

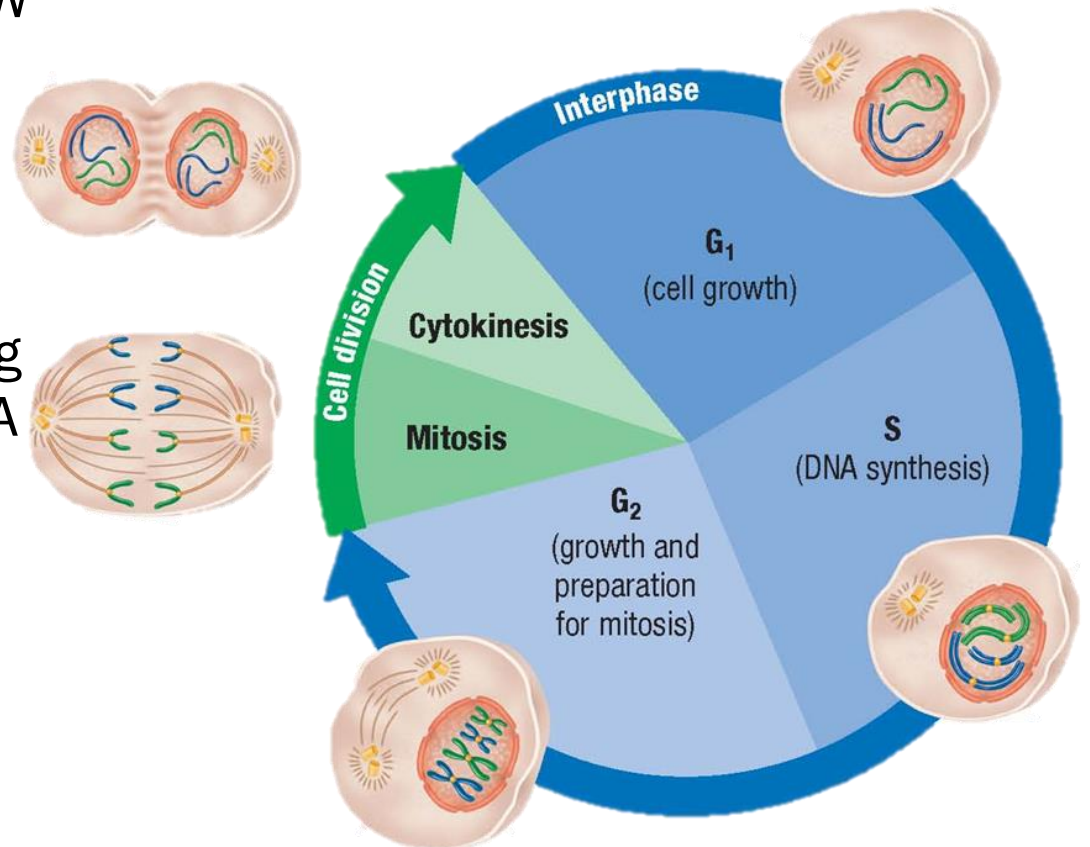
DNA REPLICATION

Objectives

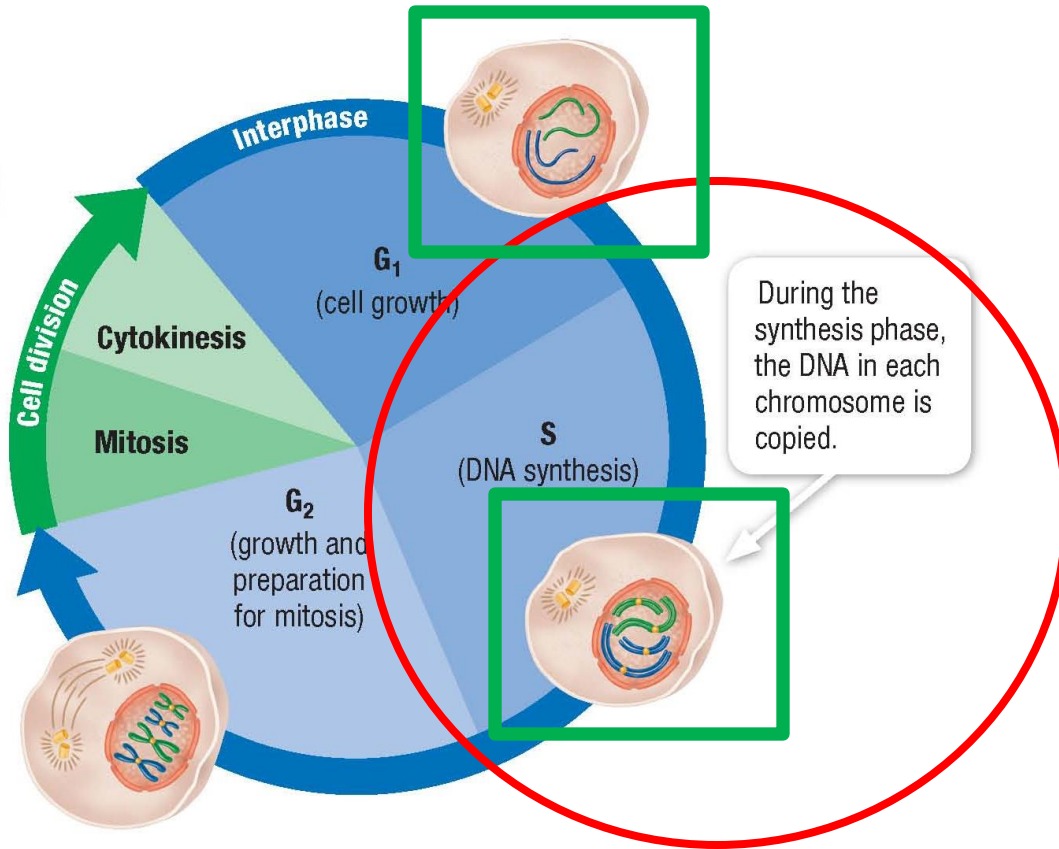
- Essential Question: Why & how do our cells reproduce DNA?
- Describe the steps of DNA replication.
- Compare the roles of DNA helicase, DNA polymerase, and ligase
- Compare the process of DNA replication in prokaryotes and in eukaryotes
- Applications of our knowledge

DNA REPLICATION

- Every time a cell goes through a cycle it must **DUPLICATE ITS DNA SO THAT WHEN IT MAKES NEW BABY CELLS THEY BOTH HAVE THE EXACT SAME DNA.**
- It happens in 'S' phase
- In this section we are going to explore the process of DNA synthesis, called DNA replication.
- This process is extremely precise and an incredibly important.



THE CELL CYCLE: THE PART WE'RE ISOLATING.



From single-copy of each chromosome



To double-copy



equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

¹Young, F. R., Gerrard, H., and Jervis, W., *Phil. Mag.*, **46**, 149 (1928).

²Longuet-Higgins, M. S., *Mos. Nat. Exp. Adv. Ser., Geophys. Supp.*, **8**, 280 (1949).

³For A.E. W. R. W. K. See *Papers in Phys. Oceanogr. Xerox*, **11** (1950).

⁴Ekman, T. W., *Adv. Mol. Adv. Phys. (Urbahar)*, **2** (1) (1953).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Twining and Corey.¹ They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining 3'-deoxyribose residues with 2',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furburg's² model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furburg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There

is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

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J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge, April 2.

¹Twining, L., and Corey, R. E., *Nature*, **171**, 149 (1953); *Proc. U.S. Nat. Acad. Sci.*, **28**, 91 (1951).

²Furburg, E., *Adv. Chem. Ser.*, **8**, 914 (1952).

³Chambers, E., for references see Zimm, B. H., Bragg, R. G., and Challa, R., *Biophys. J.*, **2**, 402 (1952).

⁴Wyck, G. B., *J. Gen. Physiol.*, **36**, 391 (1952).

⁵Arthur, W. T., *Proc. Roy. Soc. Lond. B*, **1**, *Nucleic Acids*, 88 (1948).

⁶Watson, M. R. F., and Franklin, R. E., *Biophys. J.*, **18**, 152 (1953).

Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Arthur¹) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleates. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline²⁻⁴, semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid (structure B' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 5). Arthur suggested that the strong 3.4-Å. reflection corresponded to the internucleotide repeat along the fibre axis. The ~34 Å. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflections on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown⁶ (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the n th layer line being proportional to the square of J_n , the n th order Bessel function. A straight line may be drawn approximately through



Fig. 1. Fibre diagram of deoxypentose nucleic acid from *X. laevis*. Fibre axis vertical.

the innermost maxima of each Bessel function at the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats a time along the helix there will be a meridional reflexion (J_0^2) on the n th layer line. The helical configuration produces sidebands on this fundamental frequency the effect being to reproduce the intensity distribution about the origin around the new origin, on the n th layer line, corresponding to C in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if a nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by it helices of different diameter passing through one point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner

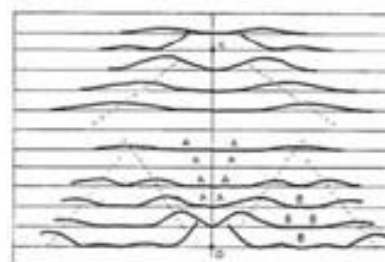


Fig. 2. Diffraction pattern of a series of helices corresponding to structure B' of deoxypentose nucleic acid. The square of Bessel functions are plotted along C on the equator and on the first, second, third and fifth layer lines for helices of the nucleotide repeat at 21 Å. diameter and nucleotide distributed along a radius, the case of a given radius being perpendicular to the radius. Also C on the fourth layer line similar functions are plotted for an outer diameter of 12 Å.

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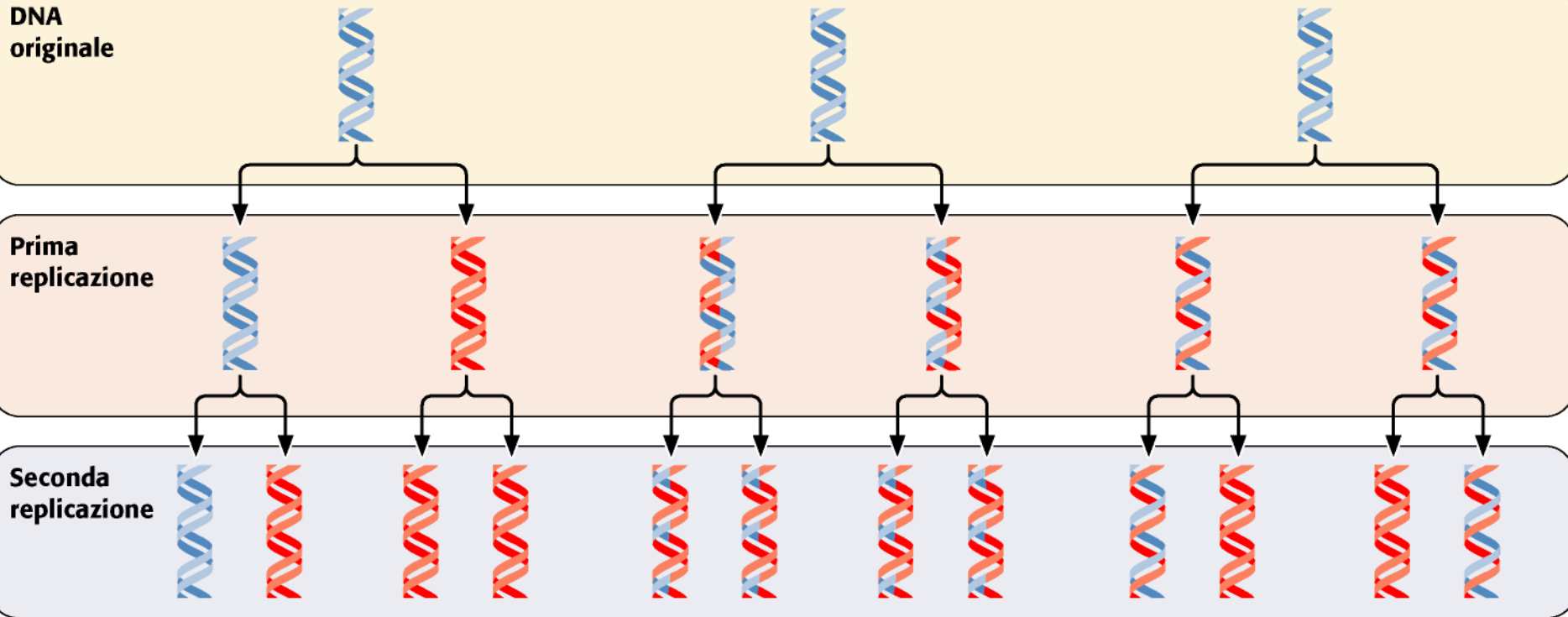
1. Pauling, L., and Corey, R. B., Nature, 171, 346 (1953); Proc. U.S. Nat. Acad. Sci., 39, 84 (1953).
2. Furberg, S., Acta Chem. Scand., 6, 634 (1952).
3. Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., Biochim. et Biophys. Acta, 9, 402 (1952).
4. Wyatt, G. R., J. Gen. Physiol., 36, 201 (1952).
5. Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
6. Wilkins, M. H. F., and Randall, J. T., Biochim. et Biophys. Acta, 10, 192 (1953).

The Three Possible DNA Replication Models

(a) Replicazione conservativa

(b) Replicazione dispersiva

(c) Replicazione semiconservativa

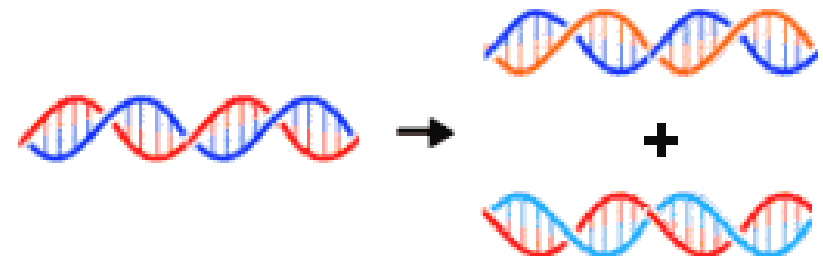


- **Conservative**- would leave the original strand intact and copy it.
- **Dispersive**-would produce two DNA molecules with sections of both old and new along each strand.
- **Semiconservative** -would produce DNA molecules with both one old strand and one new strand.

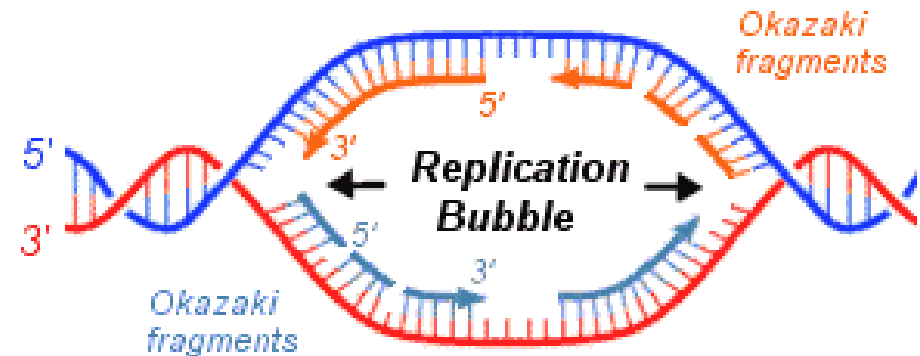
Definitions

Origin: chromosomal area where the double helix is denatured, exposing strands for production of new DNA molecules.

Replication Bubble: Chromosomal region where the DNA is single stranded from which replication proceeds in both directions.



**Semi-conservative
Replication**



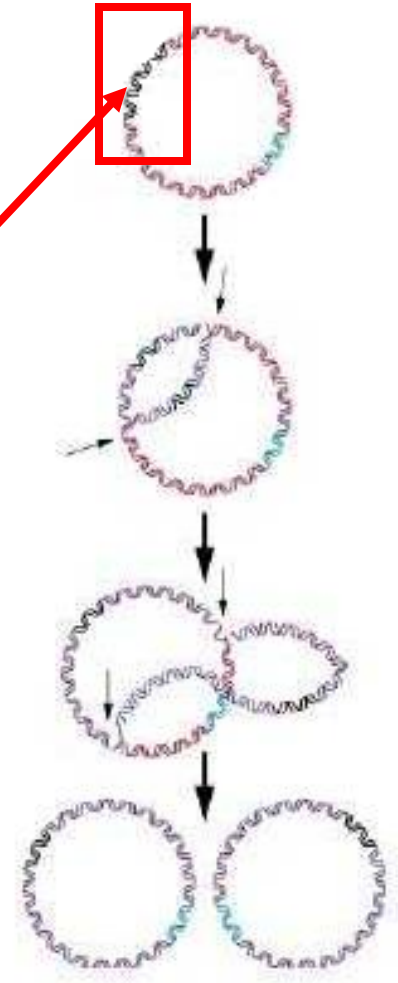
(c) 2000 Chemis

Prokaryotic and Eukaryotic Replication

- All cells have chromosomes, but eukaryotes and prokaryotes replicate their chromosomes differently.
- The main difference between prokaryote and eukaryote replication is how many **start sites** each have.
- Eukaryotic chromosomes are so long that it would take 33 days to replicate a typical human chromosome if there were only one origin of replication. As such, evolution has allowed Human chromosomes to replicate using multiple replication starting points.
- Because eukaryotic cells have multiple replication forks working at the same time, an entire human chromosome can be replicated much faster, in only about 8 hours.
- Bacteria replicate their small genomes in minutes...
- The start sites then regulate how replication proceeds in each organism type.

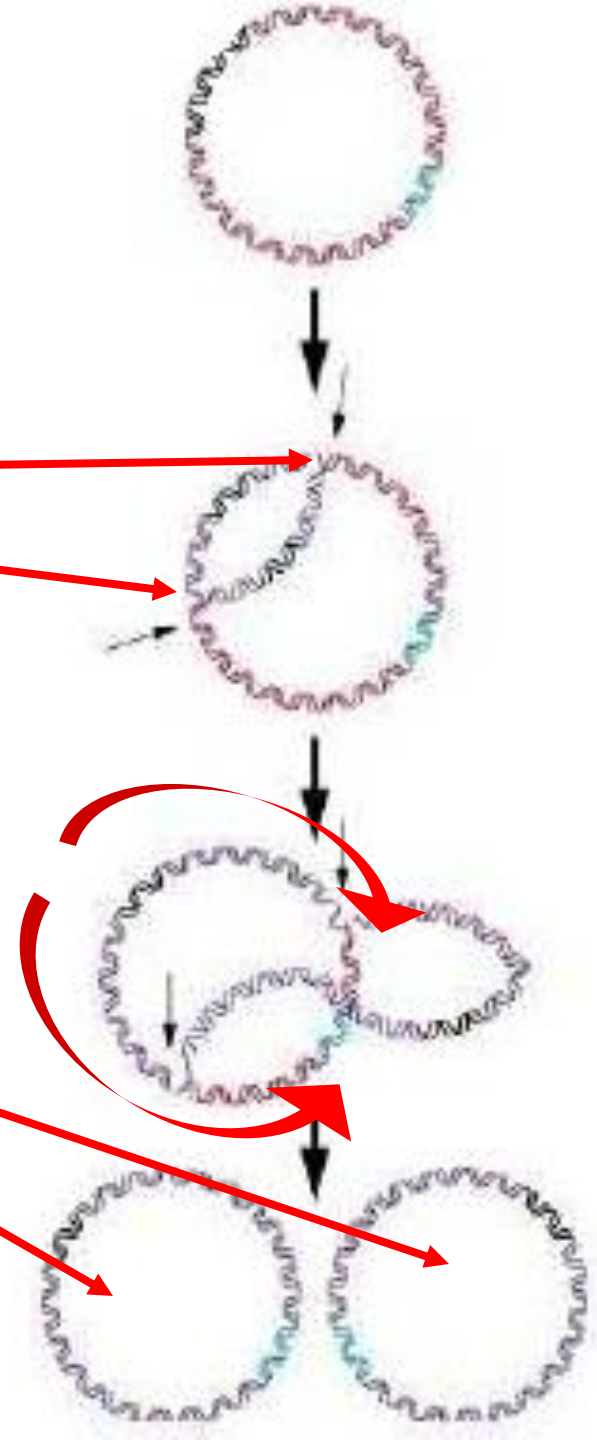
Prokaryotic DNA Replication

- Recall the structure of prokaryotic DNA.
- Does anyone remember what the structure is?
- Prokaryotic cells usually have a single chromosome which is a closed loop attached to the inner cell membrane.
- Replication in prokaryotes begins at a single site along the loop. This site is called the *origin of replication*.



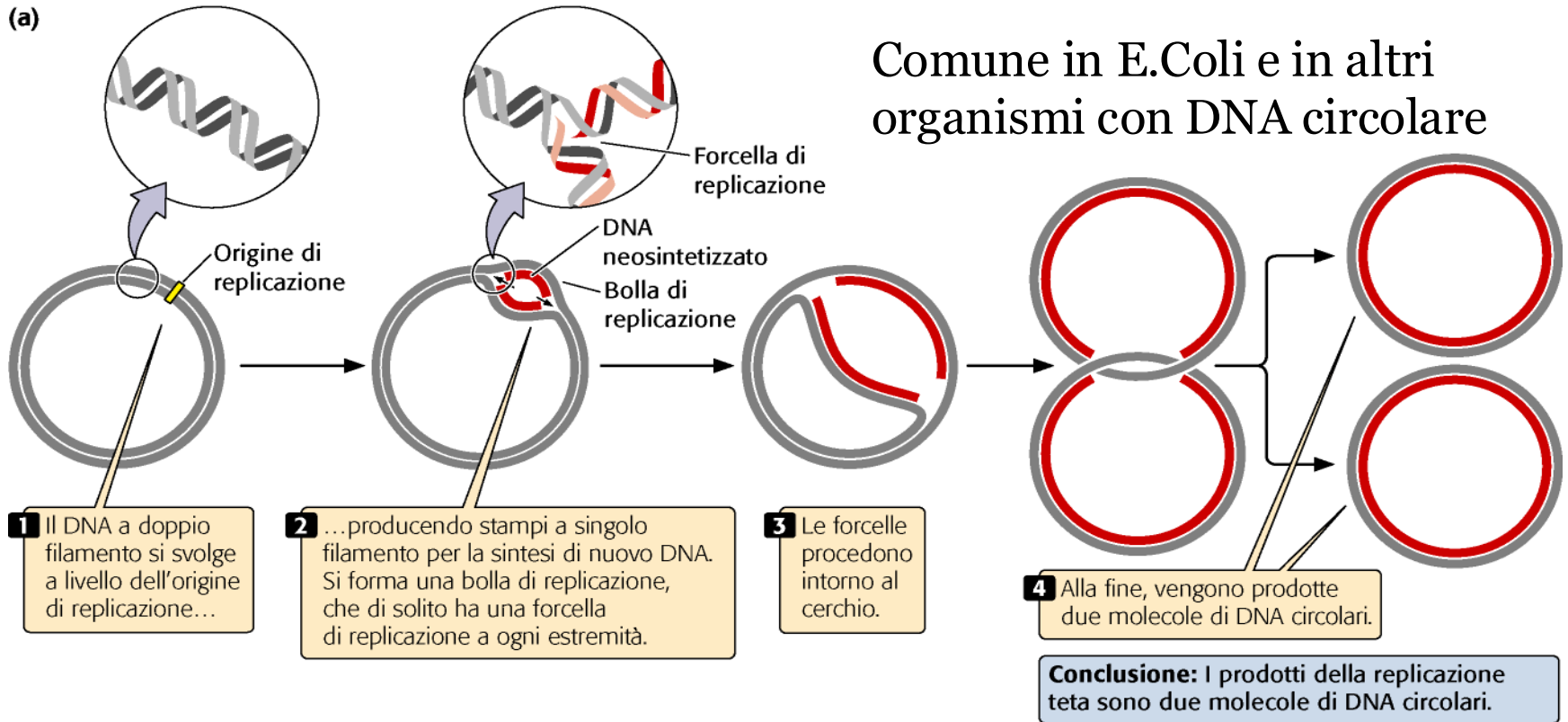
Prokaryotic Replication

- Two replication forks begin at the origin of replication.
- Replication occurs in opposite directions until the forks meet on the opposite side of the loop.
- The result is two identical loops of DNA.

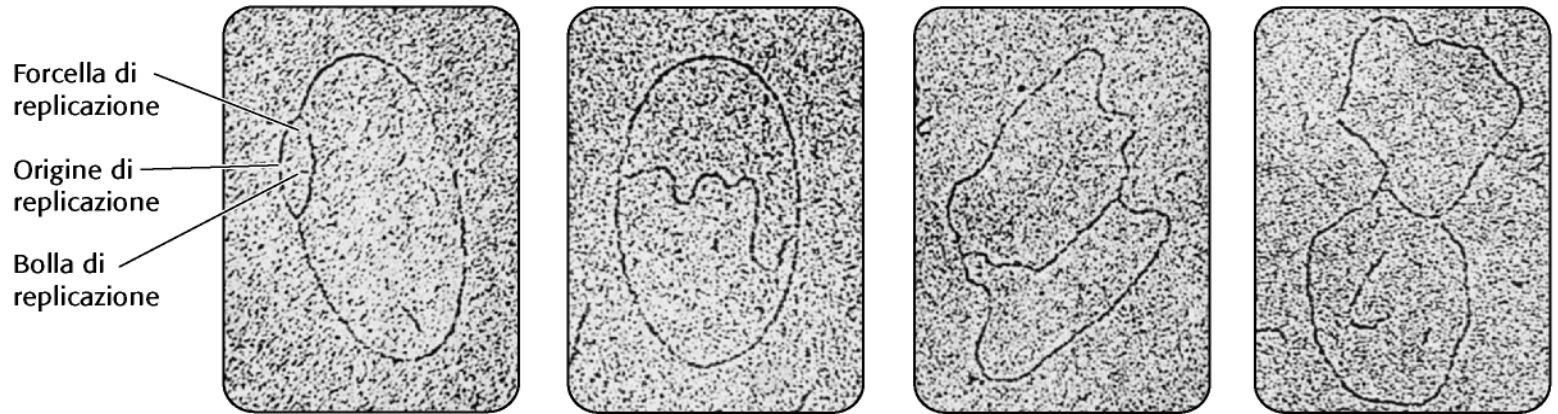


1. Replicazione teta

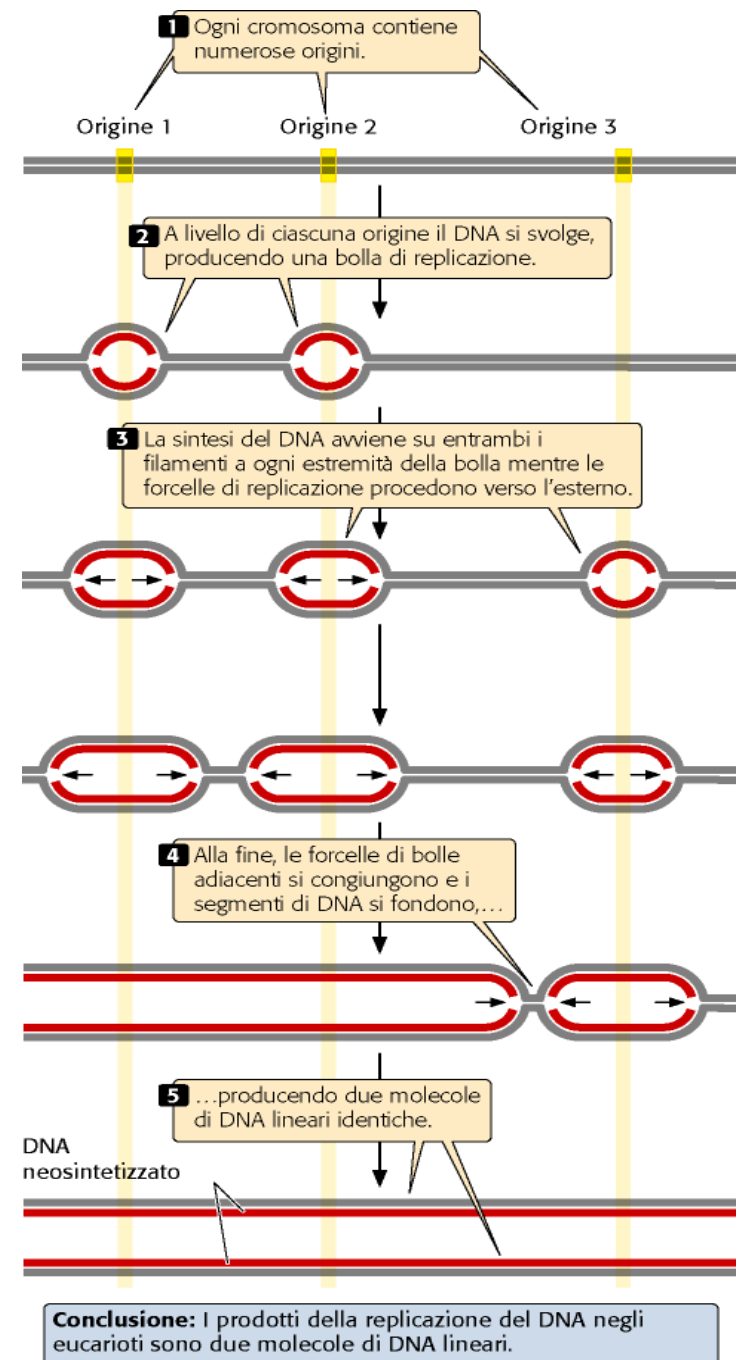
(a)



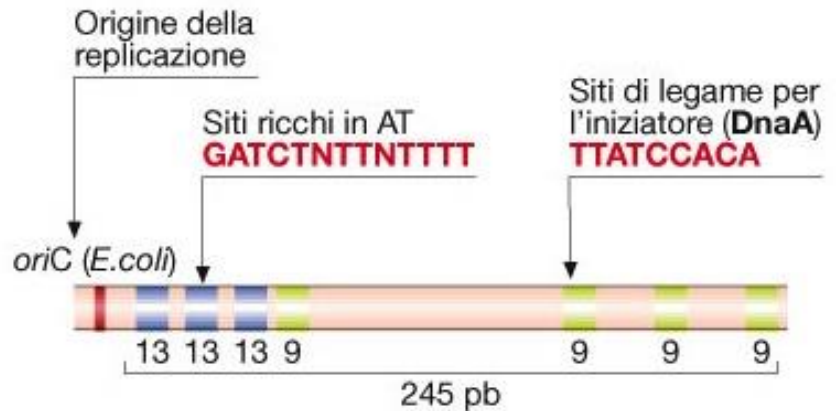
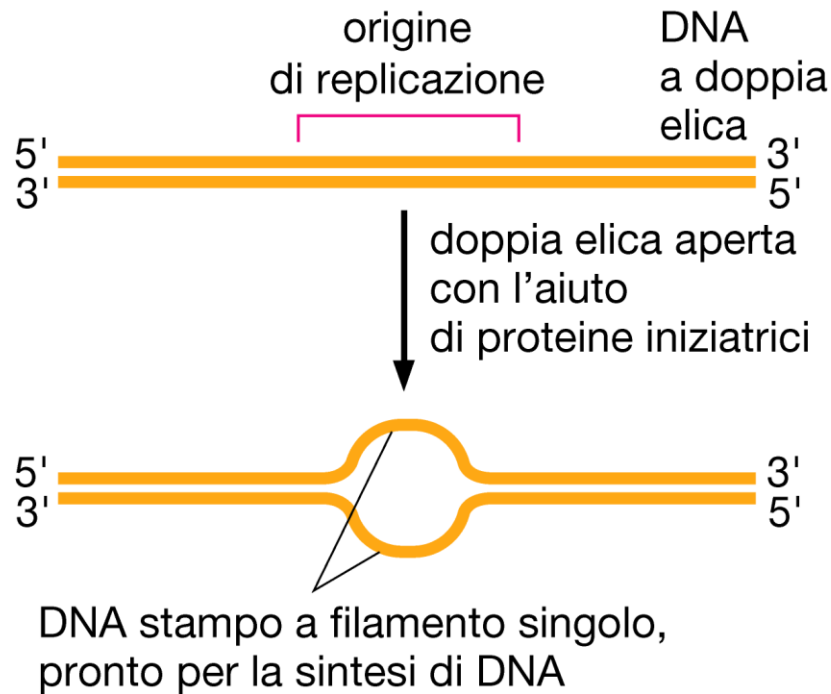
(b)



2. Linear replication (eukaryotes)



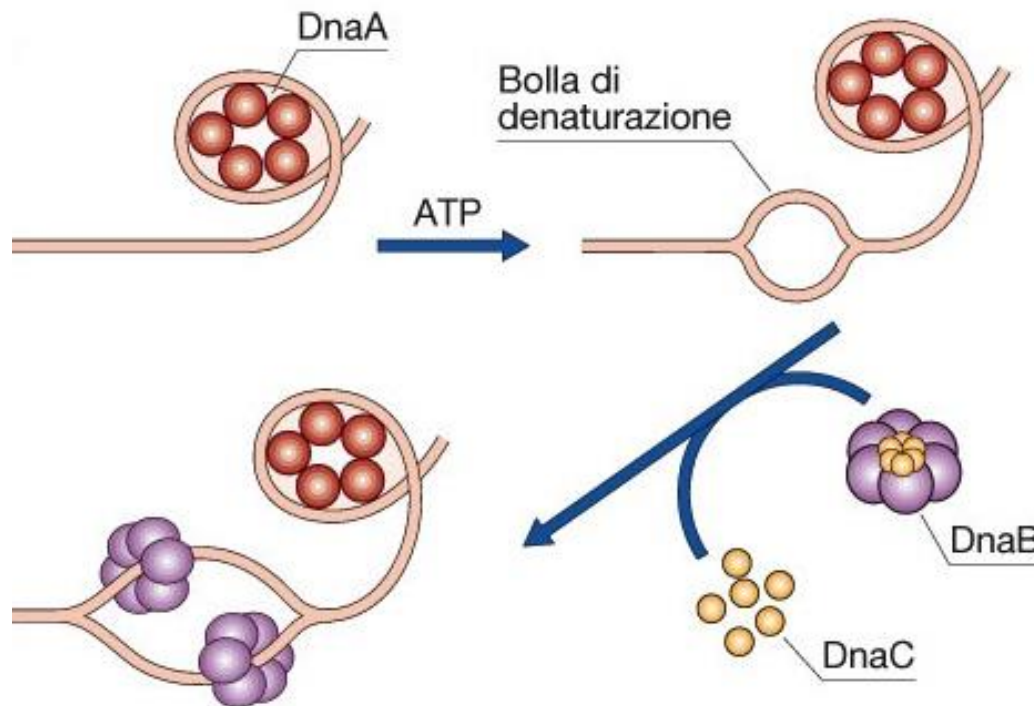
Origine di replicazione in E.Coli: *oriC*



L'origine minima di E.Coli e' lunga solo 245 bps. E' una regione ricca di A e T: contiene 4 ripetizioni di 9 pb e 3 di 13 pb molto simili tra loro. Le corte sequenze ripetute sono riconosciute da proteine specifiche nelle fasi iniziali della replicazione.

Inizio della replicazione in E.Coli

- **DnaA** riconosce *oriC* creando un core proteico attorno cui si avvolge il DNA di *oriC*. In presenza di ATP, induce la denaturazione localizzata del DNA in corrispondenza delle 3 ripetizioni di 13 pb, permettendo l'assemblaggio del complesso replicativo.
- In presenza della proteina accessoria **DnaC**, due complessi esamerici dell'elicasi replicativa (**DnaB**) vengono caricati sulla bolla di denaturazione, creando il complesso di pre-innesco e le due forcelle di replicazione bidirezionali.



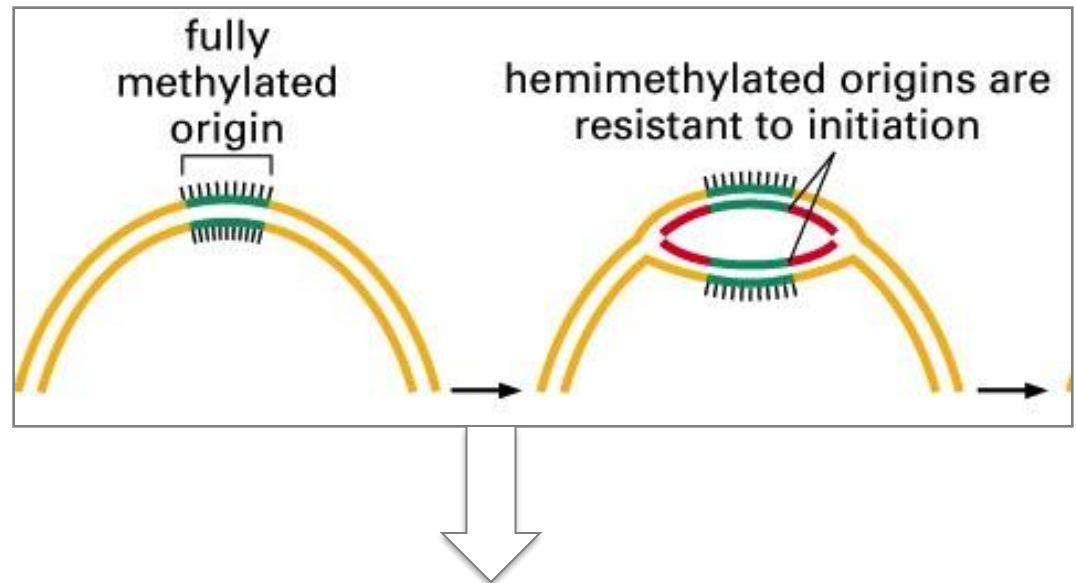
Inizio della replicazione

BATTERI: altamente regolato in quanto unico momento in cui il batterio **controlla** la replicazione

2 Livelli di regolazione:

- Interazione della proteina iniziatrice con l'origine di replicazione:
solo quando ci sono nutrienti a sufficienza;

- Origine di replicazione:
una volta usata, va incontro a un “**periodo refrattario**”, rappresentato da un **ritardo nella metilazione delle nuove A sintetizzate**, che blocca ulteriori inizi di replicazione.



Nello stato emi-metilato (transitorio!) non può funzionare da origine di replicazione, non viene usata 2 volte!

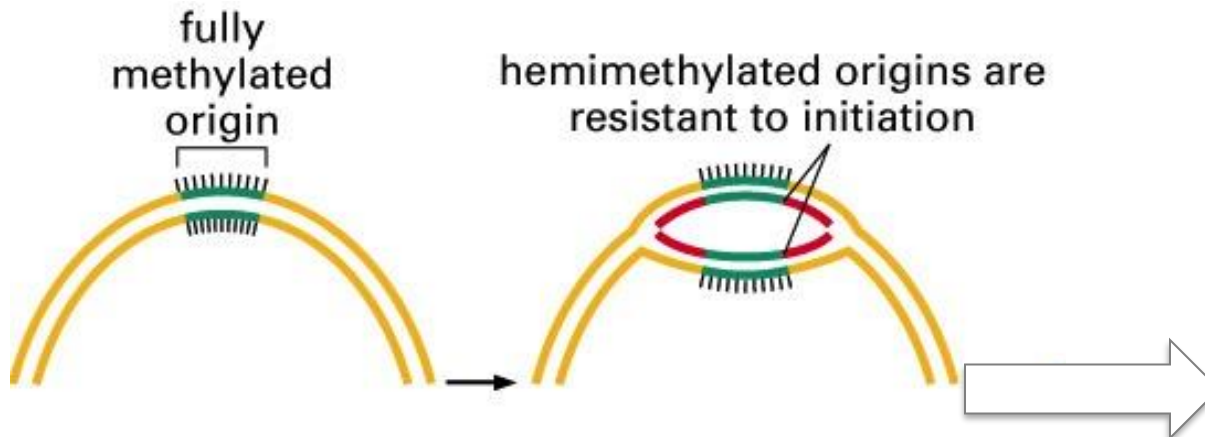
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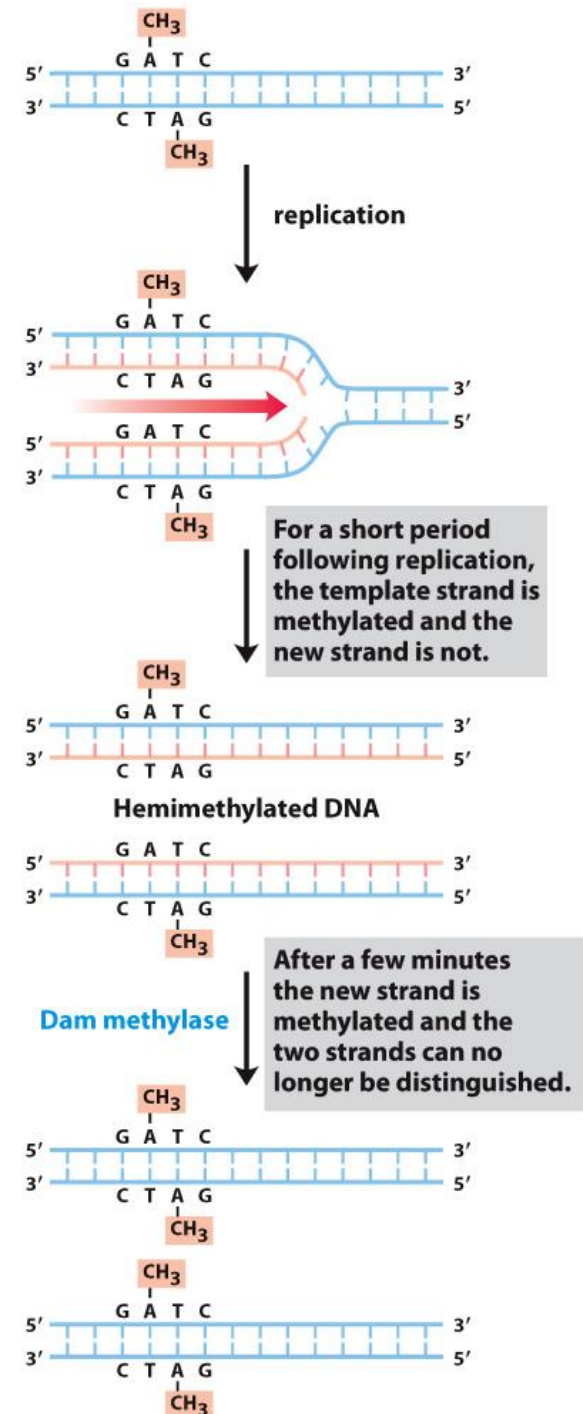


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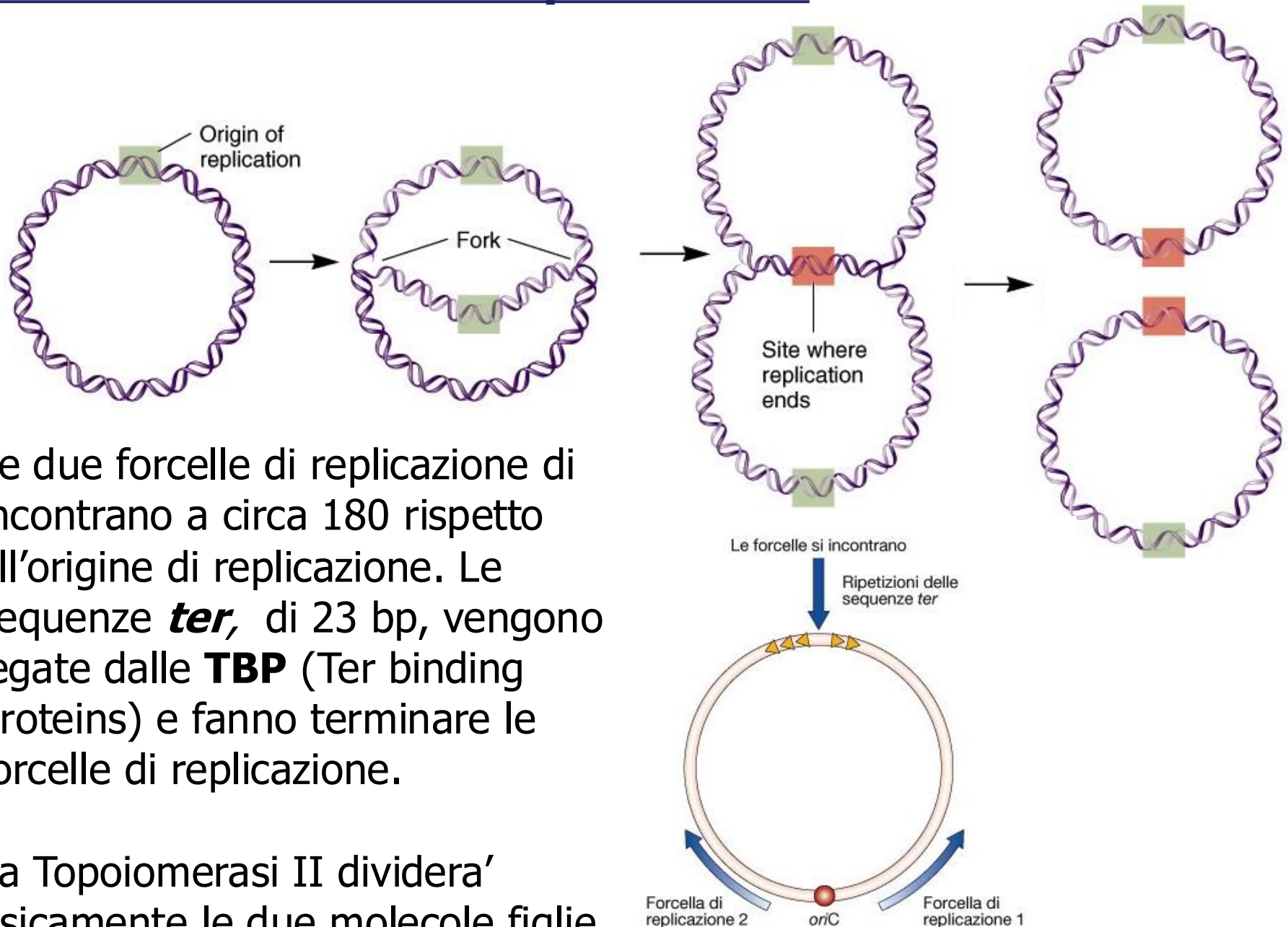
Bacterial DNA Methylation

- In *E. coli*, the parent strand is methylated.
- **Dam methylase** inserts CH_3 at adenines in the **GATC** sequence.
- Following a short period of time, the daughter strand is then methylated.
- The **newly synthesized strand is unmethylated** for a short period after synthesis.

ONLY DNA METHYLATED ON BOTH STRANDS CAN BE DUPLICATED

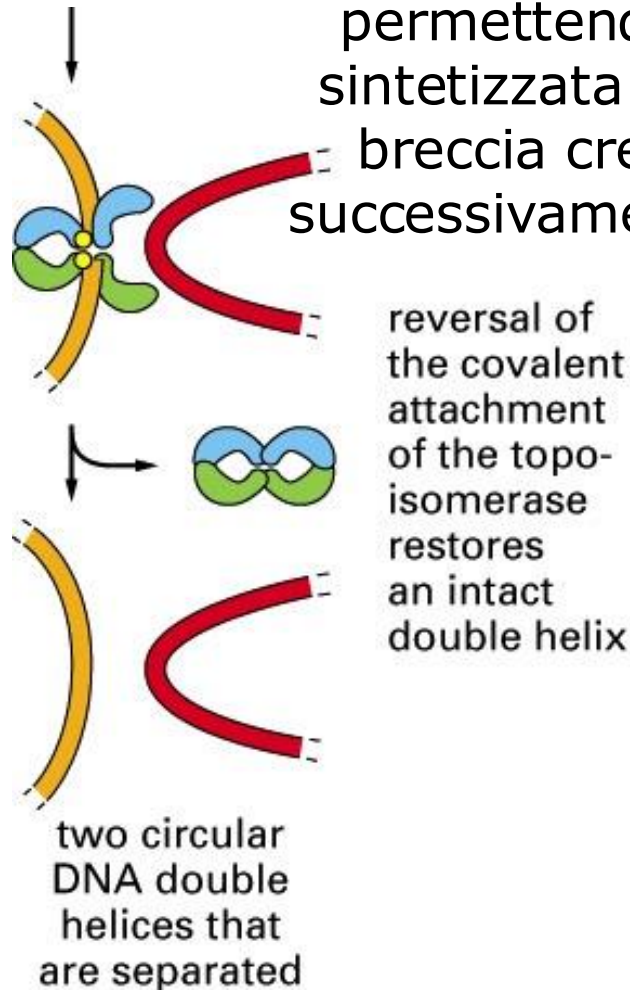
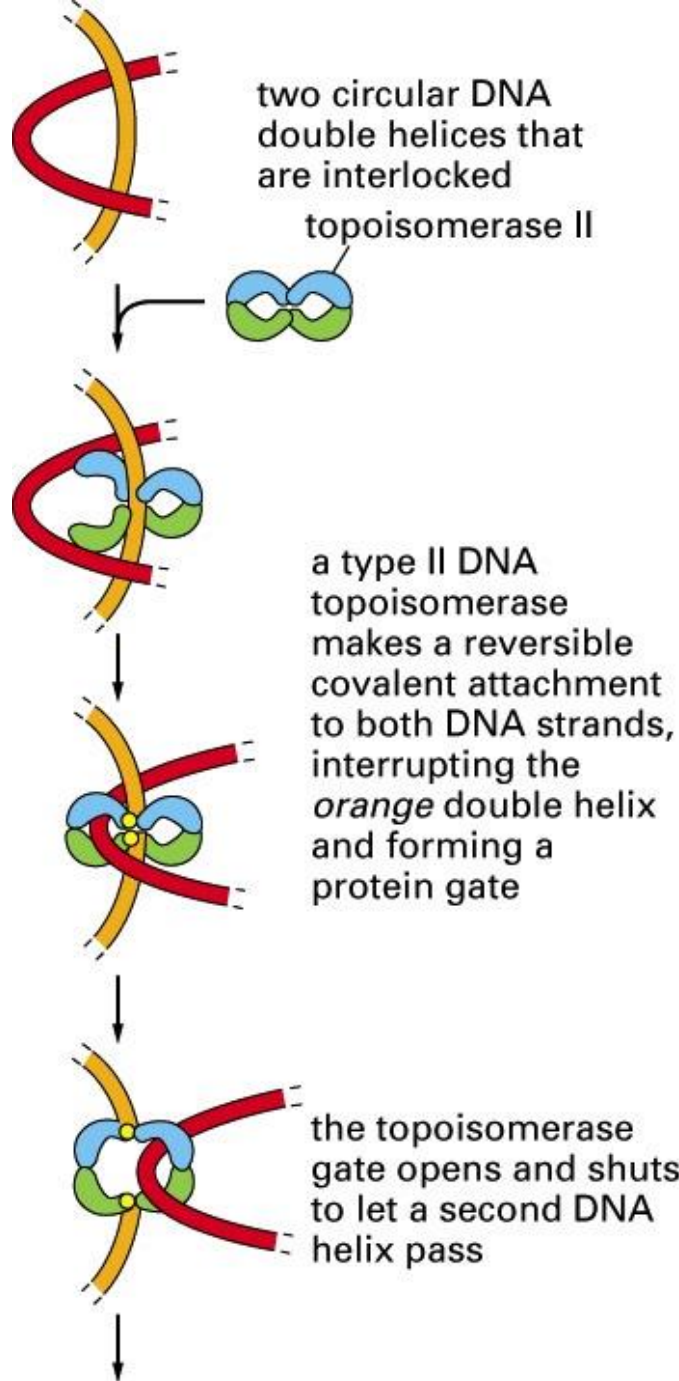


Terminazione della replicazione.



Topoisomerasi

TOPOISOMERASI II: forma un nick transiente su entrambi i filamenti di una doppia elica permettendo all'elica neo-sintetizzata di passare nella breccia creata dal taglio; successivamente salda il nick.



Serve ATP!

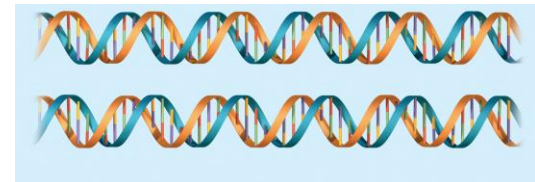
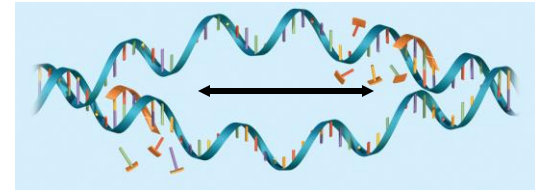
DNA Replication: THE STEPS

The process of replicating DNA is broken down into three major steps.

Your job is to know these 3 steps and be able to summarize what happens in each.

The three steps are:

1. Unwinding and Separating DNA Strands
2. Adding complimentary bases
 - a. Leading Strand:
 - b. Lagging Strand
3. Formation of Two Identical DNA molecules



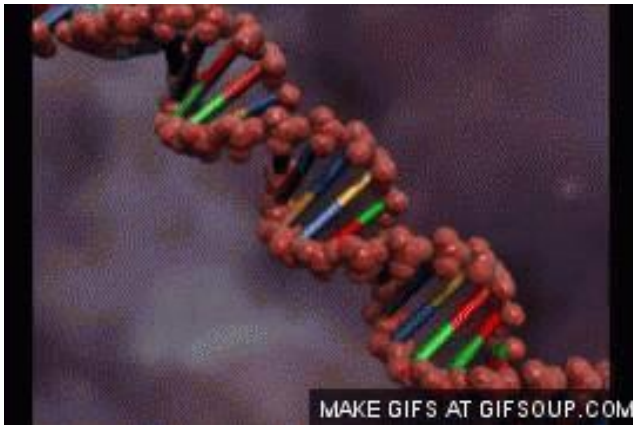
STEP1

DNA helicases unwinds & separates the original DNA double helix.

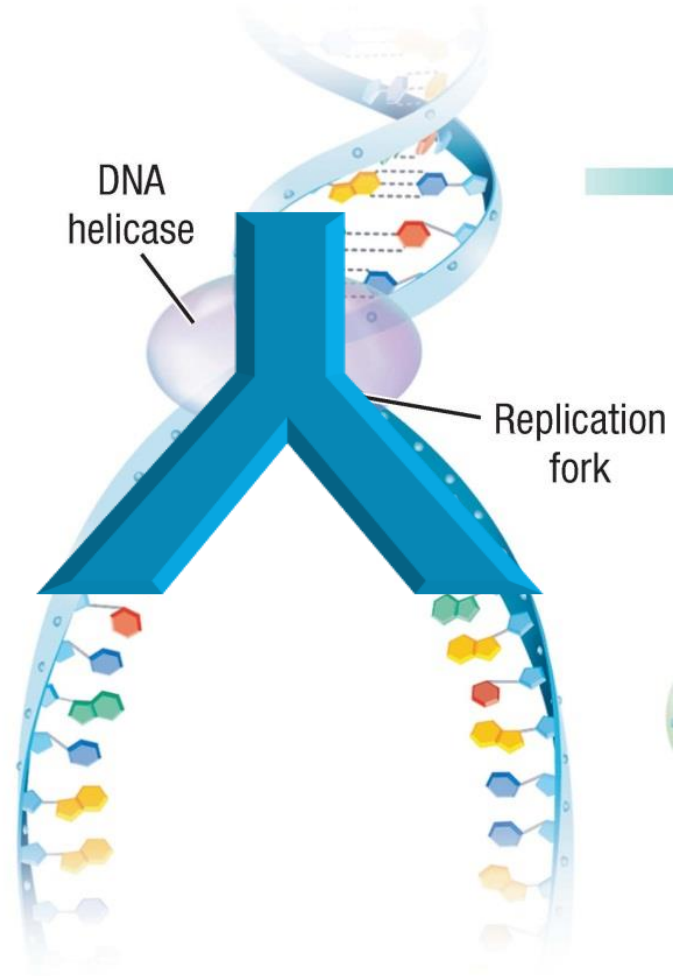
These proteins wedge themselves between the two strands of the double helix and break the hydrogen bonds between the base pairs.

Forms Replication Forks

As the double helix unwinds, the two complementary strands of DNA separate from each other and form a Y shape.



- 1 Proteins called *helicases* separate the two original DNA strands.



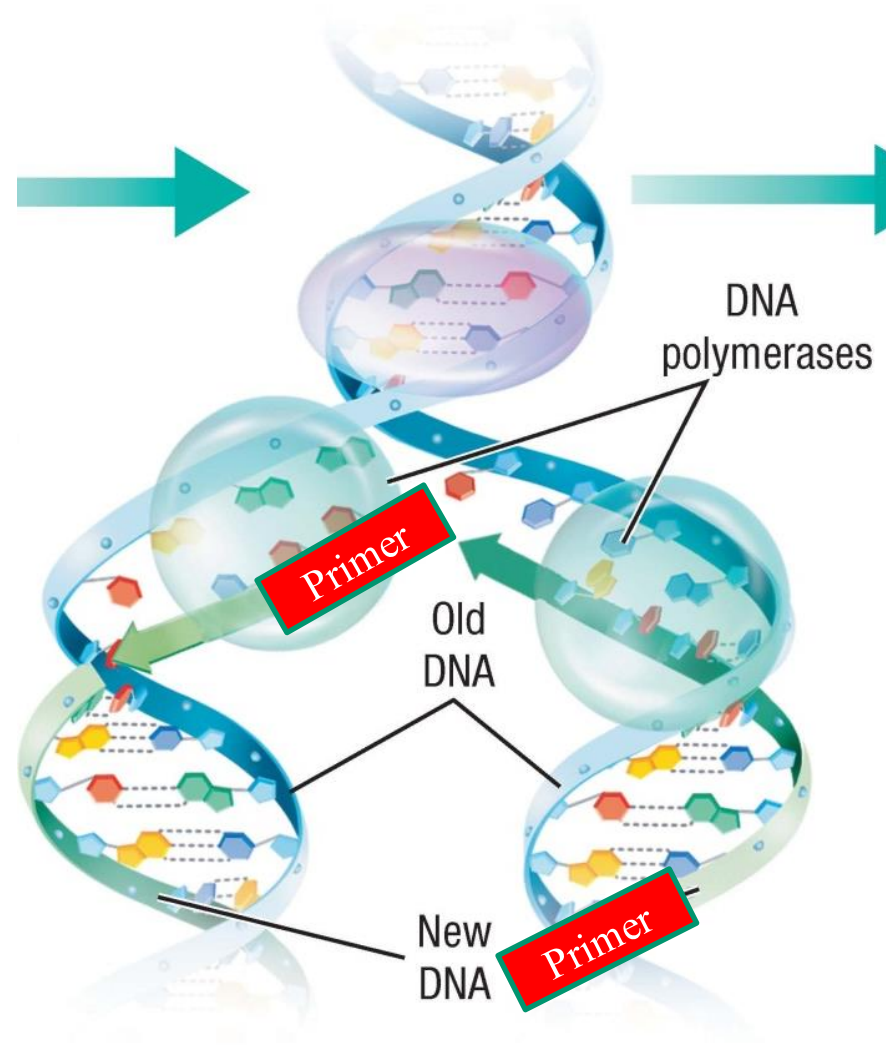
STEP2

New DNA is formed from DNA template.

RNA Primers attach to specific regions.

New nucleotides are added to the primer by the enzyme **DNA Polymerase**

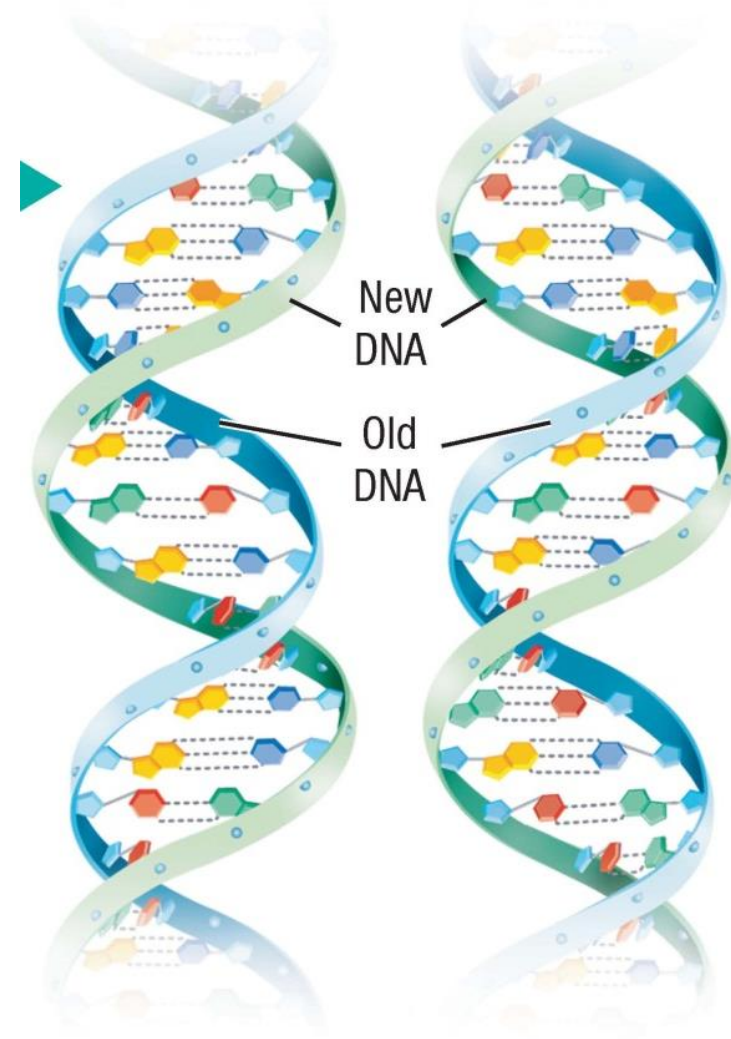
- 2 Complementary nucleotides are added to each strand by DNA polymerases.



STEP3

- The process completes when all the original bases have been paired with a new complementary nucleotide.
- Each double-stranded DNA helix is made of one new strand of DNA and one original strand of DNA.

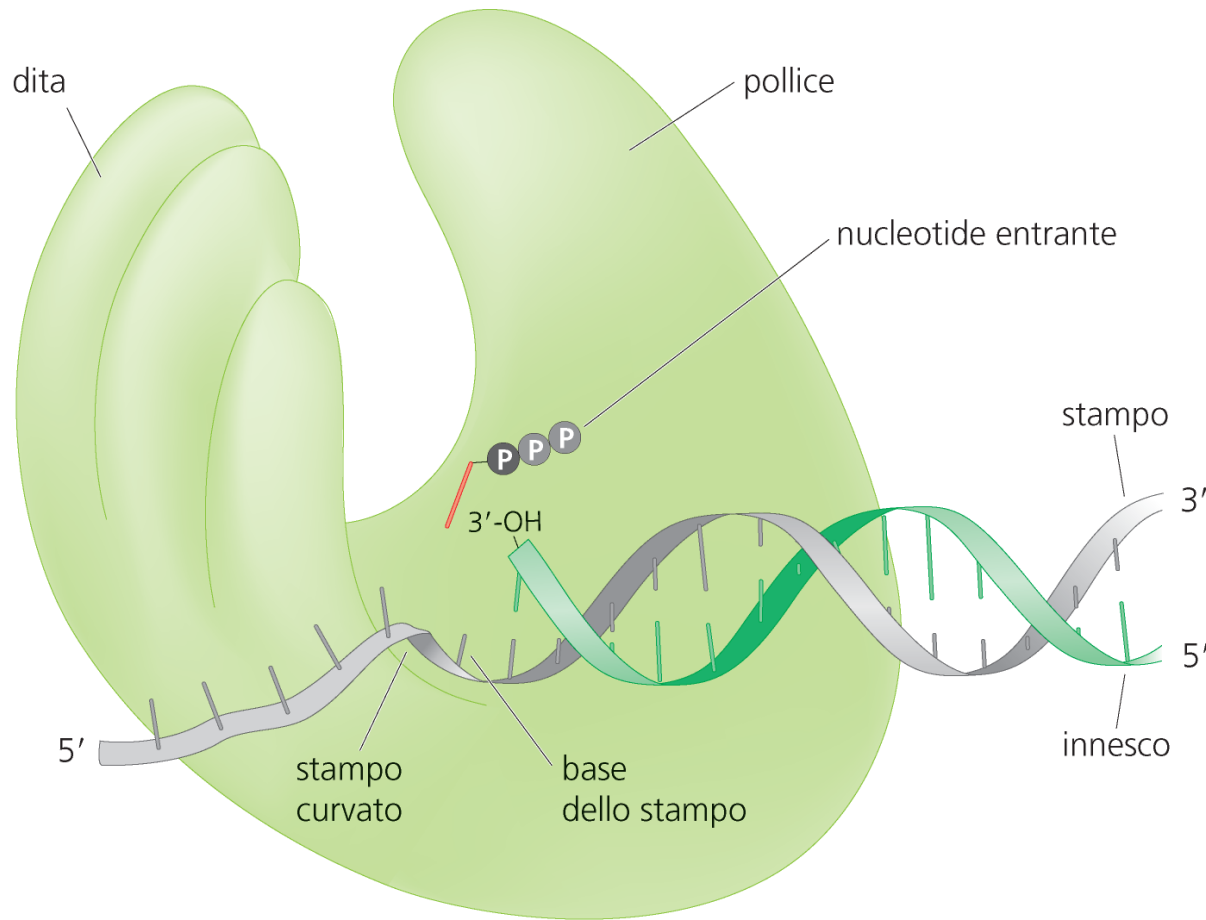
3 Two DNA molecules are formed that are identical to the original DNA molecule.



Le DNA Polimerasi

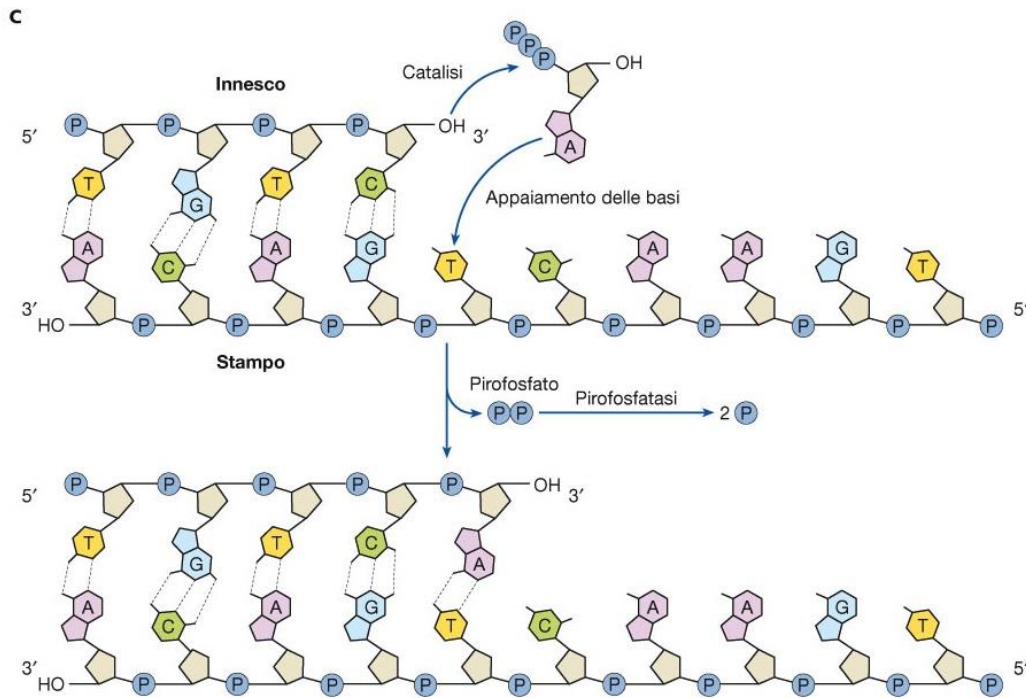
La DNA polimerasi e' un complesso multimerico formato da diverse unita' enzimatiche con diverse attivita' catalitiche.

Tutte le DNA polimerasi condividono la stessa anatomia molecolare, paragonabile a quella di una mano destra. Il complesso "innesco-stampo" attraversa il "palmo", dove viene controllata l'accuratezza dell'appaiamento tra le basi, in una scanalatura creata dalle "dita" e dal "pollice".



L'azione della DNA Polimerasi

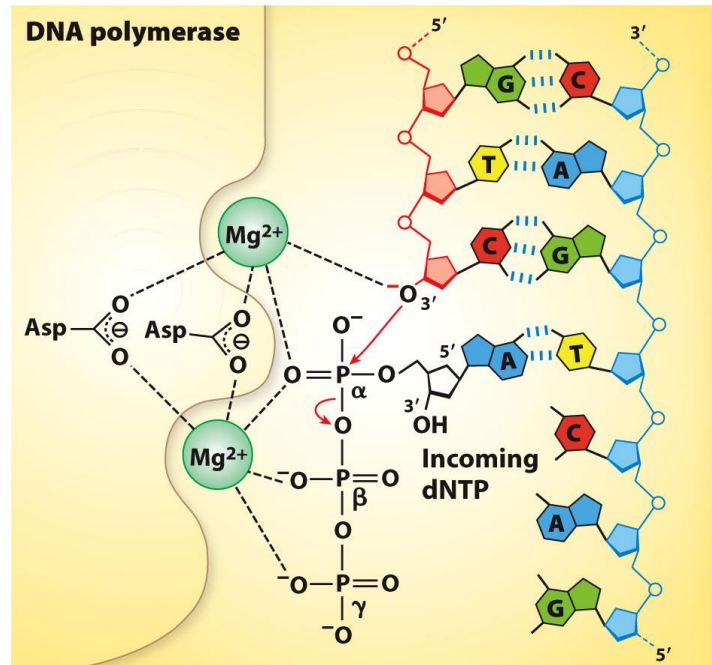
La DNA polimerasi catalizza l'aggiunta sequenziale di deossiribonucleotidi all'estremità 3'-OH di una catena polinucleotidica accoppiata ad un filamento stampo, formando un legame fosfodiesterico con il gruppo fosfato al 5' del nucleotide aggiuntivo.



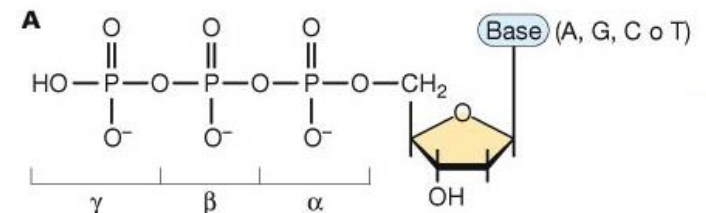
DNA synthesis

Growing strand (primer) Template strand

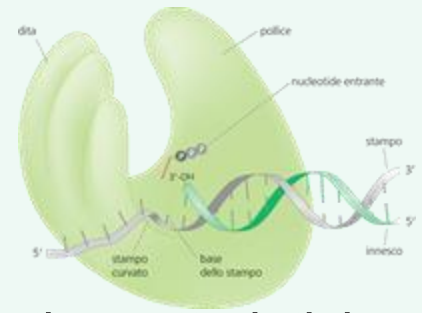
DNA polymerase



I nucleotidi entrano come nucleosidi trifosfati, apportando l'energia necessaria per la polimerizzazione, fornita dall'idrolisi di un legame fosfoanidride, con liberazione di pirofosfato. Il pirofosfato viene poi idrolizzato ulteriormente a fosfato inorganico, rendendo la reazione del tutto irreversibile.



Le DNA Polimerasi

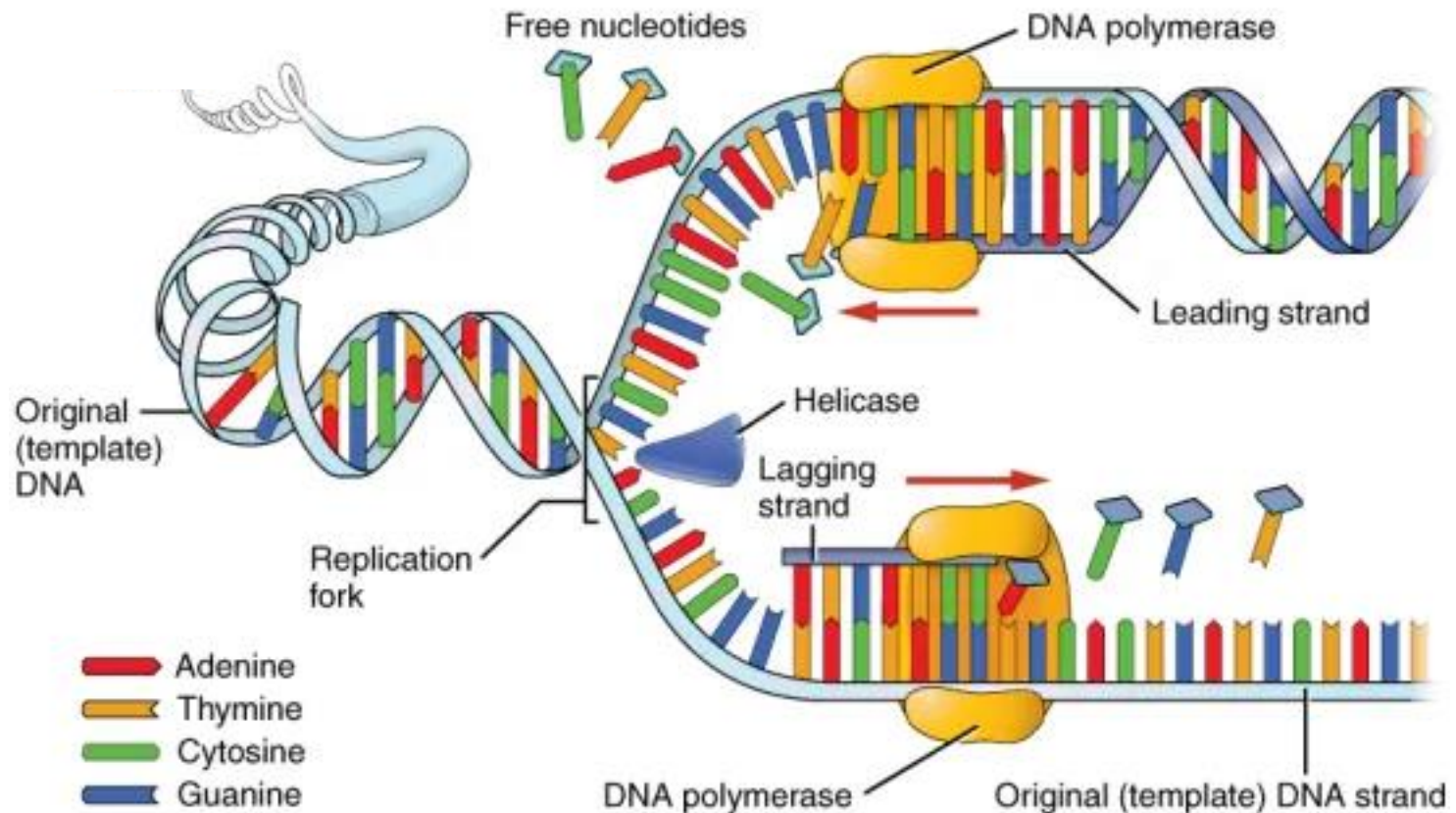


Le diverse isoforme di DNA polimerasi condividono le seguenti caratteristiche:

1. Le DNA polimerasi **non** sono in grado di rompere i legami idrogeno per separare i due filamenti di una doppia elica di DNA.
2. Tutte le DNA polimerasi **necessitano di uno stampo da copiare**, fornito dai filamenti di un' elica preesistente.
3. Tutte le DNA polimerasi sono in grado di allungare un filamento di DNA o RNA che funge da innesco, ma **non possono iniziare la sintesi di una catena ex novo**.
4. I due filamenti di una elica di DNA sono antiparalleli ($5' \rightarrow 3'$ e $3' \rightarrow 5'$) e tutte le DNA polimerasi catalizzano solo l'aggiunta di un nucleotide all'estremità $3'$ di una catena nascente.
5. In questo modo **le catene possono crescere solo in direzione $5' \rightarrow 3'$** .
6. Tutte le DNA polimerasi utilizzano come substrato solo i quattro nucleotidi trifosfato.

La Forcella di Replicazione

La forcella di replicazione è la struttura in cui avviene la duplicazione del DNA. E' formata dalla molecola di DNA parentale i cui due filamenti complementari sono parzialmente denaturati per un breve tratto.



Special proteins help to open up double helix in front of the replication fork

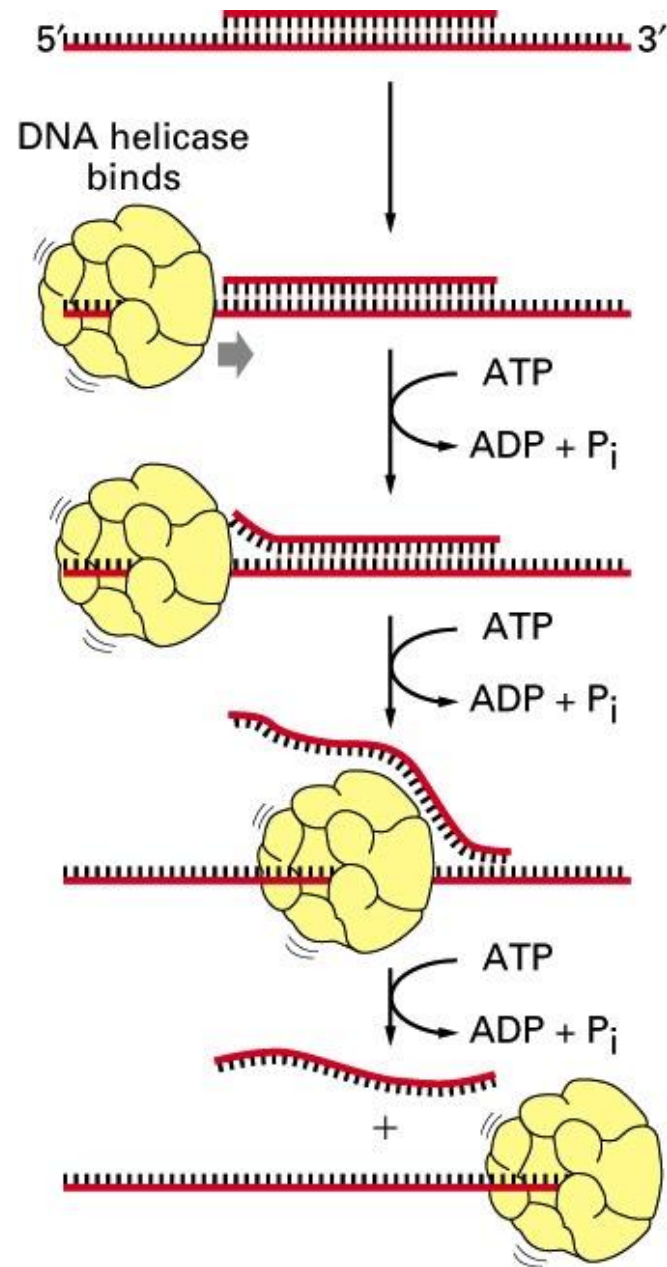
- * DNA helicases
- * Single-strand binding (SSB) proteins

DNA Helicase

An enzyme that unwinds the double helix by breaking the Hydrogen bonds between the complementary bases

La DNA elicasi si lega al DNA a singolo filamento e scorre separando i due filamenti della molecola (1000 nucleotidi/secondo).

Il movimento della DNA elicasi richiede idrolisi di ATP.



Crystal Structure of T7 Gene 4 Ring Helicase Indicates a Mechanism for Sequential Hydrolysis of Nucleotides

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Tom Ellenberger,† and Dale B. Wigley*†

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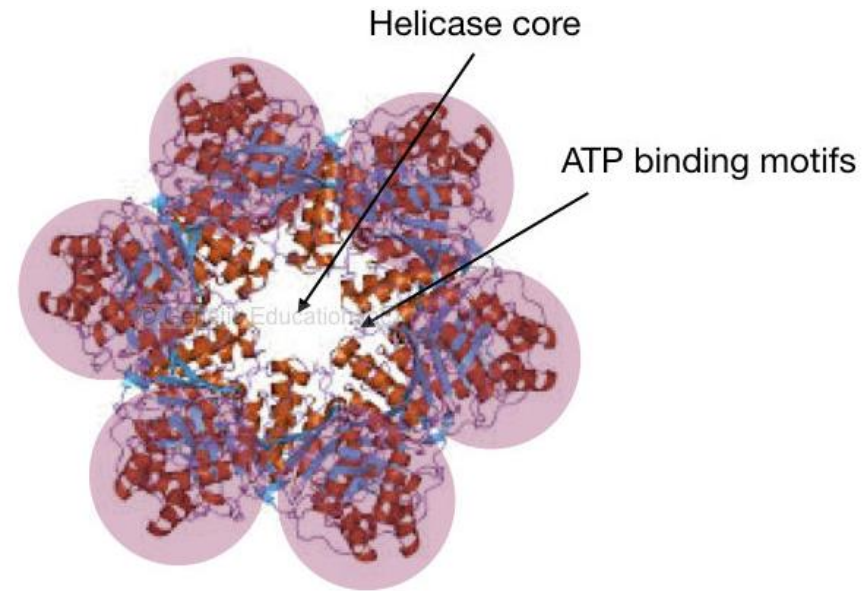
United Kingdom

†Department of Biological Chemistry

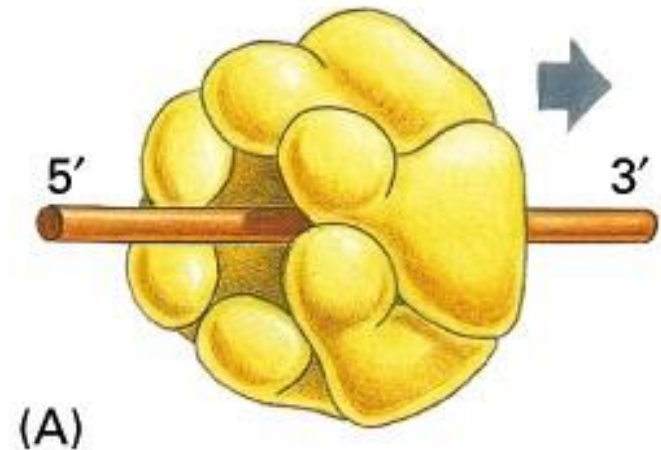
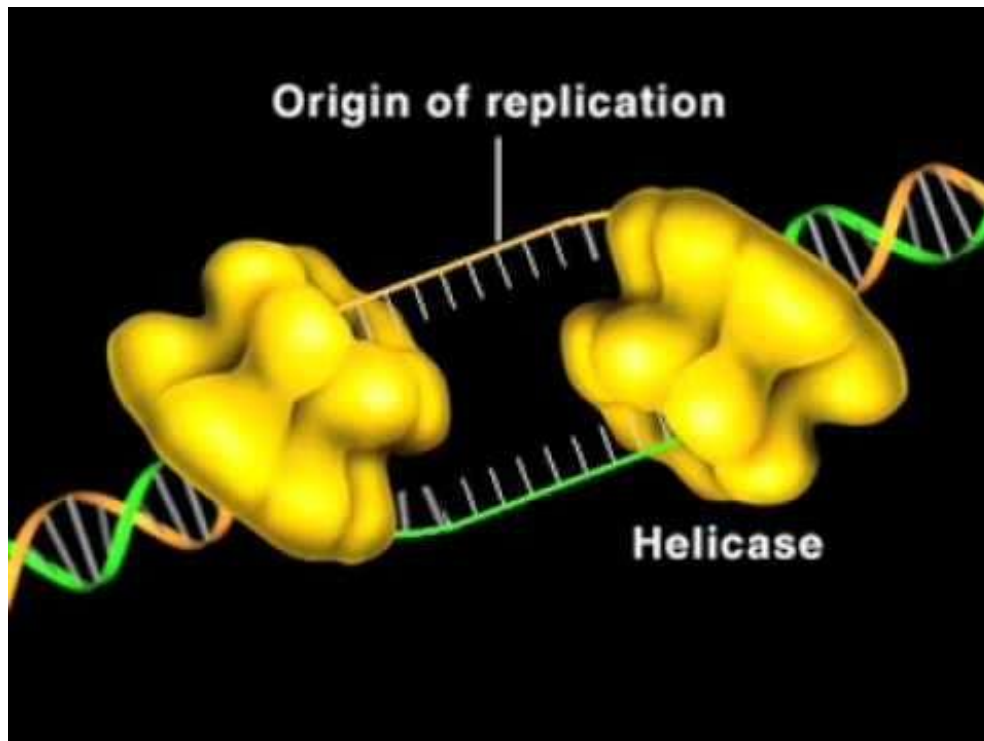
and Molecular Pharmacology

Harvard Medical School

Boston, Massachusetts 02115



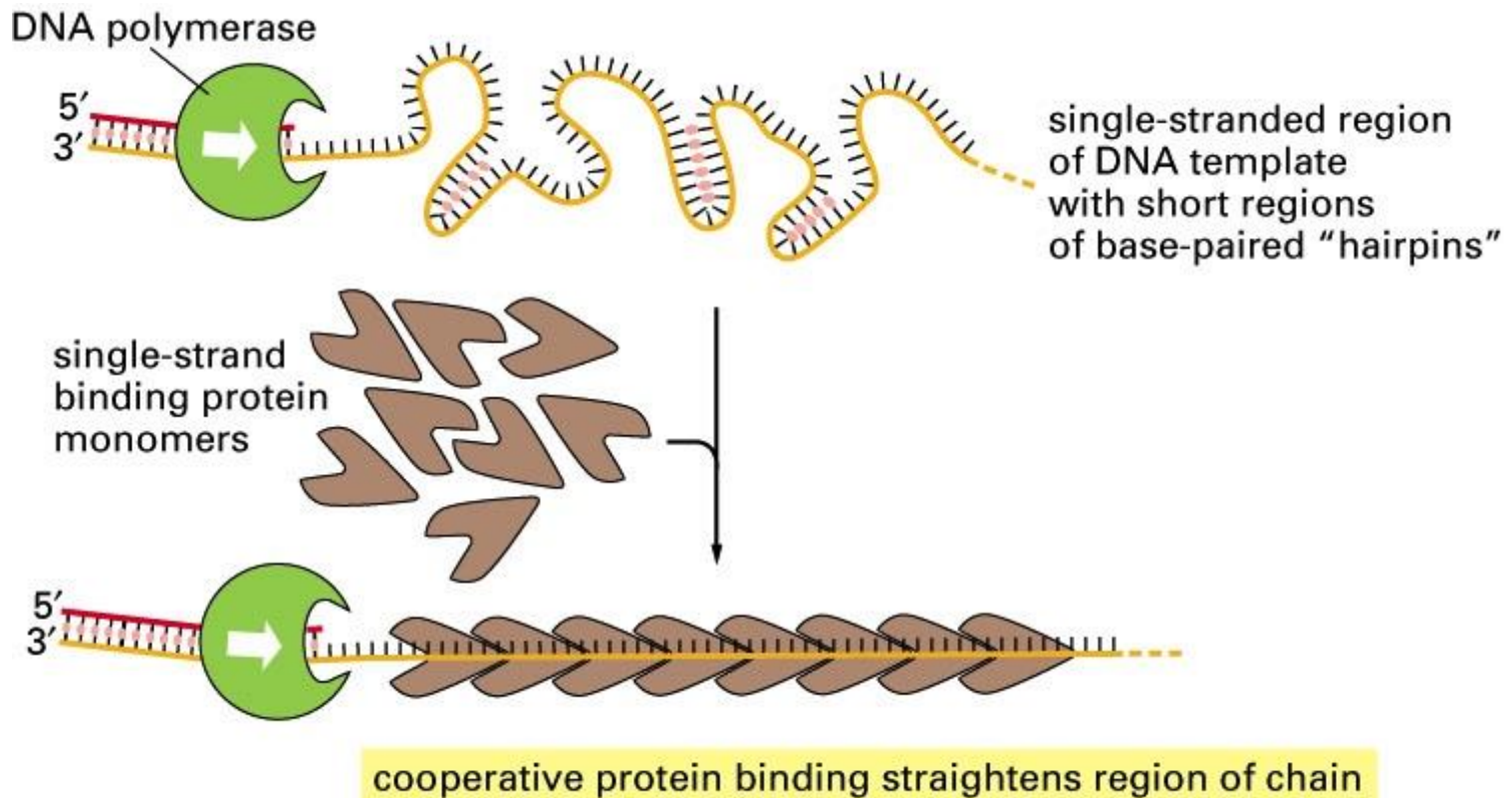
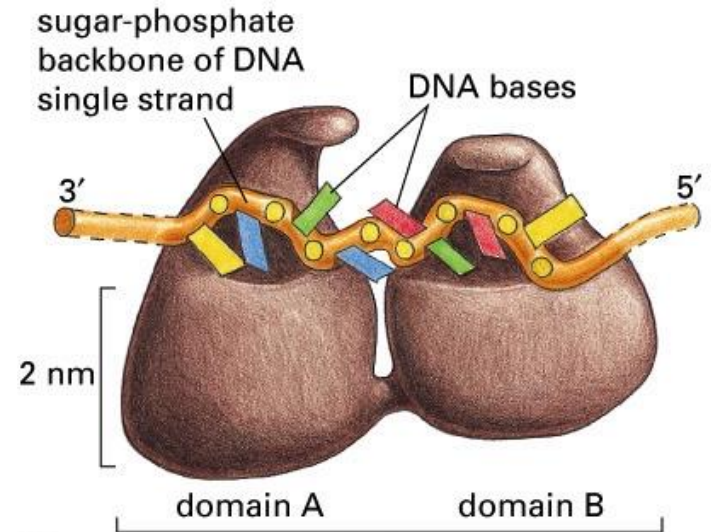
Hexameric structure of DNA helicase



SSB proteins

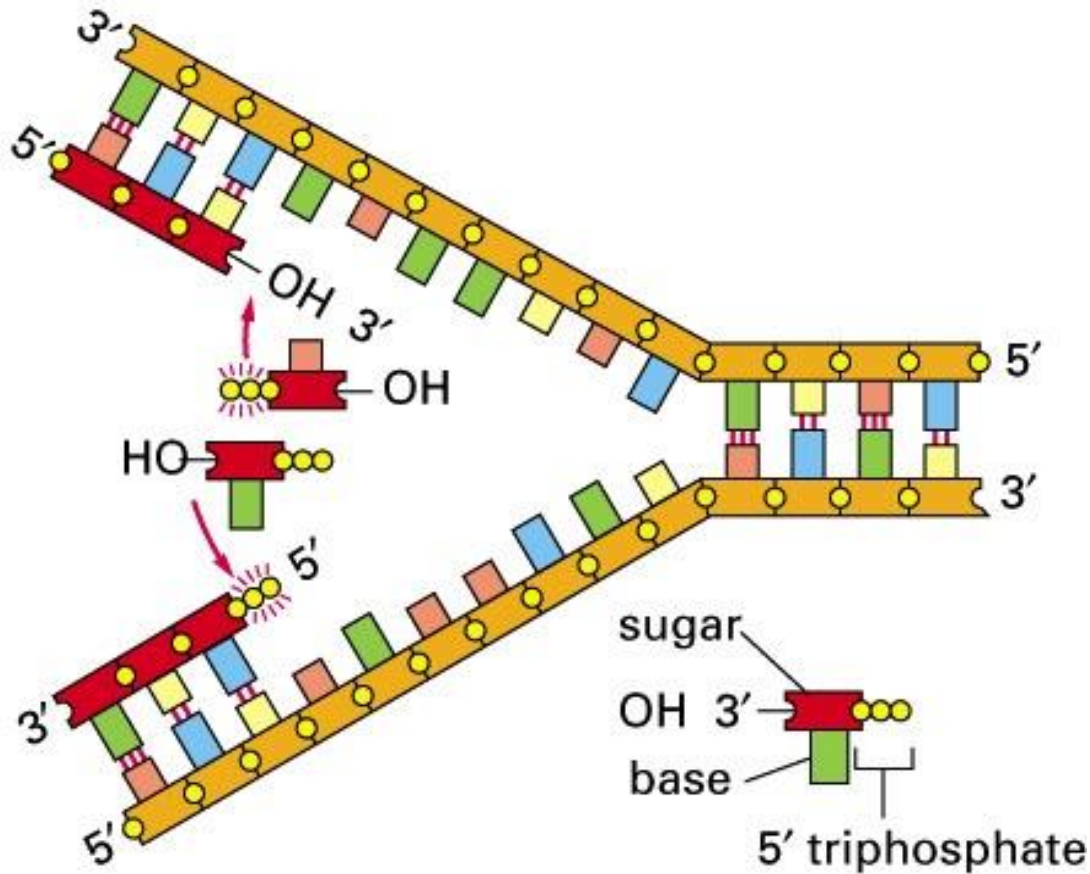
SSB proteins bind tightly to ssDNA without covering the bases.

SSB proteins are unable to open a DNA helix but aid helicases by stabilizing the unwound ss conformation



La Forcella di Replicazione

I singoli filamenti fungono da stampo per la sintesi di un nuovo filamento complementare.



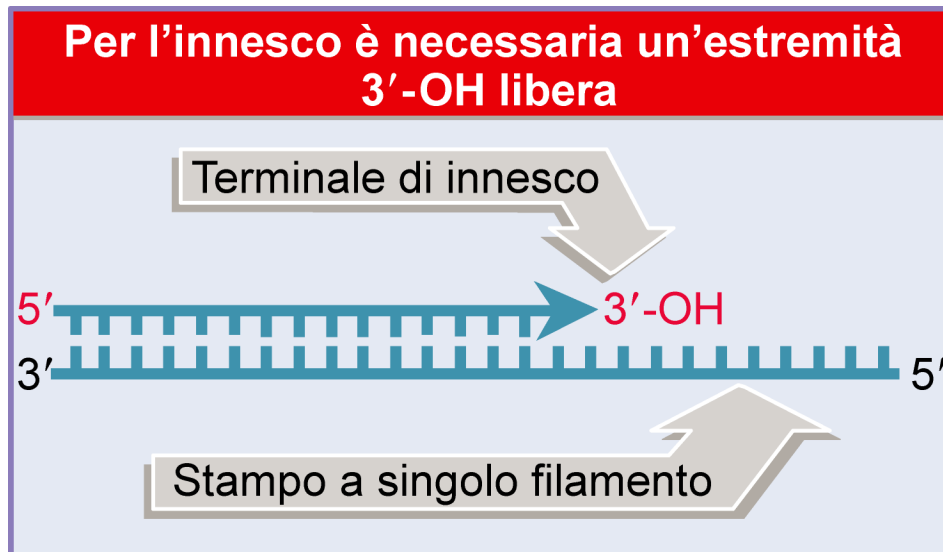
MA

La DNA polimerasi ha
attivit  di sintesi
ESCLUSIVAMENTE in
direzione 5' ->3' :
problema di sintesi
continua di uno dei due
filamenti

Le DNA Polimerasi richiedono un'estremità di innesco 3'-OH per iniziare la sintesi del DNA.



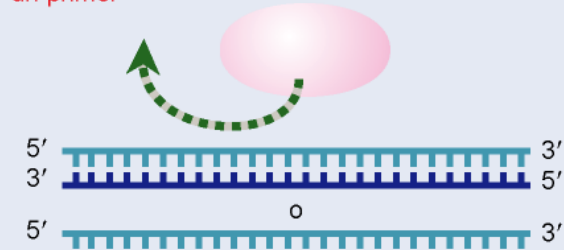
Le DNA Polimerasi richiedono un'estremità di innesco 3'-OH per iniziare la sintesi del DNA.



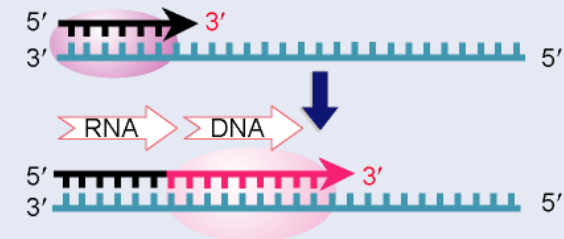
L'innesco può essere una molecola di RNA, un nick nel DNA o una proteina di innesco (virus).

Esistono molti modi per generare estremità 3'-OH

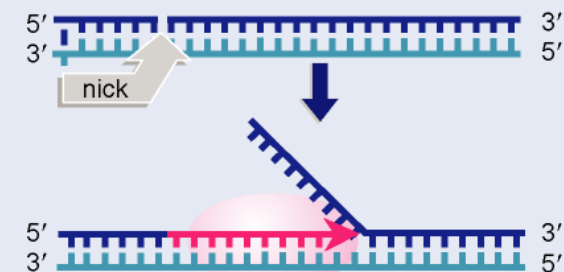
Le DNA polimerasi non possono iniziare la sintesi di DNA su DNA duplex o a singolo filamento senza un primer



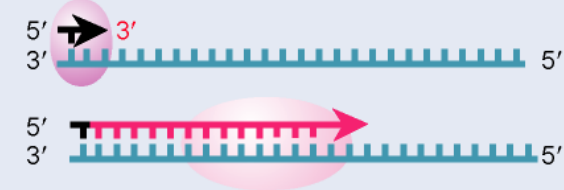
Il primer di RNA è sintetizzato o fornito per appaiamento delle basi



Il DNA duplex è tagliato per fornire l'estremità libera alla DNA polimerasi



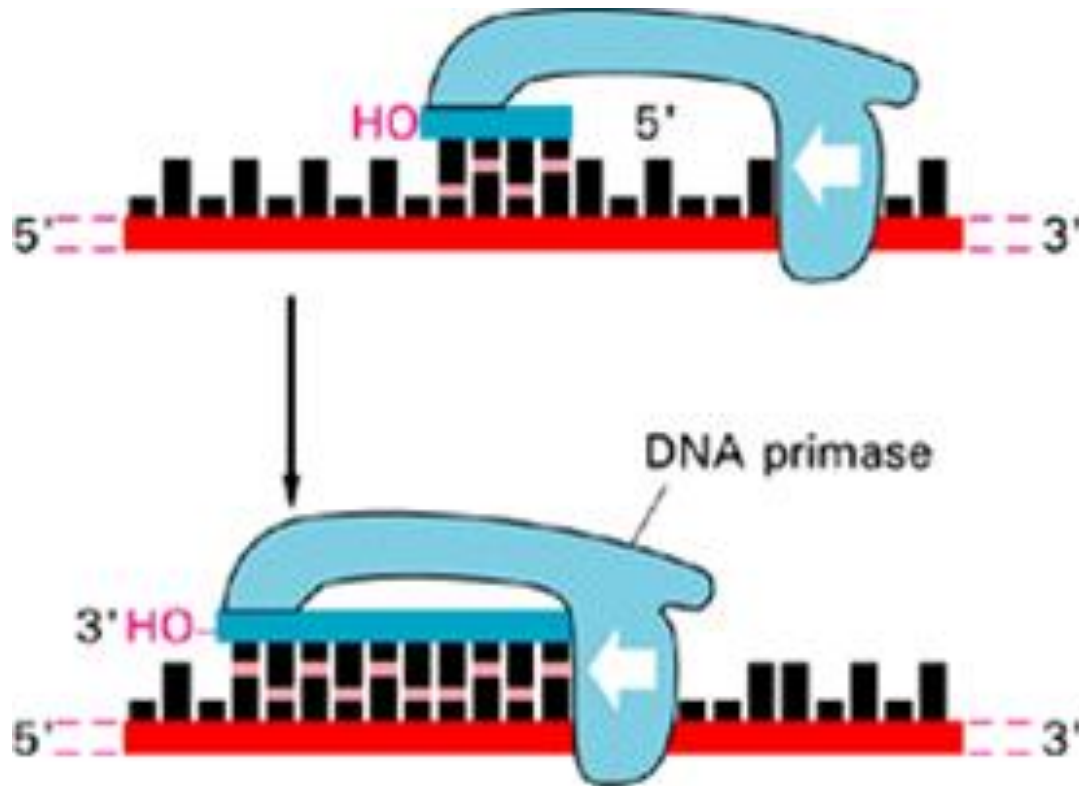
Un nucleotide di innesco è fornito da una proteina che si lega al DNA



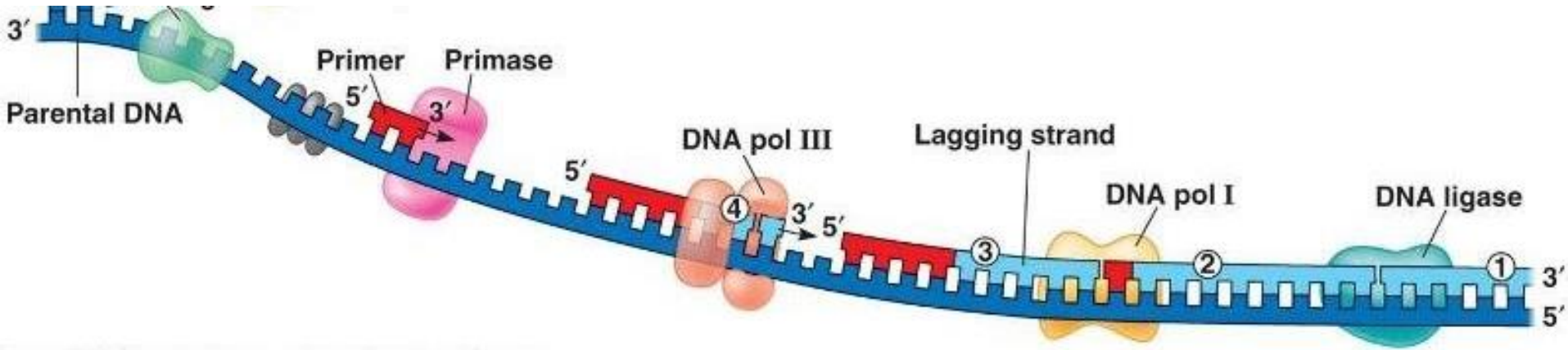
La Primasi

La primasi e' una speciale **RNA Polimerasi** che produce corti primers di RNA (circa 10 nucleotidi) che servono da innesco per la DNA polimerasi.

Al contrario della DNA polimerasi, la primasi e' in grado di iniziare una nuova catena unendo due ribonucleotidi trifosfati.



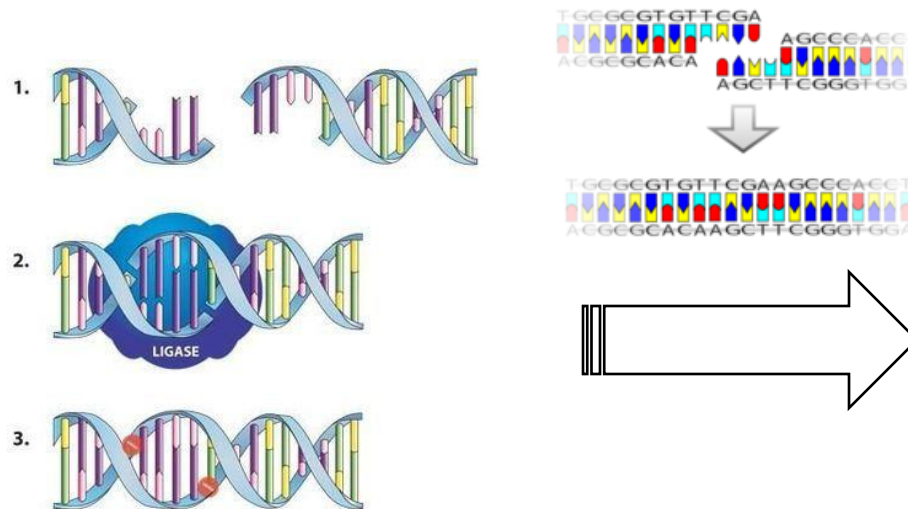
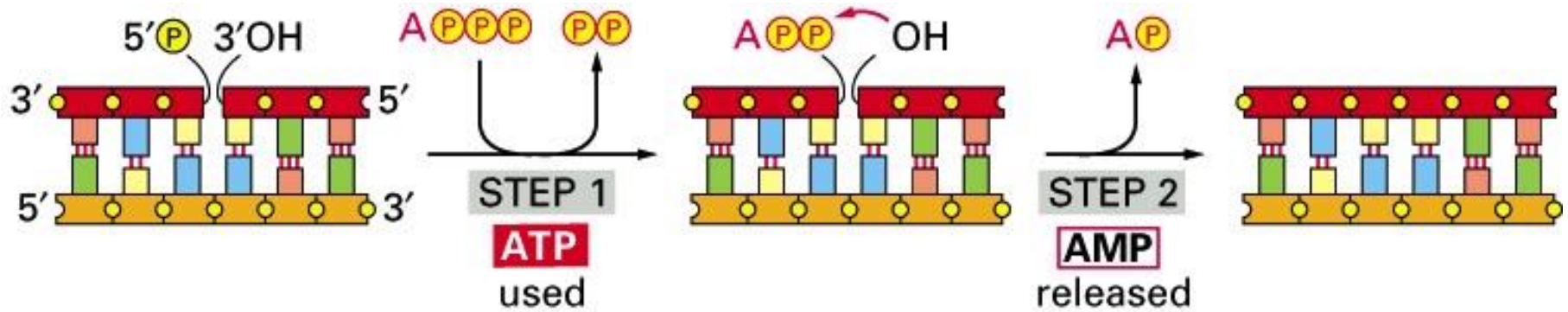
I frammenti di Okasaki



- ✓ I frammenti di Okazaki sono sintetizzati dalla DNA polimerasi III a partire degli inneschi creati dalla Primasi.
- ✓ La DNA polimerasi non e' in grado di unire i frammenti.
- ✓ I primers a RNA devono essere sostituiti, in quanto le basi U devono essere sostituite da T.
- ✓ I primers vengono degradati di una Rnasi (RNAsiH + FEN1 in eucarioti) e la DNA Polimerasi I li sostituisce con una sequenza di DNA, contenente T e non piu' U.
- ✓ I singoli frammmmenti vengono poi uniti dalla DNA Ligasi

L'azione della DNA Ligasi

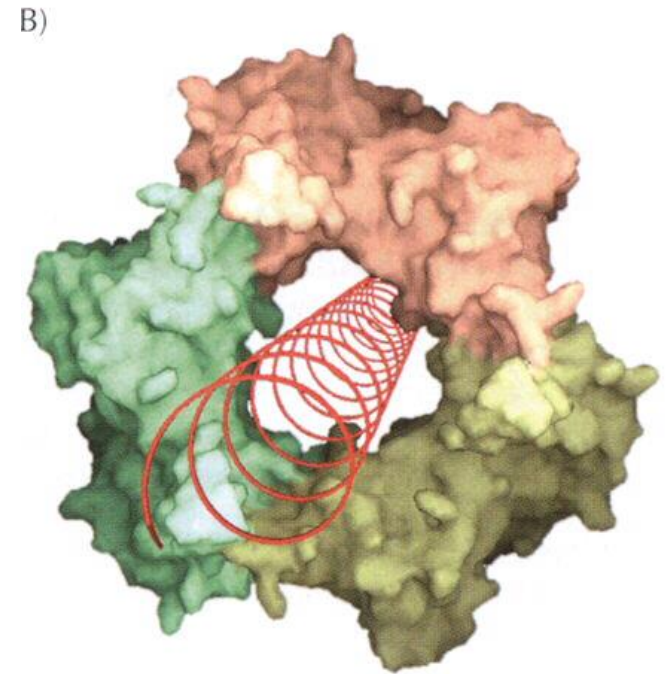
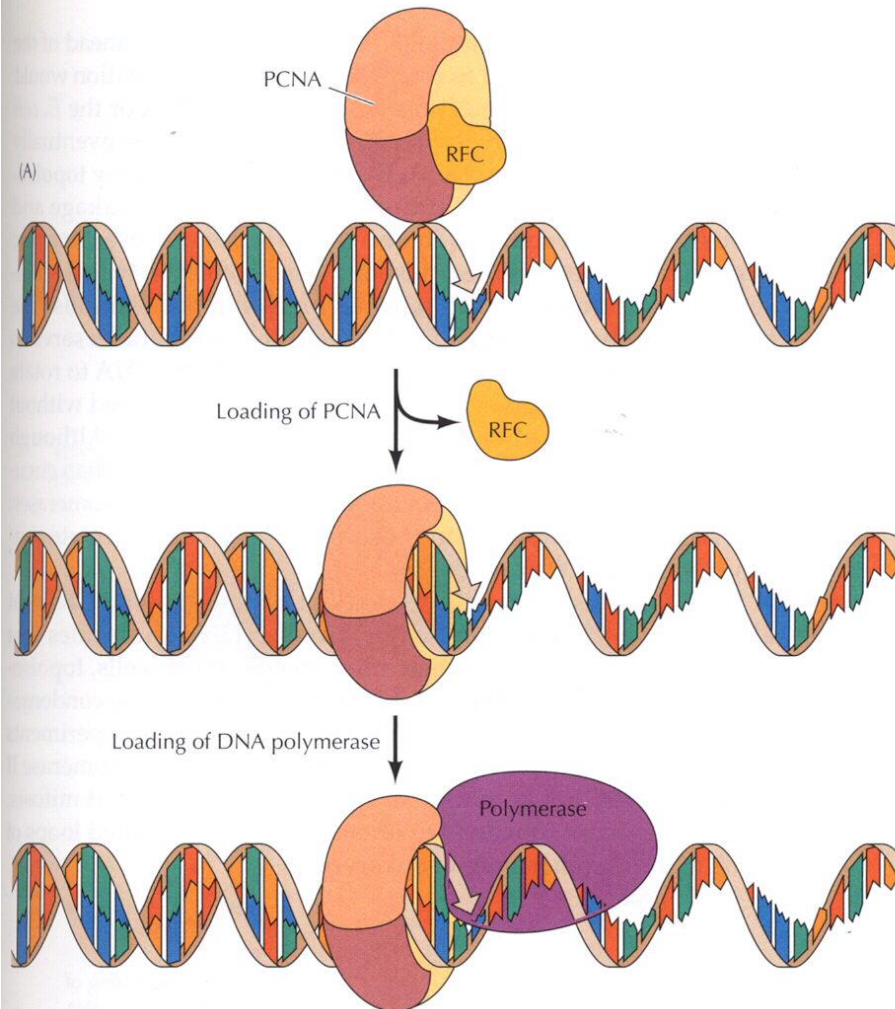
La DNA ligasi catalizza la formazione di legami fosfodiesterici tra frammenti di Okazaki



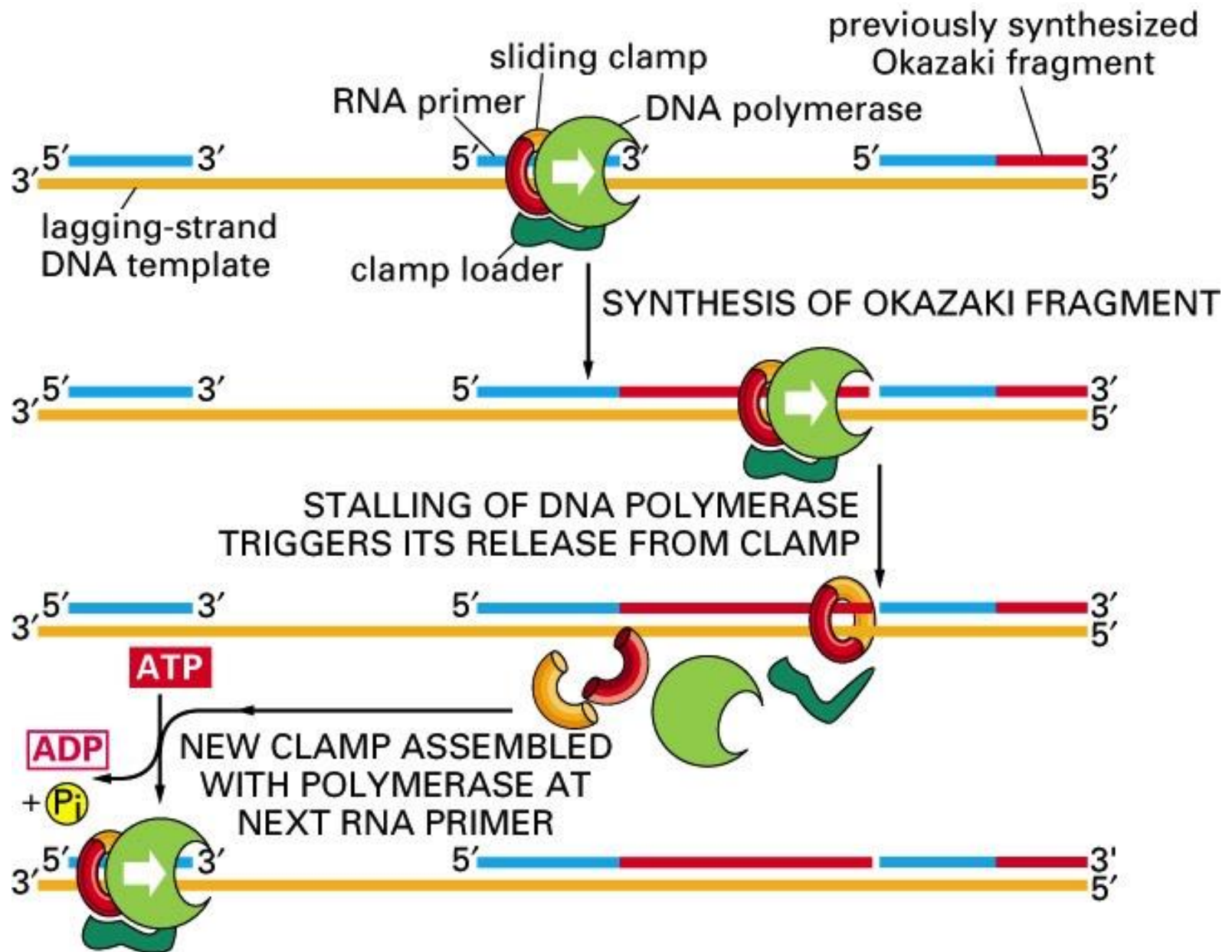
Stabilization of the replication machine

A ring clamp (PCNA) keeps the polymerase firmly on the DNA when it is moving on the dsDNA. One side of the ring binds to the back of the DNAPol and it slides along DNA as the polymerase moves on.

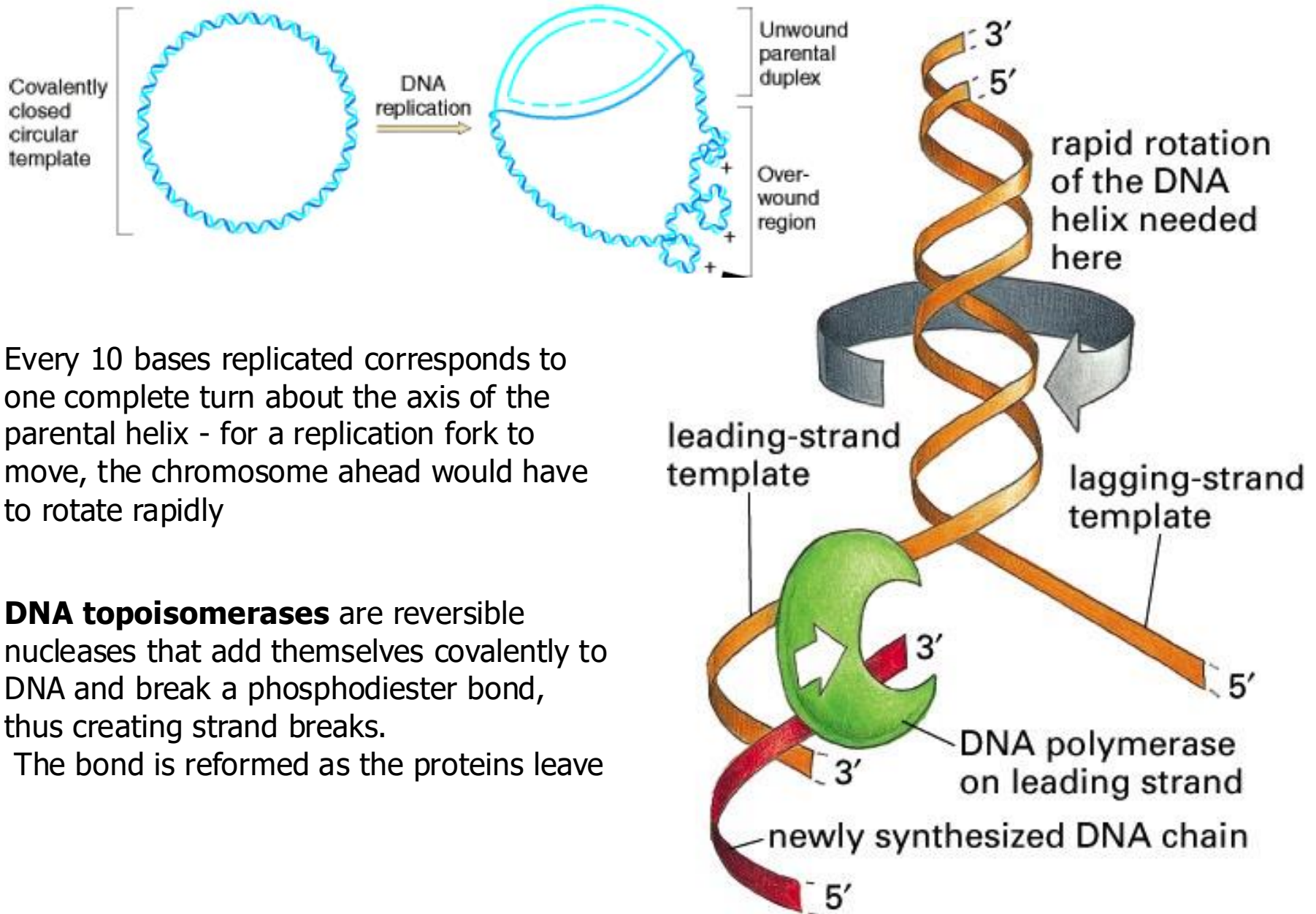
A clamp loader (**replication factor C - RFC**) catalyzes the assembly of the multisubunit clamps around the DsDNA.



around the primer-template junction requires ATP hydrolysis by a special protein complex, the **clamp loader**



The winding problem



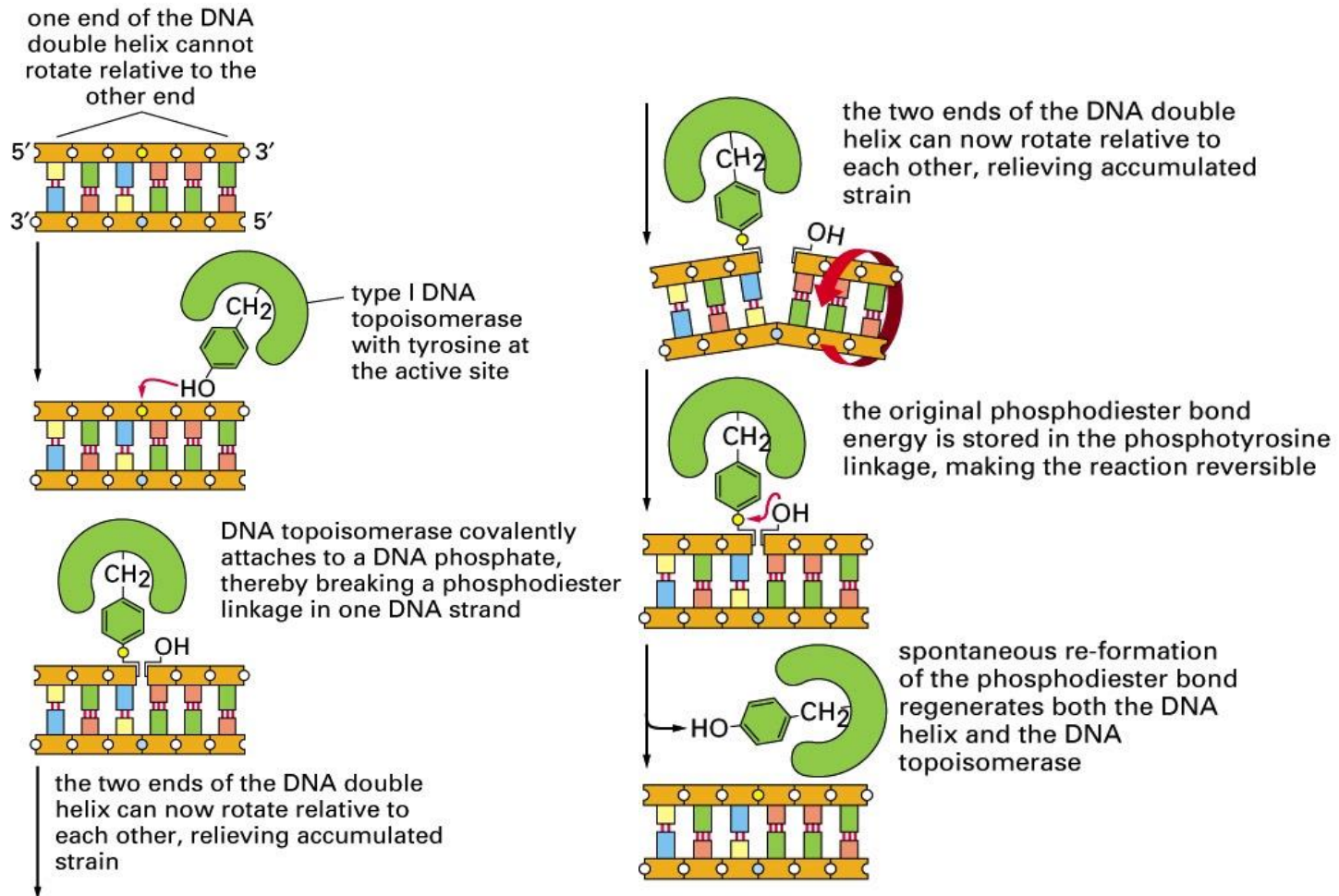
Every 10 bases replicated corresponds to one complete turn about the axis of the parental helix - for a replication fork to move, the chromosome ahead would have to rotate rapidly

DNA topoisomerases are reversible nucleases that add themselves covalently to DNA and break a phosphodiester bond, thus creating strand breaks.

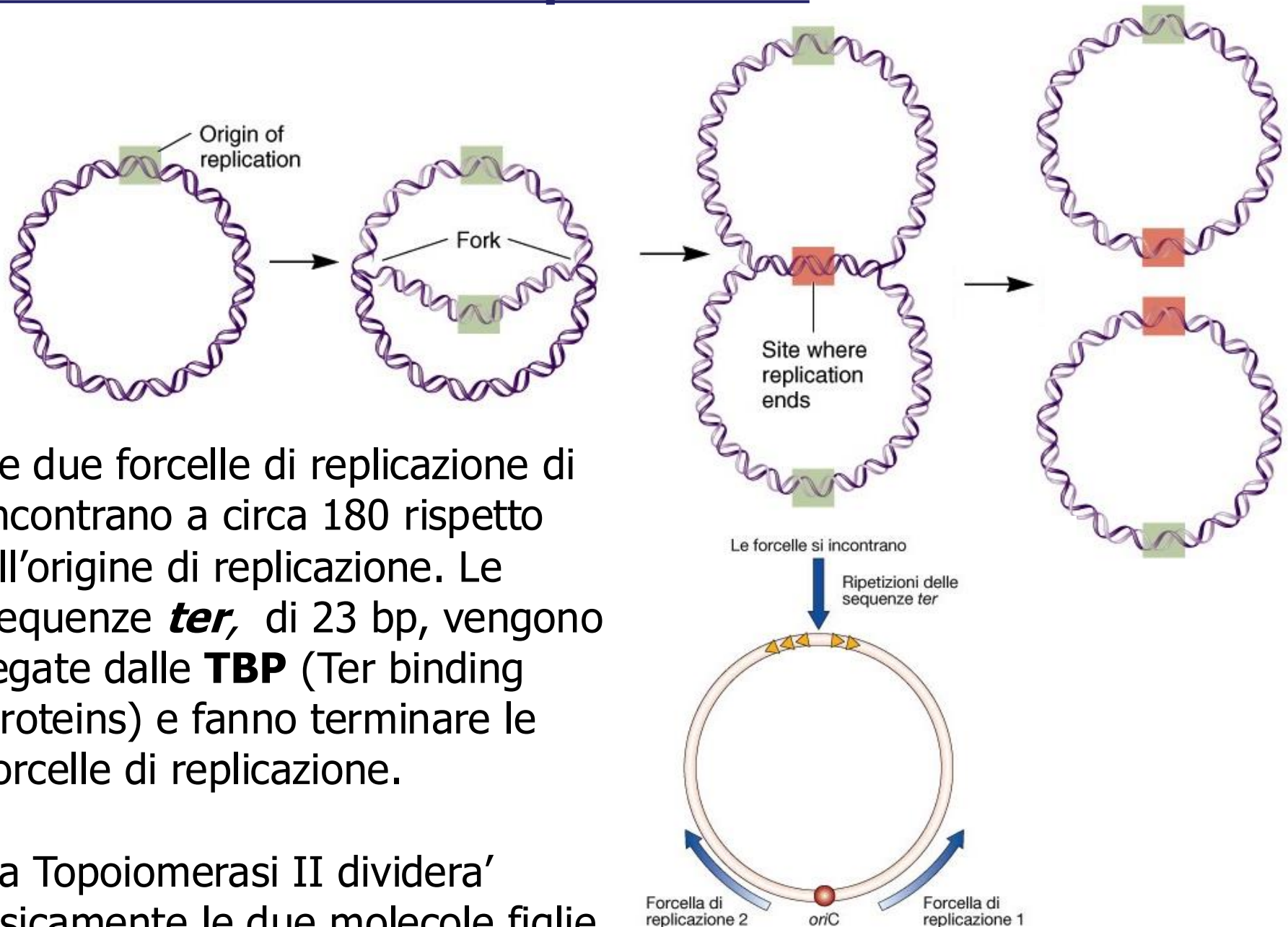
The bond is reformed as the proteins leave

Topoisomerasi

TOPOISOMERASI I: formano un nick transiente su un singolo filamento permettendo alle due parti dell'elica di ruotare liberamente l'una rispetto all'altra.



Terminazione della replicazione.

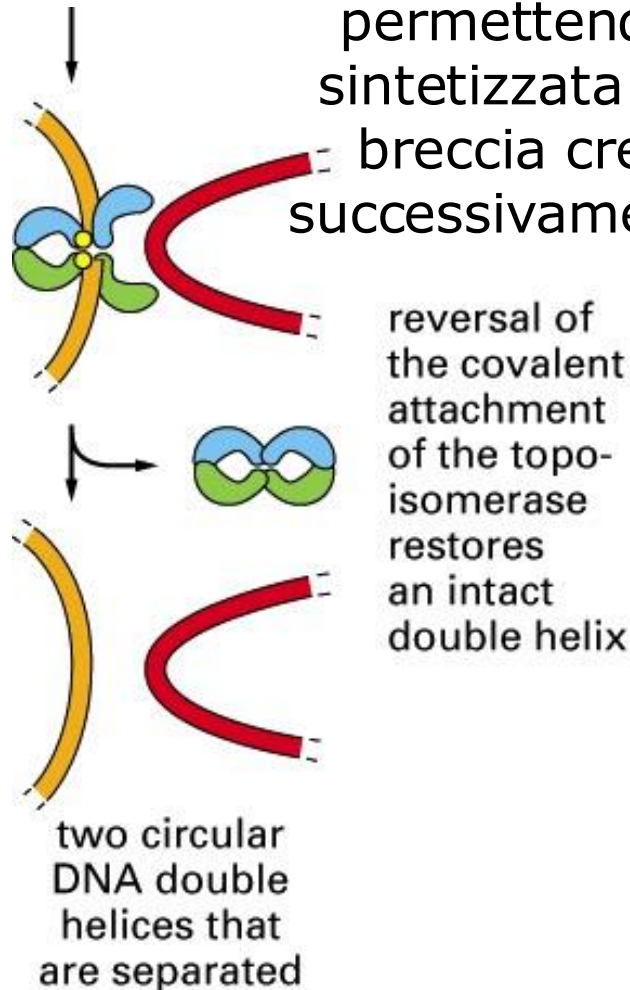
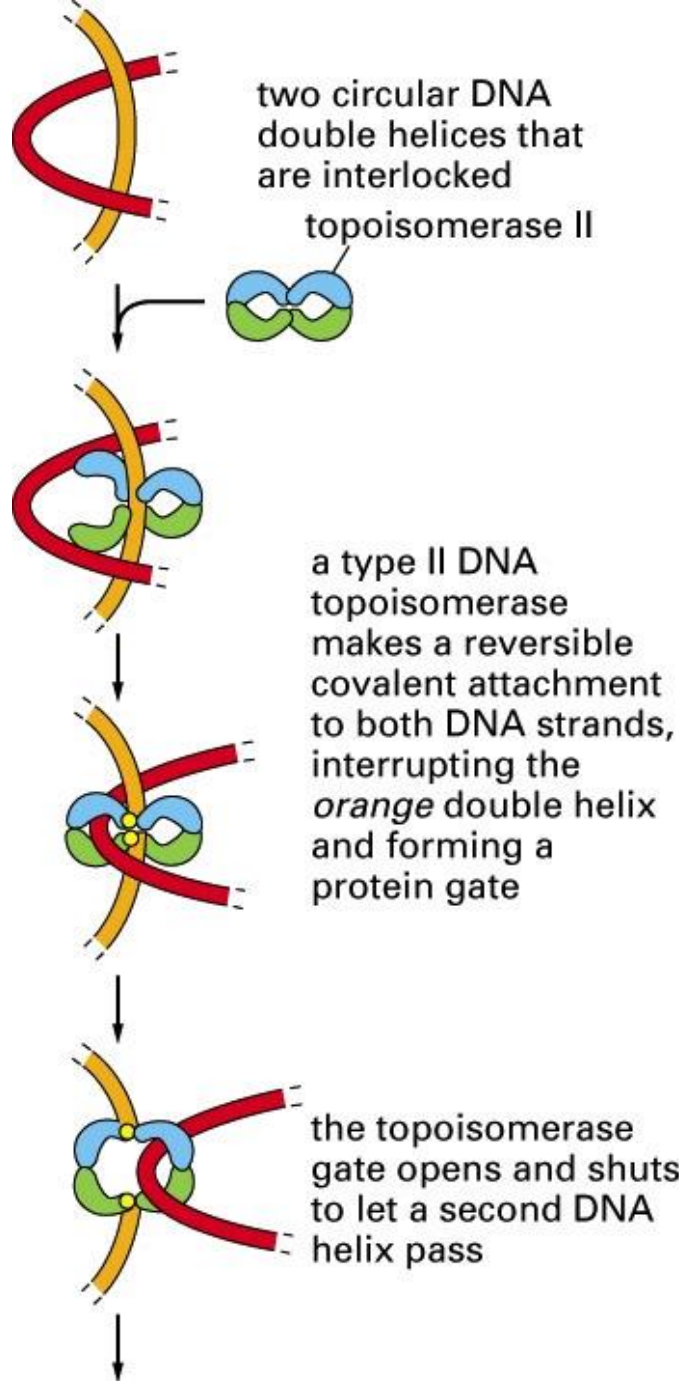


Le due forcelle di replicazione di incontrano a circa 180 rispetto all'origine di replicazione. Le sequenze ***ter***, di 23 bp, vengono legate dalle **TBP** (Ter binding proteins) e fanno terminare le forcelle di replicazione.

La Topoisomerasi II dividerà fisicamente le due molecole figlie

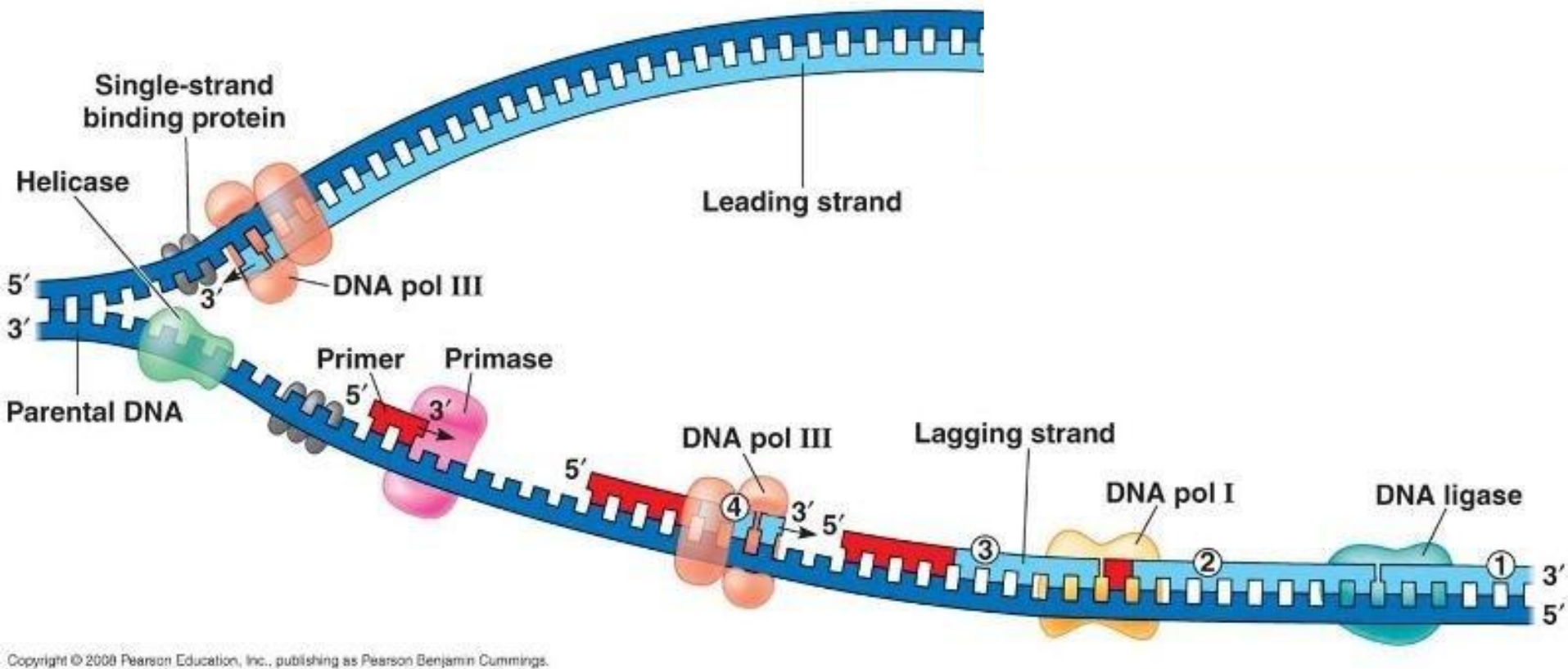
Topoisomerasi

TOPOISOMERASI II: forma un nick transiente su entrambi i filamenti di una doppia elica permettendo all'elica neo-sintetizzata di passare nella breccia creata dal taglio; successivamente salda il nick.



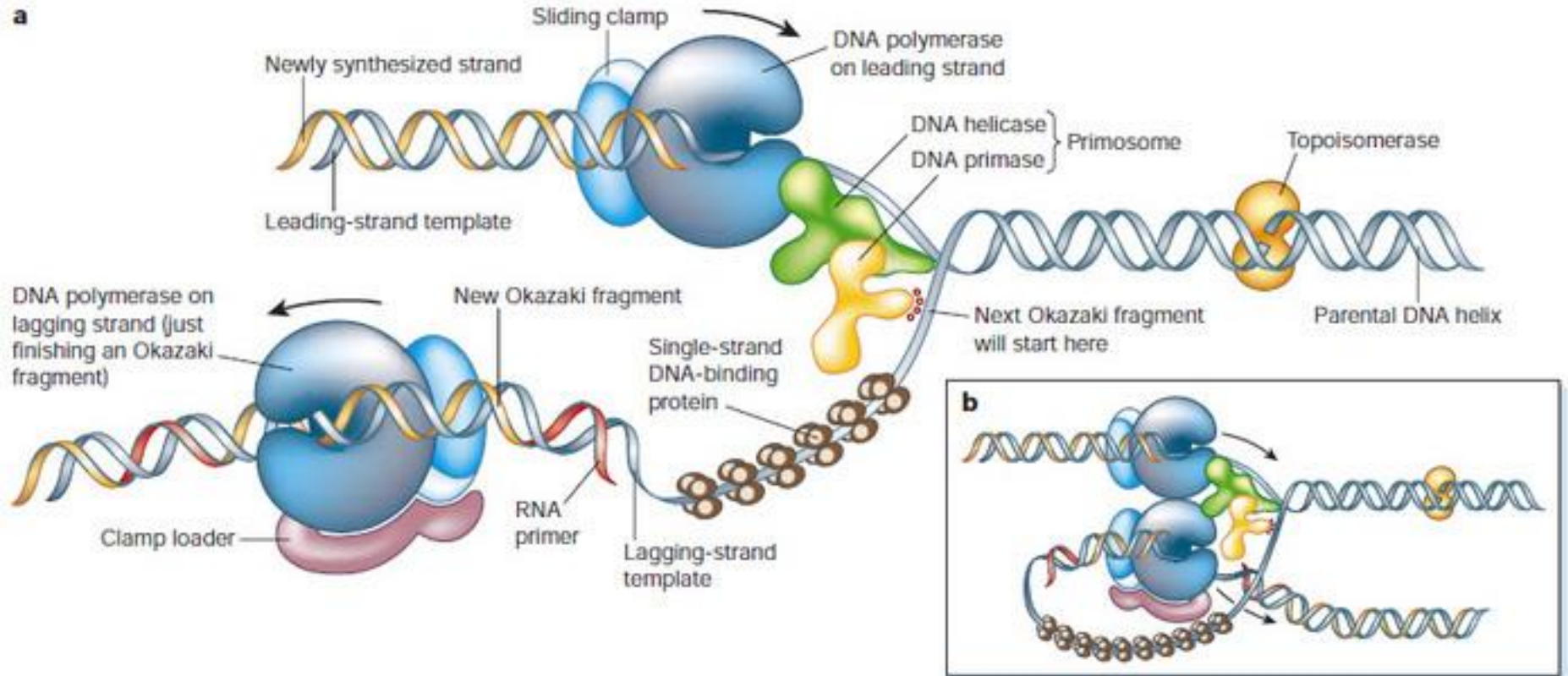
Serve ATP!

La strategia di duplicazione e' diversa sui due filamenti



Viene definito **filamento "guida" (leading)** quello in cui la sintesi di DNA procede in modo continuo, mentre il **filamento "lento" o ritardato (lagging)** è quello in cui la duplicazione avviene attraverso la sintesi di brevi segmenti di DNA, chiamati **frammenti di Okazaki**, lunghi 1000-2000 nucleotidi nei procarioti e 100-200 negli eucarioti.

The proteins at a replication fork form a multienzyme replication machine



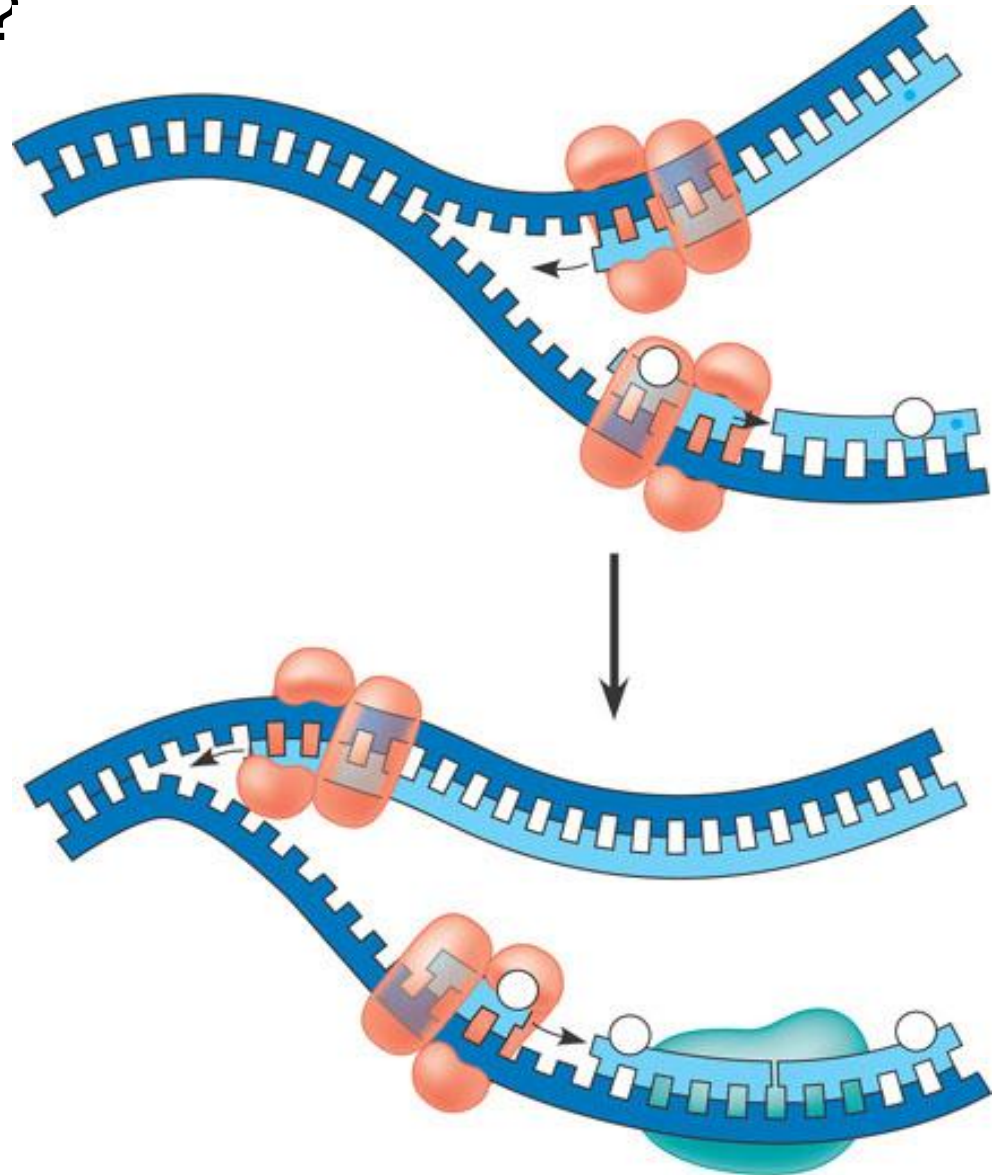
A special arrangement, with a lagging strand folded back facilitates the loading of the polymerase clamp each time that an Okazaki fragment is synthesized. The replication proteins are linked together into a single large unit, that moves rapidly along the DNA, enabling DNA to be synthesized on both sides of the replication fork in a coordinated manner

Summary

- What is DNA replication?
- Why does it happen?
- Know the steps...
- On your handout, label:

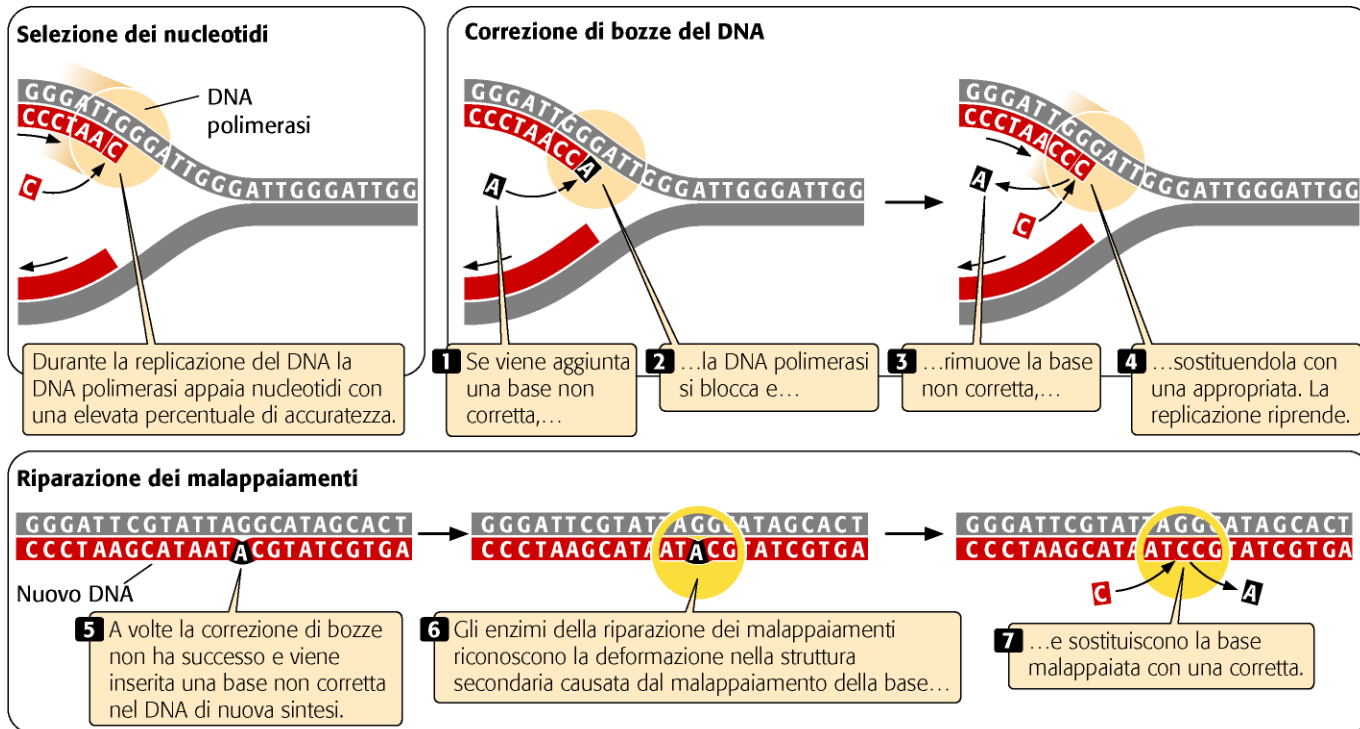
Where is...

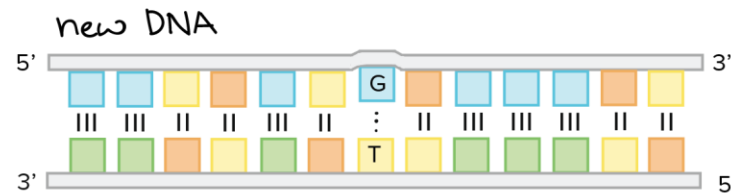
- a. Leading Strand
- b. Lagging Strand
- c. Okazaki fragments
- d. DNA Ligase
- e. Replication Fork
- f. DNA Polymerase
- g. All 3' & 5' ends.
- h. Where helicase should be



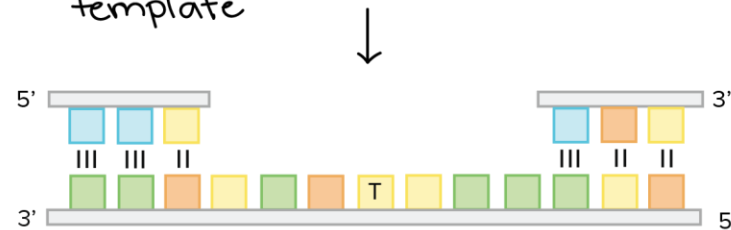
LA FEDELTA' DELLA REPLICAZIONE DEL DNA

1. -selezione dei nucleotidi
2. -correzione di bozze
3. -riparazione dei malappaiamenti

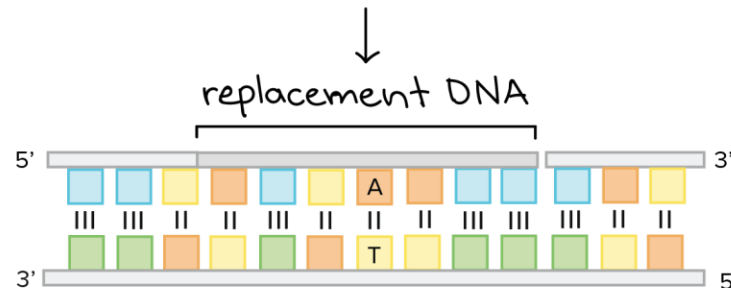




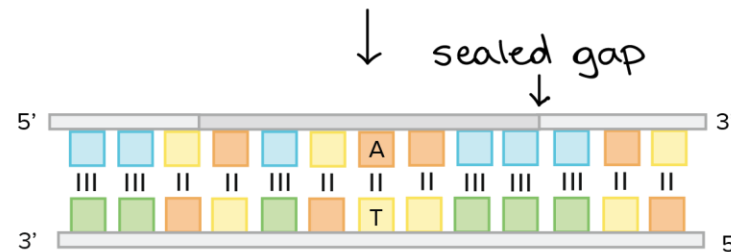
A mismatch is detected in newly synthesized DNA.



The new DNA strand is cut, and the mispaired nucleotide and its neighbors are removed.



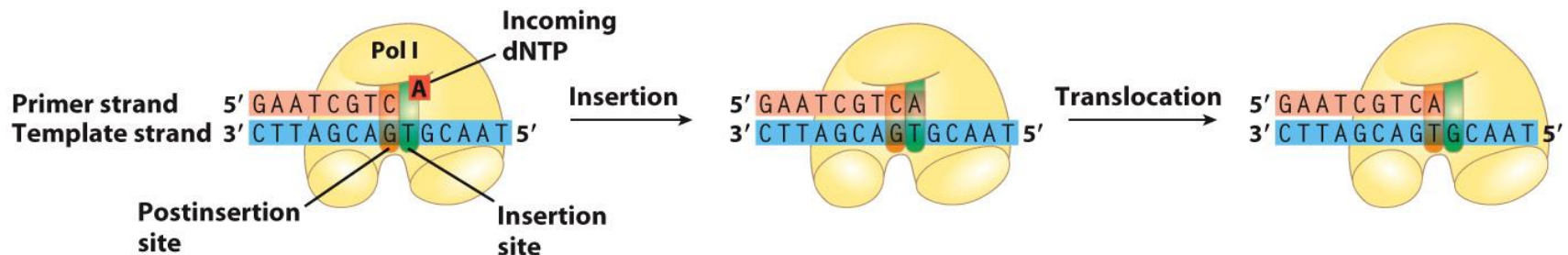
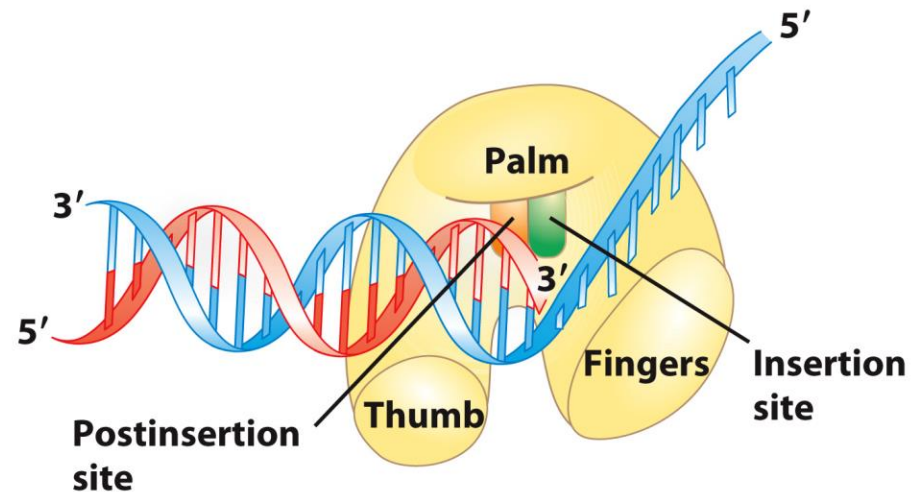
The missing patch is replaced with correct nucleotides by a DNA polymerase.



A DNA ligase seals the gap in the DNA backbone.

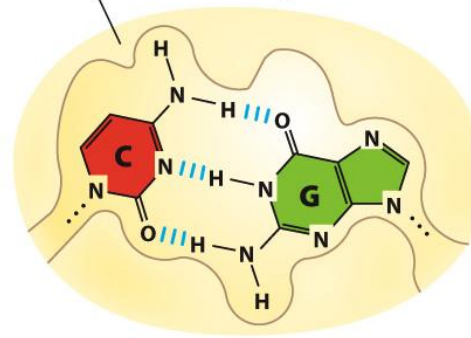
1-Selezione di nucleotidi

- DNA Pol has a pocket with two regions:
 - **insertion site**: where the incoming nucleotide binds
 - **Post-insertion site**: where the newly made base pair resides when the polymerase moves forward

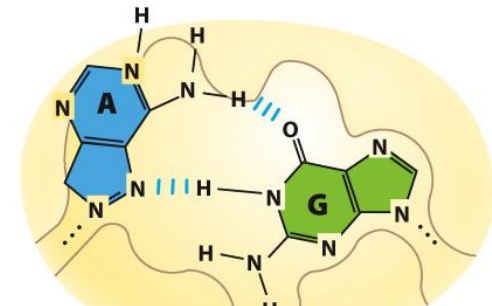
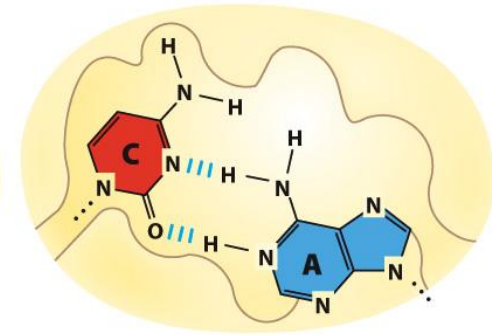
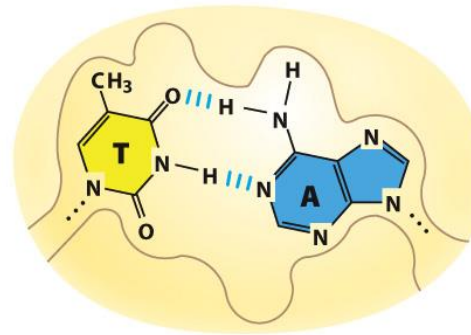
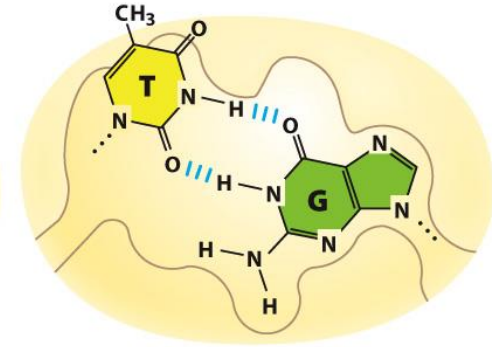


Base-Pair Geometry

(a) Correct base pairs
Active site shape



(b) Incorrect base pairs

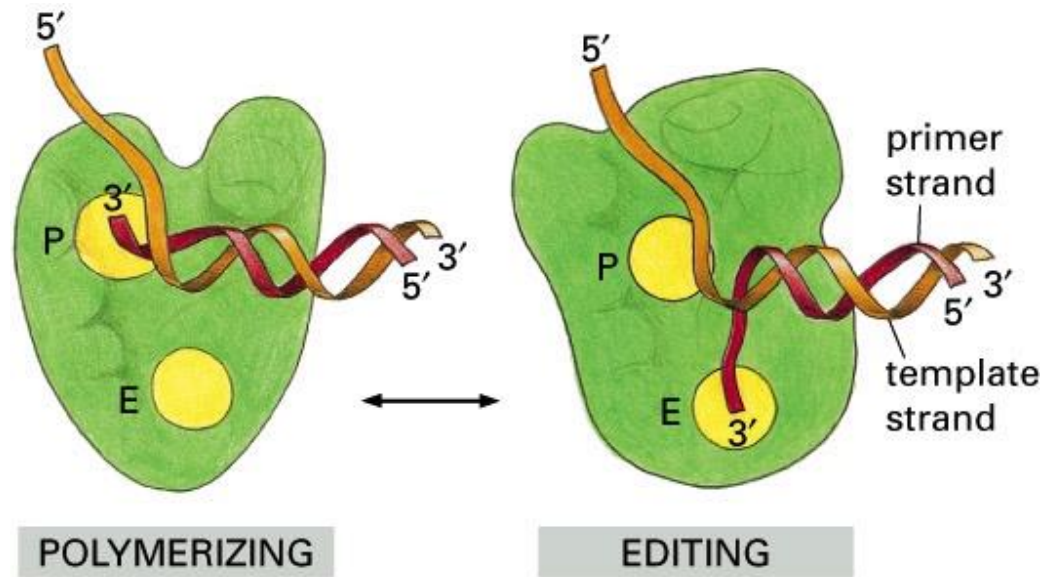


- (a) The standard A=T and G≡C base pairs have very similar geometries, and an active site sized to fit one will generally accommodate the other.
- (b) The geometry of incorrectly paired bases can exclude them from the active site, as occurs on DNA polymerase.

E se vengono commessi degli errori di incorporazione di nucleotidi?

Vari meccanismi di '**proofreading**' intervengono a vari livelli determinando l'elevata fedeltà di copiatura del DNA (1 errore ogni 10^9 nucleotidi copiati).

1. Dopo l'appaiamento del nuovo nt allo stampo, ma prima del legame covalente alla catena crescente
2. Dopo il legame covalente del nuovo nt alla catena crescente
3. Sul DNA già replicato



Dopo che un nucleotide e' entrato nel sito catalitico dell'enzima, ma **prima** che si crei il legame fosfodiesterico, la DNA Polimerasi va incontro ad un cambiamento di conformazione.

Qualora il nucleotide non si appai in maniera corretta al filamento stampo, un cambio conformazionale ne determina l'espulsione dall'enzima.

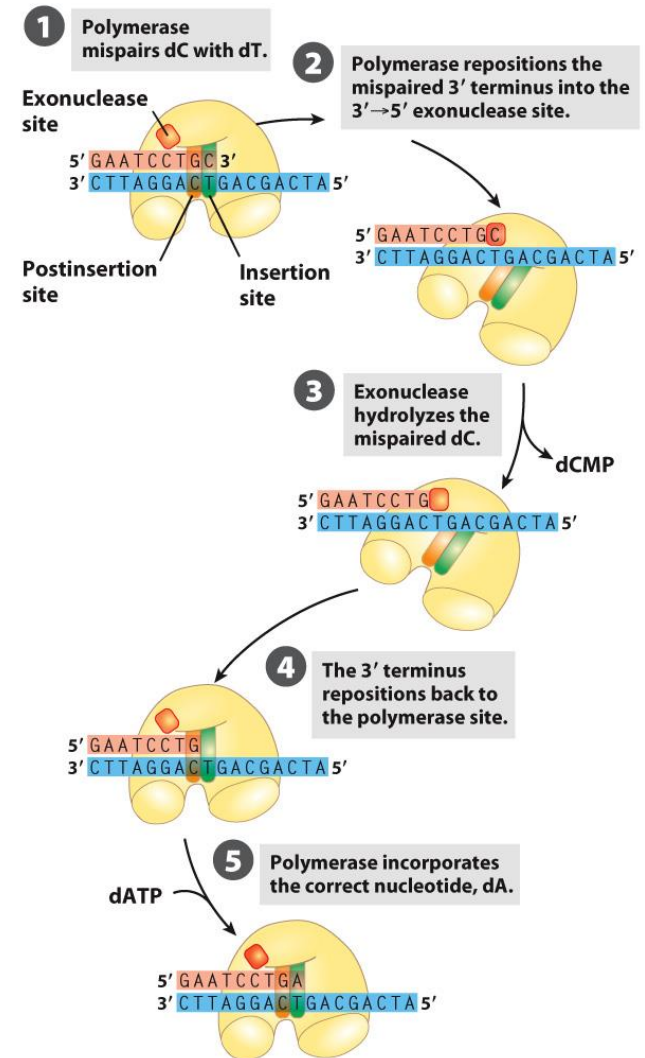
2- L'attività *proof-reading*

La DNA polimerasi possiede due attività enzimatiche distinte, espletate da due domini diversi:

- attività polimerasica in direzione 5' -3'
- attività esonucleasica in direzione 3' -5'

Quando la polimerasi rileva un errore di appaiamento tra le basi, **dopo che il legame PDE e' stato creato**, il complesso stampo-innesco si avvicina al dominio con attività esonucleasica, dove viene eliminato il nucleotide errato, permettendo alla polimerasi di riprendere velocemente la sintesi, senza provocare la dissociazione dell'intero complesso.

Questa attività *proof reading* riduce la frequenza di errore a uno ogni 10^7 nucleotidi



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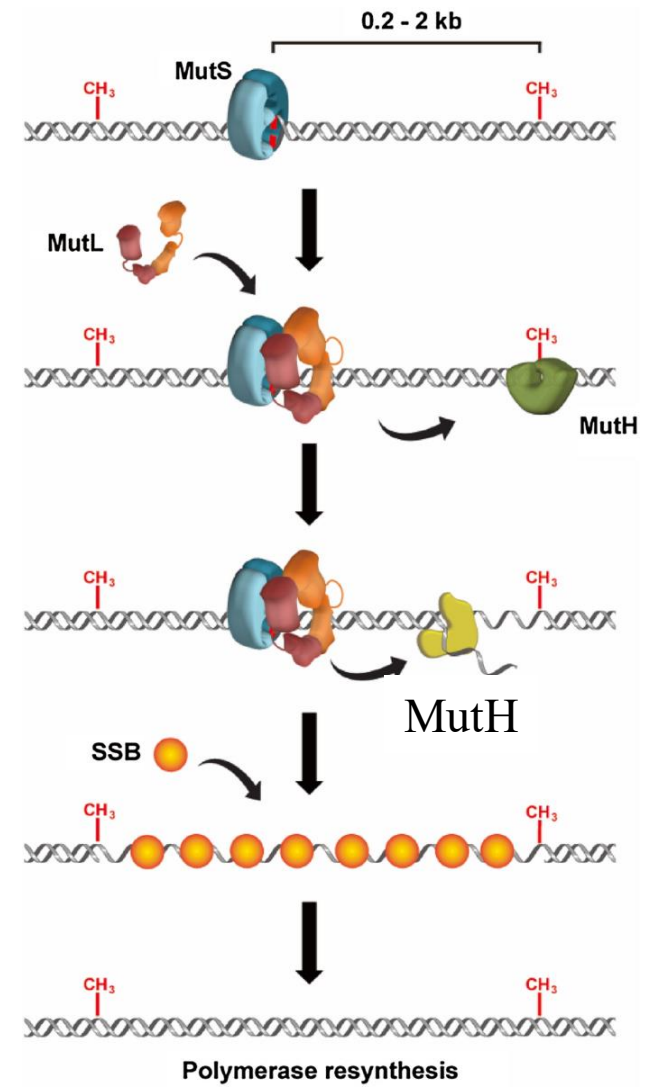
3- Sistema di riparazione degli appaiamenti sbagliati

Sistema di enzimi che rimuove i mal-appaiamenti dopo che il DNA è stato duplicato, aumentando la fedeltà di replicazione da 2 a 3 ordini di grandezza:

1. **Riconosce la distorsione dell'elica** dovuta al mismatch (distinguendo il filamento nuovo dal vecchio);
2. Taglia il segmento di DNA contenente il mismatch;
3. Risintetizza il segmento tagliato usando il filamento vecchio come stampo.

Nei procarioti MutS scorre lungo il DNA e riconosce il mismatch
MutH e MutL vengono reclutate
MutH taglia un segmento di filamento neosintetizzato, il gap viene riempito dalla DNA polimerasi e le estremità saldate dalla DNA ligasi.

MutL is a Multidomain Protein
MutL is composed of two structured domains connected by a flexible linker.
The N-terminal region of the protein (~ 330 residues) encompasses an ATPase domain that is highly conserved from *E. coli* to humans.

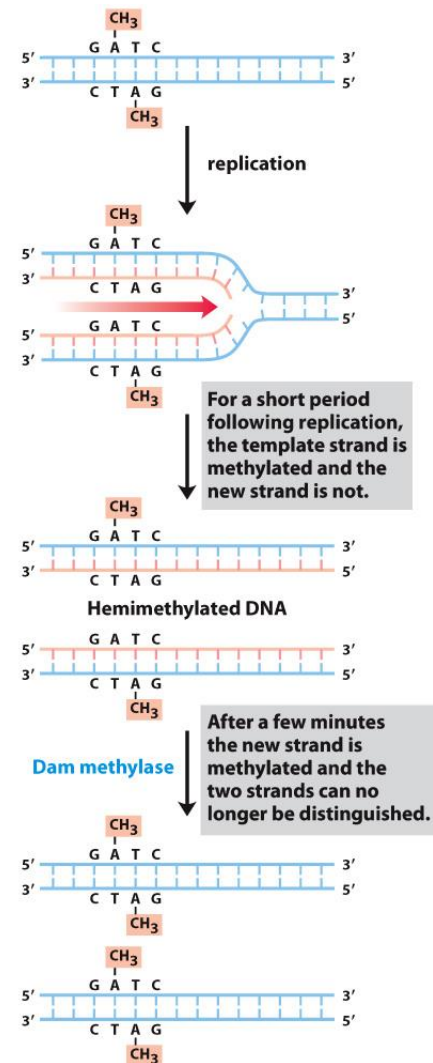


Mismatch Repair

How do repair enzymes “know” which strand is the correct one?

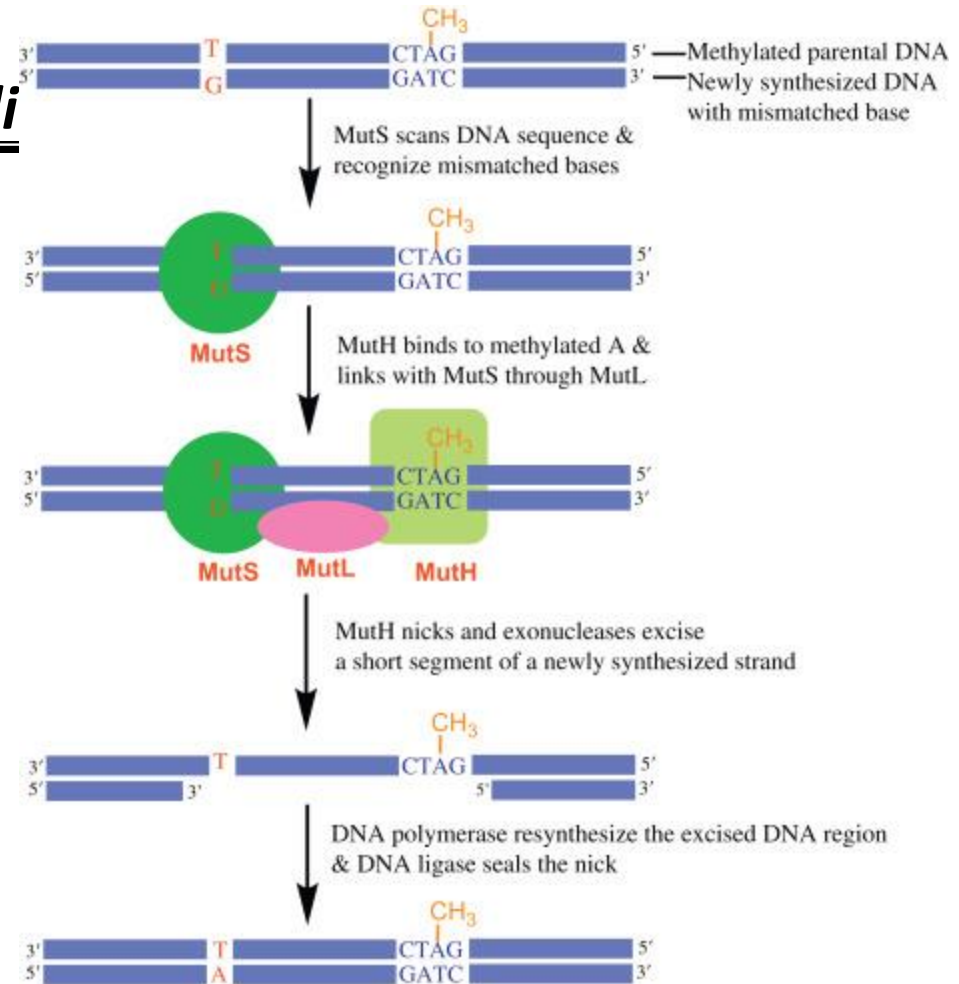
Mismatch Repair and Methylation

- In *E. coli*, the parent strand is methylated.
- **Dam methylase** inserts CH_3 at adenines in the **GATC** sequence.
- Following a short period of time, the daughter strand is then methylated.
- The **newly synthesized strand is unmethylated** for a short period after synthesis.
- Any replication errors must reside in the unmethylated strand.
- The methyl-directed mismatch repair system will **cleave the unmethylated strand** in the initial part of the repair process.

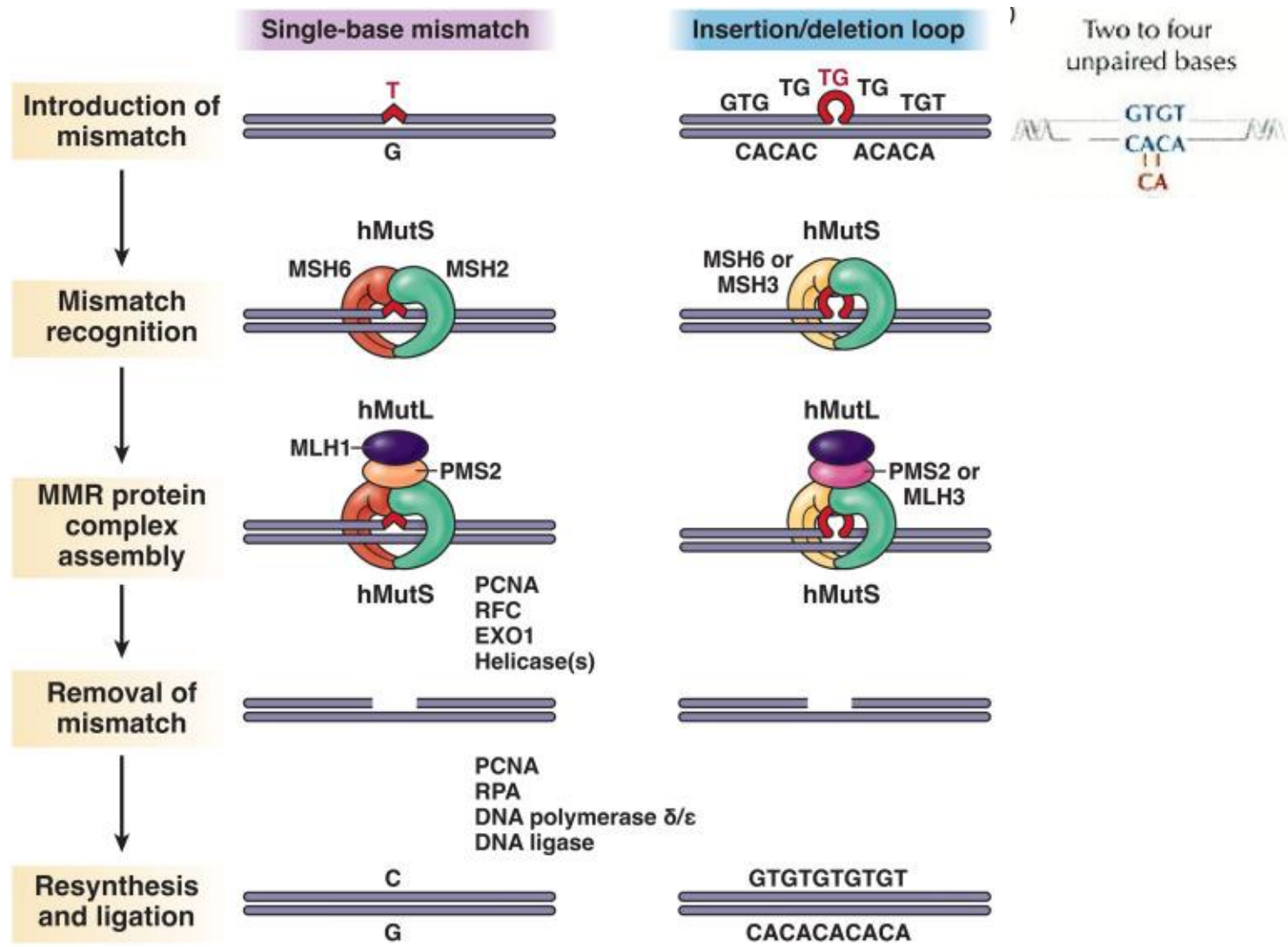


Mechanism of Mismatch Recognition and Repair in *E. coli*

- MutL and MutS proteins recognize methylated GATC.
- MutH binds to MutL-MutS-DNA complex, making a DNA loop.
- MutH cleaves the nonmethylated DNA strand on the 5'-side of the G.
- DNA unwinds and is degraded 3'→5'.
 - Helicase II (UvrD helicase), SSB, and exonucleases work to degrade the nonmethylated DNA toward the mismatch.
- The removed sequence is replaced using DNA Pol III and DNA ligase.

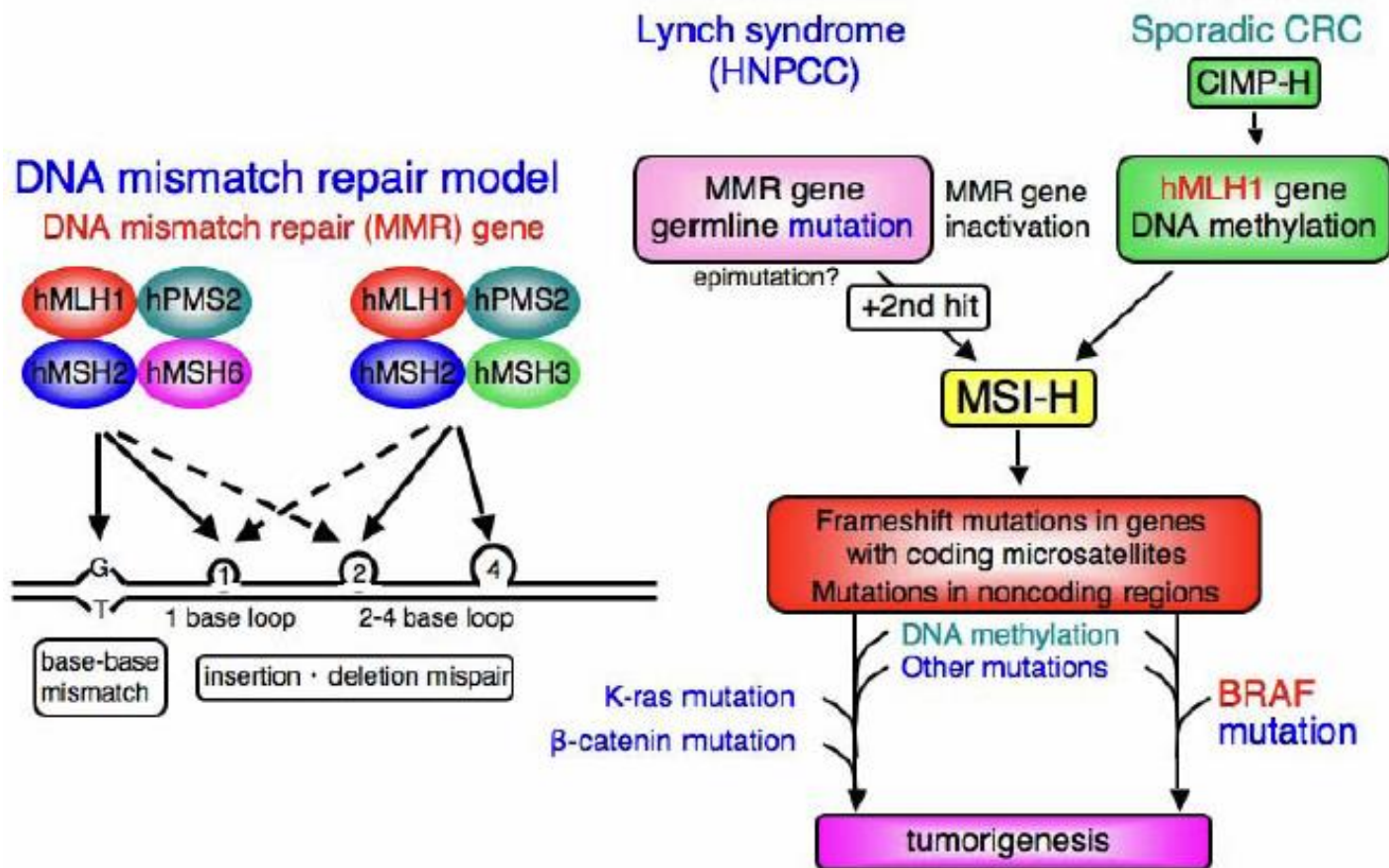


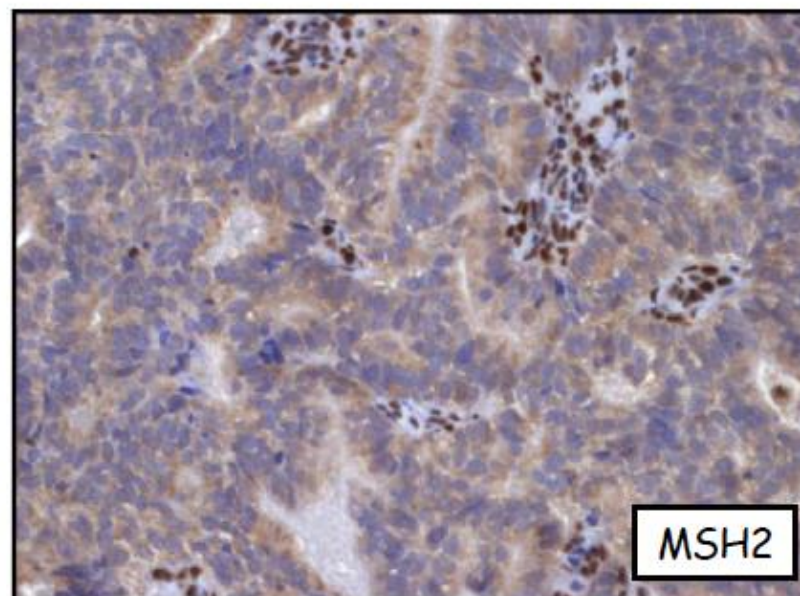
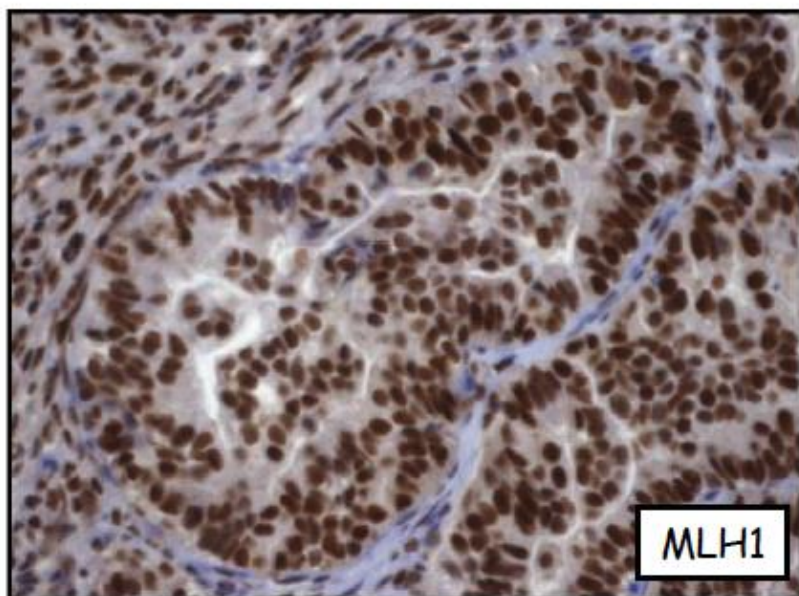
Nelle cellule umane hMSH2 e hMLH1, sono simili a MutS e MutL rispettivamente



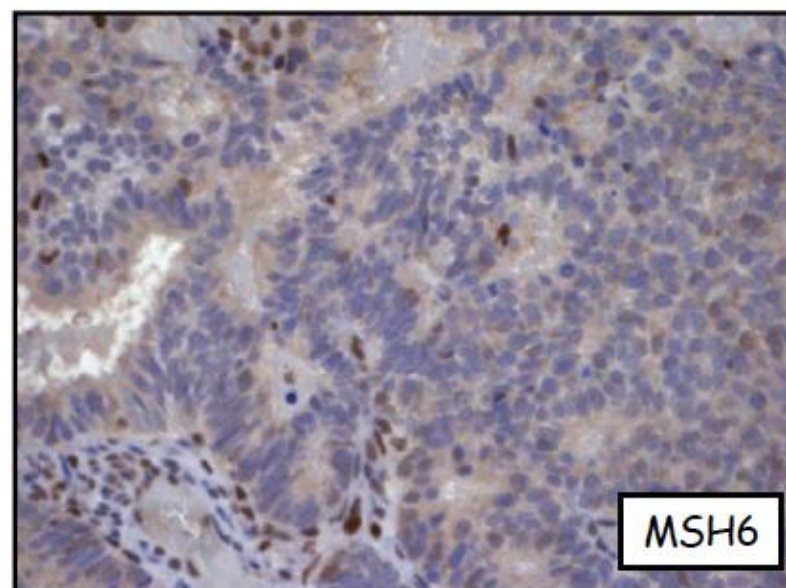
Mutazioni nei geni del mismatch repair system causano la predisposizione a certi tipi di cancro

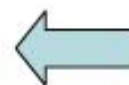
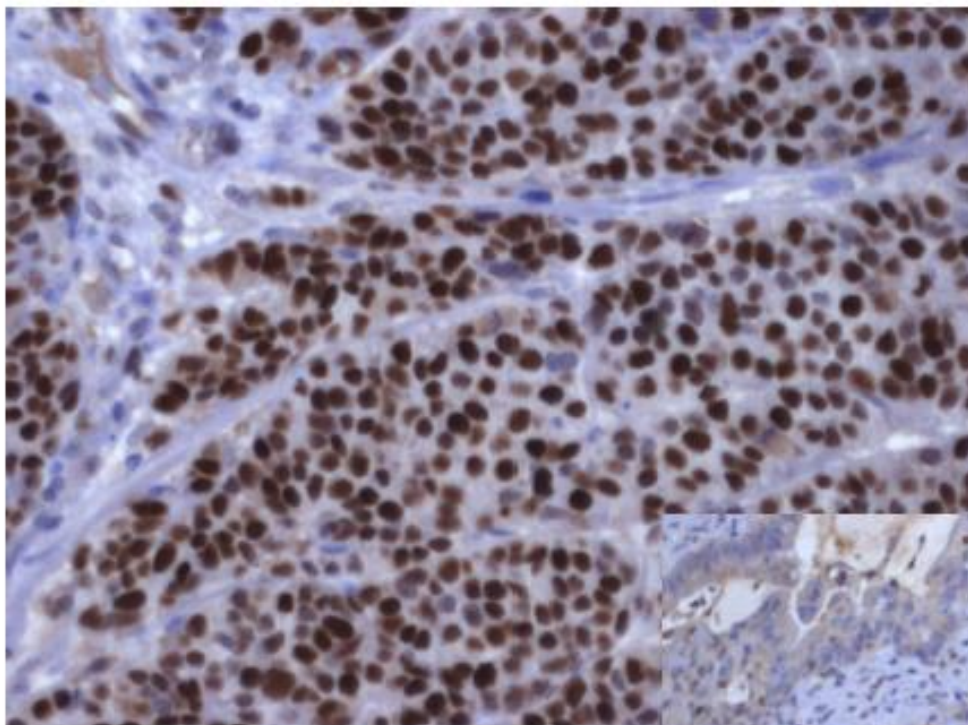
(es. HNPCC, carcinoma colonrettale ereditario non poliposico, noto come sindrome di Lynch; test predittivo: mutazioni nei geni MSH2 e MLH1).





Utero
Adenocarcinoma endometrioid
Perdita completa di espressione
di MSH2 e MSH6



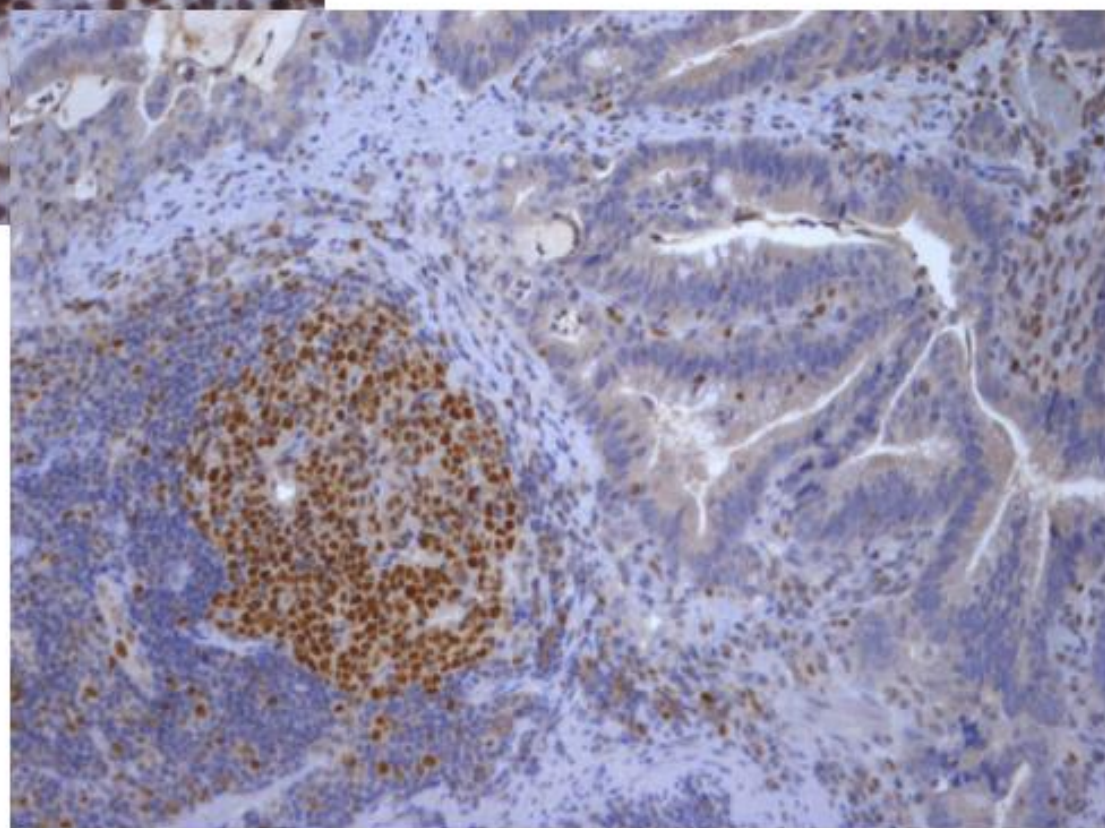


*Normale espressione
di MSH6*

*Perdita completa
di espressione
di MSH6*



Carcinoma colo-rettale



Le mutazioni nel DNA sono
sempre dannose/deleterie?

VARIABILITA' GENETICA

Cambiamenti nella sequenza del DNA



Il 99,5% della sequenza del nostro DNA nucleare è identico a quello di un altro individuo

La piccola frazione restante ($\approx 0,5\%$) è responsabile della variabilità genetica e determina tutte le nostre caratteristiche (incluso l'insorgenza di patologie)

Mutazioni

- Mutazioni **germinali** : cambiamenti “de novo” nella linea germinale (rilevanti per il genetista)
- Mutazioni **somatiche**: possono originarsi nelle cellule di tutto l'organismo.

Ogni cellula ha un patrimonio genetico differente dipendente dal numero di divisioni cellulari avvenute dal concepimento fino all'acquisizione della mutazione. Queste mutazioni in genere sono silenti in quanto mascherate dal DNA wild type presente nella restante popolazione di milioni di cellule (clinicamente importanti solo in alcune patologie e nei tumori)

Mutazioni e la loro frequenza

- Mutazioni rare (varianti alleliche presente in $< 1\%$ della popolazione umana)
- **Polimorfismo**: variante allelica presente in $> 1\%$ della popolazione umana.

Diversi tipi di polimorfismi

- Polimorfismo del singolo nucleotide (SNP)
- Polimorfismi inserzione-delezione (indels)
- Polimorfismi da inversione

TABELLA 4-2 Varianti comuni nel genoma umano

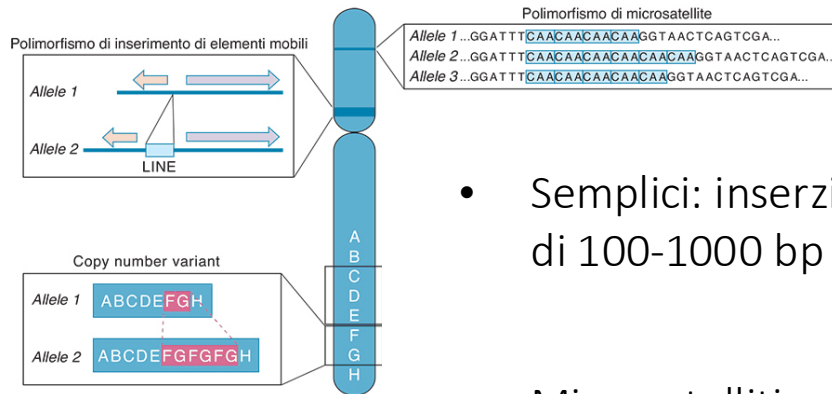
Tipo di variazione	Dimensioni approssimative	Tipo di polimorfismo	Numero di alleli
Polimorfismo del singolo nucleotide	1 bp	Sostituzione di una base in una particolare posizione del genoma	In genere 2
Inserzioni/Delezioni (indel)	1 bp fino a >100 bp	<i>Semplice</i> : presenza o assenza di un piccolo segmento di DNA di 100-1000 bp <i>Microsatelliti</i> : generalmente, unità di 2-, 3- o 4- nucleotidi ripetute in tandem 5-25 volte	<i>Semplice</i> : 2 <i>Microsatelliti</i> : tipicamente 5 o più
Copy Number Variant	10 kb fino a >1 Mb	Presenza o assenza di segmenti di DNA da 200 pb fino a 1,5 Mb	2 o più
Inversioni	Poche pb fino a >1 Mb	Un segmento di DNA con orientamento invertito	2

bp, coppia di basi; kb, coppia di chilobasi; Mb, coppia di megabasi.

SNPs

- La più semplice ed anche la più utile classe di polimorfismi si origina dalla sostituzione di una singola coppia di basi.
- Questo tipo di cambiamenti possono essere indotti da sostanze chimiche mutagene, oppure da errori durante la replicazione (forme tautomeriche delle basi) e sono denominate polimorfismi di singoli nucleotidi o SNP (snips).
- Quasi tutti gli snips sono biallelici.
- Il sequenziamento della stessa regione genomica da individui diversi permette l'identificazione di un elevato numero di SNPs.
- Il consorzio internazionale per gli SNPs, dal 2001 ha identificato, mappato e messo a disposizione su una banca dati più di 5 milioni di SNPs umani.
- Nonostante sia possibile che gli SNPs possano alterare la sequenza aminoacidica di proteine, la maggiore parte degli snips si verificano in "loci anonimi".

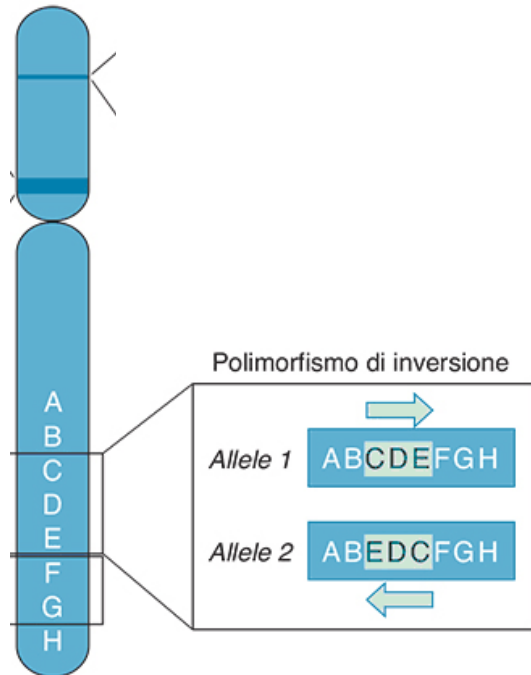
Polimorfismi inserzione-delezione (indels)



- Semplici: inserzione/delezione di piccoli segmenti di 100-1000 bp
- Microsatelliti: modulo di DNA (2, 3 o 4nt) ripetuto in tandem da una fino a decine di volte in un locus (definito anche short tandem repeat)

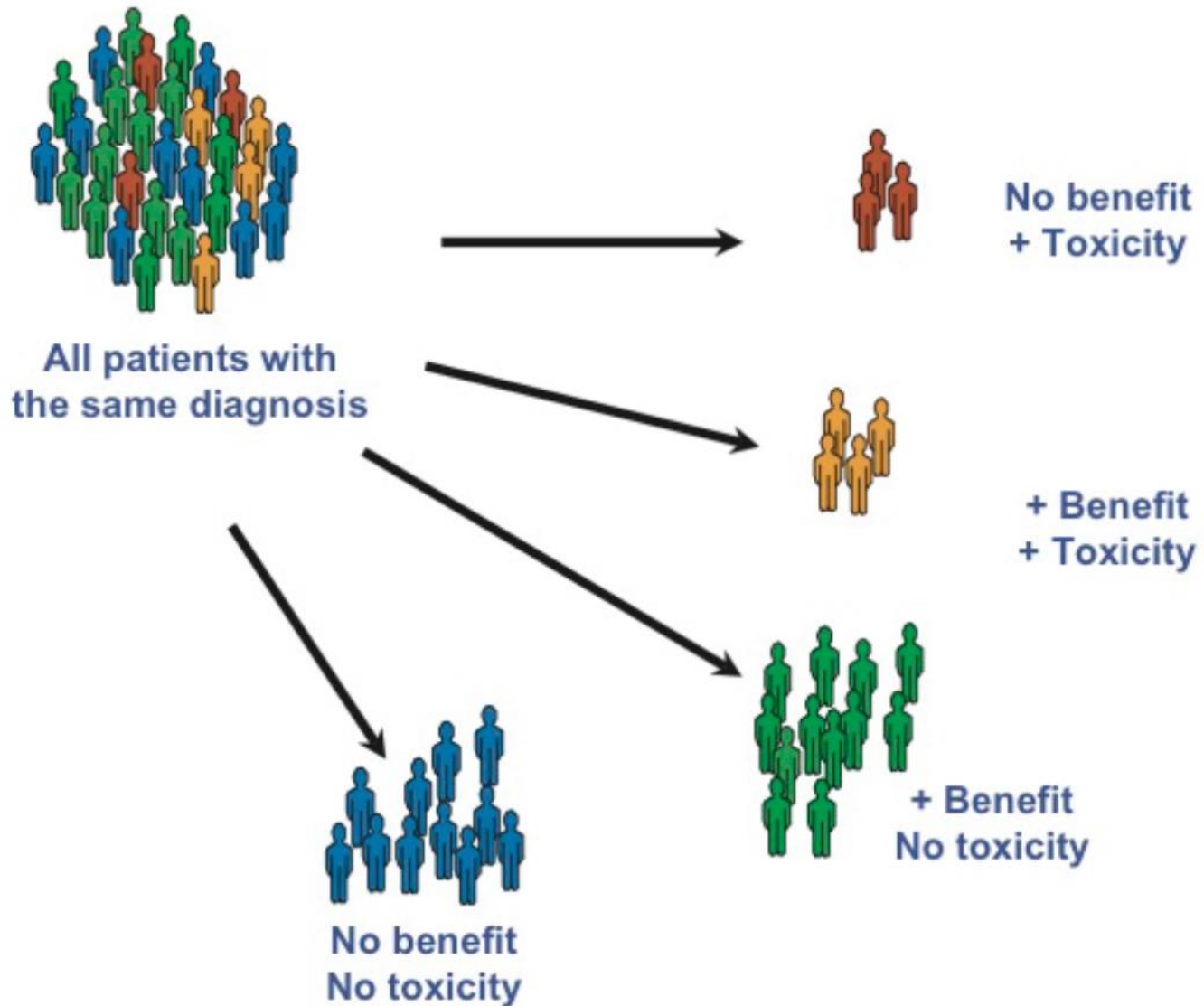
- Elementi mobili trasponibili: elementi ripetuti instabili (si spostano all'interno del genoma tramite retrotrasposizione). Hanno 2 alleli e si trovano più frequentemente nel DNA non codificante, ma non sono distribuiti in maniera uniforme nel genoma
- Copy number variants (CNVs): segmenti lunghi del genoma (1000bp fino a centinaia di migliaia di bp) presenti in un numero di variabile di copie

Polimorfismi da inversioni



Segmenti di DNA (da poche basi fino a diverse megabasi) che si presentano in orientamento inverso

Population diversity of drug response phenotypes



Why does drug response vary?

*Same symptoms,
Same findings,
Same disease?*



Different patients



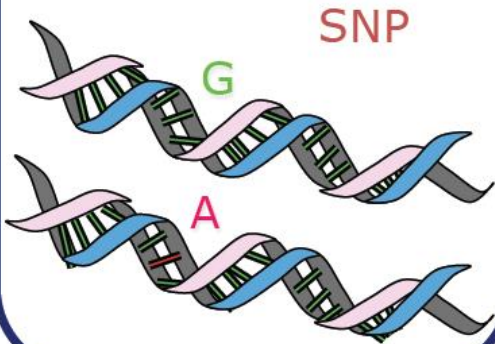
*Same drug
Same dose*

Pathogenesis and the severity of the disease being treated



Different Effects

Genetic Differences



Ethnicity
Age
Pregnancy
Genetic factors
Disease
Drug interactions
.....

Possible Reasons:
Individual variation
By chance...

Individual Variability Can Affect:

- *Ability or efficiency to transport the drug in blood vessels to the target cells*
- *The activation, usually occurring in the liver, that several drugs need*
- *The response to the direct target at the given concentration*
- *Ability or efficiency in catabolism and elimination of the drug*

Determining the likelihood of an adverse reaction