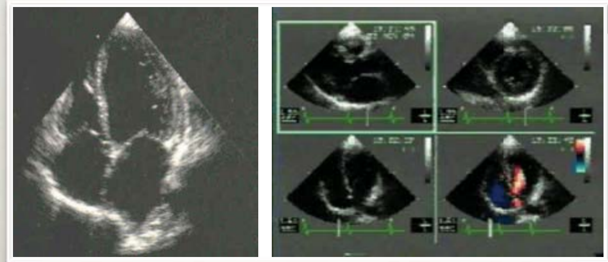
Molecular mechanisms of biological motion

Types of biological motion

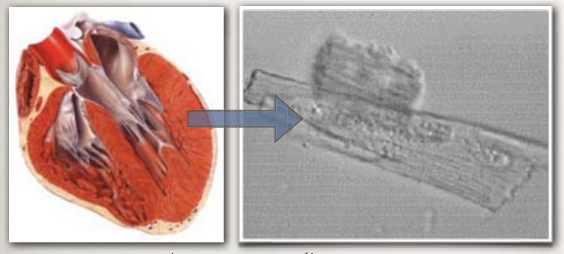
Collective motion

Body motion ("Leap of the century")



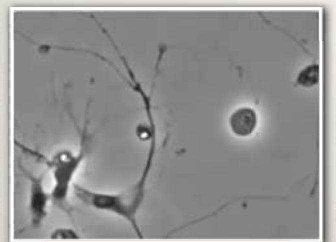
Organ motion

Types of biological motion

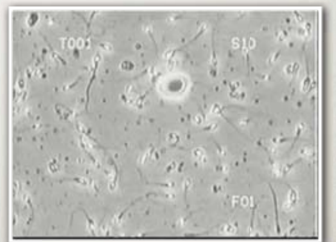


Autonomous cardiomyocyte

Dividing cell

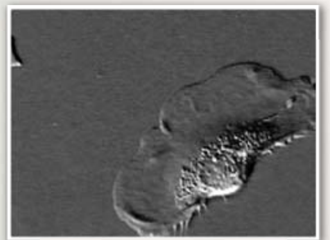


Axonal (neurite) growth

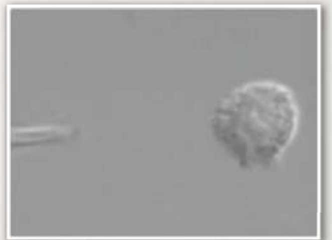


Moving spermatocytes

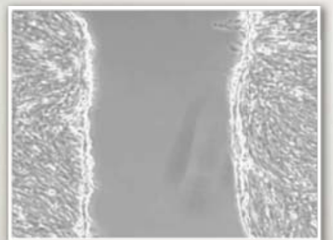
Types of biological motion



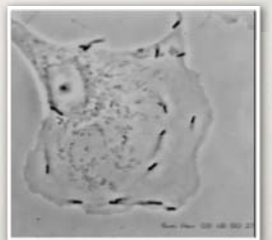
Crawling keratinocyte



Chemotaxis

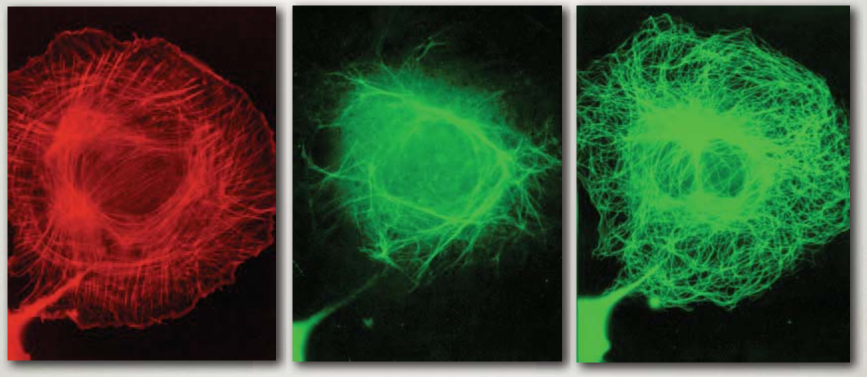


Wound healing model - collective fibroblast moevent



Intracellular movement of pathogenic Listeria bacteria

The cytoskeletal system



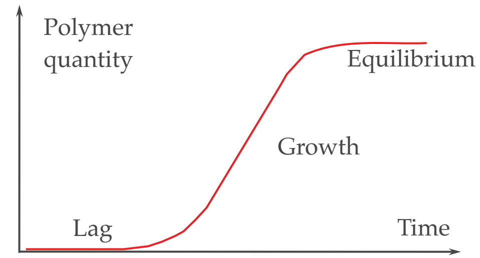
Actin (rodamin-phalloidin) — Vimentin (anti-vimentin) — Mikrotubules (GFP-tubulin)

1. Polymerization (from "smart brick" building blocks)
2. Mechanics (see following lecture)

Polymerization

Process of the assembly of monomers

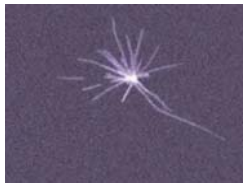
Phases of polymerization:
1. Lag phase: nucleation
2. Growth phase
3. Equilibrium phase



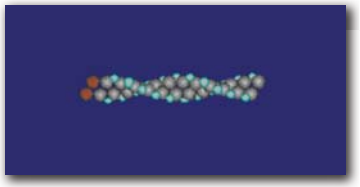
Polymerization equilibria

1. True equilibrium

2. Dynamic instability: slow growth followed by "catastrophic" depolymerization

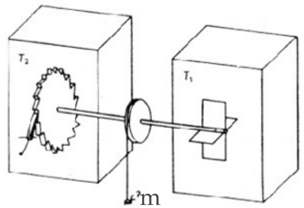


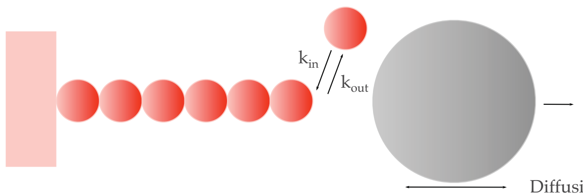
3. Treadmilling



Generation of force and displacement with filament polymerization

Brownian ratchet mechanism

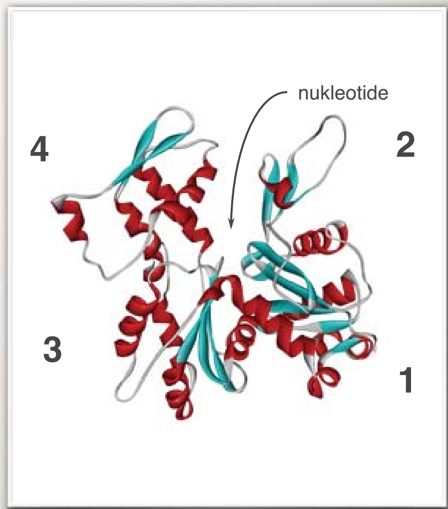




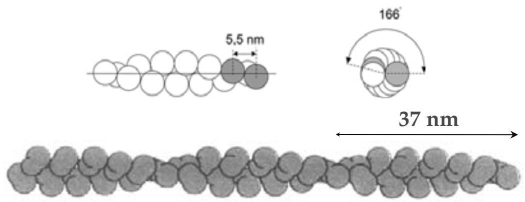
Actin monomer (G-actin)

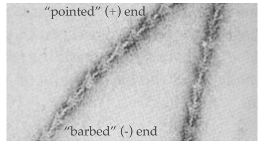
Protein of largest quantity in the eukaryotic cell (5% of total protein)
Concetration in the cell: 2-8 mg/ml (50-200 μM)

Subunit: globular (G-) actin
MW: 43 kDa, 375 amino acid residues,
1 moleule bound nucleotide (ATP or ADP)
Subdomains (4)
Genetic variability: in mammals, 6 different actins



The actin filament (F-actin)



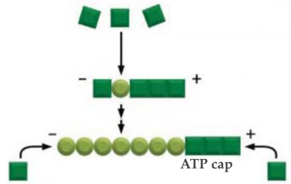


~7 nm thick, length *in vitro* exceeds 10 μm, *in vivo* 1-2 μm

Right-handed double helix.

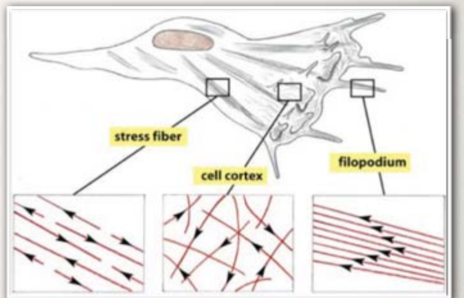
Structural polarity ("barbed", "pointed" ends)

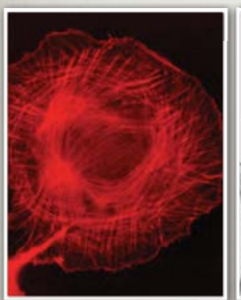
Asymmetric polymerization: ATP cap



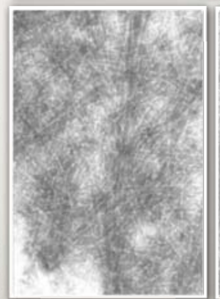
Actin in the cell

cortex
stress fibers,
cellular processes (lamellipodia, filopodia, microspikes, focal contacts, invagination)
microvillus

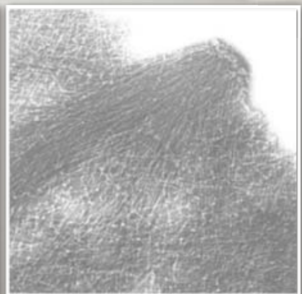




Stress fibers

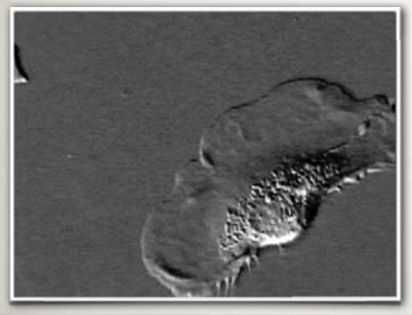


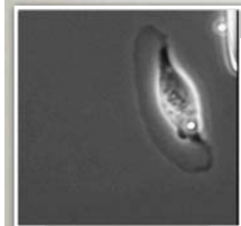
cortex

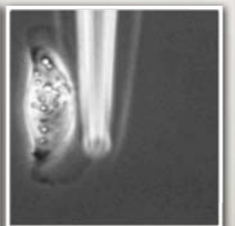


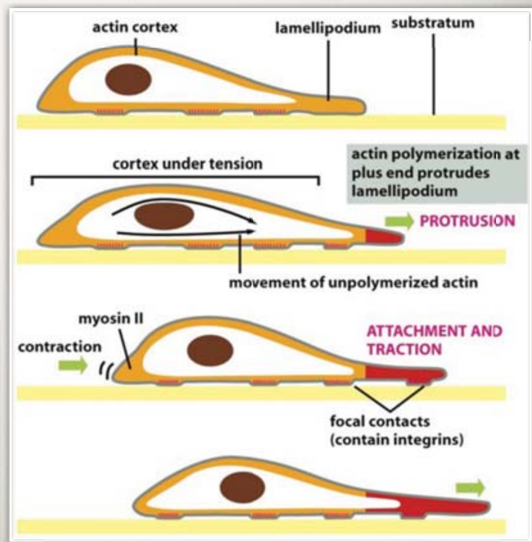
filopodium

Actin-dependent cell movement



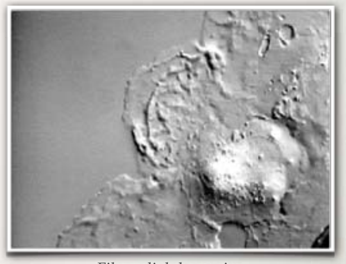






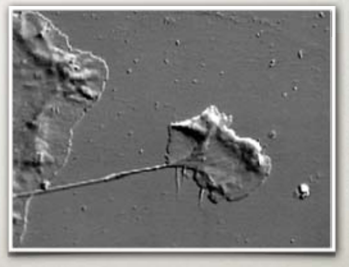
Manifestations of actin-dependent movement

Retrograde flow



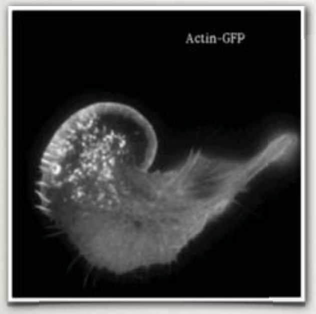
Filopodial dynamics

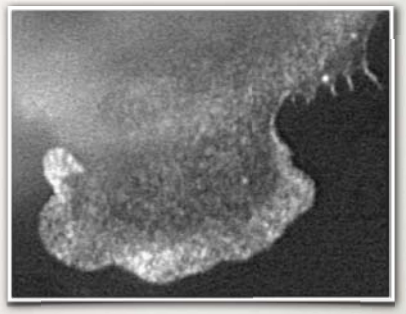
Autonomous movement of cytoplast (anuclear cell fragment)

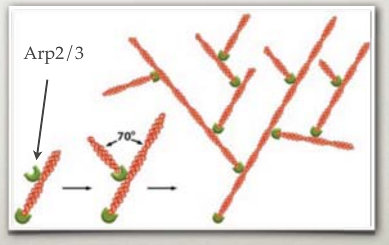


Membrane ruffling

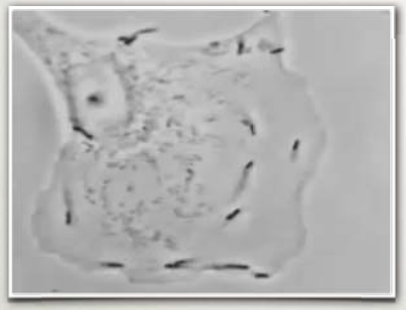
Actin dynamics in the lamellipodium



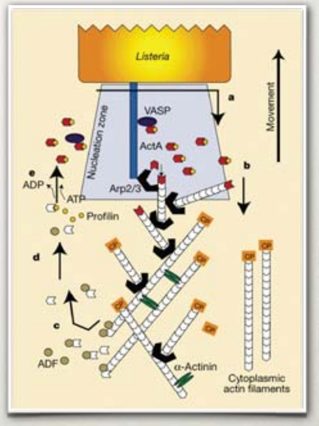




Intracellular pathogens make use of the actin system

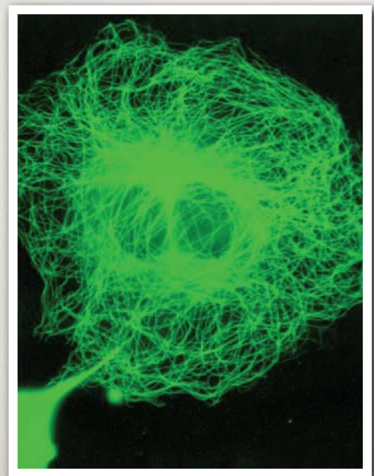


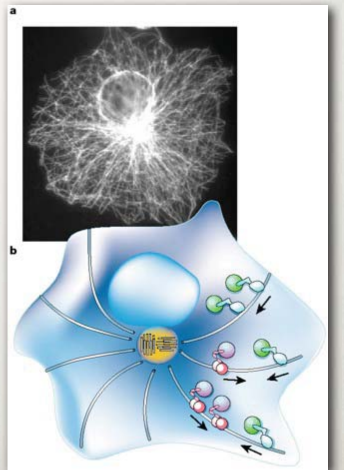
Intracellular motility of Listeria monocytogenes bacteria



Microtubular system

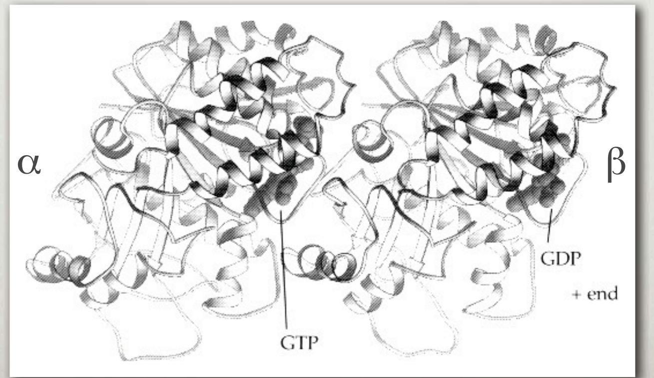
Filamentous system of eukaryotic cells composed of tubulin and its associated proteins



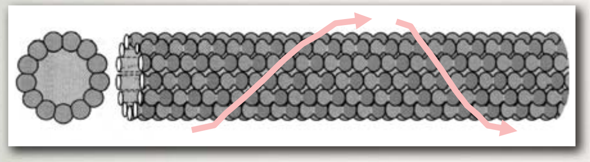


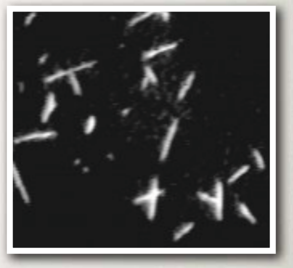
Microtubule building block: tubulin

Subunit: tubulin
10-20% of total protein in neural tissue
MW: ~50 kD: α- and β-tubulin -> heterodimer
1 molecule bound guanosine nucleotide (GTP or GDP);
exchangeable (β), and non-exchangeable (α)
Structural polarity
Genetic variability: at least 6 different α and β tubulins



The microtubule

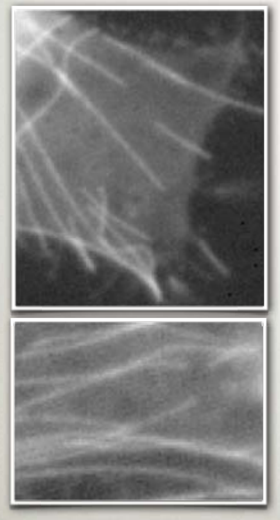




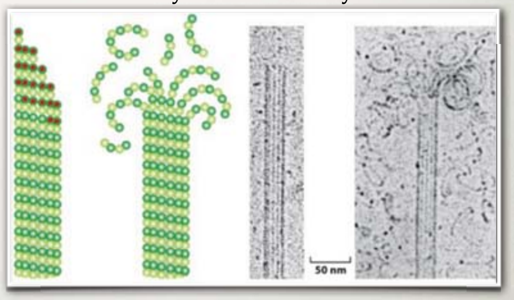
~25 nm in diameter, tubular structure
13 protofilaments
Right-handed short-pitch helix
Left-handed long-pitch helix
Structural polarity:
+end: rapid polymerization, terminated by β-subunit
-end: slow polymerization, terminated by α-subunit
GTP-cap

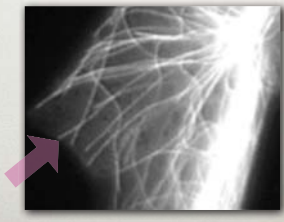
Polymerization equilibria in microtubules

Treadmilling



Dynamic instability



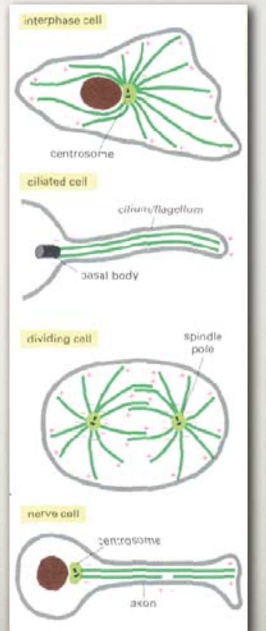


Microtubular system in the eukaryotic cell

Where?
Cytoplasm of interphase cell, axon, cilia, flagella, mitotic spindle.

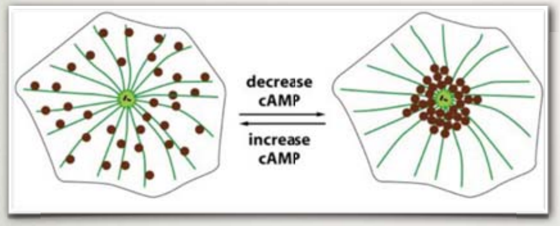
Polarity within the cell
-end in centrosome, +end in periphery.
Centrosome: 2 centrioles, centrosome matrix with γ-tubulin.

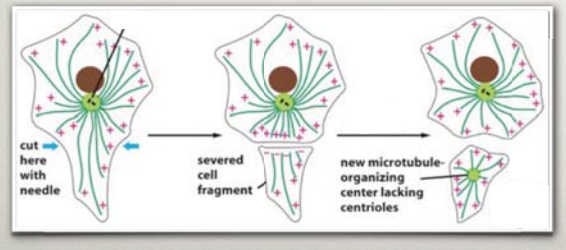
Microtubules might be involved in the commitment and fixation of cell polarity with the help of associated (capping) proteins.

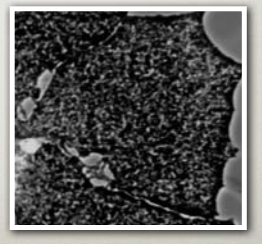


Functions of the microtubular system

1. “Highways” for motor proteins
2. Senses, monitors and finds the geometric center of the cell.
3. Motility functions (e.g., cell division)

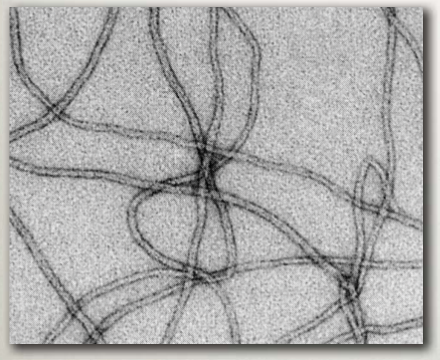


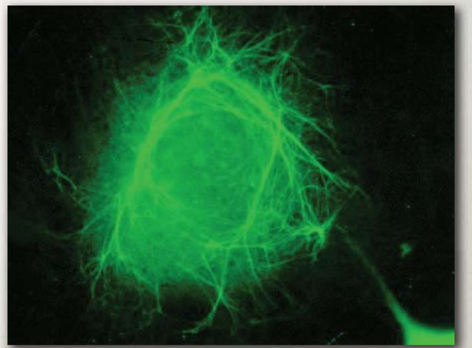




Intermediate filament system

Tissue-specific filamentous protein system composed of 8-10-nm filaments, found on most animal cell types.
Fundamental biological function is providing mechanical stability.

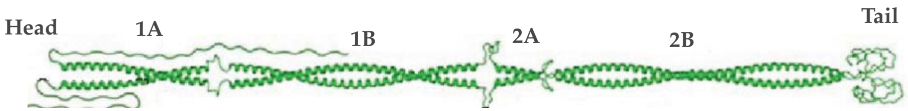




Vimentin, Vic Small

Intermediate filament building blocks

Intermediate filament dimer:



Properties:

-Chemically resistant (detergents, high ionic strength)
-Can be extracted with denaturants (e.g., urea)
-Fibrous monomer (not globular as actin or tubulin)
- -amino-terminal head
- -central rod (α-helix, heptad repeat)
- -carboxy-terminal tail
- -tissue-specific monomers differ in their terminal sequences

Structural unit of intermediate filaments: „coiled-coil” dimer

Heptad repeat, hydrophobic residues

Vimentin 1B domain dimer ribbon diagram

Vimentin 1B domain dimer wireframe diagram

Classification of intermediate filaments

Based on tissue specificity
(Classical categories)

Tissue type	Intermediate filament
Epithelium	Keratins
Muscle	Desmin
Mesenchyme	Vimentin
Glia	Glial fibrillar acidic protein (GFAP)
Nerve	Neurofilaments (NF-L, NF-M, NF-H)

Polymerization of intermediate filaments

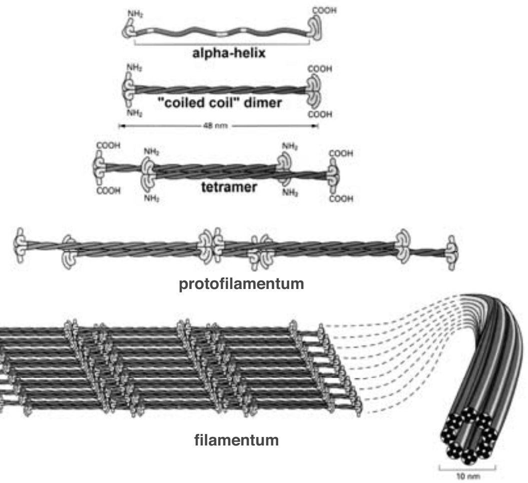
Fully polymerized state in the cell (not dynamic equilibrium)

Central rods (α-helix) hydrophobic interactions -> colied-coil dimer

2 dimers -> tetramer (antiparallel arrangement, structural apolarity)

Longitudinal association of tetramers -> protofilament

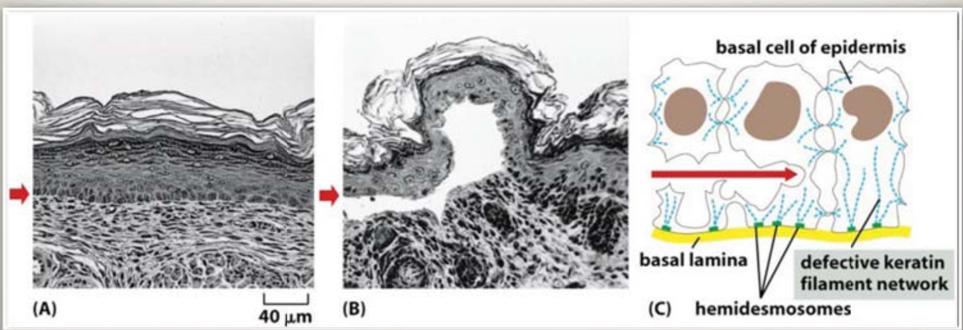
8 protofilaments -> filament



Tissue functions of intemediate filaments

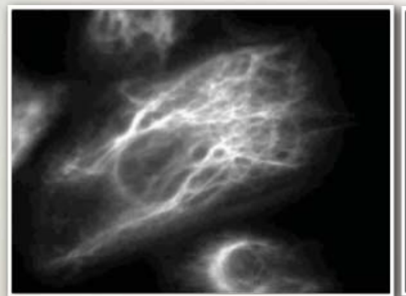
Providing mechanical stability

Epithelial cells:
- Pathology: *epidermolysis bullosa simplex*.
- Mutation in the keratin gene.
- Bullous epithelial destruction upon minor mechanical effects.



Intermediate filaments are more dynamic than earlier thought

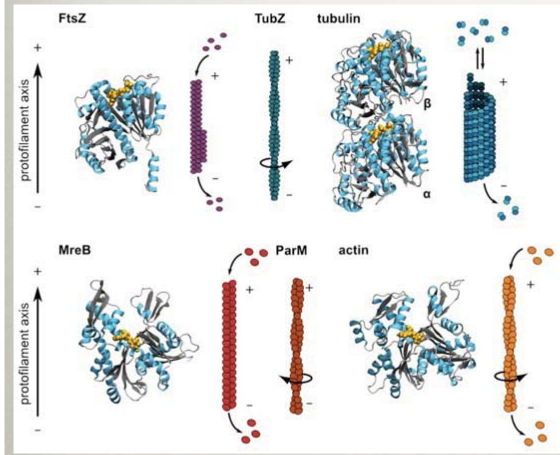
Dynamic vimentin rearrangement in the living cell



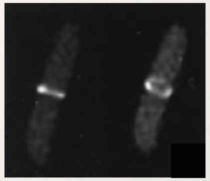
GFP-conjugated vimentin in 3T3 cell

Single filament turnover

Prokaryotic cytoskeleton



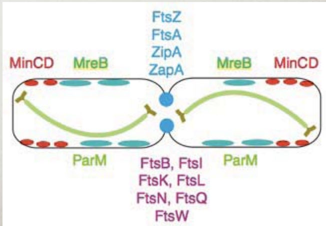
FtsZ: tubulin homolog



- Main component of the Z-ring
- Important role in cytokinesis
- Dynamic rearrangement

MreB: actin homolog

- Discovery based on sequence homology
- Helical filaments underneath cell membrane
- Role in chromosome segregation



MOTOR PROTEINS

1. Bind to specific filaments
2. Generate force and displacement
3. Convert chemical energy to mechanical

Types of motor proteins

1. Actin based

Myosins: Conventional (myosin II) and non-conventional
Myosin superfamily (I-XXIV classes). Move towards plus end.

2. Microtubule based

a. Dyneins: Ciliary (flagellar) and cytoplasmic dyneins.
Move towards the minus end along the microtubule.
b. Kinesins: Kinesin superfamily: conventional and non-conventional.
Move towards the plus end along the microtubule.
c. Dynamins: MT-dependent GTPase activity
Biological role: vacuolar protein sorting (pinchase enzymes)?

3. DNA based motors

DNA and RNA polymerases, virus capsid packaging motor, condensins
Produce force and displacement along the DNA strand

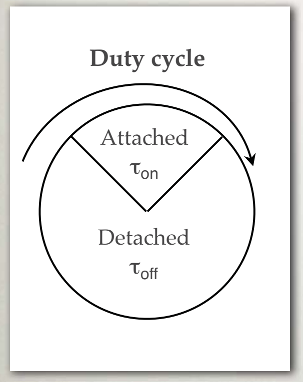
4. Rotary motors

F1F0-ATP synthase
Bacterial flagellar motor

5. Mechanoenzyme complexes

Ribosome

Duty cycle of motor proteins



"Duty ratio": $r = \frac{\delta V}{v}$

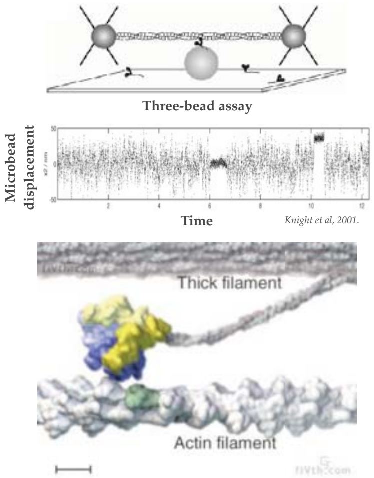
δ=working distance
V=ATPase rate
v=sliding velocity

Processive motor: r->1
E.g., kinesin, DNA-, RNA-polymerase.
Remains attached throughout most of the duty cycle.
Carries its load by itself.

Non-processive motor: r->0
E.g., myosin.
Remains detached throughout most of the duty cycle.
Works in ensembles.

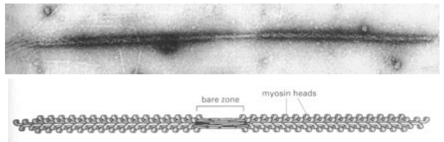
Force generated by a single motor protein: **few pN**.

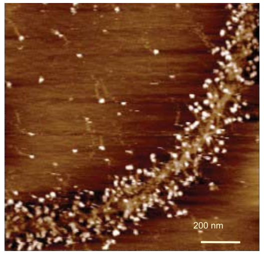
Non-processive motor proteins Myosin



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

Non-processive motors work in ensembles



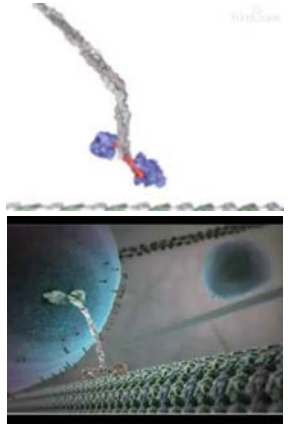


Synthetic thick filament
AFM image

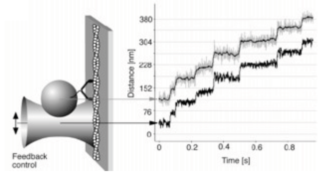
PROCESSIVE MOTOR PROTEINS

Kinesin

Step size: 8 nm
(distance between every other tubulin subunit)



Myosin V

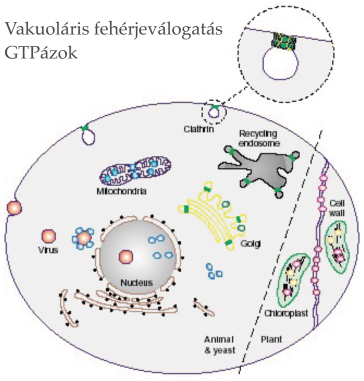


Step size: ~36 nm
(half pitch along actin helix)

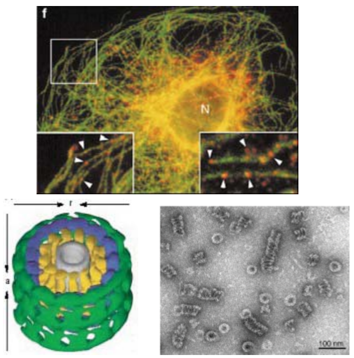
Processive motors work alone.

Dynamins

Vakuoláris fehérjeválogatás GTPázok

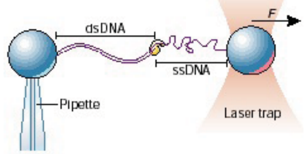


Protein	Localization	Function	Self-assembly
Dynamin	Plasma membrane (clathrin coated, caveolae), Golgi, endosomes	Vesicle formation, fission	+
Vps1	Golgi	Vesicle formation and transport	Unknown
Dnm1/Drp1/DRP-1	Mitochondria outer membrane	Mitochondrial fission & morphology	+
Mgm1/Msp1/OPA1	Mitochondria inner or outer membrane, or matrix	Mitochondrial morphology	Unknown
Phragmoplastin	Cell wall	Membrane morphology	+
ADL1	Cell wall, chloroplast	Membrane biogenesis	+
ADL2	Chloroplast	Unknown	Unknown
hGBP1	Cytoplasm	Anti-viral activity	+
Mx	Cytoplasm, nucleus	Anti-viral activity	+

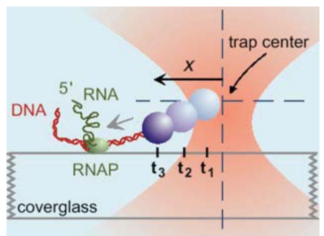


"pinchase" function

DNA Motors

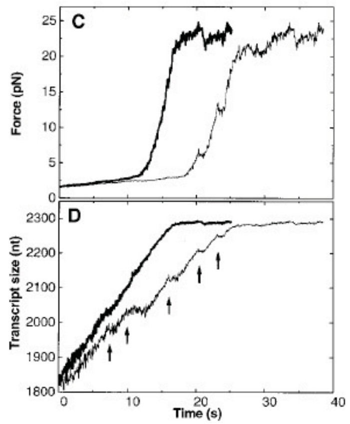


T7 DNA Polymerase



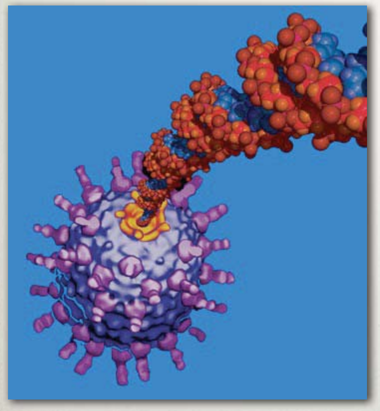
RNA Polymerase

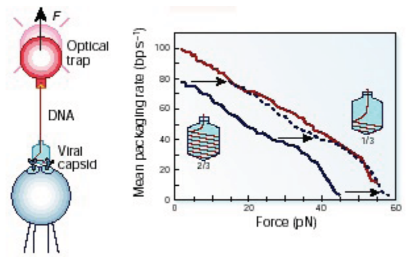
Processive motors



RNA Polymerase , Wang et al. 1998.

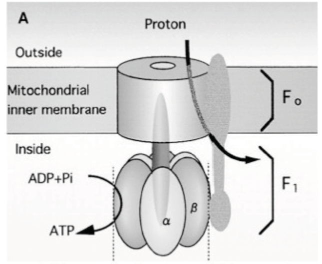
Virus portal motor
Special DNA motor

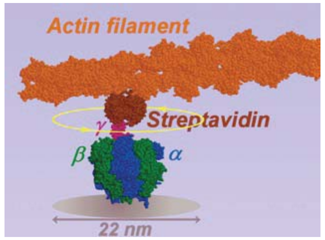


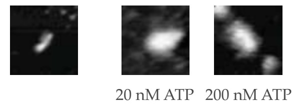


φ29 bacteriophage portal motor

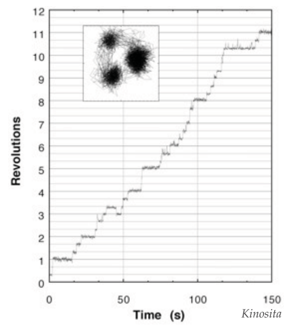
ROTARY MOTORS I:
F1F0-ATP Synthase



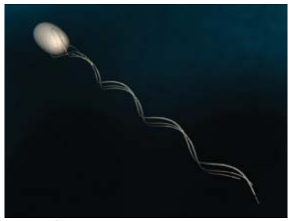


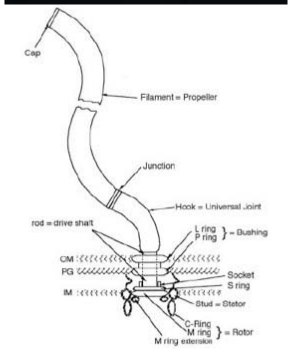


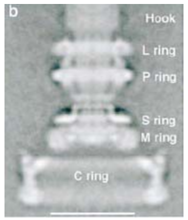
Discrete 120° rotational steps

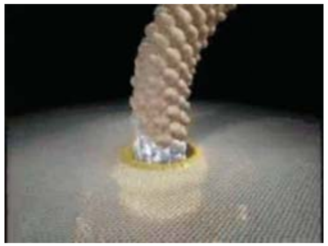


ROTARY MOTORS II:
Bacterial flagellar motor



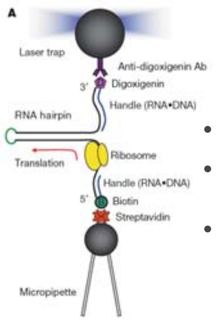






Speed: > 20000 rpm
Energy consumption: 10^{-16} W
Efficiency: > 80%
Energy source: protons

Mechanoenzyme complex
Ribosome



- 2.7 nm steps (one triplet)
- 0.078 s translocation time
- Helicase activity coupled with transocation