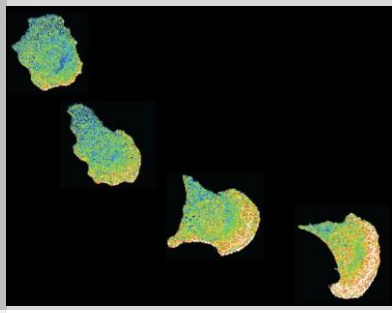
*Dynamics of cellular contacts: Cell-cell contacts and cell motility*

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# *Lecture 3: Cytoskeleton defining cell shape and organisation*

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**SLIDES ADAPTED FROM PREVIOUS VERSIONS BY**
Jeremie Rossy, Biotechnology Institute Thurgau, University of Konstanz
Urban Deutsch, Theodor Kocher Institute, University of Bern



# Structure of the lecture – Part 1
## Building knowledge on structures and function

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Introduction

**1:** Cell-cell junctions and cell migration at a glance – an introduction

Basic knowledge

**2:** Cell adhesion to extracellular matrix

**3:** Cytoskeleton: defining cell structure and membrane organisation

**4:** Molecular composition and function of cellular junctions

Function

**5:** Cell Motility : Polarization, protrusions, adhesive interactions and retraction translate into cell migration

**7a:**Cell migration in response to chemokines and synergy-inducing molecules in physiology and pathology

**7b:**Chemokine signaling pathways guiding cell migration

NEW

# OBJECTIVES OF THIS LECTURE





Recall the main structures of the cytoskeleton

CYTOSKELETON = FUNCTION

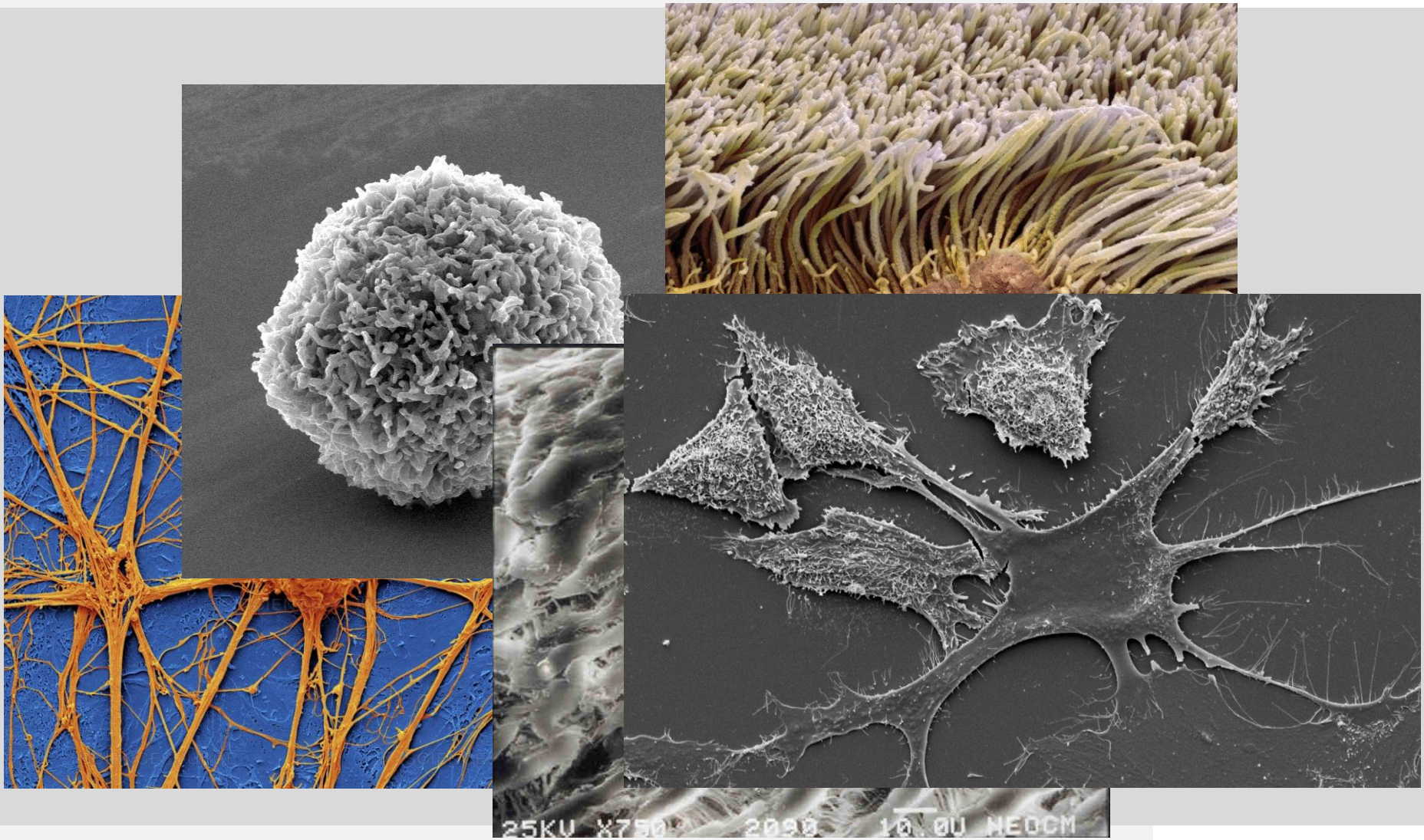
DYNAMICS

Implications for cell migration and tissue dynamics with a focus on immunology and oncology

Identify unmet needs and potential applications

# CELLS COME IN EVERY SHAPE



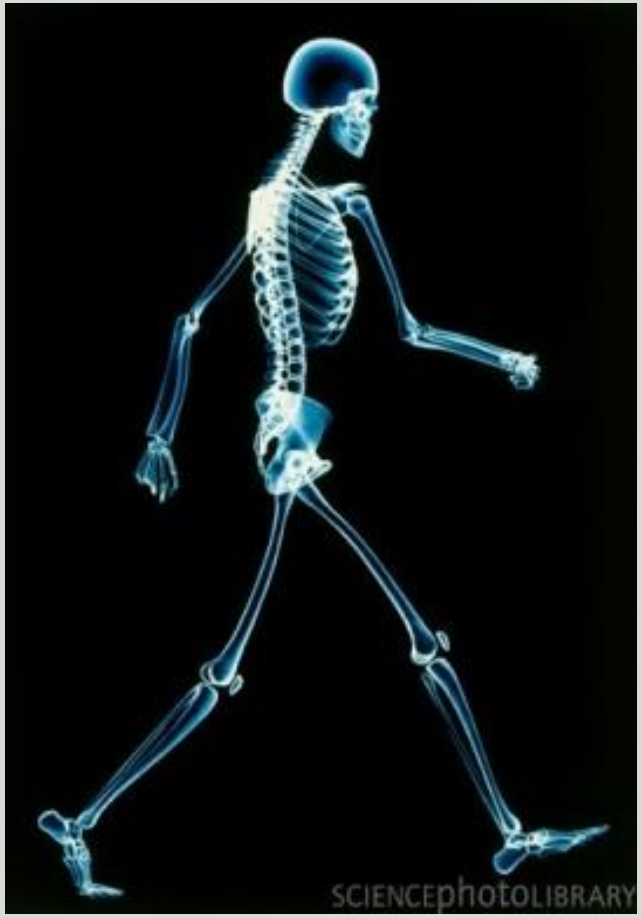


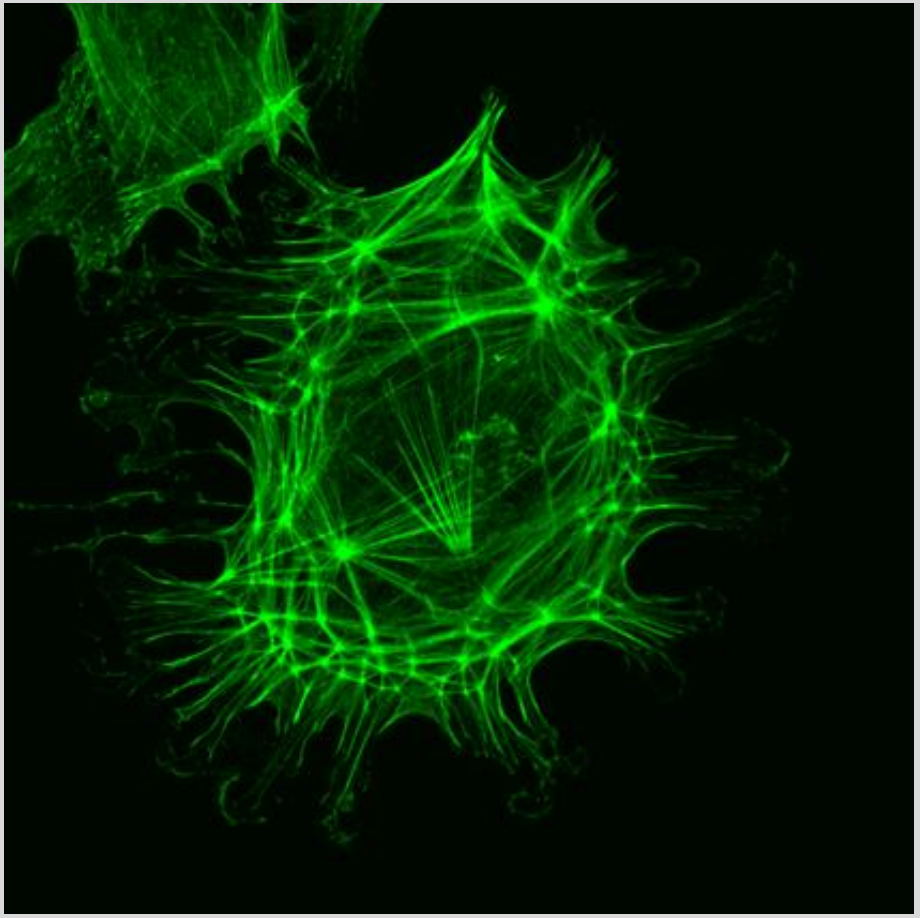
# CELLS COME IN EVERY SHAPE

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## WHAT GIVES A SHAPE?

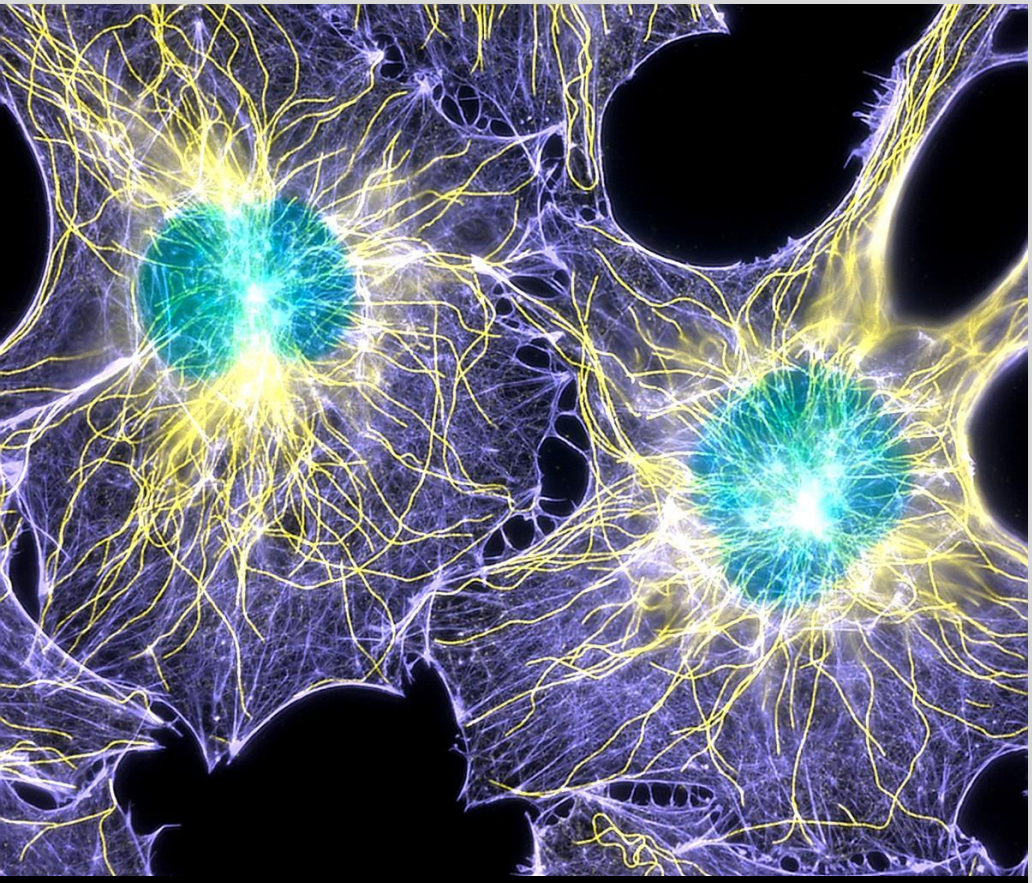
# A SCAFFOLD!



NEW

# Cytoskeleton: defining cell shape and organisation



***Mouse fibroblasts.***
*Actin filaments* *microtubules* *nuclei*
*Torsten Wittmann (2011), CIL:240. CIL. Dataset*
***https://doi.org/10.7295/W9CIL240***

WHAT IS THE MOST IMPORTANT THING IN THIS SLIDE?

NEW

# CIL: CELL IMAGE LIBRARY

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CELL IMAGE LIBRARY

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Cell Process | Cell Component | Cell Type | Organism | Microbial | Alzheimer's | Data Sets

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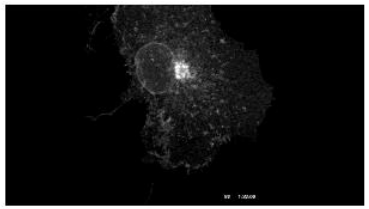
Did you miss our latest presentation from the 3rd Annual Cell Congress.
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**Proper Citation:** Cell Image Library (CIL) (RRID:SCR_003510)

## Featured Image



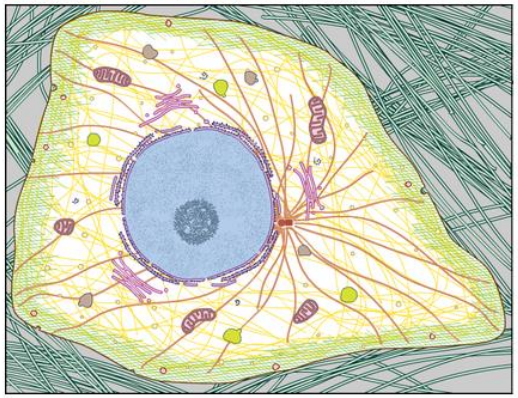
The Golgi Apparatus. In mammalian cells, the very complex architecture of the membrane system makes understanding the interrelationship of the different organelles within the cell difficult. Their dynamic relationship exists in four dimensions and is hard to capture at nanoscale resolutions. We pres...more

*Image contributed by Rachid Sougrat, Tim Mrozek, Jeremy Swan, Jennifer Lippincott-Schwartz*

## Images of Note



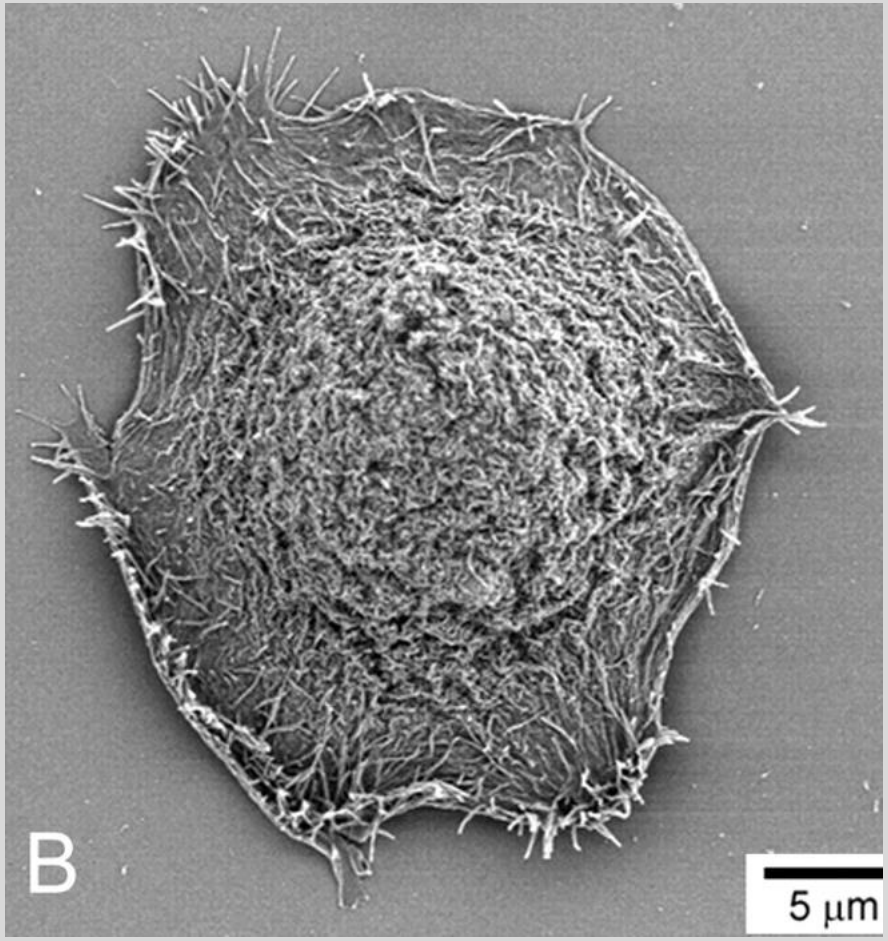
## Explore *the Eukaryotic Cell*

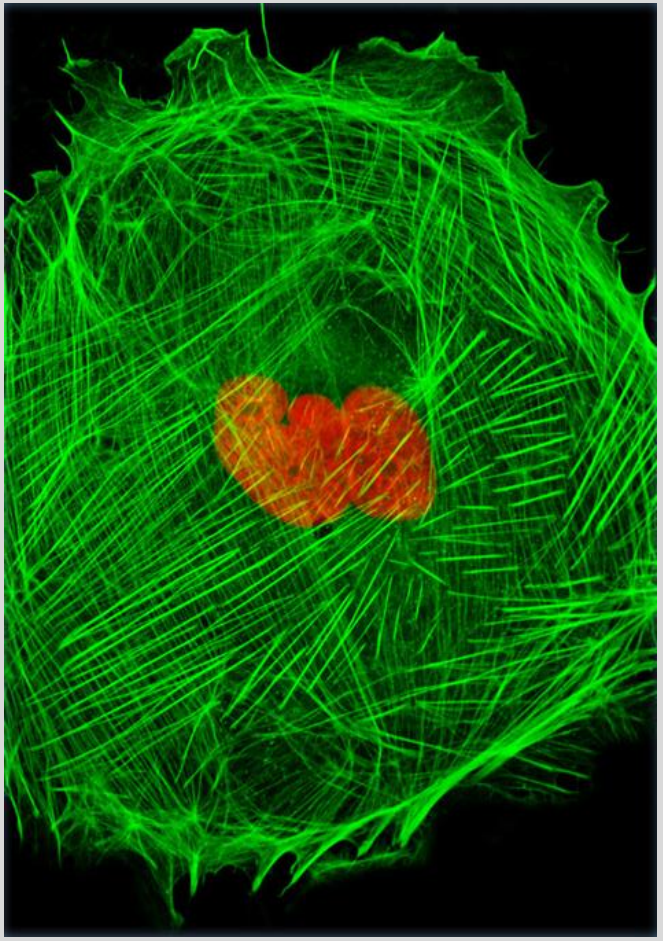


Actin Cytoskeleton
Chromosome
Endoplasmic Reticulum
Endosome
Extracellular Matrix
Golgi Apparatus
Intermediate Filament Cytoskeleton
Lysosome
Microtubule Cytoskeleton
Microtubule Organizing Centers
Mitochondrion
Nuclear Envelope
Nucleolus
Nucleus
Plasma Membrane
Ribosome
Vacuole
Vesicle

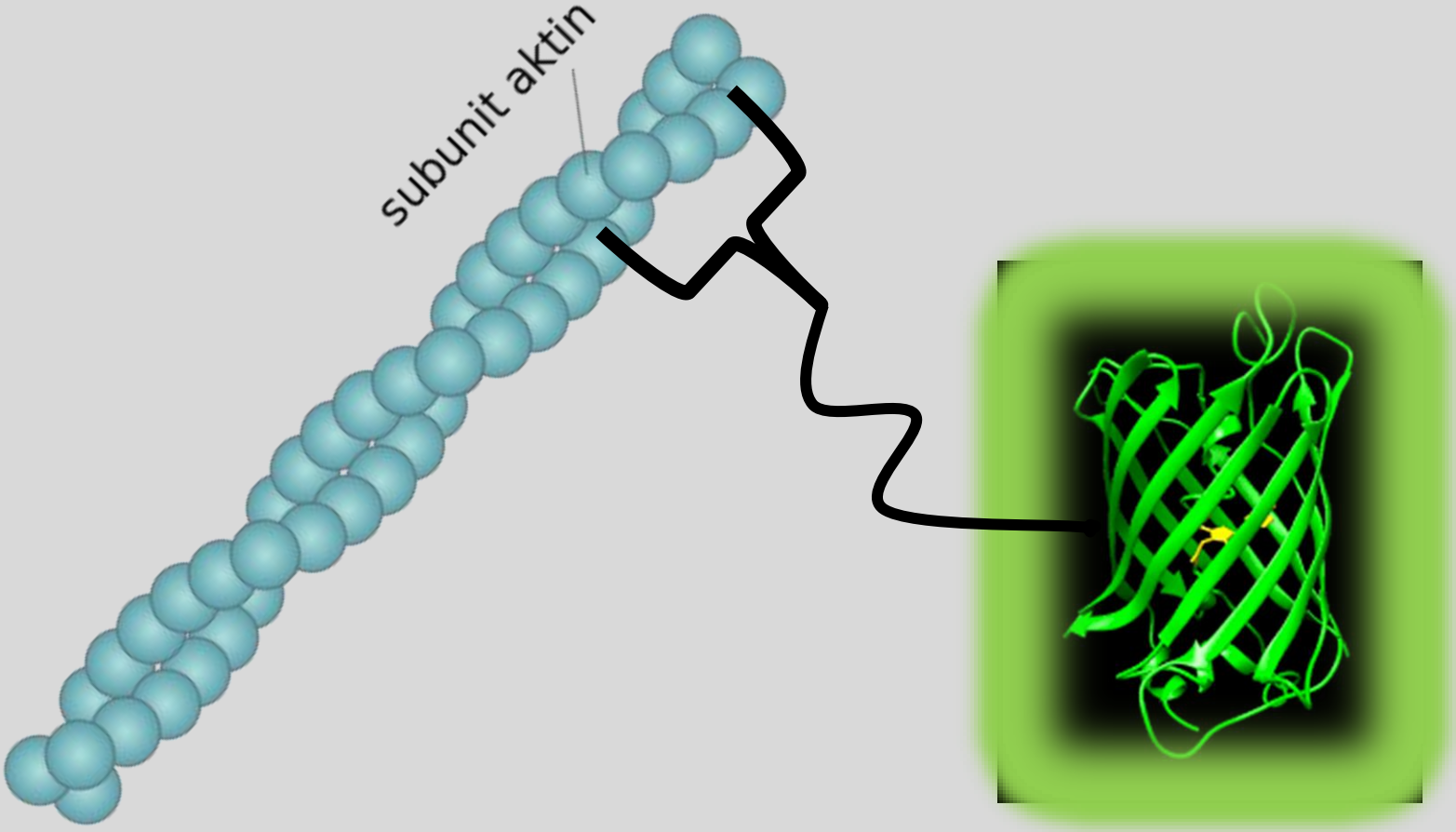
# How does a cell control its shape?



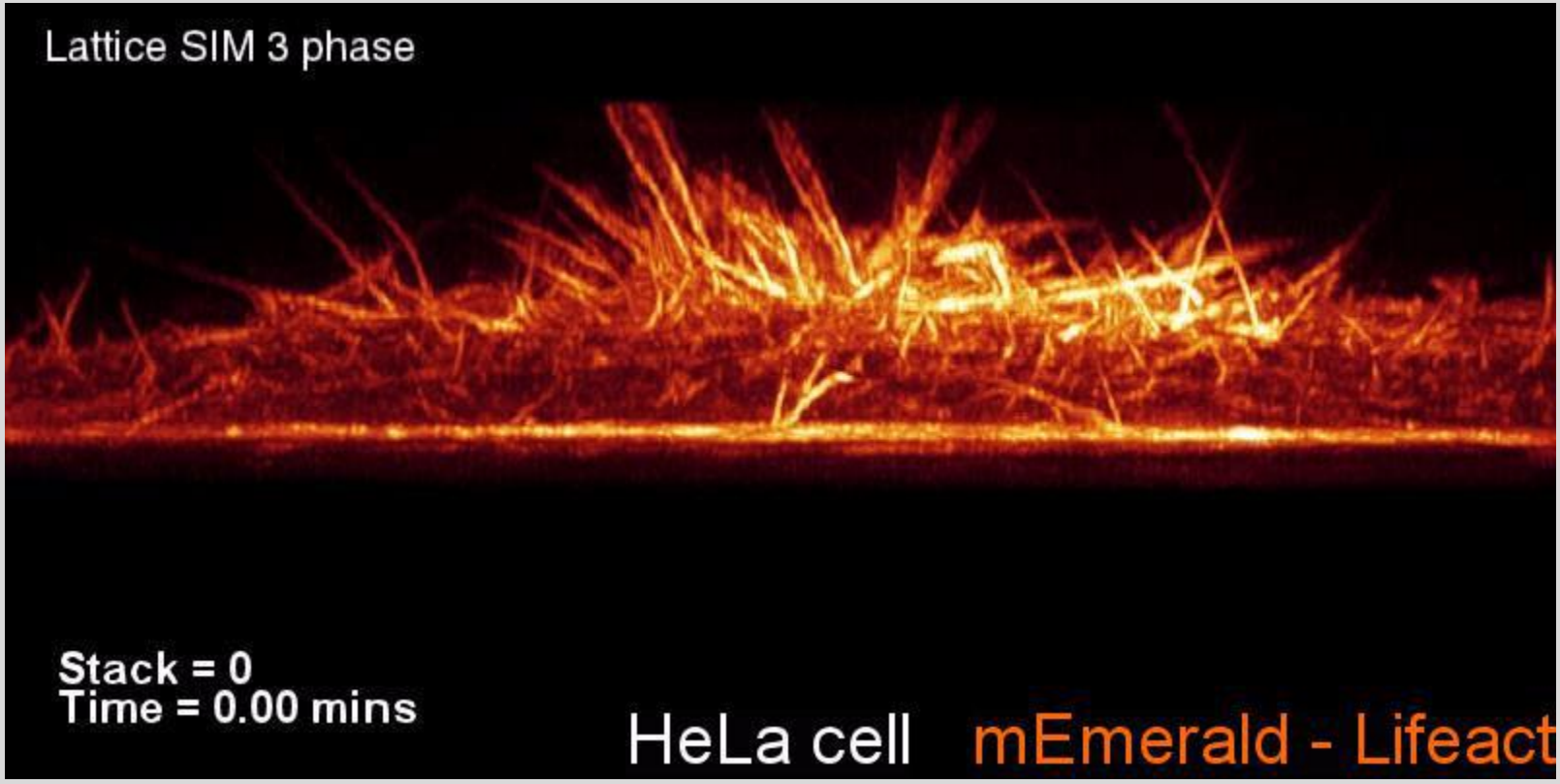


# LIFEACT: VISUALISING THE ACTIN CYTOSKELETON IN LIVE CELLS

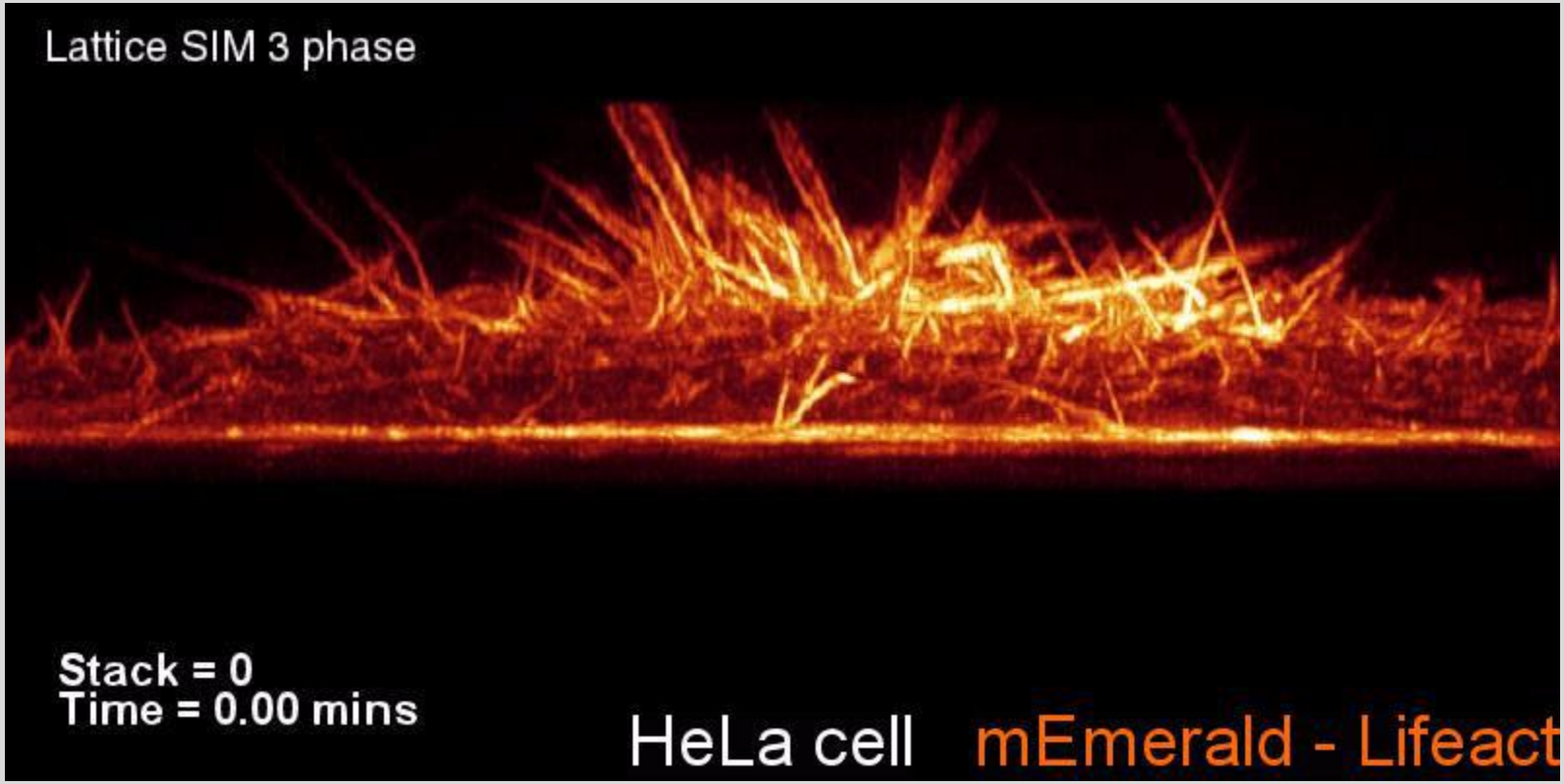


# CELLULAR STRUCTURES ARE DYNAMIC




Chen et al., Science, 2015

# CELLULAR STRUCTURES ARE DYNAMIC

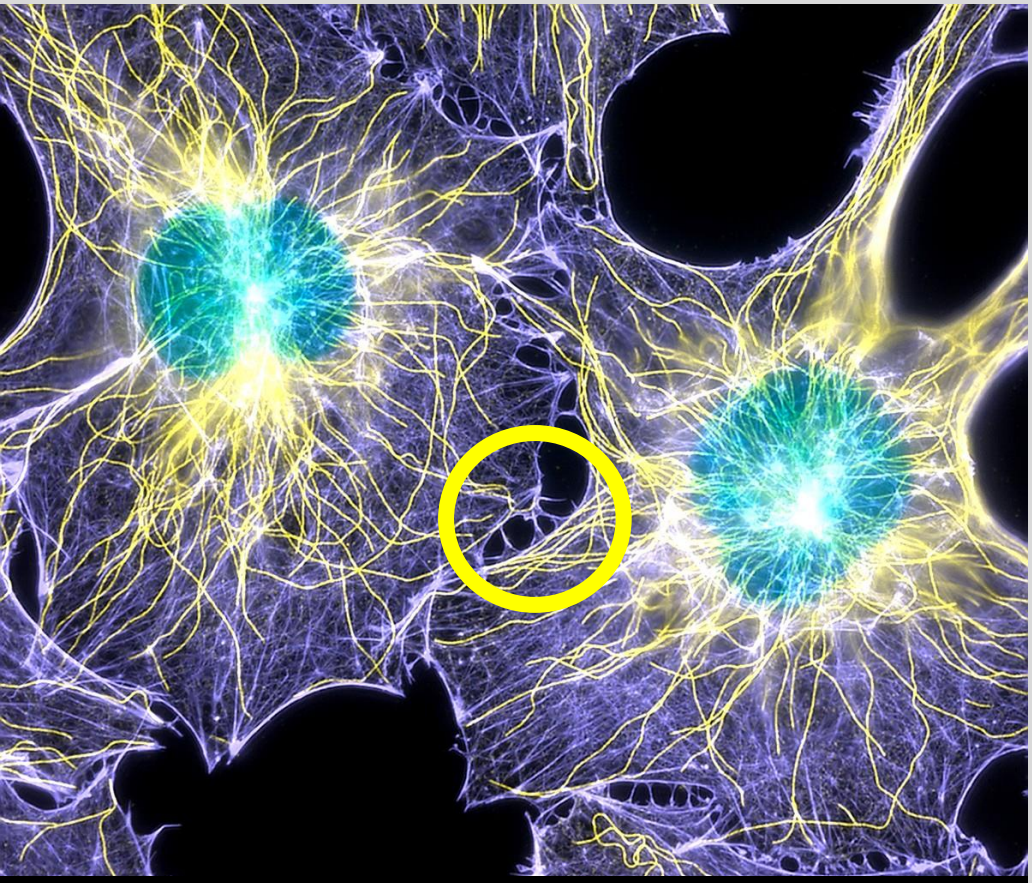



Chen et al., Science, 2015

NEW

# Cytoskeleton: defining cell shape and organisation …

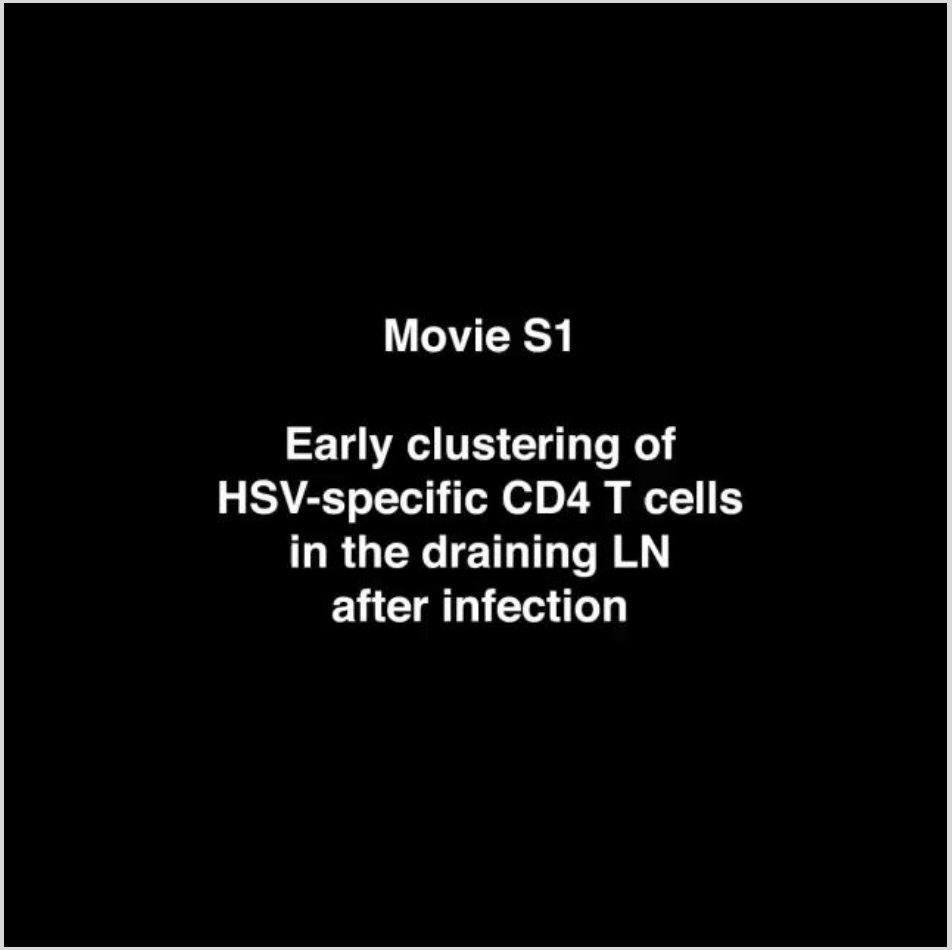
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***Mouse fibroblasts.***
*Actin filaments* *microtubules* *nuclei*
*Torsten Wittmann (2011), CIL:240. CIL. Dataset*
***https://doi.org/10.7295/W9CIL240***

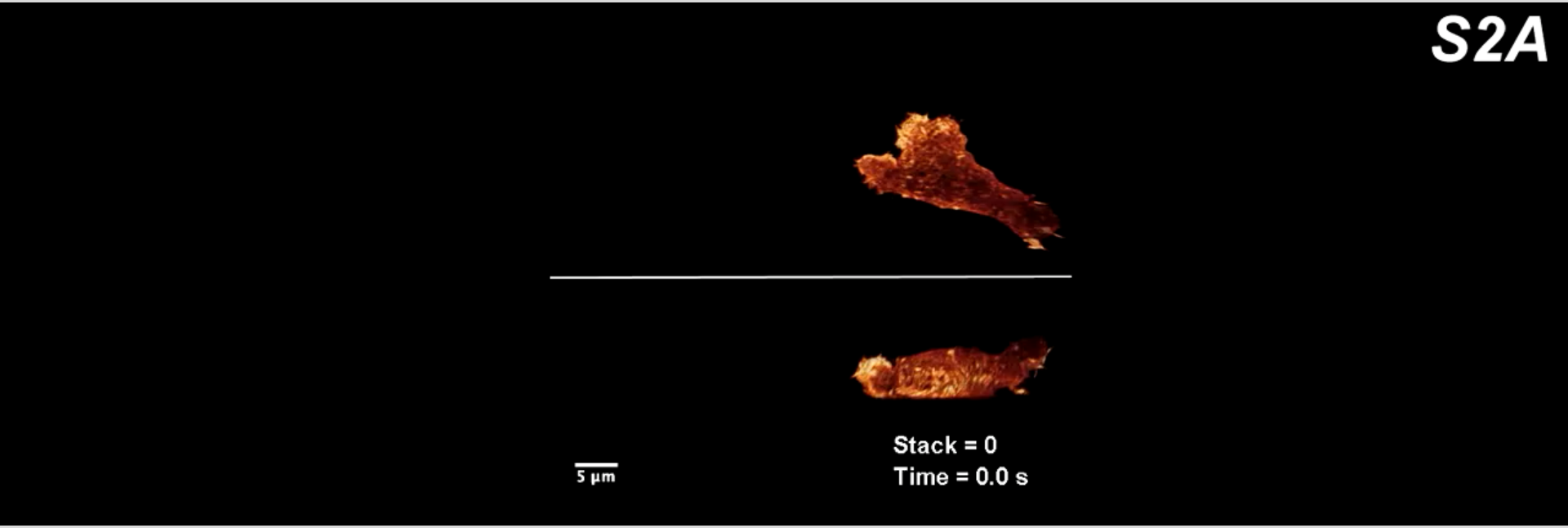
## … and function

# SOME CELLS ARE VERY DYNAMIC




Hor et al., Immunity, 2015

# THIS MEANS THAT THEIR SCAFFOLD IS DYNAMIC.




Ritter et al., Immunity, 2015

NEW

# WHAT IS THE CYTOSKELETON MADE OF?

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**Microtubules (MT)**

25 nm diameter

•Made of repeating subunits (tubulin).

•Go through rounds of polymerization and depolymerization (dynamic instability).

•Cargo trafficking, mitosis, maintenance of cell shape, movement of vilia and flagella.

**Intermediate filaments (IF)**

•10 nm diameter.

•Five major types (e.g., keratins, vimentin, neurofilaments, and nuclear lamins).

•No mix. No dynamic instability. Assembled and disassembled as needed.

•Anchoring not-motile cells, resist mechanical strain, maintena cell shape.

**Actin filaments (AF)**

•7 nm diameter.

•Made of repeating subunits, also called actin.

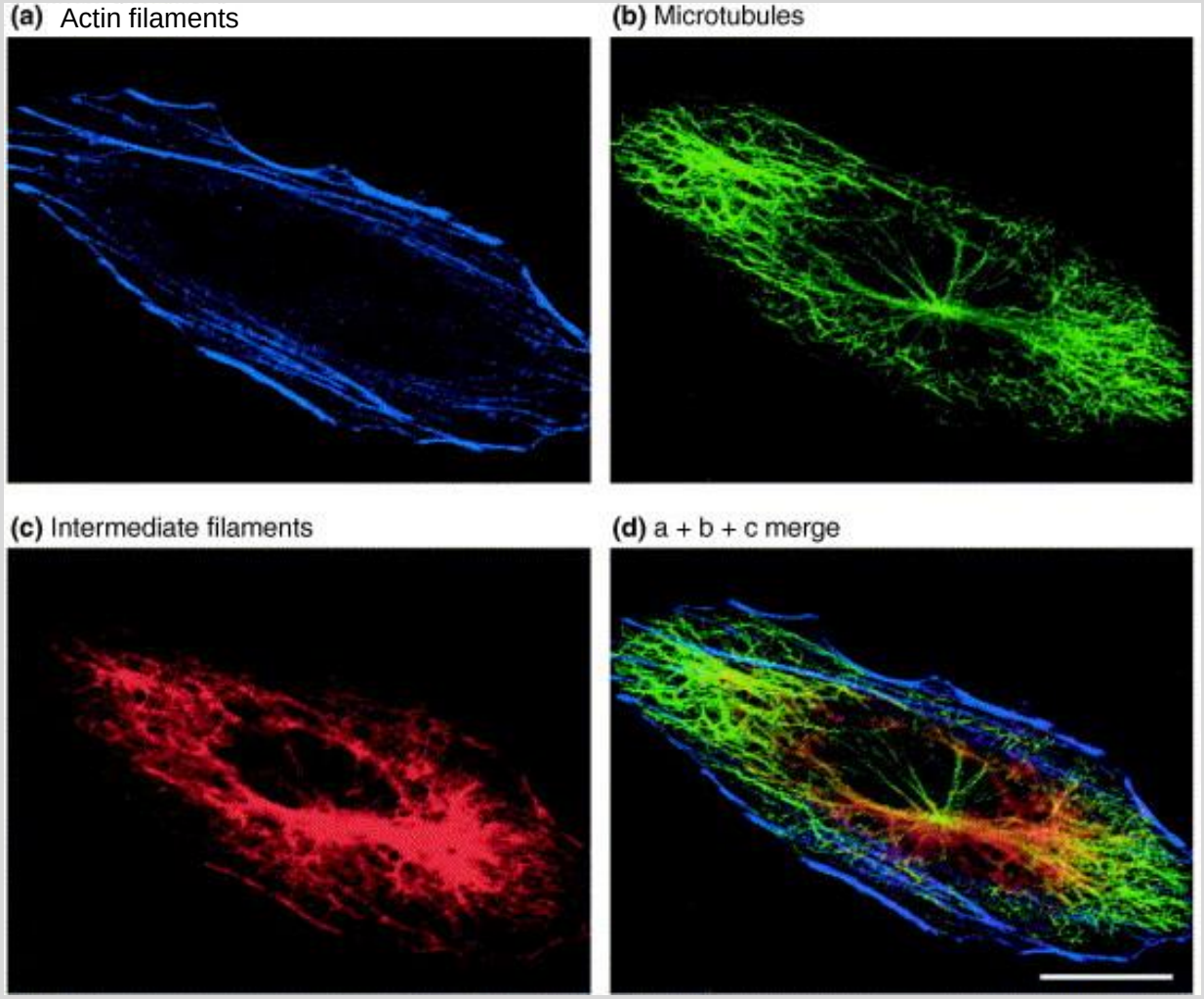
•Monomers: *g-actin; F*ilament form: *f-actin*.

•Like microtubules, they also undergo dynamic instability.

•Cellular locomotion, and shape change, adhesion, cargo trafficking, and contraction.

Omary et al. 2006. TIBS

# WHAT IS THE CYTOSKELETON MADE OF?

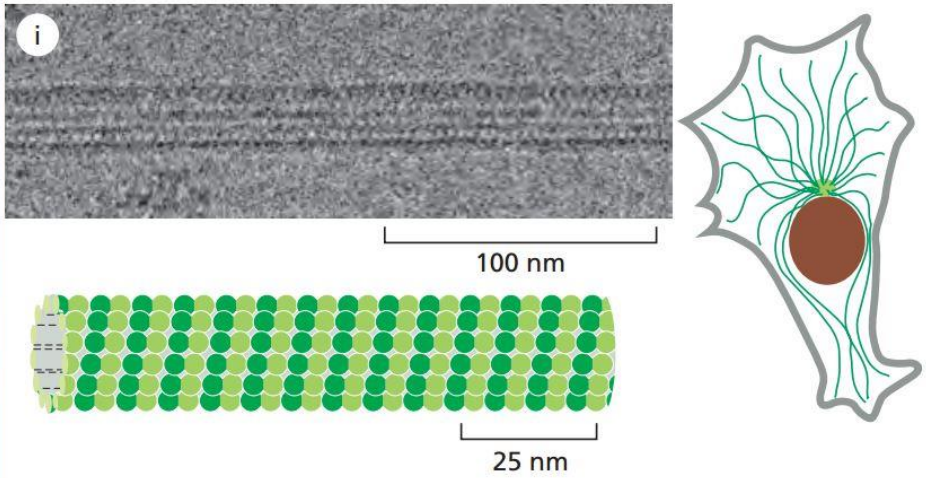


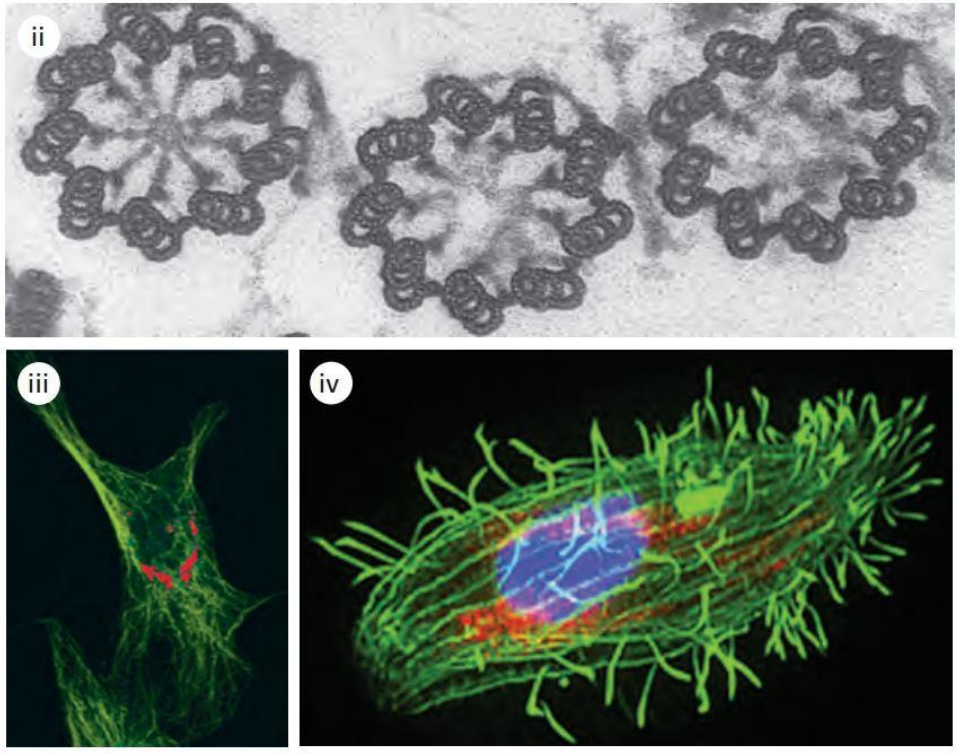

Omary et al. 2006. TIBS

# MICROTUBULES



## MICROTUBULES



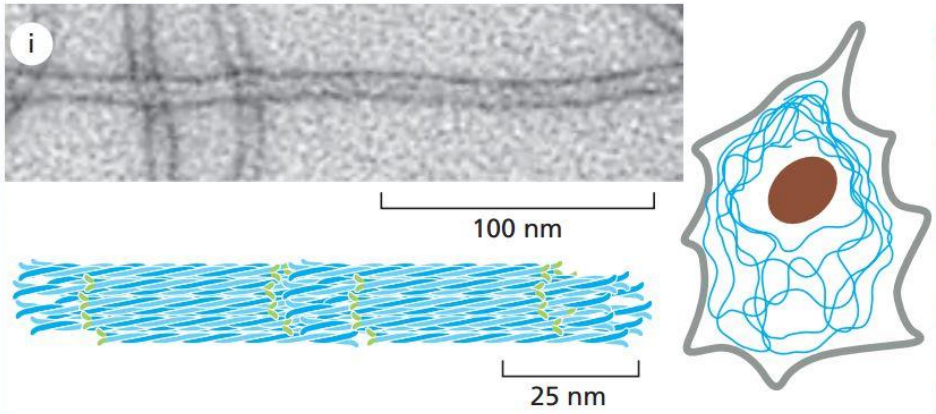

Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and frequently have one end attached to a microtubule-organizing center (MTOC) called a *centrosome*. (i) Single microtubule; (ii) cross section at the base of three cilia showing triplet microtubules; (iii) interphase microtubule array (*green*) and organelles (*red*); (iv) ciliated protozoan.

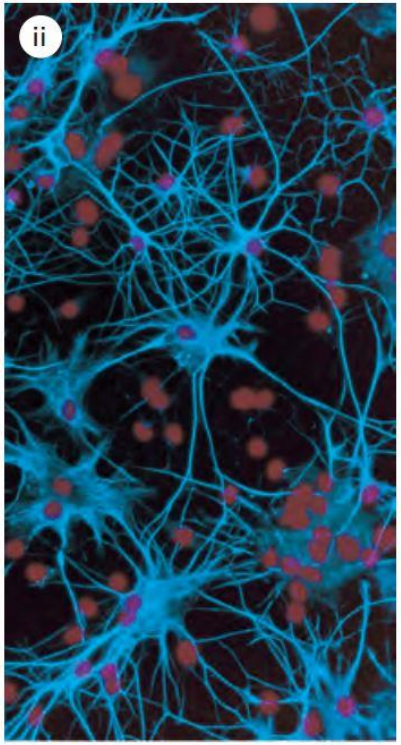
Micrographs courtesy of R. Wade (i); D.T. Woodrow and R.W. Linck (ii); D. Shima (iii); D. Burnette (iv).

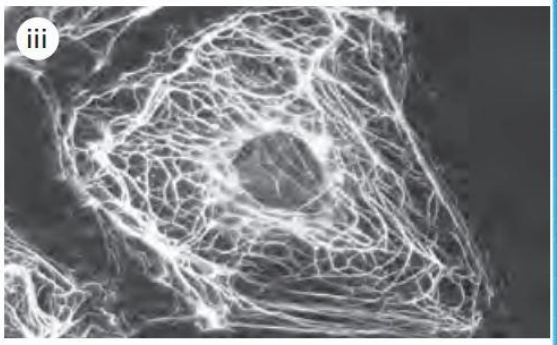
# INTERMEDIATE FILAMENTS

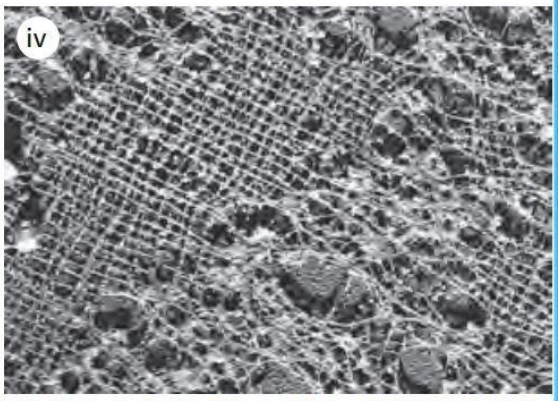


## INTERMEDIATE FILAMENTS



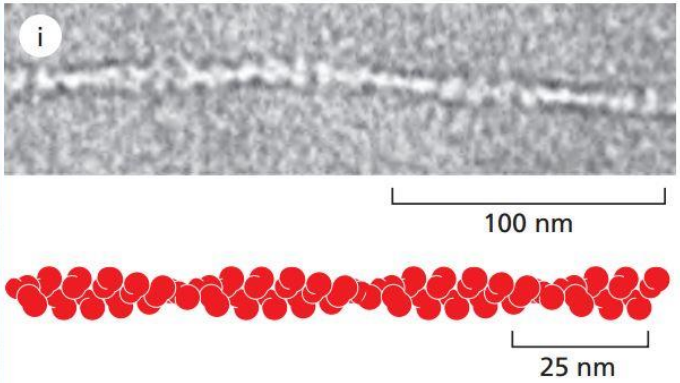

Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell–cell junction to another, thereby strengthening the entire epithelium. (i) Individual intermediate filaments; (ii) Intermediate filaments (*blue*) in neurons and (iii) epithelial cell; (iv) nuclear lamina.

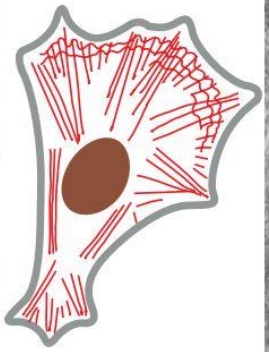
Micrographs courtesy of R. Quinlan (i); N. L. Kedersha (ii); M. Osborn (iii); U. Aebi (iv).

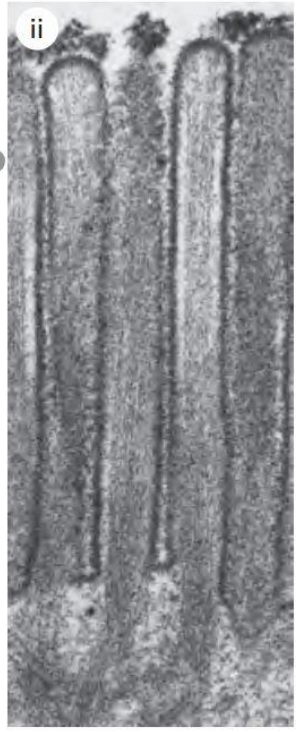
# ACTIN-FILAMENTS

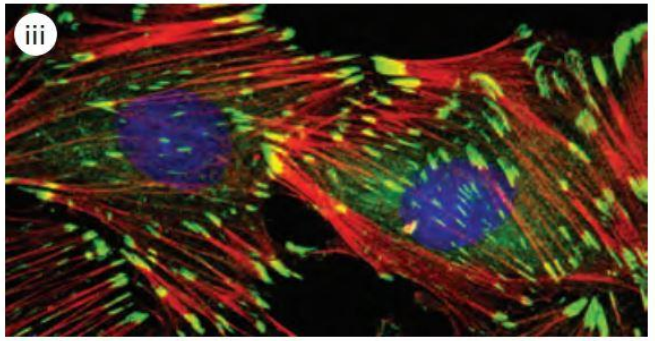


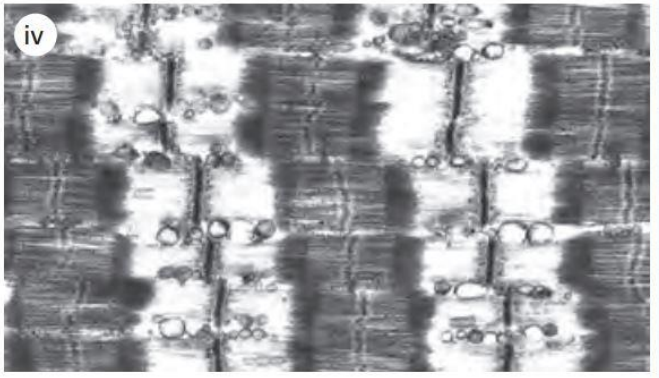
## ACTIN FILAMENTS






Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures with a diameter of 8 nm that organize into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane. (i) Single actin filament; (ii) microvilli; (iii) stress fibers (*red*) terminating in focal adhesions (*green*); (iv) striated muscle.

Micrographs courtesy of R. Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); K. Burridge (iii).

# WHAT IS THE CYTOSKELETON MADE OF

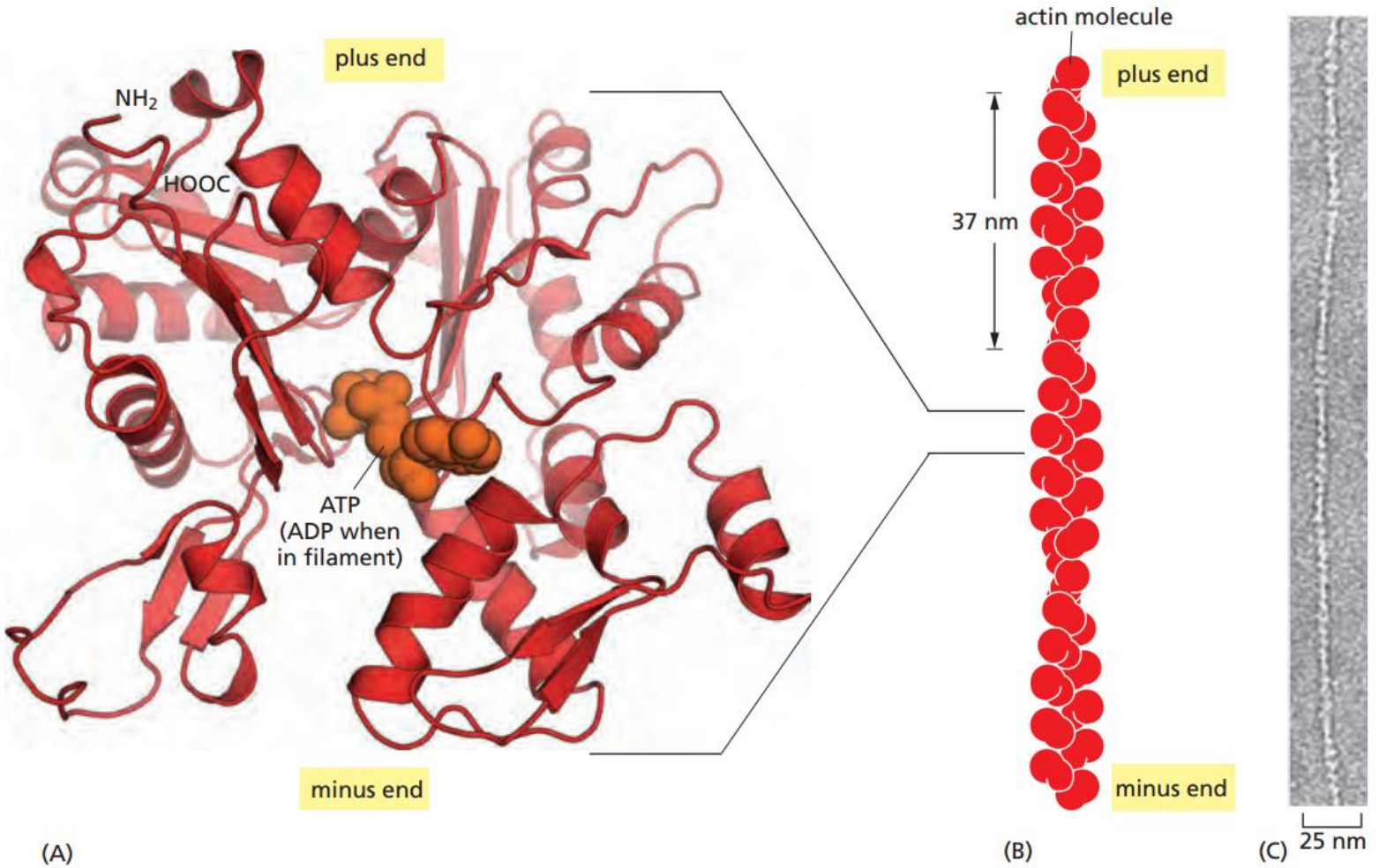
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**Filamentous (F)-actin**
- Cell surface projections: lamellipodia, filopodia
- Cell contractions
- Organisation of the plasma membrane
- Muscle fibres

**Microtubules**
- Mitotic spindle
- Core of cilia, flagella
- Transport and organisation of cellular organelles

**Intermediate filaments**
- Line the inner face of the nuclear envelope
- Connect to desmosomes and hemidesmosomes
- Hold epithelial cell sheets together
- Stabilize neuronal axons
- Structural components of hair and fingernails

# ACTIN




Alberts: Molecular Biology of the Cell, 6th edition.

NEW



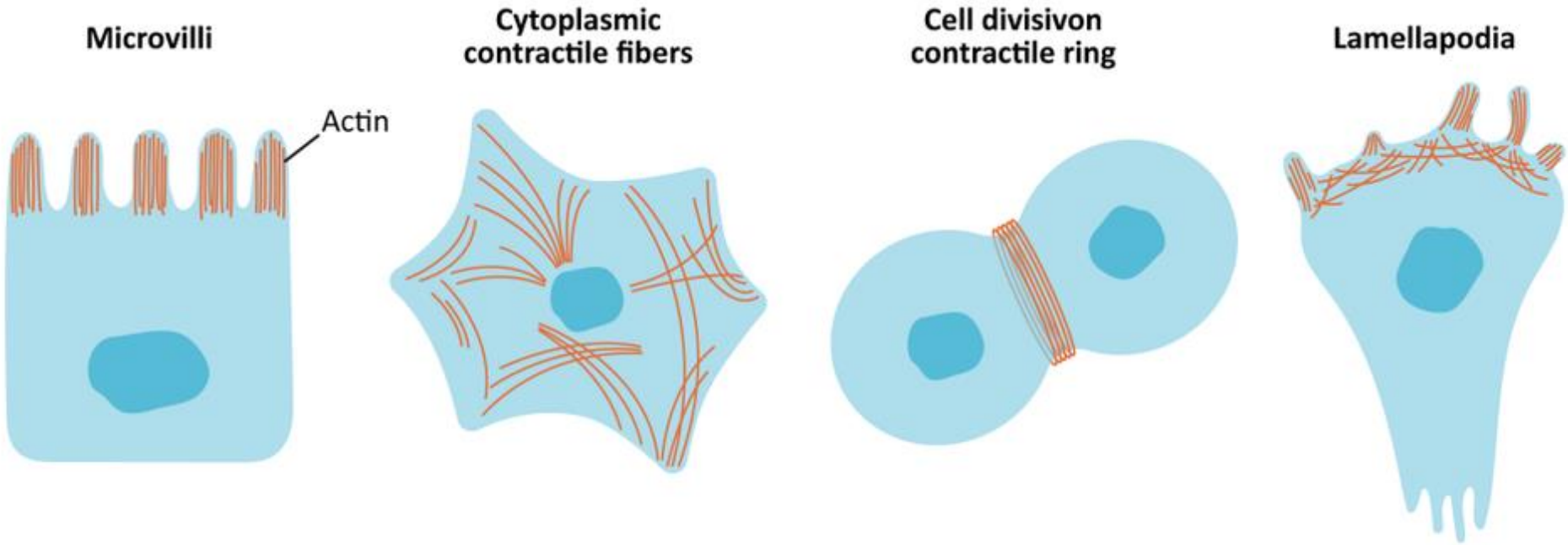
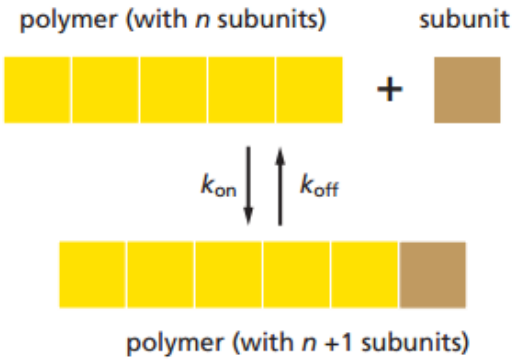



*Figure 06-21: Example cell structures formed by actin arrays in animal cells. The actin filaments are shown in orange. This image was created by Heather Ng-Cornish and is shared under a* ***CC BY-SA 4.0*** *license.*

# ACTIN DYNAMICS

## 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 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.

At this equilibrium,

$$k_{on}\,C = k_{off}$$

so that

$$C_c = \frac{k_{off}}{k_{on}} = K_d$$

(where $K_d$ is the dissociation constant; see Figure 3–44).

$C < C_c$ → depolymerization
$C > C_c$ → polymerization

(C = the actual concentration of actin monomers)

NEW

# CRITICAL CONCENTRATION

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## BANK EQUATION

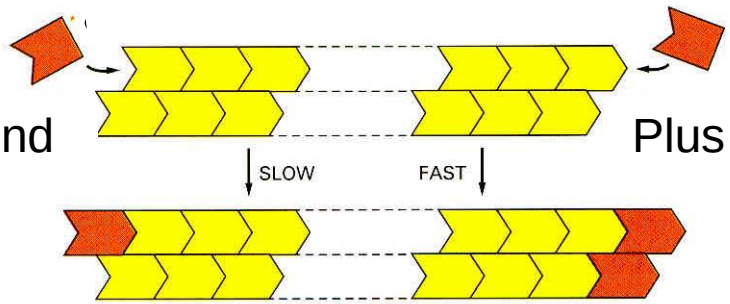
FIXED COSTS = 20 CHF / MONTH

REWARD = 0.4%

INCOME = 200 CHF / YEAR, WHAT HAPPENS?

INCOME = 200’000 CHF YEAR, WHAT HAPPENS?

# DIFFERENT ACTIN POLYMERIZATION KINETICS AT PLUS AND MINUS ENDS




**Minus end:**
**Slow** association and dissociation of actin subunits

**Plus end:**
**Rapid** association and dissociation of actin subunits (≤ 10 X faster than minus end)

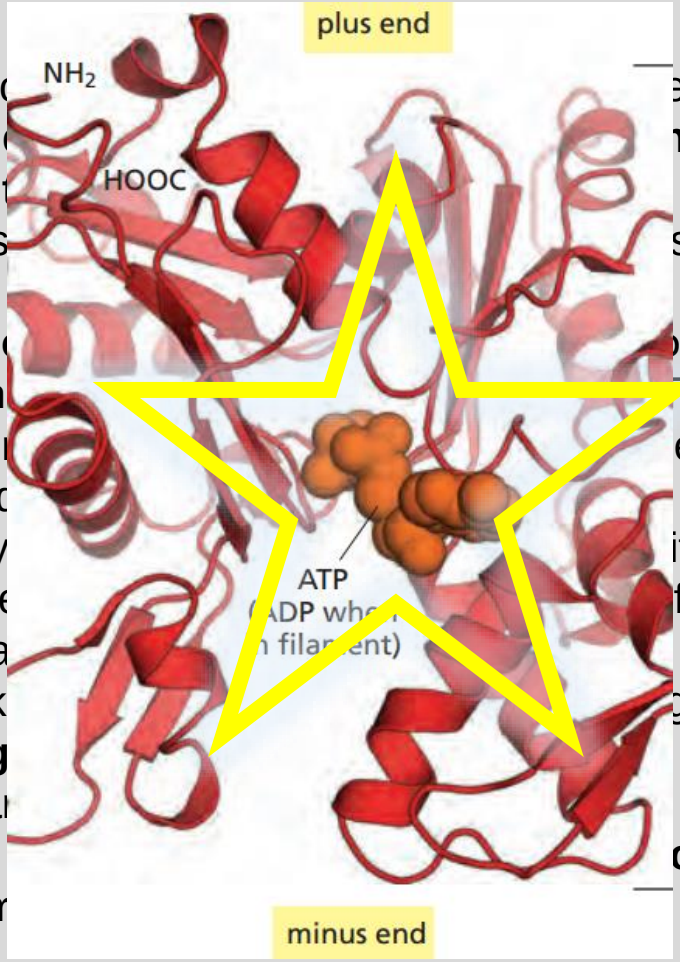
- Due to the **uniform orientation of asymmetric actin subunits** in the filament, the structures at its two ends are different
- This orientation makes the two ends of each polymer different in ways that have a profound effect on filament growth rates
- The kinetic rate constants for actin subunit association and dissociation— $k_{on}$ and $k_{off}$, respectively—**are much greater at the plus end than the minus end**
- This can be seen when an excess of purified actin monomers is allowed to assemble onto polarity-marked filaments—the plus end of the filament elongates up to ten times faster
- If filaments are rapidly diluted so that the free subunit concentration drops below the critical concentration, the plus end also depolymerizes faster

Molecular Biology of the Cell, 6th edition. Alberts

# ACTIN THREADMILLING

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- Actin can catalyze the hydr… e ATP. For free actin subunits, this hydrolysis proceeds very sl… **hen the subunits are incorporated into filamen…**
- Shortly after ATP hydrolysis… s released from each subunit, but the **ADP remains trapped …**
- Thus, **two different types …** one with the "**T form**" of the nucleotide bound (ATP), an… )
- When the nucleotide is hydr… eased by cleavage of the phosphate–phosphate bond…
- This makes the free-energy… it from the D-form polymer more negative (lower) than the fre… f a subunit from the T-form polymer (D is easier to deta…
- Consequently, the ratio of k… ger than the corresponding ratio for the **T-form** polymer (**hig…**
- **Thus, $C_c$(D) is greater tha…**
- This means that **at certain … omers, D-form polymers will shrink while T-form polym…**

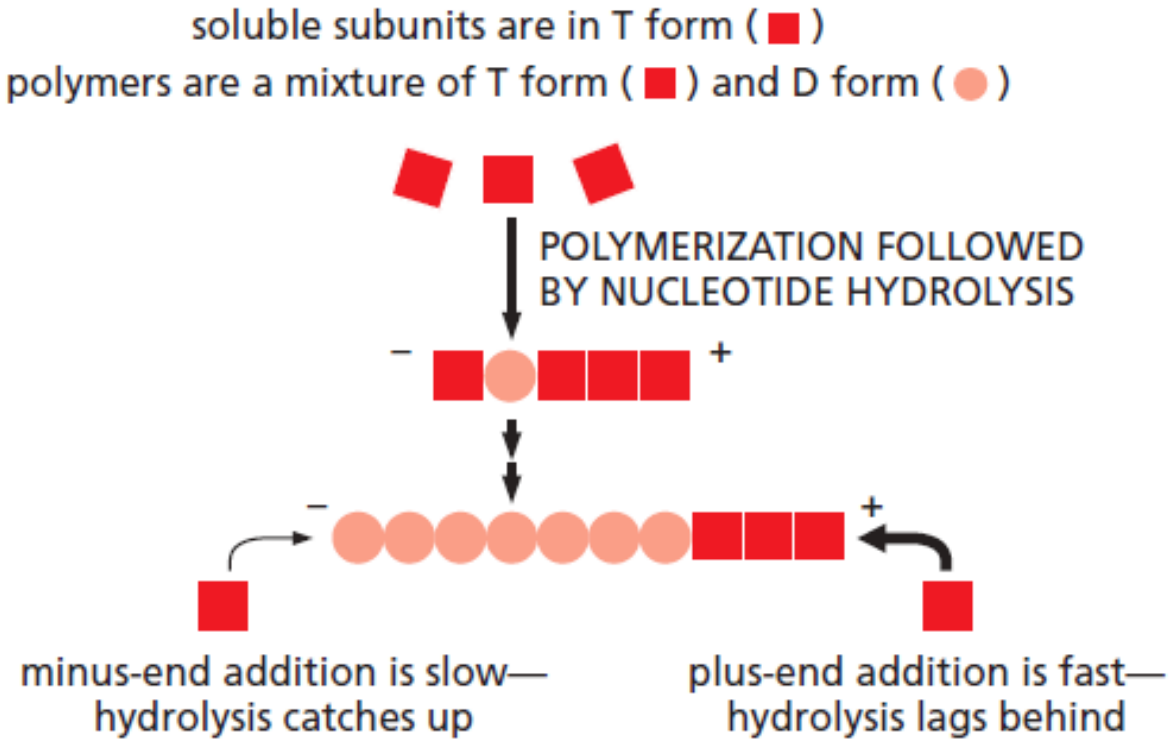



Alberts: Molecular Biology of the Cell, 6th edition.

NEW

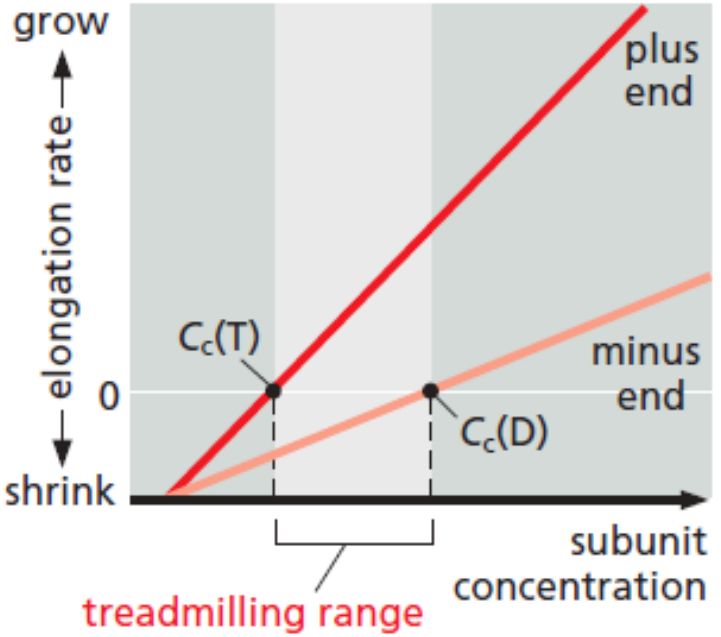
# ACTIN THREADMILLING (question in the exam!)

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$C_c(T) < C_c(D)$

(A)




(B) For $C_c(T) < C < C_c(D)$ treadmilling occurs

NEW

# ACTIN THREADMILLING (question in the exam!)

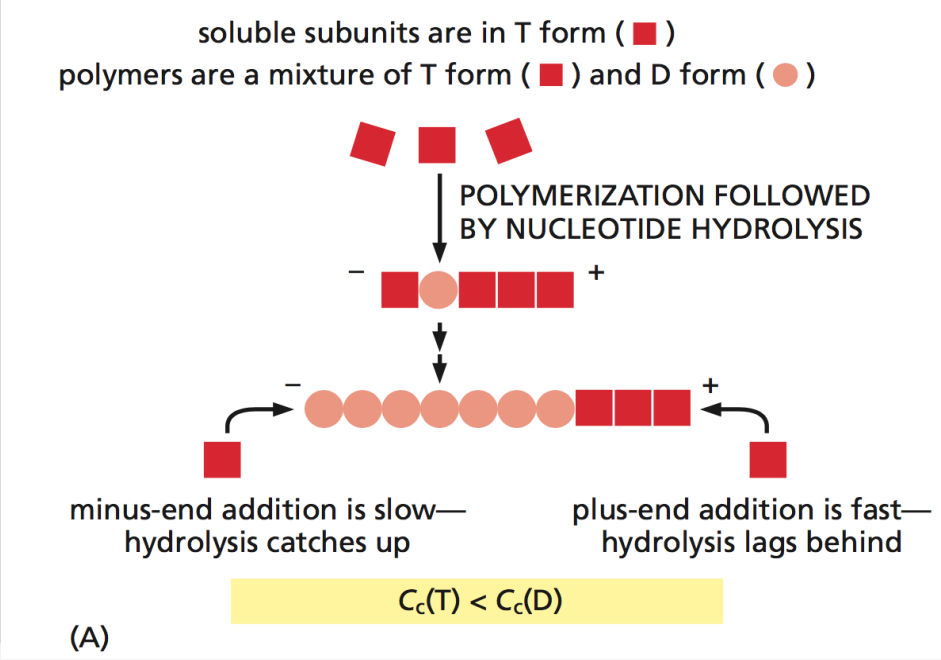
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## INTERACTIVE DEMONSTRATION

# ACTIN THREADMILLING

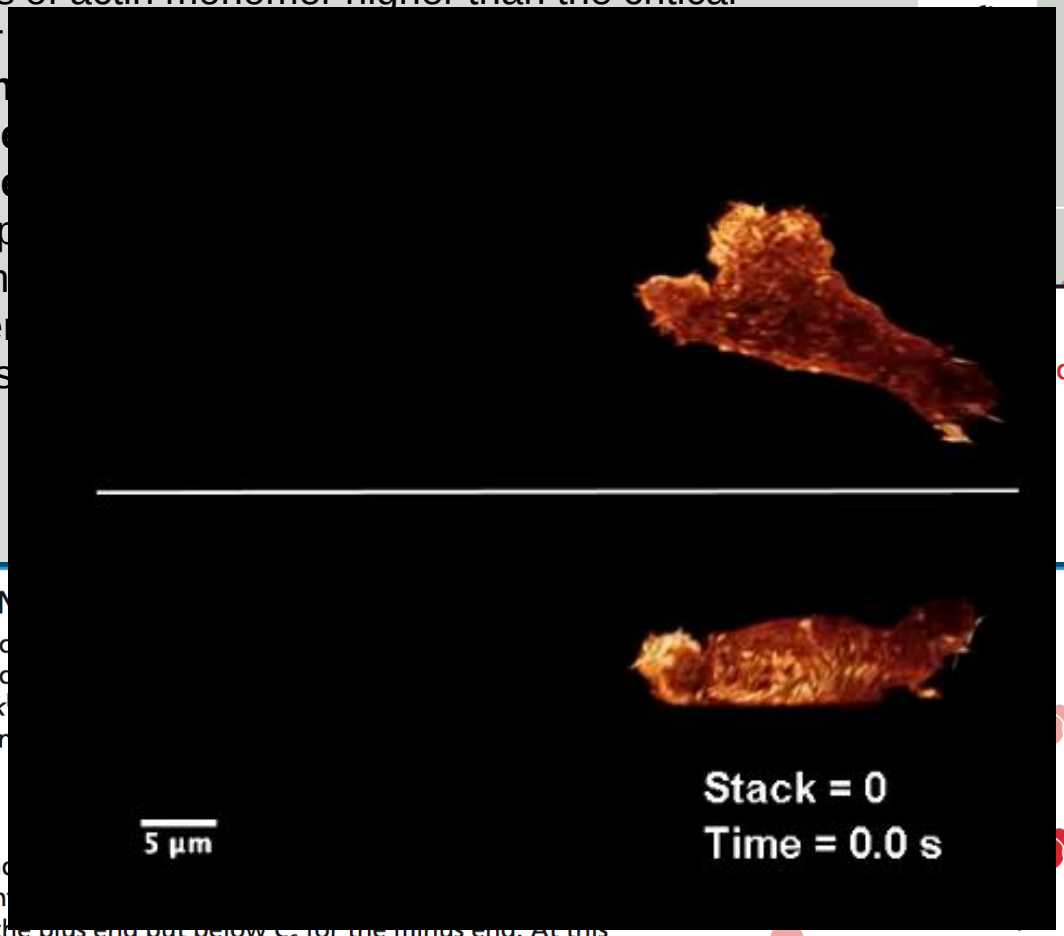
- In living cells, most soluble actin subunits are in the T form, as the free concentration of ATP is about tenfold higher than that of ADP. **However, the longer the time that subunits have been in the actin filament, the more likely they are to have hydrolyzed their ATP**
- Actin monomers in the T-form polymerize at both ends of a growing filament, and then undergo nucleotide hydrolysis within the filament
- As the filament grows, **elongation is faster than hydrolysis at the plus end**, and **the terminal actin subunits at the plus end are therefore always in the T form**
- However, **hydrolysis is faster than elongation at the minus end**, and so **terminal actin subunits at the minus end are in the D form**

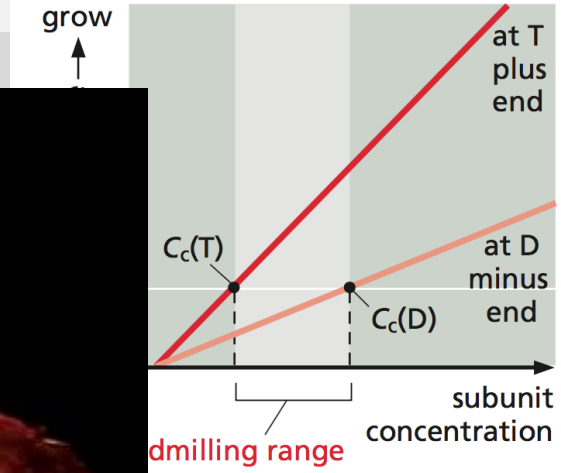
**$C_c$(D) is greater than $C_c$(T)**



# ACTIN THREADMILLING

- At concentrations of actin monomer higher than the critical concentration for
  **addition of actin**
  **hydrolysis at the**
  **hydrolysis at the**
- In this case, the p
  conformation, wh
- The filament ther
  **plus end**, while s
  **end**
- This leads to the

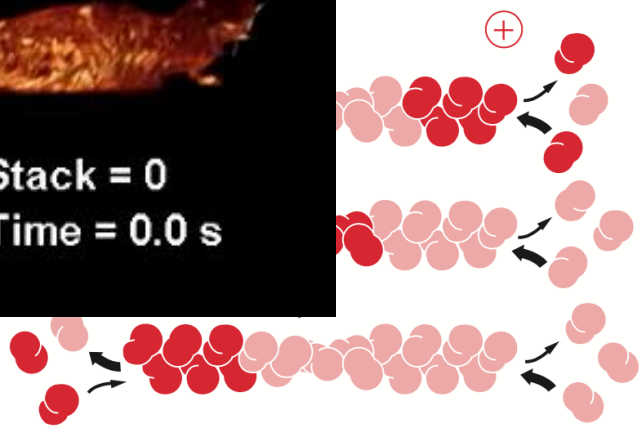



For $C_c(T) < C < C_c(D)$
treadmilling occurs

TREADMILLIN
One consequenc
formation is to c
polymer. Since $k$
$k^D_{off}/k^T_{on}$ need r

Thus, if both enc
until the concent
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 polymer maintains a constant length, even though there is a net flux of subunits through the polymer, known as treadmilling.



Alberts:

NEW

# ACTIN-ACTIVE PROTEINS





NEW

# QUESTION

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THINGS HAPPEN
OR THEY CAN BE FACILITATED TO HAPPEN

HOW ARE BIOLOGICAL PROCESSES FACILITATED?
(MAKE SOME EXAMPLES)

INTERACTIVE DEMONSTRATION 2: FORMIN AND ARP2/3

NEW

# ACTIN-ACTIVE PROTEINS



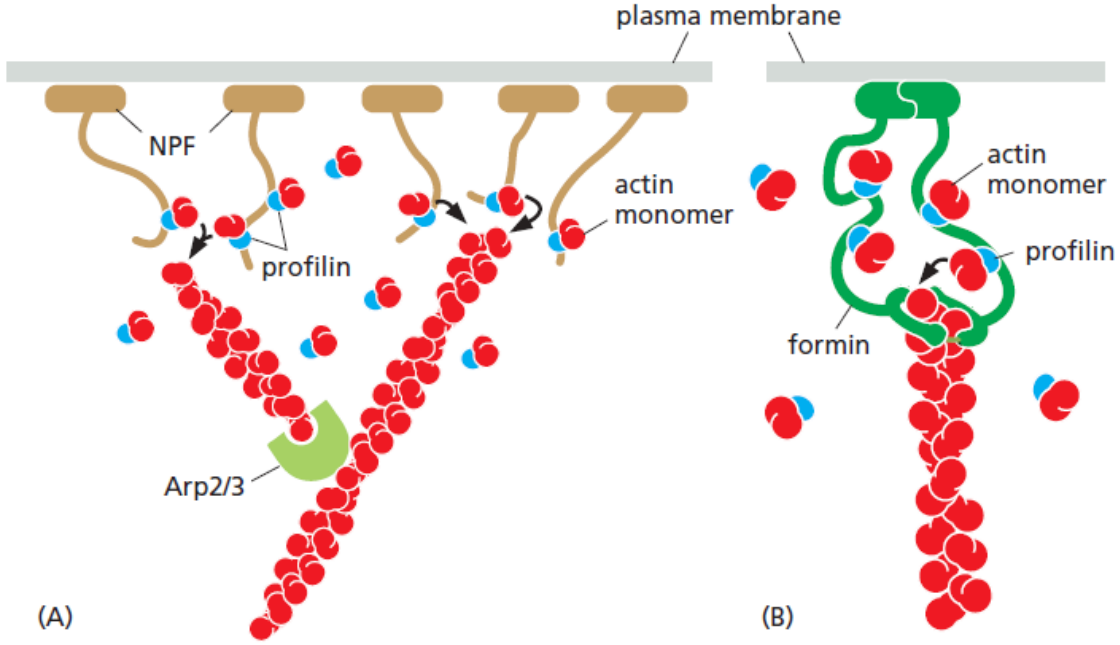
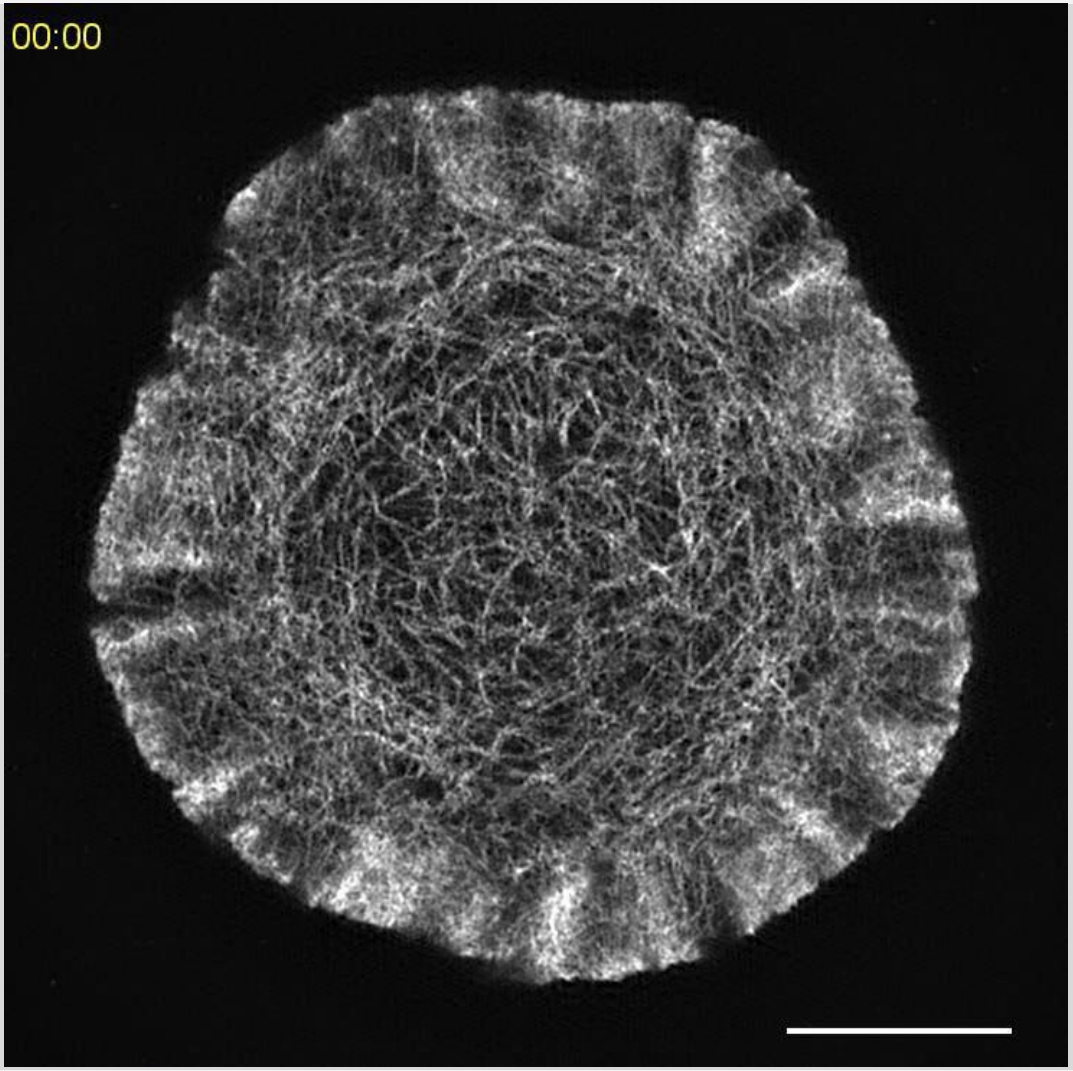



Figure 16–14 **Profilin stimulates actin filament elongation.** (A) Many nucleation-promoting factors (NPFs; *brown*) contain binding sites for profilin *(blue)*, which is bound to actin monomers. As a result, NPF activation leads not only to nucleation of branched actin filaments by Arp2/3 (*green*; see Figure 16–12), but also to rapid elongation of the new filaments. (B) Some members of the formin protein family possess whisker-like unstructured domains that contain several binding sites for profilin–actin complexes. This serves to recruit actin monomers to the growing plus end of the actin filament when formin is bound. Under some conditions, profilin can enhance the rate of actin filament elongation so that filament growth is faster than that expected for a diffusion-controlled reaction (see also Figure 3–76). Like NPFs, formin proteins inside the cell are activated to promote actin filament polymerization at membrane surfaces.

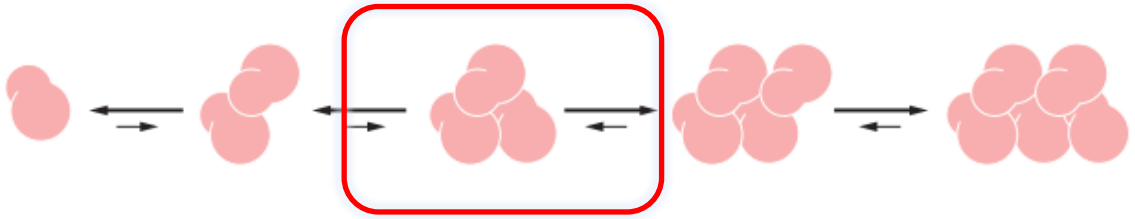
# ACTIN-ACTIVE PROTEINS



# ACTIN NUCLEATION

- **The regulation of actin filament formation is an important mechanism by which cells control their shape and movement**
- Small oligomers of actin subunits can assemble spontaneously, but they are unstable and disassemble readily because each monomer is bound to only one or two other monomers
- **For a new actin filament to form**, subunits must assemble into **an initial aggregate**, or nucleus, that is stabilized by multiple subunit–subunit contacts and can then elongate rapidly by addition of more subunits
- This process is called **filament nucleation** and can be promoted by specialized actin-binding proteins

**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.

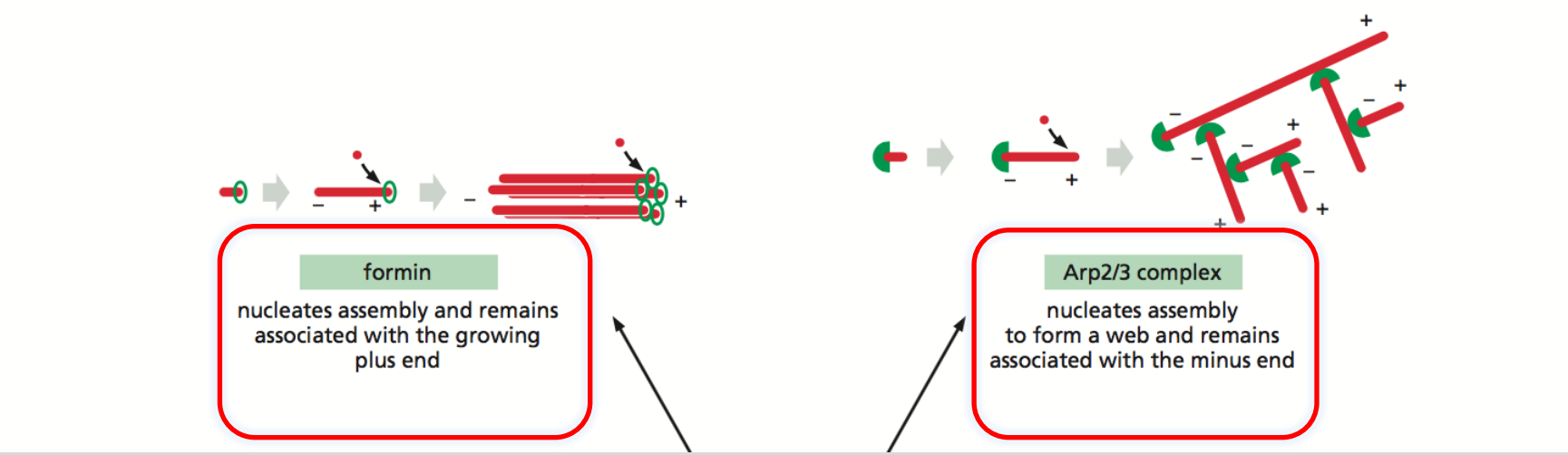
The assembly of a nucleus is relatively slow, which explains the lag phase seen during polymerization. The lag phase can be reduced or abolished entirely by adding premade nuclei, such as fragments of already polymerized microtubules or actin filaments.

3P

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# PROTEINS THAT NUCLEATE ACTIN

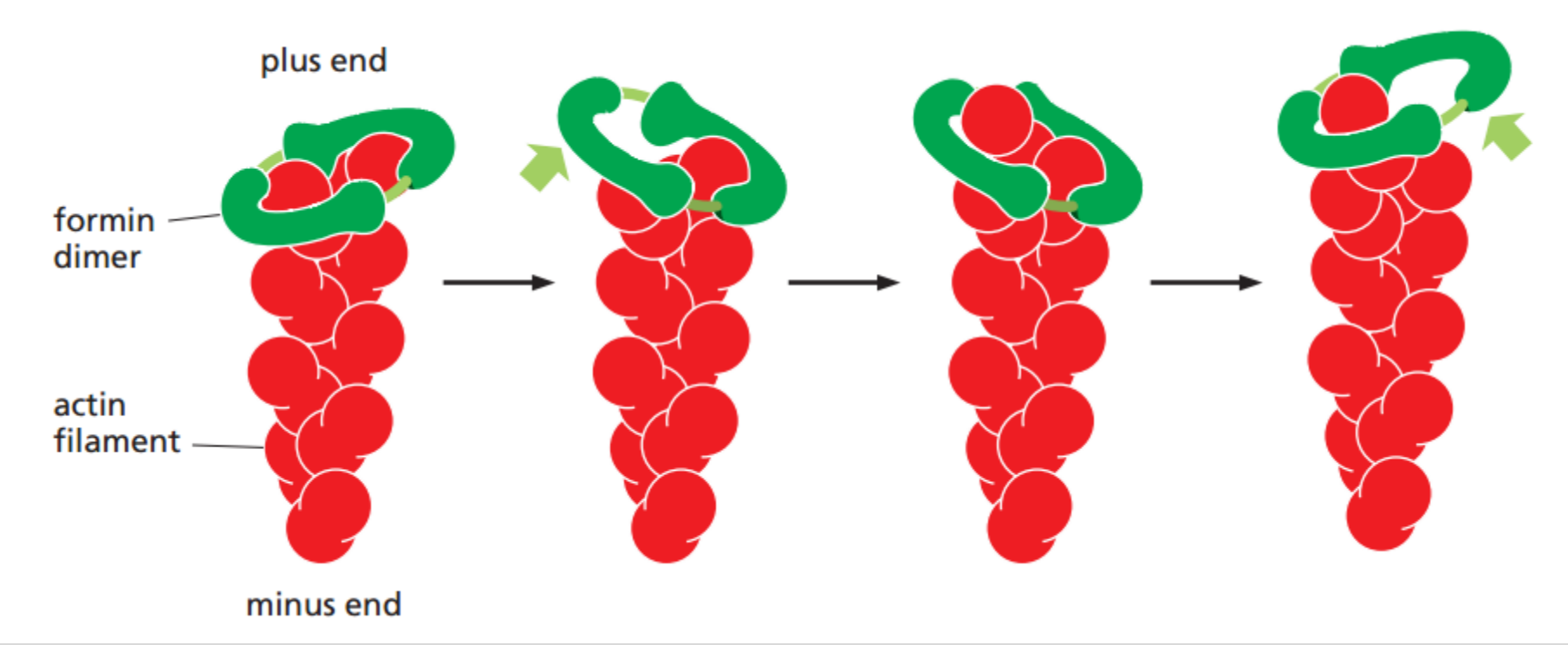
- Proteins that contain **actin monomer binding motifs** linked in tandem mediate the simplest mechanism of filament nucleation
- These actin-nucleating proteins bring several actin subunits together to form a **seed**
- In most cases, actin nucleation is catalyzed by one of two different types of factors: **the formins** and **the Arp 2/3 complex**




- Like profilin activation, actin filament nucleation by Arp 2/3 complexes and formins occurs primarily at the plasma membrane. The highest density of actin filaments in most cells is at the cell periphery
- The layer just beneath the plasma membrane is called the **cell cortex,** and the actin filaments in this region determine the shape and movement of the cell surface, allowing the cell to change its shape and stiffness rapidly in response to changes in its external environment

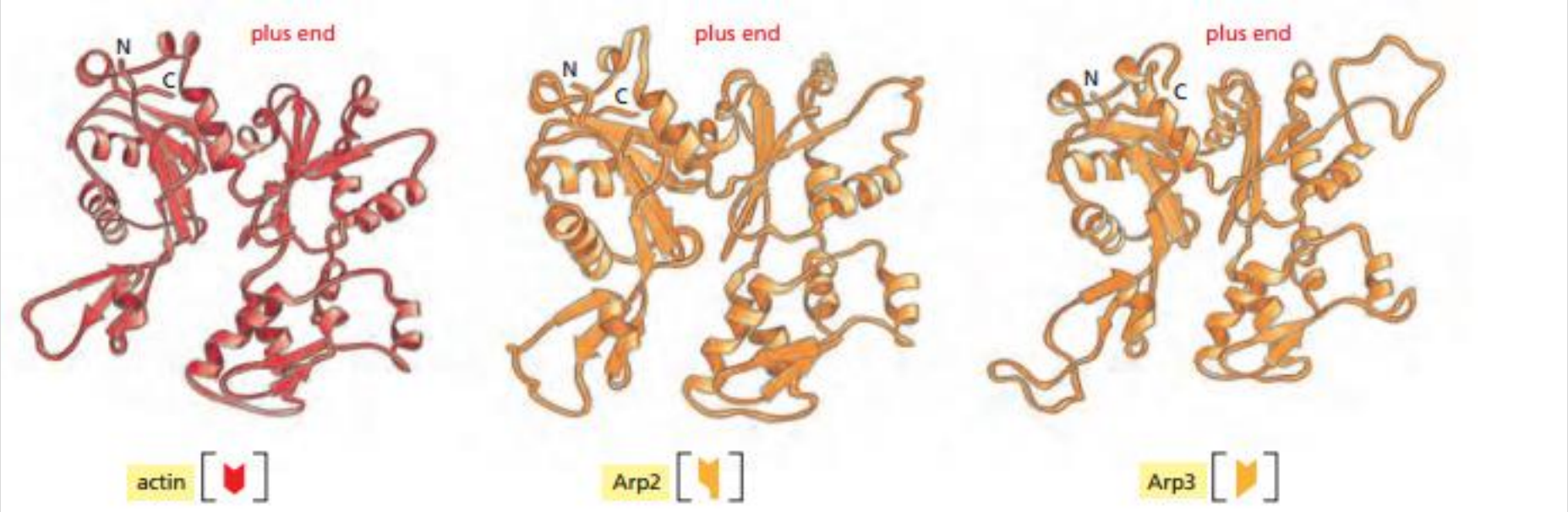
# PROTEINS THAT NUCLEATE ACTIN: FORMINS

- Formins are dimeric proteins that nucleate the growth of **straight, unbranched filaments** that can be cross-linked by other proteins to form parallel bundle
- Each formin subunit has a binding site for monomeric actin, and the formin dimer appears to **nucleate actin filament polymerization by capturing two monomers**




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# PROTEINS THAT NUCLEATE ACTIN: Arp2/3 COMPLEX




- Arp2/3 is a complex of proteins that includes two actin-related proteins, or ARPs, each of which is about 45% identical to actin
- The Arp 2/3 complex nucleates actin filament growth from the minus end, allowing rapid elongation at the plus end
- **The complex can attach to the side of another actin filament while remaining bound to the minus end of the filament that it has nucleated, thereby building individual filaments into a tree-like web**

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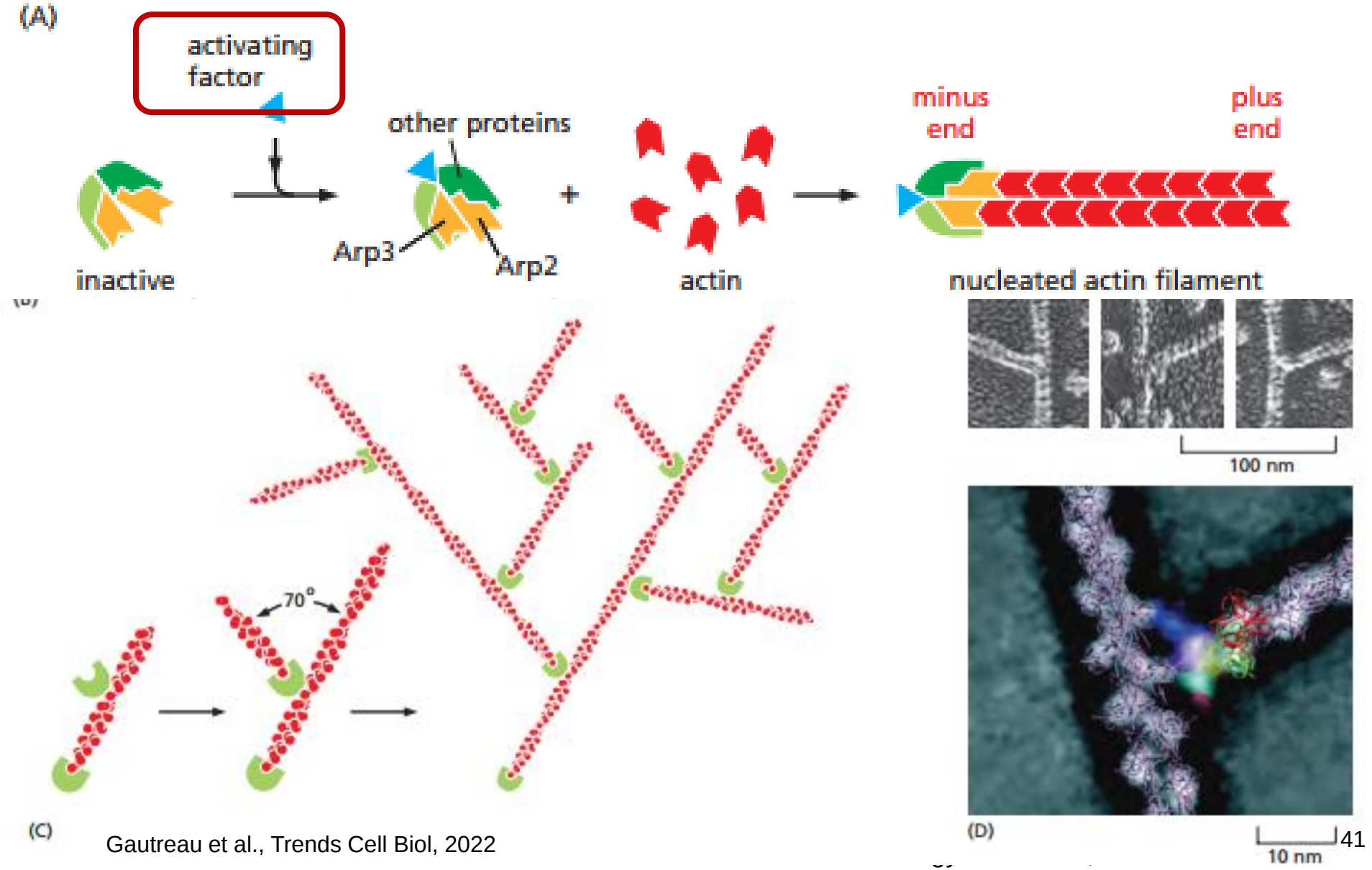
# QUESTION

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**THE ABILITY TO BIND TWO THINGS AT THE SAME TIME IS IMPORTANT IN BIOLOGY AND MEDICINE**

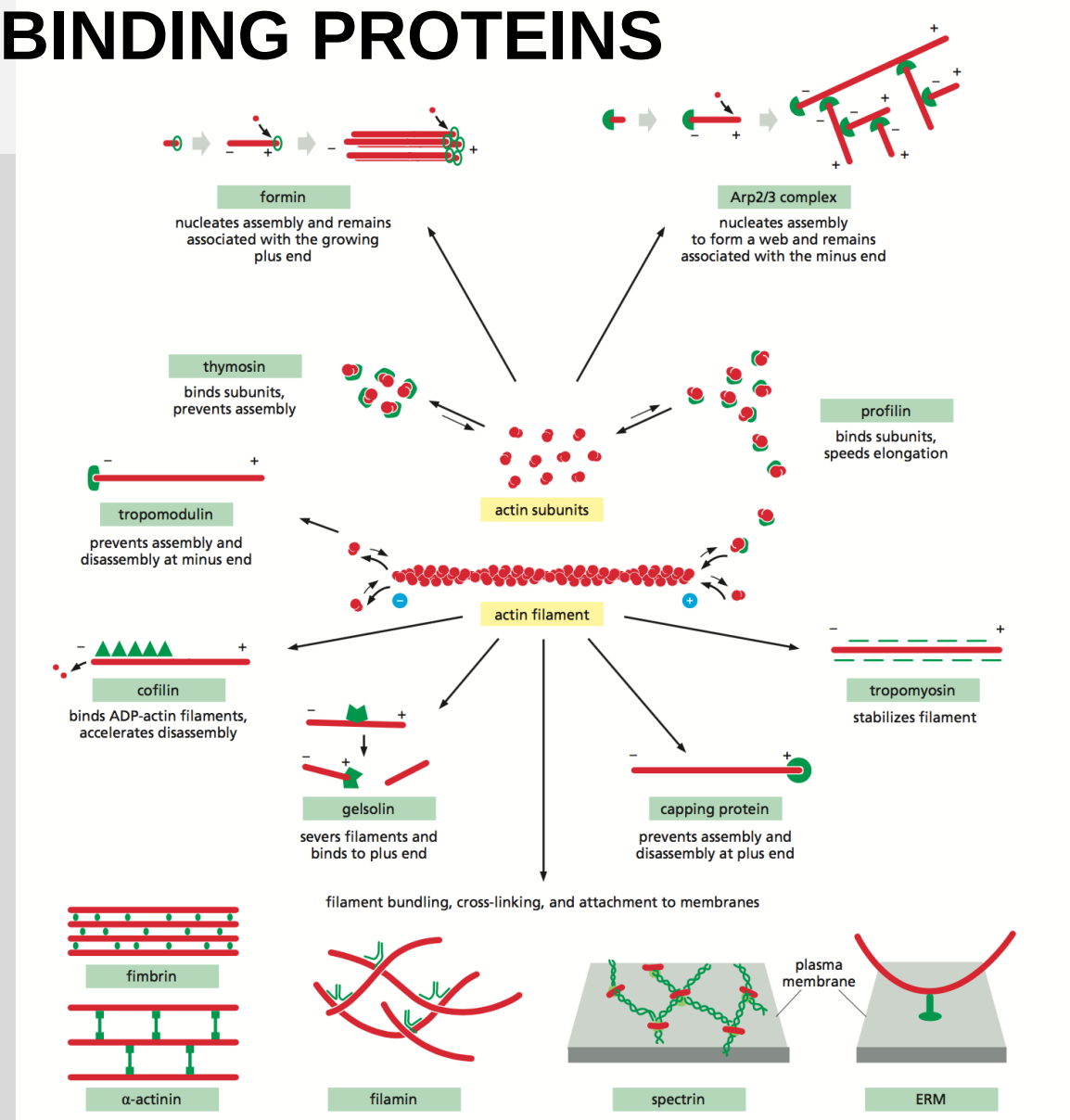
**CAN YOU MAKE AN EXAMPLE?**

# PROTEINS THAT NUCLEATE ACTIN: Arp2/3 COMPLEX



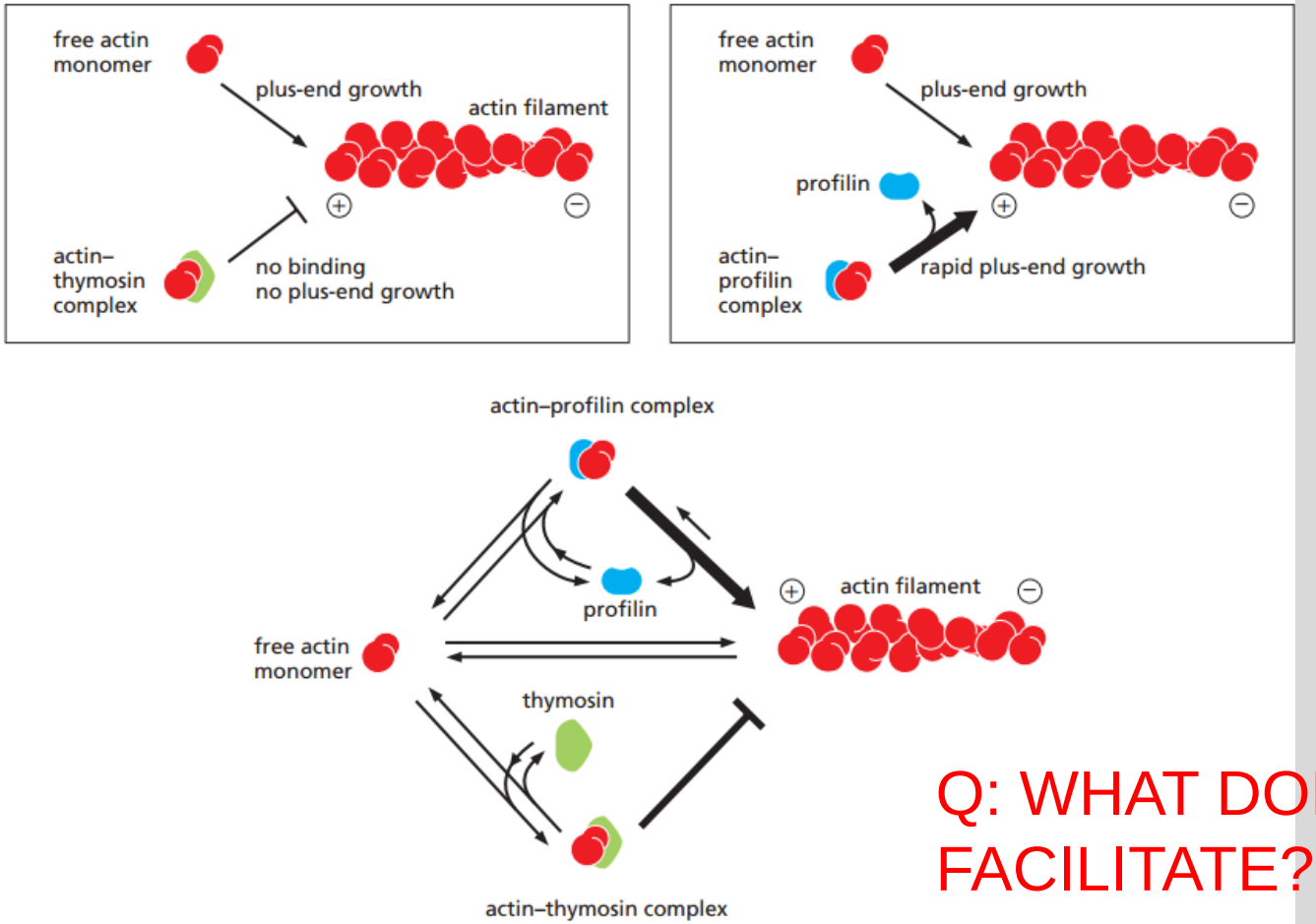

Gautreau et al., Trends Cell Biol, 2022

# ACTIN BINDING PROTEINS




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# ACTIN BINDING PROTEINS

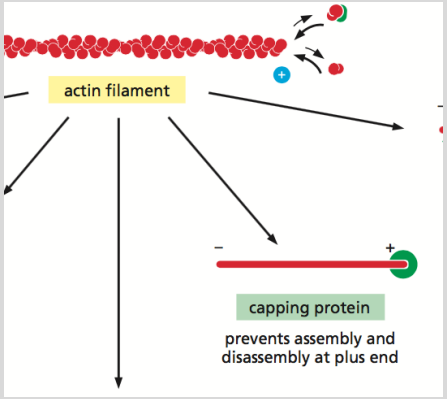


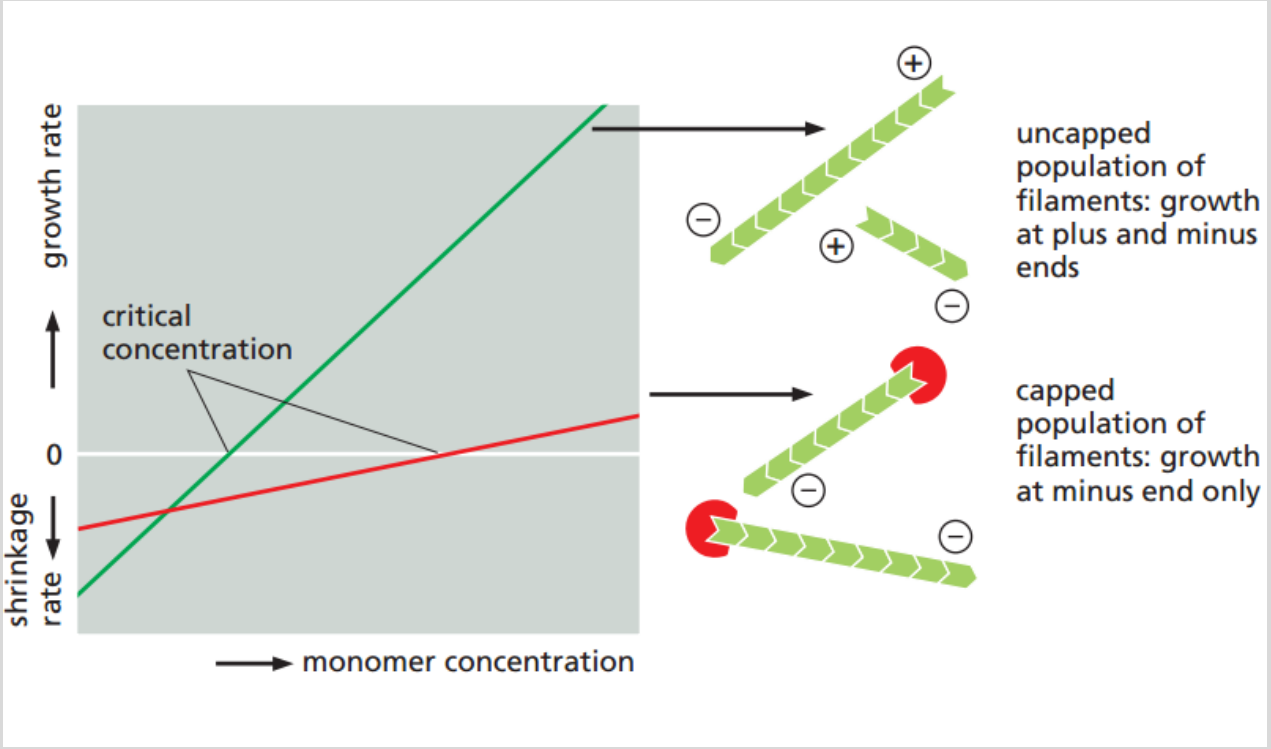

PROFILIN COMPETES WITH THYMOSIN FOR BINDING TO ACTIN MONOMERS AND PROMOTES ASSEMBLY

Q: WHAT DOES THIS FACILITATE?

Molecular Biology of the Cell, 6th edition. Alberts

# ACTIN CAPPING PROTEINS



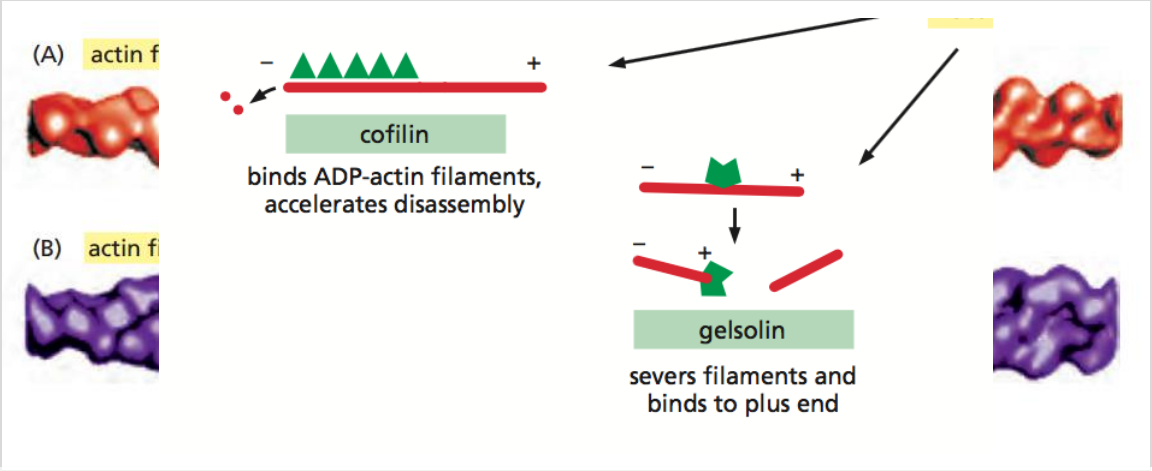




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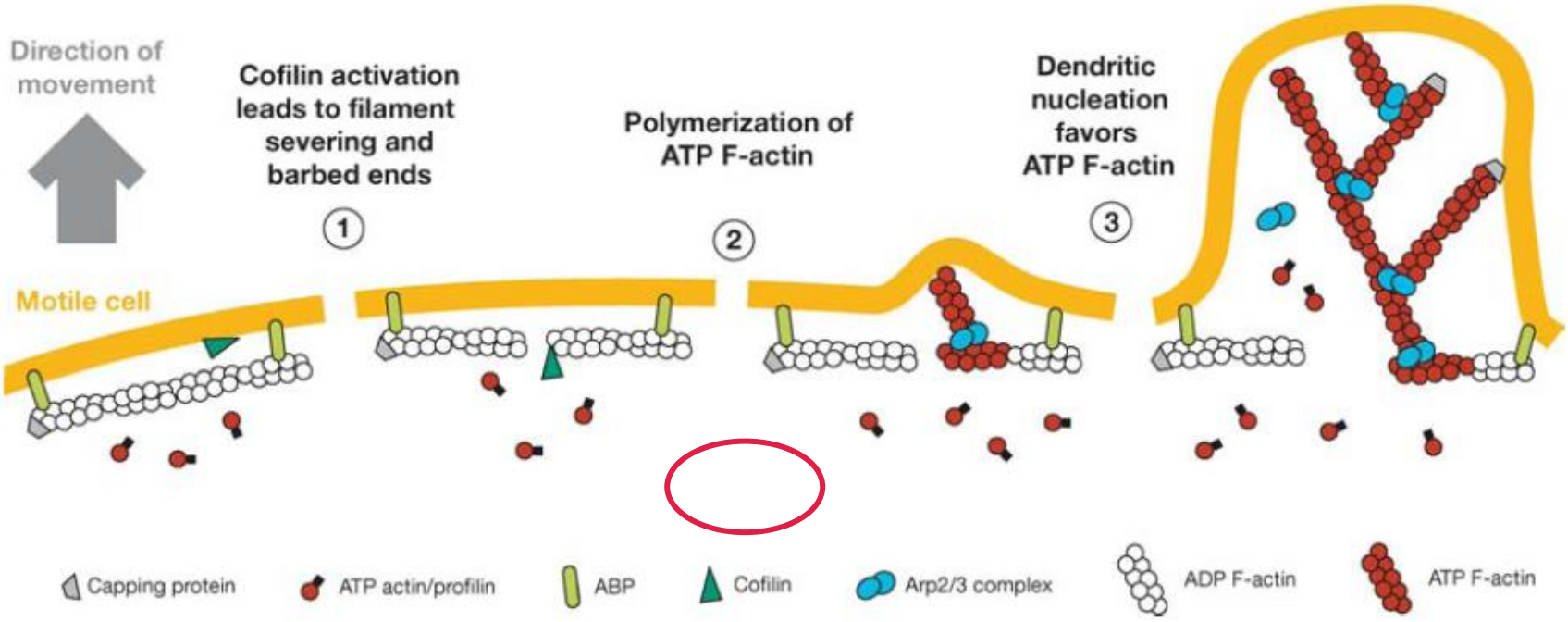
# PROTEINS THAT DESTABILISE ACTIN

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- Another important actin-filament destabilizing protein, found in all eukaryotic cells, is **cofilin**
- Cofilin binds along the length of the actin filament, forcing the filament to twist a little more tightly
- This mechanical stress **weakens the contacts between actin subunits in the filament**, making the filament more easily severed by thermal motions, generating filament ends that undergo rapid disassembly
- As a result, most of the actin filaments inside cells are shorter lived than filaments formed from pure actin in a test tube
- **Cofilin preferentially binds to ADP-containing actin** filaments rather than to ATP-containing filaments
- Since ATP hydrolysis is usually slower than filament assembly, **the newest actin filaments in the cell still contain mostly ATP and are resistant to depolymerization by cofilin**
- **Cofilin therefore tends to dismantle the older filaments in the cell**



# COFILIN PROVIDES SHORT FILAMENTS (AND/OR ACTIN MONOMERS) FOR ARP2/3-MEDIATED DENDRITIC NUCLEATION




Cofilin interacts preferentially with «aged» ADP-actin-containing filaments; Arp2/3 prefers ATP-actin-containing filaments.

Condeelis et al., 2005. Annu. Rev. Cell Dev. Biol.

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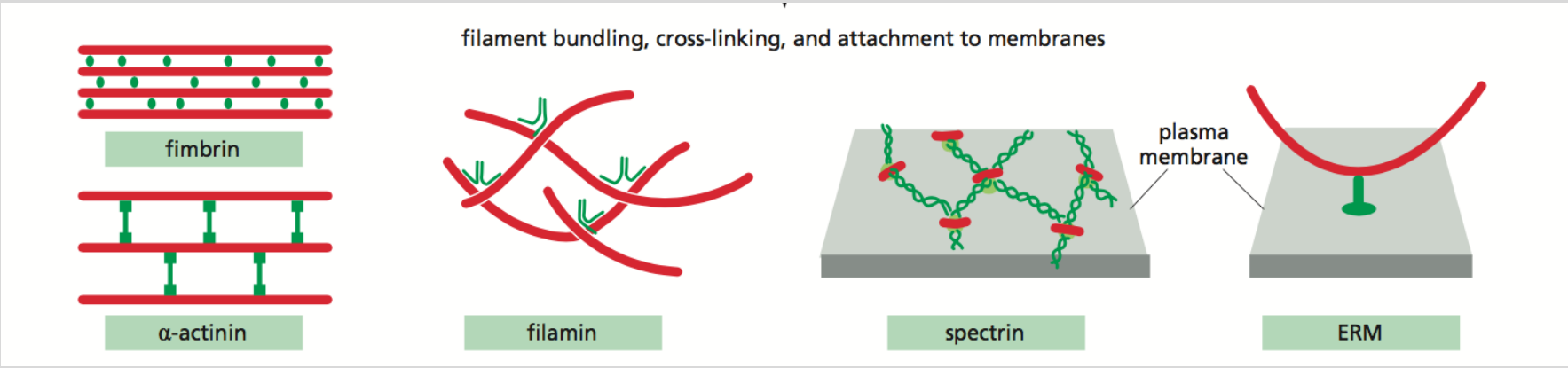
# EFFICIENCY IN CYTOSKELETON DYNAMICS

## WHO IS FASTER?



# BREAK.

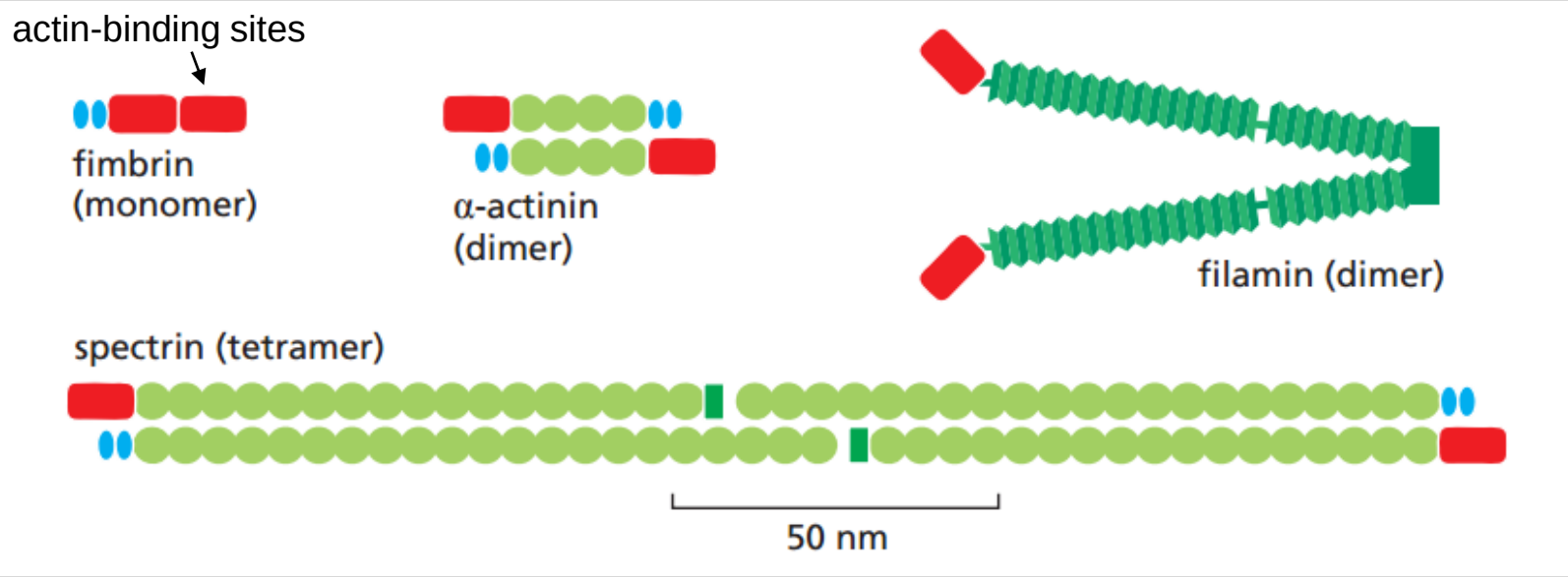
# ACTIN BUNDLING AND CROSS-LINKING




- As explained earlier, **Arp 2/3 organizes filaments into dendritic networks** by attaching filament minus ends to the side of other filaments
- Other actin filament structures are assembled and maintained by two classes of proteins: **bundling proteins**, which cross-link actin filaments into a parallel array, and **gel-forming proteins**, which hold two actin filaments together at a large angle to each other, thereby creating a looser meshwork
- Both bundling and gel-forming proteins generally **have two similar actin-filament-binding sites**, which can either be part of a single polypeptide chain or contributed by each of two polypeptide chains held together in a dimer
- **The spacing and arrangement of these two filament-binding domains determine the type of actin structure that a given cross-linking protein forms.**

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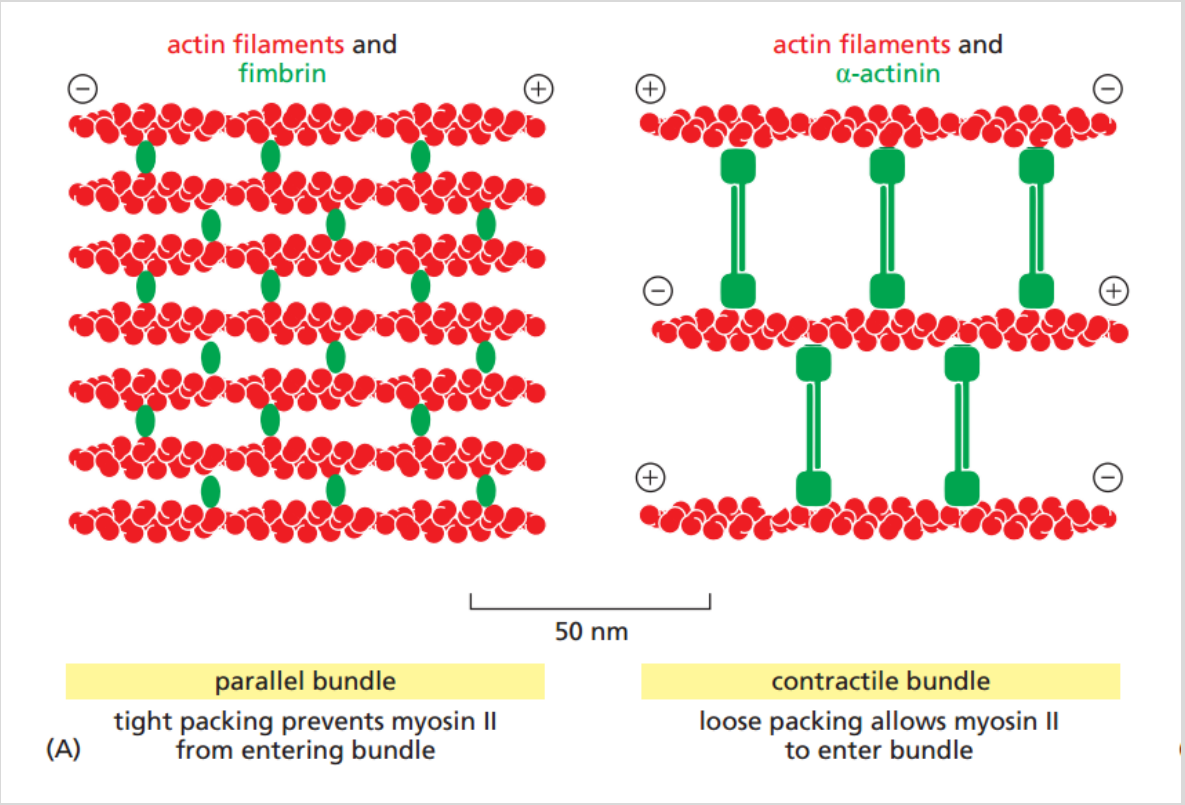
# ACTIN BUNDLING AND CROSS-LINKING

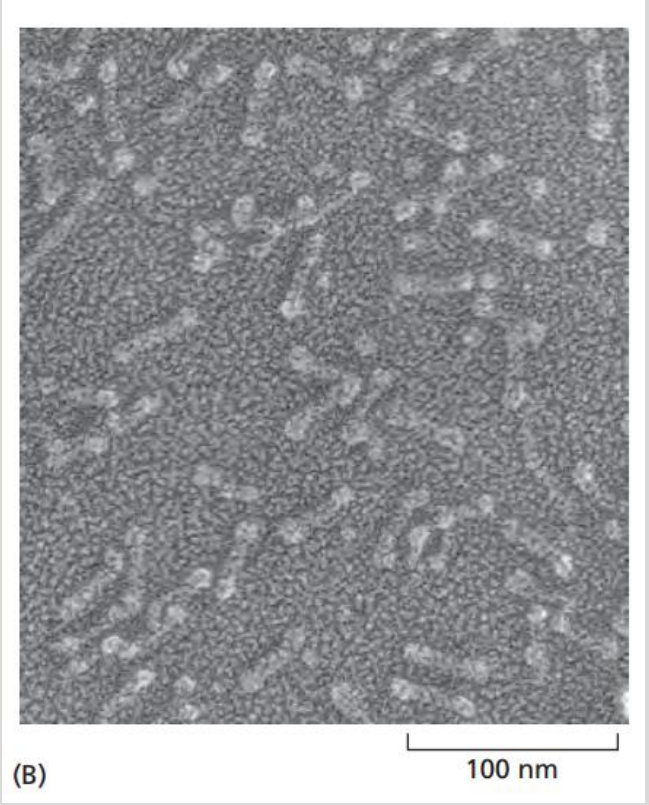



- **Fimbrin** has two directly adjacent actin binding sites, so that it holds its two actin filaments very close together (14 nm apart), aligned with the same polarity
- The two actin-binding sites in **α-actinin** are separated by a spacer around 30 nm long, so that it forms more loosely packed actin bundles
- **Filamin** has two actin-binding sites with a V-shaped linkage between them, so that it cross-links actin filaments into a network with the filaments oriented almost at right angles to one
- **Spectrin** is a tetramer of two α and two β subunits, and the tetramer has two actin binding sites spaced about 200 nm apart

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# ACTIN BUNDLING AND CROSS-LINKING



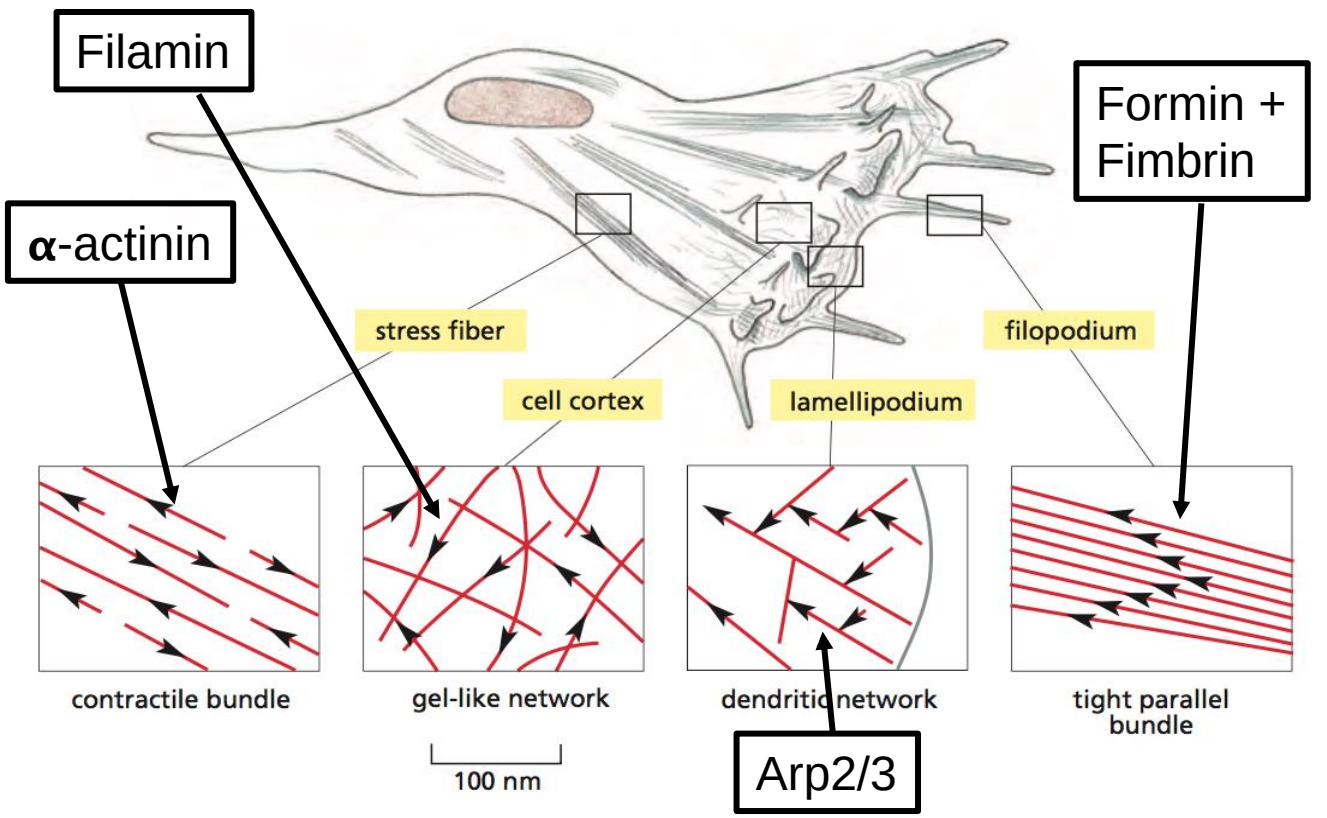




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NEW

# DIFFERENT ORGANISATIONS OF ACTIN IN THE SAME CELL





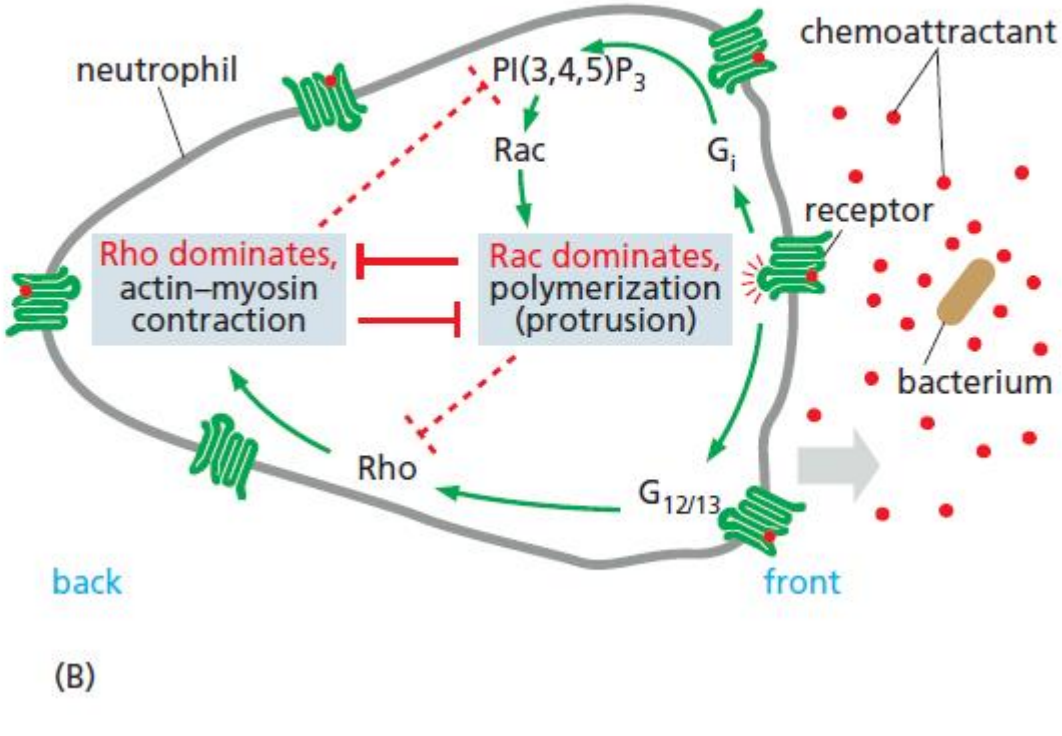

- Actin filaments cells are organized into several types of arrays: dendritic networks, bundles, and web-like (gel-like) networks
- Different structures are initiated by distinct nucleating proteins: the actin filaments of dendritic networks are nucleated by the Arp 2/3 complex; bundles are made of the long, straight filaments produced by formins
- The structural organization of different actin networks depends on specialized accessory proteins

NEW

# RELEVANCE FOR CELL MIGRATION

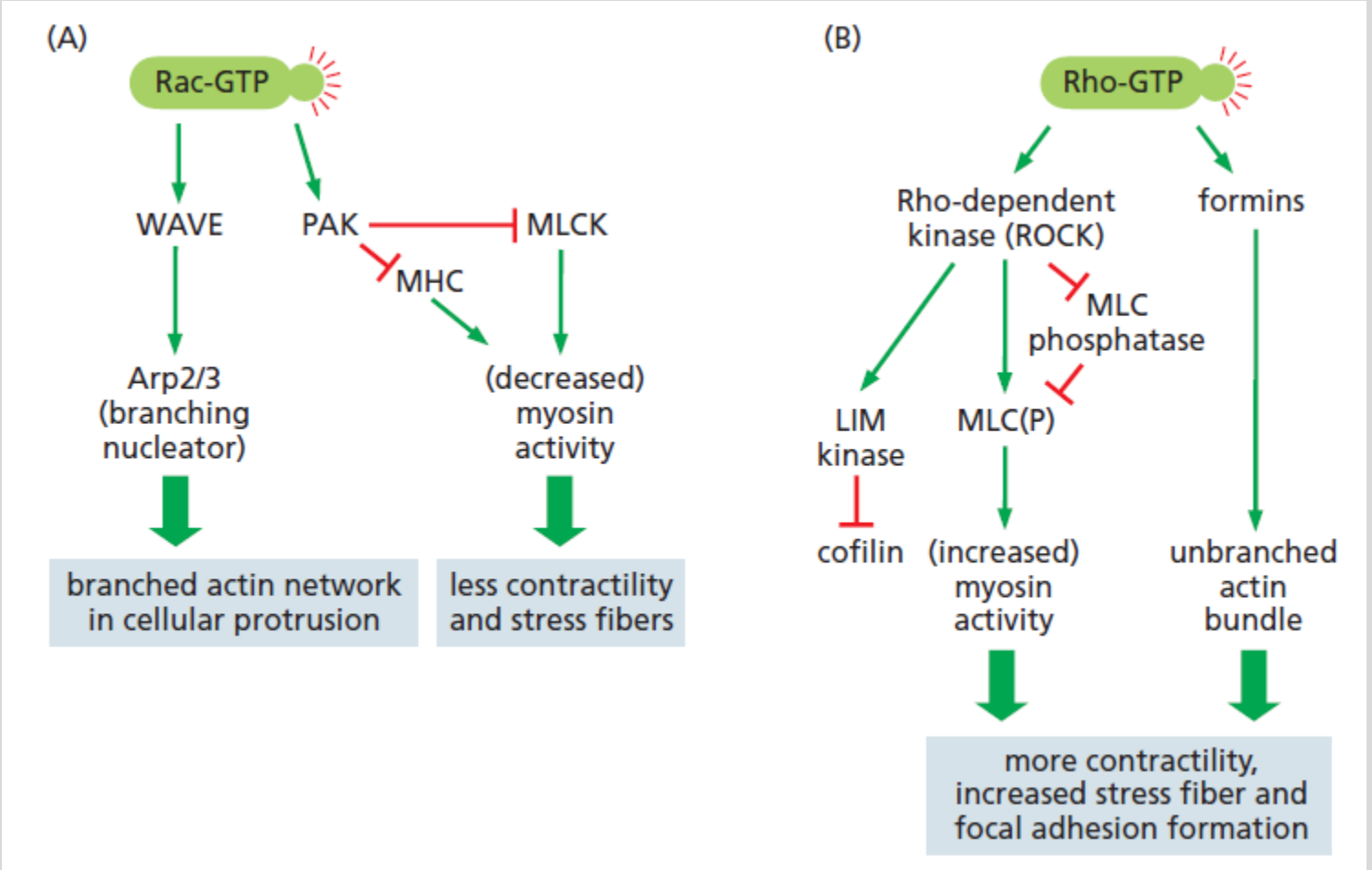




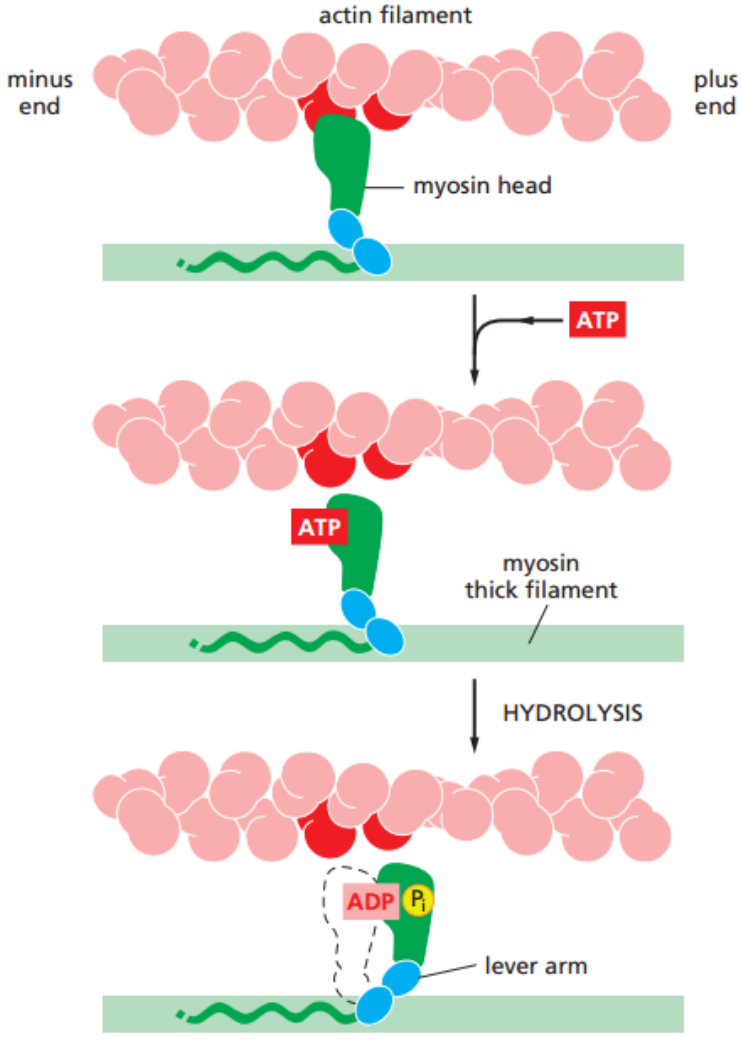



NEW

# RELEVANCE FOR CELL MIGRATION



# ACTOMYOSIN CONTRACTIONS: THE MYOSIN MOTOR



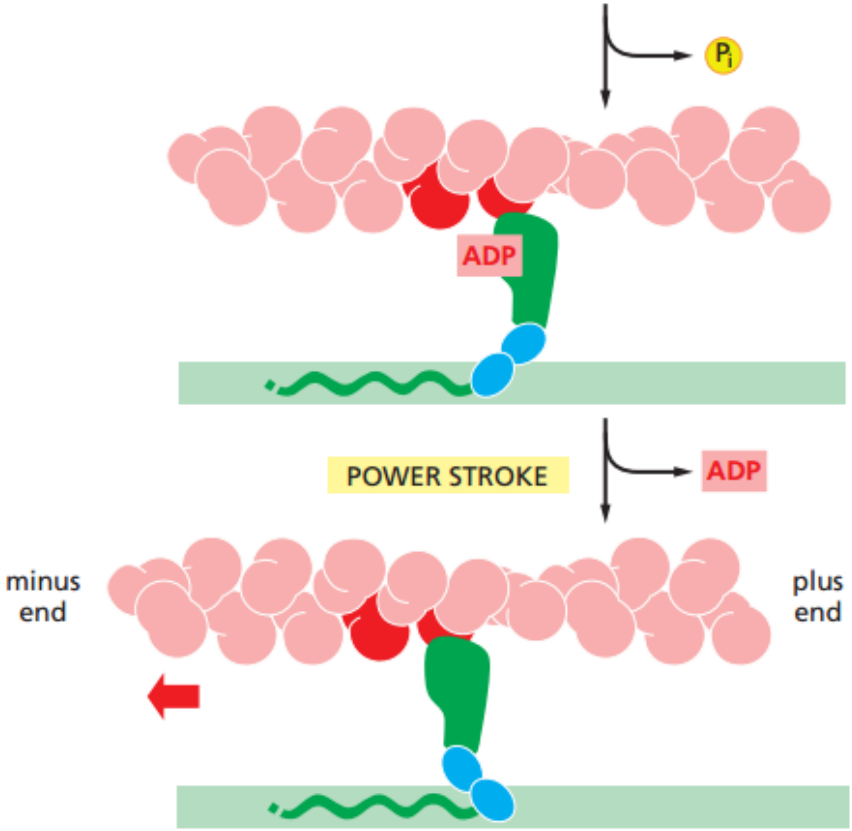

**ATTACHED** At the start of the cycle shown in this figure, a myosin head lacking a bound nucleotide is locked tightly onto an actin filament in a *rigor* configuration (so named because it is responsible for *rigor mortis*, the rigidity of death). In an actively contracting muscle, this state is very short-lived, being rapidly terminated by the binding of a molecule of ATP.

**RELEASED** A molecule of ATP binds to the large cleft on the "back" of the head (that is, on the side furthest from the actin filament) and immediately causes a slight change in the conformation of the actin-binding site, reducing the affinity of the head for actin and allowing it to move along the filament. (The space drawn here between the head and actin emphasizes this change, although in reality the head probably remains very close to the actin.)

**COCKED** The cleft closes like a clam shell around the ATP molecule, triggering a movement in the lever arm that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate ($P_i$) remain tightly bound to the protein.

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# ACTOMYOSIN CONTRACTIONS: THE MYOSIN MOTOR




**FORCE-GENERATING** Weak binding of the myosin head to a new site on the actin filament causes release of the inorganic phosphate produced by ATP hydrolysis, concomitantly with the tight binding of the head to actin. This release triggers the power stroke—the force-generating change in shape during which the head regains its original conformation. In the course of the power stroke, the head loses its bound ADP, thereby returning to the start of a new cycle.

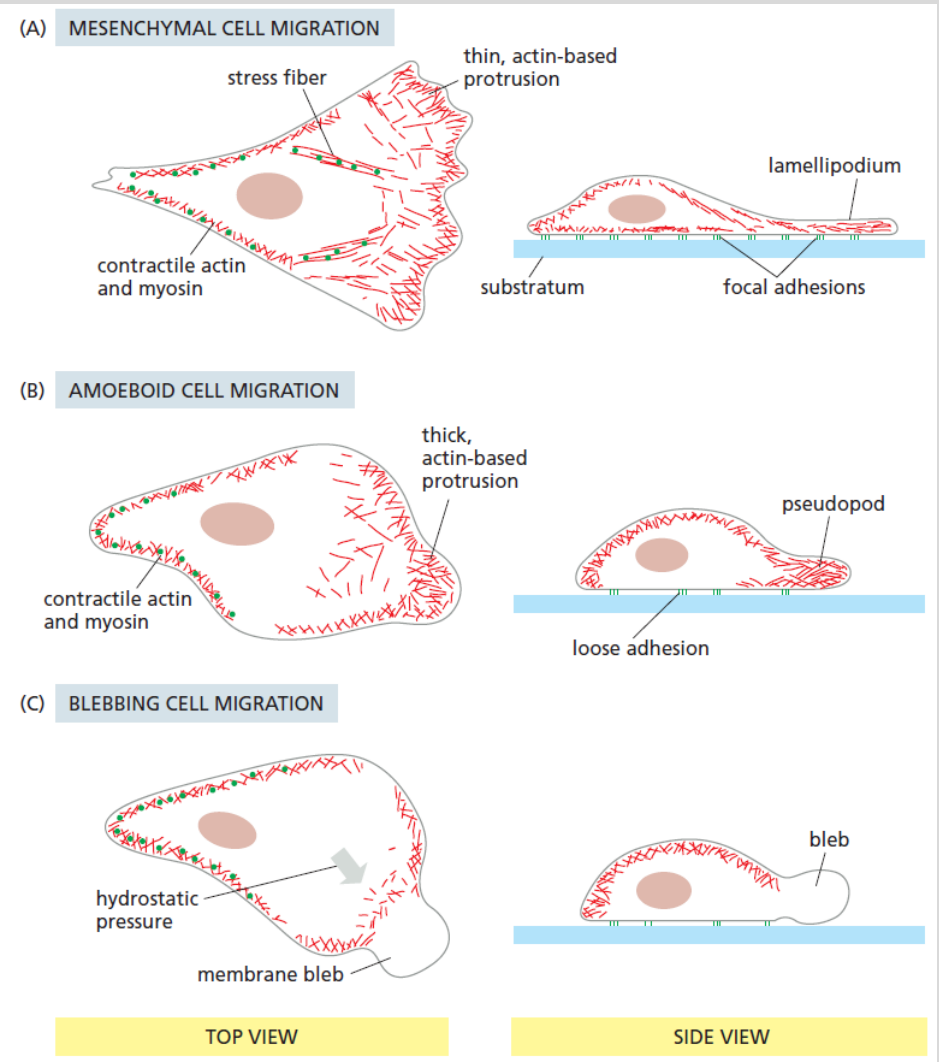
**ATTACHED** At the end of the cycle, the myosin head is again locked tightly to the actin filament in a rigor configuration. Note that the head has moved to a new position on the actin filament.

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NEW

# RELEVANCE FOR CELL MIGRATION






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NEW

# MICROTUBULES



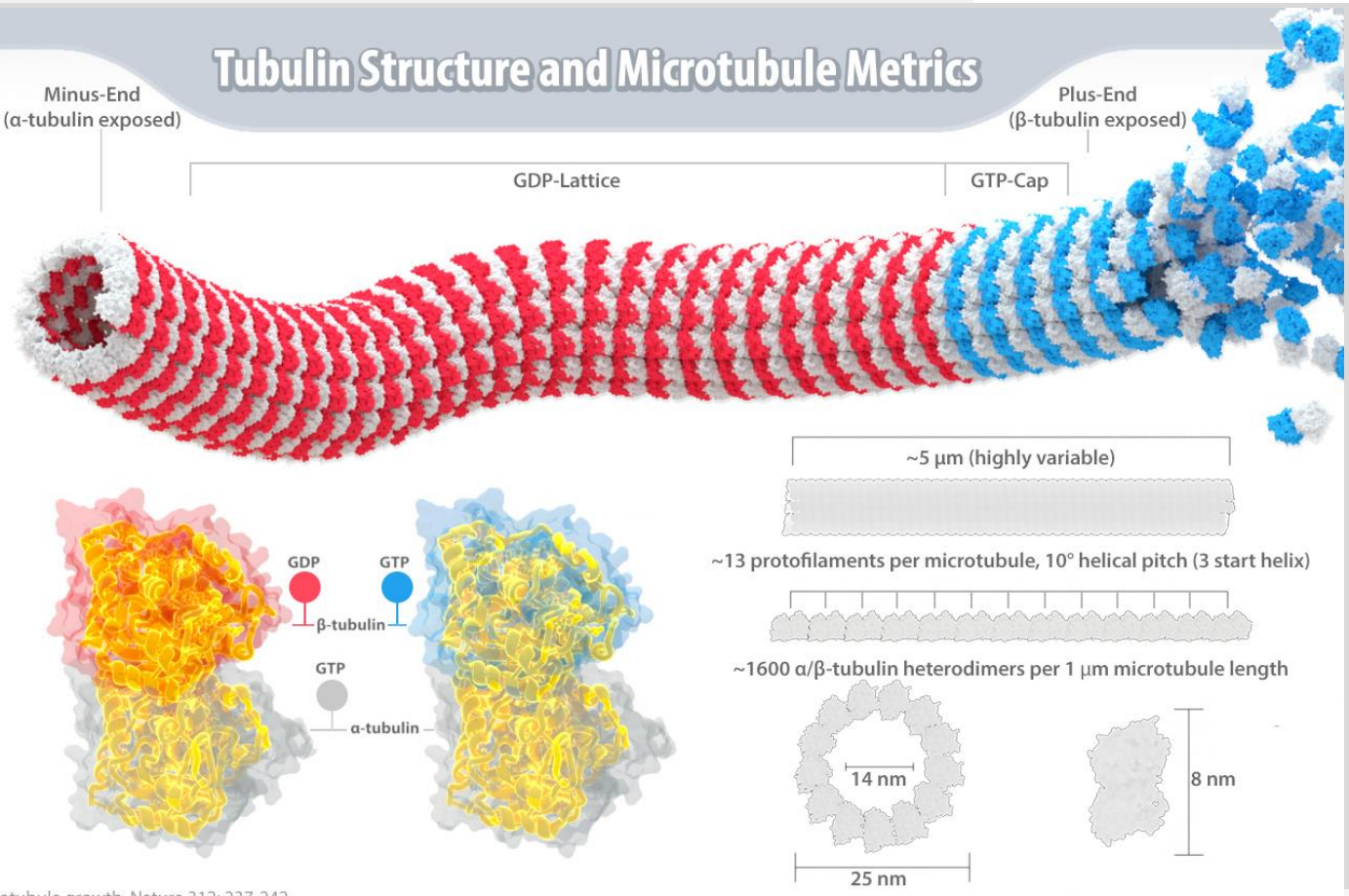


NEW

# MICROTUBULES

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## Tubulin Structure and Microtubule Metrics




**In Vitro Parameters:**

Growth rate ≈ 0.6-3 µm/min [1,2]

Shrinkage rate ≈ 20-30 µm/min [1,2]

Association rate constant ≈ 2–10 $\mu M^{-1}\ s^{-1}$ [1,2,3]

Free energy from GTP hydrolysis ≈ 7.5 kcal $mol^{-1}$ [3]

Pushing force ≈ 3-4 pN [4]

Pulling force ≈ 30-65 pN [3,4]

Persistence length ≈ 5.2 mm [5]

Critical concentration ≈ 0.1-2 mg/ml [6]

Molecular weight ≈ 110 kDa (α/β heterodimer)

Extinction coefficient ≈ 115,000 $M^{-1}\ cm^{-1}$ at 280 nm

Concentration Conversion: 1 mg/ml ≈ 10 µM

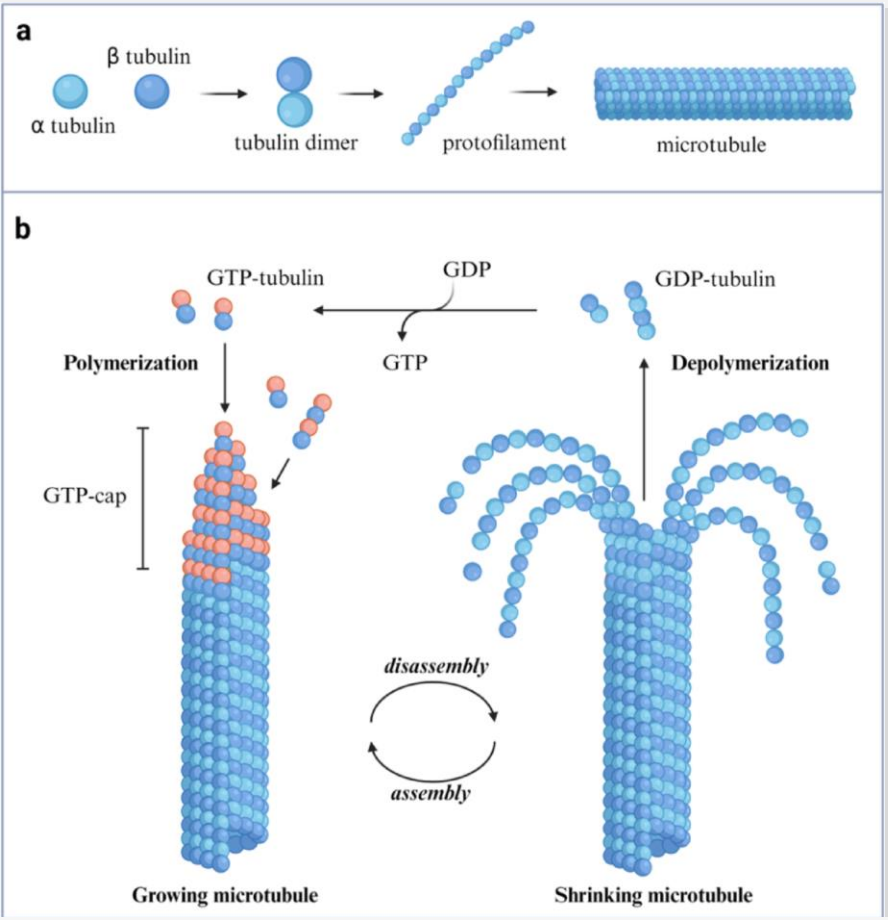

References

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2) Walker RA, O'Brien ET, Pryer NK, et al. (1988) Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. The Journal of Cell Biology 107:1437-1448.
3) Desai A, Mitchison T. (1997) Microtubule polymerization dynamics. Annual Review of Cell and Developmental Biology 13: 83-117.
4) Dogterom M and Yurke B. (1997) Measurement of the force-velocity relation for growing microtubules. Science 278: 856-860.
5) Gittes F, Mickey B, Nettleton J, and Howard J. (1993) Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. The Journal of Cell Biology 120: 923-934.
6) Mirigian M, Mukherjee K, Bane SL, Sackett DL. (2013) Chapter 14 - Measurement of in vitro microtubule polymerization by turbidity and fluorescence. Methods in Cell Biology 115, 215-229.
7) Löwe J, Li H, Downing KH, Nogales E. (2001) Refined structure of alpha beta-tubulin at 3.5 Å resolution. Journal of Molecular Biology 313: 1045-1057.


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NEW

# MICROTUBULES

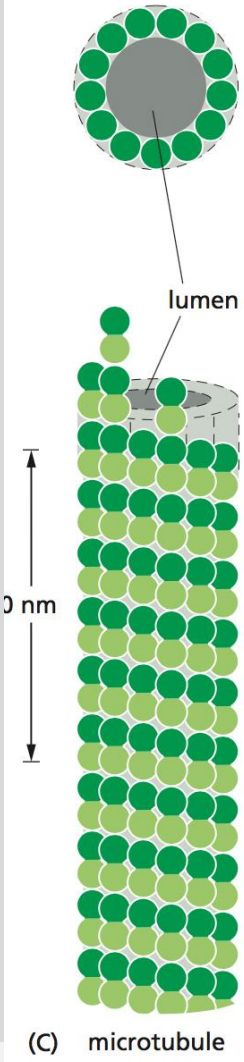


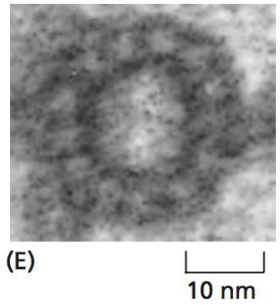

**Schematic representation of microtubule structure and dynamic behavior. a** Microtubules are usually composed of 13 protofilaments formed by repeating αβ-tubulin heterodimers arranged in a cylindrical lattice. Each tubulin subunit binds GTP, and lateral and longitudinal interactions stabilize the polymer. **b** microtubules undergo dynamic instability, alternating between phases of growth and shrinkage. This behavior is regulated by GTP hydrolysis and the presence of a stabilizing GTP cap at the plus end.

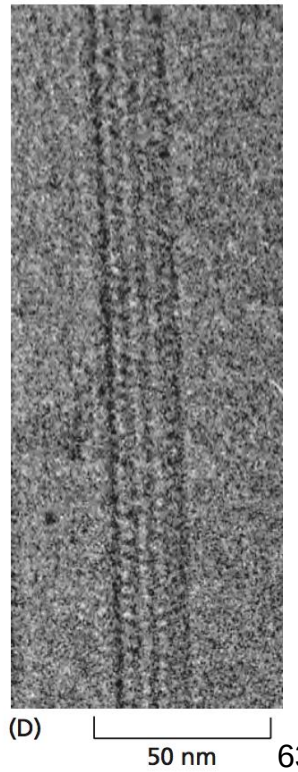
Vottero et al. J. Comp. Aided Mol. Design (2025)

# MICROTUBULES

- A microtubule is a hollow cylindrical structure built from **13** parallel **proto-filaments**, **each composed of αβ-tubulin heterodimers stacked head to tail and then folded into a tube**
- Microtubule assembly generates two new types of protein–protein contacts
- Along the longitudinal axis of the microtubule, the “top” of one β-tubulin molecule forms an interface with the “bottom” of the α-tubulin molecule in the adjacent heterodimer
- This interface is very similar to the interface holding the α and β monomers together in the dimer subunit, and the binding energy is high
- Perpendicular to these interactions, neighbouring proto-filaments form lateral contacts
- In this dimension, the main lateral contacts are between monomers of the same type (α–α and β–β)
- Because multiple contacts within the lattice hold most of the subunits in a microtubule in place, the addition and loss of sub-units occurs almost exclusively at the microtubule ends
- **These multiple contacts among subunits make microtubules stiff and difficult to bend**



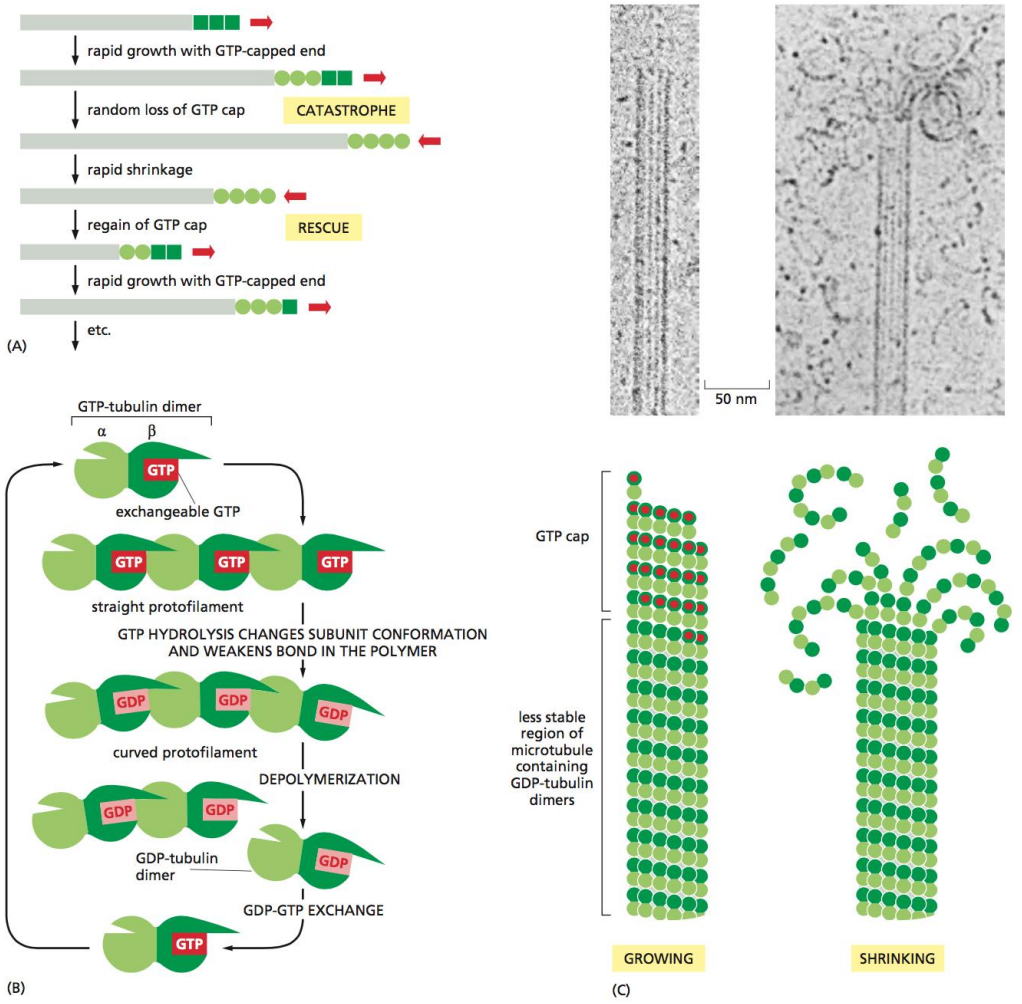



# MICROTUBULES

- Microtubule dynamics, like those of actin filaments, are profoundly influenced by the binding and hydrolysis of nucleotide—GTP in this case
- GTP hydrolysis occurs only within the β-tubulin subunit of the tubulin dimer
- Same as for actin, **GTP hydrolysis proceeds very slowly in free tubulin subunits but is accelerated when they are incorporated into microtubules**
- Following GTP hydrolysis, the free phosphate group is released and the GDP remains bound to β-tubulin within the microtubule lattice
- Thus, as in the case of actin  laments, two different types of microtubule structures can exist, one with the "**T form**" of the nucleotide bound (GTP) and one with the "**D form**" bound (GDP)

# MICROTUBULES

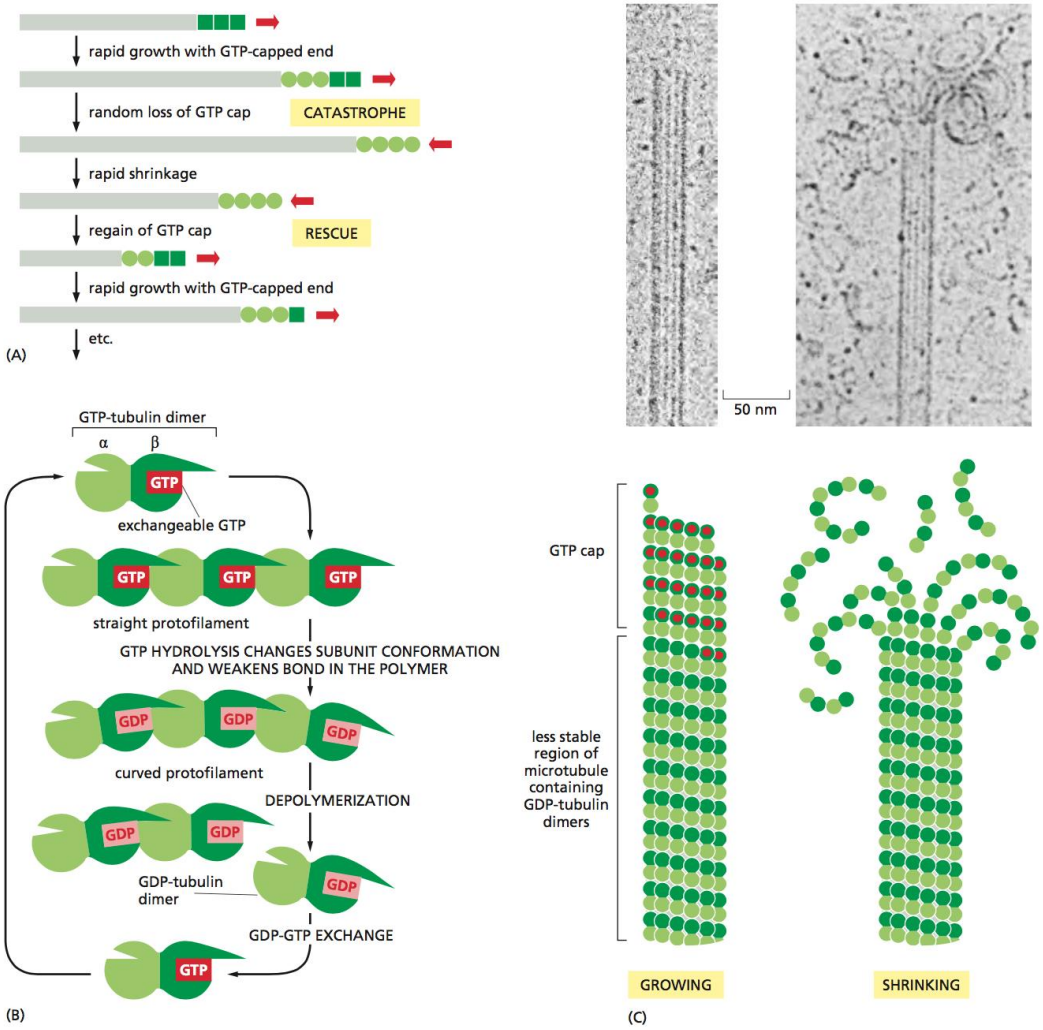
- **<u>Any end that happens to be in the T form will grow, whereas any end that happens to be in the D form will shrink</u>**
- On a single microtubule, an end might grow for a certain length of time in a T form, but then suddenly change to the D form and begin to shrink rapidly, even while the free subunit concentration is held constant
- At some later time, it might then regain a T-form end and begin to grow again
- This rapid interconversion between a growing and shrinking state, at a uniform free subunit concentration, is called **dynamic instability**
- The change from growth to shrinkage is called a **catastrophe**, while the change to growth is called a **rescue.**




Alberts: Molecular Biology of the Cell, 6th edition.

# MICROTUBULES

- The energy of nucleotide hydrolysis is stored as elastic strain in the polymer lattice, making the **free-energy change for dissociation of a subunit from the D-form polymer more negative than the free-energy change for dissociation of a subunit from the T-form polymer**
- In consequence, the ratio of $k_{off}/k_{on}$ for GDP-tubulin (its critical concentration [Cc(D)]) is much higher than that of GTP-tubulin
- **Thus, under physiological conditions, GTP-tubulin tends to polymerize and GDP-tubulin to depolymerize**

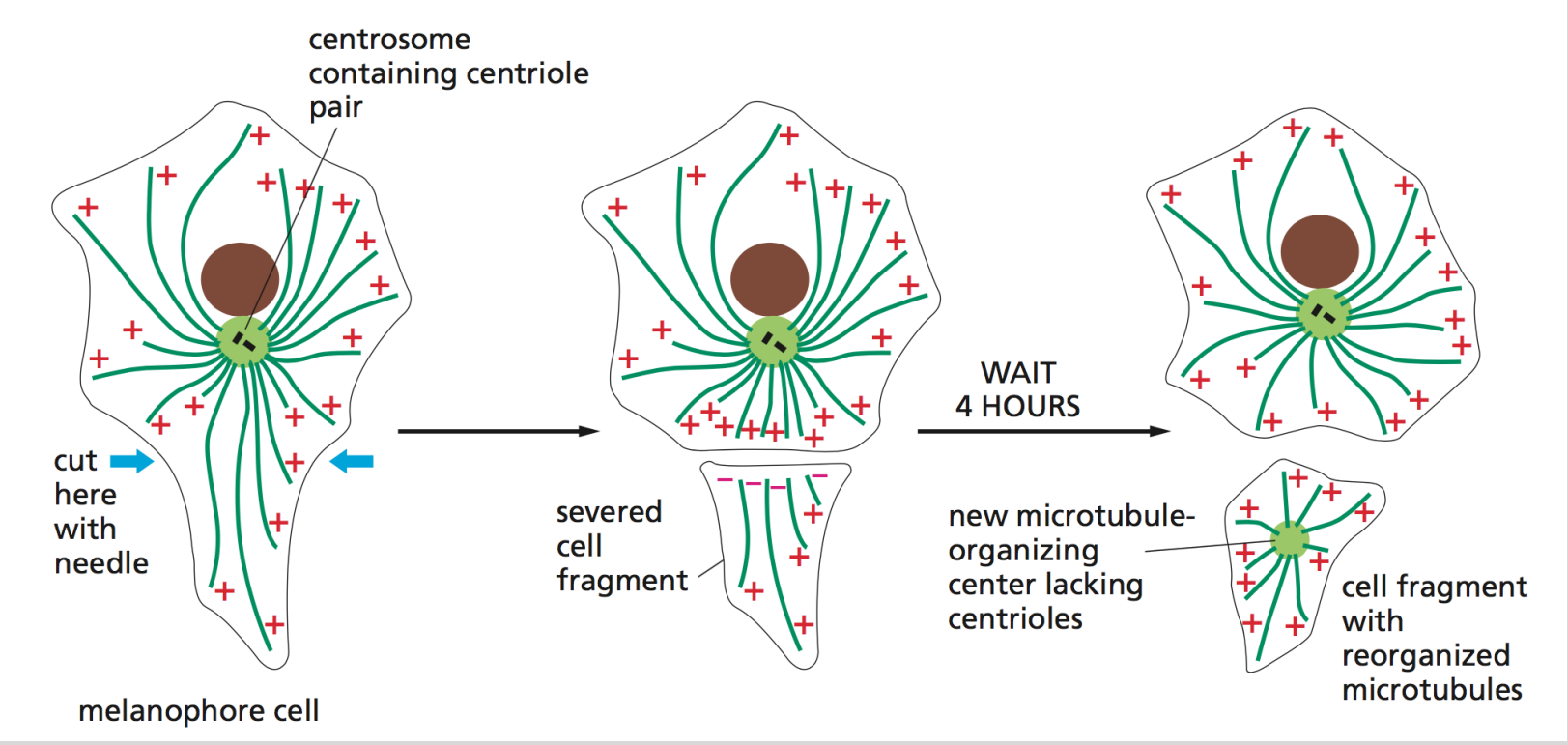



Alberts: Molecular Biology of the Cell, 6th edition.

# MICROTUBULE ORGANISING CENTRE (MTOC)

UNIVERSITÄT BERN

In cultured animal cells, the aster-like configuration of microtubules is robust, with dynamic plus ends pointing outward toward the cell periphery and stable minus ends collected near the nucleus. The system of microtubules radiating from the centrosome acts as a device to survey the outlying regions of the cell and to position the centrosome at its center




Alberts: Molecular Biology of the Cell, 6th edition.

NEW

# SEARCH AND CAPTURE MODEL

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## INTERACTIVE DEMONSTRATION

NEW

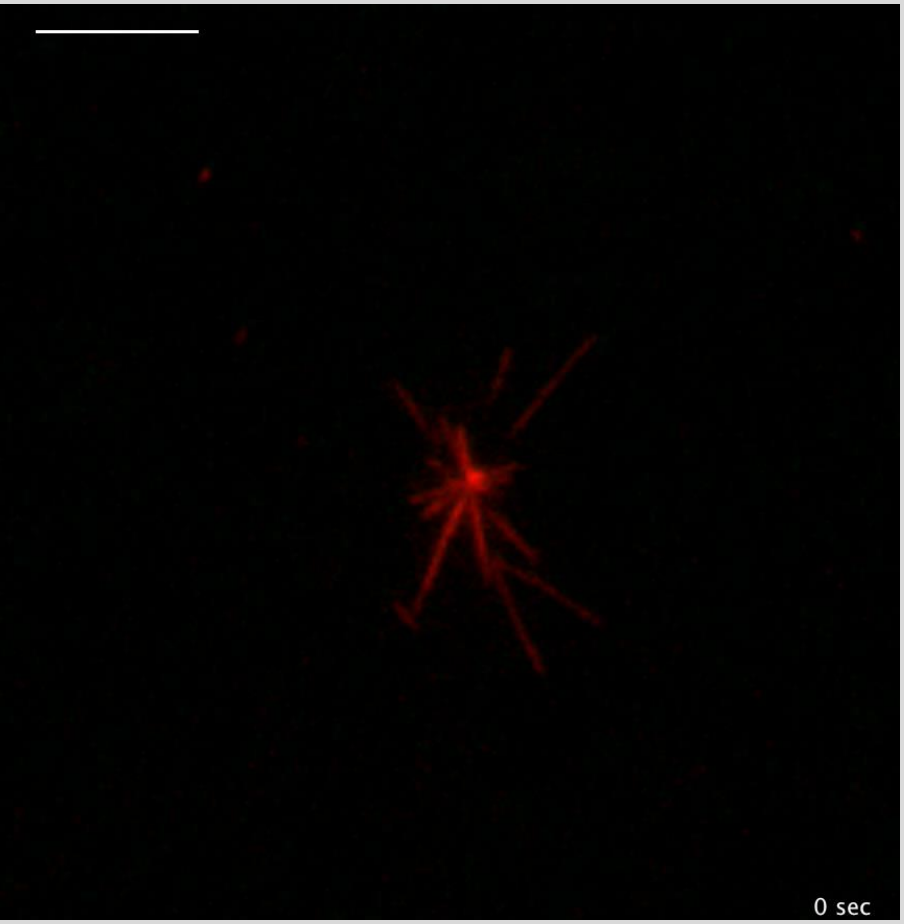
# SEARCH AND CAPTURE MODEL

UNIVERSITÄT BERN

In 1986, Marc Kirschner and Tim Mitchison proposed that microtubules use their dynamic properties of growth and shrinkage at their plus ends to **probe the three dimensional space of the cell**.

Plus ends that encounter kinetochores or sites of polarity become captured and no longer display growth or shrinkage. **In contrast to normal dynamic microtubules, which have a half-life of 5–10 minutes, the captured microtubules can last for hours**. This idea is commonly known as the "search and capture" model. Indeed, work since then has largely validated this idea

# THE CENTROSOME IS AN ACTIN-ORGANIZING CENTRE



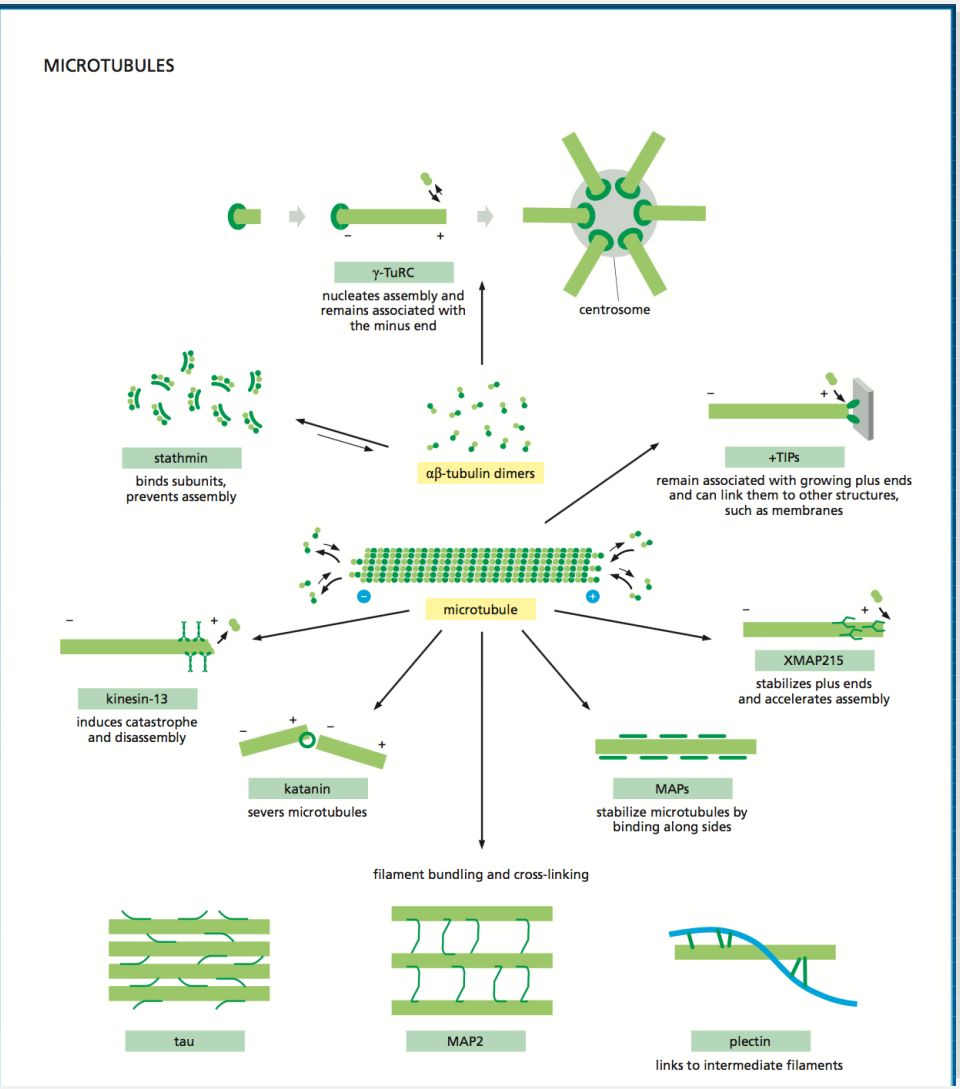

**Microtubules** **Actin**

Farina et al., Nat Cell Biol. 2016

NEW

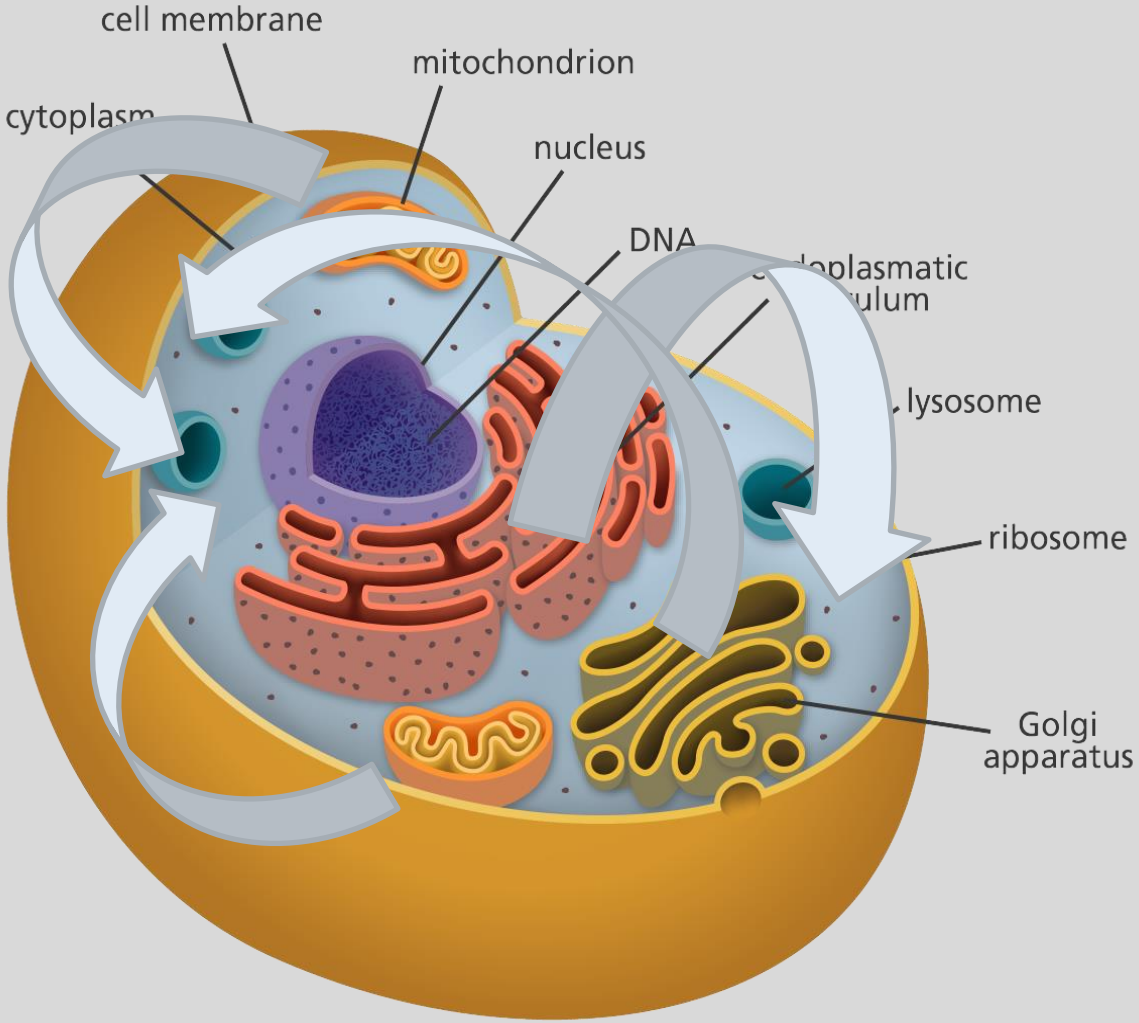
# MICROTUBULE ORGANIZING PROTEINS






- **Nucleating proteins: Gamma-tubulin** promotes new microtubule growth.
- **Stabilizing proteins: XMAP215** stabilizes microtubules and enhances growth.
- **Severing proteins: Katanin** cuts microtubules.
- **Destabilizing proteins: Kinesin-13** binds plus ends and promotes disassembly.
- **Monomer sequestration: Stathmin** binds free tubulin dimers, inhibiting growth.
- **MT-organizing proteins: Tau** and **MAP2** organize and stabilize microtubules.
- **Connecting proteins: Plectin** links microtubules with intermediate filaments.
- **Protein caps:** Stabilize microtubules and prevent growth or shrinkage.
- **Motor proteins:** Move vesicles and other cargo along microtubules.

# THE CYTOSKELETON ALSO POWERS ORGANELLE MOVEMENT

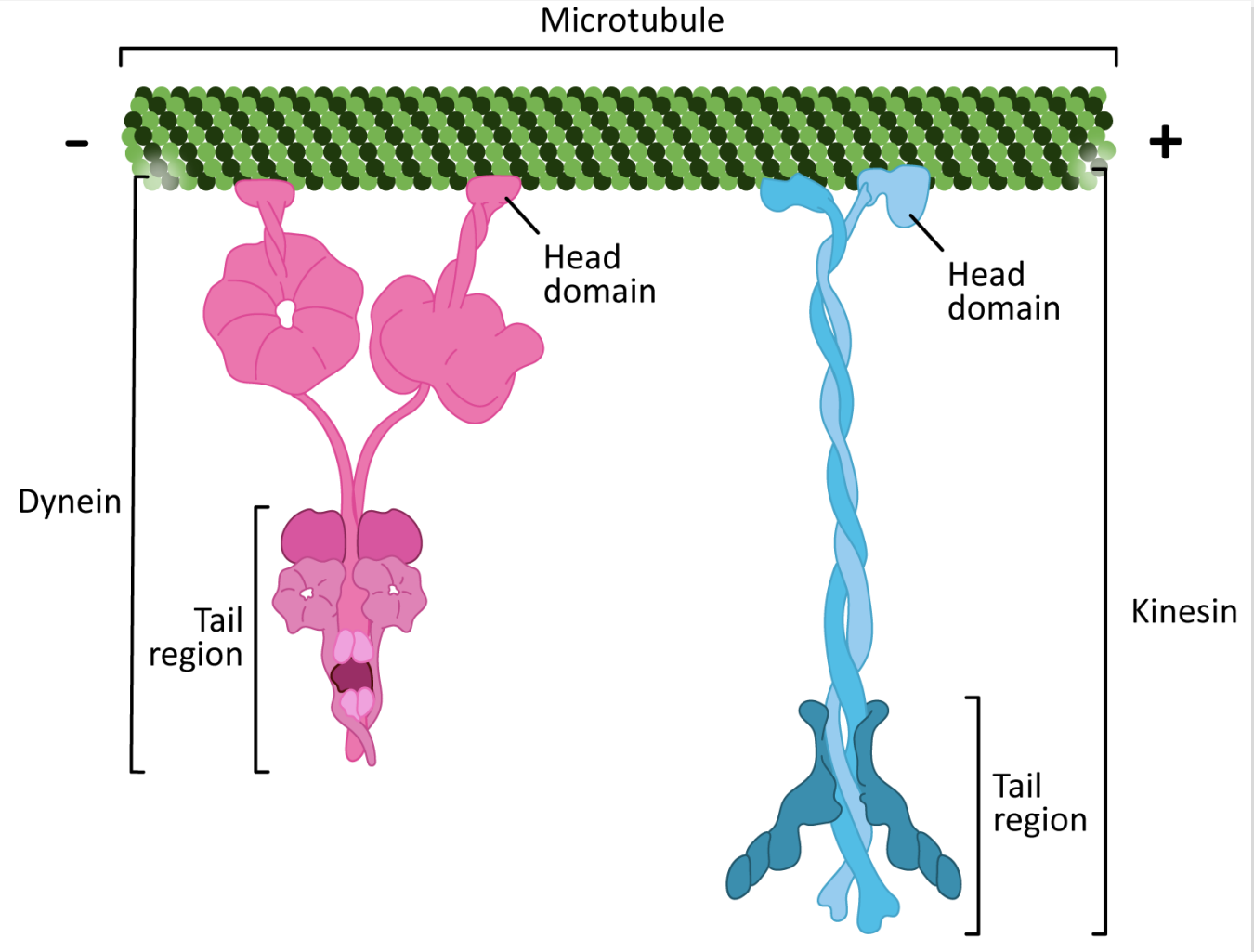



Microtubules use motor proteins to transport a cargo.

Two major classes of microtubule-based motors: **kinesins** and **dyneins**

NEW

# MT MOTOR PROTEINS

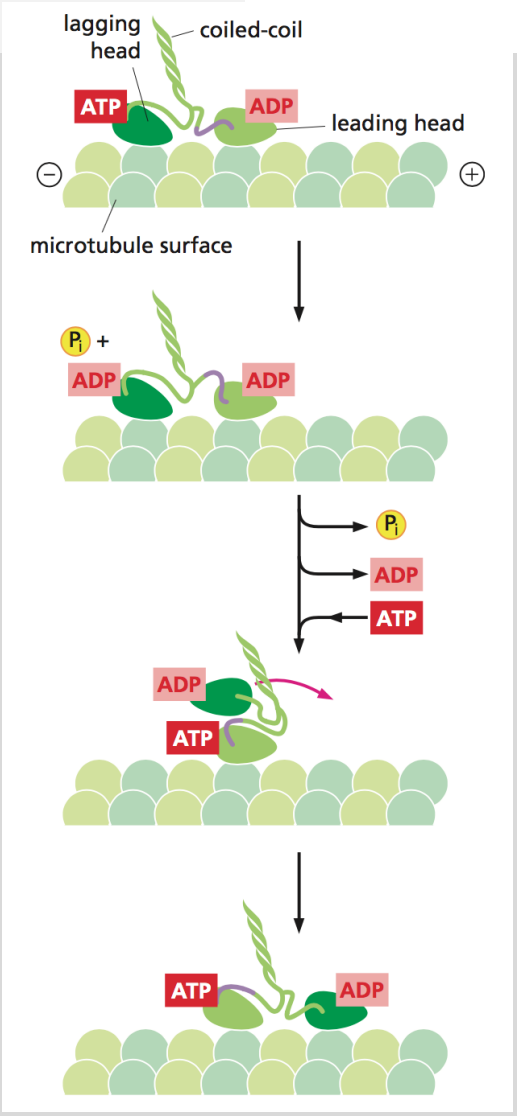



**Structural representations of kinesin and dynein on a microtubule.** The head domain binds to the microtubule reversibly (in an ATP-dependent manner). Through cycles of ATP hydrolysis, these motors are able to walk along the microtubule in a specific direction.

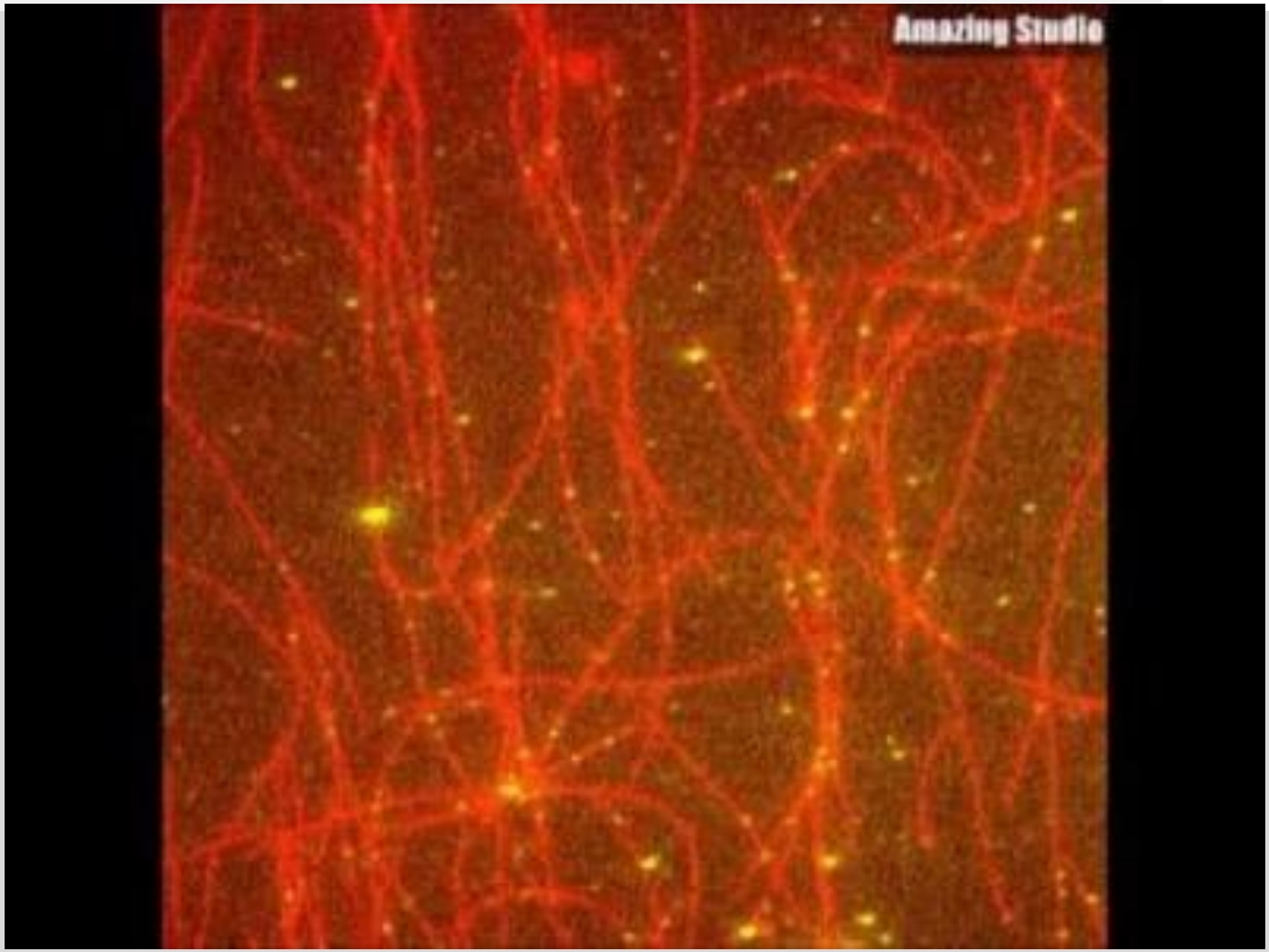
image by Heather Ng-Cornish under CC BY-SA 4.0 license.

# MICROTUBULES MOTORS: KINESIN

- Kinesin-1 is **plus-end directed motor** similar to myosin II in having two heavy chains per active motor
- These form two globular head motor domains that are held together by an elongated coiled-coil tail that is responsible for heavy-chain dimerization
- The two kinesin motor domains work in a coordinated manner; during a **kinesin "step,"** the rear head detaches from its tubulin binding site, passes the partner motor domain, and then rebinds to the next available tubulin binding site
- Using this "**hand- over-hand" motion**, **the kinesin dimer can move for long distances on the microtubule without completely letting go of its track**



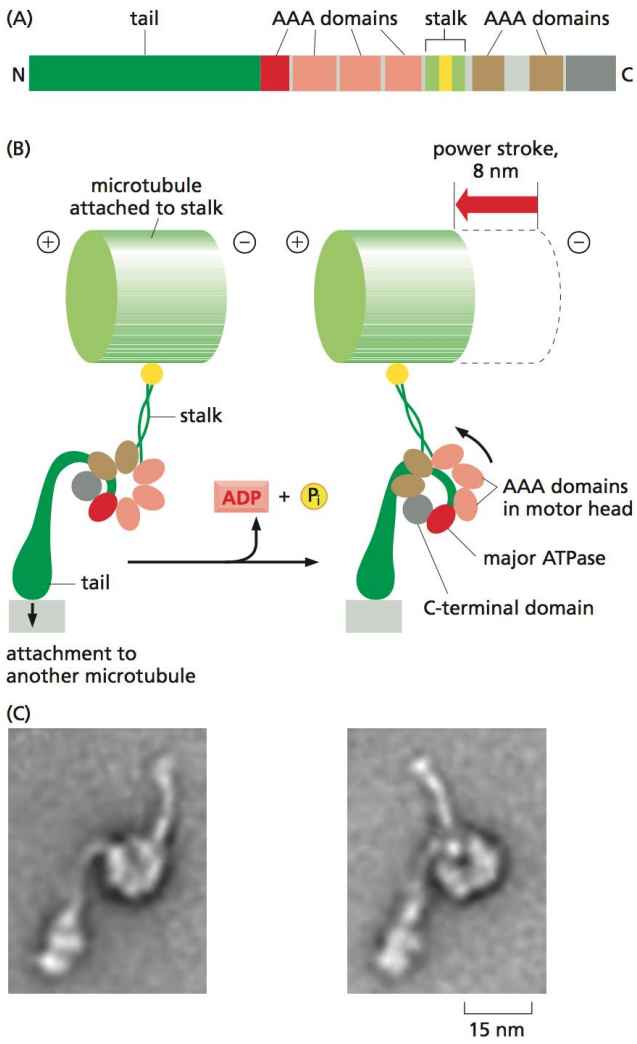
# MICROTUBULES MOTORS: KINESIN




https://www.youtube.com/watch?v=-6g5icw1Zos

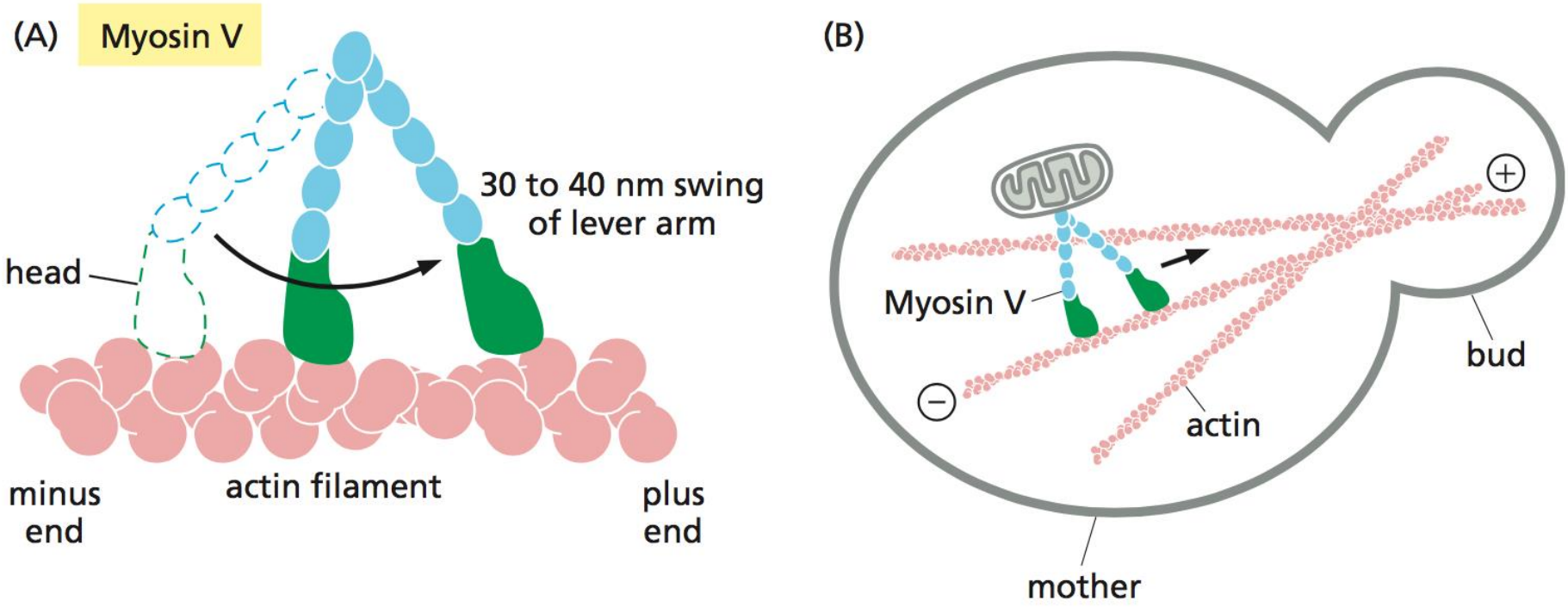
# MICROTUBULES MOTORS: DYNEIN

- The **dyneins** are a family of **minus-end directed** microtubule motors **unrelated to the kinesins**
- But dyneins still follow the general rule of coupling nucleotide hydrolysis to microtubule binding and unbinding as well as to a force-generating conformational change
- They are composed of one, two, or three heavy chains (that include the motor domain) and a large and variable number of associated intermediate, light-intermediate, and light chains
- Dyneins are the largest of the known molecular motors, and they are also among the fastest: axonemal dyneins attached to a glass slide can move micro-tubules at the rate of 14 $\mu$m/sec




Alberts: Molecular Biology of the Cell, 6th edition.

# ACTIN DOES IT TOO, USING OTHER MYOSINS

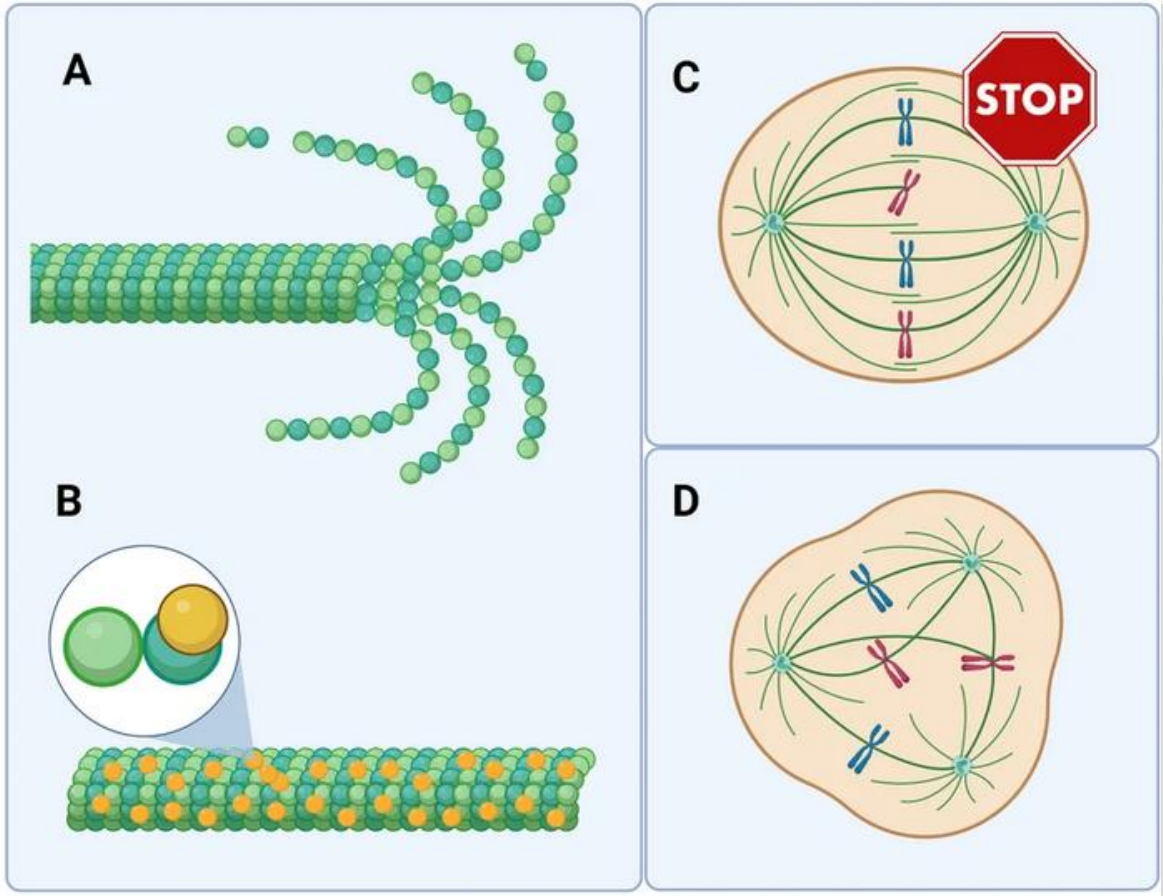



**Myosin V carries cargo along actin filaments.** (A) The lever arm of myosin V is long, allowing it to take a bigger step along an actin filament than myosin II. (B) Myosin V transports cargo and organelles along actin cables, in this example moving a mitochondrion into the growing bud of a yeast cell.

NEW

# MT TARGETING DRUGS: PACLITAXEL






Antineoplastic mechanisms of paclitaxel: Microtubules are an important and dynamic component of the cellular cytoskeleton. These structures, formed by dimers of α and β tubulin, undergo continuous polymerization and depolymerization processes to adapt to the cell's needs (A). Paclitaxel exerts its antineoplastic effect by binding to tubulin dimers (in the β subunit), which prevents microtubule depolymerization (B). This can lead to cell death through mitotic arrest (C) or through the generation of multipolar cells with deficiencies in the amount of DNA (D)

**Velasco-Gonzalez & Coffeen Nurotoxicity Research (2022)**

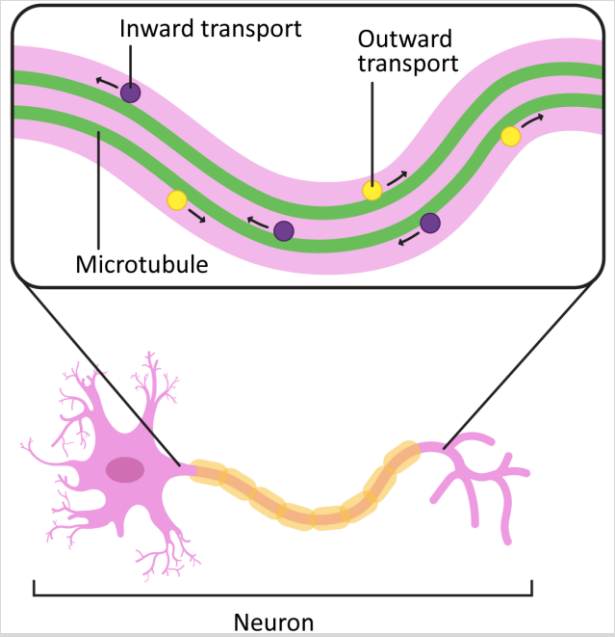
NEW

# MT TARGETING DRUGS: PACLITAXEL

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## WHICH CELLS CAN BE SENSITIVE APART FROM CANCER CELLS?

## AID: WHICH CELLS HAVE LONG ORGANELLE TRANSPORT ROUTES?

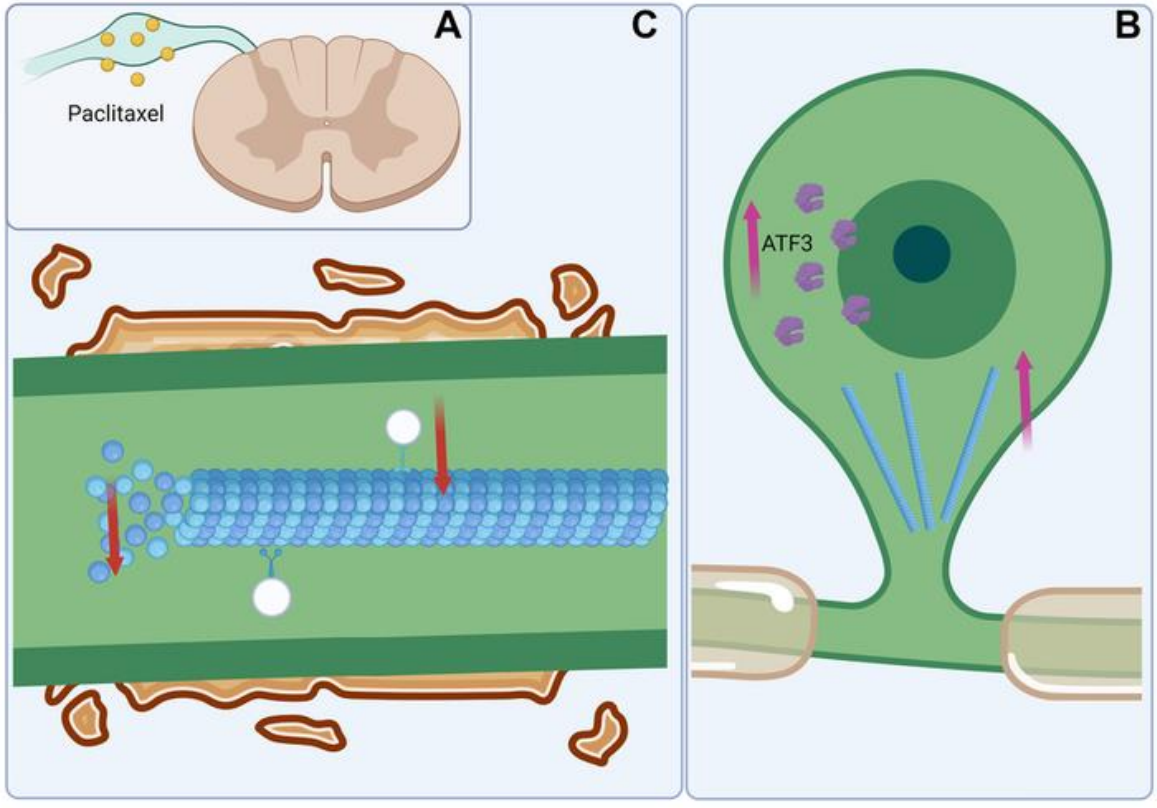



Velasco-Gonzalez & Coffeen Nurotoxicity Research (2022)

NEW

# MT TARGETING DRUGS: PACLITAXEL

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Neuromorphophysiological changes produced by paclitaxel (PAC). After administration, PAC is able to gain access to the nervous system, particularly the dorsal root ganglion (DRG), because these structures lack the protection of the blood–brain barrier (A). Within the neuronal soma, PAC is able to increase the stability of microtubules and increase the expression of the ATF3 protein (purple arrows), a marker of cell damage in neurons (B). In contrast, PAC decreases the stability of microtubules in axons, which leads to a decrease in the efficiency of axonal transport (red arrows) (C). All of the above leads to a greater probability that the neuron may suffer from degeneration of the myelin sheath and a consequent axotomy, which is particularly true for the most distal regions of the axon

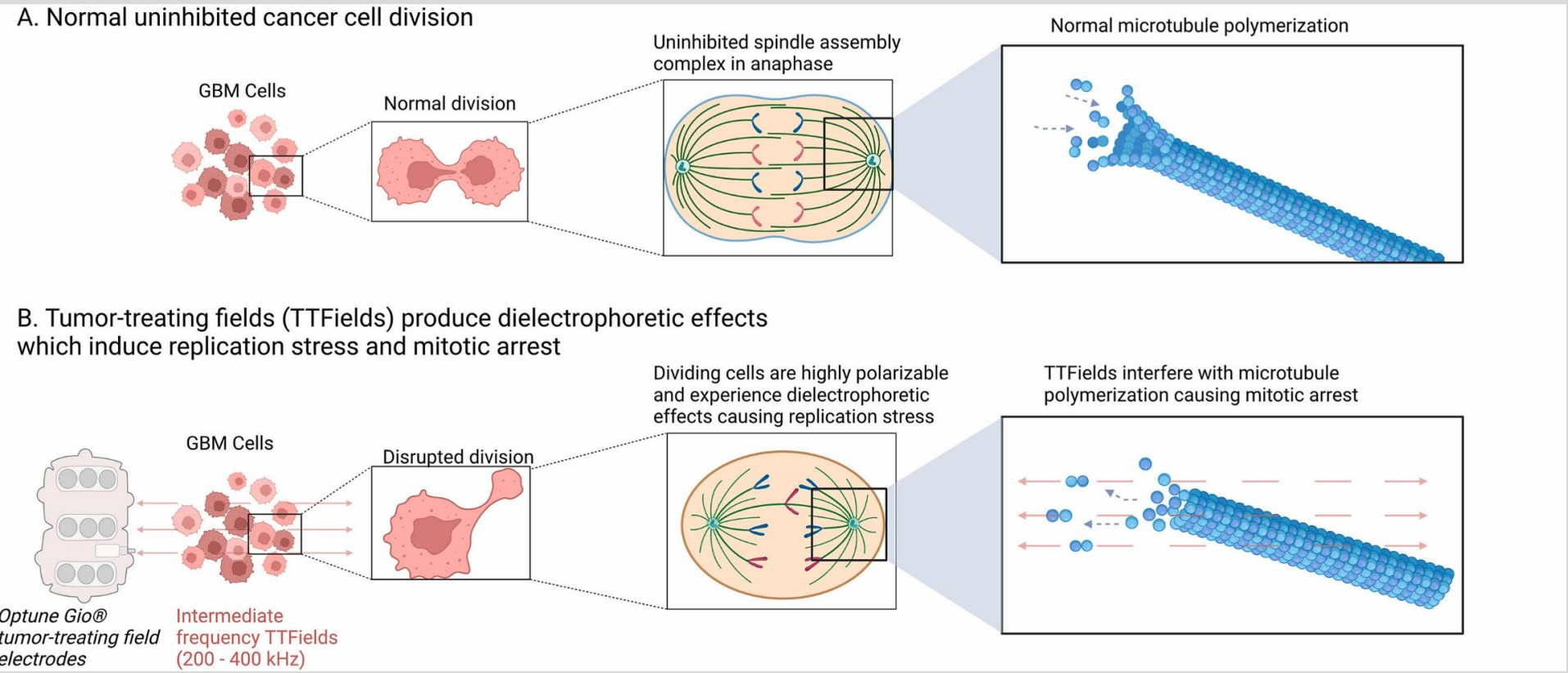
NOT SUPPOSED TO PASS BBB, BUT MORE IN PERIPHERY NEUROTOXICITY

**Velasco-Gonzalez & Coffeen Nurotoxicity Research (2022)**

NEW

# MT TTF

UNIVERSITÄT BERN




Eckert et al. Brain stimulation (2025)

# INTERMEDIATE FILAMENTS

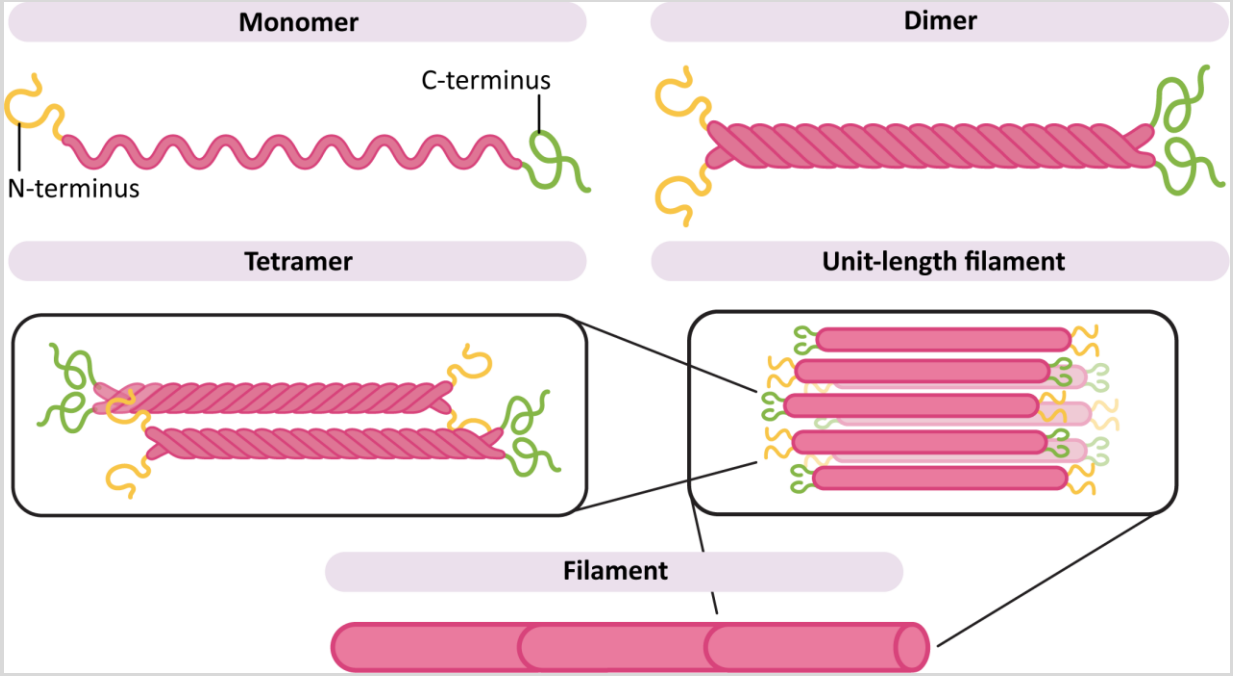


- Intermediate filaments are particularly prominent in the cytoplasm of cells that are subject to mechanical stress.
- Cytoplasmic intermediate filaments are closely related to their ancestors, the much more prevalent nuclear lamins, which are found in many eukaryotes but missing from unicellular organisms.
- The nuclear lamins form a meshwork lining the inner membrane of the nuclear envelope, where they provide anchorage sites for chromosomes and nuclear pores.
- Several times during metazoan evolution, lamin genes have apparently duplicated, and the duplicates have evolved to produce rope-like, cytoplasmic intermediate filaments.
- In contrast to the highly conserved actins and tubulin isoforms that are encoded by a handful of genes, **different families of intermediate filaments are much more diverse and are encoded by 70 different (human) genes with distinct, cell type-specific functions.**

NEW

# INTERMEDIATE FILAMENTS






- Two monomers come together to form a dimer.
- Dimers assemble in an antiparallel arrangement. This forms a tetramer.
- Tetramers assemble to form a long filament.

**Staggering** → long ropelike structure that doesn't stretch and is difficult to break.

# INTERMEDIATE FILAMENTS

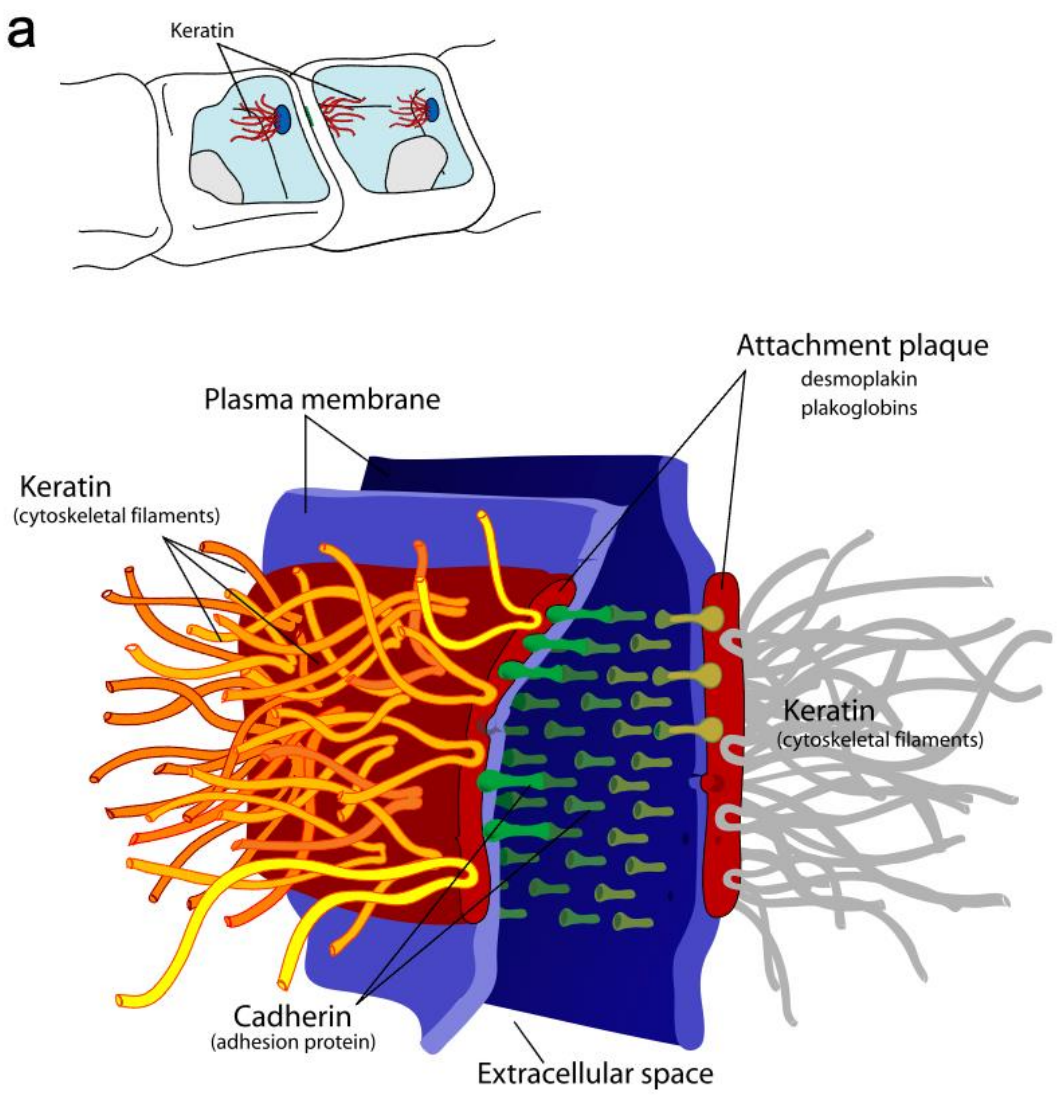
**TABLE 16–2 Major Types of Intermediate Filament Proteins in Vertebrate Cells**

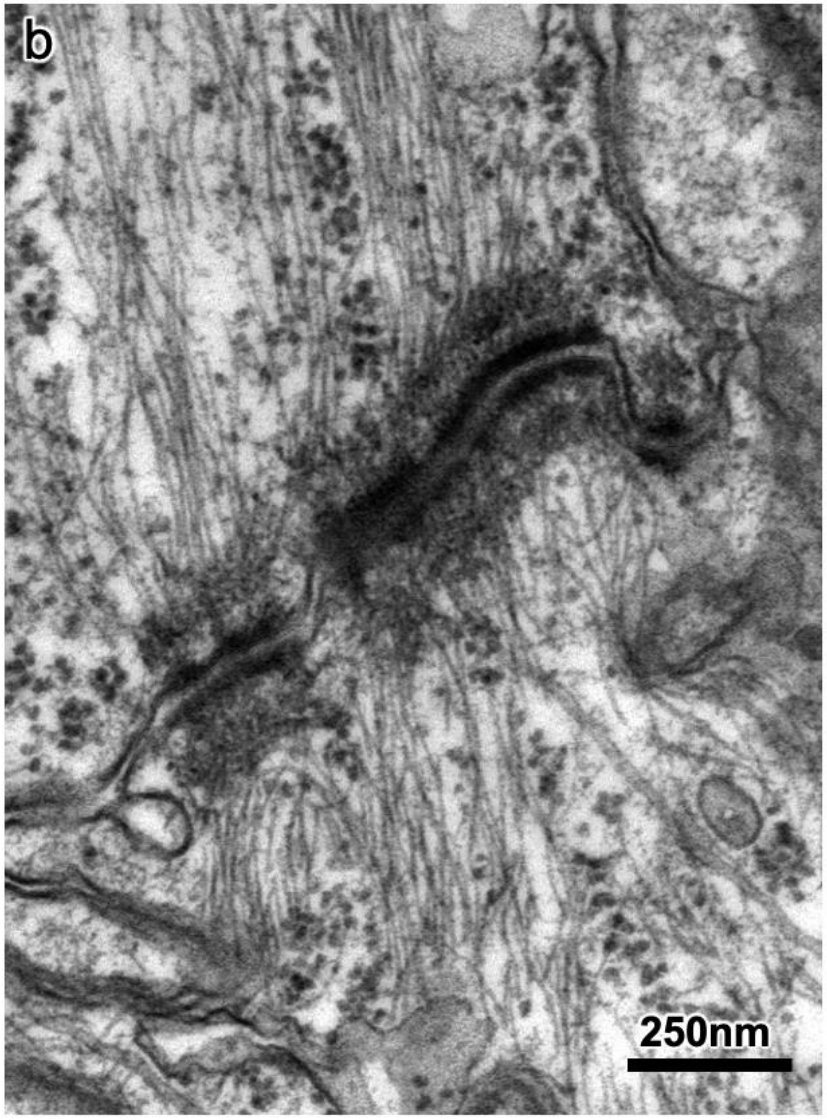
| Types of intermediate filament | Component polypeptides | Location |
|---|---|---|
| Nuclear | Lamins A, B, and C | Nuclear lamina (inner lining of nuclear envelope) |
| Vimentin-like | Vimentin | Many cells of mesenchymal origin |
| | Desmin | Muscle |
| | Glial fibrillary acidic protein | Glial cells (astrocytes and some Schwann cells) |
| | Peripherin | Some neurons |
| Epithelial | Type I keratins (acidic) | Epithelial cells and their derivatives (e.g., hair and nails) |
| | Type II keratins (neutral/basic) | |
| Axonal | Neurofilament proteins (NF-L, NF-M, and NF-H) | Neurons |

NEW

# INTERMEDIATE FILAMENTS

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NEW

# INTERMEDIATE FILAMENTS

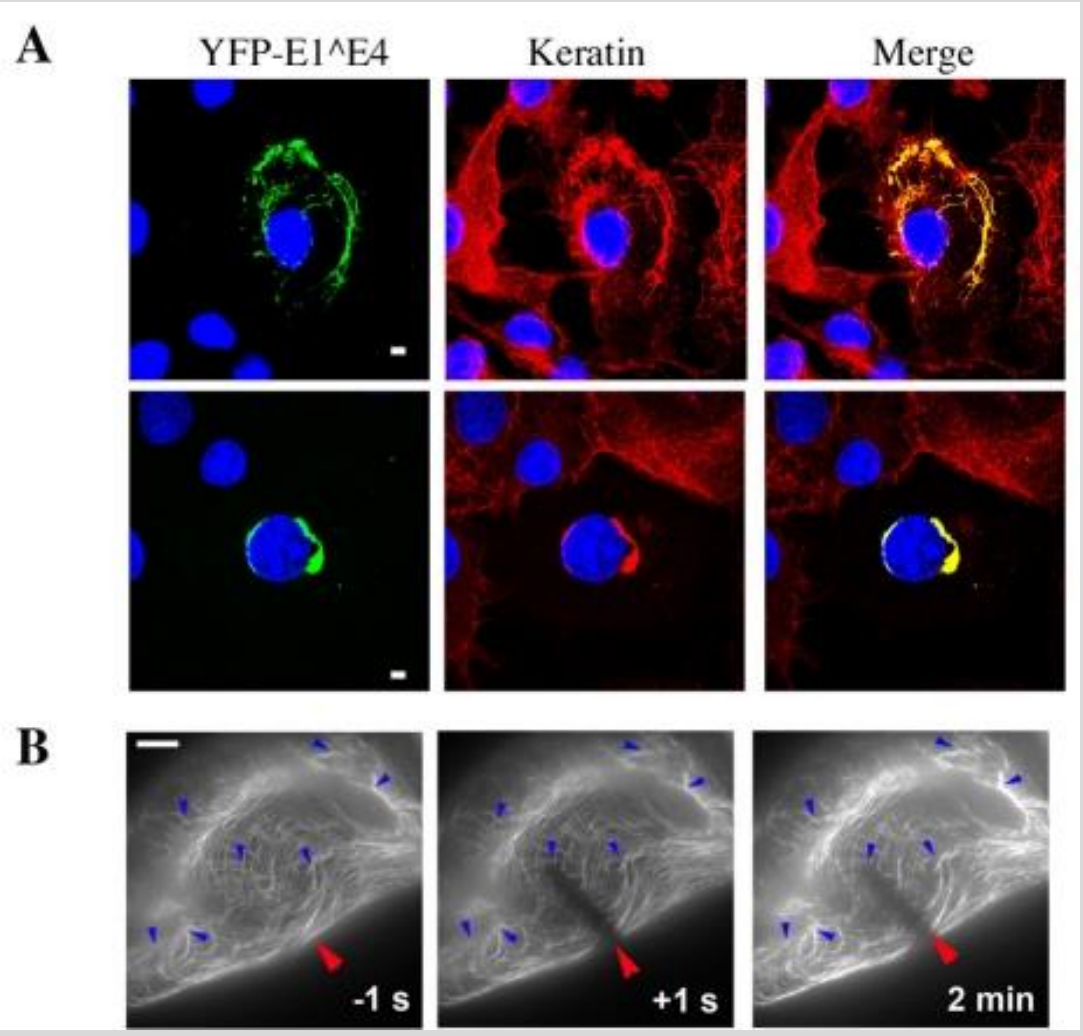
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NEW

# HPV-INDUCED KERATIN DISRUPTION

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McIntosh et al. JCS (2010)

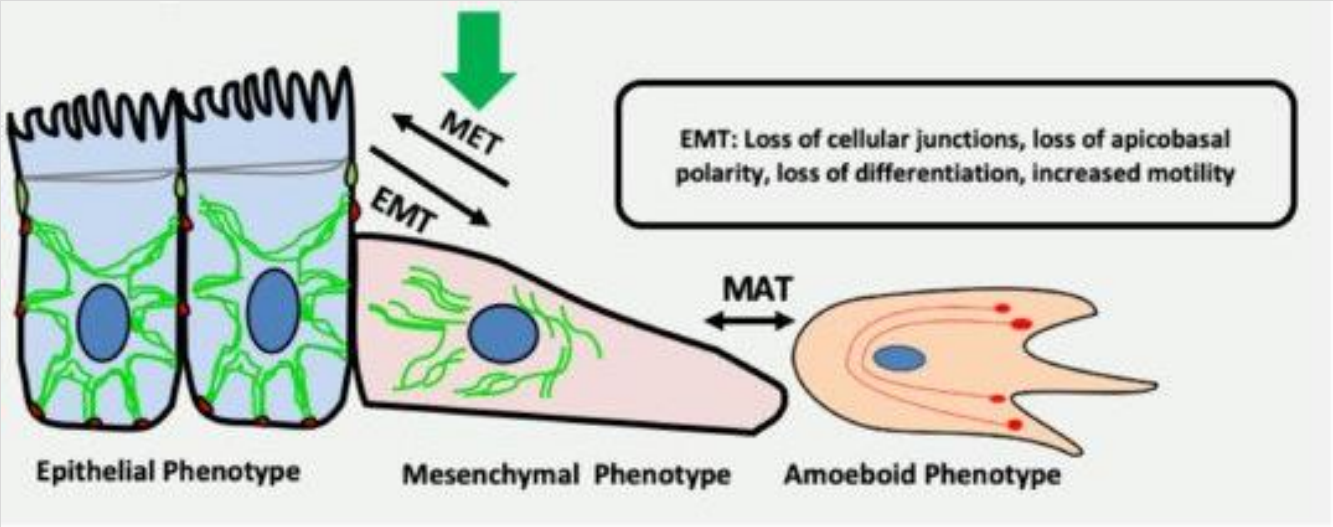
NEW

# INTERMEDIATE FILAMENTS

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During **Epithelial to Mesenchimal Transition (EMT)** vimentin and cheratin undergo a role change.

This grants tumor cells the capacity to detach from the original tissue and migrate.



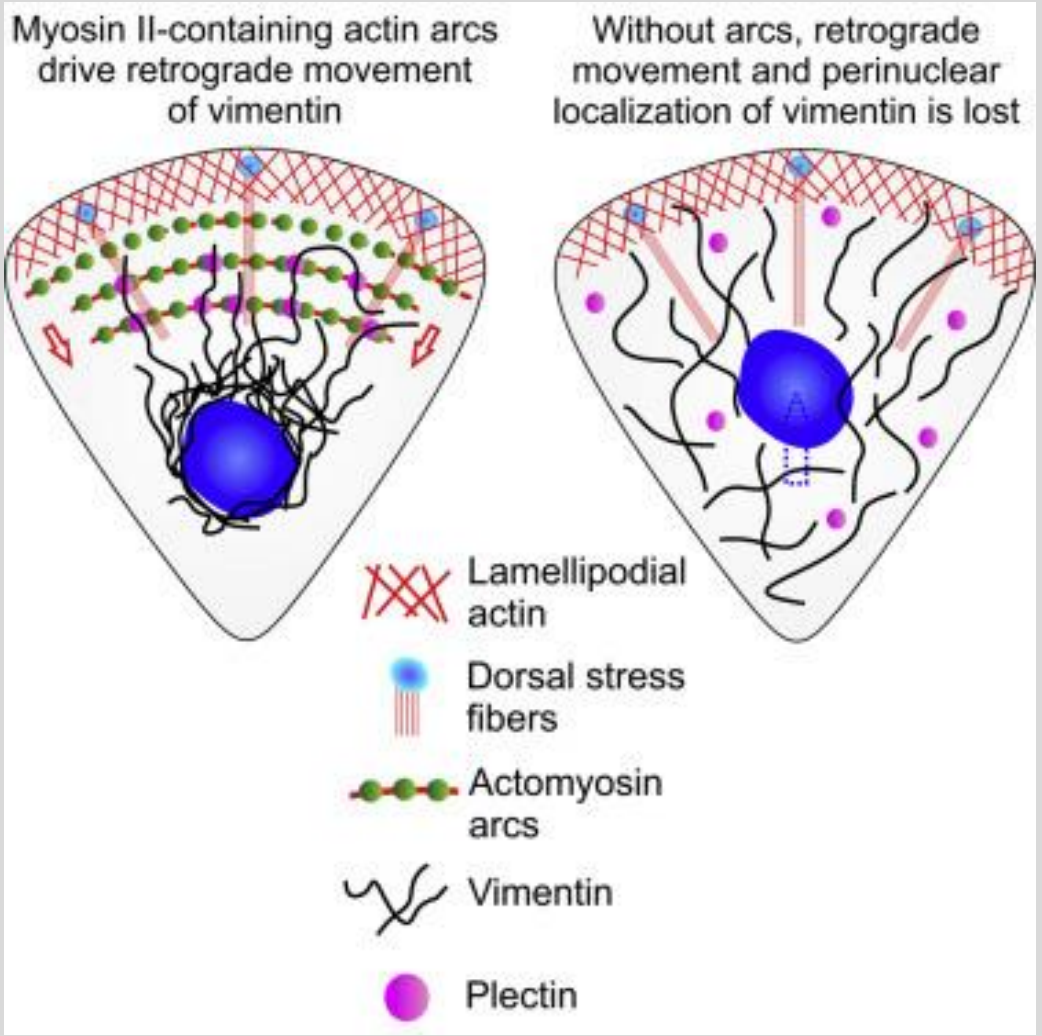

Usman et al., Vimentin at the Heart of Epithelial Mesenchymal Transition. Cancers (2021)

# INTERMEDIATE FILAMENTS: ACTIN-VIMENTIN INTERACTIONS

Jui et al. find that specific actin stress fiber structures, transverse arcs, interact with vimentin intermediate filaments and promote their retrograde flow

Consequently, myosin-II-containing arcs are important for perinuclear localization of the vimentin network in cells

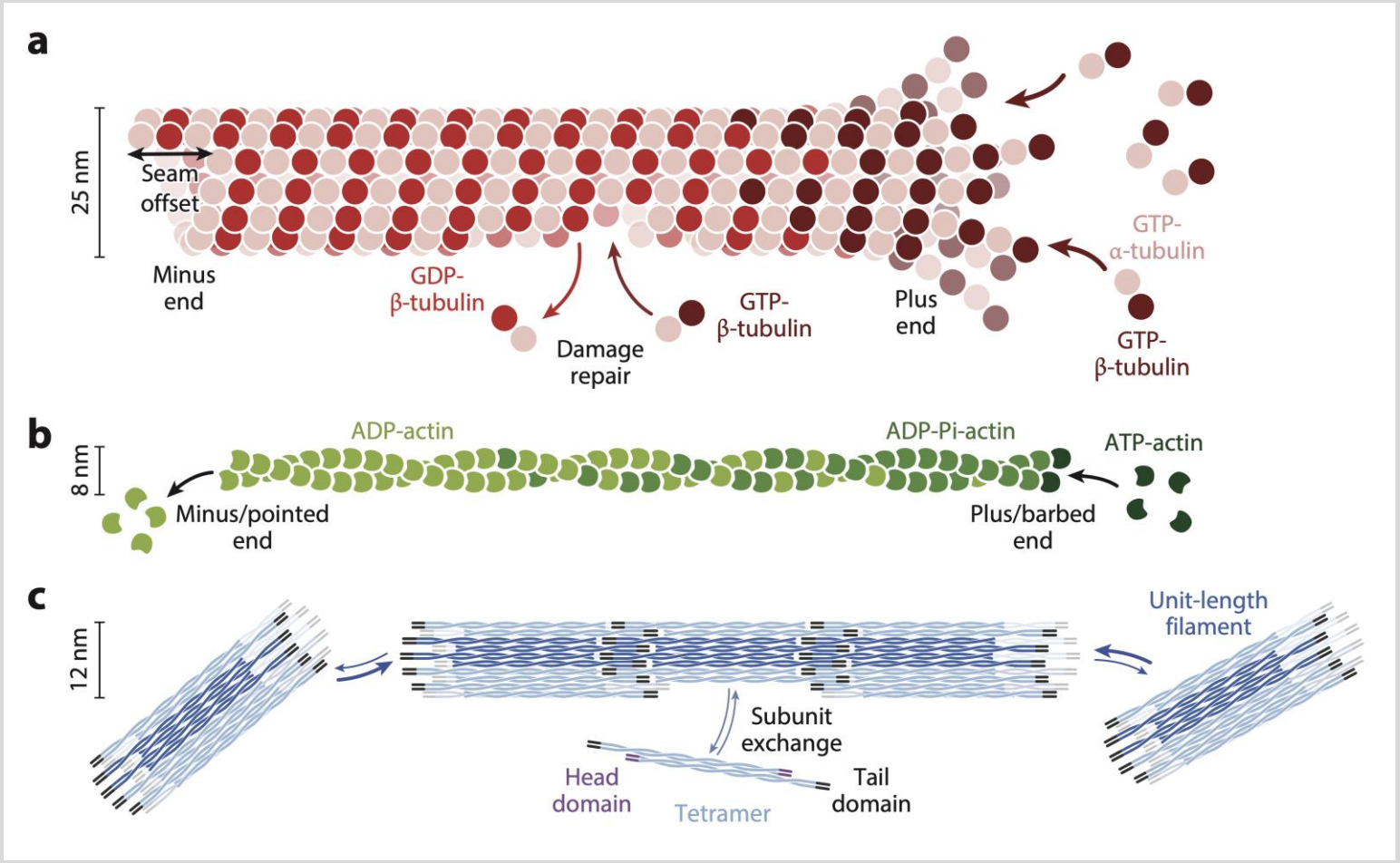
The vimentin network reciprocally restricts retrograde movement of arcs and hence controls the width of the flat lamellum at the leading edge of the cell




Jiu et al., Cell Reports 2015

NEW

# Summary of cytoskeletal structures and assembly processes.




Romet-Lemonne G, Leduc C, Jégou A, Wioland H.Annu Rev Biophys. 2025

NEW

# LAST EXERCISE



NEW

# THE END

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> THANK YOU FOR PARTICIPATING!

> QUESTIONS?

> CONTACT:

diego.pizzagalli@unibe.ch or diego.pizzagalli@supsi.ch