ENZYMES & FACTORS OF DNA REPLICATION

Replication is a very complicated process. various types of enzymes and factors are necessary for DNA replication. The entire complex has been called DNA Replicase System / the Replisome. The mg major enzymes are described below:-

A) **DNA polymerase:-** The enzymes that catalyze DNA Synthesis are called DNA polymerases. E. coli has at least 5 DNA polymerases, viz. DNA Polymerase I, II, III, IV & V.

a) **DNA polymerase I:-** In 1957, Arthur Kornberg and his co-workers isolated an enzyme from E. coli that catalyzes the covalent addition of nucleotides to pre-existing DNA chains. Initially called DNA polymerase or Kornberg's enzyme", it is now known as DNA polymerase I.

1) Structure:-

i) It is a single polypeptide with a molecular weight of 103,000 encoded by a gene called Polymerase A (Pol A).

ii) E. coli DNA pol I have three active sites having enzymatic activities. It can be proteolytically cleaved to a large or "Klenow fragment" (KF) at the C terminal, which contains both the polymerase & 3'→5' exonuclease activities, and a small fragment at the N terminal, which contains the 5'→3' exonuclease activity.

2) Functions:-

i) The enzyme requires the 5'-triphosphates of each of the four deoxy-ribonucleosides — deoxyadenosine triphosphate (dATP), ~~deoxyadenosi~~ deoxythymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) & deoxycytidine triphosphate (dCTP). It is active only in the presence of Mg^{2+} ions & pre-existing DNA. DNA polymerase I catalyzes the formation of a phosphodiester bridge between the 3'-OH at end of the primer DNA chain & the 5'-phosphate at the incoming nucleoside triphosphate with the elimination of pyrophosphate.

ii) In addition to its polymerase activity, Pol I has two independent hydrolytic activities; a 3'→5' exonuclease & a 5'→3' exonuclease activities.

⇒ The 3'→5' exonuclease activity allows polymerase I to edit its intake. If polymerase I or DNA polymerase I erroneously incorporates a mispaired nucleotide at the end of a growing DNA chain, the polymerase activity is inhibited & the 3'→5' exonuclease hydrolytically excises the offending nucleotide. This activity is called proofreading mechanism.

⇒ The 5'→3' exonuclease activity of intact DNA polymerase I can replace a segment of DNA paired to the template strand in a process known as nick translation. The 5'→3' exonuclease activity of DNA polymerase I usually excises small oligomers containing upto 10 nucleotides.

⇒ polymerase I has its most important function in lagging strand synthesis, in which it removes the RNA primers & replaces them with DNA. This

process involves the 5'→3' exonuclease & polymerase activities of Polymerase I, working in concert to excise the ribonucleotides on the 5' end of the single strand nick between the new & old Okazaki fragments & to replace them with deoxyribonucleotides. The nick is thereby translated by toward the DNA's strands' 3' end a process known as Nick translation. When the RNA has been entirely excised the nick is sealed by the action of DNA ligase, thereby linking the new & old Okazaki fragments.

iii) Polymerase I also functions in the repair of damaged DNA. Polymerase I's 5'→3' exonuclease activity excises the damaged DNA while its polymerase activity fills in the resulting single strand gap in the same way it replaces the RNA primer of Okazaki fragments.

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NOTE:- Subsequent research has shown that DNA polymerase I is not the "true replicase" in E. coli. It doesn't catalyze the semiconservative replication of E. coli chromosome. Nevertheless, DNA polymerase does perform important function in the E. coli cell, including playing a key role in chromosome replication & a central role in repairing damaged DNA.

b) **DNA polymerase II**:- DNA polymerase II is a DNA repair enzyme; but it represents a small proportion of the polymerase activity in E. coli cell.

DNA polymerase II is a single polypeptide with a molecular weight of 88,000 encoded by a gene called Pol B. DNA polymerase II has 5'→3' polymerase & 3'→5' exonuclease activities. However, it has no 5'→3' exonuclease activity.

c) **DNA polymerase III**:- DNA polymerase III is a multimeric enzyme and it is a true DNA replicase with a molecular mass of about 900,00 daltons in its complete or holoenzyme form. DNA polymerase III has 10 subunits. The minimal core that has catalytic activity in vitro contains three subunits:- α (the dnaE gene product), ε (the dnaQ gene product) & θ (the holE gene product). Addition of the τ subunit (the dnaX product) results in dimerization of the catalytic core & increased activity. The β subunit (the dnaN gene product) of DNA polymerase III forms a dimeric clamp that keeps the polymero from falling off the DNA template DNA. The β dimer forms a ring that encircles the replicating DNA molecule & allows DNA polymerase III to slide along the DNA while remaining tethered to it. The DNA polymerase III holoenzyme, which is responsible for the synthesis of both nascent DNA strands at a replication fork contains at least 20 polypeptides.

DNA polymerase III has 5'→3' polymerase & 3'→5' exonuclease activities; however, it has a 5'→3' exonuclease that is active only on single-stranded DNA.

d) **DNA polymerase IV & V**:- DNA polymerase IV & V, identified in E. coli in 199 are involved in unusual form of DNA repair.

⇒ Factors of Replication :-

a) Dna A protein :- A single complex of about 20 Dna A protein molecules bind to the four 9bp repeats in the origin, and then recognizes and successively denatures the DNA in the origin of three 13bp repeats, which are rich in A=T pairs. The process requires ATP hydrolysis. It is a dnaA gene product.

b) Dna B protein :- The hexameric DnaB protein (the product of the dnaB gene), a so called helicase, further unwinds the DNA strands in both directions in an ATP dependent manner. It consists of identical 4th residue subunits and it unwinds the strands of dsDNA by translocating along the lagging strand in the 5'→3' direction.

c) DNA Gyrase :- DNA Gyrase or Topoisomerase II is a tetramer with two α subunits encoded by the gyrA gene and two subunits specified by the gyrB gene. Strand separation creates topological stress in the helical DNA structure, which is relieved by the action of DNA gyrase. In addition to relaxing supercoiled DNA & introducing negative supercoils into DNA, topoisomerase II enzymes can separate interlocking circular molecules of DNA.

NOTE :- DNA gyrase molecules travel along the DNA ahead of the replication fork, while relieving positive supercoils, DNA gyrase cleaves both strands of the DNA duplex, passing a segment of DNA through the double-stranded break to the other side, & then sealing the cuts, a process that ~~requires~~ is driven by the energy released during ATP hydrolysis.

d) Single-strand Binding proteins (SSB proteins) :- once the DNA strands are unwound by DNA helicase, they must be kept in an extended single-stranded form for replication. They are maintained in this state by a coating of single-stranded DNA binding protein (SSB protein). In E. coli the SSB protein is encoded by the SSb gene.

The binding of SSB protein to single-stranded DNA is co-operative, that is, the binding of the first SSB monomer stimulates the binding of additional monomers at contiguous sites on the DNA chain. Because of the cooperativity of the SSB protein binding, an entire single-stranded region of DNA is rapidly coated with SSB protein. Without the SSB protein coating, the complementary strands could renature or form intrachain hairpin structures by hydrogen bonding between short segments of complementary or partially complementary nucleotide sequences. Such hairpin structures are known to hamper the activity of DNA polymerases. However, SSDNA must be stripped of SSB before it can be replicated by Pol III Holoenzyme.

e) DNA Primase :- Subsequent research has shown that each new DNA chain is initiated by a short RNA primer synthesized by DNA primase. The E. coli DNA primase is the product of the dnaG gene. In prokaryotes, these RNA primers are 10-60 nucleotides long, whereas in eukaryotes they

are shorter, only about 10 nucleotides long. The RNA primers provide the free 3'-OH's required for ~~can~~ covalent extension of polynucleotide chains by DNA polymerases.

f) DNA ligase :- The Okazaki fragments are later covalently jointed together by the enzyme DNA ligase.

DNA ligase catalyzes the formation of a phosphodiester bond between a 3'-Hydroxyl at the end of one DNA strand and a 5' phosphate at the end of another strand. The phosphate must be activated by adenylation. DNA ligases of bacteria generally use NAD^+ — a cofactor that normally functions in hydride transfer reactions — as the source of AMP activating group.

Note :- DNA ligase alone has no activity at breaks in DNA where one or more nucleotides are missing — so called Gaps. Gaps can be filled in & sealed only by the combined action of a DNA polymerase & DNA ligase.

g) Tus protein :- In E. coli the arrest of replication fork motion at Ter sites requires the action of Tus protein, a 309 residue monomer that is the product of the tus gene (for terminus utilization substance). Tus protein specifically binds to a Ter site, other where it prevents strand displacement by DnaB helicases, thereby arresting replication fork advancement.

h) DnaC protein & DnaT protein :-

DnaC protein joins the initiation complex & contributes to the formation of two bidirectional replication forks.

The DnaT protein also is present in the prepriming protein complex, but its function is unknown.

i) Other proteins :- Other proteins associated with the initiation complex at Ori are DnaJ protein, DnaK protein, PriA protein, PriB protein, PriC protein & DNA binding proteins H.U. (Histone Like Protein).