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 form of polynucleotide chain.

Thus, each strand of the daubl 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 monophosphates

(AMP, GMP, CMP, TMP) occur in nucleoplasm the activated with ATP and form triphosphate (ATP, GTP, CTP and GTP). This process known as phosphorylation and catalysed by phosphoryl

2. Initiation:

DNA replication is initiated at a defined seq. 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 replicon. In E. coli 245 b.p consisting three repeats of 13 b.p seq. and four repeats of 9 bp seq. These called 13 mers and 9mers replicai respectively. It has following steps:

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

ii) Unwinding of DNA:

Initiator protein DnaA, after binding to Ori denaturus DNA in the region of three repeats 13 b.p seq. This process require ATP and histone like protein (HU) & hexamers of helicase enzyme DnaB then

bind into each strand of unwounded DNA. Protein DnaB along with DnaC unwind the DNA bidirectionally and creates 2 potential replication fork. A topoisomerase enzyme, called DNA gyrase promotes seperation of DNA inducing negative coiling of releicase. Strand separation is maintained by single strand binding protein (SSB) by preventing renaturation of separate 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 continu as a single piece, and dirⁿ growth $5' \to 3'$, doesn't need DNA ligase) and synthesis short RNA primer at the Ori complementary to separated strand. It is short. RNA primer synthesis is essential b/of DNA polymerase can't initiate synthesis but growth DNA chain.

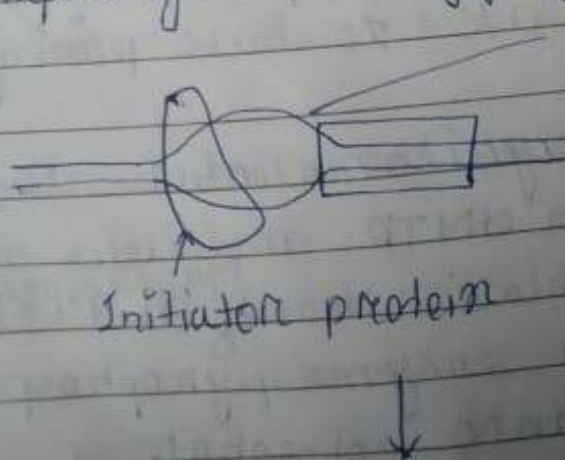
ii) <u>Base pairing</u> : Deoxyribonucleoside triphosphates pair with the appropriate N base of template DNA strands a/c to base pairing rule.

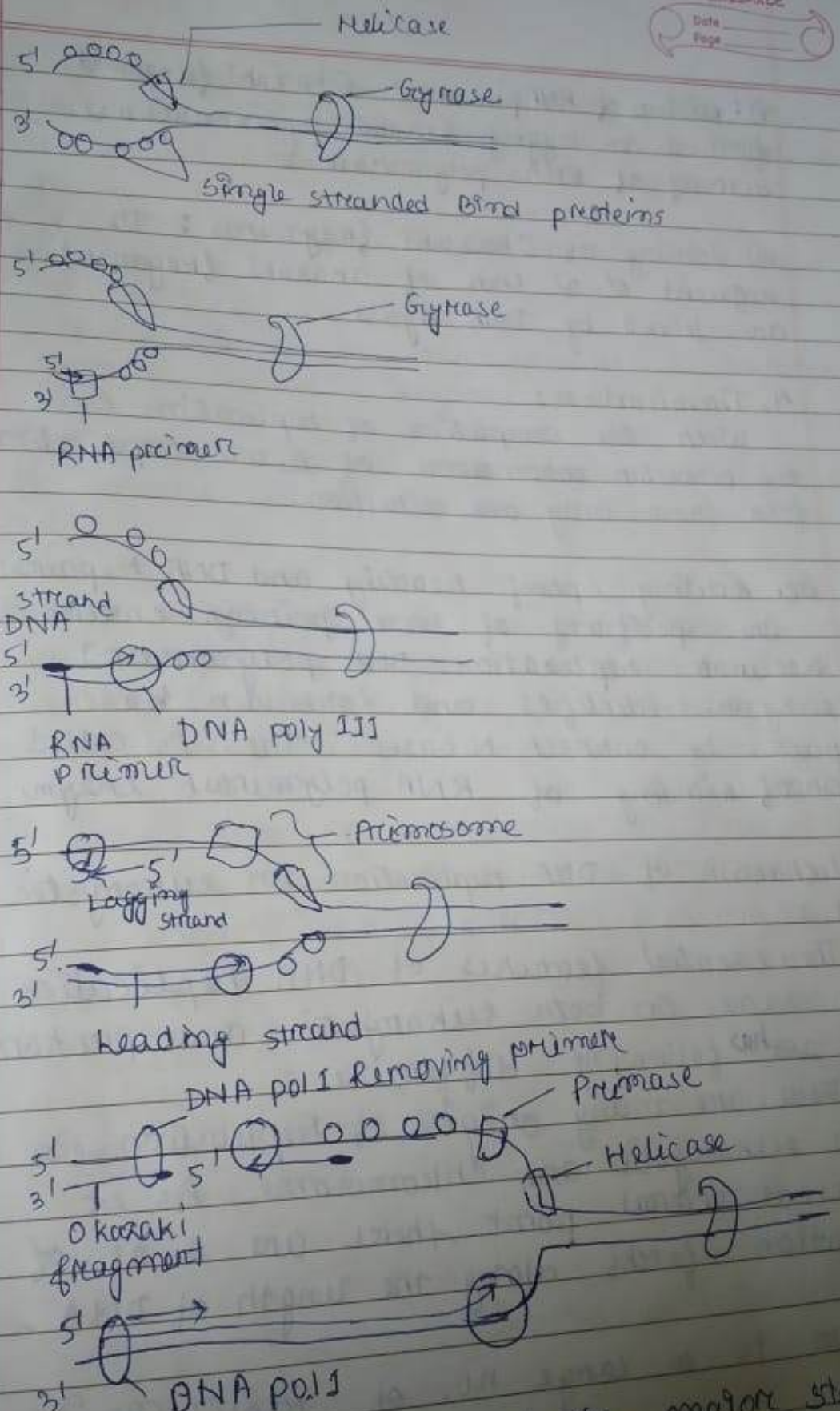
iii) Conversion of Deoxyribonucleotide Triphosphate to Monophosphate : The dNTP molecules on pairing with N base of template strand and change to deoxyribonucleotides. The enzyme pyrophosphatase hydrolyses pyrophosphate into inorganic phosphate.

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

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

a) Binding of initiator protein to Ori
b) Attachment of helicase unwound DNA separated by DNA gyrase.
c) Binding of primase on leading strand to synthesise primer RNA.
d) Initiation of DNA synthesis by adding DNA to primer RNA by DNA polymerase III.
e) Formation of RNA primer for lagging strand.
f) Discontinous synthesis of lagging strand 5'→3' producing okazaki fragments 3'→3' exonuclease.
g) Linking 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 E. 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.