Chapter 9

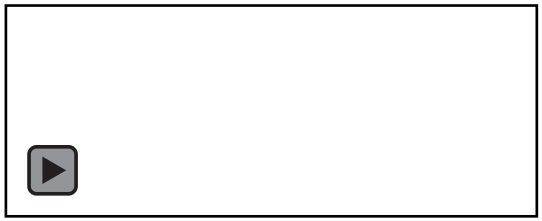
Polymerization of cytoskeletal filaments

Length of filaments

- Actin filament – 35nm in cortex to 10-100µm in stereocilia of hair cells (sensory receptors of the vertebrate inner ear)
- Microtubule – 1µm in mitotic spindle of yeast - 100µm in axons of rat neurons
- IF – few microns
- Tens of thousands of subunits in each filament
- Filaments must be multistranded because single-stranded filaments are short tend to break in the middle

Polymerization of filaments

- Assembly and disassembly of actin and microtubules occurs at the ends



- Spontaneous breakage and annealing of actin and MT in vitro is v. slow (gelsolin for actin, katanin for MT)
- Multistranded nature of actin and MT – end polymerization (energetically less favourable to break filament in the middle than to remove a subunit from the end)

Passive polymerization

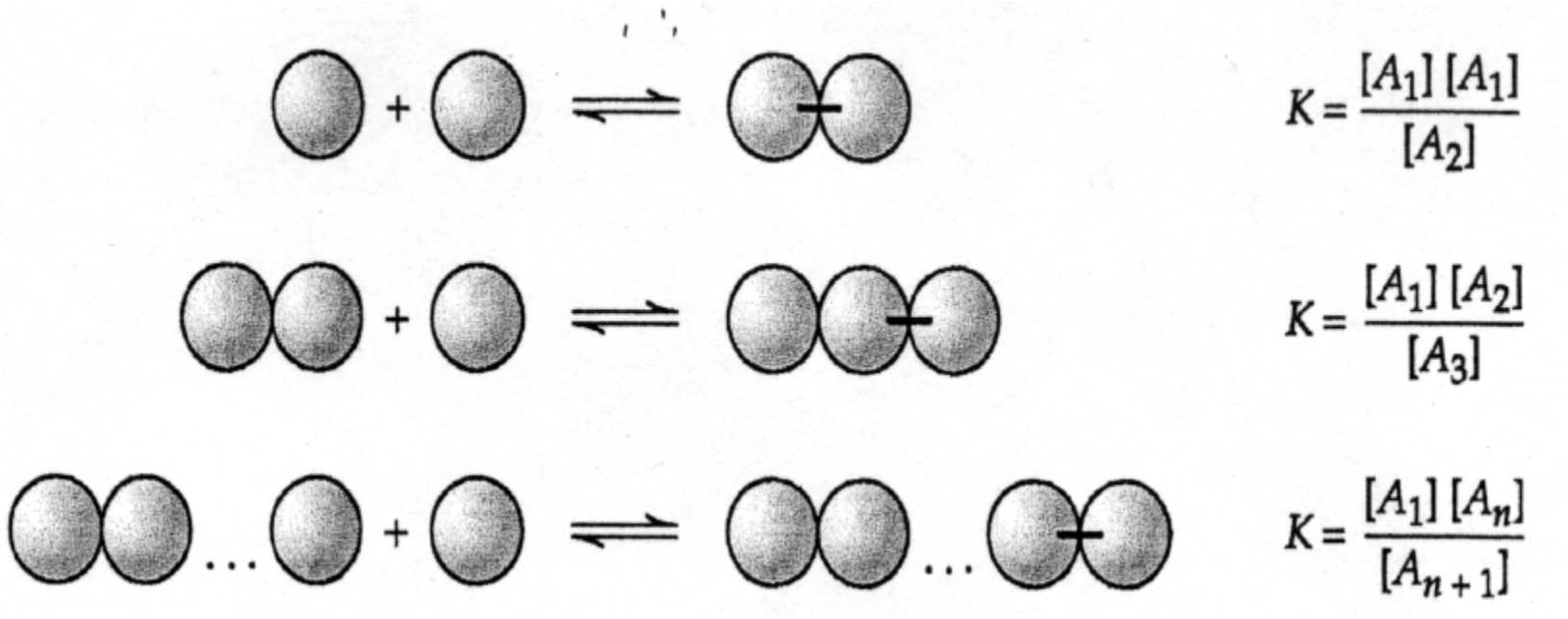
- Simple model for polymerization – no free energy during polymerization –"passive", equilibrium polymer
- Simplest is the single-stranded equilibrium polymer. Assuming all sub-unit additions have the same dissociation constant K

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

A_1 – monomer
A_n – n-mer

$$\frac{[A_n].[A_1]}{[A_{n+1}]} = K = \frac{K_{off}}{K_{on}};\ n \geq 1$$

Single-stranded/Einstein polymer



- Note: equilibrium conc. Of n-mers does not depend on the individual rates, but on the the equilibrium constant → depends on the enery difference between the subunits when they are in various states of aggregation. (Boltzmann's law)

Einstein polymer

- The Einstein polymer is crude → dissociation constants for each of the addition reactions is assumed to be equal
- However, K is expected to depend on the length of the polymer (even though differences are small)
- Dissociation constant is associated with a standard free energy change via

$$K = \exp(\frac{\Delta G^{\circ}}{k_B T}) \text{ moles/liter}$$

- ΔG^0 - Standard free energy change when the reaction occurs under standard conditions (1M concentration)
- ΔG^0 = potential energy (bond formation) + entropic energy (loss of translational and rotational energy)
- Monomer binding to long polymer loses more translational and rotational energy than monomer binding to another monomer.
- $K_{polymer\text{-}monomer} \sim 10x\ K_{monomer\text{-}monomer}$ (Hill, 1987)

Single-stranded filaments are short

- How does the average length of the polymers depend on the total concentration of subunits?
- At equilibrium, lengths of the polymers are exponentially distributed

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

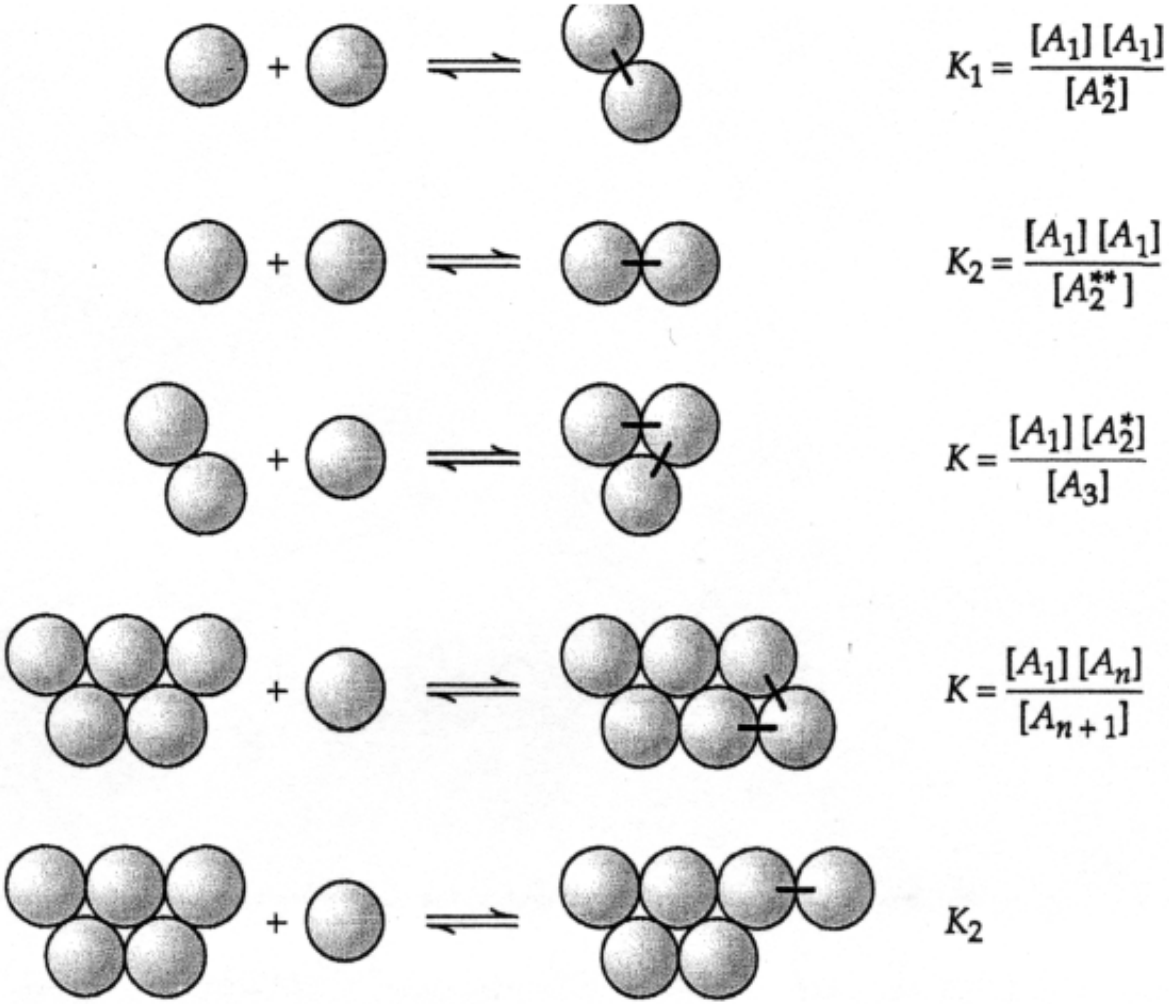
Single-stranded filaments are short

- Average length of single-stranded filament:

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

- Even when $[A_t]=100*K$, $n_{av} = 10$, but cytoskeleton contains 1000s of subunits → Total concentration of subunits must be millions of times K
- Ends of the polymers are not favourable from an energetic point of view

Multi-stranded filaments are long



Multi-stranded filaments are long

- 2 different bonds in multi-stranded filaments – one within the strands and one between
- 2 different nuclei A_2^* and A_2^{**} and three different dissociation constants K, K_1 and K_2
- Lengths of polymers exponentially distributed but n_{av} much higher:

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

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

- Eg: For actin, K = 1μM, $K_1 \approx K_2 \approx 0.1M$
- If $[A_t]=10\mu M$, then $n_{av} \approx 1000 = 2.75\mu m$ long filament.
- Ends of 2-stranded filament are energetically unfavourable → low concentration at equilibrium

Multi-stranded filaments grow and shrink at the ends

- Difference between single and multi-stranded filament is the growth and shrinkage only at the ends.
- Stable nuclei → controls the location of polymerization
- Stabilization of filament by capping is sufficient

Multi-stranded filaments grow and shrink at the ends

- Rate of elongation of multi-stranded filaments

$$\frac{dn}{dt} = k_{on}[A_1] - k_{off}$$

- Considering a single end, first annealing reactions that lead to an increase in length of an n-mer:

$$\frac{dn}{dt} = k_{on}[A_1] + 2k_{on,2}[A_2] + \cdots + mk_{on,m}[A_m] + \cdots$$

If $k_{on,m} = k_{on}$, then

$$\frac{dn}{dt} = k_{on} \sum_{m=1}^{\infty} m[A_m] = k_{on}[At]$$

- As m becomes large, translational and rotational diffusion for the n-mers becomes very slow.
- Second order annealing rates become diffusion-limited for large m and will be much slower than monomer association rate
- Therefore, growth is by monomer addition

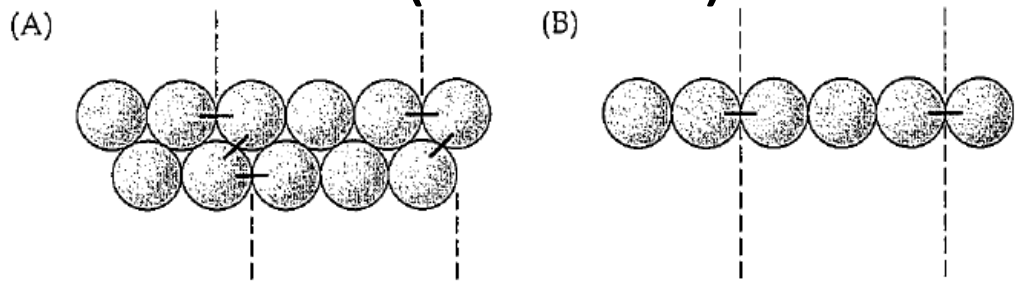
Shrinkage of multi-stranded filaments

- Change in length due to breakage

$$\frac{dn}{dt} = -k_{off} - 2k_{off} + \cdots - mk_{off,m}$$

- Dissociation constant for annealing is independent of m
- Principle of microscopic reversibility – Breakage rate is frozen out because long polymer fragments diffuse away slowly – high chance of reannealing

- Only dissociation if monomers and breaking of small oligomers will contribute significantly
- But monomers (2 bonds) more than 2-stranded filament (3 bonds)

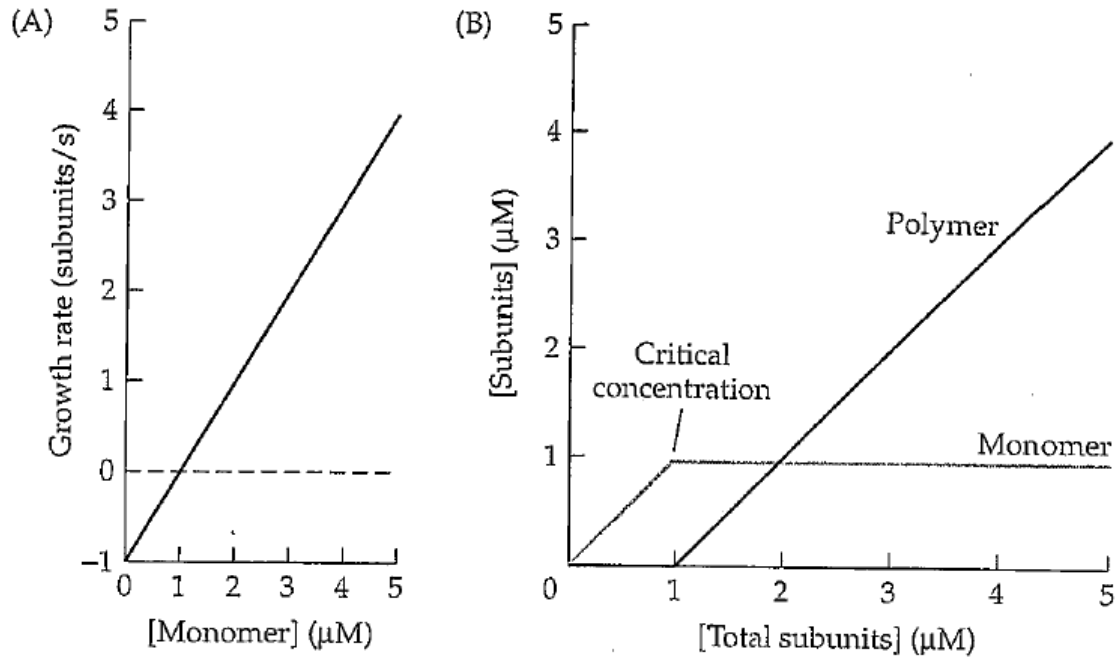


- In single-stranded filaments, both breakage and dissociation require 1 bond to be broken

Other properties of multi-stranded filaments

- Critical concentration K_c - monomer concentration at which the elongation rate is zero

$$K_c = \frac{k_{off}}{k_{on}} = K$$

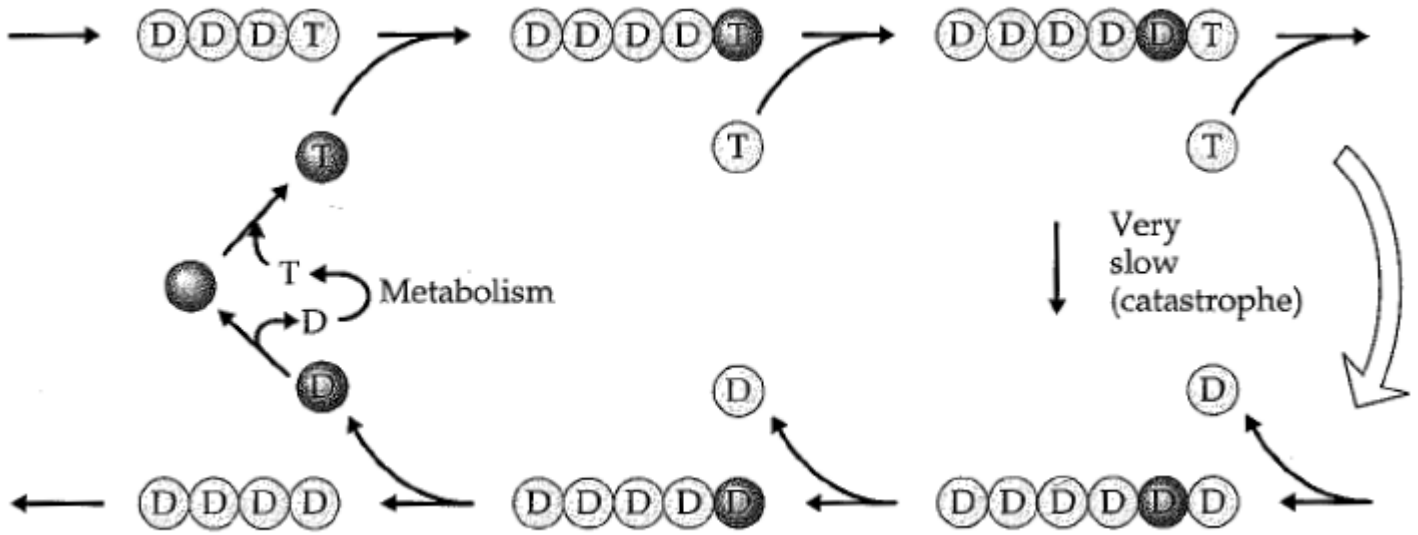


Chapter 11

Active Polymerization

- Length of an equilibrium polymer fluctuates in diffusive manner, $D = k_{on}[A_1]\delta^2$
- Leads to small change in length over time, for MT $k_{on} = 5 \times 10^6\ M^{-1}s^{-1}$, $[A_1] = 10\ \mu M$, $\delta =$ 0.6nm, SD of length over 1 min is 46nm, over 1 week is 4.7µm

Actin and tubulin hydrolysis cycles



- Lag between polymerization and hydrolysis – cap of ATP or GTP subunits at the end
- Hydrolysis of NTP in the cap – shrinkage

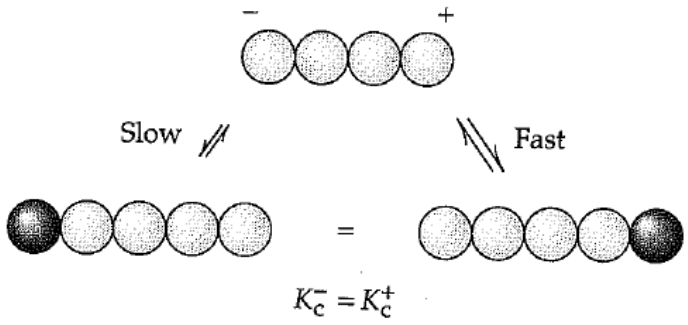
- Hydrolysis of NTP is catalyzed by polymerization → MT is GTPase activating protein for tubulin
- Exchange of NTP for NDP is catalyzed by depolymerization because exchange cannot happen in the polymer and can only happen after subunit dissociation.
- For actin, other proteins regulate the exchange of ATP for ADP on monomer (Profilin and thymosin)

Evidence for the model

- Majority of monomers have NTP bound since cytoplasmic concentration of ATP and GTP are much higher than those of ADP and GDP
- Cytoplasmic concentration of NTP-monomers is $>K_c$ – driving force for polymerization
- NTP are hydrolyzed after polymerization and phosphate released. Biochemical assays – incorporation of actin/tubulin into filament increases hydrolysis rate of ATP/GTP to $0.05s^{-1}$/ $0.2s^{-1}$
- Hydrolysis destabilizes the polymer. NDP monomers have higher K_c than NTP monomers

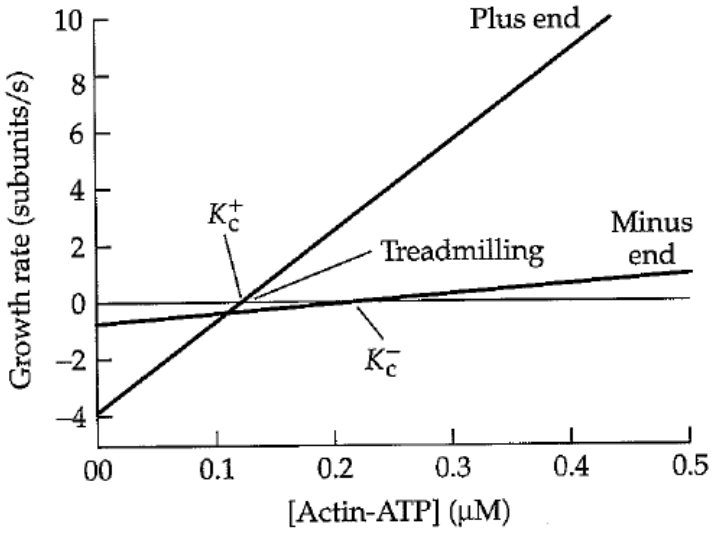
Filament Polarity

- For equilibrium polymer, K_c same at both ends → Addition of monomer to n-mer, (n+1)-mer is identical to addition at the other end (Boltzmann's law, equilibrium constants must be the same)
- But actin and MT have plus end and minus end



Treadmilling

- For both actin and tubulin, C_c at plus end is lower.
- When $C_{c+} < [A_1] < C_{c-}$ plus end grows, minus end shrinks → Treadmilling



Treadmilling

https://www.youtube.com/watch?v=xbswna2lIbk

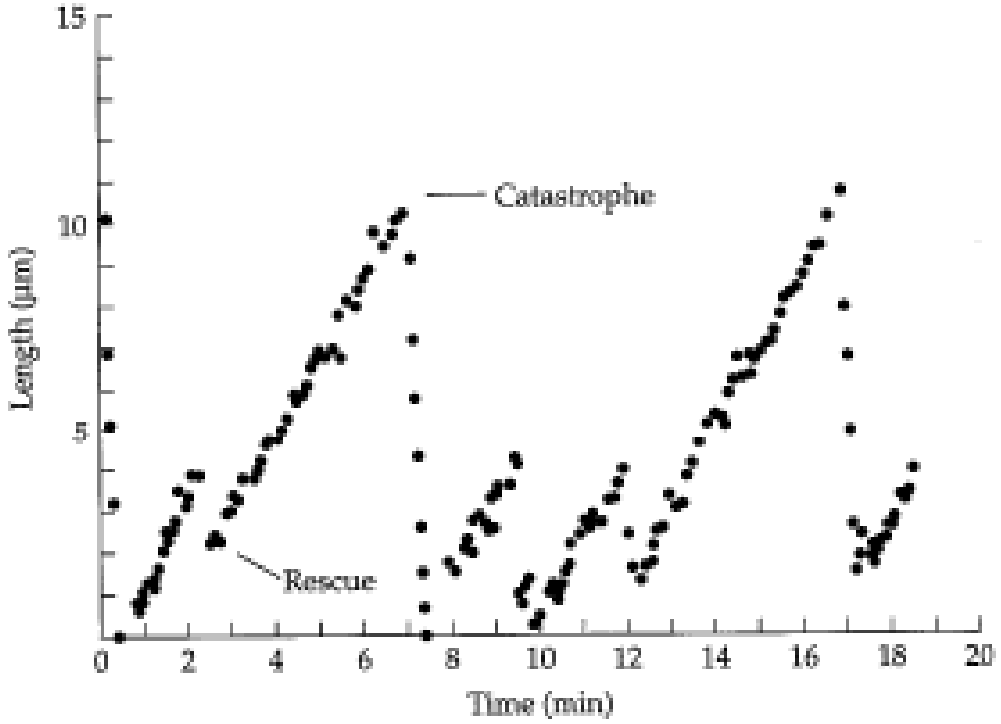
Cameron et al., JCB 2006

Nucleation

- Spontaneous nucleation is inhibited on non-equilibium polymers
- Critical concentration for nucleation would equal K_D (hydrolysis rate exceeds nucleation rate)
- If concentration of NTP monomers greater than C_c for growth on existing polymers but concentration of NDP monomers is less than K_D, growth will occur only on existing polymers

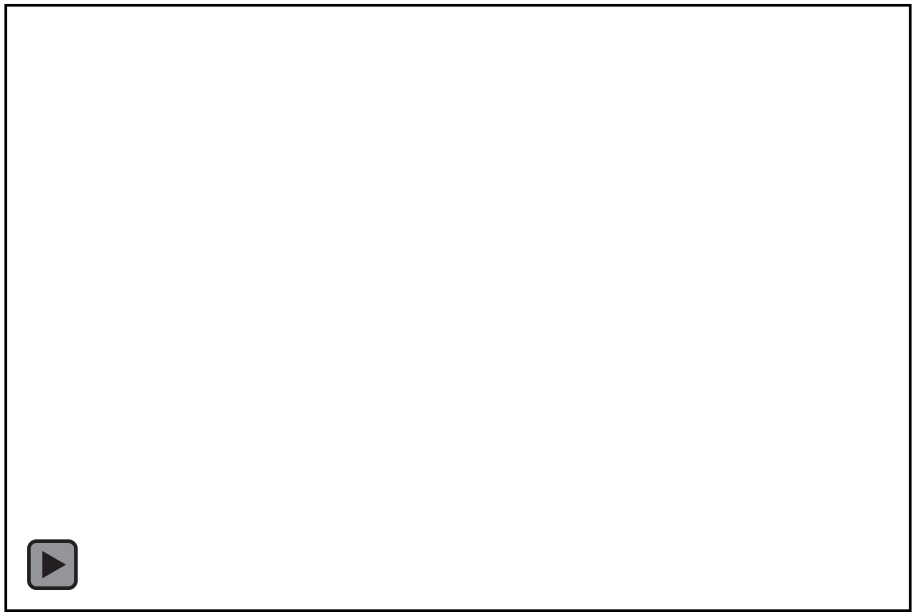
Dynamic instability

- Equilibrium polymer either growing, shrinking or at equilibrium, undergoing small diffusive fluctuations in length
- But MT switch between phases of growth and shrinkage – dynamic instability



Dynamic instability

https://www.youtube.com/watch?v=ZL3_BwrB6AM



Switching between growth and shrinkage

- Dynamic instability is described empirically by a 4-parameter model:
  - 1. Elongation rate in the growing phase v_{+}
  - 2. Shortening rate in the shrinkage phase v_{-}
  - 3. Rate of transition between growth and shrinkage (catastrophe rate) f_{+-}
  - 4. Rate of transition between shrinkage and growth (rescue rate) f_{-+}

- Bounded growth vs. unbounded growth depends on whether $f_{+-}v_{-} - f_{-+}v_{+}$ is > or < 0
- Under conditions that lead to bounded growth, mean length of microtubule

$$n_{av} \cong \frac{v_{-}v_{+}}{v_{-}f_{+-} - v_{+}f_{-+}}$$

GTP Cap model

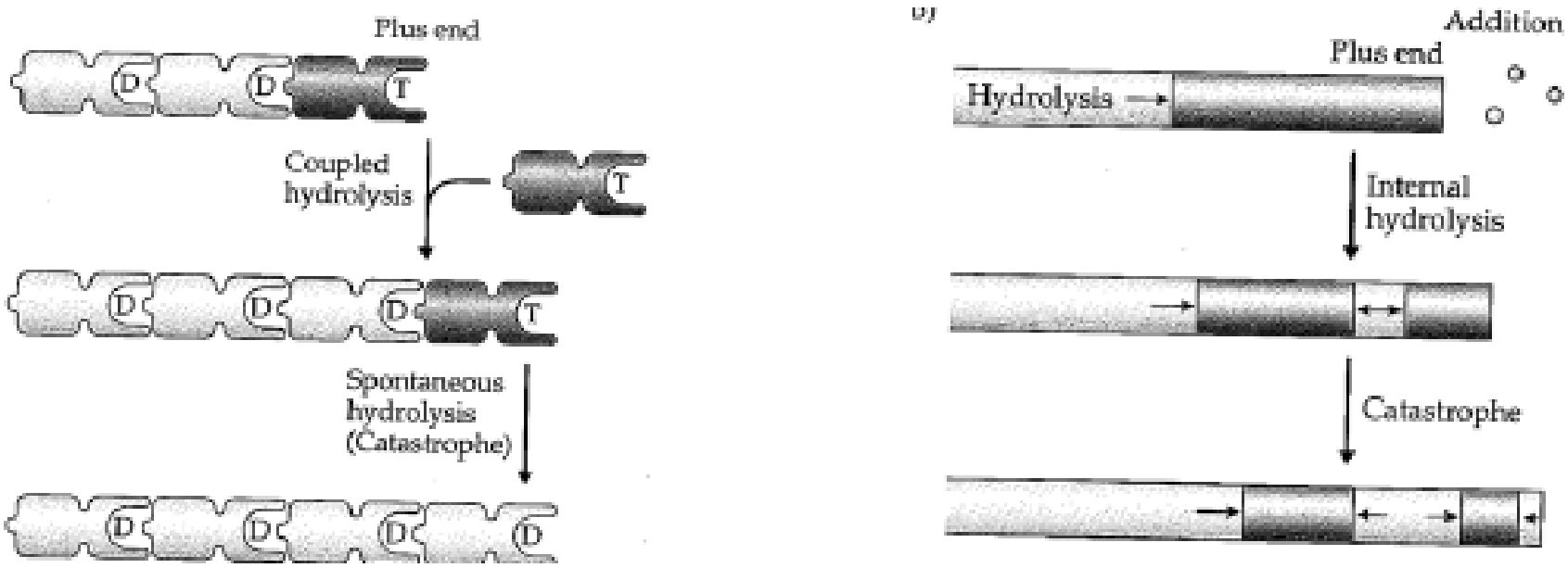
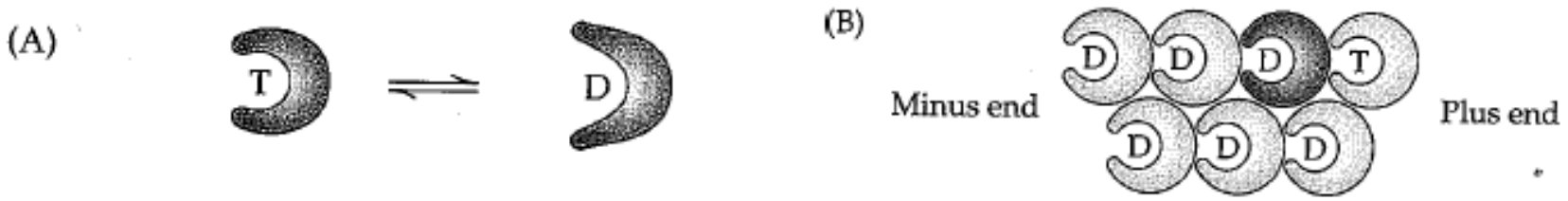


Figure 11.5 GTP-cap models for dynamic instability

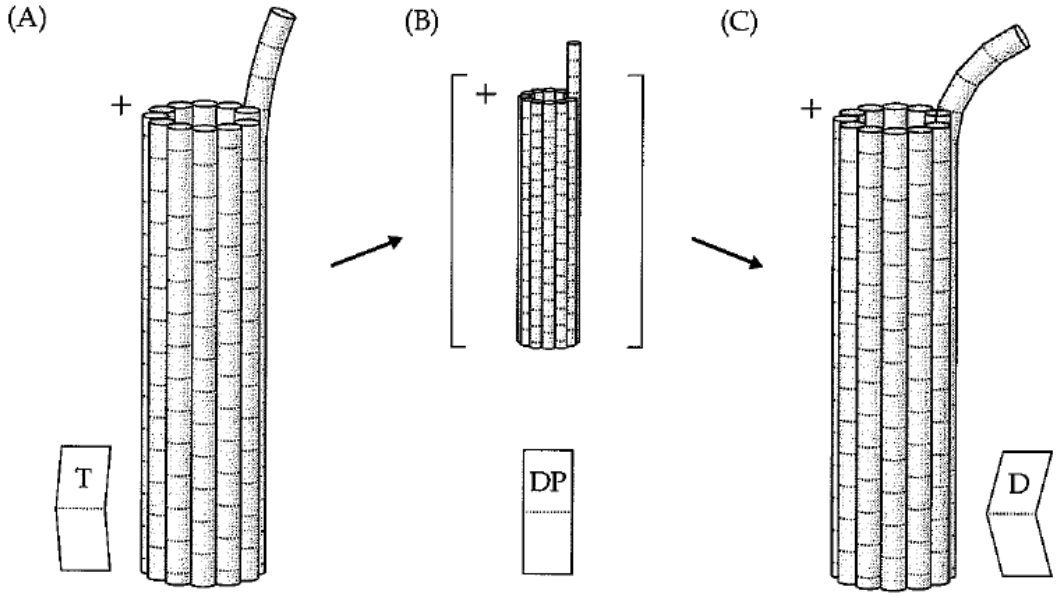
(A) The incoming subunit interacts with the nucleotide at the plus end of the microtubule and catalyzes hydrolysis. Catastrophe is caused by occasional spontaneous hydrolysis or dissociation of the terminal subunit. Only one protofilament is shown: The microtubule actually has 13 subunits. (B) There is competition between internal hydrolysis and subunit addition (top). The cap shortens abruptly when a GTP on an interior subunit spontaneously hydrolyzes and this zone of hydrolysis spreads. Catastrophe occurs when fluctuations in end growth and interior hydrolysis cause loss of the cap.

Structural changes during nucleotide hydrolysis



- When actin polymerizes, the nucleotide binding cleft closes a little
- Could explain lower critical conc. For ATP-actin if we assume ATP stabilizes the cleft in the closed state
- After hydrolysis – ADP-subunit in strained conformation (unfavourable closed state)

Tubulin structural changes during hydrolysis



- GTP dimer fairly “straight”, GDP dimer is “curved”
- Better fit for GTP dimer in the wall of the structure, so lower critical concentration.
- Transition state for GTP hydrolysis even “straighter” → catalyzed hydrolysis