NUCLEIC ACIDS – BIOCHEMISTRY MODULE 2

- Structure and Function of Nucleic Acids
- Genes and Chromosomes (brief summary)
- DNA Replication
- DNA Transcription
- Protein Synthesis

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BIOCHEMISTRY LABORATORY

- January 13th 2025, 9-11 AULA MAGNA EMATOLOGIA "M.T. CHIANTORE" - Piano Primo - Sant'Orsola - Pad. 8 - Clinical case presentations (4/5 people per group- G3 and G4)

- January 13th 2025, 14-16 AULA MAGNA EMATOLOGIA "M.T. CHIANTORE" - Piano Primo - Sant'Orsola - Pad. 8 - Clinical case presentations (4/5 people per group- G1 and G2)

- January 15th 2025, 11-13 AULA MAGNA "I MAESTRI" - Piano Primo - Sant'Orsola - Pad. 23 – Prof. Antonio Pannuti «RNA in diagnostics and therapy»

- January 17th 2025, 9-11 AULA MAGNA EMATOLOGIA "M.T. CHIANTORE" - Piano Primo - Sant'Orsola - Pad. 8 - «Mock exam – Chemistry and Biochemistry (with results discussion)»

- January 27th 2025, 9-11 AULA MAGNA EMATOLOGIA "M.T. CHIANTORE" - Piano Primo - Sant'Orsola - Pad. 8 - Dr. Akram Ghantous from the International Agency for Research on Cancer (IARC-WHO) «Epigenomics and Big Data: Linking the Environment with Health and Disease”

BIOCHEMISTRY LABORATORY

BRING YOUR LAB COAT WITH YOU

group	Experience 1	Experience 2
G1	January 20th, 2025 14:30 – 18:00	January 27th, 2025 14:30-18:00
G2	January 17th, 2025 14:30 – 18:00	January 31th, 2025 14:30-18:00
G3	January 21th, 2025 14:30 – 18:00	January 28th, 2025 14:00-18:00
G4	January 20th, 2025 9:30-13:00	January 22th, 2025 14:30-18:00

Via Umberto Terracini, 28, 40131 Bologna
Dipartimento di Ingegneria Civile, Chimica, Ambientale e dei Materiali (DICAM)
BUS #35

SEE YOU IN THE LOBBY IN FRONT OF THE RECEPTION

BIOCHEMISTRY LABORATORY

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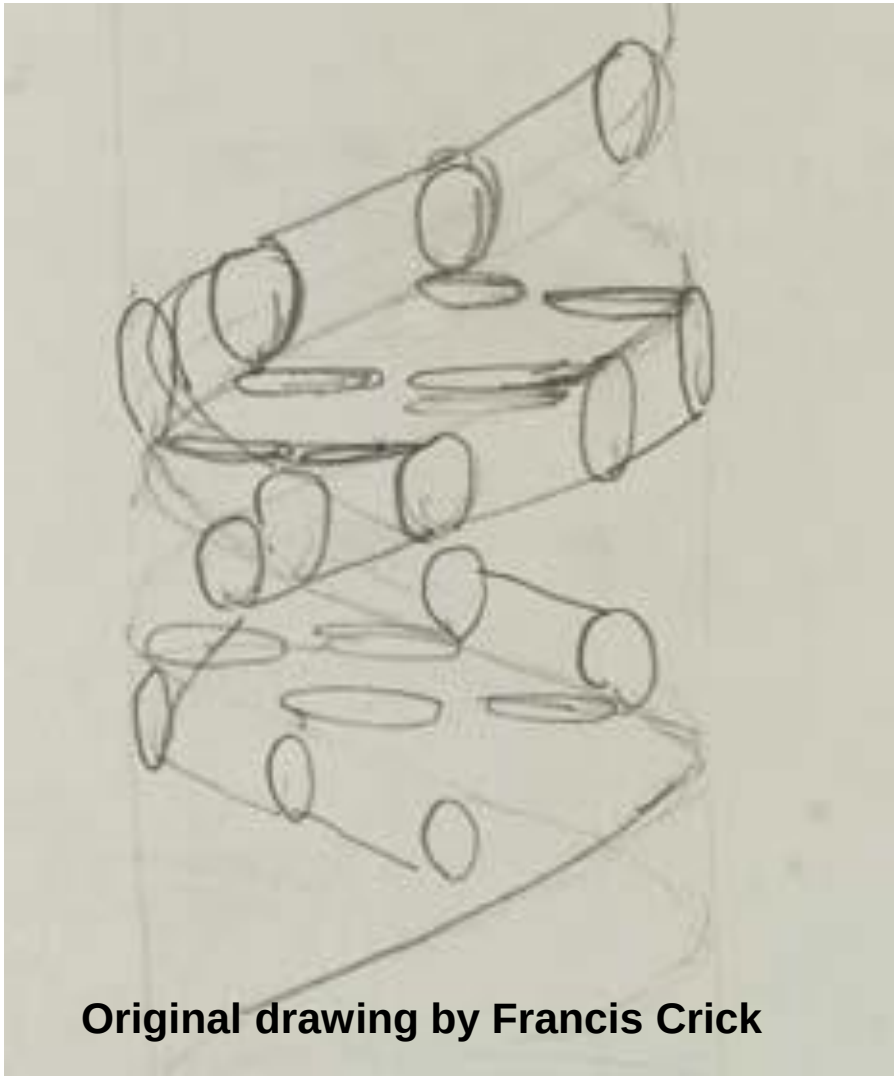
MAIN TOPICS

DNA Replication, and Repair: process by which DNA is copied with high fidelity.

RNA Synthesis and Processing: process by which the DNA genetic code is ‘read’ and transferred, forming mRNA (messenger RNA). This is the intermediate step in protein expression.

Protein Synthesis: process by which the genetic code is translated into a protein sequence, the end product of gene expression.

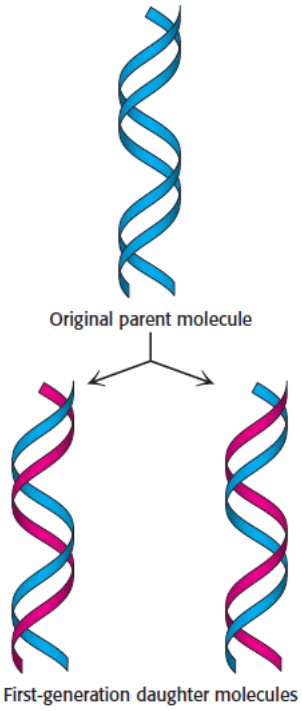
REPLICATION



Original drawing by Francis Crick

DNA —replication→ **DNA**

Replication: process by which DNA is copied with high fidelity.



REPLICATION

DNA  DNA

To replicate the human genome without mistakes, an error rate of less than 1 bp per 3 x 10^9 bp must be achieved.

Such remarkable accuracy is achieved through:

- a multilayered system of accurate DNA synthesis (which has an error rate of 1 per 10^3-10^4 bases inserted),
- proofreading during DNA synthesis (which reduces that error rate to approximately 1 per 10^6 - 10^7 bp),
- Post-replication mismatch repair (which reduces the error rate to approximately 1 per 10^9- 10^{10} bp).

DNA replication comprises three stages: Initiation, elongation, and termination

a. INITIATION

a) Proteins bind to DNA and open the double helix

b) DNA is prepared for pairing with complementary bases

b. ELONGATION

a) Proteins bind to the correct nucleotide sequences to form a continuous strand of DNA

c. TERMINATION

a) Proteins release the replication complex

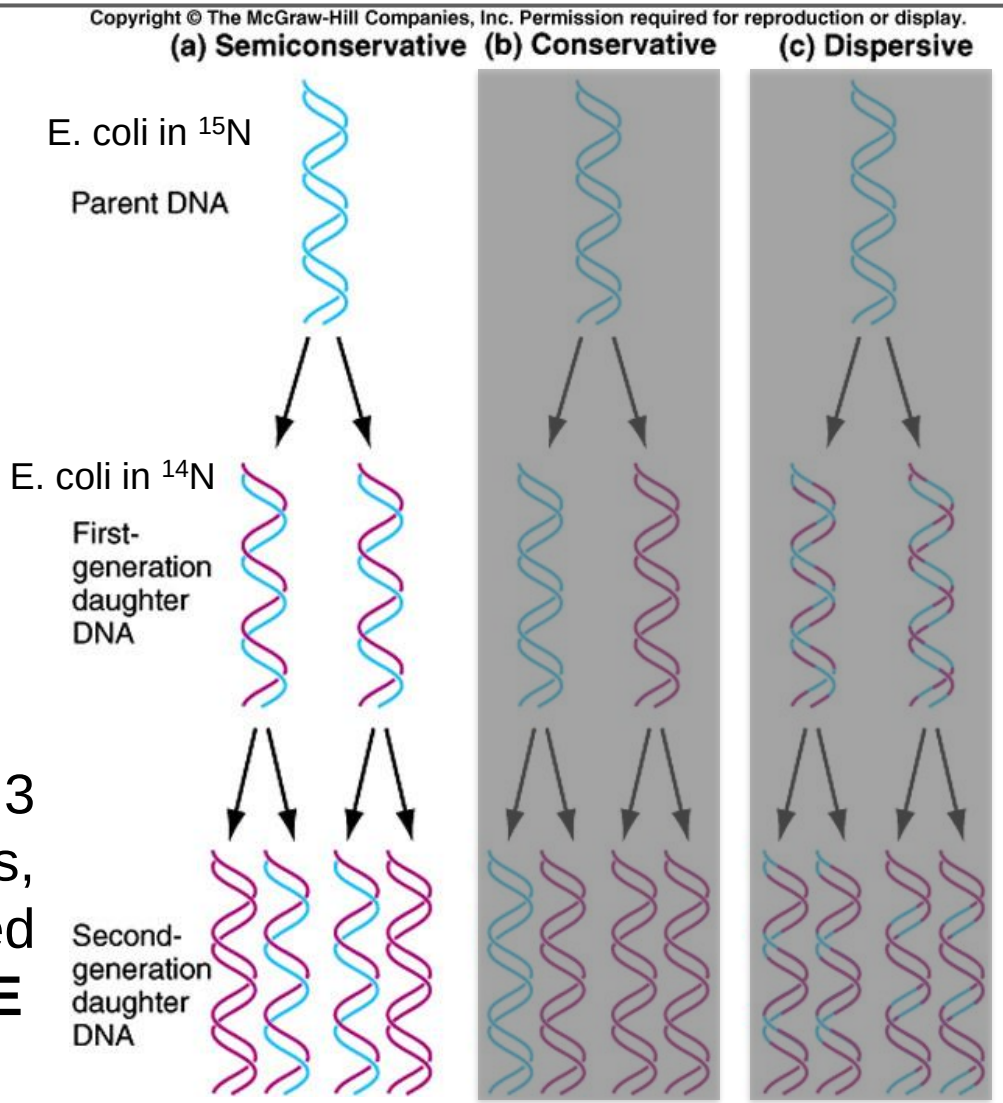
REPLICATION

Main features

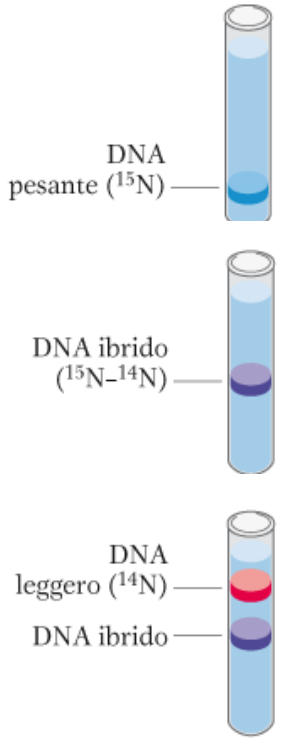
- **Semi-conservative** (each DNA strand serves as a template for the synthesis of a new strand)
- **Starts at the ORIGIN**
- **Bi-directional** (starting with a replication fork, where the DNA is separated into two strands)
- **Semi-discontinuous** (creation of fragments)
- **Always in 5‘-3’ direction**
- **Requires RNA primers** (an oligonucleotide with a free 3’-hydroxyl group that is complementary with the termination of the template to form a dsDNA)

REPLICATION

Experiments done by Meselson-Stahl (1957)
E. coli in ^{15}N



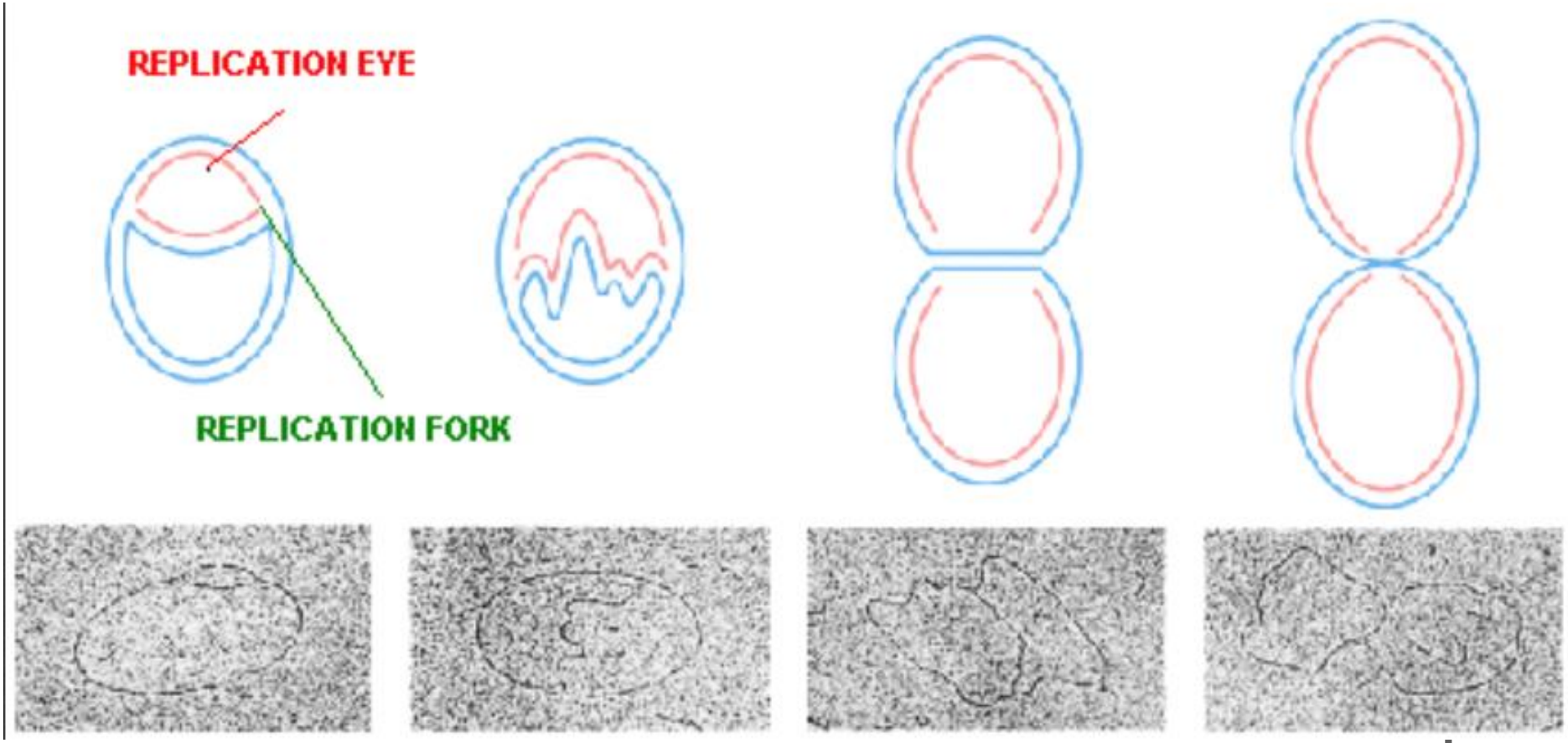
Extracted and centrifuged DNA in CsCl solution

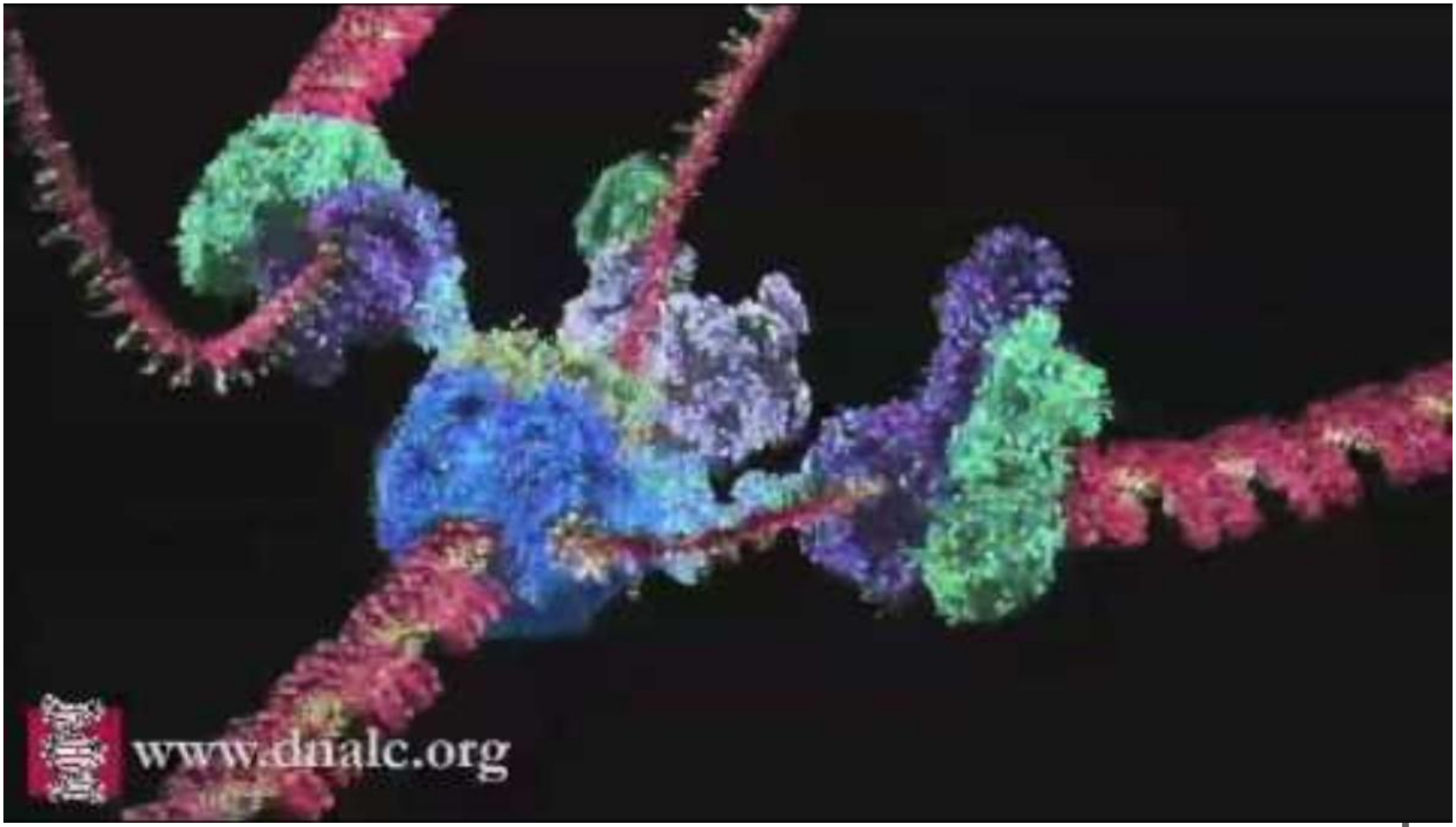


Considering the 3 possible models, replication resulted **SEMICONSERVATIVE**

REPLICATION

Cairns experiments (1962) in E. coli with radioactive thymine: replication is **bidirectional.**

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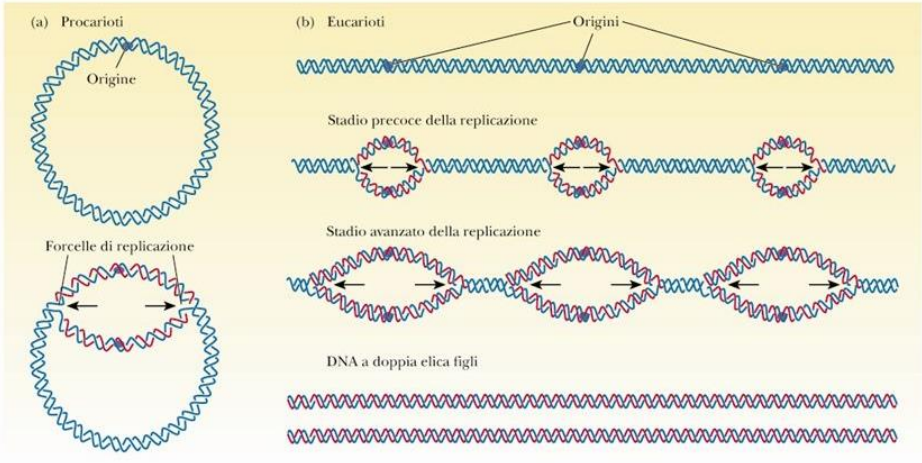
REPLICATION

Initiation proteins identify specific DNA sequences called **Sites of Origin (rich in A=T-pairs).** In prokaryotes - single site of origin, e.g. in E. coli it is **oriC**.

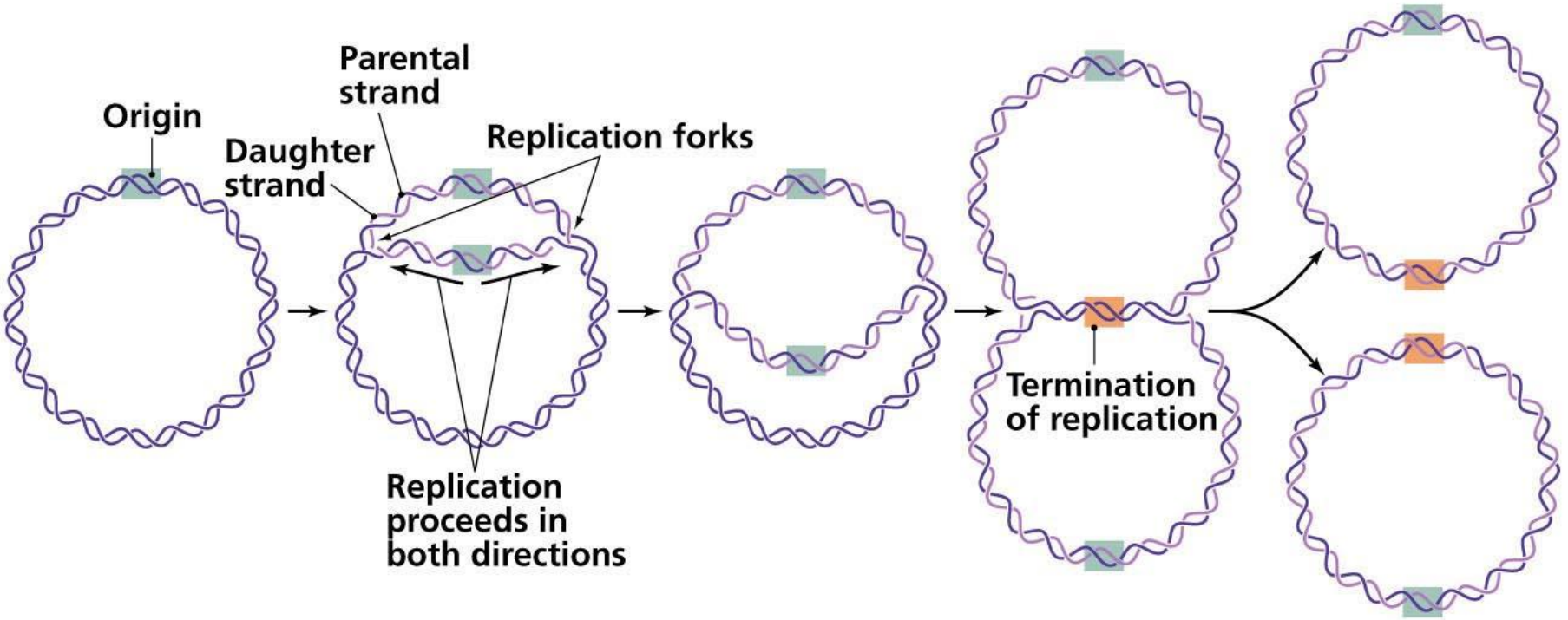
In eukaryotes - multiple sites.



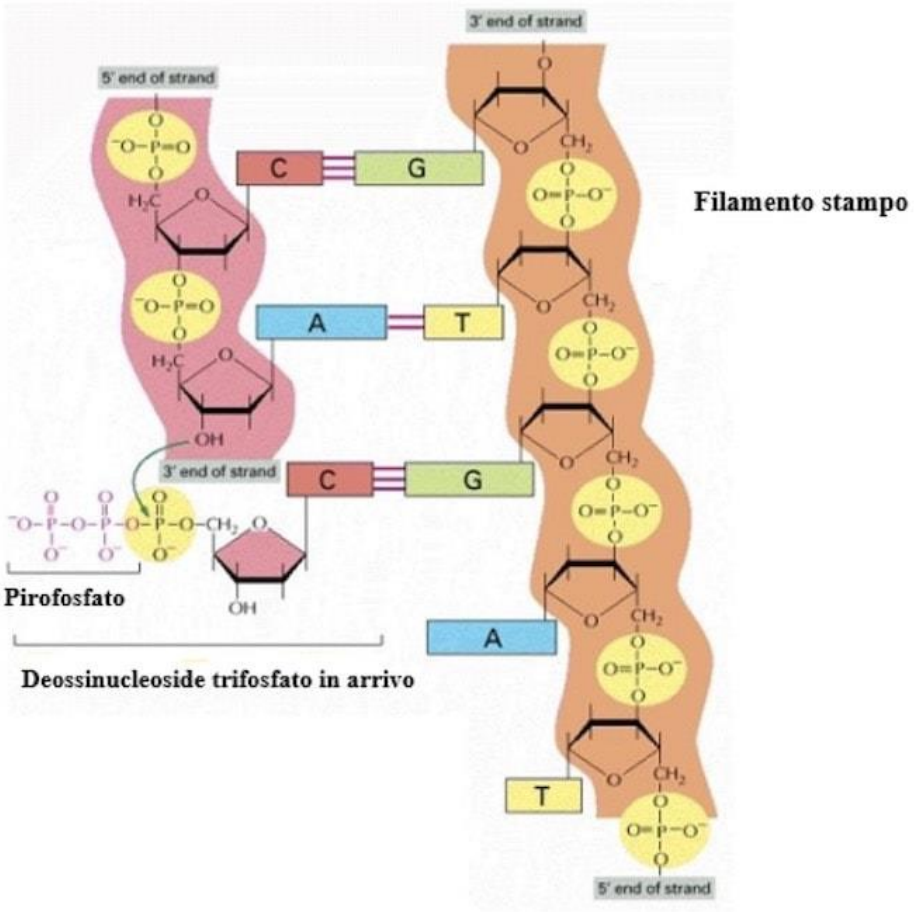
Both strands are replicated simultaneously. Replication is bidirectional (both ends of the loops have replication forks).

REPLICATION

prokaryotes



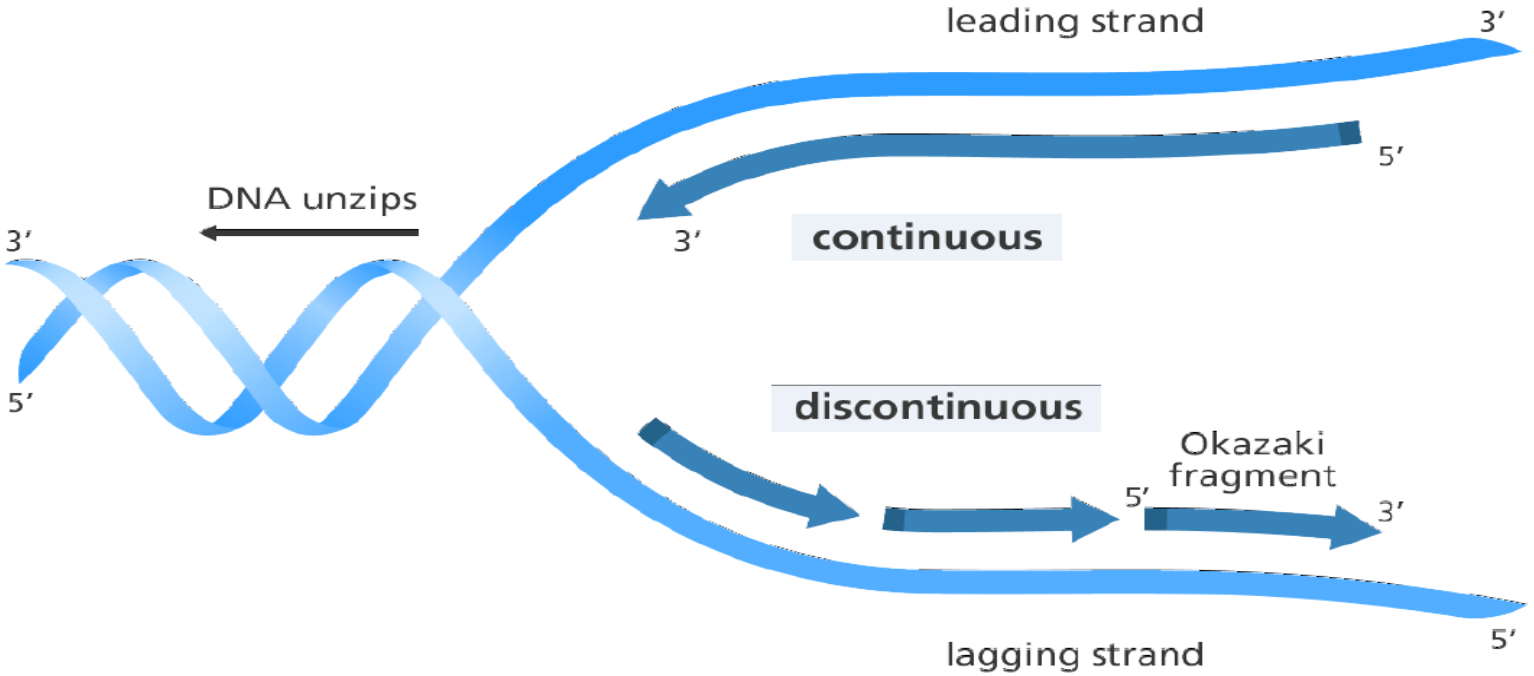
REPLICATION



Nucleotide recognition. Polymerisation catalysed by an enzyme (**DNA polymerase**). The substrates used are dNTPs.

Synthesis always occurs in the 5‘-3’ direction

REPLICATION

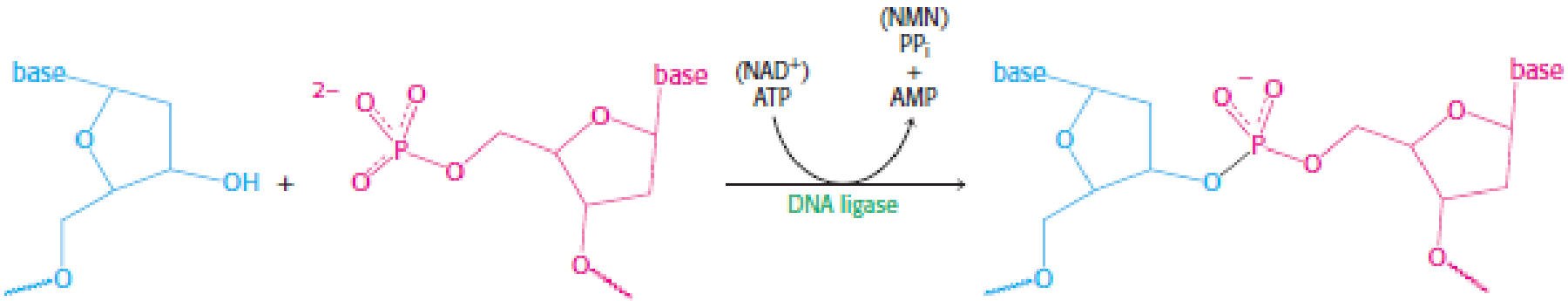


OKAZAKI fragments in the slow strand (150-200 nt in eukaryotes; 1,000-2,000 nt in bacteria). The fragments are then joined by **DNA ligase**.

REPLICATION

DNA LIGASE

DNA ligase catalyzes the formation of a phosphodiester bond between the 3'-hydroxyl group at the end of one DNA chain and the 5'-phosphoryl group at the end of the other



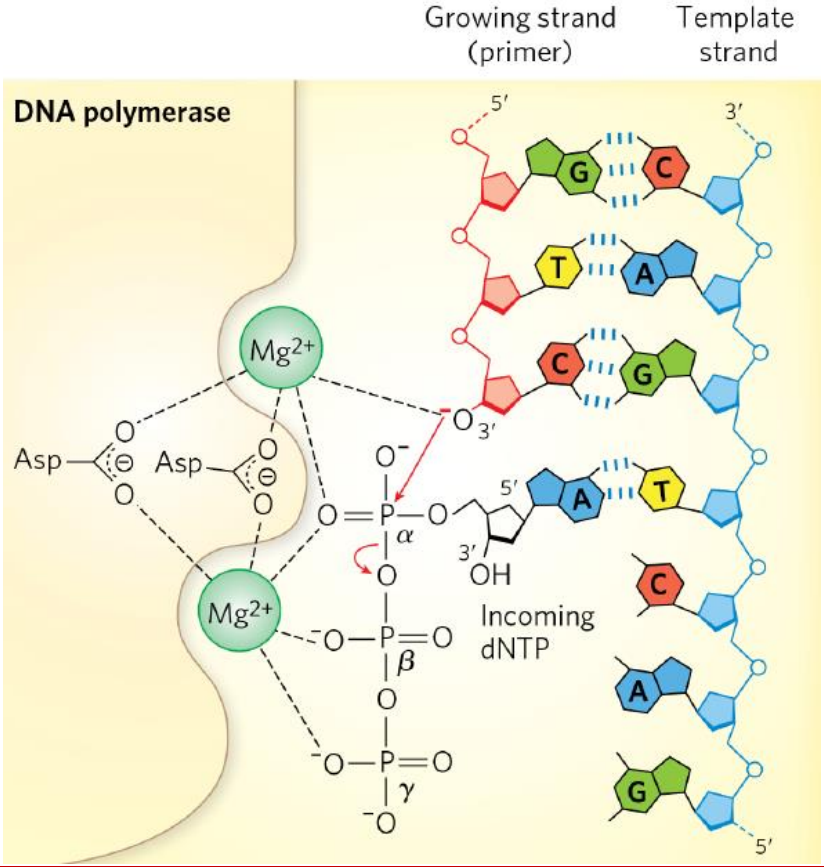
An energy source is required to drive this thermodynamically uphill reaction. In eukaryotes and archaea, ATP is the energy source.

REPLICATION

Enzymes

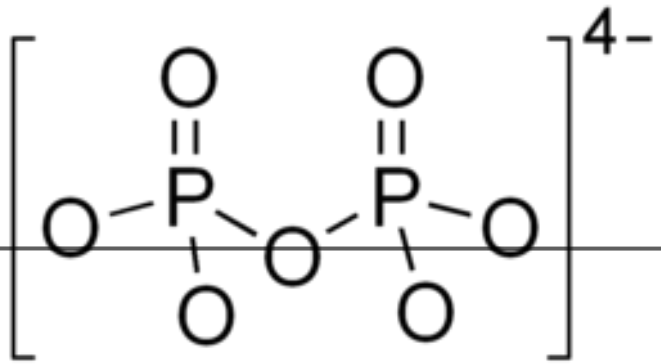
$$(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + PP_i$$

DNA polymerase



-OH in 3' attacks the α-phosphate.

Mg^{2+} ions act as cofactors stabilizing the negative charges on the deprotonated –OH moiety in 3', the phosphate backbone of DNA, and aspartate residues in the catalytic site allowing for proper strand separation during the polymerization process.



Pyrophosphate groupe (PPi)

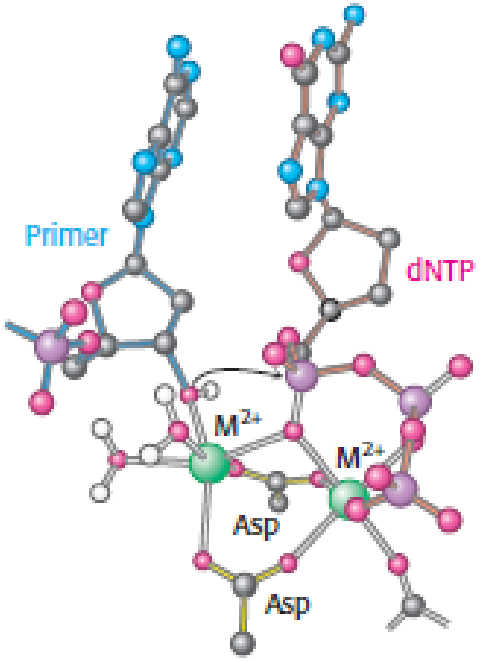


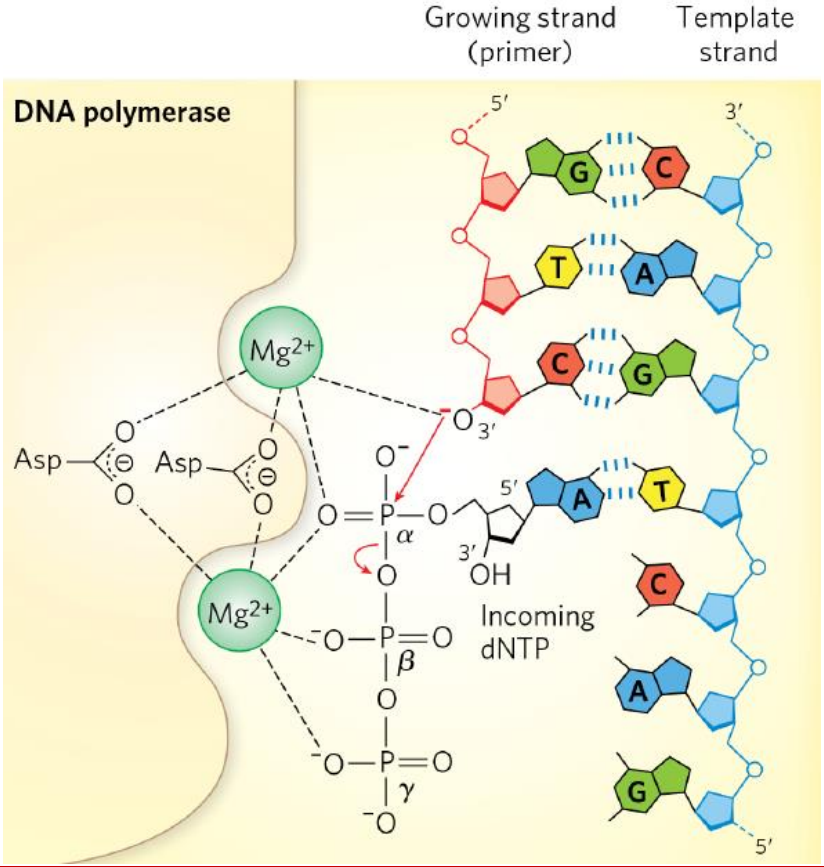
Figure 28.4 DNA polymerase mechanism. Two metal ions (typically, Mg^{2+}) participate in the DNA polymerase reaction. One metal ion coordinates the 3′-hydroxyl group of the primer, whereas the other metal ion interacts only with the dNTP. The phosphoryl group of the nucleoside triphosphate bridges between the two metal ions. The hydroxyl group of the primer attacks the phosphoryl group to form a new O–P bond.

REPLICATION

Enzymes

$$(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + PP_i$$

DNA polymerase



A phosphodiester bond is formed and a phosphoanhydride bond is broken ($\Delta G > 0$)

Interactions between bases stabilise products

Hydrolysis of pyrophosphate yields a $\Delta G < 0$

REPLICATION

DNA polymerases

- It is the enzyme responsible for DNA replication.
- Five DNA polymerases were identified in *E. coli*.

- Polymerases have different physiological roles:
  - **DNA-Pol III** is responsible for the complete duplication of the bacterial genome.
  - **DNA-Pol I** and **II** are involved in DNA repair processes.
  - **DNA-Pol IV** and **V** are involved in non-standard DNA repair processes.

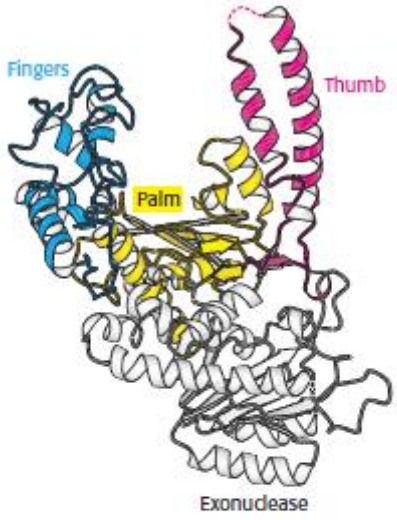


Figure 28.3 DNA polymerase structure. The first DNA polymerase structure determined was that of a fragment of *E. coli* DNA polymerase I called the Klenow fragment. *Notice* that, like other DNA polymerases, the polymerase unit resembles a right hand with fingers (blue), palm (yellow), and thumb (red). The Klenow fragment also includes an exonuclease domain that removes incorrect nucleotide bases. [Drawn from 1DPI.pdb.]

REPLICATION

DNA polymerase

It cannot initiate the synthesis of a new DNA strand: it can only elongate a pre-formed oligonucleotide fragment. So:

1) It requires a **primer**.
2) DNA chain elongation occurs only in the **5‘ - 3’ direction**.
3) It possesses a proofreading site (**3‘- 5’ exonuclease site**).

4) **DNA-Pol I** in addition to the 3‘ - 5’ exonuclease activity also possesses a 5‘ - 3’ exonuclease activity (Nick translation activity).

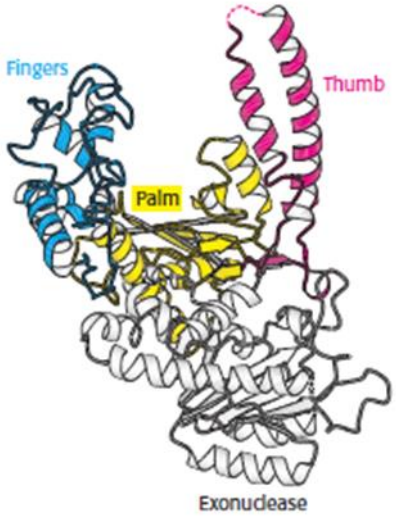


Figure 28.3 DNA polymerase structure. The first DNA polymerase structure determined was that of a fragment of *E. coli* DNA polymerase I called the Klenow fragment. *Notice* that, like other DNA polymerases, the polymerase unit resembles a right hand with fingers (blue), palm (yellow), and thumb (red). The Klenow fragment also includes an exonuclease domain that removes incorrect nucleotide bases. [Drawn from 1DPI.pdb.]

RNA primes the synthesis of DNA

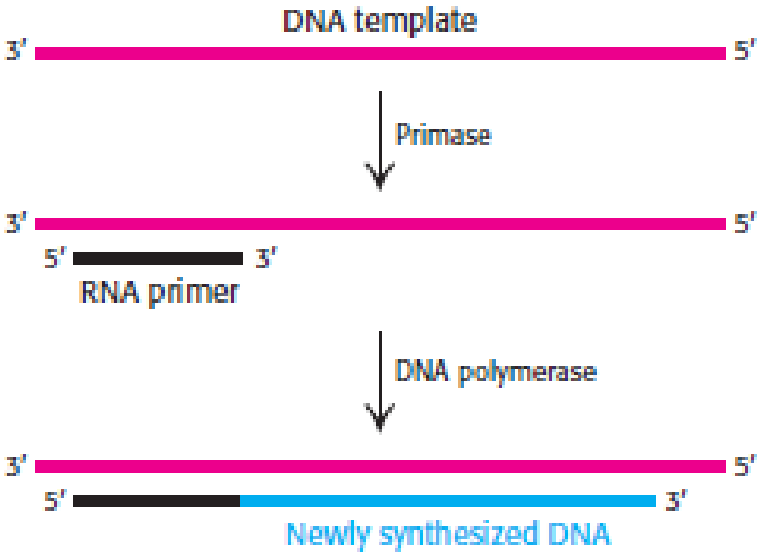


Figure 28.8 Priming. DNA replication is primed by a short stretch of RNA that is synthesized by primase, an RNA polymerase. The RNA primer is removed at a later stage of replication.

1) An RNA polymerase called *primase* synthesizes a short sequence of RNA (about five nucleotides) complementary to one of the template DNA strands.
2) DNA synthesis
3) the short sequence of RNA is removed by hydrolysis and replaced by DNA.

REPLICATION

PROTEINS involved in DNA replication in *E. coli* (replisome)

Helicase (DnaB)	unwounds DNA	300 kD	20 mol/cell
SSB	stabilizes ssDNA	75	500
DNA gyrase (topo II)	delete supercoiling	400	250
Primase	synthesis of RNA primer	60	50
DNA polymerase III	elongation	1,000	20
DNA polymerase I	primer elimination	103	300
DNA ligase	linking ssDNA fragments	74	300

The Helicase-Primase complex forms **PRIMOSOME**

REPLICATION

DNA polymerases

Arthur Kornberg et al (1955) discover the DNA polymerase (I) in *E. coli*

TABELLA 25.1 Confronto tra le DNA polimerasi di *E. coli*

	DNA polimerasi				
	I	II[a]	III	IV[a]	V[a]
Gene strutturale[b]	*polA*	*polB*	*polC* (*dnaE*)	*dinB*	*umuC*
Subunità (numero dei diversi tipi)	1	7	9	1	3
M_r	103 000	88 000[c]	1 065 400	39 100	110 000
Attività 3′→5′ esonucleasica (proofreading)	sì	sì	sì	no	no
Attività 5′→3′ esonucleasica	sì	no	no	no	no
Velocità di polimerizzazione (nucleotidi)	10-20	40	250-1000	2-3	1
Processività (nucleotidi aggiunti prima della dissociazione)	3-200	1500	≥500 000	1	6-8

Repair mechanisms

Association and dissociation determine the speed of the reaction.

PROCESSIVITY = Average no. of nt added before DNA polymerase dissociates. Variable.

REPLICATION

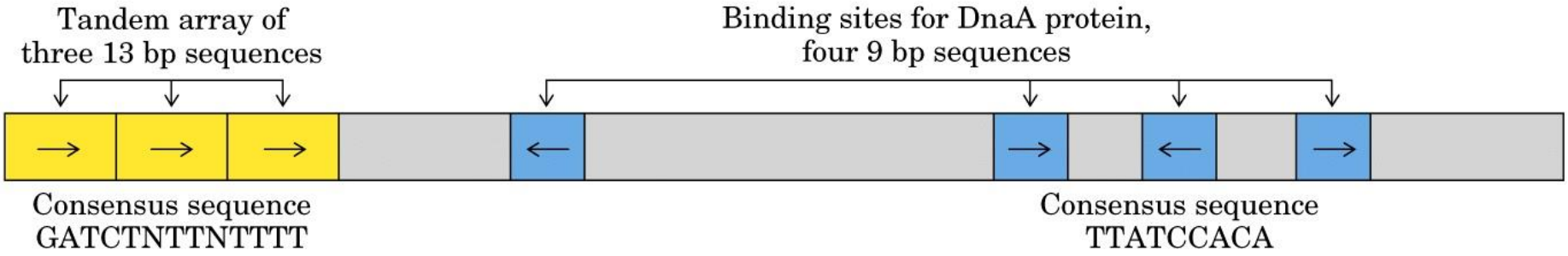
INITIATION

- Identification of the **Origin sites**.
- Positioning and action of the H**ELICASES**.
- Action of the **PRIMASE**.
- GYRASE action (**TOPOISOMERASE II**).

REPLICATION

INITIATION

1) The duplication process in E. coli begins at a specific site designated '**oriC**'. This site contains four repeated sequences of 9 base pairs (**Mer-9**) and three repeated sequences of 13 base pairs (**Mer-13**); the latter section is particularly rich in A-T pairs.



2) For a double-stranded DNA molecule to replicate, the two strands of the double helix must be separated from each other, at least locally.

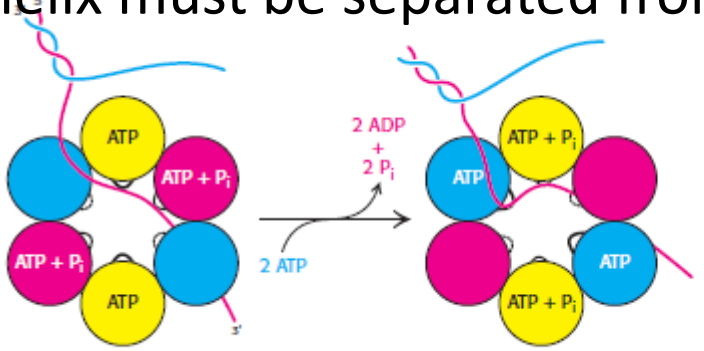
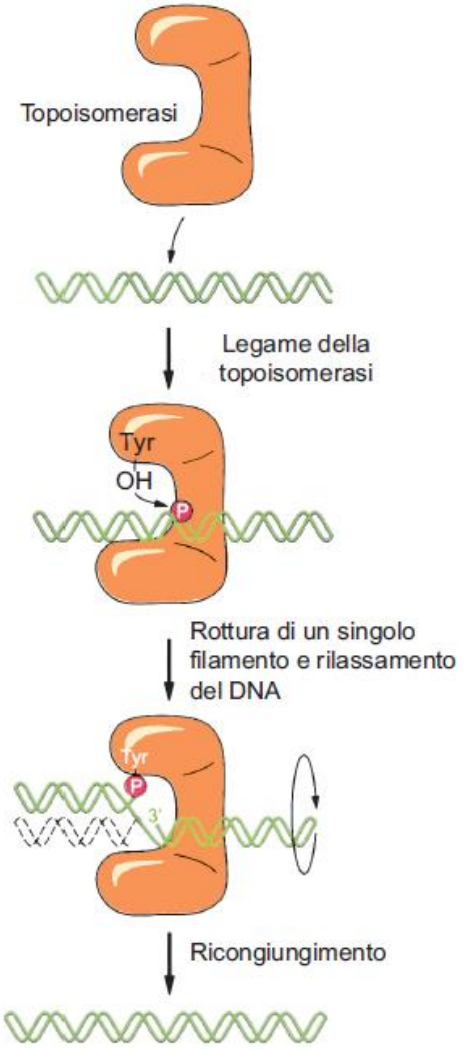


Figure 28.13 Helicase mechanism. One of the strands of the double helix passes through the hole in the center of the helicase, bound to the loops of two adjacent subunits. Two of the subunits do not contain bound nucleotides. On the binding of ATP to these two subunits and the release of ADP + P_i from two other subunits, the helicase hexamer undergoes a conformational change, pulling the DNA through the helicase. The helicase acts as a wedge to force separation of the two strands of DNA.

REPLICATION

INITIATION

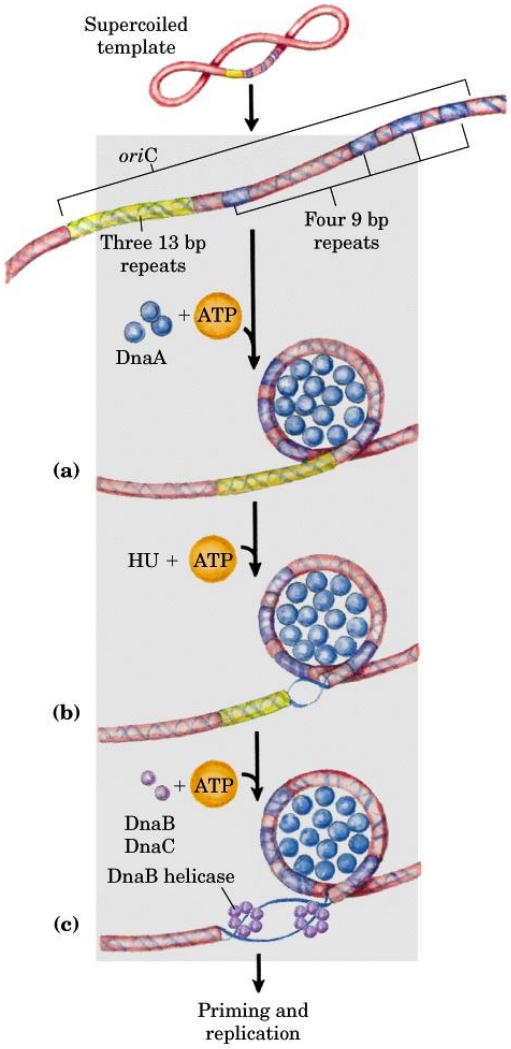
3) **Topoisomerase** eliminates supercoiling of DNA strands



3) **Topoisomerase binds** near the starting fork of a strand and reduces the twist generated by the opening of the double helix. The strand is cut and then when the DNA is relaxed, the strand is reunited.

REPLICATION

INITIATION



1. 8 molecules of **DnaA** (ATPase linked with ATP) bind **OriC site.**

2. DNA coils forming a right-handed helix that favours the opening of A-T-rich regions; the mechanism is stabilised by HU (histone like protein).

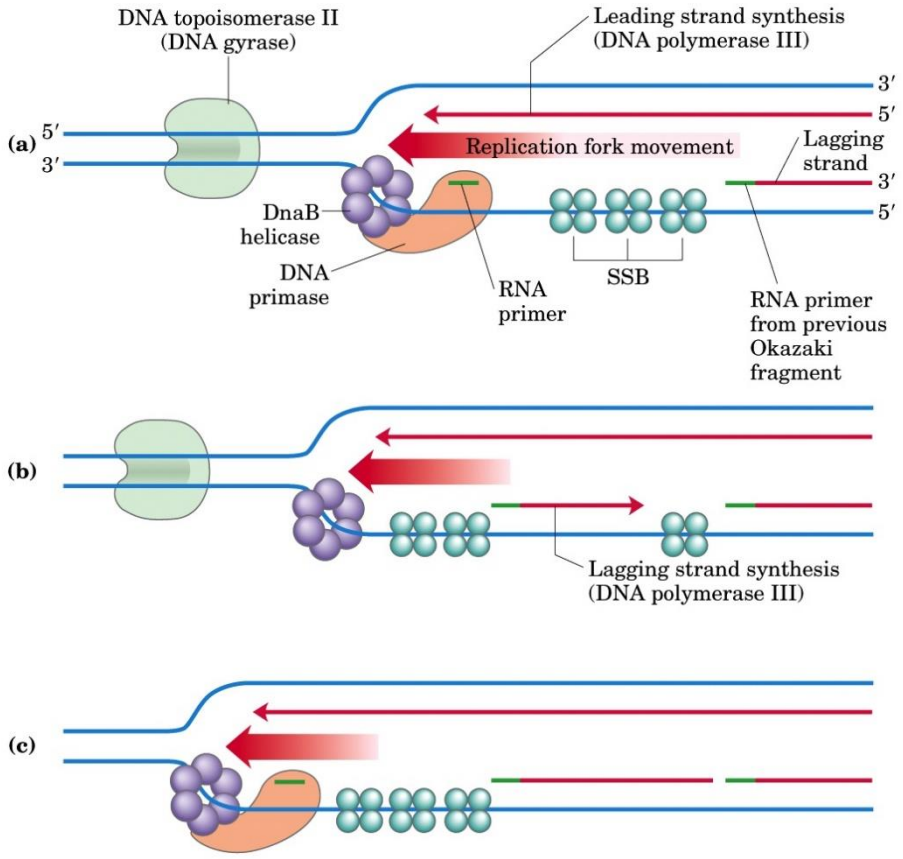
3. **DnaC** loads **DnaB Helicase** (hexamer) on the slow strand. The DnaB-DnaC interaction activates DnaB helicase. DnaC hydrolyses ATP to ADP and detaches, leaving DnaB to open the replication fork**.**

Protein DnaC is an ATPase that binds to ssDNA and DnaB (helicase) if linked with ATP

REPLICATION

INITIATION

4. **Primase** binds to **DnaB**. **SSB** proteins bind to the separated strands to stabilise them. **DNA gyrase (Topoisomerase II)** relieves tension due to supercoils.



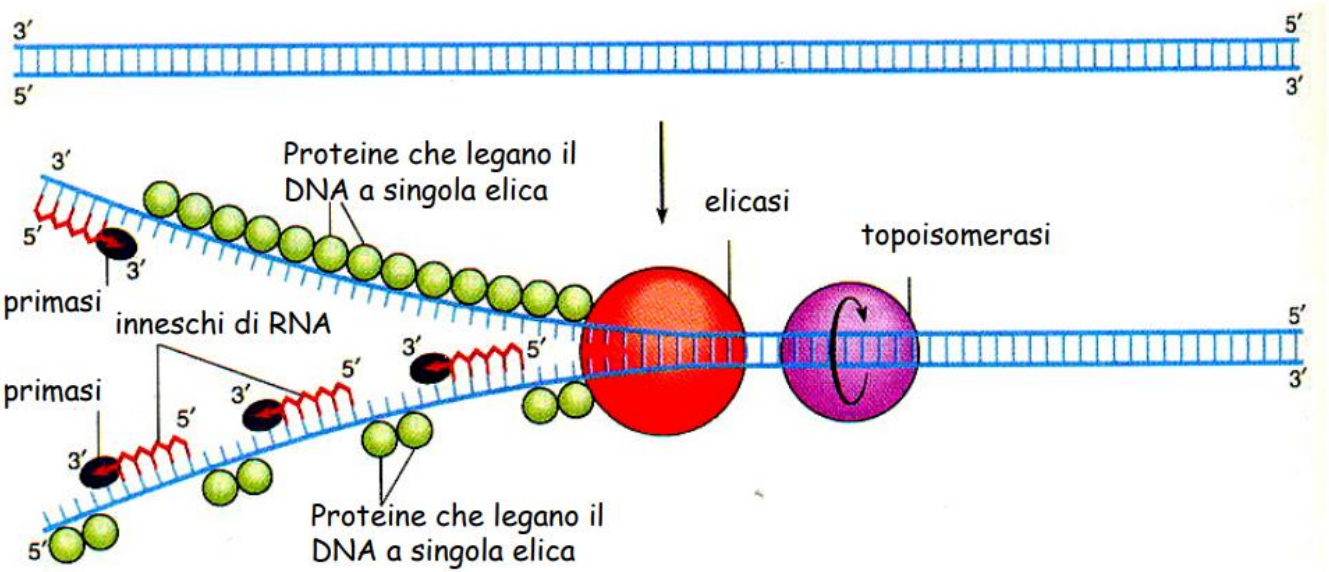
REPLICATION

INITIATION

Primase (DnaG) synthesises short sequences of RNA using DNA as a template (10-60 nt).

These RNA fragments act as primers for DNA synthesis.

In fact, DNA polymerase is incapable of starting a totally new DNA strand, but proceeds by only adding nucleotides to other nucleotides already paired in a double strand.



REPLICATION

INITIATION

When **DNA polymerase III** binds to DNA, it generates the signal for the completion of the initiation step.

The **Hda protein (ATPase)** binds to **DNA polymerase III** and interacts with the **DnaA protein causing ATP hydrolysis**.

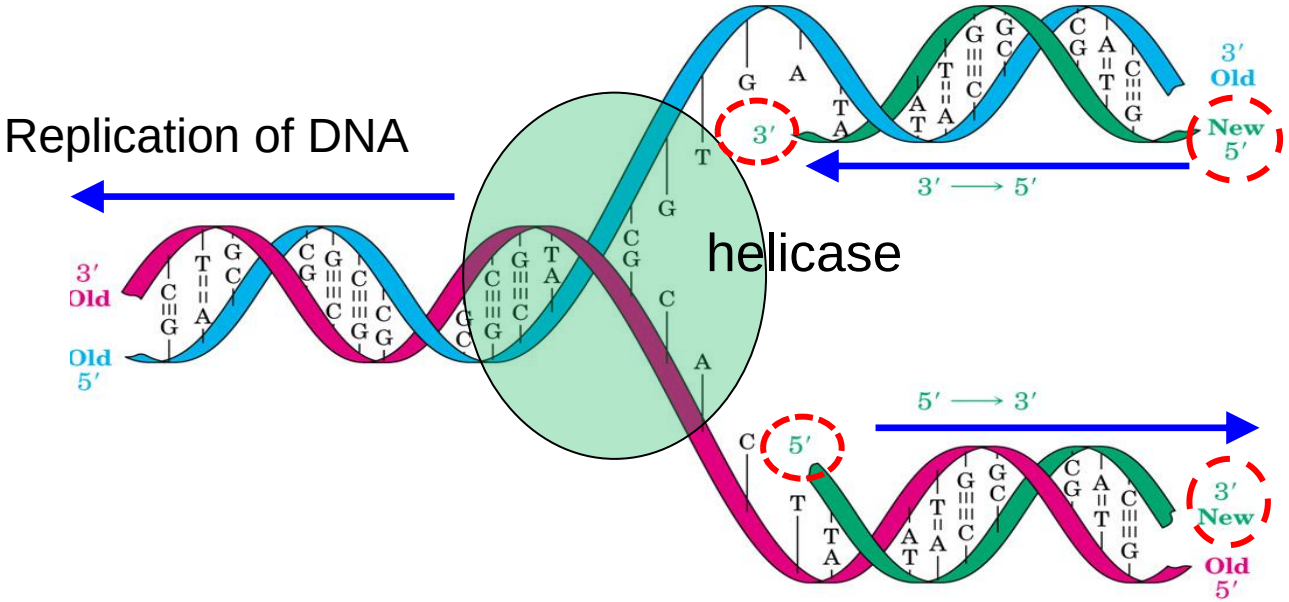
5. The **DnaA-ADP complex disassembles** from its site of origin (ATP reloading takes 20-40 minutes).

6. **DNA adenine methylase (Dam)**, methylates the N6 position of Adenine in the palindrome sequence 5'-GATC with a protective function against restriction enzymes (DNA cutting).

REPLICATION

INITIATION

Helicases expose the DNA bases (replication fork) so that replication can occur. They hydrolyse ATP to break the H-bond between the strands.

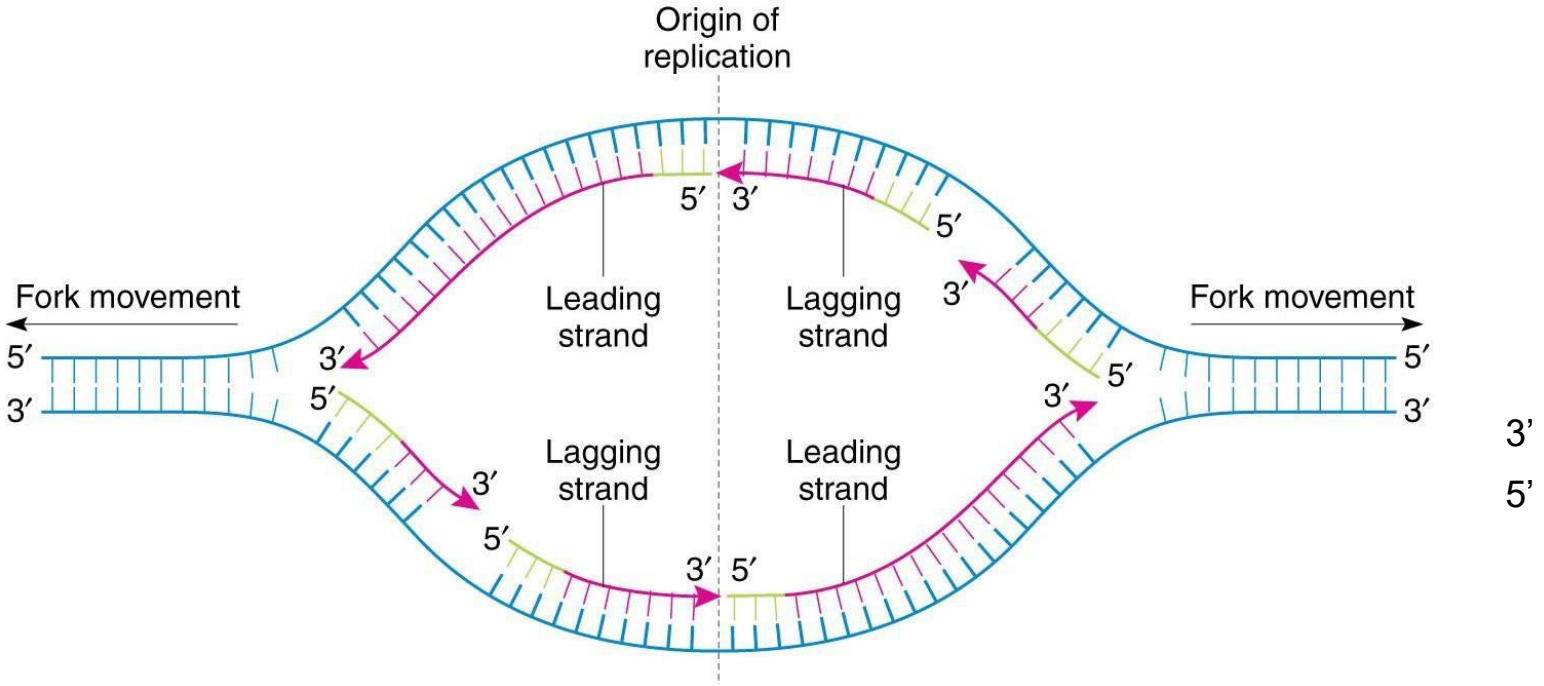


To prevent denatured strands from appearing again before replication, **DNA SINGLE STrand BINDING PROTEINS (SSB proteins)** intervene.

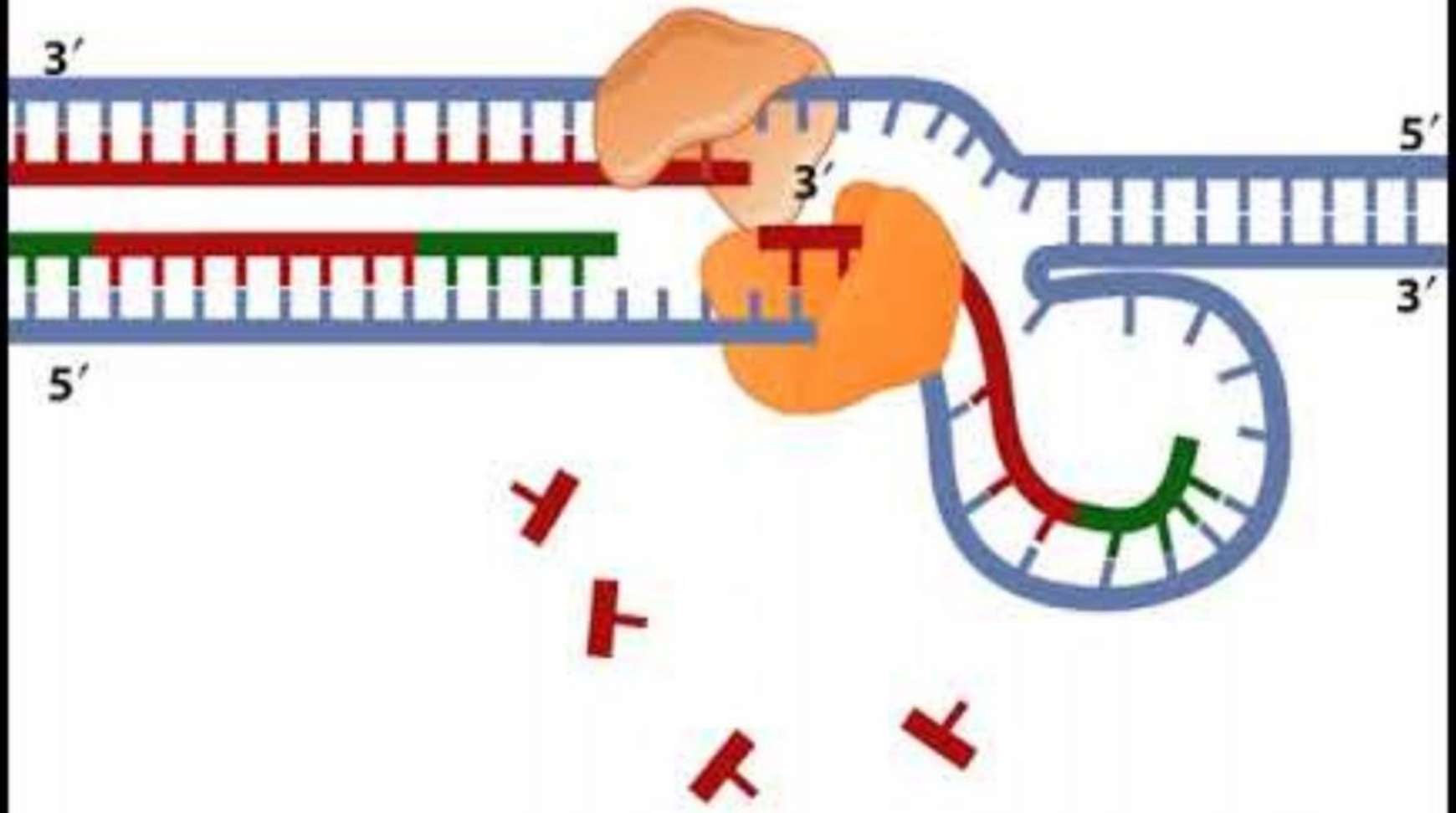
REPLICATION

ELONGATION (5’-3’)

- DNA acts just as a template.



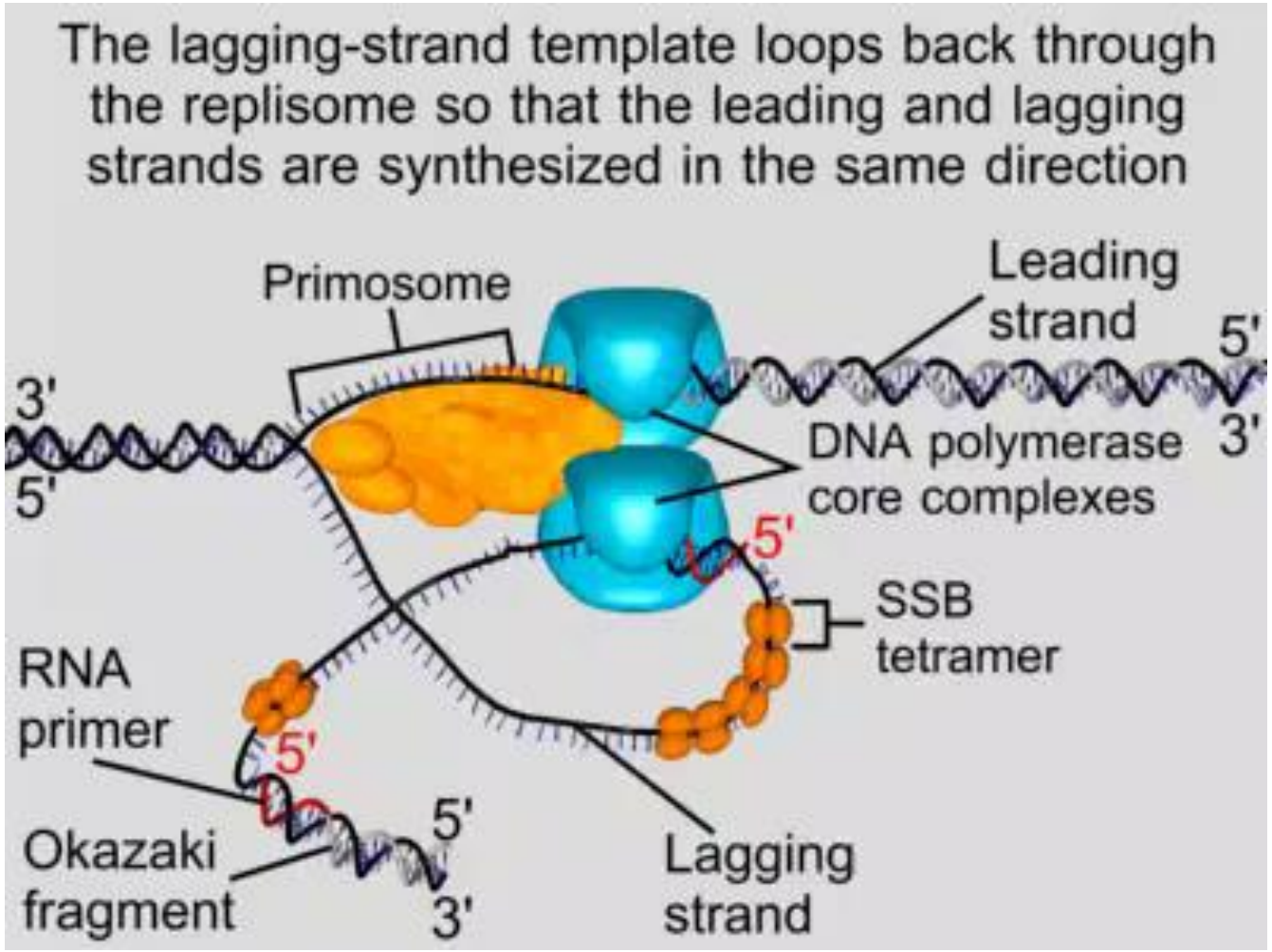
- A newly synthesised filament is slow at one replicative fork (lagging strand) and fast at the other (leading strand)



As a result, the newly synthesized lagging strand fragment loops out between the polymerase and the fork.

REPLICATION

ELONGATION

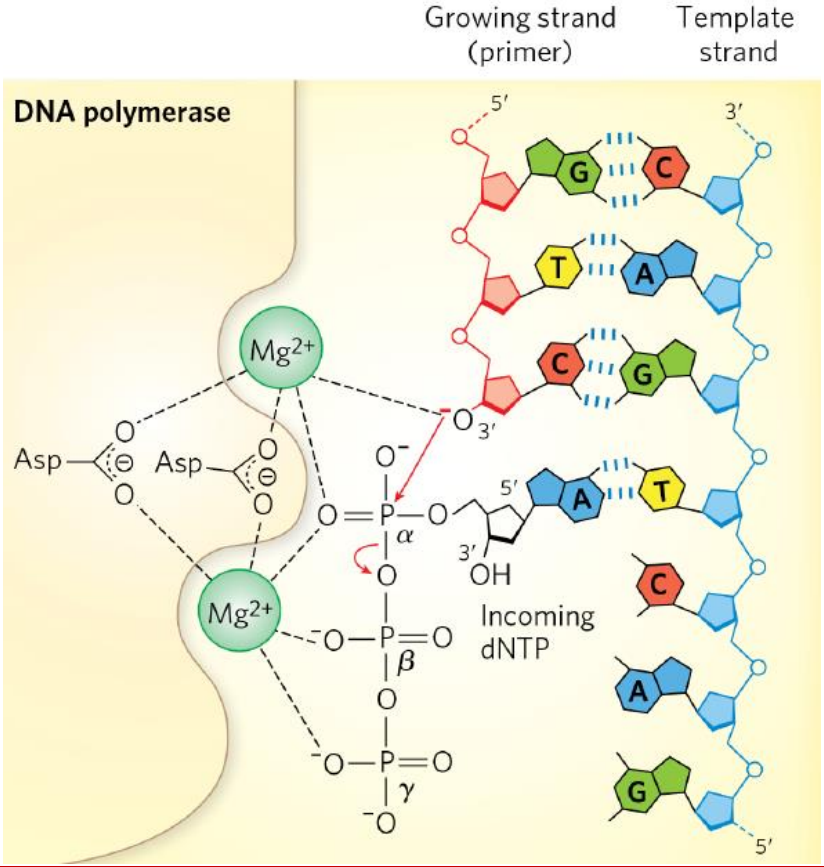


REPLICATION

ELONGATION

$$(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + PP_i$$

DNA polymerase III

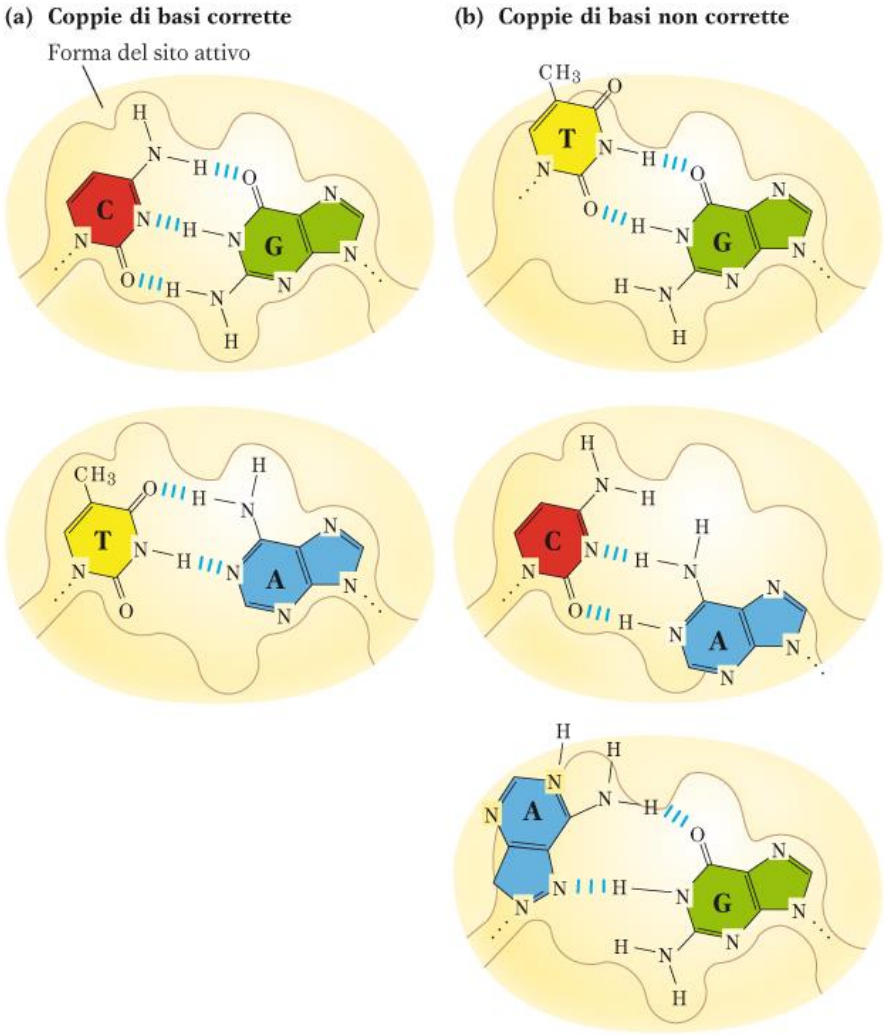


-OH in 3' attacks the α-phosphate.

Mg^{2+} ions act as cofactors stabilizing the negative charges on the deprotonated –OH moiety in 3', the phosphate backbone of DNA, and aspartate residues in the catalytic site allowing for proper strand separation during the polymerization process.

REPLICATION

ELONGATION



The geometry of nucleotide pairs contributes to the fidelity of replication.

The correct pairings:

A=T

C=G

have a very similar geometry.

Wrong pairings cannot enter the active site of the enzyme.

REPLICATION

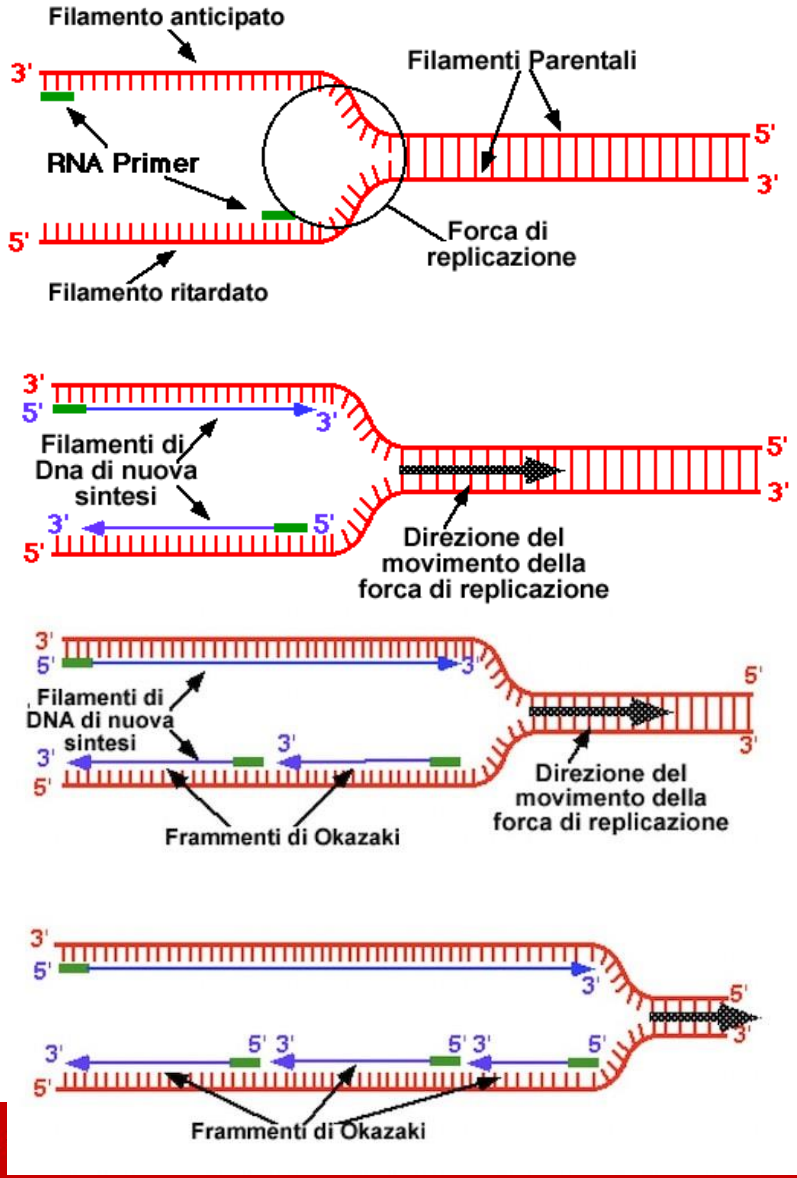
The two ‘child’ filaments are synthesized in two different ways:

Leading strand (Fast strand)

- An RNA primer is produced at Origin
- DNA pol III binds nucleotides in a 5‘-3’ direction sliding towards the fork opening

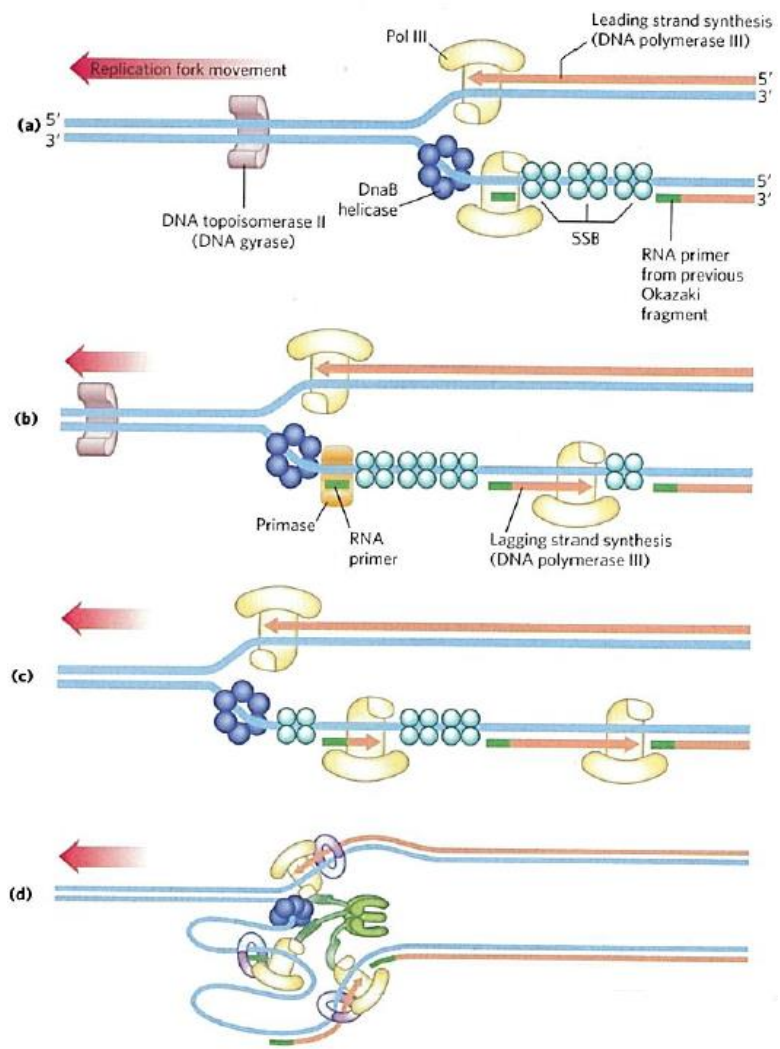
Lagging strand (slow chain)

- The synthesis is always in the 5‘-3’ direction
- Many RNA primers needed
- DNA pol III uses RNA primers to synthesize DNA fragments (1000-2000 nt).



REPLICATION

Synthesis of Okazaki fragments (OF)



a. At regular intervals, primase synthesises an RNA primer for a new OF.

b. Each primer is extended by DNA pol III.

c. DNA pol III continues up to the previous OF.

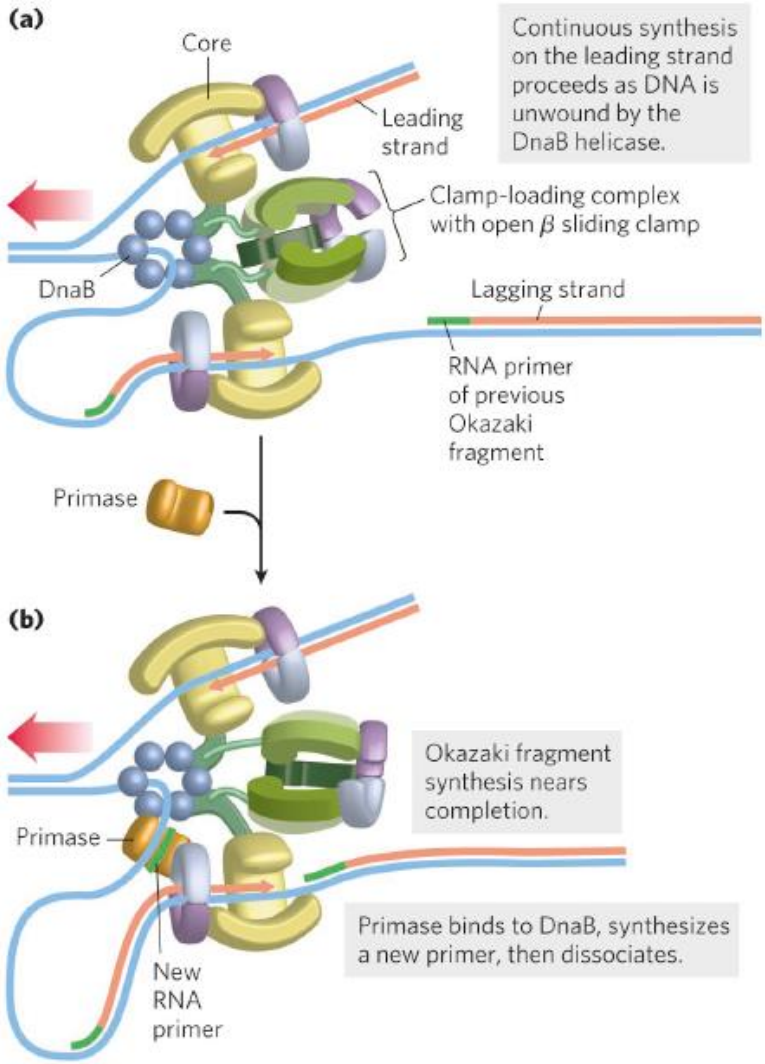
d. Each DNA pol III has 3 subunits, so it can synthesise 1 or 2 OFs simultaneously and attach to the fast strand.

REPLICATION

ELONGATION

a. DnaB (helicase) runs along the slow filament in the 5'→3' direction and unwinds the DNA

b. DnaG (primase) associates with DnaB and synthesises a small RNA primer

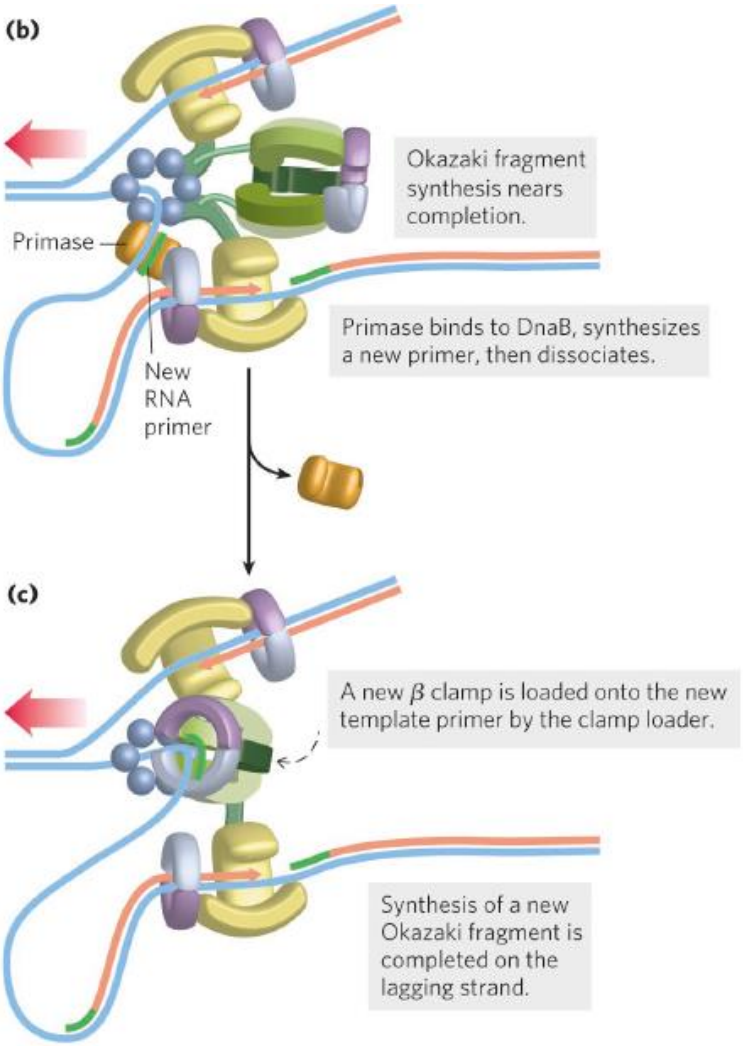


From Lehninger: Principles of Biochemistry, 7th Ed.

REPLICATION

ELONGATION

c. A sliding clamp, β subunit of DNA polymerase III, is placed on the primer

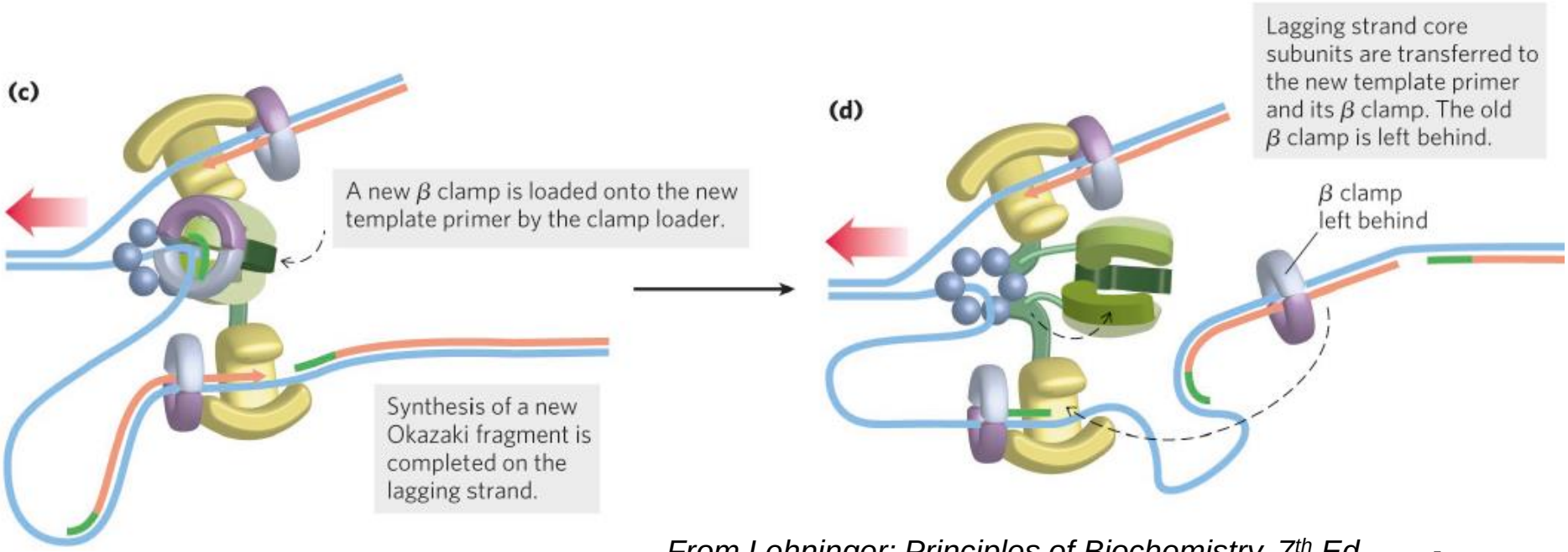


From Lehninger: Principles of Biochemistry, 7th Ed.

REPLICATION

ELONGATION

d. Replication stops after the complete synthesis of an Okazaki fragment and the sliding clamp (β subunit of DNA polymerase III) detaches

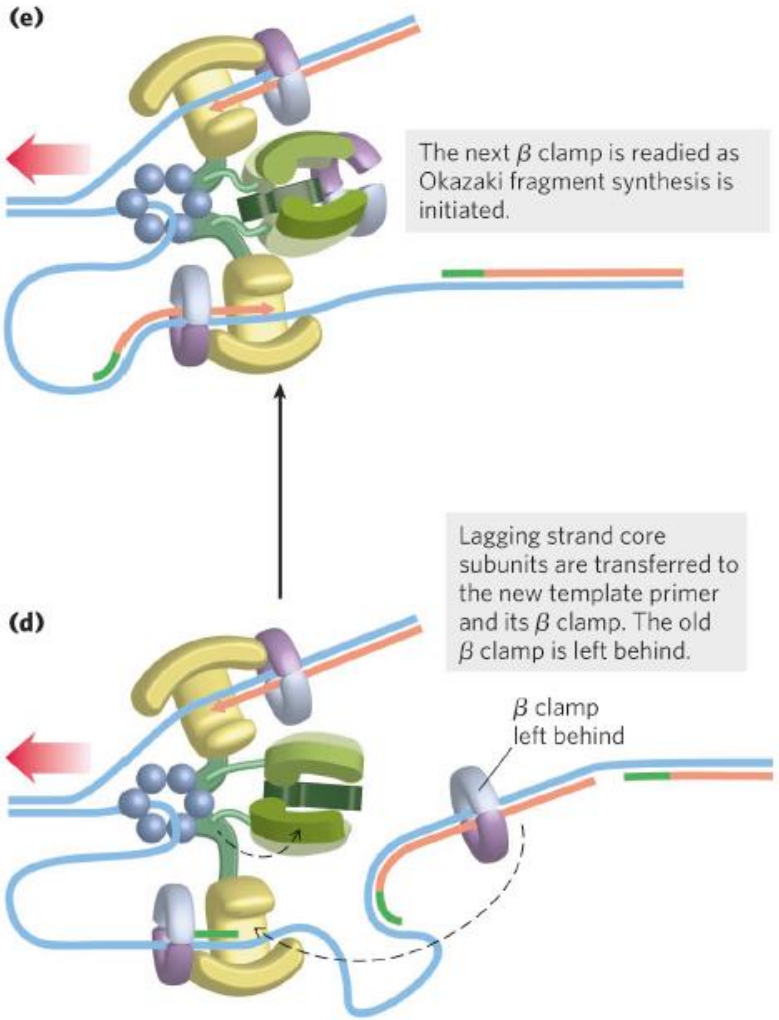


From Lehninger: Principles of Biochemistry, 7th Ed.

REPLICATION

ELONGATION

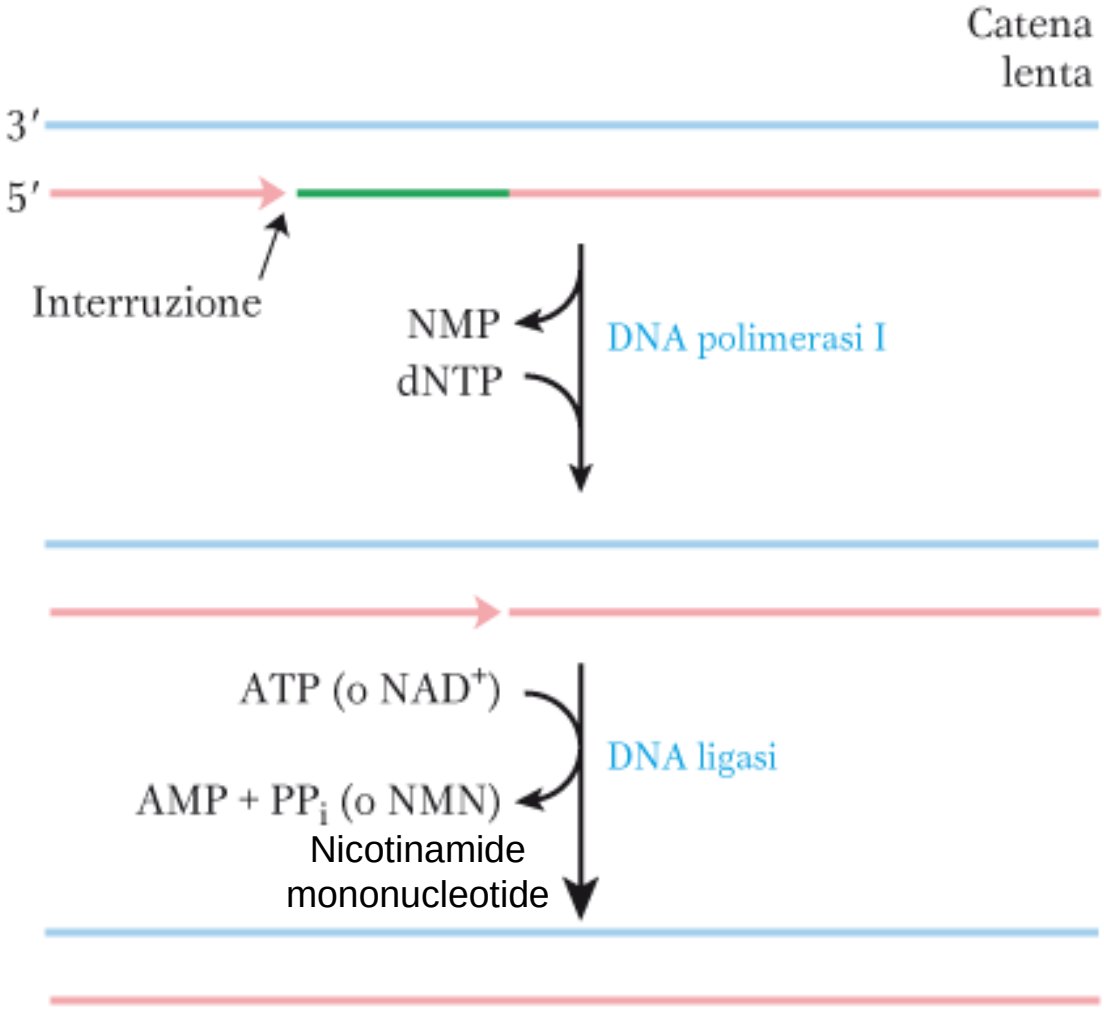
e. DNA polymerase III combines with a new sliding clamp to initiate the synthesis of a new Okazaki fragment



From Lehninger: Principles of Biochemistry, 7th Ed.

REPLICATION

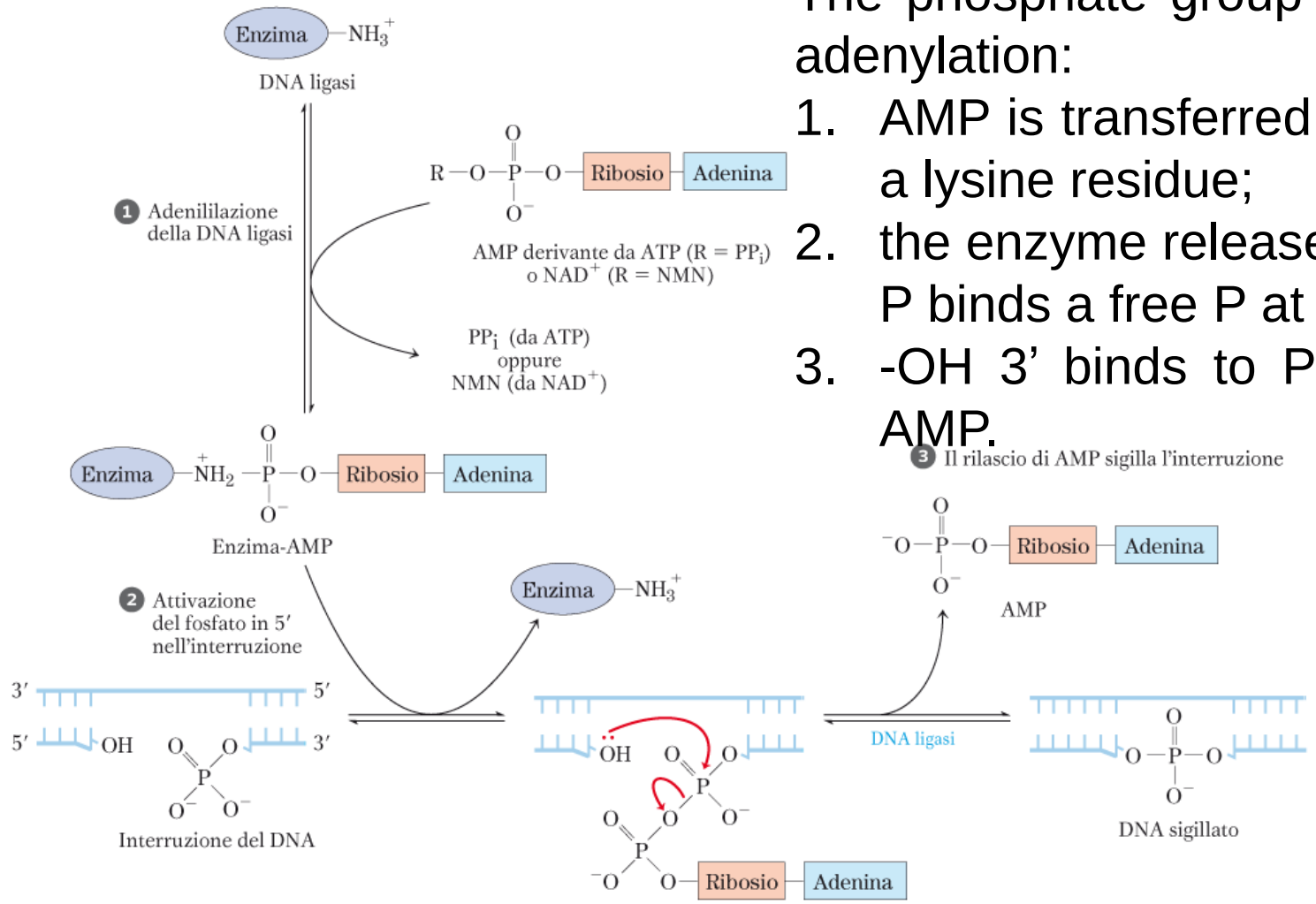
DNA ligase



DNA ligase catalyses the formation of a phosphodiester bond between the -OH at the 3‘ end of one DNA chain and the phosphate at the 5’ end of another chain. This process requires energy from the hydrolysis of **ATP** and **NAD+.**

REPLICATION

DNA ligase



The phosphate group is activated by adenylation:

1. AMP is transferred to the ligase in a lysine residue;
2. the enzyme releases AMP and the P binds a free P at 5', activating it;
3. -OH 3' binds to P and displaces AMP.

REPLICATION

TO RECAP

Topoisomerase II (introduces or eliminate supercoils DNA) – prevents DNA breakage as a result of torsion

Helicase (DnaB protein) – separates the two strands

Primase (DnaG protein) – synthesises RNA primers

Single strand binding proteins (SSB proteins)– prevent renaturation of the double strand

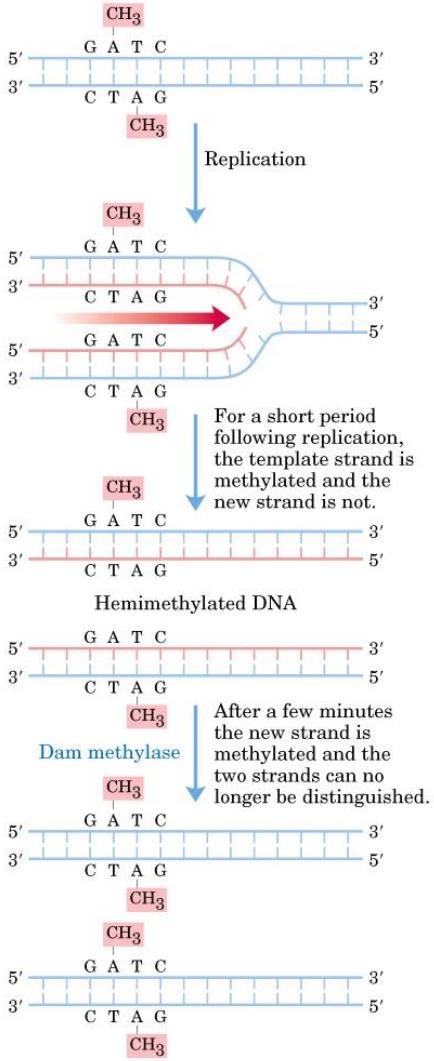
DNA polymerase III – synthesis of the new strand

DNA polymerase I - removal of RNA primers and gap filling with NTP

DNA ligase – seals cuts due to the removal of RNA primers through phosphodiester bonds

REPLICATION

methylation



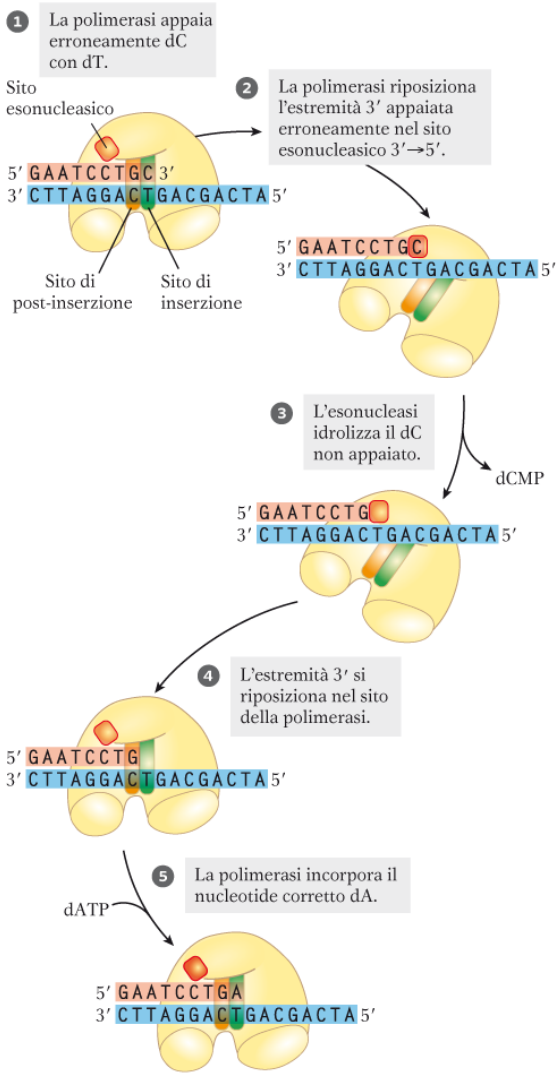
- When GATC sites in OriC are fully methylated, they allow for effective binding of DnaA, the ATPase protein essential for initiating replication.

- In contrast, when GATC sites are hemimethylated (as they are immediately after DNA replication), they do not promote initiation.

This mechanism ensures that a new round of replication does not start until the previous round is complete and the DNA is fully methylated again

REPLICATION

DNA-repair systems



DNA polymerase III has 3' → 5' exonuclease activity to correct mistakes (*proofreading activity*): the enzyme removes the incorrect nucleotide by preventing translocation.

This mechanism makes it possible to reduce base-pairing errors to $1/10^6$-10^8

REPLICATION

DNA-repair systems

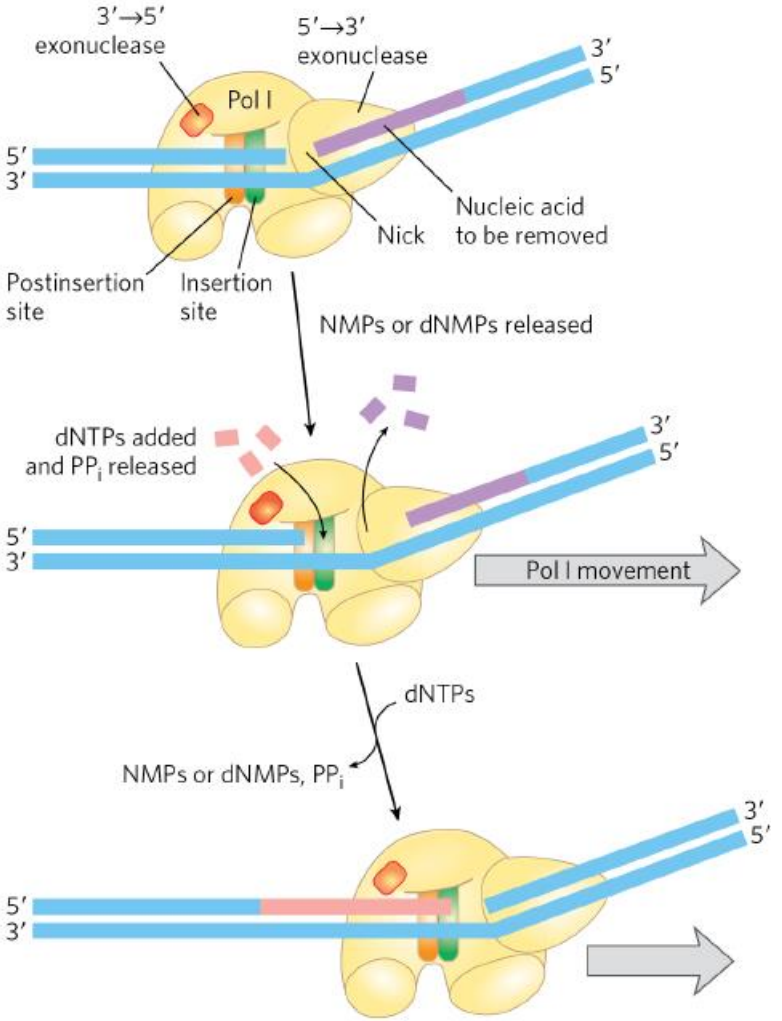
DNA polymerase I activities:

- **DNA Repair**: it has 5’ → 3’ exonuclease activity named **nick** (breakage) **translation.**

- **Removal of RNA Primers**: It is used also to remove the RNA primer

- **Polymerization Activity**: Pol I exhibits 5' to 3' polymerase activity crucial during both replication and repair processes

- **Proofreading Capability**: Pol I contains a 3' → 5' exonuclease activity, which allows it to proofread newly synthesized DNA.



REPLICATION

DNA-repair systems

Damaged proteins or RNA molecules can be replaced using the information contained in DNA.

DNA molecules are NOT replaceable.

Mutation: a permanent change in nucleotide sequence

Mutation by substitution: one bp is replaced by another

Insertion Mutation: addition of a bp

Deletion Mutation: removal of a bp

Silent mutation: mutation involving a non-coding part of DNA or not affecting the function of a gene

REPLICATION

DNA-repair systems

Types of damage

1. DAMAGE FROM REPLICATION ERRORS
2. DAMAGE BY ENVIRONMENTAL FACTORS: UV radiation, alkylating agents
3. DAMAGE FROM OXIDATIVE STRESS (ROS): formation of 8-oxoguanine

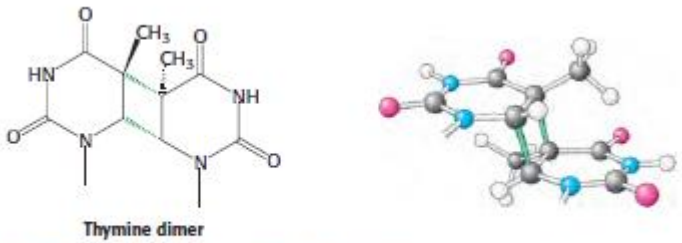


Figure 28.33 Cross-linked dimer of two thymine bases. Ultraviolet light induces cross-links between adjacent pyrimidines along one strand of DNA.

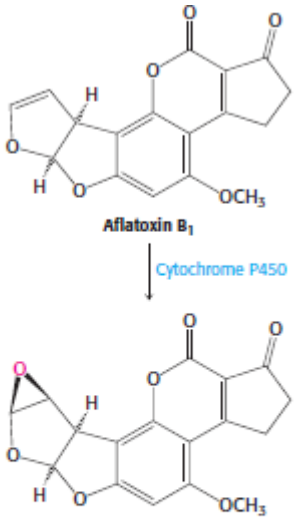


Figure 28.32 Aflatoxin activation. The compound, produced by molds that grow on peanuts, is activated by cytochrome P450 to form a highly reactive species that modifies bases such as guanine in DNA, leading to mutations.

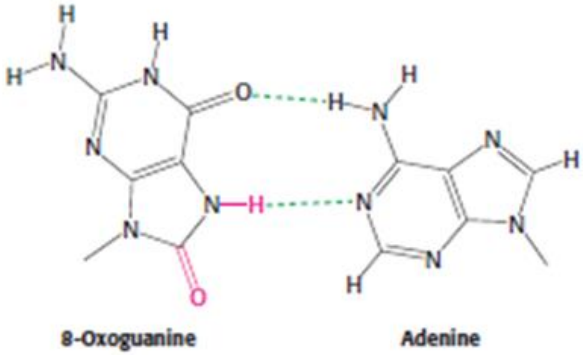


Figure 28.30 Oxoguanine–adenine base pair. When guanine is oxidized to 8-oxoguanine, the damaged base can form a base pair with adenine through an edge of the base that does not normally participate in base-pair formation.

REPLICATION

DNA-repair systems

table 25–5

Types of DNA Repair Systems in *E. coli*

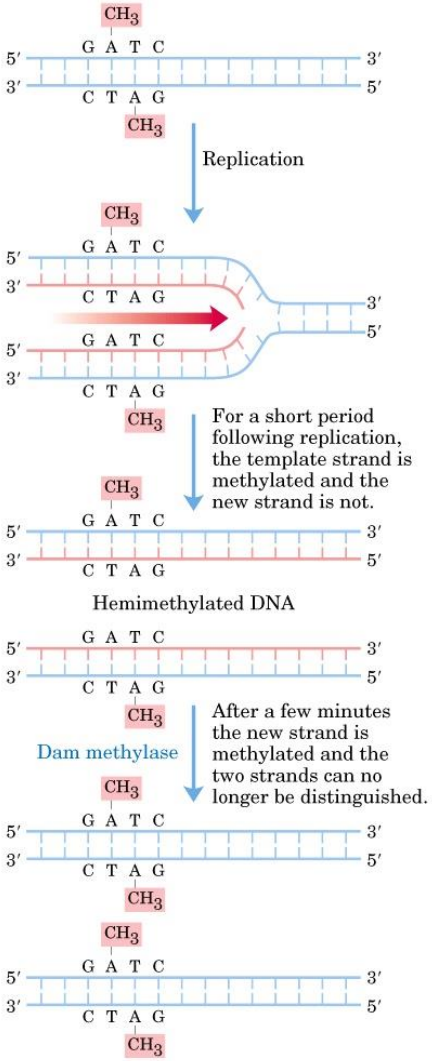
Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase	Mismatches
MutH, MutL, MutS proteins	
DNA helicase II	
SSB	
DNA polymerase III	
Exonuclease I	
Exonuclease VII	
RecJ nuclease	
Exonuclease X	
DNA ligase	
Base-excision repair	
DNA glycosylases	Abnormal bases (uracil, hypoxanthine, xanthine); 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

About 200 genes are allowed to encode proteins that repair DNA (in humans).

Many repair processes are energy inefficient (but the integrity of the genetic information is more important)

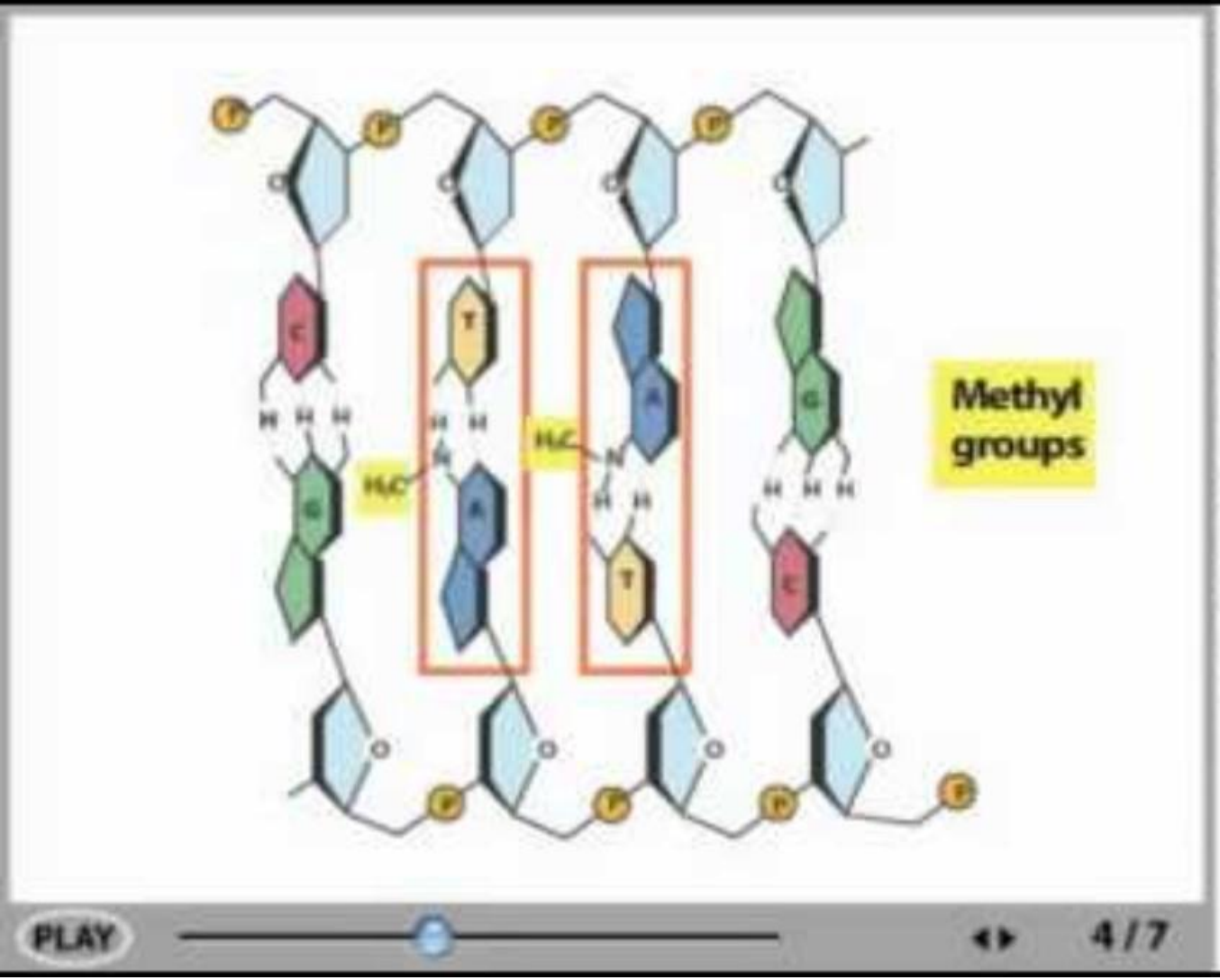
REPLICATION

Mismatch-repair system



This system scans the DNA for mismatches, in which the error is excised, removed, and replaced.

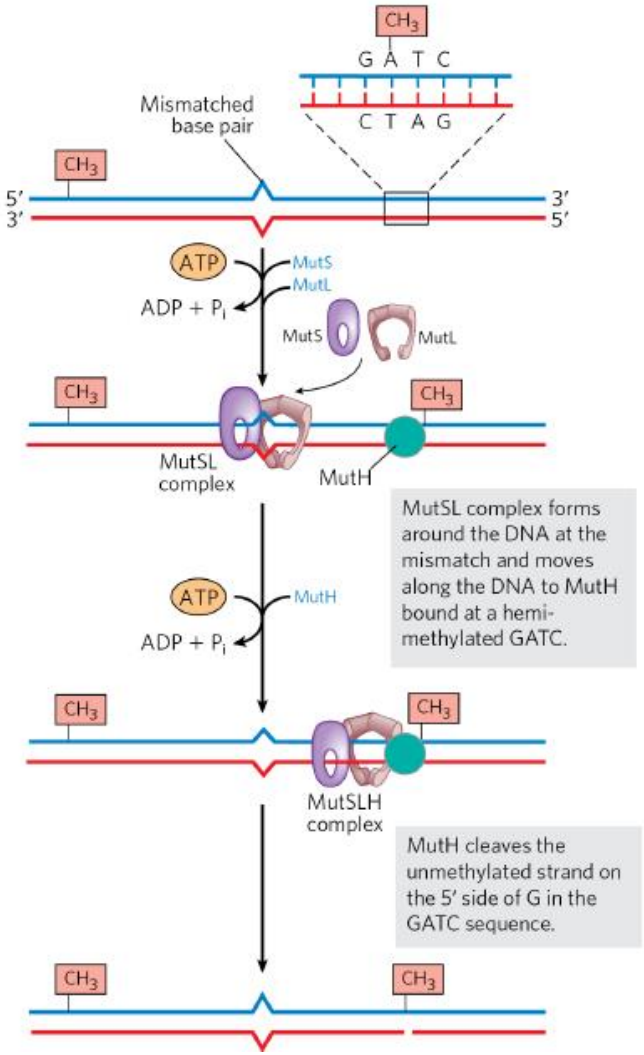
The new strand to be corrected (not yet methylated) is identified by the presence of methylated bases on the template strand.



The Dam methylase acts soon, but not immediately, after replication of a DNA sequence. Where DNA has methylation sequences, the parental strand will be methylated, but the new strand will still be unmethylated. The difference in methylation allows the cell to distinguish the correct sequence, presumably on the parental strand, from the incorrect sequence on the new strand.

REPLICATION

Methyl Directed Mismatch-repair system

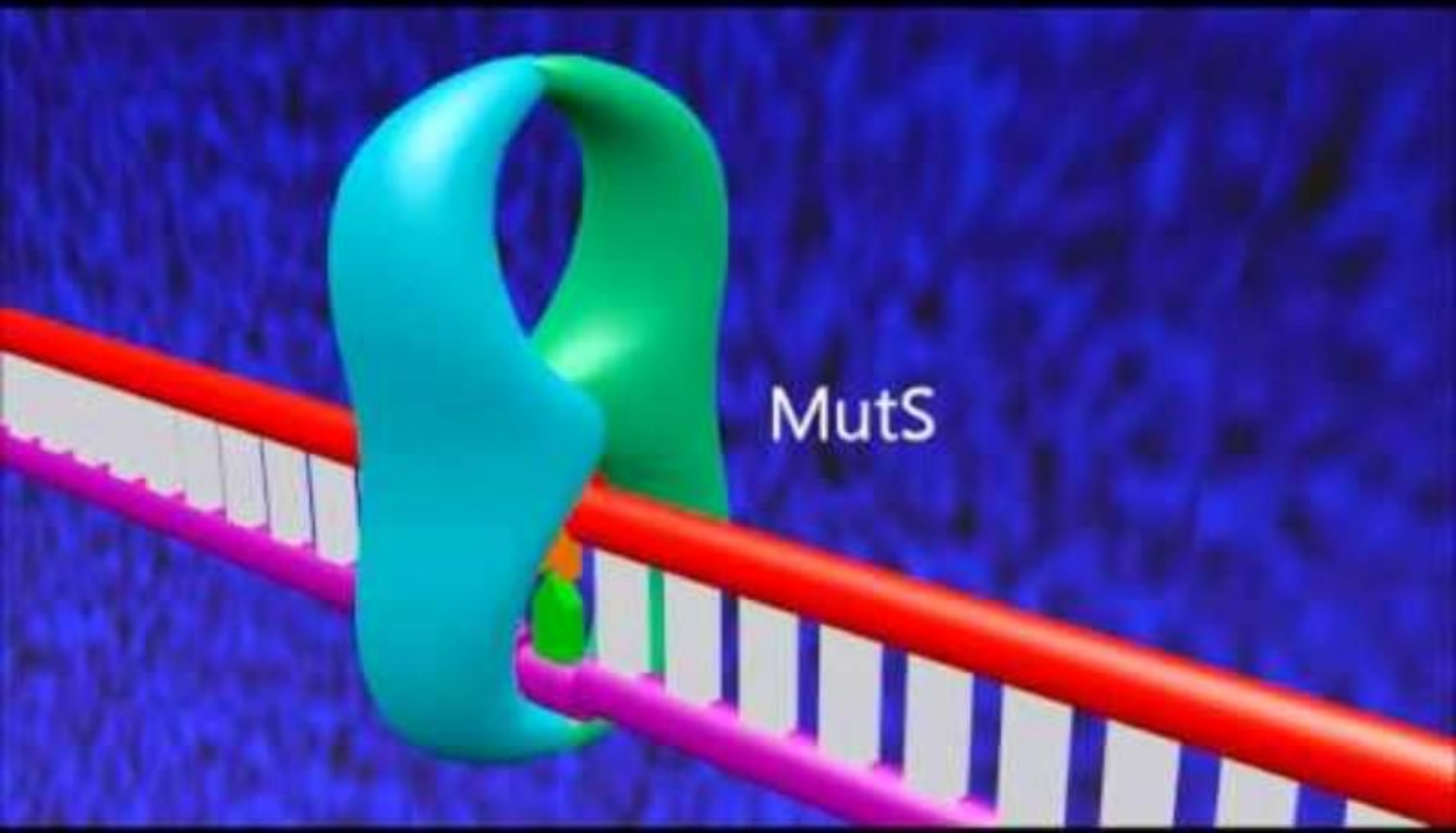


In *E. coli*, it is linked to the activity of proteins produced by the MutL, MutS, and MutH genes.

MutS recognises the mismatch;

MutH recognises a specific GATC sequence (5') and has endonuclease action; MutL binds to MutS and MutH.

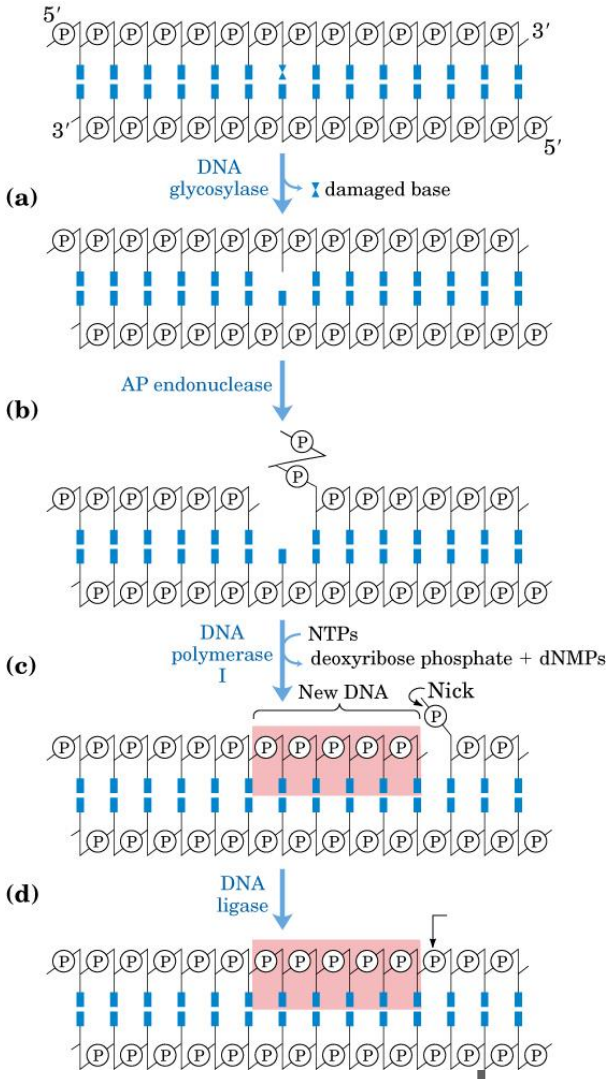
The unmethylated strand is unwound and degraded in the 3'→ 5' direction until the mismatch by exonucleases (such as ExoI or RecJ) that excise the mispaired base along with a few nucleotides beyond it. The segment is replaced by new DNA thanks to Pol III followed by ligation (ligase) to restore the integrity of the DNA strand.


MutS

REPLICATION

Base-excision repair

Mechanism of action of **DNA-N-glycosylase**: it recognises lesions in the DNA and removes the involved base by breaking the N-glycosidic bond. The removal of a base creates an apurinic or apyrimidinic (AP) site recognised by a specific endonuclease (**AP-endonuclease**) that cuts the DNA chain. DNA-Pol then takes care of the error-containing strand's deletion and replacement, and a DNA-ligase reunites the fragments.



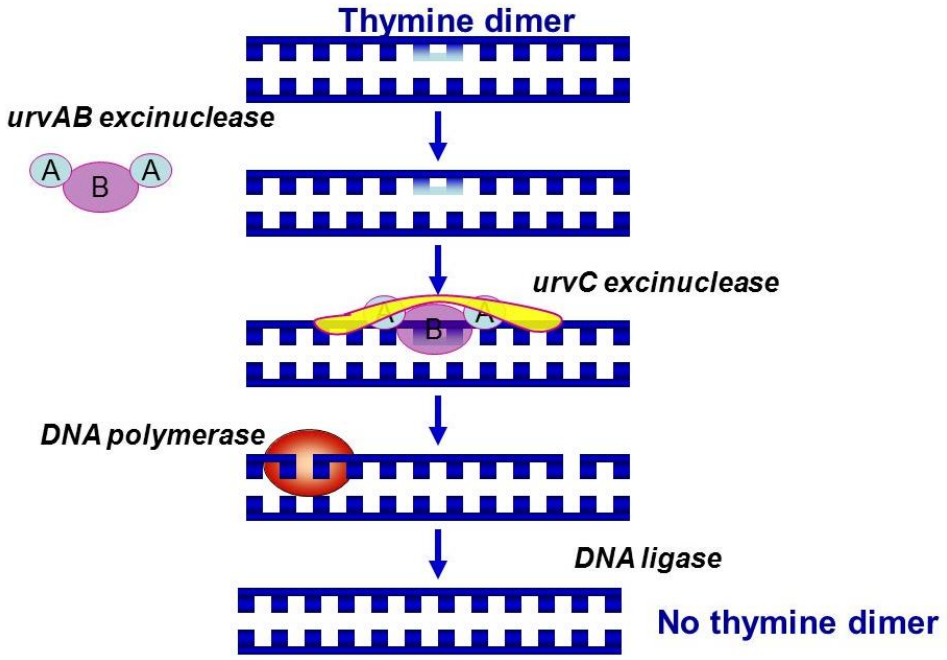
REPLICATION

Nucleotide-excision repair (NER)

Voluminous damage involving 2 to 30 nt which can include bulky adducts, thymine dimers, or other distortions in the DNA helix.

Excinucleases (endonucleases) are recruited and hydrolyse two phosphodiester bonds on both sides of the lesion, creating a single-stranded gap.

DNA polymerase reconstructs the replaced strand and DNA ligase seales the cut.



REPLICATION

Nucleotide-excision repair (NER)

Several key enzymes are involved in NER, including:

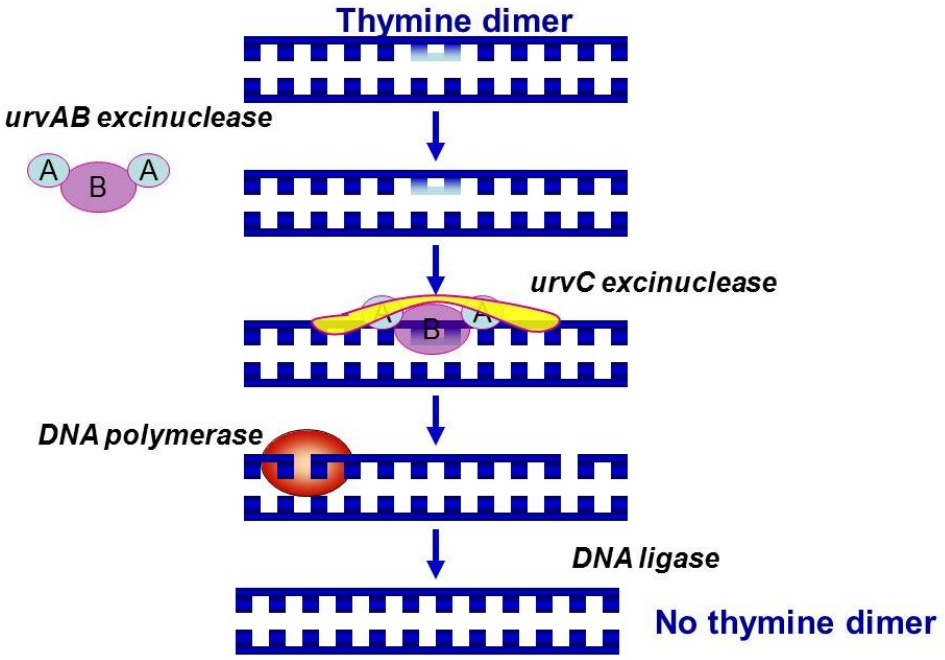
UvrA: Recognizes and binds to the damaged site.

UvrB: Joins UvrA and helps to open up the DNA around the lesion.

UvrC: Cuts the damaged strand on both sides of the lesion.

DNA Polymerase I: Fills in the gap left after excision.

DNA Ligase: Seals the newly synthesized DNA into the existing strand.

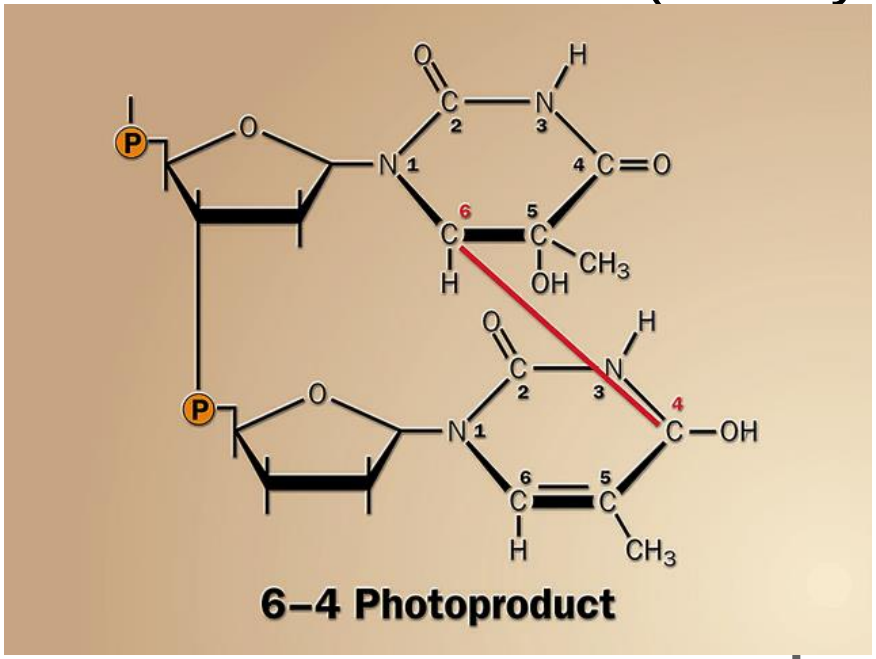


REPLICATION

Direct repair of photoproducts

DNA photoproducts caused by UV light are Pyrimidine dimers:

1. Thymine Dimers: with the formation of a cyclobutane ring structure.
2. Dimers derived from a binding between C-6 of a thymine or a cytosine and C-4 of the pyrimidine at the 3' end (usually a cytosine)



REPLICATION

Direct repair of photoproducts

Direct repair involves the direct reversal of DNA damage without the need for excision or replacement of nucleotides.

Bacteria, plants, and some non-mammalian species contain a photo reactivating enzyme called DNA photolyase, which uses light energy and acts in two stages:

1) It binds to DNA to the distorted region of DNA corresponding to the thymine dimer in a light-independent process.
2) In the presence of visible blue light (370 nm), it breaks the bonds between the two thymines.

The E. coli enzyme, a 35-kd protein that contains 2 cofactors:

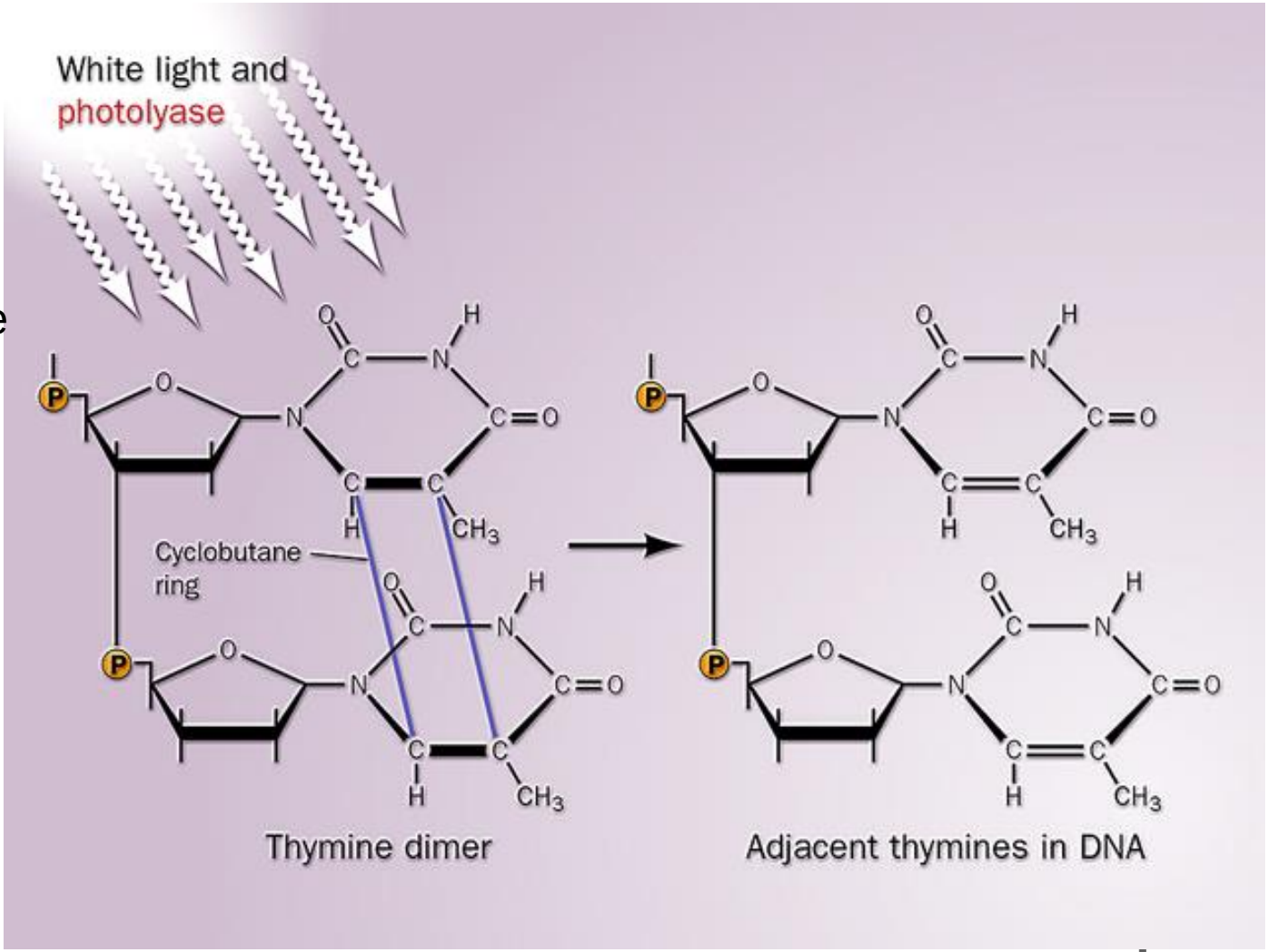
1) A flavin adenine dinucleotide (FAD)
2) N5,N10-methenyltetrahydrofolate

REPLICATION

Direct repair of photoproducts

Upon exposure to light, photolyase absorbs photons, which energizes the enzyme and allows it to break the bonds between the dimerized bases.

This restores the original bases, thus repairing the DNA without introducing any mutations.

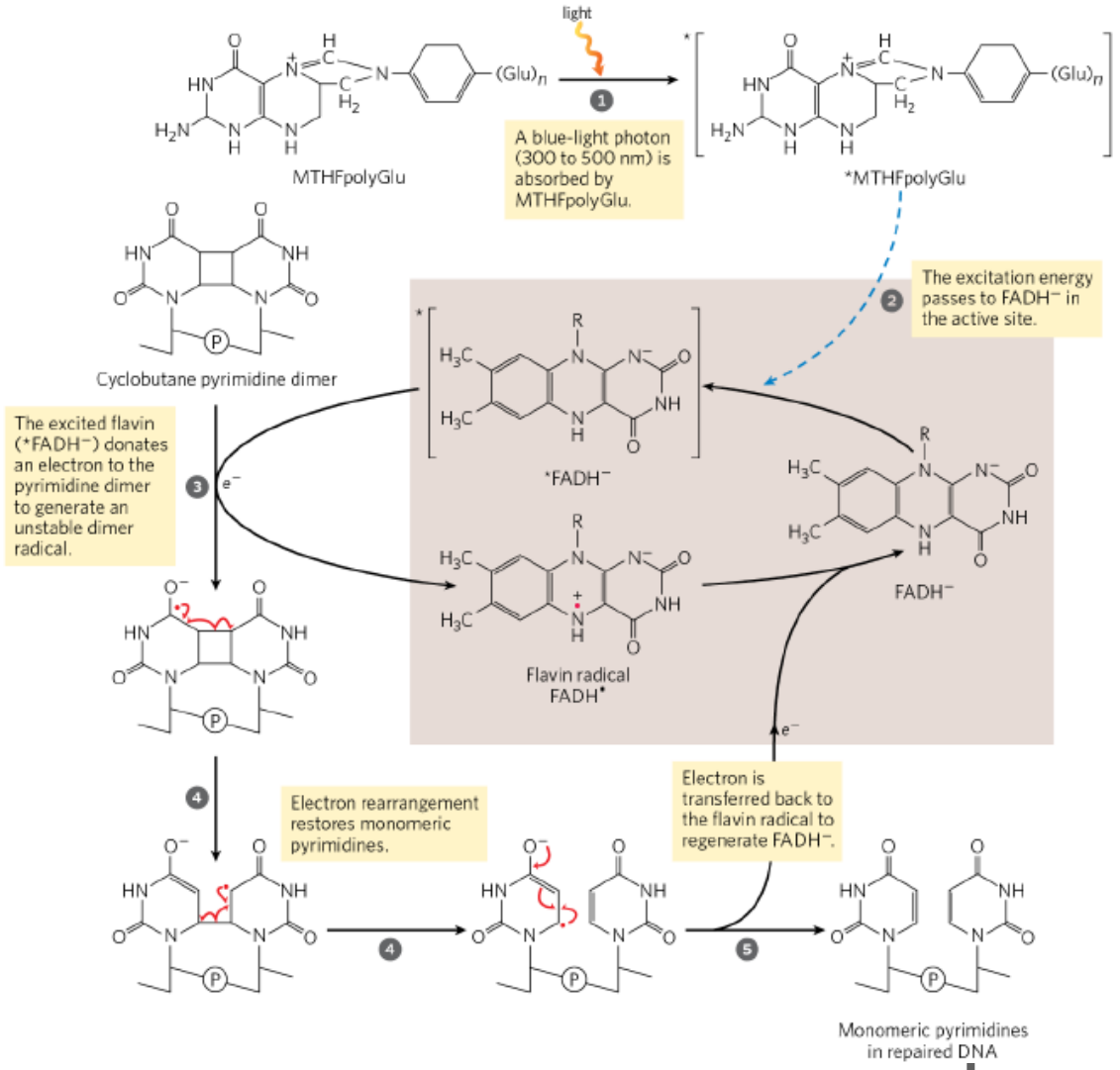


REPLICATION

Direct repair of photoproducts

DNA photolyase is NOT found in placental mammals.
Photoreactivation occurs through the formation of radical compounds.

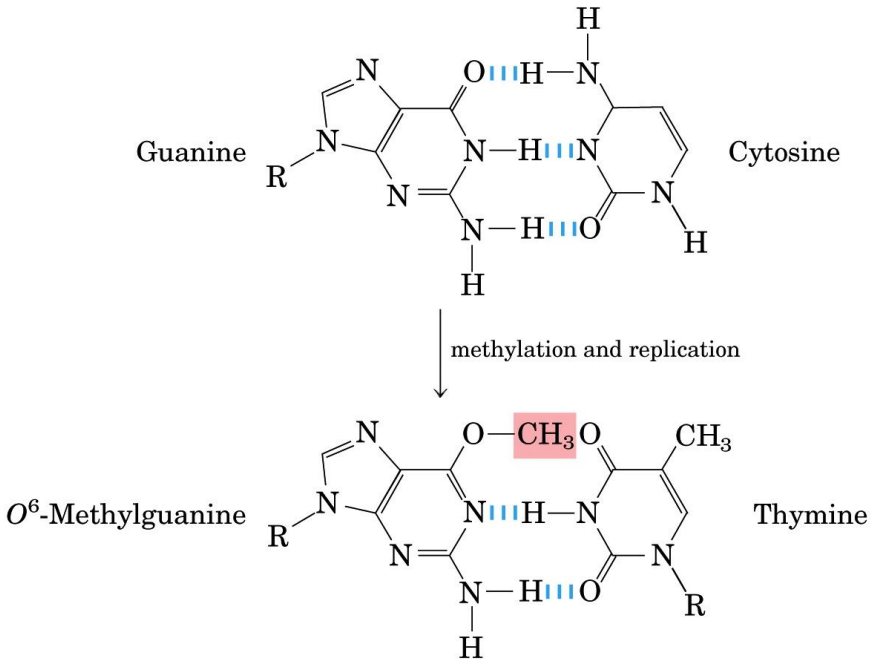
Mammals primarily rely on nucleotide excision repair (NER) to address UV-induced DNA lesions.



REPLICATION

Repairing damage from alkylating agents

They are caused by methylating or ethylating agents that introduce methyl or ethyl groups onto bases, rendering them lethal or mutagenic. The main mutagenic product is O^6methylguanine which pairs very well with Thymine facilitating the GC-GT transition.

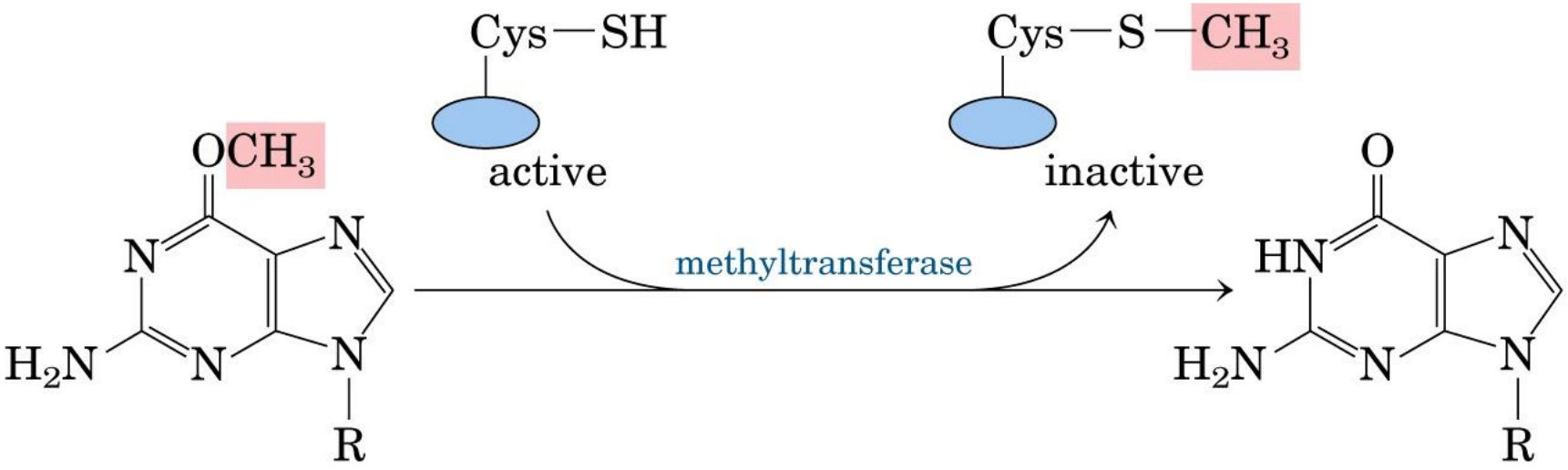


(a)

REPLICATION

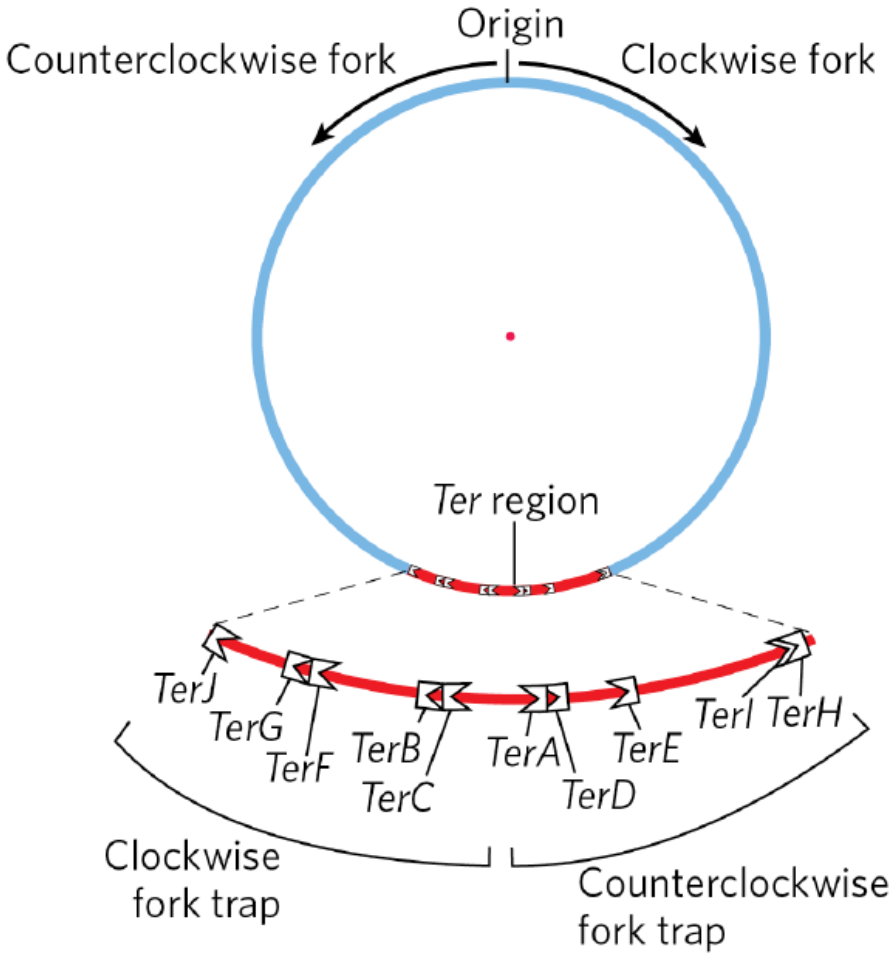
Repairing damage from alkylating agents

Enzyme **O6-methylguanine-DNA methyltransferase (MGMT) directly** repairs this type of damage: it transfers the alkyl group to a cysteine residue in its active site. The alkylated enzyme is permanently inactivated (one enzyme can only repair one damage).



REPLICATION

termination

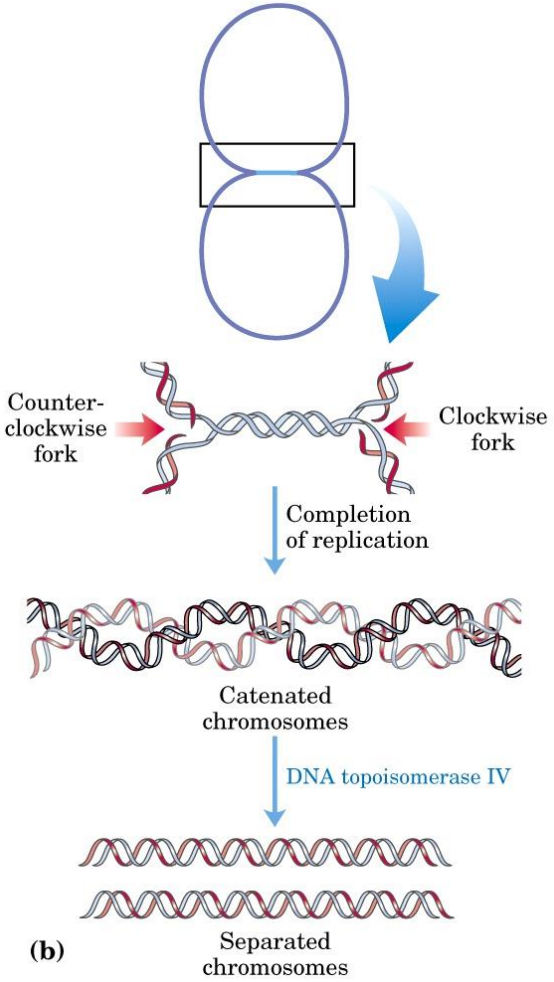


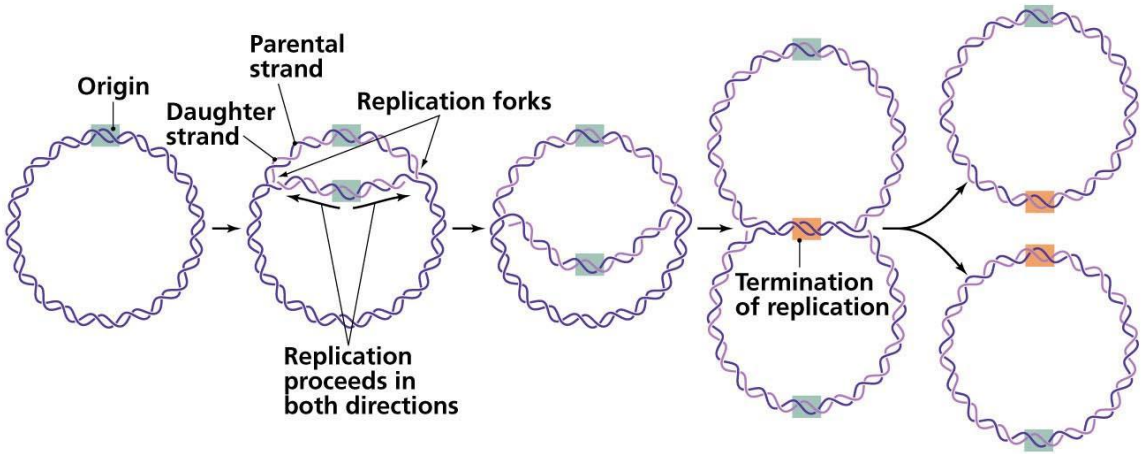
- The two replication forks of the circular chromosome of *E. coli* meet at a terminal region containing multiple copies of a sequence of 20 bp called **Ter**. There are multiple ter sites located opposite the origin of replication (oriC).
- **Tus** (terminus utilization sequence) protein binds to **Ter** and **Tus-Ter complex** stops the replication fork in one direction. The other fork locks when they meet.
- Only one Tus-Ter complex is used per cycle.

5 Ter sequences each side

REPLICATION

termination





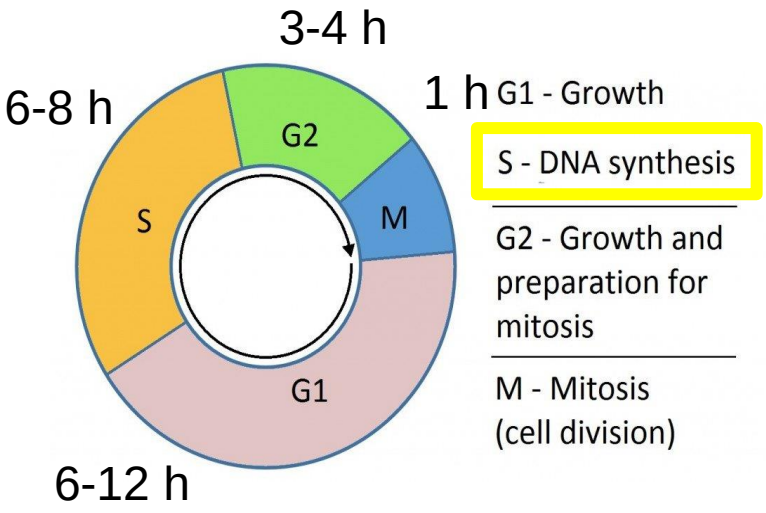
Catenanes are formed, which in *E. coli* are separated by the action of topoisomerase II.

REPLICATION

eukaryotes

Larger DNA size (over 1000 times prokaryotic DNA). Linear DNA, associated with histones in nucleosomes:

- Many origin sites (30-50k)
- Many DNA polymerases



DNA polymerase α: is associated with a primase and adds a small strand of the RNA primer to DNA.

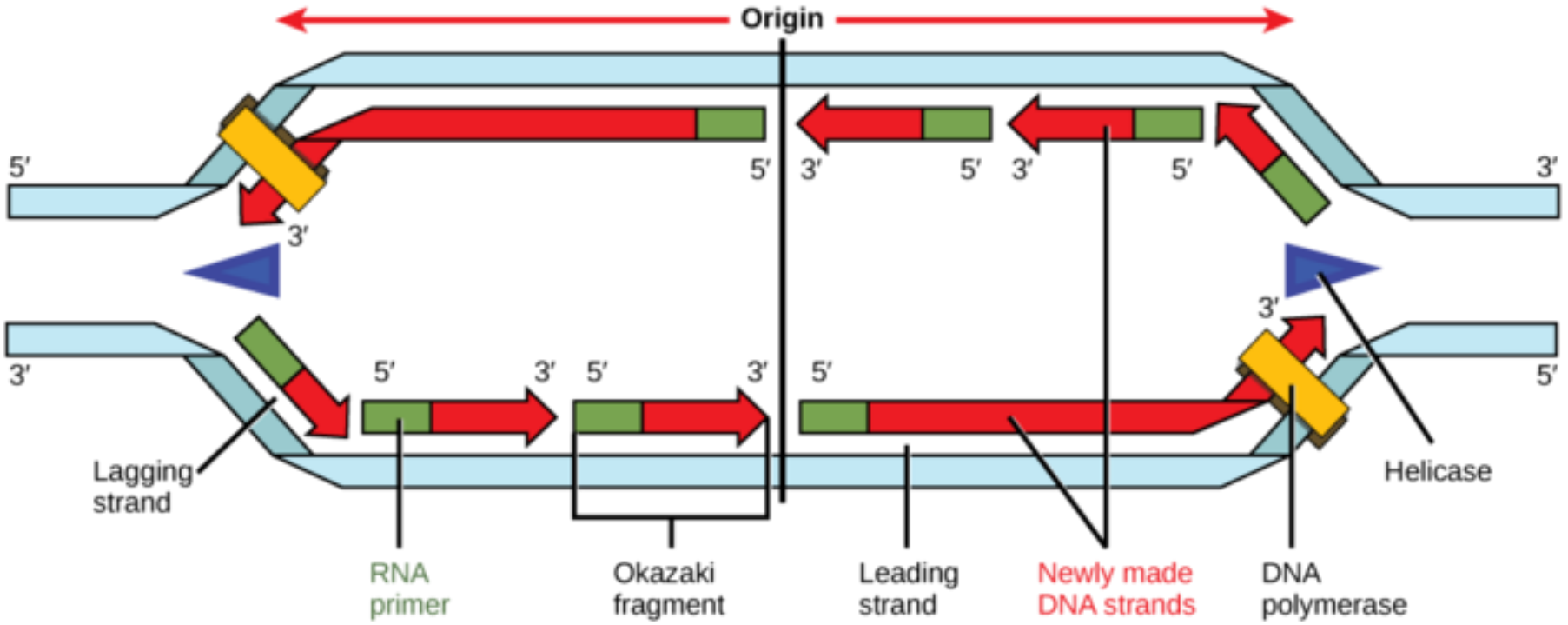
DNA polymerase β: is involved in DNA repair, particularly in base excision repair processes (BER).

DNA polymerase γ: is involved in mitochondrial DNA replication. It has both polymerase and exonuclease activities

DNA polymerase δ: It synthesises the **lagging strand.** When the polymerase reaches the previously synthesised Okazaki fragment, it continues to move along the strand, displacing the RNA primer, which is then removed by an endonuclease (FEN-1) and ribonuclease H. It has proofreading capabilities due to its 3' to 5' exonuclease activity.

DNA polymerase ε: It synthesises **the leading strand** and intervenes in DNA repair mechanisms following nucleotide excision repair processes (NER), it has proofreading activity.

DNA polymerases η, θ, ι: intervene when DNA is damaged, they are called by-pass polymerases.

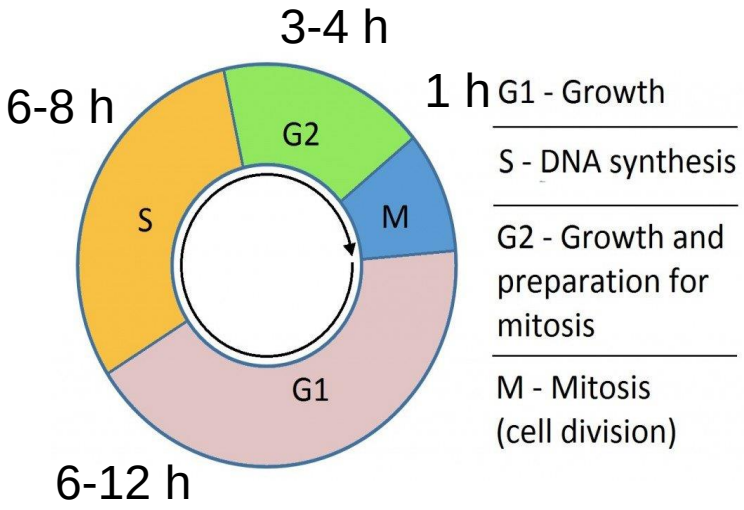


Replication is bi-directional as in bacteria.

REPLICATION

eukaryotes

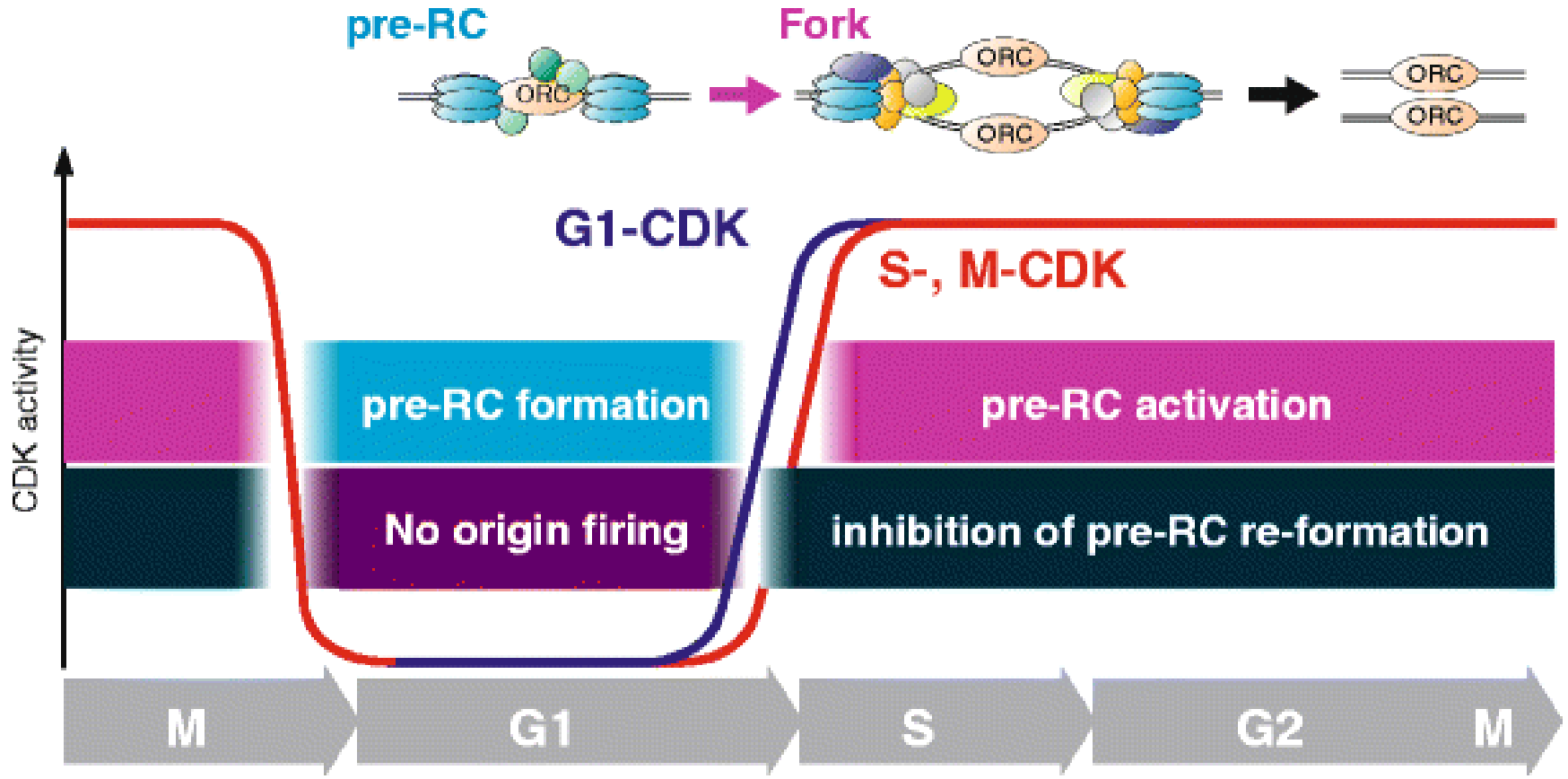
The regulation of DNA replication is given by **CYCLINES** (cell cycle-regulating proteins): the cyclins are ubiquitinated at the end of M-phase marking them for degradation by the proteasome.
The removal of cyclins allows the formation of **pre-replicative complexes (pre-RC)** in G1 phase. The formation of these complexes allows the cell to initiate replication.



Cyclins are proteins that regulate the cell cycle **by activating cyclin-dependent kinases (CDKs).** These complexes control various phases of the cell cycle, including DNA replication.

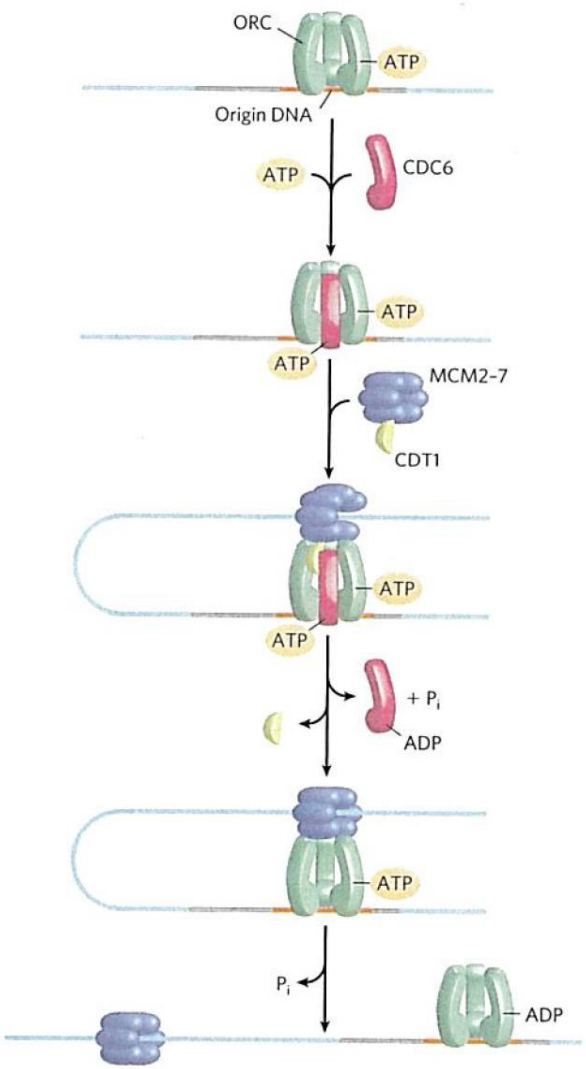
Pre-RCs consist of several proteins, including the origin recognition complex (ORC), Cdc6, and Cdt1, which prepare the DNA for replication.

Once conditions are favorable and cyclins reappear during S phase, CDKs become active again, leading to the phosphorylation of various proteins that promote the unwinding of DNA and recruitment of DNA polymerases for replication.



REPLICATION

eukaryotes



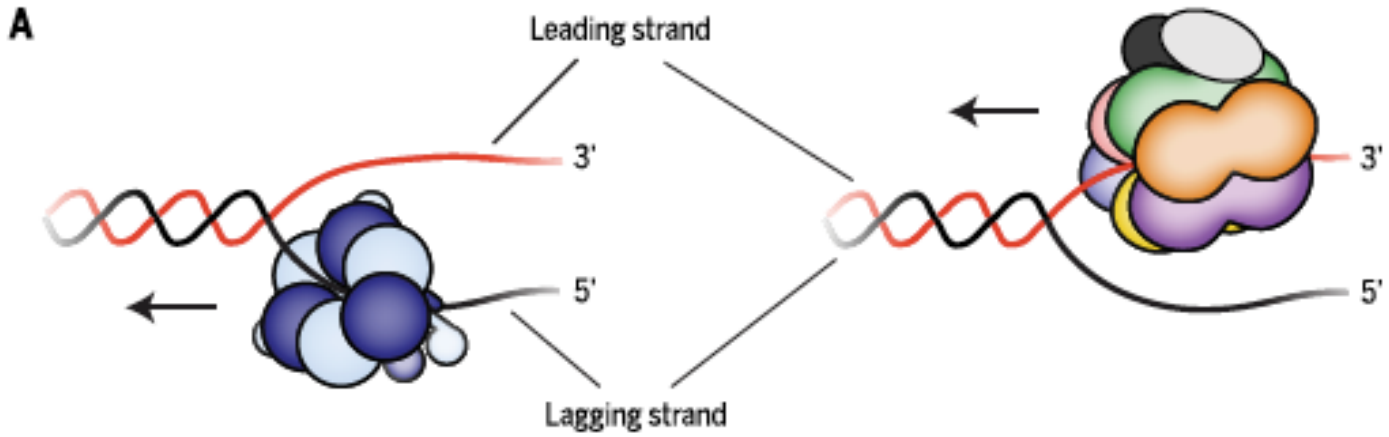
The initiation of replication occurs with the arrival of helicases (hexamers called MCM). MCM2-7 functions like the helicase DnaB but moves in the direction **3'→5'** along the leading strand.

It binds the origin site by the ORC **Origin Recognition Complex**.

ORC functions as Dna A (i.e. it has the task of recognising the origin site).

From Lehninger: Principles of Biochemistry, 7th Ed.

HELICASES



DnaB

In prokaryotes DnaB is located on the lagging strand and moves in the 5’ → 3’ direction

MCM

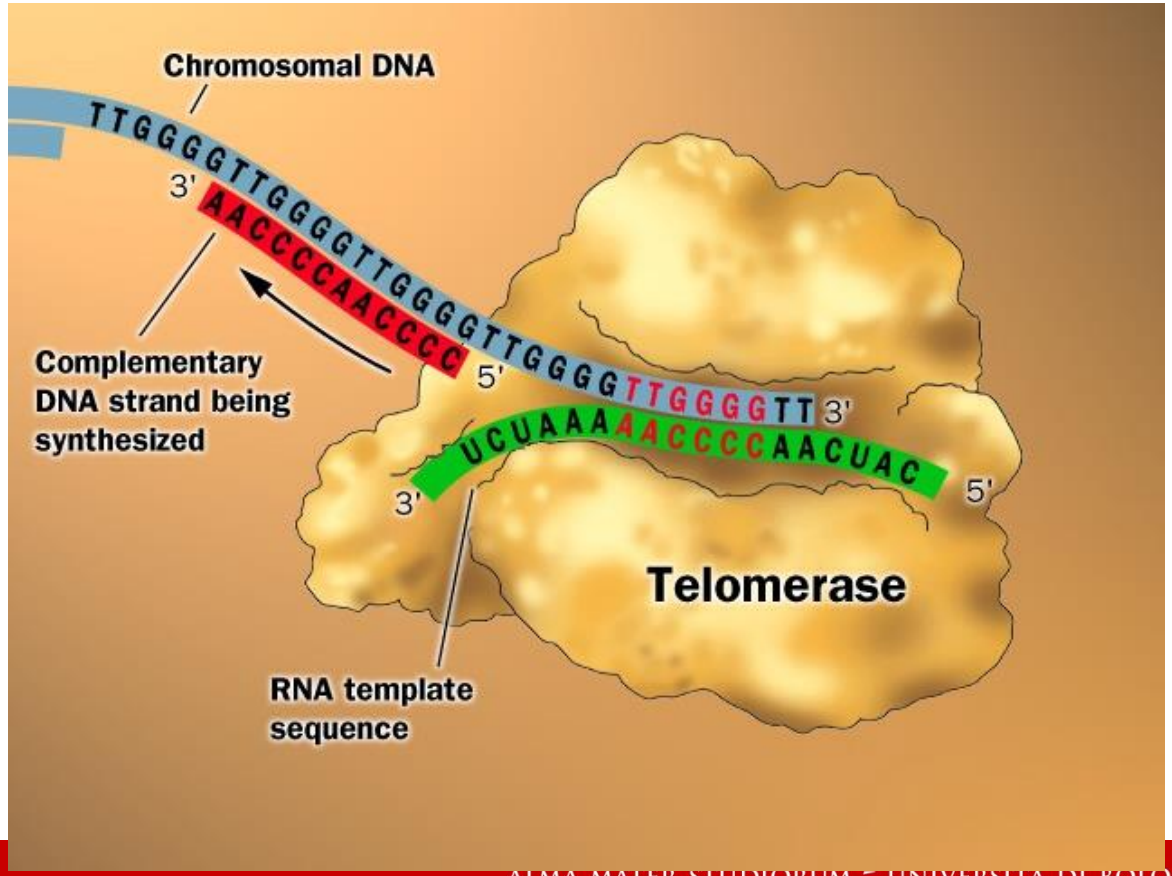
In eukaryotes Mcm2-7 is located on the fast filament and moves in the 3′ → 5′ direction

Bleichert F. (2017) Science

REPLICATION

eukaryotes

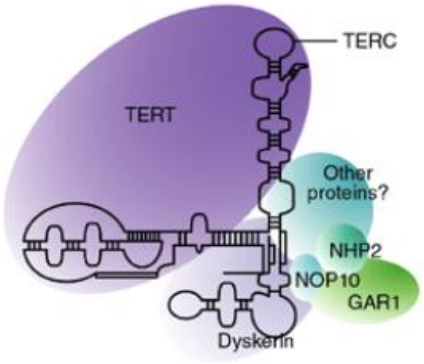
The terminal parts of linear DNA consist of non-coding redundant sequences referred to as TELOMERS and introduced by reverse transcriptases: the TELOMERASES (specialized DNA polymerases).



Telomeres are maintained by the reverse transcriptase complex "telomerase"

Chromsosome ends consist of TG-rich tandem repeats

		sequence	length
Vertebrates		*TTAGGG*	human 10 kb mouse 40 kb
Plants		*TTTAGGG*	2 – 9 kb
S. cerevisiae		$G_{(2-3)}(TG)_{(1-6)}T$	325-400 bp



TERT: protein component

TERC: non-coding RNA component

core components

Dyskerin, NOP10, NHP2, GAR1

improve activity

Telomerase resolves the end-replication Problem

Nobel Prize for Medicine 2009
'Chromosomes are protected by telomeres and the enzyme telomerase'.
Elizabeth H. Blackburn (1948),
Carol W. Greider (1961),
Jack W. Szostak (1952).

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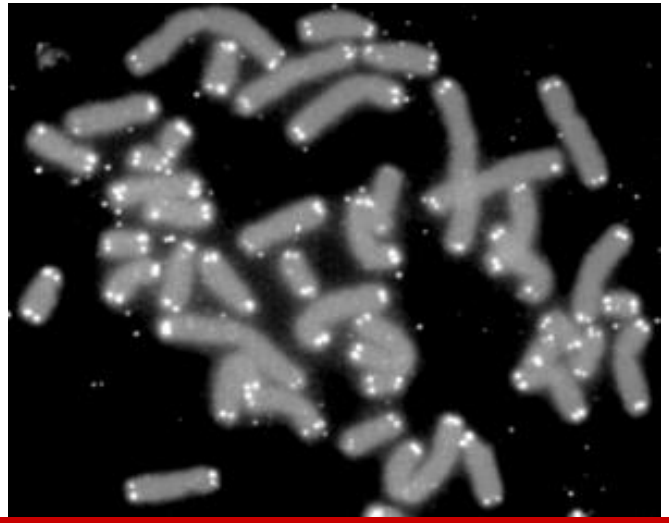
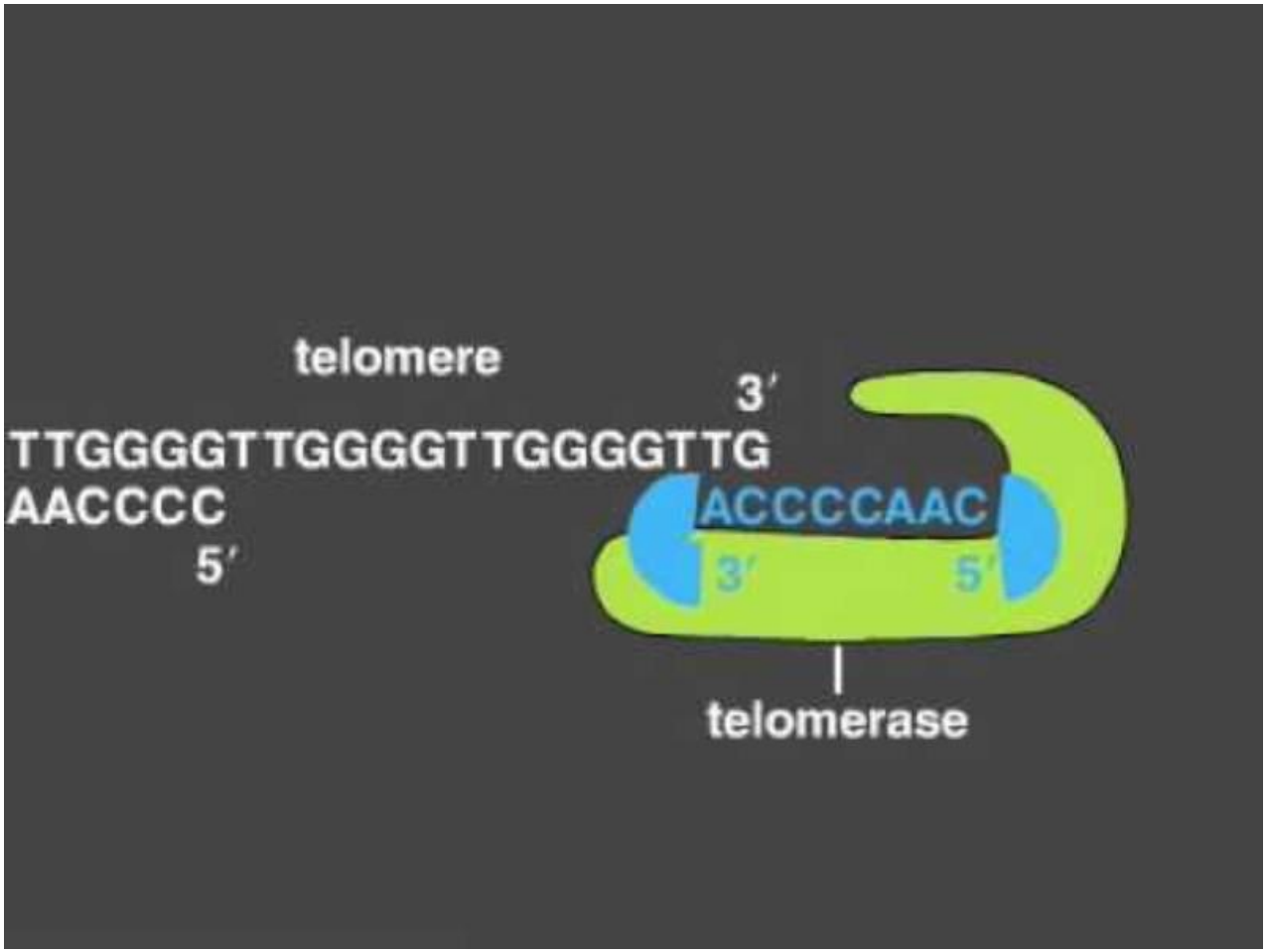
Telomers are:

- DNA repeated sequences in the terminal part of all the human chromosomes;
- They contain thousands of repetitions of sequences T_xG_y (5'-3') and C_yA_x (3'-5'); usually x e y are in the range 1 - 4
- In humans there are 92 telomeric sequences for 46 chromosomes, one at each end.




telomere
3′
TTGGGGTTGGGGTTGGGGTTG
AACCCC
5′
ACCCCAAC
3′
5′
telomerase

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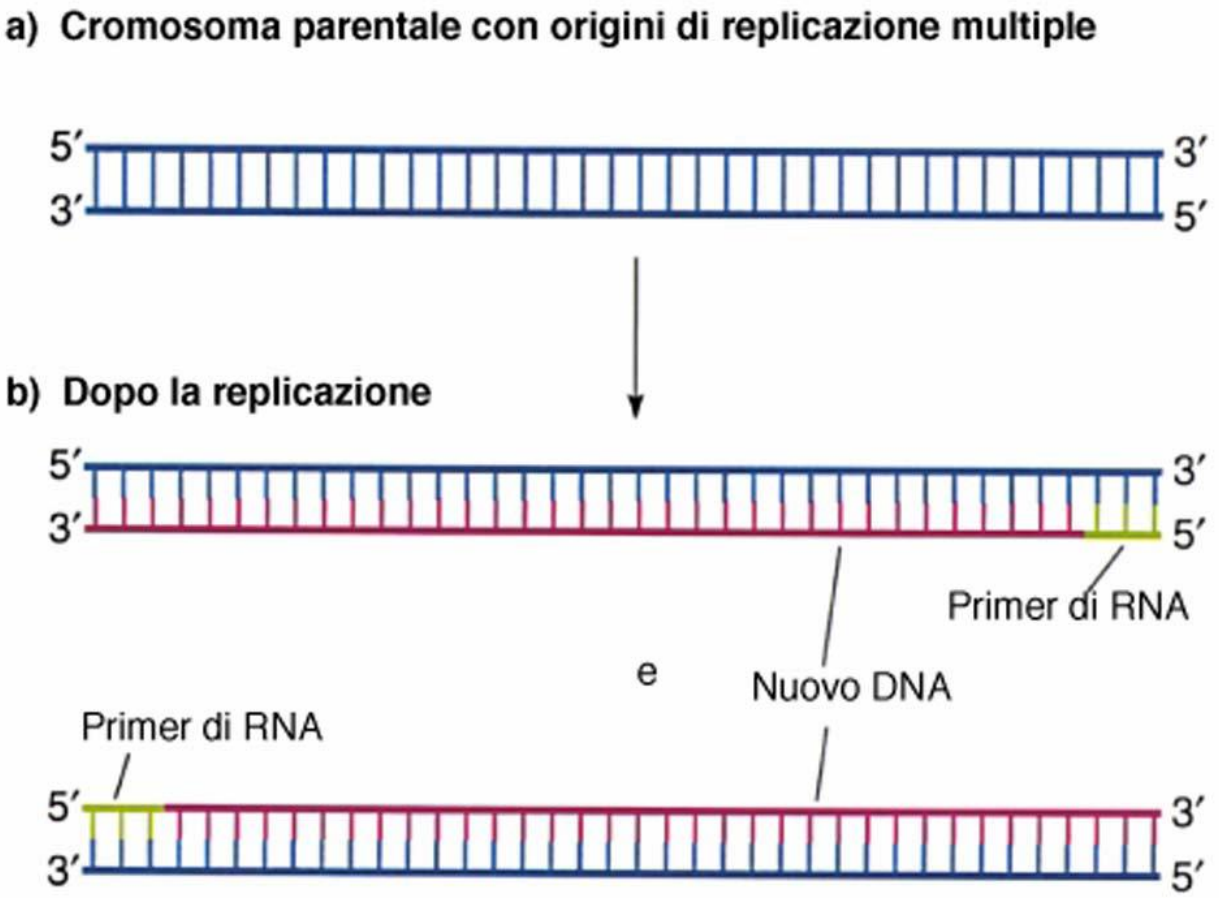
DNA polymerases synthesize new DNA strands by adding nucleotides only to the 3' hydroxyl (-OH) group of the growing strand. This means that replication can only proceed in a 5' to 3' direction.



The new DNA segments are equipped at their 5' ends with RNA primers

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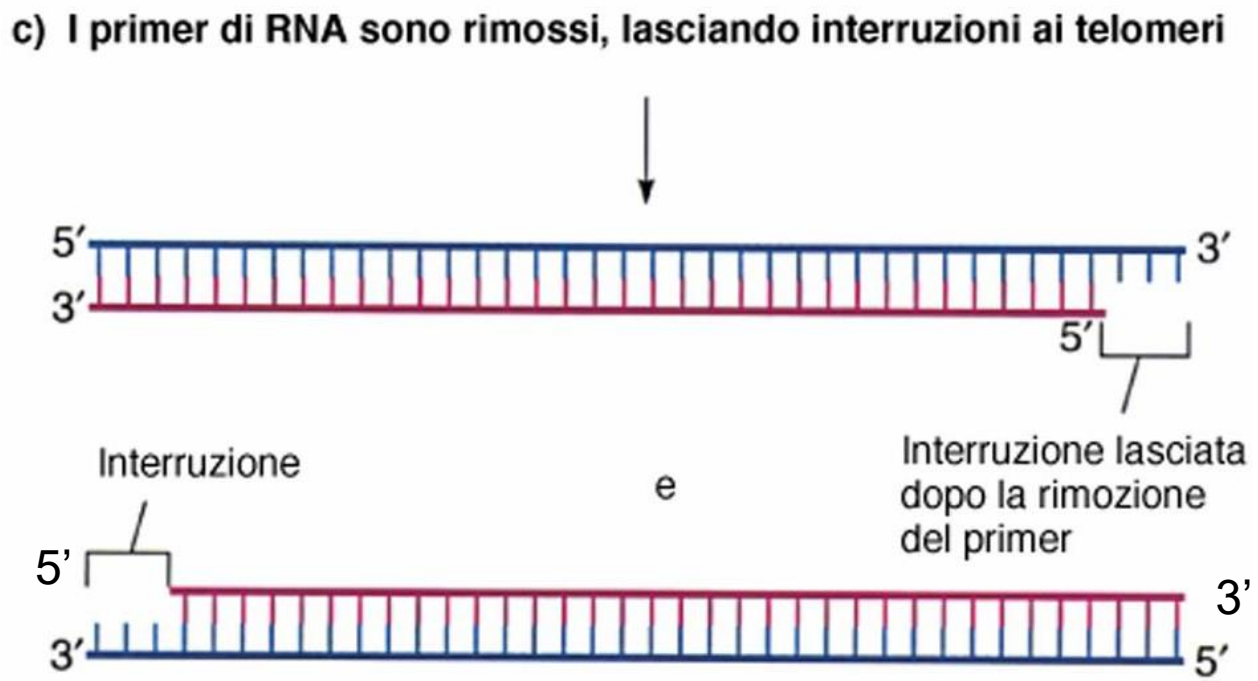
RNA primers at the 5’ end are removed resulting in a 3’ overhang of single stranded DNA that cannot be copied by Pol



Because of this, the newly formed DNA strand would be shortened with each cell division.

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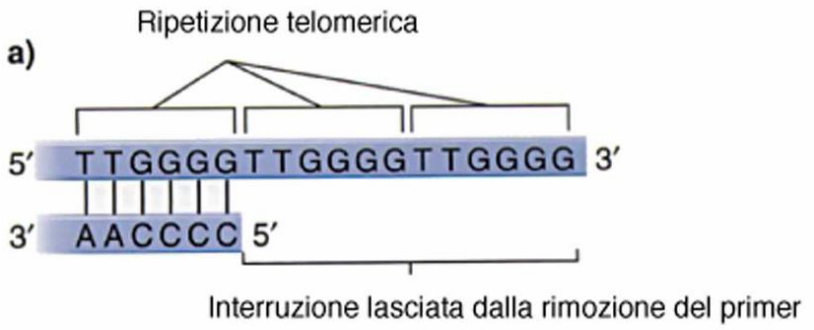
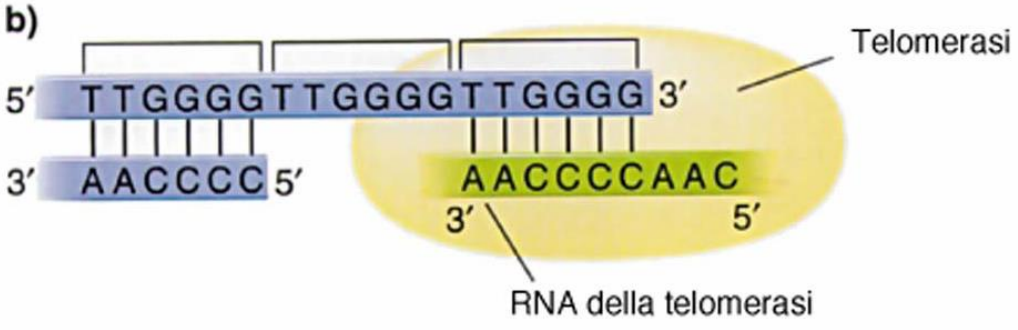
The terminal sequences of chromosomal DNA (**telomers)** contain hundreds of hexanucleotides with the sequence 5‘-TTGGGG-3’. Telomerases are INVERSE TRANSCRIPTASES (or RNA-dependent DNA polymerase) that use their own RNA fragments which contain a complementary sequence (3'-AACCCCAAC-5’) as a template for telomere elongation. It binds to the end of the 5‘-TTGGGG-3’ template strand.





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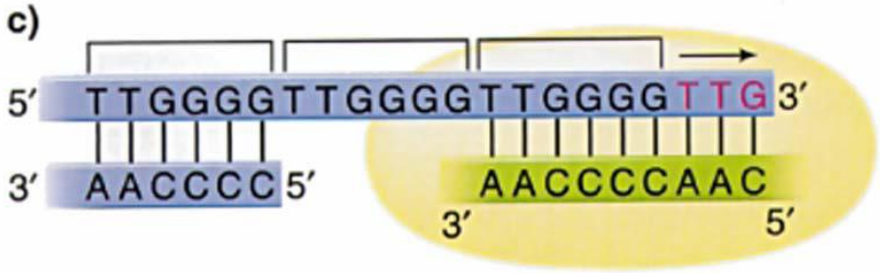
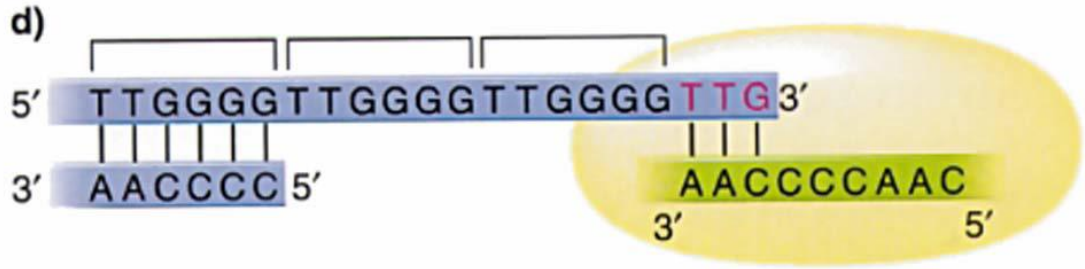
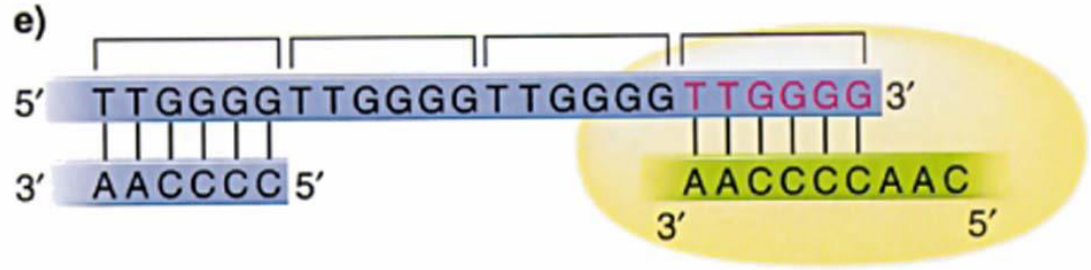


Synthesis of a three-nucleotide segment at the chromosome end, using telomerase RNA as a template.



Telomerase slippage and template binding to the extreme TTG sequence.



Synthesis of a new telomeric repeat using the RNA template. The process can be repeated several times to add more telomeric repeats

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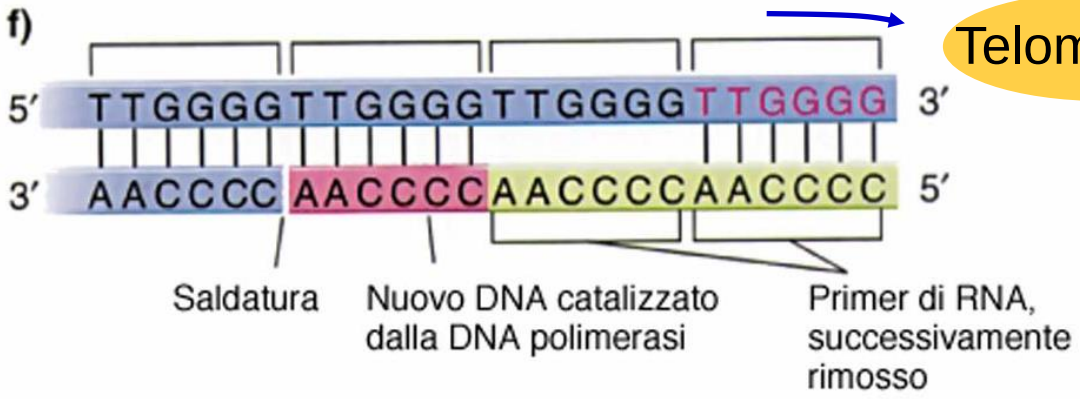
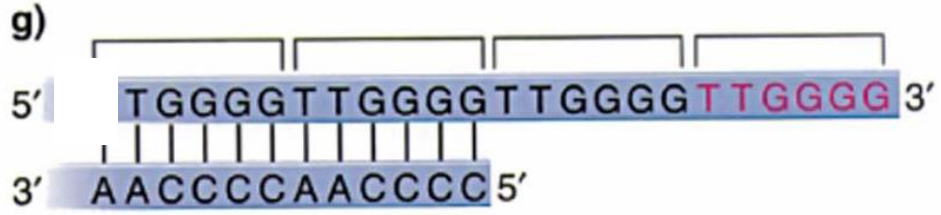


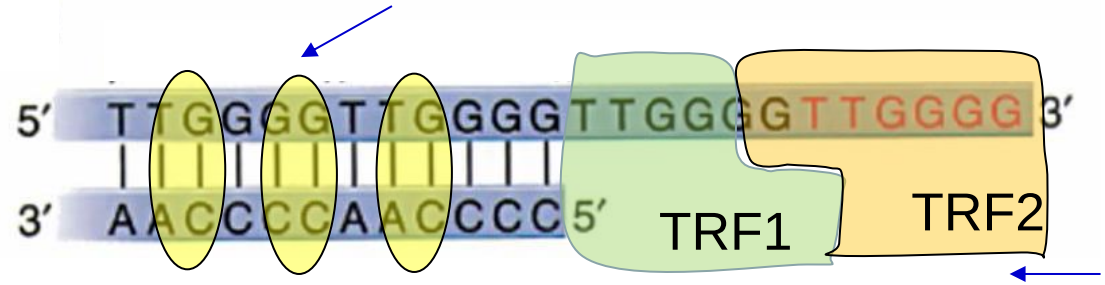


Telomerases

Telomerase dissociates from the end, a **primase (Pol α)** synthesises an RNA primer and DNA polymerase δ catalyses the synthesis of new DNA.

After removal of the primers, the result is a longer chromosome than that at the beginning.

Double-strand-binding proteins



Protein-protected end of the telomere

Loop-forming proteins

TRF=Telomeric repeat binding factor

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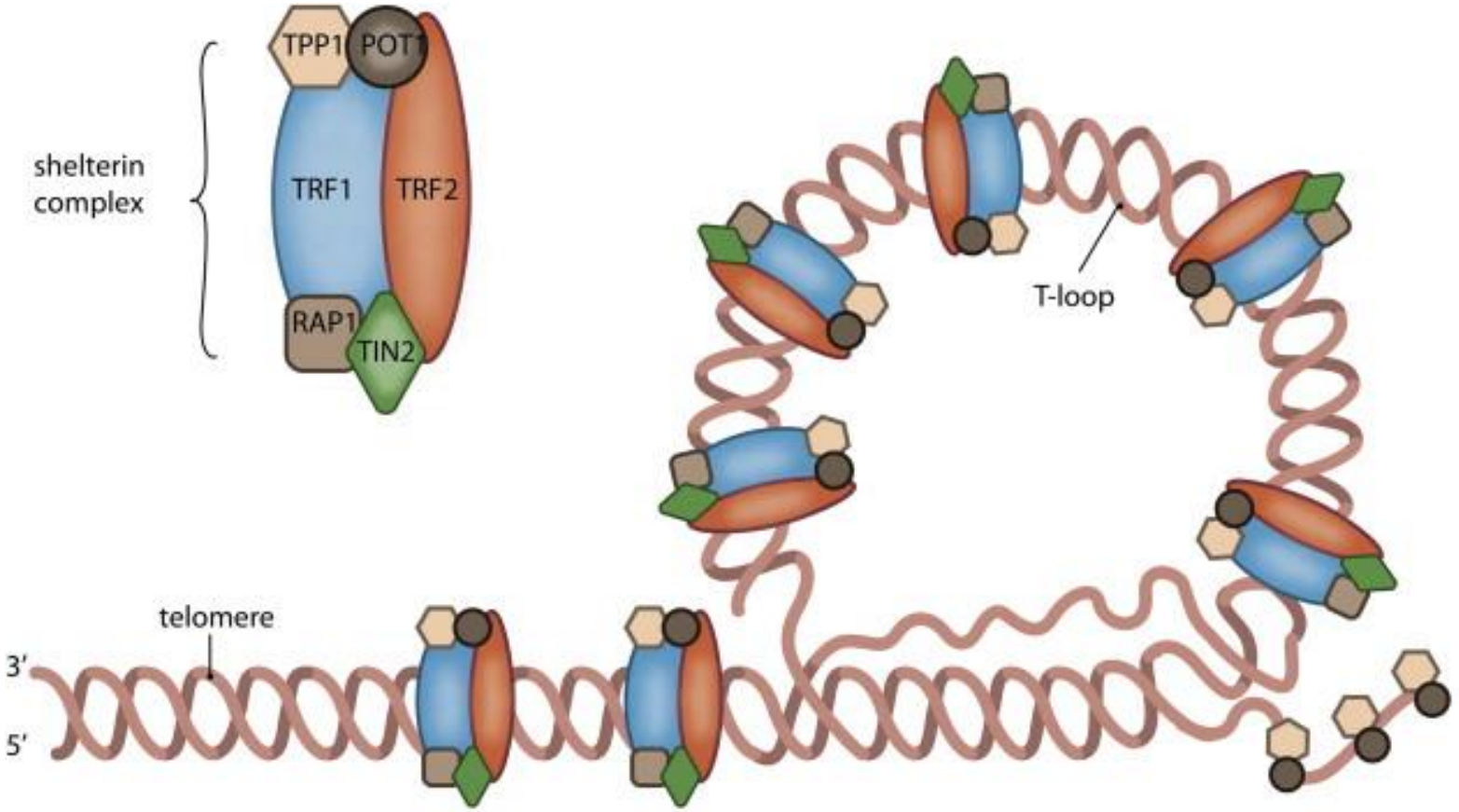

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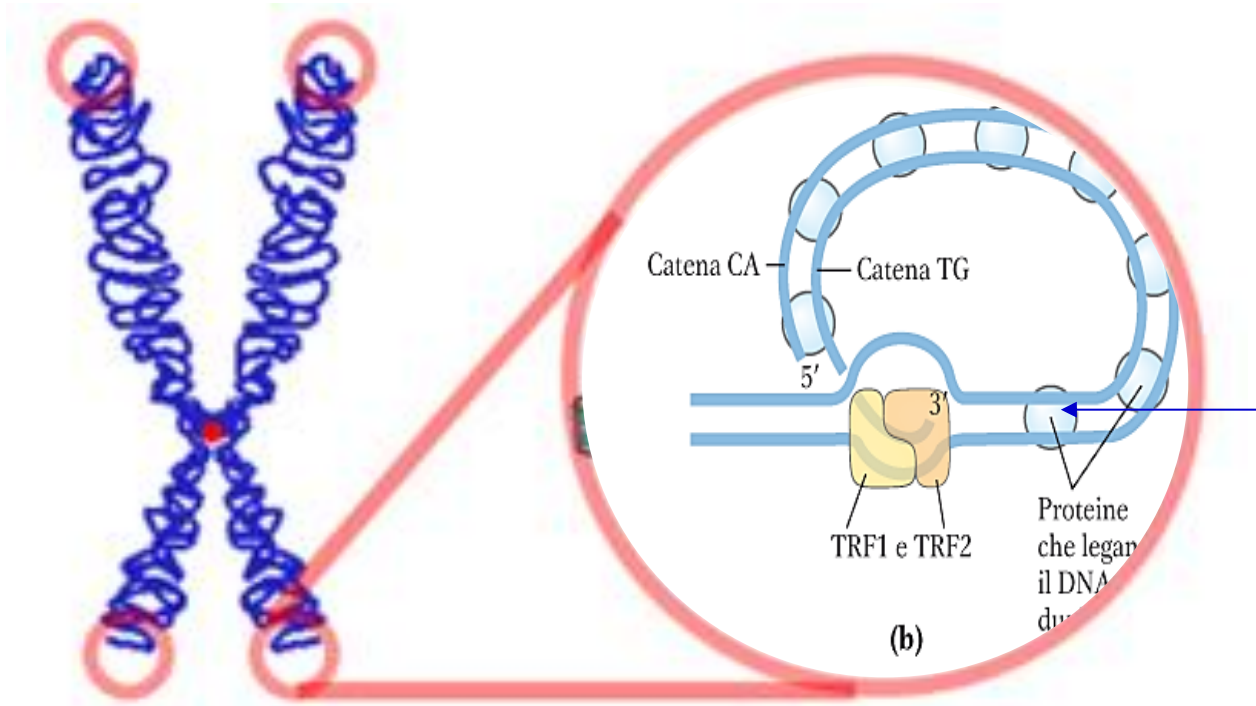
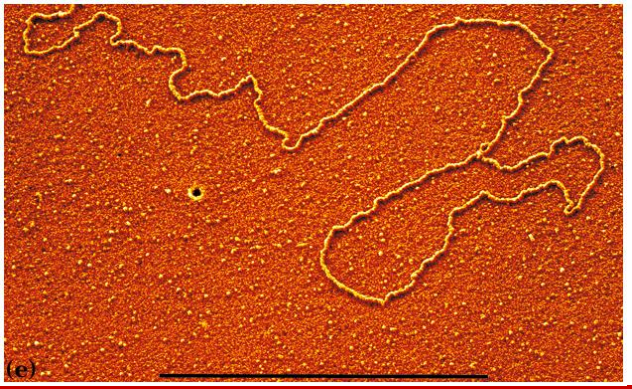


Telomere repeat binding factor 1 e 2 (TRF-1, TRF-2).



T-loop at the ends of chromosomes protects the ends from nucleases

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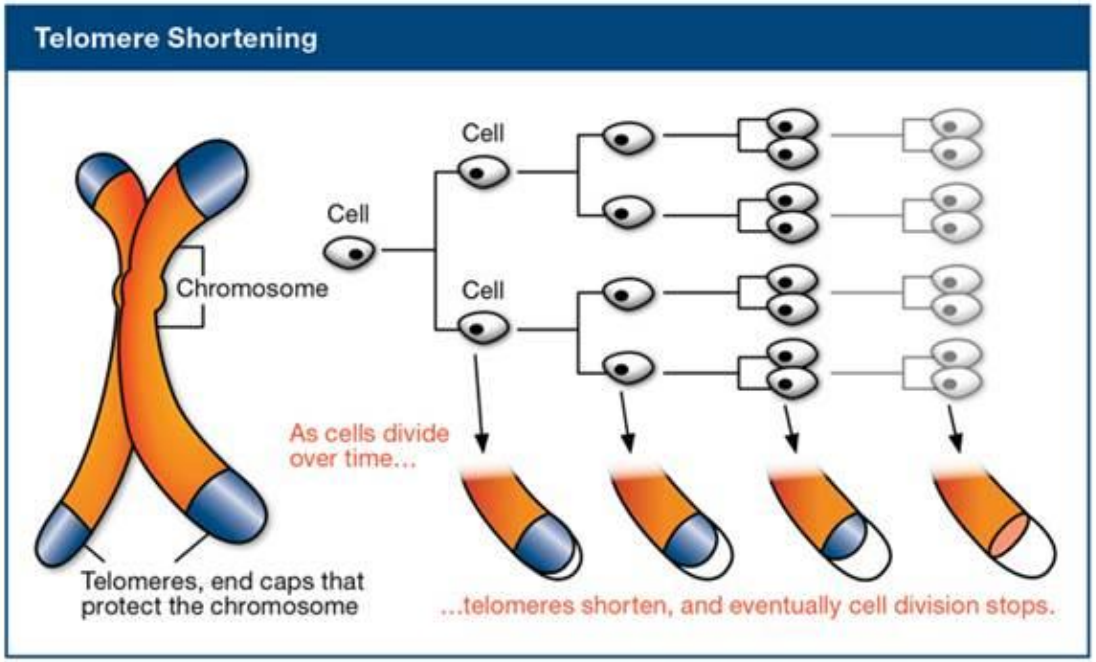
In general, in the somatic cells of eukaryotes, telomerase activity stops immediately after the cell differentiates. From this point on, the chromosomes gradually shorten with each division.
Loss of telomerase activity is a critical factor in cell ageing.



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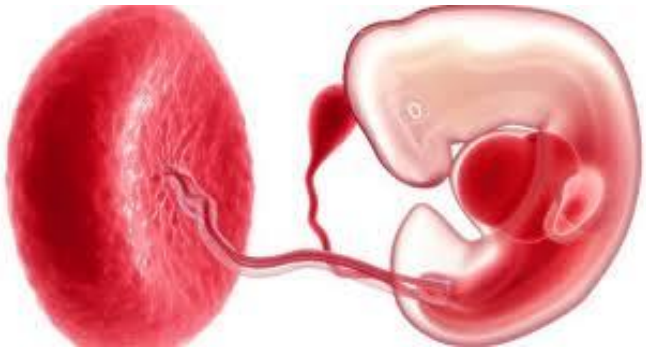
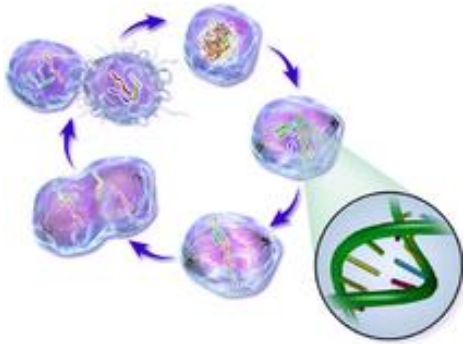
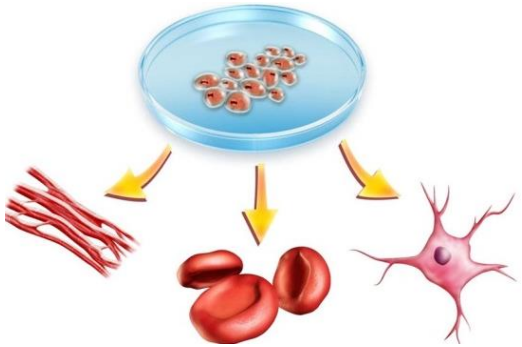
In humans, telomerase is active in foetal tissue, germinal cells, the vast majority of cancer cells and some stem cells.







Cancer cells “do not age” thanks to the continuous production of telomerase, which keeps telomeres intact.


Kahoot!