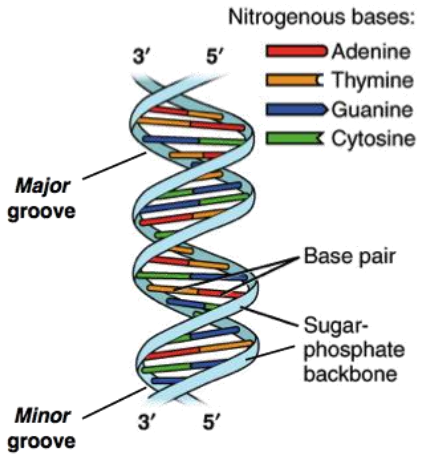
DNA:

Deoxyribonucleic acid (DNA) is a nucleic acid that is made up of three components: a deoxyribose sugar, a phosphate, and a nitrogenous base. Deoxyribonucleic acid, DNA is the genetic material via which a cell is defined. It is a long molecule containing unique codes that give instructions for the synthesis of all body proteins.

DNA structure:

- The structural model of DNA was initially proposed by James Watson and Francis Click.
- They found that DNA is a double-helical structure with two paired DNA strands with complementary nucleotide sequences.
- The double-stranded DNA molecule has two spiral nucleic acid chains that are twisted into a double helix shape. The twisting gives the DNA its compactness.
- DNA is made up of millions of nucleotides. Nucleotides are molecules that are composed of deoxyribose sugar, with a phosphate group and a nucleobase that is attached to it.
- Each nucleotide is tightly base paired with a complementary nucleotide on the opposite strand, i.e Adenine (A) paired with Thymine (T) or Guanine (G) paired with cytosine (C), and therefore one strand's sequence acts as a template for the new strand to be formed during replication.
- Nucleotides are bound to each other in strands via phosphodiester bonds forming a sugar-phosphate backbone.
- They form a bond that is between the third carbon atom on the deoxyribose sugar made up of one sugar thus it is designated as the 3′ (three prime) and the fifth carbon atom of another sugar on the next nucleotide as the 5′ (five prime).
- Any part of the sequence can be used to create or recognize its adjacent nucleotide sequence during replication.
- DNA fits within the nucleus by being closely packed into tight coils known as chromatins. The chromatins condense to form the chromosomes during cell division.
- Before DNA replication, the chromatins loosen up giving the replication machinery access to the DNA strands.



DNA Replication:

- This is a complex process that takes place during cell division, (interphase, S phase) whereby DNA makes copies (duplicates) before the cell divides through mitosis and meiosis.
- DNA replication is a semiconservative process where a parental strand (template) is used to synthesize a new complementary daughter strand using several protein elements which include enzymes and RNA molecules.
- DNA replication process uses DNA polymerase as the main enzyme for catalyzing the joining of deoxyribonucleoside 5′-triphosphates (dNTPs) forming a growing chain of DNA.
- Other proteins are also involved for initiation of the process and copying of DNA, along with proofreading capabilities to ensure the replication process takes place accurately.
- Therefore DNA replication is a process that produces identical helices of DNA from a single strand of the DNA molecule.
- DNA replication is an essential mechanism in enhancing cell growth, repair, and reproduction of an organism.

The mechanism of DNA replication:

DNA replication takes place in three major steps.

1. Opening of the double-stranded helical structure of DNA and separation of the strands
2. Priming of the template strands
3. Assembly of the newly formed DNA segments.

- During the separation of DNA, the two strands uncoil at a specific site known as the origin. With the involvement of several enzymes and proteins, they prepare (prime) the strands for duplication.
- At the end of the process, DNA polymerase enzyme starts to organize the assembly of the new DNA strands.
- These are the general steps of DNA replication for all cells but they may vary specifically, depending on the organism and cell type.
- Enzymes play a major role in DNA replication because they catalyze several important stages of the entire process.
- DNA replication is one of the most essential mechanisms of a cell's function and therefore intensive research has been done to understand its processes.
- The mechanism of DNA replication is well understood in Escherichia coli, which is also similar to that in eukaryotic cells.
- In E.coli, DNA replication is initiated at the oriClocus (oriC), to which DNAA protein binds while hydrolyzing of ATP takes place.

DNA replication enzymes and Proteins:

DNA polymerase

- DNA polymerases are enzymes used for the synthesis of DNA by adding nucleotide one by one to the growing DNA chain. The enzyme incorporates complementary amino acids to the template strand.
- DNA polymerase is found in both prokaryotic and eukaryotic cells. They both contain several different DNA polymerases responsible for different functions in DNA replication and DNA repair mechanisms.

DNA Helicase enzyme

- This is the enzyme that is involved in unwinding the double-helical structure of DNA allowing DNA replication to commence.

- It uses energy that is released during ATP hydrolysis, to break the hydrogen bond between the DNA bases and separate the strands.
- This forms two replication forks on each separated strand opening up in opposite directions.
- At each replication fork, the parental DNA strand must unwind exposing new sections of single-stranded templates.
- The helicase enzyme accurately unwinds the strands while maintaining the topography on the DNA molecule.

DNA primase enzyme
This is a type of RNA polymerase enzyme that is used to synthesize or generate RNA primers, which are short RNA molecules that act as templates for the initiation of DNA replication.

DNA ligase enzyme
This is the enzyme that joins DNA fragments together by forming phosphodiester bonds between nucleotides.
Exonuclease
These are a group of enzymes that remove nucleotide bases from the end of a DNA chain.
Topoisomerase
- This is the enzyme that solves the problem of the topological stress caused during unwinding.
- They cut one or both strands of the DNA allowing the strand to move around each other to release tension before it rejoins the ends.
- And therefore, the enzyme catalysts the reversible breakage it causes by joining the broken strands.
- Topoisomerase is also known as DNA gyrase in E. coli.

Telomerase
This is an enzyme found in eukaryotic cells that adds a specific sequence of DNA to the telomeres of chromosomes after they divide, stabilizing the chromosomes over time.

DNA Replication Steps/Stages:

Initiation:-
- This is the stage where DNA replication is initiated.
- DNA synthesis is initiated within the template strand at a specific coding region site known as origins.
- The origin sites are targeted by the initiator proteins, which recruit additional proteins that help in the replication process to form a replication complex around the DNA origin.
- There are several origin sites on which DNA replication is initiated and they are all known as replication forks.
- The formed replication complex contains the DNA helicase enzyme whose function is to unwind the double helix, exposing the two strands, which act as templates for replication.
- The mechanism of DNA helicase enzyme is by hydrolyzing the ATP that is used to form the bonds between the nucleobases, thus breaking the bond that holds the two strands.
- Additionally, during initiation DNA primase enzyme synthesizes small RNA primers that kick-start the function of DNA polymerase.
- DNA polymerase enzyme functions by growing the new DNA daughter strand.

Elongation:-

- This is the phase where the DNA polymerase grows the new DNA daughter strand by attaching to the original unzipped template strand and the initiating short RNA primer.
- The DNA polymerase is able to synthesize a new strand that matches the template, by extending the primer via the addition of free nucleotides to the 3′ end.

- One of the templates reads in the 3′ to 5′ direction, and therefore, the DNA polymerase synthesizes the new strand in the 5′ to 3′ direction, which is known as the leading strand.
- Along the template strand, DNA primase synthesizes a short RNA primer at the beginning of the template in the 5′ to 3′ direction, which initiates the DNA polymerase to continue synthesizing new nucleotides, extending the new DNA strand.
- The other template (5′ to 3′) is elongated in an antiparallel direction, by the addition of short RNA primers which are filled with other joining fragments, forming the newly formed lagging strand. These short fragments are known as the Okazaki fragments.
- The synthesis of the lagging strand is discontinuous since the newly formed strand is disjointed.
- The RNA nucleotides from the short RNA primers must be removed and replaced by DNA nucleotides, which are then joined by the DNA ligase enzyme.

Termination:-

- After the synthesis and extension of both the continuous and discontinued stands, an enzyme knows as exonuclease removes all RNA primers from the original strands.
- The primers are replaced with the right nucleotide bases.
- While removing the primers, another type of exonuclease proofread the new stands, checking, removing, and replacing any errors formed during synthesis.
- DNA ligase enzyme joins the Okazaki fragments to form a single unified strand.
- The ends of the parent strand consist of a repetition of DNA sequences known as telomeres which act as protective caps at the ends of chromosomes preventing the fusion of nearby chromosomes.
- The telomeres are synthesized by a special type of DNA polymerase enzyme known as telomerase.
- It catalyzes the telomere sequences at the end of the DNA.
- On completion, the parent and complementary strand coil into a double helical shape, producing two DNA molecules each passing one strand from the parent molecule and one new strand.

Okazaki fragments:-

- The two DNA strands run in opposite or antiparallel directions, and therefore to continuously synthesize the two new strands at the replication fork requires that one strand is synthesized in the 5’to3′ direction while the other is synthesized in the opposite direction, 3’to 5′.
- However, DNA polymerase can only catalyze the polymerization of the dNTPs only in the 5’to 3’direction.
- This means that the other opposite new strand is synthesized differently. But how?
- By the joining of discontinuous small pieces of DNA that are synthesized backward from the direction of movements of the replication fork. These small pieces or fragments of the new DNA strand are known as the Okazaki Fragments.
- The Okazaki fragments are then joined by the action of DNA ligase, which forms an intact new DNA strand known as the lagging strand.
- The lagging phase is not synthesized by the primer that initiates the synthesis of the leading strand.
- Instead, a short fragment of RNA serves as a primer (RNA primer) for the initiation of replication of the lagging strand.
- RNA primers are formed during the synthesis of RNA which is initiated de novo, and an enzyme known as primase synthesizes these short fragments of RNA, which are 3-10 nucleotides long and complementary to the lagging strand template at the replication fork.
- The Okazaki fragments are then synthesized by the extension of the RNA primers by DNA polymerase.
- However, the newly synthesized lagging strand is that it contains an RNA-DNA joint, defining the critical role of RNA in DNA replication.

Replication Fork Formation and its function:-

- The replication fork is the site of active DNA synthesis, where the DNA helix unwinds and single strands of the DNA replicates.
- Several sites of origin represent the replication forks.
- The replication fork is formed during DNA strand unwinding by the helicase enzyme which exposes the origin of replication. A short RNA primer is synthesized by primase and elongation done by DNA polymerase.
- The replication fork moves in the direction of the new strand synthesis. The new DNA strands are synthesized in two orientations, i. e 3′ to 5′ direction which is the leading strand, and the 5′ to 3′ orientation which is the lagging strand.
- The two sides of the new DNA strand (leading and lagging strand) are replicated in two opposite directions from the replication fork.
- Therefore the replication fork is bi-directional.

Leading Strand

- The leading strand is the new DNA strand that is continuously synthesized by the DNA polymerase enzyme.
- It is the simplest strand that is synthesized during replication.
- The synthesis starts after the DNA strand has unzipped and separated. This generates a short piece of RNA known as a primer, by the DNA primase enzyme.
- The primer binds to the 3′ end (start) of the strand, thus initiating the synthesis of the new strand (leading strand).
- The synthesis of the leading strand is a continuous process.

The Lagging Strand

- This is the template strand (5′ to 3′) that is synthesized in a discontinuous manner by RNA primers.
- During the synthesis of the leading strand, it exposes small, short strands, or templates that are then used for the synthesis of the Okazaki fragments.
- The Okazaki fragments synthesize the lagging strand by the activity of DNA polymerase which adds the pieces of DNA (the Okazaki fragments) to the strand between the primers.
- The formation of the lagging strand is a discontinuous process because the newly formed strand (lagging strand) is the fragmentation of short DNA strands.

DNA replication in prokaryotes:-

Initiation:-

- DNA replication begins from origin. In E coli, replication origin is called OriC which consists of 245 base pair and contains DNA sequences that are highly conserved among bacterial replication origin. Two types of conserved sequences are found at OriC, three repeats of 13 bp (GATRCTNTTNTTTT) and four/five repeats of 9 bp (TTATCCACA) called 13 mer and 9 mer respectively.
- About 20 molecules of DNA A proteins binds with 9 mer repeats along with ATP which causes DNA to wraps around DNAA protein forming initial complex. The DNA A protein and ATP trigger the opening of 13 mer repeats froming open complex.
- Two copies of DNAB proteins (helicase) binds to 13 mer repeats. This binding is facilitated by another molecule called DNAC. The DNAB-DNAC interaction causes DNAB ring to open which binds with each of the DNA strand. The hydrolysis of bound ATP release DNAC leaving the DNAB bound to the DNA strand.

- The binding of helicase is key step in replication initiation. DNA B migrates along the single stranded DNA in 5'-3' direction causing unwinding of the DNA.
- The activity of helicase causes the topological stress to the unwounded strand forming supercoiled DNA. This stress is relieved by the DNA topoisomerase (DNA gyrase) by negative supercoiling. Similarly, single stranded binding protein binds to the separated strand and prevents reannealing of separated strand and stabilize the strand.
- The DNA polymerase cannot initiate DNA replication. So, at first primase synthesize 10±1 nucleotide (RNA in nature) along the 5'-3' direction. In case of E.coli primer synthesized by primase starts with ppp-AG-nucleotide. Primer is closely associated with DNAB helicase so that it is positioned to make RNA primer as ssDNA of lagging strand.

2. Elongation:

i. Leading strand synthesis:

- Leading strand synthesis is more a straight forward process which begins with the synthesis of RNA primer by primase at replication origin.
- DNA polymerase III then adds the nucleotides at 3'end. The leading strand synthesis then proceed continuously keeping pace with unwinding of replication fork until it encounter the termination sequences.

ii. Lagging strand synthesis:

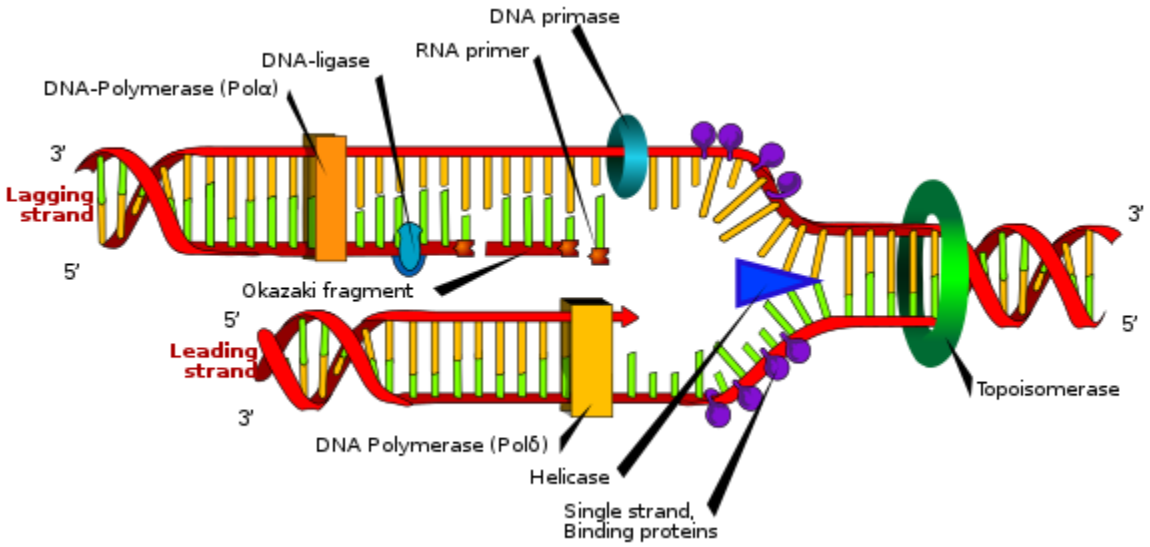
- The lagging strand synthesized in short fragments called Okazaki fragments. At first RNA primer is synthesized by primase and as in leading strand DNA polymerase III binds to RNA primer and add dNTPS.
- On this level the synthesis of each okazaki fragments seems straight forward but the reality is quite complex.

Mechanism of Lagging strand synthesis

- The complexity lies in the co-ordination of leading and lagging strand synthesis. Both the strand are synthesized by a single DNA polymerase III dimer which accomplished the looping of template DNA of lagging strand synthesizing Okazaki fragments.
- Helicase (DNAB) and primase (DNAG) constitute a functional unit within replication complex called primosome.
- DNA pol III use one set of core sub unit (Core polymerase) to synthesize leading strand and other set of core sub unit to synthesize lagging strand.
- In elongation steps, helicase in front of primase and pol III, unwind the DNA at the replication fork and travel along lagging strand template along 5'-3' direction.
- DNAG primase occasionally associated with DNAB helicase synthesizes short RNA primer. A new B-sliding clamp is then positioned at the primer by B-clamp loading complex of DNA pol III.
- When the Okazaki fragments synthesis is completed, the replication halted and the core sub unit dissociates from their sliding clamps and associates with new clamp. This initiates the synthesis of new Okazaki fragments.
- Both leading and lagging strand are synthesized coordinately and simultaneously by a complex protein moving in 5'-3' direction. In this way both leading and lagging strand can be replicated at same time by a complex protein that move in same direction.
- Every so often the lagging strands must dissociates from the replicosome and reposition itself so that replication can continue.
- Lagging strand synthesis is not completes until the RNA primer has been removed and the gap between adjacent Okazaki fragments are sealed. The RNA primer are removed by exonuclease activity (5'-3') of DNA pol-I and replaced by DNA. The gap is then sealed by DNA ligase using NAD as co-factor.

Termination:

- Eventually the two replication fork of circular E. coli chromosome meet at termination recognizing sequences (ter).
- The Ter sequence of 23 bp are arranged on the chromosome to create trap that the replication fork can enter but cannot leave. Ter sequences function as binding site for TUS protein.
- Ter-TUS complex can arrest the replication fork from only one direction. Ter-TUS complex encounter first with either of the replication fork and halt it. The other opposing replication fork halted when it collide with the first one. This seems the Ter-TUS sequences is not essential for termination but it may prevents over replication by one fork if other is delayed or halted by a damage or some obstacle.
- When either of the fork encounter Ter-TUS complex, replication halted.
- Final few hundred bases of DNA between these large protein complexes are replicated by not yet known mechanism forming two interlinked (cataneted) chromosome.
- In E. coli DNA topoisomerase IV (type II) cut the two strand of one circular DNA and segregate each of the circular DNA and finally join the strand. The DNA finally transfer to two daughter cell.



REFERENCES:

- **Diagrams from Khan`s Academy**
- **Gupta. P.K (2019), Cell and Molecular Biology, Rastogi Publications, Fifth edition.**
- **https://techbullion.com/dna-polymerase-market-register-a-revenue-cagr-of-6-4-during-the-forecast-period-2021-2031/**