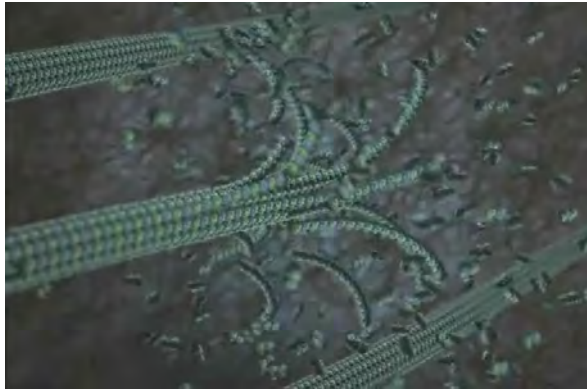
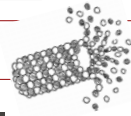


3. biopolymers - polymerization



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

me239 mechanics of the cell

1

biopolymers

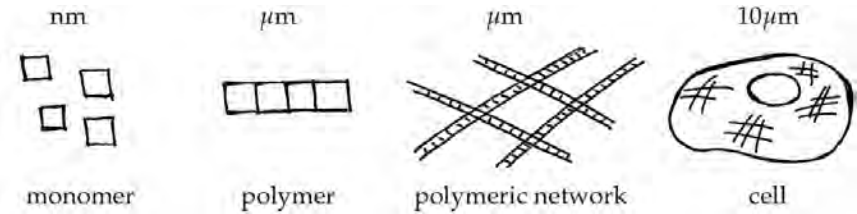
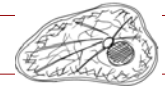
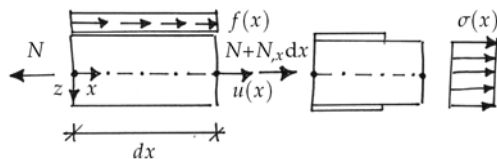


Figure 3.1. Biopolymers. Characteristic length scales on the cellular and subcellular level.

3.1 biopolymers - motivation

2

axial deformation - tension



$EA u_{,xx} + f = 0$ with EA ... axial stiffness
cross section area $A = \pi r^2$

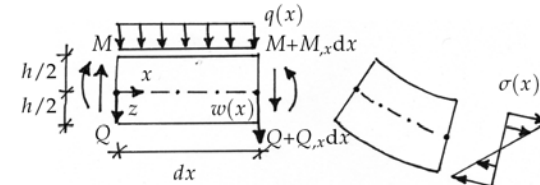
	r	A	E	EA
microtubule	12.5 nm	491 nm ²	1.9·10 ⁹ N/m ²	93·10 ⁻⁸ N
intermediate filament	5.0 nm	79 nm ²	2.0·10 ⁹ N/m ²	15·10 ⁻⁸ N
actin filament	3.5 nm	39 nm ²	1.9·10 ⁹ N/m ²	7·10 ⁻⁸ N

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

3.3 biopolymers - energy

3

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 constituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

3.3 biopolymers - energy

4

concept of persistence length



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

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

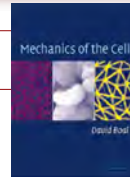
	r	E	EI	$A = [EI]/[kT]$
microtubule	12.5 nm	$1.9 \cdot 10^9 \text{N/m}^2$	$364 \cdot 10^{-25} \text{Nm}^2$	8.800 mm
intermediate filament	5.0 nm	$2 \cdot 10^9 \text{N/m}^2$	$10 \cdot 10^{-25} \text{Nm}^2$	0.240 mm
actin filament	3.5 nm	$1.9 \cdot 10^9 \text{N/m}^2$	$2 \cdot 10^{-25} \text{Nm}^2$	0.048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

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

3.3 biopolymers - entropy

5

concept of persistence length



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

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]

3.4 biopolymers - entropy

7

concept of persistence length



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

- 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**

3.4 biopolymers - entropy

6

concept of persistence length



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

- 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]

3.4 biopolymers - entropy

8

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]

3.2 biopolymers - polymerization

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microtubules - polymerization

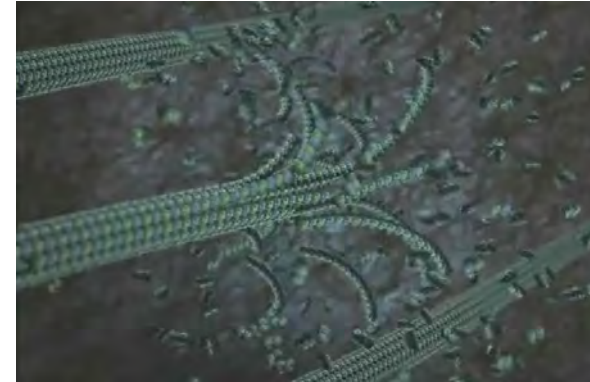
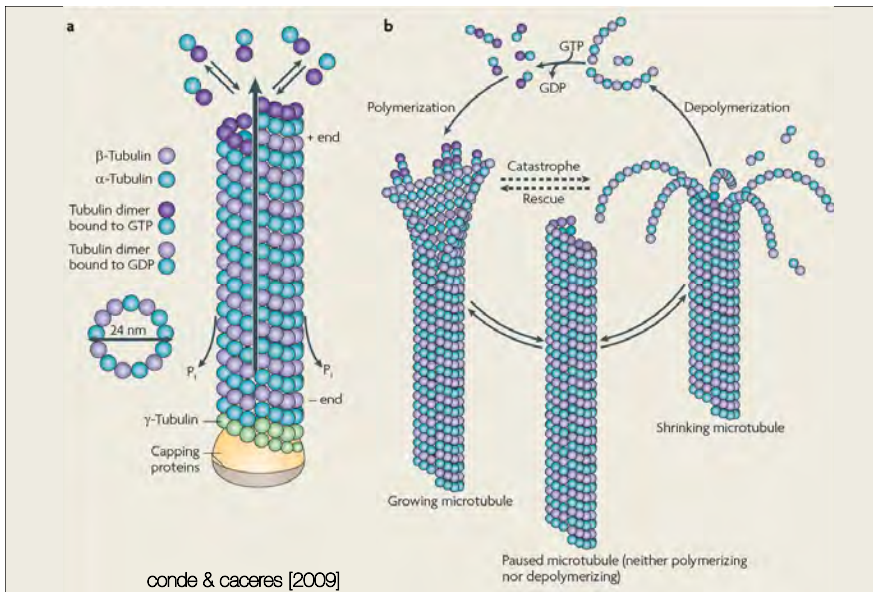


Figure 1.4.4 While the plus ends of some microtubules extend toward the plasma membrane, proteins stabilize the curved conformation of protofilaments from other microtubules, causing their rapid plus end depolymerization.

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

3.2 biopolymers - polymerization

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3.2 biopolymers - polymerization

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microtubules - polymerization

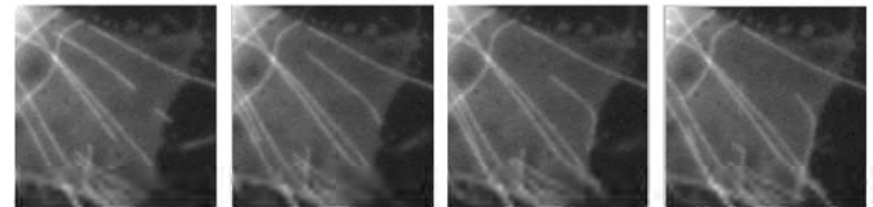


Figure 3.2: In vivo time sequence of fluorescently tagged cytoplasmic microtubules. Microtubules are nucleated and anchored by their minus ends at the centrosome and turn over by depolymerization and repolymerization at their plus ends. Free microtubules move toward the periphery by treadmilling growing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

rodionov & borisy [1997]

3.2 biopolymers - polymerization

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microtubules - dynamic instability

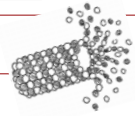


Figure 1.6.1 Microtubules continually grow from this centrosome added to a cell extract. Quite suddenly, however, some microtubules stop growing and then shrink back rapidly, a behavior called dynamic instability.

the cell, albers et al. [2008]

3.2 biopolymers - polymerization

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microtubules - dynamic instability

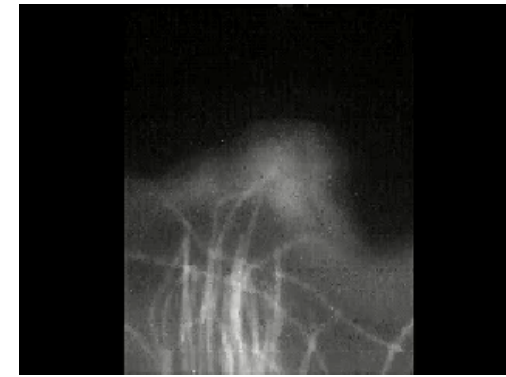
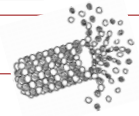


Figure 1.6.2 Governed by the principles of dynamic instability, microtubules constantly extend into the leading edge of a migrating cell and retract again. Superposed on the dynamic microtubule cytoskeleton (red), the membrane network of the endoplasmic reticulum (green) exhibits its own dynamic behavior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

the cell, albers et al. [2008]

3.2 biopolymers - polymerization

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microtubules - dynamics *in vivo*

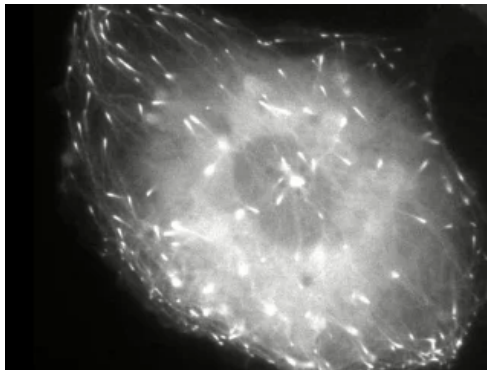
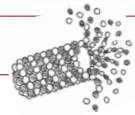


Figure 1.6.3 EB1 is a protein that binds to the GTP-tubulin cap at the growing ends of microtubules. Cells expressing an GFP-EB1 fusion protein reveal the spectacular dynamics of the microtubule cytoskeleton. Note that many but not all microtubules in this cell grow from the centrosome. Only the ends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

the cell, albers et al. [2008]

3.2 biopolymers - polymerization

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polymerization of idealized polymers

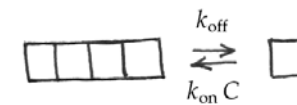
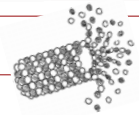


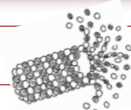
Figure 3.3: Model of idealized polymerization with addition and removal of subunits.

$$\begin{aligned} \frac{dn}{dt} &= +k_{\text{on}} C && \dots \text{ monomer capture} && \frac{dn}{dt} &= +k_{\text{on}} C - k_{\text{off}} \\ \frac{dn}{dt} &= -k_{\text{off}} && \dots \text{ monomer release} && & \\ &&& \text{critical free monomer concentration} && & \\ \frac{dn}{dt} &= +k_{\text{on}} C_{\text{crit}} - k_{\text{off}} \stackrel{!}{=} 0 && \text{thus} && C_{\text{crit}} &= \frac{k_{\text{off}}}{k_{\text{on}}} \end{aligned}$$

3.2 biopolymers - polymerization

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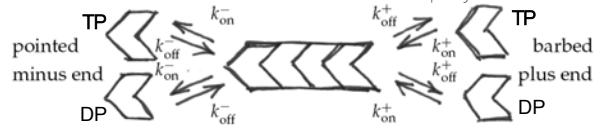
polymerization of actin and tubulin



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 **hydrolyzed 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.



3.2 biopolymers - polymerization

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polymerization of actin and tubulin

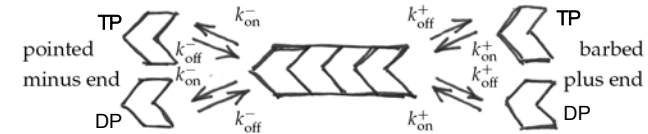
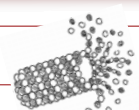


Figure 3.4: Eight rate constants for polymerization capture and release in non-symmetric actin and microtubules filaments.

	k_{on}^+ [1/(μ Ms)]	k_{off}^+ [1/s]	k_{on}^- [1/(μ Ms)]	k_{off}^- [1/s]	C_{crit}^+ μ M	C_{crit}^- μ M
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

3.2 biopolymers - polymerization

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polymerization of microtubules



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

polymerization of microtubules



Polymerization of microtubules Determine the time that it takes for a plus end of a microtubule to grow $5\mu\text{m}$ from the centrosome to the cell membrane. Assume a monomer concentration of $C = 10\mu\text{M}$ and a tubulin dimer length of 8nm . Use the growth equation $dn/dt = k_{on}C - k_{off}$ with the parameters of GTP-tubulin, i.e., $k_{on}^+ = 8.9/(\mu\text{Ms})$ and $k_{off}^+ = 44/\text{s}$ $dn/dt = [8.9 \cdot 10 - 44] / \text{s} = 45/\text{s}$. The required number of tubulin dimers is $n = 5\mu\text{m} / 8\text{nm} = 5000/8 = 625$. The required time follows from $dn/dt = 45/\text{s} = t/n$, thus $t = n / [dn/dt] = 625 / 45 \text{ s} = 13.89 \text{ s}$. It takes 13.89 seconds for the microtubule to grow from the centrosome to the cell membrane. But how long would it take for the polymer to shrink to zero length? Let's assume microtubule shrinks only from the membrane end. Then, $dn/dt = k_{on}C - k_{off}$ with the parameters of GDP-tubulin, i.e., $k_{on}^+ = 0/(\mu\text{Ms})$ and $k_{off}^+ = 733/\text{s}$. Then $dn/dt = [0 - 733] / \text{s} = -733/\text{s}$. With $n = 625$, the required time is $t = n / [dn/dt] = -625 / -733 \text{ s} = 0.85 \text{ s}$. Polymer shrinkage takes 0.85 s. In this example, polymer shrinkage is approximately 16 times faster than polymer growth.

3.2 biopolymers - polymerization

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3.2 biopolymers - polymerization

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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 treadmilling. 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.

3.2 biopolymers - polymerization

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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.

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

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

3.2 biopolymers - polymerization

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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.

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

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

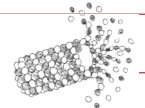
$$C_{\text{std}} = \frac{k_{\text{off}}^+ + k_{\text{off}}^-}{k_{\text{on}}^+ + k_{\text{on}}^-} = \frac{1.4 + 0.8}{11.6 + 1.3} \mu\text{M} = 0.17 \mu\text{M} \quad \text{steady state concentration}$$

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

3.2 biopolymers - polymerization

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polymerization of actin and tubulin



ON RATES AND OFF RATES

A linear polymer of protein molecules, such as an actin filament or a microtubule, assembles (polymerizes) and disassembles (depolymerizes) by the addition and removal of subunits at the ends of the polymer. The rate of addition of these subunits (called monomers) is given by the rate constant k_{on} , which has units of $\text{M}^{-1} \text{sec}^{-1}$. The rate of loss is given by k_{off} (units of sec^{-1}).

polymer (with n subunits) + subunit

k_{on} k_{off}

polymer (with $n+1$ subunits)

THE CRITICAL CONCENTRATION

The number of monomers that add to the polymer (actin filament or microtubule) per second will be proportional to the concentration of the free subunit ($k_{\text{on}}C$), but the subunits will leave the polymer end at a constant rate (k_{off}) that does not depend on C . As the polymer grows, subunits are used up, and C is observed to drop until it reaches a constant value, called the **critical concentration** (C_c). At this concentration the rate of subunit addition equals the rate of subunit loss.

At this equilibrium,

$$k_{\text{on}} C = k_{\text{off}}$$

so that

$$C_c = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{1}{K}$$

(where K is the equilibrium constant for subunit addition; see Figure 3-44).

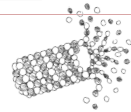
alberts, johnson, lewis, raif, roberts, walter [2002]



3.2 biopolymers - polymerization

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polymerization of actin and tubulin



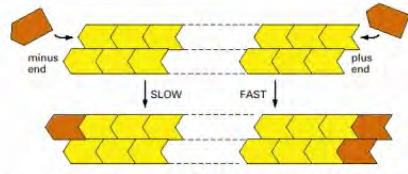
PLUS AND MINUS ENDS

The two ends of an actin filament or microtubule polymerize at different rates. The fast-growing end is called the **plus end**, whereas the slow-growing end is called the **minus end**. The difference in the rates of growth at the two ends is made possible by changes in the conformation of each subunit as it enters the polymer.



This conformational change affects the rates at which subunits add to the two ends.

Even though k_{on} and k_{off} will have different values for the plus and minus ends of the polymer, their ratio k_{off}^+/k_{on}^+ —and hence C_c^+ —must be the same at both ends for a simple polymerization reaction (no ATP or GTP hydrolysis). This is because exactly the same subunit interactions are broken when a subunit is lost at either end, and the final state of



the subunit after dissociation is identical. Therefore, the ΔG for subunit loss, which determines the equilibrium constant for its association with the end, is identical at both ends: if the plus end grows four times faster than the minus end, it must also shrink four times faster. Thus, for $C > C_c$, both ends grow; for $C < C_c$, both ends shrink. The nucleoside triphosphate hydrolysis that accompanies actin and tubulin polymerization removes this constraint.

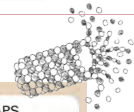
alberts, johnson, lewis, raff, roberts, walter [2002]

THE CELL

3.2 biopolymers - polymerization

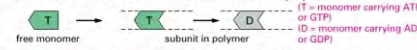
25

polymerization of actin and tubulin



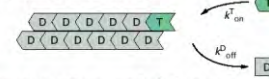
NUCLEOTIDE HYDROLYSIS

Each actin molecule carries a tightly bound ATP molecule that is hydrolyzed to a tightly bound ADP molecule soon after its assembly into polymer. Similarly, each tubulin molecule carries a tightly bound GTP that is converted to a tightly bound GDP molecule soon after the molecule assembles into the polymer.



Hydrolysis of the bound nucleotide reduces the binding affinity of the subunit for neighboring subunits and makes it more likely to dissociate from each end of the filament (see Figure 16-11 for a possible mechanism). It is usually the **D** form that adds to the filament and the **T** form that leaves.

Considering events at the plus end only:



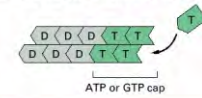
As before, the polymer will grow until $C = C_c$. For illustrative purposes, we can ignore k_{on}^D and k_{off}^T since they are usually very small, so that polymer growth ceases when

$$k_{on}^T C = k_{off}^D \quad \text{or} \quad C_c = \frac{k_{off}^D}{k_{on}^T}$$

This is a steady state and not a true equilibrium, because the ATP or GTP that is hydrolyzed must be replenished by a nucleotide exchange reaction of the free subunit ($D \rightarrow T$).

ATP CAPS AND GTP CAPS

The rate of addition of subunits to a growing actin filament or microtubule can be faster than the rate at which their bound nucleotide is hydrolyzed. Under such conditions, the end has a "cap" of subunits containing the nucleoside triphosphate—an ATP cap on an actin filament or a GTP cap on a microtubule.



DYNAMIC INSTABILITY and **TREADMILLING** are two behaviors observed in cytoskeletal polymers. Both are associated with nucleoside triphosphate hydrolysis. Dynamic instability is believed to predominate in microtubules, whereas treadmilling may predominate in actin filaments.

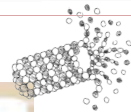
alberts, johnson, lewis, raff, roberts, walter [2002]

THE CELL

3.2 biopolymers - polymerization

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polymerization of actin and tubulin

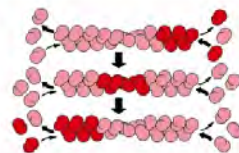


TREADMILLING

One consequence of the nucleotide hydrolysis that accompanies polymer formation is to change the critical concentration at the two ends of the polymer. Since k_{on}^+ and k_{off}^+ refer to different reactions, their ratio k_{off}^+/k_{on}^+ need not be the same at both ends of the polymer, so that:

$$C_c^+ (\text{minus end}) > C_c^+ (\text{plus end})$$

Thus, if both ends of a polymer are exposed, polymerization proceeds until the concentration of free monomer reaches a value that is above C_c^+ for the plus end but below C_c^+ for the minus end. At this steady state, subunits undergo a net assembly at the plus end and a net disassembly at the minus end at an identical rate. The polymer maintains a constant length, even though there is a net flux of subunits through the polymer, known as **treadmilling**.



DYNAMIC INSTABILITY

Microtubules depolymerize about 100 times faster from an end containing GDP tubulin than from one containing GTP tubulin. A GTP cap favors growth, but if it is lost, then depolymerization ensues.



Individual microtubules can therefore alternate between a period of slow growth and a period of rapid disassembly, a phenomenon called **dynamic instability**.

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

THE CELL

3.2 biopolymers - polymerization

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