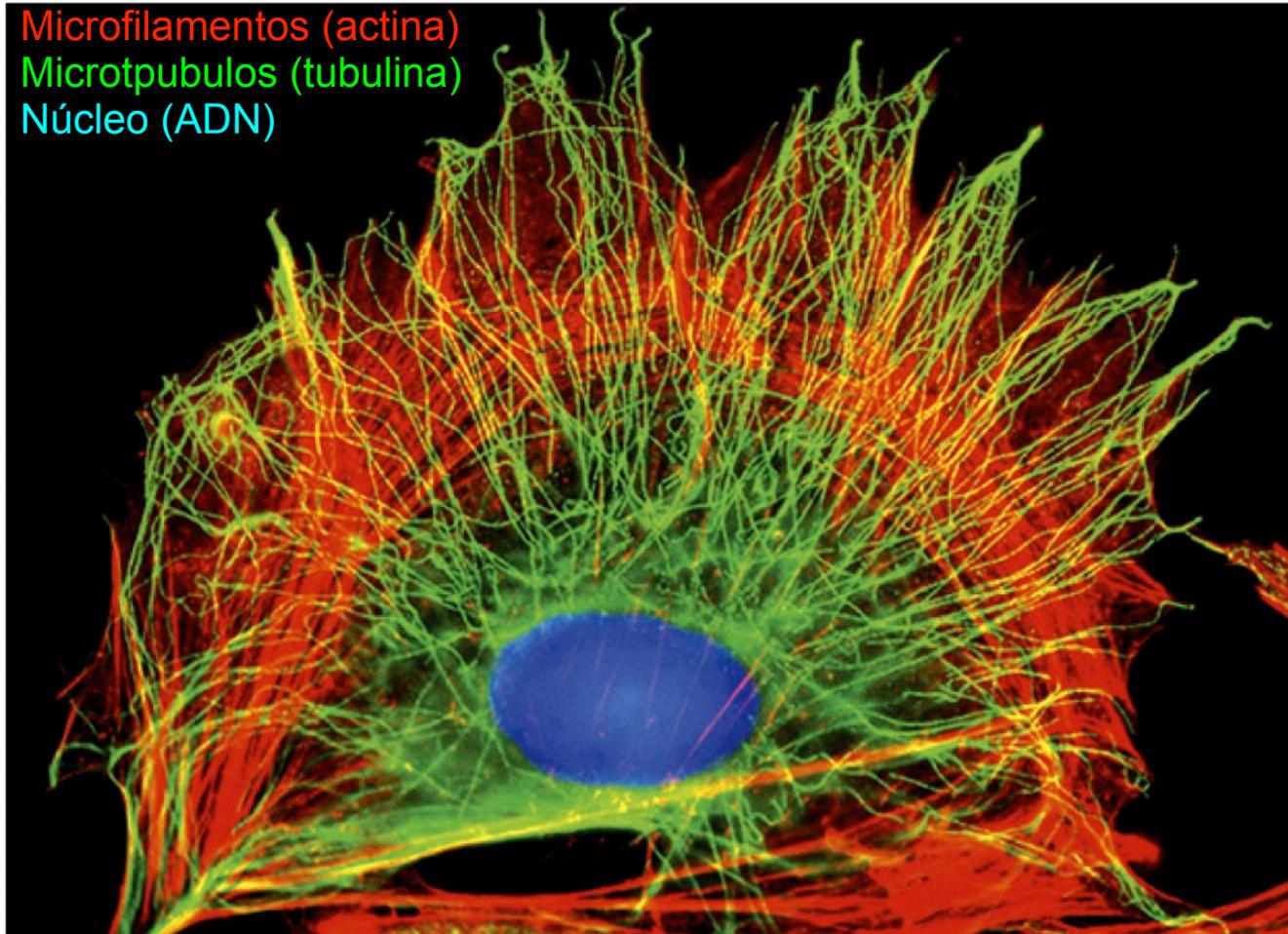


# Citoesqueleto y Uniones celulares



Microfilamentos (actina)  
Microtubulos (tubulina)  
Núcleo (ADN)

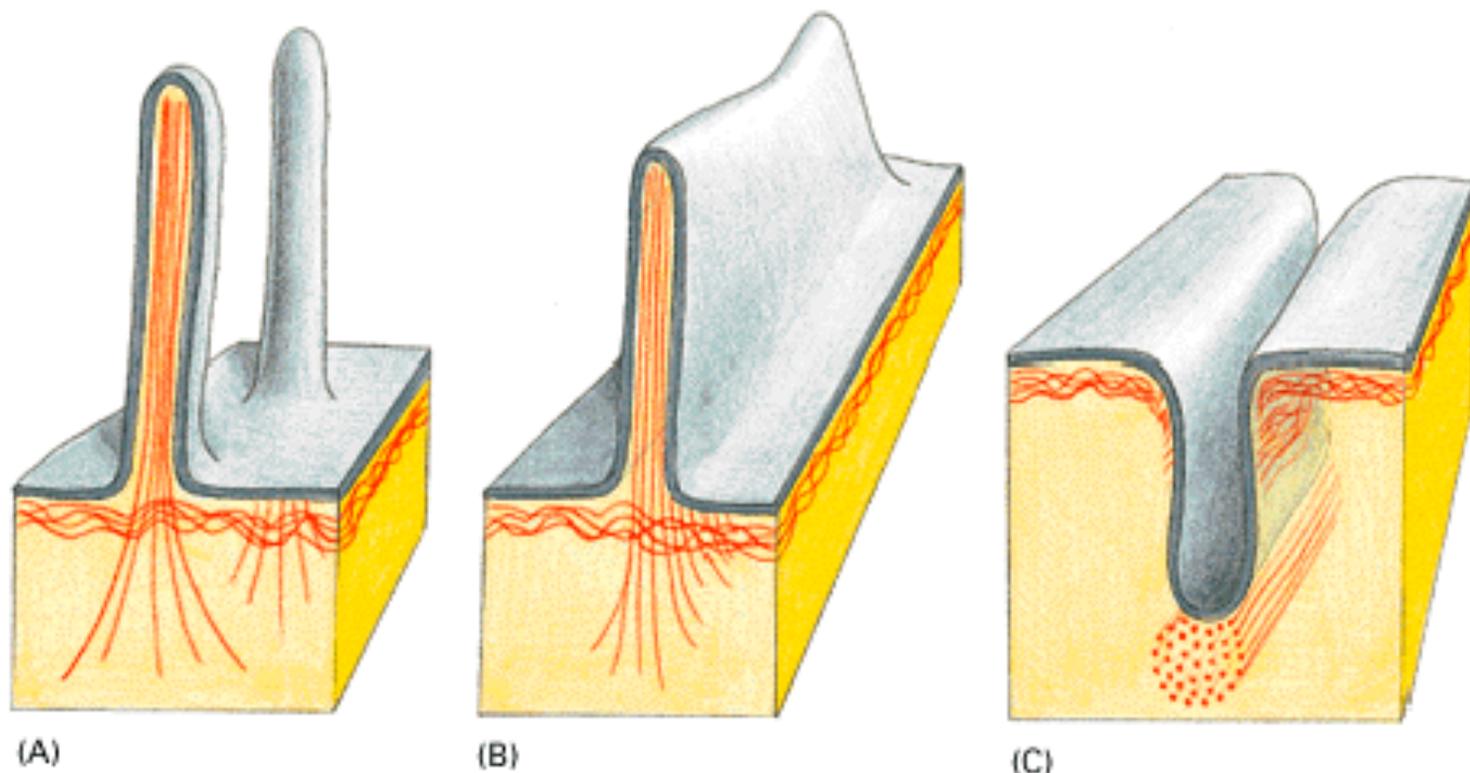
10  $\mu\text{m}$

# Funciones del Citoesqueleto



## DAR FORMA

- Apoyo estructural de la célula:
  - El citoesqueleto permite que la célula **adopte una forma** y que la modifique.
  - **Movimiento celular** (movimiento ameboide, cilios y flagelos, contracción muscular, conos de crecimiento)
- Armazón Interno:
  - Mantención de la posición de los organelos al interior de la célula. (núcleo, mitocondrias, ribosomas, etc)
  - Movimiento de materiales y organelos. (movimiento de membranas, transporte de vesículas, invaginación de la membrana, transporte de ARNm, etc...)
- Transducción de señales:
  - Contacto con la membrana plasmática.
  - Anclaje de las moléculas de adhesión.



**Figure 16-9.** Actin filaments often shape the plasma membrane of animal cells. Three examples of plasma membrane changes caused by the cortical network of actin filaments. (A) Thin, spiky protrusions such as microspikes form on the surface of cells by the assembly of supporting bundles of actin filaments anchored in the cell cortex. (B) Sheetlike extensions, called lamellipodia, also form on the surface, in this case supported by a flattened web of actin filaments rather than discrete bundles. (C) Invaginations of the cell surface, as occur during cell division, are produced by a contractile bundle of actin filaments associated with the motor protein myosin

# Conceptos claves

**¡El citoesqueleto es dinámico!**

## **Existen 3 tipos básicos de redes filamentosas**

- Microtúbulos (filamentos de 25 nm)
- Microfilamentos (filamentos de 7nm)
- Filamentos Intermedios (filamentos de 8-12 nm)

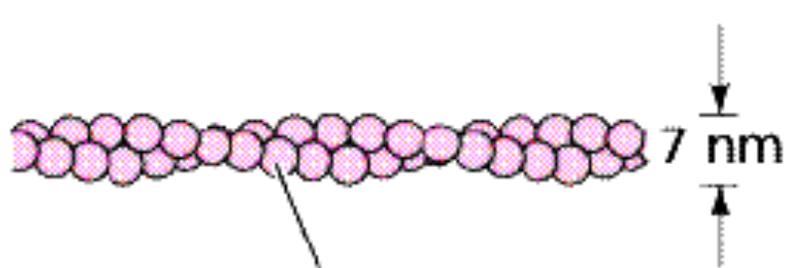
## **Características generales**

- La estructura fibrilar es producto del ensamblaje de muchas subunidades.
- El paso limitante es la polimerización.
  - Cinta transportadora (microfilamentos ppal%)
  - Inestabilidad Dinámica (microtúbulos ppal%)

## **La función es regulada por proteínas accesorias:**

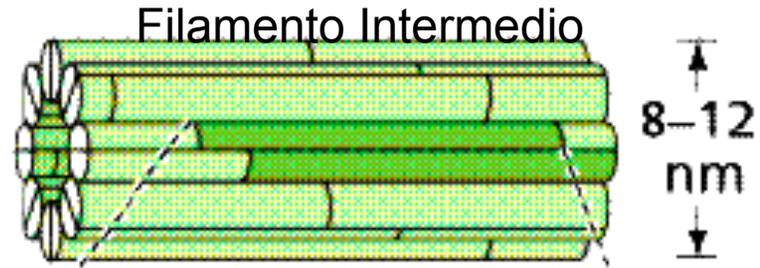
- Secuestradoras de subunidades.
- Proteínas que se unen al costado del filamento
- Proteínas que tapan un extremo (capping)

# COMPONENTES DEL CITOESQUELETO



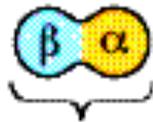
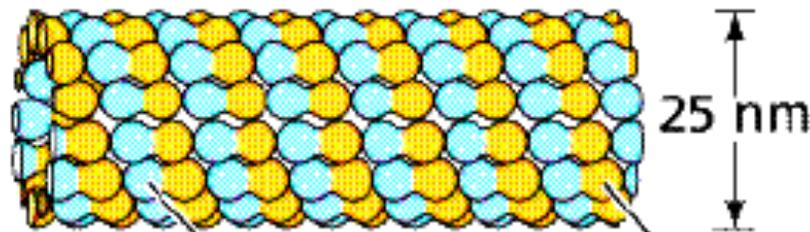
Actin monomer

Sub Unidades  
GLOBULARES



Fibrous subunit

Sub Unidades  
FIBRILARES



Tubulin dimer

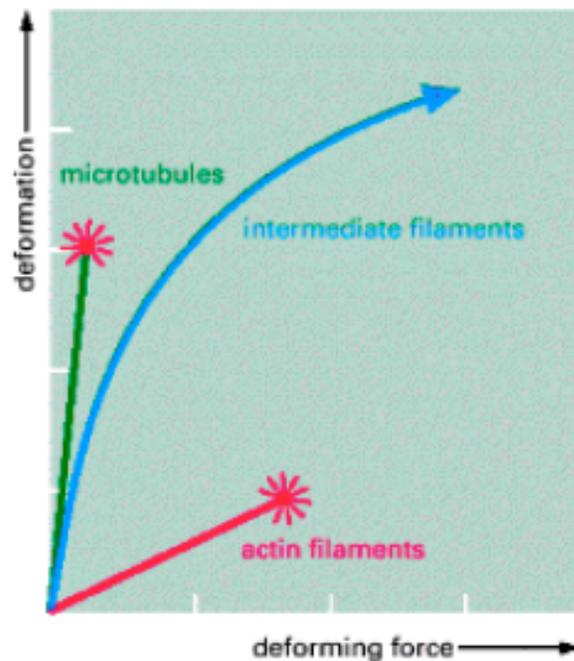
Sub Unidades  
GLOBULARES

$\beta$ -Tubulin  
monomer

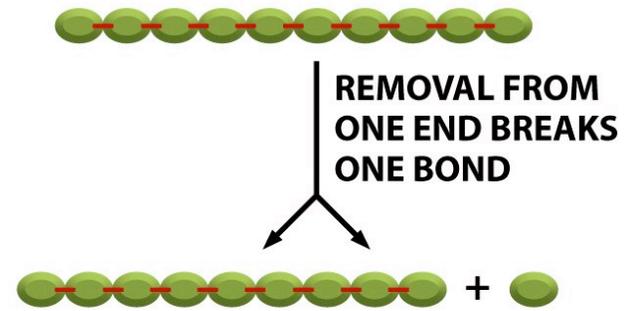
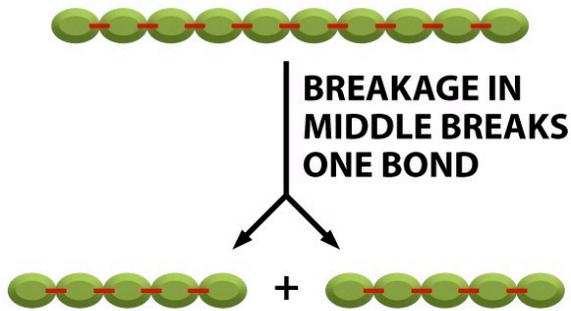
$\alpha$ -Tubulin  
monomer

ESTRUCTURA TERCEARIA VS.  
CUATERNARIA

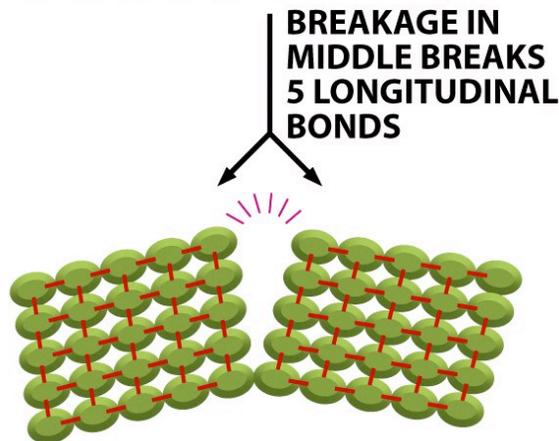
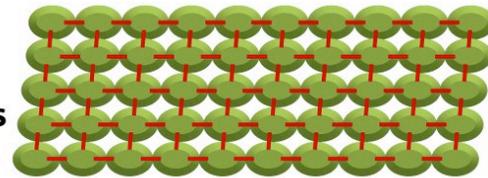
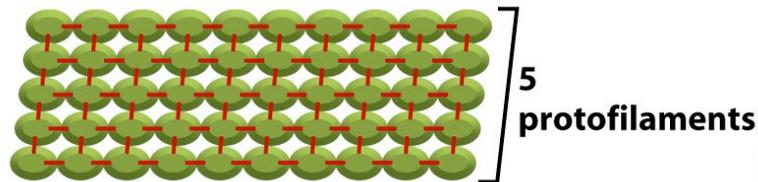
## ¿Por qué tres tipos de filamentos diferentes?



**Figure 16-17. Mechanical properties of actin, tubulin, and intermediate filament polymers.** Networks composed of microtubules, actin filaments, or a type of intermediate filament called vimentin, all at equal concentration, were exposed to a shear force in a viscometer, and the resulting degree of stretch was measured. The results show that microtubule networks are easily deformed but that they rupture (indicated by *red starburst*) and begin to flow without limit when stretched beyond 150% of their original length. Actin filament networks are much more rigid, but they also rupture easily. Intermediate filament networks, by contrast, are not only easily deformed, but they withstand large stresses and strains without rupture; they are thereby well suited to maintain cell integrity.

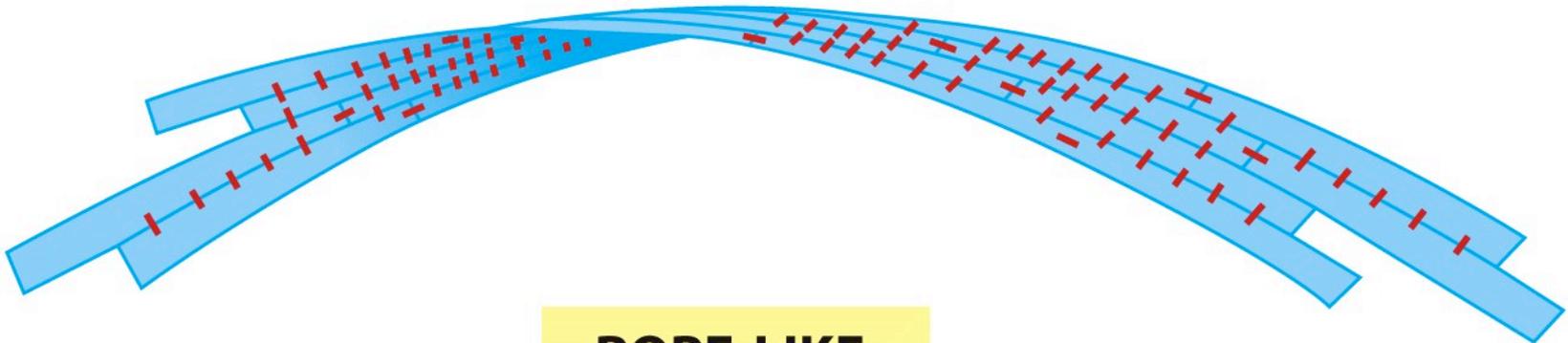
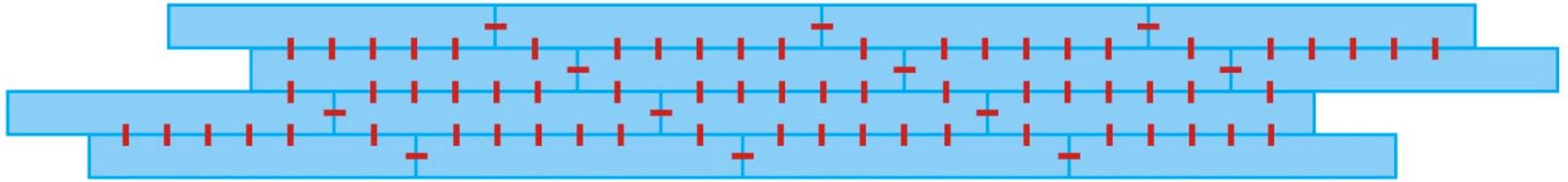


**SINGLE PROTOFILAMENT: THERMALLY UNSTABLE**



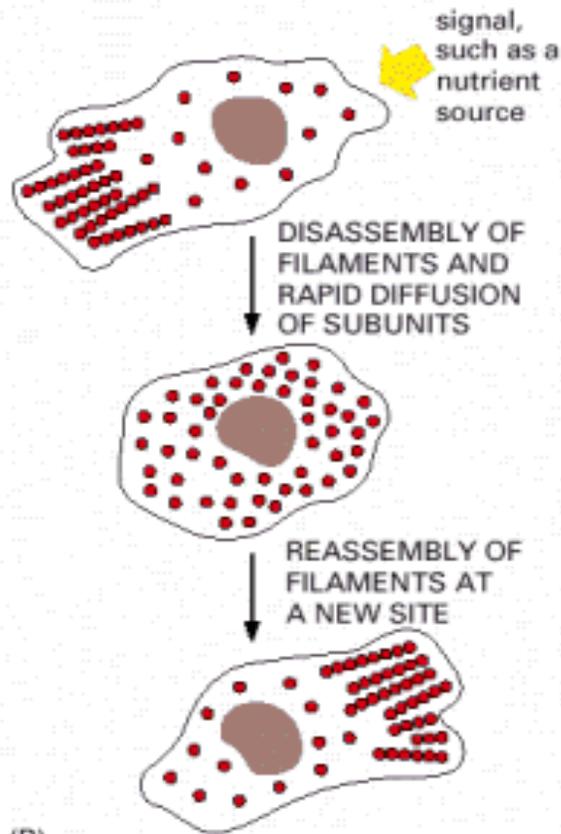
**MULTIPLE PROTOFILAMENTS: THERMALLY STABLE**

**staggered long subunits: lateral contacts dominate**

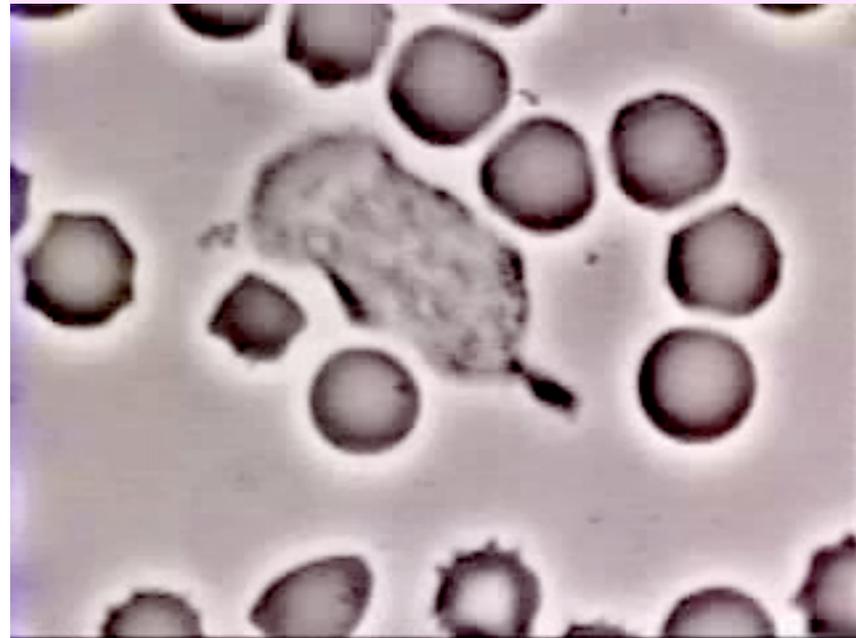


**ROPE-LIKE  
PROPERTIES**

# Dinamismo del Citoesqueleto

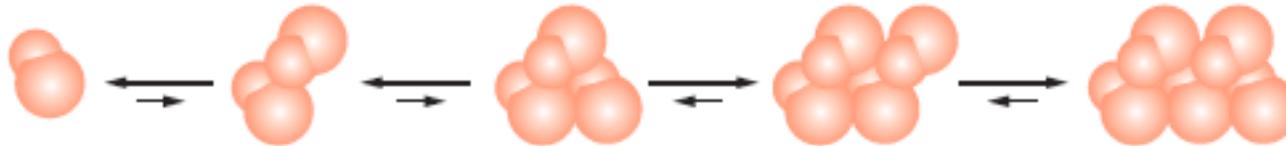


- Construcción en base a monómeros
  - monómeros muy abundantes
  - las subunidades no están unidas covalentemente.
- Proteínas accesorias
  - regulan el sitio y velocidad de nucleación de filamentos
  - Regulan el equilibrio entre **polimerización y despolimerización.**



# Nucleación

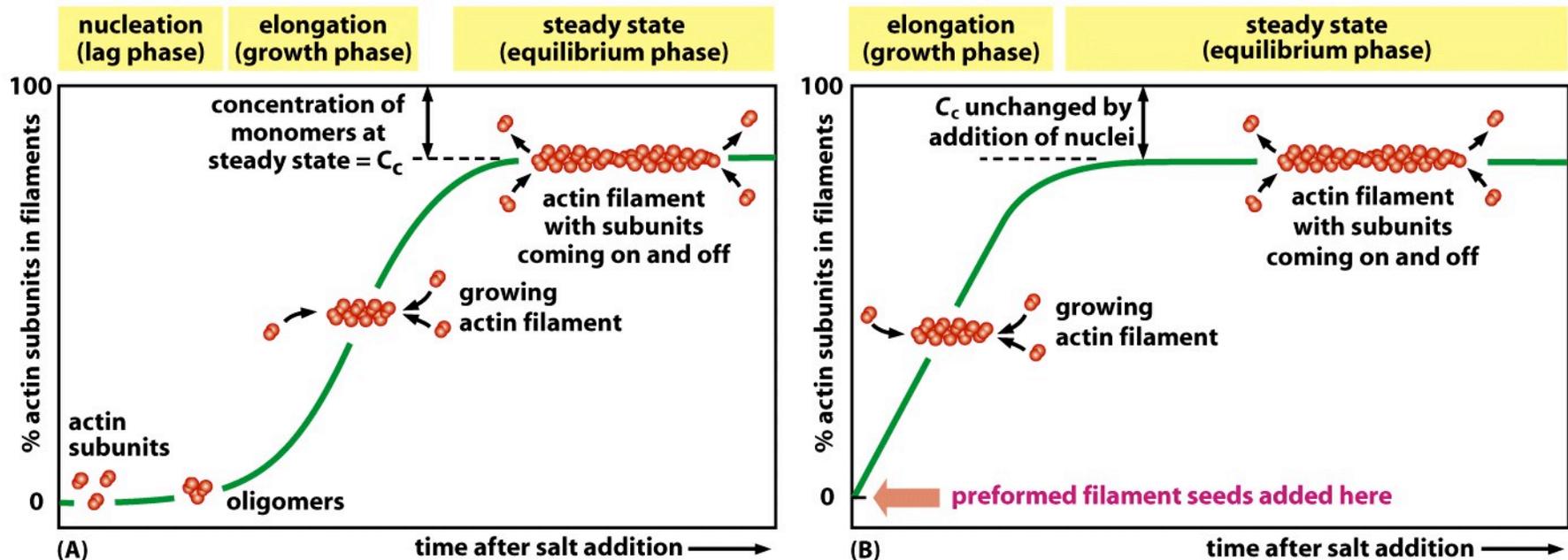
Los polímeros se estabilizan por los múltiples enlaces entre las subunidades. En el caso de actina, dos sub U son poco estables, un trímero aumenta la estabilidad.



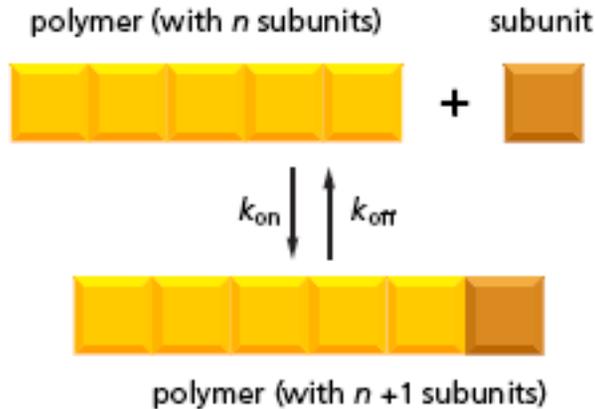
Trímero

Puede adicionar monómeros y crecer.

La aparición de núcleos es el paso limitante en el crecimiento de los filamentos

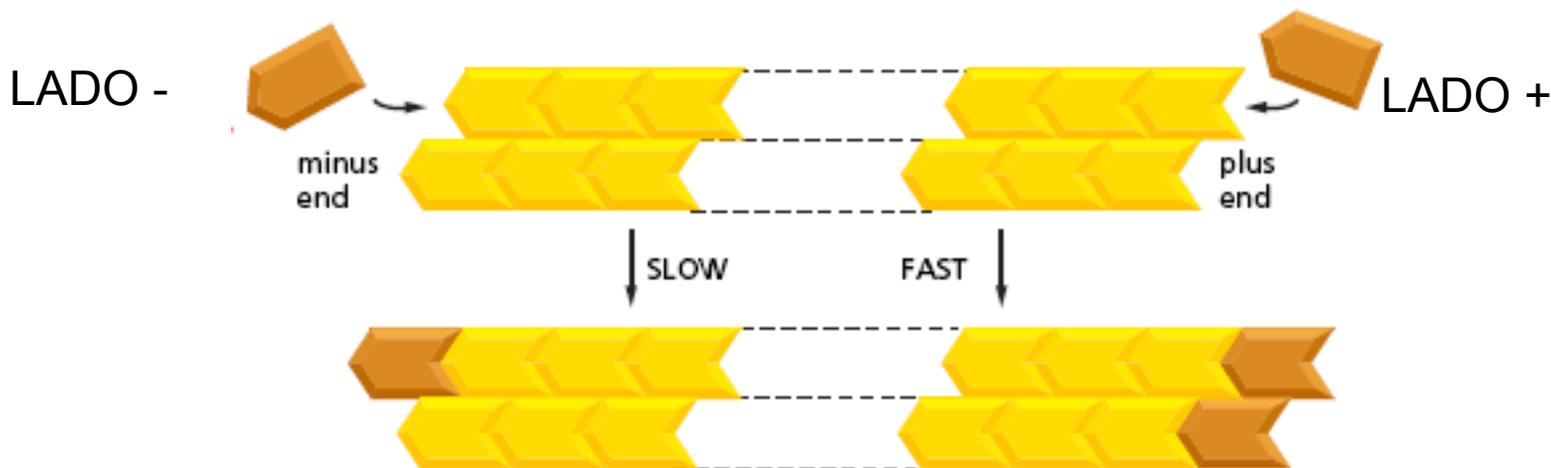


# polimerización y. despolimerización



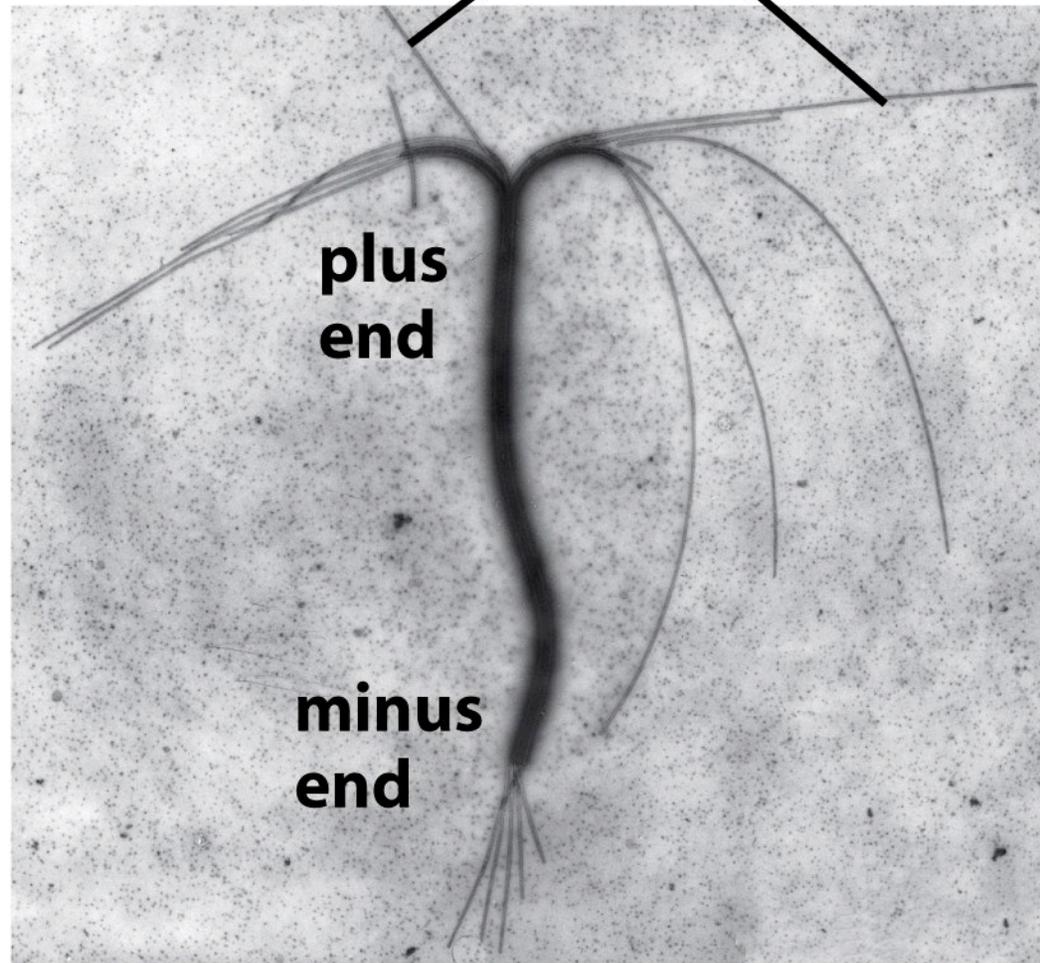
Los conceptos de lado positivo y negativo hacen referencia donde ocurren más rápido los procesos.

**NO** SE REFIERE A QUE LOS MONOMEROS SE INCORPORAN POR UN LADO Y SALEN POR OTRO



Los monómeros se adicionan más rápido del lado positivo (+) que del negativo (-)

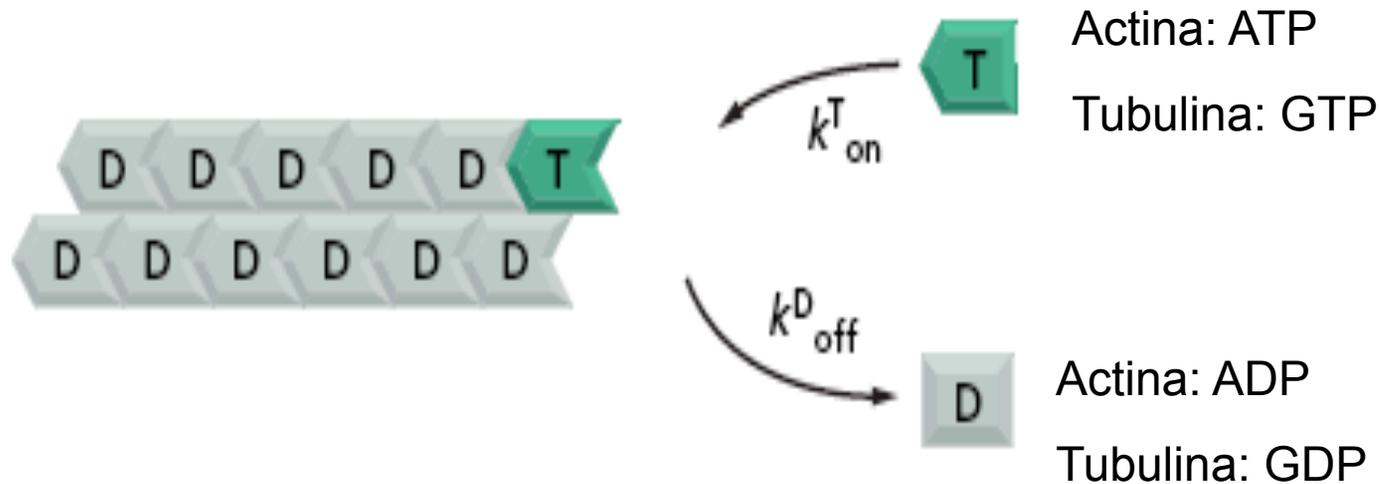
**newly formed microtubules**



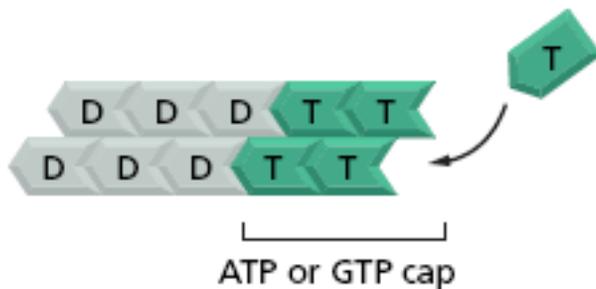
**plus  
end**

**minus  
end**

**1  $\mu$ m**



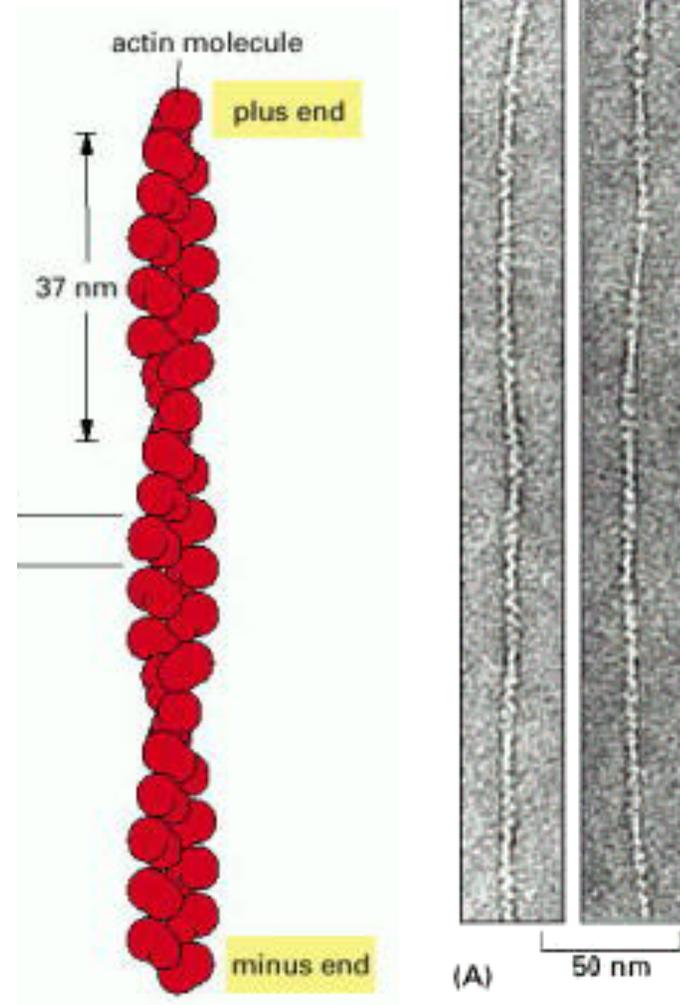
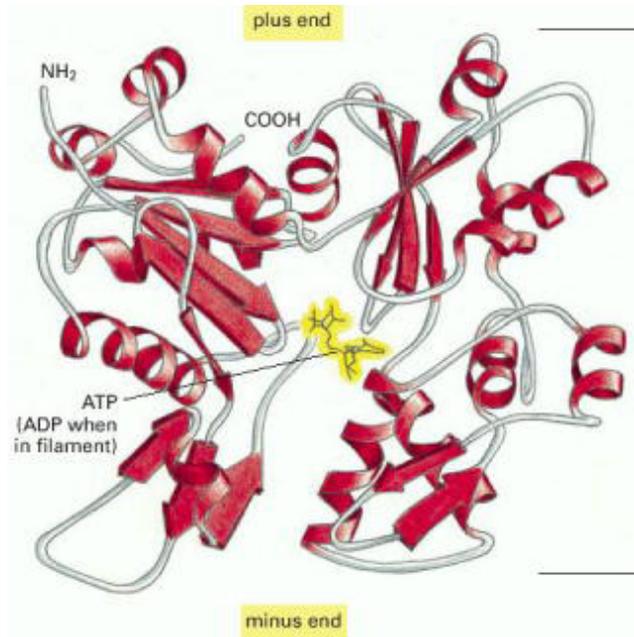
¿qué significa el cambio ATP por ADP?



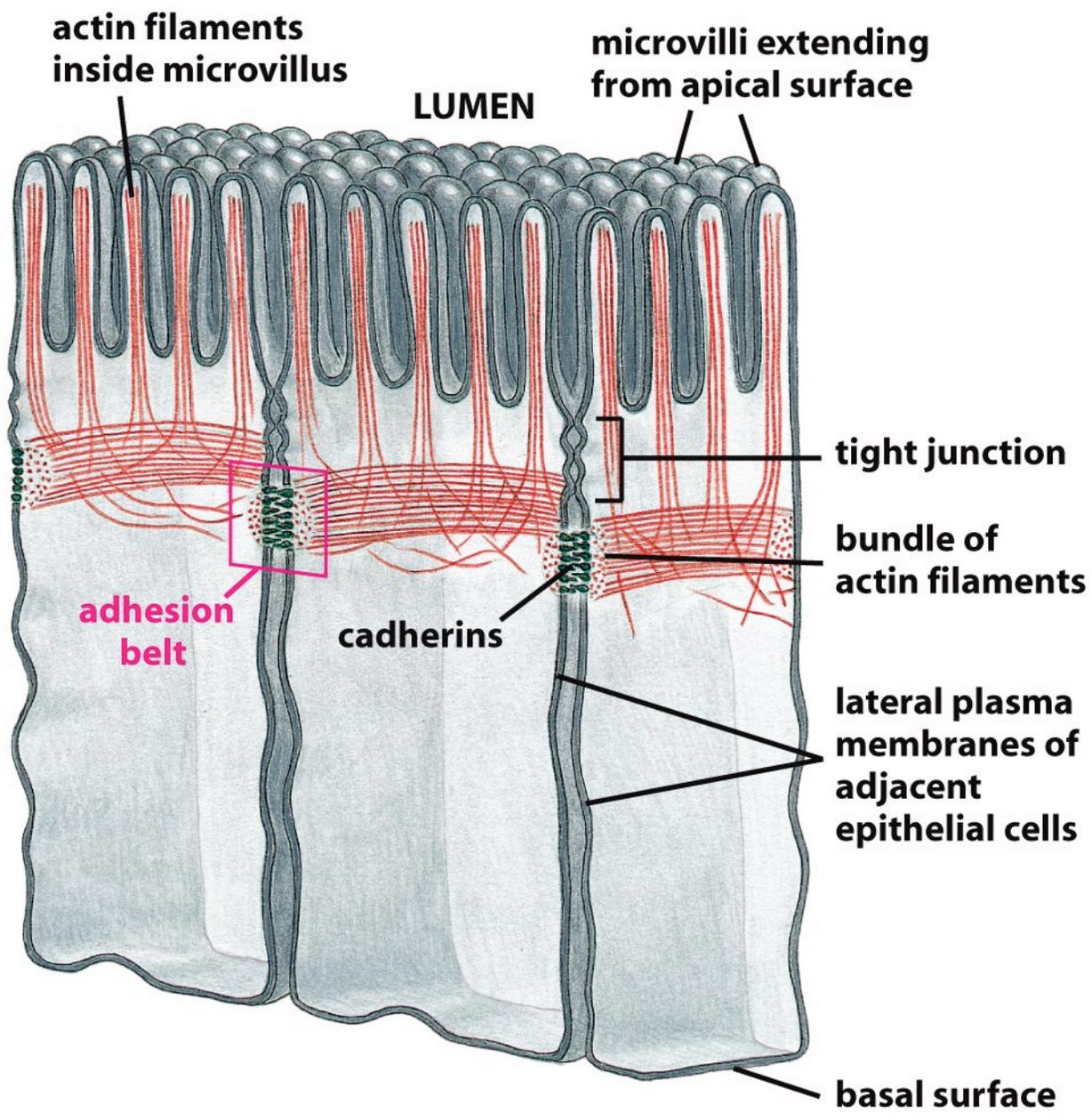
“Cap” (tapa)

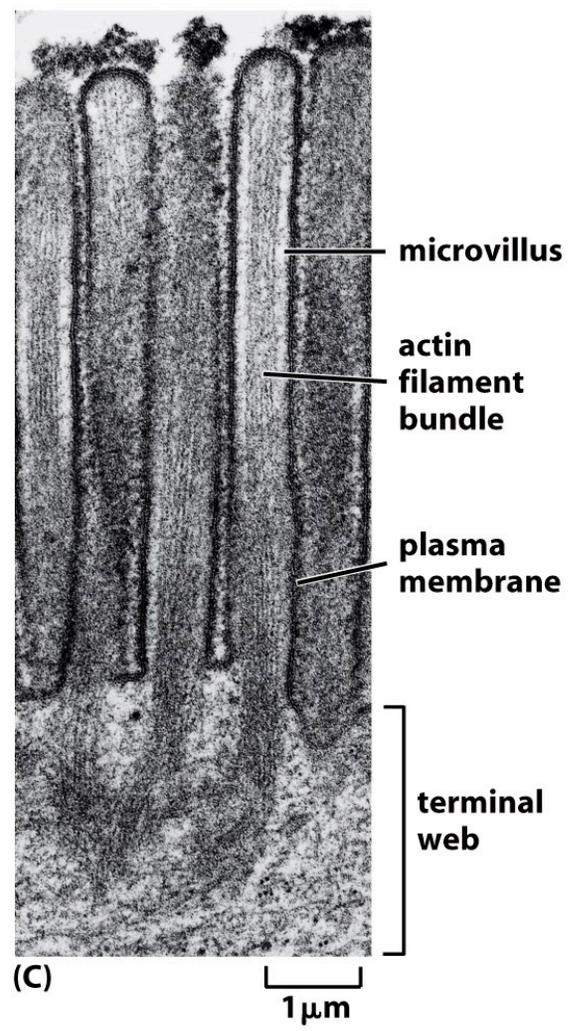
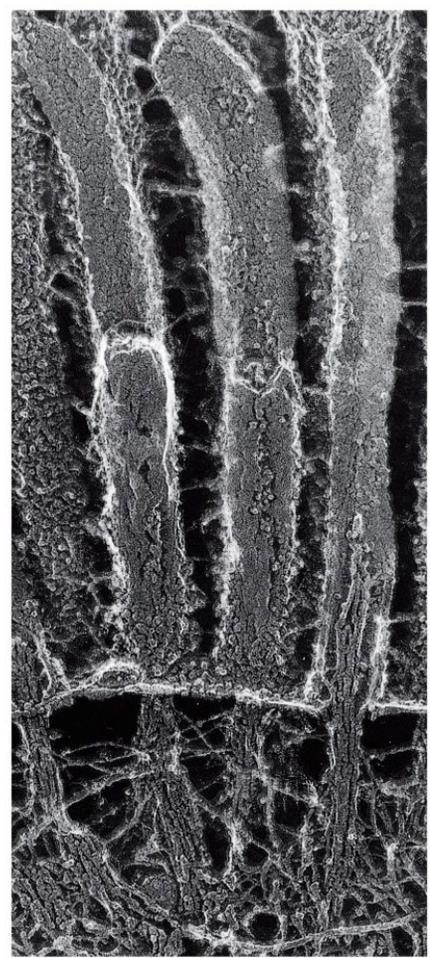
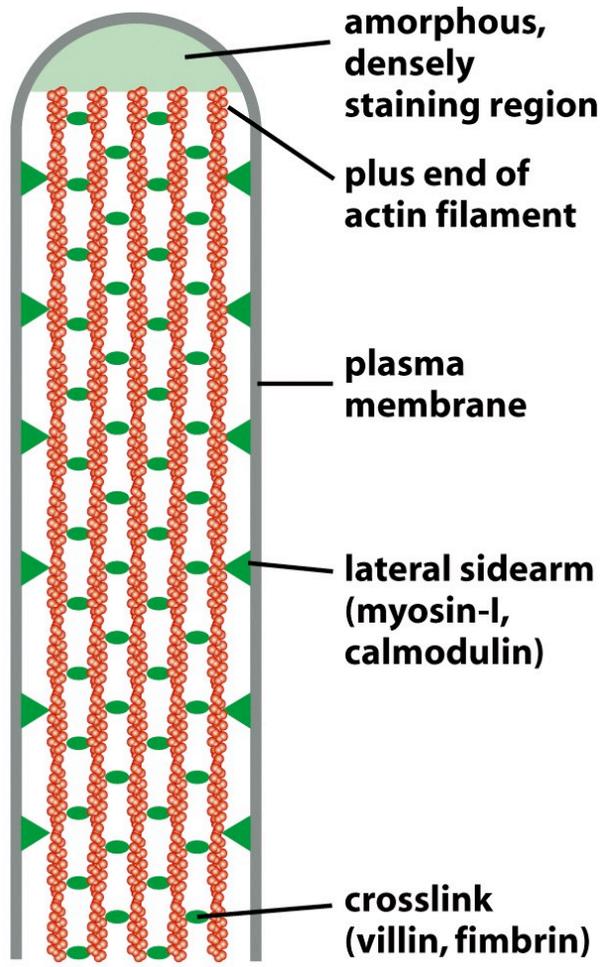
La rapidez de adición de subU puede ser más rápida que la hidrólisis del ATP o GTP. Esto forma una tapa de subunidades unidas a ATP o GTP (y con mayor afinidad por otras subU).

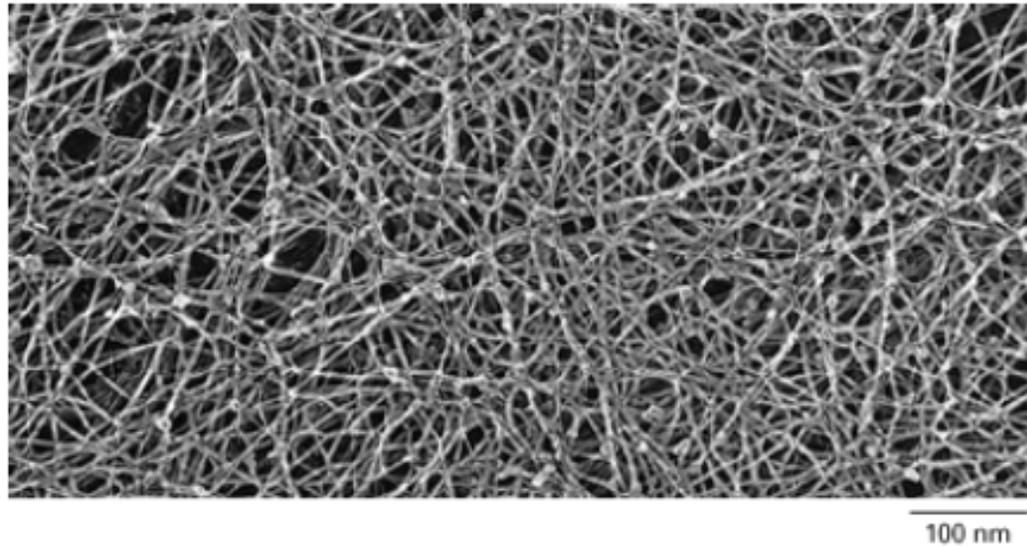
# Microfilamentos



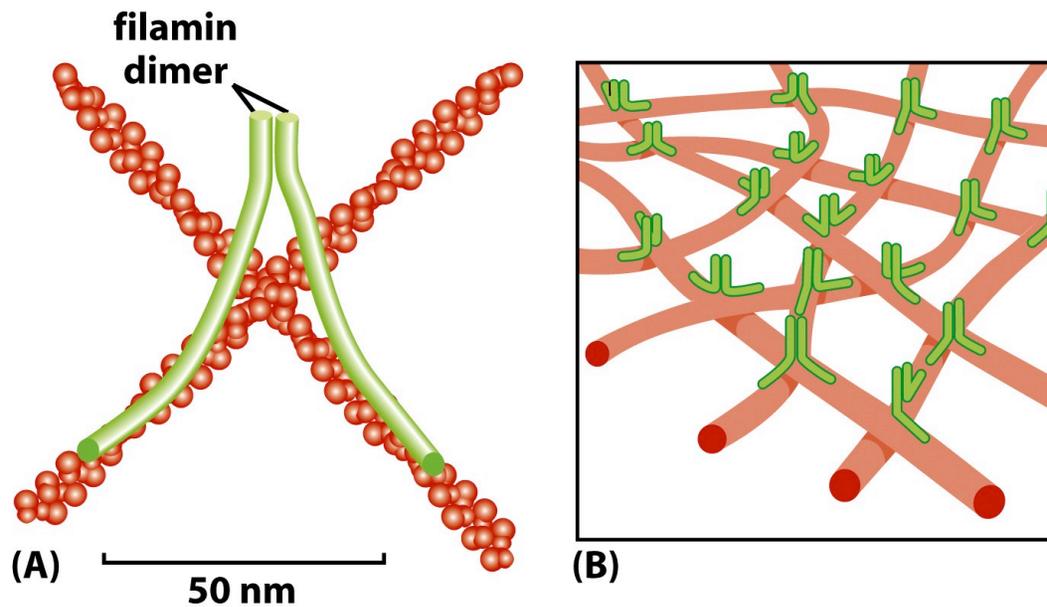
- **conservados** (homólogo en bacterias: MreB)
- *G-actina* (monómero, actina globular) se ensambla a *F-actina*, un polímero helicoidal **polarizado**
- Ensamblaje de los filamentos depende de la hidrólisis de ATP (**requiere energía**)
- Forman filamentos, redes y geles (3D)

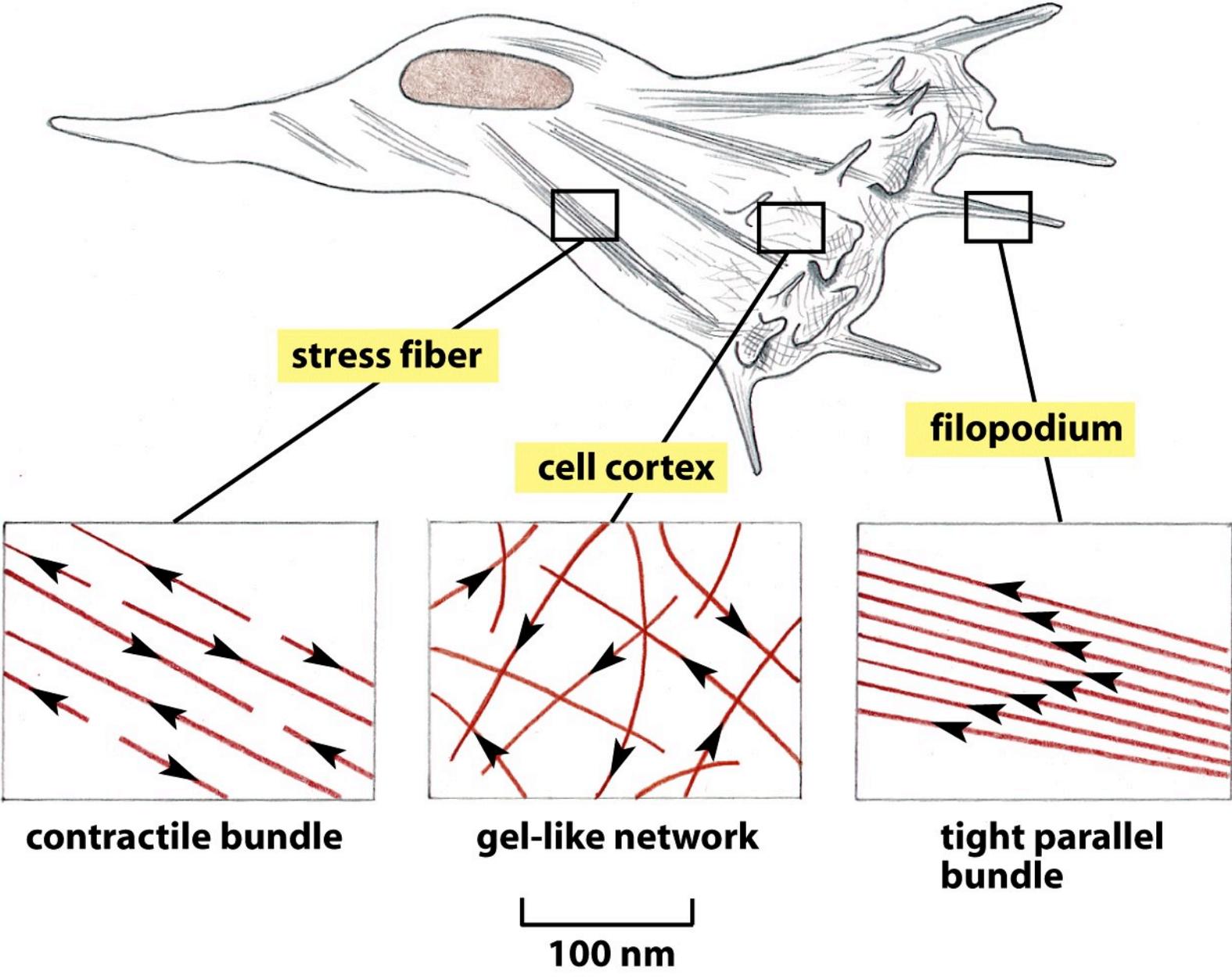






**Figure 1-26.** Actin. A network of actin filaments underlying the plasma membrane of an animal cell is seen in this electron micrograph prepared by the deep-etch technique. (Courtesy of John Heuser.)





**stress fiber**

**cell cortex**

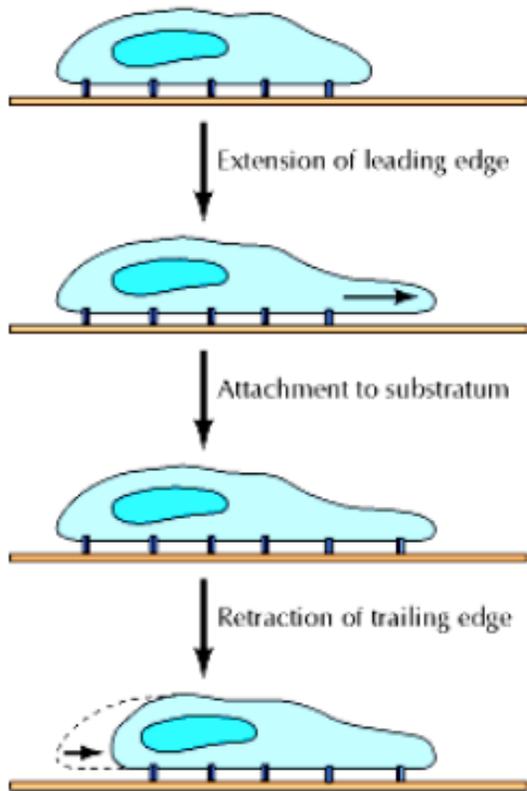
**filopodium**

**contractile bundle**

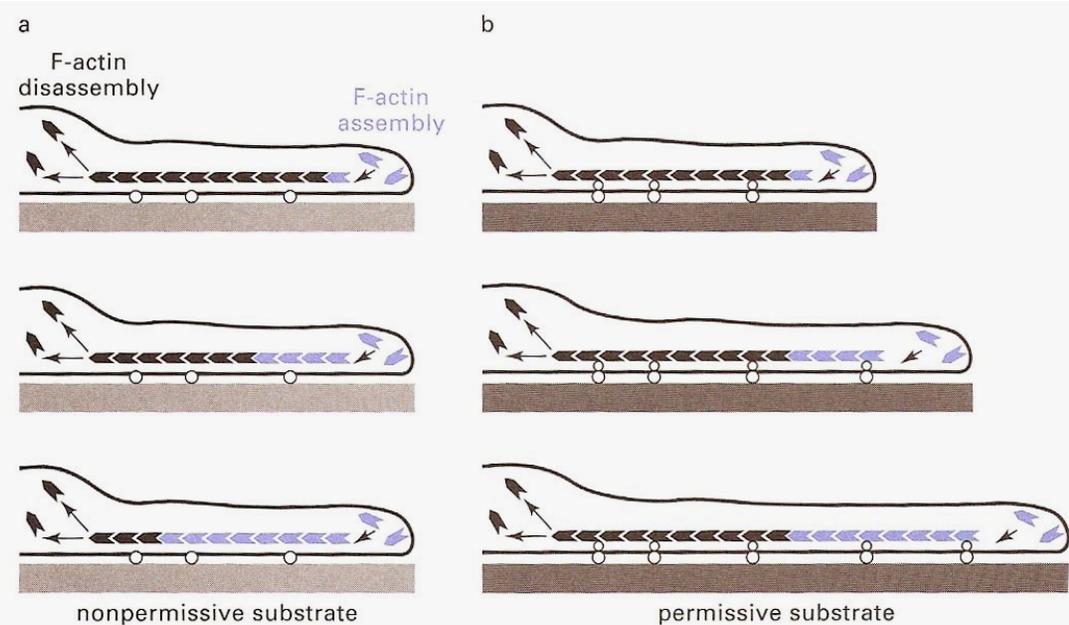
**gel-like network**

**tight parallel bundle**

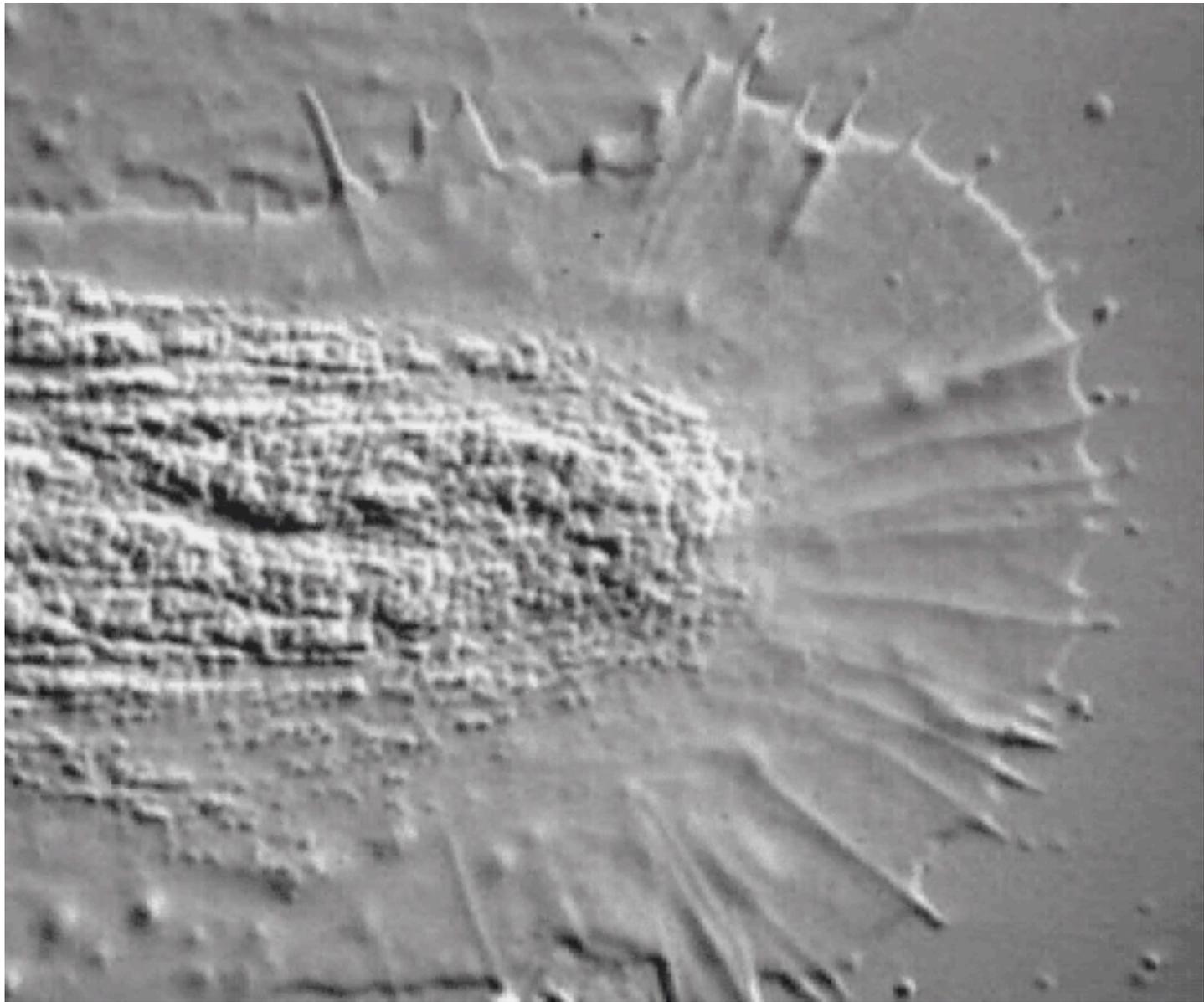
**100 nm**

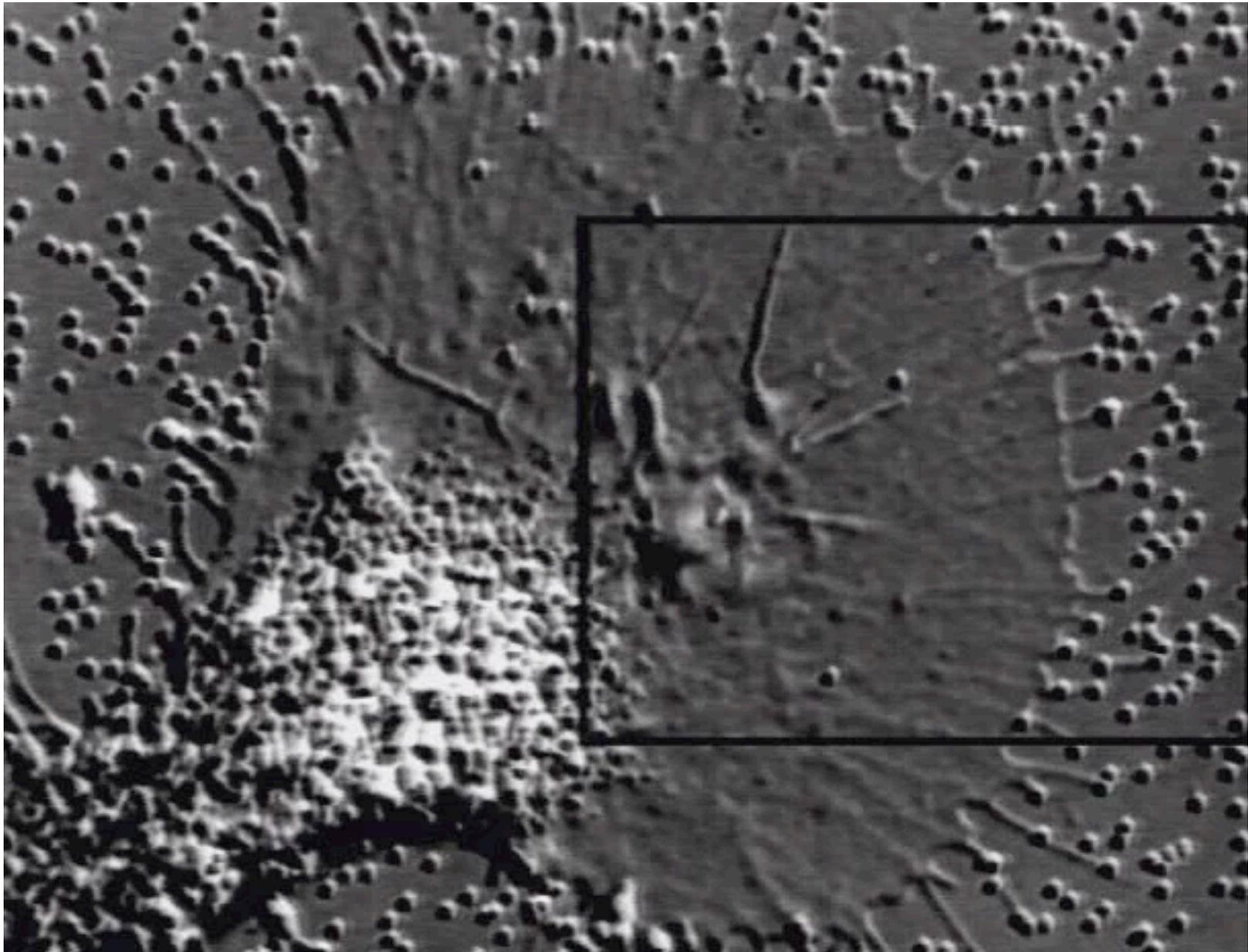


**Figure 11.30. Cell crawling**  
 The crawling movements of cells across a surface can be viewed as three stages of coordinated movements: (1) extension of the leading edge, (2) attachment of the leading edge to the substratum, and (3) retraction of the rear of the cell into the cell body.

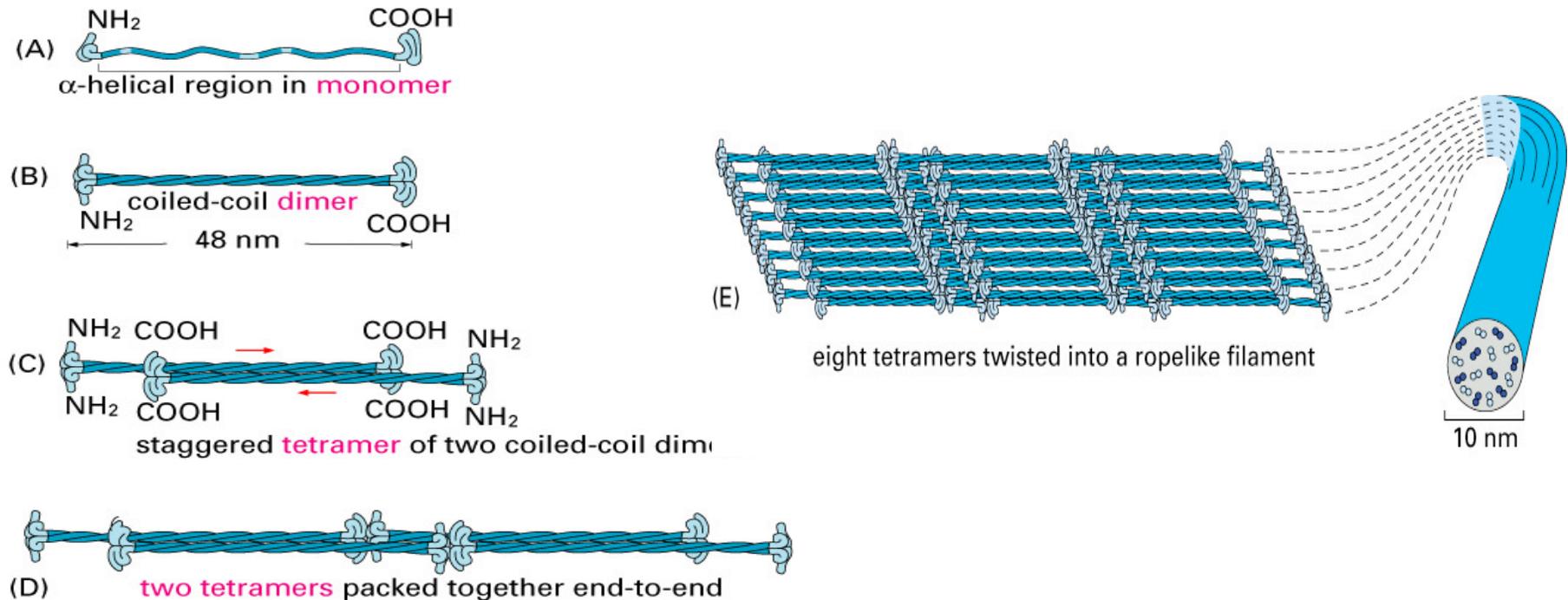


**Figure 17-4. Actin assembly in the lamellipodium.** *a:* Experiments by Paul Forscher and colleagues suggest that on a surface that is nonpermissive for axon elongation, F-actin filaments are not closely linked to the surface, leading to persistent retrograde flow of newly assembled F-actin. *b:* When the F-actin cytoskeleton is coupled to the substrate, polymerization of F-actin at the leading edge leads to forward extension of the growth cone.



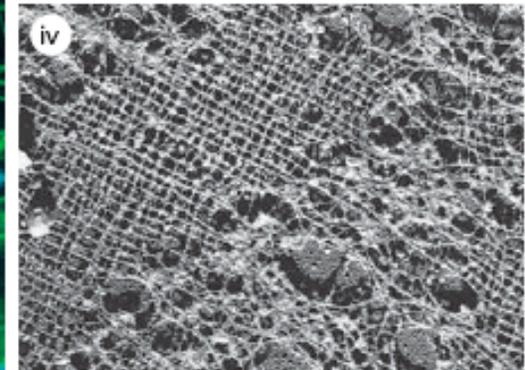
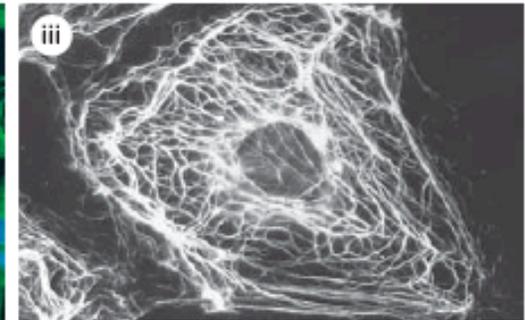
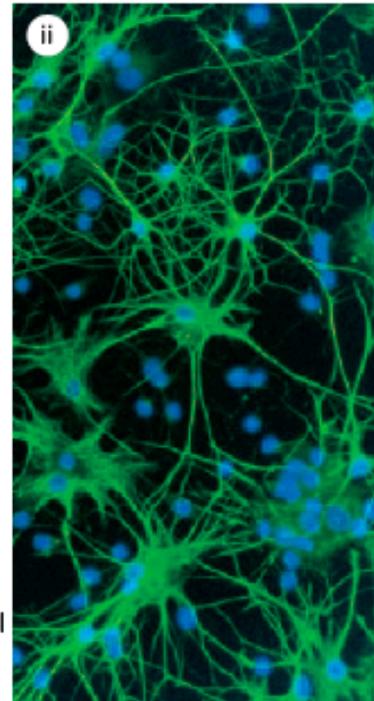
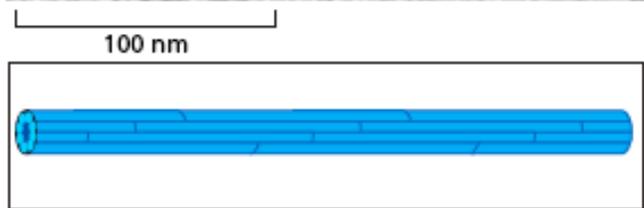


# Filamentos Intermedios



- **NO conservados**: no se encuentran en todos los eucariontes
- **heterogéneos**: específicos de acuerdo al tejido
- NO se requiere energía para su ensamblaje, **auto asociación lateral**
- Filamentos **NO polarizados**

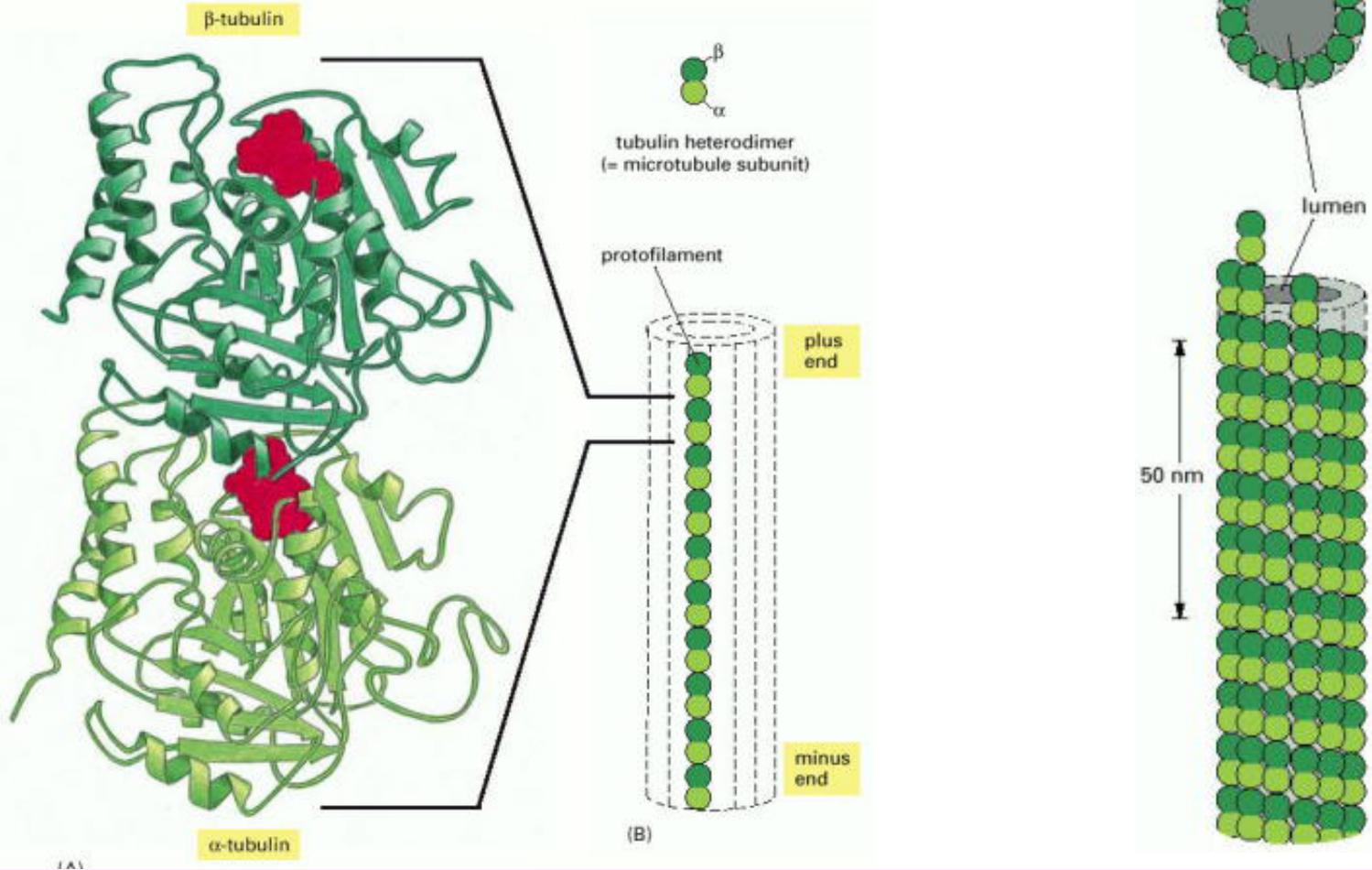
## INTERMEDIATE FILAMENTS



**Intermediate filaments** are ropelike fibers with a diameter of around 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.

Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

# Microtubulos

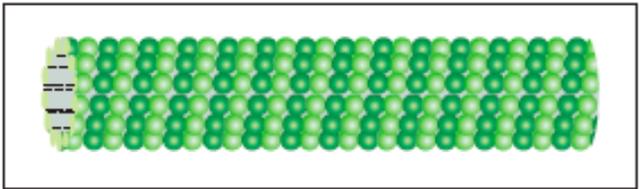


- **conservados** (Existe un homólogo en bacterias: FtsZ)
- heterodímeros ( $\alpha$ -tubulina y  $\beta$ -tubulina unidas por un GTP), forman 13 protofilamentos polarizados
- Solo se hidroliza el GTP unido a  $\alpha$ -tubulina (**requiere energía**)

## MICROTUBULES



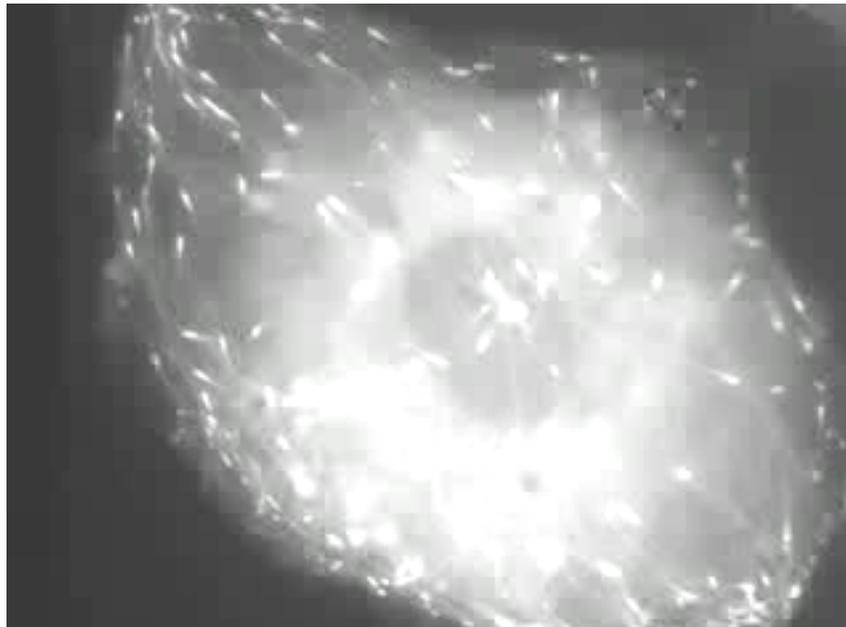
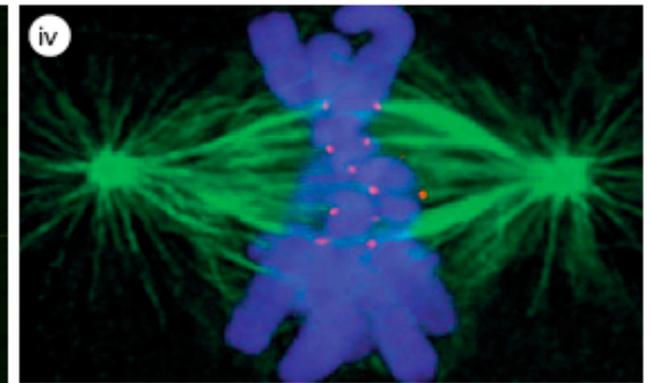
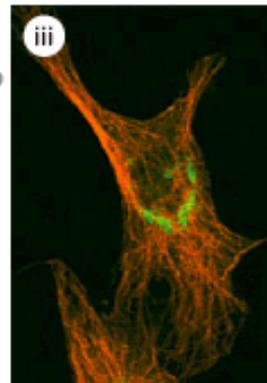
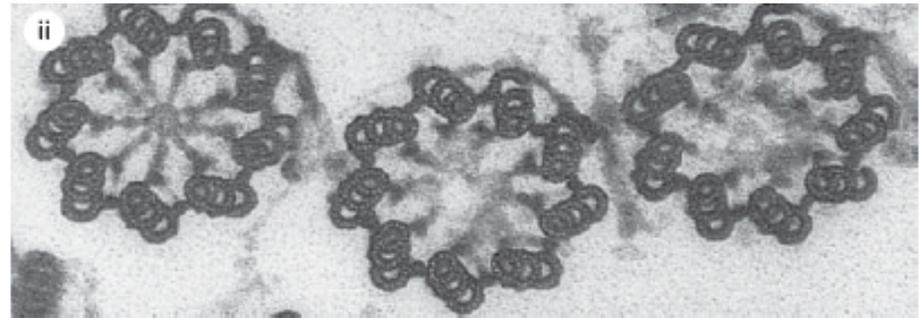
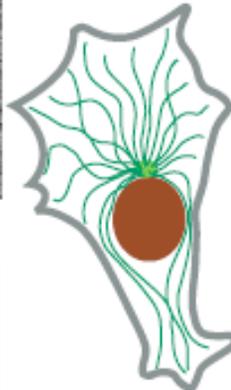
100 nm



25 nm

**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 typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*.

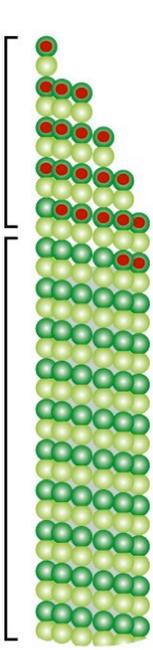
Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (iii); A. Desai (iv).



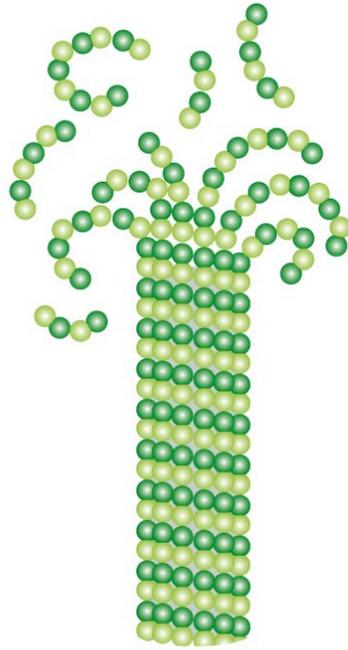
EB1-EGFP se une al CAP-fosforilado. Si se pierde el CAP, se pierde la marca.

Comparar con tubulina-EGFP

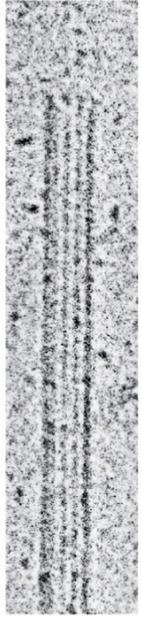
GTP cap  
less stable region of microtubule containing GDP-tubulin dimers



GROWING



SHRINKING

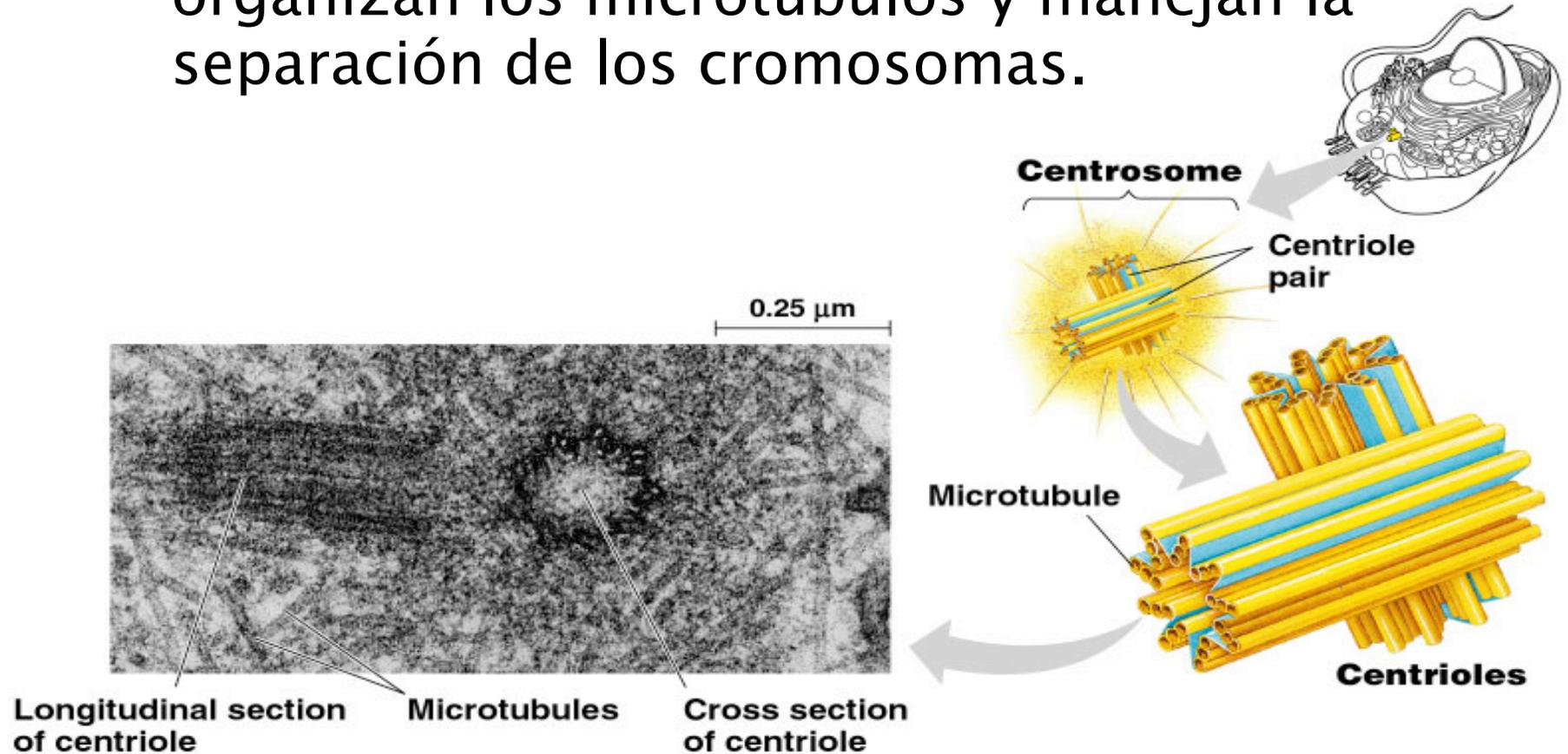


50 nm



# Centriolos

- División celular
  - En células animales un par de centriolos organizan los microtúbulos y manejan la separación de los cromosomas.



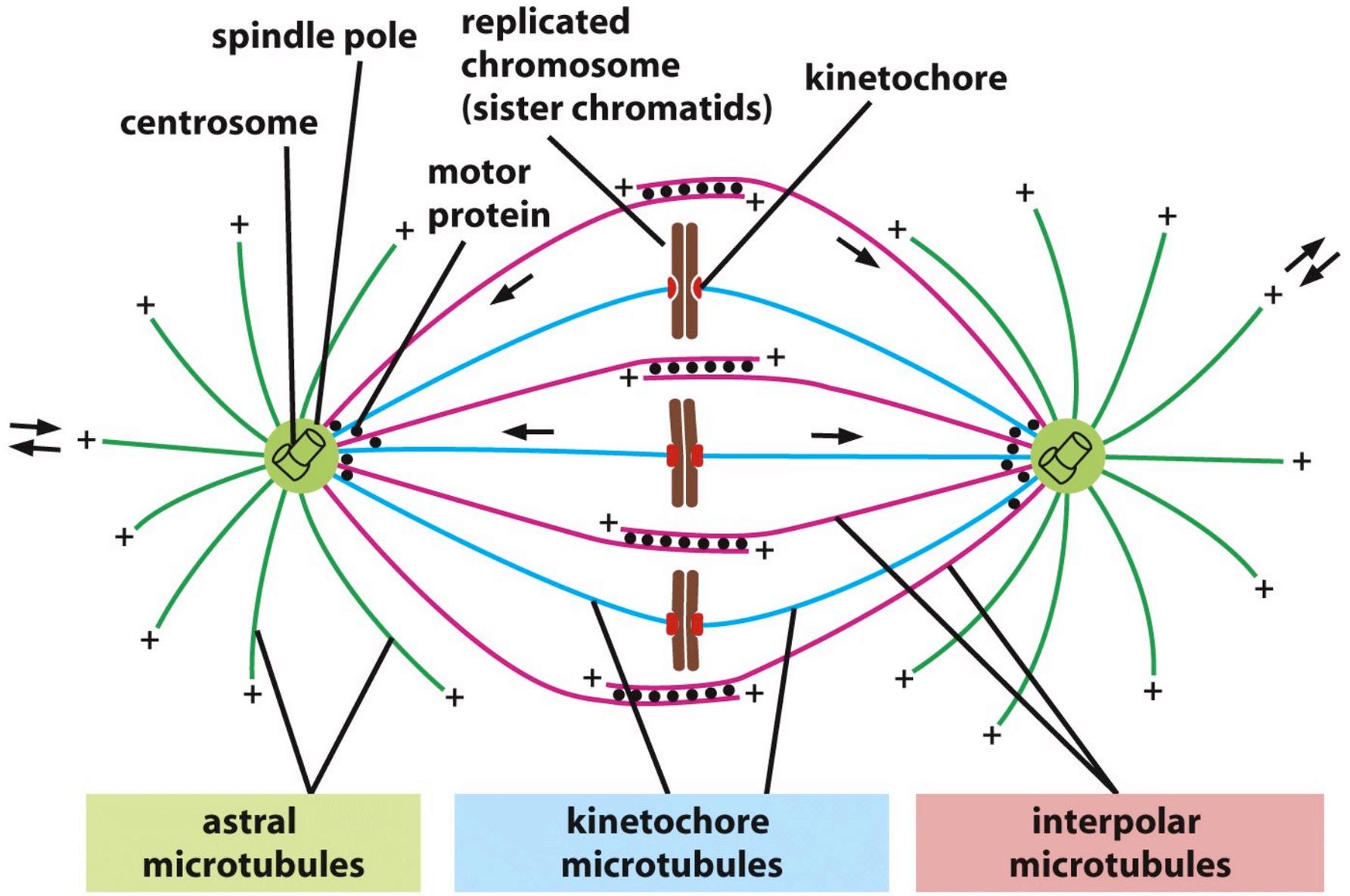
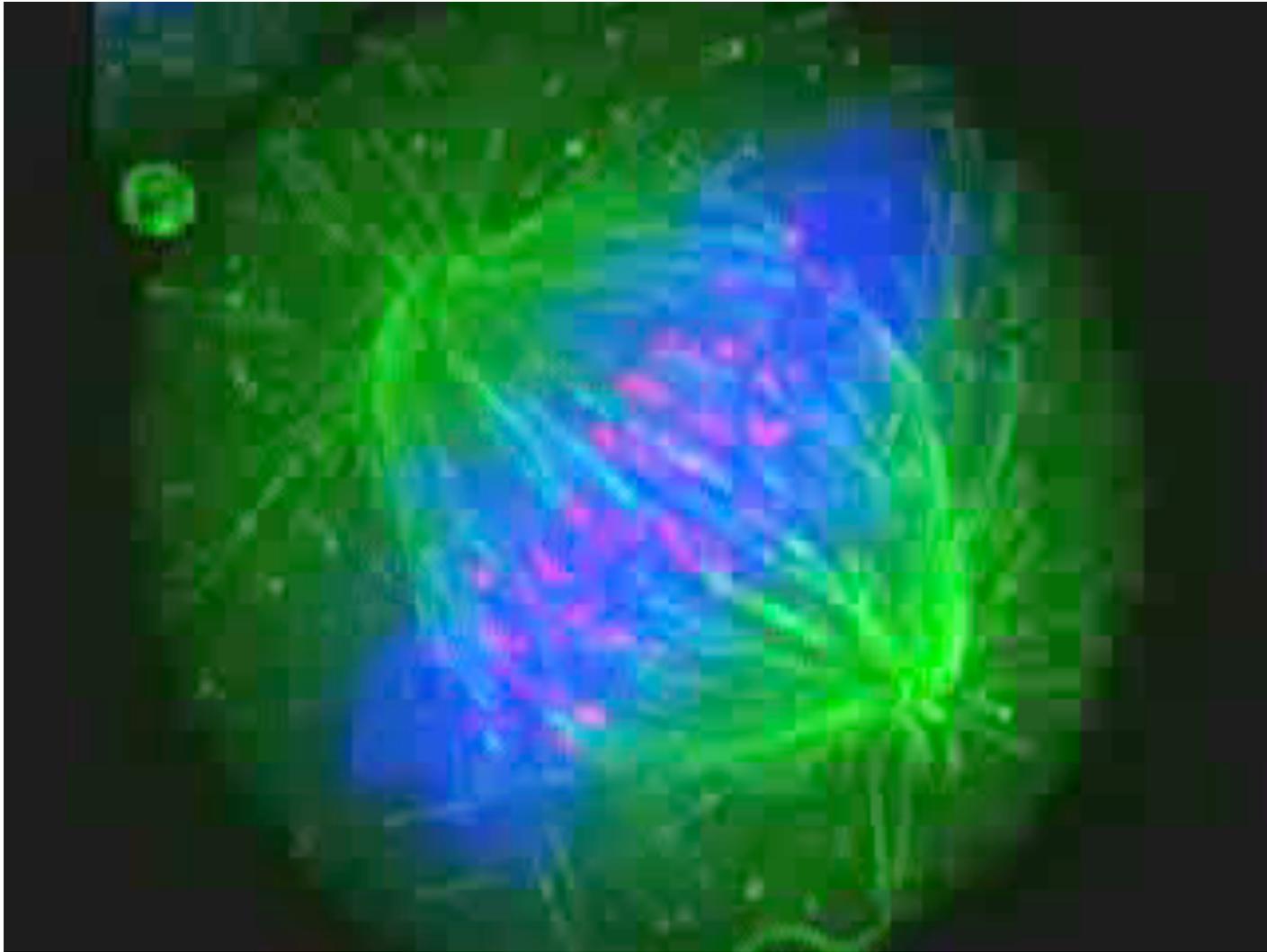


Figure 16-85a Molecular Biology of the Cell 5/e (© Garland Science 2008)



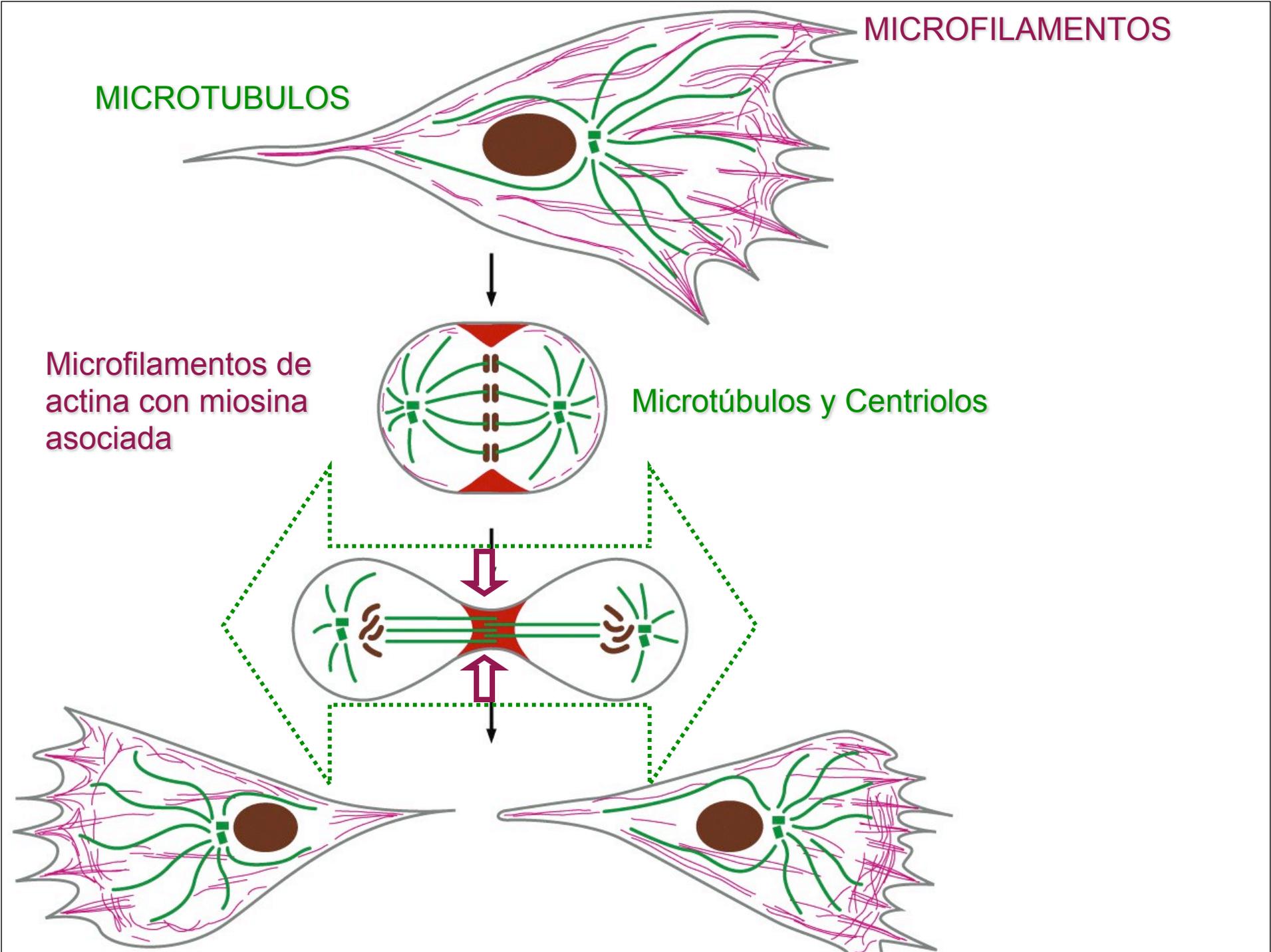
# Drogas que afectan los filamentos de actina y/o los microtúbulos

## **ACTINA-ESPECIFICAS**

- Faloidina se une a y estabiliza los filamentos
- Citocalasina forma un CAP en los extremos positivos
- Swinholdina corta los filamentos
- Latrunculina se une a las subunidades y evita su polimerización (secuestra)

## **MICROTUBULO-ESPECIFICAS**

- Taxol: se une a y estabiliza los microtúbulos (¿por qué sirve contra el cancer?)
- Colchicina. Colcemida, se unen a las subunidades y evitan su polimerización (secuestran)
- Vinblastina, vincristina, se unen a las subunidades y evitan su polimerización (secuestran)
- Nocodazol se unen a las subunidades y evitan su polimerización (secuestra)



# Proteínas Motoras

El movimiento se genera por el cambio conformacional producido por la hidrólisis del ATP.

El dominio “cabeza” determina el filamento al que se unen y la dirección en la que se mueven.

El dominio “cola” determina la carga o la estructura a la que se asocian.

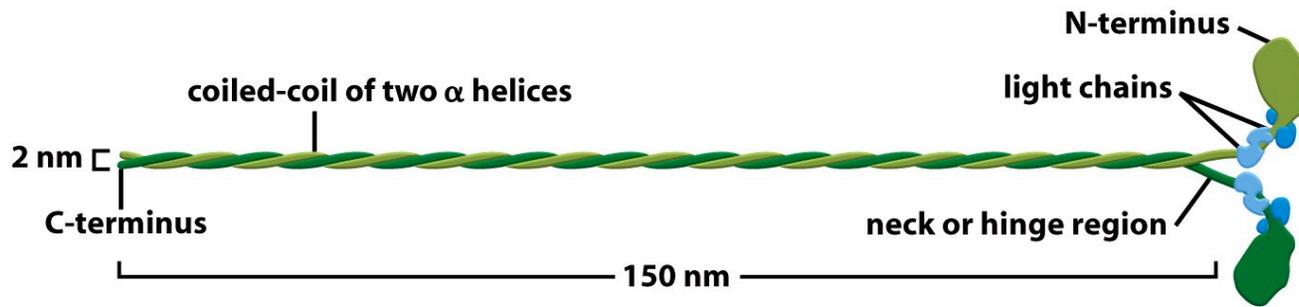


Figure 16-54a Molecular Biology of the Cell 5/e (© Garland Science 2008)

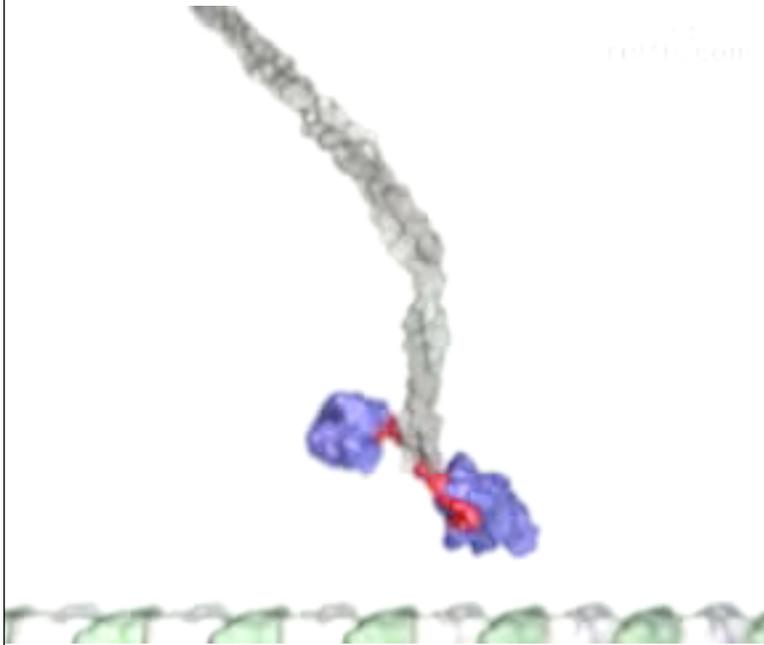
Sobre microfilamentos: Miosinas

Sobre Microtúbulos:

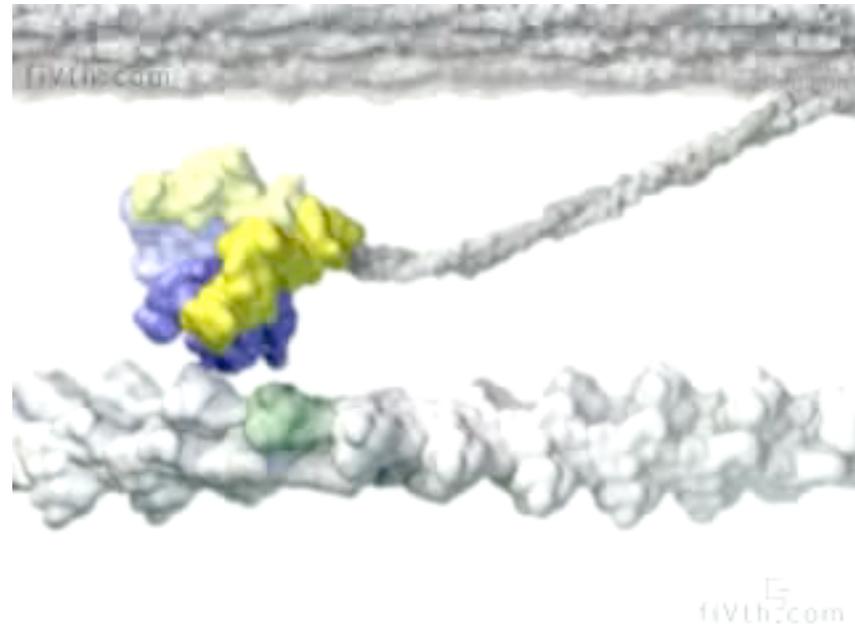
**Kinesinas:** avanzan hacia el lado (+), se ALEJAN del centro organizador de Microtúbulos (centrosoma).

**Dineínas:** avanzan hacia el lado (-). Van en dirección del centrosoma.

## Kinesina



## Miosina



Animaciones de Alberts 2008. Disponibles en U-Cursos

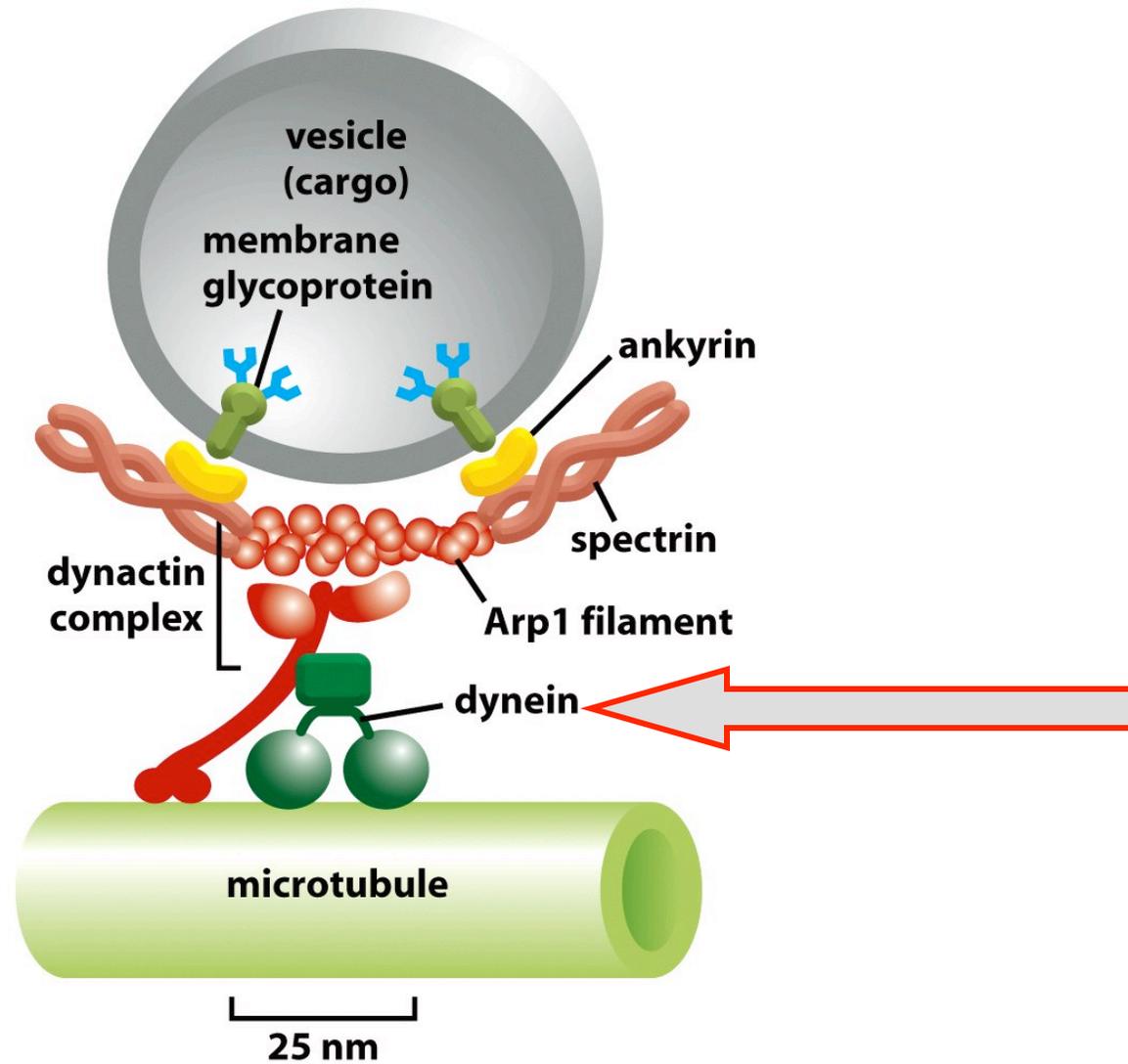


Figure 16-67 Molecular Biology of the Cell 5/e (© Garland Science 2008)

## Cilios & flagelos

- ◆ Cubiertos de Membrana plasmática.
- ◆ Centro **axonema**: complejo de microtúbulos y proteínas asociadas. Notablemente **Dineína**.

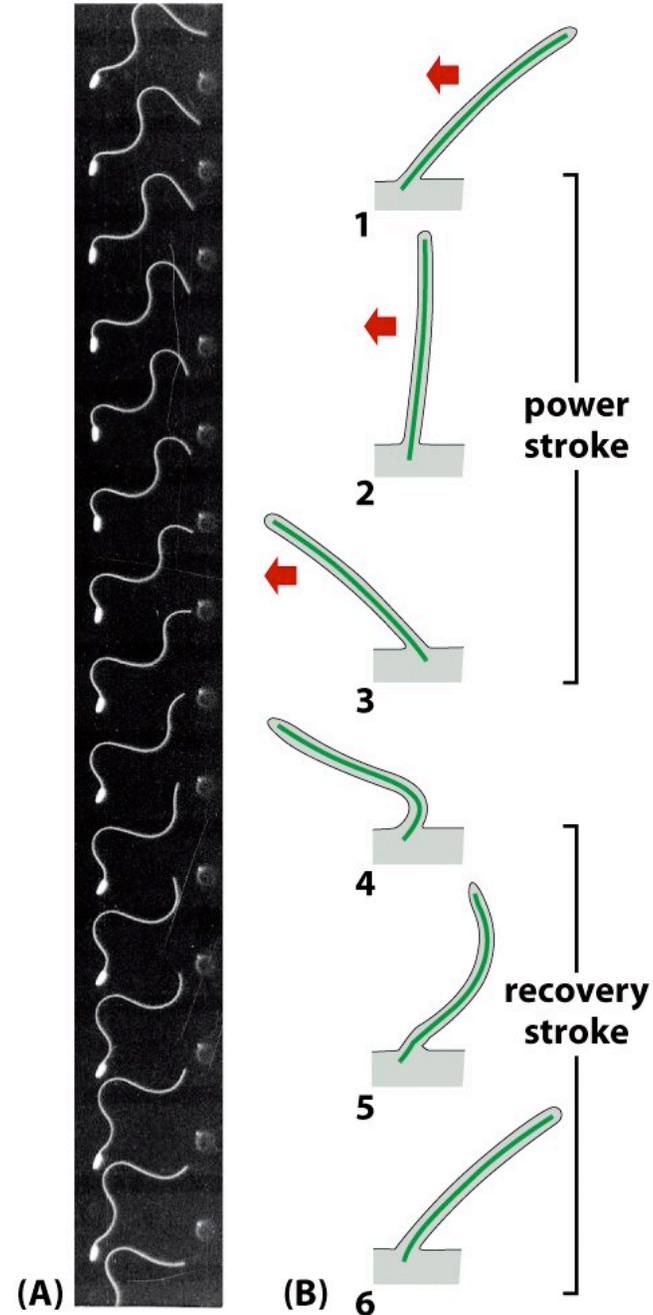
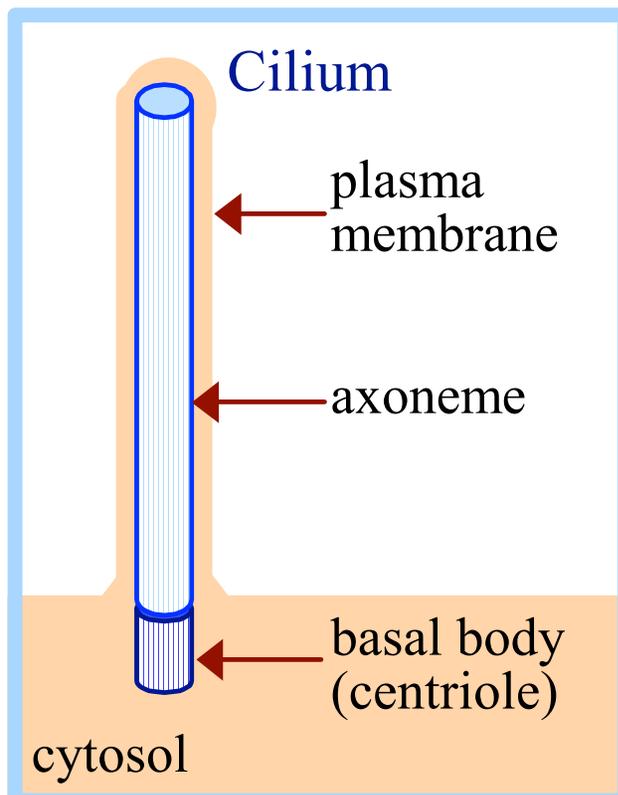
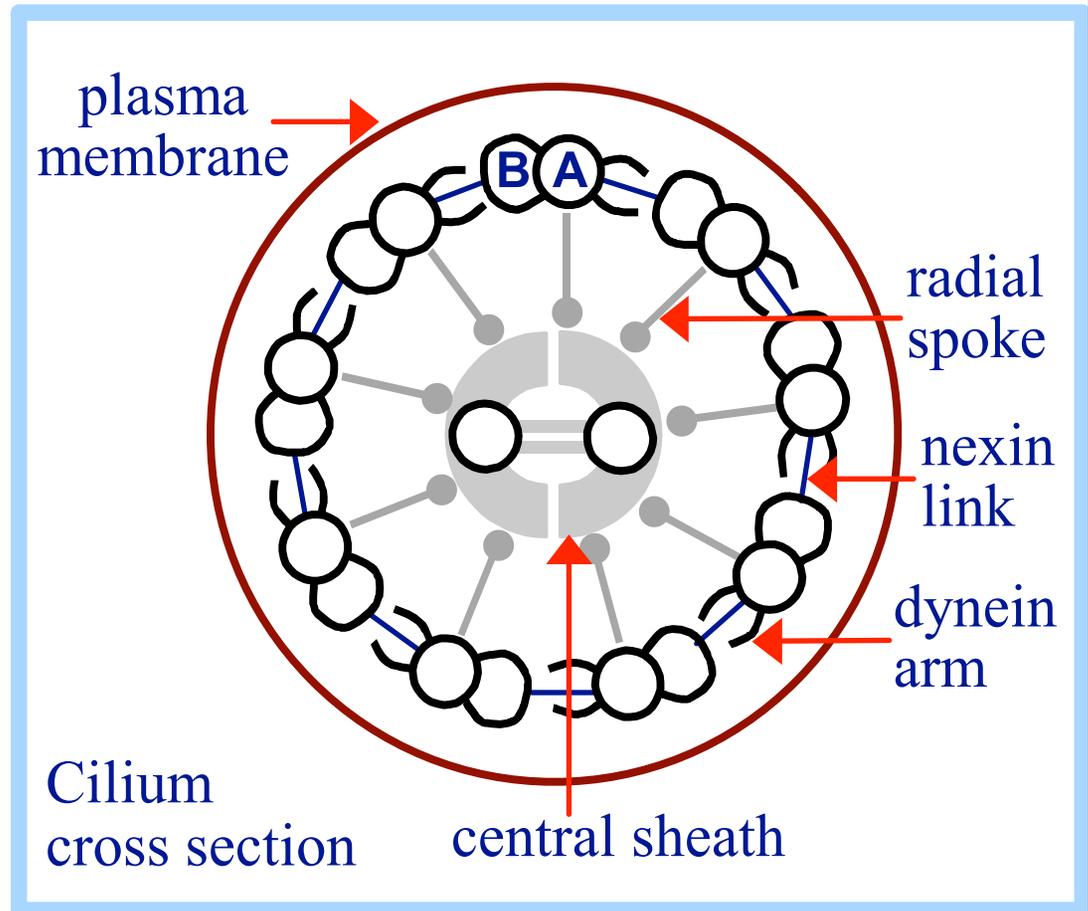
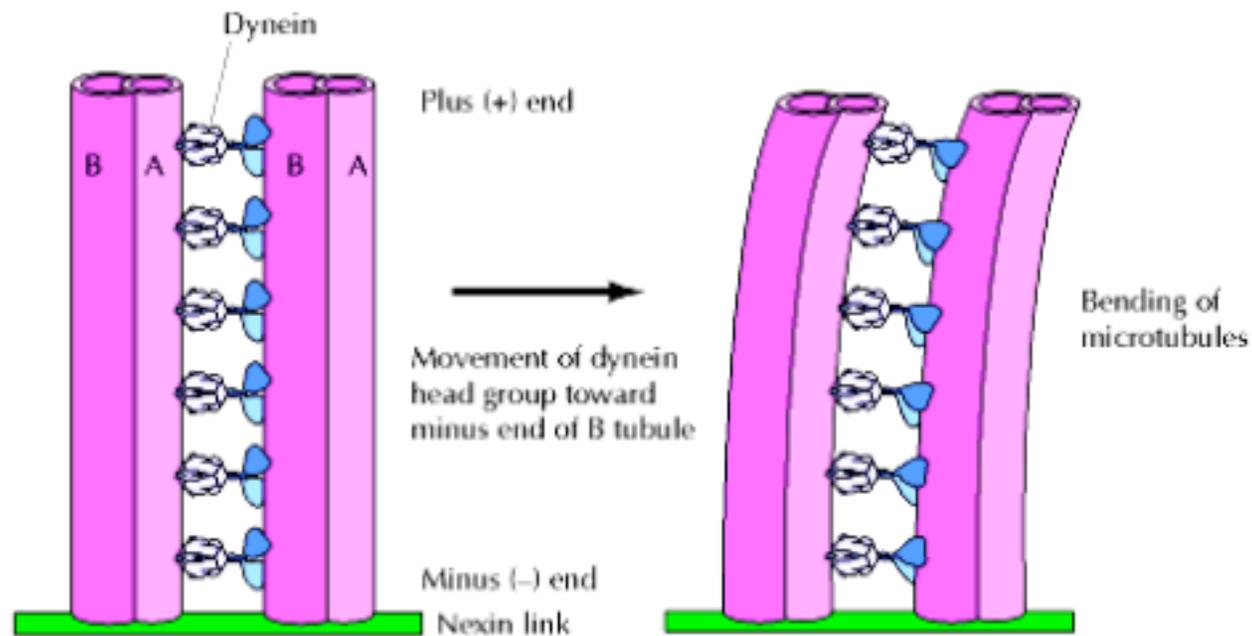


Figure 16-80 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Un **axonema** incluye:

- **Nueve dobletes** de microtubúlos en la perifería.
- El túbulo A posee brazos de **dineína**.
- Dos microtúbulos centrales, encapsulados
- Uniones radiales y de nexina.





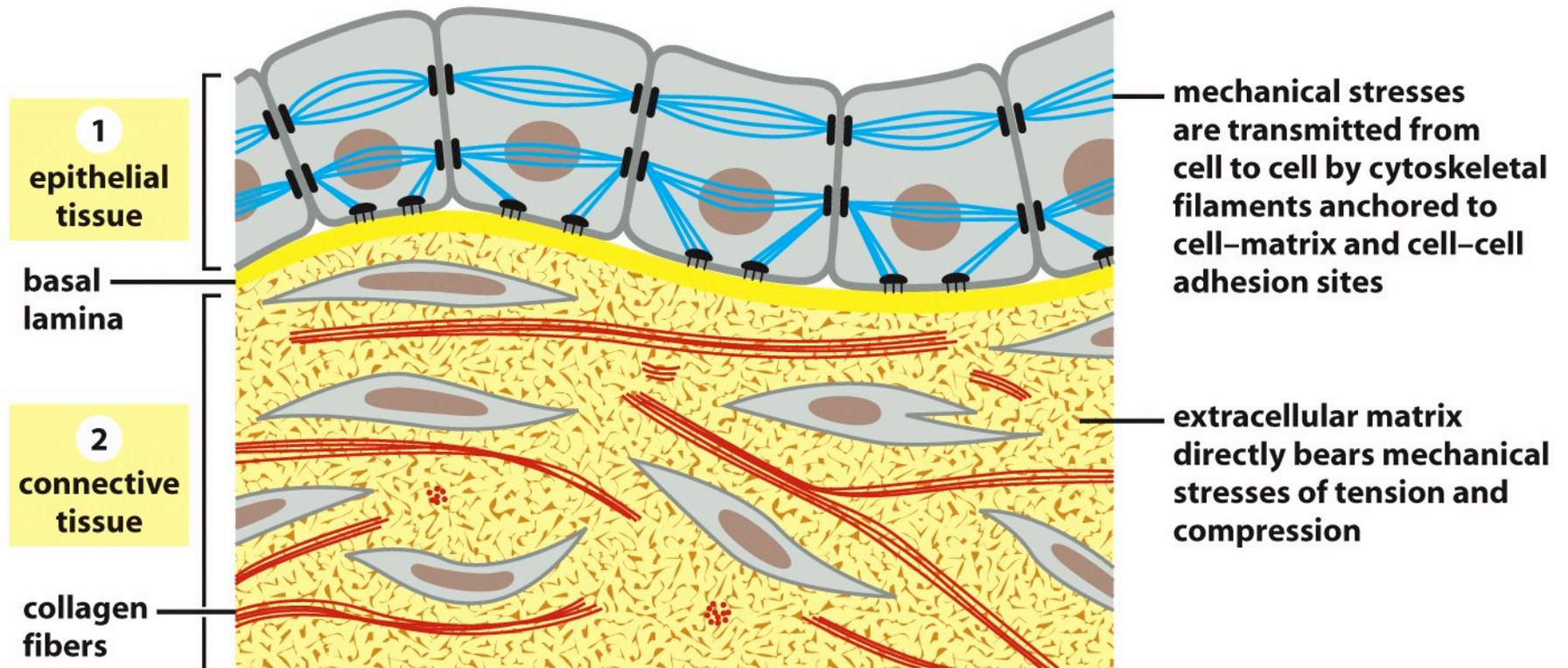
**Figure 11.53. Movement of microtubules in cilia and flagella** The bases of dynein arms are attached to A tubules, and the motor head groups interact with the B tubules of adjacent doublets. Movement of the dynein head groups in the minus end direction (toward the base of the cilium) then causes the A tubule of one doublet to slide toward the base of the adjacent B tubule. Because both microtubule doublets are connected by nexin links, this sliding movement forces them to bend.

# Contracción Muscular

- Al final de la clase o será materia de la primera clase de tejidos.
- Sigamos con Uniones Celulares



# UNIONES CELULARES



El modelo más utilizado son las células epiteliales debido a que presentan polaridad, sin embargo, hay múltiples casos de gran importancia. Por ejemplo, las células musculares deben poder anclarse a su medio con mucha fuerza.

**APICAL**

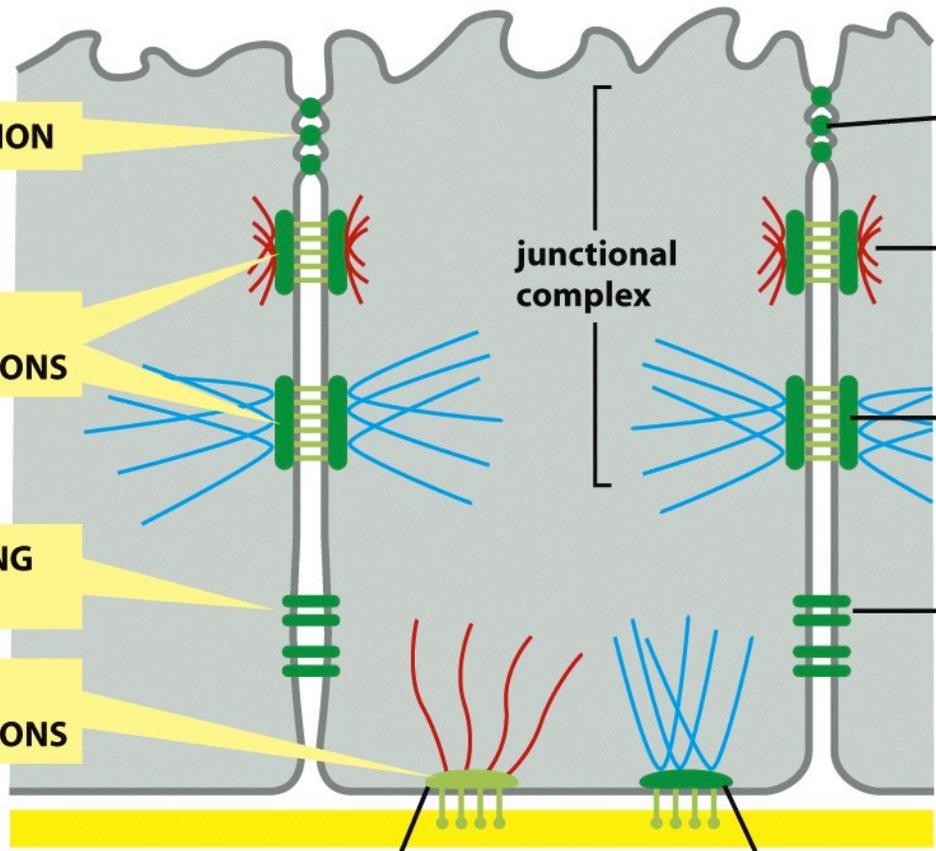
**OCCCLUDING JUNCTION**

**CELL-CELL ANCHORING JUNCTIONS**

**CHANNEL-FORMING JUNCTIONS**

**CELL-MATRIX ANCHORING JUNCTIONS**

**BASAL**



tight junction seals gap between epithelial cells

adherens junction connects actin filament bundle in one cell with that in the next cell

desmosome connects intermediate filaments in one cell to those in the next cell

gap junction allows the passage of small water-soluble molecules from cell to cell

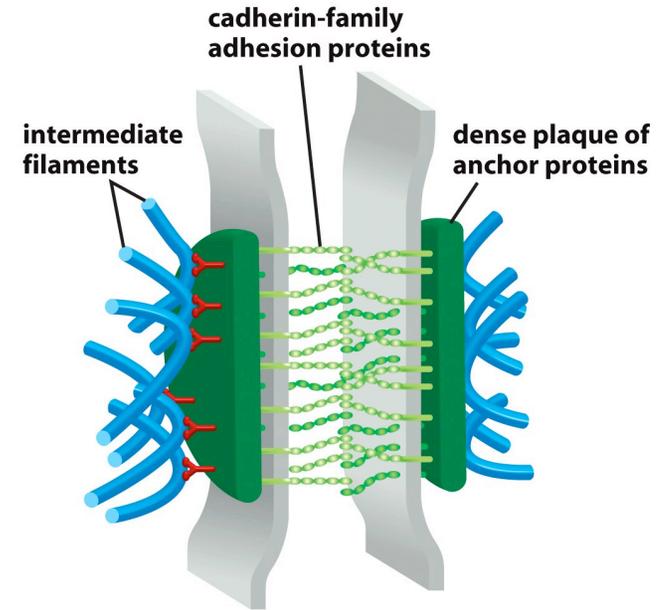
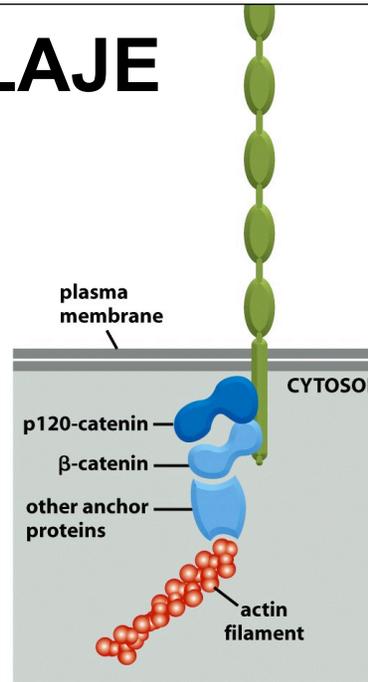
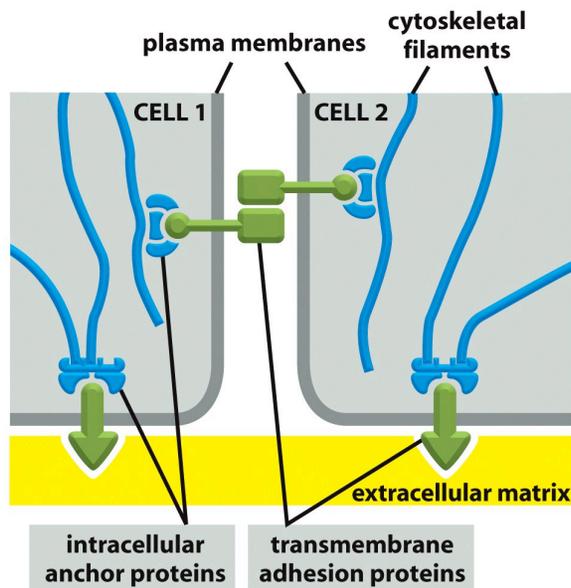
actin-linked cell-matrix adhesion anchors actin filaments in cell to extracellular matrix

hemidesmosome anchors intermediate filaments in a cell to extracellular matrix

junctional complex

Figure 19-3 *Molecular Biology of the Cell* (© Garland Science 2008)

# UNIONES DE ANCLAJE



**Table 19-2 Anchoring Junctions**

JUNCTION	TRANSMEMBRANE ADHESION PROTEIN	EXTRACELLULAR LIGAND	INTRACELLULAR CYTOSKELETAL ATTACHMENT	INTRACELLULAR ANCHOR PROTEINS
<i>Cell-Cell</i>				
adherens junction	cadherin (classical cadherin)	cadherin in neighboring cell	actin filaments	$\alpha$ -catenin, $\beta$ -catenin, plakoglobin ( $\gamma$ -catenin), p120-catenin, vinculin, $\alpha$ -actinin
desmosome	cadherin (desmoglein, desmocollin)	desmoglein and desmocollin in neighboring cell	intermediate filaments	plakoglobin ( $\gamma$ -catenin), plakophilin, desmoplakin
<i>Cell-Matrix</i>				
actin-linked cell-matrix adhesion	integrin	extracellular matrix proteins	actin filaments	talin, vinculin, $\alpha$ -actinin, filamin, paxillin, focal adhesion kinase (FAK)
hemidesmosome	integrin $\alpha 6\beta 4$ , type XVII collagen (BP180)	extracellular matrix proteins	intermediate filaments	plectin, dystonin (BP230)

# Uniones estrechas (zonula occludens o tight junctions)

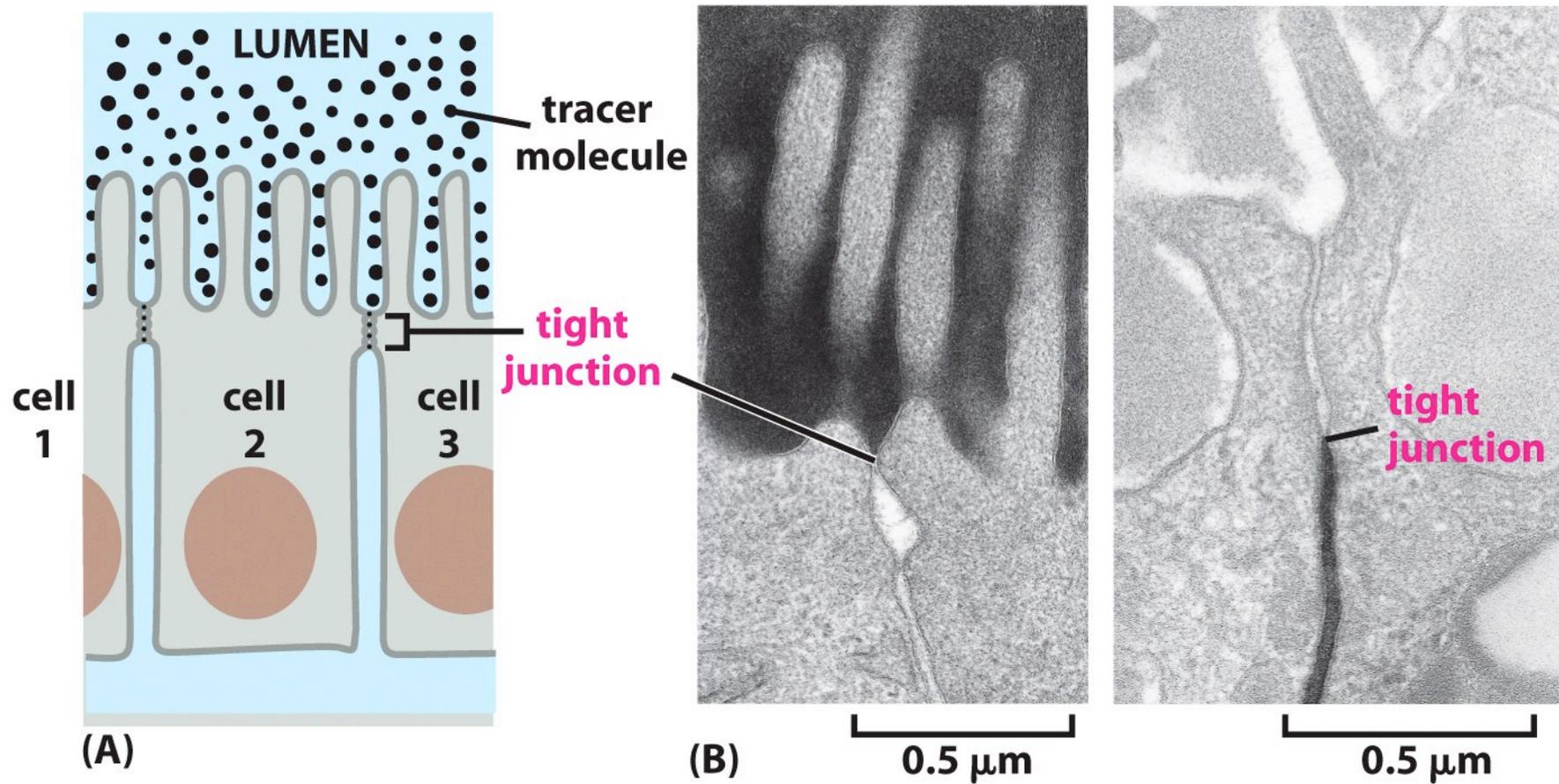
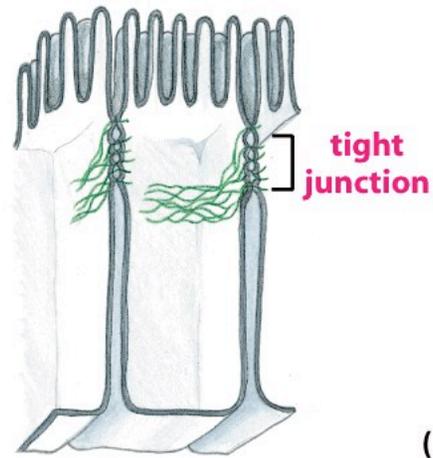
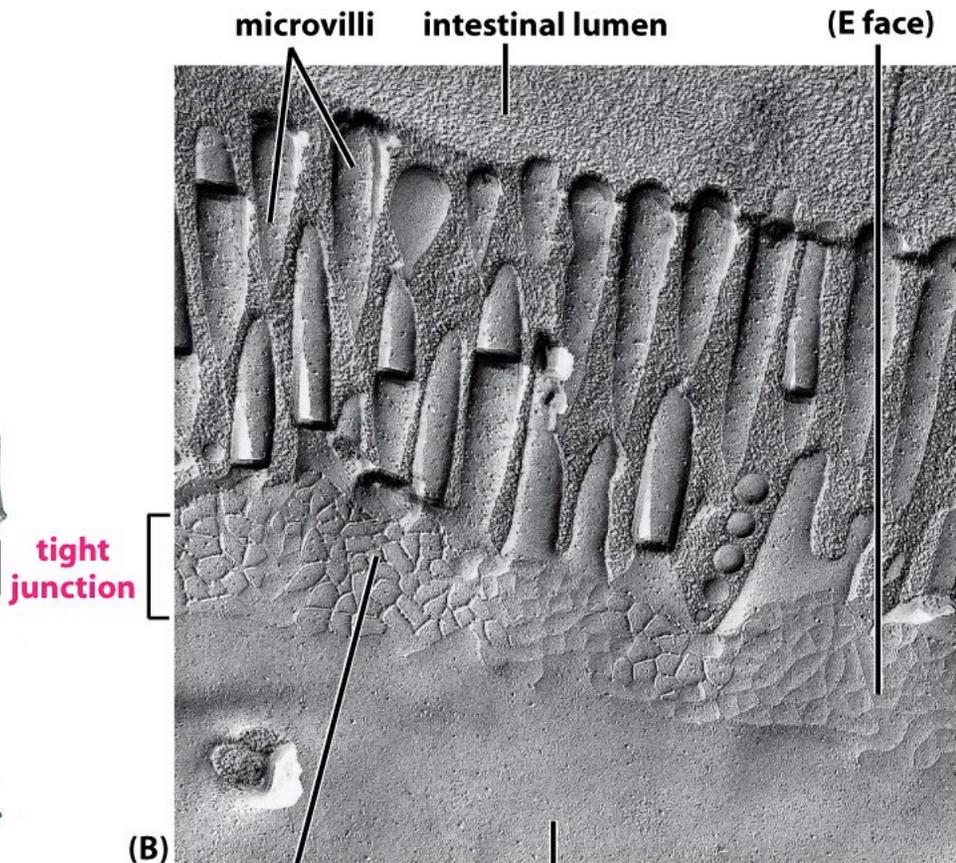


Figure 19-24 *Molecular Biology of the Cell* (© Garland Science 2008)



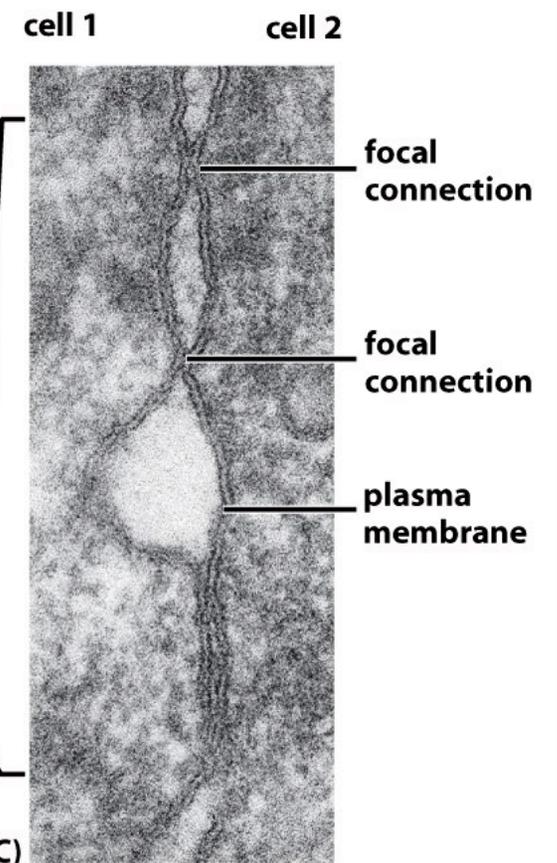
(A)



(B)

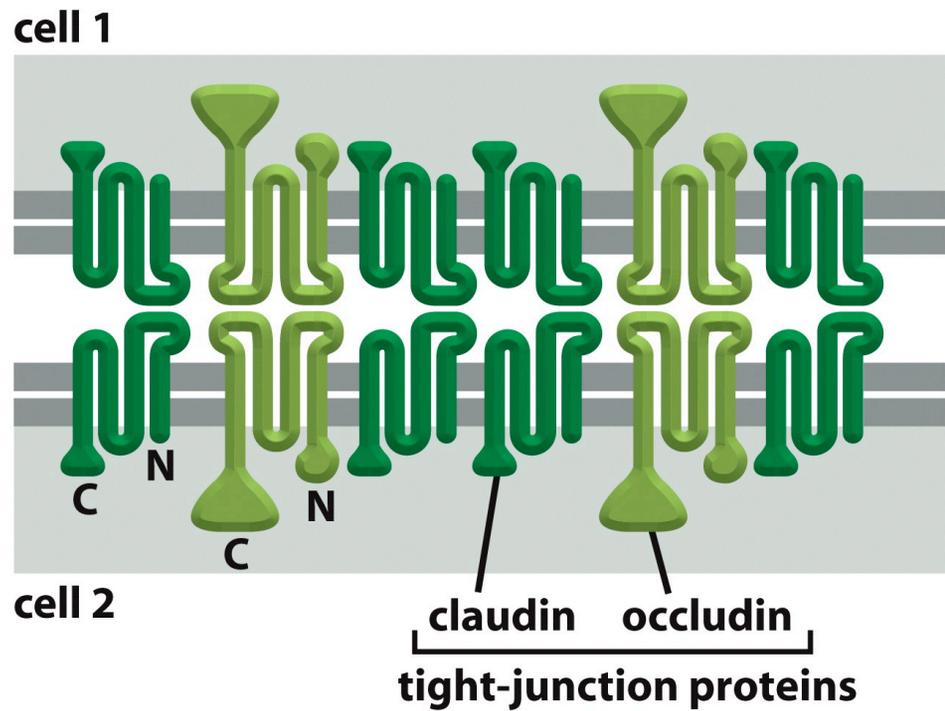
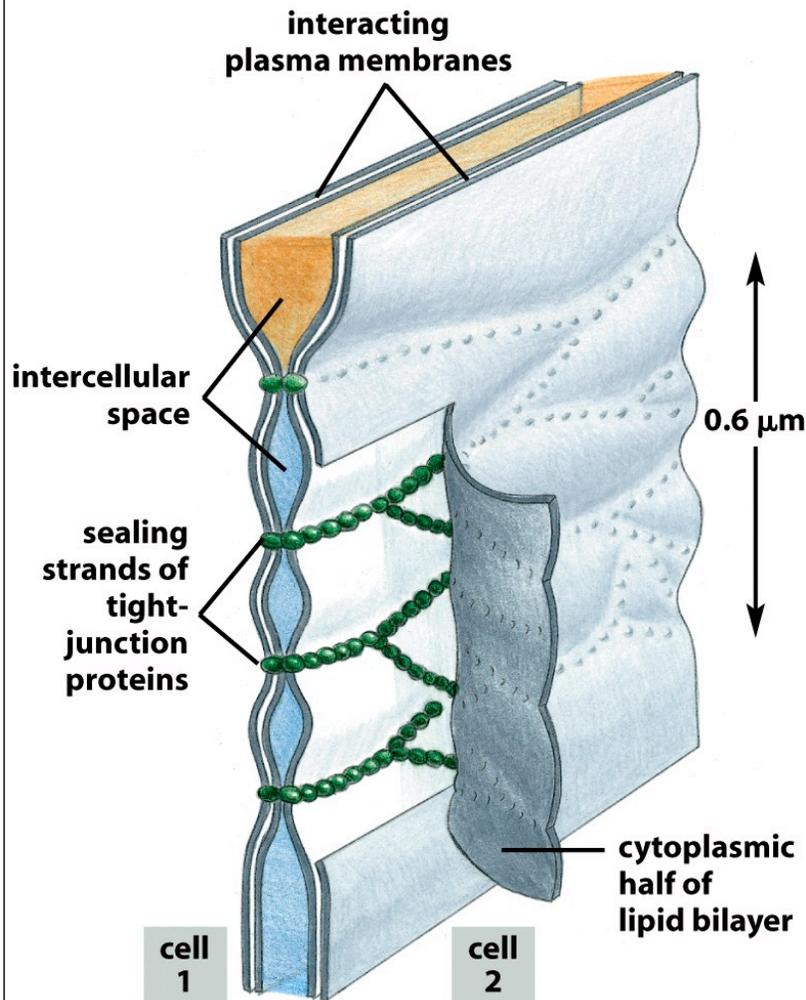
ridges of transmembrane particles forming sealing strands (P face) lateral plasma membrane

0.5  $\mu\text{m}$



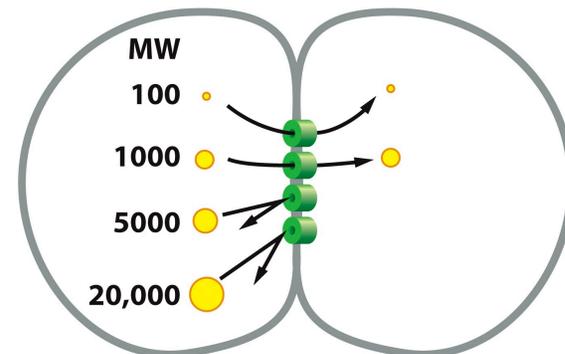
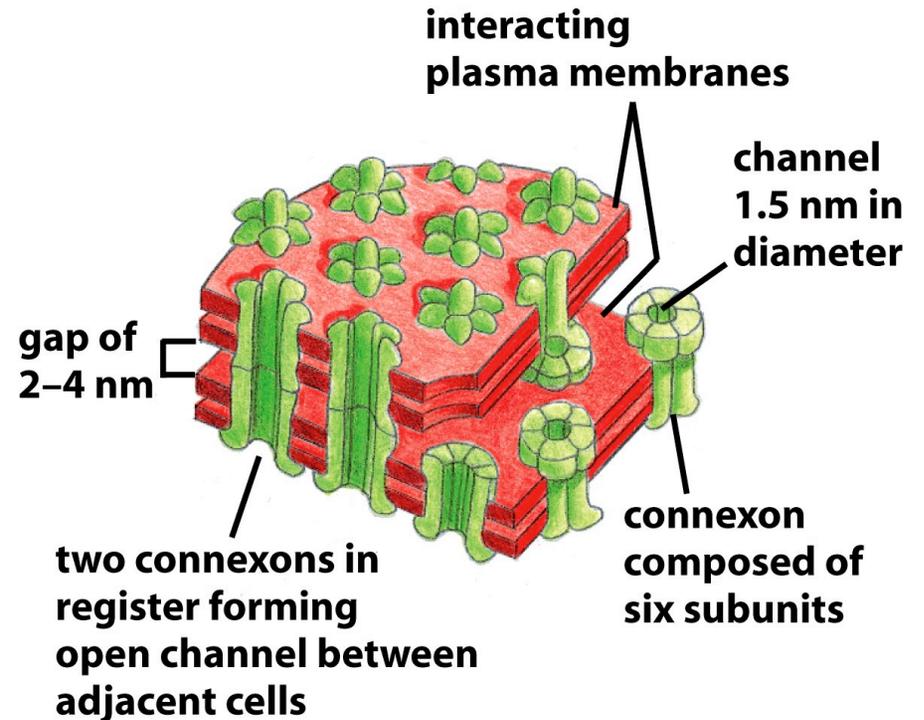
(C)

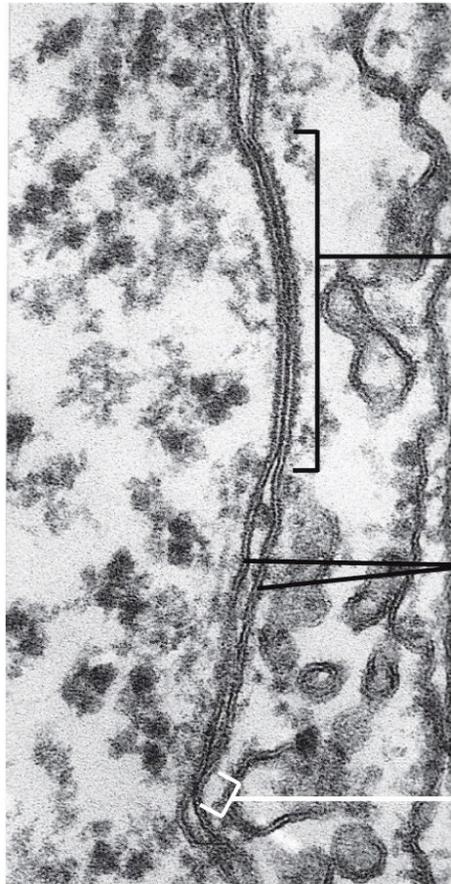
50 nm



# Uniones comunicantes

- Uniones en Hendidura (gap junctions) de células animales.
  - Conexión directa célula-célula.
  - Capacidad selectiva de paso
  - Regulación de apertura
  - Reconocimiento entre diferentes células
- Plasmodesmos de células vegetales.





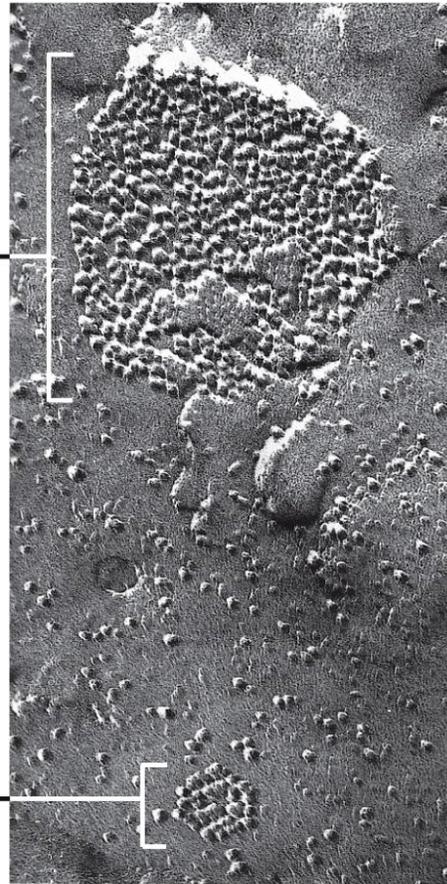
(A)

100 nm

large  
gap  
junction

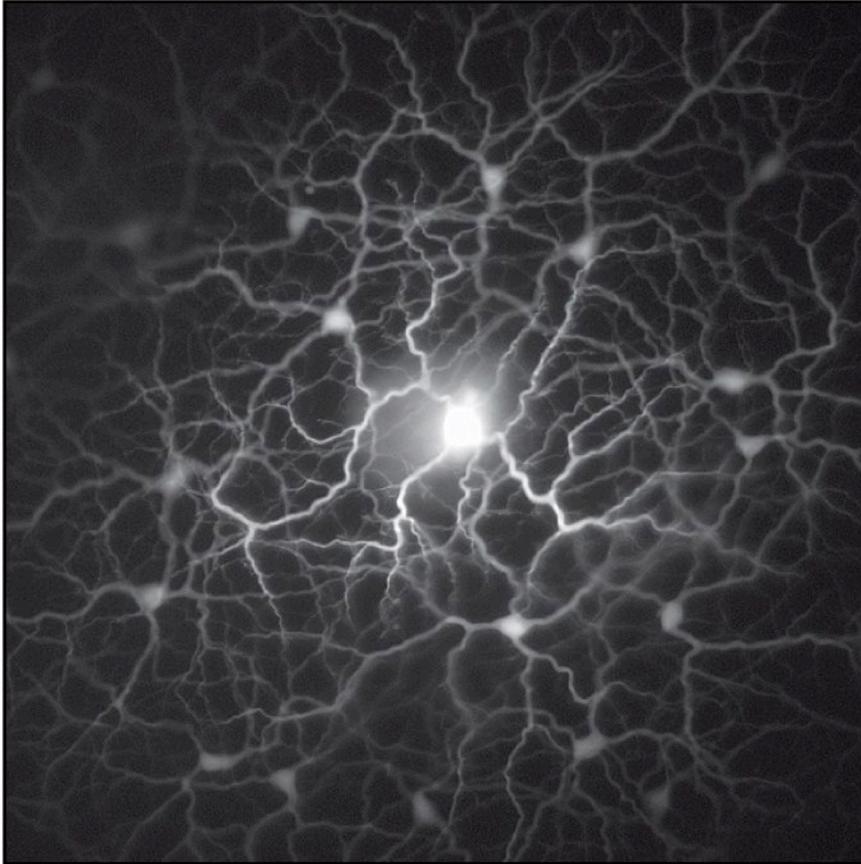
membranes

small  
gap  
junction

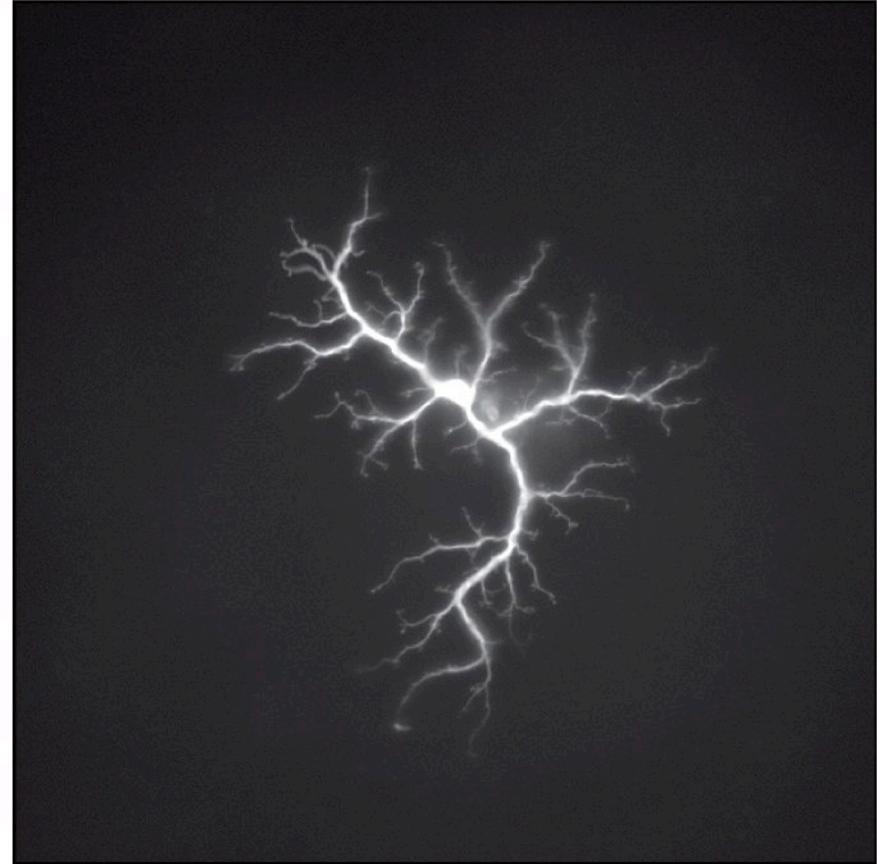


(B)

100 nm



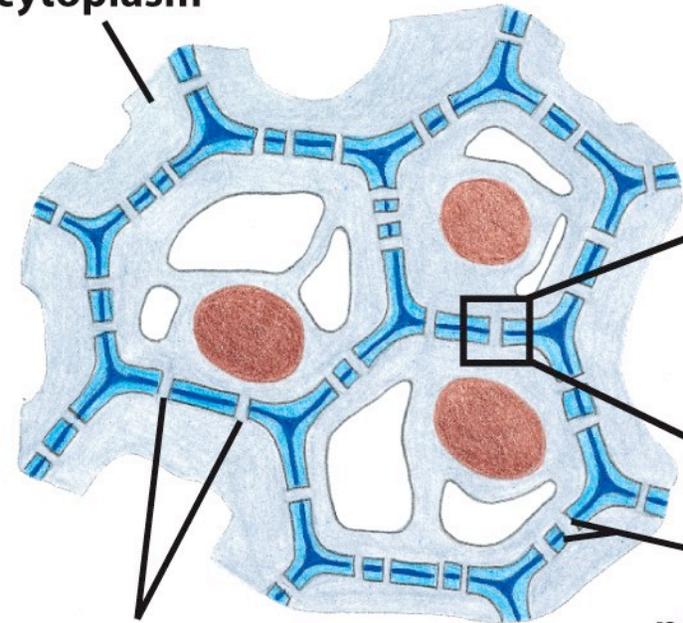
**(A)**



**(B)**

- A. Neurona inyectada con “amarillo de lucifer”, colorante que atraviesa las uniones en hendidura y marca otras neuronas.
- B. Neurona pre-tratada con dopamina (neurotransmisor), no deja pasar el colorante. Cierra sus Uniones en hendidura.

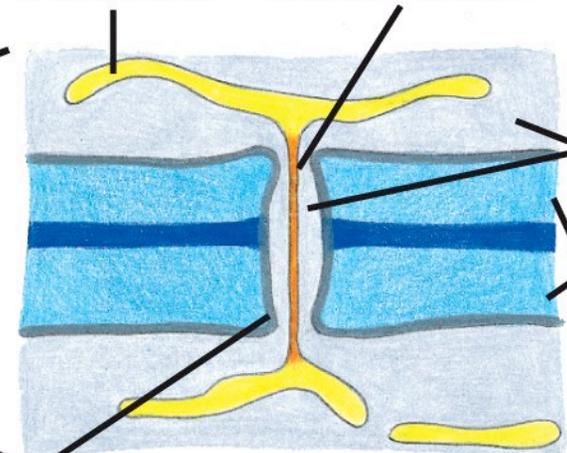
cytoplasm



plasmodesmata

(A)

smooth  
endoplasmic  
reticulum



plasma membrane lining  
plasmodesma, connecting  
two adjacent cells

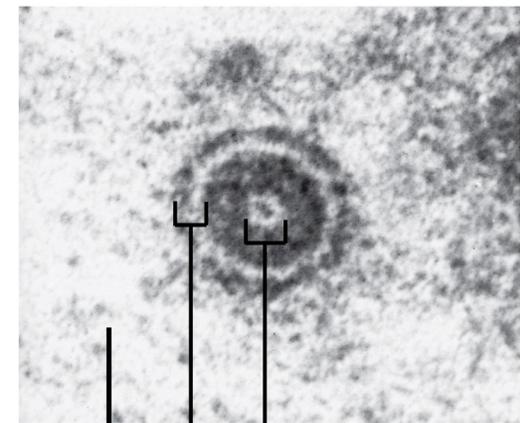
(B)

100 nm

cytosol

cell walls  
of adjacent  
plant cells

# Plasmodesmos



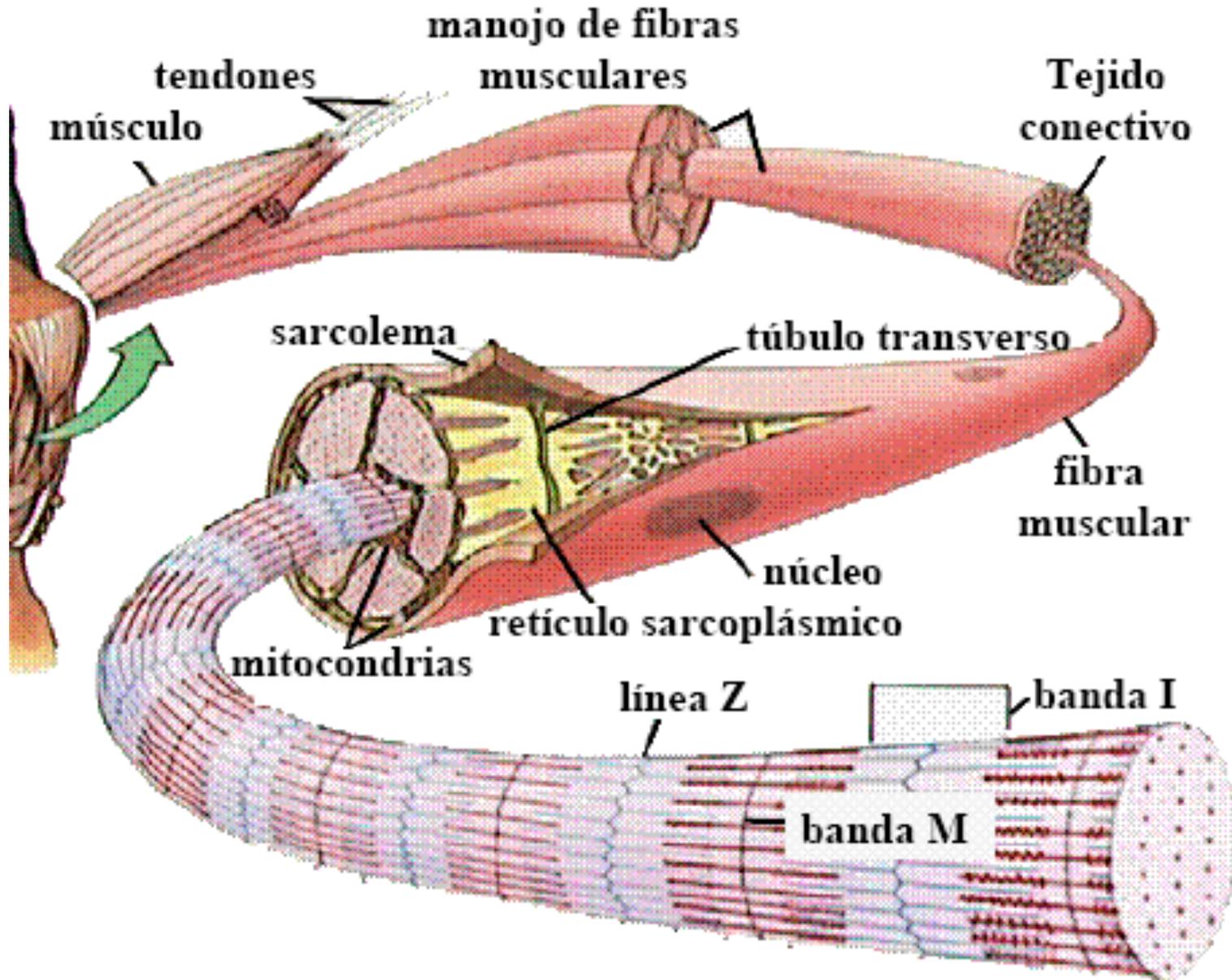
cell  
wall

desmotubule

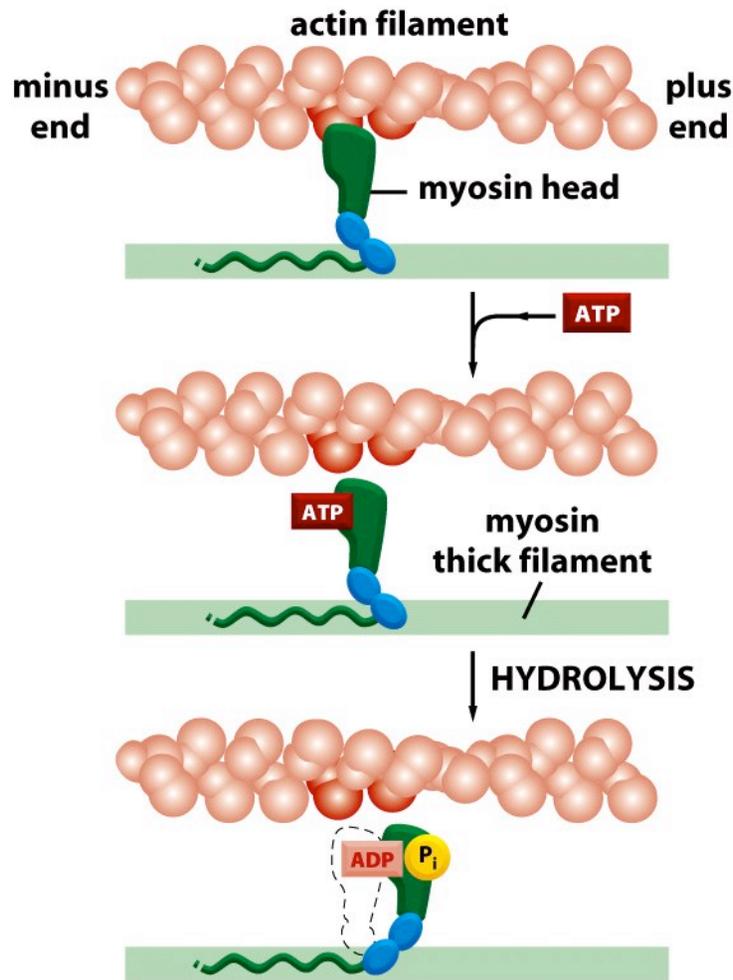
plasma membrane

25 nm

# Contracción Muscular



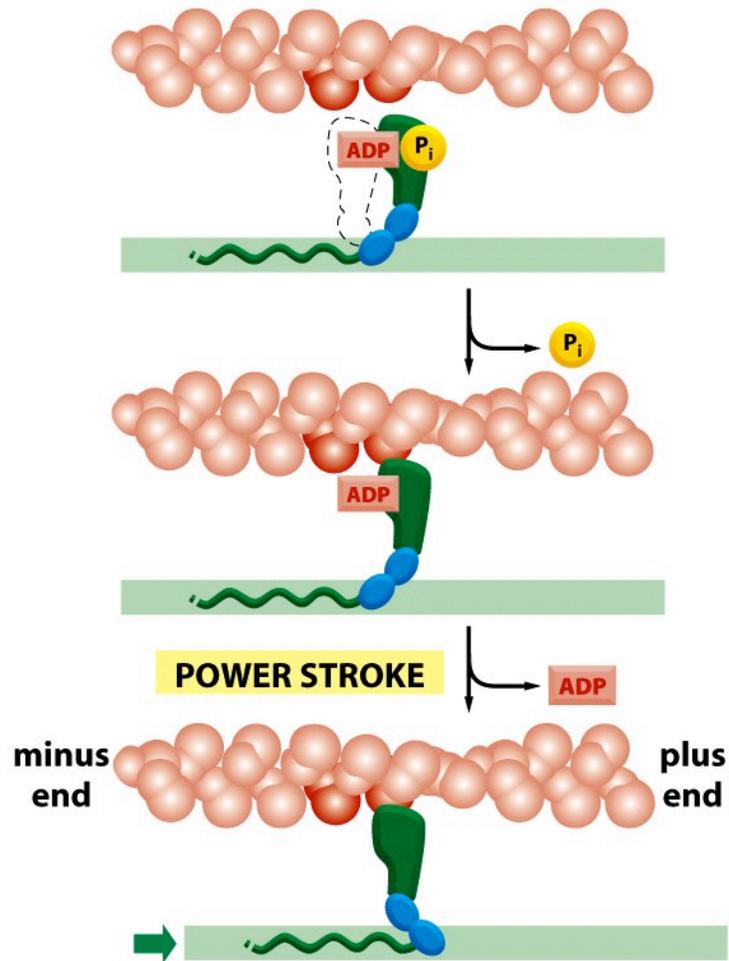
# Contracción Muscular



**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 domains that make up the actin-binding site. This reduces the affinity of the head for actin and allows 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.)

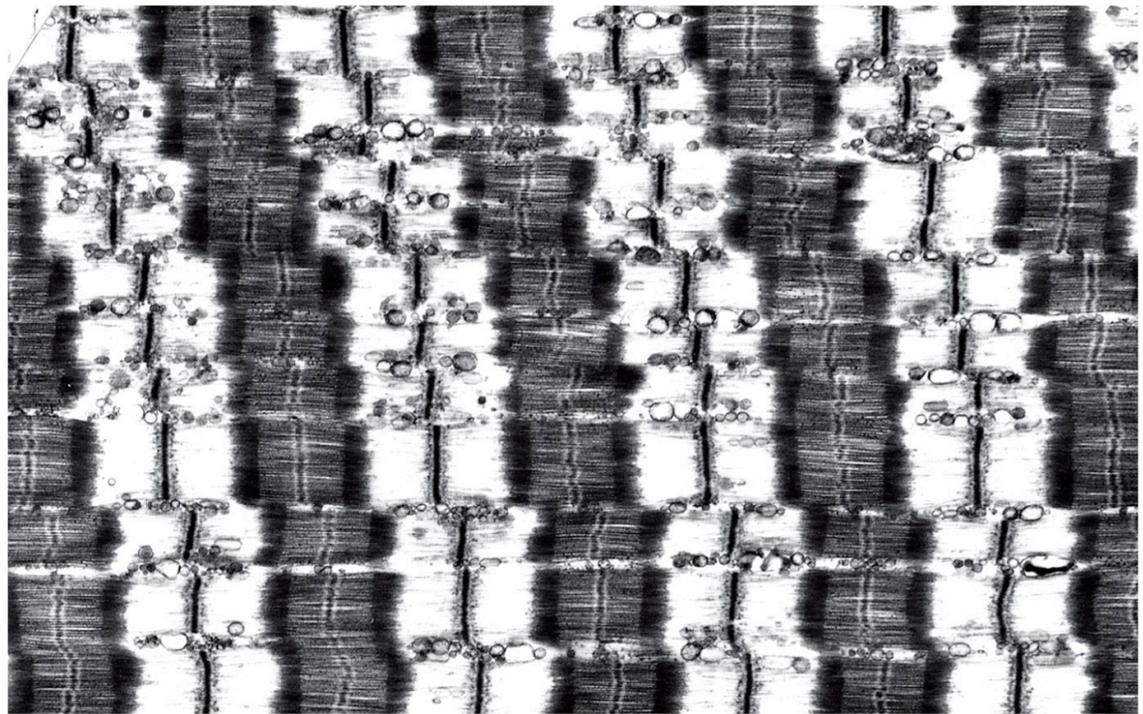
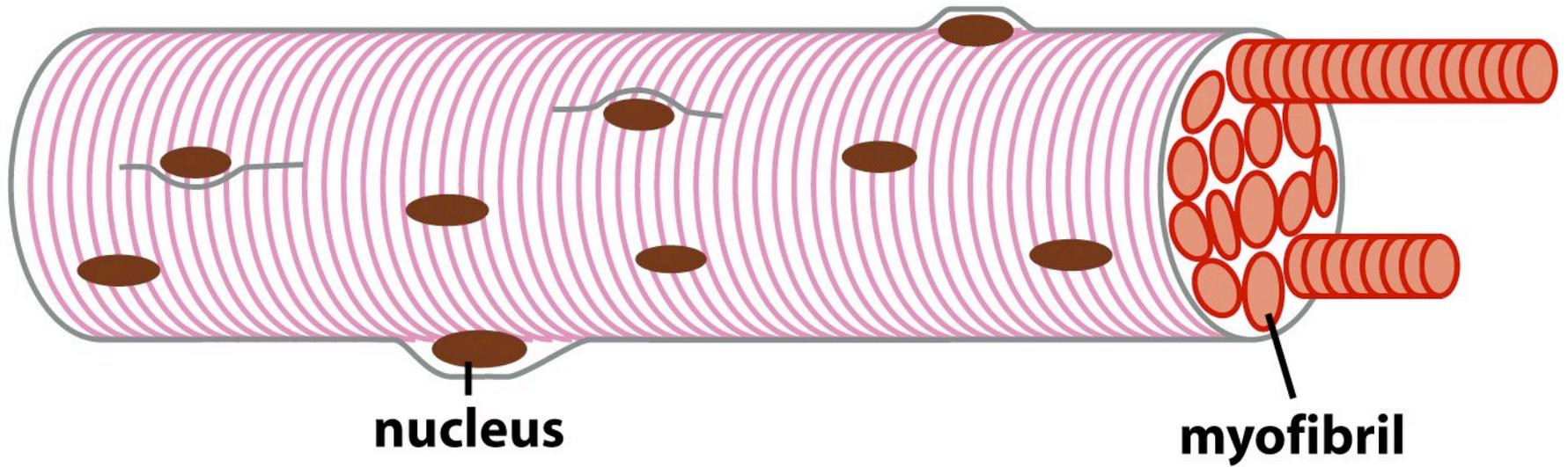
**COCKED** The cleft closes like a clam shell around the ATP molecule, triggering a large shape change that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate ( $P_i$ ) produced remain tightly bound to the protein.



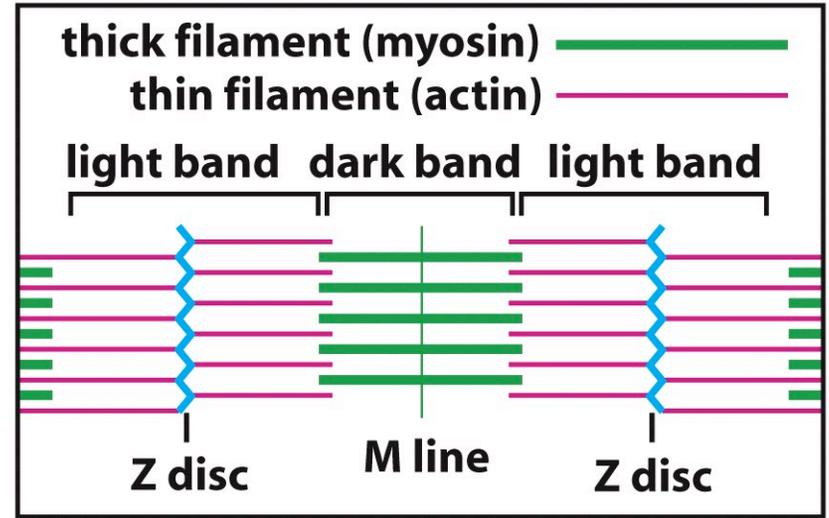
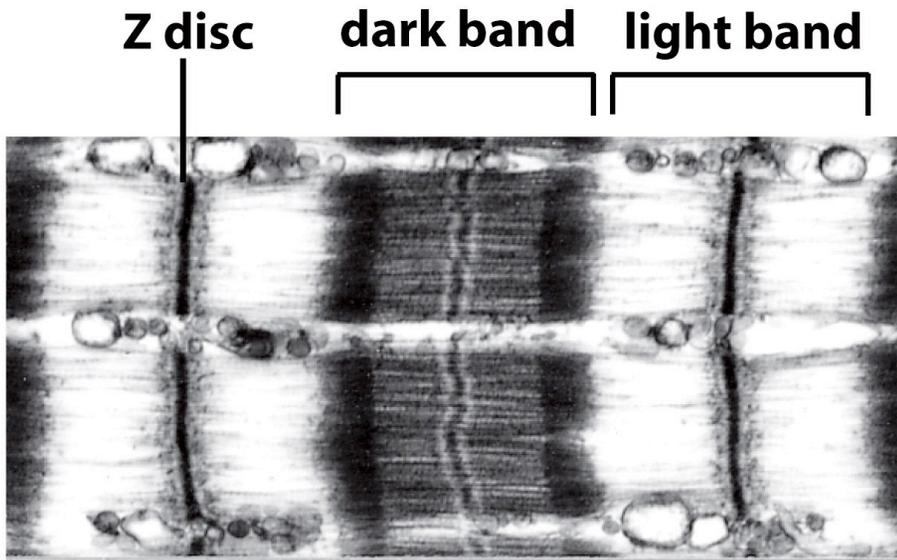
**COCKED** The cleft closes like a clam shell around the ATP molecule, triggering a large shape change that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate ( $P_i$ ) produced remain tightly bound to the protein.

**FORCE-GENERATING** A 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.

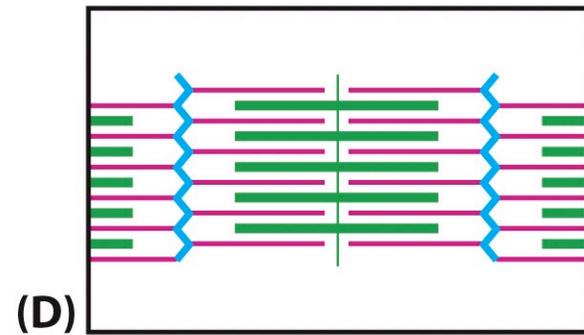
**ATTACHED** At the end of the cycle, the myosin head is again locked tightly to the actin filament in a rigor configuration. Note that the head has moved to a new position on the actin filament.



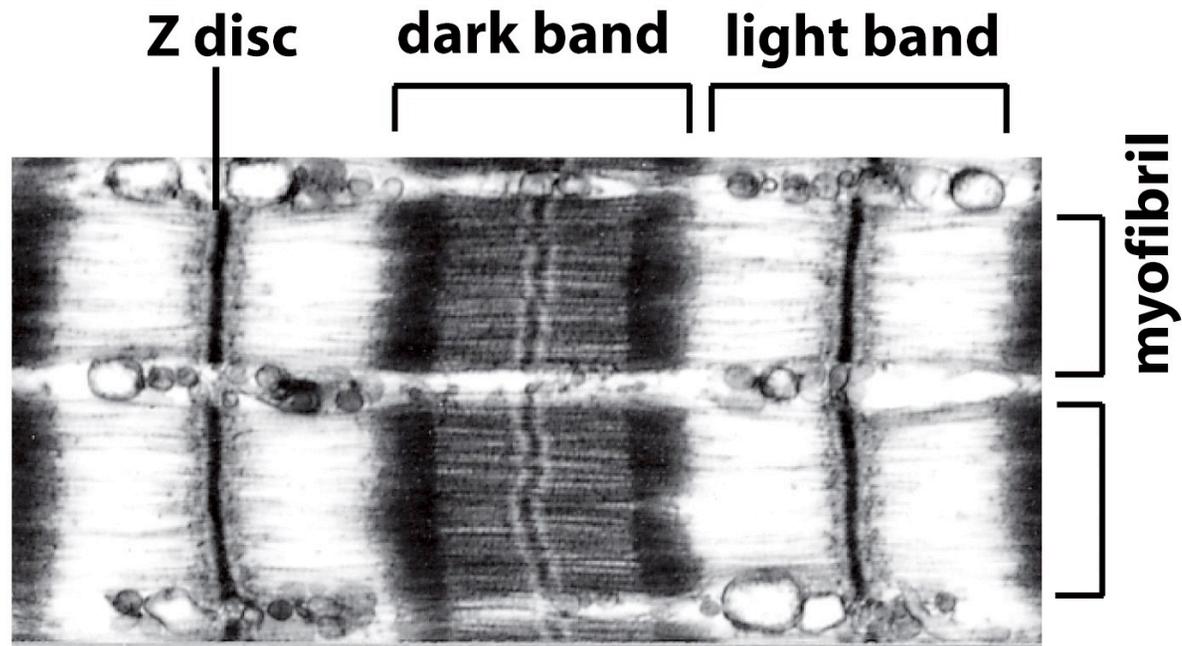
2  $\mu$ m



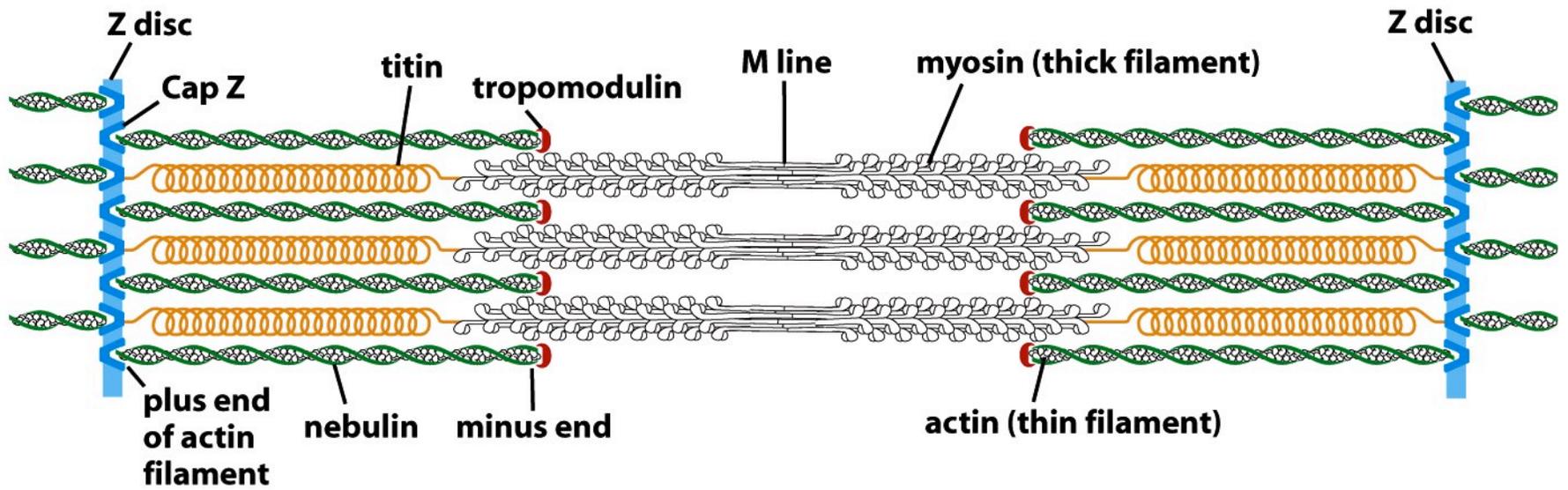
(C)



(D)



**one sarcomere**



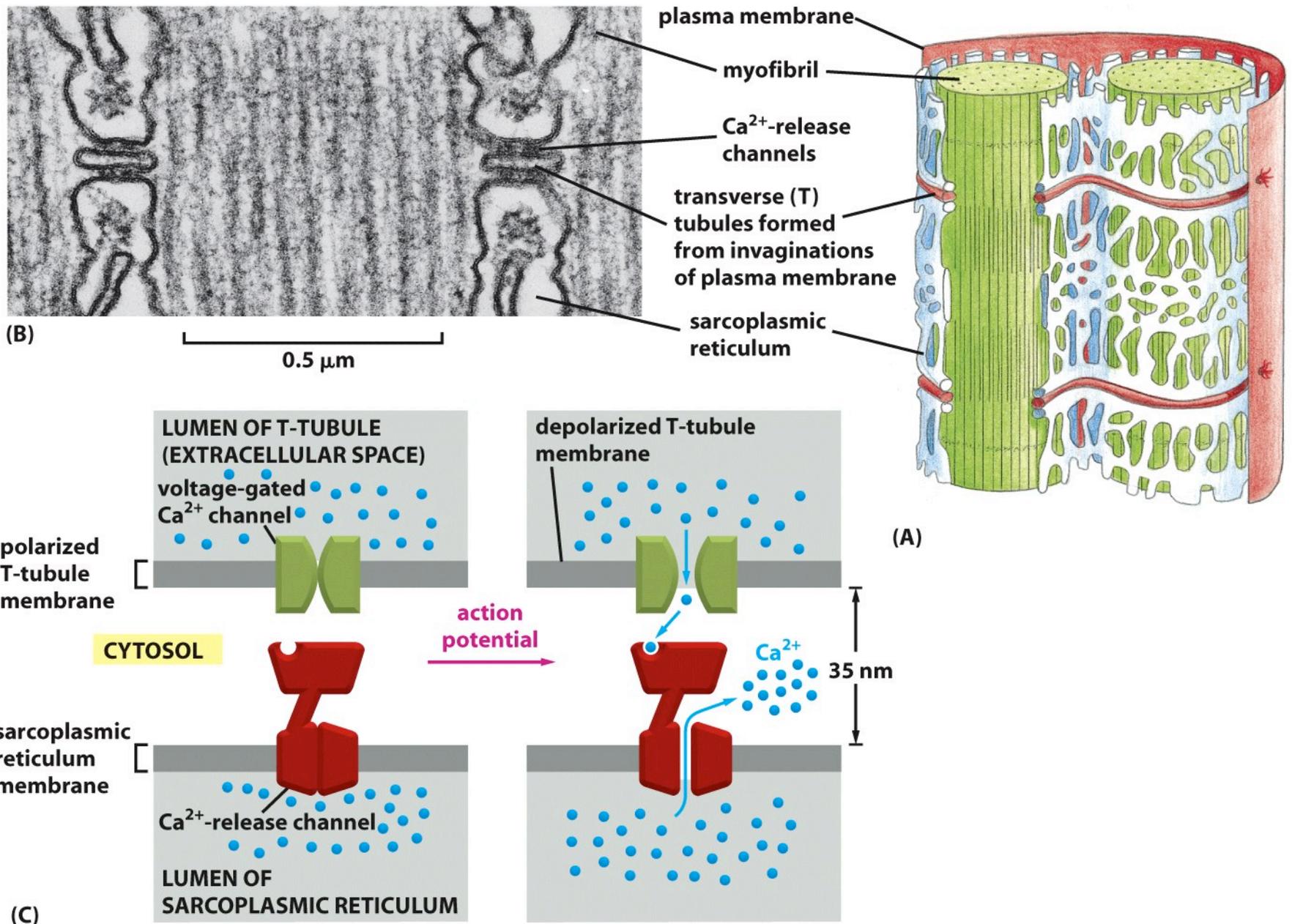


Figure 16-77 *Molecular Biology of the Cell* (© Garland Science 2008)

