UNIT 3: DNA & GENETICS       TOPIC B: DNA REPLICATION

Essential Idea(s)

- The structure of DNA is ideally suited to its replication.

IB Assessment Statements and Class Objectives:

2.7 U1 The replication of DNA is semiconservative and depends on complimentary base pairing.

- Describe the meaning of “semiconservative” in relation to DNA replication.
- Explain the role of complementary base pairing in DNA replication.

2.7 S2 Analysis of Meselson and Stahl’s results to obtain support for the theory of semi-conservative replication of DNA.

- Compare dispersive, conservative, and semi-conservative replication.
- Predict experimental results in the Meselson and Stahl experiment if DNA replication was dispersive, conservative, or semi-conservative.

2.7 NOS Obtaining of evidence for scientific theories – Meselson and Stahl obtained evidence for the semi-conservative replication of DNA.

- Describe the procedure of the Meselson and Stahl experiment.
- Explain how the Meselson and Stahl experiment demonstrated semi-conservative DNA replication.

2.7 U2 Helicase unwinds the double helix and separates the two strands by breaking hydrogen bonds.

- State why DNA strands must be separated prior to replication.
- Outline two functions of helicase.
- State the role of the origin of replication in DNA replication.
- Contrast the number of origins in prokaryotic cells to the number in eukaryotic cells.

2.7 U3 DNA polymerase links nucleotides together to form a new strand, using a pre-existing strand as a template.

- Describe the movement of DNA polymerase along the DNA template strand.
- Describe the action of DNA polymerase III in pairing nucleotides during DNA replication.

3.5 U2 PCR can be used to amplify small amounts of DNA.

- State the function of PR.
- Describe the selectivity of PCR.

2.7 A1 Use of Taq DNA polymerase to produce multiple copies of DNA rapidly by the polymerase chain reaction (PCR)

- Outline the process of PCR
- Explain the use of Taq DNA polymerase in the PCR
DNA Replication

Cells carry out the process of **DNA replication** (DNA duplication) prior to cell division (mitosis and meiosis). This process ensures that each resulting cell is able to receive a complete set of genes from the original cell. After the DNA has replicated, each chromosome is made up of two chromatids, which are joined at the centromere. DNA replication is **semi-conservative**; each chromatid contains half original (parent) DNA and half new (daughter) DNA. The two chromatids will become separated during cell division to form two separate chromosomes. During DNA replication, new nucleotides become added at a region called the **replication fork**. The position of the replication fork moves along the chromosome as the replication progresses. This whole process occurs simultaneously for each chromosome of a cell and the entire process is tightly controlled by enzymes.

**Step 1: Unwinding the DNA molecule**
A normal chromosome consists of an unreplicated DNA molecule. Prior to cell division, this long molecule of double-stranded DNA must be replicated. It is untwisted at high speed at its replication fork by two enzymes: one, called **helicase**, unwinds the parental strands and the other then relieves the strain that this generates by cutting, winding and rejoining the DNA strands.

**Step 2: Making new DNA strands**
The formation of new DNA is carried out mostly by an enzyme complex called **DNA polymerase**, and a series of proteins that cause the two strands to break apart. Nucleotides are assembled in a continuous fashion on one strand of the DNA while on the other side fragments are created which are later joined to form one continuous length.

**Step 3: Rewinding the DNA molecule**
Each of the two new double-helix DNA molecules has one strand of the original DNA (dark gray and white) and one strand that is newly synthesized (patterned). The two DNA molecules rewind into their 'corkscrew' double-helix shape again. The replication of DNA is semi-conservative, with each new double helix containing one parent strand and one newly synthesized strand. The new chromosome has actually twice as much DNA as a regular (non-replicated) chromosome. The two chromatids will become separated in the cell division process to form two separate chromosomes.
1. State the purpose of DNA replication: **complete set of genes passed on**

2. Summarize the three main steps involved in DNA replication:
   (a) **Unwind DNA molecule**
   (b) **Make new DNA strands**
   (c) **RepliDNA molecule**

3. For a cell with 22 chromosomes, state how many chromatids would exist following DNA replication: **44**

4. Discuss the importance of enzymes in DNA replication: **Enzymes complete the steps. Helicase unwinds the DNA molecule and DNA polymerase actually builds the new strands.**

5. DNA replication occurs during the S (synthesis) phase of the cell cycle. This is part of a larger phase called interphase. It is the phase in which the cell is not dividing (in mitosis).

   The light micrograph (right) shows a section of cells in an onion root tip. These cells have a cell cycle of approximately 24 hours. The cells can be seen to be in various stages of the cell cycle. By counting the number of cells in the various stages it is possible to calculate how long the cell spends in each stage of the cycle.

   Count and record the number of cells in the image which are undergoing mitosis and those that are in interphase. Estimate the amount of time a cell spends in each phase.

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of cells</th>
<th>% of total cells</th>
<th>Estimated time in stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

6. Match the statements in the table below to form complete sentences, then put the sentences in order to make a coherent paragraph about DNA replication and its role:

   The enzymes also proofread the DNA during replication... **...is required before mitosis or meiosis can occur.**
   DNA replication is the process by which the DNA molecule... **...by enzymes.**
   Replication is tightly controlled... **...to correct any mistakes.**
   After replication, the chromosome... **...and half new (daughter) DNA.**
   DNA replication... **...during mitosis**
   The chromatids separate... **...is copied to produce identical DNA strands.**
   A chromatid contains half original (parent) ... **...is made up of two chromatids.**

   Write the complete paragraph here: **[Blank]**
Enzyme Control of DNA Replication

The sequence of enzyme controlled events in DNA replication is shown below (1-5). Although shown as separate, many of the enzymes are found clustered together as enzyme complexes. These enzymes are also able to 'proof-read' the new DNA strand as it is made and correct mistakes. The polymerase enzyme can only work in one direction, so that one new strand is constructed as a continuous length (the leading strand) while the other new strand is made in short segments to be later joined together (the lagging strand). Note that the nucleotides are present as deoxyribonucleoside triphosphates. When they are hydrolyzed, energy released is used to incorporate the nucleotide into the growing strand.

DNA replication occurs during interphase of the cell cycle at an astounding rate. As many as 4000 nucleotides per second are replicated. This explains how under ideal conditions, bacterial cells with as many as 4 million nucleotides, can complete a cell cycle in about 20 minutes.

1. What is the purpose of DNA replication? ________pass on a complete set of genes________

2. Summarize the steps involved in DNA replication (on the previous activity):
   (a) Step 1: ________unwind DNA molecule________
   (b) Step 2: ________make new DNA strands________
   (c) Step 3: ________rewind DNA molecule________

3. Explain the role of the following enzymes in DNA replication:
   (a) Helicase: ________splits/unwinds DNA molecule________
   (b) DNA polymerase I: ________digests RNA primer; replaces with DNA________
   (c) DNA polymerase III: ________extends RNA primer with comp. DNA________
   (d) Ligase: ________joins fragments together________

4. Determine the time it would take for a bacteria to replicate its DNA (see note in diagram above): ________20 min________
Review of DNA Replication

The diagram below summarizes the main steps in DNA replication. You should use this activity to test your understanding of the main features of DNA replication, using the knowledge gained in the previous activity to fill in the missing information. You should attempt this from what you have learned, but refer to the previous activity if you require help.

**Purpose of DNA Replication**

DNA replication creates a chromosome with two chromatids

- Parent chromosome
- Chromatid
- Centromere
- Replicated chromosome
- Parent DNA is made up of two strands coiled into a double helix

**The Replication Fork**

Nucleotide symbols

- G
- C
- A
- T

DNA base pairing rule

- G pairs with C
- A pairs with T

The replication fork (A-F):

- DNA
- Replication fork
- DNA polymerase
- Parent DNA
- Daughter DNA
- Free nucleotide

1. In the white boxes provided in the diagram, state the base pairing rule for making a strand of DNA:

2. Identify each of the structures marked with a letter (A-F):
   - A: DNA
   - B: Replication fork
   - C: DNA polymerase
   - D: Parent DNA
   - E: Daughter DNA
   - F: Free nucleotide

3. Match each of the processes (1-4) to the correct summary of the process provided below:
   - 2. Unwinding of parent DNA double helix
   - 3. Free nucleotides occupy spaces alongside exposed bases
   - 4. DNA strands are joined by base pairing

- Unzipping of parent DNA
### Polymerase Chain Reaction (PCR)

<table>
<thead>
<tr>
<th>Step</th>
<th>What is it?</th>
<th>Why is it necessary?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denature</td>
<td>$95^\circ C$&lt;br&gt;DNA splits polymerase</td>
<td>makes templates</td>
</tr>
<tr>
<td>Anneal</td>
<td>$55-65^\circ C$&lt;br&gt;primers bind polymerase forms copies</td>
<td>gets replication going</td>
</tr>
<tr>
<td>Extend</td>
<td>$72^\circ C$</td>
<td>completes strand</td>
</tr>
<tr>
<td>Repeat</td>
<td>N/A</td>
<td>make many copies</td>
</tr>
</tbody>
</table>

### Taq Polymerase
- What does it do?
  - *amplifies target DNA sequences*
- Where is it found in nature?
  - *hot springs/bacteria*
- How is it unique from other DNA polymerases?
  - *withstands other hot temps*
- Why is this feature important in PCR?
  - *hot temps needed to split DNA*