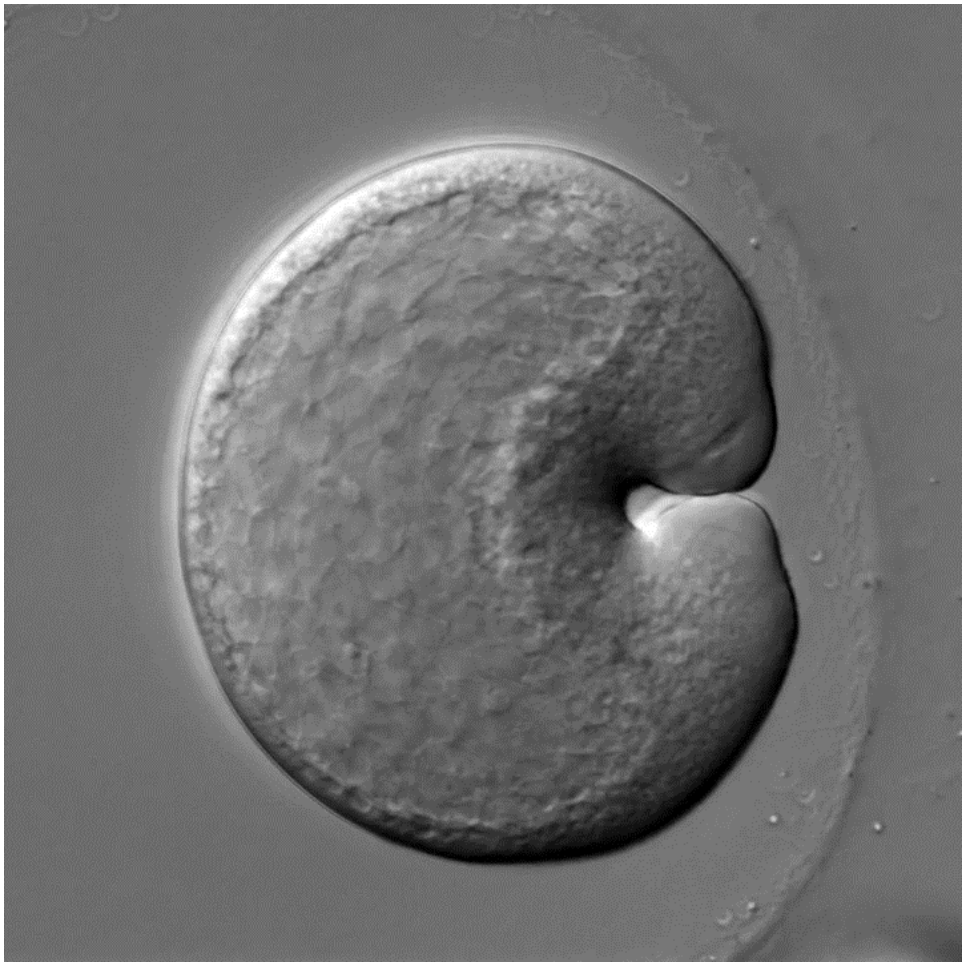
Ciclo celular I



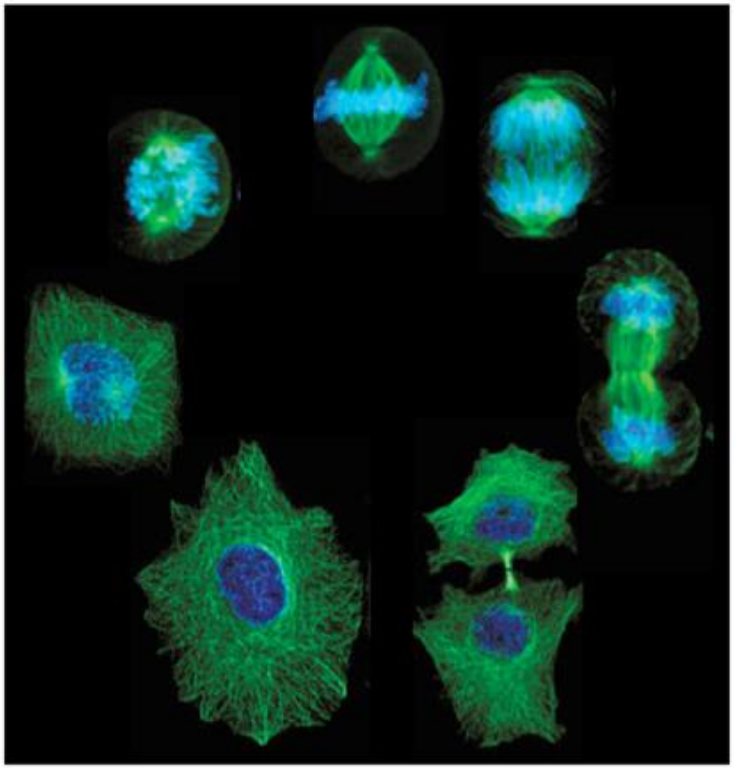
https://youtu.be/Zxk7bH2H6hQ

Gonzalo Aparicio
gaparicio@fcien.edu.uy

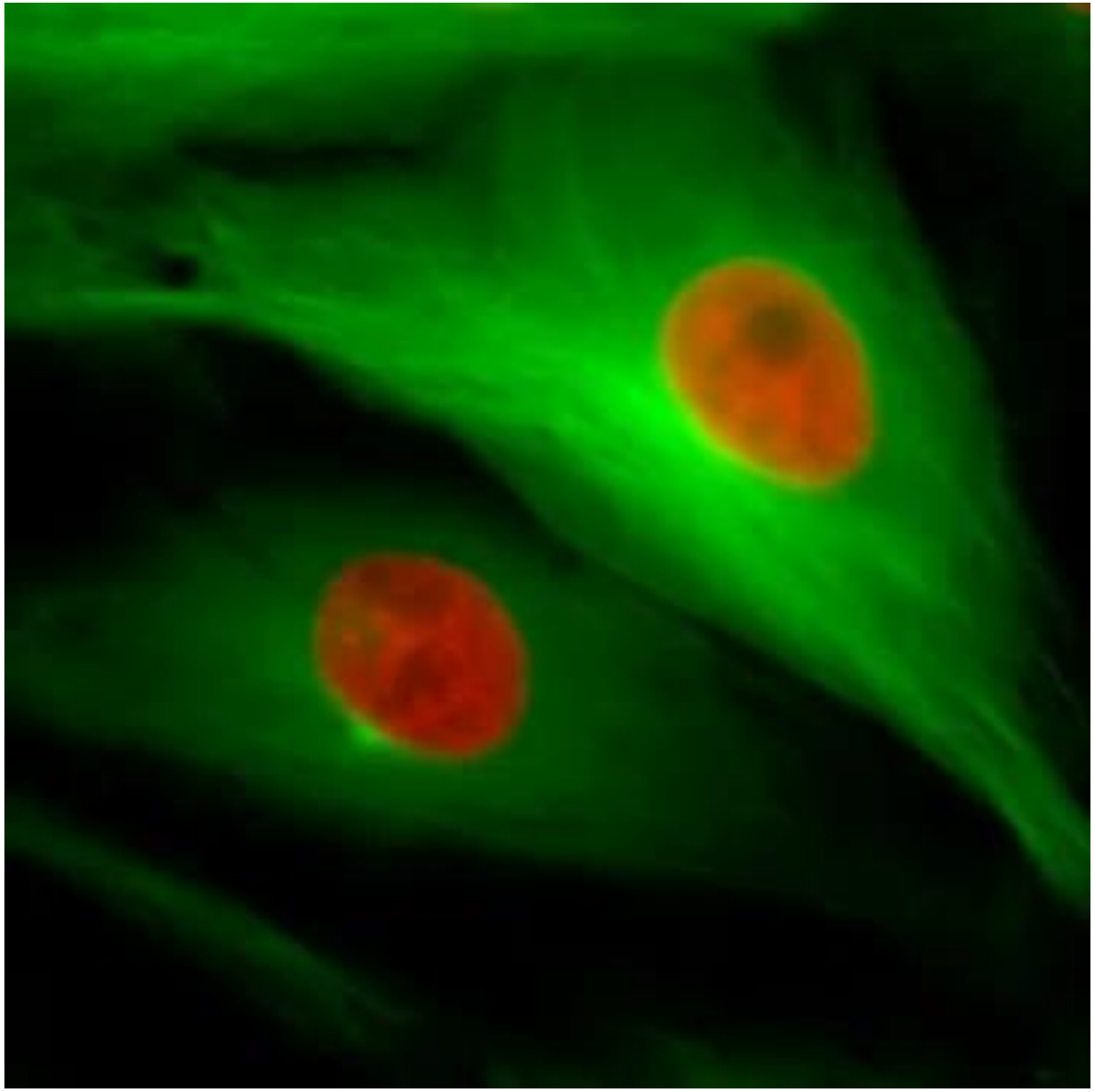
Rudolf Virchow
(1821–1902)

'Omnis cellula e cellula'

Toda célula deriva de otra célula



Todos los seres vivos, desde los organismos unicelulares hasta los metazoarios, descienden de un ancestro común por rondas sucesivas de crecimiento y divisiones.



https://youtu.be/rym83phFUI0

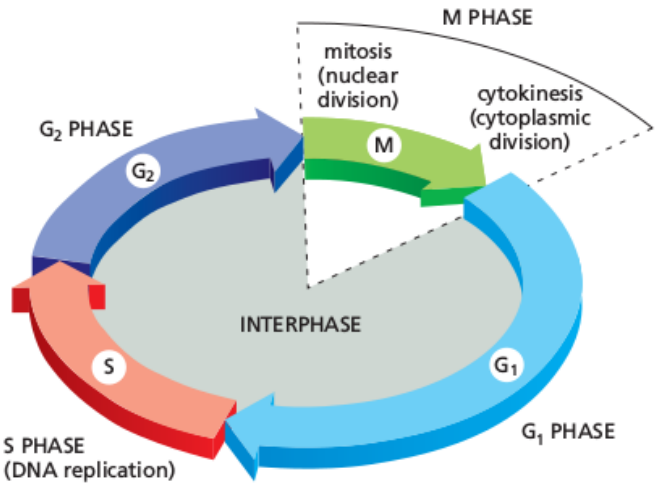
Problemas que enfrenta una célula a la hora de dividirse

Debe generar todos sus componentes celulares en cantidades suficientes para poder repartirlos entre las células hijas.

Por ejemplo, deben existir copias suficientes de organelos (**mitocondrias, ER, ribosomas**, etc)

Para estructuras presentes en número limitados (**cromosomas, centrosoma**) una maquinaria especial debe generarse para asegurar la correcta segregación entre las células hijas.
El **huso mitótico**

Ciclo celular



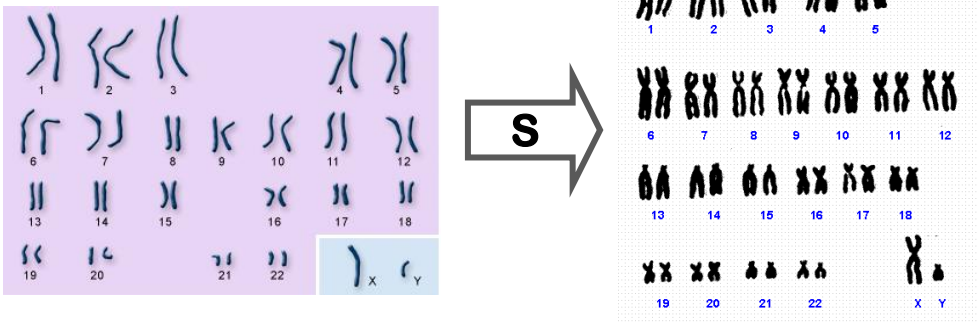
Interfase:

G1 y G2:

- Crecimiento celular
- Duplicación de organelos.
- Duplicación del centrosoma
- Chequeo de condiciones internas y externas

S:

duplicación fidedigna del ADN:



G1 G2

Fase M

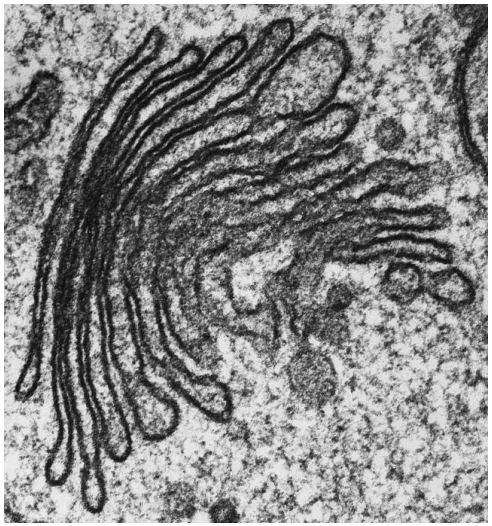
- **Mitosis** (división nuclear)
- **Citocinesis** (división del citoplasma)

Ciclo celular

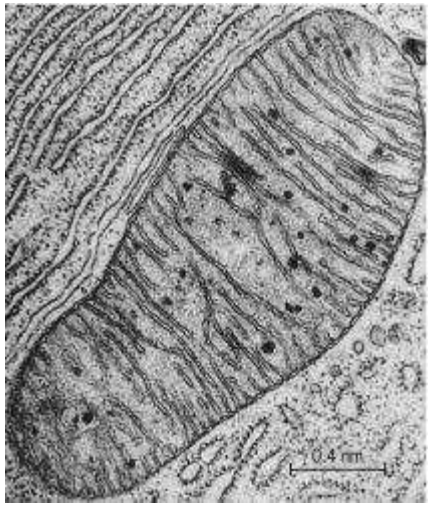
Interfase:

- G1
- S
- G2

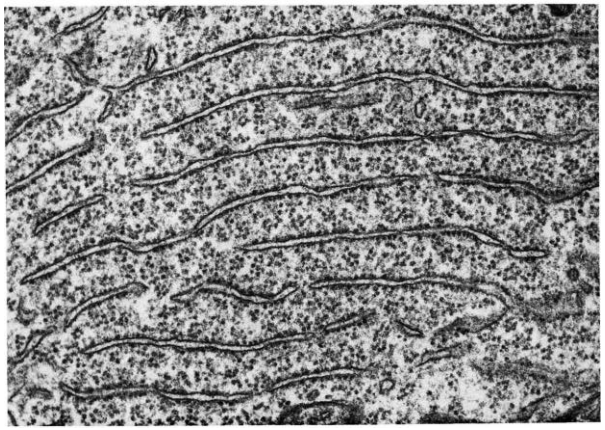
Interfase: duplicación de organelos



Aparato de Golgi

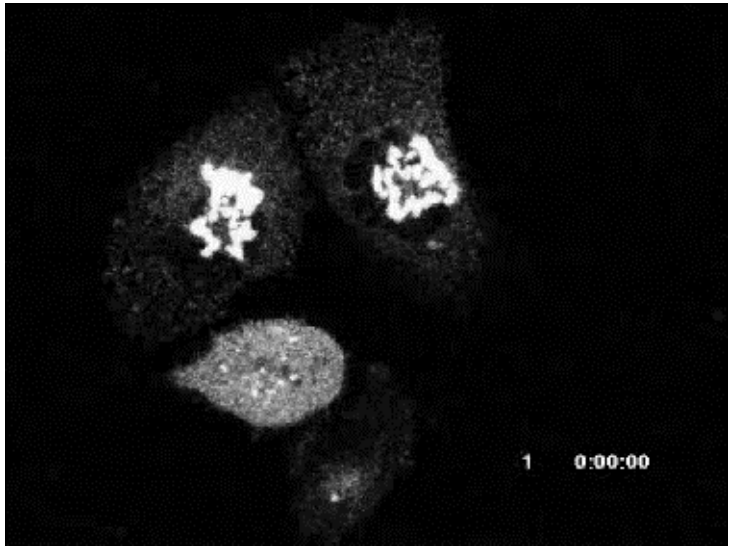


Mitocondrias



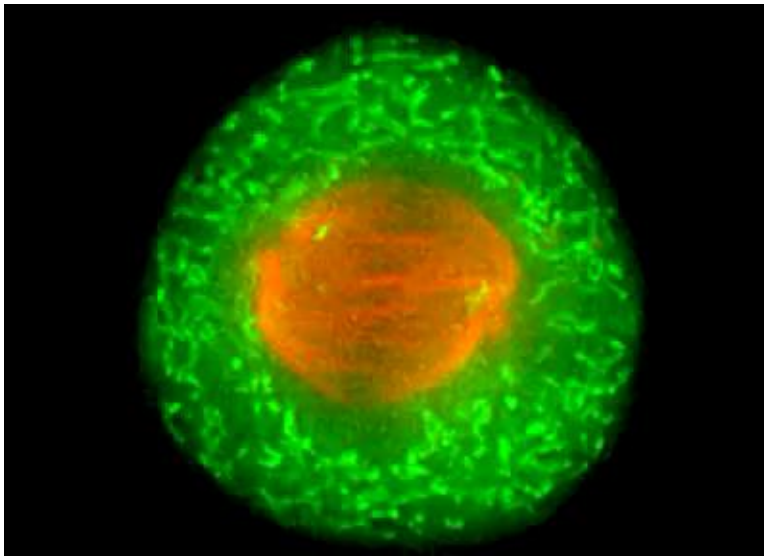
Retículo endoplásmico rugoso

Dinámica de los organelos durante el ciclo celular



Aparato de Golgi (blanco)

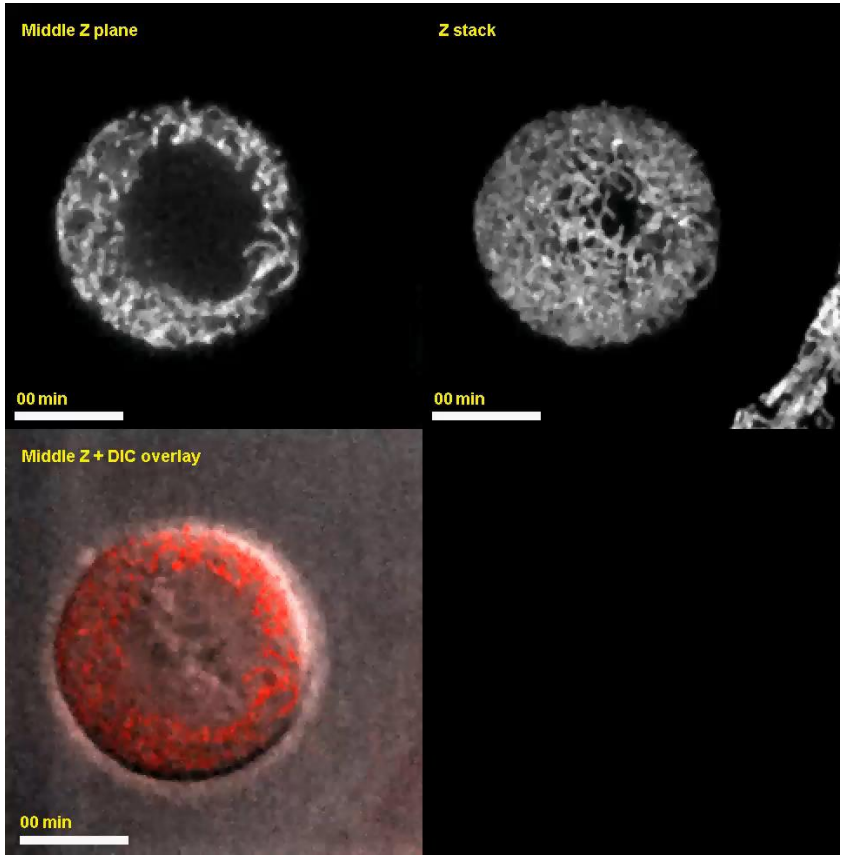
https://youtu.be/WI3fnTfWy5M



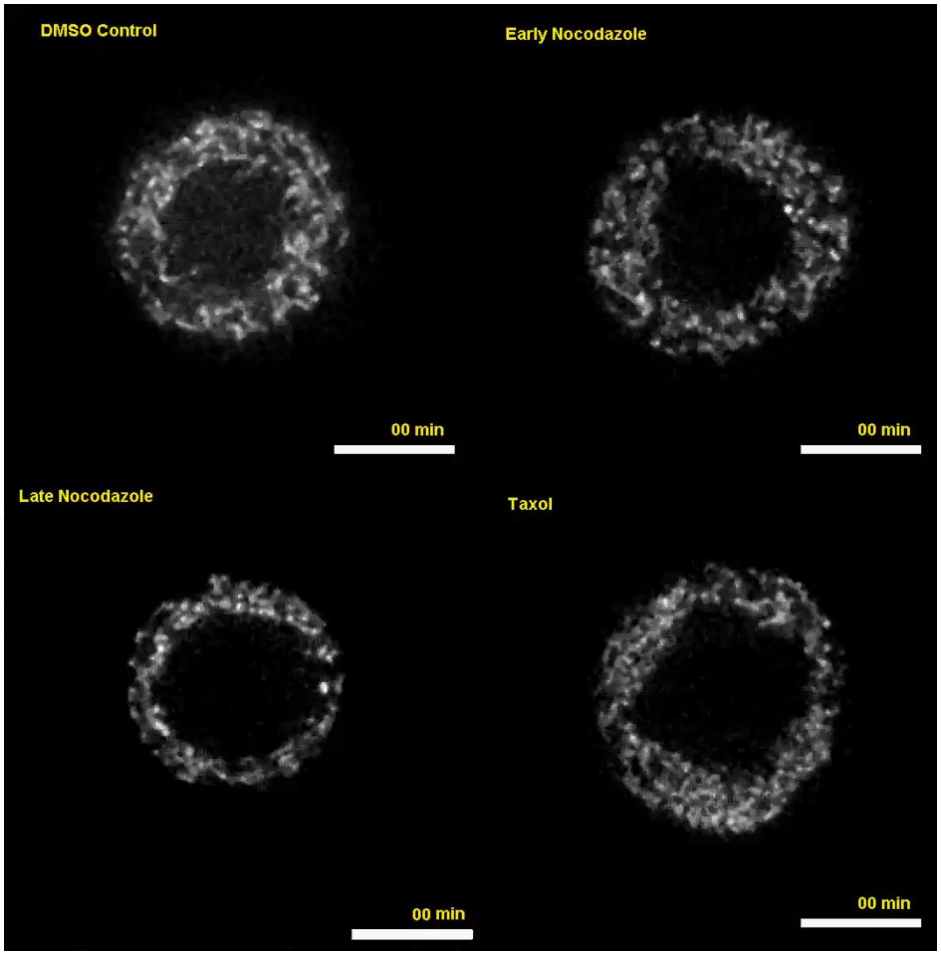
Mitocondrias (verde)
Microtúbulos (rojo)

https://youtu.be/1mp64Og1zLo

Dinámica de los organelos durante el ciclo celular



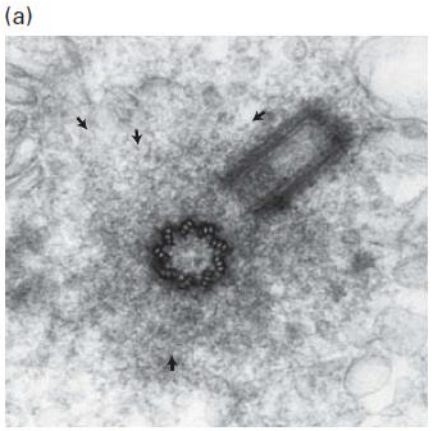
https://youtu.be/YoyPRnlvpxk

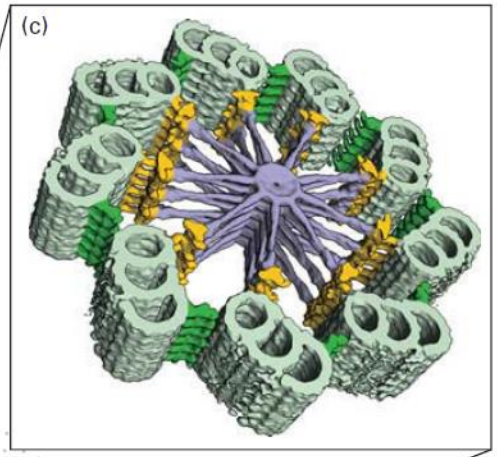


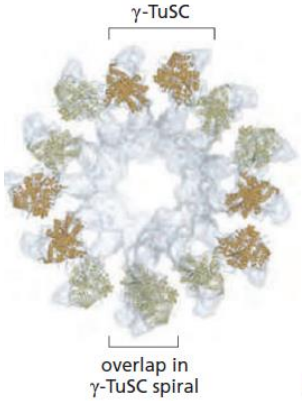
https://www.youtube.com/watch?v=QMOw8e6SlyQ

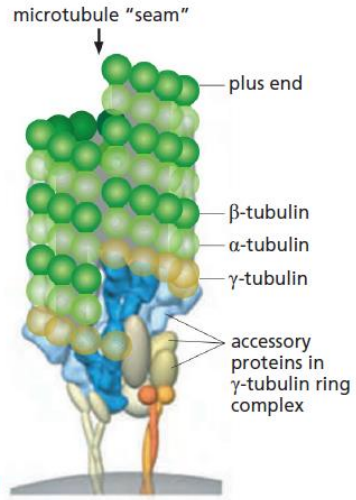
Lawrence and Mandato, 2013

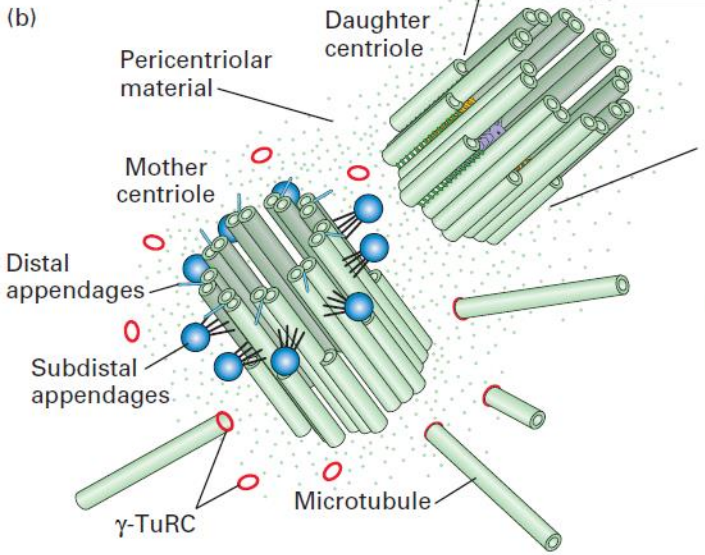
Centrosoma

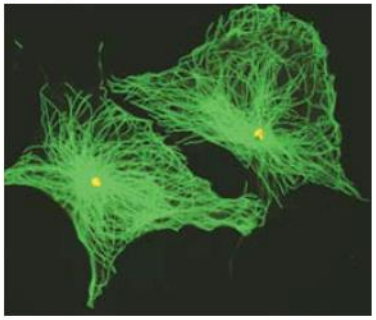

(a)

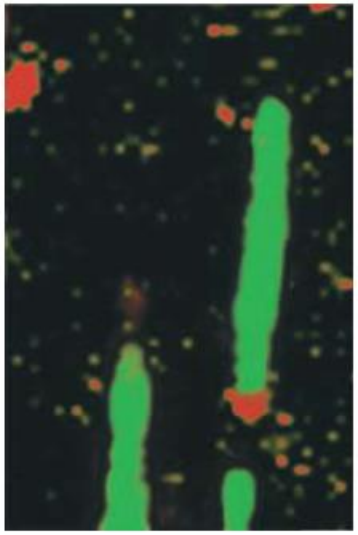

(c)


γ-TuSC
overlap in
γ-TuSC spiral

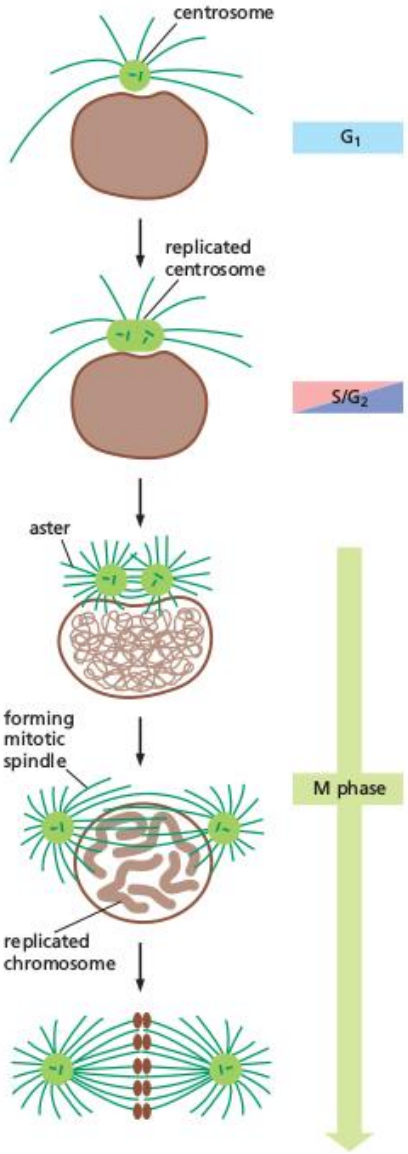

microtubule "seam"
plus end
β-tubulin
α-tubulin
γ-tubulin
accessory
proteins in
γ-tubulin ring
complex
(C)

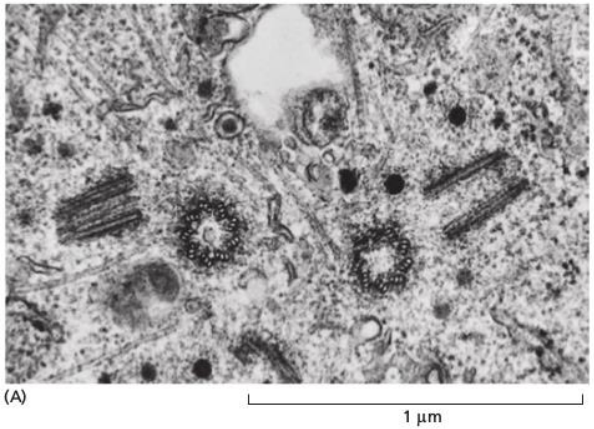

(b)
Daughter
centriole
Pericentriolar
material
Mother
centriole
Distal
appendages
Subdistal
appendages
γ-TuRC
Microtubule

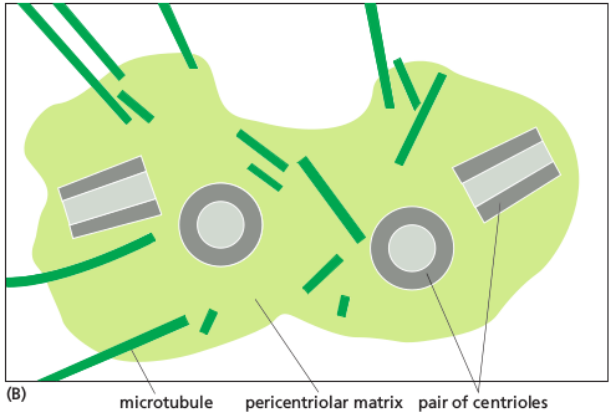

(d)



Duplicación del centrosoma



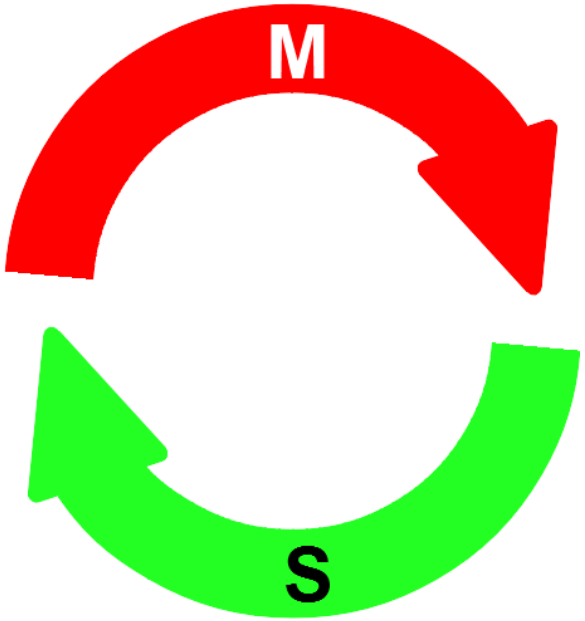




La duplicación comienza entre la fase S y G2 del ciclo

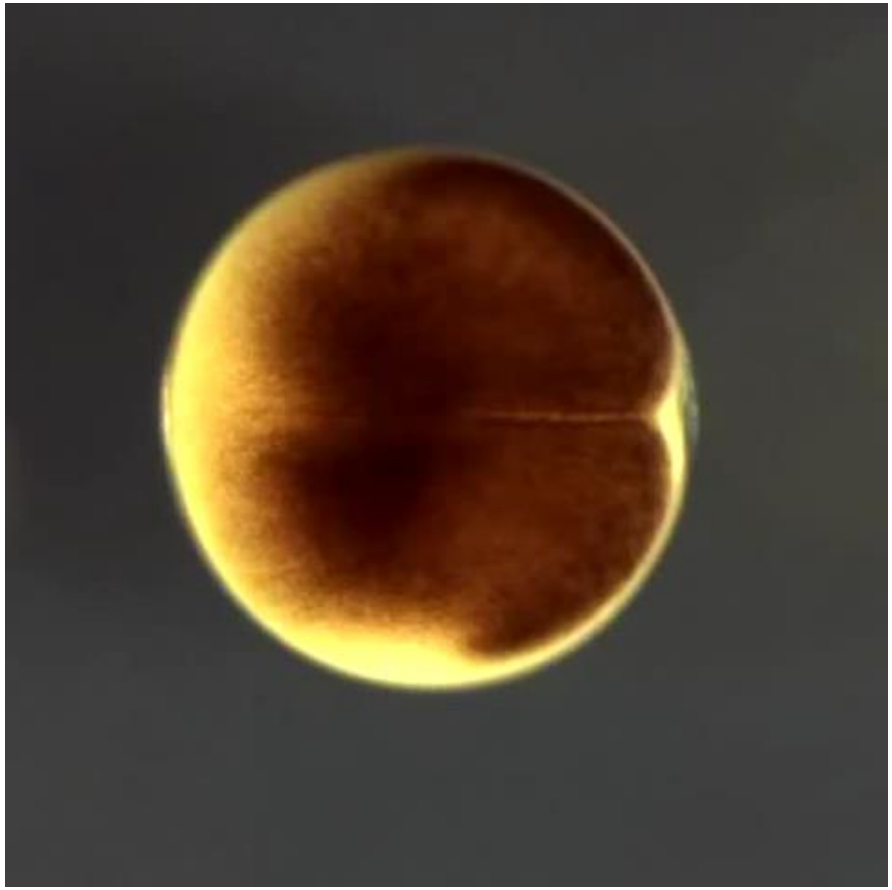
División sin crecimiento

División sin crecimiento



Ciclo Bifásico

https://www.youtube.com/watch?v=GO5YN_t1fqw



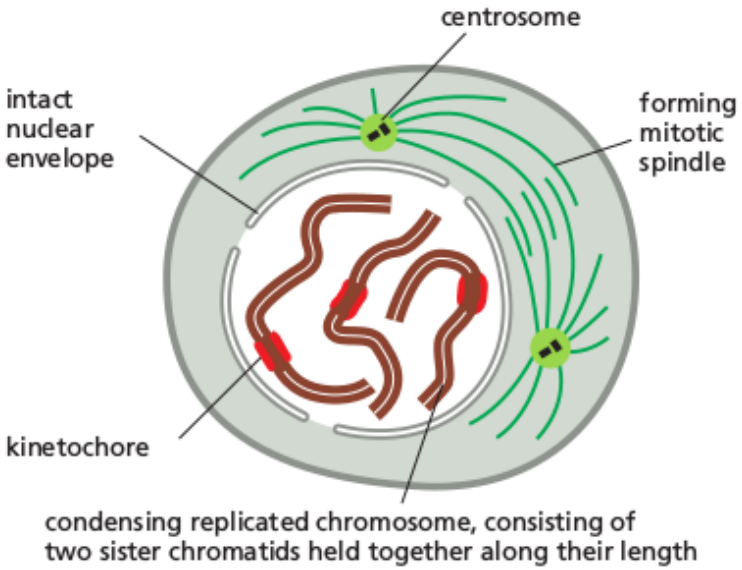
Embrión de anfibio

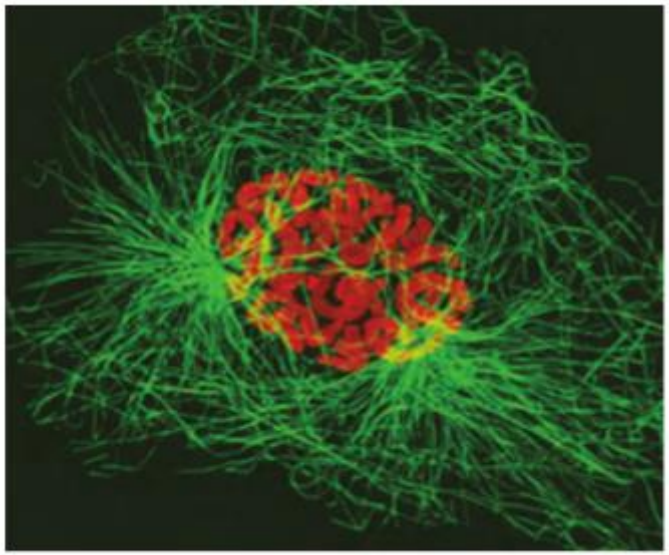
Ciclo celular

Fase M

- **Mitosis** (división nuclear)
  - Profase
  - Prometafase
  - Metafase
  - Anafase
  - Telofase
- **Citocinesis** (división del citoplasma)

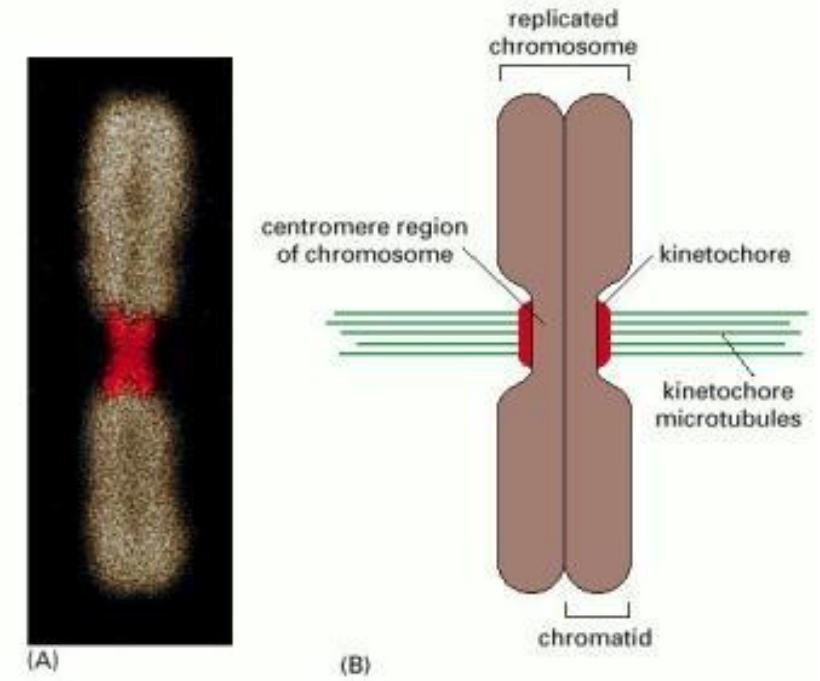
Profase

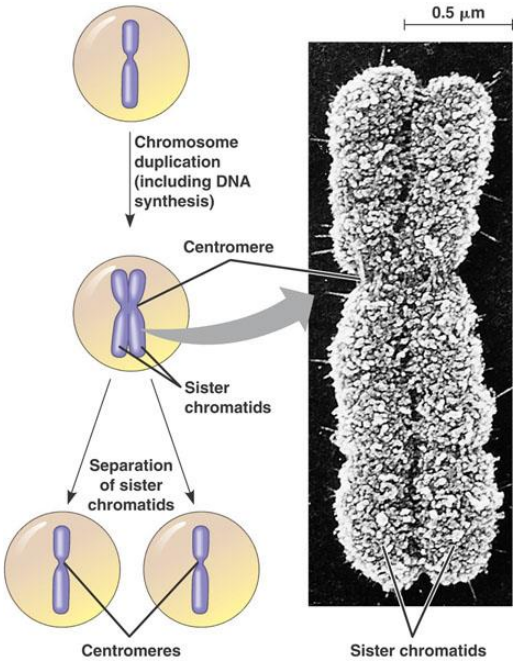




Los cromosomas replicados se condensan.
Fuera del núcleo, el huso mitótico se ensambla entre ambos centrosomas.

Cromosoma mitótico

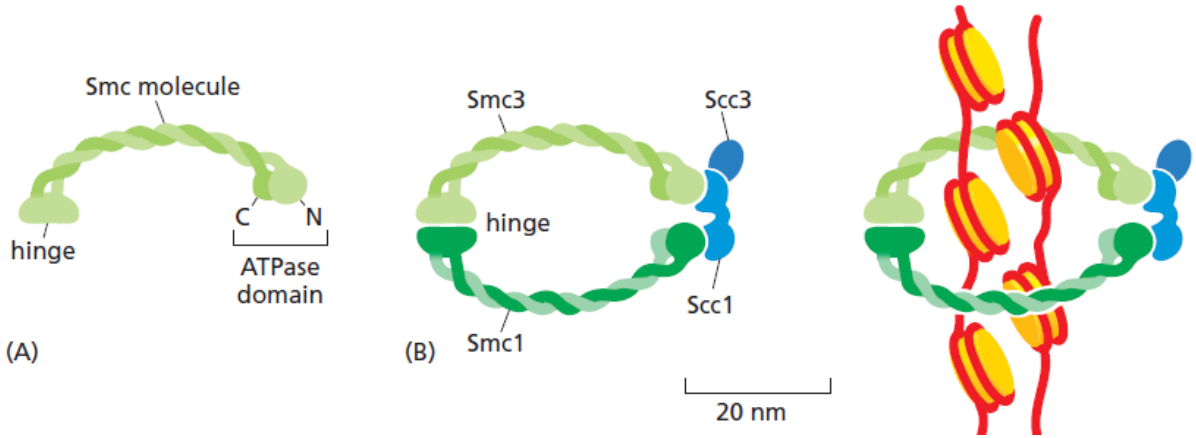


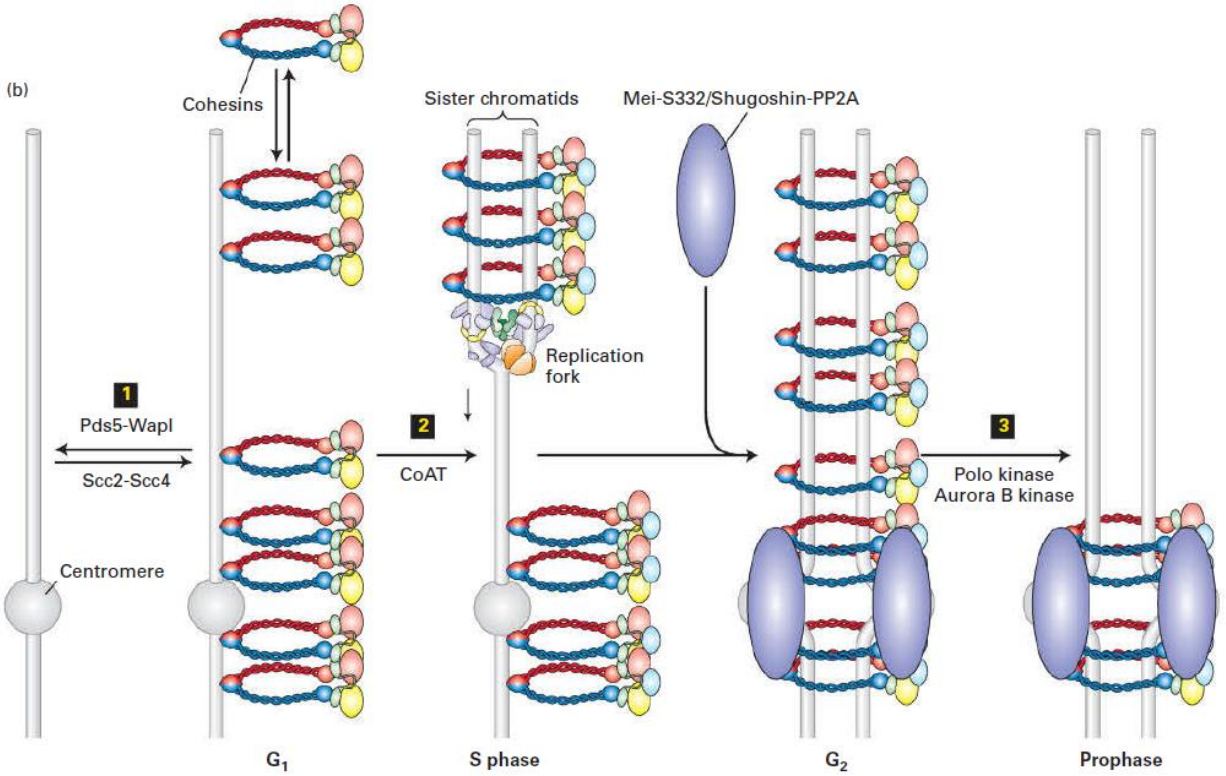


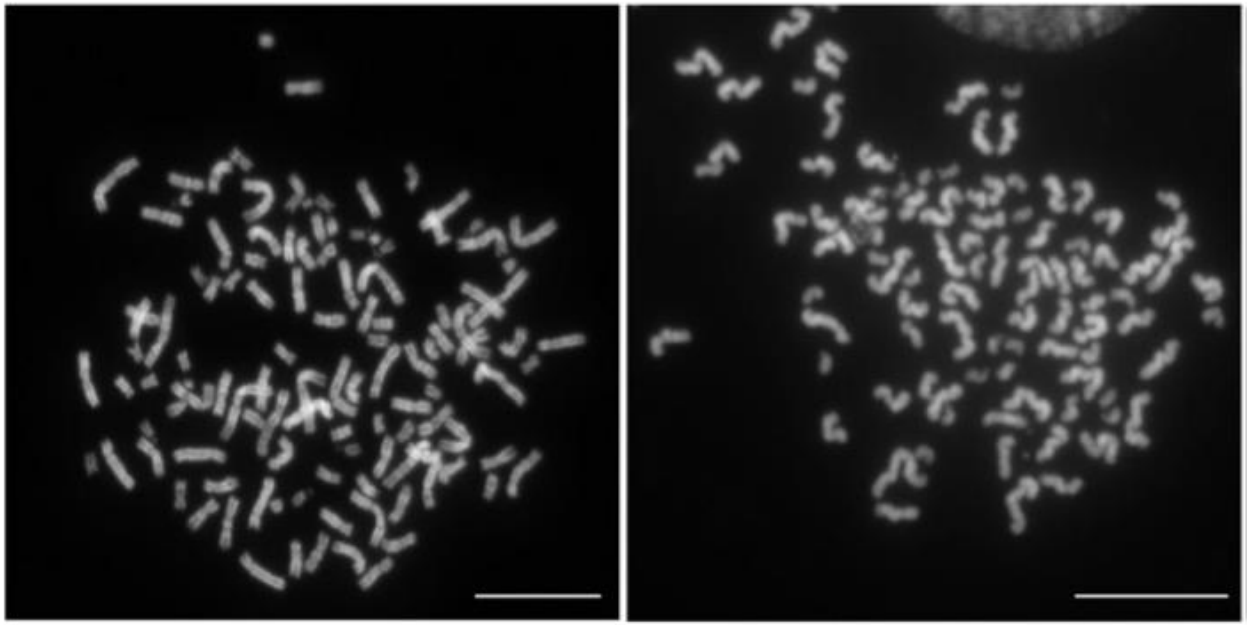
Cromátidas hermanas sintetizadas en fase S y unidas por **cohesinas**

Cinetocoro: estructura proteica sobre los **centrómeros** de las cromátidas hermanas

Cohesinas


Smc molecule
hinge
C
N
ATPase domain
(A)
Smc3
Scc3
hinge
Scc1
Smc1
(B)
20 nm

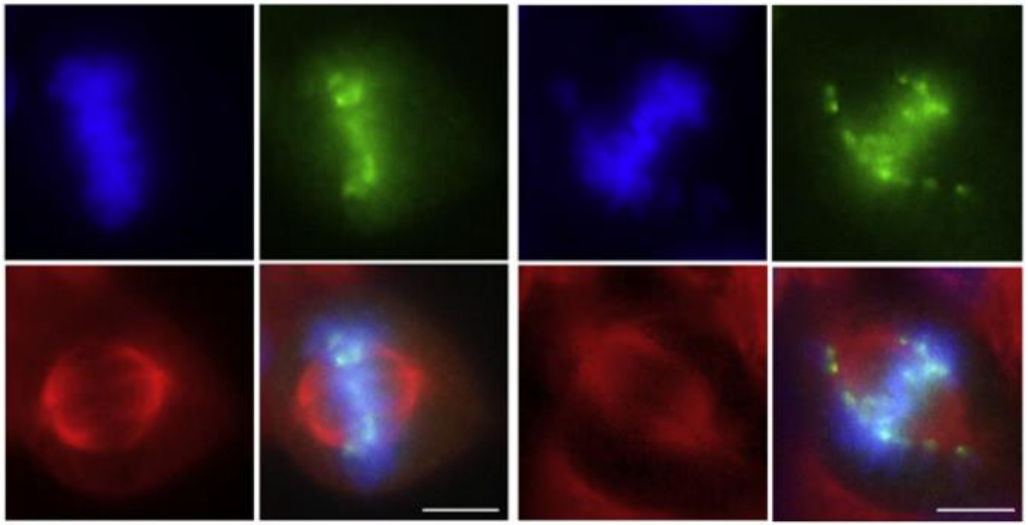

(b)
Cohesins
Sister chromatids
Mei-S332/Shugoshin-PP2A
Replication fork
1
Pds5-Wapl
Scc2-Scc4
2
CoAT
3
Polo kinase
Aurora B kinase
Centromere
G1
S phase
G2
Prophase



Normal

Complete loss of cohesion

Blue: DAPI Green: INCENP Red:α-Tubulin

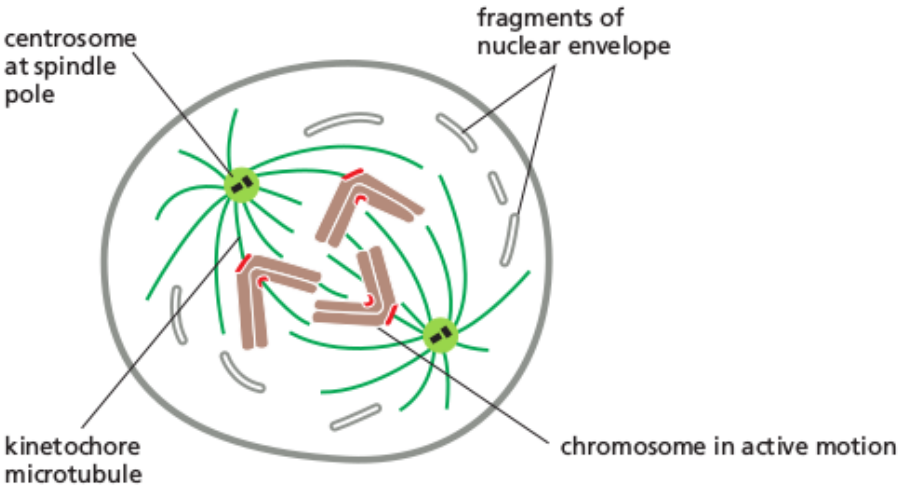


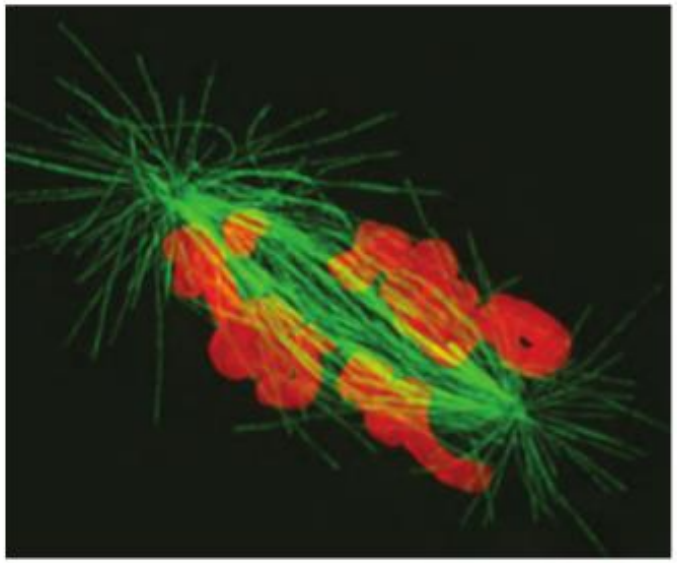
Normal

Scattered chromosomes

Zhang et al., 2008

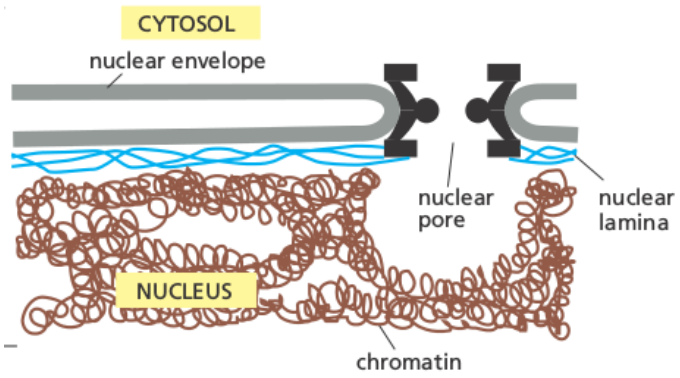
Prometafase

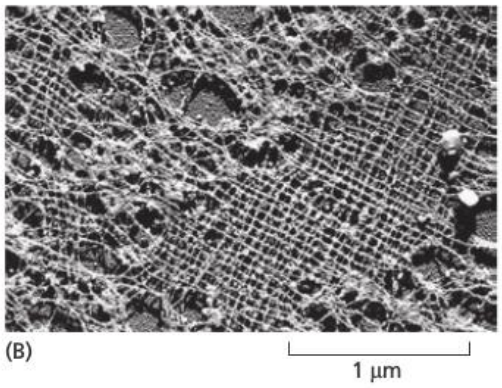


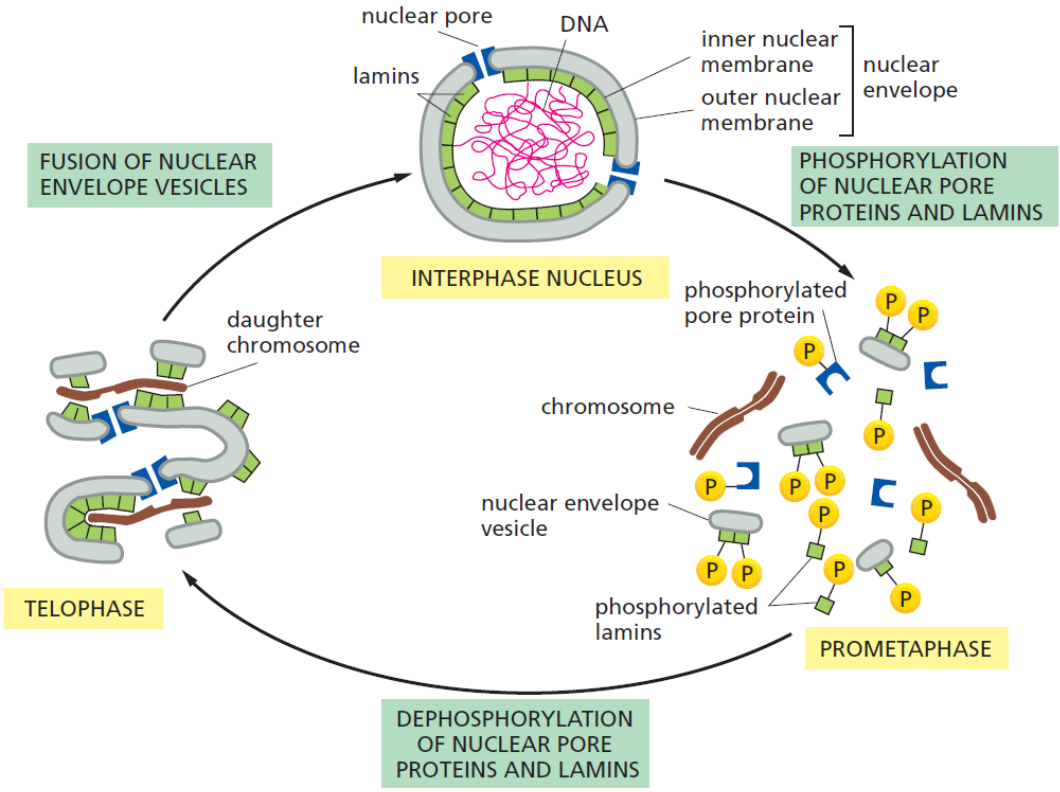


Desensamblado de la envoltura nuclear.
Los cromosomas se unen al huso mitótico

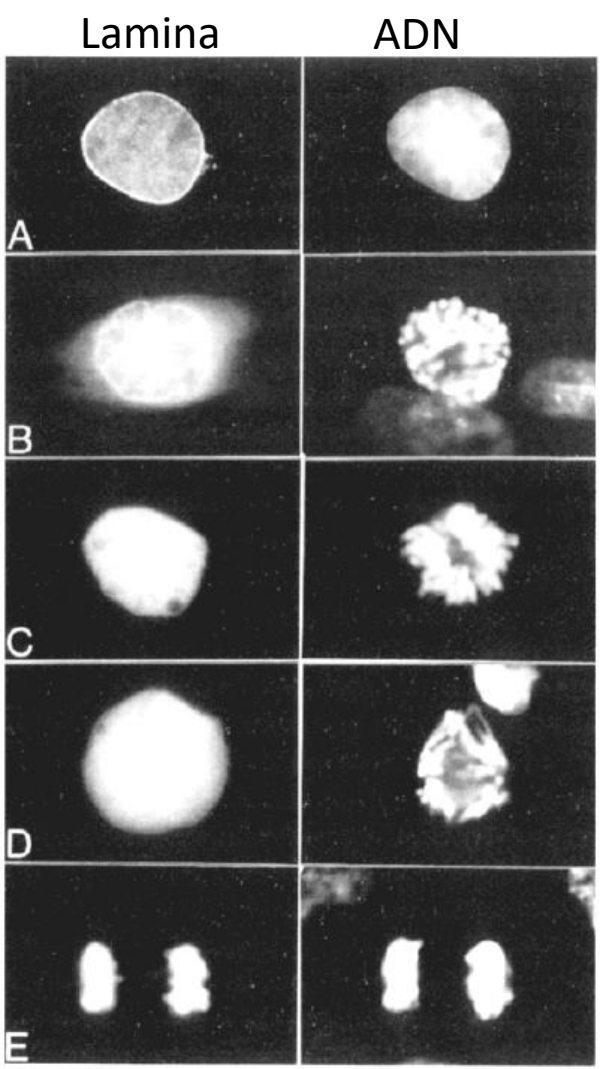
Fosforilación y desensamblado de la lámina nuclear

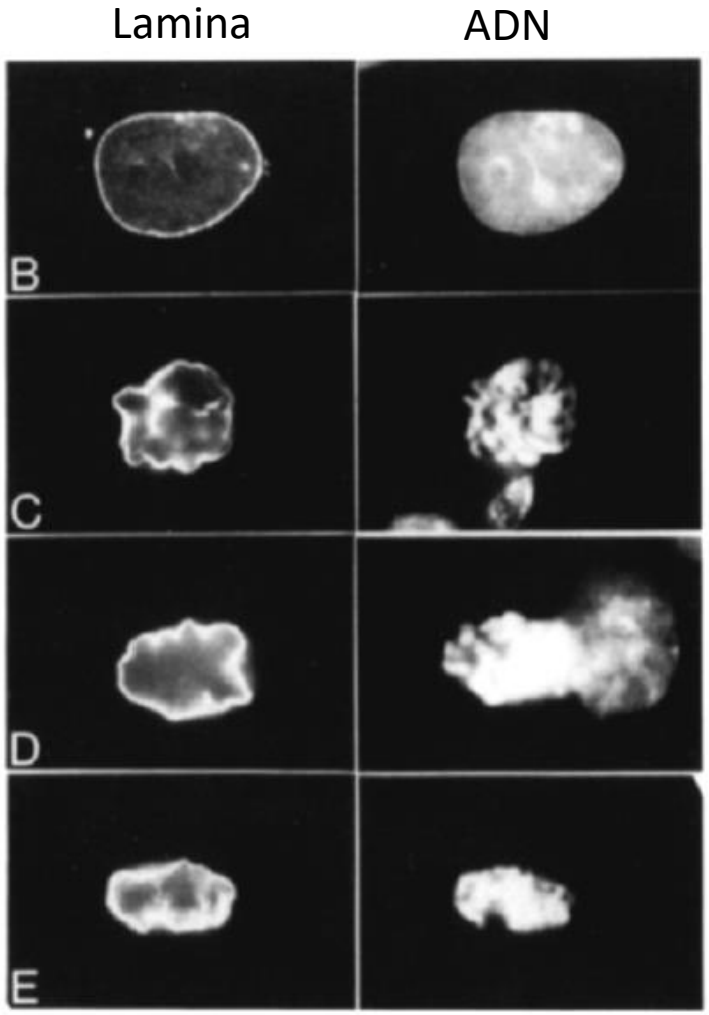




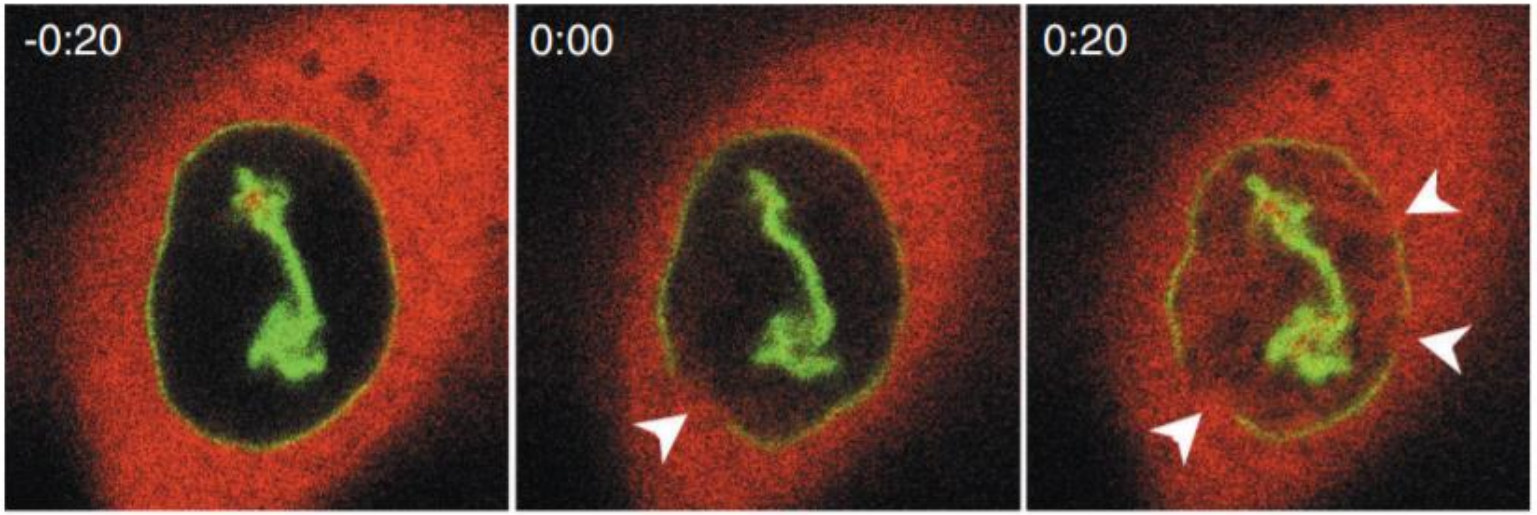


Fosforilación y desensamblado de la lámina nuclear

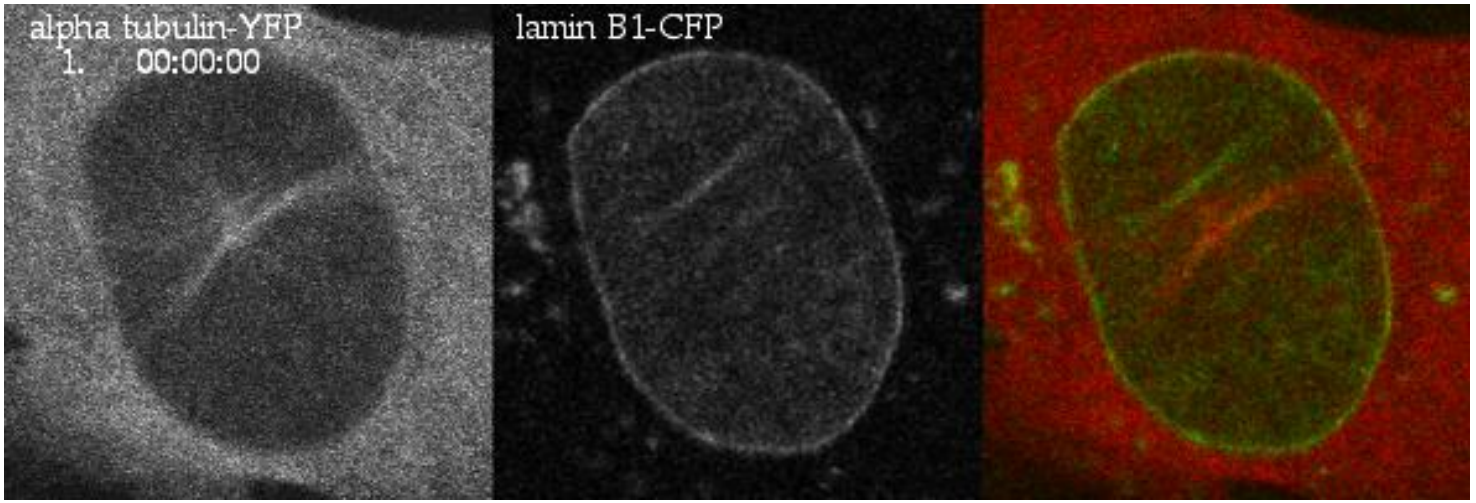




Heald and Mckeon, 1990

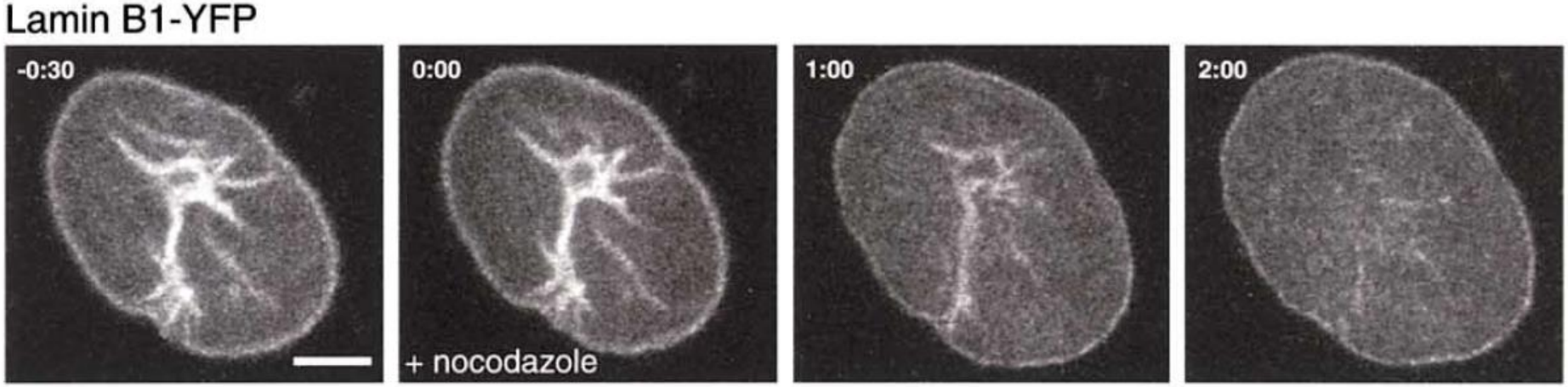


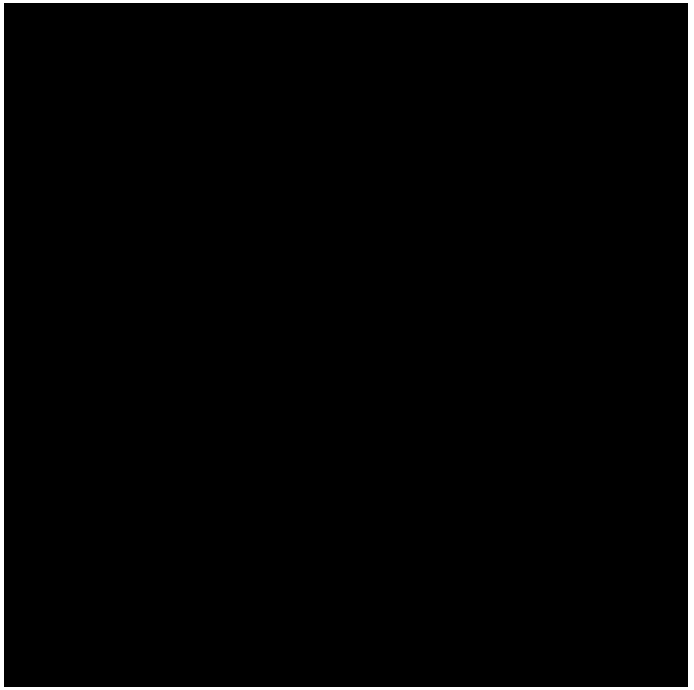
Lamina-GFP/Dextrano 500KDa



Tubulina-YFP **Lamina-CFP** https://youtu.be/lXNREhSRCYQ

Beaudouin et al., 2002



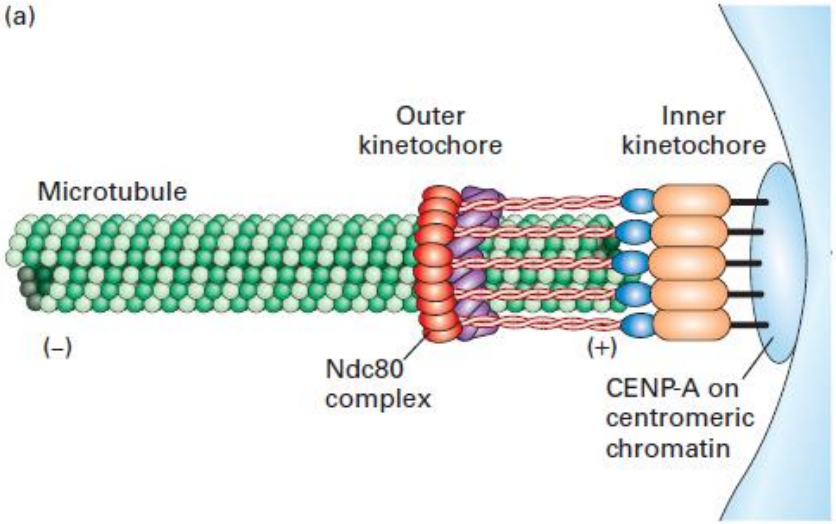


Lamina-GFP + Nocodazole

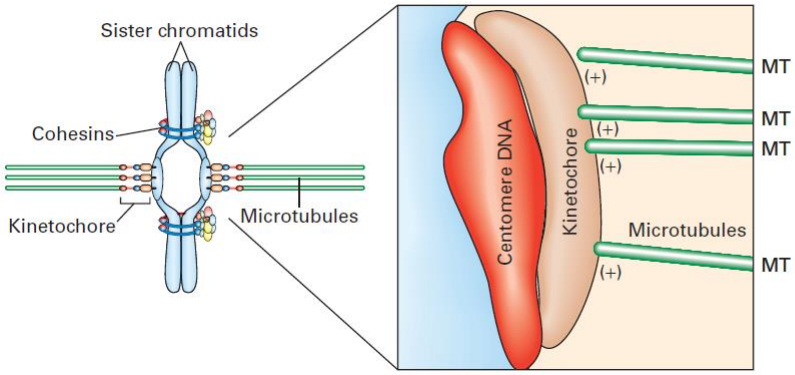
https://youtu.be/rPUFsEUWCCc

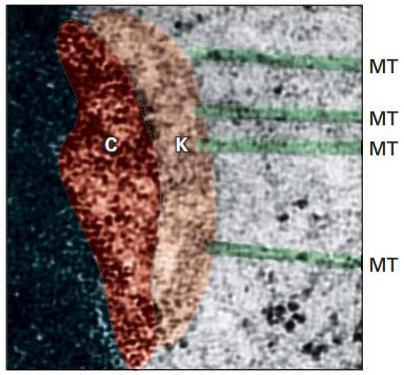
Beaudouin et al., 2002

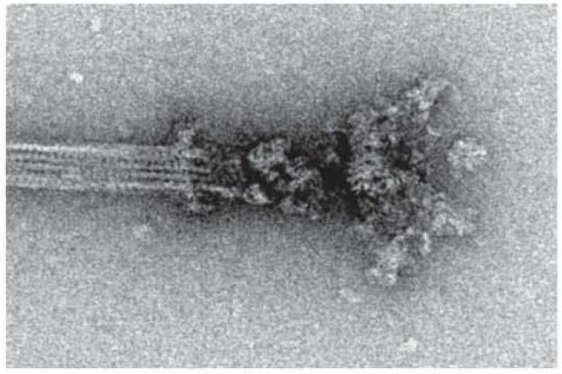
Cromosoma mitótico: Cinetocoro



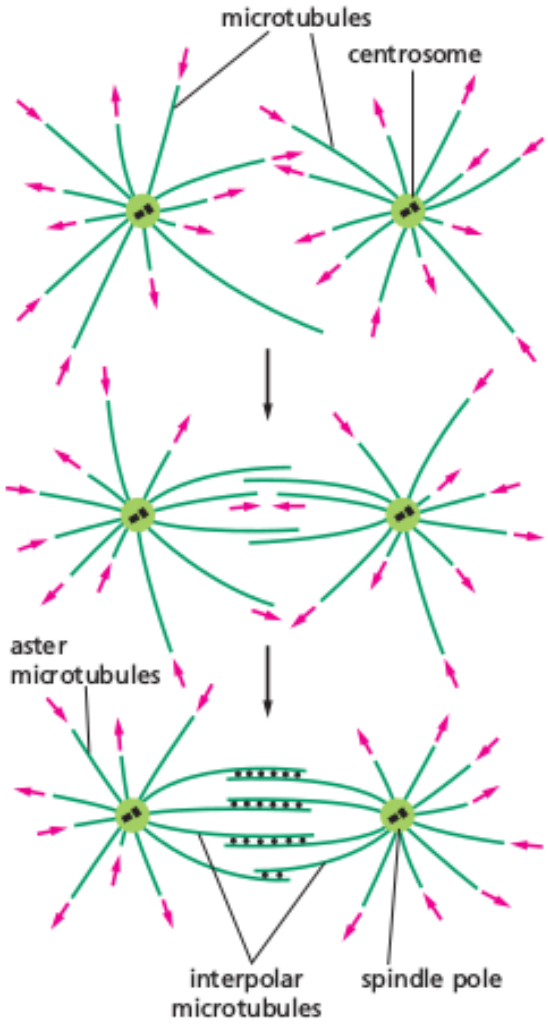
CENP-A
Histona H3 específica del centrómero

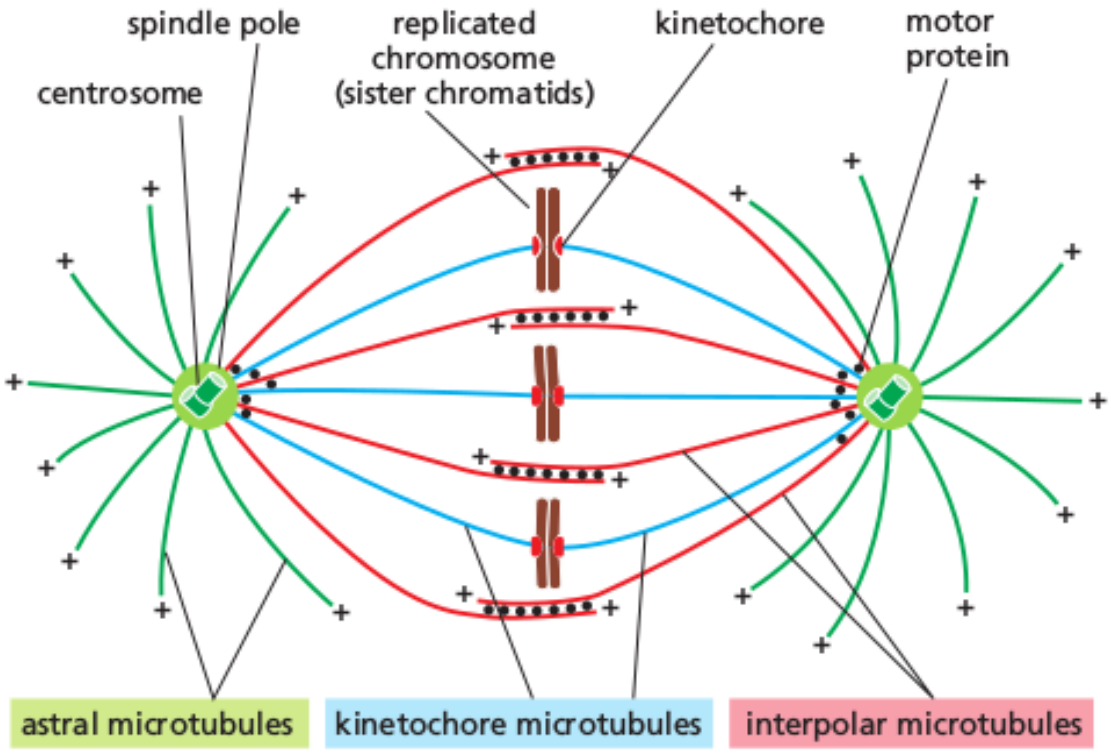


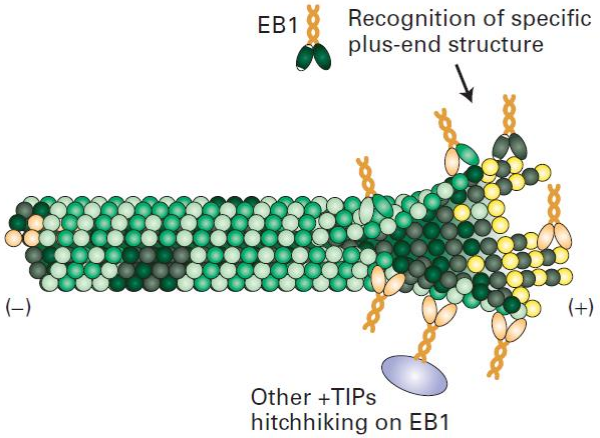


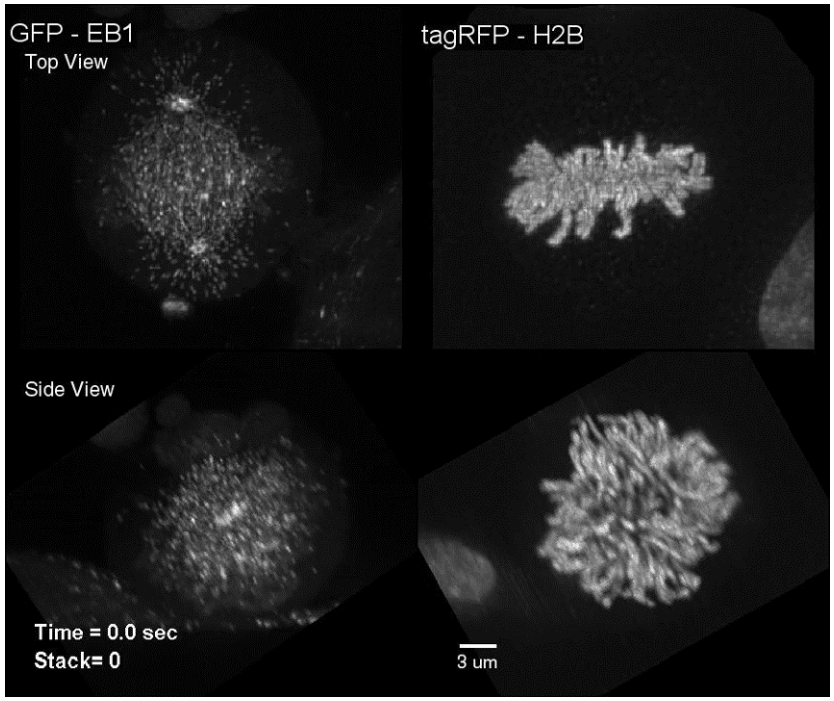


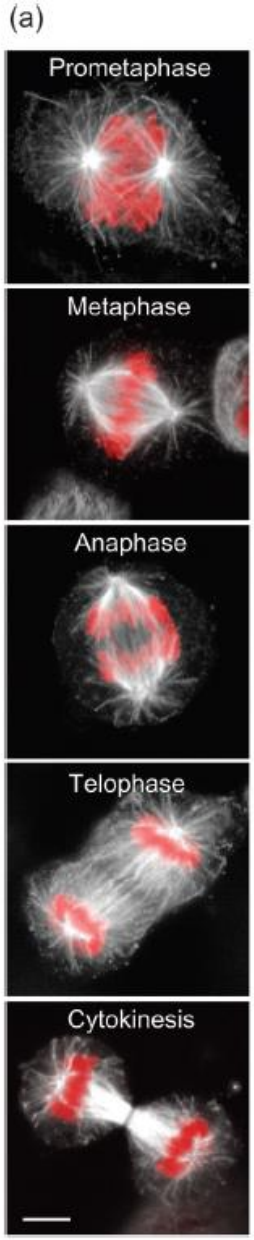
Huso mitótico

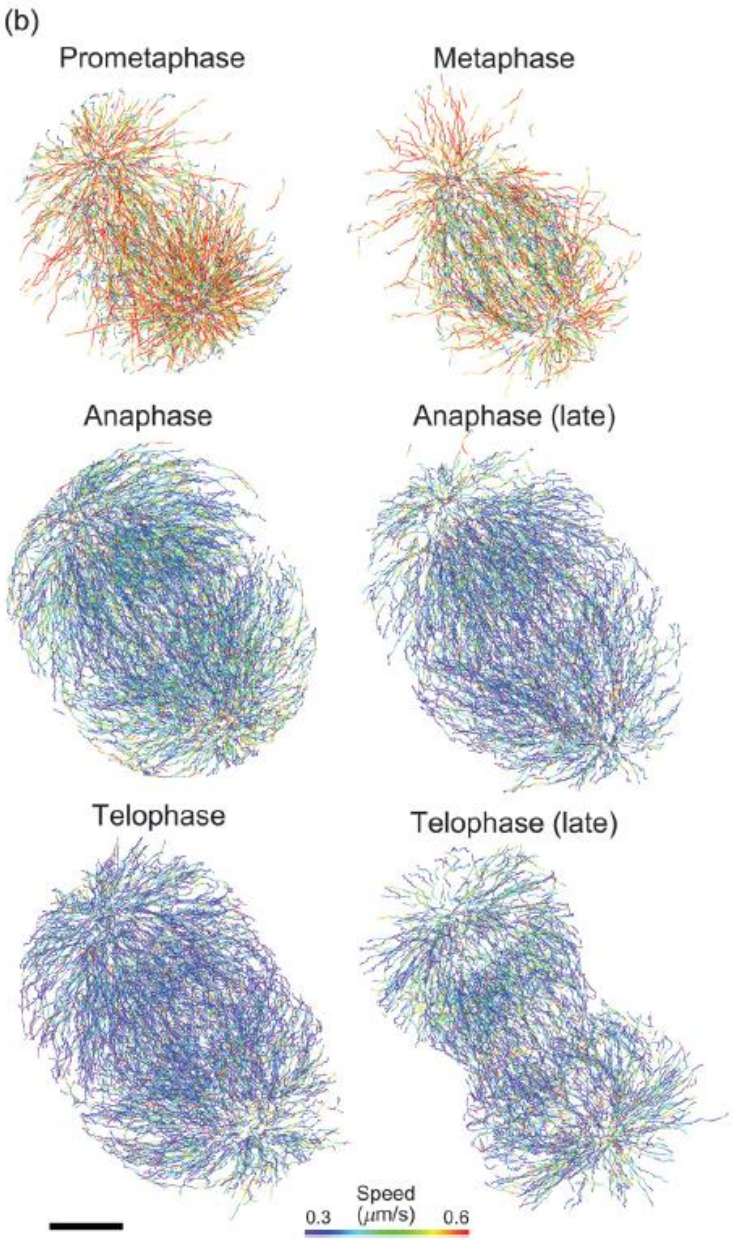






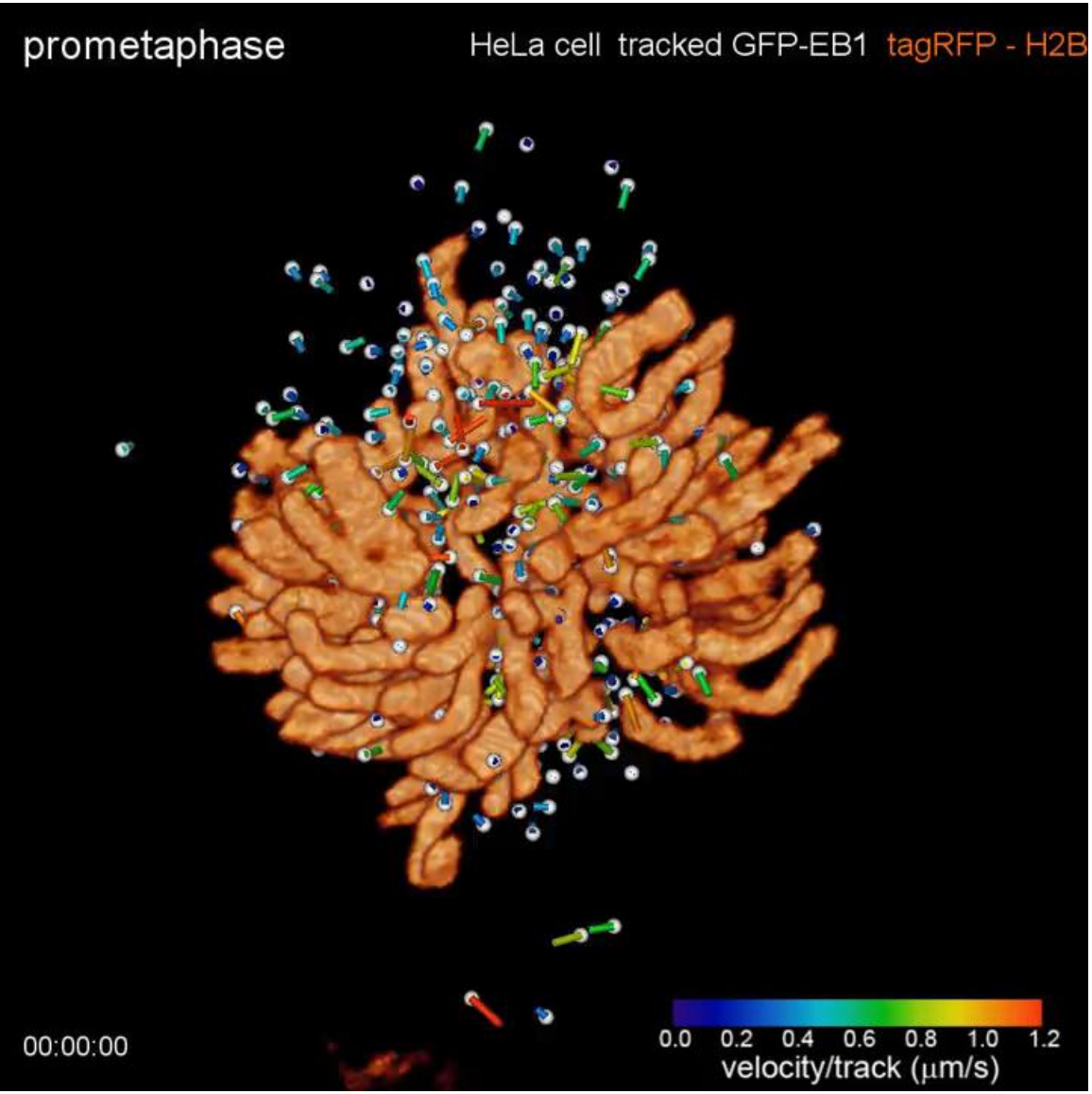






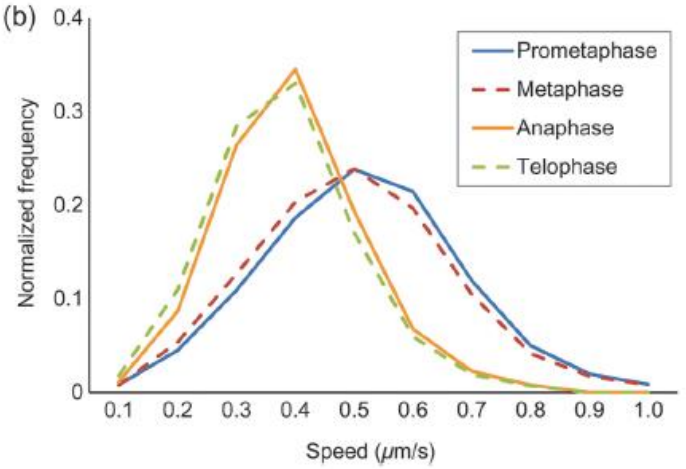
https://vimeo.com/album/3098015/video/109402221

Yamashita et al., 2015



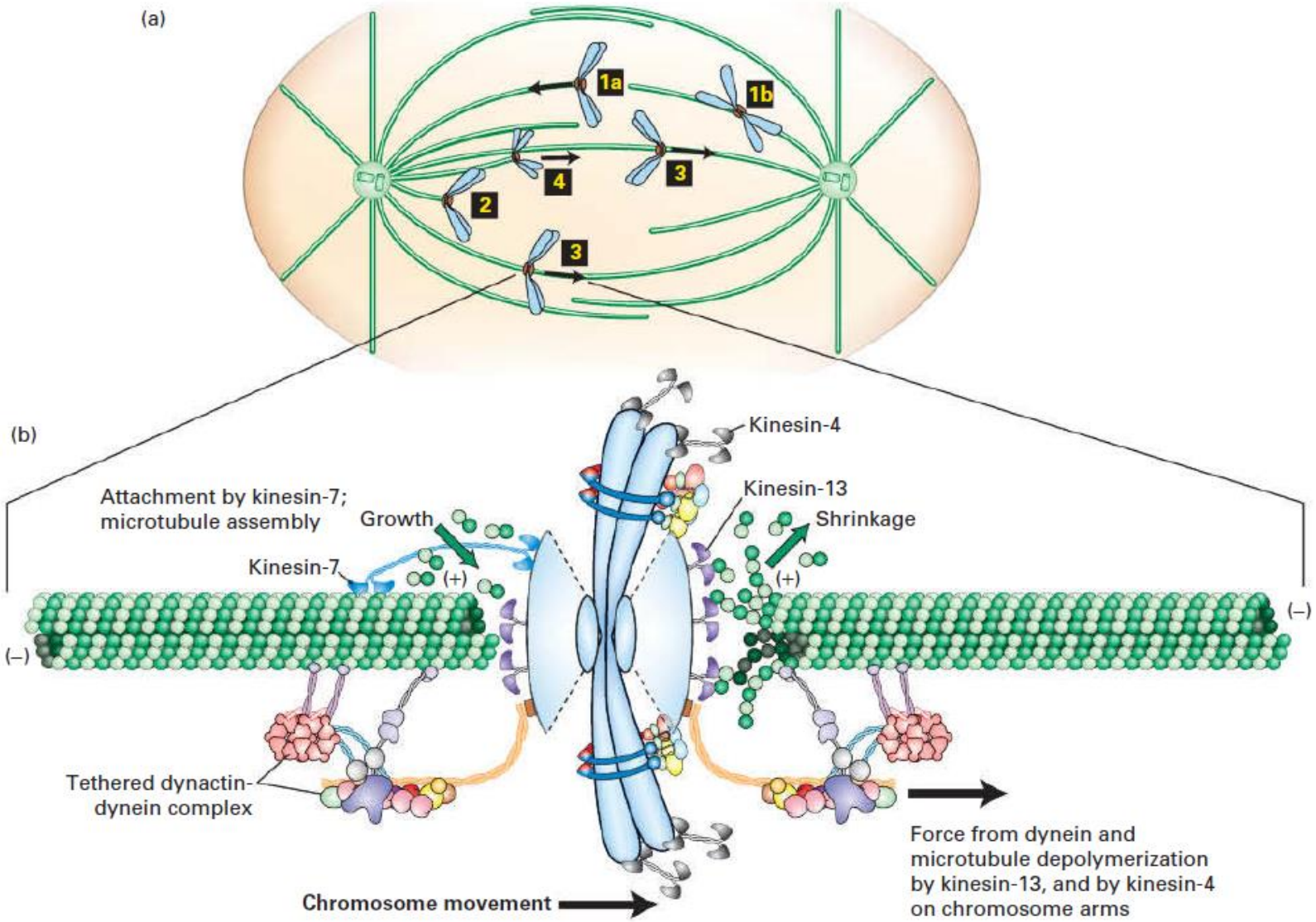
(a)

	Prometaphase	Metaphase	Anaphase	Telophase
Cell number	3	3	3	2
Spot count	62869	44876	142031	93462
Track count	11079	8374	18964	12191
Average speed (μm/s)	0.465	0.449	0.346	0.332
S.D.	0.165	0.165	0.119	0.121

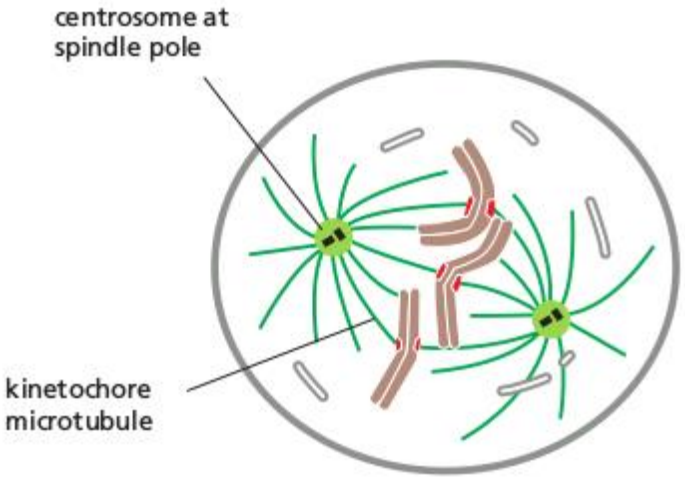


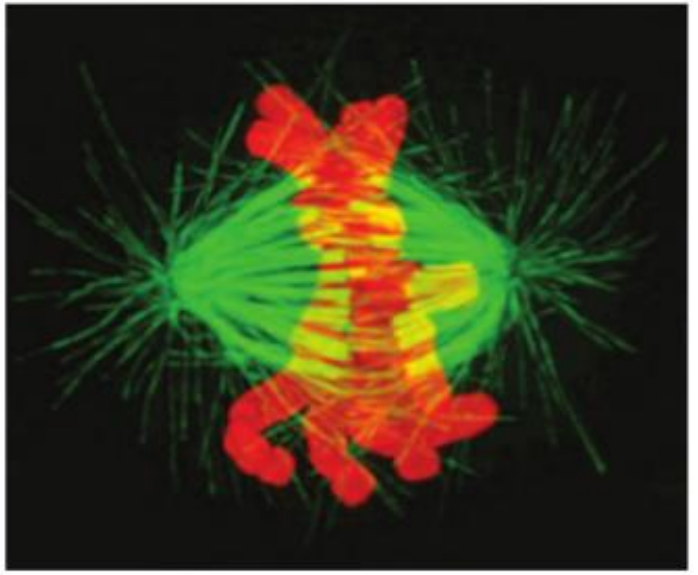
https://vimeo.com/album/3098015/video/109402304

Yamashita et al., 2015

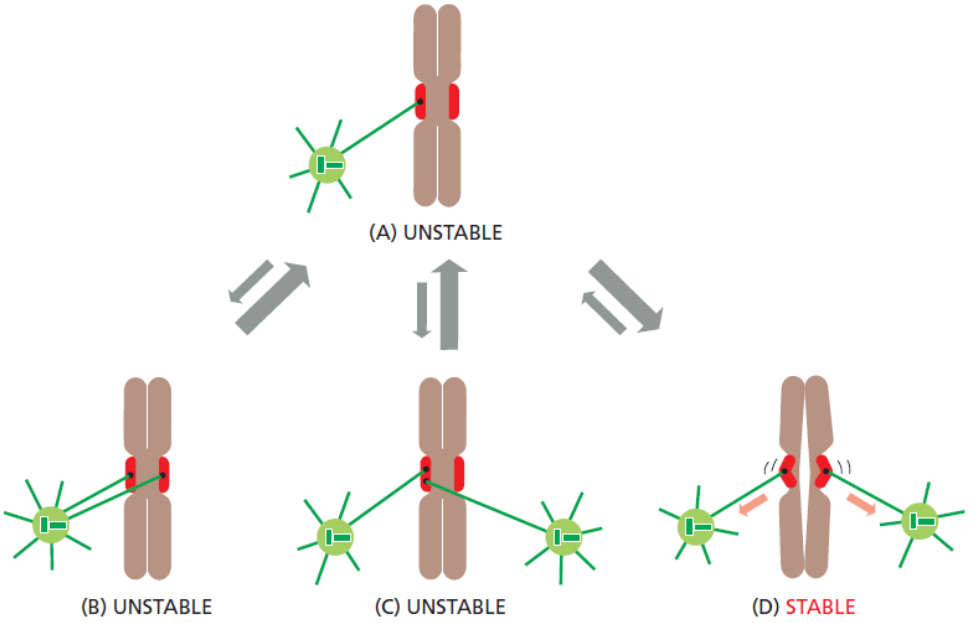

(a)
1a
1b
2
3
4
3
(b)
Kinesin-4
Kinesin-13
Attachment by kinesin-7;
microtubule assembly
Growth
Shrinkage
Kinesin-7
(+)
(+)
(–)
(–)
Tethered dynactin-
dynein complex
Force from dynein and
microtubule depolymerization
by kinesin-13, and by kinesin-4
on chromosome arms
Chromosome movement

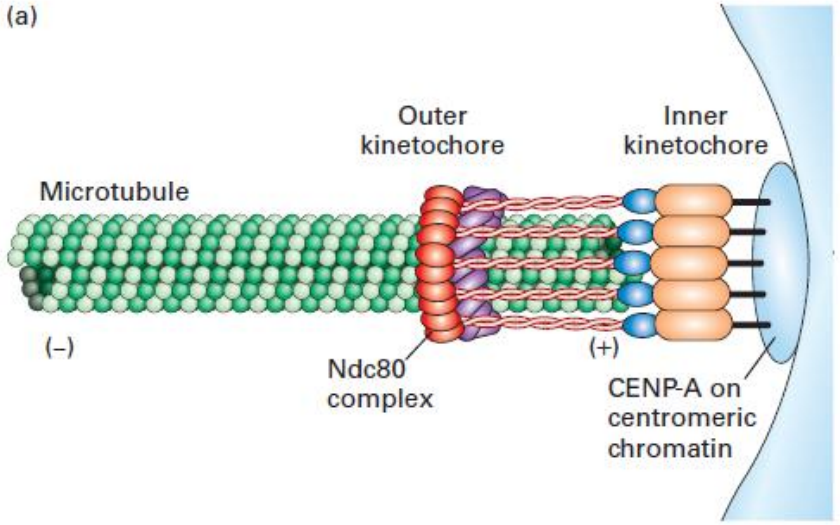
Metafase

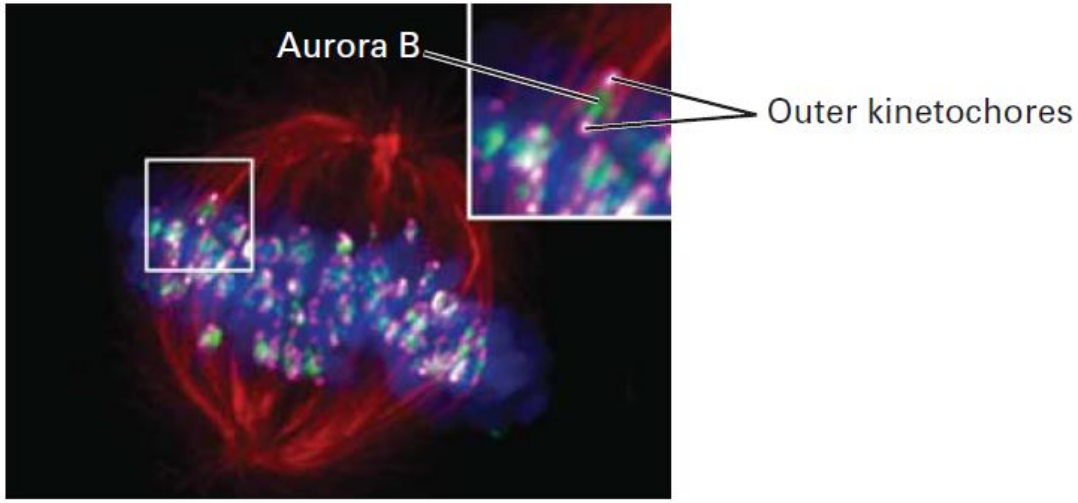


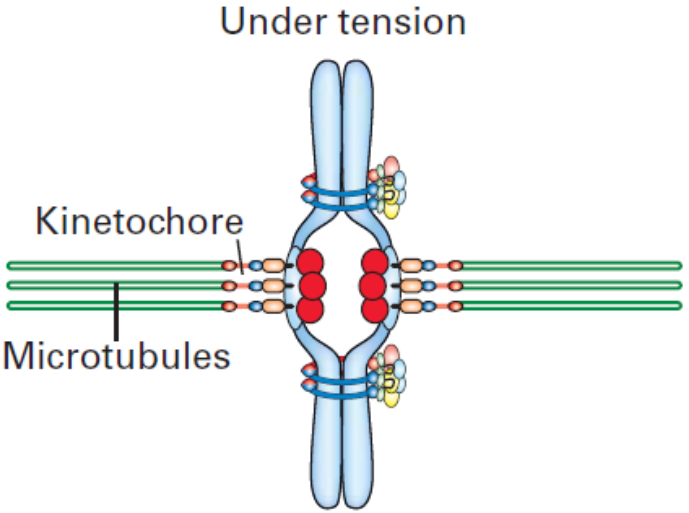


Los cromosomas son alineados en la placa metafásica.

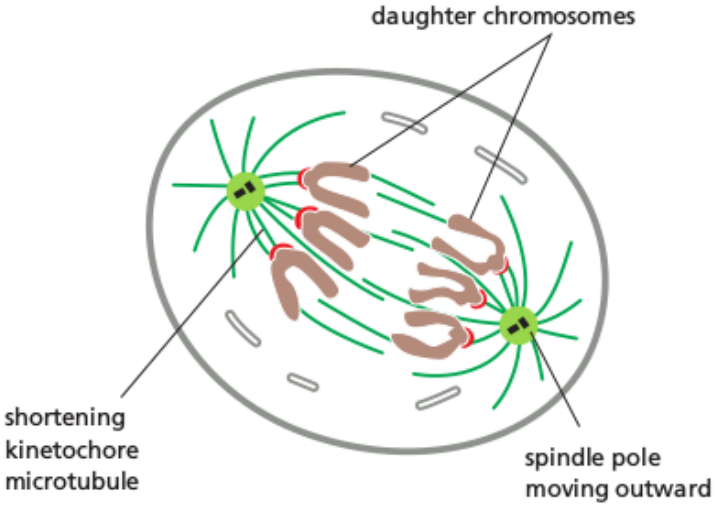

(A) UNSTABLE
(B) UNSTABLE
(C) UNSTABLE
(D) STABLE

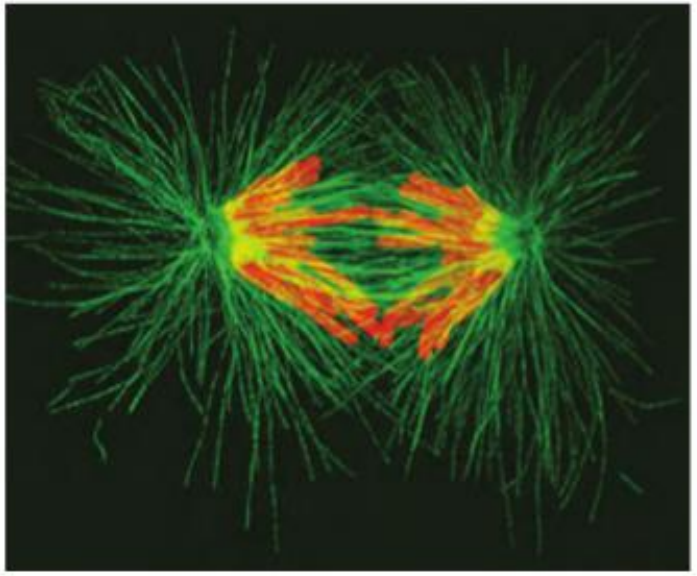

(a)
Outer kinetochore
Inner kinetochore
Microtubule
(–)
(+)
Ndc80 complex
CENP-A on centromeric chromatin


Aurora B
Outer kinetochores


Under tension
Kinetochore
Microtubules

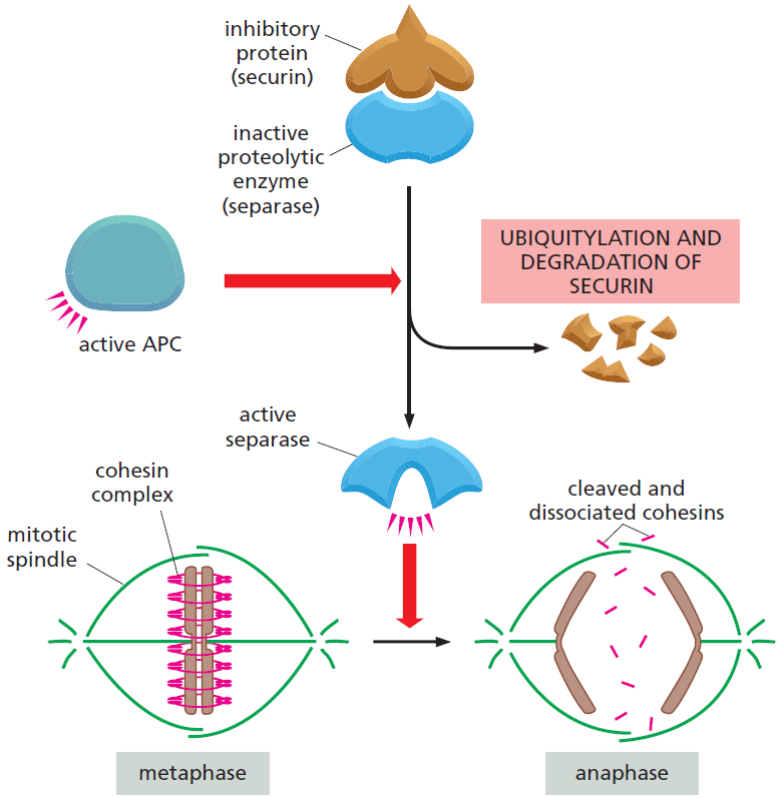
Anafase

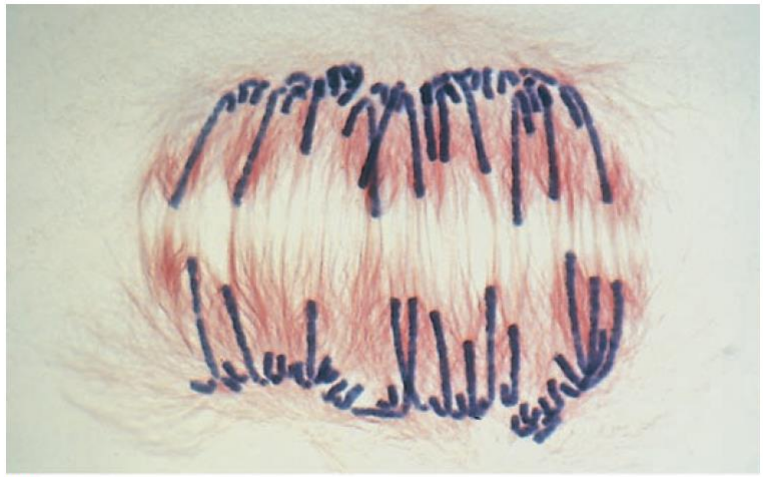


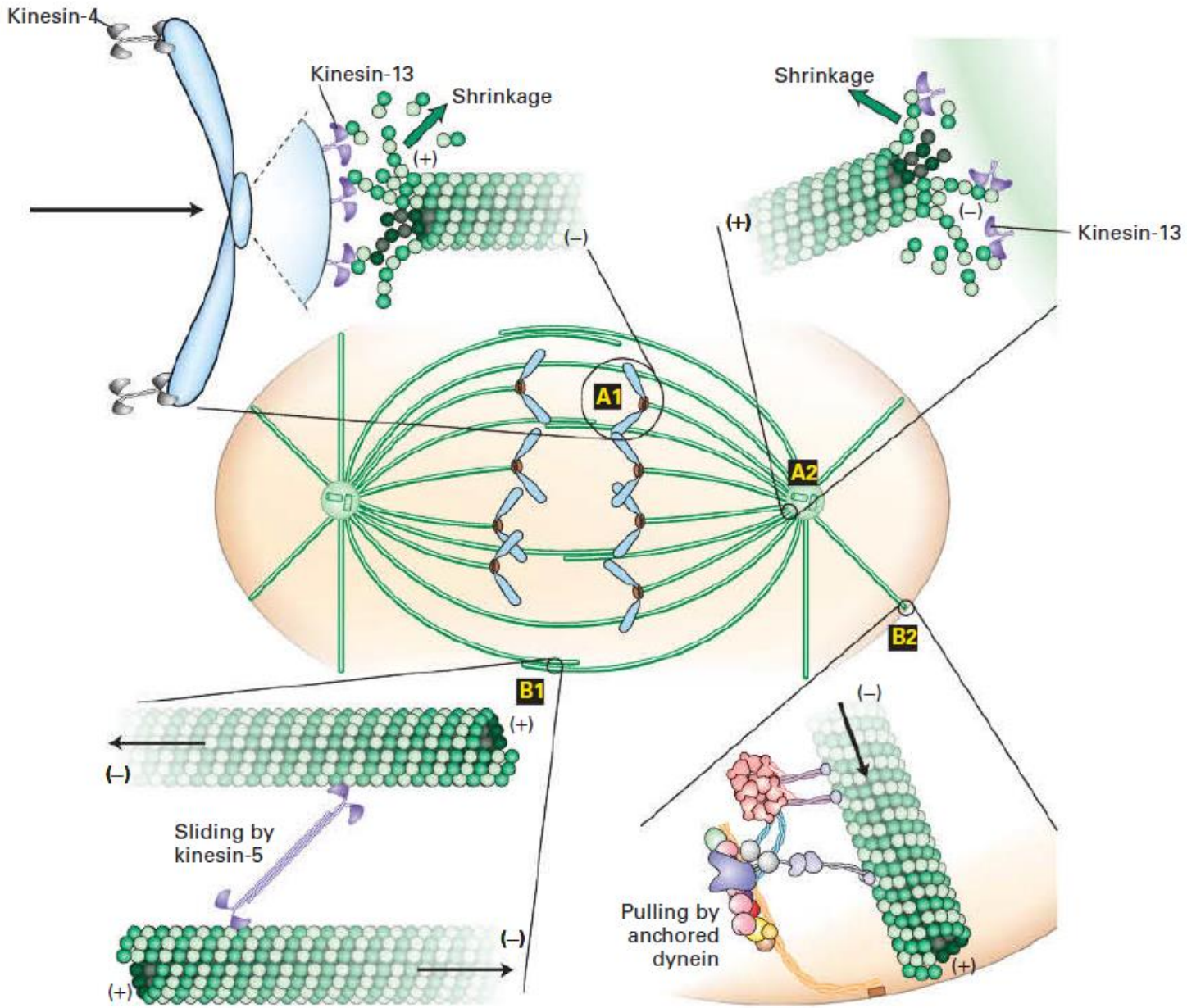


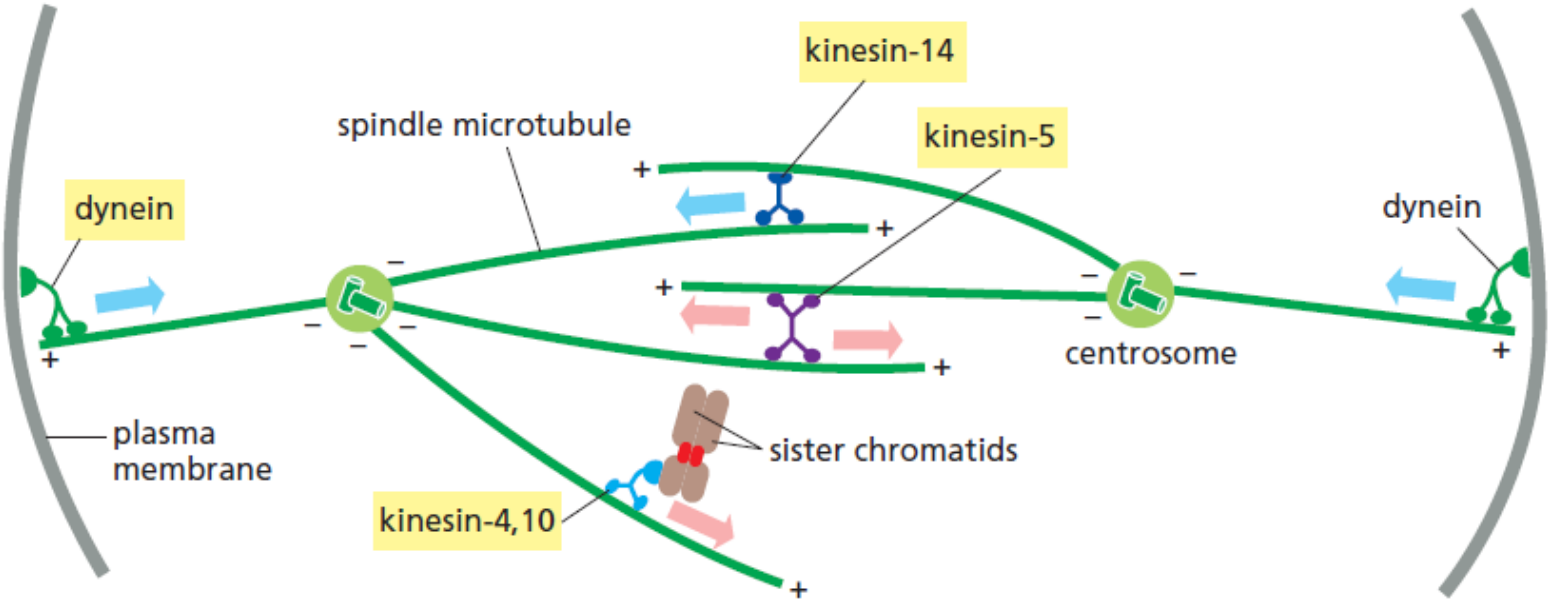
Las cromátidas hermanas se separan y cada una es empujada hacia un polo del huso mitótico

Separación de las cromátidas hermanas

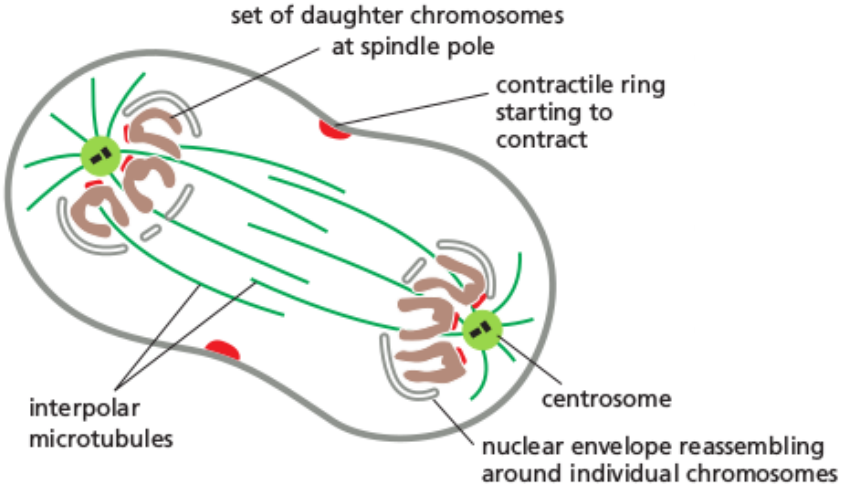


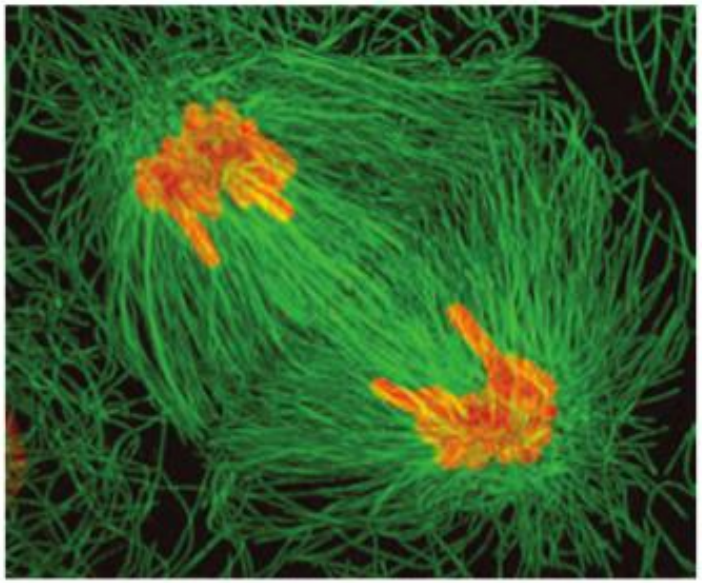



Kinesin-4
Kinesin-13
Shrinkage
(+)
(–)
Shrinkage
(+)
(–)
Kinesin-13
A1
A2
B2
B1
(+)
(–)
Sliding by
kinesin-5
(–)
(+)
(–)
Pulling by
anchored
dynein
(+)


kinesin-14
spindle microtubule
kinesin-5
dynein
dynein
centrosome
plasma
membrane
sister chromatids
kinesin-4,10

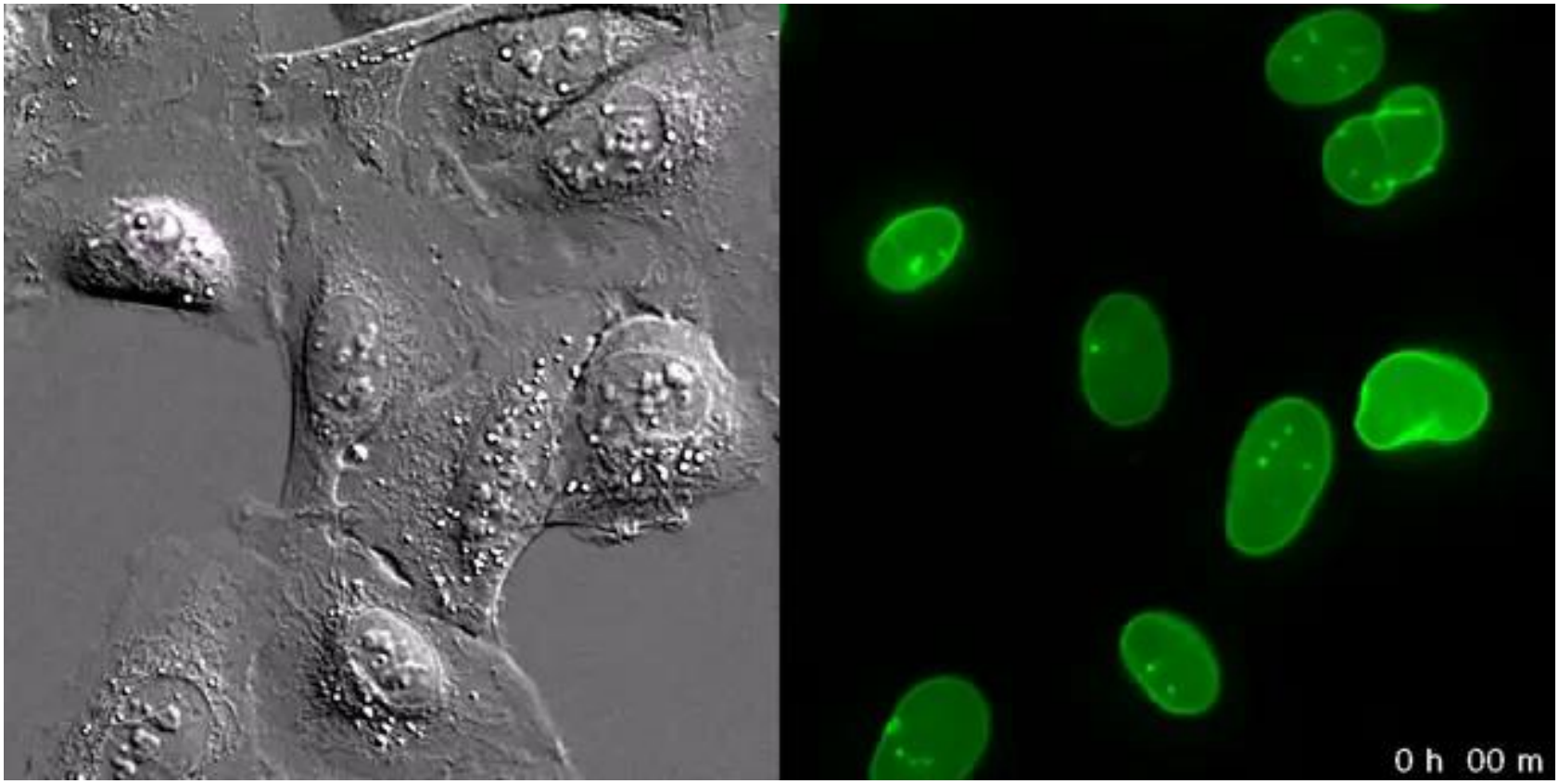
Telofase





Los cromosomas llegan a sus respectivos polos y comienza el re-ensamblado de la envoltura nuclear

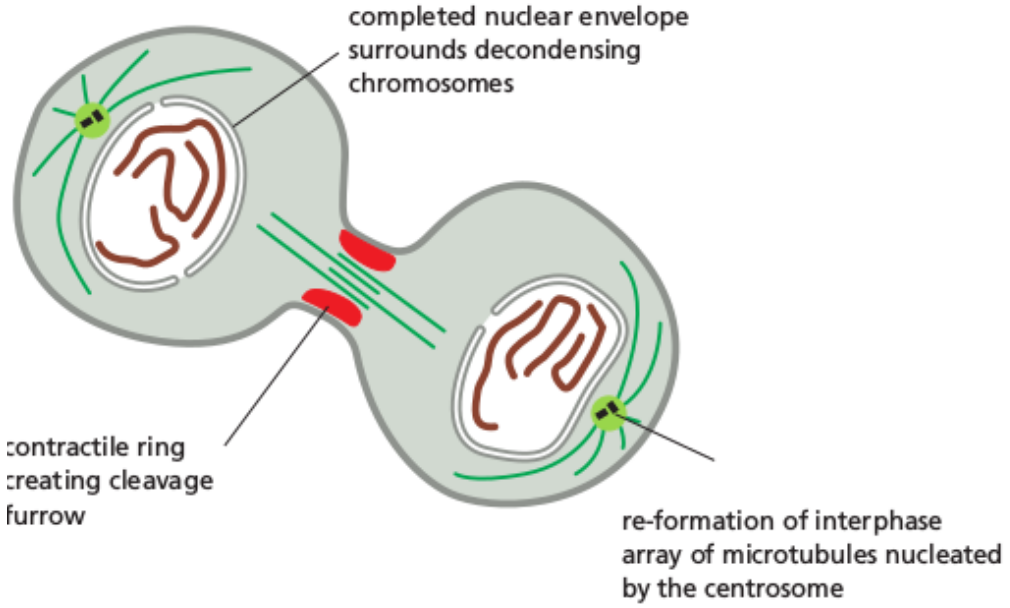
Re-ensamblado de la envoltura nuclear

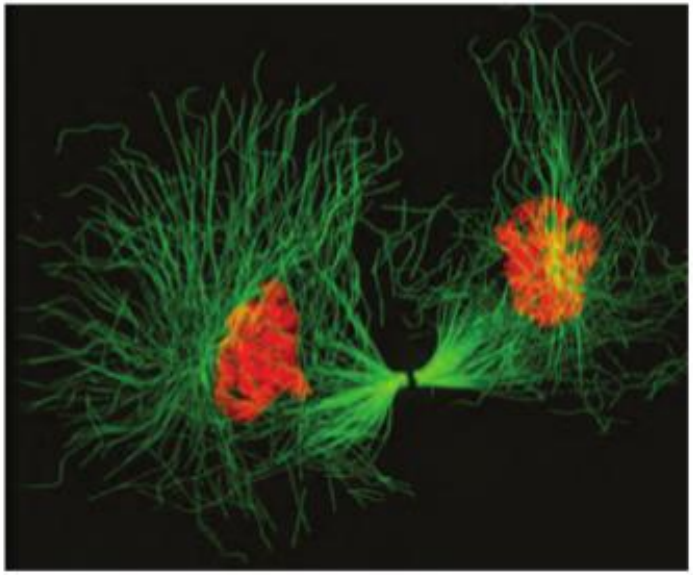


https://www.youtube.com/watch?v=34hoegpIiY4

Lamina-GFP

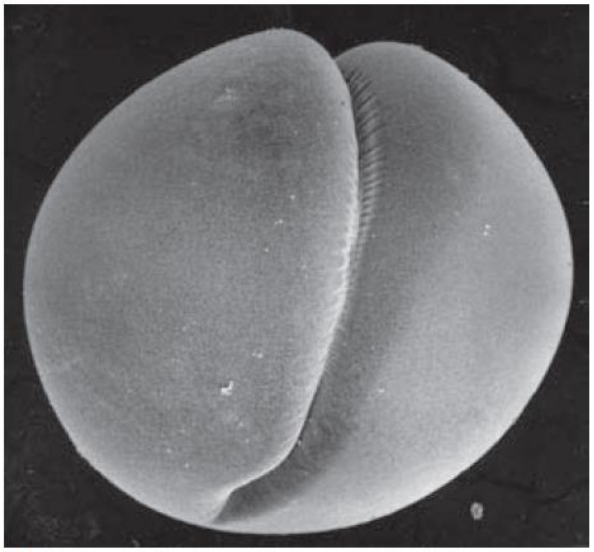
Citocinesis

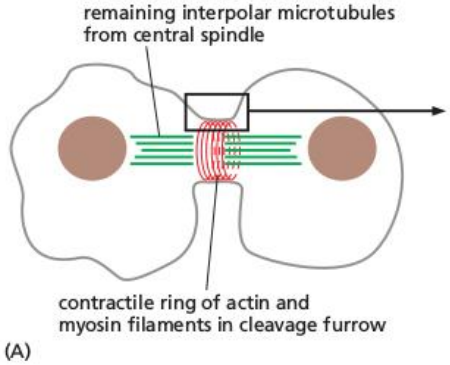


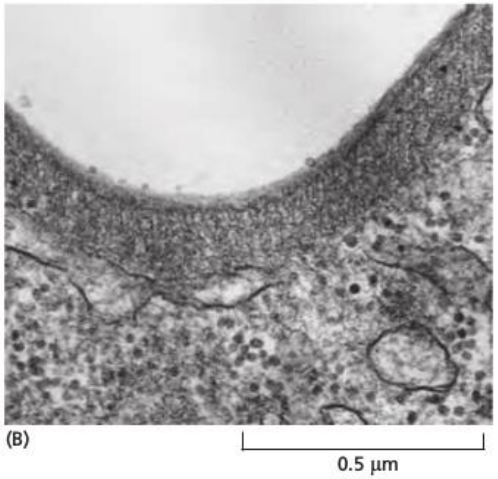


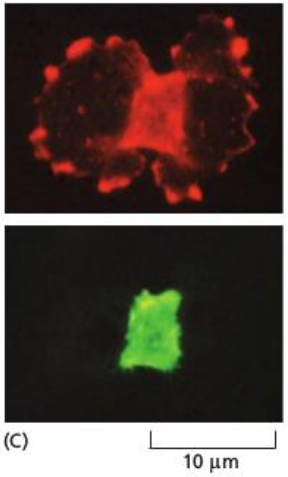
EL citoplasma es dividido en dos por un anillo contráctil de actina y miosina

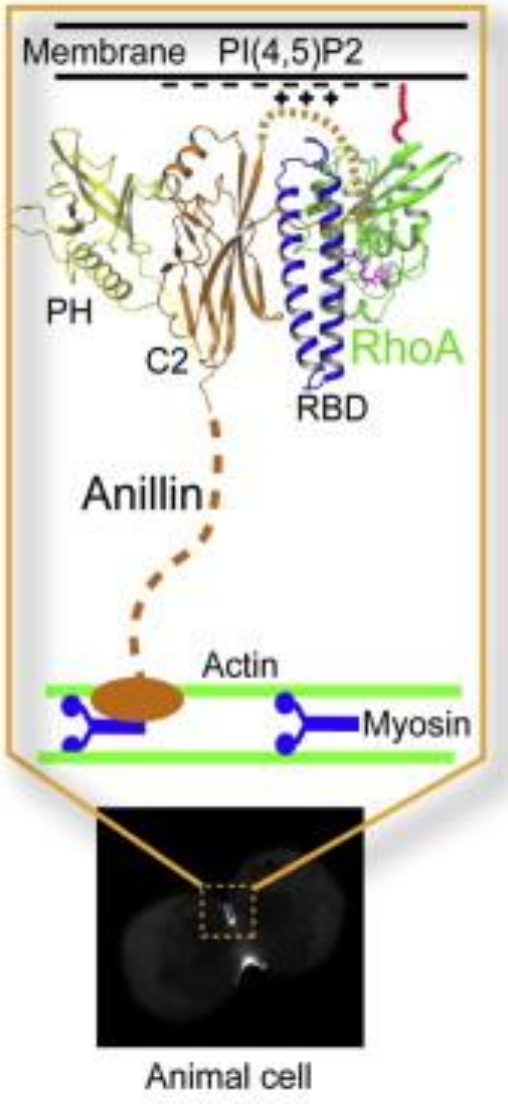
Anillo contráctil



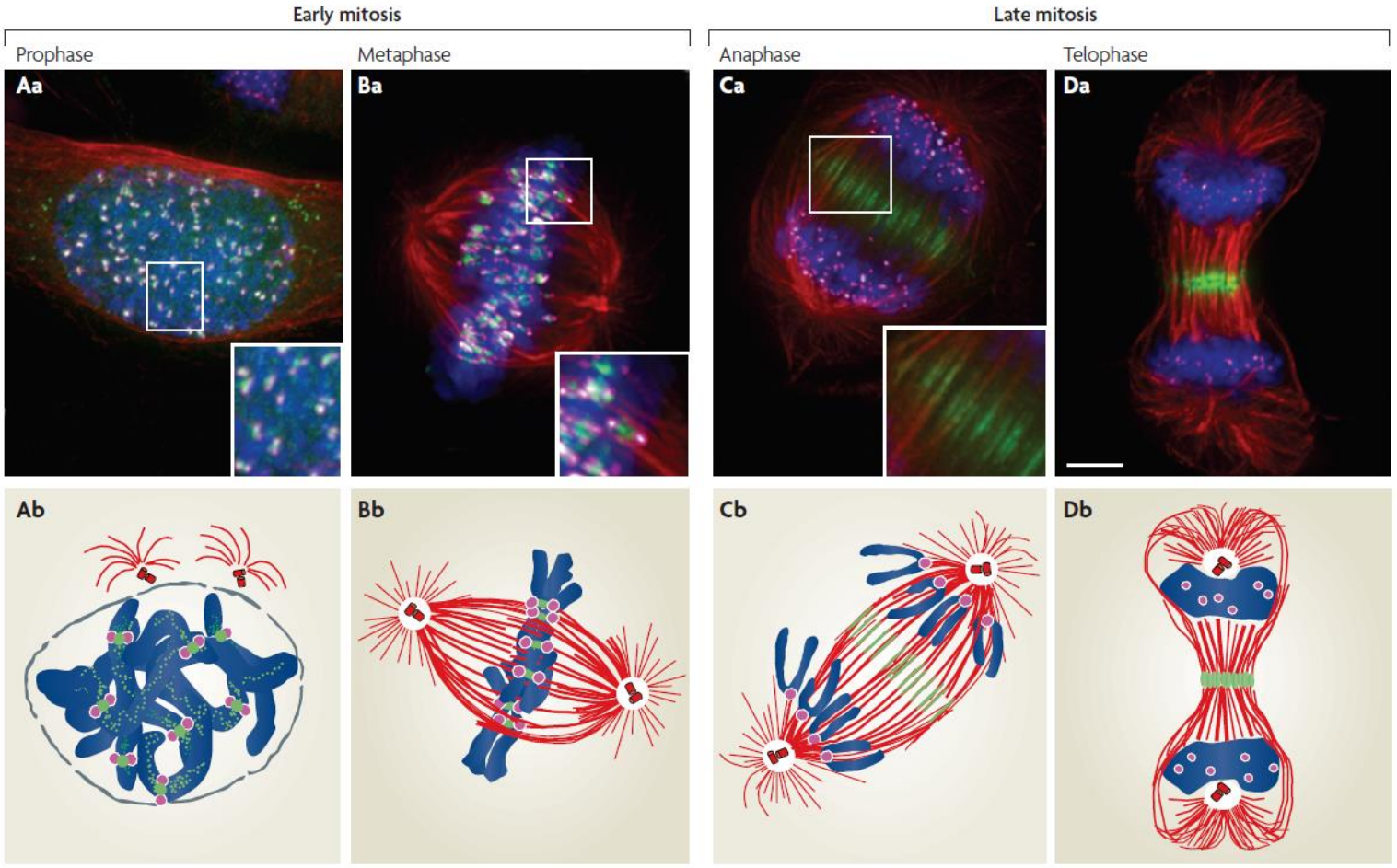








¿Dónde colocar el anillo?

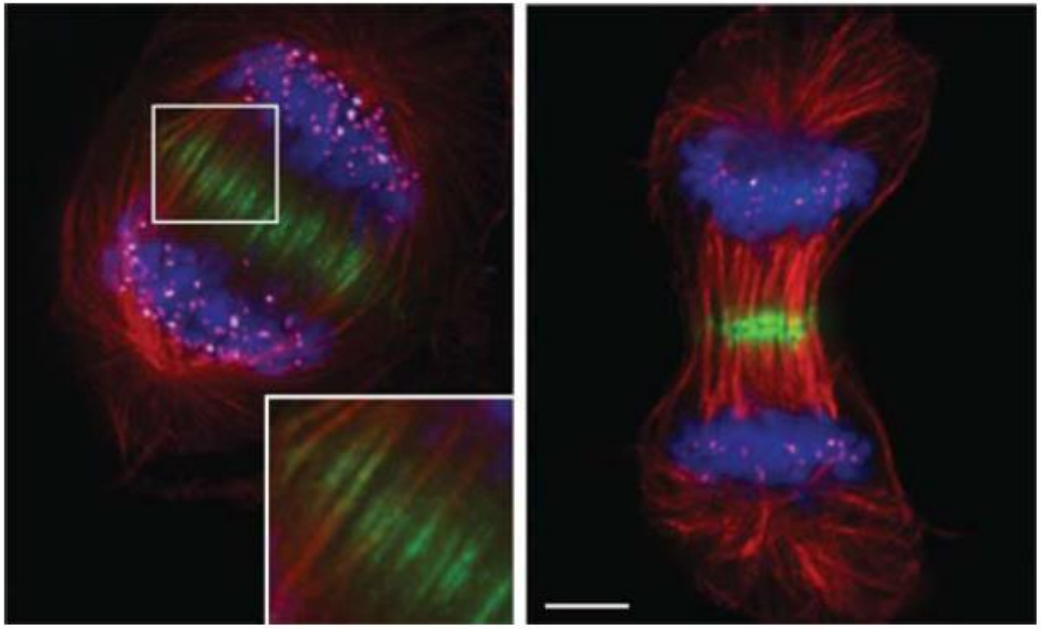


Chromosomal passenger complex (CPC)

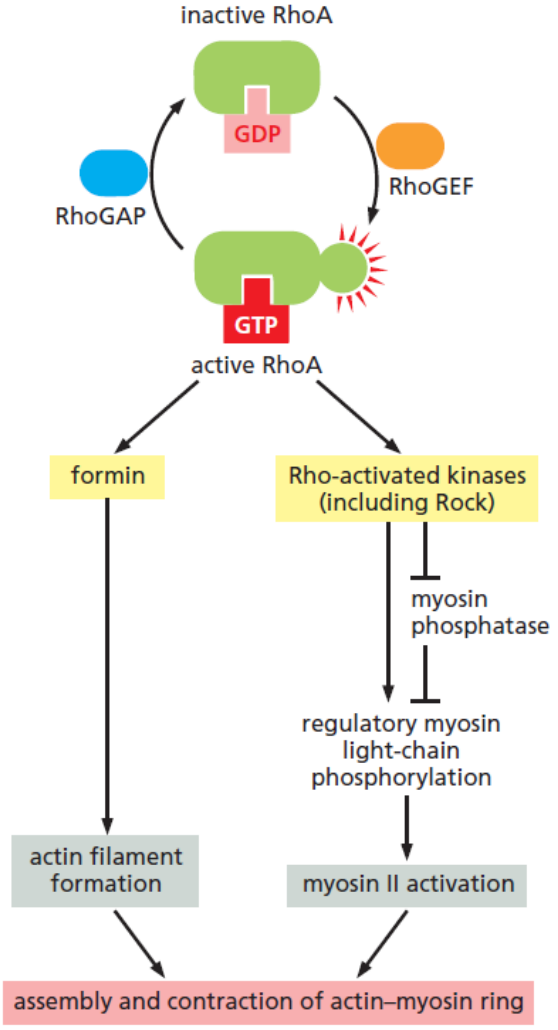
Aurora B
Microtúbulos
ADN
Cinetocoro

Ruchaud et al., 2007

¿Dónde colocar el anillo?

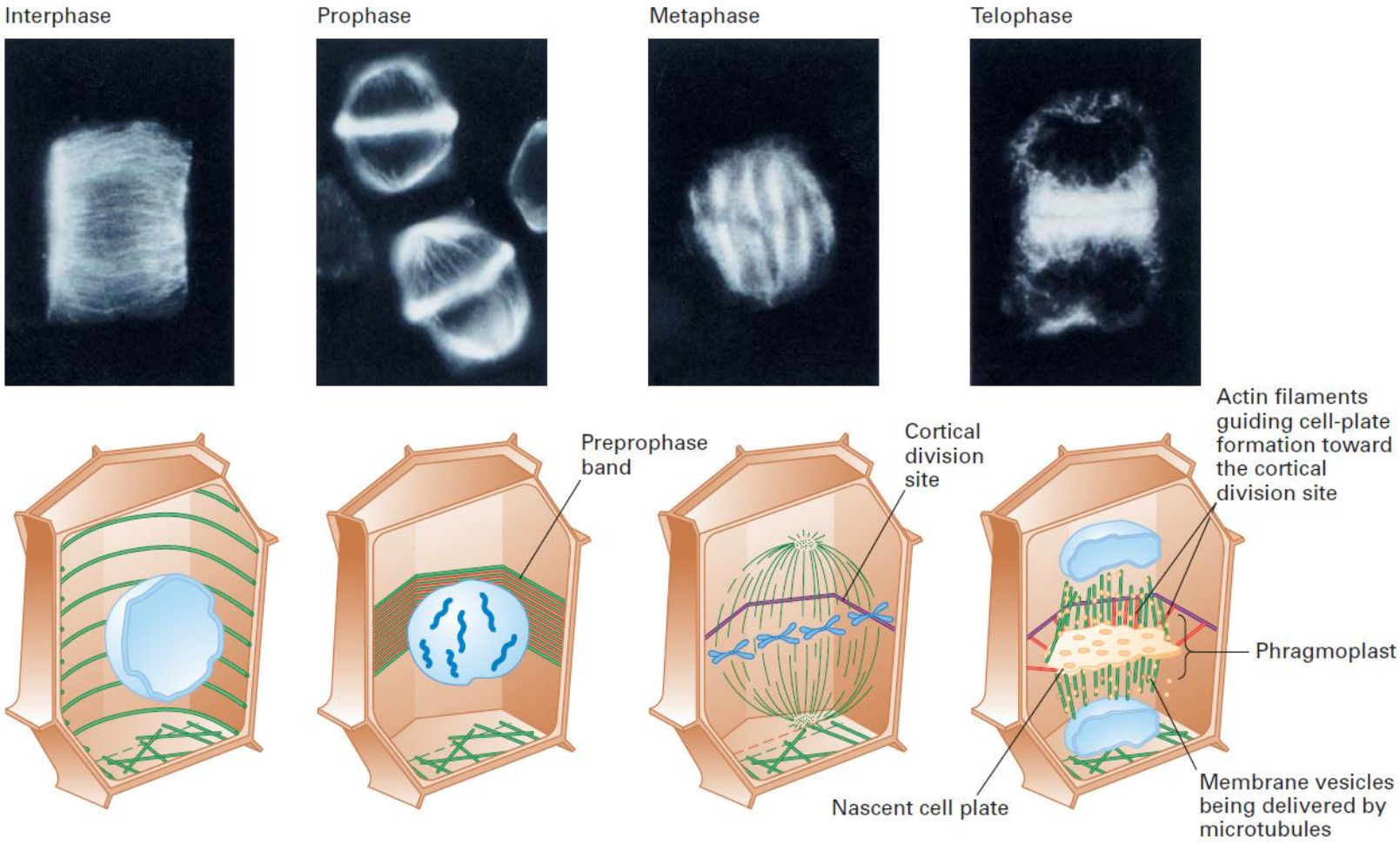


Anaphase Telophase

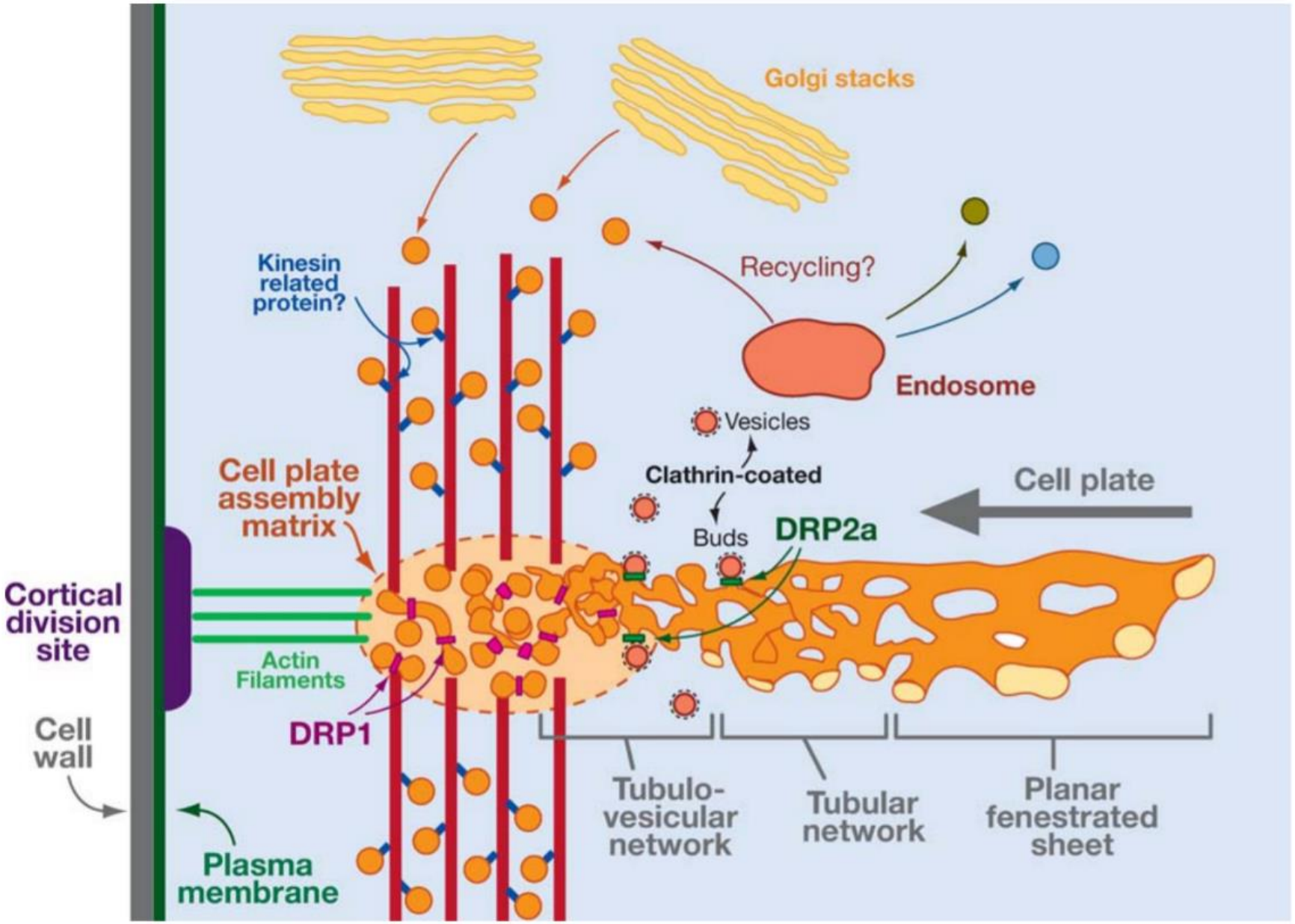


Chromosomal passenger complex (CPC)

Citocinesis en plantas



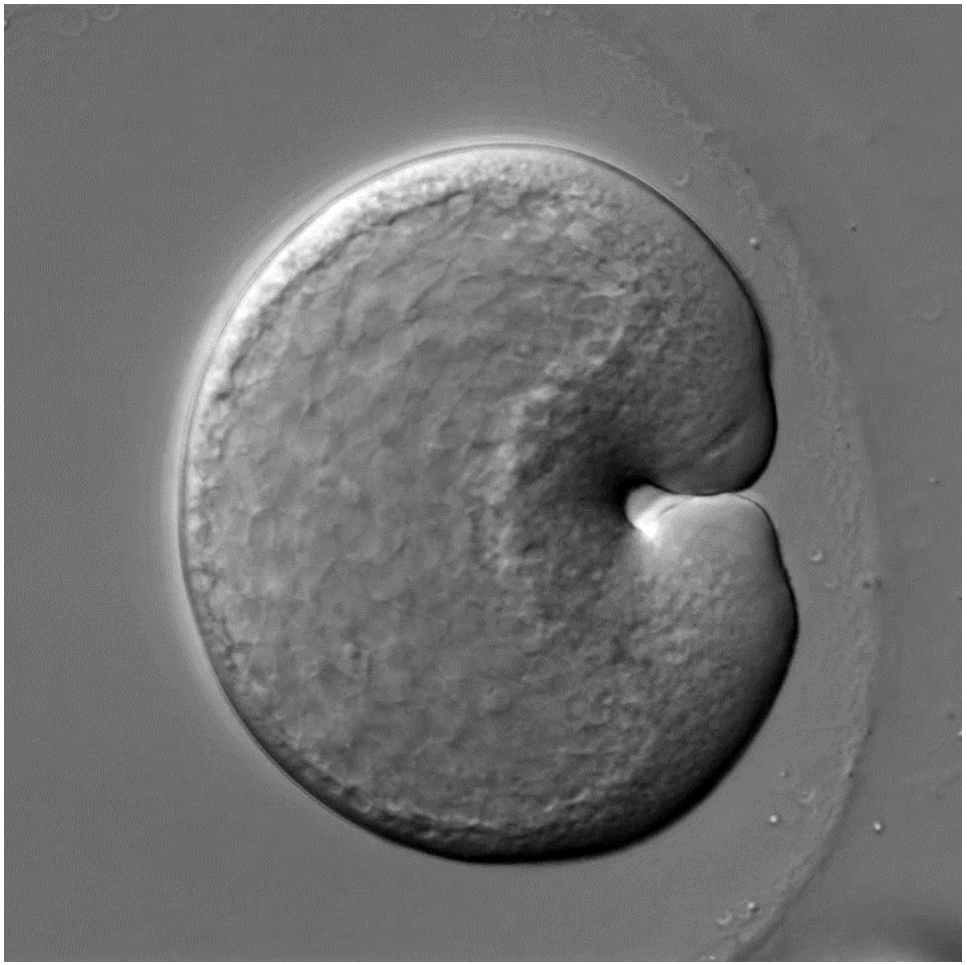
Fragmoplasto: vesículas derivas del Aparato de Golgi


Golgi stacks
Kinesin related protein?
Recycling?
Endosome
Vesicles
Clathrin-coated
Buds
Cell plate assembly matrix
Cell plate
DRP2a
Cortical division site
Actin Filaments
DRP1
Cell wall
Plasma membrane
Tubulo-vesicular network
Tubular network
Planar fenestrated sheet

Citocinesis en plantas

https://www.youtube.com/watch?v=an-L6TzoDbE&feature=youtu.be

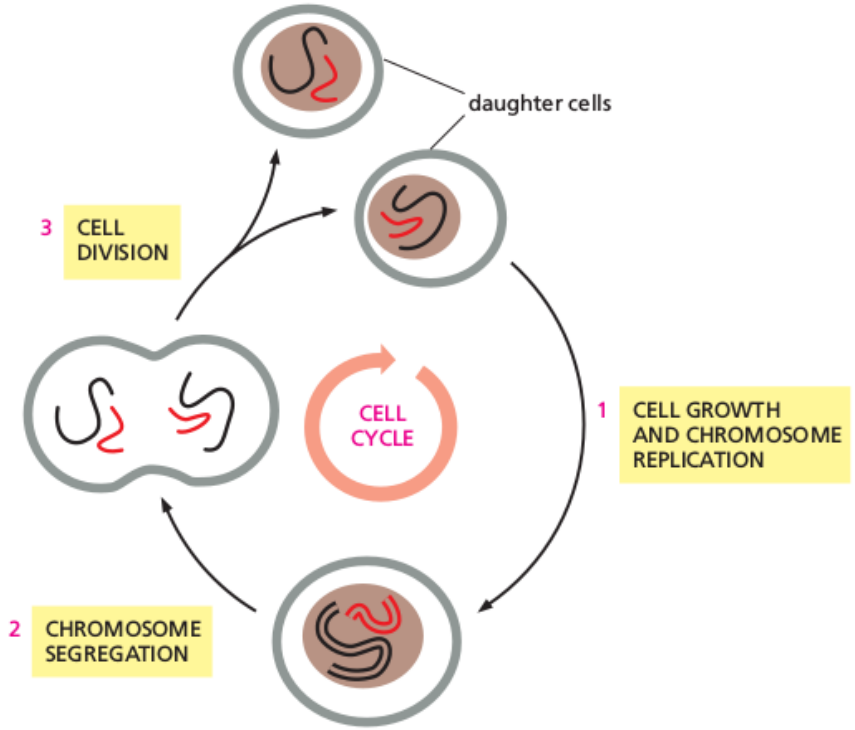
Ciclo celular II

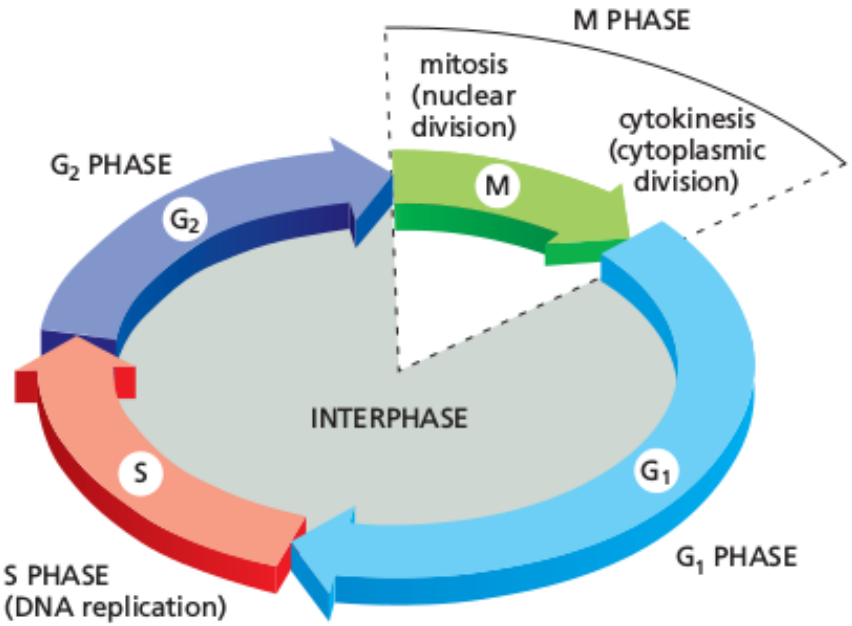


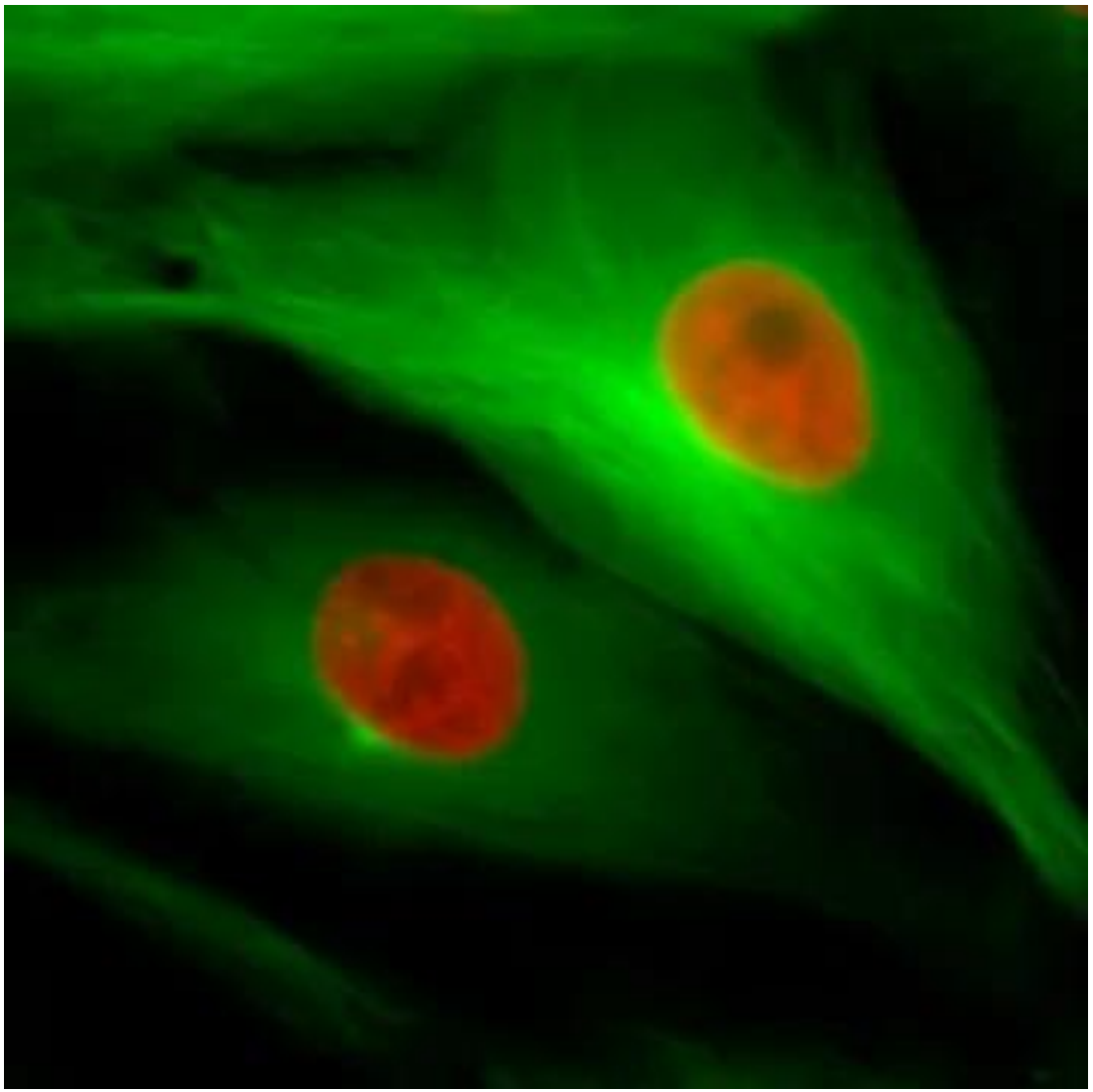
https://youtu.be/Zxk7bH2H6hQ

Gonzalo Aparicio
gaparicio@fcien.edu.uy

Fases del ciclo celular







https://youtu.be/rym83phFUI0

¿Como se regula el ciclo celular?

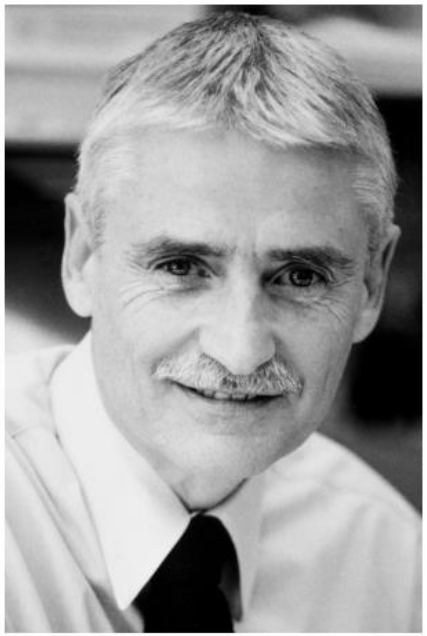


Photo from the Nobel Foundation archive.

Leland H. Hartwell

Prize share: 1/3

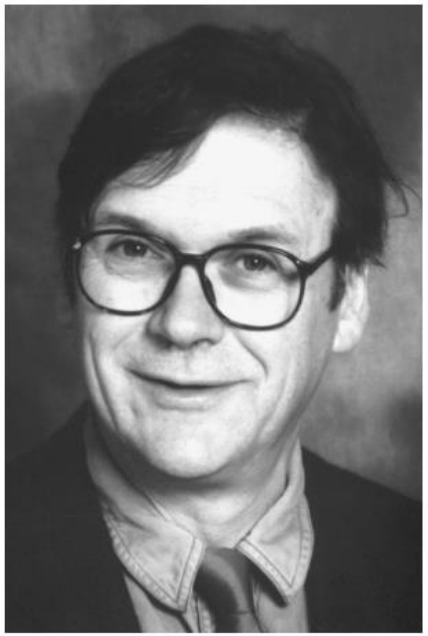


Photo from the Nobel Foundation archive.

Tim Hunt

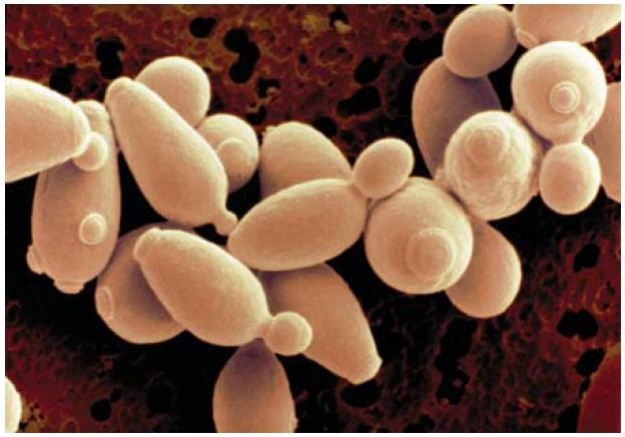
Prize share: 1/3

Photo from the Nobel Foundation archive.

Sir Paul M. Nurse

Prize share: 1/3

Saccharomyces cerevisiae



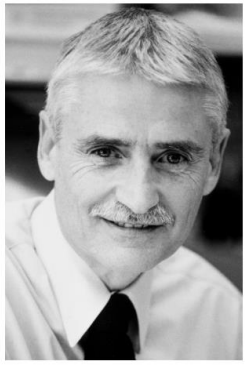


Photo from the Nobel Foundation archive.

Leland H. Hartwell

Schizosaccharomyces pombe

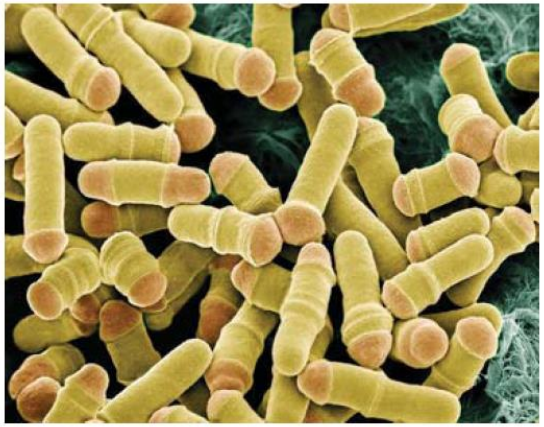
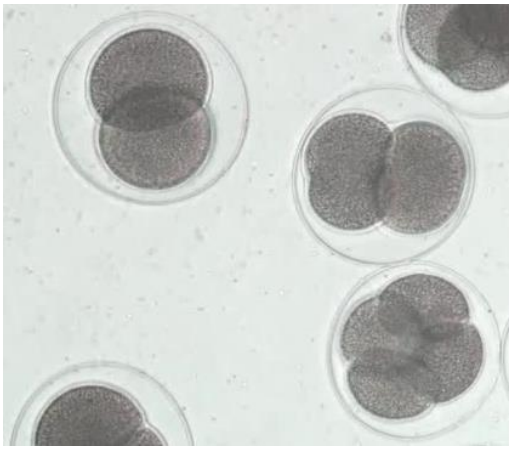


Photo from the Nobel Foundation archive.

Sir Paul M. Nurse

Arbacia punctulata



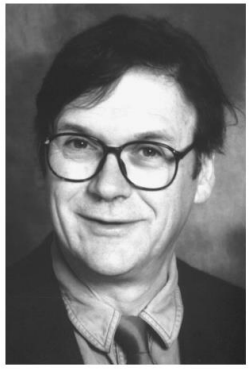
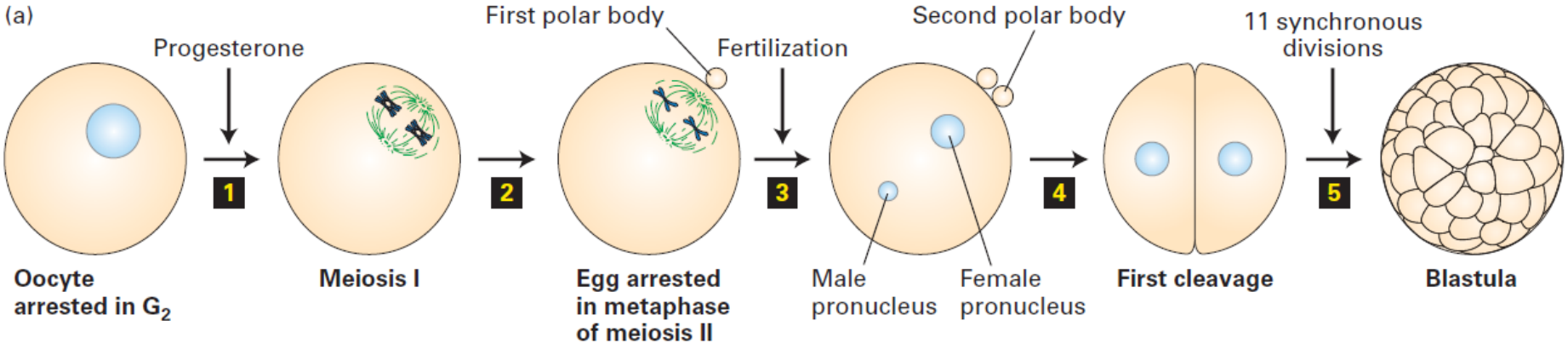
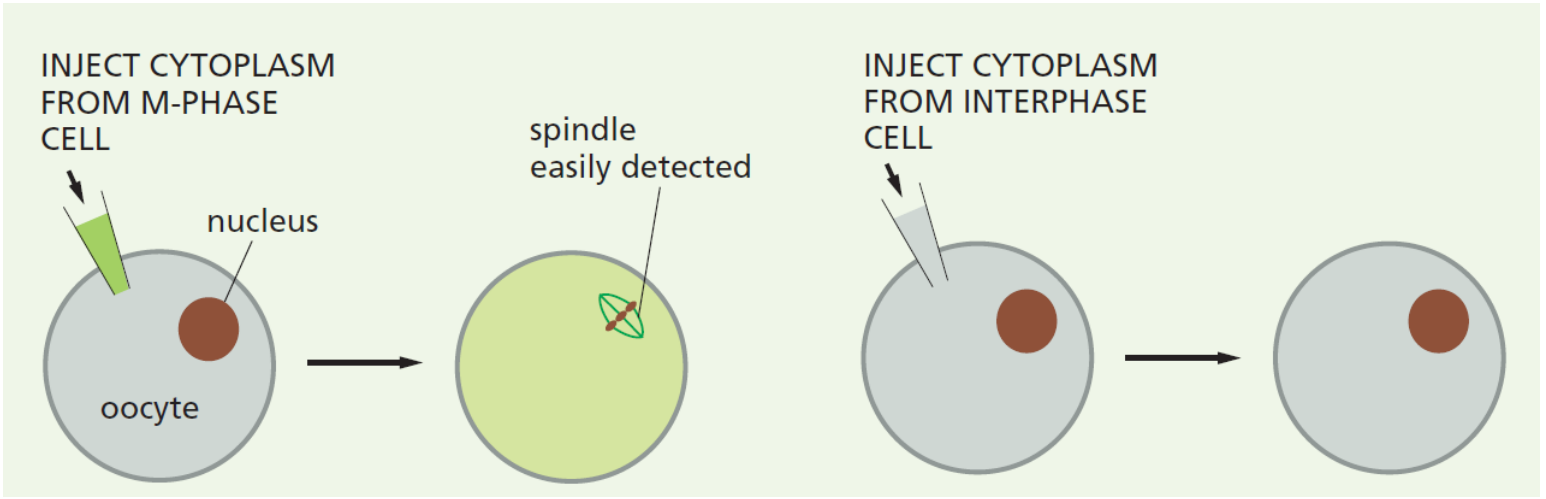


Photo from the Nobel Foundation archive.

Tim Hunt


(a)
Progesterone
1
Oocyte arrested in G_2
Meiosis I
2
First polar body
Egg arrested in metaphase of meiosis II
Fertilization
3
Second polar body
Male pronucleus
Female pronucleus
4
First cleavage
11 synchronous divisions
5
Blastula

Yosio Masui y Clement Markert (1971)



Entrada a Fase M

No entra a Fase M

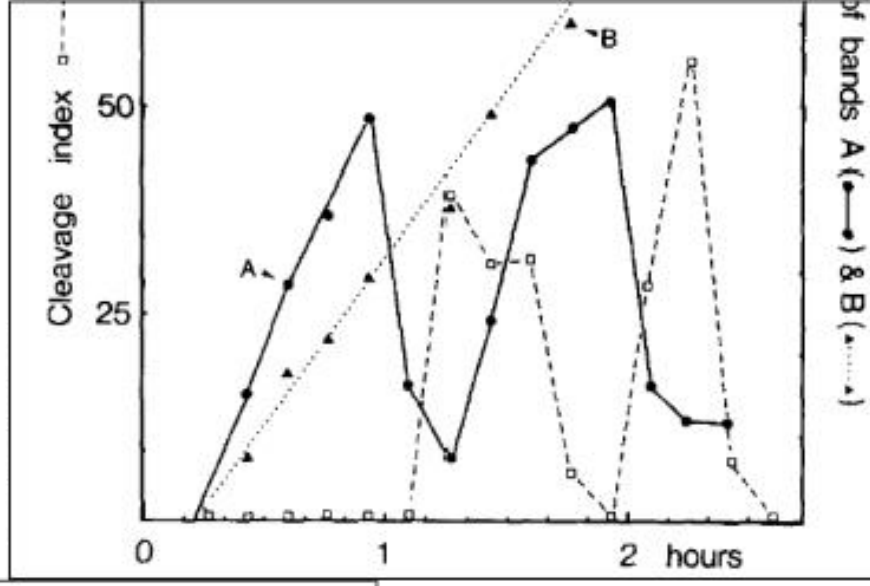
Factor Promotor de la Maduración (FPM)

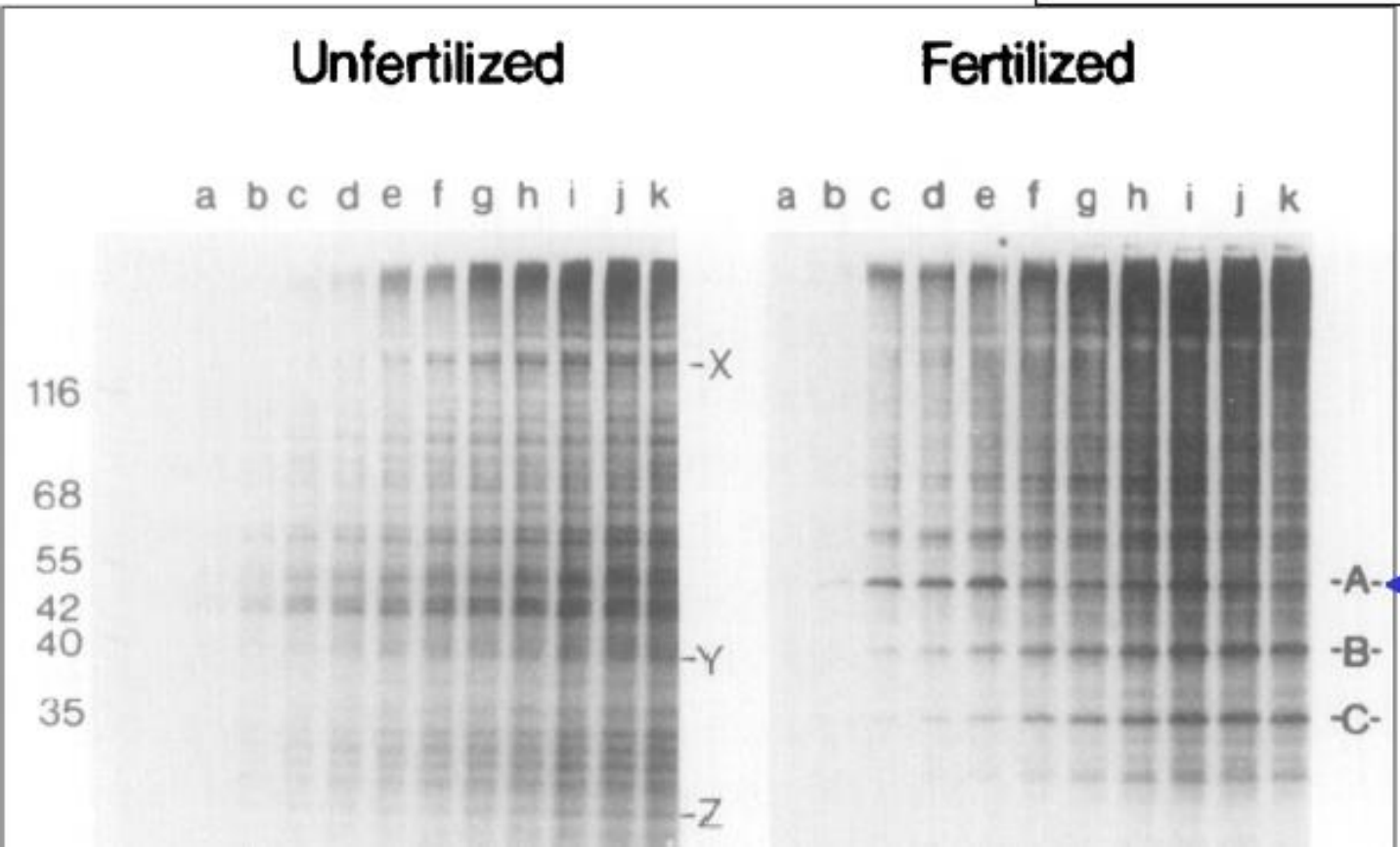
Existen señales en el citoplasma que regulan el ciclo celular

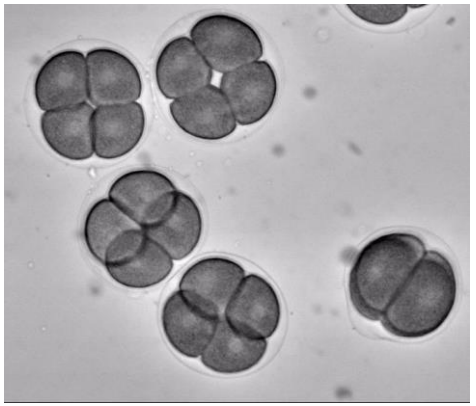
Cell, Vol. 33, 389–396, June 1983, Copyright © 1983 by MIT

Cyclin: A Protein Specified by Maternal mRNA in Sea Urchin Eggs That Is Destroyed at Each Cleavage Division

Tom Evans,* Eric T. Rosenthal,† Jim Youngblom,‡ Dan Distel,§ and Tim Hunt‖
Marine Biological Laboratory
Woods Hole, Massachusetts 02543





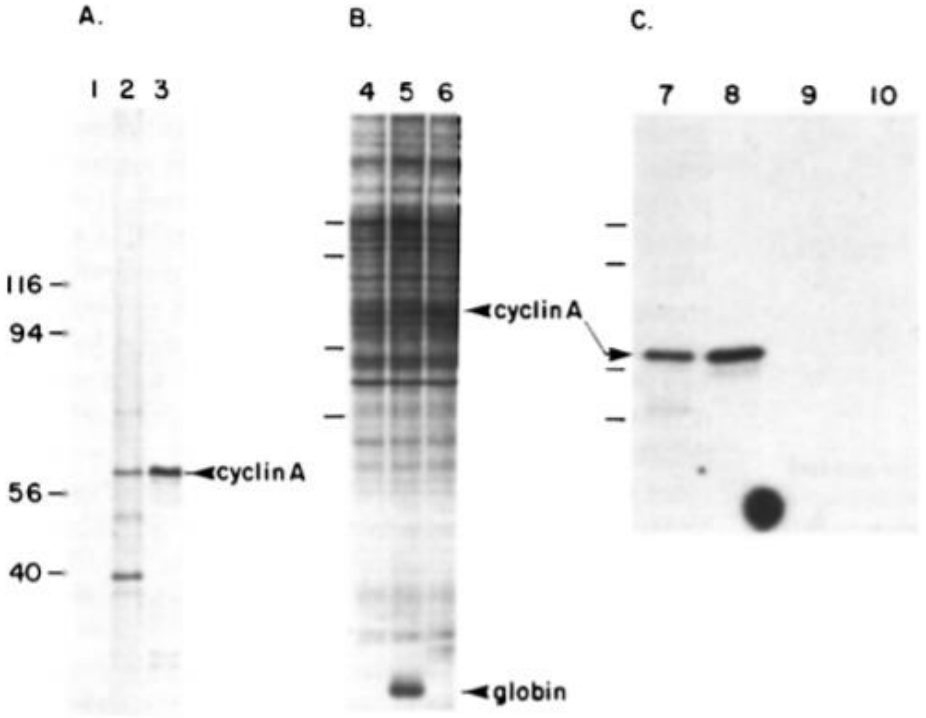


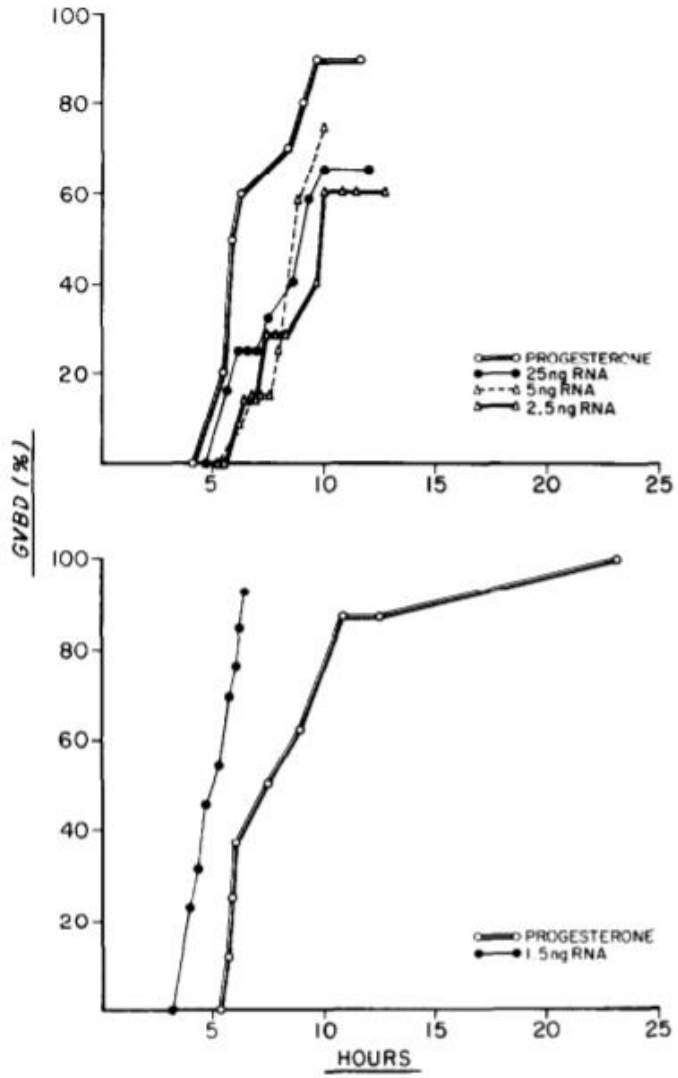
Ciclina

Cell, Vol. 47, 861–870, December 26, 1986, Copyright © 1986 by Cell Press

The Clam Embryo Protein Cyclin A Induces Entry into M Phase and the Resumption of Meiosis in Xenopus Oocytes

Katherine I. Swenson,* Kevin M. Farrell,† and Joan V. Ruderman





Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 3009–3013, May 1988
Cell Biology

Purification of maturation-promoting factor, an intracellular regulator of early mitotic events

(cell cycle/mitosis/protein phosphorylation)

MANFRED J. LOHKA*, MARIANNE K. HAYES†, AND JAMES L. MALLER

Department of Pharmacology, University of Colorado School of Medicine, Denver, CO 80262

Communicated by Raymond L. Erikson, December 22, 1987 (received for review October 10, 1987)

ABSTRACT **Maturation-promoting factor causes germinal vesicle breakdown when injected into *Xenopus* oocytes and can induce metaphase in a cell-free system. The cell-free assay was used to monitor maturation-promoting factor during its purification from unfertilized *Xenopus* eggs. Ammonium sulfate precipitation and six chromatographic procedures resulted in a preparation purified >3000-fold that could induce germinal vesicle breakdown within 2 hr when injected into cycloheximide-treated oocytes. Proteins of 45 kDa and 32 kDa were correlated with fractions of highest activity in both assays. These fractions contained a protein kinase activity able to phosphorylate the endogenous 45-kDa protein, as well as histone H1, phosphatase inhibitor 1, and casein. The highly purified preparations described here should help to identify the mechanism of action of maturation-promoting factor and to elucidate the role of protein kinases in the induction of metaphase.**

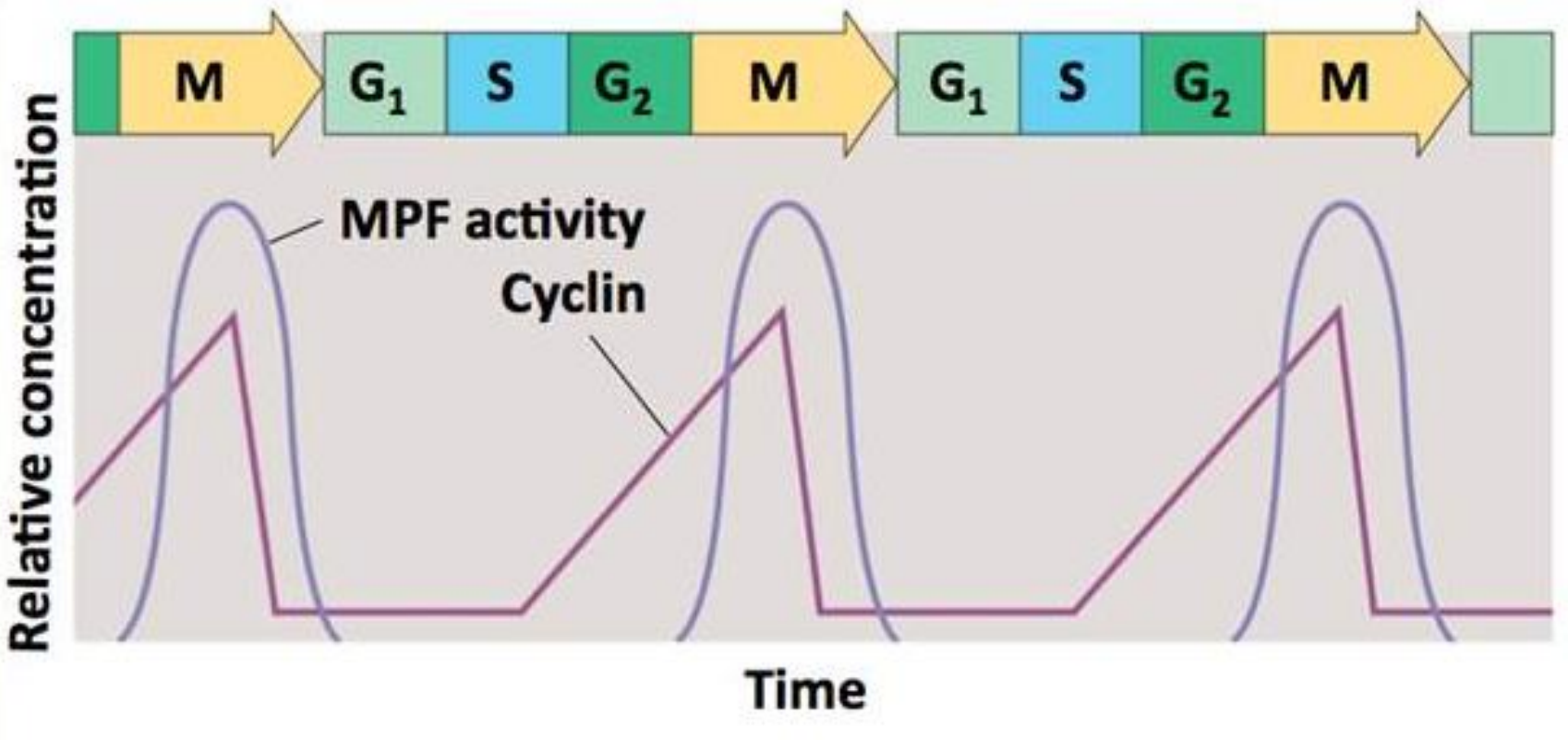
of MPF in the cell cycle, the protein(s) responsible for activity has not been identified as yet.

A cell-free system from amphibian eggs has been d oped in which nuclei can be induced to undergo early m events by the addition of crude or partially purified p rations of MPF (14–16). Experiments in this cell-free sy implicated protein phosphorylation in the mechanis action of MPF (17), results consistent with the observa (18, 19) of increased protein phosphorylation when appears during oocyte maturation or after MPF injection hypothesis that a protein kinase is involved in the regul of the $G_2 \rightarrow M$ transition is supported by observatio increased phosphorylation of histones, lamins, and proteins during M phase (20, 21). Furthermore, in ye least two of the mutations that cause arrest in G_2 affect encoding protein kinase activities (22–24). As genes enc proteins of similar sequence can also be detected in

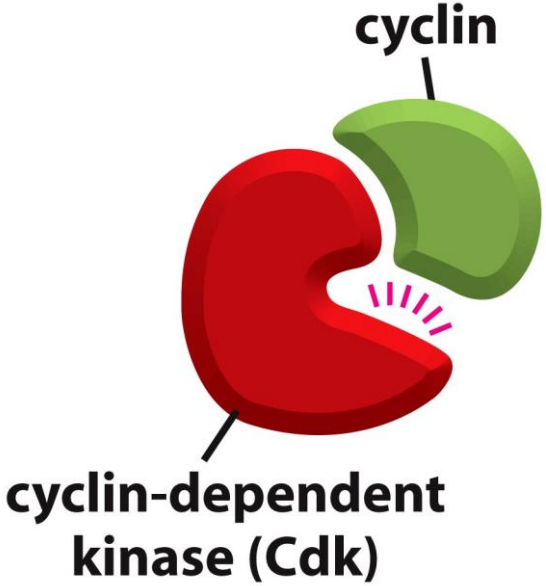
Componentes de mpf:

-32KDa con actividad quinasa

-45 KDa sin actividad quinasa


M
G1
S
G2
M
G1
S
G2
M
MPF activity
Cyclin
Relative concentration
Time

Ciclinas y quinasas dependientes de ciclinas (CDKs)



Conservación evolutiva en eucariotas

ciclina + CDK = heterodímero activo

⇩

activación / inactivación
proteínas blanco (fosforilación)

⇩

entrada y salida coordinada
de las fases del ciclo

Ciclinas y quinasas dependientes de ciclinas (CDKs)

NATURE VOL. 327 7 MAY 1987 ARTICLES 31

Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*

Melanie G. Lee & Paul Nurse

Cell Cycle Control Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, UK

cyclin-dependent kinase (Cdk)

activación / inactivación proteínas blanco (fosforilación)

entrada y salida coordinada de las fases del ciclo

Las quinasas dependientes de ciclinas son activas únicamente cuando se encuentran formando complejo con su ciclina reguladora

Diferentes tipos de complejos ciclina-CDK inician diferentes eventos del ciclo celular

G1-CDK y **G1/S CDK** ⇨ entrada al ciclo celular

M-CDK ⇨ entrada a mitosis

Múltiples mecanismos son utilizados para asegurar que las diferentes CDKs estén activas únicamente en los estadios que promueven

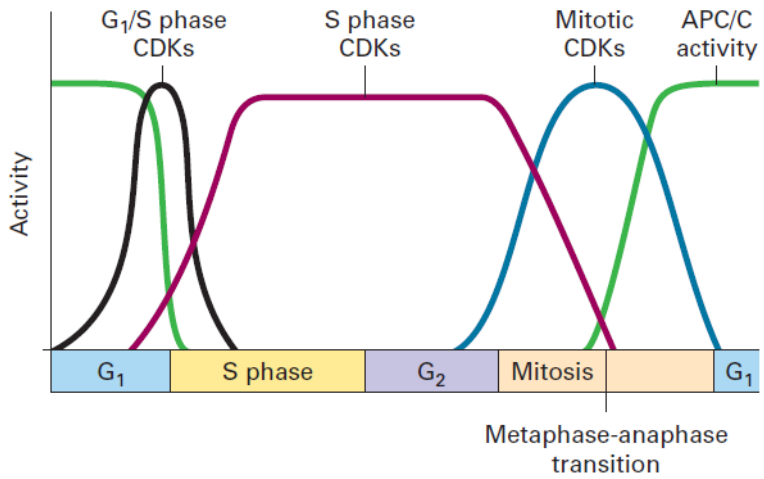
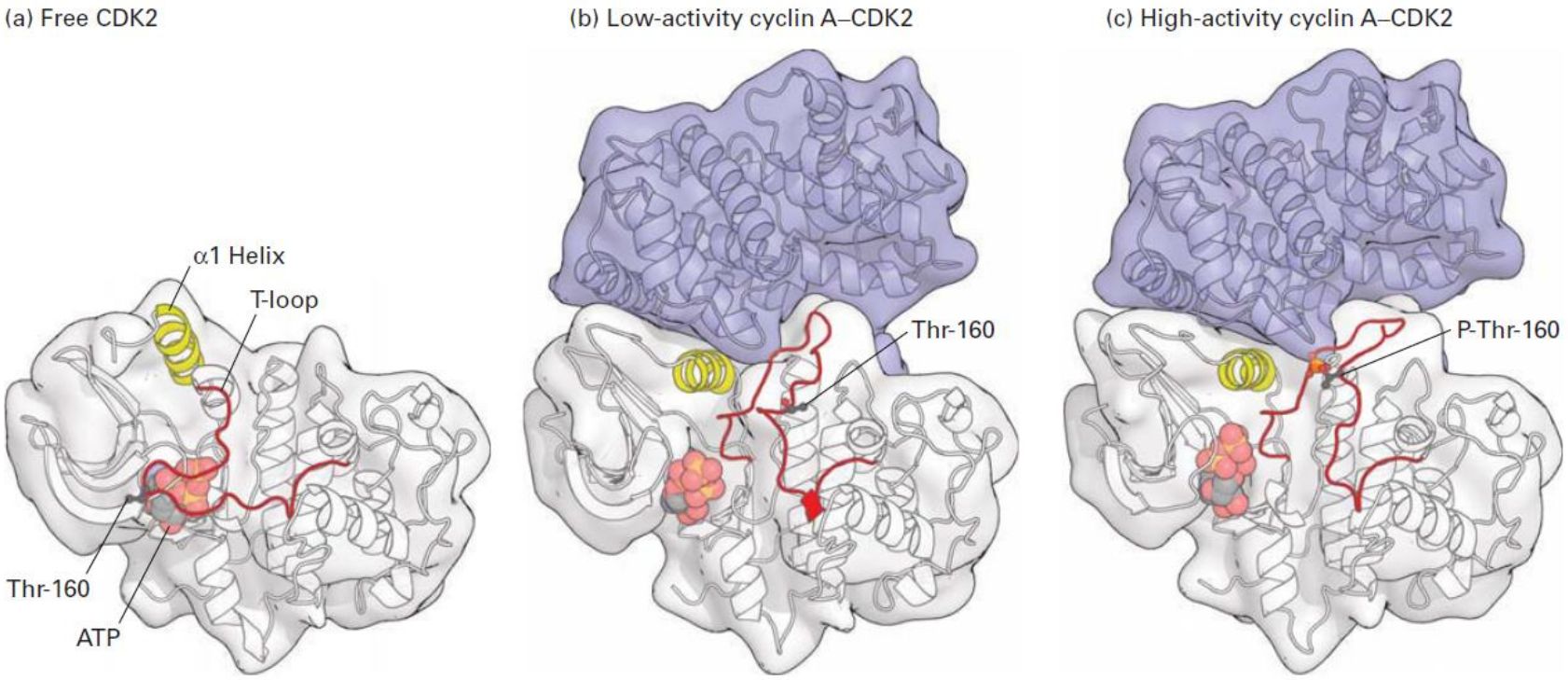


TABLE 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

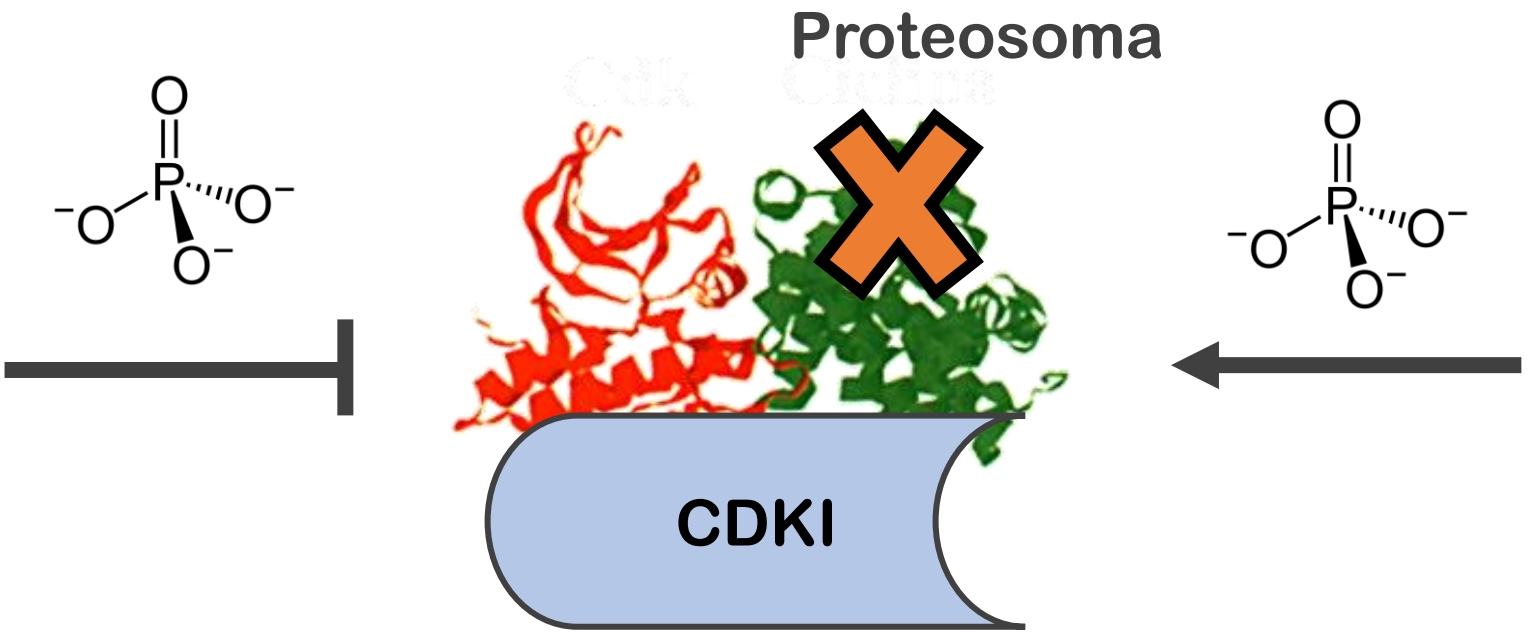
	Vertebrates		Budding yeast	
Cyclin–Cdk complex	Cyclin	Cdk partner	Cyclin	Cdk partner
G_1-Cdk	Cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	Cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	Cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	Cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

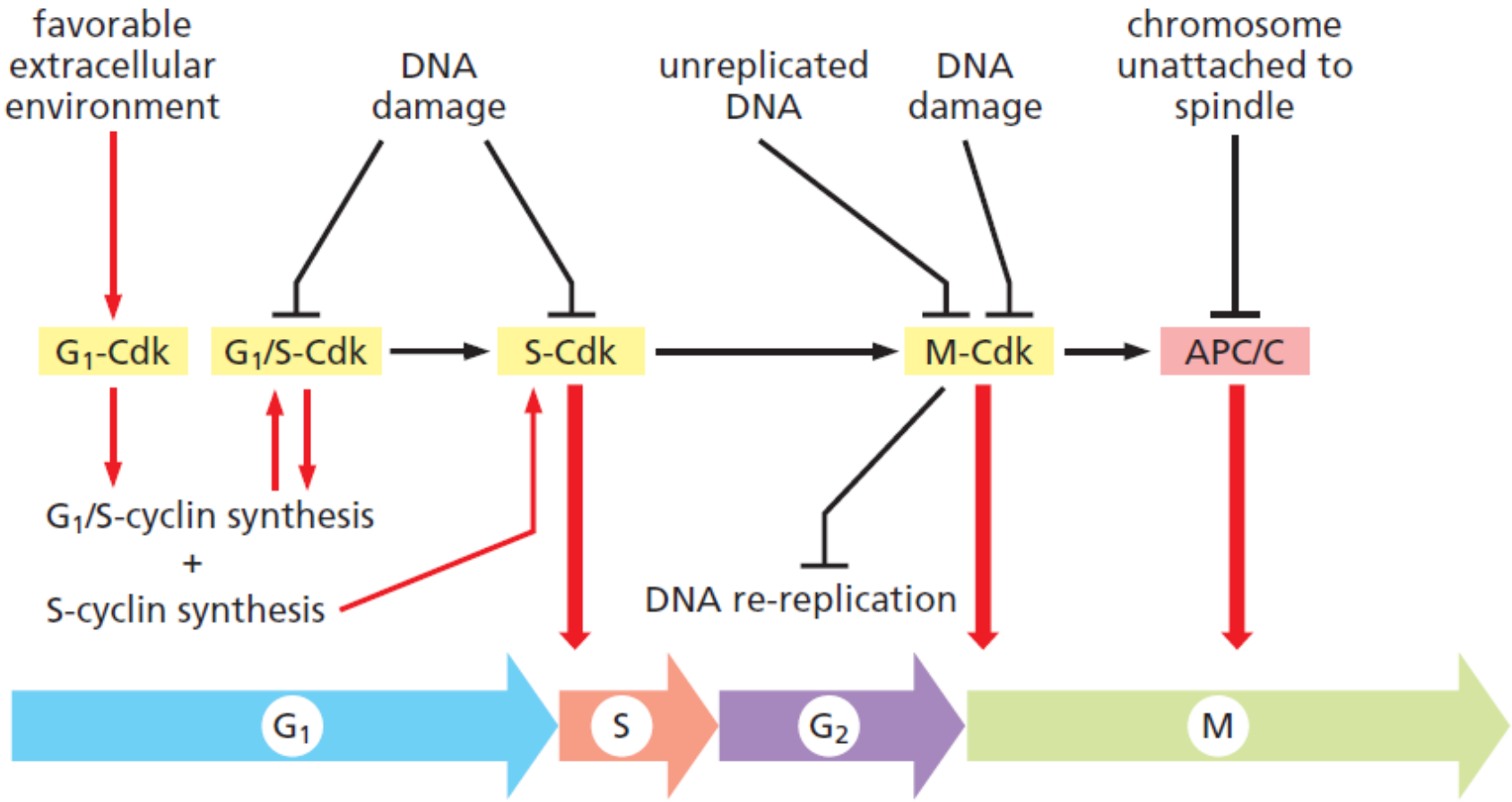
* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

Activación del complejo Ciclina-CDK

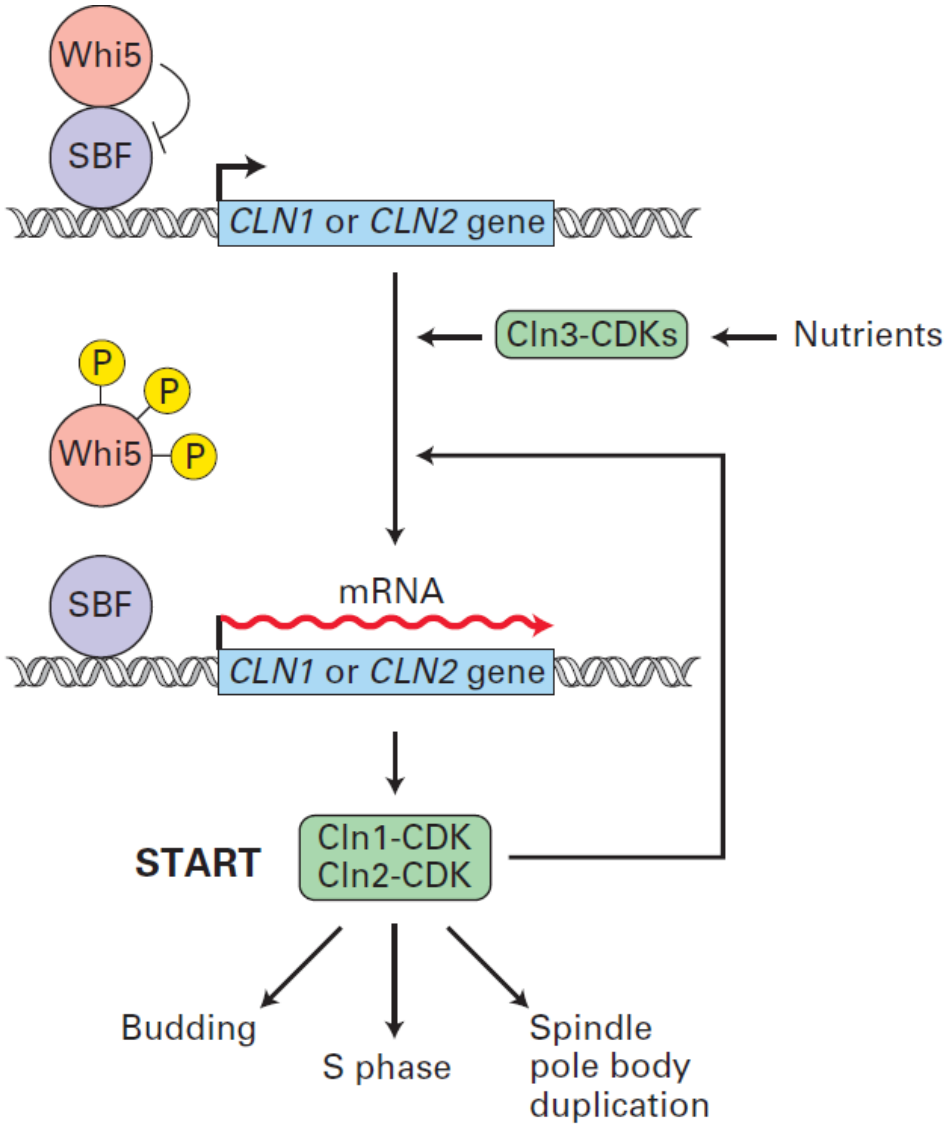


Múltiples vías de regulación de la actividad del complejo Ciclina-CDK

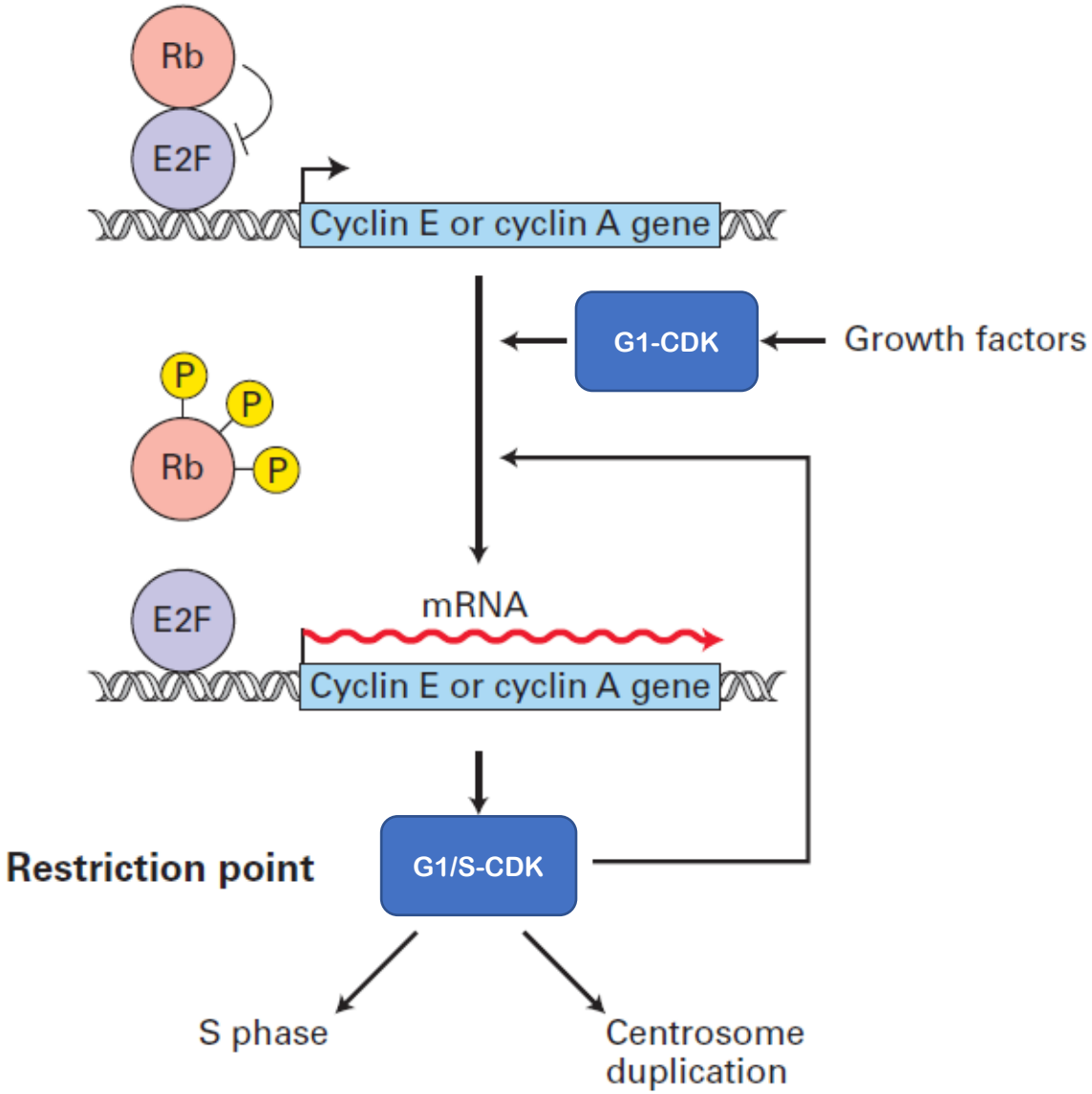


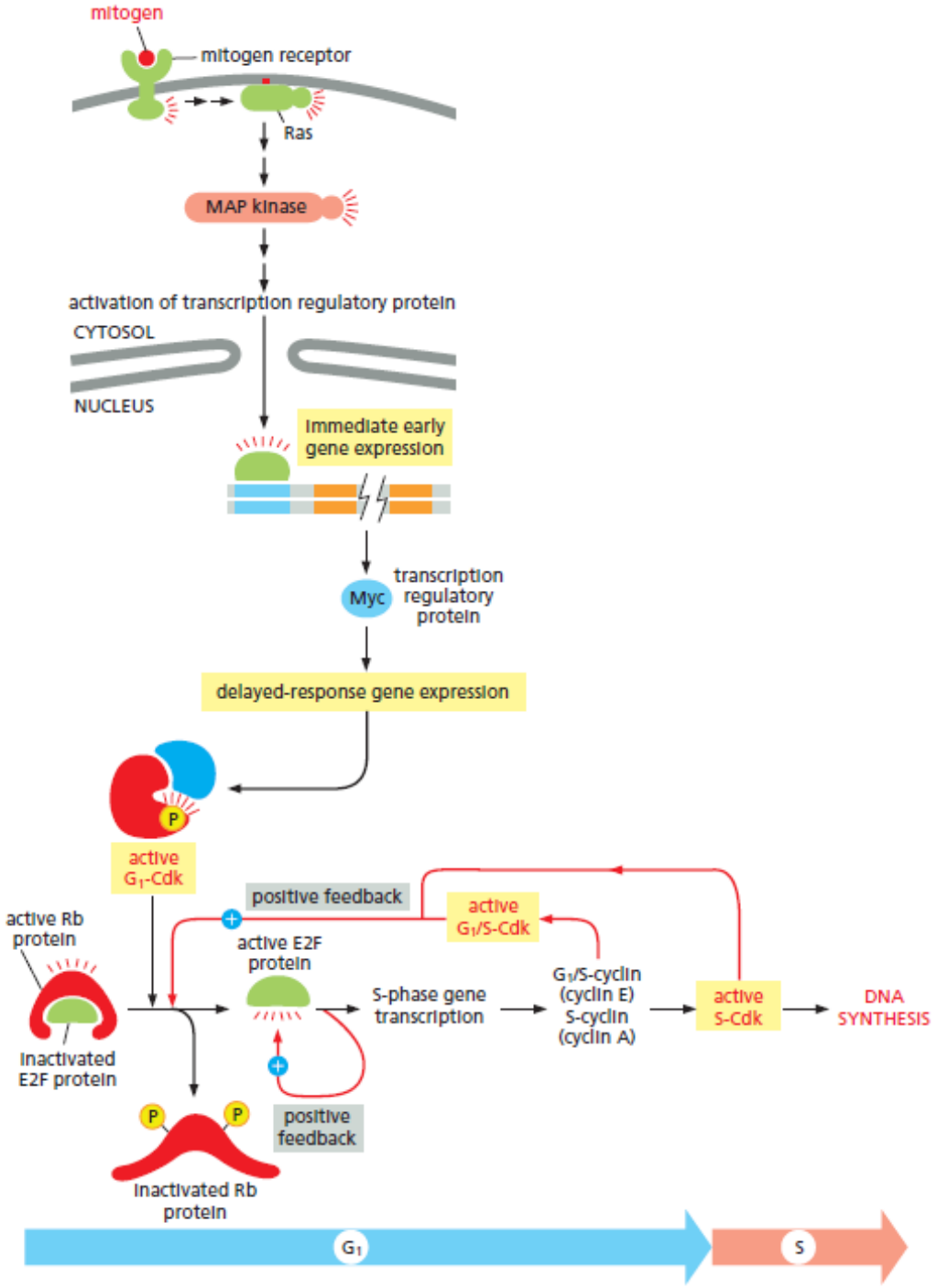

favorable extracellular environment
DNA damage
unreplicated DNA
DNA damage
chromosome unattached to spindle
G1-Cdk
G1/S-Cdk
S-Cdk
M-Cdk
APC/C
G1/S-cyclin synthesis
+
S-cyclin synthesis
DNA re-replication
G1
S
G2
M

Entrada al ciclo

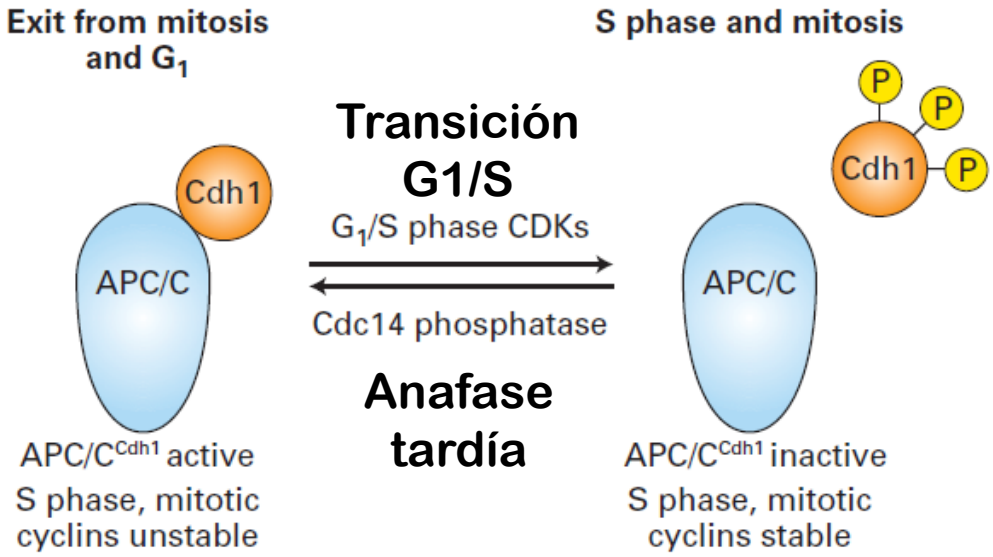


Entrada al ciclo

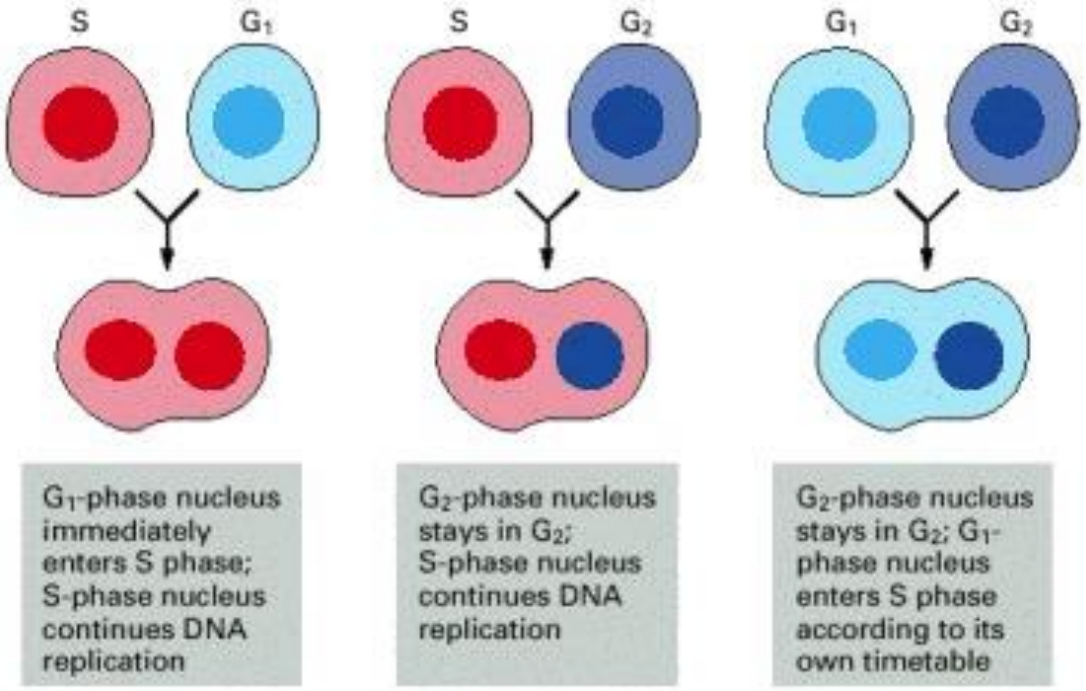


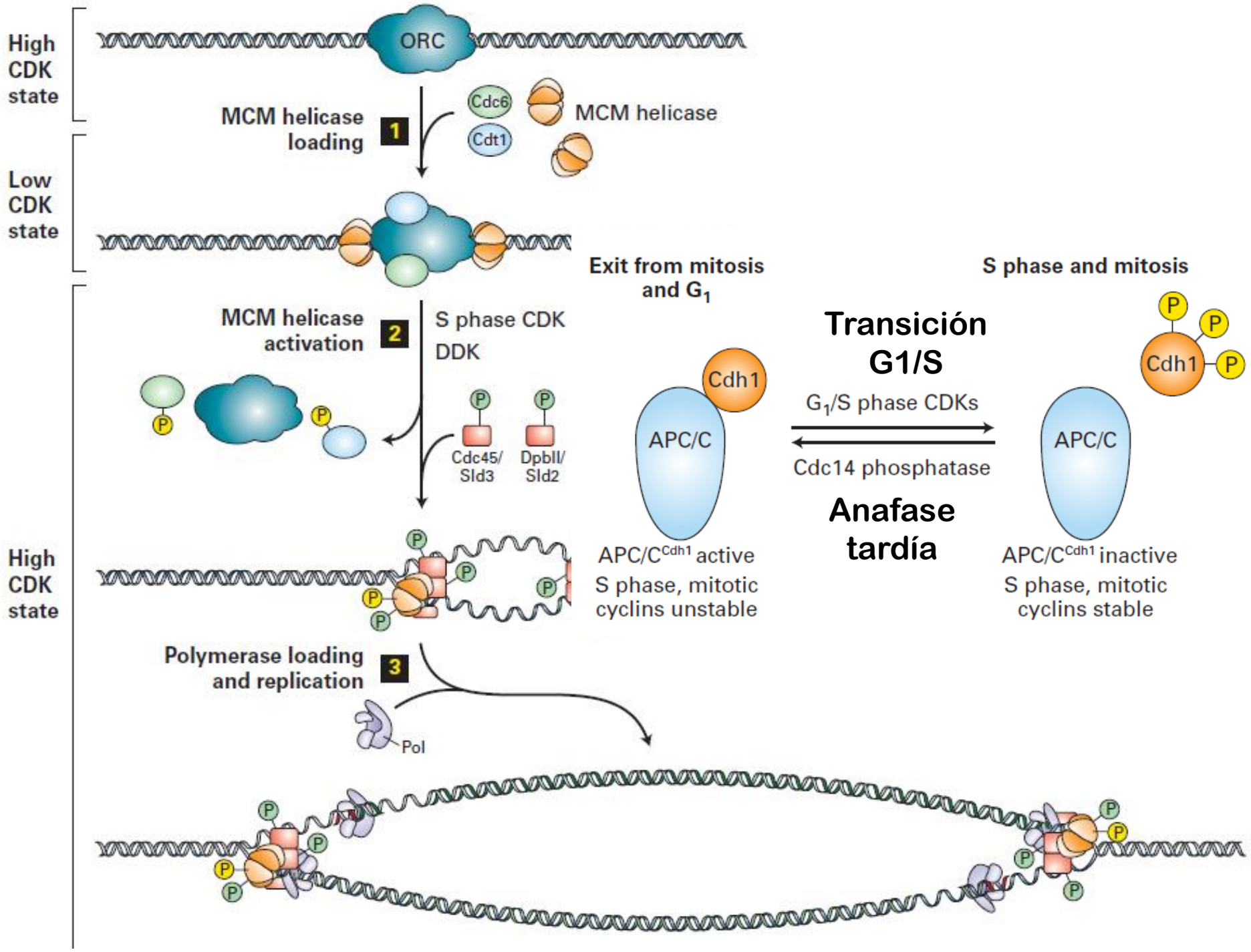

mitogen
mitogen receptor
Ras
MAP kinase
activation of transcription regulatory protein
CYTOSOL
NUCLEUS
Immediate early gene expression
Myc
transcription regulatory protein
delayed-response gene expression
P
active G1-Cdk
positive feedback
active G1/S-Cdk
active Rb protein
active E2F protein
S-phase gene transcription
G1/S-cyclin (cyclin E) S-cyclin (cyclin A)
active S-Cdk
DNA SYNTHESIS
Inactivated E2F protein
P
P
positive feedback
Inactivated Rb protein
G1
S

Entrada a fase S

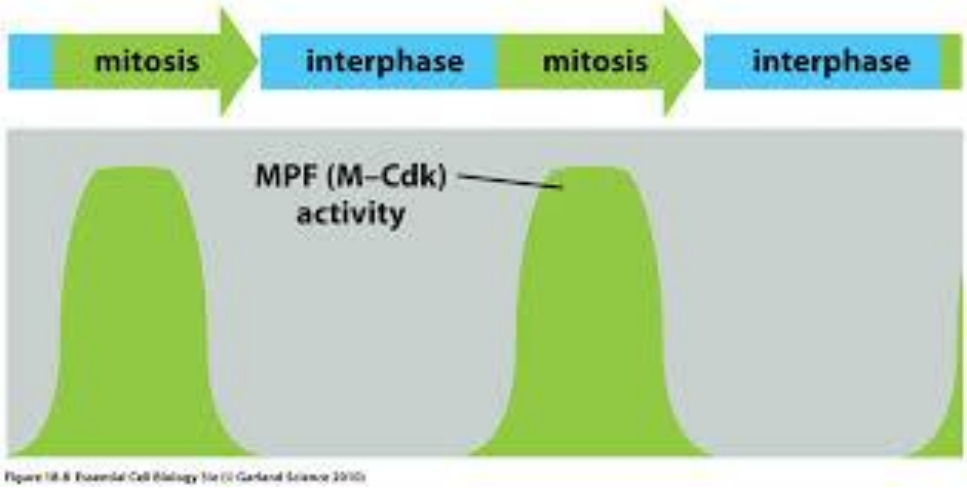


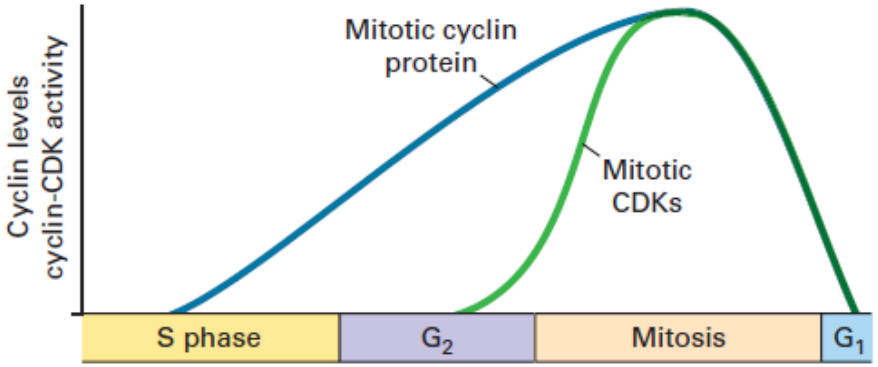
Inactivación del inhibidor de S-CDK


S
G1
S
G2
G1
G2
G1-phase nucleus immediately enters S phase; S-phase nucleus continues DNA replication
G2-phase nucleus stays in G2; S-phase nucleus continues DNA replication
G2-phase nucleus stays in G2; G1-phase nucleus enters S phase according to its own timetable

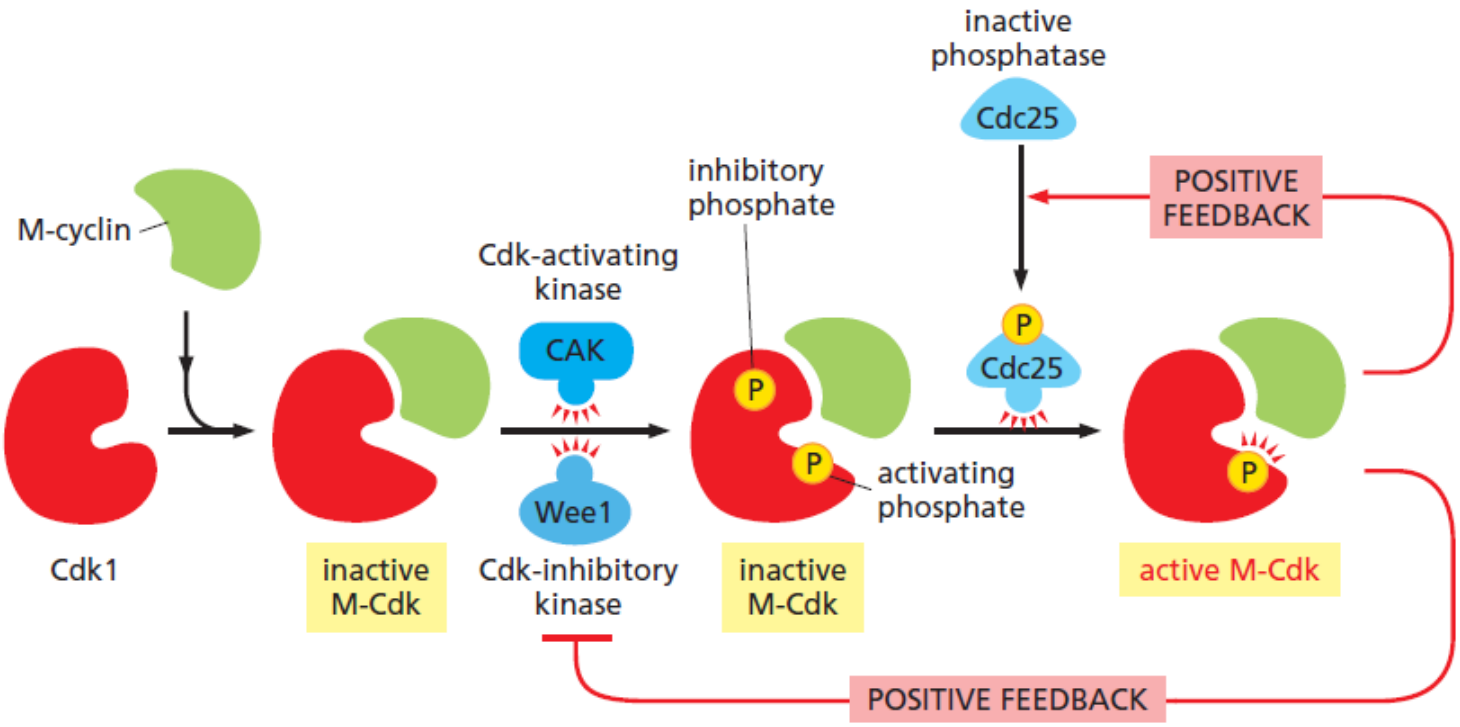

High CDK state
ORC
MCM helicase loading
1
Cdc6
Cdt1
MCM helicase
Low CDK state
MCM helicase activation
2
S phase CDK
DDK
Cdc45/ Sld3
Dpbll/ Sld2
High CDK state
Polymerase loading and replication
3
Pol
Exit from mitosis and G1
Cdh1
APC/C
APC/C^Cdh1 active
S phase, mitotic cyclins unstable
Transición G1/S
G1/S phase CDKs
Cdc14 phosphatase
Anafase tardía
S phase and mitosis
Cdh1
APC/C
APC/C^Cdh1 inactive
S phase, mitotic cyclins stable

¿Como se genera la entrada a mitosis?

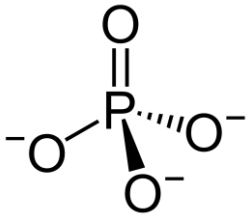


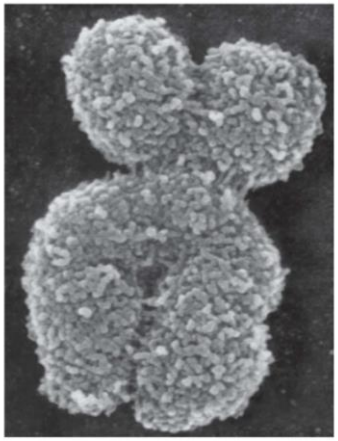


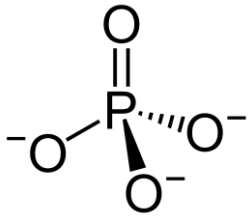
Entrada a mitosis

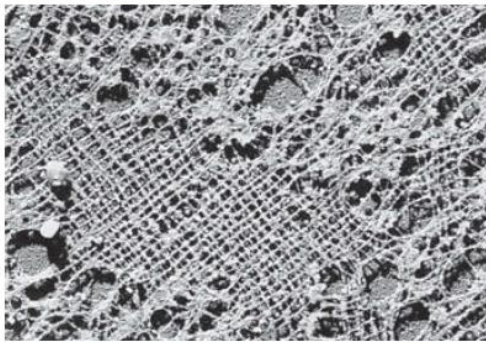


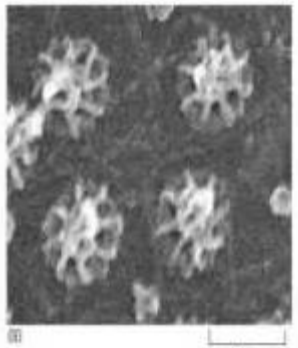
M-CDK

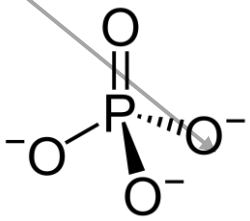


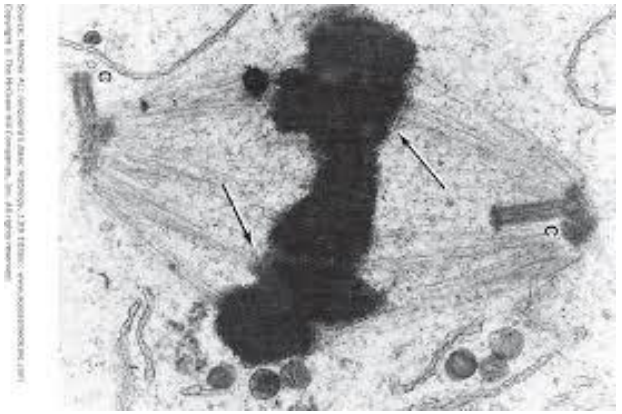


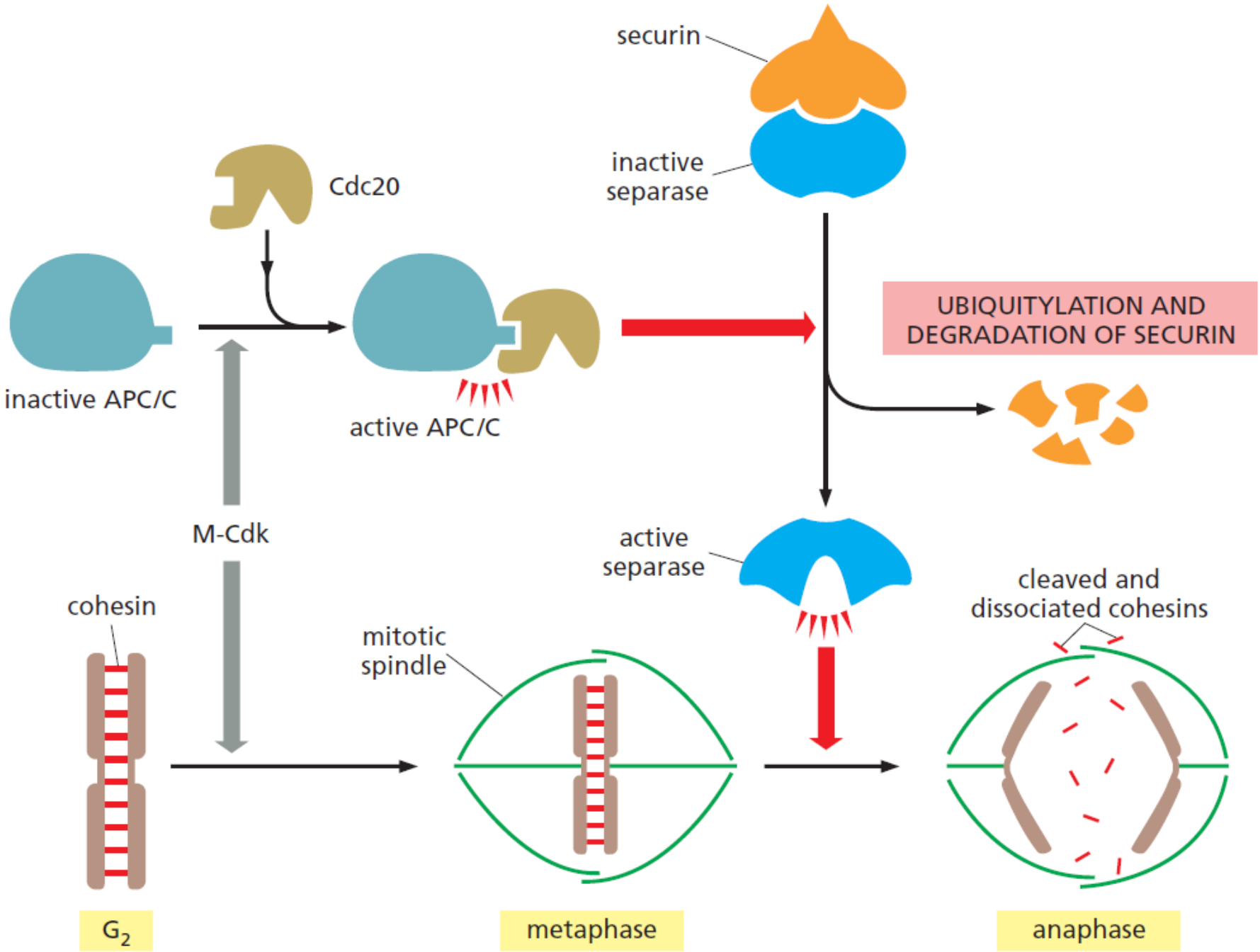


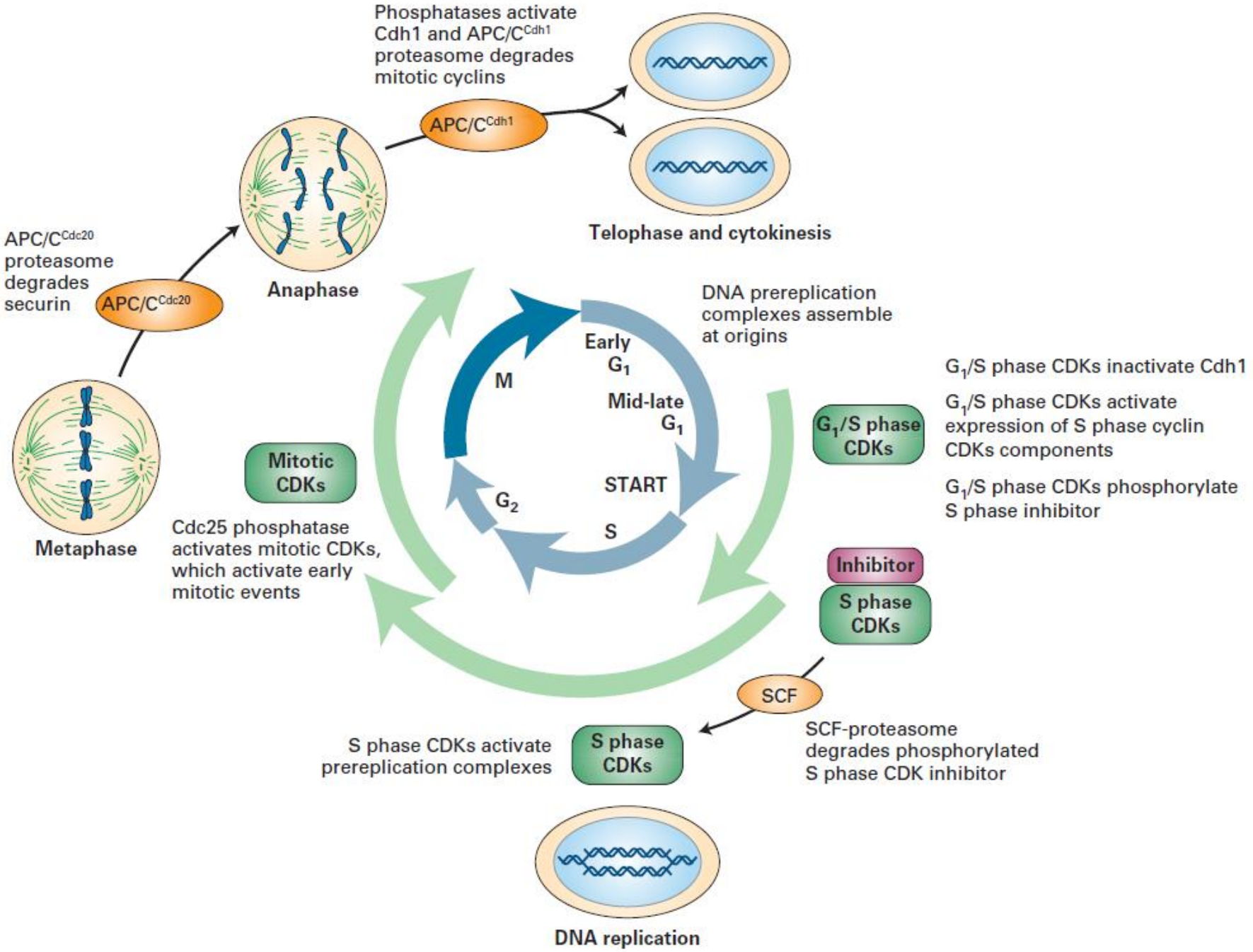




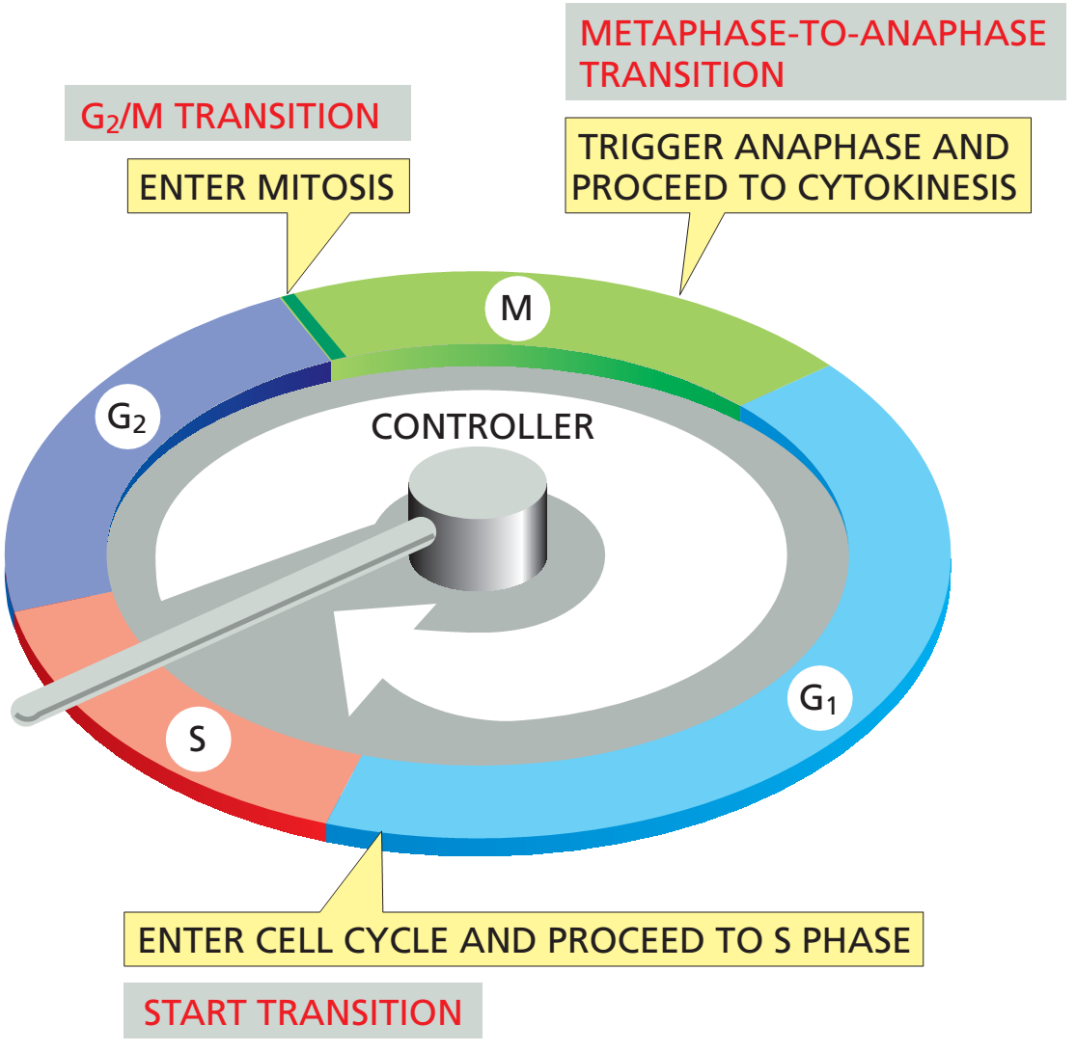




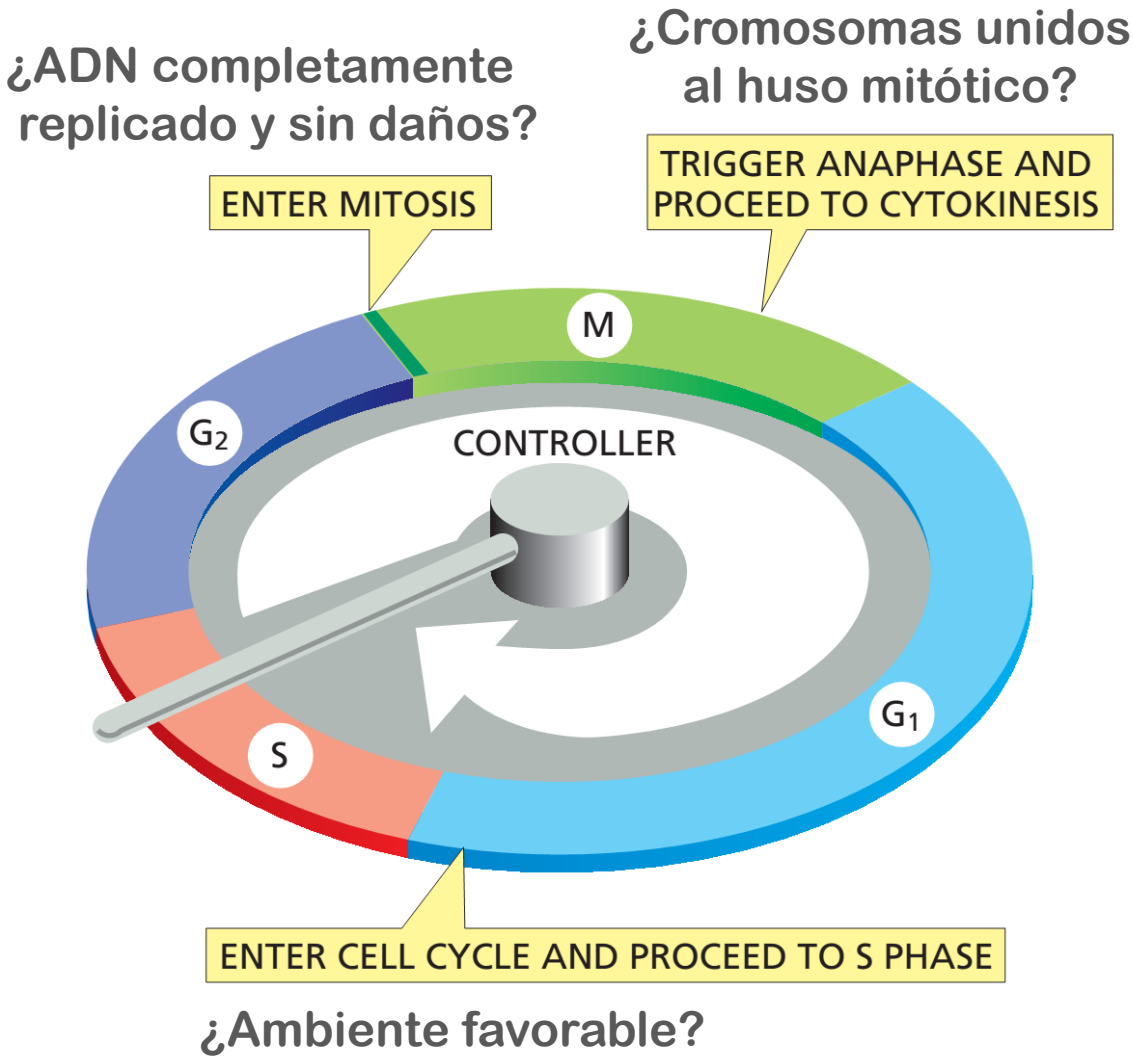

securin
inactive separase
Cdc20
inactive APC/C
active APC/C
UBIQUITYLATION AND DEGRADATION OF SECURIN
M-Cdk
active separase
cohesin
mitotic spindle
cleaved and dissociated cohesins
G2
metaphase
anaphase


Phosphatases activate Cdh1 and APC/C^Cdh1 proteasome degrades mitotic cyclins
APC/C^Cdh1
Telophase and cytokinesis
Anaphase
APC/C^Cdc20 proteasome degrades securin
APC/C^Cdc20
Metaphase
M
Early G1
Mid-late G1
START
G2
S
DNA prereplication complexes assemble at origins
G1/S phase CDKs
G1/S phase CDKs inactivate Cdh1
G1/S phase CDKs activate expression of S phase cyclin CDKs components
G1/S phase CDKs phosphorylate S phase inhibitor
Mitotic CDKs
Cdc25 phosphatase activates mitotic CDKs, which activate early mitotic events
Inhibitor
S phase CDKs
SCF
SCF-proteasome degrades phosphorylated S phase CDK inhibitor
S phase CDKs
S phase CDKs activate prereplication complexes
DNA replication

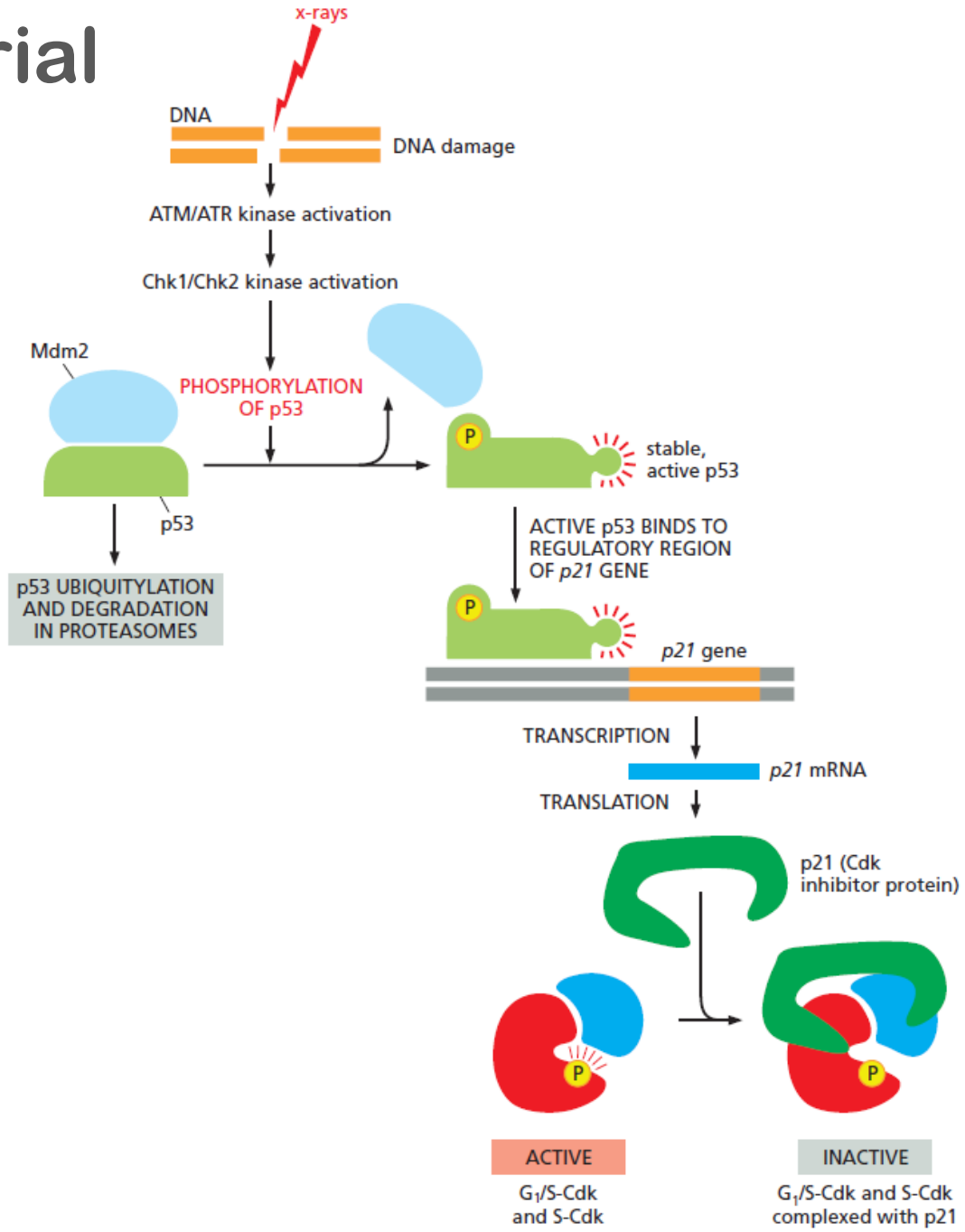
Control de ciclo celular



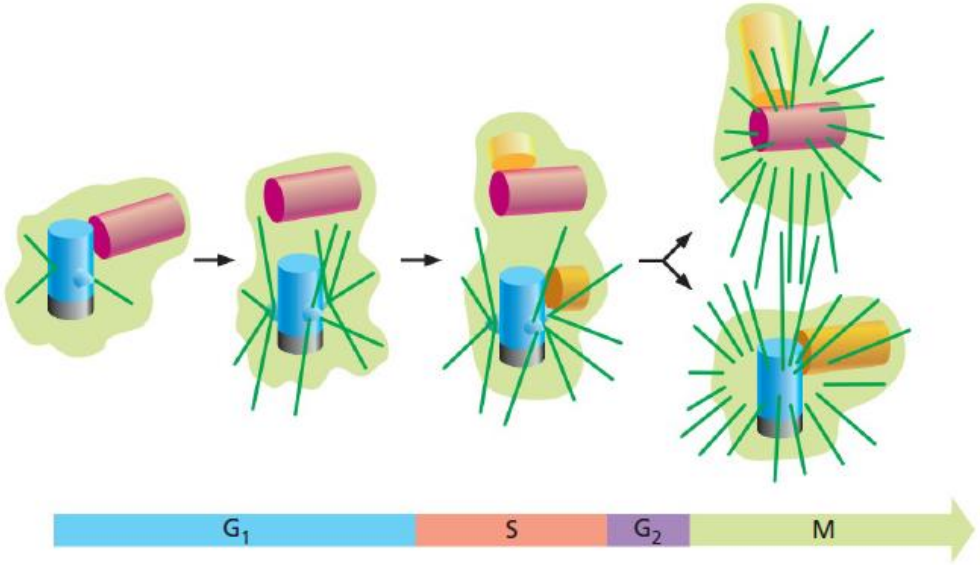
Control de ciclo celular

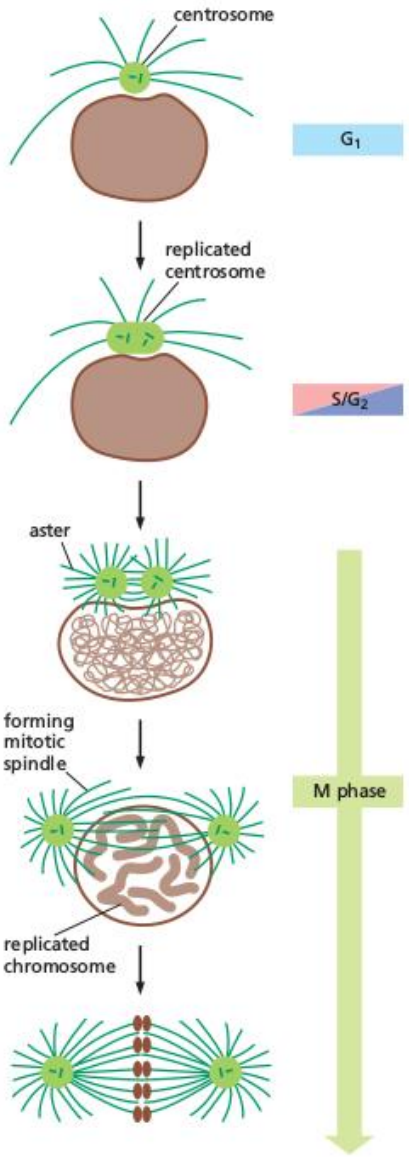


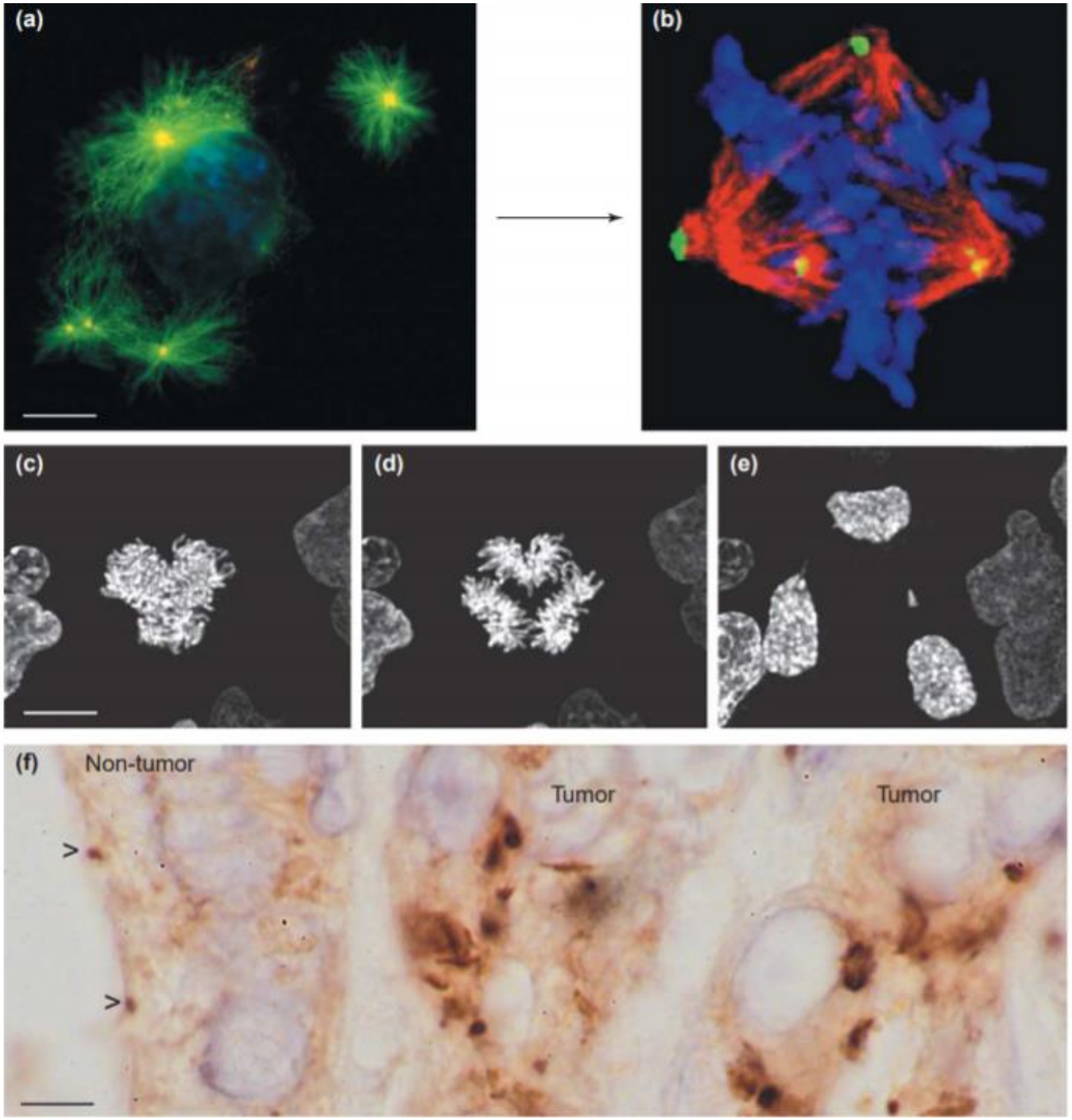
Control daño material genético



Duplicación del centrosoma: G1/S CDK

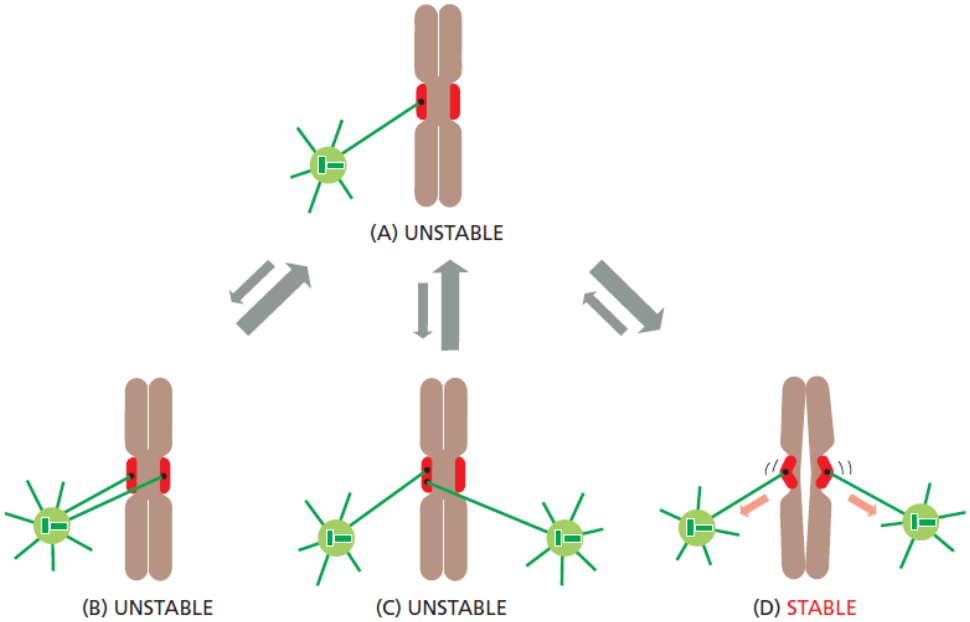


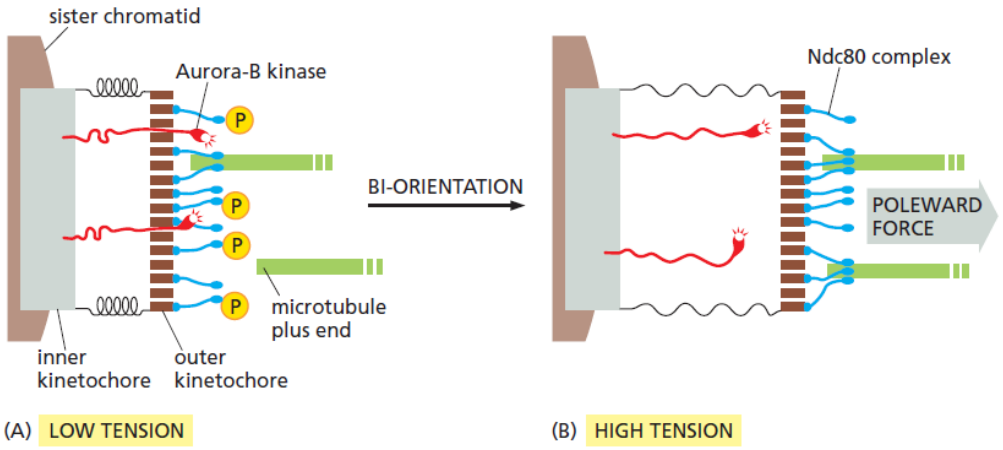




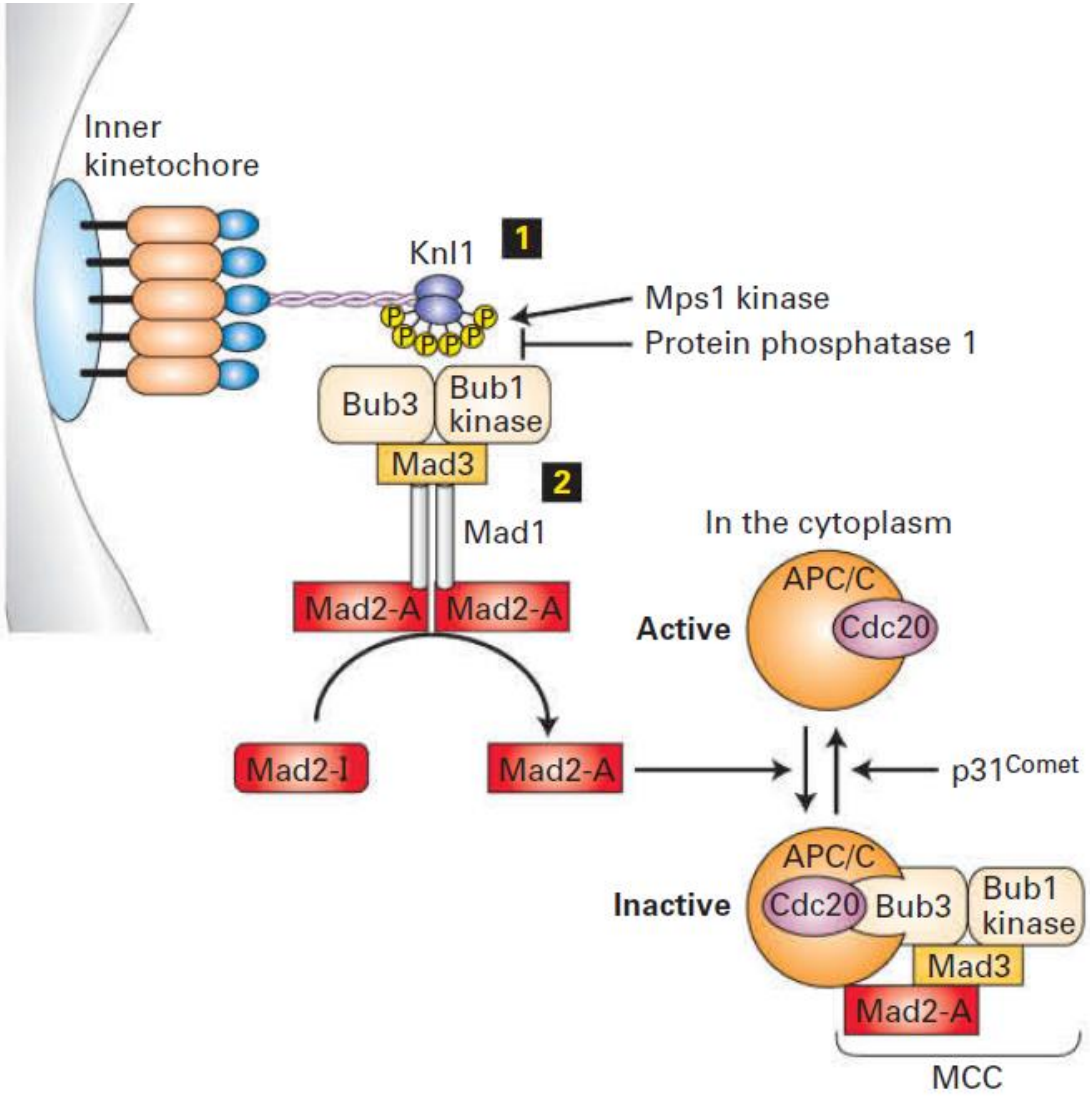
Doxsey et al., 2005

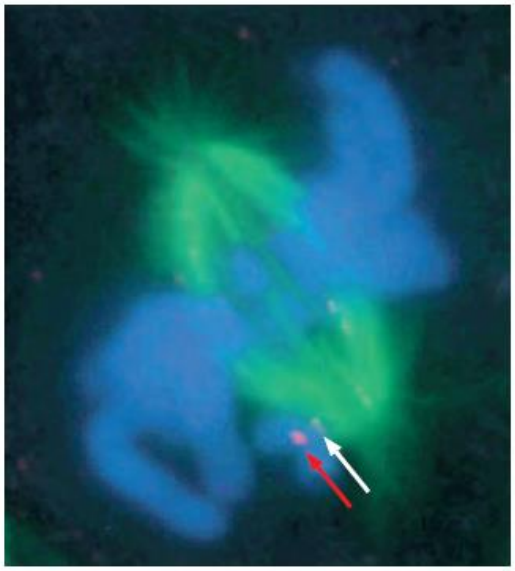
Punto de control: Unión huso mitótico - Cinetocoro



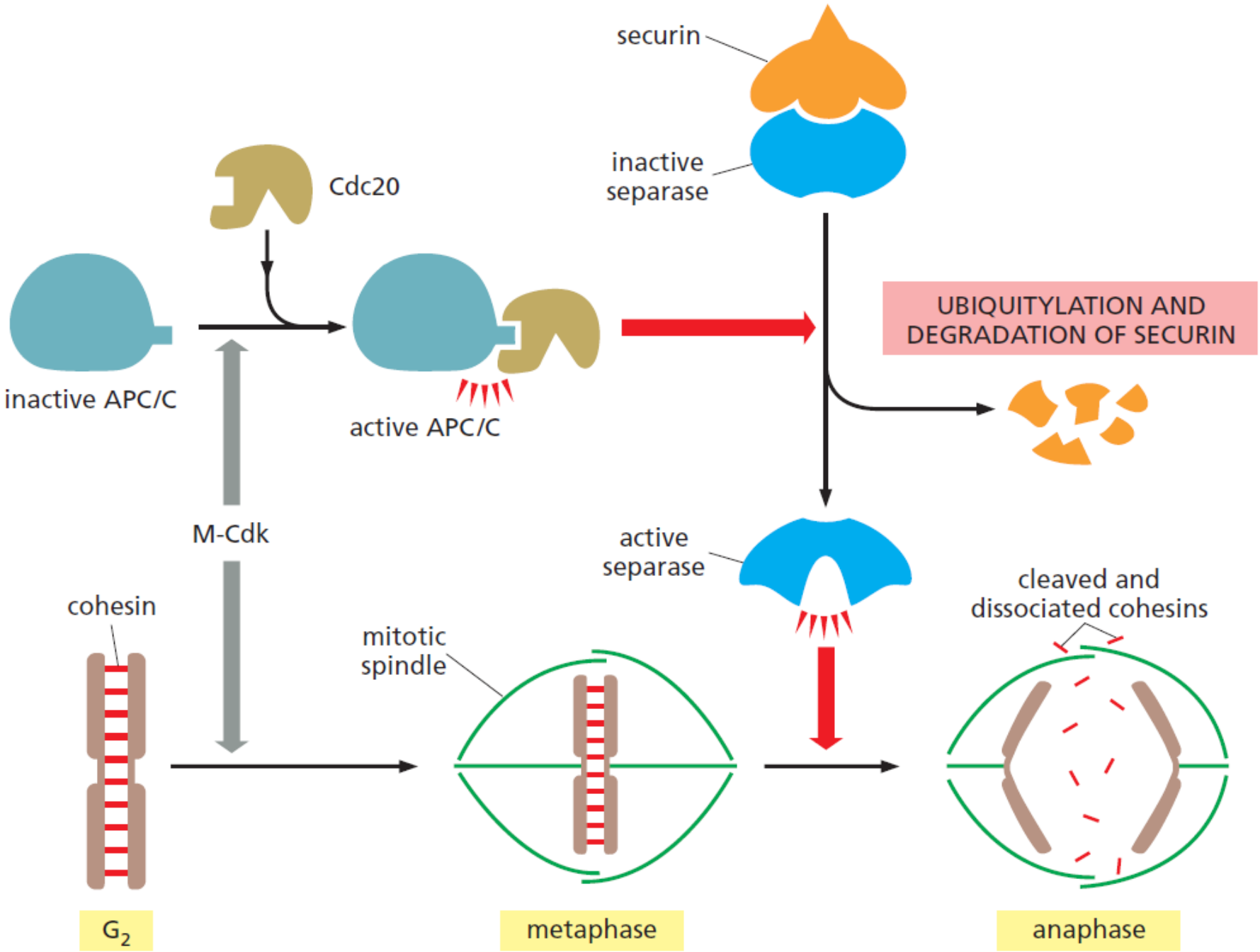


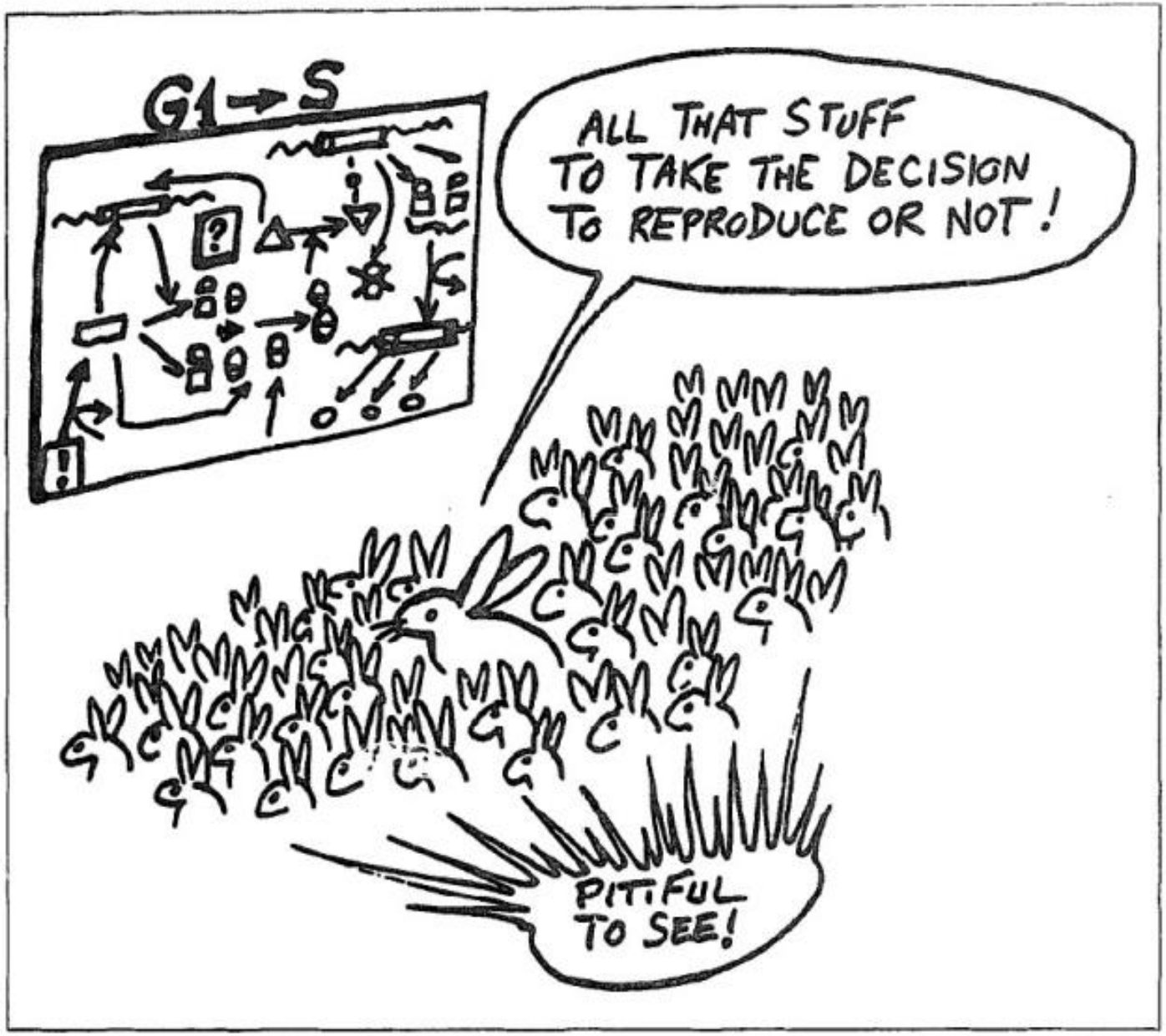
Punto de control: Unión huso mitótico-cinetocoro





Mad2


securin
inactive separase
Cdc20
inactive APC/C
active APC/C
UBIQUITYLATION AND DEGRADATION OF SECURIN
M-Cdk
active separase
cohesin
mitotic spindle
cleaved and dissociated cohesins
G2
metaphase
anaphase


G1→S
?
!
ALL THAT STUFF TO TAKE THE DECISION TO REPRODUCE OR NOT!
PITIFUL TO SEE!