DNA Replication

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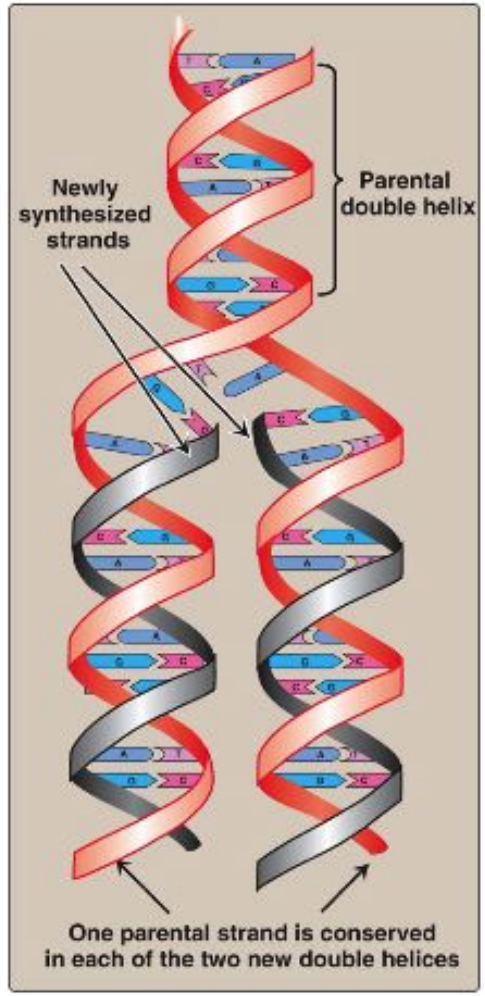
Important metabolic functions of the DNA

- Self-Replication
- Repair of damages
- Recombination – re-arrangement of genetic information
- Transcription of the genetic information to mRNA

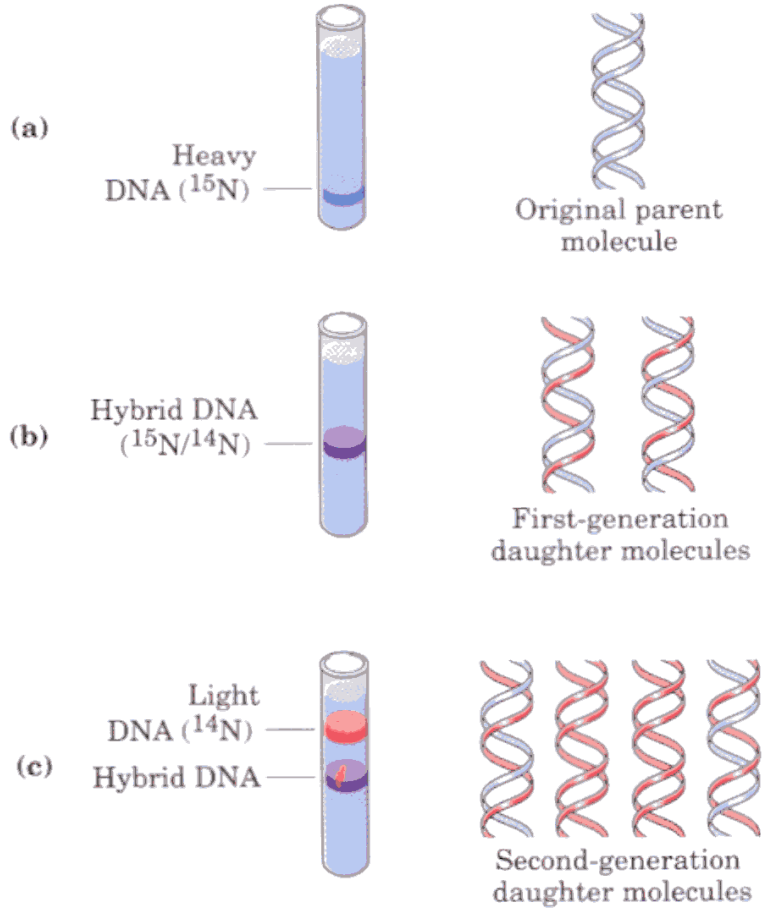
DNA replication



- **Replication**: the process during which a DNA molecule is synthesized ("daughter" molecule), identical to an existing ("parental") DNA molecule.

- **DNA replication is a semi conservative process:**Each DNA strand serves as a "template" for the synthesis of a new strand, producing two DNA molecules, each one with one new strand and one old strand.

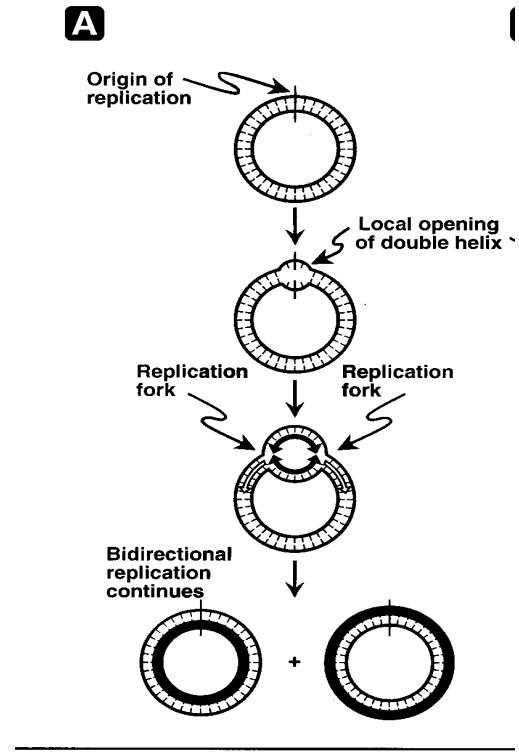
DNA replication is a semi conservative process (the Meselson-Stahl experiment)



- Each DNA strand serves as a “template” for the synthesis of a new strand, producing two DNA molecules, each one with one new strand and one old strand.

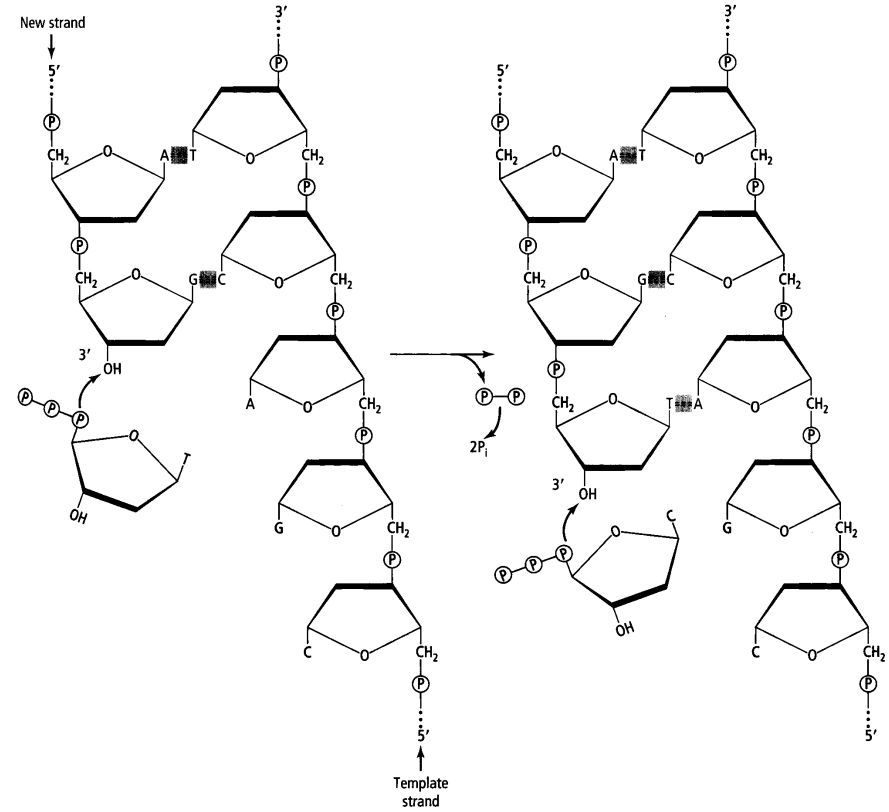
Fundamental Rules of Replication

- DNA replication is **semi-conservative**
- Always initiates at a unique point, called an "**origin**"
- It is usually bidirectional
- Always proceeds in the 5' to 3' direction
- Requires a "**template**" and a "**primer**"



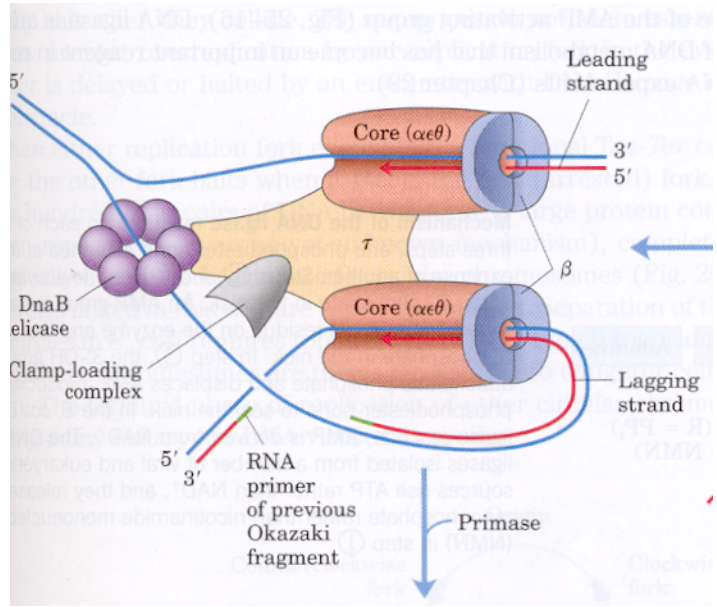
Template-directed synthesis of DNA by **DNA polymerase**

- DNA polymerase catalyzes the synthesis of DNA in the 5’ ⟶ 3’ direction.
- Precursors for DNA synthesis are deoxyribonucleoside triphosphates.
- DNA polymerase requires single-stranded DNA template, and a **primer** (a short double –stranded region with a free OH group at the 3’ end).
- **Phosphodiester bonds** are formed by linking the proximal phosphate of the nucleotide to the 3’ -hydroxy group at the end of the growing strand.
- The pyrophosphate which is released during the formation of the phosphodiester bond is cleaved to inorganic phosphate by pyrophosphatases.



The DNA polymerase III of *E. coli*

- It is the major enzyme of DNA replication.
- Large (>1 MDa) multisubunit (10 subunits) enzyme.
- Pol III clamps around primed template in a reaction that requires ATP hydrolysis by the β subunit. This way the Pol III has unlimited processivity (>500,000)
- Rate of elongation ~1000 nt per sec.
- Pol III alone does not hydrolyze ATP and has a processivity of 10 to 15 nt.
- DNA pol III has the following activities:
  - DNA polymerase (5' to 3')
  - 3' to 5' exonuclease (proofreading)



Properties of the DNA polymerases

- **Chain elongation (5’ to 3’ polymerization)**:
  the rate (nucleotides per sec) at which polymerization occurs.
- **Processivity:**
  number of nucleotides added to the nascent chain before the enzyme disengages from the template.
- **Proofreading (3’ to 5’ exonuclease activity):**
  identification and correction of copying errors.
- **5’ to 3’ exonuclease activity:**
  Excision of primers

The DNA polymerase I of *E. coli*

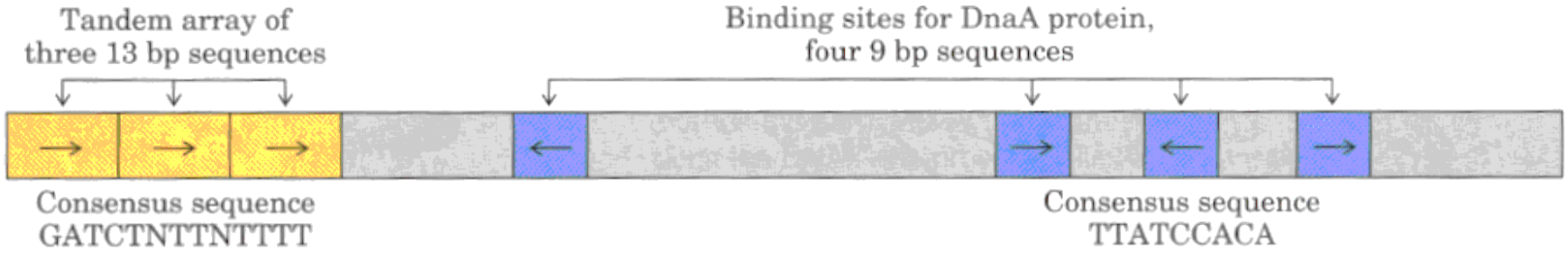
- **Slow polymerization rate: 16 to 20 nt/sec**
- **Low processivity :3-200 nt**
- **DNA pol I has three types of activities:**
  - **DNA polymerase (5’ to 3’)- 600nt/min**
  - **3’ to 5’ exonuclease (proofreading)**
  - **5’ to 3’ exonuclease (excision of the RNA primer)**
- **The fragment that remains after the 5’ to 3’ exonuclease activity has been removed is known as Klenow fragment**

- **Completes chain synthesis between Okazaki fragments on the lagging strand. Does mostly host clean-up functions during replication, recombination and repair.**

The DNA polymerase II of *E. coli*

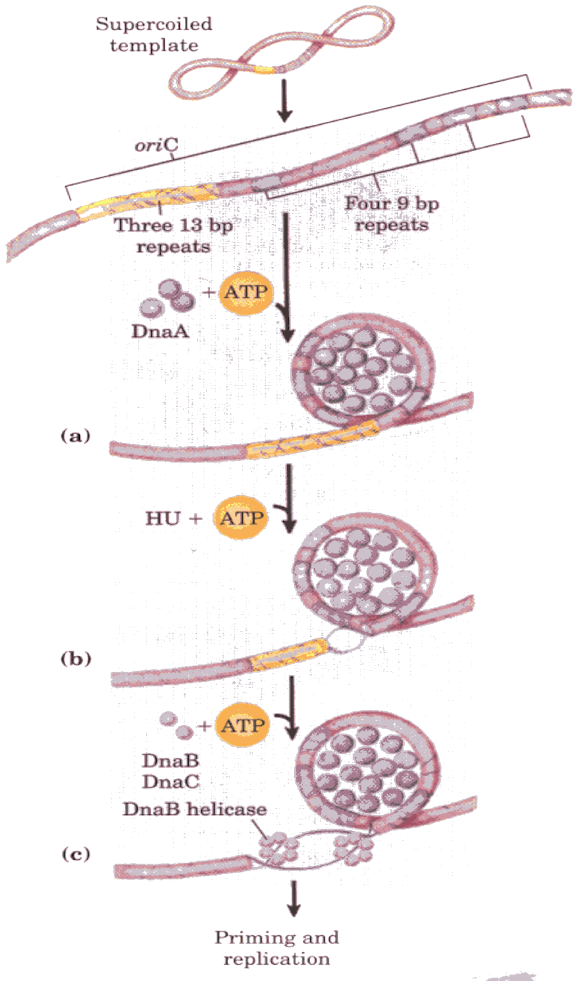
- **Polymerization rate 40 nt/sec**
- **Intermediate processivity: 1,500 nt**
- **DNA pol II has two types of activities:**
  - **DNA polymerase (5’ to 3’)- 600nt/min**
  - **3’ to 5’ exonuclease (proofreading)**
- **Involved in DNA repair.**

The *oriC* of *E.coli*



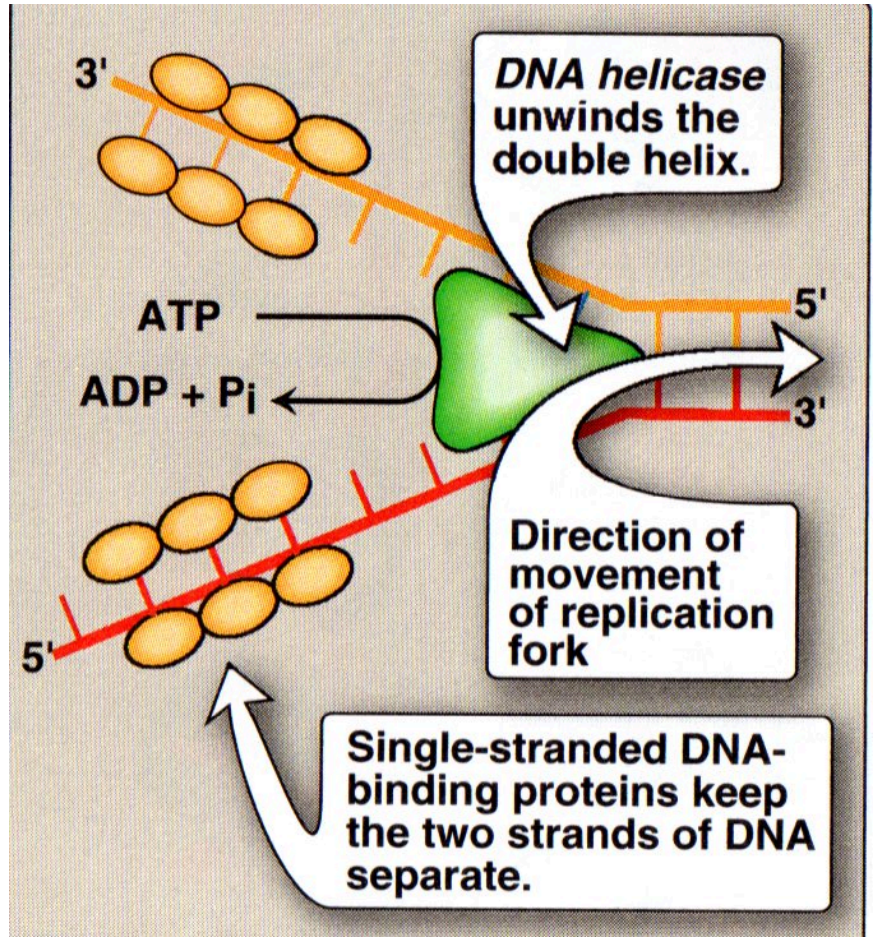
The *oriC* of *E. coli* consists of 245 bp which contain elements that are highly conserved among bacterial replication origins.

Initiation of replication

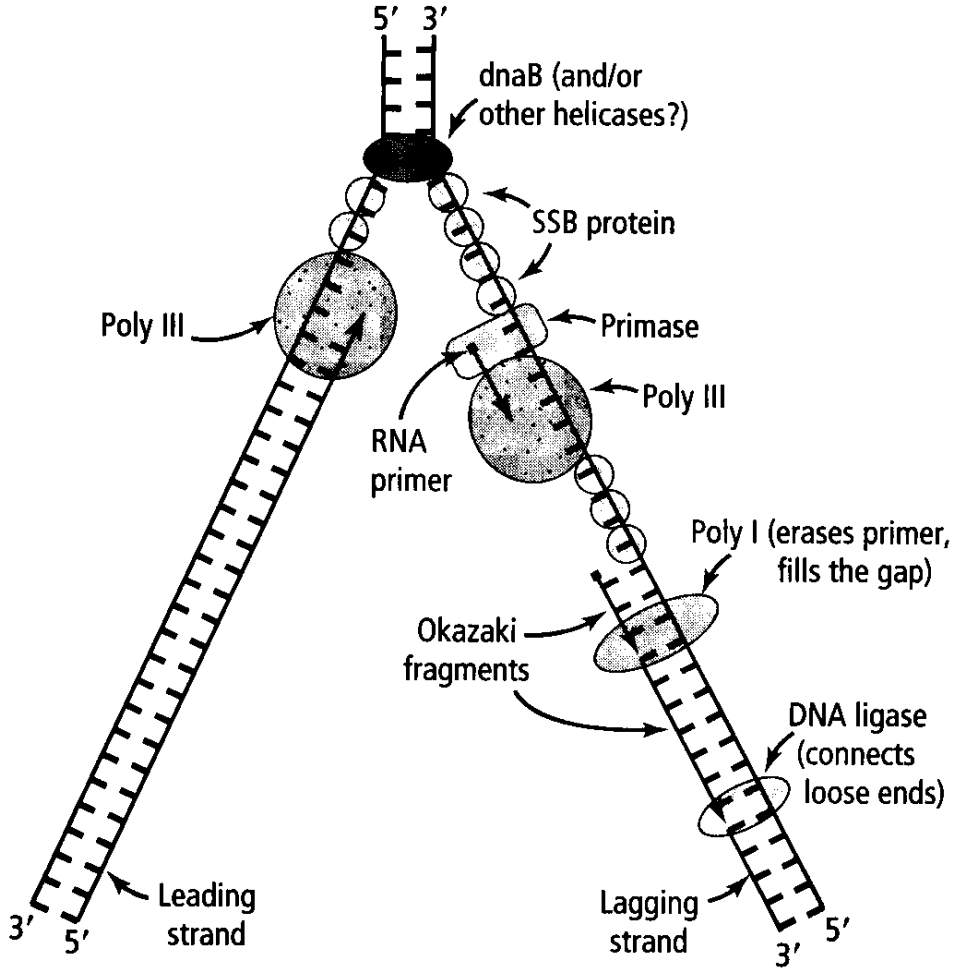


- DnaA protein (20 to 40 monomers) recognizes and binds to the 9 bp repeats in *oriC*
- Negatively supercoiled DNA loops around the DnaA monomers with the help of HU
- The three AT-rich 13bp repeats “melt” in a reaction that requires ATP and DnaA
- DnaB (helicase) and DnaC bind to the “melted” region formimg the “prepriming complex”
- Single-Stranded-Binding proteins (SSB) keep the DNA strands separated and protect them from nucleases

The replication fork



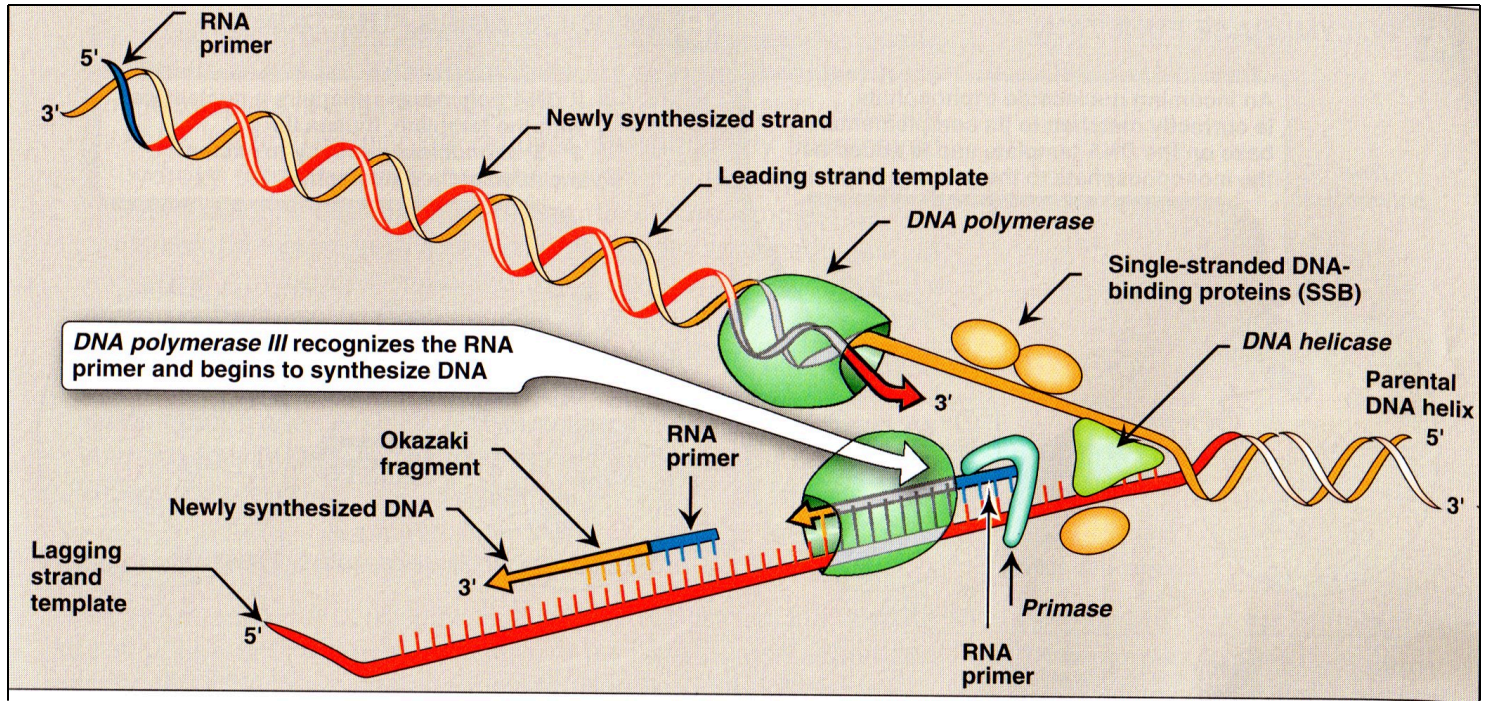
Elongation of the leading and lagging strands.



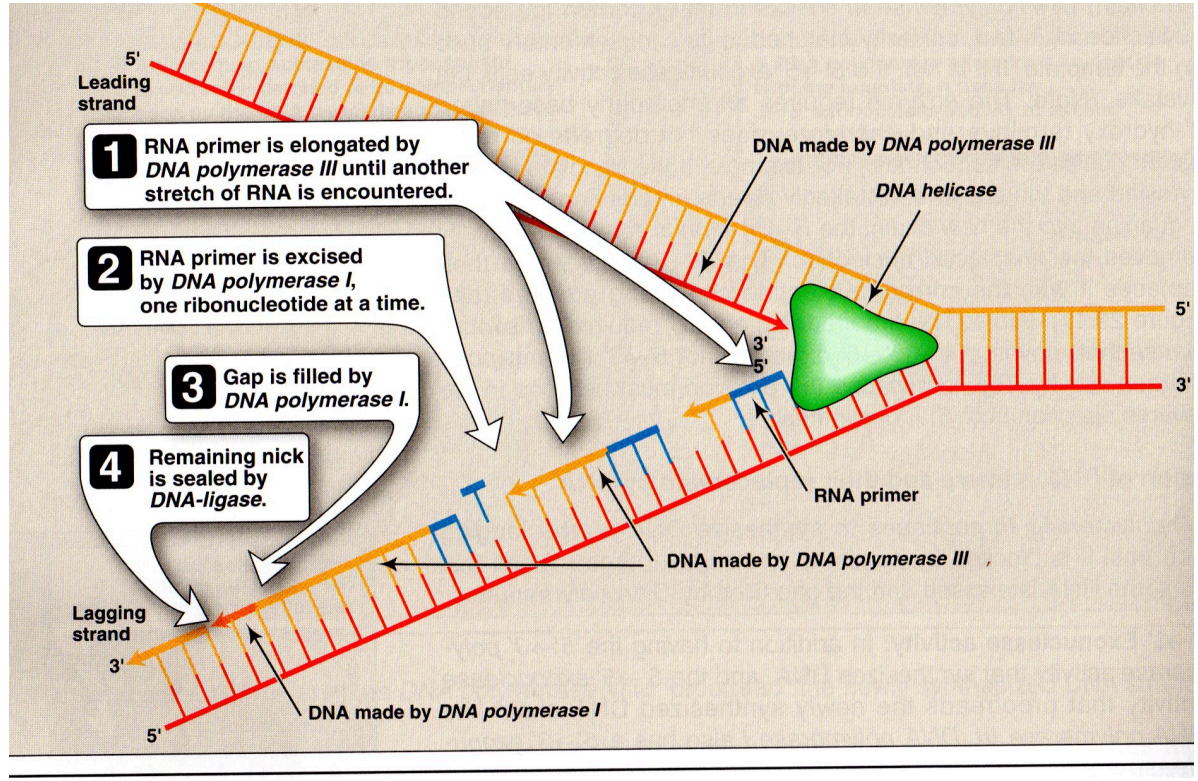
Additional enzymatic activities involved in DNA synthesis:

- **Primase** : initiates synthesis of RNA primers.
- **Primosome**: prepriming complex plus primase
- **Ligase**: seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand by catalyzing the formation of phosphodiester bond. ATP is required.
- **3'→ 5' Exonuclease activity** of DNA pol. III and I: hydrolytically removes misplaced nucleotides from the 3' end of the growing strand.
- **5'→ 3' Exonuclease activity** of DNA pol I. Excises the RNA primer.

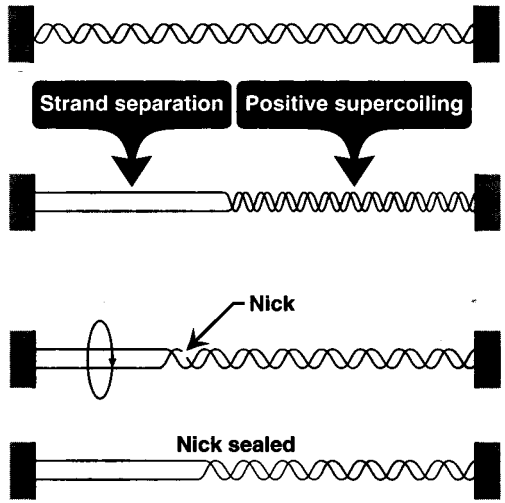
Elongation of the leading and lagging strands.



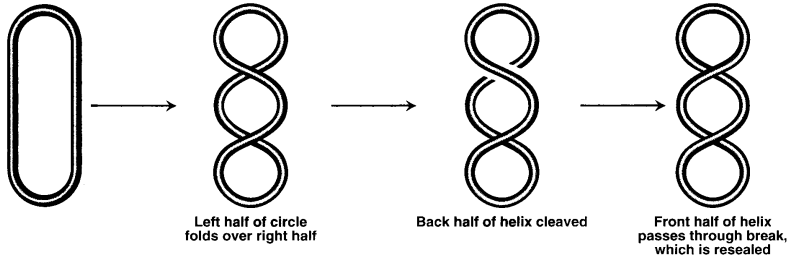
Excision of RNA primers and filling of the gaps.



The problem of DNA supercoils



Mechanism of action of Type I topoisomerase

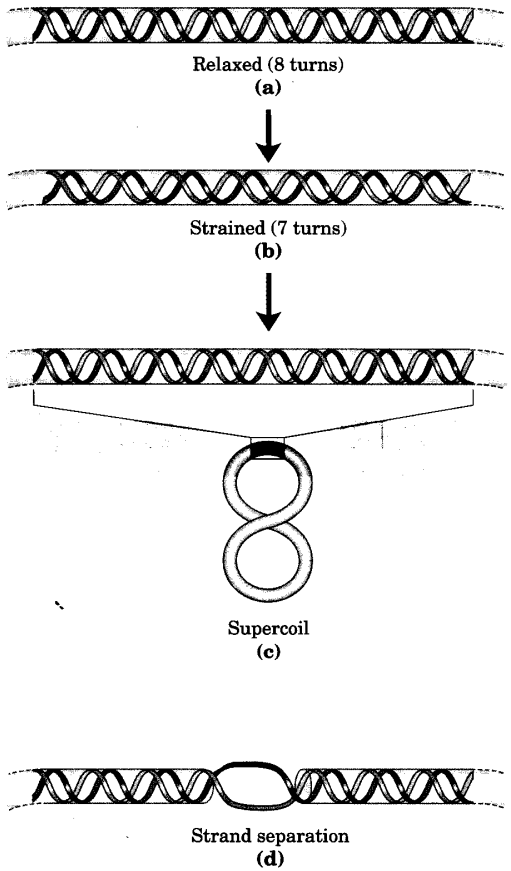


Mechanism of action of Type II topoisomerase

Type I topoisomerases:
- cut a single strand of the double helix
- have both nuclease and ligase activities
- do not require ATP
- relaxes negative supercoils in bacteria, negative and positive supercoils in eukaryotic cells.

Type II topoisomerases:
- bind to both strands of the double helix
- relaxes both negative and positive supercoils in prokaryotic and eukaryotic cells
- required for the separation of the interlocked molecules of DNA following chromosomal replication
- require ATP
- anticancer drugs such as **etoposide** target the human type II topoisomerase

Effects of negative supercoiling (underwining)



DNA gyrase:

-Unusual type II topoisomerase found in *E. coli*.

-introduces negative supercoils in to resting circular DNA, facilitating future replication

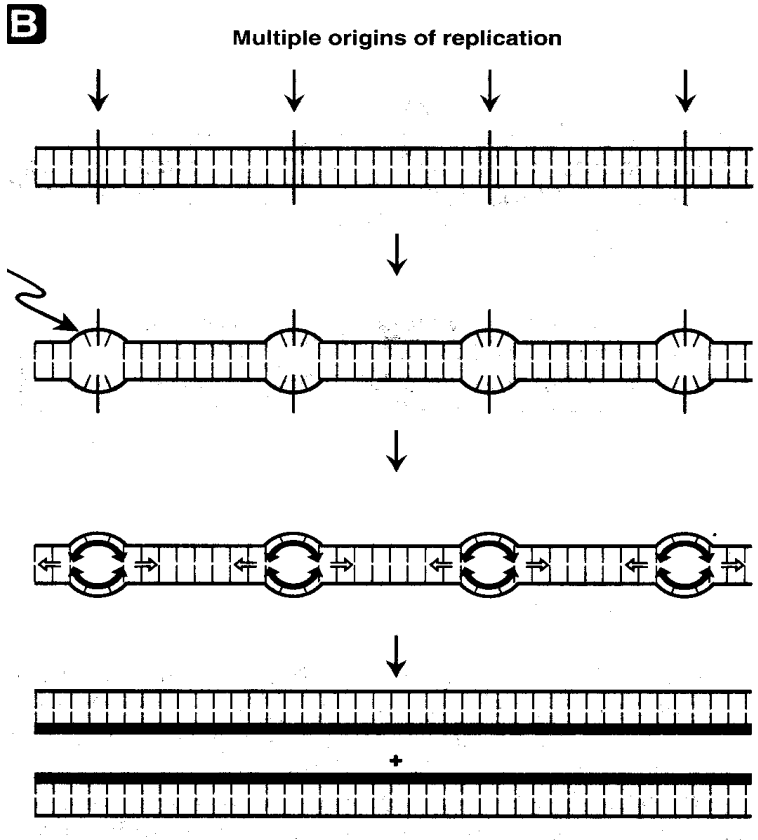
- requires ATP

- inactivated by **quinolones**

Regulation of replication in *E.coli*

- The rate of replication is regulated at the phase of initiation, such that replication occurs only once in each cell cycle.
- The mechanism of regulation of replication initiation in *E.coli* involves methylation of the adenine in conserved pallindromic sequence 5' -GATC-3' at the *oriC* (about 11 of them) by Dam methylase.
- The hemimethylated DNA strands interact with the plasma membrane and are therefore sequestered.
- After cell division, the *oriC* is released from the plasma membrane and must be again methylated in order to bind DnaA and initiate replication.

Replication in eukaryotic cells



Human chromosomal DNA has multiple sites of origin of replication, (replicators) one every ~30,000 to 300,000 bp

Initiation of replication all eukaryotic cells requires the ORC (origin recognition complex) protein.

Rate of replication fork movement in eukaryotes is 50 nt/sec, about 20 slower than in *E. coli*.

Eukaryotic DNA polymerases

Polymerase	Function	Proofreading
Pol α	Contains primase Initiates DNA synthesis	-
Pol β	Repair	-
Pol γ	Replicates mitocondrial DNA	+
Pol δ	Elongates leading strands and Okazaki fragments	+
Pol ε	Repair	+

Telomerase

- Linear eukaryotic chromosomes become shorter with each replication because the removal of the RNA primer at the 5’ end leaves single-stranded DNA that is susceptible to degradation by nucleases
- Telomeres are repetitive DNA sequences (mostly T’s and G’s) complexed with proteins which are found at the ends of the eukaryotic chromosomes.
- Their function is to protect the ends of the chromosomes from getting shorter with each chromosomal replication
- Chromosomal shortening is associated with aging and death.
- Cells that do not age (germ-line cells, cancer cells) express telomerase, a special kind of reverse-transcriptase that is responsible for replacing DNA from the telomeres.
- Telomerase is an attractive target for anti-cancer chemotherapy.

Nucleoside analogues inhibit DNA synthesis

- Nucleoside analogues carry modifications in the sugar portion of the nucleoside
- They inhibit DNA synthesis, therefore can be used to slow down the division of rapidly growing cells.
- Clinical applications:
  - Anticancer chemotherapy: cytarabine (araC)
  - Antiviral drugs: zidovudine (AZT), vidarabine (araA).