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Deoxyribonucleic acid (DNA)

DNA is a genetic material in human and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA). Genetic material must be able to do three things: Replicate so that it can be transmitted to the next generation, store information and undergo change (mutation) to provide genetic variability. DNA meets all three of these criteria.

Structure of DNA:

DNA is a double helix. It is composed of two strands that spiral about each other. Each strand is a polynucleotide because it is composed of a series of nucleotides. A nucleotide is a molecule composed of three subunits: phosphoric acid (phosphate), a pentose sugar (deoxyribose), and a nitrogen-containing base (either adenine [A], cytosine [C], guanine [G], or thymine [T].

Looking at just one strand of DNA, notice that the phosphate and sugar molecules make up a backbone and the bases project to one side. Put the two strands together, and DNA resembles a ladder. The bases are held together by hydrogen bonding: A pairs with T by forming two hydrogen bonds, and G pairs with C by forming three hydrogen bonds, or vice versa. This is called **complementary base pairing.**

These **complementary paired** bases are important to the functioning of DNA. Remember that adenine and guanine are **purines** (purine is a structure with two rings), and cytosine and thymine are **pyrimidines** (pyrimidine has one ring). The two strands of DNA are antiparallel, meaning they run in opposite directions.

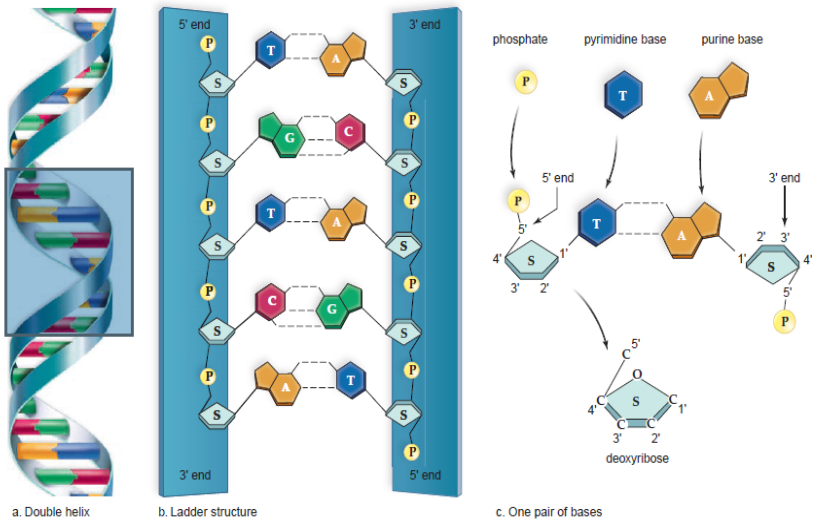

Figure 1 (A, B & C): The structure of DNA.

DNA Replication models:

There are three different mechanisms to produce the net of DNA replication, these mechanisms are shown in Figure (2). **The first one is referred to as a conservative model**, according to this hypothesis, both two strands of parental DNA remain together following DNA replication, in this model, the original arrangement of parental strand is completely conserved, while the two newly made daughter strands are also together following replication**. The second mechanism is called a semi conservative model**, the double strand DNA is a half conserved following the replication process (the double strand DNA contains one parent strand and one daughter strand. **The last mechanism, dispersive**, the model proposes that segments of parental DNA and newly made DNA are interspersed with both strands following the replication process.

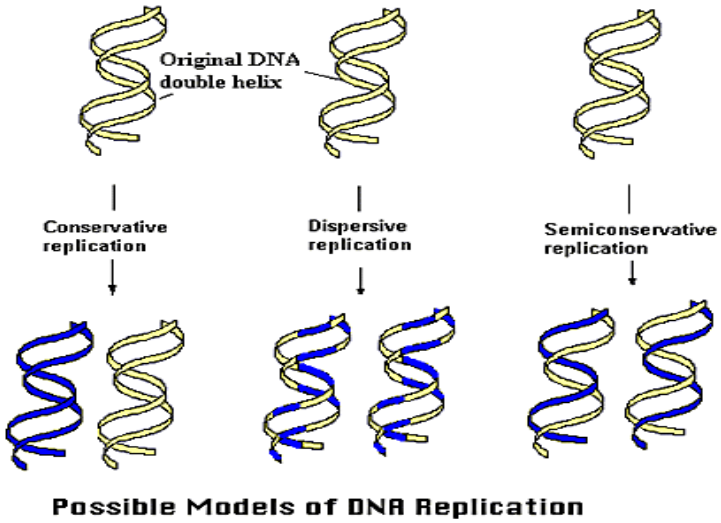

Figure 2: Possible models of DNA replication

Replication of DNA:

DNA Replication is a process in which the DNA within a cell makes an exact copy of itself during cell division. During the S phase of interphase during mitosis when DNA is replicated, the double-stranded structure of DNA allows each original strand to serve as a **template** for the formation of a complementary new strand. DNA replication is termed ***semiconservative*** because each new double helix has one original strand and one new strand.

I. General Features of Replication

A. Semi-Conservative

B. Starts at Origin

C. Bidirectional

D. Semi-Discontinuous

II. Identifying Proteins and Enzymes of Replication

III. Detailed Examination of the Mechanism of Replication

A. Initiation B. Priming

C. Elongation D. Proofreading and Termination

1. The DNA synthesis begins at a site termed the **origin of replication**.
2. Synthesis of DNA proceeds **bidirectionally** around the bacterial chromosome. As the 2 DNA strands open at the origin, replication bubbles form. Prokaryotes (bacteria) have a single bubble, while Eukaryotic chromosomes have MANY bubbles.
3. The first step in DNA replication is to '**unzip'** the double helix structure of the DNA molecule. This is carried out by an enzyme called **helicase, which** breaks the hydrogen bonds holding the complementary bases of DNA together (A with T, C with G).
4. The separation of the two single strands of DNA creates a **'Y' shape called a replication 'fork**'. The two separated strands will act as **templates** for making the new strands of DNA.
5. Single strand Binding Proteins **(SSB)** keep the two strands from re-annealing (coming back together).
6. Enzyme **Topoisomerase** attaches to the 2 forks of the bubble to relieve stress on the DNA molecule as it separates.
7. Before new DNA strands can form, A short piece of RNA called **a primer** (produced by an enzyme called **primase**) presents to start the addition of new nucleotides.
8. One of the strands is oriented in the 3- to 5- direction (towards the replication fork), this is the leading strand. The other strand is oriented in the 5' to 3' direction (away from the replication fork), this is the lagging strand. As a result of their different orientations, the two strands are replicated differently:

	Leading strand	**Lagging strand**
10	A primer comes along and binds to the end of the leading strand. The primer acts as the starting point for DNA synthesis.	Numerous RNA primers bind at various points along the lagging strand.
11	DNA polymerase binds to the leading strand and then 'walks' along it, adding new complementary nucleotide bases (A, C, G and T) to the strand of DNA in the 5' to 3' direction.	Chunks of DNA, called Okazaki fragments, are then added to the lagging strand also in the 5' to 3' direction.
12	This sort of replication is called continuous.	This sort of replication is called dis- continuous.

13. Once all of the bases are matched up (A with T, C with G), an enzyme called **exonuclease** strips away the primer(s). The gaps where the primer(s) were are then filled by yet more complementary nucleotides.
14. The **enzyme Ligase** joins the Okazaki fragments together to make one strand.
15. The new strand proofreads to make sure there are no mistakes in the new DNA sequence.
16. The result of DNA replication is two DNA molecules consisting of one new and one old chain of nucleotides. This is why DNA replication is described as semi-conservative, half of the chain is part of the original DNA molecule, half is brand new.
17. Following replication, the new DNA automatically winds up into a double helix.
18. There are now 2 identical double helices of DNA.

Proofreading New DNA:

•DNA polymerase initially makes about 1 in 10,000 base pairing errors

•Enzymes proofread and correct these mistakes

•The new error rate for DNA that has been proofread is 1 in 1 billion bases pairing errors

Several enzymes are used in the process of bacterial DNA replication?

1**. DNA gyrase** (a type of DNA topoisomerase): is used to relax supercoils

2. **A helicase** is needed to separate the strands. It requires ATP.

a) **A single stranded binding protein** binds to single stranded DNA, preventing the re-formation of the double stranded molecule.

3**. Primase**: specialized RNA polymerase used to synthesize 15-50 bp primers needed for DNA replication.

4**. DNA polymerase I**: DNA repair and minor role in DNA replication.

5. **DNA polymerase III**: large protein complex enzymes used to synthesize complementary DNA strands. Each DNA polymerase can extend DNA chains by adding nucleotides one at a time to a free 3'-OH end. The base added depends on the template strand. Errors can occur every 100,000 bases/replication.

6. **DNA polymerases II, IV, V**: used for DNA repair.

7**. DNA ligase**: used to form covalent bonds between Okazaki fragments on the lagging strand.

Basic Principles of Eukaryotic DNA Replication

1. Each eukaryotic chromosome contains many replicons.
2. In eukaryotic cell replication only occurs during the S phase of the cell cycle.
3. Unlike bacteria, eukaryotic chromosomes form linear replicons.
4. There are problems associated with linear replicons.

a) DNA polymerase won't bind to only one nucleotide at the end of the chain.

Several enzymes are used in the process of eukaryotic DNA replication

- 1. DNA topoisomerases I and II:
- 2. A helicase.
- 3. Primase.
- 4. DNA polymerase a
- 5. DNA polymerase d.
- 5. DNA polymerase e
- 6. DNA ligase.
- 7. Exonuclease