


WELCOME TO MOLECULAR BIOLOGY AND GENETICS

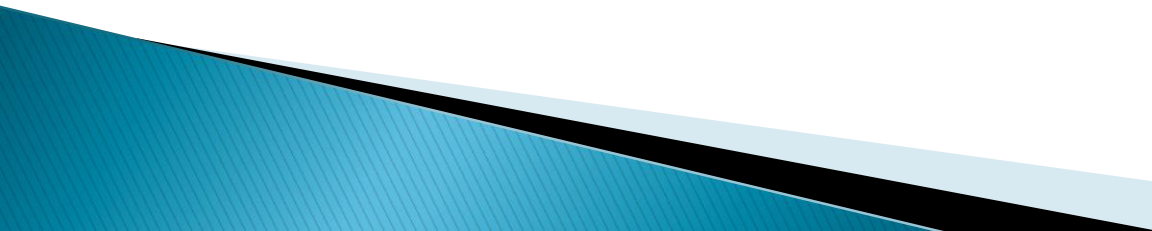
BIO1400/BIO1412




Course Content

- ▶ Objective
 - ▶ 1. Explain the basic principles of molecular biology and genetics.
 - ▶ 2. Describe the role of gene regulation in cellular function.
 - ▶ 3. Explain the chromosomal theory of inheritance.
 - ▶ 4. Discuss classical Mendelian and post-Mendelian genetics
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Topics

- ▶ **Molecular Biology**
 - ▶ DNA replication in prokaryotes: Semi-conservative replication,
 - ▶ Transcription: mRNA, tRNA and rRNA transcription in prokaryotes (Initiation, elongation and termination)
 - ▶ Translation: Protein synthesis; properties of the genetic code;
 - ▶ Regulation of gene expression: Significance; the Lac operon
 - ▶ Mutagens
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- ▶ **GENETICS:**
 - ▶ Chromosomal theory of inheritance
 - ▶ Cell division
 - ▶ Introduction to genetics
 - ▶ Mendelian Genetics
 - ▶ Gene interactions
 - ▶ Sex determination and sex-linkage
 - ▶ Gene Mutations
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DNA REPLICATION

Objectives

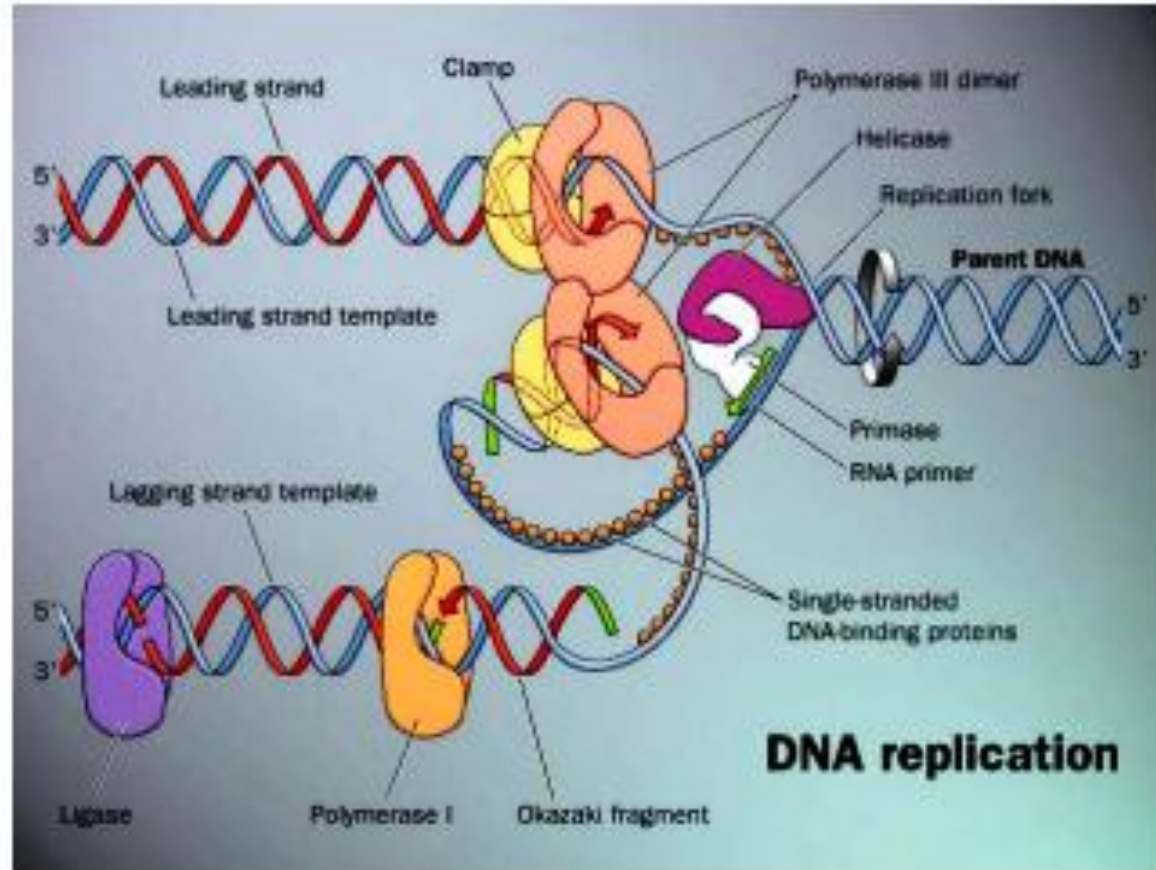
- ▶ You should be able to:
 - ▶ 1) define DNA replication.
 - ▶ 2) Describe the models proposed to explain DNA replication.
 - ▶ 3) Explain the different stages of prokaryotic DNA replication
 - ▶ 4) Differentiate between the synthesis of the leading strand and that of the lagging strand

Replication

- ▶ The process involves the making of new DNA strands using old DNA.
- ▶ The new strands are complementary in sequence to the original strand.
- ▶ The process of making new strands is catalyzed by DNA polymerases.
- ▶ These add nucleotides to each nascent DNA strand.

- ▶ Replication of the DNA molecule begins with the breaking of hydrogen bonds between base pairs of the double helix.
 - ▶ The two halves of a DNA molecule unwind and come apart.
 - ▶ There are three hypotheses that attempt to explain DNA replication.
 - ▶ Polymerases use bases on the template strand as guides to form the Watson and Crick pairs where A and T form double bonds and C and G form triple bonds.
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DNA replication is performed by a multienzyme complex >1 MDa



DNA
Nucleotides

Replisome:

DNA polymerases

Helicase

Primase

SSBs

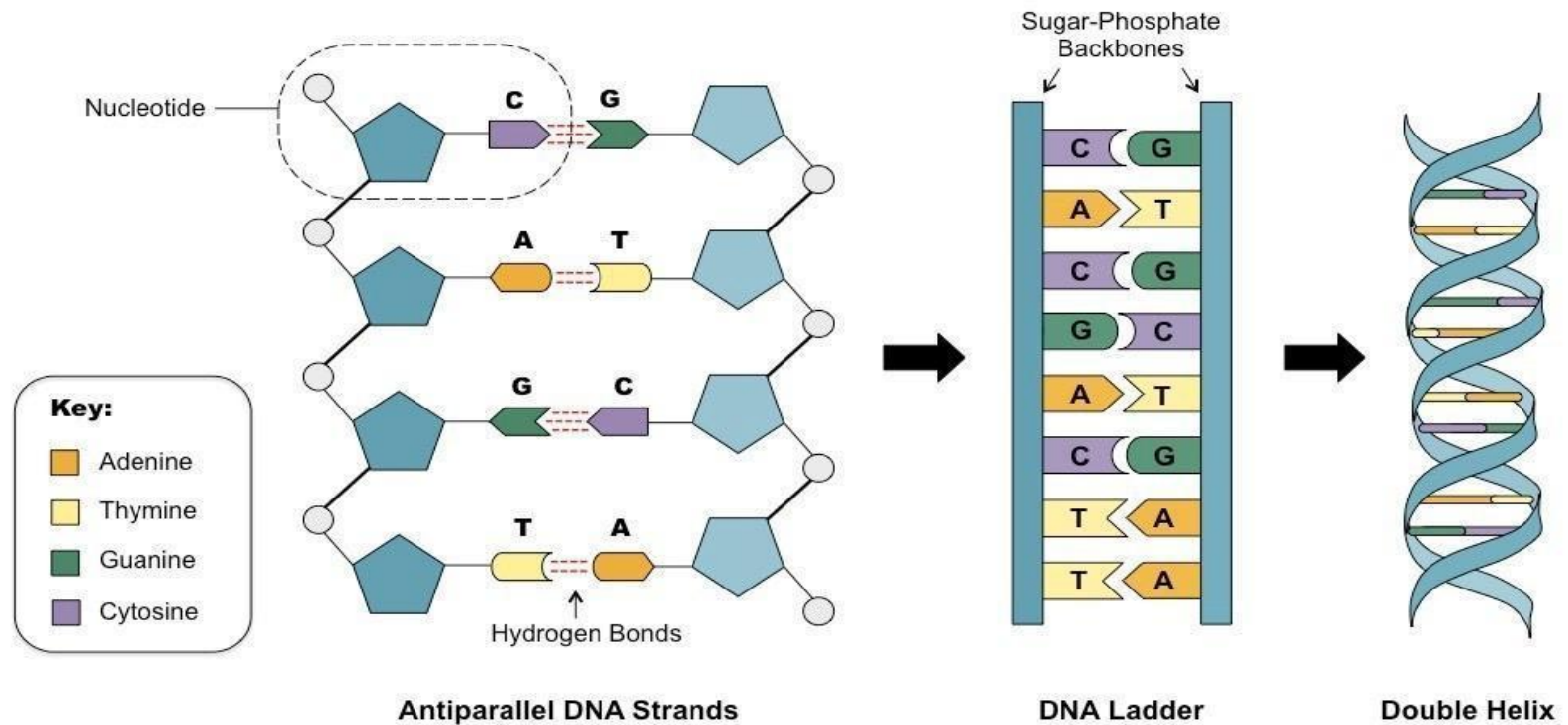
DNA ligase

Clamps

(Topoisomerases)

Historical

- ▶ Three models were originally proposed.
- ▶ These were conservative, dispersive and semi-conservative replication.



Each original strand functions as template for DNA synthesis

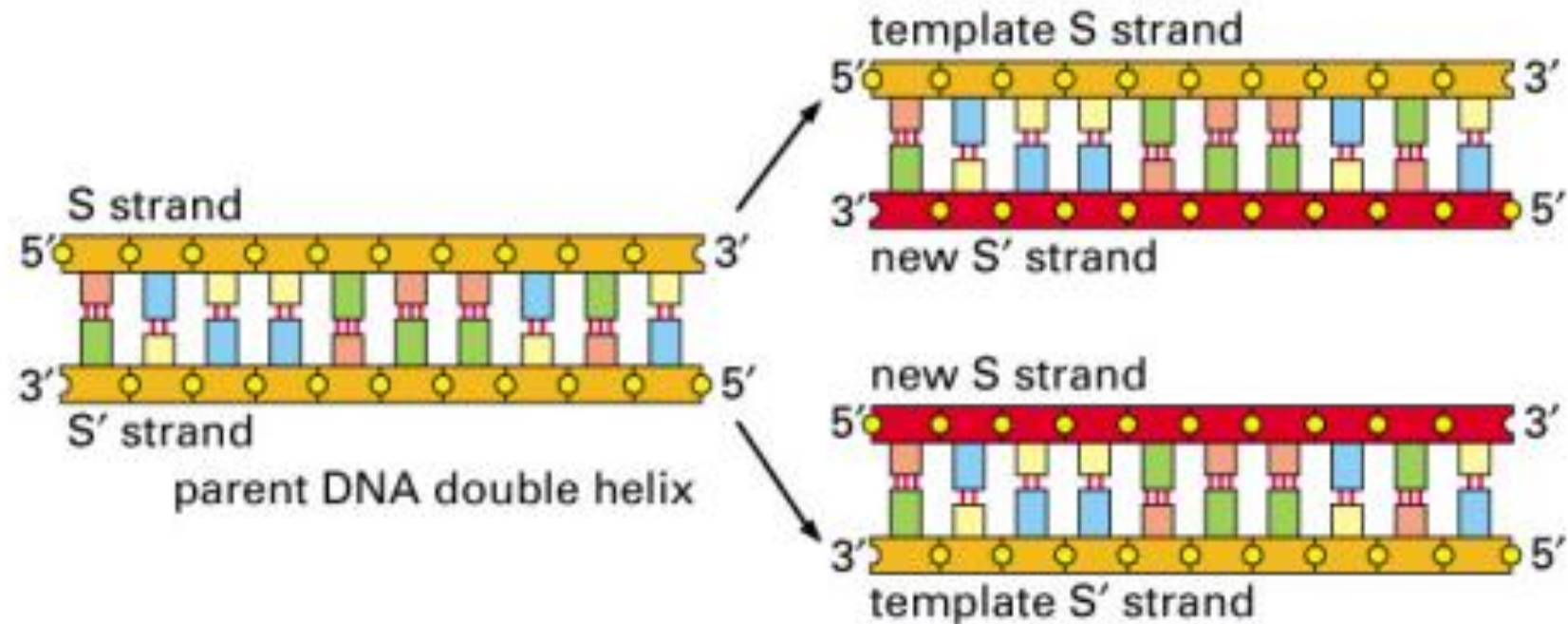




Figure 5-2. Molecular Biology of the Cell, 4th Edition.


History

- ▶ **Semi-conservative** model, the two parental strands separate and each makes a copy of itself.
 - ▶ The two daughter molecules each comprises one old and one new strand after one replication cycle.
 - ▶ After two cycles, two of the **DNA** molecules consist only of new material, while the other two contain one old and one new strand.
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History

- ▶ In the **conservative** model, the parental molecule directs synthesis of an entirely new double-stranded molecule.
 - ▶ After one round of replication, one molecule is conserved as two old strands.
 - ▶ This is repeated in the second round.
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History

- ▶ In the **dispersive** model, material in the two parental strands is distributed more or less randomly between two daughter molecules.
 - ▶ In the model old material is distributed symmetrically between the two daughters molecules.
 - ▶ Other distributions are possible.
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“The Most Beautiful Experiment in Biology”

Meselson & Stahl:
Semiconservative Replication of DNA



Matthew Meselson

(Molecular Biologist & Geneticist)

- ▶ Born May 24th, 1930 in Denver, Colorado
- ▶ Labs in garage & basement
- ▶ Graduated high school 2 years early
- ▶ University of Chicago (chemistry),
California Institute of Technology (Ph.D.
under Linus Pauling → x-ray
crystallography, density centrifugation)
- ▶ Since 1960–Professor at Harvard



Franklin Stahl

(Molecular Biologist & Geneticist)

- ▶ Born October 8th, 1929 in Boston, Massachusetts
- ▶ Studied at Harvard (A.B.) & University of Rochester (Ph.D.)
- ▶ Researched at California Institute Technology & University of Missouri
- ▶ Currently professor at University of Oregon



Woods Hole

- ▶ 1954: Meselson went to Woods Hole to be a teaching assistant
- ▶ Stahl was a post-doctoral fellow at Woods Hole, where he was studying molecular biology techniques
- ▶ Throughout the summer, Meselson and Stahl had discussions on theory and experiments
 - Shared similar ideas and were keen to work together
 - Stahl received a postdoctoral position in Caltech
 - Particularly interested in building off of Watson and Crick's DNA model

The DNA Model

- ▶ Watson and Crick's DNA Model (1953):
 - Double-stranded helix
 - Right-handed
 - Nucleotides bases form interior; sugar phosphate backbone
 - Aligning with Chargaff's rule → each purine paired with a pyrimidine (A-T, G-C sequences)

***Watson and Crick speculated that the specific base pairings were indicative of a precise mode of DNA replication**

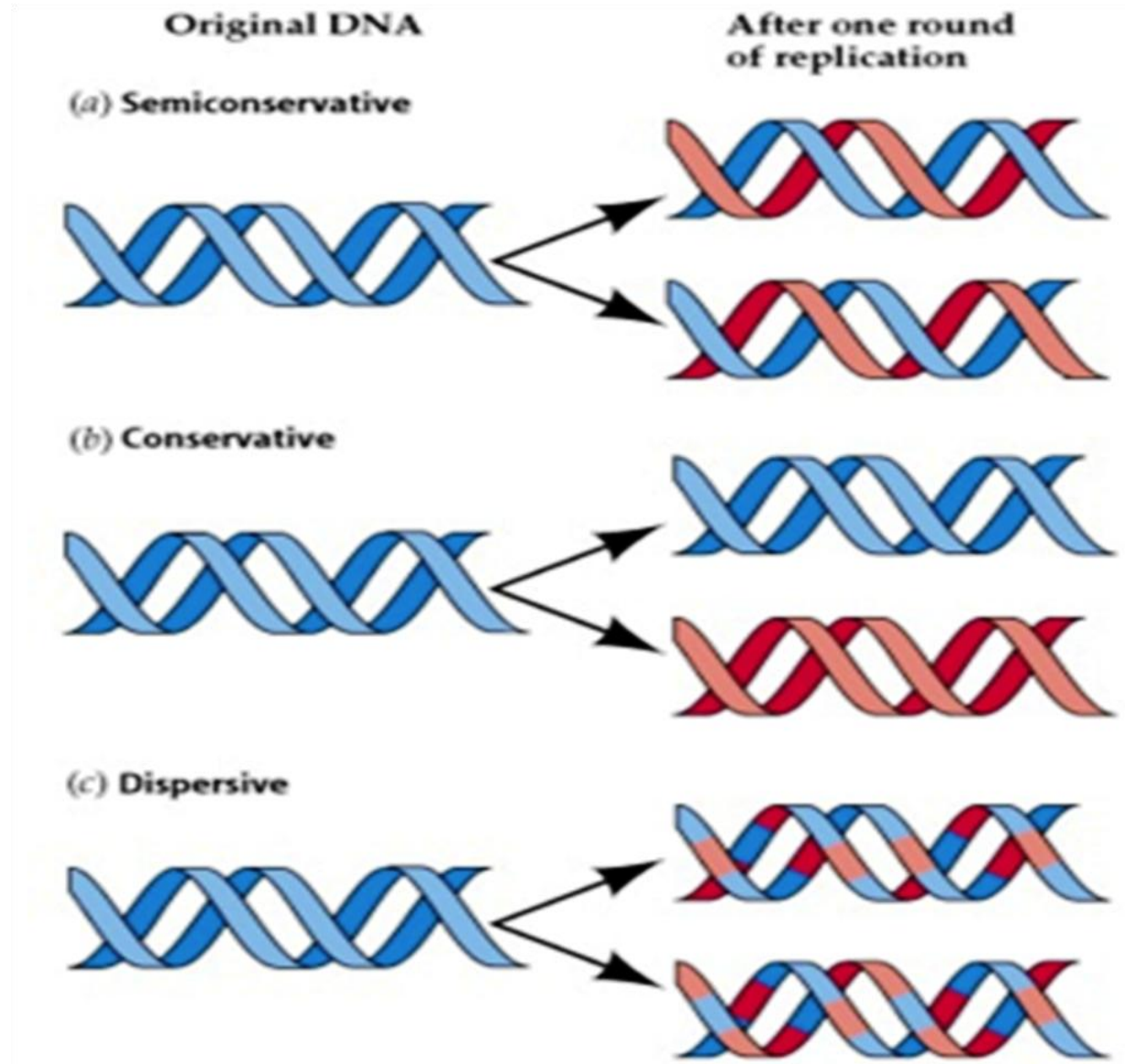
Meselson and Stahl sought to prove Watson and Crick's hypothesis.

3 Major Hypotheses for DNA Replication

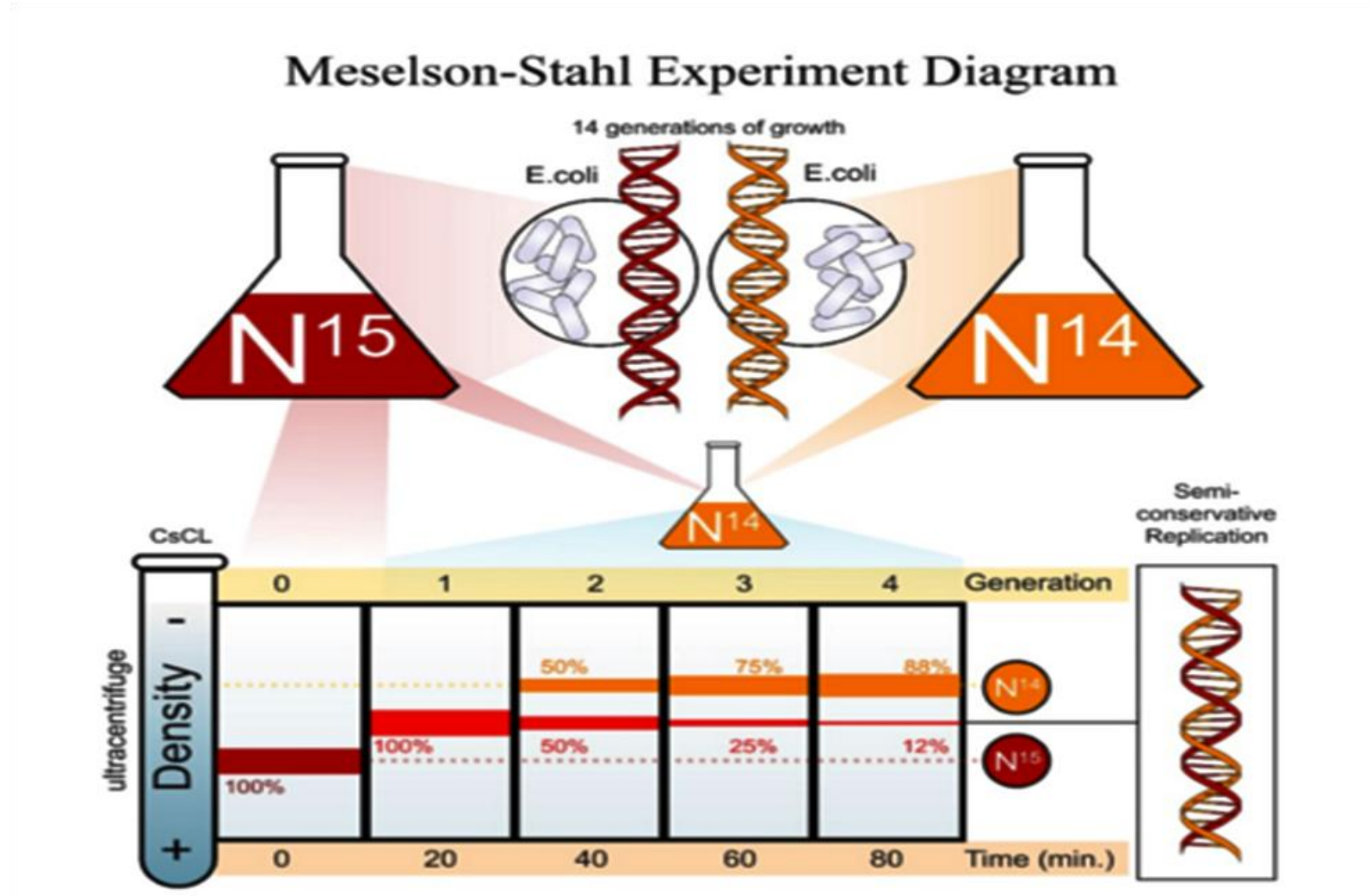
Semiconservative: each strand serves as template for replication

Conservative: intact double helix is copied in its entirety

Dispersive: replication results in hybrids consisting of old and new DNA



Meselson and Stahl's Experiment (1958)



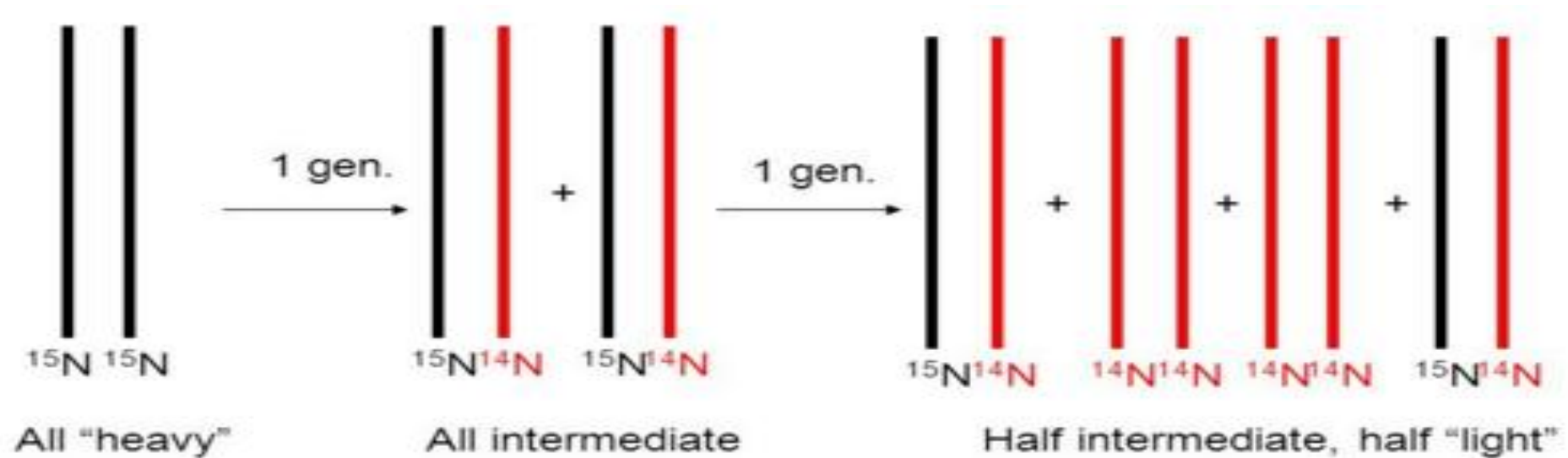
Overview

- ▶ Utilization of isotope labels to differentiate between parent and daughter DNA
 - Chose ^{14}N (common, lighter) and ^{15}N (rare, heavier) → key element of DNA
- Technique of cesium chloride equilibrium density gradient centrifugation
 - Separation of molecules based on density
- Goal: detect whether new nitrogen atoms appear on 1 or both daughter strands

Methodology

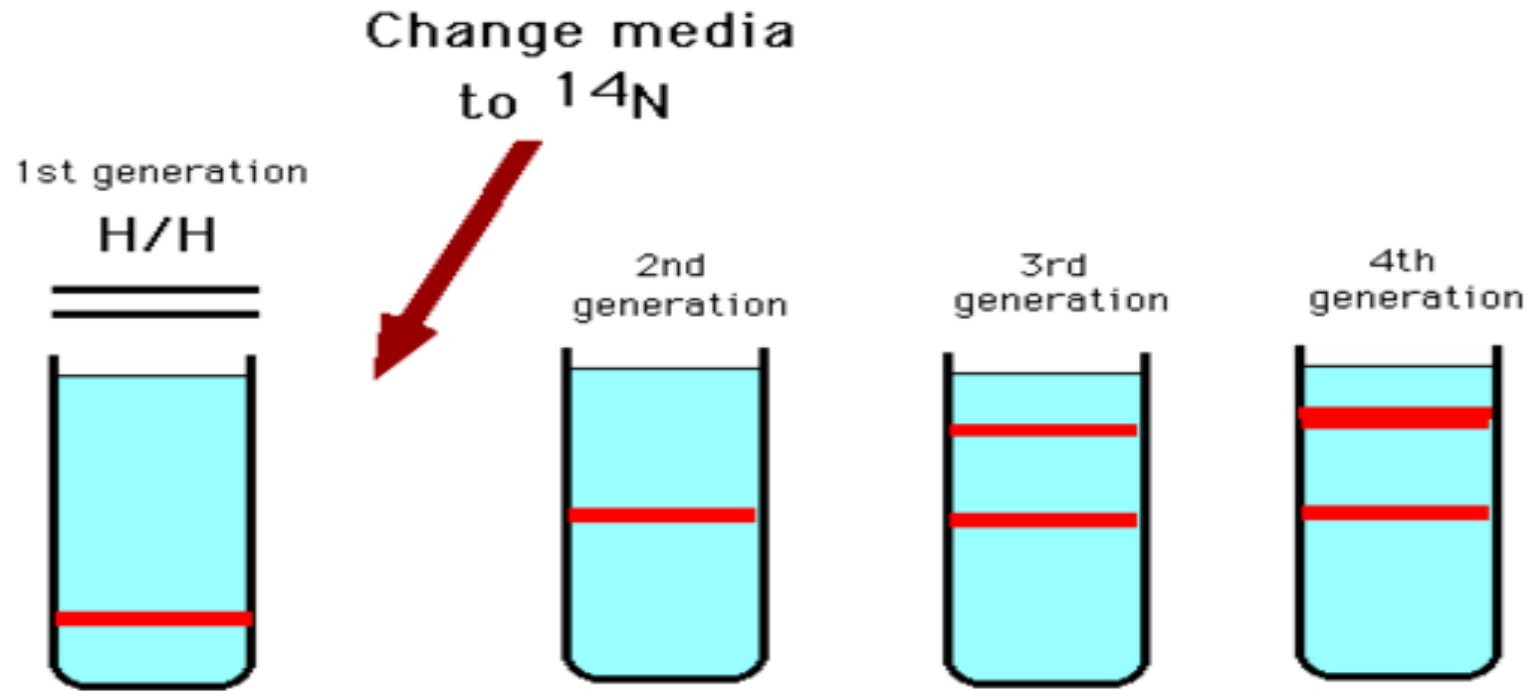
- ▶ Grew 14 generations *E. coli* in ^{15}N (dense, would be at bottom of centrifuge)
- ▶ Changed medium to ^{14}N → from this point forward all DNA replicated in this medium
- ▶ Periodically sampled the DNA grown in the ^{14}N medium by means of equilibrium density gradient centrifugation to compare the percentage of ^{15}N to ^{14}N
 - Samples mixed with cesium chloride, centrifuged and allowed to settle
 - http://www.phschool.com/science/biology_place/biocoach/dnarep/cscl.html

Results



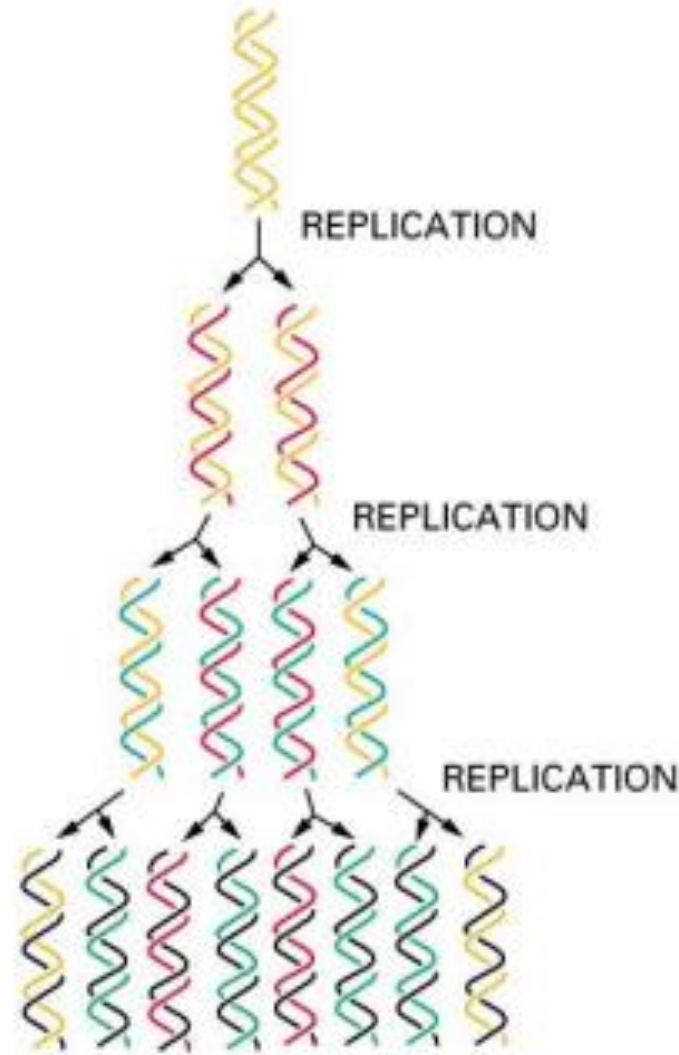
- 1) DNA made up of only ^{15}N formed a single band
- 2) First replication band located at midpoint between ^{15}N and ^{14}N
- 3) Second generation had two bands (1 at midpoint, 1 at ^{14}N); same two bands seen subsequently

Semiconservative Replication



Meselson and Stahl elucidated the core mechanism of DNA replication

After each replication cycle, DNA is doubled



DNA extracted and centrifuged to equilibrium in CsCl density gradient

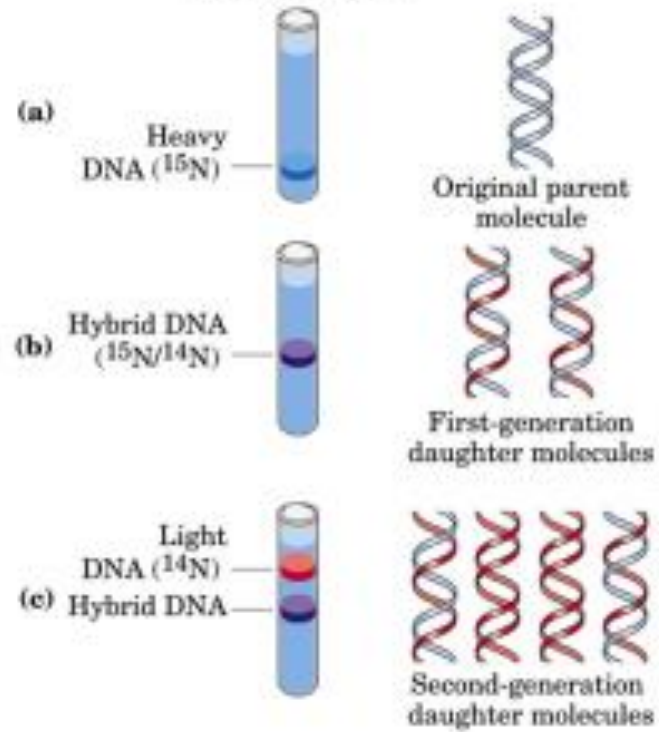
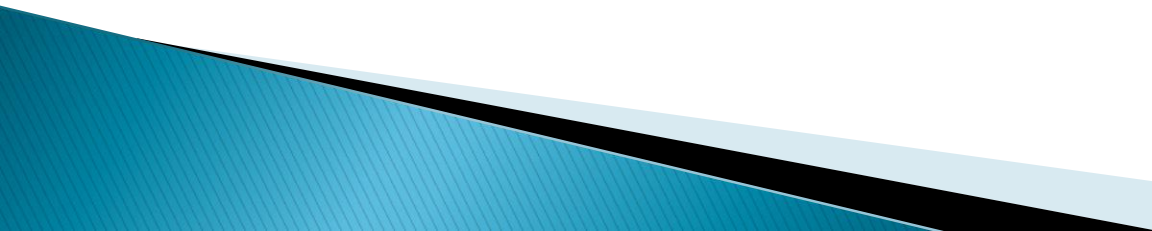


Figure 5-5. Molecular Biology of the Cell, 4th Edition.

Contributions to Biotechnology

- ▶ “Most Beautiful Experiment” → forming predictions based on various models and pinpointing which matches experimental data
 - Addressed all models simultaneously
- ▶ Demonstrated effective, unique mode of isotope visualization
 - Labeling not based on radioactivity but rather density (gradient analyzed by means of centrifugation)
 - Heavy isotopes are now widely used to label and analyze molecules

Advancing the Field of Molecular Biology

- ▶ Semiconservative DNA replication has been confirmed in every other studied species
 - ▶ Understanding semiconservative DNA replication is crucial to elucidating functionality of cells and organisms
 - Genetic material must replicate completely and accurately during divisions
 - ▶ Furthered knowledge on mechanisms of DNA and its interactions with enzymes (i.e. helicase)
 - ▶ Having a grasp on proper DNA replication allows for identification of abnormalities underlying defects
 - ▶ Insights into heredity
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Sources

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