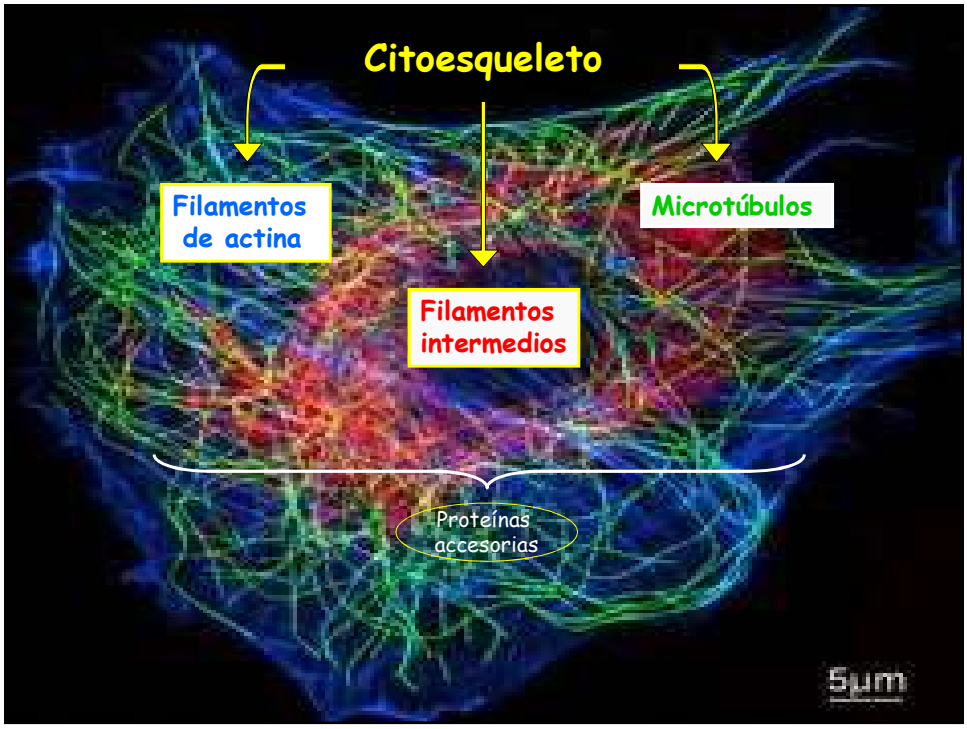
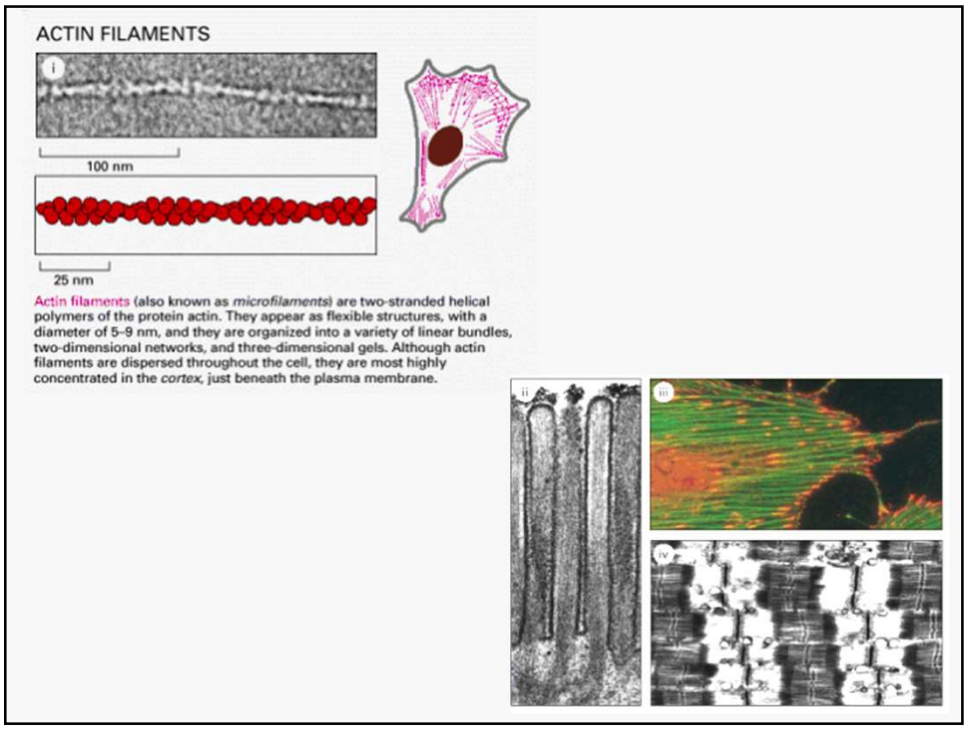
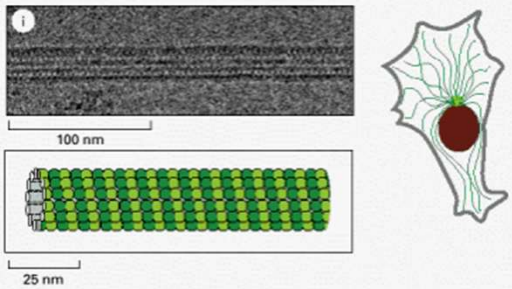

Citoesqueleto
Filamentos de actina
Microtúbulos
Filamentos intermedios
Proteínas accesorias
5µm

1

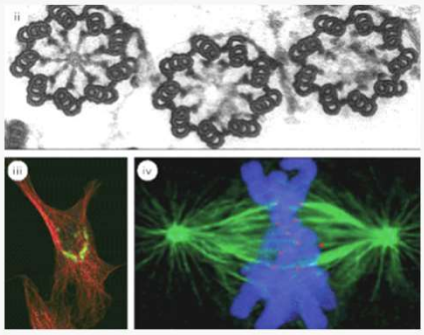

ACTIN FILAMENTS
100 nm
25 nm
Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

2

MICROTUBULES

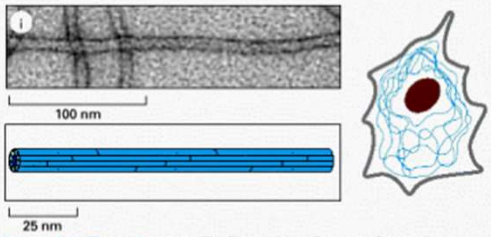


Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a

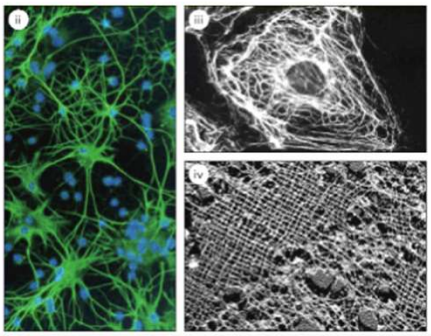


3

INTERMEDIATE FILAMENTS

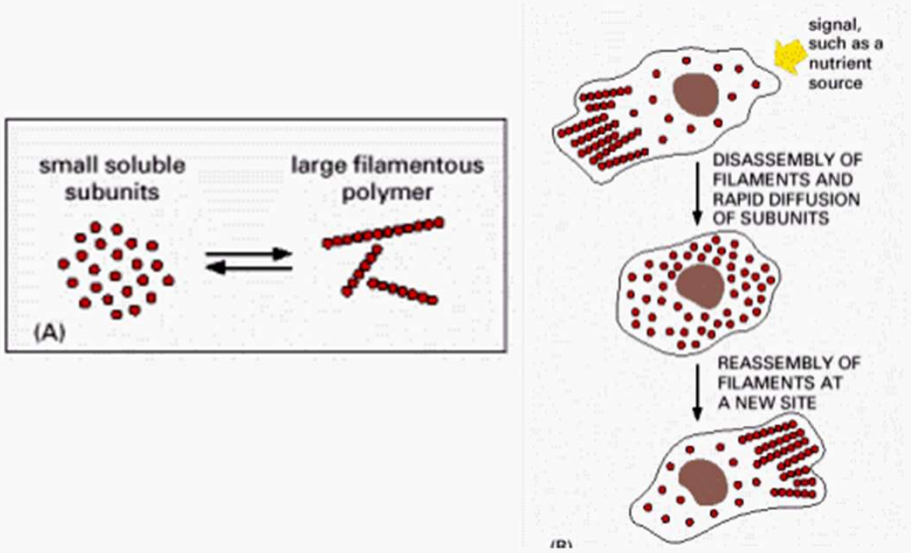


Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types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



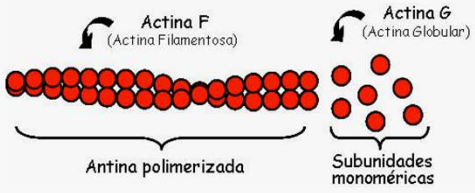
4

Citoesqueleto: estructura dinámico

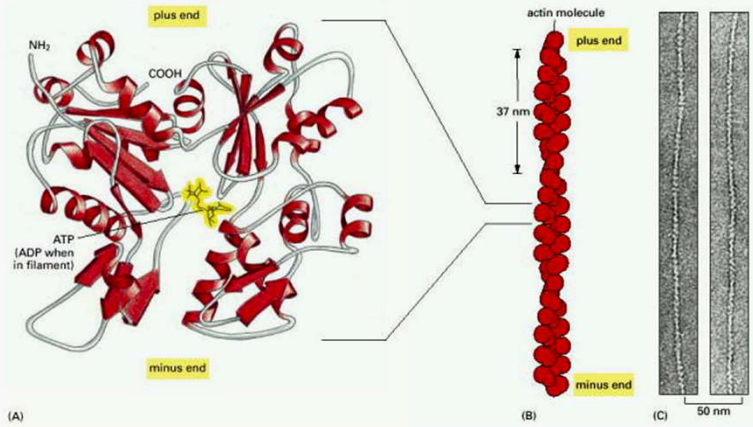


5

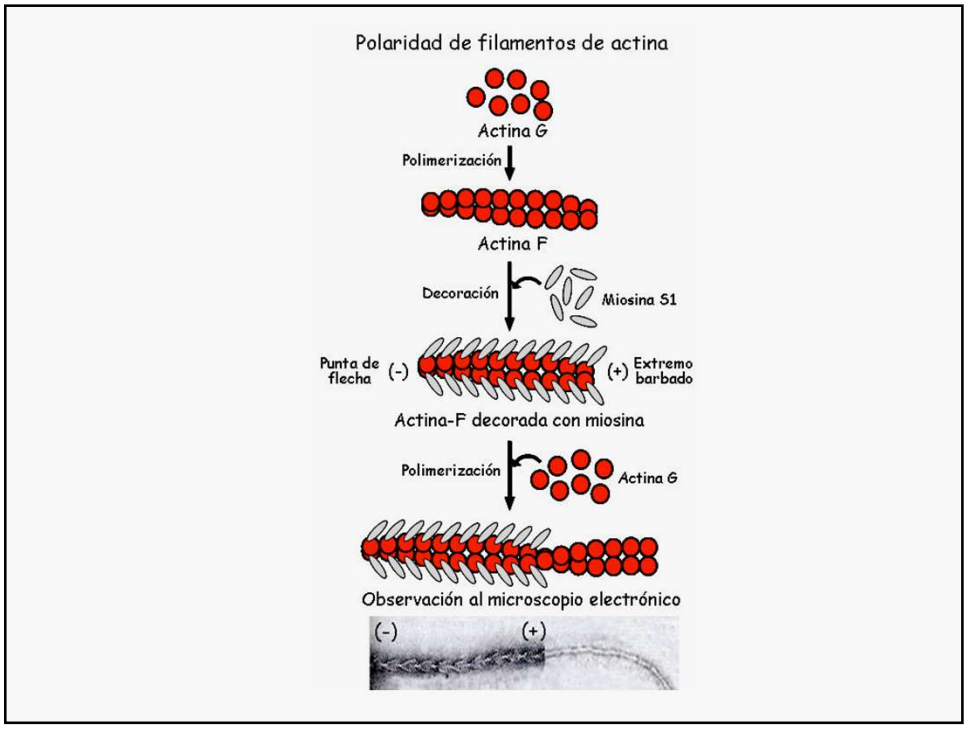
Filamentos de actina



Polaridad



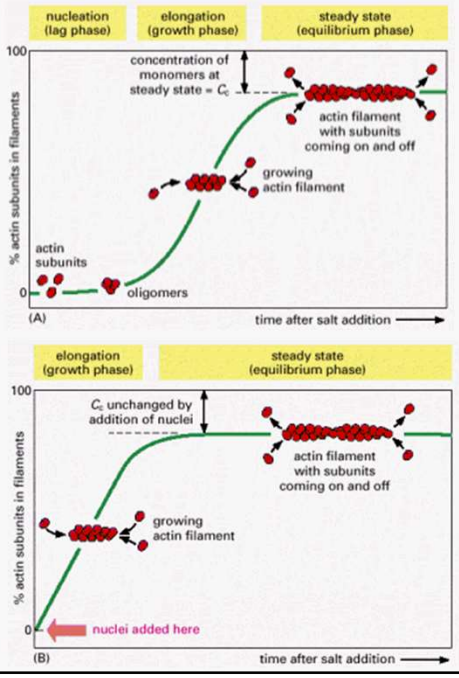
6



7

Polimerización de actina in vitro

$$Cc = \frac{K \text{ pérdida de subunidades}}{K \text{ adición de subunidades}}$$

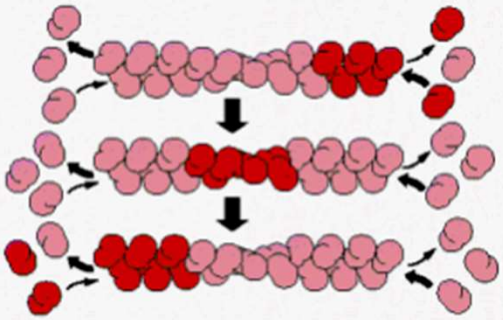


8

Treadmilling: flujo neto de subunidades a lo largo del polímero

$$Cc = \frac{K \text{ pérdida de subunidades}}{K \text{ adición de subunidades}}$$

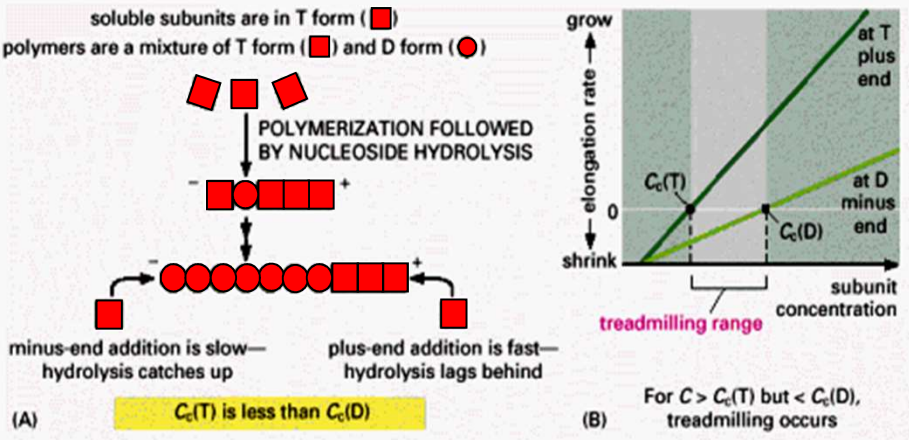
C_c (minus end) > C_c (plus end)



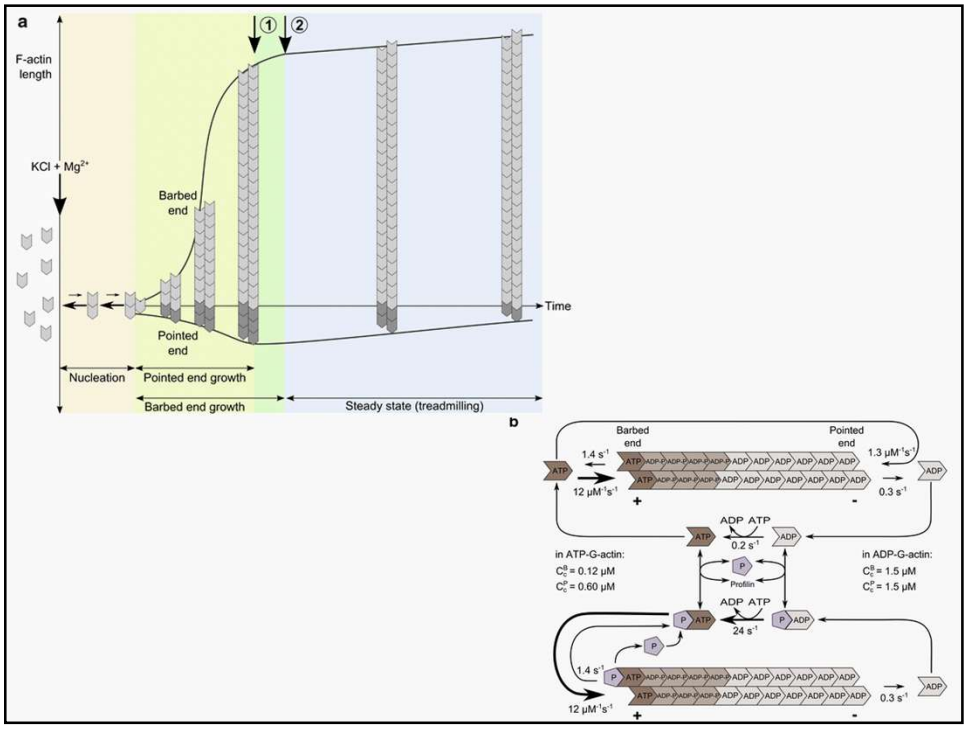
9

Actina: enzima que hidroliza ATP; muy activa cuando esta incorporada al filamento

La vel. relativa de hidrólisis y la vel. de adición de subunidades determina si la subunidad en el extremo del filamento se encuentra en la forma T o forma D



10



11

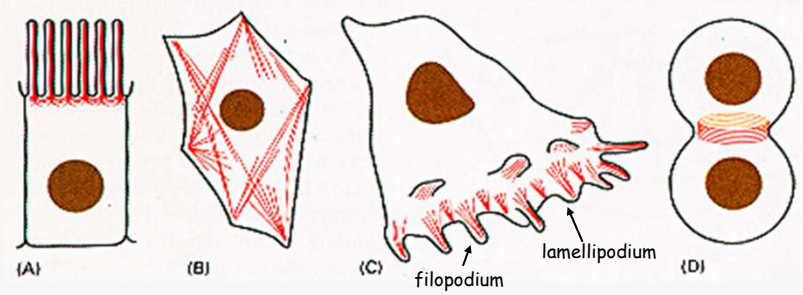
Diferentes estructuras y funciones de filamentos de actina

Microvilli: intestinal cells

Contractile bundles: cytoplasm

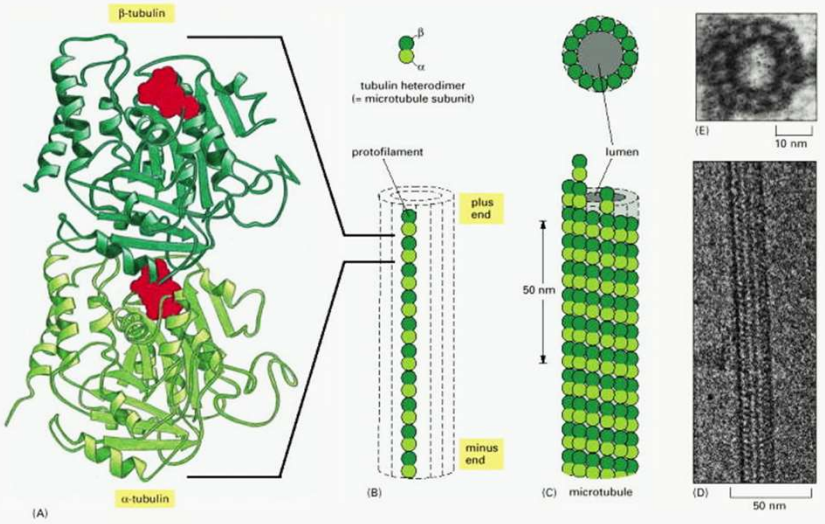
Lamellipodia and filopodia: leading edge of migrating cells

Contractile ring: between dividing cells



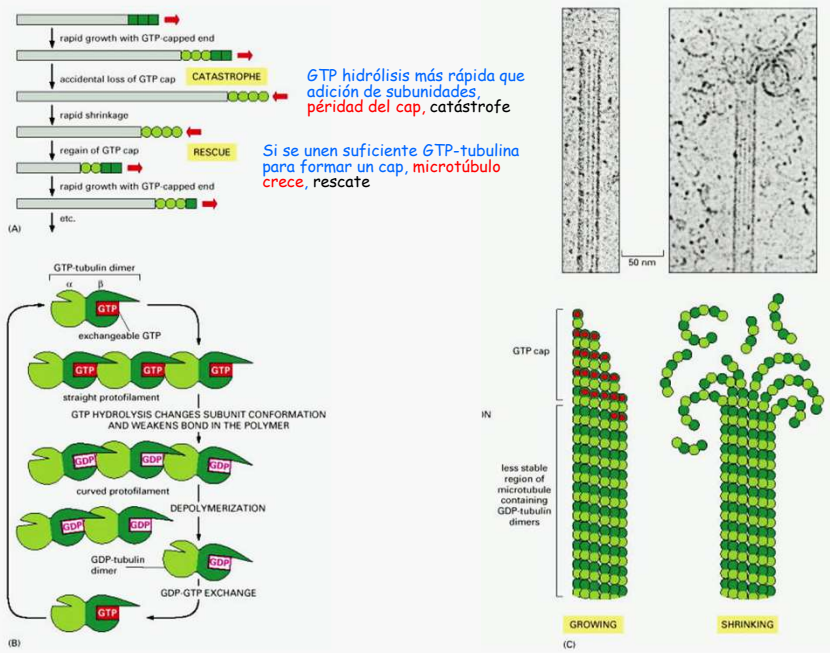
12

Microtúbulos



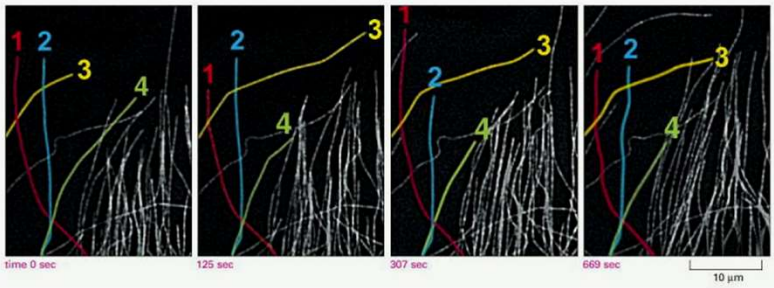
13

Inestabilidad dinámica



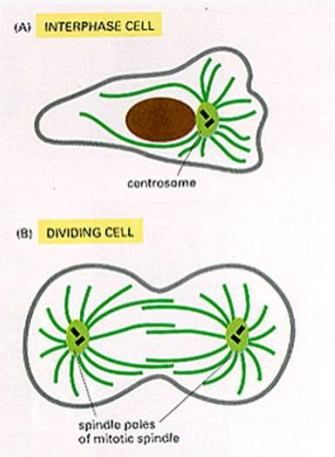
14

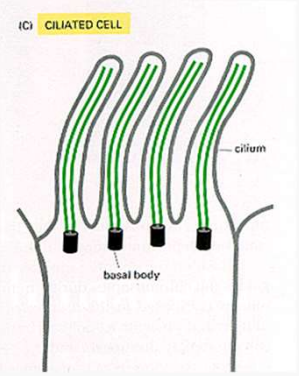
Inestabilidad dinámica en una célula viva



15

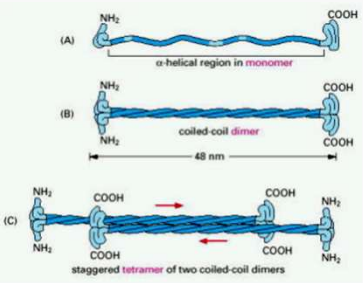
Diferentes estructuras y funciones de microtúbulos

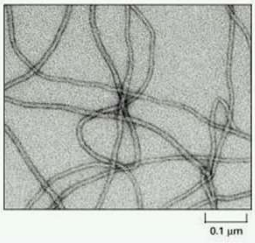




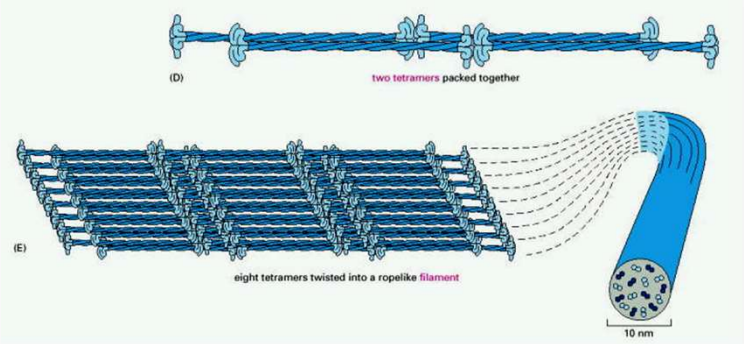
16

Filamentos intermedios





—> subunidad,
extremos = —> polímero NO polar



17

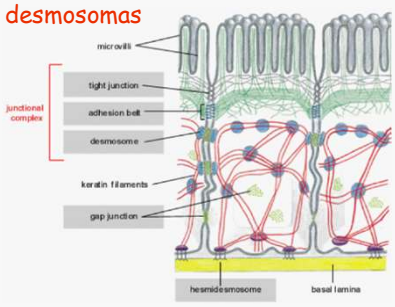
- FI amplia variedad de tipos: variaciones de secuencia en las diferentes isoformas
- Dominio α-hélice: motivos repetidos compuestos por 7 aa (tb. de 11aa), forman "coiled-coil", similares en las ≠ isoformas
- Dominios globulares N y C- terminales, varían entre las ≠ isoformas
- FI varían dentro de un mismo tipo celular y dentro de una célula: heterodímeros

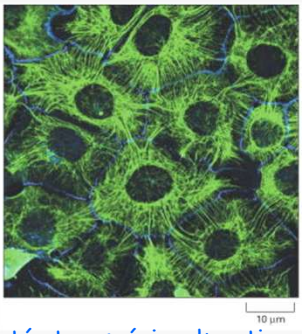
Principales tipos de proteínas de FI en células de vertebrados

TYPES OF IF	COMPONENT POLYPEPTIDES	CELLULAR LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	- vimentin - desmin - glial fibrillary acidic protein - peripherin	- many cells of mesenchymal origin - muscle - glial cells (astrocytes and some Schwann cells) - some neurons
Epithelial	- type I keratins (acidic) - type II keratins (basic)	epithelial cells (20 k. in ≠ types of human epithelia cells) and their derivatives (10 k) (e.g., hair and nails)
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

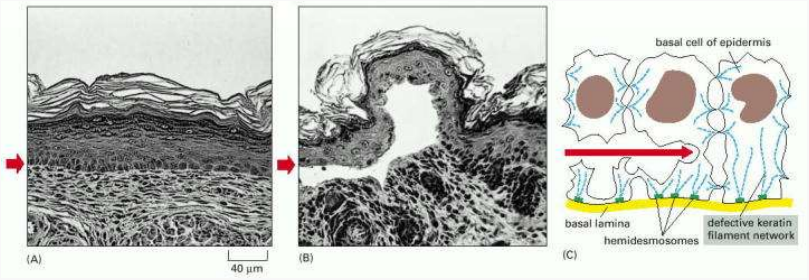
18

Filamentos de keratina en células epiteliales: desmosomas





Ampollas causadas por mutaciones en keratinas: ratón transgénico, keratina truncada en los extremos en N y C (≈ epidermólisis bulbosa simple: mutaciones en el dominio central)

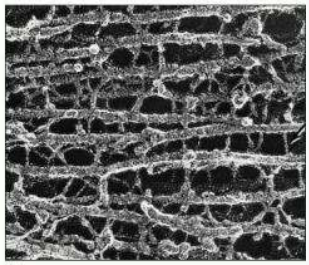


19

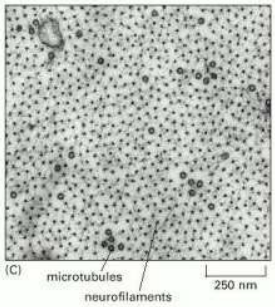
Dos tipos de FI (neurofilamentos) en células del sistema nervioso

- Heteropolímeros: NF-L:NF-M o NF-L:NF-H
- C-terminal de NF-M o NF-H: largo —> uniones cruzadas entre NF dinámicos: incorporación lateral o terminal de sudunidades —> crecimiento de axón en diámetro y longitud
- Esclerosis lateral amiotrófica: acumulación y ensamble anormal de NF en el cuerpo y axón de neuronas motoras —> debilidad y atrofia muscular

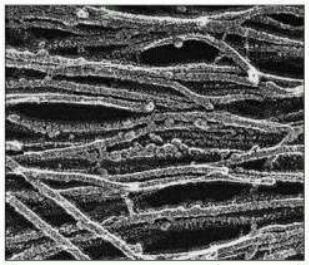
Neurofilamentos en axón: puentes cruzados C-terminal NF-H (Freeze-etch)



Sección transversal de un axón: NF y Mts



Filamentos gliales en células gliales: lisos y pocos cross-bridges (Freeze-etch)



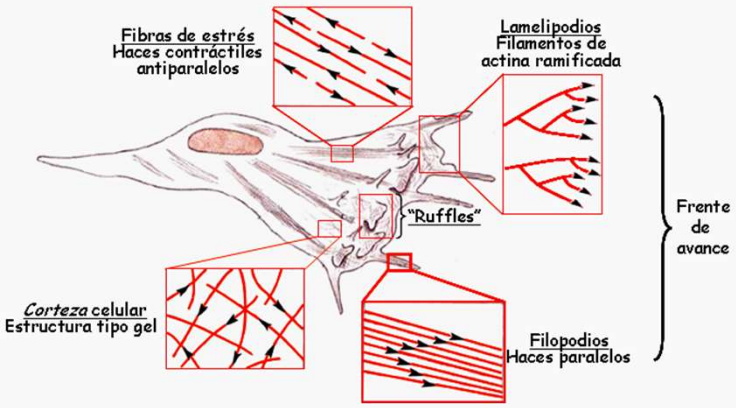
20

Nucleación de actina

- Interacción con la membrana: corteza celular (cell cortex): forma y movimiento de la superficie celular

- Microvellosidades, filipodios y lamelipodios

Dinámica y estructuración de los microfilamentos en una célula en movimiento



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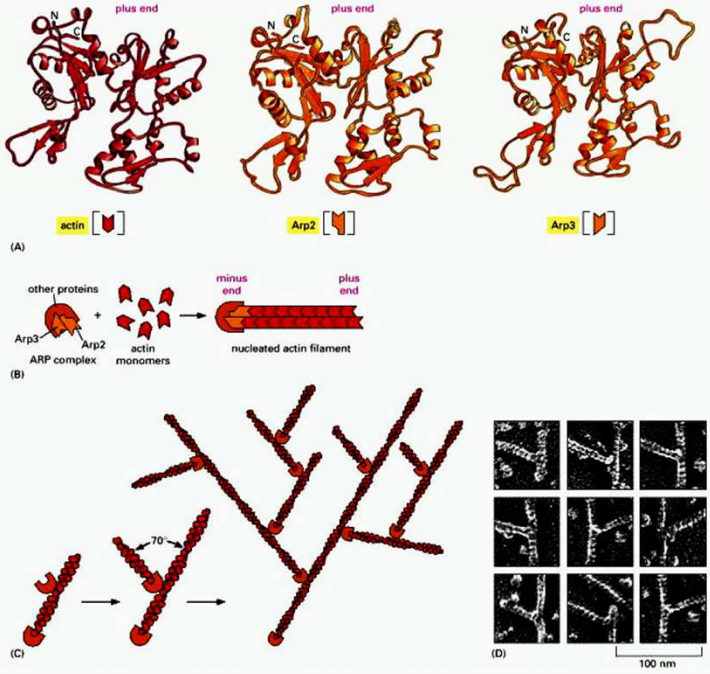
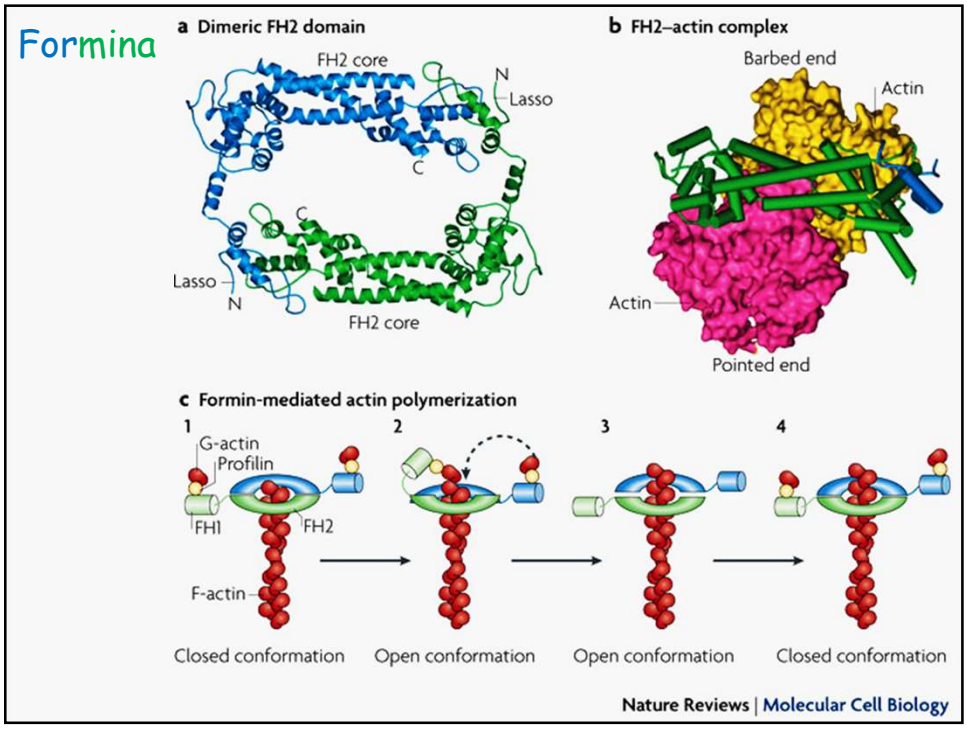
Complejo Arp2/3



22


Formina
a Dimeric FH2 domain
FH2 core
N
Lasso
C
C
Lasso
N
FH2 core
b FH2–actin complex
Barbed end
Actin
Actin
Pointed end
c Formin-mediated actin polymerization
1
2
3
4
G-actin
Profilin
FH1
FH2
F-actin
Closed conformation
Open conformation
Open conformation
Closed conformation
Nature Reviews | Molecular Cell Biology

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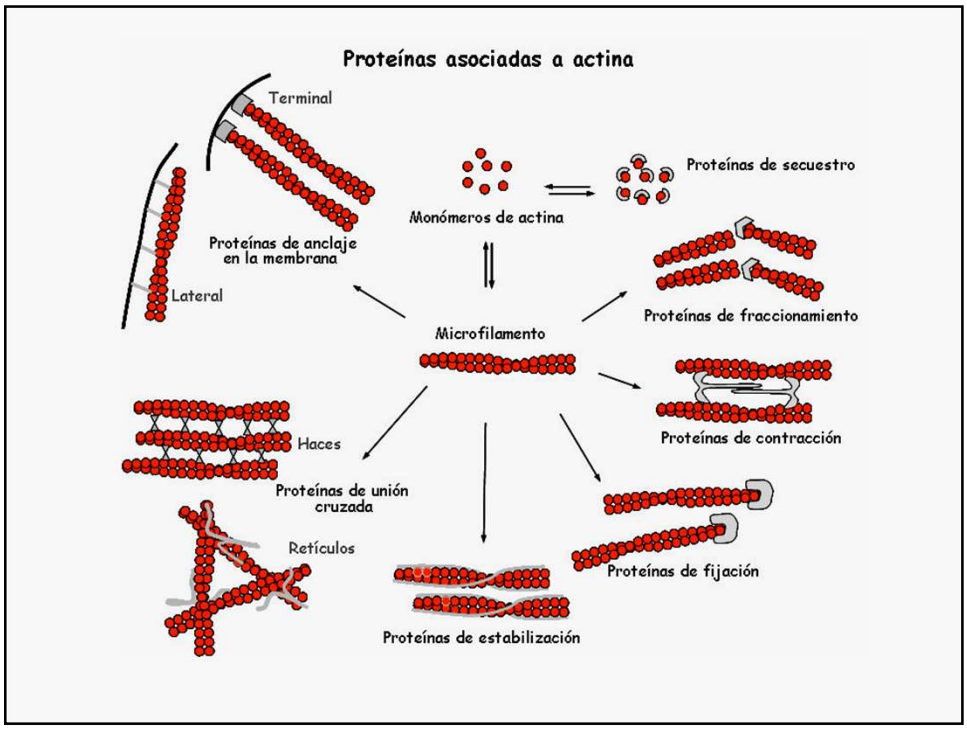
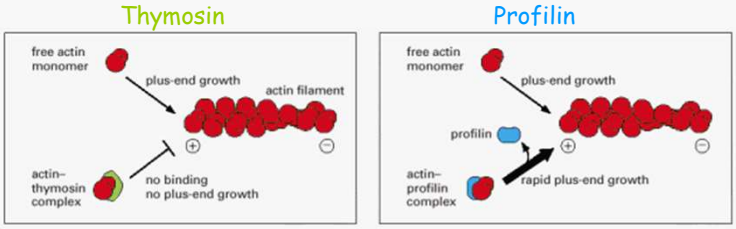

Proteínas asociadas a actina
Terminal
Proteínas de anclaje en la membrana
Lateral
Monómeros de actina
Proteínas de secuestro
Microfilamento
Proteínas de fraccionamiento
Haces
Proteínas de unión cruzada
Retículos
Proteínas de contracción
Proteínas de fijación
Proteínas de estabilización

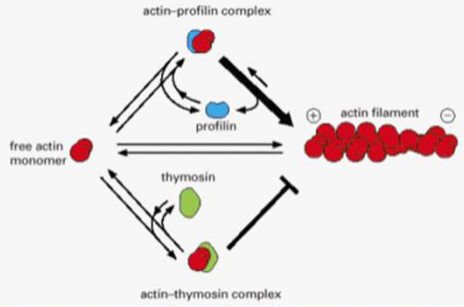
24

Proteínas que se unen a actina-G: proteínas de secuestro



Actina, unida a timosina, esta en un estado cerrado: no puede hidrolizar ni intercambiar el nucleótido unido

Profilina se une al extremo opuesto al bolsillo de ATP —> bloquea sitio de unión al extremo - del filamento. El complejo puede unirse al extremo +

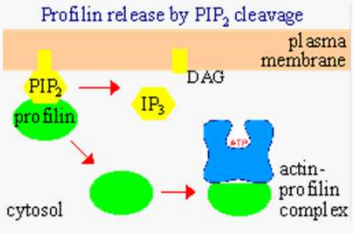


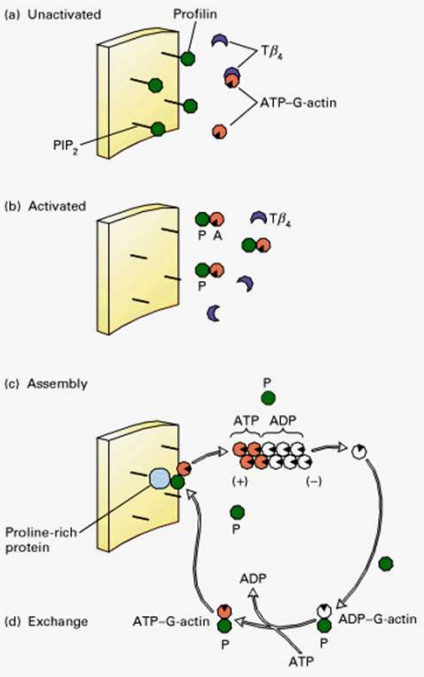
PROFILIN COMPETES WITH THYMOSIN FOR BINDING TO ACTIN MONOMERS AND PROMOTES ASSEMBLY

25

Regulación de profilina

phosphatidyl inositol (in membrane)
↓ kinases
PIP_2
Phospholipase C (signal activated)
IP_3 — diacylglycerol
causes Ca^{++} release from ER — activates Protein Kinase C





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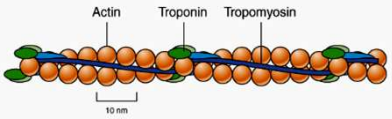
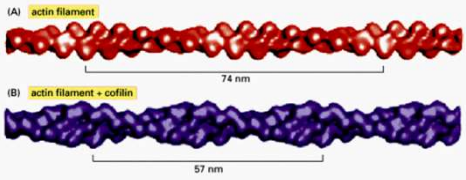
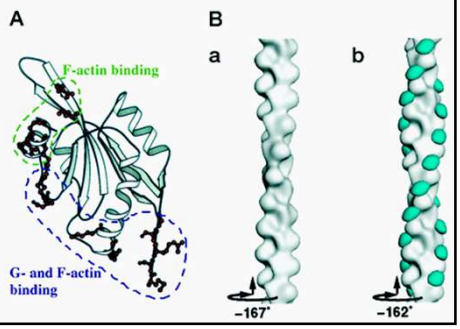
Proteínas que se unen lateralmente a los filamentos: estabilizan o desestabilizan

Tropomiosina:- se une cada 7 subunidades:
previene unión de otras proteínas
- estabiliza actina-F



Cofilina (ADF): - se une al actina-F: desestabiliza.
- también se une a actina-G
- se une preferentemente a actina-ADP: filamentos más viejos más sensibles
- filamentos más nuevos: actina-ATP —> más resistentes





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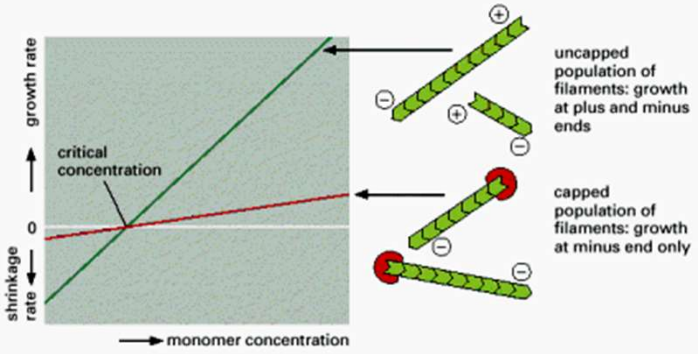
Proteínas que se unen a los extremos de filamentos: proteínas de fijación ("capping proteins)



CapZ: extremo (+) —> inhibe polimerización de filamentos
(PIP2 inactiva la proteína fijadora —> "uncapping")
Tropomudulina en músculo: extremo (-) de filamentos cubiertos con tropomiosina —> estabilización de filamentos

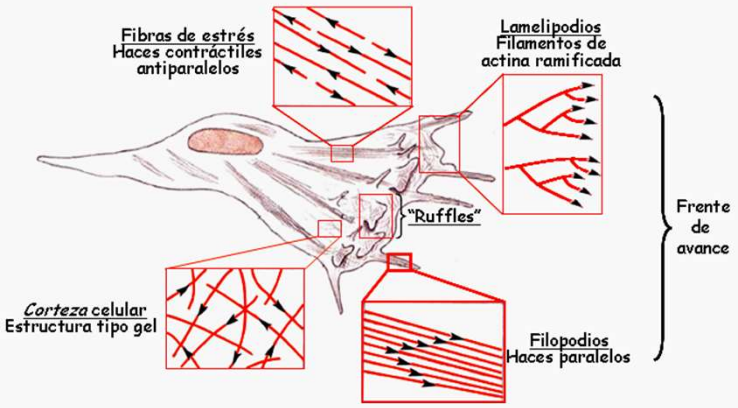
ARP2/3: extremo (-). En células típicas es posible que ARP se despegue —> entremos (-)

28

Proteínas de unión cruzada ("cross-linking proteins")

- proteínas formadoras de haces ("bundling proteins"): actina-F forman haces
- proteínas formadoras de retículos("gel-forming proteins"): actina-F forma retículos similares a geles

Dinámica y estructuración de los microfilamentos en una célula en movimiento



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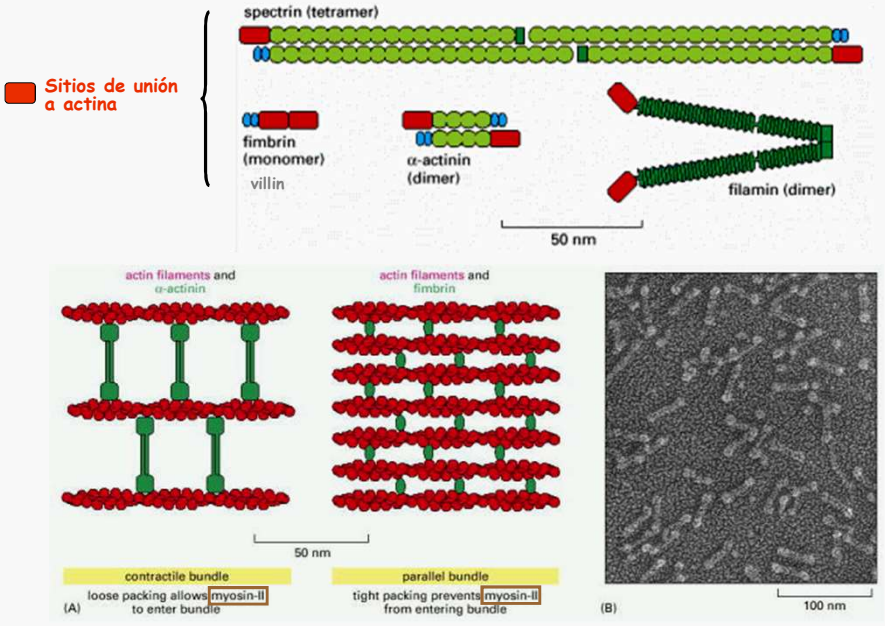
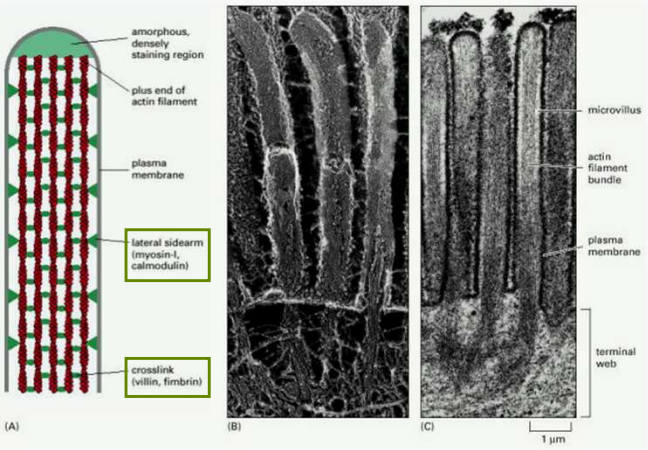
Estructura molecular de proteínas de unión cruzada

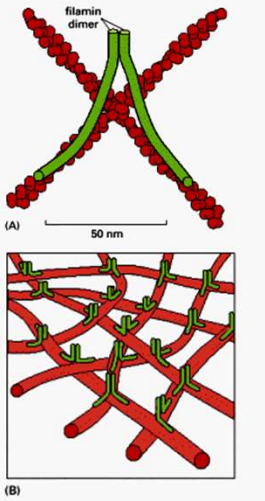


30

Fimbrina y villina



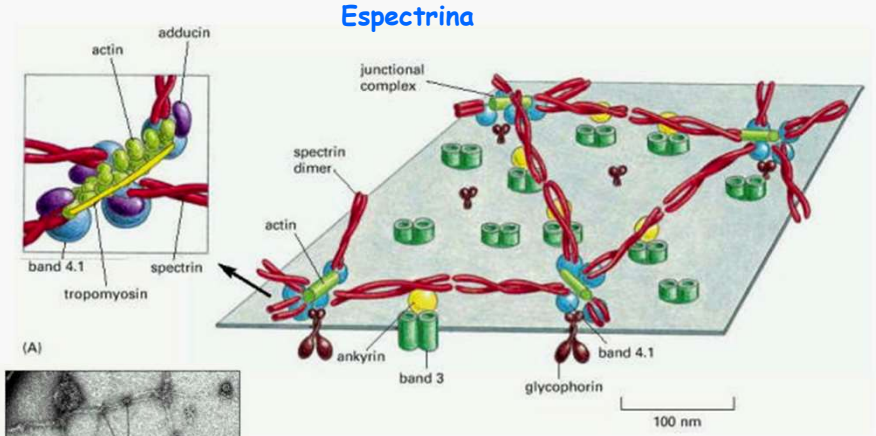
Filamina

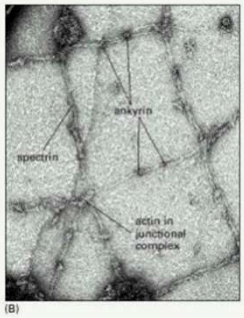


Consistencia gel viscoso

31

Espectrina





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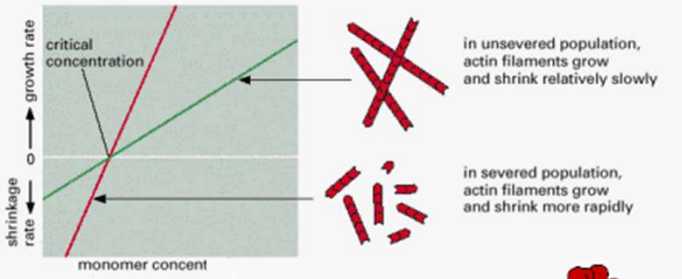
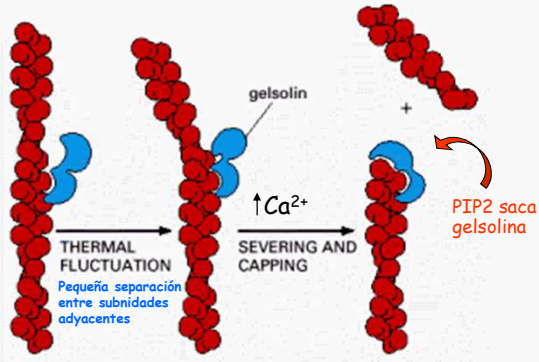
Proteínas de fraccionamiento ("severing proteins")



Gelsolina



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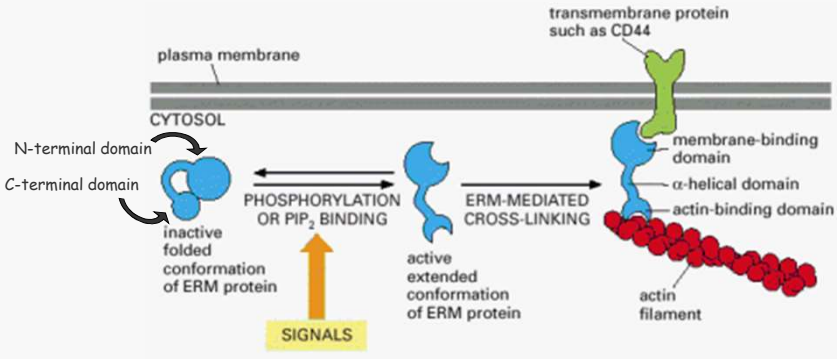
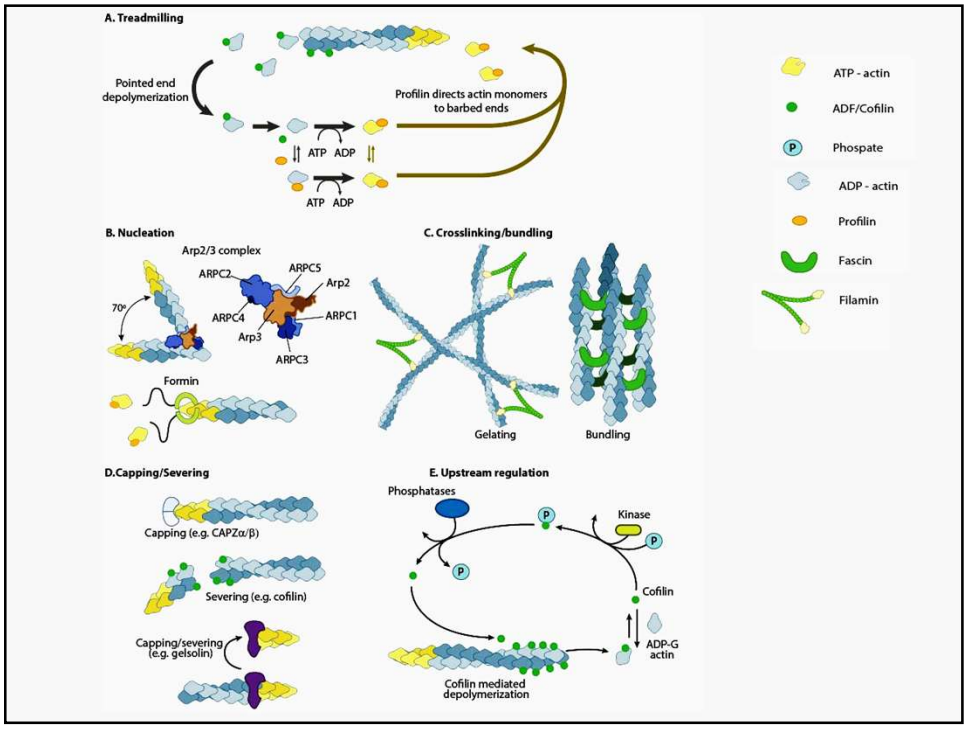
Adhesión celular: interacción entre el citoesqueleto y el medio extracelular

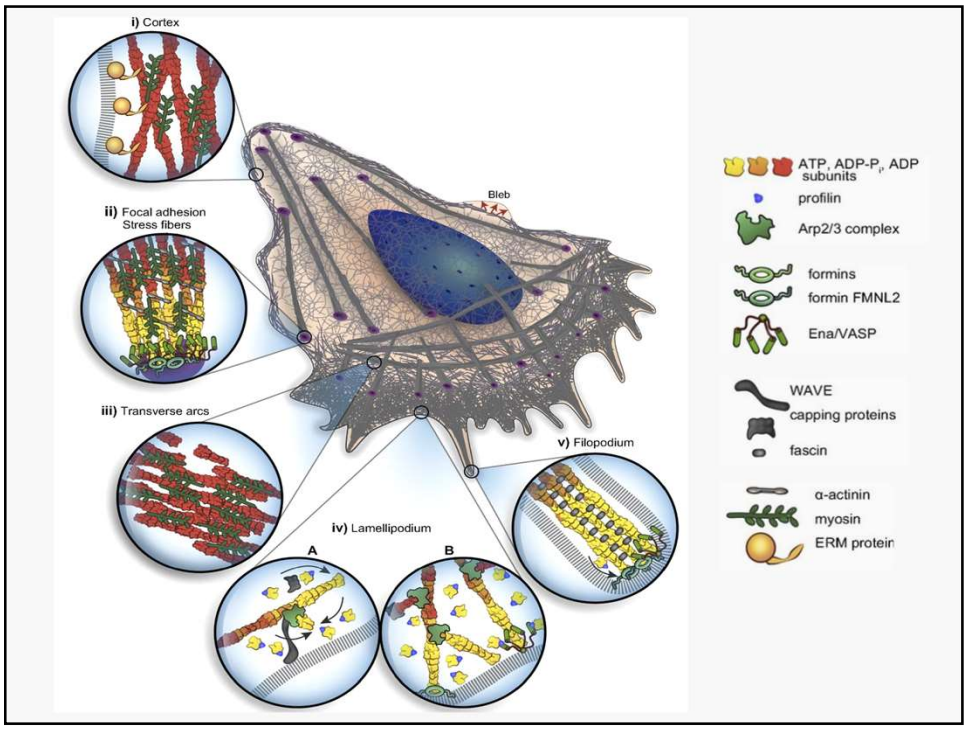
Proteínas ERM (ezrin, radixin, moesin): asocian actina-F a la membrana plasmática



34


A. Treadmilling
Pointed end depolymerization
Profilin directs actin monomers to barbed ends
ATP ADP
ATP ADP
B. Nucleation
Arp2/3 complex
ARPC2
ARPC5
Arp2
70°
ARPC4
ARPC1
Arp3
ARPC3
Formin
C. Crosslinking/bundling
Gelating
Bundling
D.Capping/Severing
Capping (e.g. CAPZα/β)
Severing (e.g. cofilin)
Capping/severing (e.g. gelsolin)
E. Upstream regulation
Phosphatases
Kinase
P
Cofilin
ADP-G actin
Cofilin mediated depolymerization
ATP - actin
ADF/Cofilin
Phospate
ADP - actin
Profilin
Fascin
Filamin

35


i) Cortex
Bleb
ii) Focal adhesion
Stress fibers
iii) Transverse arcs
v) Filopodium
iv) Lamellipodium
A
B
ATP, ADP-Pi, ADP subunits
profilin
Arp2/3 complex
formins
formin FMNL2
Ena/VASP
WAVE
capping proteins
fascin
α-actinin
myosin
ERM protein

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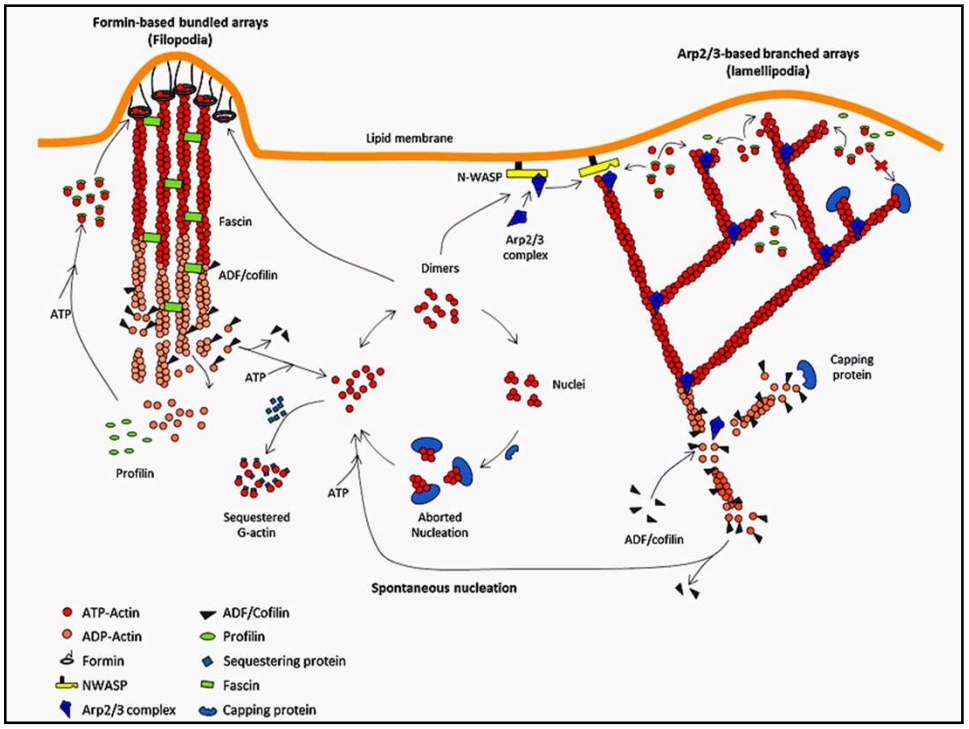

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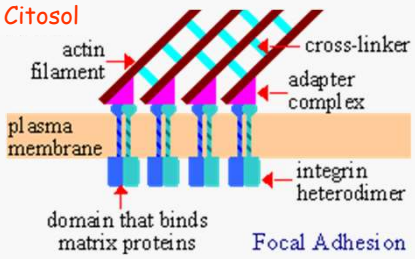
Adhesiones focales

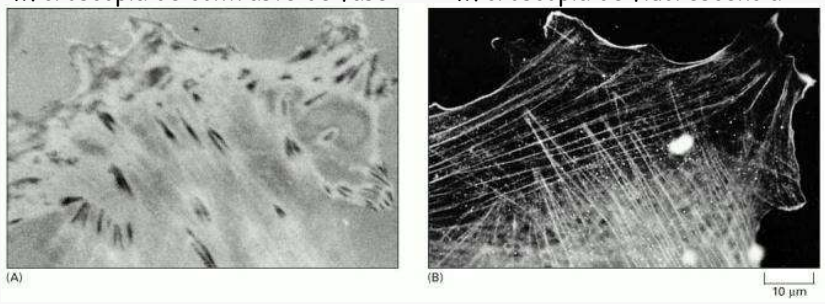


α-actinina
talina

Medio extracelular: matriz extracelular

Microscopía de contraste de fase

Microscopía de fluorescencia



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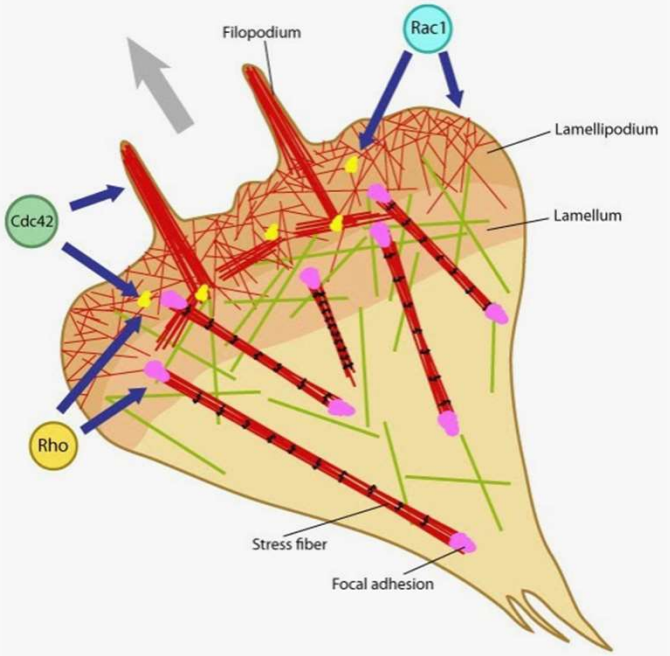
GTPsas monoméricas Rho: reguladores del citoesqueleto



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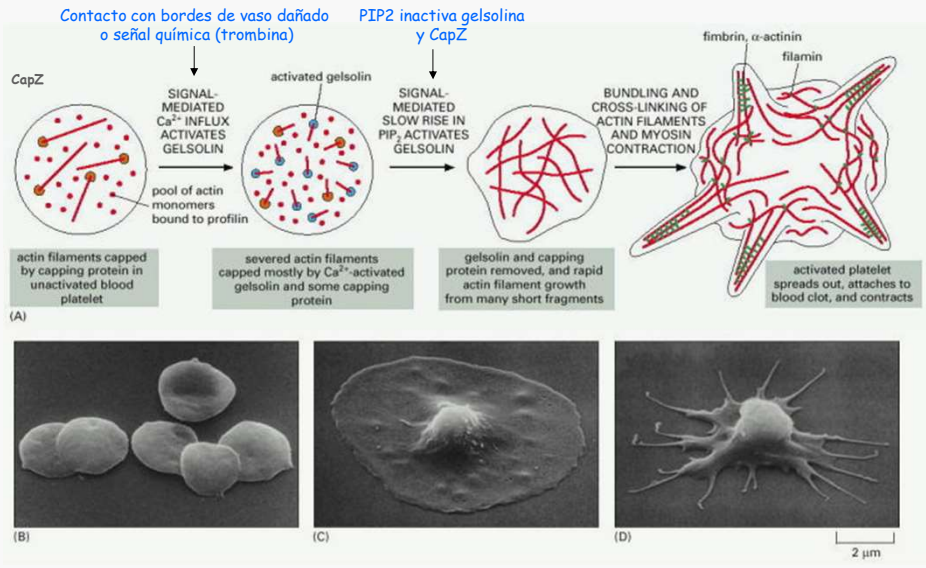
Activación de plaquetas



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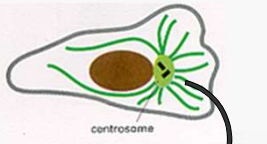
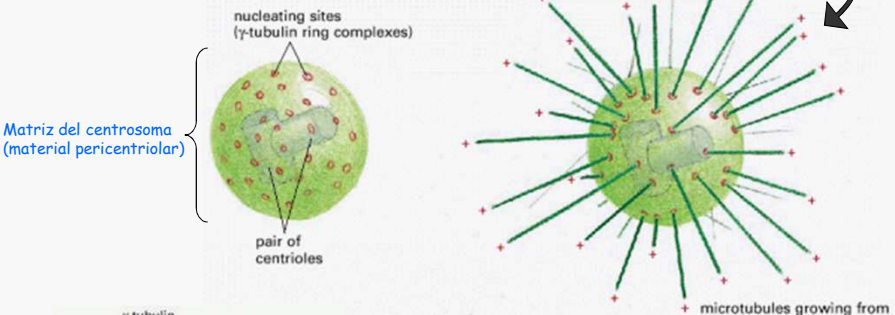
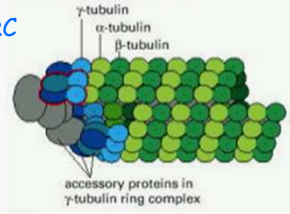
Nucleación de microtúbulos

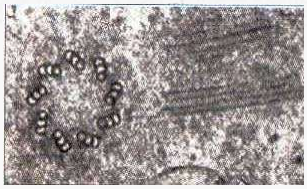
Centro organizador de Mts (MTOC): centrosoma
Complejo de anillo de γ-tubulina (γ-TuRC)





γ-TuRC





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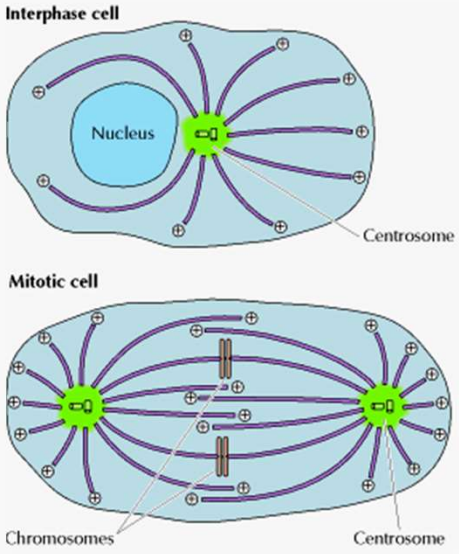
Centrosoma



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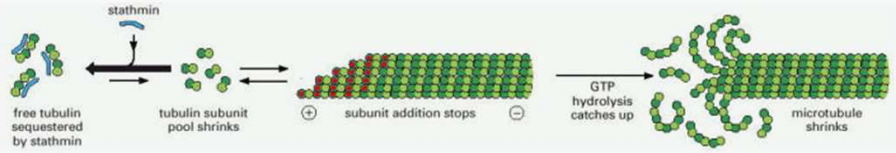
Statmina: proteína secuestradora de tubulina



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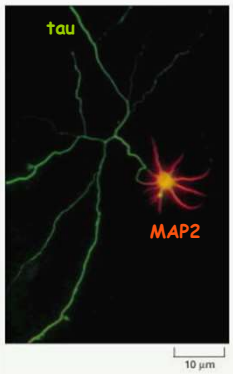
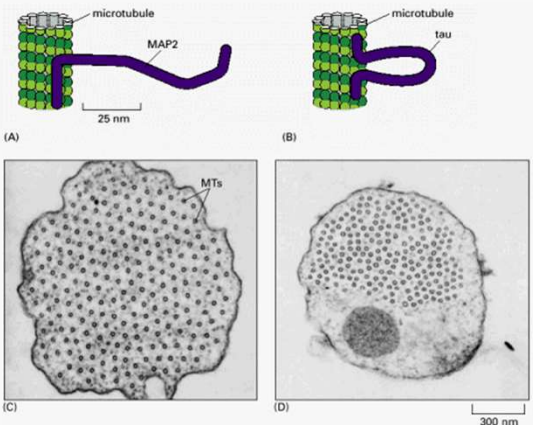
Proteínas que se unen lateralmente a los Mts

Proteínas asociadas a microtúbulos (MAPs)

- Estabilidad (in vitro estabilizan oligómeros de tubulina
- asociación a otros componentes celulares



Microscopía de fluorescencia. Distribución de: Tau en el axón (tb. en dendritas) y MAP2 en cuerpo y dendritas



Microscopía electónica. Sección transversal de células que sobreexpresan MAP2 (A) o tau (B) donde se muestran haces de Mts.

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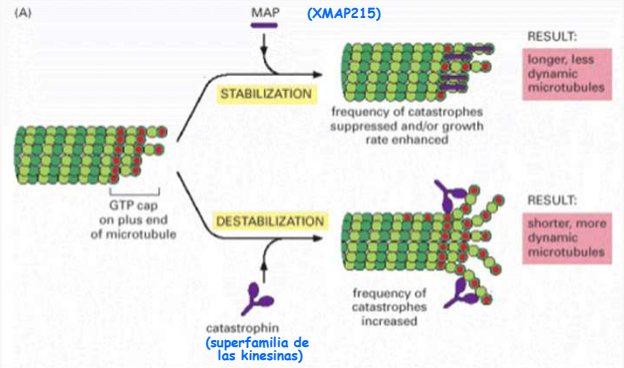
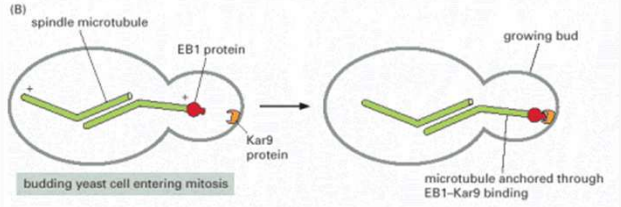
Proteínas que interaccionan con los extremos de los Mts

Efecto sobre la inestabilidad dinámica de Mts



Control del posicionamiento de Mts (huso mitótico hacia el "bud" en crecimiento)



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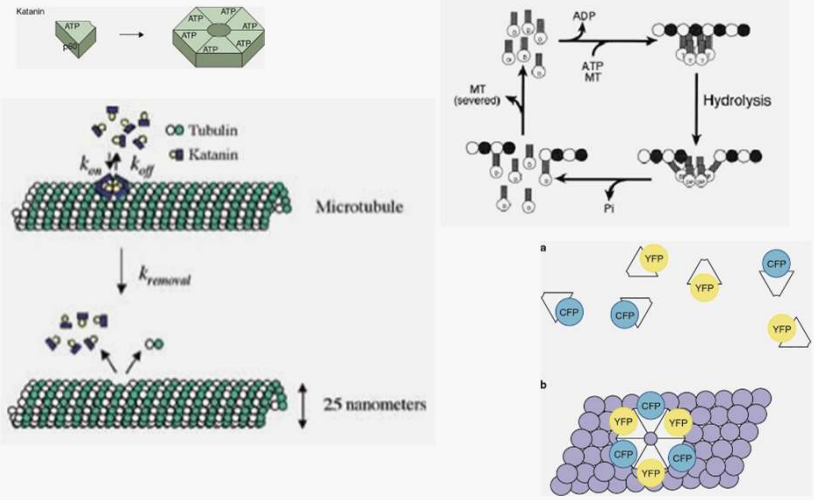
Proteínas de fraccionamiento ("severing proteins") para Mts

Katanina: - subunidad menor, ATPasa y "severing"
- subunidad mayor, transporta a centrosoma
- libera Mts del MTOC
- rápida despolimerización de Mts en los polos del huso mitótico



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