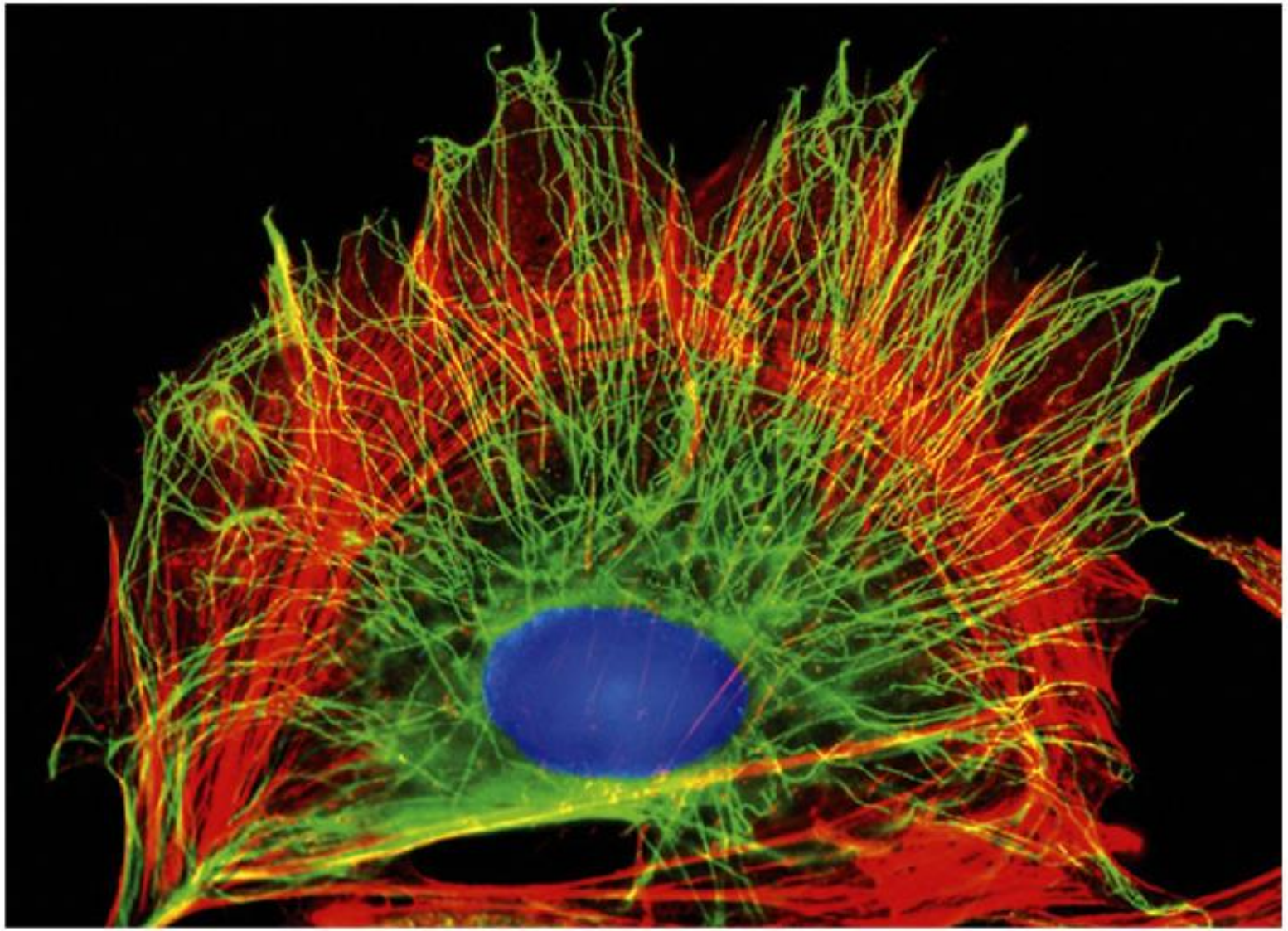
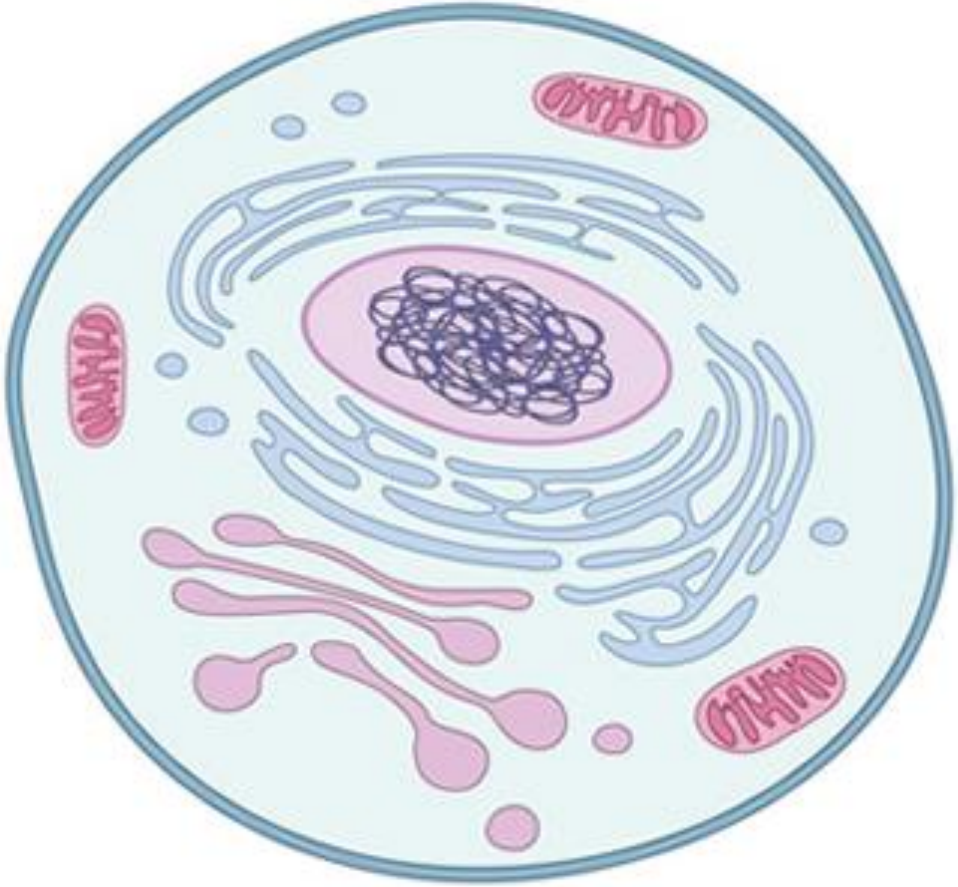
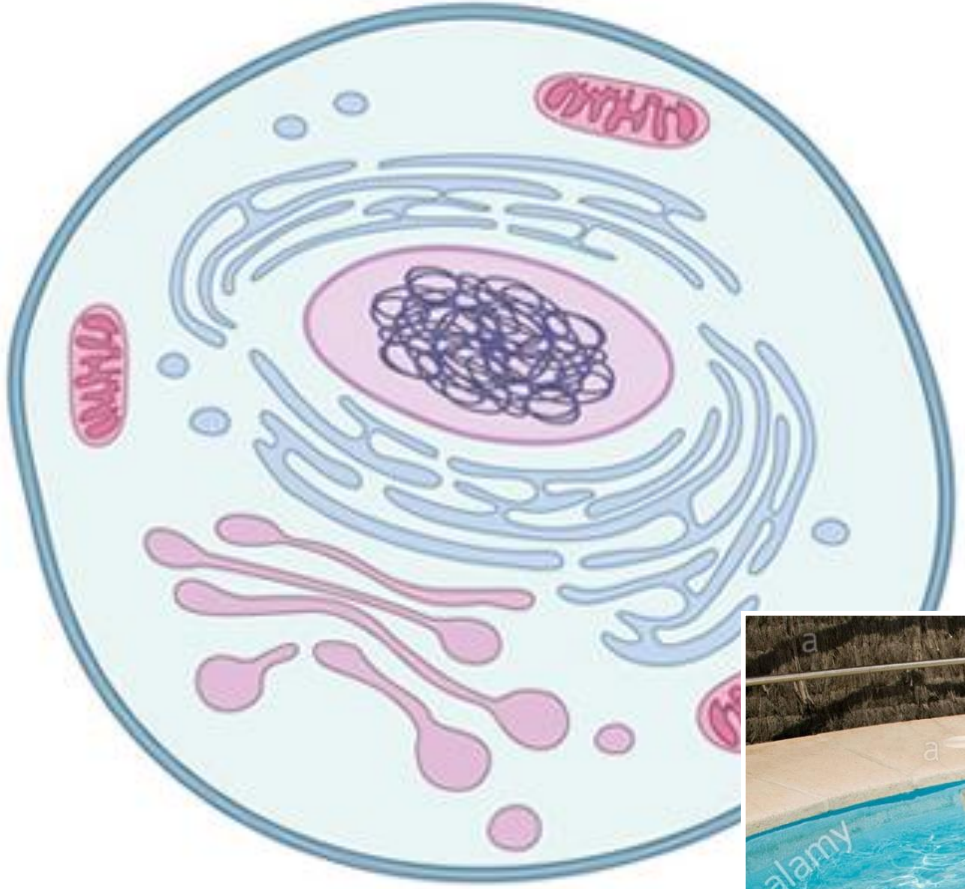
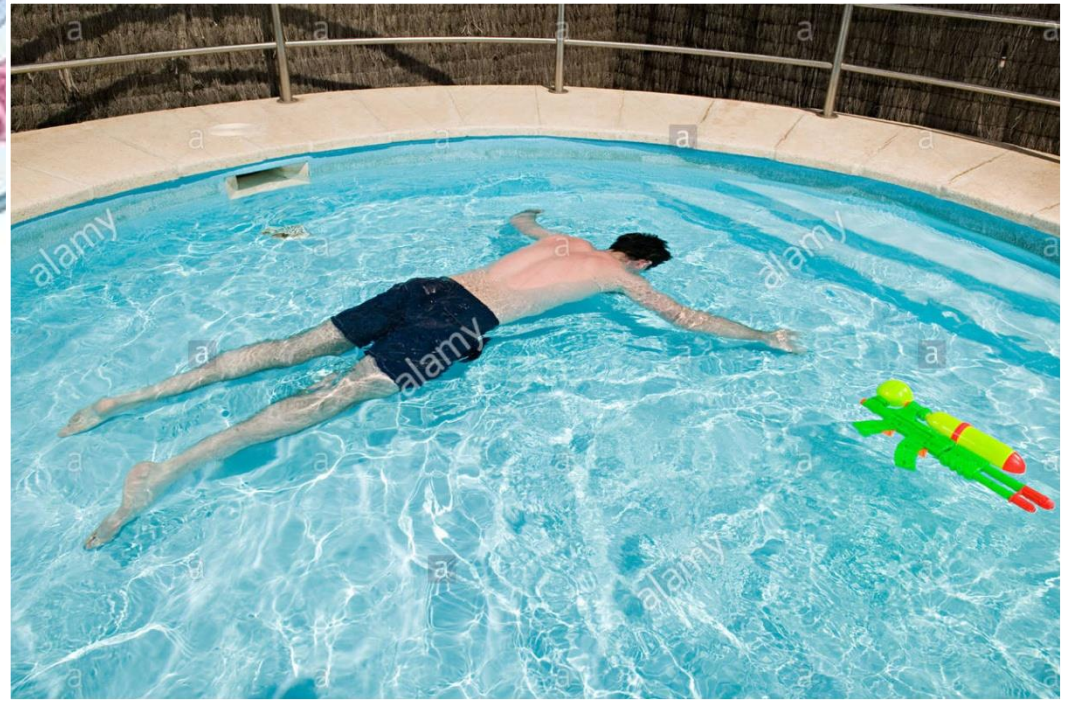
El Citoesqueleto



Uriel Koziol – ukoziol@fcien.edu.uy






alamy

Funciones del citoesqueleto

- **Forma celular**

- **Motilidad celular**
(migración, contracción, cilias)

- **Transporte intracelular**
(vesículas, moléculas, cromosomas, organelos)

- **Organización y polaridad celular**

- **Resistencia mecánica**

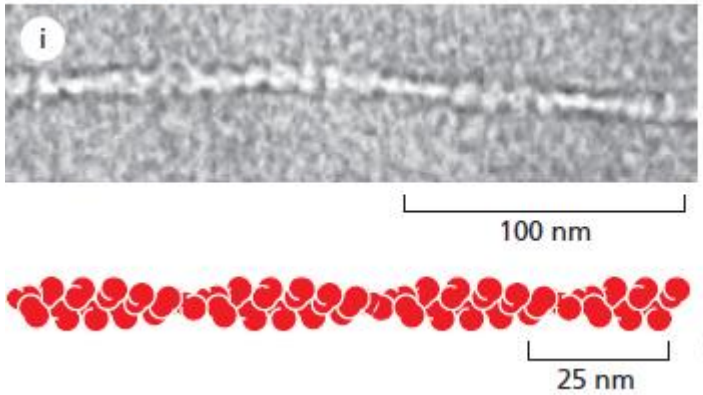
Tres familias de filamentos proteicos

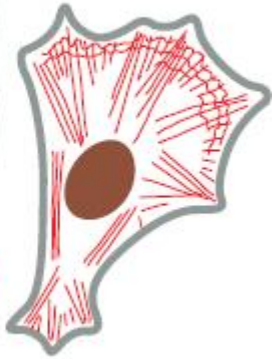
- **Filamentos de actina (microfilamentos)**
  Forma celular, proyecciones, motilidad, contractilidad.
  También transporte intracelular, resistencia mecánica.

- **Microtúbulos**
  Transporte intracelular, localización de organelos.
  Estructura y movimiento de cilias.

- **Filamentos intermedios**
  Resistencia mecánica

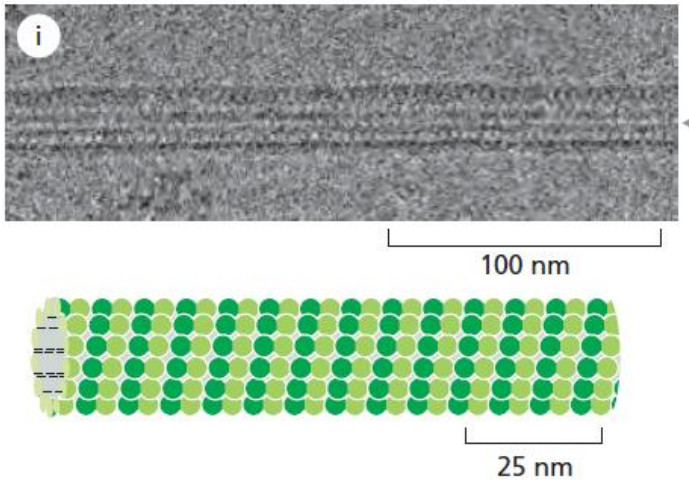
ACTIN FILAMENTS

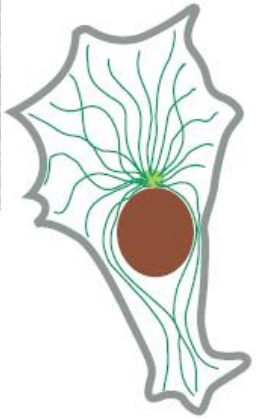




Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures with a diameter of 8 nm that organize into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane. (i) Single actin filame

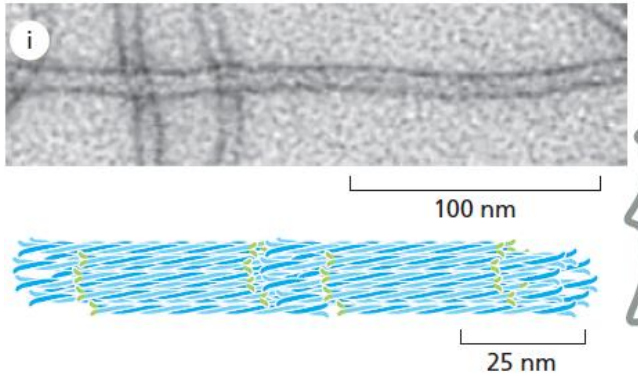
MICROTUBULES





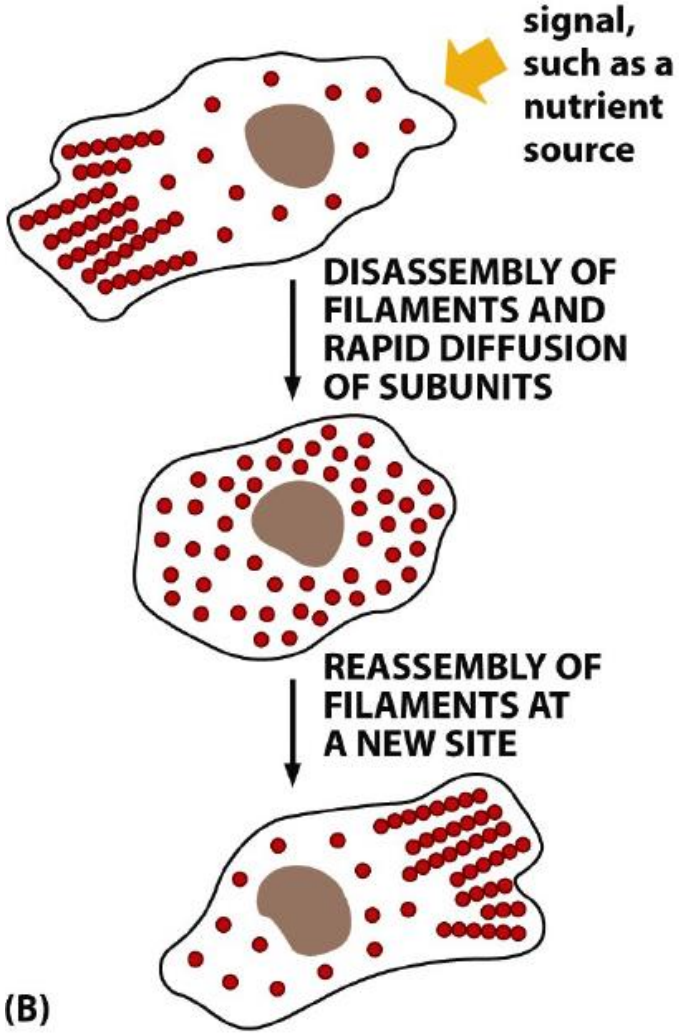
Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and frequently have one end attached to a microtubule-organizing center (MTOC) called a *centrosome*. (i) Single microtubule

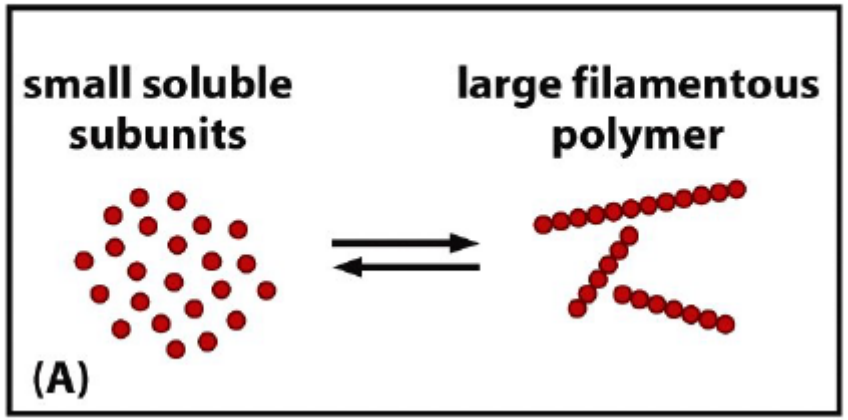
INTERMEDIATE FILAMENTS

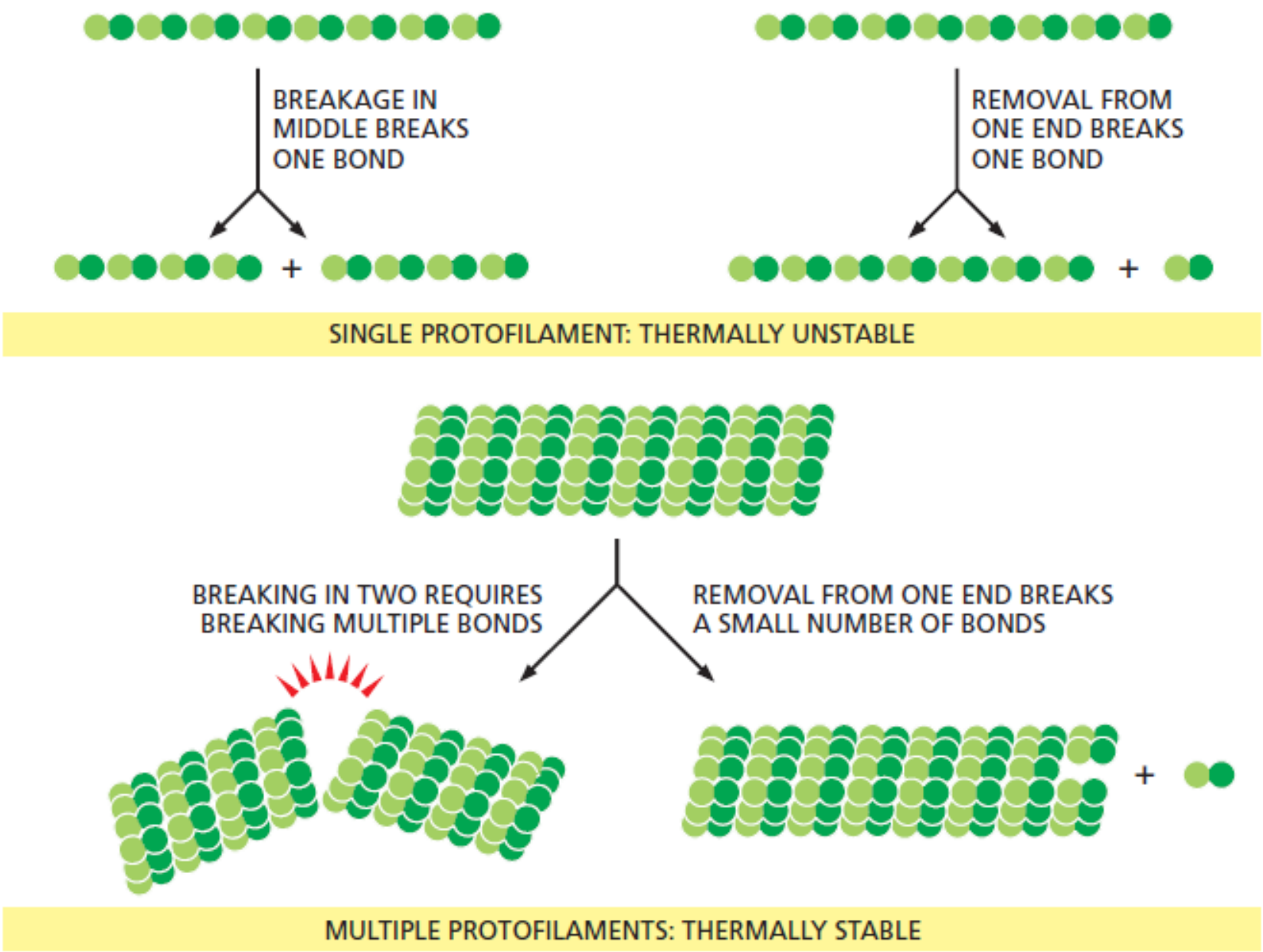


Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types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

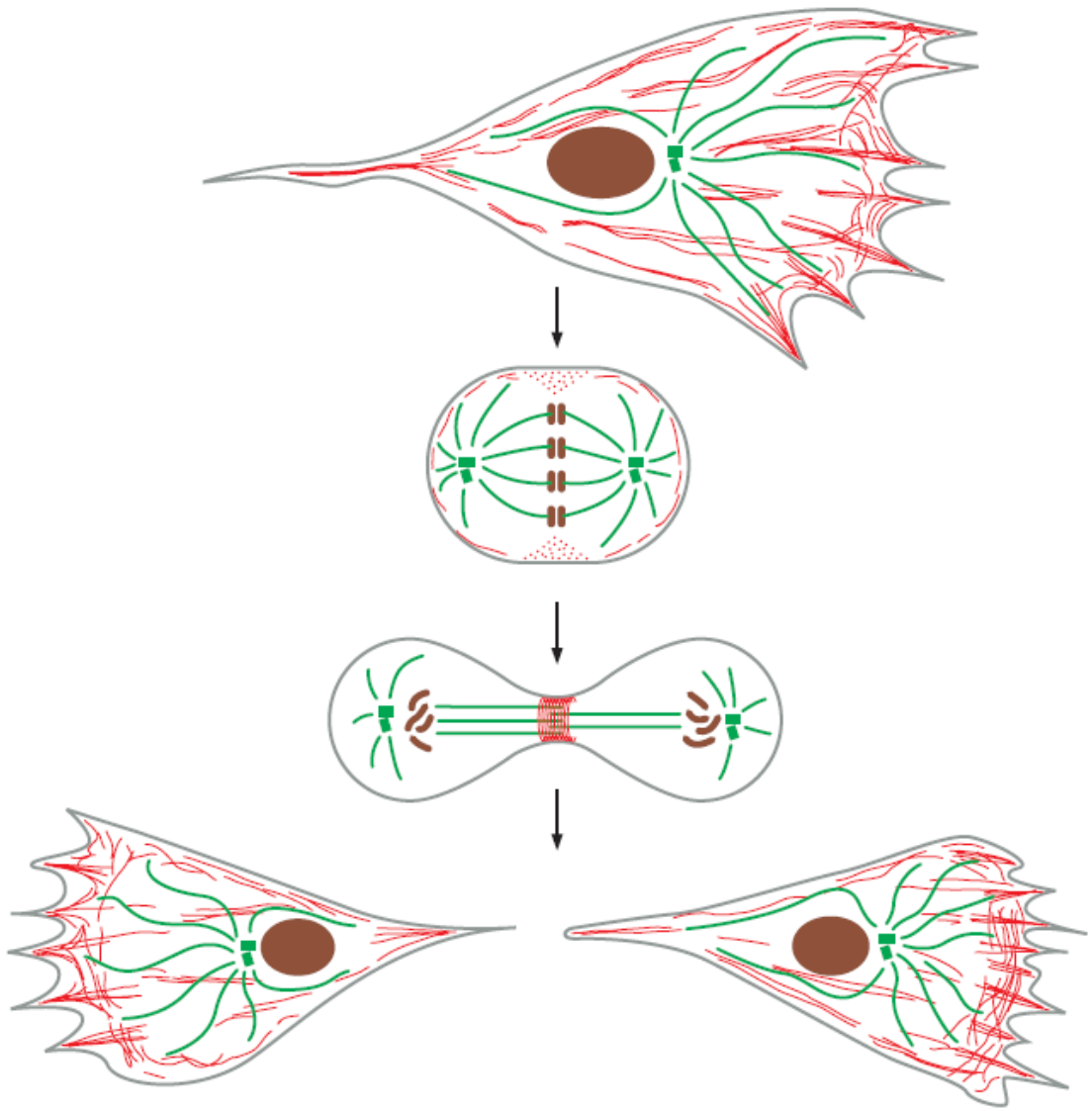
Algunas características en común



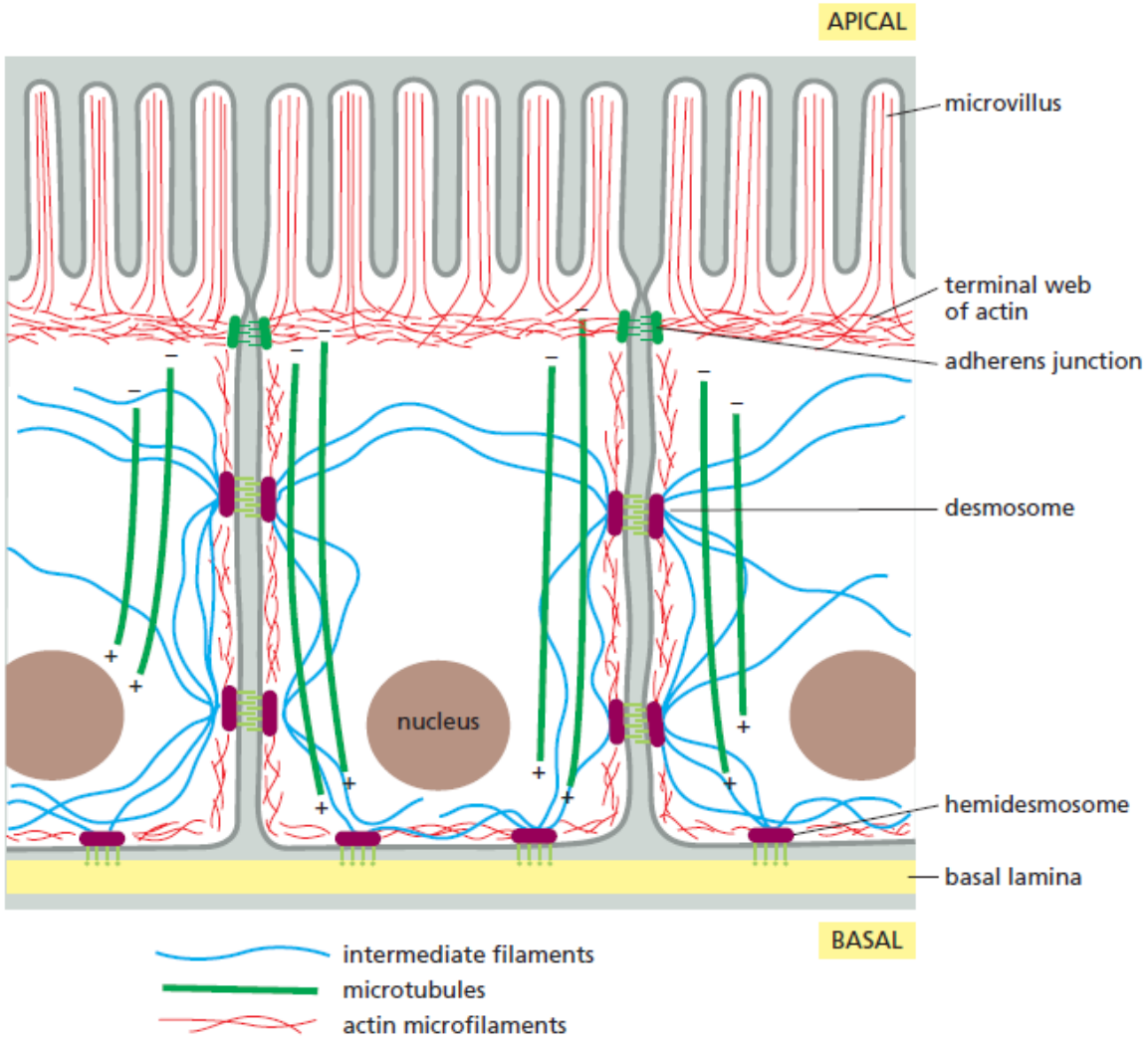


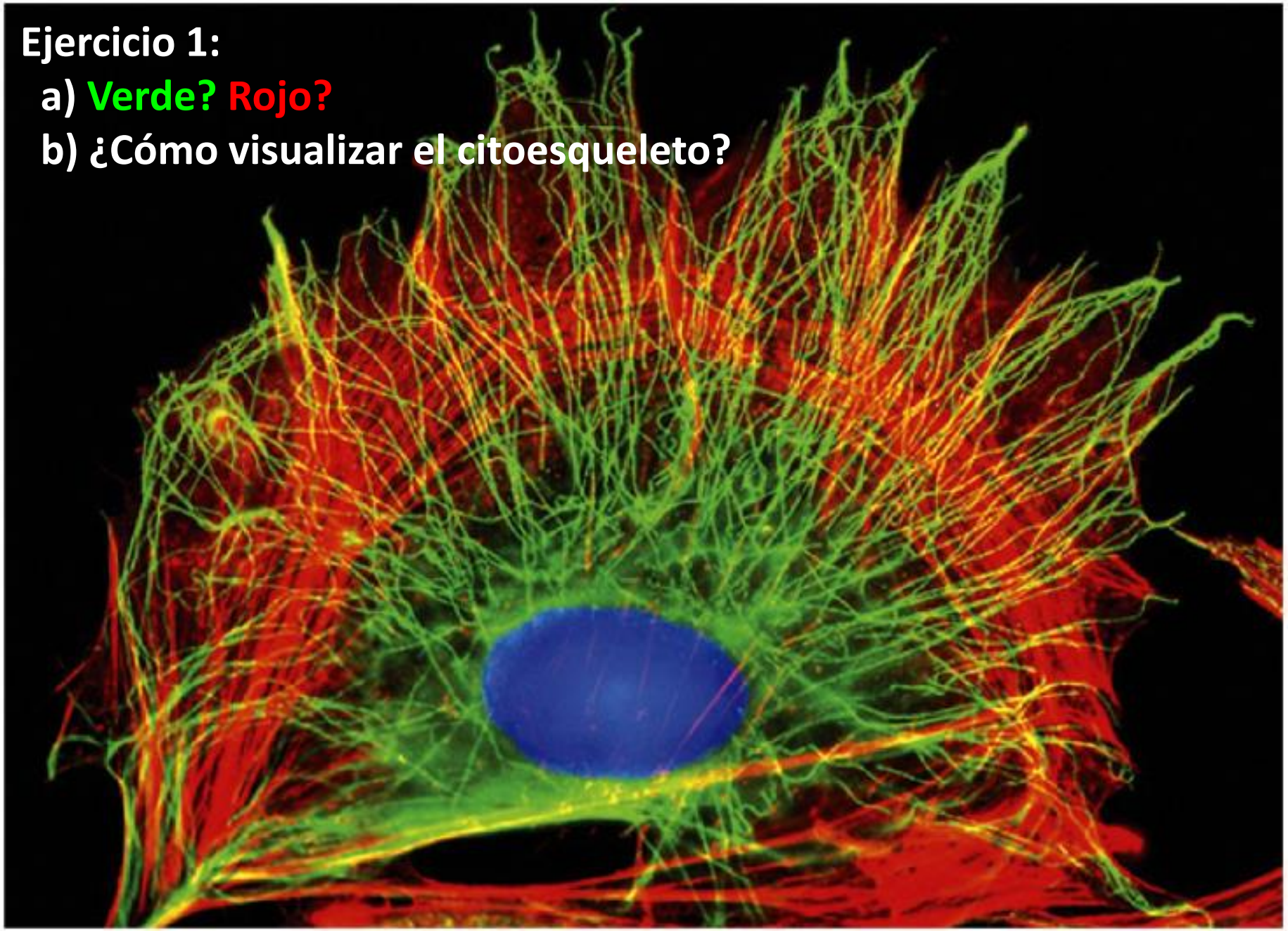

BREAKAGE IN MIDDLE BREAKS ONE BOND
REMOVAL FROM ONE END BREAKS ONE BOND
SINGLE PROTOFILAMENT: THERMALLY UNSTABLE
BREAKING IN TWO REQUIRES BREAKING MULTIPLE BONDS
REMOVAL FROM ONE END BREAKS A SMALL NUMBER OF BONDS
MULTIPLE PROTOFILAMENTS: THERMALLY STABLE

Dinámica del citoesqueleto : ciclo celular

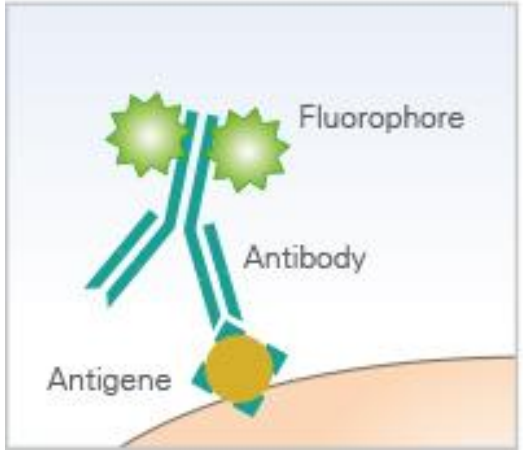


Diferencias en la organización del citoesqueleto entre diferentes tipos celulares

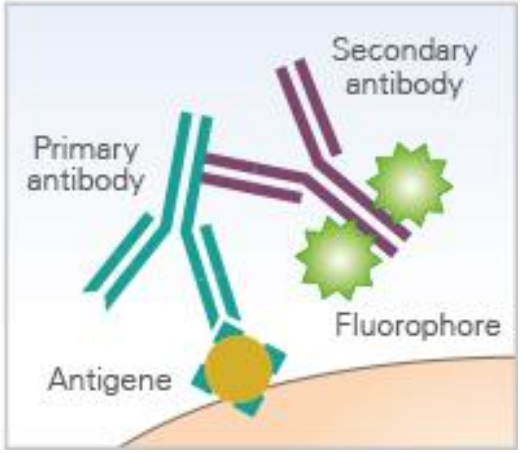



Ejercicio 1:
a) Verde? Rojo?
b) ¿Cómo visualizar el citoesqueleto?

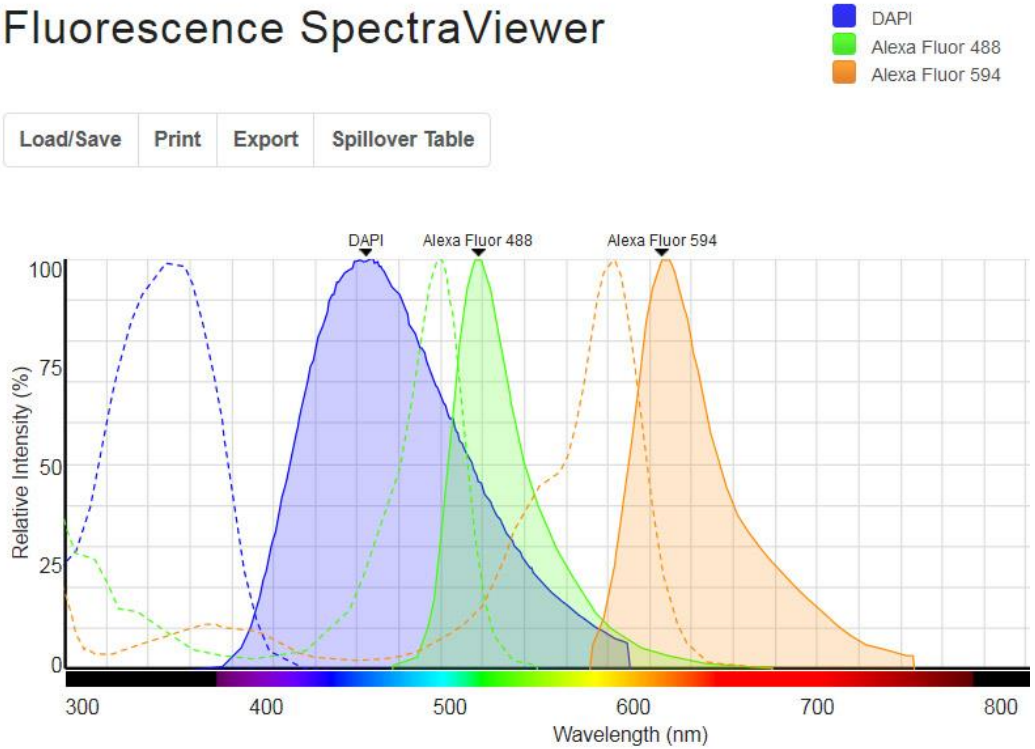
Direct Immunofluorescence



Indirect Immunofluorescence



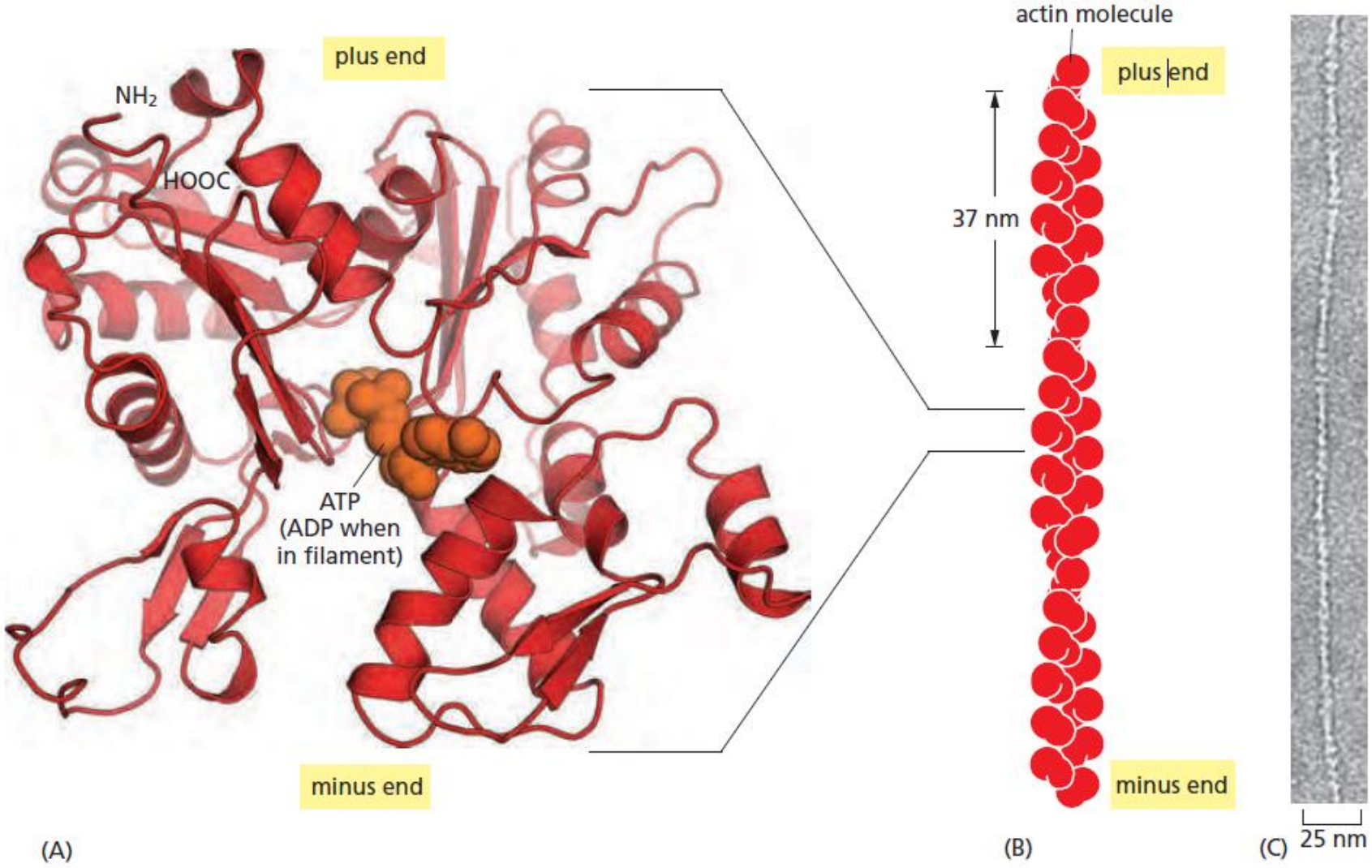
Fluorescence SpectraViewer

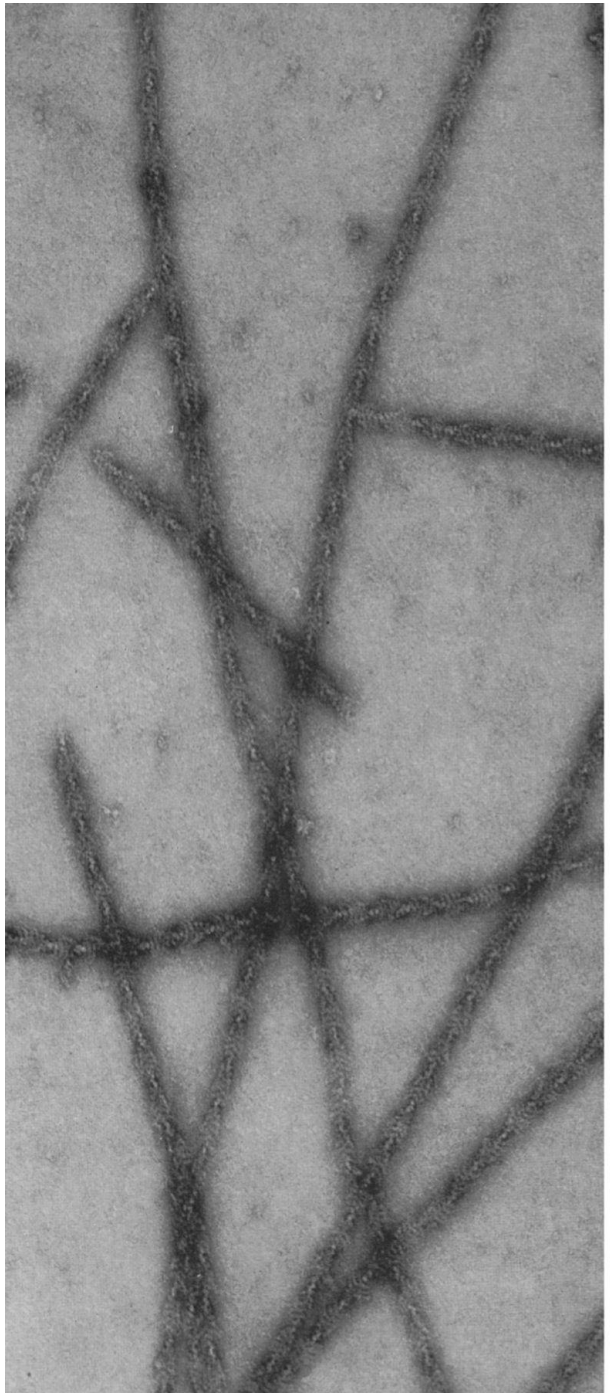


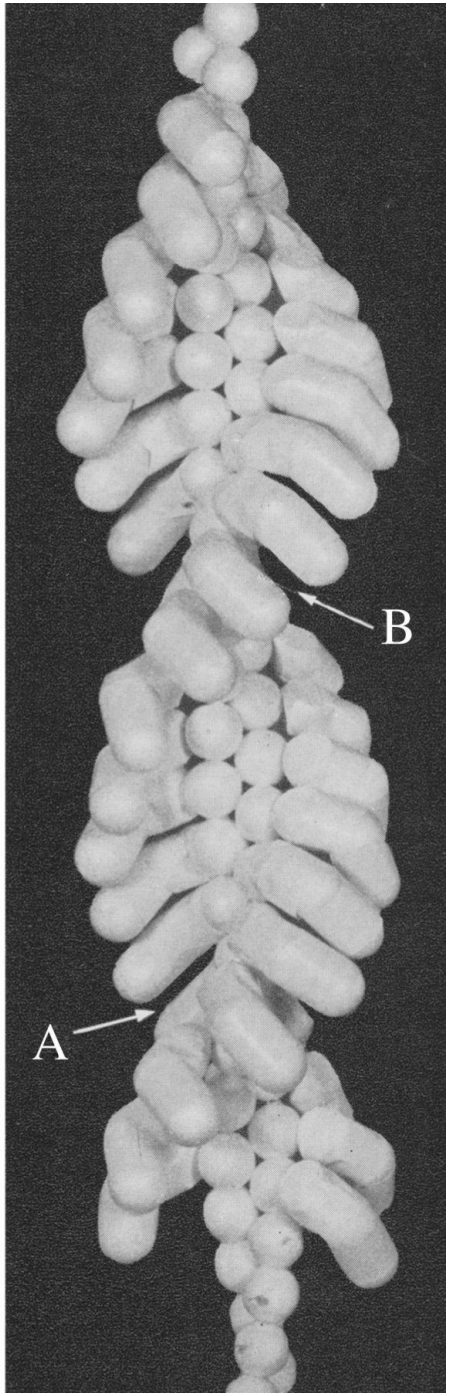
Actina

Actina G – 375 AA

Actina F

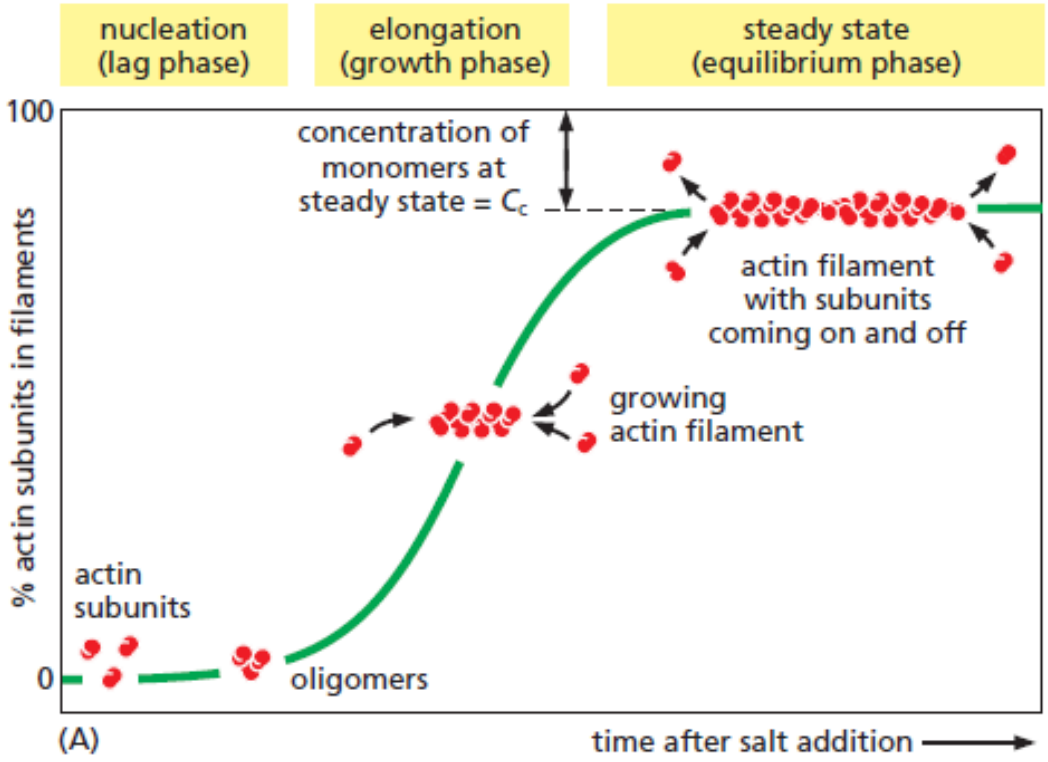


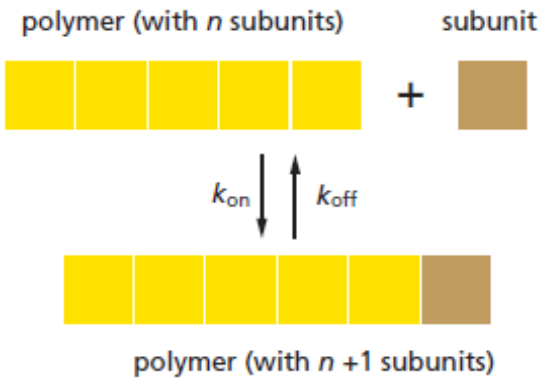



B
A

Minus (pointed) end
Actina
Cabezas
de miosina
Plus (barbed) end

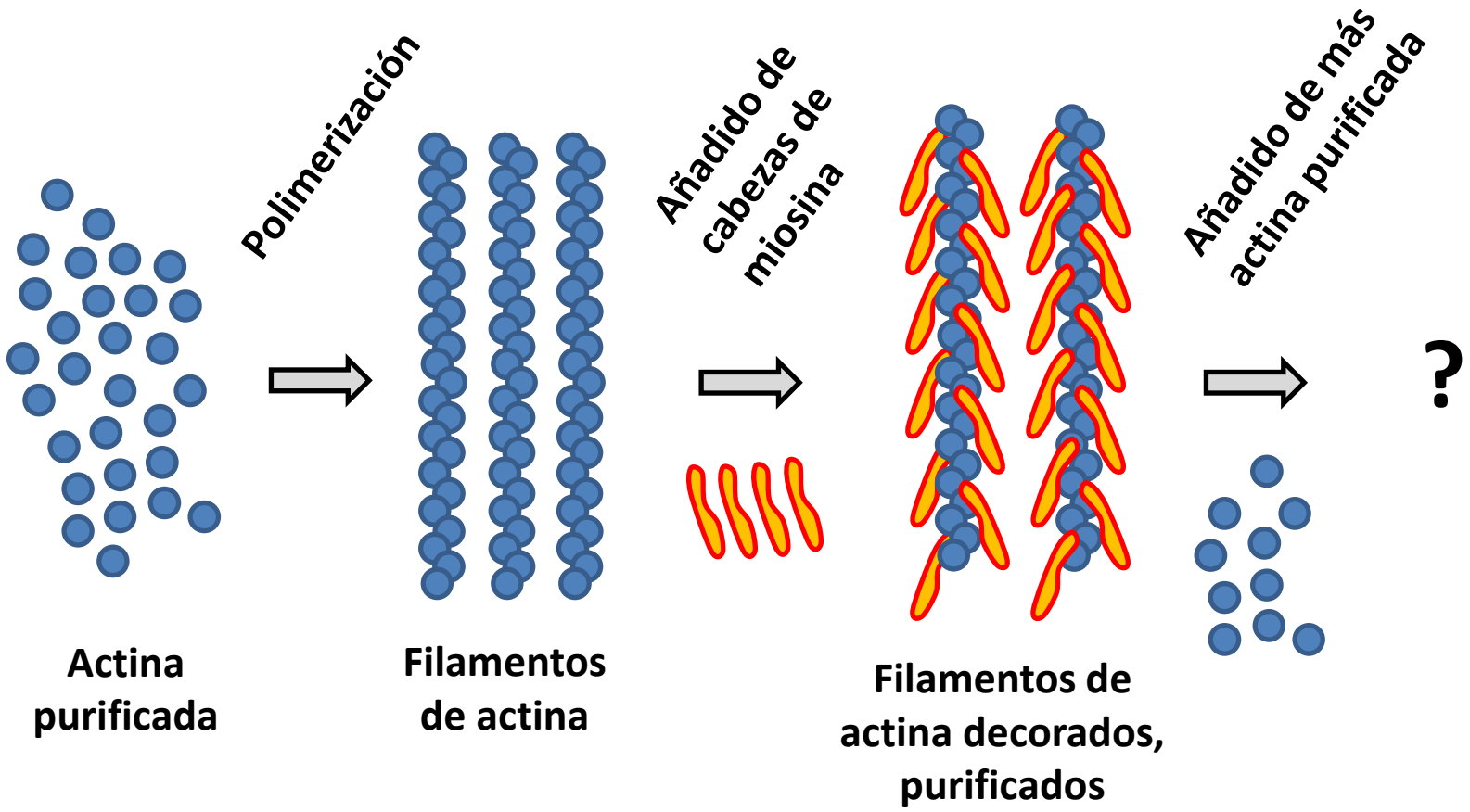
Polimerización de actina *in vitro*



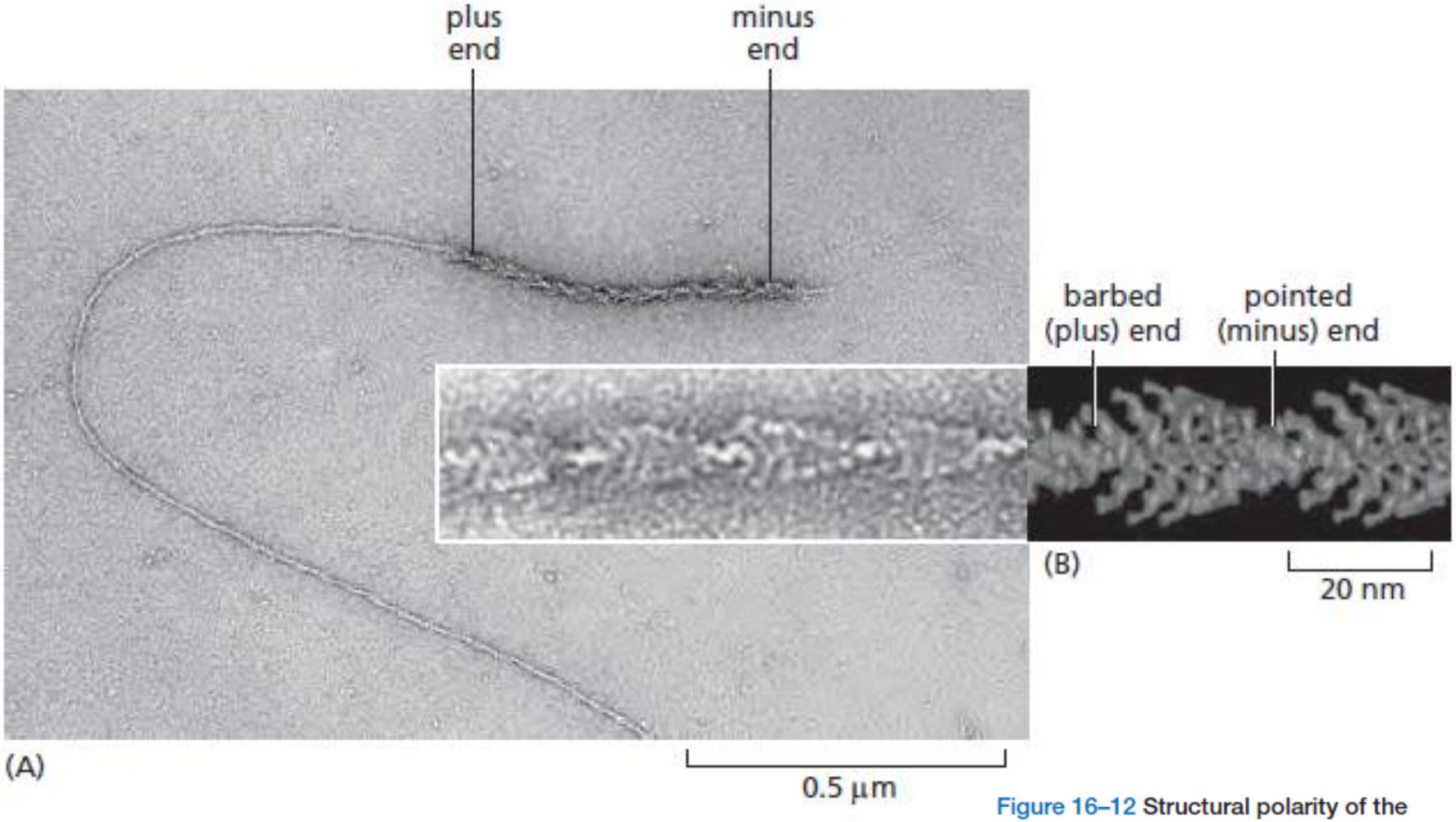


En equilibrio (sin añadir ATP)

Experimento: diferentes propiedades de los extremos (+) y (–) de la Actina



¿Qué esperan observar?



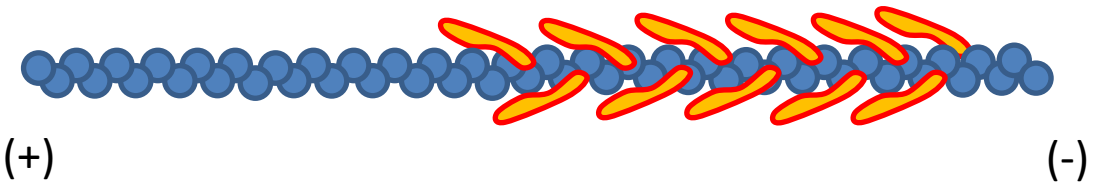
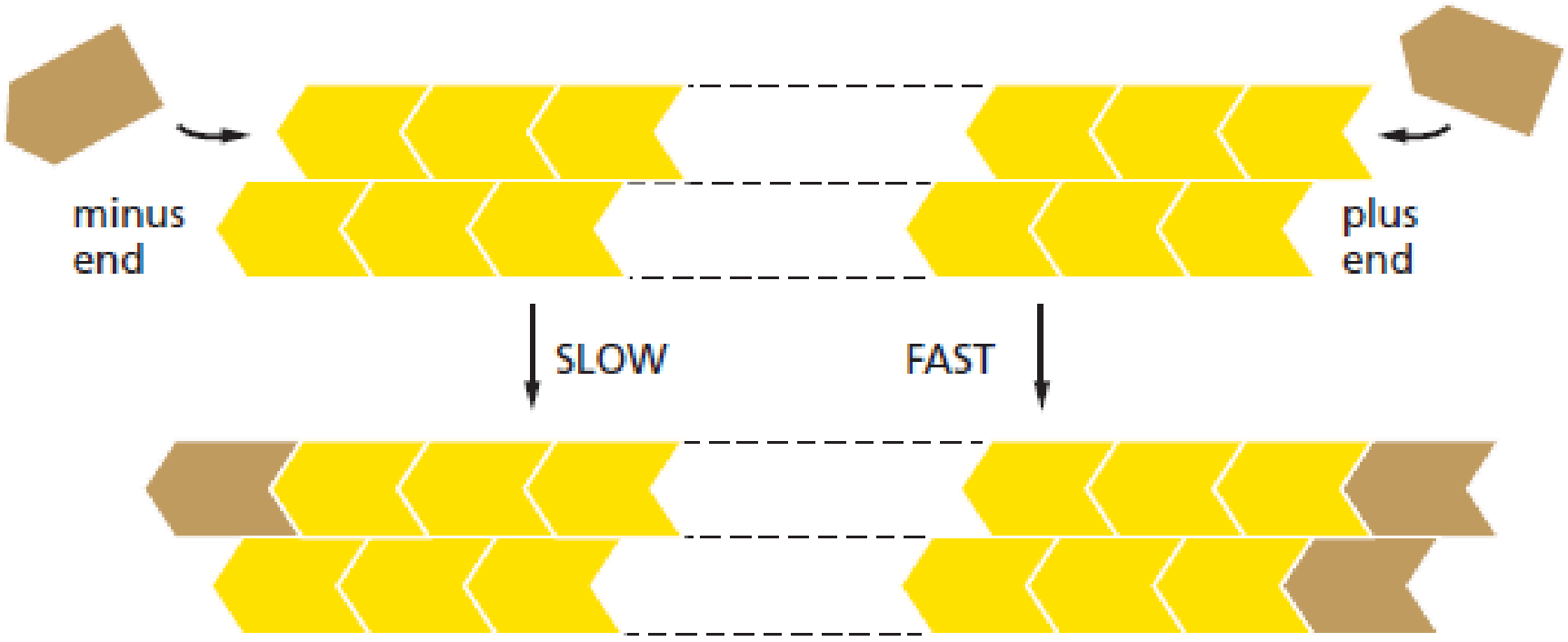
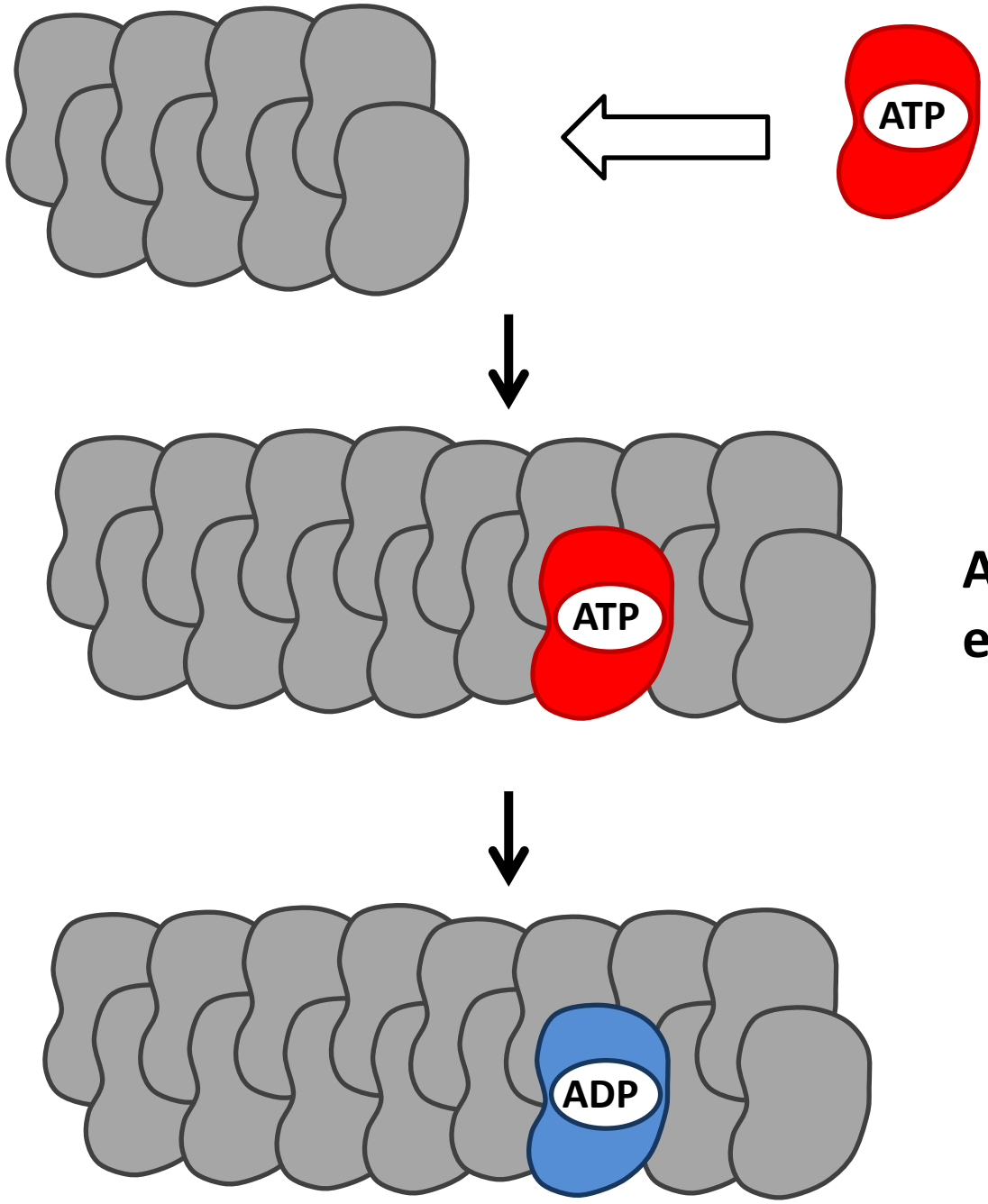


Figure 16–12 Structural polarity of the actin filament. (A) This electron micrograph shows an actin filament polymerized from a short actin filament seed that was decorated with myosin motor domains, resulting in an arrowhead pattern. The filament has grown much faster at the barbed (plus) end than at the pointed (minus) end. (B) Enlarged image and model showing the arrowhead pattern. (A, courtesy of Tom Pollard; B, adapted from M. Whittaker, B.O. Carragher and K.A. Milligan, *Ultramicro.* 54:245–260, 1995.)

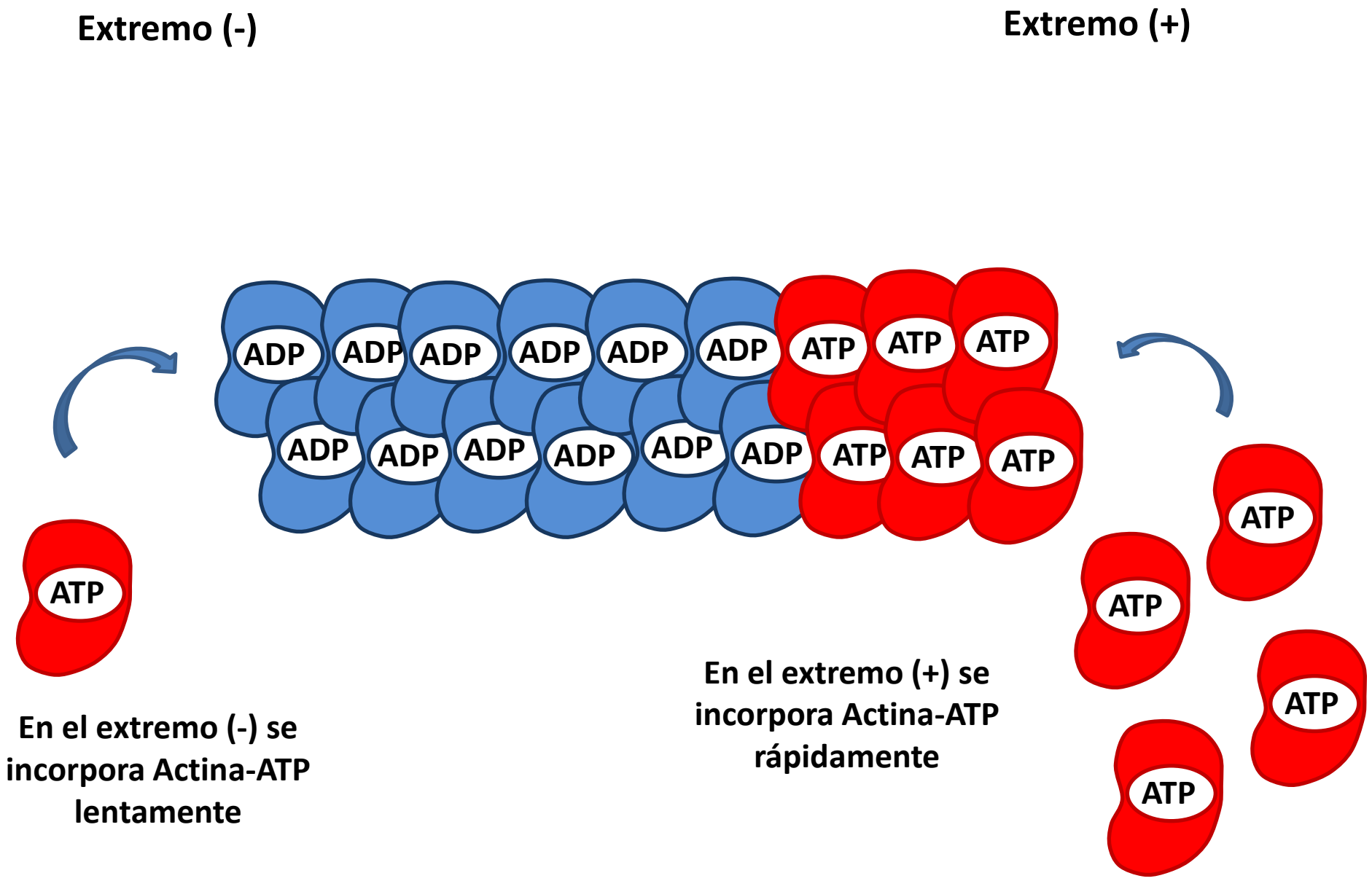

minus
end
plus
end
SLOW
FAST



Actina-ATP
(monómero libre; K_{on} alta)

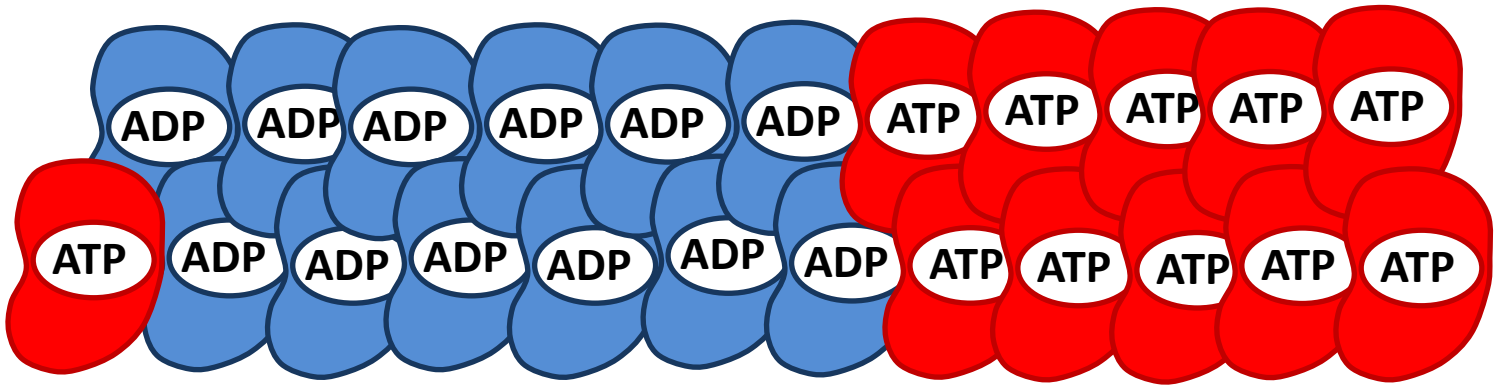
Actividad ATPasa de actina estimulada en el filamento

Actina-ADP
(K_{off} alta; filamento más inestable)


Extremo (-)
Extremo (+)
ADP
ATP
En el extremo (-) se incorpora Actina-ATP lentamente
En el extremo (+) se incorpora Actina-ATP rápidamente

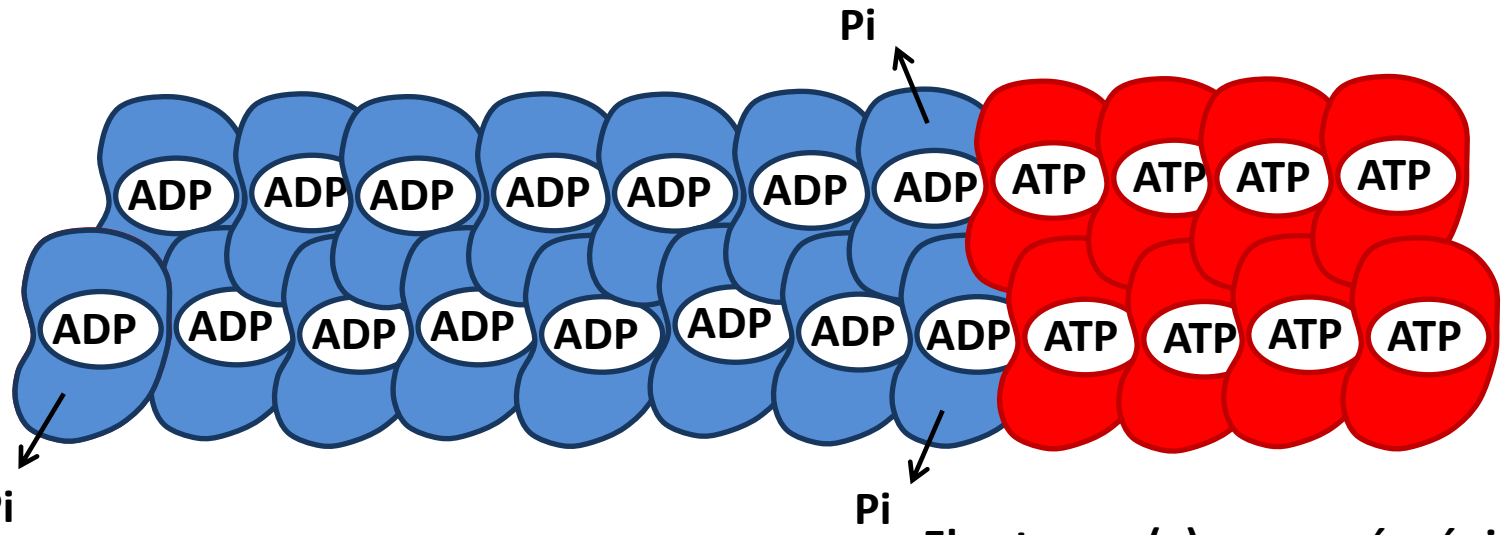
Extremo (-)

Extremo (+)



Extremo (-)

Extremo (+)



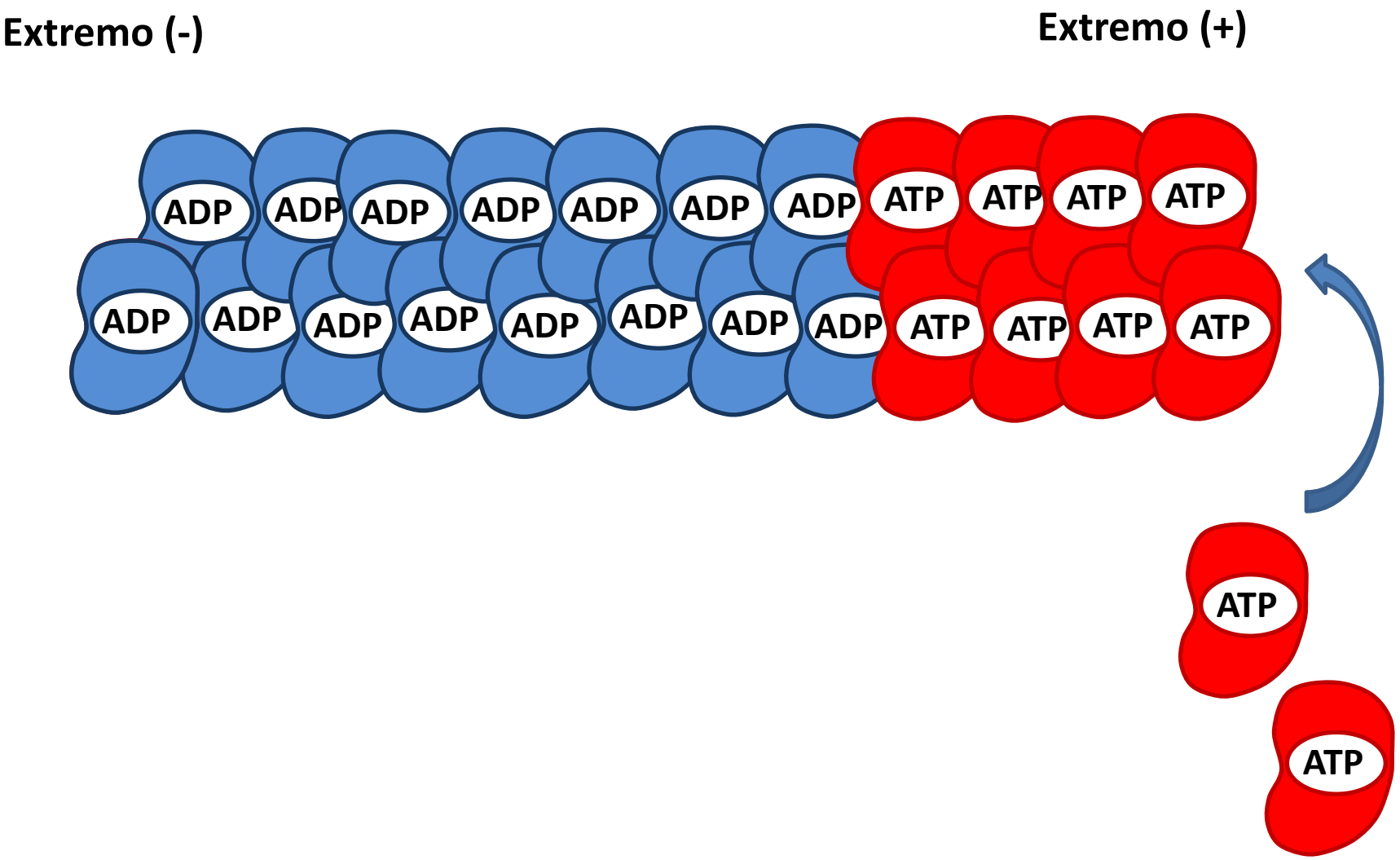
El extremo (-) crece más lento de lo que ocurre la hidrólisis de ATP

El extremo (+) crece más rápido de lo que ocurre la hidrólisis de ATP

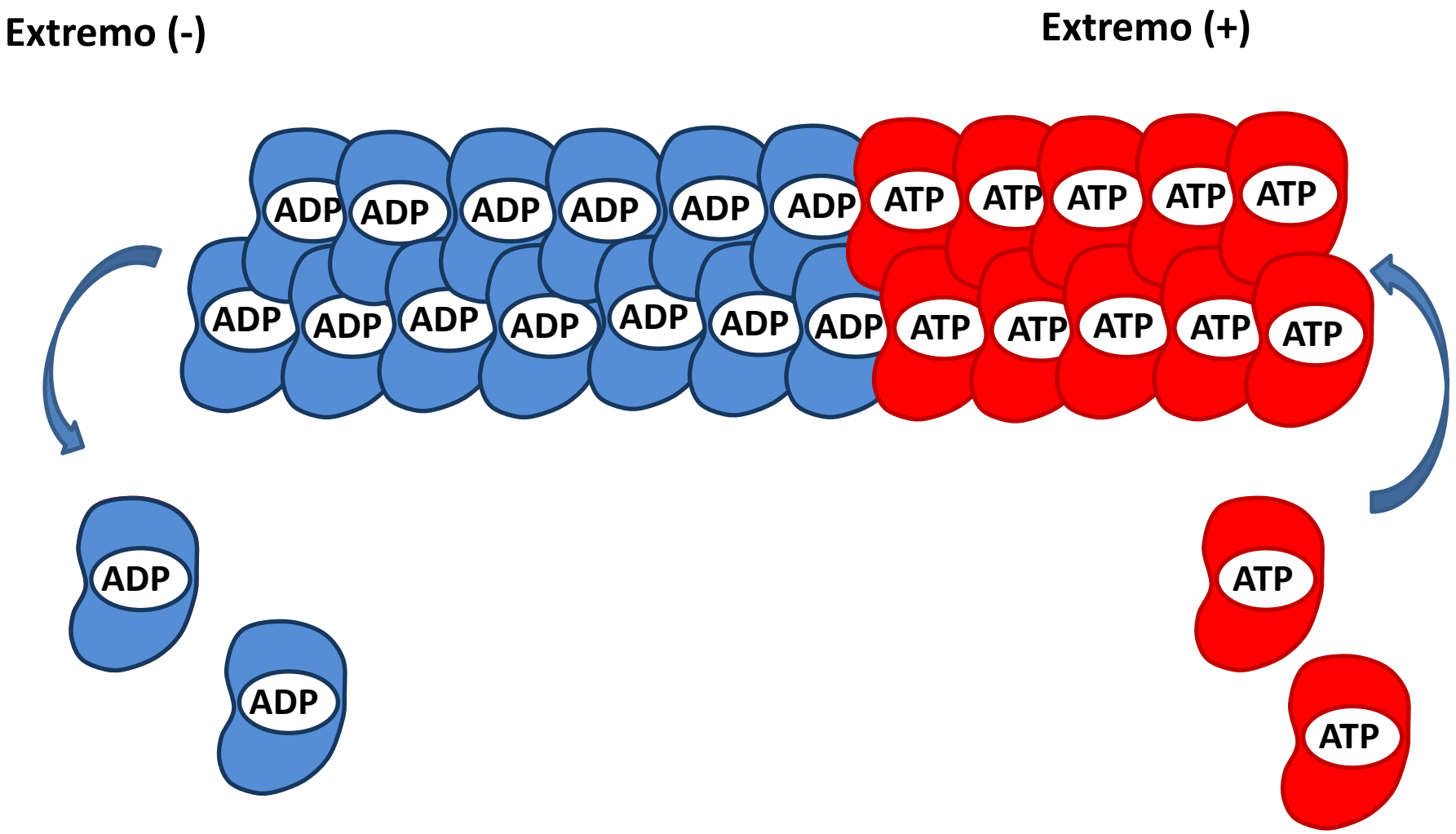
CAPUCHÓN DE ACTINA-ATP en extremo (+)

***Cc* diferentes en ambos extremos!**

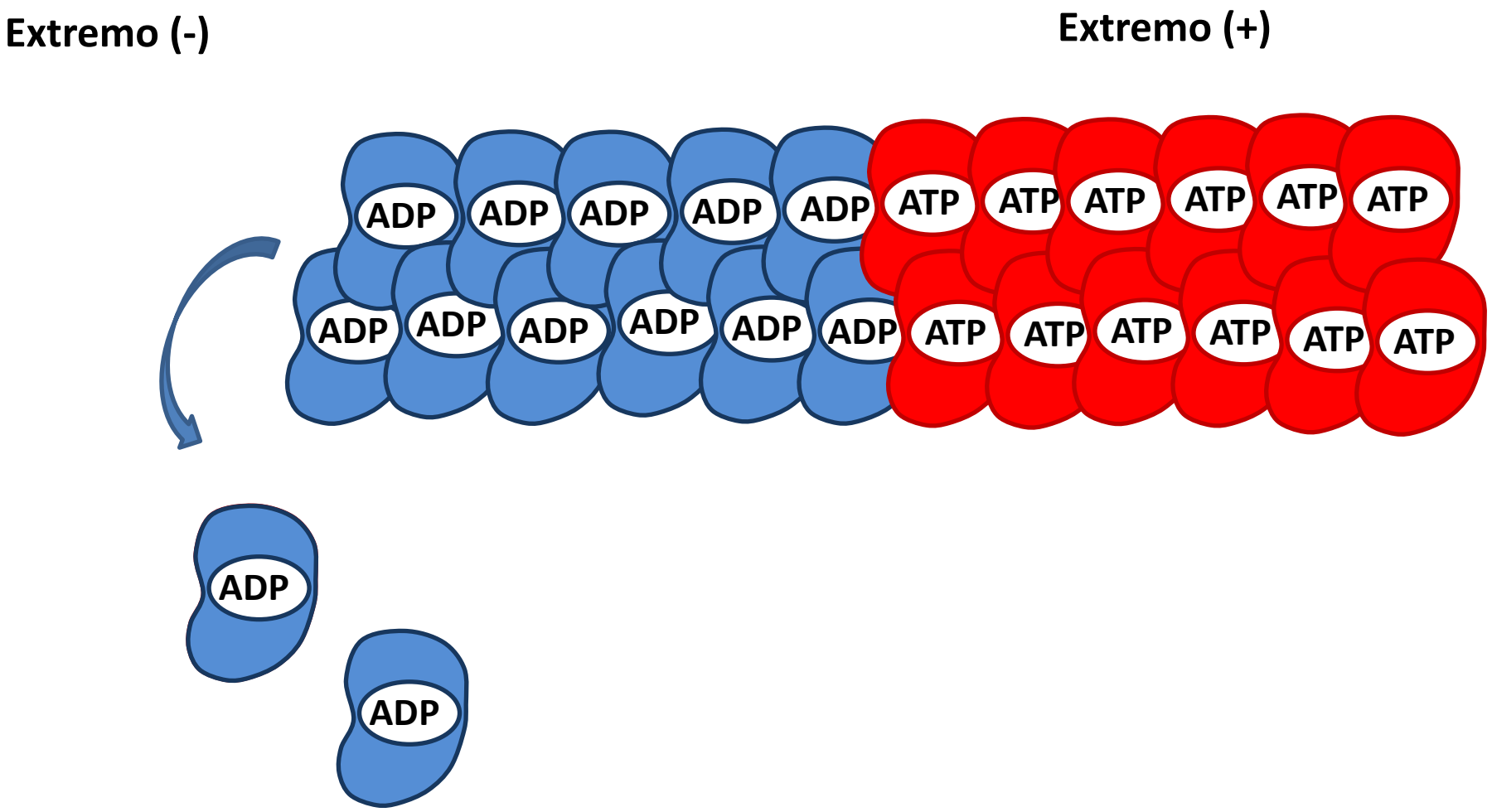
Recambio rotatorio (treadmilling)



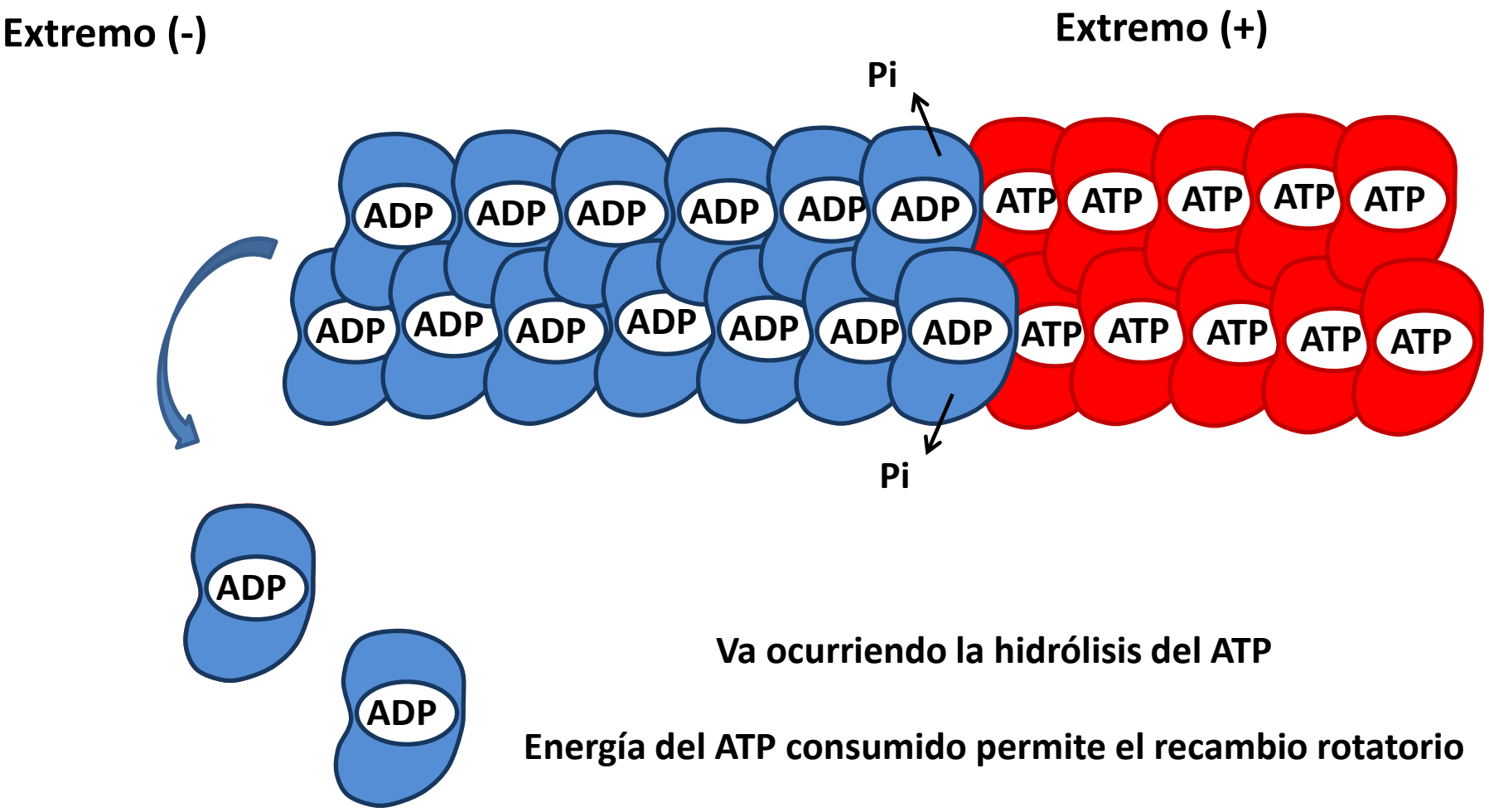
Recambio rotatorio (treadmilling)



Recambio rotatorio (treadmilling)

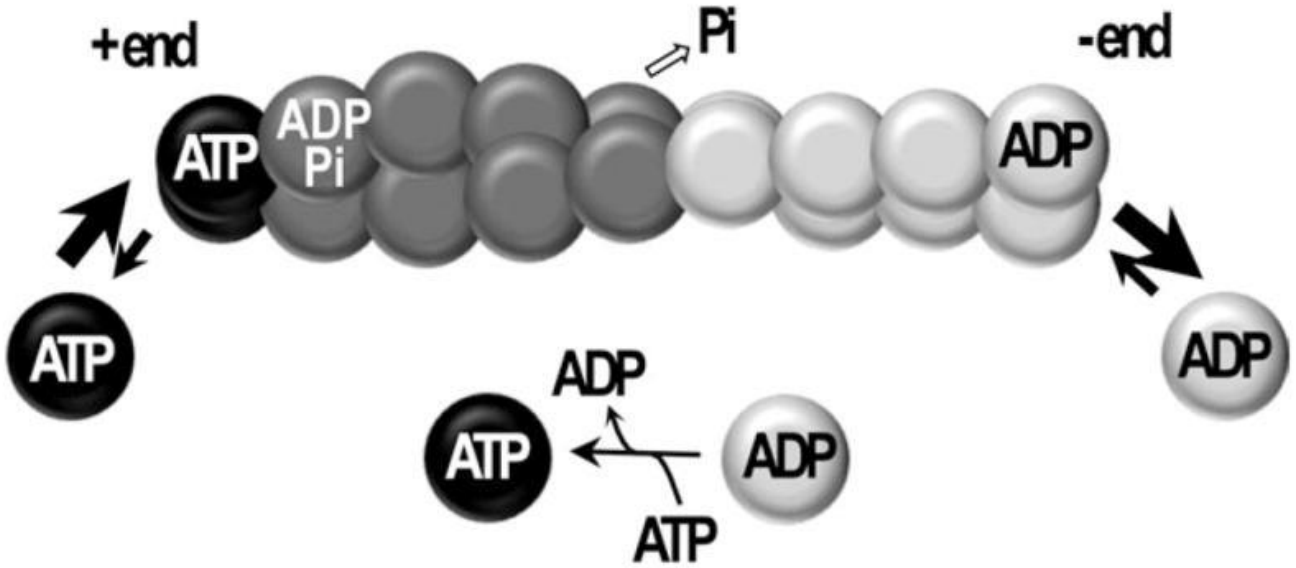


Recambio rotatorio (treadmilling)



Va ocurriendo la hidrólisis del ATP

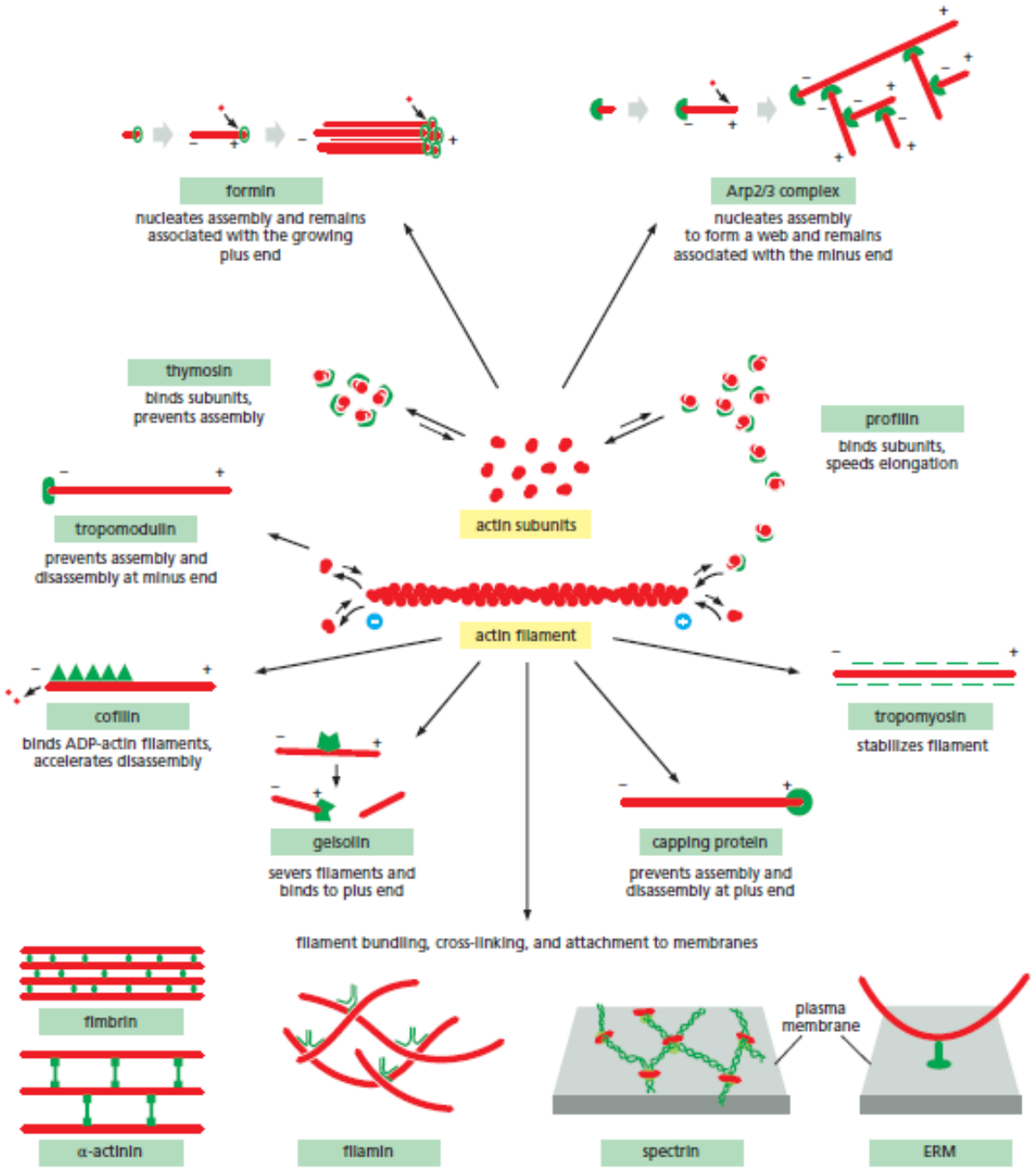
Energía del ATP consumido permite el recambio rotatorio


+end
Pi
-end
ATP
ADP
Pi
ADP
ATP
ADP
ADP
ATP
ADP
ATP

Life Fitness

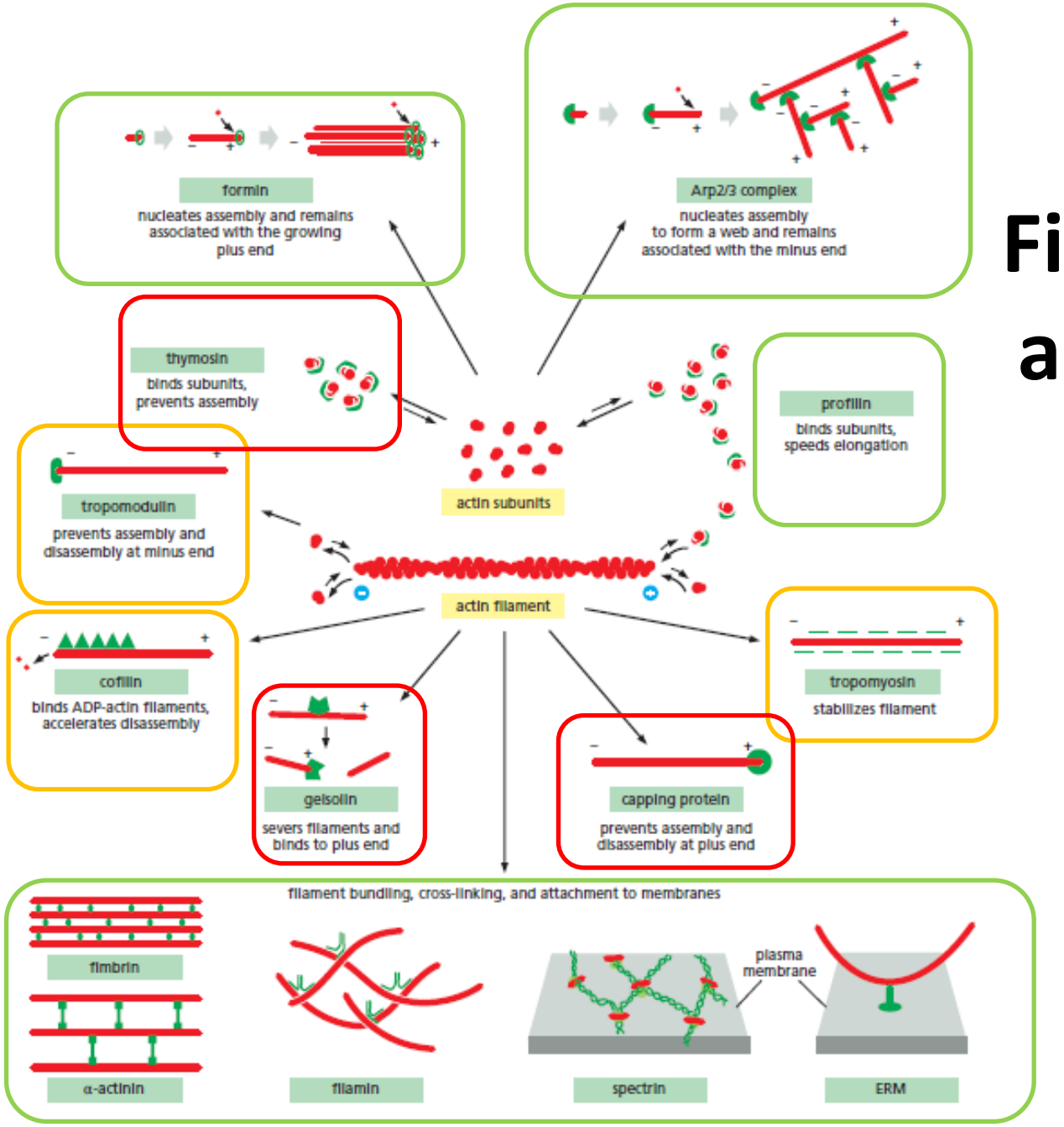
Hasta aquí, vimos las propiedades de la actina en forma pura en un tubo de ensayo…

Pero… ¿cómo se regula la formación de filamentos en la célula?



Filamentos de actina *in vivo*

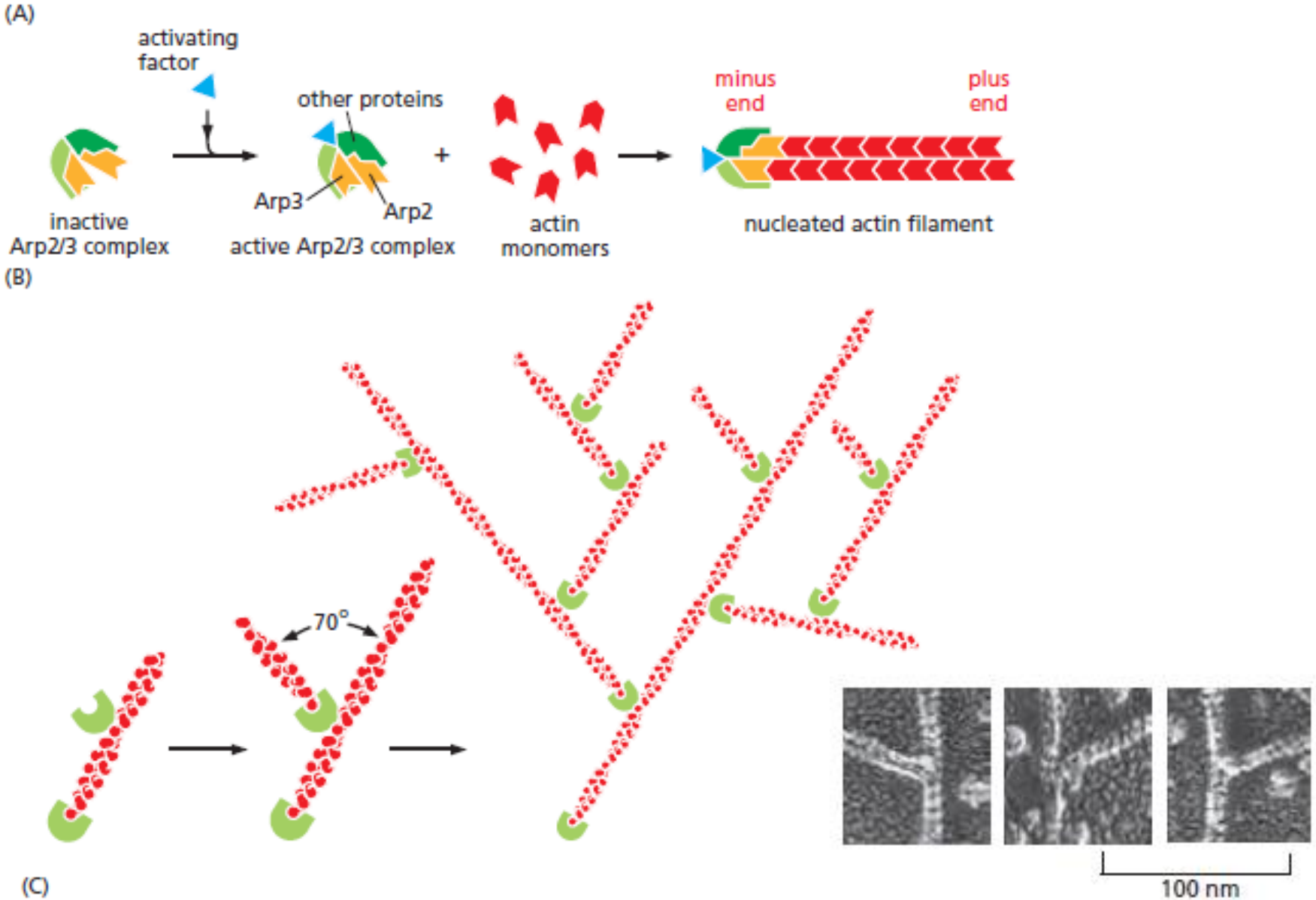
Roles de proteínas accesorias

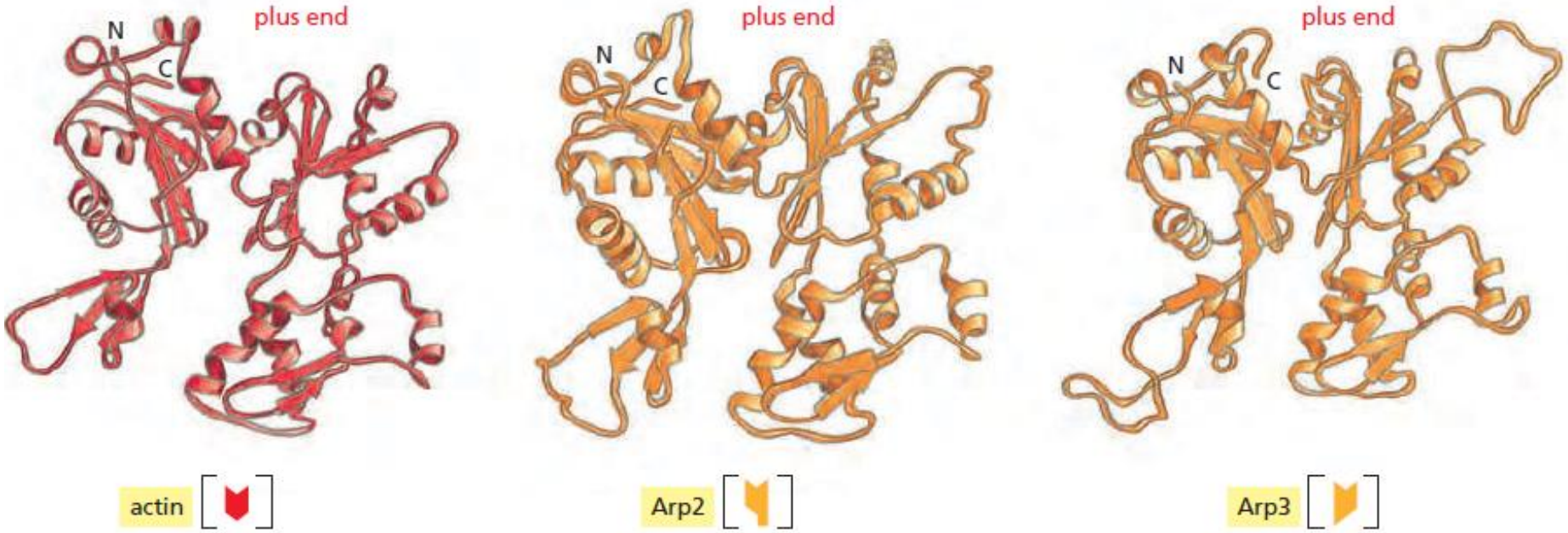


Filamentos de actina *in vivo*

Roles de proteínas accesorias

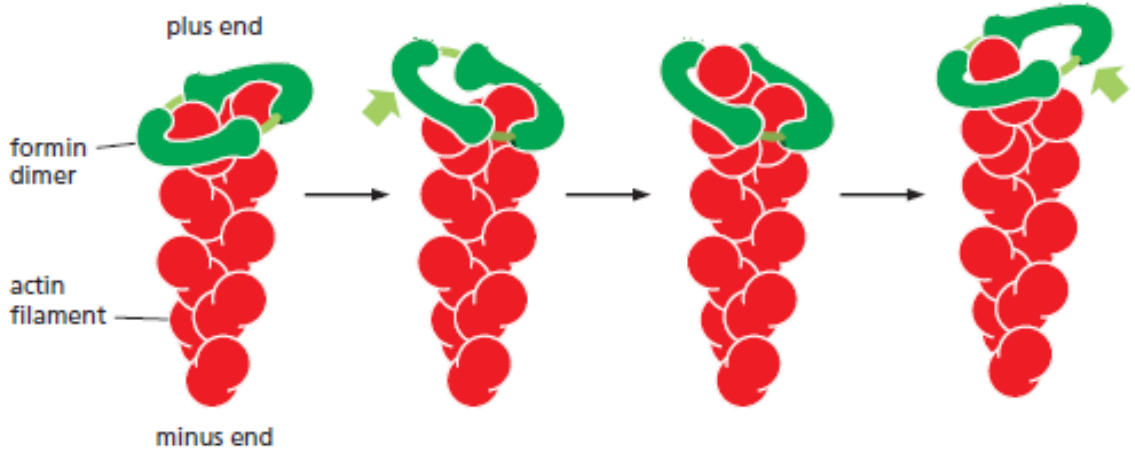
Nucleación ramificada por ARP2/3





A)

Nucleación por Forminas en haces paralelos



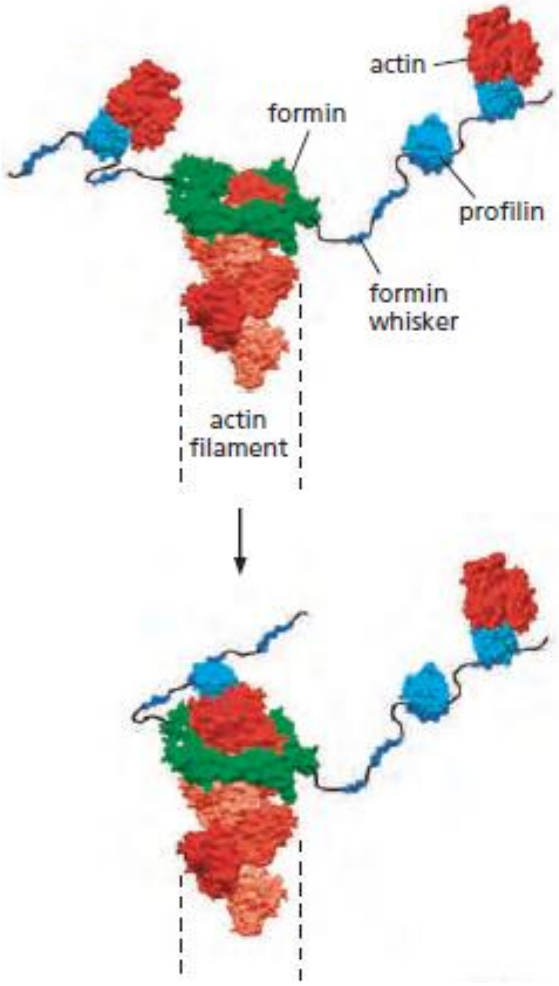
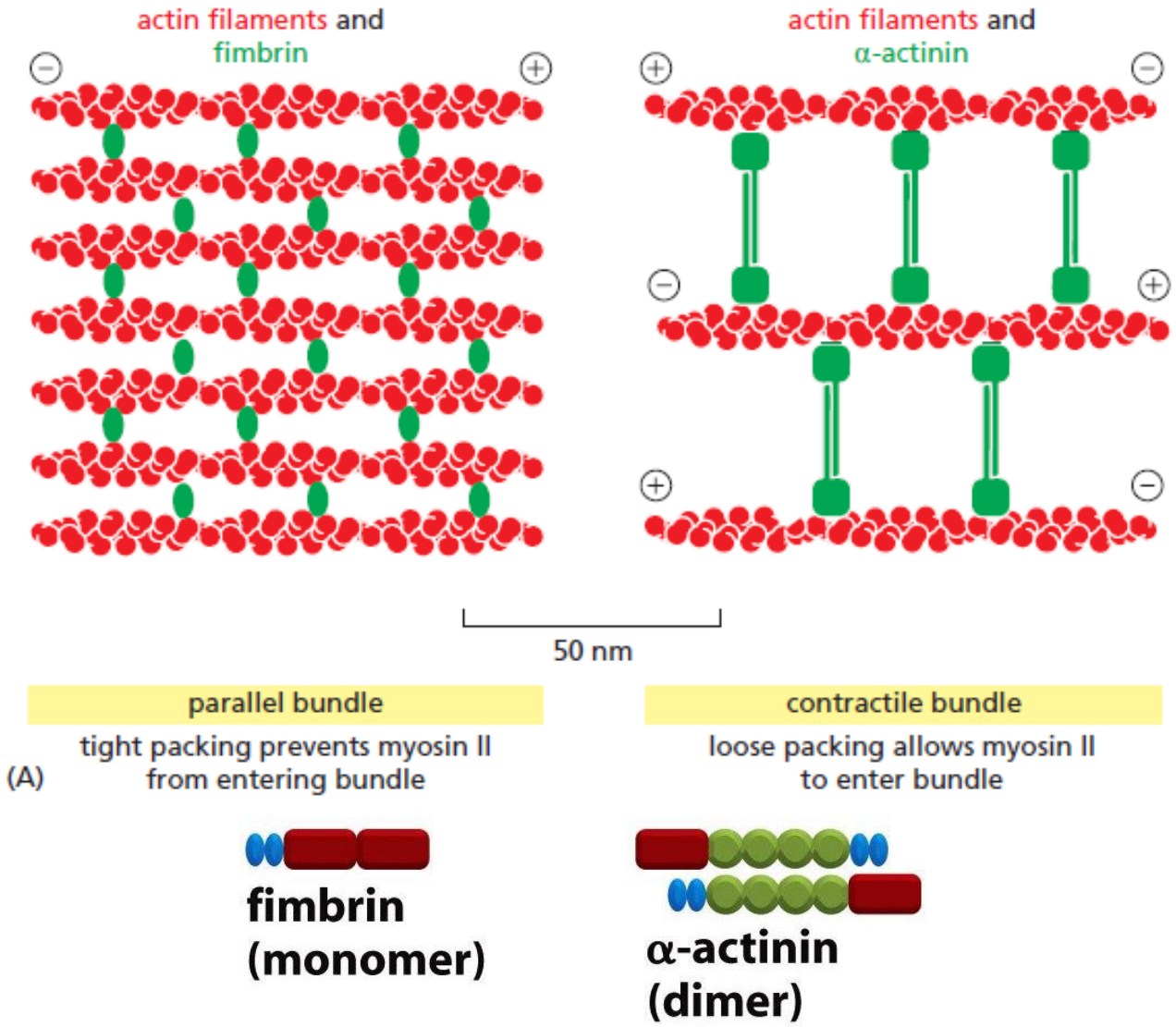
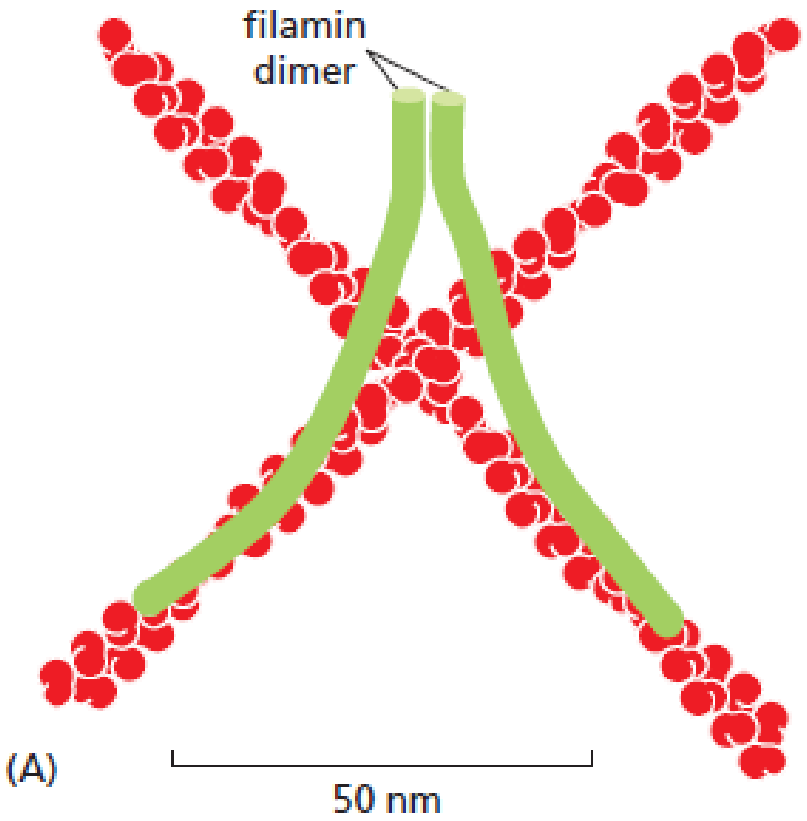


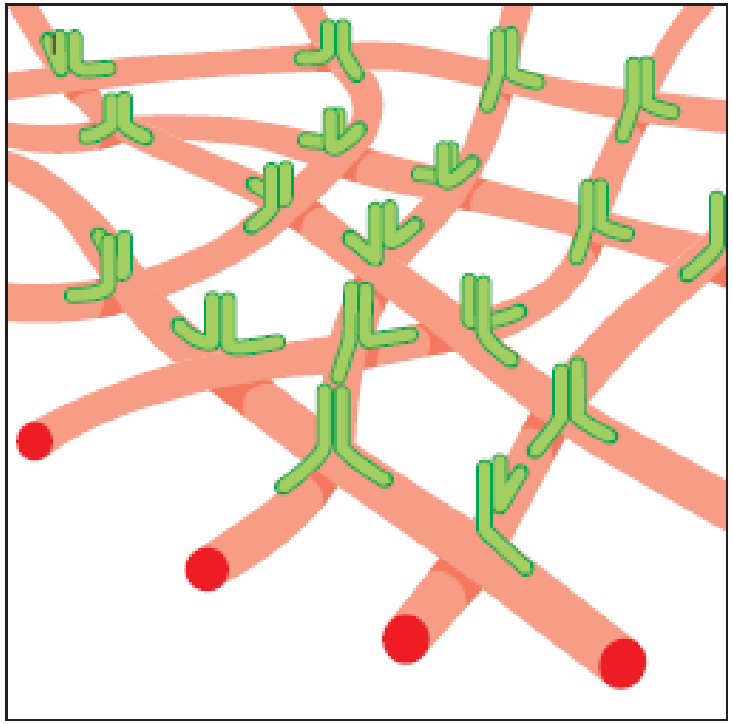
Figure 16–17 Actin elongation mediated by formins. Formin proteins *(green)* form a dimeric complex that can nucleate the formation of a new actin filament *(red)* and remain associated with the rapidly growing plus end as it elongates. The formin protein maintains its binding to one of the two actin subunits exposed at the plus end as it allows each new subunit to assemble. Only part of the large dimeric formin molecule is shown here. Other regions regulate its activity and link it to particular structures in the cell. Many formins are indirectly connected to the cell plasma membrane and aid the insertional polymerization of the actin filament directly beneath the membrane surface.

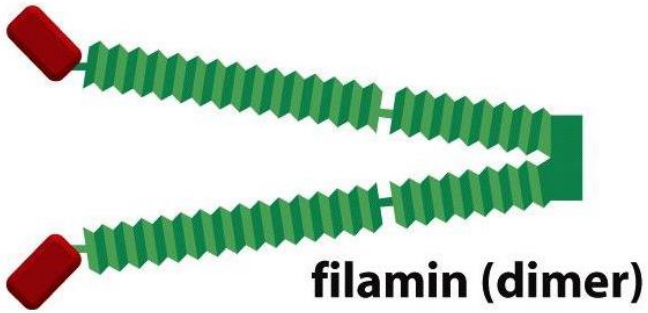
Entrecruzamiento de filamentos de actina en haces paralelos



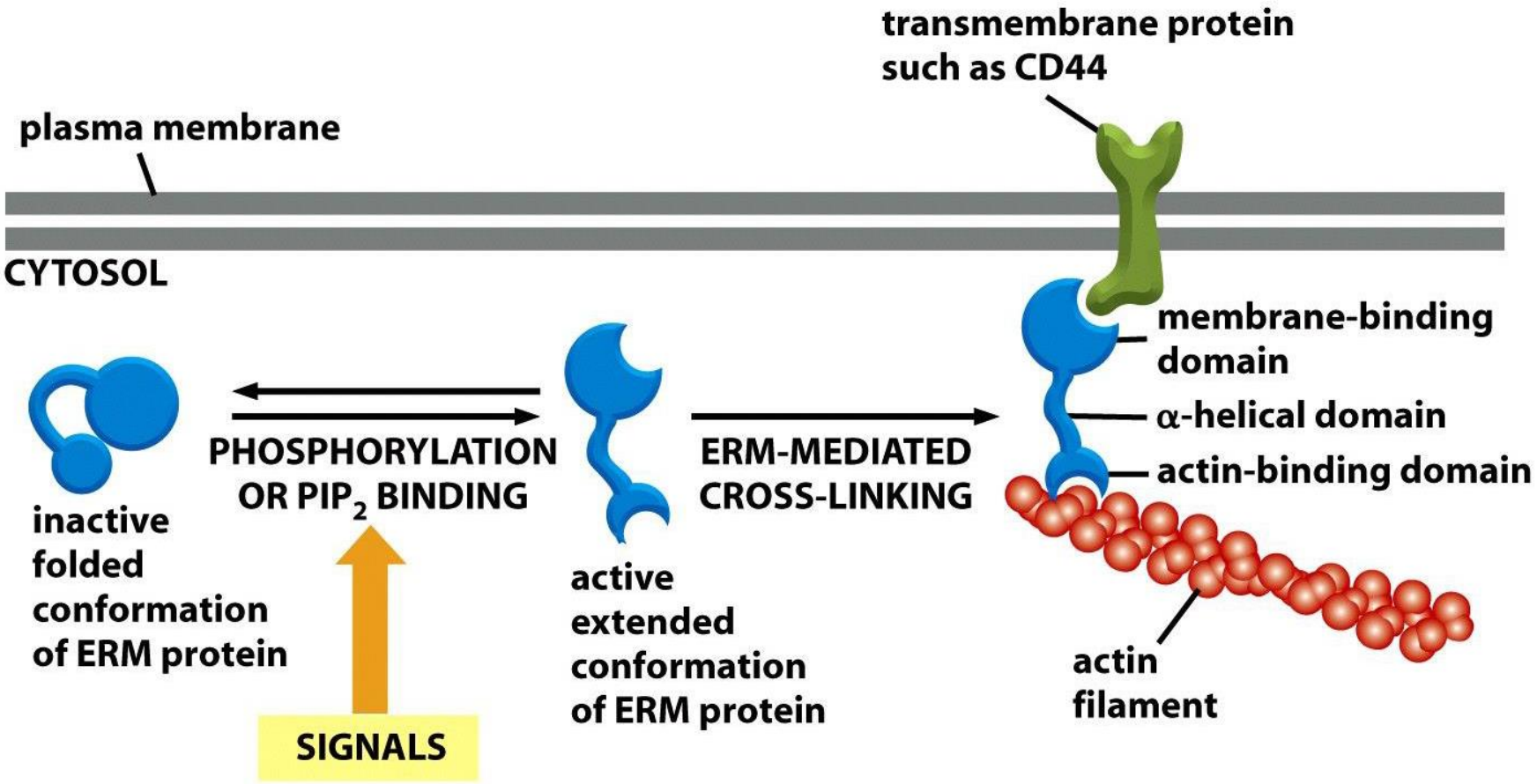
Entrecruzamientos por filaminas – formación de geles



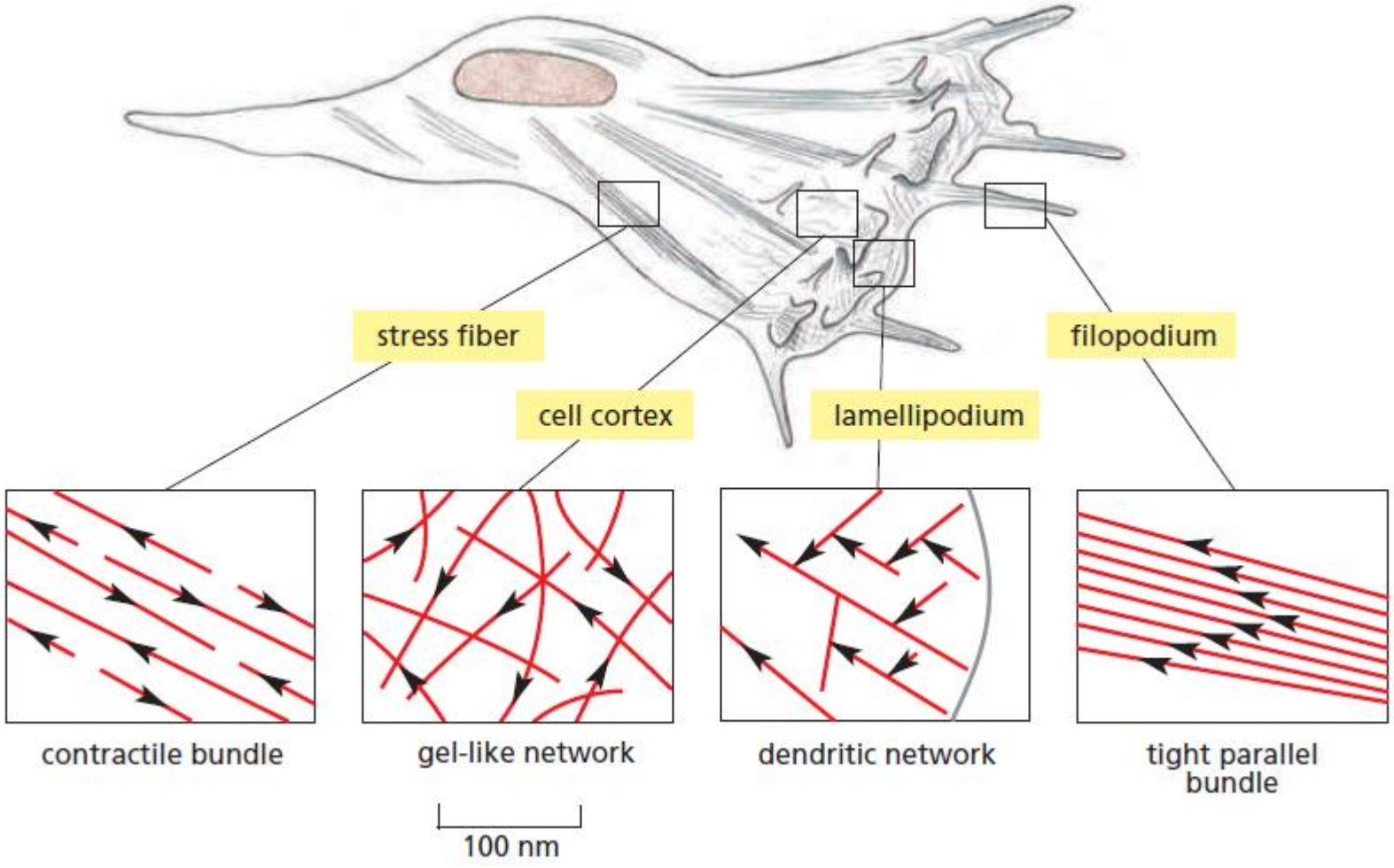




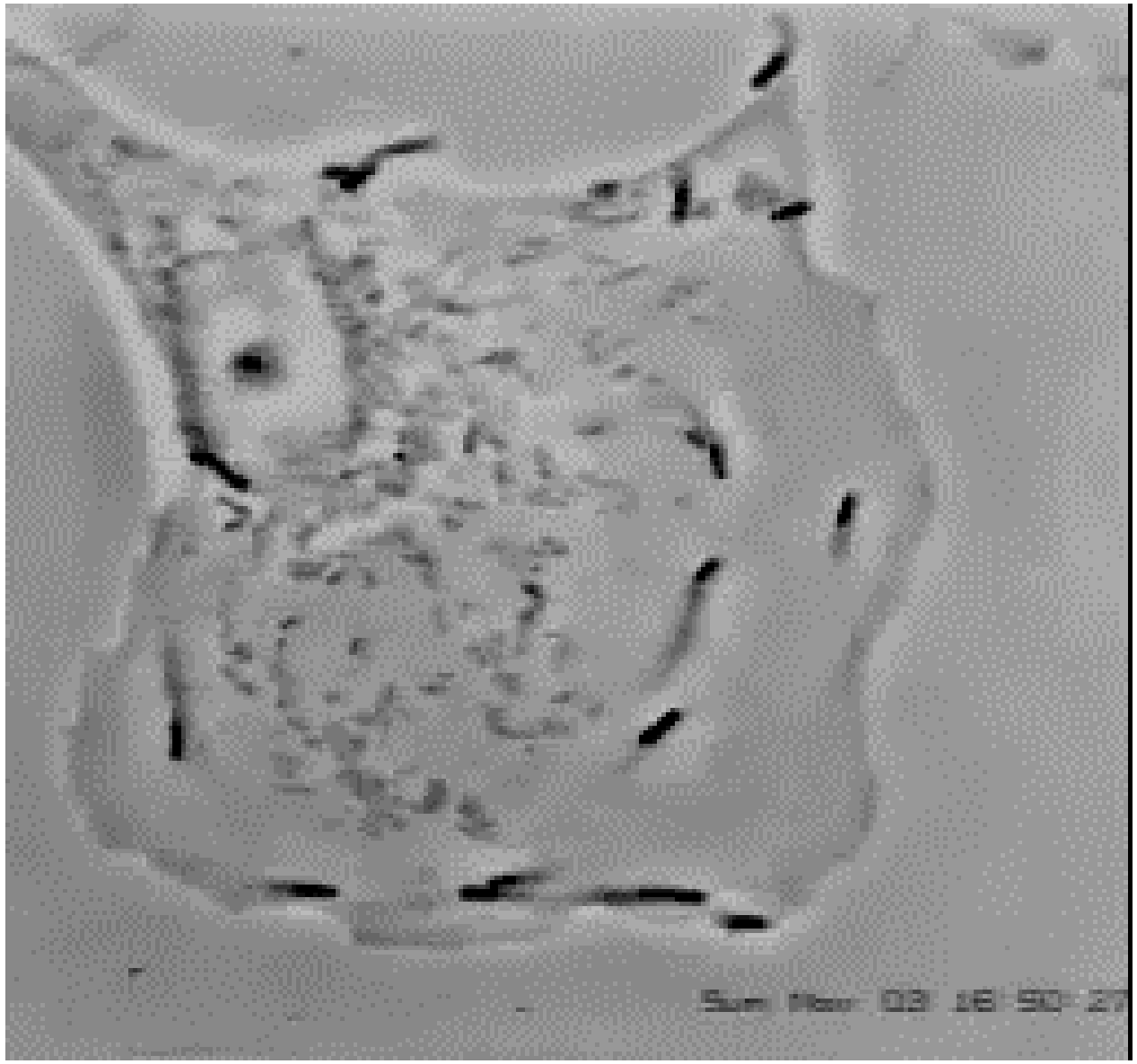
Interacciones entre el citoesqueleto de actina y la membrana plasmática



Ejemplo: migración celular (teórico 18)



TALLER: Infección intracelular por *Listeria monocitogenes*



https://www.youtube.com/watch?v=UUjFuxFTBrI

¿Como determinar si la motilidad intracelular de *Listeria* depende de la polimerización de Actina?

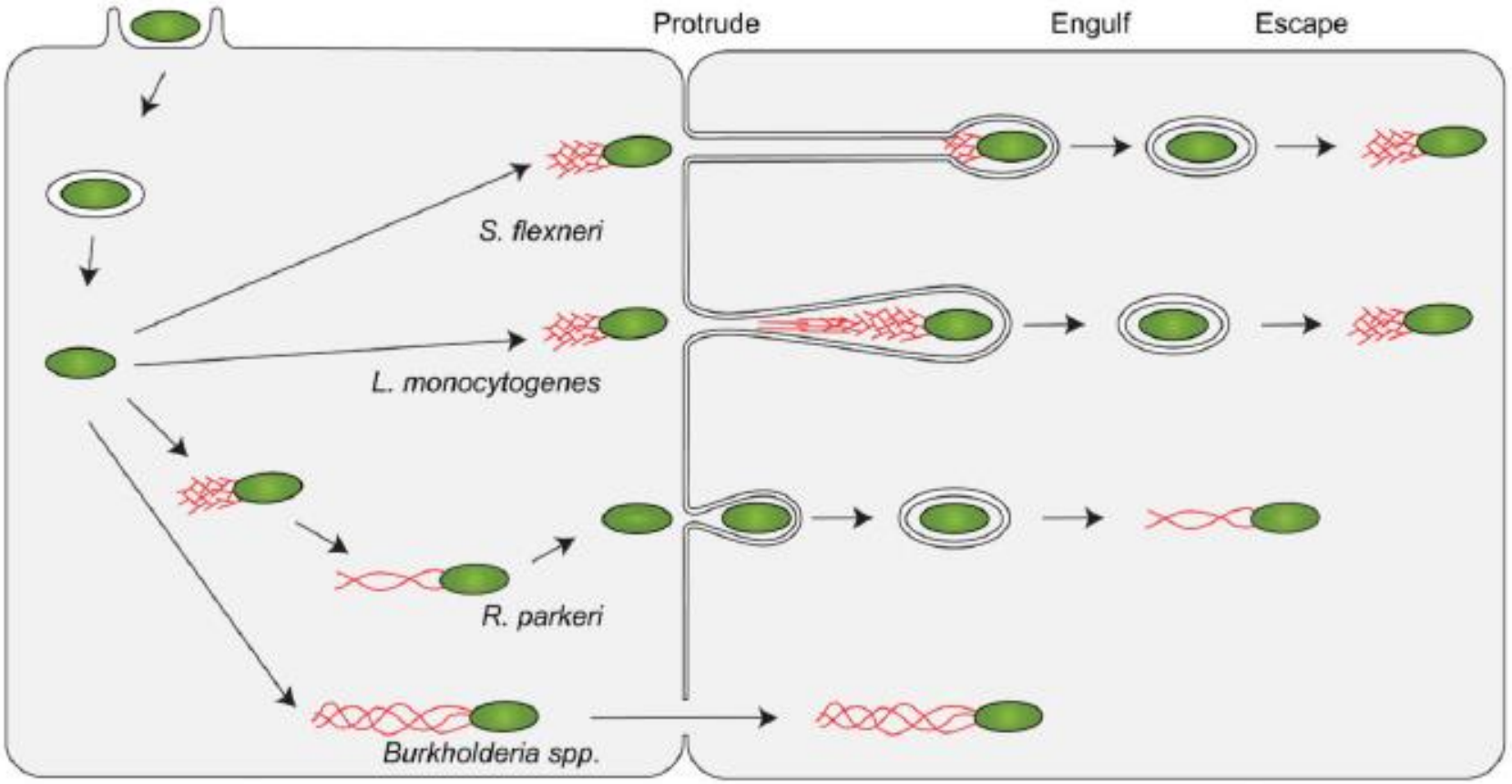
¿Como determinar si la motilidad intracelular de *Listeria* depende de la polimerización de Actina?

TABLE 16–1 Chemical Inhibitors of Actin and Microtubules			
Chemical	Effect on filaments	Mechanism	Original source
Actin			
Latrunculin	Depolymerizes	Binds actin subunits	Sponges
Cytochalasin B	Depolymerizes	Caps filament plus ends	Fungi
Phalloidin	Stabilizes	Binds along filaments	*Amanita* mushroom

Polimerización de actina en una célula viva infectada

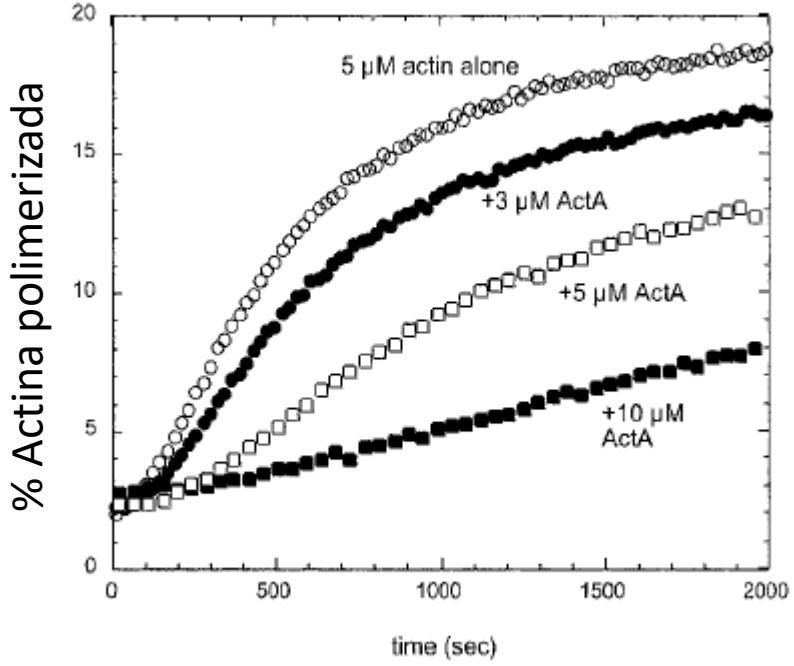
Cómo podemos ver in vivo la distribución de la actina?

https://www.youtube.com/watch?v=bF-7YXgQK14


Protrude
Engulf
Escape
S. flexneri
L. monocytogenes
R. parkeri
Burkholderia spp.

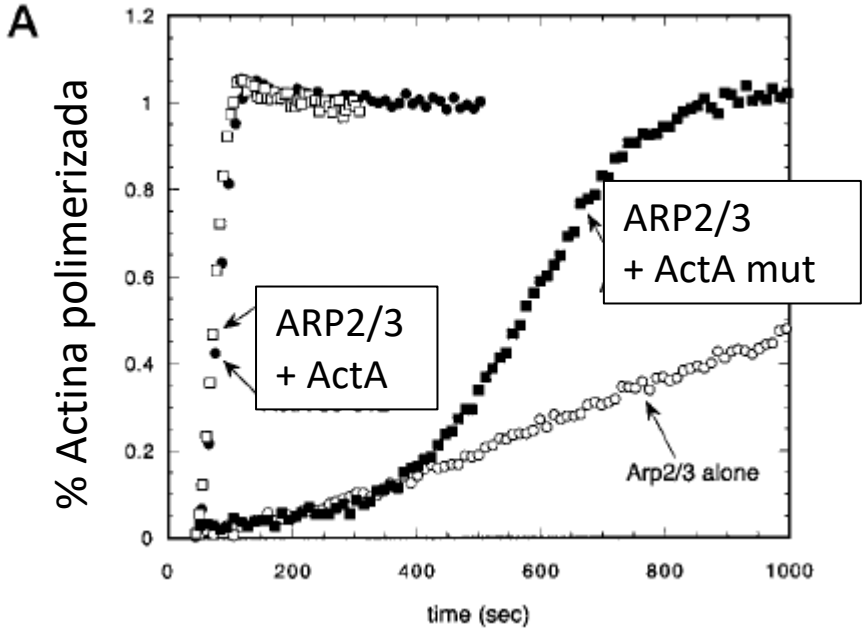
Ejercicio: ActA – proteína de *Listeria*, esencial para la formación de “cometas”

Experimento 1:
Actina purificada + ActA purificada
Polimerización *in vitro*



Tiempo

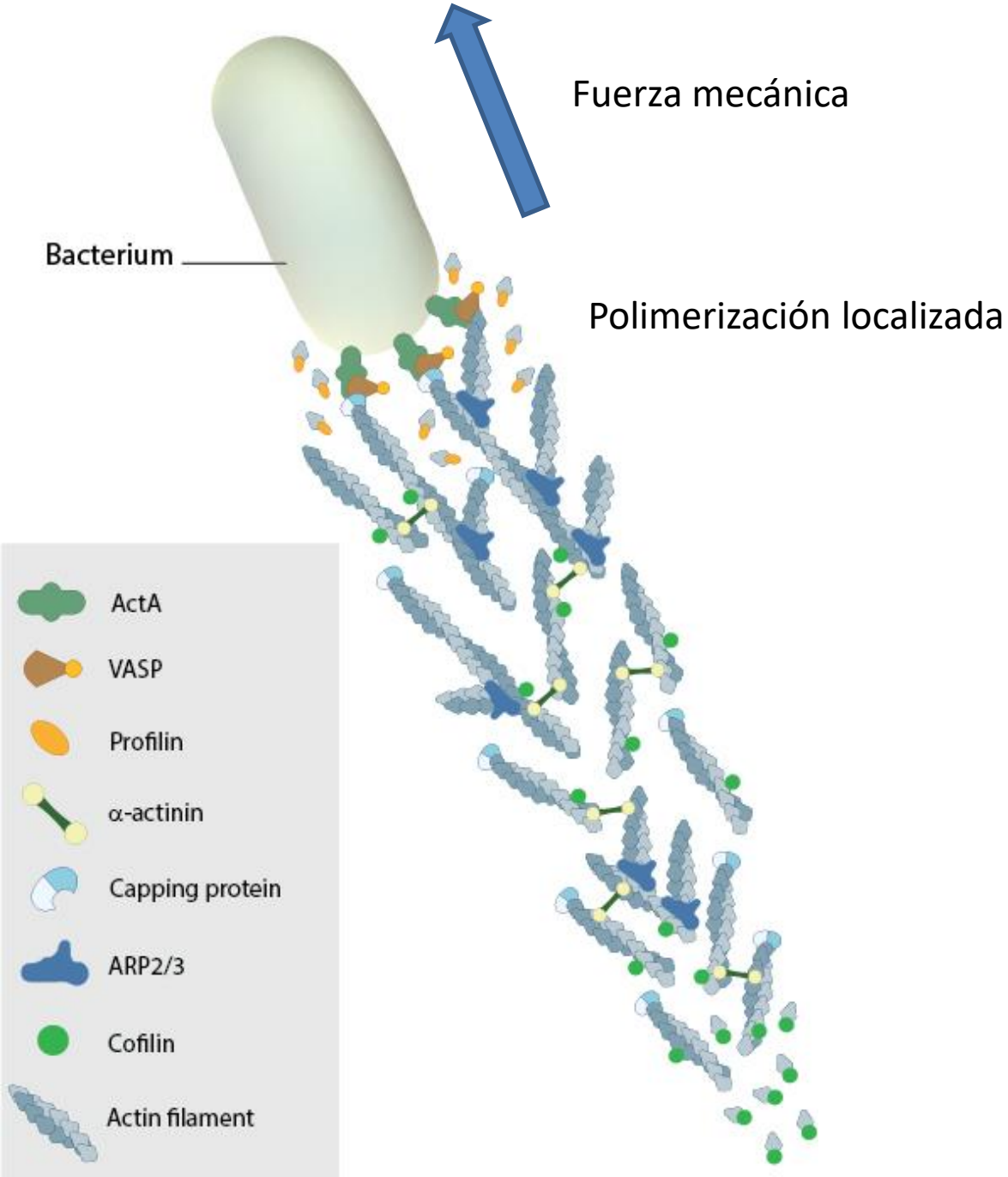
Experimento 2:
Actina purificada + ARP2/3
+ ActA purificada
Polimerización *in vitro*



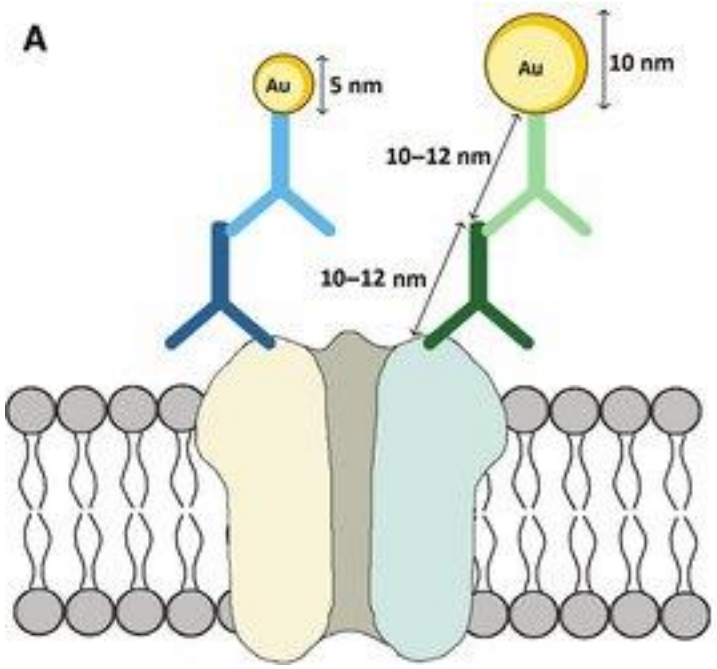
Tiempo

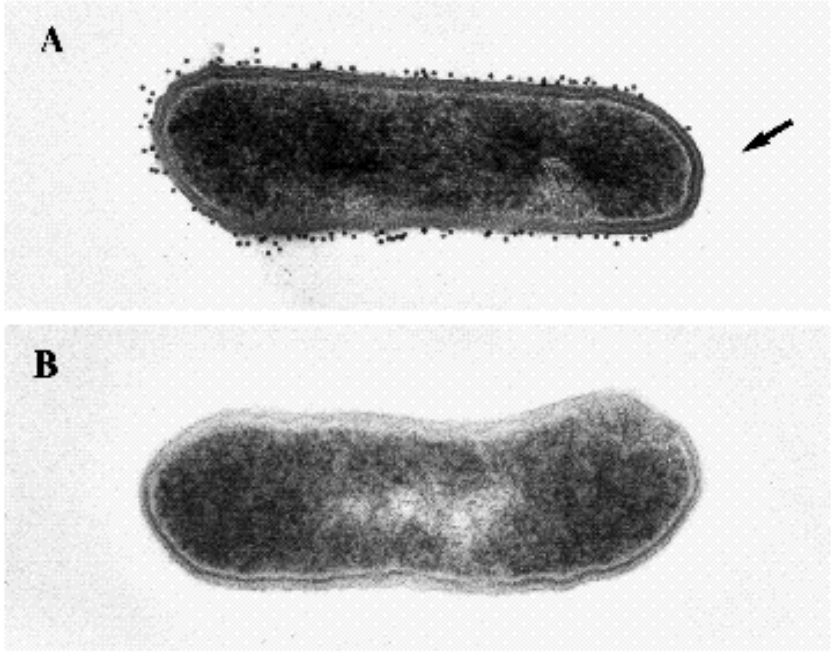
¿Cómo promueve ActA la formación de cometas?

Zalevsky et al 2001


Fuerza mecánica
Bacterium
Polimerización localizada
ActA
VASP
Profilin
α-actinin
Capping protein
ARP2/3
Cofilin
Actin filament

¿Cómo promueve ActA la formación POLARIZADA de cometas?

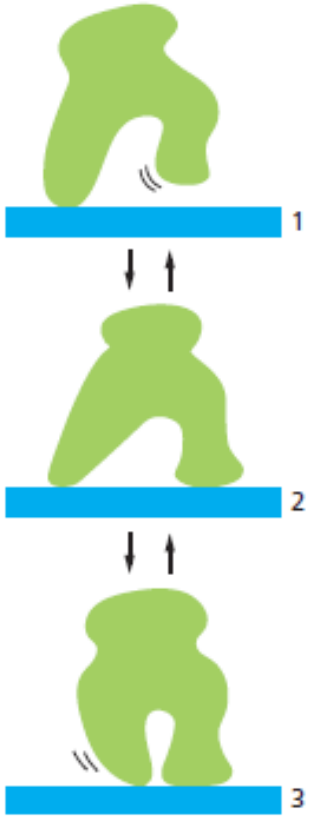


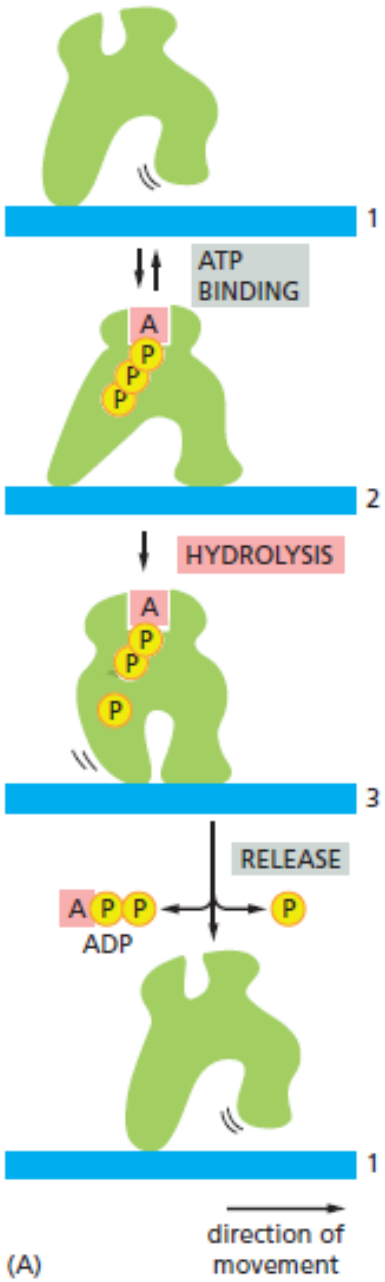


Proteínas motoras

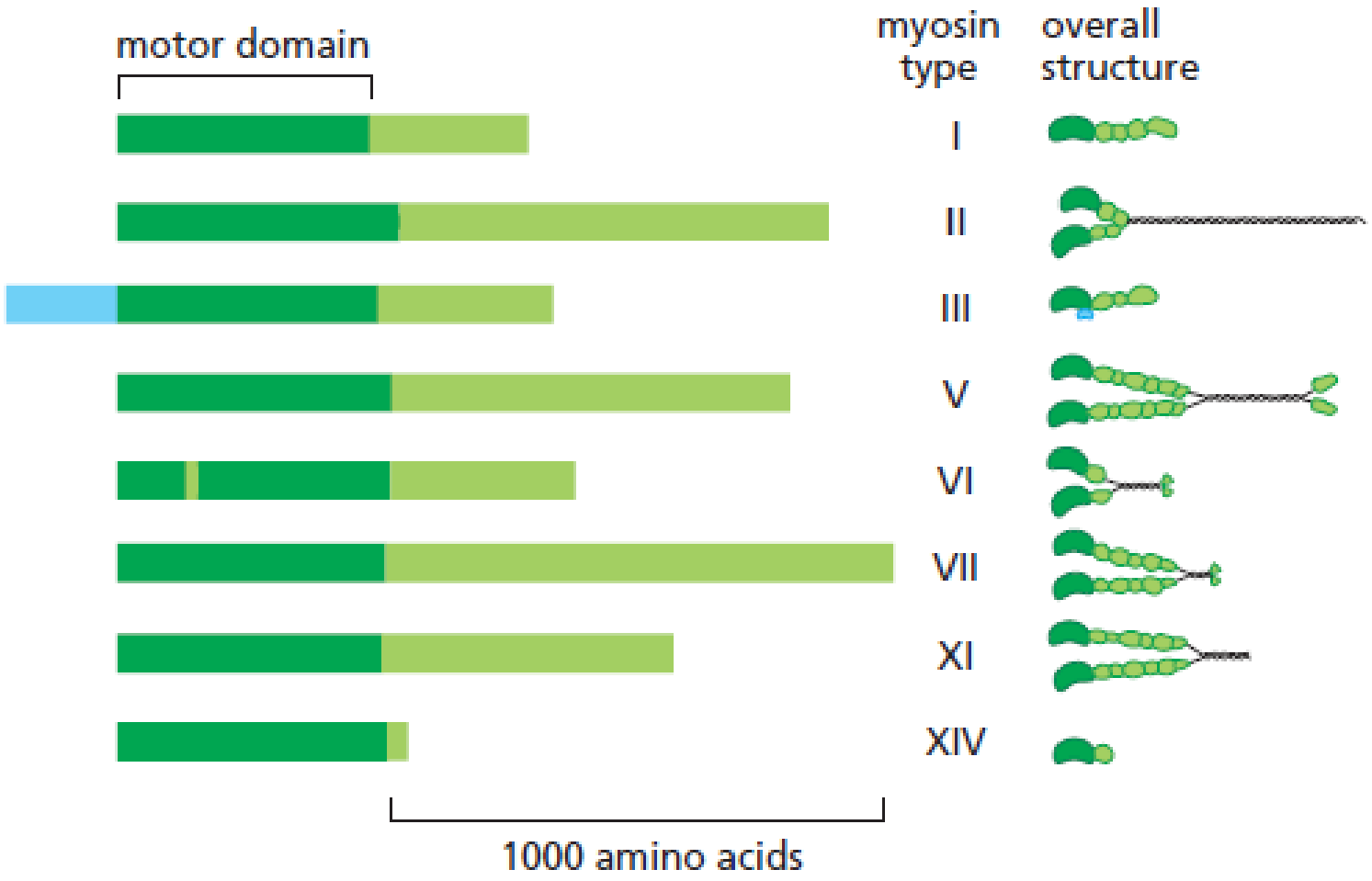
Unen a filamentos del citoesqueleto, y acoplan energía de hidrólisis de ATP a movimiento direccional sobre los mismos

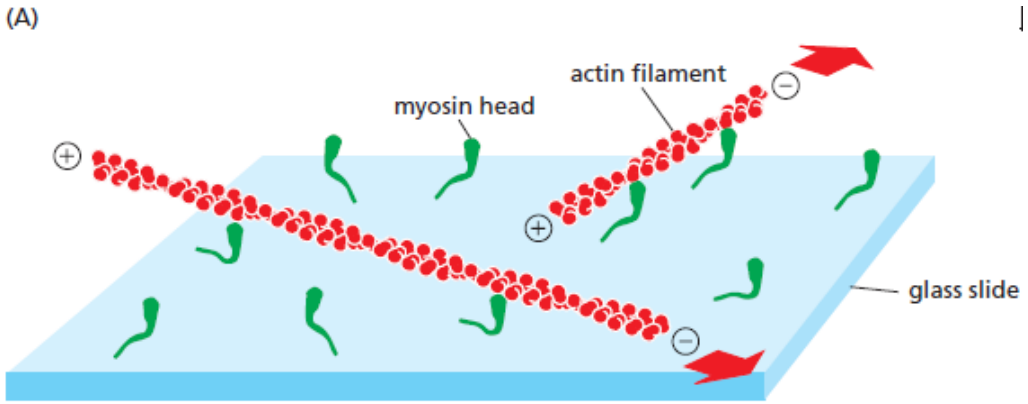
En el caso de Actina, motores moleculares de la familia de las **Miosinas**, que “caminan” hacia el extremo (+) de los microfilamentos.


1
2
3

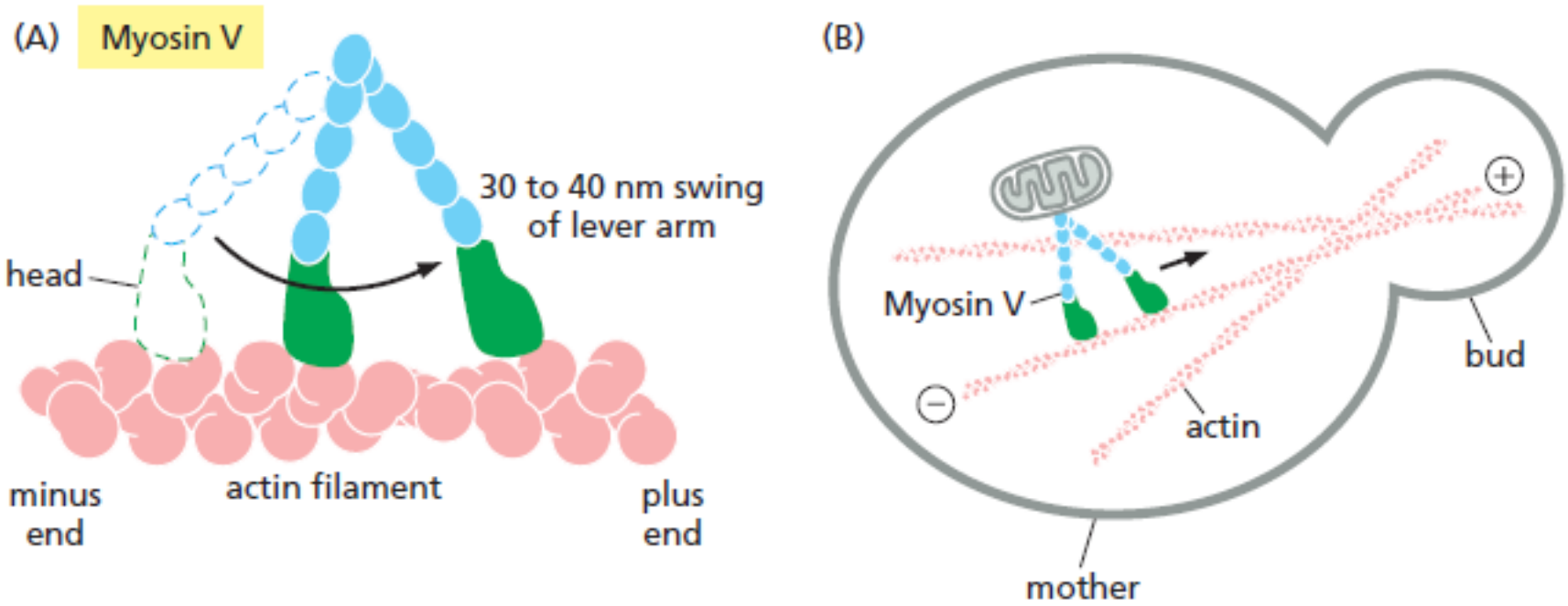

1
ATP
BINDING
A
P
P
P
2
HYDROLYSIS
A
P
P
P
3
RELEASE
A P P
ADP
P
1
direction of
movement
(A)

Diferentes tipos de motores tipo miosina tienen diferentes funciones en la célula

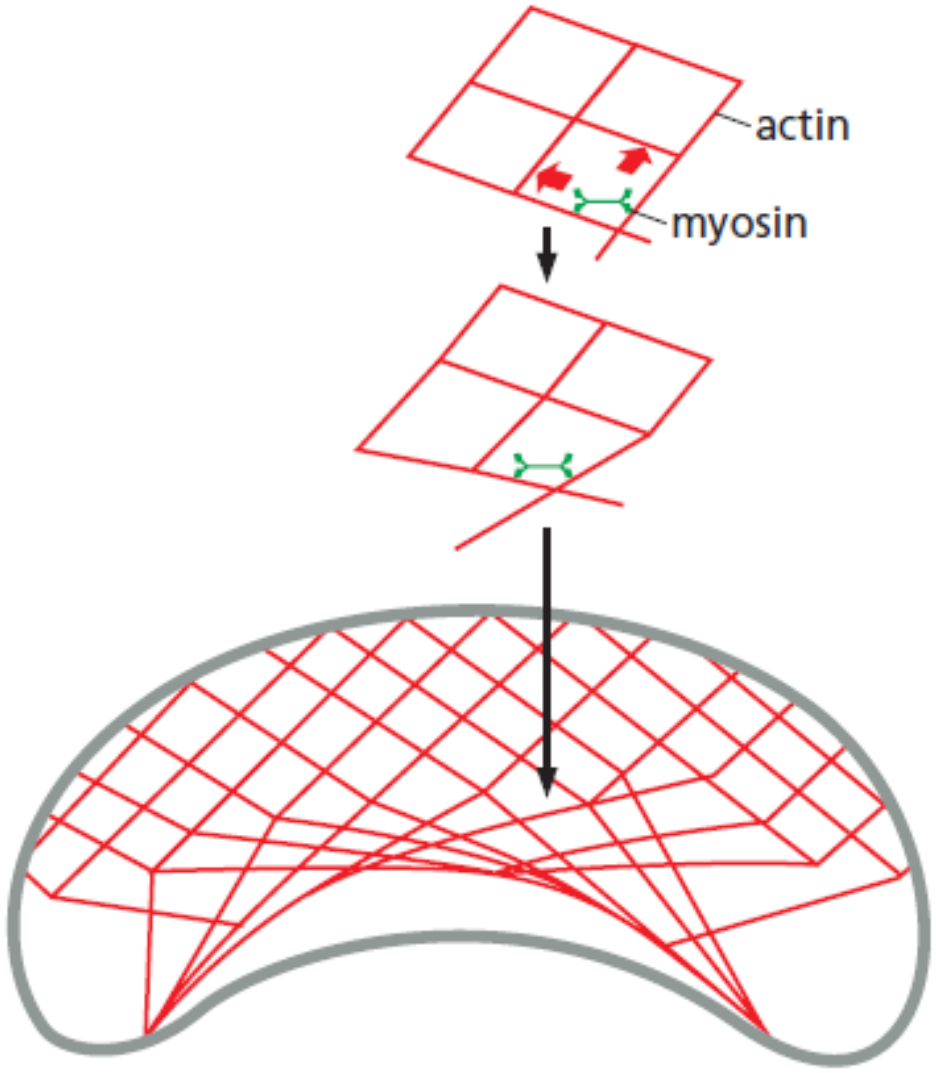



(A)
actin filament
myosin head
glass slide

Miosina V


(A)
Myosin V
30 to 40 nm swing
of lever arm
head
minus
end
actin filament
plus
end
(B)
Myosin V
actin
bud
mother

Miosina II



(A)

TO BE
CONTINUED...