DNA REPLICATION

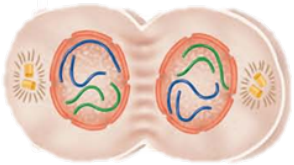
Objectives

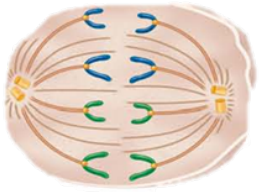
- Essential Question: Why & how do our cells reproduce DNA?

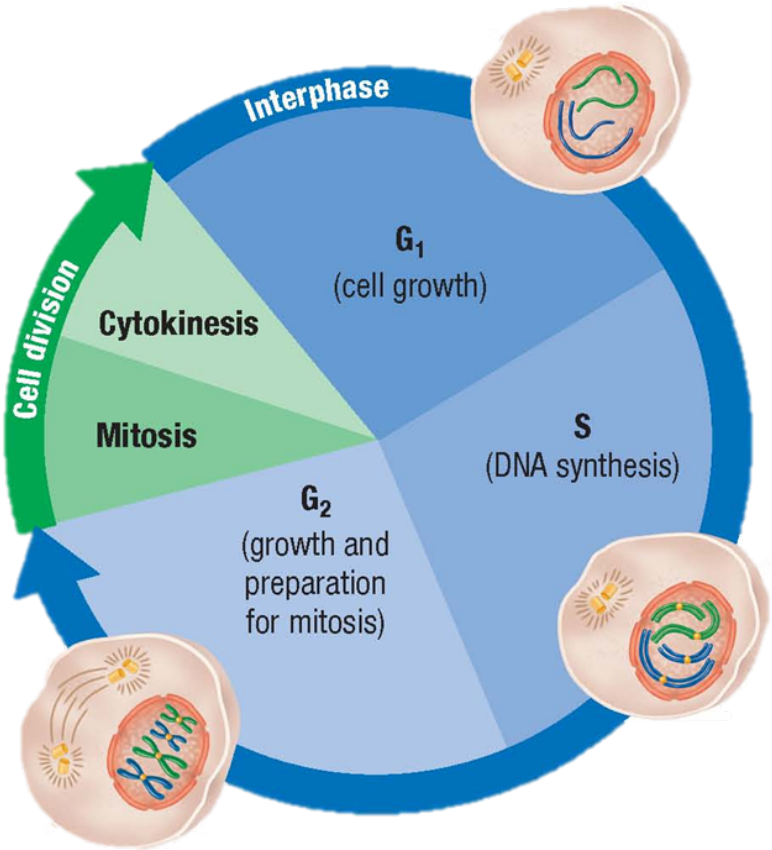
- Describe the steps of DNA replication.
- Compare the roles of DNA helicase, DNA polymerase, and ligase
- Compare the process of DNA replication in prokaryotes and in eukaryotes
- Applications of our knowledge

DNA REPLICATION

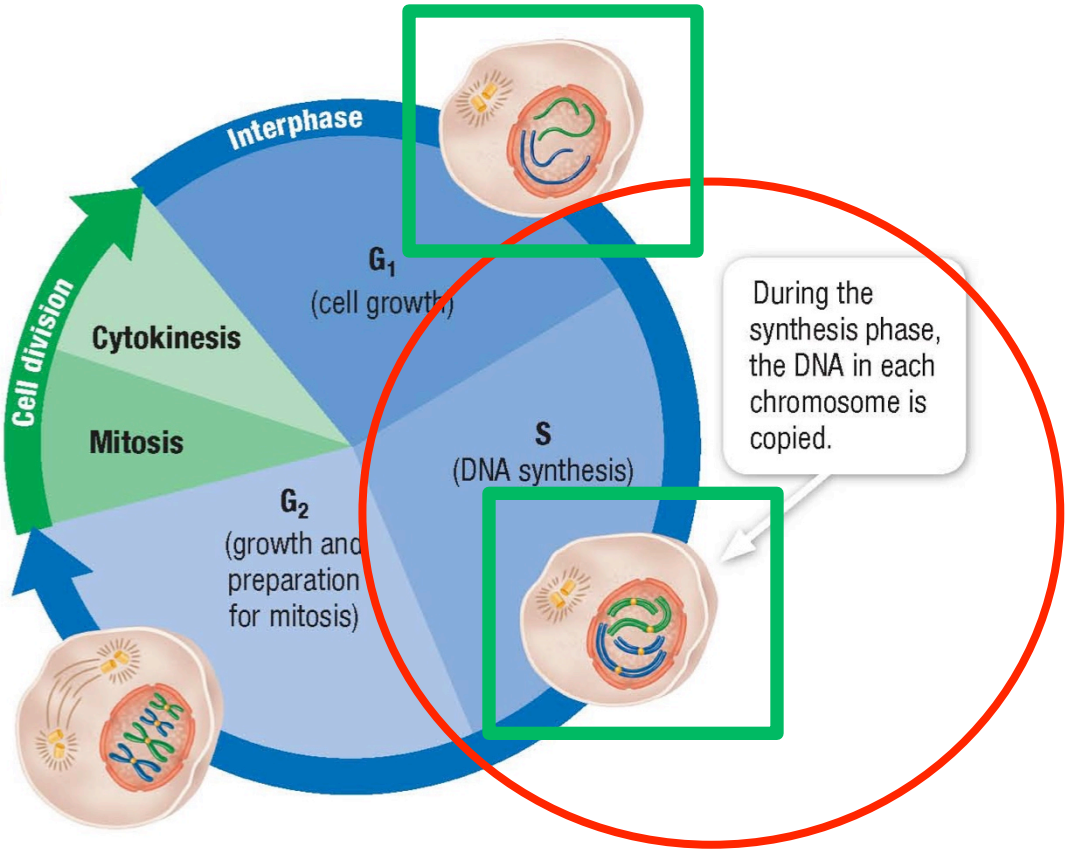
- Every time a cell goes through a cycle it must DUPLICATE ITS DNA SO THAT WHEN IT MAKES NEW BABY CELLS THEY BOTH HAVE THE EXACT SAME DNA.
- Happens in ‘S’ phase
- In this section we are going explore the process of DNA synthesis, called DNA replication.
- This process is extremely precise and an incredibly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



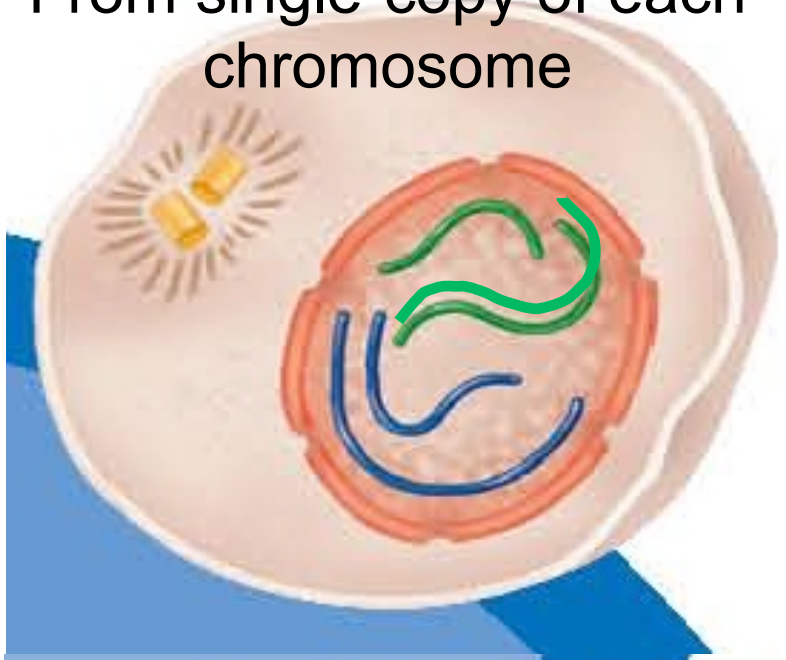




THE CELL CYCLE: THE PART WE'RE ISOLATING.



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.

[1] Young, F. B., Gerard, R., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (Stockholm), **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

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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.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

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 β-D-deoxyribofuranose residues with 3′,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 Furberg's[2] 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 Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. 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 A. The distance of a phosphorus atom from the fibre axis is 10 A. 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.

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

[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).

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. Astbury[1]) 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 nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[2,3], 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 (*ref. ref.* 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the internucleotide repeat along the fibre axis. The ~ 34 A. 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 reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown[4] (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

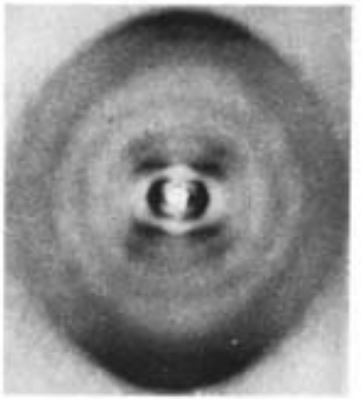

Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B*, wet. Fibre axis vertical

the innermost maxima of each Bessel function as the origin. The angle this line makes with the equat is roughly equal to the angle between an element the helix and the helix axis. If a unit repeats *n* tim along the helix there will be a meridional reflexi (J_n^2) on the *n*th layer line. The helical configurati produces side-bands on this fundamental frequen the effect[4] being to reproduce the intensity distributi about the origin around the new origin, on the *n* layer line, corresponding to *C* in Fig. 2.

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

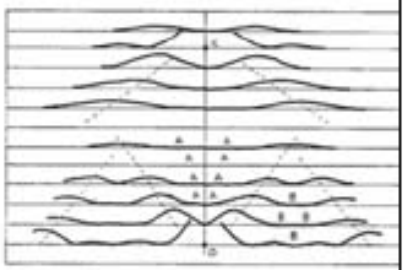

Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About *C* on the tenth layer line similar functions are plotted for an outer diameter of 12 A.

Nature, Vol. 171, p.737, April 25, 1953

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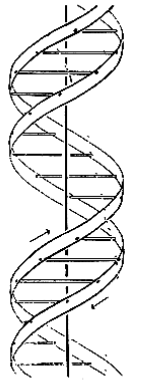

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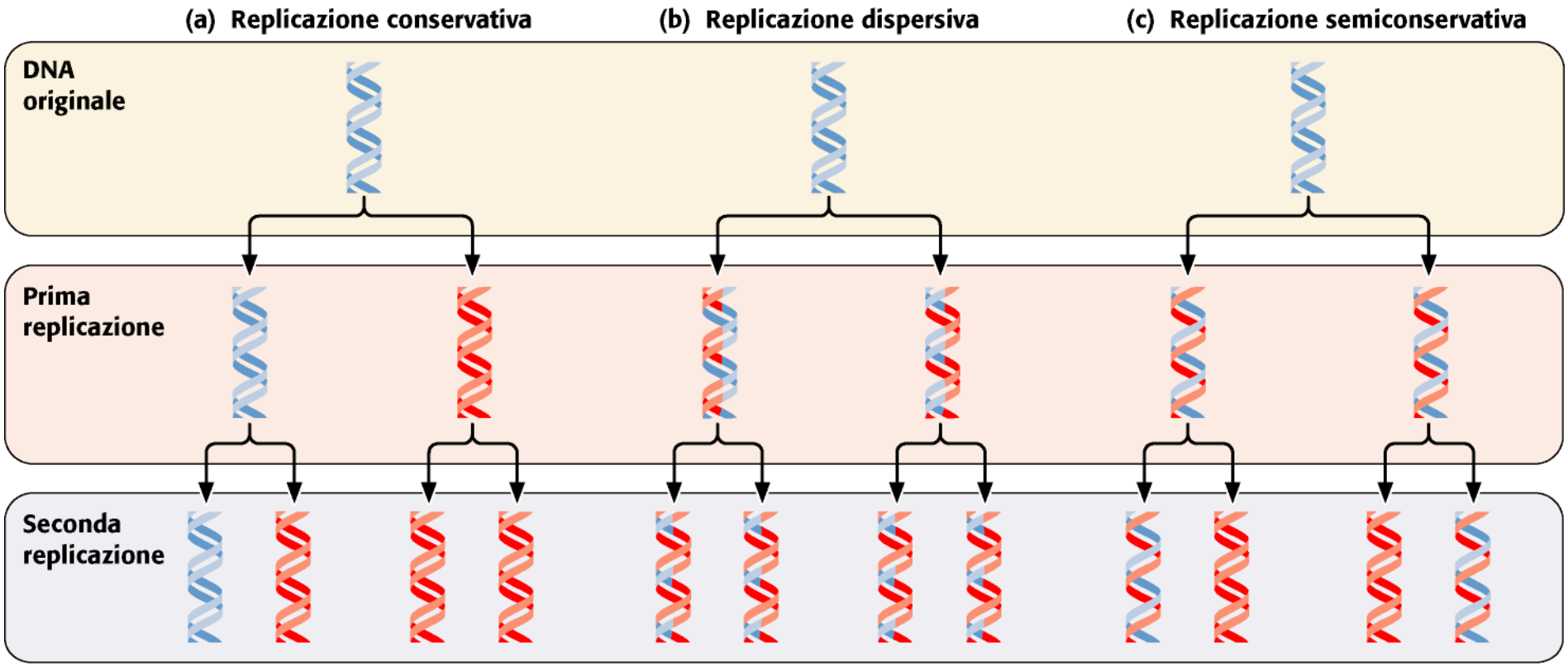
The Three Possible DNA Replication Models

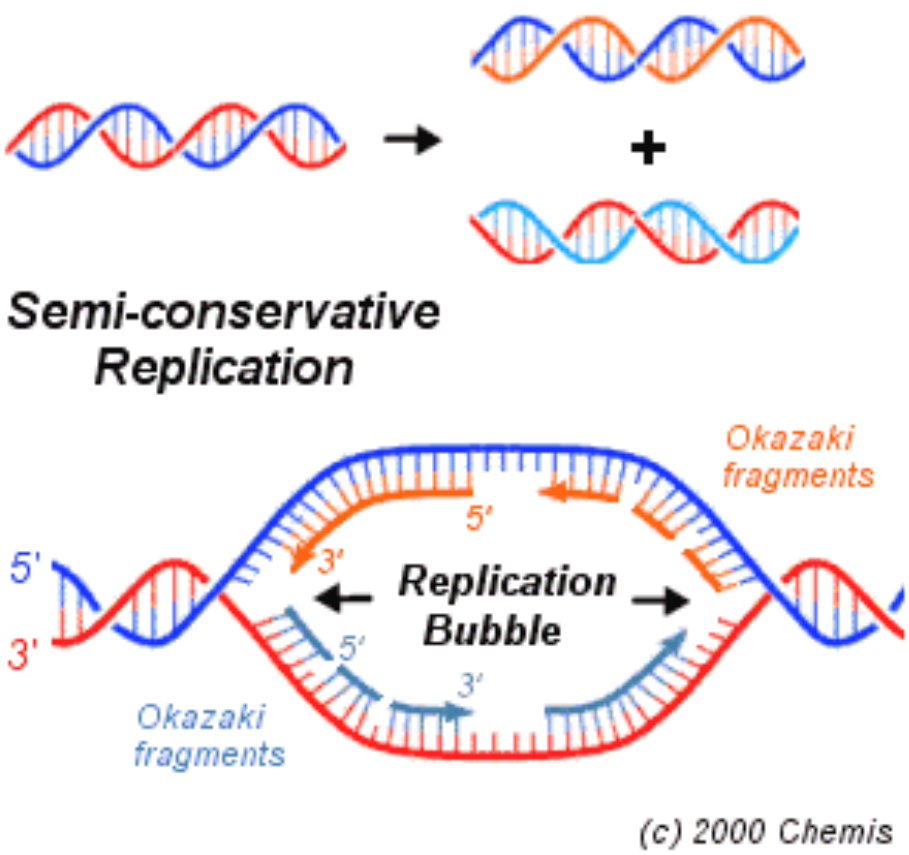


- Conservative- would leave the original strand intact and copy it.
- Dispersive-would produce two DNA molecules with sections of both old and new along each strand.
- Semiconservative –would produce DNA molecules with both one old strand and one new strand.

Definitions

Origin: chromosonal 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.

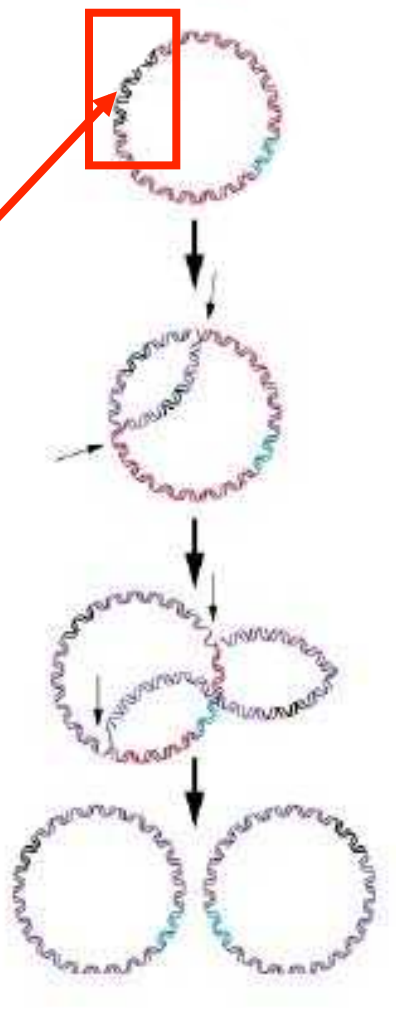


Prokaryotic and Eukaryotic Replication

- 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.

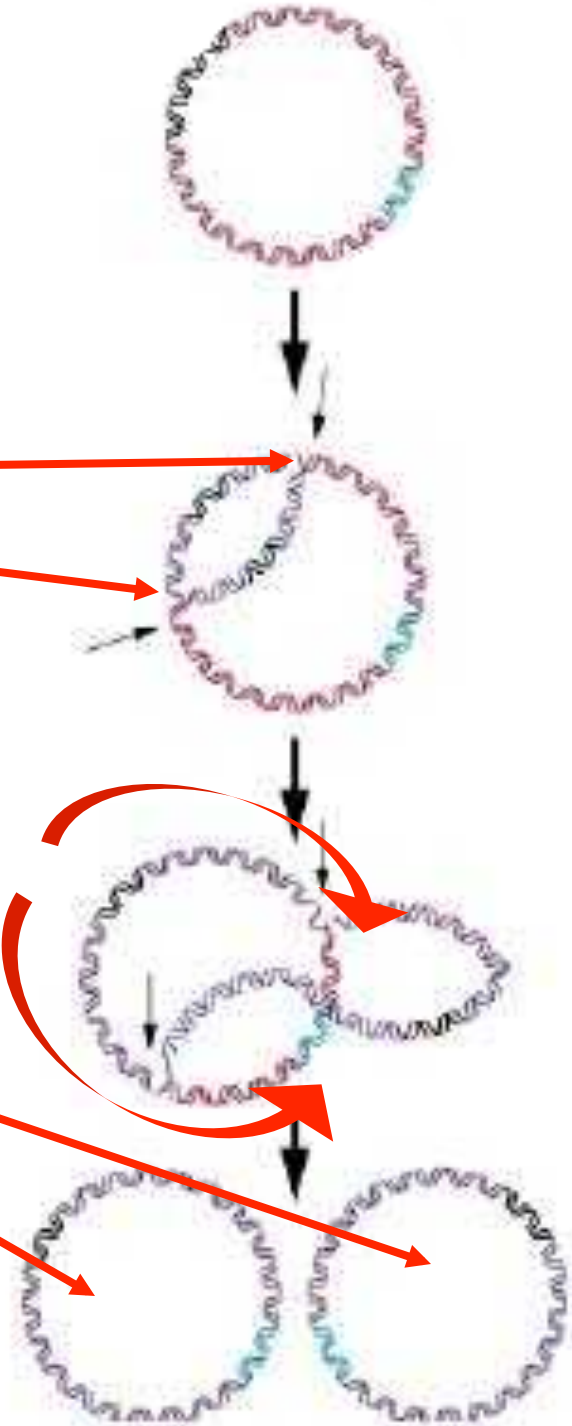
Prokaryotic DNA Replication

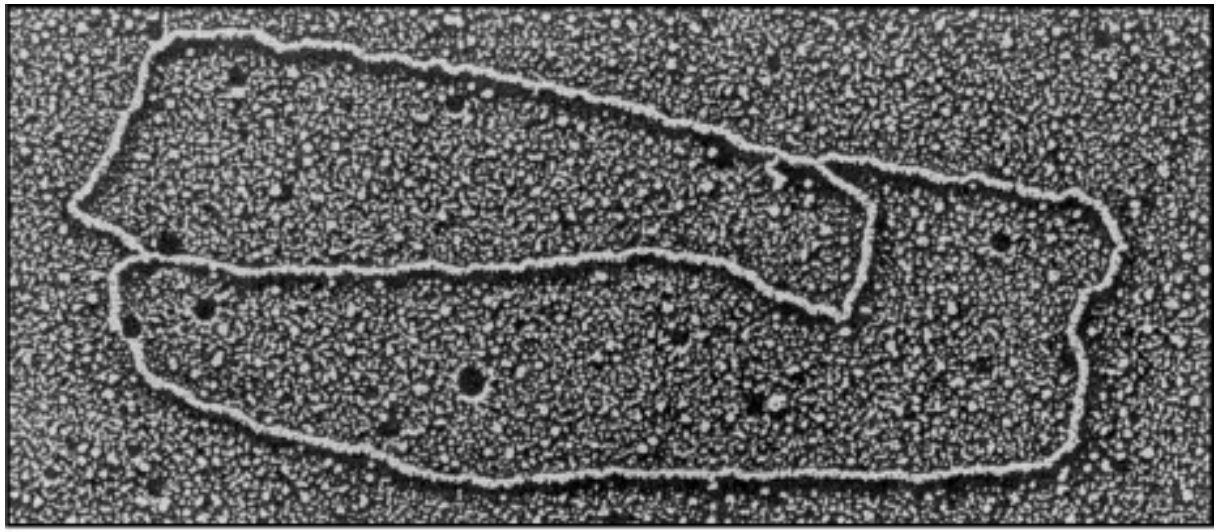
- Recall the structure of prokaryotic DNA.
- Does anyone remember what the structure is?
- Prokaryotic cells usually have a single chromosome which is a closed loop attached to the inner cell membrane.
- Replication in prokaryotes begins at a single site along the loop. This site is called the *origin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

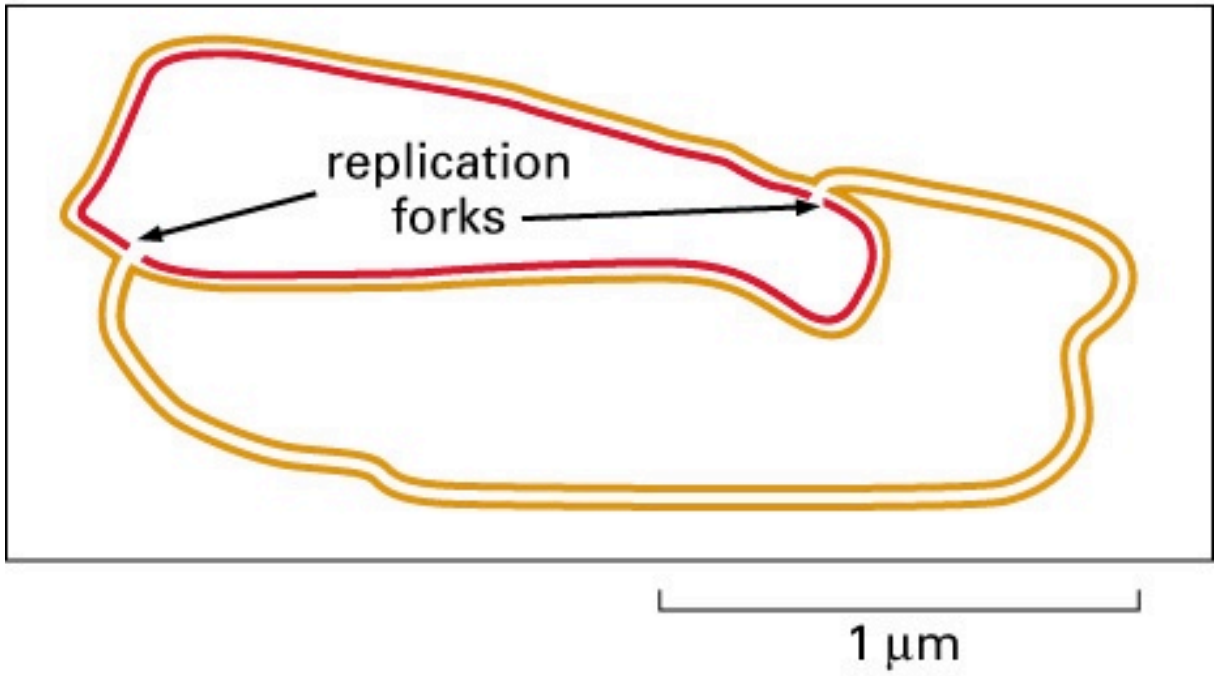


Prokaryotic Replication

- 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.



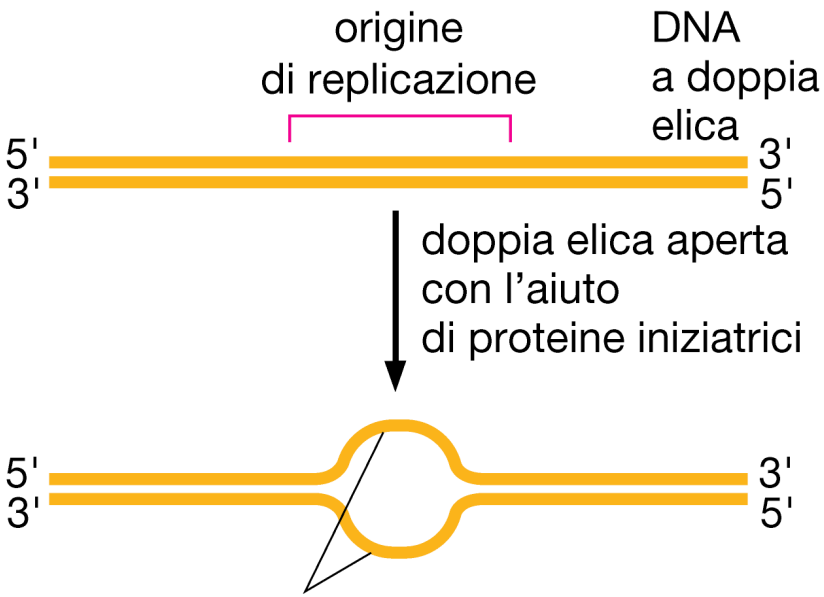


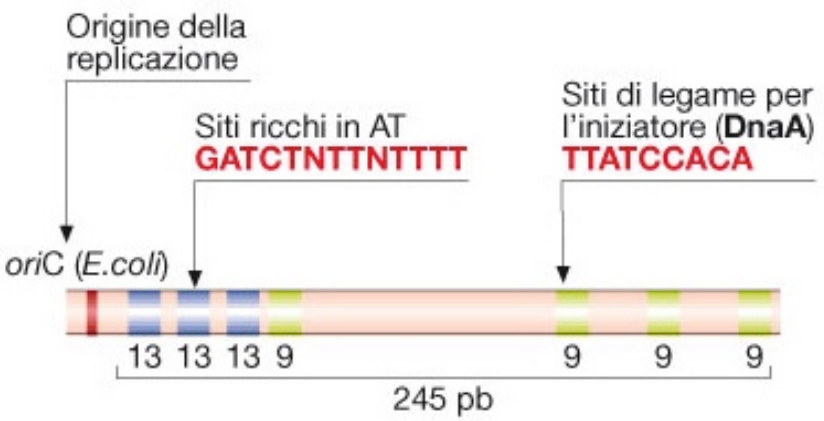


Al microscopio elettronico si distinguono delle biforcazioni a Y, dette **FORCELLE REPLICATIVE**. In questi punti la macchina replicatrice si muove lungo il DNA, aprendo i due filamenti e copiando l'informazione.

Le forcelle viaggiano rapidissime, circa 1000 coppie di nucleotidi al secondo nei batteri e 100 coppie di nucleotidi al secondo nell'uomo (verosimilmente per le difficolta' poste dalla struttura piu' complessa della cromatina)

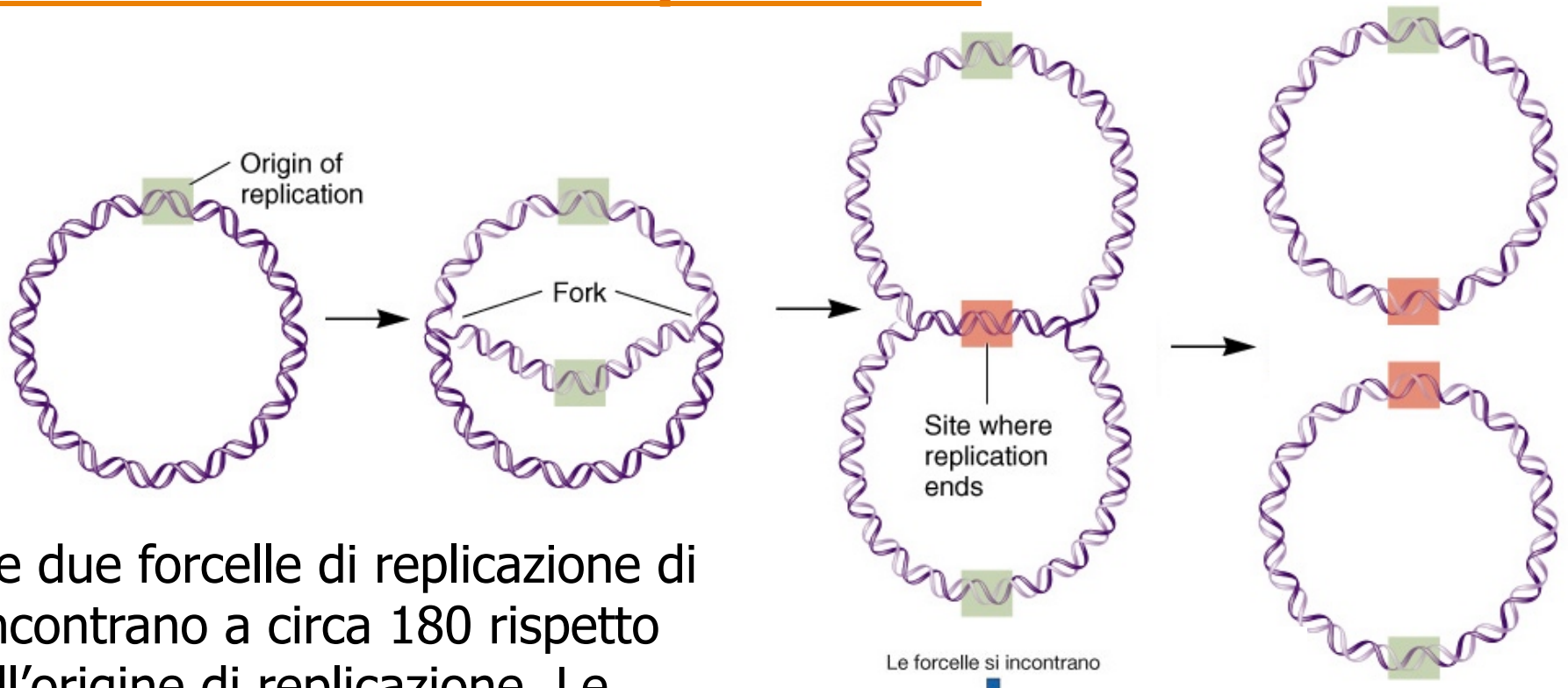
Origine di replicazione in E.Coli: ***oriC***





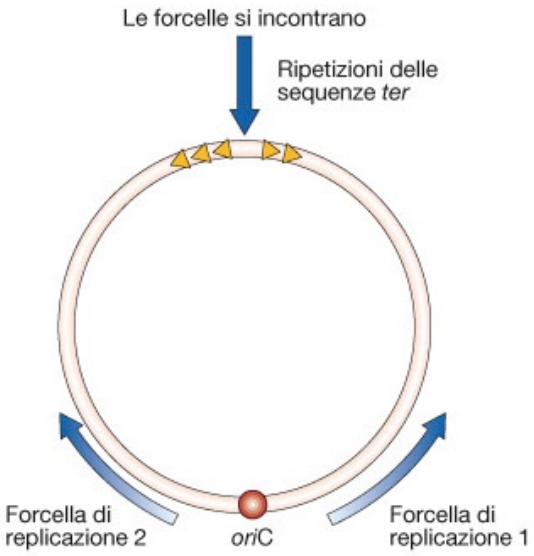
L'origine minima di E.Coli e' lunga solo 245 bps.
E' una regione ricca di A e T: contiene 4 ripetizioni di 9 pb e 3 di 13 pb molto simili tra loro.
Le corte sequenze ripetute sono riconosciute da proteine specifiche nelle fasi iniziali della replicazione.

Terminazione della replicazione.

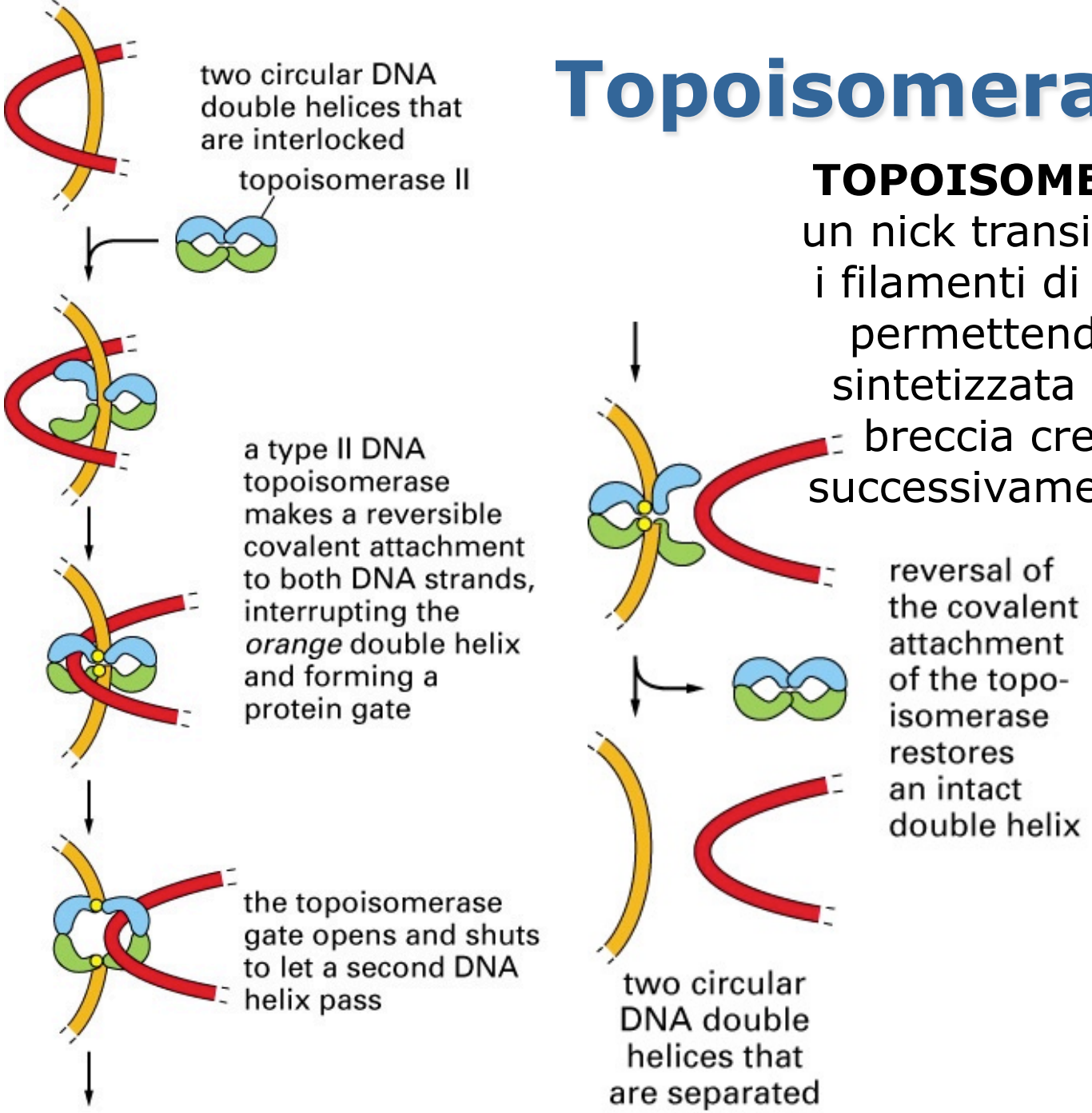


Le due forcelle di replicazione di incontrano a circa 180 rispetto all'origine di replicazione. Le sequenze ***ter***, di 23 bp, vengono legate dalle **TBP** (Ter binding proteins) e fanno terminare le forcelle di replicazione.

La Topoiomerasi II dividera' 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!

DNA Replication Properties

- **Three fundamental rules of replication**
  - Replication is semiconservative
  - Replication begins at an origin and proceeds (usually) bidirectionally
  - Synthesis of new DNA occurs in the 5'→3' direction and is semidiscontinuous

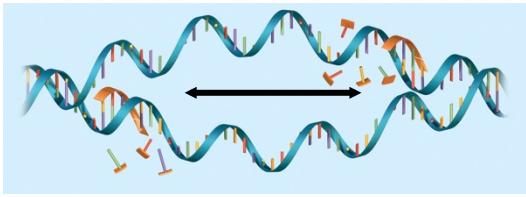
DNA Replication: THE STEPS

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.

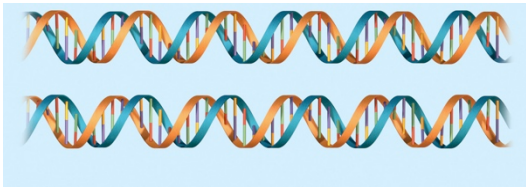
The three steps are:

1. Unwinding and Separating DNA Strands



2. Adding complimentary bases
   a. Leading Strand:
   b. Lagging Strand

3. Formation of Two Identical DNA molecules



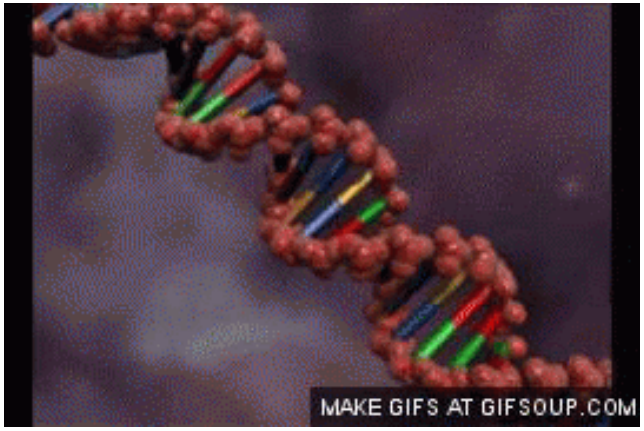
STEP1

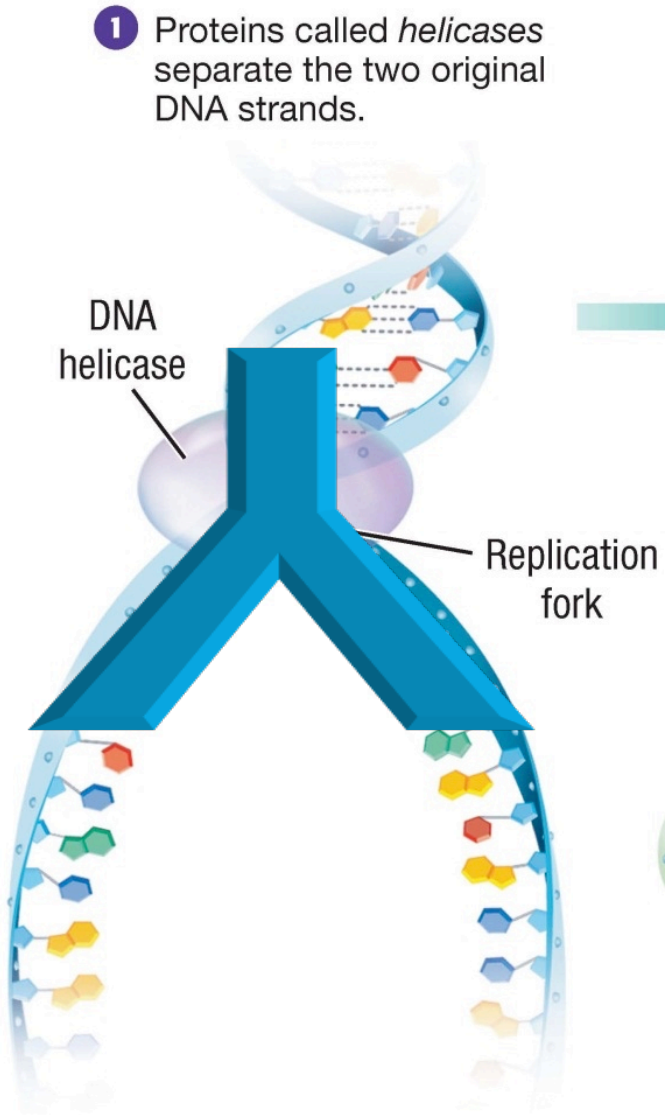
DNA helicases unwinds & separates the original DNA double helix.

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.



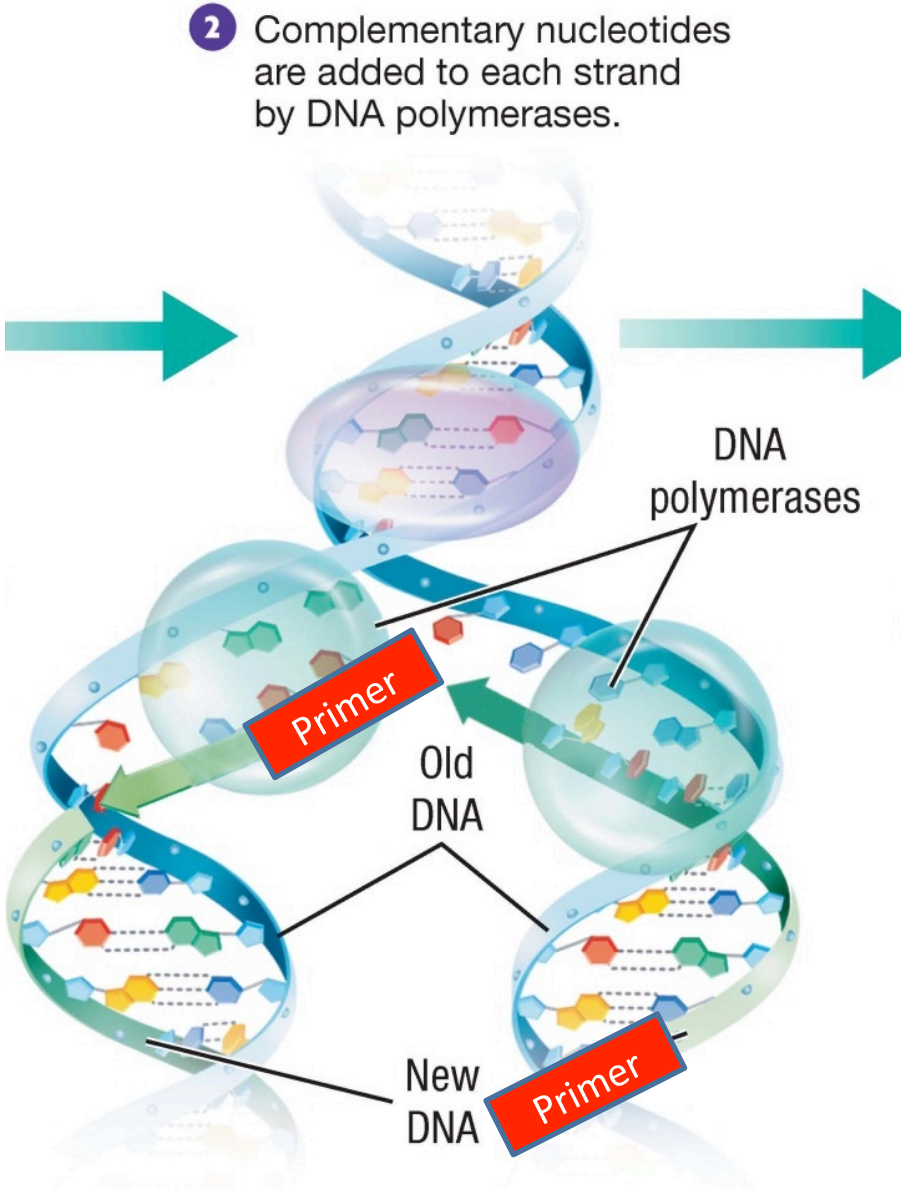


STEP2

New DNA is formed from DNA template.

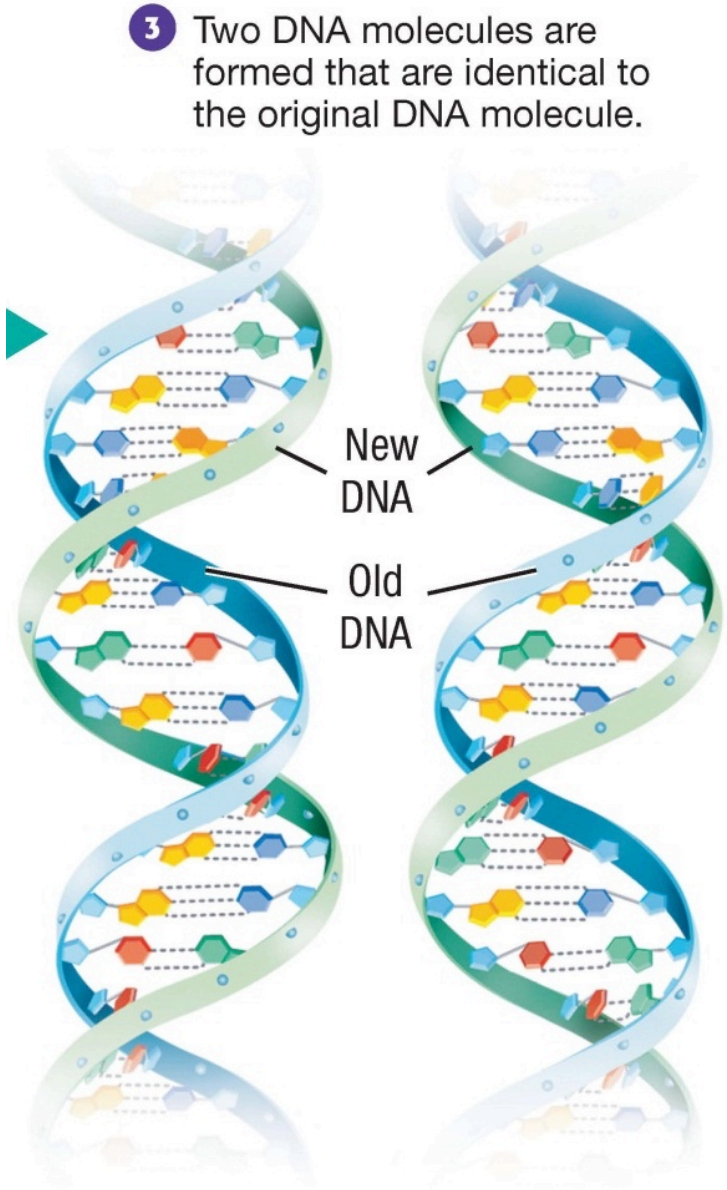
RNA Primers attach to specific regions.

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



STEP3

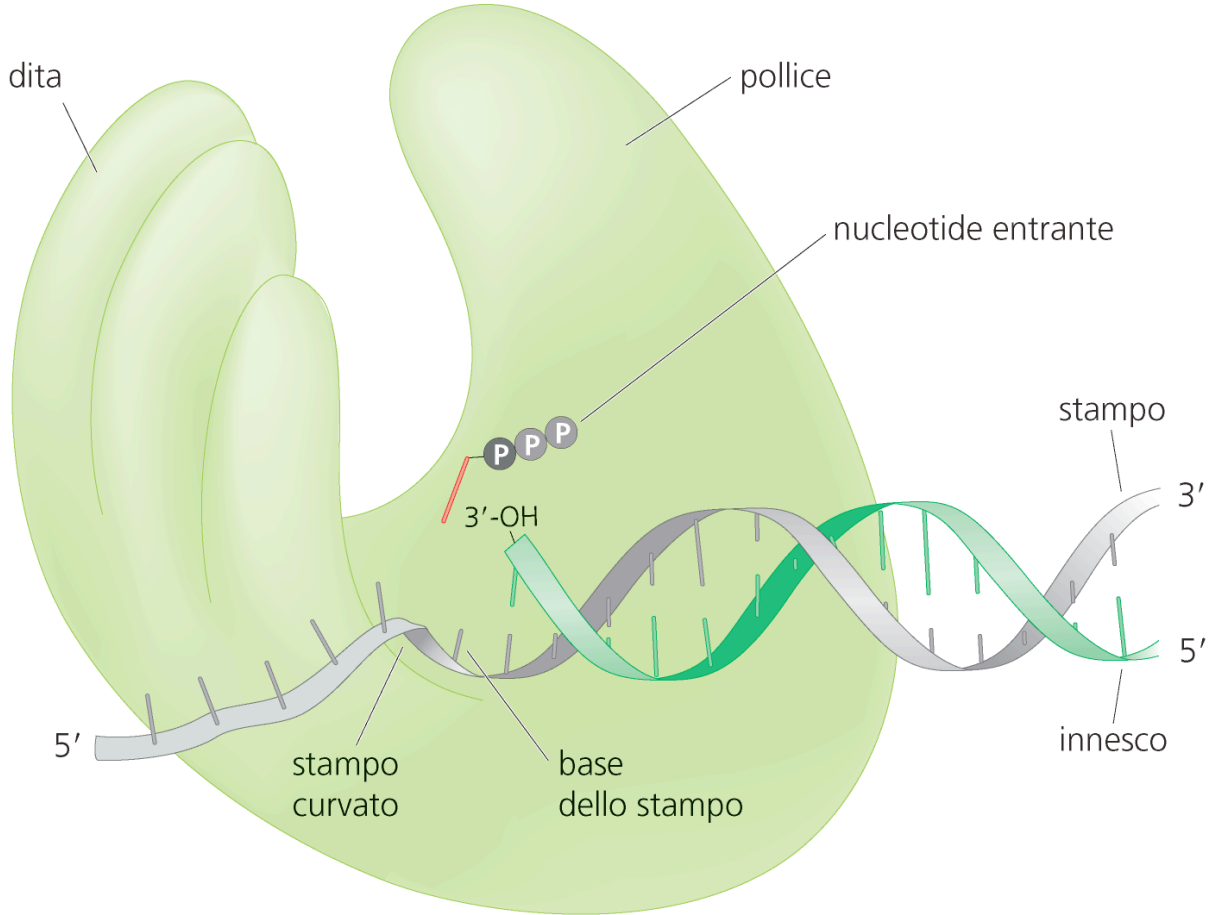
- The process completes when all the original bases have been paired with a new complementary nucleotide.
- Each double-stranded DNA helix is made of one new strand of DNA and one original strand of DNA.



Le DNA Polimerasi

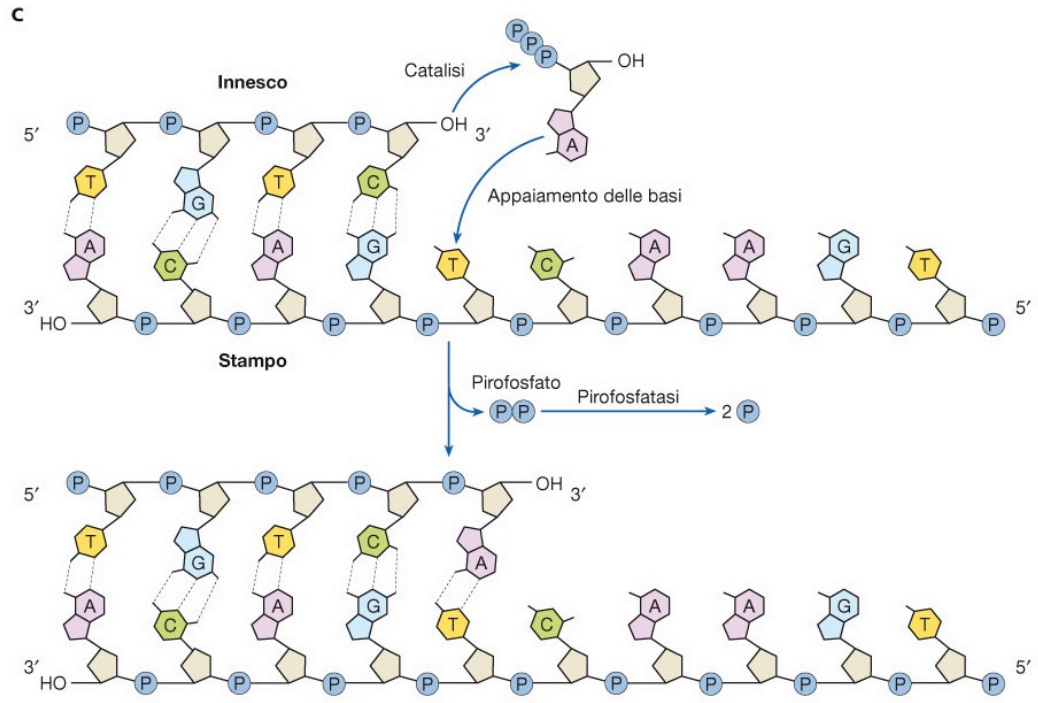
La DNA polimerasi e' un complesso multimerico formato da diverse unita' enzimatiche con diverse attivita' catalitiche.

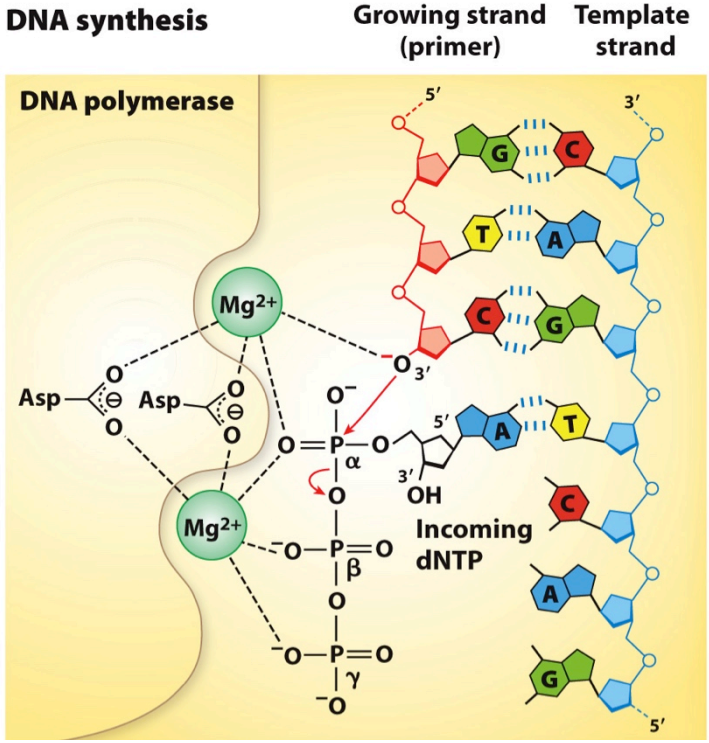
Tutte le DNA polimerasi condividono la stessa anatomia molecolare, paragonabile a quella di una mano destra. Il complesso "innesco-stampo" attraversa il "palmo", dove viene controllata l'accuratezza dell'appaiamento tra le basi, in una scanalatura creata dalle "dita" e dal "pollice".



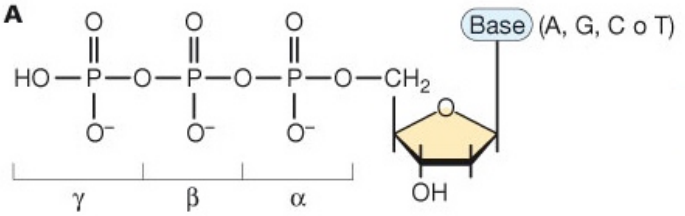
L'azione della DNA Polimerasi

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

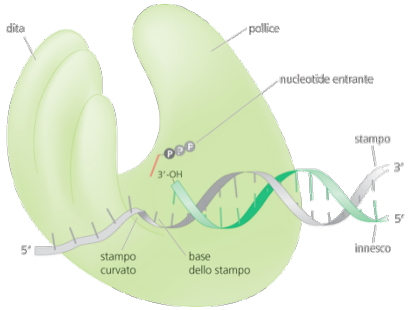




I nucleotidi entrano come nucleosidi trifosfati, apportando l'energia necessaria per la polimerizzazione, fornita dall'idrolisi di un legame fosfoanidride, con liberazione di pirofosfato. Il pirofosfato viene poi idrolizzato ulteriormente a fosfato inorganico, rendendo la reazione del tutto irreversibile.



Le DNA Polimerasi



Le diverse isoforme di DNA polimerasi condividono le seguenti caratteristiche:

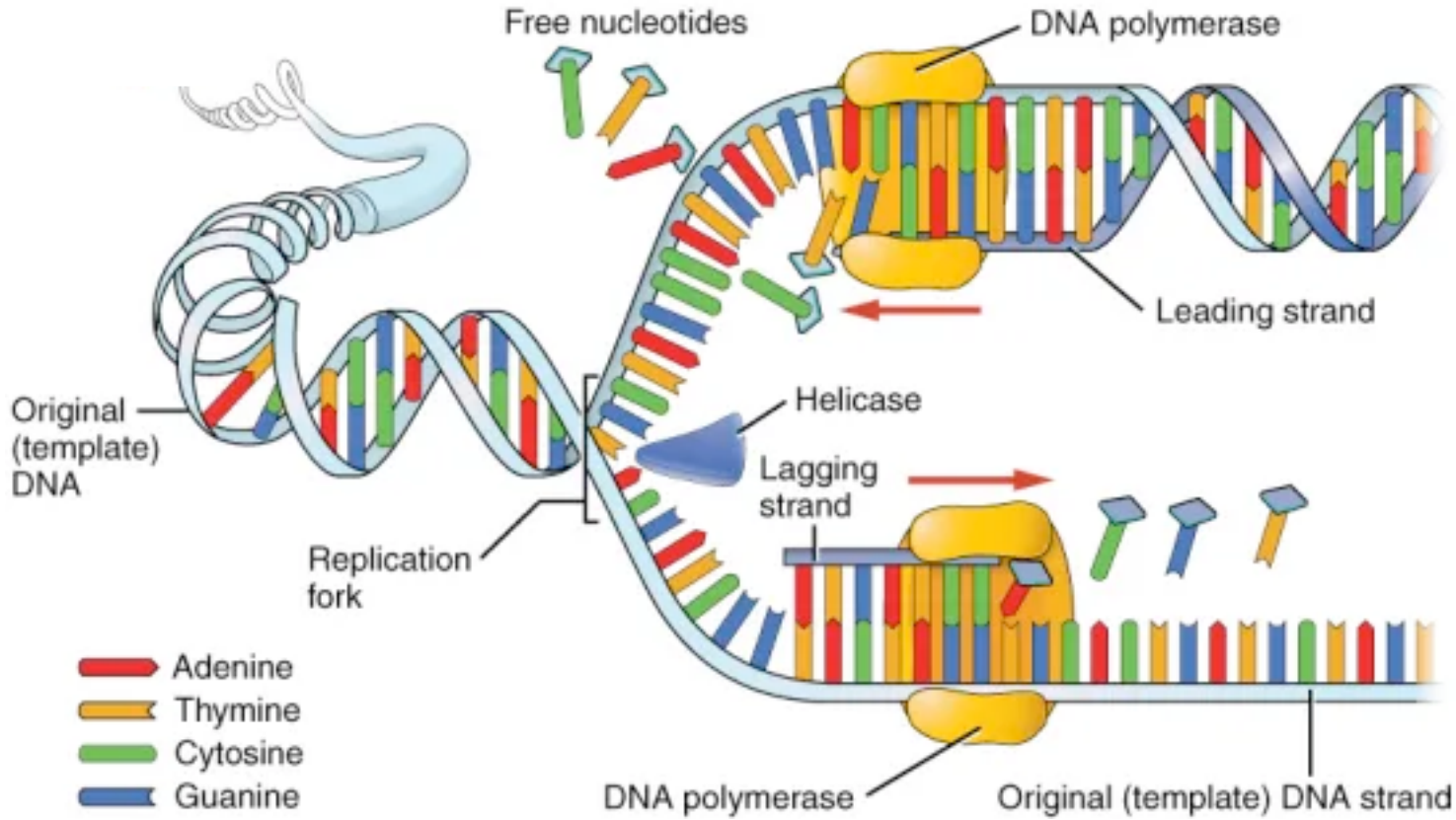
1. Le DNA polimerasi **non** sono in grado di rompere i legami idrogeno per separare i due filamenti di una doppia elica di DNA.
2. Tutte le DNA polimerasi **necessitano di uno stampo da copiare**, fornito dai filamenti di un' elica preesistente.
3. Tutte le DNA polimerasi sono in grado di allungare 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'— 3' e 3' — 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' — 3'**.
6. Tutte le DNA polimerasi utilizzano come substrato solo i quattro nucleotidi trifosfato.

Requirements for *E. Coli* DNA Replication

- *E. coli* requires over 20 enzymes and proteins.
- **The set is called the replisome.**
- Includes:
  - helicases (use ATP to unwind DNA strands)
  - topoisomerases (relieve the stress caused by unwinding)
  - DNA-binding proteins to stabilize separated strands
  - primases to make RNA primers
  - DNA ligases to seal nicks between successive nucleotides on the same strand (i.e., Okazaki fragments)

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.

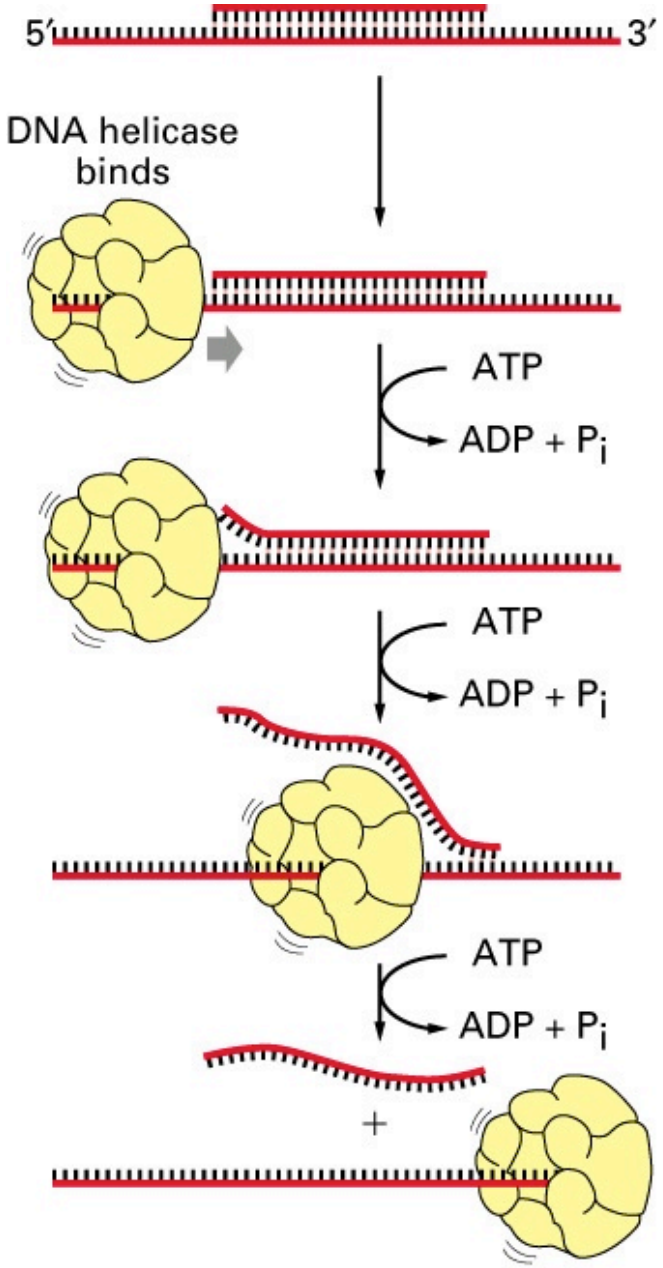


Special proteins help to open up double helix in front of the replication fork

* 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.

Cell, Vol. 101, 589–600, June 9, 2000, Copyright ©2000 by Cell Press

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

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Harvard Medical School
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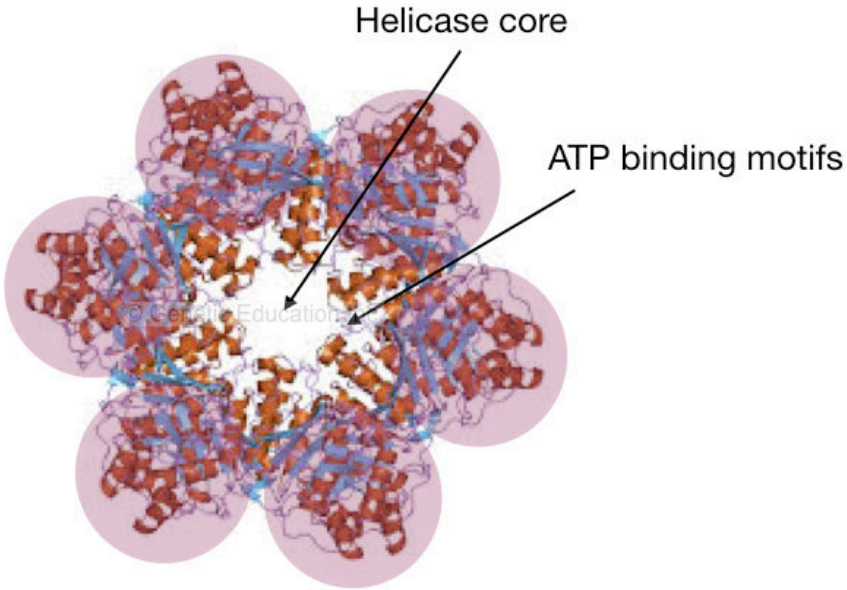
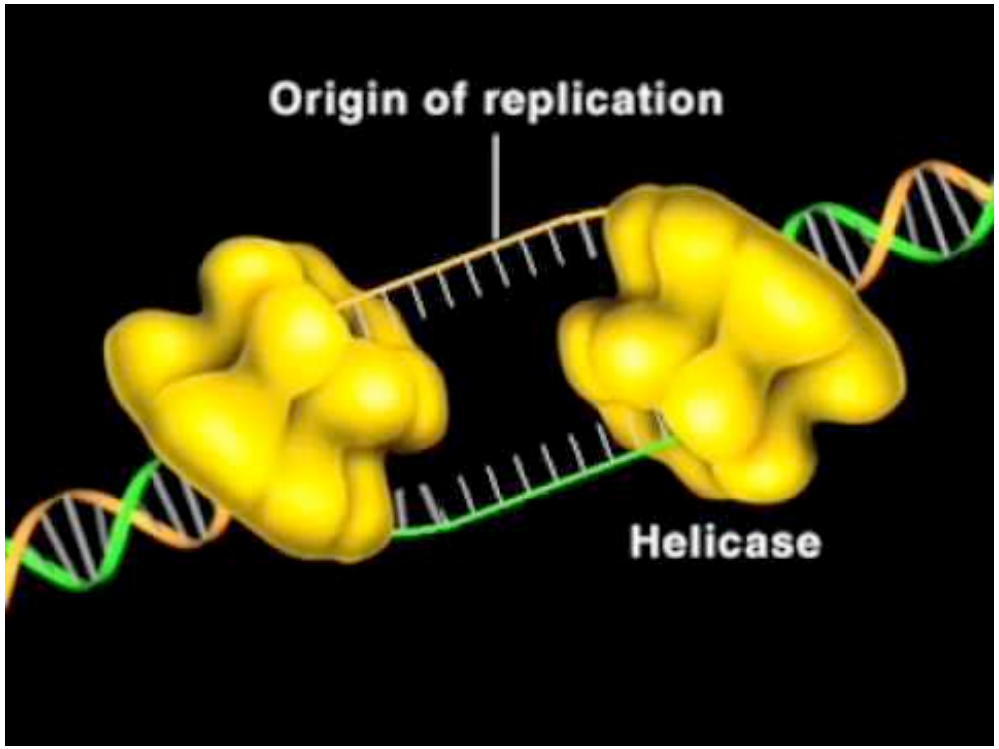
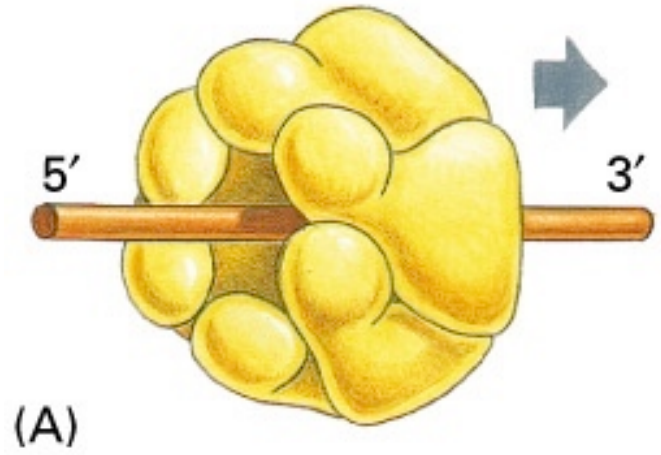




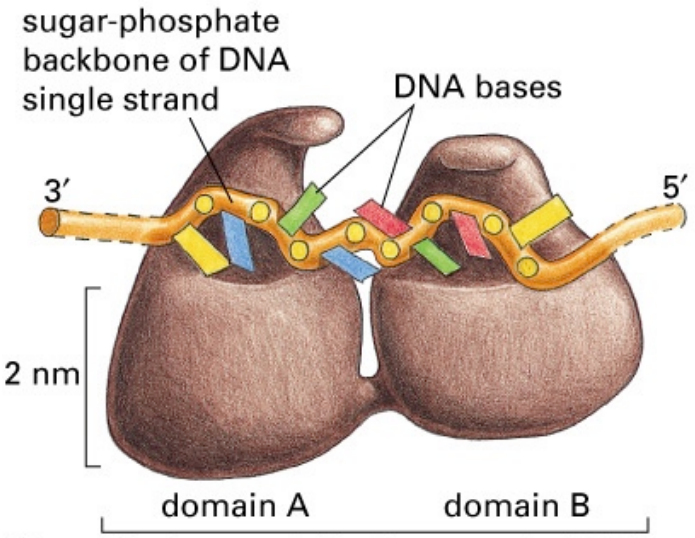
Hexameric structure of DNA helicase

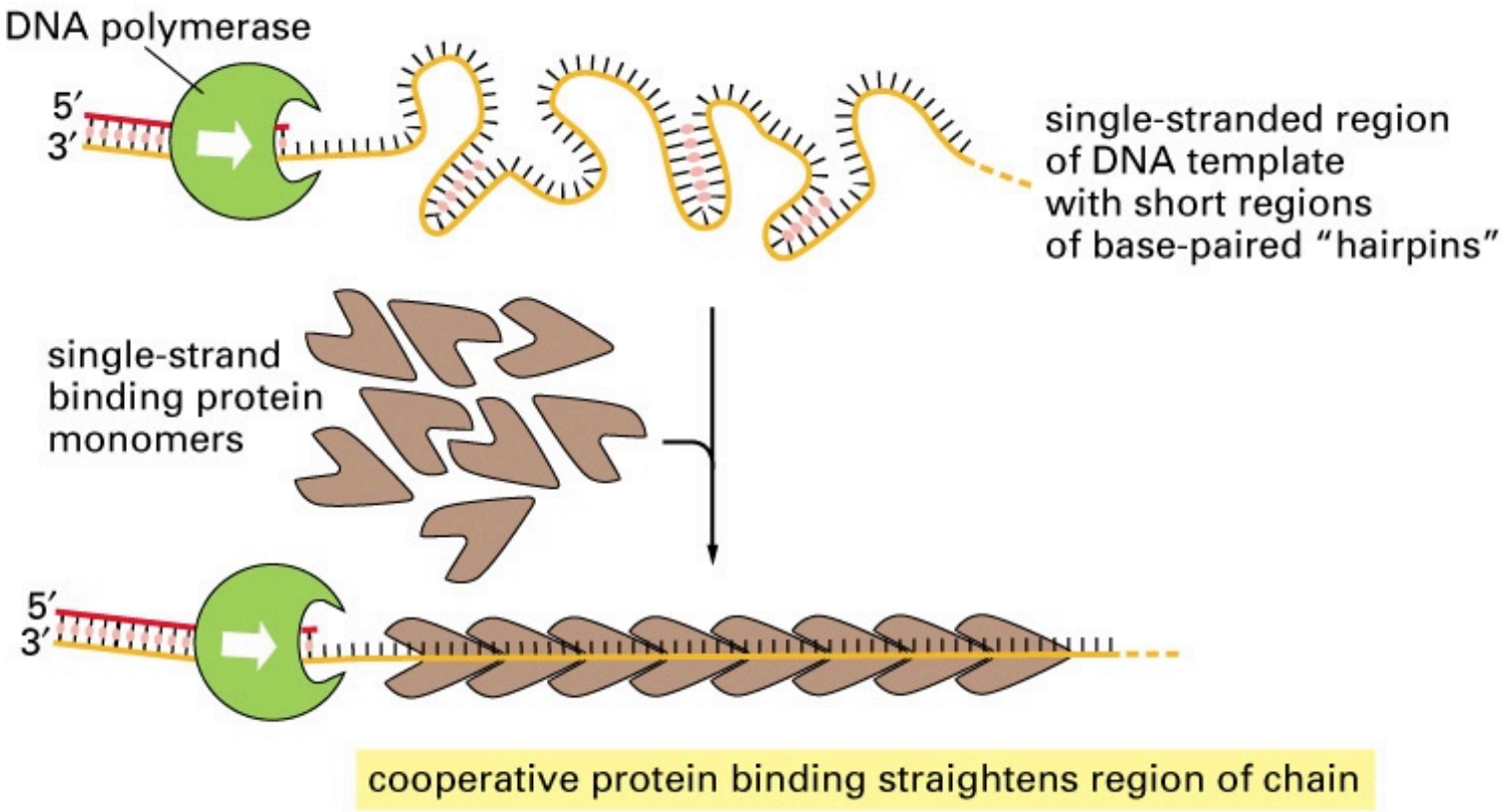


SSB proteins

SSB proteins bind tightly to ssDNA without covering the bases.

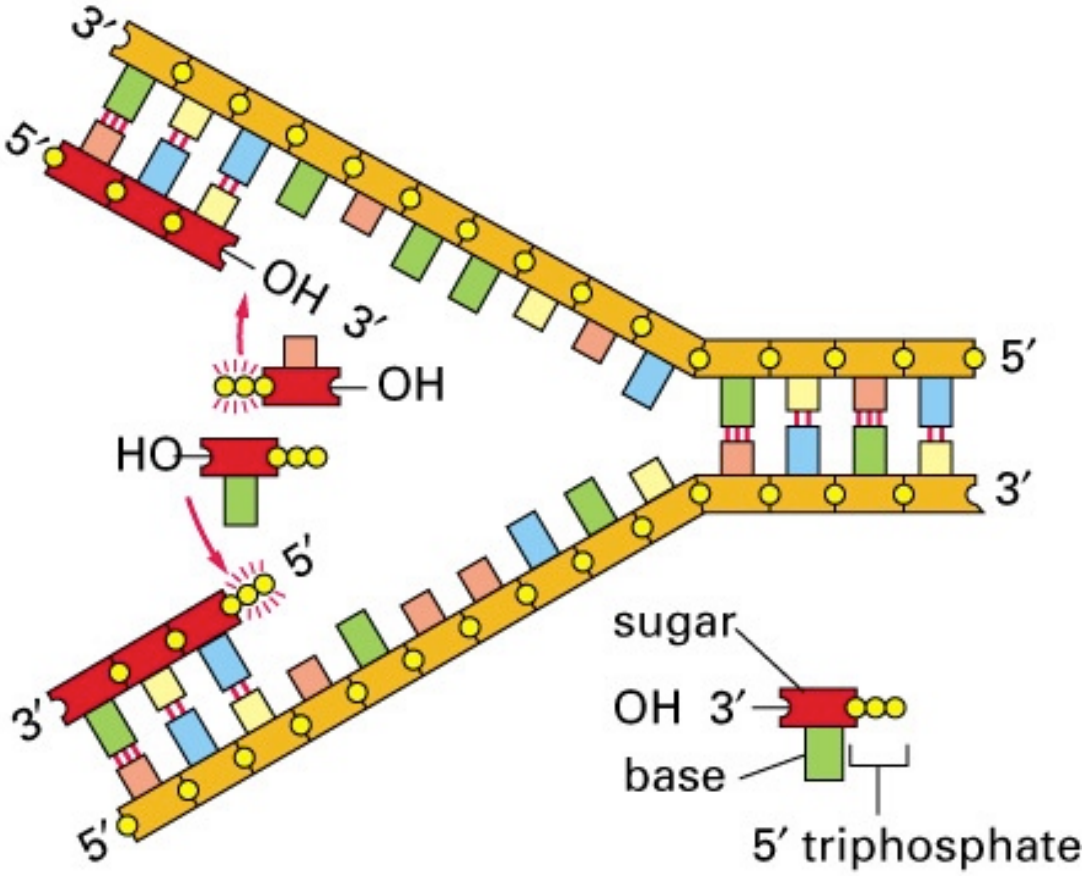
SSB proteins are unable to open a DNA helix but aid helicases by stabilizing the unwound ss conformation





La Forcella di Replicazione

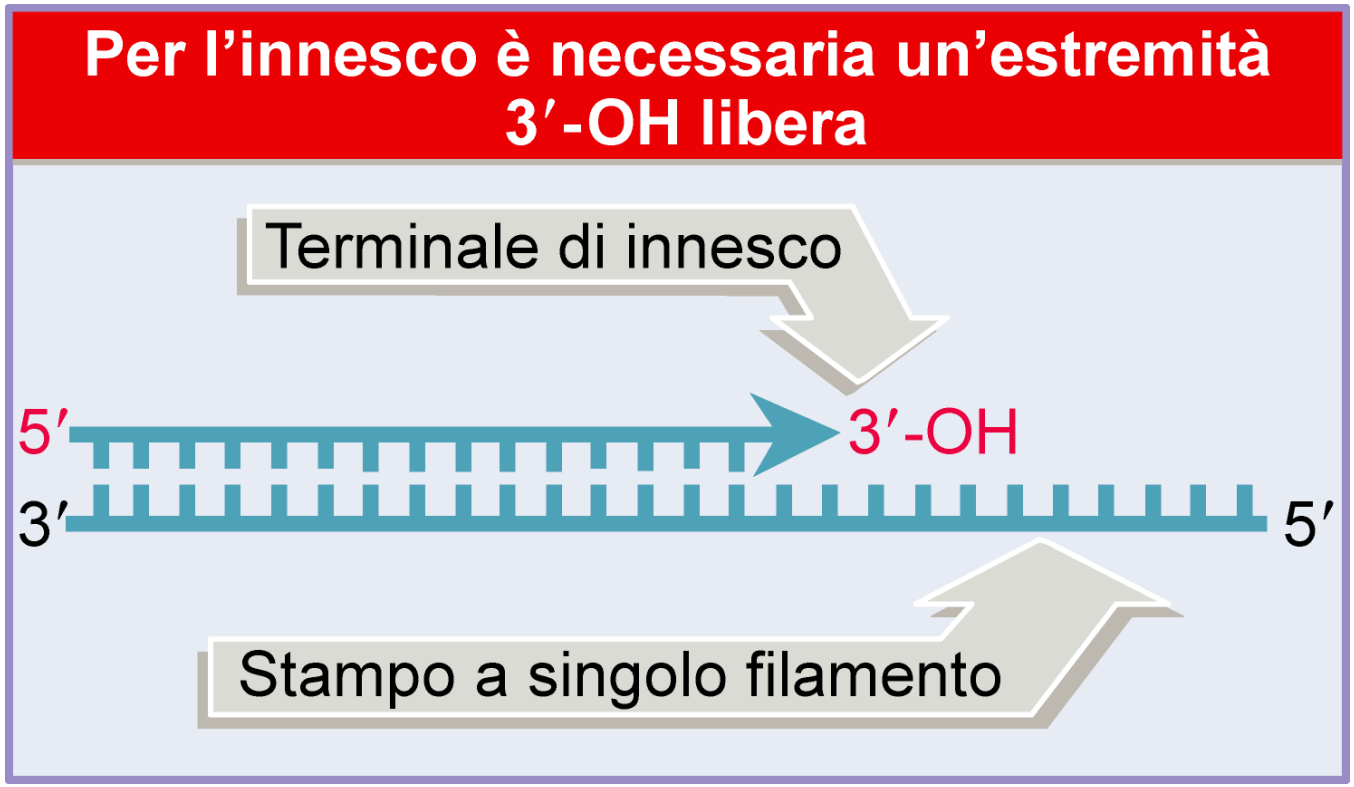
I singoli filamenti fungono da stampo per la sintesi di un nuovo filamento complementare.



MA

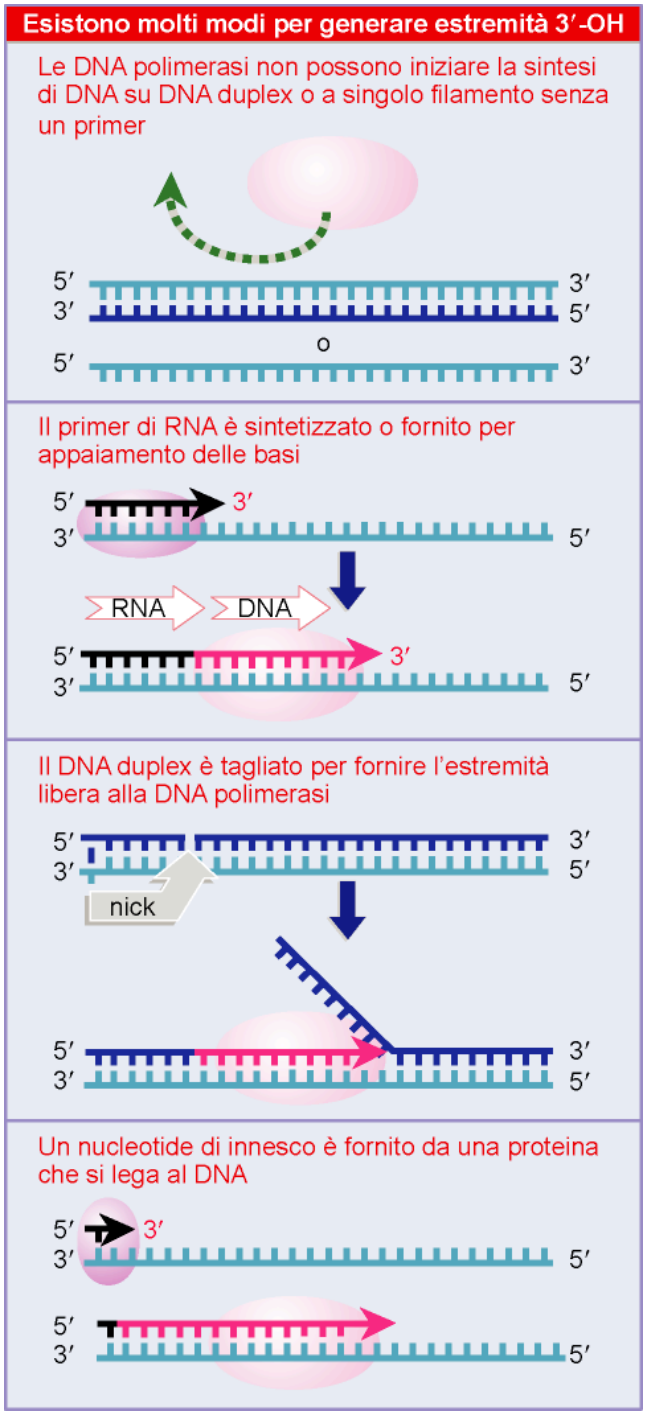
La DNA polimerasi ha attivita' di sintesi ESCLUSIVAMENTE in direzione 5' ->3 ':
problema di sintesi continua di uno dei due filamenti

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



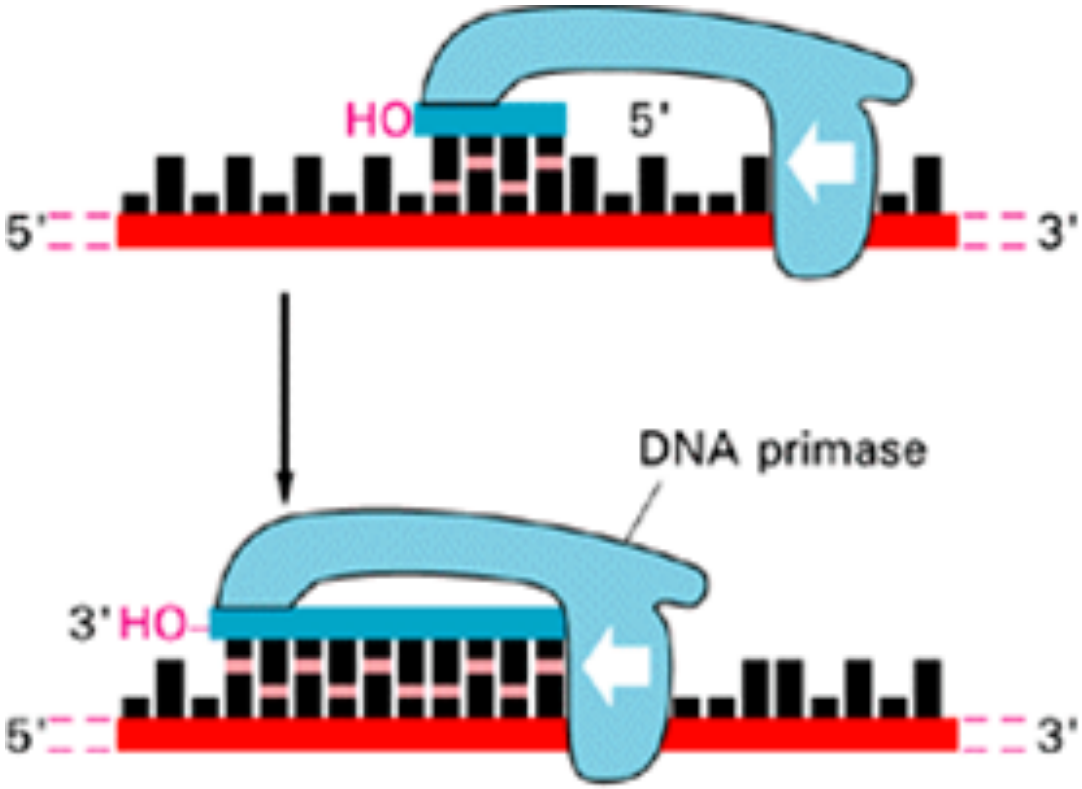
Le DNA Polimerasi richiedono un'estremita' di innesco 3'-OH per iniziare la sintesi del DNA.

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

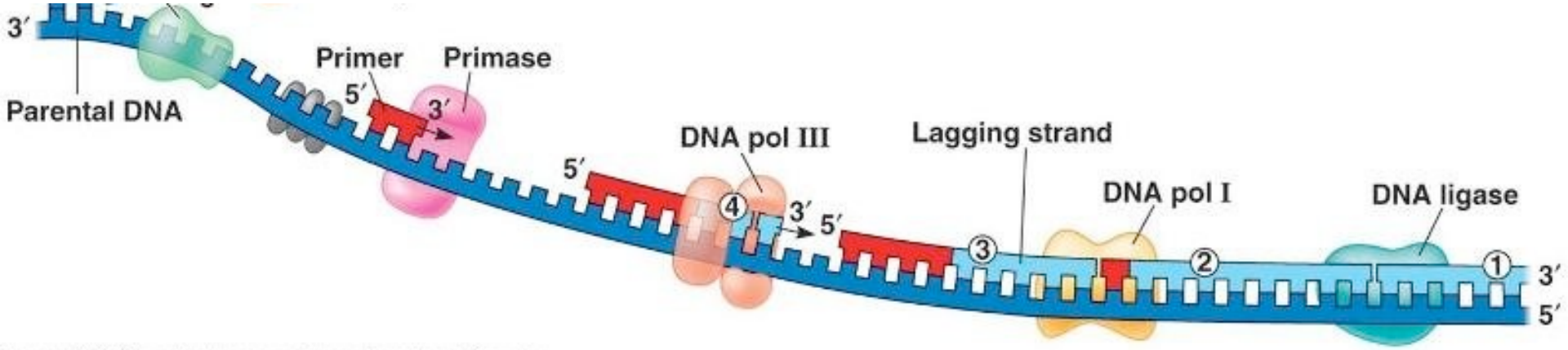


La Primasi

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.



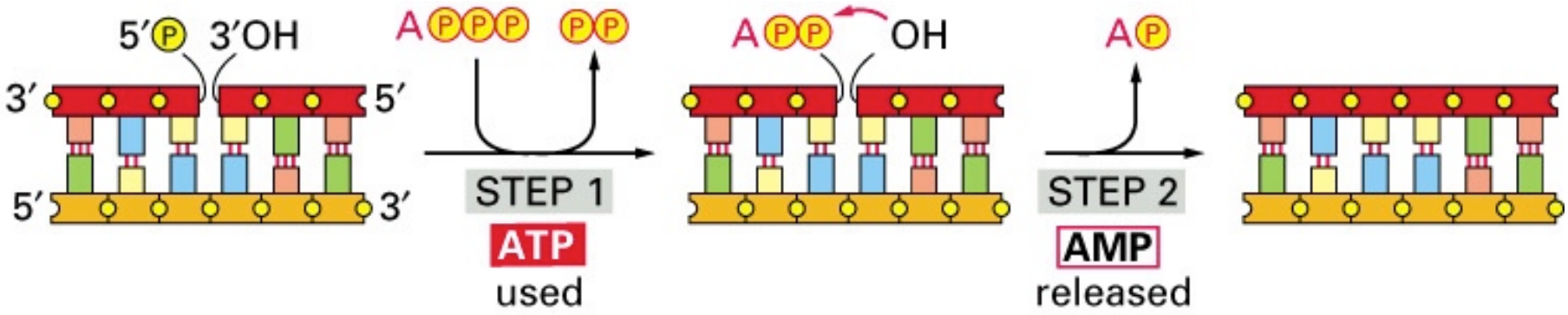
I frammenti di Okasaki

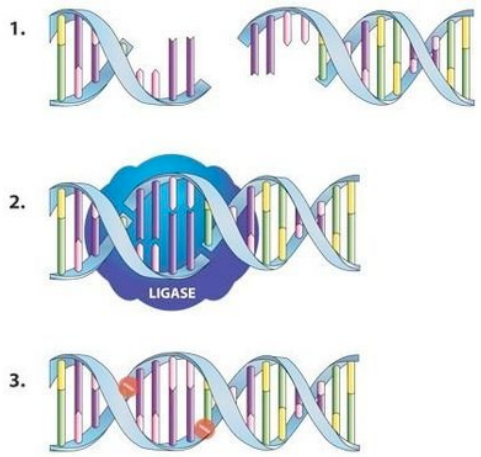


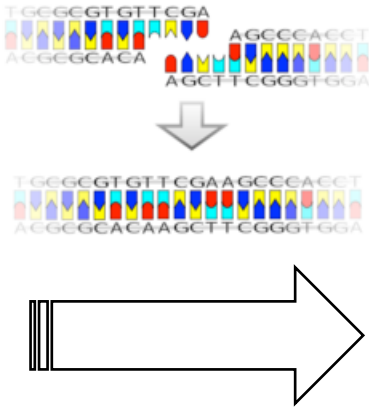
- ✓ I frammenti di Okazaki sono sintetizzati dalla DNA polymerasi III a partire degli inneschi creati dalla Primasi.
- ✓ La DNA polymerasi non e' in grado di unire i frammenti.
- ✓ I primers a RNA devono essere sostituiti, in quanto le basi U devono essere sostituite da T.
- ✓ I primers vengono degradati 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 frammmenti vengono poi uniti dalla DNA Ligasi

L'azione della DNA Ligasi

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



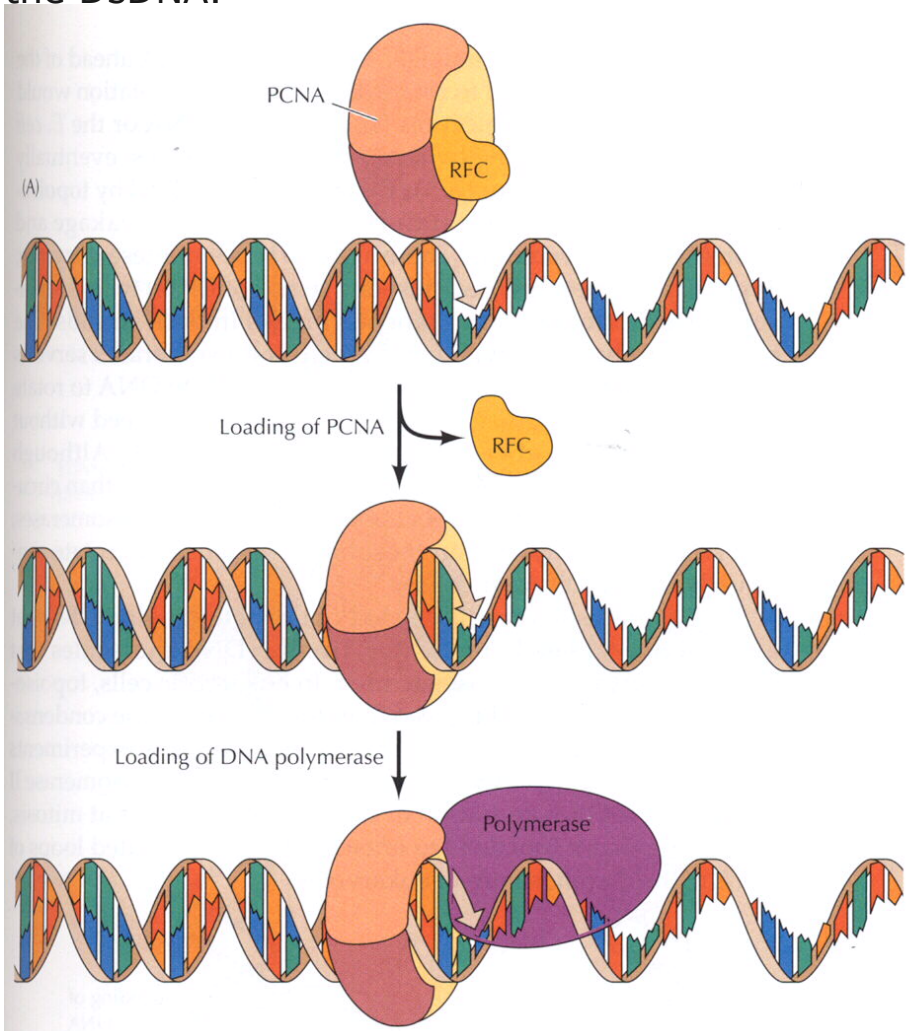


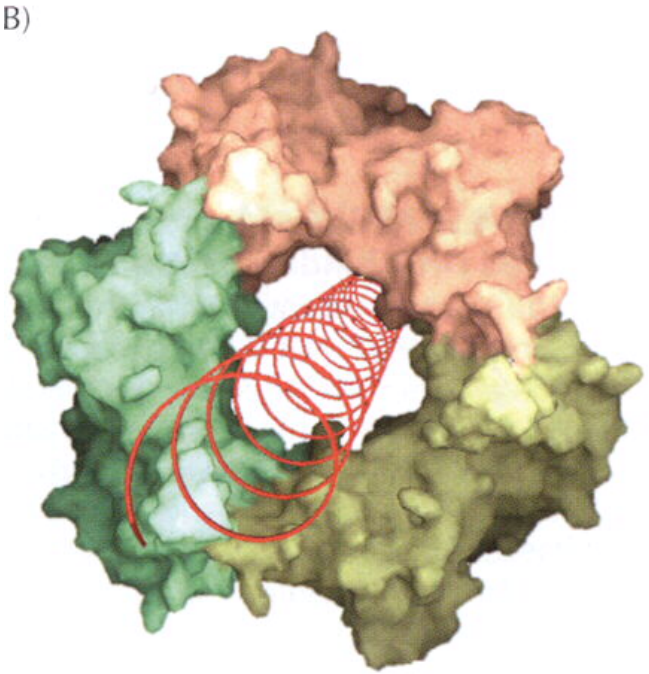


Stabilization of the replication machine

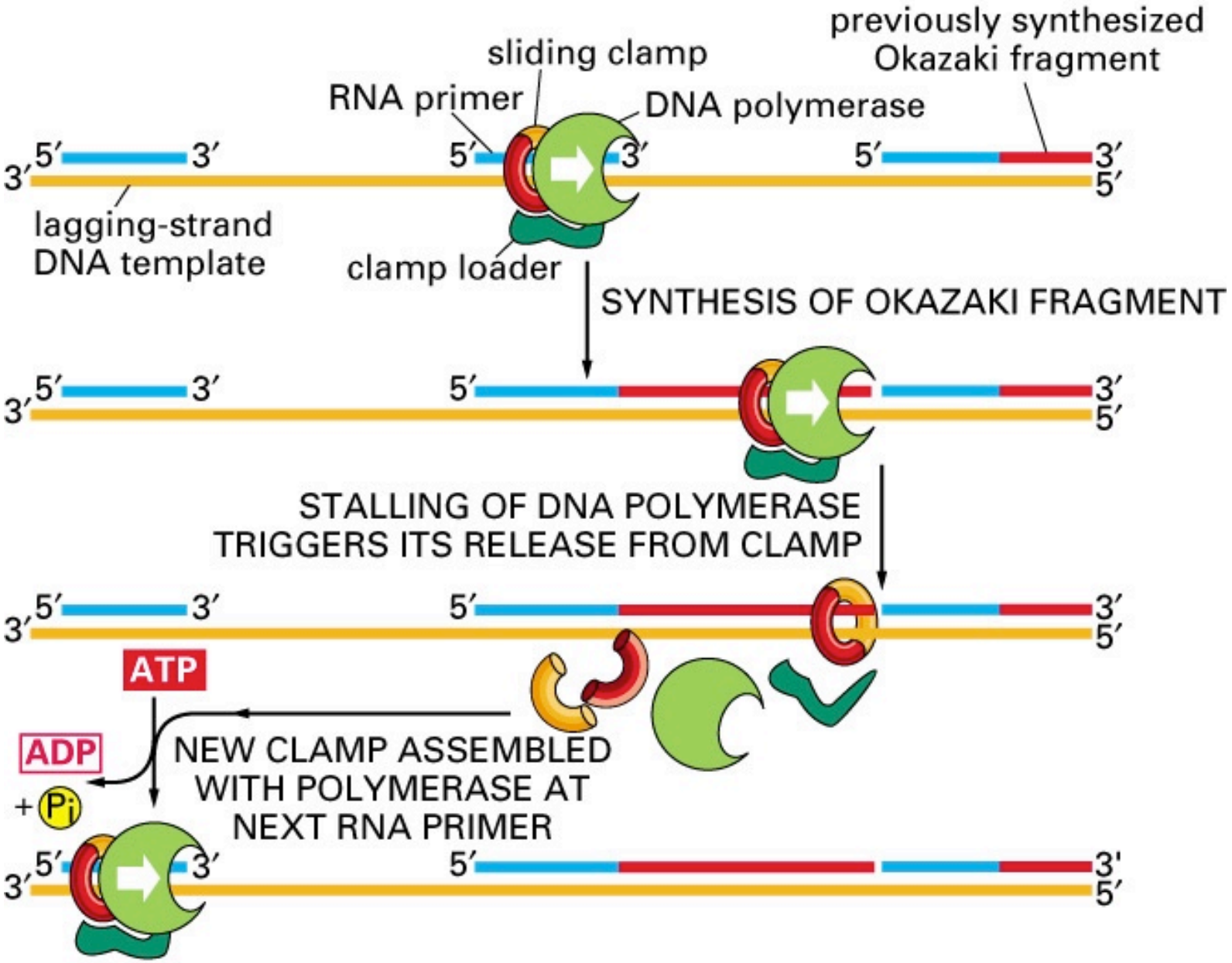
A ring clamp (PCNA) keeps the polymerase firmly on the DNA when it is moving on the dsDNA. One side of the ring binds to the back of the DNApol and it slides along DNA as the polymerase moves on.

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

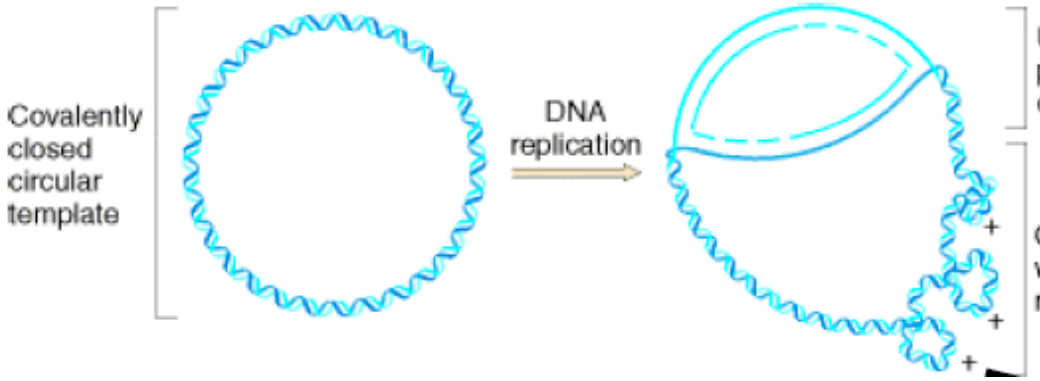




around the primer-template junction requires ATP hydrolysis by a special protein complex, the **clamp loader**

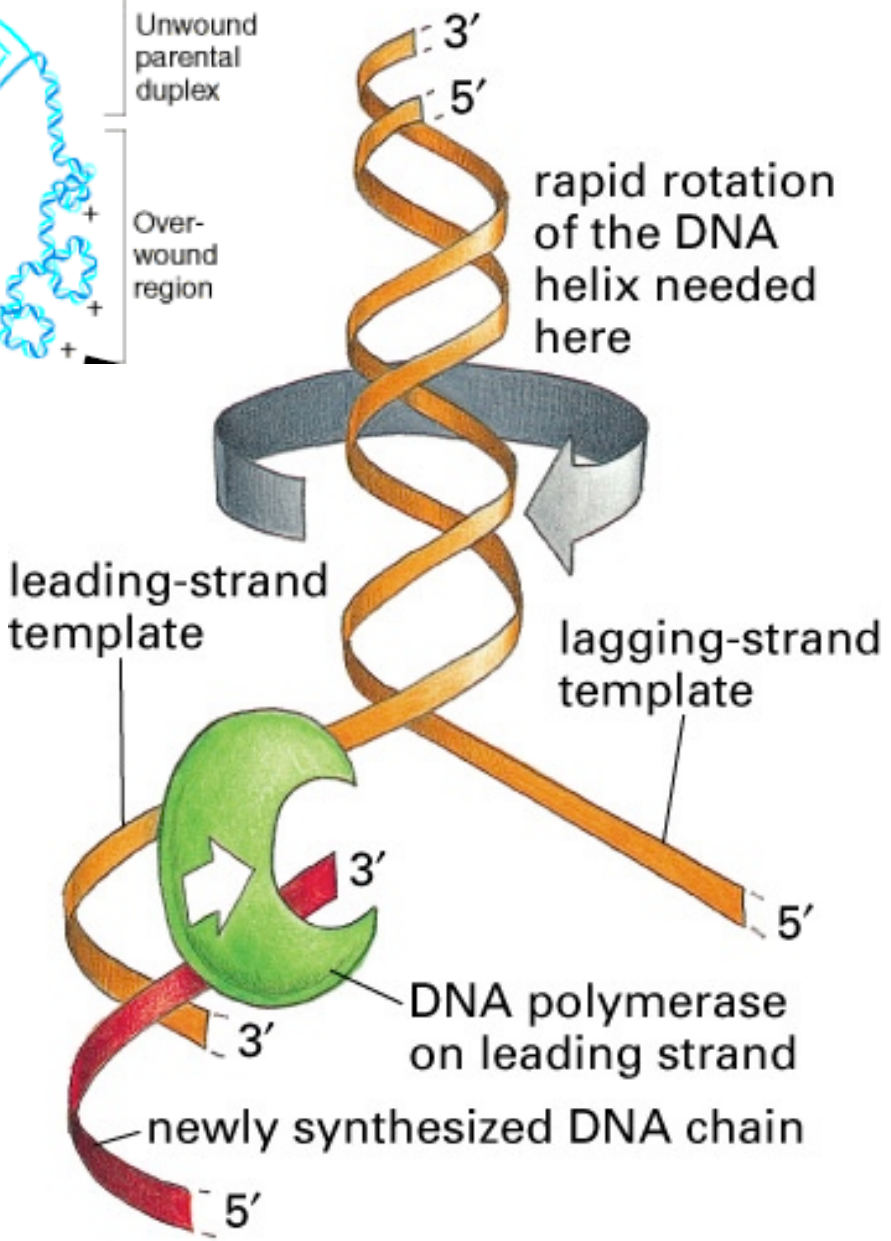

previously synthesized
Okazaki fragment
sliding clamp
RNA primer
DNA polymerase
5′
3′
3′
5′
5′
3′
3′
5′
lagging-strand
DNA template
clamp loader
SYNTHESIS OF OKAZAKI FRAGMENT
STALLING OF DNA POLYMERASE
TRIGGERS ITS RELEASE FROM CLAMP
ATP
ADP
+ Pi
NEW CLAMP ASSEMBLED
WITH POLYMERASE AT
NEXT RNA PRIMER

The winding problem



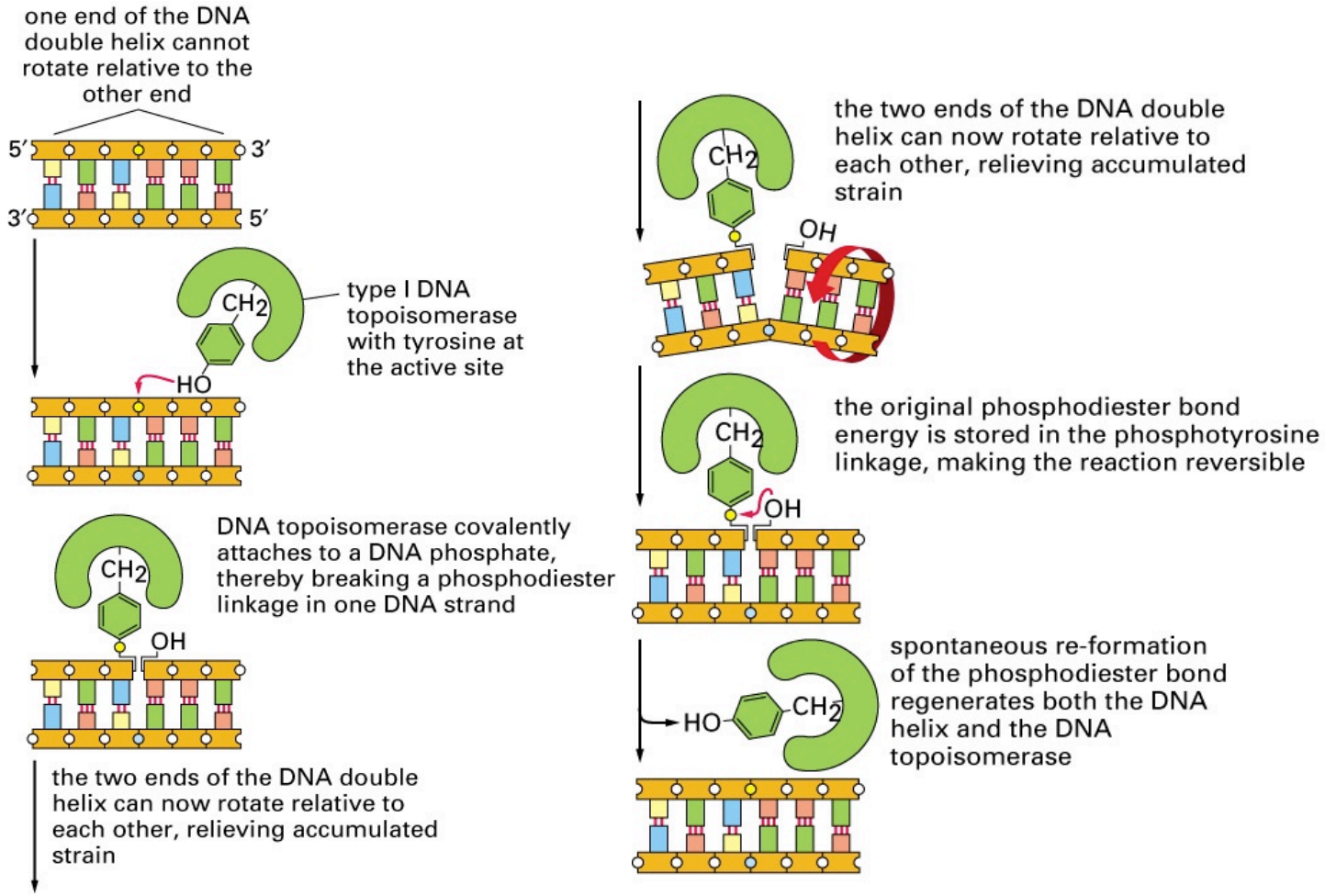
Every 10 bases replicated corresponds to one complete turn about the axis of the parental helix - for a replication fork to move, the chromosome ahead would have to rotate rapidly

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

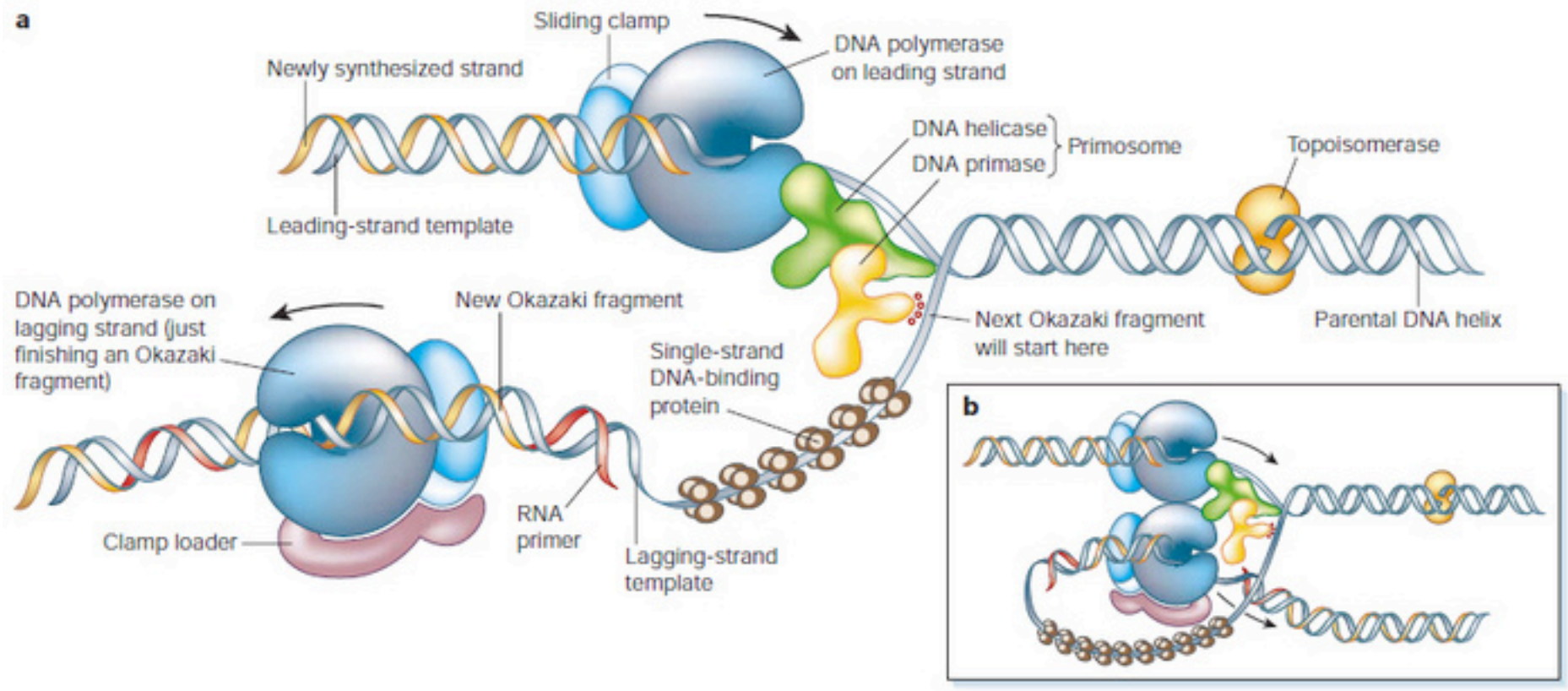


Topoisomerasi

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



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

DNA Replication

DNA is anti-parallel because of the bi-directionality of DNA.
One side goes 3' to 5'. The other 5' to 3'.
New nucleotides can only be added to the 3' end of the existing chain.

One side (the top pictured here) is the LEADING STRAND. It has its new strand continuously synthesized as helicase unwinds more DNA.
The other side, the LAGGING STRAND (on bottom) is discontinuous replication because it the bases are oriented in the wrong direction.

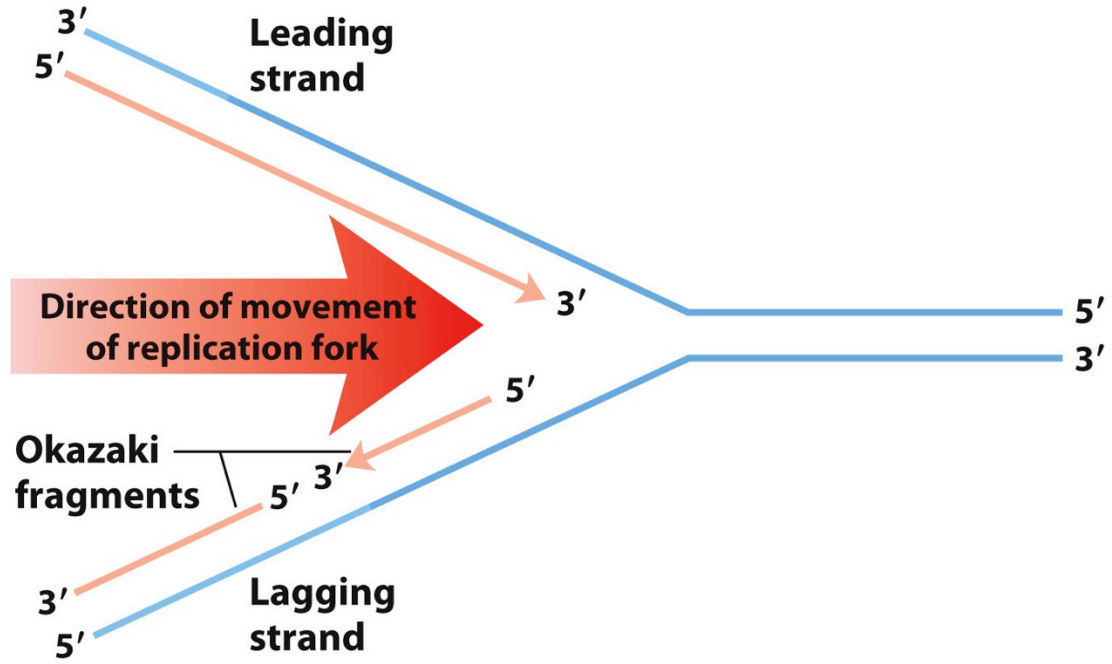
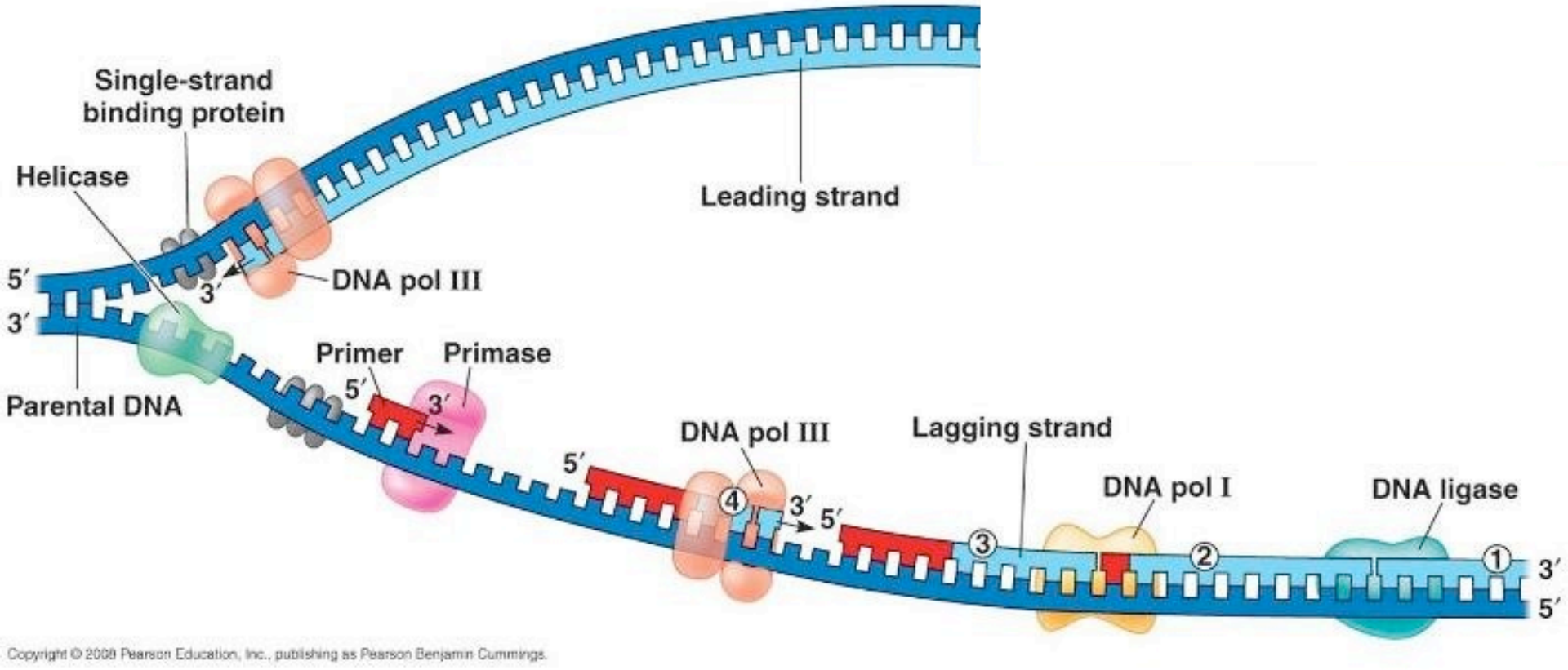


Figure 25-4
Lehninger Principles of Biochemistry, Seventh Edition

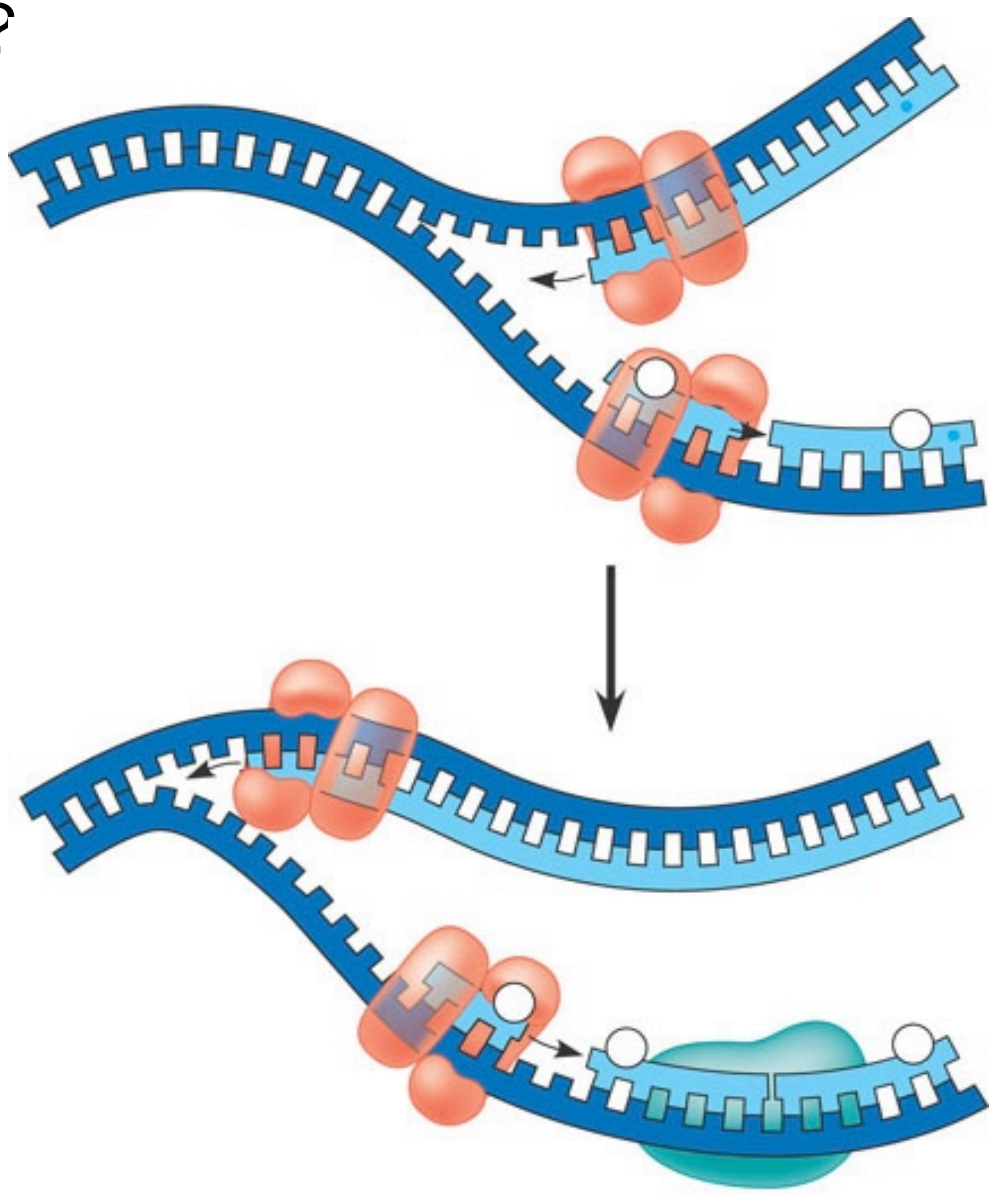
La strategia di duplicazione e' diversa sui due filamenti



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.

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