DNA POLYMERASES

BY

J.PALLAVI

DNA POLYMERASES:

- DNA polymerases are a group of enzymes required for DNA synthesis. Arthur Kornberg purified and characterized DNA polymerase from E.coli for the first time. It is a single-chain polypeptide now known as DNA polymerase-I. Scientists have now found five DNA polymerases in E. coli.

DNA Polymerase Definition:

- “DNA Polymerases are a group of enzymes that catalyse the synthesis of DNA during replication.”

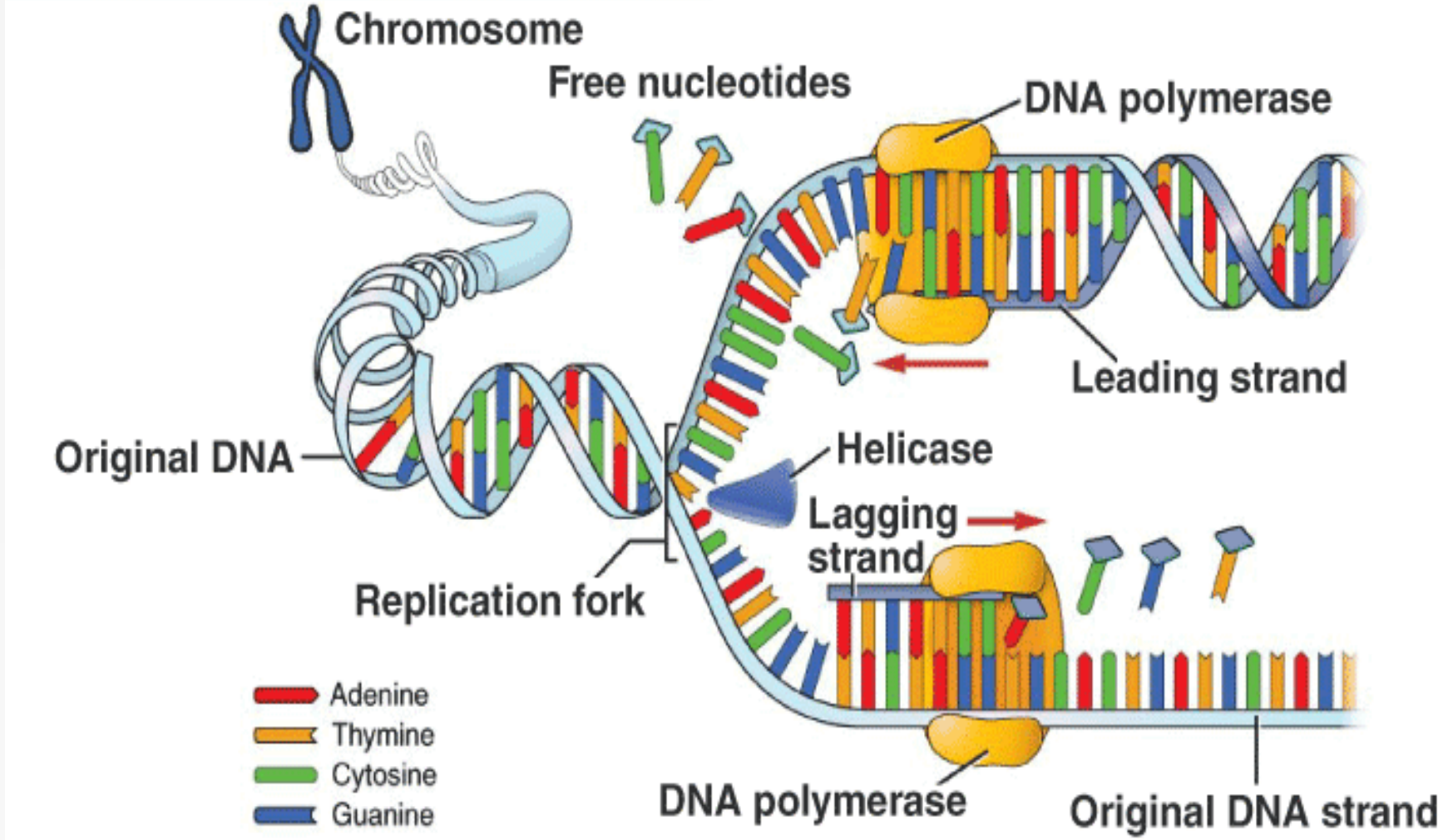
- The main function of DNA polymerases is to duplicate the DNA content of a cell during cell division. They do so by adding nucleotides at 3’-OH group of the growing DNA strand.

DNA Polymerase Function:

Replication:

- The main function of the DNA polymerase is to synthesize DNA by the process of replication.
- It is an important process to maintain and transfer genetic information from one generation to another.
- DNA polymerase works in pairs, replicating two strands of DNA. They add deoxyribonucleotides at the 3′-OH group of the growing DNA strand.
- The DNA strand grows in 5’→3’ direction by their polymerisation activity. **Adenine pairs with thymine** and **guanine pairs with cytosine**. DNA polymerases cannot initiate the replication process and they need a primer to add to the nucleotides.

DNA REPLICATION



- DNA polymerase III is the main enzyme responsible for replication in prokaryotes. In eukaryotes, DNA polymerase **δ** is the main enzyme for replication.

- DNA polymerase I removes the RNA primer by 5’→3’ exonuclease activity and replaces the primer by its polymerase activity in the lagging strand.

Repair:

- The replication process is a humongous task and it is important to maintain the integrity of the genome. Apart from replication errors, DNA repair is the continuous process to rectify any errors in the genome due to DNA damage. There are various mechanisms by which DNA is repaired.

Proofreading:

- DNA replication is not perfect and there occurs an error after every 104 to 105 nucleotides added. Removing the incorrect nucleotide sequence or mismatched nucleotides from the newly synthesised strand is very important for the functionality of proteins, which can even lead to cancer. DNA polymerases remove incorrect pairs by exonuclease activity. They move one step back and remove the mismatched pair by 3’→5’ exonuclease activity. This is known as proofreading.

- DNA polymerases are also involved in the post-replication DNA repair processes and also in translesion synthesis by which DNA polymerase copies unrepaired part of the DNA blocking the progression of replication.

- Let’s learn in detail about different types of DNA polymerases and their functions.

- DNA Polymerase Structure and Types
- The structure of most of the DNA polymerases resembles a hand, which is holding active sites. The active site of the enzyme has two parts. At the insertion site, nucleotides are added. After adding, the newly formed base-pair migrates to the post-insertion site.

- Prokaryotic DNA Polymerase Types and Function
- There are five DNA polymerases identified in E.coli. All the DNA polymerases differ in structure, functions and rate of polymerization and processivity.

- DNA Polymerase I is coded by polA gene. It is a single polypeptide and has a role in recombination and repair. It has both 5’→3’ and 3’→5’ exonuclease activity. It removes the RNA primer from lagging strand by 5’→3’ exonuclease activity and also fills the gap.

- DNA Polymerase II is coded by polB gene. It is made up of 7 subunits. Its main role is in repair and also a backup of DNA polymerase III. It has 3’→5’ exonuclease activity.

- DNA Polymerase III is the main enzyme for replication in E.coli. It is coded by polC gene. The polymerization and processivity rate is maximum in DNA polymerase III. It also has proofreading 3’→5’ exonuclease activity.

- DNA polymerase III of E.coli is made up of a total of 13 subunits, which comprises 9 different types of subunits.

- It consists of two core domains made up of α, ϵ, and θ subunits. It is attached to the γ complex or clamp-loading complex, which is made up of five subunits, $\tau 2\gamma\delta\delta$’. Additional subunits χ and ψ are attached to the clamp-loading complex. β subunits make two clamps with a dimer each. They increase the processivity of the DNA polymerase III.

- DNA Polymerase IV is coded by dinB gene. Its main role is in DNA repair during SOS response, when DNA replication is stalled at the replication fork. DNA polymerase II, IV and V are translesion polymerases.

- DNA Polymerase V is also involved in translesion synthesis during SOS response and DNA repair. It is made up of UmuC monomer and UmuD dimer.

- Eukaryotic DNA Polymerase Types and Function
- Like prokaryotic cells, eukaryotic cells also have many DNA polymerases, which perform different functions, e.g. mitochondrial DNA replication, nuclear DNA replication, etc. The nuclear DNA replication is mainly done by DNA polymerase delta and alpha. There are at least 15 DNA polymerases identified in human beings.
- DNA polymerase delta – It is the main enzyme for replication in eukaryotes. It also has 3’→5’ exonuclease activity for proofreading.
- DNA polymerase alpha – The main function of DNA polymerase α is to synthesize primers. The smaller subunit has a primase activity. The largest subunit has polymerization activity. It forms a primer for Okazaki fragments, which is then extended by DNA polymerase delta.
- DNA polymerase epsilon – The main function is DNA repair. It removes primers for Okazaki fragments from the lagging strand.
- DNA polymerase gama – It is the main replicative enzyme for mitochondrial DNA.

- After adding a nucleotide, the DNA polymerase can either dissociate or move along to add more nucleotides. It depends on the processivity of DNA polymerase and it differs in different DNA polymerases.
- Replication is a highly accurate process and even the change in a single nucleotide can cause mutation. To avoid this there are two mechanisms by which DNA polymerases ensure that there are no discrepancies.
- The geometry of the active sites allows only the correct nucleotide base pairs to fit. But this is not sufficient and it is seen that it can add an incorrect nucleotide after correctly adding 104 to 105 nucleotides.
- To correct this type of errors, DNA polymerase has 3’→5’ exonuclease activity. DNA polymerase checks each of the added nucleotides and removes the nucleotide if there is a mismatch. This is known as proofreading. In DNA polymerase I, there are different active sites for polymerizing and proofreading functions.

- How does DNA Polymerase work?
- The reaction is phosphoryl group transfer. The 3’-OH group of the growing strand acts as a nucleophile and attacks the incoming deoxyribonucleoside triphosphate at the alpha-phosphorus, leading to phosphodiester bond formation. Inorganic phosphate is released in the reaction.

- $(dNMP)n + dNTP \rightarrow (dNMP)n+1 + PPi$

- All the DNA polymerases require two Mg ions at the active site. It is important to note that DNA polymerase can only add nucleotides at the 3′ end of the growing strand, that is why replication always occurs in the 5’→3’ direction. They cannot initiate the formation of new DNA.

- They need a template strand, which guides the polymerisation reaction. They also need a primer for their action as they can only add nucleotides at 3’ OH group. The primer can be a short segment of RNA, DNA or both. Generally, the primer is an RNA oligonucleotide in the living system.

DNA LIGASES:

- DNA ligases indicate the basic class of enzymes necessary for all entities to sustain structural integrity of the genome.
- This enzyme connects two strands of DNA together as a result of association between phosphate group of one strand and deoxyribose group on the other strand.
- The DNA ligase is functional in joining the Okazaki fragments that take shape on the lagging strand while the DNA replicates.
- The DNA ligase is able to join two of the DNA fragments as a result of formation of a phosphodiester bond between them with the help of a molecule of energy.
- Cellular phenomena of recombination, DNA replication and repair generate breaks in the phosphate backbone DNA structure.
- It compromises genome’s stability posing a threat to the loss of genetic content in addition to the introduction of the deleterious chromosomal mutations.

- The catalytic activity of DNA ligases eventually repairs DNA breaks by forming phosphodiester bonds between adjacent nucleotides in duplex DNA.
- The DNA ligase activity necessitates a nucleotide cofactor following a unique 3-step reaction mechanism involving covalent modification.
- The process includes covalent alteration of the DNA substrate and ligase enzyme.
- The DNA ligase can either be used for the introduction of genes of interest into the plasmid vectors or for the creation of fusion genes by uniting one gene into the other. This phenomenon is referred to as ligation.
- Ligation can be carried out on lengths of DNA having sticky or blunt ends following restriction digests.
- The DNA fragments in the blunt end ligation are directly joined by the DNA ligase.

DNA Ligase – Function:

- The importance of DNA ligases to maintain genomic integrity is immense.
- It does so by joining the breaks in the DNA’s phosphodiester backbone occurring while recombination and replication takes place in addition to the result of DNA damage and its repairing.
- The primary role of DNA ligase is to ligate two strands of DNA, which could be single or double strands despite the fact that ligases are differently used for various purposes in vitro and vivo processes.

Role of DNA ligase in replication:

- 4 different daughter single-stranded DNA molecules are produced in the process of replication from a single DNA duplex.
- To bring about complete replication, various enzymes perform a range of activities. Replication of DNA is initiated with the introduction of the RNA primer via primase enzyme.
- Primer’s 3′ end is used as the initial point to add nucleotides by the DNA polymerase at the leading strand.
- The process terminates at the lagging strand through the synthesis of the Okazaki fragments.
- When the process is about to complete, the primer is extracted out and loaded with nucleotides in the gaps between the Okazaki fragments by the DNA polymerase. However, the strands so produced are yet to be joined.

- The role of DNA ligase here is to fill gaps by producing phosphodiester links between the gaps once the primer between the Okazaki fragments is removed.
- Though ligation 5′ end of a strand and 3′ end of another end are joined through the elimination of the pyrophosphate from the triphosphate.
- On the contrary, the DNA replication uses ligation, the same is not applicable on blunt ended ds DNA or double stranded DNA.

Role of ligases in recomibination DNA technology:

- DNA ligases I,II, or IV are used in cloning experiments.
- Two types of DNA ends are generated by restriction digestion, they are blunt or sticky ends.
- For various molecular biological techniques, different ends are generated.

LIGASE TYPES:

- DNA ligase I - It ligates the nascent DNA on the lagging strand.
- DNA ligase II- These are not deemed to be trie ligases as it does not possess its own gene.
- DNA ligase III: Participates in the DNA repair, in nucleotide exclusionrepair.
- DNA ligase IV:It joins ds DNA and does take part in repair mechanism.