STUDY MATERIAL

VIVEKANANDA COLLEGE THAKURPUKUR

NAAC ACCREDITED GRADE—'A'

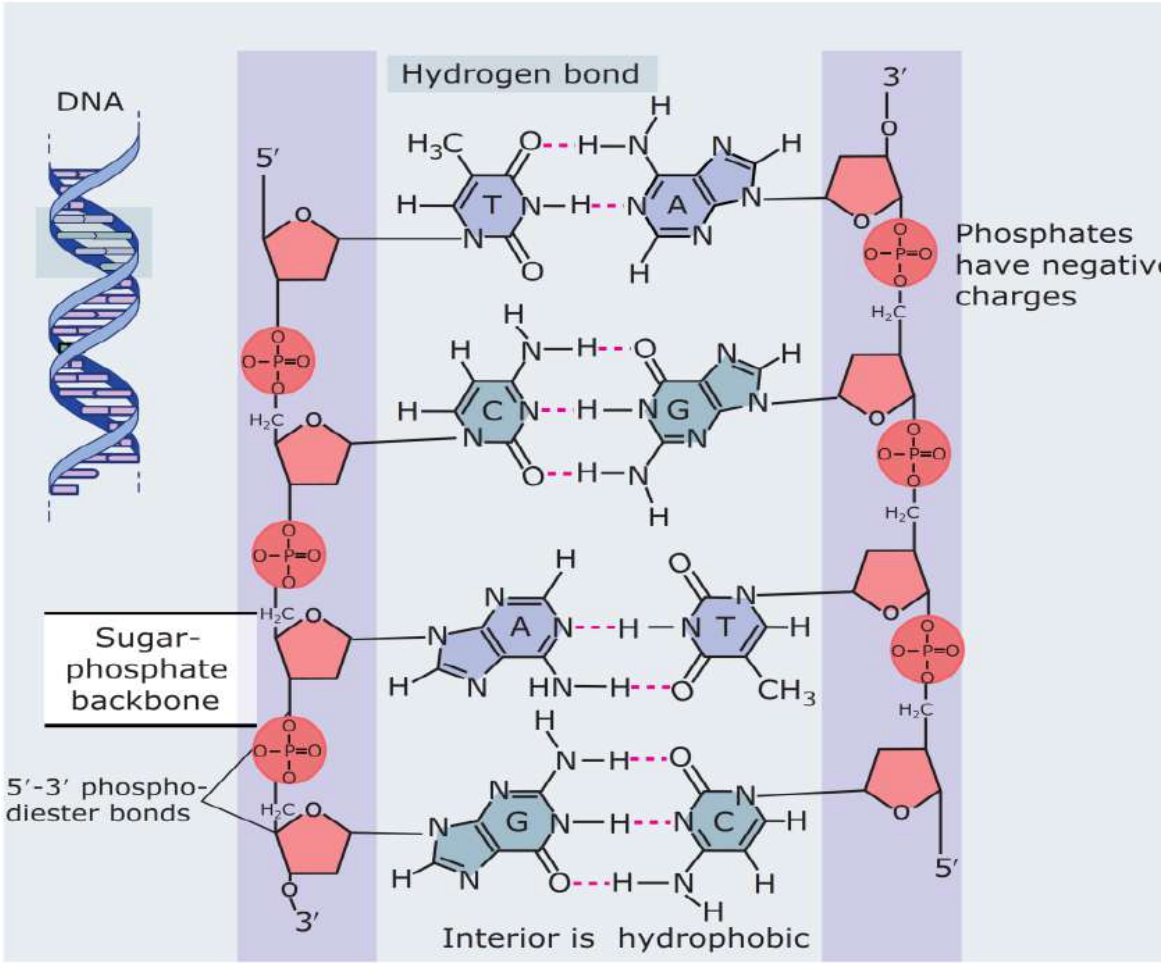
Subject: Zoology

Topic: **Model of DNA Replication** and **DNA Polymerization**

[CC2(Molecular Biology) of Semester 1]

Name of the Teacher: Dr. Malabika Bhattacharjee

Duplication → DNA —Transcription→ RNA —Translation→ Protein



Semiconservative DNA Replication

1. **Watson and Crick DNA model** implies a mechanism for replication:
   a. Unwind the DNA molecule.
   b. Separate the two strands.
   c. Make a complementary copy for each strand.

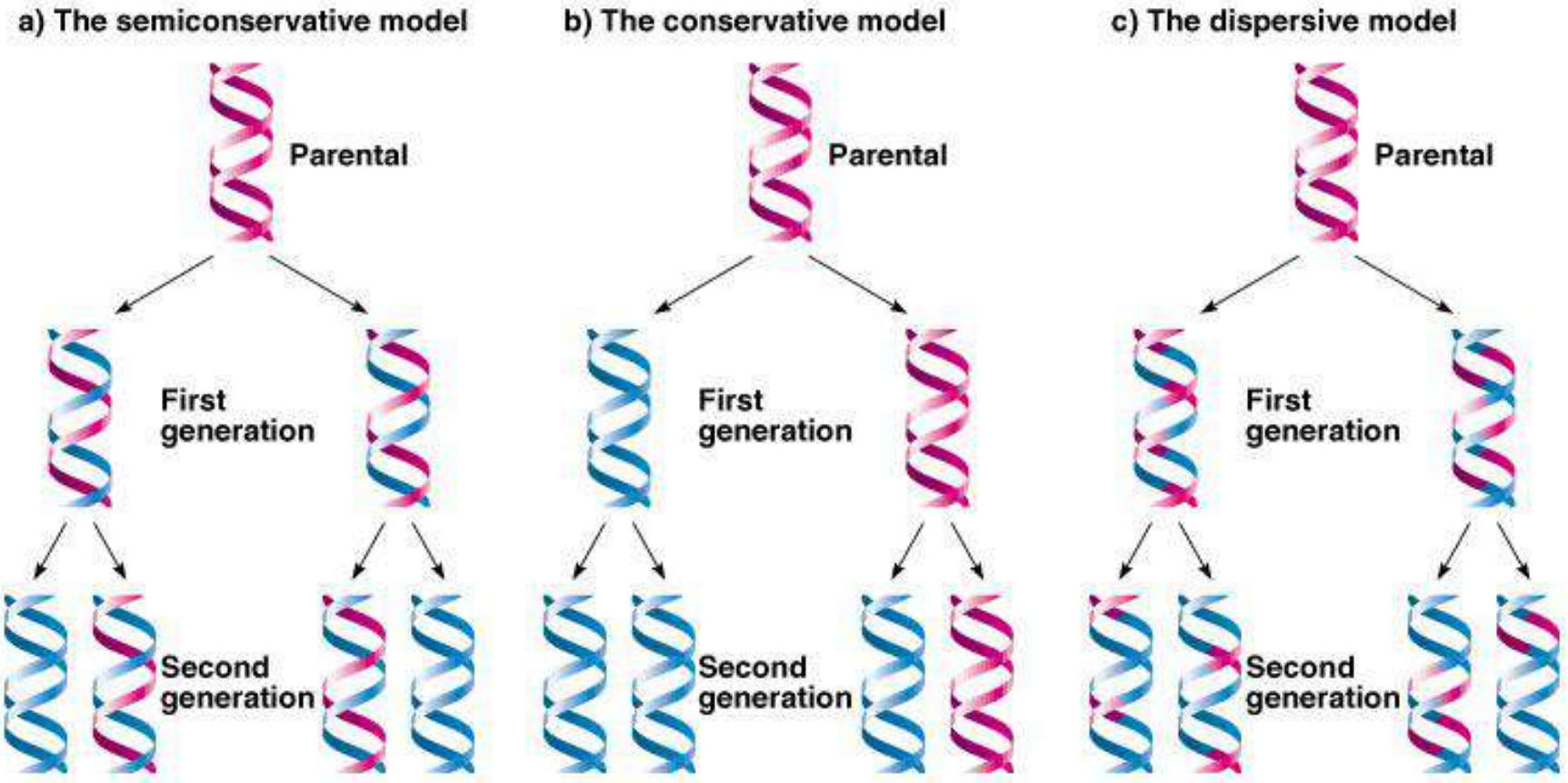
2 .Three possible models were proposed for DNA replication:

a. Conservative model proposed both strands of one copy would be entirely old DNA, while the other copy would have both strands of new DNA.

b. Dispersive model was that dsDNA might fragment, replicate dsDNA, and then reassemble, creating a mosaic of old and new dsDNA regions in each new chromosome.

c. Semiconservative model is that DNA strands separate, and a complementary strand is synthesized for each, so that sibling chromatids have one old and one new strand. This model was the winner in the Meselson and Stahl experiment.

3 Models for the Replication of DNA

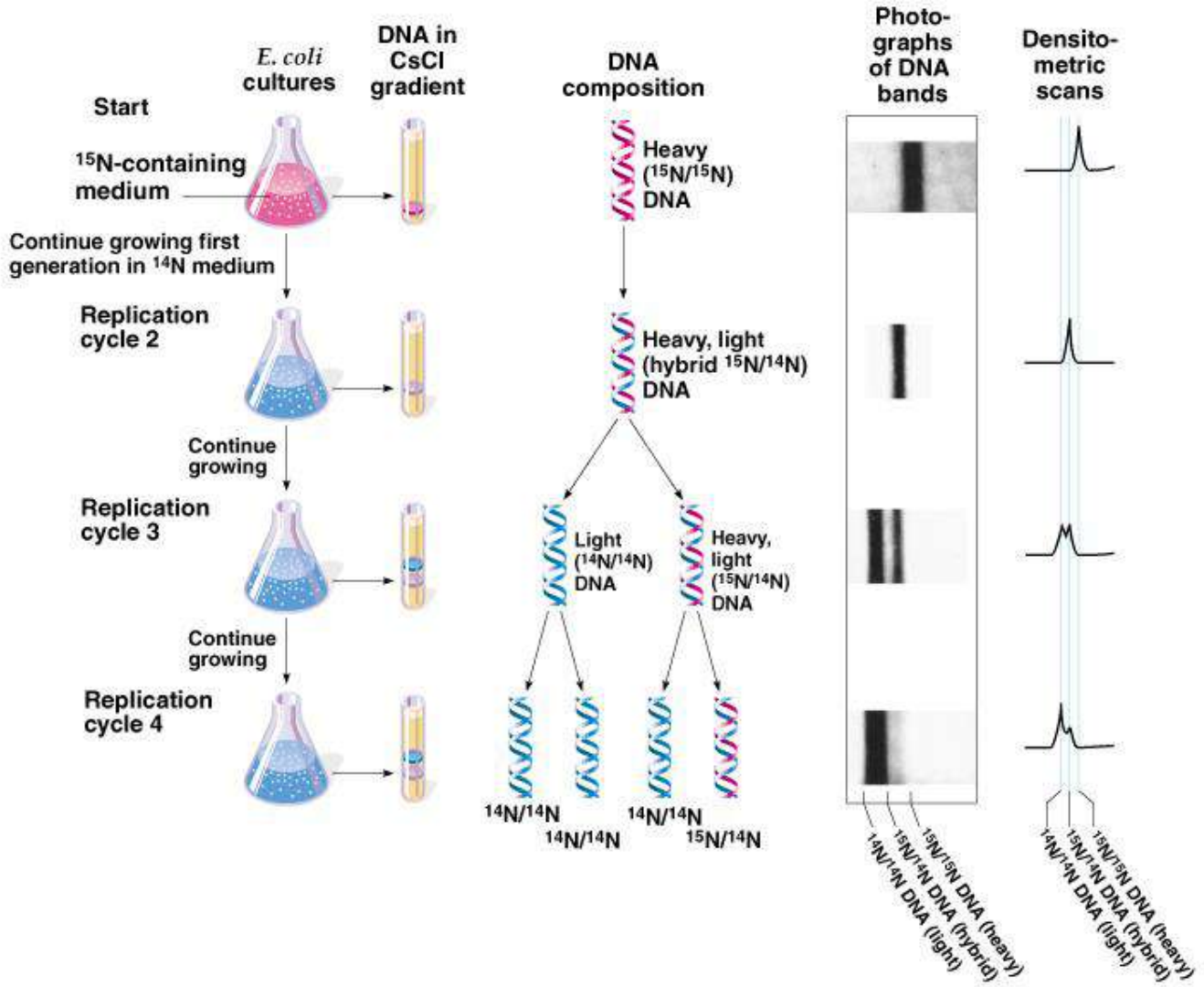


The Meselson-Stahl Experiment

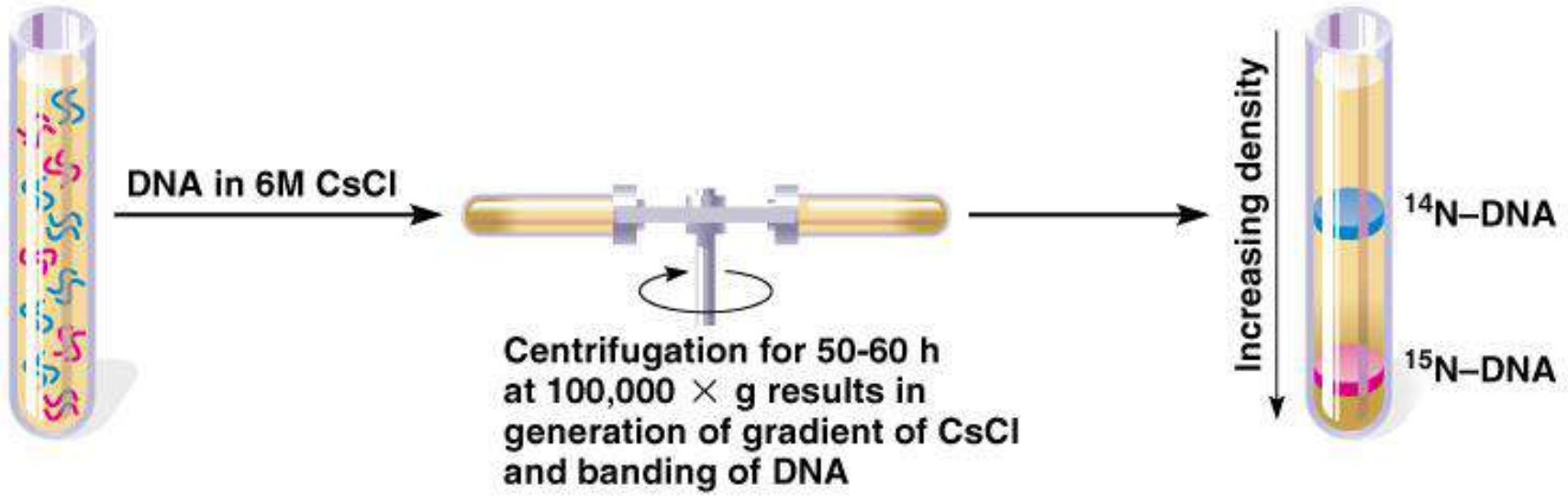
1. Meselson and Stahl (1958) grew *E. coli* in a heavy (not radioactive) isotope of nitrogen, ^{15}N in the form of $^{15}NH_4Cl$. Because it is heavier, DNA containing ^{15}N is more dense than DNA with normal ^{14}N, and so can be separated by CsCl density gradient centrifugation.

2. Once the *E. coli* were labeled with heavy ^{15}N, the researchers shifted the cells to medium containing normal ^{14}N, and took samples at time points. DNA was extracted from each sample and analyzed in CsCl density gradients

The Meselson-Stahl experiment, which showed that DNA replicates semi-conservatively



Equilibrium centrifugation of DNA of different densities in a cesium chloride density gradient



3. After one replication cycle in normal ^{14}N medium, all DNA had density intermediate between heavy and normal. After two replication cycles, there were two bands in the density gradient, one at the intermediate position, and one at the position for DNA containing entirely ^{14}N.

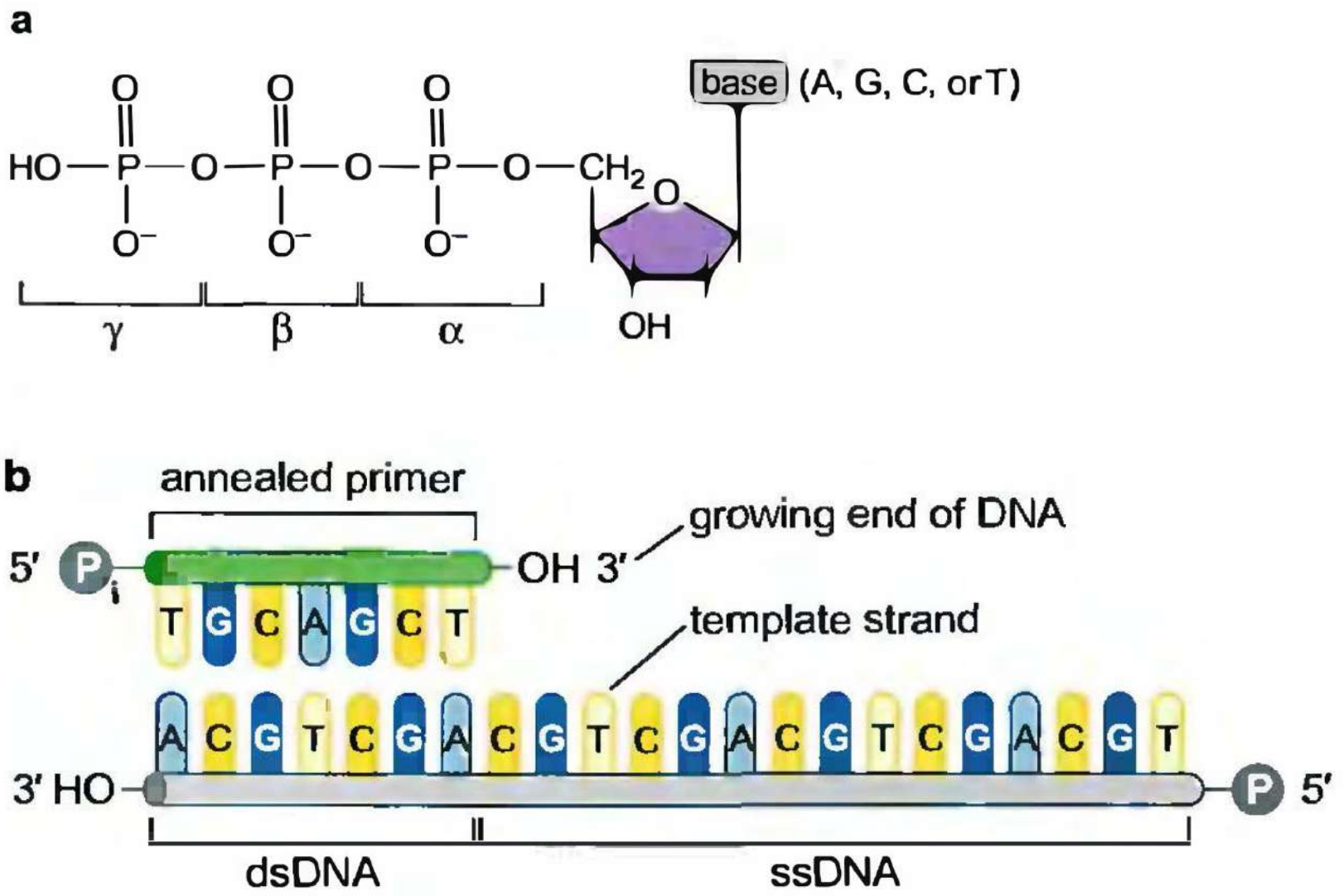
4. **Results compared with the three proposed models:**

   a. Does not fit conservative model, because after one generation there is a single intermediate band, rather than one with entirely ^{15}N DNA and another with entirely ^{14}N DNA.

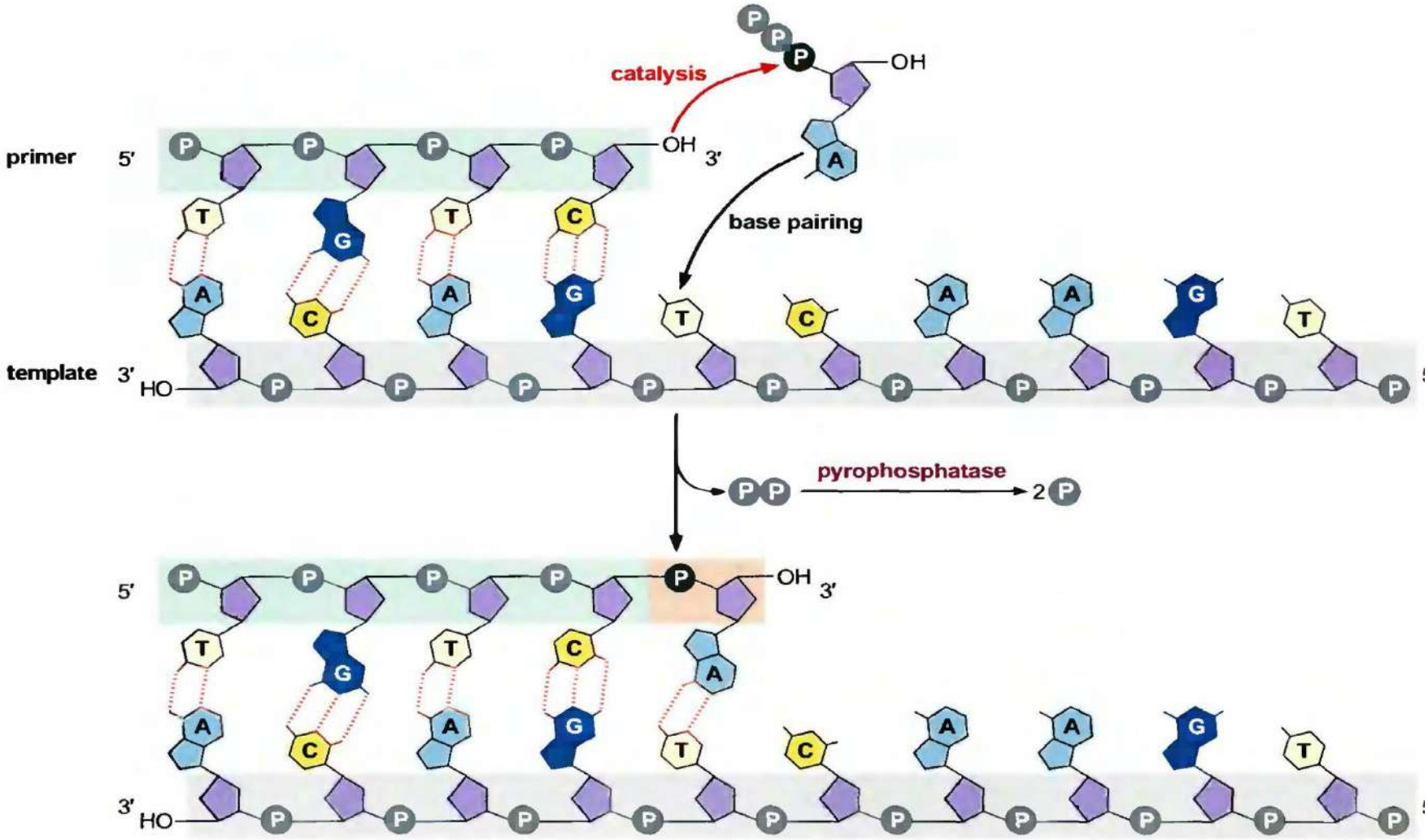
   b. The dispersive model predicted that a single band of DNA of intermediate density would be present in each generation, gradually becoming less dense as increasing amounts of ^{14}N were incorporated with each round of replication. Instead, Meselson and Stahl observed two bands of DNA, with the intermediate form decreasing over time.

   c. **The semiconservative model fits the data very well.**

Raw materials for Replication

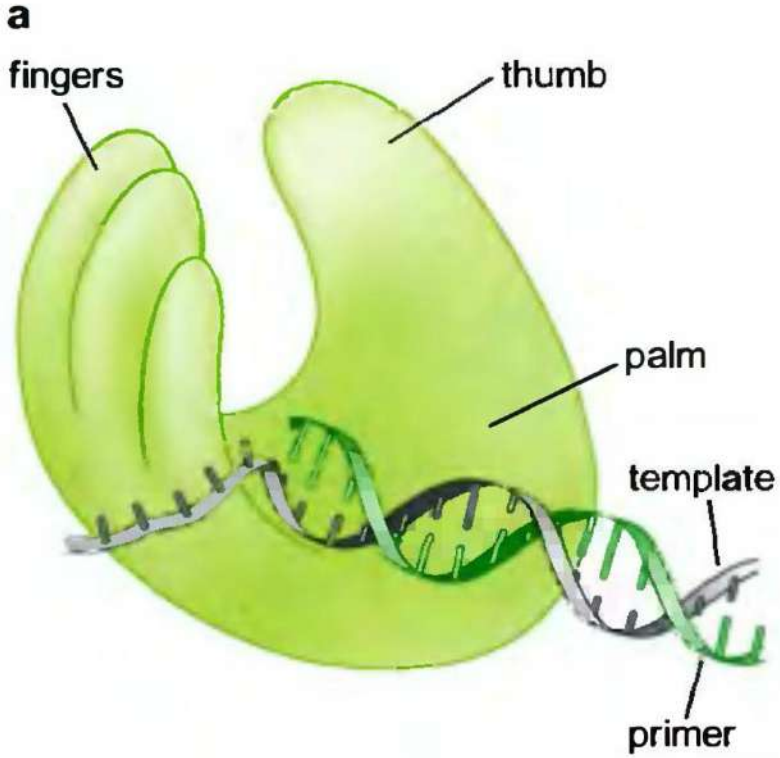


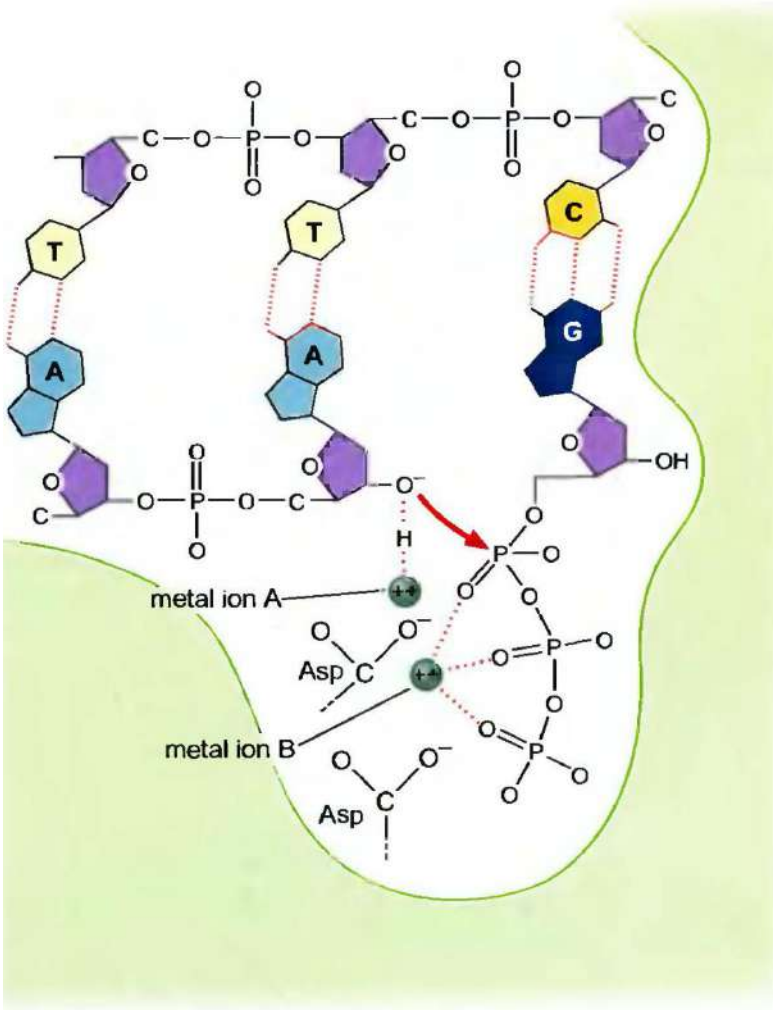
Addition of dNTPs



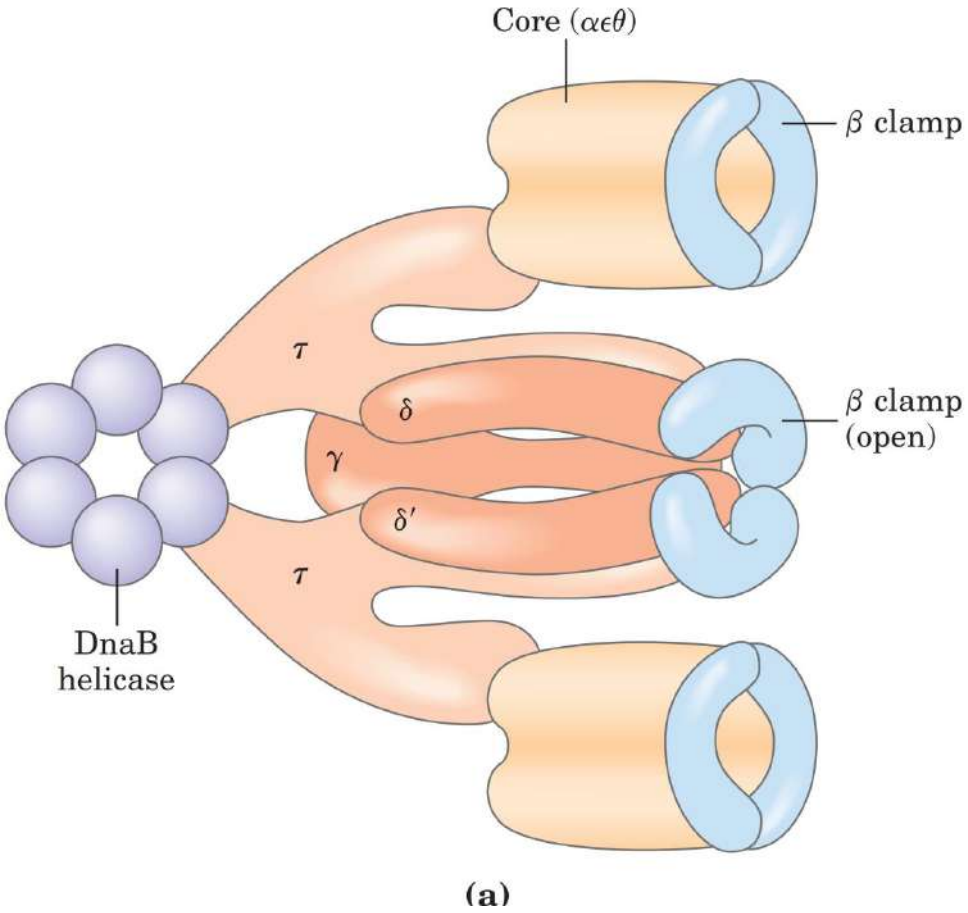
DNA Polymerases, the DNA Replicating Enzymes-Active Center Cleft

First isolation of an enzyme involved in DNA replication was in 1955. **Arthur Kornberg** won the 1959 Nobel Prize in Physiology or Medicine for this work



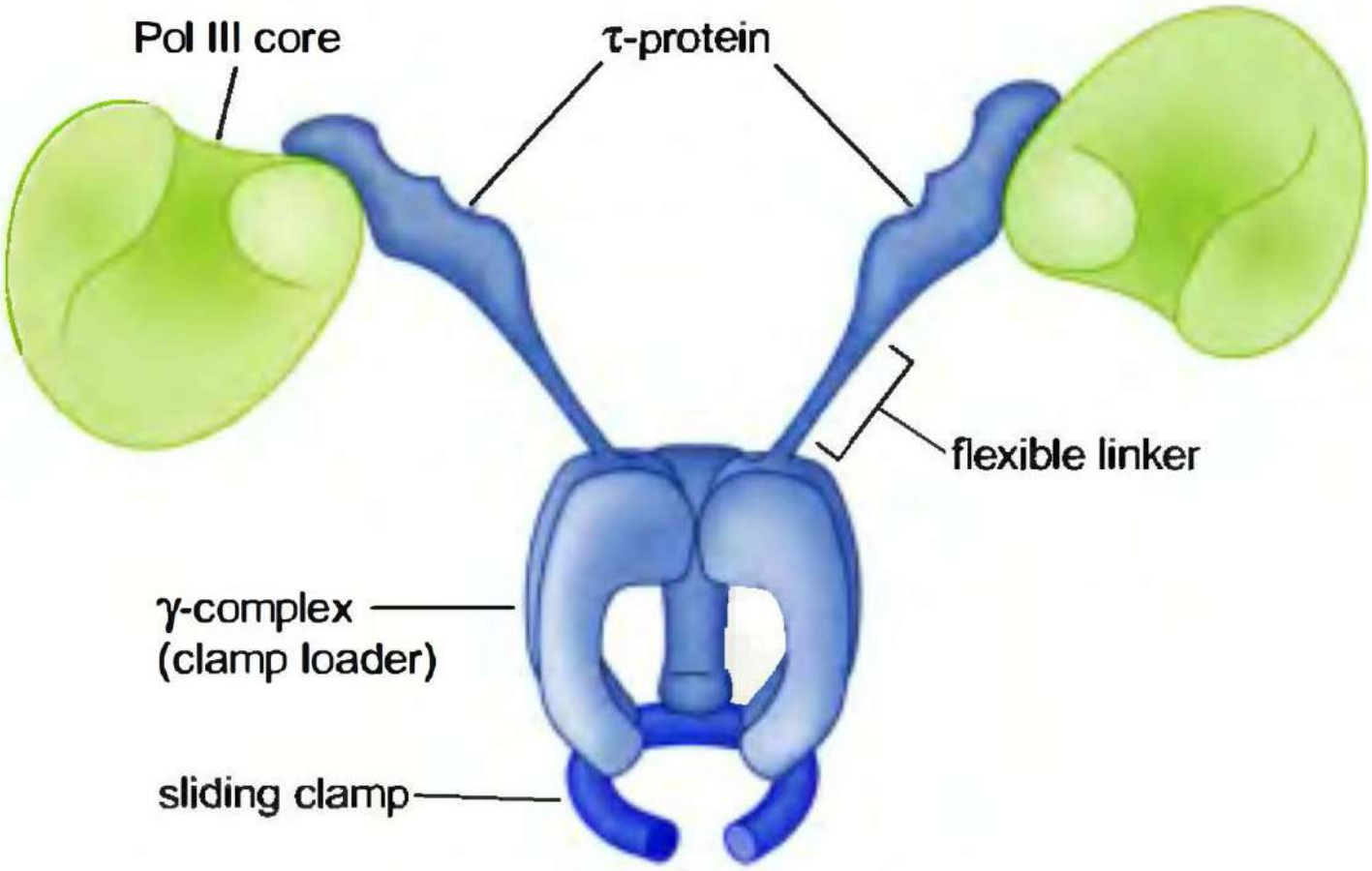


DNA Polymerase-Subunits

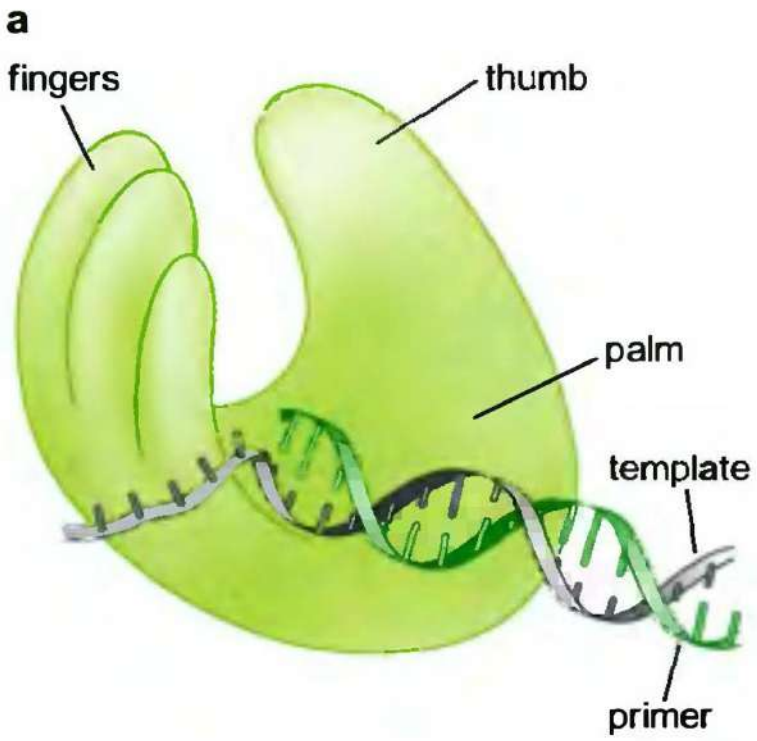


1. DNA polymerase III has ten types of subunits.
2. Its polymerization and proofreading activities reside in its α and ε (epsilon) subunits, respectively.
3. The θ subunit associates with α and ε to form a core polymerase, which can polymerize DNA but with limited processivity.
4. Two core polymerases can be linked by another set of subunits, a clamp-loading complex, or γ complex, consisting of five subunits of four different types $\tau_2\gamma\delta\delta'$.
5. The core polymerases are linked through the τ (tau) subunits.
6. Two additional subunits, χ (chi) and ψ (psi), are bound to the clamp-loading complex.
7. The entire assembly of 13 protein subunits (nine different types) is called DNA polymerase III.

DNA Polymerase-Holoenzyme and the sliding Beta Clamp



DNA Polymerases- Functional domains



Palm domain

The palm domain is composed of a β sheet and contains the primary elements of the catalytic site. In particular, this region of DNA polymerase binds two divalent metal ions (typically Mg^{+2} or Zn^{+2}) that alter the chemical environment around the correctly base-paired dNTP and the 3 '0H of the primer.

Finger domain:

Several residues located within the fingers bind to the incoming dNTP. Once a correct base pair is formed between the incoming dNTP and the template, the finger domain moves to enclose the dNTP. This closed form of the polymerase hand stimulates catalysis by moving the incoming nucleotide in close contact with the catalytic metal ions.

Thumb domain

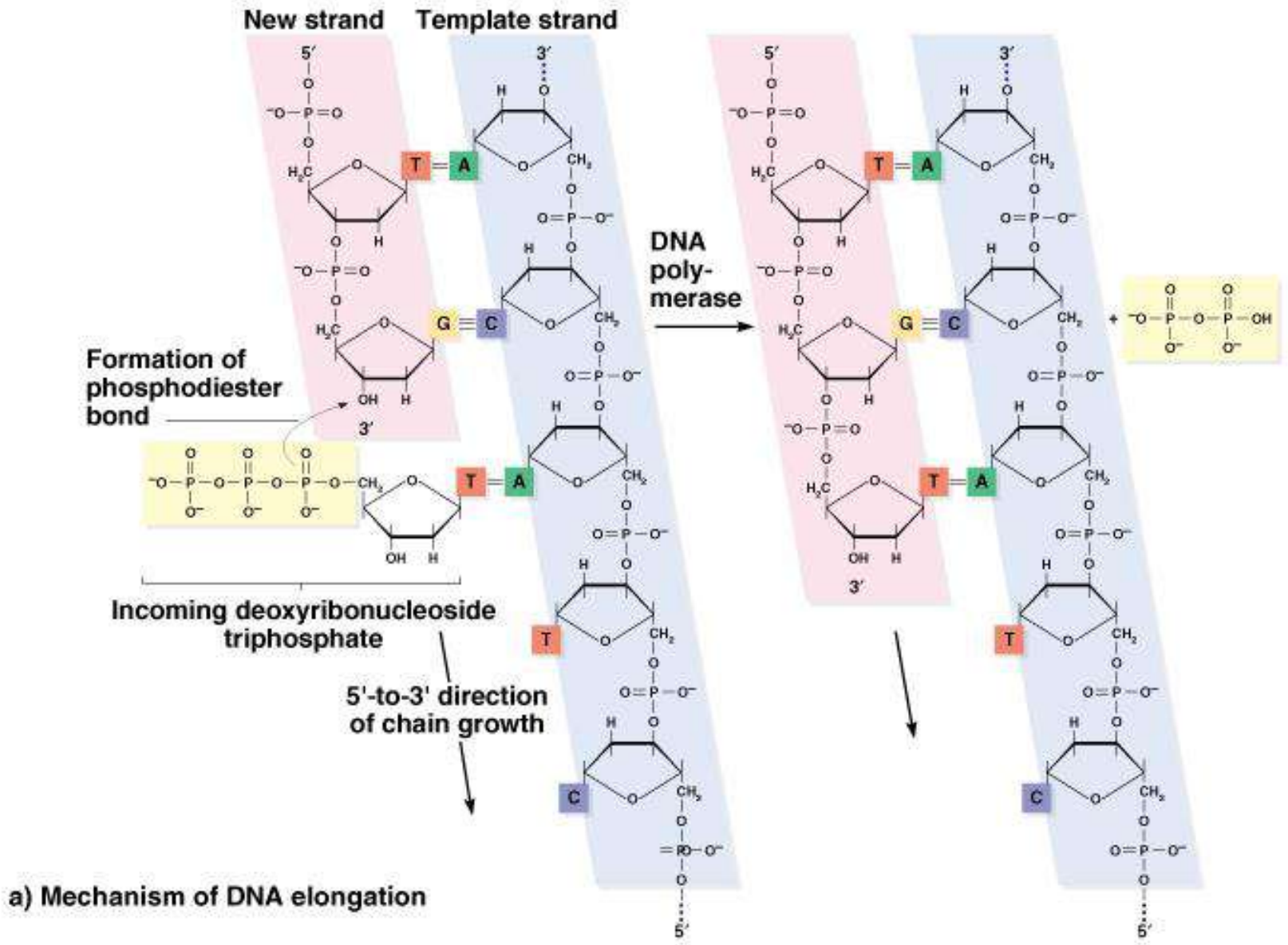
It maintains the correct position of the primer and the active site.

Second, the thumb helps to maintain a strong association between the DNA polymerase and its substrate.

Roles of DNA Polymerases

1. All DNA polymerases link dNTPs into DNA chains. Main features of the reaction:
   a. An incoming nucleotide is attached by its **5'-phosphate group to the 3'-OH of the growing DNA chain**. Energy comes from the dNTP releasing two phosphates. The DNA chain acts as a primer for the reaction.
   b. The incoming nucleotide is selected by its ability to hydrogen bond with the complementary base in the template strand. The process is fast and accurate.
   c. DNA polymerases synthesize only from **5' to 3'.**
2. The enzyme Kornberg isolated was believed to be the only DNA polymerase in E. coli. However, mutations in this gene (*polA1*) were not lethal, indicating that other DNA polymerases must exist in *E. coli*.

DNA chain elongation catalyzed by DNA polymerase



The properties of DNA polymerases

5. The properties of known *E. coli* DNA polymerases are:
   a. DNA polymerase I is a single peptide encoded by *polA* and used for DNA replication. Replicates DNA in the 5'→3' direction. Has 5'→3' exonuclease activity to remove nucleotides from 5' end of DNA or from an RNA primer.
   b. DNA polymerase II is a single peptide encoded by *polB*. Used for **DNA repair.**
   c. DNA polymerase III has three polypeptide subunits in the catalytic core of the enzyme: α (encoded by the *dnaE* gene), ε(*dnaQ*), and θ(*holE*). Holoenzyme has an additional six different polypeptides. Replicates DNA in the 5'→3' direction.
   d. DNA polymerase IV is encoded by the *dinB* gene, and is used in DNA repair.
   e. DNA polymerase V is encoded by *umuDC*, and is used in DNA repair.

6. *E. coli* DNA polymerases used for DNA replication (DNA polymerase I and DNA polymerase III) have 3'→5' exonuclease (proofreading) activity.

Bidirectional Model of DNA Replication

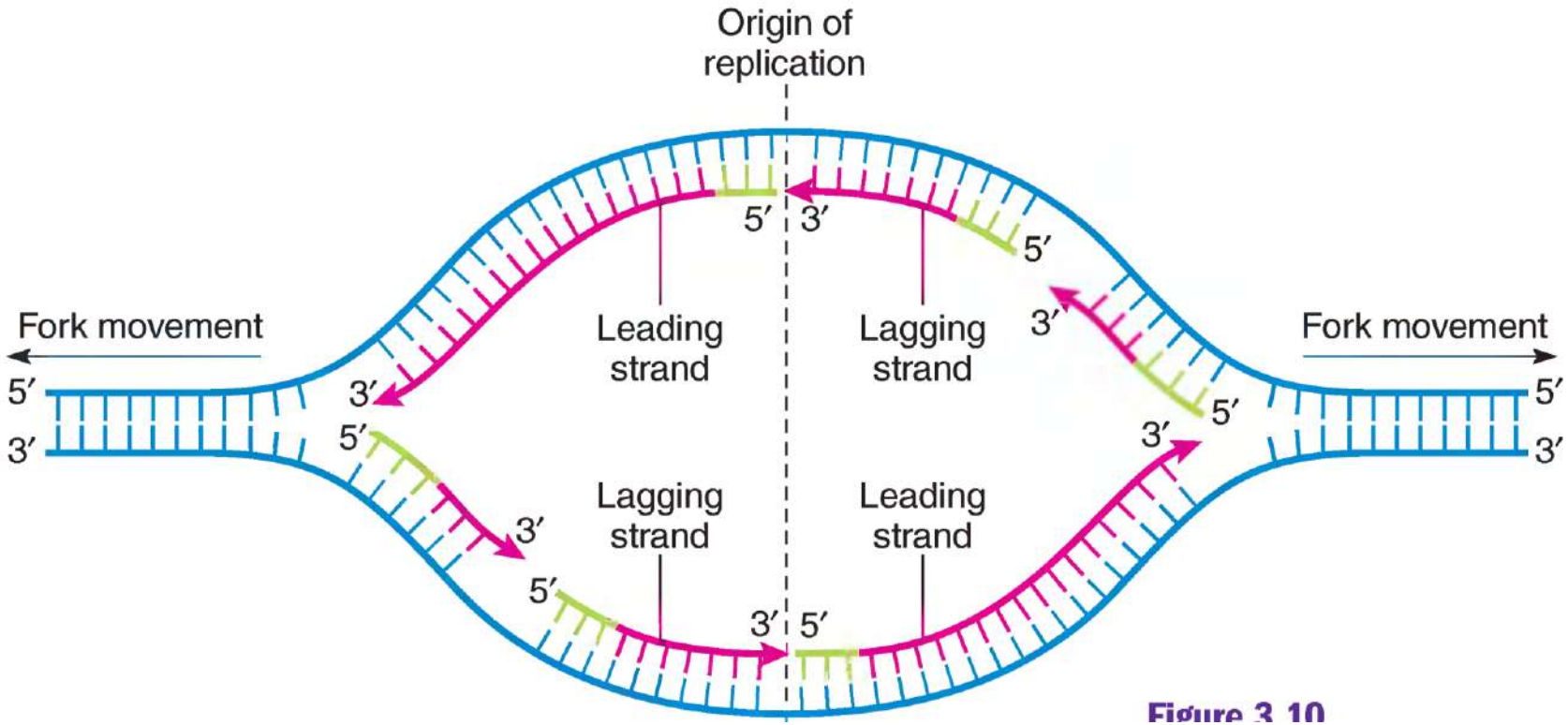


Figure 3.10

REFERENCE

- RUSSELL iGENETICS
- WATSON Molecular Biology of Gene
- LEWIN Cell