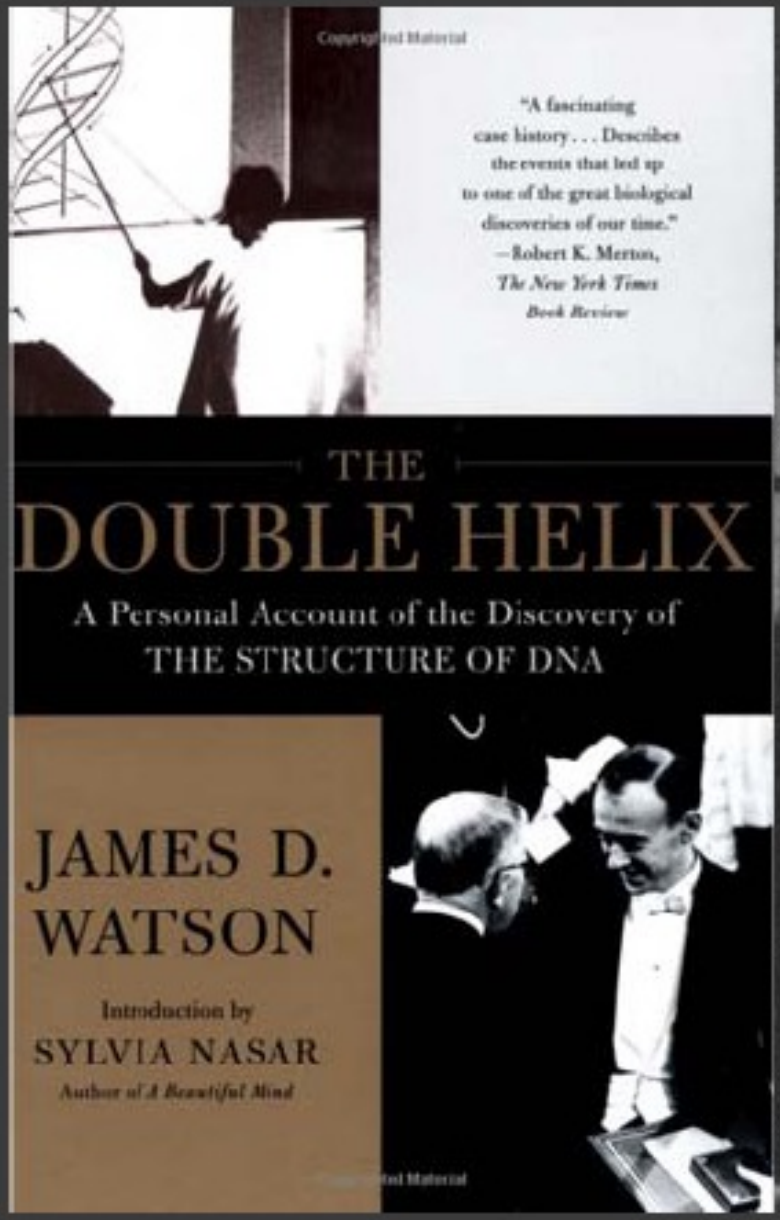
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"A fascinating case history . . . Describes the events that led up to one of the great biological discoveries of our time."
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THE
DOUBLE HELIX
A Personal Account of the Discovery of THE STRUCTURE OF DNA
JAMES D. WATSON
Introduction by
SYLVIA NASAR
Author of A Beautiful Mind
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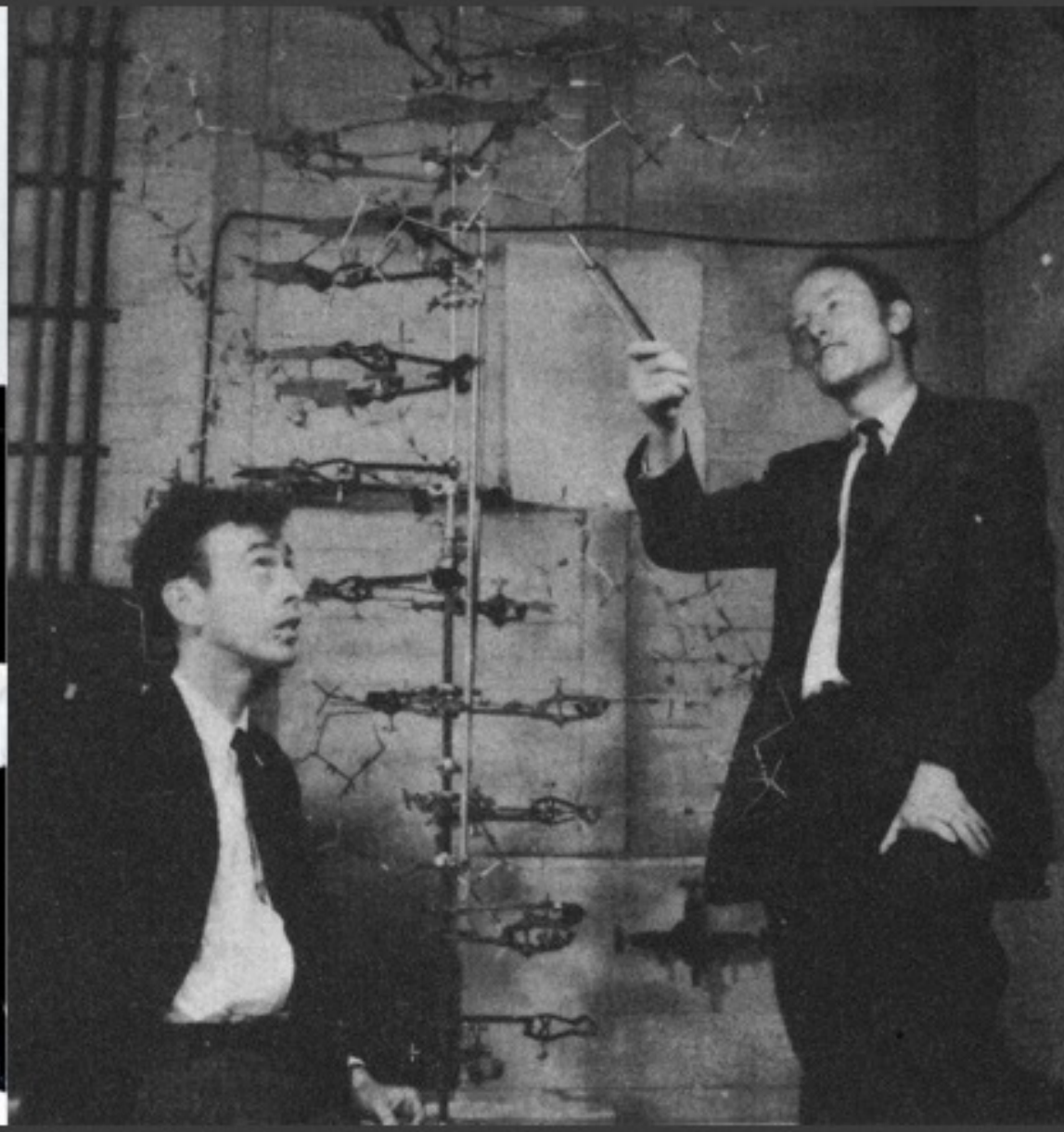
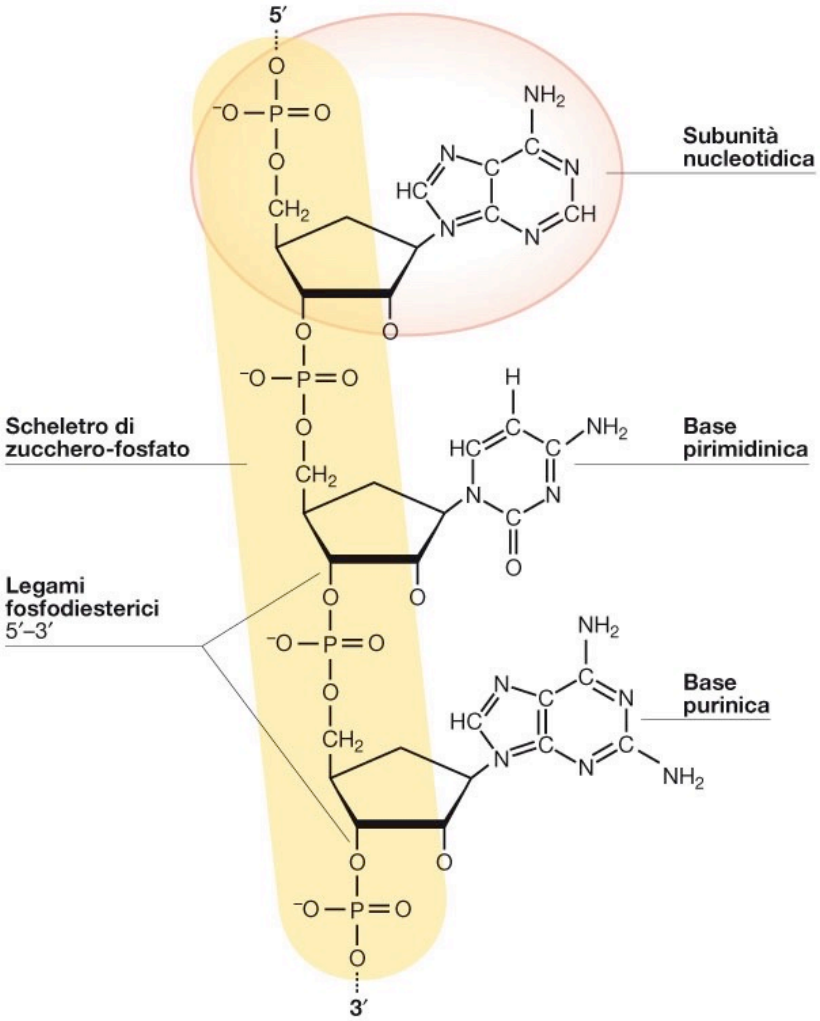
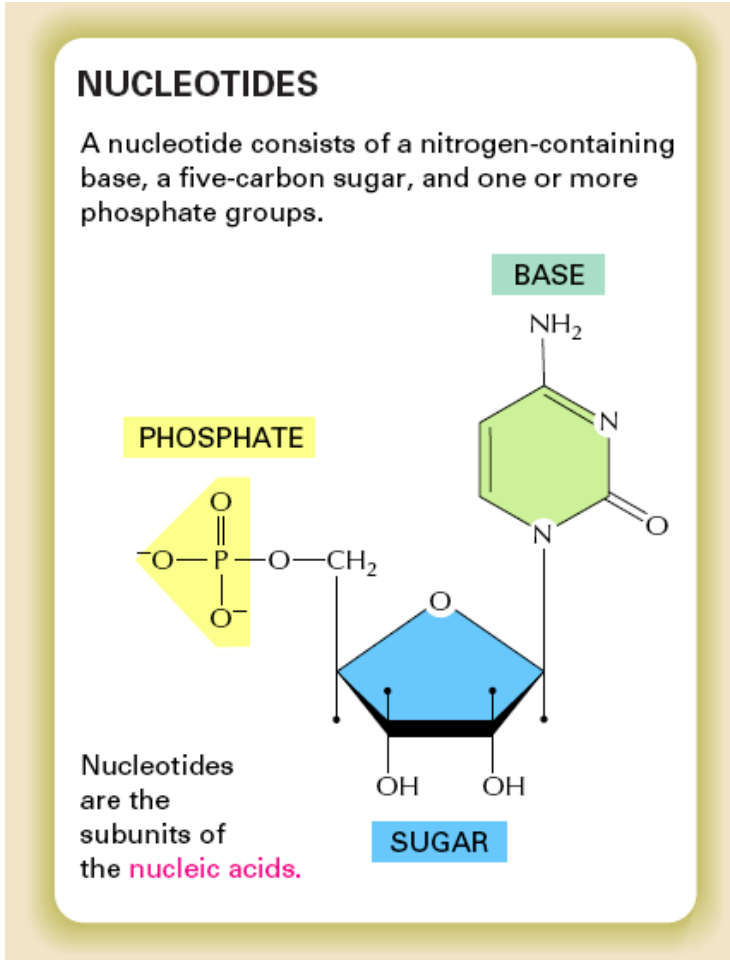


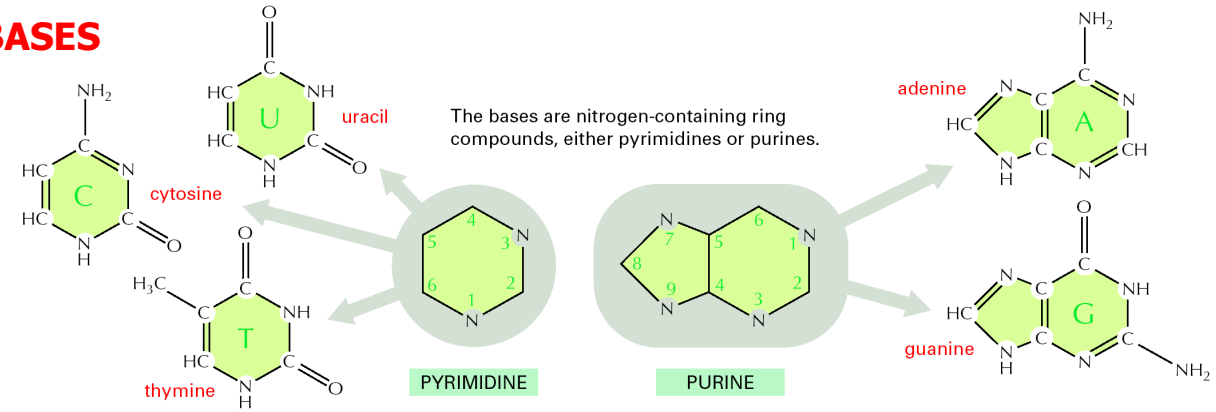
The DNA building blocks are the NUCLEOTIDES





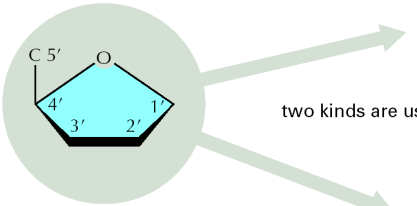
The DNA building blocks are the NUCLEOTIDES

BASES

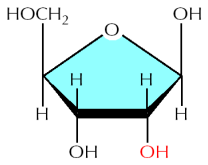


SUGARS

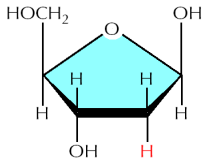
PENTOSE
a five-carbon sugar



two kinds are used



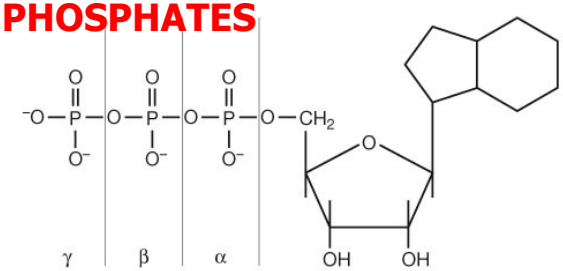
β-D-ribose
used in ribonucleic acid

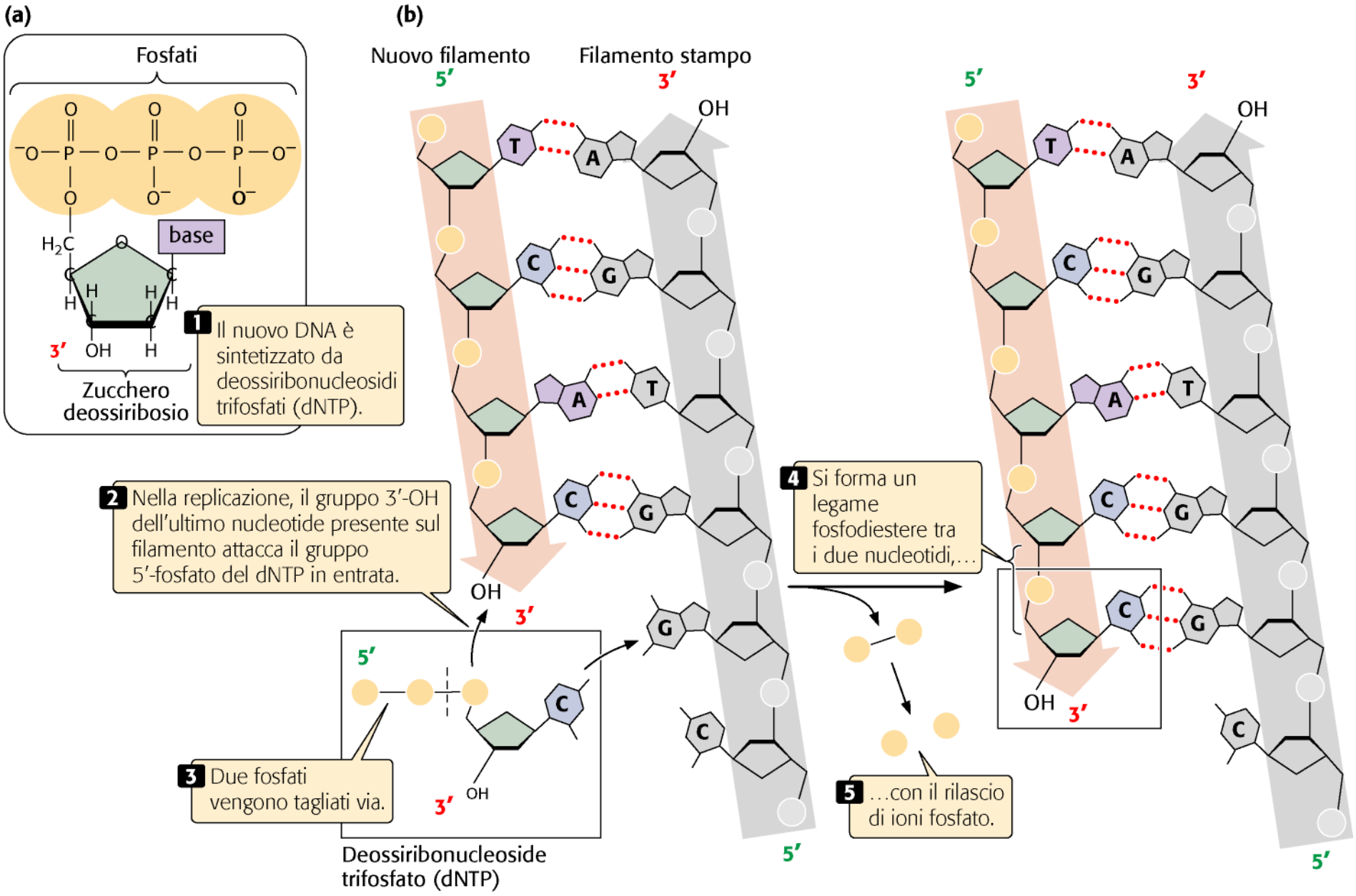


β-D-2-deoxyribose
used in deoxyribonucleic acid

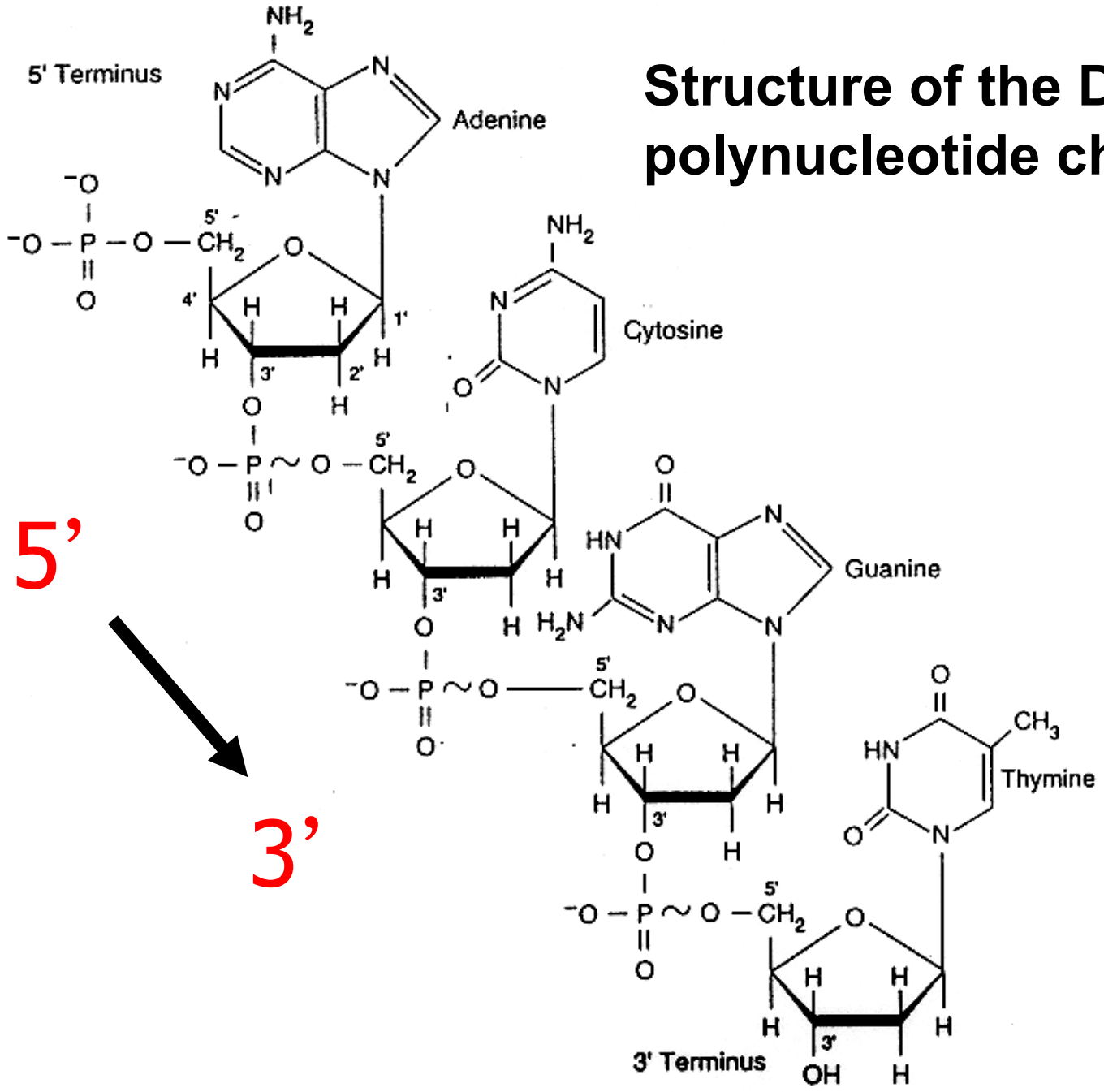
Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.

PHOSPHATES



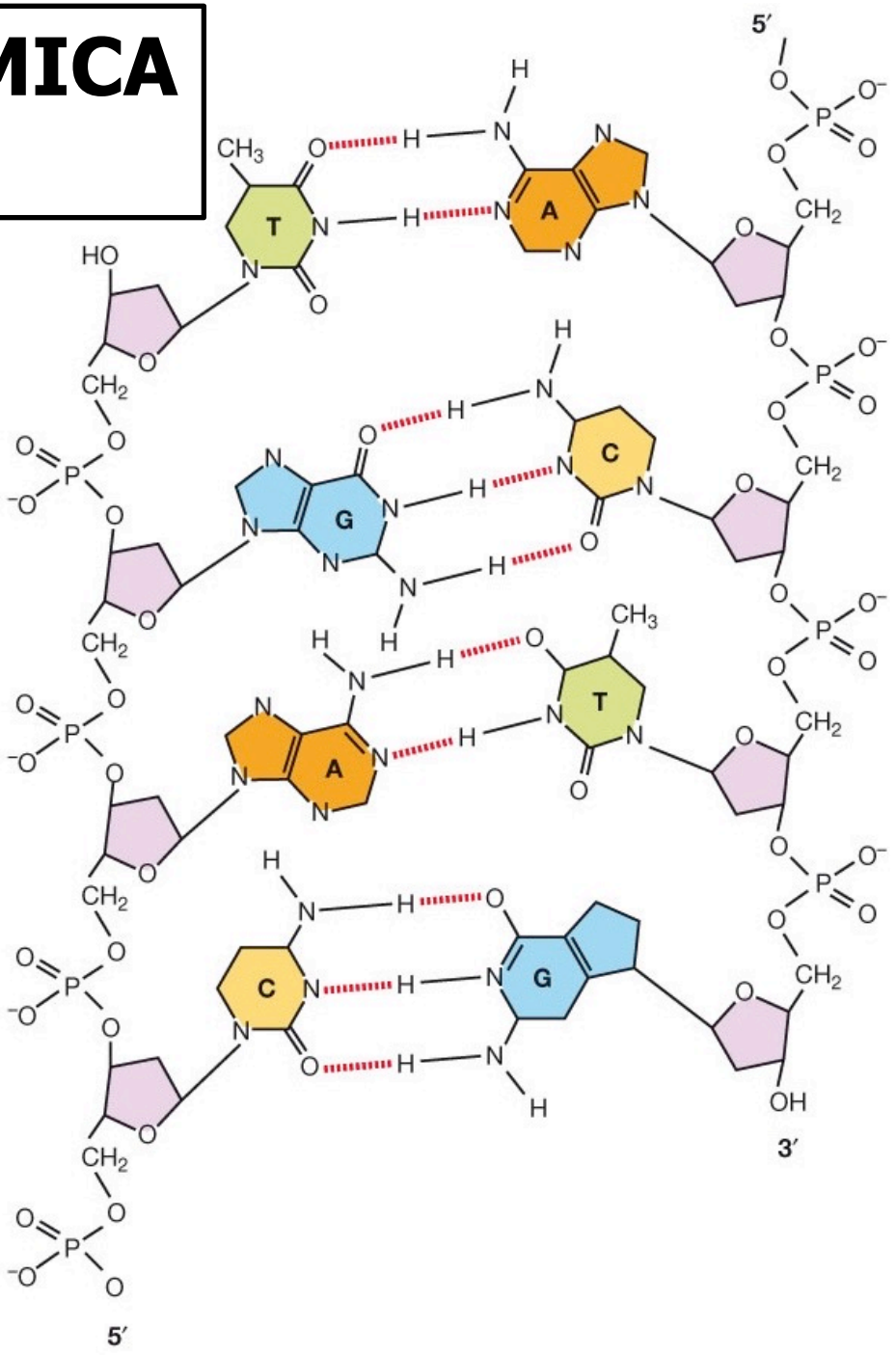

(a)
Fosfati
base
Zucchero deossiribosio
1 Il nuovo DNA è sintetizzato da deossiribonucleosidi trifosfati (dNTP).
(b)
Nuovo filamento
Filamento stampo
2 Nella replicazione, il gruppo 3′-OH dell'ultimo nucleotide presente sul filamento attacca il gruppo 5′-fosfato del dNTP in entrata.
3 Due fosfati vengono tagliati via.
Deossiribonucleoside trifosfato (dNTP)
4 Si forma un legame fosfodiestere tra i due nucleotidi,…
5 …con il rilascio di ioni fosfato.

Structure of the DNA polynucleotide chain

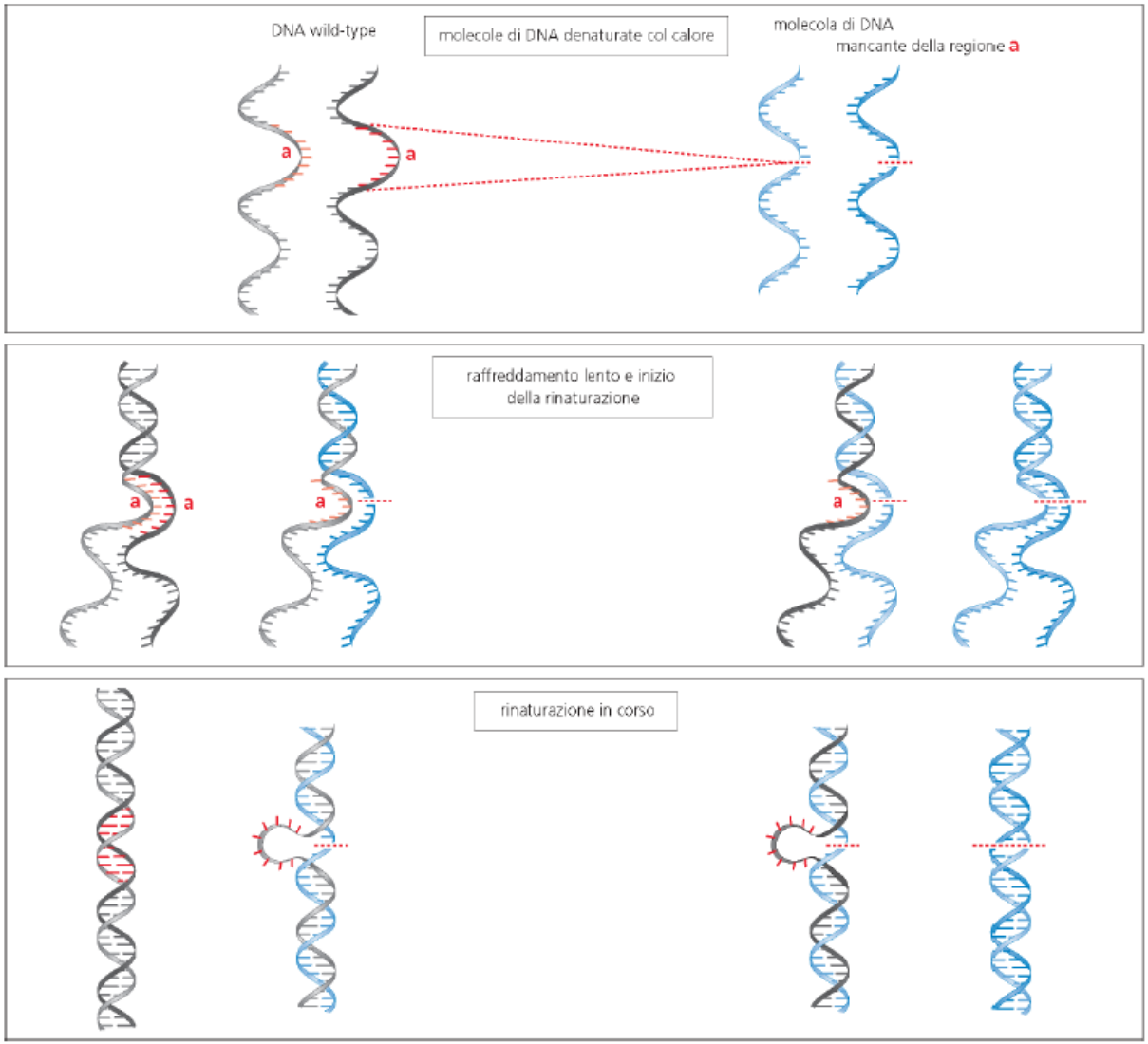


LA STRUTTURA CHIMICA DEL DNA

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.

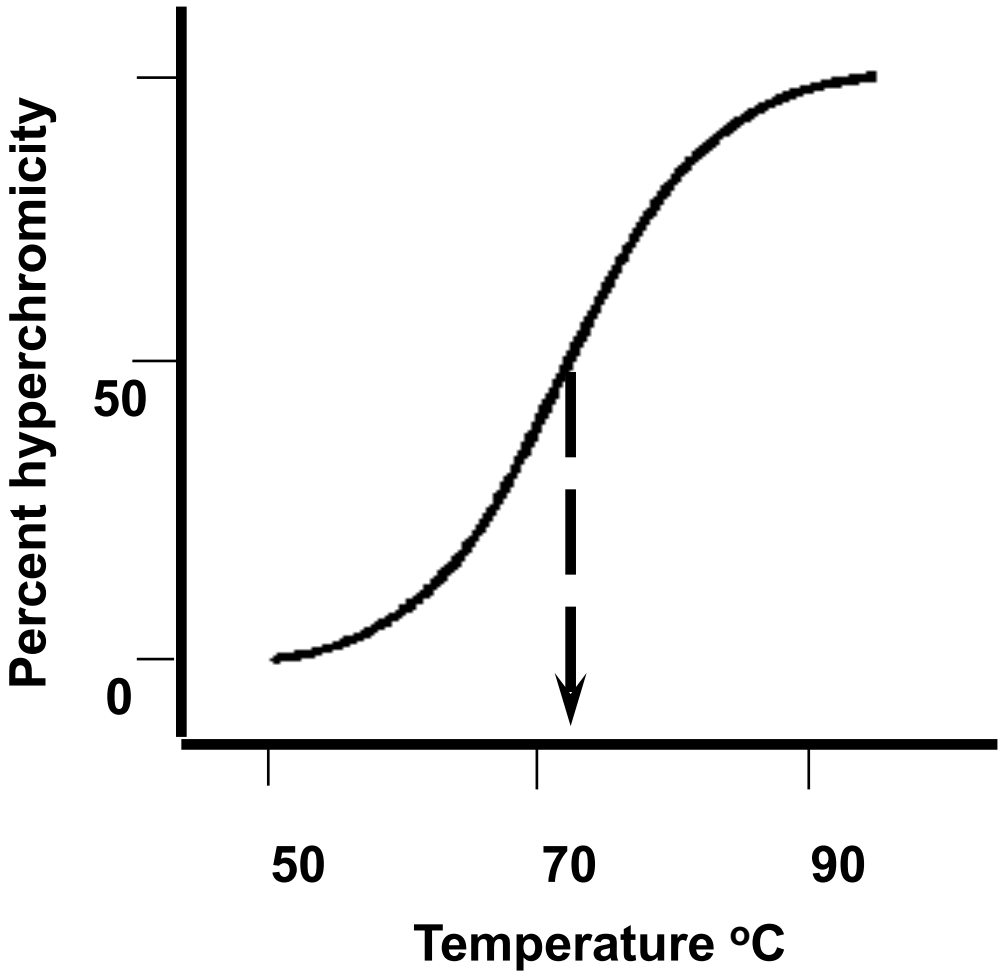
Se si ritorna lentamente alle condizioni di partenza il DNA può rinaturarsi e formare molecole ibride (ibridazione del DNA)

Stabilità termodinamica del duplex di DNA e sua denaturazione

Fattori intrinseci: composizione in basi, peso molecolare.

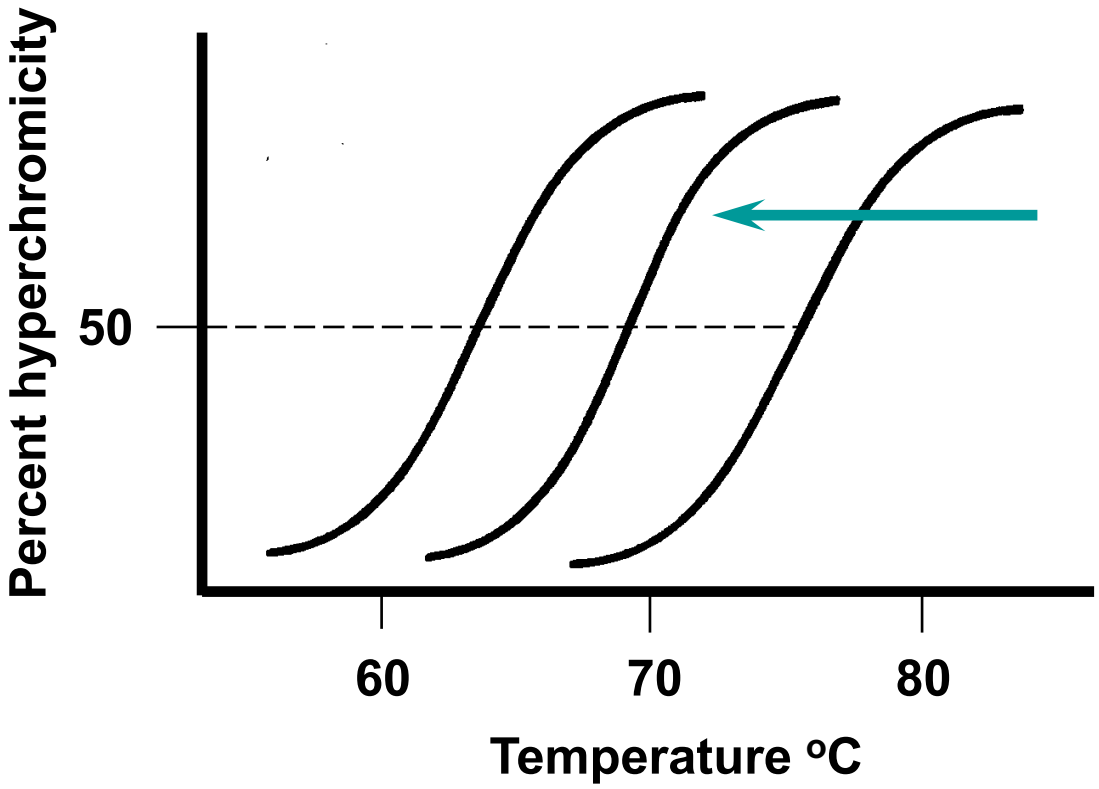
Fattori estrinseci: temperatura, pH, forza ionica.

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

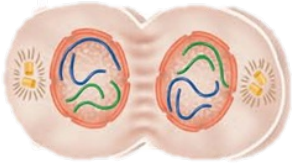
Objectives

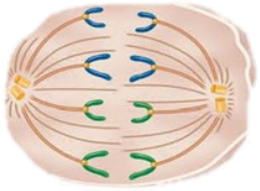
- Essential Question: Why & how do our cells reproduce DNA?

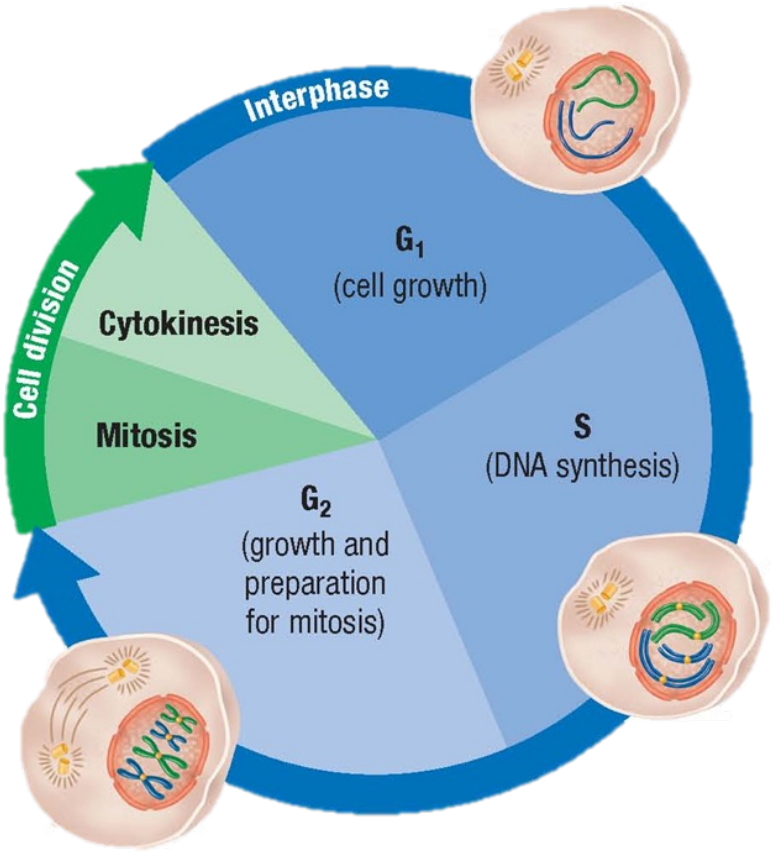
- Describe the steps of DNA replication.
- Compare the roles of DNA helicase, DNA polymerase, and ligase
- Compare the process of DNA replication in prokaryotes and in eukaryotes
- Applications of our knowledge

DNA REPLICATION

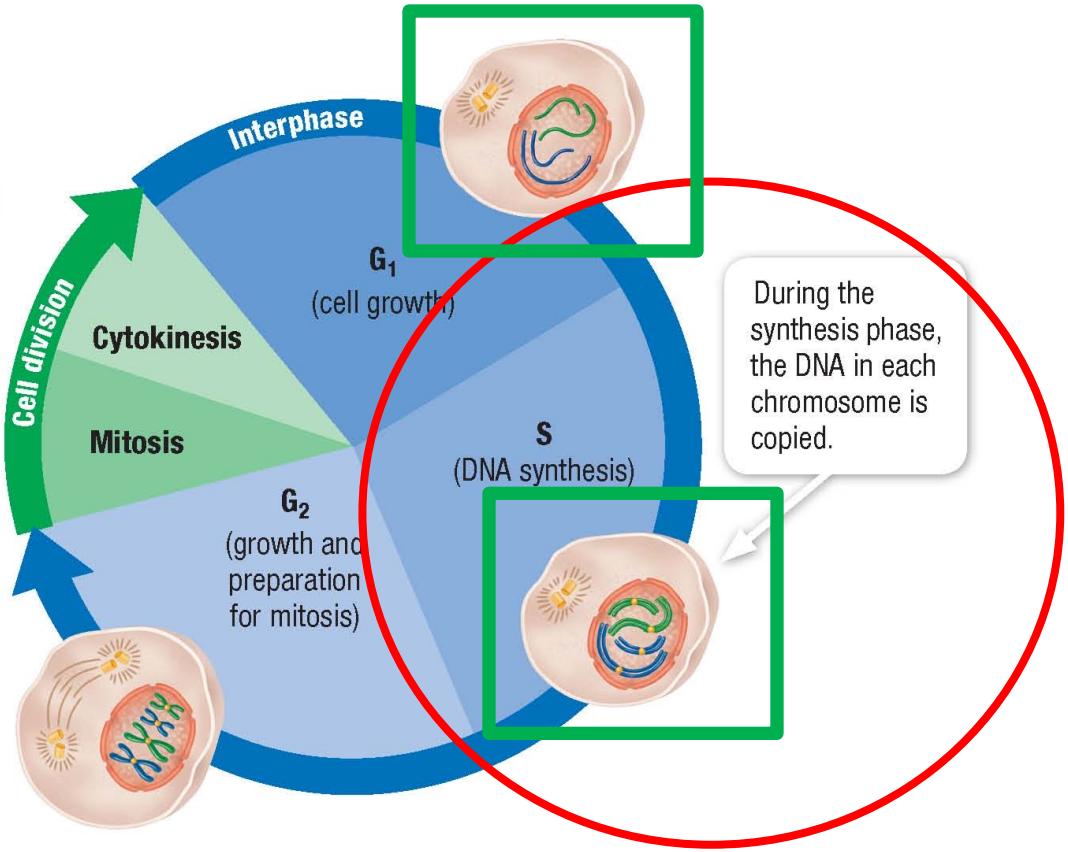
- Every time a cell goes through a cycle it must DUPLICATE ITS DNA SO THAT WHEN IT MAKES NEW BABY CELLS THEY BOTH HAVE THE EXACT SAME DNA.
- It happens in ‘S’ phase
- In this section we are going explore the process of DNA synthesis, called DNA replication.
- This process is extremely precise and an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







THE CELL CYCLE: THE PART WE'RE ISOLATING.



From single-copy of each chromosome

To double-copy

1952

KING'S College LONDON

King's College London

Franklin–Wilkins Building

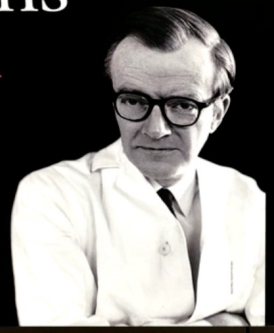
Wilkins

Professor Maurice

Biophysicist

Maurice Wilkins (1916–2004), Alec Stokes and Herbert Wilson of the Randall Institute at King's College London made crucial contributions to the discovery of the structure of DNA in the early 1950s.

Wilkins and his colleagues later verified the Cambridge hypothesis about the structure of the molecule, and Wilkins received the Nobel Prize with Crick and Watson in 1962 for his work on DNA over many years.



Pattern

Searching for the shape of DNA

Clearly a helix

1950 Work on DNA by Maurice Wilkins and Ray Gosling pre-dated that of Rosalind Franklin. In summer 1950, using a moistened sample of DNA fibres, they obtained the clearest pictures so far of the diffraction pattern of DNA, using an X-ray diffraction camera they had modified by filling it with hydrogen to reduce background scattering. In the same year their colleague Alec Stokes suggested that the patterns obtained by their images showed that the molecule was helical in structure.

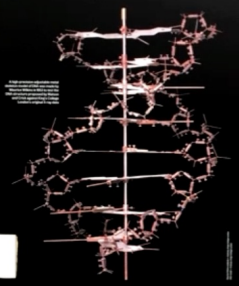
Double helix

Telling the story of life

Heredity

The discovery of the double helix structure of deoxyribonucleic acid (DNA) in 1953 revealed the physical and chemical basis of how heredity works: both how characteristics are passed down through the generations and how they are expressed in individual organisms.

The discovery led to advances in biology and medicine which have fundamentally changed aspects of the human condition and our response to the natural world.



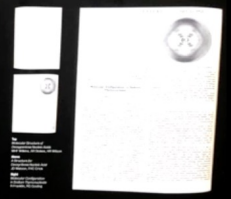
Nature magazine

Historic papers

Publishing research

1953 Two of the three historic papers about the structure of DNA published in *Nature* magazine on 25 April 1953 were written by scientists from the Randall Institute at King's College London.

The Institute was the first to bring together physicists, chemists and biologists and to apply physical techniques to the solution of biological problems. King's scientists continue to play a leading role in the scientific developments that have spiralled out of the DNA discovery, particularly in the areas of medicine, dentistry, genetics, psychiatry, neurosciences, medical law and ethics.



Franklin

Dr Rosalind

Biophysicist

The research of Rosalind Franklin (1920–58) at King's College London was essential to the discovery of the structure of the DNA molecule and helped to lay the foundations of structural molecular biology.

Her systematic X-ray diffraction study of DNA provided clear evidence of the helical structure of the molecule, and she produced the first analytical demonstration of this structure.

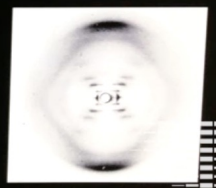
Photo 51

X-Ray diffraction

An historic photograph

1952 The X-Ray diffraction image of the DNA molecule taken by Rosalind Franklin and PhD student Ray Gosling at King's College London in 1952 can claim to be one of the world's most important photographs.

It demonstrated the helical structure of DNA and enabled James Watson and Francis Crick of Cambridge to build the first model of the molecule in 1953.



Franklin-Wilkins Building, King's College London

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] Van 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

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 Pauling 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.

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.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric form (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).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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.

[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[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 (*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

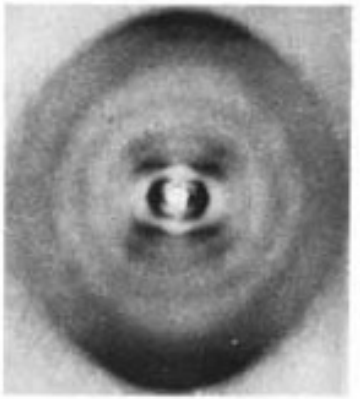


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B*, with fibre axis vertical

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 u 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 ea point are the same. Summation of the correspondi Bessel functions gives reinforcement for the inte

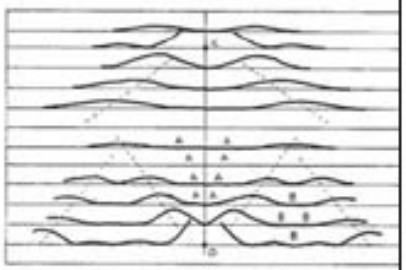


Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 24 A. diameter and remainder distributed 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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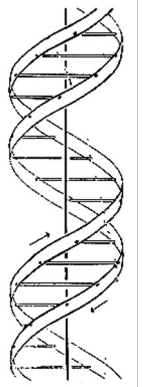

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

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. The are joined together in pairs, 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).

In other words, if an adenine forms one member of a pair, on either chain, then on th ese assumptions the other member must be thymine ; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on t he other chain is automatically determined.

It has been found experimentally (3,4) that the ratio of the amounts of adenine to thymine, and the ration of guanine to cytosine, are always bery close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact. The previously published X-ray data (5,6) on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E . Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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

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

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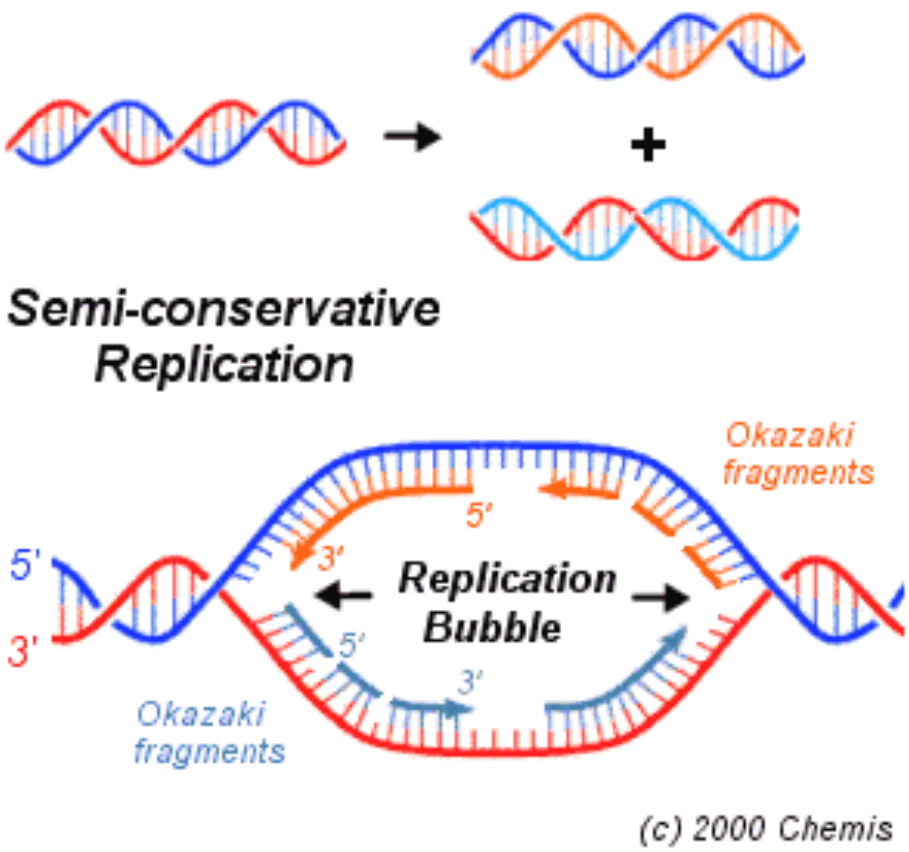
Definitions

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.

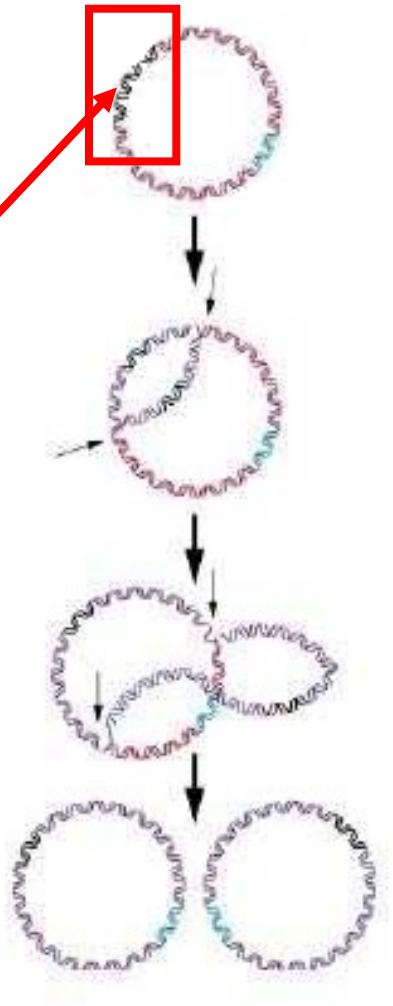


Prokaryotic and Eukaryotic Replication

- 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.

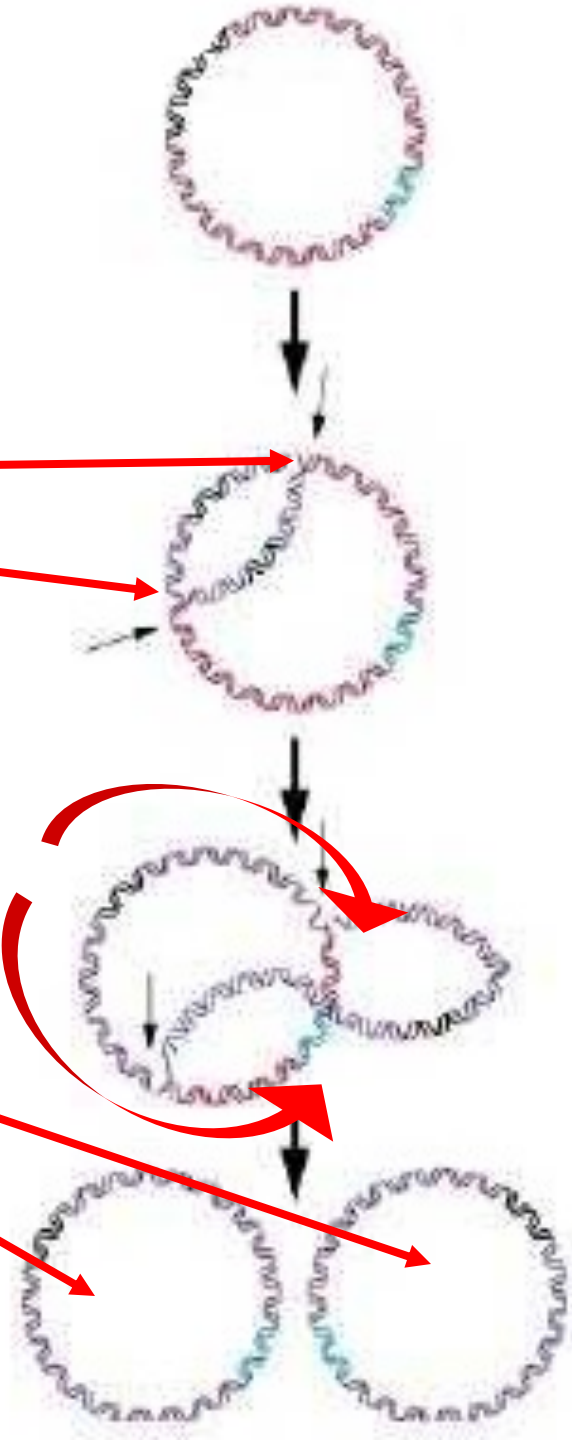
Prokaryotic DNA Replication

- 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*.

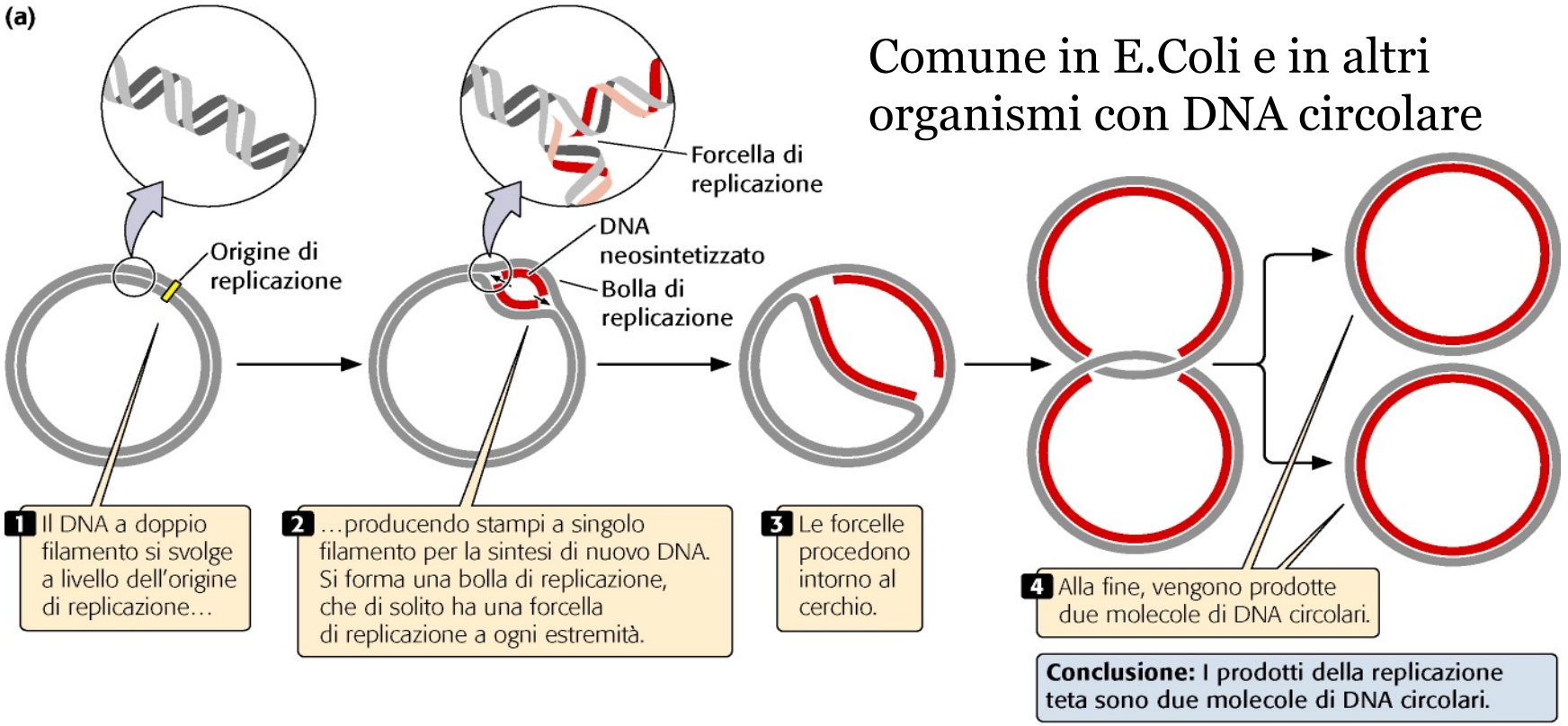


Prokaryotic Replication

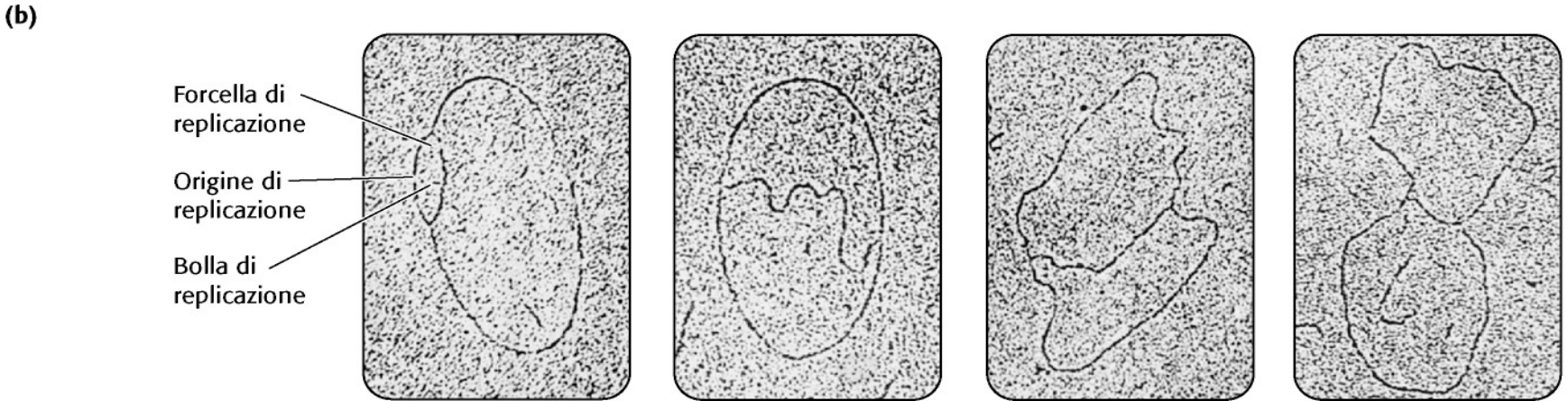
- Two replication forks begin at the origin of replication.
- Replication occurs in opposite directions until the forks meet on the opposite side of the loop.
- The result is two identical loops of DNA.



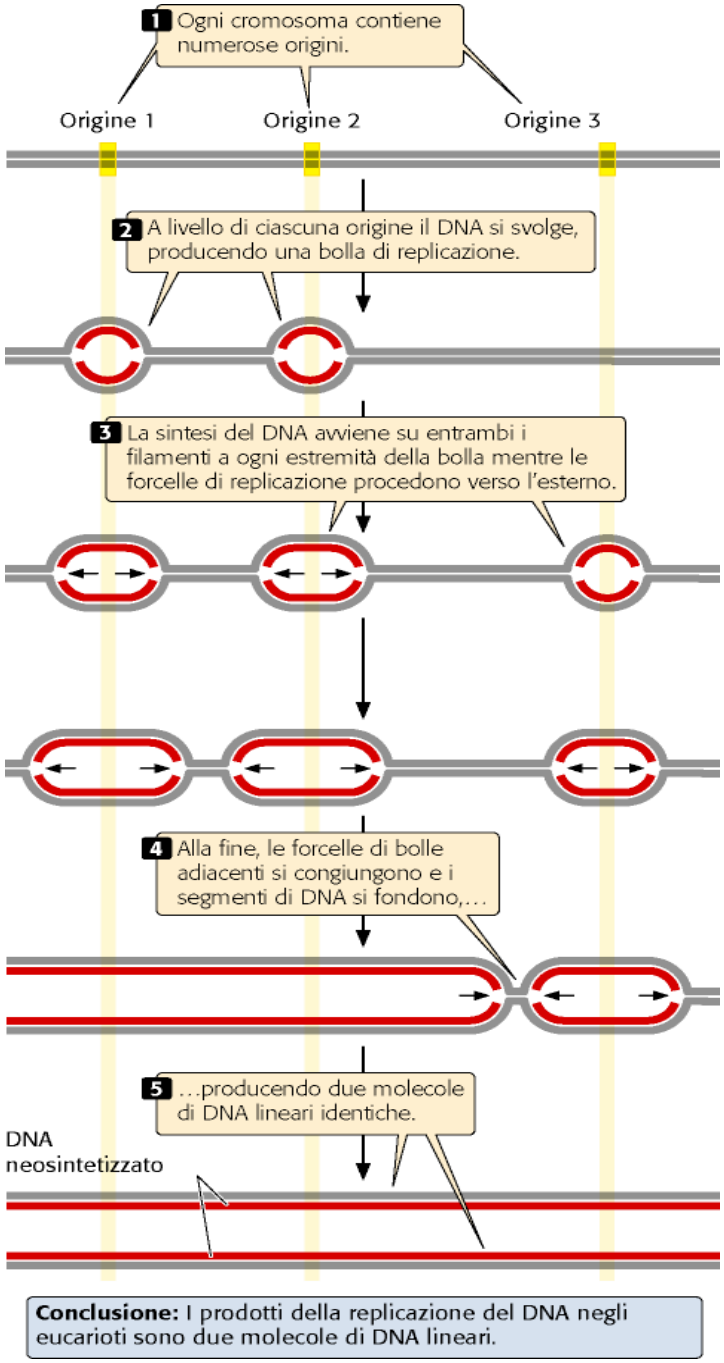
1. Replicazion teta



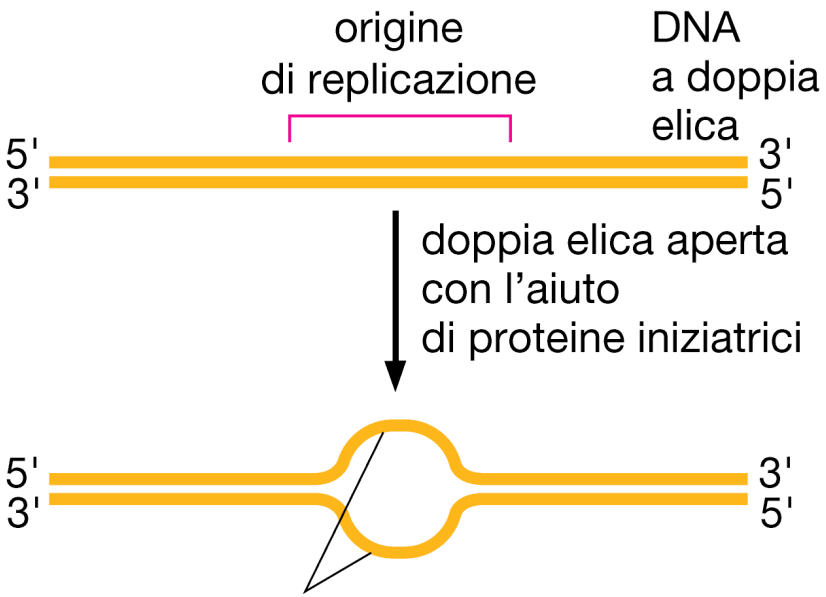
Comune in E.Coli e in altri organismi con DNA circolare

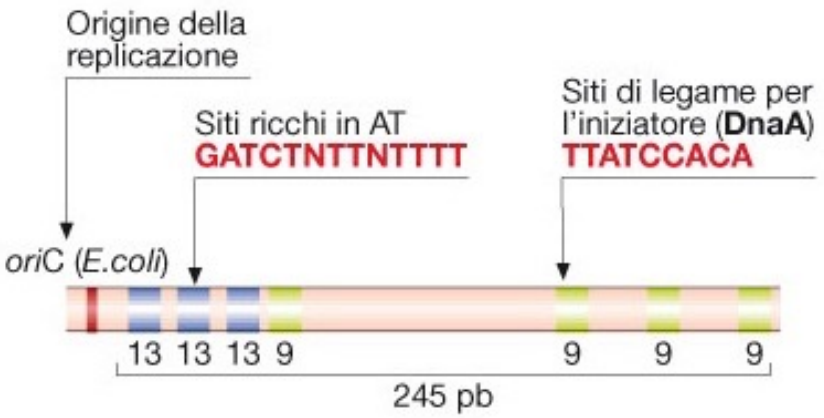


2. Linear replication (eukaryotes)



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



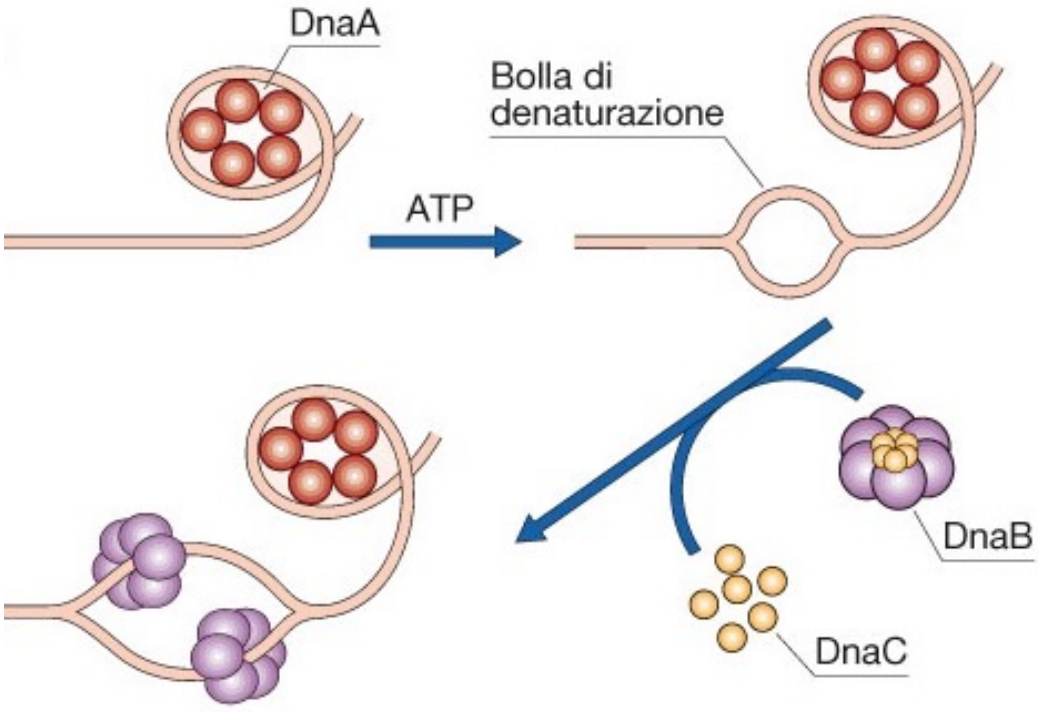


L'origine minima di E.Coli e' lunga solo 245 bps.
E' una regione ricca di A e T: contiene 4 ripetizioni di 9 pb e 3 di 13 pb molto simili tra loro.
Le corte sequenze ripetute sono riconosciute da proteine specifiche nelle fasi iniziali della replicazione.

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.



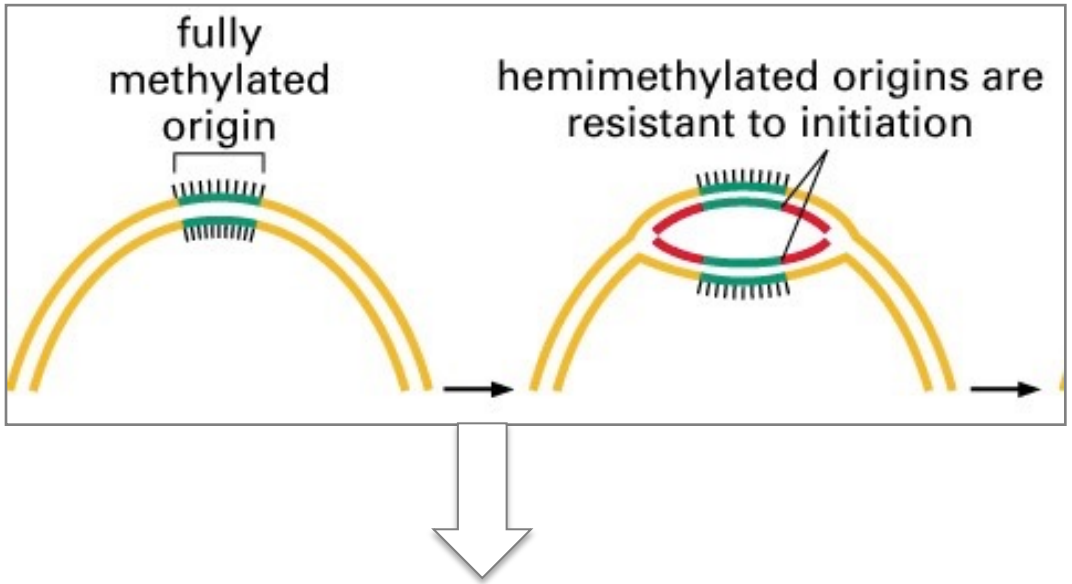
Inizio della replicazione

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!

Inizio della replicazione

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

2 Livelli di regolazione:

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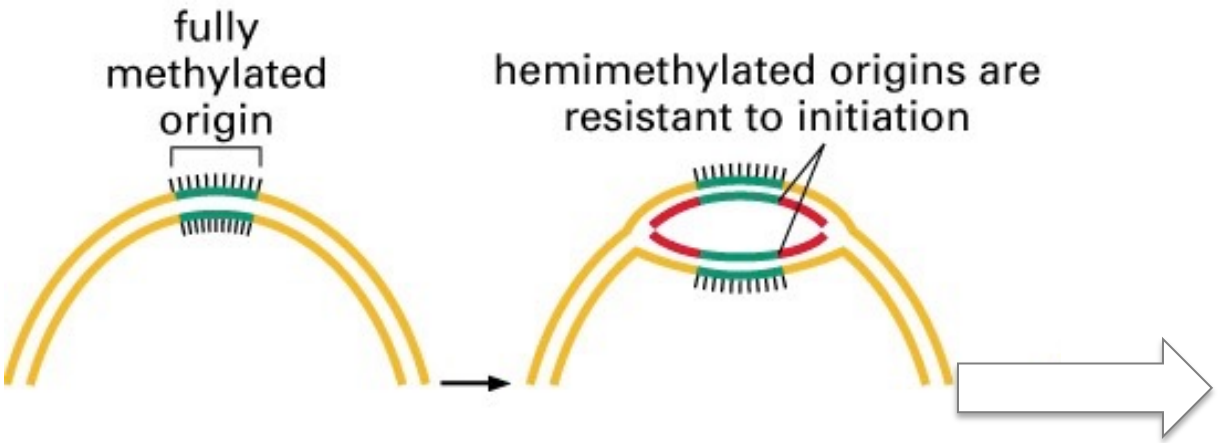
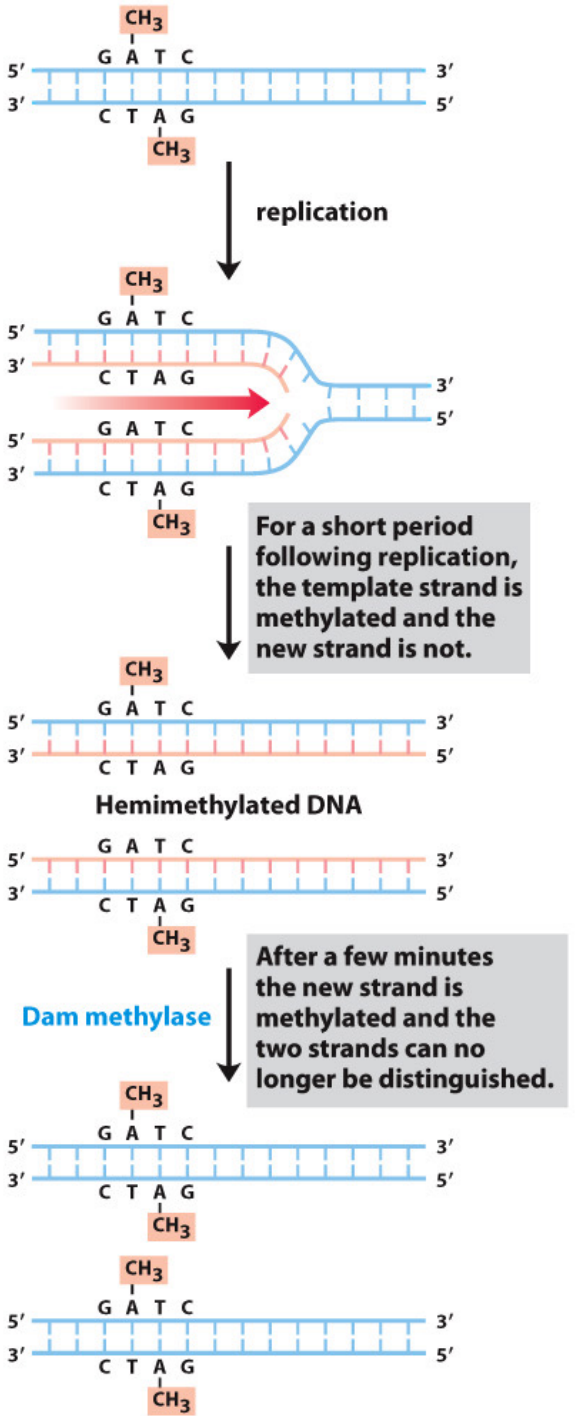


Nello stato emi-metilato (transitorio!) non può funzionare da origine di replicazione, non viene usata 2 volte!

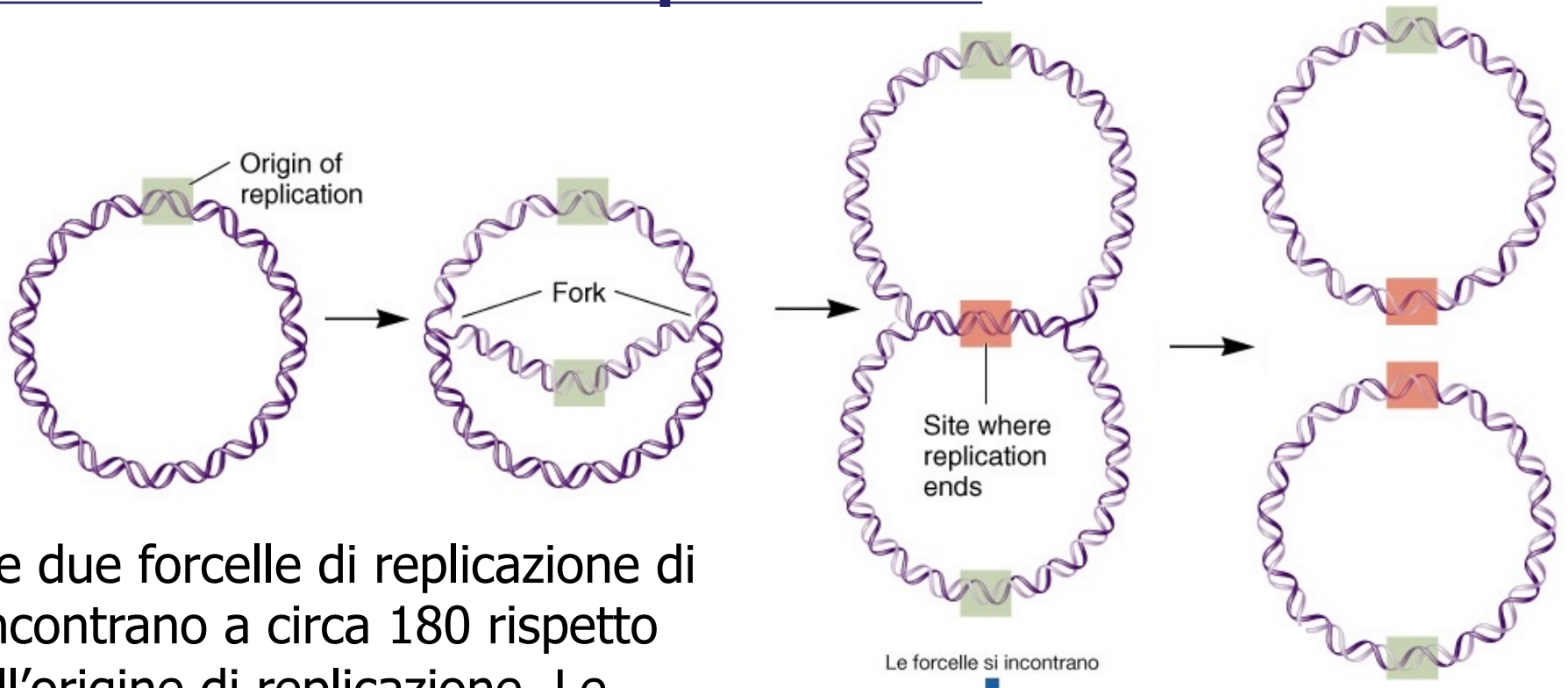
Bacterial DNA Methylation

- 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

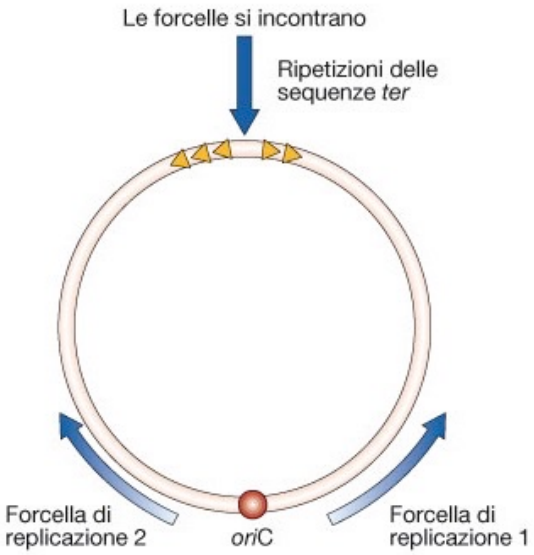


Terminazione della replicazione.

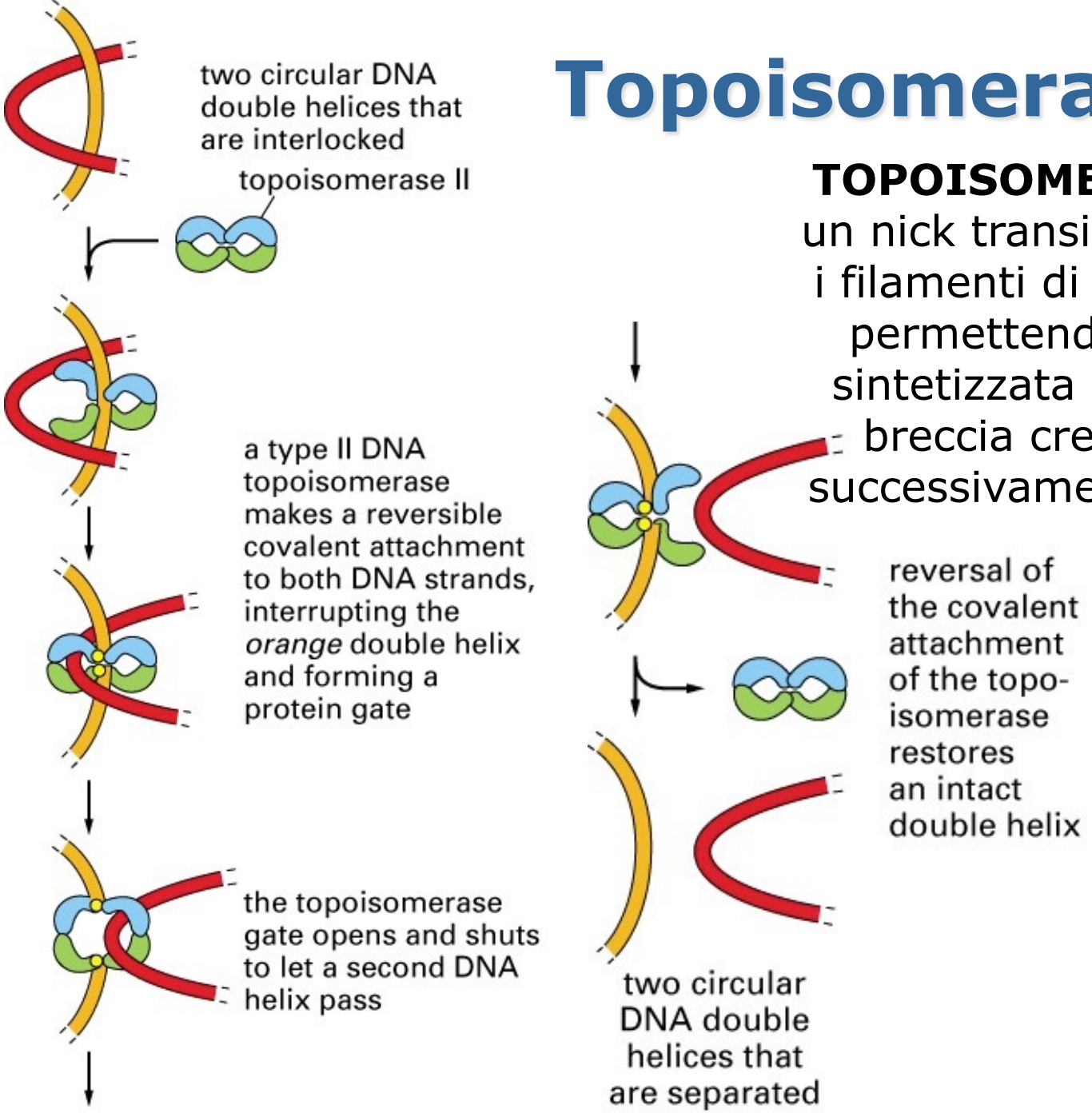


Le due forcelle di replicazione di incontrano a circa 180 rispetto all'origine di replicazione. Le sequenze ***ter***, di 23 bp, vengono legate dalle **TBP** (Ter binding proteins) e fanno terminare le forcelle di replicazione.

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

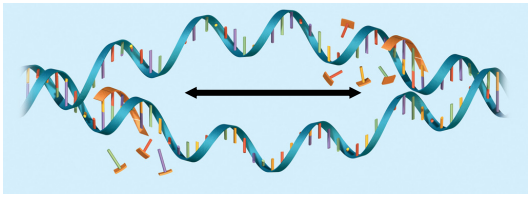
DNA Replication: THE STEPS

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.

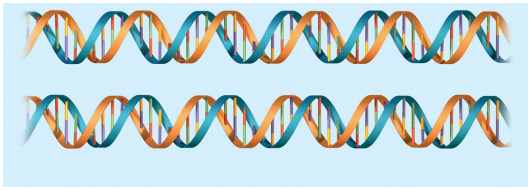
The three steps are:

1. Unwinding and Separating DNA Strands



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

3. Formation of Two Identical DNA molecules



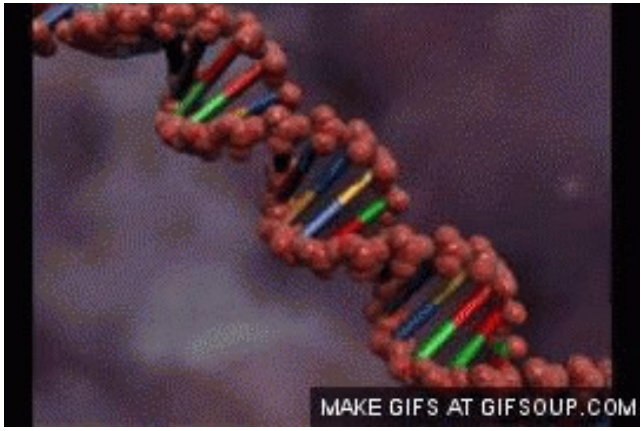
STEP1

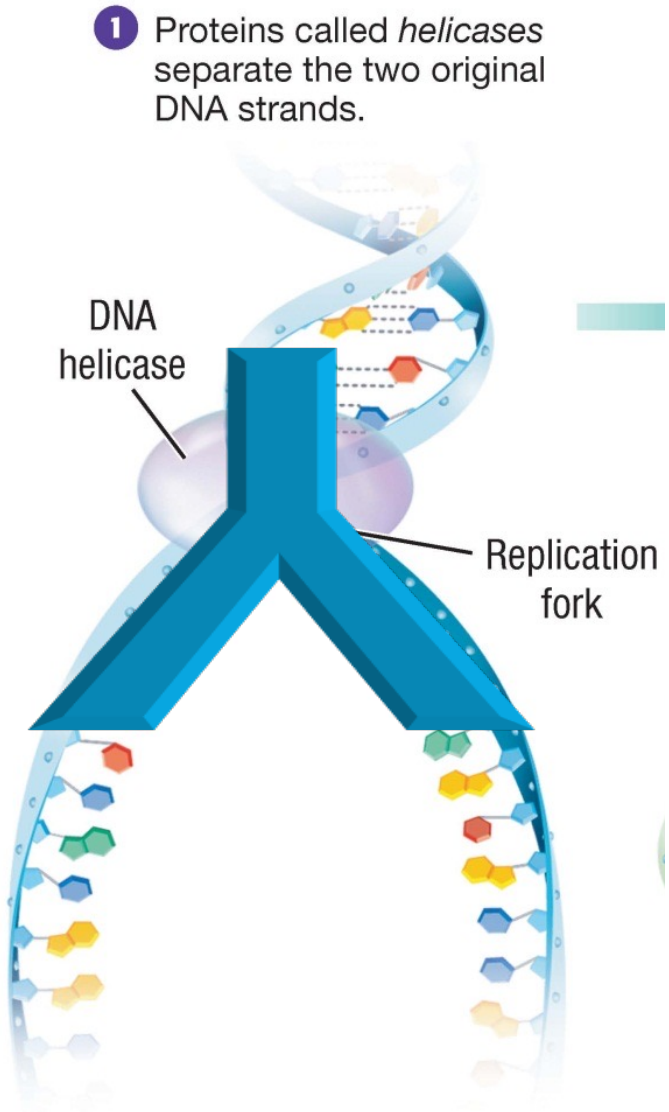
DNA helicases unwinds & separates the original DNA double helix.

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.



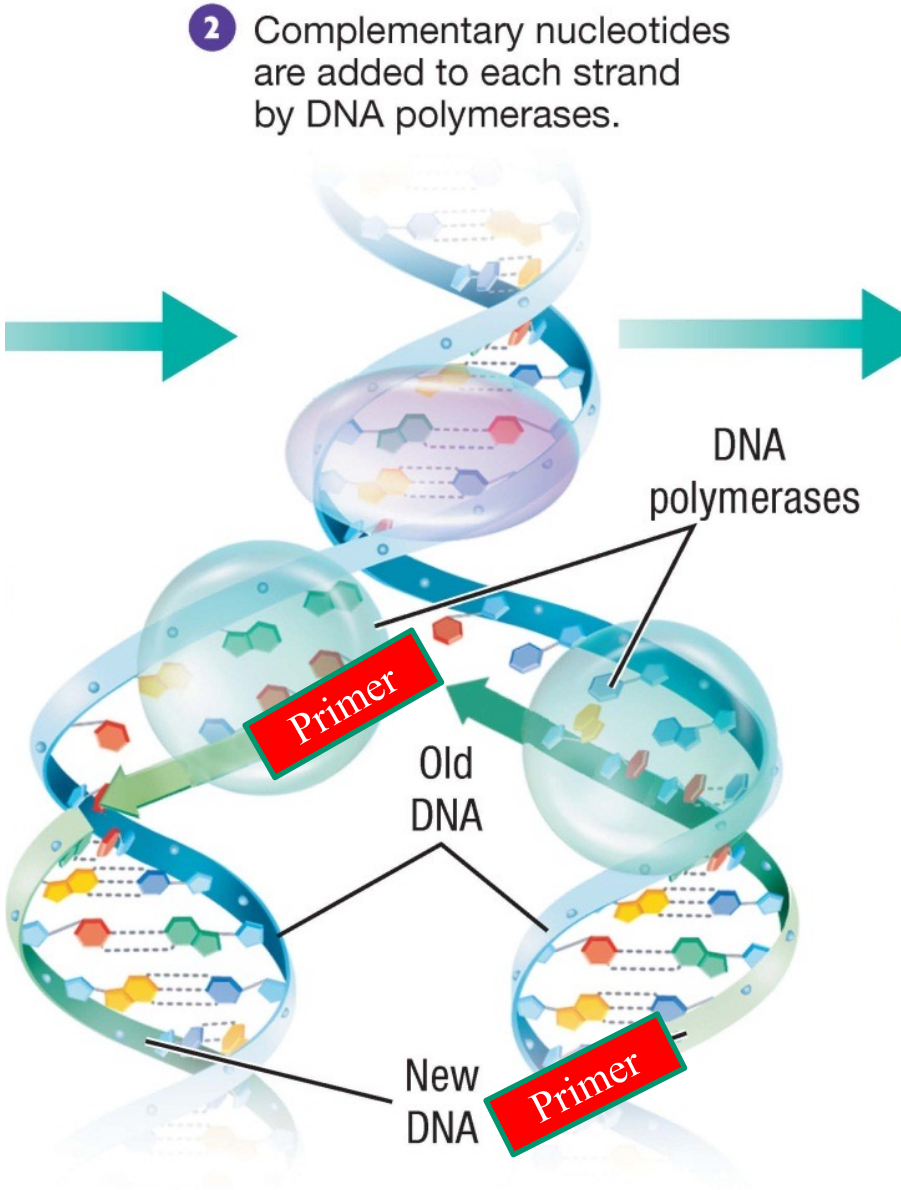


STEP2

New DNA is formed from DNA template.

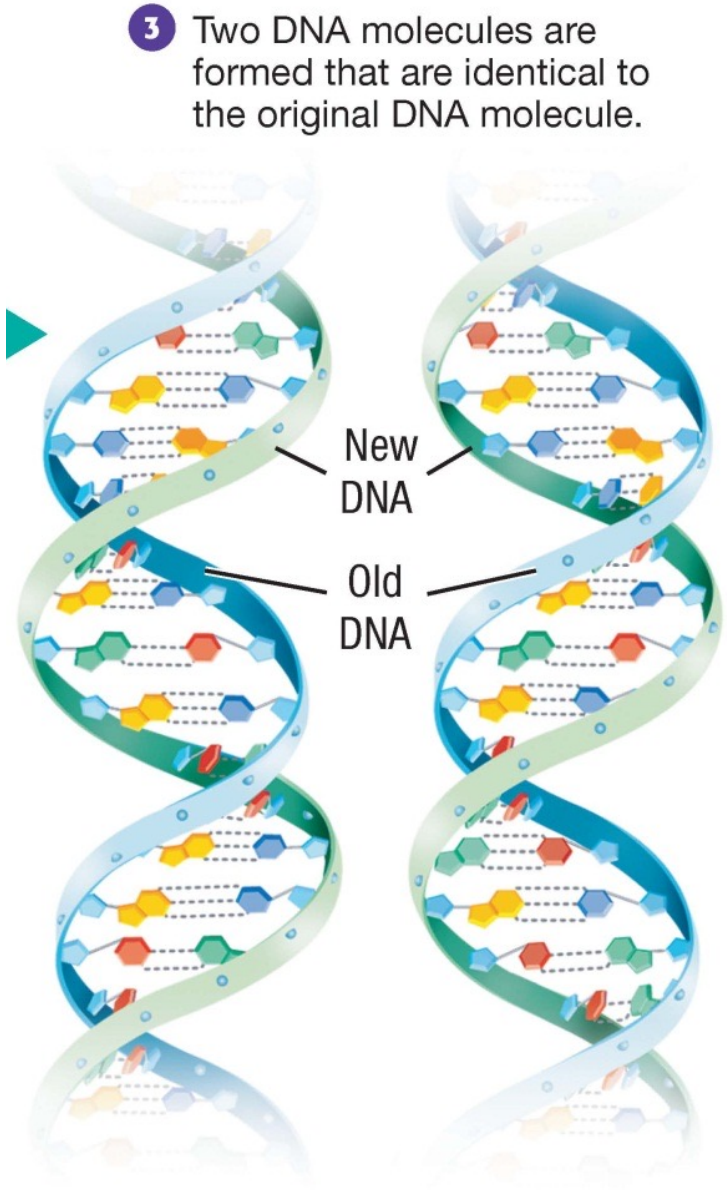
RNA Primers attach to specific regions.

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



STEP3

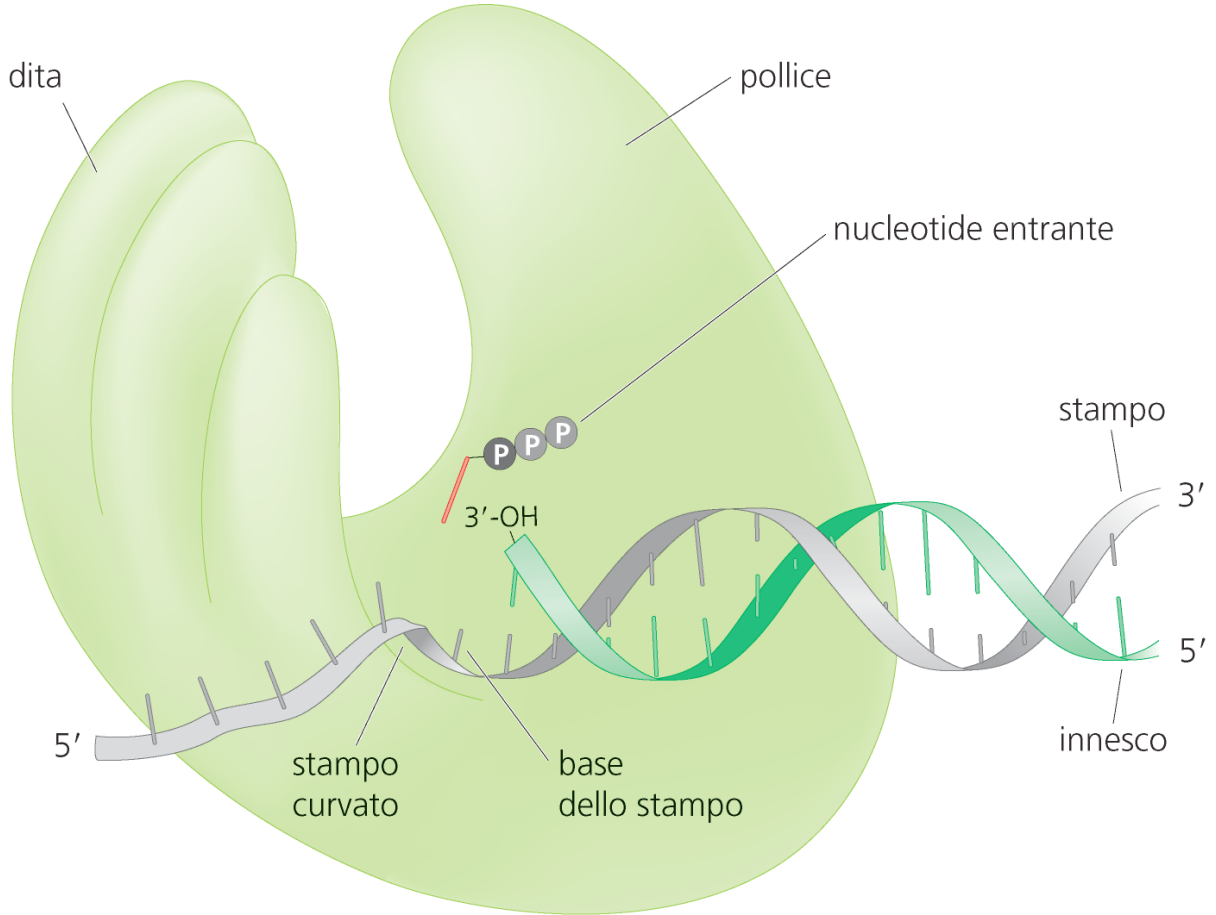
- The process completes when all the original bases have been paired with a new complementary nucleotide.
- Each double-stranded DNA helix is made of one new strand of DNA and one original strand of DNA.



Le DNA Polimerasi

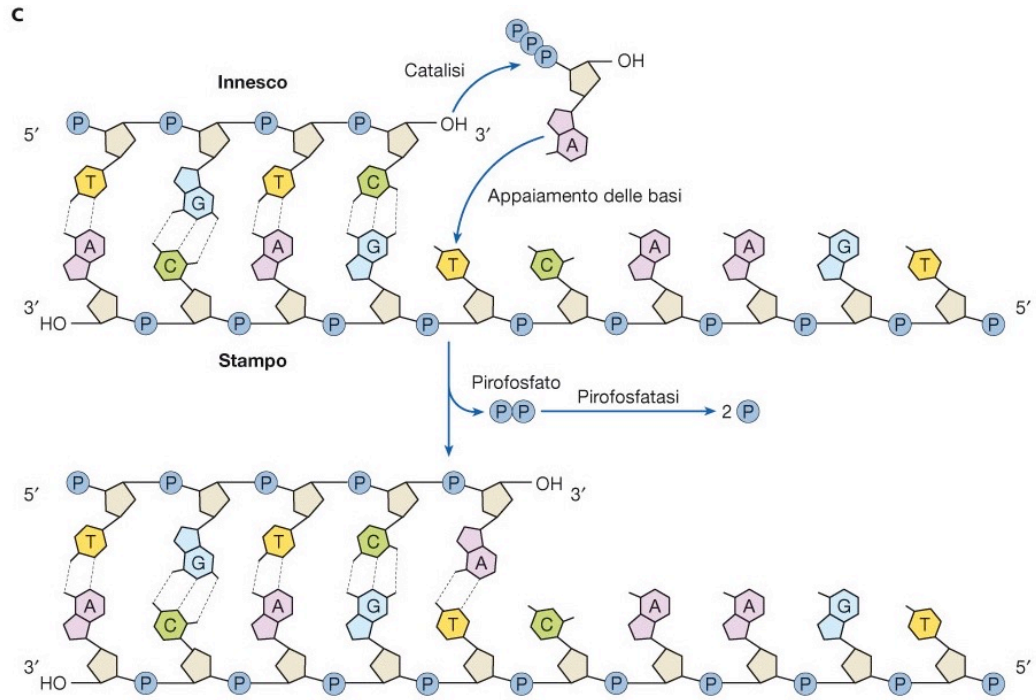
La DNA polimerasi e' un complesso multimerico formato da diverse unita' enzimatiche con diverse attivita' catalitiche.

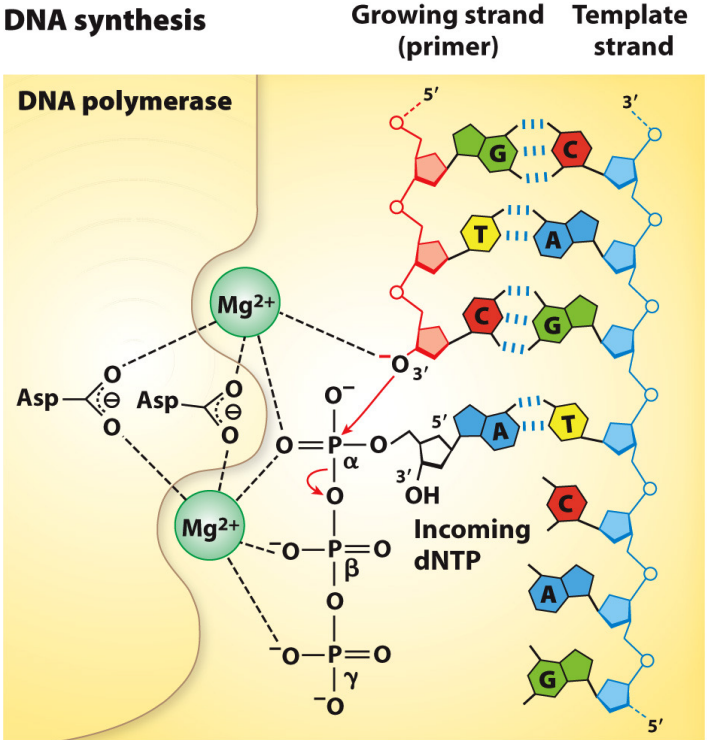
Tutte le DNA polimerasi condividono la stessa anatomia molecolare, paragonabile a quella di una mano destra. Il complesso "innesco-stampo" attraversa il "palmo", dove viene controllata l'accuratezza dell'appaiamento tra le basi, in una scanalatura creata dalle "dita" e dal "pollice".



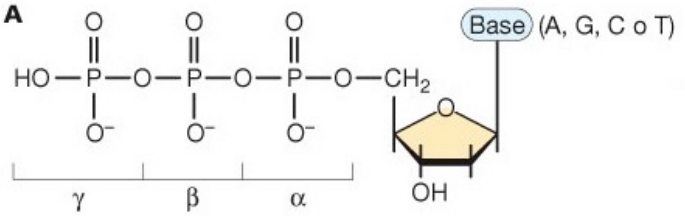
L'azione della DNA Polimerasi

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.

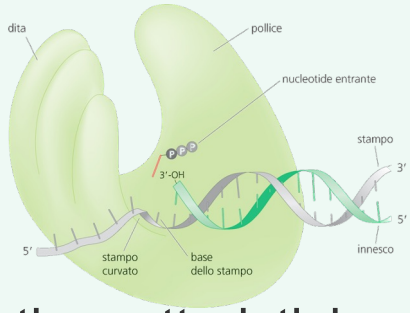




I nucleotidi entrano come nucleosidi trifosfati, apportando l'energia necessaria per la polimerizzazione, fornita dall'idrolisi di un legame fosfoanidride, con liberazione di pirofosfato. Il pirofosfato viene poi idrolizzato ulteriormente a fosfato inorganico, rendendo la reazione del tutto irreversibile.



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.

There Are at Least Five DNA Polymerases in *E. Coli*

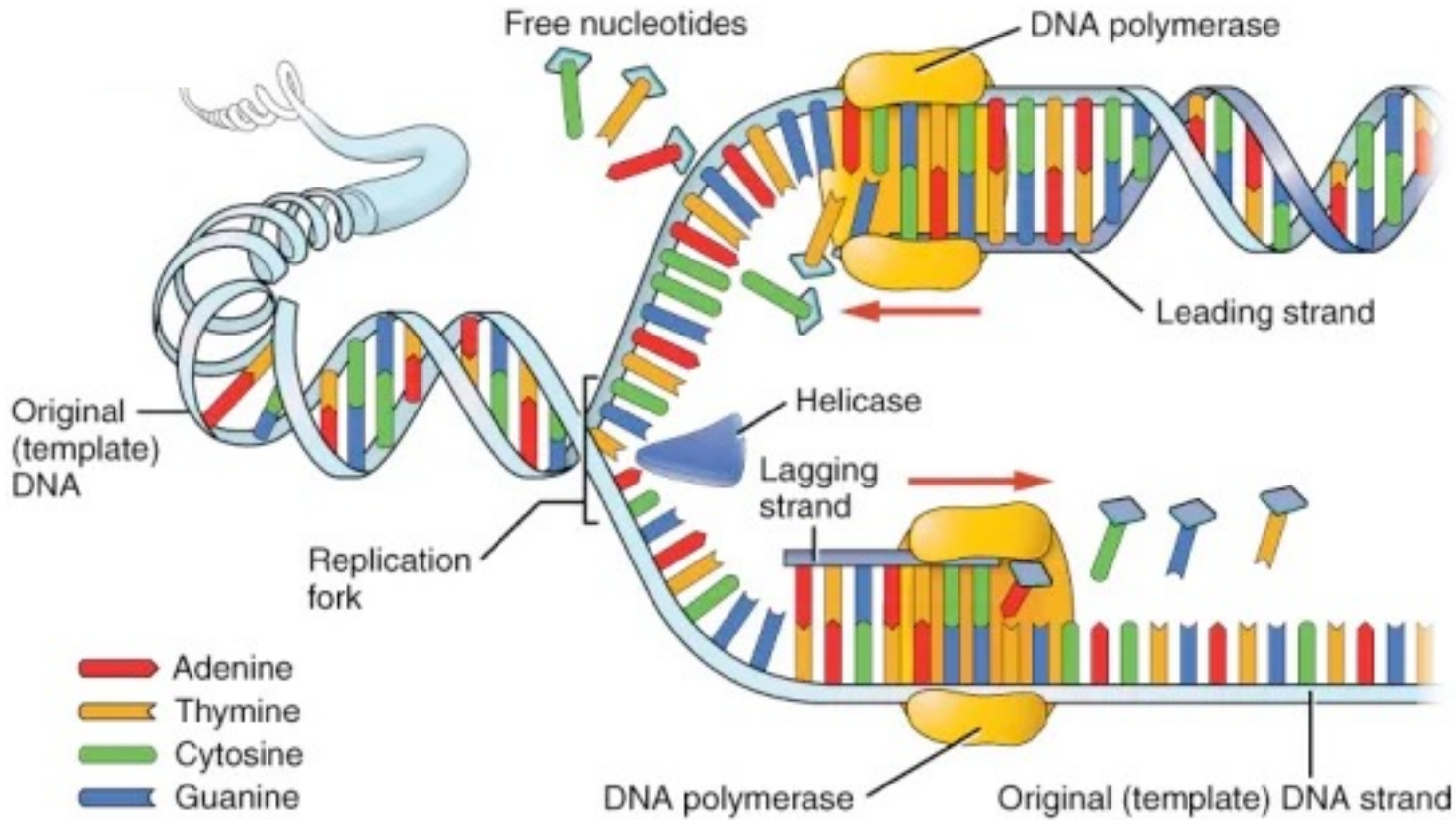
- DNA polymerase I is abundant but is not ideal for replication.
  - rate (600 nucleotides/min) is slower than observed for replication fork movement
  - has low processivity
  - primary function is in clean-up
- DNA polymerase III is the principal replication polymerase.
- DNA polymerases II, IV, and V are involved in DNA repair.

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.

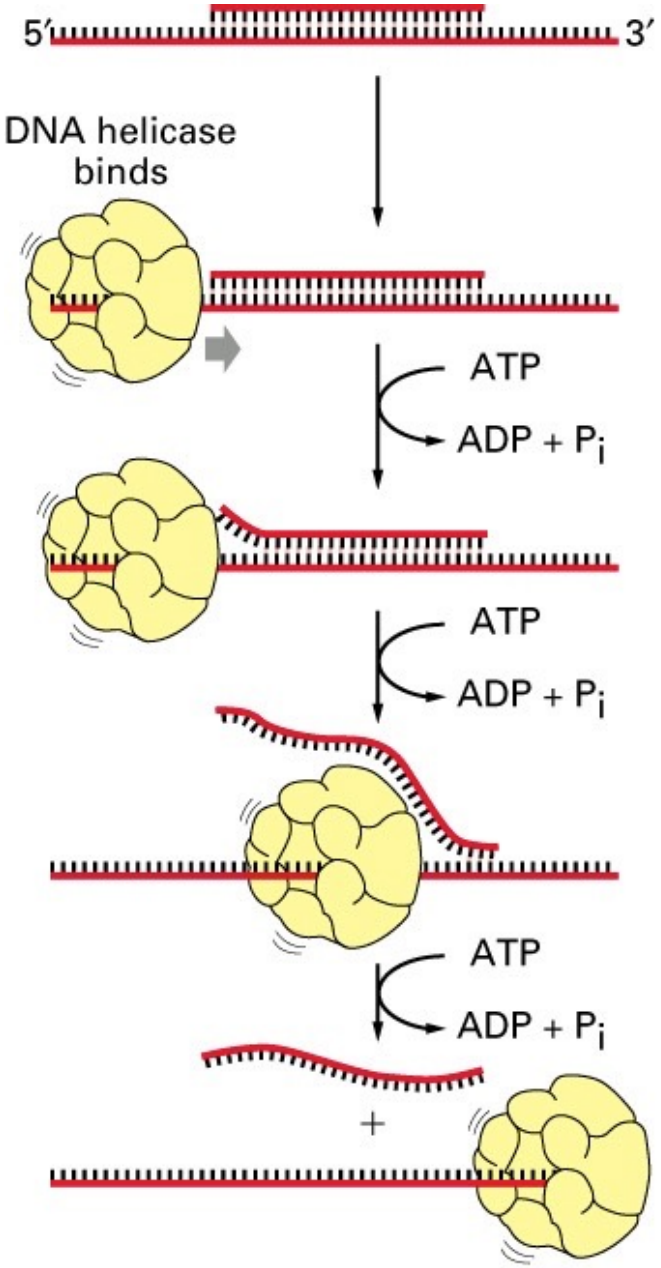


Special proteins help to open up double helix in front of the replication fork

* 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.

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

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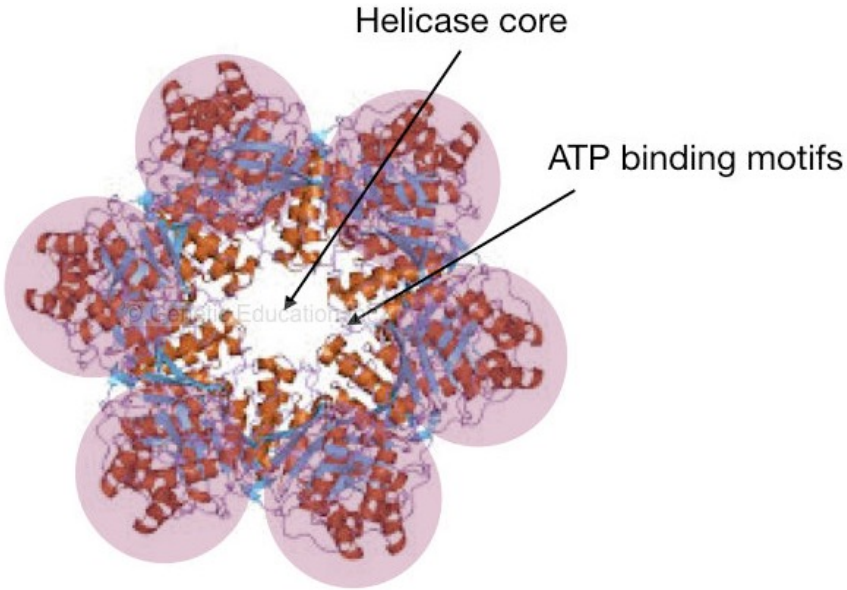
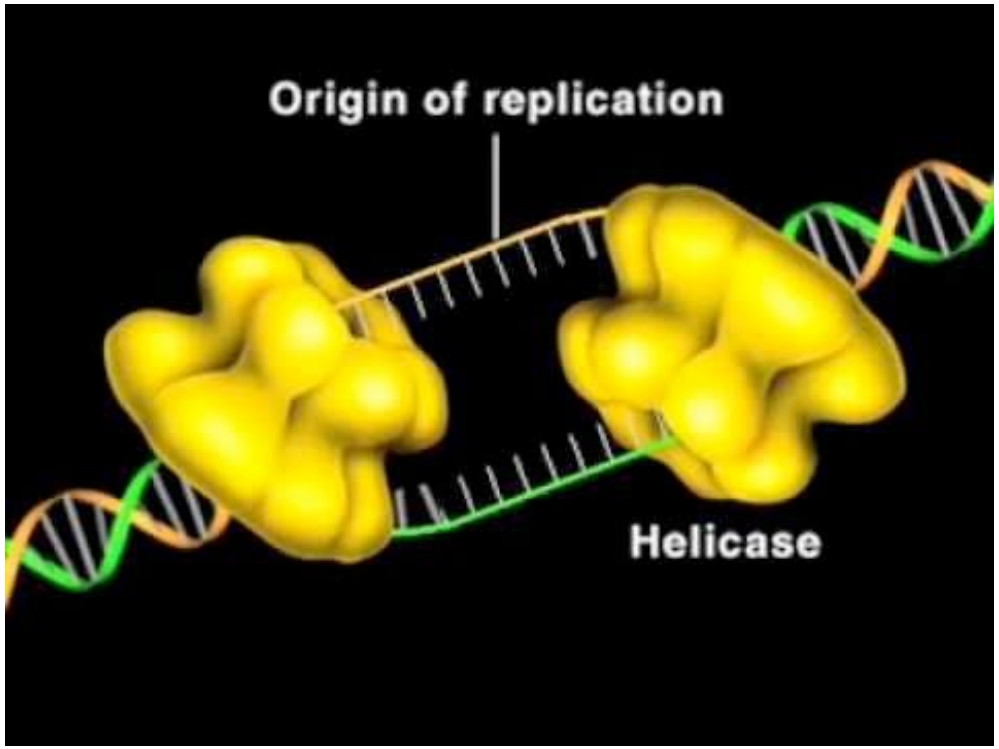
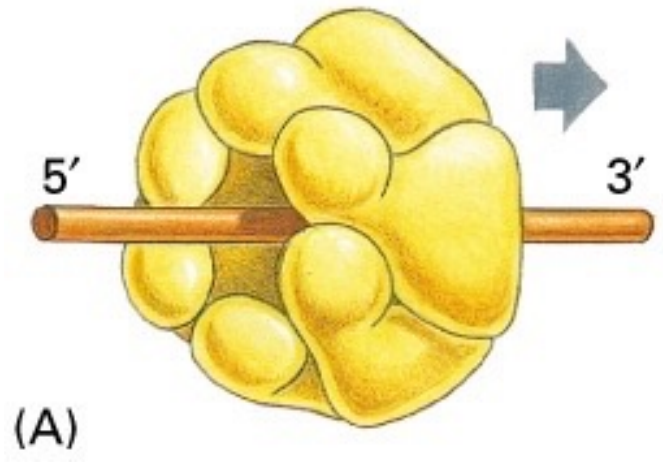




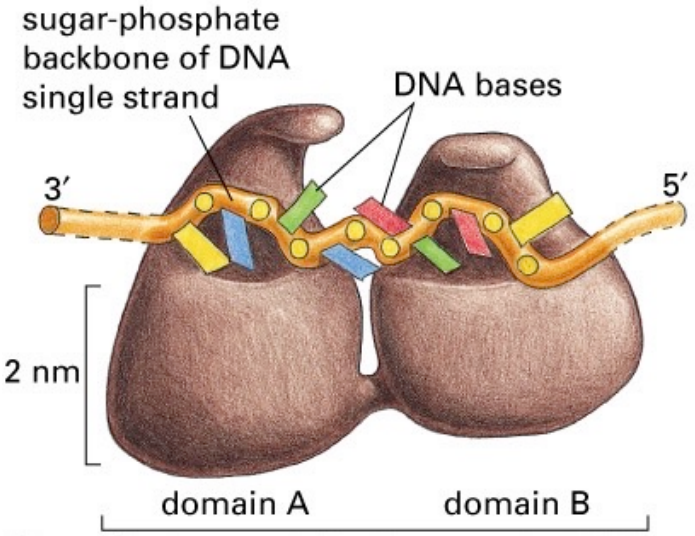
Hexameric structure of DNA helicase

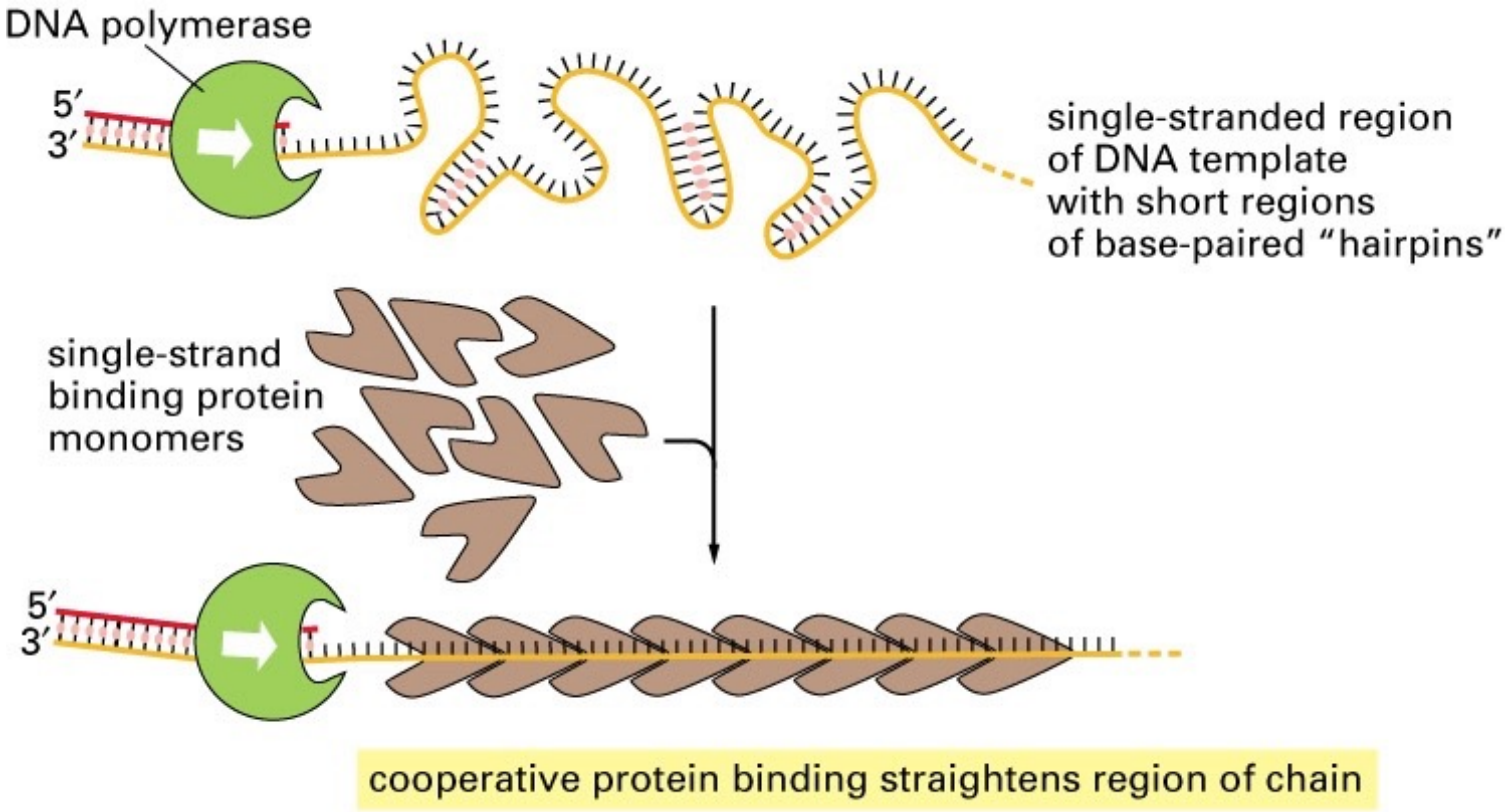


SSB proteins

SSB proteins bind tightly to ssDNA without covering the bases.

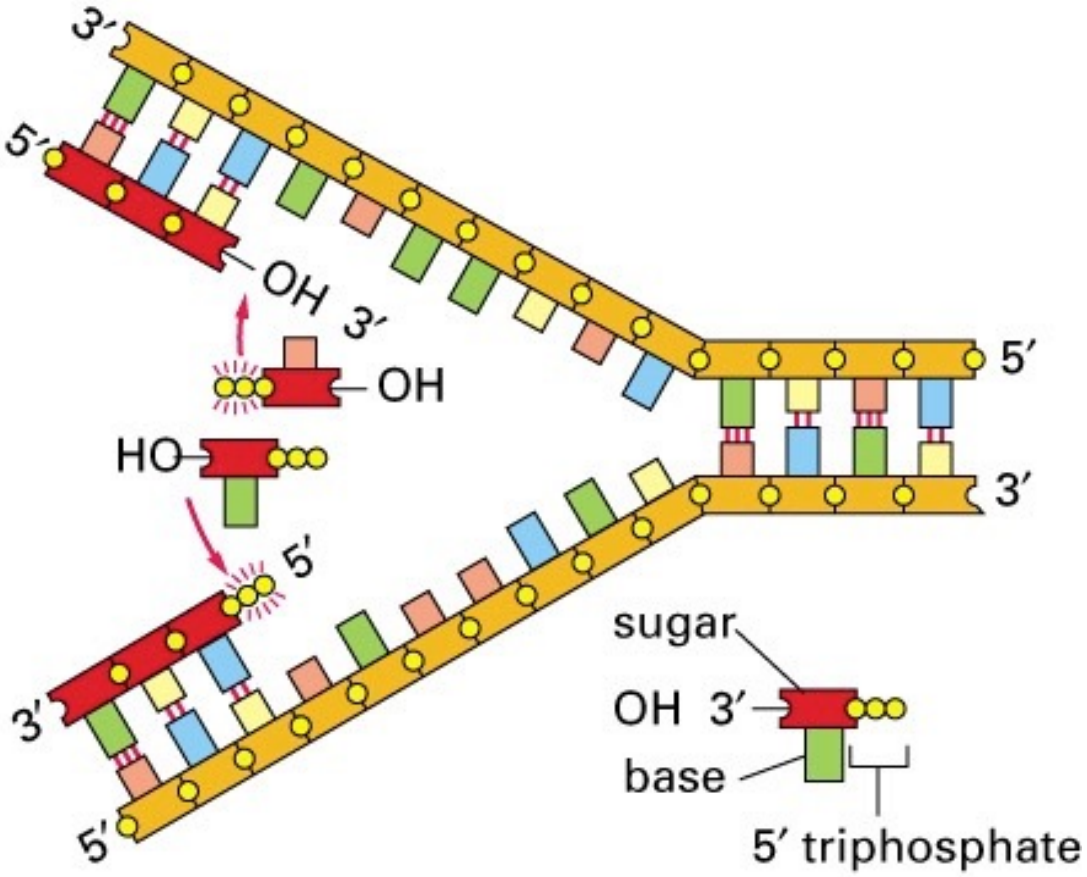
SSB proteins are unable to open a DNA helix but aid helicases by stabilizing the unwound ss conformation





La Forcella di Replicazione

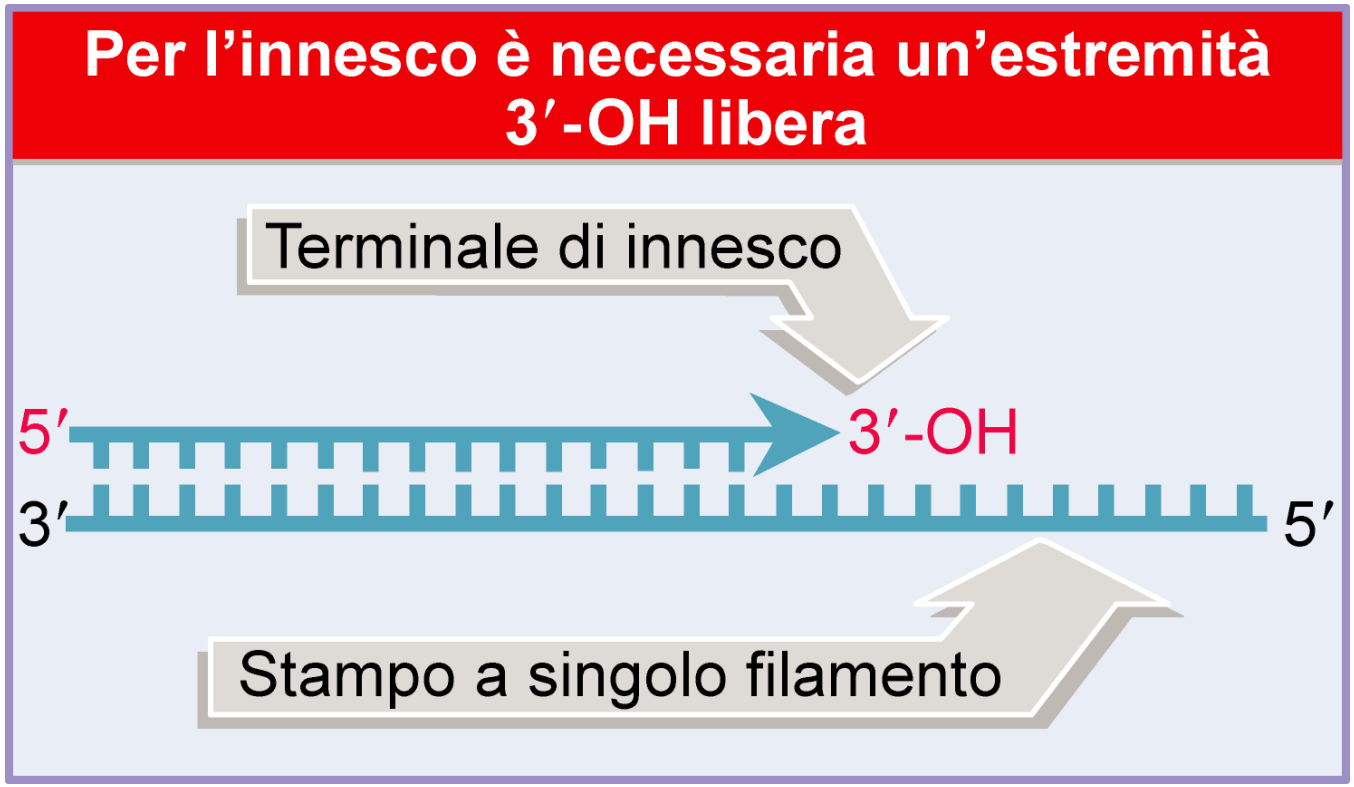
I singoli filamenti fungono da stampo per la sintesi di un nuovo filamento complementare.



MA

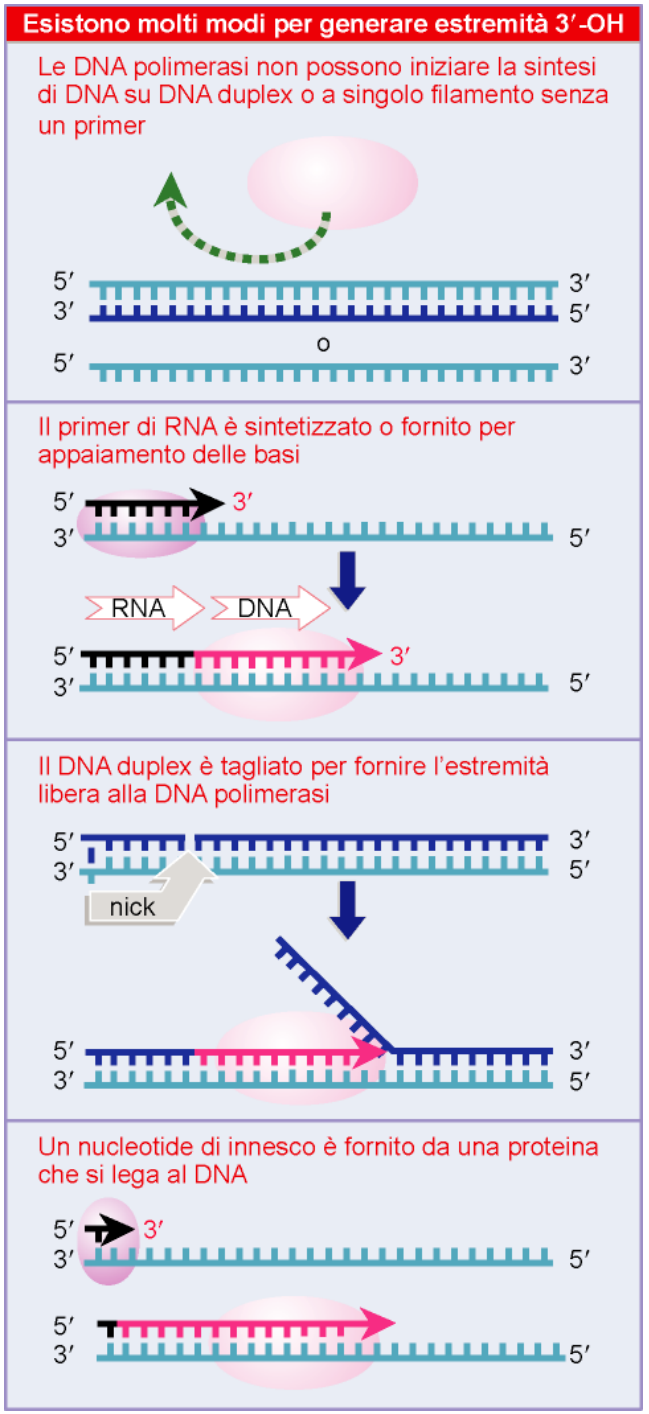
La DNA polimerasi ha attivita' di sintesi ESCLUSIVAMENTE in direzione 5' ->3 ':
problema di sintesi continua di uno dei due filamenti

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



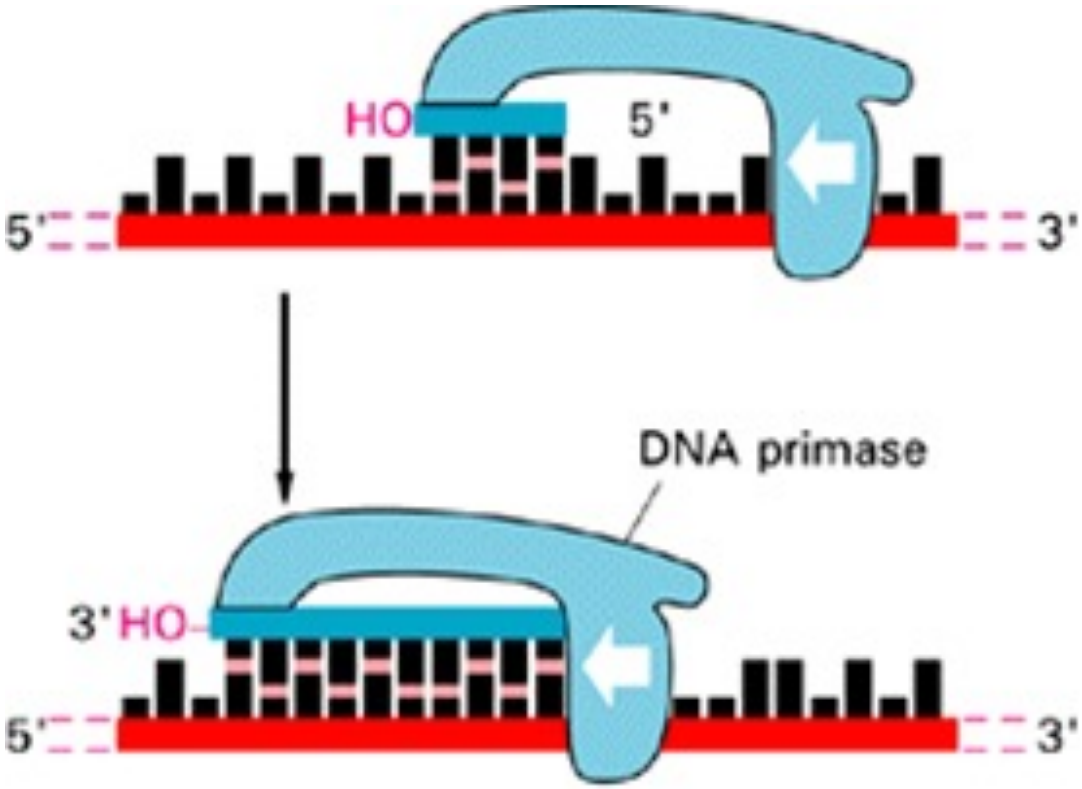
Le DNA Polimerasi richiedono un'estremita' di innesco 3'-OH per iniziare la sintesi del DNA.

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

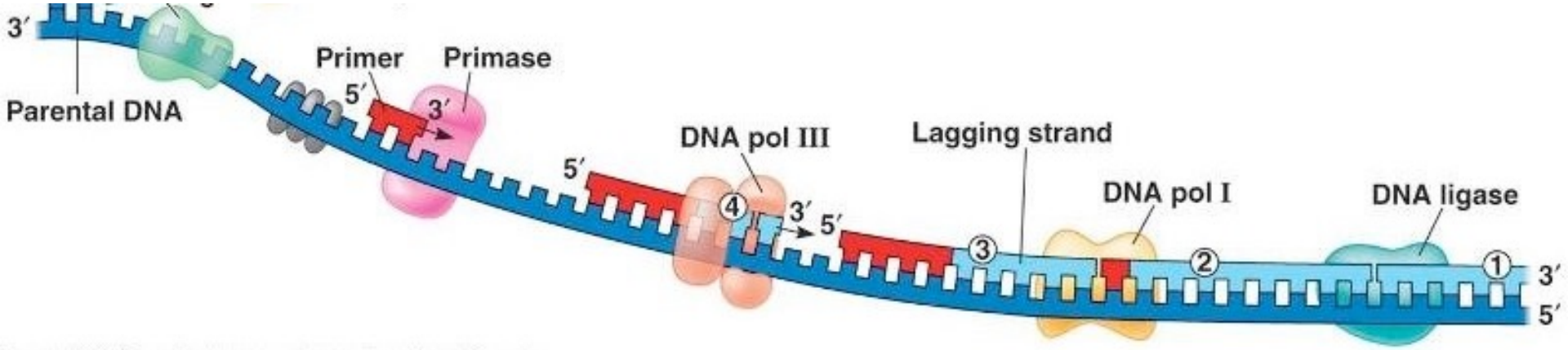


La Primasi

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.



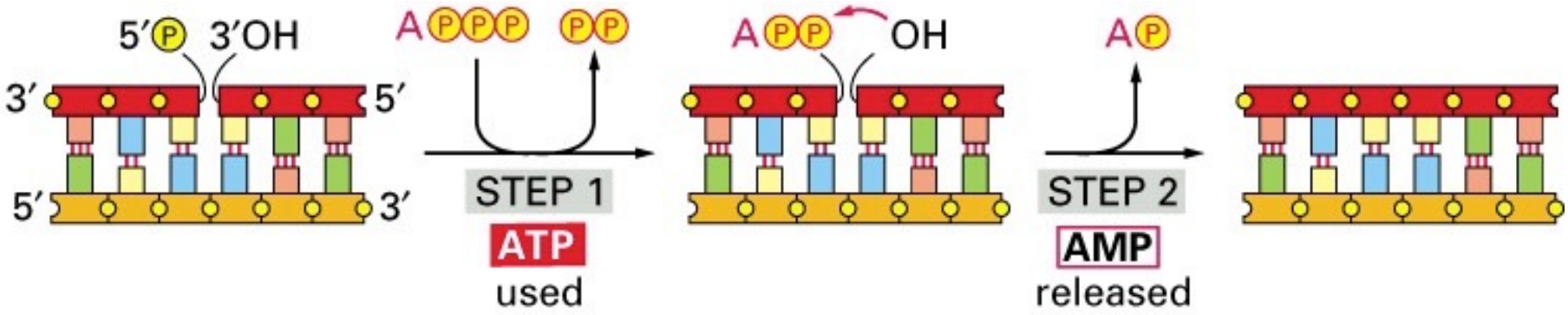
I frammenti di Okasaki

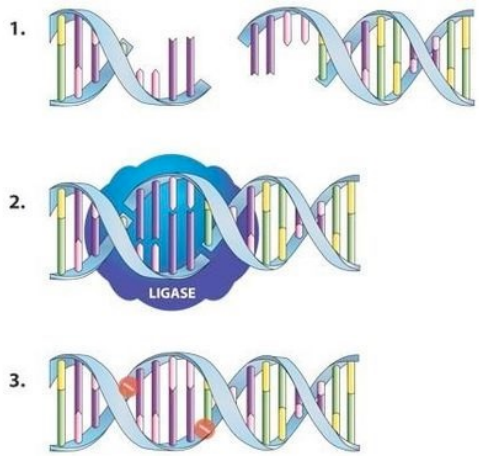


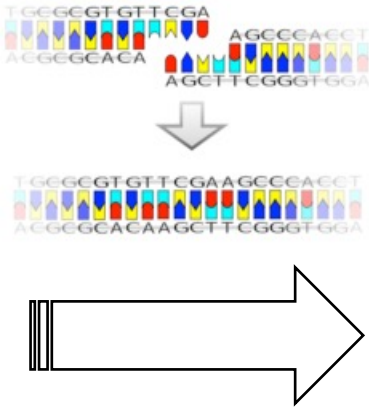
- ✓ I frammenti di Okazaki sono sintetizzati dalla DNA polymerasi III a partire degli inneschi creati dalla Primasi.
- ✓ La DNA polymerasi non e' in grado di unire i frammenti.
- ✓ I primers a RNA devono essere sostituiti, in quanto le basi U devono essere sostituite da T.
- ✓ I primers vengono degradati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



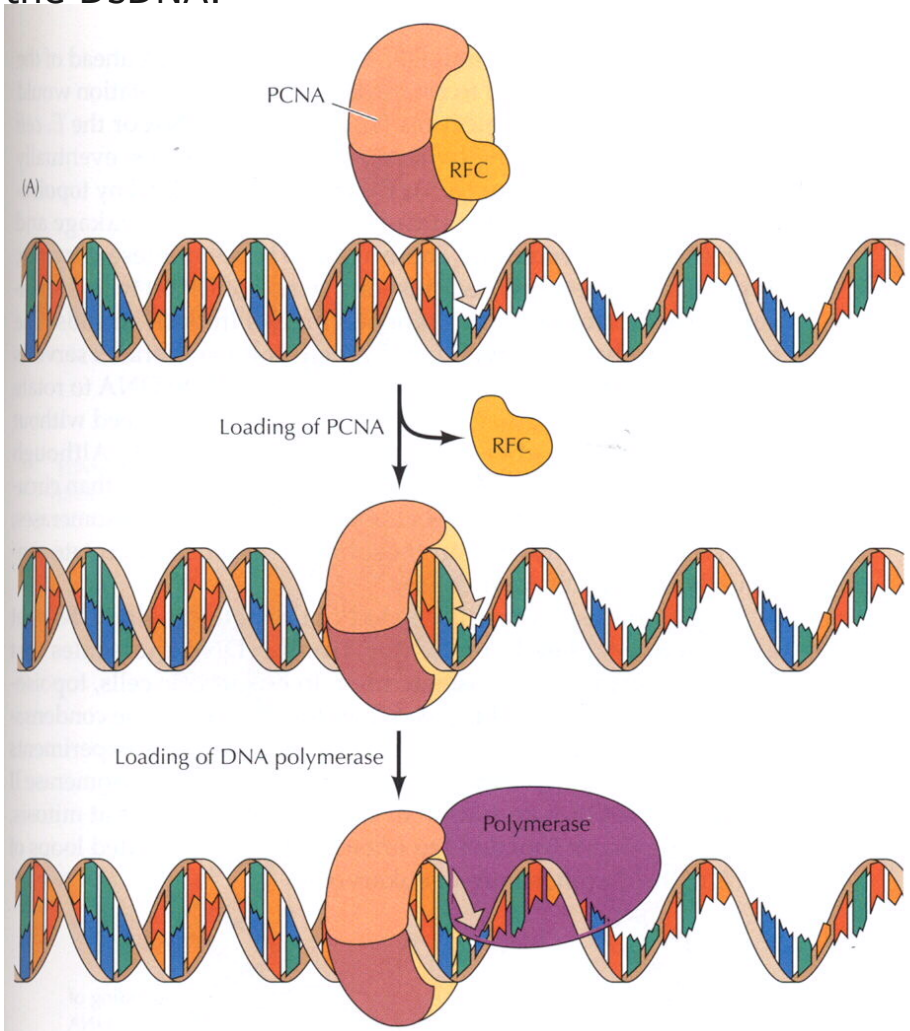


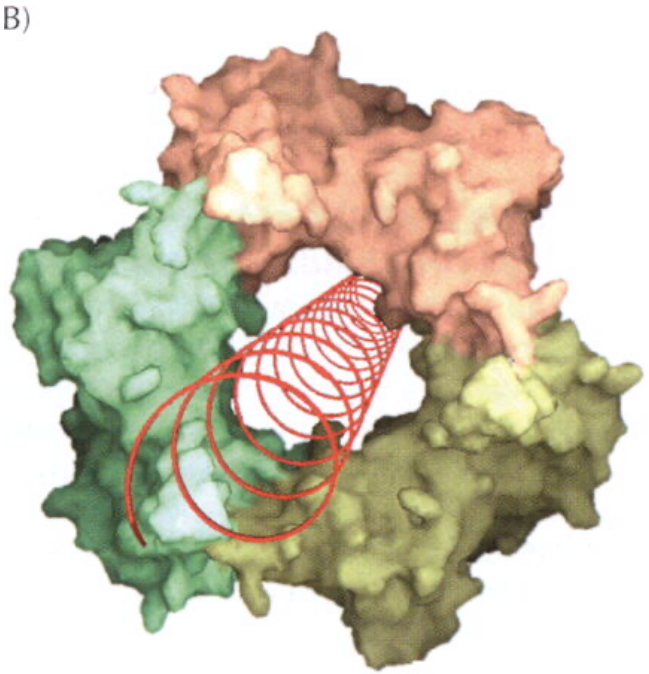


Stabilization of the replication machine

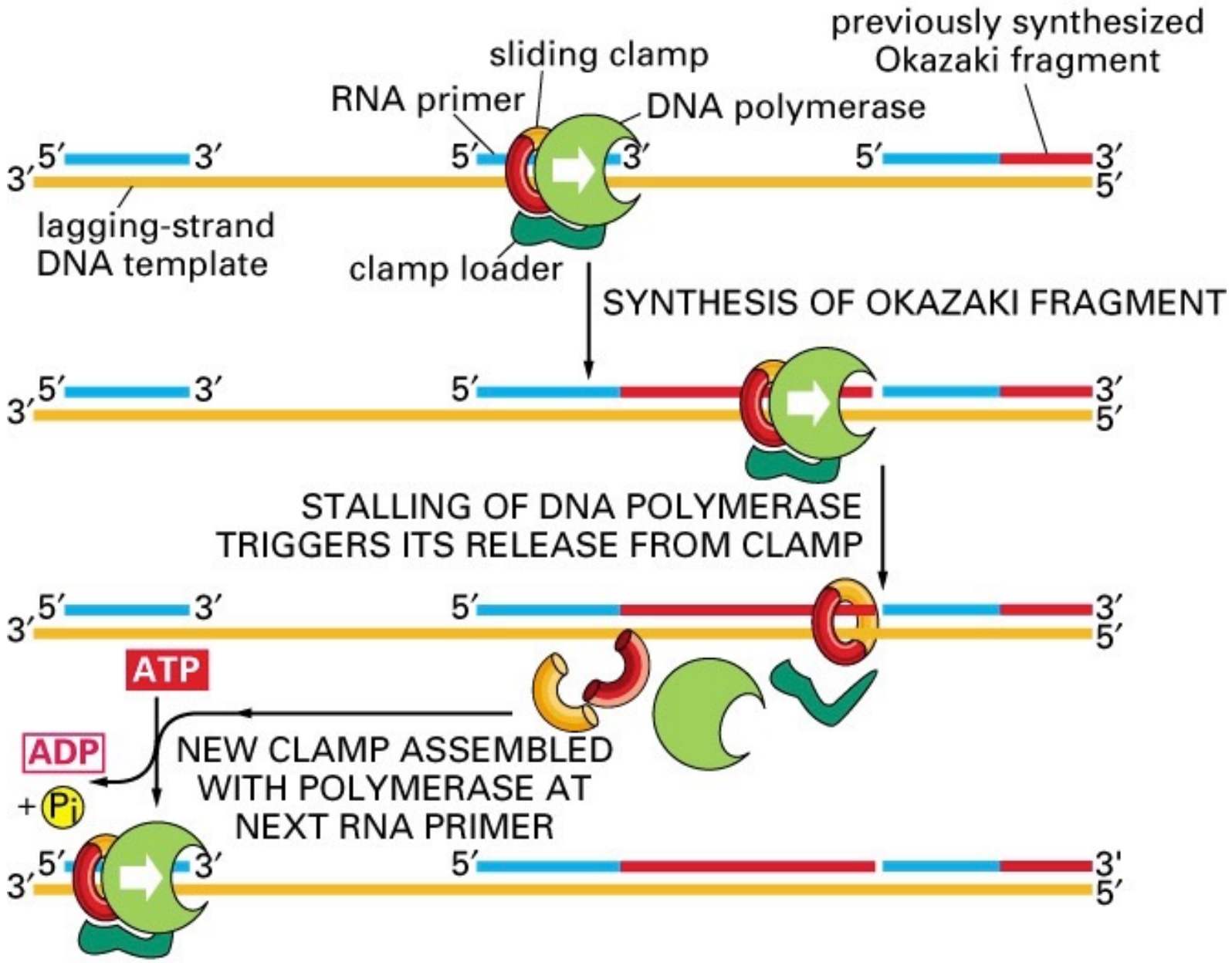
A ring clamp (PCNA) keeps the polymerase firmly on the DNA when it is moving on the dsDNA. One side of the ring binds to the back of the DNApol and it slides along DNA as the polymerase moves on.

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

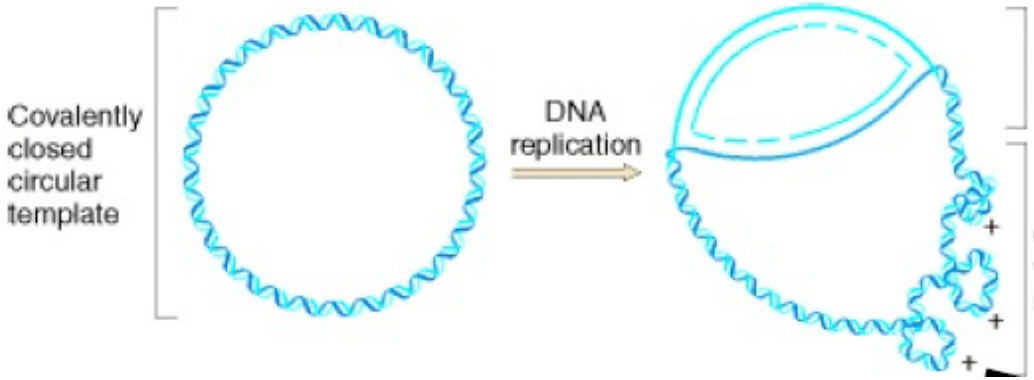




around the primer-template junction requires ATP hydrolysis by a special protein complex, the **clamp loader**

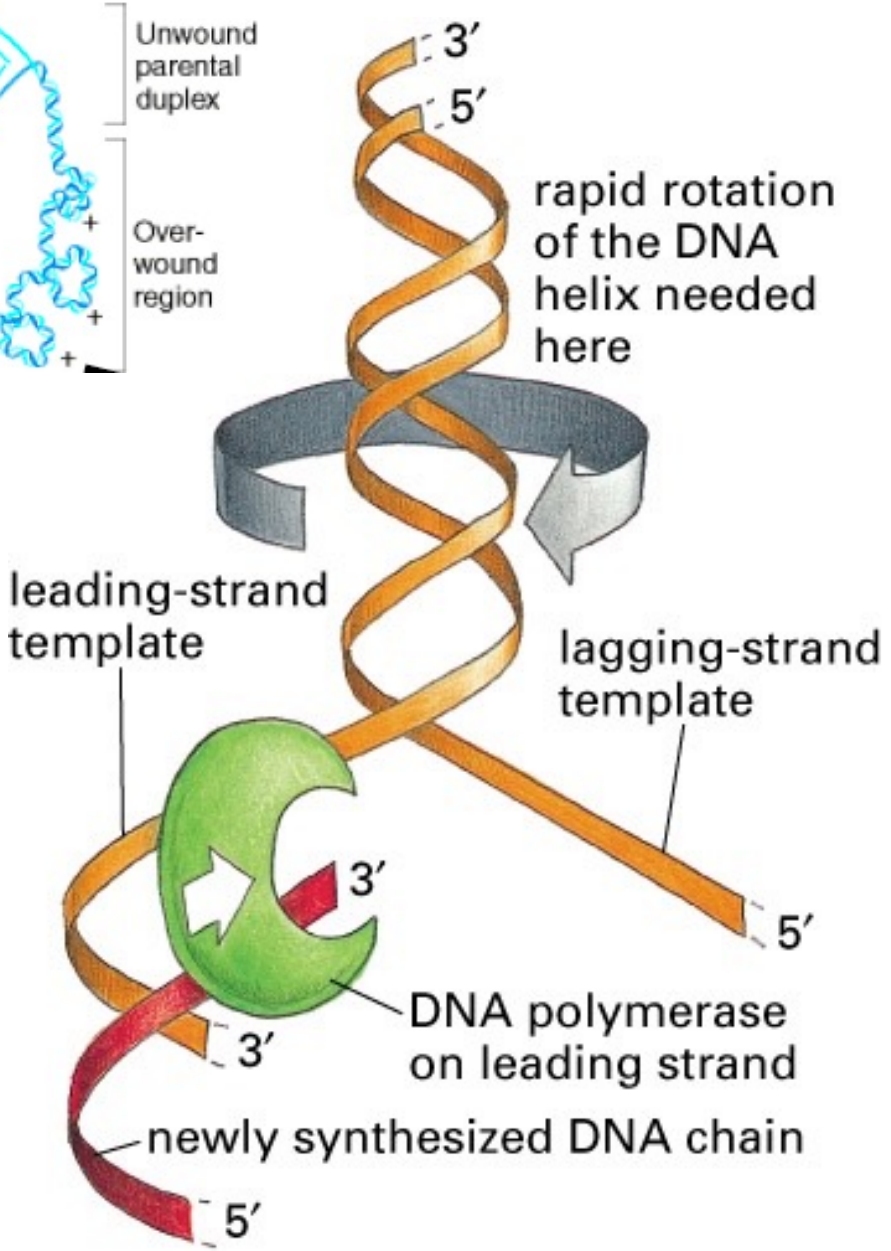

previously synthesized
Okazaki fragment
sliding clamp
RNA primer
DNA polymerase
5′
3′
3′
5′
5′
3′
3′
5′
lagging-strand
DNA template
clamp loader
SYNTHESIS OF OKAZAKI FRAGMENT
STALLING OF DNA POLYMERASE
TRIGGERS ITS RELEASE FROM CLAMP
ATP
ADP
+ Pi
NEW CLAMP ASSEMBLED
WITH POLYMERASE AT
NEXT RNA PRIMER

The winding problem



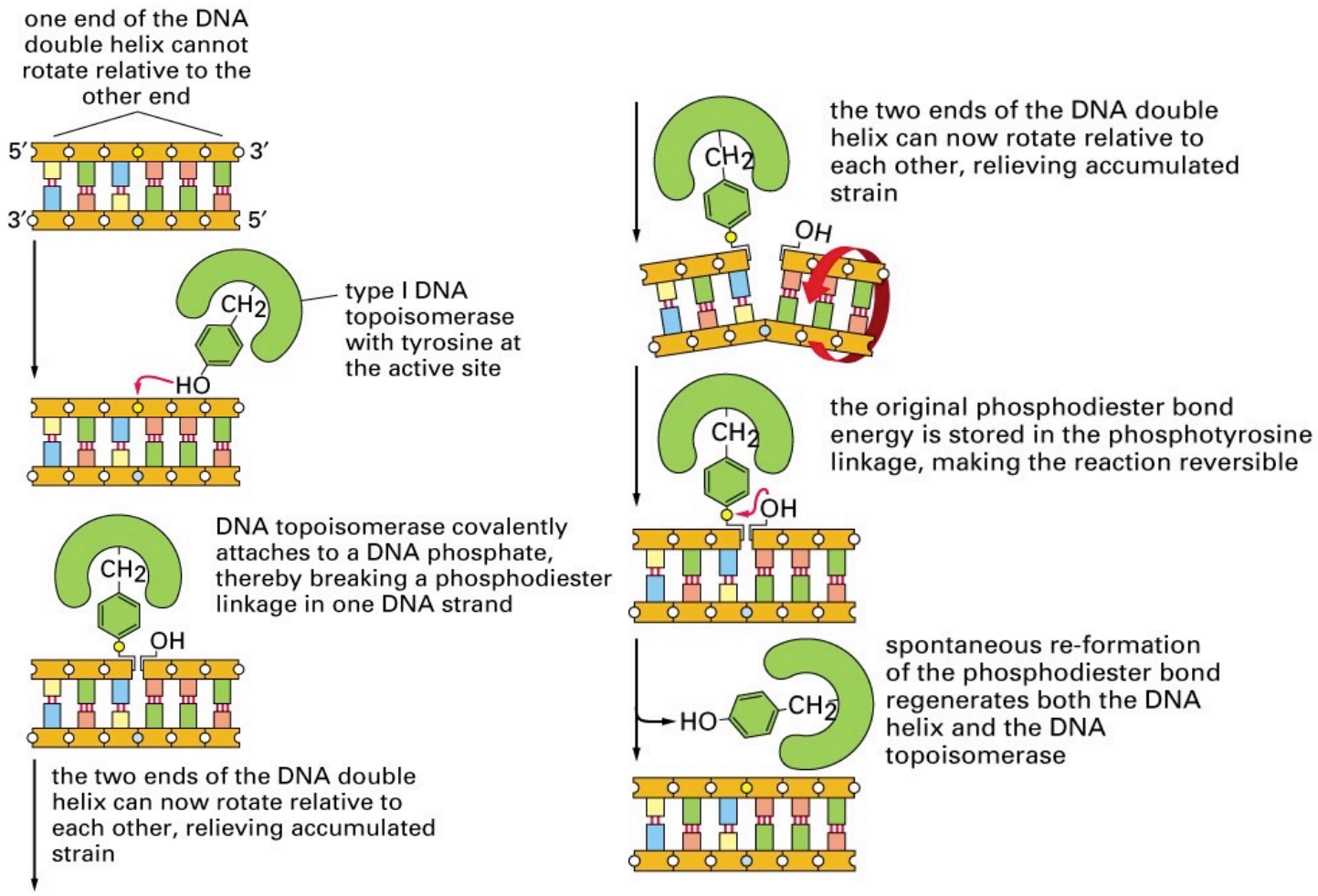
Every 10 bases replicated corresponds to one complete turn about the axis of the parental helix - for a replication fork to move, the chromosome ahead would have to rotate rapidly

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

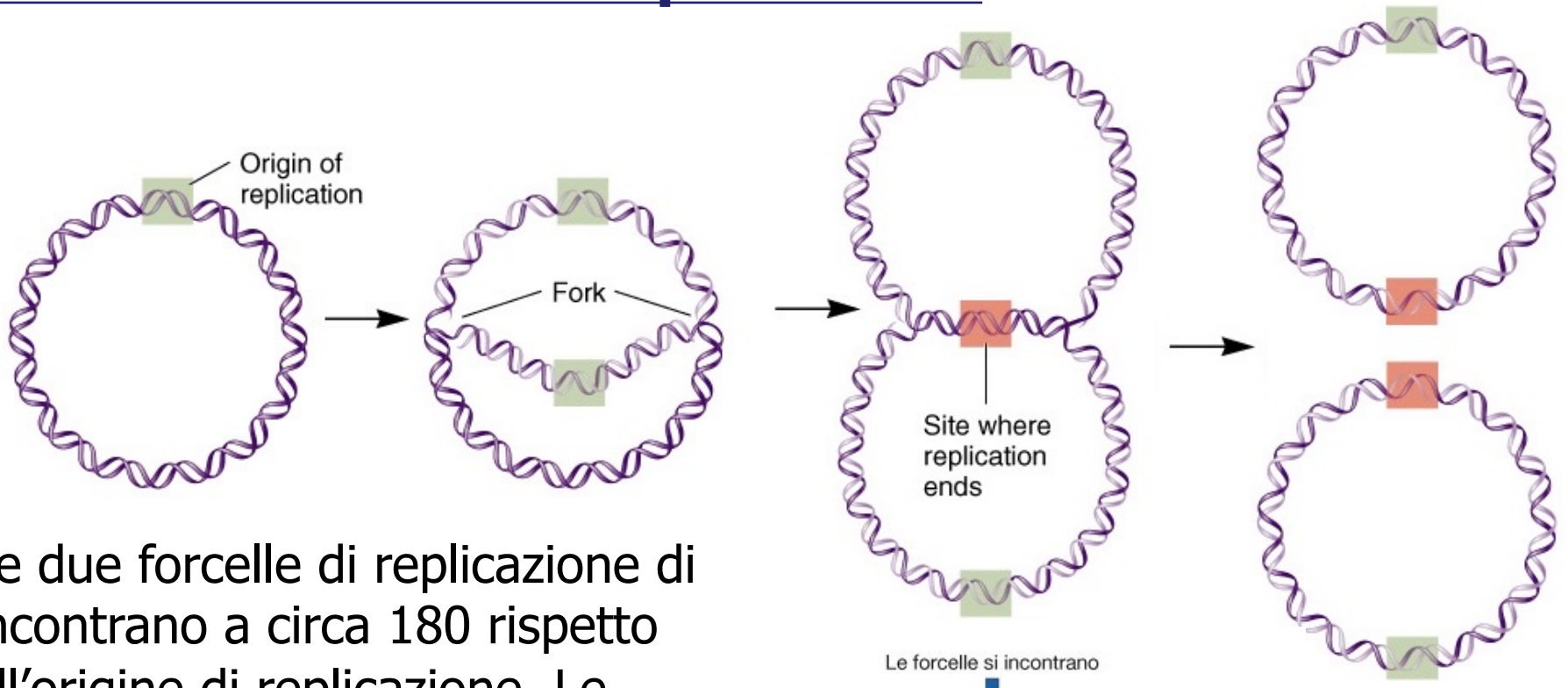


Topoisomerasi

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

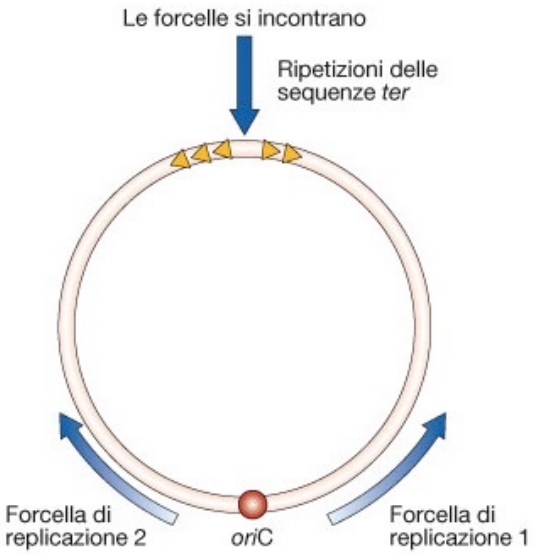


Terminazione della replicazione.

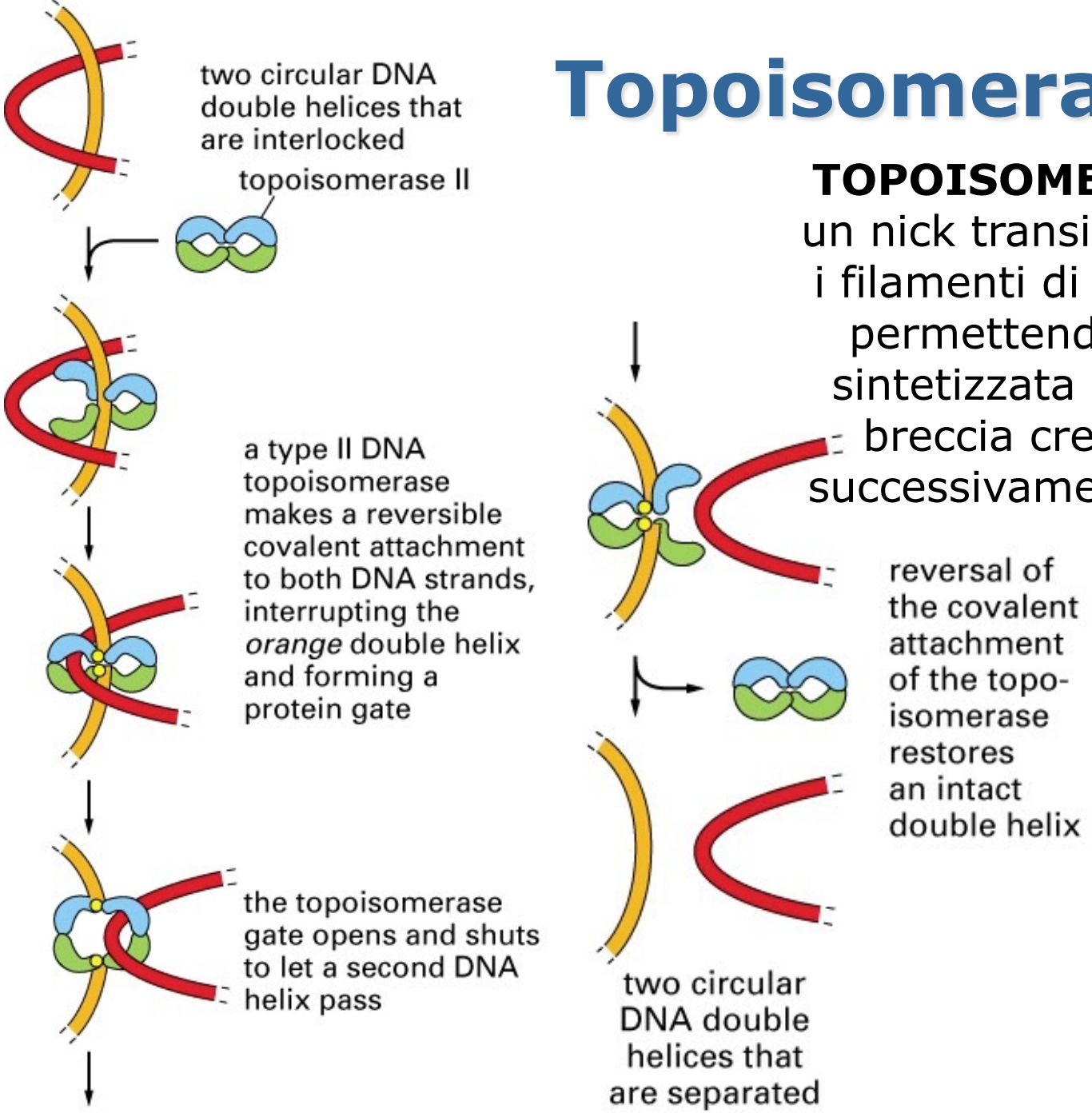


Le due forcelle di replicazione di incontrano a circa 180 rispetto all'origine di replicazione. Le sequenze ***ter***, di 23 bp, vengono legate dalle **TBP** (Ter binding proteins) e fanno terminare le forcelle di replicazione.

La Topoiomerasi II dividera' fisicamente le due molecole figlie



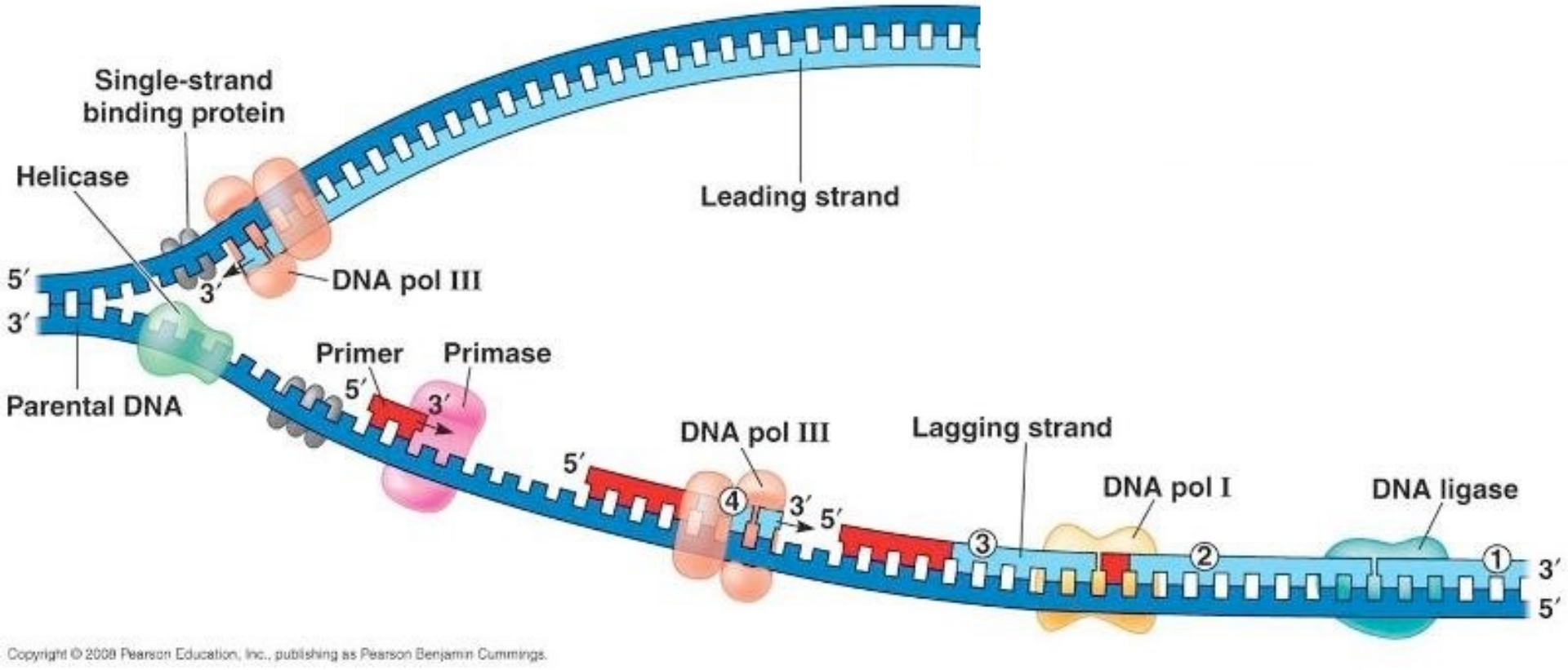
Topoisomerasi



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.

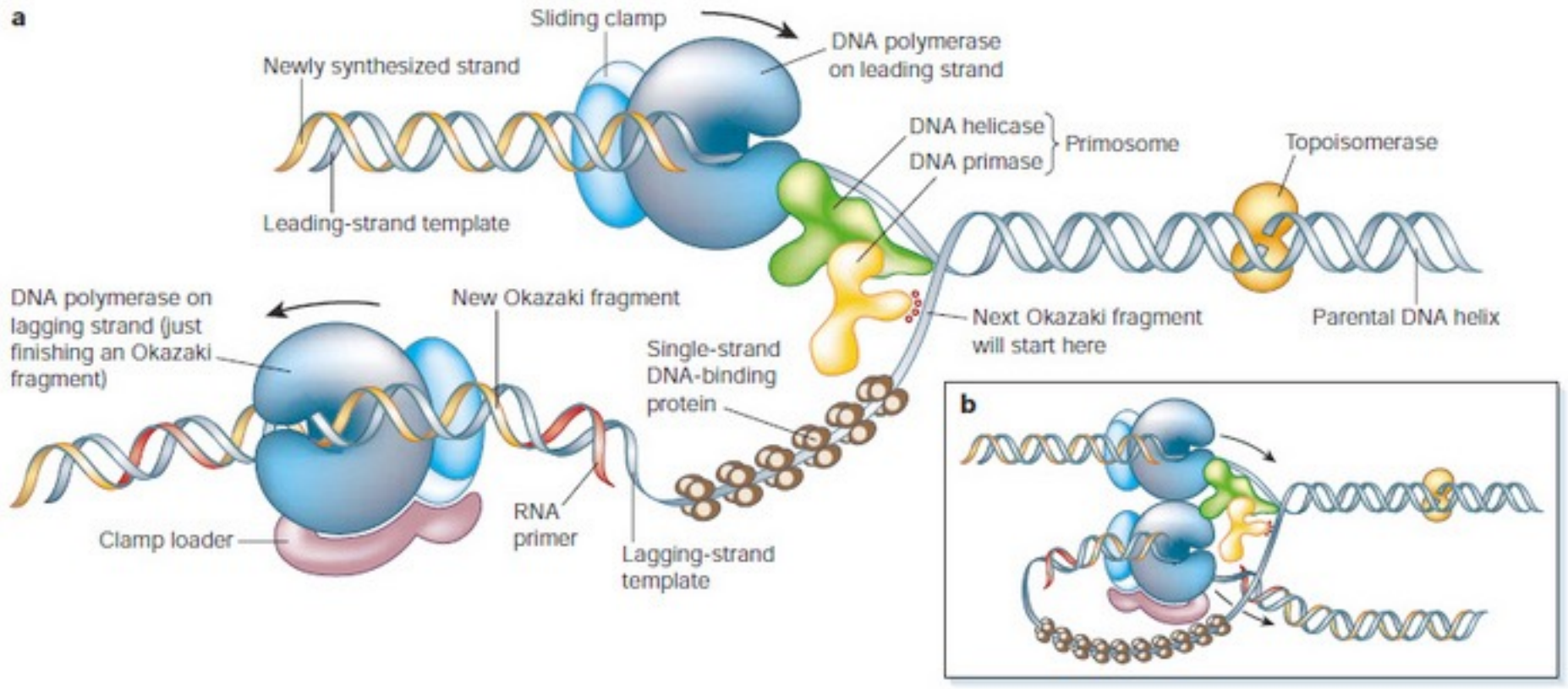
Serve ATP!

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.

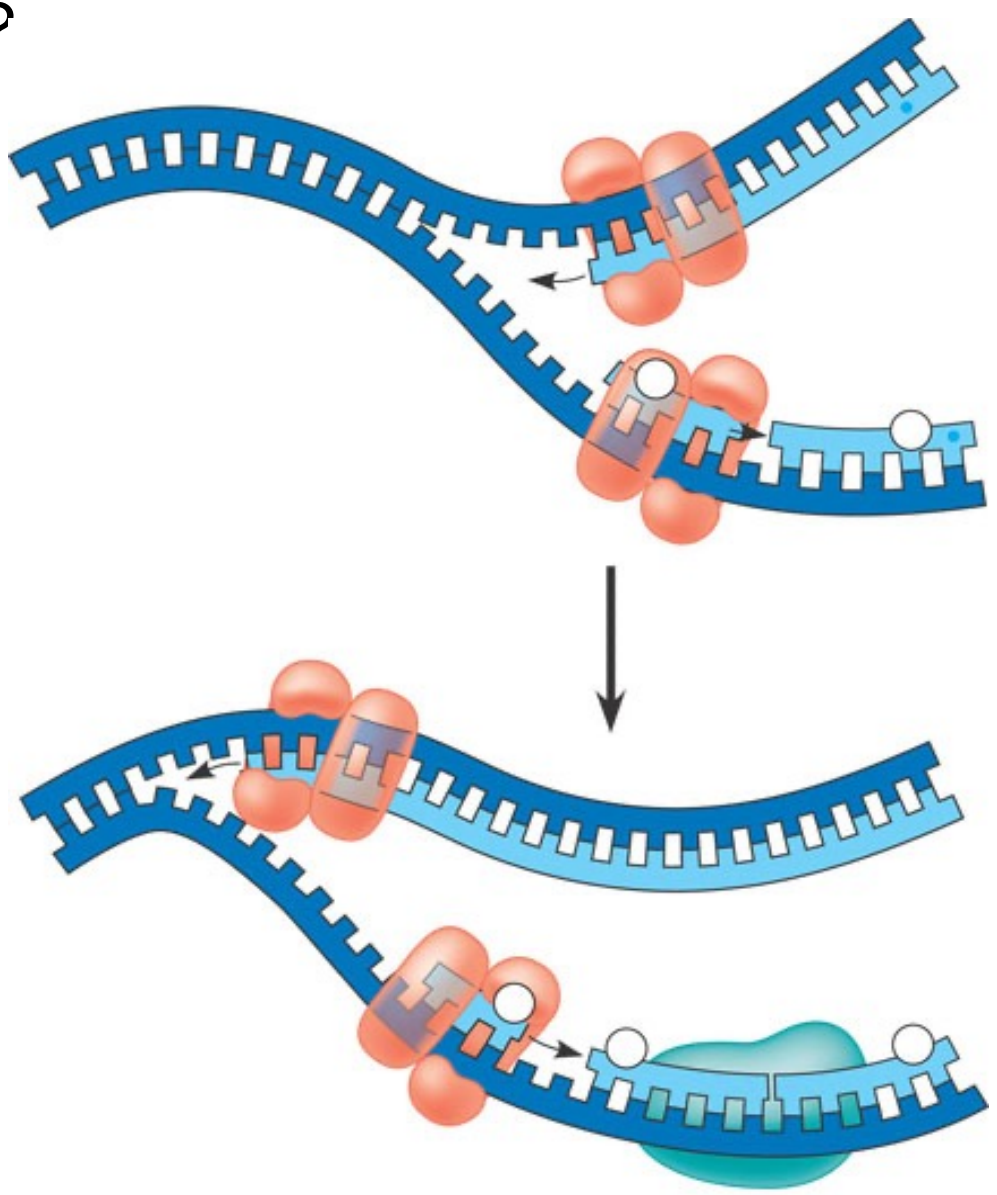
The proteins at a replication fork form a multienzyme replication machine



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


DNA