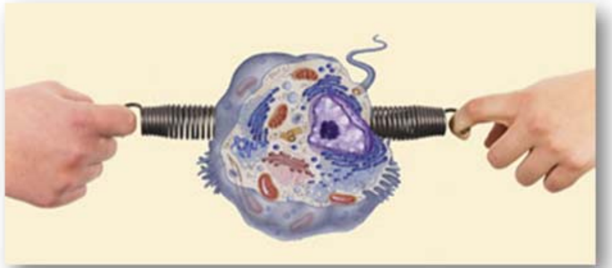
Cytoskeletal system. Motor proteins. Molecular mechanisms of biological motion.



http://www.rsc.org/images/b901714c-400-FOR-TRIDION_tcm18-152053.jpg

Balázs Kiss

MTA-SE Molecular Biophysics Research Group,
Department of Biophysics and Radiation Biology

20. February, 2014

Lecture outline

TB. pages 346-356.

- cytoskeleton
  - history
  - polymer mechanics
  - measurement methods
  - polymerization
  - actin

  10 min pause

  - microtubuli
  - intermediate filaments
- motor proteins
  - types
  - duty cycle
  - animations

POSSIBLE final exam questions

The cytoskeletal system. Polymerization of cytoskeletal filaments.

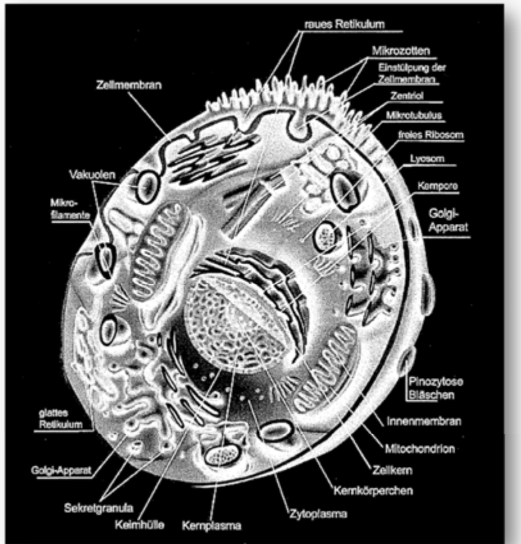
Actin filament system. Actin-dependent biological motion.

Microtubular system. Microtubule-dependent biological motion.

Intermediate filament system.

Motor proteins. Duty cycle. Processivity.

Historical overview



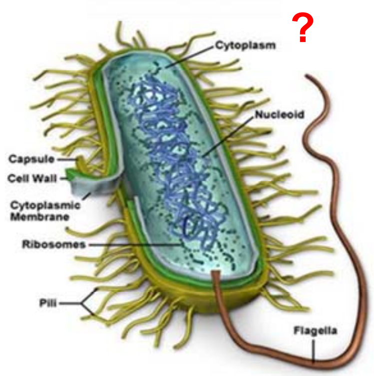
old approach:

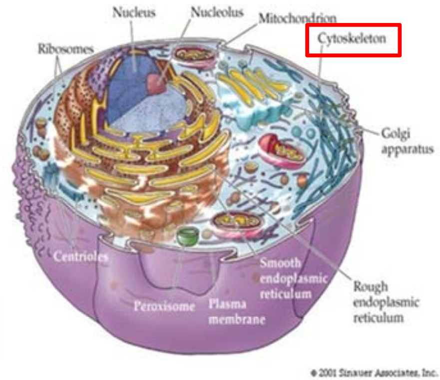
- cell: colloidal system
- membrane-bound structures, organelles
- cytoskeleton: 1-2% volume percent of the cell

„big names”:

- Nikolai Koltsov (1903): the shape of cells is determined by a network of tubules
- Rudolph Peters (1929): protein mosaic coordinates cytoplasmic biochemistry
- Paul Wintrebert (1931):
- *cytosquelette*

Prokaryotes ⟶ Eukaryotes





prokaryotic „cytoskeleton”: analogous to the eukaryotic cytoskeleton

The cytoskeleton

- dynamic skeleton of eukaryotic cells
- long end-to-end distance (=„filament"):
- network building at low concentrations

<u>three main filament classes:</u>

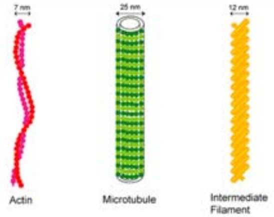
A. thin (actin) (d ~ 7 nm)
B. intermediate (d ~ 10 nm)
C. microtubule (d ~ 25 nm)

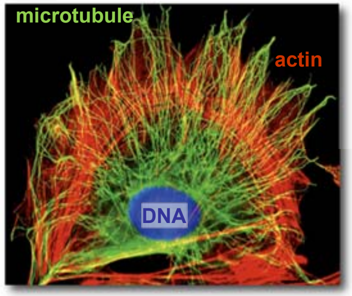
+ associated proteins

<u>polymerization:</u> monomer subunits

<u>role:</u>

A. motility, metamorphosis
B. mechanical stabilization
C. cell division, transport

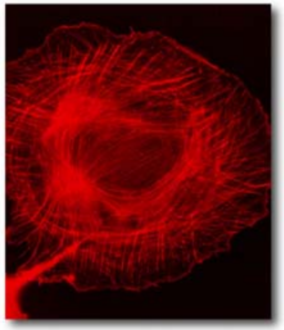




The filaments of the cytoskeleton

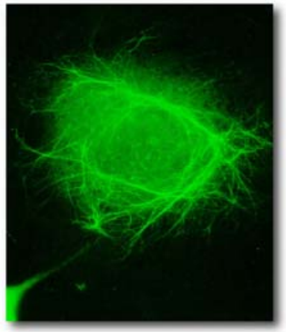
(visualized by fluorescence microscopy)

THIN (actin)



actin
(rhodamin-phalloidin)

INTERMEDIATE



vimentin
(anti-vimentin)

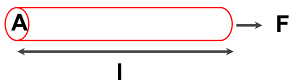
MICROTUBULE

microtubuli
(GFP-tubulin)

Polymer mechanics: Hookean elasticity

- the stiffness or spring constant (D = F/Δl) is not only material-dependent
- the stiffness (D) depends on the shape of the body and the direction of the force

<u>Longitudinal stiffness:</u>

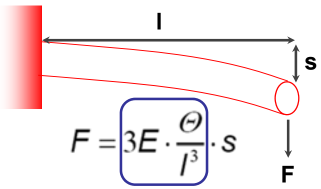


$$F = \boxed{E \cdot \frac{A}{l}} \Delta l$$

D

E: elastic modulus (Young modulus)

<u>Bending stiffness:</u>

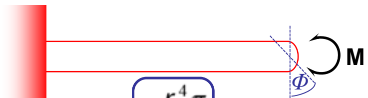


$$F = \boxed{3E \cdot \frac{\Theta}{l^3}} \cdot s$$

D

θ: flexural modulus (bending modulus)

<u>Torsional stiffness:</u>



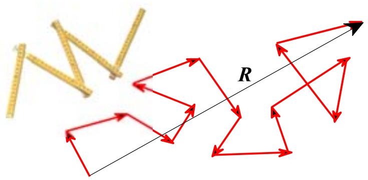
$$M = \boxed{G \frac{r^4 \pi}{2l}} \phi$$

D

G: shear modulus (modulus of rigidity)

Polymer mechanics: FJC, WLC

„Freely Jointed Chain"



polymer built up from elementary vectors (*N* pieces, one elementary vector maintains its orientation within the chain)

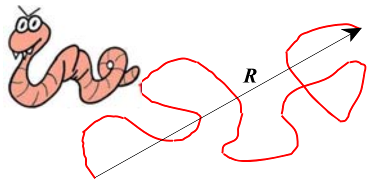
l = correlation length

(average length of one elementary vector)

Nl = *L* = contour length

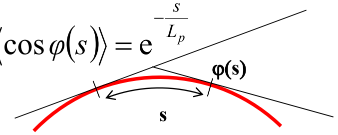
R = end-to-end distance

„Worm-Like Chain"



L_p = persistence length (measure of the bending rigidity; the ability of the polymer to maintain its direction)

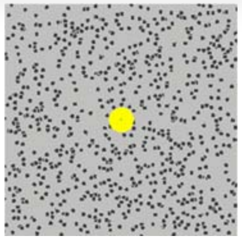
$$\langle \cos \varphi(s) \rangle = e^{-\frac{s}{L_p}}$$



- if s<<L_p: cos(φ)~1, and φ(s)~0˚
- if s>>L_p: cos(φ)~0, thus φ(s) is between 0˚ and 360˚

n.b.: thermodynamics chapter (later during the semester)

Excursion: Brownian motion



$$\frac{1}{2}mv^2 = \frac{3}{2}k_BT$$ (for ideal gases)

The small (black) particles move randomly with different velocities and impart momentum to the bigger one (yellow). As a consequence, the yellow particle performs random walk.

Excursion: Entropy

characterizes the disorder / thermodynamic probability of a state in a system

„thermal" excitation

investing work

Polymer mechanics: „thermal" elasticity

$$L_p = \frac{E\Theta}{k_BT}$$

Θ : : flexural modulus (second moment of inertia)

– for a rod with circular cross-section: $\Theta = r^4\pi/4$

rigid chain

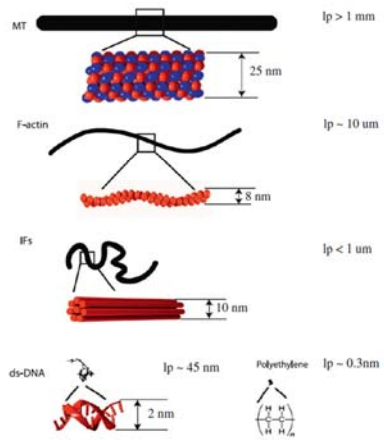
$L_p >> L$

semiflexible chain

$L_p \sim L$

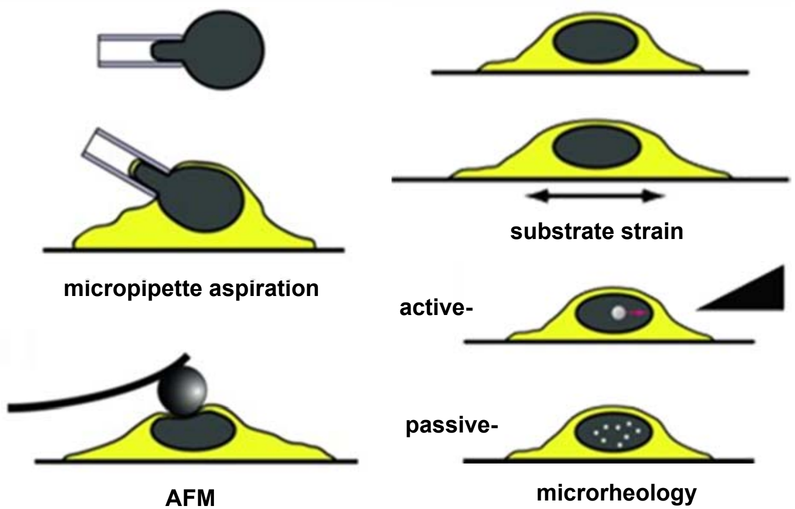
flexible chain

$L_p << L$



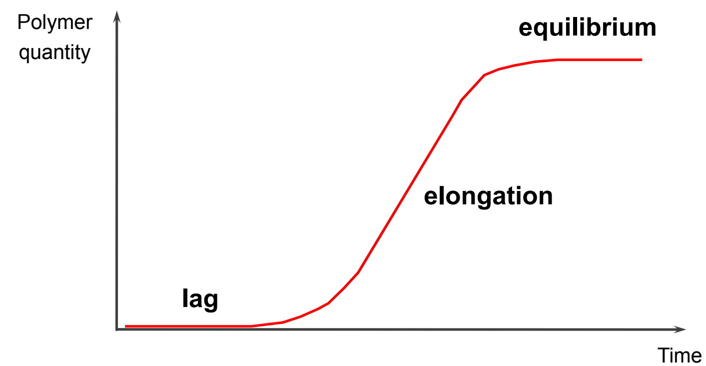
Mechanical measurement methods

(applicable to the nucleus or the whole cell)



Phases of polymerization

1. **Lag phase: nucleation**
2. **Elongation**
3. **Equilibrium**

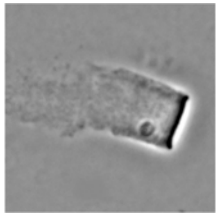


Polymerization equilibria

1. **real equilibrium:**

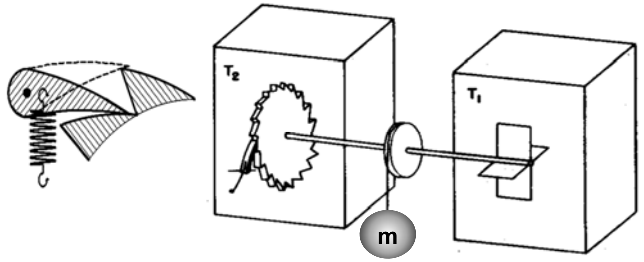
2. **dynamic instability:** continuous, slow elongation followed by a catastrophic depolymerization

3. **treadmilling:**

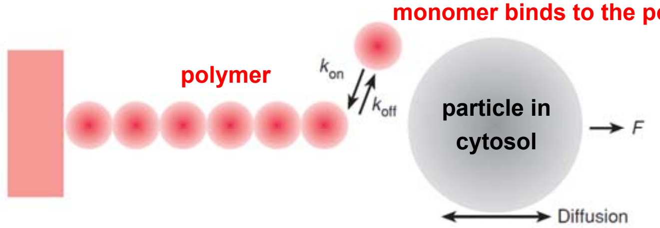


Force generation by polymerization

(Brownian ratchet mechanism)

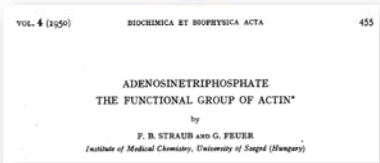


only works if $T_1>T_2$



Actin monomer (G-actin)

- **actin: 5% of all proteins present in eukaryotic cells,**
- **monomer unit: globular (G-) actin,**
- **43 kDa molecule weight, 4 subdomains (covalent bonds),**
- intracellular concentration: 2-8 mg/mL (50-200 µM): corresponds to ~25 nm average intermolecular distance in solution…
- **1 bound adenosine nucleotide / monomer (ATP or ADP).**



VOL. 4 (1950) BIOCHIMICA ET BIOPHYSICA ACTA 455

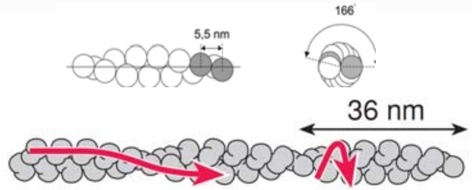
ADENOSINETRIPHOSPHATE THE FUNCTIONAL GROUP OF ACTIN*

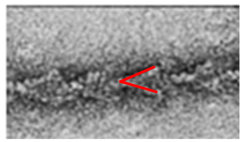
by

F. B. STRAUB AND G. FEUER

Institute of Medical Chemistry, University of Szeged (Hungary)

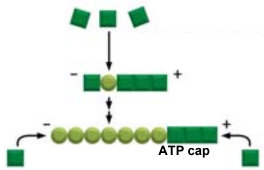
Actin filament (F-actin)





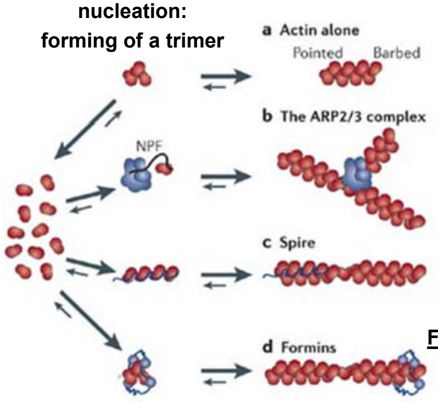
Structure, properties:

- **~7 nm diameter, *in vitro* length: several 10 µm, *in vivo* length ~1-2 µm; right-handed long-pitch helix; left-handed short-pitch helix,**
- **semiflexible polymer (L_p: ~10 µm),**
- **structural polarity:**
  - **barbed end: +,**
  - **pointed end: -.**
- **asymmetric polymerization: ATP cap (at the + end).**



Nucleation factors (associated proteins) of actin

nucleation: forming of a trimer



spontaneous nucleation is improbable (actin dimer is instable)

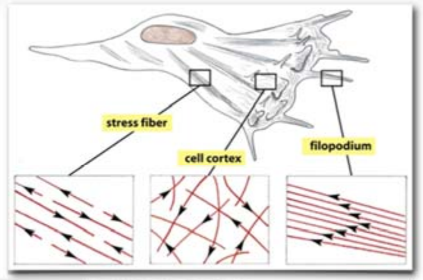
<u>Arp2/3:</u> mimics an actin dimer or trimer, generates y-branched actin network

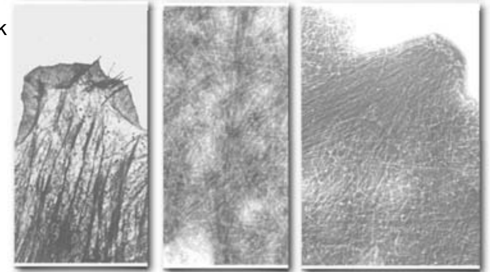
<u>Spire:</u> stabilizes 4 longitudinally-aligned G-actin

<u>Formins:</u> stabilizes actin dimer / trimer in its dimerized state, remains bound to the +end („formin cap")

Occurrence of actin filaments

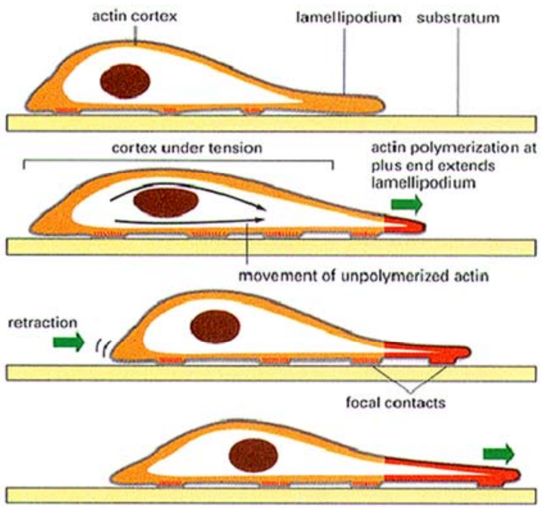
- **<u>cortex</u> (periphery of the cell),**
- **<u>stress fibers:</u> cross-linked actin network with myosin-II motors** (originally: tension initiates its formation); **provides mechanical force for cell adhesion**
- **cytoplasmic projections:**
  - **<u>lamellipodium:</u>** 2D actin network on the mobile edge of the cell, propels the cell forward
  - **<u>filopodium</u>: (microspikes)** forms focal adhesion with the substrate,
  - **<u>microvillus:</u>** increases the surface area of cells; absorption, secretion, mechanotransduction

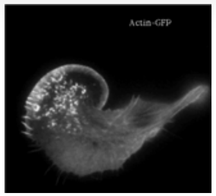




stress fibers **cortex** **filopodium**

Steps of cell locomotion





1. protrusion

2. adhesion:
focal contacts
(integrins and actin bundles)

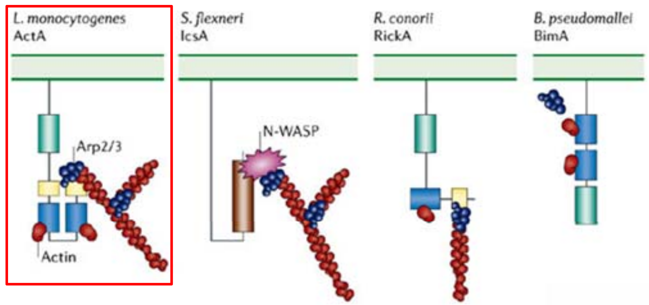
3. traction + retraction

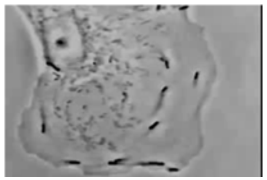
4. lamellipodium outgrowth

Intracellular locomotion of bacteria

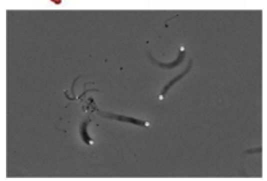
(they use the actin network for „travelling")

bacteria:
binding protein:






Listeria monocytogenes

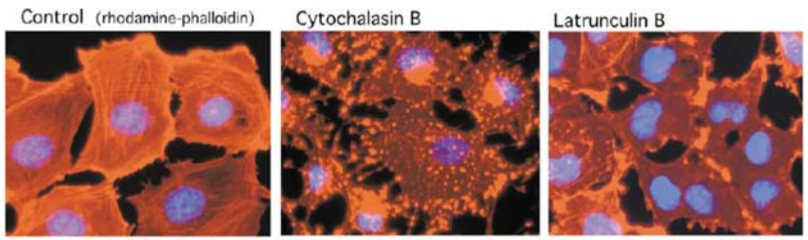


WASP + Arp2/3-functionalized silica microbeads

Actin as target in medicine

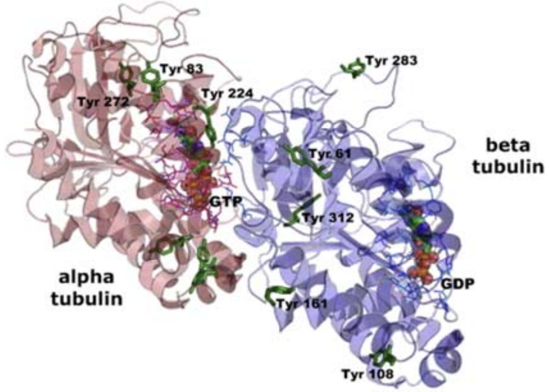
(mechanism of effect: inhibition of actin dynamics)

substance	target	disease / application
phalloidin (death cap)	binds to F-actin, inhibits polymerization	mushroom poisoning
cytochalasin (chalasis: relaxation)	inhibits monomer incorporation	experimental tumor therapy
latrunculin	actin monomer (nucleotide-binding cleft)	experimental tumor therapy

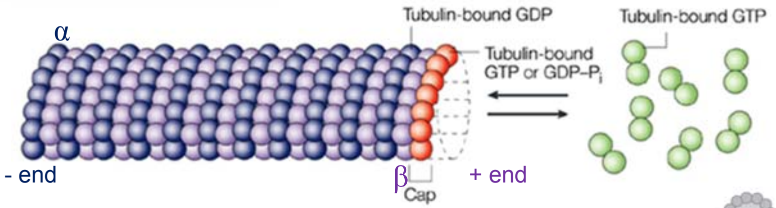


The microtubular system

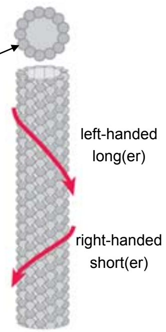
- subunit: <u>tubulin</u> (α- and β-tubulin), ~ 50 kDa
- in neural tissue: up to 10-20% of the total protein
- 1 guanosine nucleotide (GTP or GDP) / subunit
- the nucleotide might be exchangeable (β) or not exchangeable (α)



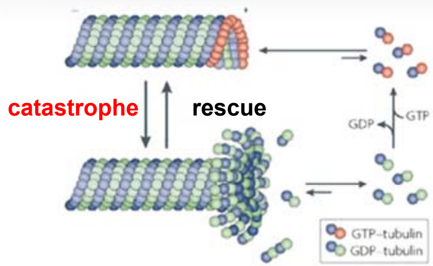
The microtubule

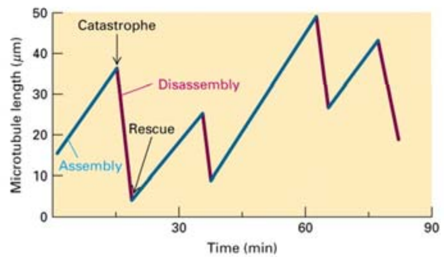


- ~25 nm in diameter, hollow, helical
- the filament tube is composed of 13 protofilaments
- rigid chain (L_p: several mm!)
- structural polarity:
  - <u>+ end:</u> fast polymerization, terminated by β-subunit
  - <u>- end:</u> slow polymerization, terminated by α-subunit
- GTP-cap



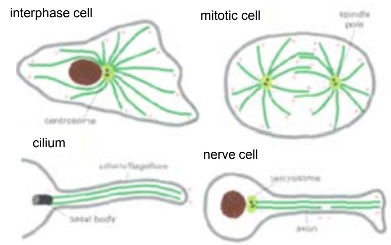
Microtubular dynamics, occurrence





Where does it occur in eukaryotes?

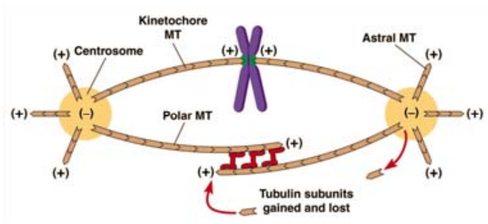
- cytoplasm of interphase cells,
- axon,
- cilium, flagellum,
- mitotic spindle of mitotic cells.

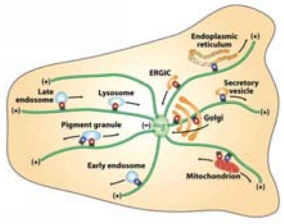


Localization and function of microtubules

Specific polarity pattern inside the cell:

- in the centrosome: - end, on the periphery: + end,
- <u>centrosome:</u> 2 centrioles, centrosome matrix containing γ-tubulin,
- maintaining the polarity of the cell with the help of associated proteins.





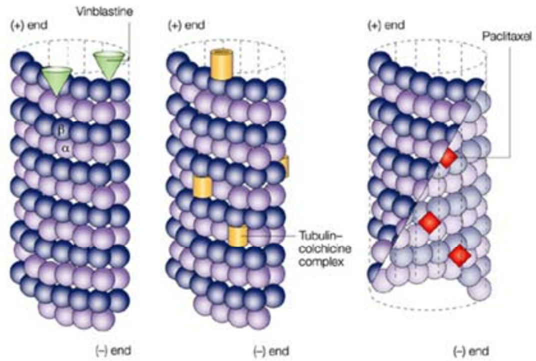
Functions:

1. „motorways” for motor proteins,
2. senses, finds and monitors the geometric centre of the cell,
3. motility (cell division).

Microtubuli as targets in medicine

(mechanism of effect: inhibition of microtubule dynamics)

substance	target	disease / application
vinblastin	+ end of the microtubule	cancer therapy
colhicine	tubulin dimer	gout
paclitaxel	interior surface of MT	cancer therapy

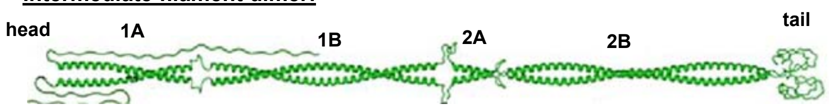


Intermediate filaments

- **diameter: 8-10 nm,**
- **chemically resistant,**
- **fibrous monomer (non-globular), polymerization does not require ATP/GTP**
- **tissue-specific monomers differ in their tail sequence / structure:**

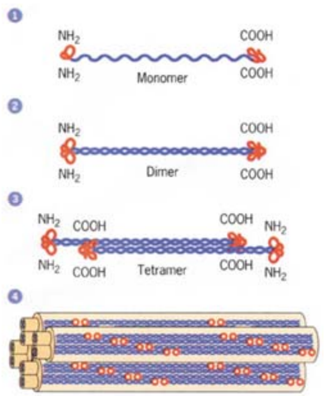
epithelium	keratins (α, β)
muscle	desmin
connective tissue	vimentin
glia	glial fibrillary acidic protein (GFAP)
nerve	neurofilaments (L, M, H)

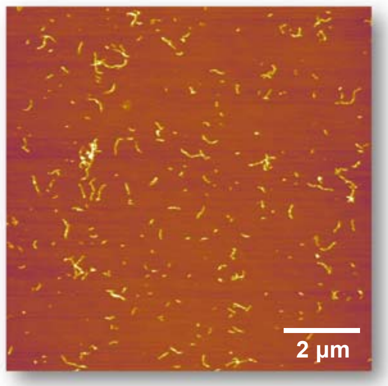
<u>Intermediate filament dimer:</u>



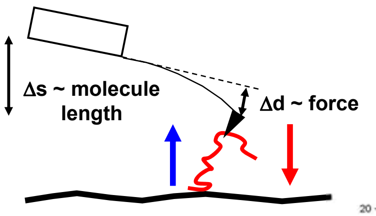
Polymerization of intermediate filaments

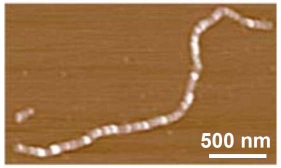
- **in cells they are usually present in their fully-polymerized state,**
- **no polymerization-depolymerization dynamism,**
- **depolymerization is regulated via phosphorylation.**

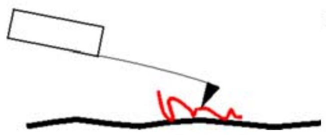


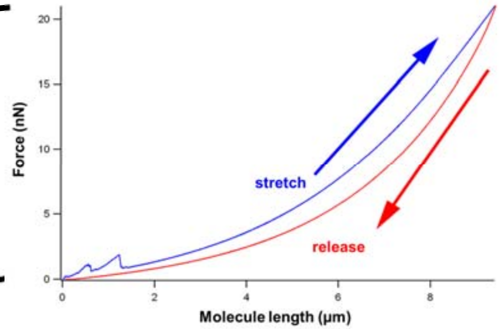


Stretching a single IF (desmin) with AFM



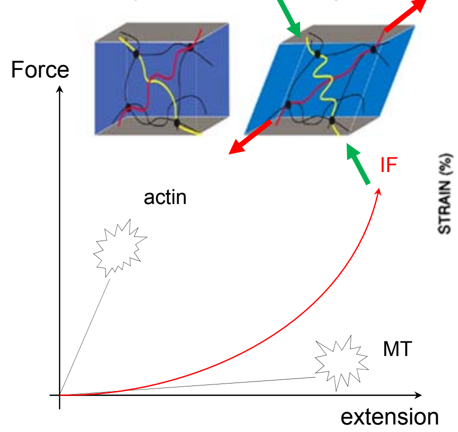


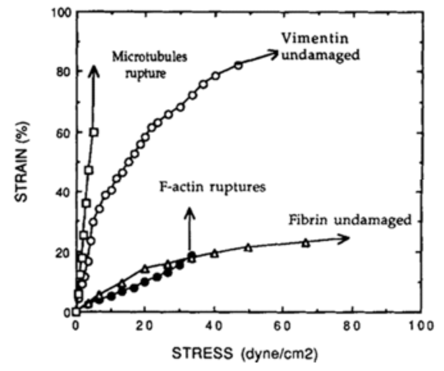




Hypothetic role of IFs: mechanical stabilization

- nonlinear elasticity: strain hardening at high forces
- polymer network: negative normal stress (green arrows)



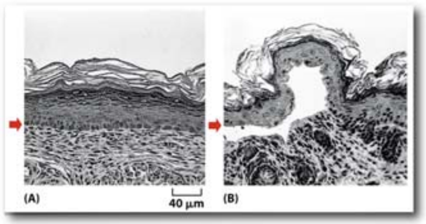


1 Pa = 10 dyne/cm^2

Tissue-specific role of intermediate filaments

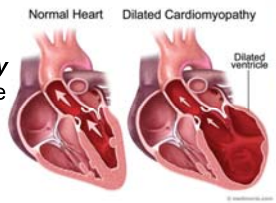
in epithelial cells:

- **clinical picture: *epidermolysis bullosa simplex*.** Defect in anchoring between the epidermis and dermis, resulting in friction and skin fragility: blisters in the skin and mucosal membranes.
- **cause: keratin gene mutation**



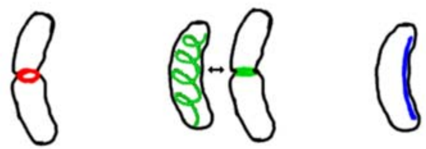
in cardiac tissue:

- **clinical picture:** ***cardiomyopathy*** (deterioration of the function of the myocardium = the heart muscle)
- **cause: desmin gene mutation**



Point of interest: prokaryotic cytoskeleton

	cell division	cell polarity	cell shape
eukaryotes	tubulin	actin	intermediate filaments
prokaryotes	FtsZ	MreB	CreS



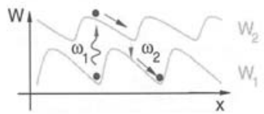
Motor

a cyclic working device capable of converting energy into movement (force and thus displacement)

$E_x \rightarrow W$

E_x :

- mechanical energy
- chemical energy
- electric energy
- thermal energy

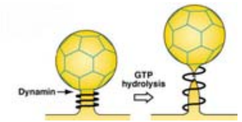


Motor proteins

- non-equilibrium systems (the displacement coupled to the chemical reaction is strictly directed)
- velocities: 0.01-100 μm/s
- step sizes: 0.3-40 nm
- forces: 1-60 pN
- fuel: hydrolysis of ATP or similar macroergic compound, (e.g.: coupled to polymerization): 54 kJ/mol energy = 22 k_bT
- efficiency: 50-100% (!!!)

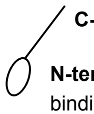
Types of motor proteins

1. **Actin-based:**
   - **myosins:** conventional (myosin-II) and non-conventional;
   - myosin superfamily (classes I-XXIV), move towards plus end.
2. **Microtubule-based:**
   - **dyneins:** ciliary (flagellar) and cytoplasmic dyneins;
     - move towards the minus end.
   - **kinesins:** kinesin superfamily: conventional and non-conventional;
     - move towards the plus end.
   - **dynamins:** MT-dependent GTPase activity;
     - biological role: vacuolar protein sorting



3. **DNA-based mechanoenzymes:**
   - DNA and RNA **polymerases**, virus capsid **packaging motor**;
   - produce force and displacement along the DNA strand.
4. **Rotary motors:**
   - F1F0-ATP synthase,
   - bacterial flagellar motor.
5. **Mechanoenzyme complexes**
   - ribosome

Duty cycle of motor proteins 1.

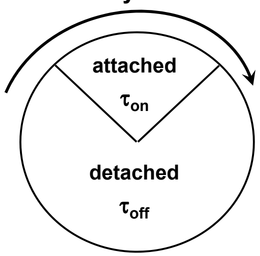


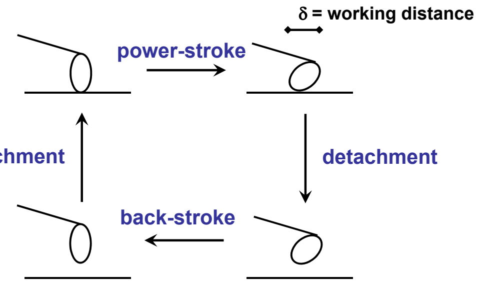
C-terminus: functional binding site (e.g.: cargo)

N-terminus: globular head: motor domain (binds and hydrolyzes nucleotide), binding site for the respective cytoskeletal filament

ATP hydrolysis cycle

tight coupling: 1 ATP is being hydrolized per 1 cycle





motor protein conformational change: ~μs

Duty cycle of motor proteins 2.

duty ratio (r):

$$r = \frac{\tau_{on}}{\tau_{on} + \tau_{off}} = \frac{\tau_{on}}{\tau_{total}}$$

sliding velocity:

$$v = \frac{\delta}{\tau_{on}} \xrightarrow{\text{attached time:}} \tau_{on} = \frac{\delta}{v}$$

cycle time:

$$\tau_{total} = \frac{1}{V}$$

$$r = \frac{\delta V}{v}$$

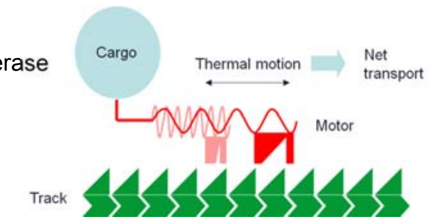
δ=working distance or step size; V=ATP-ase rate; sliding velocity

Processive motor: r ~ 1

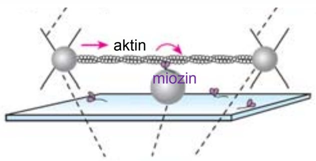
- e.g.: kinesin, DNA , RNA-polimerase
- carries its load by itself

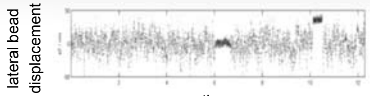
Non-processive motor: r ~ 0

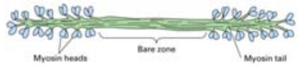
- e.g.: myosin-II
- works in ensembles



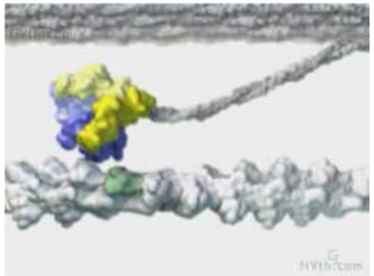
Non-processive motors: myosin-II



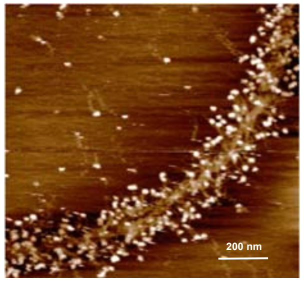




myosin-II filaments working in ensemble: **thick filament**



step size: 5.5 nm
(distance between neighboring actin subunits)

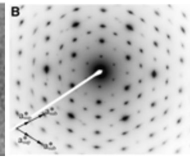


AFM image of synthetic thick filament

Relaxation: how were the animations made?

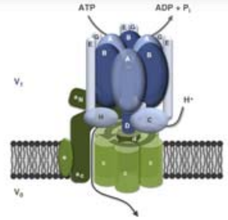
→

EM

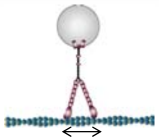


crystallography

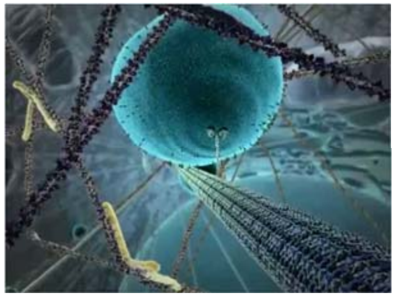
→



Processive motors



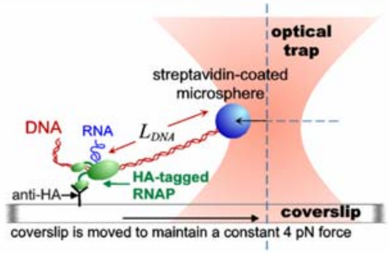
myosin-V walking on F-actin: high-speed AFM image: 36 nm step size

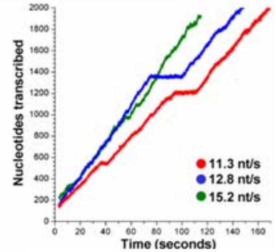


kinesin: 8 nm step size
(distance between every other tubulin subunits)

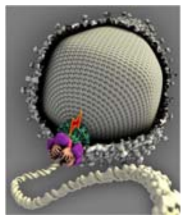
dynein: stochastic and coordinated stepping („drunken sailor")

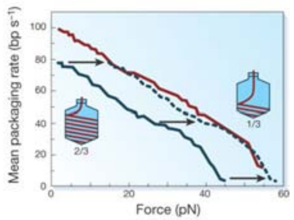
Nucleic acid-based motors

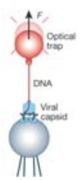




RNA polymerase: transcription with constant rate interrupted by pauses



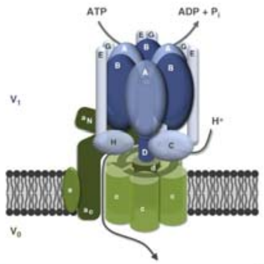


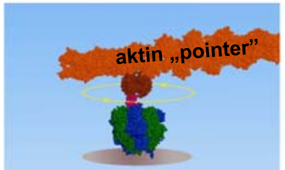


portal motor: bacteriophage virus DNA is packed into the viral capsid

Rotary motors

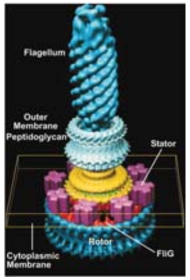
F1F0 ATP-synthase

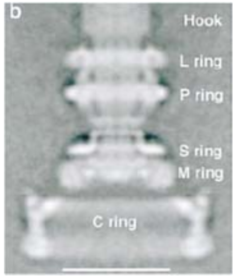




discrete, 120° rotational steps:

Bacterial flagellar motor





- speed: > 20000 RPM
- power: 10^{-16} W
- efficiency: > 80%
- energy source: protons

Thank You for Your attention!