CELL MECHANICS

1. Introduction - L1

2. Physical principles

2.1. Forces at molecular and cell level - L2

2.2. Thermal forces, diffusion, and chemical forces;
and Single molecule experiments – L3, L4, L5, L6

3. Mechanics of the Cytoskeleton and Mechnaotransduction

3.1. Motor proteins - types, working principle – L8-9

3.2. Force generation by the cytoskeleton and cell motility – L10

3.3. Cellular mechanotransduction (basic principles and examples) –

4. Experimental techniques to study cell mechanics

4.1. Optical, magnetic and acoustic tweezers L7, L11

4.2. Super-resolution optical microscopy techniques (STED, PALM) - L12

4.3. Lab visit and experimental optical tweezers – cell mechanics session at CNR-IOM – January 2021

OUTLINE

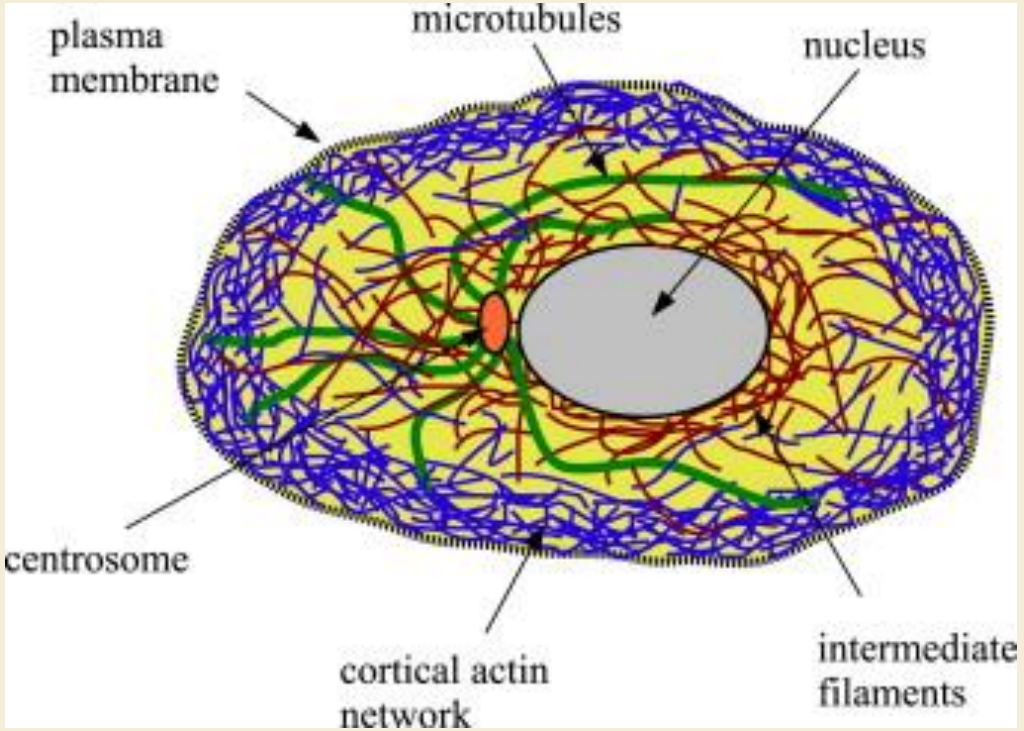
- Cytoskeleton filaments: types, structure, localization and function
- Cytoskeleton filaments polymerization and depolymerization.
- Forces generated by polymerization / depolymerization
- Cell motility induced by cytoskeleton filaments, examples.

1. Howard Book, Ch. 9 Polymerization of Cytoskeleton Filaments; Ch. 11 Active polymerization; Ch 10 Force generation by cytoskeletal filaments
2. Alberts Book Ch 16. Self assembling

The **cytoskeleton** is a **self assembly** and **dynamic structure of protein filaments.**

The **cytoskeleton** has several critical functions as:

- determines the cell shape
- provides mechanical strength and resistance to shear stress
- determines the position of the cell organelles
- supports cell motility and locomotion
- supports cell division
- provides a track-like system that directs the movement of organelles and other substances within cells.

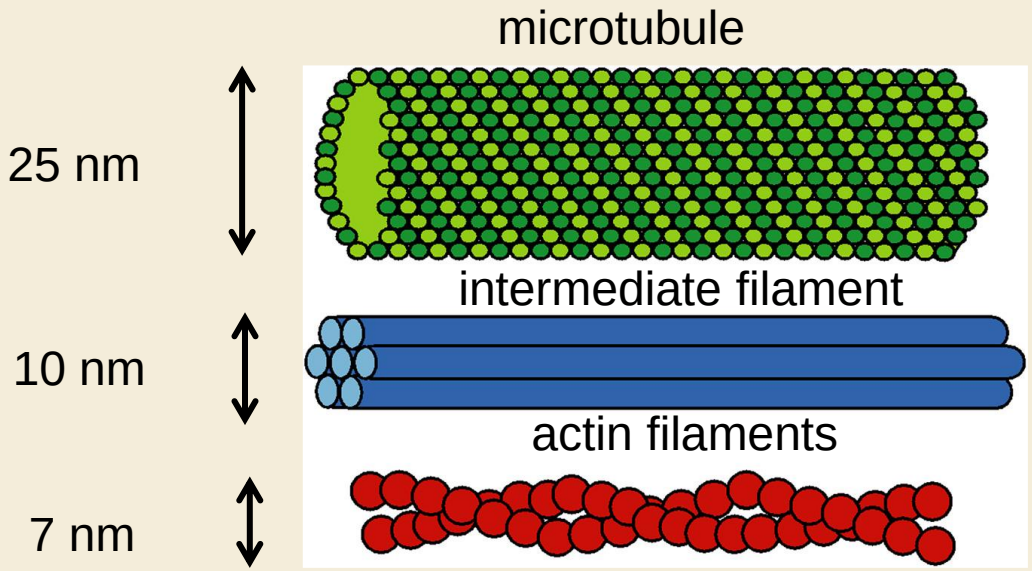


Cytoskeletal filaments:

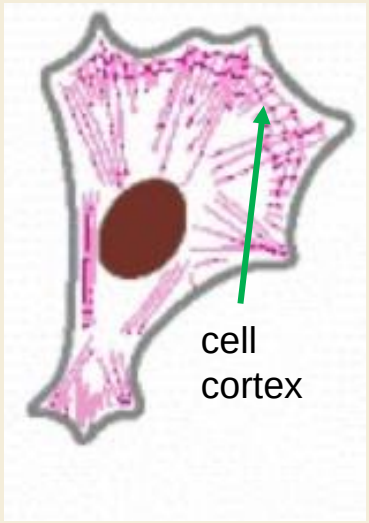
Intermediate filaments : provide mechanical strength and resistance to shear stress.

Microtubules: determine the positions of membrane-enclosed organelles and direct intracellular transport.

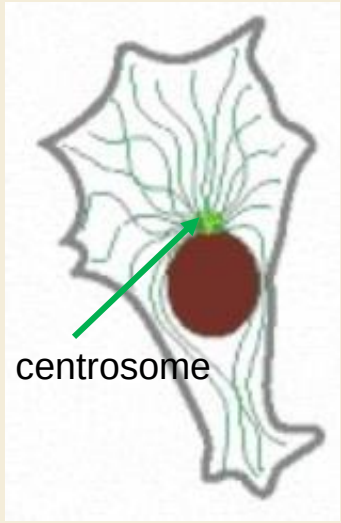
Actin filaments: determine the shape of the cell's surface and are necessary for cell locomotion.



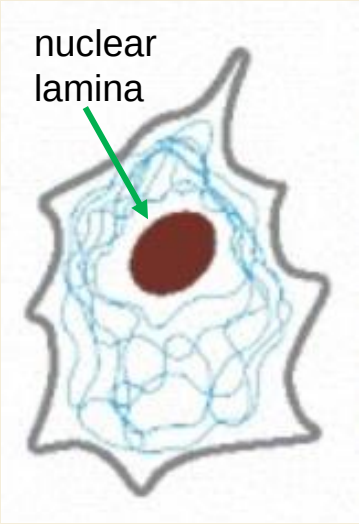
Localization of the cytoskeletal filaments in the cell



actin filaments

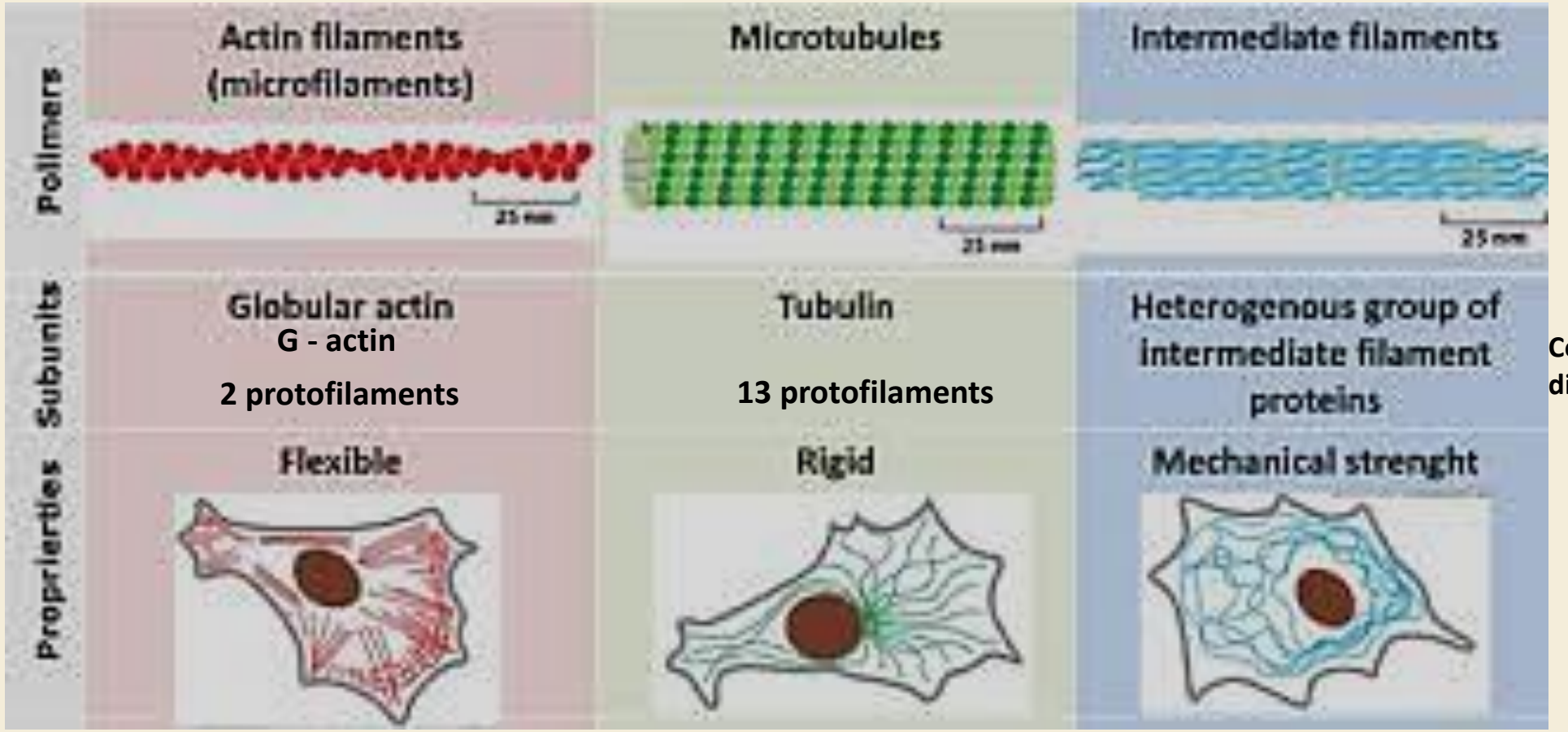


microtubules



intermediate filaments

Cytoskeletal filaments / subunits:



All three types of cytoskeletal polymer filaments form as **helical assemblies of subunits / protofilaments**, which self-associate, using a combination of end-to-end and side to side protein contacts.

Subunit self association and growing are based on two types of reactions:

- Polymerization / depolymerization
- ATP or GTP hydrolysis

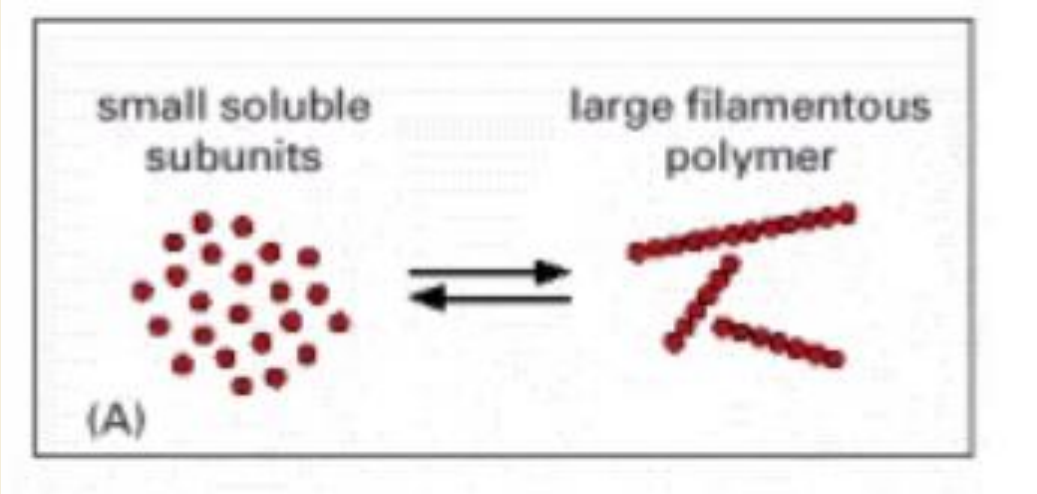
Moreover, filaments dynamics and networking are regulated by accessory proteins.

The **stability and mechanical properties** of each type of filaments depend on differences in the **structure** of the subunits and the **strength of the attractive forces** between them.

The three types of **cytoskeletal "polymers"** are held together by **weak noncovalent interactions**, which means that their assembly and disassembly can occur rapidly, without covalent bonds being formed or broken.

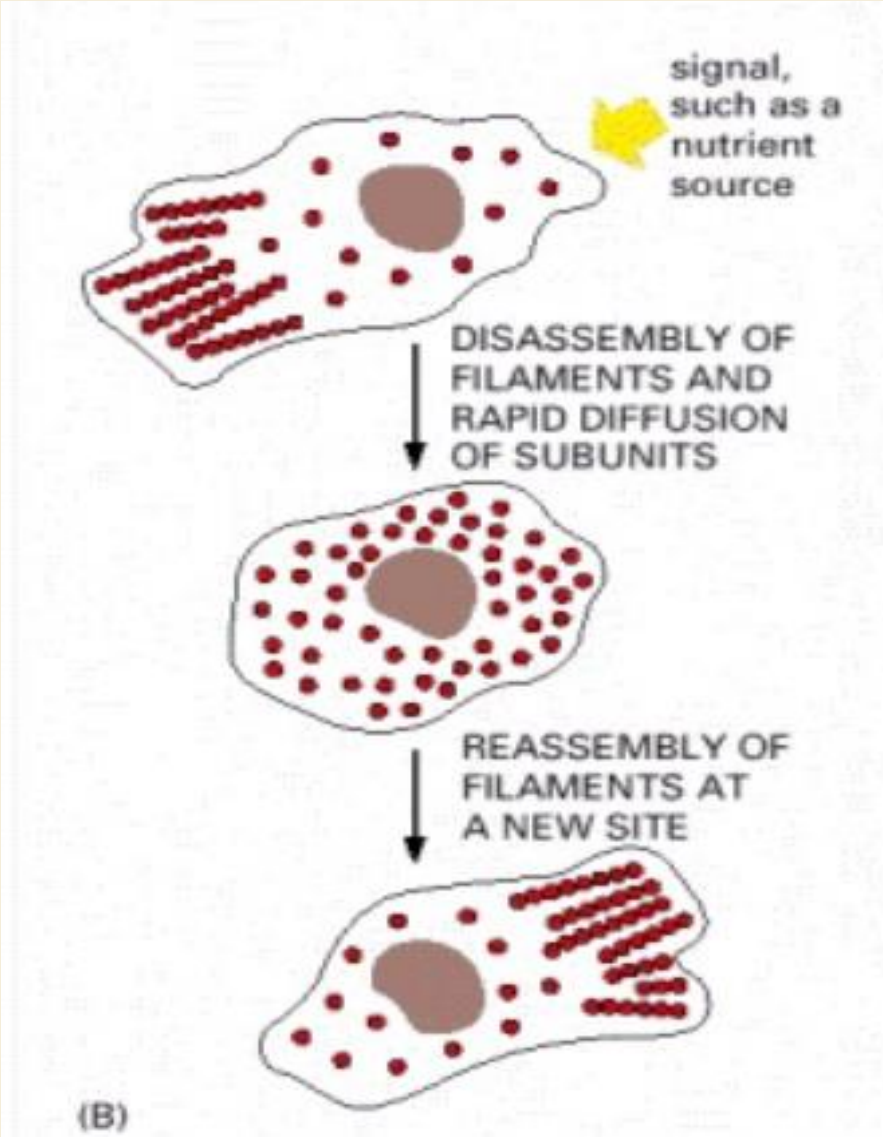
In contrast, many biological polymers—including **DNA, RNA, and proteins**—are held together by **covalent linkages** between their subunits.

A cytoskeletal filament is constructed from protein subunits by polymerization



The formation of protein filaments from much smaller protein subunits (nm size) allows regulated filament assembly and disassembly to reshape the cytoskeleton / cell shape.

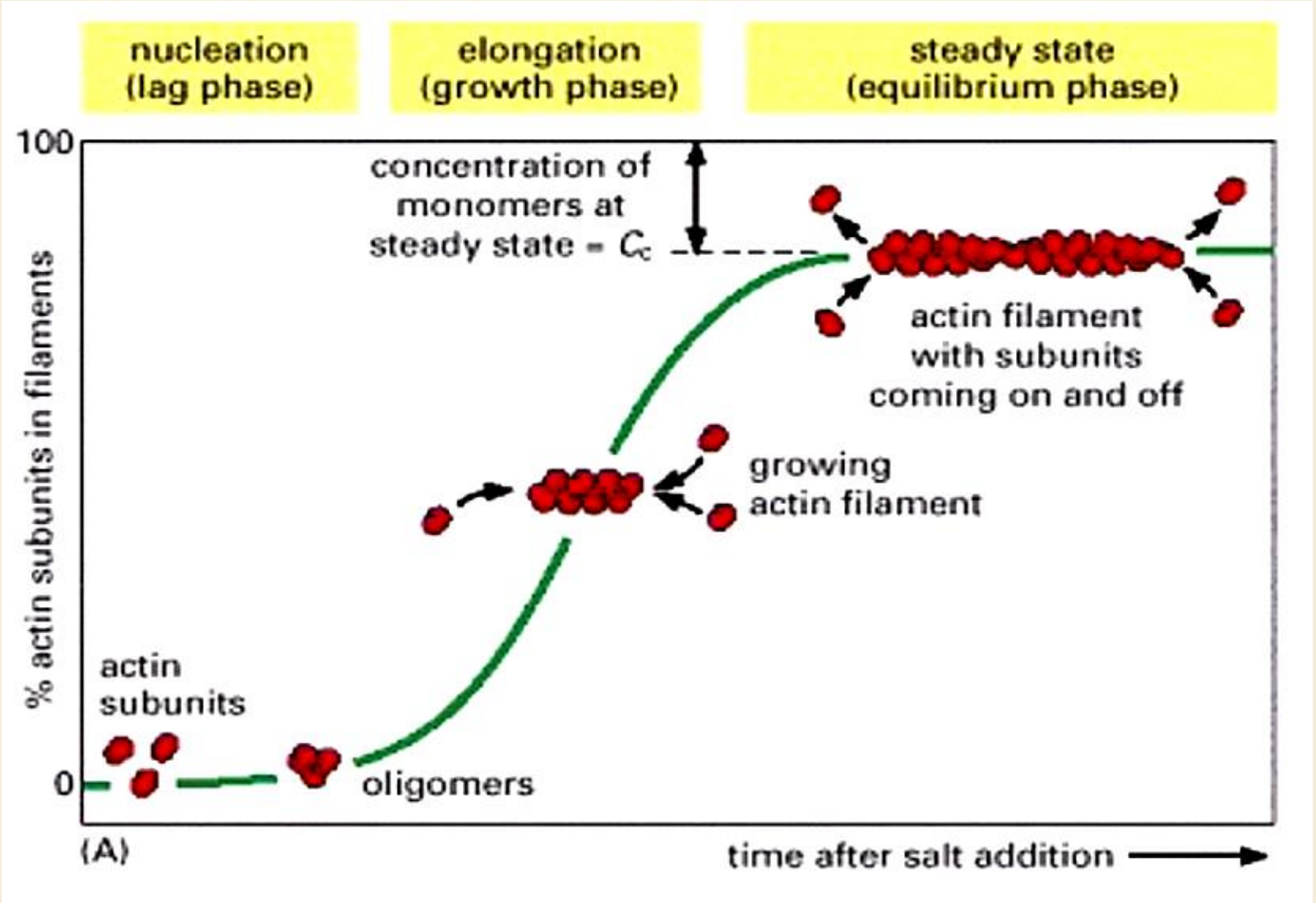
Schematic of the reorganization of the cytoskeleton in a cell in response to an external signal.



Example of the reorganization of the cytoskeleton in a neuron in response to an external signal.

Amin L. et al J. Cell Science 2016

The time course of actin polymerization

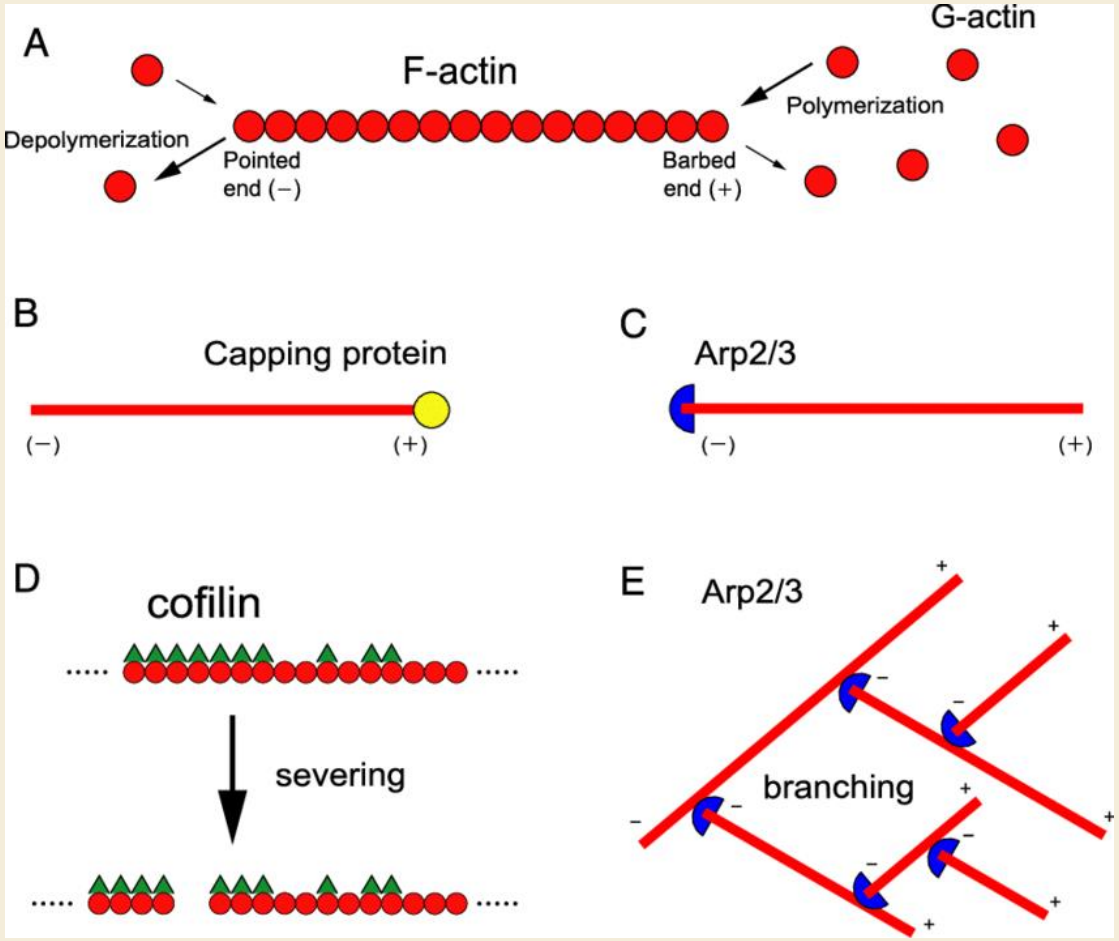


Three phases:

- Nucleation,
- Growth,
- Equilibrium.

To accelerate the formation of the long actin filaments, preformed fragments of actin filaments can be added at the beginning to act as nuclei for filament growth.

Actin filament kinetics and the role of accessory proteins



F-actin is dominantly polymerized and de-polymerized at its barbed and pointed ends, respectively (A) → Polar filaments.

Accessory proteins:

- capping protein (B) and Arp2/3 (C) are bound to the barbed and pointed ends, respectively, to terminate the polymerization and de-polymerization reactions. Arp2/3 can attach to the side of a parent filament, leading to branched networks of F-actins.
- Profilin (not shown) - actin binding protein that binds to actin monomers and catalyzes the exchange of ADP for ATP, promoting polymerization and growth of the actin filament.
- Cofilin or actin depolymerizing factor - actin binding protein that binds to the minus end of an actin filament (specifically associating with ADP-actin), promoting depolymerization. Severing of F-actin (D) is induced when two neighboring subunits of F-actin are bound by cofilin.

Accessory proteins function:

- determine the sites of assembly of new filaments,
- regulate the partitioning of polymer proteins between filament and subunit forms
- change the kinetics of filament assembly and disassembly
- harness energy to generate force,
- link filaments to one another or to other cellular structures such as organelles and the plasma membrane

Passive polymerization and active polymerization

Passive polymerization (Equilibrium polymer)

Consider only the polymerization/depolymerization, ignoring that actin and tubulin are nucleotide triphosphatases, i.e. ignore the free energy derived from ATP/GTP hydrolysis.

Single strand vs **double strand** models – allow to understand the average length and stability of filaments in cell.

Passive polymerization (Equilibrium polymer)

Single-stranded model

A_1 – the monomer

A_n – the n-mer

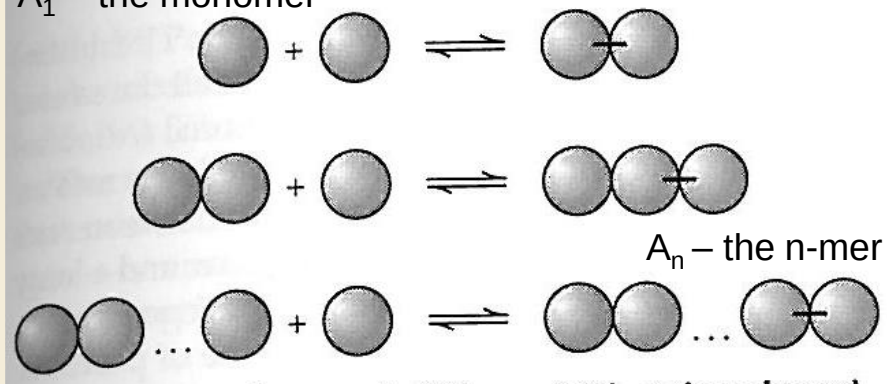


Figure 9.1 Single-stranded filament (Einstein polymer)

Individual subunit-addition reaction:

$$A_n + A_1 \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} A_{n+1}$$

equilibrium constant K_{eq} :

$$K_{eq} = \frac{[A_{n+1}]}{[A_n] \cdot [A_1]}$$

dissociation constant K:

$$K = 1 / K_{eq} = \frac{k_{off}}{k_{on}}$$

Assume identic dissociation constants K for all reactions:

$$K = \frac{[A_1]\,[A_1]}{[A_2]}$$

$$K = \frac{[A_1]\,[A_2]}{[A_3]}$$

$$K = \frac{[A_1]\,[A_n]}{[A_{n+1}]}$$

[An] – concentration of polymers with n-mers.

Exponential distribution

verifies ←

$$[A_n] = K \exp\left(-\frac{n}{n_0}\right)$$

n_0 – ct related to $[A_1]$

$a_n = e^{-\frac{n}{n_0}} \rightarrow [A_n] = a_n \cdot K$

$n_0 = -\frac{1}{\ln a_1}$

total number of subunits

(1) $a_t = \sum_1^\infty n a_n = \frac{a_1}{(1-a_1)^2}$

the average length of the polymers

(2) $n_{av} = \sum_2^\infty n p_n = \frac{\sum_2^\infty n a_n}{\sum_2^\infty a_n} = 1 + \frac{1}{1-a_1}$

(1), (2) $\Longrightarrow$ $a_t \gg 1$ $\quad n_{av} \cong \sqrt{a_t} = \sqrt{\frac{[A_t]}{K}}$ 9.1 appendix Howard

Q: How does the average length $\boldsymbol{n_{av}}$ of the polymers depend on the total concentration of subunits $\boldsymbol{[A_t]}$ available for polymerization ?

$$n_{av} \cong \sqrt{\frac{[A_t]}{K}}$$

The average length, n_{av} of the polymer filaments increases slowly with the total concentration $[A_t]$

$$n_{\mathrm{av}} \cong \sqrt{\frac{[A_t]}{K}}$$

The average length, n_{av} of the polymer filaments for the single stranded model increases slowly with the total concentration $[A_t]$

Even when $[A_t] >> K$ the average length of the single-stranded filaments is still modest:

Ex: for $[A_t] = 100\ K$, the average length of the polymer → $n_{av} = 10$ only !

But the cytoskeleton filaments are thousands subunits length. This would require → $[A_t] > 10^6\ K$!

Considering the concentrations of actin and tubulin:

For actin, total concentration $[At] \sim 200$ uM, and the dissociation constant $K = 0.1$ uM → n_{av} = sqrt(2000) ≈ 45

For tubulin $[At] \sim 20$ uM vs $K \sim 10\ uM$ → $n_{av} \approx 1.4$

Conclusion: The single stranded model fails because, i.e. it does not fit the reality !

The single-strand predicts short filaments, mainly because of the assumption:

"same K" a monomer can decrease the free energy of the system equally well by associating with another monomer as it can by the associating with a long polymer.

Two-stranded model

Actin filaments and microtubules are multi-stranded, and multi-stranded filaments are inherently longer.

In a two-stranded filaments model, there are two different classes of bonds, one within the strands and one between the strands → there are two different nuclei A_2* and A_2** and three different dissociation constants: *K, K_1* and *K_2,* with *K << K_1 , K_2*

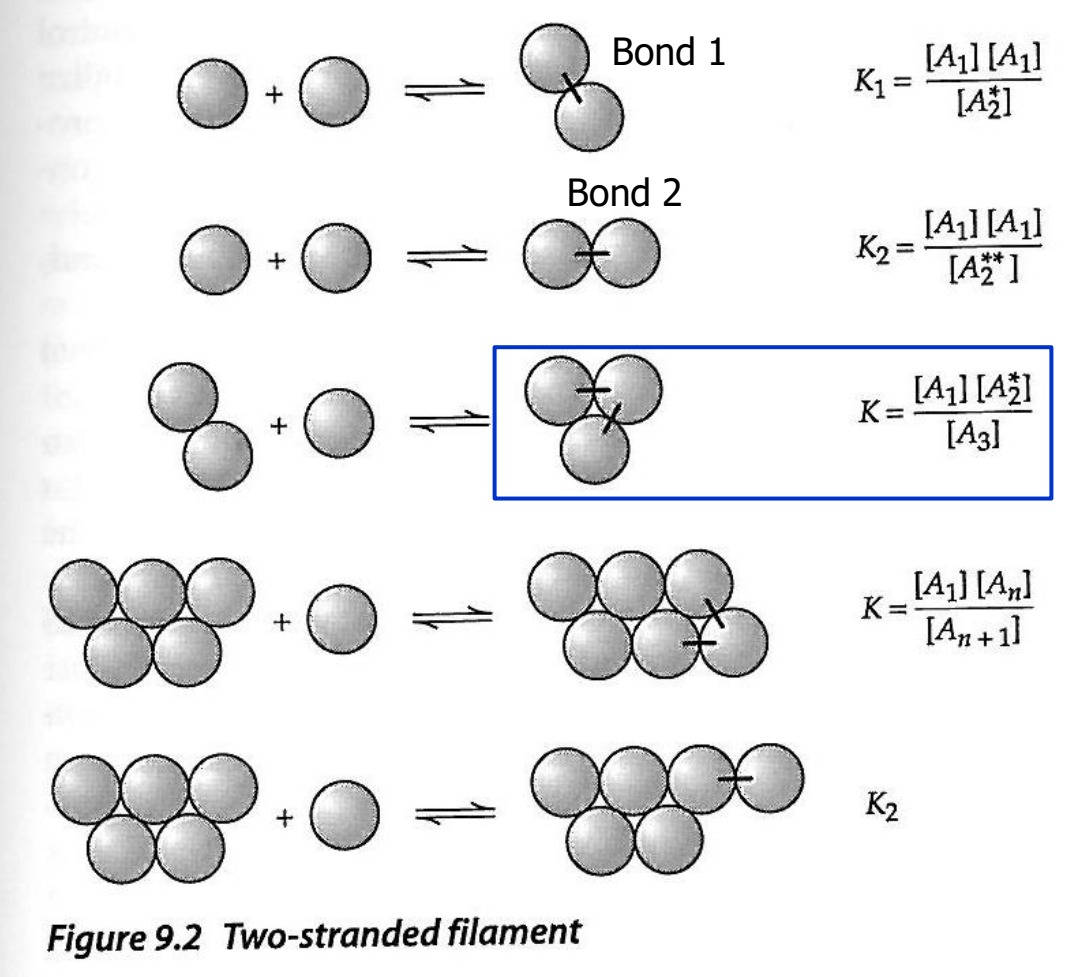


Figure 9.2 Two-stranded filament

The average length, n_{av} , of the two-stranded filaments:

$$n_{av} \cong \sqrt{\frac{K_1}{K}}\sqrt{\frac{[A_t]}{K}}$$

(*demo annex 9.2, Howard*)

Ex for actin: *[At] = 200 uM >> K= 0.1 uM, K_1=K_2= 0.1 M*

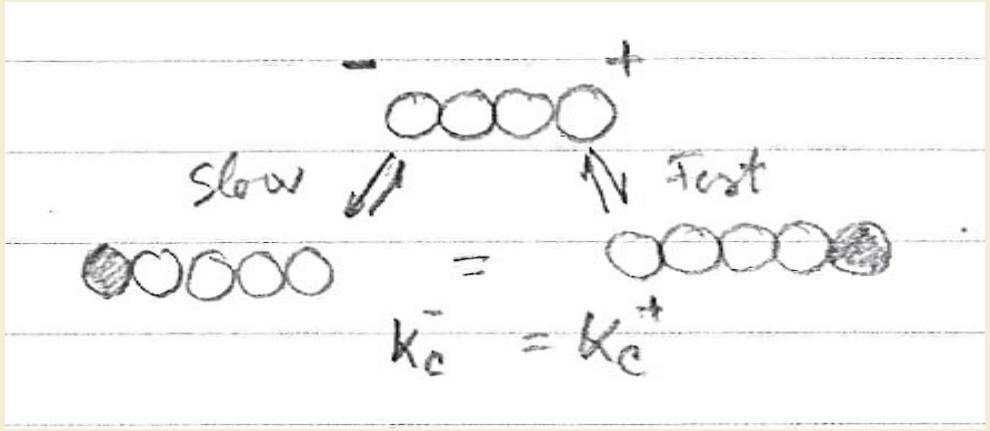
→ **n_{av} ≈ 4.5 x 10^3** , correspondig to a 12.4 um long actin filament

The two-stranded model predicts filament lengths consistent with the polymer length in the cell.

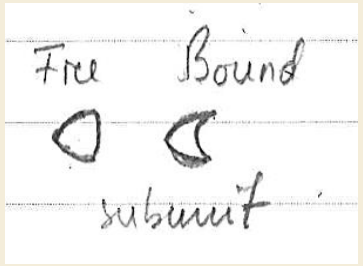
Two stranded filaments are longer than single stranded filaments because the ends of a two stranded filament are energetically unfavorable, so there will be only a low concentration of them at equilibrium.

Polar filaments – plus and minus ends

An important constraint on passive polymerization (equilibrium polymer) is that the critical concentration must be the same at both its ends.



Although the rates at the two ends might be different, the critical concentrations must be the same.
The end with **faster kinetics** is referred to **plus end**, while the other 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 binds another subunit i.e. enters the polymer.

ATP hydrolysis that accompanies actin polymerization removes this constraint.

Active polymerization

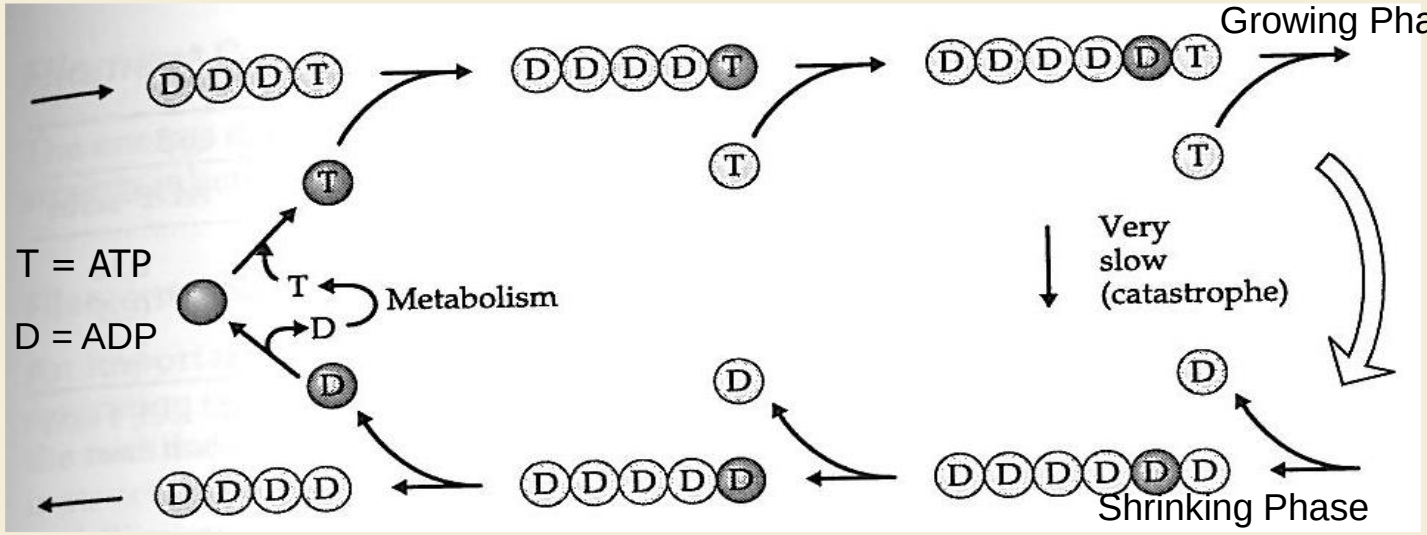
Cytoskeletal filaments are not at equilibrium in cells → fast addition and substraction of subunits

Nonequilibrium requires an energy source → hydrolysis of ATP (actin) and GTP (tubulin)

The polymerization cycle for actin / tubulin

Polymerization and hydrolysis are coupled:

- ATP Hydrolysis is catalyzed by polymerization
- The exchange of NTP for NDP is catalyzed by depolymerization



During the polymerization process, ATP that is bound to G-actin is hydrolyzed to ADP that is bound to F-actin. The hydrolysis reaction occurs on the F-actin subsequent to the polymerization reaction in two steps:

cleavage of ATP followed by the slower release of inorganic phosphate (Pi).

As a result, at high rates of filament growth a transient cap of ATP-actin subunits exists at the ends of elongating filaments, and at steady state a stabilizing cap of ADP.Pi-actin subunits exists at the barbed ends of filaments. Cleavage of ATP results in a highly stable filament with bound ADP.Pi, and release of Pi destabilizes the filament. Thus, these two steps of the hydrolytic reaction provide potential mechanisms for regulating the monomer-polymer transition.

Table 11.1 Rate constants for actin polymerization and depolymerization

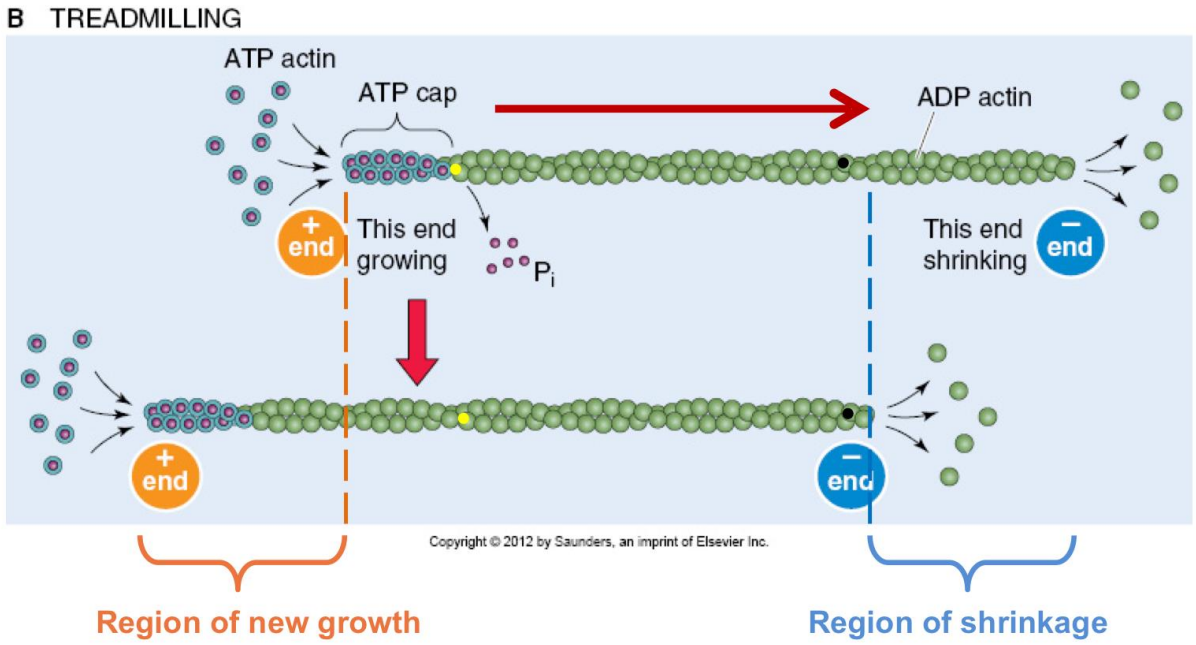
Rate constant or equilibrium constant	Plus end		Minus end	
	ATP-actin	ADP-actin	ATP-actin	ADP-actin
k_{on} ($\mu M^{-1} \cdot s^{-1}$)	11.6	3.8	1.3	0.16
k_{off} (s^{-1})	1.4	7.2	0.8	0.27
K_c	0.12	1.9	0.6	1.7

The plus ends have lower critical concentration *Kc* than the minus ends . $K_c^- > K_c^+$

The plus end is expected to grow while the minus end is expected to shrink.

Howard book, table 11.1

Actin Filaments: Treadmilling



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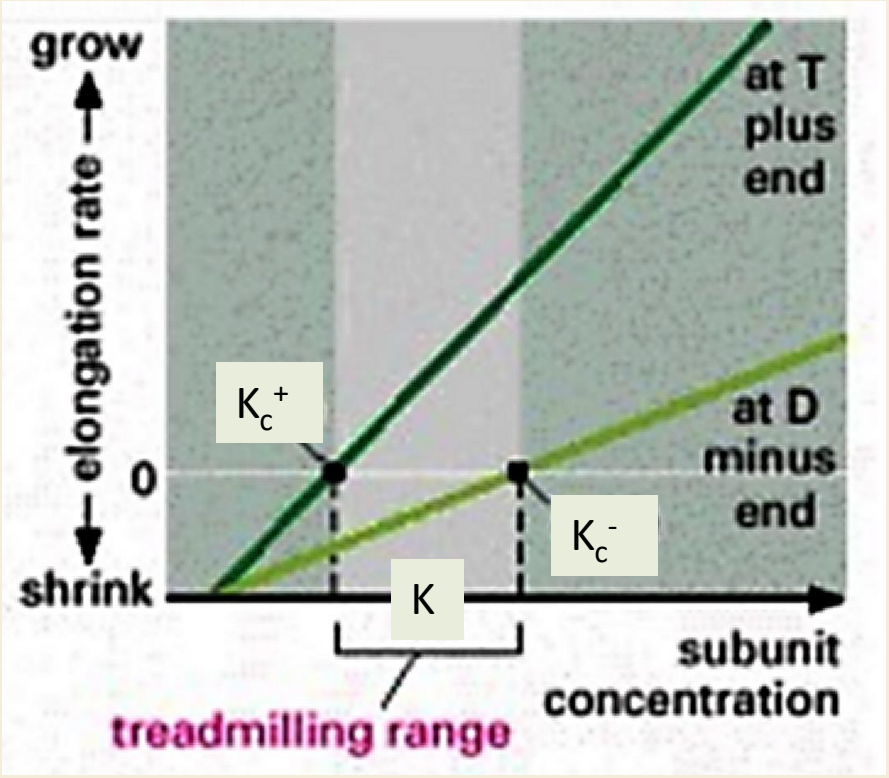
Boron and Boulpaep, fig. 2-12B



If both ends are exposed, polymerization proceeds until the concentration of free monomer reaches a value K: $K_c^+ < K < K_c^-$

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.

Intermediate filaments - types

TYPES OF IF	COMPONENT POLYPEPTIDES	CELLULAR LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	vimentin	many cells of mesenchymal origin
	desmin	muscle
	glial fibrillary acidic protein	glial cells (astrocytes and some Schwann cells)
	peripherin	some neurons
Epithelial	type I keratins (acidic)	epithelial cells and their
	type II keratins (basic)	derivatives (e.g., hair and nails)
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

Table 8.4 Young's moduli of biological materials

Material	Young's modulus (GPa)	Notes
Proteins		
Actin	2	Filament persistence length, L_p = 15 μm
Tubulin	2	L_p = 6 mm
Coiled coil	2	L_p = 100–200 nm
IF protein	2 (longitudinal)	Hydrated keratins and filaments. L_p ~1 μm
Flagellin	1	L_p = 0.5–1 mm
Silk	~5	Silk-moth cocoon (depends on humidity)
Collagen	~2	Tendons and ligaments
Abductin	0.004	Hinge ligament of molluscan shell
Resilin	0.002	Hinge of insect flight system
Elastin	0.002	Vertebrate smooth muscle and ligaments

OUTLINE

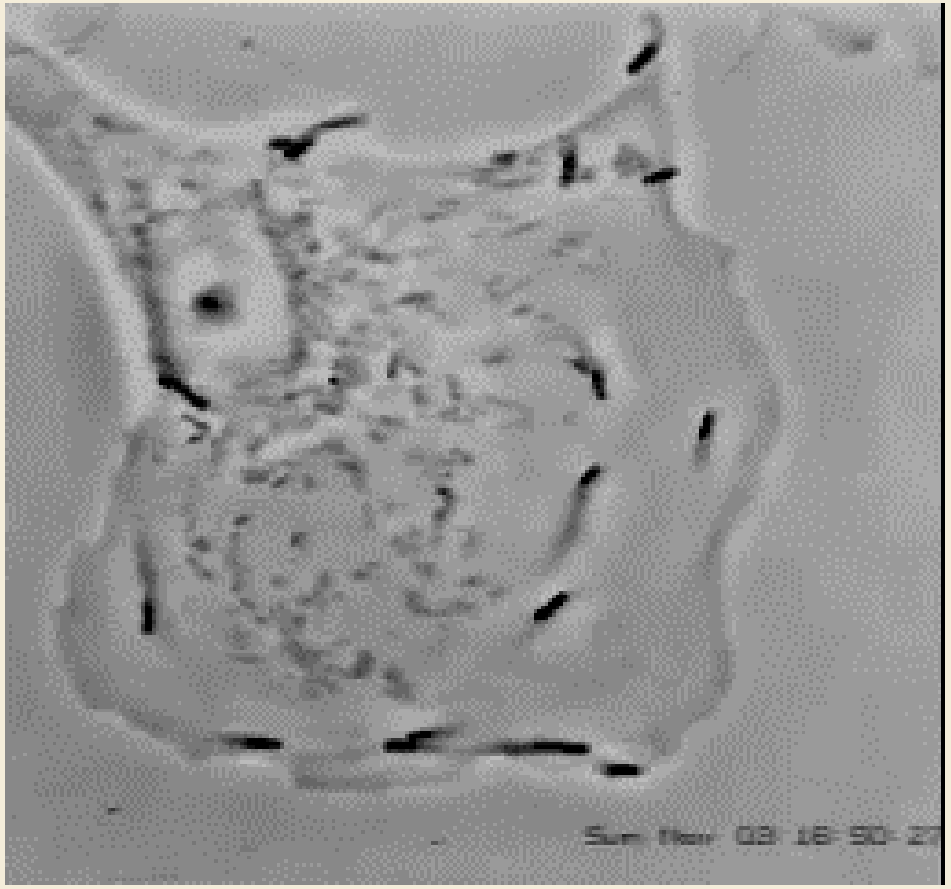
- Cytoskeleton filaments: types, structure, localization and function
- Cytoskeleton filaments polymerization and depolymerization.
- **Forces generated by polymerization / depolymerization**
- Cell motility induced by cytoskeleton filaments, examples.

  - **Is a polymer able to do work ?**
  - **How big the force generated by actin/ tubulin polymerization can be ?**
  - **How fast is the polymerization ?**

Example in which actin polymerization generates forces in cells : motility of intracellular viruses and bacteria

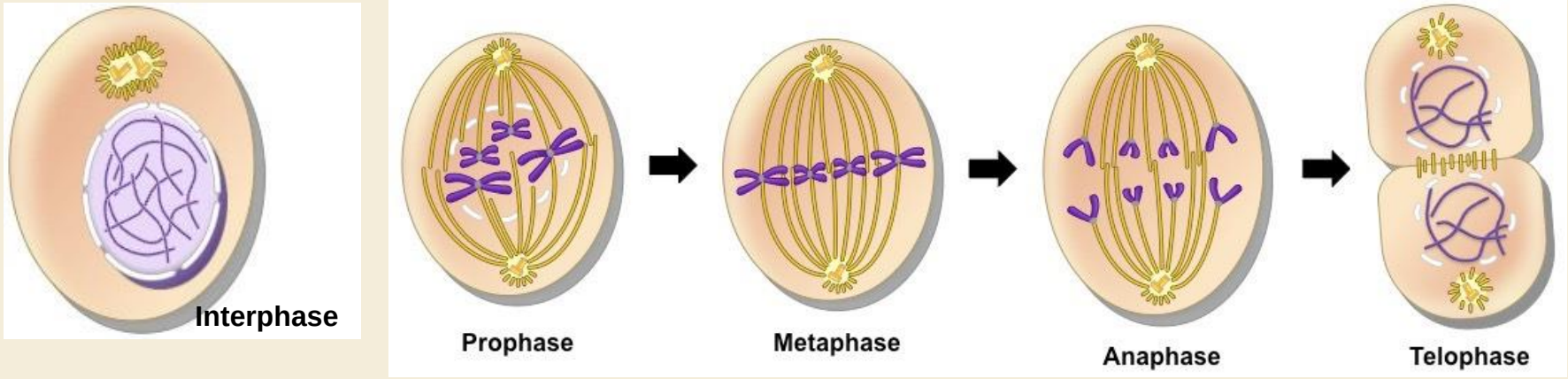
Listeria monocytogenes (gram positive bacteria)

v= 1 um / s



Example in which actin microtubule polymerization generates force in cells:

anaphase and telophase in Mitosis.



In **anaphase**, sister chromatids (now called chromosomes) are pulled toward opposite poles.
In **telophase**, chromosomes arrive at opposite poles, and nuclear envelope material surrounds each set of chromosomes.
Finally, in cytokenesis, the two daughter cells are separated.

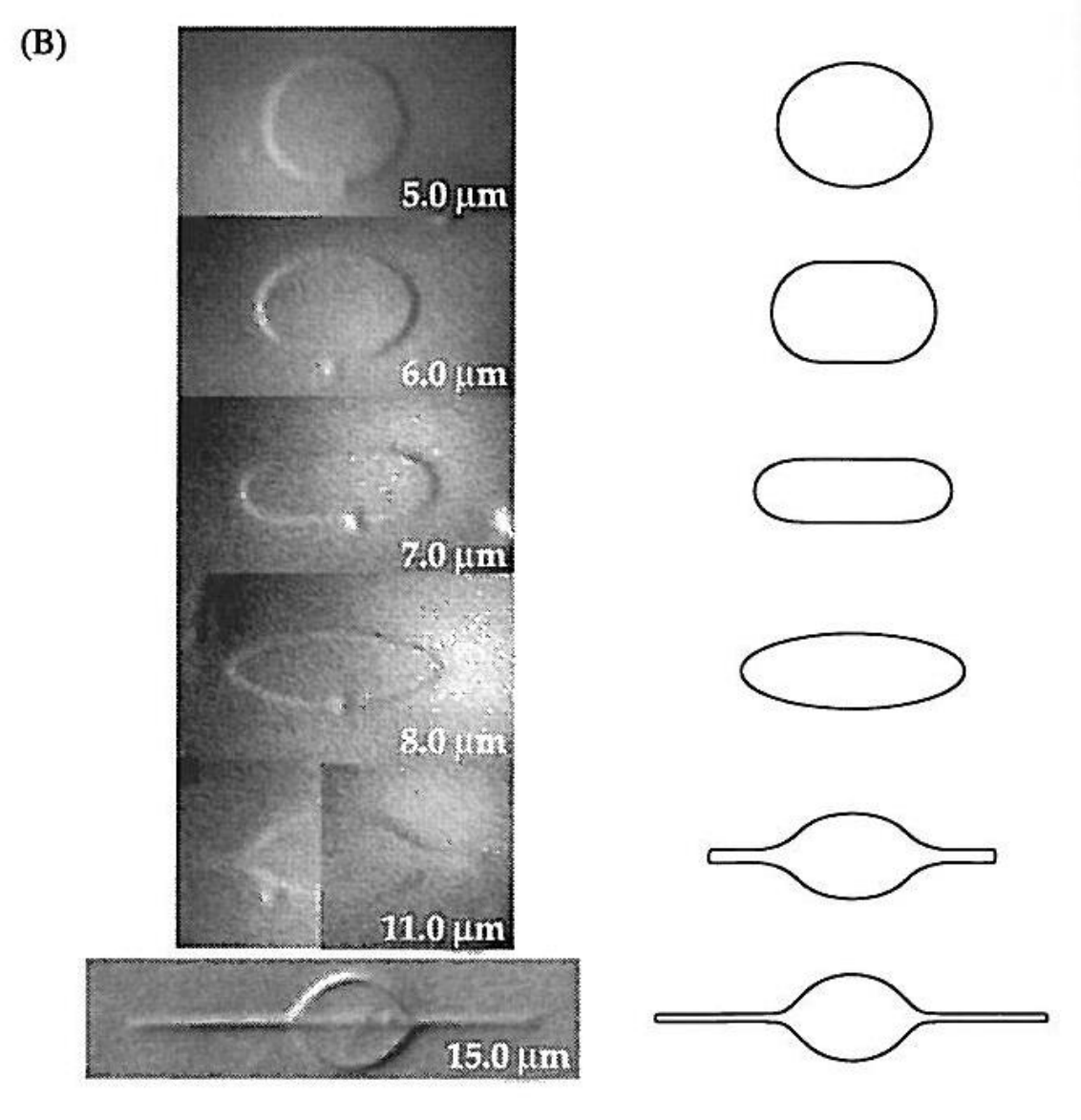
Example of polymerization- driven force generation

in vitro: polymerization of microtubules inside liposomes.

Forces of 2-8 pN are sustained before buckling of polymerizing microtubules.

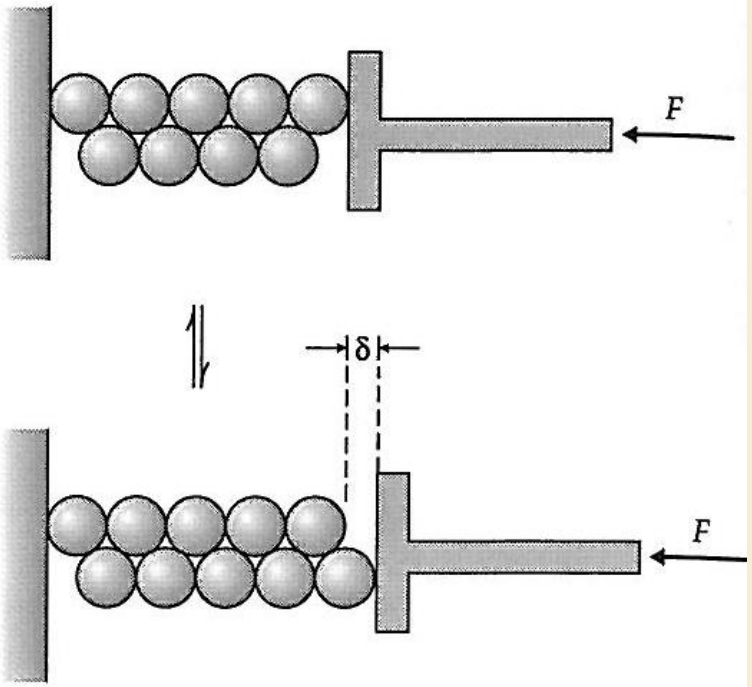
<u>This demonstrates that force generation can take place without additional proteins</u>

(even though in the cell other proteins are involved, as we know).



How much force can be generated by polymerization and depolymerization ?

adding a compressive force opposing the polymerization of a polymer



Polymerization against the force *F* → the mechanical energy of the n+1 mer exceeds that of the n-mer by ***F δ***.

Ex. Actin: δ = 5.5 /2 = 2.75 nm (monomer size / 2 - the number of strands);
Microtubules: δ = 8 /13 ≈ 0.6 nm (monomer size / 13 – number of monom / ring)

At equilibrium in the presence of force, application of Boltzmann's law gives a dissociation constant *K(F)*:

$$K(F) = K_c \exp\left(\frac{F \cdot \delta}{kT}\right)$$

K(F) - the concentration at which there is no net elongation of the filament.

Kc - the critical concentration in the absence of force.

At quilibrium concentration [A1] with force, the system is at equilibrium (i.e. there is no net polymerization) when ***F= F_{eq}***:

$$F_{eq} = \frac{kT}{\delta} \ln \frac{[A_1]}{K_c}$$

Ex: for a concentration [A1] of monomer [A1] = 100 Kc → $F_{eq} \approx 7$ pN for actin and $F_{eq} \approx 30$ pN for microtubule

Some comments:

1. **The equilibrium force is independent of the polymerization mechanism**.
   i.e. does not matter whether the polymer pushes directly on the particle or whether the polymer is coupled to the particle via accessory proteins (e.g. Arp2/3 complex for actin, kinetochore for microtubules).

2. **The maximum force exerted by a real polymer will be less than the equilibrium force.**
   i.e. polymerization might be so slow at higher forces that growth is effectively stalled at forces significantly smaller than the equilibrium force (polymerization stalls at forces < 7 pN for actin and 30 pN for microtubules).

3. **Are the cytoskeletal filaments strong enough to exert forces while polymerizing ?**
   Polymerization-based motility requires that the filaments be in compression, and there is only so much compressive force that a filament can withstand before it buckles. This does not represent a problem until the filaments are not too long (< 1 um).

The polymerization mechanism can generate sufficiently large forces to account for cellular processes. Both actin and microtubules filaments are rigid enough to support the polymerization forces that are observed in cells.

Another question is: **is the polymerization fast enough** ?

To answer this question, kinetic mechanisms by which polymerization is coupled to force generation should be considered.

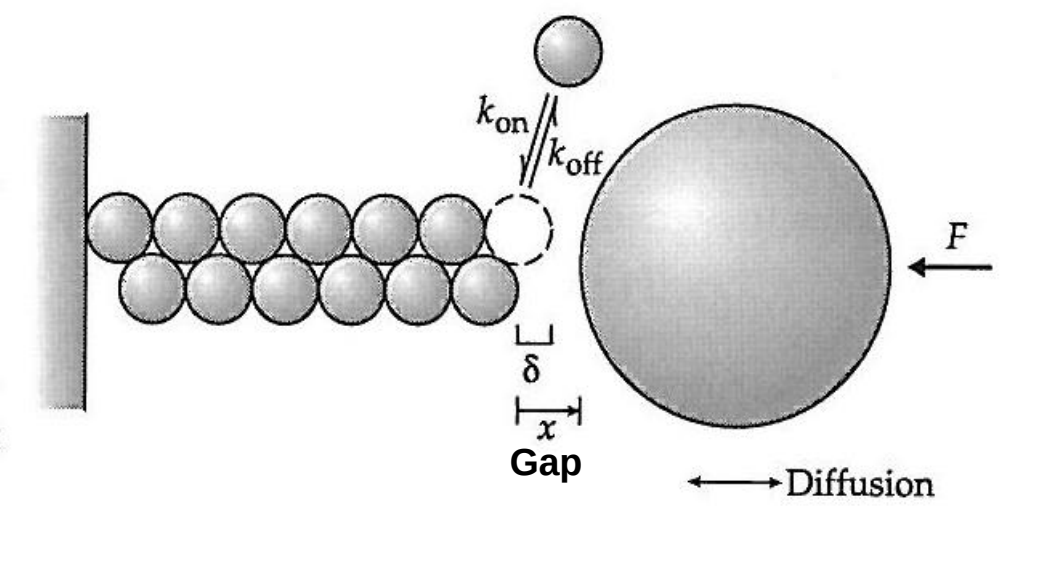
It is difficult to envision how the end of a growing or shrinking filament could push or pull on a particle.
e.g. how a depolymerizing polymer could maintain the contact with the particle being pulled ?

A possible answer are the **accessory proteins** which may **connect** the end of **the filament** to **the particle**.
However, experiments in vitro demonstrated that the additional proteins are not a necessary condition for force generation.

This has lead to the idea that in the case of a growing polymer pushing against an opposing force, the particle being pushed must be able to undergo thermal noise sufficiently large to unblock the adjacent filament end and permit subunit addition. → mechanism termed a Brownian ratchet model.

Brownian ratchet model

Brownian ratchet mechanism for force generation by polymerization.



Assumption: the transition rate constants k_{on} and k_{off} do not depend on the gap size.

Due to diffusive motion of the particle, the transition state from off <-> on has the gap fully open → model similar with Kramers like mechanism→ **reaction diffusion equation**.

<u>Two possible cases:</u>

If **diffusion** is very **fast**, the process is **reaction limited**:
the creation of a sufficiently large gap occurs frequently, but the probability that the gap will be filled is low because the on-rate for subunit addition is low.

If **diffusion is slow**, the process is **diffusion limited**:
the rate-limiting step is the actual creation of a gap. Once a gap is created, a subunit will always drop in.

At **high-enough forces**, polymerization will become **reaction limited** because the lifetime of the open state will be very short.

Annex. 10.1 Howard – model formulation as a reaction diffusion equation

Reaction-limited polymerization

The diffusion coefficient *D* is very large and the probability *p(x)* that the gap distance equals *x* is:

(which means that Boltzmann's law holds).

F> 0, is the compressive force to the left

$$p(x) = \left(\frac{F}{kT}\right) \exp\left(-\frac{Fx}{kT}\right)$$

We can think of the gap exploring all possible positions as though the particle were in equilibrium, and **only very occasionally** does a monomer drop in.

The **elongation rate** is given by:

$$v = \delta \frac{dn_{\text{av}}}{dt} = \delta\left(k_{\text{on}}[A_1]e^{-\frac{F\delta}{kT}} - k_{\text{off}}\right)$$

Equation – appendix 10.1

the growth rate **(k_{on})** is weighted by the probability that the **Gap** > **δ** (which depends on the applied force *F*), while shrinking **(k_{off})** is not affected by the applied force ***F*.**

At equilibrium (v=0) the equation reduces to the equilibrium force equation:

because $K_c = k_{off} / k_{on}$

$$F_{\text{eq}} = \frac{kT}{\delta} \ln \frac{[A_1]}{K_c}$$

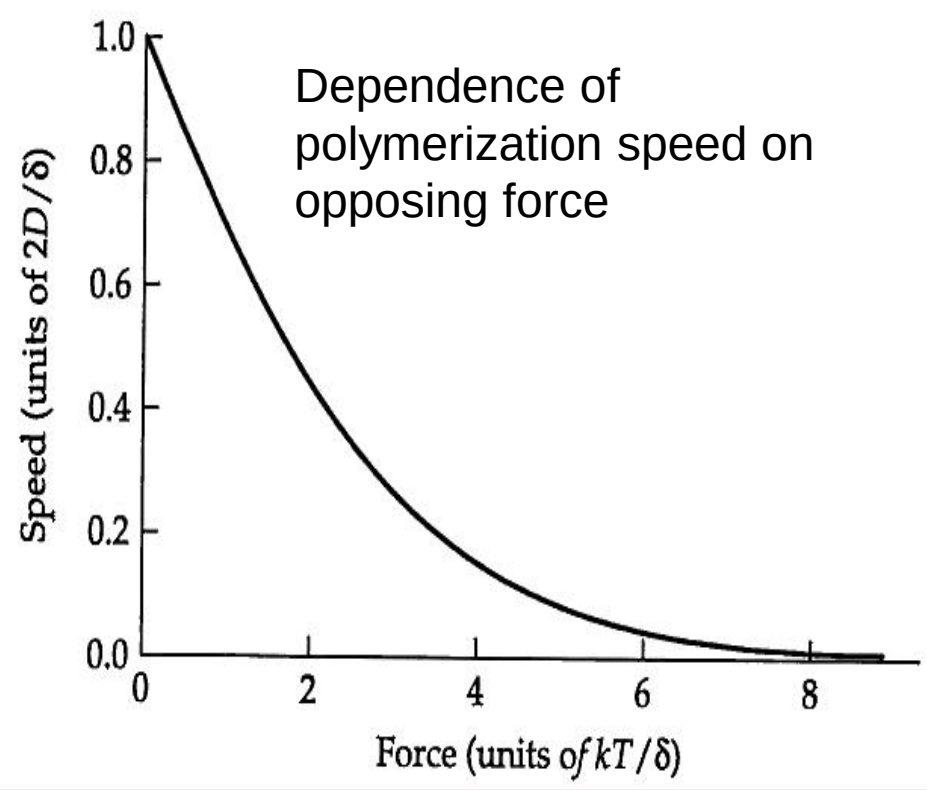
The first term in the elongation rate decreases e-fold for every force step $\Delta F = KT/\delta$;

Ex: Actin: ΔF = 1.45 pN; Microtubule: ΔF = 6.7 pN

Annex. 10.1 Howard – model formulation as a reaction diffusion equation

Diffusion limited polymerization : the diffusion coefficient, *D*, is very small.

Assumption: the monomer addition reaction is so fast that almost as soon as a gap opens up, a monomer will drop in. The **polymerization rate** is approximately **equal to the rate of gap creation**, which is the time it takes the particle to diffuse a distance δ against the force (the mean passage problem), whose solution leads to the elongation rate, v:



$$v = \delta \frac{dn_{av}}{dt} \cong \frac{2D}{\delta} \frac{(F\delta/kT)^2/2}{e^{F\delta/kT} - 1 - F\delta/kT}$$

D - diffusion coefficient for the particle.

In the case of very low external force, Fδ << KT, the diffusion-limited elongation rate approaches v → 2D/δ.

When the force increases, the speed drops approximately exponentially.

Annex. 10.1 Howard – model formulation as a reaction diffusion equation

Diffusion limited polymerization

If, in the case of zero external force, the elongation rate is diffusion limited, the drag force against which the polymerization mechanism acts is:

$$F_{\text{drag}} = \gamma \cdot v = \gamma \cdot \frac{dn_{\text{av}}}{dt} \cdot \delta = \frac{kT}{D}\frac{2D}{\delta} = \frac{2kT}{\delta}$$

$\gamma = kT / D$

For an actin filament $F_{drag} \approx 3$ pN, for a microtubule $F_{drag} \approx 13$ pN.

These are force values similar to the equilibrium forces.

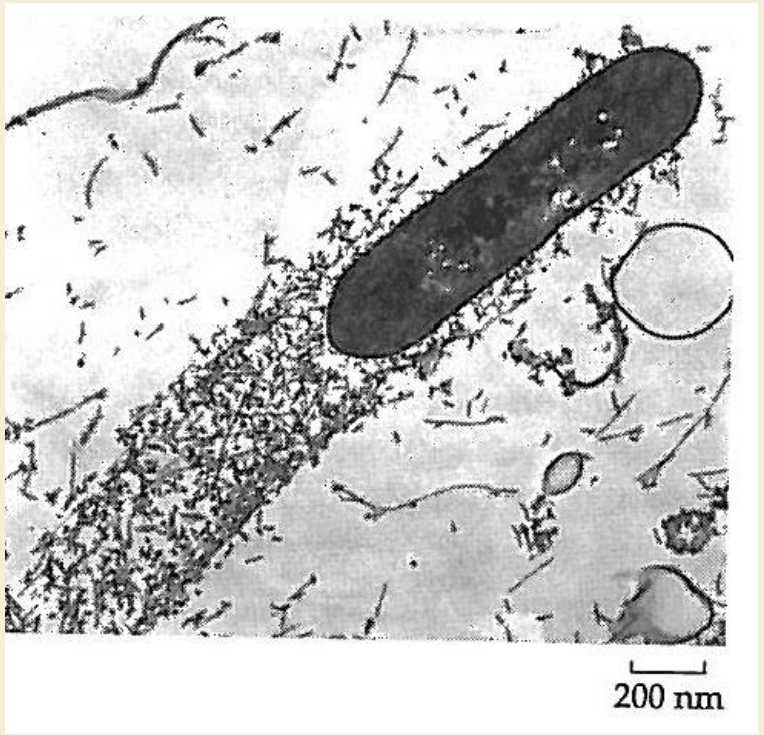
Examples of motility driven by actin polymerization

***Example 10.1 Actin polymerization is fast enough and powerful enough to drive the movement of* Listeria** The fastest movements of *Listeria* are ~1 μm/s, corresponding to a growth rate of 360 monomers/s. This is consistent with a polymerization mechanism because the on-rate for actin binding to the end of a filament is 12 $\mu M^{-1} \cdot s^{-1}$ (see Table 11.1), and the total actin concentration in nonmuscle cell is ~200 μM (Bray, 1992); if 15% of this actin is free to polymerize (i.e., 30 μM), a speed of 1 μm/s can be attained (see Equation 10.3). 30 μM is about three hundred times higher than the critical concentration for ATP-actin measured in vitro (see Table 11.1); a maximum (equilibrium) force of ~8 pN per actin filament is therefore possible (Equations 10.2 and 10.3). Because the actin filaments in the tails are crisscrossed, rather than being in line with the direction of movement, even high forces are possible (the factor is ~1/cosθ, where θ is the angle of the filament with respect to the direction of motion). Given that a transverse section through the tail contains several filaments (see Figure 10.1B), total forces up to 100 pN are therefore possible. It should be noted that in order for the bacterium to be propelled forward, the actin-containing tail must somehow be anchored within the cell, perhaps via crosslinking to the existing cytoskeleton.

Reaction-limited polymerization

$$v = \delta \frac{dn_{av}}{dt} = \delta \left(k_{on}[A_1] e^{-\frac{F\delta}{kT}} - k_{off} \right)$$

$$F_{eq} = \frac{kT}{\delta} \ln \frac{[A_1]}{K_c}$$



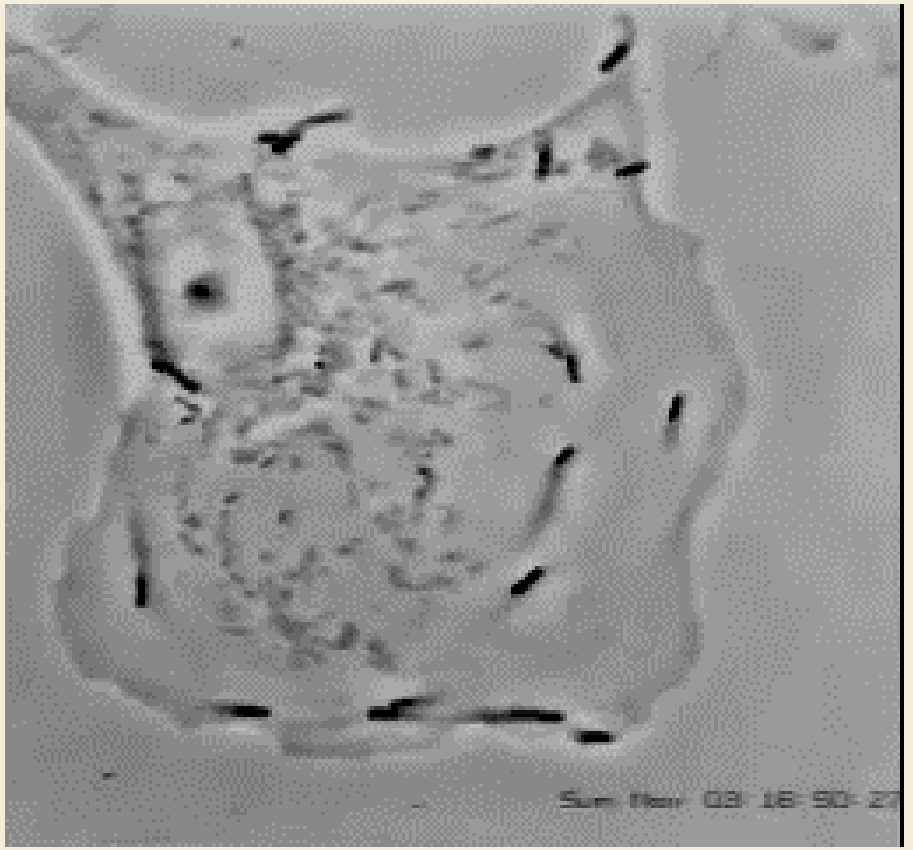
EM of Listeria, showing the actin filaments in the tail

Diffusion limited polymerization

$v \rightarrow 2D/\delta$

$$F_{drag} = \gamma \cdot v = \gamma \cdot \frac{dn_{av}}{dt} \cdot \delta = \frac{kT}{D}\frac{2D}{\delta^2}\delta = \frac{2kT}{\delta}$$

***Example 10.2 The diffusion-limited speed of* Listeria** For a 1-μm-long bacterium being pushed by actin polymerization through water, the diffusion-limited maximum speed is 360 μm/s (= $2D/\delta$, where using $D = 0.5$ $\mu m^2/s$, $\delta = 2.75$ nm), well in excess of the measured speeds of 0.1 to 1 μm/s. This suggests that *Listeria*'s motion may not be diffusion limited. However, if the mobility of a bacterium in cytoplasm is only 1/100 to 1/1000 that in water, the diffusion-limited speed is reduced by a corresponding factor to a value more nearly equal to the observed speeds. If the motion were diffusion limited, the force would equal ~3 pN per filament, by Equation 10.5. This is of the same order of magnitude as the maximum reaction-limited force (Example 10.1). It is interesting that *Listeria* moves at different speeds in different cell types (Dabiri et al., 1990): The three-fold difference could arise from differences in the monomeric actin concentration if the motion were reaction limited, or to differences in "viscosity" (i.e., different mobilities of the bacteria) if the motion were diffusion limited.



PNAS

Direct measurement of force generation by actin filament polymerization using an optical trap

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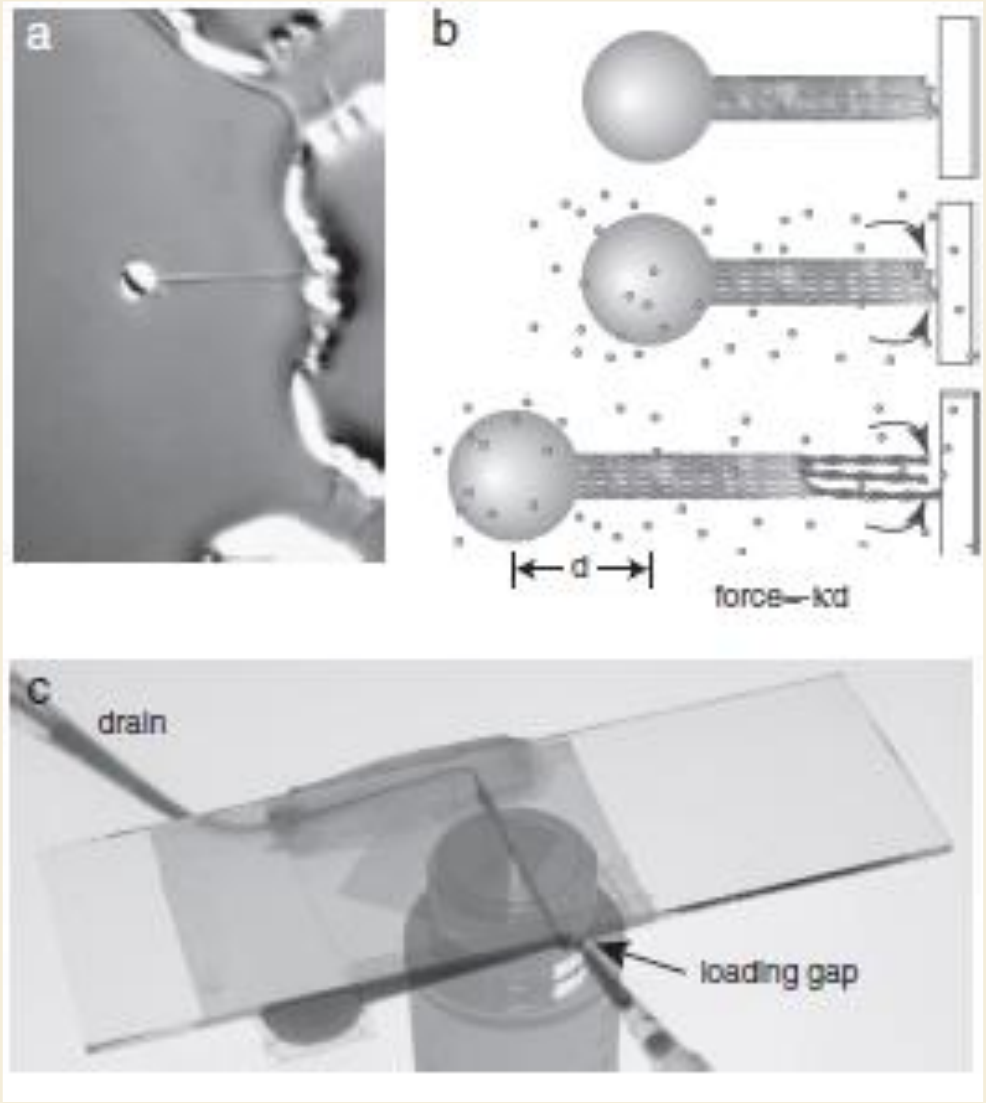
Edited by James A. Spudich, Stanford University School of Medicine, Stanford, CA, and approved November 9, 2006 (received for review August 15, 2006)

Actin filament polymerization generates force for protrusion of the leading edge in motile cells. In protrusive structures, multiple actin filaments are arranged in cross-linked webs (e.g. lamellipodia) or parallel bundles (as in filopodia).

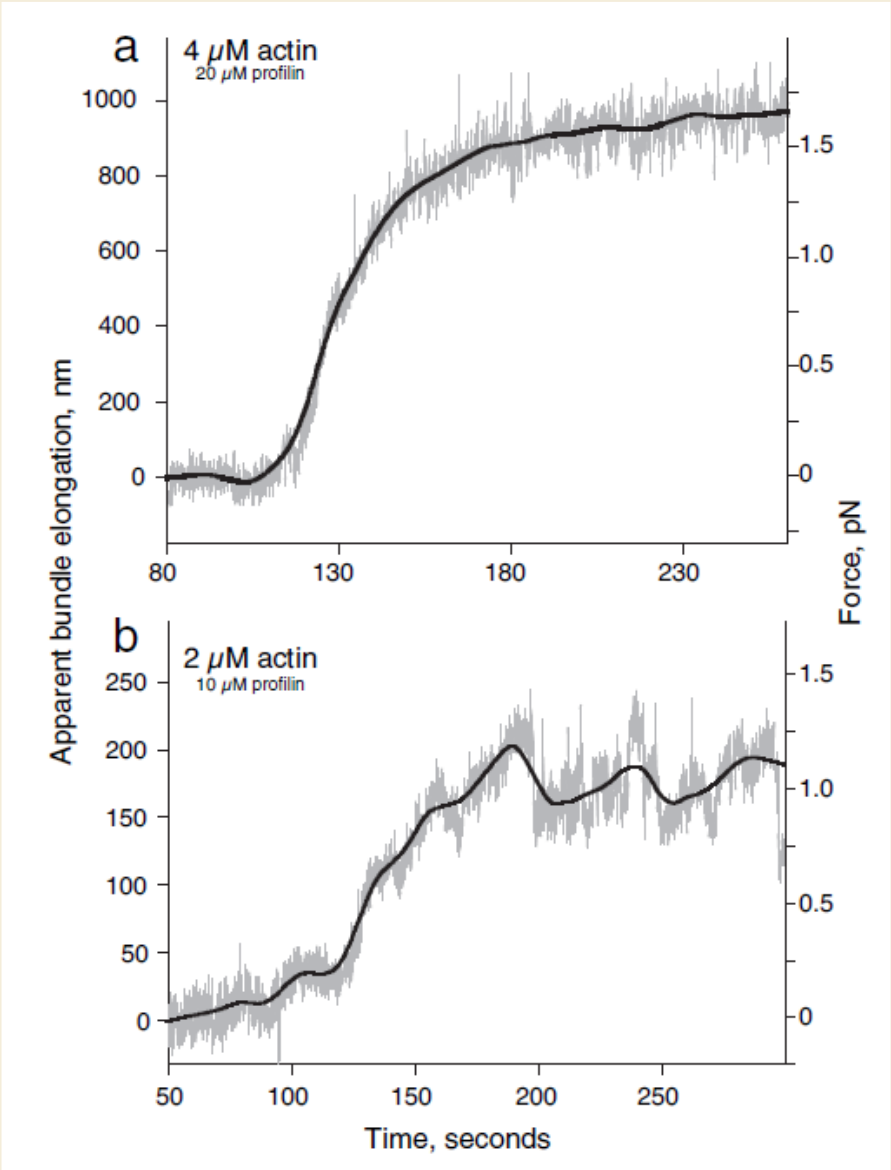
Authors have used an optical trap to directly measure the forces generated by elongation of a **few parallel-growing actin filaments brought into opposition with a rigid barrier**, **mimicking the geometry of filopodial protrusion**.

They find that the **growth of approximately 8 actin parallel-growing filaments** can be stalled by relatively **small applied load forces on the order of 1 pN**, consistent with the theoretical load required to stall the elongation of a single filament under their conditions. Indeed, large length fluctuations during the stall phase indicate that only the longest actin filament in the bundle is in contact with the barrier at any given time.

These results suggest that force generation by small actin bundles is limited by a dynamic instability of single actin filaments, and therefore living cells must use actin-associated factors to suppress this instability to generate substantial forces by elongation of parallel bundles of actin filaments.

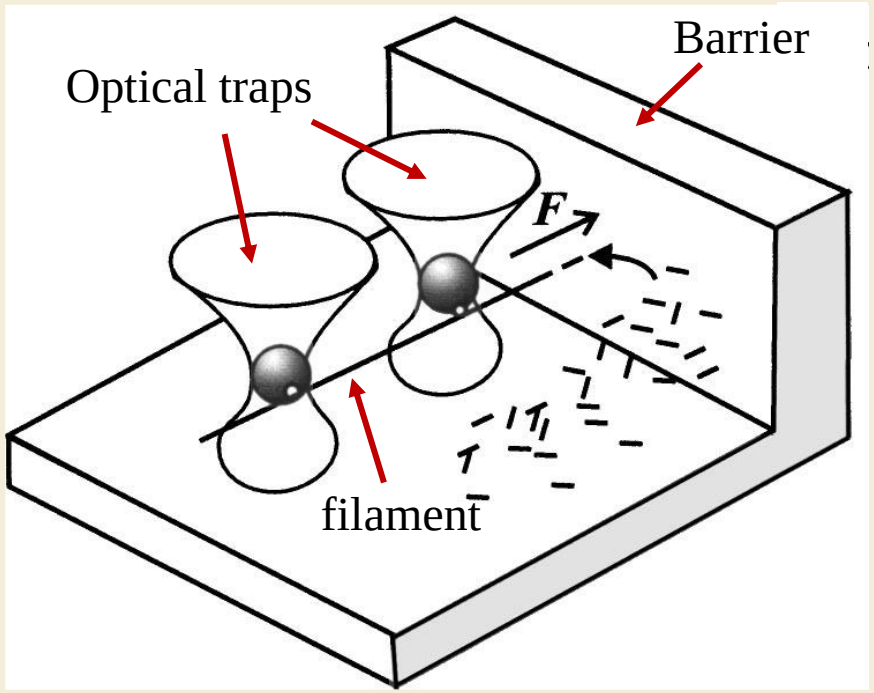


Experimental setup. (a) Bead-acrosomal bundle held in keyhole trap, next to microfabricated barrier.
(*b*) The sequence of events for an experiment.
(*c*) Flow cell used in the experiments.

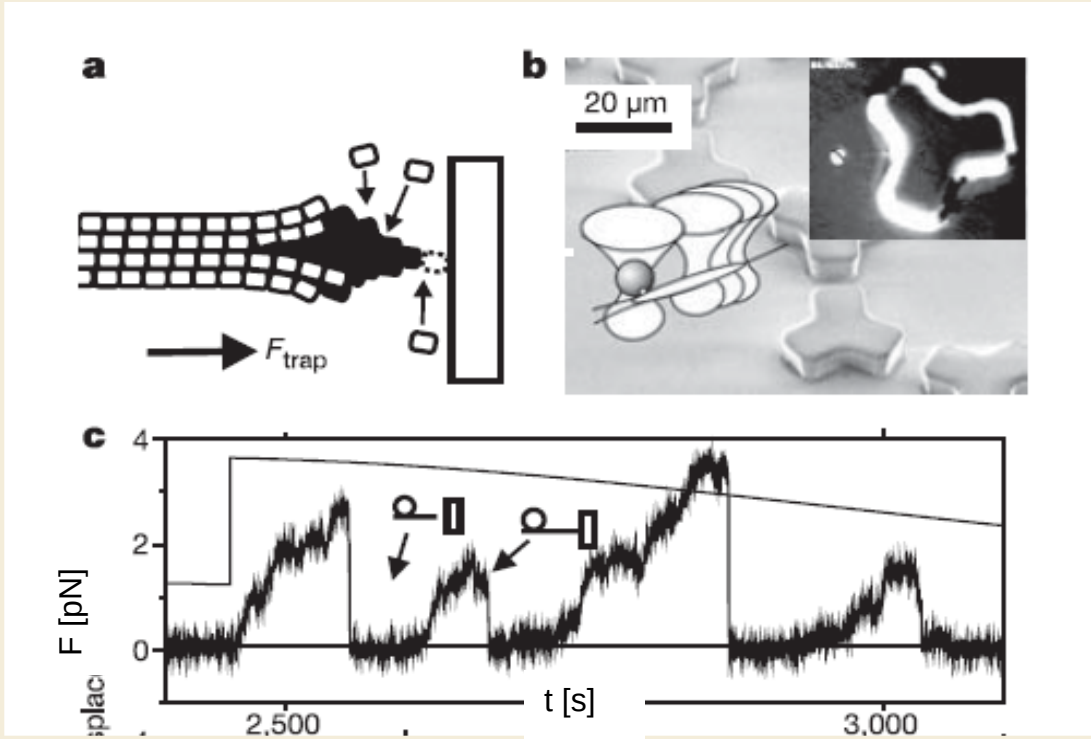


Force measurement for actin filament growth with monomeric actin at 2 (*b*) and 4 (*a*) uM.

Optical trap technique for measuring actin / microtubule polymerization forces



A growing polymerizing filament is trapped in front of a rigid barrier. Elongation of the filament pushes the connected beads backward in the traps, from which the pushing force *F* is inferred. The second trap is necessary for stabilization of the pushing direction.
2003_APL_Kerssemakers + Dogterom



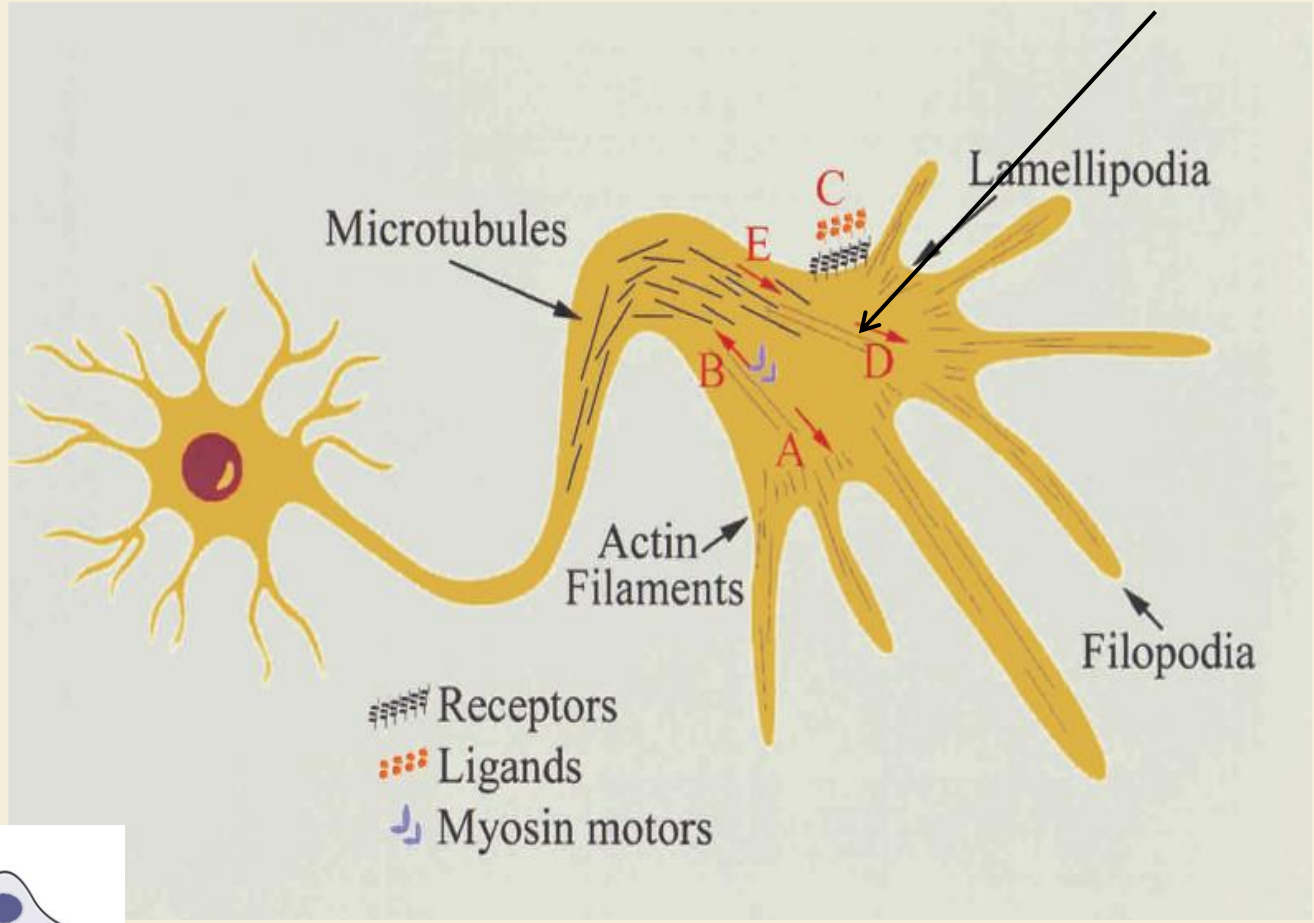
(a) Measuring scheme for microtubules polymerization
(b) Physical implementation of the barrier
(c) Force measurement during growth and shrinking of the filament
2006_Nature_Kerssemakers + Dogterom

All the measurements above were performed on “artificial” cytoskeletal filaments (actin and microtubules)

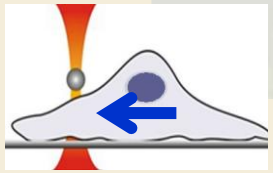
i.e. NOT IN A LIVE CELL !

Axon guidance - neural development

The **Growth Cone** (GC) is a dynamic structure at the tip of a developing neurite seeking its synaptic target. Normal sensation, motor control and behavior depend on correct wiring up of neuronal circuits during fetal development.

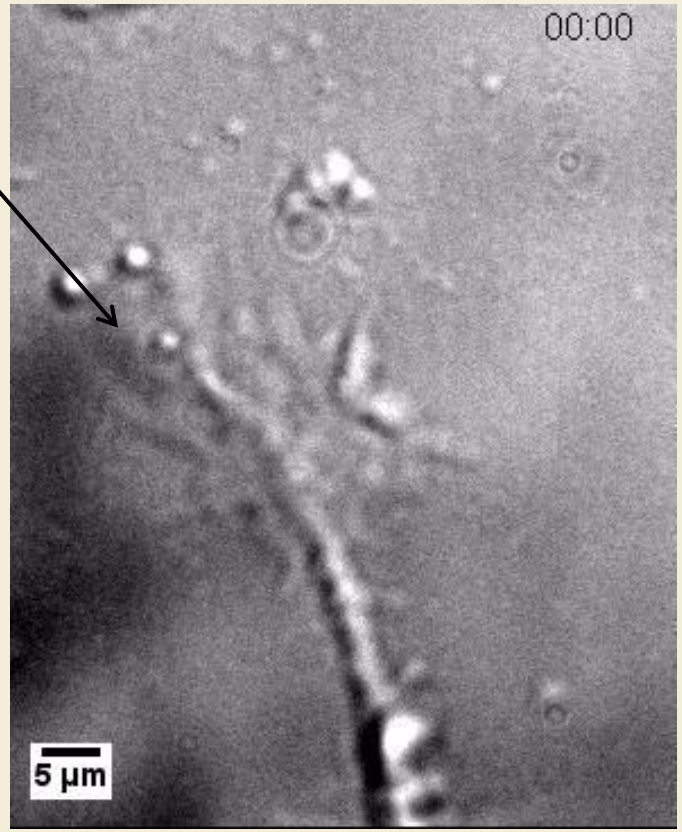


J.L. Goldberg, Genes and Dev. ***17*** 941 (2003)



Growth cone (GC)

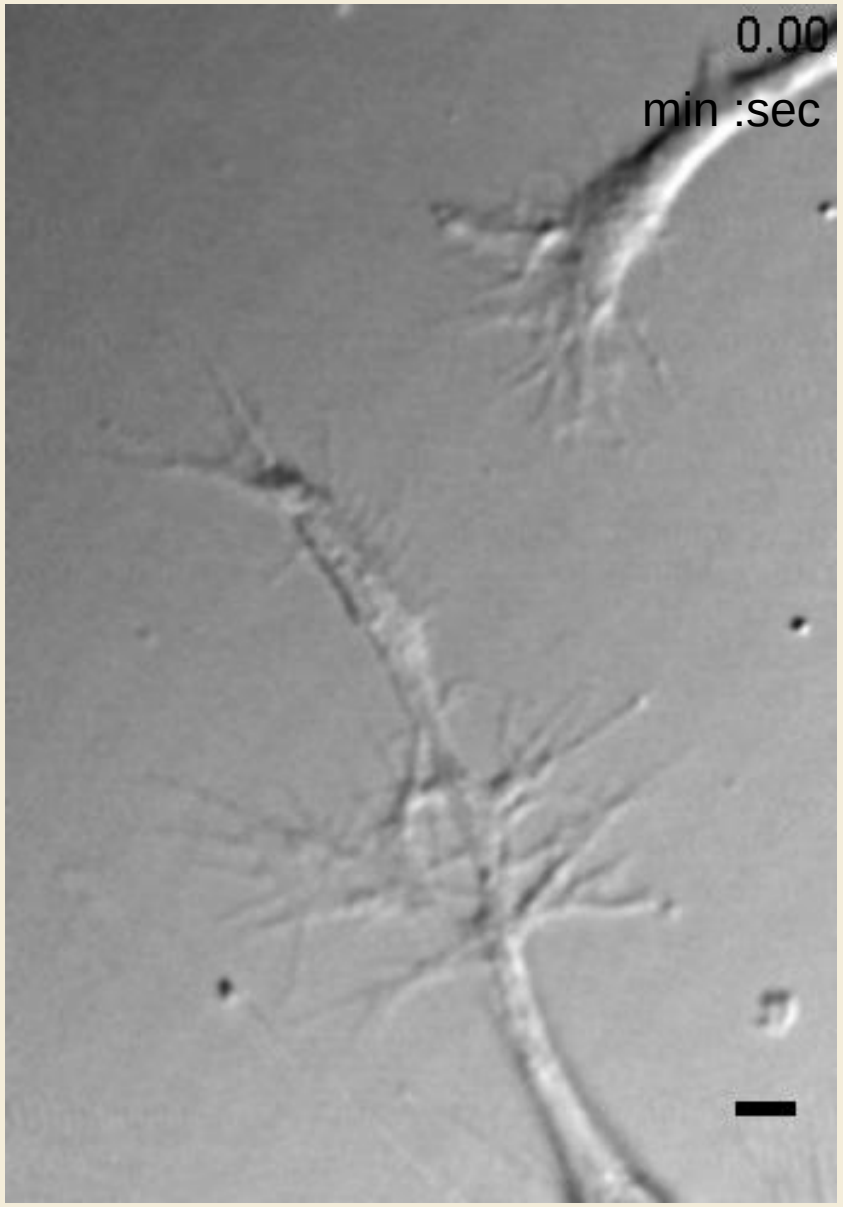
min:sec



Hippocampal neuron from mouse (2 DIV) stimulated with BDNF at t= 5 min.

(2 DIV = 2 Days In Vitro)

Neuronal development



Neurons release biochemical cues which are intercepted and interpreted by their nearby neurons.

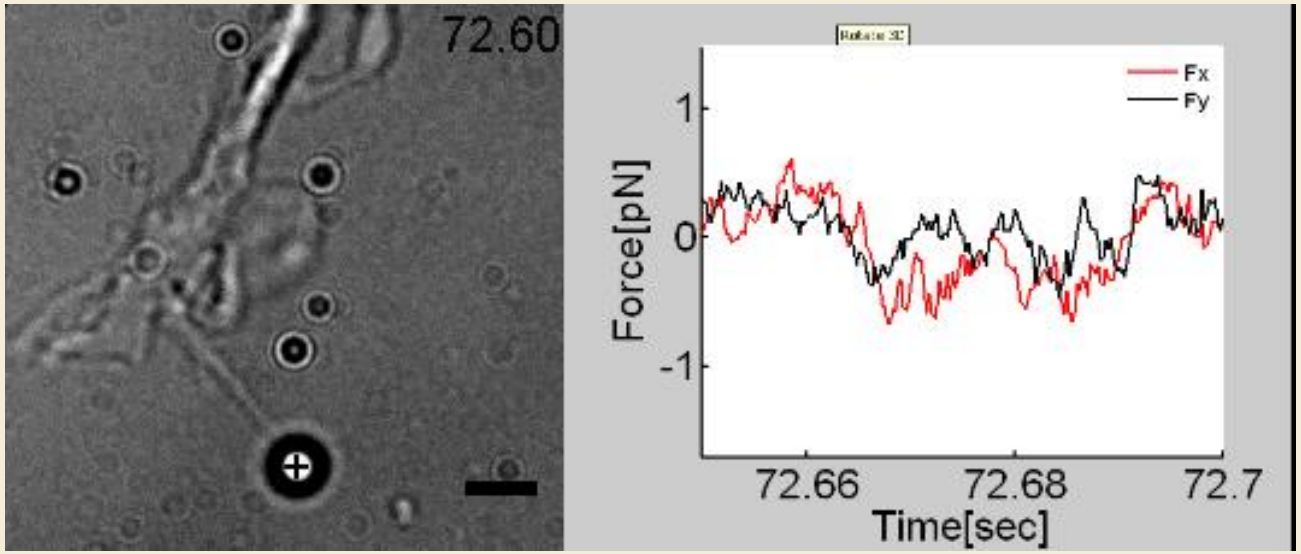
➢ The **Growth Cone (GC)** searches and detects molecular signposts that are displayed by the nearby developing neuron and the environment.

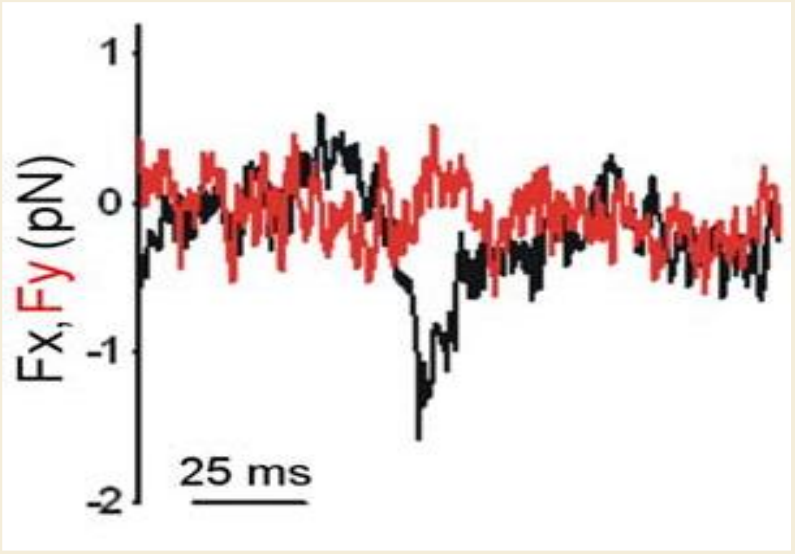
➢ **GC** responds to these signs by advancing, pausing and turning until it reaches its proper destination

Scale Bar = 2 μm
Acquisition freq= 1 frame every 5 s

F. Difato *et al* (2006) OM-Lab & SISSA

Force exerted by Filopodia



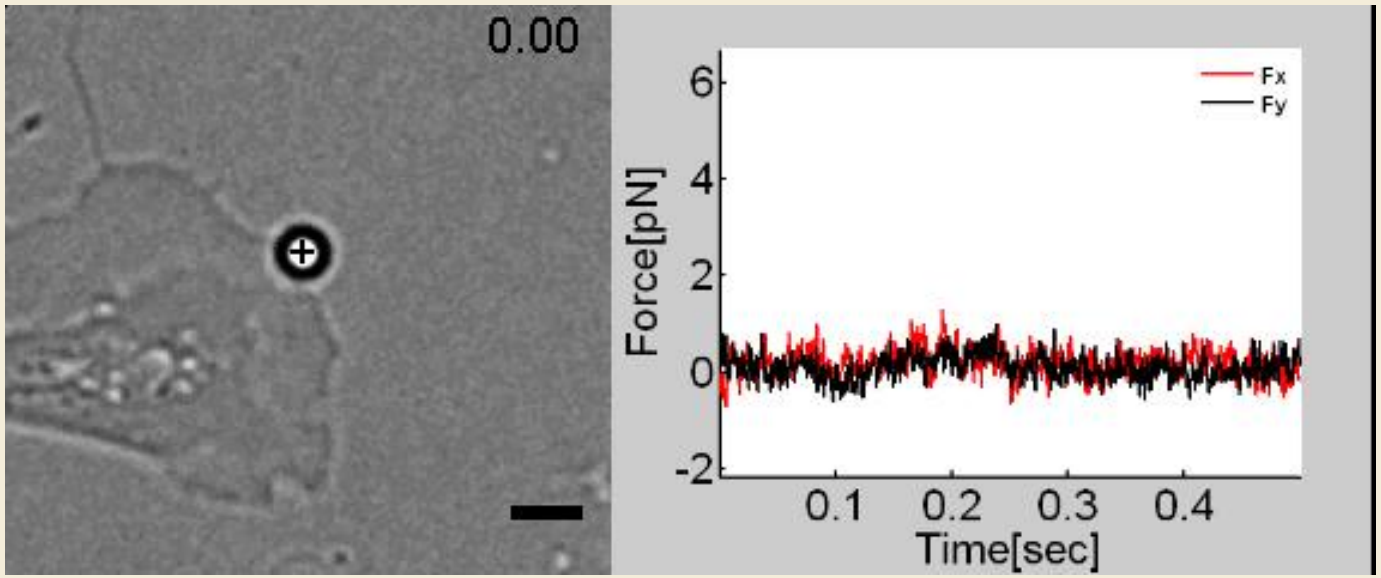


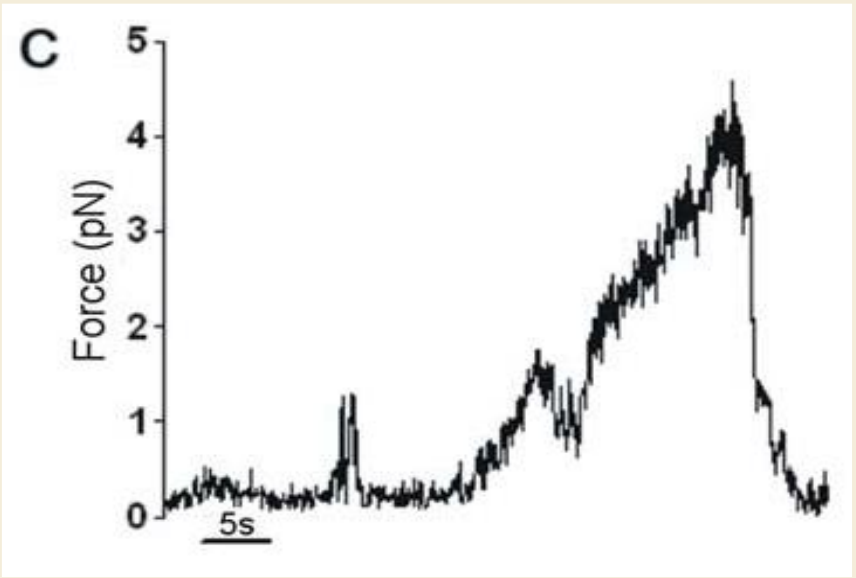
Acquisition rate: 20Hz; Scale Bar = 2μm; Time in seconds

Acquisition rate : 4KHz, Subsampled at : 2KHz

2007_PlosOne_Cojoc; 2013_Int J Mol Sci_Difato

Force exerted by Lamellipodia





Acquisition rate: 20Hz; Scale Bar = 2μm; Time in seconds

Acquisition rate : 4KHz, Subsampled at: 2KHz

Development of forces larger than 3 pN requires microtubule polymerization

Cells crawling across a solid surface

In animals, **almost all cell locomotion** occurs by **crawling**, with the exception of swimming sperm.

Examples:

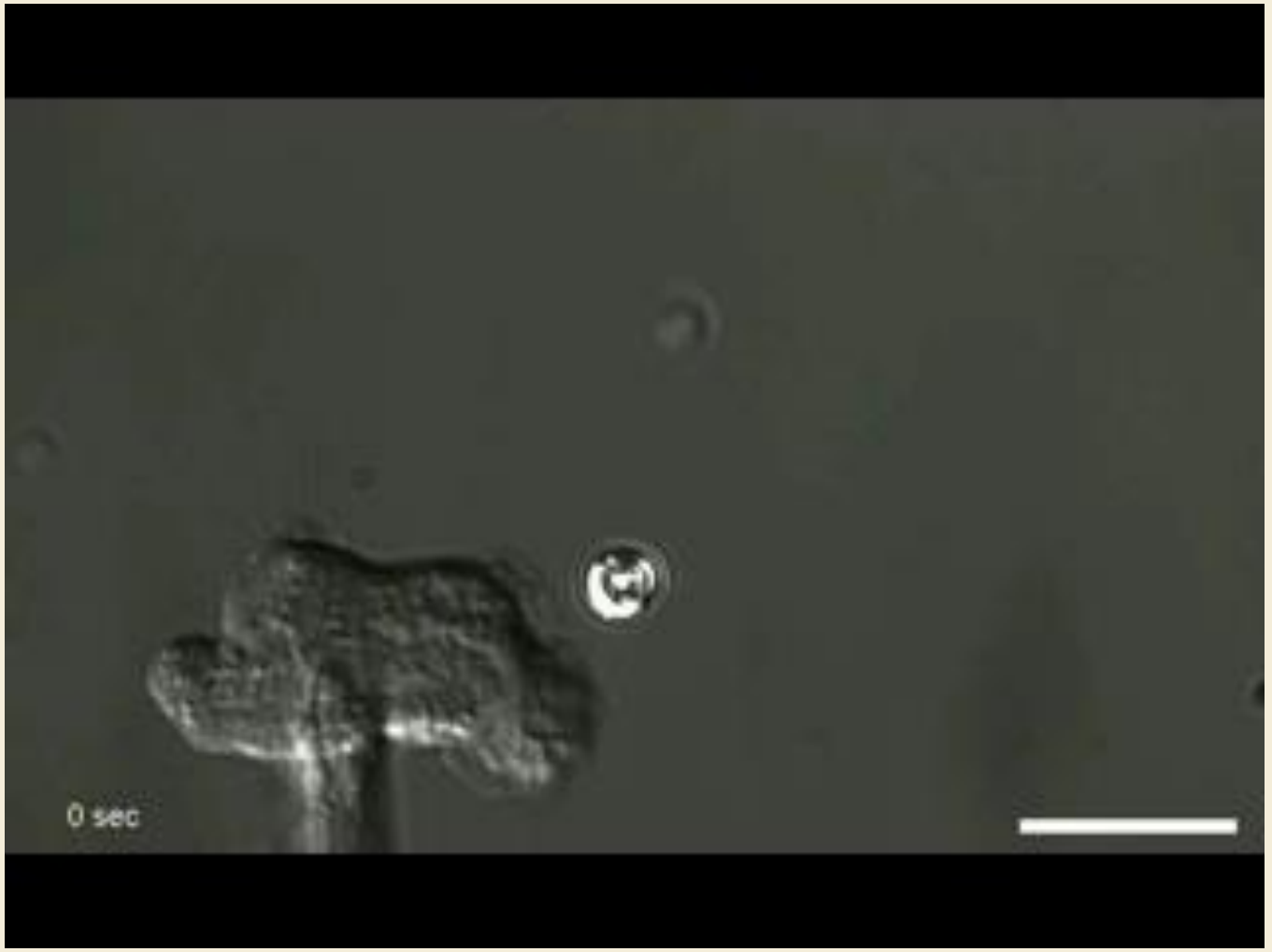
1. During **embryogenesis**, the structure of an animal is created by the **migrations of individual cells** to specific target locations and by the **coordinated movements of whole epithelial sheets**.

Long-distance crawling is fundamental to the **construction** of the entire **nervous system**: it is in this way that the actin-rich **growth cones** at the advancing tips of developing axons **travel to their eventual synaptic targets**, guided by combinations of soluble signals and signals bound to cell surfaces and extracellular matrix along the way.

2. The **adult animal** is also seething with crawling cells.

- **Macrophages** and **neutrophils** crawl to sites of infection and engulf foreign invaders as a critical part of the innate immune response.
- **Fibroblasts** can migrate through connective tissues, remodeling them where necessary and helping to rebuild damaged structures at sites of injury.
- **Cell crawling** also has a role in many **cancers**, when cells in a primary tumor invade neighboring tissues and crawl into blood vessels or lymph vessels and are thereby carried to other sites in the body to form metastases.

Neutrophil Cell crawling induced by a chemoattractant released from an optical manipulated source



Dufresne et al Nat. Methods 2008

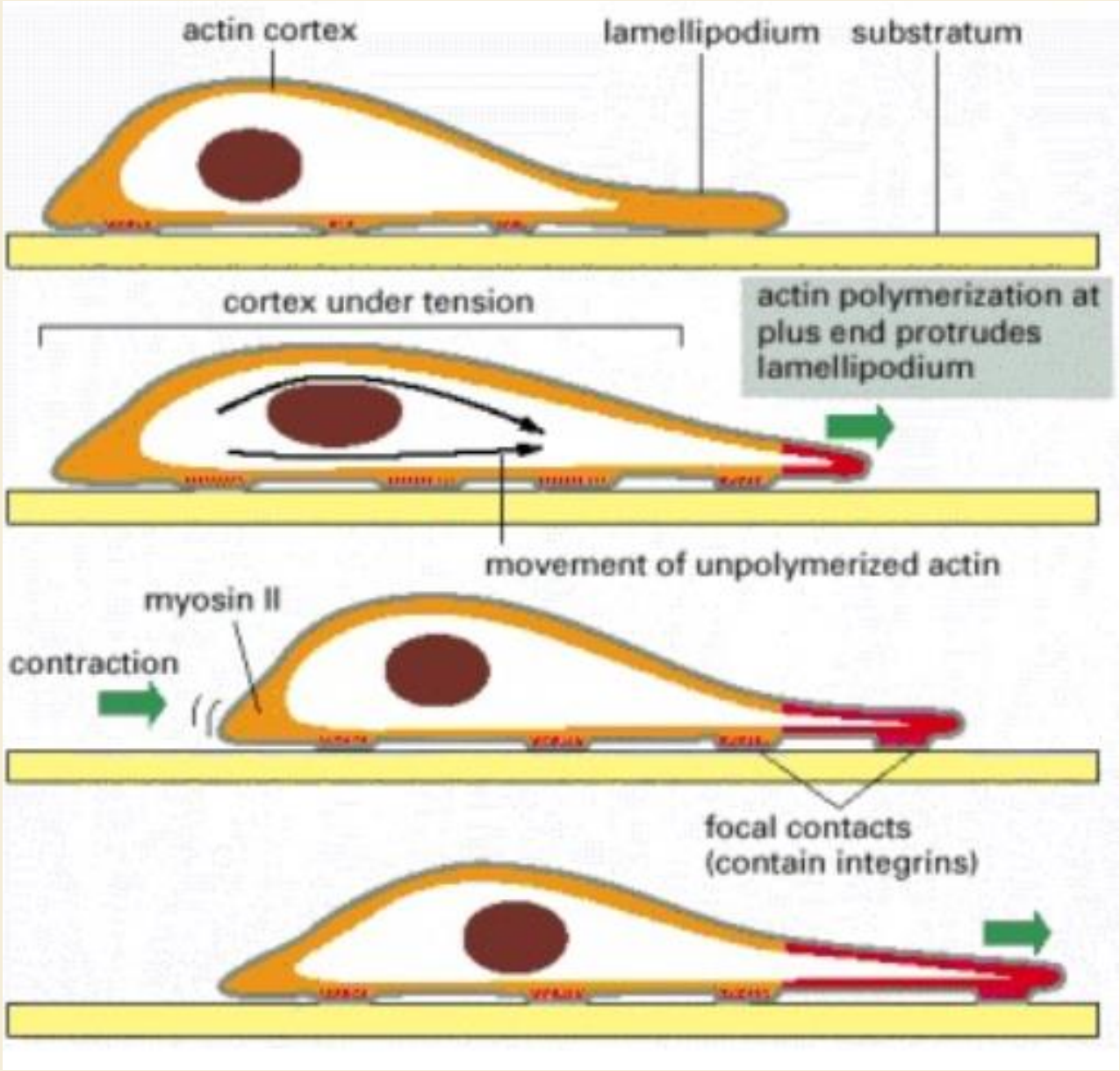
Cells Can Crawl Across A Solid Substratum

Cell crawling is a highly complex integrated process, dependent on the actin-rich cortex beneath the plasma membrane.

Three distinct activities are involved:

- **protrusion**, in which actin-rich structures are pushed out at the front of the cell;
- **attachment**, in which the actin cytoskeleton connects across the plasma membrane to the substratum;
- **traction**, in which the bulk of the trailing cytoplasm is drawn forward.

Cells Can Crawl Across A Solid Substratum



A model of how forces generated in the actin-rich cortex move a cell forward

The actin-polymerization-dependent **protrusion** and firm **attachment** of a lamellipodium at the leading edge of the cell moves the edge and stretches the actin cortex.

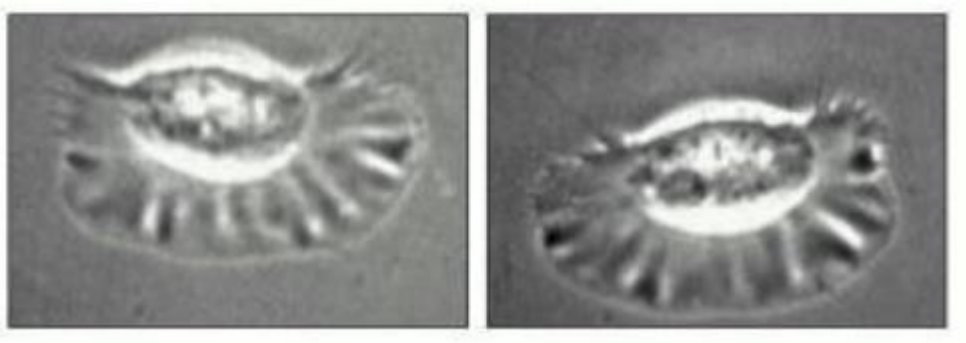
Contraction at the rear of the cell propels the body of the cell forward to relax some of the tension (**traction**).

New **focal contacts** are made at the front, and old ones are disassembled at the back as the cell crawls forward.

The same cycle can be repeated, moving the cell forward. The newly polymerized cortical actin is shown in red.

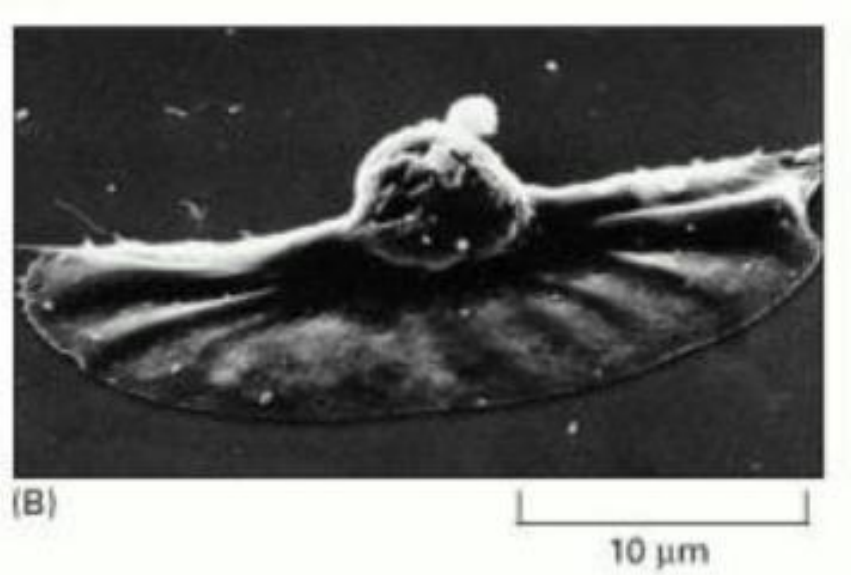
Lamellipodia contain all the machinery that is required for cell motility.

Migratory keratocytes from a fish epidermis.

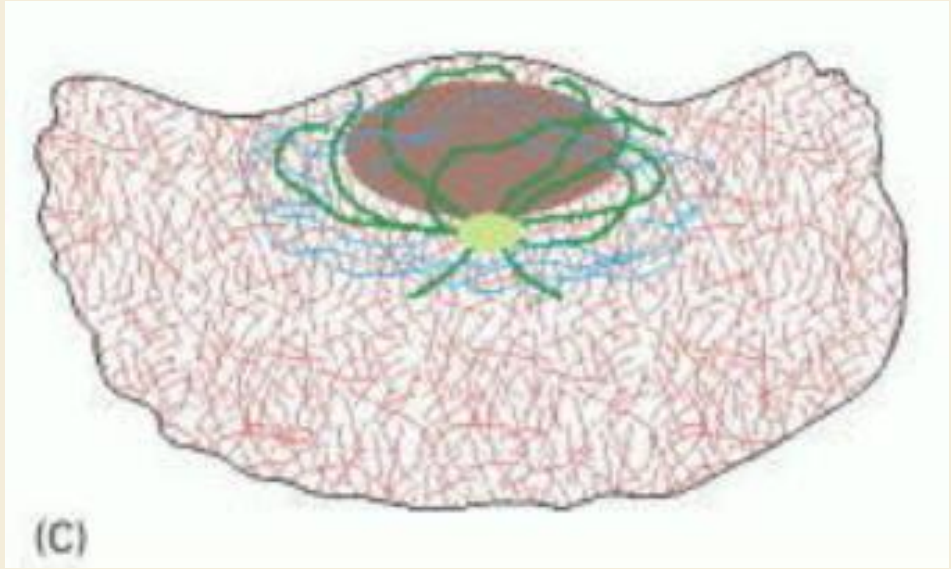


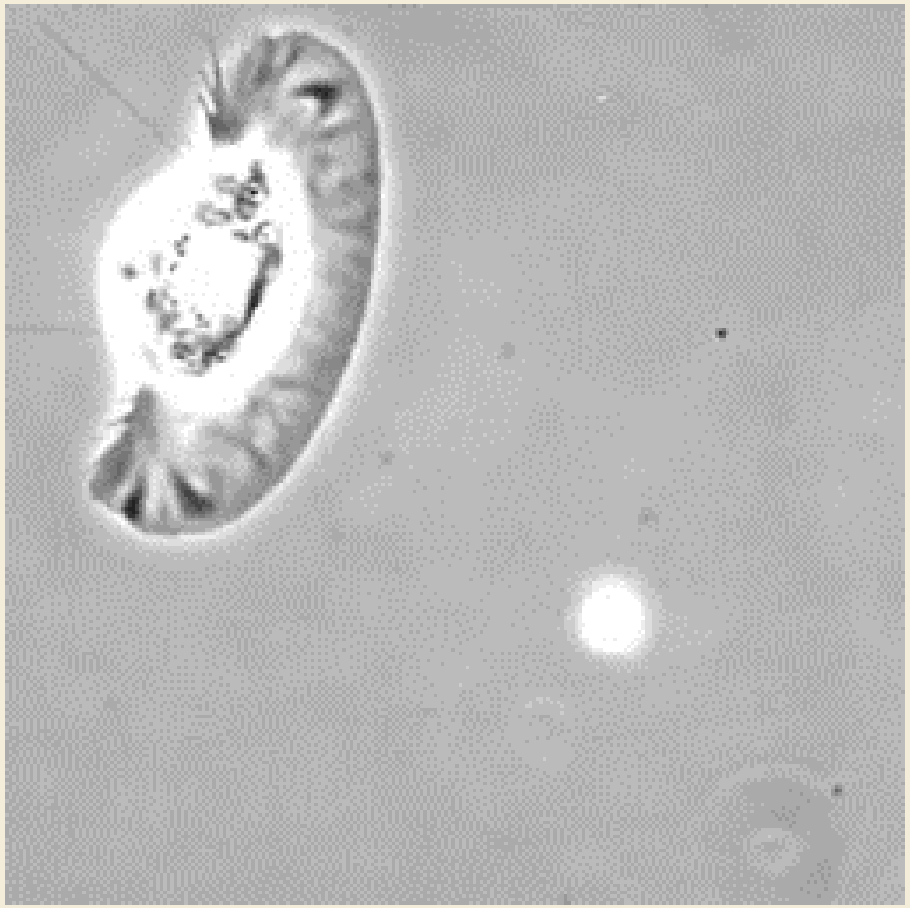
Light micrographs of a keratocyte in culture, taken about 15 sec apart. This cell is moving at about 15 µm/sec.

Keratocyte seen by scanning electron microscopy, showing its broad, flat lamellipodium and small cell body, including the nucleus



Distribution of cytoskeletal filaments in this cell.
Actin filaments *(red)* fill the large lamellipodium and are responsible for the cell's rapid movement.
Microtubules *(green)* and intermediate filaments *(blue)* are restricted to the regions close to the nucleus.





The cell is crawling on a glass surface coated with 4 µg/ml PLL-PEG-RGD.

The cell is fan-shaped, with clearly defined lead and trailing edges, and moves persistently in one direction.

The movie is at 30× real time.