Prokaryotic DNA Polymerase (3)

In eubacteria such as E. coli, there are five different DNA dependent DNA polymerases inscribed by roman numerals I to V. However, collectively prokaryotes may have many more types of polymerases which we have not discussed here.

DNA polymerase I: Pol I also known as the **Kornberg enzyme**, was the first DNA polymerase identified in E. coli. Arthur Kornberg's discovery of this enzyme earned him a Nobel Prize in 1959. This enzyme is a monomeric protein consisting of 928 amino acid residues. It possesses three main enzymatic activities: 5'→3' polymerase activity, 5'→3' exonuclease activity, and 3'→5' exonuclease activity. The 5'→3' exonuclease activity is distinct from the other two activities. Proteases such as subtilisin or trypsin can split DNA polymerase I into two fragments: a larger C-terminal or Klenow fragment, containing both polymerase and 3'→5' exonuclease activities, and a smaller N-terminal fragment, which carries the 5'→3' exonuclease activity. The 3'→5' exonuclease activity functions in proofreading DNA. DNA polymerase I has low processivity, meaning it adds about 200 nucleotides per DNA-binding event, and a slow polymerization rate of approximately 20 nucleotides per second. Its functions include primer removal (using the 5'→3' exonuclease activity), gap filling (using the 5'→3' polymerase activity), and DNA repair.

DNA Polymerase II: DNA polymerase II (Pol II) is a member of the B family of DNA polymerases, and is made up of 783 amino acids and 7 subunits. DNA polymerase II was discovered by Thomas Kornberg (the son of Arthur Kornberg) and Malcolm E. Gefter in 1970 while further elucidating the role of Pol I in E. coli DNA replication. This enzyme serves primarily as a backup enzyme in DNA replication. It possesses 5′→3′ DNA synthesis capability and 3′→5′ exonuclease proofreading activity. Pol II interacts with various binding partners

shared with DNA polymerase III to enhance its fidelity and processivity. Unlike many other polymerases, Pol II functions as a monomer, with distinct sections colloquially referred to as the palm, fingers, and thumb, which envelop a DNA strand. The palm contains catalytic residues coordinating with metal ions necessary for its function. Pol II is present in higher quantities in the cell compared to Pol III, around 30-50 copies. It plays a role in DNA replication, especially when Pol III encounters difficulties or becomes inactive. Pol II is proficient in repairing inter-strand cross-links, which are caused by damaging chemicals like nitrogen mustard and psoralen, making it essential for maintaining genomic integrity. Although it may not be as fast as Pol III, Pol II's abilities, including proofreading and processing mismatches, contribute to its effectiveness in DNA repair and replication.

DNA Polymerase III: DNA polymerase III is the main enzyme responsible for DNA replication, forming a complex consisting of 10 different polypeptides (α, ε, θ, τ, γ, χ, ψ, β, δ, δ'). It is characterized by a remarkably high polymerization rate, about 1000 nucleotides per second, and high processivity. Processivity refers to the average number of nucleotides added before the polymerase dissociates from the template, with higher processivity enhancing the rate of DNA synthesis. During replication at the replication fork, DNA pol III exists as a holoenzyme complex. This "holoenzyme" comprises two copies of a catalytic core (each containing α, ε, and θ subunits), two copies of a dimerization component (τ), two copies of a processivity component (each a homodimer of b-subunit forming a ring-shaped structure and acting as a sliding clamp), and one copy of a clamp loader (a five-subunit subassembly – γ, δ, χ, ψ, δ collectively called the **γ-complex**). Each catalytic core consists of three subunits: α (with 5'→3' polymerase activity), ε (with 3'→5' exonuclease activity for proofreading), and θ (which enhances the proofreading activity of ε). In the DNA pol III holoenzyme, two catalytic cores are linked by a clamp loader (γ-complex) to form DNA polymerase III. Each catalytic core interacts with one β-clamp (or sliding clamp), sliding along the DNA molecule to increase processivity. The loading of the β-clamp onto the DNA is facilitated by the g-complex (clamp loader). The dimerization component (τ) ensures that the two catalytic cores at the replication fork are linked together, forming an asymmetrical dimer.

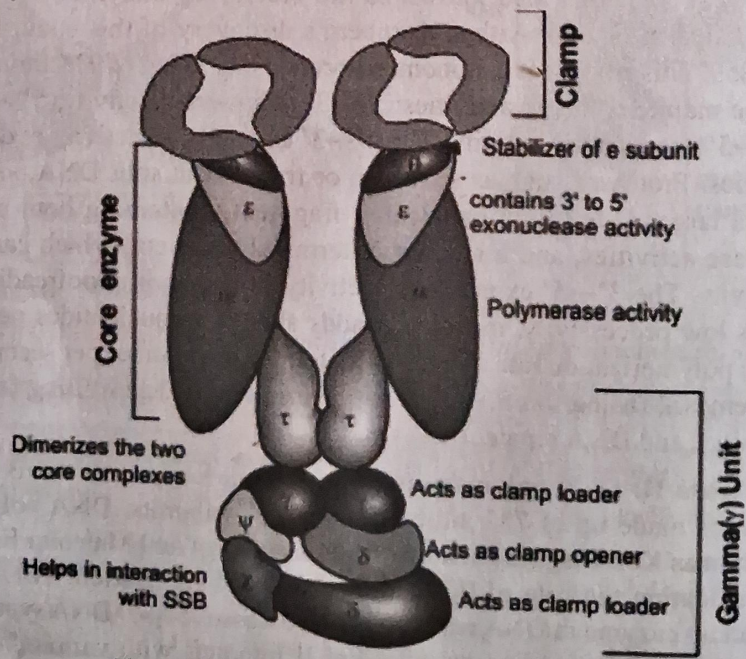

Fig. 2.4. *Structure of DNA polymerase III of E coli.*

In addition to the three key enzymes, E coli also has two more DNA polymerase IV and V, which are specialized enzymes involved in DNA repair and translesion synthesis. They can polymerize DNA in the 3' to 5' direction but lack 5' to 3' exonuclease activity, meaning they cannot proofread during DNA synthesis. These polymerases are activated by the SOS response and play crucial roles in maintaining genomic integrity. DNA polymerase IV primarily functions to stall DNA polymerase III at the replication fork, allowing time for DNA repair processes to occur. Additionally, both DNA polymerases IV and V are involved in DNA translesion synthesis, where they copy unrepaired or mutated parental sequences, facilitating the synthesis of daughter strands in damaged DNA regions.