The Cytoskeleton

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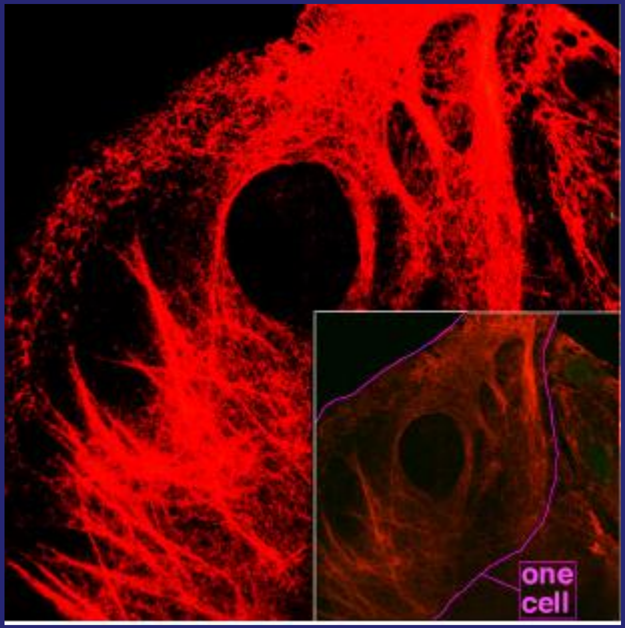
Biology, 2024

Content of the present lecture

1. Chemical and physical structure of cytoskeleton
2. Intermediate filaments, microtubules, centrosom, actin filaments
3. Self-assembly and dynamic structure of cytoskeletal filaments
4. How cells regulate their cytoskeletal filaments
5. Molecular motors

Scientists in Brno

prof. MUDr. Oldřich Nečas, DrSc.



Nečas, O et al.: Cytoskelet. Academia, 1991

The cytoskeleton – fibrillar structures

Cytoskeleton

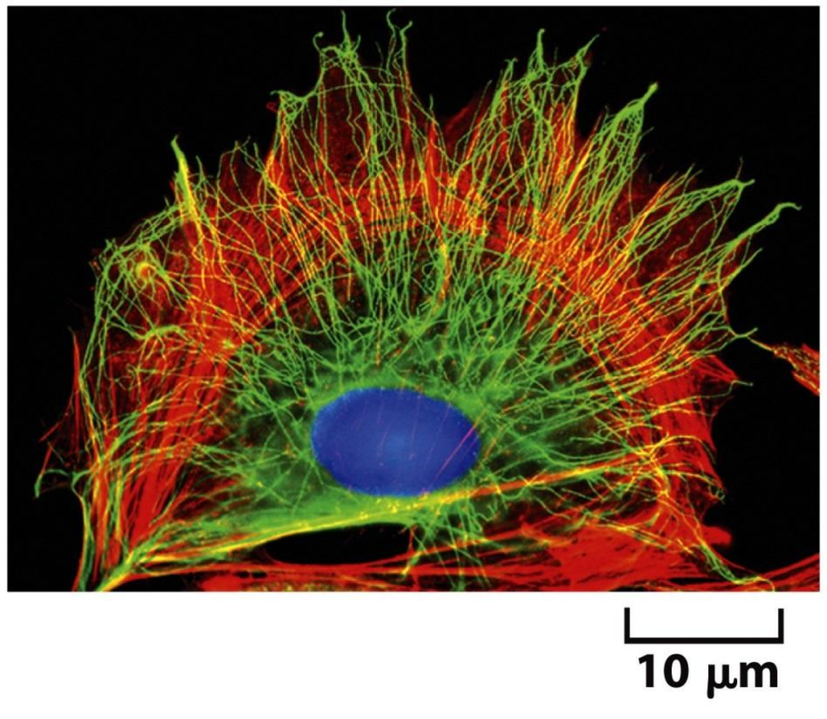
the system of proteins filaments and tubules which main functions are <u>transport</u> of substances and cell components, <u>cell support</u> and participation on <u>cell division</u>

System of filaments situated in cytoplasm and nucleus

The cytoskeleton – fibrillar structures

Fixed and labelled cell in culture

Microtubules (green)



Actin filaments (red)

DNA in nucleus (blue)

Major types of cytoskeleton filaments

Microtubules

(determine the positions of membrane-enclosed organelles and direct nuclear transport)

Microfilaments, actin filaments

(determine the shape of the cell's surfaces + cell locomotion)

Intermediate filaments

(provide mechanical strength)

Cytoskeleton is dynamic structure

?

The cytoskeleton – fibrillar structures

Cytoskeleton

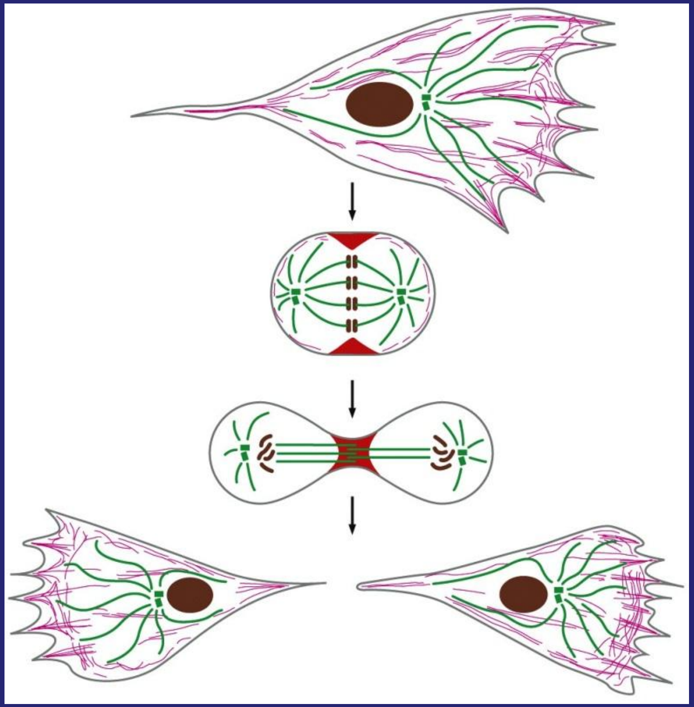
the dynamic system of proteins filaments and tubules which main functions are <u>transport</u> of substances and cell components, <u>cell support</u> and participation on <u>cell division</u>

System of filaments situated in cytoplasm and nucleus

Rapid changes in cytoskeleton

Cytoskeleton

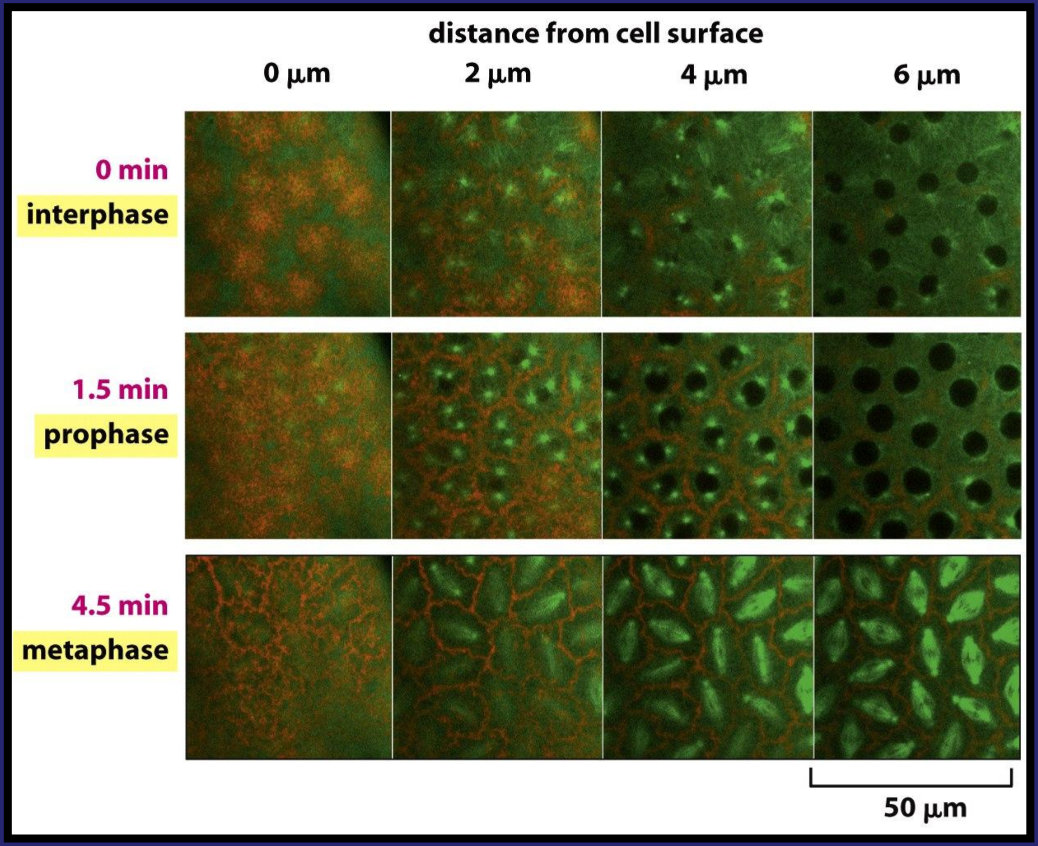
is reorganised by rapid changes, for example during cell division



Crawling fibroblast with polarized dynamic <u>actin</u> cytoskeleton (<u>red</u>). The polarization is assisted by <u>microtubule</u> cytoskeleton (<u>green</u>). Chromosomes are drawn brown.

Rapid changes in cytoskeleton

Rapid changes in cytoskeletal organisation observed during the development of *Drosophila* early embryo



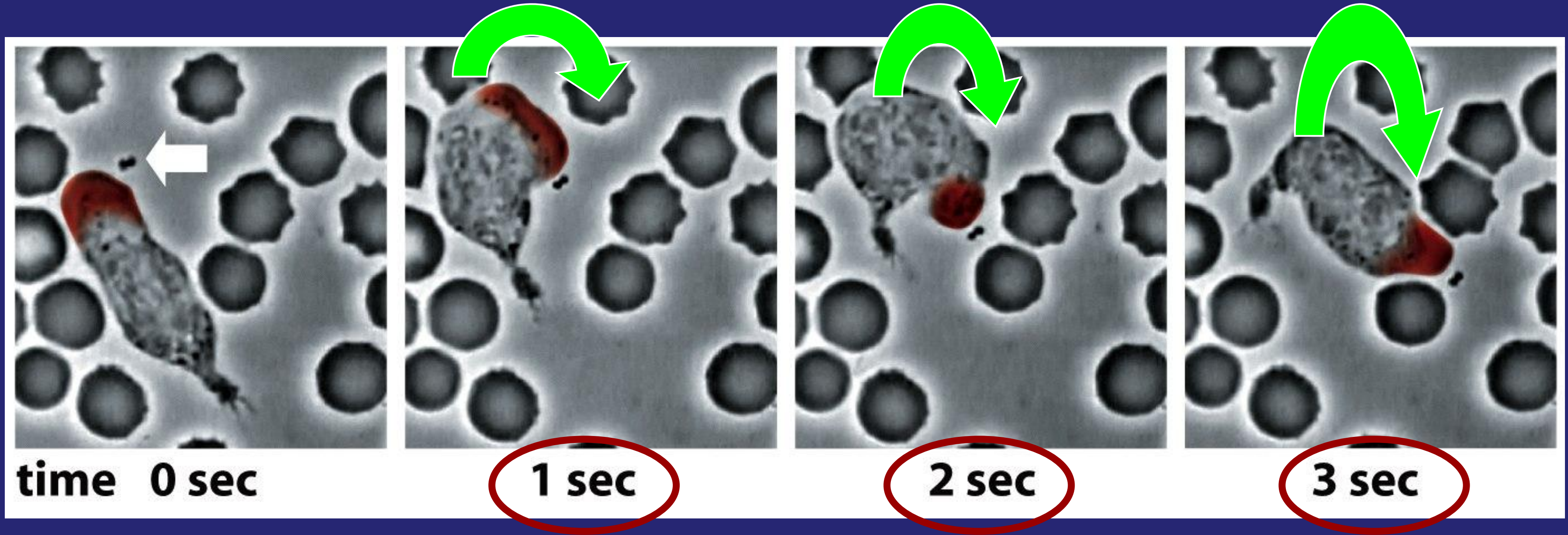
Actin filaments = red

Microtubules = green

A neutrophil in pursuit of bacteria

Clump of bacteria (white arrow) is about to be captured by a neutrophil

As the bacteria move, the neutrophil quickly reassembles the dense actin network at the leading edge (red) to push toward the location of the bacteria



Cytoskeleton can form also stable structures

- **stable structures are typical for such cells that have achieved a <u>stable</u>, <u>differentiated morphology</u>**
- **typically neurons or epithelial cells**

Although the <u>actin bundles</u> must maintain their stable organisation for the entire lifetime of the animal, the <u>individual actin filaments</u> remain strikingly dynamic and are continuously remodelled and replaced on average every 48 hours

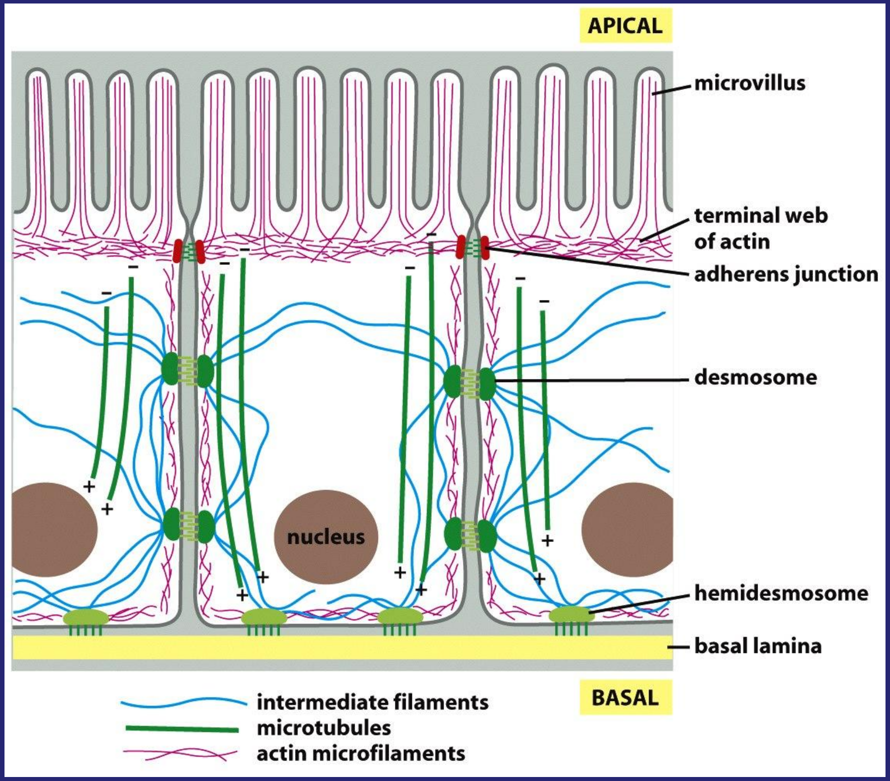
Cytoskeleton is responsible for large-scale cellular polarity

Besides forming stable specialised cell surface protrusions

Polarity enabling cells to tell the difference between
- **top and bottom**
- **front and back**

Polarized epithelial cells maintain the critical functions between
- **apical surface = absorbs nutrients**
- **basolateral surface = transfer nutrients through plasma membrane to the bloodstream**

Cytoskeleton in polarized epithelia



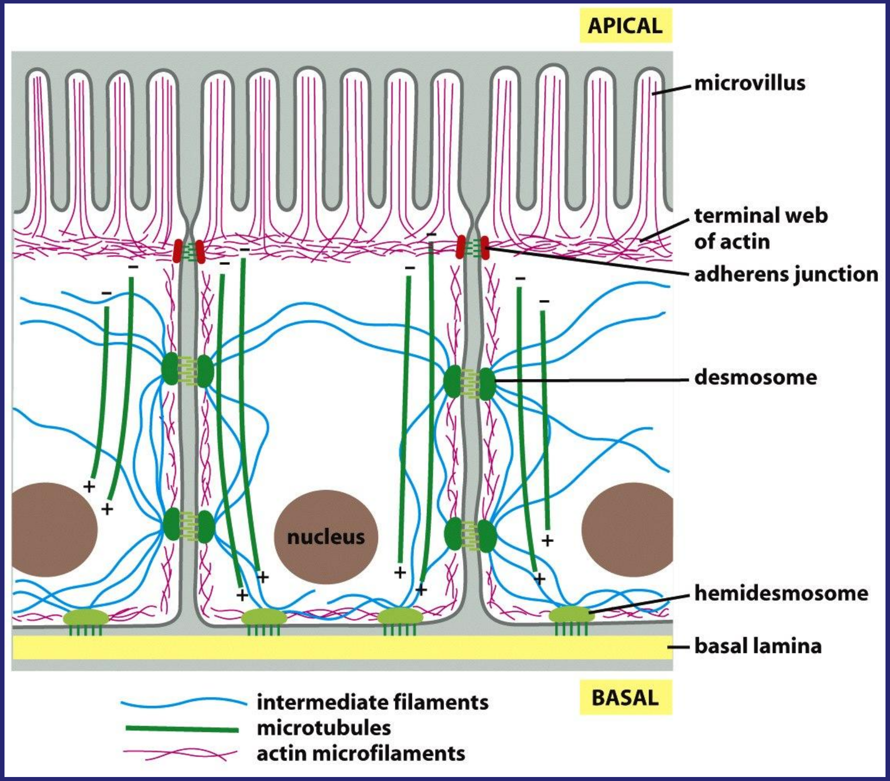
Apical surface form microvilli that increase the cell surface (formed by actin filaments, red)

Below the microvilli band of actin filaments form cell-cell junctions

Intermediate filaments (blue) are anchored to other structures

All the components of the cytoskeleton cooperate to produce the characteristic shapes of specialized cells

Cytoskeleton in polarized epithelia



Apical surface form microvilli that increase the cell surface (formed by actin filaments, red)

Below the microvilli band of actin filaments form cell-cell junctions

Intermediate filaments (blue) are anchored to other structures

Microtubules (green) provide a global coordinate system

The cytoskeleton functions

Structural support of the eukaryotic cell

- **mechanical stability of the cell**
- **cell shape**
- **internal arrangement (spatial organization) of the organelle**

Enables the cell movement

Regulate the cell movement

Where one can find the cytoskeleton?

1) Cytoskeleton components are freely available in the cell
2) They create organelles and their parts
3) They participate on building of eukaryotic flagellum, centrioles, spindle body, etc.
4) Microtubules freely penetrate the whole cell
5) Actin filaments form dense network directly under the cell surface

Major types of cytoskeleton filaments

Microtubules

(determine the positions of membrane-enclosed organelles and direct nuclear transport)

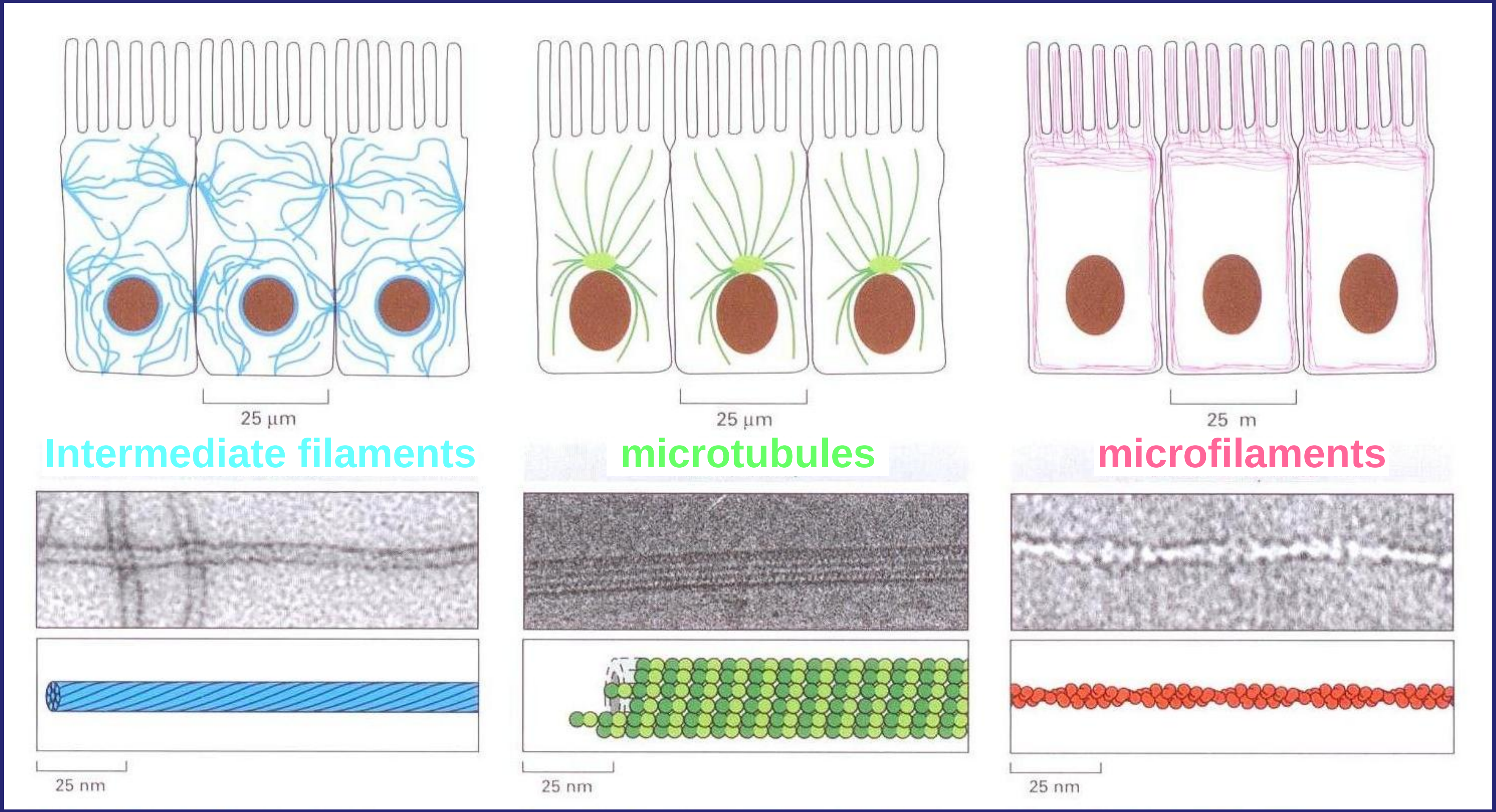
Microfilaments, actin filaments

(determine the shape of the cell's surfaces + cell locomotion)

Intermediate filaments

(provide mechanical strength)

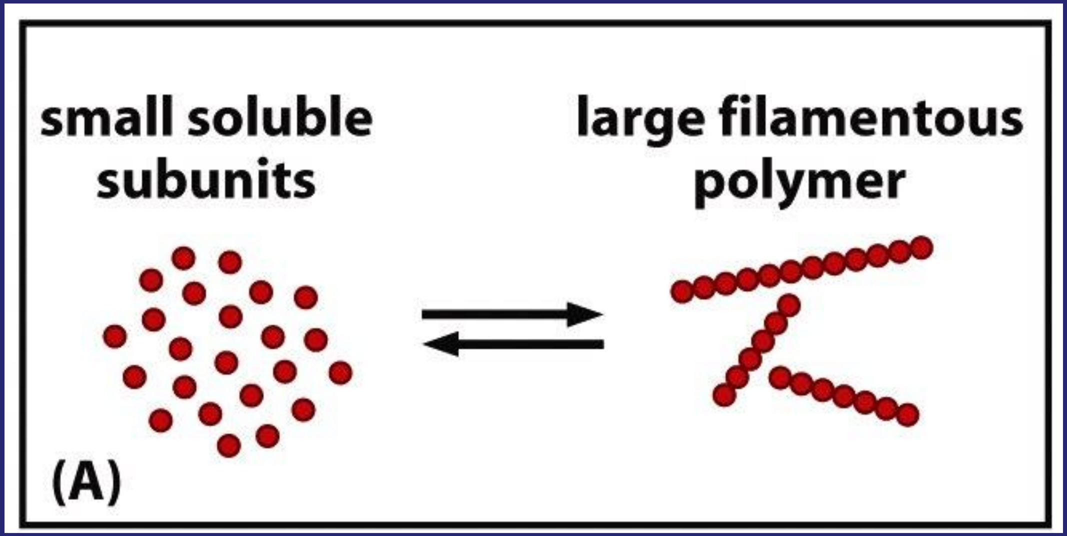
Topography of filaments



Cytoskeleton filaments are constructed from smaller protein subunits

Cytoskeleton during changes

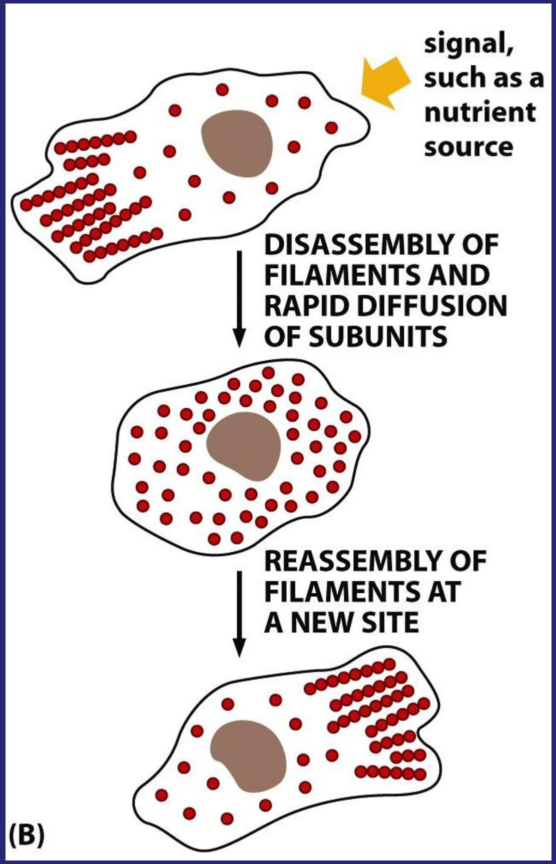
The formation of protein filaments from much smaller subunits allows regulated filament assembly and disassembly to reshape the cytoskeleton



Filament formation from a small protein subunits

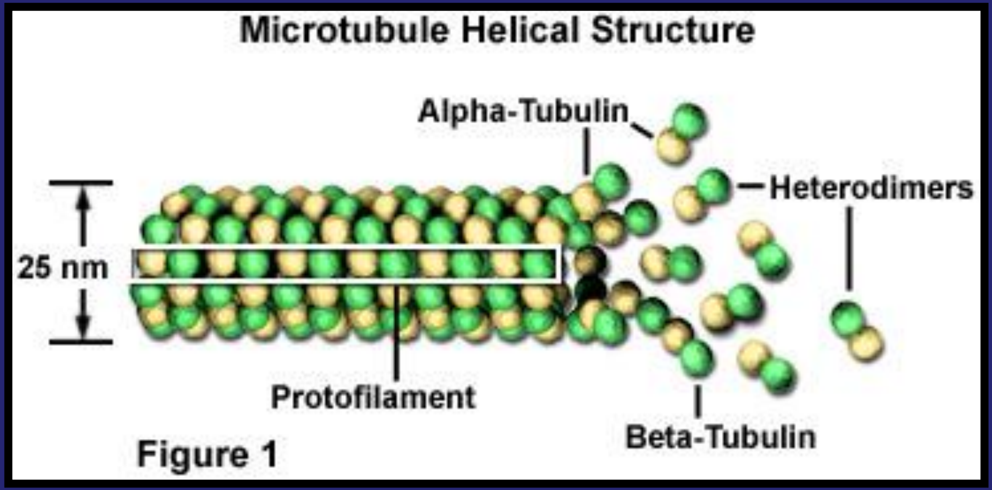
Cytoskeleton reorganization

Rapid reorganization of the cytoskeleton in a cell in response to an external signal

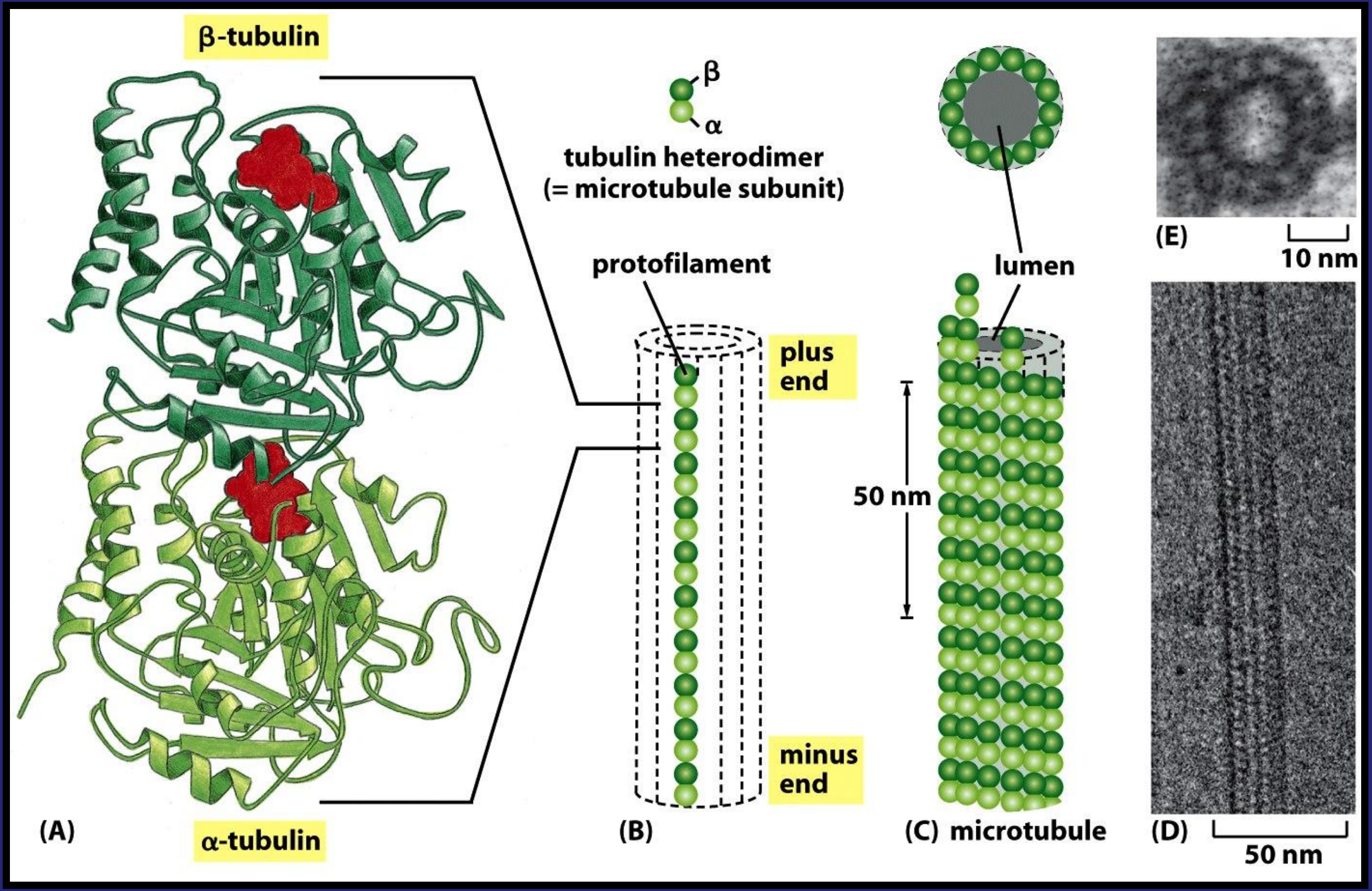


Microtubules

- are long, hollow cylinders made of the protein tubulin
- have an outer diameter of 25 nm
- consist of 13 parallel protofilament
- protofilaments are polymers of tubulin α and β, which form heterodimers



Structure of microtubules

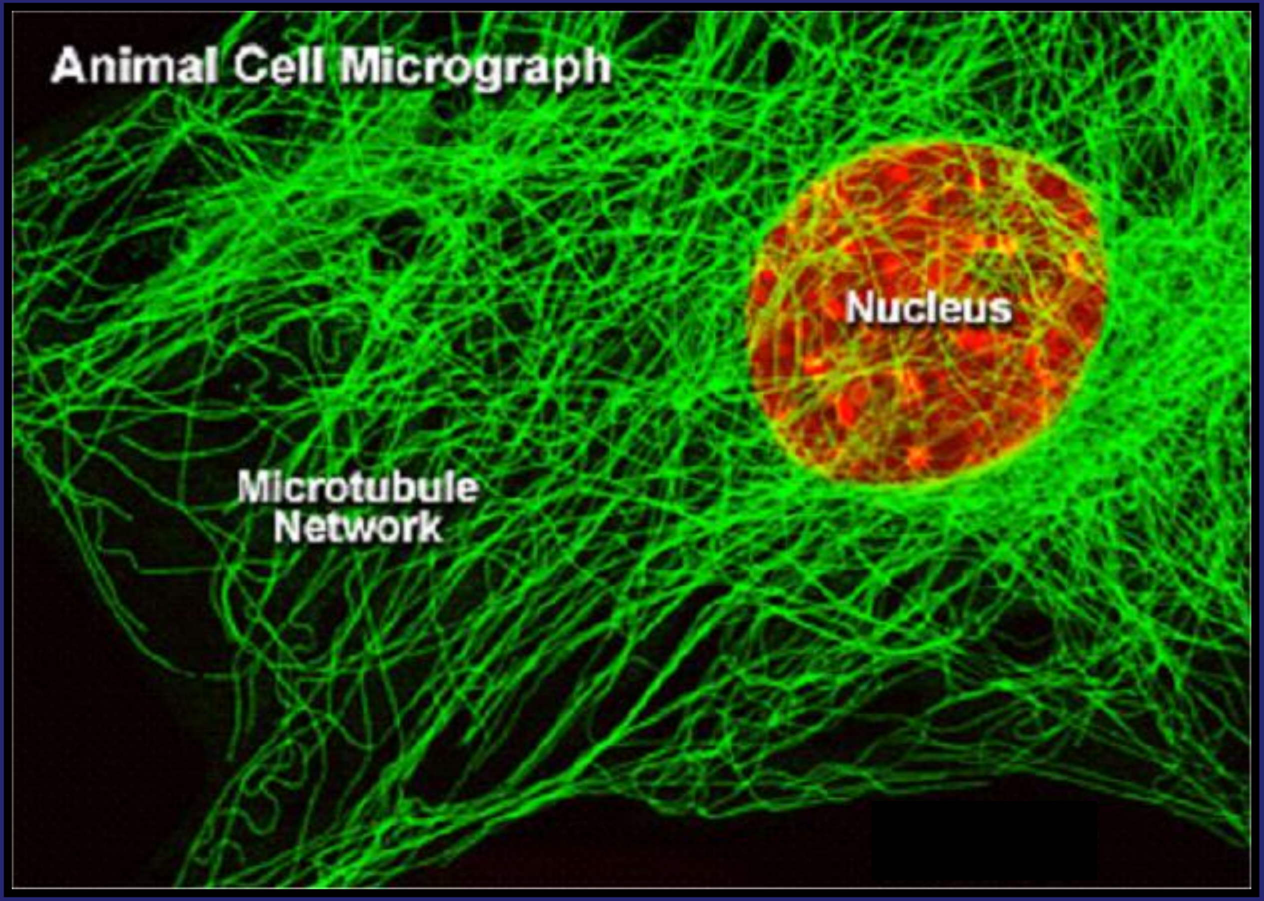


Functions of microtubules

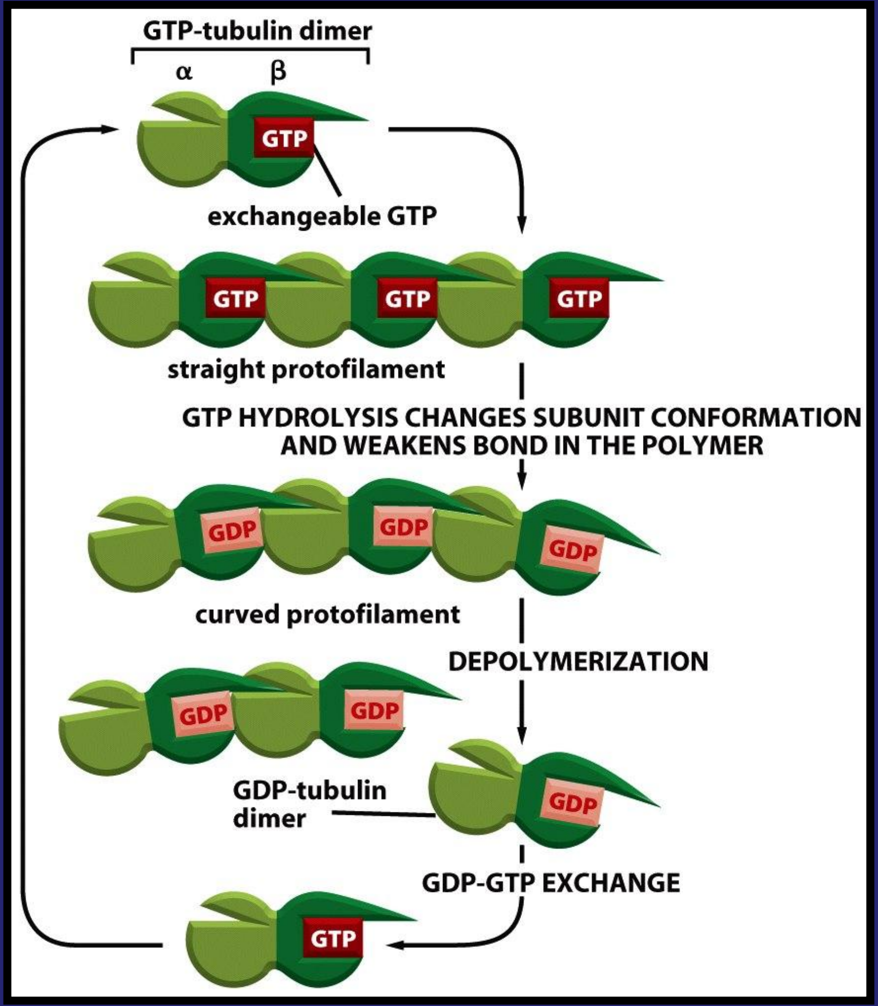
- microtubules are long and straight and typically have one end attached to a single microtubule-organising centre called a centrosome

- they run out the centrosome to outer part of cell
- organise organelle movement and determine the positions of membrane-enclosed organelles
- direct nuclear transport
- help forming cell shape and cause as a support skeleton of cell

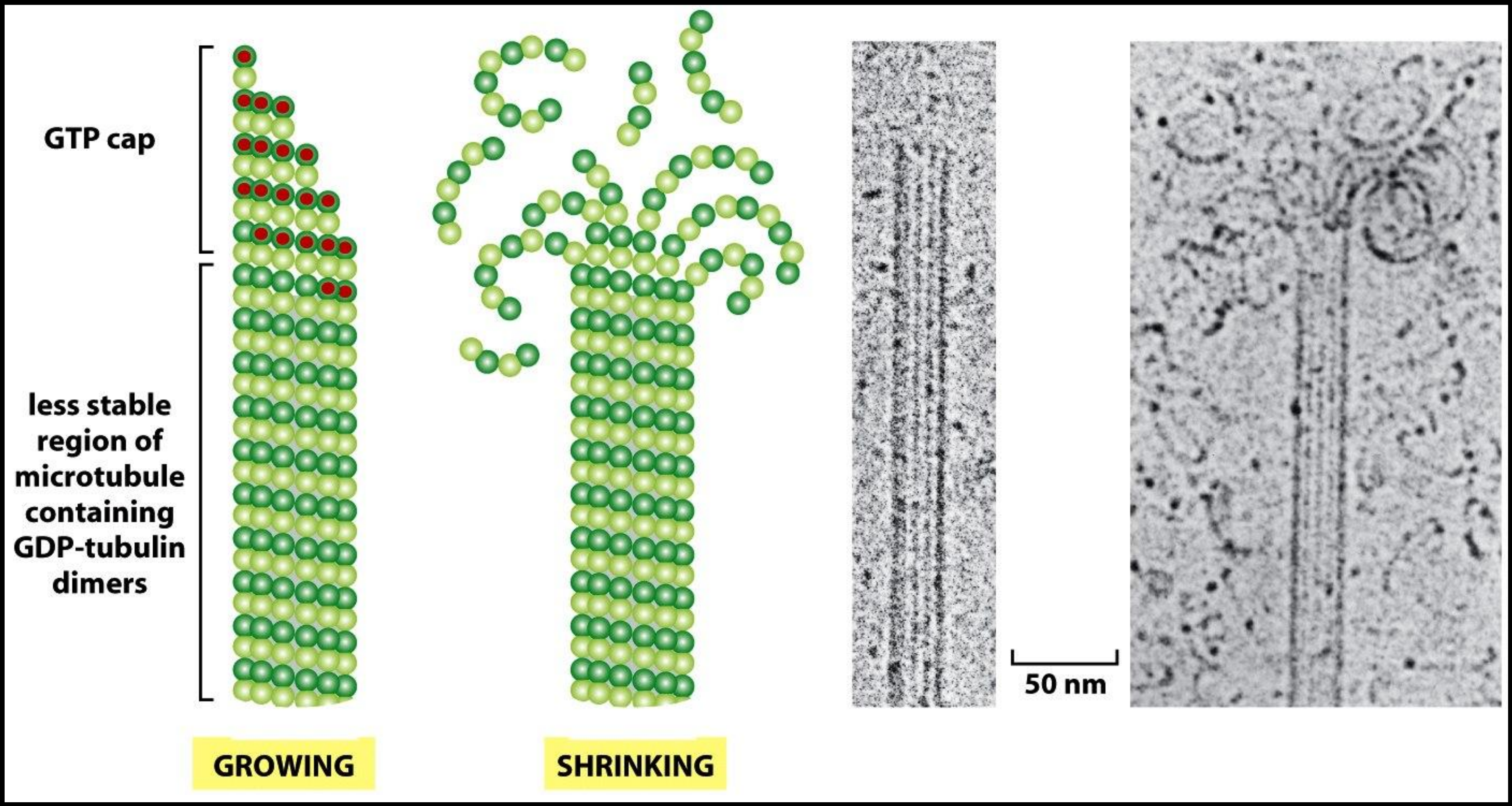
Microscopy of microtubules



Dynamic instability of microtubule end



Growing and shrinking of microtubule end



Microtubules and colchicine

- Colchicine is so named „<u>mitotic toxin</u>“

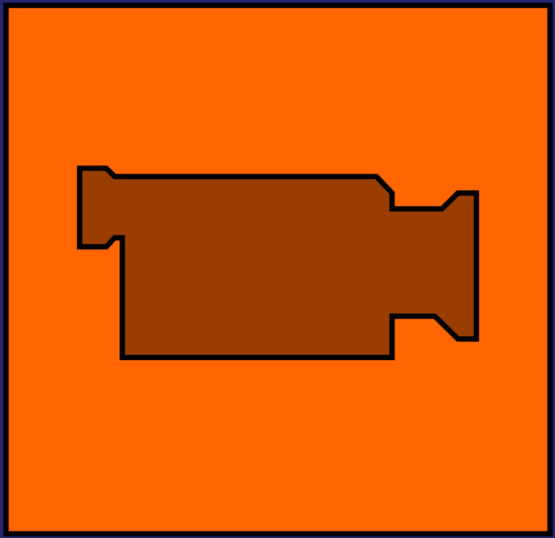
1) It inhibits the polymerisation of tubuline protomers
   → inhibits mitotic spindle formation
   → stops mitosis in metaphase

2) It stops the cell movements → slow down leukocyte movement → curing of acute phase of holarthitis

- The similar effects have vinblastine and vincristine from *Vinca rosea*
- Taxans from *Taxus brefifolia* accelerate tubules formation, stabilised them and inhibit depolymerisation

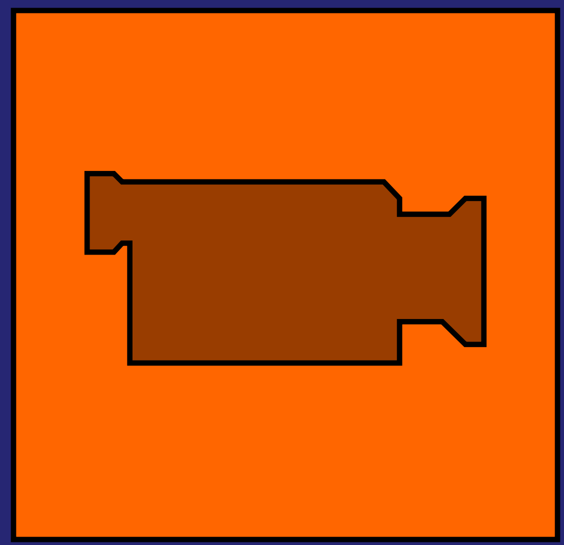
Microtubules

http://www.youtube.com/watch?v=5rqbmLiSkpk&feature=related



Cytoskeleton microtubules.wmv

Microtubule dynamics



16.5-microtubule_dynamics.mov

Actin filaments, microfilaments

- two-stranded helical polymers of the protein actin
- they appear as flexible structures with a diameter of 5-9 nm

- are organised into a variety of linear bundles, two-dimensional networks, and three-dimensional gels
- actin filaments are dispersed through the cell
- but most highly are concentrated in the cortex, just beneath the plasma membrane

Actin filaments functions

- <u>mechanically supporting function</u>
- altogether with myosin form the <u>contractile apparatus</u>
- responsible for a lot of type of <u>intracellular movements</u>
  - the cytoplasm flux
  - the cell protrusions and invaginations forming
- on higher levels the actin and myosin are the components of muscle cells

Microfilaments structure

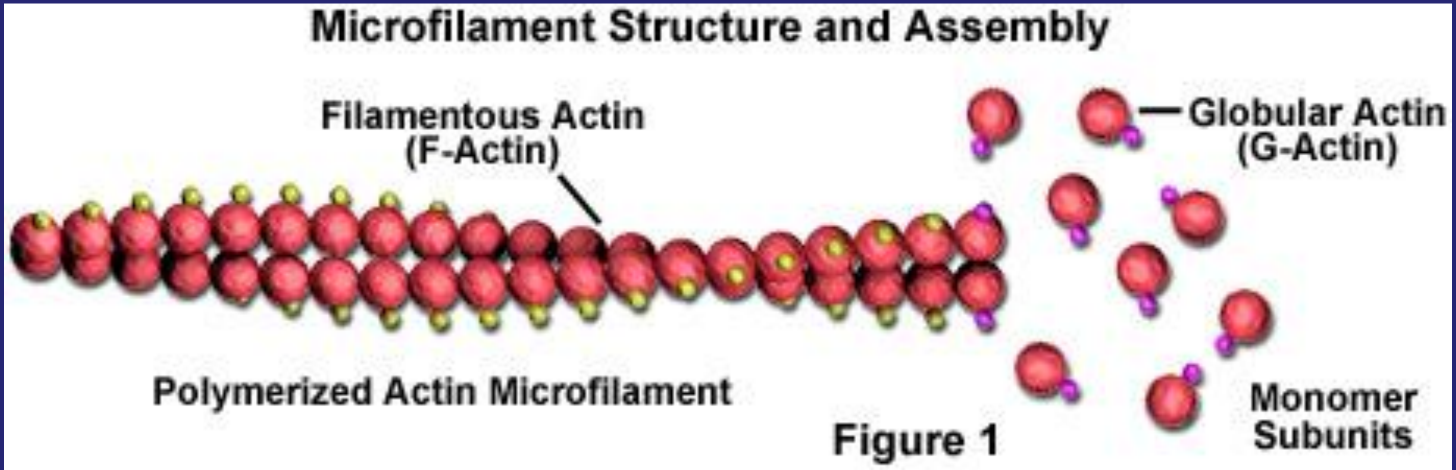


Figure 1

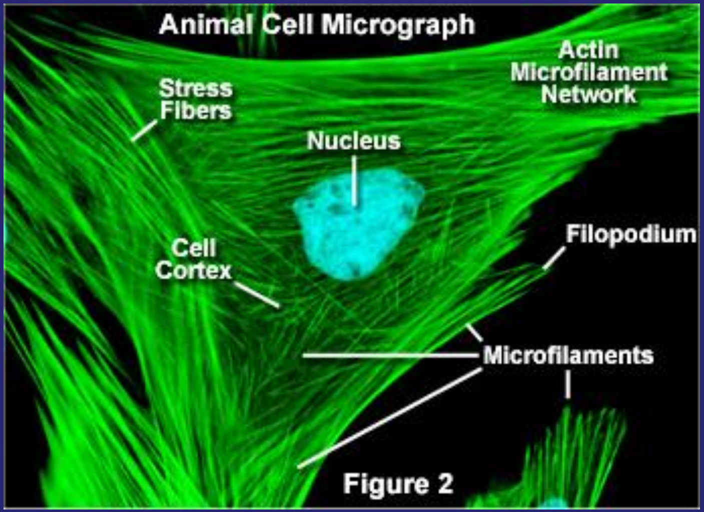
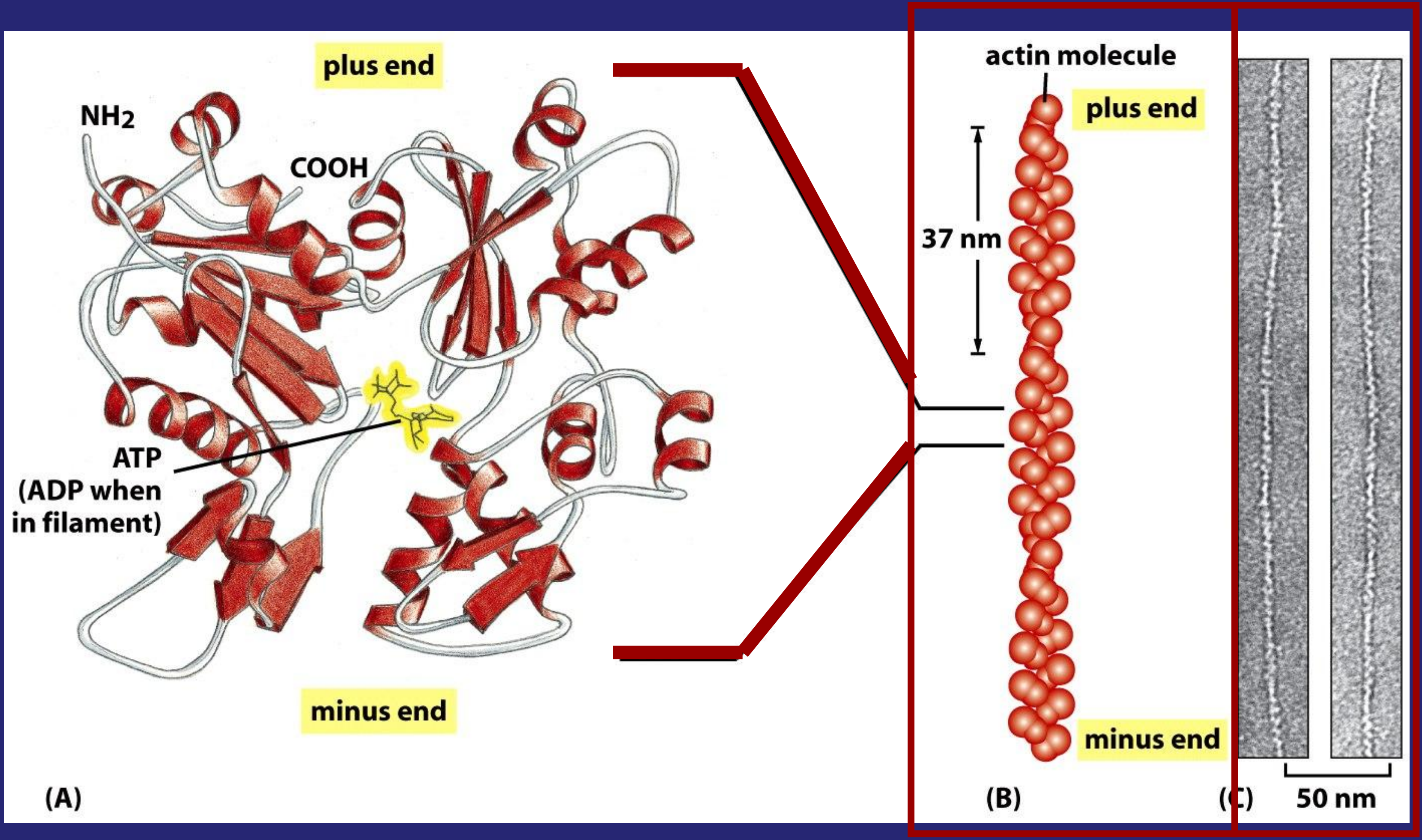


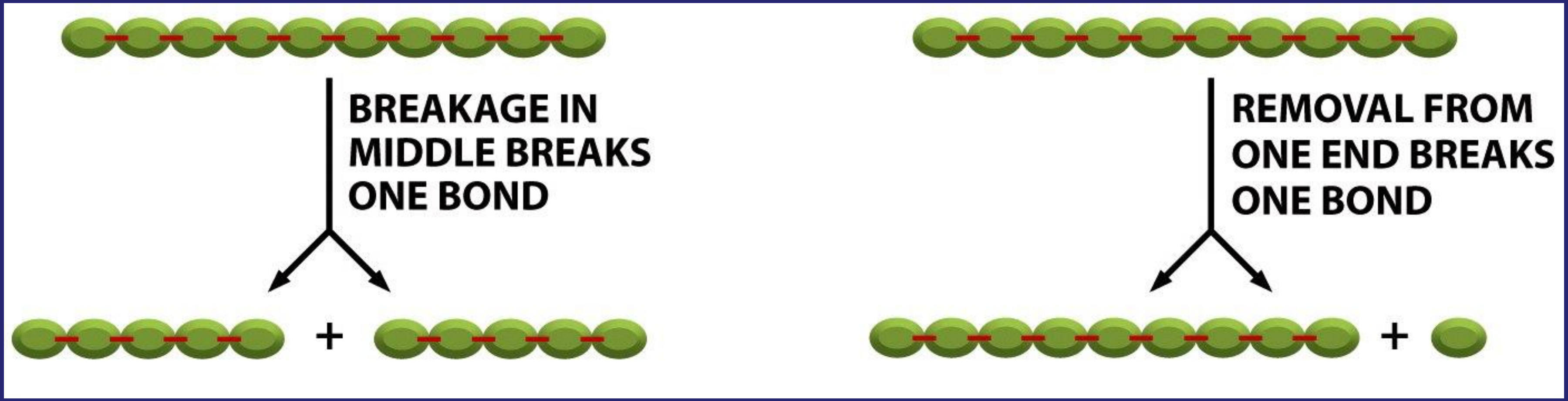
Figure 2

Microfilaments structure



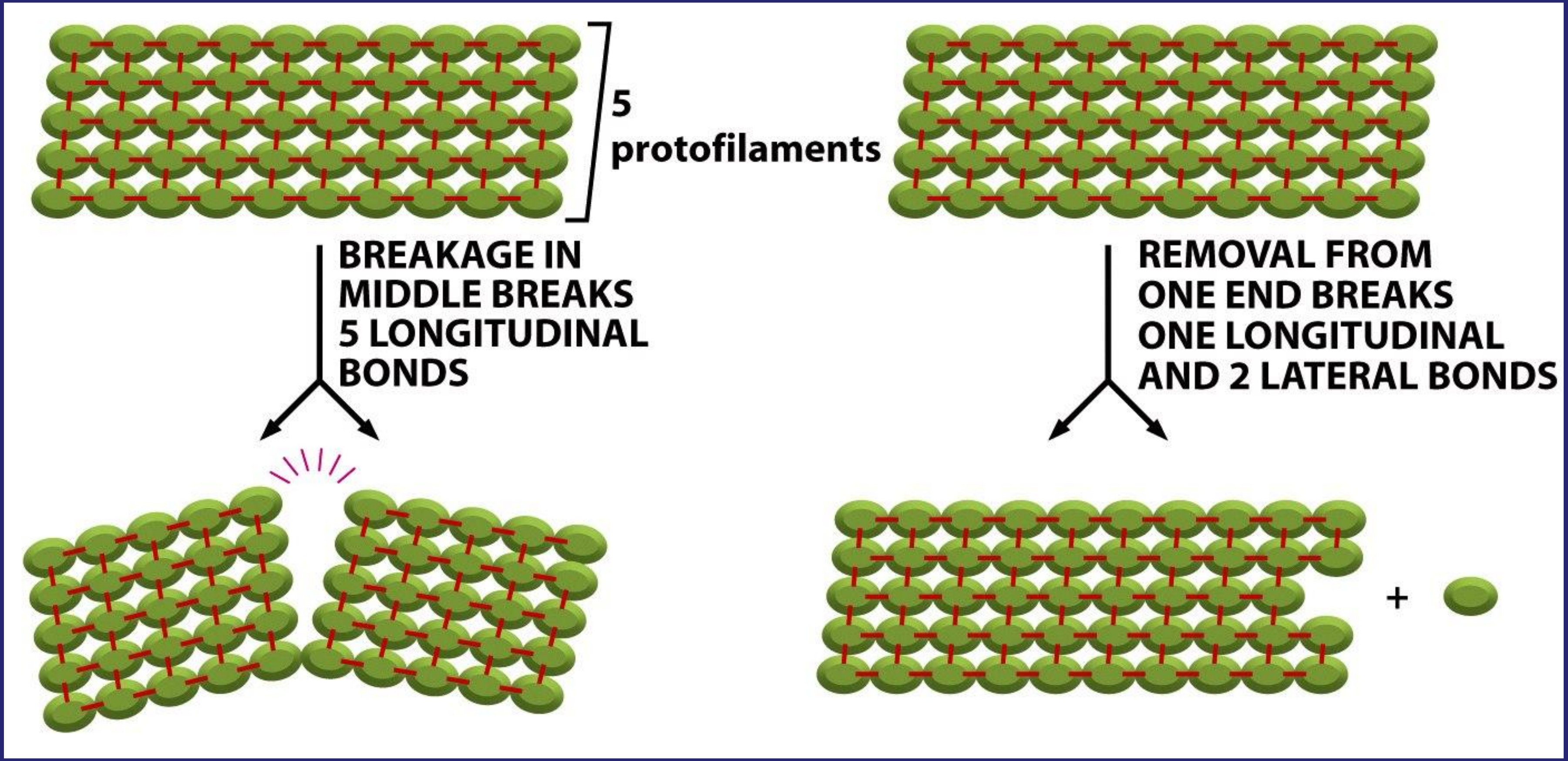
Filaments resist thermal breakage

Single protofilament: thermally unstable



Filaments resist thermal breakage

Multiple protofilament: thermally stable



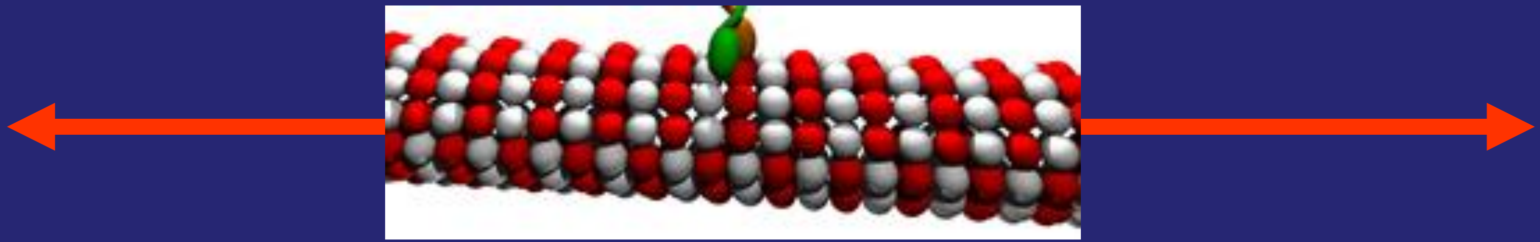
Molecular motors

- bind to polarized cytoskeletal filament and use the energy derived from repeated cycles of ATP hydrolysis to move steadily along it
- transport shipment along microtubules
- organise movement of organelles, vesicles, etc.
- function is strongly connected with ATP hydrolysis
- kinesins and cytoplasmic dyneins

Kinesin and dynein

Kinesin

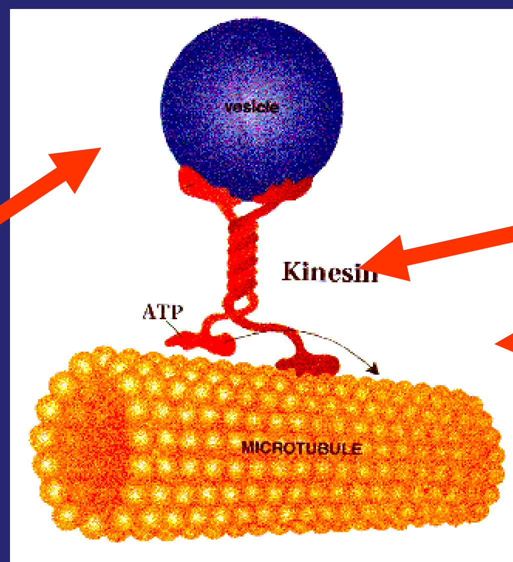
Movement to plus end of microtubule, from centrosome to cell periphery



Dynein

Movement to minus end of microtubule, to centrosome

Kinesin



kinesin (dimer)

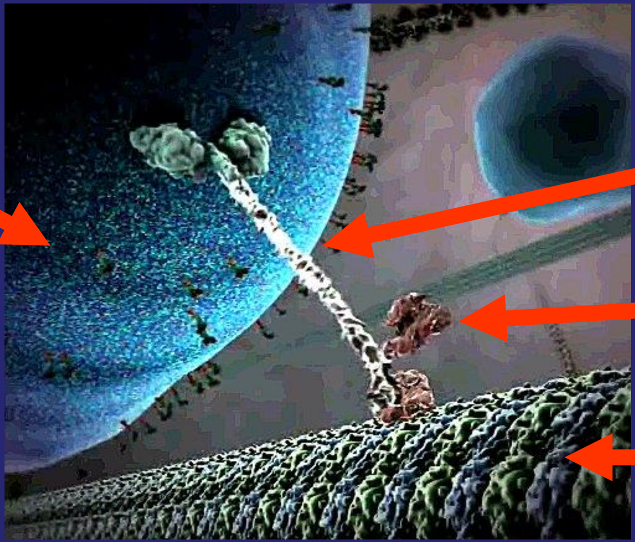
ATP

microtubule

organelle

Kinesin

organelle

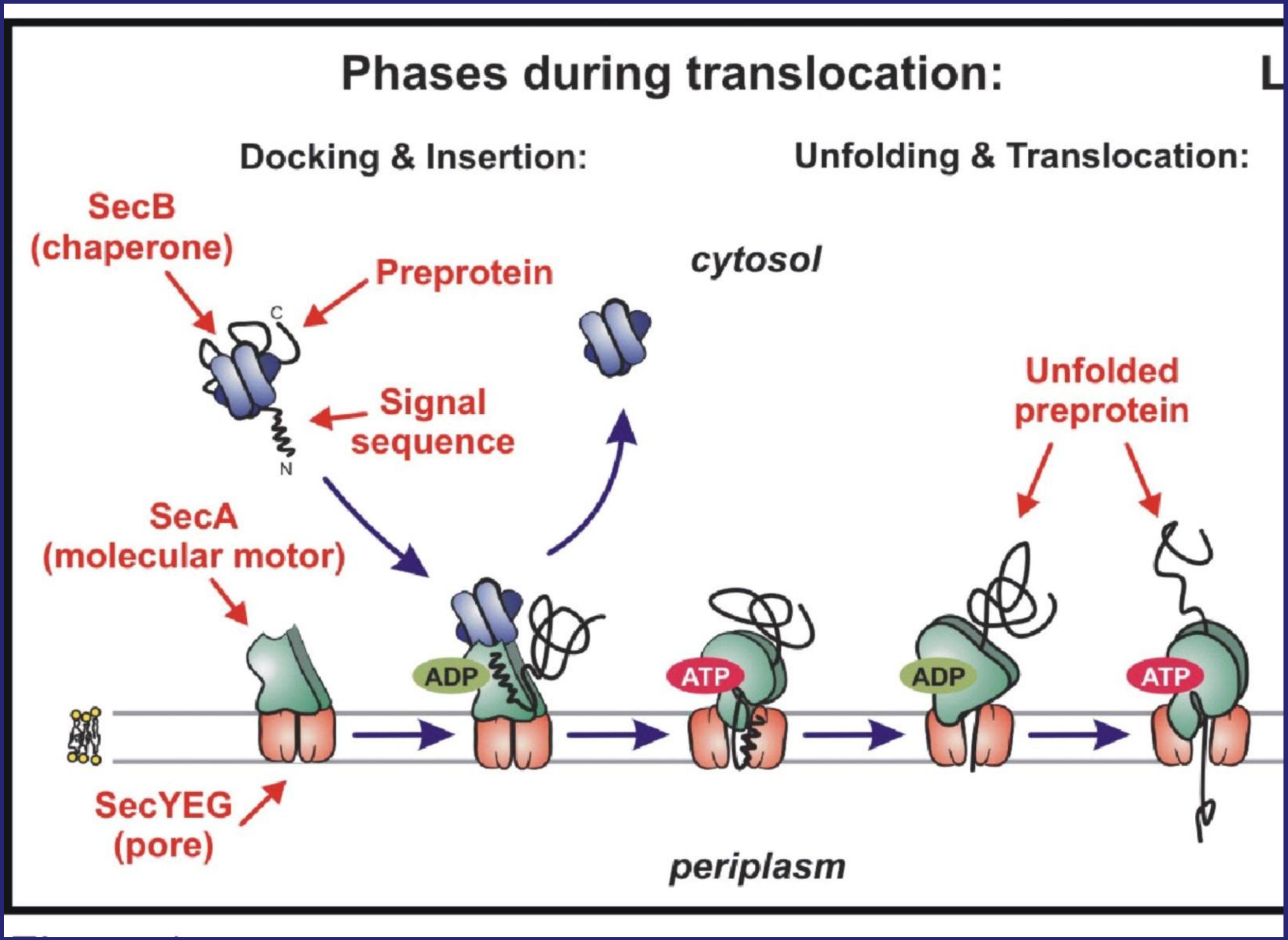


kinesin (dimer)

ATP

microtubule

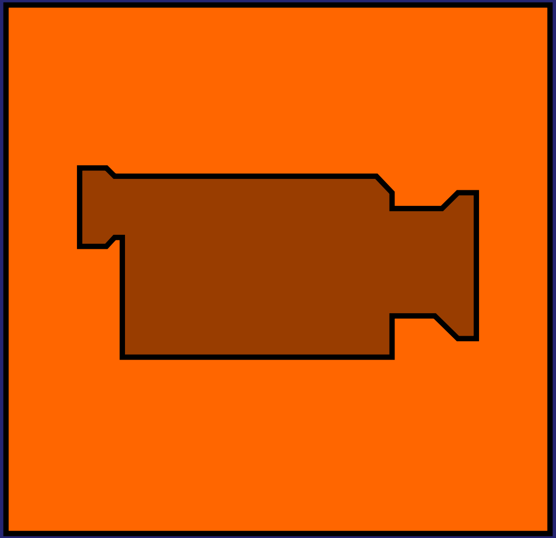
Phases during translocation



http://www.biomach.org/Molecular_Devices/Biological_Motors/Ion_Channels_and_Nanopores/SEC_YEG.jpg

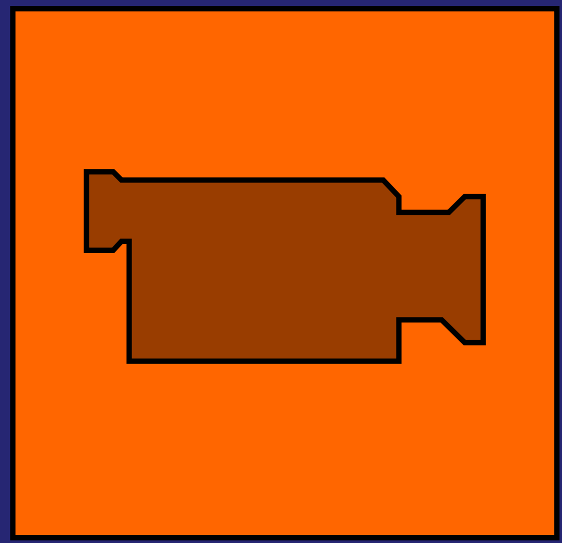
Kinesin explanation

http://www.youtube.com/watch?v=lLxlBB9ZBj4



Kinesin.avi + 16.7 Kinesin.mov

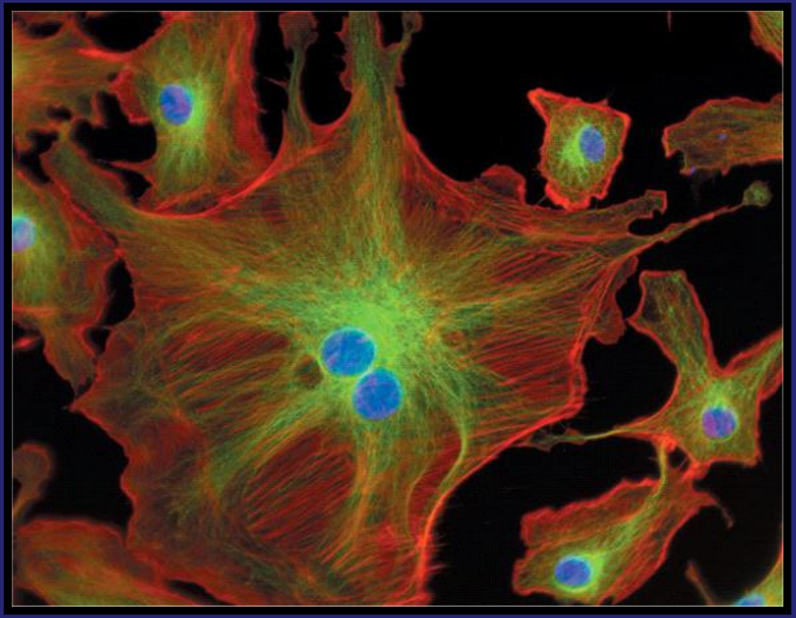
Organelle movement



16.6-organelle_movement.mov

Intermediate filaments

- rope-like fibbers with a diameter of around 10-15 nm
- made by intermediate filament proteins
- different sizes and constitutions in different cell types; also in the same cells of different animal species



- extend across the cytoplasm giving cells mechanical strength
- in an epithelia strength the entire epithelium
- nuclear lamina just beneath the inner nuclear membrane

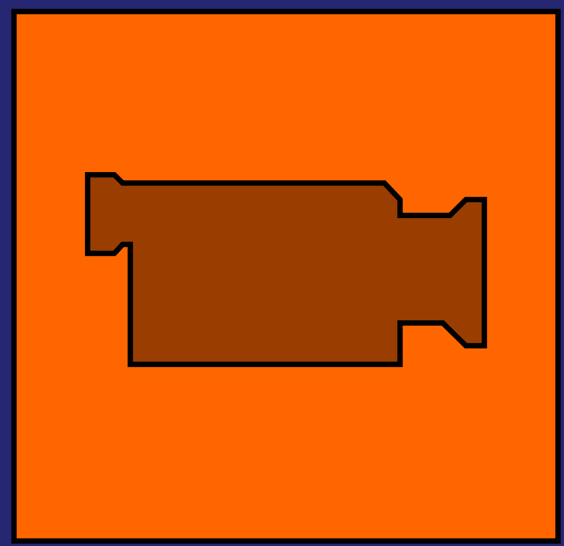
Types of intermediate filaments

According to basic protein subunit

1) **Vimentin-like = cells of mesenchymal origin**
2) **Desmin – muscle cells**
3) **Neurofilament proteins = neurons**
4) **Glial fibrillary acidic proteins = glial cells**
5) **Cytokeratins = epithelial cells and their derivatives (hair, feathers, nails, skin, …)**

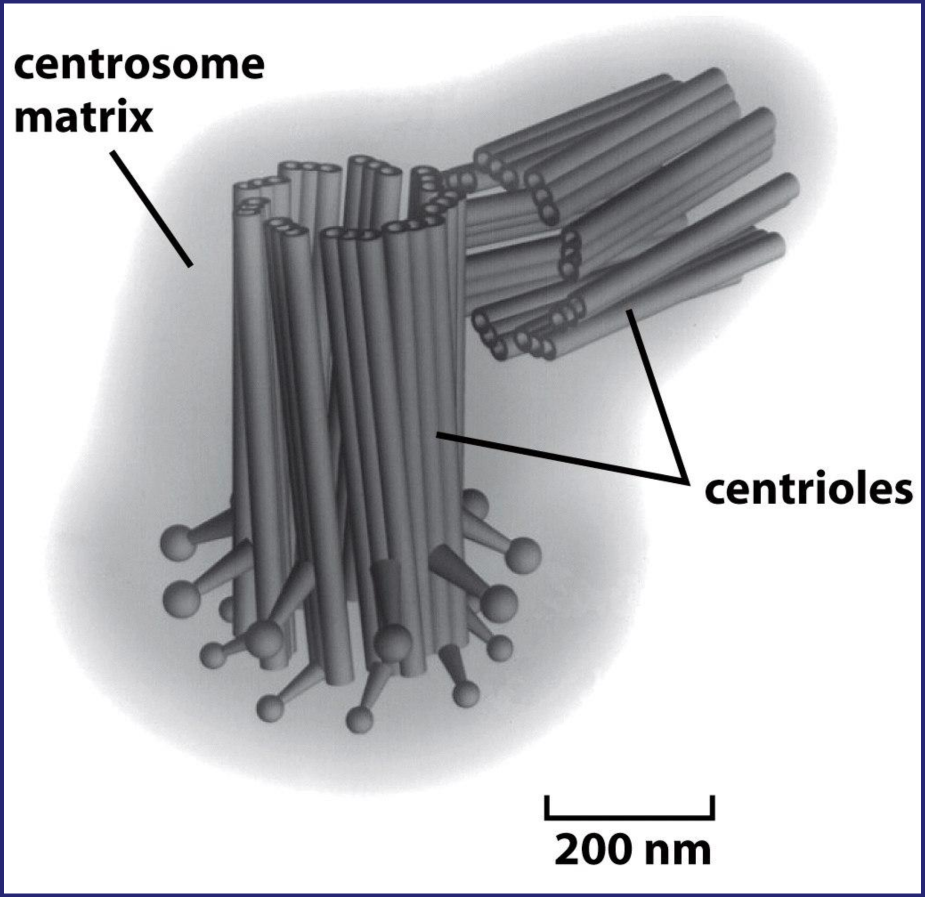
Intermediate filament

http://www.youtube.com/watch?v=FoDniO676Dw



Intermediate filaments.wmv

Centrosome – mitotic spindle body



Centrosome function

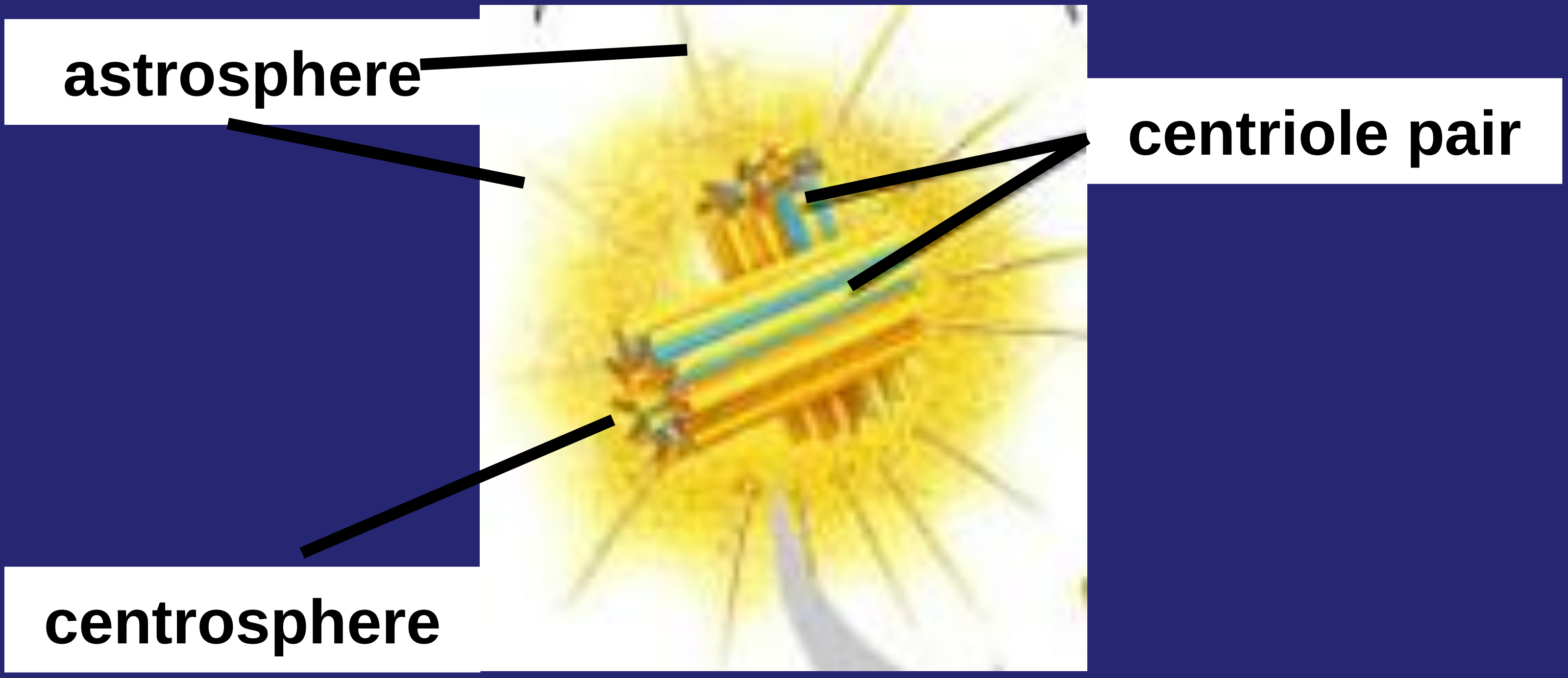
- organelle of animal cells and the cell of lower plant
- associated with the nuclear membrane
- consists of microtubules and associated proteins

- serves as the main microtubule organizing centre
- participate in separation of chromosomes during nucleus division
- undermine orientation of chromosomes and their movement to poles of mitotic spindle

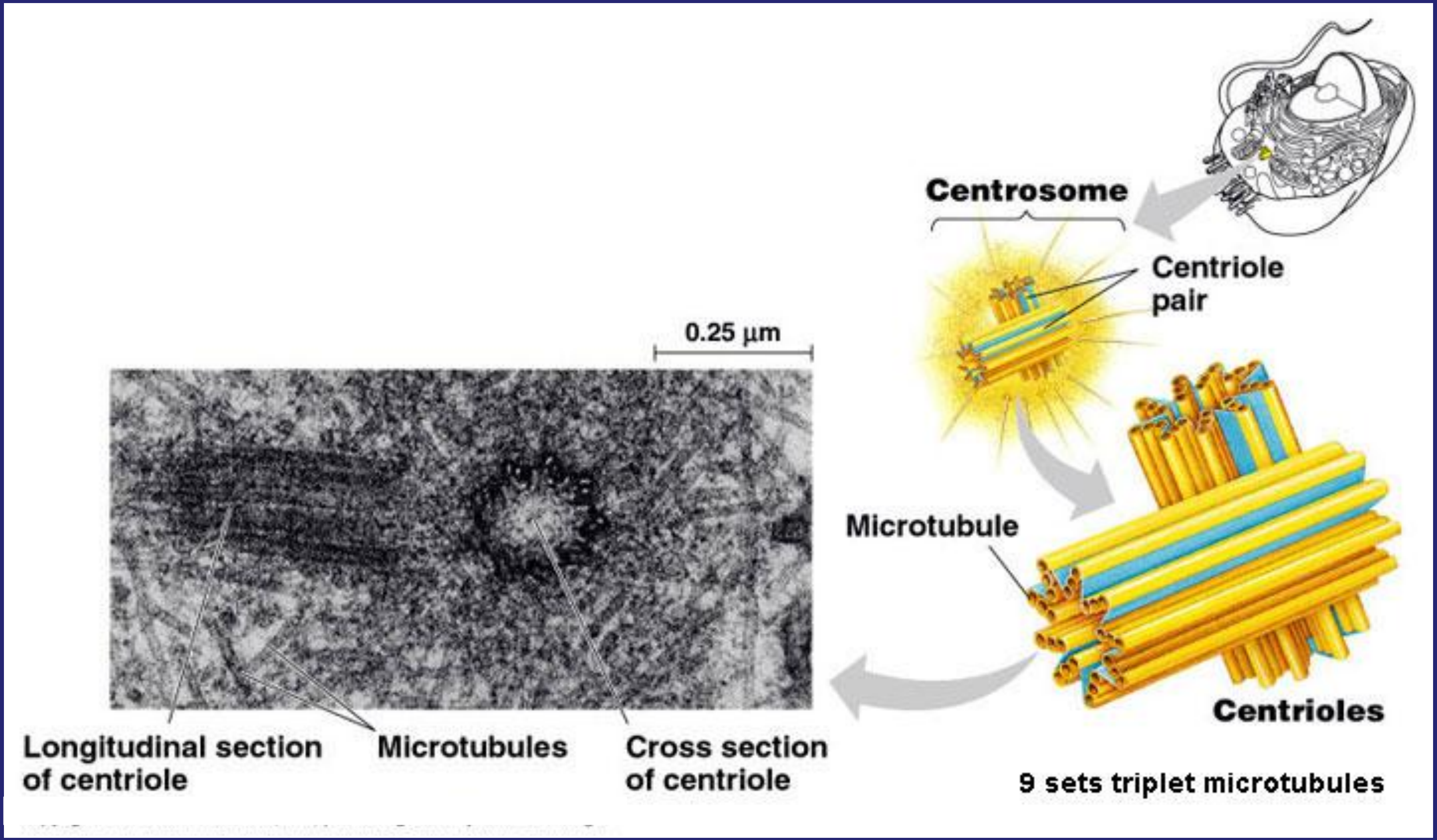
- during egg cell maturation disappears
- it is transport to zygote by sperm

Centrosome structure

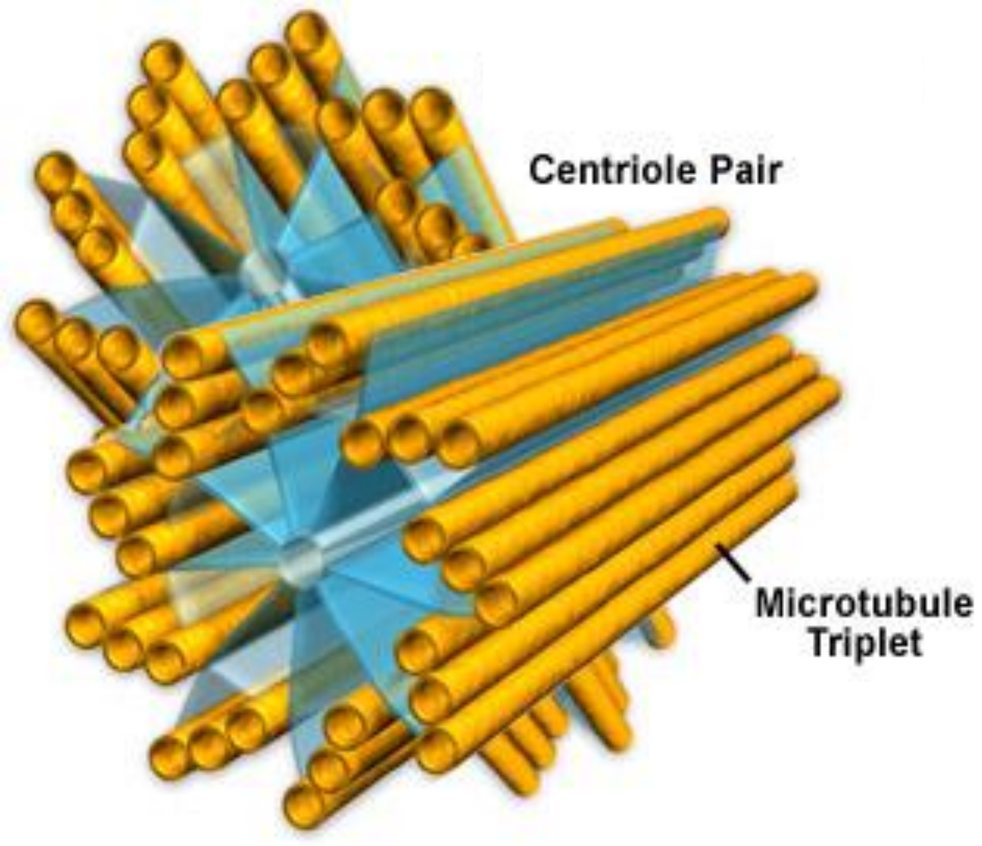
- **centriole** - central body
- **centrosphere** - dense unstructured net surrounding the centriole
- **astrosphere** - slurry fibers of cytoplasm protruding from the centrosphere



Details of centriole structure

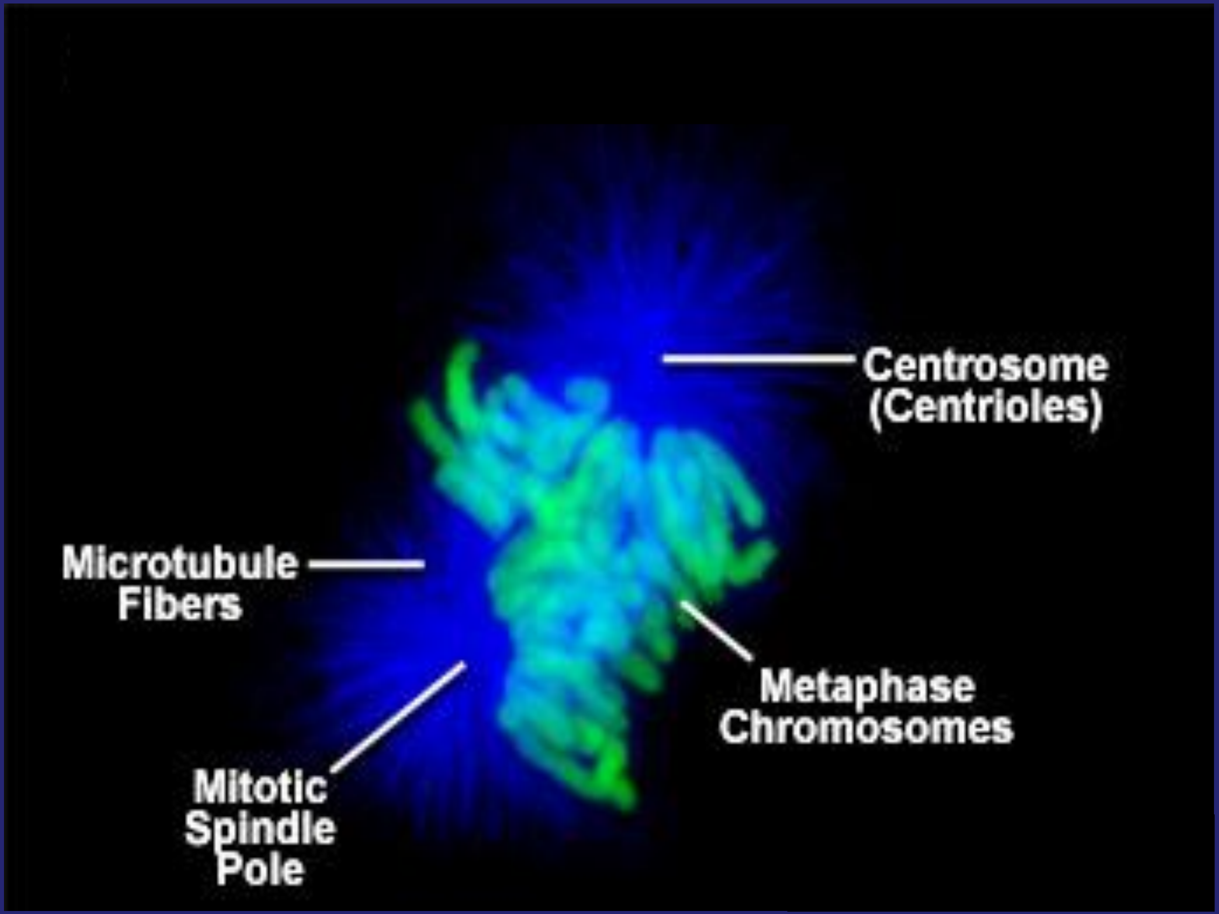


Centriole structure



A total of 9 microtubule triplets is in each centriole

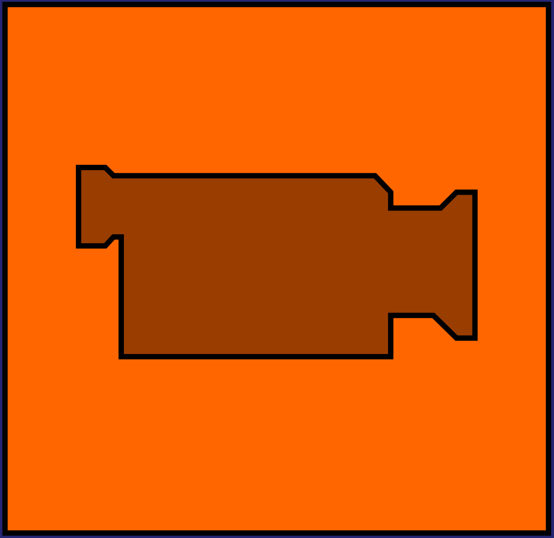
Centriole during mitosis



More information on …

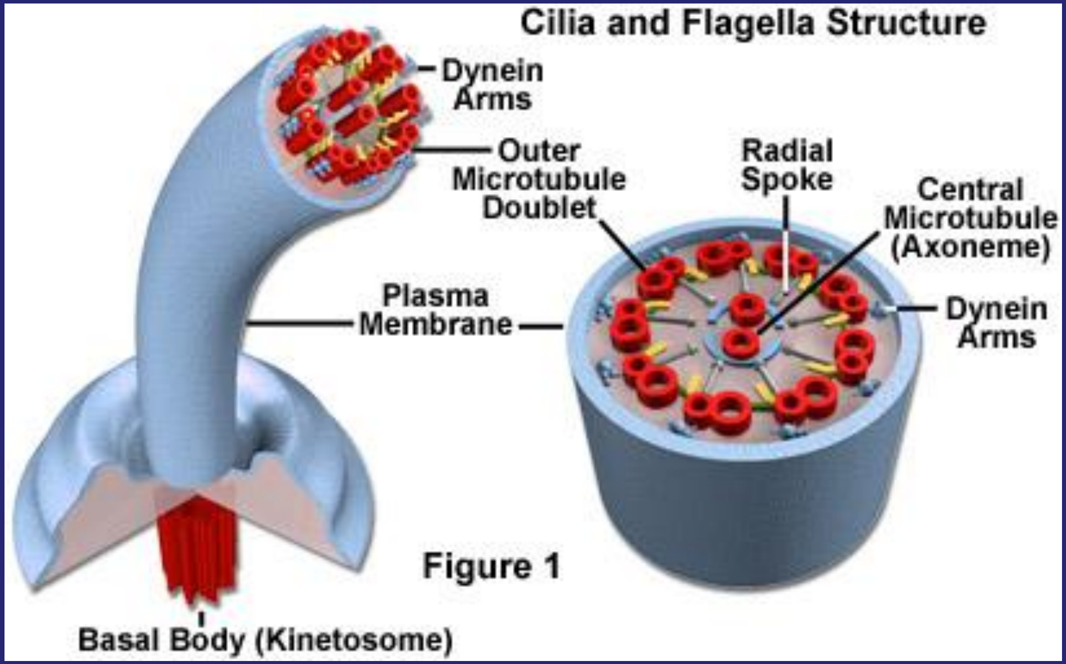
http://www.nature.com/nrm/journal/v2/n9/slideshow/nrm0901_688a_F1.html

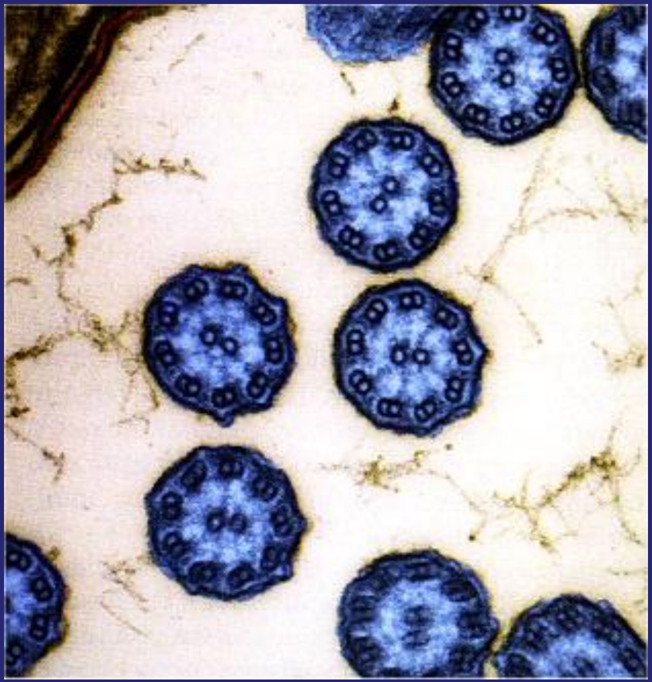
Mitotic spindle



17.7-mitotic_spindle.mov

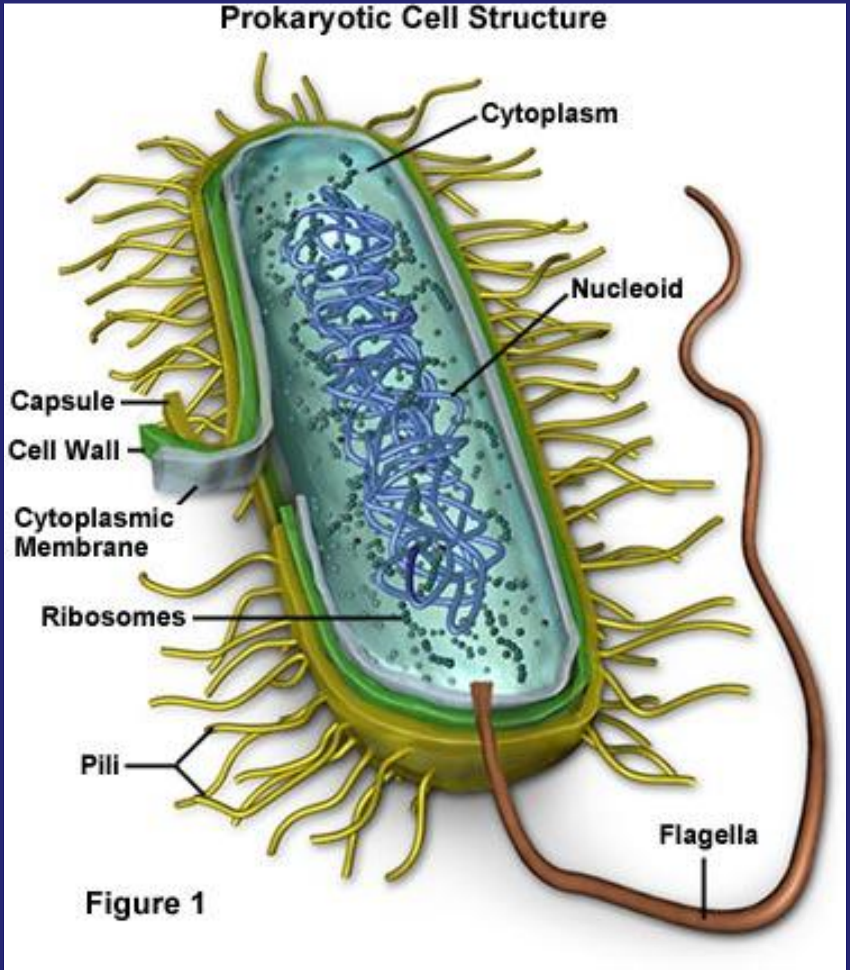
Flagellum of eukaryotic cells





9 pairs of microtubules from protein tubulin + protein dynein

Cytoskeleton in prokaryotic cells?



Cytoskeleton in prokaryotic cells?

- **the cytoskeleton was considered to be specific feature of eukaryotic cell**
- **it was not possible to find anything inside of prokaryotic cells either by electron microscope**

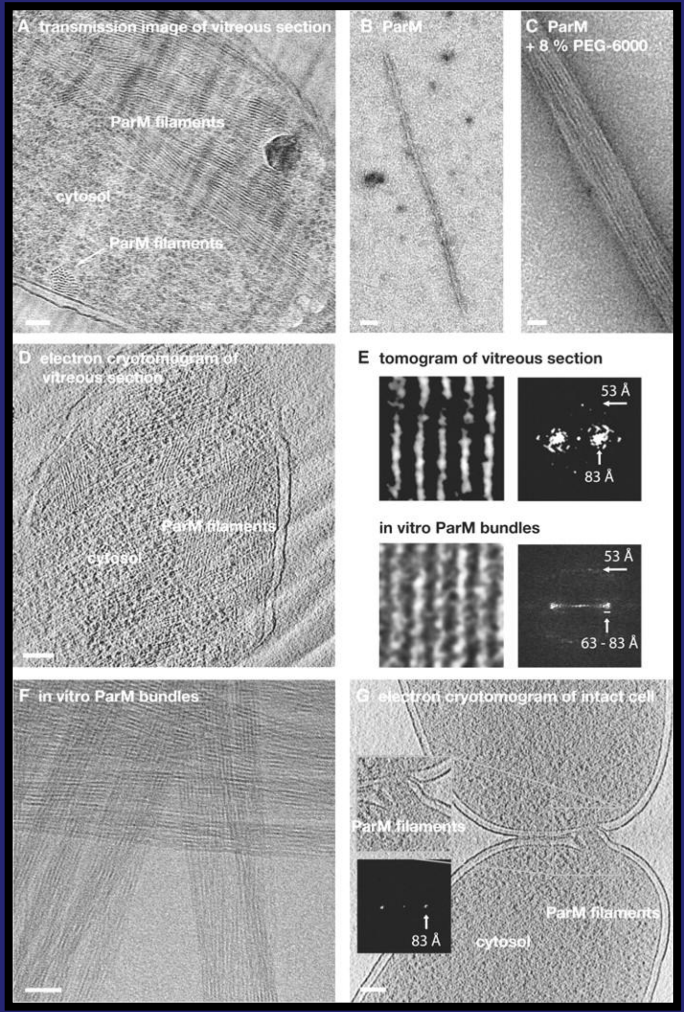
! JANUARY 2009 !

Salje et al. found cytoskeletal filaments responsible for DNA segregation during division

The method used was cryo-electron microscopy

Direct observation of bundles

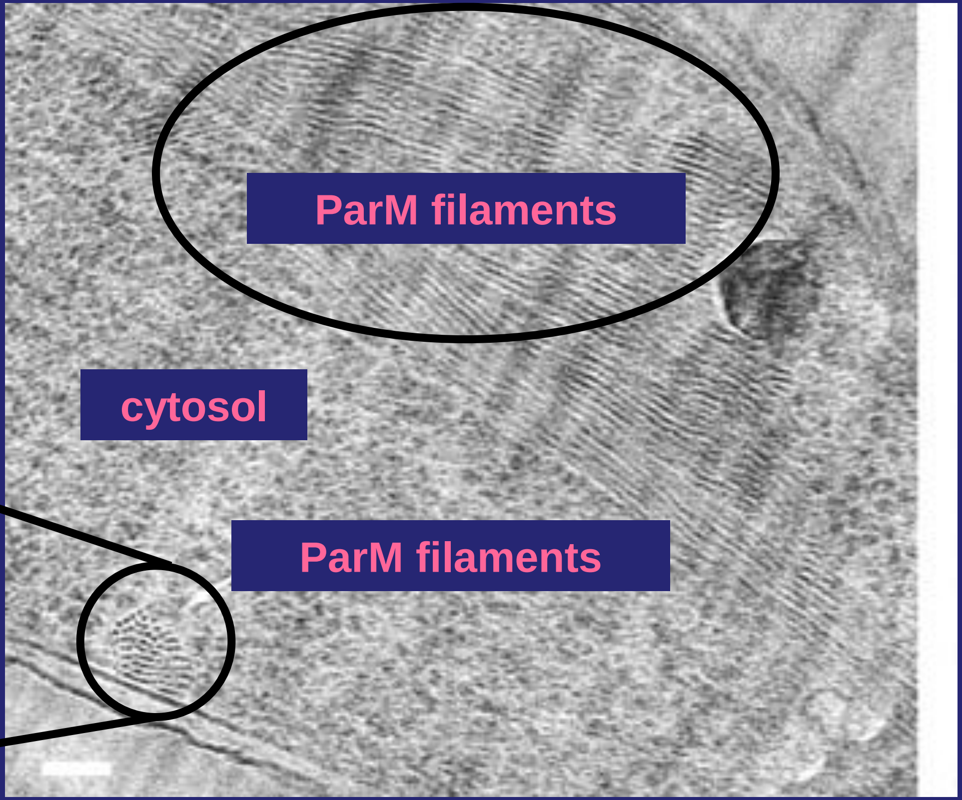
ParM filaments in frozen *E. coli* cells



J. Salje et al., Science 323, 509 -512 (2009)

Direct observation of bundles

Transmission image of a vitreous cryosection of a cell containing overexpressed ParM filaments



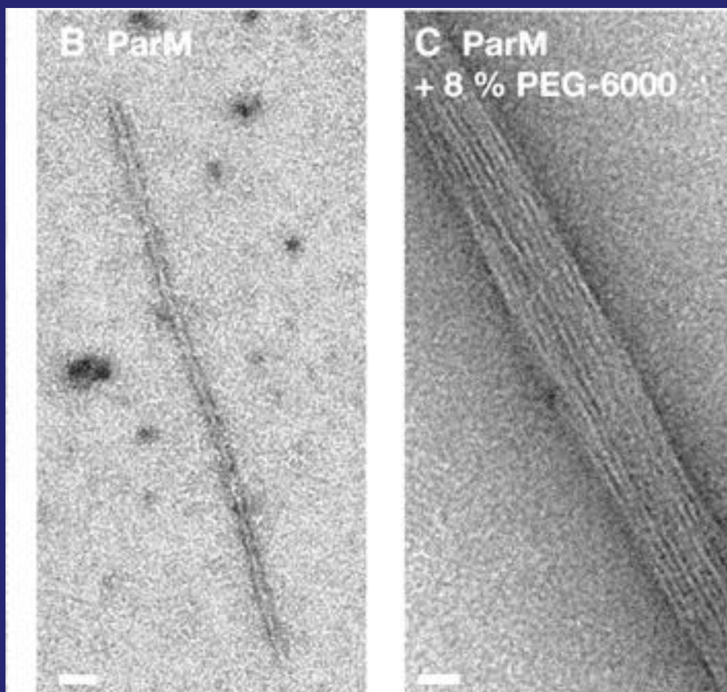
view over the longitudinal axis

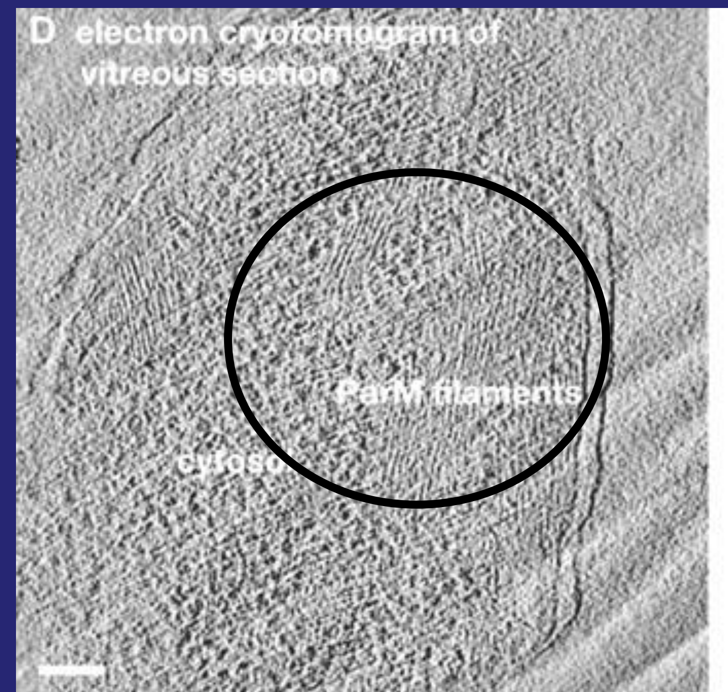
J. Salje et al., Science 323, 509 -512 (2009)

Direct observation of bundles

detail of the filament

electron cryotomogram

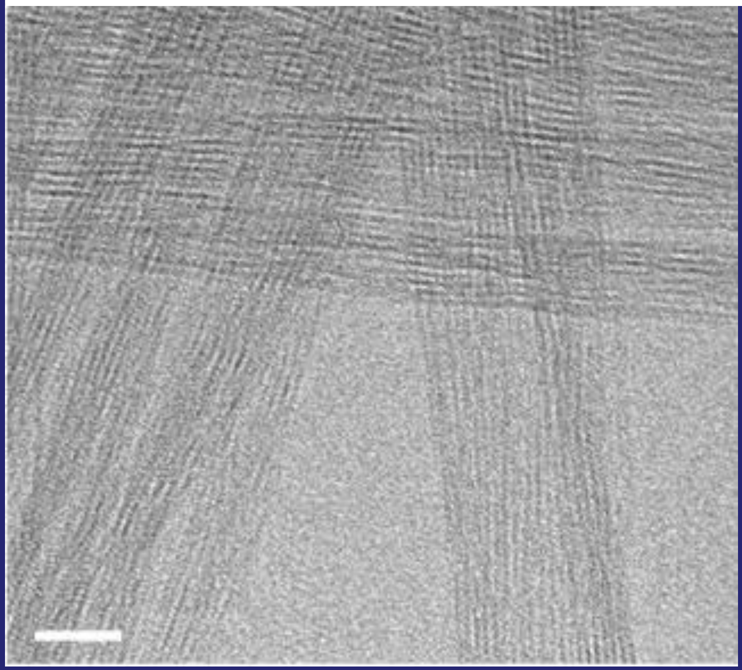




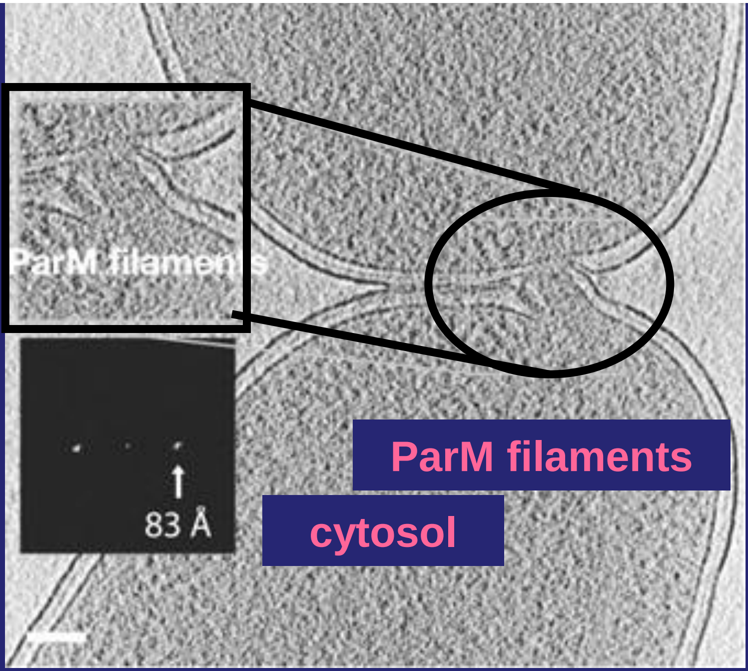
J. Salje et al., Science 323, 509 -512 (2009)

Direct observation of bundles

in vitro **ParM bundles**



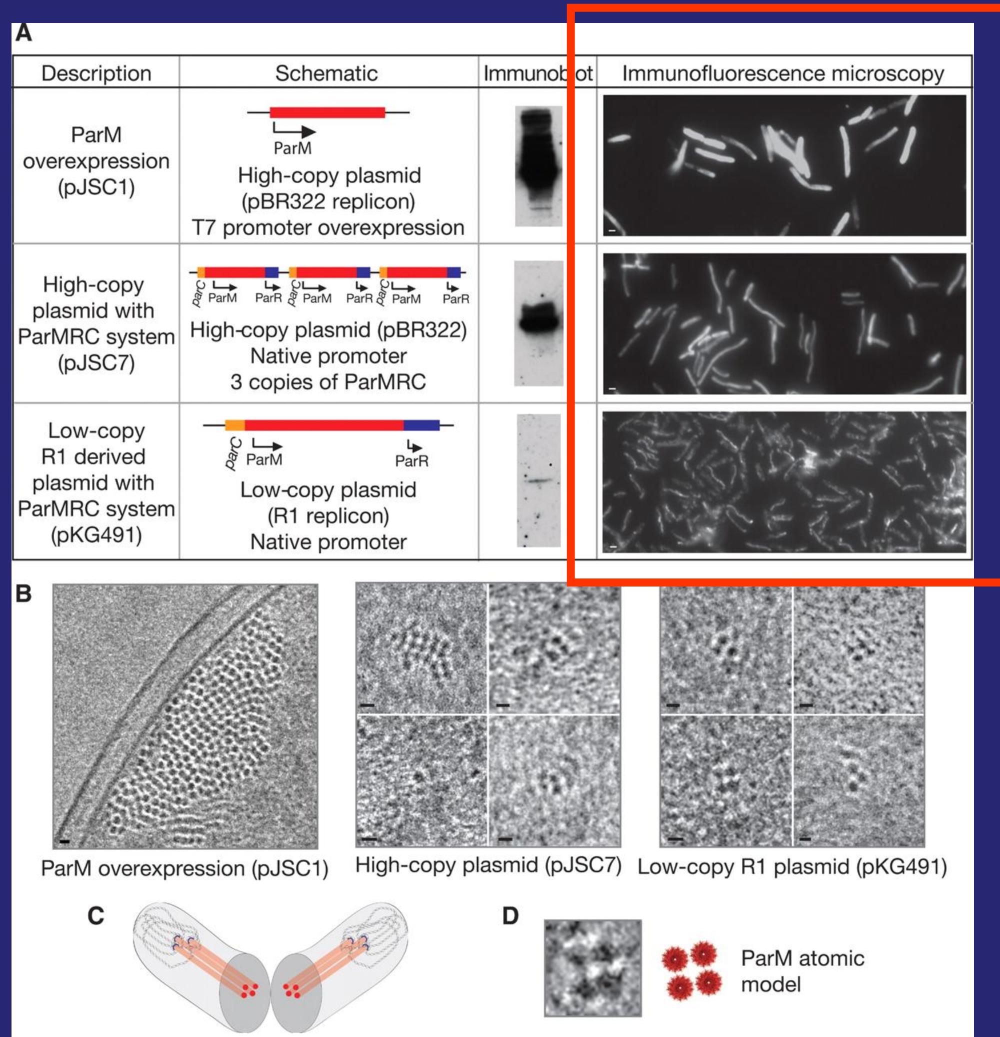
cryotomogram of intact cell



J. Salje et al., Science 323, 509 -512 (2009)

Direct observation of bundles

Small bundles of ParM filaments are involved in plasmid-DNA segregation

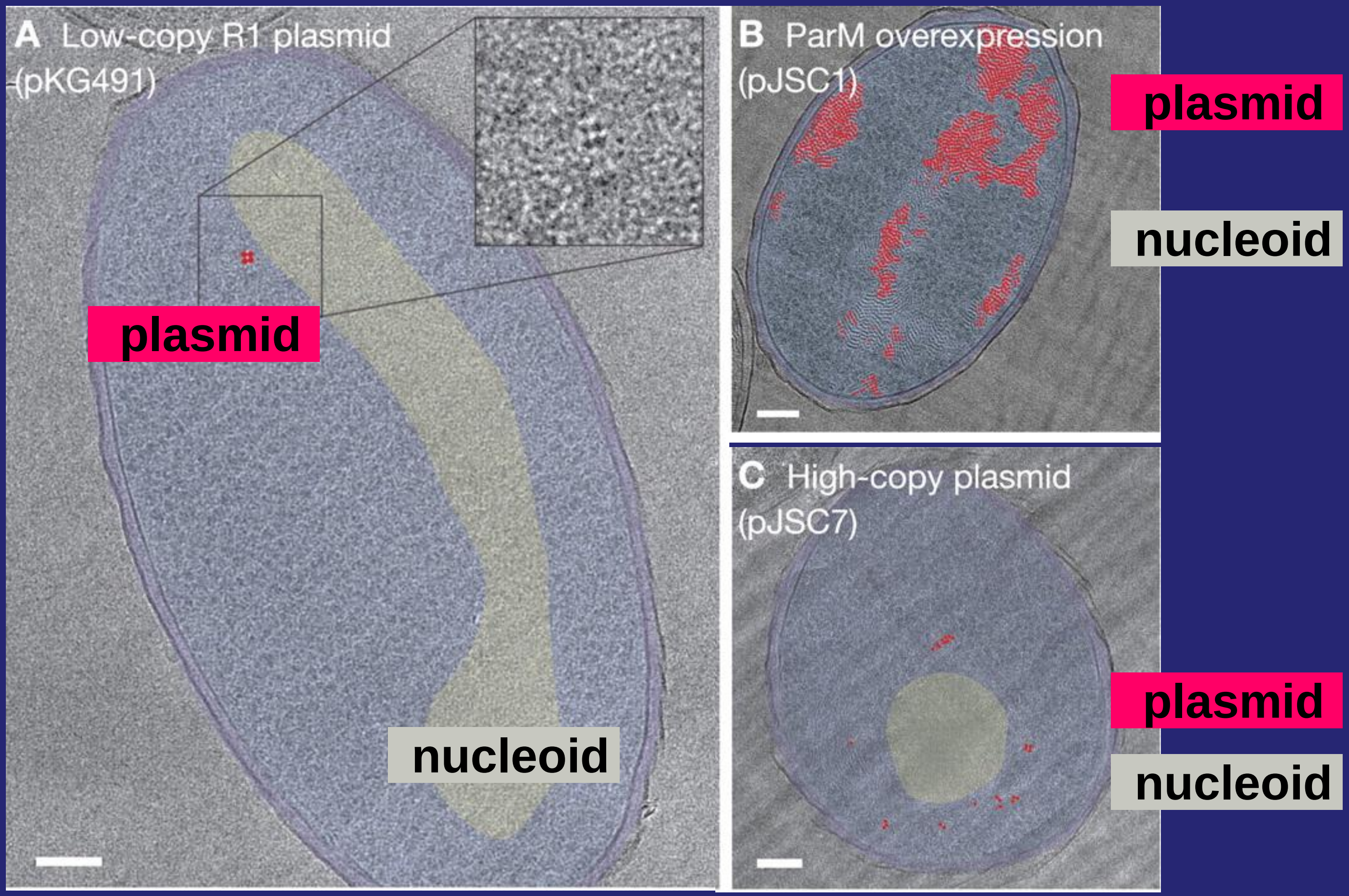


J. Salje et al., Science 323, 509 -512 (2009)

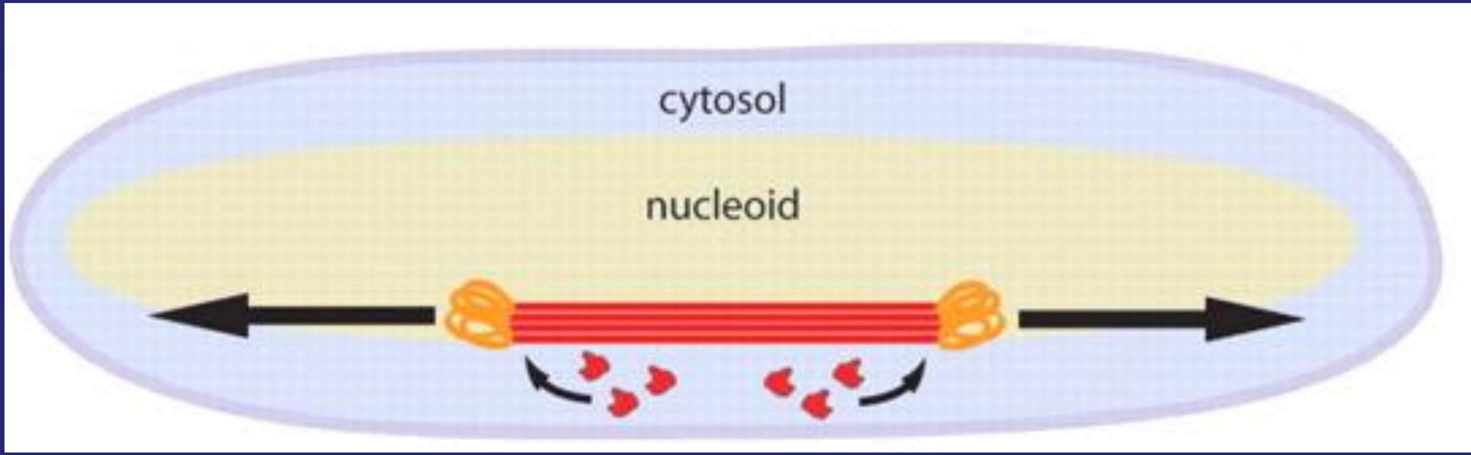
Science AAAS

Direct observation of bundles

Bundles of ParM filaments involved in R1 plasmid DNA segregation lie at the periphery of the nucleoid and may indicate plasmid capture therein



Model of plasmid DNA segregation



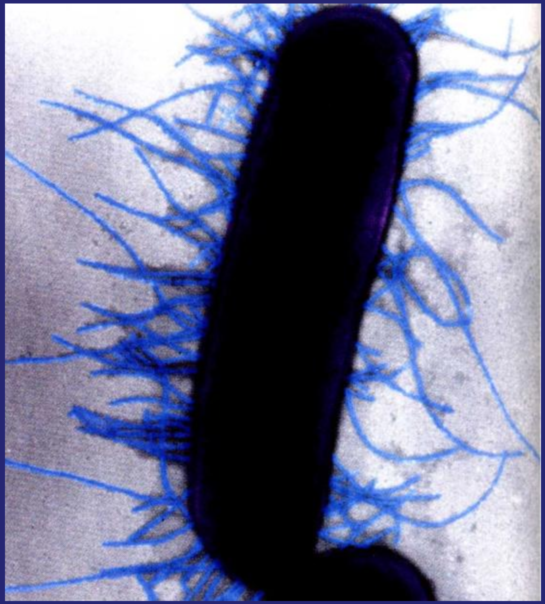
- filament ParM bundles which enables plasmid segregation lie at the nukleoid periphery

- plasmid molecules are entrapped here and are subsequently distributed into daughter cells

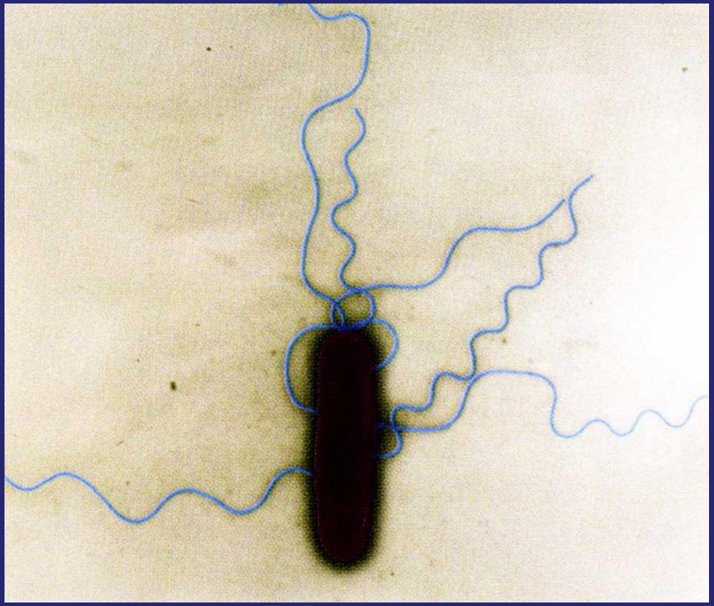
J. Salje et al., Science 323, 509 -512 (2009)

Microtubules in prokaryotic cell

→ pili, flagella

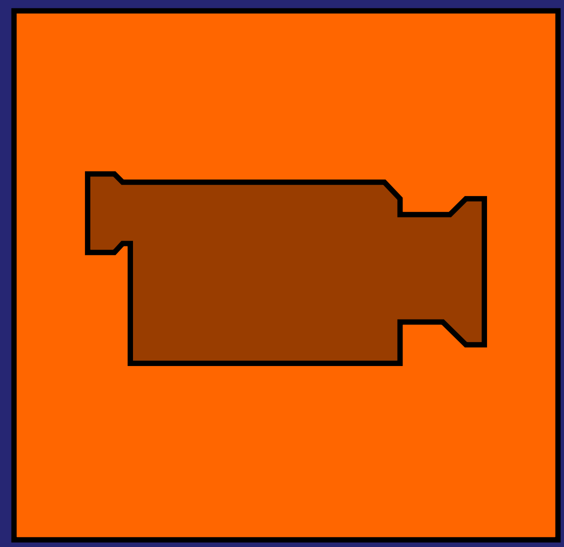


pilus of *E. coli*



flagellum of r. *Salmonella*

Flagellum



14.6 bacterial flagellum.mov

Organisms' movement

Who is moving faster, macro or micro organisms?

Organism	Km/h	Body length/s
Cheetah		
Human		
Bacterium		

Organisms' movement

Who is moving faster, macro or micro organisms?

Organism	Km/h	Body length/s
Cheetah	111	
Human	37,5	
Bacterium	0,00015	