Citoesqueleto y Uniones celulares

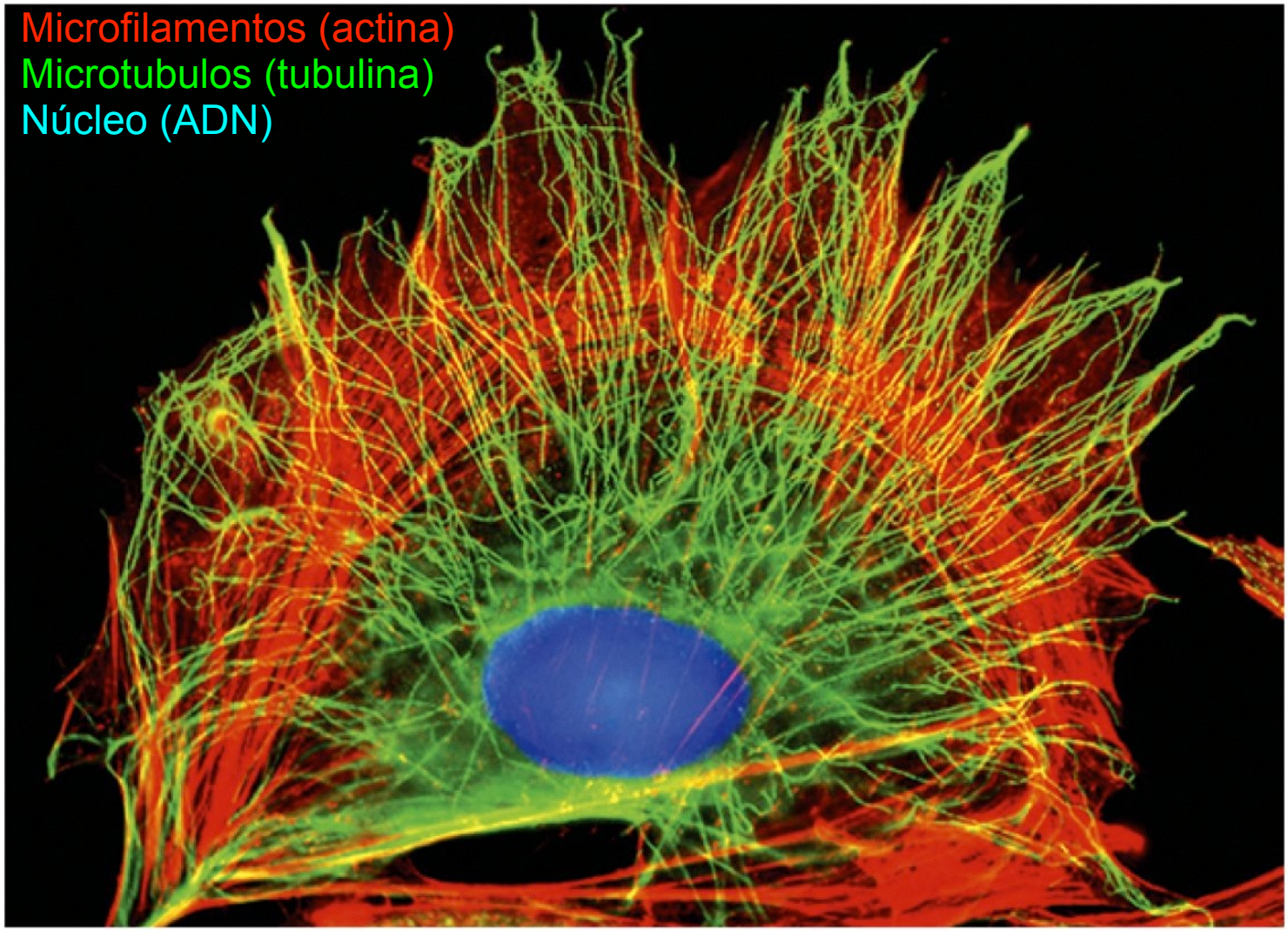
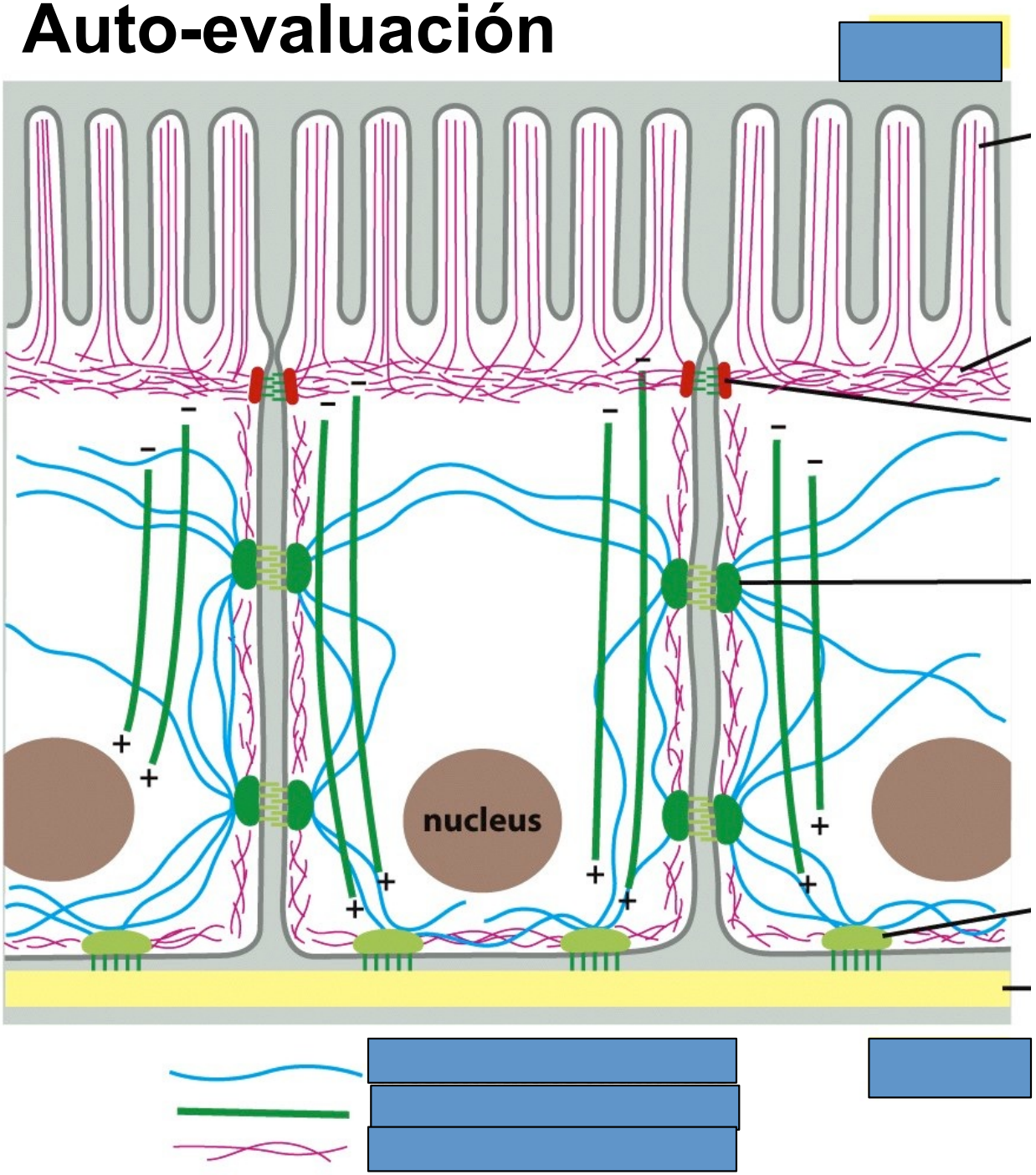


Figure 16-1 *Molecular Biology of the Cell* (© Garland Science 2008)

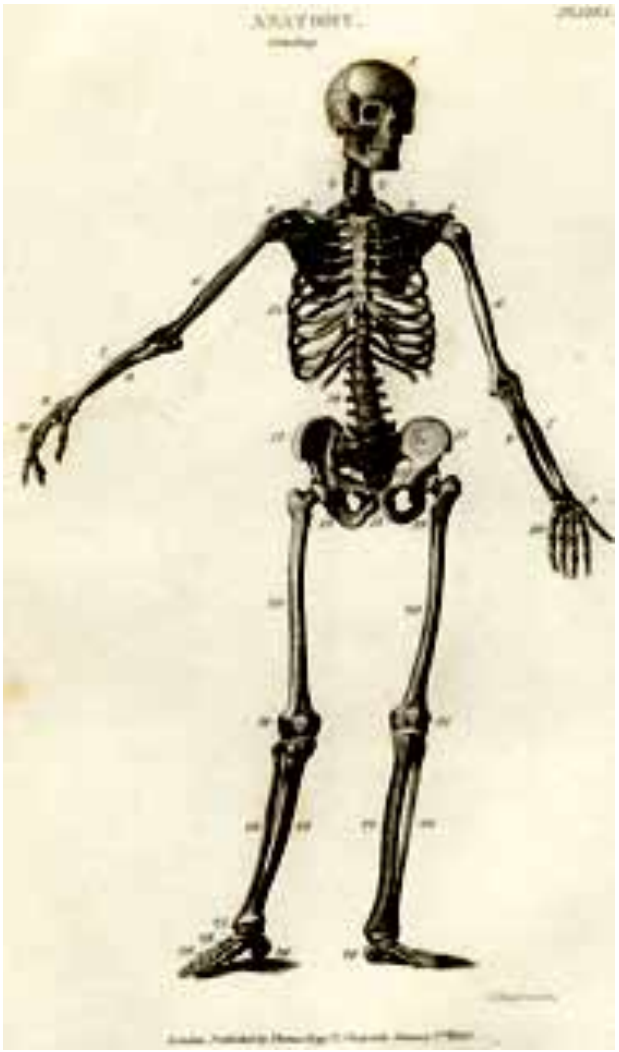
Auto-evaluación



1. ¿Qué estamos viendo?
2. ¿Cuántas estructuras puede rotular?
3. ¿Cuál es la función de cada una?
4. Que proteínas son las que componen la estructura rotulada?

Figure 16-5 *Molecular Biology of the Cell* (© Garland Science 2008)

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.

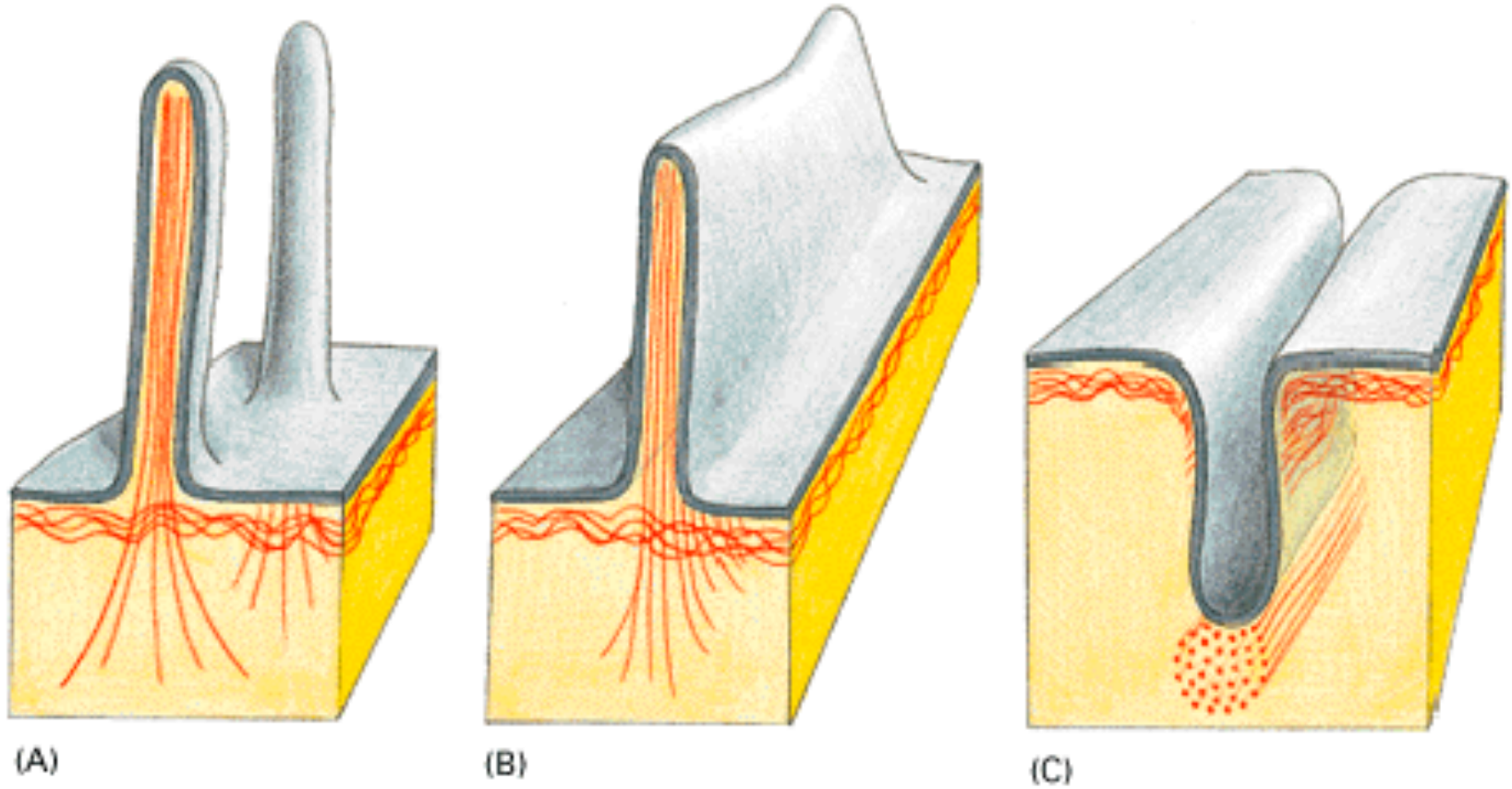


Figure 16-9. Actin filaments often shape the plasma membrane of animal cells. Three examples of plasma membrane changes caused by the cortical network of actin filaments. (A) Thin, spiky protrusions such as microspikes form on the surface of cells by the assembly of supporting bundles of actin filaments 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)

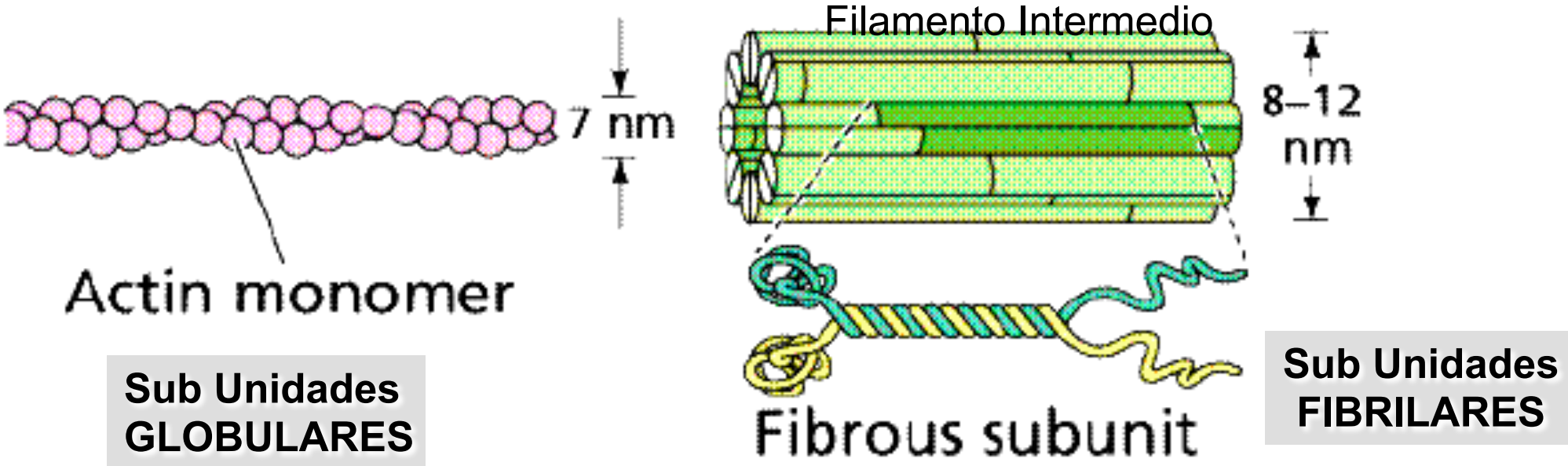
Características generales

- 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:

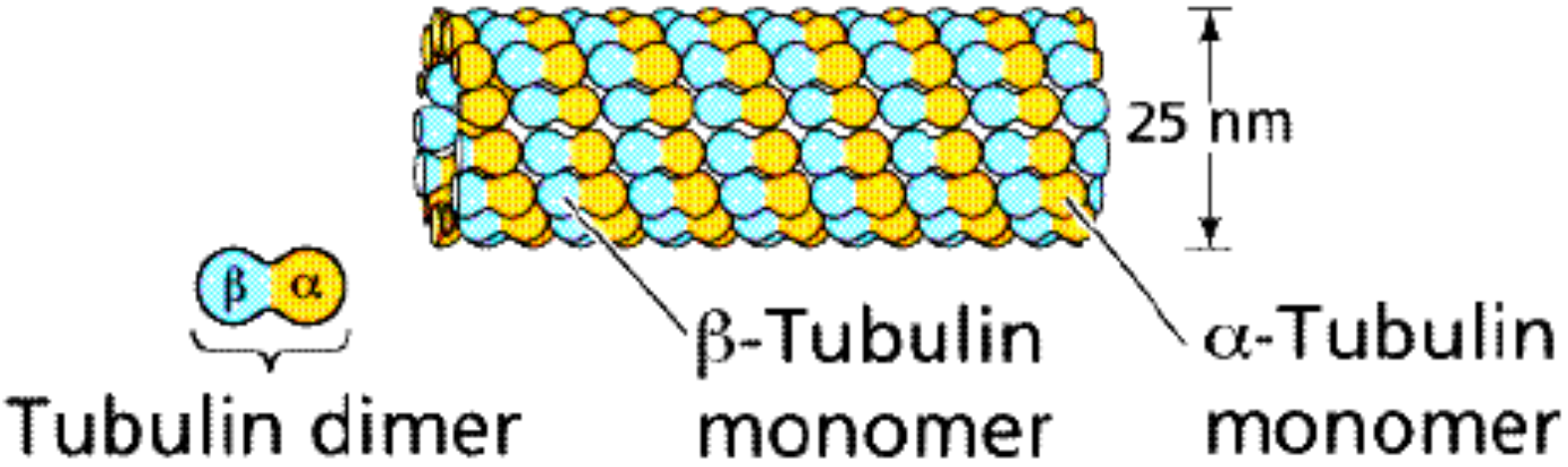
- Secuestradoras de subunidades.
- Proteínas que se unen al costado del filamento
- Proteínas que tapan un extremo (capping)

COMPONENTES DEL CITOESQUELETO



Sub Unidades GLOBULARES

Sub Unidades FIBRILARES



Sub Unidades GLOBULARES

ESTRUCTURA TERCEARIA VS. CUATERNARIA

¿Por qué tres tipos de filamentos diferentes?

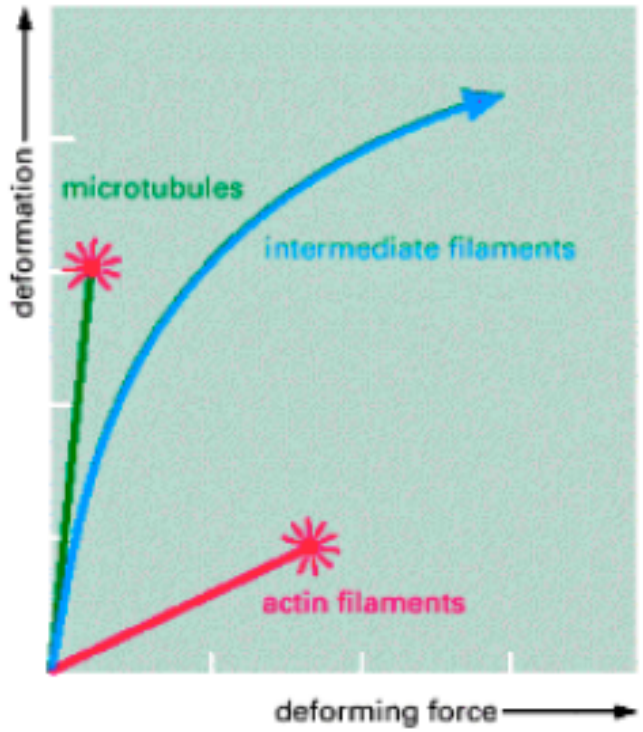
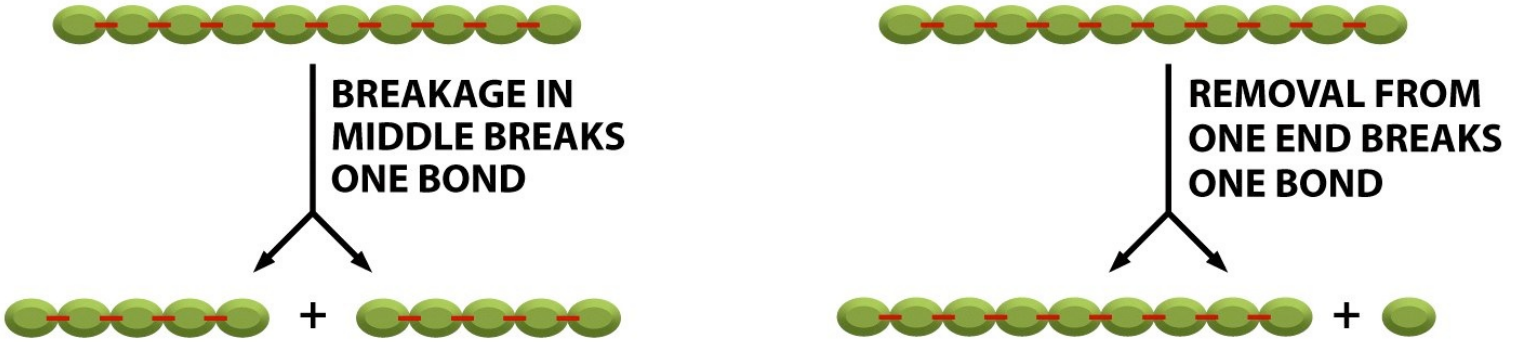
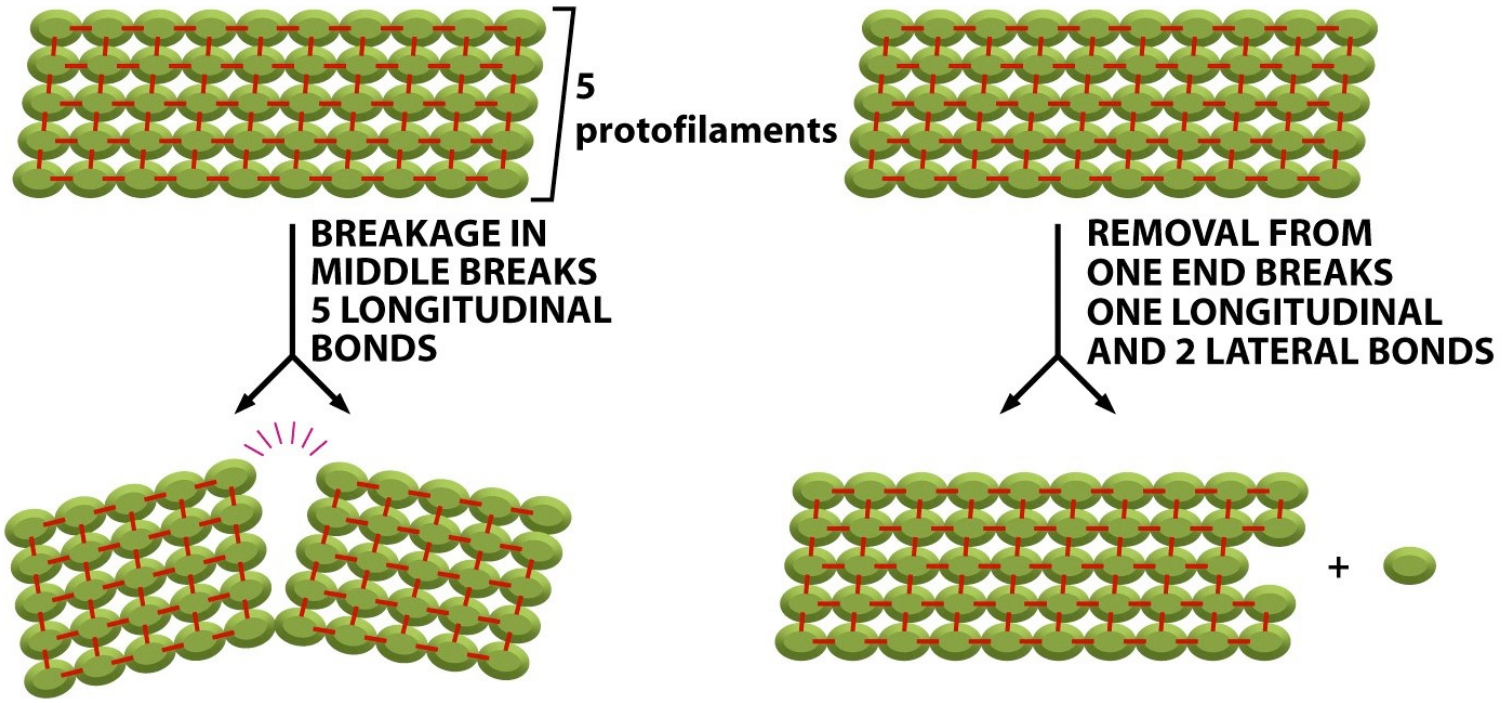


Figure 16-17. Mechanical properties of actin, tubulin, and intermediate filament polymers. Networks composed of microtubules, actin filaments, or a type of intermediate filament called vimentin, all at equal concentration, were exposed to a shear force in a viscometer, and the resulting degree of stretch 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.

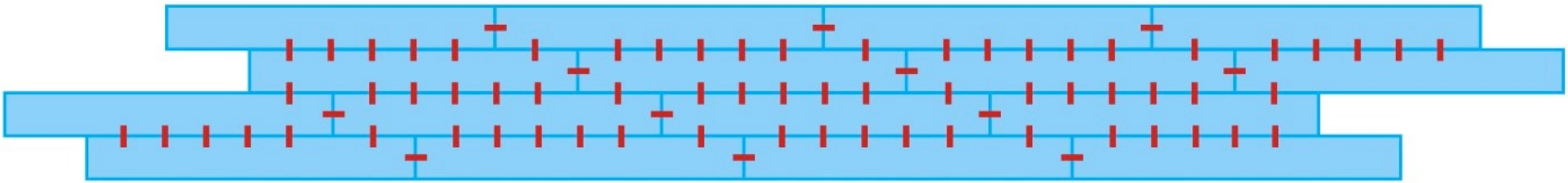


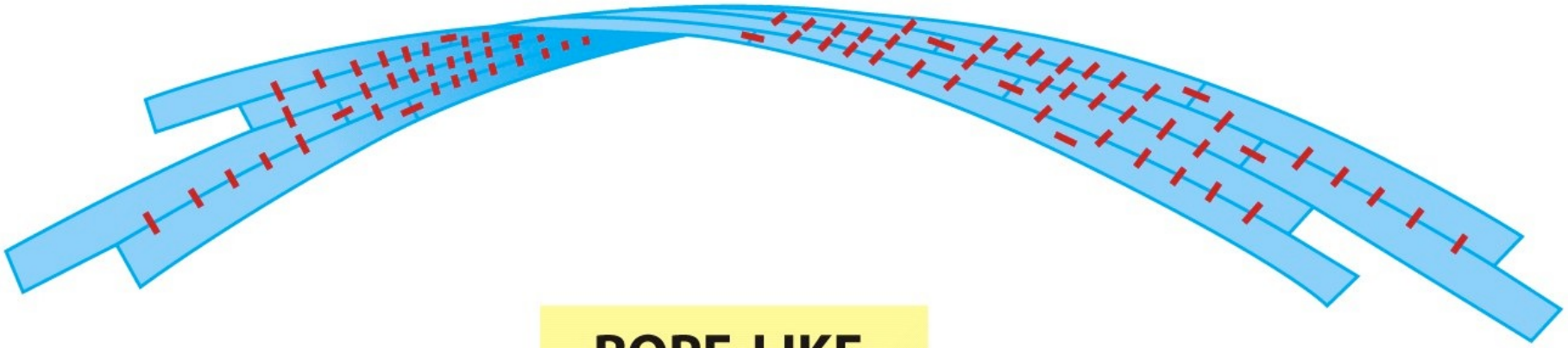
SINGLE PROTOFILAMENT: THERMALLY UNSTABLE



MULTIPLE PROTOFILAMENTS: THERMALLY STABLE

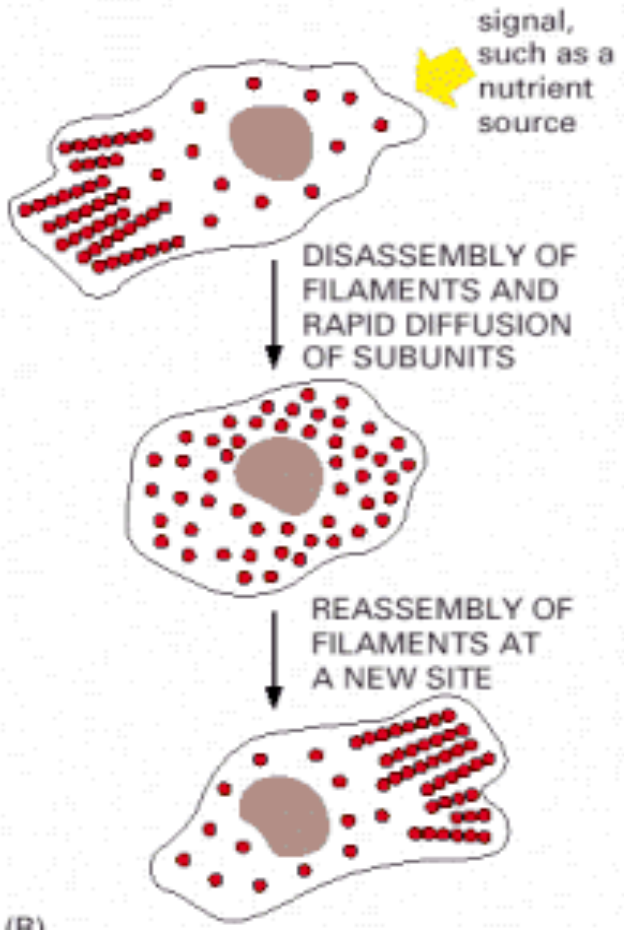
staggered long subunits: lateral contacts dominate





ROPE-LIKE PROPERTIES

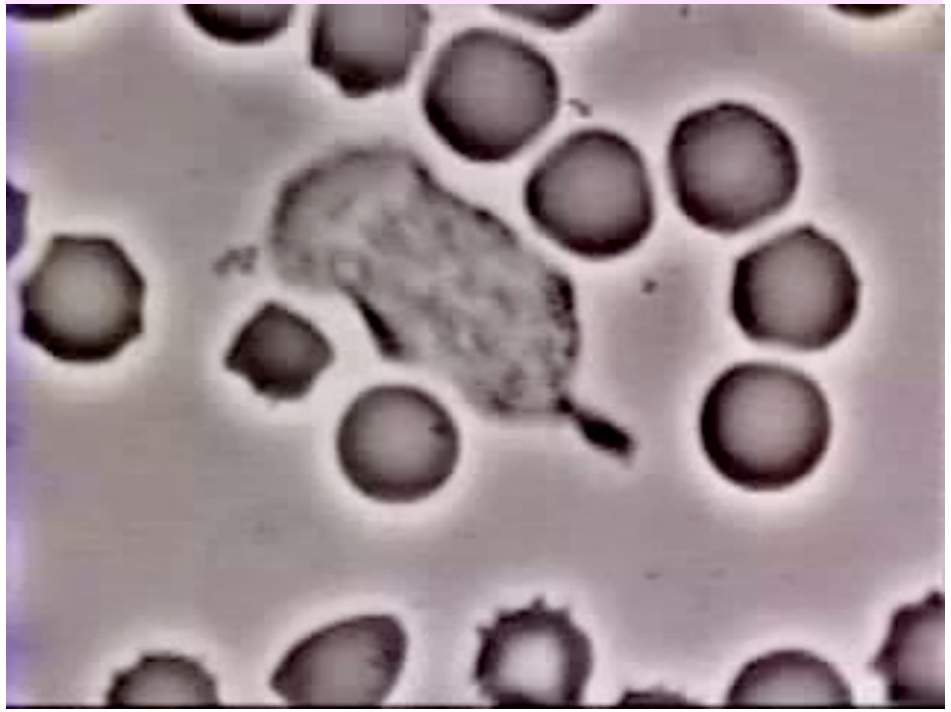
Dinamismo del Citoesqueleto



- <u>Construcción en base a monómeros</u>
  - monómeros muy abundantes
  - las subunidades no están unidas covalentemente.

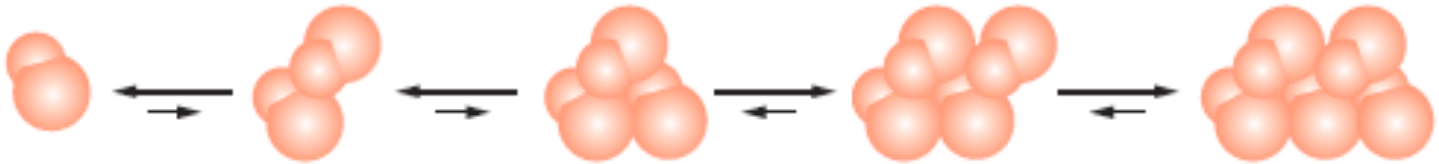
- <u>Proteínas accesorias</u>
  -regulan el sitio y velocidad de nucleación de filamentos

  -Regulan el equilibrio entre **polimerización** y **despolimerización**.



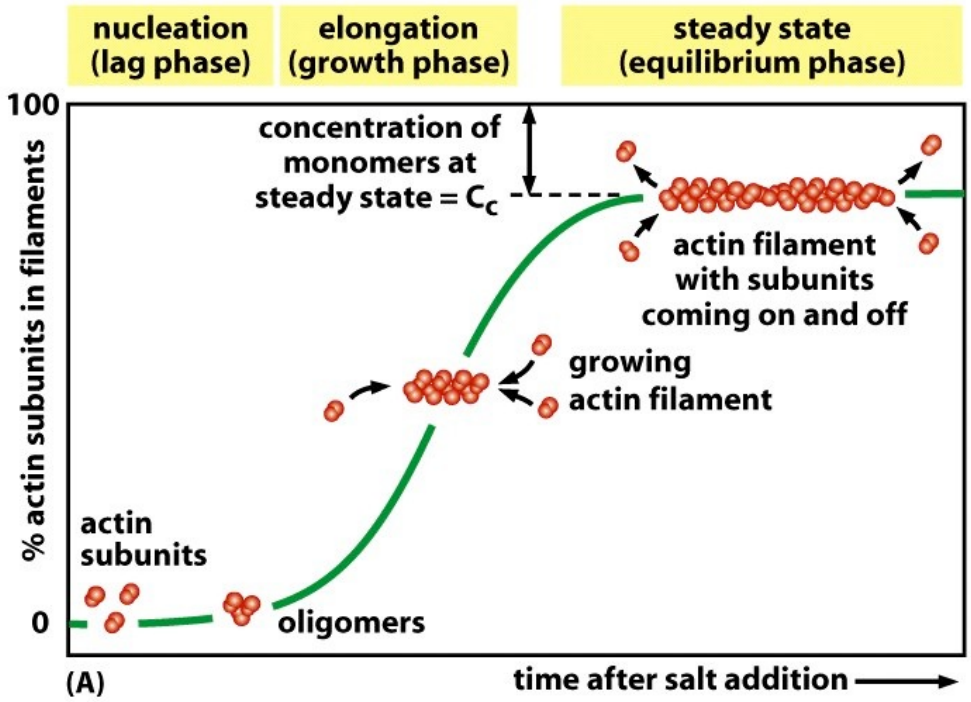
Nucleación

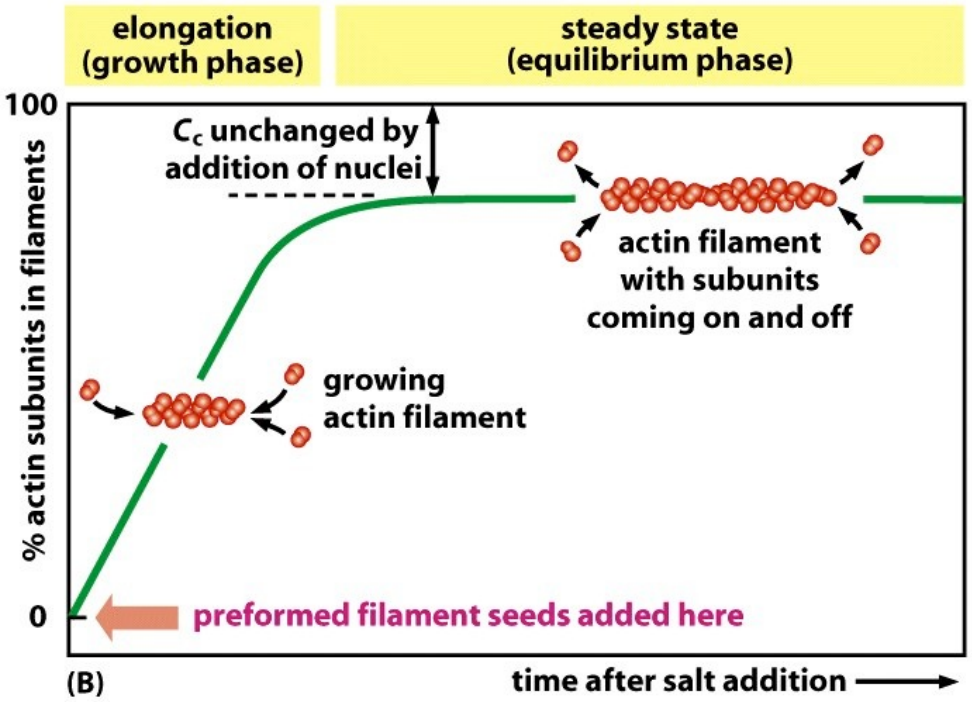
Los polímeros se estabilizan por los múltiples enlaces entre las subunidades. En el caso de actina, dos sub U son poco estables, un trímero aumenta la estabilidad.



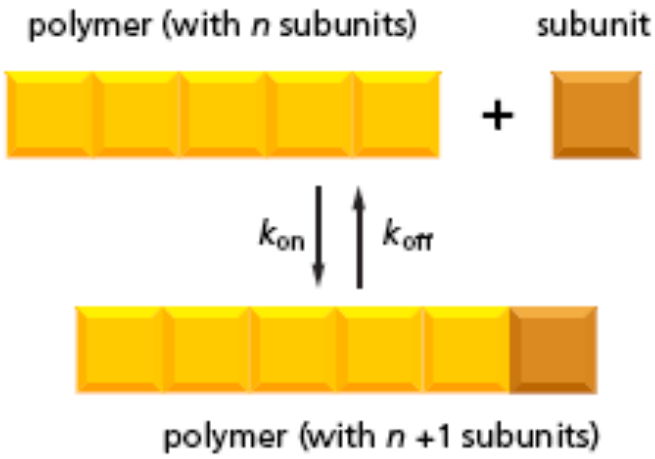
Trímero

Puede adicionar monómeros y crecer.
La aparición de núcleos es el paso limitante
en el crecimiento de los filamentos





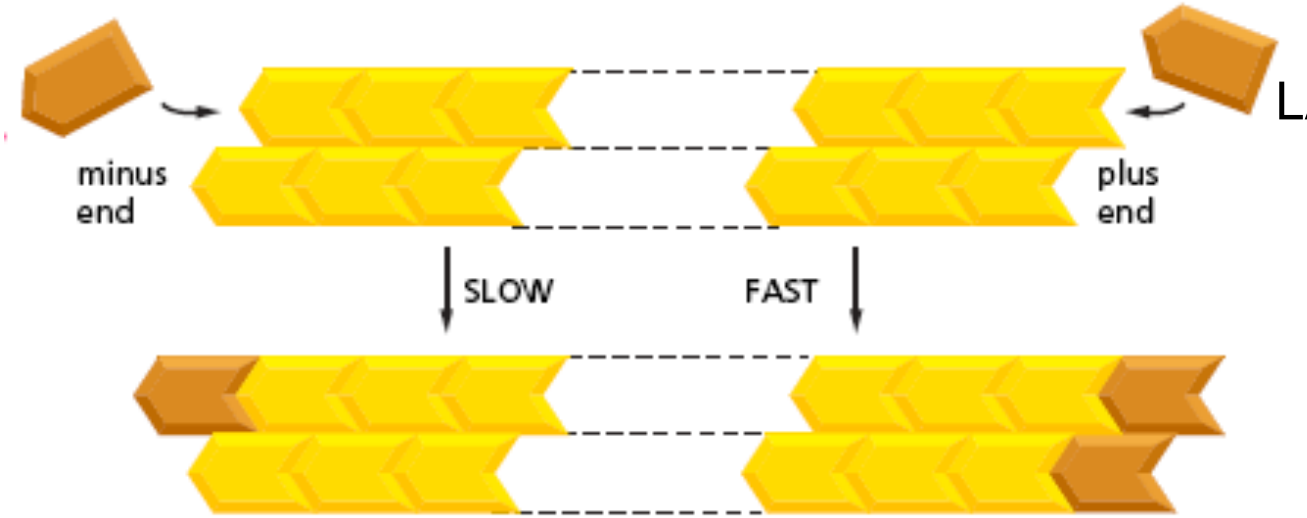
polimerización y. despolimerización



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

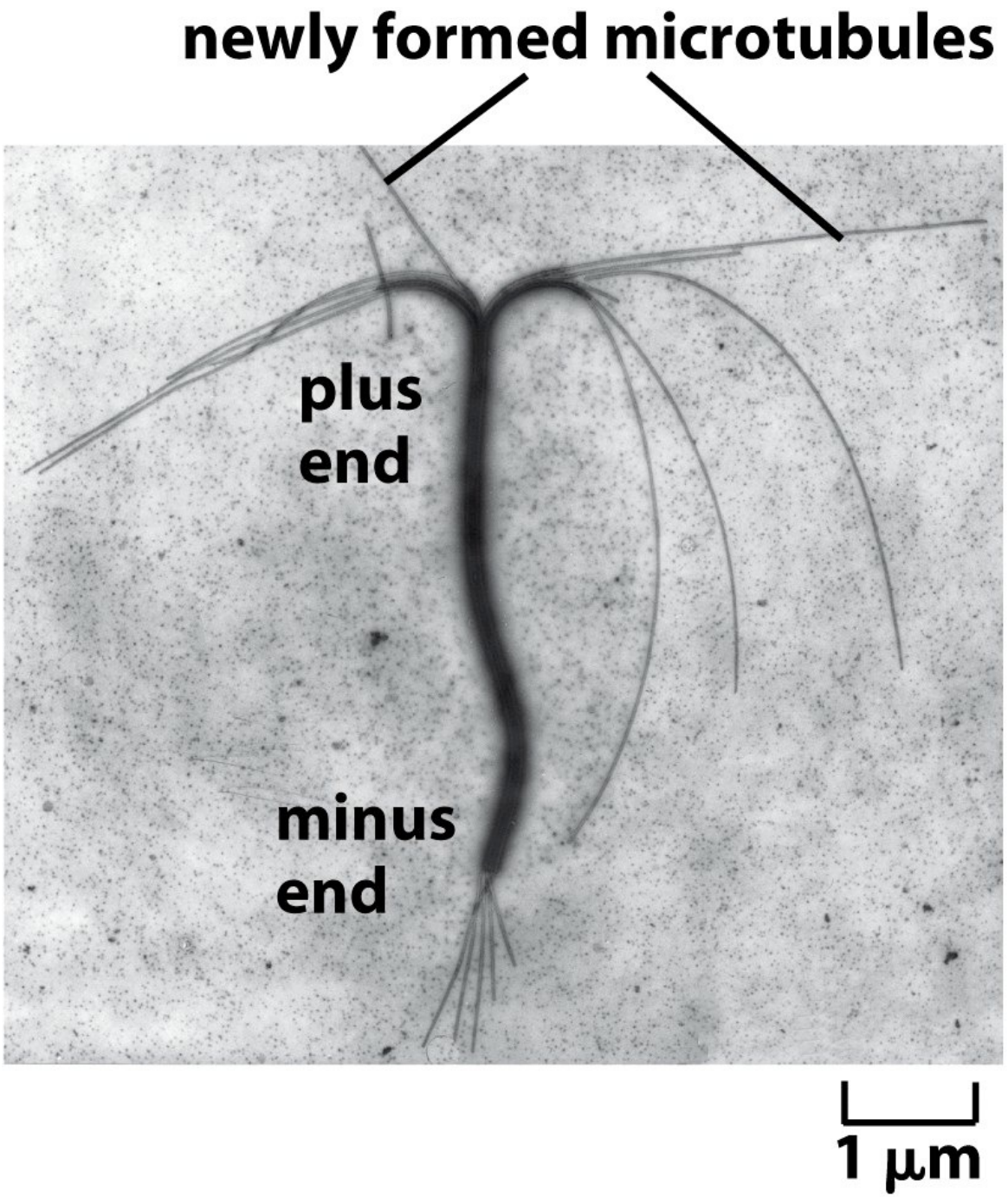
NO SE REFIERE A QUE LOS MONOMEROS SE INCORPORAN POR UN LADO Y SALEN POR OTRO

LADO -



LADO +

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


newly formed microtubules
plus
end
minus
end
1 μm

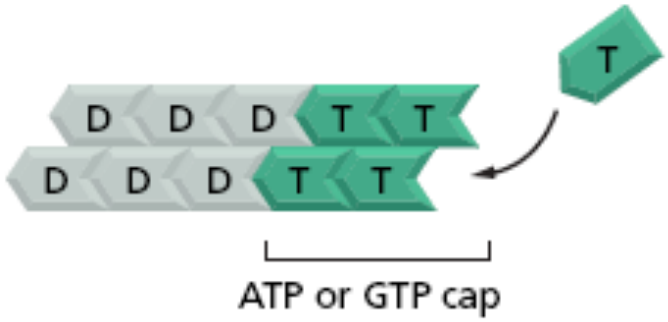
Actina: ATP

Tubulina: GTP

Actina: ADP

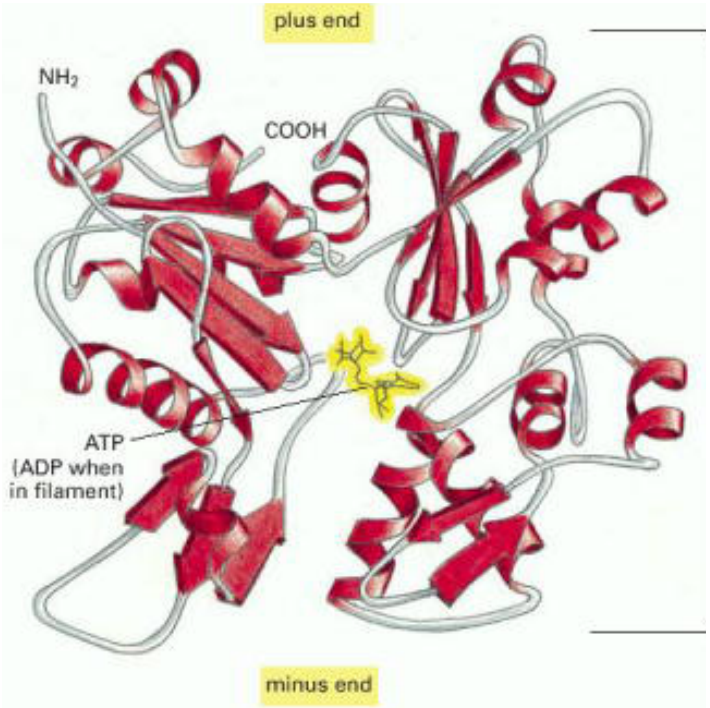
Tubulina: GDP

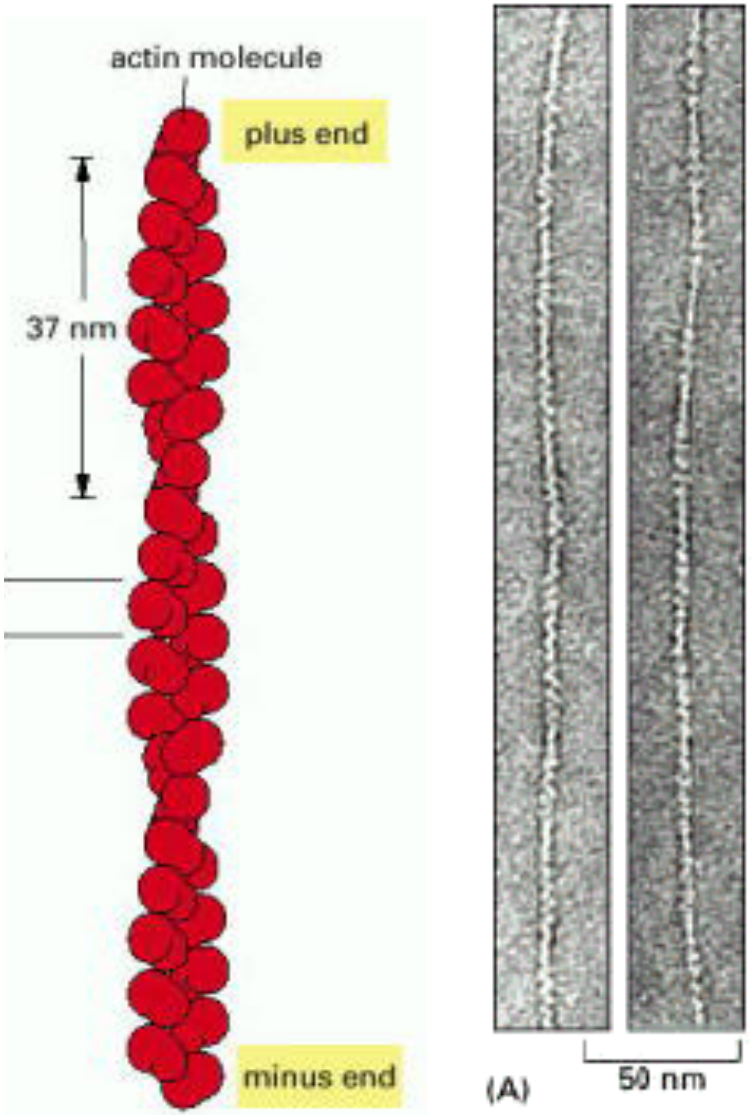
¿qué significa el cambio ATP por ADP?



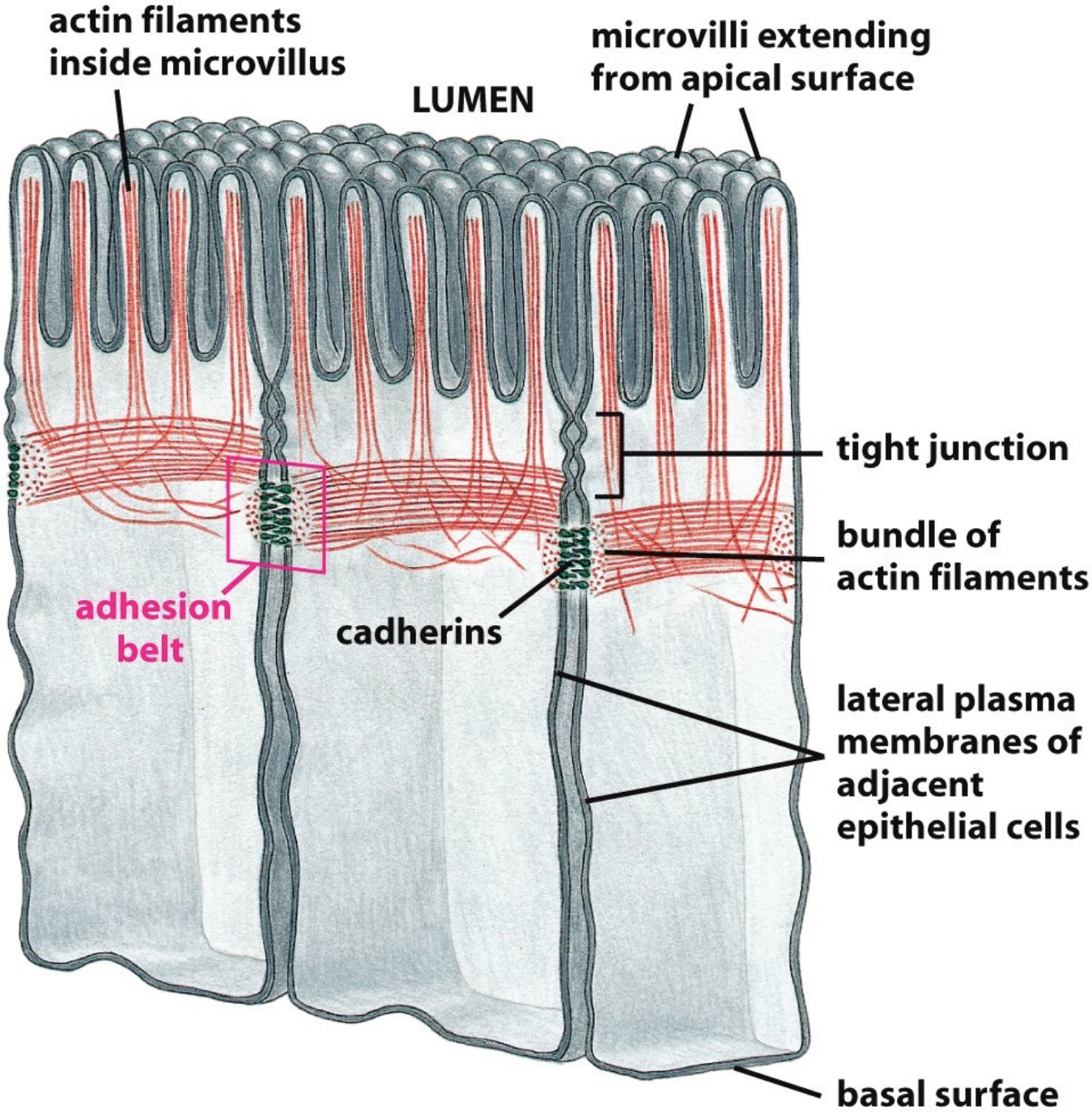
“Cap” (tapa)
La rapidez de adición de subU puede ser más rápida que la hidrólisis del ATP o GTP. Esto forma una tapa de subunidades unidas a ATP o GTP (y con mayor afinidad por otras subU.

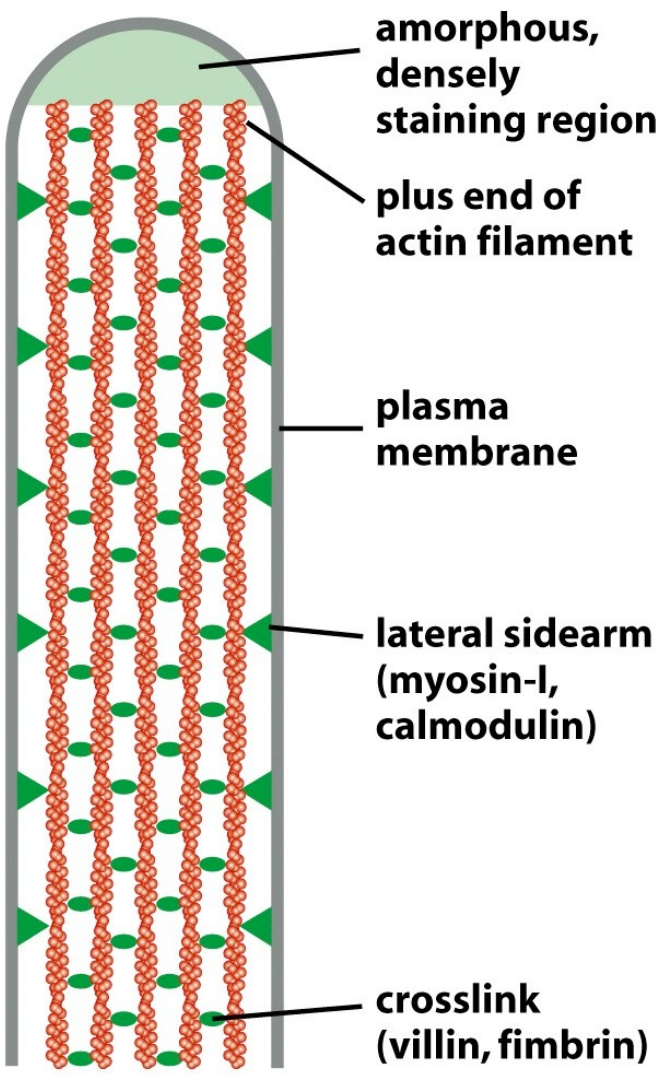
Microfilamentos

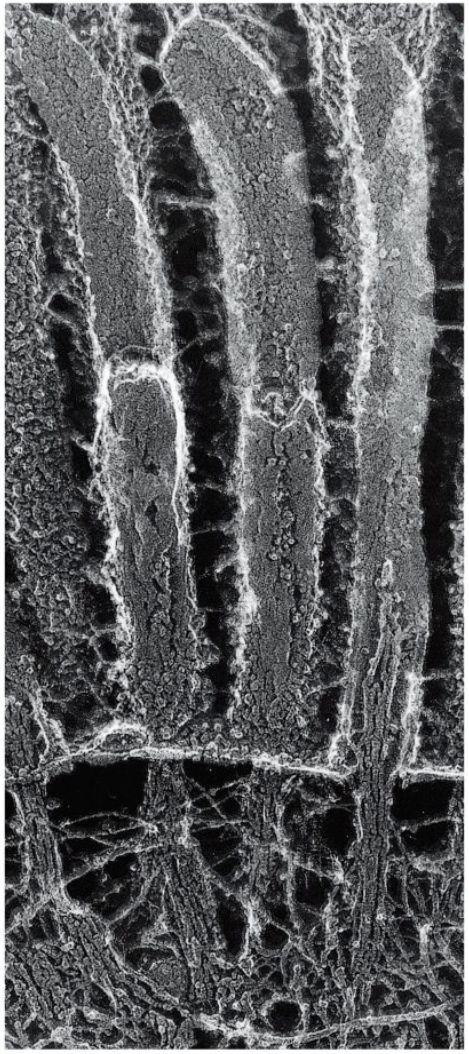




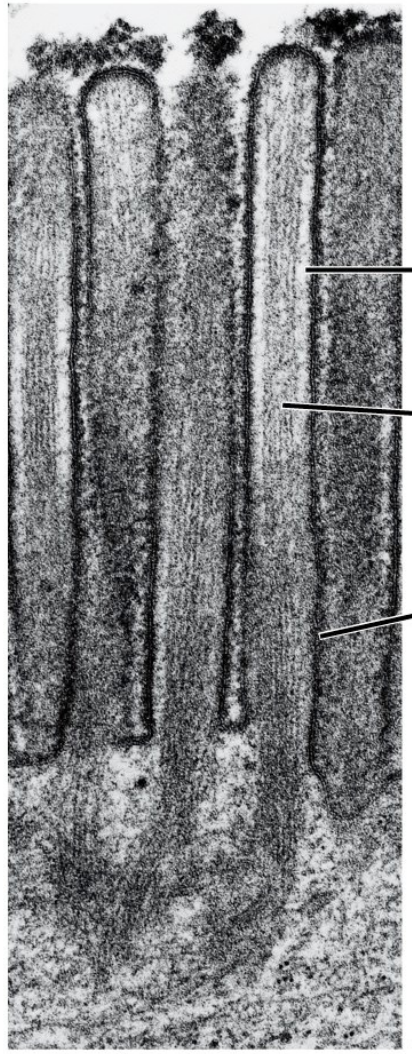
- conservados (homólogo en bacterias: MreB)
- *G-actina* (monómero, actina globular) se ensambla a *F-actina*, un polímero helicoidal **polarizado**
- Ensamblaje de los filamentos depende de la hidrólisis de ATP (requiere energía)
- Forman filamentos, redes y geles (3D)


actin filaments
inside microvillus
LUMEN
microvilli extending
from apical surface
tight junction
bundle of
actin filaments
adhesion
belt
cadherins
lateral plasma
membranes of
adjacent
epithelial cells
basal surface


amorphous, densely staining region
plus end of actin filament
plasma membrane
lateral sidearm (myosin-I, calmodulin)
crosslink (villin, fimbrin)



(B)


microvillus
actin filament bundle
plasma membrane
terminal web

(C)

1 μm

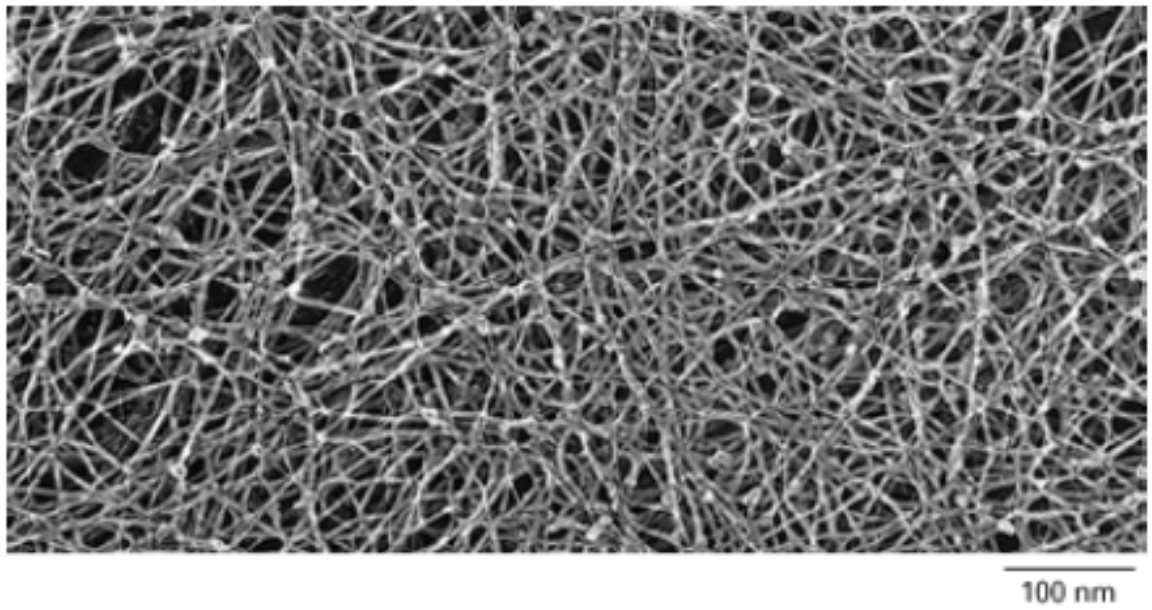
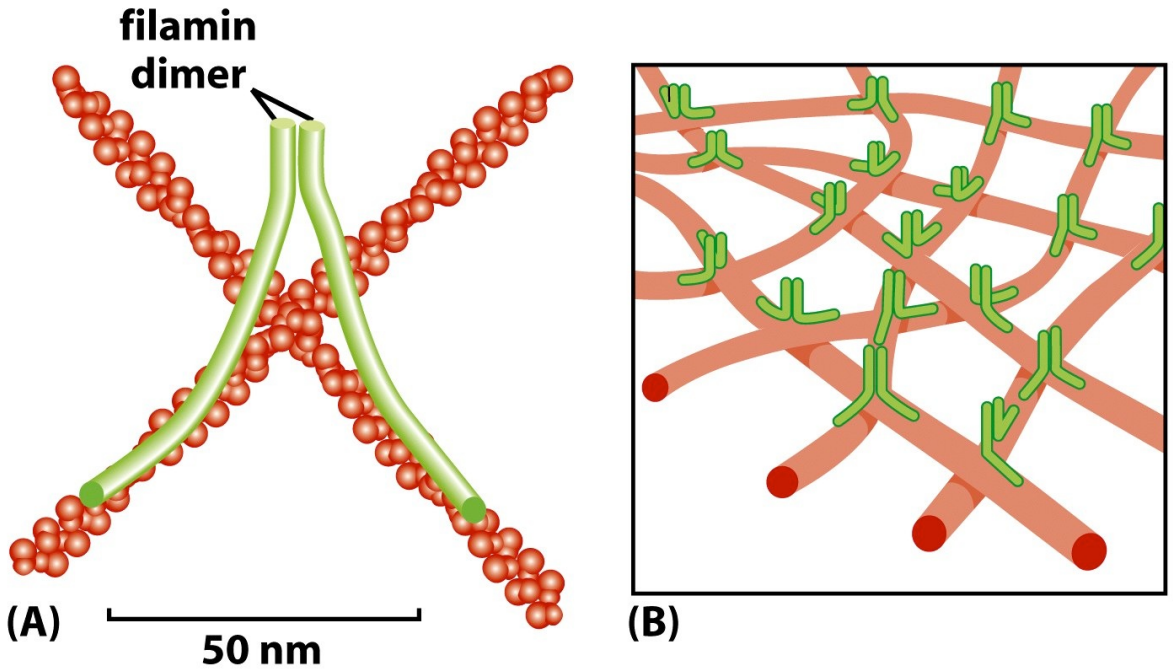
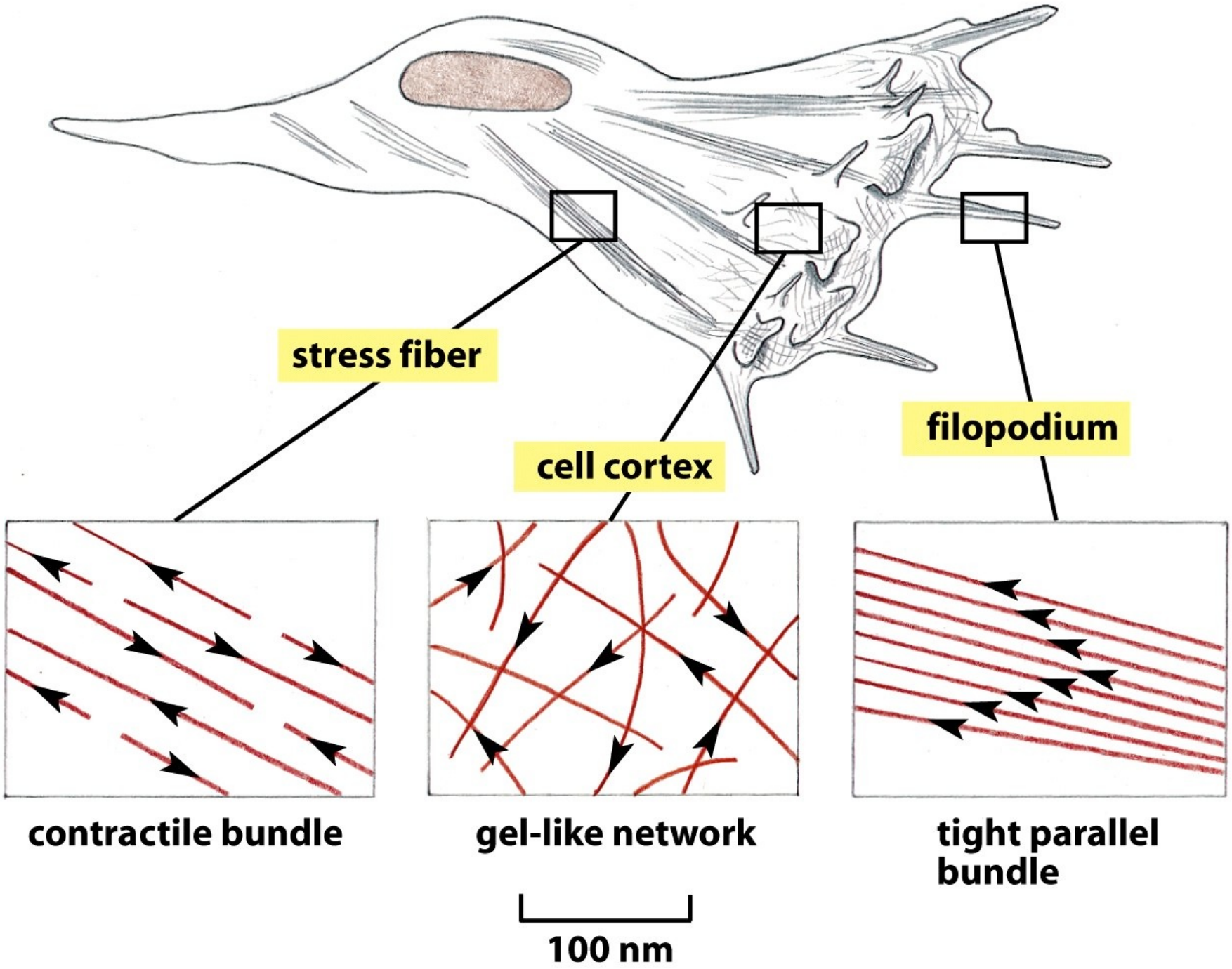


Figure 1-26. Actin. A network of actin filaments underlying the plasma membrane of an animal cell is seen in this electron micrograph prepared by the deep-etch technique. (Courtesy of John Heuser.)




stress fiber
cell cortex
filopodium
contractile bundle
gel-like network
tight parallel bundle
100 nm

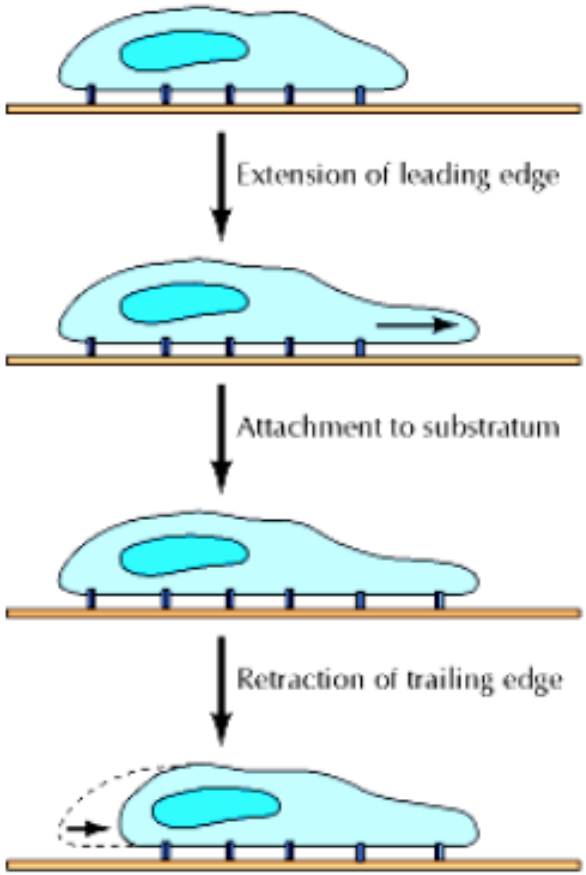


Figure 11.30. Cell crawling The crawling movements of cells across a surface can be viewed as three stages of coordinated movements: (1) extension of the leading edge, (2) attachment of the leading edge to the substratum, and (3) retraction of the rear of the cell into the cell body.

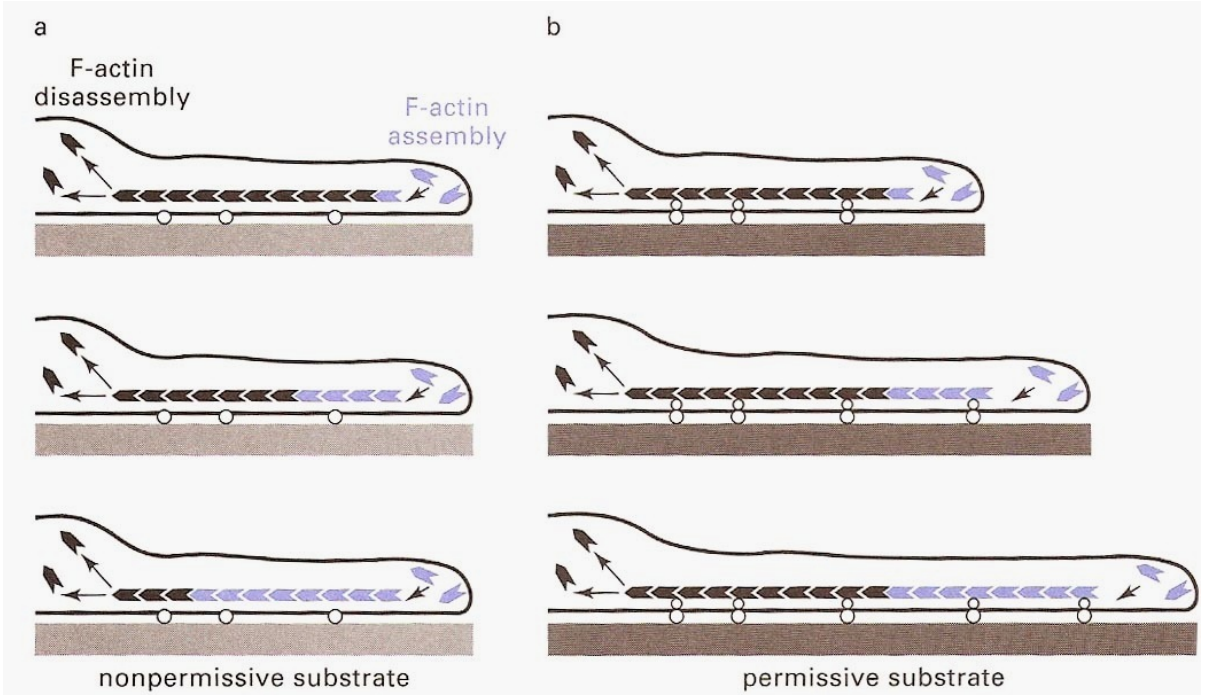
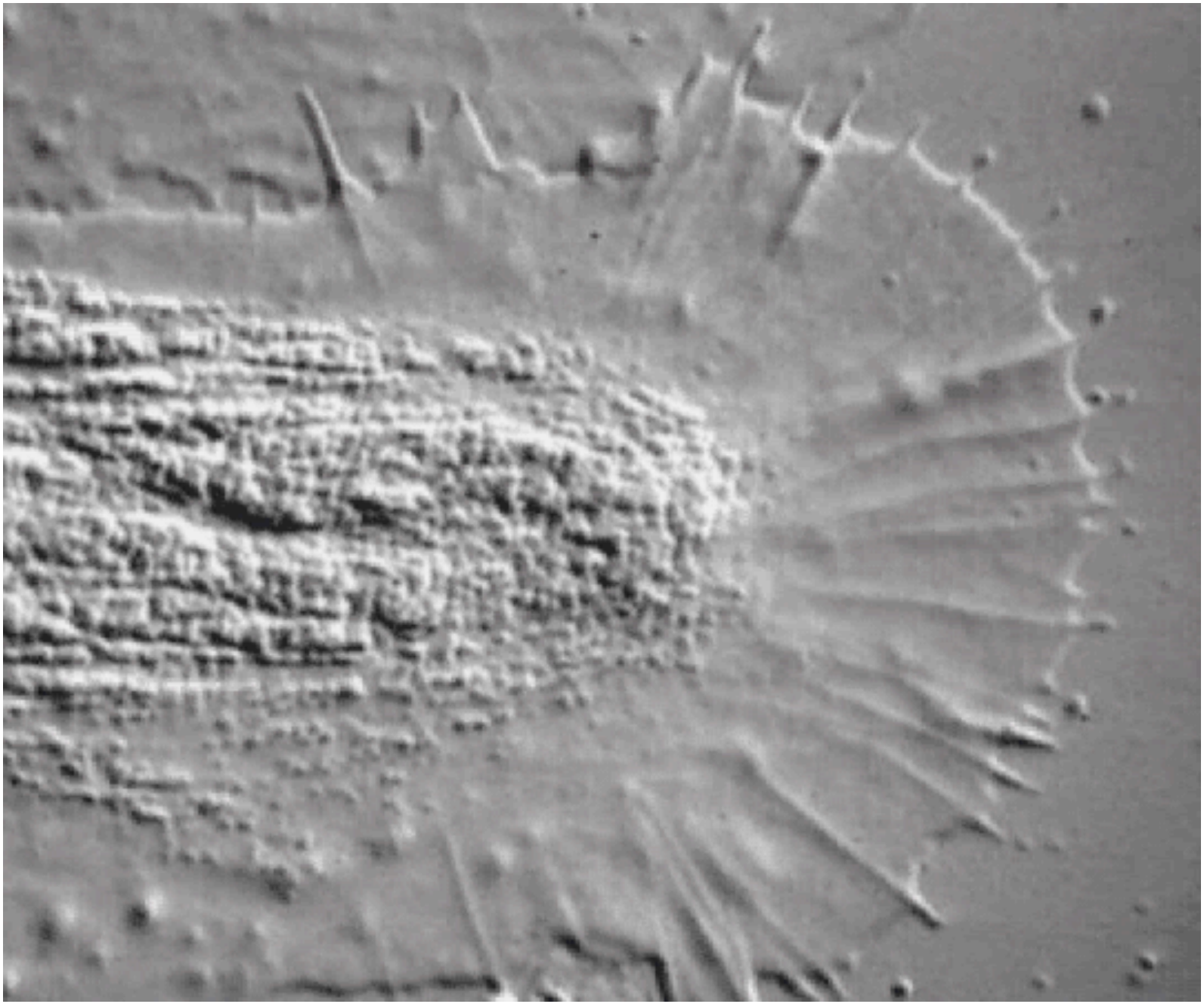
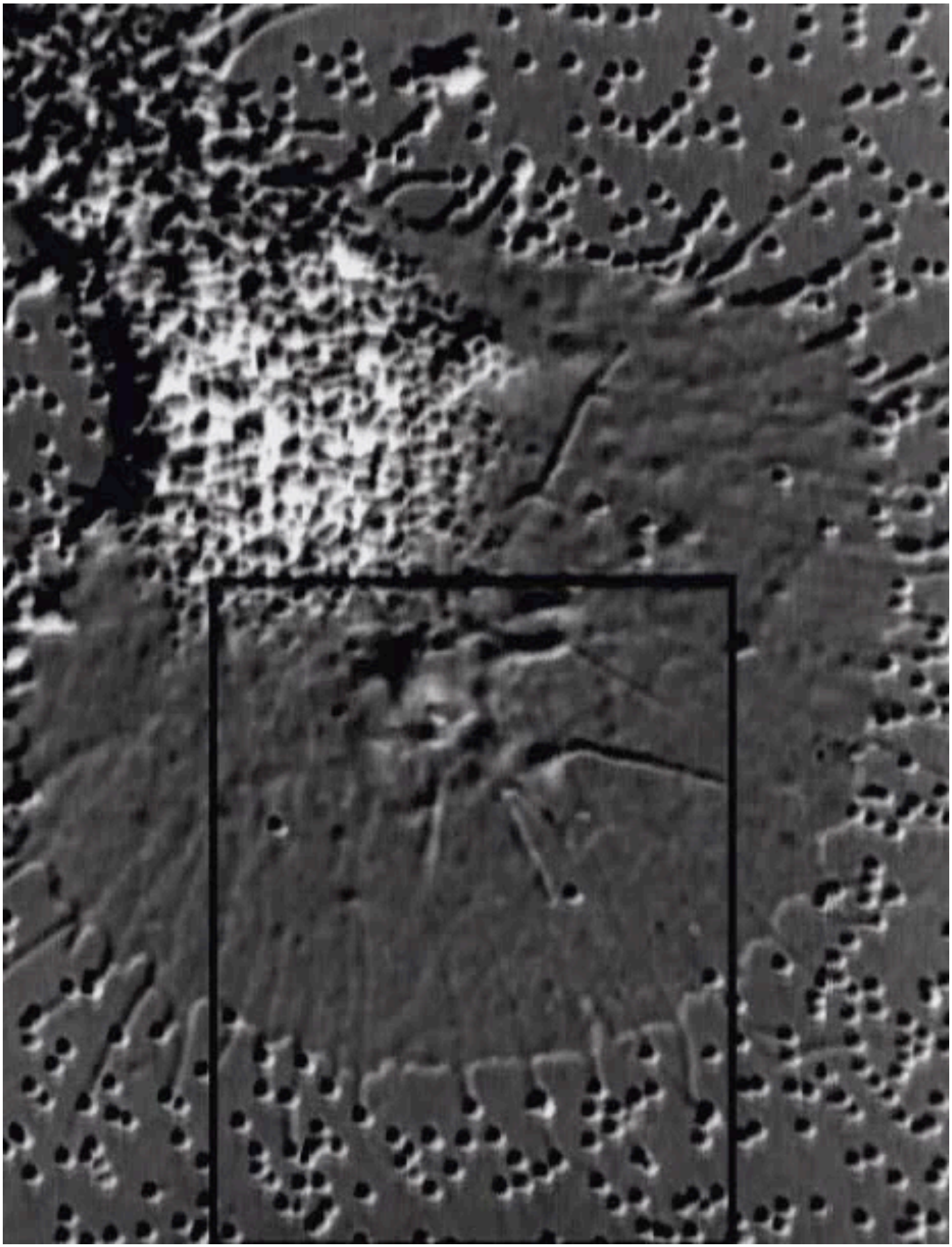
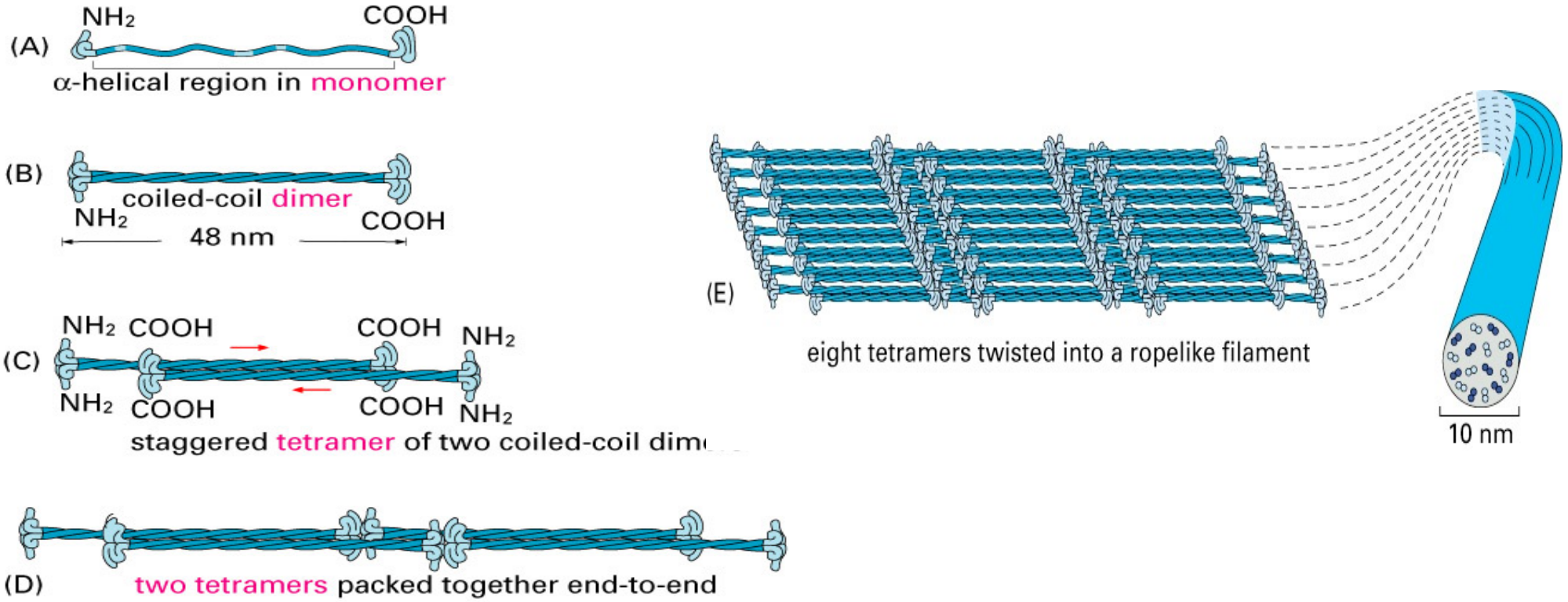


Figure 17–4. Actin assembly in the lamellipodium. *a*: Experiments by Paul Forscher and colleagues suggest that on a surface that is nonpermissive for axon elongation, F-actin filaments are not closely linked to the surface, leading to persistent retrograde flow of newly assembled F-actin. *b*: When 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

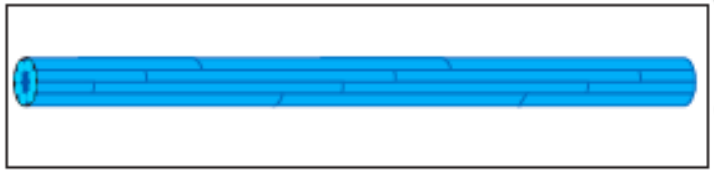


- NO conservados: no se encuentran en todos los eucariontes
- heterogéneos: específicos de acuerdo al tejido

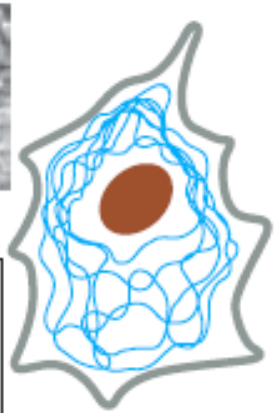
- NO se requiere energía para su ensamblaje, auto asociación lateral
- Filamentos NO polarizados

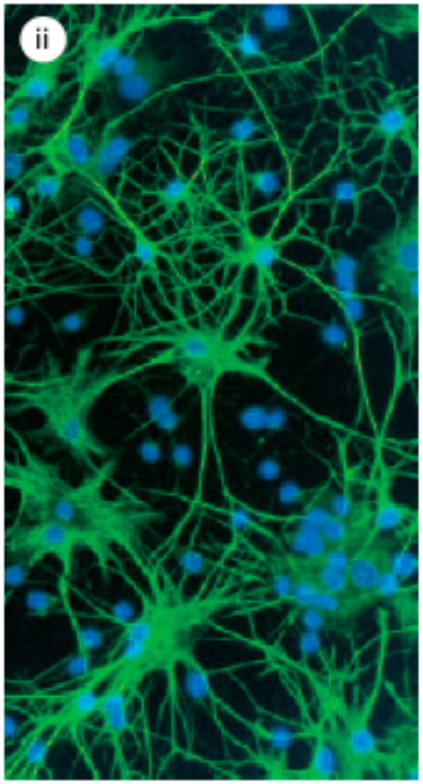
INTERMEDIATE FILAMENTS

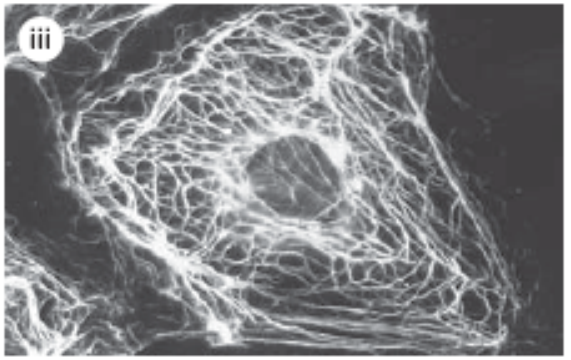
100 nm

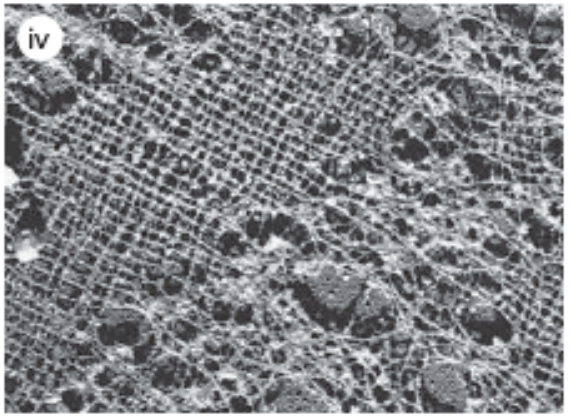


25 nm





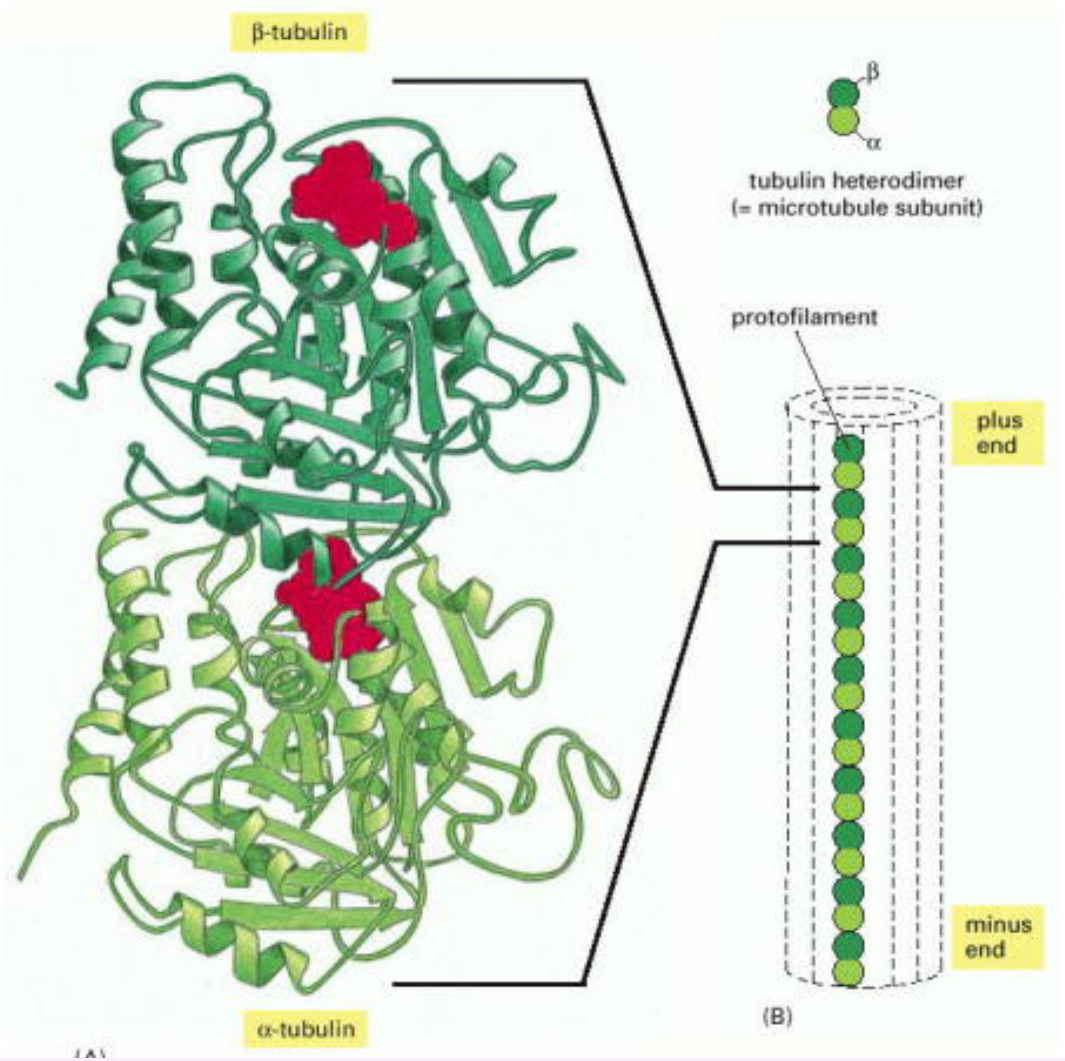


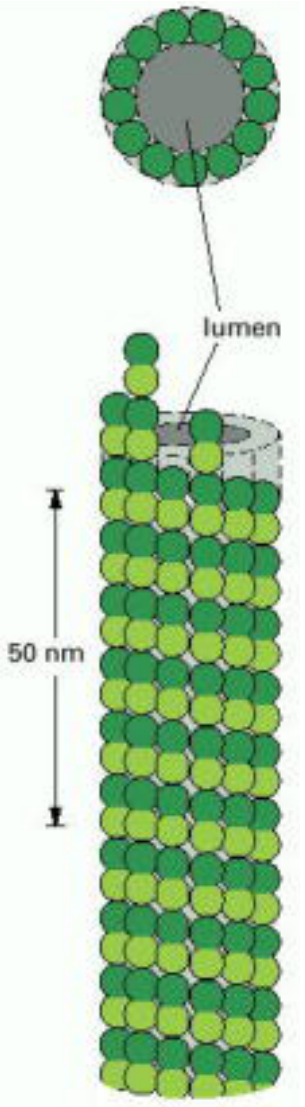


Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types 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).

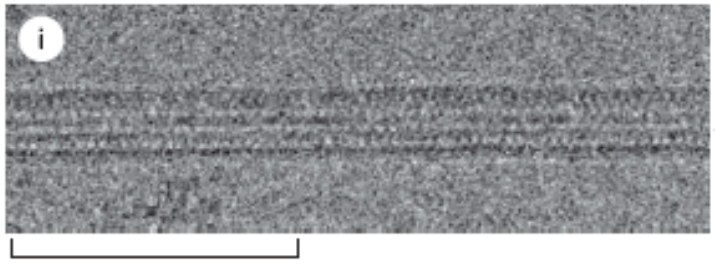
Microtubulos

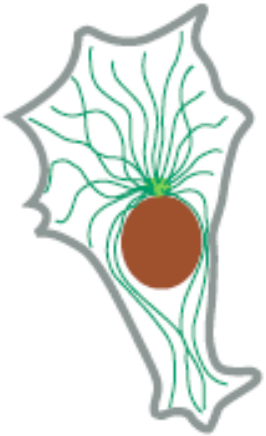


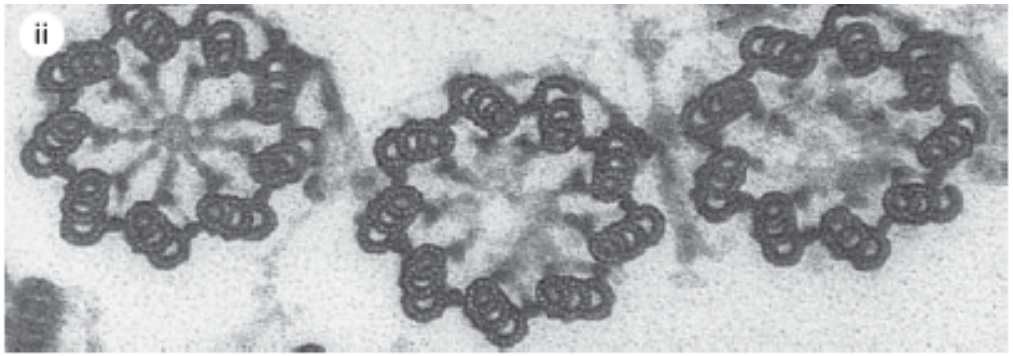


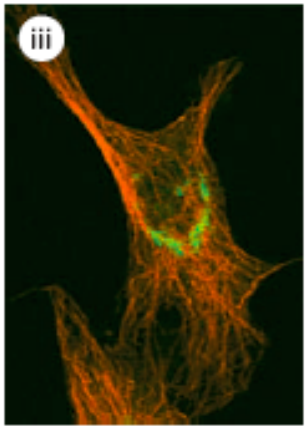
- conservados (Existe un homólogo en bacterias: FtsZ)
- heterodímeros (α-tubulina y β-tubulina unidas por un GTP), forman 13 protofilamentos polarizados
- Solo se hidroliza el GTP unido a α-tubulina (requiere energía)

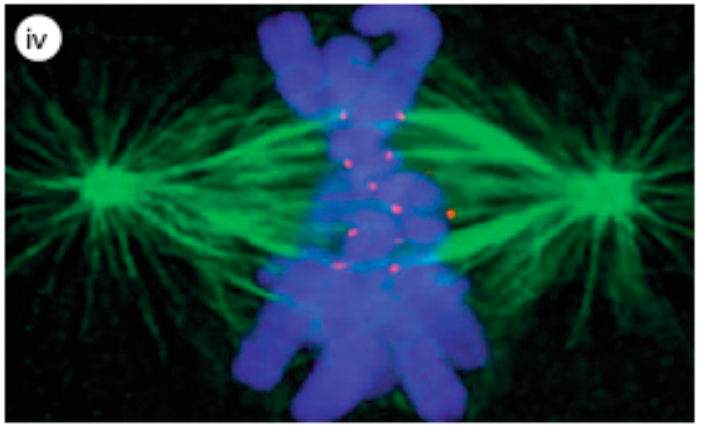
MICROTUBULES





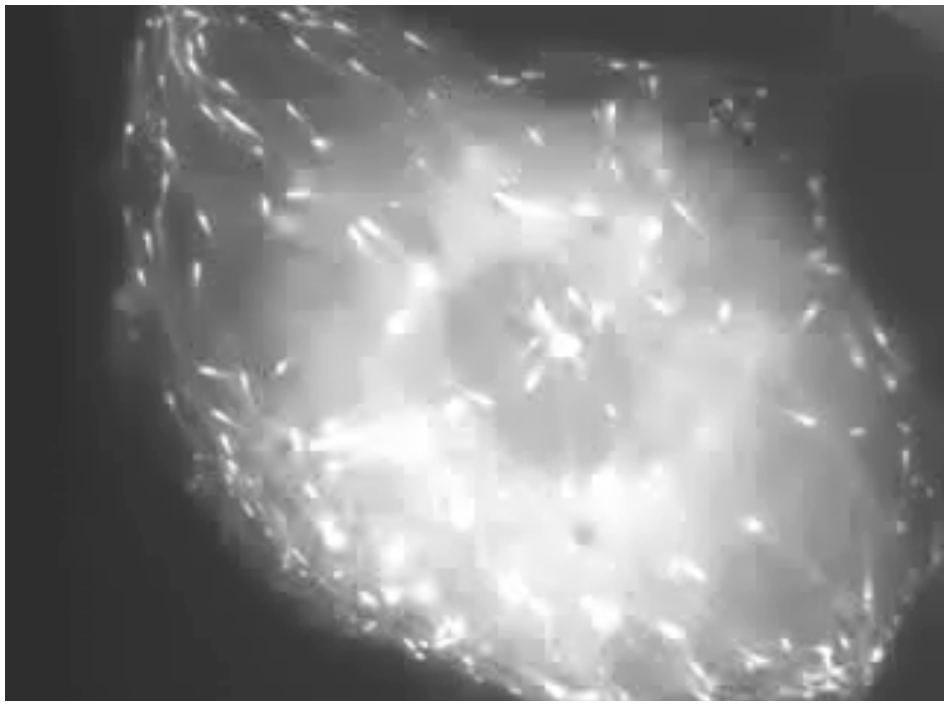






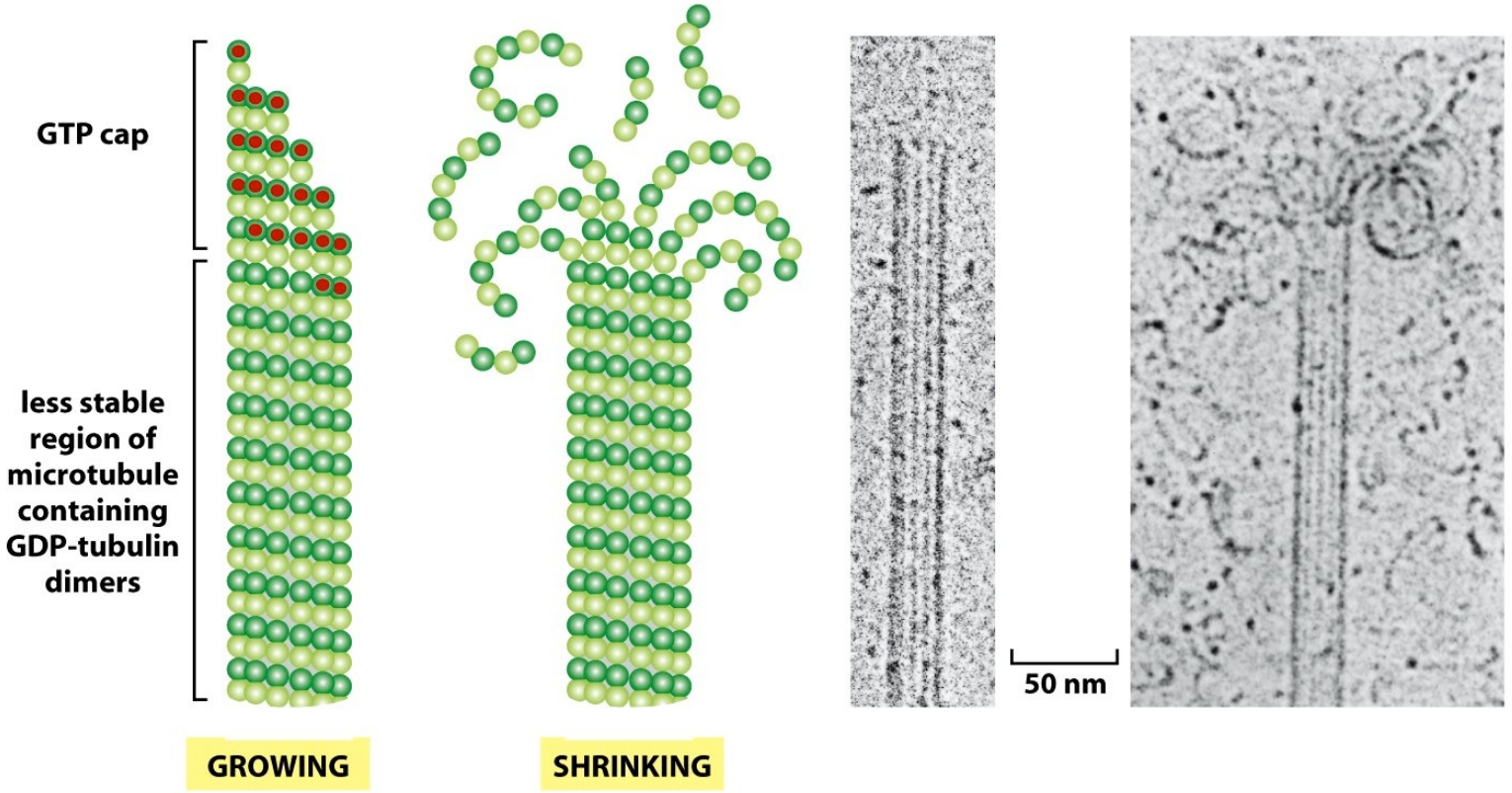
Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*.

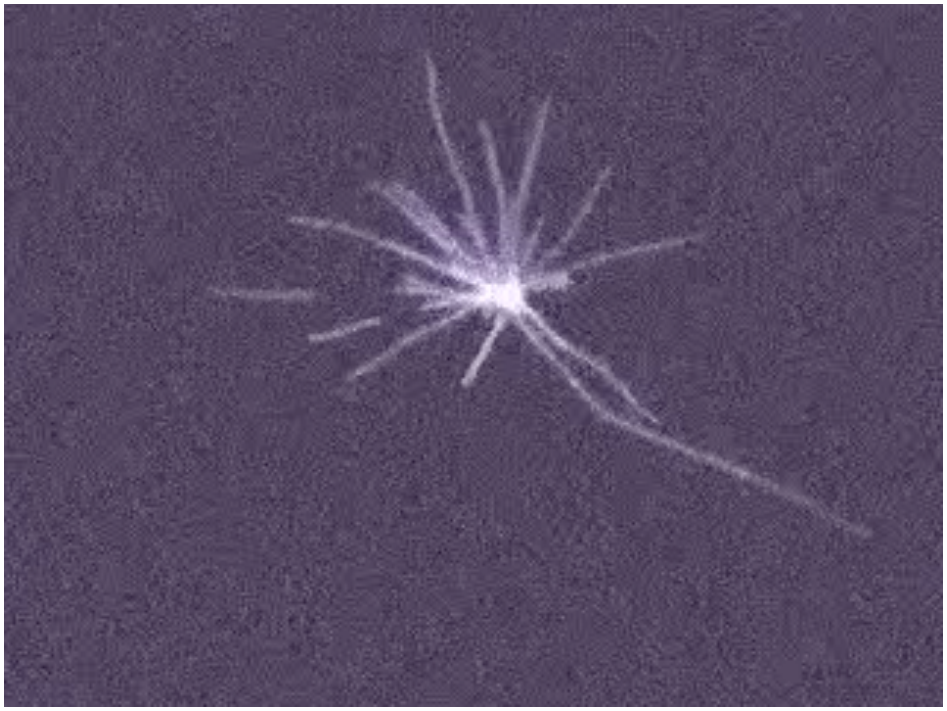
Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (iii); A. Desai (iv).



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

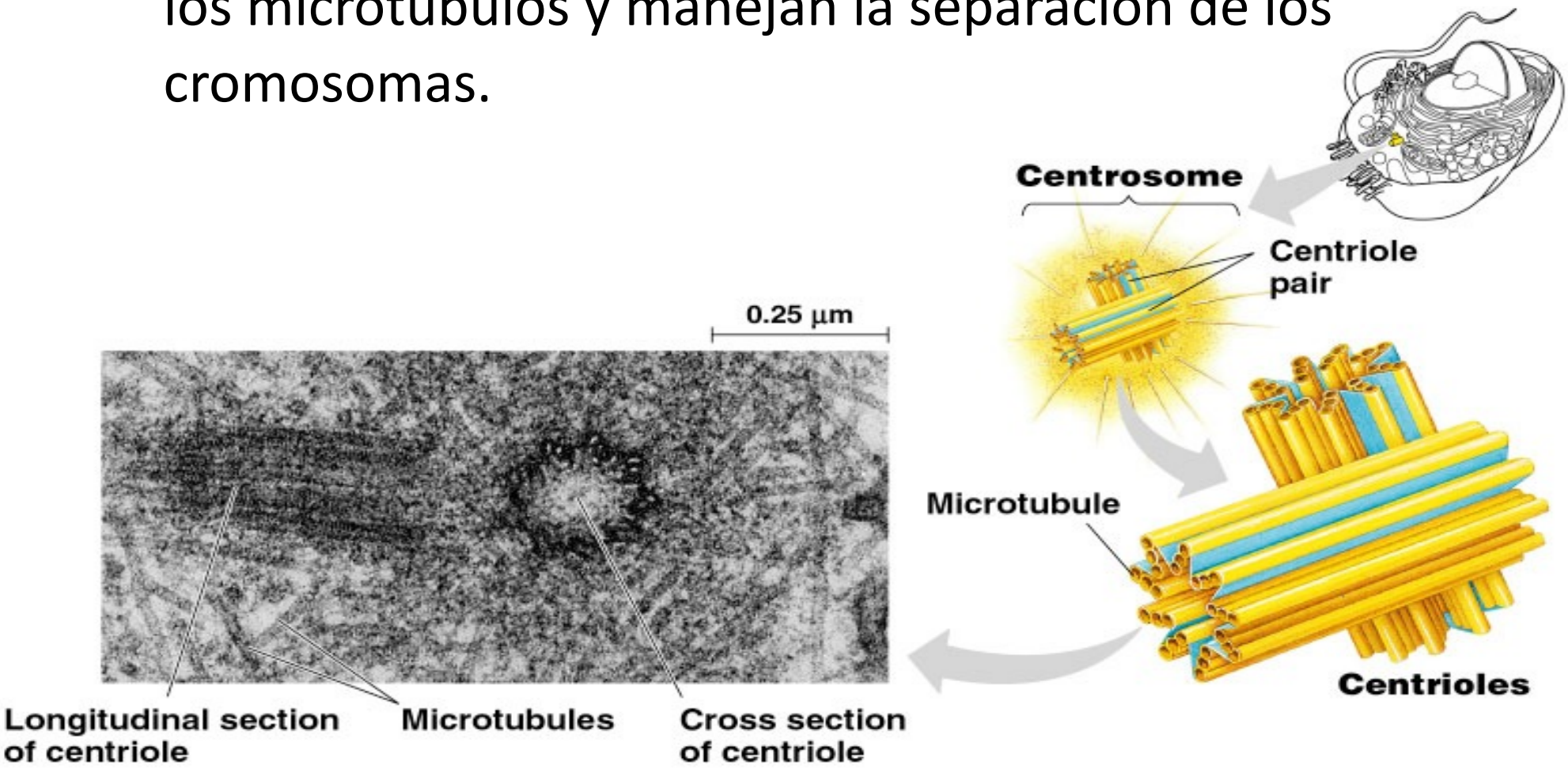
Comparar con tubulina-EGFP


GTP cap
less stable region of microtubule containing GDP-tubulin dimers
50 nm
GROWING
SHRINKING



Centriolos

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



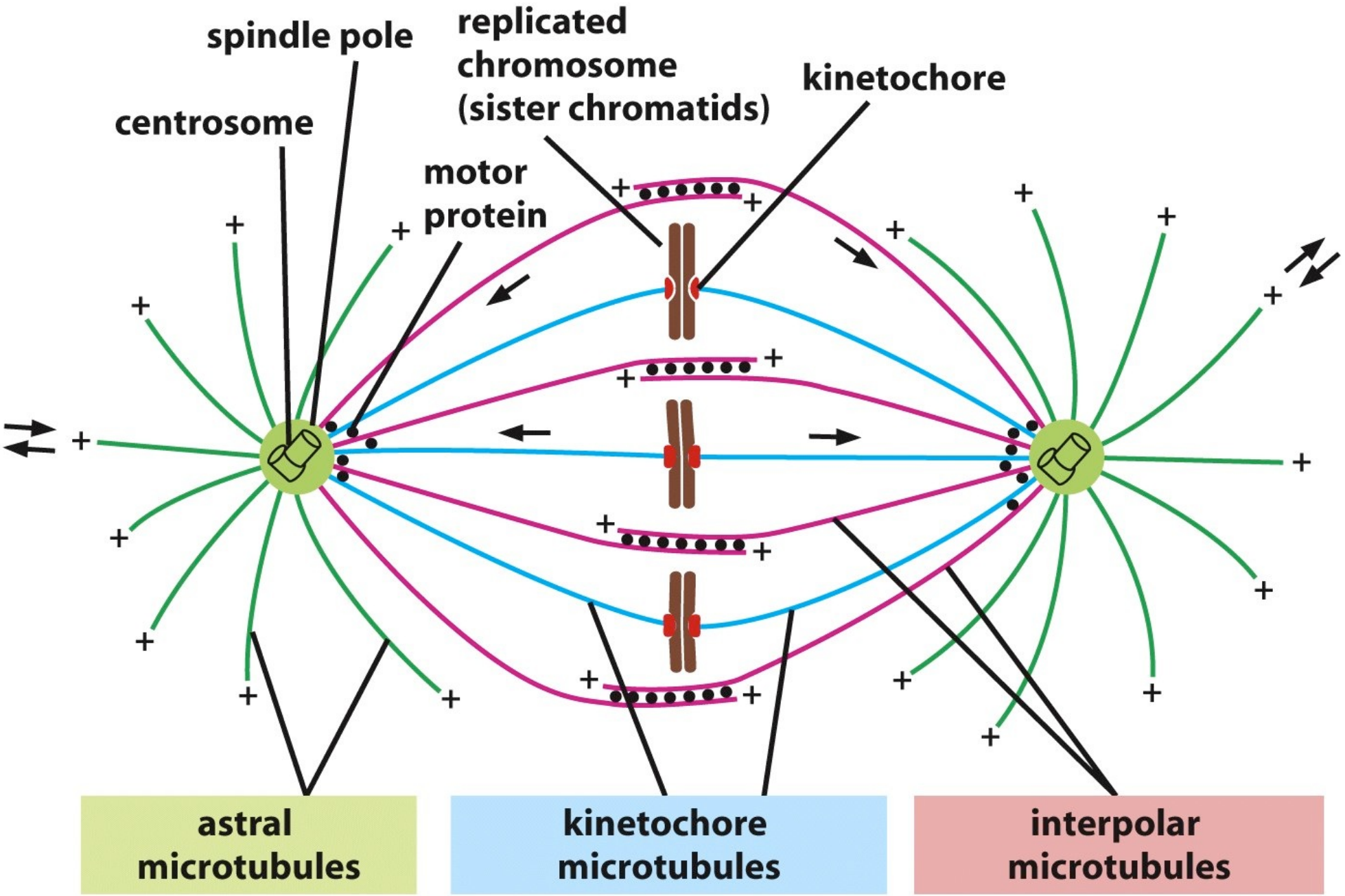
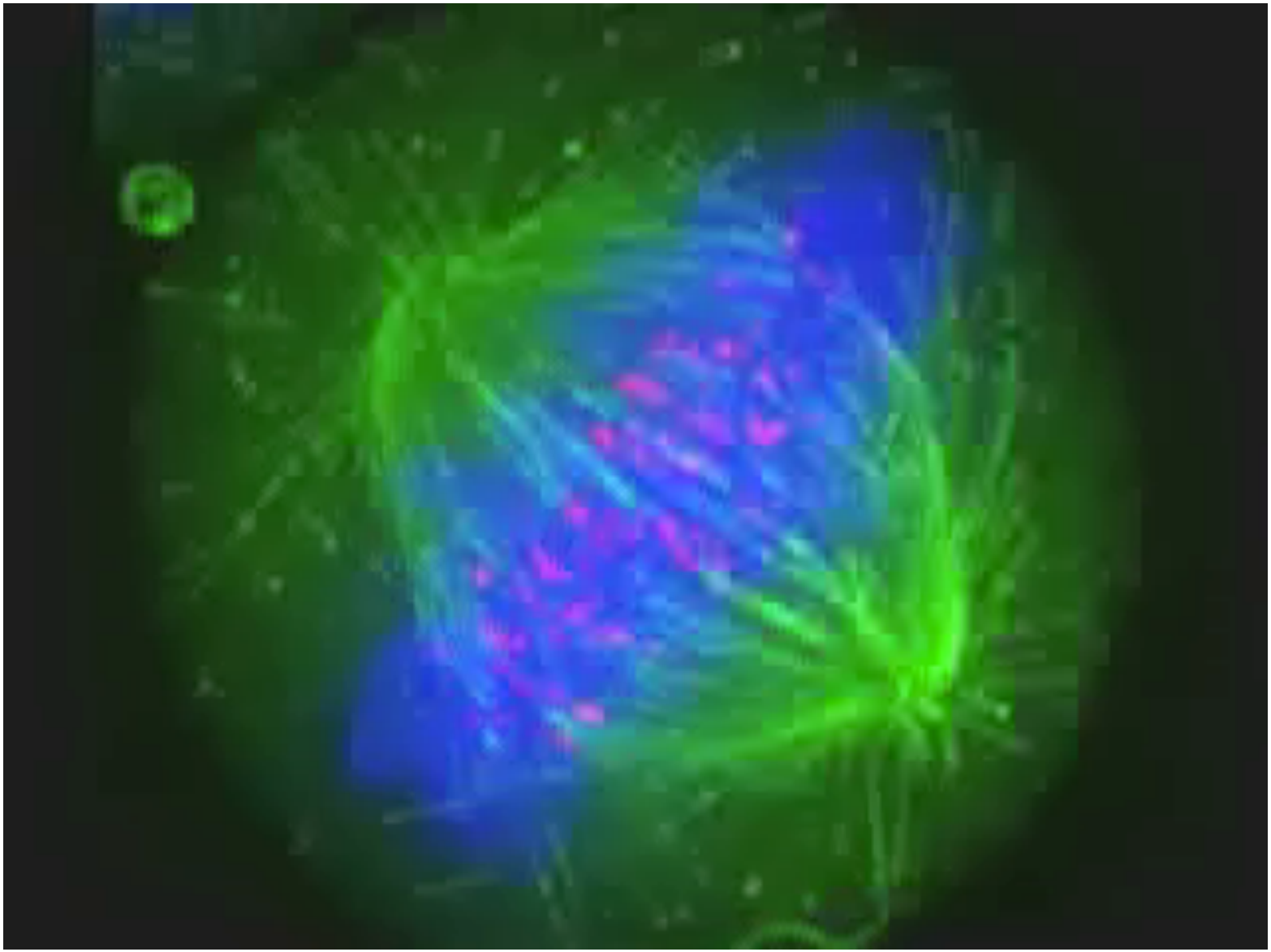


Figure 16-85a Molecular Biology of the Cell 5/e (© Garland Science 2008)



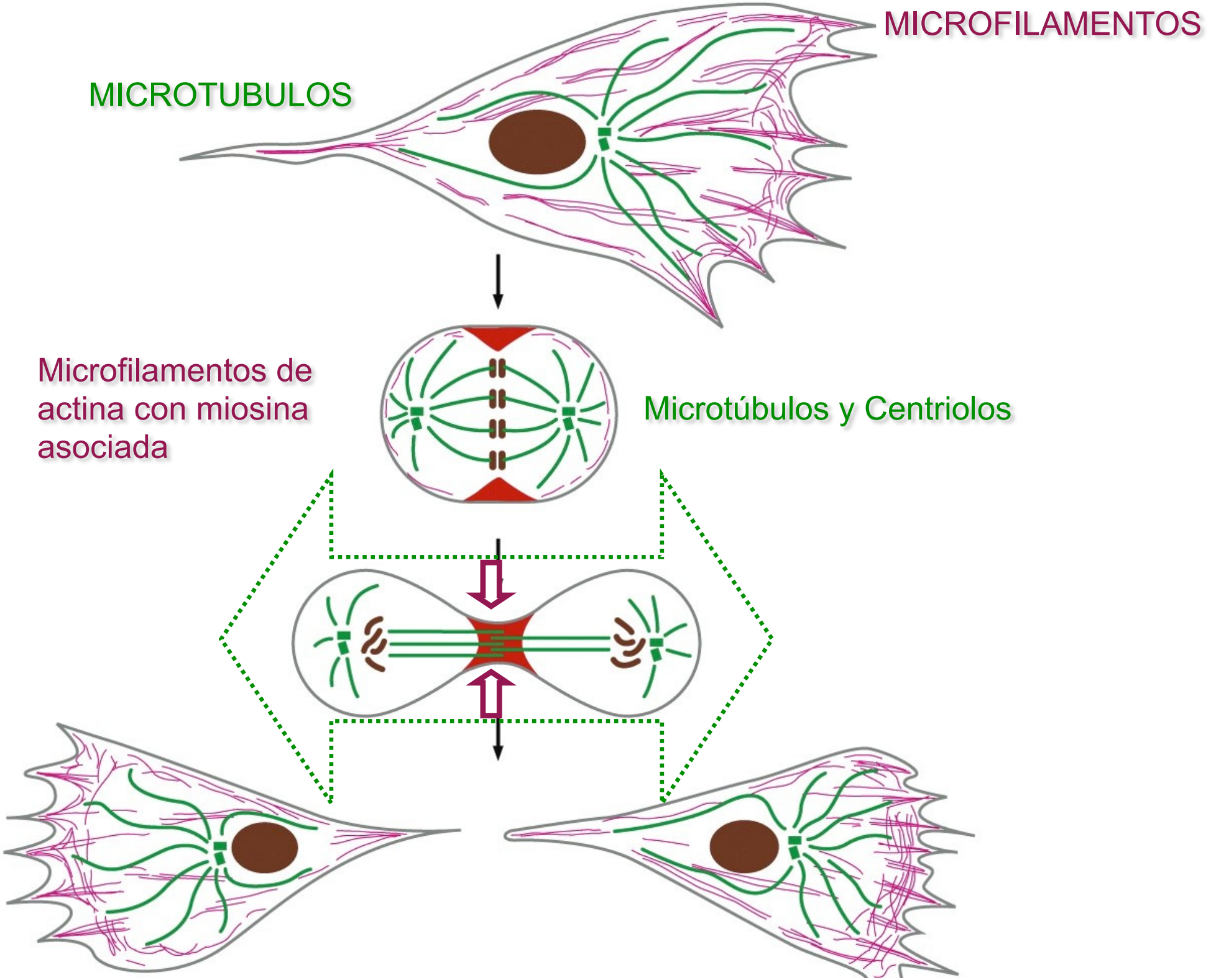
Drogas que afectan los filamentos de actina y/o los microtúbulos

ACTINA-ESPECIFICAS

- Faloidina se une a y estabiliza los filamentos
- Citocalasina forma un CAP en los extremos positivos
- Swinholidina 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)


MICROFILAMENTOS
MICROTUBULOS
Microfilamentos de actina con miosina asociada
Microtúbulos y Centriolos

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.

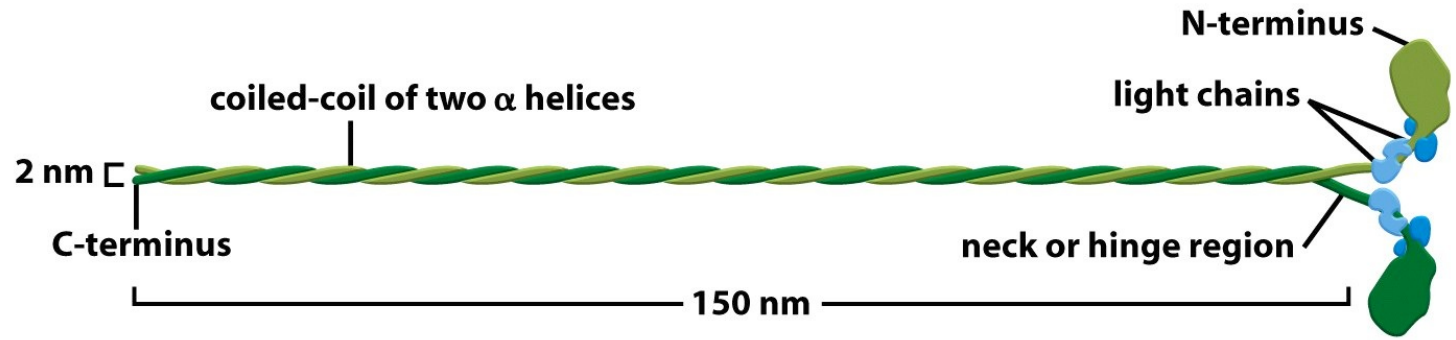


Figure 16-54a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Sobre microfilamentos: Miosinas

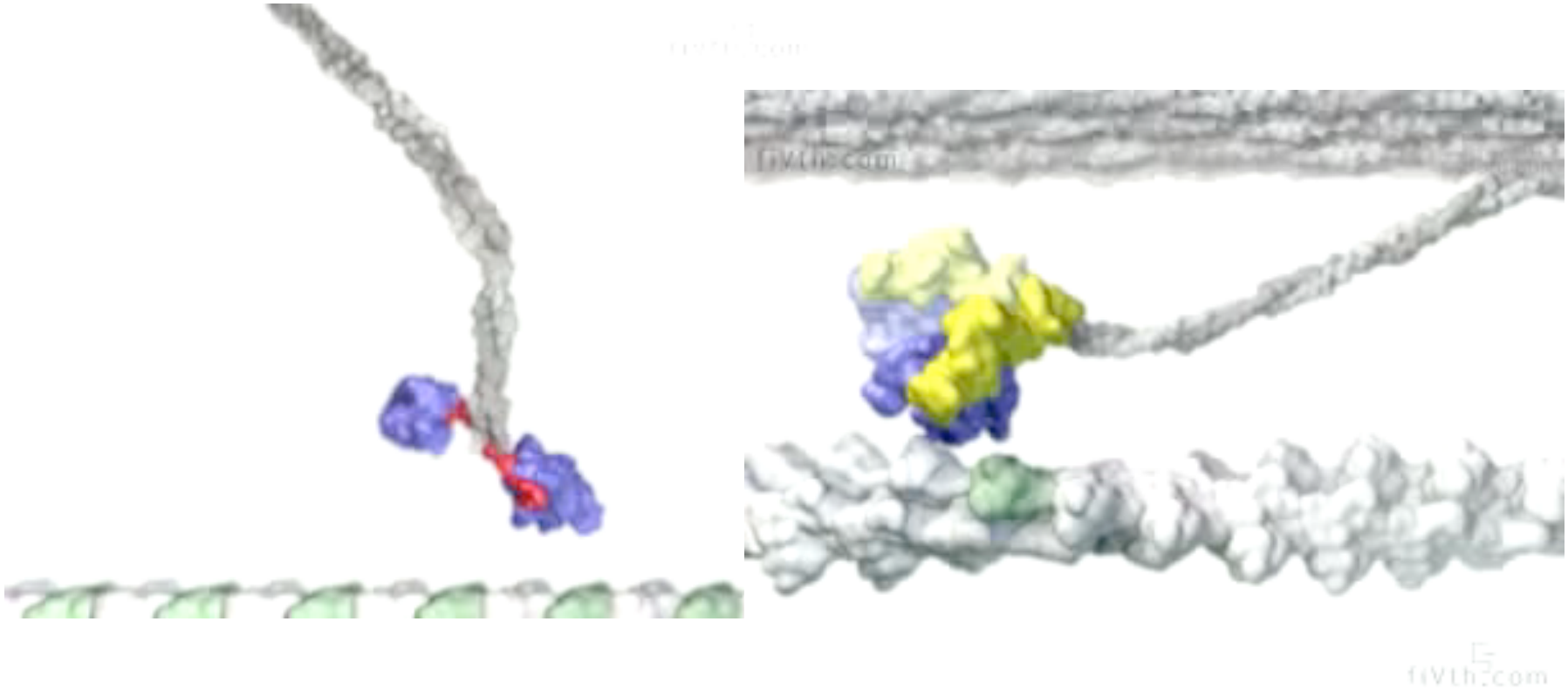
Sobre Microtúbulos:

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

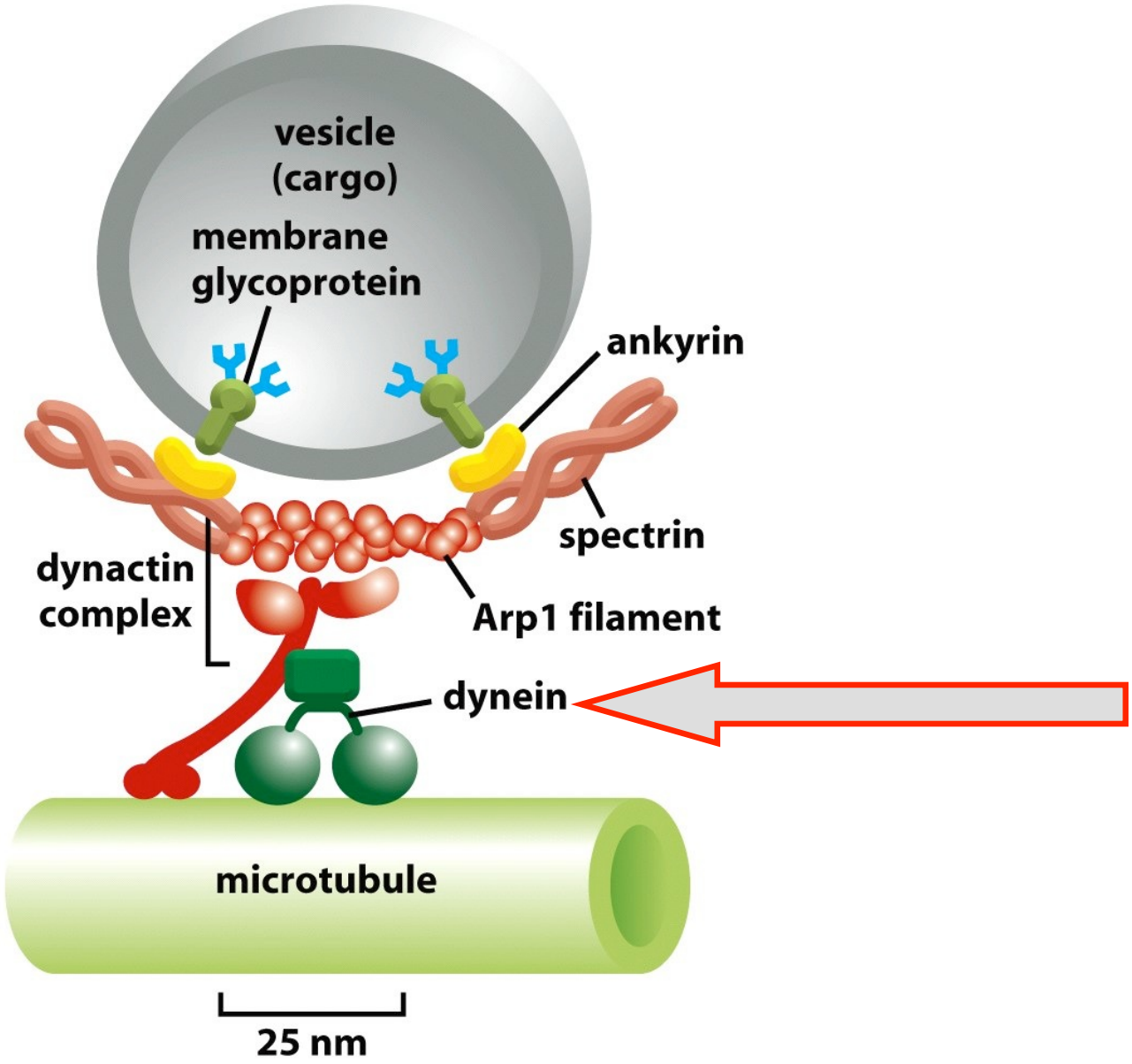
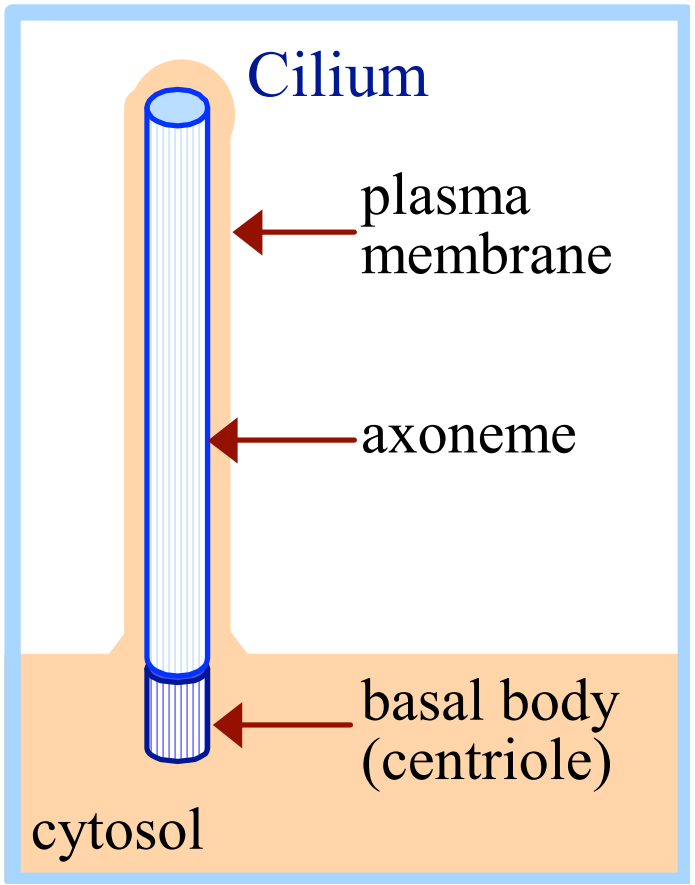


Figure 16-67 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Cilios & flagelos

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



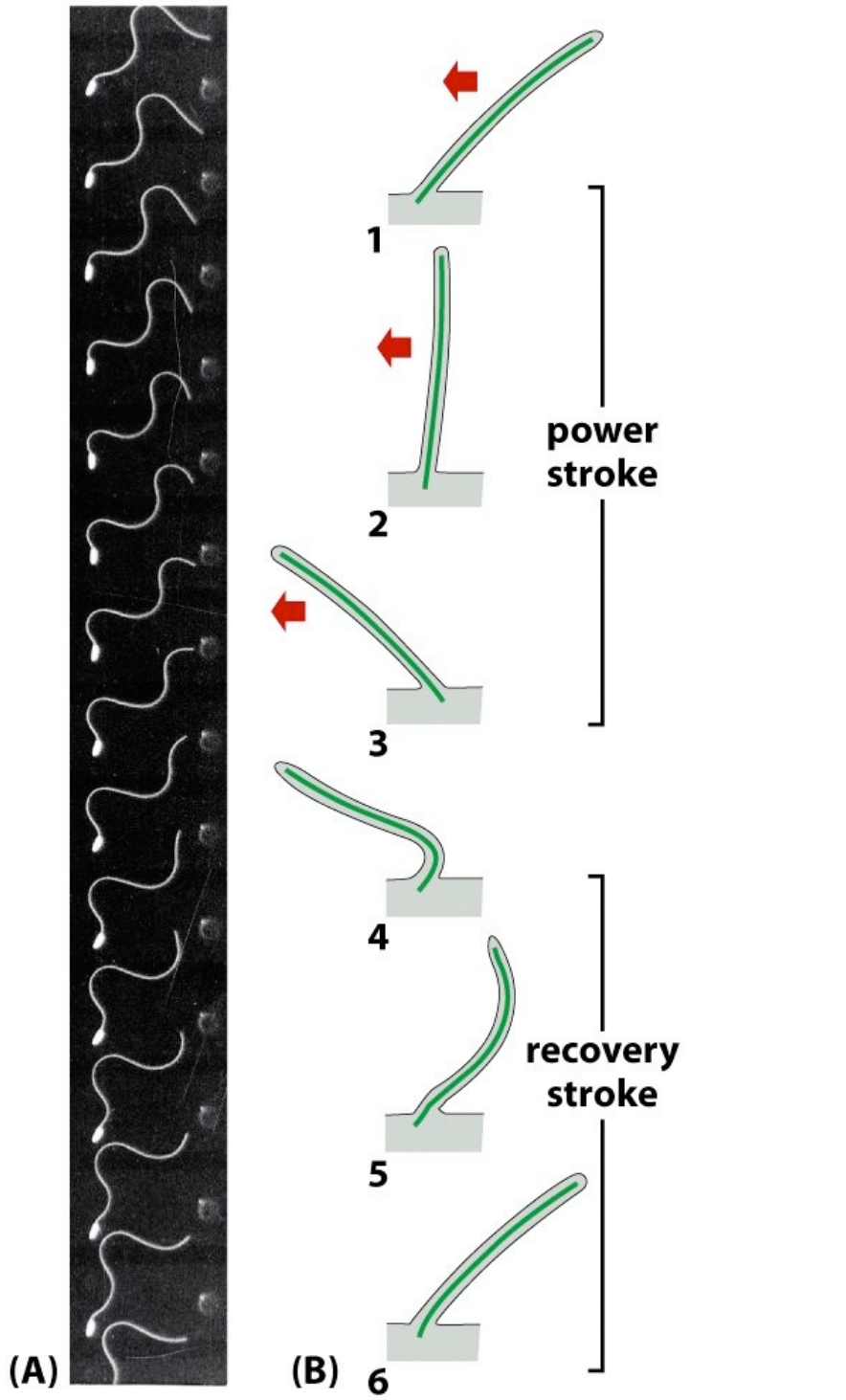
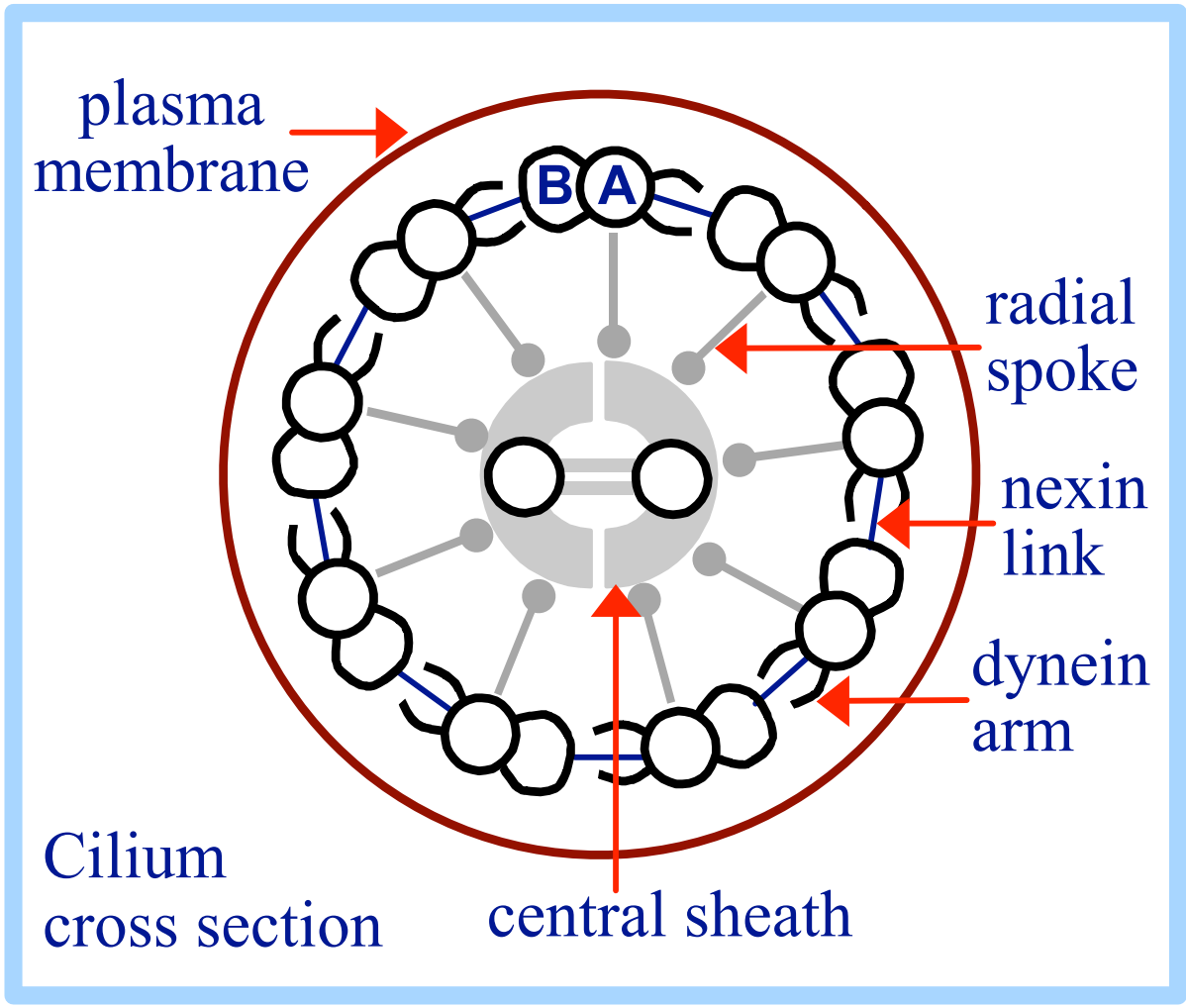


Figure 16-80 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Un **axonema** incluye:

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



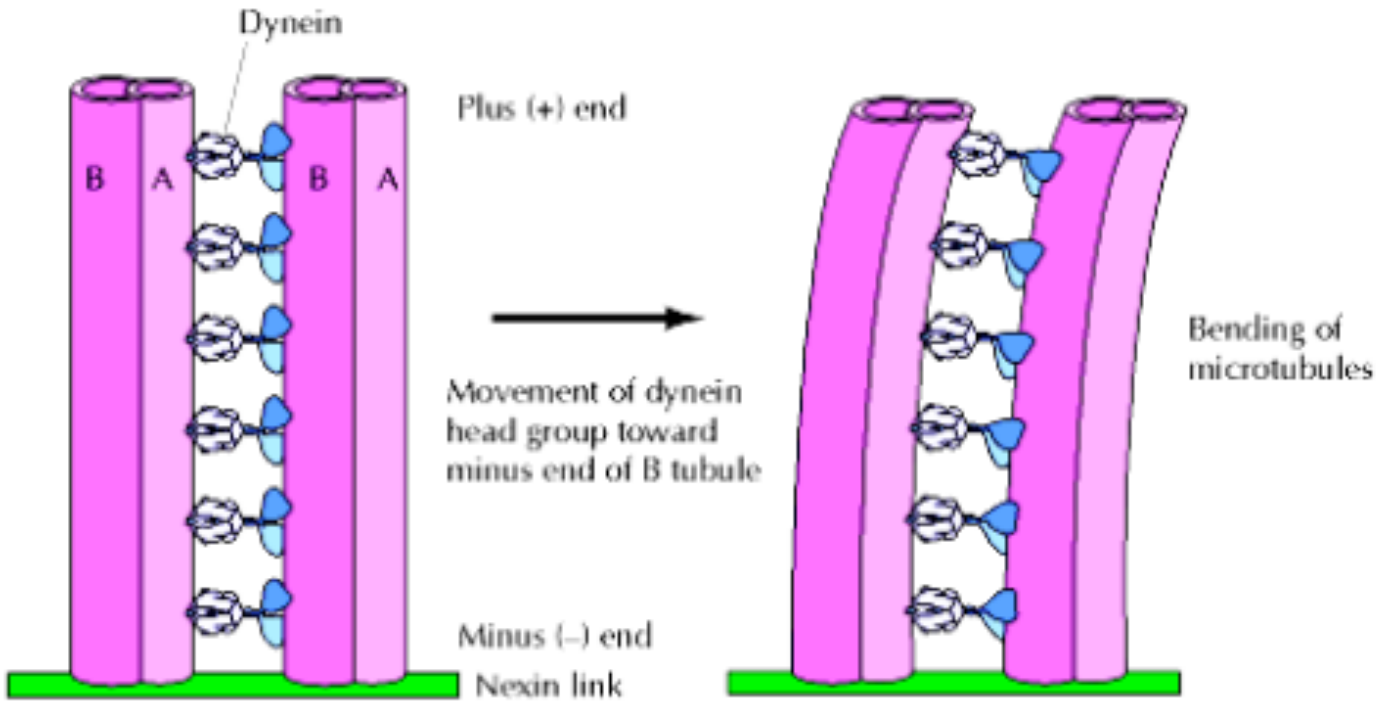
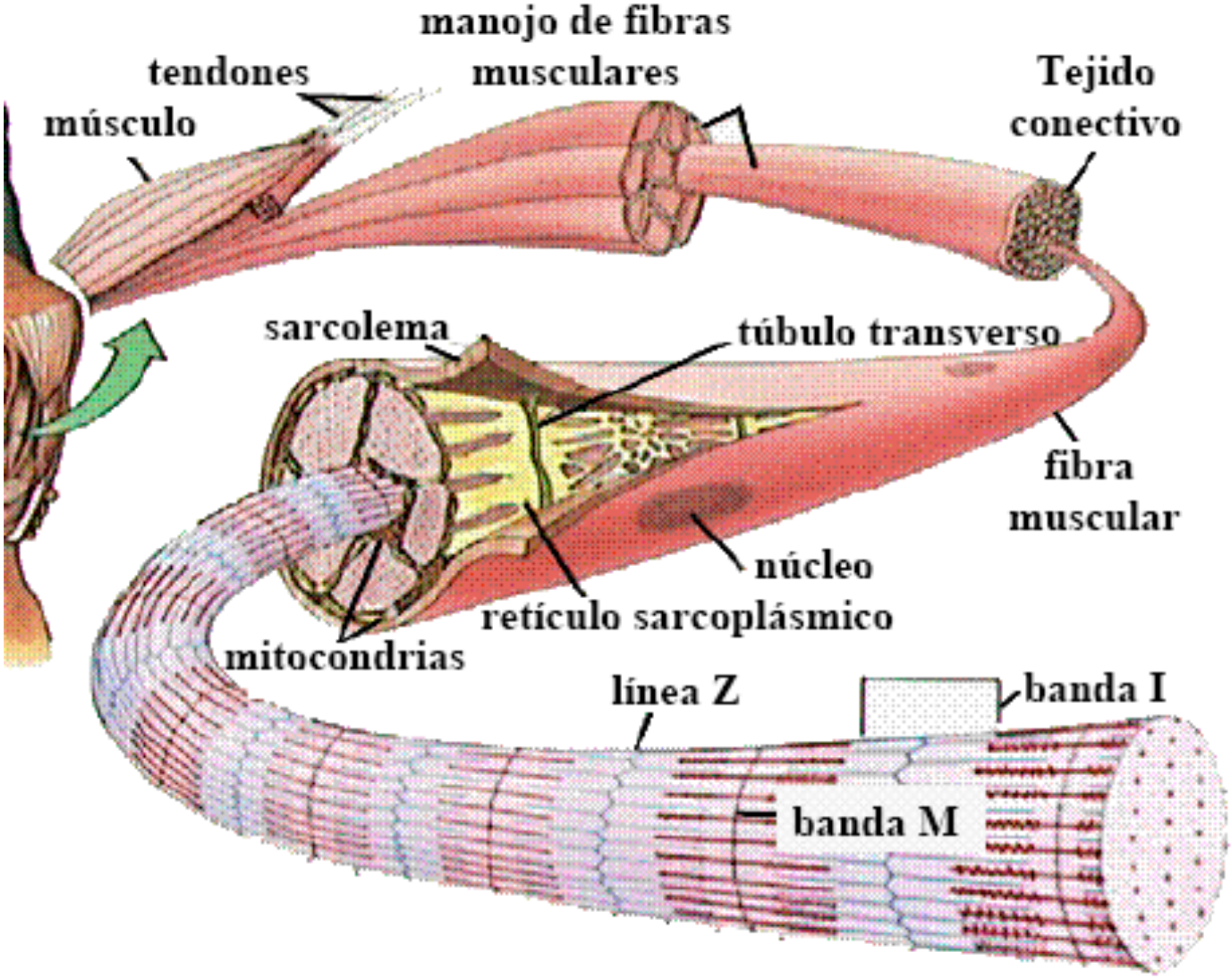
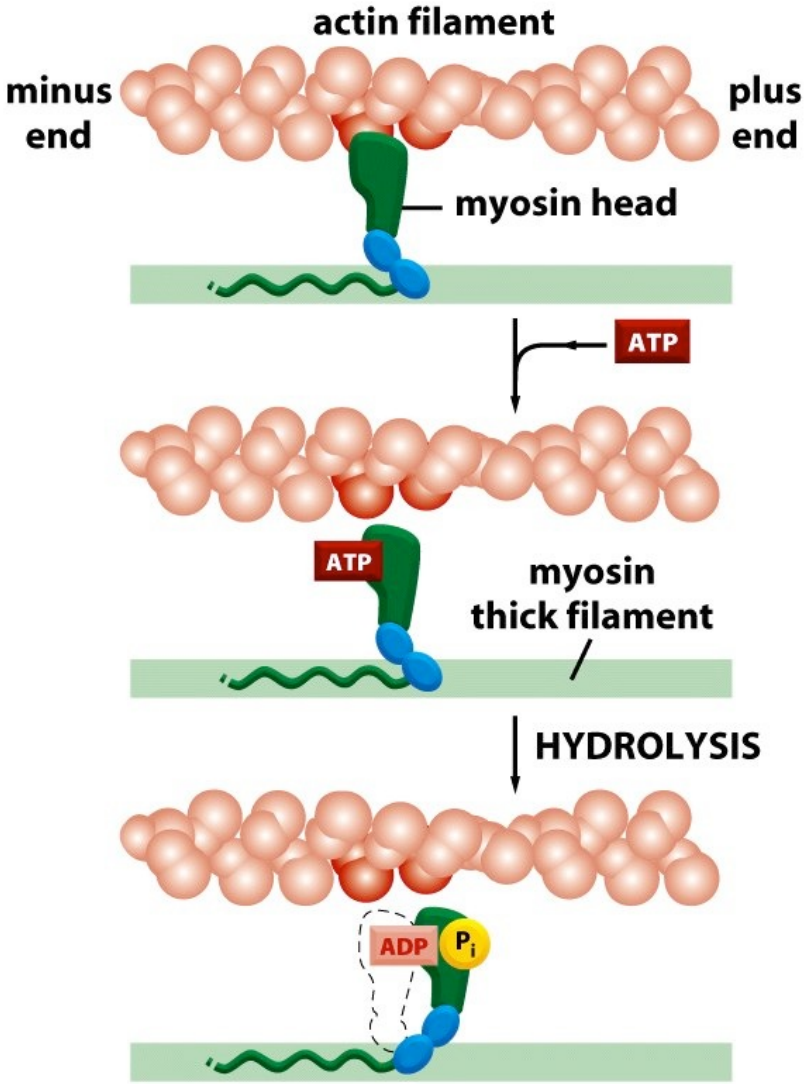


Figure 11.53. Movement of microtubules in cilia and flagella The bases of dynein arms are attached to A tubules, and the motor head groups interact with the B tubules of adjacent doublets. Movement of the dynein head groups in the minus end direction (toward the base of the cilium) then causes the A 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



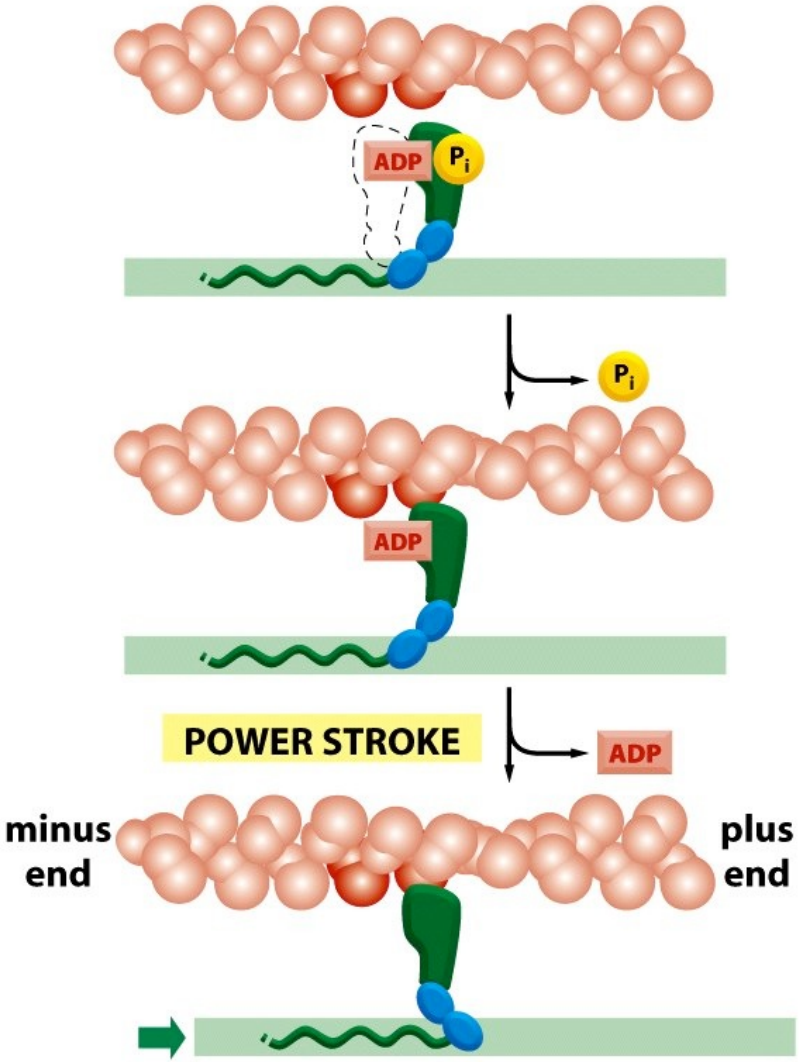
Contracción Muscular



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.)

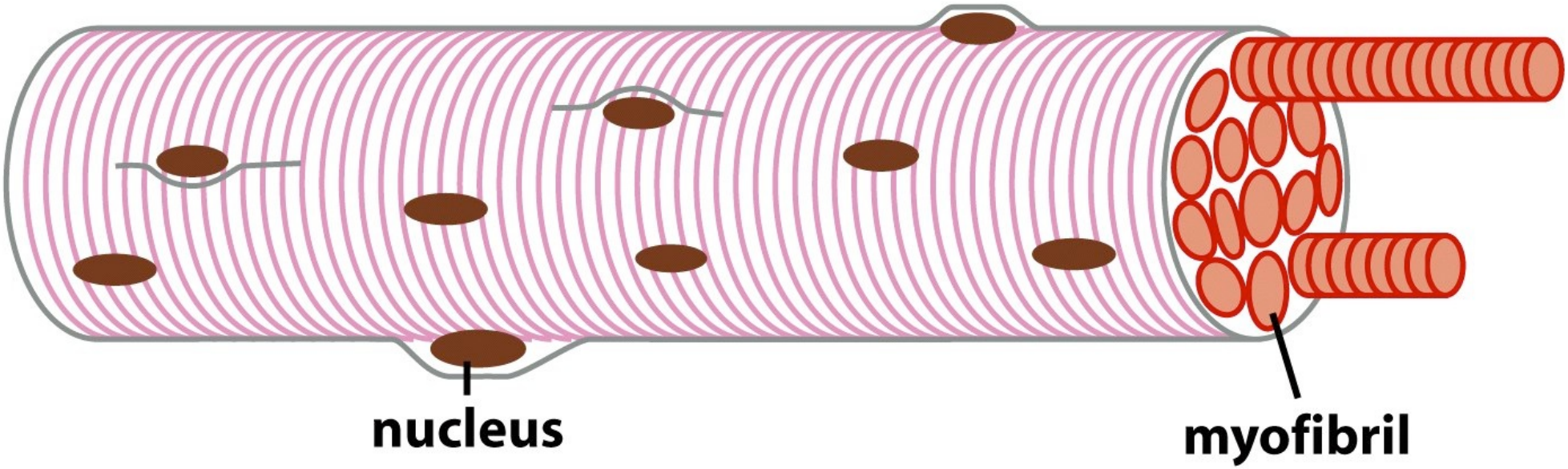
COCKED The cleft closes like a clam shell around the ATP molecule, triggering a large shape change that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (P_i) produced remain tightly bound to the protein.

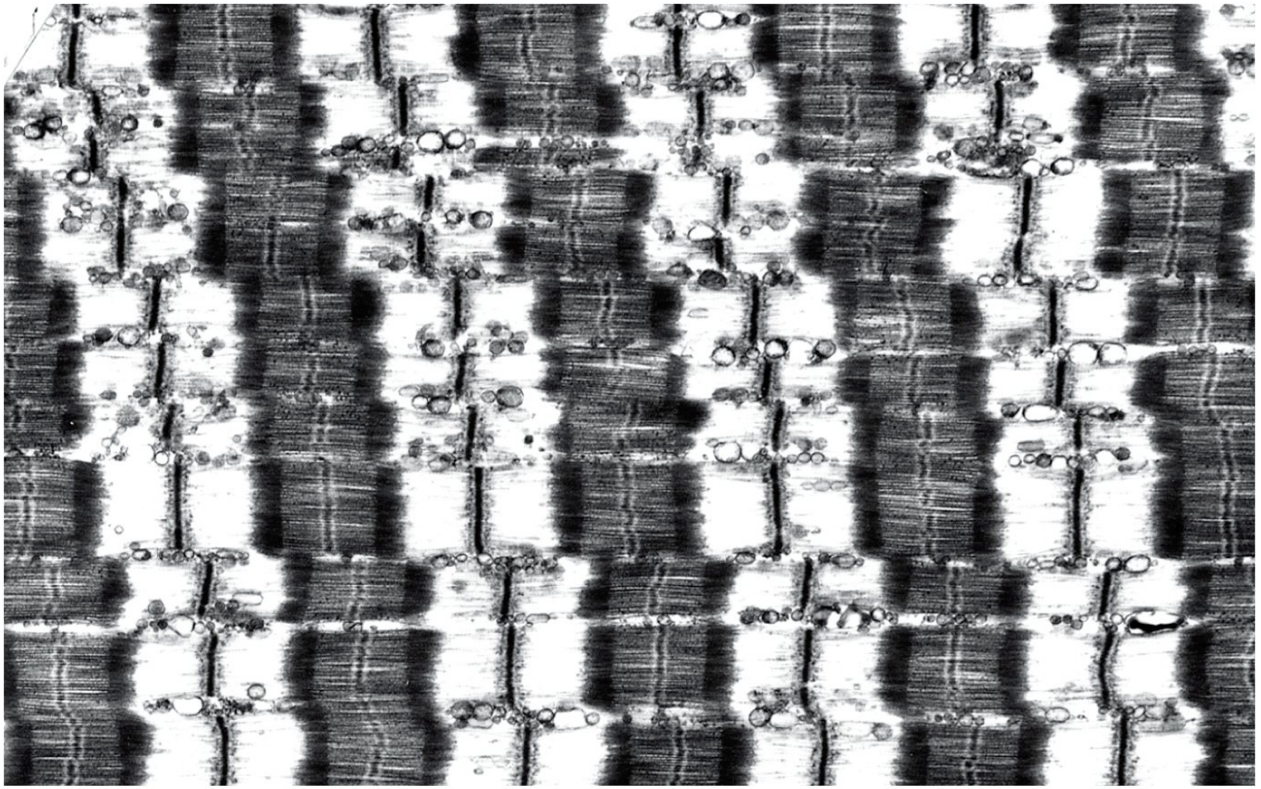


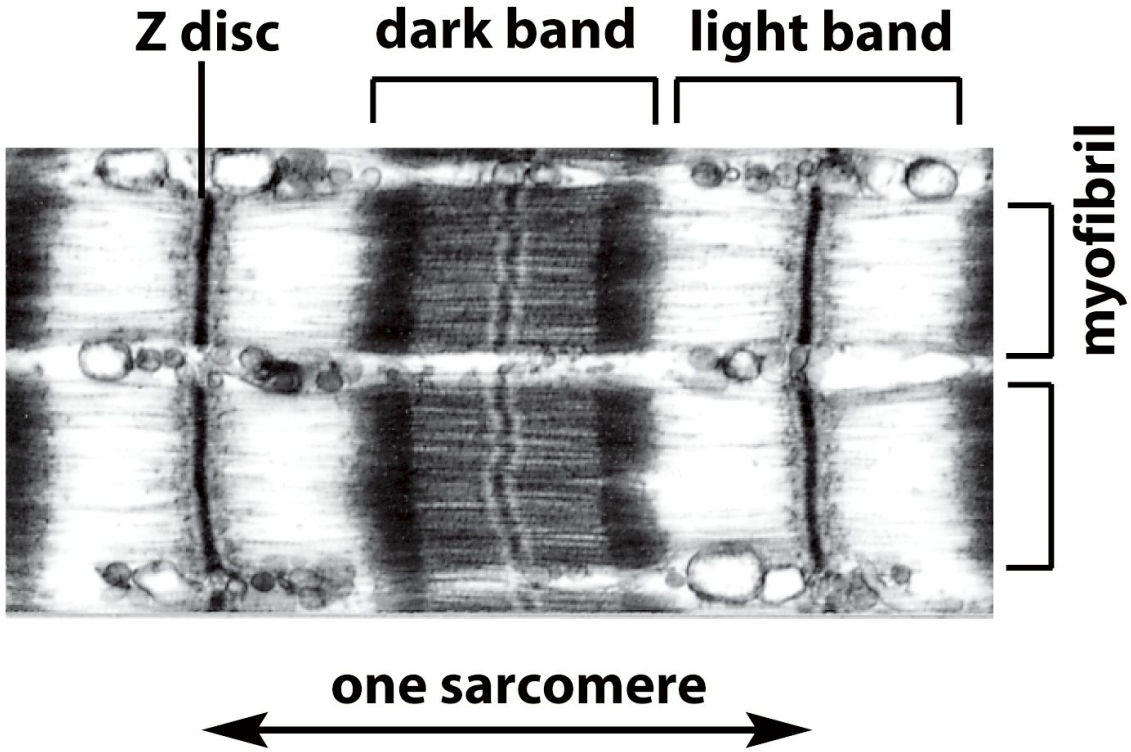
COCKED The cleft closes like a clam shell around the ATP molecule, triggering a large shape change that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (P_i) produced remain tightly bound to the protein.

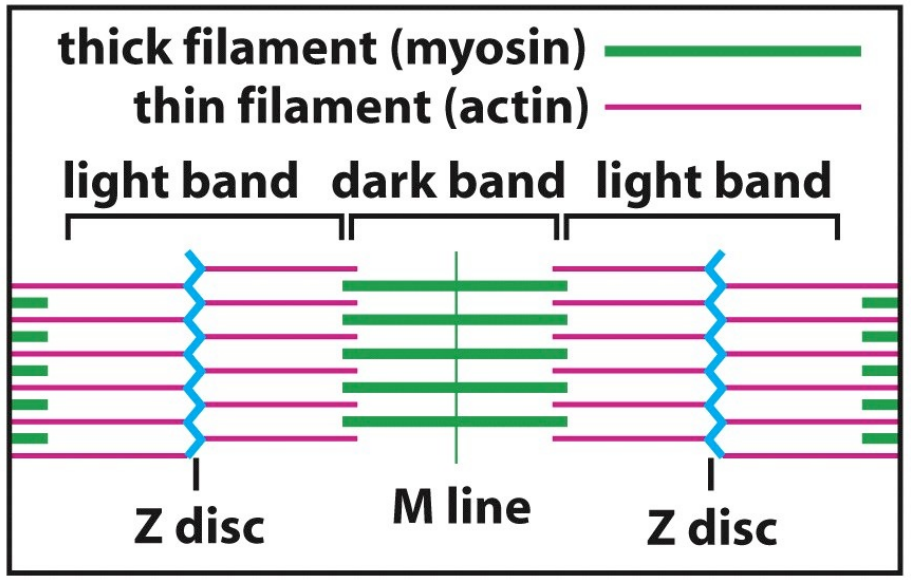
FORCE-GENERATING A weak binding of the myosin head to a new site on the actin filament causes release of the inorganic phosphate produced by ATP hydrolysis, concomitantly with the tight binding of the head to actin. This release triggers the power stroke—the force-generating change in shape during which 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.

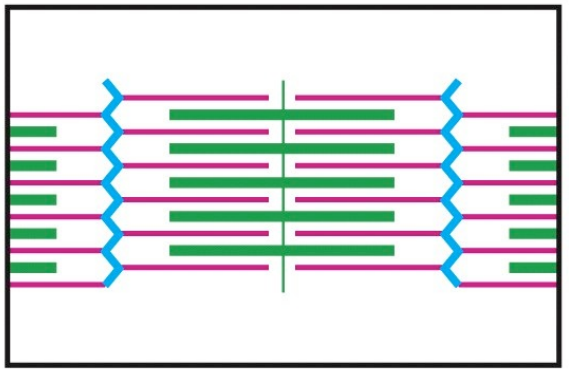

nucleus
myofibril


2 μm

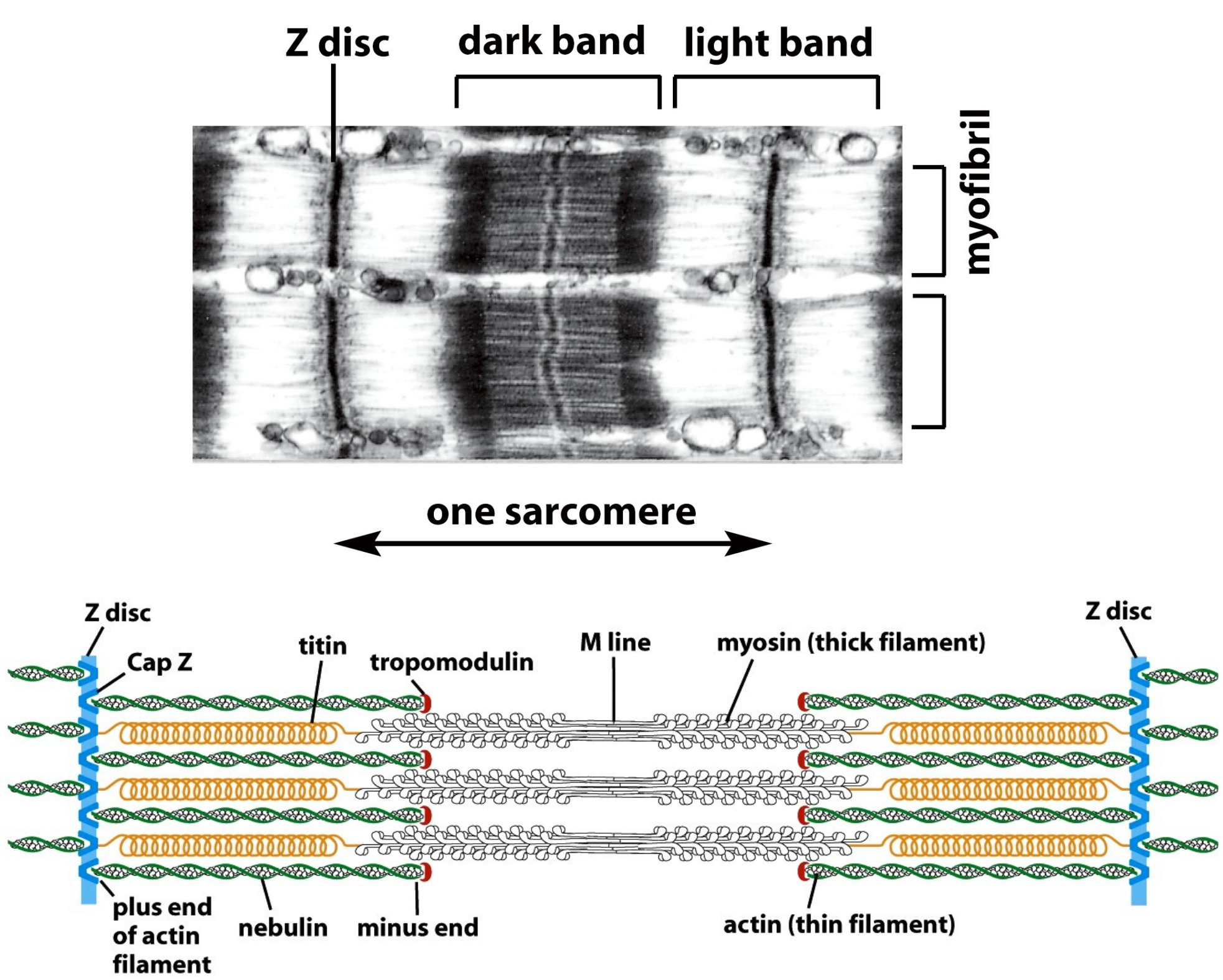

Z disc
dark band
light band
myofibril
one sarcomere


thick filament (myosin)
thin filament (actin)
light band
dark band
light band
Z disc
M line
Z disc

(C)



(D)


Z disc
dark band
light band
myofibril
one sarcomere
Z disc
Cap Z
titin
tropomodulin
M line
myosin (thick filament)
Z disc
plus end of actin filament
nebulin
minus end
actin (thin filament)

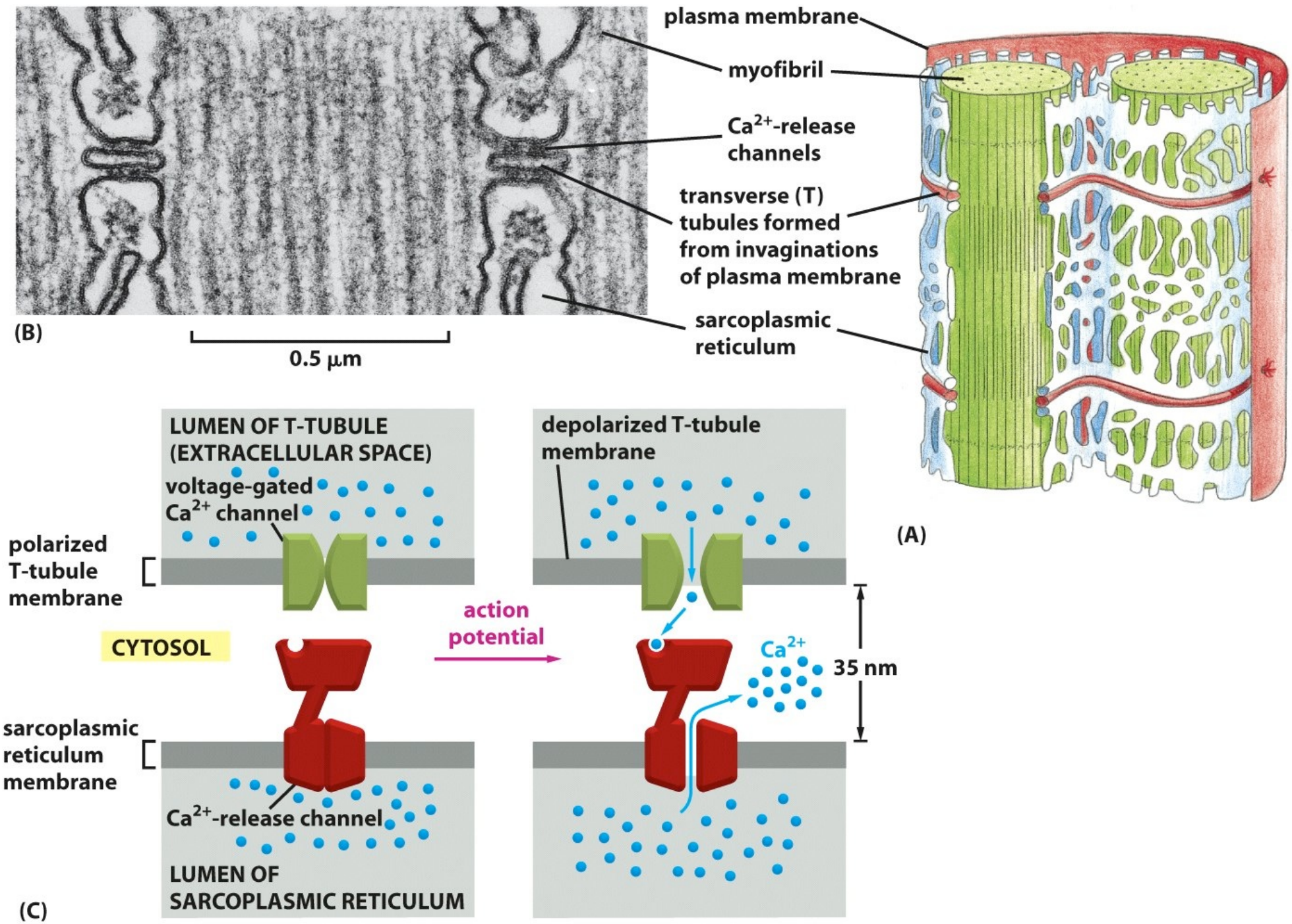
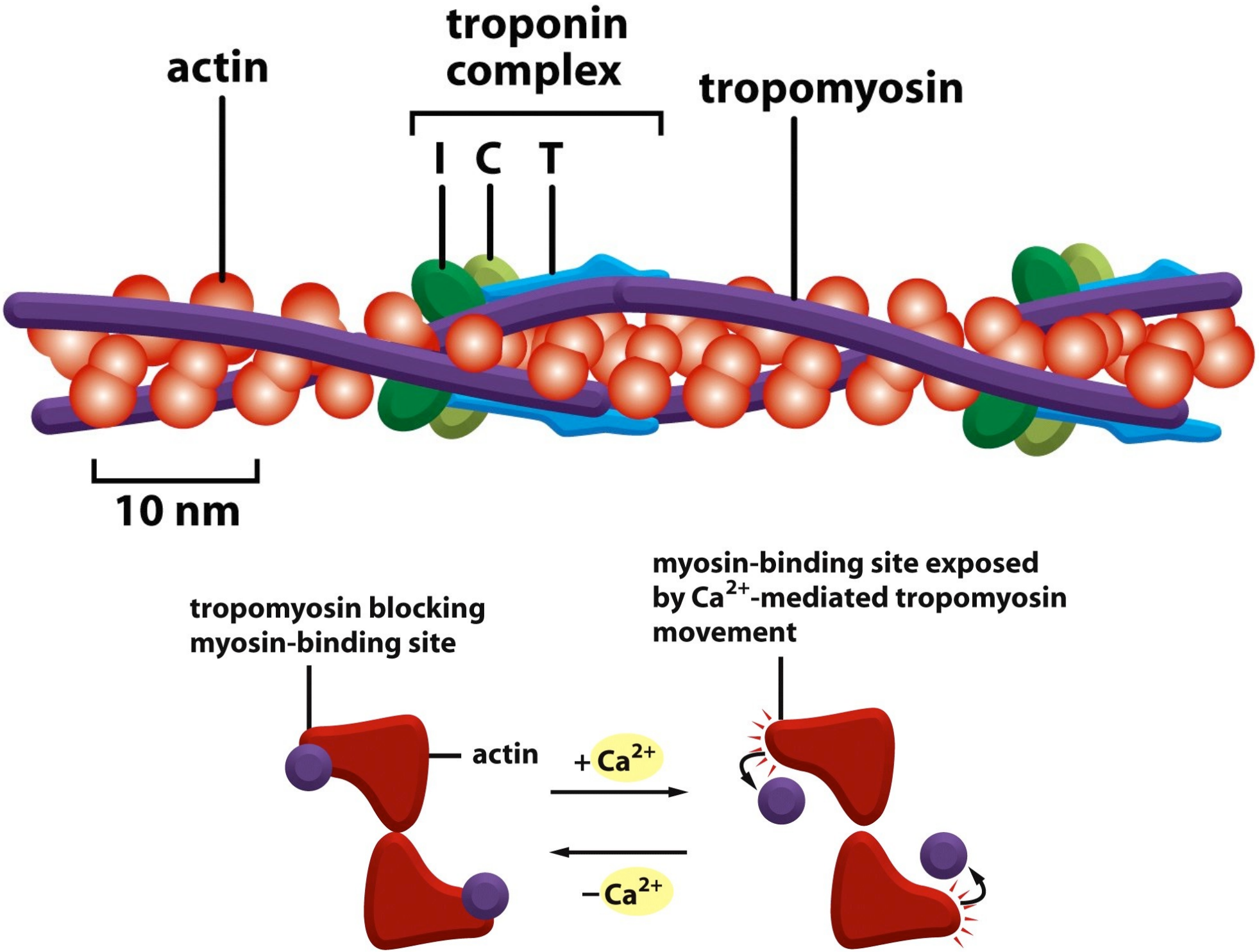


Figure 16-77 *Molecular Biology of the Cell* (© Garland Science 2008)


actin
troponin complex
I C T
tropomyosin
10 nm
tropomyosin blocking myosin-binding site
actin
+ Ca2+
– Ca2+
myosin-binding site exposed by Ca2+-mediated tropomyosin movement