Bharathidasan University

Tiruchirappalli – 620024

Tamil Nadu, India

Programme: M.Sc., Biochemistry

Course Title: Molecular Biology

Course Code:BC202CR

Unit 2

Enzymes in Replication

Dr.S.Maneemegalai

Associate Professor

Enzymes in DNA replication

- Replication in E.coli requires 20 or more different enzymes and proteins.
- This entire complex has been termed as **DNA replicase system or Replisome**

- **Helicase**: DNA strands are separated by the helicase enzyme. This enzyme move along the DNA and separate the strands using chemical energy from ATP.

- **Topoisomerase:** These enzymes can produce a variety of topological changes in DNA. It produces negative superhelicity and the removal of superhelicity.
- In replication strand separation creates topological stress in the helical DNA structure which are relieved by the action of topoisomerases.

- DNA binding proteins: The separated strands during replication are stabilized by DNA binding proteins.

- Primase: In DNA replication, before the synthesis of new DNA strand, a short segment of RNA known as primer must be present. This primer is synthesized by the enzyme primase.

- DNA polymerase I: The RNA primers in the new DNA strand is removed and replaced by DNA.This is accomplished by DNA polymerase I. This enzymes functions are enhanced by its 5’ →3’exonuclease activity.

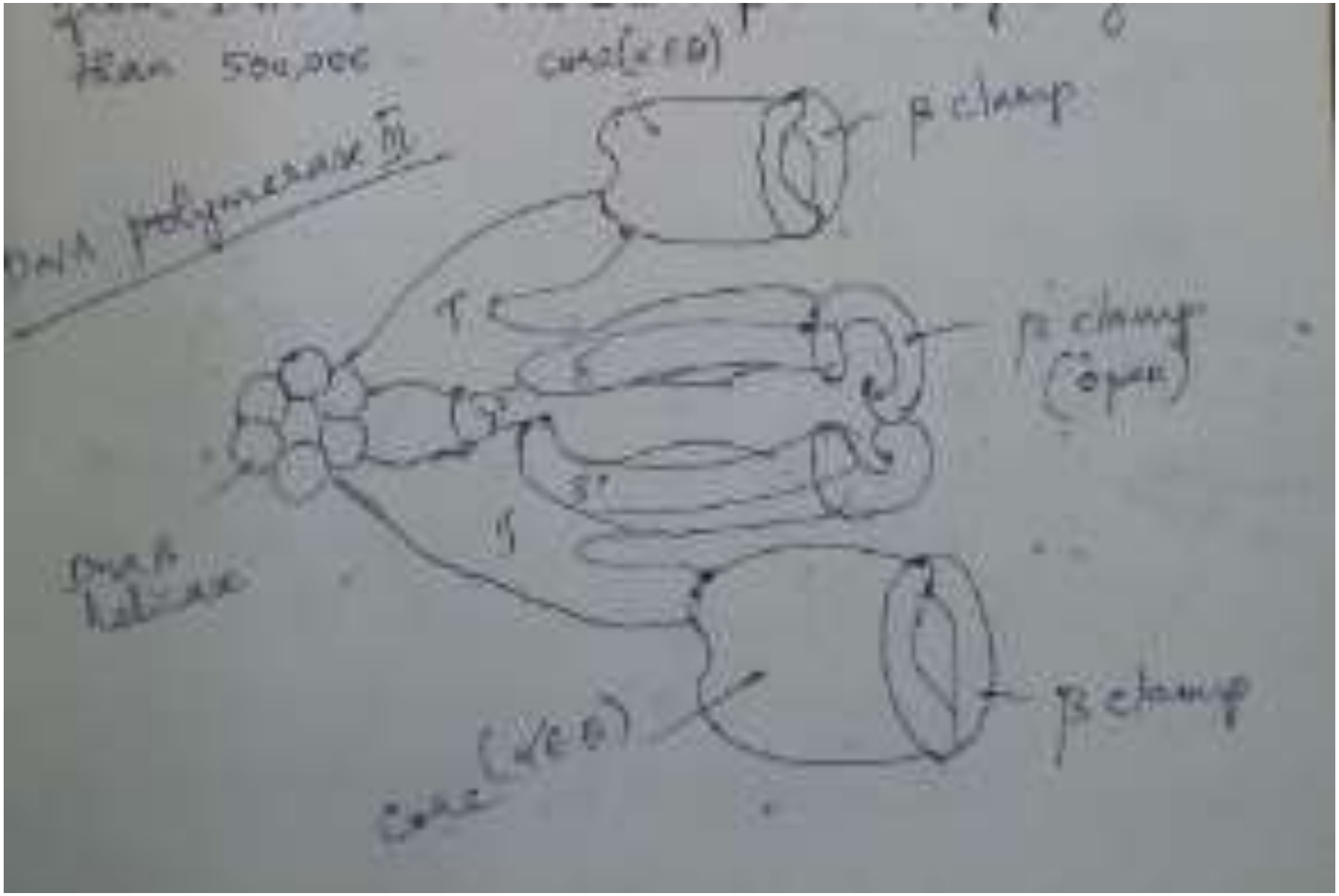
- DNA ligases: The nicks or breaks on the phosphodiester bond of the DNA back bone are sealed by DNA ligases.

- DNA Polymerases III: DNA polymerase III is the principal replication enzyme in E.coli. DNA polymerase III is a complex enzyme having ten types of subunit.
- Its polymerization and proof reading activities reside in its α and ε (epsilon) subunits, respectively. The ɵ subunit associates with α and ε to form a core polymerase, which can polymerize DNA but with limited processivity.

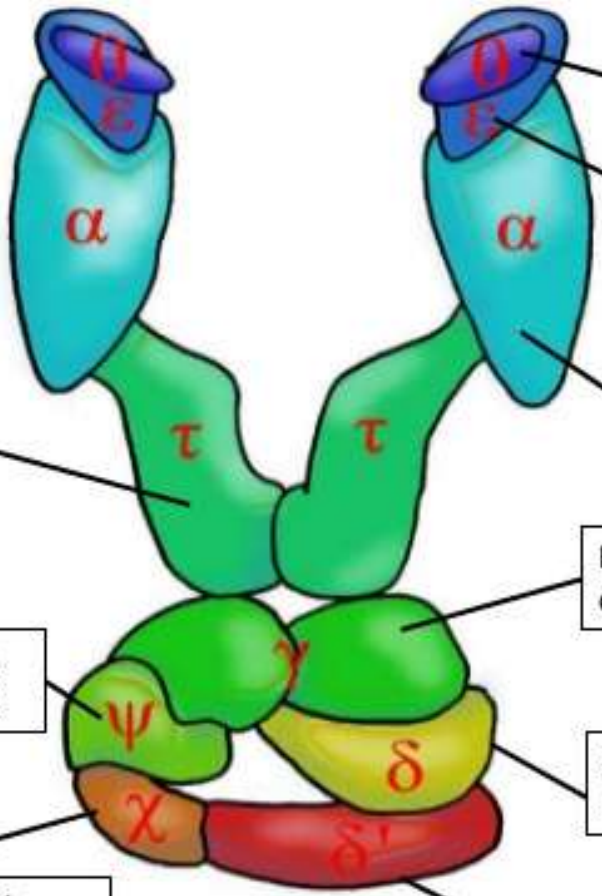
- Two core polymerases can be linked by another set of subunits, a clamp complex or γ complex. It consists of five subunits of four different types.Τ2γδδ’.
- The core polymerases are linked through the Τ (tau) subunits.
- Two additional subunits χ (chi) and ψ (psi), are bound to the clamp loading complex.

- The entire assembly of 13 protein subunits (nine different types) is called DNA polymerase III.
- DNA polymerase III can polymerize DNA but with a much lower processivity. The necessary increase in processivity is provided by the addition of the β units. Four β subunits complete the DNA polymerase III holoenzyme.

- The β subunits associate in pairs to form donut shaped structures that encircle the DNA and act like clamps.
- Each dimer associates with a core subassembly of polymerase III and slides along the DNA as replication proceeds.The β sliding clamp prevents the dissociation of DNA polymerase III from DNA and increases processivity to greater than 50000.


β clamp
DNA polymerase III
5'
3'
β clamp
(open)
β clamp

Schematic of DNA Polymerase III



Encoded by **holE** gene, stimulates the ε proof-reading activity

Encoded by **dnaQ** gene, has 3'->5' exonuclease activity

Encoded by **dnaE** gene, has the polymerase activity

Encoded by **dnaX**, which acts to dimerize two of the core enzymes (α, ε, and θ subunits).

Encoded by **dnaX**, which acts as the clamp loader

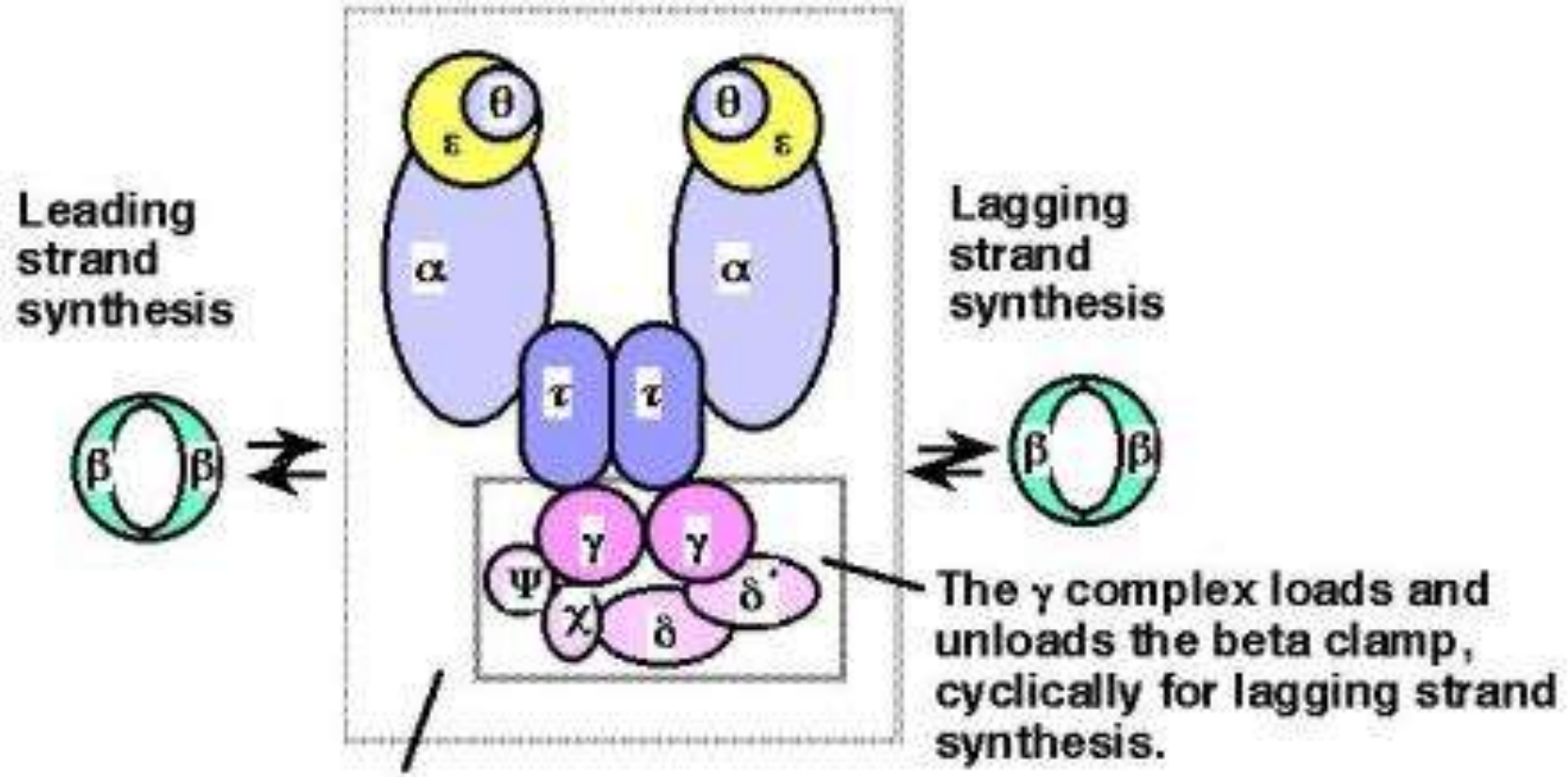
Encoded by **holD**, which helps in the interaction with γ and X

Encoded by **holA,** which acts as a clamp opener

Encoded by **holC**, which helps in interaction with SSB

Encoded by **holB**, which acts as a clamp loader

Source: www.en.wikipedia.org


Leading strand synthesis
Lagging strand synthesis
θ
ε
α
τ
γ
Ψ
χ
δ
δ'
β
The γ complex loads and unloads the beta clamp, cyclically for lagging strand synthesis.
Pol III* subassembly lacks the beta sliding clamp.

Function of DNA polymerase III

- DNA is synthesized by DNA polymerase III. It adds the complementary nucleotide to the existing growing strand free 3'hydroxyl group. The fundamental reaction is a phosphoryl group transfer.
- The nucleophile is the 3' – hydroxyl group of the nucleotide at the 3'end of the growing strand.Nucleophile attack occurs at the α phosphorous of the incoming deoxy nucleoside 5' – triphosphate.

- Inorganic pyrophosphate is released in the reaction.
- $(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + ppi$

  DNA Lengthened DNA

$$\underset{\text{DNA}}{(\text{dNMP})_n} + \text{dNTP} \longrightarrow \underset{\substack{\text{Lengthened}\\ \text{DNA}}}{(\text{dNMP})_{n+1}} + \text{PP}_i \qquad (24\text{–}1)$$

where dNMP and dNTP are deoxynucleoside 5′-monophosphate and 5′-triphosphate, respectively.