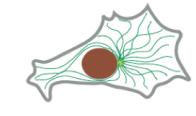
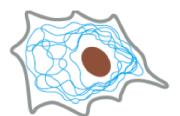


# Cytoskeleton dynamics

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	Actin Filaments	Microtubules	Intermediate Filaments
			
Subunit	actin monomer	tubulin dimer of $\alpha$ -tubulin and $\beta$ -tubulin	cell type dependent
Molecular weight (subunit)	42 kDa	50 kDa	40 – 210 kDa
Nucleotide binding	ATP/ADP	GTP/GDP	–
Form of polymer	double-stranded helix	hollow tube of 13 parallel filaments	staggered coiled-coil
Diameter of filament	~7 nm	~25 nm	8 – 10 nm
Motor proteins	myosins	kinesins & dyneins	–

- Microtubules use GTP instead of ATP
- Actin filament is double-stranded helix
- Microtubular hollows contain 13 tubulin components
- Microtubules and Actin Filaments are **Polarised and Dynamic!**
  - Polarised: have + and - ends: the two ends are not symmetric nor identical
  - Dynamic: created by polymerisation of their subunits, and are rapidly assembled and disassembled in cells
  - Intermediate filaments are neither polarised nor dynamic

## Natural cytoskeleton-targeting toxins

Toxin	Effect	Mechanism	Application
<b>Actin-binding</b>			
Latrunculin (red sea sponge)	Inhibits actin polymerisation	Binds to actin monomers	Research on cytoskeleton function
Phalloidin (Amantina mushroom)	Stabilises actin filaments	Binds to polymerised actin	Visualisation of actin for microscopy
Cytochalasins (mycotoxin)	Inhibit actin polymerisation	Cap filament plus ends	Research on cytoskeleton function
<b>Tubulin-binding</b>			
Colchicine (Colchicum autumnale)	Inhibits tubulin polymerisation	Binds to tubulin dimer	Gout treatment
Paclitaxel (pacific yew bark)	Stabilises microtubules	Binds to microtubules	Anti-cancer drug

These toxins demonstrate that the cytoskeleton is composed of very dynamic and highly regulated structures, as stabilization of these is toxic

## Actin Filament

### ▼ Actin monomer & existent form variation

#### ▼ monomer

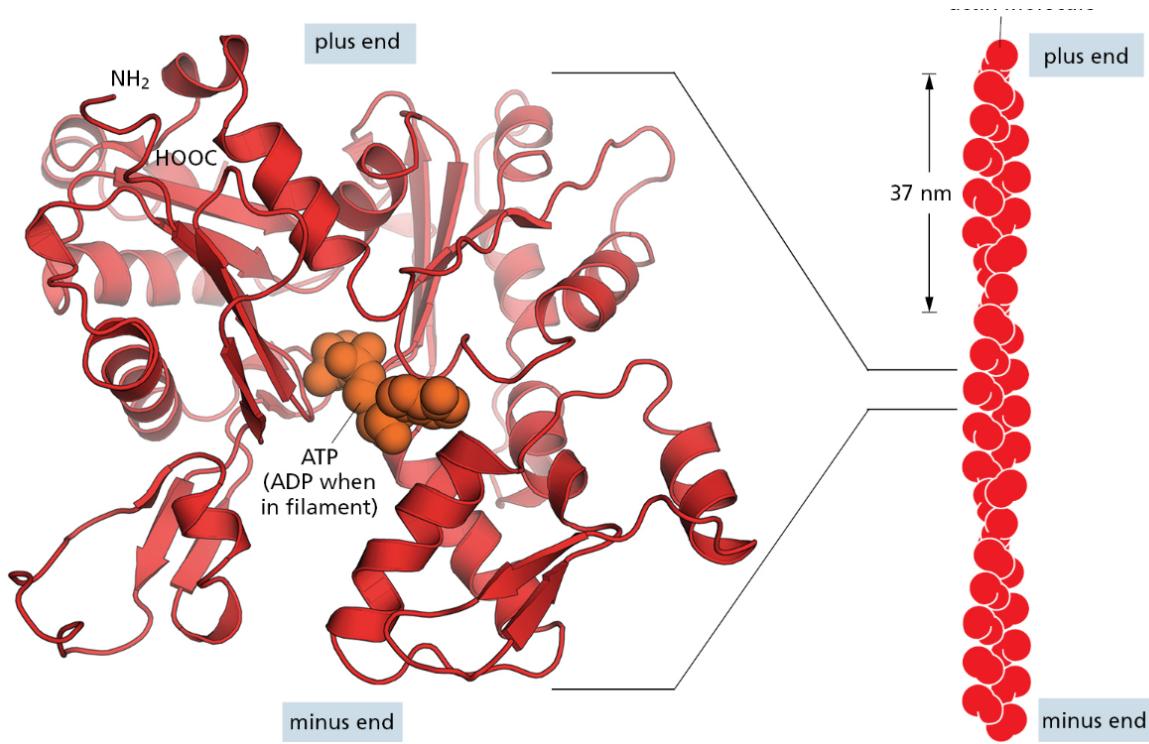
alpha-actin → ONLY muscle

beta-actin → non-muscle cells

gamma-actin → muscle & smooth muscle

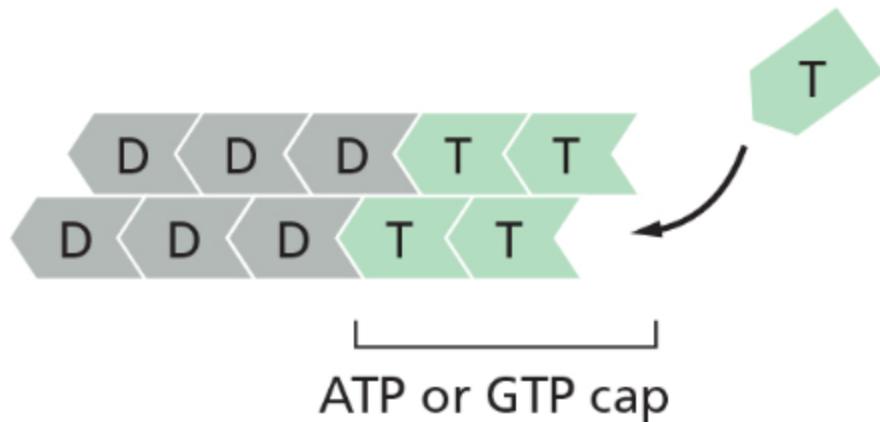
#### ▼ Exist Form

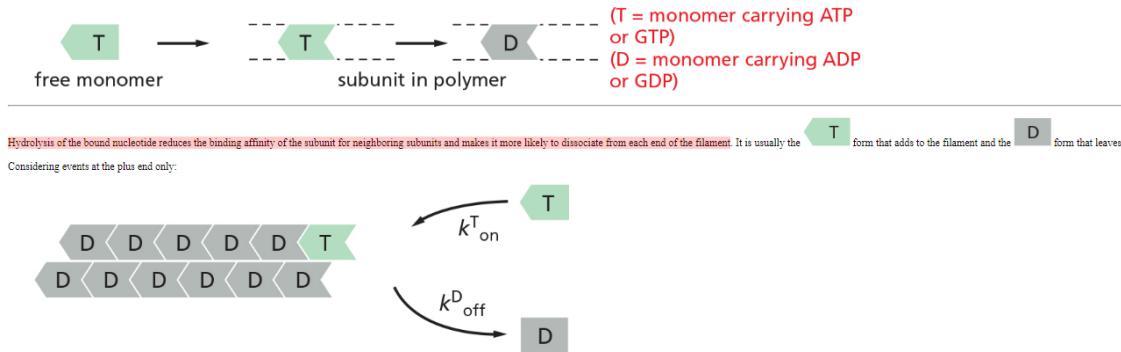
- monomer subunits: G-actin (globular)
- polymerised filaments: F-actin
- bundles (higher structures)



▼ Actin filament assembly — important mechanism by which cells control shapes and movements

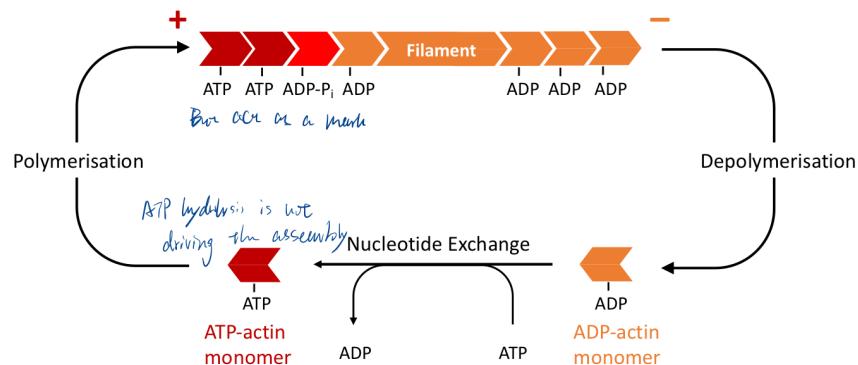
- each monomer (G-actin) is a polypeptide carrying ATP or ADP (when in filament)
  - within the filament, the subunits are positioned with their ATP-binding cleft directed toward the minus end





## Actin filament assembly

- Actin assembly requires ATP-bound actin monomers
- Hydrolysis of ATP occurs in incorporated subunits, lags rate of assembly and favours disassembly of the subunit
  - ATP-actin monomers preferentially assemble
  - ADP-actin monomers preferentially disassemble
- Assembly at plus-end, net disassembly at minus-end
- ATP caps are created on a growing filament, when the rate of subunit addition is higher than the rate of ATP hydrolysis



## Cc — critical concentration: free monomer subunit

Cc. As explained in Panel 16–2, the value of the critical concentration is equal to the rate constant for subunit loss divided by the rate constant for subunit addition; that is,  $C_c = k_{off}/k_{on}$ , which is equal to the dissociation constant,  $K_d$ , and the inverse of the equilibrium constant,  $K$ .

$$r_{on} = [k_{on} \cdot 2 \text{ monomer}] \cdot [\text{monomer}] = M \cdot s^{-1}$$

$$k_{on} = M^{-1} \cdot s^{-1}$$



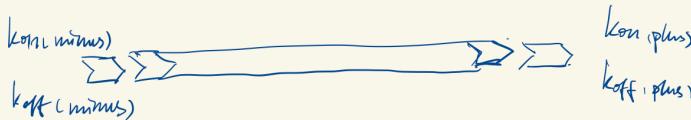
$$r_{off} = [k_{off} \cdot 2 \text{ polymer}] = M \cdot s^{-1}$$

$$\therefore k_{off} = s^{-1}$$

$$C_c = \frac{k_{off}}{k_{on}} = \frac{K_d}{\cancel{M}} \Rightarrow \text{in equilibrium.}$$

$$\Rightarrow \underline{[C_c]_{on} = k_{off}}$$

$C_c$ : constant concentration of free units - (in equilibrium).



plus end: fast-growing end.

minus end: slow-growing end.

In equilibrium: (?)

although  $k_{on}$  &  $k_{off}$  at both ends would be different,

$\frac{k_{on}}{k_{off}}$  ratio should be identical (when no ATP hydrolysis)

$C_c$  at both ends are identical

$$\therefore \text{When } C > C_c \Rightarrow \frac{k_{on}}{k_{off}} > \text{at equilibrium } \frac{k_{on}}{k_{off}} (K_d) \Rightarrow \text{both ends grow}$$

$$C < C_c \Rightarrow \frac{k_{on}}{k_{off}} < \text{at equilibrium } \frac{k_{on}}{k_{off}} \Rightarrow \text{both ends shrink}$$

This equilibrium does not stand bc there's ATP/GTP hydrolysis when addition of avin monomer & subunit

Hydrolysis makes the monomer more likely to dissociate from the filament bc of conformational change  $\Rightarrow k_{on} & k_{off} \rightarrow k_{on}^D & k_{off}^D$

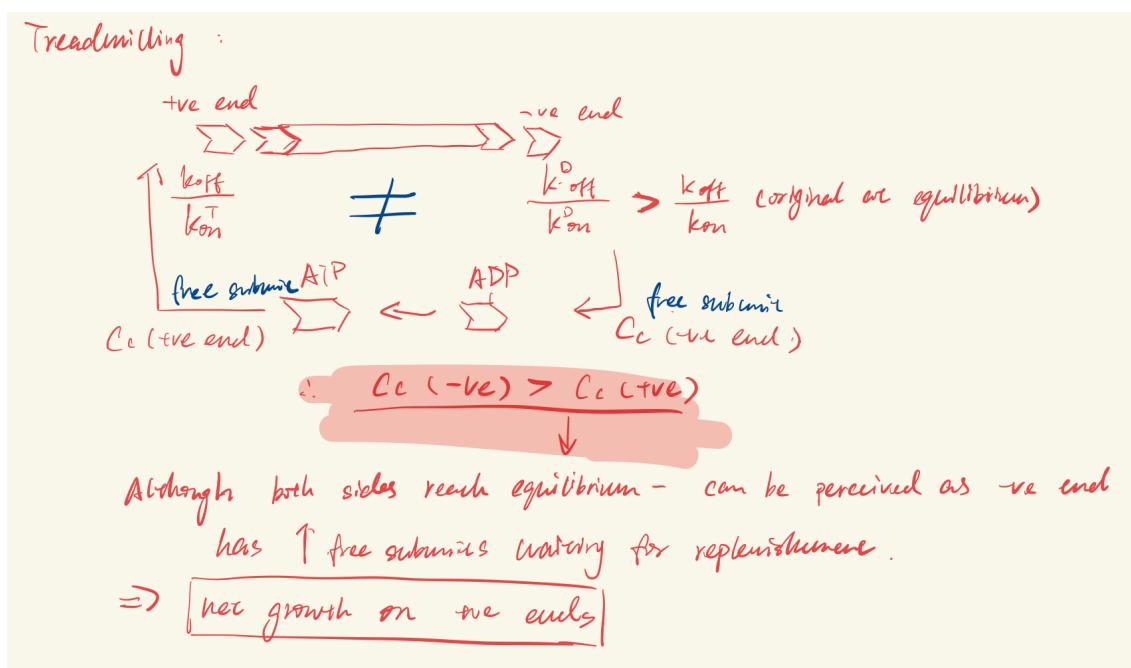
$$\therefore C_c = \frac{k_{off}^D}{k_{on}^D}$$

(?)  $\therefore$  This represent the steady state, but we the true equilibrium:

bc also need to consider the rate of replenishment of ATP/GTP-bound monomer from ADP/GDP-bound form

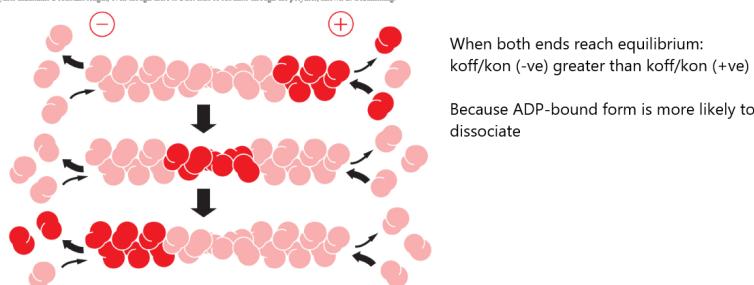
- Dynamic instability and Treadmilling are two behaviours observed in cytoskeletal polymers & both are associated with ATP/GTP hydrolysis
    - dynamic instability is predominated in microtubules
    - treadmilling may be predominated in actin filaments

### ▼ Treadmilling



- when both ends are at equilibrium:  $C_c(+\text{end}) < C_c(-\text{end})$  — there is an increase in the **free subunits : polymerisation proceeds**

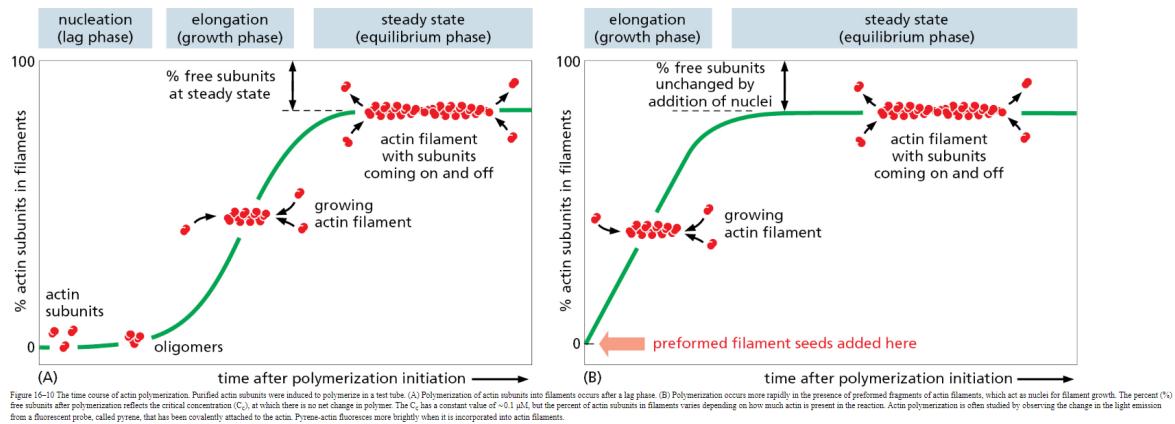
Thus, if both ends of a polymer exhibit an exposed, polymerization proceeds until the concentration of free monomer reaches a value that is above  $C_c$  for the plus end but below  $C_c$  for the minus end. At this steady state, subunits undergo a net assembly at the plus end and a net disassembly at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



- polymerisation ceases at AT STEADY STATE: the concentration of free subunits should be greater than  $C_c(+end)$  and smaller than  $C_c(-end)$

- Thus this will be perceived as NET assembly at +end and NET disassembly at -end
- BUT there is no net growth of the length
  - Because at the +end: there are more association than dissociation; at the -end: there are more dissociation than association → effects cancel out

## ▼ Assembly Dynamics:



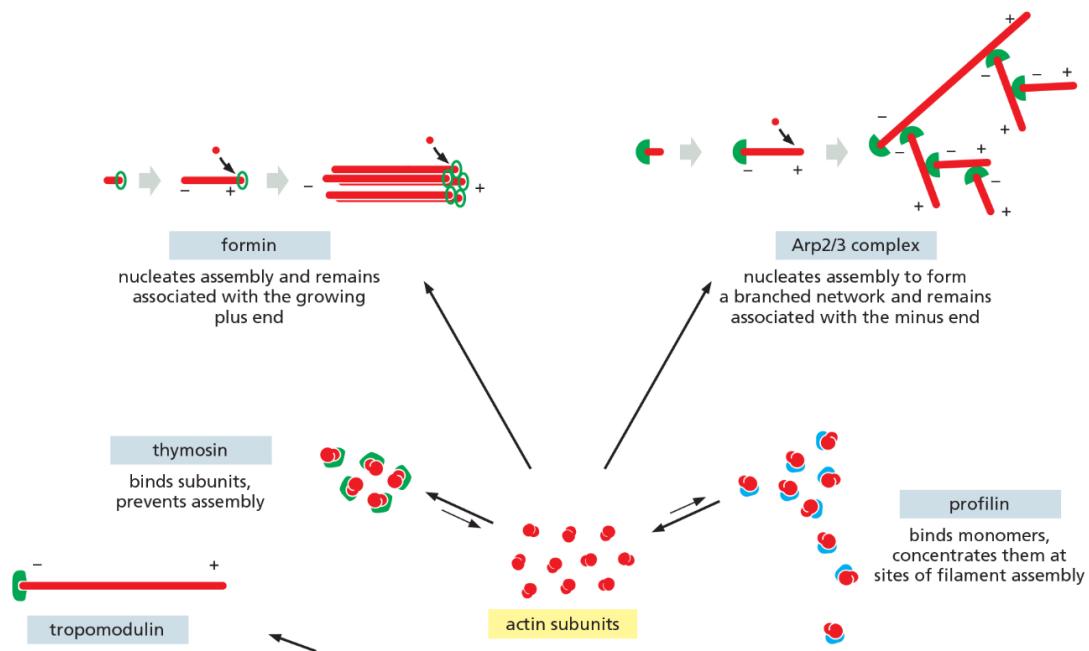
- When polymerization is initiated, this results in a **lag phase**:  
no filaments are observed → oligomers are unstable and likely to dissociate back to monomers.
- During this lag phase, however, the small, unstable oligomers gradually succeed in making the transition to a more stable form that resembles an actin filament → **success in nucleation**
- **Rapid filament elongation** during which subunits are added quickly to the ends of the **nucleated filaments**
- Finally, as the **concentration of actin monomers declines**, the system approaches a **steady state** at which the **rate of addition of new subunits to the filament ends exactly balances the rate of subunit dissociation**.

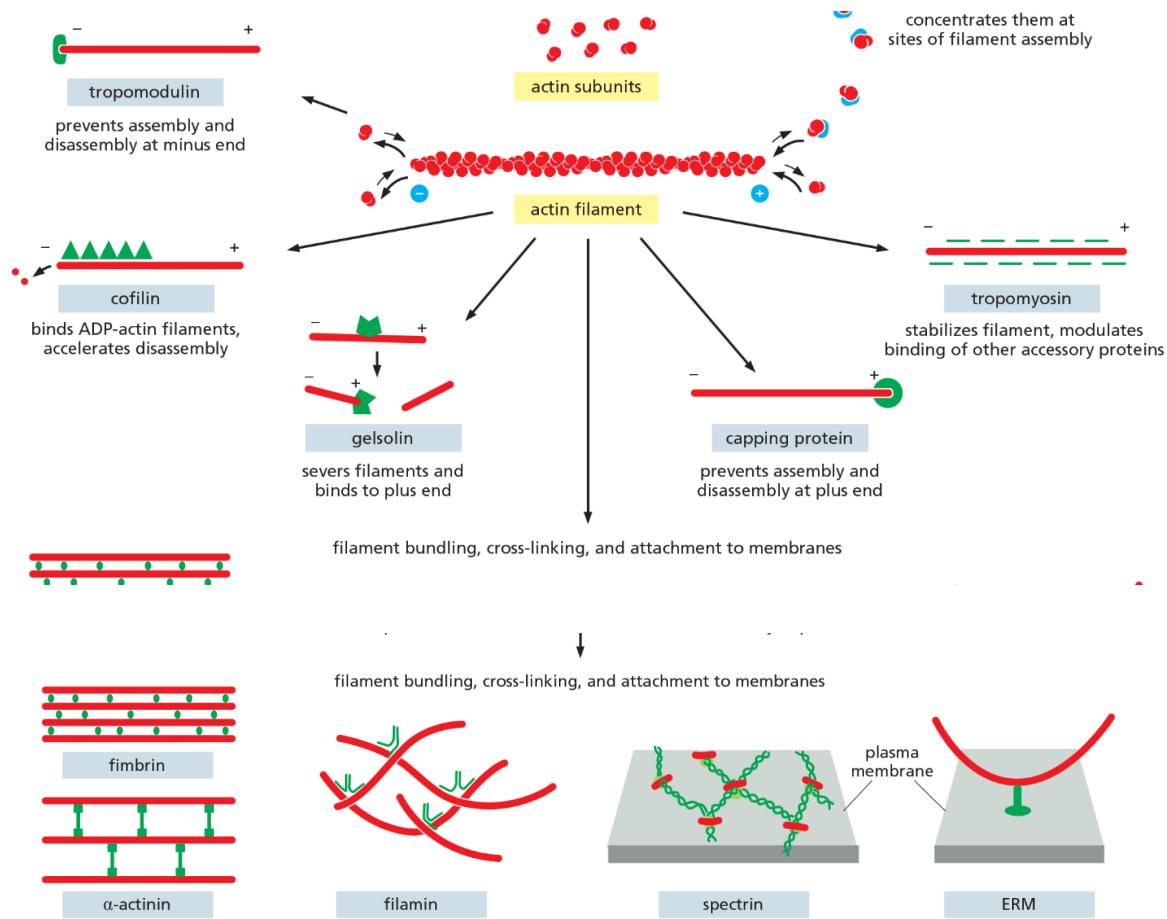
The concentration of free subunits left in solution at this point is called the critical concentration

- In a test tube, the C<sub>c</sub> for actin polymerization—that is, the free actin monomer concentration at which the fraction of actin in the polymer stops increasing—is about 0.1 μM. Inside the cell, the concentration of unpolymerized actin is much higher than this, and the cell has evolved mechanisms to prevent most of its monomeric actin from assembling into filaments

### ▼ Assembly factors & components

- In the cell, C<sub>c</sub> is much higher than the C<sub>c</sub> tested in test-tubes:
  - In the cell, the polymerisation is forced not to be spontaneous — always the case in cell → energy-costing!



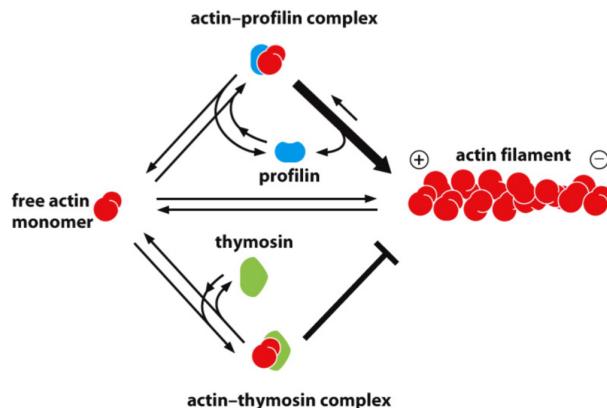


### ▼ From monomers to polymers

- thymosin prevents the polymerisation
- profilin competes for actin monomer → free them from thymosin to promote assembly

## 1) Regulation of free monomer pool

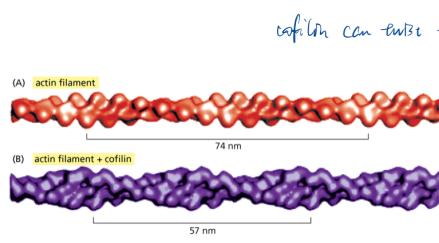
- In cells, spontaneous actin assembly is unfavoured and almost never occurs
- Instead, actin monomers are sequestered by thymosin, which inhibits assembly
- Thereby, thymosin maintains a large pool of monomers at cellular concentrations much higher than  $c_c$ , as they are unavailable for assembly
- Profilin competes for actin monomer binding, thereby freeing it from thymosin and promoting assembly
- Assembly is regulated by actin-binding proteins in space and time according to cellular needs



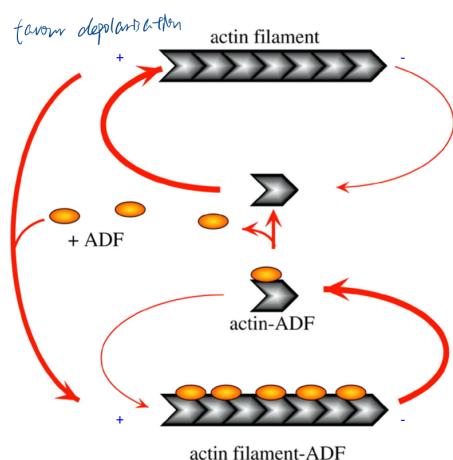
## ▼ Elongation & Depolymerisation

- Cofilin → promotes depolymerisation (by inducing twists) + ADF (actin depolymerising factor)

### 2) Regulation of elongation/depolymerisation: Actin Depolymerising Factor (ADF)/cofilin



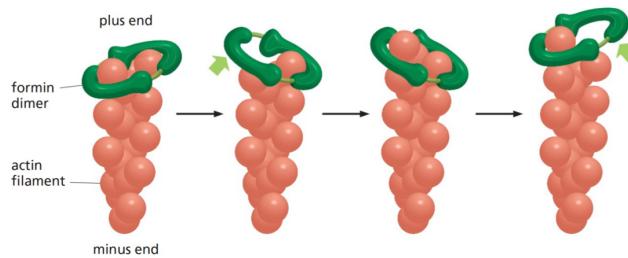
- Cofilin binding to the actin filament induces a twisting of the double helix
- This destabilises the filament, making it more susceptible to disassembly
- In addition, ADP-actin dissociates more easily from cofilin-bound filaments



- Formin → promotes nucleation & elongation
  - Dimer

- Always attach to the +ve end
- Capture two actin monomers

## 2) Regulation of elongation/depolymerisation: Formin

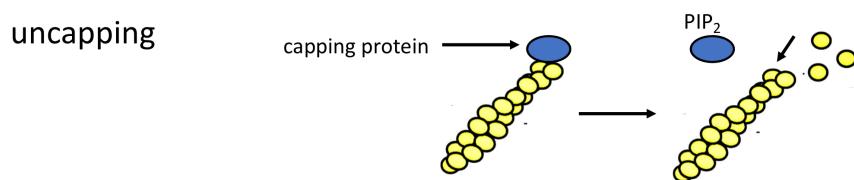


- Formin dimers capture two actin monomers and allows for the association of new subunits, thereby enhancing filament growth *keep "walking along" the +ve end*
- Other than other nucleating factors, formin stays associated with the growing plus end

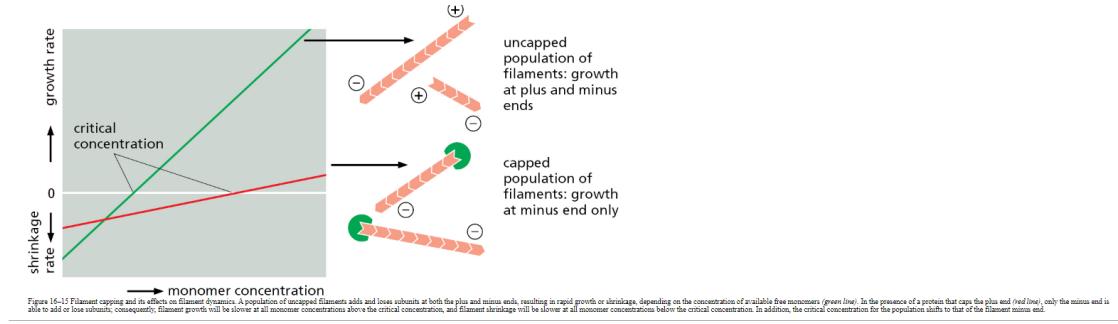
## ▼ Capping — terminate the dynamics

- capping protein → cap at + end
- tropomodulin → cap at - end

## 3) Regulation of elongation/capping:



Capping protein: usually located at cell surface : when interaction with  $\text{PIP}_2$  → decap → dynamic restarts



- Capping will increase the critical concentration
  - A population of uncapped filaments adds and loses subunits at both the plus and minus ends, resulting in rapid growth or shrinkage, depending on the concentration of available free monomers (green line). In the presence of a protein that caps the plus end (red line), only the minus end is able to add or lose subunits; consequently, filament growth will be slower at all monomer concentrations above the critical concentration, and filament shrinkage will be slower at all monomer concentrations below the critical concentration. In addition, the critical concentration for the population shifts to that of the filament minus end.

## ▼ Filament construction & rearrangement