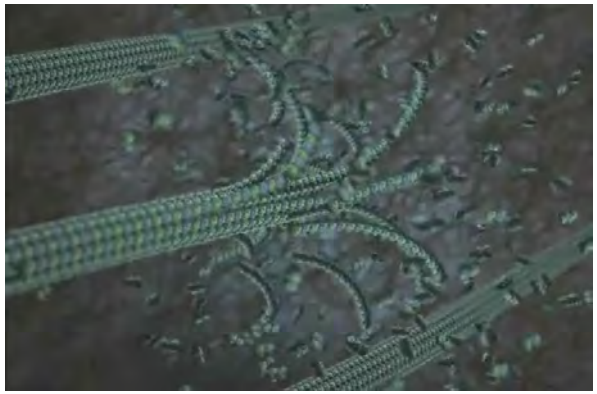
3. biopolymers - polymerization



the inner life of a cell, viel & lue, harvard [2006]

biopolymers

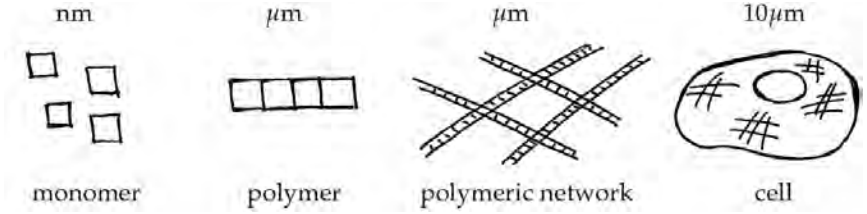
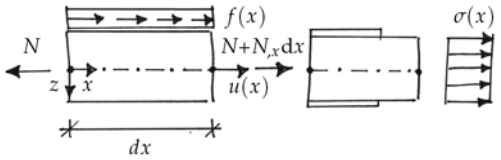


Figure 3.1. Biopolymers. Characteristic length scales on the cellular and sucellular level..

axial deformation - tension



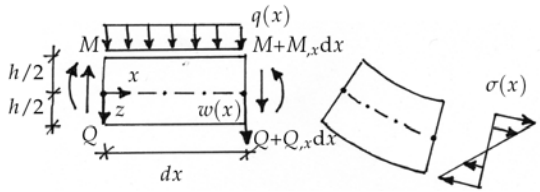
$EA\,u_{,xx} + f = 0$ with EA ... axial stiffness

cross section area $A = \pi r^2$

	r	A	E	EA
microtubule	12.5 nm	491 nm^2	1.9·10^9 N/m^2	93·10^{-8} N
intermediate filament	5.0 nm	79 nm^2	2.0·10^9 N/m^2	15·10^{-8} N
actin filament	3.5 nm	39 nm^2	1.9·10^9 N/m^2	7·10^{-8} N

Table 3.1: Axial stiffness EA of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

transverse deformation - bending



$q = EI\,w_{,xxxx}$ with EI ... bending stiffness

for circular cross sections $I = \pi r^4 / 4$

	r	I	E	EI
microtubule	12.5 nm	19,175 nm^4	1.9·10^{9}N/m^2	364·10^{-25}Nm^2
intermediate filament	5.0 nm	491 nm^4	2·10^{9}N/m^2	10·10^{-25}Nm^2
actin filament	3.5 nm	118 nm^4	1.9·10^{9}N/m^2	2·10^{-25}Nm^2

Table 3.2: Bending stiffness of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

concept of persistence length

- stiffer filaments are straighter ∝ bending stiffness EI
- cooler filaments are straighter ∝ inverse temperature kT

$$A = \frac{EI}{kT} \quad \ldots \quad \text{persistence length}$$

	r	E	EI	$A = [EI]/[kT]$
microtubule	12.5 nm	$1.9 \cdot 10^9 \mathrm{N/m^2}$	$364 \cdot 10^{-25} \mathrm{Nm^2}$	8.800 mm
intermediate filament	5.0 nm	$2 \cdot 10^9 \mathrm{N/m^2}$	$10 \cdot 10^{-25} \mathrm{Nm^2}$	0.240 mm
actin filament	3.5 nm	$1.9 \cdot 10^9 \mathrm{N/m^2}$	$2 \cdot 10^{-25} \mathrm{Nm^2}$	0.048 mm

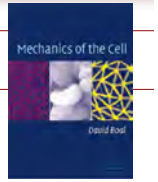
Table 3.6: Persistence lengths of major consituents of cytoskeleton at room temperature: microtubules, intermediate filaments and actin filaments

concept of persistence length

$$A = \frac{EI}{kT} \quad \ldots \quad \text{persistence length}$$

- informally, for pieces of the polymer that are **shorter** than the persistence length, the molecule behaves rather like a **flexible elastic rod**, while for pieces of the polymer that are much **longer** than the persistence length, the properties can only be described statistically, like a three-dimensional **random walk**
- formally, the persistence length is defined as the **length over which correlations** in the direction of the **tangent are lost**
- the persistence length is the **distance** which we should travel from one end of the chain **to bend it 90 degrees**

concept of persistence length



$$A = \frac{EI}{kT} \quad \ldots \quad \text{persistence length}$$

one way of **quantifying** the amplitude of the shape **fluctuations** at finite temperature is finding the typical distance along the rod over which it undergoes a significant change in direction: flexible rods change direction over shorter distances than stiff rods. this length scale must be **directly proportional to** the flexural rigidity ***EI*** and **inversely proportional to** the temperature ***kT***. in fact, the combination of EI/kT has the units of a length, and is defined as the persistence length of the filament.

suggested reading: 2.1 filaments in the cell / 2.5 elasticity and cellular filaments
mechanics of the cell, boal [2002]

concept of persistence length

$$A = \frac{EI}{kT} \quad \ldots \quad \text{persistence length}$$

- the persistence length is a measure of the length scale over which a **polymer remains roughly straight**
- the persistence length is a measure of the **competition between** the **entropic** parts of the free energy randomizing the orientation of the polymer and the **energetic** cost of bending.
- the persistence length is the scale over which the **tangent-tangent correlation function decays** along the chain

suggested reading: 8.2 macromolecules as random walks / 10.2.2 beam theory and the persistence length
physical biology of the cell, phillips, kondev, theriot [2009]

actin - polymerization

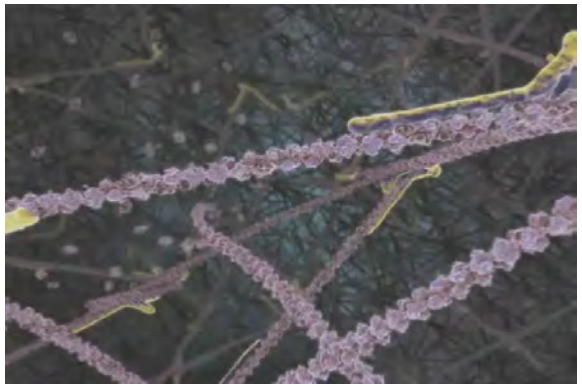


Figure 1.4.2 The actin network is a very dynamic structure with a continuous directional polymerization and disassembly. Filaments, capped at their minus ends by a protein complex, grow away from the plasma membrane by the addition of actin monomers to their plus end.

the inner life of a cell, viel & lue, harvard [2006]

microtubules - polymerization

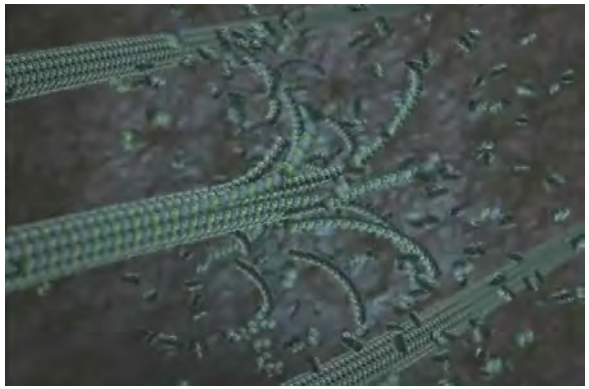
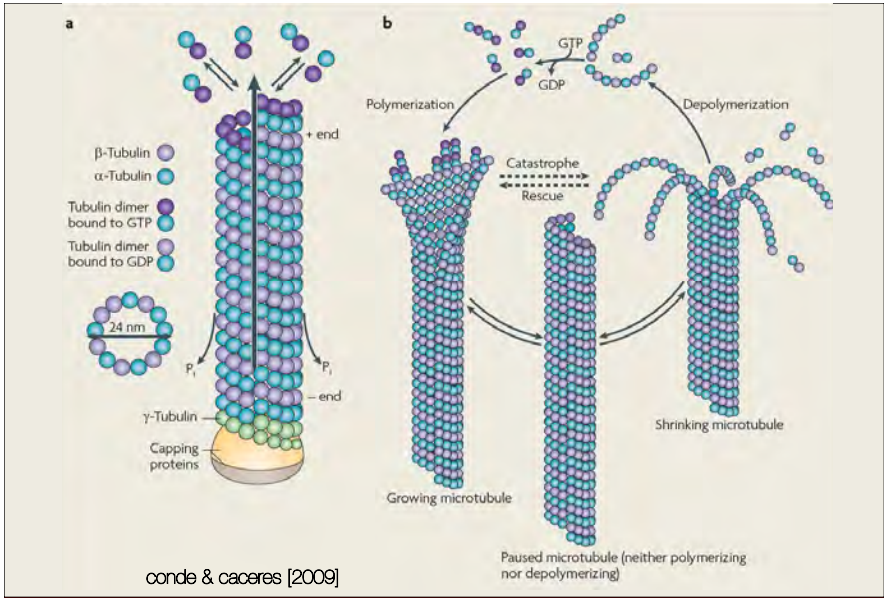


Figure 1.4.4 While the plus ends of some microtubules extend toward the plasma membrane, proteins stabilize the curved conformation of protofilaments from other microtubules, causing their rapid plus end depolymerization.

the inner life of a cell, viel & lue, harvard [2006]



conde & caceres [2009]

microtubules - polymerization

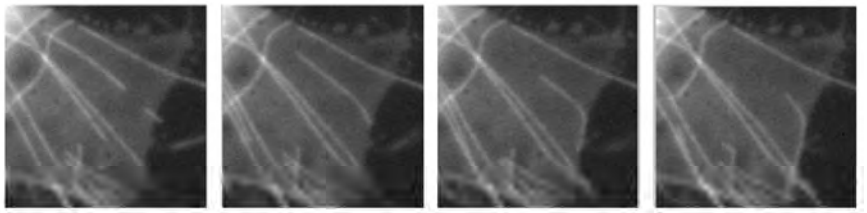


Figure 3.2: In vivo time sequence of fluorescently tagged cytoplasmic microtubules. Microtubules are nucleated and anchored by their minus ends at the centrosome and turn over by depolymerization and repolymerization at their plus ends. Free microtubules move toward the periphery by treadmilling growing at the plus end while shrinking at the minus end [37]

rodionov & borisy [1997]

microtubules - dynamic instability

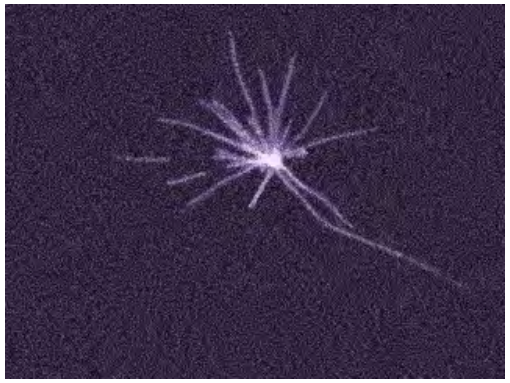


Figure 1.6.1 Microtubules continually grow from this centrosome added to a cell extract. Quite suddenly, however, some microtubules stop growing and then shrink back rapidly, a behavior called dynamic instability.

the cell, albers et al. [2008]

microtubules - dynamic instability

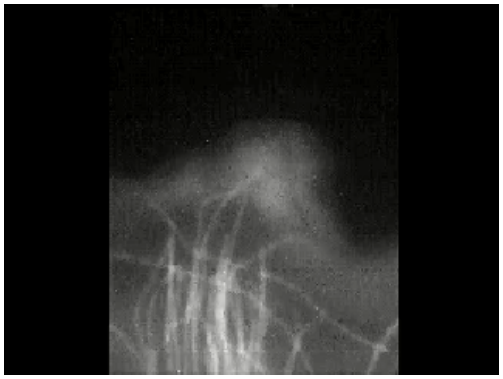


Figure 1.6.2 Governed by the principles of dynamic instability, microtubules constantly extend into the leading edge of a migrating cell and retract again. Superposed on the dynamic microtubule cytoskeleton (red), the membrane network of the endoplastic reticulum (green) exhibits its own dynamic behavior as tubes are extended by motor proteins on the microtubule tracks.

the cell, albers et al. [2008]

microtubules - dynamics *in vivo*

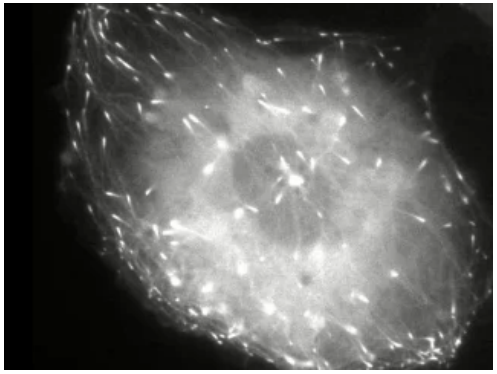


Figure 1.6.3 EB1 is a protein that binds to the GTP-tubulin cap at the growing ends of microtubules. Cells expressing an GFP-EB1 fusion protein reveal the spectacular dynamics of the microtubule cytoskeleton. Note that many but not all microtubules in this cell grow from the centrosome. Only the ends of the growing microtubules are visible; those that are static or shrinking have lost their GTP-tubulin caps and do not bind to EBI.

the cell, albers et al. [2008]

polymerization of idealized polymers

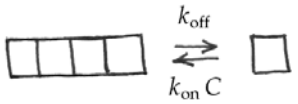


Figure 3.3: Model of idealized polymerization with addition and removal of subunits.

$$\frac{\mathrm{d}n}{\mathrm{d}t} = +k_{\text{on}}\,C \qquad \text{... monomer capture}$$

$$\frac{\mathrm{d}n}{\mathrm{d}t} = -k_{\text{off}} \qquad \text{... monomer release}$$

$$\frac{\mathrm{d}n}{\mathrm{d}t} = +k_{\text{on}}\,C - k_{\text{off}}$$

critical free monomer concentration

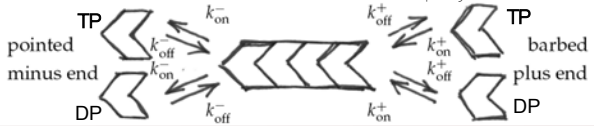
$$\frac{\mathrm{d}n}{\mathrm{d}t} = +k_{\text{on}}\,C_{\text{crit}} - k_{\text{off}} \doteq 0 \qquad \text{thus} \qquad C_{\text{crit}} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

polymerization of actin and tubulin

in reality, polymerization is more complex for two reasons. for actin and tubulin,

- subunits are **not symmetric**, i.e., they add to the two ends of the filament with preferred orientation giving rise to an **oriented filament**
- free subunits **carry triphosphate** nucleotide, ATP (actin) and GTP (tubulin) which is **hydrolized to diphosphate**, ADP/GDP, after polymerization

as a consequence, the two ends of a polymer grow at different rates. the faster growing end is often referred to as the + end or barbed end, the slower growing end as the - end or pointed end. this implies, that 2^3=8 rate constants are needed to describe actin or tubulin polymerization.



polymerization of actin and tubulin

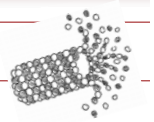


Figure 3.4: Eight rate constants for polymerization capture and release in non-symmetric actin and microtubules filaments.

	k_{on}^+ [1/(μMs)]	k_{off}^+ [1/s]	k_{on}^- [1/(μMs)]	k_{off}^- [1/s]	C_{crit}^+ μM	C_{crit}^- μM
ATP-actin	11.60	1.40	1.30	0.80	0.12	0.62
ADP-actin	3.80	7.20	0.16	0.27	1.90	1.70
GTP-tubulin	8.90	44.00	4.30	23.00	4.90	5.30
GDP-tubulin	0	733	0	915	n/a	n/a

Table 3.1: Measured rate constants of actin filaments and microtubules

polymerization of microtubules

Polymerization of microtubules Determine the time that it takes for a plus end of a microtubule to grow 5μm from the centrosome to the cell membrane. Assume a monomer concentration of $C = 10\mu$M and a tubulin dimer length of 8nm. Use the growth equation $dn/dn = k_{on}C - k_{off}$ with the parameters of GTP-tubulin,

polymerization of microtubules

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polymerization of actin - treadmilling

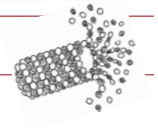


Figure 3.5: During treadmilling, monomers move along the filament from the plus end to the minus end while the overall filament might remain at constant length.

Treadmilling The special situation when one end of a filament polymerizes while the other end depolymerizes is referred to as treatmilling. Treadmilling can be observed in many cellular cytoskeletal filaments, especially in actin filaments and microtubules. The filament grows at the plus or barbed end while, at the same, it shrinks at the minus or pointed end. From the outside, it seems as if segments of the filament move across the cytosol [1]. In general, treadmilling may occur at different rates at both ends. At a particular concentration at which the speed of growth at the plus end is equal to the rate of growth at the minus end the net length of the treadmilling filament remains unchanged. This is state is called steady-state treadmilling.

polymerization of actin - steady state treadmilling

Figure 3.5: During treadmilling, monomers move along the filament from the plus end to the minus end while the overall filament might remain at constant length.

$$C_{crit}^{-} = \frac{k_{off}^{-}}{k_{on}^{-}} = \frac{0.8}{1.3}\,\mu M = 0.62\,\mu M \qquad C_{crit}^{+} = \frac{k_{off}^{+}}{k_{on}^{+}} = \frac{1.4}{11.6}\,\mu M = 0.12\,\mu M$$

$$\frac{dn^{+}}{dt} + \frac{dn^{-}}{dt} = k_{on}^{+} C_{std} - k_{off}^{+} + k_{on}^{-} C_{std} - k_{off}^{-} \doteq 0 \qquad C_{std} = \frac{k_{off}^{+} + k_{off}^{-}}{k_{on}^{+} + k_{on}^{-}}$$

polymerization of actin - steady state treadmilling

Figure 3.5: During treadmilling, monomers move along the filament from the plus end to the minus end while the overall filament might remain at constant length.

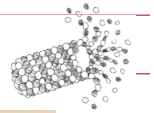
$$C_{crit}^{-} = \frac{k_{off}^{-}}{k_{on}^{-}} = \frac{0.8}{1.3}\,\mu M = 0.62\,\mu M \qquad C_{crit}^{+} = \frac{k_{off}^{+}}{k_{on}^{+}} = \frac{1.4}{11.6}\,\mu M = 0.12\,\mu M$$

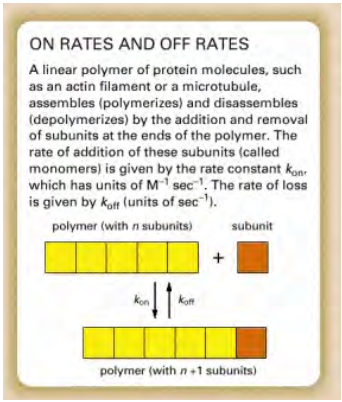
$$\frac{dn^{+}}{dt} + \frac{dn^{-}}{dt} = k_{on}^{+} C_{std} - k_{off}^{+} + k_{on}^{-} C_{std} - k_{off}^{-} \doteq 0 \qquad C_{std} = \frac{k_{off}^{+} + k_{off}^{-}}{k_{on}^{+} + k_{on}^{-}}$$

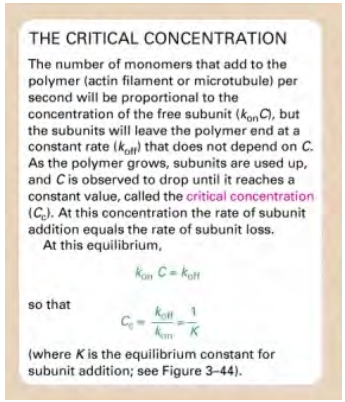
$$C_{std} = \frac{k_{off}^{+} + k_{off}^{-}}{k_{on}^{+} + k_{on}^{-}} = \frac{1.4 + 0.8}{11.6 + 1.3}\,\mu M = 0.17\,\mu M \quad \text{steady state concentration}$$

$$C_{crit}^{+} = 0.12\,\mu M \leq C_{std} = 0.17\,\mu M \leq C_{crit}^{-} = 0.62\,\mu M$$

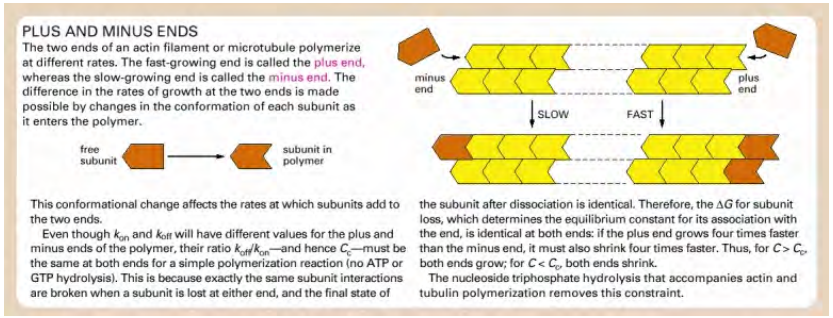
polymerization of actin and tubulin







alberts, johnson, lewis, raff, roberts, walter [2002]



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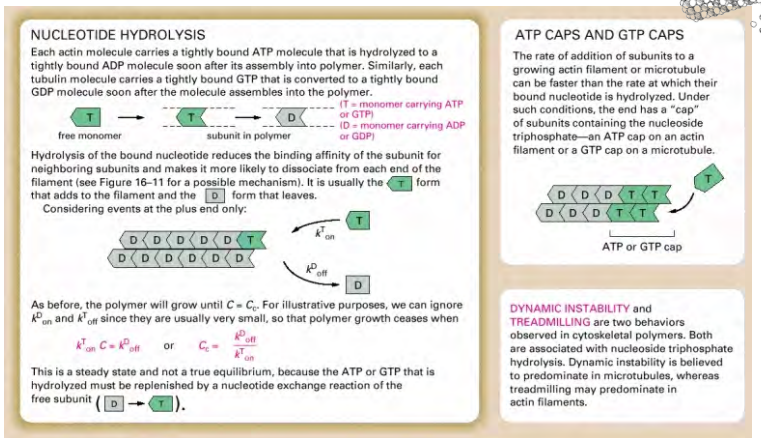

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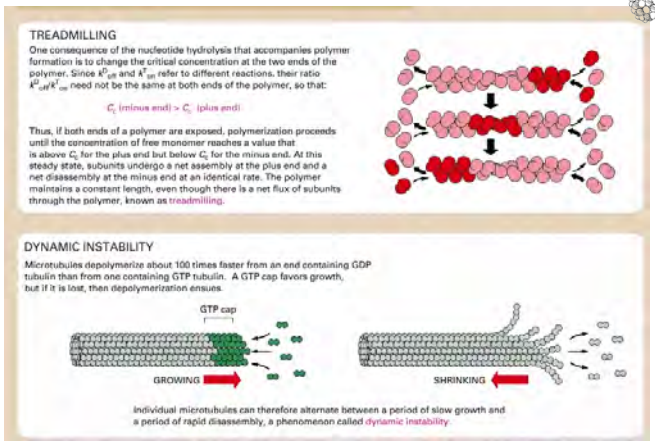

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