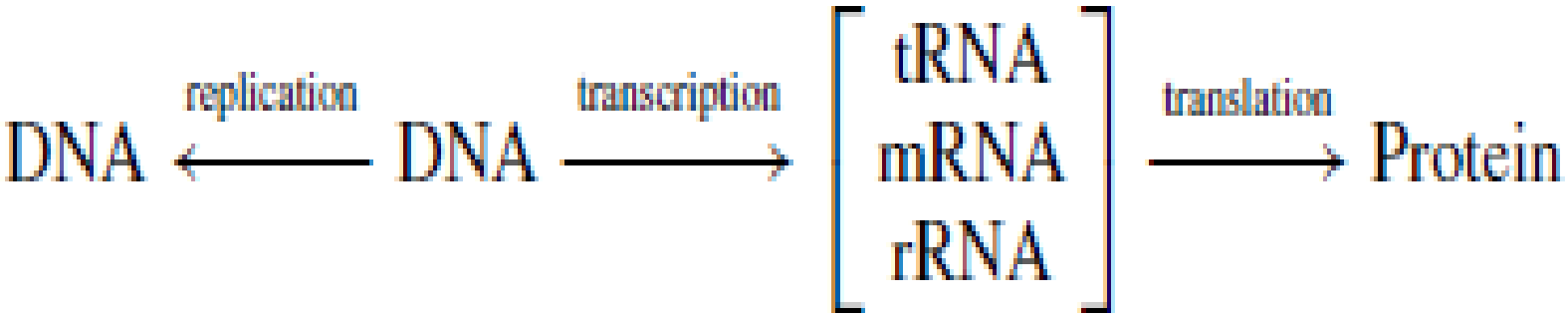
وراثة الأحياء الدقيقة
Microbial Genetics

أساسيات في علم الوراثة
Fundamentals of Genetics
Lecture 3

Genetic Materials المادة الوراثية

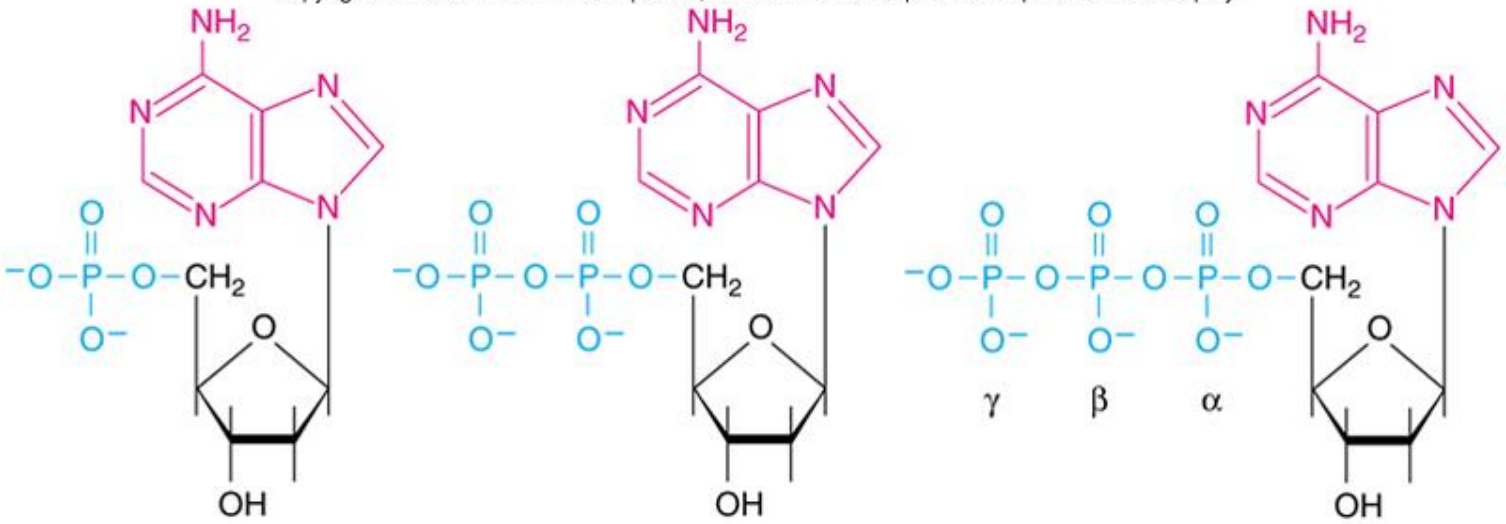
- **Important Processes in Genetics**:
  - ***DNA Replication***: The sequence of a nucleotides in a DNA molecule serves as a template to copy itself, so two identical copies of the DNA helix are formed.
  - ***Transcription***: The sequence of nucleotides in a DNA molecule serves as a template for the synthesis of an RNA molecule; typically, only a small segment of the DNA is copied. This is the first step in gene expression.
  - ***Translation*****:** The sequence of nucleotides in an RNA molecule serves to direct the assembly of amino acids into a protein chain on a ribosome. This is the second step in gene expression.

Genetic Materials المادة الوراثية



DNA Linkage

- Nucleotides are nucleosides with a phosphate group attached through a phosphodiester bond
- Nucleotides may contain one, two, or even three phosphate groups linked in a chain



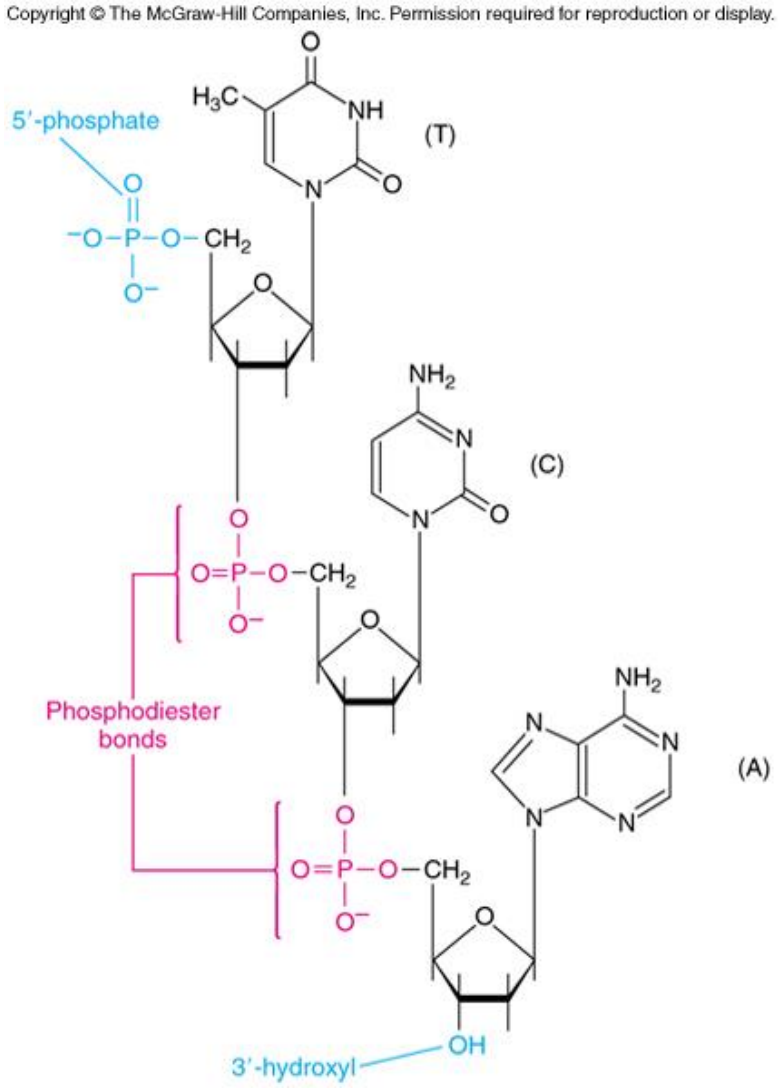
Deoxyadenosine-5′-monophosphate (dAMP)

Deoxyadenosine-5′-diphosphate (dADP)

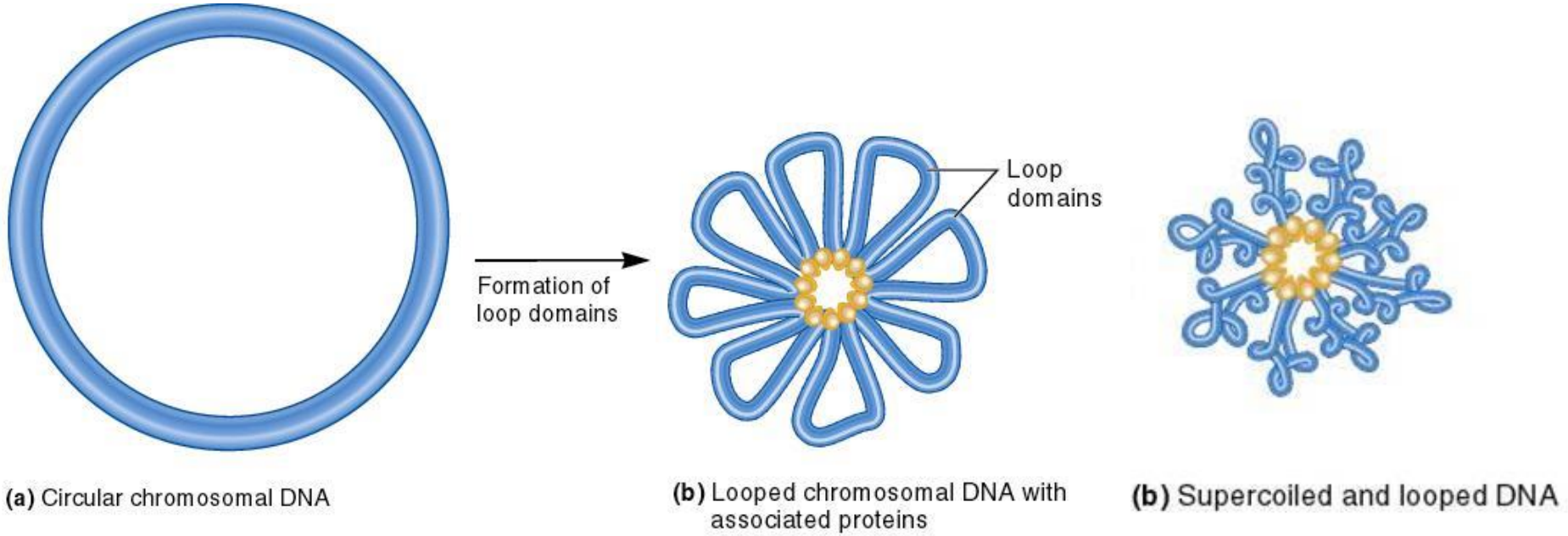
Deoxyadenosine-5′-triphosphate (dATP)

Tri-nucleotide

- The example trinucleotide has polarity
  - Top of molecule has a free 5’-phosphate group = **5’ end**
  - Bottom has a free 3’-hydroxyl group = **3’ end**



Bacterial Chromosomes

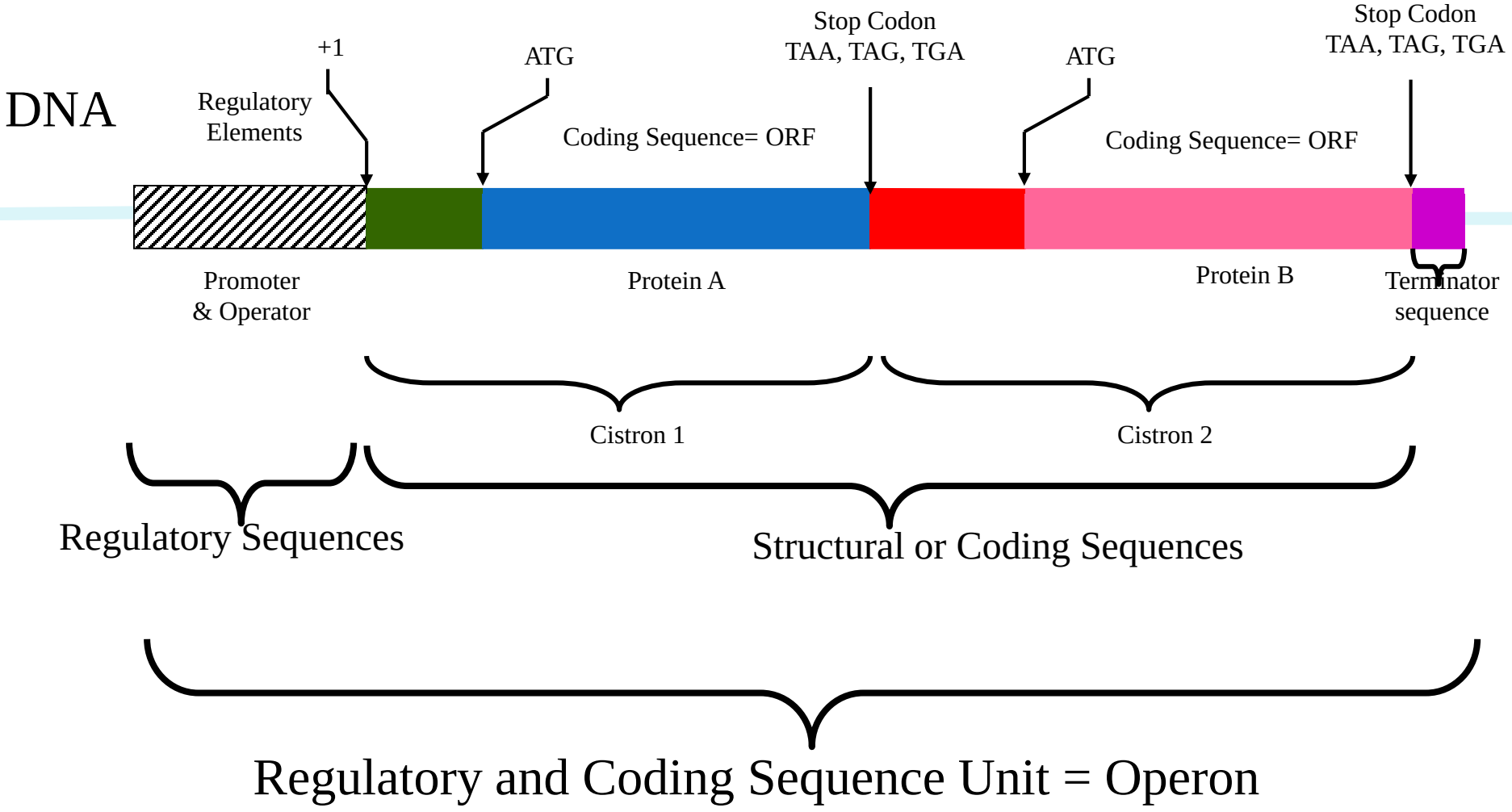


- Chromosomal DNA is compacted ~ 1000 fold to fit within cell.

Genetic Materials المادة الوراثية

- **Gene**
  - Contemporary understanding:
    - A segment on a DNA molecule
    - Usually at a specific location (locus) on a chromosome or plasmid
    - Characterized by its nucleotide sequence
  - **Genes play three notable roles:**
    - To encode the nucleotide sequences of mRNA, which in turn encodes the amino acid sequences of proteins
    - To encode the nucleotide sequences of tRNA or rRNA
    - To regulate the expression of other genes
  - **Mutation**:
    - Change in the nucleotide sequence of a gene, usually resulting from an error during DNA replication.

Prokaryotic Gene (Operon) Structure



Genetic Materials المادة الوراثية

- **Phenotype**
  - The appearance or visible characteristics of a trait in an individual
  - Mutation in a gene responsible for a phenotype may cause a change in the phenotype.
  - Typically, more that one gene is responsible for a phenotype.
- **Genotype**
  - The genetic make-up of an individual with reference to one or more specific genes
  - A genotype is designated by using symbols to represent the mutations of the gene

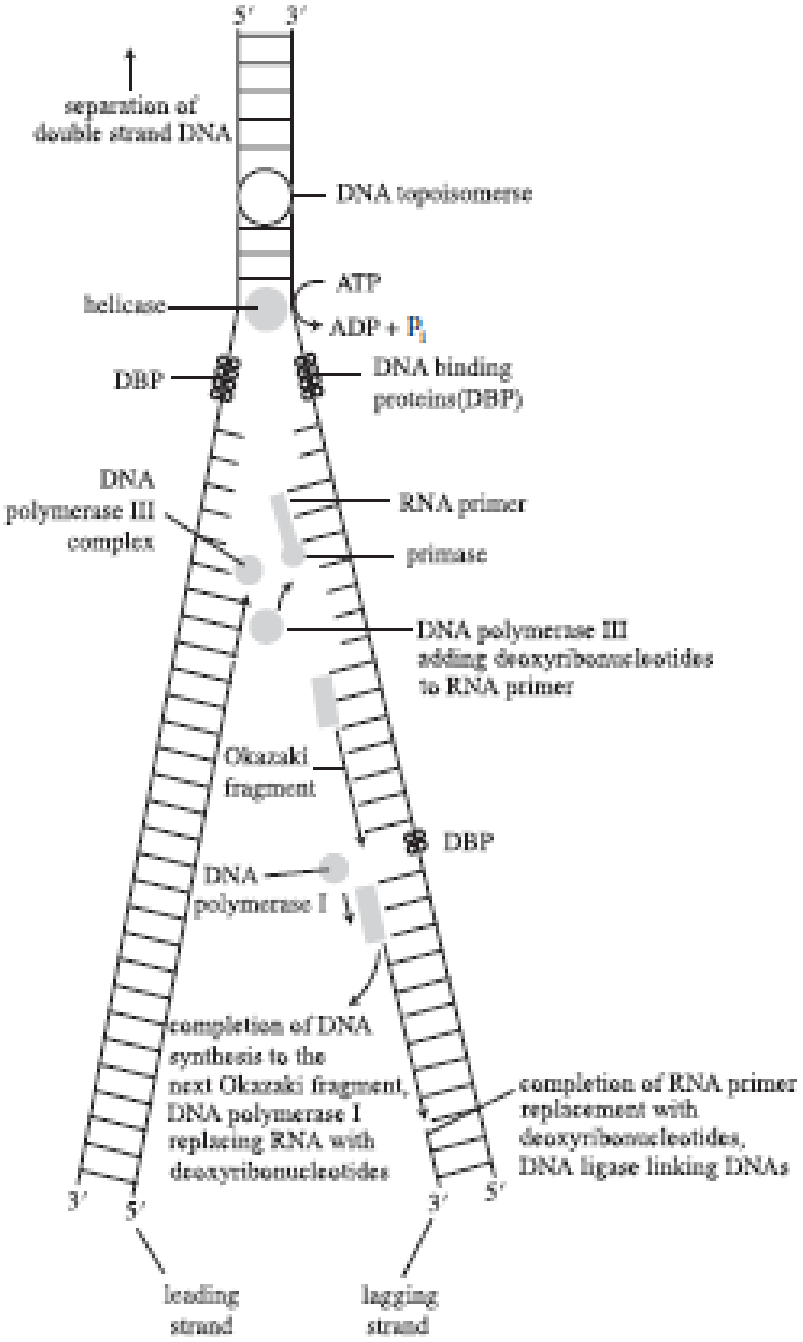
Deoxyribonucleic acid (DNA) Replication

- DNA synthesis is a complex process involves a range of enzymes referred to as the **DNA replicase system** or "**replisome**".
- Over 30 different proteins are involved in DNA replication forms a complex "replisome".
- DNA topoisomerase (gyrase) adjusts supercoiled DNA to a relaxed form.
- The **D**NA **b**inding **p**rotein (**DBP)** or **h**elix **d**estabilizing **p**rotein (**HDP**).
- Since DNA is synthesized in the direction of 5'–3',
  - Continuously in leading strand.
  - Discontinuously in the lagging strand in segments (Okazaki fragments)- 1000–2000 nucleotides.

Model of DNA Replication

- The bacterial chromosome (4,639,221 bp) replicates at a rate of 800 to 1000 nucleotides per second.
- Steps involve in the current replication model:

- Prepriming (Primosome)-hexameric helicase.
- Unwinding- DNA relaxation.
- Priming- a signal for the primase (RNA primer 10 nt).
- *β*-clamp loading- RNA formed and DNA polymerase III starts.
- Completion of lagging strand.
- Proofreading- exonuclease proofreading activity of the DNA polymerase holoenzyme.
- Replacing the primer- RNA removal and replaced with DNA (RNAse H and DNA Pol I).
- Repairing single-strand nicks on the lagging strand- DNA ligase.


5′ 3′
separation of double strand DNA
DNA topoisomerse
ATP
helicase
ADP + Pi
DBP
DNA binding proteins(DBP)
DNA polymerase III complex
RNA primer
primase
DNA polymerase III adding deoxyribonucleotides to RNA primer
Okazaki fragment
DBP
DNA polymerase I
completion of DNA synthesis to the next Okazaki fragment, DNA polymerase I replacing RNA with deoxyribonucleotides
completion of RNA primer replacement with deoxyribonucleotides, DNA ligase linking DNAs
3′ 5′
3′ 5′
leading strand
lagging strand

Deoxyribonucleic acid (DNA) Replication

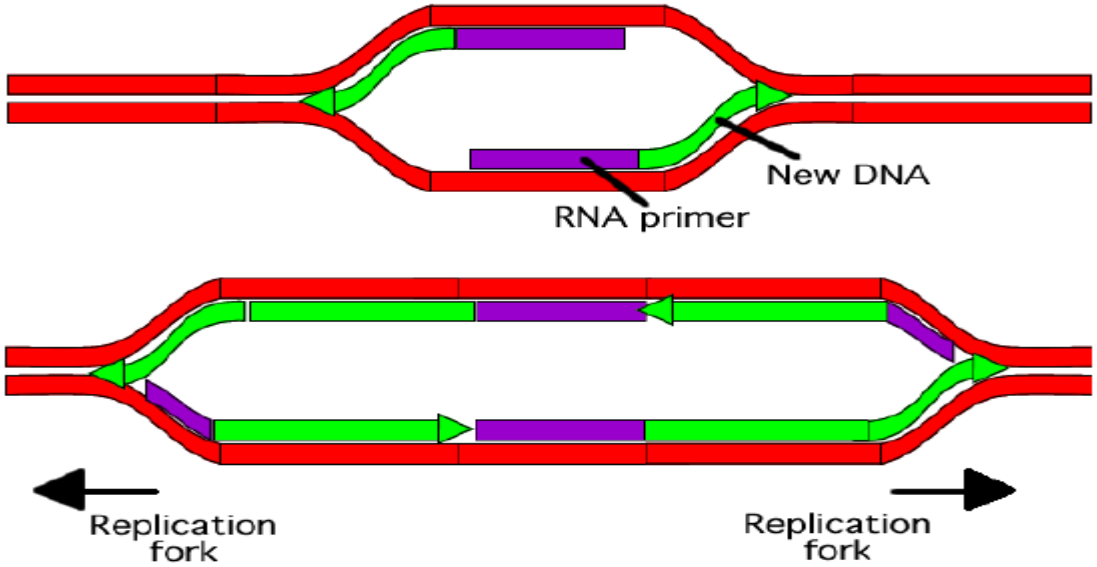
- **DNA polymerase**:
  - *Escherichia coli* has three separate DNA polymerases, I, II and III.
  - DNA polymerase activity in the direction of 5'–3'.
  - DNA polymerase III synthesizes the **Okazaki fragment** (RNA primer).
  - DNA polymerase I removes the RNA primer-replaced by DNA.
  - DNA ligase links Okazaki fragments after the primer RNA is completely replaced with DNA.
- **Train Versus Factory Model:** Two general models have been proposed for DNA replication 1- DNA polymerase moves along the DNA, 2- DNA polymerase is stationary.

Deoxyribonucleic acid (DNA) Replication

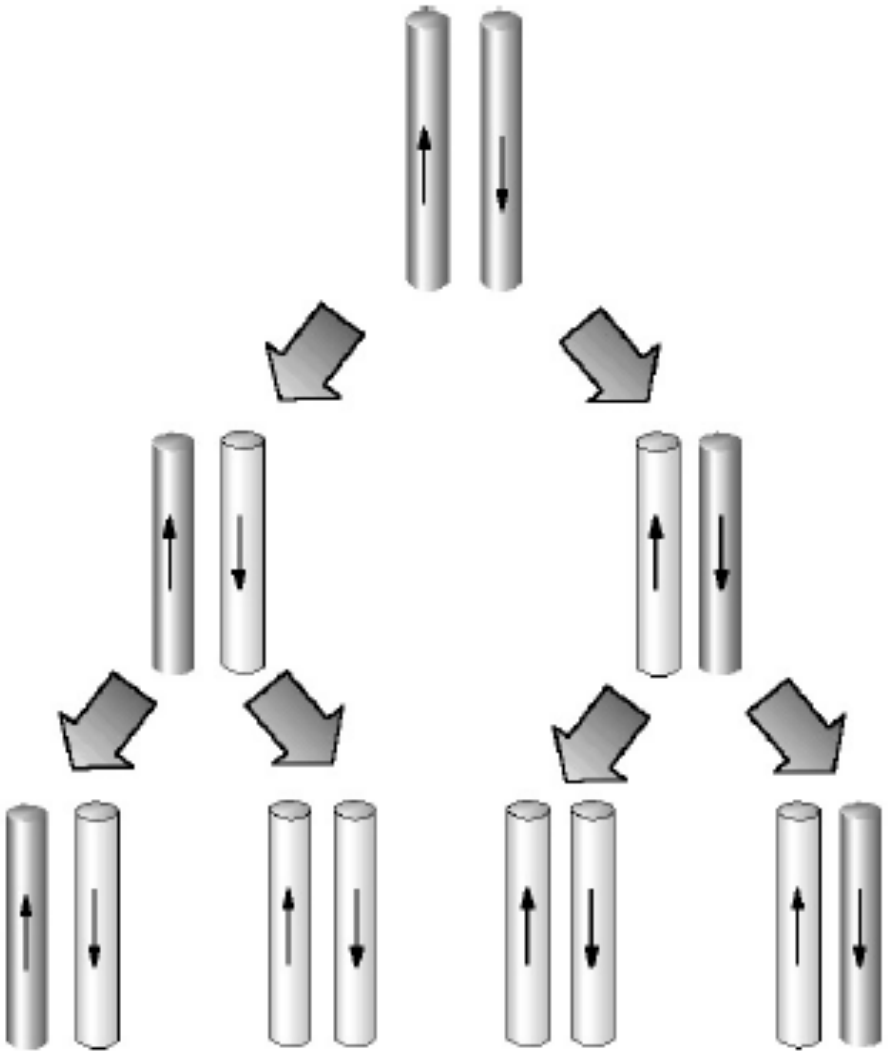
- **Post-replicational modification:**
  - Bacterial DNA contains 5-methyl cytosine and 6-methyl adenine- DNA methyltransferase.
  - Restriction enzymes- types I, II and III.
  - Types I and III- Hydrolyze the DNA sequence and methylate adenine and cytosine- “restriction-modification enzymes”.
  - Type II restriction enzymes- hydrolyzing activity.
  - ***S***-**a**denosyl**m**ethionine (SAM)- the methyl group donor for the DNA modification reaction.
- Topoisomerase converts the modified DNA into a supercoiled form.

Deoxyribonucleic acid (DNA) Replication

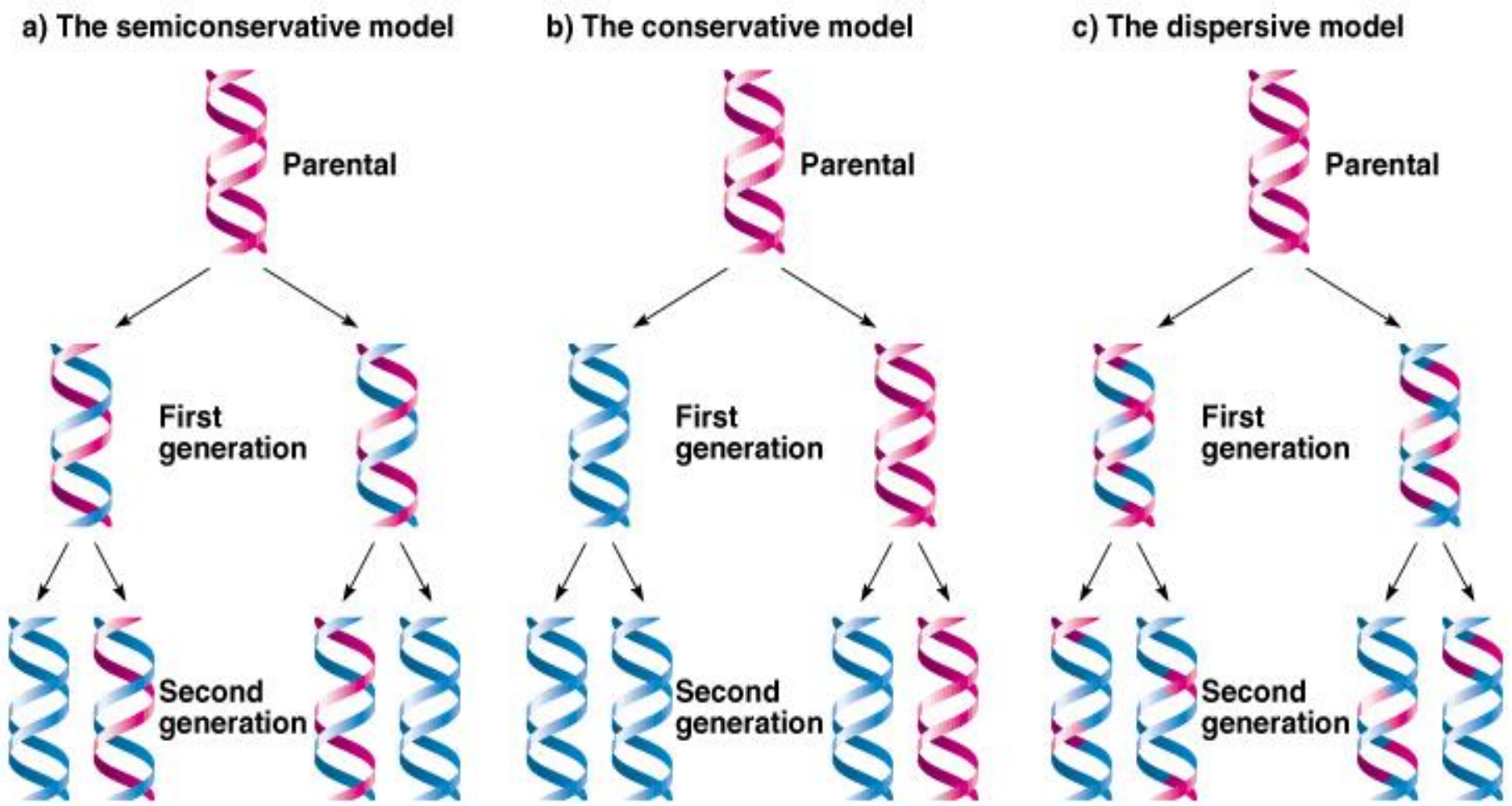
- DNA replication proceeds in both directions from the replication origin to the terminator sequence
- The two replication forks meet at the terminator region, the daughter chromosomes are separated by the enzyme “topoisomerase IV”.



DNA Replication: Semi-conservative



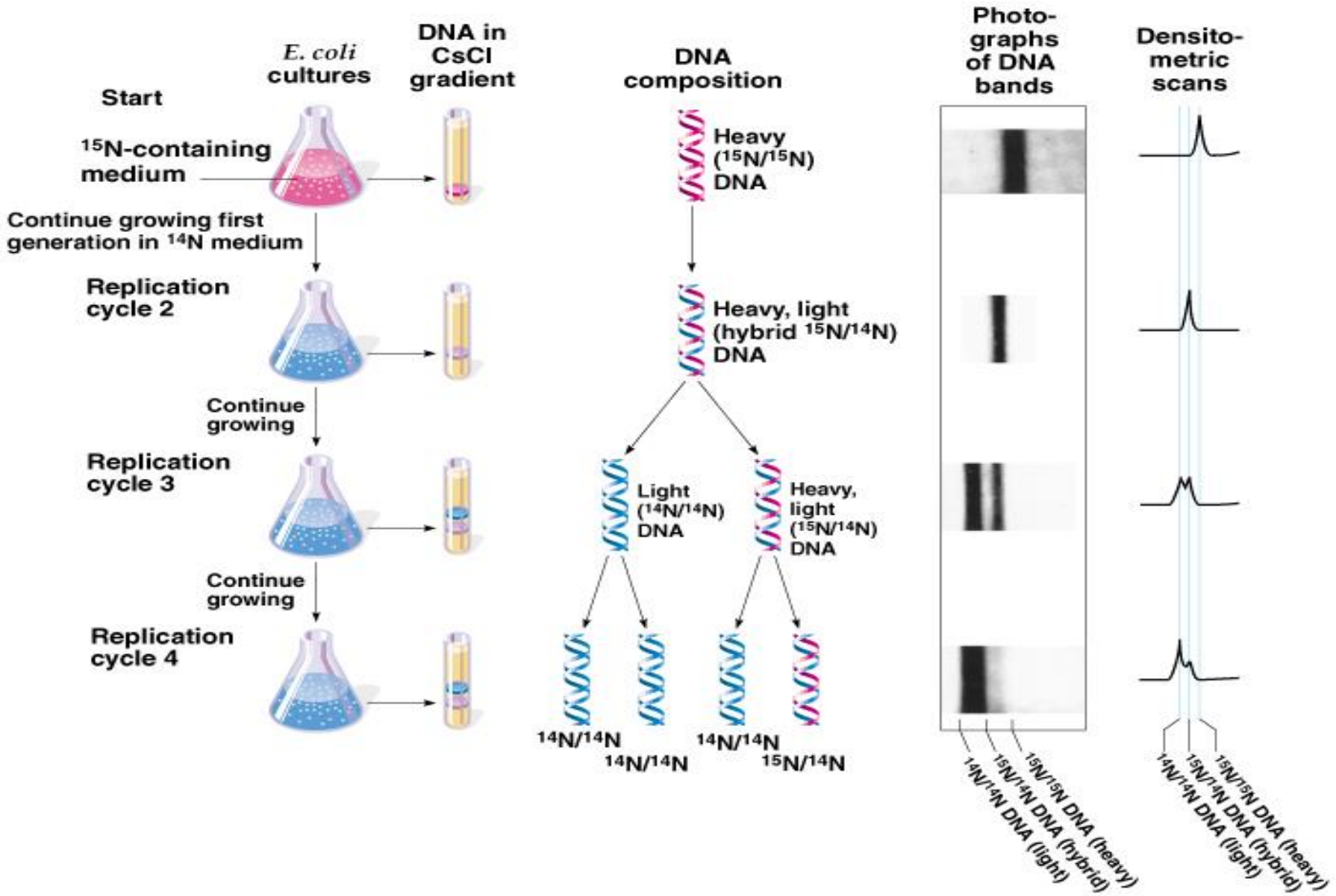
- **Three possible models were proposed for DNA replication:**

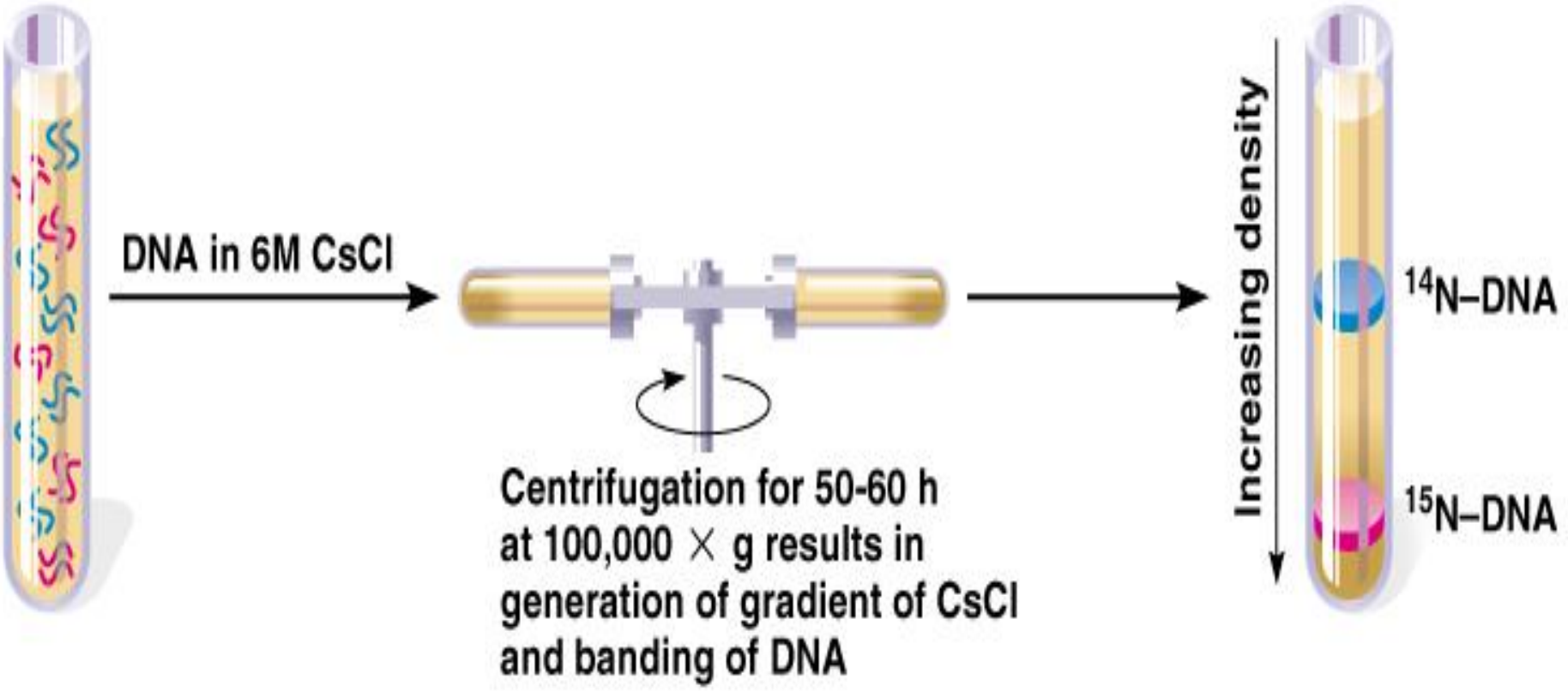


The Meselson-Stahl Experiment

• 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 Cesium Chloride (CsCl) density gradient centrifugation.

• 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 .


E. coli cultures
DNA in CsCl gradient
Start
15N-containing medium
Continue growing first generation in 14N medium
Replication cycle 2
Continue growing
Replication cycle 3
Continue growing
Replication cycle 4
DNA composition
Heavy (15N/15N) DNA
Heavy, light (hybrid 15N/14N) DNA
Light (14N/14N) DNA
Heavy, light (15N/14N) DNA
14N/14N
14N/14N
14N/14N
15N/14N
Photo-graphs of DNA bands
Densito-metric scans
14N/14N DNA (light)
15N/14N DNA (hybrid)
15N/15N DNA (heavy)
14N/14N DNA (light)
15N/14N DNA (hybrid)
15N/15N DNA (heavy)


DNA in 6M CsCl
Centrifugation for 50-60 h
at 100,000 × g results in
generation of gradient of CsCl
and banding of DNA
Increasing density
^{14}N–DNA
^{15}N–DNA

DNA Polymerases, the DNA Replicating Enzymes

- 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.
- Enzyme originally called the Kornberg enzyme, now known as DNA Polymerase I.

❖ **Four components are required in the mechanisms of DNA synthesis:**

1. dNTPs: dATP, dTTP, dGTP, dCTP (deoxyribonucleoside triphosphates).

2. DNA template.

3. DNA polymerase (*Kornberg enzyme*).

4. Mg^{2+} (optimizes DNA polymerase activity).

Deoxyribonucleic acid (DNA) Replication

- http://www.dnatube.com/search/?search_id=DNA+REPLICATION

QUESTIONS??