RNA replication

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General Objectives:

1. To understand the fundamental process and purpose of DNA replication.
2. To analyze the different models of DNA replication (semiconservative, conservative, dispersive) and identify which one is biologically correct.
3. To identify the key roles of enzymes and proteins involved in replication (e.g., Helicase, Polymerase, Ligase, Primase).
4. To explain the sequential steps of the replication process: Replication Fork Formation, Primer Binding, Elongation (on leading and lagging strands), and Termination.
5. To differentiate between the replication of the leading and lagging strands (including understanding Okazaki fragments).

The Process of DNA Replication

Why Replicate DNA?

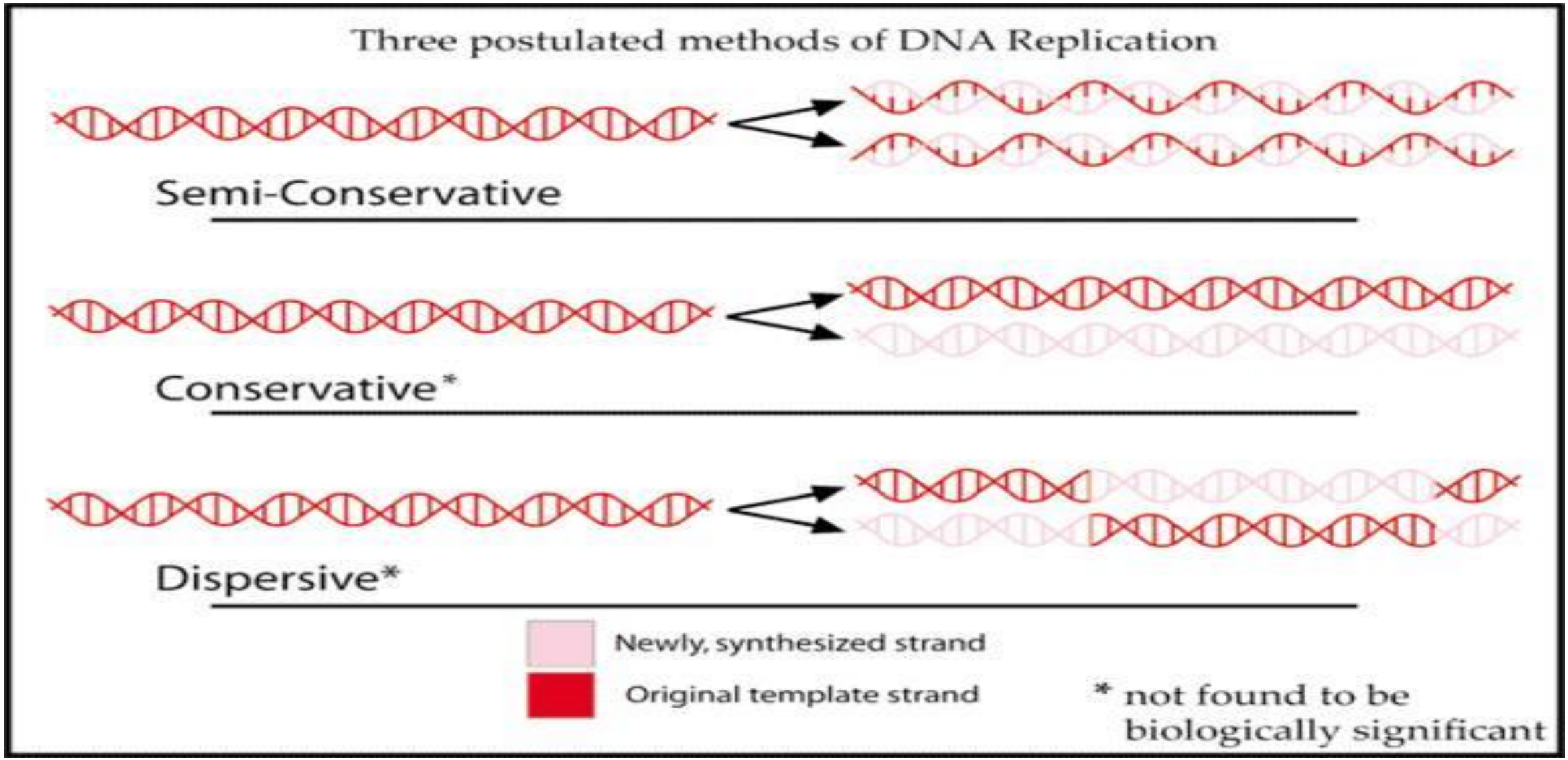
DNA is the genetic material that defines every cell. Before a cell duplicates and is divided into new daughter cells through either mitosis or meiosis, biomolecules and organelles must be copied to be distributed among the cells. DNA, found within the nucleus, must be replicated in order to ensure that each new cell receives the correct number of chromosomes. The process of DNA duplication is called DNA replication. Replication follows several steps that involve multiple proteins called replication enzymes and RNA. DNA replication is semiconservative, meaning that each strand in the DNA double helix acts as a template for the synthesis of a new, complementary strand.

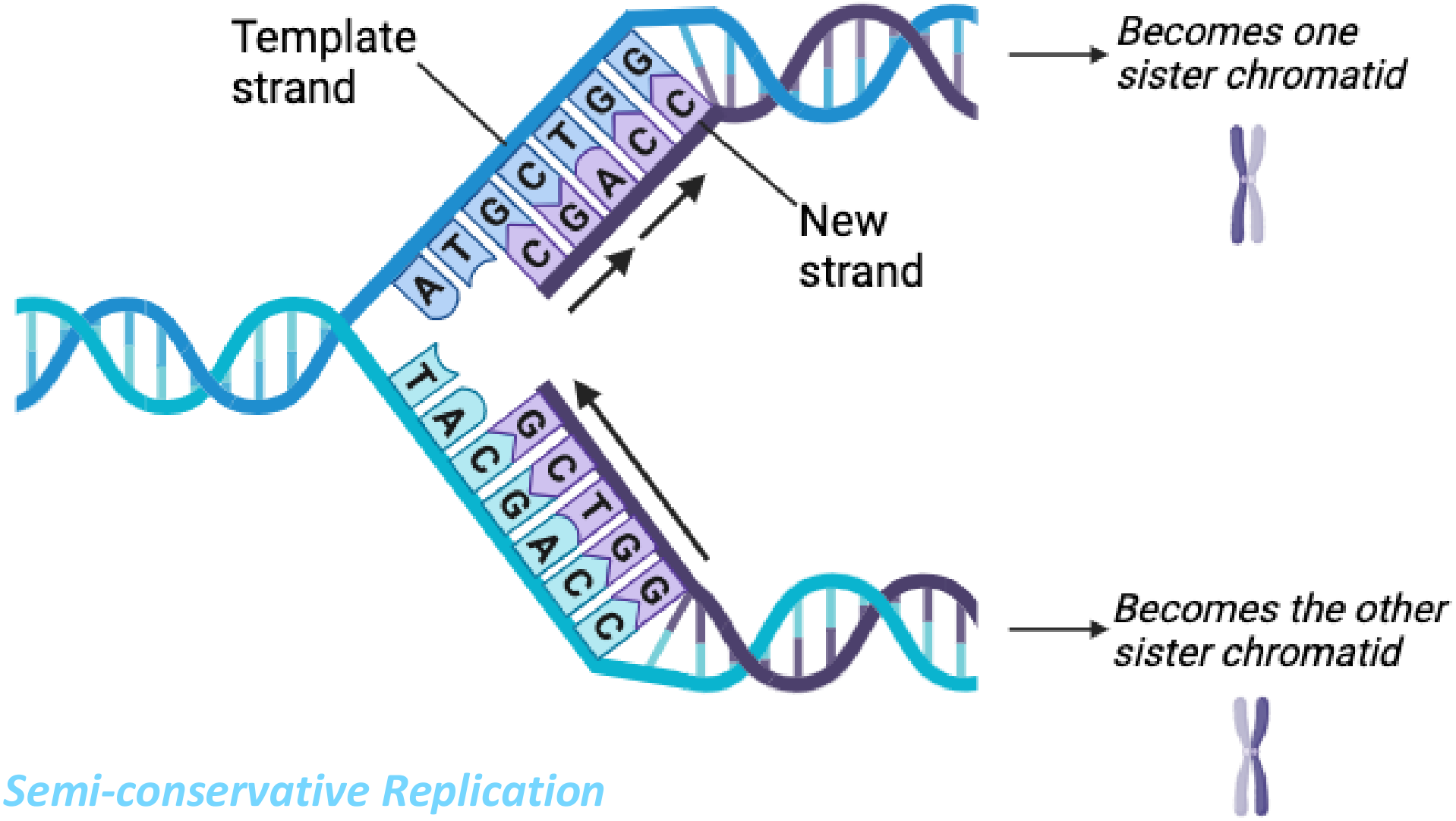
This process takes us from one starting molecule to two "daughter" molecules, with each newly formed double helix containing one new and one old strand. In a sense, that's all there is to DNA replication! But what's actually most interesting about this process is how it's carried out in a cell. Cells need to copy their DNA very quickly, and with very few errors (or risk problem such as cancer). To do so, they use a variety of enzymes and proteins, which work together to make sure DNA replication is performed smoothly and accurately.

DNA Replication Models :

DNA replication derives its name from the fact that this mechanism of transcription was one of three models originally proposed for DNA replication:

- **Semiconservative replication** would produce two copies that each contained one of the original strands of DNA and one new strand. Semiconservative replication is beneficial to DNA repair. During replication, the new strand of DNA adjusts to the modifications made on the template strand.
- **Conservative replication** would leave the two original template DNA strands together in a double helix and would produce a copy composed of two new strands containing all of the new DNA base pairs.
- **Dispersive replication** would produce two copies of the DNA, both containing distinct regions of DNA composed of either both original strands or both new strands. The strands of DNA were originally thought to be broken at every tenth base pair to add the new DNA template. Eventually, all new DNA would make up the double helix after many generations of replication.


Three postulated methods of DNA Replication
Semi-Conservative
Conservative*
Dispersive*
Newly, synthesized strand
Original template strand
* not found to be biologically significant



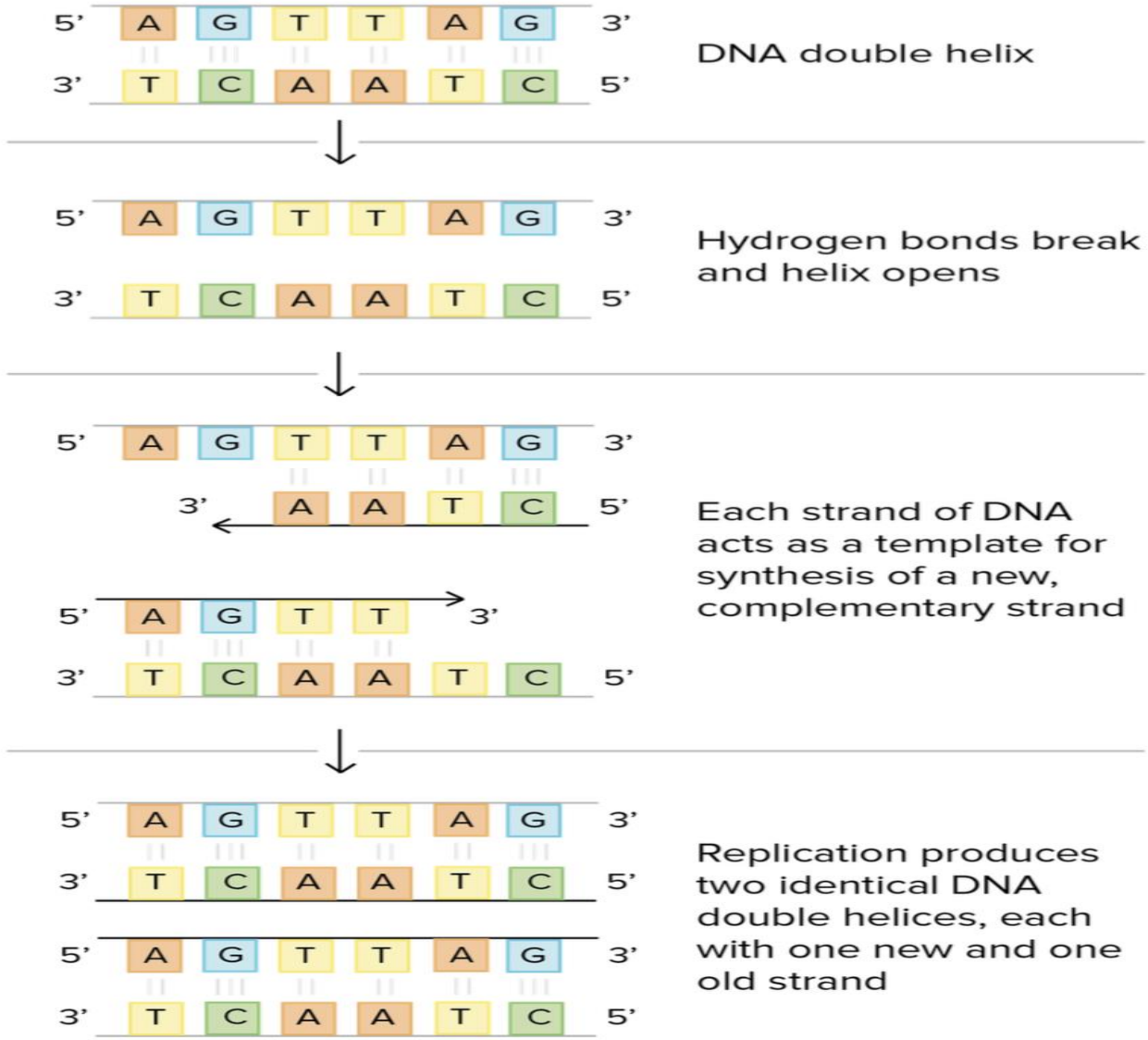
Semi-conservative Replication

DNA polymerase

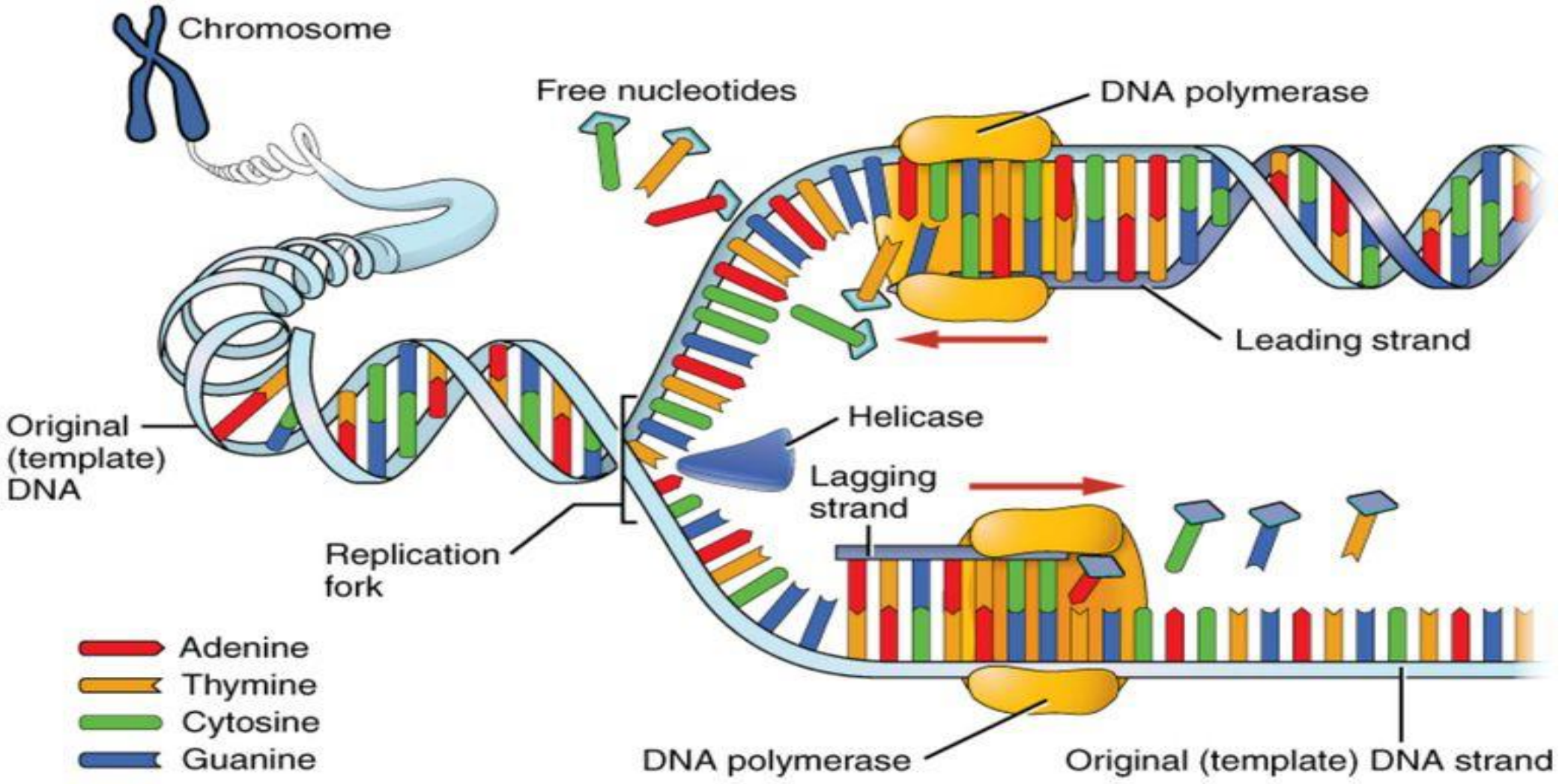
One of the key molecules in DNA replication is the enzyme DNA polymerase. DNA polymerases are responsible for synthesizing DNA: they add nucleotides one by one to the growing DNA chain, incorporating only those that are complementary to the template.

Here are some key features of DNA polymerases:

- ✓ They always need a template.
- ✓ They can only add nucleotides to the 3' end of a DNA strand.
- ✓ They can't start making a DNA chain from nothing, but require a pre-existing chain or short stretch of nucleotides called a primer.
- ✓ They proofread, or check their work, removing the vast majority of "wrong" nucleotides that are accidentally added to the chain .

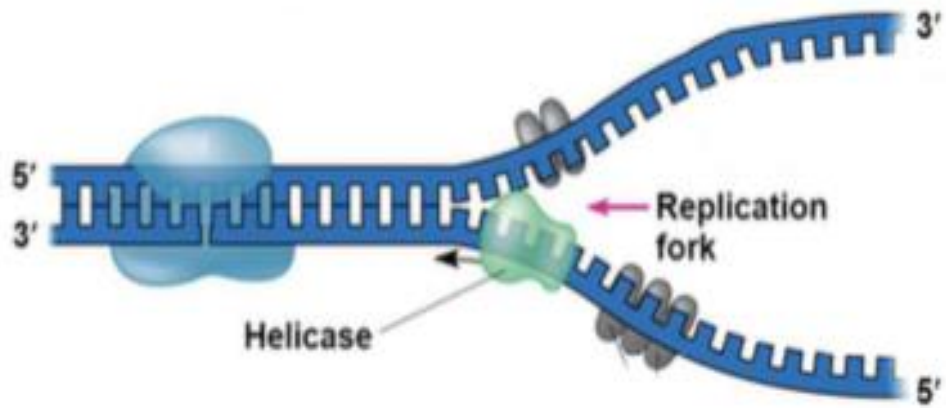

5’ A G T T A G 3’
3’ T C A A T C 5’
DNA double helix
Hydrogen bonds break and helix opens
Each strand of DNA acts as a template for synthesis of a new, complementary strand
Replication produces two identical DNA double helices, each with one new and one old strand

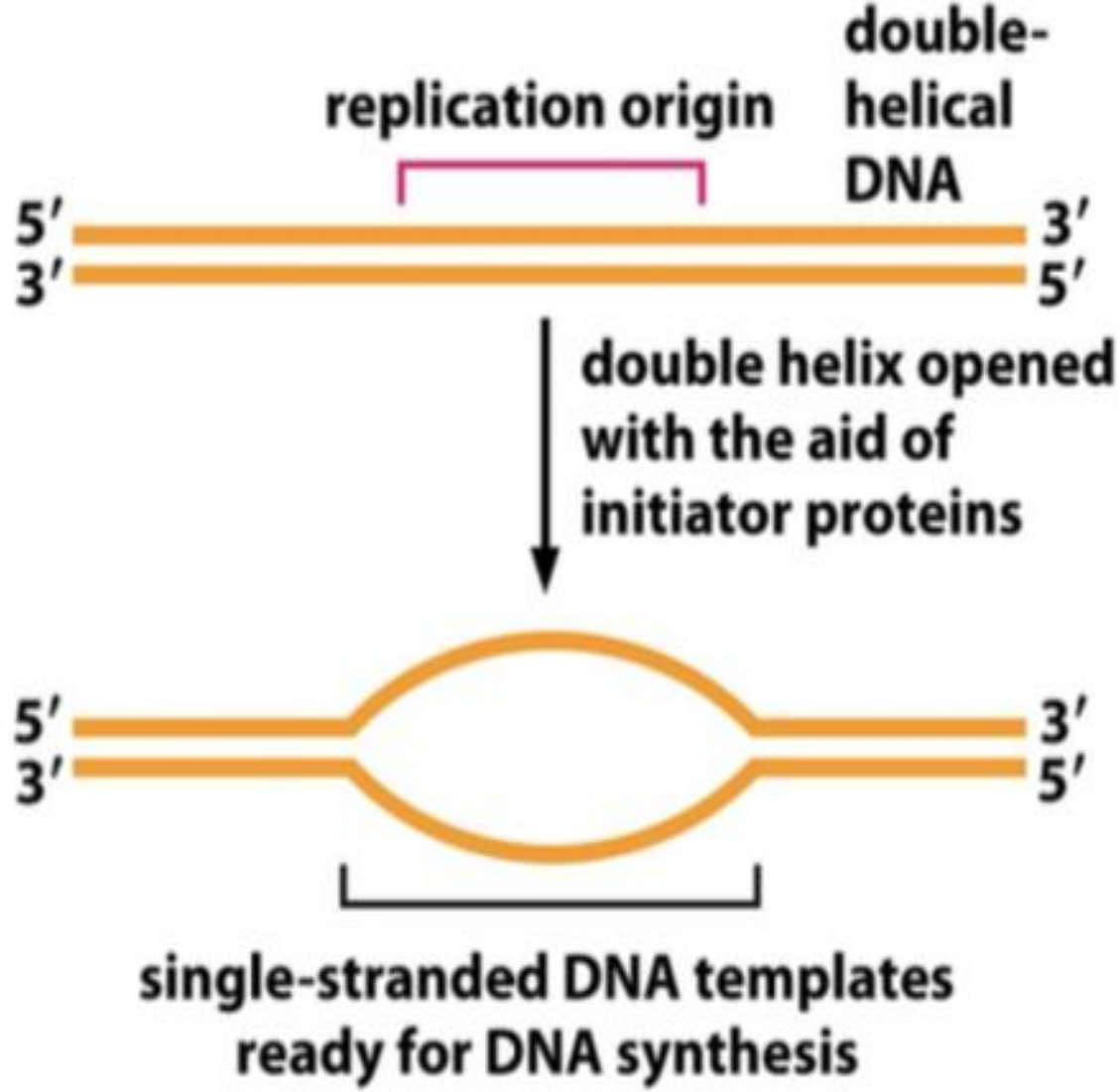
Preparation For Replication



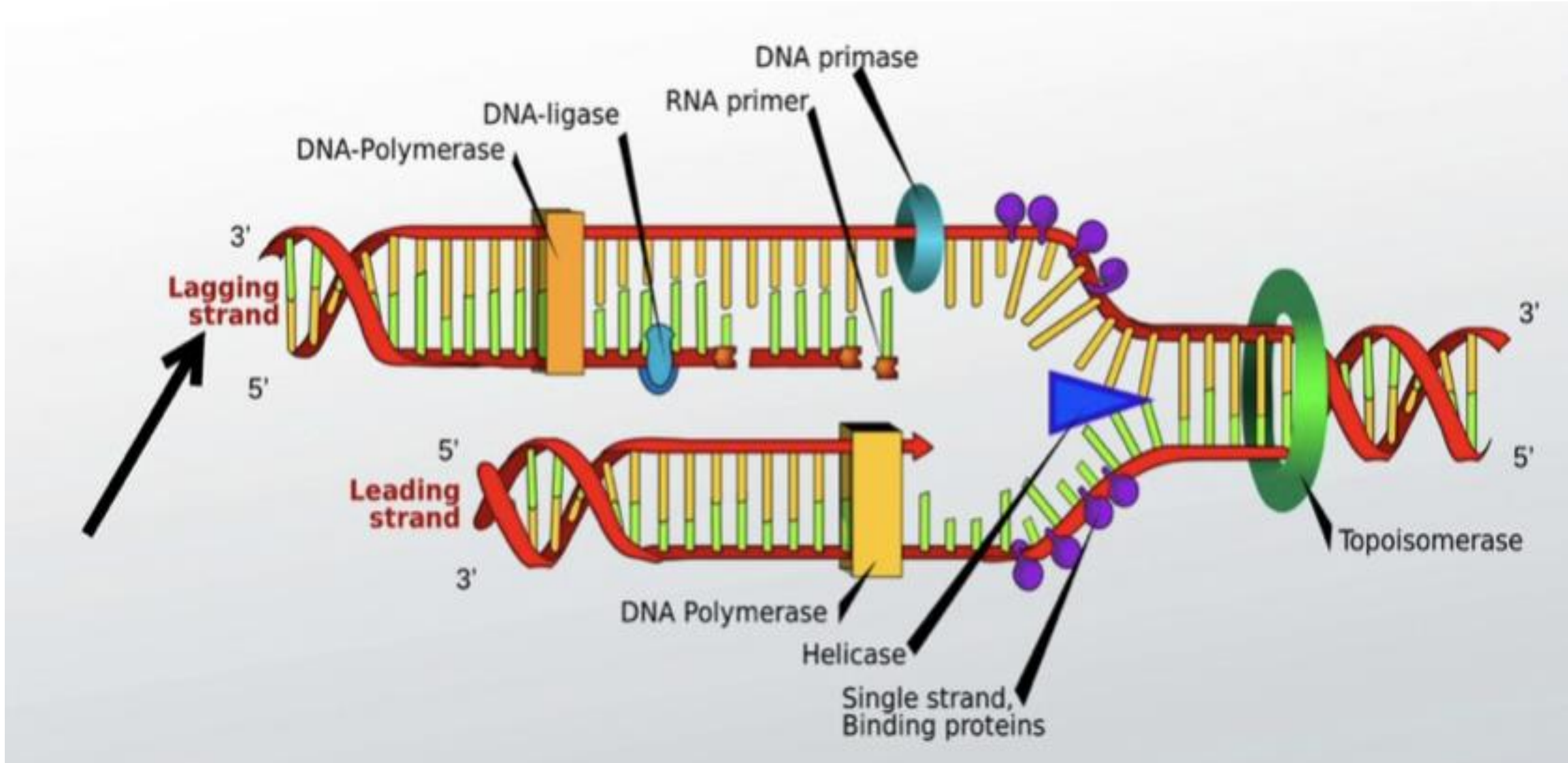
Step 1: Replication Fork Formation

Before DNA can be replicated, the double stranded molecule must be “unzipped” into two single strands. As we mentioned in the previous lecture 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. The initiation point where the splitting starts is called *“origin of replication”*


5′
3′
3′
5′
Replication fork
Helicase


replication origin
double-helical DNA
5′
3′
3′
5′
double helix opened with the aid of initiator proteins
5′
3′
3′
5′
single-stranded DNA templates ready for DNA synthesis

DNA is directional in both strands, signified by a 5' and 3' end. This notation signifies which side group is attached the DNA backbone. The **5' end** has a phosphate (P) group attached, while the **3' end** has a hydroxyl (OH) group attached. 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.


DNA primase
RNA primer
DNA-ligase
DNA-Polymerase
3'
Lagging strand
5'
5'
Leading strand
3'
3'
5'
Topoisomerase
DNA Polymerase
Helicase
Single strand,
Binding proteins

REPLICATION BEGINS

Step 2: Primer Binding

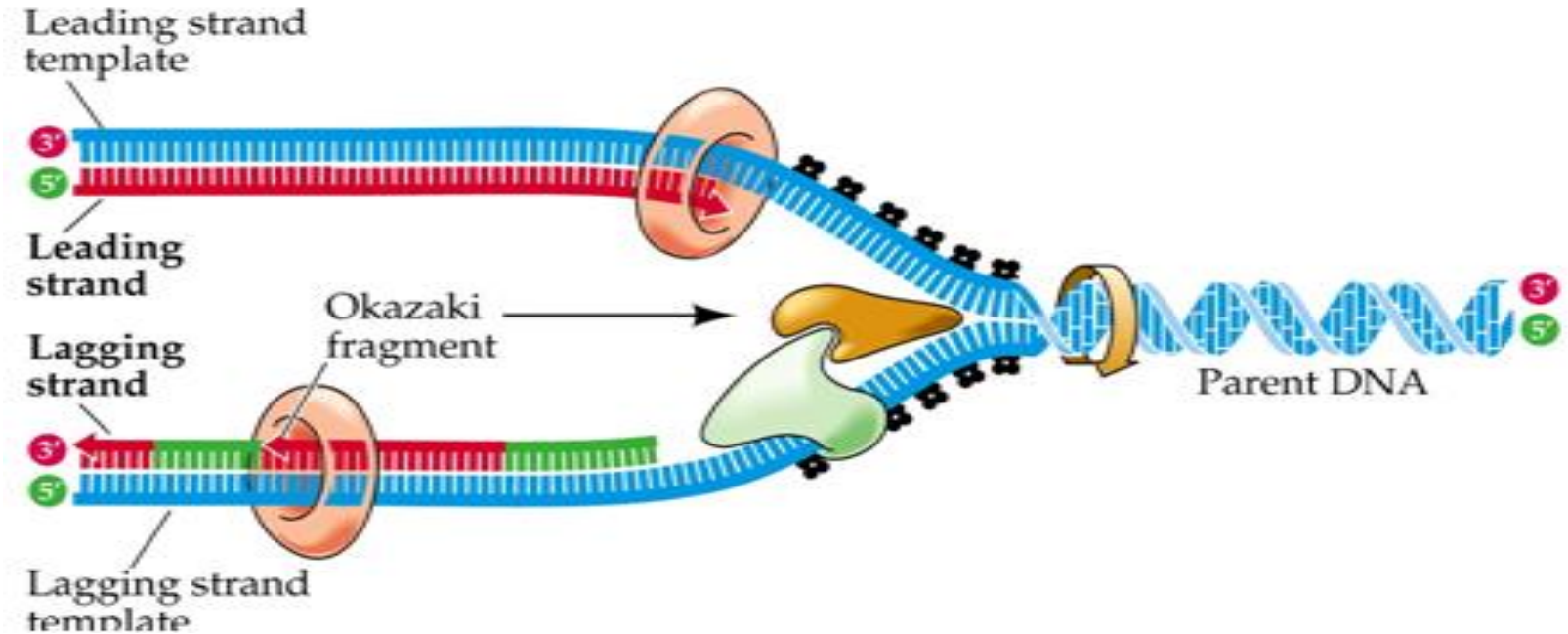
The leading strand is the simplest to replicate. Once the DNA strands have been separated, a short piece of RNA called a **primer** binds to the 3' end of the strand. The primer always binds as starting point for replication . Primers are generated by the enzyme **DNA primase**

Step 3: Elongation

The elongation process is different for the 5'-3' and 3'-5' template.

1.a) 5'-3' Template;

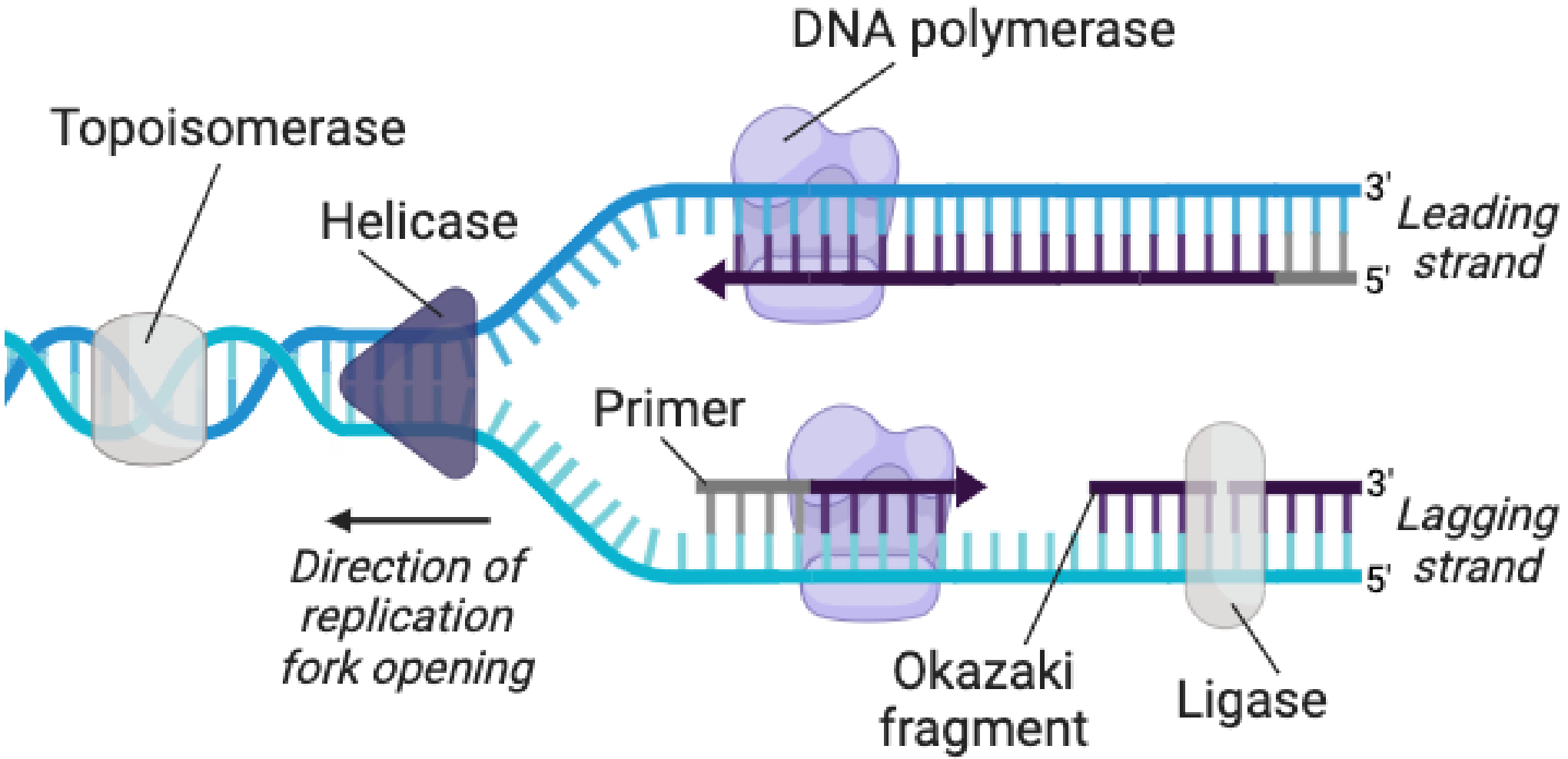
The 5'-3' proceeding daughter strand (i.e. uses a 5'-3' template) is called ***leading strand*** because DNA polymerase (ä) can read the template and continuously adds nucleotides (completed or complementary to the nucleotides of the template, for example Adenine opposite to Thymine... etc.).



1.b) 3’-5’ Template;

The 3’-5’ template cannot be read by DNA polymerase (ä). In the lagging strand the RNA Primase adds more RNA Primers. The DNA polymerase (ä) reads the template and lengthens the bursts. The gap between two RNA primers is called “Okazaki Fragments”. Named for the Japanese scientist who discovered them. The leading strand can be extended from one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments.

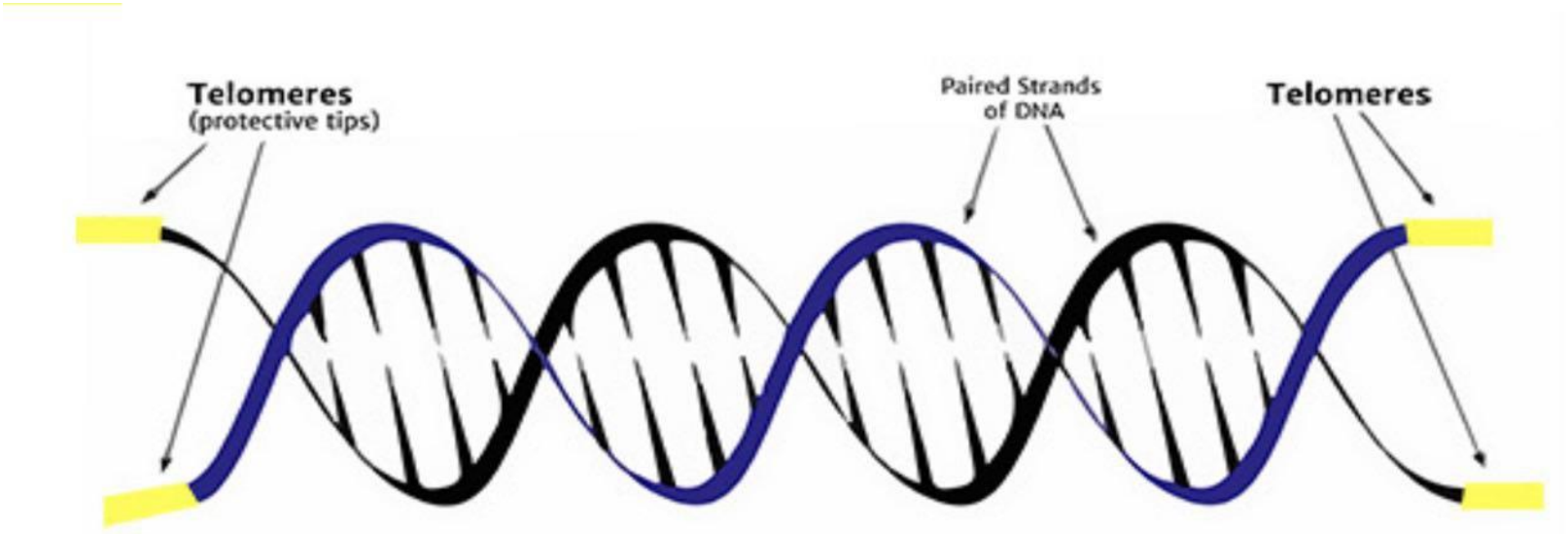
The RNA primers are necessarily for DNA polymerase (ä) to bind Nucleotides to the 3’ end of them. The daughter strand is elongated with the binding of more DNA nucleotides.


DNA polymerase
Topoisomerase
Helicase
3'
Leading strand
5'
Primer
3'
Lagging strand
5'
Direction of replication fork opening
Okazaki fragment
Ligase

Step 4: Termination

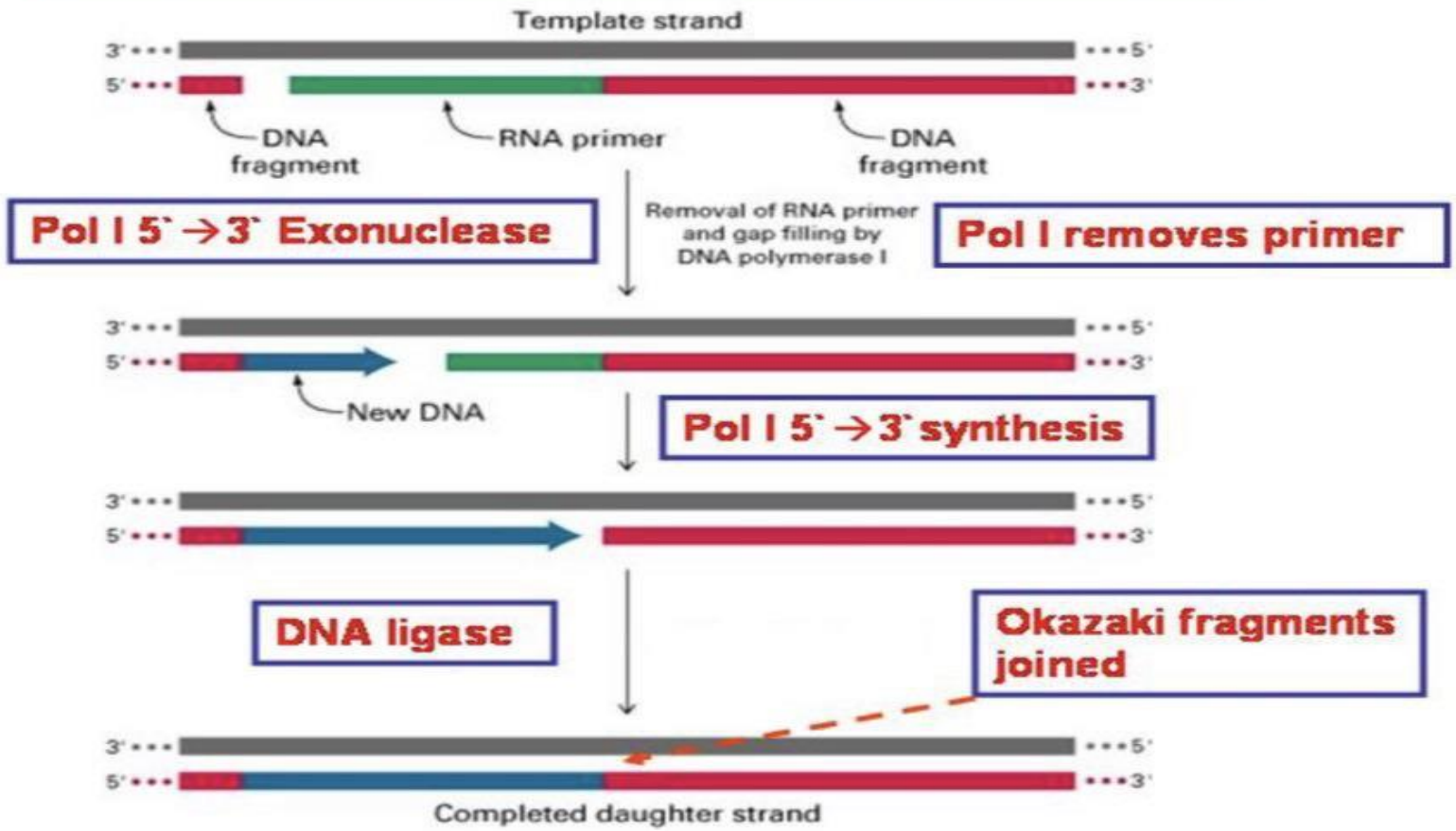
Once both the continuous and discontinuous strands are formed, an enzyme called **exonuclease** removes all RNA primers from the original strands. These primers are then replaced with appropriate bases. Another exonuclease "proofreads" the newly formed DNA to check, remove and replace any errors. Another enzyme called **DNA ligase** joins Okazaki fragments together forming a single unified strand.

The ends of the parent strands consist of repeated DNA sequences called **telomeres**.



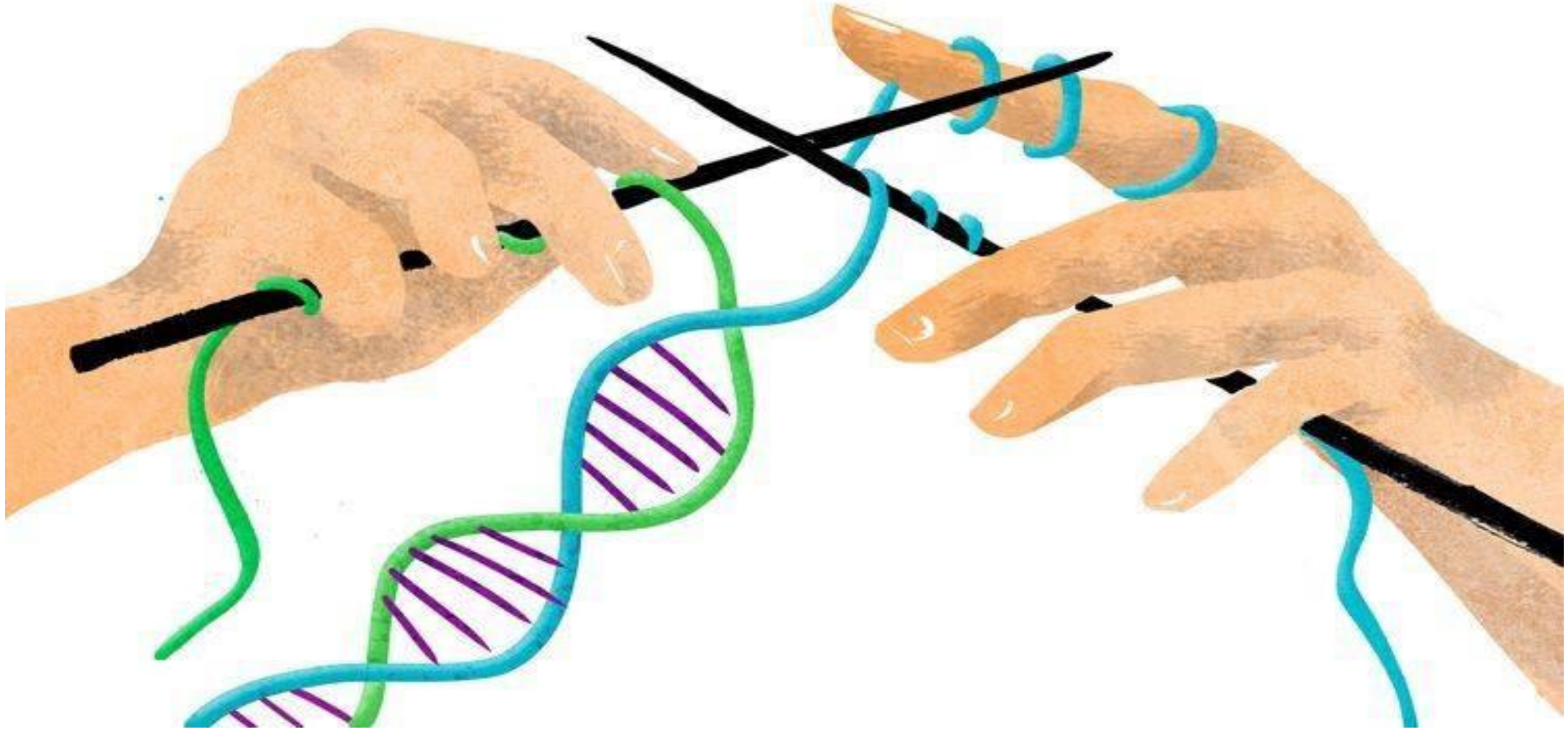
A special type of DNA polymerase enzyme called **telomerase** catalyzes the synthesis of telomere sequences at the ends of the DNA. Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing. Once completed, the parent strand and its complementary DNA strand 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.

Termination of DNA synthesis



- **DNA helicase** - unwinds and separates double stranded DNA as it moves along the DNA. It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.
- **The replication fork** is a very active area where DNA replication takes place. It is created when DNA helicase unwinds the double helix structure of the DNA.
- **DNA primase** - a type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that 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.

- **Okazaki Fragments**; are short sequences of DNA nucleotides which are synthesized discontinuously and later linked together by the enzyme DNA ligase to create the lagging strand during DNA replication.
- **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.
- **The origin of replication**- (also called the replication origin) is a particular sequence in a genome at which replication is initiated. This can either involve the replication of DNA in living organisms such as prokaryotes and eukaryotes, or that of DNA or RNA in viruses, such as double-stranded RNA viruses.



Thank you for Listening