COLLEGE OF SCIENCE
AL-MUSTAQBAL UNIVERSITY
DEPARTMENT OF BIOCHEMISTRY
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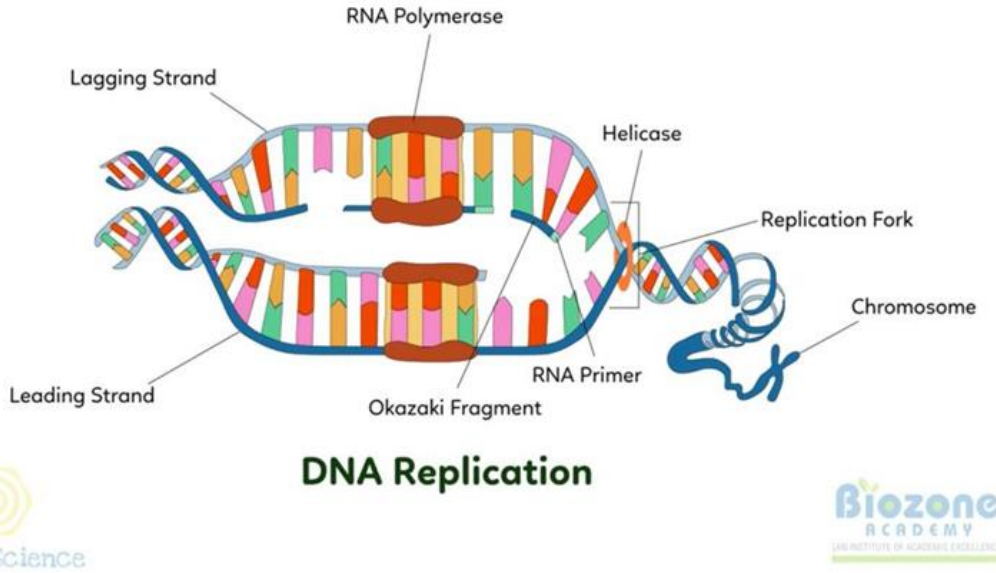


What are the Steps involved in DNA Replication?

The complete process of DNA Replication involves the following steps:

1. Recognition of initiation point

– DNA replication starts at a specific point called initiation point or origin where replication fork begins. This is a nucleotide sequence of 100 to 200 pairs of bases.

Specific initiation proteins recognize the initiation site on DNA. Such proteins along with DNA directed RNA polymerase initiate the synthesis of RNA primer for the formation of DNA chain. Prokaryotic chromosomes usually possess one initiation point or replication fork., whereas the eukaryotic chromosomes may possess several replication forks.

Nicks are produced by the endonuclease enzyme.

2. Unwinding of DNA –

When the DNA duplex molecule is cut open (nicked) to form a bubble or fork the unwinding proteins get attached at the point of nick which helps in the separation of the strands of the DNA duplex.

3. Template DNA –

The single stranded DNA on which the new DNA is synthesized is called template DNA.

4. RNA Primer –

Pre formed polynucleotide chain is necessary to start the synthesis of DNA. RNA polymerase synthesizes RNA primer on template DNA. In the absence of RNA primer the DNA replication is irregular.

5. Chain Elongation –

New DNA strand is formed due to DNA polymerase III enzyme. This enzyme adds nucleotides in 5’ to 3’ direction. This activity of DNA polymerase is called polymerizing.

6. Replication forks –

Due to opening of the DNA strand a replication fork is formed.

Okazaki fragments –Polymerizing activity of polymerase III enzyme takes place only in 5’ to 3’ direction. Thus one of the two strands of DNA having 3’ to 5’ polarity gets continuous synthesis of DNA, hence called continuous strand. The other strand having 5’ to 3’ polarity gets synthesis of DNA in small fragments called Okazaki fragments, after the name of the scientist who first discovered them.

Since the synthesis of the strand takes place in fragments, the strand is called the discontinuous strand. The synthesis of DNA on this strand is opposite to the movement of replication fork.

Thus this strand is called the lagging strand, while the continuous strand is called the leading strand.

Ligation – RNA primers are exited out once the replication is finished. The gaps formed are sealed by polynucleotide ligase enzyme. This enzyme is active in the presence of NADP, in the case of prokaryotes and ATP in the case of eukaryotes.

Replication can be unidirectional or bi directional.

7. Proof reading –

DNA replication is a very complex process. If any error is made during the replication it may lead to mutation. The DNA polymerase I and polymerase III act as proof readers of the newly formed DNA. They move along the new synthesized DNA, read mistakes formed due to improper base pairing and correct those through 3’ to 5’ exonuclease activity.

8. Removal of RNA primer and completion of DNA strand –

when the okazaki fragments are formed most of the lagging strand is duplicated. The RNA primer is removed by DNA polymerase I which synthesizes a short segment of complimentary DNA to seal the gap. Polymerase I remove the primer one nucleotide at a time and replace it with complimentary deoxyribonucleotide.

9. Joining of fragments –

At the end the fragments are joined by DNA ligate that forms a phosphodiesterase bond between 3’ OH end of the growing strand and 5’ end of the okazaki fragment.

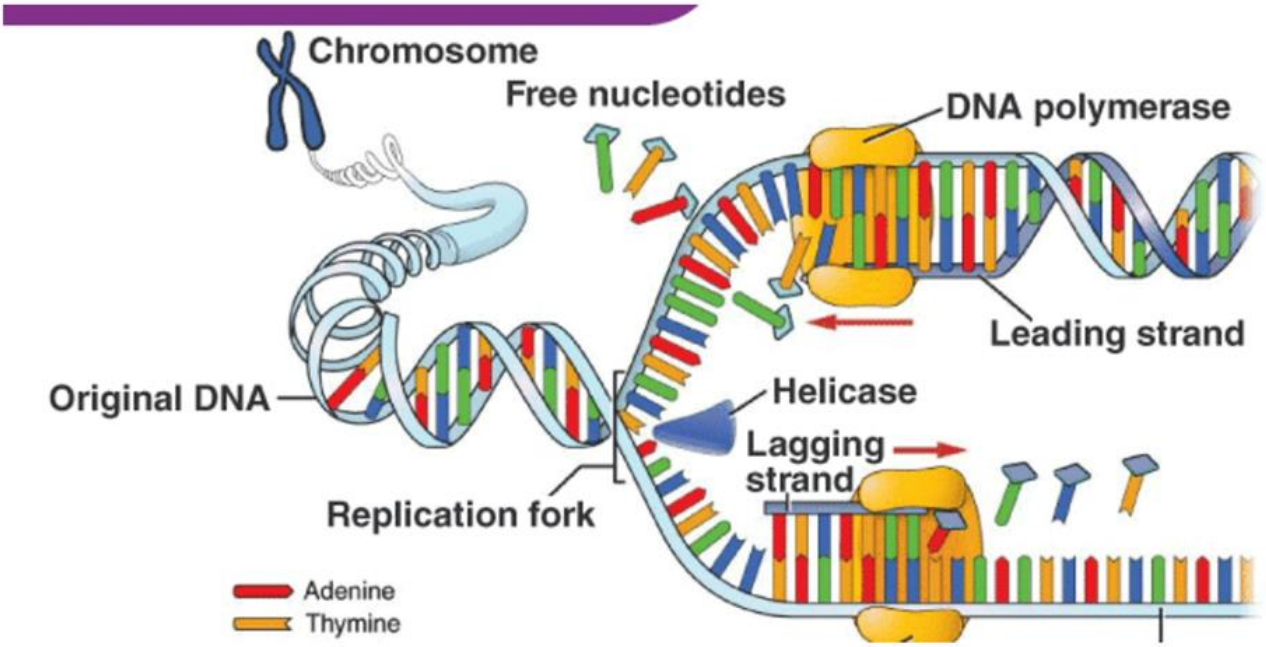


Table 12.4 Components required for replication in bacterial cells

Component	Function
Initiator protein	Binds to origin and separates strands of DNA to initiate replication
DNA helicase	Unwinds DNA at replication fork
Single-strand-binding proteins	Attach to single-stranded DNA and prevent reannealing
DNA gyrase	Moves ahead of the replication fork, making and resealing breaks in the double-helical DNA to release torque that builds up as a result of unwinding at the replication fork
DNA primase	Synthesizes short RNA primers to provide a 3′-OH group for attachment of DNA nucleotides
DNA polymerase III	Elongates a new nucleotide strand from the 3′-OH group provided by the primer
DNA polymerase I	Removes RNA primers and replaces them with DNA
DNA ligase	Joins Okazaki fragments by sealing nicks in the sugar–phosphate backbone of newly synthesized DNA

DNA replication:

DNA replication is the process of making new copies of double-stranded DNA by synthesising new

DNA strands.

DNA replication is a bidirectional process as the strands of DNA are antiparallel, i.e., 3'-5' in one strand and 5'-3' in another stand.

It duplicates the DNA inside the cell during cell division.

Due to the process of DNA replication, each daughter cell gets an equal amount of DNA.

The process of DNA replication helps in the inheritance process by transfer of the genetic material from one generation to another.

Therefore it is required for the growth, repair, and regeneration of tissues in living organisms.

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. In eukaryotic cells, such as animal cells and plant cells, DNA replication occurs in the S phase of interphase during the cell cycle. The process of DNA replication is vital for cell growth, repair, and reproduction in organisms.

Key Takeaways

Deoxyribonucleic acid, commonly known as DNA, is a nucleic acid that has three main components: a deoxyribose sugar, a phosphate, and a nitrogenous base.

Since DNA contains the genetic material for an organism, it is important that it be copied when a cell divides into daughter cells. The process that copies DNA is called replication.

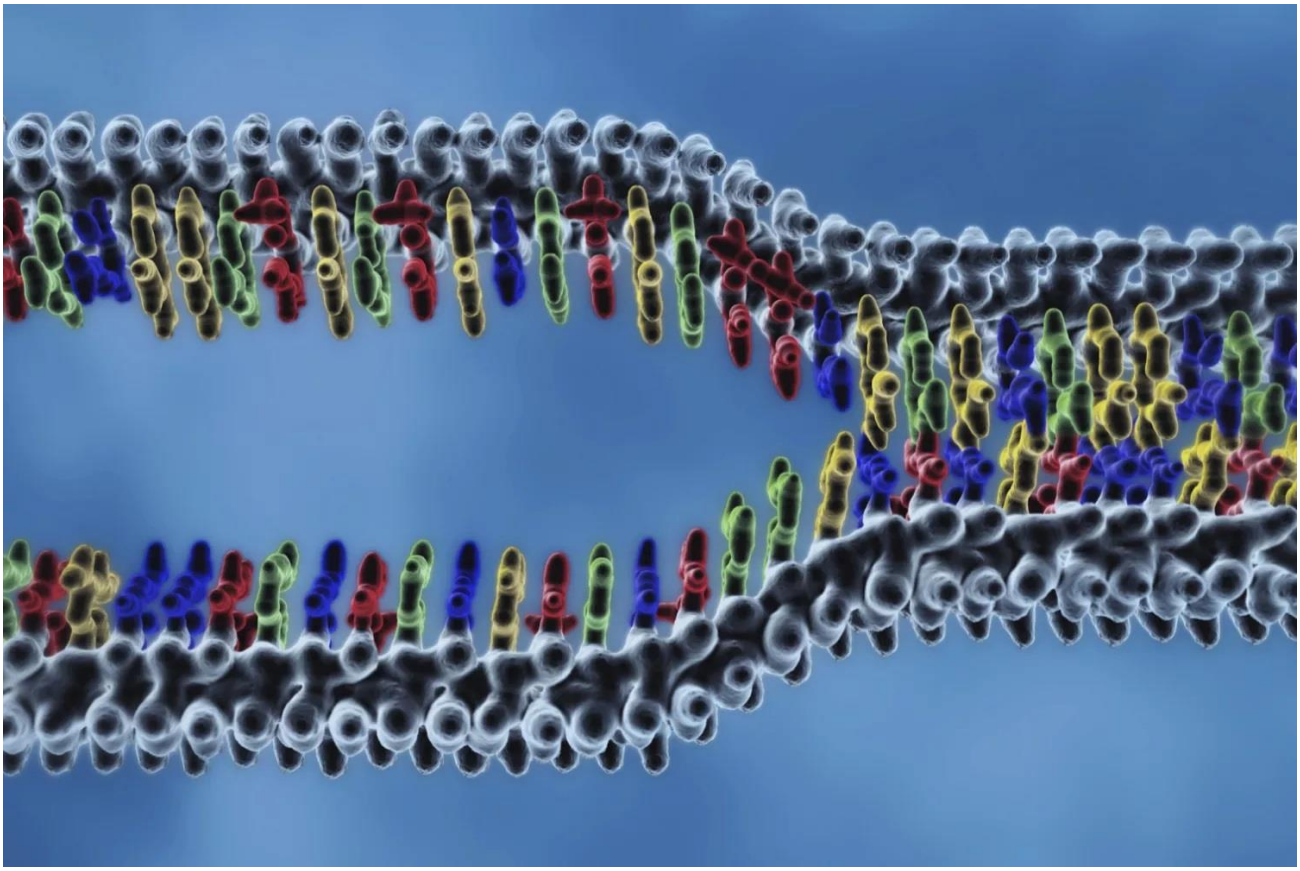
Replication involves the production of identical helices of DNA from one double-stranded molecule of DNA.

Enzymes are vital to DNA replication since they catalyze very important steps in the process.

The overall DNA replication process is extremely important for both cell growth and reproduction in organisms. It is also vital in the cell repair process.

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. Double-stranded 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.



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 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.

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 the starting point for replication. Primers are generated by the enzyme DNA primase.

DNA Replication: Elongation

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 in bacteria and human cells. In bacteria such as E. coli, polymerase III is the main replication enzyme, while polymerase I, II, IV and V are responsible for error checking and repair. DNA polymerase III binds to the strand at the site of the primer and begins adding new base pairs complementary to the strand during replication. 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 on the leading strand, 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

DNA replication would not occur without enzymes that catalyze various steps in the process. Enzymes that participate in the eukaryotic DNA replication process include:

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.

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.

Topoisomerase or DNA Gyrase - unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.

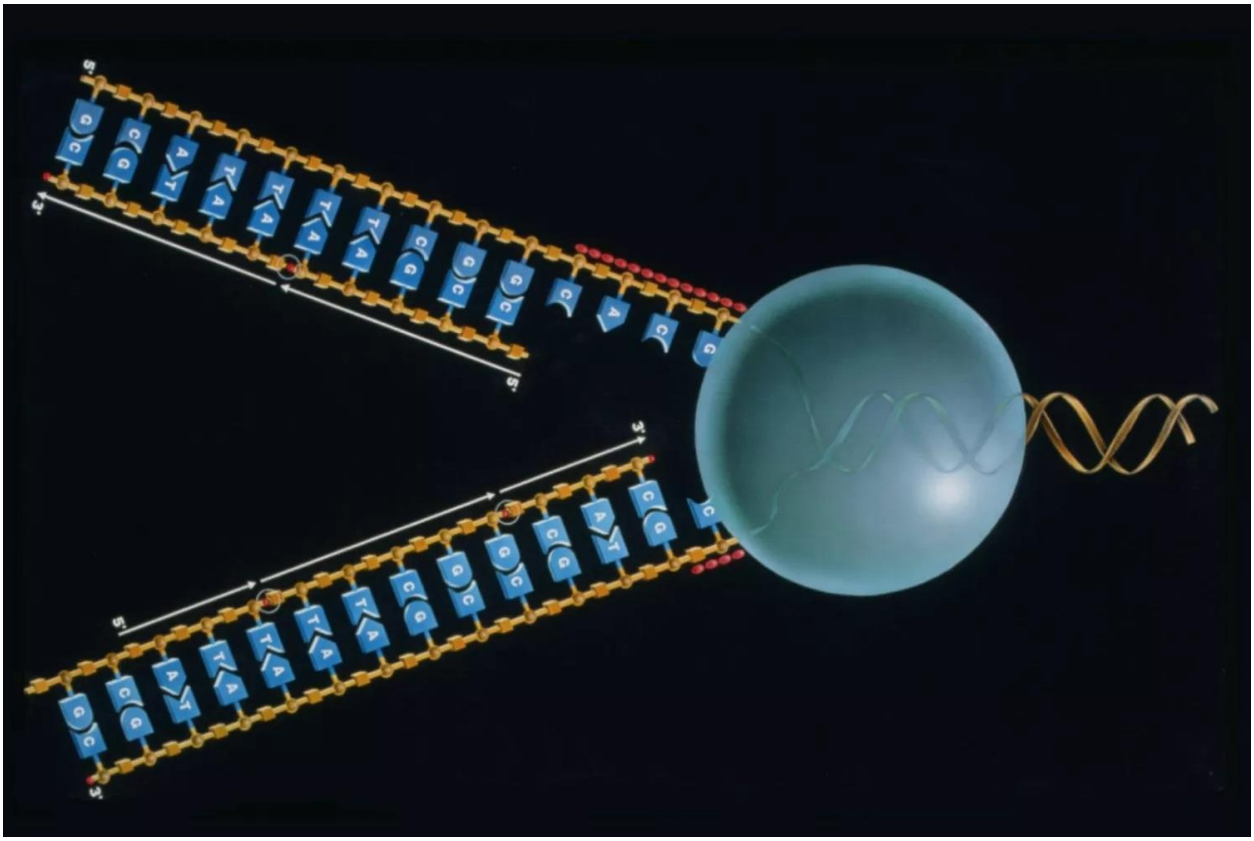
Exonucleases - group of enzymes that remove nucleotide bases from the end of a DNA chain.

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.



DNA replication exhibit polarity.

للقديم

3 ⇝ 5	5 ⇝ 3
1. replication occurs at the same direction of helicase	1. replication occurs at the opposite direction of helicase
2. continous	2. Semi-discontinous
3. RNA primer → not common	3. RNA primer → common
⊙ « Leading strand »	⊙ « Lagging strand »
4. no OKAZAKI FRAGMENTS	4. has OKAZAKI FRAGMENTS

→ OKazaki Fragments: الأجزاء المقطوعة في شريط DNA الجديد في اتجاه 5 → 3 للشريط القديم