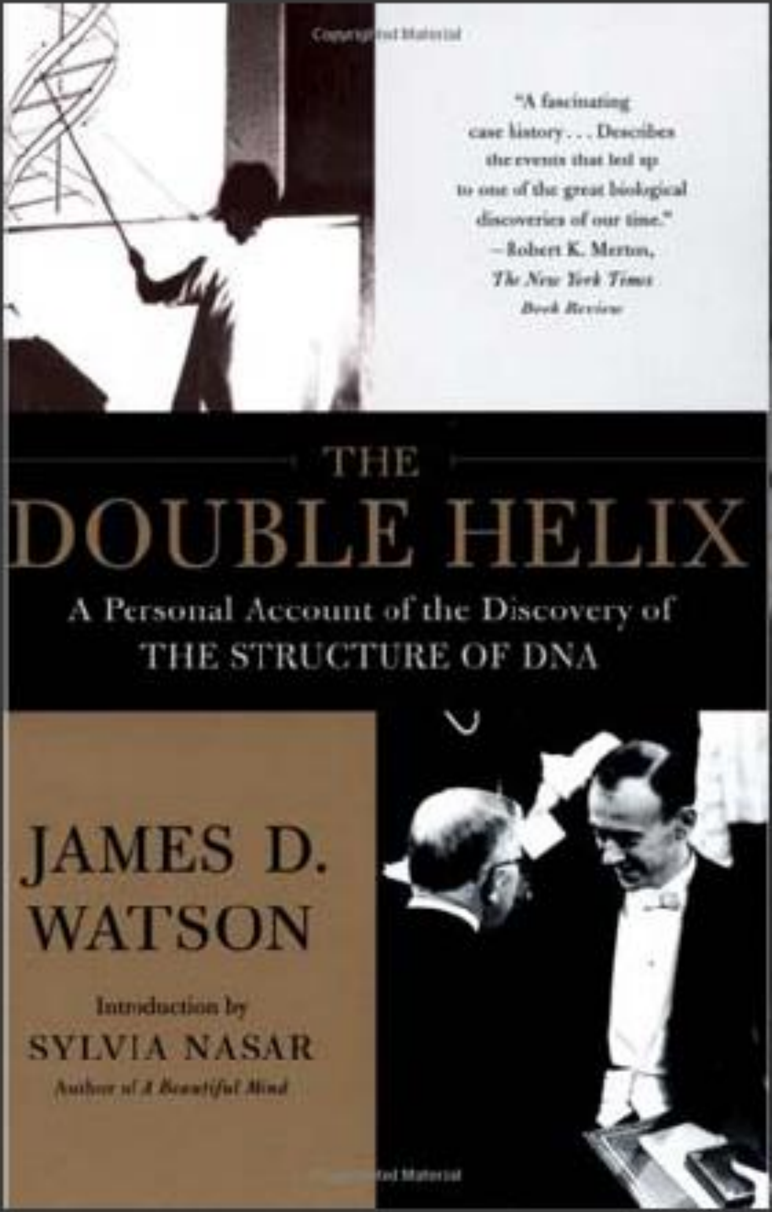


"A fascinating
case history . . . Describes
the events that led up
to one of the great biological
discoveries of our time."
–Robert K. Merton,
*The New York Times
Book Review*

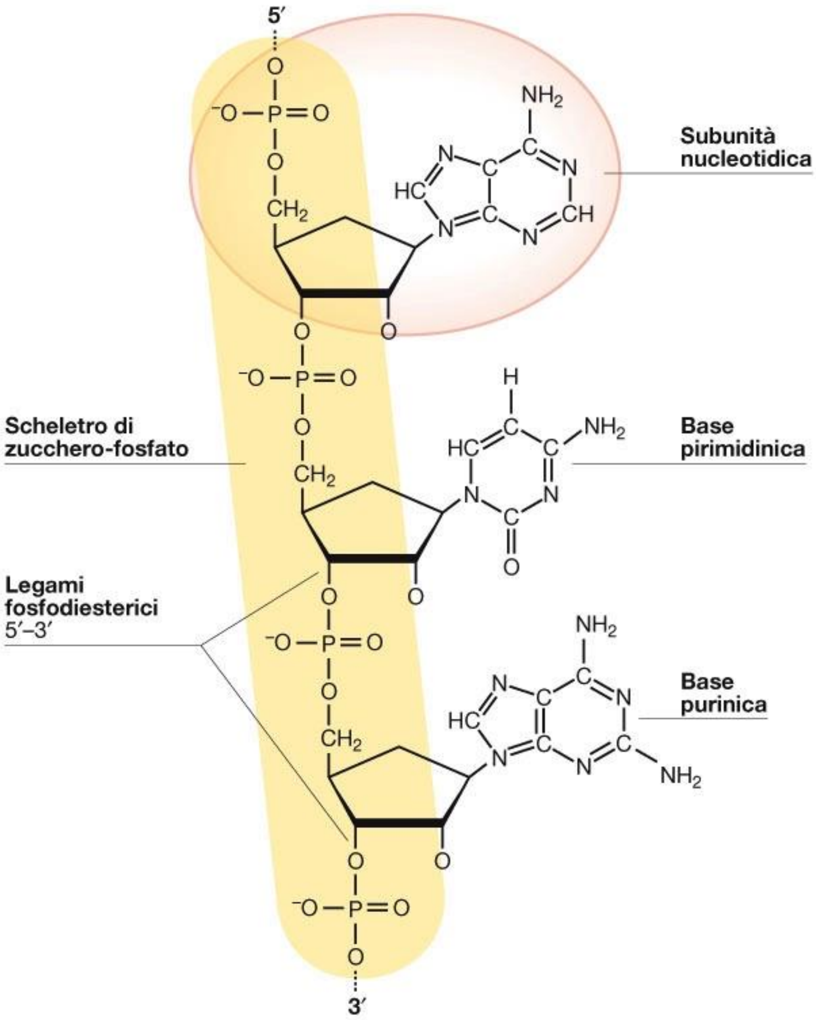
THE DOUBLE HELIX

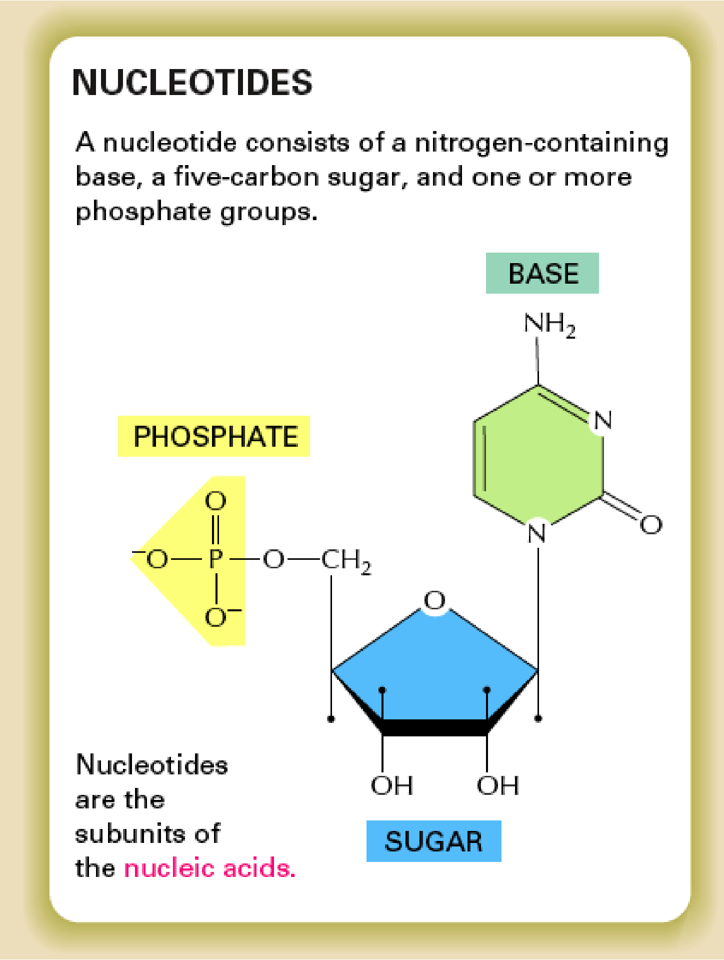
A Personal Account of the Discovery of
THE STRUCTURE OF DNA

JAMES D.
WATSON

Introduction by
SYLVIA NASAR
Author of A Beautiful Mind

The DNA building blocks are the NUCLEOTIDES





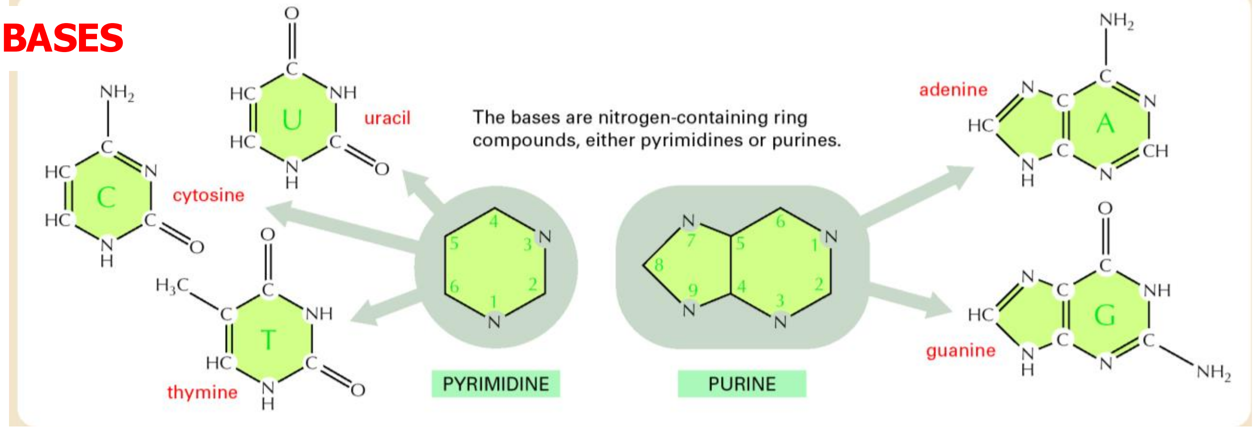
NUCLEOTIDES

A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.

Nucleotides are the subunits of the nucleic acids.

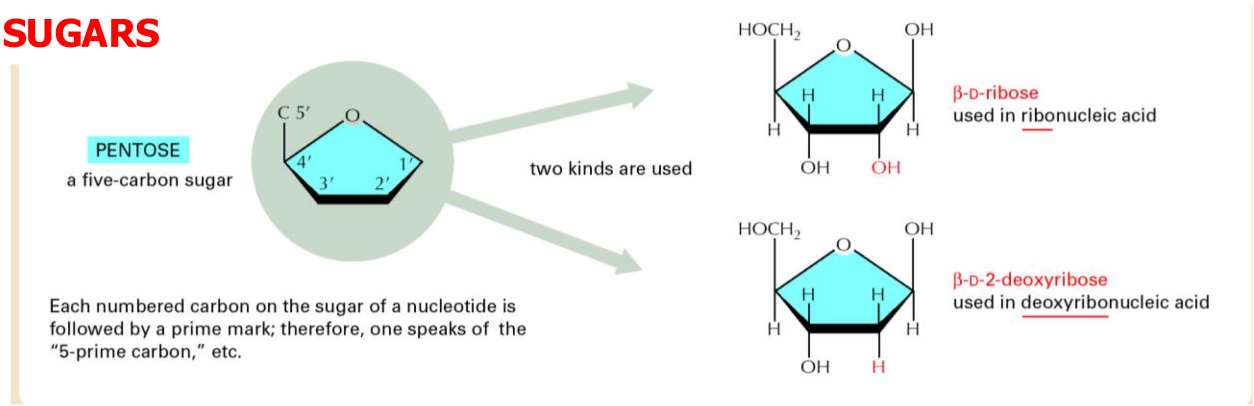
The DNA building blocks are the NUCLEOTIDES

BASES



The bases are nitrogen-containing ring compounds, either pyrimidines or purines.

SUGARS



PENTOSE
a five-carbon sugar

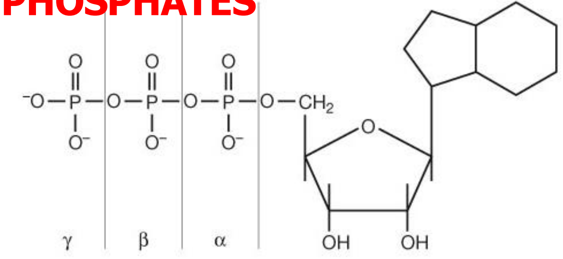
two kinds are used

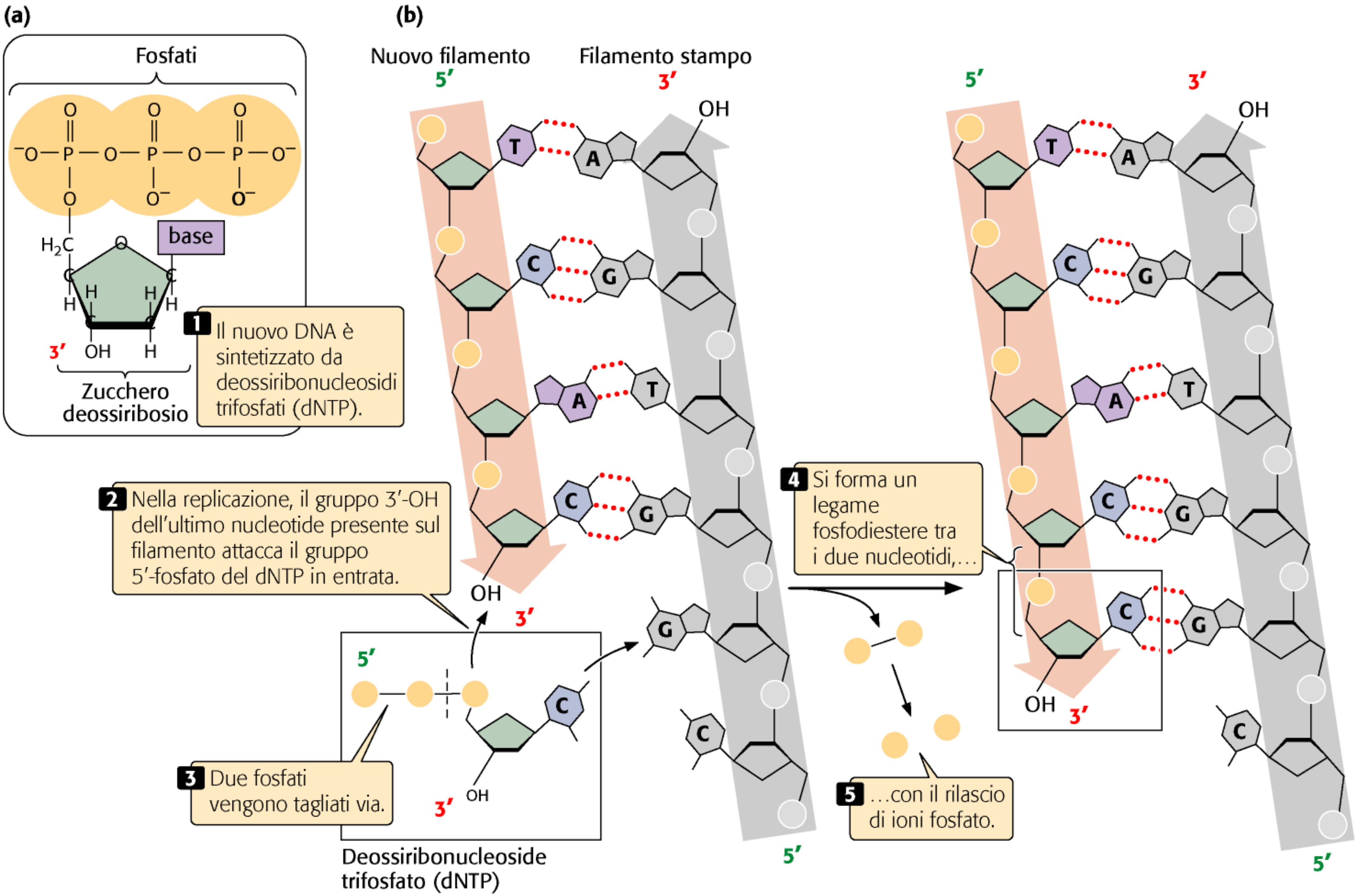
β-D-ribose
used in ribonucleic acid

β-D-2-deoxyribose
used in deoxyribonucleic acid

Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.

PHOSPHATES





1 Il nuovo DNA è sintetizzato da deossiribonucleosidi trifosfati (dNTP).

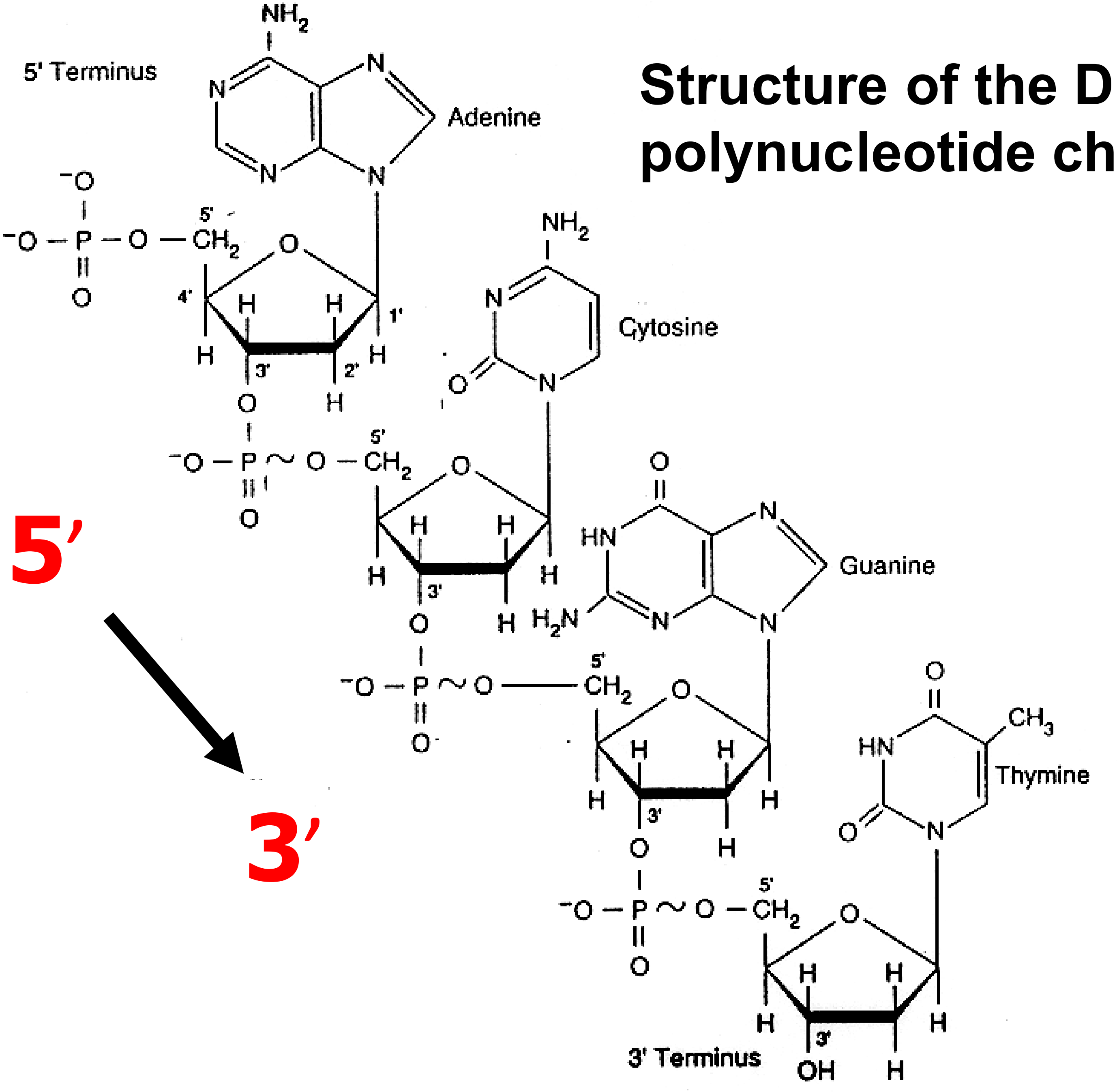
2 Nella replicazione, il gruppo 3′-OH dell'ultimo nucleotide presente sul filamento attacca il gruppo 5′-fosfato del dNTP in entrata.

3 Due fosfati vengono tagliati via.

4 Si forma un legame fosfodiestere tra i due nucleotidi,…

5 …con il rilascio di ioni fosfato.

Structure of the DNA polynucleotide chain

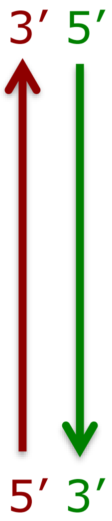


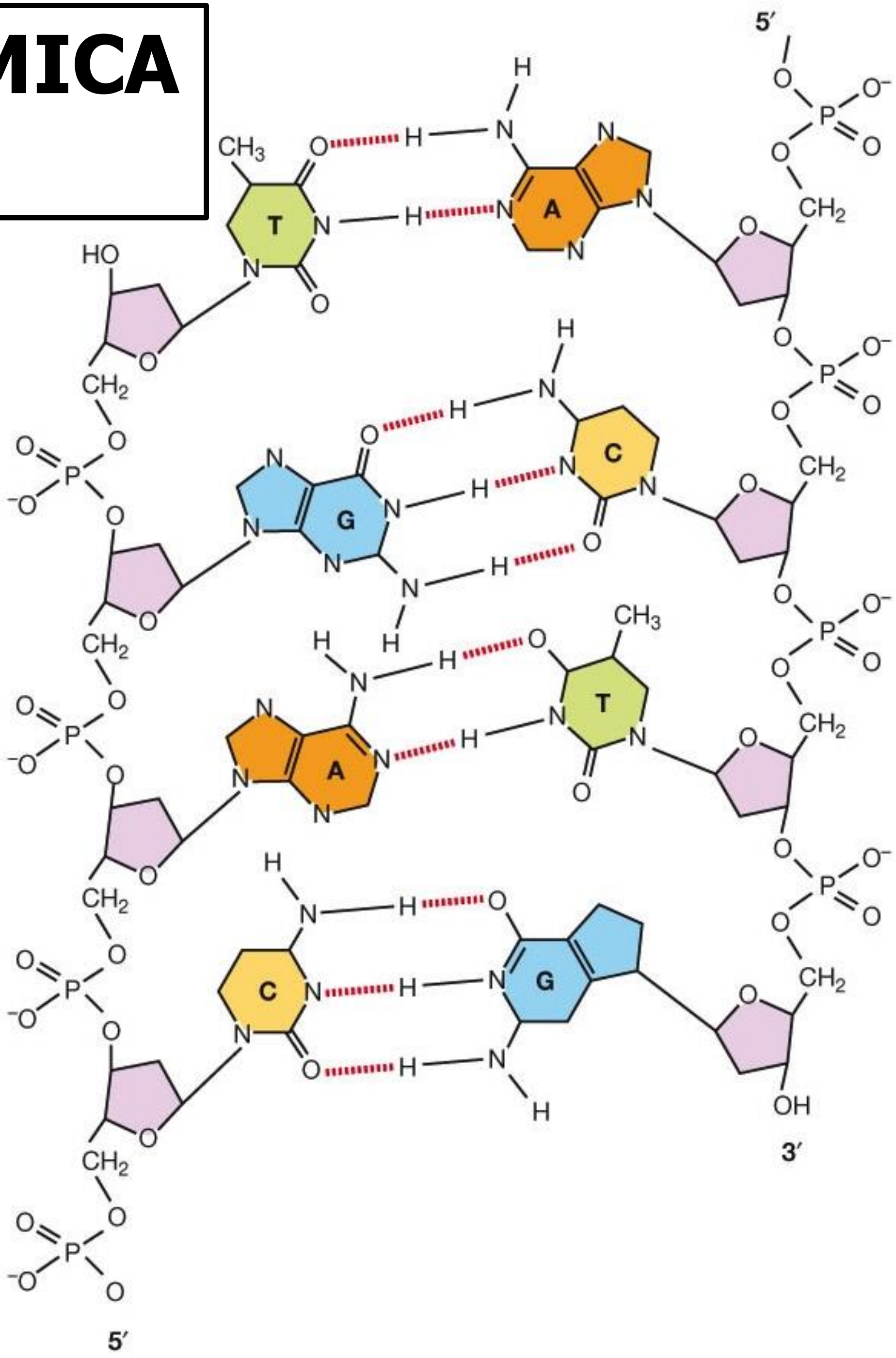
5′

3′

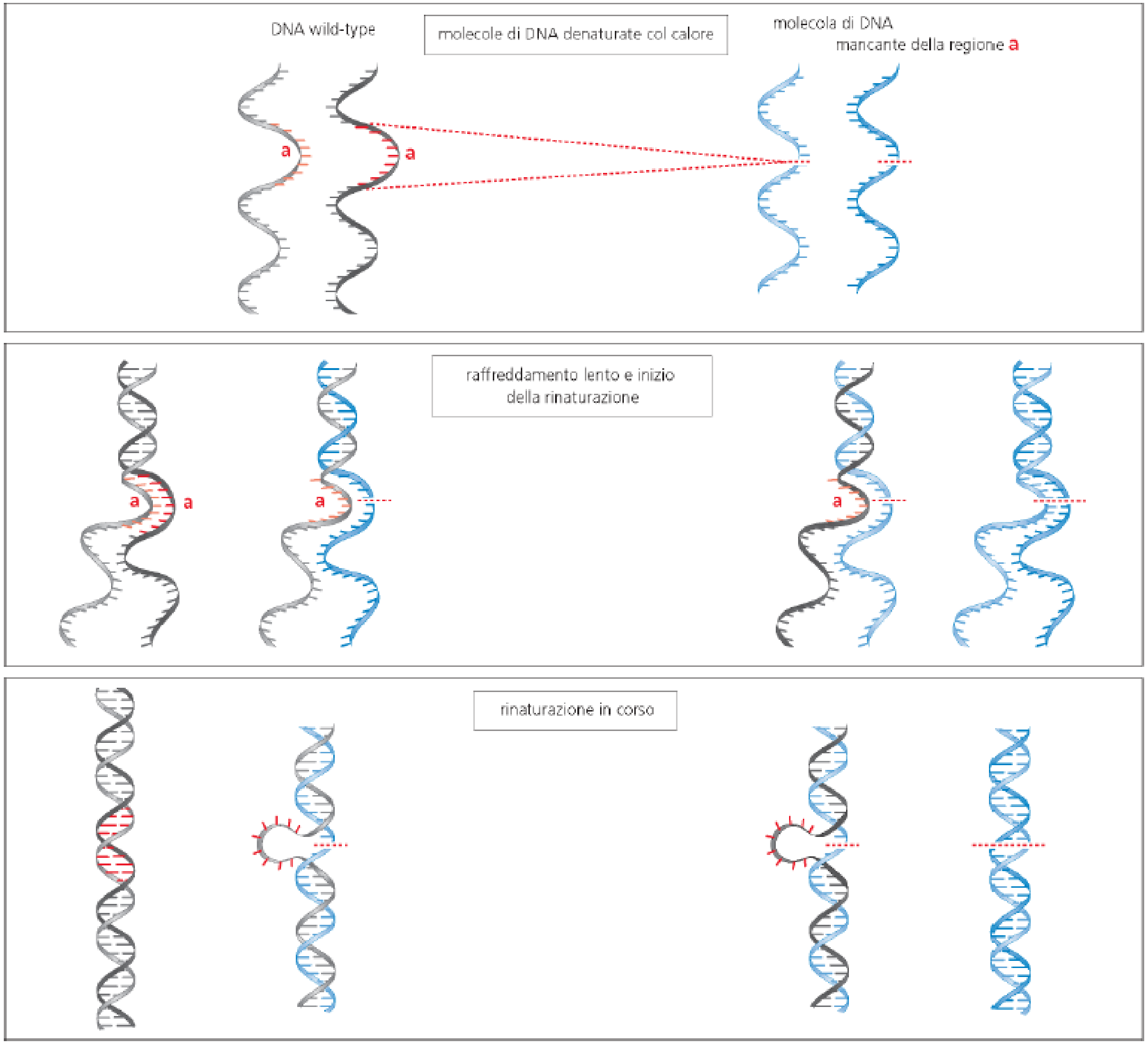
LA STRUTTURA CHIMICA DEL DNA

I 2 filamenti sono ANTIPARALLELI





I due filamenti di DNA possono separarsi e riassociarsi



Se il DNA viene riscaldato (100°) o posto a pH elevato si può denaturare.

Se si ritorna lentamente alle condizioni di partenza il DNA può rinaturarsi e formare molecole ibride (ibridazione del DNA)

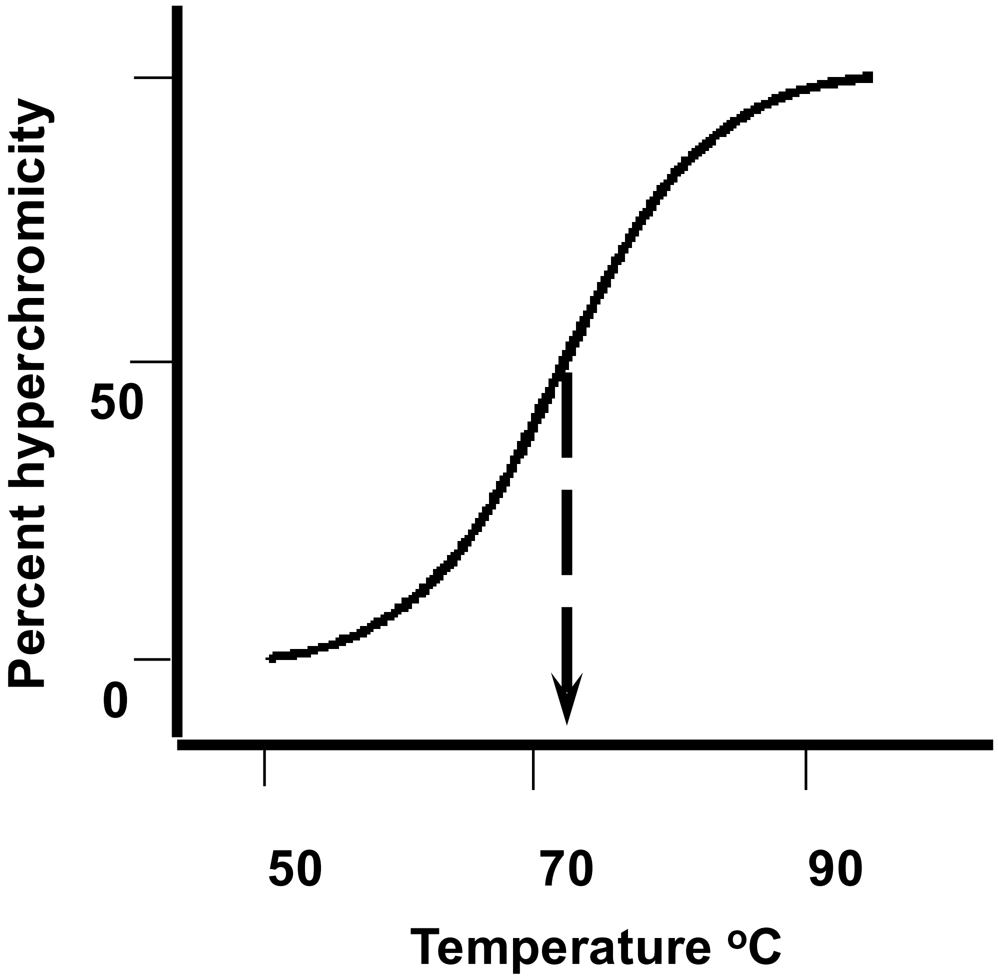
Watson et al., BIOLOGIA MOLECOLARE DEL GENE, 6/E, Zanichelli editore S.p.A. Copyright © 2009

Stabilità termodinamica del duplex di DNA e sua denaturazione

Fattori intrinseci: composizione in basi, peso molecolare.

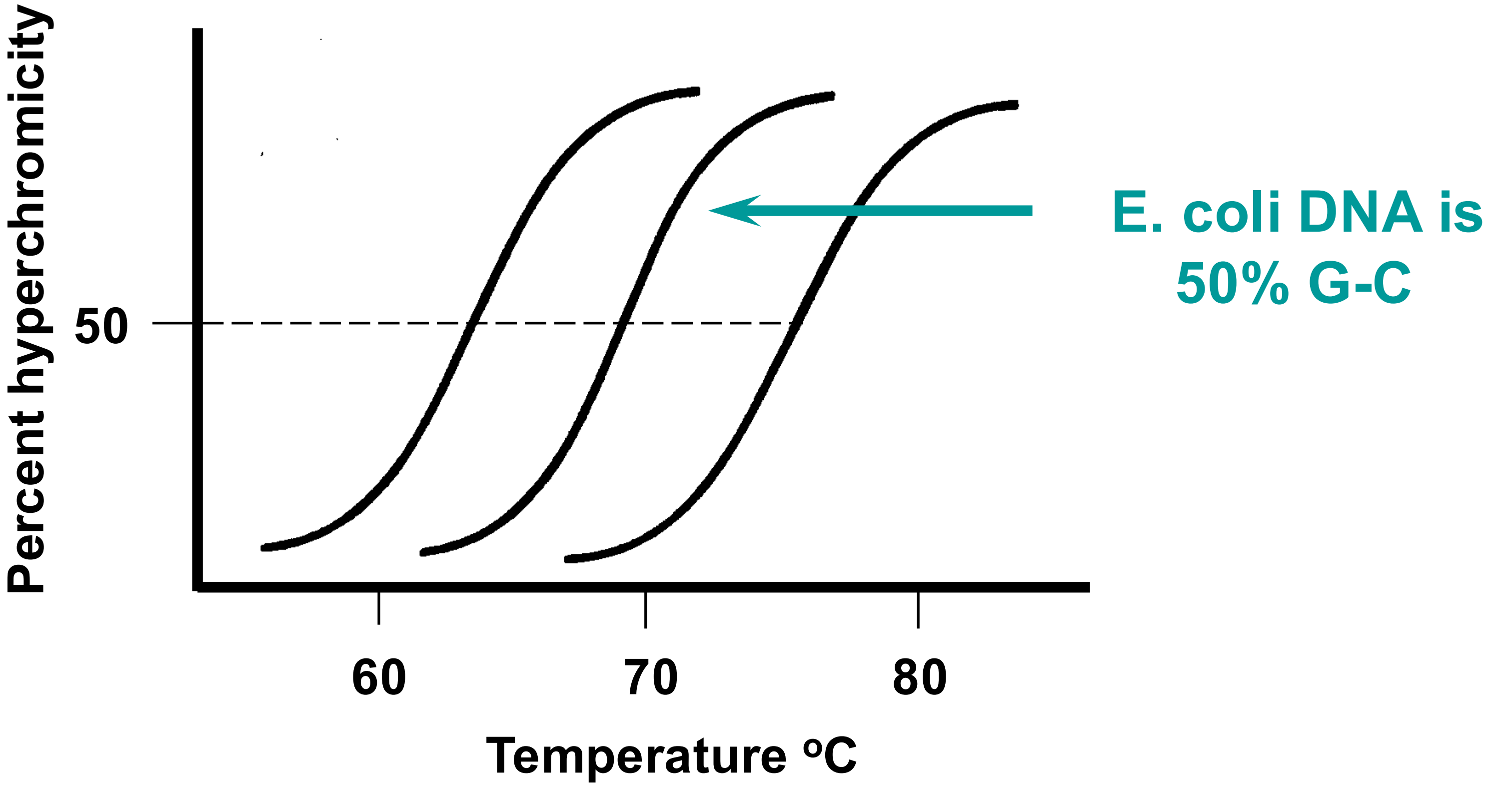
Fattori estrinseci: temperatura, pH, forza ionica.

DNA melting curve



- T_m is the temperature at the midpoint of the transition

T_m is dependent on the G-C content of the DNA



Average base composition (G-C content) can be determined from the melting temperature of DNA

DNA REPLICATION

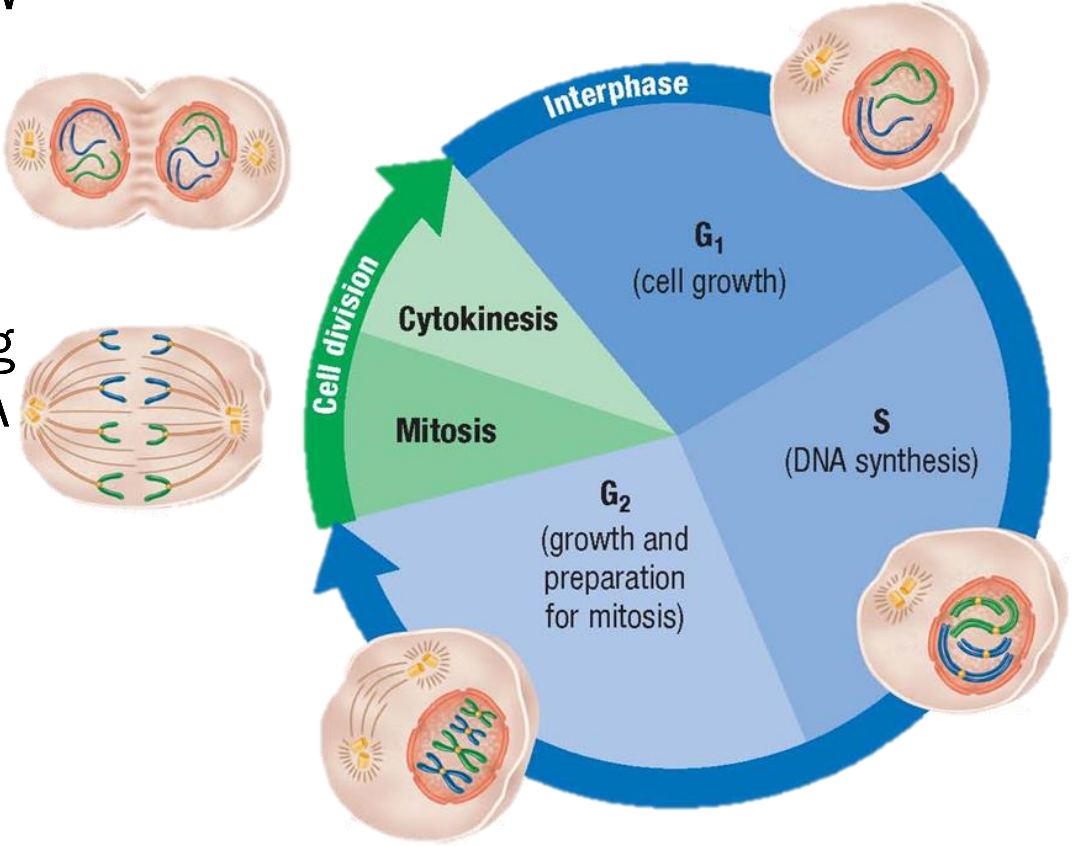
Objectives

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

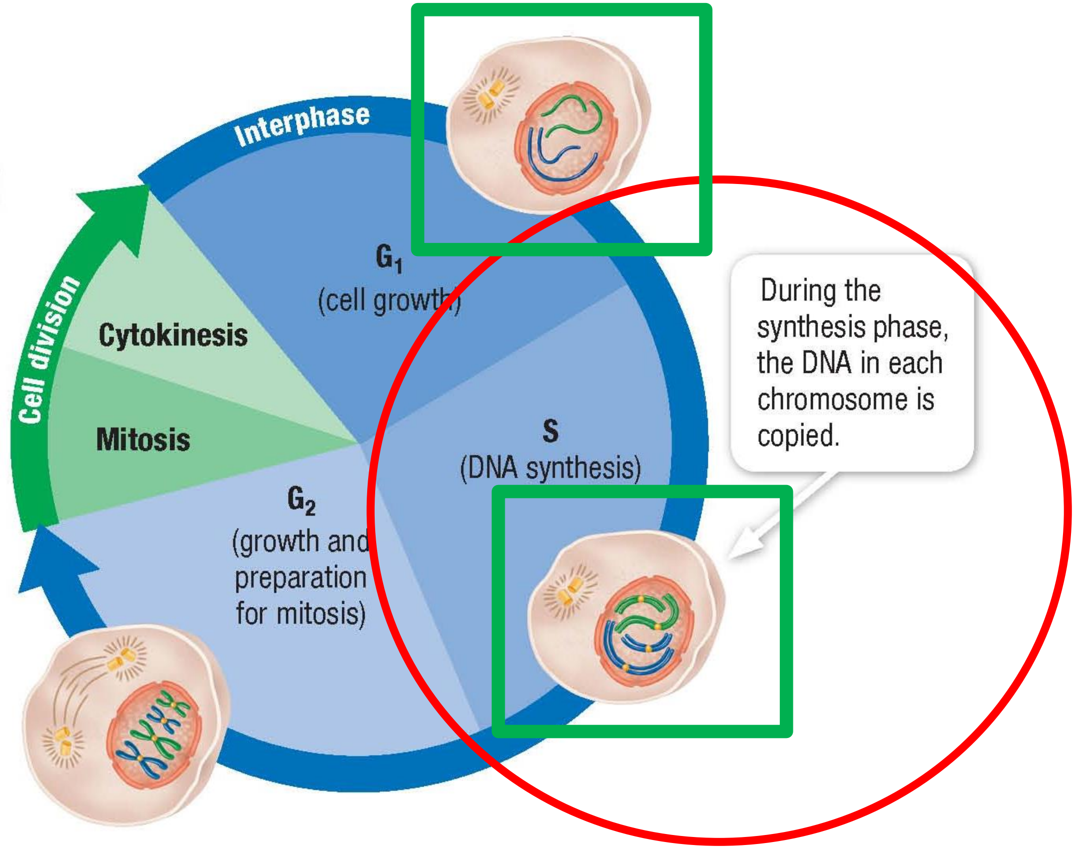
- Describe the steps of DNA replication.
- Compare the roles of DNA helicase, DNA polymerase, and ligase
- Compare the process of DNA replication in prokaryotes and in eukaryotes
- Applications of our knowledge

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 explore the process of DNA synthesis, called DNA replication.
- This process is extremely precise and an incredibly important.

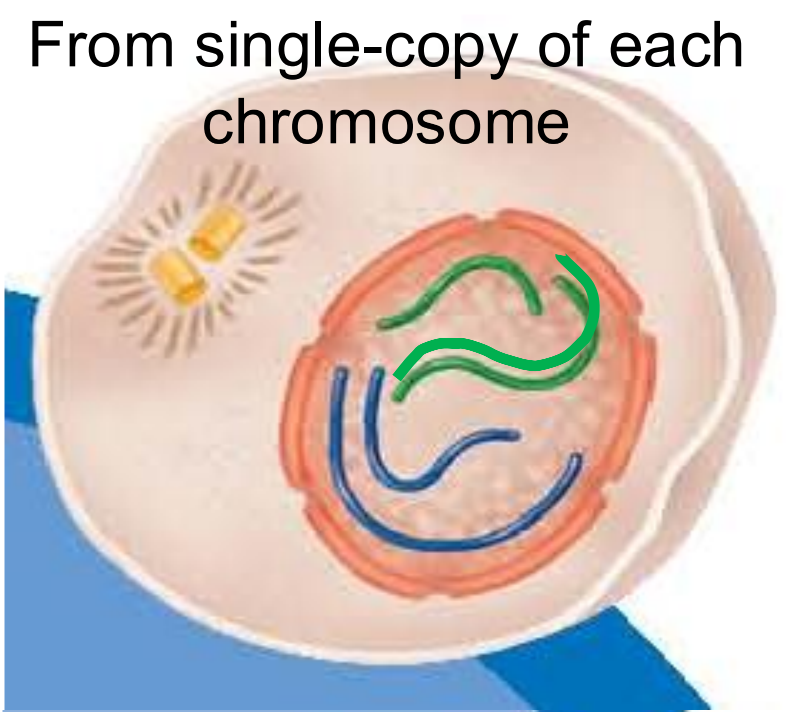


THE CELL CYCLE: THE PART WE'RE ISOLATING.



During the synthesis phase, the DNA in each chromosome is copied.

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., Gerrard, H., 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).

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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).

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[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[1-3], 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 (cf. 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.

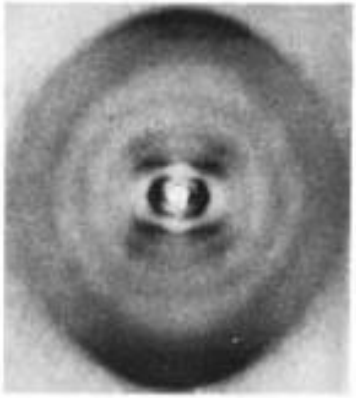

Fig. 1. Fibre diagram of deoxypentose nucleic acid from B. col
Fibre axis vertical

Diffraction by Helices

It may be shown[5] (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 the innermost maxima of each Bessel function a 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_0^2) on the *n*th layer line. The helical configurati produces side-bands on this fundamental frequenc the effect[5] 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 th nucleotide consists of a unit having circular symmet about an axis parallel to the helix axis, the who 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 th helix axis, the phases of radiation scattered by th helices of different diameter passing through eac point are the same. Summation of the correspondin Bessel functions gives reinforcement for the inne

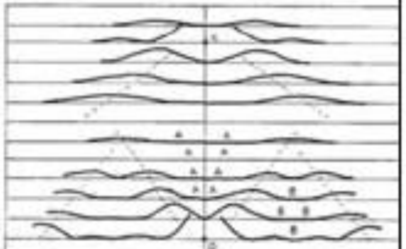

Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 Å. diameter and remainder distributed 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 Å.

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

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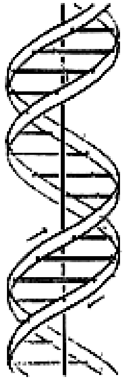

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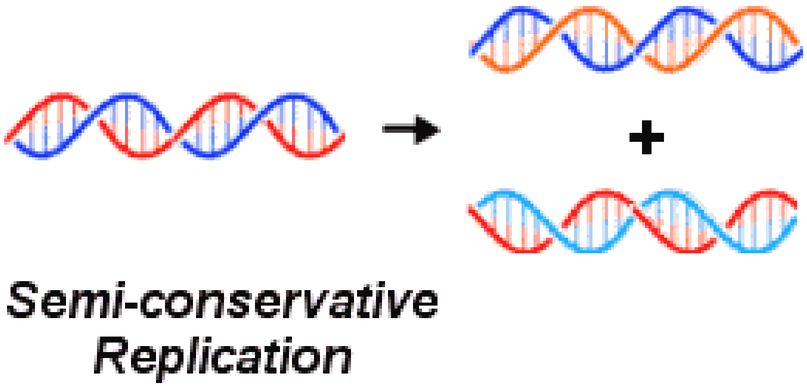
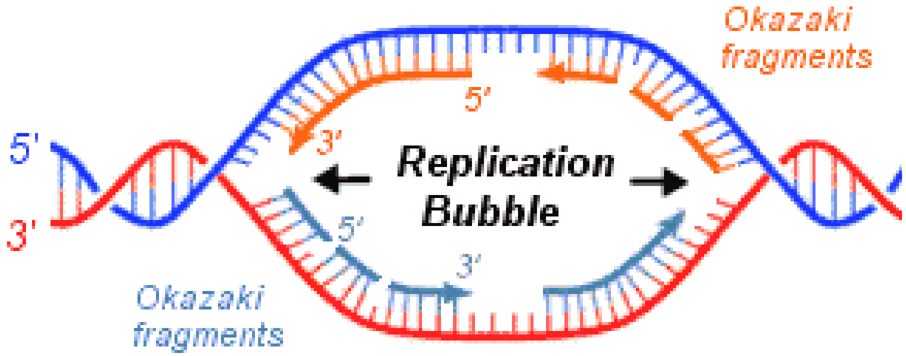
Definitions

<u>Origin</u>: chromosonal area where the double helix is denatured, exposing strands for production of new DNA molecules.

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





(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.

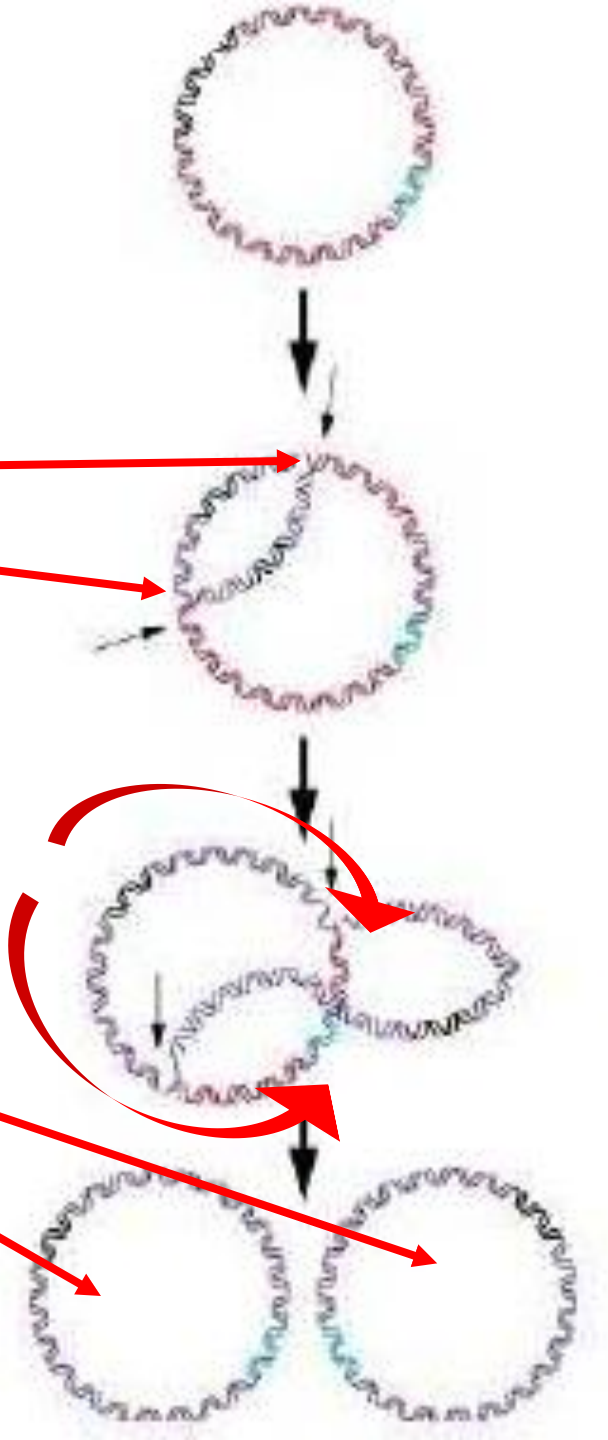
- Bacteria replicate their small genomes in minutes…

- 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*.

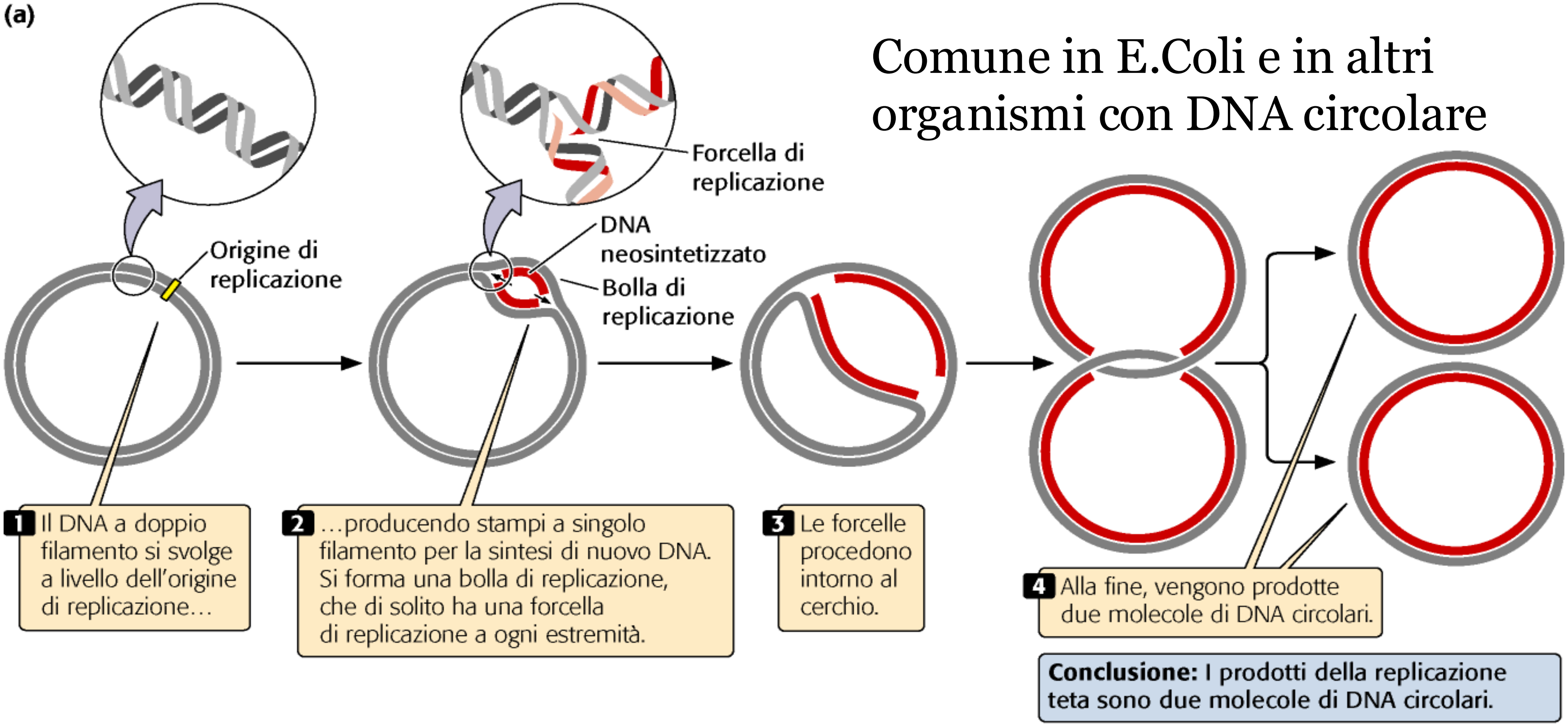
Prokaryotic Replication



- 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. Replicazion teta

Comune in E.Coli e in altri organismi con DNA circolare



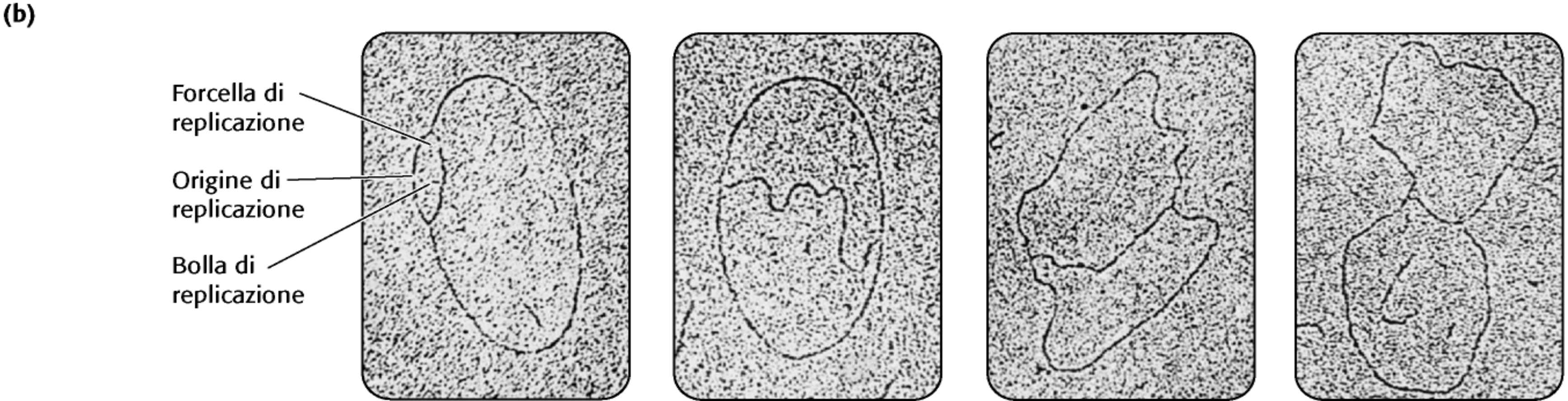
1 Il DNA a doppio filamento si svolge a livello dell'origine di replicazione…

2 …producendo stampi a singolo filamento per la sintesi di nuovo DNA. Si forma una bolla di replicazione, che di solito ha una forcella di replicazione a ogni estremità.

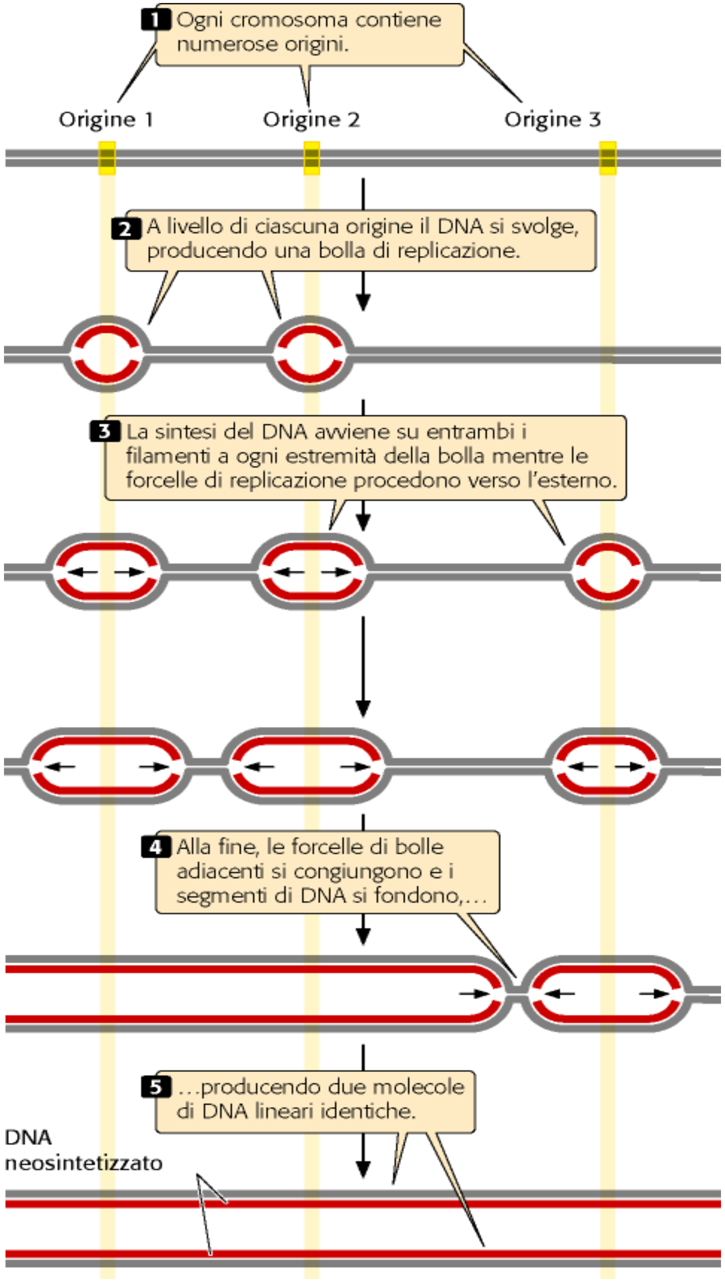
3 Le forcelle procedono intorno al cerchio.

4 Alla fine, vengono prodotte due molecole di DNA circolari.

Conclusione: I prodotti della replicazione teta sono due molecole di DNA circolari.



2. Linear replication (eukaryotes)



1 Ogni cromosoma contiene numerose origini.

2 A livello di ciascuna origine il DNA si svolge, producendo una bolla di replicazione.

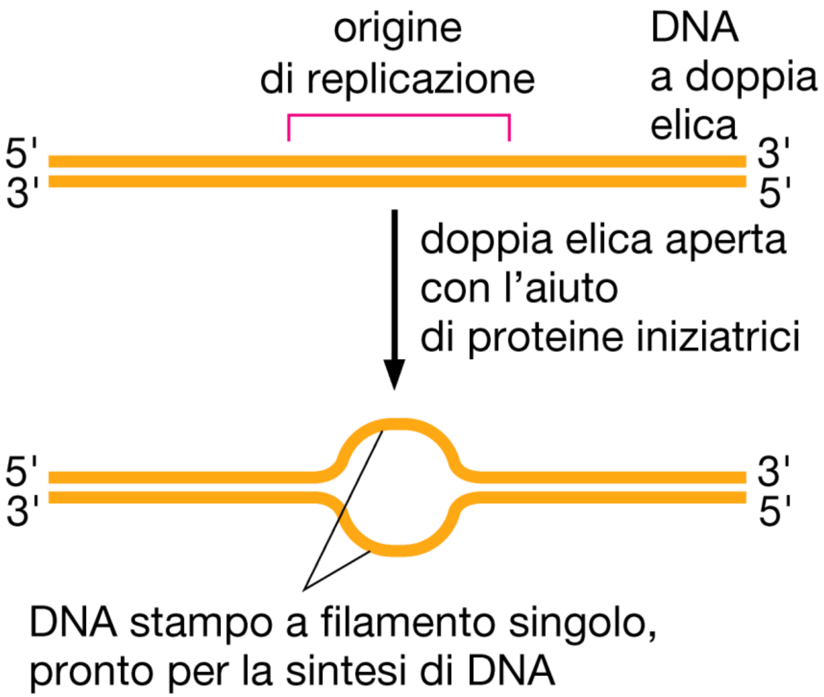
3 La sintesi del DNA avviene su entrambi i filamenti a ogni estremità della bolla mentre le forcelle di replicazione procedono verso l'esterno.

4 Alla fine, le forcelle di bolle adiacenti si congiungono e i segmenti di DNA si fondono,…

5 …producendo due molecole di DNA lineari identiche.

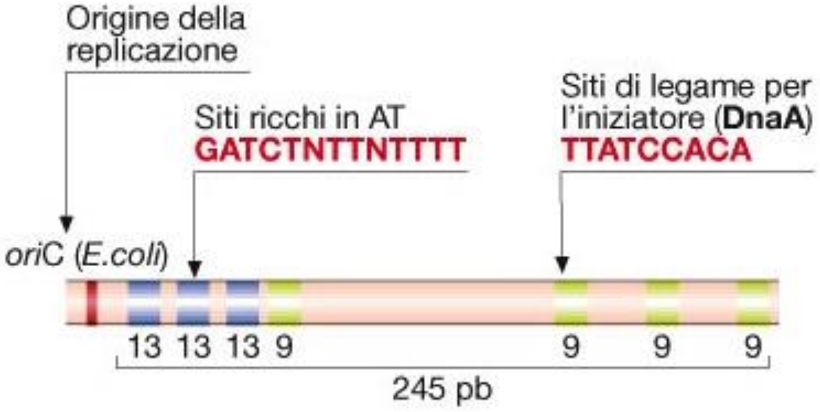
Conclusione: I prodotti della replicazione del DNA negli eucarioti sono due molecole di DNA lineari.

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



doppia elica aperta con l'aiuto di proteine iniziatrici

DNA stampo a filamento singolo, pronto per la sintesi di DNA

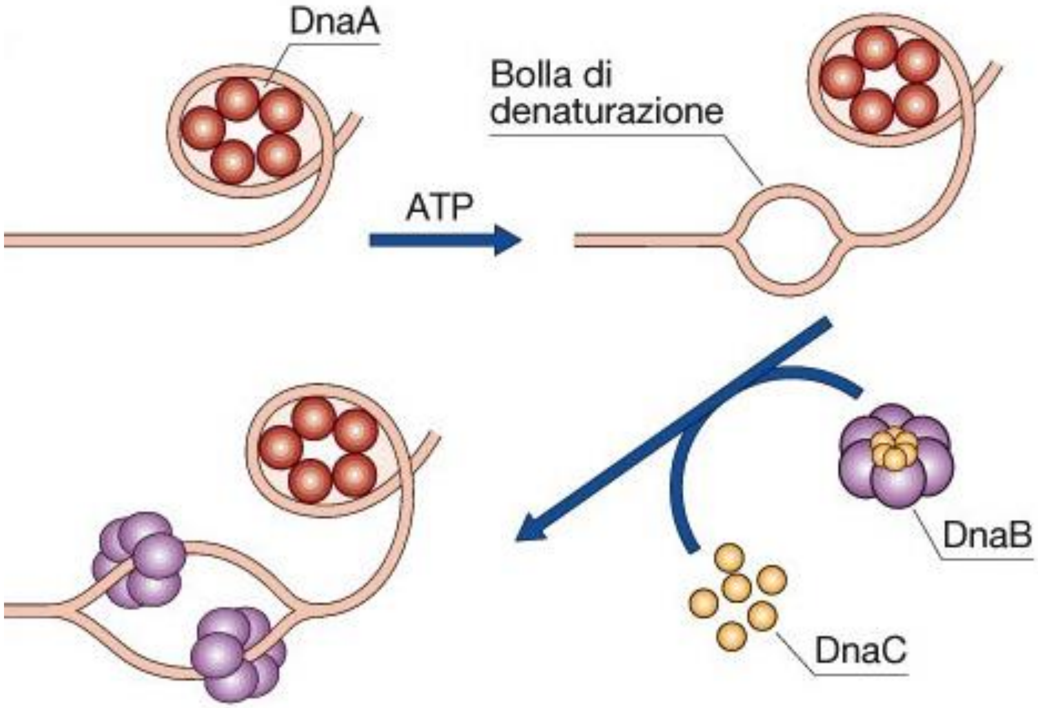


L'origine minima di E.Coli e' lunga solo 245 bps.
E' una regione ricca di A e T: contiene 4 ripetizioni di 9 pb e 3 di 13 pb molto simili tra loro.
Le corte sequenze ripetute sono riconosciute da proteine specifiche nelle fasi iniziali della replicazione.

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.



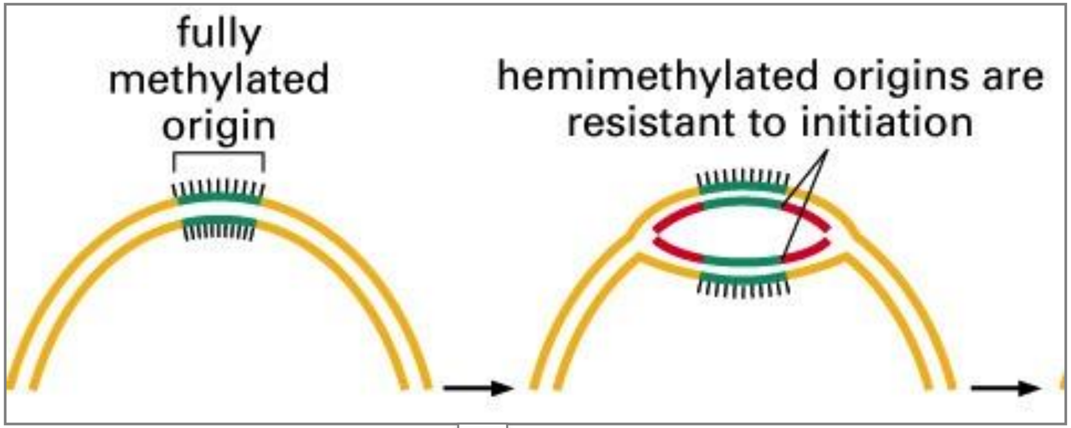
Inizio della replicazione

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!

Inizio della replicazione

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

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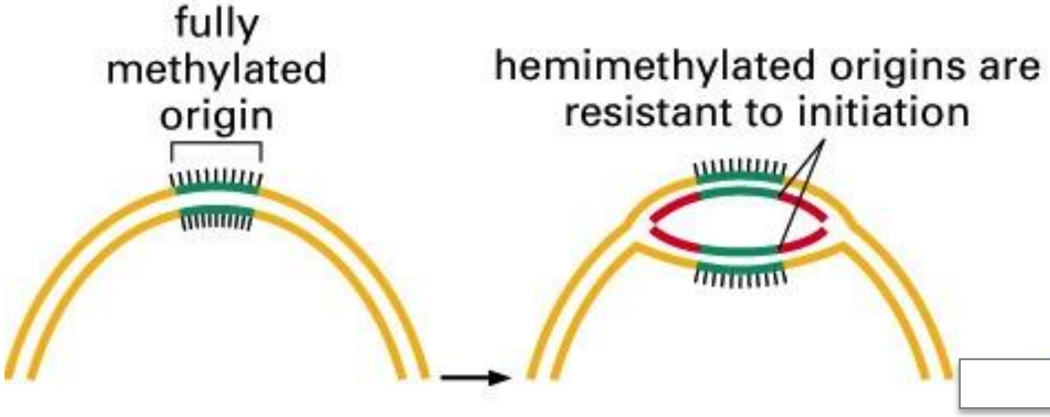
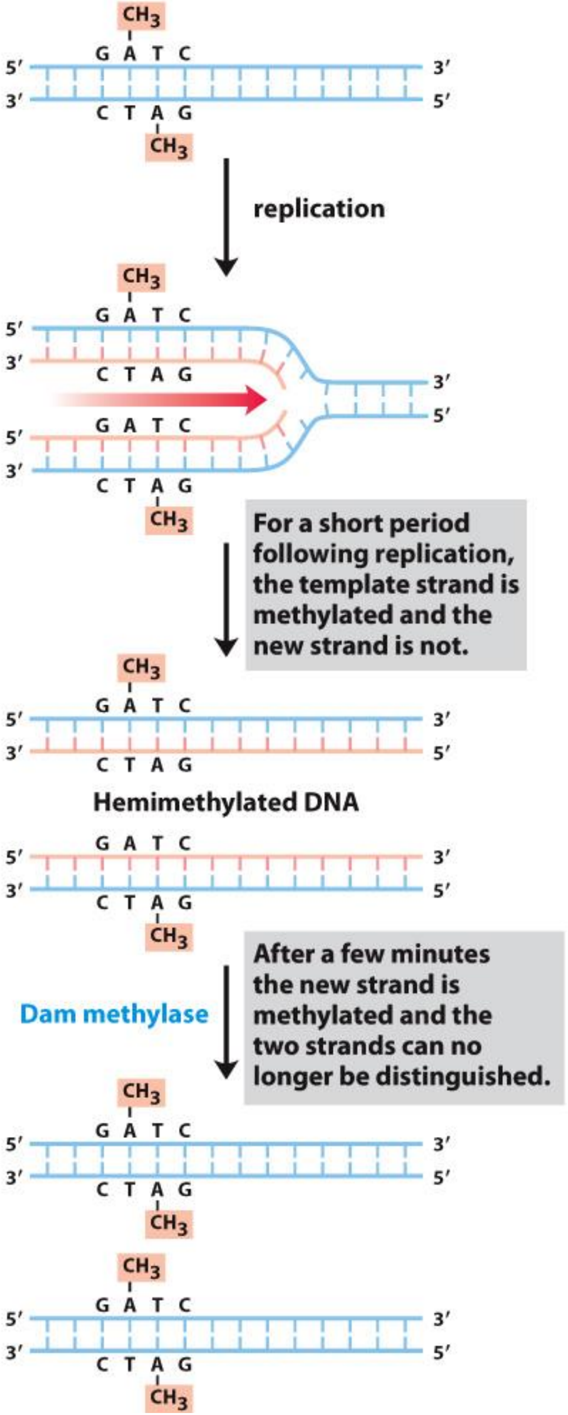


Nello stato emi-metilato (transitorio!) non può funzionare da origine di replicazione, non viene usata 2 volte!

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.

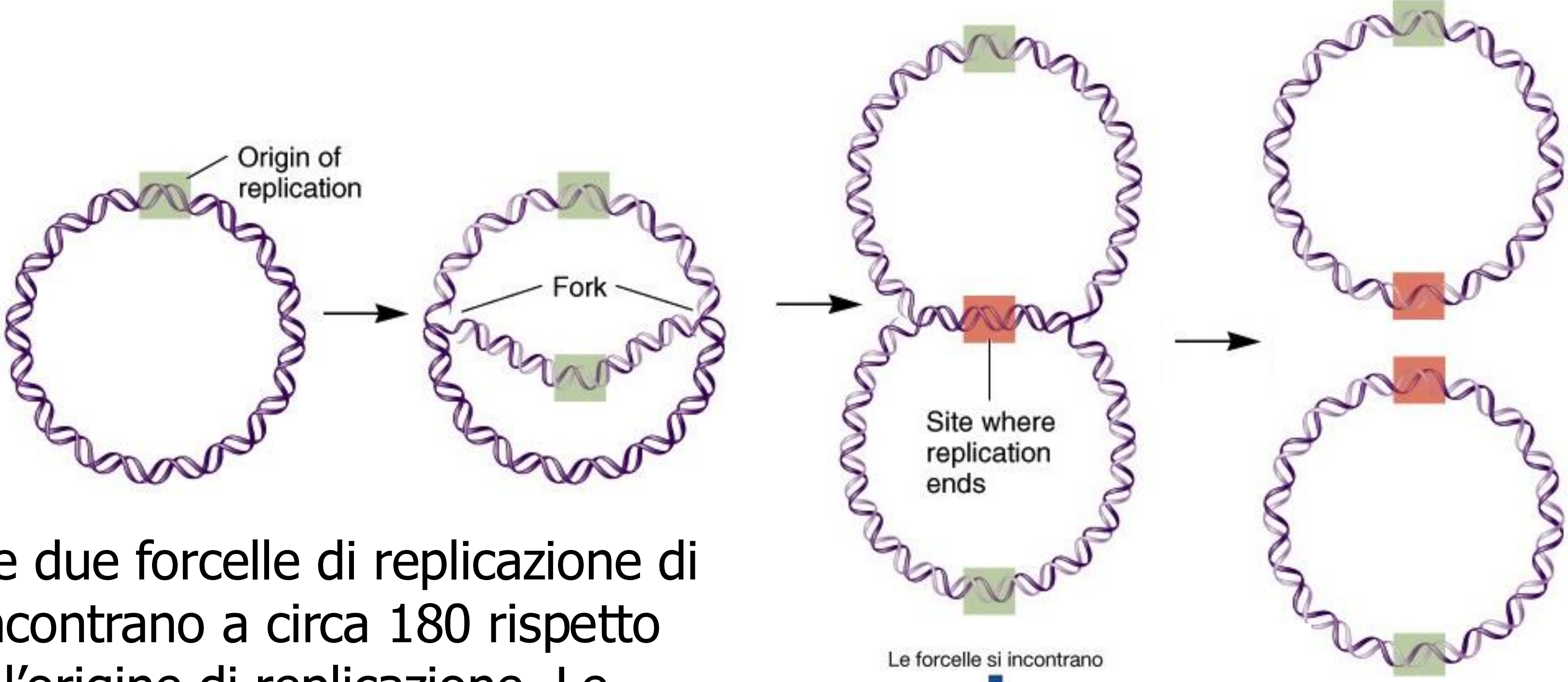
ONLY DNA METHYLATED ON BOTH STRANDS CAN BE DUPLICATED



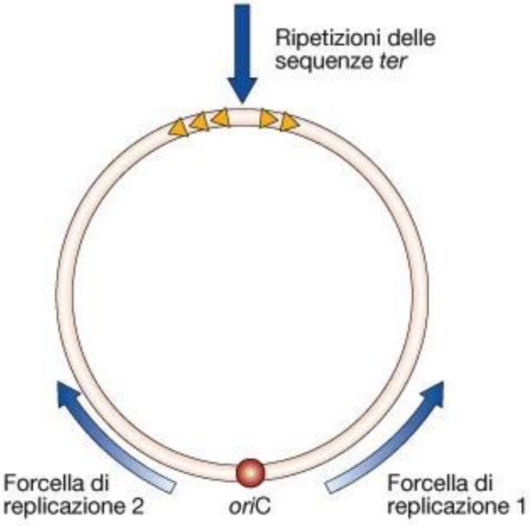
For a short period following replication, the template strand is methylated and the new strand is not.

After a few minutes the new strand is methylated and the two strands can no longer be distinguished.

Terminazione della replicazione.

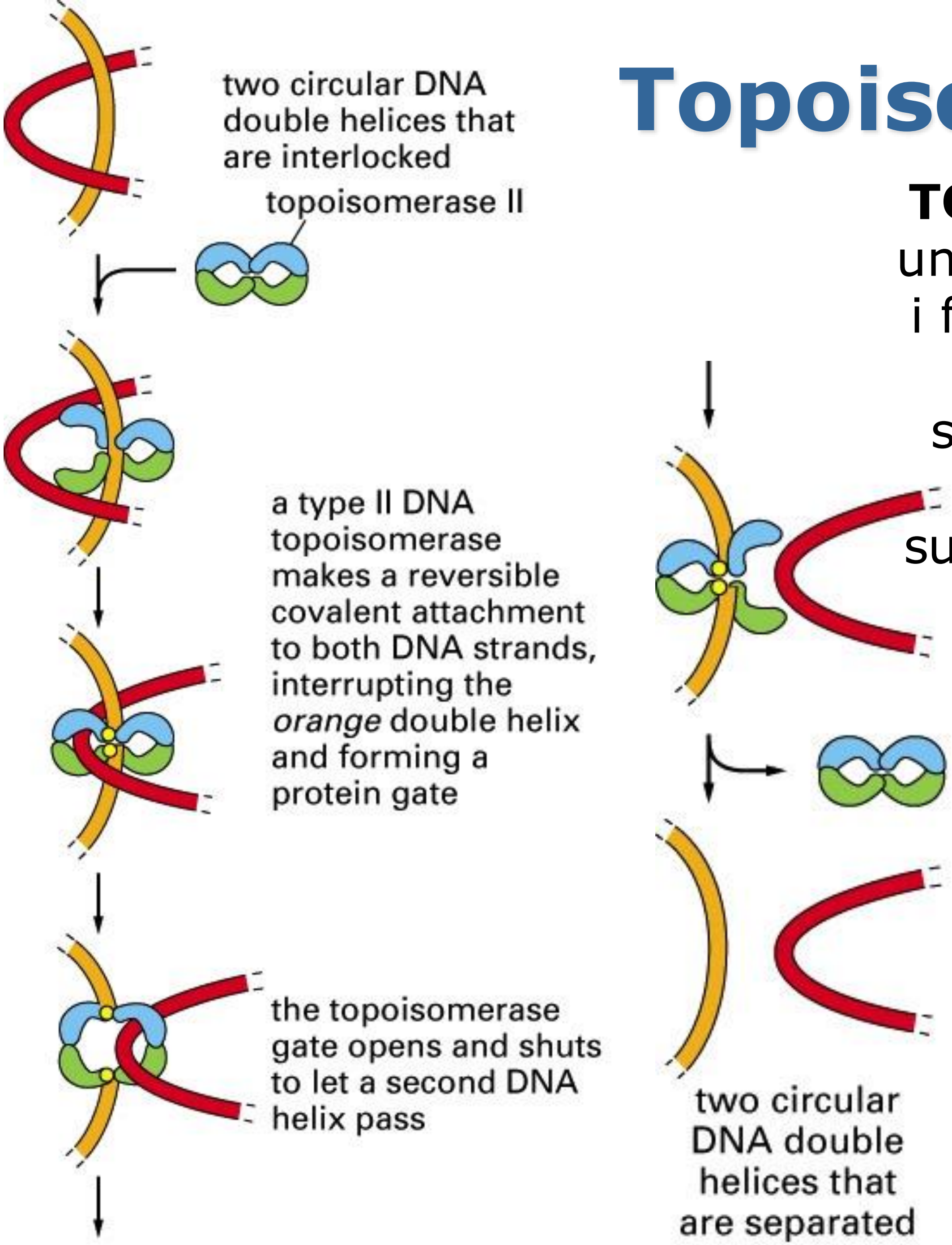


Le due forcelle di replicazione di incontrano a circa 180 rispetto all’origine di replicazione. Le sequenze ***ter***, di 23 bp, vengono legate dalle **TBP** (Ter binding proteins) e fanno terminare le forcelle di replicazione.



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!

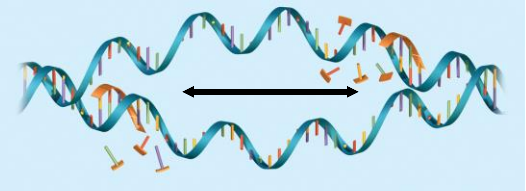
DNA Replication: THE STEPS

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

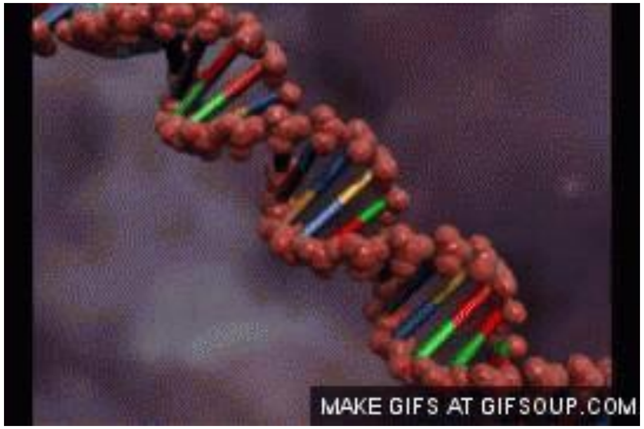
STEP1

DNA helicases unwinds & separates the original DNA double helix.

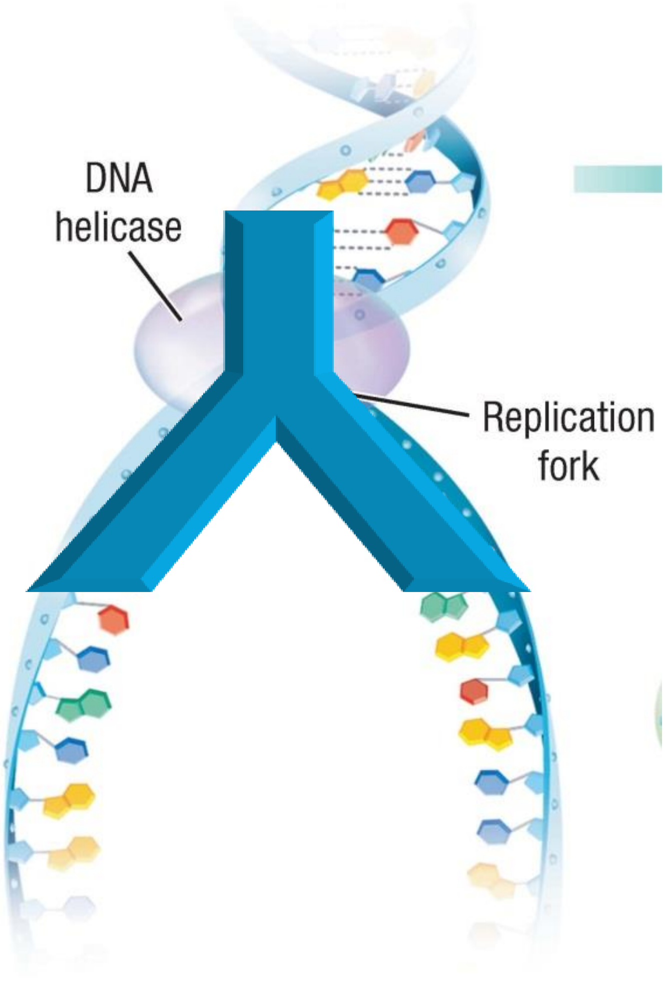
These proteins wedge themselves between the two strands of the double helix and break the hydrogen bonds between the base pairs.

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.

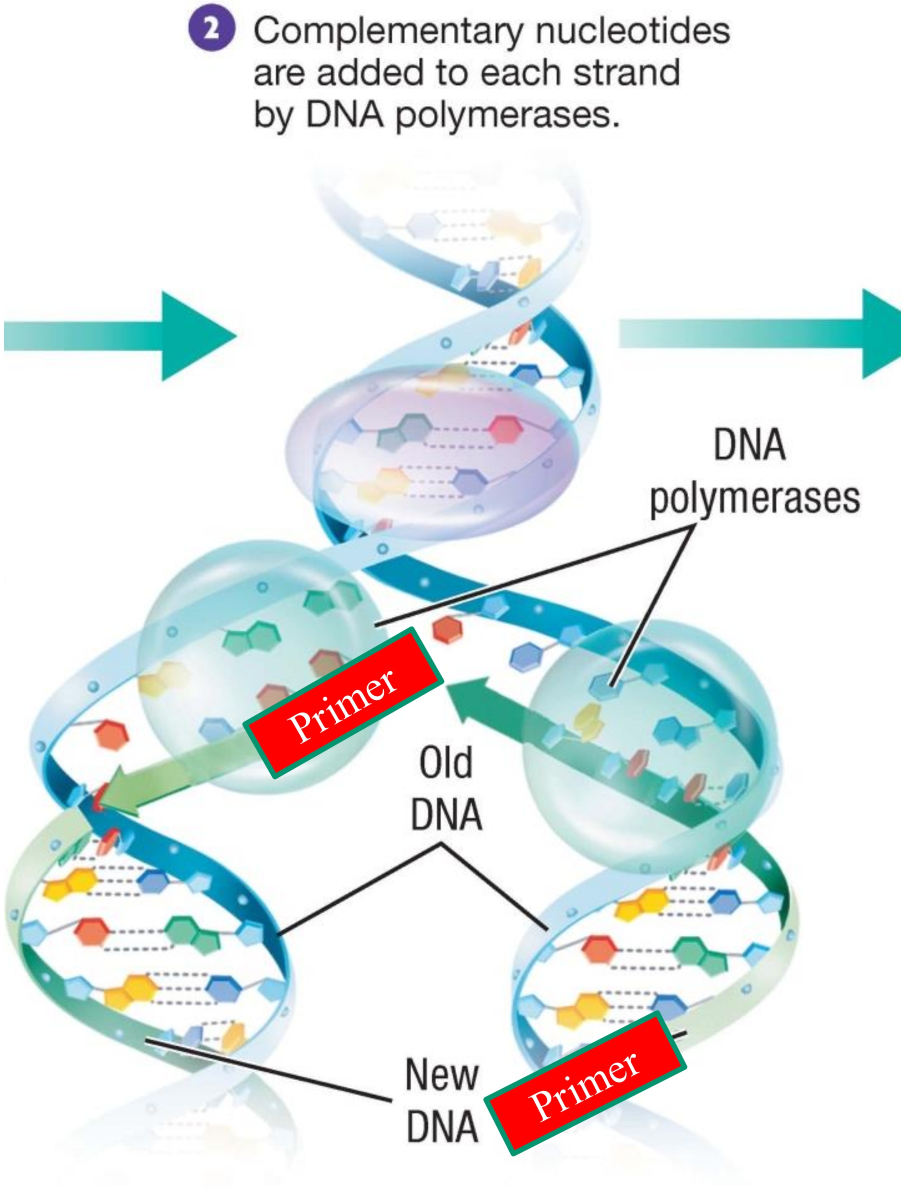


STEP2

New DNA is formed from DNA template.

RNA Primers attach to specific regions.

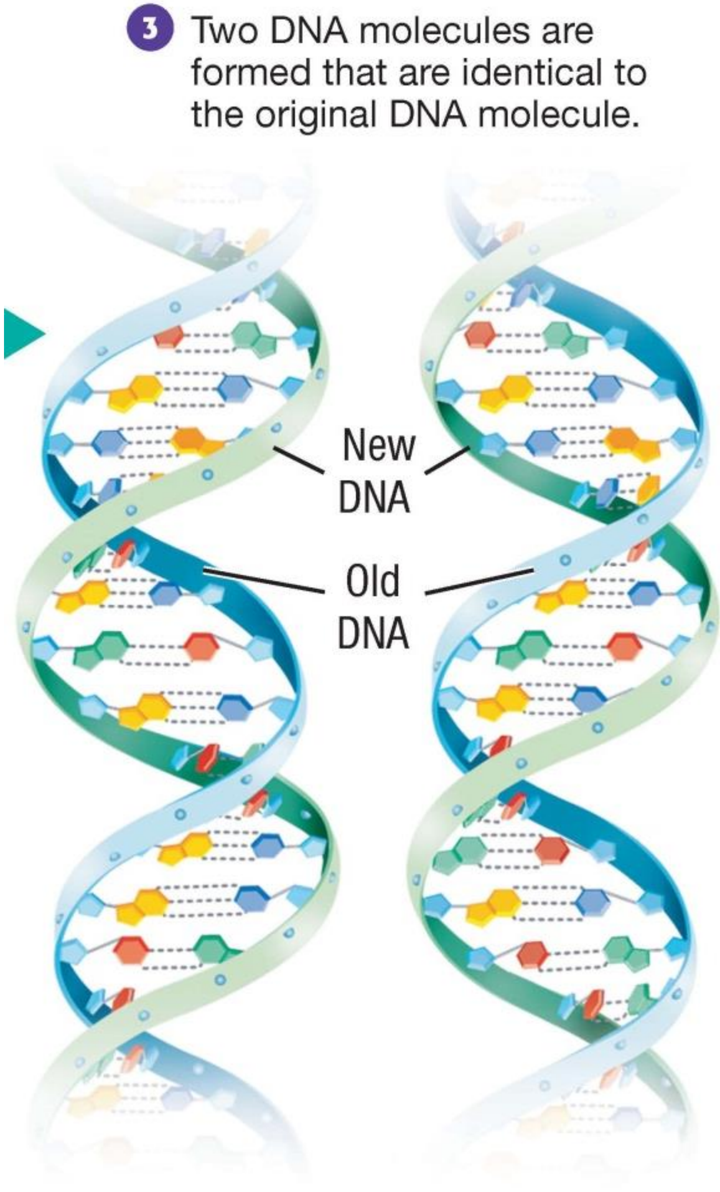
New nucleotides are added to the primer by the enzyme DNA Polymerase



STEP3

- The process completes when all the original bases have been paired with a new complementary nucleotide.

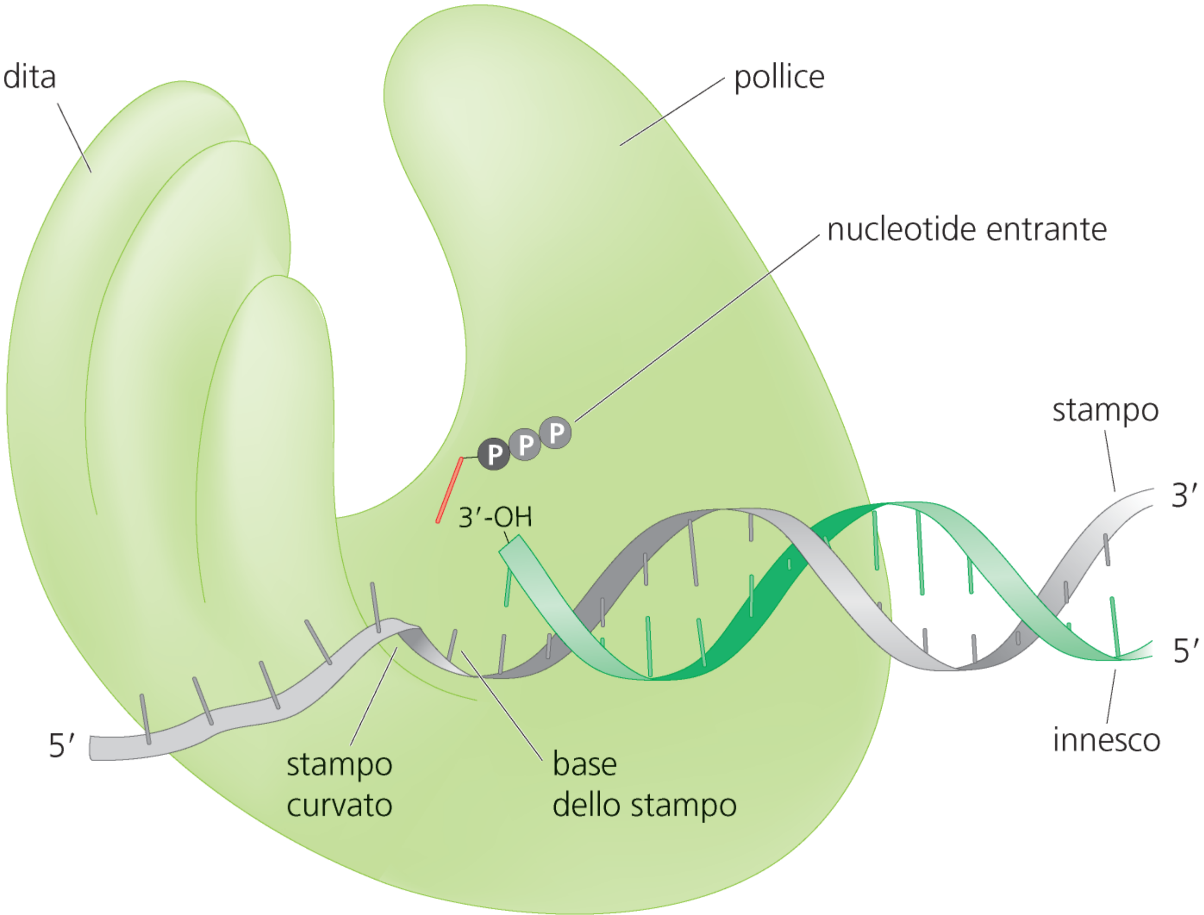
- Each double-stranded DNA helix is made of one new strand of DNA and one original strand of DNA.



Le DNA Polimerasi

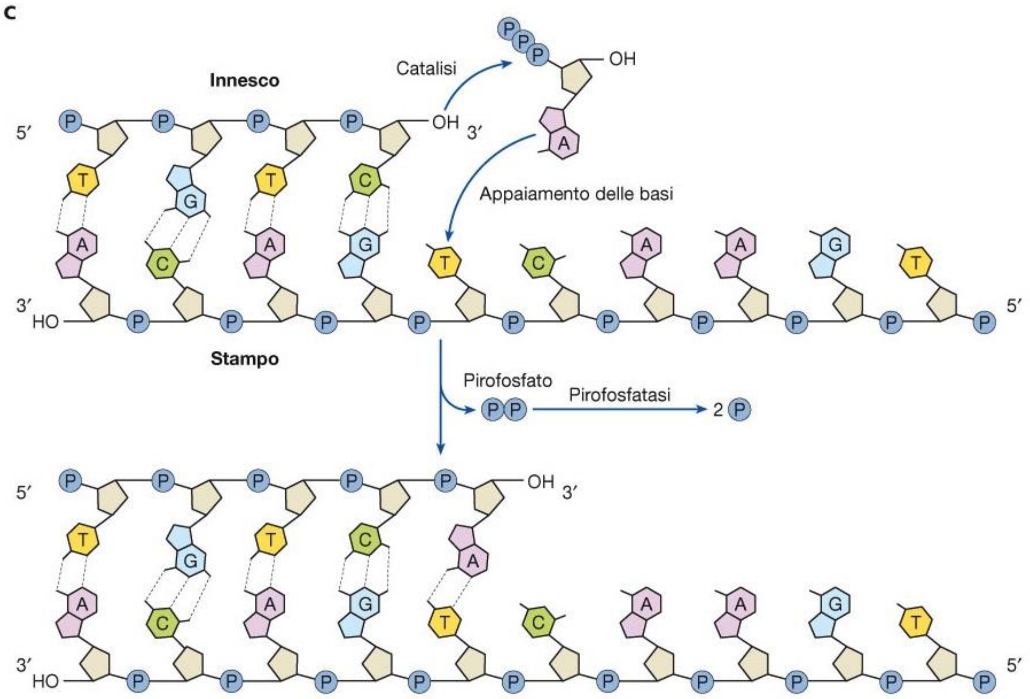
La DNA polimerasi e’ un complesso multimerico formato da diverse unita’ enzimatiche con diverse attivita’ catalitiche.

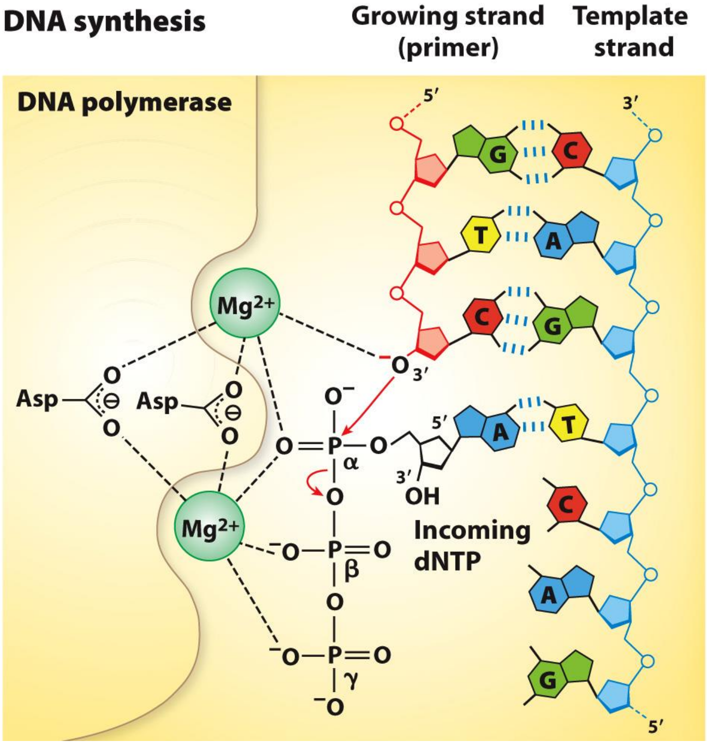
Tutte le DNA polimerasi condividono la stessa anatomia molecolare, paragonabile a quella di una mano destra. Il complesso “innesco-stampo” attraversa il “palmo”, dove viene controllata l’accuratezza dell’appaiamento tra le basi, in una scanalatura creata dalle “dita” e dal “pollice”.



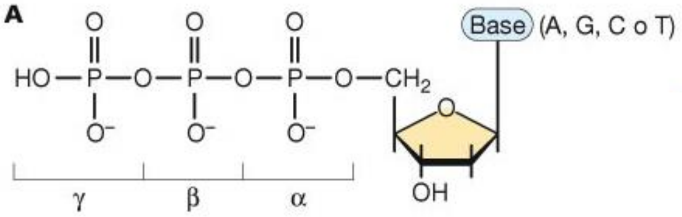
L'azione della DNA Polimerasi

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.

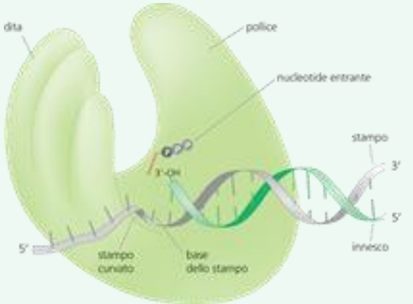




I nucleotidi entrano come nucleosidi trifosfati, apportando l'energia necessaria per la polimerizzazione, fornita dall'idrolisi di un legame fosfoanidride, con liberazione di pirofosfato. Il pirofosfato viene poi idrolizzato ulteriormente a fosfato inorganico, rendendo la reazione del tutto irreversibile.



Le DNA Polimerasi

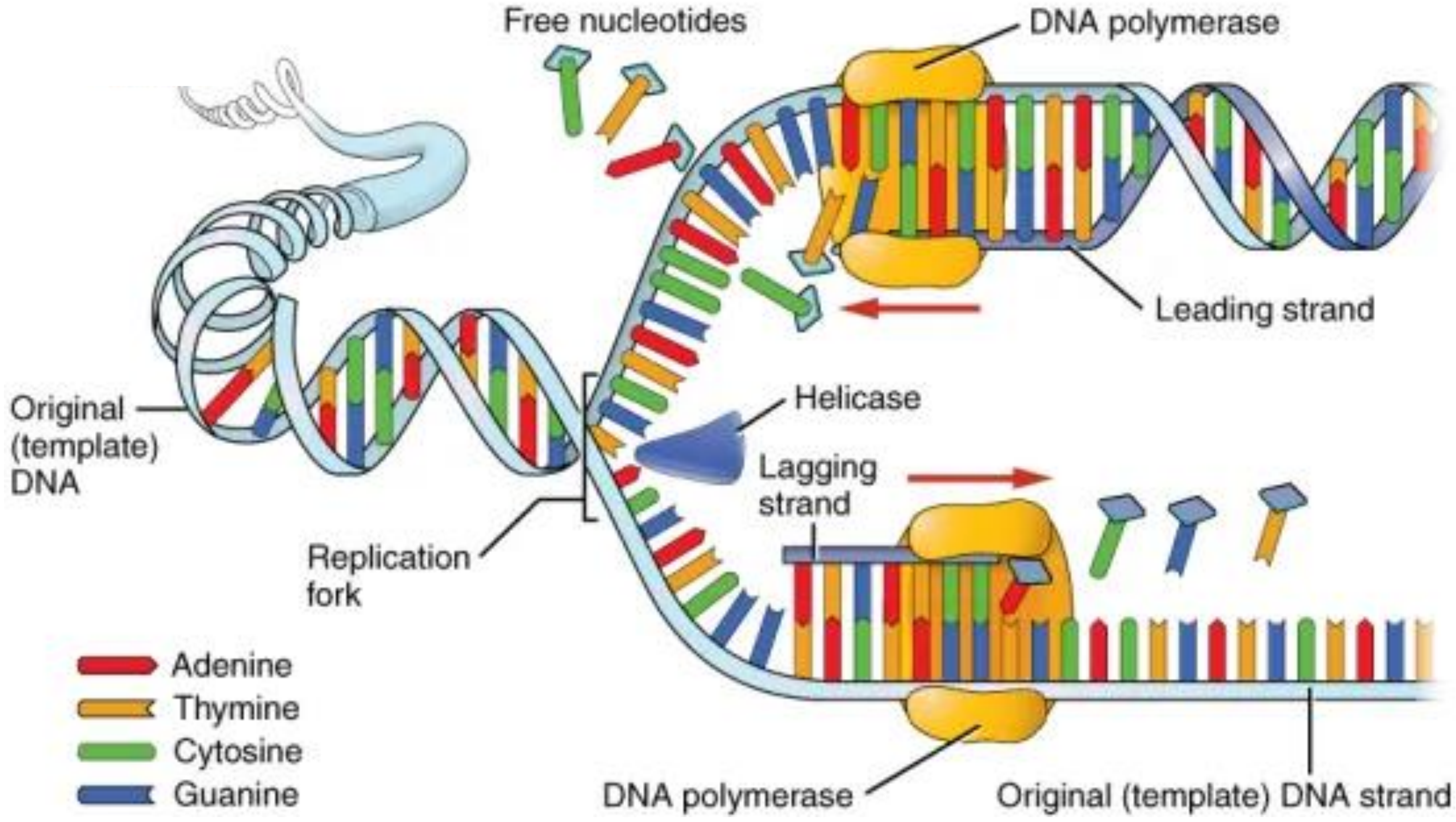


Le diverse isoforme di DNA polimerasi condividono le seguenti caratteristiche:

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.

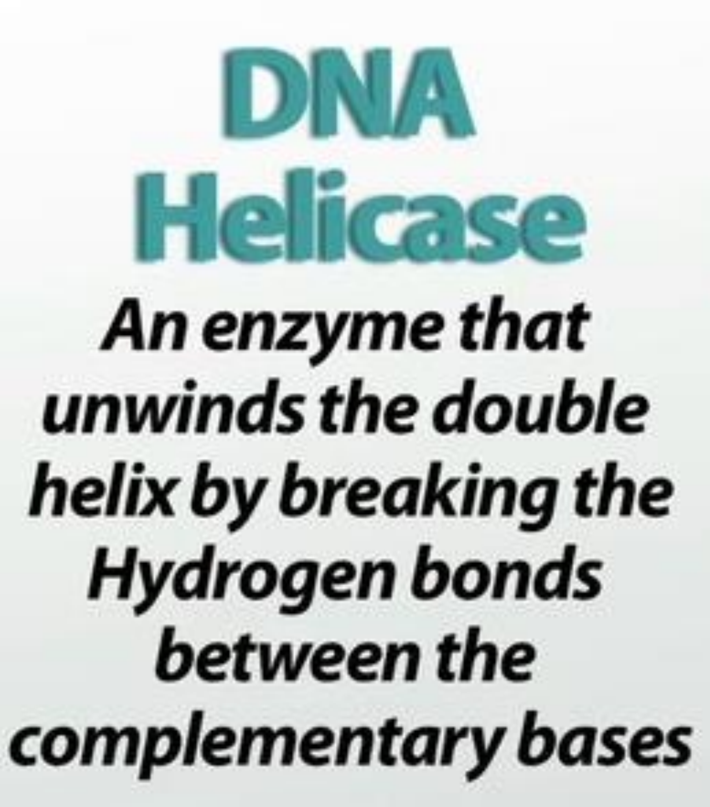
La Forcella di Replicazione

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

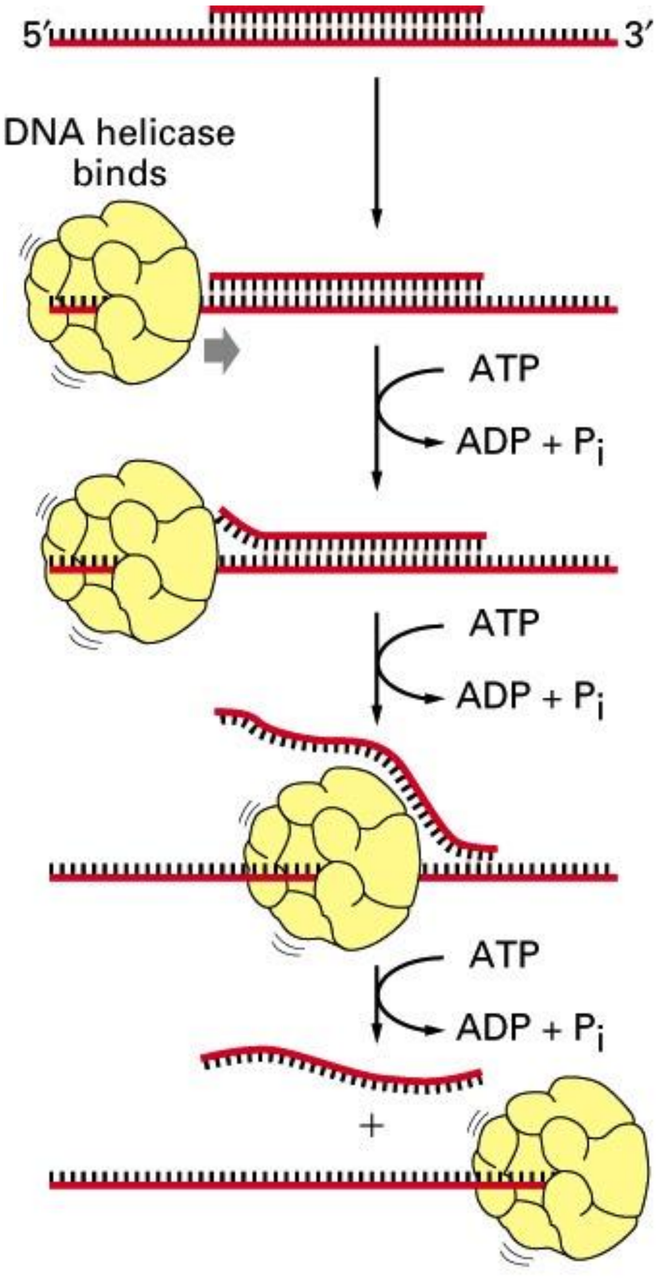


DNA Helicase

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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Tom Ellenberger,[†] and Dale B. Wigley[*‡]
[*]Sir William Dunn School of Pathology
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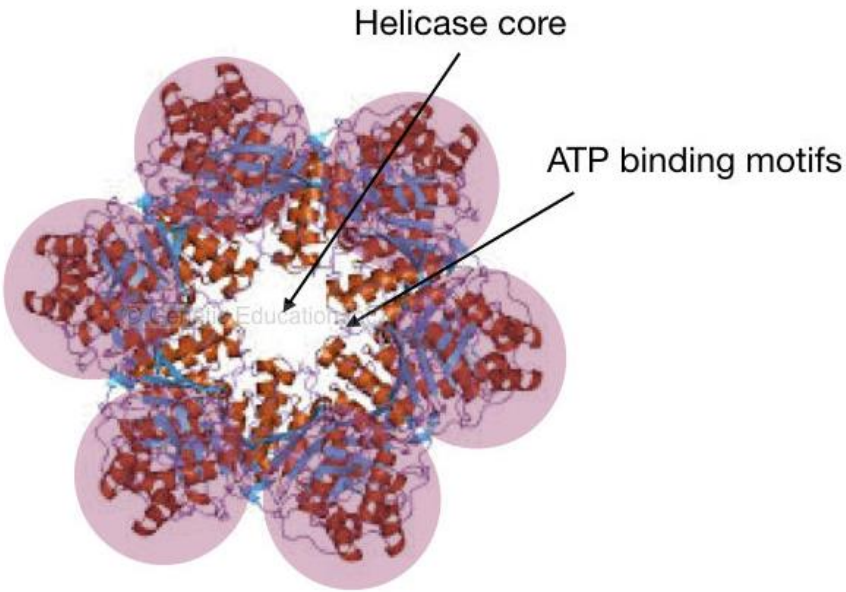
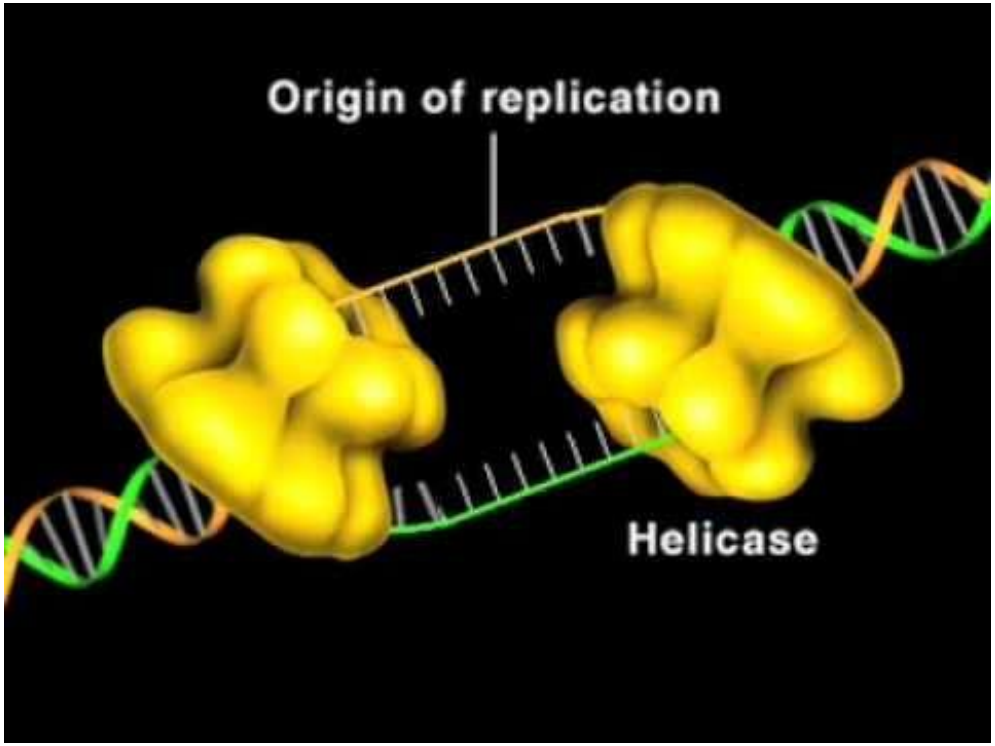
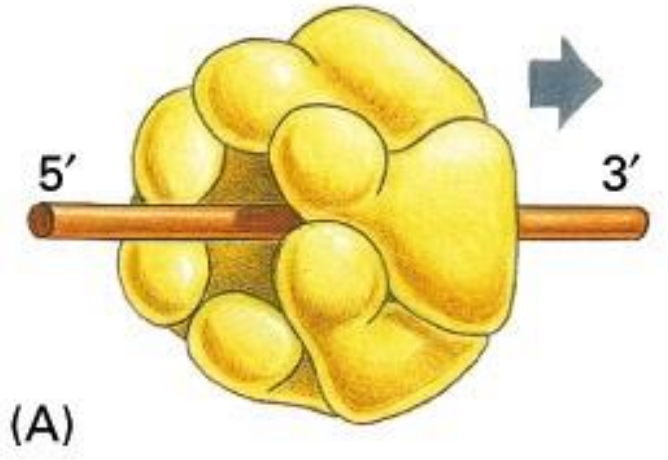




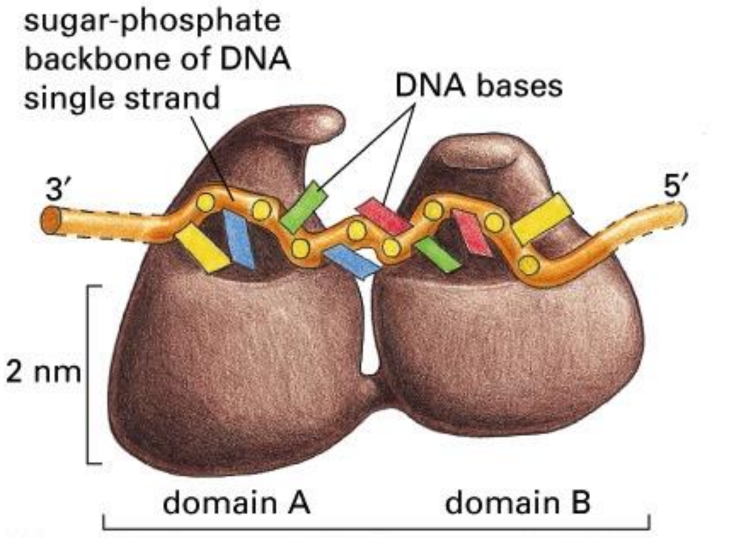
Hexameric structure of DNA helicase

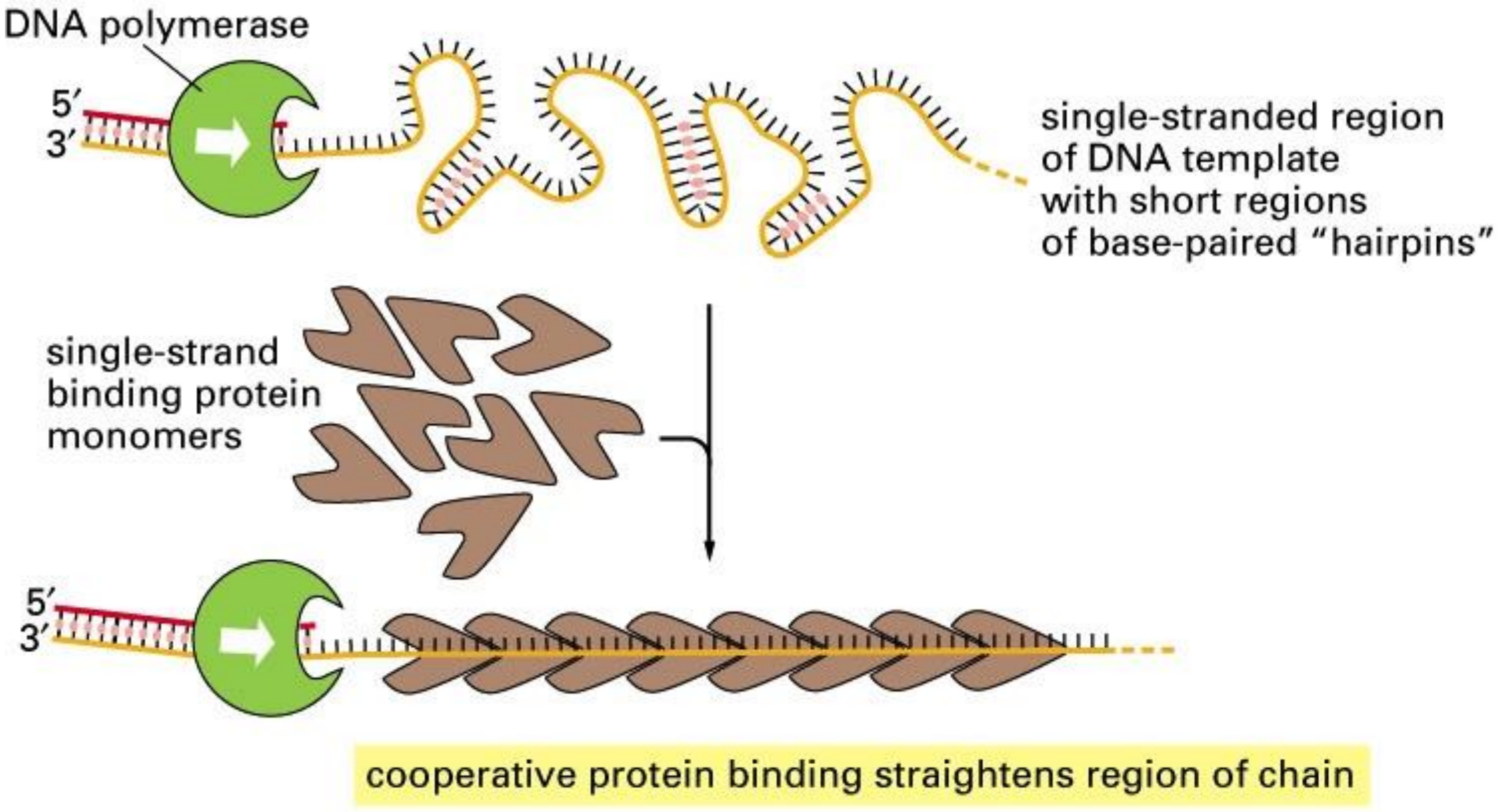


SSB proteins

SSB proteins bind tightly to ssDNA without covering the bases.

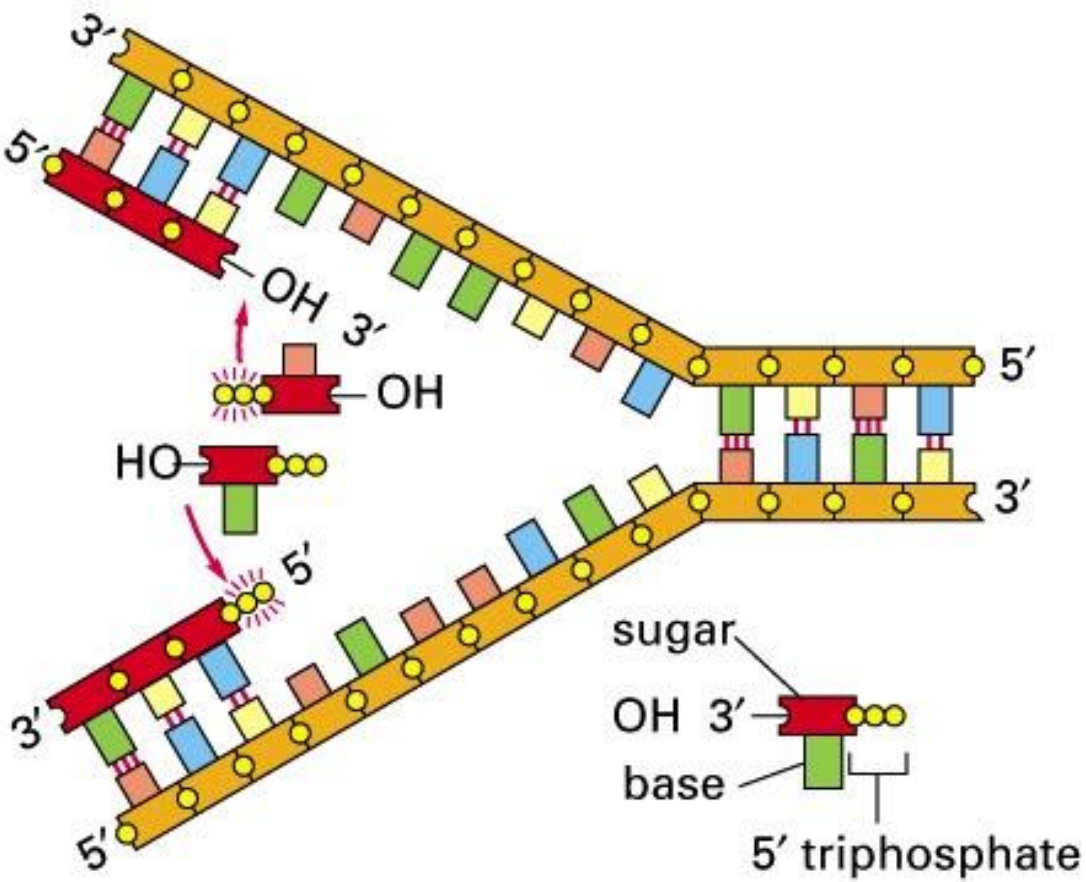
SSB proteins are unable to open a DNA helix but aid helicases by stabilizing the unwound ss conformation





La Forcella di Replicazione

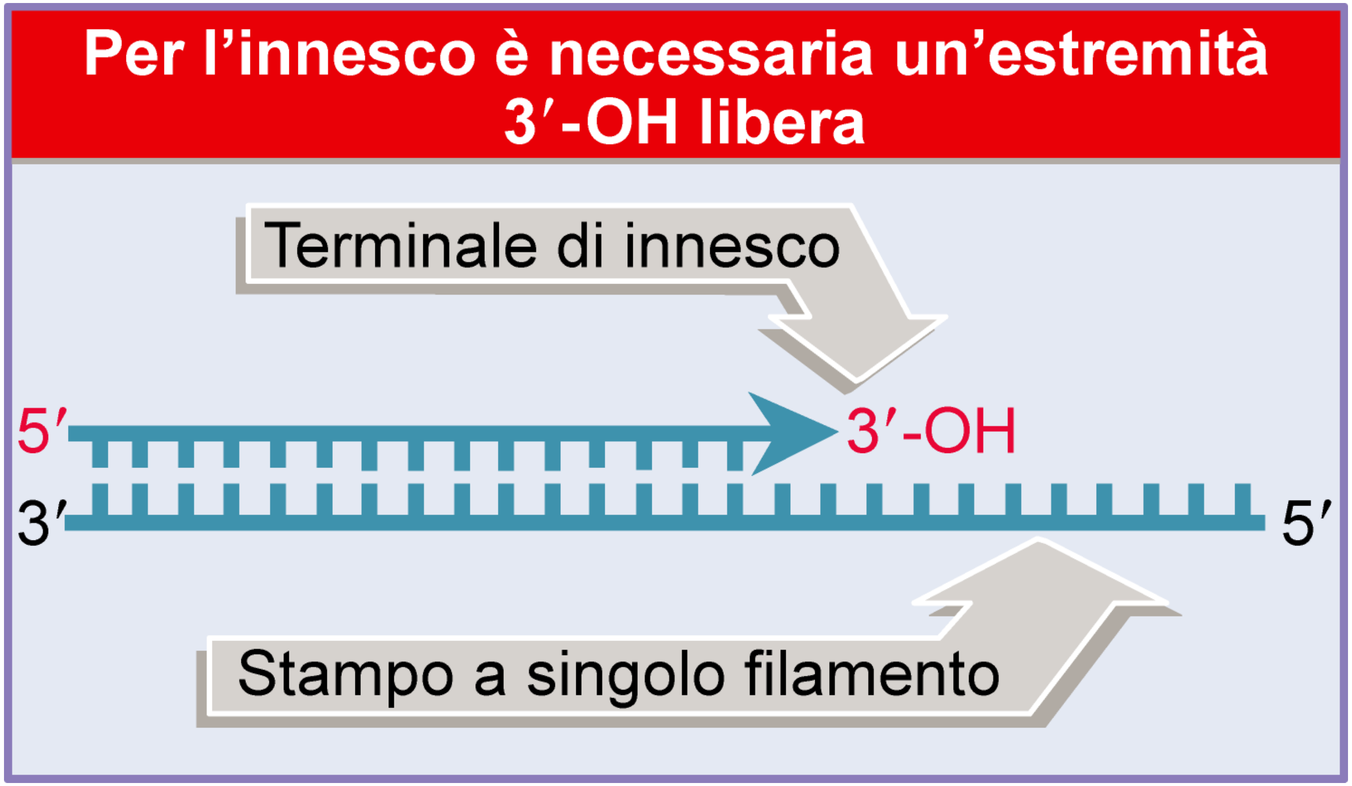
I singoli filamenti fungono da stampo per la sintesi di un nuovo filamento complementare.



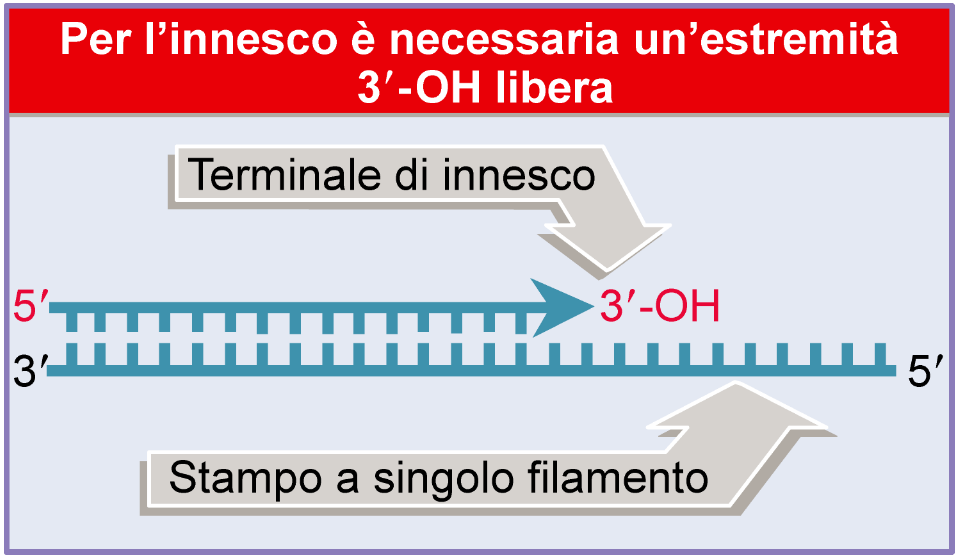
MA

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

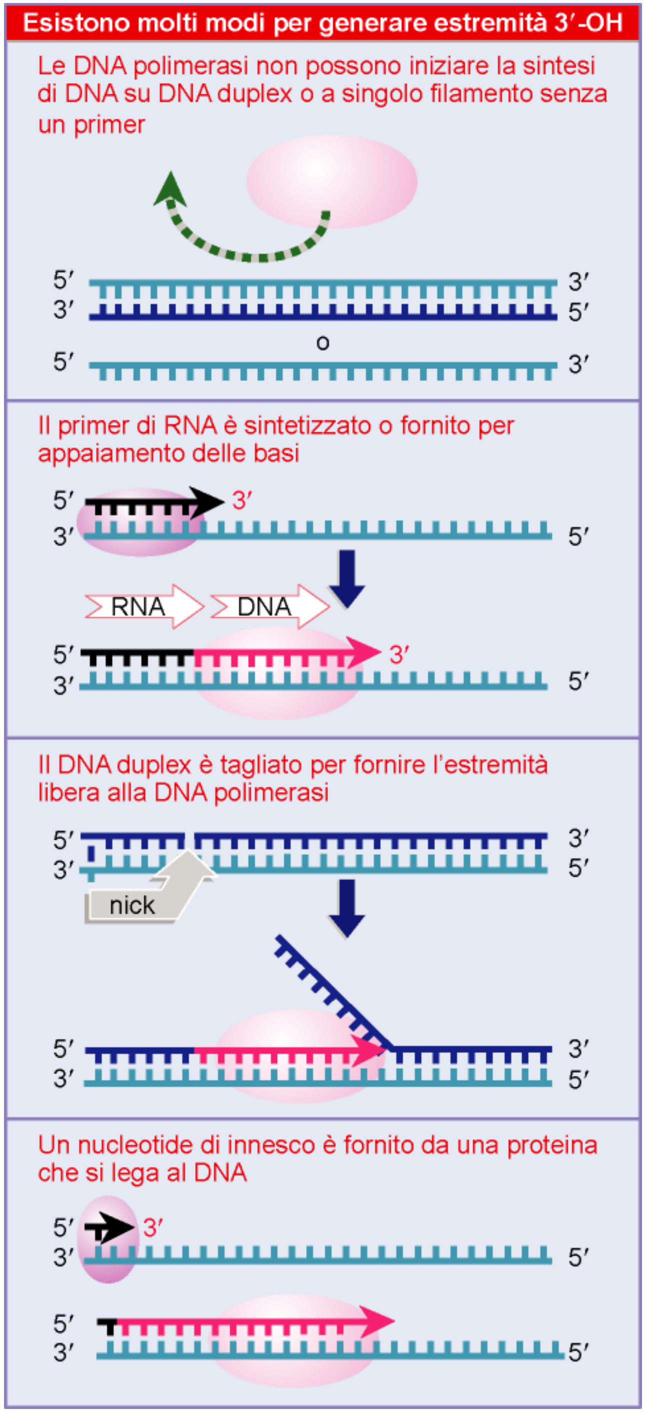
Le DNA Polimerasi richiedono un’estremita’ di innesco 3’-OH per iniziare la sintesi del DNA.


Per l’innesco è necessaria un’estremità 3′-OH libera

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



Per l’innesco è necessaria un’estremità 3′-OH libera



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

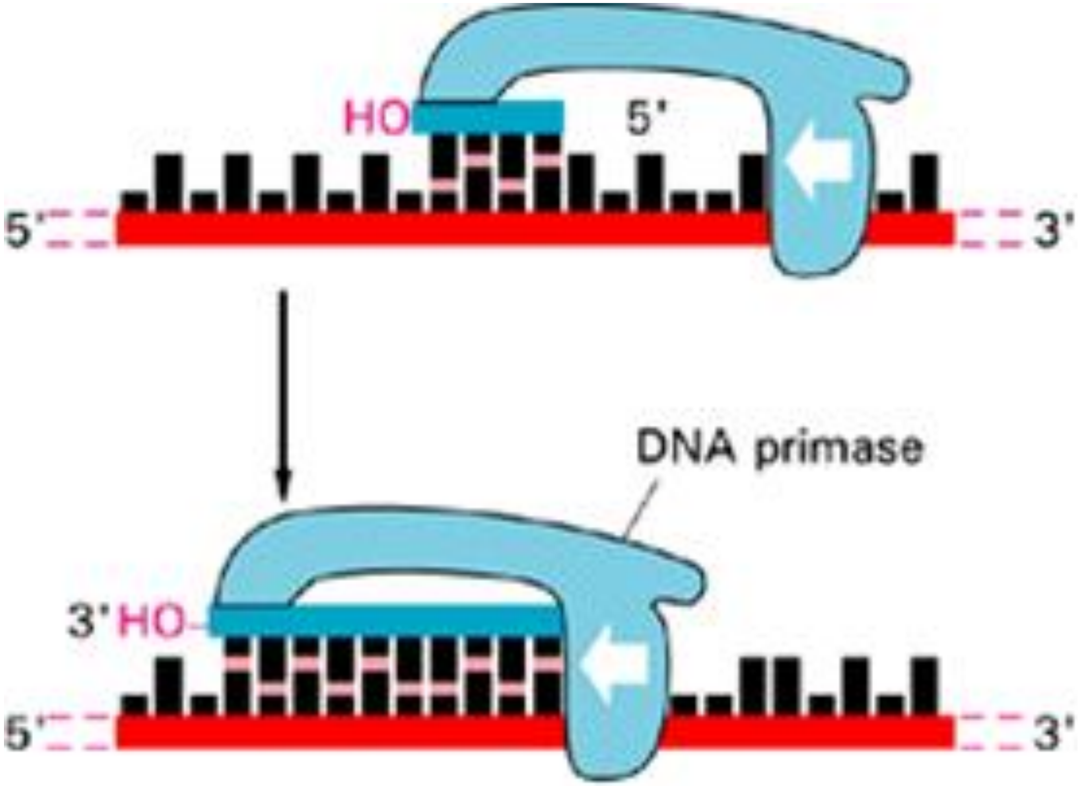
Il DNA duplex è tagliato per fornire l’estremità libera alla DNA polimerasi

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

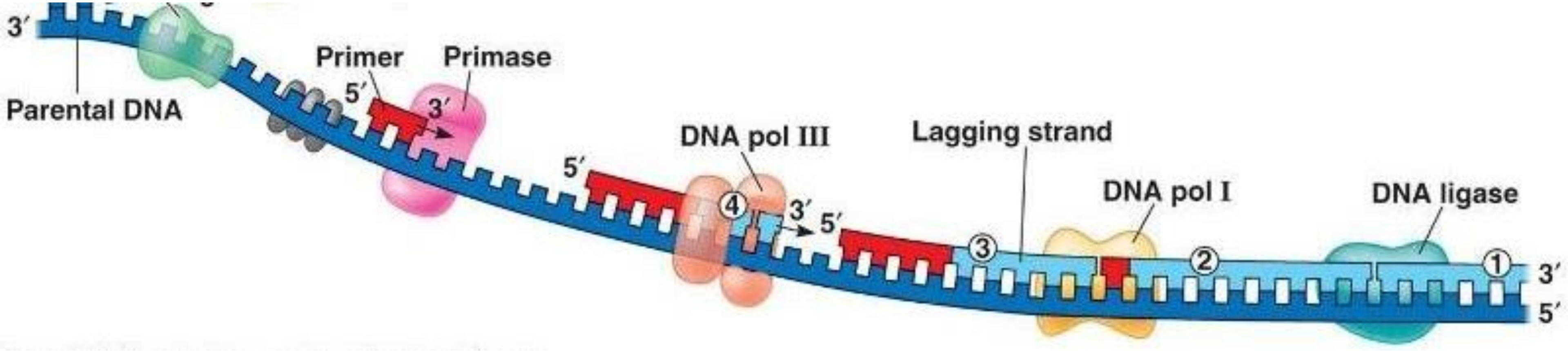
L’innesco puo’ essere una molecola di RNA, un nick nel DNA o una proteina di innesco (virus).

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.

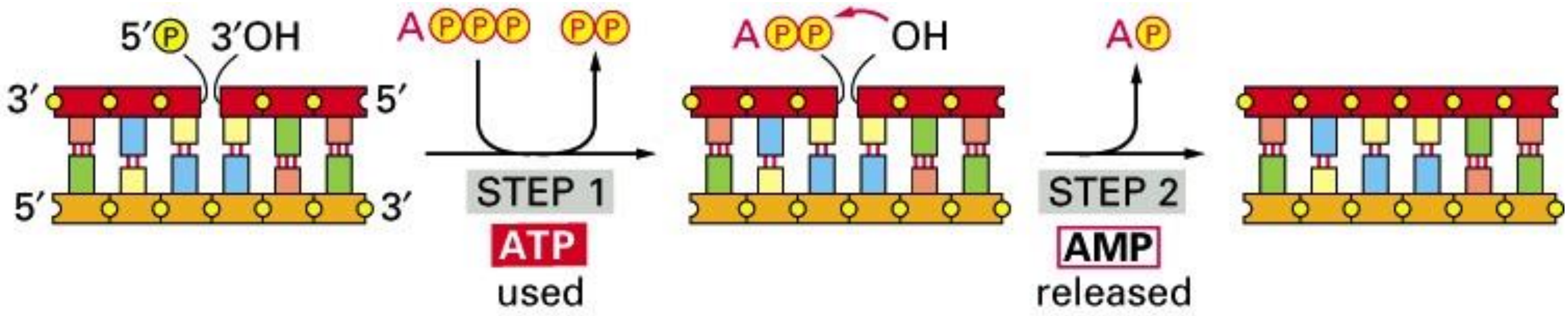
- ✓ I primers a RNA devono essere sostituiti, in quanto le basi U devono essere sostituite da T.

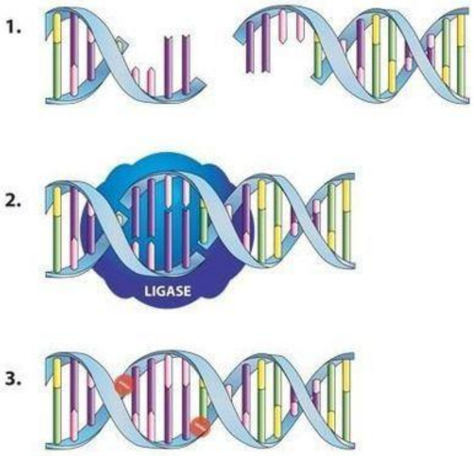
- ✓ I primers vengono degradati di una Rnasi (RNAsiH + FEN1 in eucarioti) e la DNA Polimerasi I li sostituisce con una sequenza di DNA, contenente T e non piu’ U.

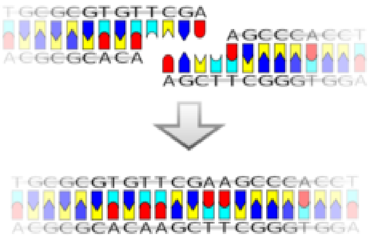
- ✓ I singoli frammmenti vengono poi uniti dalla DNA Ligasi

L'azione della DNA Ligasi

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



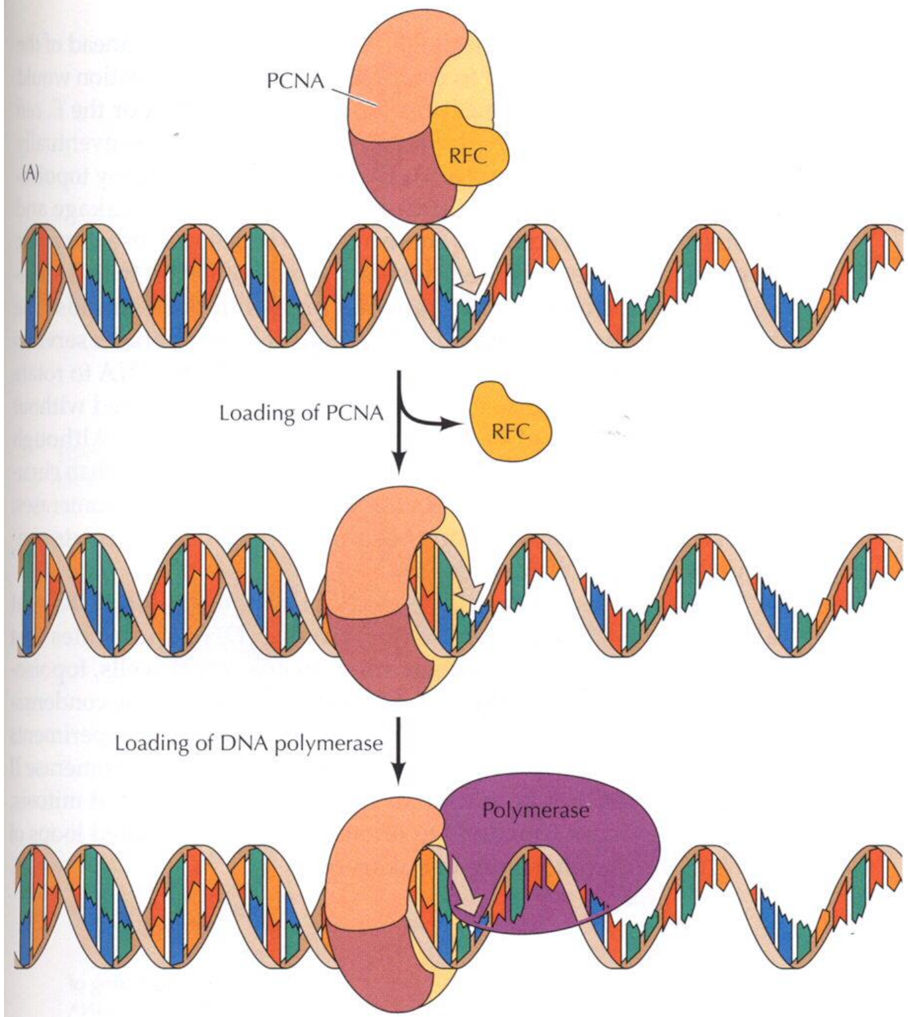


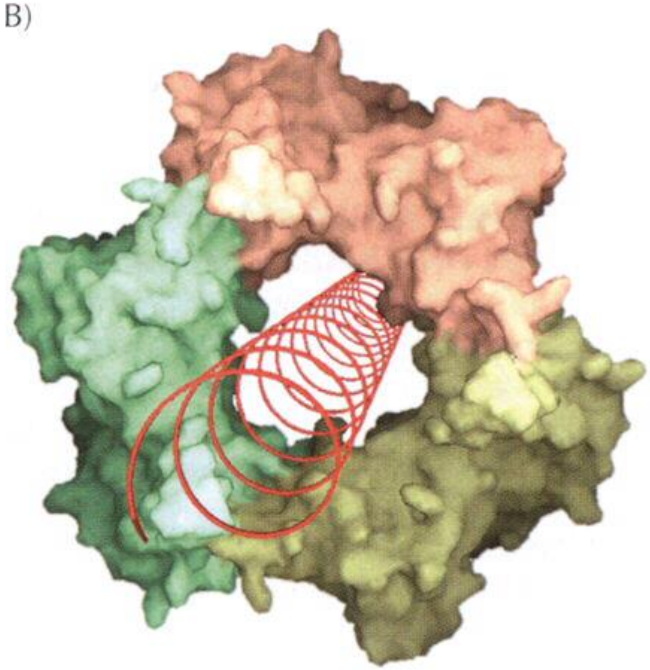


Stabilization of the replication machine

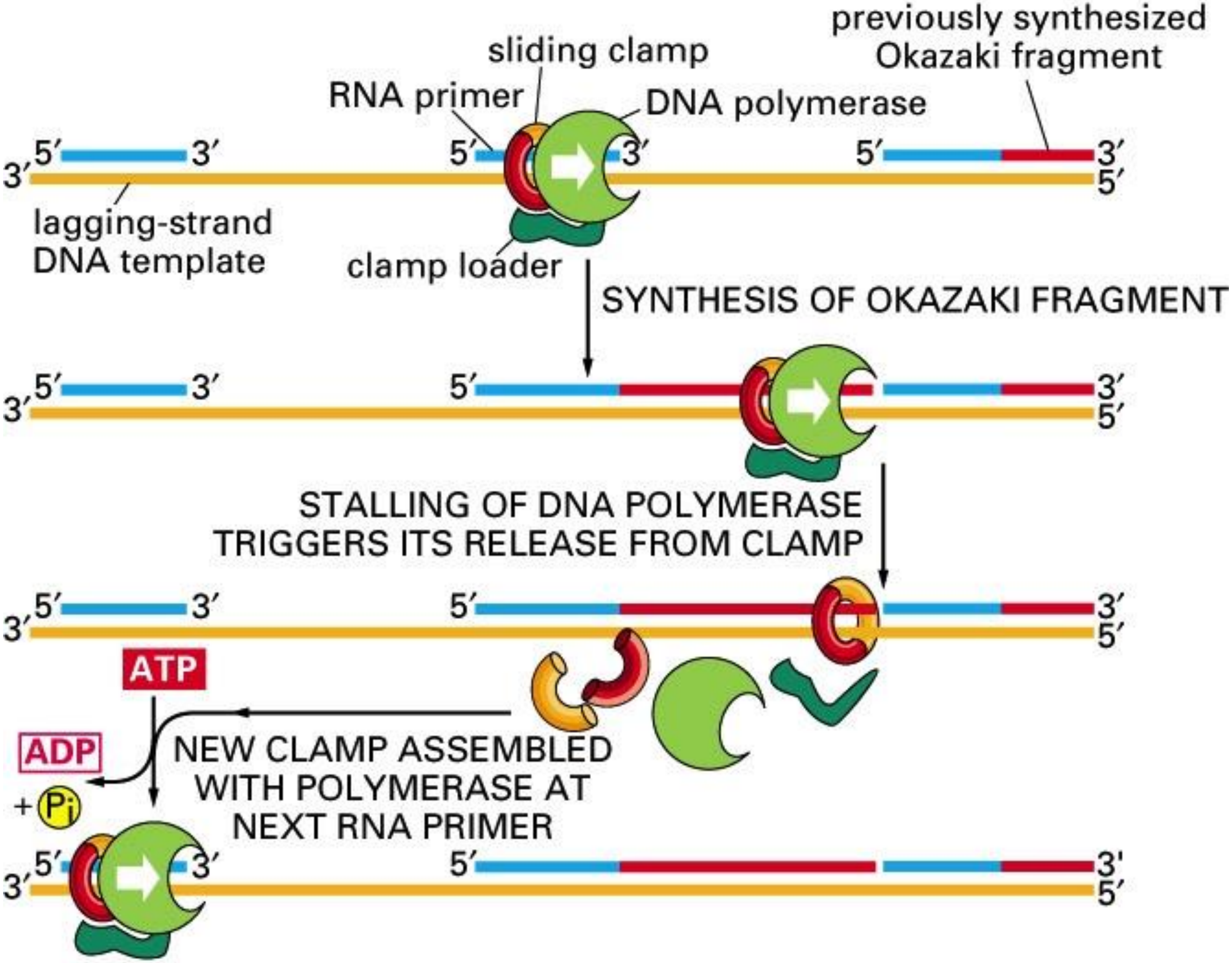
A ring clamp (PCNA) keeps the polymerase firmly on the DNA when it is moving on the dsDNA. One side of the ring binds to the back of the DNApol and it slides along DNA as the polymerase moves on.

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

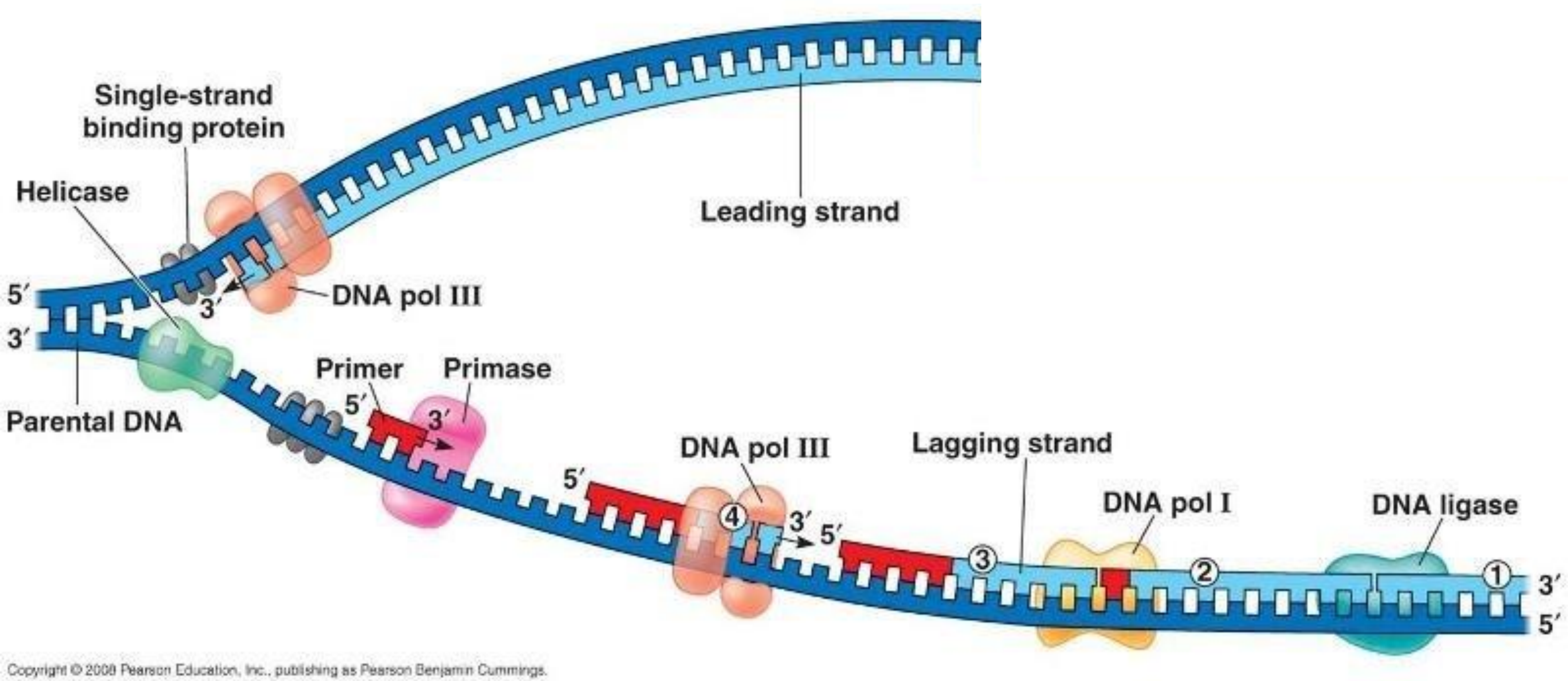




around the primer-template junction requires ATP hydrolysis by a special protein complex, the **clamp loader**

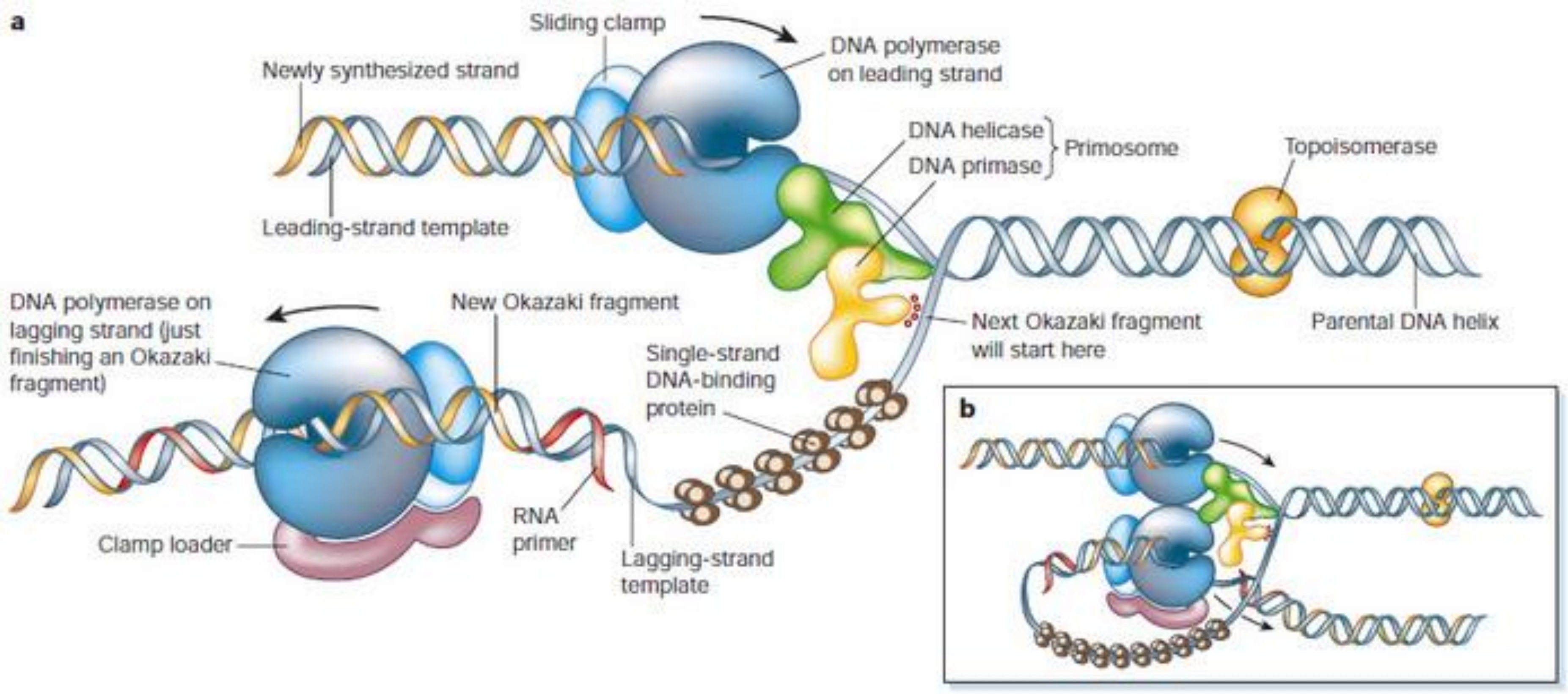

previously synthesized Okazaki fragment
sliding clamp
RNA primer
DNA polymerase
5′
3′
lagging-strand DNA template
clamp loader
SYNTHESIS OF OKAZAKI FRAGMENT
STALLING OF DNA POLYMERASE TRIGGERS ITS RELEASE FROM CLAMP
ATP
ADP
+ Pi
NEW CLAMP ASSEMBLED WITH POLYMERASE AT NEXT RNA PRIMER

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.

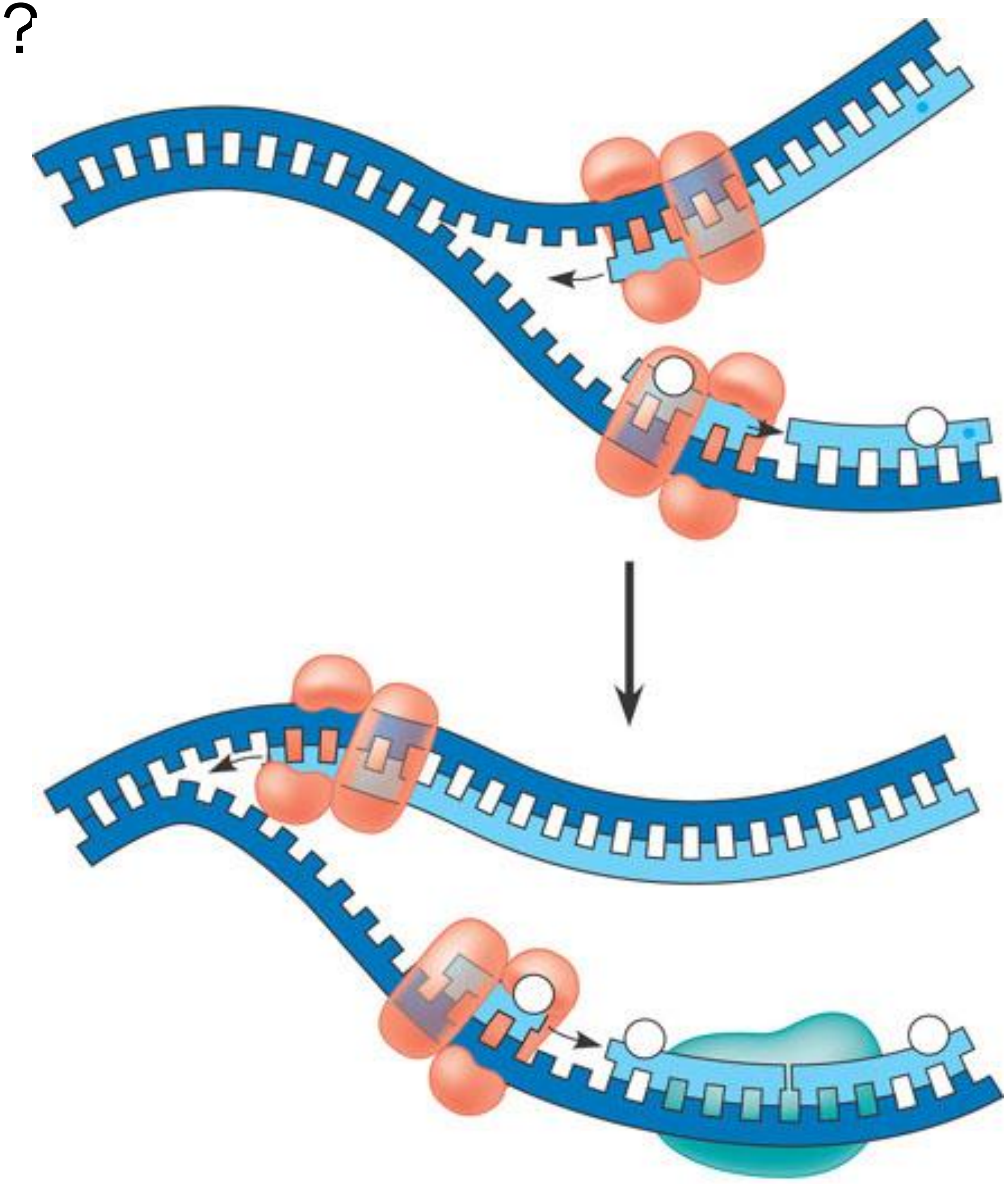
The proteins at a replication fork form a multienzyme replication machine



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:

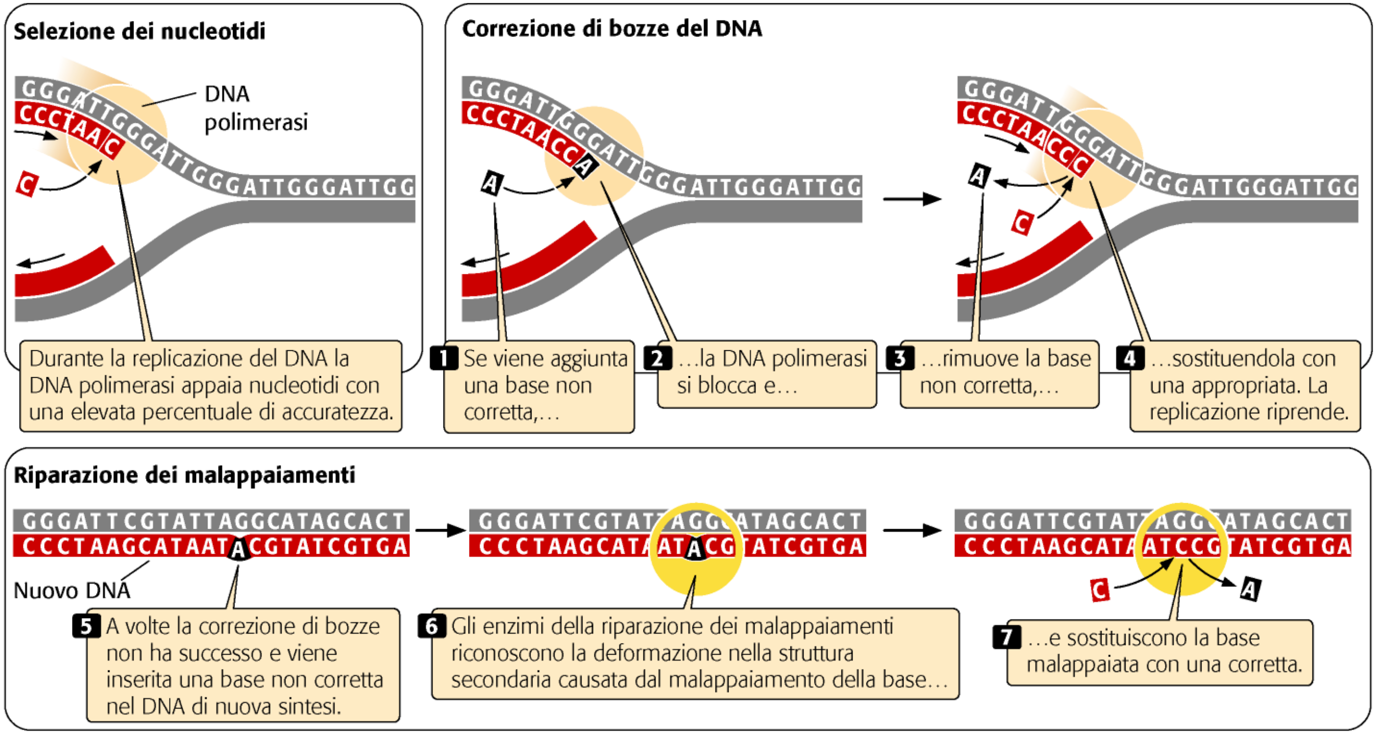


Where is…

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



Selezione dei nucleotidi

Durante la replicazione del DNA la DNA polimerasi appaia nucleotidi con una elevata percentuale di accuratezza.

Correzione di bozze del DNA

1 Se viene aggiunta una base non corretta,…

2 …la DNA polimerasi si blocca e…

3 …rimuove la base non corretta,…

4 …sostituendola con una appropriata. La replicazione riprende.

Riparazione dei malappaiamenti

5 A volte la correzione di bozze non ha successo e viene inserita una base non corretta nel DNA di nuova sintesi.

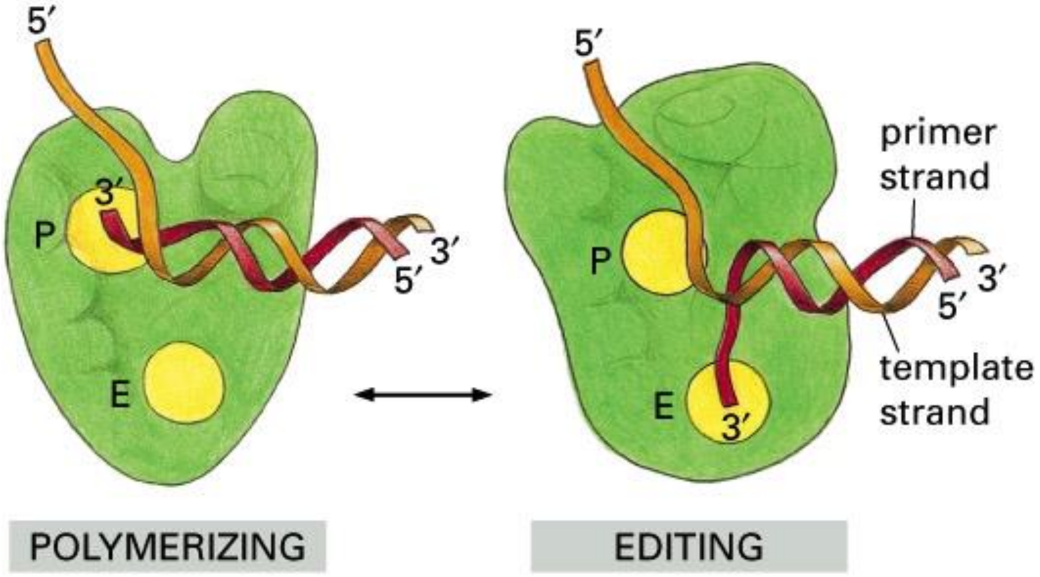
6 Gli enzimi della riparazione dei malappaiamenti riconoscono la deformazione nella struttura secondaria causata dal malappaiamento della base…

7 …e sostituiscono la base malappaiata con una corretta.

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 fosfodiestereo, 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.

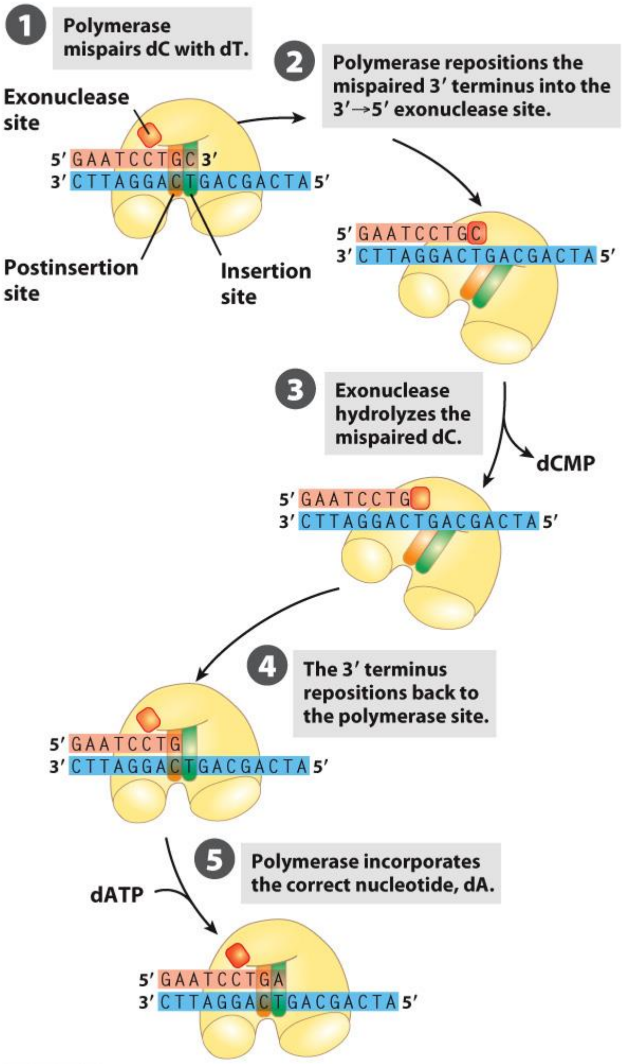
2- L’attivita’ *proof-reading*

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

- attivita’ polimerasica in direzione 5’-3’
- attivita’ 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 attivita’ esonucleasica, dove viene eliminato il nucleotide errato, permettendo alla polimerasi di riprendere velocemente la sintesi, senza provocare la dissociazione dell’intero complesso.

Questa attivita’ *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 fedelta’ di replicazione da 2 a 3 ordini di magnitudine:

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.

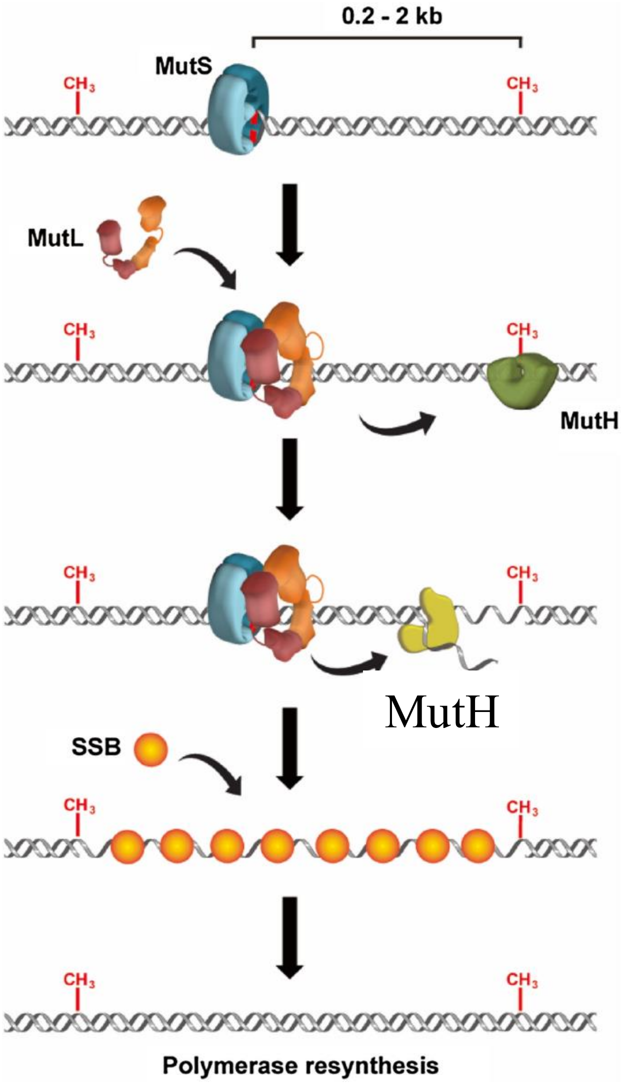
Nei procarioti MutS scorre lungo il DNA e riconosce il mismatch

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.

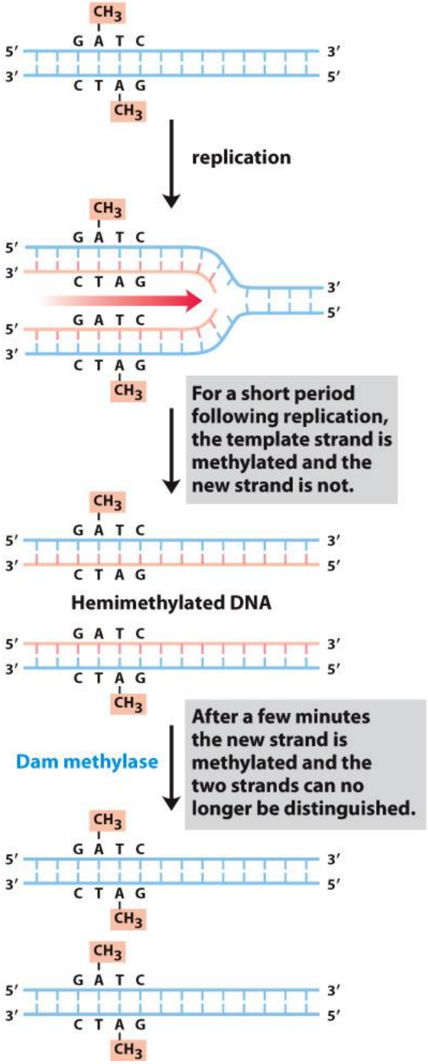


Mismatch Repair

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.

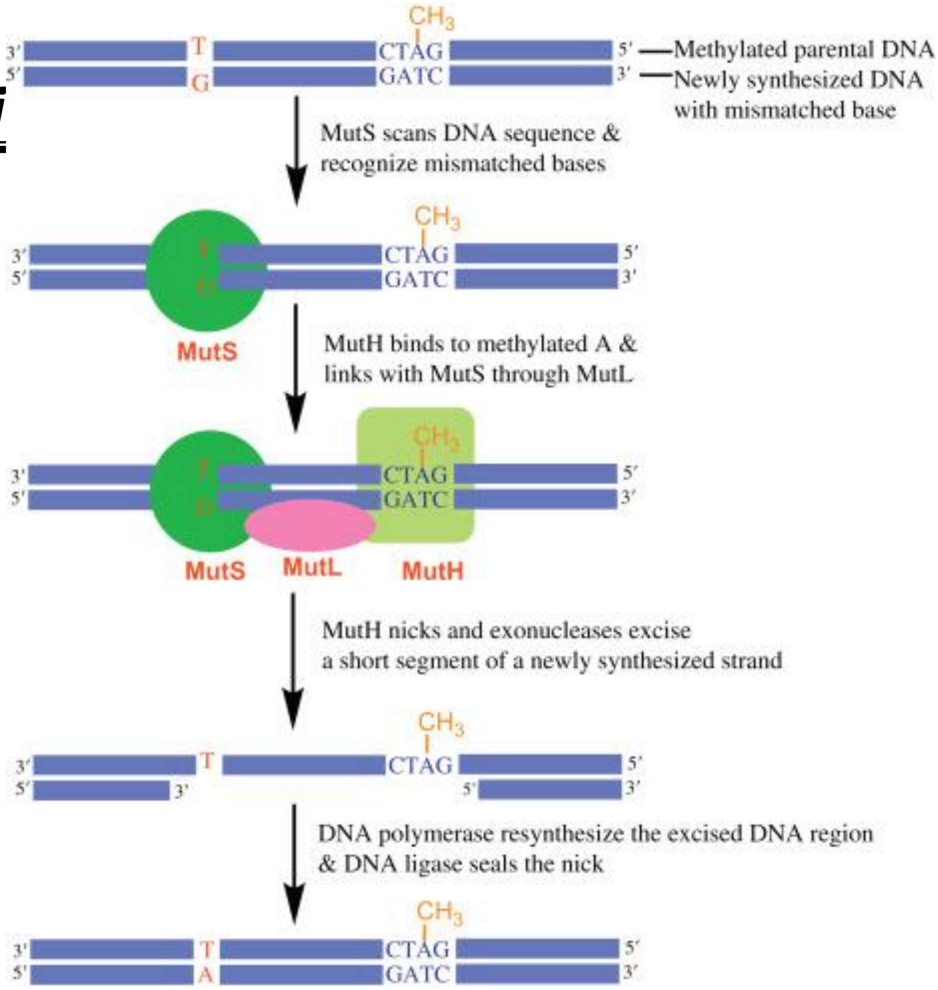


For a short period following replication, the template strand is methylated and the new strand is not.

After a few minutes the new strand is methylated and the two strands can no longer be distinguished.

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.



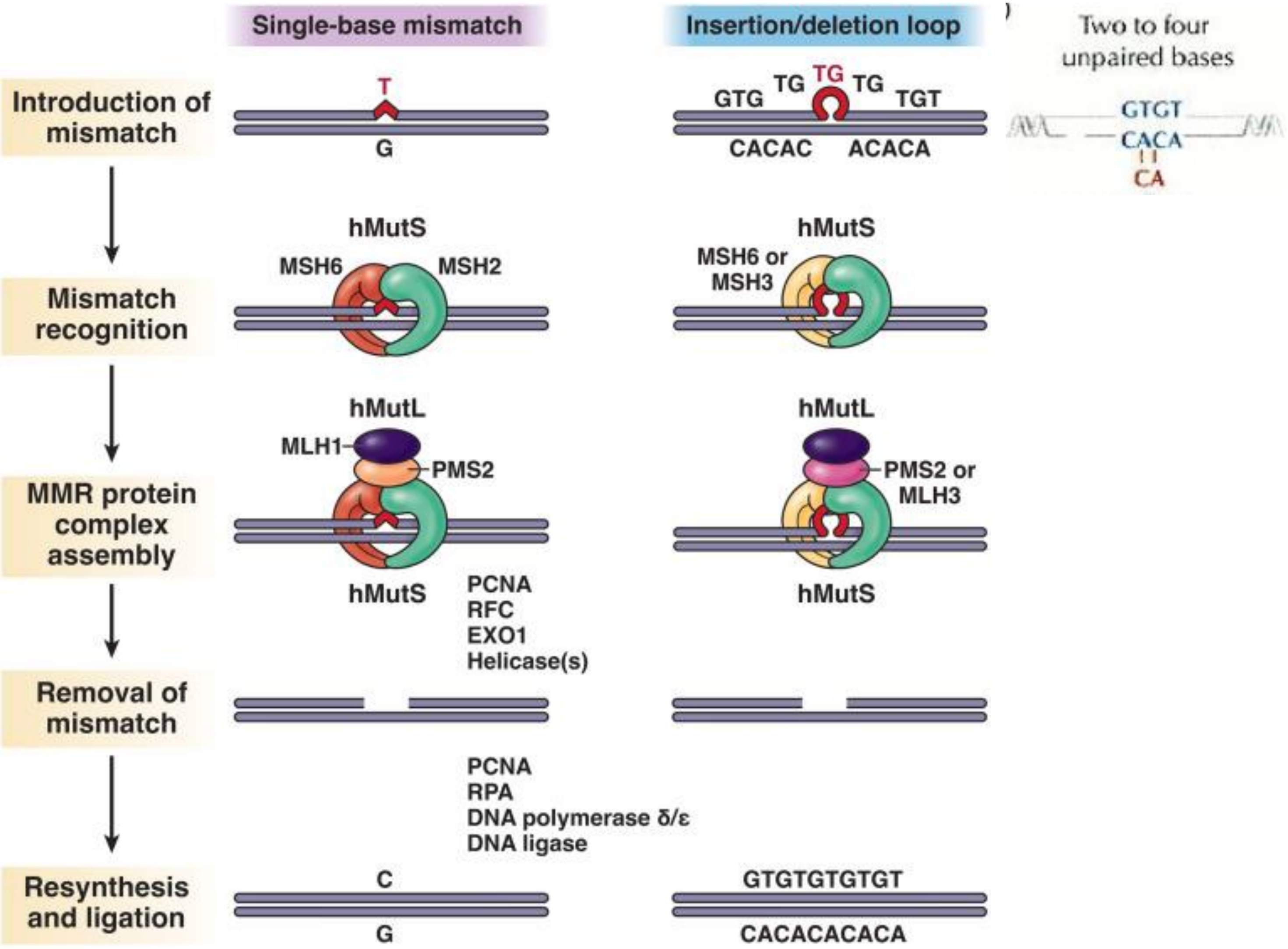
MutS scans DNA sequence & recognize mismatched bases

MutH binds to methylated A & links with MutS through MutL

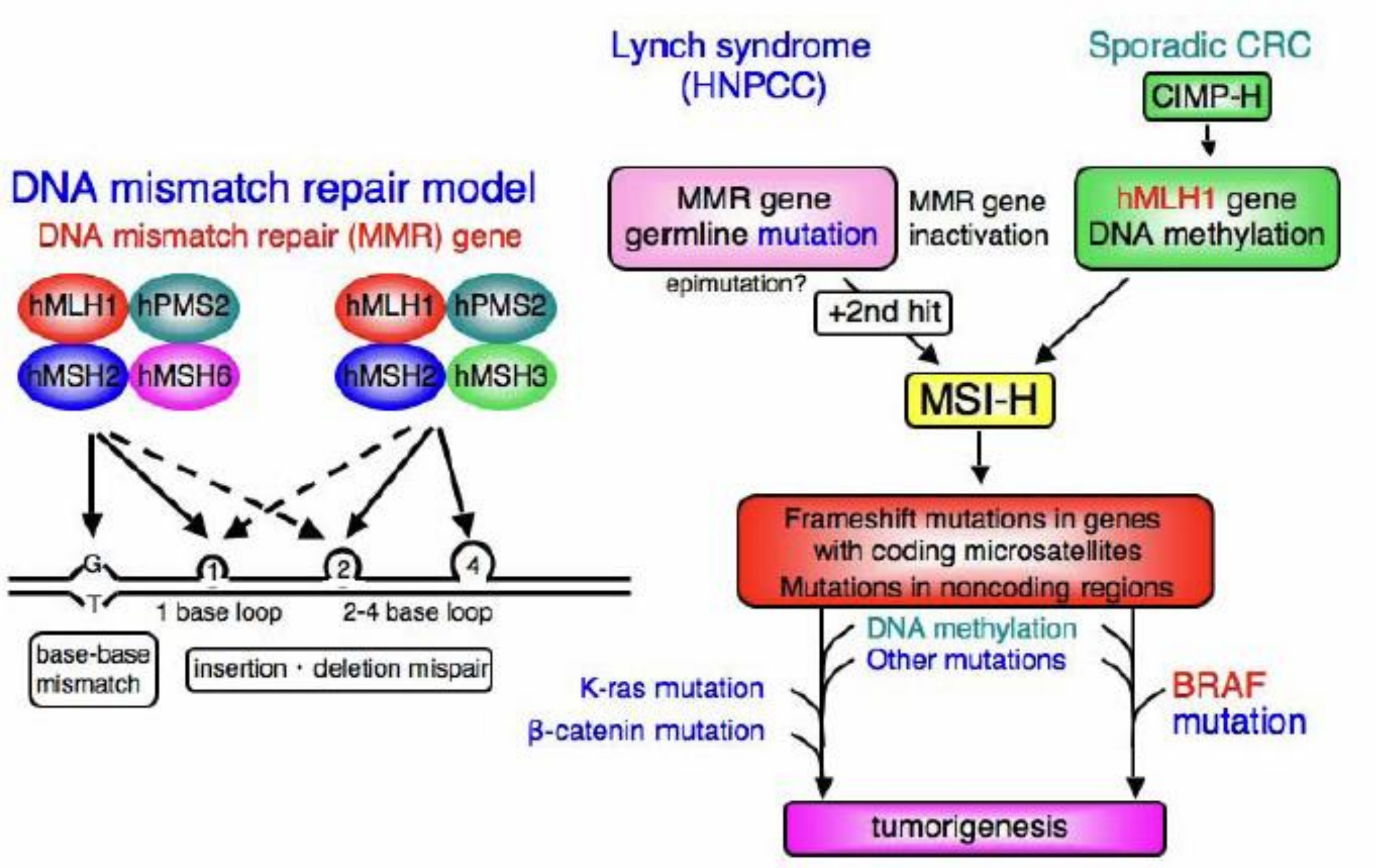
MutH nicks and exonucleases excise a short segment of a newly synthesized strand

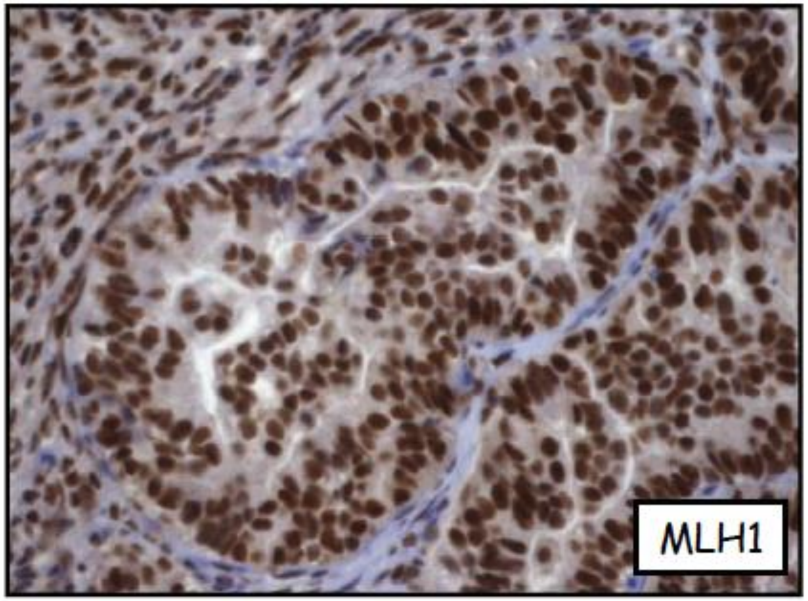
DNA polymerase resynthesize the excised DNA region & DNA ligase seals the nick

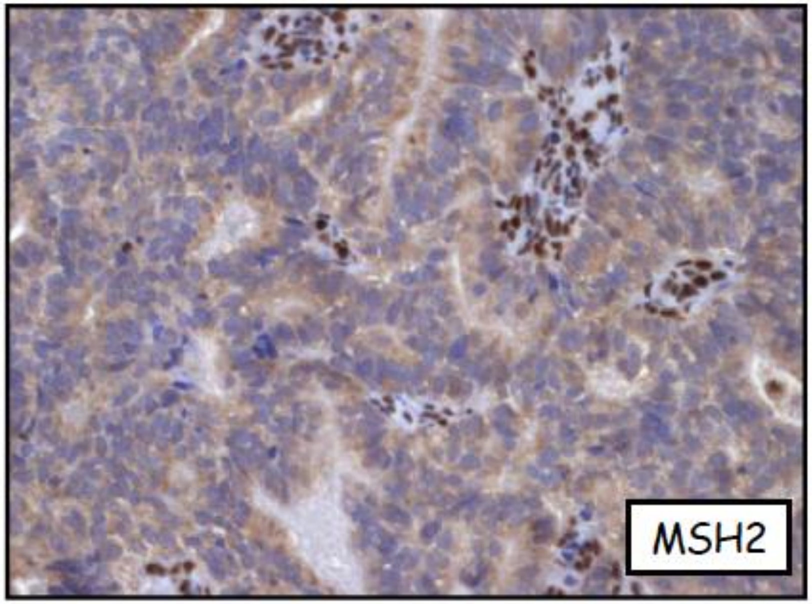
Nelle cellule umane hMSH2 e hMLH1, sono simili a MutS e MutL rispettivamente

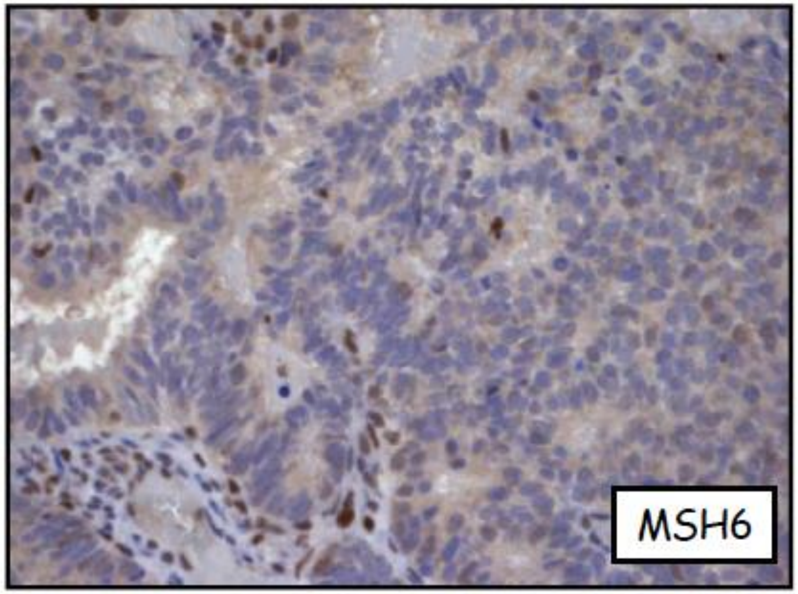


Mutazioni nei geni del mismatch repair system causano la predisposizione a certi tipi di cancro
(es. HNPCC, carcinoma colonrettale ereditario non poliposico, noto come sindrome di Lynch; test predittivo: mutazioni nei geni MSH2 e MLH1).

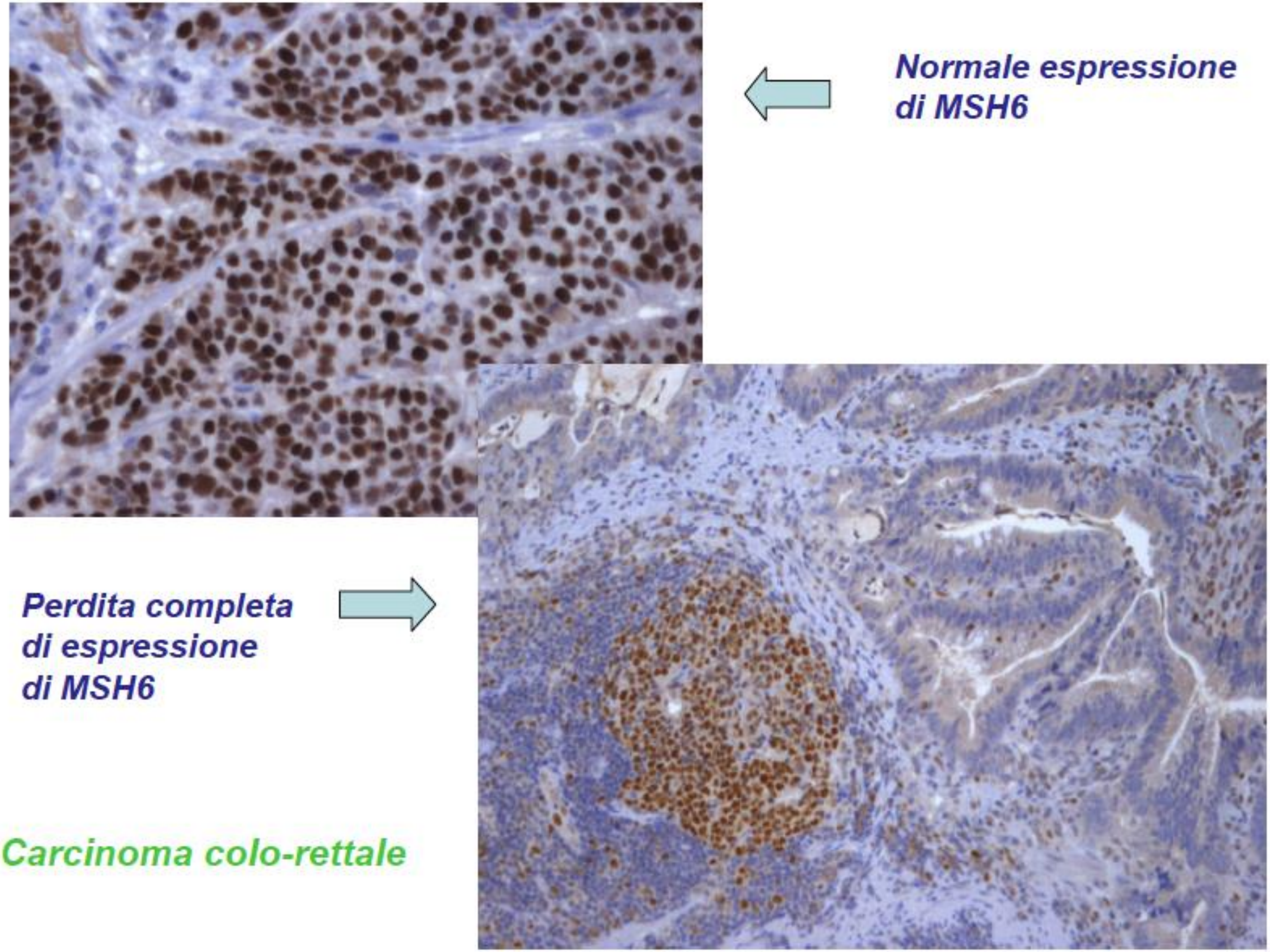








Utero
Adenocarcinoma endometrioide
Perdita completa di espressione
di MSH2 e MSH6



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 (≈ 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.