MOLECULAR BIOLOGY

Basics and Concepts: What is what?

Part-I, II(a,b): Characteristics and enzymes in DNA Replication.

Based on :

(i) Molecular Biology **Weaver Robert F.** (5ed.,2012)

(ii) Biochemistry **Garrett R.H., Grisham C.M.**(2ed., 1999)

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- Replication is the process of synthesis of DNA molecule using strands of pre existing DNA as templates.

- Thus replication is **DNA dependent DNA polymerization.**

- Due to the ability to replicate the DNA is the most preferred genetic material.

- With few differences the basic mechanism of replication is same prokaryotes and eukaryotes as in both of these groups the replication show following characteristic features:
  - Replication is **bidirectional**
  - Replication is **semi-conservative**.
  - Replication is **semi discontinuous**

Replication Is Bidirectional

- Replication of DNA begins at one or more unique sites called origin(s) of replication and proceeds in both directions from this origin.

- Thus bidirectional replication involves two replication forks which move in opposite direction.

- Very few exception of this phenomenon are known example include certain bacteriophage chromosomes (λ phage, ColE1DNA, ϕX174 and plasmid DNAs)

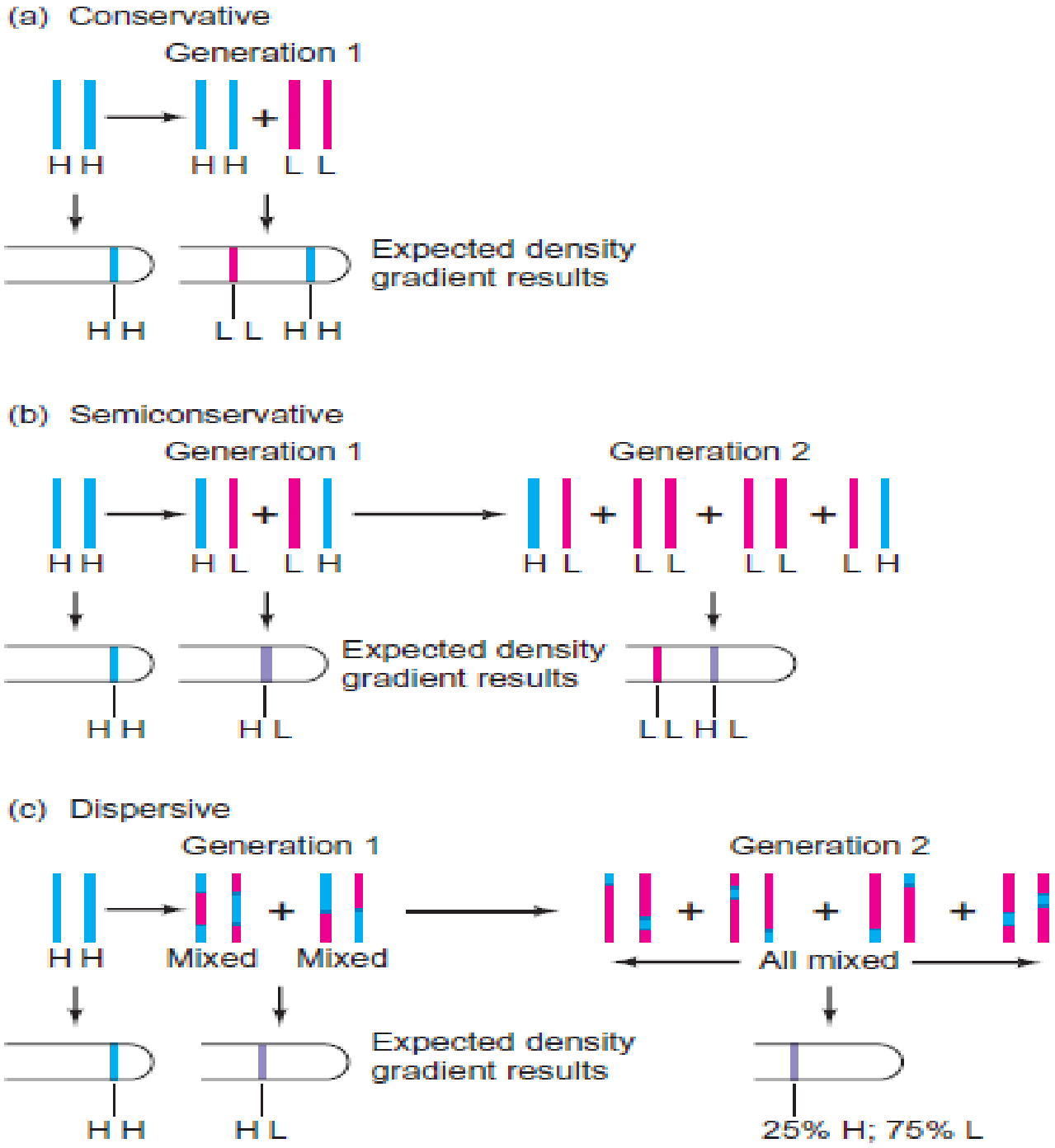
Replication is Semi-Conservative

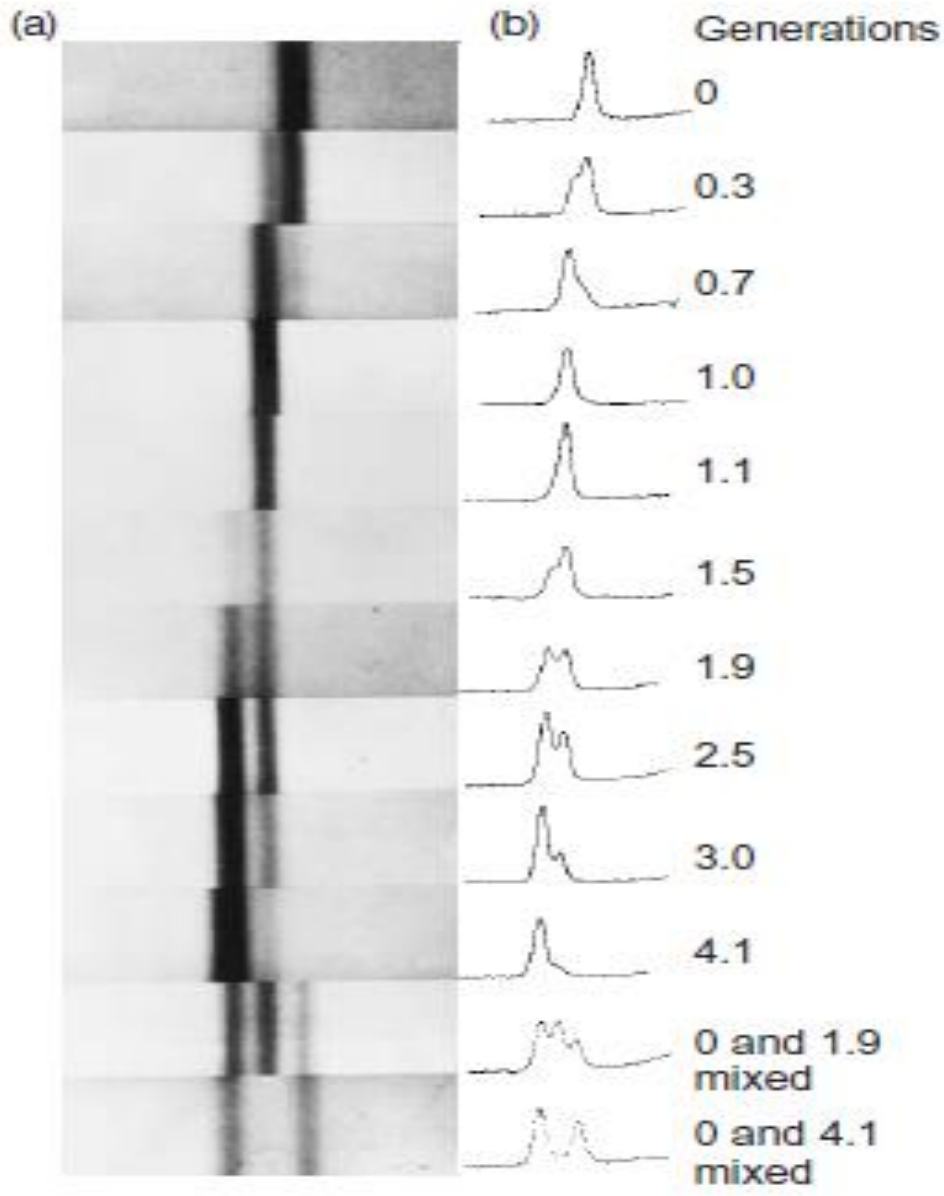
- Semi-conservative replication depends upon unwinding of DNA double helix to expose both the strand to be employed as templates in polymerase reaction.
- The experimental evidences supporting semiconservative model were provided by Methu Meselson and Franklin Stahl (1958).

The Meselson & Stahl Experiments

- In their experiment cells of bacteria ***Eucherichia coli*** were grown for many generations in medium containing $^{15}NH_4Cl$ as the only source of nitrogen. Thus all nitrogenous bases of DNA contained ^{15}N. It was confirmed by CsCl density gradient ultra centrifugation.

  (this technique can resolve macromolecule deferring in density by 0.01 g/ml)
- Then a tenfold excess of $^{14}NH_4Cl$ was added as main source of nitrogen.


(a) Conservative
Generation 1
H H
H H
L L
Expected density gradient results
H H
L L
H H
(b) Semiconservative
Generation 1
Generation 2
H H
H L
L H
H L
L L
L L
L H
Expected density gradient results
H H
H L
L L
H L
(c) Dispersive
Generation 1
Generation 2
H H
Mixed
Mixed
All mixed
Expected density gradient results
H H
H L
25% H; 75% L



Results of the experiment performed by Meselson & Stahl

- DNA was isolated from the cells collected at appropriate intervals and analyzed.

- In first generation all the DNA molecules had same density which was lower than that of **^{15}N DNA** but higher than **^{14}N DNA.** This finding rejects the conservative mode of replication (for which there should have been two types of molecules one with density corresponding to ^{15}N DNA and other with ^{14}N DNA).

- To make sure that the mode of replication is not disruptive the DNA was further analyzed.

- In second generation two types of molecules were found. Density of half of the molecules were found between **^{14}N DNA and ^{15}N DNA** while density of other half molecules was found corresponding to **^{14}N DNA.**

- **This experiment not only proves the semiconservative nature of replication but also confirms that DNA is made up of two strands of equal sizes.**

Replication is Semi-discontineous

- At each replication fork the two strands of the DNA are used as templates and both are replicated by DNA Polymerase.
- DNA plolymerase uses ssDNA as a template and makes a complementary (reverse complimentary rather) by polymerizing deoxynucleotides.

- The DNA polymerase can synthesize the new DNA strand only in 5’⟶ 3’ direction reading the template in 3’⟶5’ direction.

- So how does DNA poly. Copy the parent strand that runs in a 5’⟶ 3’ direction.

- Two strands are synthesized in different ways ;

➢ The template strand in 3’⟶5’ is read and replicate continuously (leading strand).

➢ The other template strand running in 5’⟶3’ direction is replicated in discontinuously (lagging strand).

Lagging Strand: The Okazaki fragmsent

- Tuneko and Rejii Okazaki (1968) gave experimental proof of semi discontinuous replication.
- They exposed a rapidly dividing E. coli culture to H^3 labeled thymidine for 30 seconds and quickly collected the cells.
- They found that some portion of the labels were incorporated in nucleic acid appeared in short ssDNA chains just 1000- 2000 nt. long (Okazaki fragments). While other labels were part of large DNA molecule.