DISCUSSION SECTIONS BY STUDENT NUMBER ENDING IN ODD NUMBERS 2-3, EVEN 3-4

Methods for Studying the Cell Cycle

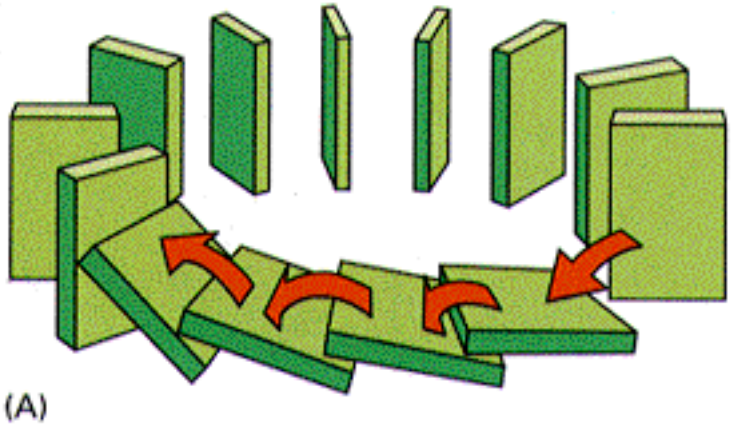
cell fusion
live and fixed imaging

genetics
biochemistry
in vitro systems

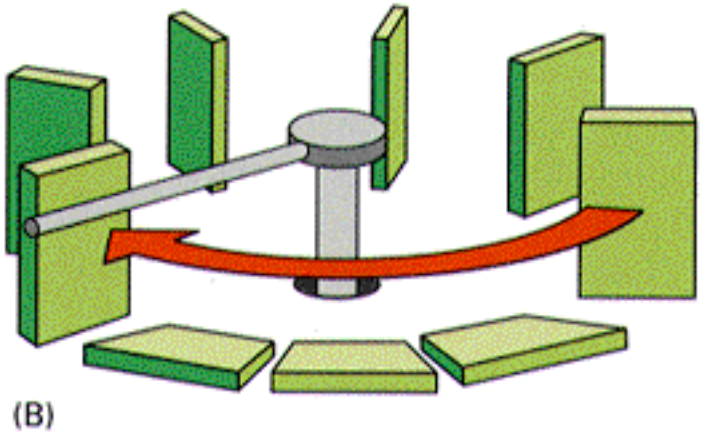
inhibitors of cellular processes (transcription, replication, microtubules)

Genetic Screens: Yeast ‘Cell Division Cycle’ (CDC) Mutants

‘Dominos’ sequential, dependent events

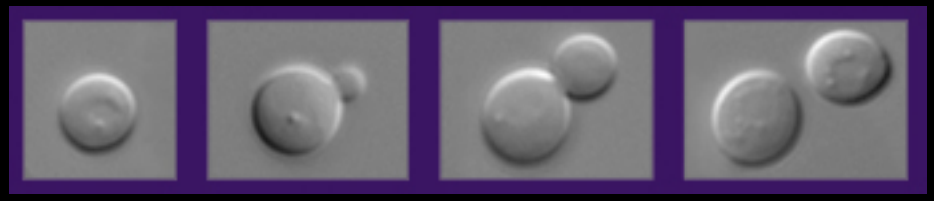


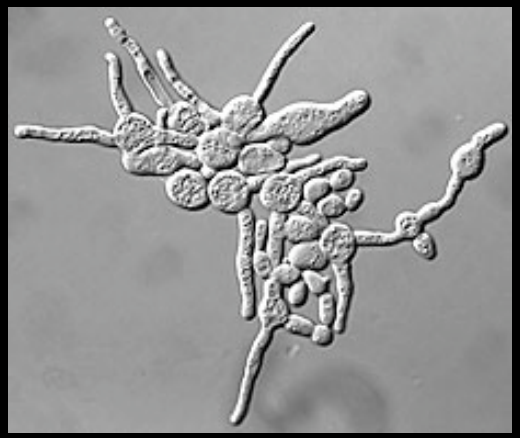
‘Oscillator’ central controller



can individual protein mutations block steps or whole process ?

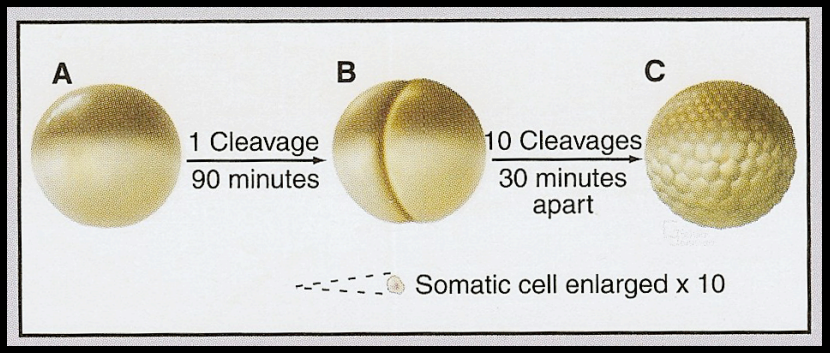
Genetic Screens: Yeast 'Cell Division Cycle' (CDC) Mutants

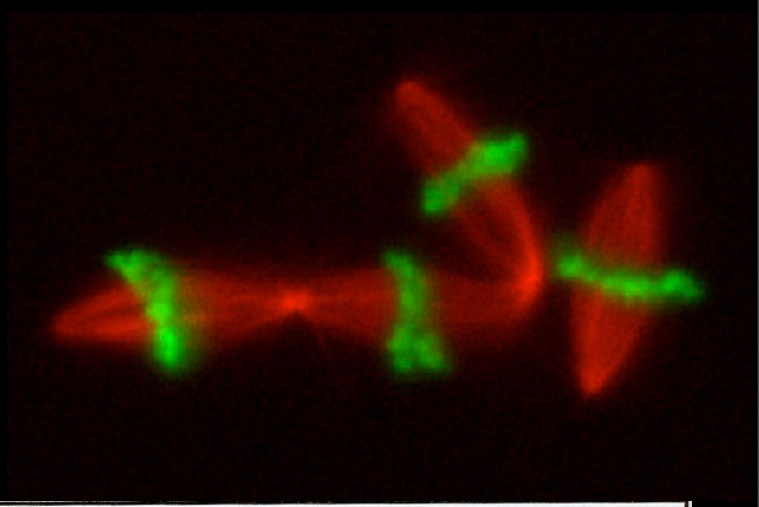


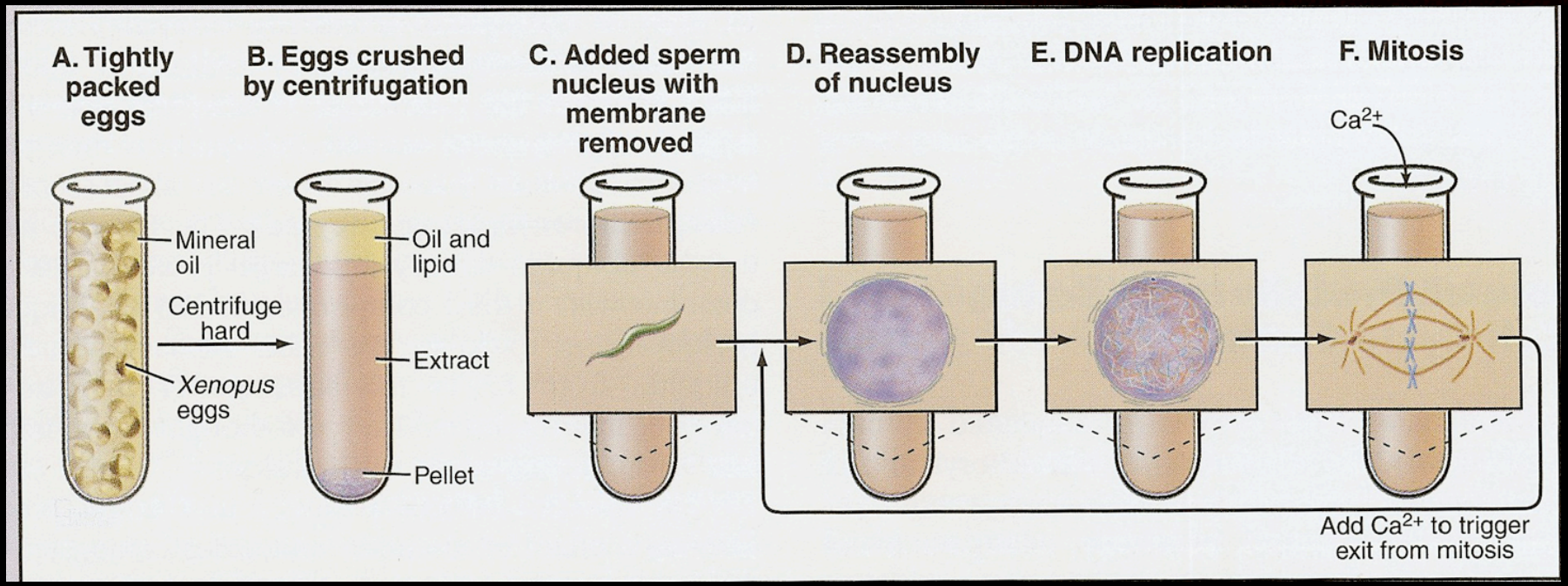


- Lee Hartwell (cerevisiae); Paul Nurse (pombe)
- Goal: find mutants unable to transit the cell cycle
- Why yeast?
  - Cell shape --> cell cycle stage
  - Grow as haploids (easier to find mutants), or diploids (can do genetics)
- Problem:
  - the screen is for cells that can't grow
- Solution:
  - temperature sensitive mutants
  - Replica plating

In vitro Dissection of the Cell Cycle - Xenopus Egg Extracts







use to isolate proteins present at particular stages
manipulate proteins-deplete and observe changes to cell cycle

Lecture 2

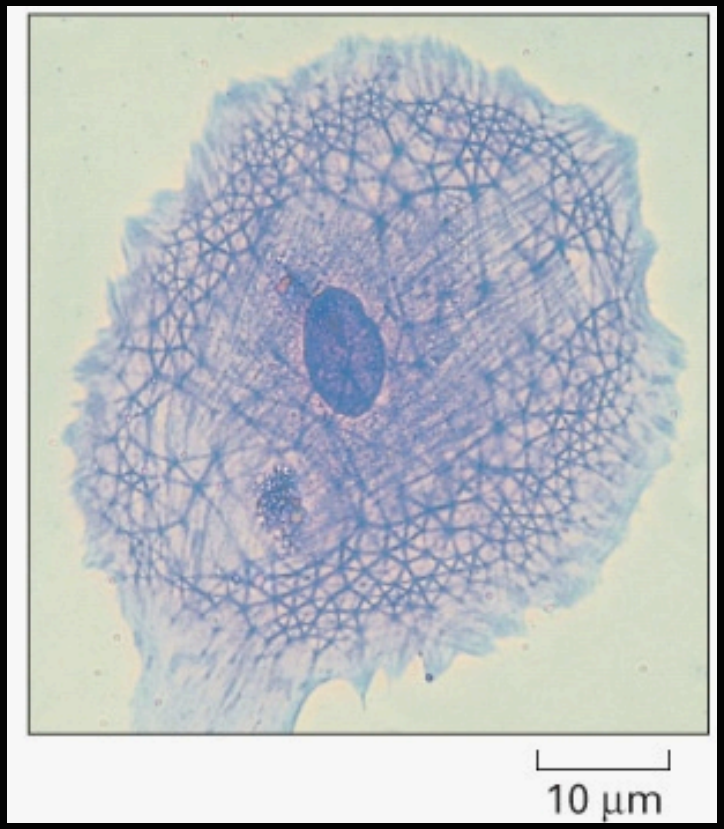
Introduction to the Cytoskeleton

Outline:

Composition of the cytoskeleton

Polymer Dynamics in theory

Polymer Dynamics in cells



Paper: Identification of pathways regulating cell size and cell-cycle progression by RNAi

Paul Nurse "Controlling the Cell Cycle" !!!
Thu 4 PM, 100 GPBB

Roles of the Cytoskeleton

Structural scaffold - cell shape, spatial organization

Dynamic assemblies - movement and force production:

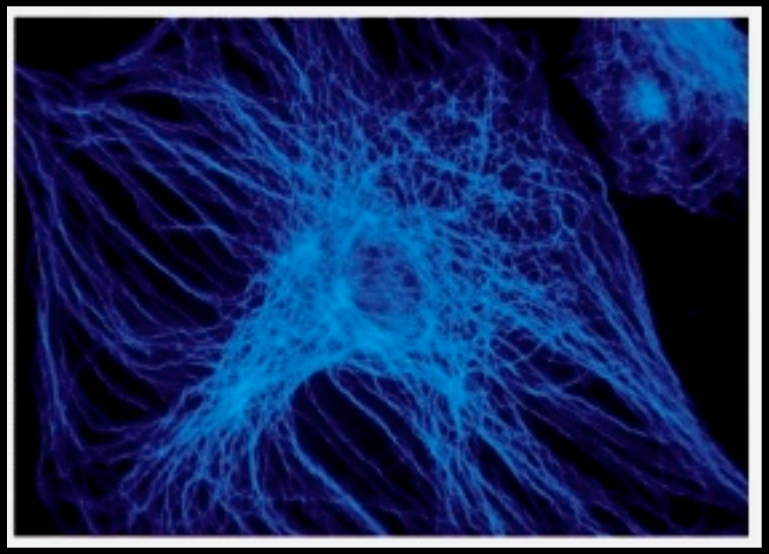
- cell migration
- cell division
- intracellular traffic
- contraction

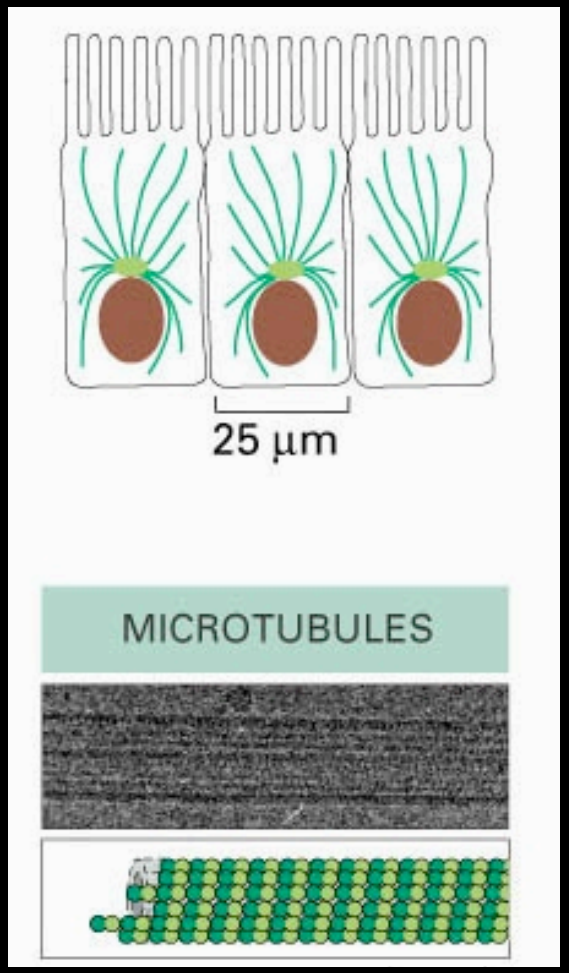
cytoskeletal functions often involve motor proteins

3 major elements of the cytoskeleton

microtubules

- α/β tubulin dimers
- 25 nm diameter
- relatively stiff –
  hollow, 13 protofilaments

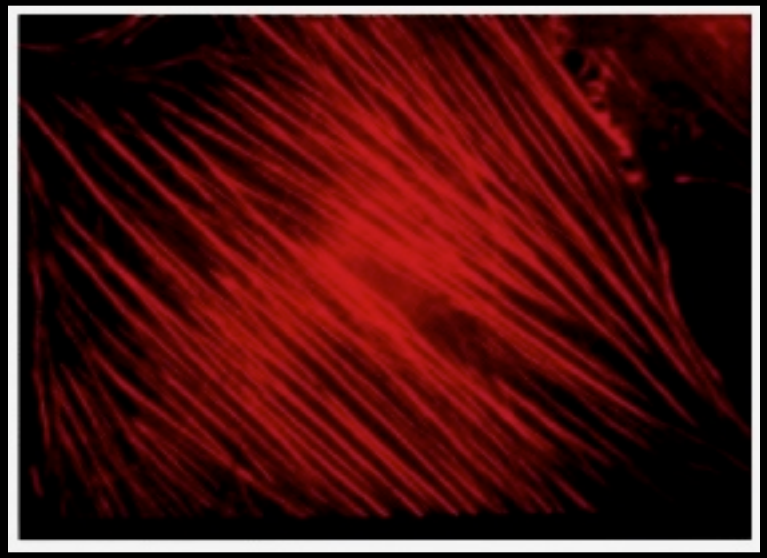


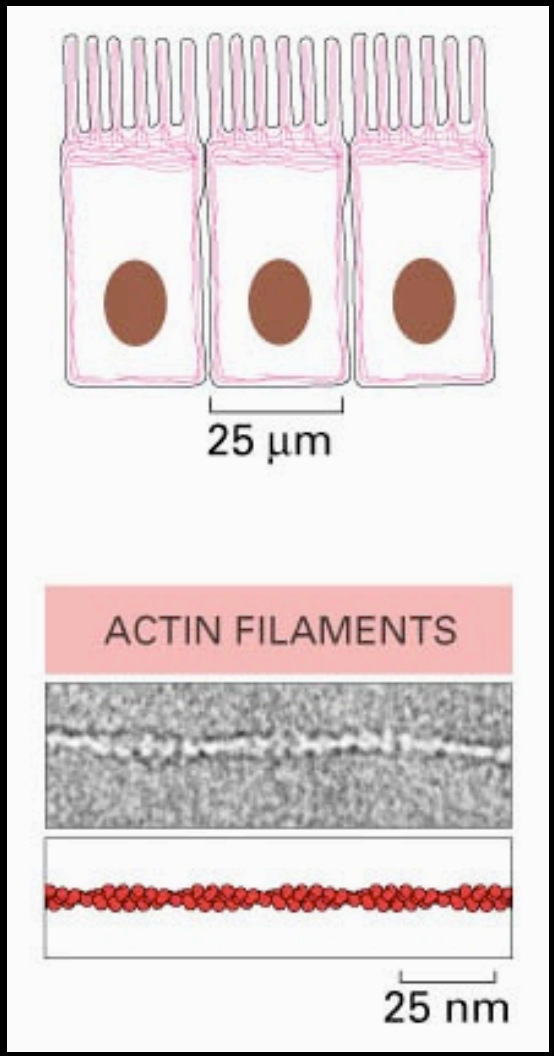


3 major elements of the cytoskeleton

microfilaments = actin filaments

- actin monomers
- 7 nm diameter
- more flexible – 2 helicies





3 major elements of the cytoskeleton

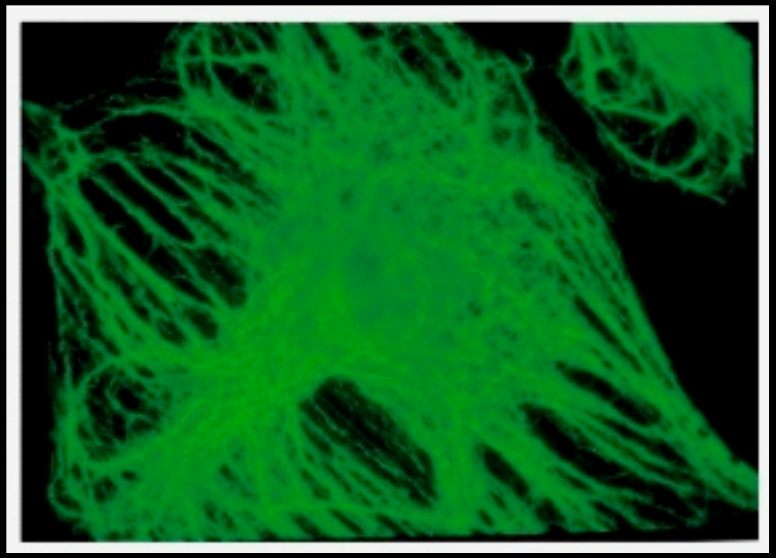
intermediate filaments

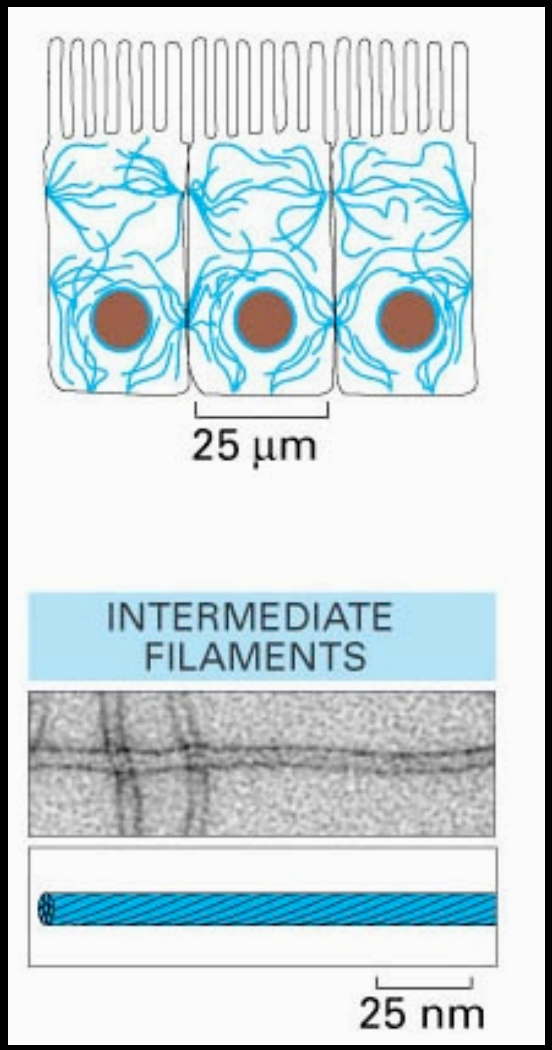
10 nm diameter

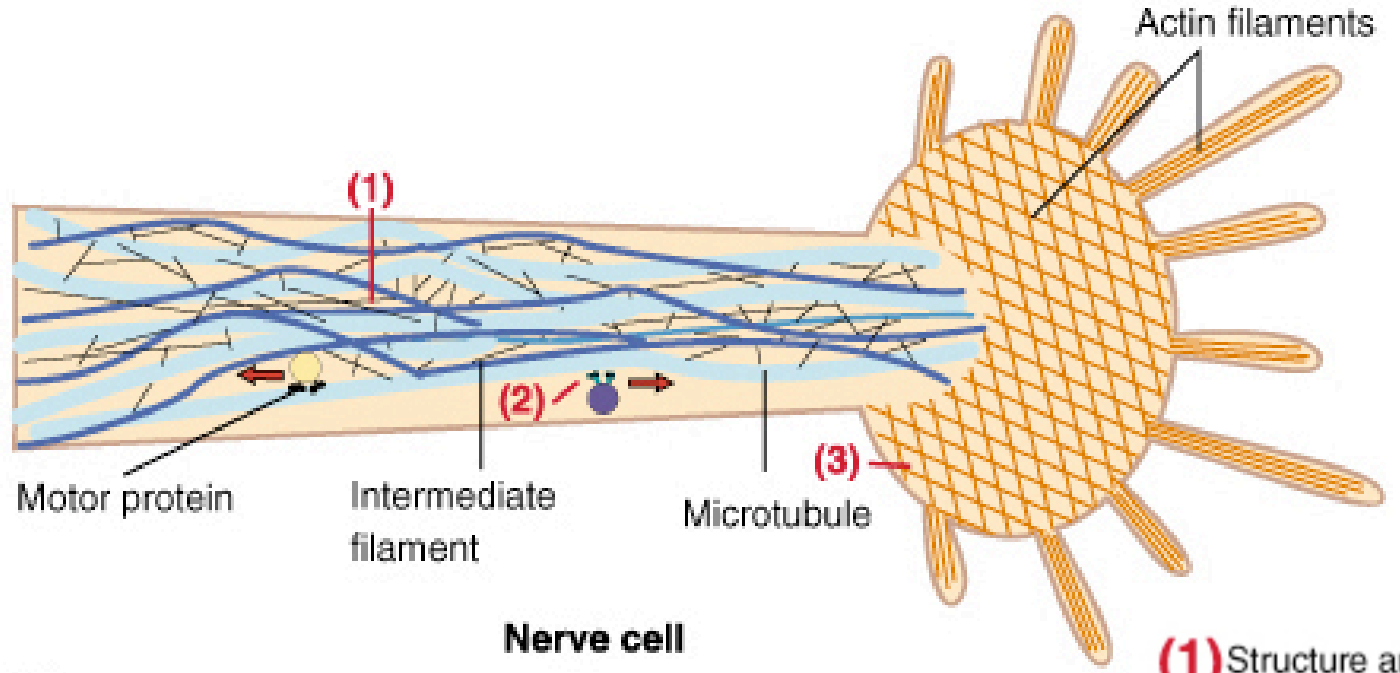
fibrous – resistant to shear forces

structural – prominent in cells subject to mechanical stress

vimentin, nuclear lamins







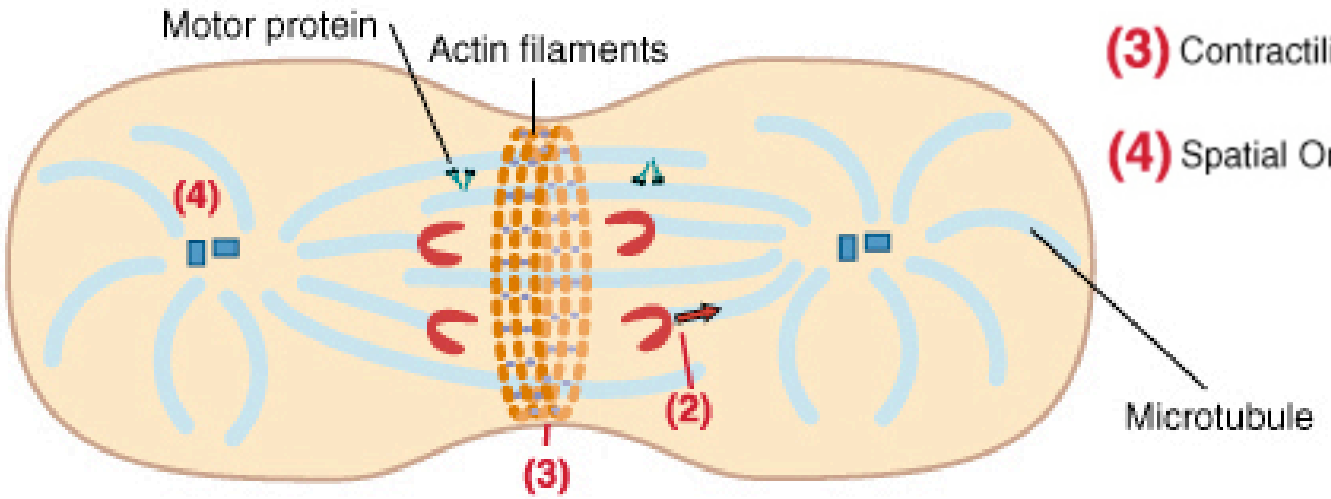
(b)

(1) Structure and Support

(2) Intracellular Transport

(3) Contractility and Motility

(4) Spatial Organization



(c)

Introduction to polymer dynamics: 3 cases

1) simple equilibrium polymers

2) polar polymers: asymmetric subunits undergo conformational change during assembly

actin and microtubules

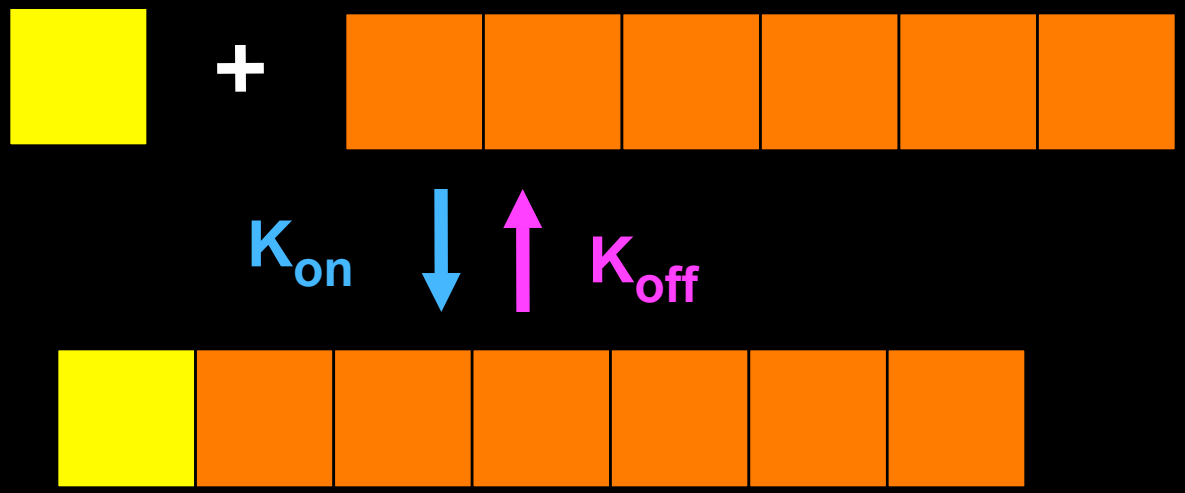
3) complex polymers: non-equilibrium

subunit nucleotide hydrolysis (energy input)

Simple Equilibrium Polymer

assembles & disassembles by addition & loss of subunits at ends

rates = K_{on} and K_{off}

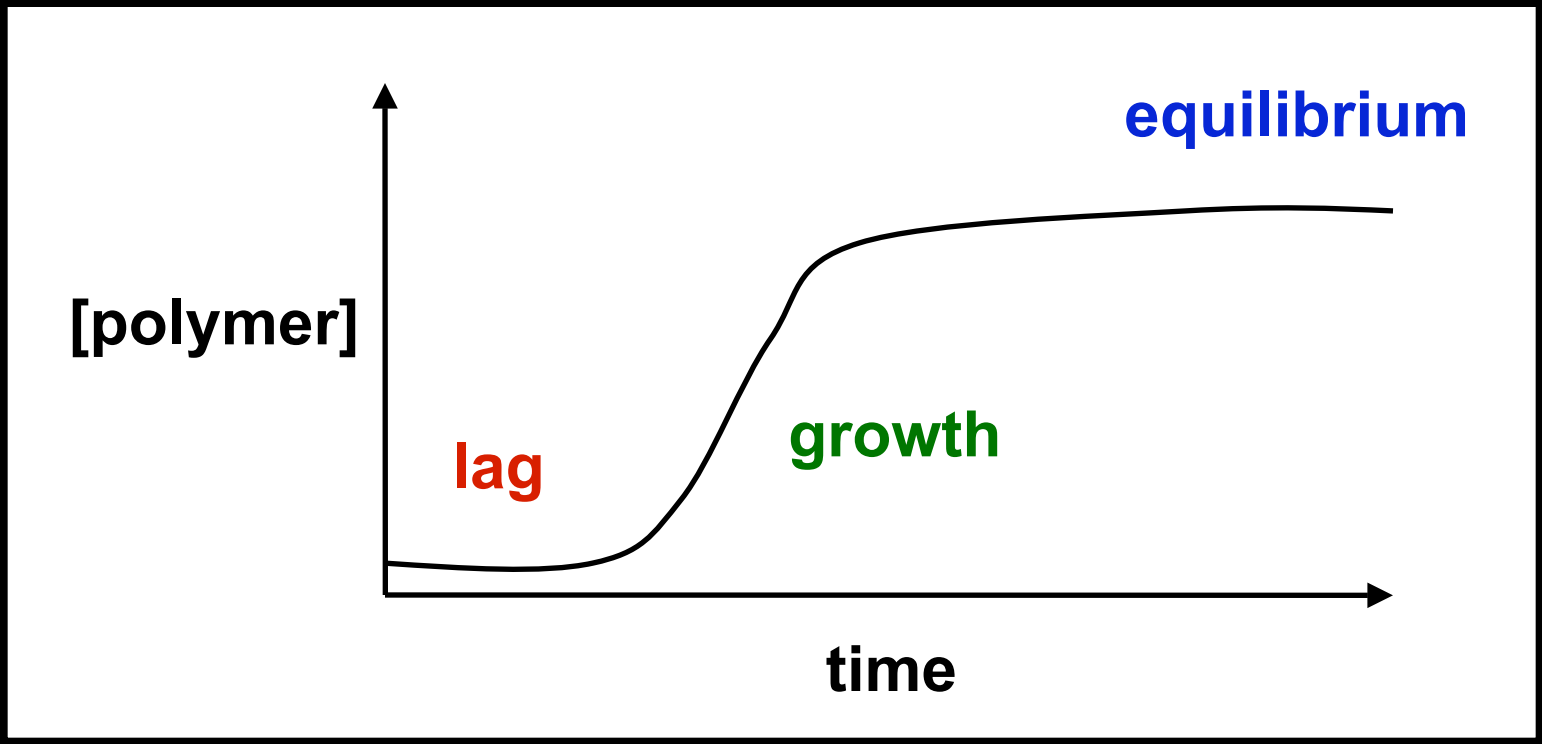


K_{on} depends on concentration of subunit, units of $M^{-1}sec^{-1}$

K_{off} does not (unimolecular), units of sec^{-1}

Polymer assembly timecourse

1) lag due to kinetic barrier to nucleation
2) growth
3) equilibrium

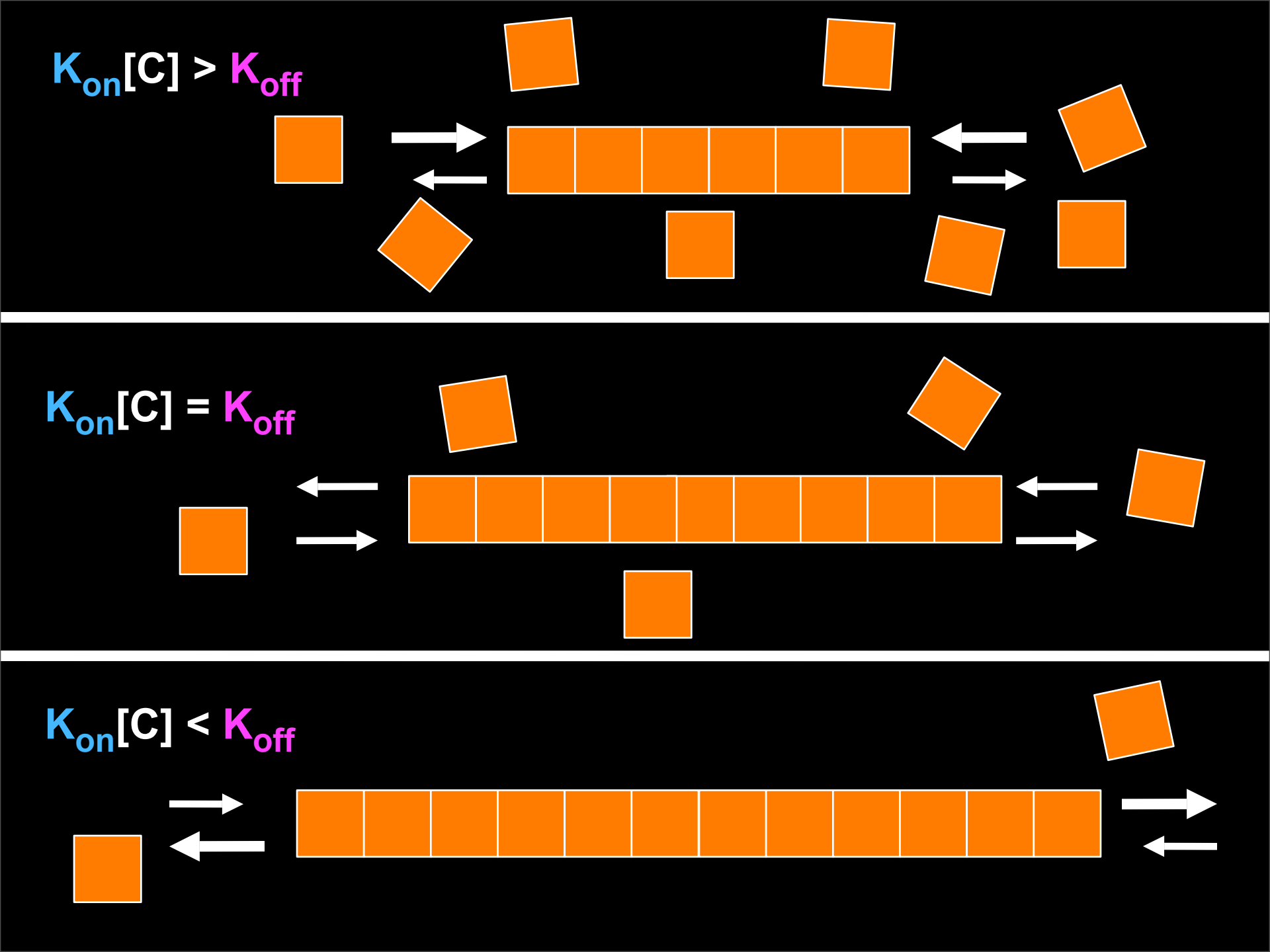


rate of subunit addition = rate of loss

polymer grows as time proceeds

subunit concentration drops until $K_{on}[C] = K_{off}$

[C] = critical concentration Cc ($M^{-1}sec^{-1}[M] = sec^{-1}$)


$K_{on}[C] > K_{off}$
$K_{on}[C] = K_{off}$
$K_{on}[C] < K_{off}$

Critical Concentration

Concentration of free subunits at which rate of subunit addition = rate of loss

Above Cc net growth, below net shrinkage

Equilibrium constant K_{eq} determined by change in free energy between free subunits and polymer

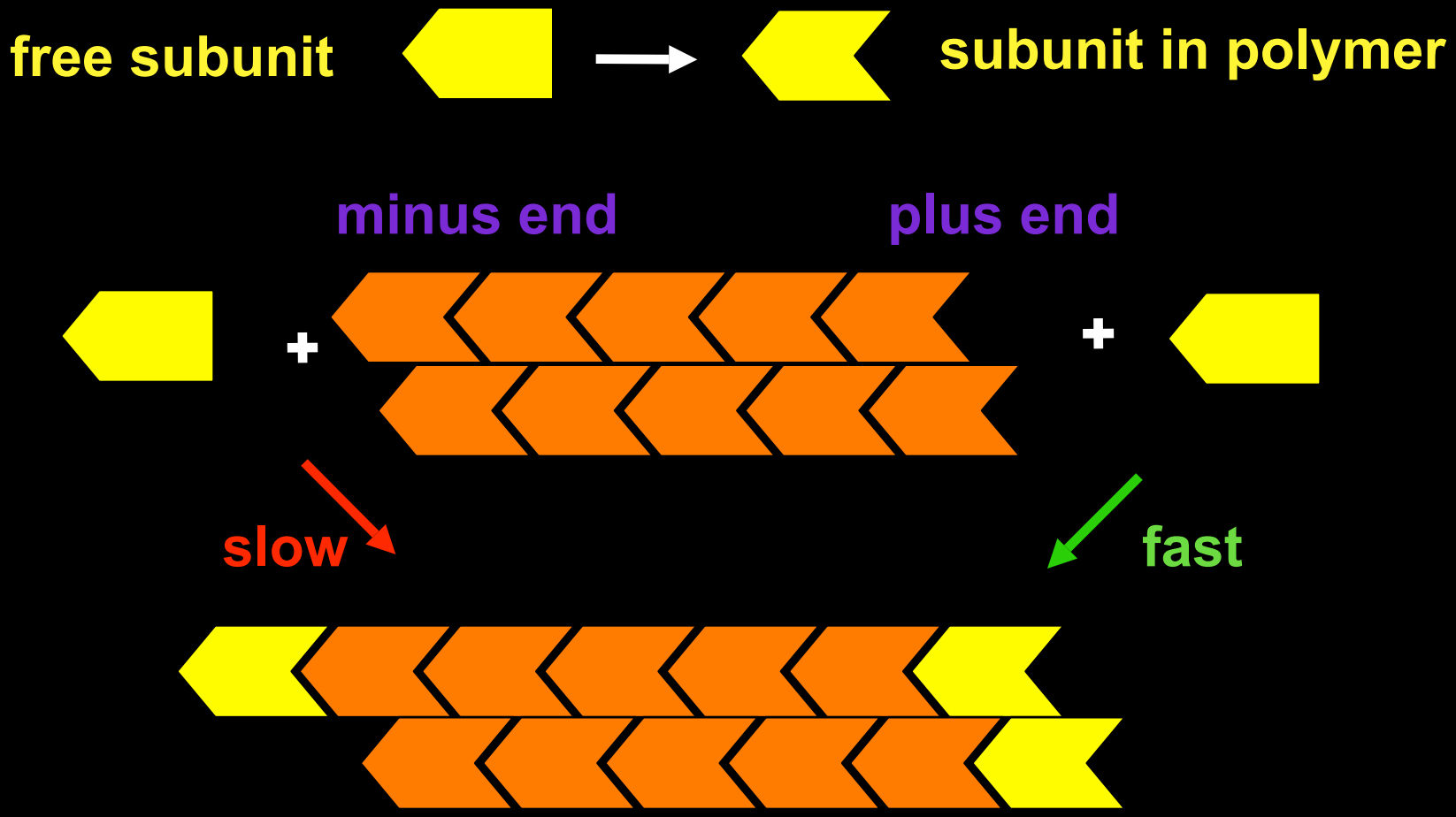
$K_{eq} = K_{on}/K_{off} = 1/Cc$

Polar Polymer

Two ends polymerize and depolymerize at different rates

BECAUSE

subunit conformation changes as it incorporates into the polymer



Plus and minus ends

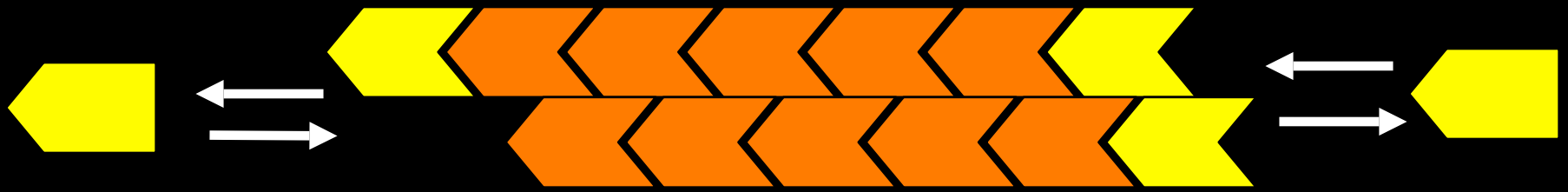
different K_{on} and K_{off}

BUT !

K_{off} / K_{on} ratio or Cc must be the same for both ends:

> the same interactions are broken when a subunit dissociates from either end

> the final state of the subunit is identical



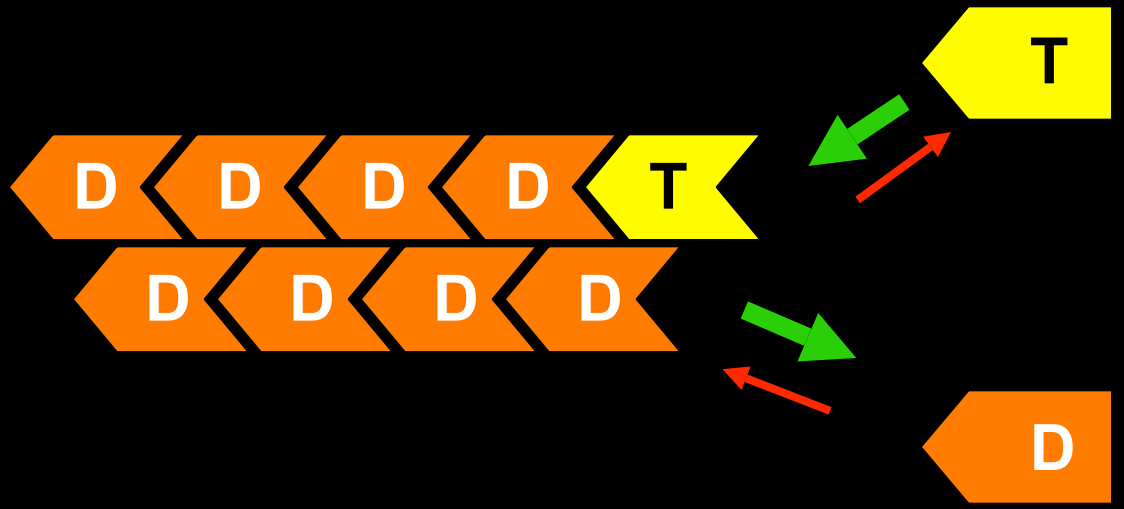
If the plus end grows 3 times faster
it must also shrink 3 times faster.
above Cc both ends grow, below Cc, both shrink

Complex Polymer (non-equilibrium): microtubules, actin filaments

due to nucleotide hydrolysis upon assembly of subunit into polymer

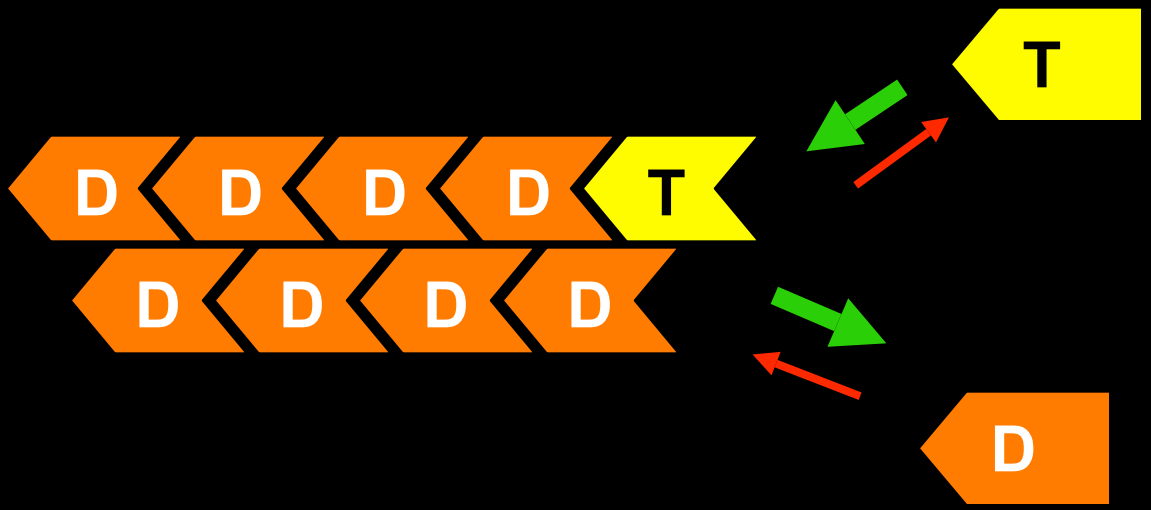
D= nucleotide diphosphate

T= nucleotide triphosphate



nucleotide hydrolysis reduces binding affinity

Complex Polymer properties



internal subunits have different dynamic properties than the ends

T form binds, D form dissociates

$$K_{Ton} >> K_{Don} \quad K_{Doff} >> K_{Toff}$$

Css = "steady state" concentration

$$K_{Ton}[C] = K_{Doff}$$

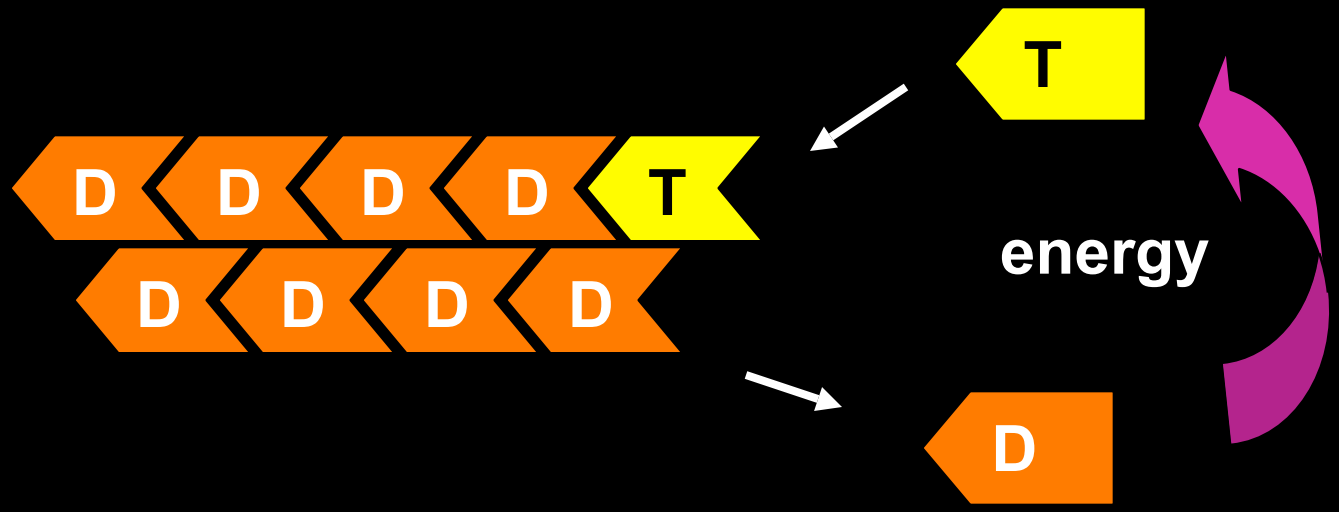
$$Css = K_{Doff} / K_{Ton}$$

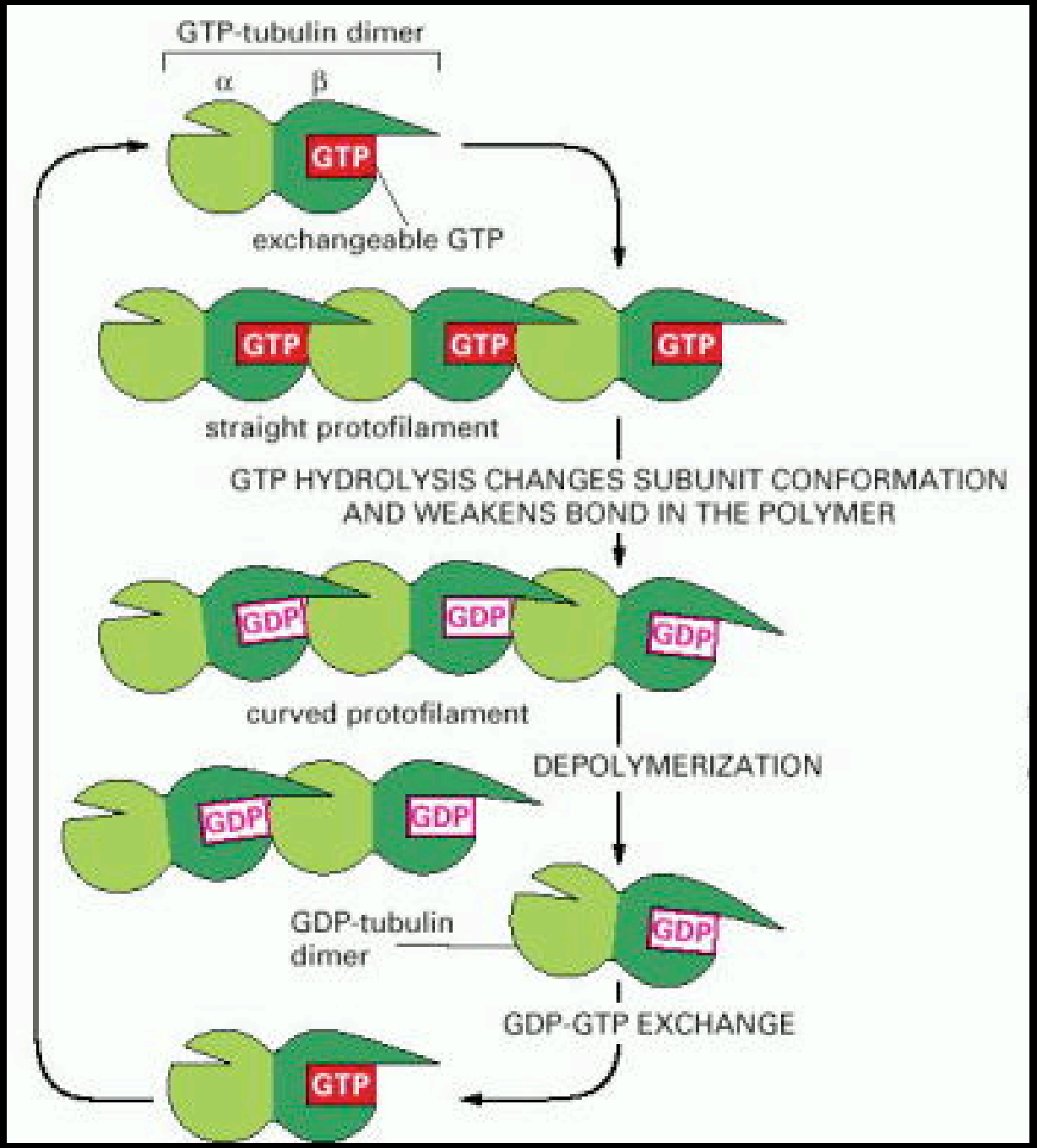
Steady State Dynamics

no longer true equilibrium, rather steady state
because
ATP or GTP subunits must be replenished

D=diphosphate

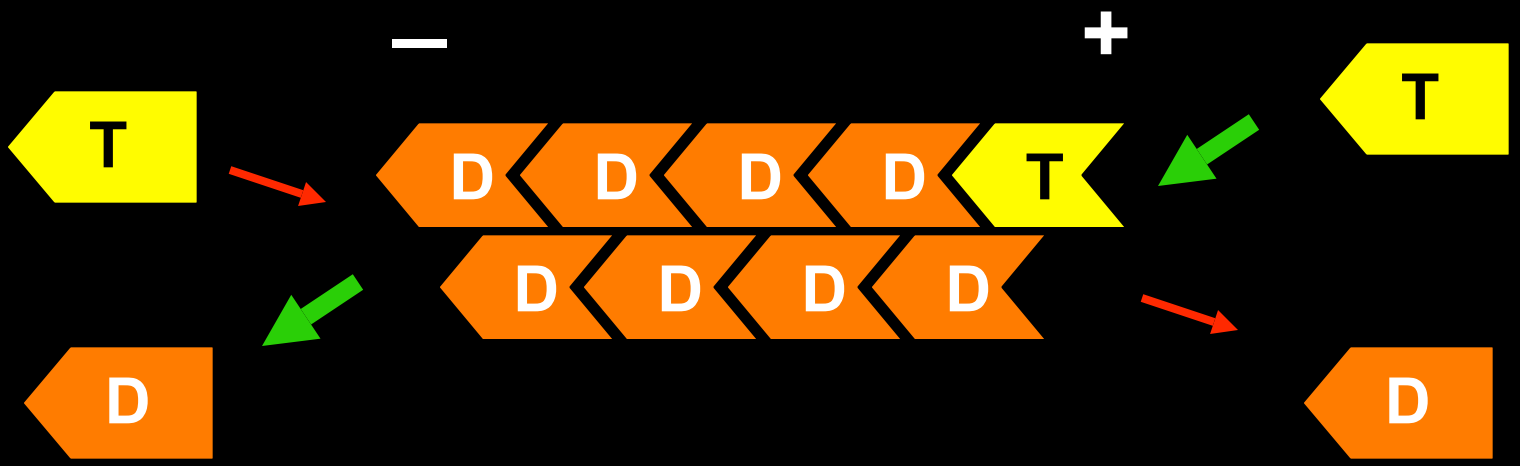
T=triphosphate




GTP-tubulin dimer
α
β
GTP
exchangeable GTP
GTP
GTP
GTP
straight protofilament
GTP HYDROLYSIS CHANGES SUBUNIT CONFORMATION
AND WEAKENS BOND IN THE POLYMER
GDP
GDP
GDP
curved protofilament
DEPOLYMERIZATION
GDP
GDP
GDP-tubulin
dimer
GDP
GDP-GTP EXCHANGE
GTP

Consequences for polymer dynamics

treadmilling (actin and microtubules)



two different reactions at each end of the polymer

critical concentration different

Cc(- end) > Cc(+ end)

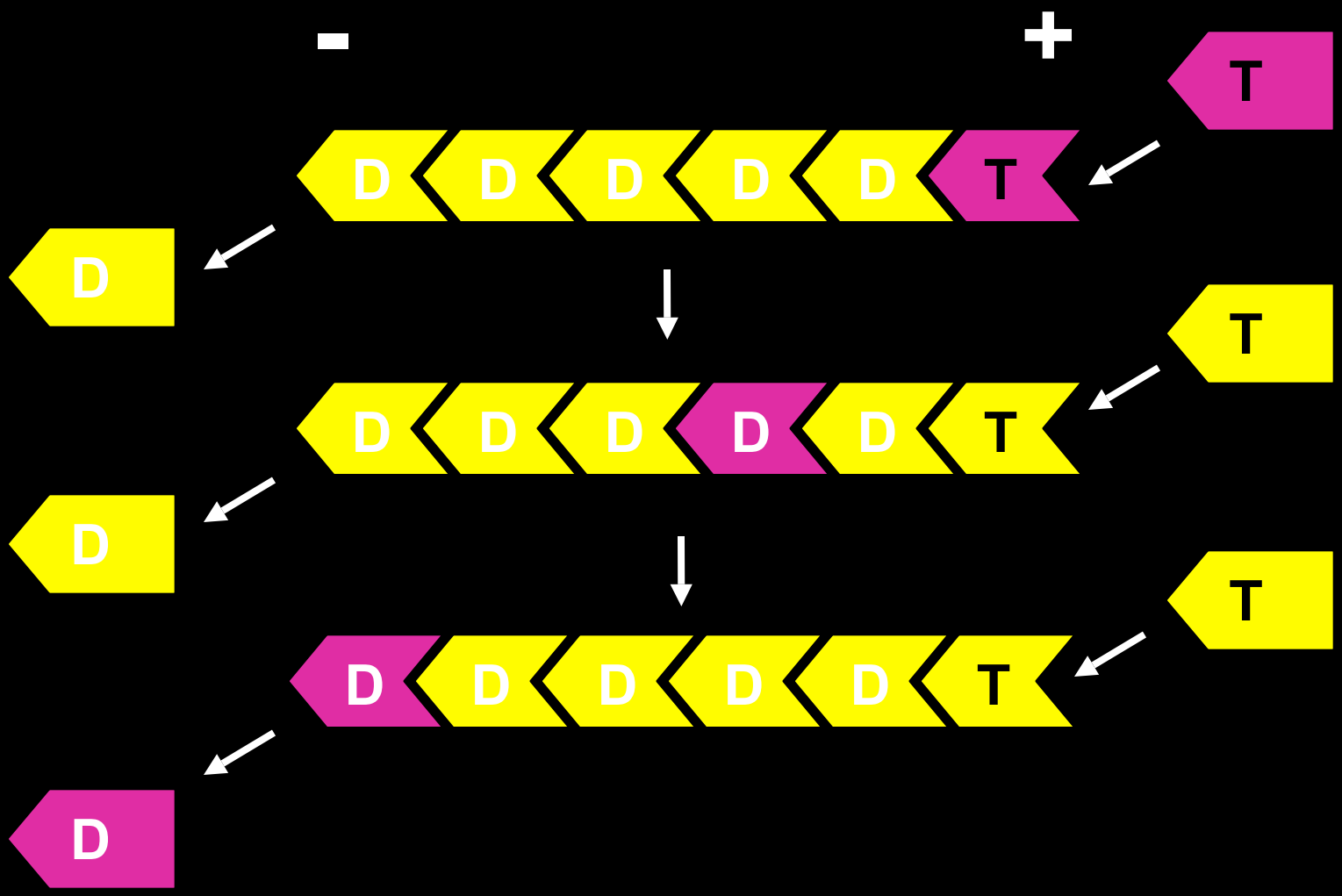
Treadmilling

both ends exposed
Steady state occurs at concentration between
Cc(- end) and Cc(+ end)

net assembly at the plus end
net disassembly at the minus end

subunits “flux” through the polymer

Treadmilling



Dynamic instability (microtubules)

subunit addition is faster than nucleotide hydrolysis

CAP of GTP-tubulin on polymer ends

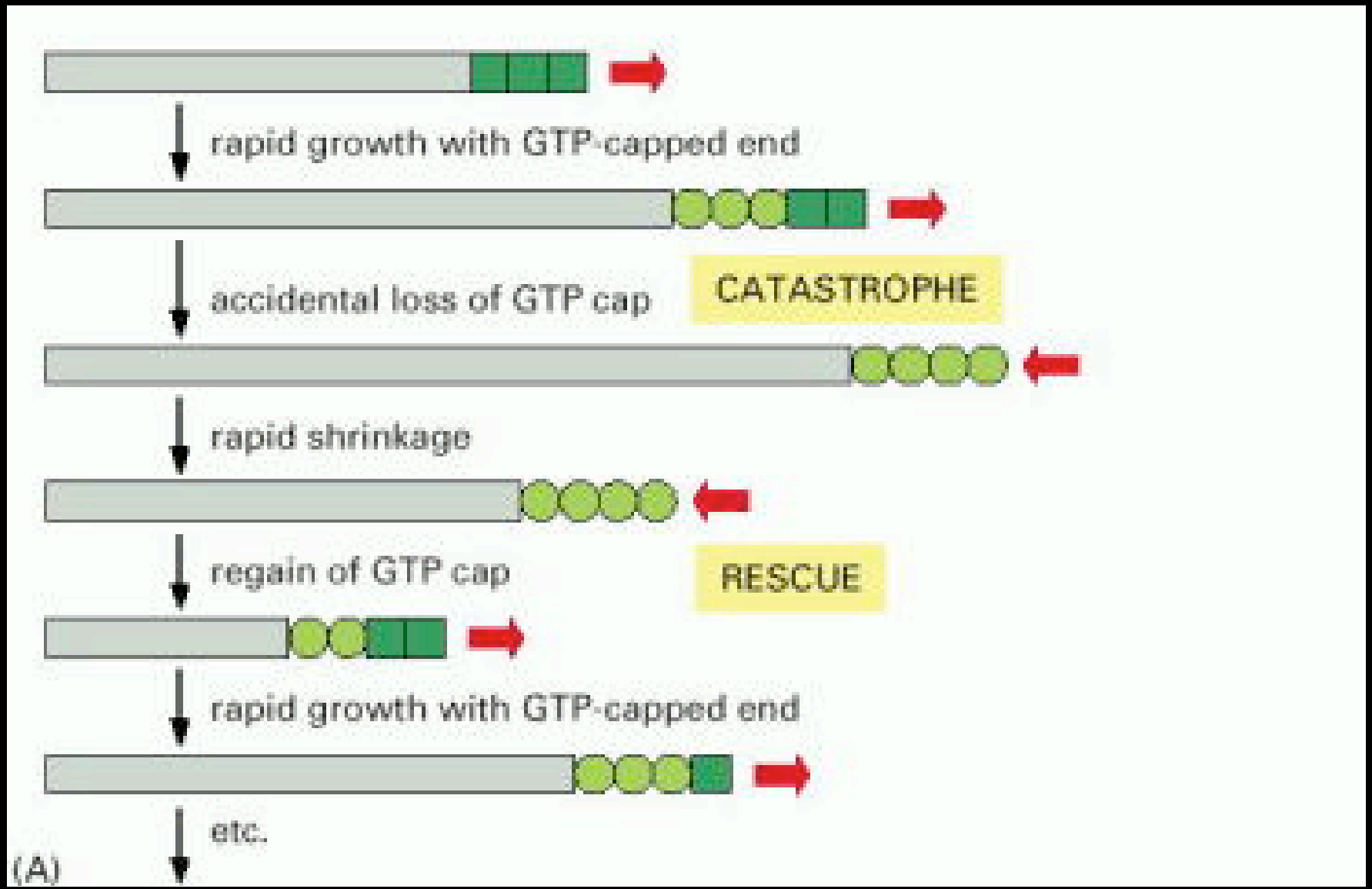
$K_{Doff} >> K_{Toff}$: GTP CAP favors growth

GTP CAP present: growth

GTP CAP lost: rapid disassembly

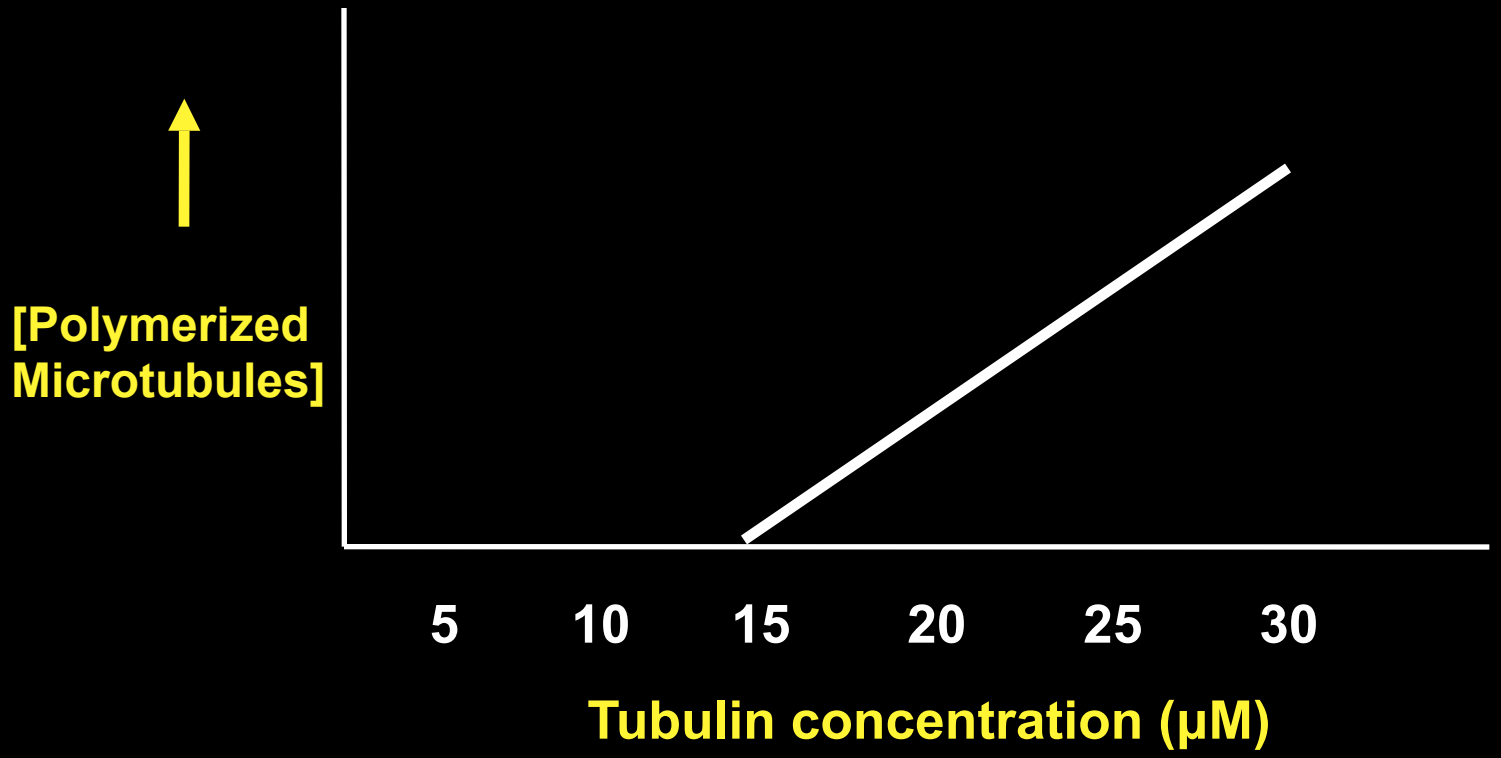
stochastic (unpredictable) transitions

frequency correlates with tubulin concentration


rapid growth with GTP-capped end
accidental loss of GTP cap
CATASTROPHE
rapid shrinkage
regain of GTP cap
RESCUE
rapid growth with GTP-capped end
etc.
(A)

Classic experiments by Mitchison and Kirschner 1984

microtubules nucleated from seeds - no lag



1) determine steady state concentration (Css) = 14 μM

2) dilution experiment:

grow microtubule seeds

dilute into tubulin solution above or below Css

wait 10 minutes

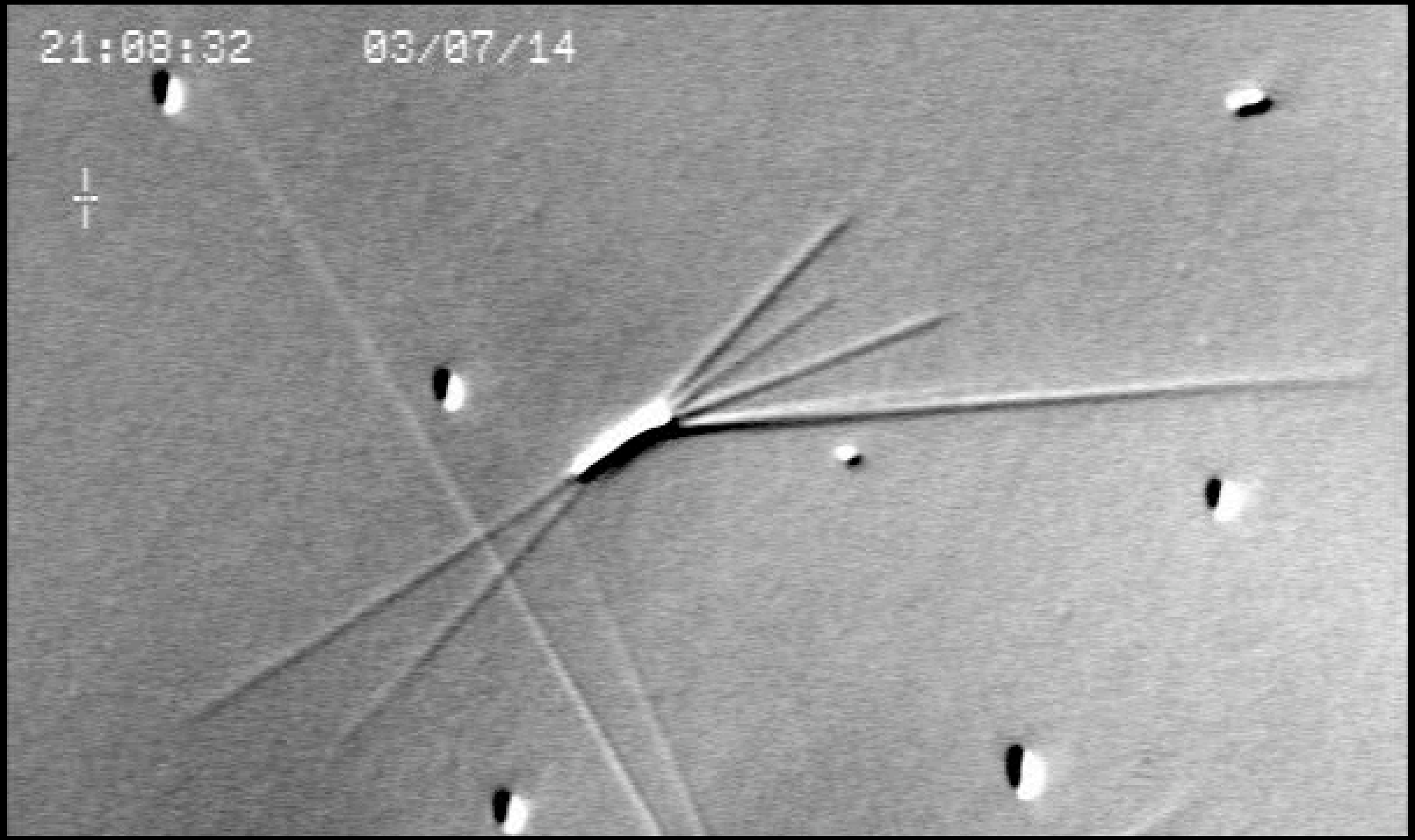
measure Mt number-concentration, Mt length

(spun onto EM grids)

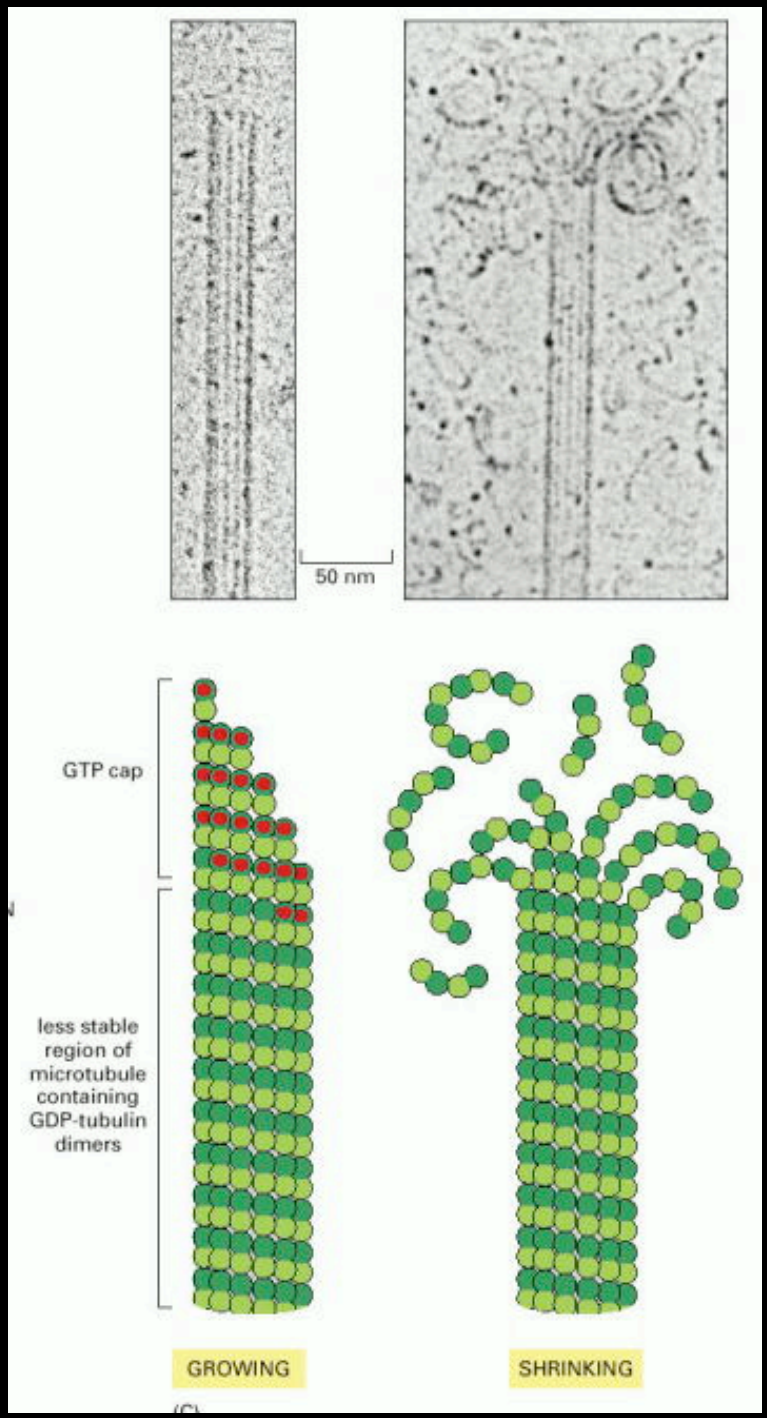
	#concentration:	x10^8/ml	
	before dilution	15 uM	7.5 uM
average length: (μM)	32	32	15
	18	40	22

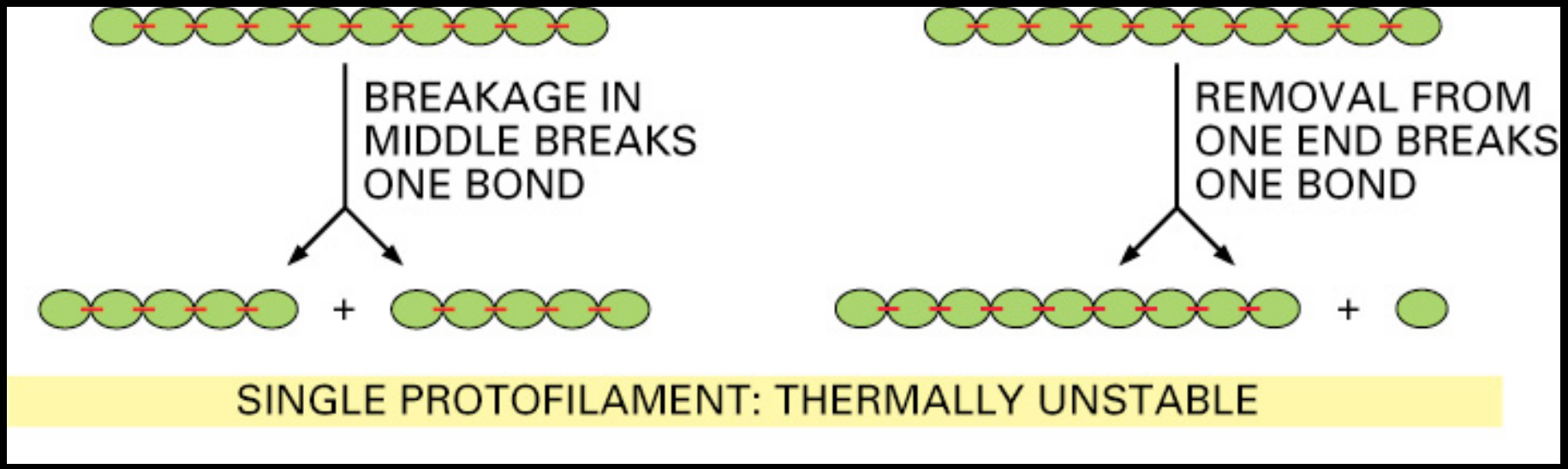
size is dependent on the concentration of tubulin

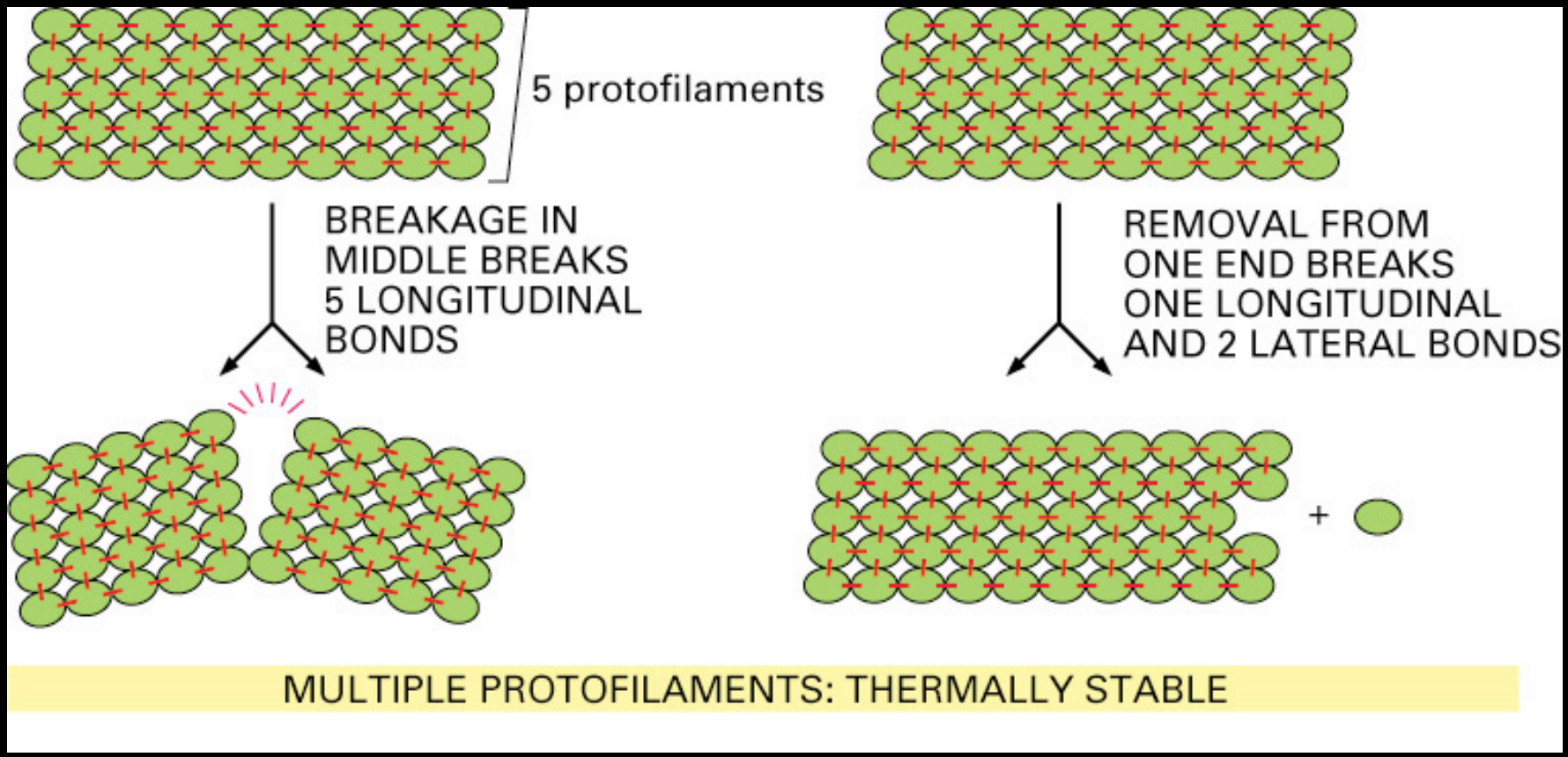
Dynamic instability in vitro



Microtubules are really....
tubes, not simple polymers




BREAKAGE IN
MIDDLE BREAKS
ONE BOND
REMOVAL FROM
ONE END BREAKS
ONE BOND
+
+
SINGLE PROTOFILAMENT: THERMALLY UNSTABLE


5 protofilaments
BREAKAGE IN
MIDDLE BREAKS
5 LONGITUDINAL
BONDS
REMOVAL FROM
ONE END BREAKS
ONE LONGITUDINAL
AND 2 LATERAL BONDS
+
MULTIPLE PROTOFILAMENTS: THERMALLY STABLE

Summary

simple equilibrium:

exchange only at the ends

turnover only with dramatic changes in subunit concentration

non-equilibrium:

Dynamic instability

Treadmilling

complete and rapid polymer turnover at steady state

energy required

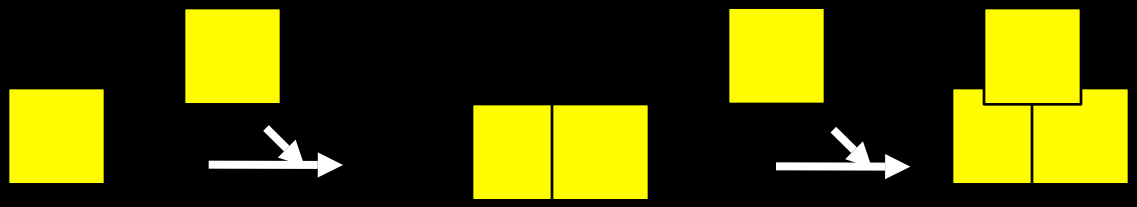
Polymer properties regulated in cells

1) nucleation
2) polarity
3) dynamics

1) Nucleation: kinetic barrier - slow step

trimer for actin

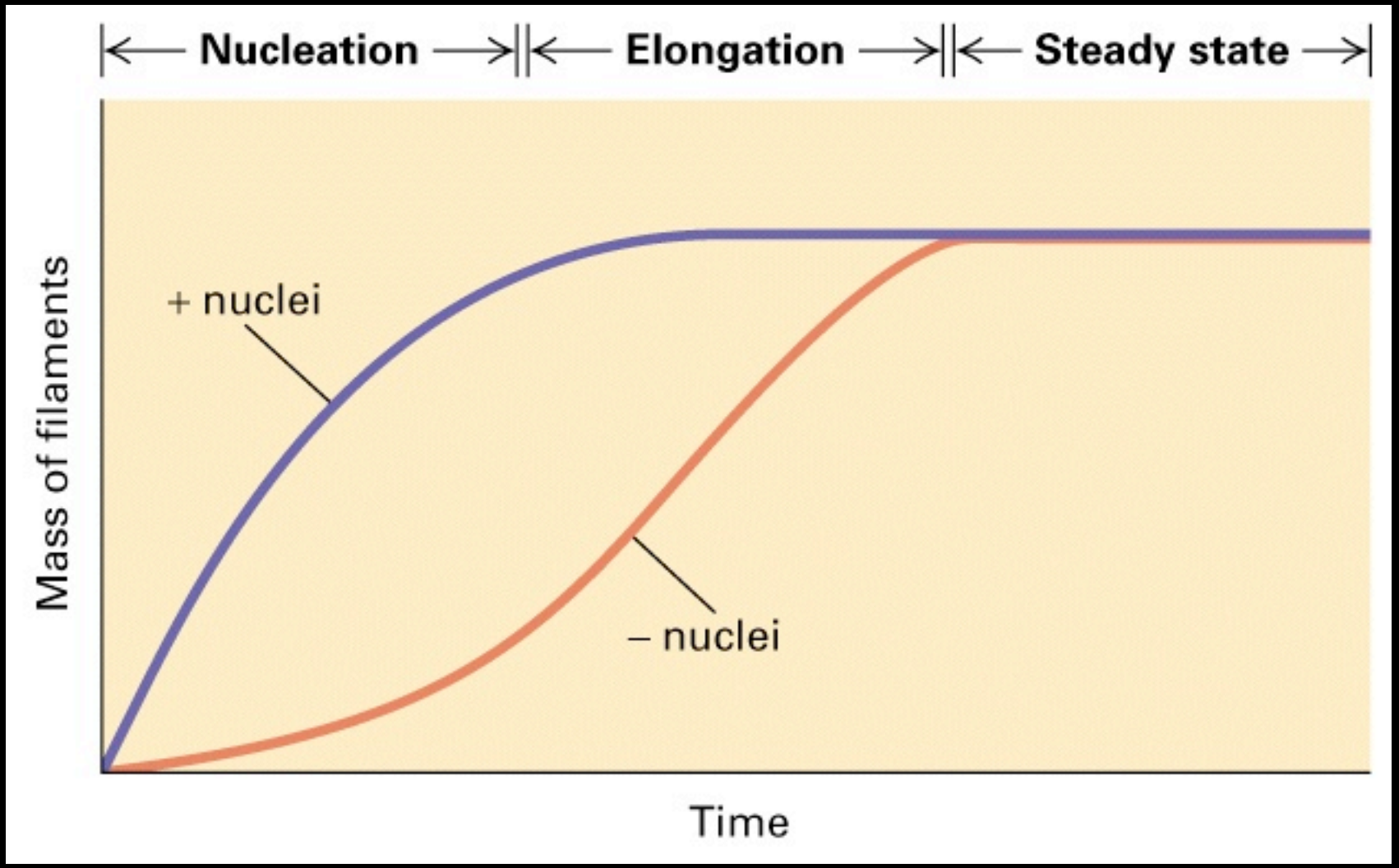
more complex
for microtobules



monomer

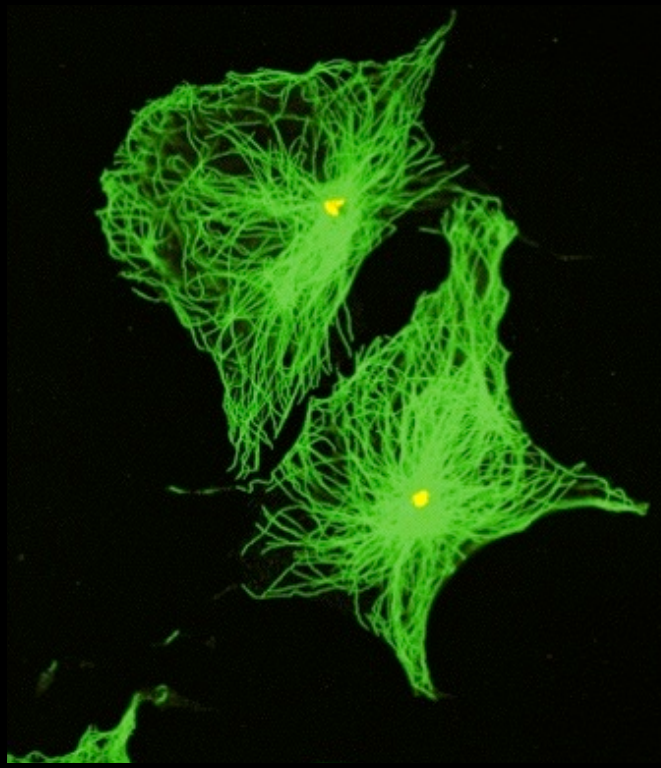
dimer

trimer =
nucleation site


Nucleation
Elongation
Steady state
Mass of filaments
+ nuclei
– nuclei
Time

Nucleating factors in cells

Microtubules: **centrosomes**



Actin: protein complexes (Arp2/3)

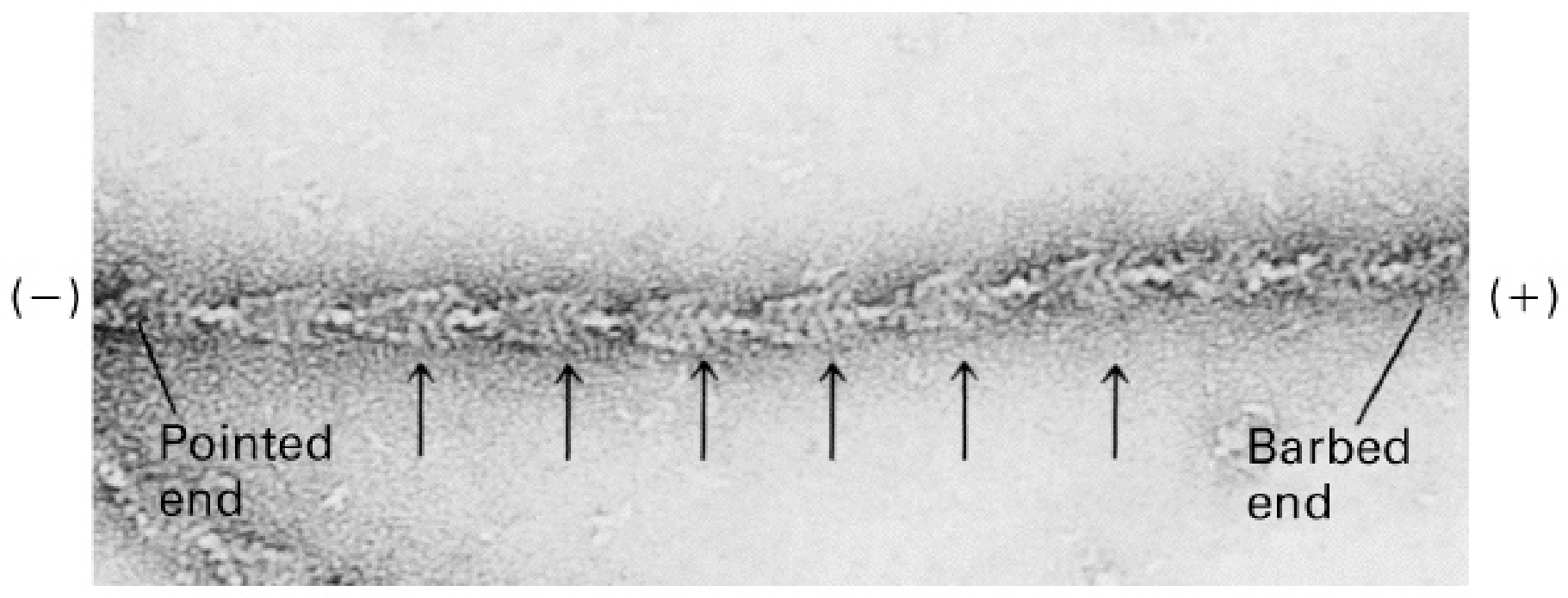
2) Polarity: due to asymmetry of subunits

structural polarity of polymer lattice

visualized by decoration of actin filaments and microtubules

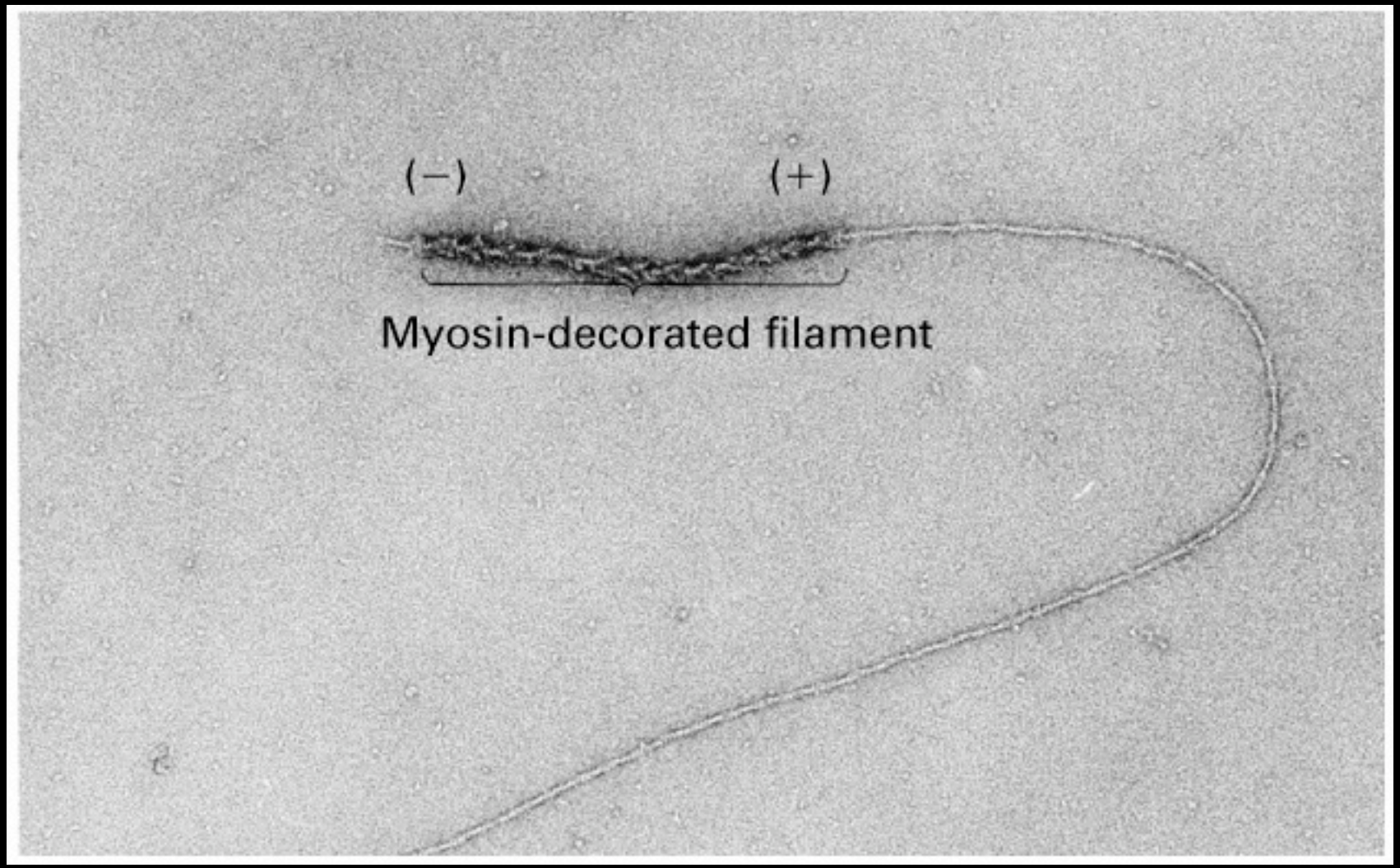
allows cell to generate asymmetric structures and shapes
basis of motility

Actin lattice polarity revealed



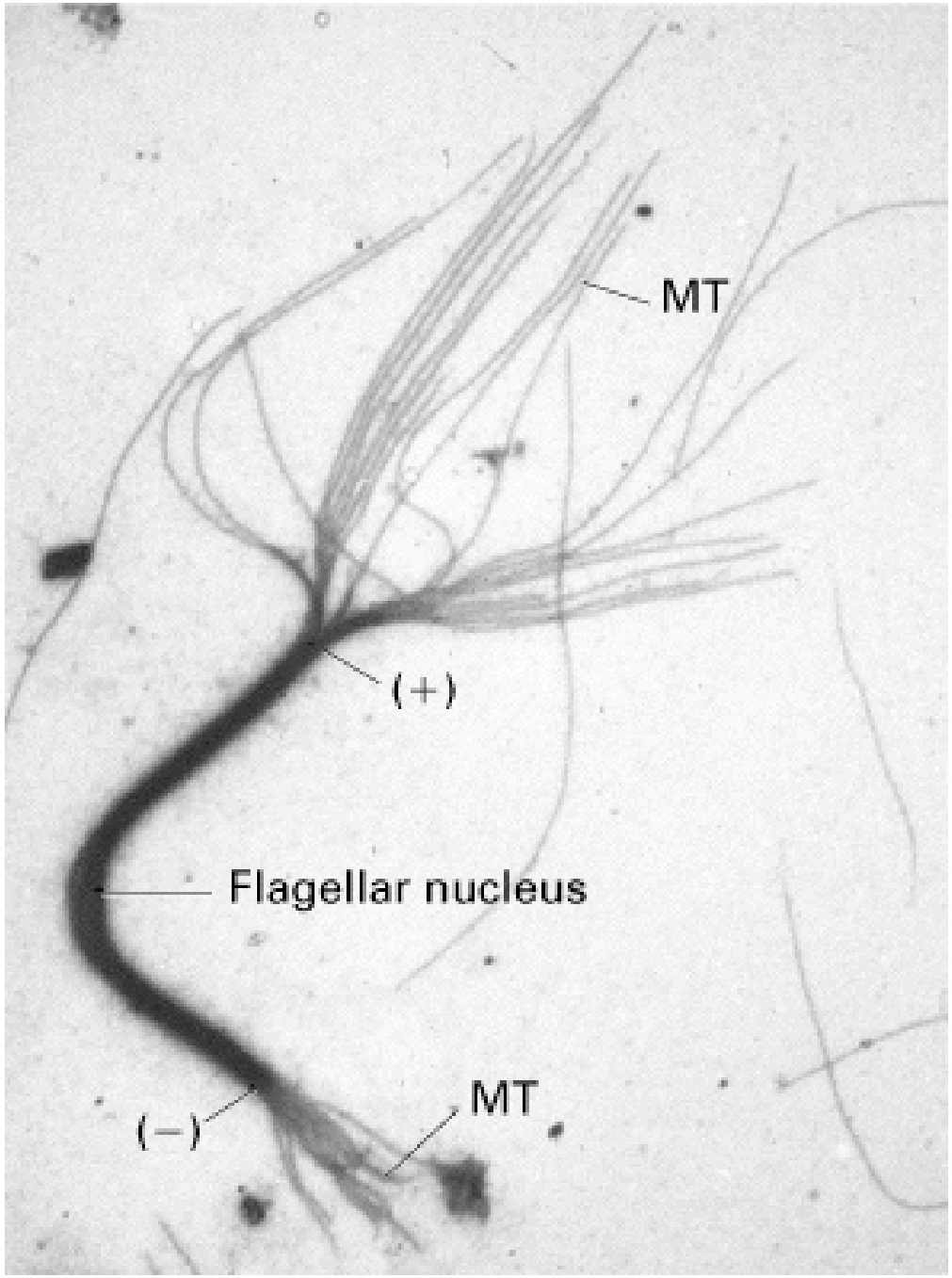
Actin decorated with myosin subfragment 1

actin structural and dynamic polarity revealed



Microtubule lattice polarity revealed by hook direction

Microtubules decorated and viewed in cross section



microtubule
dynamic polarity revealed

Motor proteins recognize polymer asymmetry

mechanochemical enzymes

- hydrolyze ATP to move along filament
- produce force or carry cargo

polarity generates directionality

more on motors in later lectures ...

3) dynamics

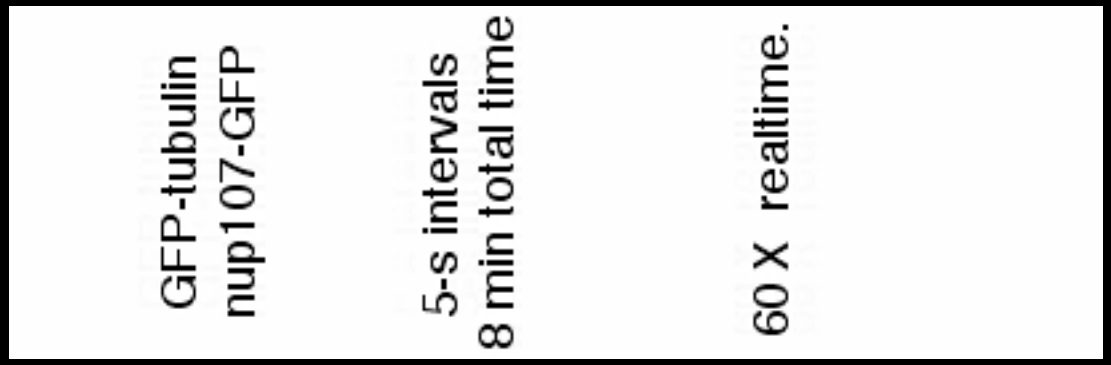
regulated by many cellular factors

end-capping
subunit sequestering
polymer binding proteins

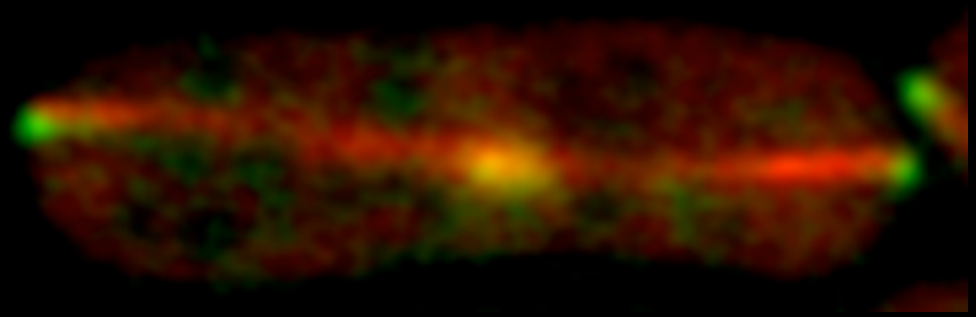
regulators also regulated

MT dynamics in *pombe*

role in nuclear positioning and deformation

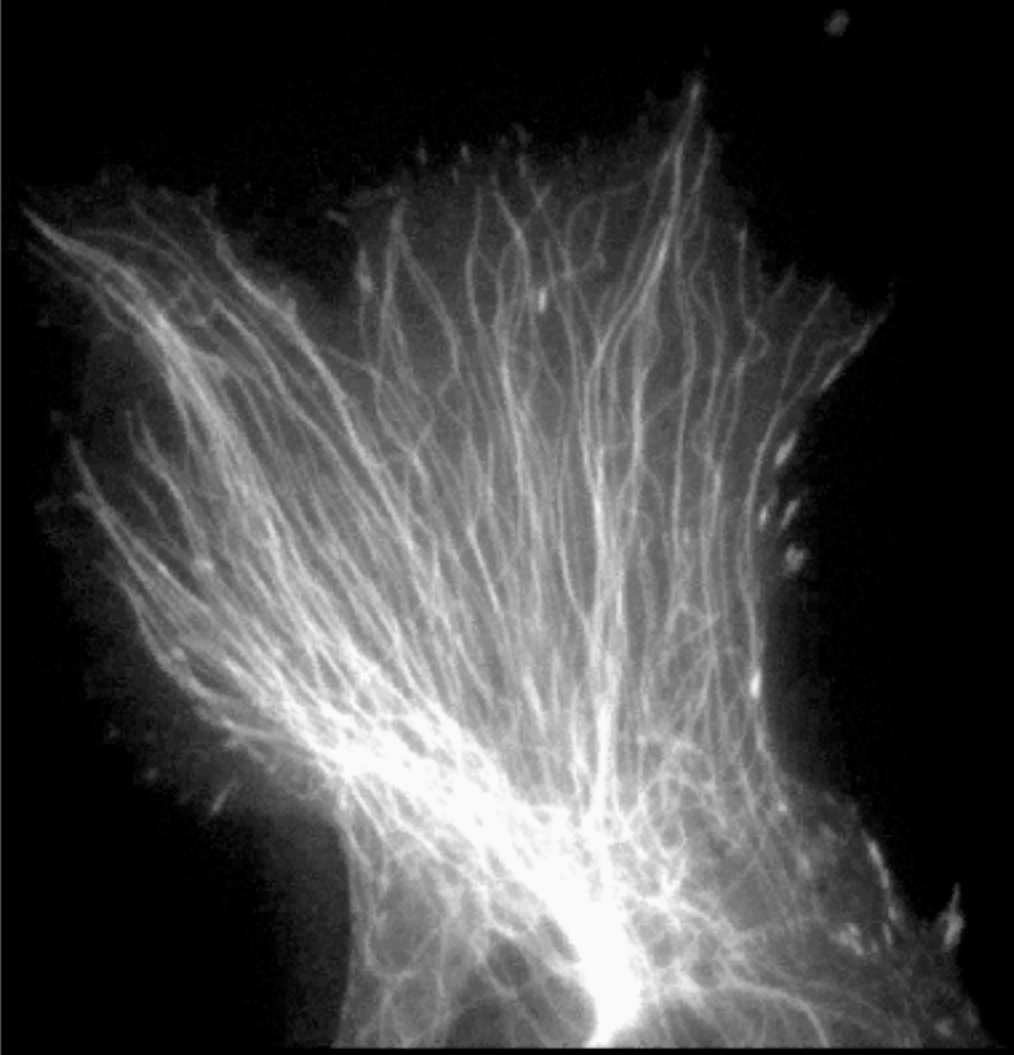


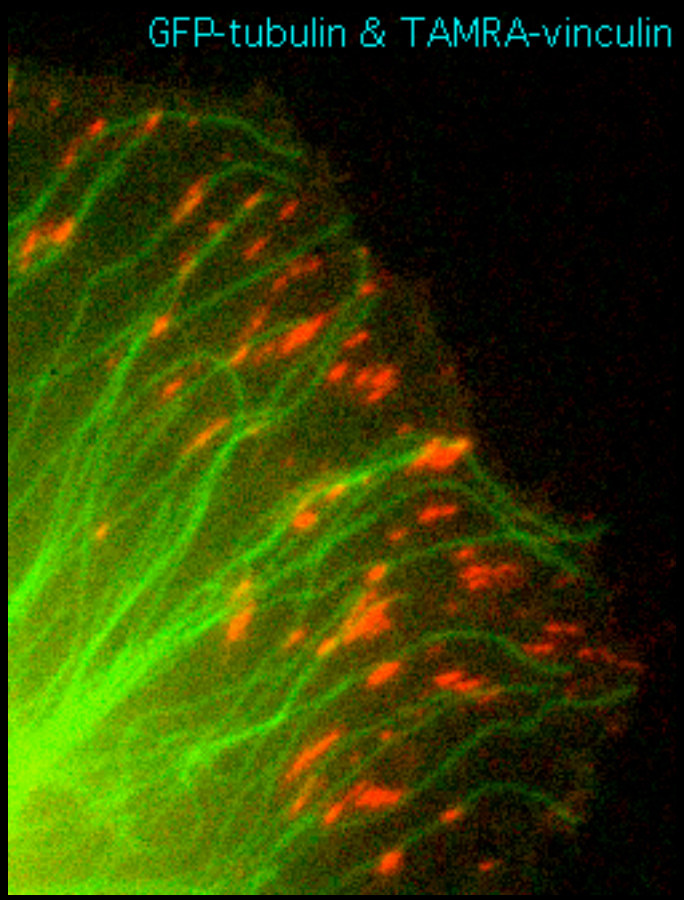
movement of kinesin motors on growing and shrinking MTs



MT dynamics in vertebrate cells

impact of destabilization of actin





Dynamic Instability