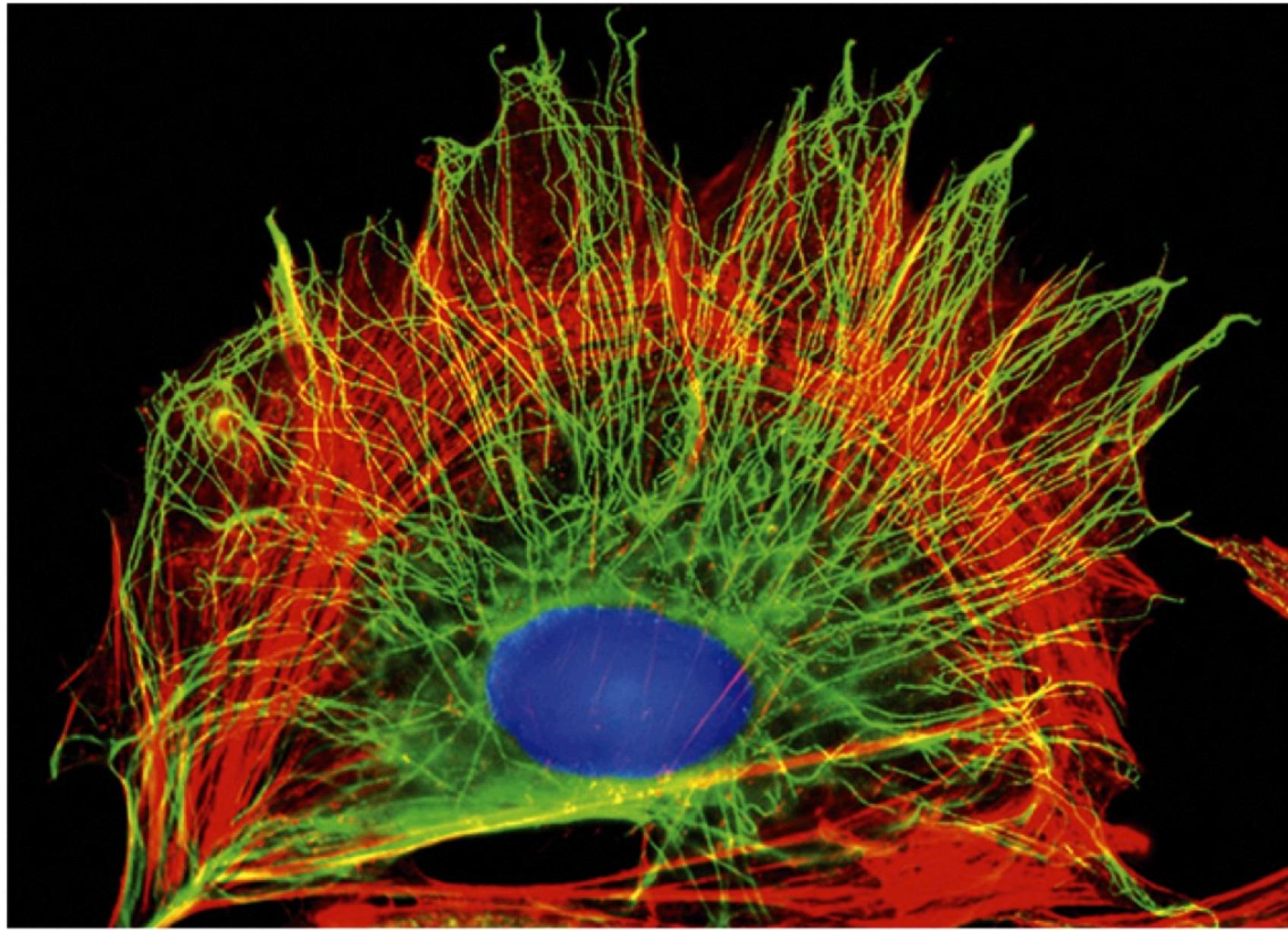
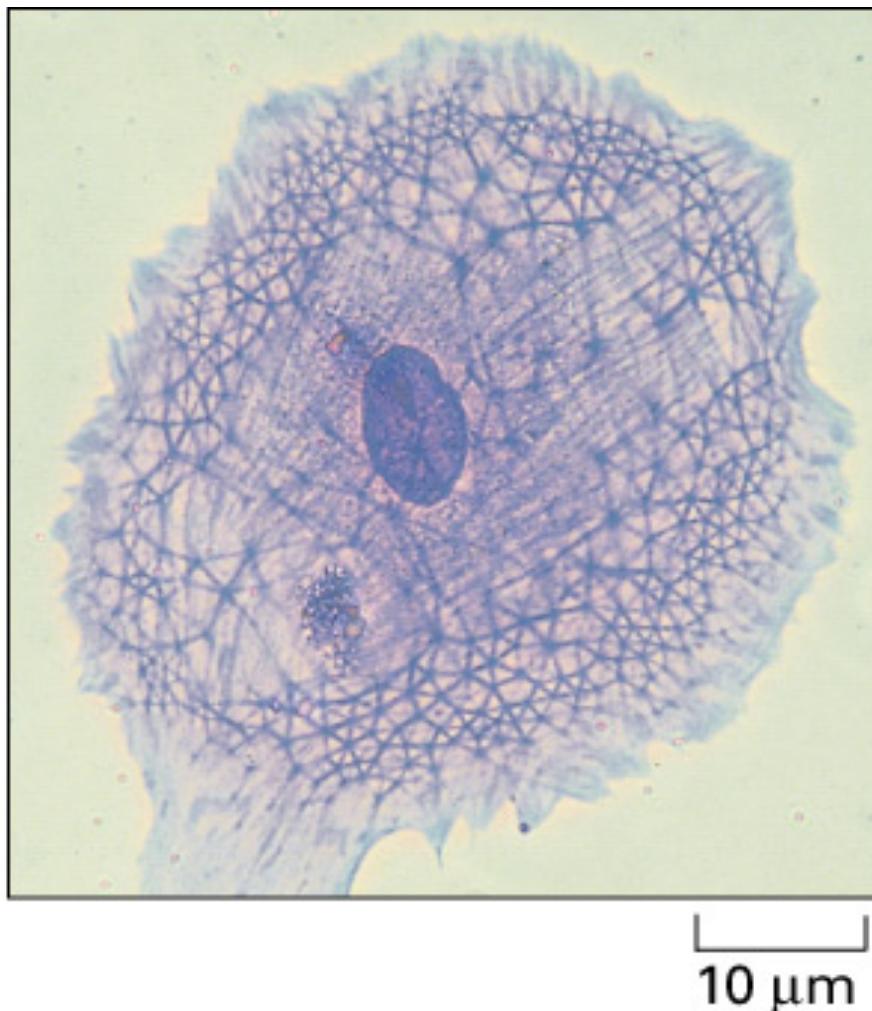


# Introduction to the Cytoskeleton



# The Cytoskeleton

Coomassie Blue stain of a cell: a general protein stain



**Actin filaments**

**Microtubule filaments**

**Intermediate filaments**

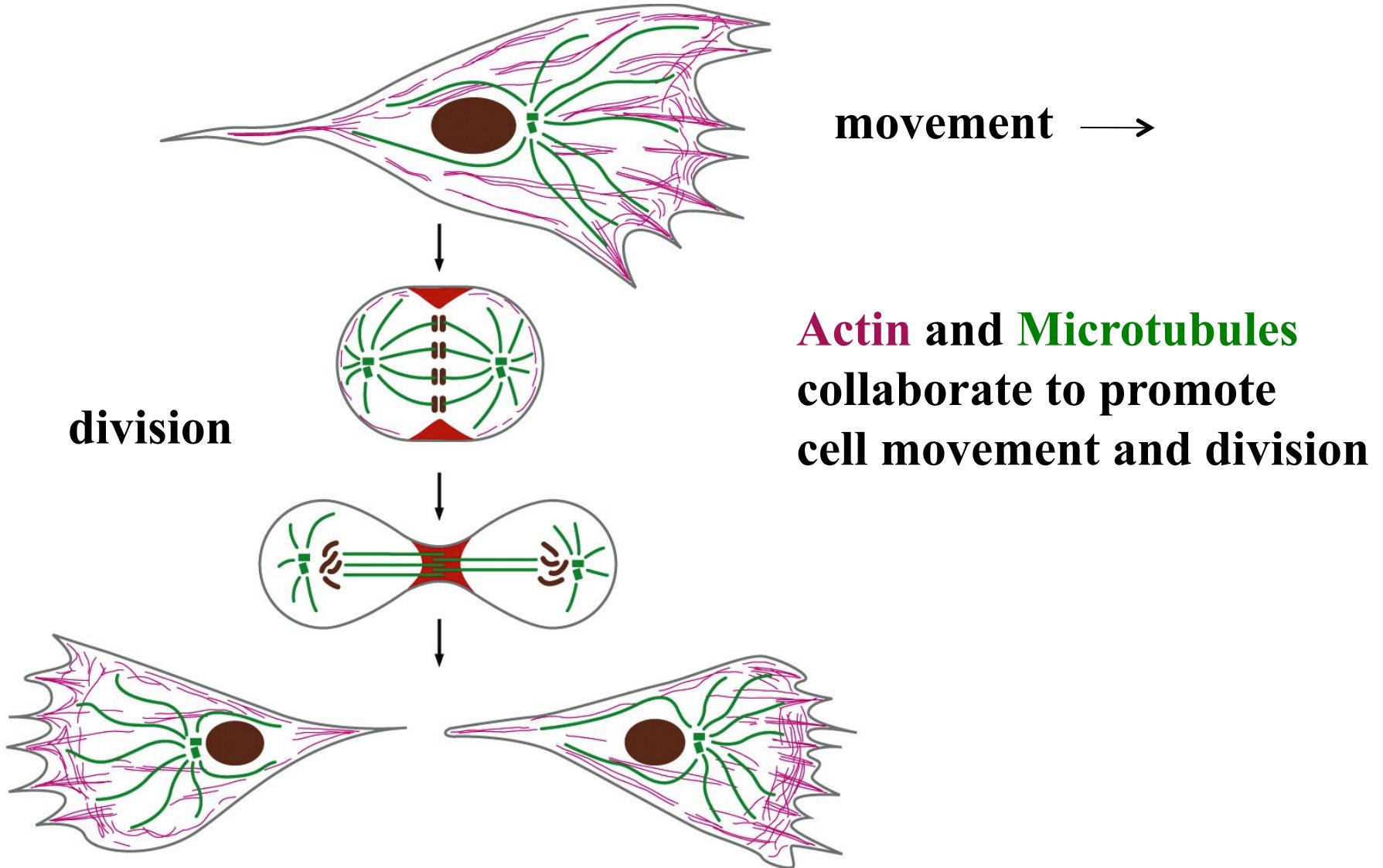
## Key Points

What are the functions of different cytoskeletons?

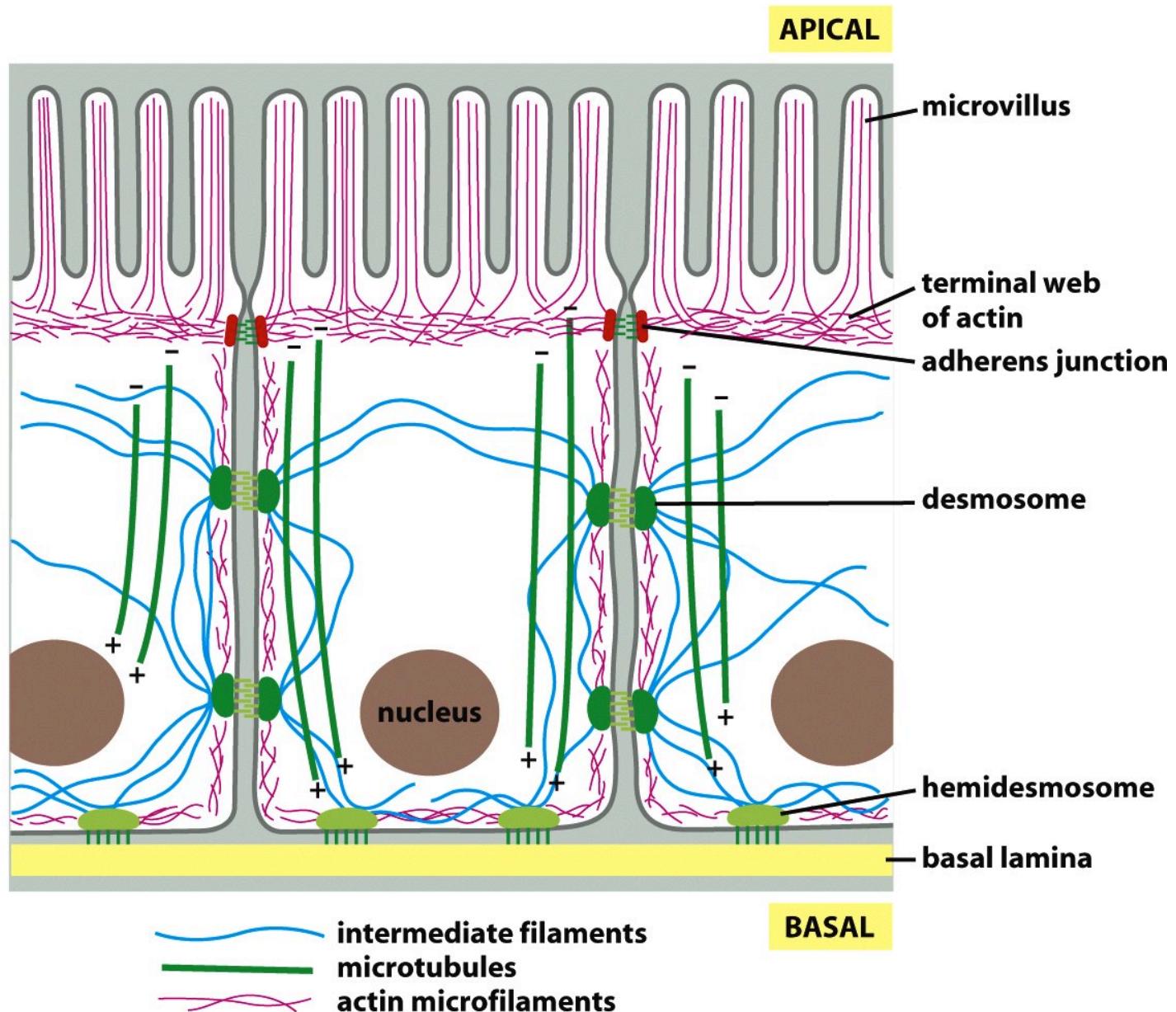
&

What are the intrinsic properties  
(i.e. those related to the filaments themselves)  
governing the assembly/disassembly of different cytoskeletons?

# The Cytoskeleton is Involved in Numerous Cellular Events



# Organization of Cytoskeleton in a Polarized Epithelial Cell



# An Overview of Cytoskeleton

**Introduction of different types of cytoskeleton**

**Assembly/disassembly of cytoskeletal filaments**

# Cytoskeletal Filaments are Non-Covalent Polymers

individual linear subunits



Subunits are joined end to end to form protofilaments



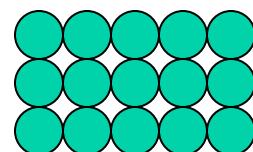
Multiple protofilaments are linked to form filaments



individual subunits

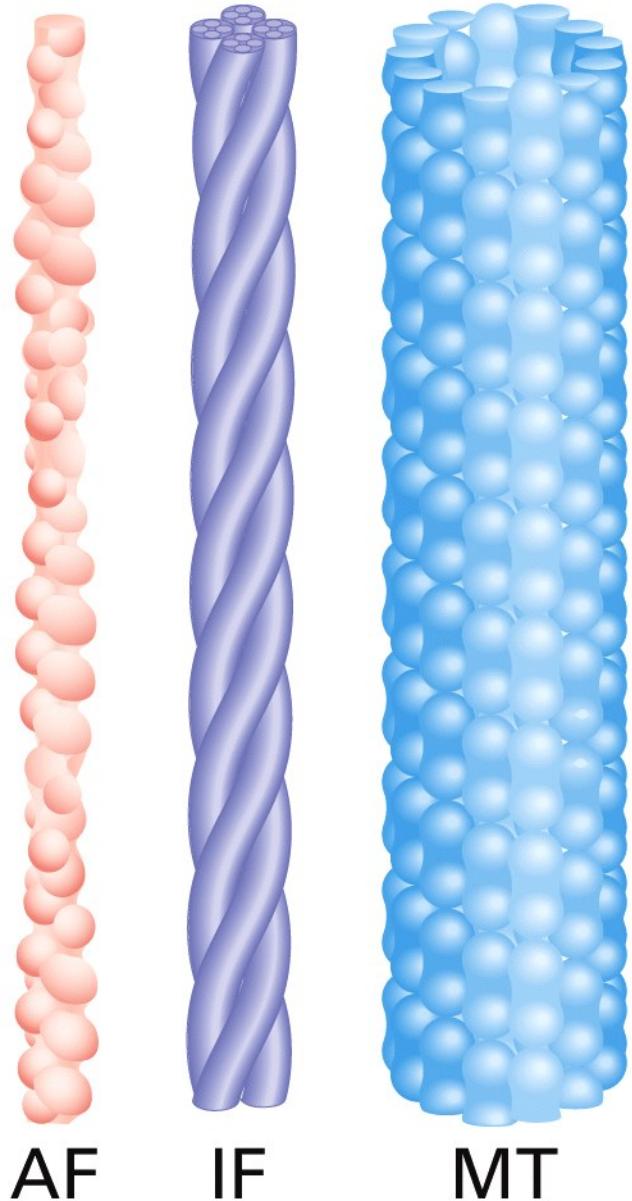


protofilament



filament

# Three Types of Cytoskeletal Filaments



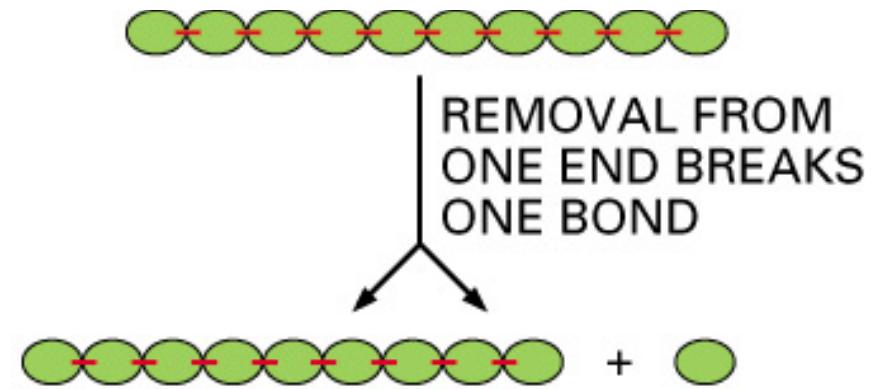
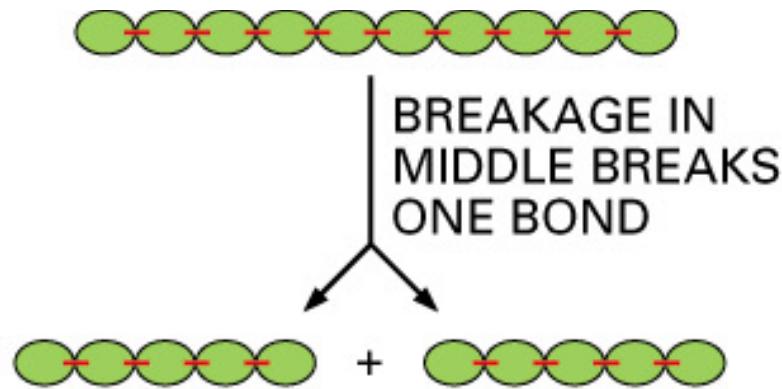
**actin filaments (microfilaments, F-actin, AF):**  
present in all eukaryotic cells  
two twisted protofilaments forming a coil  
filament of 8-9 nm in diameter

**intermediate filaments (IF):**  
present in only animal cells  
alpha-helical protofilaments forming a rope  
filament of 10 nm in diameter  
(there are many subtypes)

**microtubule filaments (MT):**  
present in all eukaryotic cells  
in most cases: 13 protofilaments forming a  
hollow tube filament of 24 nm in diameter

# Why Link Multiple Protofilaments to Form Filaments?

One protofilament is not thermally stable in the middle or ends



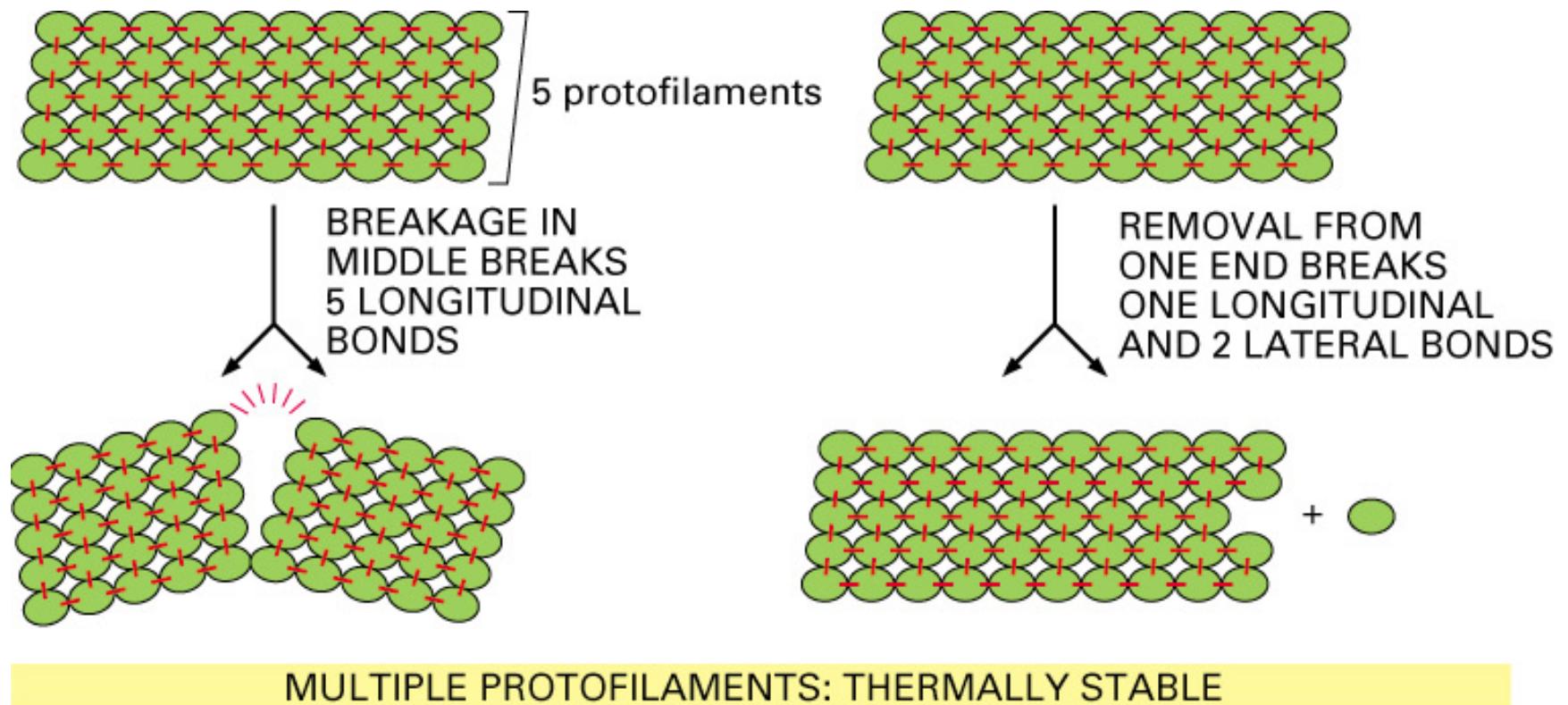
SINGLE PROTOFILAMENT: THERMALLY UNSTABLE

One can easily break the protofilament in the middle  
or from the end by destroying only one interaction  
(Note that “bond” here refers to non-covalent interactions)

# Why Link Multiple Protofilaments to Form Filaments?

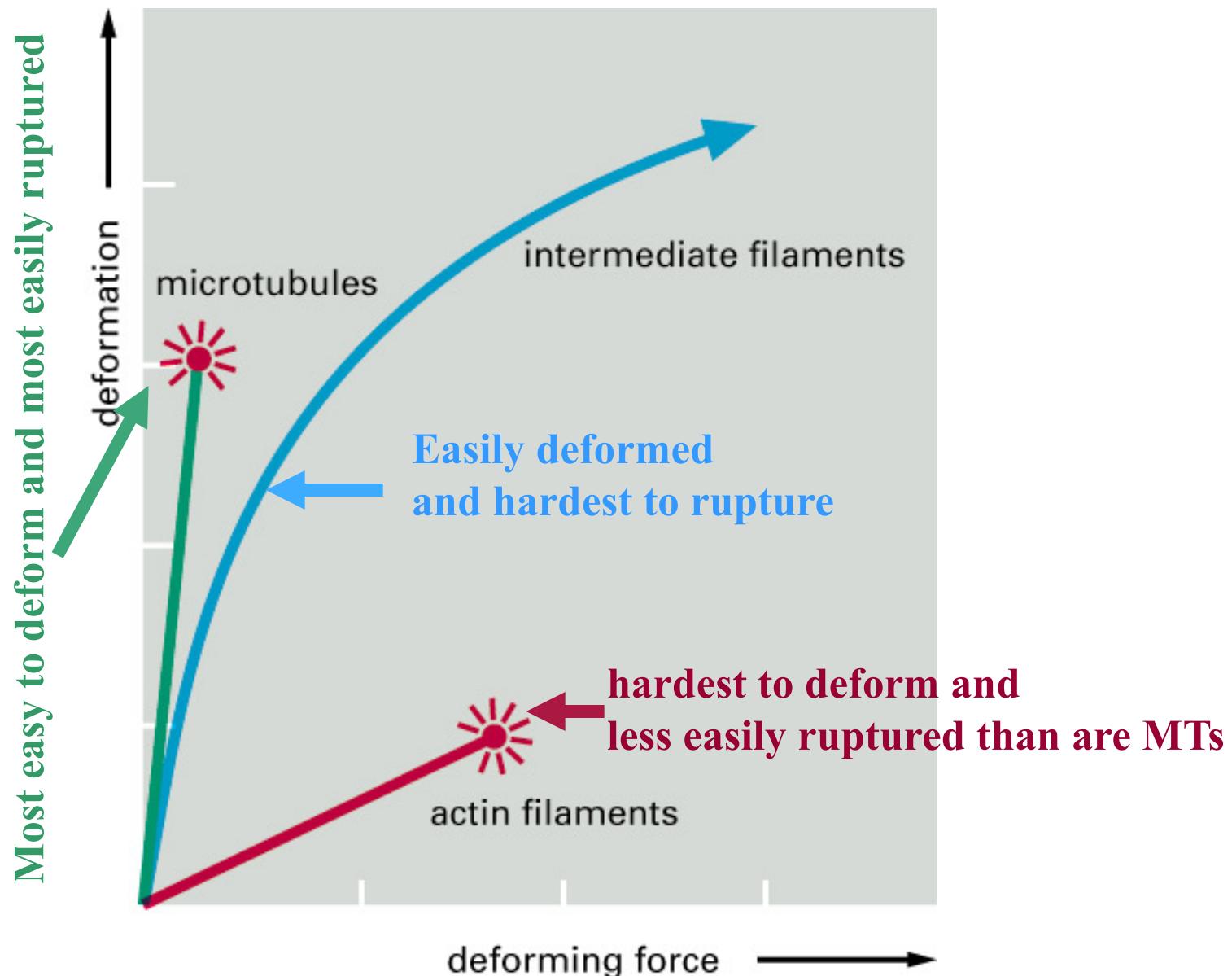
It makes the structure stronger.

It also makes the structure more dynamic since it is easier to remove the subunits from the ends than from the middle



Filaments are thermally stable in the middle but are dynamic at the ends

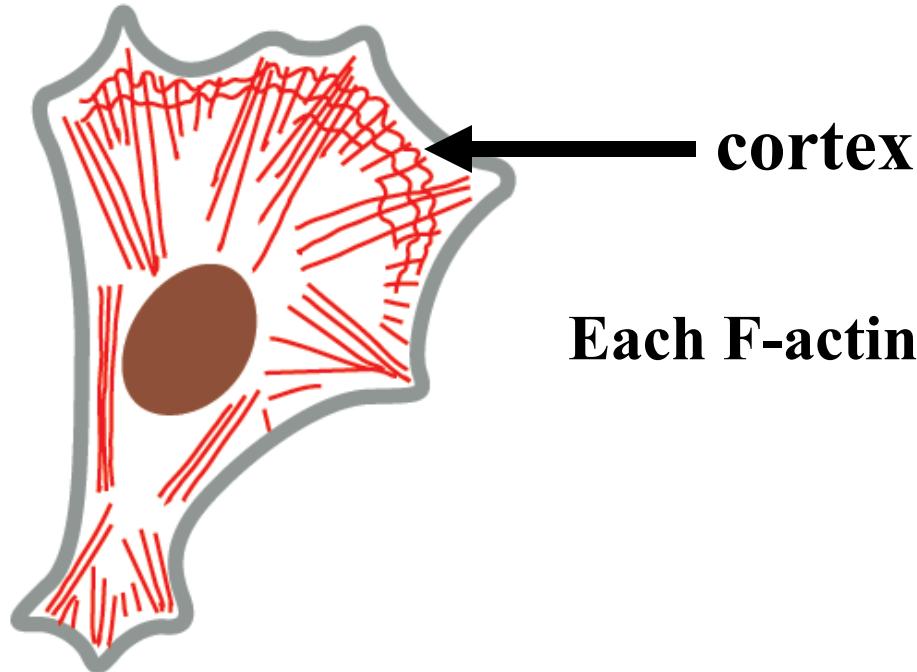
# Different Mechanical Properties of F-Actin, MT, and IF



# Actin Filaments (F-actin)

Highly dynamic

Present in different shapes: linear bundles, 2D networks, and 3D gels  
most enriched in the cortex (the area below the PM)



Each F-actin has polarity (+ and – ends)

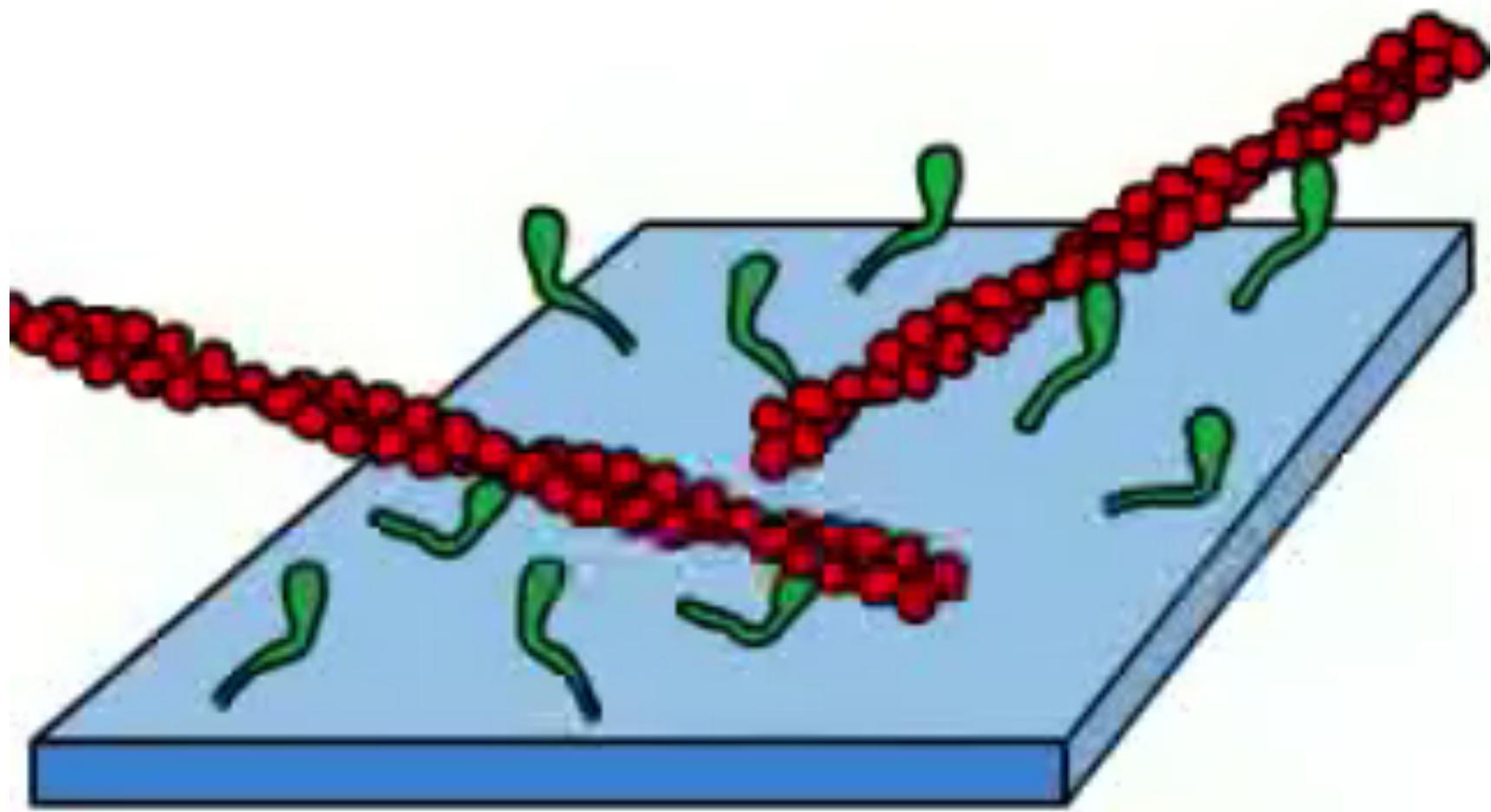
determines cell shape

functions in cell migration and

short-range intracellular transport: by the motor protein myosins.

Most myosins move toward the plus end  
but some move toward the minus end.

## Actin Movement by Myosin Motors: Movie



# Actin Monomers & Actin Filaments

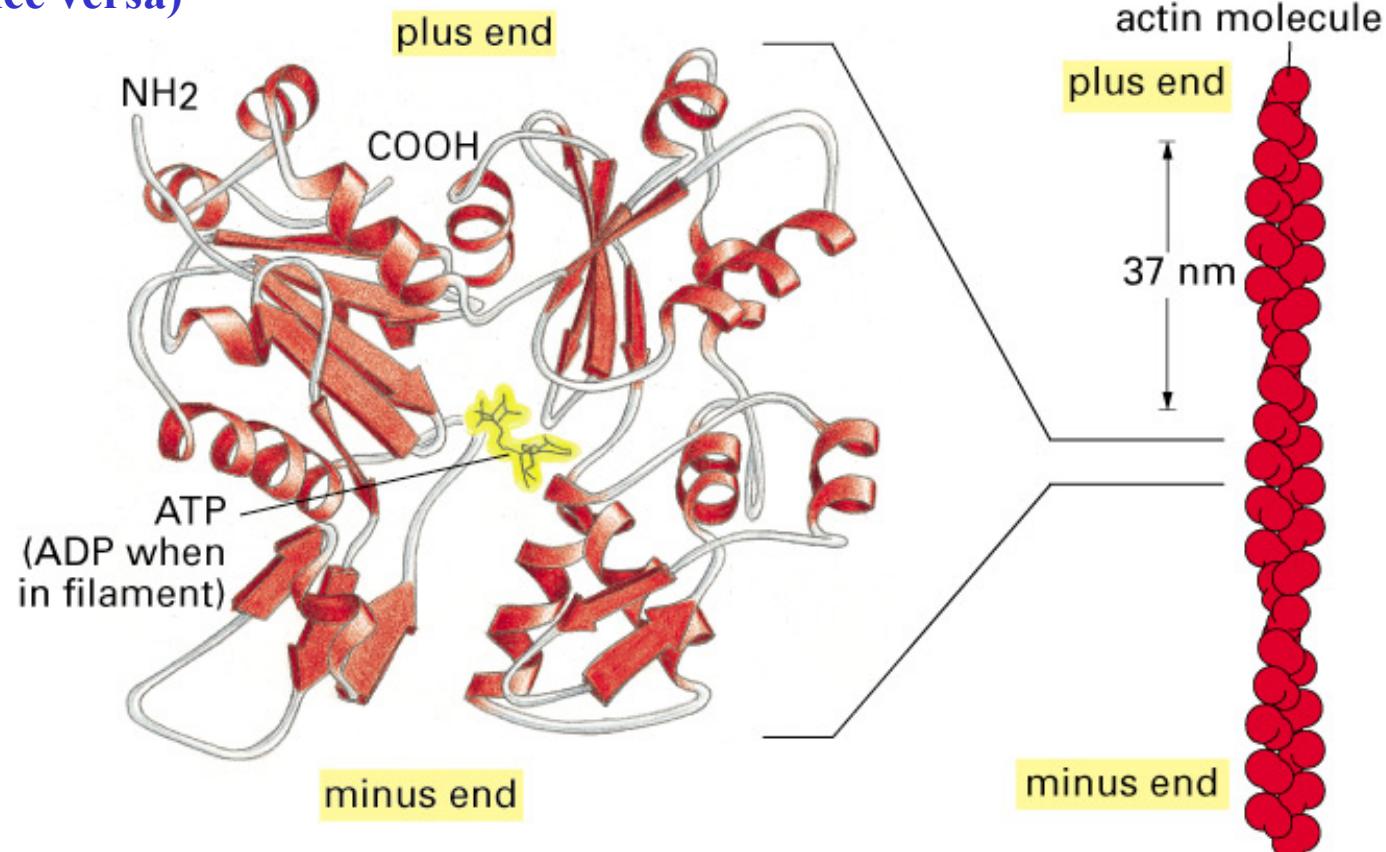
## Monomer (G-actin)

Actin is an ATPase.

When in monomers or “new” filaments:  
ATP bound.

When in “old” filaments: ADP bound.

Two ends have different properties  
(+end can be only connected to – end  
and vice versa)



## Filament (F-actin)

The plus and minus ends of two adjacent monomers are connected to form a protofilament. The two parallel protofilaments then wind into a helix via the lateral contact. Polarity is maintained in F-actin.

# Intermediate Filament (IF)

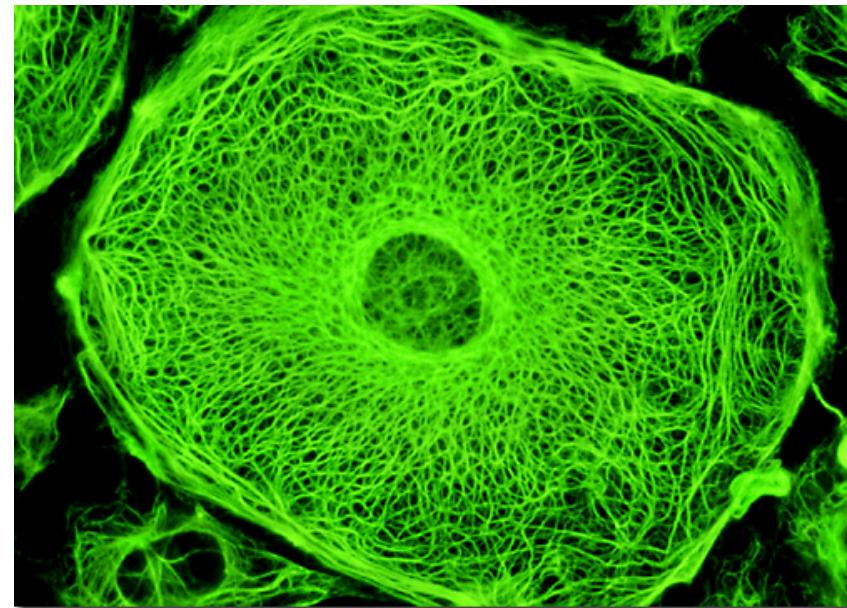
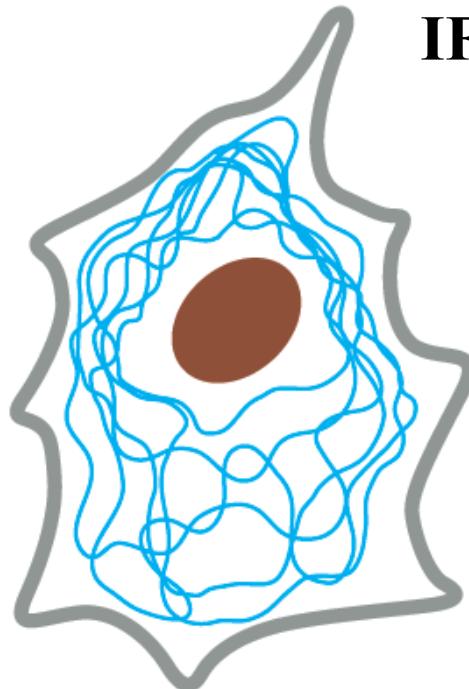
many different subtypes of IF exist

one type forms the nuclear lamina

other types extend across the cytoplasm

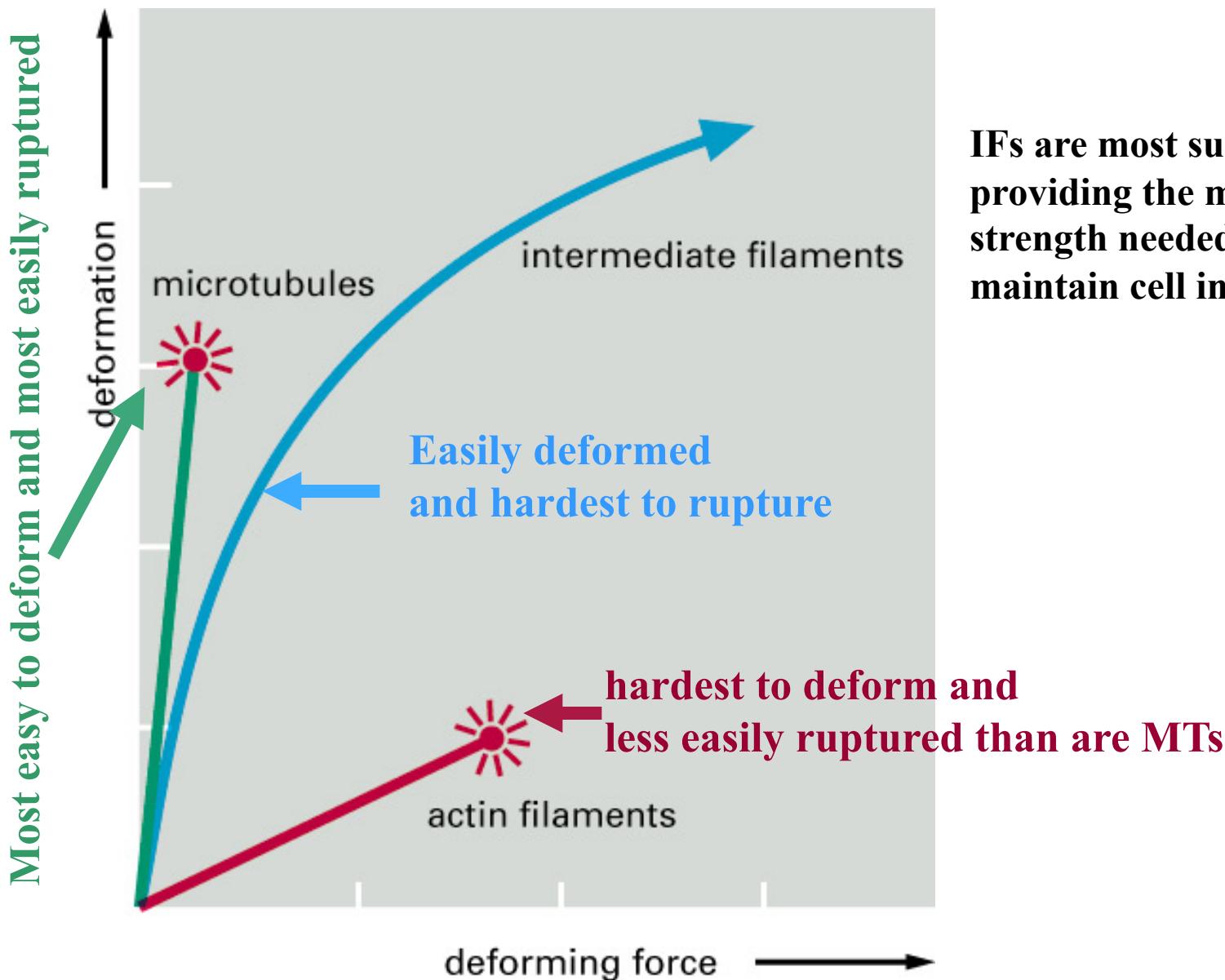
a less dynamic structure compared to F-actin and MT

IFs do not have polarity



IF provides mechanical strength and structural integrity to cells

# Different Mechanical Properties of F-Actin, MT, and IF



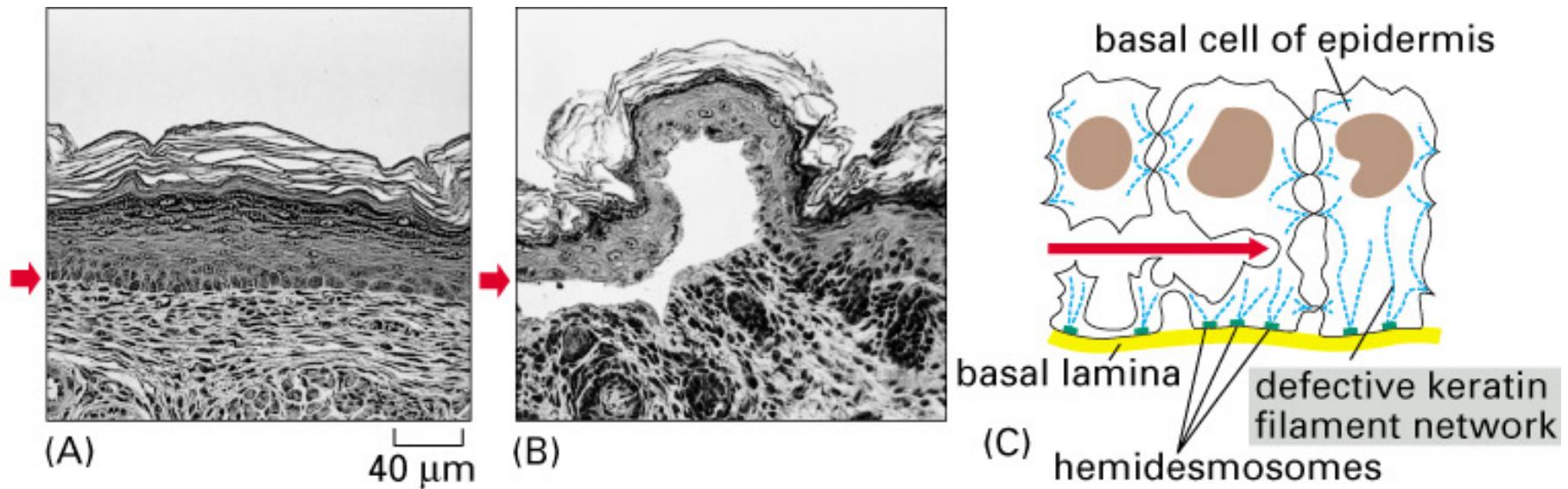
IFs are most suitable for providing the mechanical strength needed to maintain cell integrity

# Major Types of IF Filament Proteins in Vertebrate Cells

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) type II keratins (basic)	epithelial cells and their derivatives (e.g., hair and nails)
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

# Skin Blistering Caused by Mutations in Keratin Genes

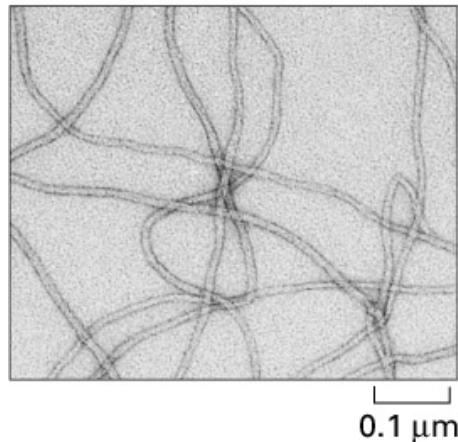
Keratin forms a type of IF in epithelial cells. Expression of a truncated keratin gene in a transgenic mouse leads to a skin disorder. The blisters are caused by the rupturing of cells located on the basal layer of the epidermis.



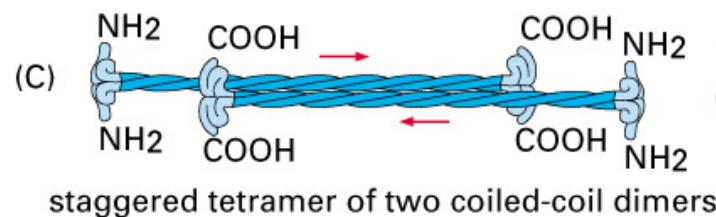
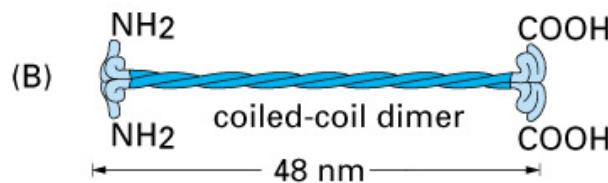
## Human Epidermolysis Bullosa Simplex:

Commonly due to mutations in genes encoding proteins called keratin 5 and keratin 14a, and reflects group of genetic conditions that cause the skin to be very fragile and to blister easily.

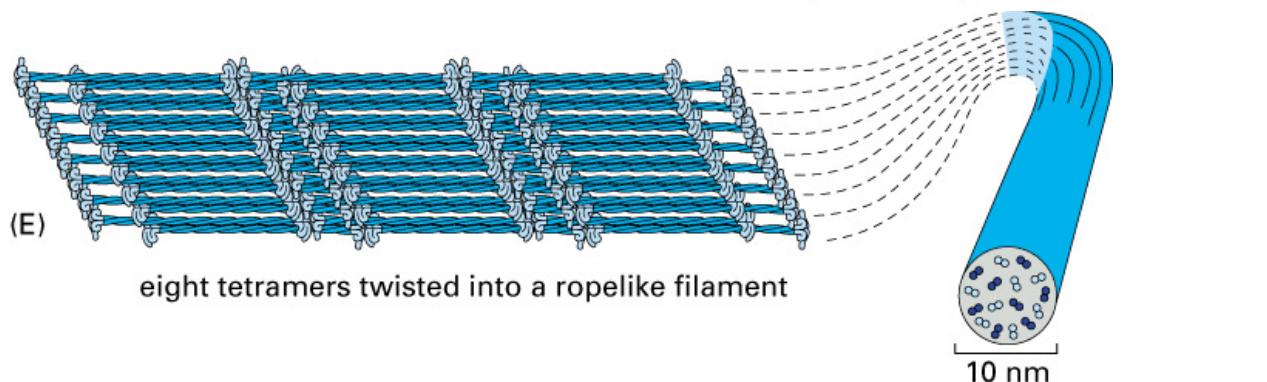
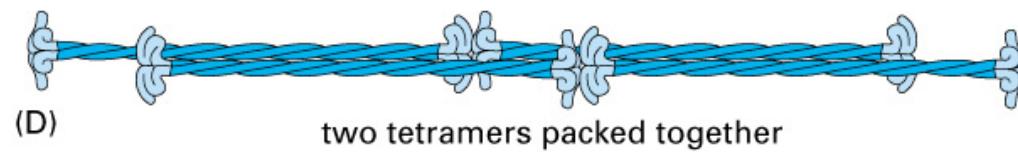
# A Model of Intermediate Filament Formation



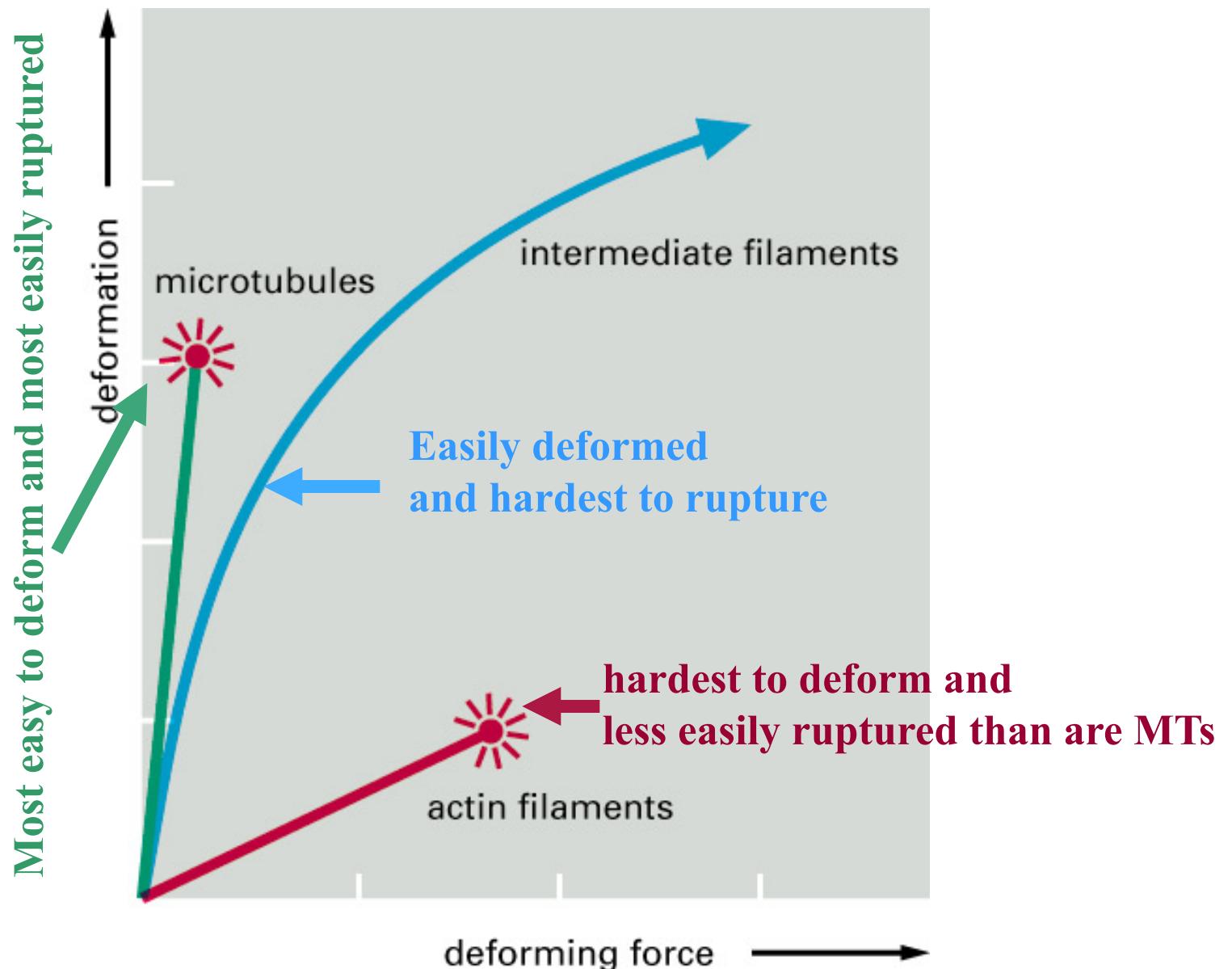
0.1  $\mu\text{m}$



**anti-parallel arrangement  
(so no polarity is generated)**



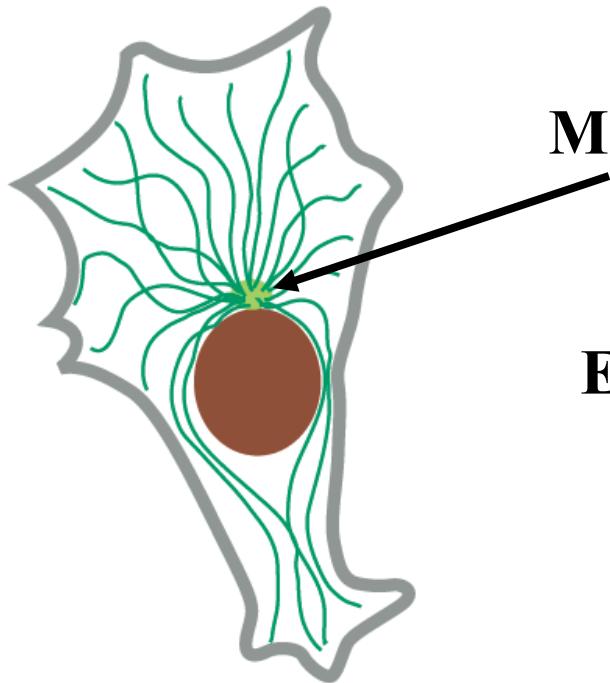
# Different Mechanical Properties of F-Actin, MT, and IF



# Microtubules (MT)

Highly dynamic structure.

During interphase periods of cell function (vs. cell division/mitosis):  
One end (minus end) is attached to the MTOC (often perinuclear),  
while the other end (plus end) extends to the cell periphery



MTOC: Microtubule Organizing Center

Each MT has polarity (+ and – ends)

long-range intracellular transport  
determining positions of organelles

} via two types of motor proteins  
(kinesin: to plus end; dynein: to minus end)

segregation of chromosomes during mitosis

# Tubulin Heterodimers & Microtubules

**tubulin heterodimer ( $\alpha\beta$ ):**

Tubulin is a GTPase (for both  $\alpha$  and  $\beta$ )

When in dimers or “new filaments:

GTP bound  $\beta$

When in “old” filaments: GDP bound  $\beta$

**microtubules:**

The plus and minus ends of two adjacent monomers are connected to form a protofilament. 13 parallel protofilaments then form a hollow tube w/ polarity maintained

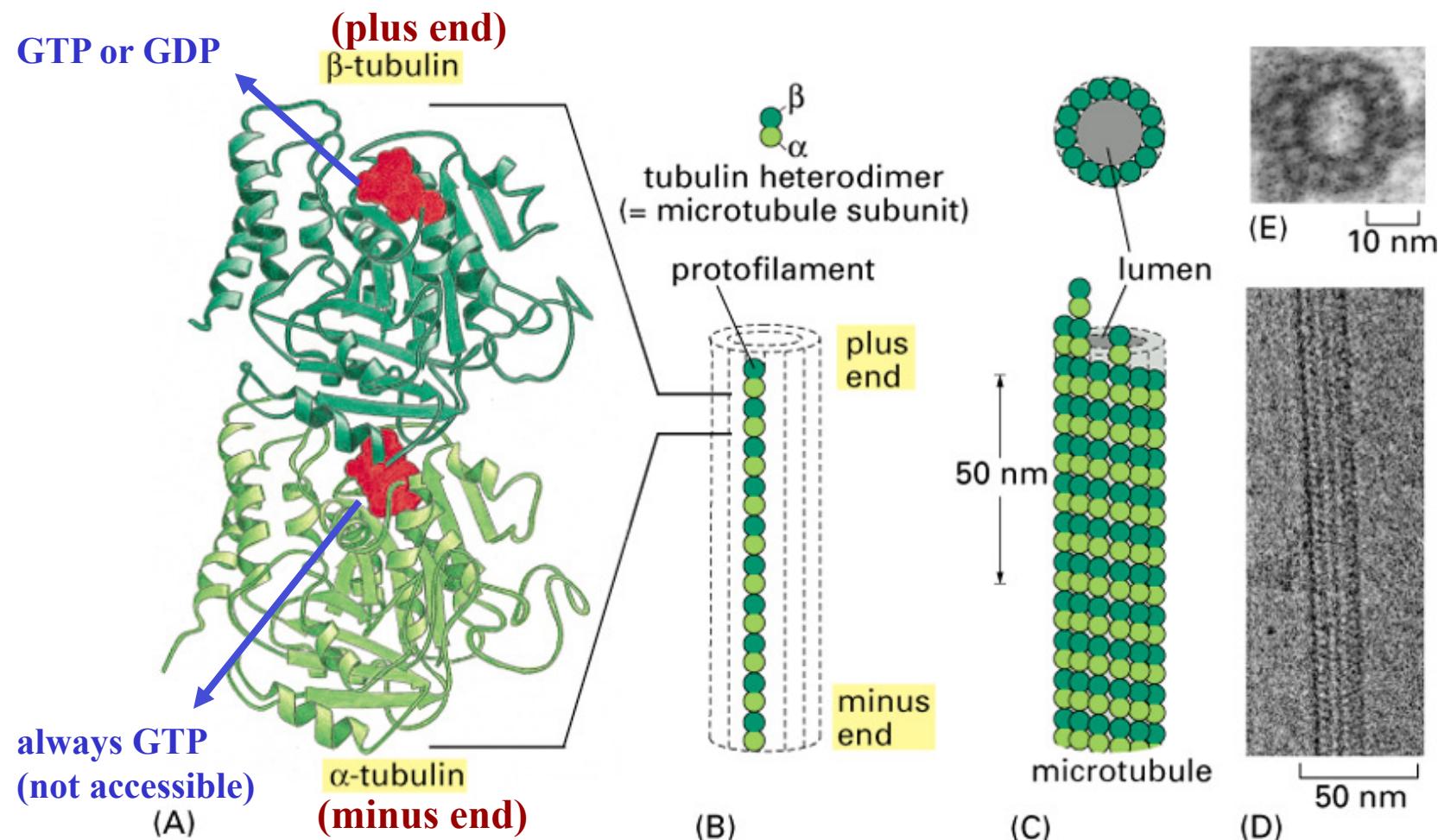


Figure 16–6. Molecular Biology of the Cell, 4th Edition.

# An Overview of Cytoskeleton

## Introduction of different types of cytoskeleton

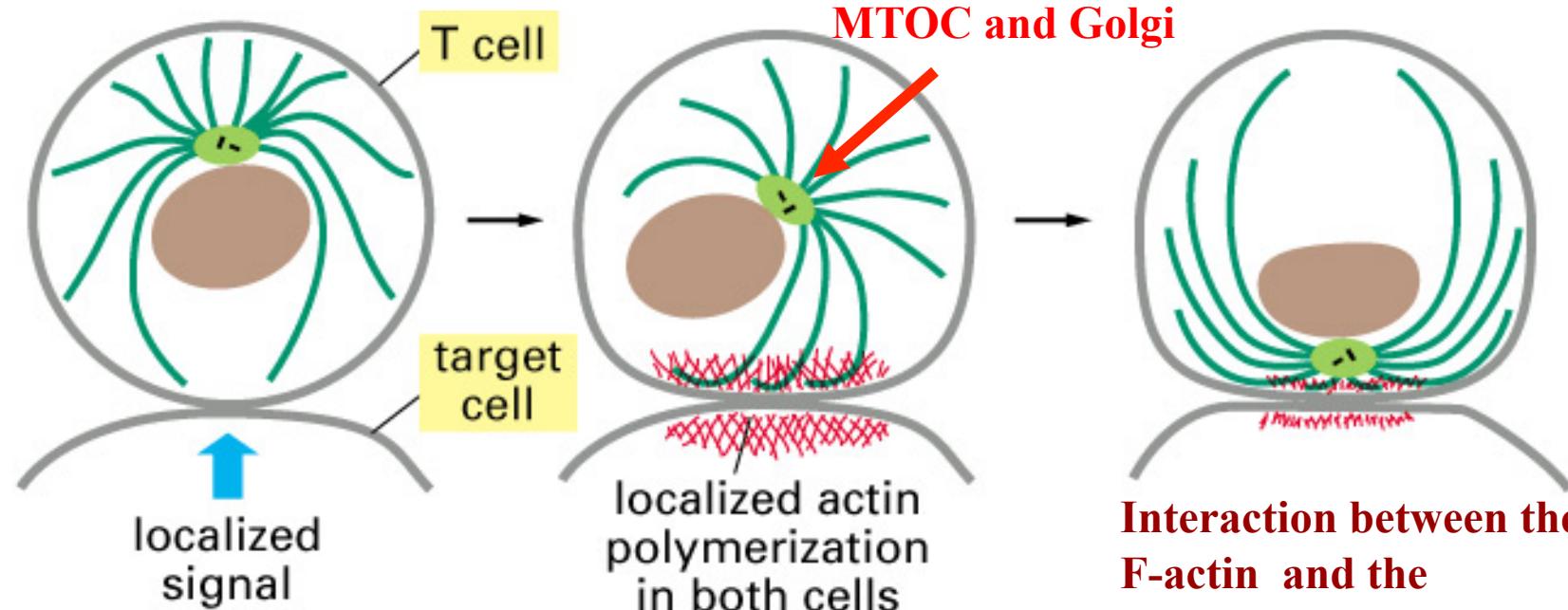
**Assembly/disassembly (focused on F-actin and MT)**

**All cytoskeleton filaments can be considered dynamic  
and their assembly /disassembly can be regulated**

# Why study cytoskeletal assembly/disassembly?

Role of F-actin and microtubule dynamics in T-cell function as an example

Local exocytosis of lytic granules allows cytotoxic T Cells to concentrate the killing machinery into the target cells.

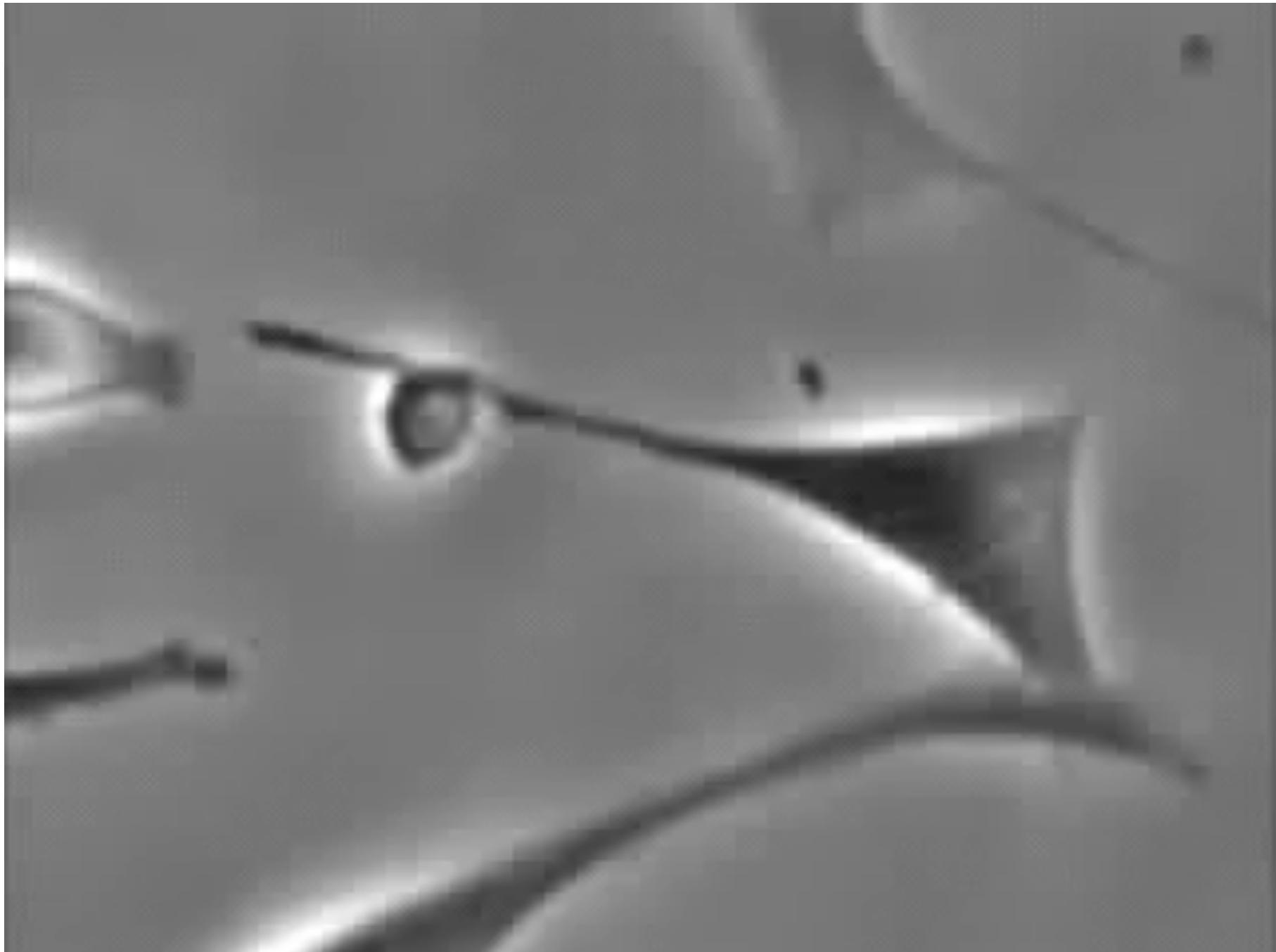


Foreign antigen on the surface of a target cell is recognized by the T-cell receptor. This recognition produces a local signal.

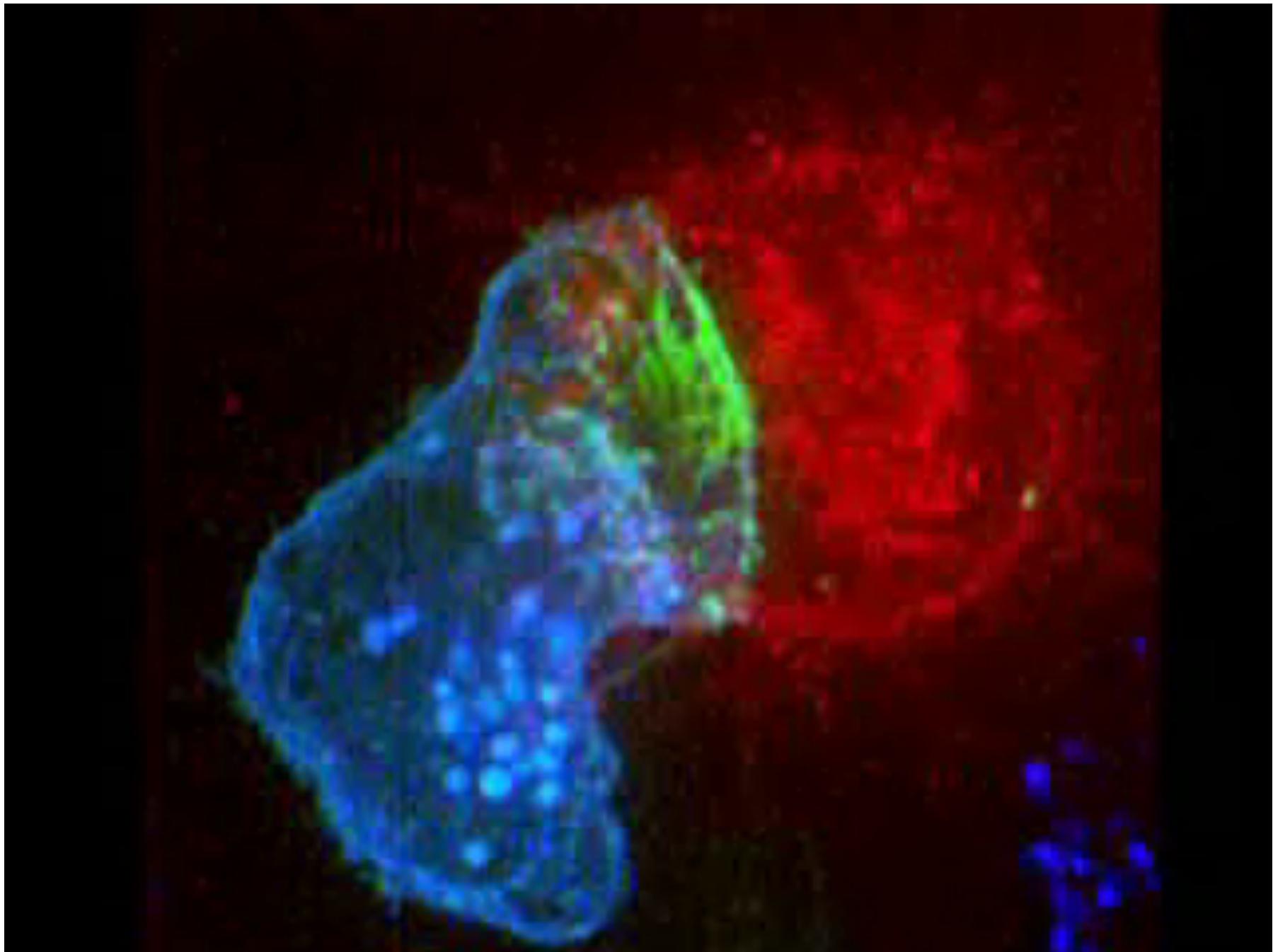
This signal induces actin polymerization at the contact site to form F-actin.

Interaction between the F-actin and the microtubule leads to the reorientation of MTOC and Golgi. The dynamic microtubules then become stabilized on the contact site to allow delivery of lytic granules to the site.

## Cytotoxic T Cells Lyse Their Targets: movie



## The “T Cell Synapse”: movie



# **Intrinsic Factors Controlling Cytoskeletal Dynamics**

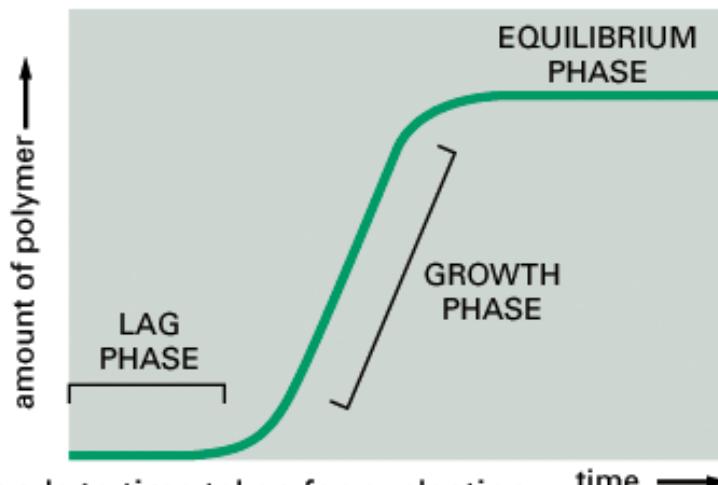
# Cytoskeleton Assembly/Disassembly

## “In-Vitro” Polymerization

Three are three time-course phases for filament assembly starting from G-actin monomers or tubulin dimers

### TIME COURSE OF POLYMERIZATION

The assembly of a protein into a long helical polymer such as a cytoskeletal filament or a bacterial flagellum typically shows the following time course:



The lag phase corresponds to time taken for nucleation.

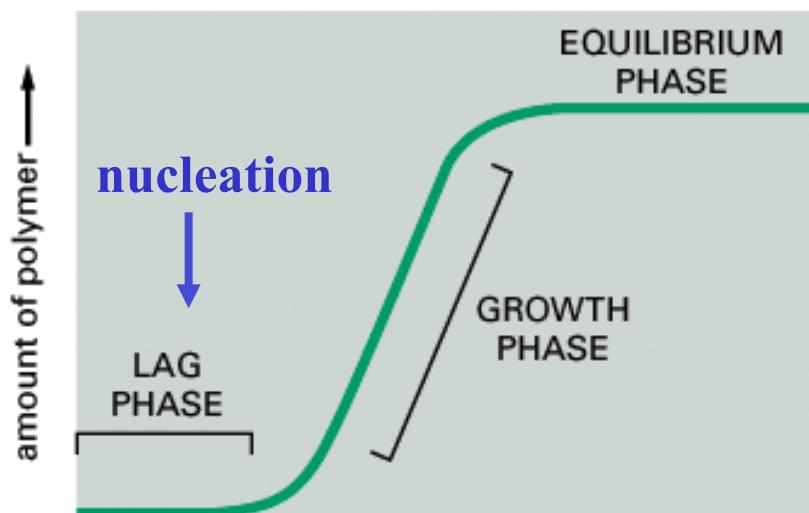
The growth phase occurs as monomers add to the exposed ends of the growing filament, causing filament elongation.

The equilibrium phase, or steady state, is reached when the growth of the polymer due to monomer addition is precisely balanced by the shrinkage of the polymer due to disassembly back to monomers.

# The Lag phase corresponds to the nucleation of filaments

## TIME COURSE OF POLYMERIZATION

The assembly of a protein into a long helical polymer such as a cytoskeletal filament or a bacterial flagellum typically shows the following time course:



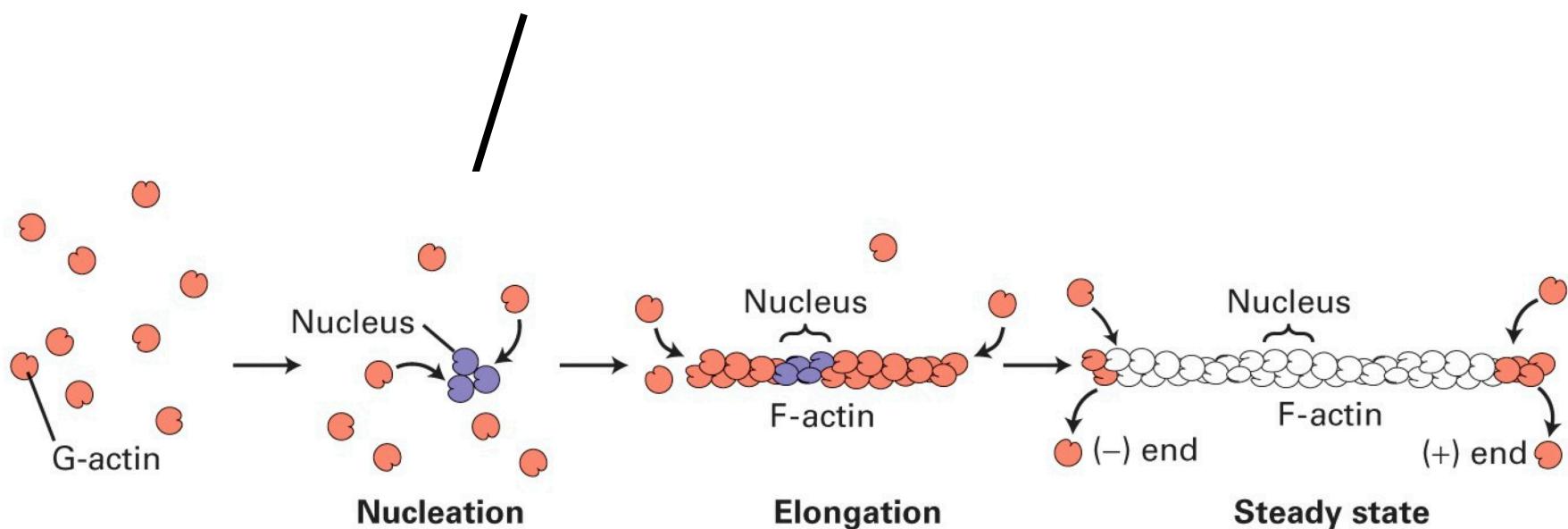
The lag phase corresponds to time taken for nucleation. time →

The growth phase occurs as monomers add to the exposed ends of the growing filament, causing filament elongation.

The equilibrium phase, or steady state, is reached when the growth of the polymer due to monomer addition is precisely balanced by the shrinkage of the polymer due to disassembly back to monomers.

# Nucleation is Rate-Limiting in F-Actin Formation

**Nucleation phase:**  
G-actin or tubulin subunits  
must form a seed (or nucleus)  
before elongation takes place  
(the rate-limiting step – cause of the lag period)



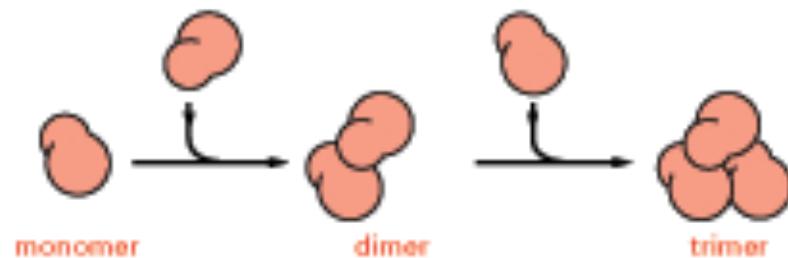
# Why is the nucleation step rate-limiting?

For the F-actin assembly, a trimer of G-actin is needed to begin polymerization

The probability of forming a trimer is much lower than that of forming a dimer  
(as in the case of elongation)

## NUCLEATION

A helical polymer is stabilized by multiple contacts between adjacent subunits. In the case of actin, two actin molecules bind relatively weakly to each other, but addition of a third actin monomer to form a trimer makes the entire group more stable.

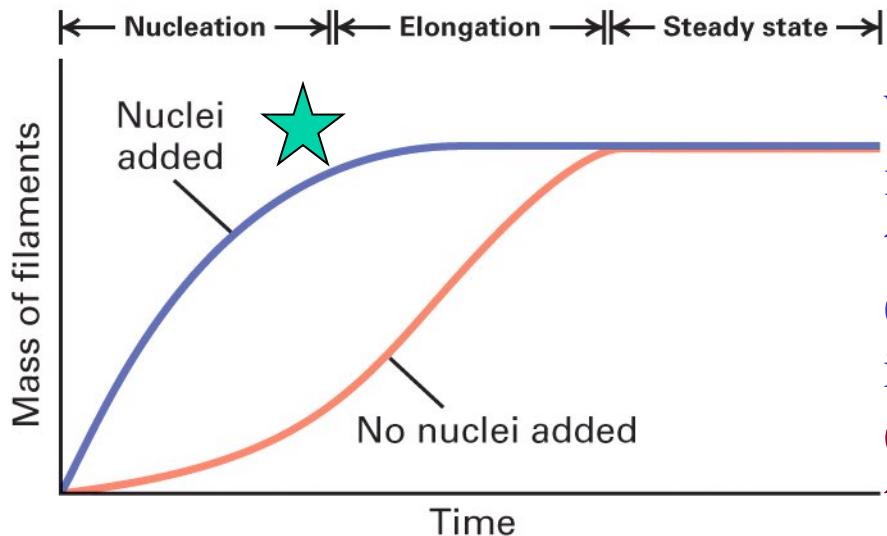
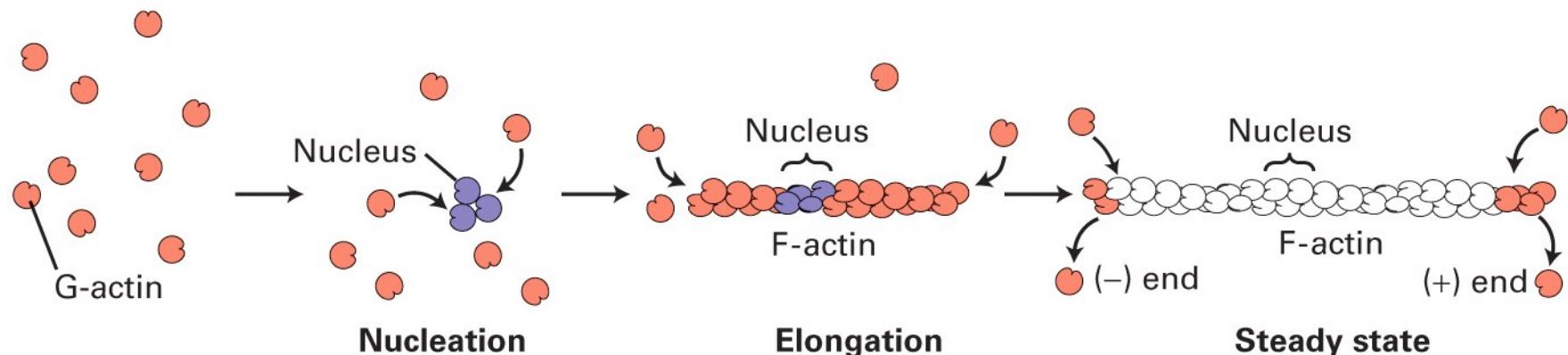


Further monomer addition can take place onto this trimer, which therefore acts as a **nucleus** for polymerization. For tubulin, the nucleus is larger and has a more complicated structure (possibly a ring of 13 or more tubulin molecules)—but the principle is the same.

The assembly of a nucleus is relatively slow, which explains the lag phase seen during polymerization. The lag phase can be reduced or abolished entirely if premade nuclei, such as fragments of already polymerized microtubules or actin filaments, are added.

For MT assembly, the number of tubulin subunits required is different but the principle is the same.

# However, the nucleation phase only affects the kinetics of polymerization, not the steady-state level

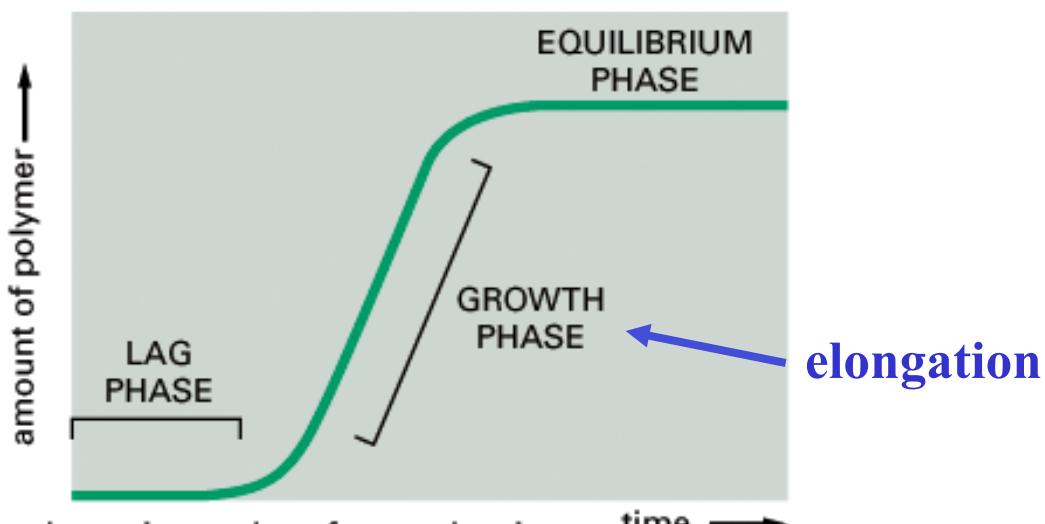


When nuclei are added into the assembly reaction or when a filament already exists, the steady state may be reached sooner. (however, the degree of polymerization is not affected by the addition of nuclei) (seeds or nuclei function in a way similar to that of a catalyst in a chemical reaction)

# The Growth Phase Corresponds to Filament Elongation

## TIME COURSE OF POLYMERIZATION

The assembly of a protein into a long helical polymer such as a cytoskeletal filament or a bacterial flagellum typically shows the following time course:



The lag phase corresponds to time taken for nucleation.

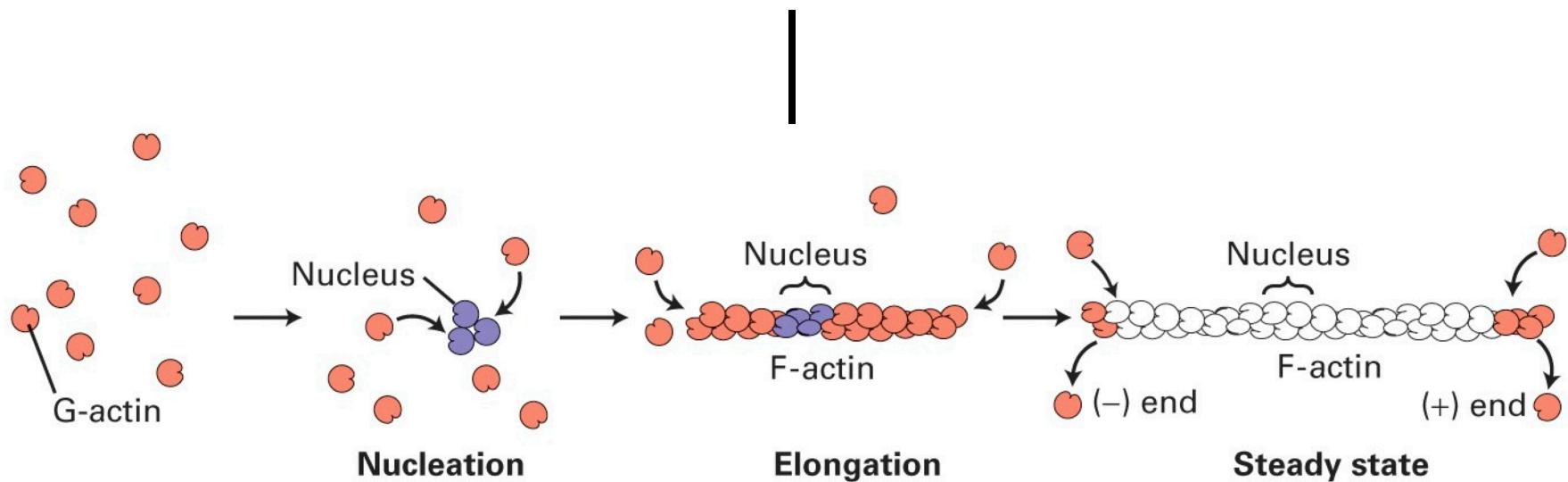
The growth phase occurs as monomers add to the exposed ends of the growing filament, causing filament elongation.

The equilibrium phase, or steady state, is reached when the growth of the polymer due to monomer addition is precisely balanced by the shrinkage of the polymer due to disassembly back to monomers.

# Nucleation, Elongation & Steady state

elongation

on rate > off rate



# Elongation Phase: On Rate and Off Rate

$K_{on}$  - rate constant for the addition of a subunit to the filament end

$K_{off}$  - rate constant for the removal of a subunit from the filament end

C - free subunit concentration (G-actin or tubulin dimer)

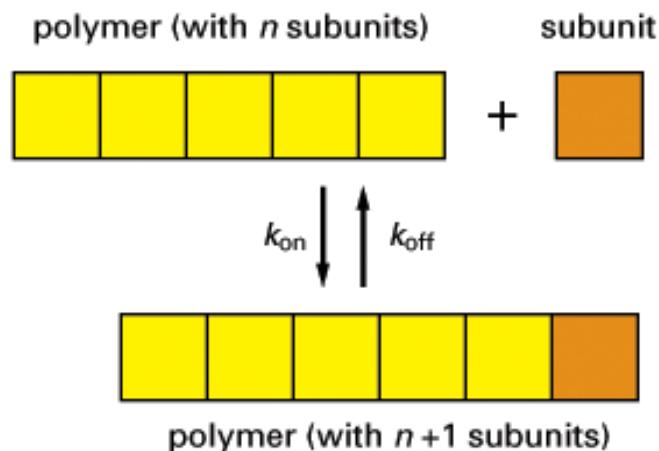
$$\text{on rate} = K_{on} \times C$$

$$\text{off rate} = K_{off}$$

during elongation, on rate > off rate

## ON RATES AND OFF RATES

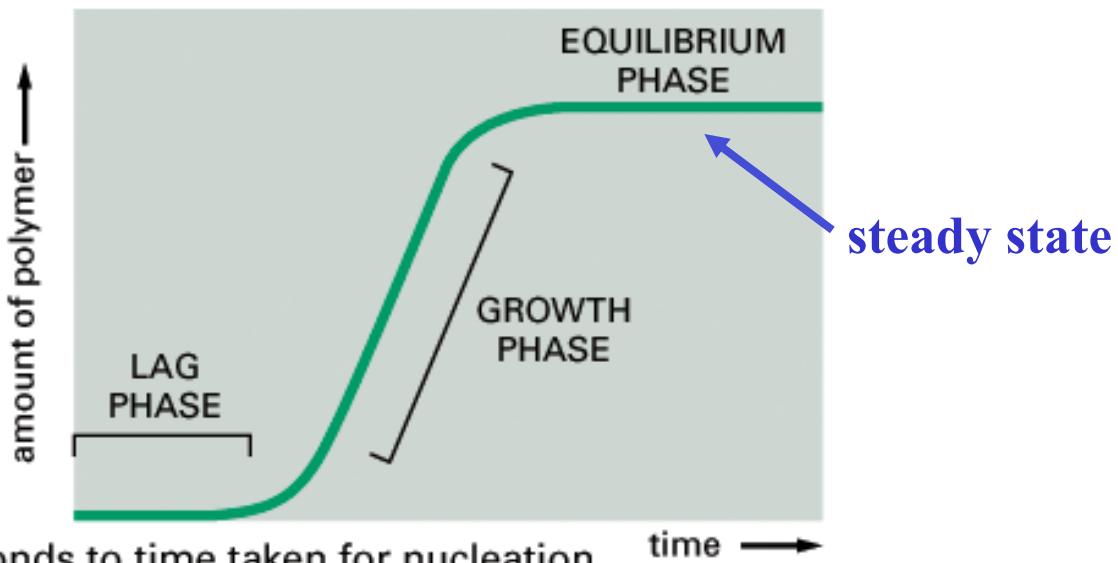
A linear polymer of protein molecules, such as an actin filament or a microtubule, assembles (polymerizes) and disassembles (depolymerizes) by the addition and removal of subunits at the ends of the polymer. The rate of addition of these subunits (called monomers) is given by the rate constant  $k_{on}$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



# The Equilibrium Phase Corresponds to the Steady State

## TIME COURSE OF POLYMERIZATION

The assembly of a protein into a long helical polymer such as a cytoskeletal filament or a bacterial flagellum typically shows the following time course:



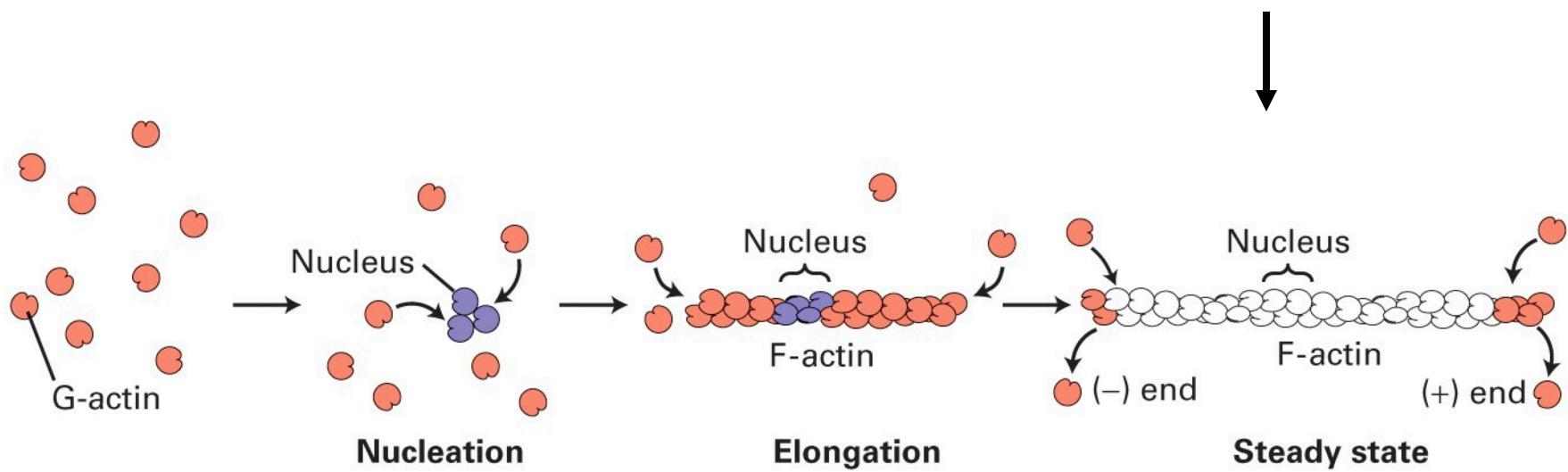
The lag phase corresponds to time taken for nucleation.

The growth phase occurs as monomers add to the exposed ends of the growing filament, causing filament elongation.

The equilibrium phase, or steady state, is reached when the growth of the polymer due to monomer addition is precisely balanced by the shrinkage of the polymer due to disassembly back to monomers.

# Nucleation, Elongation & Steady State

Steady state phase  
on rate = off rate



# Steady State Phase & Critical Concentration (Cc)

## The definition of Cc:

Cc is the concentration of free subunits at which:

the on rate = the off rate

$$K_{on} \times C_c = K_{off}$$

## The significance of Cc:

If  $C > C_c$ :

$$K_{on} \times C > K_{on} \times C_c = K_{off}$$

on rate > off rate (favors polymerization)

If  $C < C_c$ :

$$K_{on} \times C < K_{on} \times C_c = K_{off}$$

on rate < off rate (favors depolymerization)

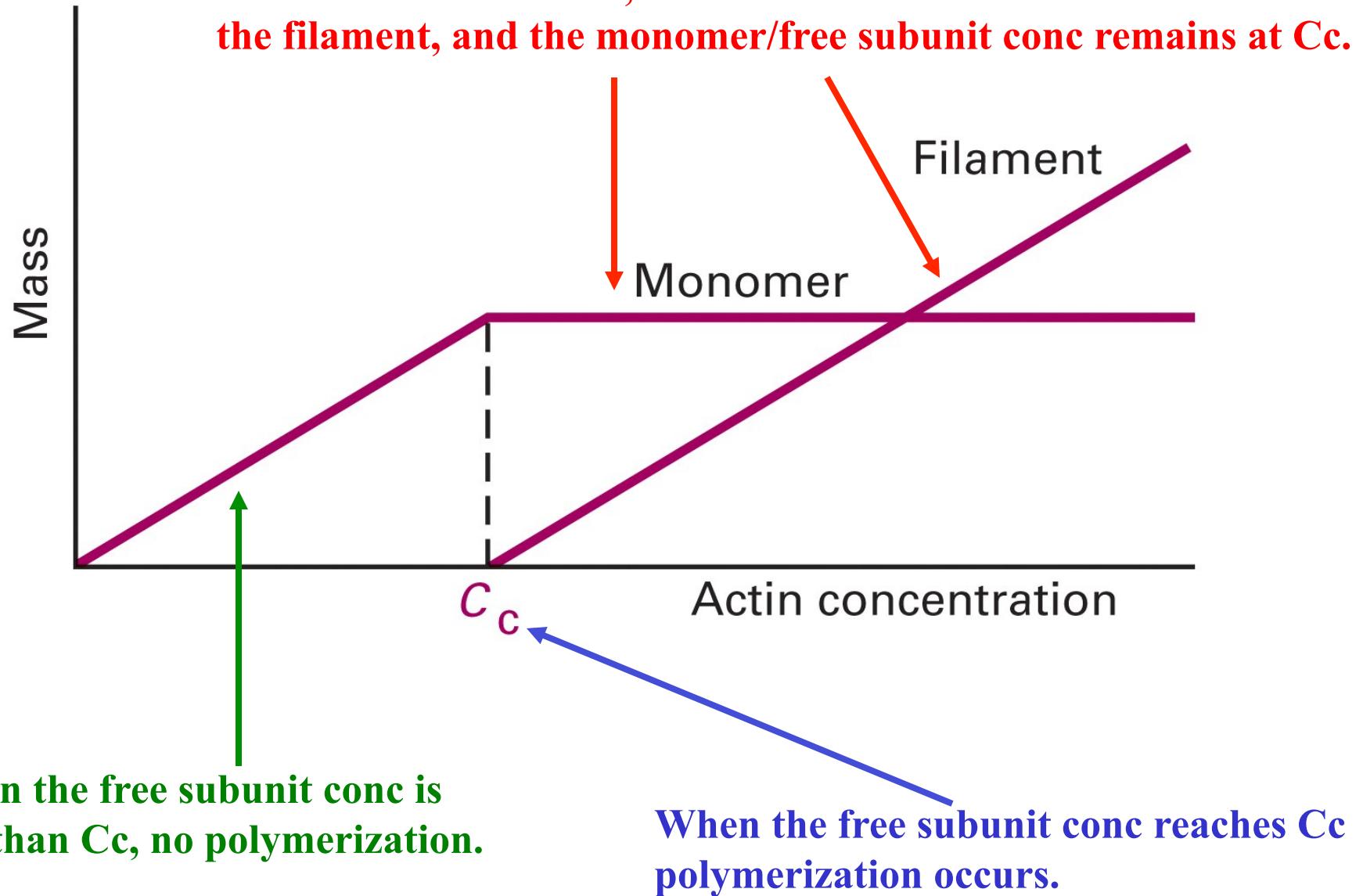
Do the plus and minus ends have the same Kon, Koff and Cc?

## 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 ( $k_{on}C$ ), 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.

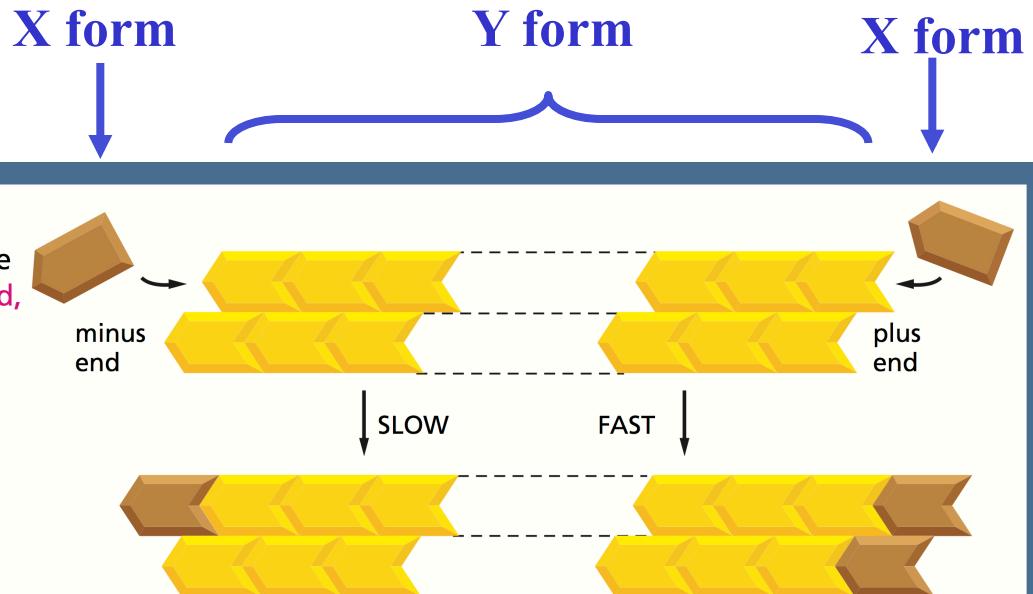
The difference between the free subunit concentration and the  $C_c$  determines whether a filament will grow or shrink

Once the  $C_c$  is reached, the excess subunits will be added to the filament, and the monomer/free subunit conc remains at  $C_c$ .



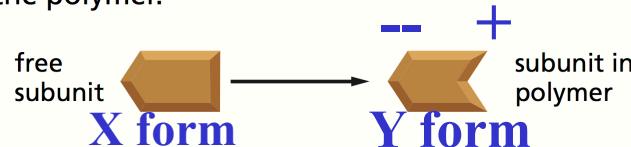
# The Compounding Factor: The two ends of F-actin or microtubules are not equivalent

**Reason 1: A conformational change of each subunit occurs when it enters the filament**



## 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  $\Delta G$  for subunit

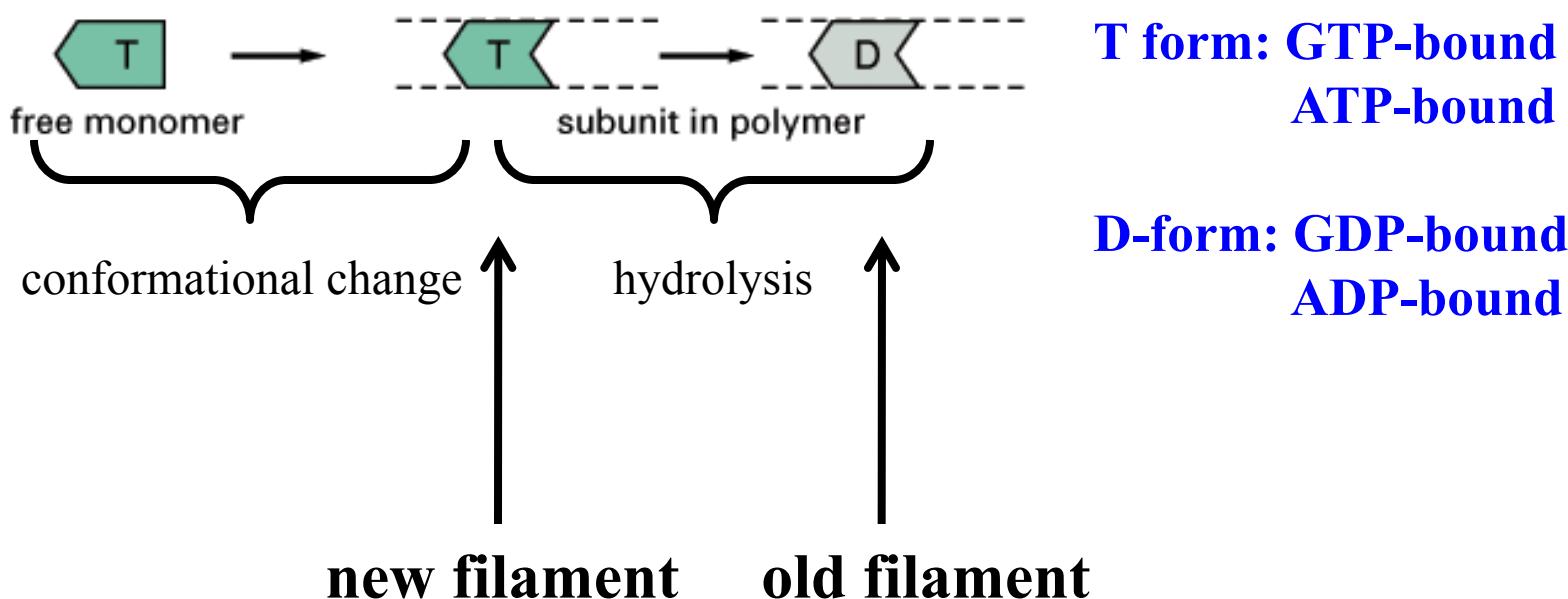
loss, which determines the equilibrium constant for its association with the end, is identical at both ends: if the plus end grows four times faster than the minus end, it must also shrink four times faster. Thus, for  $C > C_c$ , both ends grow; for  $C < C_c$ , both ends shrink.

The nucleoside triphosphate hydrolysis that accompanies actin and tubulin polymerization removes this constraint.

# The Compounding Factor: The two ends of F-actin or microtubules are not equivalent

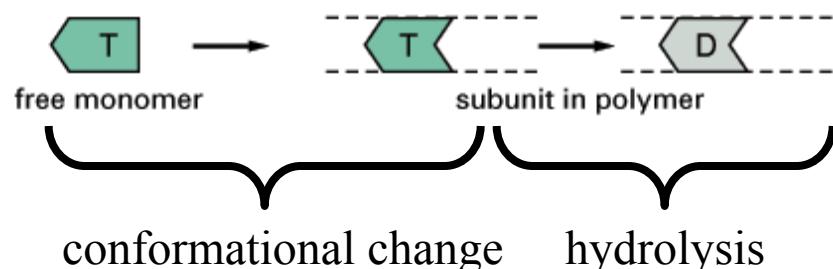
Reason 2: ATP or GTP hydrolysis differentially modifies the behaviors of two ends

After a subunit is added to a filament, its ATP (for actin) or GTP (for tubulin) will be eventually hydrolyzed due to a conformational change.

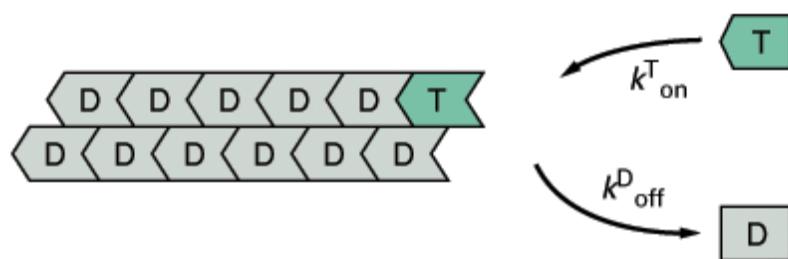


# This ATP/GTP Hydrolysis Leads to Two Consequences

- (1) The D form has a lower affinity for the neighboring subunits and thus a higher  $K_{off}$  (i.e. higher Cc)
- (2) The T form is more likely to be found at the plus end and thus Cc (+) is often less than Cc (-)



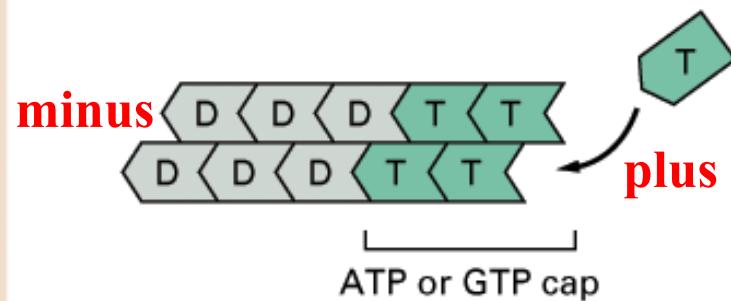
**T form has a lower Cc**



**D form has a higher Cc**

## 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.



# **Key Concepts**

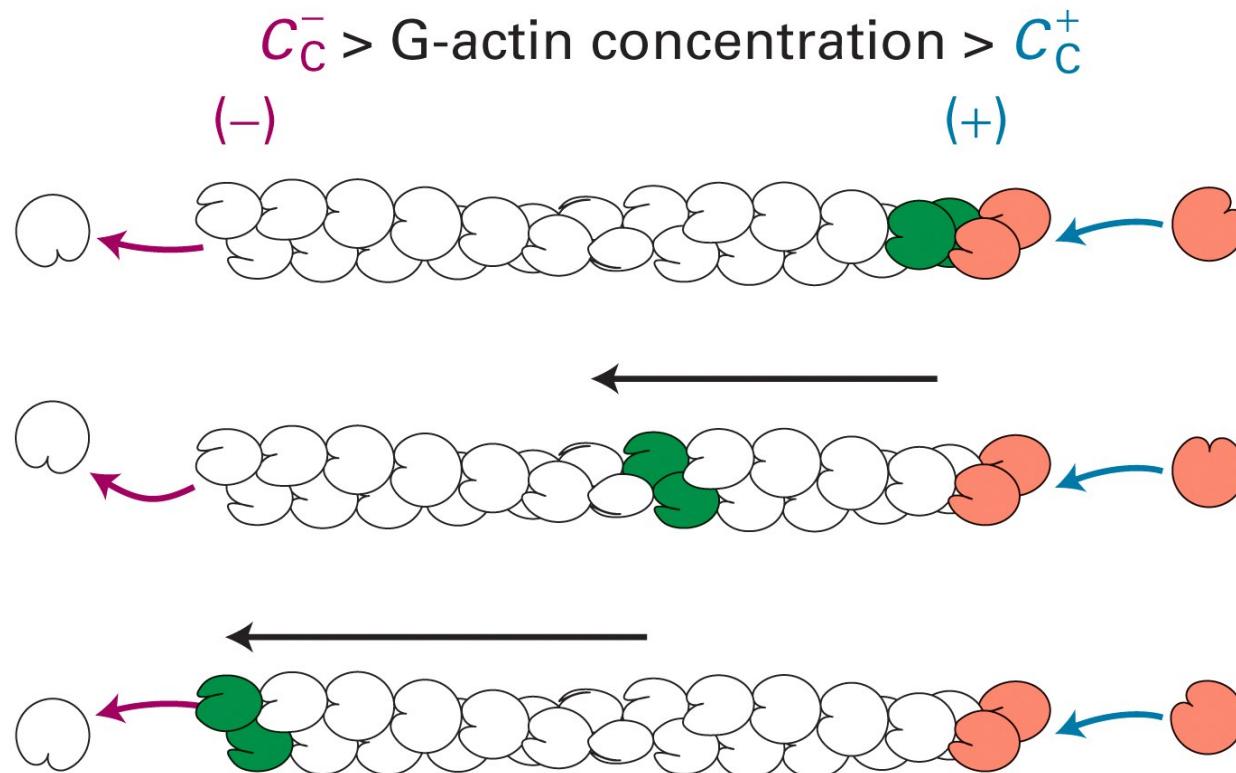
**The physical meaning of Cc**

**The plus end is more dynamic**

**The T cap has a lower Cc than the D form**

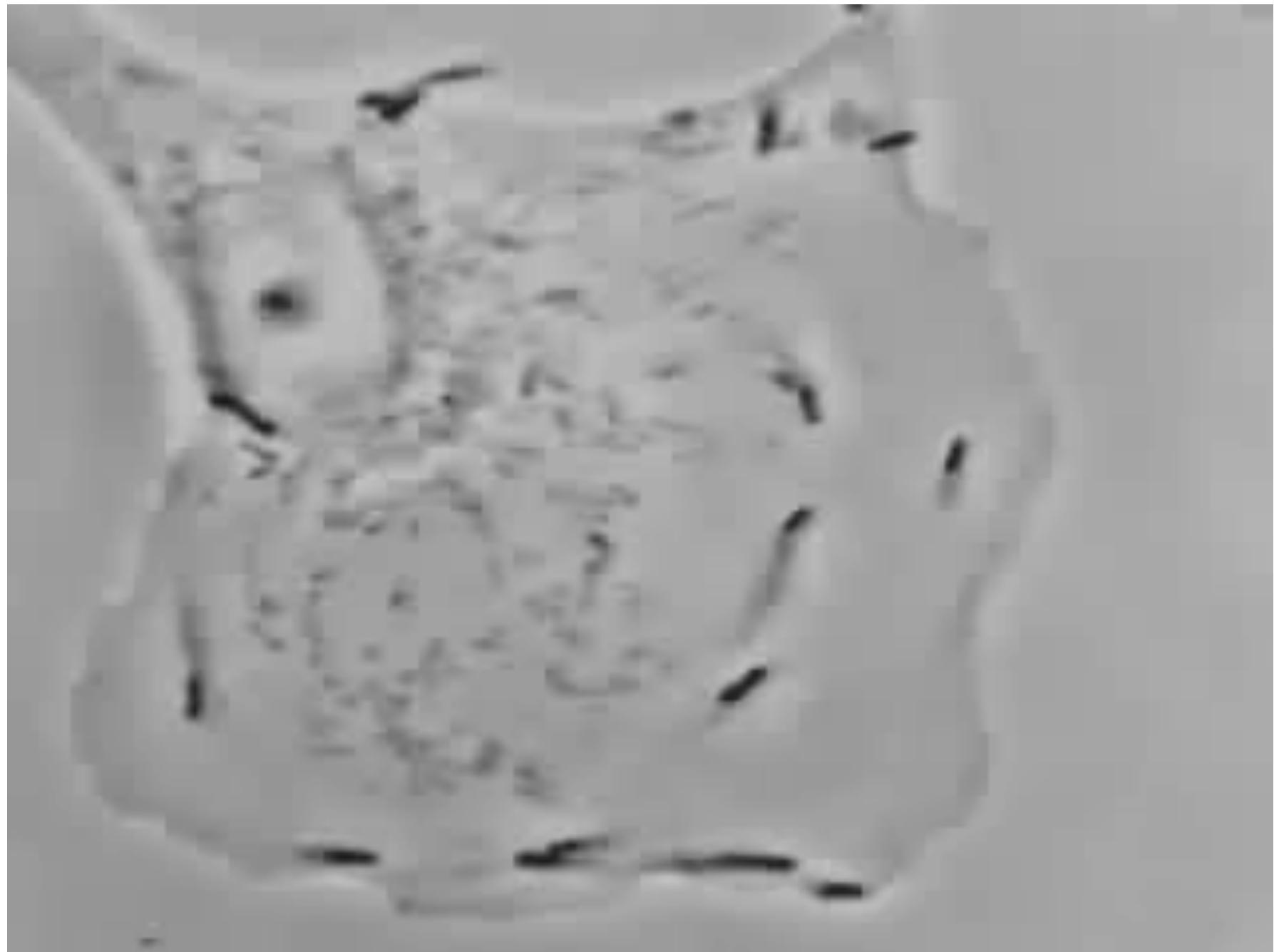
**What are the implications?**

# One Implication: Treadmilling

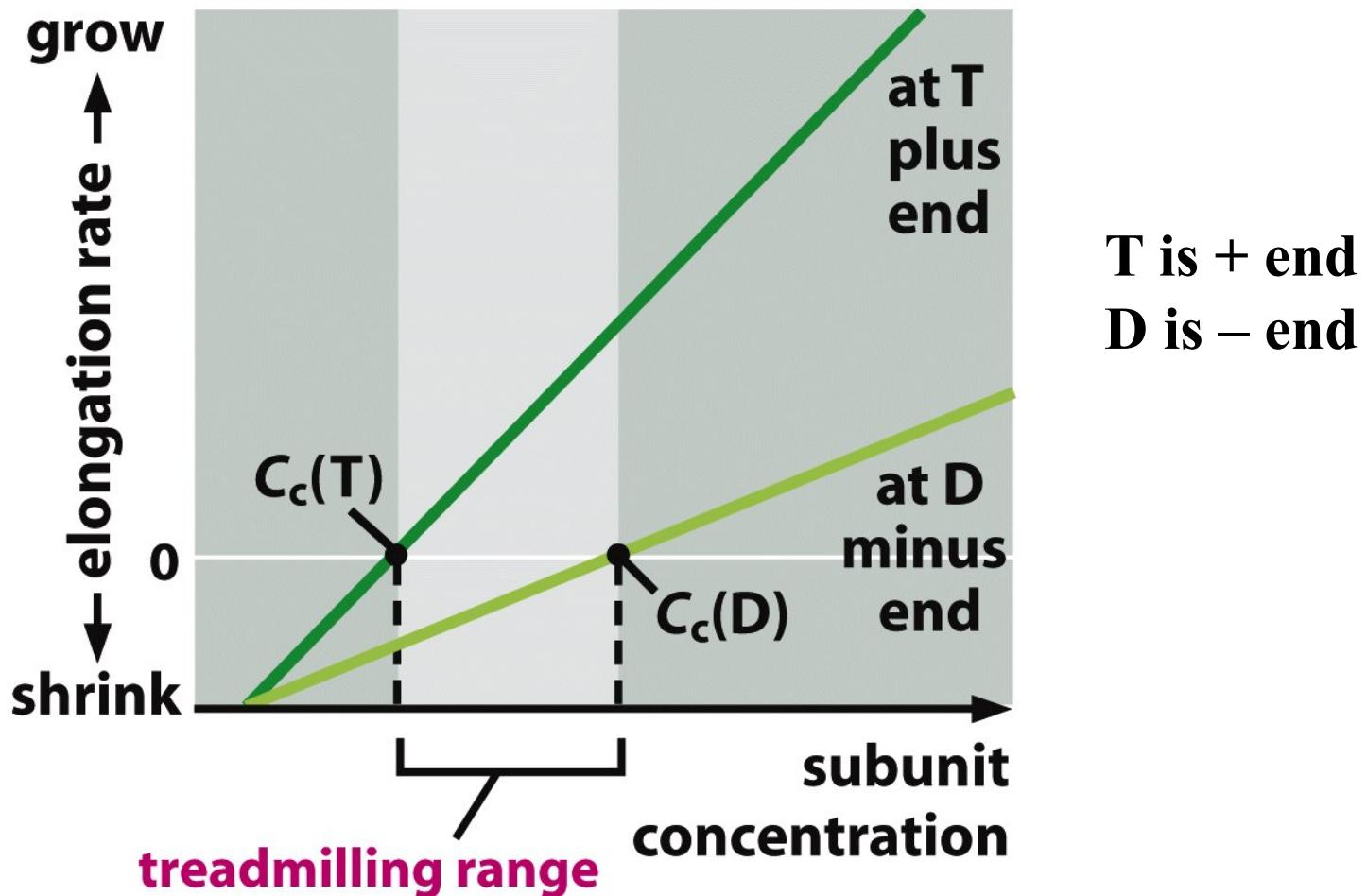


**Under the above condition, there will be a net assembly of F-actin at the plus end and a net disassembly at the minus end. This phenomena is called treadmilling.**

## **Listeria Uses Actin Polymerization (treadmilling) for Movement: Movie**



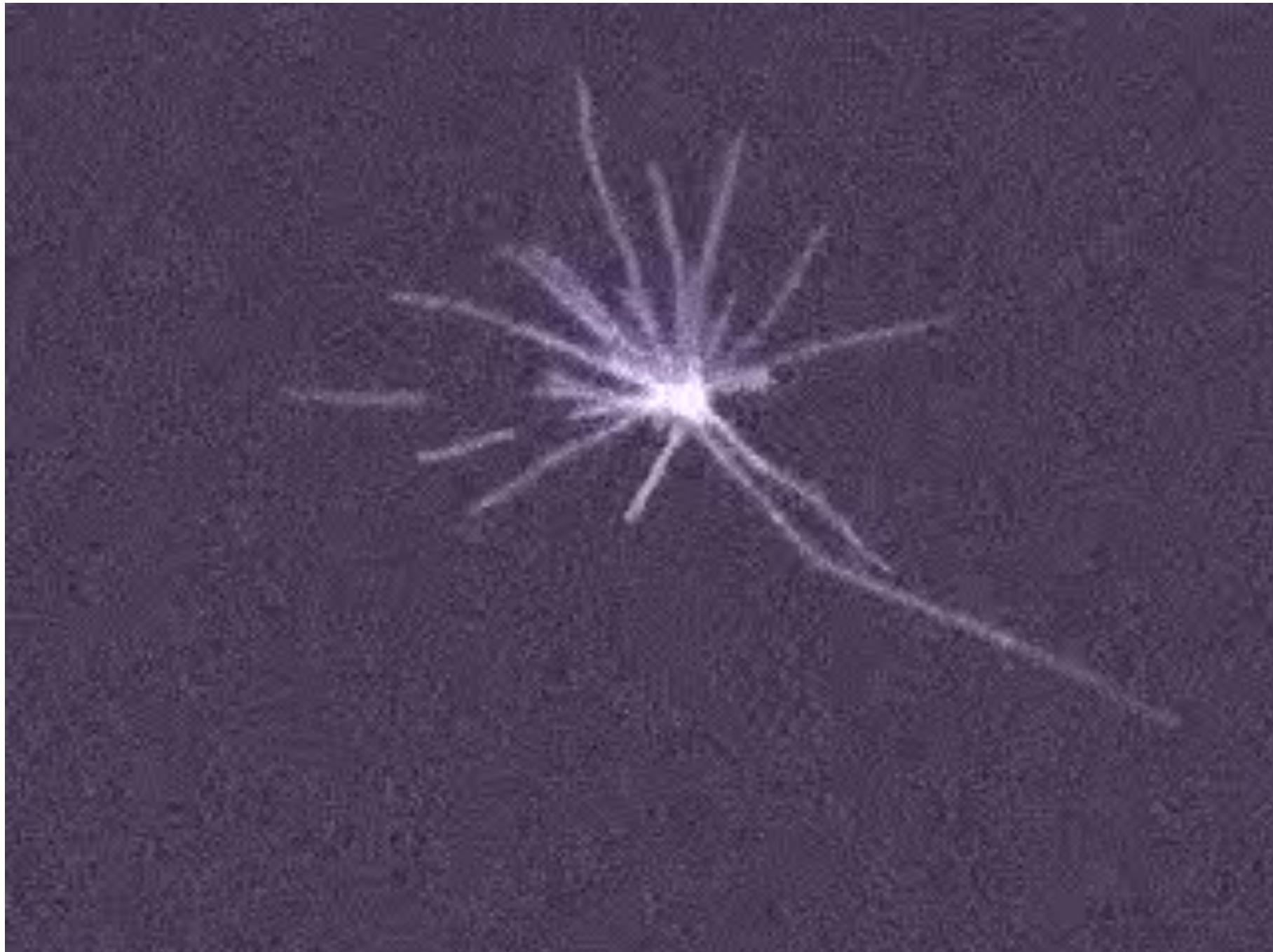
# Treadmilling



For  $C_c(T) < C < C_c(D)$

treadmilling occurs

## **Microtubules: Dynamic Instability: movie**

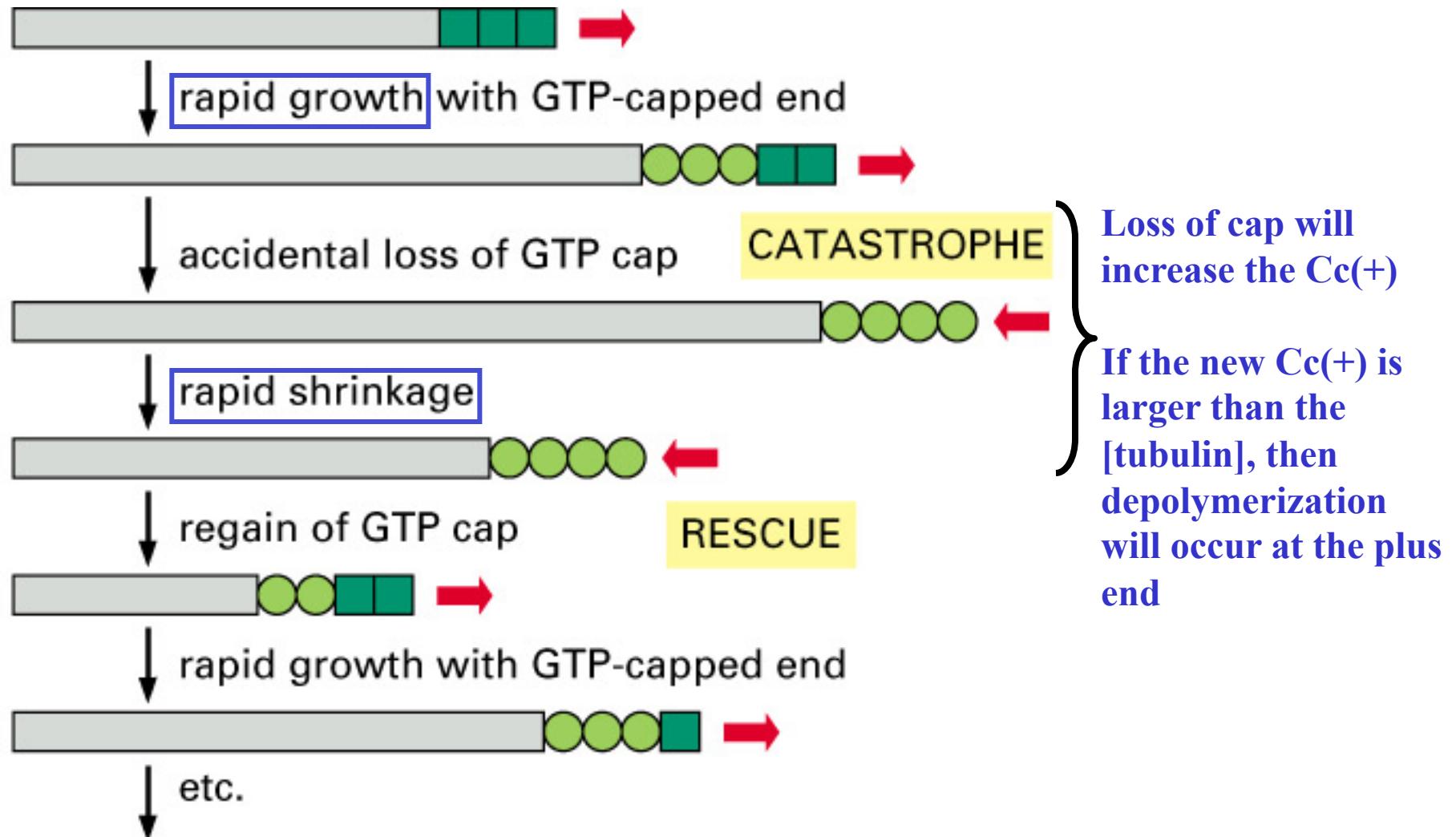


## **Microtubules – ER: movie**

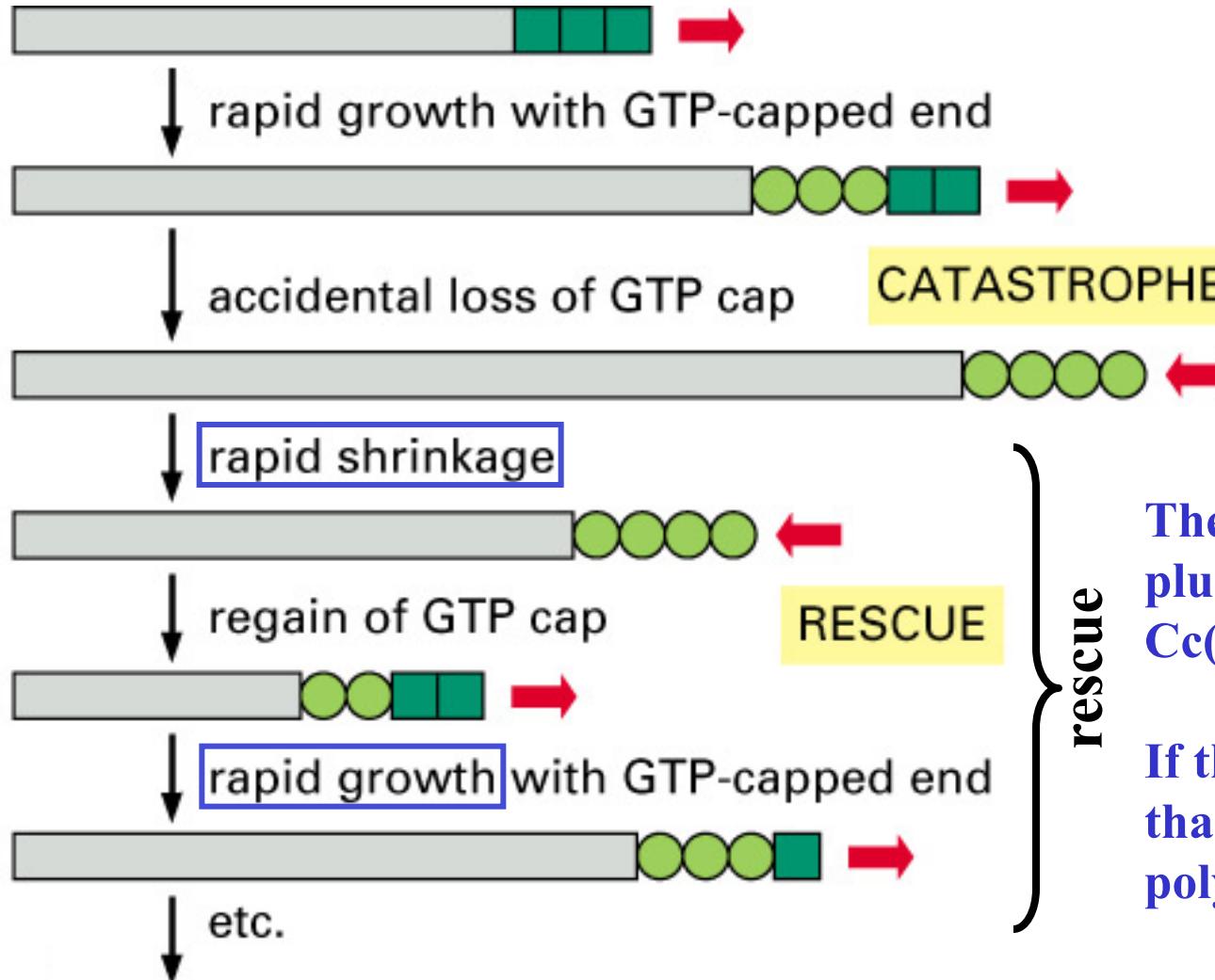


# Another Implication of Dynamic Instability (“Catastrophe”)

Catastrophe (rapid loss of filament) occurs after the removal of GTP or ATP cap



# Catastrophe can be Rescued if a Cap is Regained at the Plus End



The addition of a cap to the plus end will decrease the  $C_c(+)$  again.

If the new  $C_c(+)$  is smaller than the [tubulin], then polymerization will occur