

DNA replication:

Watson and Crick give double helical structure of DNA replication. In the process of replication weak H bonds b/w the nitrogenous base of nucleotide separate so that two polynucleotide strand of DNA also separate and uncoil. The strand thus separated are complementary to one another. Each nucleotide of separated chain attracts its complementary nucleotide from the cell cytoplasm. The sugar radicals unite through phosphate and formⁿ of polynucleotide chain.

Thus, each strand of the double helix DNA serve as template. This method of DNA replication is described as semi-conservative method.

Mechanism of DNA replication in Prokaryotes:
In E. coli replication involves following steps:

1. Activation of amino A:
The deoxyribonucleoside monophosphate

(AMP, GMP, CMP, TMP) occur in nucleosides. They are activated with ATP and form triphosphate (ATP, GTP, CTP and UTP) by this process known as phosphorylation and catalysed by phosphorylase.

2. Initiation :

DNA replication is initiated at a defined sequence of nucleotides, called initiation point or Origin of replication (OriC). There is only one origin of replication per chromosome. Each point of origin along with the part of DNA to be replicated from it forms one replication. In *E. coli* 245 b.p consisting three repeats of 13 b.p seq. and four repeats of 9 b.p seq. These called 13mers and 9mers, repeated respectively. It has following steps :

i) Recognition of Initiation Point : Specific initiator protein Dna A recognise the initiation point on DNA. A single complex of 20 molecules of Dna A protein bind to the four repeats of 9 b.p seq. in one of the strand of duplex and initiates the separation of interwound polynucleotide chain.

ii) Unwinding of DNA :

Initiator protein Dna A, after binding to Ori denatures DNA in the region of three repeats 13 b.p seq. This process require ATP and histone like proteins (HLP) & hexamers of helicase enzyme Dna B then

bind into each strand of unwound DNA. Protein DnaB along with DnaI unwind the DNA bidirectionally and creates a potential replication fork. A topoisomerase enzyme, called DNA gyrase promotes separation of DNA inducing negative coiling of helix. Strand separation is maintained by single strand binding proteins (SSB) by preventing reannealing of separated strand and stabilizes the template.

3. Synthesis of New strand of DNA:

i) RNA priming or Formⁿ of RNA primer:

The primase enzyme (DNA directed RNA polymerase) binds to leading strand (grows continuous as a single piece, and dirⁿ growth 5' → 3', doesn't need DNA ligase) and synthesizes short RNA primer at the Ori complementary to separated strand. It is short. RNA primer is essential b/c of DNA polymerase can't initiate synthesis but grows DNA chain.

ii) Base pairing: DN Deoxyribonucleoside triphosphates pair with the appropriate N. base of template DNA strands acc to base pairing rule.

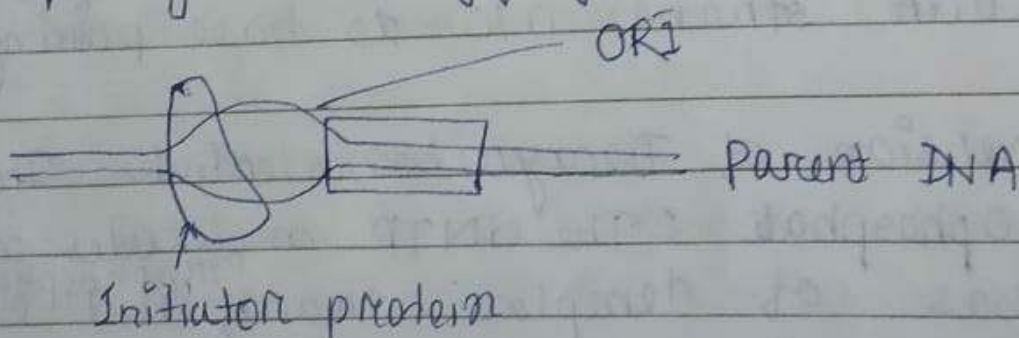
iii) Conversion of Deoxyribonucleoside Triphosphate to Monophosphate: The dNTP molecules on pairing with N base of template strand and change to deoxyribonucleotides. The enzyme pyrophosphatase hydrolyses into inorganic phosphate.

1³
4
2
3

7N) Formation of New DNA chains on RNA primers (Polymerisation of DNA): The energy is released polynucleotide chain. The process is catalysed by DNA polymerase along with Mn^{2+} and Mg^{2+} ions.

The new strand formed in the $5' \rightarrow 3'$ direction $3' \rightarrow 5'$. The addition is affected by DNA polymerase III. It is shown below:

- Binding of initiator protein to Ori
- Attachment of helicase unwound DNA separated by DNA gyrase
- Binding of primase on leading strand to synthesise primer RNA.
- Initiation of DNA synthesis by adding DNA to primer RNA by DNA polymerase III.
- Formⁿ of RNA primer for lagging strand.
- Discontinuous synthesis of lagging strand $5' \rightarrow 3'$ producing okazaki fragments $5' \rightarrow 3'$ exonuclease.
- Joining of okazaki fragments by DNA ligase completing the lagging strand of DNA.



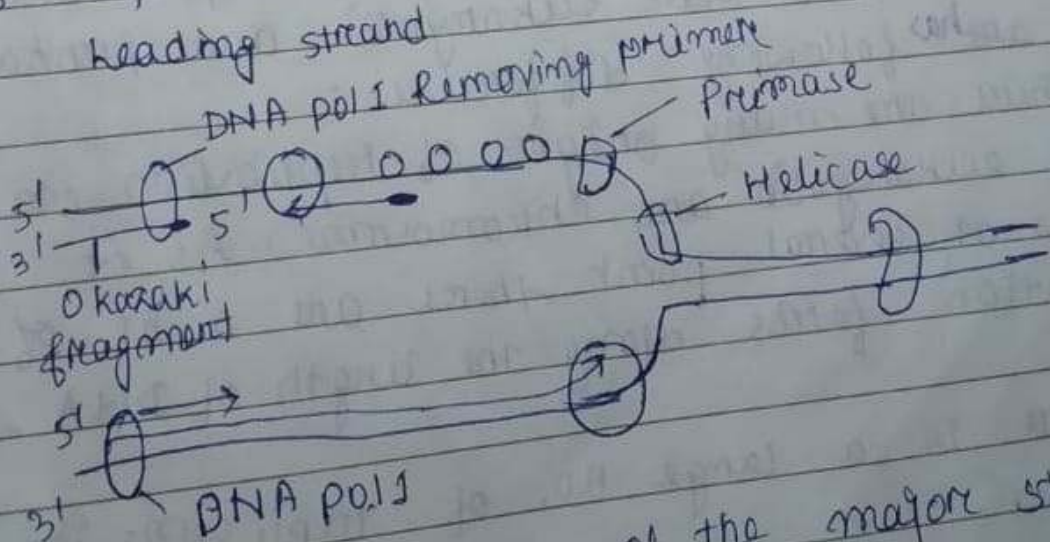
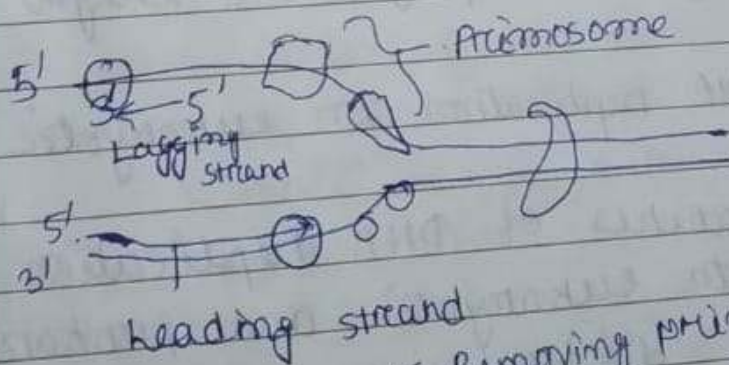
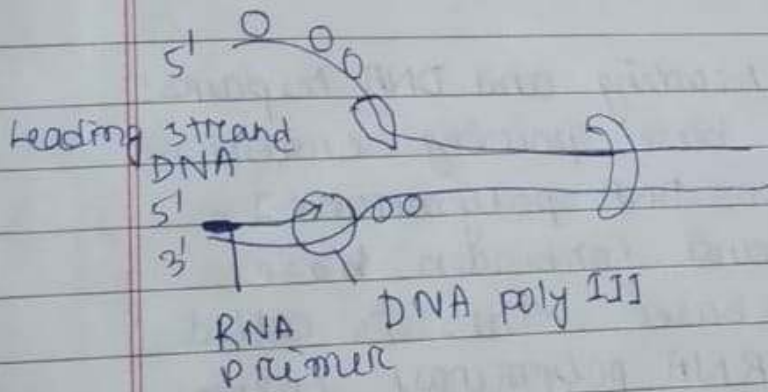
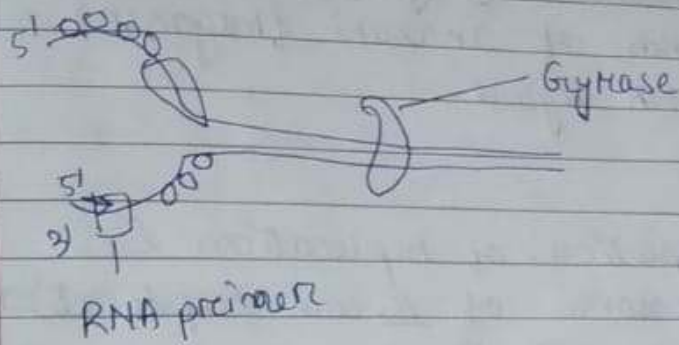
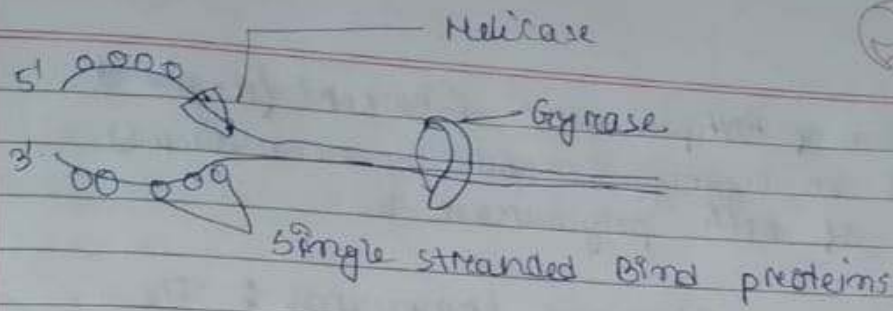


Fig: Summary of the major steps in DNA replication

v) Excision of RNA primer: Okazaki fragment formed in lagging strand by exonuclease activity of DNA polymerase-1.

vi) Joining of Okazaki fragments: The adjacent 5'-3' ends of Okazaki fragment are joined by DNA ligase.

4. Termination:

With the completion of replication in the circular chromosome of K. coli replication fork from only one direction.

5. Editing / proof reading and DNA repairs: The specificity of base pairing ensures accurate replication, DNA polymerase-I enzymes identifies and forbidden base pairs by correct N bases. This is called proof reading of RNA polymerase enzyme.