STRUCTURE OF RNA & REPLICATION

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STRUCTURE OF RNA

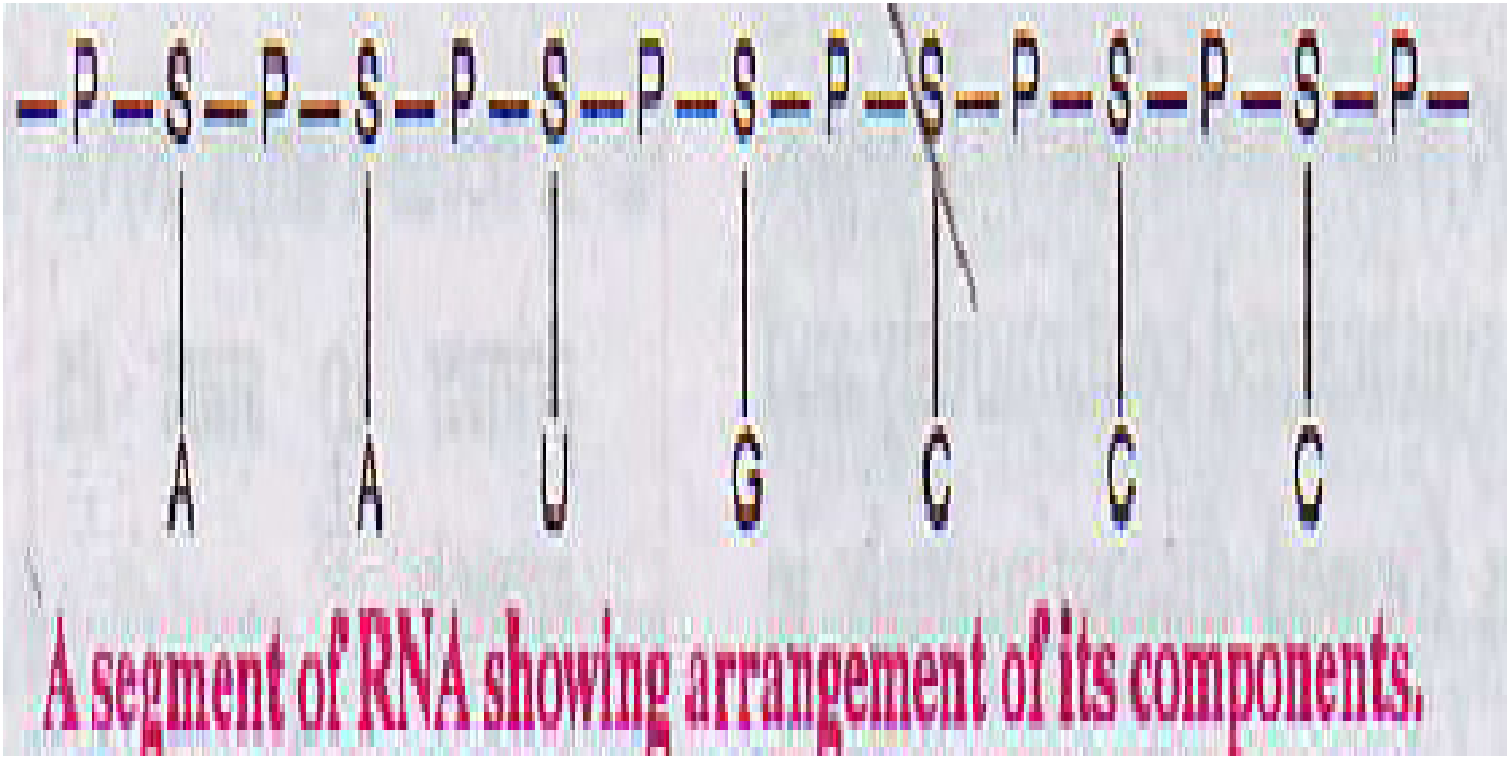
- Long unbranched,single stranded polymer of ribonucleotide units.

- A ribonucleotide unit has:
- 5-Carbon ribose sugar.

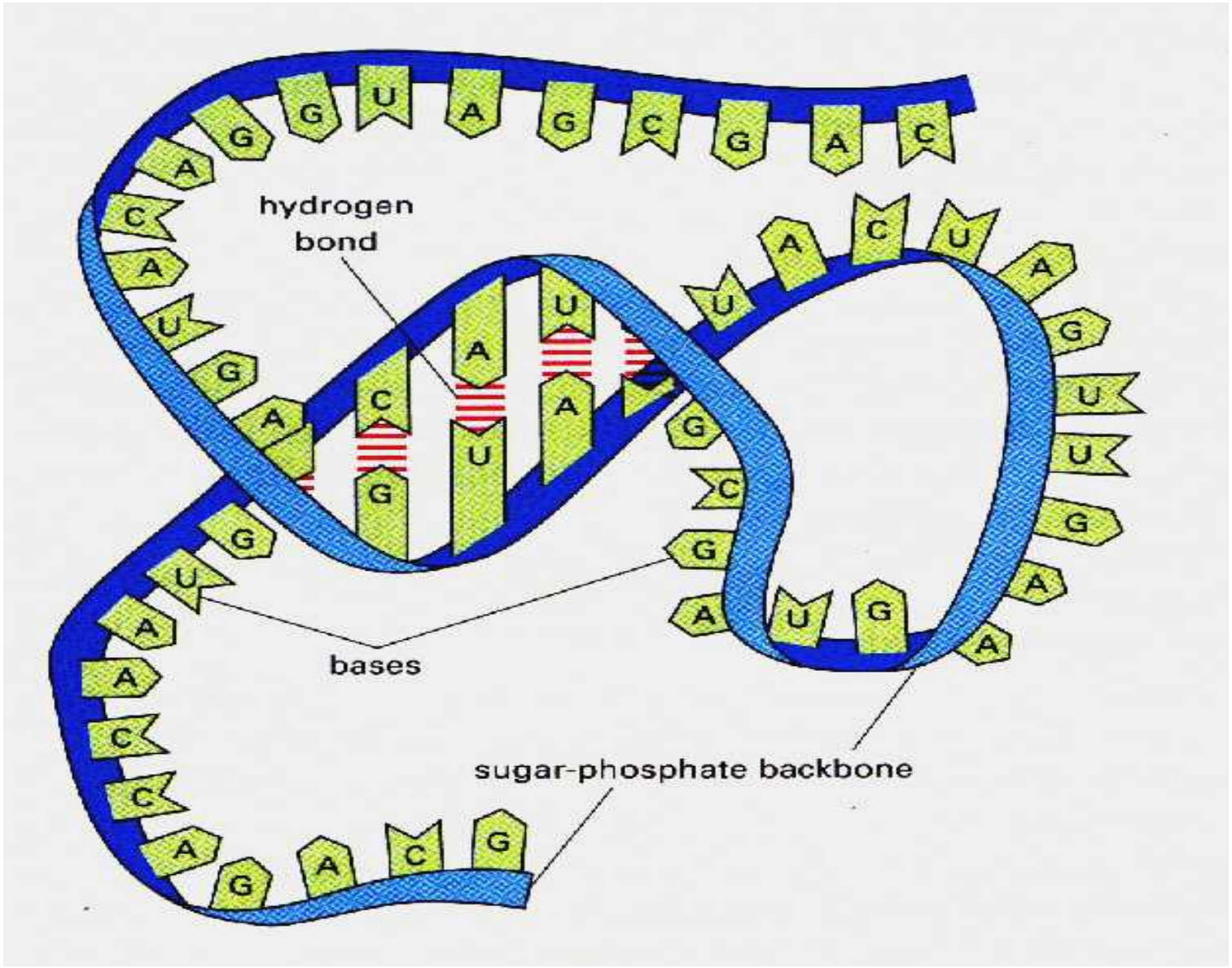
- Nitrogen Base:
- Purines: Adenine &Guanine.
- Pyrimidines: Uracil & Cytosine.

- Phosphate group.

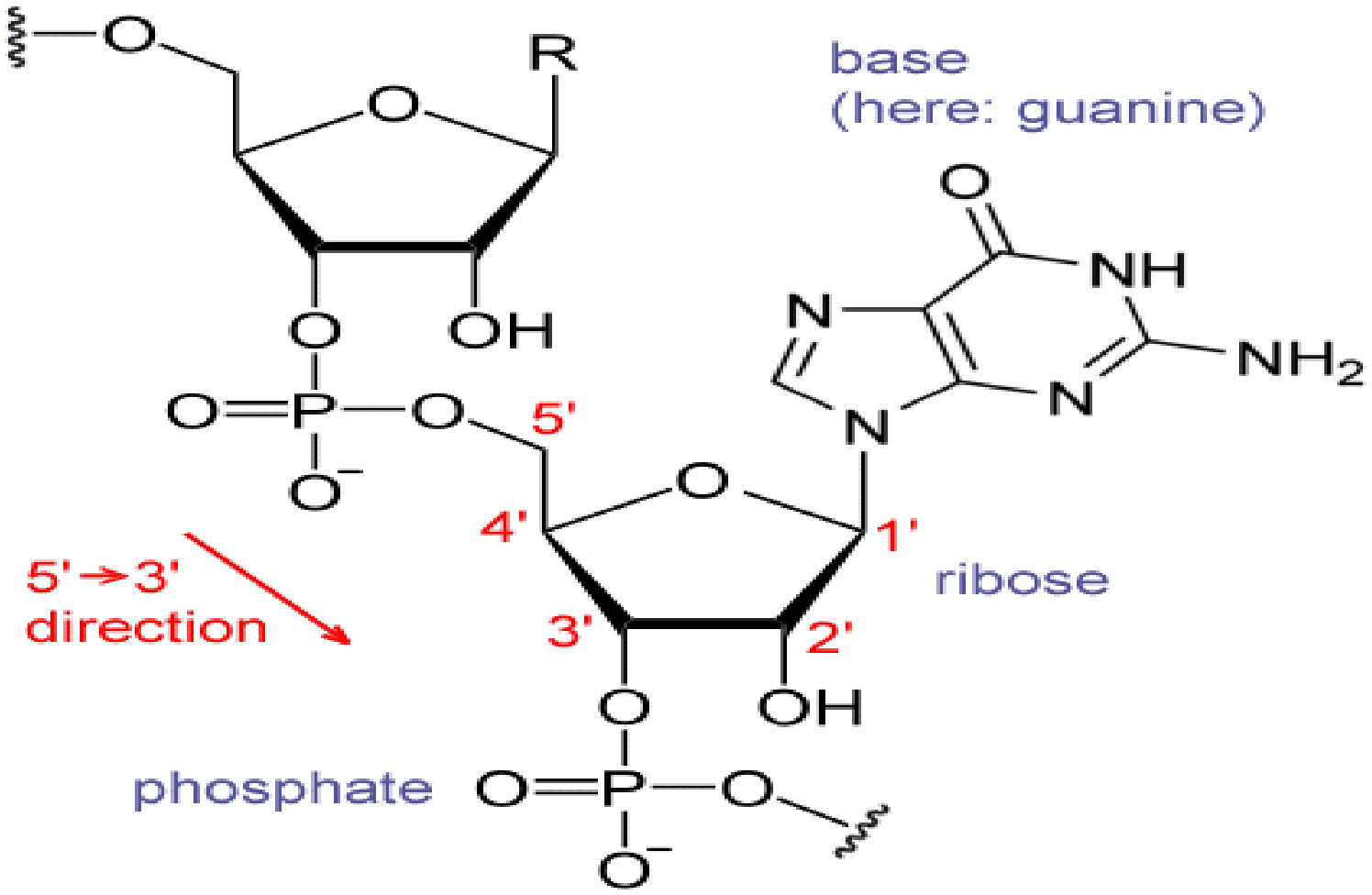
STRUCTURE OF RNA



A segment of RNA showing arrangement of its components.

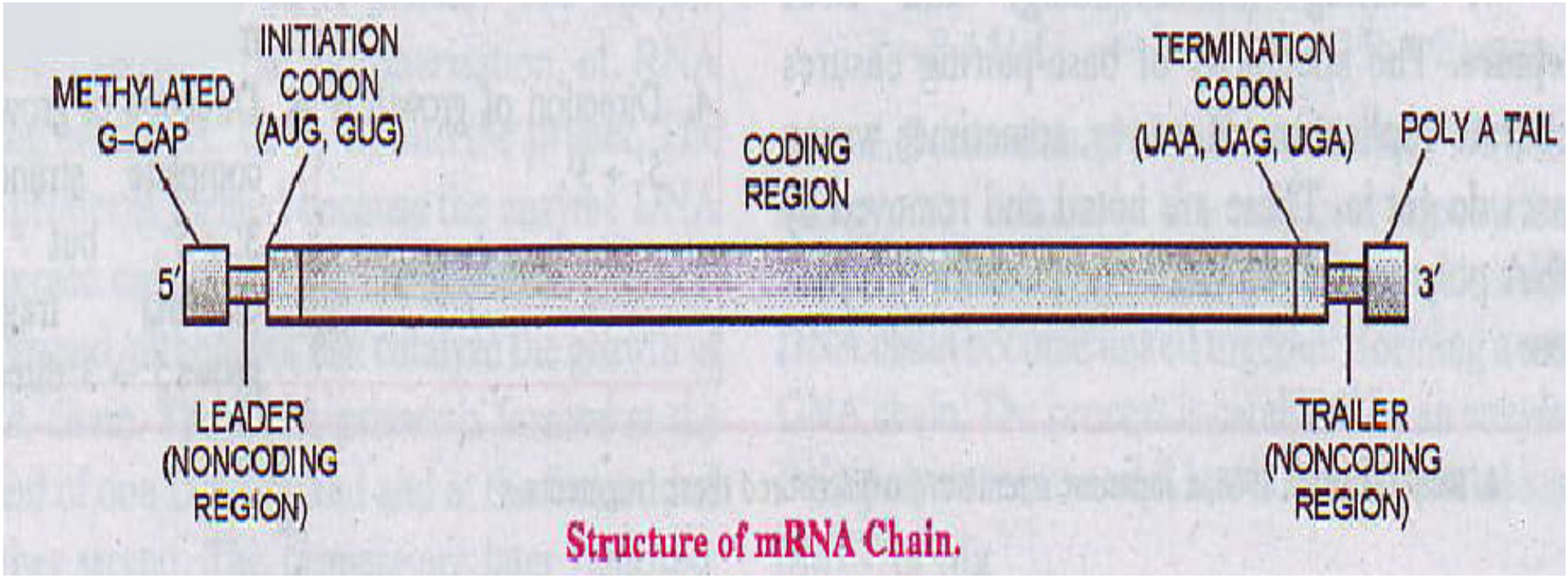

hydrogen
bond
bases
sugar-phosphate backbone

CHEMICAL STRUCTURE OF RNA



TYPES OF RNA

- **Messenger RNA [mRNA]:**
- Forms 5% of total RNA.
- Linear & largest of all RNA's
- Carries message from DNA about sequence of particular amino acids to form polypeptide.



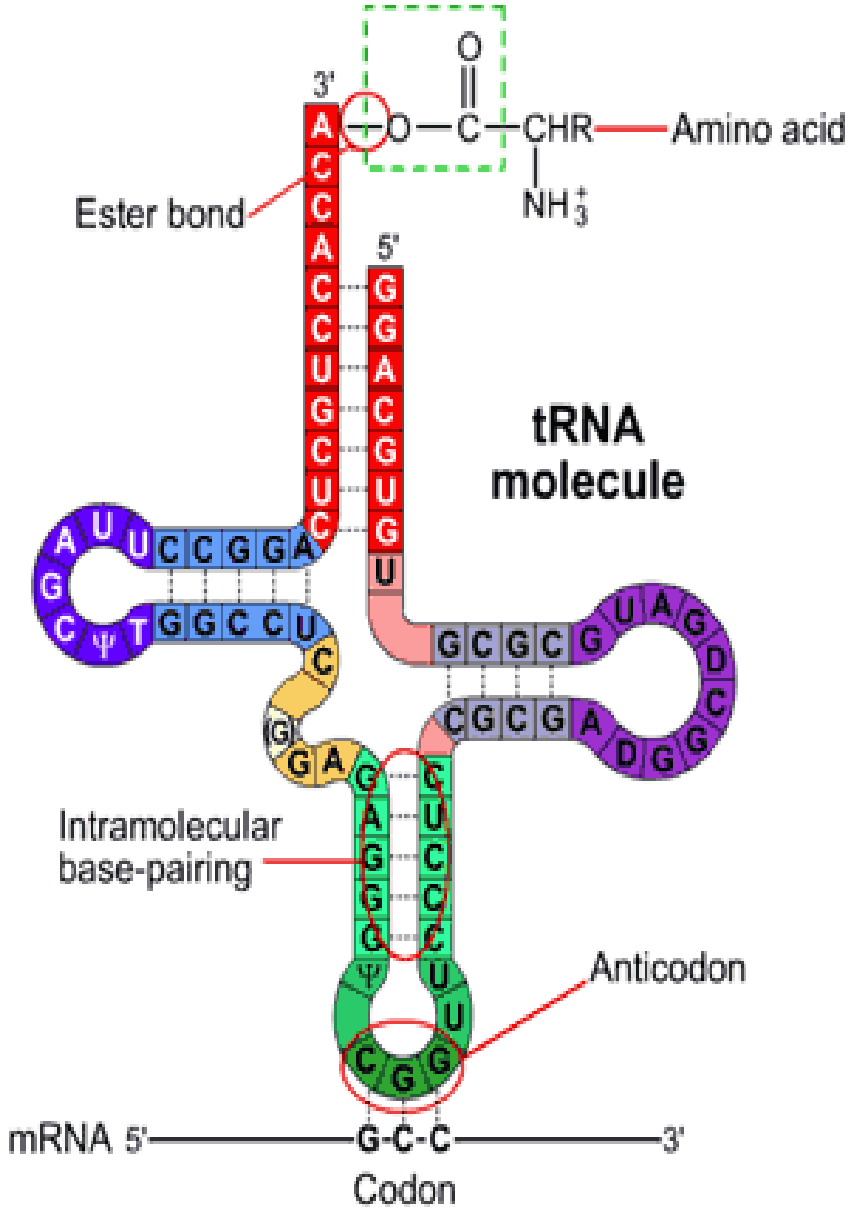
Structure of mRNA Chain.

- **Transfer RNA [tRNA]:** forms 15% of total RNA

- Smallest of all RNA types & clover leaf shaped.

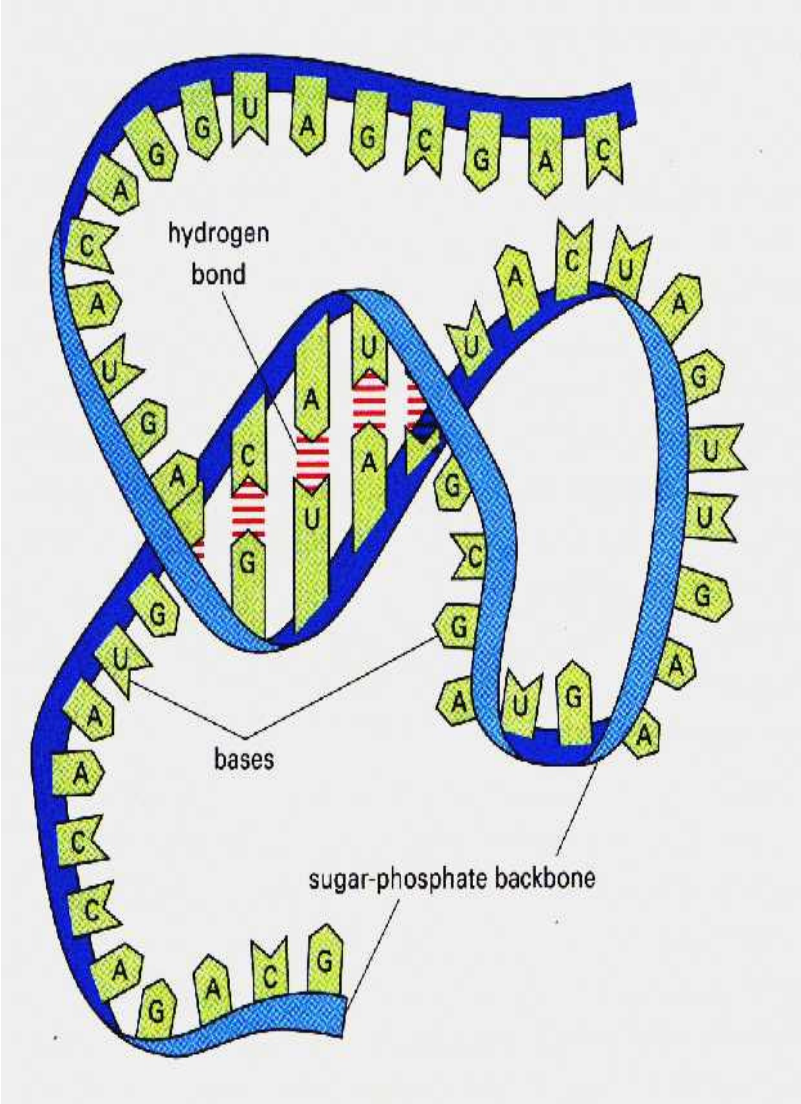
- 3'end as carrier end where specific amino acid joins

- Opposite to carrier end is recognition end having three triplets called anticodon.

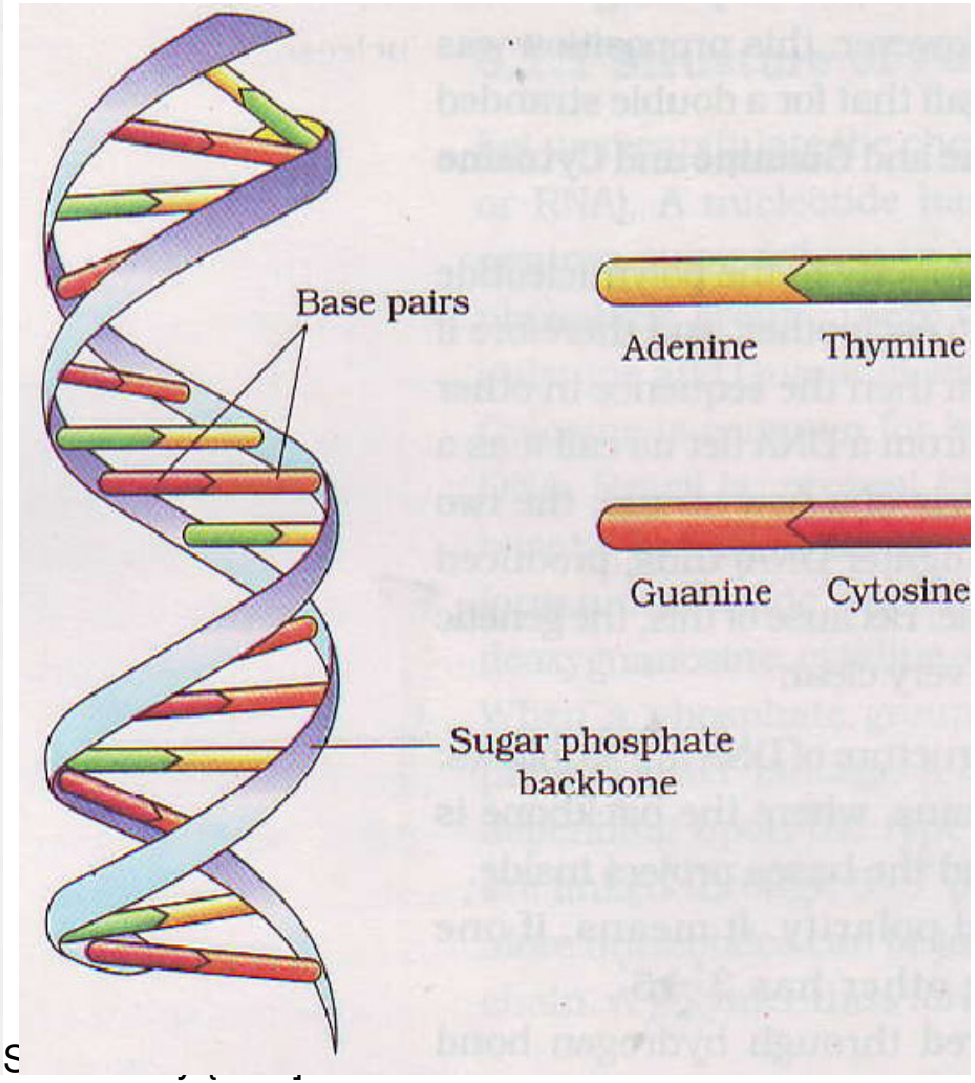


- Enzyme site for charging enzyme.
- Ribosome site opposite to enzyme site for attachment of ribosome
- **Ribosomal RNA [r RNA]**:
- Forms 80% of total RNA.
- Greatly coiled molecule.
- Along with Proteins,forms larger & smaller subunits of ribosome.

- RNA


hydrogen
bond
bases
sugar-phosphate backbone

- DNA


Base pairs
Adenine
Thymine
Guanine
Cytosine
Sugar phosphate
backbone

STEPS IN PROTEIN SYNTHESIS

- There are three main steps in protein synthesis :-

  1) Replication

  2) Transcription

  3) Translation

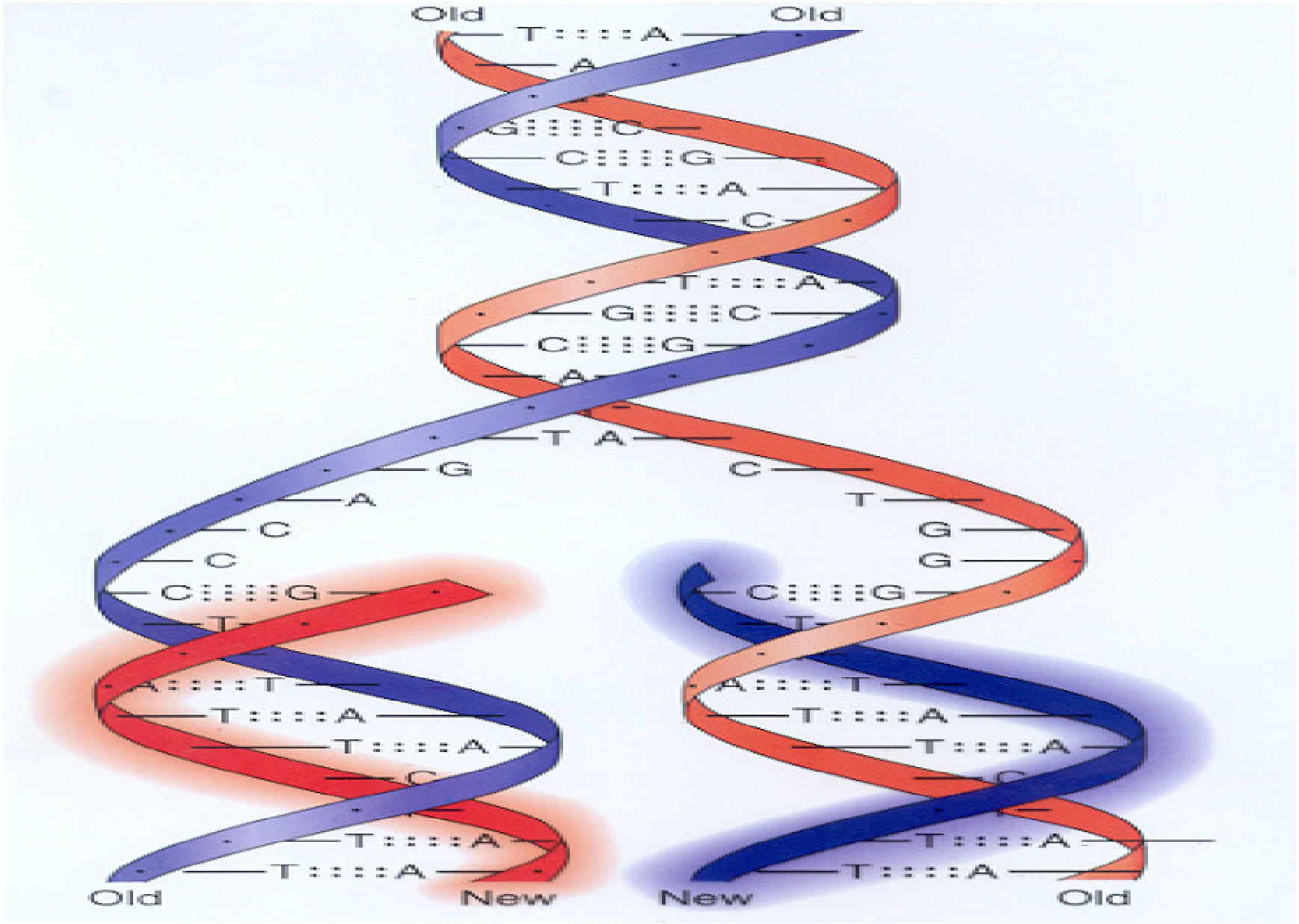
REPLICATION

- DNA replication is the process whereby an entire double-stranded DNA is copied to produce a second, identical DNA double helix.
- **Site of occurrence** :
- In Eukaryotes occurs in the nucleus
- In Prokaryotes occurs in the cytoplasm .

- Time of occurrence :
- Replication takes place during the s-phase of the cell cycle . The stimulus which starts the process at this time and stops it at other time is not fully known.

MODE OF REPLICATION

- **REPLICATION IS SEMI CONSERVATIVE**
- The structure of DNA was suggested by *Watson and crick*. They proposed that when the DNA is to be replicated:
- The two strands of the DNA molecule uncoil and separate from each other, but remain intact, as each serve as the template for the synthesis of a complementary strand.


Old
Old
Old
New
New
Old

- When the replication process is complete, each daughter DNA molecule consist of one old (parent) strand and one new strand .

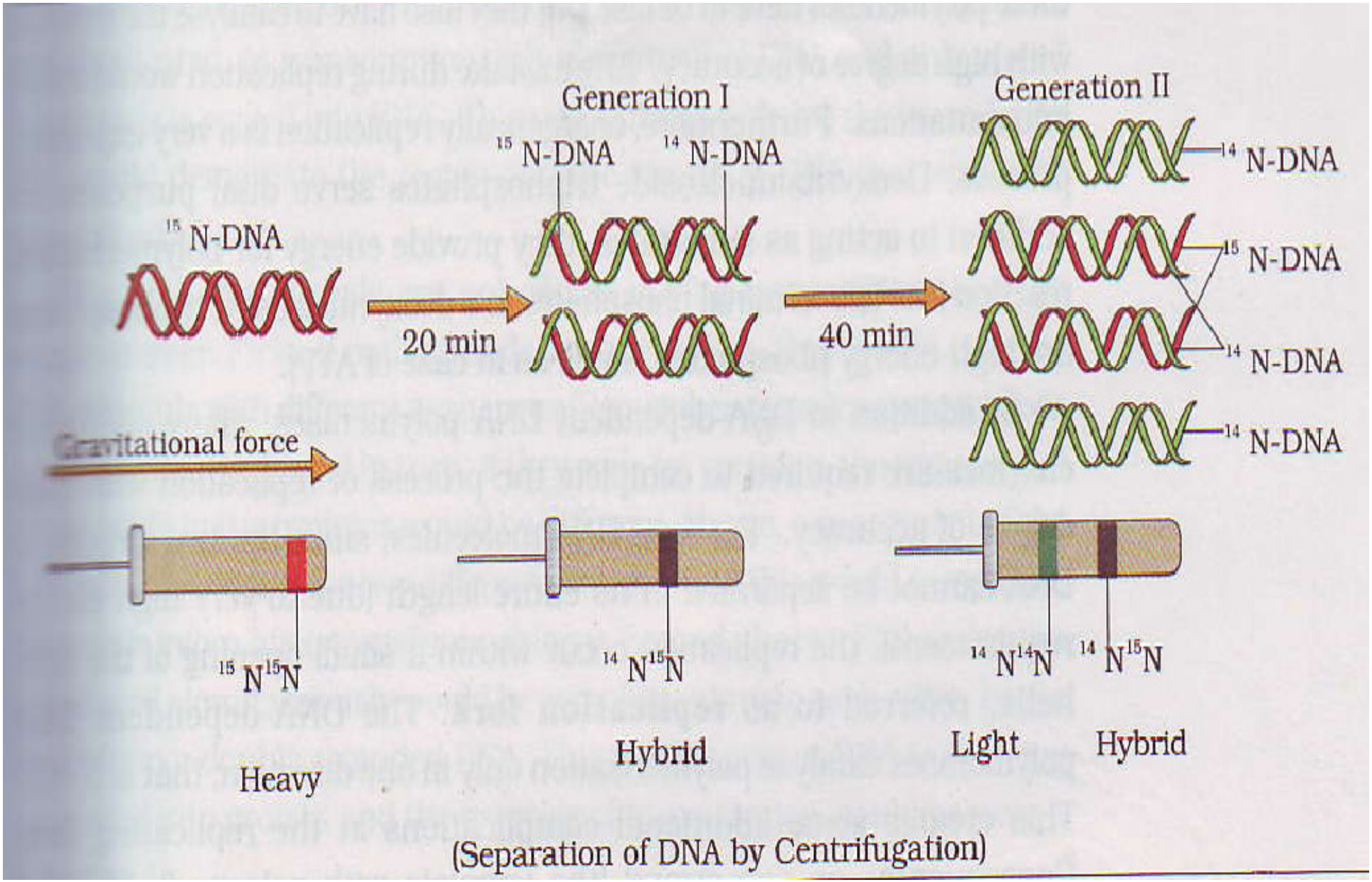
- This mode of replication is described as **semi conservative.**

- **Evidence for semiconservative replication**
- Came from the experiments of. Meselson and stahl in 1958.

- They grew E.coli in a medium containing heavy nitrogen isotope 15N for many generations

- Obtained a population that had uniformly 15N DNA , which was heavier than the DNA obtained from E.coli grown in 14N medium .

SEMICONSERVATIVE REPLICATION



- E.coli cells with 15N DNA were than transferred to a medium having 14N isotope. DNA molecules of first generation cells were found hybrid (15N-14N) .this suggests the semiconservative mode of replication .

- The daughter cells when divided again the second generation cells were found to have :

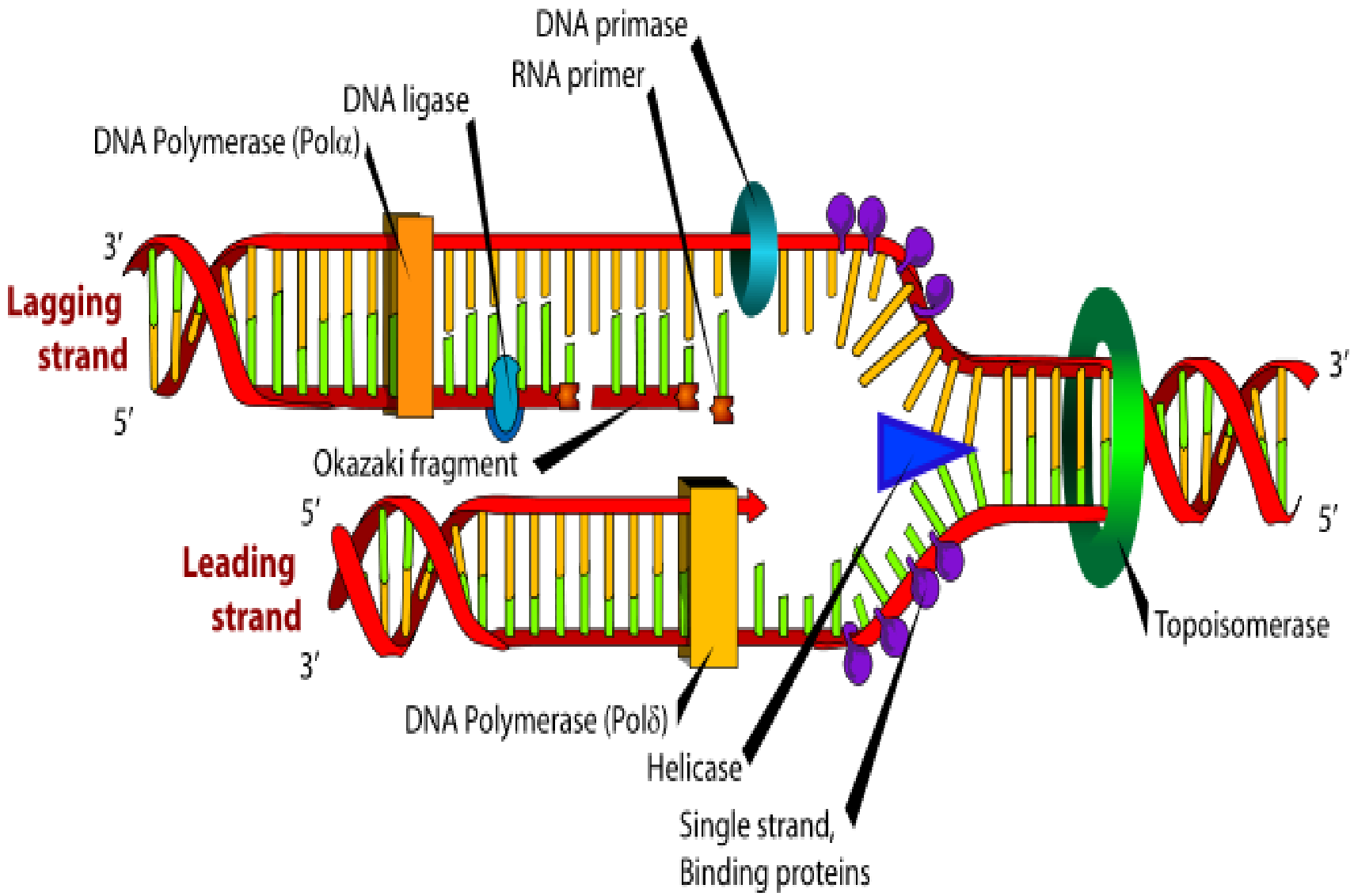
  50% 15N-14N hybrid DNA and 50% 14N-14N DNA, this again confirms the semiconservative mode of replication .

MECHANISM OF DNA REPLICATION

- It is multi step process
- Activation of deoxyribonucleotides
- Unwinding of DNA double helix
- Formation of RNA primer
- Base pairing
- Conversion to deoxyribonucleotide monophosphate
- Formation of new DNA chain
- Editing (proof reading) and DNA repair

DNA REPLICATION PROTEINS

- **Helicase-unwinds DNA double helix into two individual strands**
- **Single stranded binding protein or SSB's prevent DNA strands to form double helix.**
- **Primase or RNA Polymerase synthesises short RNA primers needed to start replication.**

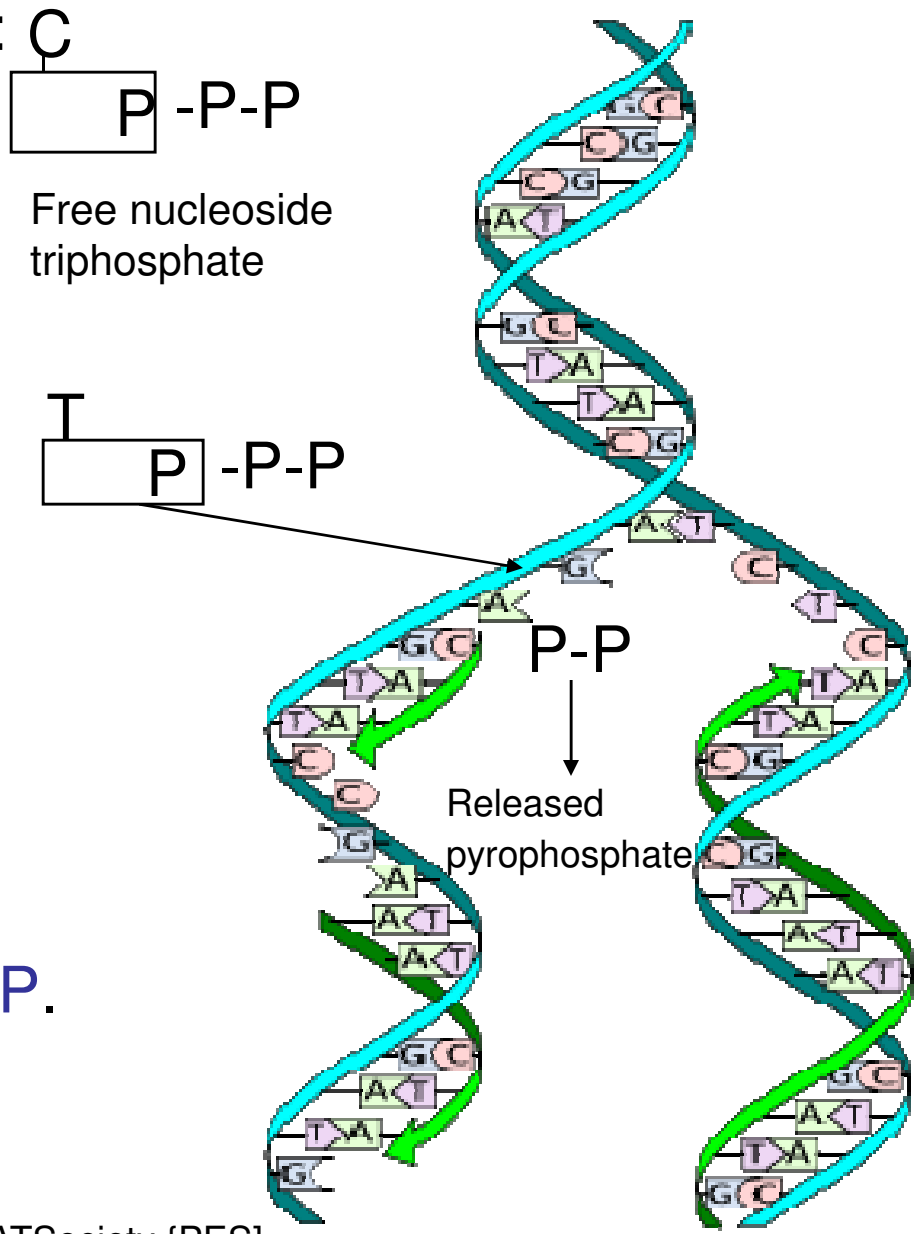

DNA primase
RNA primer
DNA ligase
DNA Polymerase (Polα)
3′
Lagging strand
5′
Okazaki fragment
5′
Leading strand
3′
DNA Polymerase (Polδ)
Helicase
Single strand, Binding proteins
Topoisomerase
3′
5′

- **DNA Polymerase strings nucleotides together to form a DNA strand.**
- **RNAse H remove RNA primer that began DNA strand synthesis.**
- **DNA Ligase links short DNA segments together to create continuous DNA strand**

1. Activation of deoxyribonucleotides :

a. Four types of deoxyribonucleoside monophosphates(deAMP, deGMP, deCMP, deTMP) found in the nuclear sap , serve as raw material.

b. They are activated by union with ATP, process catalyzed by enzyme phosphorylase resulting in deATP,deGTP,deCTP,deTTP.



2. Unwinding of parent DNA bases :

a. **Helicases** unwinds the DNA helix by breaking of weak hydrogen bonds between complimentary bases.

b. **SSB proteins** or helix stabilizing protein stabilizes the chain in single stranded form to reduce the energy needed for unwinding the DNA helix

c. **Topoisomerases** may cut and rejoin one strand of DNA to facilitate uncoiling.

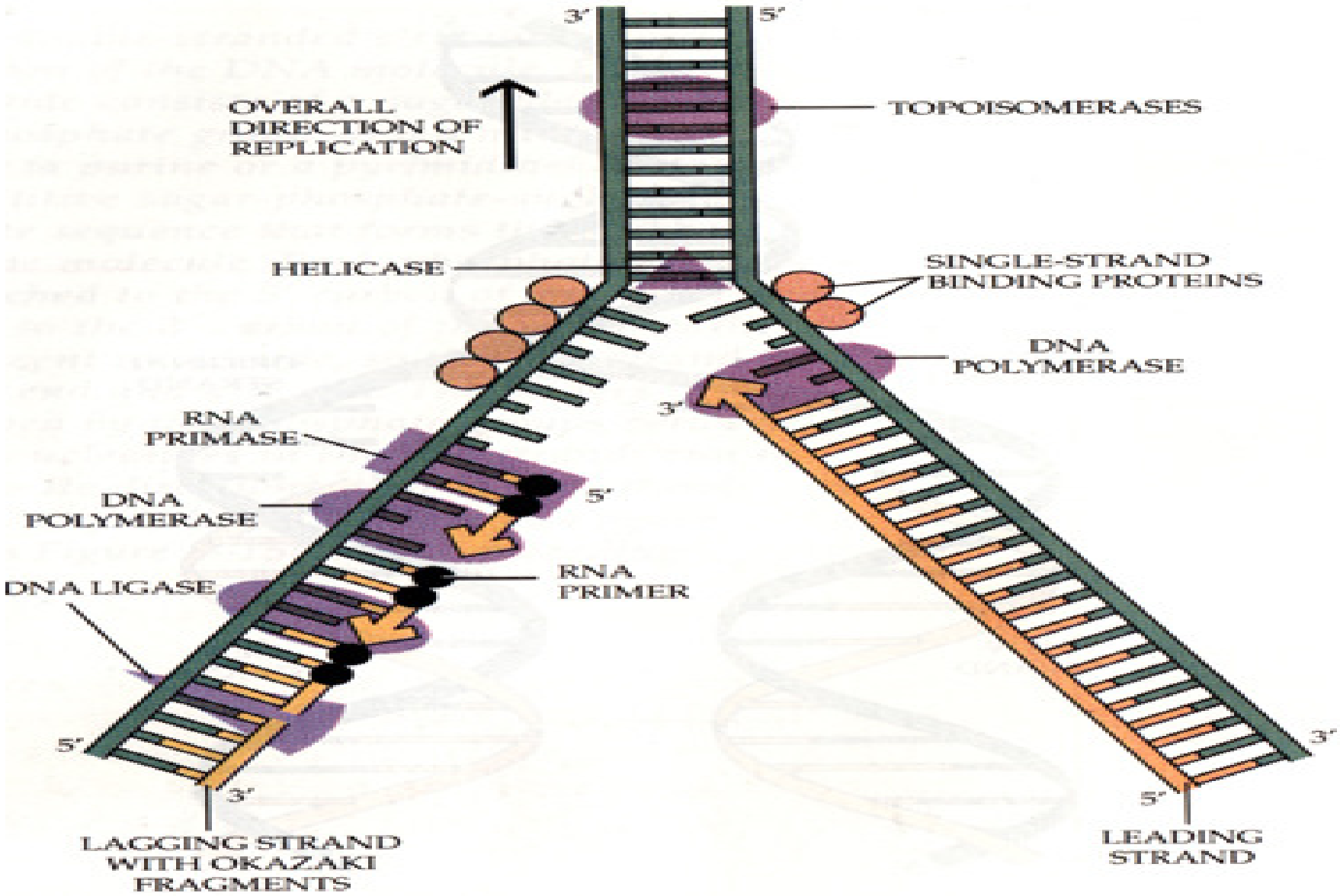
d. In prokaryotes, the enzymes **gyrases** do the work of helicases and topoisomerases .

3. Formation of RNA primer

1. Unzipping of the DNA double strand forms a Y – In eukaryotes DNA strand separates at specific points called ***origins of replication*** , but there is a single origin called ***Ori*** in prokaryotes.

2. Y-shaped structure called the ***replication fork*** .

3. A short chain of RNA (**RNA primer**) is formed on the DNA template at the 5' end ,this is catalyzed by enzyme primase.

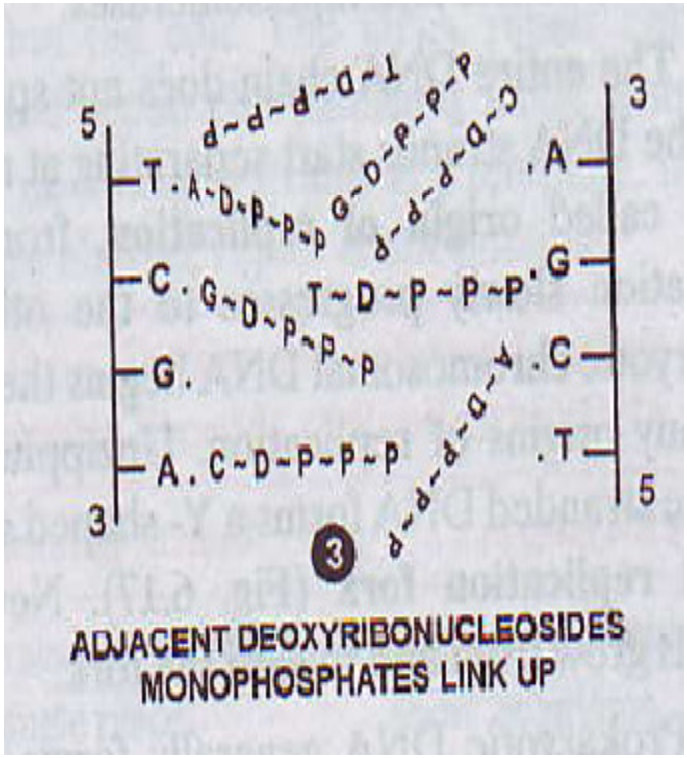

3′
5′
OVERALL DIRECTION OF REPLICATION
TOPOISOMERASES
HELICASE
SINGLE-STRAND BINDING PROTEINS
DNA POLYMERASE
3′
RNA PRIMASE
5′
DNA POLYMERASE
RNA PRIMER
DNA LIGASE
5′
3′
3′
5′
LAGGING STRAND WITH OKAZAKI FRAGMENTS
LEADING STRAND

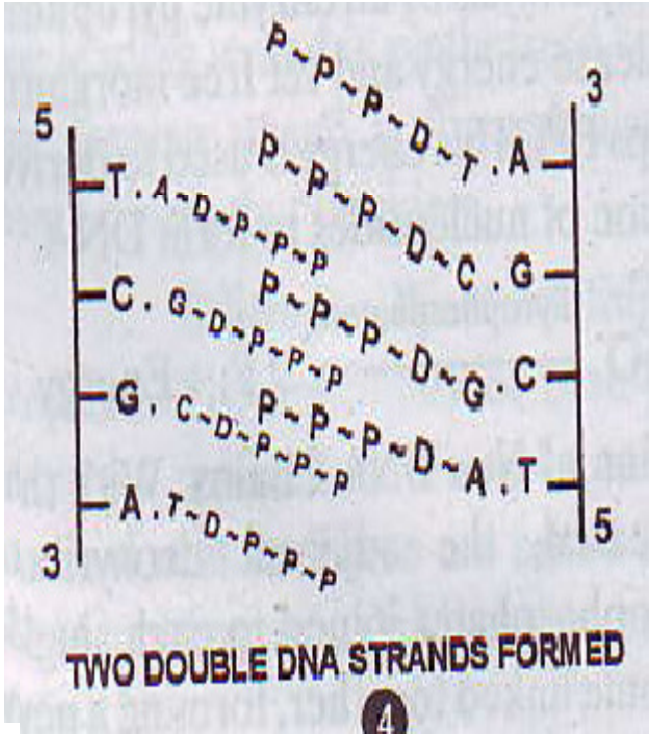
4. RNA primer is required because DNA primase cannot initiate the synthesis of a new DNA strand .

5. RNA primers are formed at the free end of one strand and the fork end of the other strand because it can initiate only at the 5’ end .

6. Primers are later removed and the gaps are filled with deoxyribonucleotides to make the DNA strands continuous.

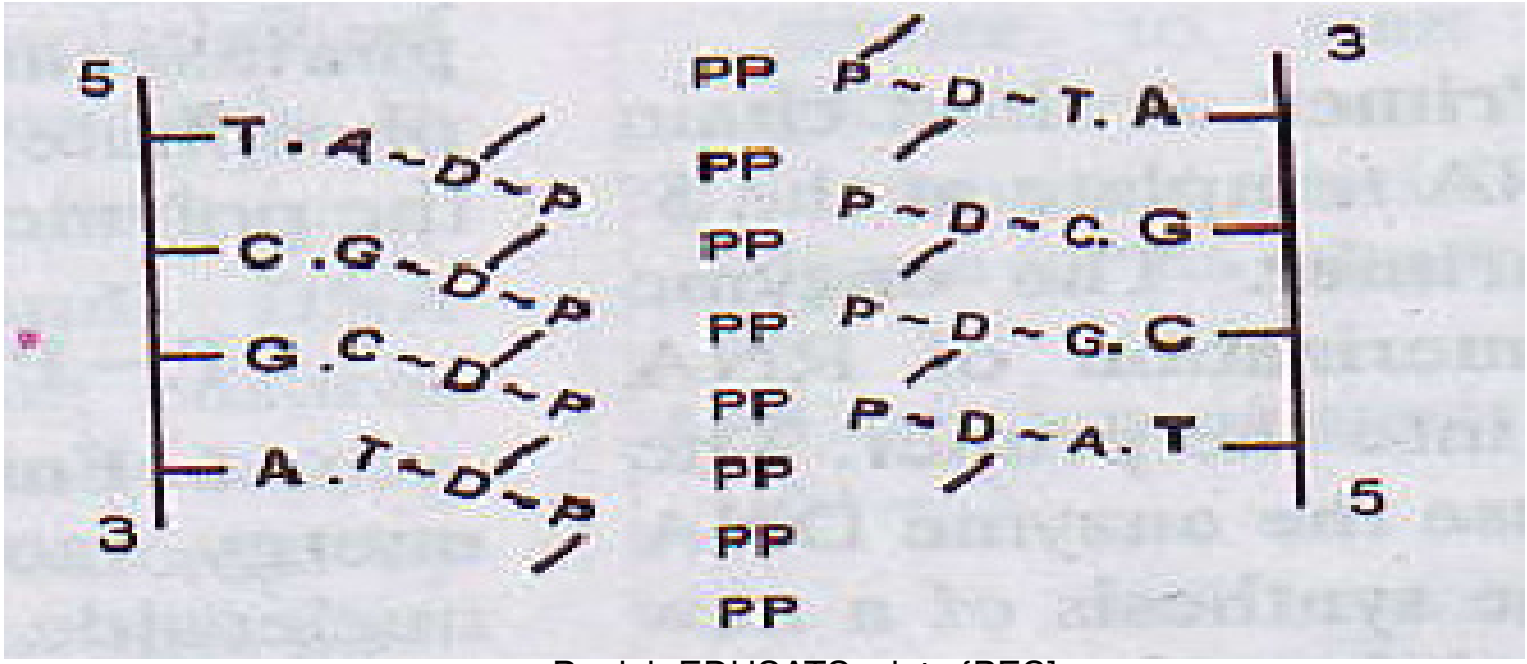
- # Base pairing:
- Deoxyribonucleoside triphosphate of one strand get joined to appropriate nitrogen bases of both single DNA chains.
- Nitrogen bases bind acc. to base pairing rule of Watson & Crick
- A-T,T-A,C-G, G-C.





- # Conversion to Deoxyribonucleoside Monophosphates:
- Deoxyribonucleoside triphosphate joined to each singleDNA chain breaks off high energy bonds &set free Pyrophosphate[P~P]

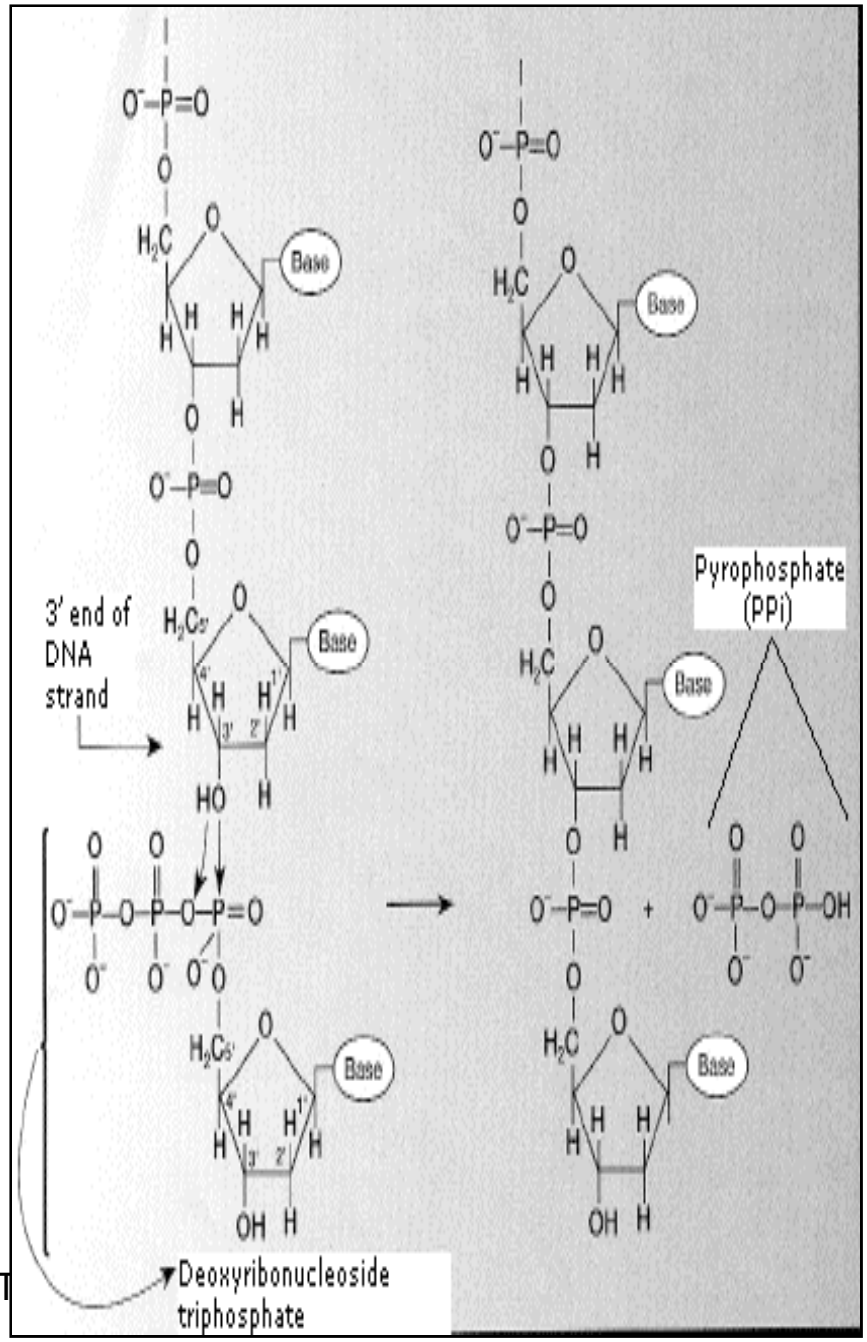
- Pyrophosphate undergo hydrolysis & release energy used to derive polymerisation of nucleosides to form DNA.



- DNA replication begins with the "unzipping" of the parent molecule as the hydrogen bonds between the base pairs are broken.

- The second and third phosphates are removed together as molecule of **pyrophosphate** (**PPi**).

- When the process is complete, two DNA molecules have been formed identical to each other and to the parent molecule



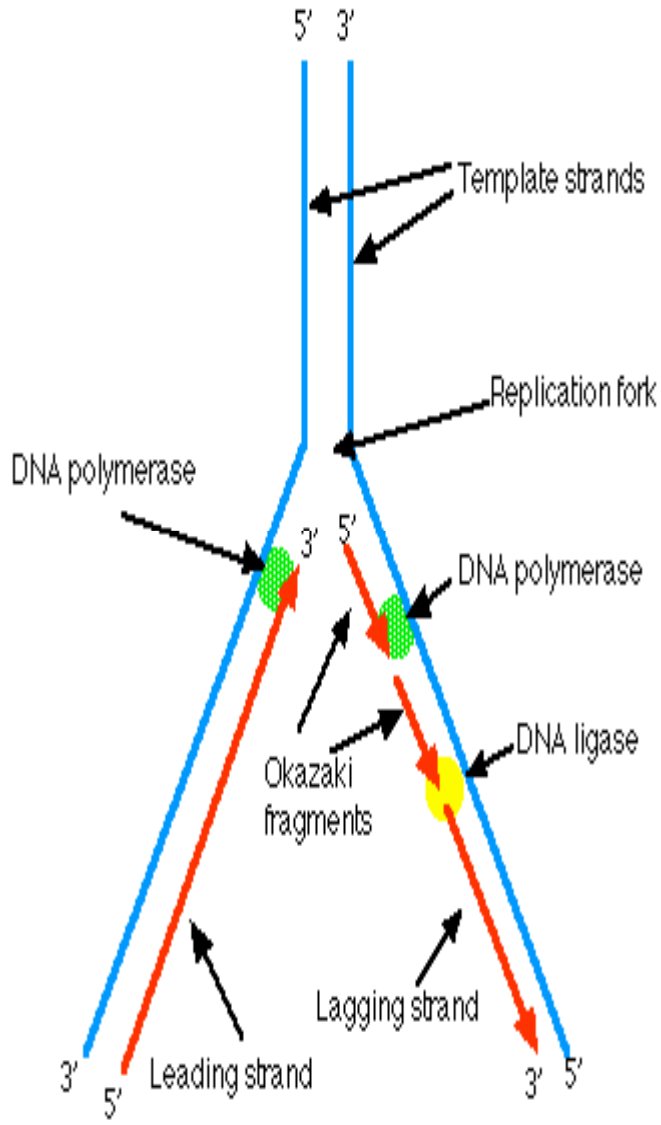
Formation of new DNA chain

- The adjacent deoxyribonucleoside monophosphates get linked together forming a new DNA chain with the help of energy released due to hydrolysis of pyrophosphates
- Process is catalyzed by enzyme DNA polymerase, metal ions Mn++ and Mg++
- The two double DNA chains inherits one single chain from the mother chain which serves as the code specific template for the formation of new single chain
- Thus genetic code is faithfully transmitted from one DNA generation to the next

Leading and Lagging strands

- The double helix consists of two antiparallel DNA strands with complementary 5’ to 3’ strands running in opposite directions.

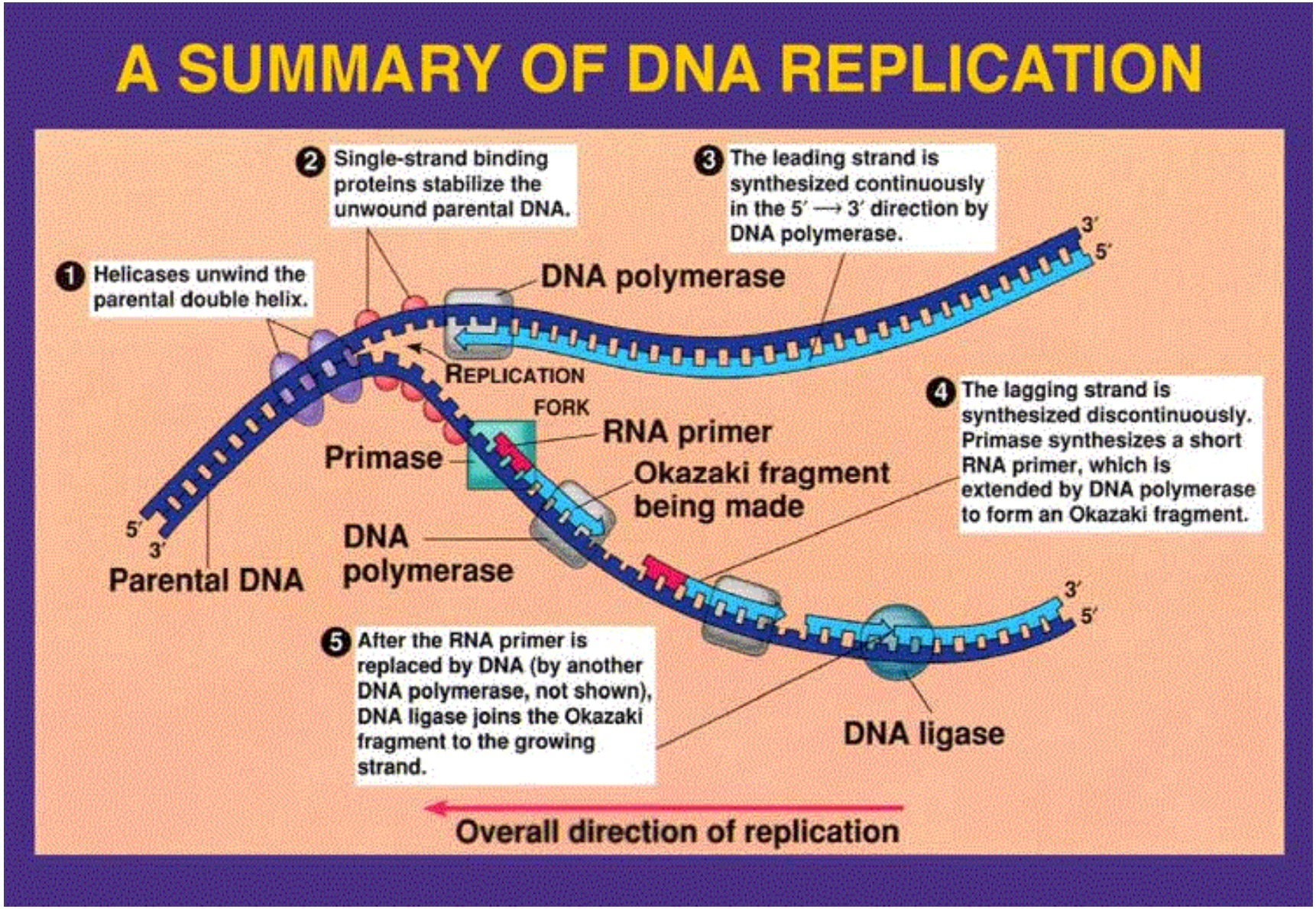
- Polymerase enzymes can synthesize nucleic acid strands only in the 5’ to 3’ direction,



- One new strand is formed in a continuous stretch in the 5’- 3’ direction,called **leading strand**.

- The other strand is formed in short DNA segments in the 5’-3’ direction starting from RNA primers, these are called okazaki fragments.

- The okazaki fragments are later joined together by enzyme DNA ligase forming a **lagging strand.**


A SUMMARY OF DNA REPLICATION
1 Helicases unwind the parental double helix.
2 Single-strand binding proteins stabilize the unwound parental DNA.
3 The leading strand is synthesized continuously in the 5′ → 3′ direction by DNA polymerase.
4 The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
5 After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.
DNA polymerase
REPLICATION FORK
RNA primer
Primase
Okazaki fragment being made
DNA polymerase
Parental DNA
DNA ligase
3′
5′
5′
3′
3′
5′
Overall direction of replication

7.EDITING AND DNA REPAIR

- Though specificity of base paring ensures accurate replication, however some wrong bases do get in, which are noted and removed by **DNA polymerase**

- The abnormal regions of DNA resulting from mutation are cleaved by E**nzymes nucleases.**

- The **DNA polymerase** resynthesizes the missing segments of DNA strand using the intact DNA strand as the template.

- The **DNA ligase** joins the new and old segments of the strand under repair .

- This makes the damaged DNA strand normal.

- Each daughter double DNA molecule becomes spirally coiled to form a double helix.

THANKS

Punjab EDUSATSociety {PES]