Molecular Genetics

1. THE STRUCTURE OF NUCLEIC ACIDS

History of the studying of the structure of nucleic acids

- 1868 – F.Miescher isolated nuclei and identified phosphorus-containing substance, which was called “nuclein”. Now it is called “nucleoprotein”.
- 1872 – F.Miescher found that heads of salmon sperm cells contain acid substance. Now it is called “nucleic acid”.
- 1944 – O.Avery, C.McLeod, and M.McCarty identified deoxyribonucleic acid as the carrier of genetic information .
- 1953 – J.Watson and F.Crick created the model of the DNA structure.

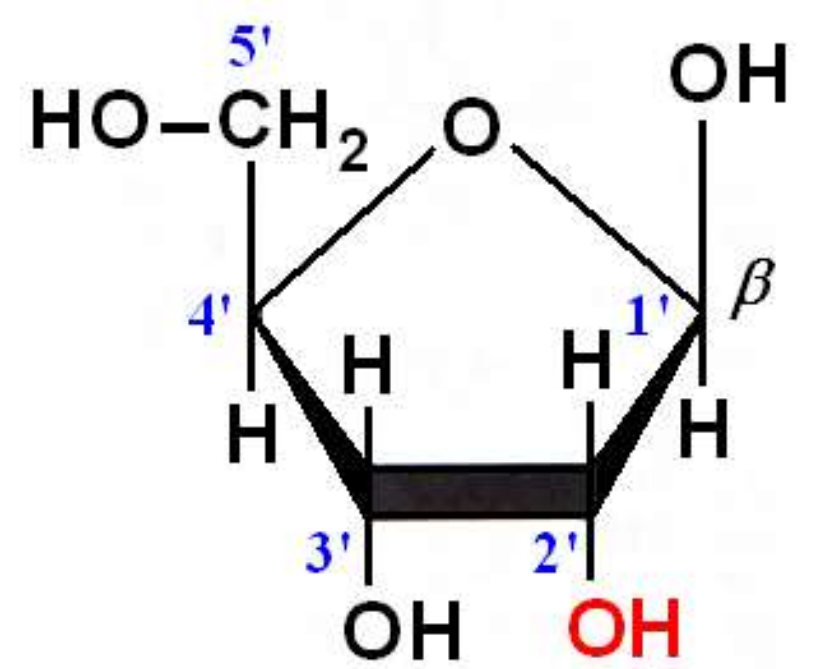
The nucleotide structure

Nucleotides are composed of a five-carbon sugar to which are attached one or more phosphate groups and a nitrogen-containing base.

In the case of the nucleotides in RNA, the sugar is ribose. In the case of the nucleotides in DNA, the sugar is deoxyribose.

The nucleotide structure:

sugar

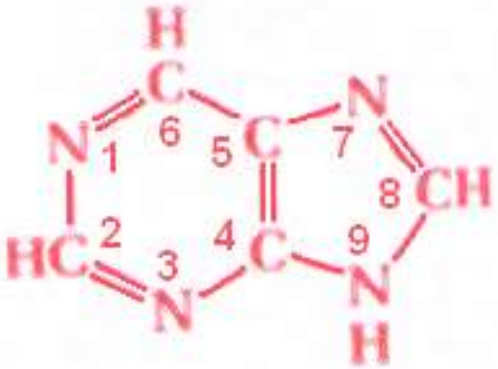


β-D-ribose

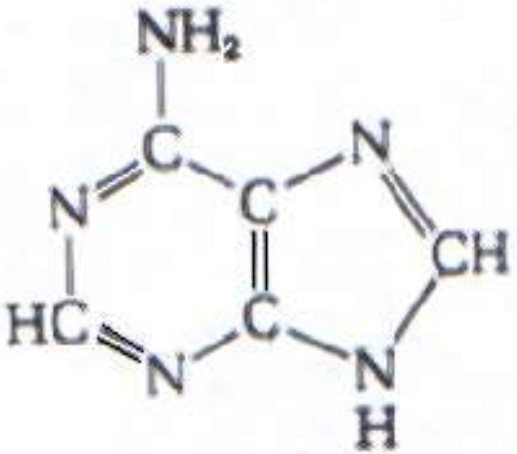
β-D-deoxyribose

The nucleotide structure:

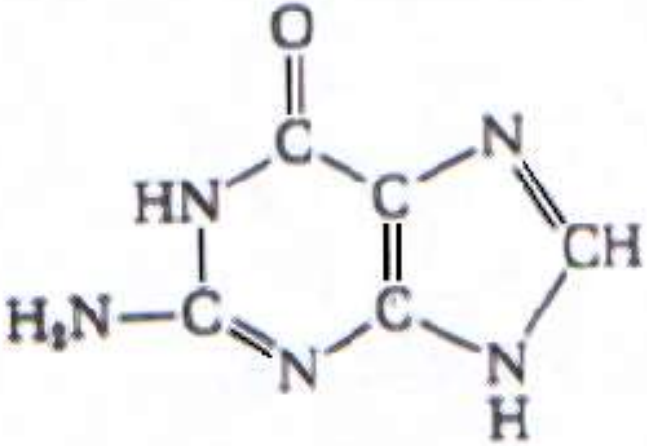
purine nitrogen-containing bases



purine



adenine

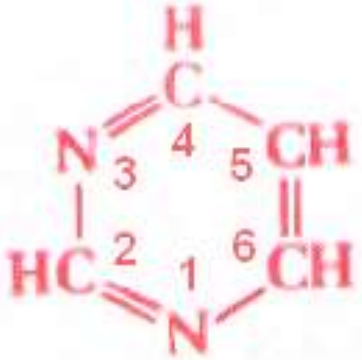


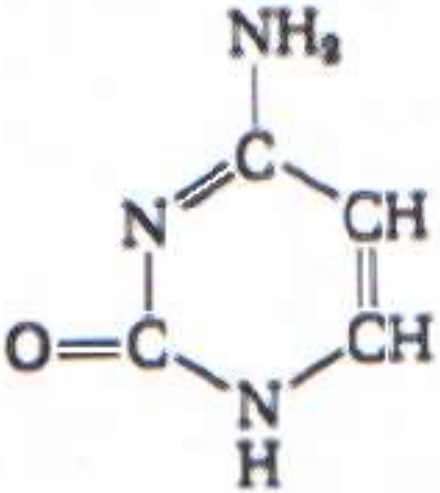
guanine

The nucleotide structure:

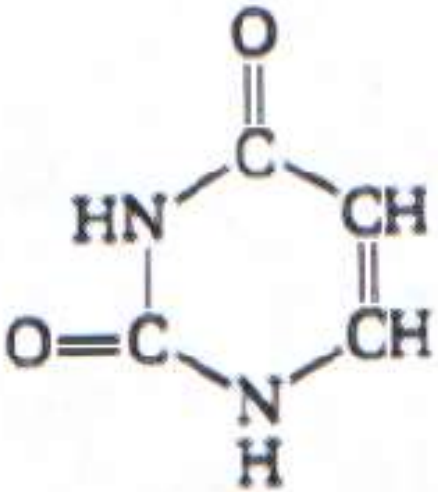
pyrimidine nitrogen-containing bases

pyrimidine

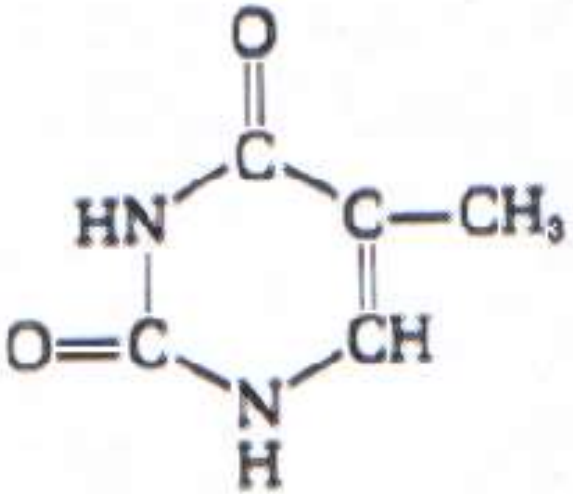




cytosine

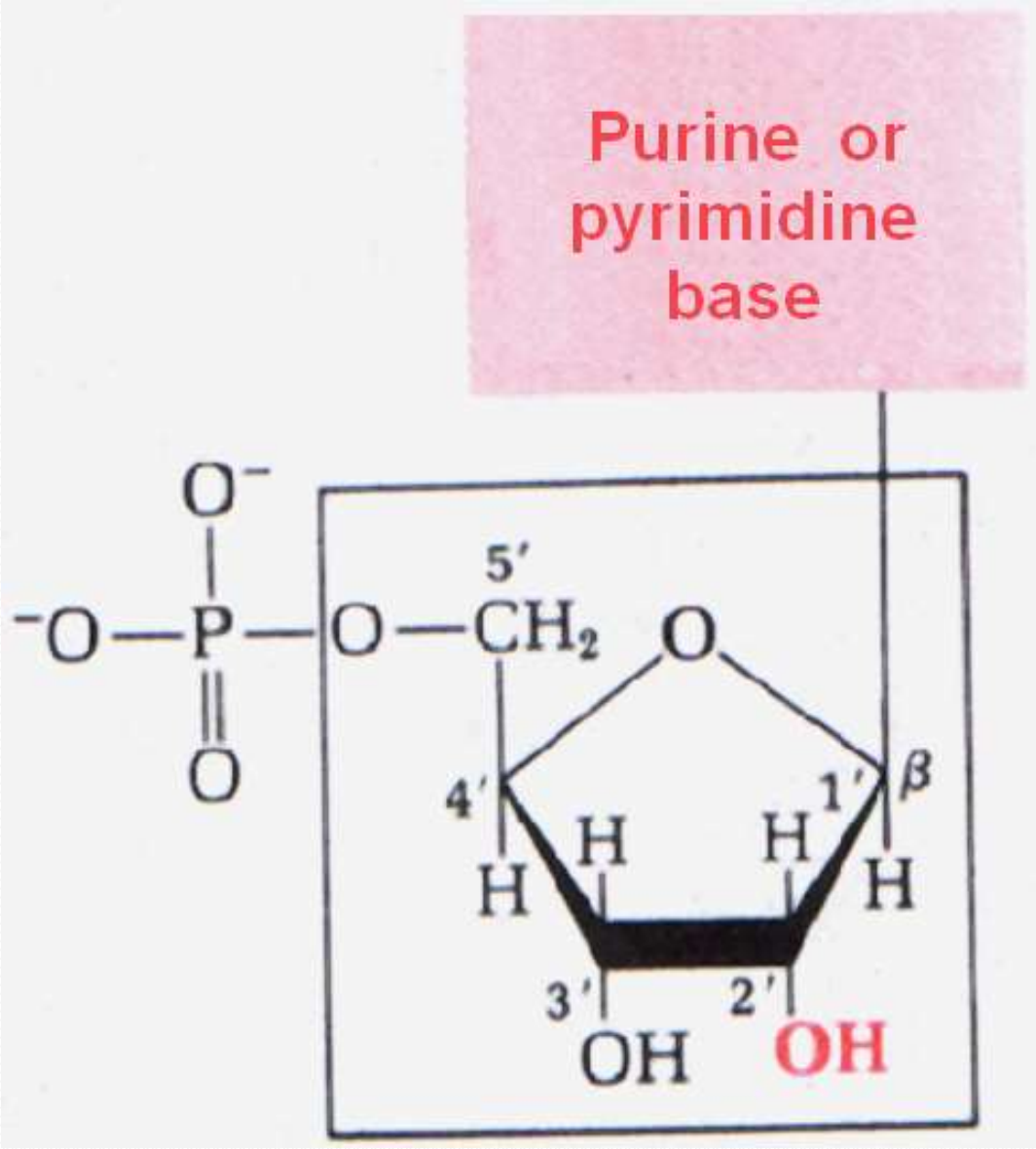


uracil



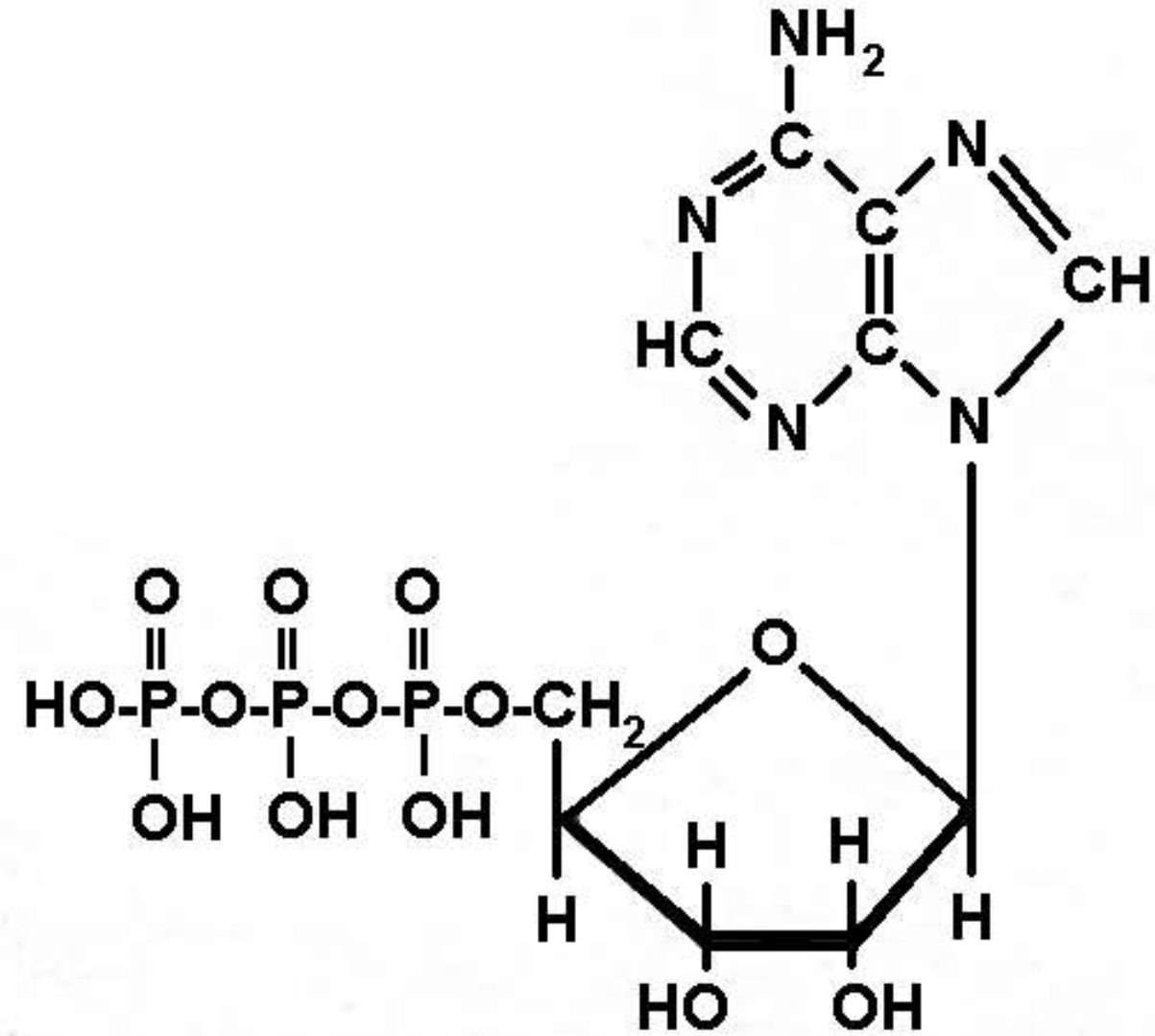
thymine

structure



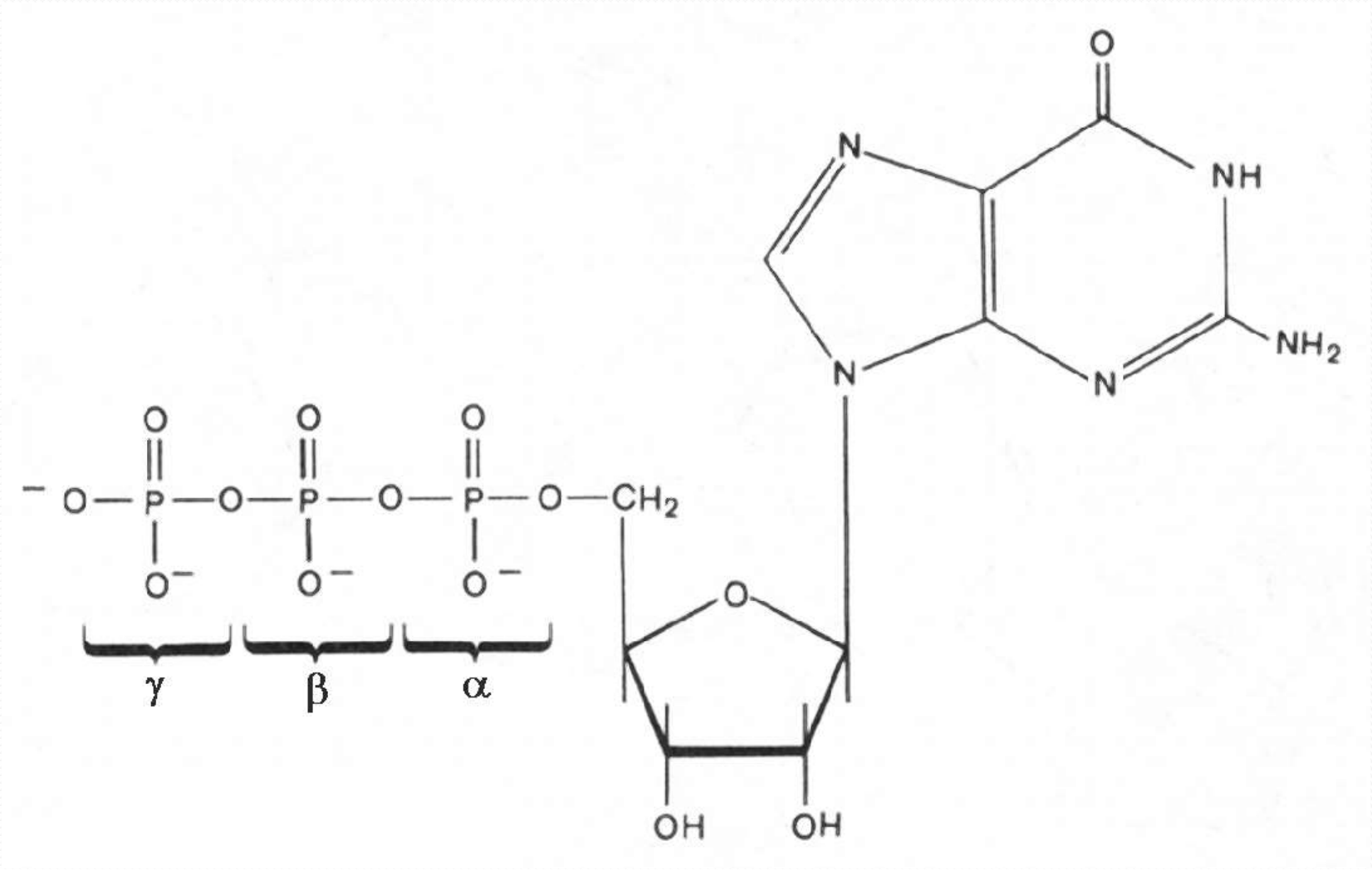
structure:

ATP

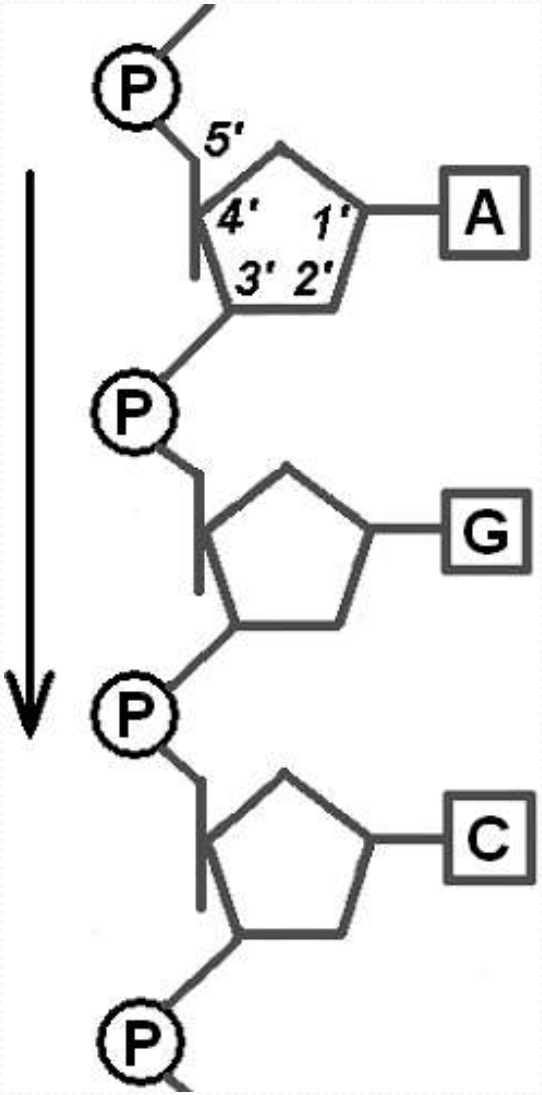


structure:

GTP



Primary Structure of DNA



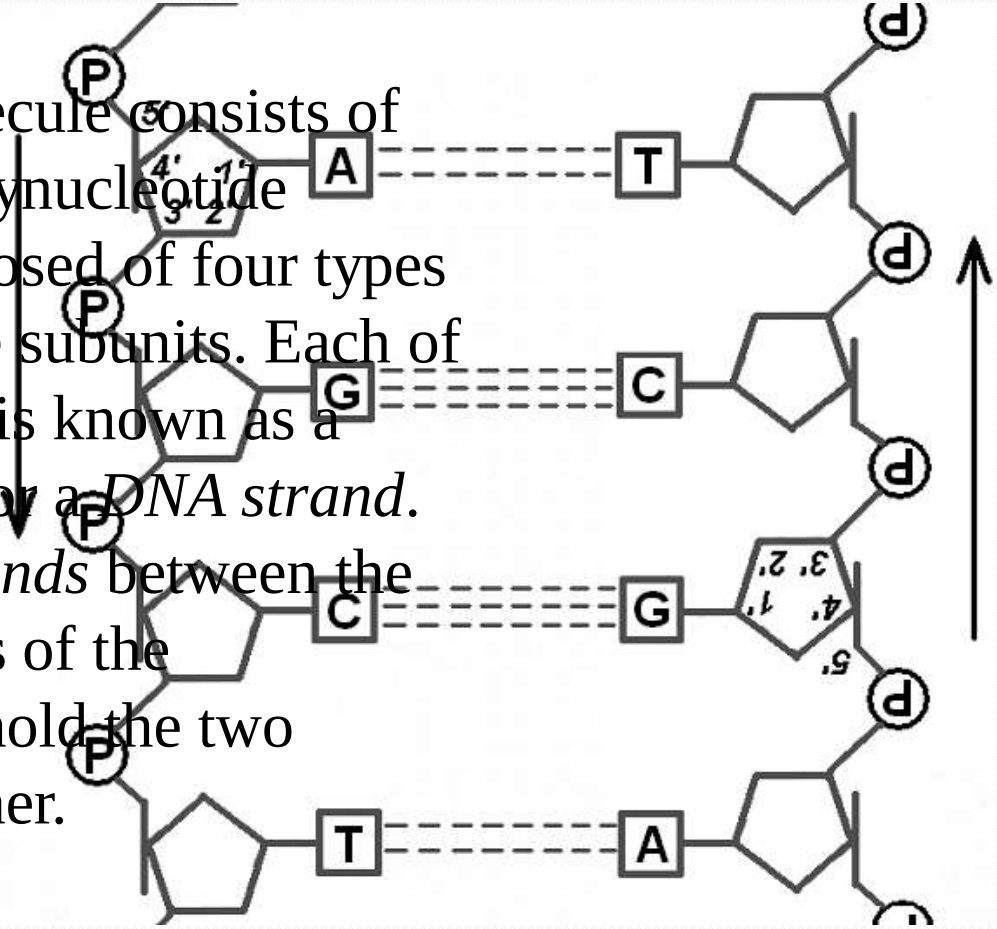
The nucleotides are covalently linked together in a chain through the sugars and phosphates, which thus form a "backbone" of alternating sugar-phosphate-sugar-phosphate.

The way in which the nucleotide subunits are lined together gives a DNA strand a chemical polarity. DNA strand has the 5′ phosphate on one terminus and the 3′ hydroxyl on the other. This polarity in a DNA chain is indicated by referring to one end as the 3′ end and the other as the 5′ end.

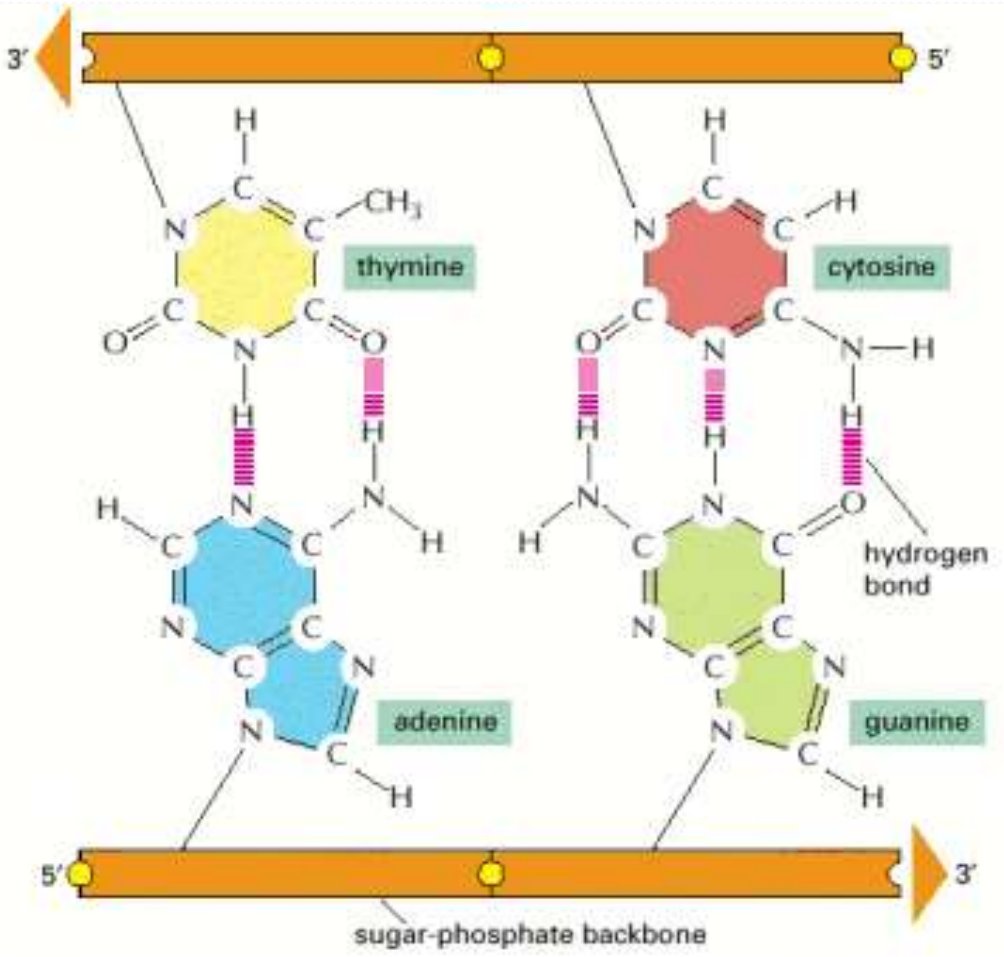
The nucleotides are linked together covalently by *phosphodiester bonds* (i.e. C–O–P–O–C) through the 3′-hydroxyl (–OH) group of one sugar and the 5′-phosphate (P) of the next. Thus, each polynucleotide strand has a chemical polarity; that is, its two ends are chemically different. The 3′ end carries an unlinked –OH group attached to the 3′ position on the sugar ring; the 5′ end carries a free phosphate group attached to the 5′ position on the sugar ring.

Secondary Structure of DNA

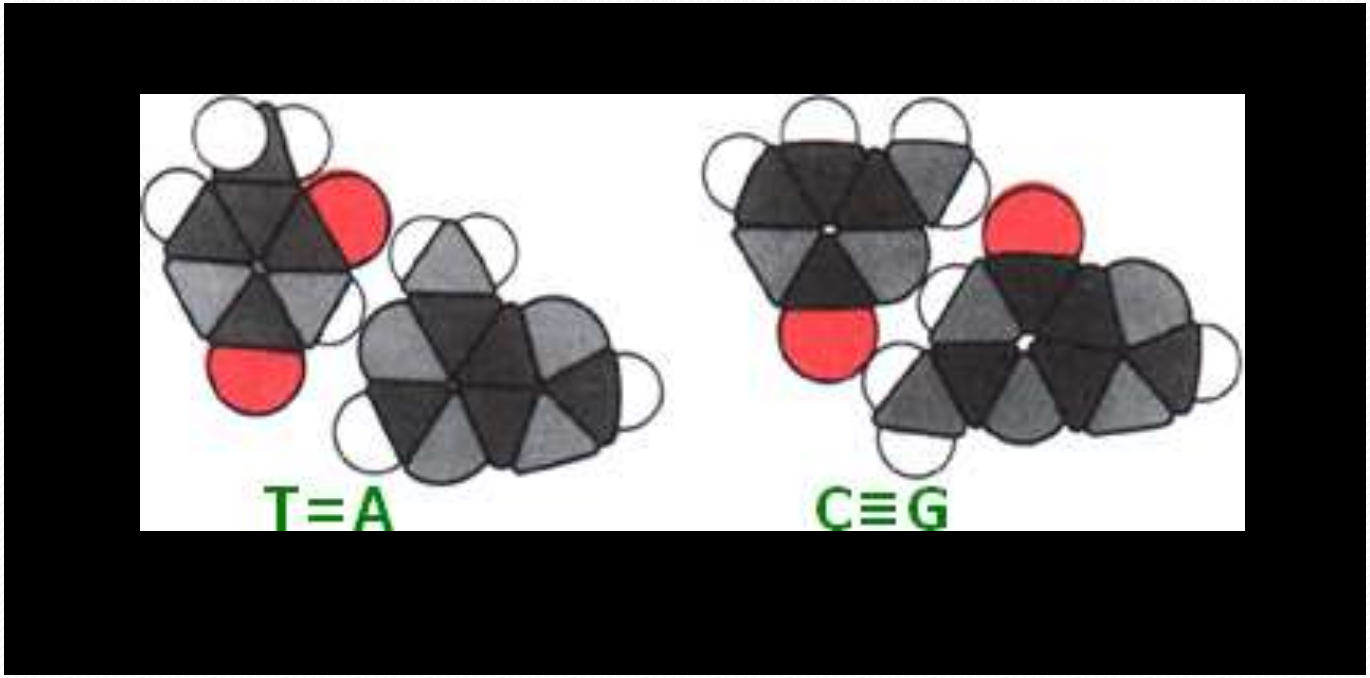
- A DNA molecule consists of two long polynucleotide chains composed of four types of nucleotide subunits. Each of these chains is known as a *DNA chain*, or a *DNA strand*. *Hydrogen bonds* between the base portions of the nucleotides hold the two chains together.

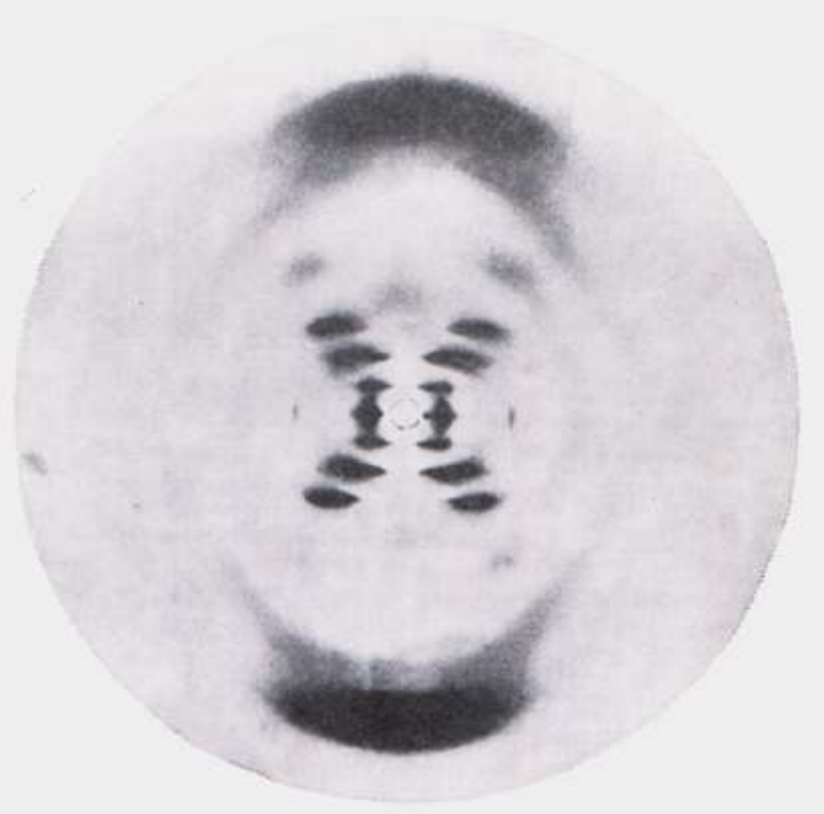


- A always pairs with T, and G with C (*complementary base-pairing*). Two hydrogen bonds form between A and T, while three form between G and C. The bases can pair in this way only if the two polynucleotide chains that contain them are *antiparallel* to each other.



T-A and C-G pairs





DNA roentgenogram was received in 1952 by Rosalind Franklin in the laboratory headed by M.Wilkins.

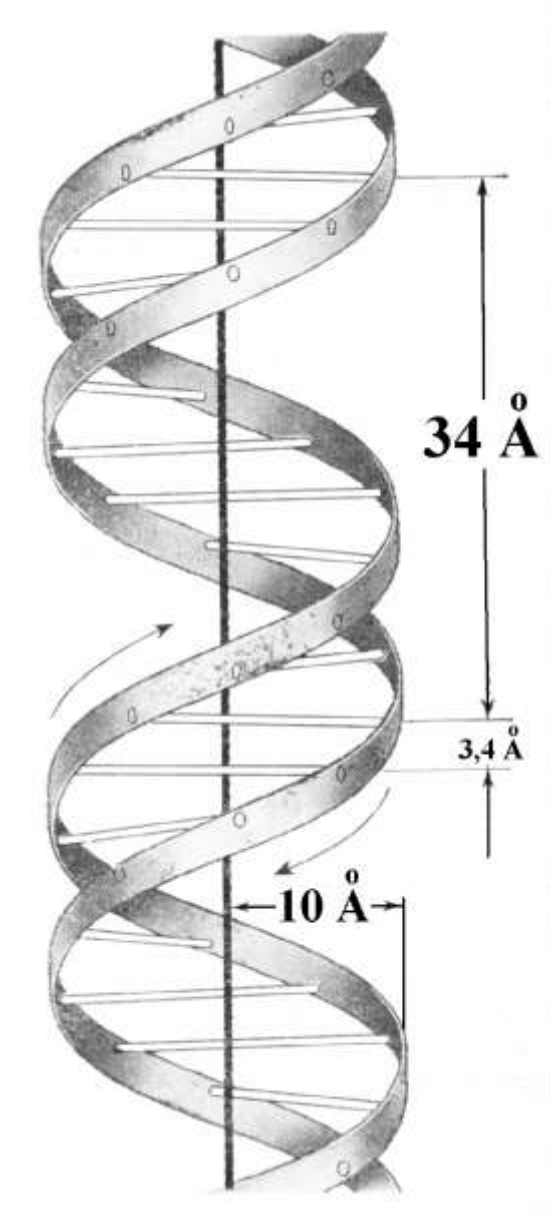
- Cross-like structure in the centre of this photo proves that DNA is composed of the polymer wound into a helix.
- Two large dark structures correspond the distance of 3.4 angstrom (thickness of one nucleotide).

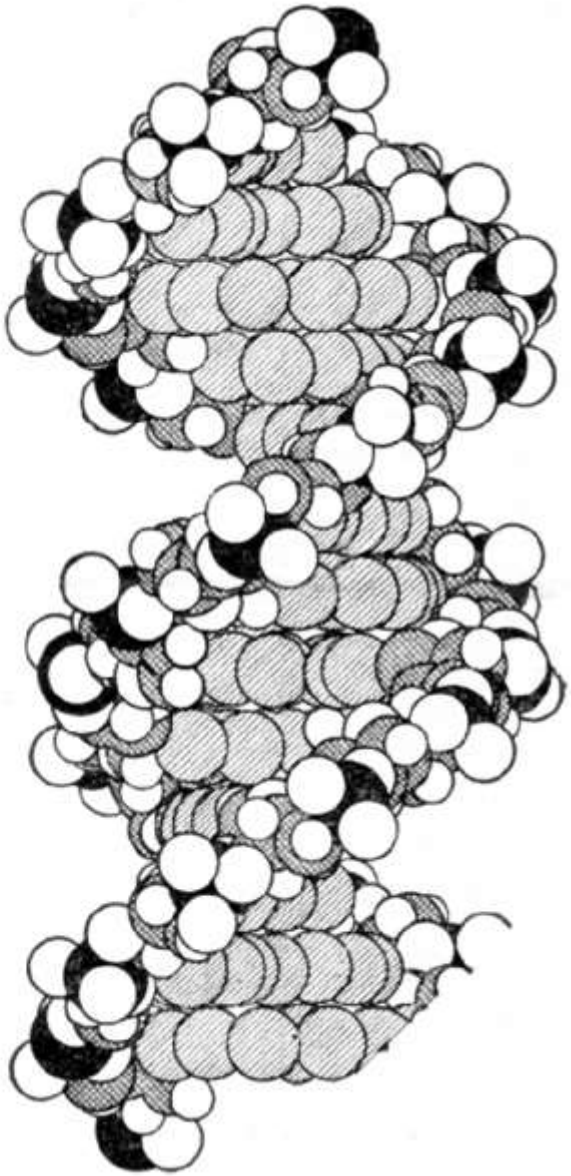
The observation that DNA was double-stranded was of crucial significance and provided one of the major clues that led to the Watson–Crick structure of DNA. Only when this model was proposed did DNA's potential for replication and information encoding become apparent.

The three-dimensional structure of DNA – **the double helix** – arises from the chemical and structural features of its two polynucleotide chains.

J.Watson and F.Crick near the model of the DNA

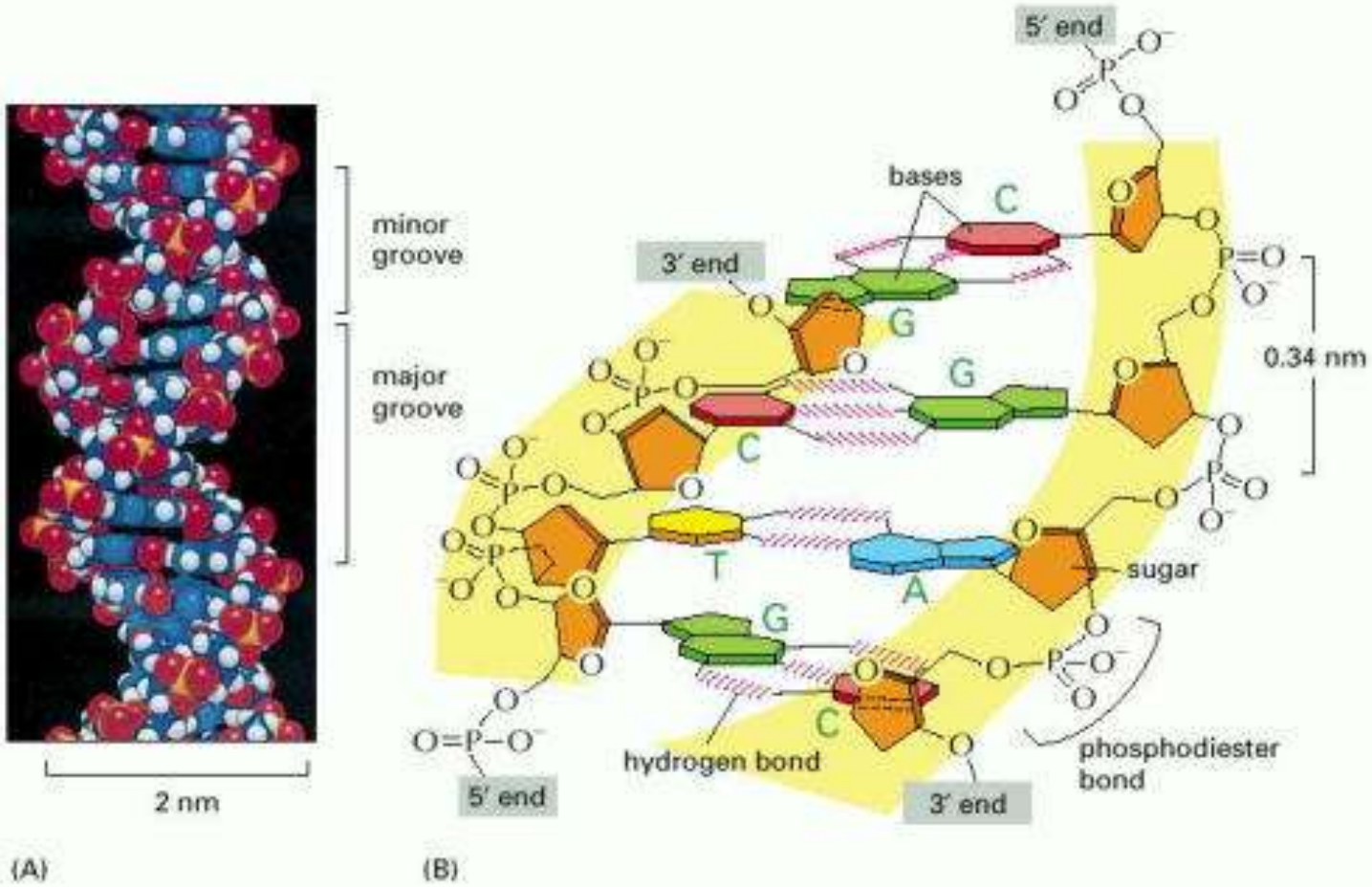
Secondary Structure of DNA

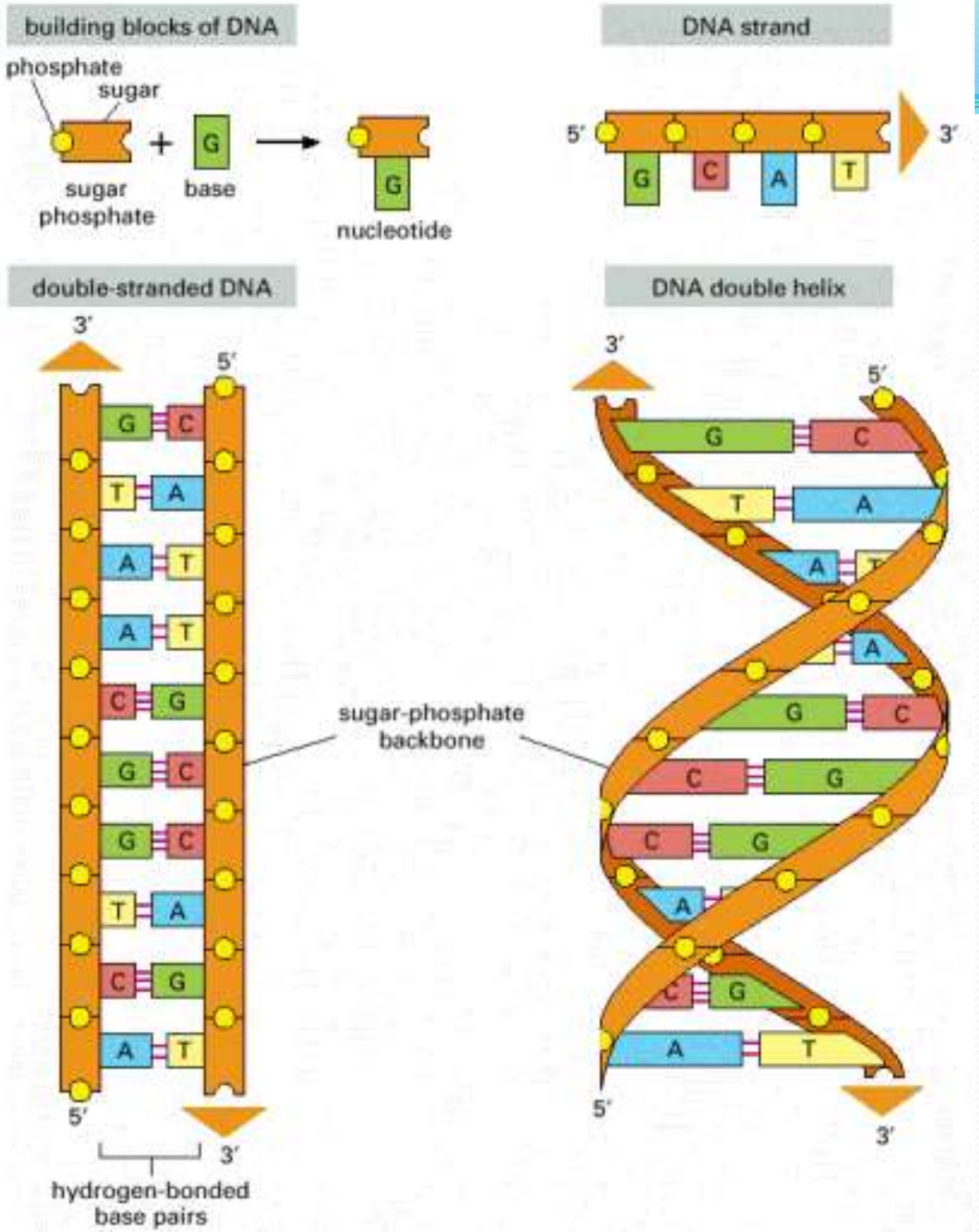




Each turn of DNA is made up of 10.4 nucleotide pairs and the center-to-center distance between adjacent nucleotide pairs is 3.4Å. The coiling of the two strands around each other creates two grooves in the double helix. The wider groove is called the major groove, and the smaller the minor groove.

All the bases are on the inside of the double helix, and the sugar-phosphate backbones are on the outside. Bases are hydrophobic (don't like water) and the sugar-phosphate back-bone is hydrophi-lic (likes water).

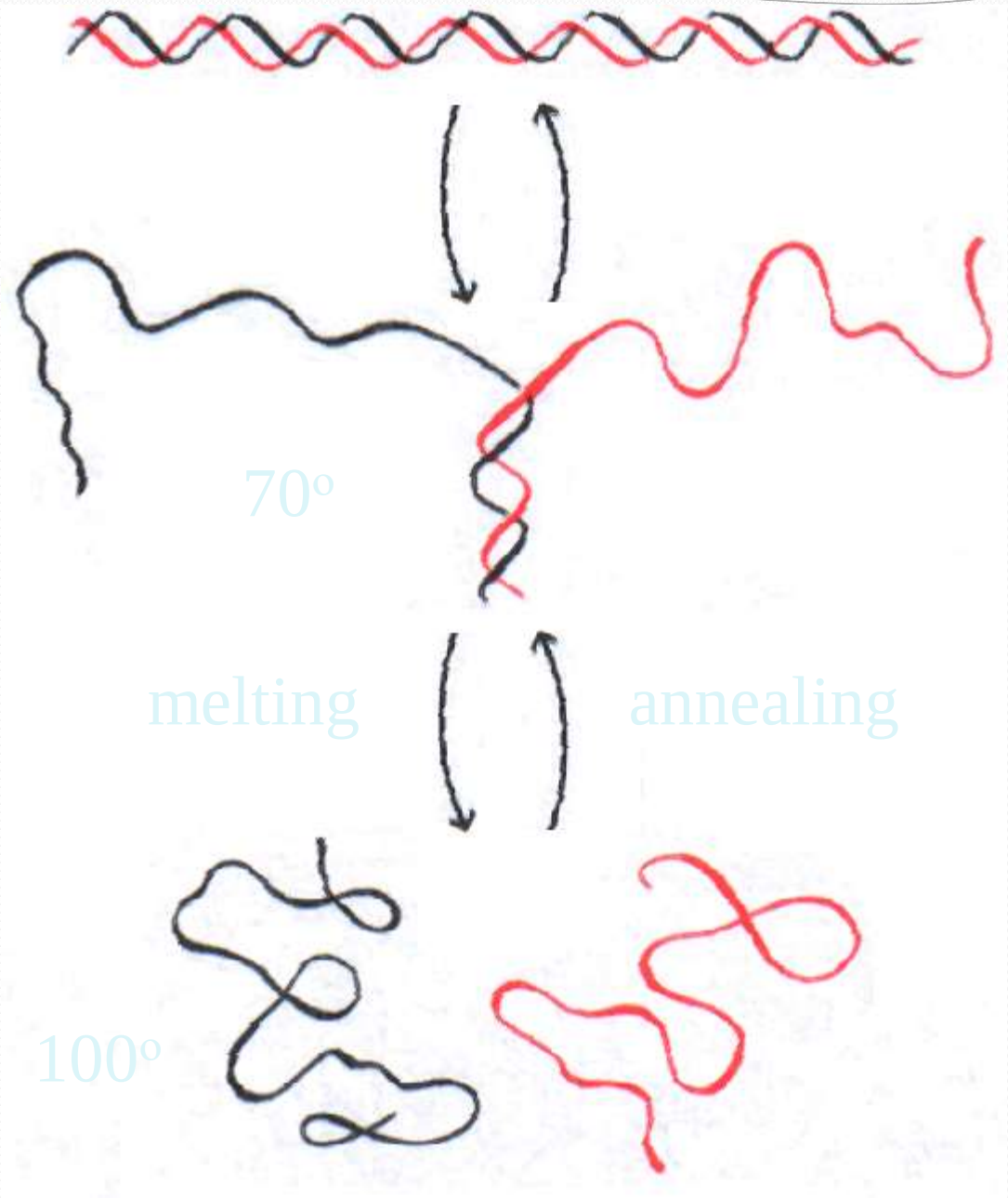



building blocks of DNA
phosphate
sugar
sugar
phosphate
base
nucleotide
DNA strand
5′
3′
G
C
A
T
double-stranded DNA
DNA double helix
sugar-phosphate
backbone
hydrogen-bonded
base pairs

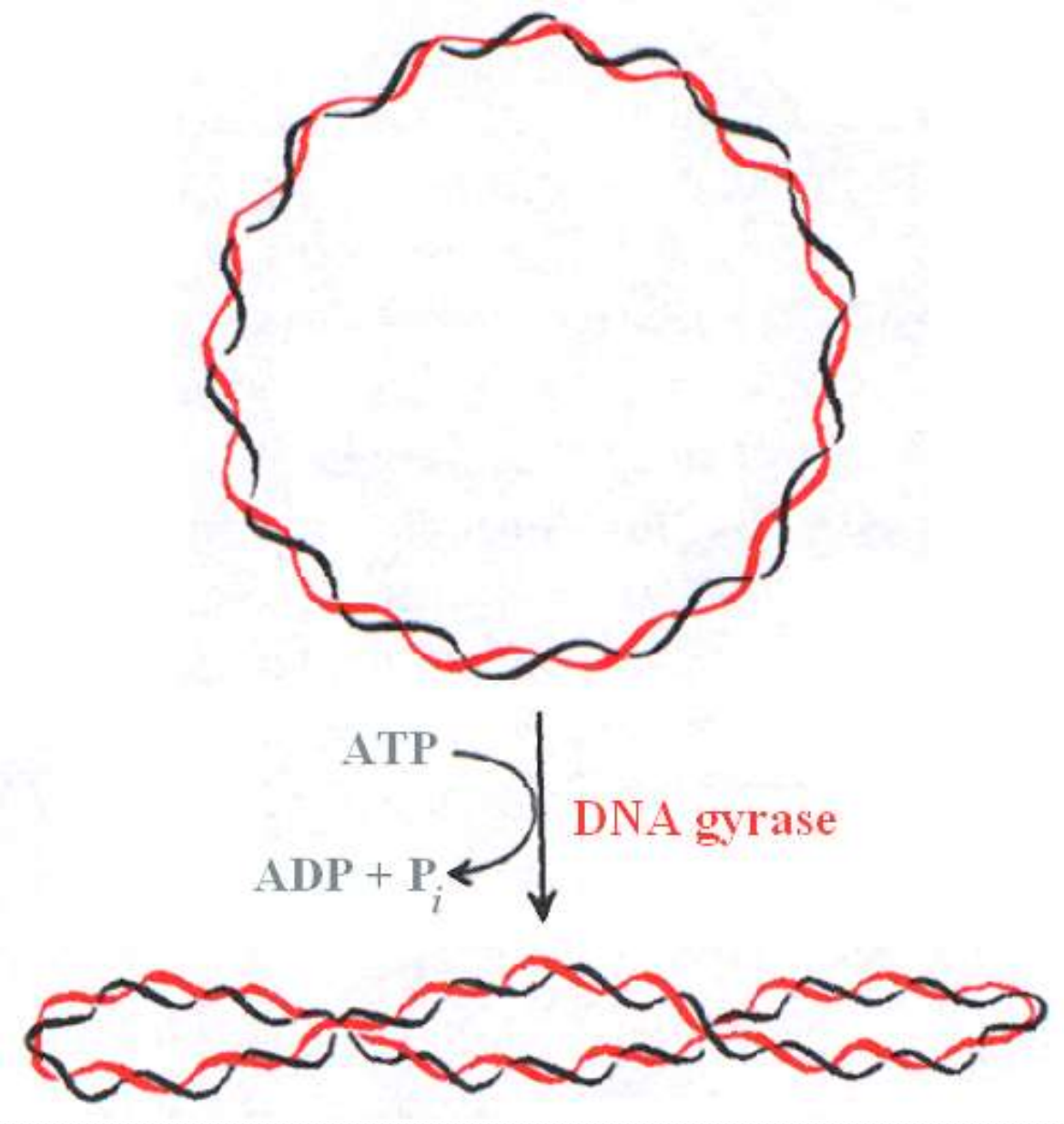
1962, Nobel Prize: F.Crick, J.Watson, and M.Wilkins.

Francis Crick and James Watson

DNA



Supercoiling of DNA



Molecular Genetics

DNA Function	*Cellular Process Which Provides DNA Functioning*
1. Information Storage	DNA Repair
2. Transfer of Information	DNA Replication
3. Regulation of Cellular Processes	Transcription & Translation

Molecular Genetics

2. **DNA REPAIR**

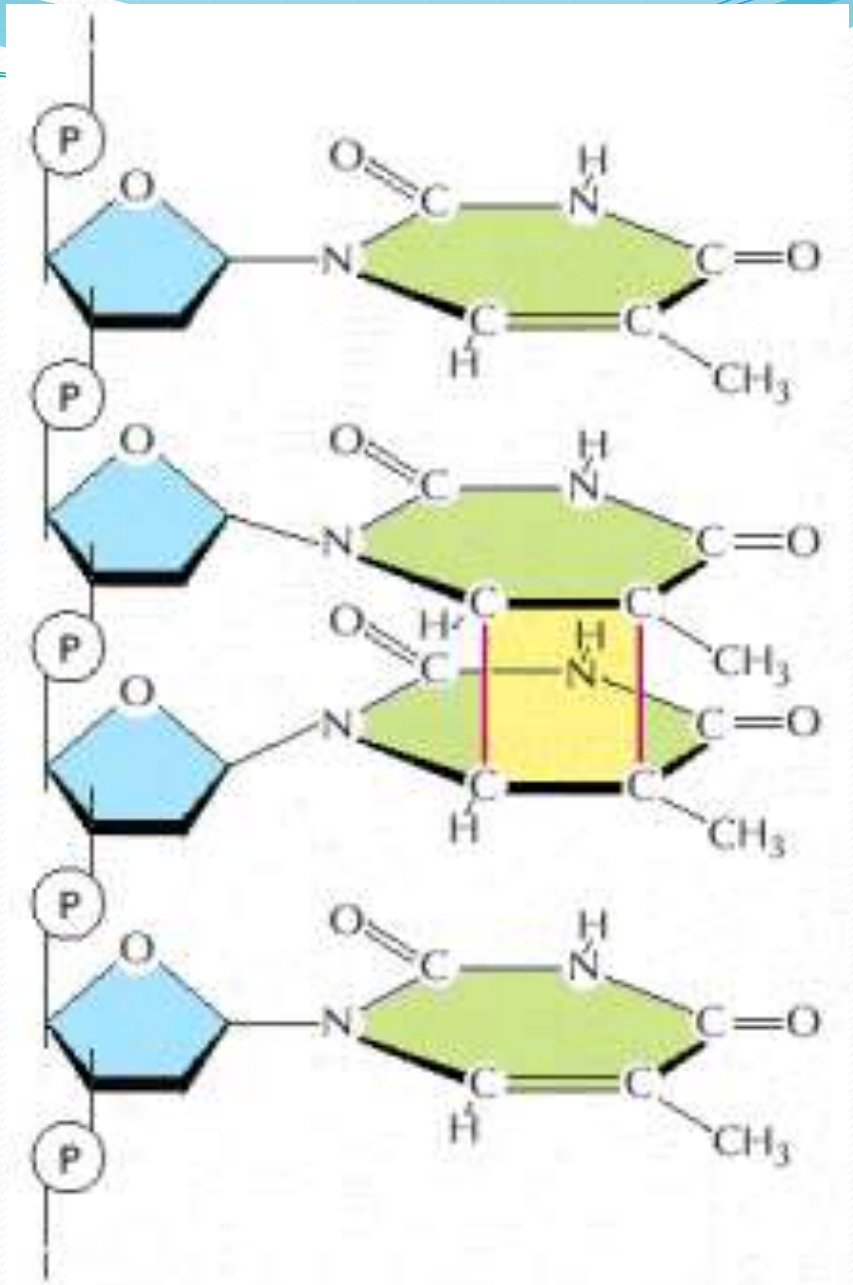
DNA inside cells is repeatedly damaged by chemicals and radiation from the environment, as well as by thermal accidents and reactive molecules.

Maintaining genetic stability requires mechanisms for repairing the many accidental lesions that occur continually in DNA.
Of the thousands of random changes created every day in the DNA of a human cell by heat, metabolic accidents, radiation of various sorts, and exposure to substances in the environment, only a few accumulate as mutations in the DNA sequence.
Special cellular mechanism – **DNA repair** – is responsible for keeping all changes in the DNA structure to a minimum.

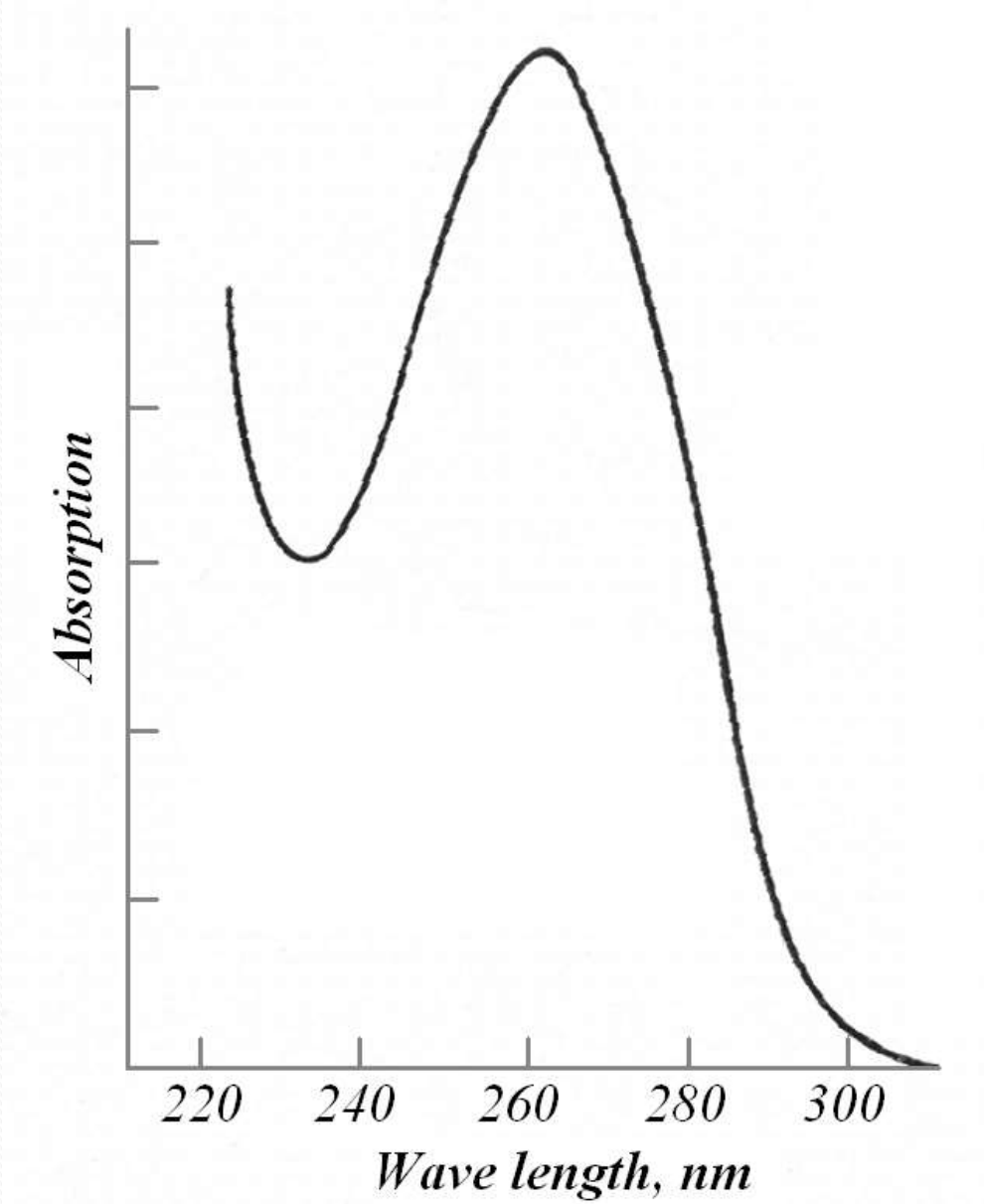
DNA undergoes such major changes as:

- Depurination – about 5000 purine bases (adenine and guanine) are lost every day from the DNA.
- Deamination – the amino group is removed from bases spontaneously.
- Methylation – the methyl group is added to bases.

- The thymine dimer.
  This type of damage is introduced into DNA in cells that are exposed to ultraviolet irradiation (as in sunlight). Ultraviolet radiation from the sun can produce a covalent linkage between two adjacent pyrimidine bases in DNA to form, for example, thymine dimers. A similar dimer will form between any two neighboring pyrimidine bases (C or T residues) in DNA.



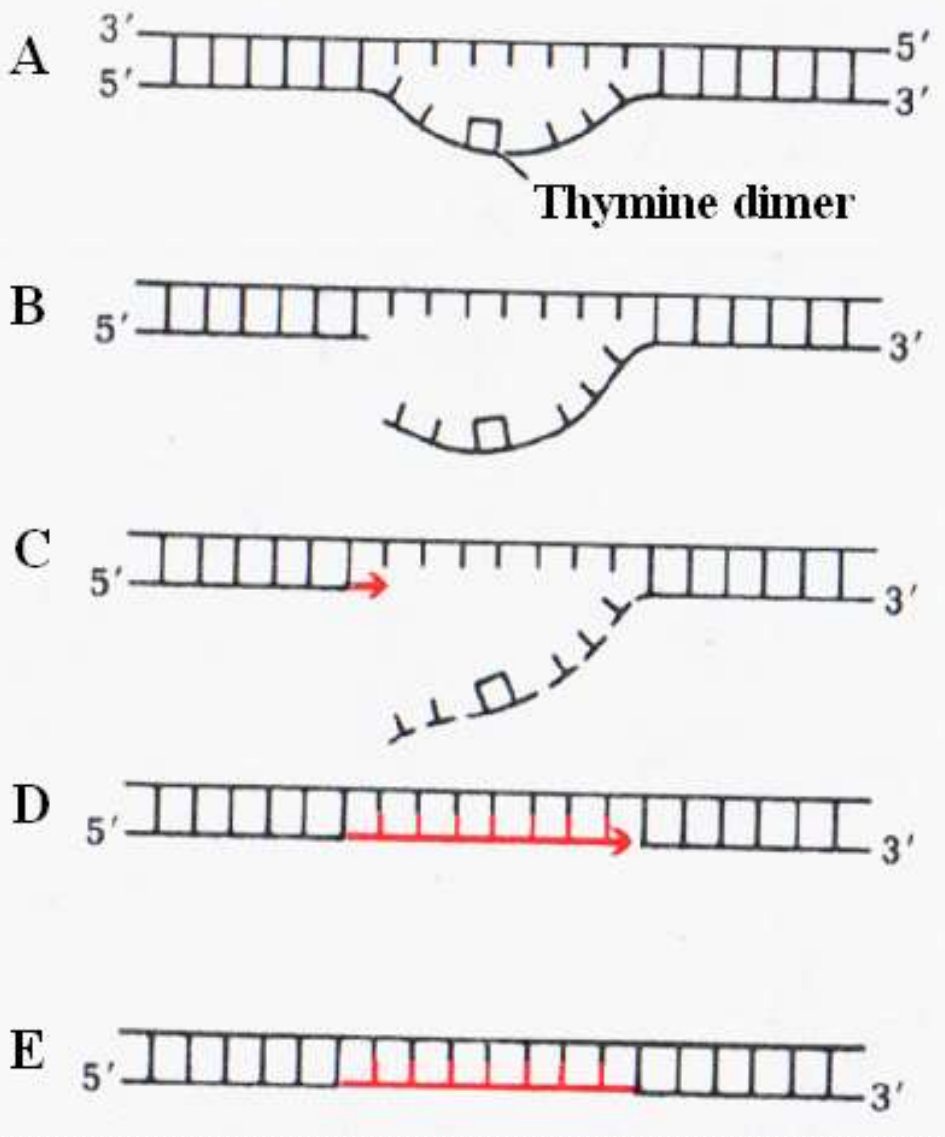
Absorption of UV irradiation



If left uncorrected when the DNA is replicated, most of these changes would be expected to lead either to the deletion of one or more base pairs or to a base-pair substitution in the daughter DNA chain. The mutations would then be propagated throughout subsequent cell generations as the DNA is replicated. Such a high rate of random changes in the DNA sequence would have disastrous consequences for an organism.

The double-helical structure of DNA is ideally suited for repair because it carries two separate copies of all the genetic information – one in each of its two strands. Thus, when one strand is damaged, the complementary strand retains an intact copy of the same information, and this copy is generally used to restore the correct nucleotide sequences to the damaged strand.
There are multiple pathways for DNA repair, using different enzymes that act upon different kinds of lesions.

Nucleotide excision repair



A large multienzyme complex scans the DNA for a distortion in the double helix.
Once a bulky lesion has been found, the phosphodiester backbone of the abnormal strand is cleaved, and an oligonucleotide containing the lesion is peeled away from the DNA double helix. The large gap produced in the DNA helix is then repaired by DNA polymerase (red chain) and DNA ligase (it ligates new and old chains).

DNA undergoes another changes as:

- One-Strand Breaks – are repaired by DNA-ligase.

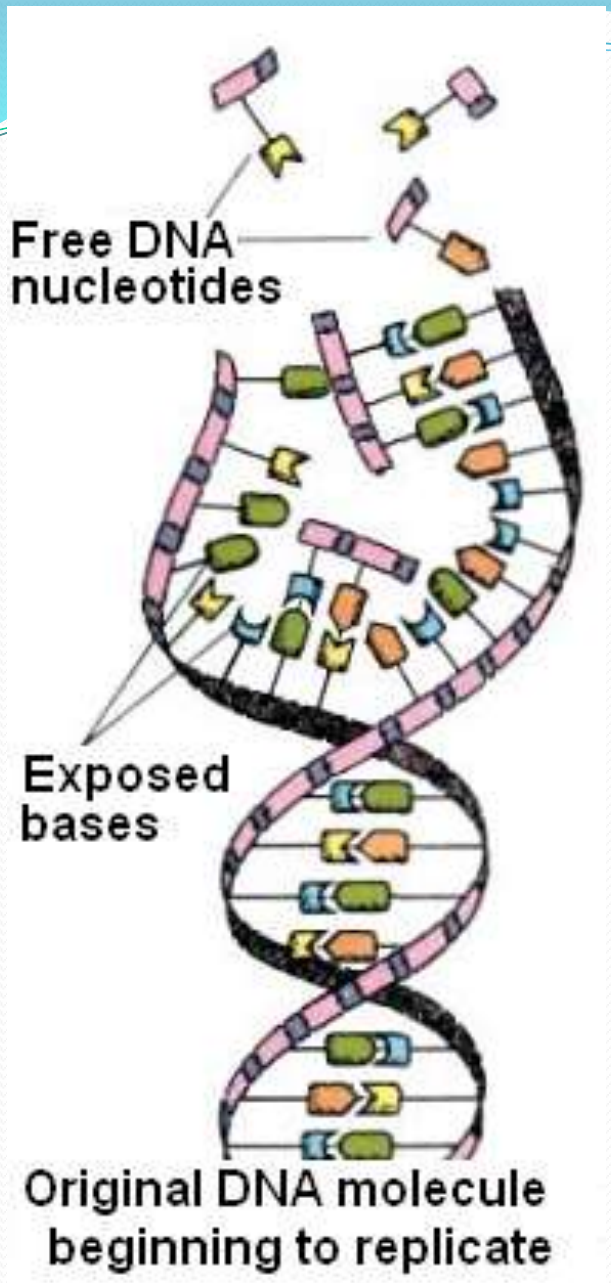
- Double-Strand Breaks – potentially dangerous type of DNA damage occurs when both strands of the double helix are broken. They cann't be repaired.

Molecular Genetics

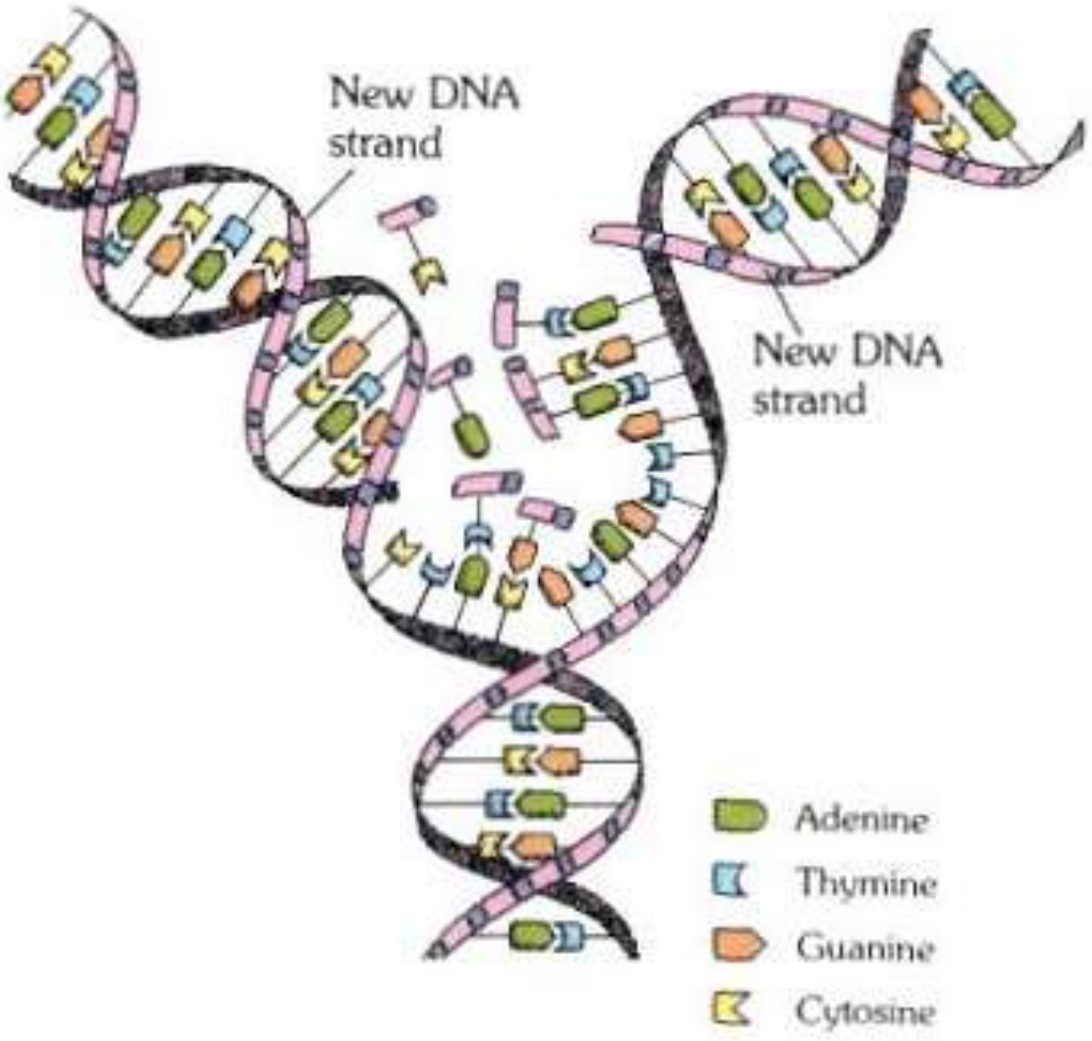
3. DNA REPLICATION

- When a cell reproduces to form two cells, each new cell receives exact copies of the DNA molecules found in the original cell. The copies carry the same instructions as in the original DNA molecules. The structure of DNA molecules enables the molecules to make exact copies of themselves in a process called **DNA replication**.

- DNA replication is the process of accurate duplication of vast quantities of genetic information. It must occur before a cell can produce two genetically identical daughter cells, during interphase.

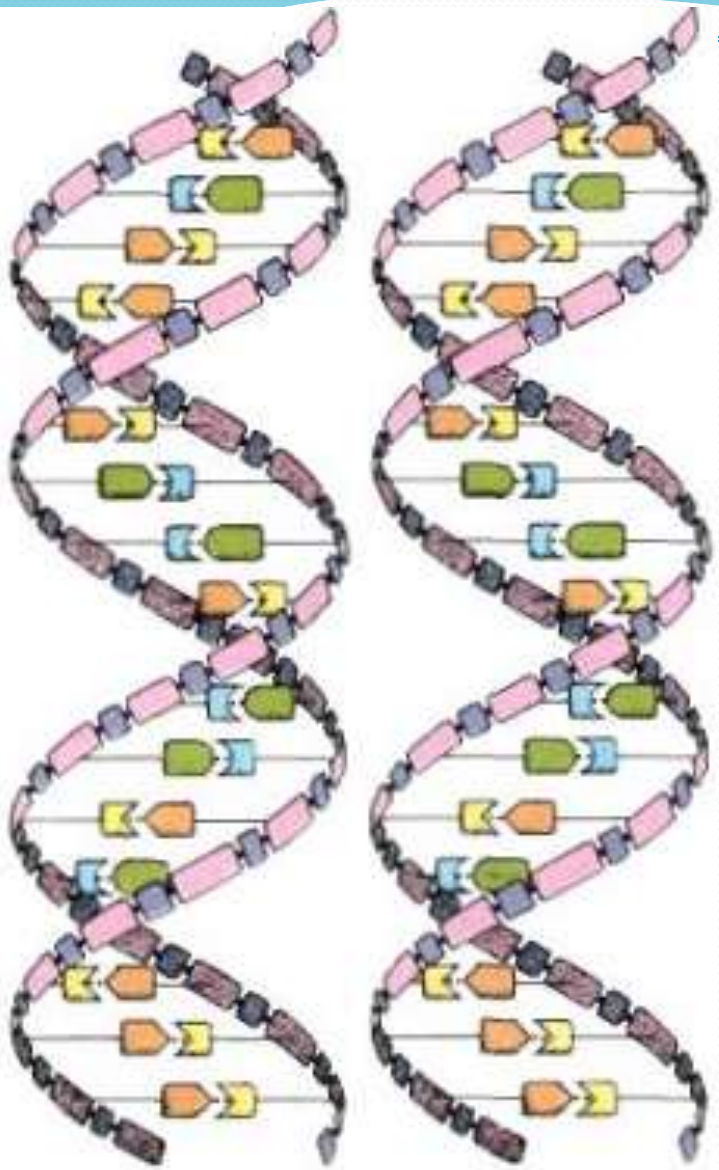


DNA replication begins when part of the DNA molecule unwinds. The hydrogen bonds in that part of the molecule break and the two complementary strands separate, exposing two rows of nitrogen bases. Free DNA nucleotides are present in the nucleus. The nitrogen bases of the free nucleotides begin to match up and form hydrogen bonds with the exposed bases on each strand of the DNA molecule. Adenines and thymines pair together, and cytosines and guanines pair together.



Two new DNA strands forming

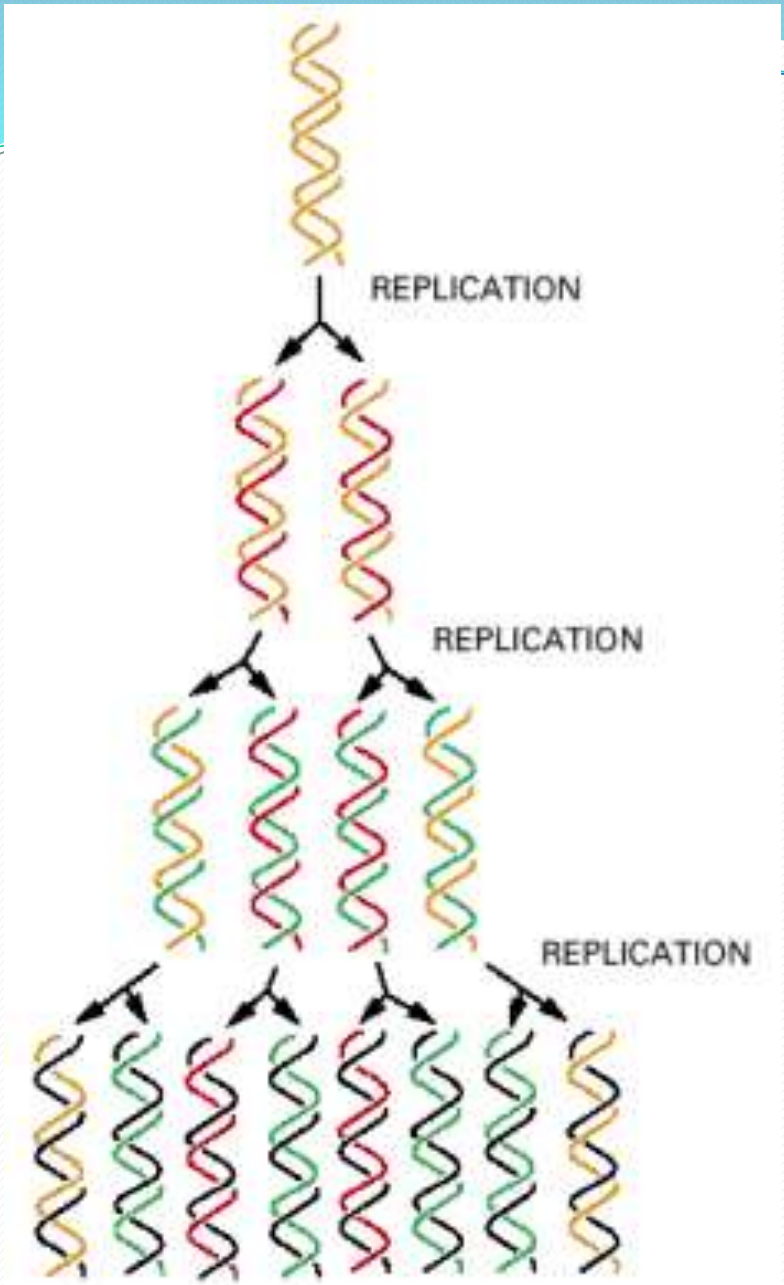
The free nucleotides are bonded together covalently and begin to form two new DNA strands. Notice that each strand of the original DNA molecule acts as a pattern, or *template*, for the synthesis of a new DNA strand.



Two DNA molecules

The process of replication continues all the way through the original DNA molecule. Each original strand makes a copy that is complementary to itself. The final product is two new DNA molecules that are identical to the original DNA molecule and contain the same information.

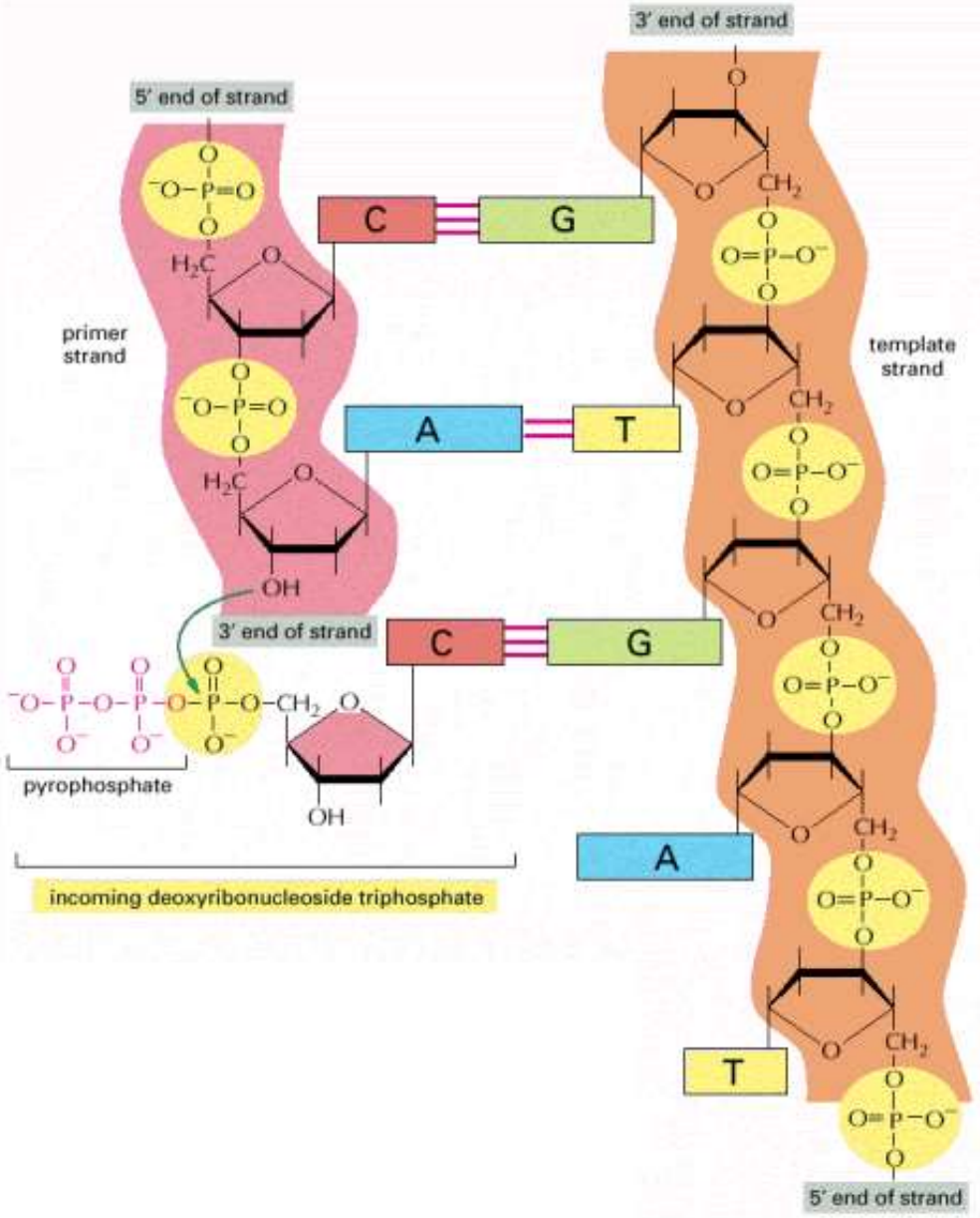
The process in which the nucleotide sequence of a DNA strand is copied by complementary base-pairing into a complementary DNA sequence is called *DNA templating*.



The semiconservative nature of DNA replication.

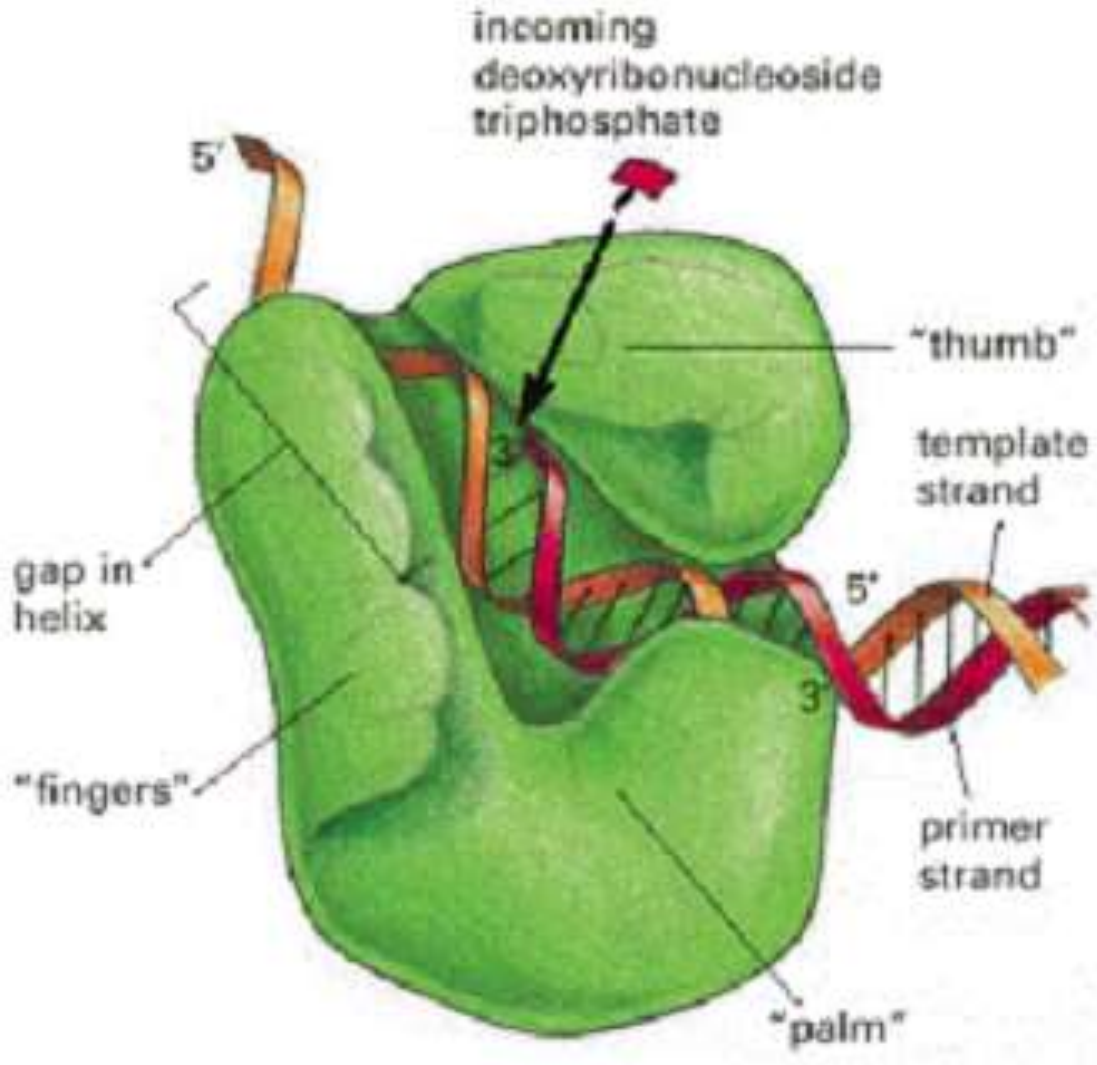
In a round of replication, each of the two strands of DNA is used as a template for the formation of a complementary DNA strand. The original strands therefore remain intact through many cell generations.

The chemistry of DNA synthesis



The addition of a deoxyribonucleotide to the 3′ end of a polynucleotide chain (the *primer strand*) is the fundamental reaction by which DNA is synthesized. As shown, base-pairing between an incoming deoxyribonucleoside triphosphate and an existing strand of DNA (the *template strand*) guides the formation of the new strand of DNA and causes it to have a complementary nucleotide sequence. Pyrophosphate is released.

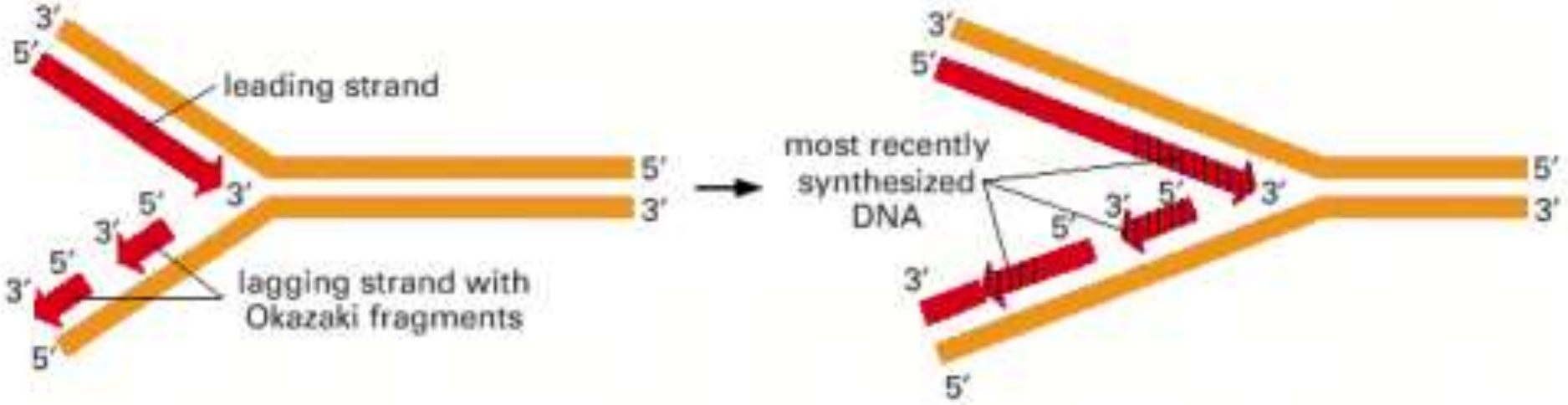
The chemistry of DNA synthesis



DNA synthesis is catalyzed by *DNA polymerase*. This enzyme was discovered in *E. coli* in 1957 (see figure).

A. Kornberg and S. Ochoa were awarded by Nobel Prize in 1959.

The chemistry of DNA synthesis



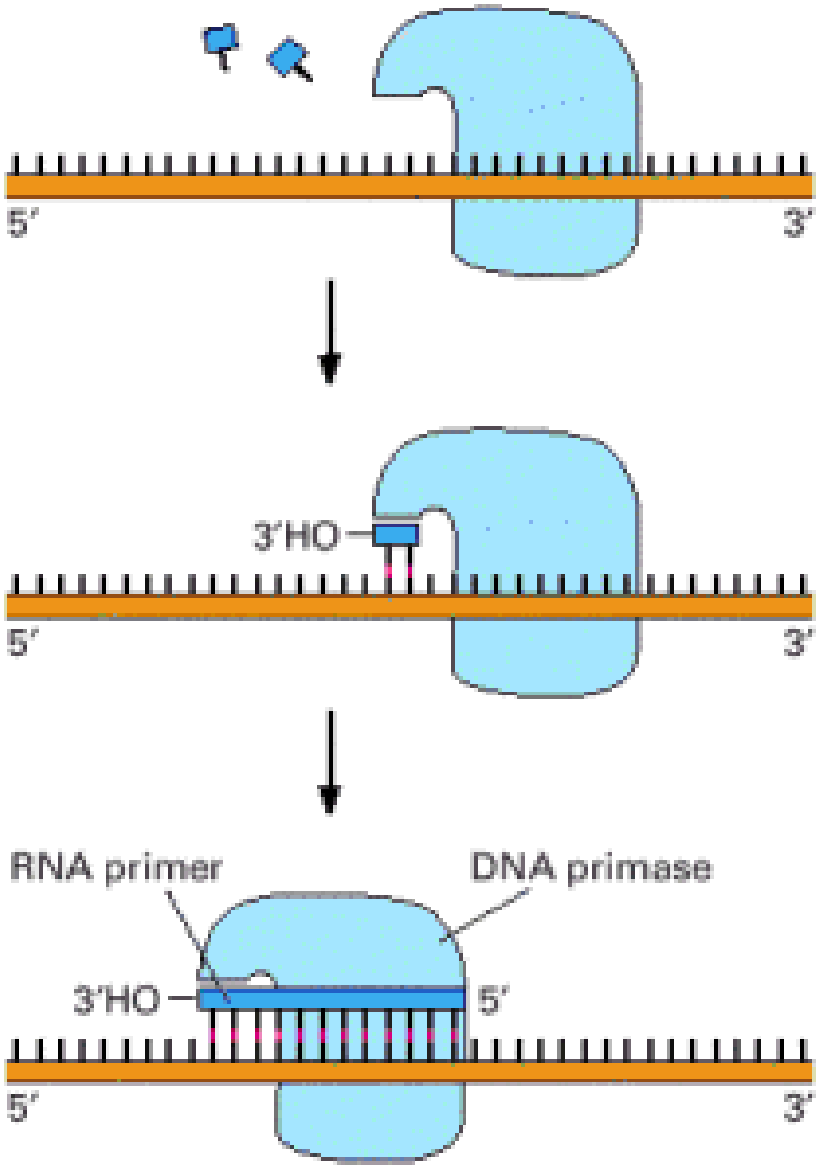
A localized region of replication moves progressively along the parental DNA double helix. Because of its Y-shaped structure, this active region is called a *replication fork*. DNA polymerase can polymerize DNA chain in the 5′-to-3′ direction only. But the two DNA strands have the antiparallel orientation. A replication fork therefore has an asymmetric structure. The DNA daughter strand that is synthesized continuously is known as the *leading strand*. The daughter strand that is synthesized discontinuously is known as the *lagging strand*.

The chemistry of DNA synthesis

- Lagging-strand DNA synthesis at the growing replication fork occurs by small pieces of DNA now commonly known as *Okazaki fragments*. These fragments are 1000–2000 nucleotides long in prokaryotes and 100–200 nucleotides long in eucaryotes.

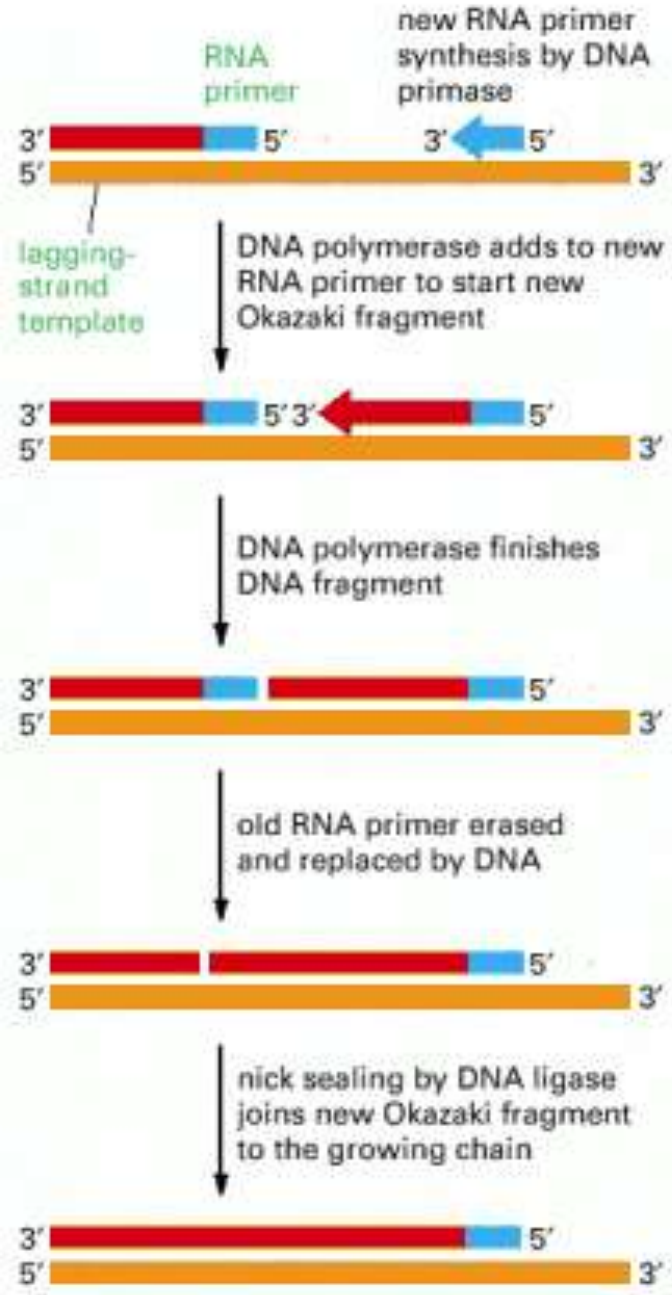
- The Okazaki fragments were shown to be polymerized only in the 5′-to-3′ chain direction and to be joined together after their synthesis to create long DNA chains.

The chemistry of DNA synthesis



- A special nucleotide-polymerizing enzyme synthesizes short RNA primer molecules on the lagging strand. This enzyme called *DNA primase* uses ribonucleoside triphosphates to synthesize short *RNA primers*.
- The primase synthesizes a short polynucleotide in the 5′-to-3′ direction and then stops, making the 3′ end of this primer available for the DNA polymerase.

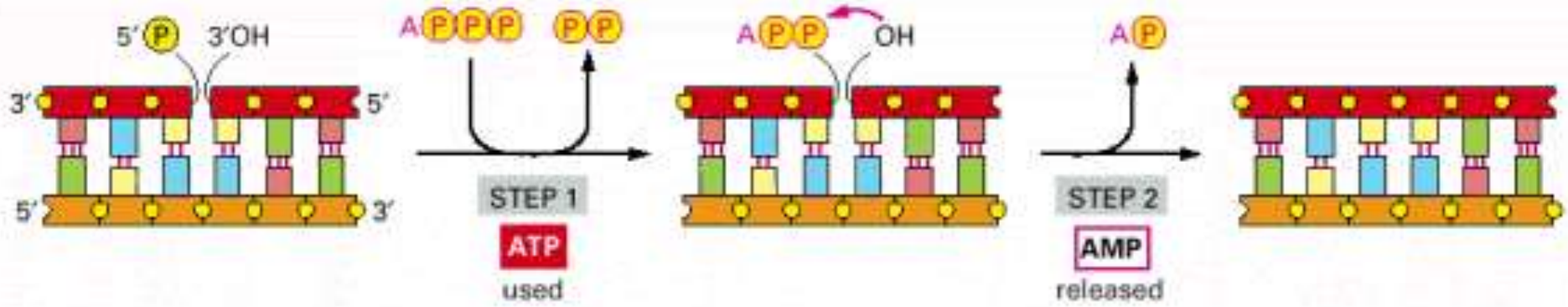
The chemistry of DNA synthesis



In bacteria:

- ✓ An RNA primer can be elongated by the DNA polymerase III at this end to begin an Okazaki fragment.
- ✓ To produce a continuous DNA chain from the many DNA fragments made on the lagging strand, each primer is erased by DNA polymerase I, which fills in gaps by nucleotides.
- ✓ An enzyme called *DNA ligase* then joins the 3′ end of the new DNA fragment to the 5′ end of the previous one to complete the process.

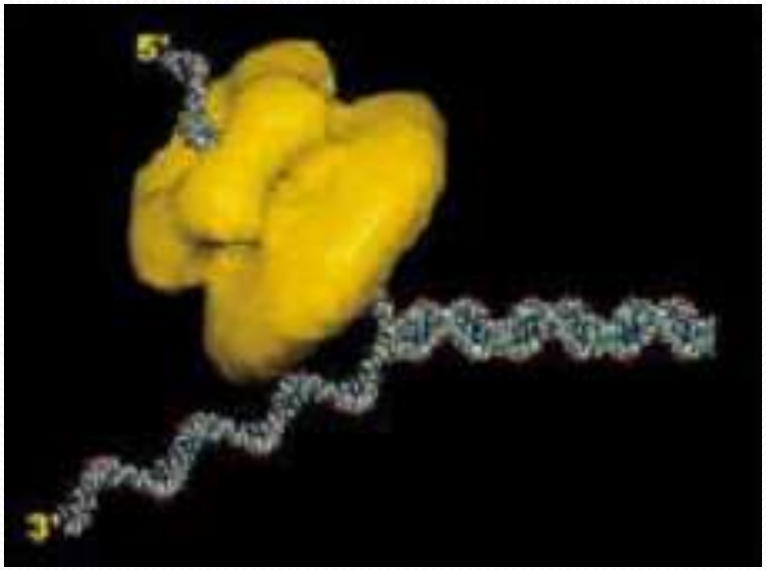
The chemistry of DNA synthesis

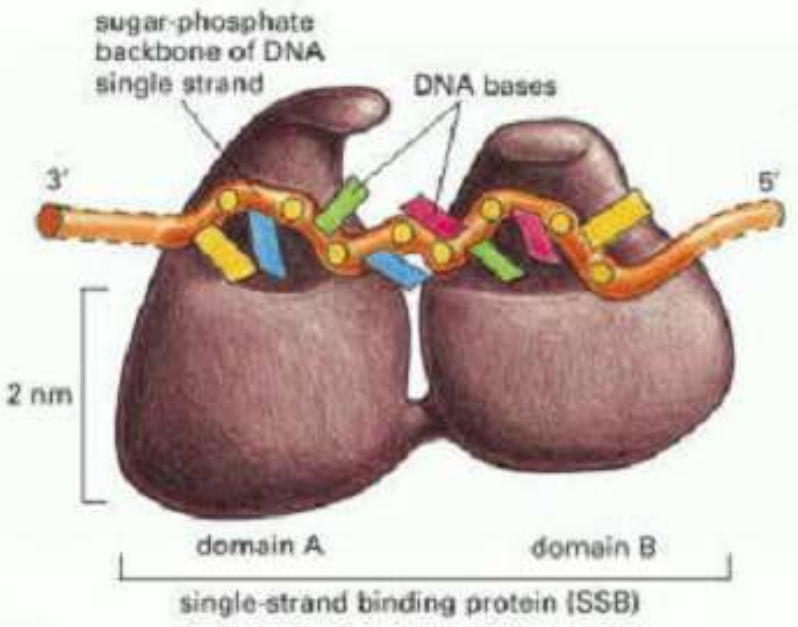


DNA ligase uses a molecule of ATP to activate the 5′ end at the nick (step 1) before forming the new bond (step 2).

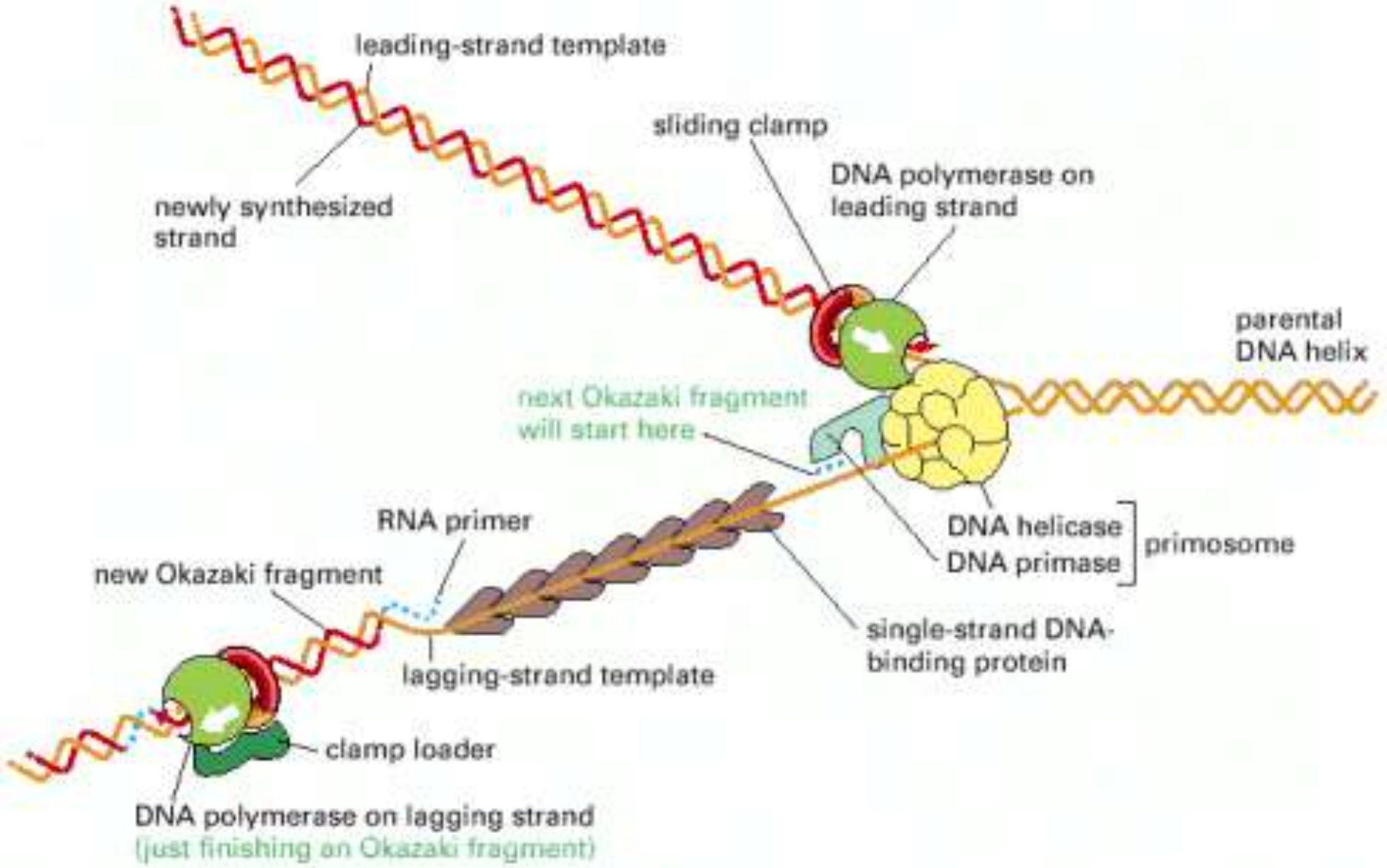
Special Proteins Help to Open Up the DNA Double Helix in Front of the Replication Fork

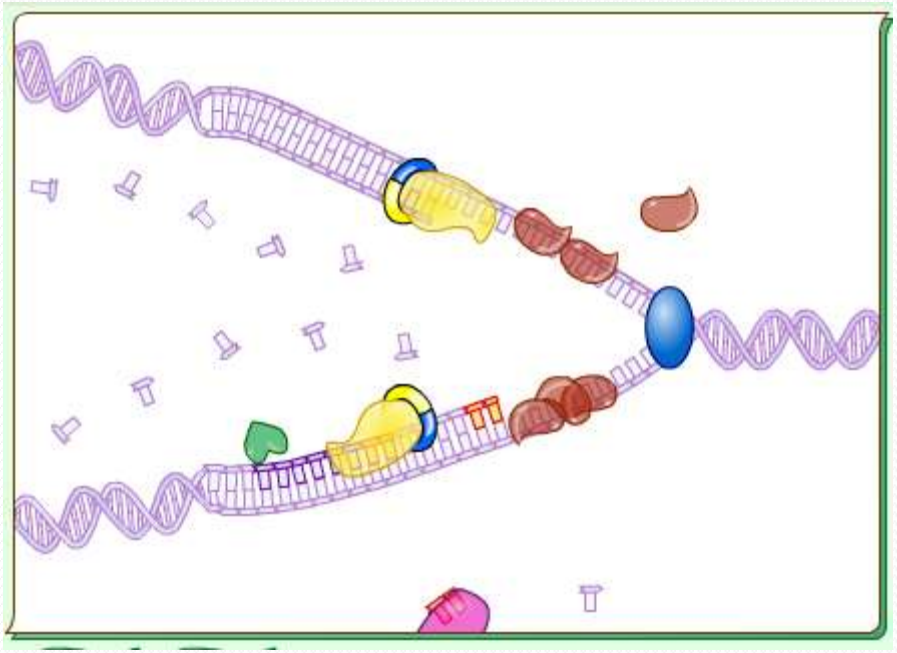
- ✓ *DNA helicases* catalyze the unwinding of the template DNA helix. DNA is synthesized at rates of up to 1000 nucleotide pairs per second.



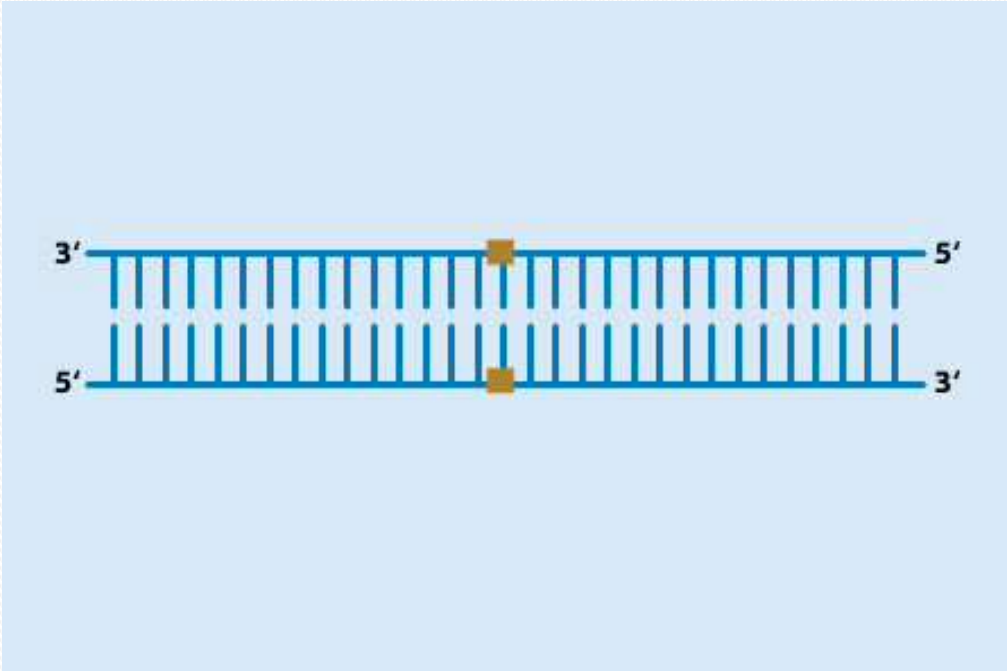


- ✓ Single-strand DNA-binding (SSB) proteins stabilize the unwound, single-stranded conformation.


leading-strand template
sliding clamp
DNA polymerase on leading strand
newly synthesized strand
parental DNA helix
next Okazaki fragment will start here
RNA primer
DNA helicase
DNA primase
primosome
new Okazaki fragment
single-strand DNA-binding protein
lagging-strand template
clamp loader
DNA polymerase on lagging strand
(just finishing an Okazaki fragment)



Animation: **DNA Replication**



The High Fidelity of DNA Replication Requires Several Proofreading Mechanisms

- The fidelity of copying DNA during replication is such that only about 1 mistake is made for every 10^9 nucleotides copied.

- This fidelity is much higher than one would expect, on the basis of the accuracy of complementary base-pairing (an error frequency of about 1 in 10^4). If the DNA polymerase did nothing special when a mispairing occurred between an incoming deoxyribonucleoside triphosphate and the DNA template, the wrong nucleotide would often be incorporated into the new DNA chain, producing frequent mutations. The high fidelity of DNA replication, however, depends not only on complementary base-pairing but also on several "proofreading" mechanisms that act sequentially to correct any initial mispairing that might have occurred.