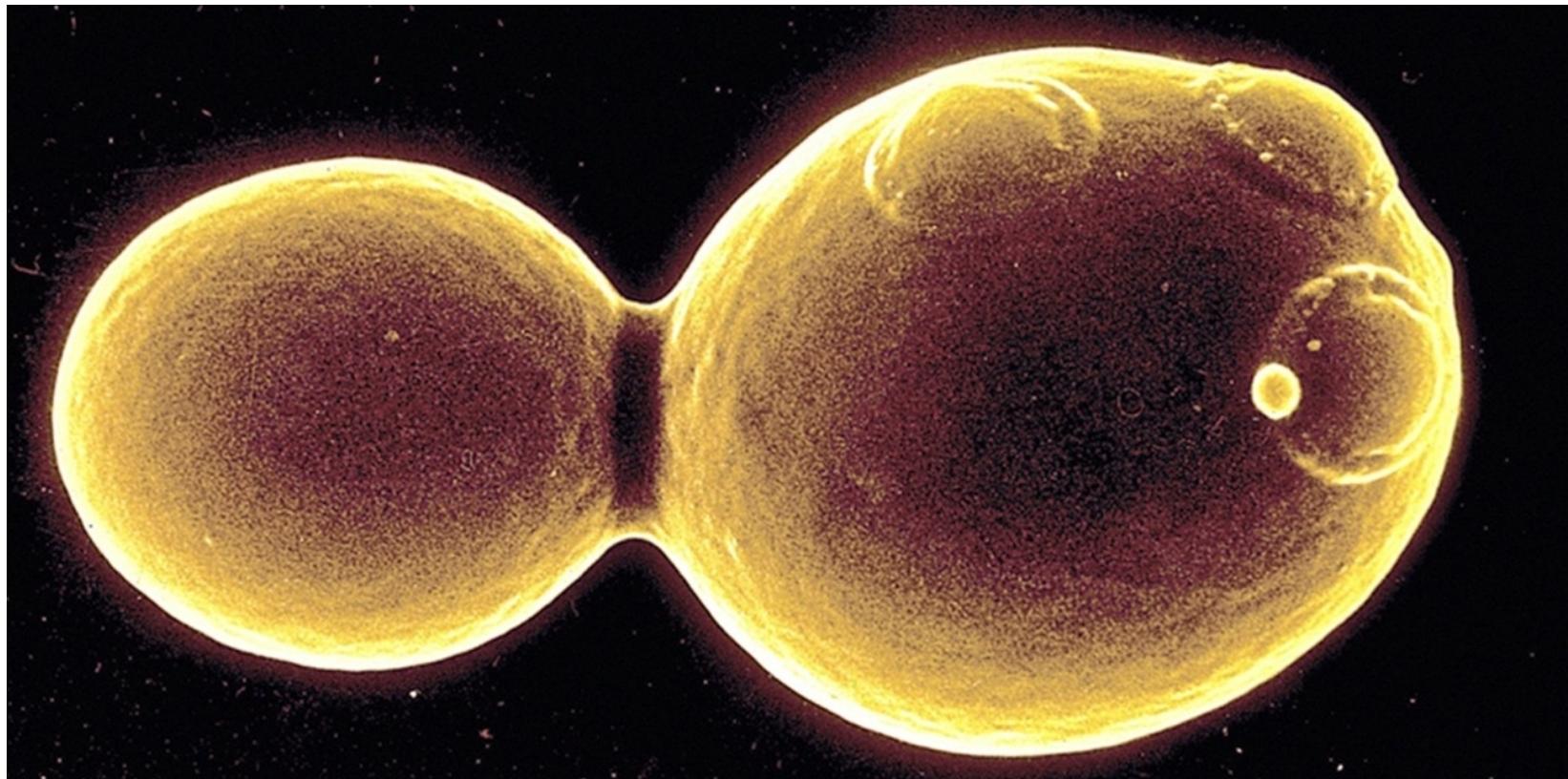


YEAST BUDDING

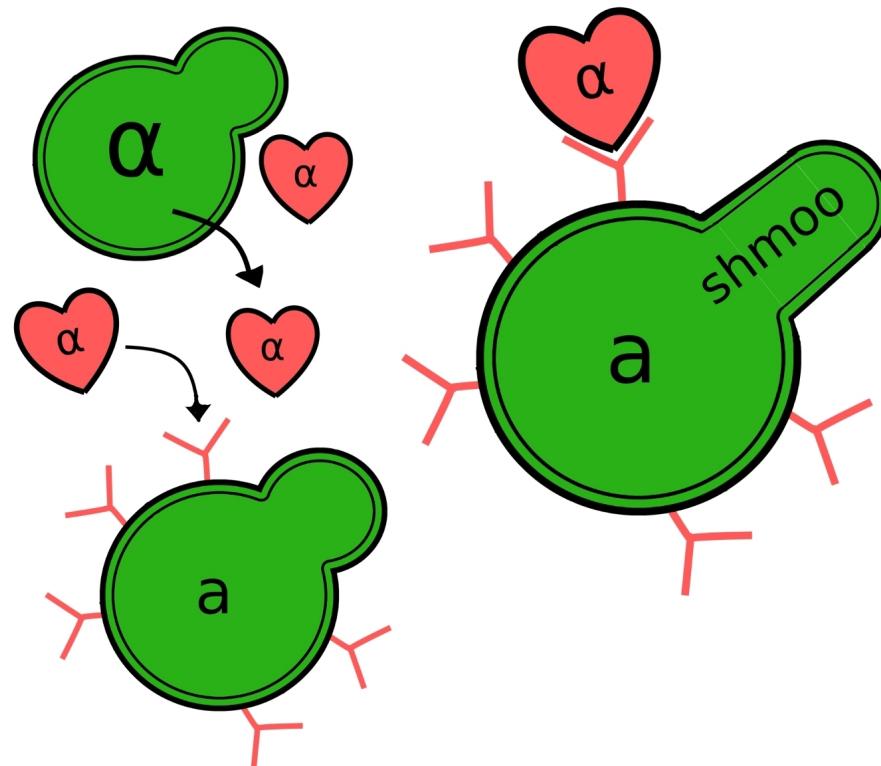
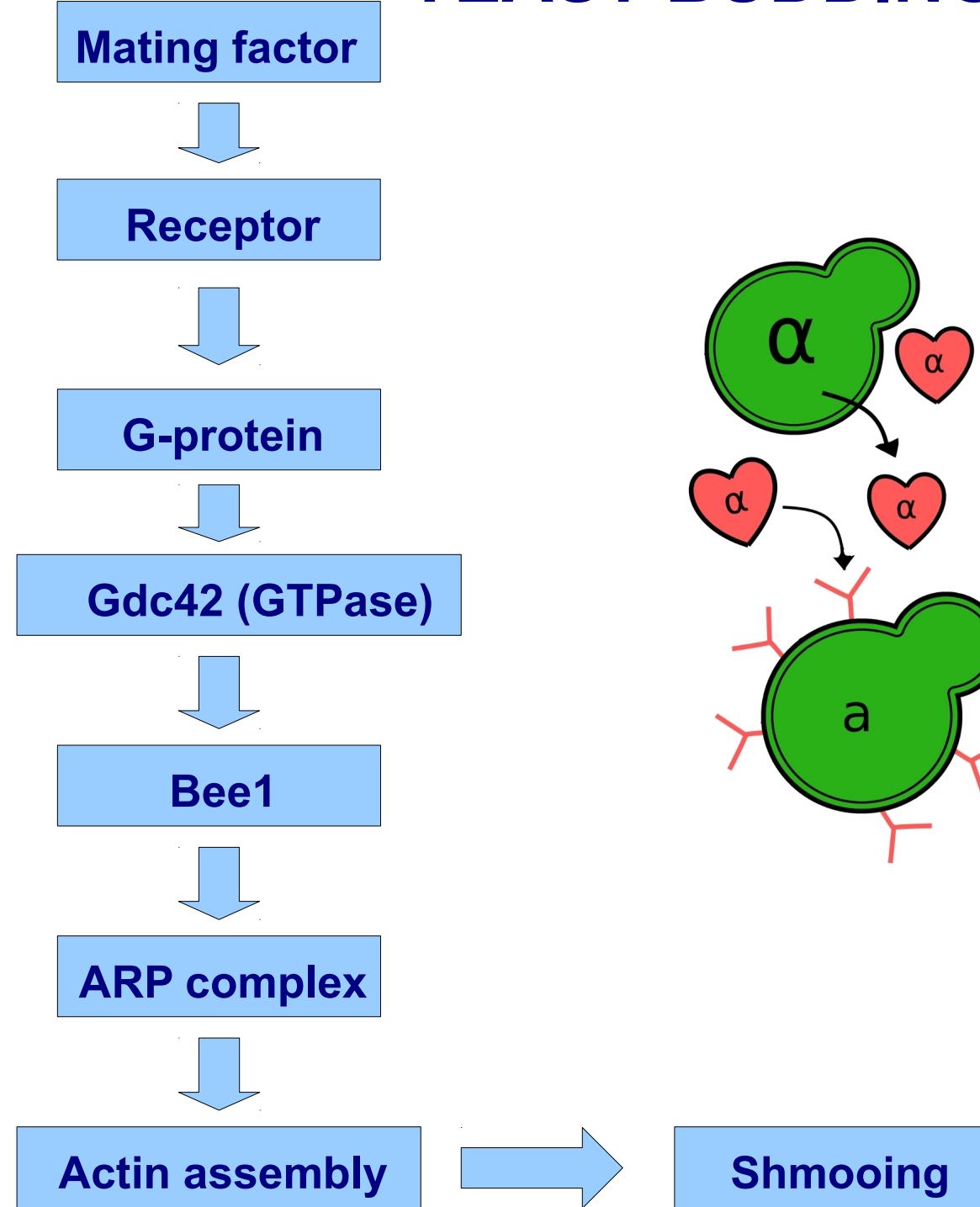


1. Budding 2. Conjugation 3. Spore

YEAST BUDDING

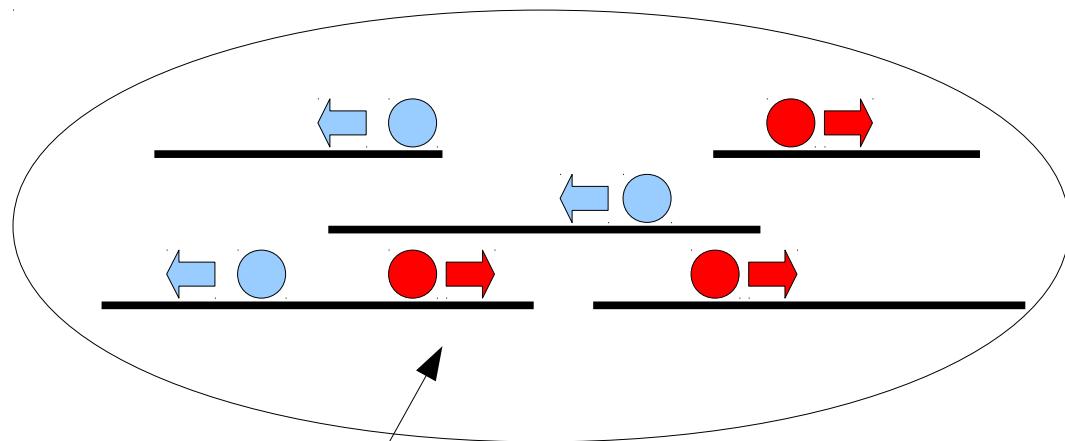
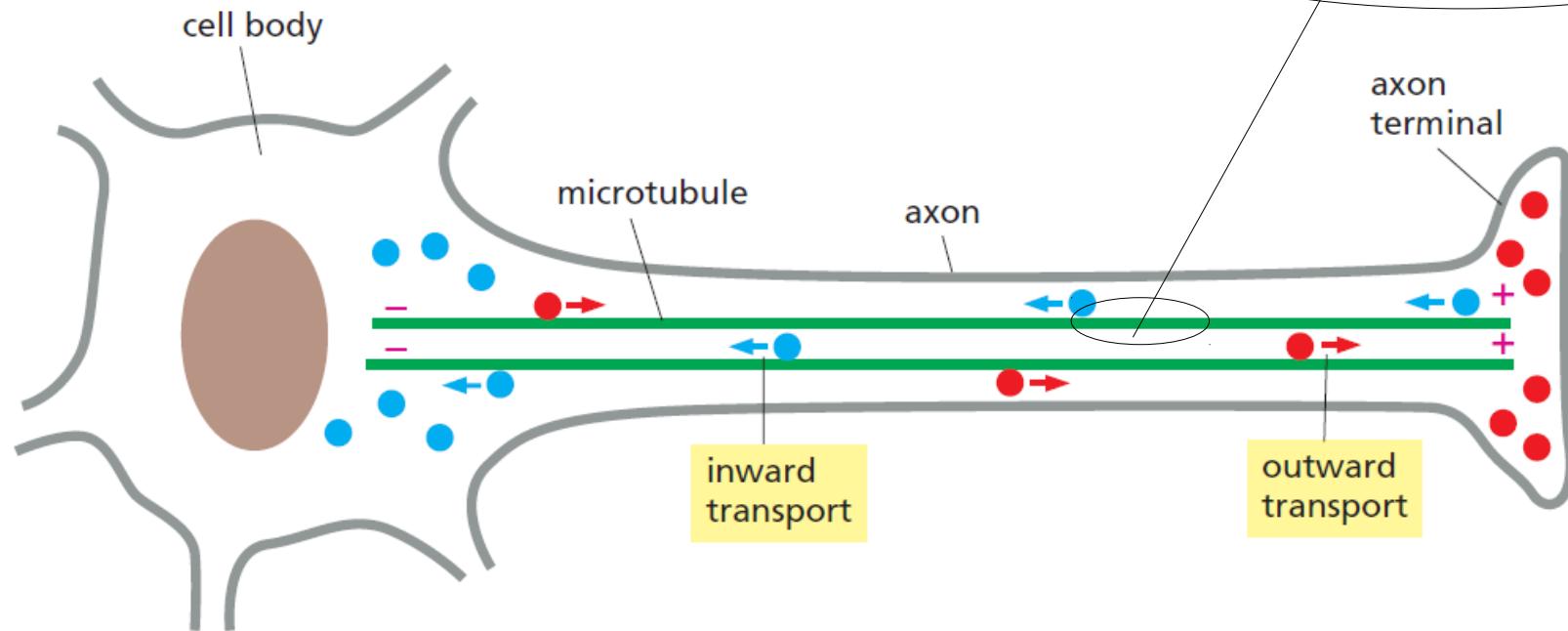


YEAST BUDDING



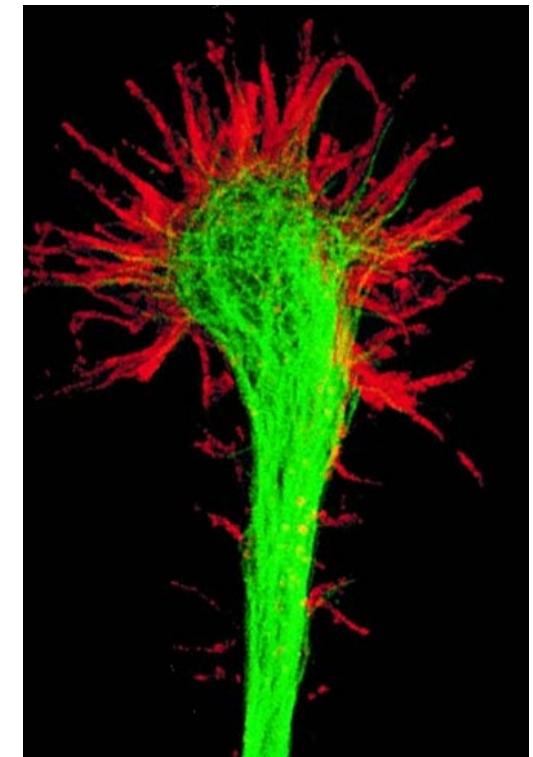
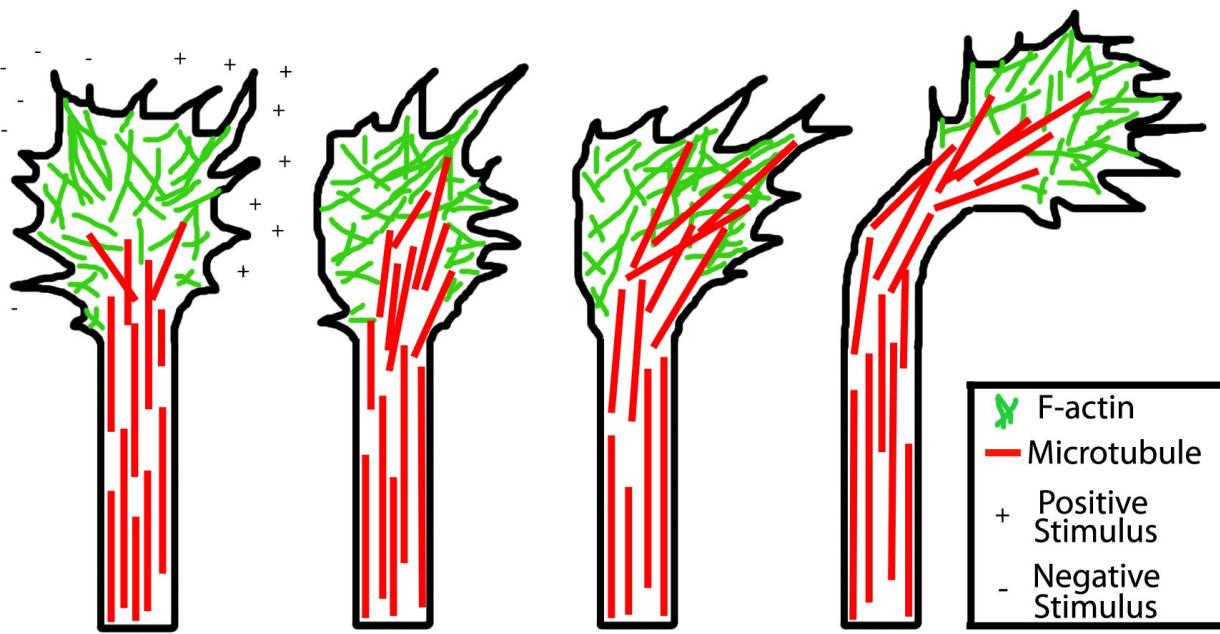
NEURON SPECIALIZATION

- Axons are too long to provide transport with microtubules
- Overlapping fragments of similarly oriented microtubules
- Transport:
 - anterograde/outward (kinesin)
 - retrograde/inward (dynein)

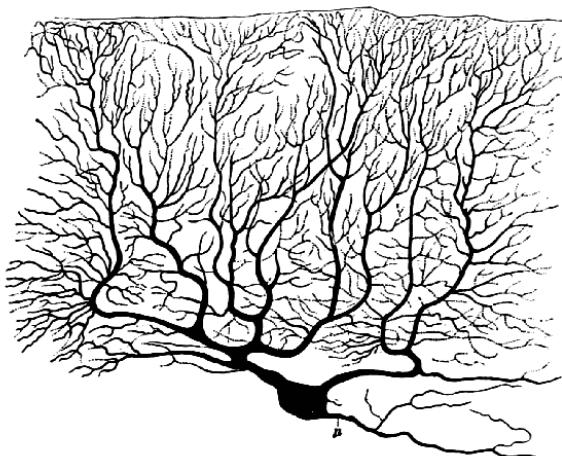


NEURON SPECIALIZATION

- Actin filaments => growth cone
- AF, IF, MT collaboration



Growth cone



Purkinje cell

LECTURES 10-11: CELL ORGANIZATION III

- Introduction to cytoskeleton
- Intermediate filaments
- Microtubules
- Actin filaments
- Muscle contraction
- Regulation of cytoskeleton biogenesis
- Polarity of the cell and cytoskeleton:
 - cell crawling (adhesion and traction)
 - chemotaxis
 - yeast budding
 - neuron specialization

