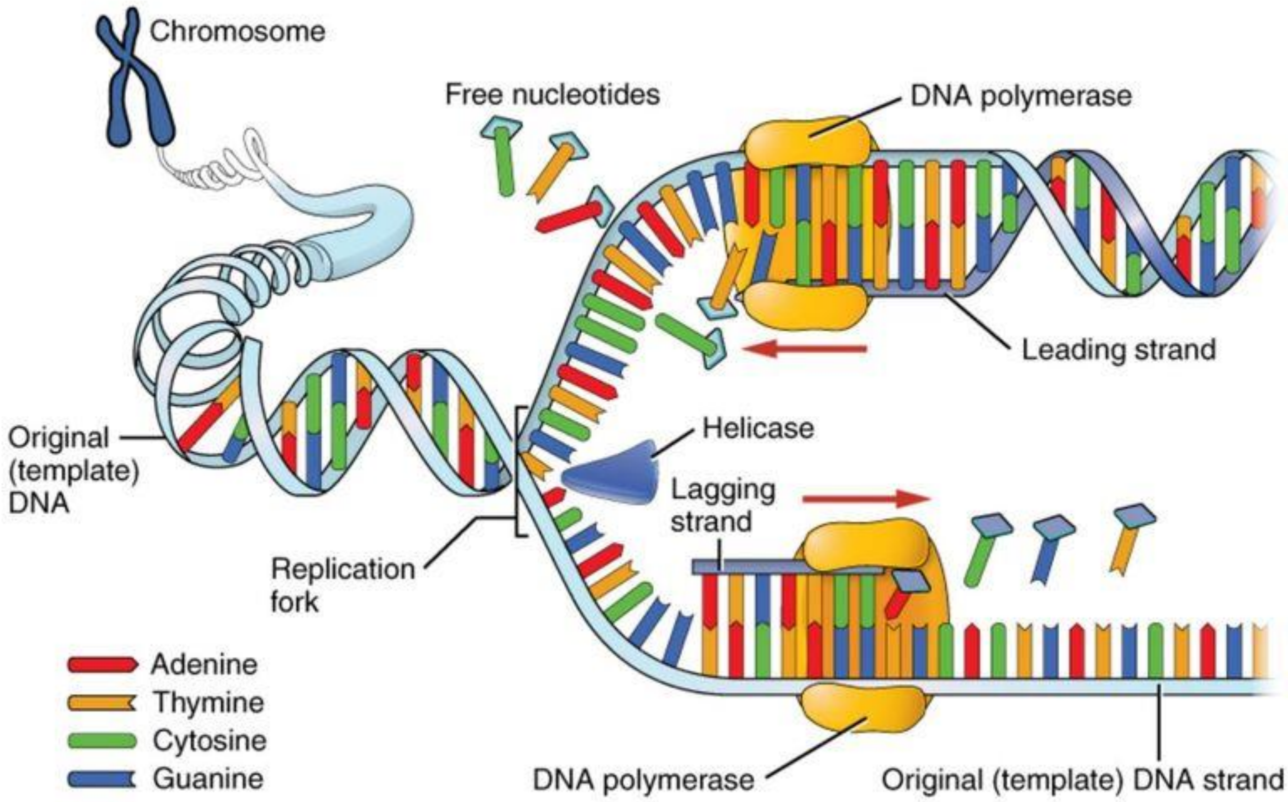
DNA Replication



THE PROCESS OF DNA REPLICATION

DNA is the genetic material that defines every cell. Whenever a cell duplicates and divides into two new daughter cells, all the organelles must be copied as well, including molecules like DNA housed in the nucleus. This process of duplication is called DNA replication. Replication follows several steps that involve multiple proteins called replication enzymes and RNA. In eukaryotic cells, such as animal cells and plant cells, DNA replication occurs in the S phase of interphase during the cell cycle.

DNA STRUCTURE

DNA or deoxyribonucleic acid is a type of molecule known as a nucleic acid. It consists of a 5-carbon deoxyribose sugar, a phosphate, and a nitrogenous base. DNA consists of two spiral nucleic acid chains that are twisted into a double helix shape. This twisting allows DNA to be more compact. In order to fit within the nucleus, DNA is packed into tightly coiled structures called chromatin. Chromatin condenses to form chromosomes during cell division. Prior to DNA replication, the chromatin loosens giving cell replication machinery access to the DNA strands.

PREPARATION

Step 1: Replication Fork Formation

Before DNA can be replicated, the double stranded molecule must be “unzipped” into two single strands. DNA has four bases called **adenine (A)**, **thymine (T)**, **cytosine (C)** and **guanine (G)** that form pairs between the two strands. Adenine only pairs with thymine and cytosine only binds with guanine. In order to unwind DNA, these interactions between base pairs must be broken. This is performed by an enzyme known as DNA **helicase**. DNA helicase disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the replication fork. This area will be the template for replication to begin.

DNA is directional in both strands, signified by a 5' and 3' end. This notation refers to which side group is attached the DNA backbone. The 5' end has a phosphate (P) group while the 3' end has a hydroxyl (OH) group. This directionality is important for replication as it only progresses in the 5' to 3' direction. However, the replication fork is bi-directional; one strand is oriented in the 3' to 5' direction (leading strand) while the other is oriented 5' to 3' (lagging strand). The two sides are therefore replicated with two different processes to accommodate the directional difference.

REPLICATION

Step 2: Primer Binding

The leading strand is the simplest to replicate. Once DNA has been separated, a short piece of RNA called a **primer** binds to the 3' end of the strand. The primer always binds as the starting point for replication.

Step 3: Elongation

Enzymes known as **DNA polymerases** are responsible creating the new strand by a process called elongation. There are five different known types of DNA polymerases found in bacteria and human cells. In E. coli, polymerase III is the main replication enzyme, while polymerase I, II, IV and V are responsible for error checking and repair. Therefore, during replication DNA polymerase III binds to the strand at the site of the primer and begins adding new base pairs complementary to the strand. In eukaryotic cells, polymerases alpha, delta, and epsilon are the primary polymerases involved in DNA replication. Because replication proceeds in the 5' to 3' direction, the newly formed strand is continuous.

The lagging strand begins replication by binding with multiple primers. Each primer is only several bases apart. DNA polymerase then adds pieces of DNA, called Okazaki fragments, to the strand between primers. This process of replication is discontinuous as the newly created fragments are disjointed.

Step 4: Termination

Once both the continuous and discontinuous strands are formed, an enzyme called exonuclease removes all RNA primers from the original strands. These primers are then replaced with appropriate bases. Another exonuclease "proofreads" the newly formed DNA to check, remove and replace any errors. Another enzyme called **DNA ligase** joins Okazaki fragments together forming a single unified strand. The ends of the linear DNA present a problem as DNA polymerase can only add nucleotides in the 5′ to 3′ direction. The ends of the parent strands consist of repeated DNA sequences called telomeres. Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing. A special type of DNA polymerase enzyme called telomerase catalyzes the synthesis of telomere sequences at the ends of the DNA. Once completed, the parent strand and its complementary DNA strand coils into the familiar double helix shape. In the end, replication produces two DNA molecules, each with one strand from the parent molecule and one new strand.

REPLICATION ENZYMES

Enzymes that participate in the eukaryotic DNA replication process include:

- **DNA helicase** - unwinds and separates double stranded DNA as it moves along the DNA.
- **Primase** - RNA polymerase that generates RNA primers. Primers act as templates for the starting point of DNA replication.
- **DNA polymerases** - synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.
- **Topoisomerase** - unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.
- **Exonucleases** - group of enzymes that remove nucleotide bases from the end of a DNA chain.
- **DNA ligase** - joins DNA fragments together by forming phosphodiester bonds between nucleotides.

DNA REPLICATION SUMMARY

DNA replication is the production of identical DNA helices from a single double-stranded DNA molecule. Each molecule consists of a strand from the original molecule and a newly formed strand. Prior to replication, the DNA uncoils and strands separate. A replication fork is formed which serves as a template for replication. Primers bind to the DNA and DNA polymerases add new nucleotide sequences in the 5′ to 3′ direction. This addition is continuous in the leading strand and fragmented in the lagging strand. Once elongation of the DNA strands is complete, the strands are checked for errors, repairs are made and telomere sequences are added to the ends of the DNA.