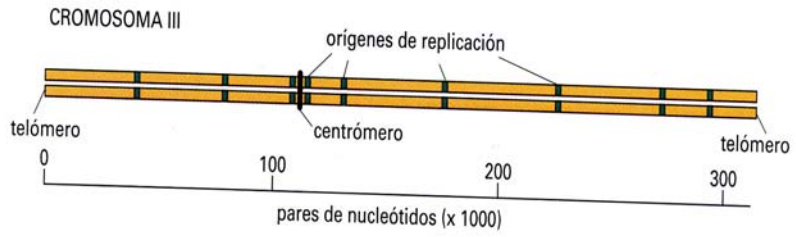
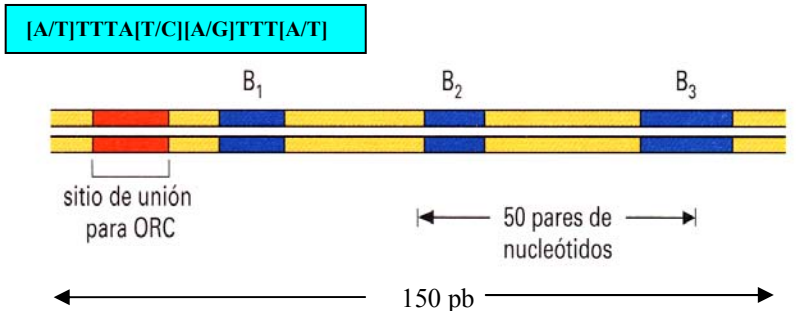
Replicación en eucariotas

Origen de replicación:



Origenes de replicación del DNA en el cromosoma III de S. cerevisiae. Este cromosoma uno de los cromosomas eucarióticos más pequeño que se conoce tiene un total de 180 genes y nueve origenes de replicación.



Este origen de replicación en levadura comprende unos 150 pb. Tiene un sitio de unión para ORC, complejo de proteínas que se une a cualquier origen de replicación. Este origen tiene otros sitios de unión (B1, B2 y B3) para otras proteínas que pueden diferir respecto a otros orígenes. En eucariótas más complejos existen ORC similares que inician la replicación del DNA, pero no están tan bien caracterizados.

*DNA replication initiates from defined origin sequences, also called as **autonomously replicating sequences (ARS)** because of their ability to allow episomal replication of plasmid DNA. **ARSs consist of an A element and a variable number of B elements**.
***The A element** is essential for origin function and it contains an ARS core consensus sequence (ACS) **5'-[A/T]TTTA[T/C][A/G]TTT[A/T]-3'**, an essential determinant for the binding of the origin recognition complex (ORC). ***The ORC consist of six subunits, recognizes the origin sequence in an ATP dependent manner and is necessary for the initiation of DNA replication**.
***The B elements** flanking the A element are less conserved in sequence, but functionally equivalent elements can be found from distinct origins**.**
***In ARS1**, the best characterized ARS in *S. cerevisiae*, any two of the three B elements are sufficient for a functional origin. The B1 element is next to the A element and partially protected by ORC binding.
***The B3** contains a binding site for **a transcription factor Abf1**, whose binding enhances origin utilization.
*The function of **the B2** element is not fully understood, but the loss of the ARS activity together with the increase in helical stability in B2 deletion mutants implies a role as a **DNA unwinding element (DUE)**.
*In the fission yeast *S. pombe,* the origins of DNA replication are much larger and more difficult to define. They contain AT-rich sequence blocks that are important for ARS activity, but lack any well-conserved consensus sequence**.**
***In higher eukaryotes, ORC proteins are conserved, but initiation is largely determined in a sequence independent manner and DNA replication may start from alternative sites on the relative large initiation zone.**

Características consenso de los orígenes de replicación.

- **Segmentos de DNA singulares que contienen múltiples secuencias repetidas cortas**
- **Estas unidades repetitivas cortas son reconocidas por proteínas multiméricas que se unen al origen**
- **Las regiones de origen suelen contener un segmento rico en AT**

DNA polimerasas eucarióticas

Clasificación

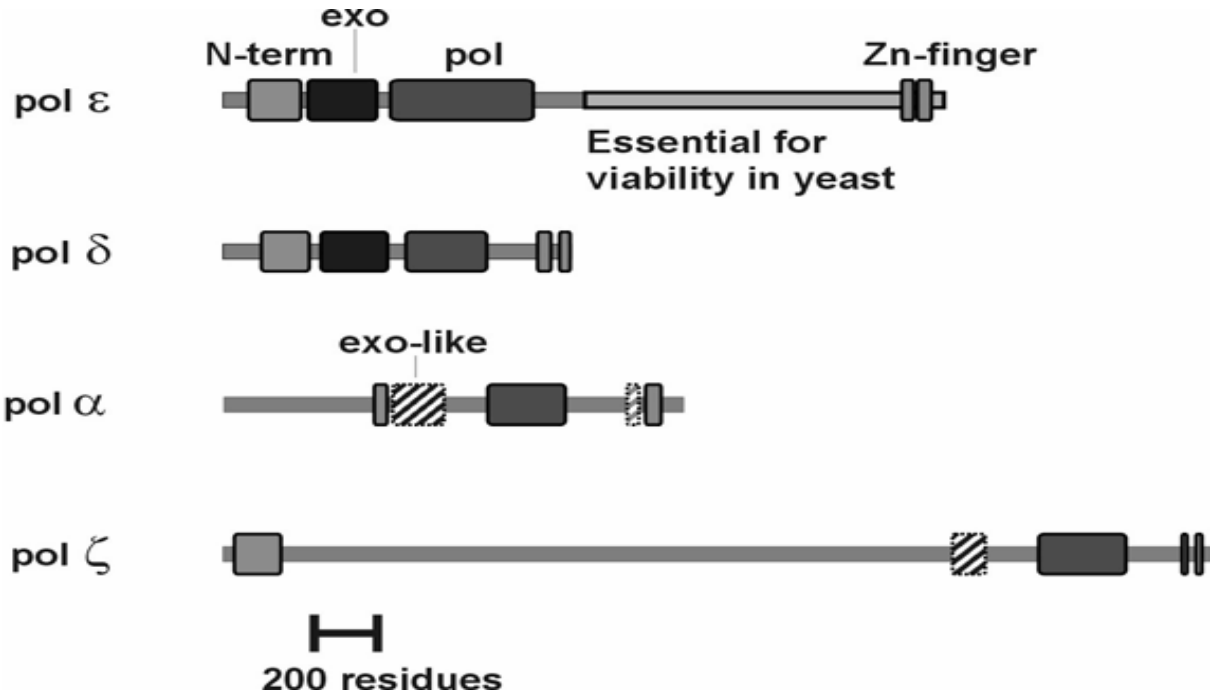
Eukaryotic DNA polymerases

Greek name	HUGO name	Class	Subunit composition (kDa)[1]	Proposed main function(s)[2]
α (Alpha)	**Pol a**	**B**	**166 (167)**	**Replication, repair (DSBR), telomere maintenance,**
			66 (79)	**S phase checkpoint control**
			59 (62)	
			50 (48)	**Priming in replication**
δ (Delta)	**Pol d**	**B**	**124 (125)**	**Replication, repair (MMR, BER, NER, DSBR), recombination, telomere maintenance, S phase checkpoint control**
			51 (55)	
			51 (40)	
			12	
ε (Epsilon)	**Pol e**	**B**	**262 (256)**	**Replication, repair (BER, NER, DSBR), recombination, transcriptional silencing, S phase checkpoint control**
			60 (79)	
			17 (23)	
			12 (22)	
ζ (Zeta)	Pol z	B	353 (173)	Translesion synthesis
			24 (29)	
γ (Gamma)	**Pol g**	**A**	**140 (144)**	**Mitochondrial DNA replication**
			55	
θ (Theta)	Pol q	A	198	Interstrand cross-link repair?
β (Beta)	**Pol b**	**X**	**38**	**Repair (BER)**
λ (lambda)	Pol l	X	63 (68)	Meiotic repair
μ (mu)	Pol m	X	55	Somatic hypermutation
σ (Sigma)	Pol σ1 (Trf4)	X	57 (66)	Establishment of sister chromatid cohesion
	Pol σ2 (Trf5)	X	60 (73)	Establishment of sister chromatid cohesion
η (eta)	Pol h	Y	78 (72)	Translesion synthesis
ι (iota)	Pol i	Y	80	Translesion synthesis, mutagenesis?
κ (Kappa)	Pol k	Y	99	Translesion synthesis
	Rev1	Y	138 (112)	Translesion synthesis

[1]Calculated from cDNAs for the human polypeptides. The mass of the *S .cerevisiae* orthologue is shown in parentheses. [2]BER = base excision repair, DSBR = double strand break repair, MMR = mismatch repair, NER = nucleotide excision repair. HUGO = The human genome nomenclature committee.

DNA polymerases can be classified in six main groups based upon phylogenetic relationships with E. coli Pol I (class A), E. coli Pol II (class B), E.coli Pol III (class C), Euryarchaeotic Pol II (class D), human Pol β (class X), and E.coli UmuC/Din B and eukaryotic RAD 30/xeroderma pigmentosum variant (class Y). All known eukariotic enzymes are either class A, B, X or class Y enzymes.

Estructura



The comparison of conserved motifs between B family pols. (Húbscher et al. 2000)

Regulación

- **Niveles de expresión**
- **Fosforilación**

Comparison of the regulation of mammalian pol α and polδ

Pol subunit	Expression	Phosphorylation
Pol α subunit A	G_0/G_1: 8-fold up in steady-state mRNA	G_1/S: substrate for Cyclin E/Cdk2, Cdc7/Dbf4
	11-fold up in *de novo* protein	
	G_1/S: < 2-fold up in steady-state mRNA	S/G_2: substrate for Cyclin A/Cdk2 on T174, S209, T219
	no change in immunoreactive protein	
Pol α subunit B	G_0/G_1: 10-fold up in steady-state mRNA	G_1/S: substrate for Cyclin E/Cdk2
	G_1/S: no significant change	S/G_2: substrate for Cyclin A/Cdk2
Pol δ subunit A	G_0/G_1: 10-fold up in steady-state mRNA	G_1/S: substrate for Cyclin D3/Cdk4,
	G_1/S: 3-fold up in steady-state mRNA	Cyclin E/Cdk2
	2-fold up in immunoreactive protein	
Pol δ subunit B	G_0/G_1: no significant change?	?

Data from Wahl *et al.* 1988, Miyazawa *et al.* 1993, Dehde *et al.* 2000, Voitenleitner *et al.* 1999, Weinreich & Stillman 1999, Schub *et al.* 2001, Yang *et al.* 1992, Zeng *et al.* 1994, Wu *et al.* 1998, Iyer *et al.* 1999.

Replicación del SV40 "In Vitro"

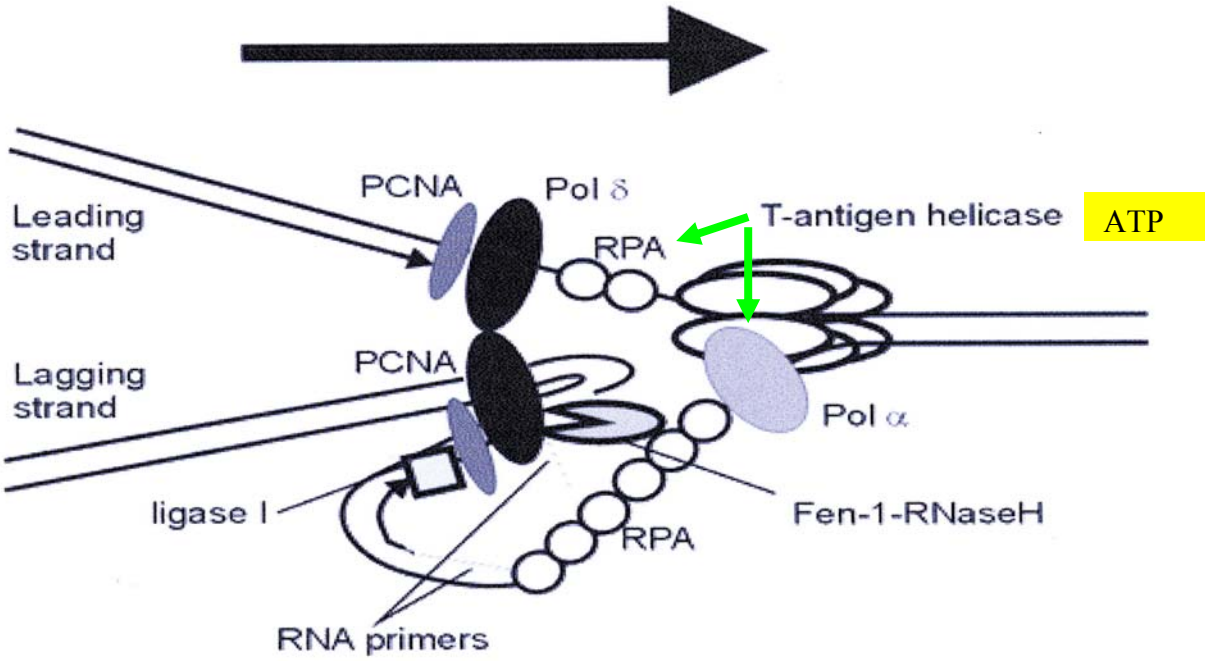
Table 1 Functions of DNA replication fork proteins

Proteins	Functions
RPA	Single-stranded DNA binding; stimulates DNA polymerases; facilitates helicase loading
PCNA	Stimulates DNA polymerases and RFC ATPase
RFC	DNA-dependent ATPase; primer-template DNA binding; stimulates DNA polymerases; PCNA loading
Pol α/primase	RNA-DNA primer synthesis
Pol δ/ε[a]	DNA polymerase; 3′–5′ exonuclease
FEN1	Nuclease for removal of RNA primers
RNase HI	Nuclease for removal of RNA primers
DNA ligase I	Ligation of DNA
T antigen[b]	DNA helicase; primosome assembly

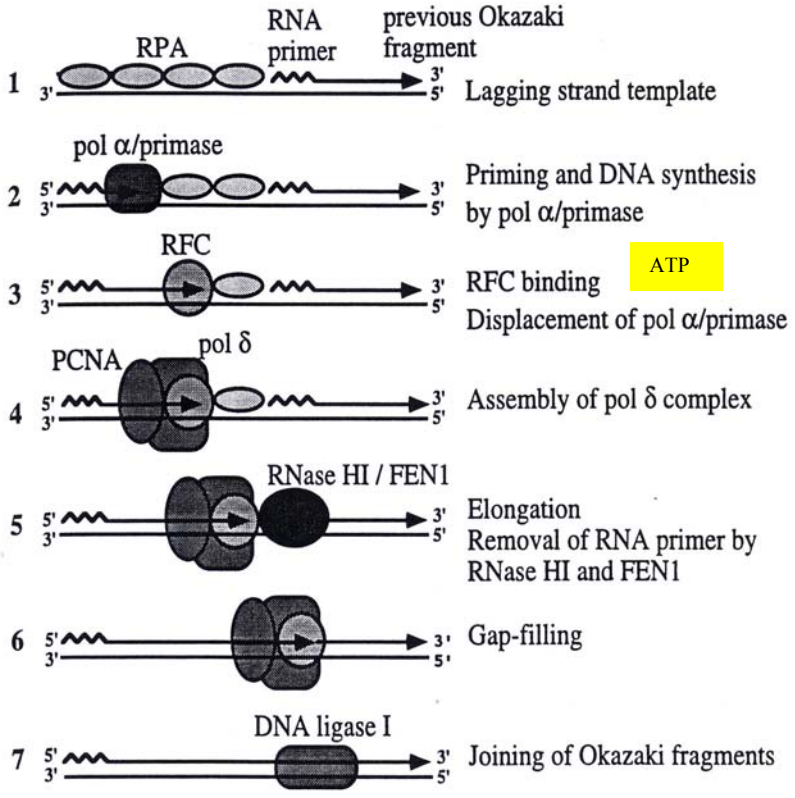
[a]A specific function of DNA polymerase ε in replication has not been assigned, although it is known to be essential for S-phase progression in *S. cerevisiae* (196, 197).
[b]T antigen is required for the replication of SV40 DNA. Its functional equivalent in eukaryotic cells has not been identified.

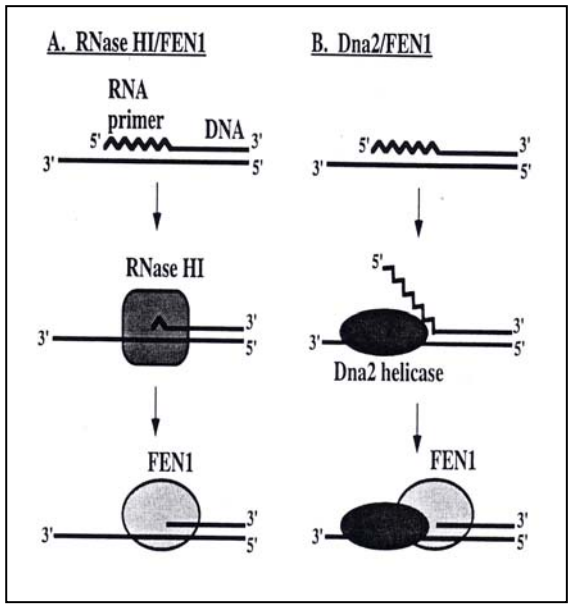
The large T-antigen (T-ag), is a multifunctional 708 aa phosphoprotein with ssDNA and sequence specific dsDNA binding, ATPase and helicase activities. First, T-ag recognizes and binds to the origin of replication as a double hexamer. This binding is negatively and positively regulated by phosphorylation *in vivo*, and indeed, SV40 DNA replication *in vivo* is tightly restricted to the S phase. Secondly, T-ag catalyses the melting of the DNA duplex. Thirdly, it recruits the replication protein A (RPA) and DNA polymerase alpha-primase (pol α) onto the origin. RPA is a eukaryotic single strand binding protein that protects and stabilizes ssDNA and promotes DNA unwinding. Fourthly, T-ag acts as a replicative helicase that unwinds DNA in front of the replication fork.
Pol α primes the leading strand as well as every Okazaki fragment of the lagging strand by synthesizing a short RNA-DNA primer that consists of a ca. 10 nt long RNA with a 20-30 nt long DNA extension
The polymerase switch occurs both on the leading and lagging strands. RNA-DNA primers synthesized by pol α are elongated to long nascent products on the leading strand template and to ca. 300 bp Okazaki-fragments on lagging strand template by pol δ. The loading of pol δ requires the presence of a primer recognition complex that consists of proliferating cell nuclear antigen (PCNA), replication factor C (RFC) and ATP. PCNA is a processivity factor for pols δ and ε and forms "a sliding clamp" around the DNA. PCNA cannot assemble on covalently closed circular DNA itself, but needs a "clamp loader" – RFC. The primer recognition complex competes with pol α on binding to the primer end and replaces pol α in an ATP dependent manner. This reaction is mediated by RPA that associates with RFC and releases pol α.
The maturation of Okazaki fragments into continuous DNA is catalysed in a series of reactions, where RNaseH first cleaves the RNA moiety endonucleolytically except for the last 3' ribonucleotide that is removed by the exonuclease activity of Fen-1, also known as maturation factor 1 (MF1). The gap is filled by pol δ and finally the fragments are covalently linked by ligase I. Pol ε is dispensable in SV40 DNA replication, but is able to partially replace pol δ. Finally, topoisomerases I and II are needed for removing the positive supercoiling in front of the replication fork and the negative supercoiling behind the fork as well as for the decatenation of daughter chromosomes.

A model for the structure of the SV40 replication fork

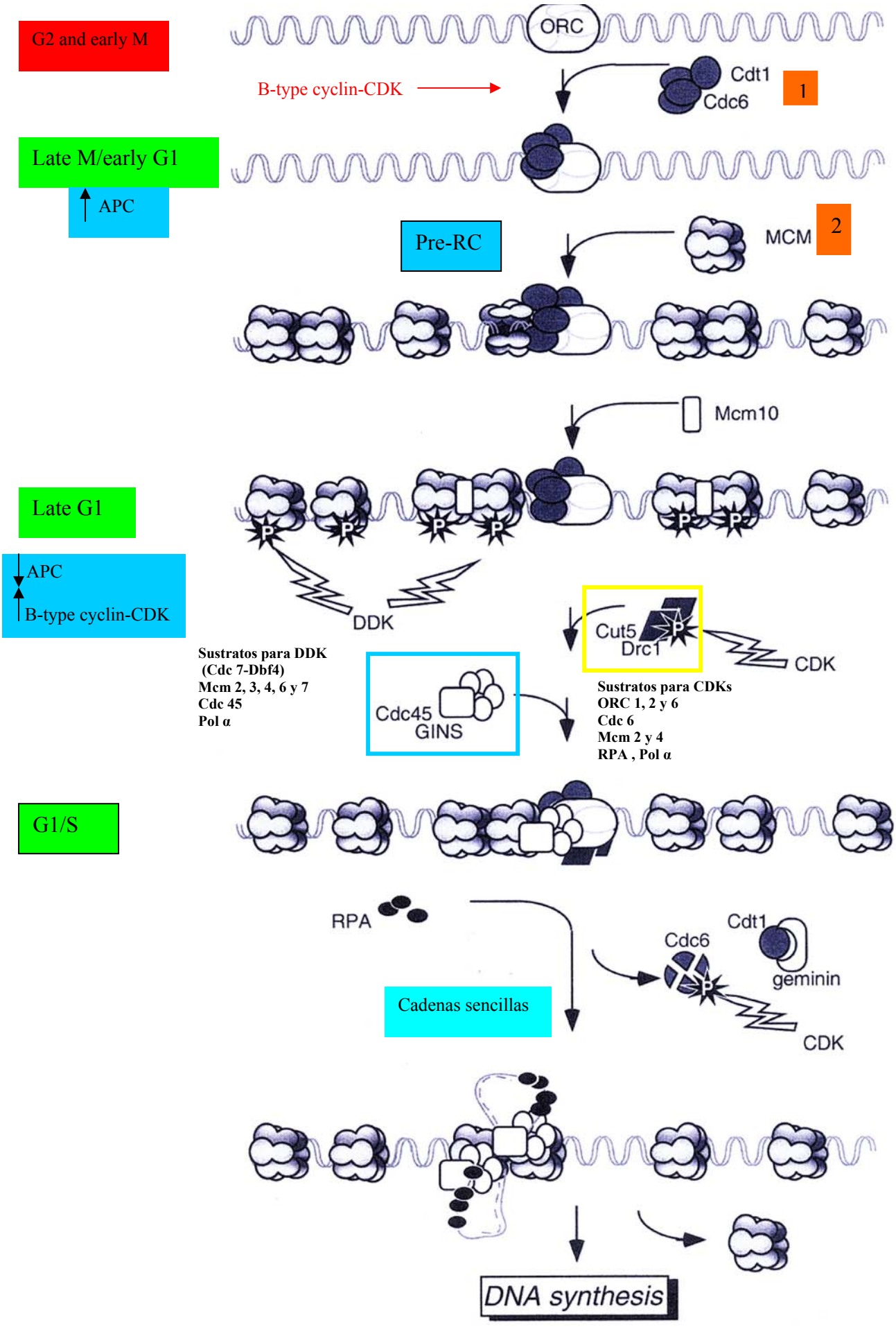


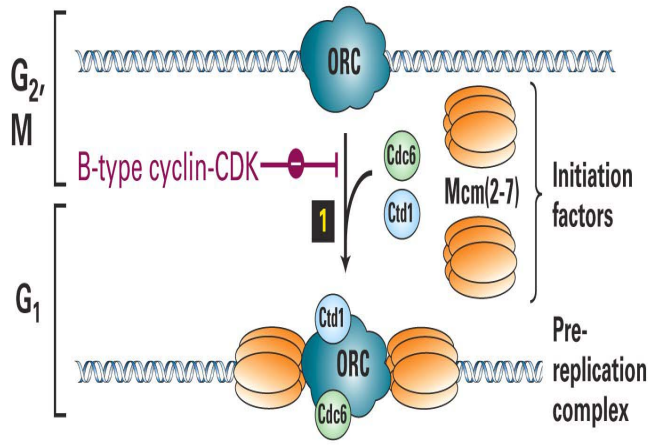
Polymerase switching and maturation of Okazaki fragments on a lagging-strand DNA template

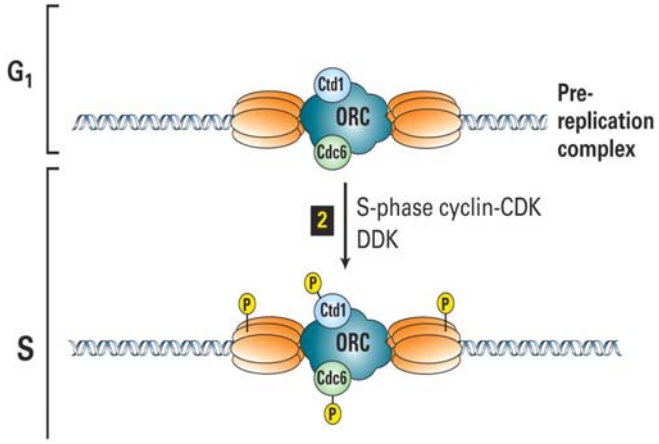


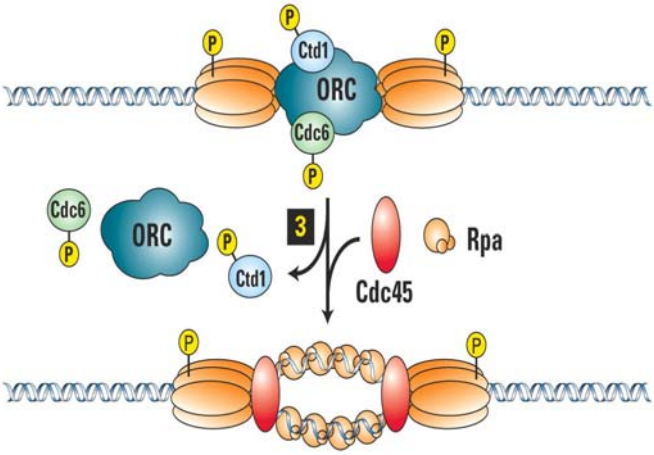


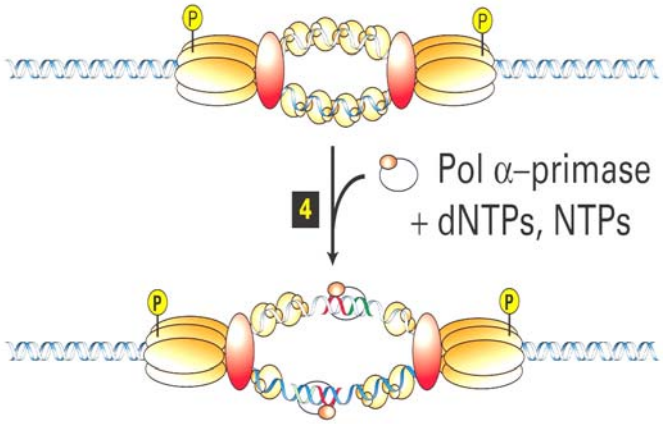
Two mechanism for the removal of RNA primers

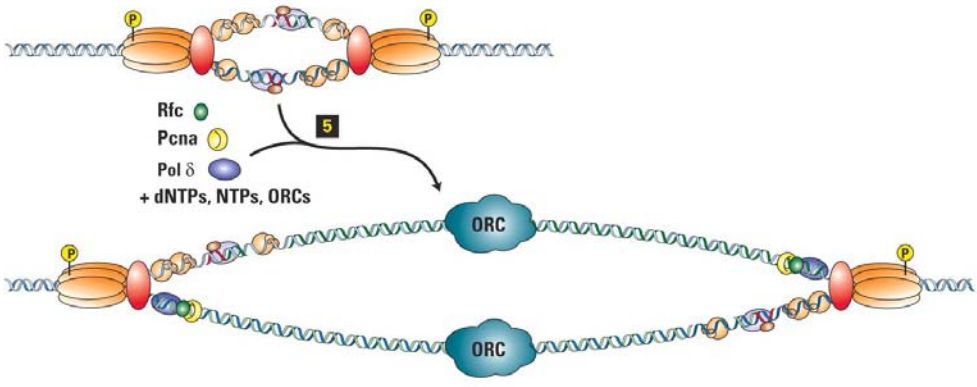

G2 and early M
ORC
B-type cyclin-CDK
Cdt1
Cdc6
1
Late M/early G1
APC
Pre-RC
MCM
2
Mcm10
Late G1
APC
B-type cyclin-CDK
DDK
Cut5
Drc1
CDK
Sustratos para DDK
(Cdc 7-Dbf4)
Mcm 2, 3, 4, 6 y 7
Cdc 45
Pol α
Cdc45
GINS
Sustratos para CDKs
ORC 1, 2 y 6
Cdc 6
Mcm 2 y 4
RPA , Pol α
G1/S
RPA
Cadenas sencillas
Cdt1
Cdc6
geminin
CDK
DNA synthesis

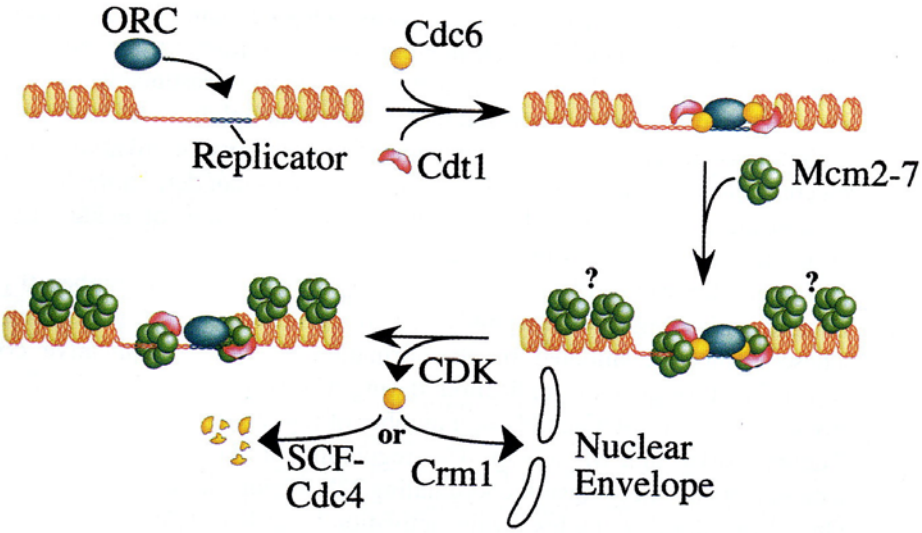

G2/M
ORC
B-type cyclin-CDK
Cdc6
Ctd1
1
Mcm(2-7)
Initiation factors
G1
Ctd1
ORC
Cdc6
Pre-replication complex

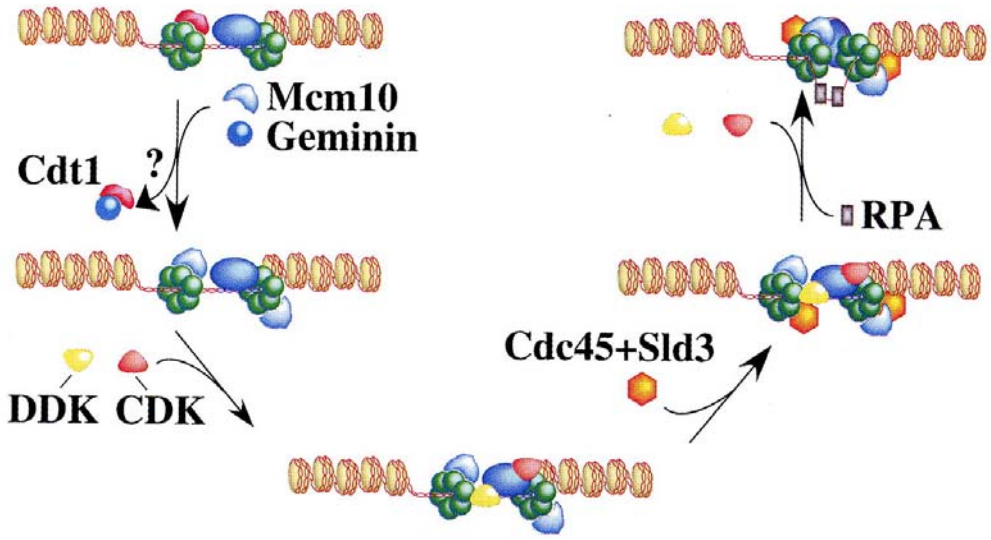

G1
Ctd1
ORC
Cdc6
Pre-replication complex
2
S-phase cyclin-CDK
DDK
S
P
Ctd1
ORC
Cdc6


P
Ctd1
ORC
Cdc6
Cdc6
ORC
3
Rpa
Cdc45


4
Pol α–primase
+ dNTPs, NTPs


Rfc
Pcna
Pol δ
+ dNTPs, NTPs, ORCs
5
ORC
ORC





Sustratos para DDK
(Cdc 7-Dbf4)
Mcm 2, 3, 4, 6 y 7
Cdc 45
Pol α

Sustratos para CDKs
ORC 1, 2 y 6
Cdc 6
Mcm 2 y 4
RPA , Pol α
Sld2(Drc1) → Dpb11(Cut5)

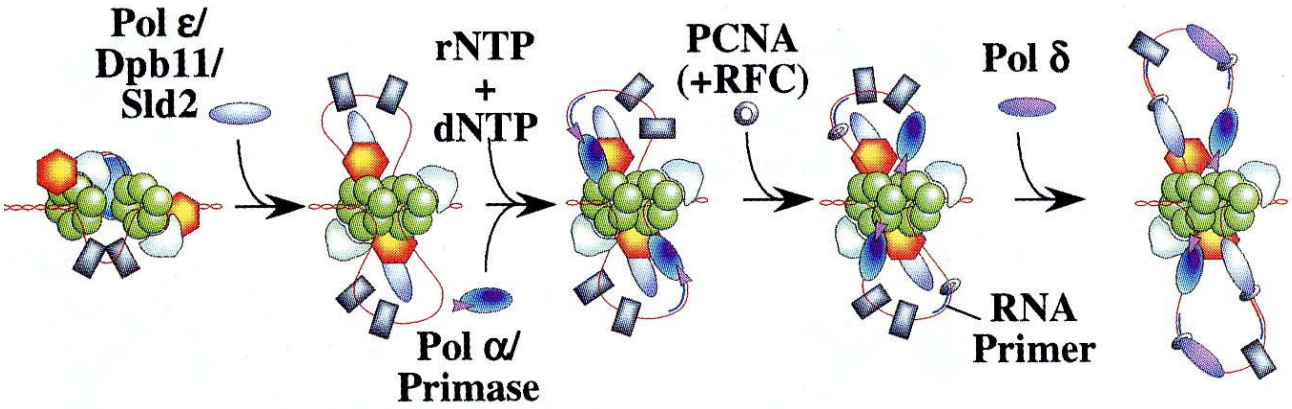
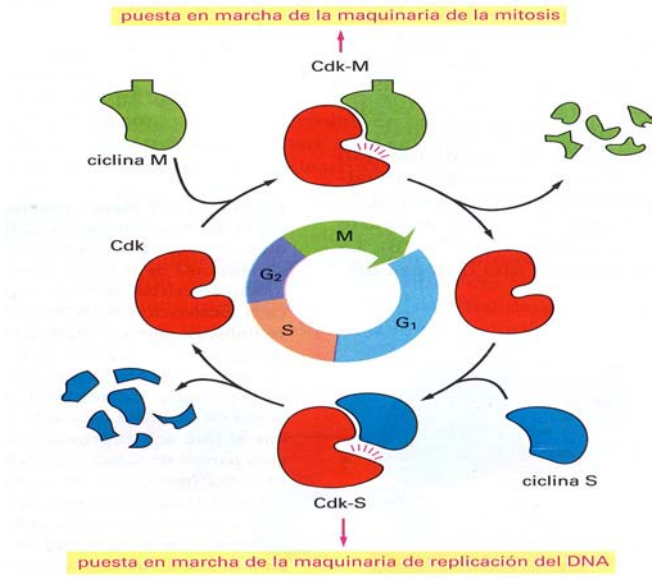
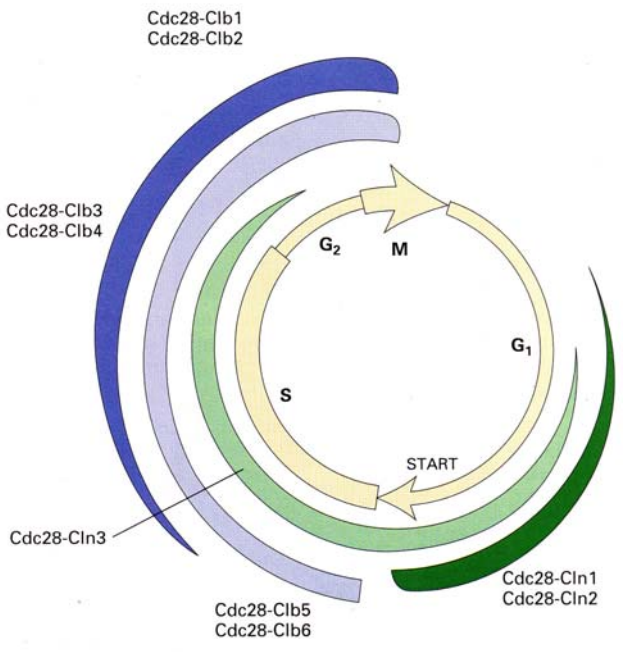


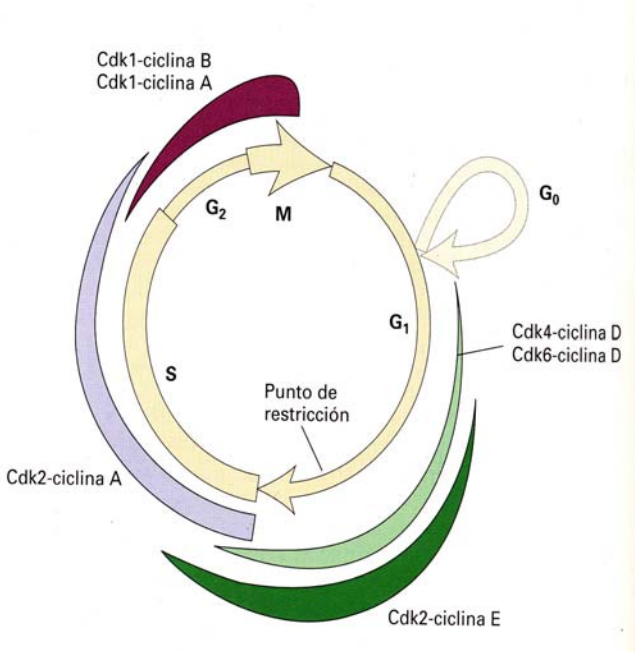
TABLA 17–1 Principales ciclinas y Cdk presentes en los vertebrados y en la levadura de gemación

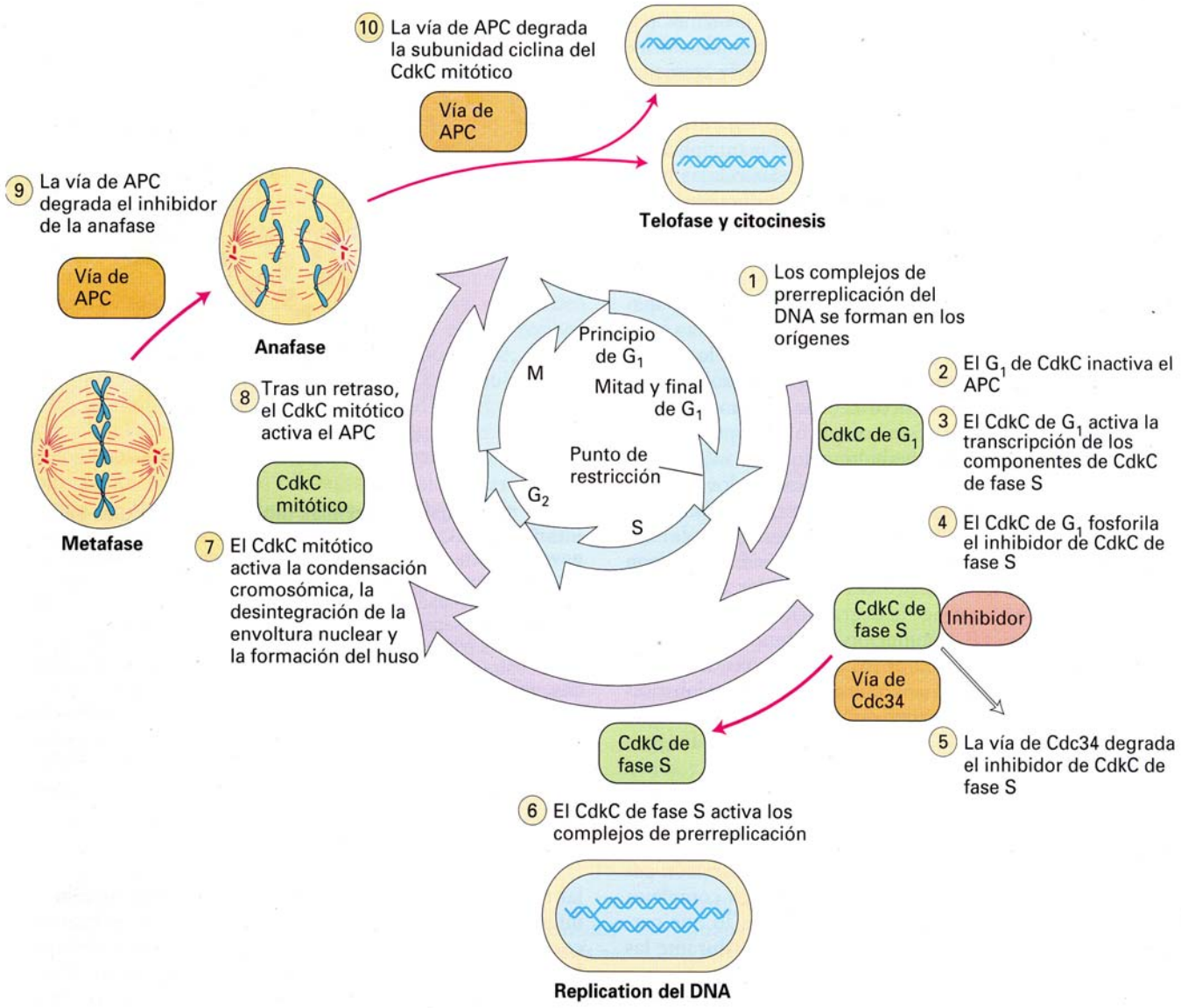
COMPLEJO CDK-CICLINA	VERTEBRADOS CICLINA	VERTEBRADOS CDK ASOCIADA	LEVADURA DE GEMACIÓN CICLINA	LEVADURA DE GEMACIÓN CDK ASOCIADA
Cdk-G_1	ciclina D*	Cdk4, Cdk6	Cln3	Cdk1**
Cdk-G_1/S	ciclina E	Cdk2	Cln1, 2	Cdk1
Cdk-S	ciclina A	Cdk2	Clb5, 6	Cdk1
Cdk-M	ciclina B	Cdk1**	Clb1, 2, 3, 4	Cdk1

* Hay tres ciclinas D en mamíferos (ciclinas D1, D2 y D3).
** El nombre original de la Cdk1 fue Cdc2 tanto en vertebrados como en la levadura de fisión, y Cdc28 en la levadura de gemación.








10 La vía de APC degrada la subunidad ciclina del CdkC mitótico
Vía de APC
9 La vía de APC degrada el inhibidor de la anafase
Vía de APC
Telofase y citocinesis
Anafase
Metafase
1 Los complejos de prerreplicación del DNA se forman en los orígenes
2 El G_1 de CdkC inactiva el APC
3 El CdkC de G_1 activa la transcripción de los componentes de CdkC de fase S
4 El CdkC de G_1 fosforila el inhibidor de CdkC de fase S
CdkC de G_1
Principio de G_1
Mitad y final de G_1
Punto de restricción
S
G_2
M
8 Tras un retraso, el CdkC mitótico activa el APC
CdkC mitótico
7 El CdkC mitótico activa la condensación cromosómica, la desintegración de la envoltura nuclear y la formación del huso
CdkC de fase S
Inhibidor
Vía de Cdc34
5 La vía de Cdc34 degrada el inhibidor de CdkC de fase S
CdkC de fase S
6 El CdkC de fase S activa los complejos de prerreplicación
Replication del DNA

Comparison of cellular/viral replication proteins in the three kingdoms of life

Replication protein	Eubacteria[1]	Eukaryotes	SV40[2]	HSV1[3]	Archaea
Origin recognition & unwinding[4]	DnaA*	ORC*	T-ag*	UL9	Cdc6?[5]*
Licensing/helicase loading	DnaC*	Cdc6/Cdc18	T-ag	UL8[7]	Cdc6
Licensing, helicase[6]	DnaB	Mcm2-3-4-5-6-7*	T-ag	UL5[7]	Mcm
Primase	DnaG	Pol α-primase	Pol α-primase	UL52[7]	primase
ssDNA binding & stabilization	SSB	RPA	RPA	UL29	RPA
Processivity clamp[8]	β -subunit of Pol III	PCNA	PCNA	UL42	PCNA
Clamp loader[9]	γ -complex of Pol III*	RFC*	RFC	-	RFC
DNA polymerase	PolC, PolA(Pol III, Pol I)	PolB(Pol α, Pol δ, Pol ε)	PolB(Pol α, Pol δ)	UL30	PolB, PolD
Maturation nuclease	RnhA	Fen1	Fen1		Fen1
Ligase	Ligase	Ligase I	Ligase I		LigaseI

[1]Nomenclature and data based on Escherichia coli.

[2]SV40 (Simian virus 40) uses the replication machinery of the host with a single viral encoded protein, T-ag.

[3]HSV1 (Herpes simplex virus 1) represents viruses that encode most of the proteins needed in replication.

[4]DnaA accumulates onto the origin as a homomultimer, ORC as a heterohexamer, T-ag as a double homohexamer, and UL9 as a dimer.

[5]Cdc6/Cdc18 is ATP-dependent loader of Mcm proteins. It shows sequence homology to Orc proteins. In Archaea it possibly acts also as a origin recognition protein.

[6]DnaB and T-ag are essentially replicative helicases with mono and double homohexameric structure. Mcms are heteromultimers in eukaryotes, but homomultimers at least in some Archaea. They are required for loading of pols and a role as a replicative helicase has been proposed.

[7]HSV1 UL8, UL5 and UL52 protein form a heterotrimeric complex with helicase and primase activities.

[8]β -subunit dimer and PCNA trimer, although non-homologous in sequence, form similar ring-structure around DNA and function as a processivity factor for pol. UL42 is unrelated, and does not form ring-structure, but has intrinsic affinity on dsDNA.

[10]γ −complex is a heteropentamer of (γ , δ, δ', χ, ψ). RFC is a heteropentamer of one large and four small subunits. In eukaryotes small subunits are distinct, but in Archaea all four are identical.

*** These proteins belong to the AAA^{+}-ATPase superfamily.**

How important is DNA repair?

- DNA is the only biomolecule that is specifically repaired. All others are replaced.

- > 100 genes participate in various aspects of DNA repair, even in organism with very small genomes.

- Cancer is caused by mutations. In most cases, "genetic instability" (elevated mutation rate) is required to permit accumulation of sufficient mutations to generate cancer during a human lifetime. DNA repair mechanisms promote genomic stability and prevent cancer. Many, perhaps most, cancers are at least partially attributable to defects in DNA repair.

- Replication errors. Another major source of potential alterations in DNA is the generation of mismatches or small insertions or deletions during DNA replication. Although DNA polymerases are moderately accurate, and most of their mistakes are immediately corrected by polymerase-associated proofreading exonucleases, nevertheless the replication machinery is not perfect. As we shall see later, efficient repair mechanisms correct most of these problems.

- Note that many types of DNA damage are generated spontaneously, in some cases at very high frequency. Thus defects in DNA repair systems are likely to cause problems even in the absence of additional damage caused by environmental factors.

TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411**:366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509**:49.

Mutaciones y reparación del DNA

Mutaciones

Alteraciones en la estructura del DNA que dan lugar a variaciones permanentes en la información genética codificada por la molécula.

Clasificación

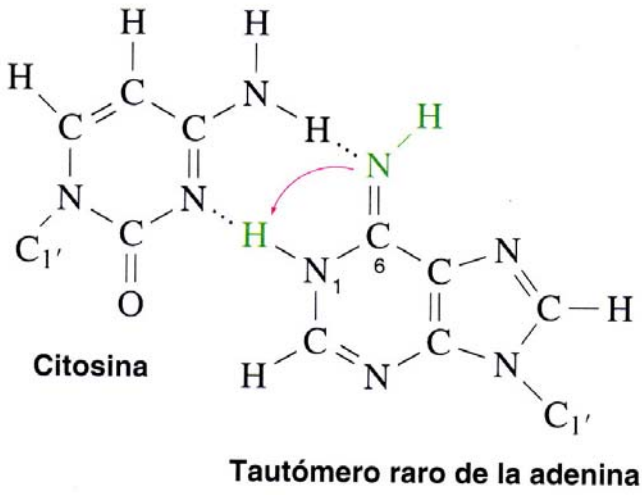
1) **Sustitución de un par de bases por otro**. Son las mas frecuentes y son de dos tipos:

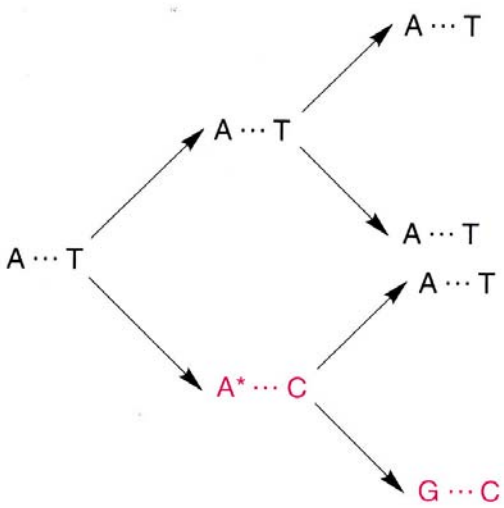
a) Transiciones. Sustitución de una purina por otra purina o de una pirimidina por otra pirimidina
b) Transversiones. Sustitución de una purina por una pirimidina o viceversa.

2) Eliminación o inserción de un par de bases

Mutaciones espontáneas:

1- Tautomería. Debido a las formas tautoméricas transitorias de las bases se pueden dar apareamientos de bases atípicos. Ejemplo:

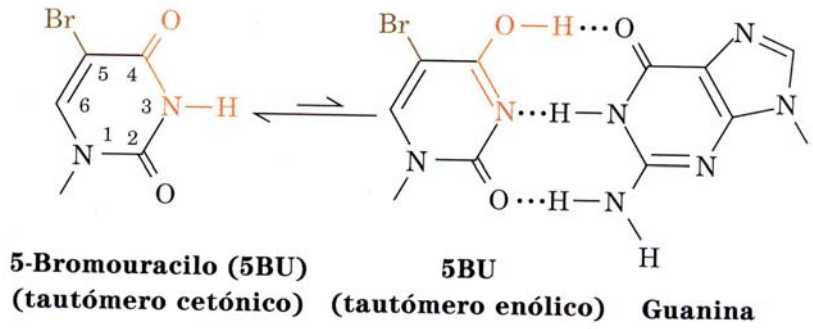




El tautómero menos frecuente de la adenina se empareja con citosina en lugar de hacerlo con timina. El tautómero se forma por el salto de un protón desde el grupo 6 amino al N-1

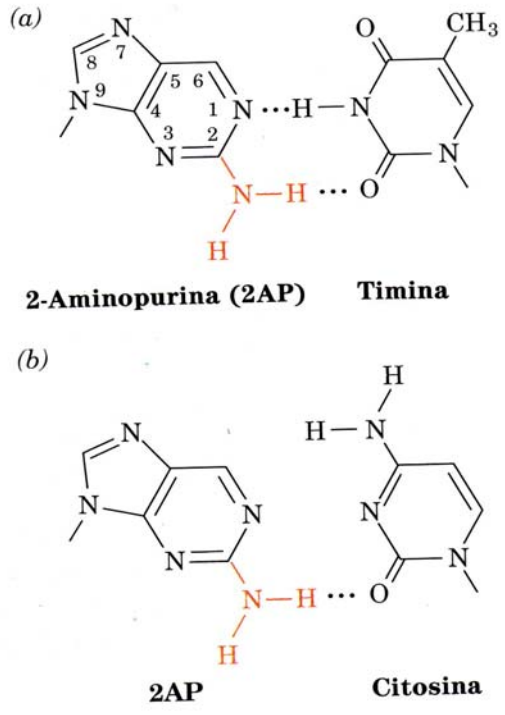
El emparejamiento del tautómero menos frecuente de la adenina A^* con citosina, origina el par de bases G . C en la generación siguiente

2- Análogos de bases que se incorporan al DNA: El 5-bromouracilo y la 2-amino purina



La forma cetónica del 5-bromouracilo (izquierda) es su tautómero más frecuente. Sin embargo, a menudo asume la forma enólica (derecha), la cual aparea con la guanina.

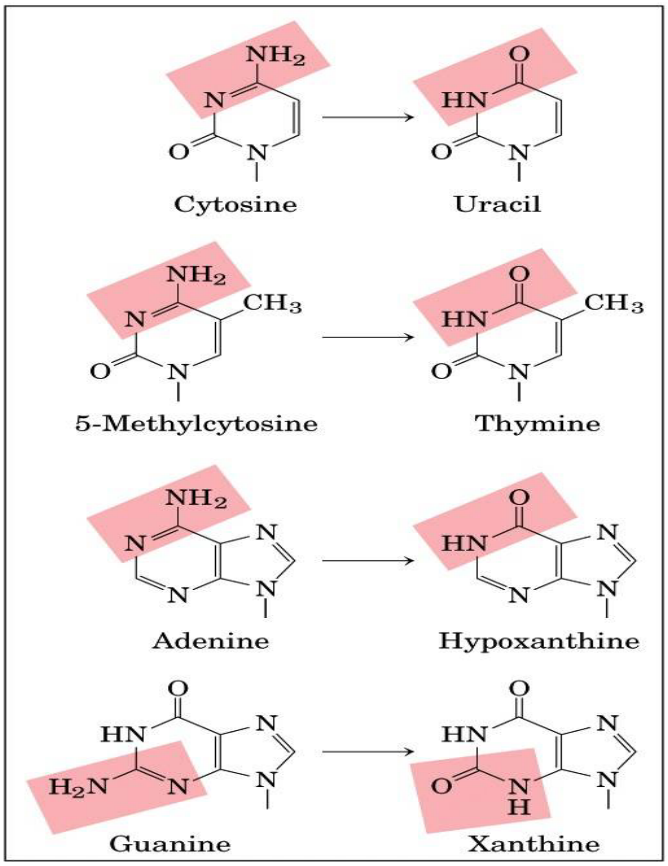
Transiciones A . T → G . C y G . C → A .T



El análogo de adenina 2-aminopurina se aparea normalmente con (a) timina, pero en ocasiones lo hace también con citosina (b).

Transiciones A . T → G . C y G .C → A . T

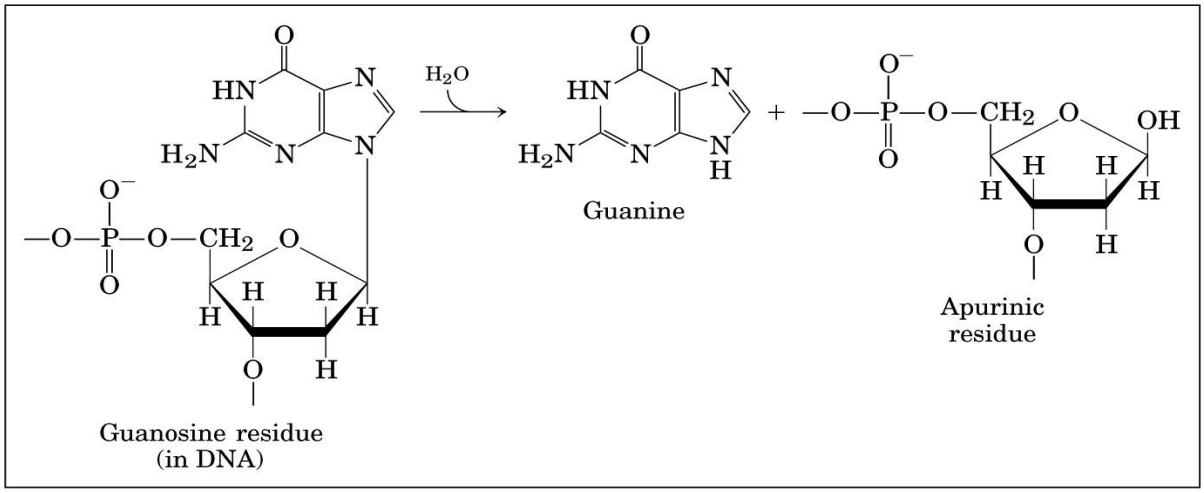
3- Transformaciones no enzimáticas del DNA: desaminaciones y pérdidas de bases
Modificaciónes espontáneas de las bases del DNA, con el consecuente cambio de bases



Deamination
(a)

Frecuencia de desaminación de las bases

Citosina $1/10^7$/24h → 100 mut/día /cel.
Adenina 1 mut/día/cel
Guanina " " " "



Depurination
(b)

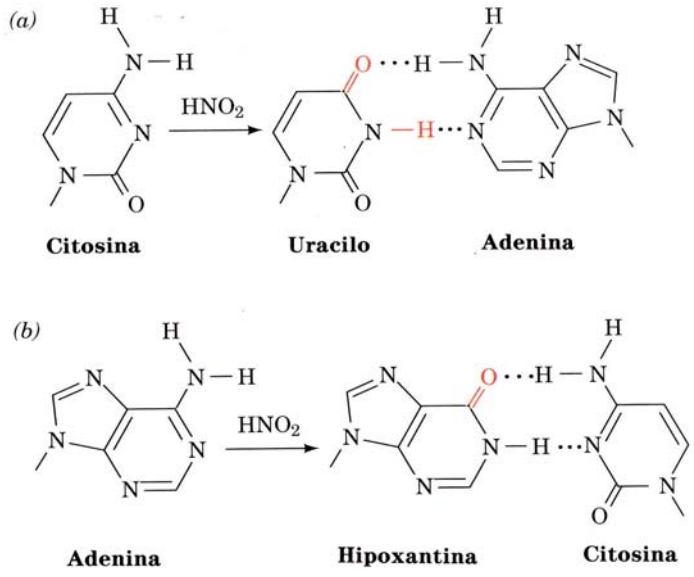
(Hidrólisis del enlace N-β-glucosídico)
The glycosyl bond linking DNA bases with deoxyribose is labile under physiological conditions. Within a typical mammalian cell, several thousand purines and several hundred pyrimidines are spontaneously lost per haploid genome per day (purines $1/10^5$/24h → 10.000/cell). Loss of a purine or pyrimidine base creates an apurinic/apyrimidinic (AP) site (also called an abasic site).

4- Agentes químicos que provocan algunas lesiones en el DNA

Muchos agentes químicos ambientales, incluyendo los naturales (frecuentes en los alimentos que consumimos), pueden modificar también las bases del DNA. Existen dos clases principales **1) agentes desaminantes**, en particular el ácido nitroso **HNO_2**, o compuestos que pueden dar lugar a la formación metabólica del ácido nitroso o nitritos, y **2) agentes alquilantes**, por adición de grupos metilos u otros grupos alquílicos. Además, con frecuencia el metabolismo normal lleva a la alquilación. En este sentido se ha mostrado que la S-adenosilmetionina, el dador biológico normal de grupos metilo, reacciona accidentalmente con el DNA para producir bases alquiladas como la 3-metiladenina a una velocidad de cien de veces por genoma haploide de mamífero. La alquilación ocurre sobre las posiciones más nucleofílicas.

1) agentes desaminantes

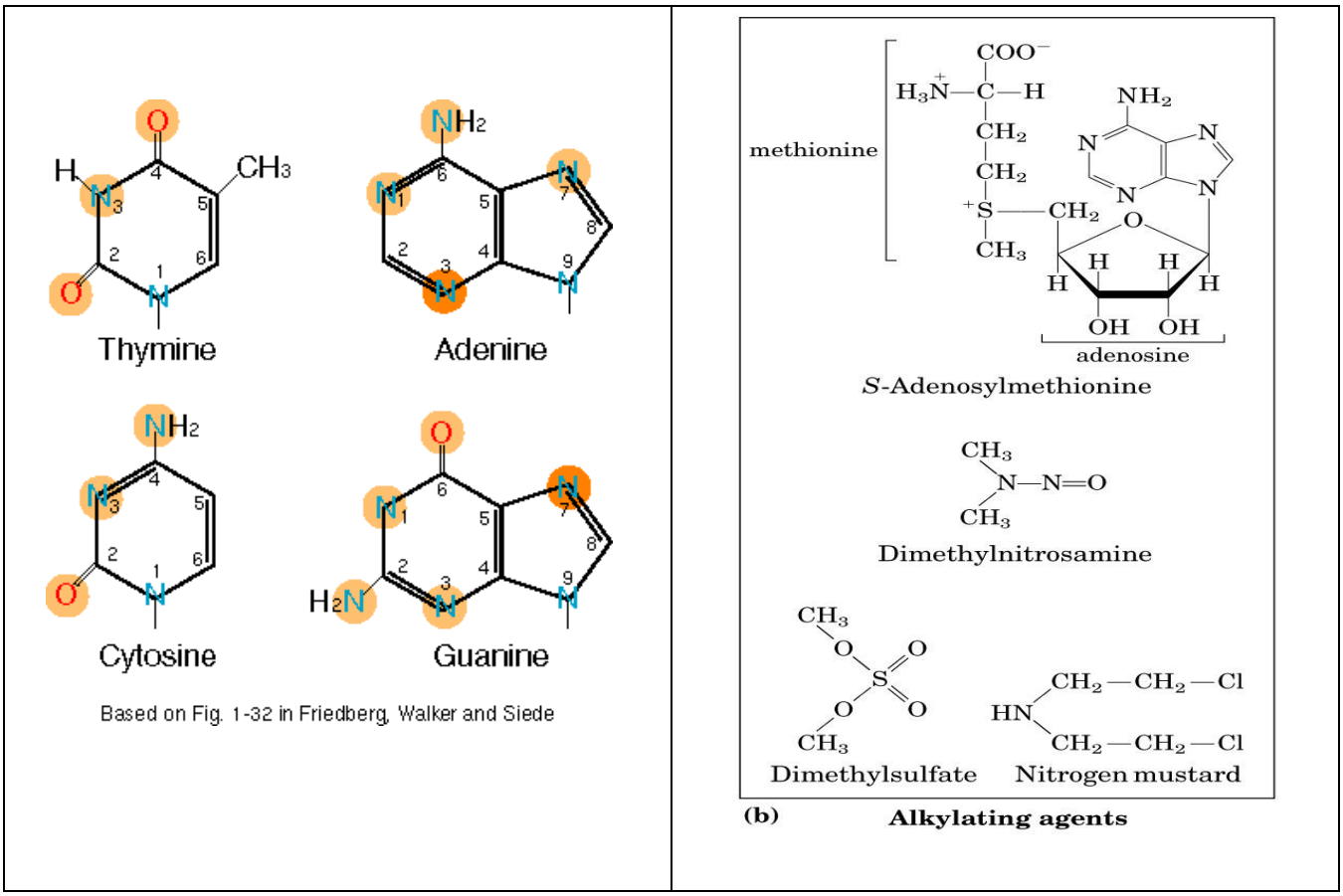
Precursores del ácido nitroso		
$NaNO_2$ Nitrito sódico	**$NaNO_3$** Nitrato sódico	**R^1** **N-N=O** **R^2** Nitrosamina



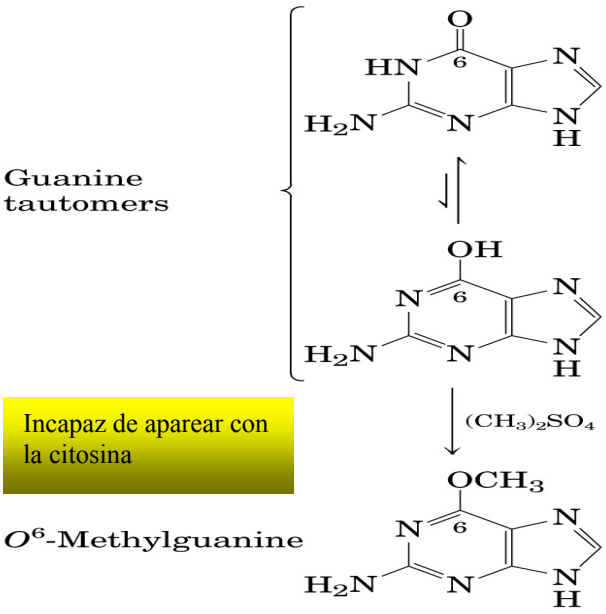
La reacción con el ácido nitroso convierte (a) la citosina en uracilo, que aparea con la adenina: y (b) la adenina en hipoxantina, que aparea con la citosina.

Estas reacciones provocan transiciones tanto:
A . T → G . C como G . C → A . T

2) agentes alquilantes



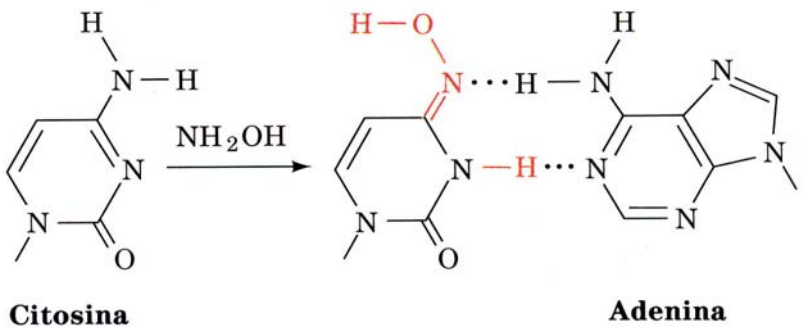
La utilización de compuestos alquilantes provoca frecuentemente la aparición de **transversiones**. **La alquilación de la posición N7 de un nucleótido de purina provoca su posterior despurinación.** El hueco que queda en la secuencia es rellenado por un sistema enzimático de reparación que corrige errores. Las transversiones surgen cuando la purina perdida es sustituida por una pirimidina.



3- Procesos oxidativos. Agua oxigenada, iones hidroxilos y superóxidos producen oxidación de la desoxirribosa, de las bases e incluso hasta la rotura de las cadenas.

4- Agentes cancerígenos

a) La hidroxilámina es mutágeno altamente específico reacciona casi exclusivamente con la citosina para dar un producto que forma un par de bases con la adenina



La reacción de la hidroxilamina convierte a la citosina en un derivado que se aparea con la adenina.
Transiciones G . C → A . T

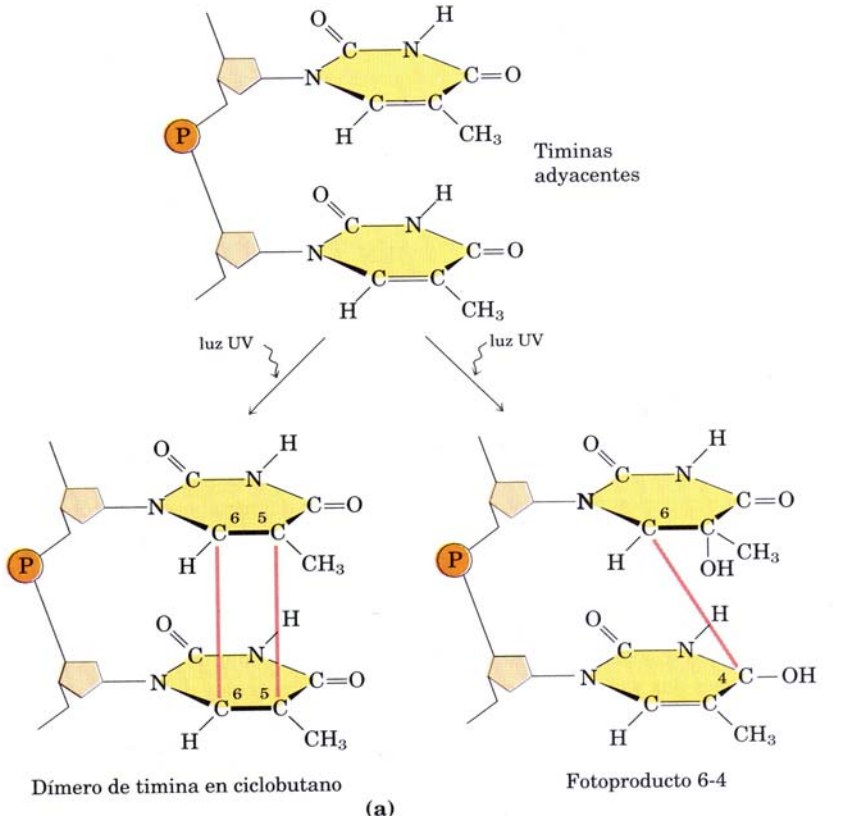
b) Una clase diferente de mutaciones se produce por efecto de **moléculas aromáticas planas como las acridinas**, que se intercalan entre los planos de los pares de bases produciendo la inserción o eliminación de uno o más pares de bases. La distancia entre dos pares de bases consecutivos se duplica como consecuencia del intercalamiento de una de estas moléculas entre ellas. La replicación de este DNA distorcionado puede dar lugar a la inserción o delección ocasional de uno o más nucleótidos en el polinucleótido de nueva síntesis.

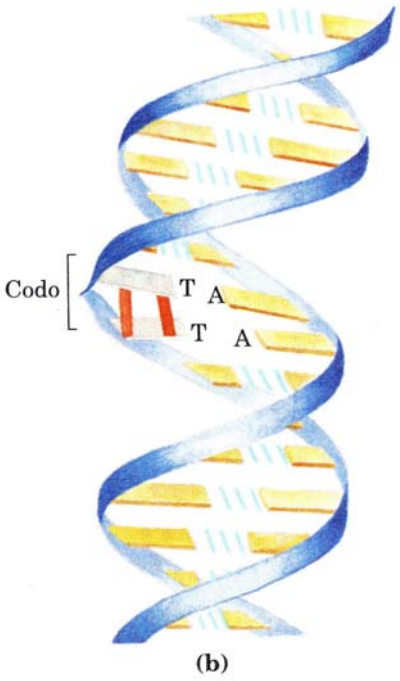
c) **Enlaces cruzados entre cadenas.** Por la unión a bases en ambas cadenas de agentes alquilantes bifuncionales tales como los psoralenos pueden enlazarse ambas cadenas. Estos enlaces cruzados pueden también generarse por UV y radiación ionizante.

d) **Enlaces cruzados entre proteínas-DNA**. Los agentes alquilantes bifuncionales y la radiación pueden también formar enlaces cruzados entre las cadenas de DNA (generalmente vía las bases) y las moléculas de proteína.

Lesiones causadas por la radiación.

La luz ultravioleta es absorbida por las bases de los ácidos nucleicos, y el influjo de energía resultante puede inducir cambios químicos.
-Dímeros de timina y Fotoproducto 6-4 (principal causante de las mutaciones inducídas por la luz UV.)
-Rotura de cadenas. Ionizing radiation can generate both single strand nicks and double-strand breaks: Both types of break must be repaired.





Formación de dímeros de pirimidina inducída por la luz UV. (a) Un tipo de reacción (a la izquierda) da como resultado la formación de un anillo de ciclobutano en el que están implicados los C-5 y C-6 de dos residuos adyacentes de pirimidina. Una reacción alternativa (a la derecha) da como resultado un enlace entre el C-6 de una pirimidina y el C-4 del vecino; (b) la formación de un dímero de pirimidina de ciclobutano introduce un codo en el DNA.

Las células tienen multiples sistemas de reparación

table 25–5

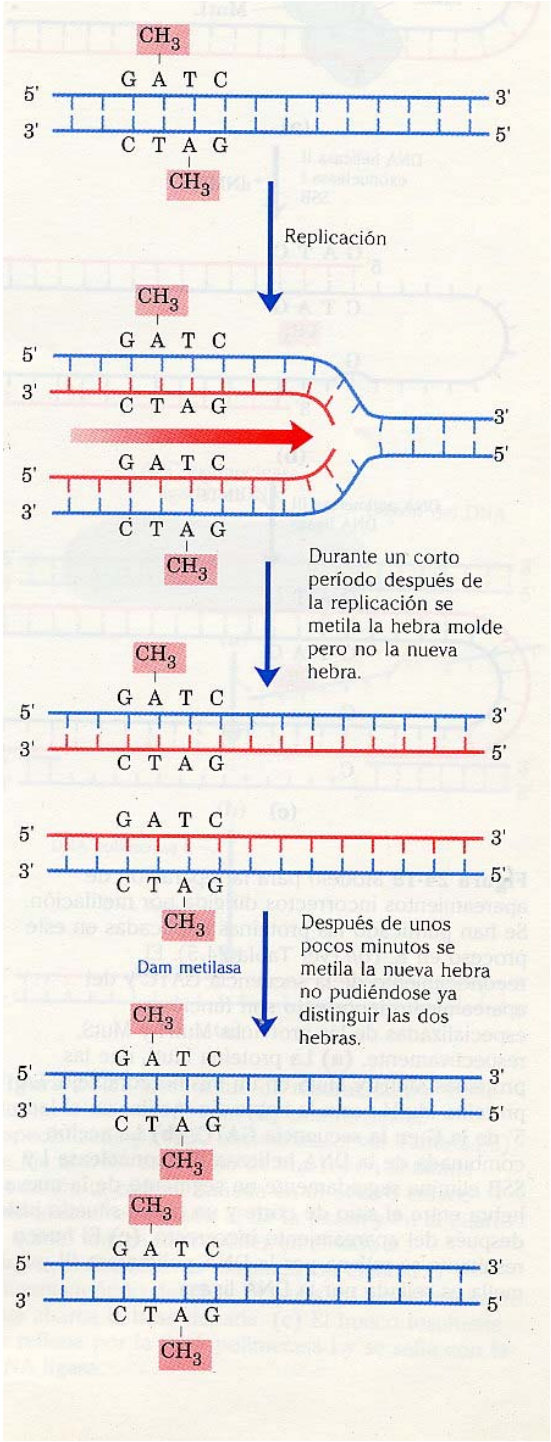
Types of DNA Repair Systems in *E. coli*

Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase	Mismatches
MutH, MutL, MutS proteins	
DNA helicase II	
SSB	
DNA polymerase III	
Exonuclease I	
Exonuclease VII	
RecJ nuclease	
Exonuclease X	
DNA ligase	
Base-excision repair	
DNA glycosylases	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; pyrimidine dimers in some other organisms
AP endonucleases	
DNA polymerase I	
DNA ligase	
Nucleotide-excision repair	
ABC excinuclease	DNA lesions that cause large structural changes (e.g., pyrimidine dimers)
DNA polymerase I	
DNA ligase	
Direct repair	
DNA photolyases	Pyrimidine dimers
O^6-Methylguanine-DNA methyltransferase	O^6-Methylguanine

Sistemas de reparación del DNA

a) Reparación de apareamientos incorrectos (marcaje de la cadena a reparar)

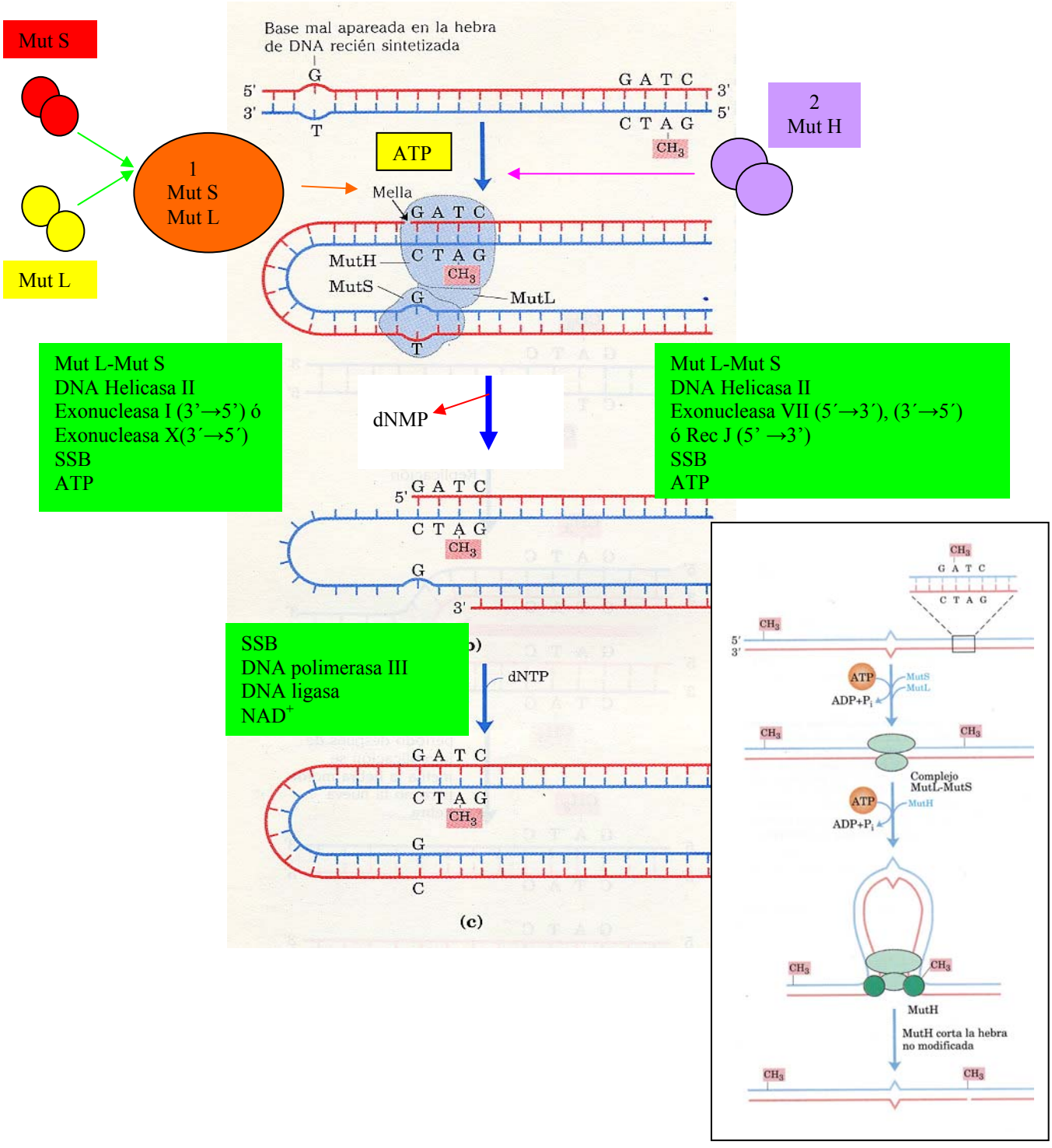
La metilación de las cadenas de DNA puede servir para distinguir las hebras parentales (moldes) de las cadenas recién sintetizadas en el DNA de E. coli. Esta función es crítica para la reparación de apareamientos incorrectos en el DNA. La metilación tiene lugar en la posición 6 de la adenina dentro de la secuencia 5´-GATC y el sistema que lo realiza es el Dam.



Reparación de apareamientos incorrectos dirigida por metilación

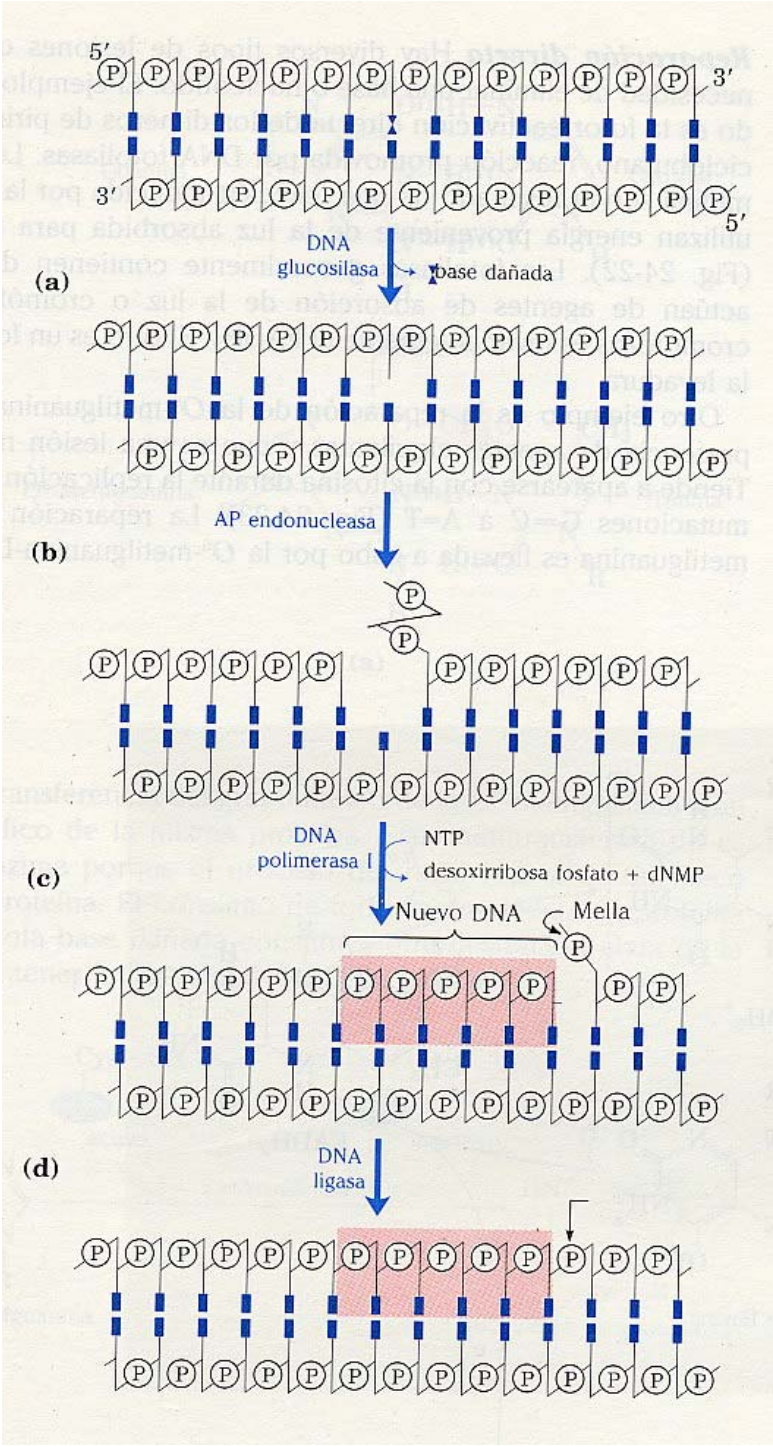
Las proteínas **Mut S, Mut H y Mut L** son los elementos claves dentro del sistema. Este sistema de reparación repara correctamente los errores a una distancia entre 1000 pb y 6000 pb de una secuencia GATC semimetilada.

Mut S reconoce los apareamientos incorrectos; 97 KD, dimérico ó multimérico (hexámero)
Mut L proteína de unión entre Mut S y Mut H; 70 KD, homodímero
Mut H reconoce la secuencia GATC; 25 KD, monomérico ó dimérico. Actúa como endonucleasa produciendo una mella con especificidad de secuencia, cortando la cadena no metilada por el lado 5' de la G en la secuencia GATC.



Las glucosilasas eliminan las bases alteradas

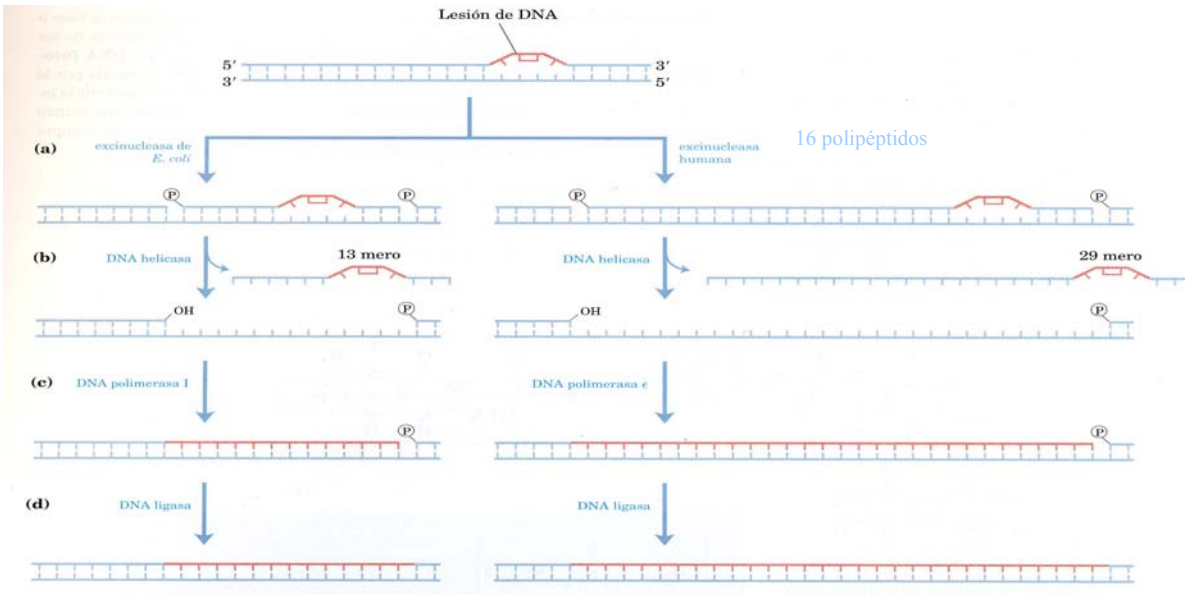
Las células poseen un conjunto de DNA glucosilasas, cada una de las cuales reconoce el enlace glucosídico correspondiente a un tipo específico de nucleótido alterado, dejando así el resto de desoxirribosa en el esqueleto de la doble hélice. Estos sitios apurínicos o apirimidínicos (AP) se crean también en condiciones fisiológicas, por hidrólisis espontánea del enlace glucosídico . En ambos casos, el resto de desoxirribosa es cortado en uno de los lados por una endonucleasa AP, la desoxirribosa y varios restos adyacentes son eliminados por la acción de la DNA polimerasa I o alguna otra exonucleasa celular, y el hueco rellenado por la polimerasa y sellado por la ligasa.

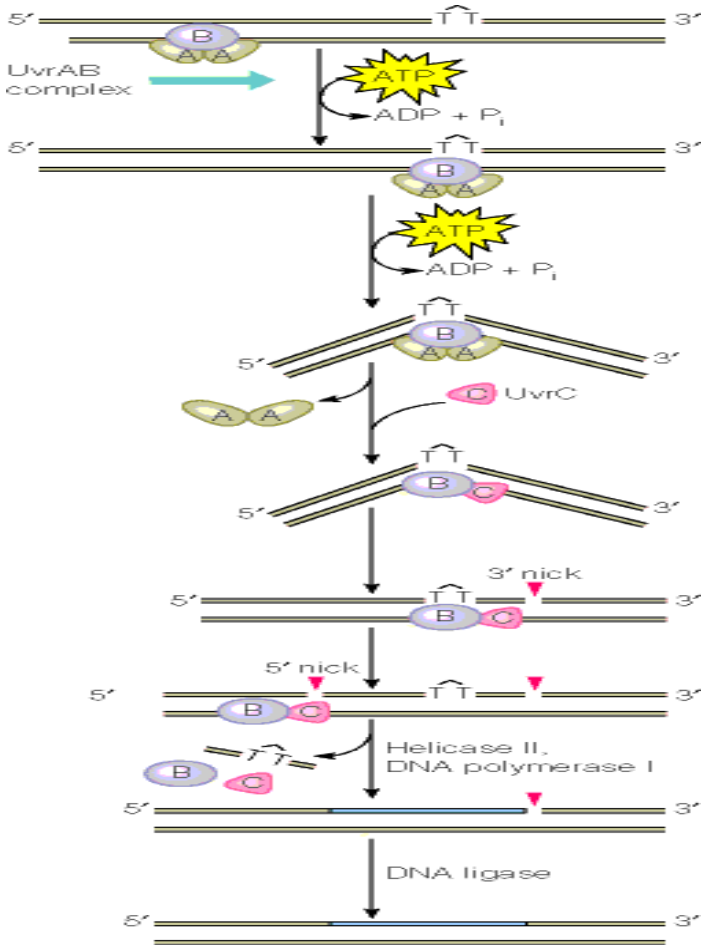


Reparación por escisión de nucleótidos

Sistema ABC escinucleasas: genes Uvr (A, B y C)

Un complejo de proteínas (A_2B) avanza a lo largo del DNA hasta que llega a un dímero de timina u otra zona dañada, en donde se detiene y obliga al DNA a doblarse. A continuación se disocia Uvr A, lo que permite la unión de UvrC a UvrB. El complejo BC corta a ambos lados del dímero (8 nucleótidos hacia el lado 5' y 4-5 hacia el lado 3'). La helicasa II (**Uvr D**), la polimerasa y la ligasa eliminan el dodecámero dañado y lo sustituyen por nuevo DNA.

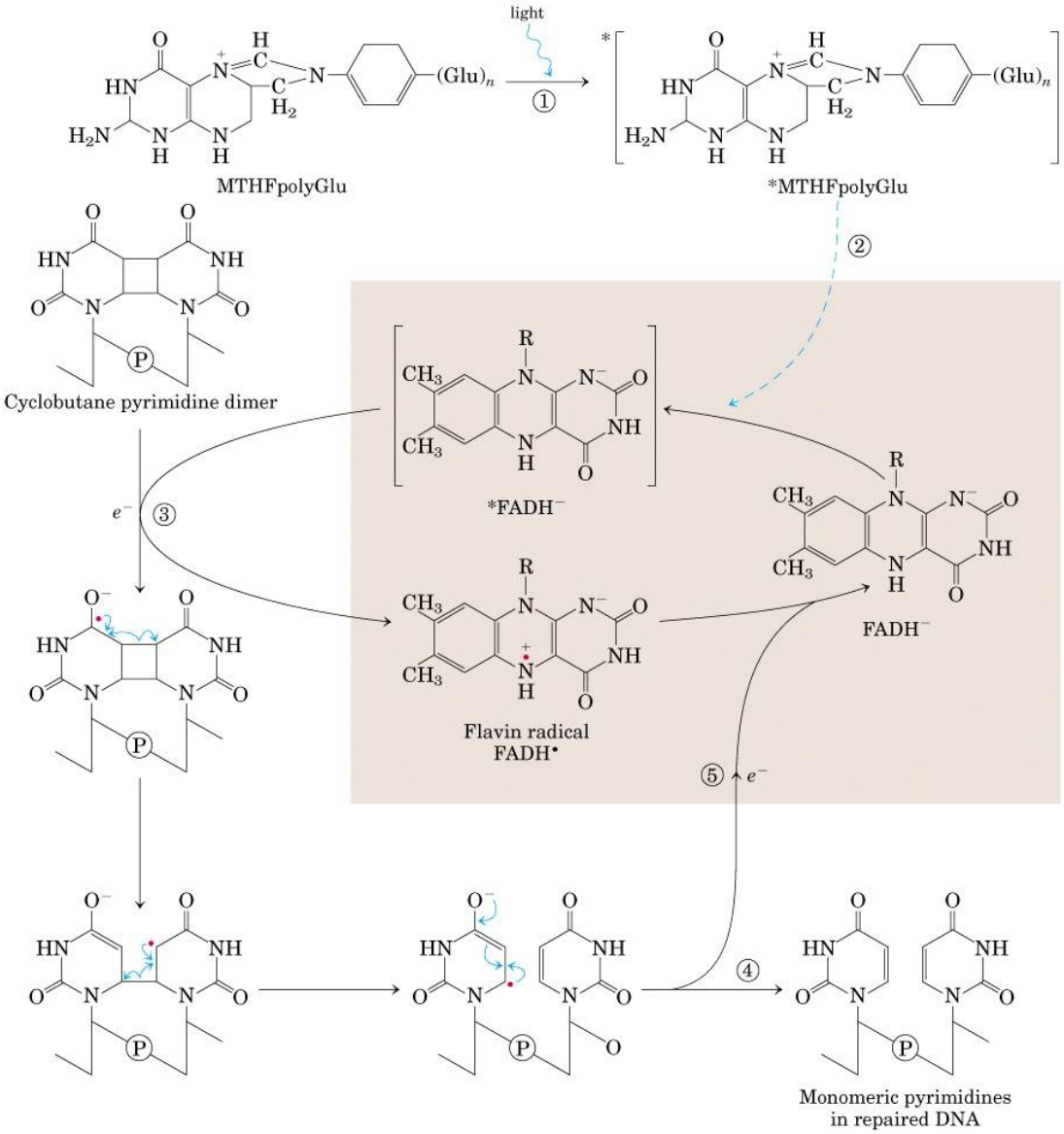




↑ Mecanismo de reparación por escisión de nucleótidos en E. coli y en humanos. El mecanismo general de reparación por escisión de nucleótidos es similar en todos los organismos. **(a)** Una escinucleasa se une al DNA en los lugares donde hay lesiones de importancia. **(b)** La escinucleasa corta la hebra dañada a ambos lados de la lesión y la helicasa elimina el segmento de DNA. **(c)** El hueco resultante es rellenado por la DNA polimerasa I y **(d)** la mella restante es sellada por la DNA ligasa.

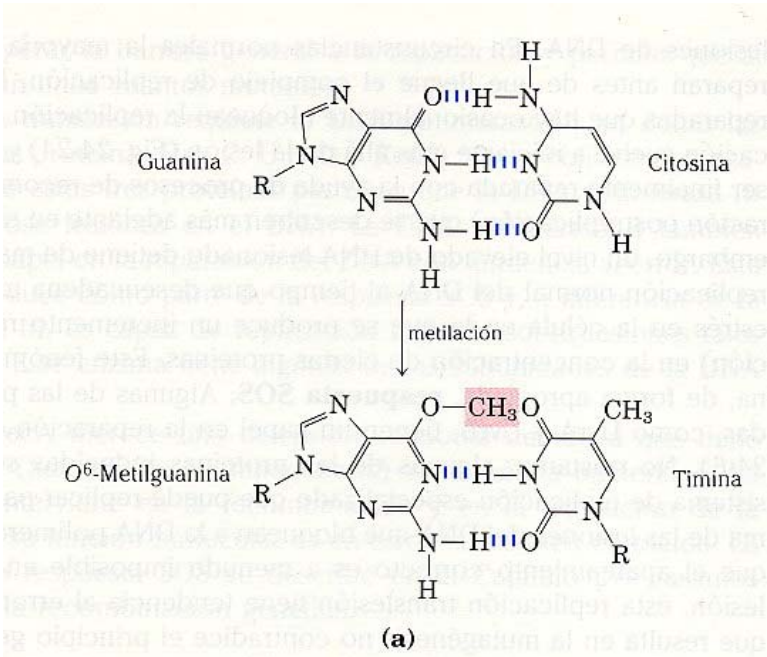
Reparación directa

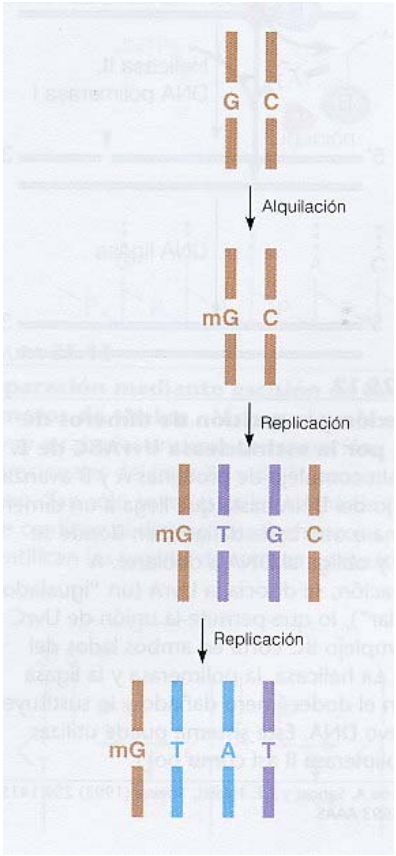
Hay diversos tipos de lesiones que se reparan sin necesidad de eliminar una base o un nucleótido. El ejemplo mejor caracterizado es la fotorreactivación directa de los dímeros de pirimidina que forman ciclobutano, reacciones catalizadas por las fotoliasas. Las fotoliasas generalmente contienen dos cromóforos que actúan de agentes de absorción de luz. Uno de ellos es invariablemente el $FADH_2$. El otro en E. coli y levaduras es el 5, 10-meteniltetrahidrofolato, ambos se complementan en términos de las longitudes de onda a las que absorben de manera eficiente. La mayor parte de la energía luminosa fotorreactivante se absorbe por el folato que la transfiere al $FADH_2$. La forma excitada resultante $FADH_2$* transfiere un electrón al dímero de pirimidina, este último con un radical libre es inestable degradándose para formar pirimidinas monoméricas y regenerándose nuevamente el $FADH_2$.

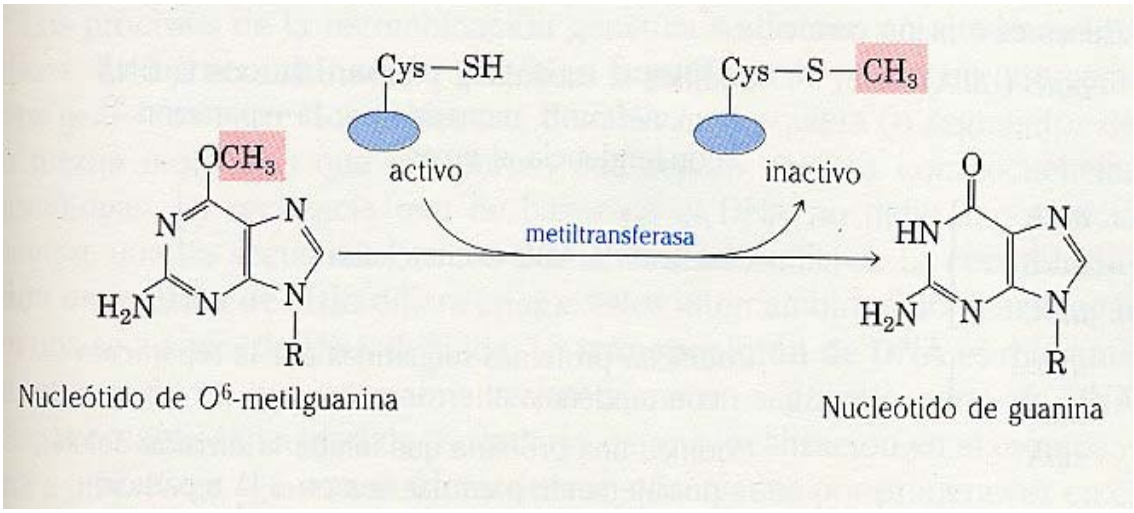


Alquiltransferasas desalquilan las bases alquiladas

Ejemplo: la O^6-metilguanina metiltransferasa, la cual transfiere directamente el grupo alquilo a uno de sus propios restos de cisteína inactivandose.



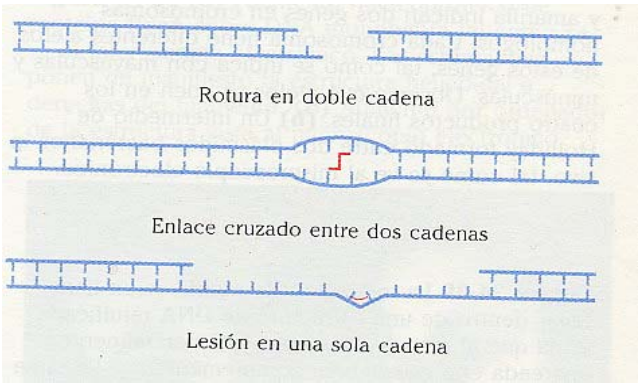


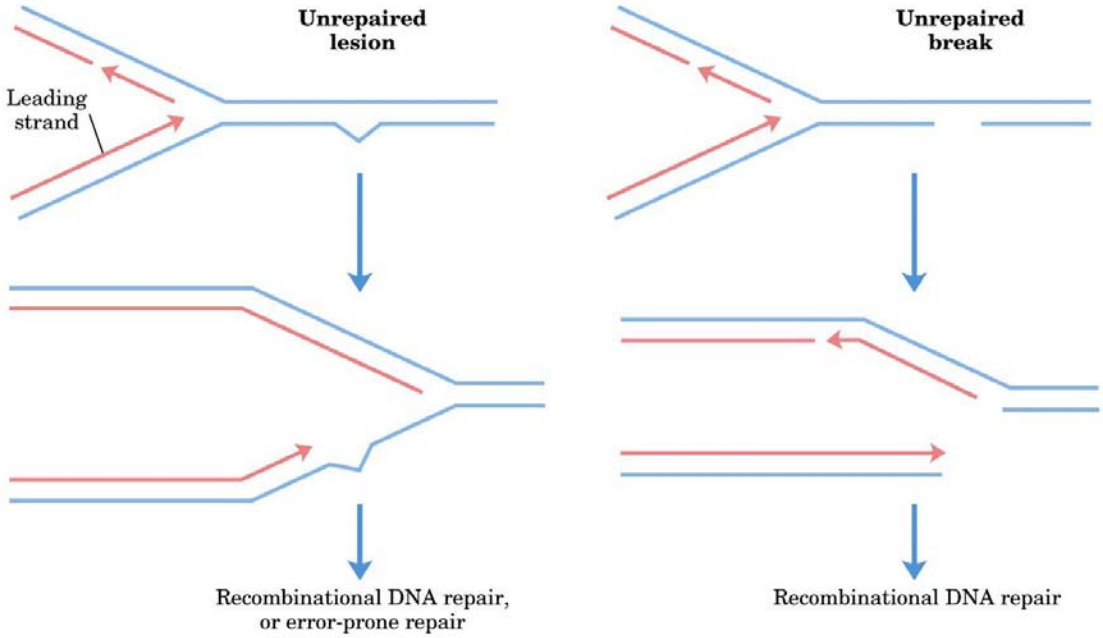


Sistemas de reparación durante la replicación

La interacción de las horquillas de replicación con lesiones del DNA induce la recombinación o la reparación propensa al error.

a) Reparación por recombinación
b) Inducción del sistema SOS



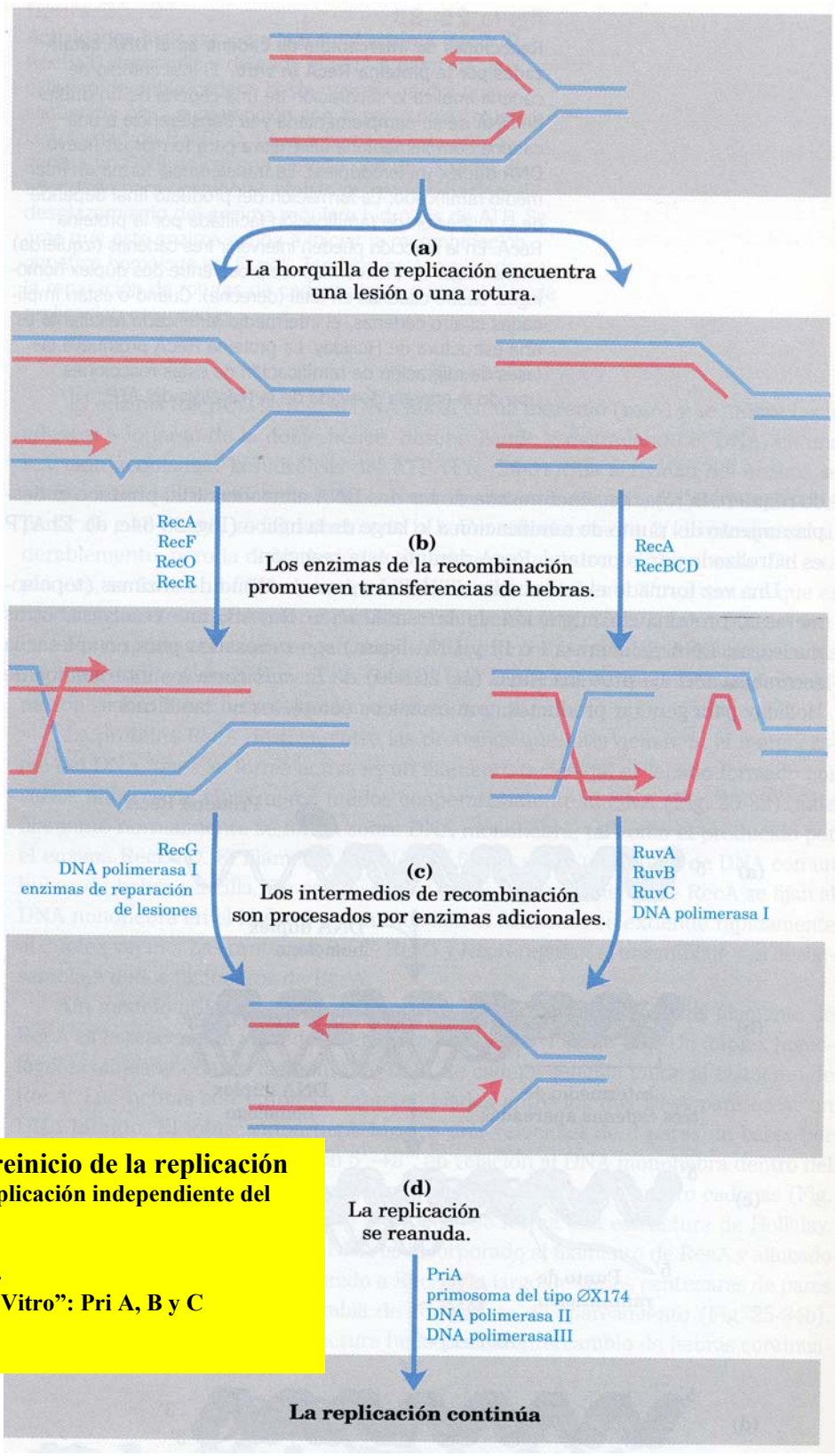


La recombinación homóloga es una vía de reparación del DNA

Si la horquilla de replicación se encuentra una lesión sin reparar ésta se detiene por lo general reemprendiéndose nuevamente la replicación en un punto más lejano del cromosoma. La lesión se deja atrás en un segmento monohebra sin replicar, y que no puede ser reparada por los mecanismos vistos hasta el momento por carecer de molde. Una de las vías posibles para repararlo es la vía recombinacional.

Huecos de cadena sencilla

Rotura de doble cadena



Primosoma de reinicio de la replicación
(iniciación de la replicación independiente del origen ORI^c)

Primosoma ØX174
siete proteínas "In Vitro": Pri A, B y C
Dna B, C, G y T

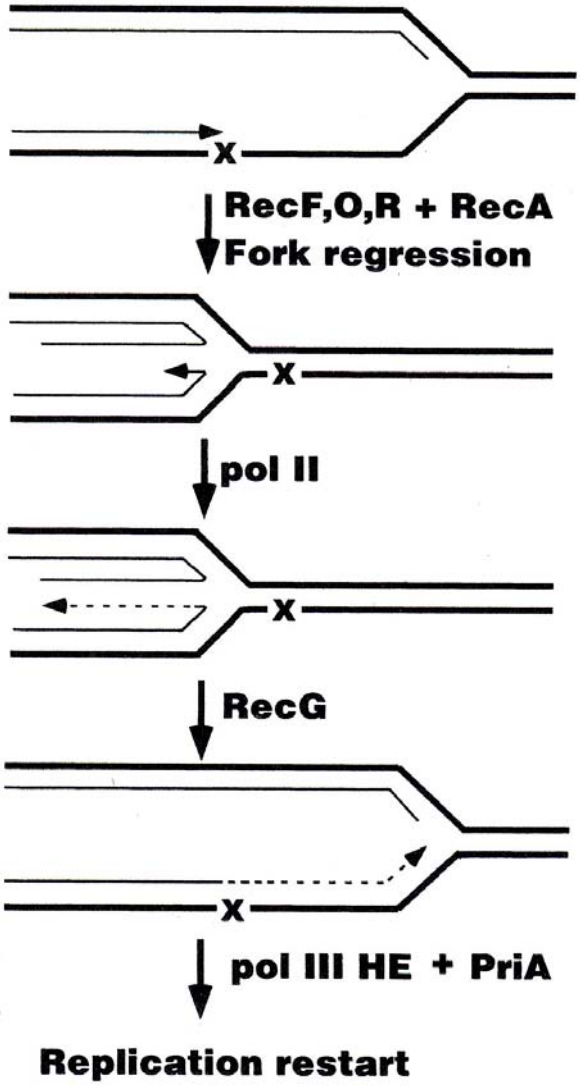


Figure 5 Model of error-free replication restart involving *E. coli* pol II. Replicative DNA synthesis is blocked by DNA template damage (**X**). Pol III dissociates from the damaged primer-template strand while synthesis continues on the undamaged strand. Regression of the replication fork occurs in the presence of RecF, O, and R proteins, thereby providing an undamaged template strand for pol II to copy that contains the correct coding information at the DNA damage site. RecG is required for progression of the fork past the lesion site and PriA is then involved in reconstituting the replication fork.

Sistema SOS

Un nivel elevado de DNA lesionado detiene de manera efectiva la replicación normal de DNA al tiempo que desencadena una respuesta de estrés en la célula en que se produce un incremento regulado (inducción) en la concentración de ciertas proteínas. Este fenómeno se denomina respuesta SOS. Algunas de las proteínas inducidas son parte de un sistema de replicación especializado que puede replicar pasando por encima de las lesiones del DNA que bloquean a la DNA polimerasa III. Debido a que el apareamiento correcto es a menudo imposible en el sitio de una lesión, esta replicación tras lesión es propensa al error.

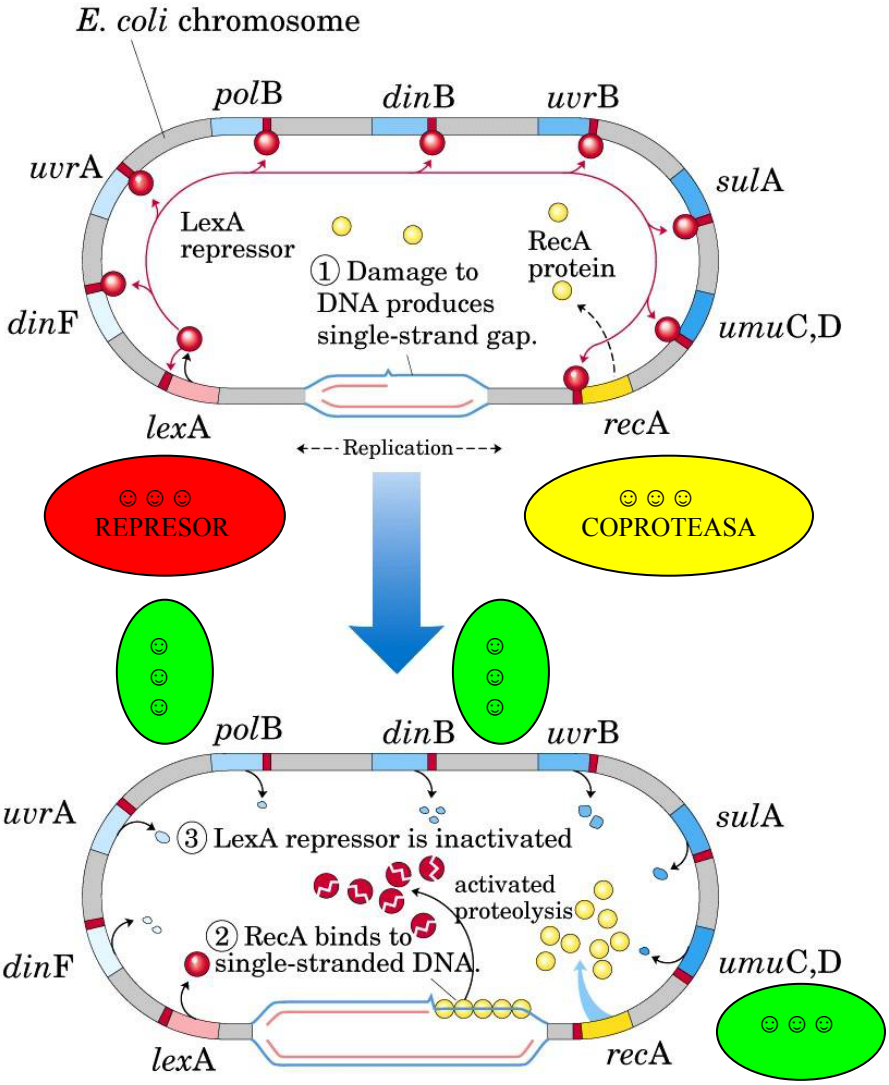
table 25–6

Genes Induced as Part of the SOS Response in *E. coli*

Gene name	Protein encoded and/or role in DNA repair
Genes of known function	
*pol*B (*din*A)	Encodes polymerization subunit of DNA polymerase II, required for replication restart in recombinational DNA repair
*uvr*A, *uvr*B	Encode ABC excinuclease subunits UvrA and UvrB
*umu*C, *umu*D	Encode DNA polymerase V
*sul*A	Encodes protein that inhibits cell division, possibly to allow time for DNA repair
*rec*A	Encodes RecA protein required for error-prone repair and recombinational repair
*din*B	Encodes DNA polymerase IV
Genes involved in DNA metabolism, but role in DNA repair unknown	
ssb	Encodes single-stranded DNA-binding protein (SSB)
*uvr*D	Encodes DNA helicase II (DNA-unwinding protein)
*him*A	Encodes subunit of integration host factor, involved in site-specific recombination, replication, transposition, regulation of gene expression
*rec*N	Required for recombinational repair
Genes of unknown function	
*din*D	
*din*F	

Note: Some of these genes and their functions are further discussed in Chapter 28.

Mecanismo de la respuesta SOS



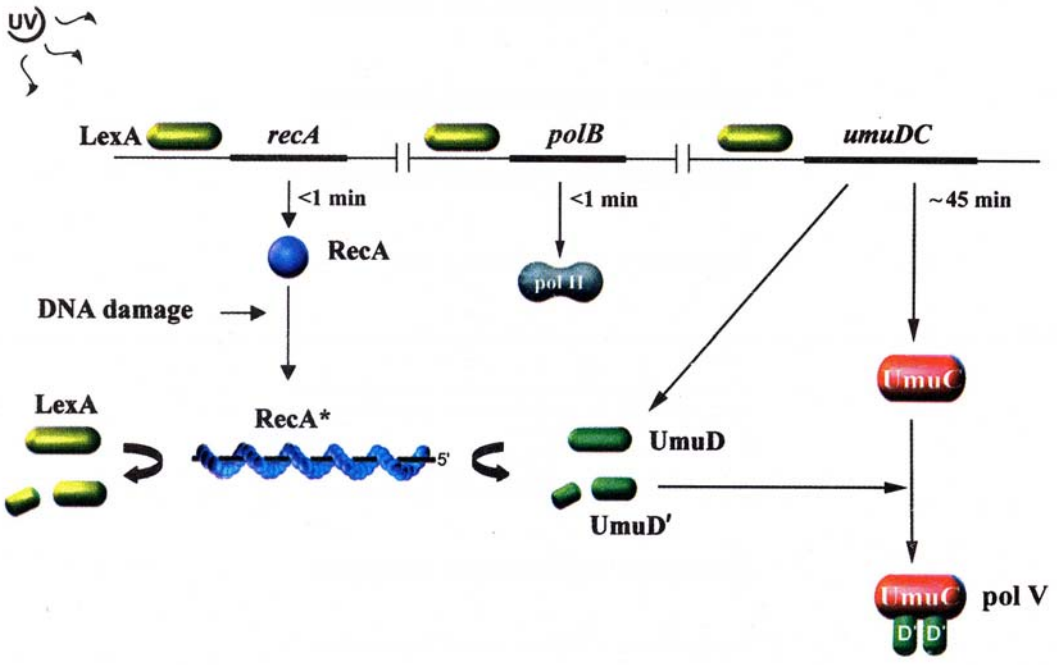


Figure 2 UV induction of SOS in *E. coli*. Binding of the LexA repressor (yellow) to regulatory operators upstream of SOS genes limits their expression under normal growth conditions. RecA (blue) is induced shortly after (<1 min) irradiation with UV, and becomes activated by binding to regions of single-stranded DNA to form a nucleoprotein filament, RecA* (blue helix). RecA* acts as a coprotease in the autocleavage of LexA, allowing induction of the SOS genes. In a similar reaction taking place on RecA*, UmuD (green) is cleaved between residues 24 and 25 of its amino-terminal end to form the mutagenically active carboxy-terminal fragment UmuD′ (26a). Two molecules of UmuD′ combine with one UmuC (red) to form pol V ($UmuD'_2C$). *PolB*, encoding pol II, is expressed early (<1 min post UV) and is involved in error-free replication restart (see Figure 5). Pol V appears much later (~45 min post UV); it copies persisting UV lesions to generate targeted SOS mutagenesis.

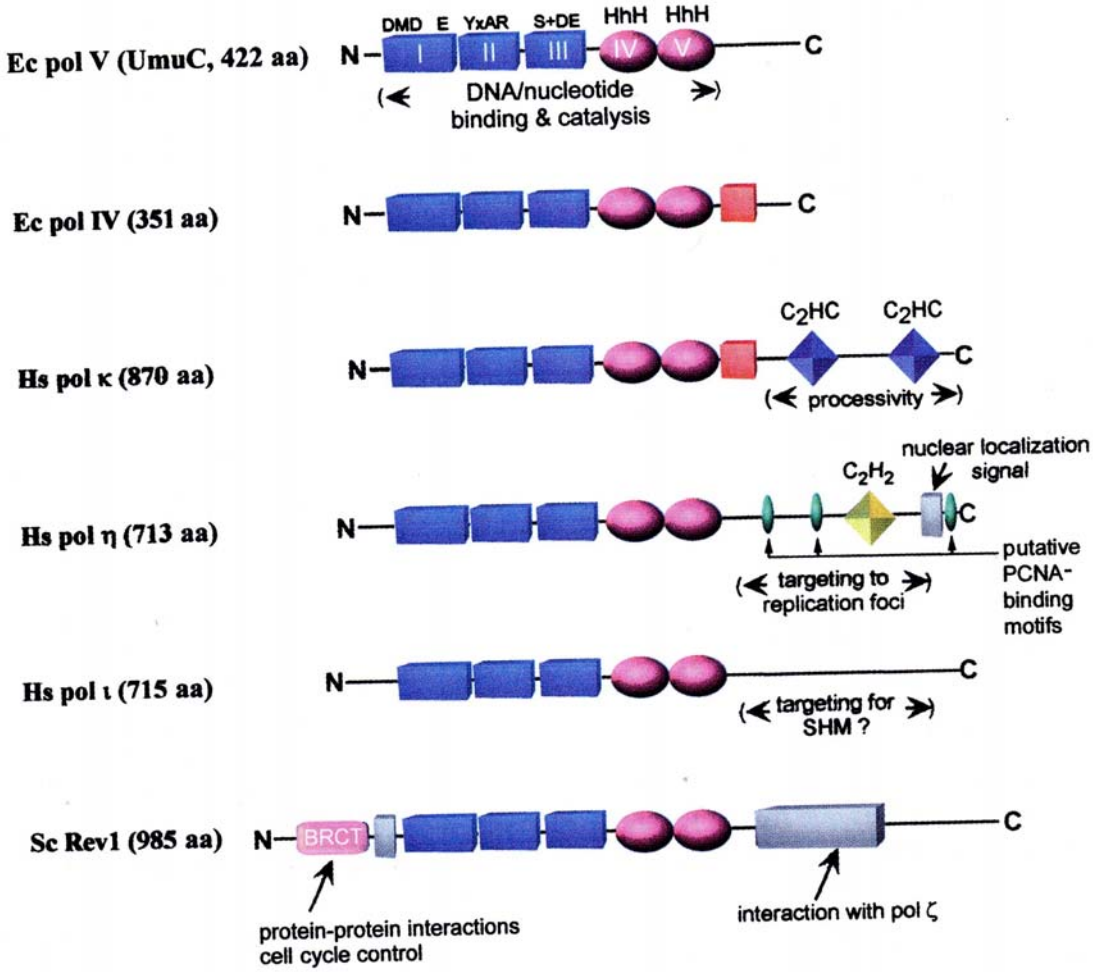


Figure 1 Domain structure of Y-family DNA polymerases. Conserved and unique domains are represented for *E. coli* pol V (UmuC), *E. coli* pol IV (DinB), human pol κ (DinB), human pol η (XPV or Rad30A), human pol ι (Rad30B), and *S. cerevisiae* Rev1. The highly conserved domains I–III (blue rectangles) contain catalytic residues, and IV–V (red ovals) contain helix-hairpin-helix motifs (HhH). Amino acid clusters involved in Mg^{2+} binding and catalysis, based on site-specific mutational analysis, are indicated above domains I–III in UmuC, the least conserved family member. The DinB subgroup contains a short conserved motif (orange square) present from *E. coli* to humans. The C_2HC zinc-binding motif (blue diamond) is involved in DNA binding, and the C_2H_2 zinc-binding motif (yellow diamond) is required for targeting to replication foci, perhaps via interactions with proliferating cell nuclear antigen (PCNA). (SHM, somatic hypermutation; aa, amino acids.)

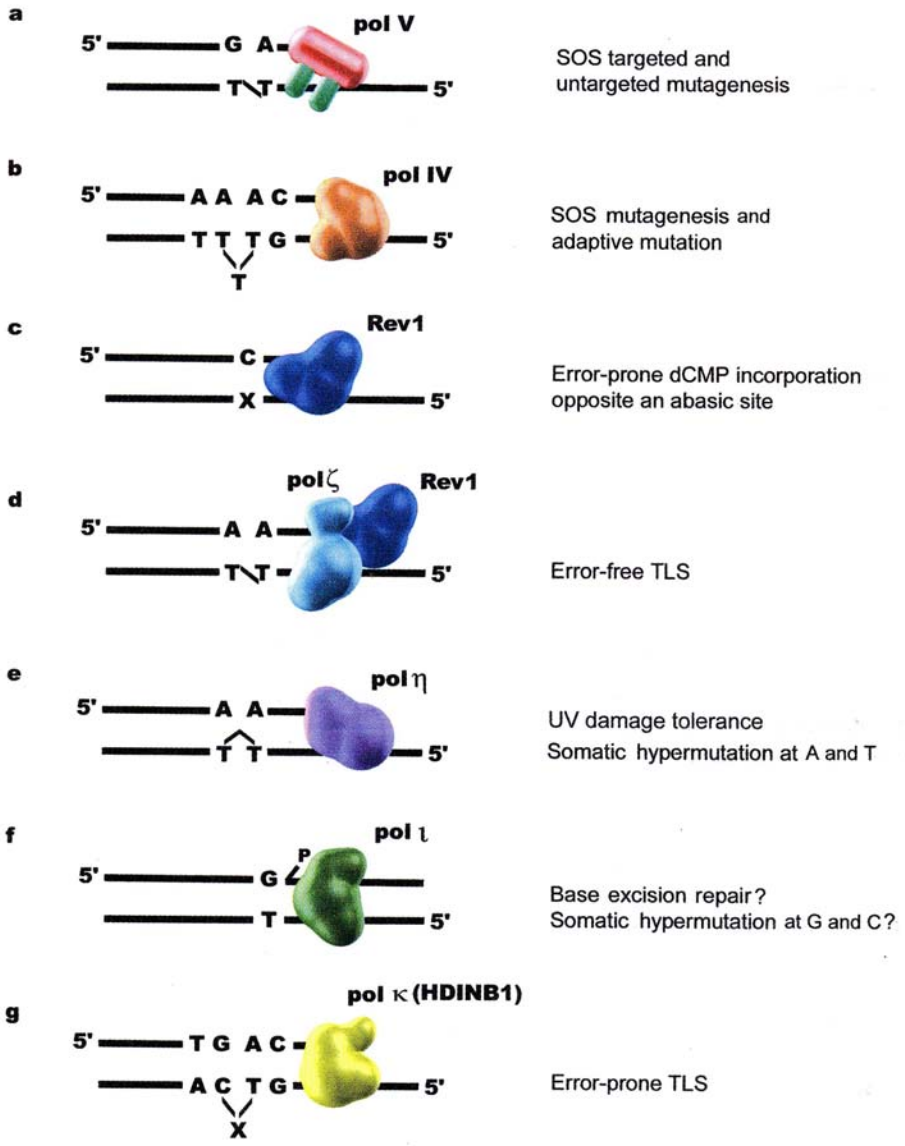


Figure 6 Biochemical properties of the EP pols. (*a*) Error-prone translesion synthesis (TLS) by *E. coli* pol V results in misincorporation of G opposite the 3′ T of a TT (6–4) photoproduct, leading to A → G transition mutations. (*b*) Misaligned primer-template ends are extended efficiently by *E. coli* pol IV, leading to frameshift mutations. (*c*) The DNA-dependent dCMP transferase activity of Rev1 protein incorporates C opposite an abasic template site. (*d*) Pol ζ, a B-family pol, efficiently incorporates two A nucleotides opposite a TT (6–4) photoproduct in vitro, resulting in error-free bypass of the lesion, dependent on the presence of Rev1 protein. (*e*) DNA polymerase η catalyzes error-free replication across a TT *cis-syn* photodimer by incorporating two A nucleotides, thereby avoiding mutation and offering protec-

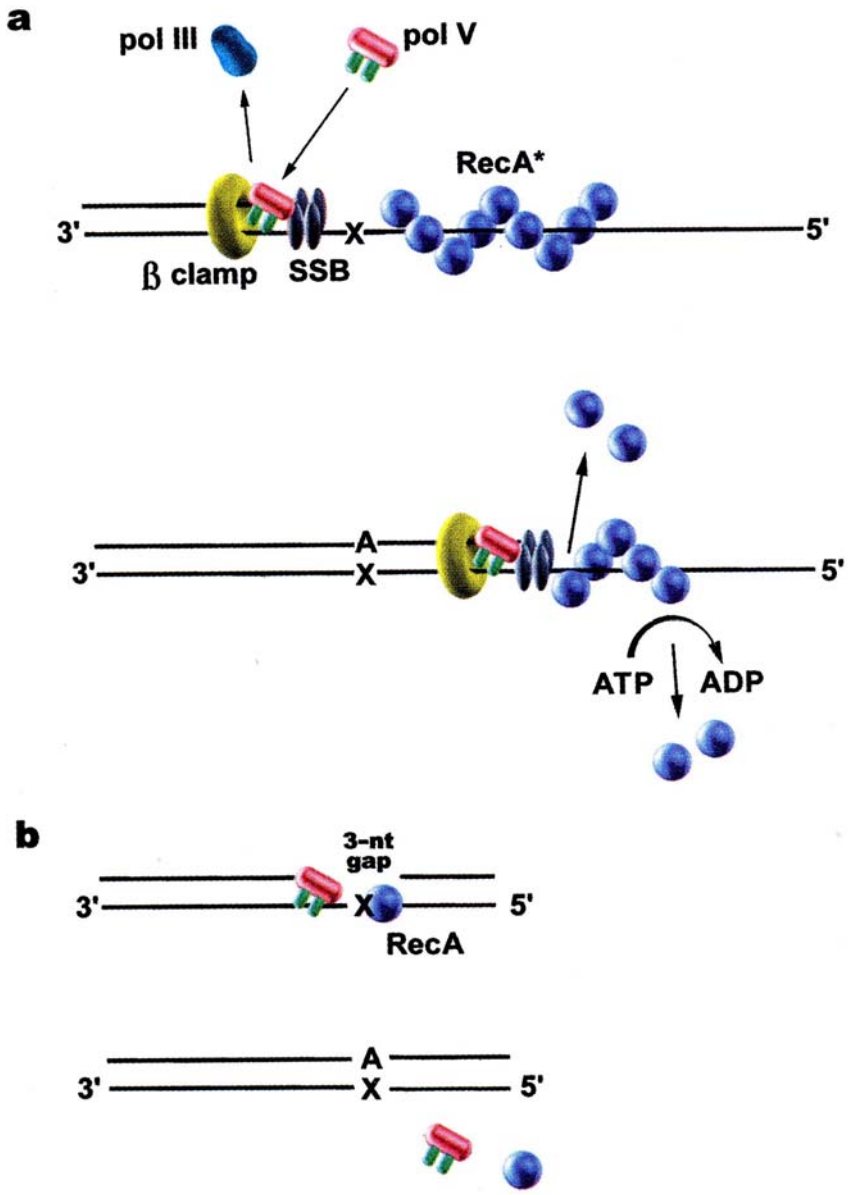


Figure 4 Models of *E. coli* pol V catalysis of error-prone translesion synthesis. (*a*) A cowcatcher model involving a RecA nucleoprotein filament (RecA*). Replicative pol III stalls when encountering a template lesion (**X**), dissociates from the 3′-primer end, and is replaced by pol V. The activity and binding affinity of pol V are strongly stimulated by the presence of RecA, SSB, and β sliding clamp. The continued unwinding action of dnaB helicase (Figure 3) allows formation of a RecA nucleoprotein filament ahead of the lesion. The filament assembles in a 5′ → 3′ direction on ssDNA, advancing to the DNA damage site. Pol V + SSB operate as a locomotive cowcatcher to strip RecA from the DNA template in a 3′ → 5′ direction immediately ahead of an advancing pol V molecule. The cowcatcher stripping reaction does not involve ATP hydrolysis and takes place concurrently with the "standard" 5′ → 3′ filament disassembly reaction requiring ATP hydrolysis. The p/t DNA is composed of a 30-nucleotide (nt) primer annealed to a 120-nt template. (*b*) Translesion synthesis requires the presence of RecA but not a RecA nucleoprotein filament. Pol V–cata-

lyzed TLS taking place within a 3-nt gap requires the presence of RecA and ATPγS, but not SSB. The TLS gap-filling reaction does not occur within a 2-nt gap, although the 2-nt gap can be filled in the absence of a lesion by pol V alone. The p/t DNA is composed of a 120-nt template annealed to an oligonucleotide primer and "downstream" oligonucleotide, forming a gap of either 3 or 2 nt.

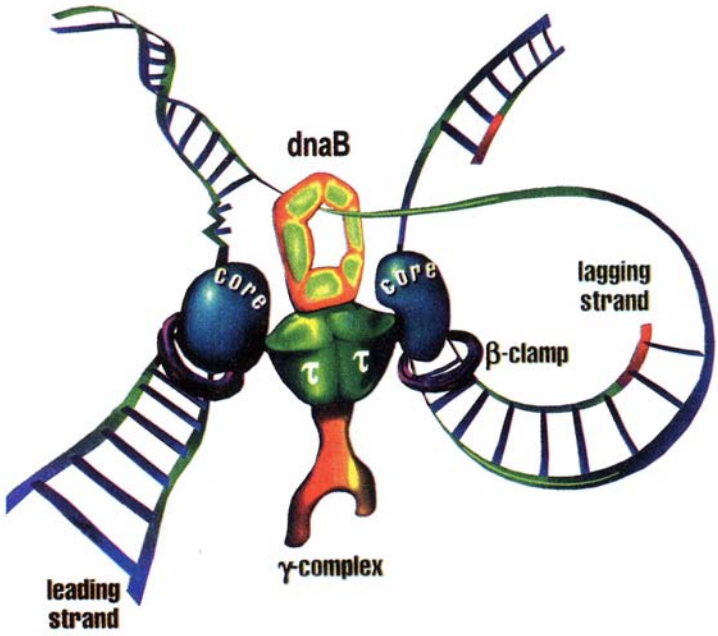


Figure 3 *E. coli* replisome and DNA replication as proposed by the trombone model. The τ dimer (green) links two pol III core molecules (blue), one for leading- and the other for lagging-strand synthesis. The coupled leading- and lagging-strand reactions are interrupted in the presence of DNA damage (indicated by a distortion immediately ahead of the polymerase core on the leading-strand track), presumably causing disassembly of the replication complex. EP pols such as pol V or pol IV then take over from the pol III core to synthesize past the damage site. Reconstitution of the replisome with the pol III core occurs following translesion synthesis. Shown as part of the replisome are the pol III core (composed of three subunits: α polymerase, ϵ exonuclease proofreading, and θ subunits); the γ complex required for loading the β-dimer sliding clamp onto DNA (five subunits); and the DnaB helicase. Not shown is SSB (single-stranded-DNA-binding protein), which coats ssdNA regions ahead of the replication fork. The pol III holoenzyme (HE) is composed of a pol III core + β/γ + τ. Lagging-strand RNA Okazaki fragment primers appear in red.

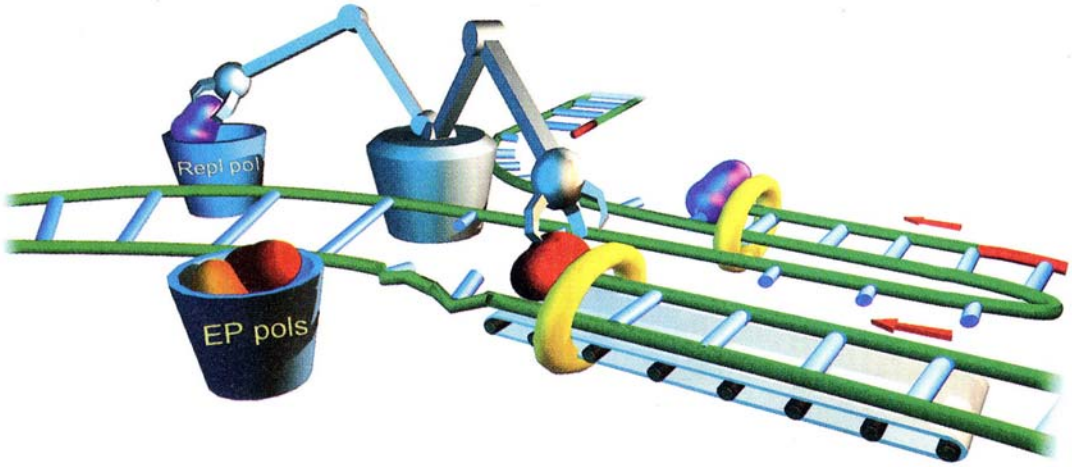


Figure 8 DNA repair factory model. The cartoon depicts a stationary replication-repair complex encountering damaged DNA rolled along as on a conveyer belt, in contrast to the more common textbook illustrations of DNA polymerase and accessory proteins moving along a stationary DNA molecule (Figure 3). A lesion is shown on the leading strand of the DNA template (green rod) as a distortion in the template track. The replication fork collapses when confronting the lesion, and leading- and lagging-strand synthesis become uncoupled. The leading-strand replicative polymerase (Repl pol, purple dumbbell) that was initially attached to the sliding clamp (yellow doughnut) cannot copy past the lesion and is replaced by an EP pol (red dumbbell). The bucket housing different EP pols symbolizes the high local concentrations of polymerases chosen at random to copy the damaged template strand. Lagging-strand synthesis continues on the undamaged template strand using the Repl pol. Okazaki fragments primed by RNA oligomers (red rods) are shown on the newly synthesized portion of the lagging strand.