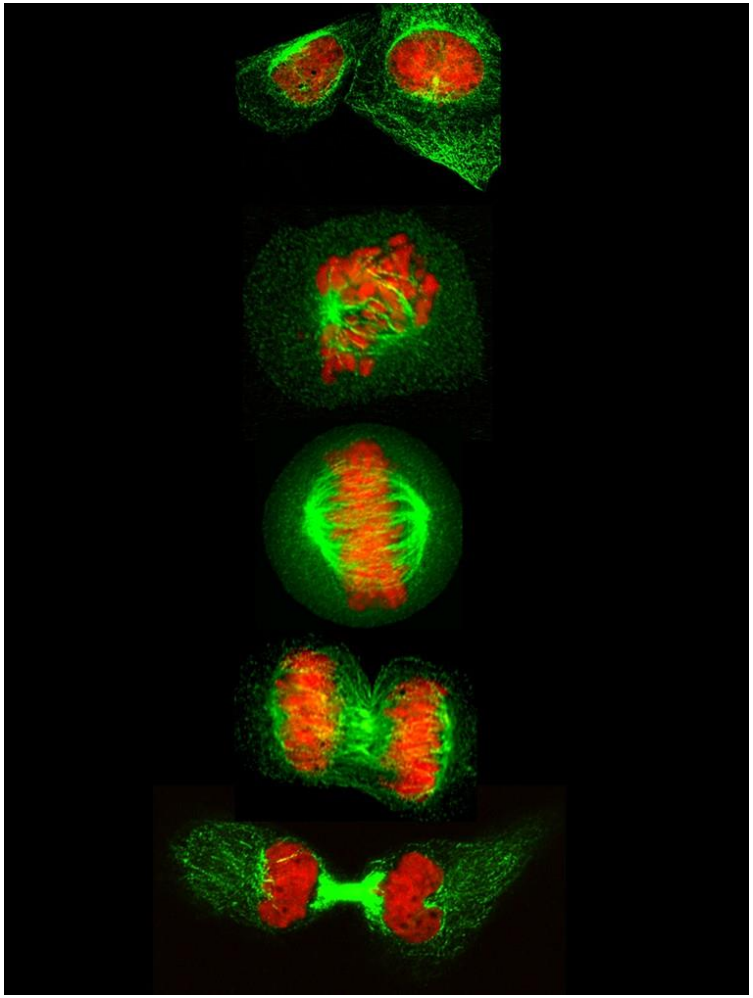
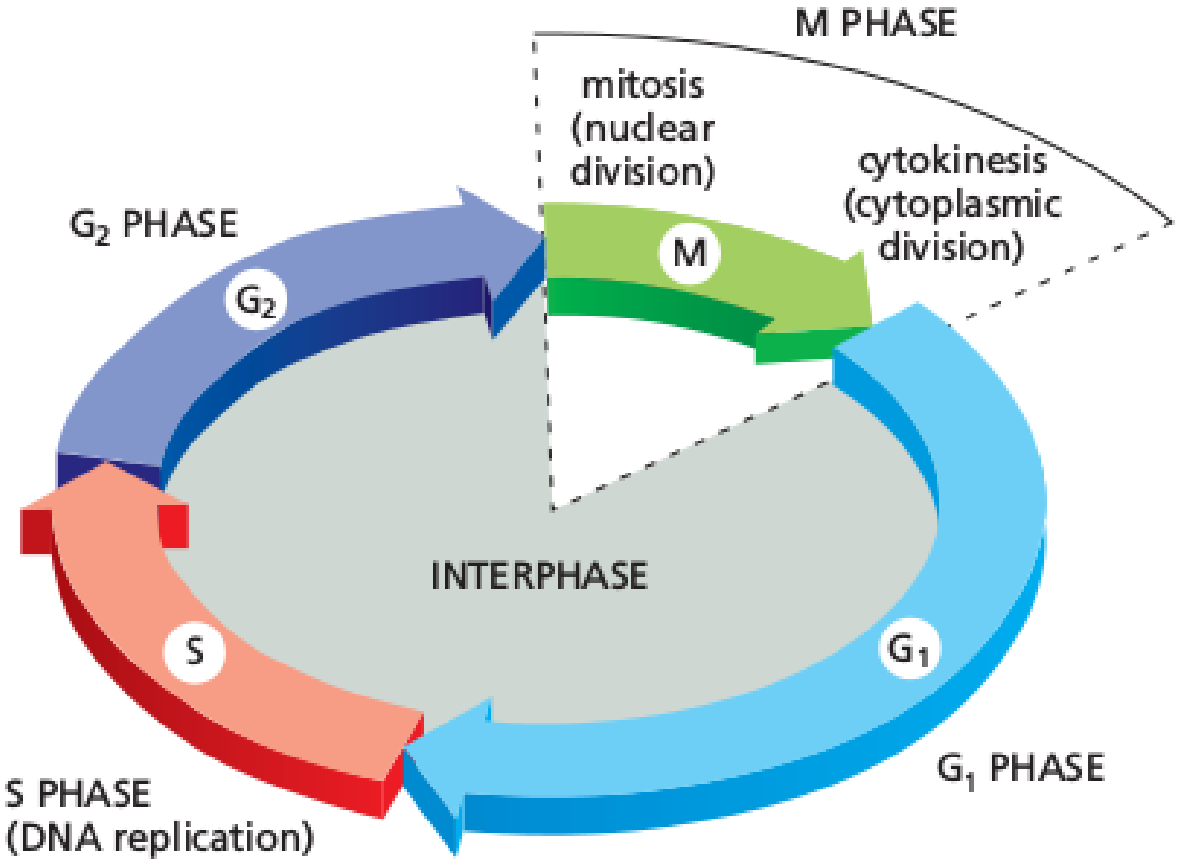
Ciclo Celular II



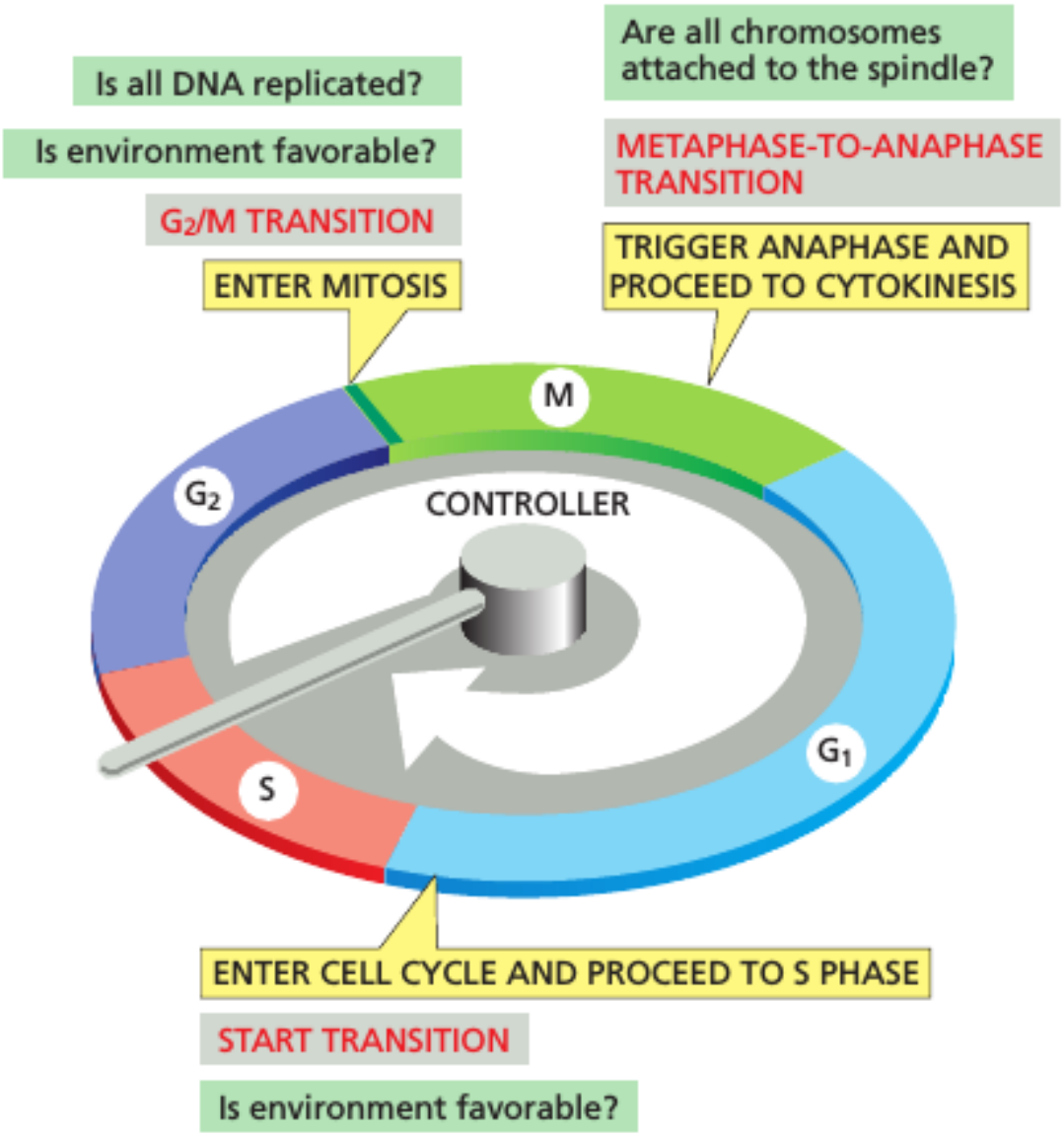
Cecilia Mathó
cmatho@fcien.edu.uy

Fases del ciclo celular



¿Cómo se controla el ciclo celular?

¿Cuáles son los puntos de control?


Are all chromosomes attached to the spindle?
Is all DNA replicated?
METAPHASE-TO-ANAPHASE TRANSITION
Is environment favorable?
G_2/M TRANSITION
TRIGGER ANAPHASE AND PROCEED TO CYTOKINESIS
ENTER MITOSIS
M
G_2
CONTROLLER
G_1
S
ENTER CELL CYCLE AND PROCEED TO S PHASE
START TRANSITION
Is environment favorable?

The Nobel Prize in Physiology or Medicine 2001

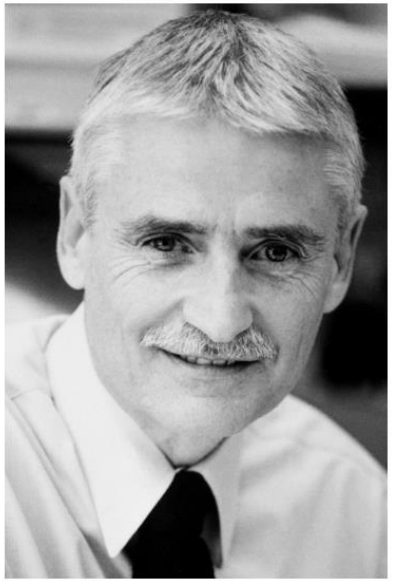


Photo from the Nobel Foundation archive.

Leland H. Hartwell

Prize share: 1/3

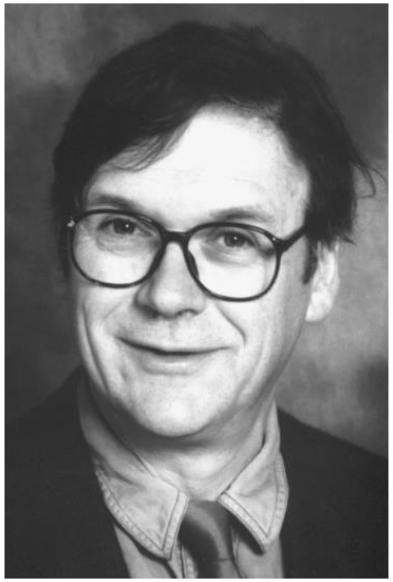


Photo from the Nobel Foundation archive.

Tim Hunt

Prize share: 1/3

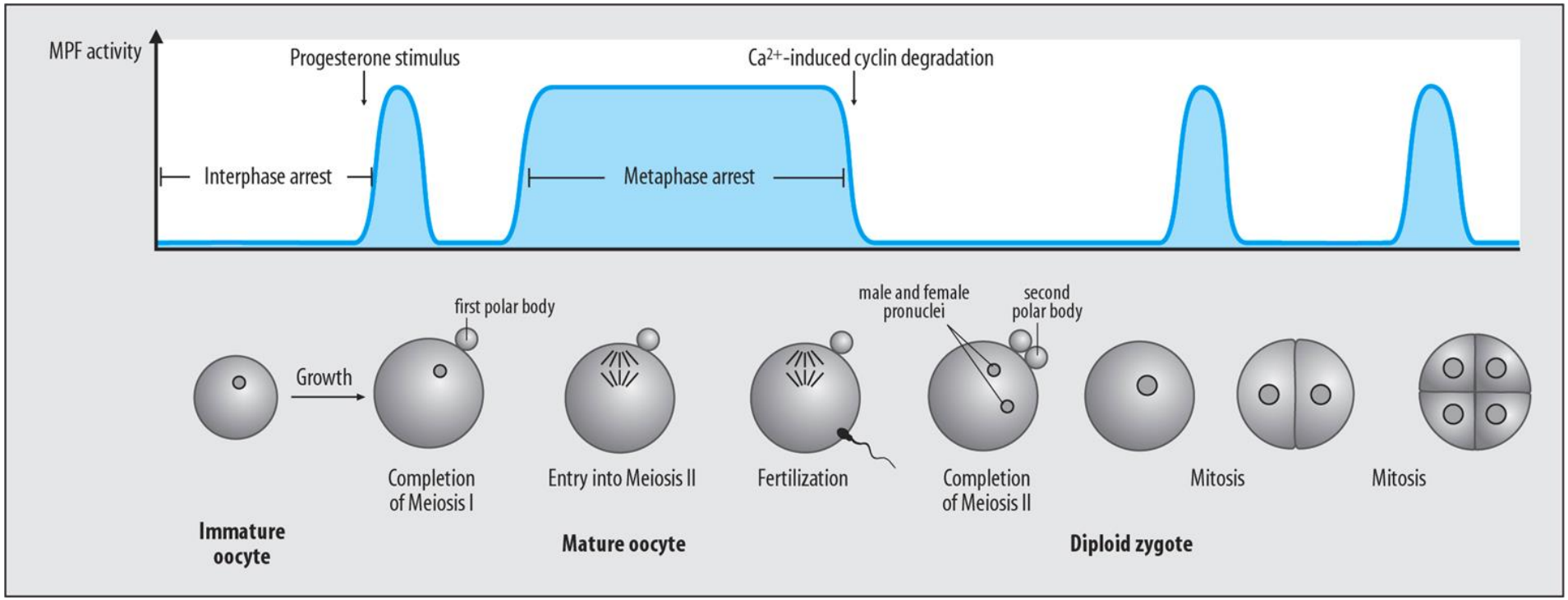
Photo from the Nobel Foundation archive.

Sir Paul M. Nurse

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2001 was awarded jointly to Leland H. Hartwell, Tim Hunt and Sir Paul M. Nurse "for their discoveries of key regulators of the cell cycle."

Experimentos iniciales



Experimentos iniciales

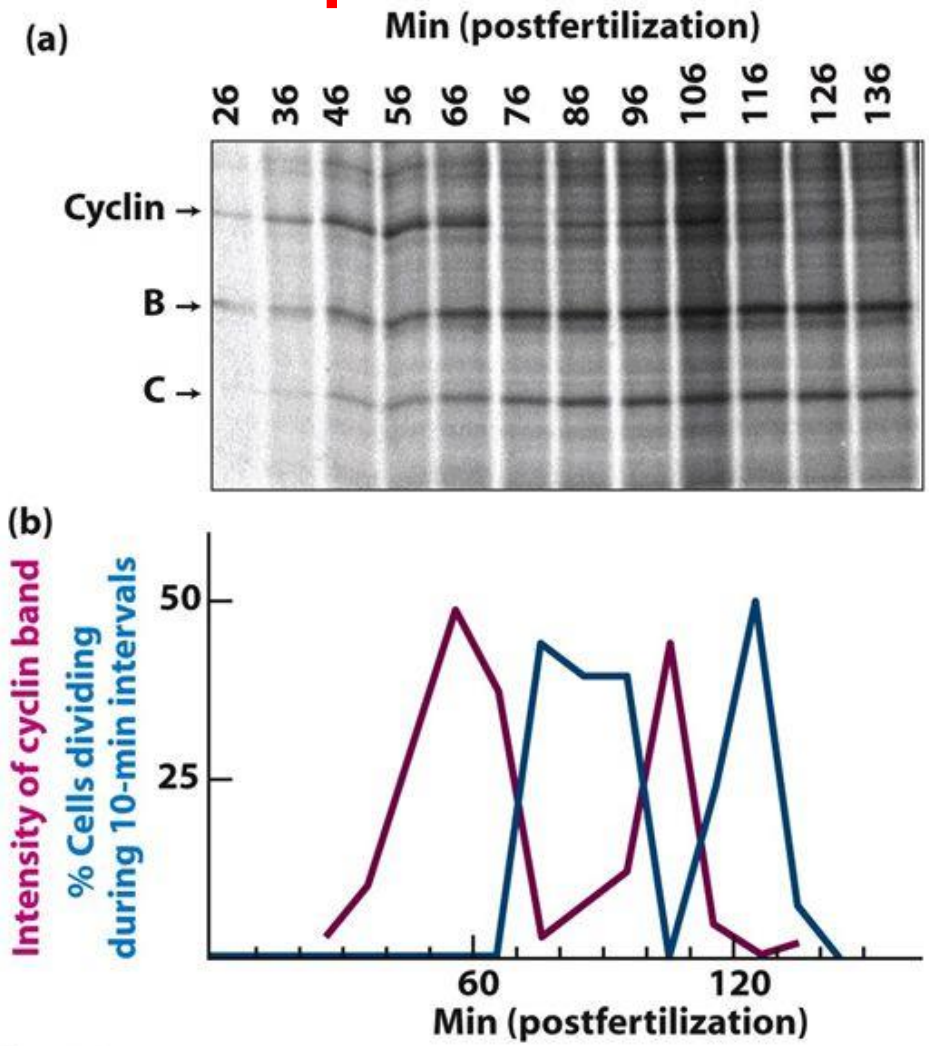
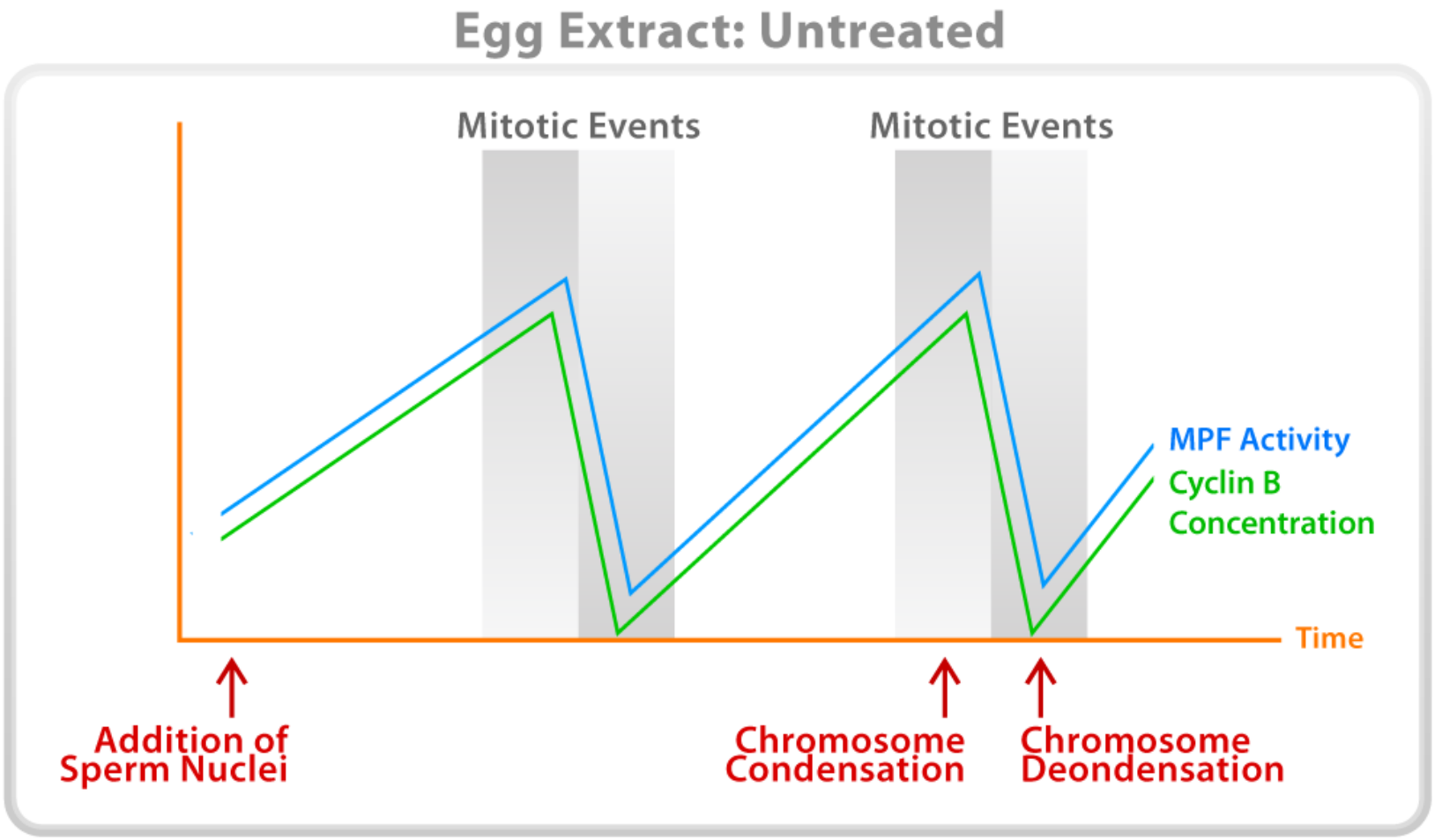


Figure 20-8
Molecular Cell Biology, Sixth Edition

Mitotic Cyclin Was First Identified in Early Sea Urchin Embryos

EXPERIMENTAL FIGURE 20-8
Autoradiography permits the detection of cyclical synthesis and destruction of mitotic cyclin in sea urchin embryos

Experimentos iniciales



Experimentos iniciales

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

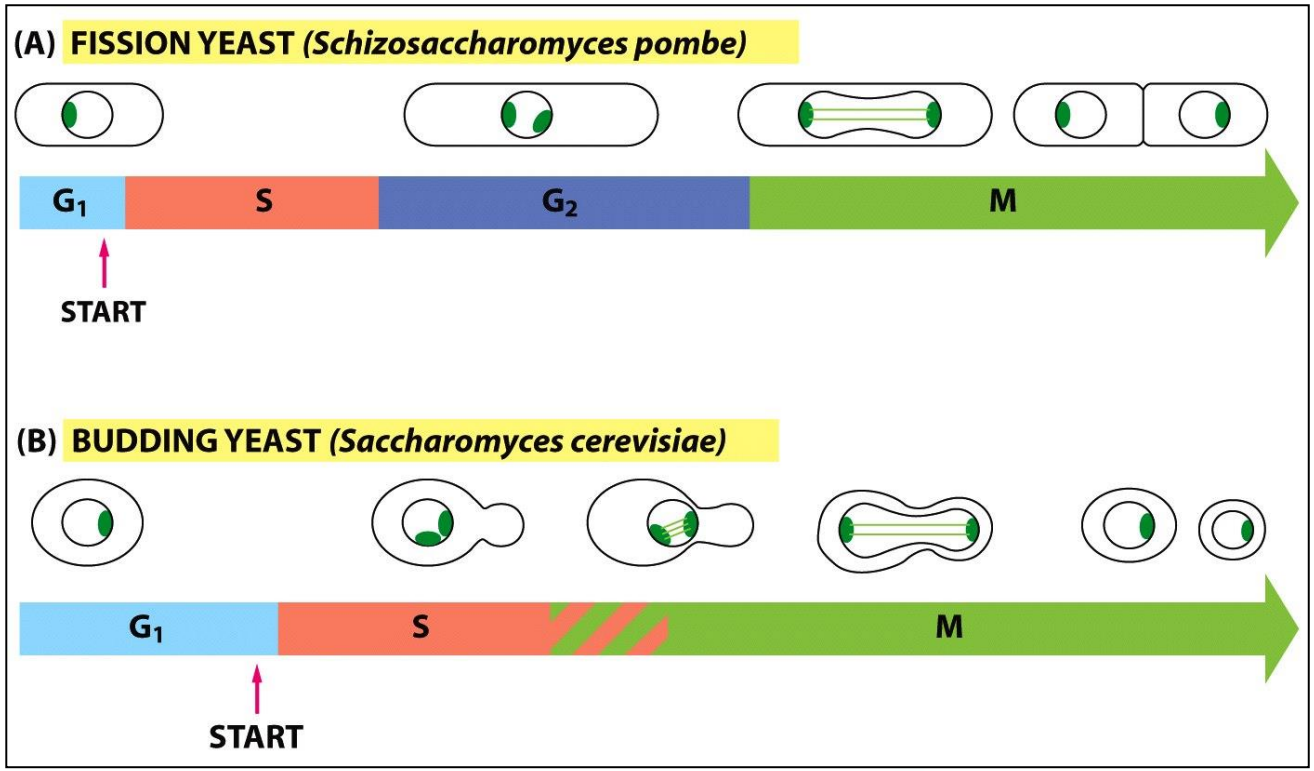
¿Qué es MPF?

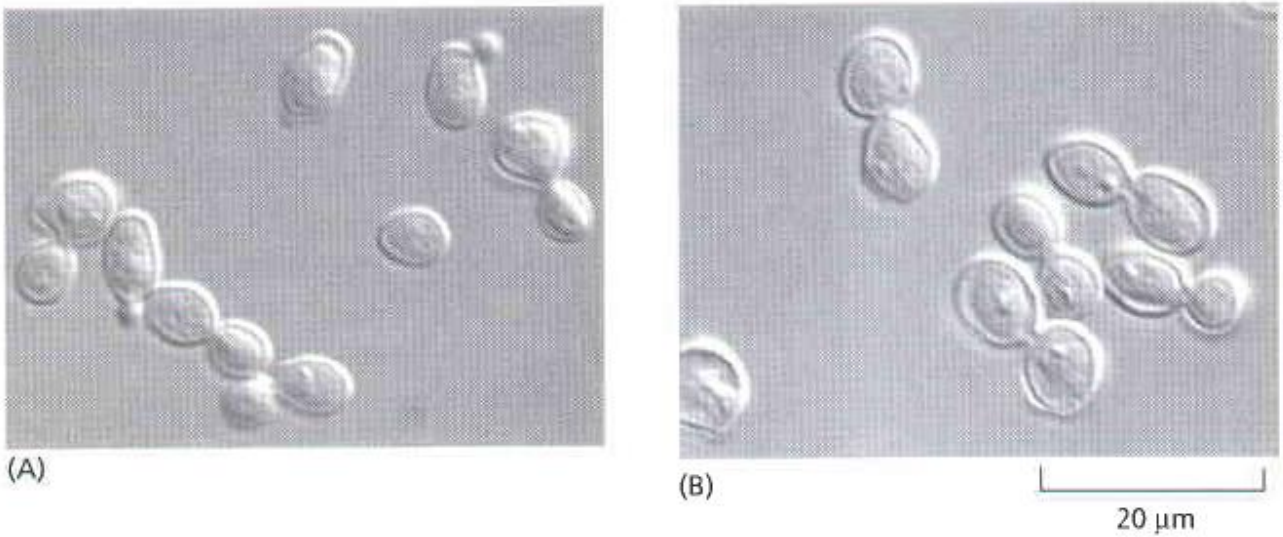
Componentes de mpf:

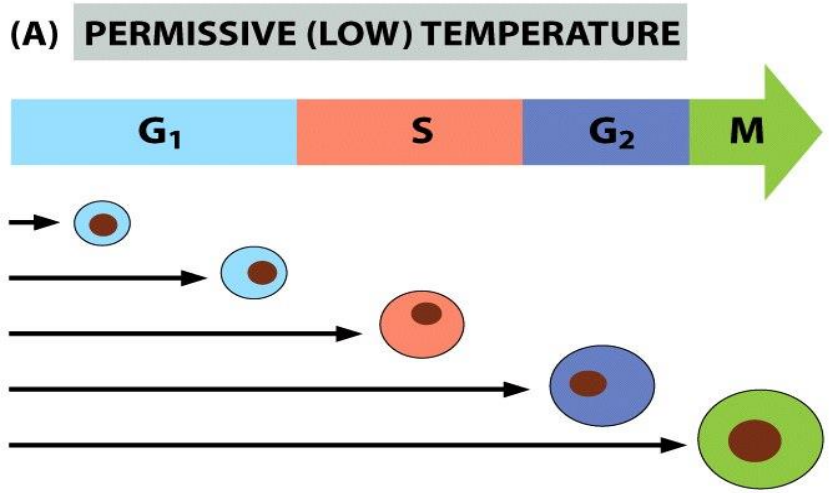
-32KDa con actividad quinasa

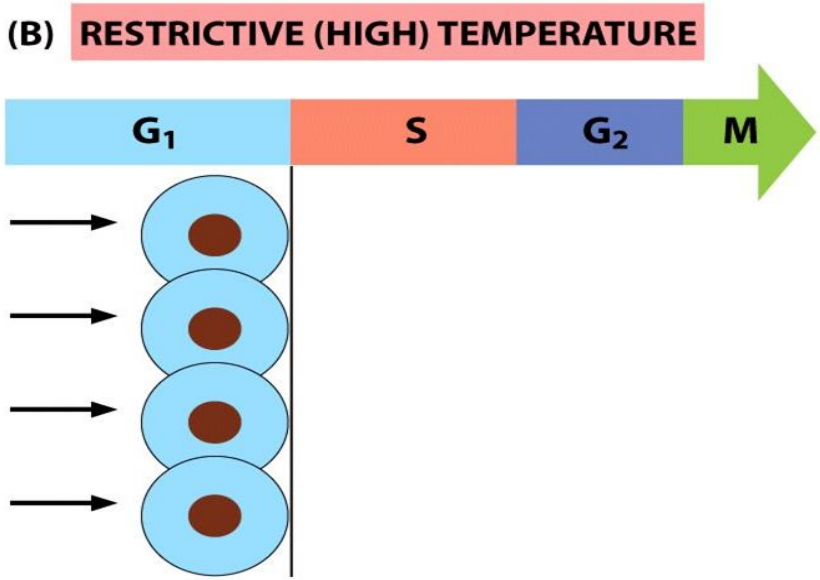
-45 KDa sin actividad quinasa

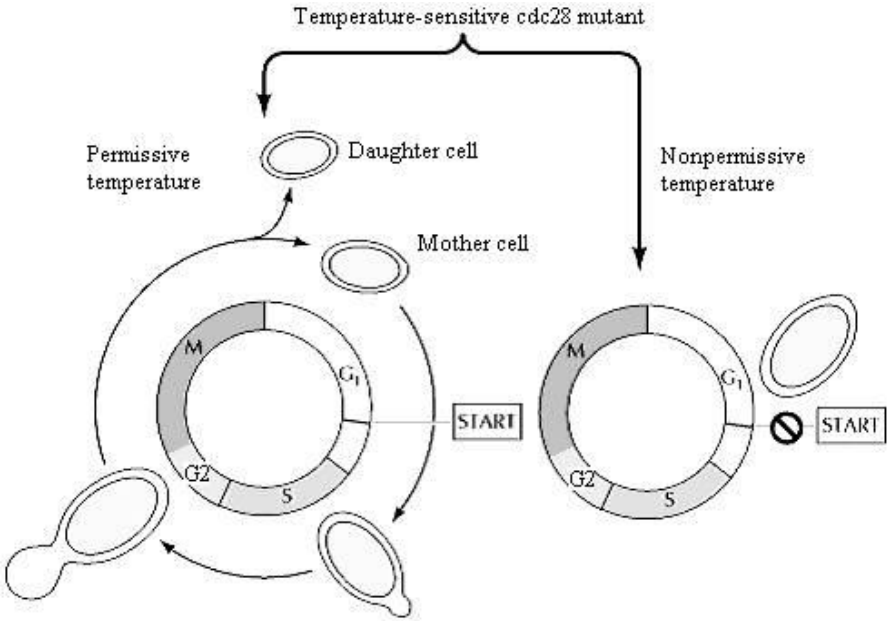
Descubrimiento de componente quinasa de mpf en levadura





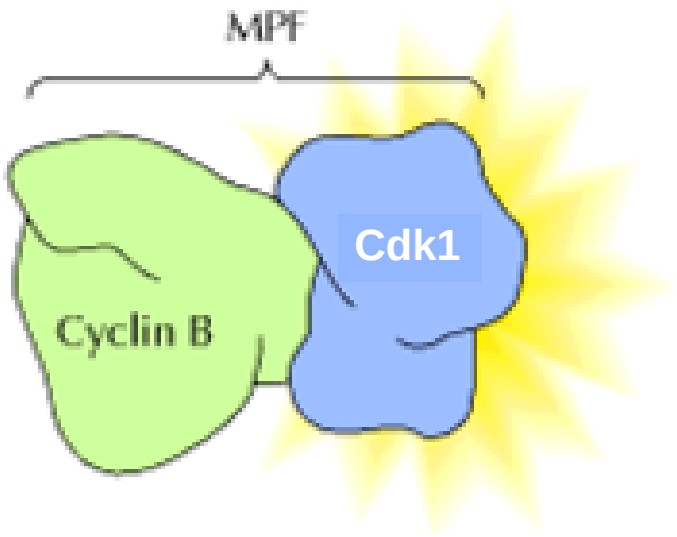






Mutantes en levadura fueron denominados cdc (cell division cycle)

MPF: complejo ciclina-cdk

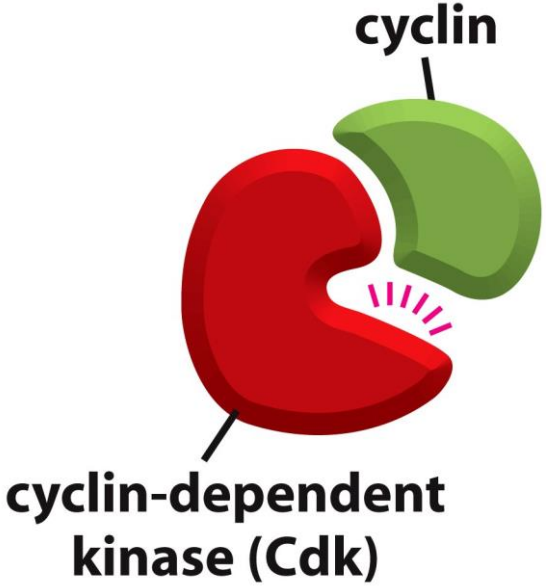


Cdk **Ciclina**

Cdk: “kinasa dependiente de ciclina”

Cdk1 = *cdc2* = *cdc28*

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

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

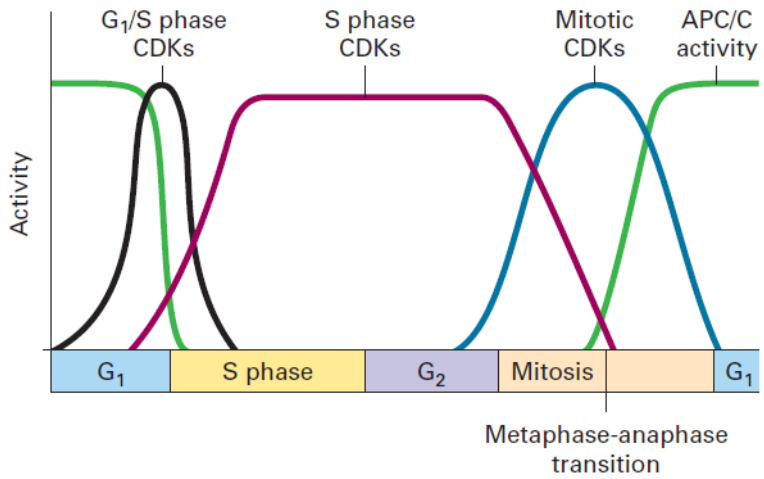
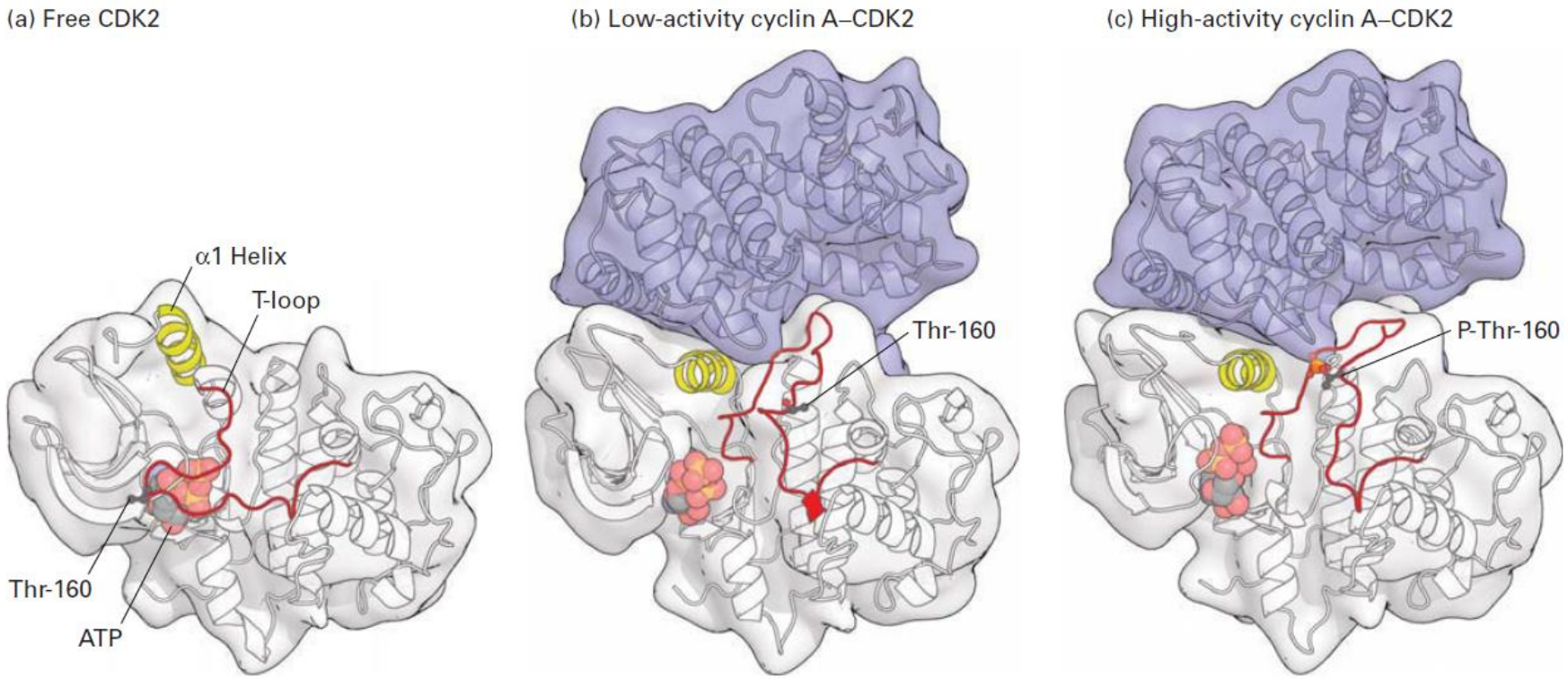


TABLE 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

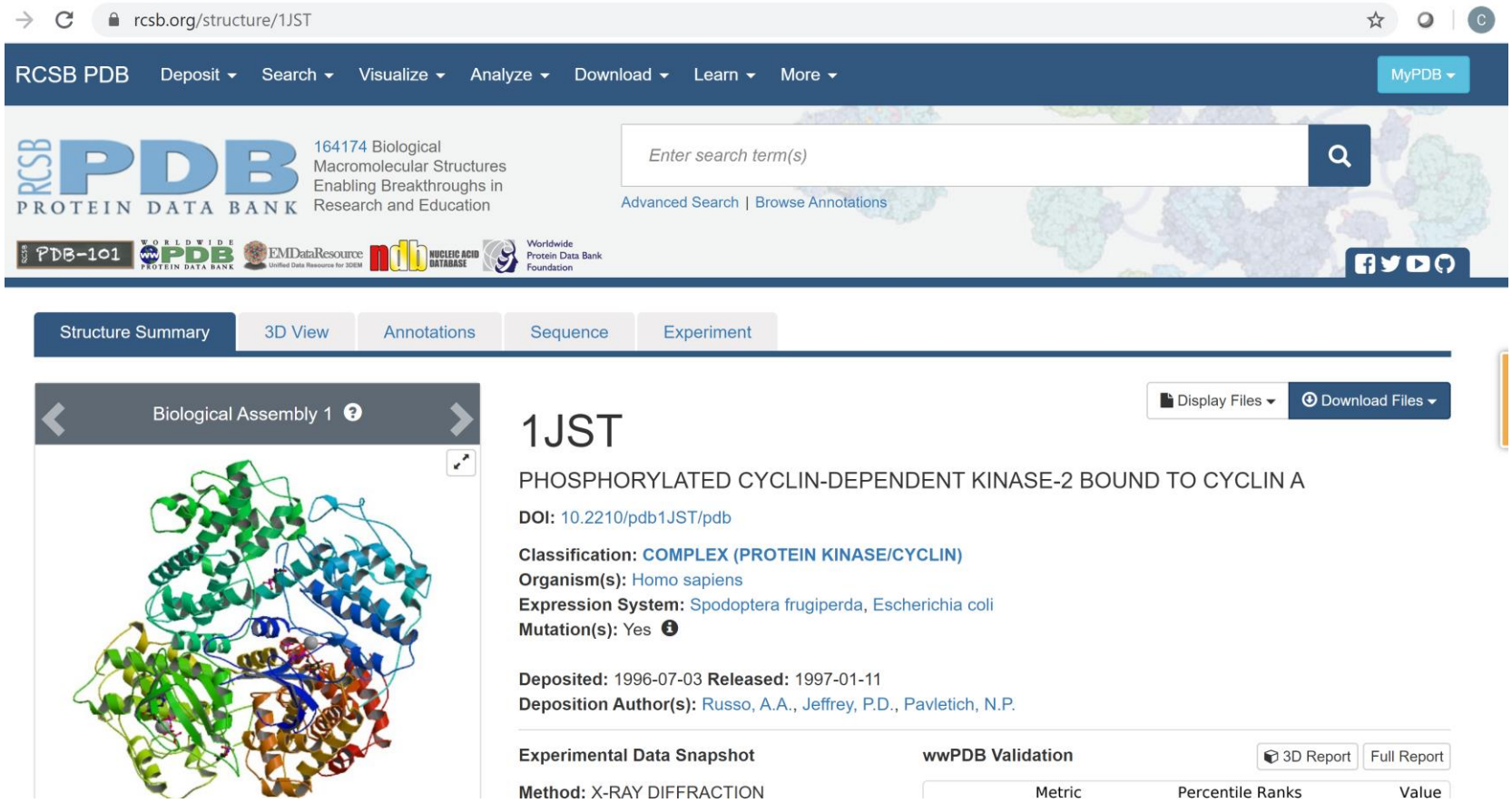
	Vertebrates		Budding yeast	
Cyclin–Cdk complex	Cyclin	Cdk partner	Cyclin	Cdk partner
G_1-Cdk	Cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	Cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	Cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	Cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

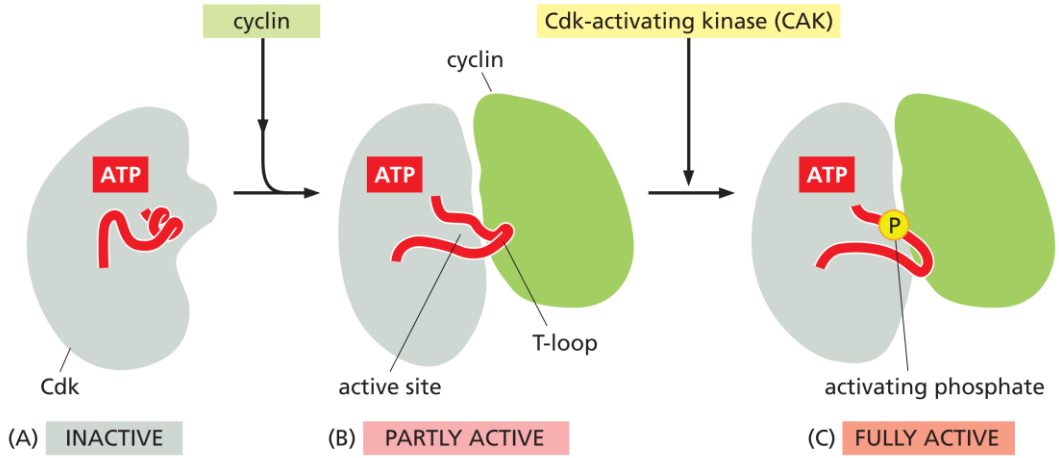
* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

Activación del complejo Ciclina-CDK



https://www.rcsb.org/structure/1JST





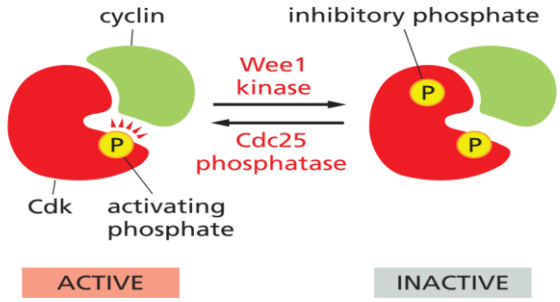


Figure 17–13 The regulation of Cdk activity by phosphorylation. The active cyclin–Cdk complex is turned off when the kinase Wee1 phosphorylates two closely spaced sites above the active site. Removal of these phosphates by the phosphatase Cdc25 activates the cyclin–Cdk complex. For simplicity, only one inhibitory phosphate is shown. CAK adds the activating phosphate, as shown in Figure 17–12.

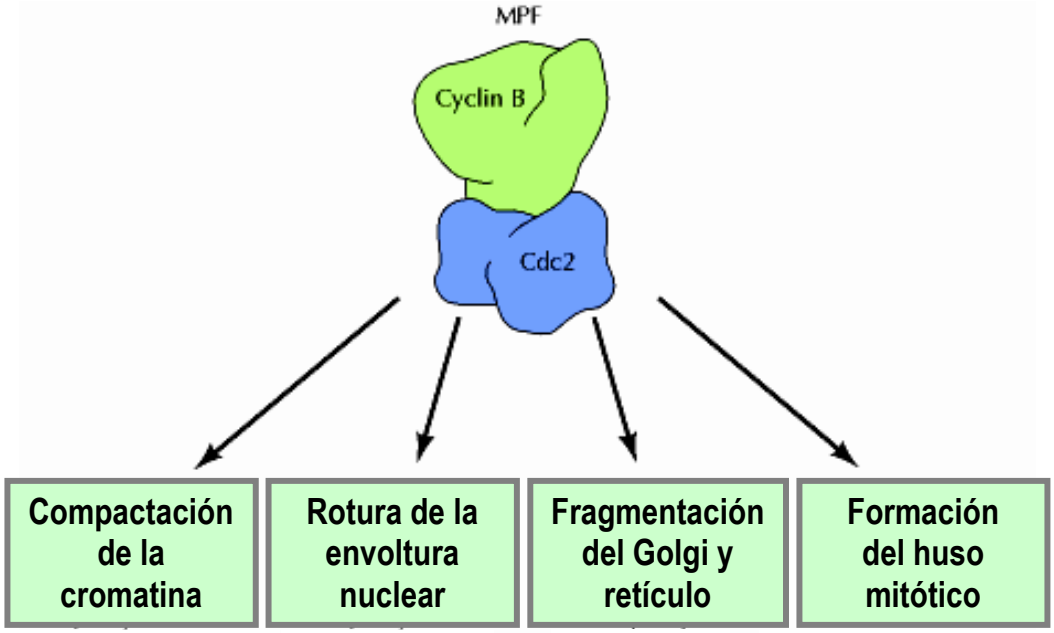
Regulación de la actividad del complejo Ciclina-CDK

TABLE 19-2 Regulators of Cyclin-CDK Activity

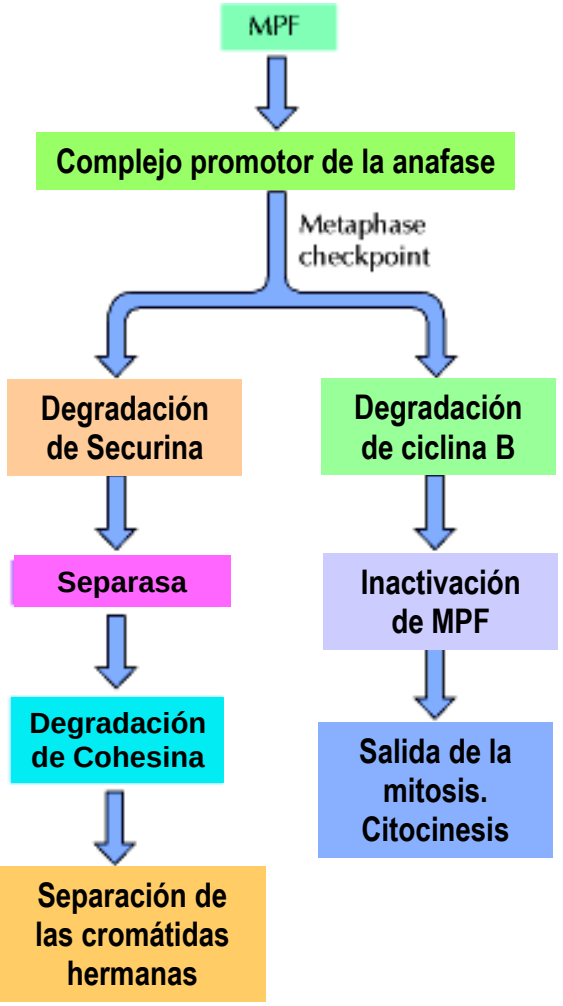
Type of Regulator	Function
Kinases and Phosphatases	
CAK kinase	Activates CDKs
Wee1 kinase	Inhibits CDKs
Cdc25 phosphatase	Activates CDKs
Cdc14 phosphatase	Activates Cdh1 to degrade mitotic cyclins
Cdc25A phosphatase	Activates vertebrate S phase CDKs
Cdc25C phosphatase	Activates vertebrate mitotic CDKs
Inhibitory Proteins	
Sic1	Binds and inhibits S phase CDKs
CKIs $p27^{KIP1}$, $p57^{KIP2}$, and $p21^{CIP}$	Bind and inhibit CDKs
INK4	Binds and inhibits G_1 CDKs
Rb	Binds E2Fs, preventing transcription of multiple cell cycle genes
Ubiquitin-Protein Ligases	
SCF	Degradation of phosphorylated Sic1 or $p27^{KIP1}$ to activate S phase CDKs
APC/C^{Cdc20}	Degradation of securin, initiating anaphase. Induces degradation of B-type cyclins
APC/C^{Cdh1}	Degradation of B-type cyclins in G_1 and geminin in metazoans to allow loading of replicative helicases on DNA replication origins

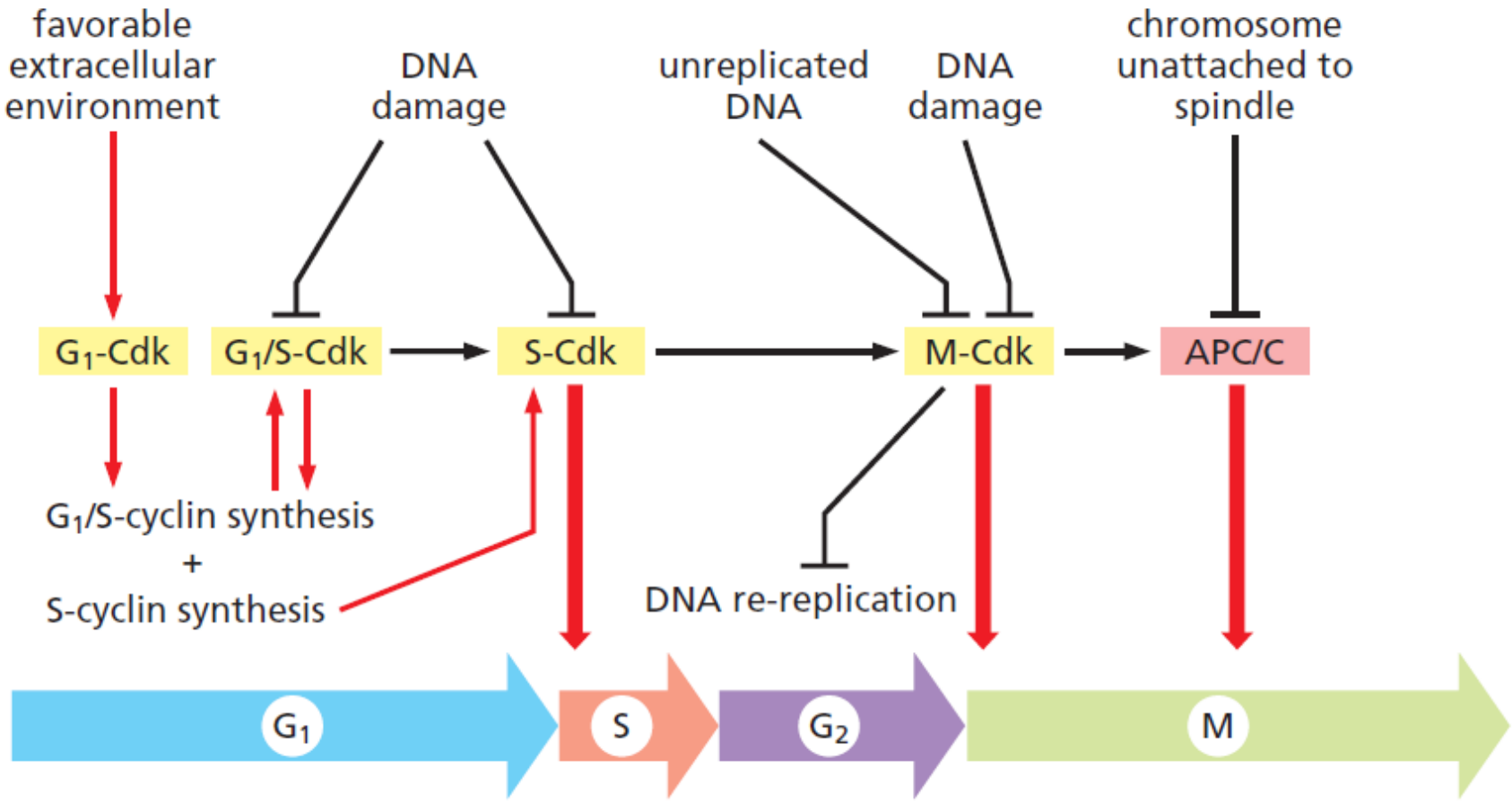
Ejemplo :
Blancos de la actividad M-Cdk (ciclina B-Cdk1)

Fosforilación directa



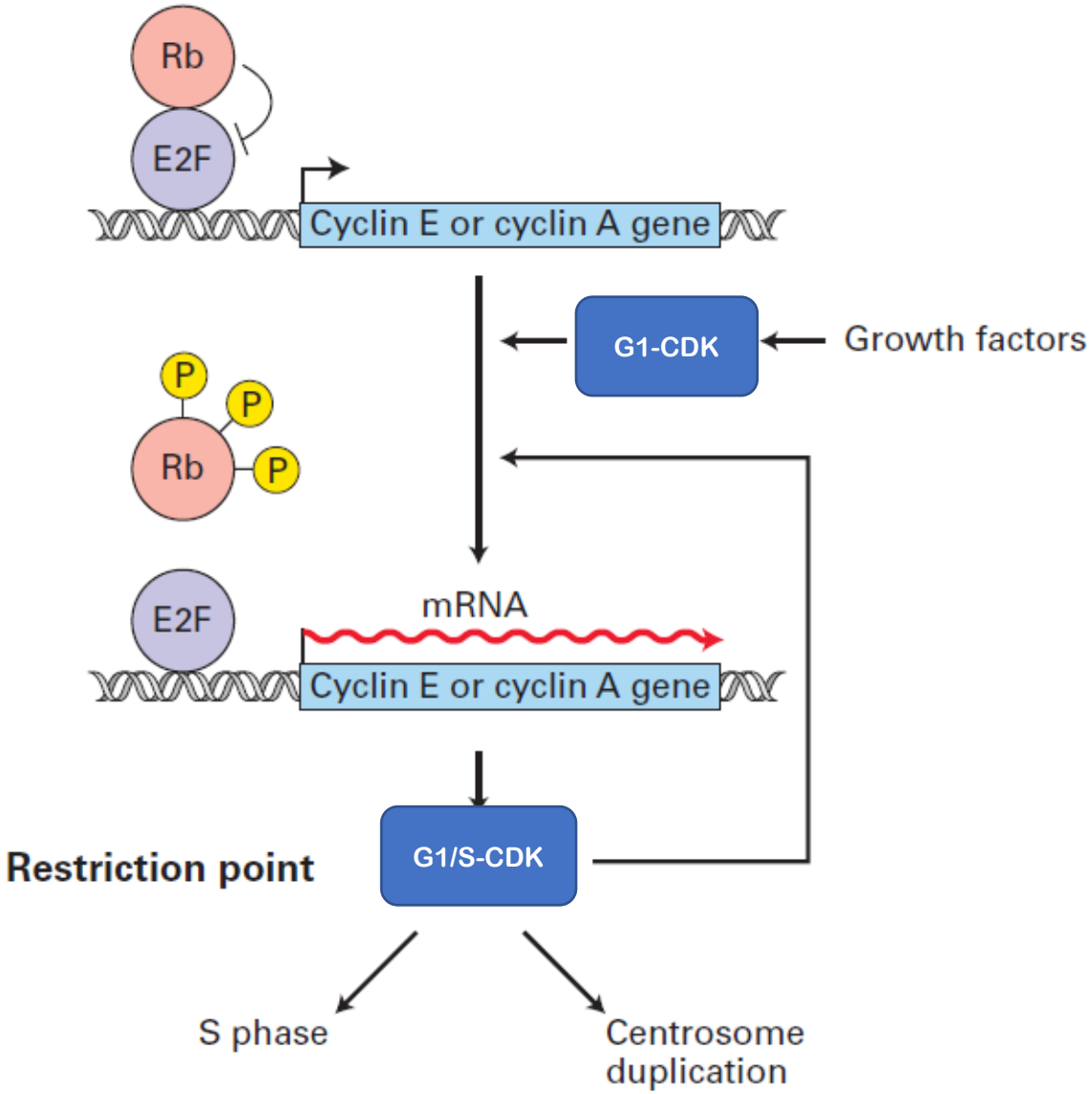
Activación de proteólisis



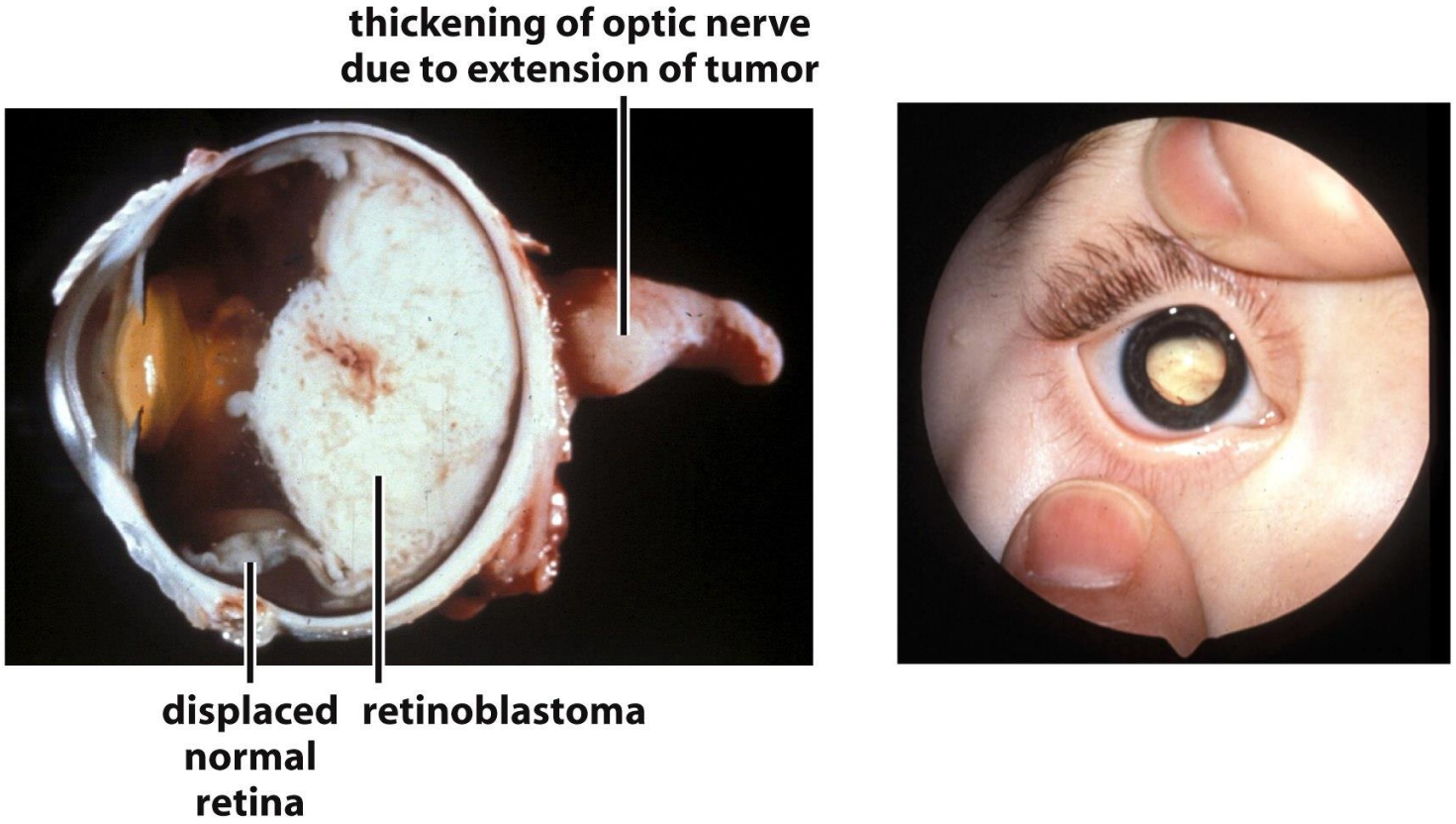

favorable extracellular environment
DNA damage
unreplicated DNA
DNA damage
chromosome unattached to spindle
G1-Cdk
G1/S-Cdk
S-Cdk
M-Cdk
APC/C
G1/S-cyclin synthesis
+
S-cyclin synthesis
DNA re-replication
G1
S
G2
M

Rb= proteína del retinoblastoma

Entrada al ciclo



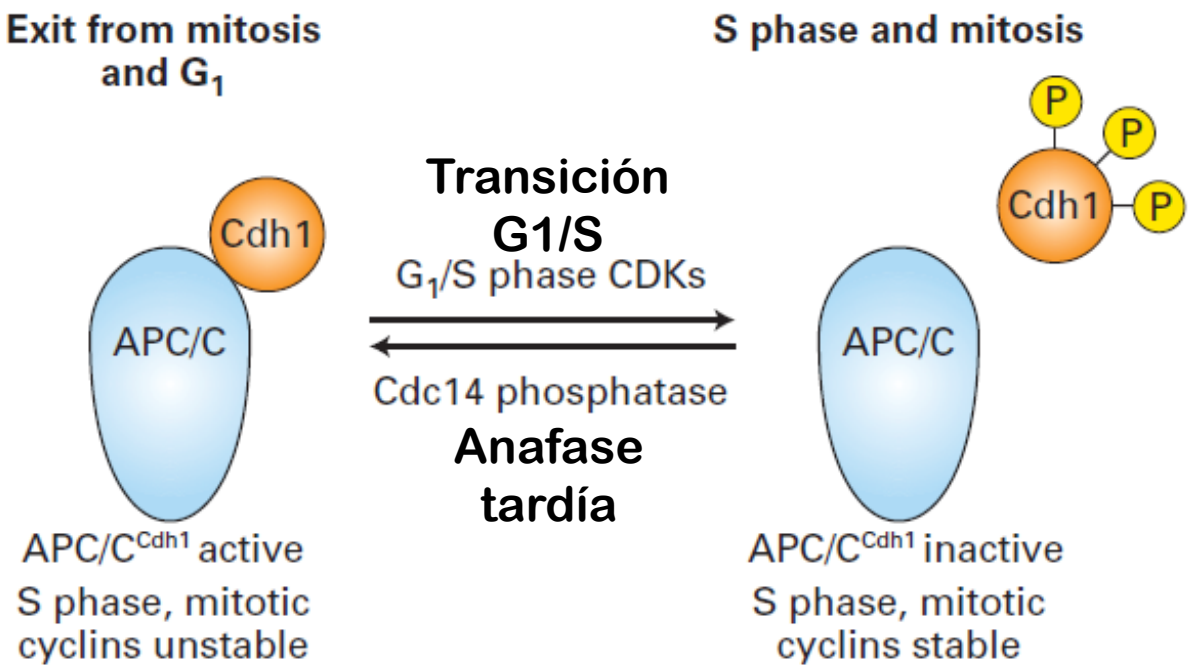
Retinoblastoma



https://ghr.nlm.nih.gov/condition/retinoblastoma#genes

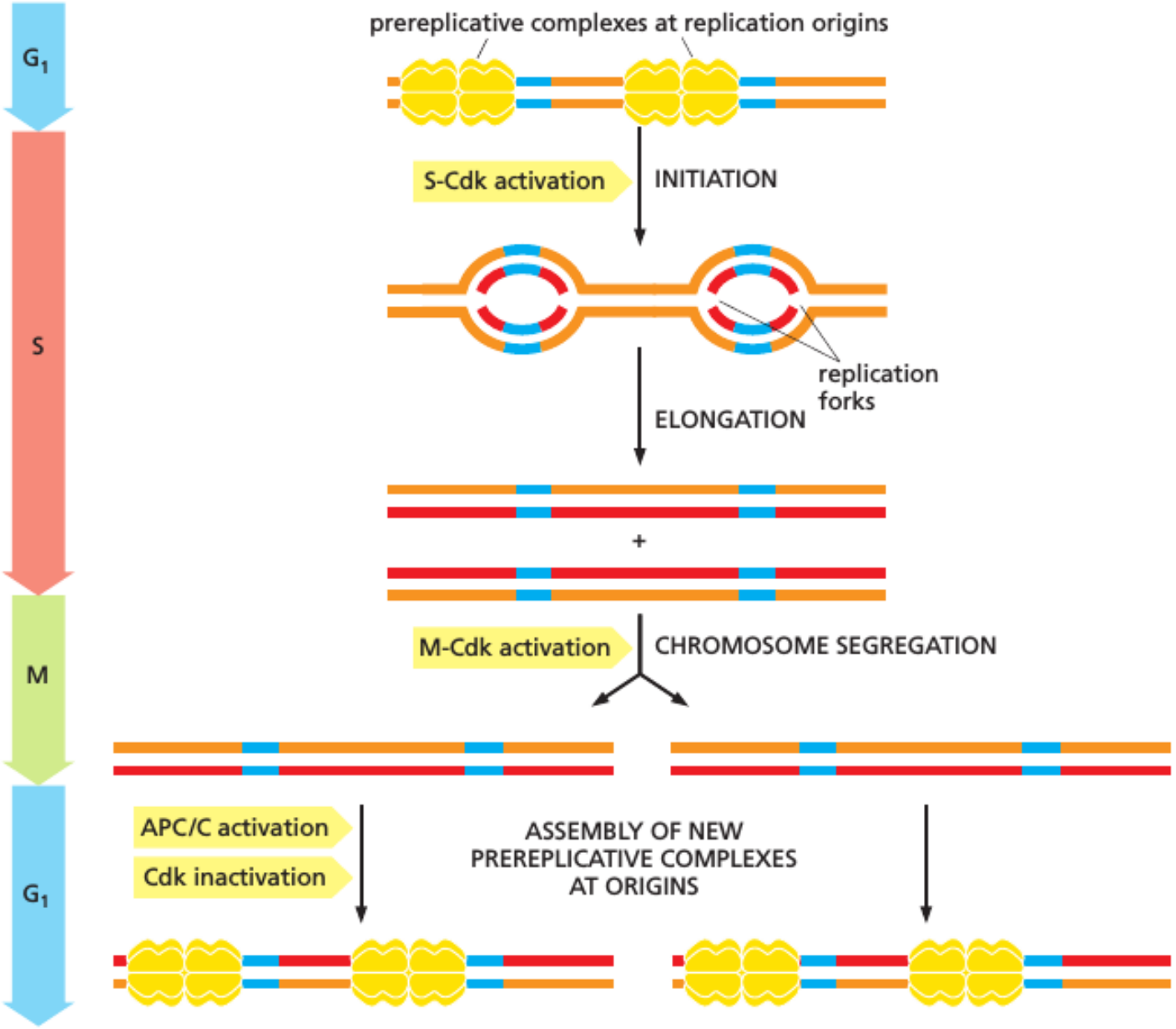
Figure 7.4b *The Biology of Cancer* (© Garland Science 2007)

Entrada a fase S

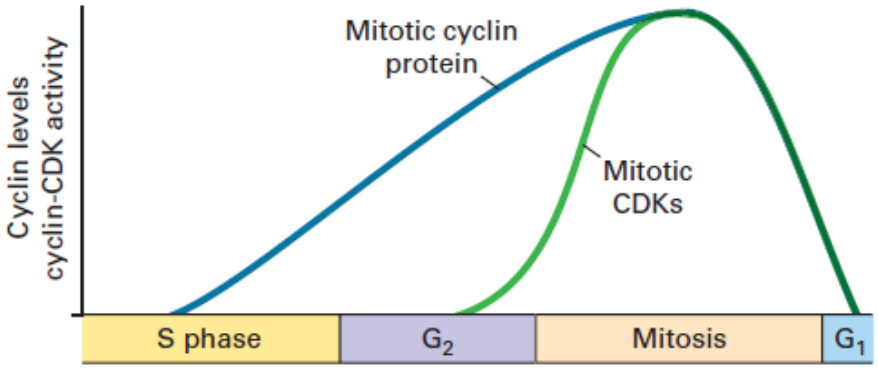


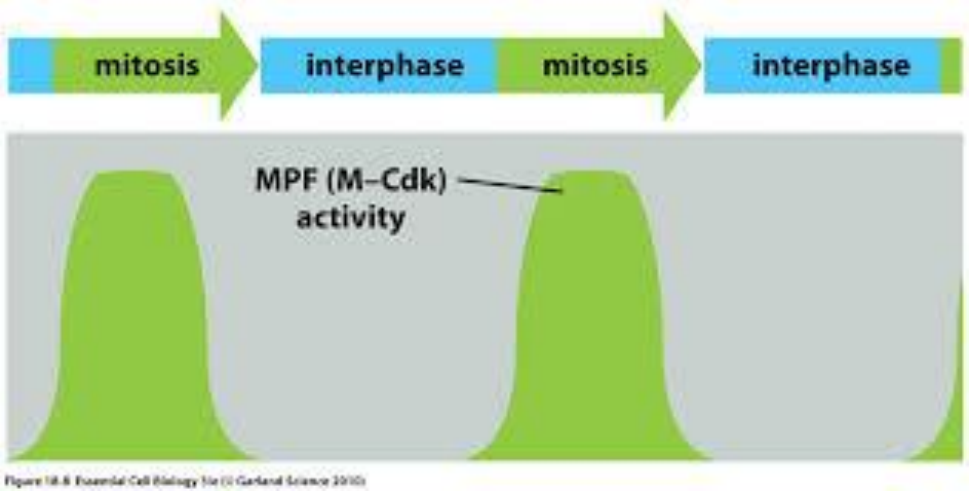
Inactivación del inhibidor de S-CDK

Regulación de la replicación del ADN

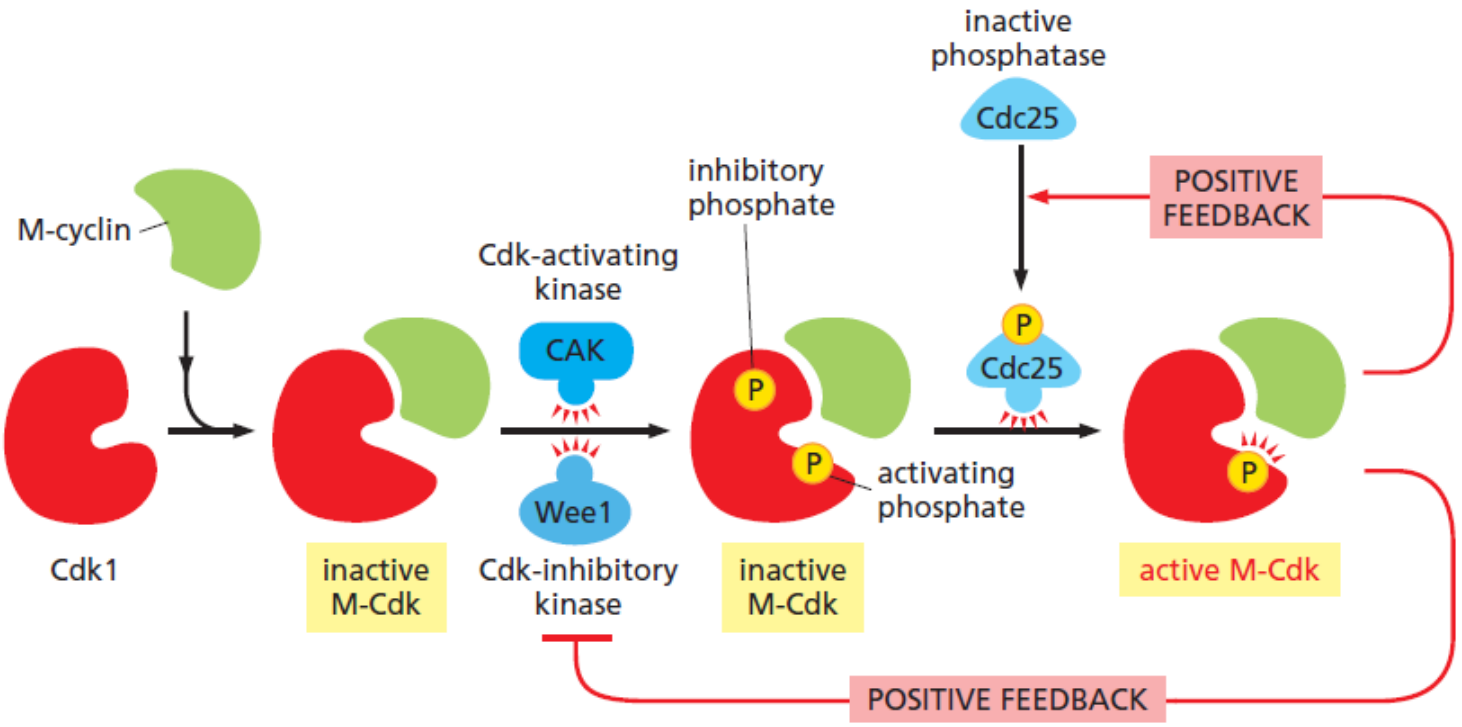


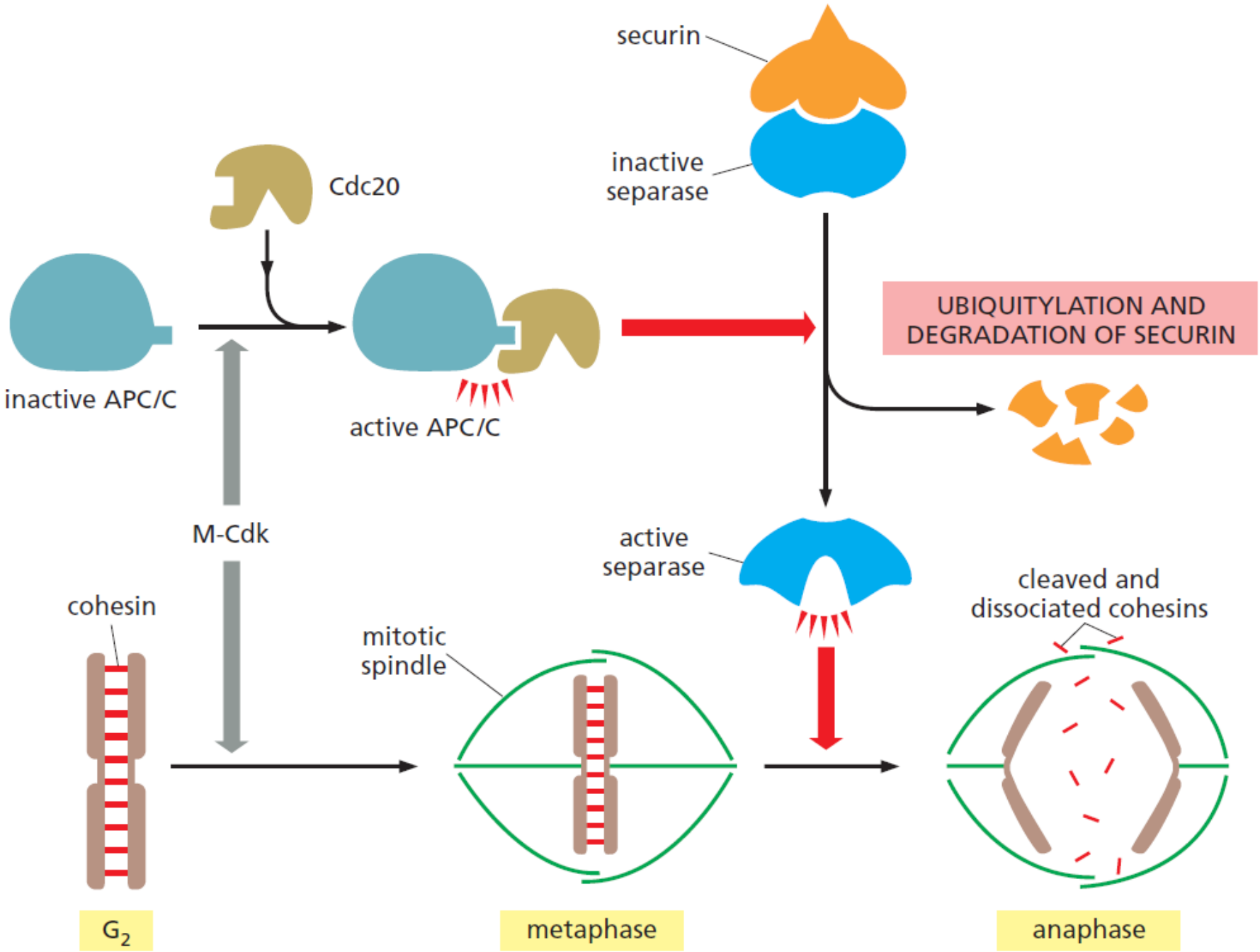
¿Cómo se genera la entrada a mitosis?



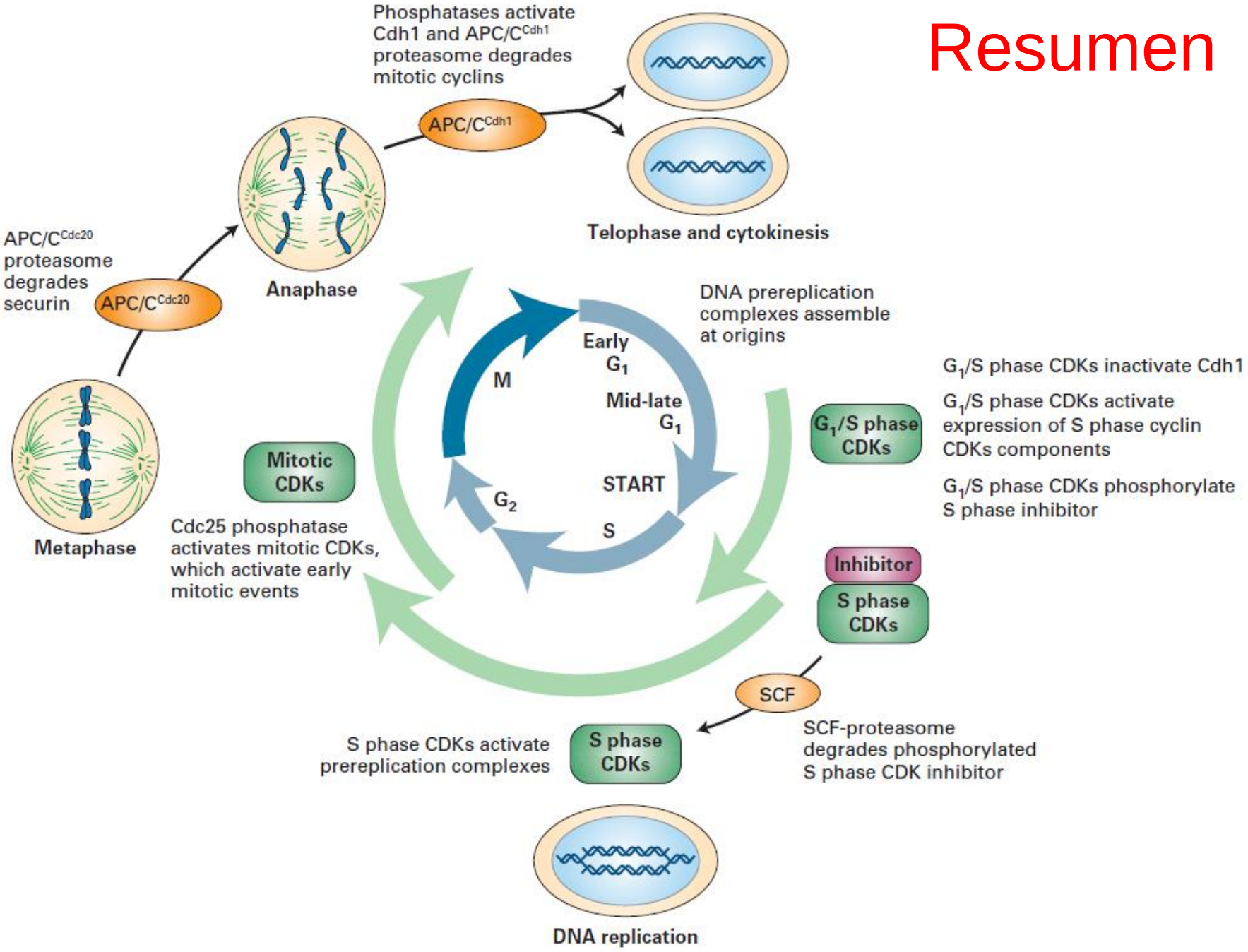


Entrada a mitosis




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

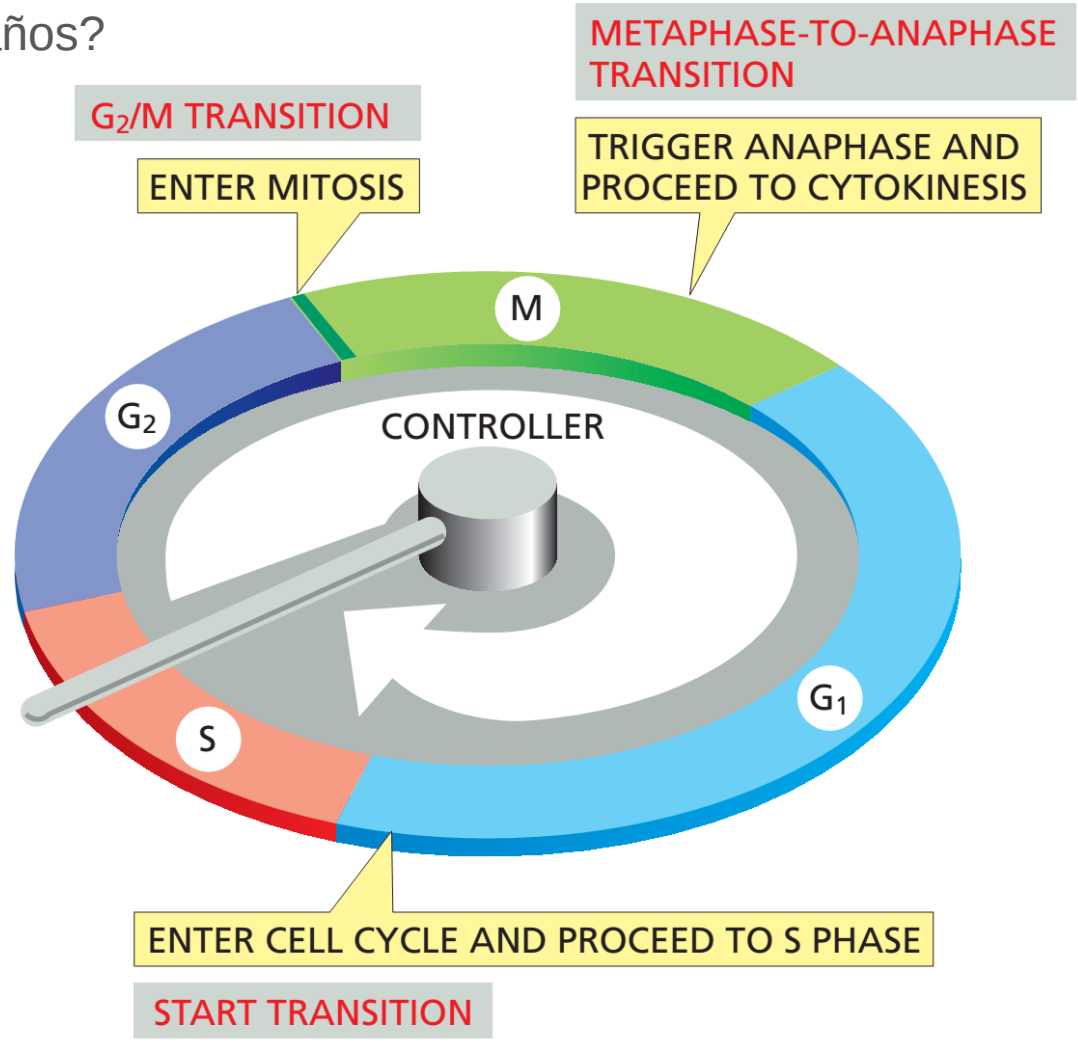
Resumen


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
DNA prereplication complexes assemble at origins
Early G1
Mid-late G1
START
S
G2
M
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

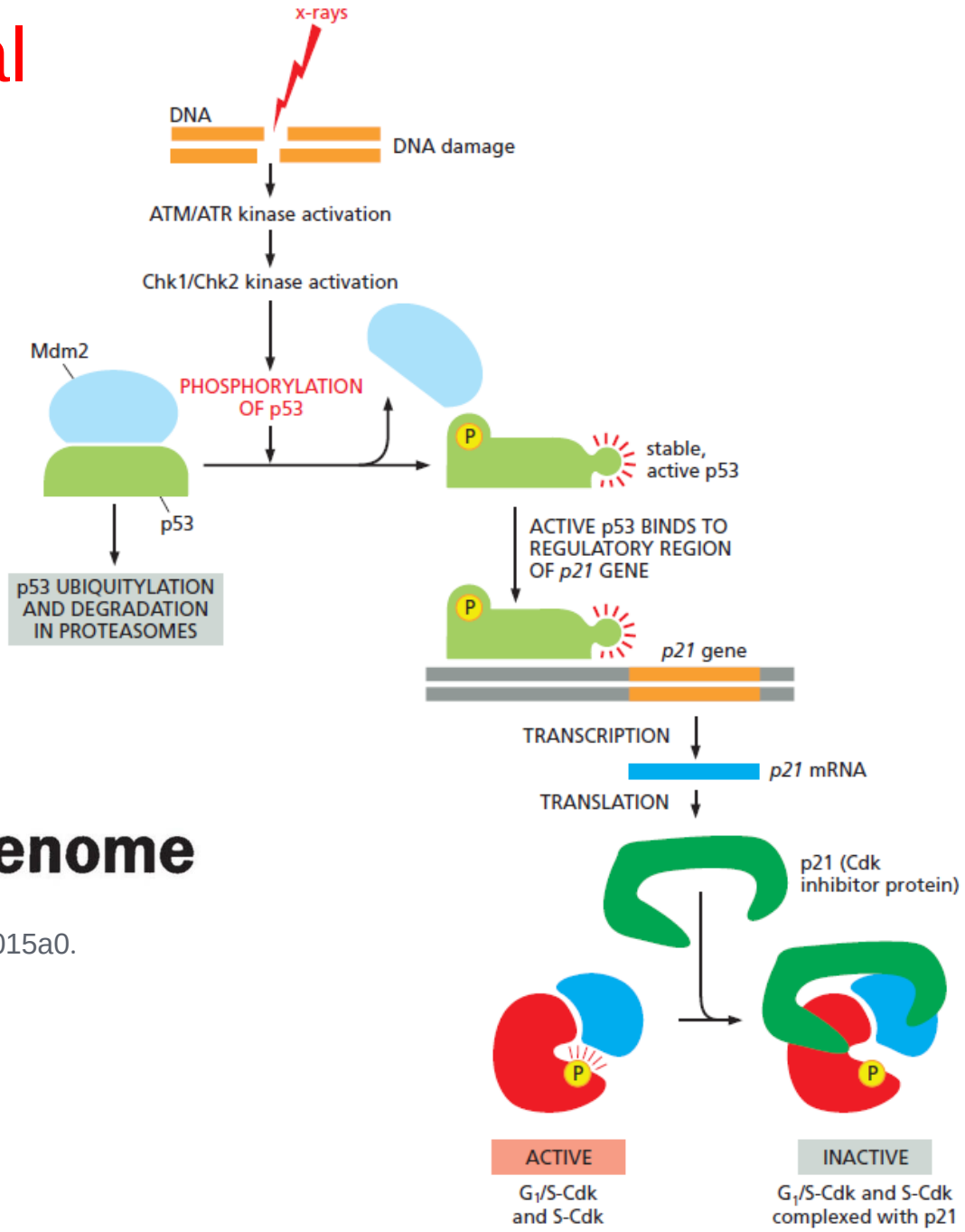
¿ADN completamente replicado y sin daños?

¿Cromosomas unidos al huso mitótico?



¿Ambiente favorable?

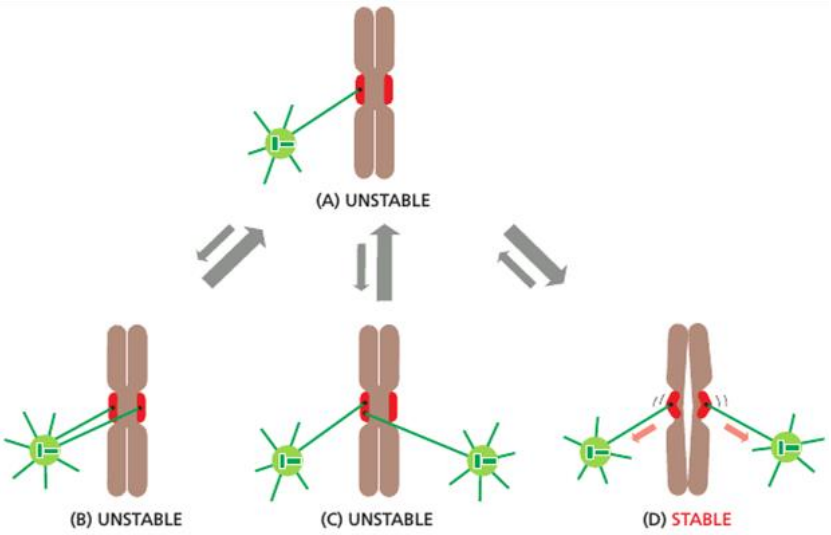
Control daño material genético

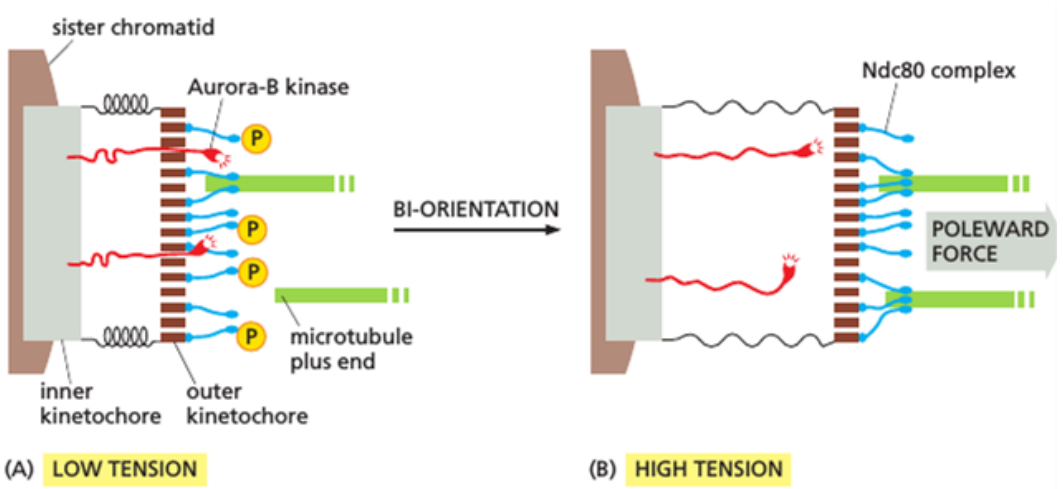


p53, guardian of the genome

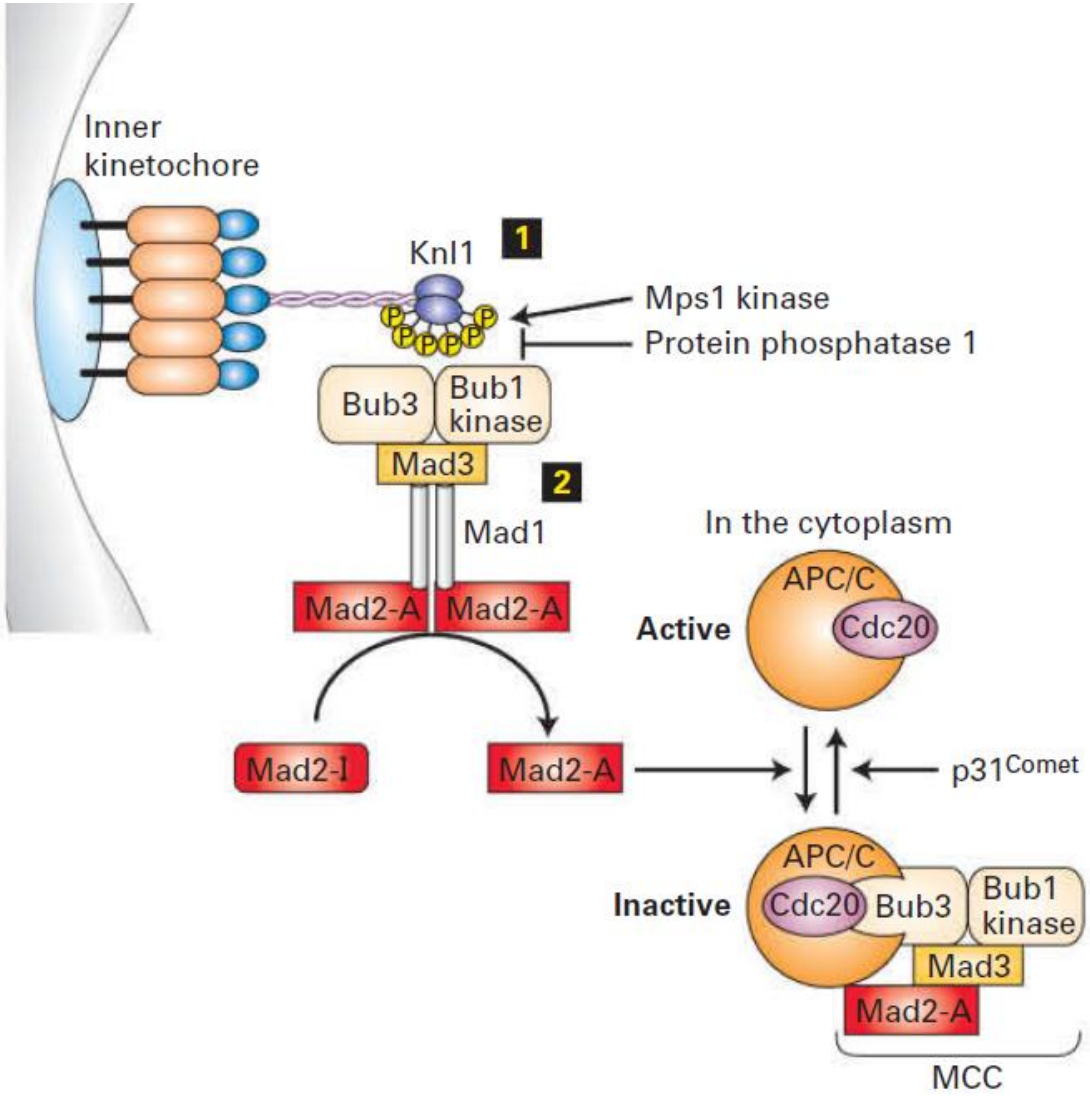
Nature, 1992 Jul 2;358(6381):15-6. doi: 10.1038/358015a0.

Punto de control: Unión huso mitótico - Cinetocoro

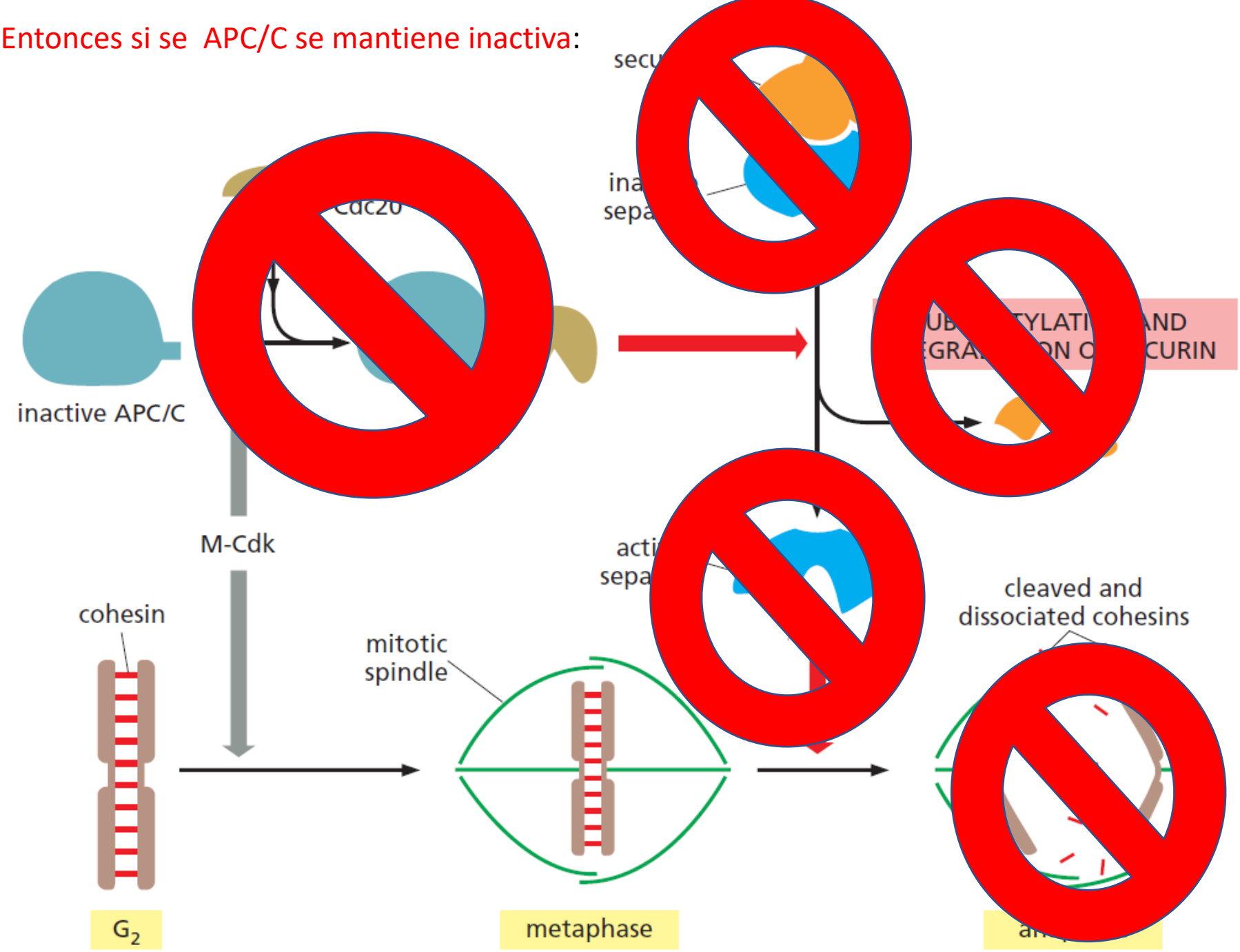




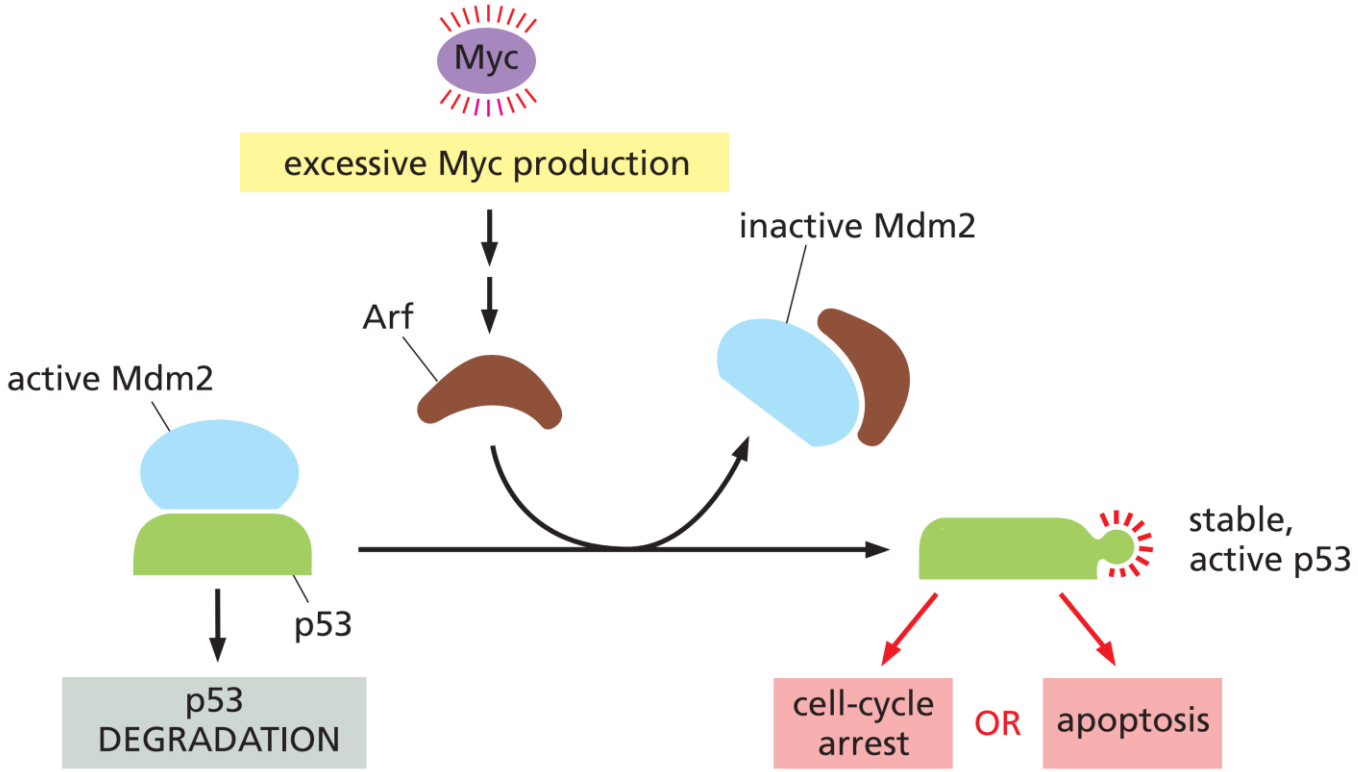
Punto de control: Unión huso mitótico - Cinetocoro



Entonces si se APC/C se mantiene inactiva:



Exceso de señales de proliferación pueden causar detención del ciclo celular o apoptosis



Excepción: cáncer

ARTICLE

Cell Cycle Control and Cancer

Leland H. Hartwell* and Michael B. Kastan

Multiple genetic changes occur during the evolution of normal cells into cancer cells. This evolution is facilitated in cancer cells by loss of fidelity in the processes that replicate, repair, and segregate the genome. Recent advances in our understanding of the cell cycle reveal how fidelity is normally achieved by the coordinated activity of cyclin-dependent kinases, checkpoint controls, and repair pathways and how this fidelity can be abrogated by specific genetic changes. These insights suggest molecular mechanisms for cellular transformation and may help to identify potential targets for improved cancer therapies.

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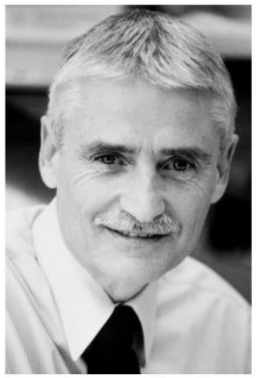

Photo from the Nobel Foundation archive.
Leland H. Hartwell

Table 8.3 Molecular changes in human cancers leading to deregulation of the cell cycle clock

Specific alteration	Clinical result
Alterations of pRb	
Inactivation of the *Rb* gene by mutation	retinoblastoma, osteosarcoma, small-cell lung carcinoma
Methylation of *Rb* gene promoter	brain tumors, diverse others
Sequestration of pRb by Id1, Id2	diverse carcinomas, neuroblastoma, melanoma
Sequestration of pRb by the HPV E7 viral oncoprotein	cervical carcinoma
Alteration of cyclins	
Cyclin D1 overexpression through amplification of *cyclin D1* gene	breast carcinoma, leukemias
Cyclin D1 overexpression caused by hyperactivity of *cyclin D1* gene promoter driven by upstream mitogenic pathways	diverse tumors
Cyclin D1 overexpression due to reduced degradation of cyclin D1 because of depressed activity of GSK-3β	diverse tumors
Cyclin D3 overexpression caused by hyperactivity of *cyclin D3* gene	hematopoietic malignancies
Cyclin E overexpression	breast carcinoma
Defective degradation of cyclin E protein due to loss of hCDC4	endometrial, breast, and ovarian carcinomas
Alteration of cyclin-dependent kinases	
CDK4 structural mutation	melanoma
Alteration of CDK inhibitors	
Deletion of 15^{INK4B} gene	diverse tumors
Deletion of 16^{INK4A} gene	diverse tumors
Methylation of $p16^{INK4A}$ gene promoter	melanoma, diverse tumors
Decreased transcription of $p27^{Kip1}$ gene because of action of Akt/PKB on Forkhead transcription factor	diverse tumors
Increased degradation of $p27^{Kip1}$ protein due to Skp2 overexpression	breast, colorectal, and lung carcinomas, and lymphomas
Cytoplasmic localization of $p27^{Kip1}$ protein due to Akt/PKB action	breast, esophagus, colon, thyroid carcinomas
Cytoplasmic localization of $p21^{Cip1}$ protein due to Akt/PKB action	diverse tumors
Multiple concomitant alterations by Myc, N-myc or L-myc	
Increased expression of Id1, Id2 leading to pRb sequestration	diverse tumors
Increased expression of cyclin D2 leading to pRb phosphorylation	diverse tumors
Increased expression of E2F1, E2F2 E2F3 leading to expression of cyclin E	diverse tumor
Increased expression of CDK4 leading to pRb phosphorylation	diverse tumors
Increased expression of Cul1 leading to $p27^{Kip1}$ degradation	diverse tumors
Repression of $p15^{INK4B}$ and $p21^{Cip1}$ expression allowing pRb phosphorylation	diverse tumors

Table 8.3 *The Biology of Cancer* (© Garland Science 2007)

Table 8.4 Alteration of the cell cycle clock in human tumors A plus sign indicates that this gene or gene product is altered in at least 10% of tumors analyzed. Alteration of gene product can include abnormal absence or overexpression. Alteration of gene can include mutation and promoter methylation. More than one of the indicated alterations may be found in a given tumor.

Tumor type	Gene product or gene						% of tumors with 1 or more changes
	Rb	Cyclin E1	Cyclin D1	$p16^{INK4A}$	$p27^{Kip1}$	CDK4/6	
Glioblastoma	+	+		+	+	+/+	>80
Mammary carcinoma	+	+	+	+	+	+/	>80
Lung carcinoma	+	+	+	+	+	+/	>90
Pancreatic carcinoma			[a]		+		>80
Gastrointestinal carcinoma	+	+	+[b]	+	+	+/[e]	>80
Endometrial carcinoma	+	+	+	+	+	+/	>80
Bladder carcinoma	+	+	+	+	+		>70
Leukemia	+	+	+	+[c]	+	+/	>90
Head and neck	+		+	+	+	+/	>90
Lymphoma	+	+	+[d]	+[c]	+	/+	>90
Melanoma		+	+	+	+	+/	>20
Hepatoma	+	+	+	+[c]	+	+/[e]	>90
Prostate carcinoma	+	+	+	+	+		>70
Testis/ovary	+	+	+[b]	+	+	+/	>90
Osteosarcoma		+		+		+/	>80
Other sarcomas		+	+	+	+	/+	>90

[a] Cyclin D3 is up-regulated in some tumors.
[b] Cyclin D2 is up-regulated in some tumors.
[c] $p15^{INK4B}$ also found to be absent in some tumors.
[d] Cyclin D2 and D3 also found up-regulated in some lymphomas.
[e] CDK2 also found to be up-regulated in some tumors.

Adapted from M. Malumbres and M. Barbacid, *Nat. Rev. Cancer* 1:222–231, 2001.

Table 12.3 Mutated, methylated, and overexpressed genes in cancer cells that perturb chromosomal stability

Gene	Function of gene product	Consequence of alteration in cancer cells
BUB1	spindle assembly checkpoint	progress through mitosis, even in the presence of microtubule inhibitors[a]
MAD1[b]	spindle assembly checkpoint	large-scale aneuploidy
MAD2[b,c]	spindle assembly checkpoint	premature entrance into anaphase[d], aneuploidy
Securin	attachment of sister chromatids	nondisjunction of chromosomes[e]
ATM	chromosome segregation	defective metaphase-anaphase transition
Aurora-A,-B,-C	separation of chromatids at anaphase	premature entrance into anaphase[d]
CHFR	spindle assembly checkpoint	nondisjunction, chromosome loss[e]
14-3-3σ	DNA damage checkpoint	segregation of unrepaired chromosomes

[a]Microtubule inhibitors such as colchicine and nocodazole block the assembly of mitotic spindle fibers.
[b]Mad1 and Mad2 form complexes at the kinetochore that prevent chromatid separation until complexes with spindle fibers have been properly formed.
[c]The *MAD2* gene is transcriptionally repressed in a number of solid tumors and is frequently mutated in gastric carcinomas. Mice that are heterozygous at the *Mad2* locus (i.e., are *Mad2*$^{+/-}$) develop lung cancers as adults.
[d]Premature entrance into anaphase can lead to loss of entire chromosomes.
[e]Nondisjunction is the failure of sister chromatids to separate at anaphase.

Table 12.3 *The Biology of Cancer* (© Garland Science 2007)

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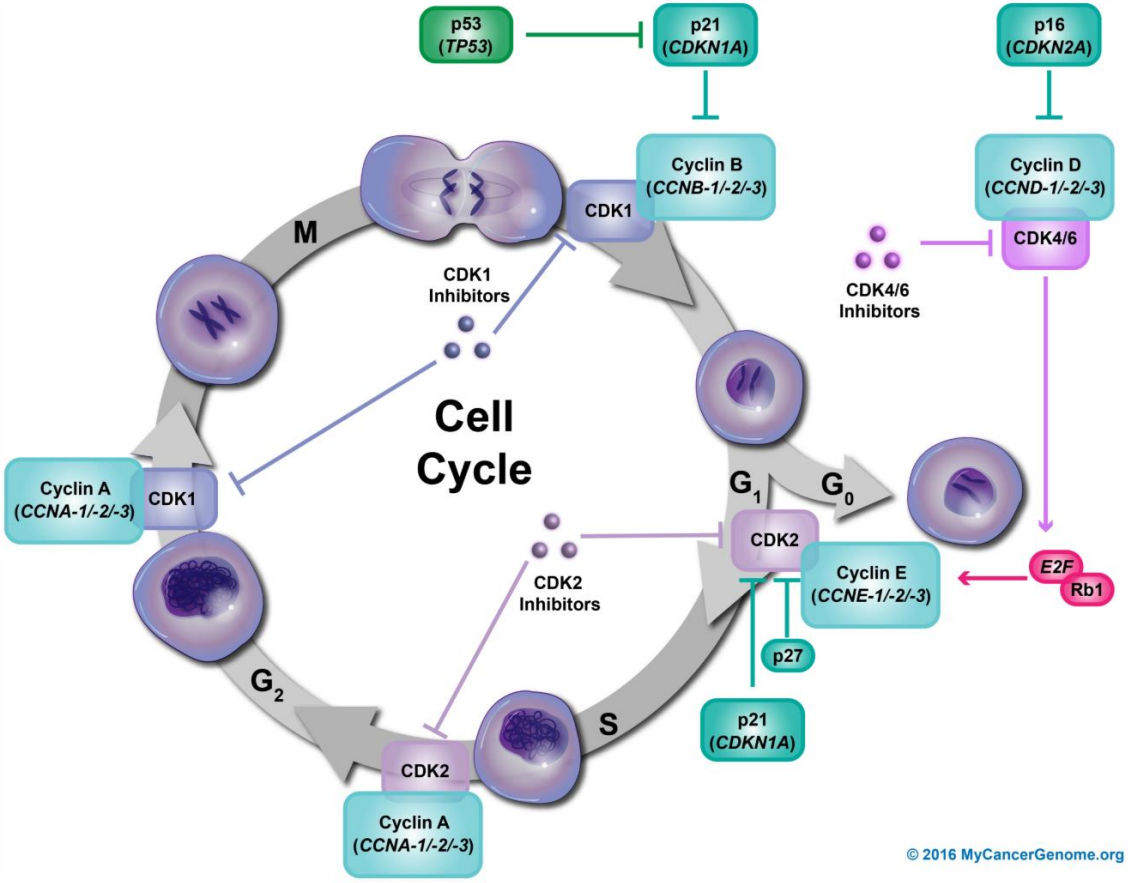
Overview

The cell cycle involves regulated cell growth, replication, and division. The cell cycle includes four distinct phases: G1 (gap phase 1), S (DNA synthesis), G2 (gap phase 2), and M (mitosis). Cell cycle regulation (both activation and inhibition) is dependent upon specific cell cycle checkpoints, which prevent abnormal cell cycle activation and continuation. For example, the G2/M checkpoint ensures that cells containing damaged DNA do not enter mitosis. These cell cycle checkpoints are controlled by the coordinated action of CDK+cyclin binding pairs including CDK4/6+cyclin D, RB1/E2F, CDK2+cyclin E, CDK2+cyclin A, CDK1+cyclin A, CDK1+cyclin B. [1]



https://www.mycancergenome.org/content/pathways/cell-cycle-control/

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Pathways upstream of cell cycle control pathway:	JAK/STAT signaling, kinase fusions, MAP kinase signaling, PI3K/AKT1/MTOR, and receptor tyrosine kinase/growth factor signaling
Drug categories targeting cell cycle control pathway:	CDK inhibitors, CDK1 inhibitors, CDK2 inhibitors, and CDK4/6 inhibitors

› Biomarker-Directed Therapies

View Therapies for Cell cycle control

› Clinical Trials

View Clinical Trials for Cell cycle control

https://www.mycancergenome.org/content/pathways/cell-cycle-control/

Práctico 8 : Ciclo Celular

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