CELL BIOLOGY

6 REPLICATION OF DNA

6-1 INTRODUCTION

The process by which a DNA molecule produces its identical copies is described as *DNA replication*. It is a type of self duplication or self reproduction of DNA, where two daughter molecules are formed from a single DNA molecule.

6-2 DNA REPLICATION

Theoretically, three possible modes of DNA replication are possible. They are :

1. Dispersive replication : The two strands of parent DNA break randomly and produce several pieces. These pieces replicate and reunite to form new daughter DNA molecules. These new DNA molecules contain a mixture of old and new nucleotides scattered along, the chains. The daughter molecules can be described as *hybrids*. This mechanism is neither accepted nor proved experimentally.

2. Conservative replication : After replication, one daughter DNA contains the original two strands of the parent molecule. While the other daughter molecule contains two newly synthesized strands. This method is also not accepted.

3. Semi conservative replication : This method of DNA replication was proposed by Watson and Crick. Because of the specificity of base pairing, the sequence of bases along one chain automatically determines the base sequence along the other. Thus, each chain of the double helix can serve as a template for the synthesis of the complementary strand. More precisely semi - conservative' means half of the DNA is conserved i.e; only one strand is synthesized and the other half of the original DNA is retained.

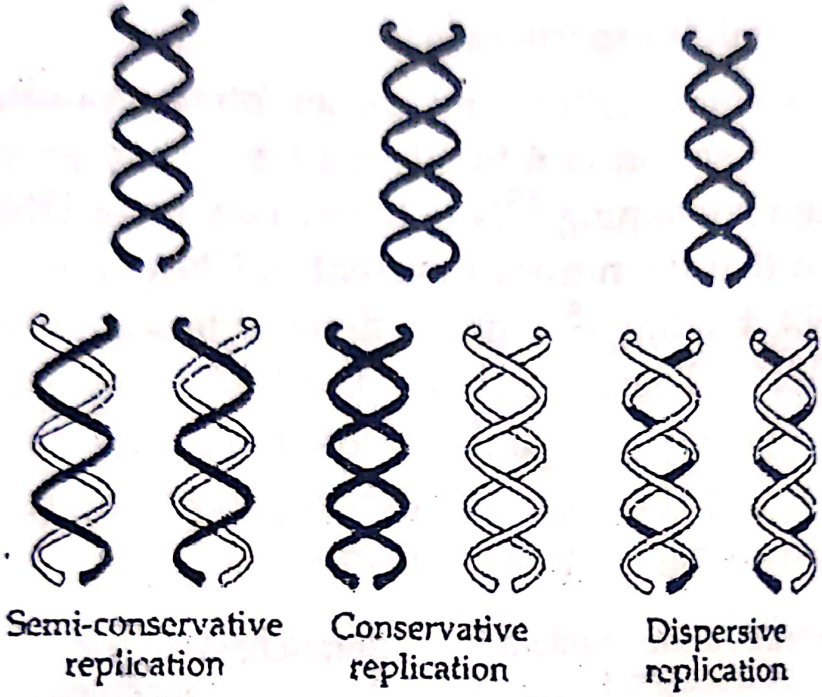


Fig. 6·1 : Three models for DNA replcation

The main features of this model of DNA replication are as follows :

(1) Progressive separation of the two strands of DNA molecule undergoing replication.

(2) Complementary base - pairing of the bases located in the single - stranded region with the appropriate free deoxyribonucleotides, and

(3) Formation of phosphodiester linkages between the neighbouring deoxyribonucleotides, there by producing the new strand.

(4) This ensures that the base sequences of the new strands are strictly complementary to those of the old strands.

(5) Each DNA molecule produced by replication has one 'old' and one 'new' strand.

Since, each of the two double helices conserves only one of the parent polynucleotide strands, the process is said to be *semi - conservative.*

6-4 MECHANISM OF DNA REPLICATION

DNA replication is a complex event and includes the following 3 major phases : (1) Initiation, (2) Elongation and (3) Termination. Replication occurs inside the chromosomes during interphase. The parent DNA strands serve as templates for the synthesis of new. DNA strands DNA replication occurs by semi conservative method. It is catalysed by the enzyme DNA polymerase.

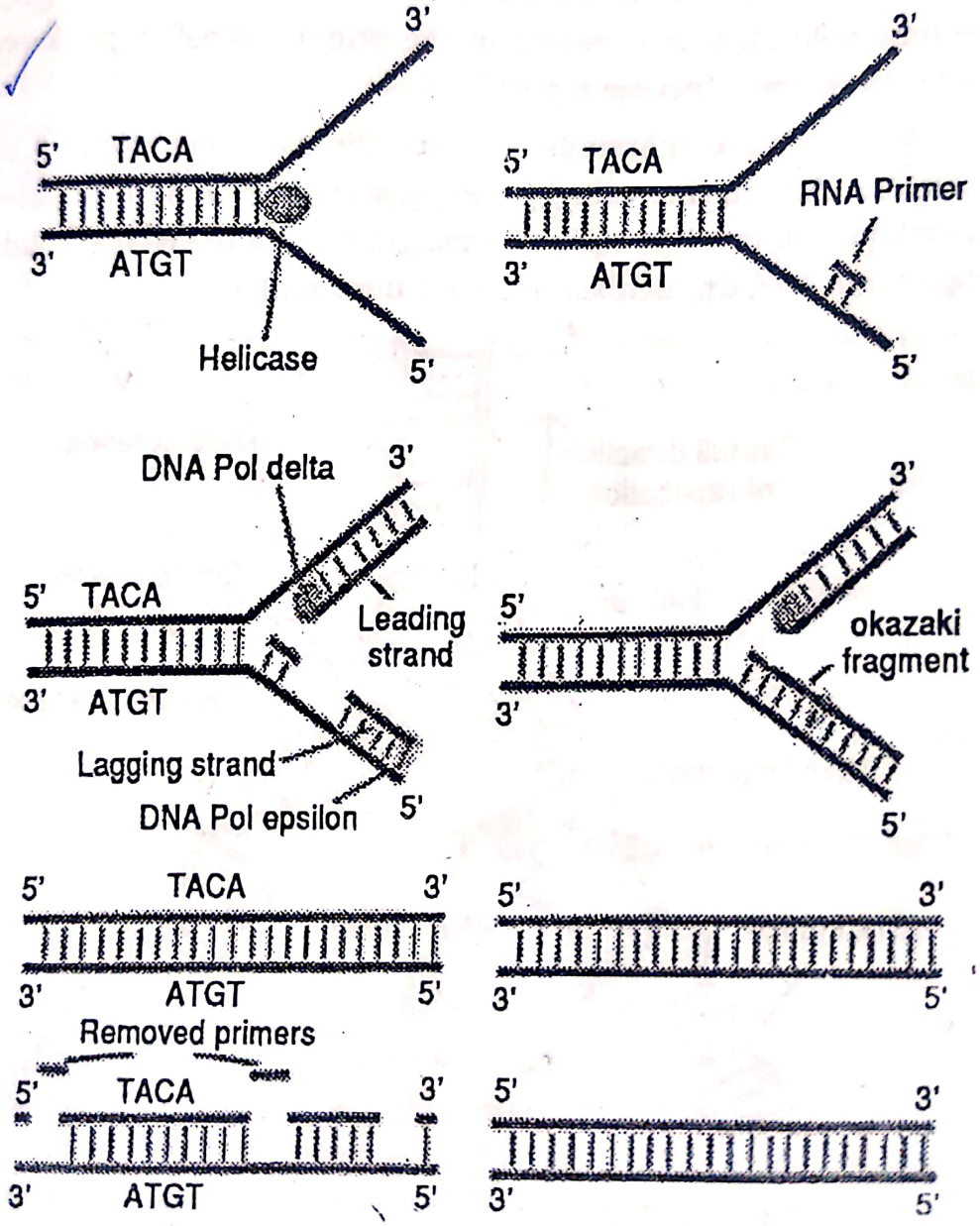


Fig. 6·4 : Steps in DNA replication

. Initiation :

(i) DNA replication begins at certain unique and fixed points called "Origin" (ori).

(ii) Two enzymes *DNA gyrase* and *DNA helicase*, bind to the origin points and induce the unwinding and separation of complementary strands of DNA double helix. This separation is known as *DNA melting*.

(iii) Melting of DNA produces two Y- shaped foks at origin, one fork is located at each end of the origin. When replication begins, these forks become *replication forks*.

(iv) As the two strands separate, the bases are exposed to enzymes. An enzyme called *RNA polymerase* or *primase* initiates transcription of the strand (3' → 5') and generates a 10 - 60 nucleotide long *primer RNA* (transcribed in 5' → 3' direction).

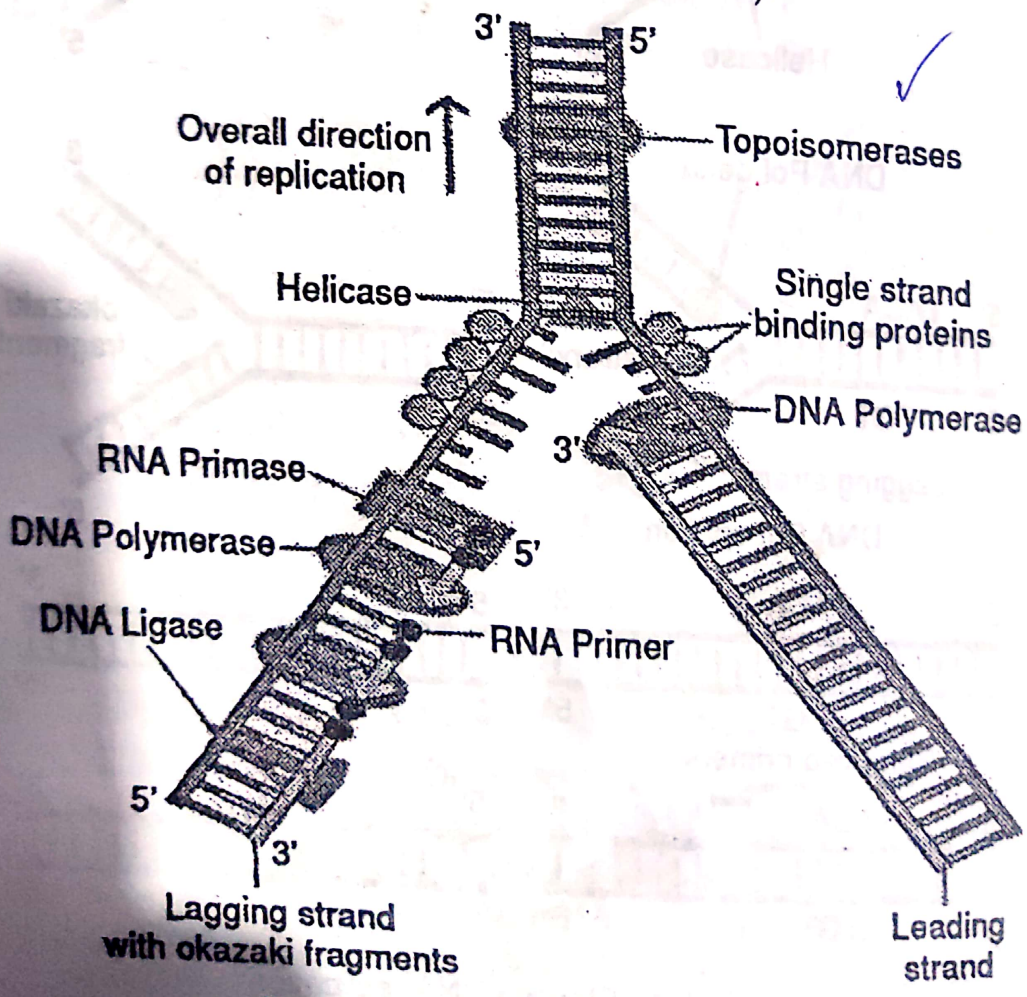


Fig. 6·5 : Semi discontinuous Types

2. Elongation :

(v) The free 3' — OH of this primer RNA provides the initiation point for the synthesis of new DNA strand. Deoxy ribonucleotides are added to the 3' - OH group of the last ribonucleotide of the RNA primer. This leads to elongation of the primer nuclotides in the 5' → 3' direction. This is mediated by DNA polymerase III.

This enzyme requires the fee 3' — OH of a pre - existing polynucleotide for the initiation of DNA replication.

(vi) DNA polymerase progressively adds deoxyribo nucleotides to the free 3' – OH of this growing polynucleotide chain. Consequently, the replication of 3'→5' strand of a DNA molecule proceeds continuously.

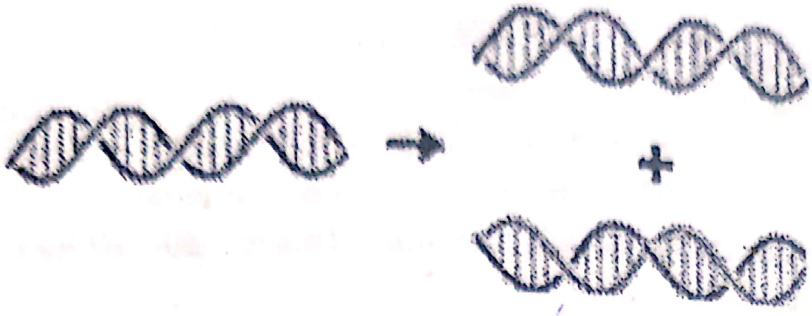
(vii) The replication of second strand (5' → 3' strand) of the DNA molecule is discontinuous. It begins some what later than that of the 3' → 5' strand. Therefore the 3' → 5' strand of DNA molecule in known as the *leading strand*, while the 5' → 3' strand is termed as the *lagging strand*.

(viii) The helicase enzyme progressively unwinds the duplex and the replication fork moves along like a bubbe.

(ix) When replication of the 3'→ 5' strand has progressed for sometime, primase initiates the synthesis of RNA primer on the 5' → 3' strand close to the replication fork (away from the origin site). The primer synthesis begins close to the replication fork and progesress towards the origin. The 3' → OH of this primer RNA provides the initiation point for DNA polymerase to catalyse replication of the *'lagging strand'*. Obviously, the replication of lagging strand proceeds from the replication fork towards the origin, i.e. its direction is opposite to that of the leading strand.

(x) The replication of the lagging strand (5' → 3') generates small polynucleotide fragments called *Okazaki fragments*.

(xi) The RNA primer associated with the newly synthesized okazaki fragments is digested by DNA polymerase I in prokaryotes. This enzyme also catalyzes the filling of gaps so generated in the new strands. The okazaki fragments are joined together by the enzyme *Polynucleotide ligase* to form a long polynucleotide chain.



Semi-conservative Replication

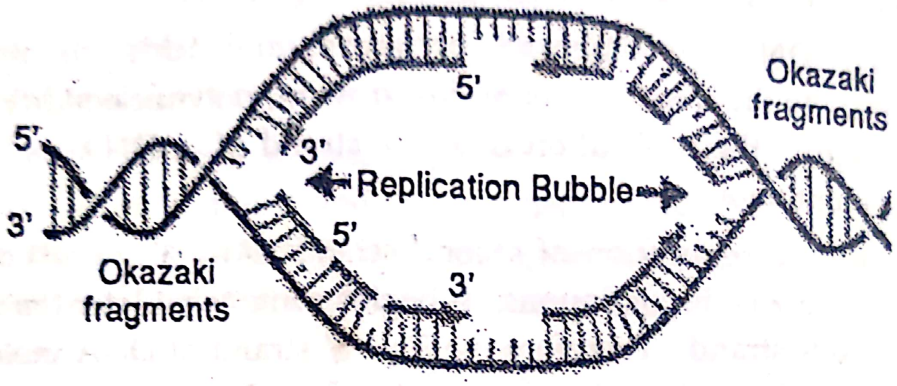


Fig. 6·6 : Bi-directional replication of DNA

3. Termination :

(xii) In *E. coli*, the termination is signalled by specific sequenes called *ter* elements. They serve as binding site for protein *Tus*. The *Tus* protein binds to *ter* element and stops helicase enzyme from unwinding DNA. This stops the movement of the replication fork. The leading strand is replicated upto the *ter* elements. While the lagging strand replication is stopped 50 - 100 bp before the *ter* element.

6-5 ENZYMES INVOLVED IN DNA REPLICATION

(1) DNA Polymerase : DNA polymerase is the chief enzyme of DNA replication. Its activity was discovered by Kornberg in 1956. There are atleast three types of DNA polymerases in *E.coli*. They are : DNA polymease I (Pol I), II (Pol II) and III ((Pol III). All the DNA polymerases require the following : (i) a template DNA strand. (2) a short primer (either RNA or DNA), and (3) a free 3' - OH in the primer. They add one nucleotide at a time to the free 3'OH of the primer, and extend the primer chain in 5' → 3' direction.

(A) DNA polymerase I : This enzyme was first purified by Kornberg in 1956. Hence it is also called Kornberg enzyme. This

enzyme has three activities, which appear to be located in different parts of the molecule.

(i) A polymerase activity, which catalyses chain growth in the 5' → 3' direction.

(ii) A 3' → 5' exonuclease activity, which removes mismatched bases (DNA proof reading)

(iii) A 5' → 3' exonuclear activity, which degrades double - stranded DNA (excision repair). An exonuclease digests nucleic acids from one end (it does not cut DNA internally).

DNA polymerase I is encoded by gene *pol* A, and has a single polypeptide chain. This can initiate DNA replication *in vitro* at a nick in a DNA duplex.

(B) DNA polymerase II : This enzyme repairs the damaged DNA. It has 5' → 3' polymerase and 3' → 5' exonuclease activities.

(C) DNA polymerase III : This enzyme is responsible for DNA replication *in vivo*. It has 5' → 3' polymerase and 3' → 5' exonuclease activities. It is composed of several subunits : α_2, θ_2, ε_2, r, x, ψ, δ, δ', T_2 and β_4. Both leading and lagging strands are elongated by DNA polymerase III holoenzyme. This multi subunit complex is a dimer, one half synthesing the leading strand and the other the lagging stand. Having two polymereses in a single complex

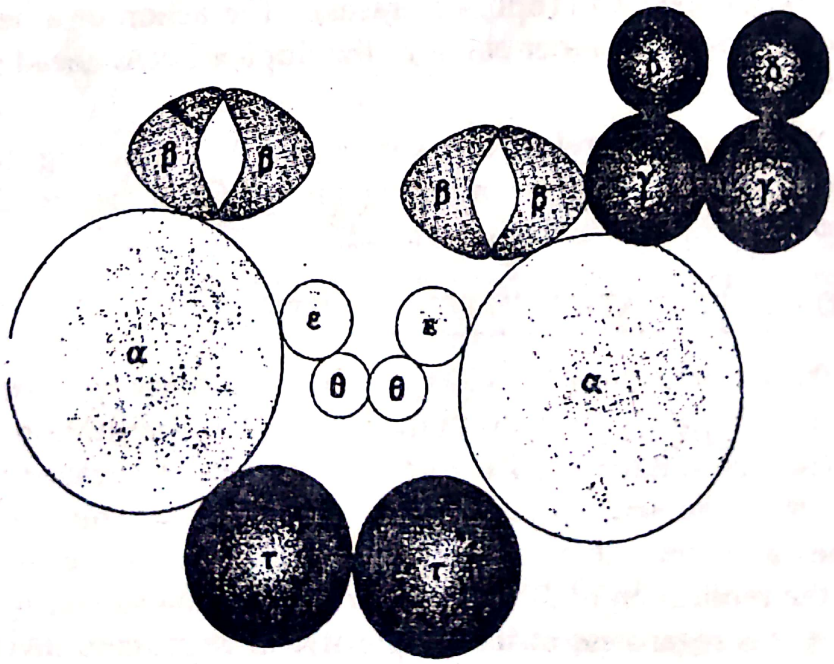


Fig. 6-7 : Components of DNA polymerase III

ensures both strands are synthesized at the same rate. Both halves of the dimer contain a α *subunit*, the actual polymerase and an ε *subunit* which is a 3' → 5' *proof reading* exonuclease. The β subunits clamp the polymerase to the DNA. The remaining subunits in each half are different and may allow the holoenzyme to synthesize short and long structures of DNA on the lagging and leading strands, respectively. Once the lagging strand primers have been elongated by DNA Polymere III, they are removed and the gaps are filled by *DNA polymerase I.*

In vivo, the DNA polymerase III holoenzyme dimer, the primasome and the DNA helicases are believed to be physically associated in a large complex called a **replisome** which synthesises DNA at a rate of 900 bp per sec.

The enzyme is assembled at the replication fork as follows :

(i) First, the γ – δ complex (subunits γ δ δ' x ψ) and a pairs sub unit of β recognize the primed template and binds to it.

(ii) They now attach to the catalytic cored - (αθε subunits).

(iii) Subunit τ now joins the complex. It brings two more β subunits and another catalytic core to the complex. This generates a DNA polymerase III holoenzyme.

2. DNA helicases : These are ATP - dependent unwinding enzymes which promote separation of the two parental strands and establish replication fork.

3. DNA gyrases (Topoisomerases) : The action of a helicase introduces a positive super coil into the duplex DNA, ahead of the replication fork.

DNA gyrases relax the super coil by attaching to the transiently super coil duplex, nicking (cutting) one of the strands and rotating it through the unbroken strand.