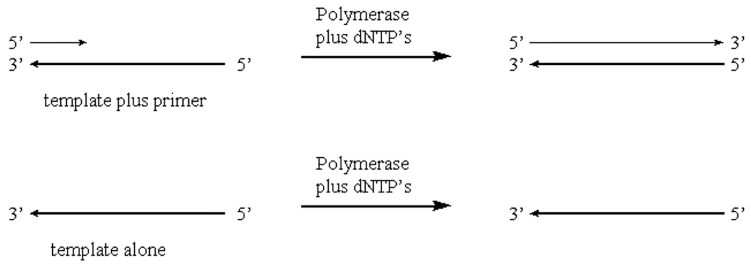
DNA Replication in prokaryotes (E. coli)

In E. coli, **DNA polymerase** characteristics:

- Polymerase will only elongate an existing polynucleotide. It cannot initiate polynucleotide formation:



Figuren: DNA Polymerase activity

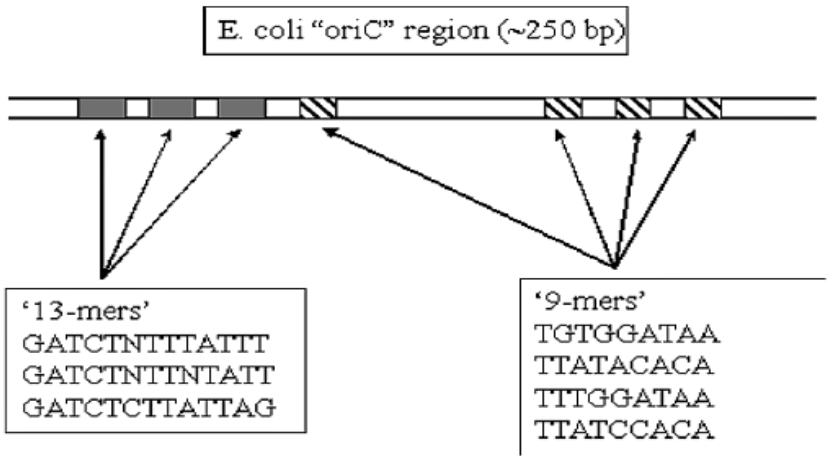
- Polymerase will catalyze polymerization of nucleotides only in one direction (5'>3') via a phosphodiester bond between a 3' hydroxyl and 5' phosphate group.
- DNA polymerase is unable to unwind duplex DNA to separate the two strands which need to be copied.

E. coli genome is circular duplex DNA of approximately 4 x 106 base pairs (i.e. 4 Mb)

- The genome has a **single origin of replication.**
- DNA duplication in E. coli begins at a specific site in the DNA called "**oriC**"

Ori C is a region of DNA approximately 240 nucleotides long

- It contains repetitive **9-base pair** and **13-base** pair sequences (known as the **'9-mer'** and **'13-mer'** regions).
- These sequences are AT rich regions, which melt at lower temperatures than DNA containing GC pairs.
- These regions are postulated to help melt the DNA duplex in the oriC region for initiation of DNA replication



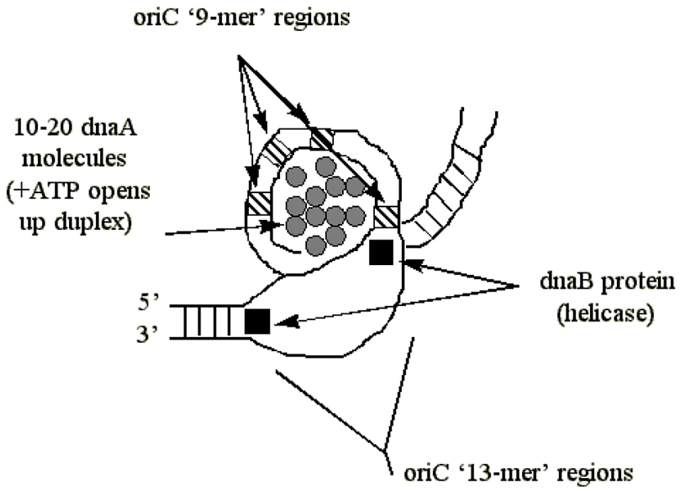
dnaA protein:-

- dnaA protein binds to the **'9-mer'** region in **oriC** and forms a multimeric complex with 10-20 protein subunits (i.e. at a single oriC region there will be bound 10-20 dnaA protein molecules).
- Binding requires ATP.
- Further addition of ATP was observed to result in a melting and opening up of the DNA duplex in the oriC region. This was determined by addition of S1 nuclease (like mung bean, but will also cut DNA at the site of an internal nick), which resulted in cleavage of DNA at the site of oriC

dnaB protein:-

- The dnaB protein has been identified as a **helicase**. A helicase moves along a DNA strand opening up the duplex to melt and separate the DNA strands.
- dnaB protein binds to the single stranded DNA in the general region of the oriC DNA segment.
- Binding requires ATP as well as the dnaC (the dnaC protein).
- After helicase/dnaC binds to the DNA, the **dnaC** protein is released.
- Two helicases bind at the oriC region, one helicase on each strand of the DNA

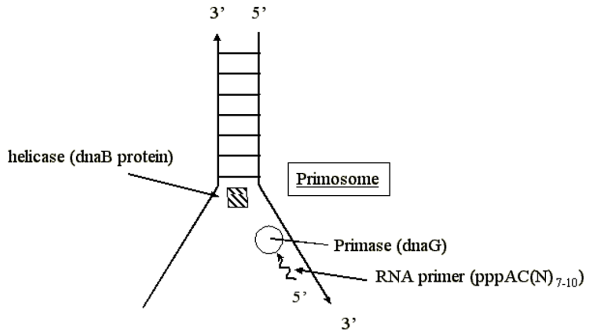
This stage represents the prepriming complex



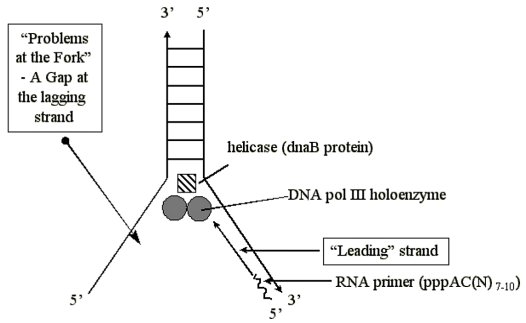
- Separated strands in the oriC region are prevented from reannealing by the binding of **single-stranded binding protein** (**ssb proteins**).

The **dnaG protein**:

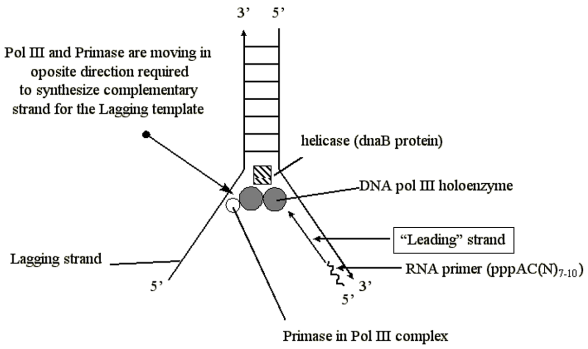
- The dnaG gene protein is called **primase**.
- Primase catalyzes synthesis of short RNA molecules that function as primers for DNA synthesis by E. coli **DNA polymerase III (pol III**).
- Primase binds to dnaB protein at oriC and forms a **primosome**.
- The primase within the primosome complex provides RNA primers for synthesis of both strands of duplex DNA.



- After synthesis of the 9-12 mer **RNA primer**, **DNA Pol III holoenzyme** enters the **replication fork** and is able to utilize the RNA as a primer for DNA synthesis.
- As the replication fork opens up, the **leading strand** synthesis can continue, but a gap develops in the **lagging strand**



- **DNA Pol III is a large multicomplex enzyme (holoenzyme) which is somewhat dimeric in nature (there are two polymerase active sites). The two active polymerase sites in Pol III could actually function to synthesize both nascent strands at the fork. However, the synthesis of the lagging template strand would be in the opposite direction to the movement of the Pol III complex.**



- **Primase can bind to the Pol III complex, but the arrangement of the DNA strand as it passes through the Pol III/primase complex is quite unique. It forms a loop structure such that primase and the Pol III active site can accomplish discontinuous synthesis of the lagging template strand even though the general**

direction of the Pol III complex is opposite to the require direction of DNA synthesis

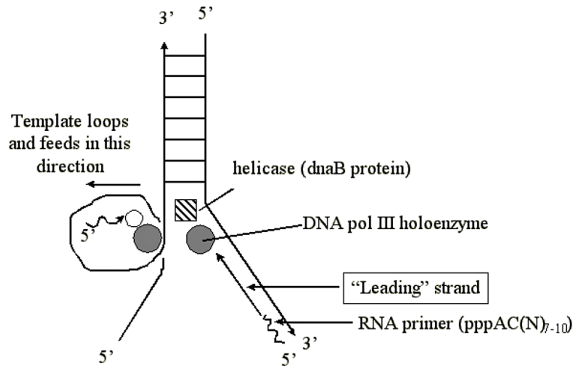


Figure : Loop for Pol III activity

- **After primase makes another primer on the lagging template, the adjacent Pol III active site can extend the primer (incorporating dNTP's) by utilizing the same loop structure and feeding the template through in the direction shown.**

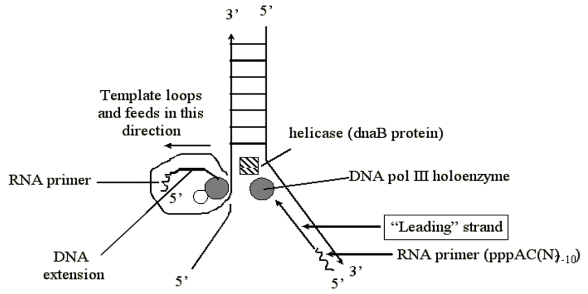


Fig:- Synthesis of lagging strand

- The lagging strand loop cannot be fed through the Pol III complex forever, and after a nascent DNA strand is synthesized the loop is released and a new one is formed using the opened template DNA further up the fork

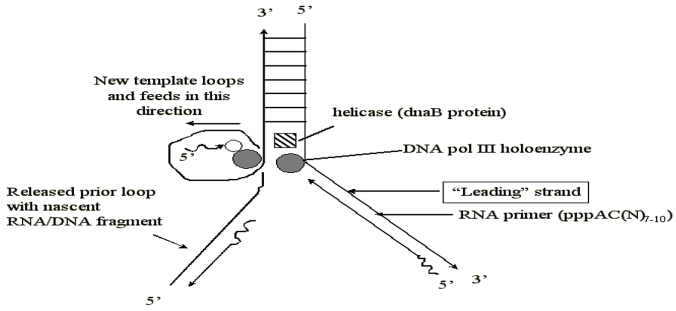
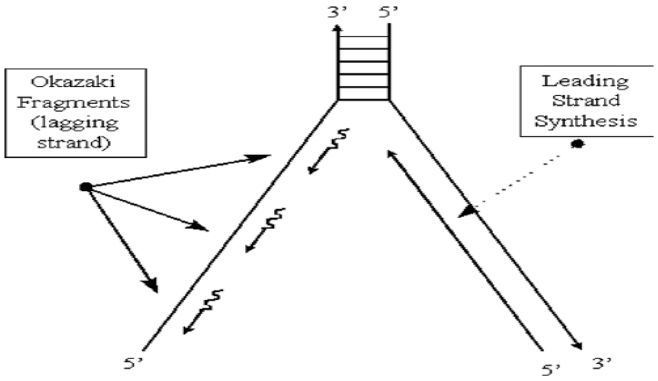


Figure: Continuation of lagging strand synthesis

As synthesis continues:

- there will be a single continuous DNA strand on the leading strand
- there will be a series of short fragments on the lagging strand, containing both RNA and DNA, called **Okazaki fragments**.



How are these RNA/DNA fragments converted into one long continuous DNA strand? The RNA could be removed by a polymerase which has 5'->3' exonuclease activity, however, Pol III lacks this activity.

- DNA Pol I does have 5'->3' exonuclease activity
- It can extend DNA synthesis via nick-translation.
- The nick-translation activity results in degradation of the RNA primers.
- The end result is a series of "nicks" in the lagging strand, now 100% DNA:

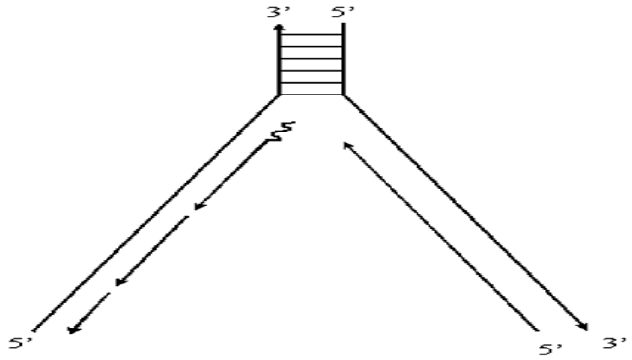


Figure: Nicks in lagging strand

- DNA Pol I leaves and **DNA ligase** then joins these discontinuous DNA fragments to form a continuous DNA duplex on the lagging strand

Termination of DNA replication

- Specific termination sites of DNA replication exist in E. coli.
- Termination involves the binding of the tus gene product (tus protein).
- This protein may act to prevent helicase from unwinding DNA (will therefore halt pol III and pol I action).
- DNA replication produces two interlocking rings which must be separated.
- This is accomplished via the enzyme topoisomerase

Summary of steps in E. coli DNA Synthesis

- **dnaA protein melts duplex in the oriC region.**
- **dnaB (helicase), along with dnaC and ATP binds to replication fork (dnaC protein exits).1 (Pre-priming complex)**
- **Single strand binding protein (ssb protein) binds to separated strands of DNA and prevents reannealing.**
- **Primase complexes with helicase, creates RNA primers (pppAC(N)7-10) on the strands of the open duplex2 (Primase+helicase constitute the Primosome).**
- **After making the RNA primers, DNA pol III holoenzyme comes in and extends the RNA primer (laying down dNTP's) on the leading strand.**
- **As the replication fork opens up (via helicase + ATP action) leading strand synthesis is an uninterrupted process, the lagging strand experiences a gap.**
- **The gap region of the lagging strand can wind around one active site unit of the Pol III complex, and bound Primase initiates an RNA primer in the gap region3.**
- **On the lagging strand, Pol III extends the RNA primer with dNTP's as the lagging template strand is looped through the Pol III complex**
- **After synthesis of a nascent fragment the lagging strand loop is released and the single strand region further up near the replication fork is subsequently looped through the Pol III complex.**
- **Steps 7-9 are repeated.**
- **Meanwhile, Pol I removes the RNA primer regions of the Okazaki fragments via 5' to 3' exonuclease activity (nick translation**
- **Pol I exits and ligase joints the DNA fragments (on lagging strand).**