DNA REPLICATION IN PROKARYOTES

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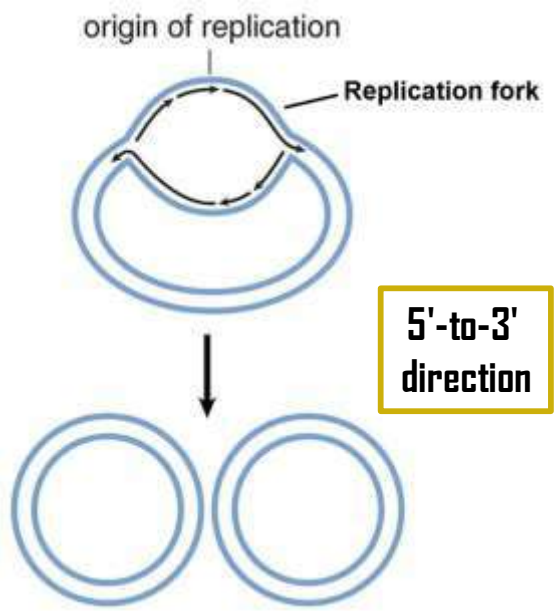
Introduction

- The prokaryotic chromosome is a circular molecule.
- They are supercoiled structure that are suspended in cytoplasm.
- They are negatively supercoiled during normal growth.
- Most prokaryotes contain single circular genome, however some have multiple copies of genome, such as *Vibrio cholerae (Cholera)* contains 2 genomes (Trucksis et. al., 1998) and *Borrelia burgdorferi* (Lyme disease) contains up to 11 copies of genome (Ferdows & Barbour, 1989).
- *Borrelia burgdorferi* genome are not supercoiled as that of *Escherichia coli* (*E. coli*); rather; their DNA strands are diffused throughout the cell (Hinnebusch & Bendich, 1997).
- In addition to genome, most prokaryotes also contain linear or circular small DNA molecules known as plasmid, an extrachromosomal DNA molecule that encode nonessential genes. It may be found in one or multiple copies in the bacteria. *Borrelia* may contain up to 20 plasmids (Fraser *et al*., 1997).
- Plasmids are much smaller than genome often contain only 1500 kb and replicate independently.
- Replication, which is a duplication of chromosome, starts from a sequence known as the origin of replication (point at which the DNA opens up) in prokaryotes.

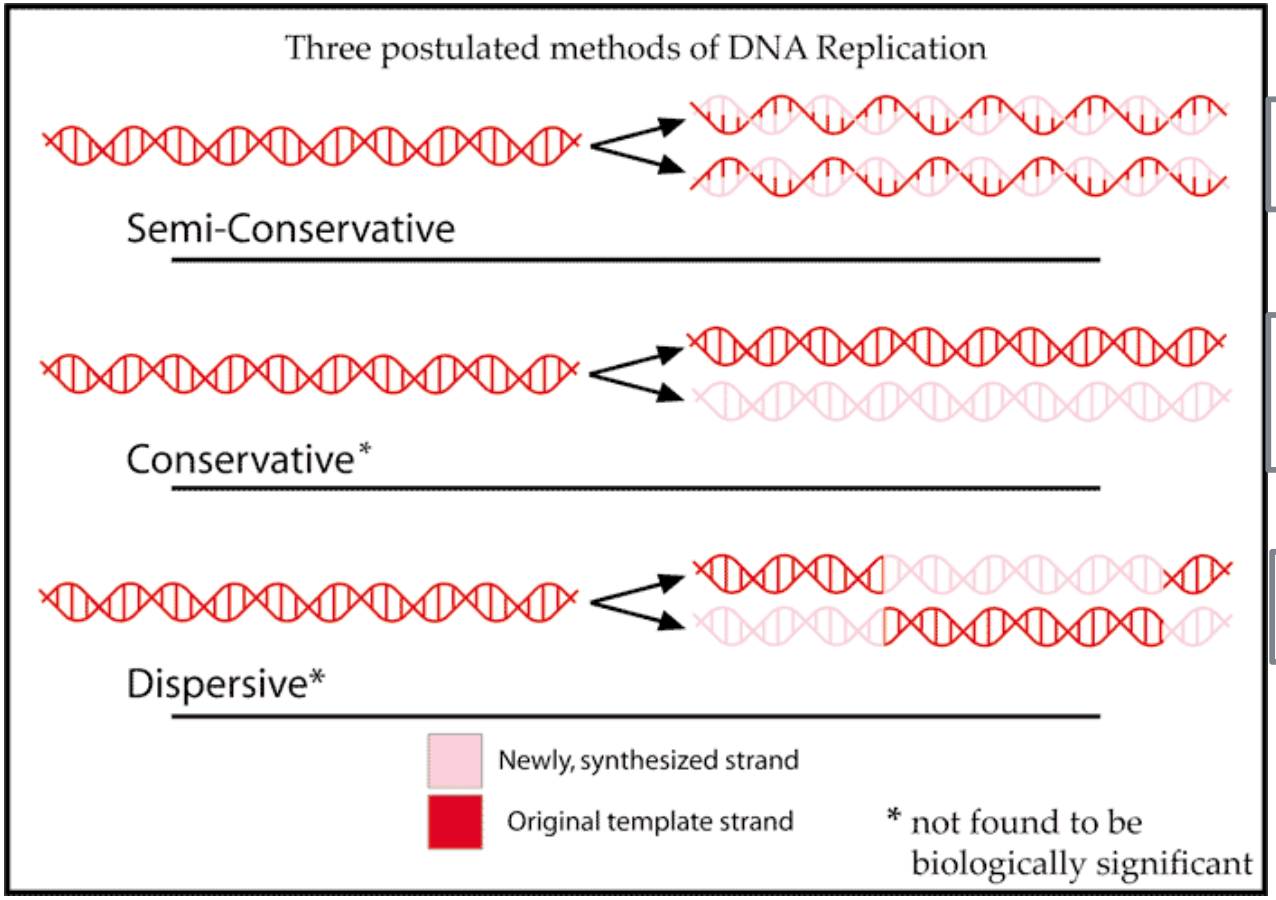
Introduction

- The specific structure of the replication origin varies somewhat from species to species, but all share some common characteristics such as high AT content.
- Prokaryotes have single replication origin per circular chromosome except Archaea have several replication origin. It is called as OriC in *E. coli*.
- It is called as theta replication as the structure resembles the Greek alphabet theta (θ).
- The replication in prokaryotes occurs in three steps:
  1.) **Initiation:** at replication origin
  2.) **Elongation:** at replication fork
  3.) **Termination:** at termination sequence
- Replication is bidirectional Y-shaped formation of replication fork runs in both directions.
- Replication is semi-continuous:
  Leading strand – continuous
  Lagging strand – fragmented **(Okazaki fragments)**



Mode of replication

- Replication is performed by semiconservative mode, i.e., one strand of DNA is conserved (template strand) while other strand is not conserved.



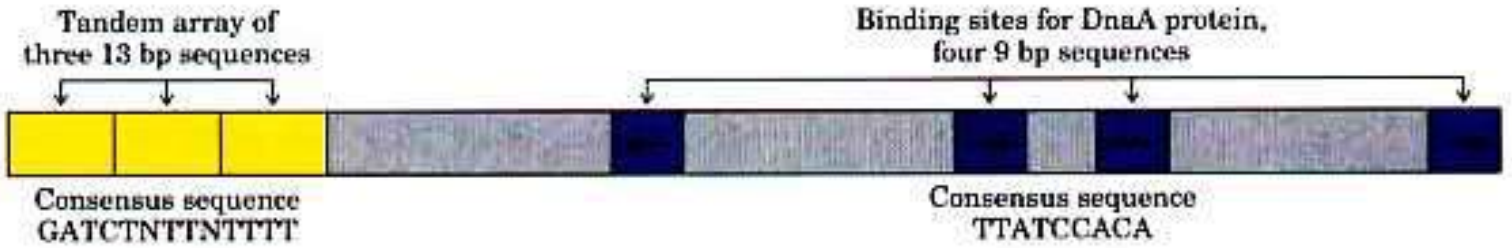
Meselson and Stahl, All cells

Donnianni and Symington, and Saini et al.

No evidence of its occurrence in nature

Initiation

- DNA replication starts with the formation of replication origin which is highly conserved among bacteria.
- The oriC replication origin of E. coli 245 base pairs.
- The key sequences oriC are two series of short repeats; three repeats of a 13 base pair sequence and four repeats of a 9 base pair sequence.



- DnaA protein binds to 9-mer DnaA box consensus sequence, 5′-TTATnCACA-3′ and results in unwinding of DNA at oriC. ***(DiaA complex : DiaA-DnaA complex)***
- Unwinding results in the recognition of DNA by other replication proteins that act subsequently in the initiation process.
- Thereafter, integration host factor binds to its single site, which causes a sharp (120–180°) bend in the double helix (Swinger and Rice, 2004).
- A complex consisting of *oriC*, IHF, DiaA, and oligomeric ATP-DnaA is considered to make up the initiation complex in *E. coli* (Keyamura et al., 2007, 2009).
- This complex finally leads to the loading of DNA helicases and thereby the formation of replisomes.

Initiation

Unbinding of DNA by helicases

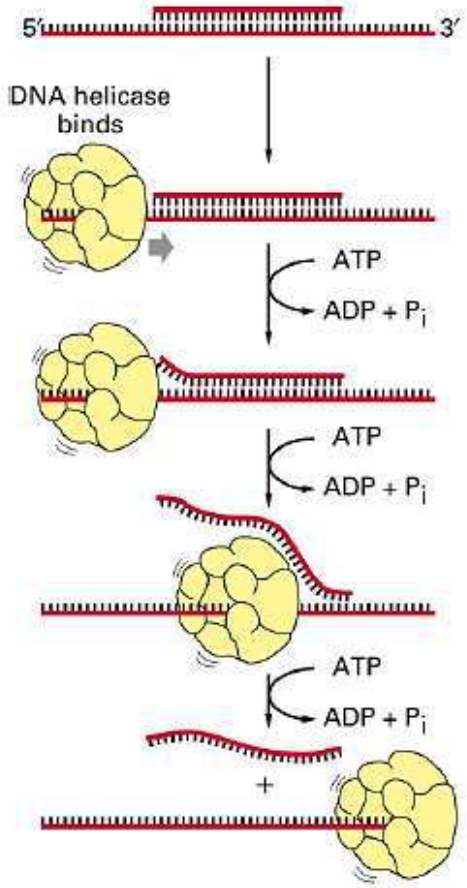


Figure 5–15. Molecular Biology of the Cell, 4th Edition.

An enzyme that unwinds the double helix by breaking the hydrogen bonds between the complimentary bases

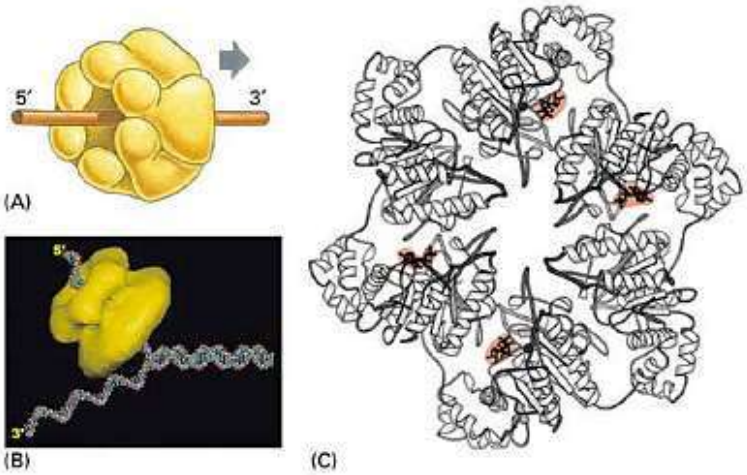
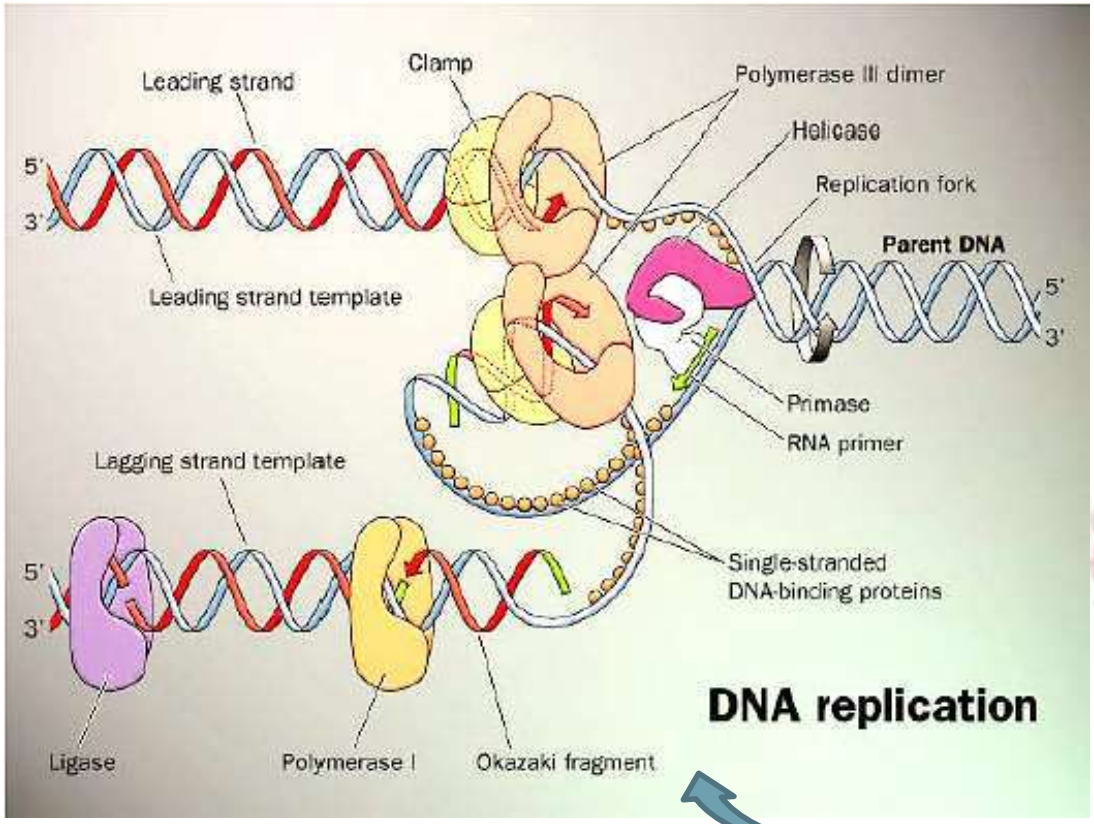
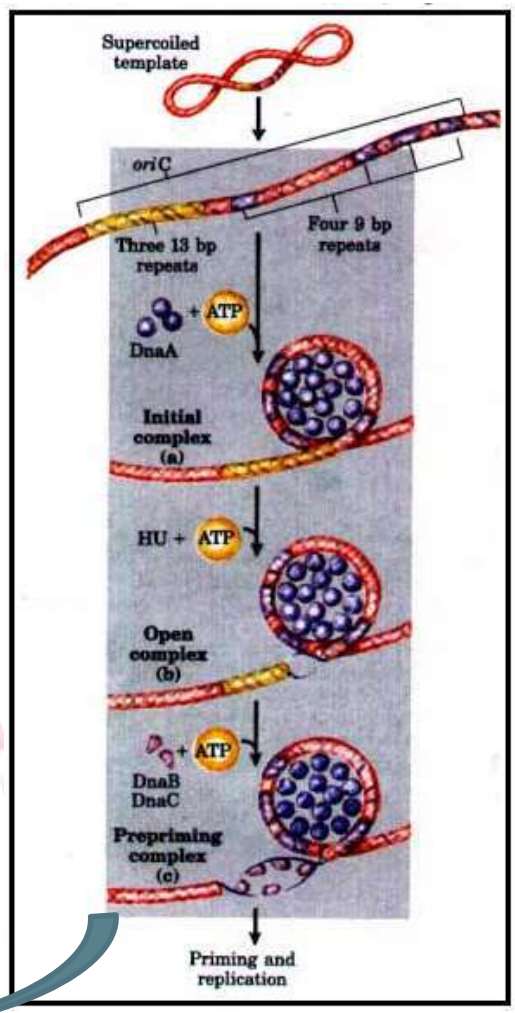


Figure 5–16. Molecular Biology of the Cell, 4th Edition.

Initiation

- Replisome – DNA ploymerases, helicases, SSBs, DNA ligase, clamps (e.g. topoisomerases).
- Replisome is a multienzyme complex of >1MDa.

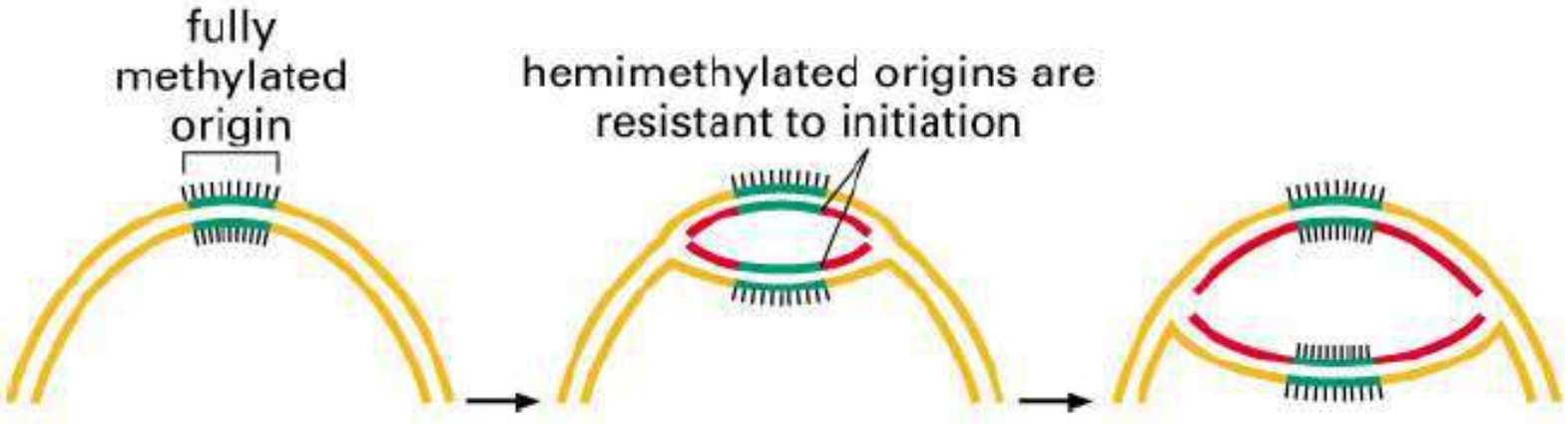




http://www.bioinfo.org.cn/book/biochemistry/chapt24/bio3.htm

Initiation

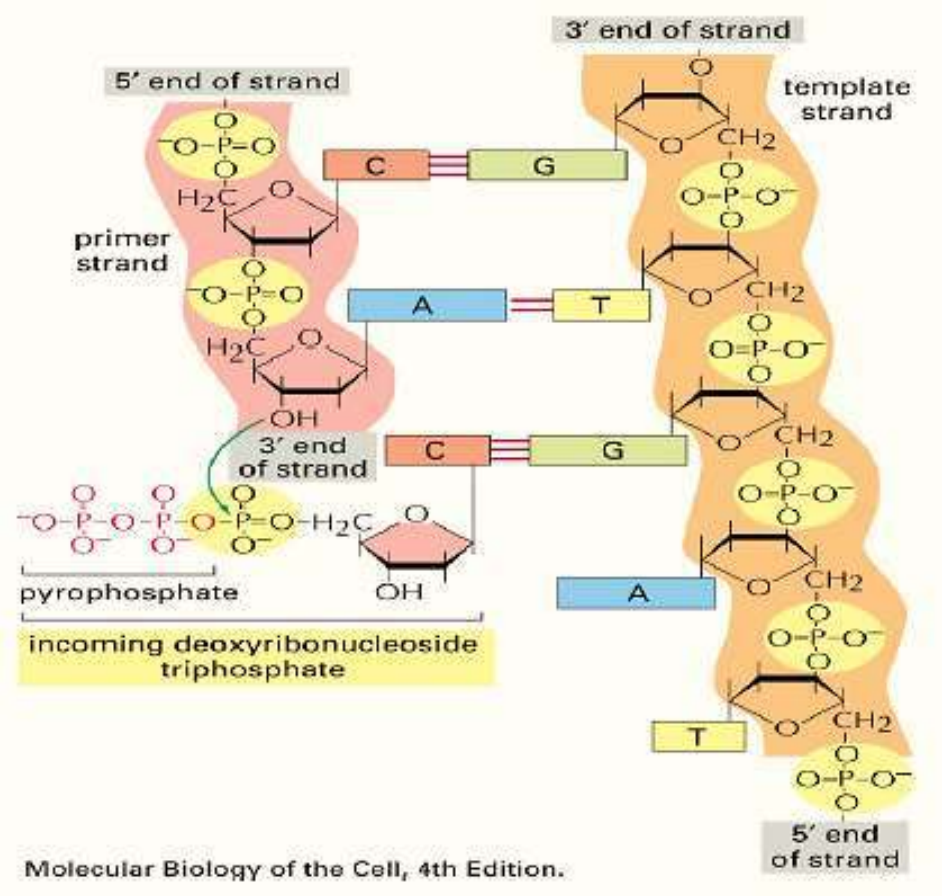
Regulation of initiation of replication



Molecular Biology of the Cell, 4th Edition.

Elongation

- Elongation of new DNA at a replication fork.
  - It is catalyzed by enzymes called DNA polymerases, which add nucleotides to the 3' end of a growing strand.



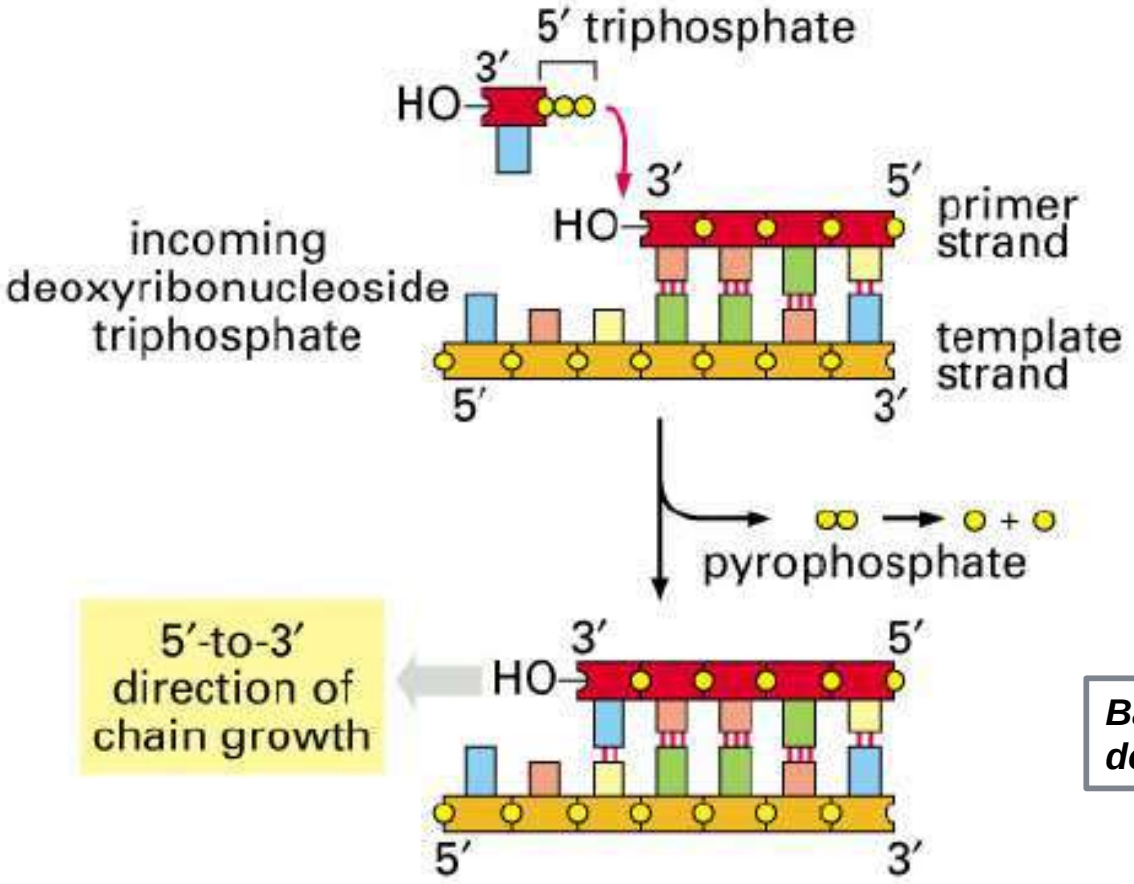
DNA polymerase adds nucleotides to the deoxyribose (3') ended strand in a 5' to 3' direction

Accuracy
1 error in 1 billion bases

Speed
500 nt/s in bacteria
50 nt/s in mammals

Elongation

- DNA is synthesized in 5´to 3´direction



Molecular Biology of the Cell, 4th Edition.

Bacterial chromosome doubles in 40 min

Elongation

RNA polymerase or DNA primase

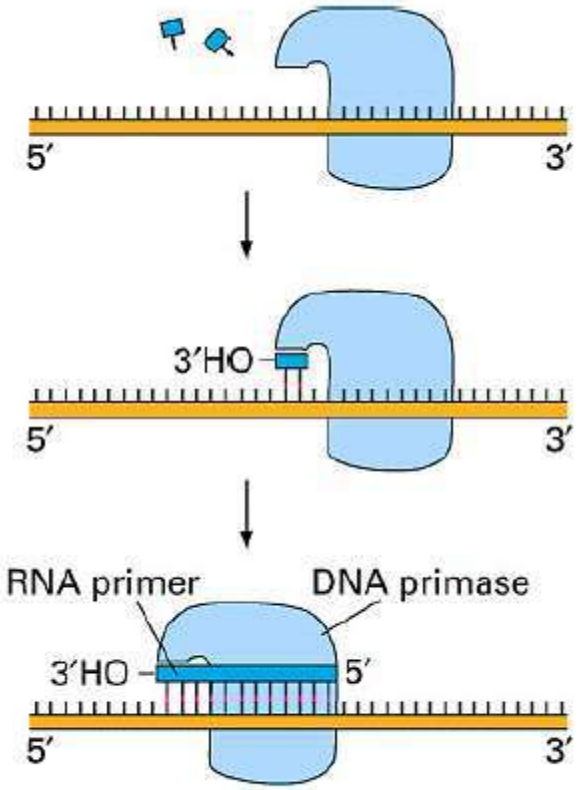


Figure 5–12. Molecular Biology of the Cell, 4th Edition.

- ***Makes the 10 nt RNA primer required for start of replication in the beginning of leading strand.***
- ***In beginning of each Okazaki-Fragment, it synthesizes primer sequences of ribose nucleotide.***

Elongation

- Topoisomerase in initiation complex prevents over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up by breaking the strand.

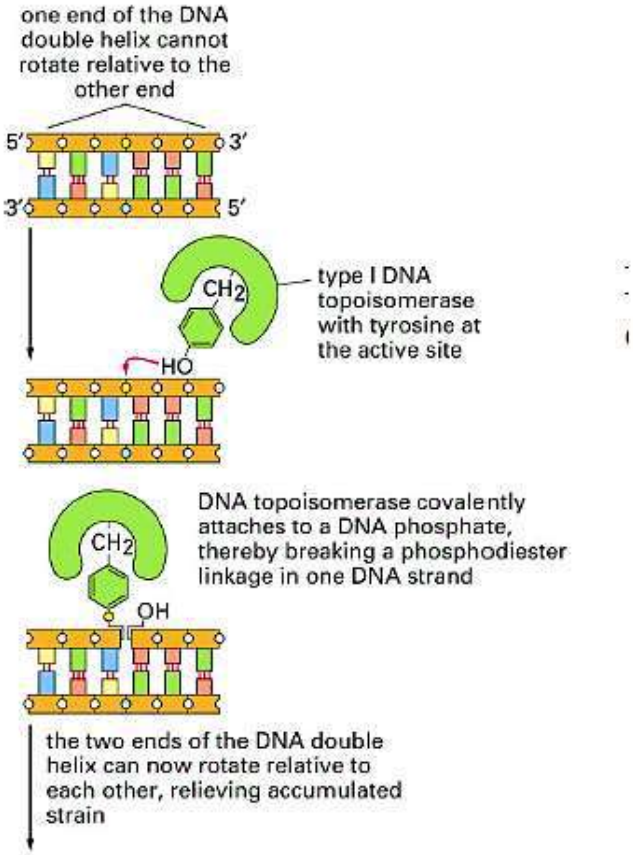


Figure 5–25 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

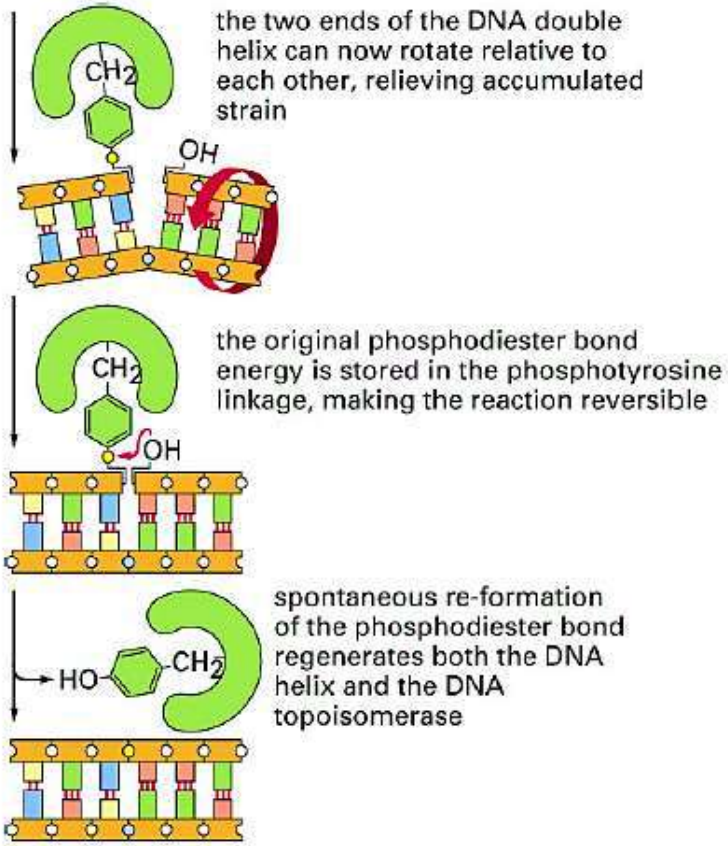
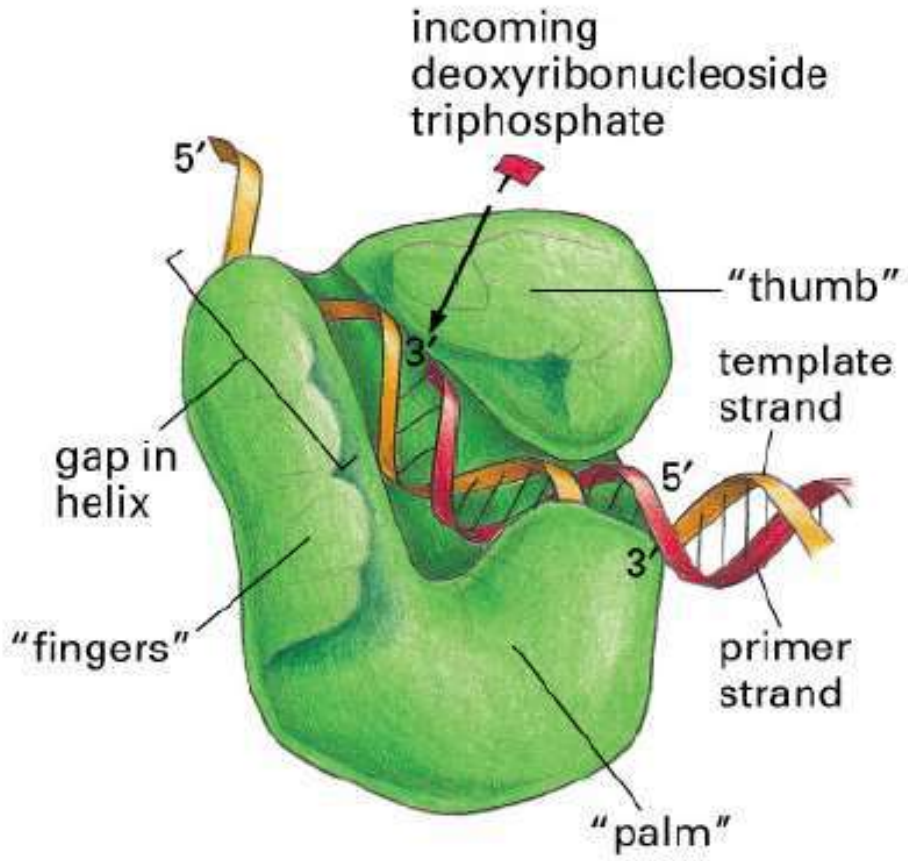


Figure 5–25 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Elongation

- Nucleotides are added by the DNA polymerase.



Molecular Biology of the Cell, 4th Edition.

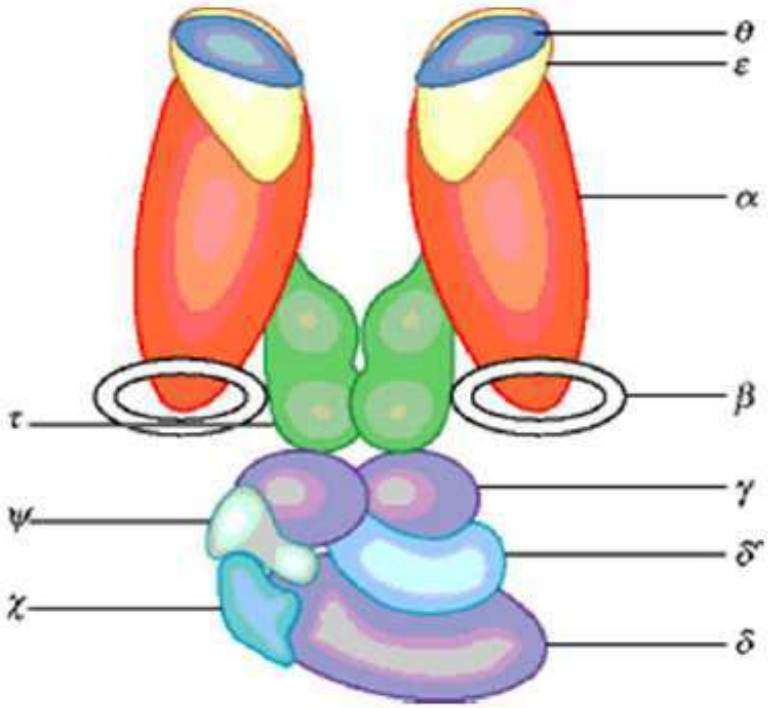
DNA polymerase requires:

1. ***A free 3'-OH group supplied by RNA Primer for start of polymerisation***
2. ***Mg2+ ions for activity in active site***

3. A template to copy

Elongation

- Nucleotides are added by the DNA polymerase. DNA polymerase is a protein complex like DNA polymerase III composed of 10 subunits as shown below.



Subunit	function
θ	not known
ε	3' exonuclease
α	polymerase
β	clamp
τ	dimerisation
γ	clamp loader
δ	
ψ	
χ	

Structure of DNA polymerase III (dnaX)

Elongation

Binding of SSBs proteins to DNA

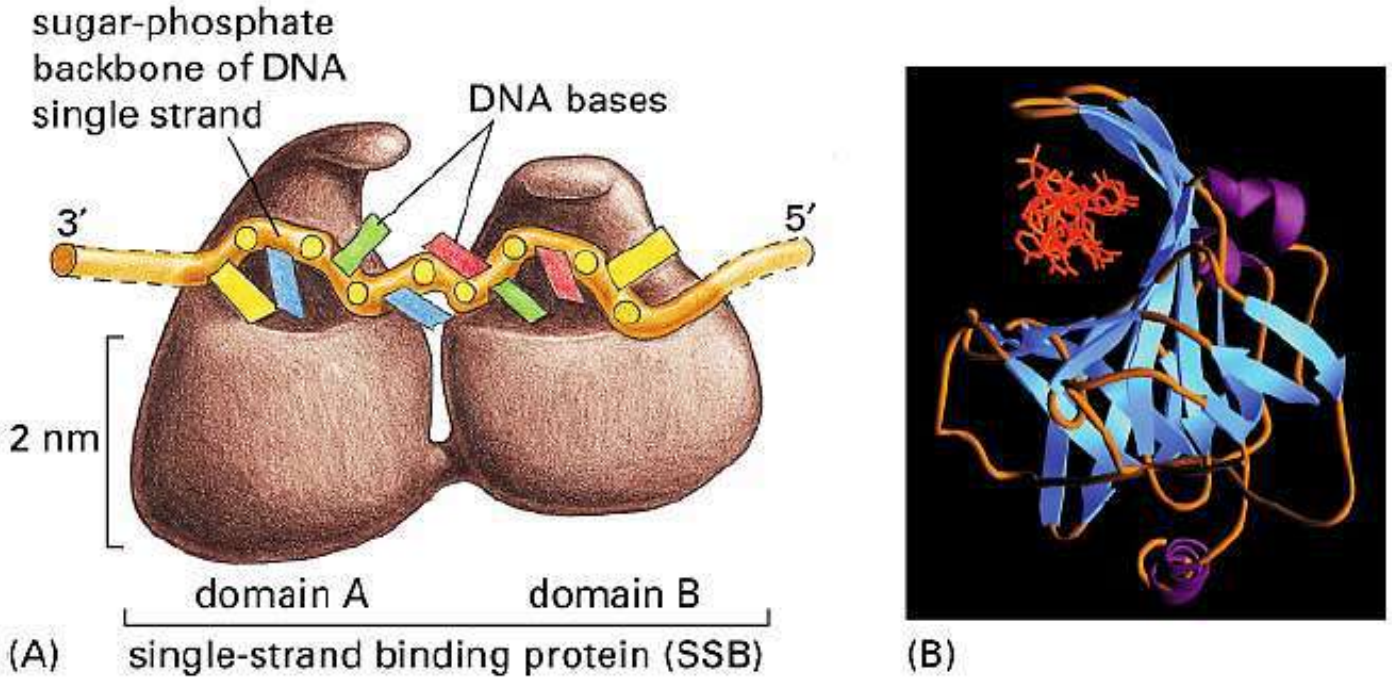


Figure 5–18. Molecular Biology of the Cell, 4th Edition.

Elongation

Binding of SSBs proteins to DNA

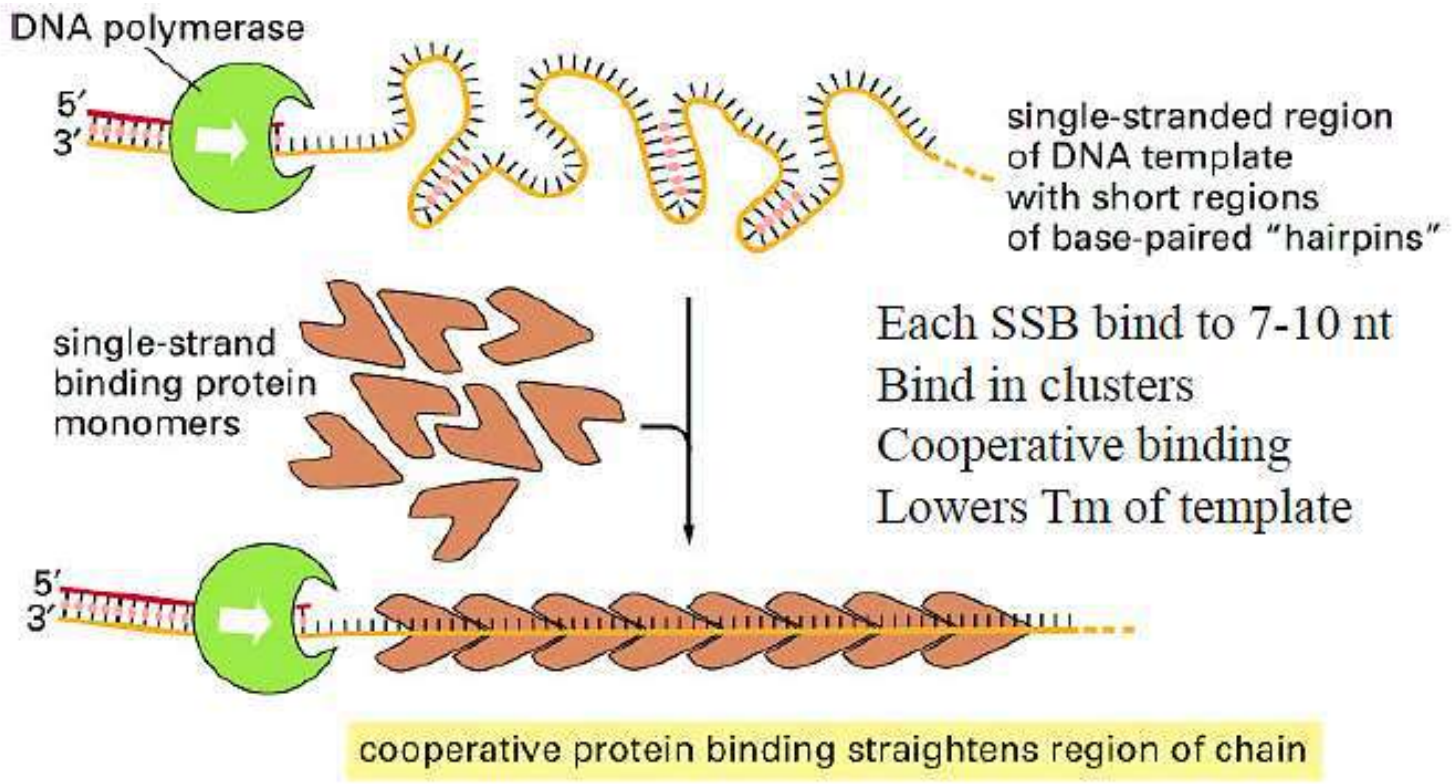


Figure 5–17. Molecular Biology of the Cell, 4th Edition.

Elongation

***E. coli* contains multiple DNA polymerases**

	DNA poly I	DNA poly II	DNA poly III
Number/cell	400	100	10
Speed (nt/s)	16-20	2-5	250-1000
3' exonuclease	Yes	Yes	No
5' exnuclease	Yes	No	No
Processitivity	3-200	10000	500000
Role	DNA repair RNA primer removal	DNA repair	Replication

- DNA poly I was found by Arthur Kornberg at mid 1950's
- It has three enzymatic activities:
  - Polymerase activity
  - 3' to 5' exonuclease activity
  - 5' to 3' exonuclease activity

Klenow enzyme is lacking one subunit responsible for the 5' to 3' exonuclease activity.

Elongation

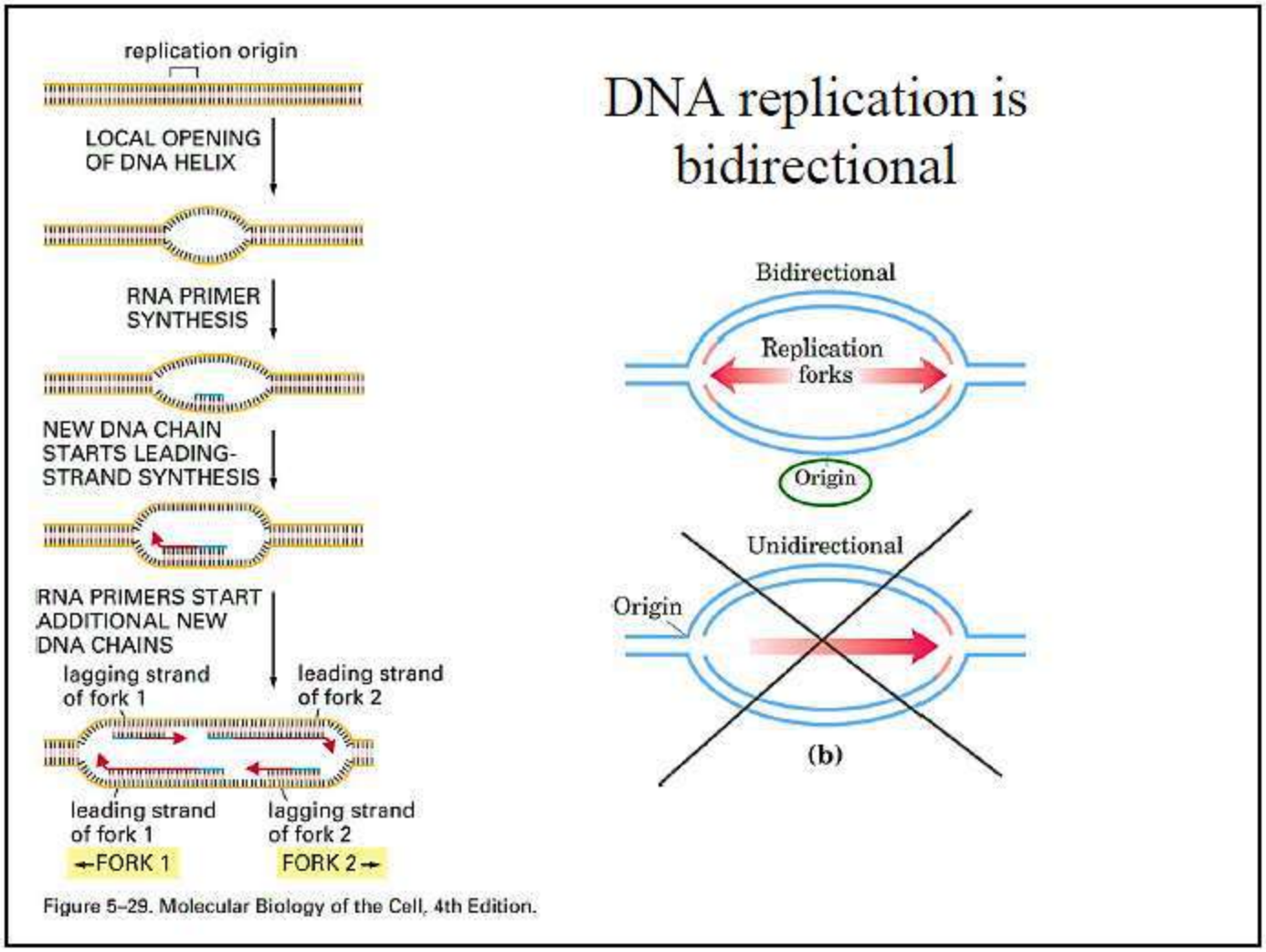


Figure 5–29. Molecular Biology of the Cell, 4th Edition.

Elongation

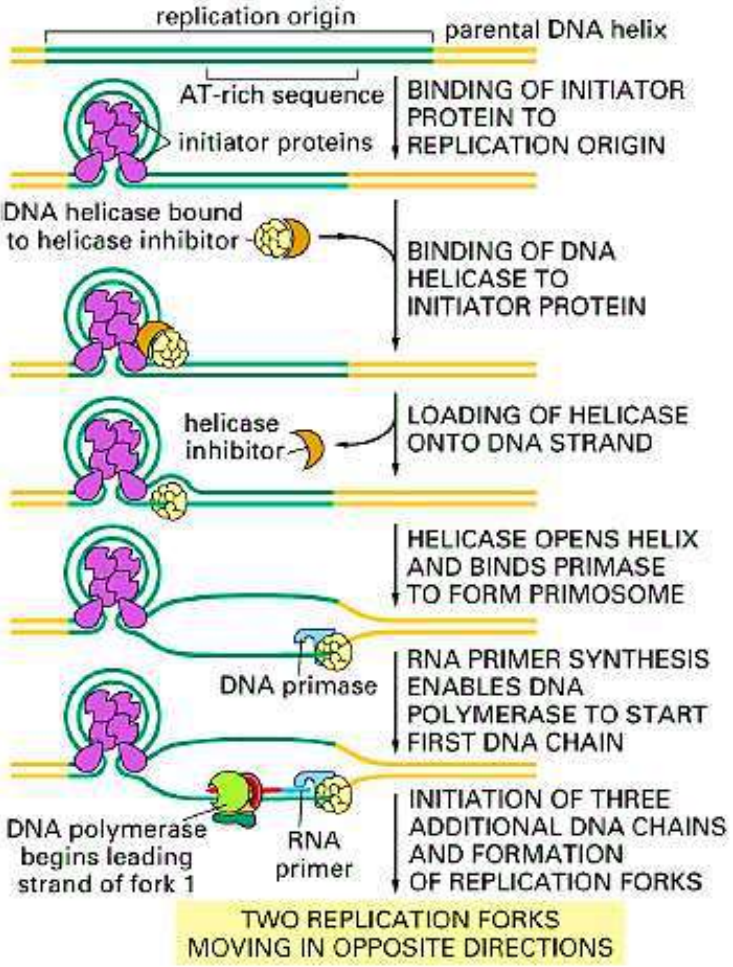
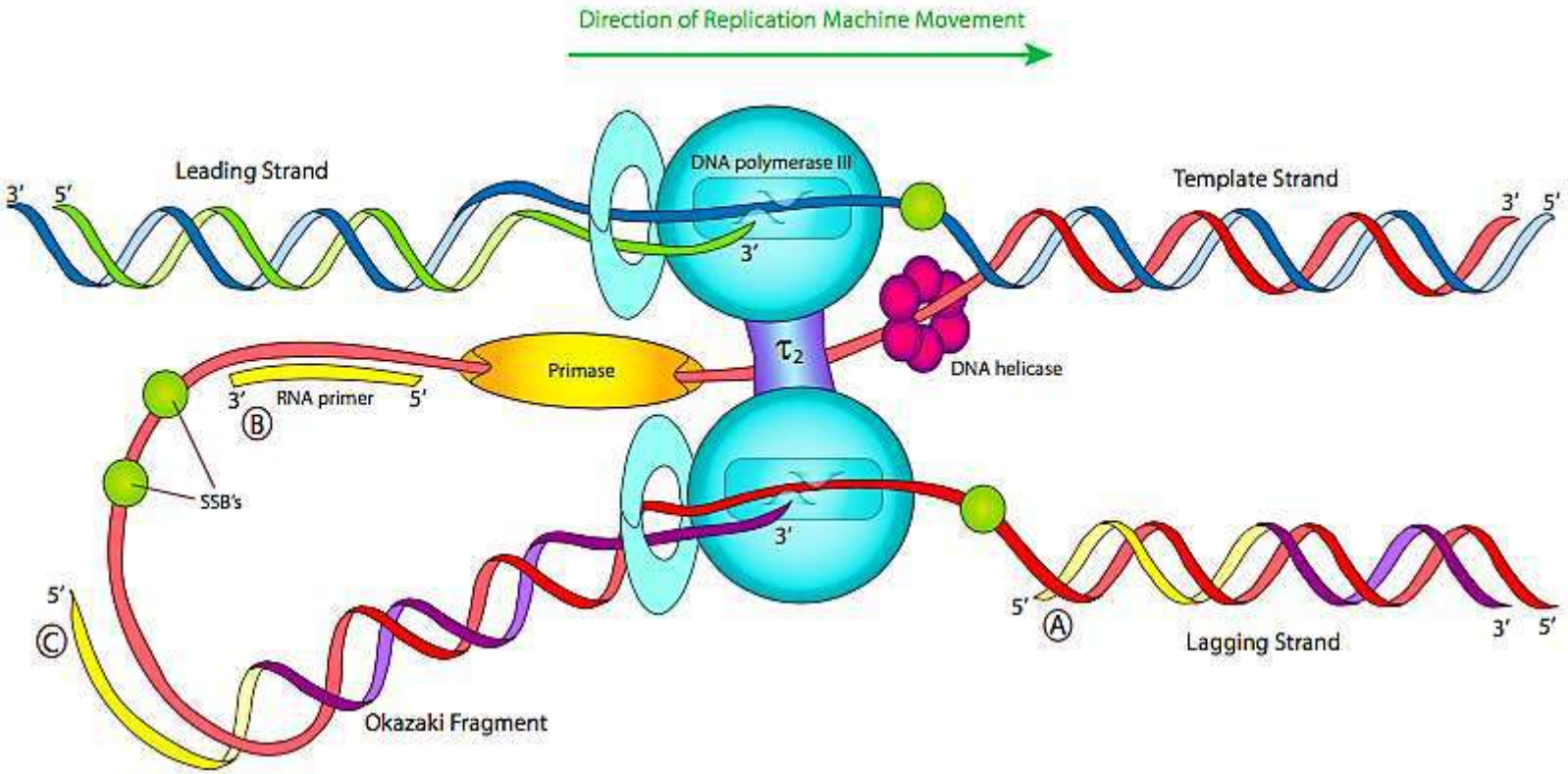


Figure 5–31. Molecular Biology of the Cell. 4th Edition.

Initiation of replication at the replication origin

Elongation

- DNA is synthesized in the replication fork in 5’ to 3’ direction



Elongation

- DNA is synthesized in the replication fork in 5’ to 3’ direction

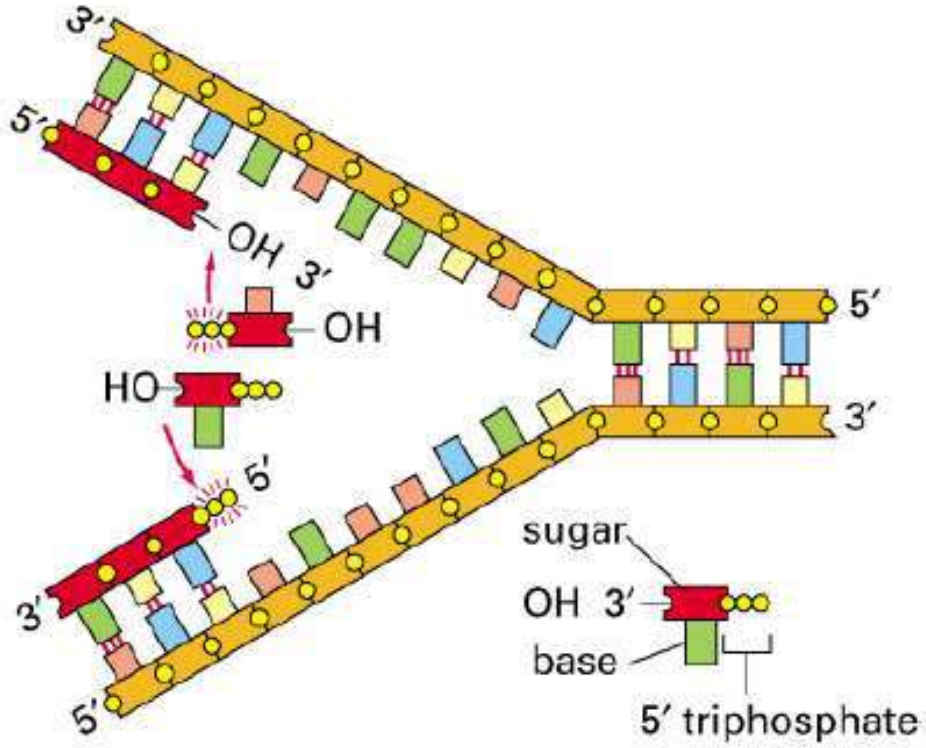


Figure 5–7. Molecular Biology of the Cell, 4th Edition.

- Here, a primer sequence is added with complementary RNA nucleotides by RNA polymerase called primase, which are then replaced by DNA nucleotides.
- DNA polymerase adds DNA nucleotides to the 3′ end of the newly synthesized polynucleotide strand.
- The template strand specifies which of the four DNA nucleotides (A, T, C, or G) is added at each position along the new chain.
- Only the nucleotide complementary to the template nucleotide at that position is added to the new strand.
- Once DNA replication is finished, the daughter molecules are made entirely of continuous DNA nucleotides, with no RNA portions.

Elongation

- Leading strand synthesis is continuous whereas lagging strand is synthesized in fragments

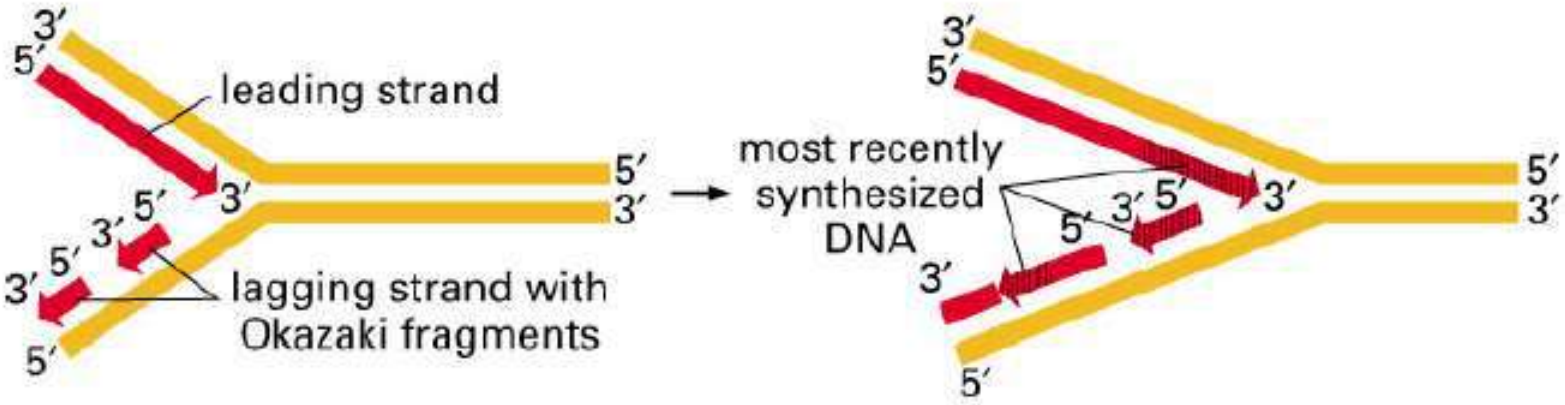


Figure 5–8. Molecular Biology of the Cell, 4th Edition.

- DNA polymerase can only synthesize new strands in the 5′ to 3′ direction.
- The “leading strand” is synthesized continuously toward the replication fork as helicase unwinds the template double-stranded DNA.
- The “lagging strand” is synthesized in the direction away from the replication fork and away from the DNA helicase unwinds.
- This lagging strand is synthesized in pieces because the DNA polymerase can only synthesize in the 5′ to 3′ direction. The pieces are called as Okazaki fragments
- Length of Okazaki fragments in prokaryotes are 1000-2000 nt.

Elongation

- **Error correction** or **proofreading:** Any error that has been left during the replication is corrected by DNA polymerase.
- Error repair is achieved with the exonuclease activity of DNA polymerase.

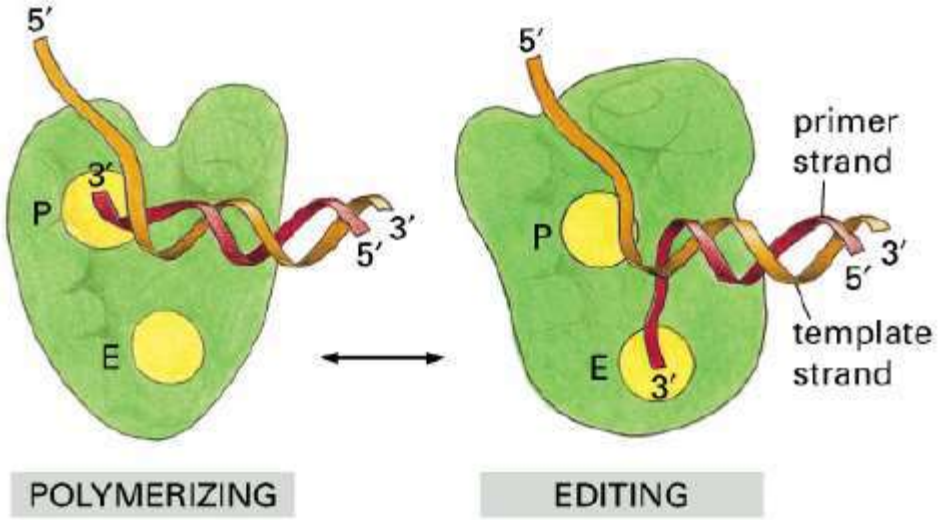


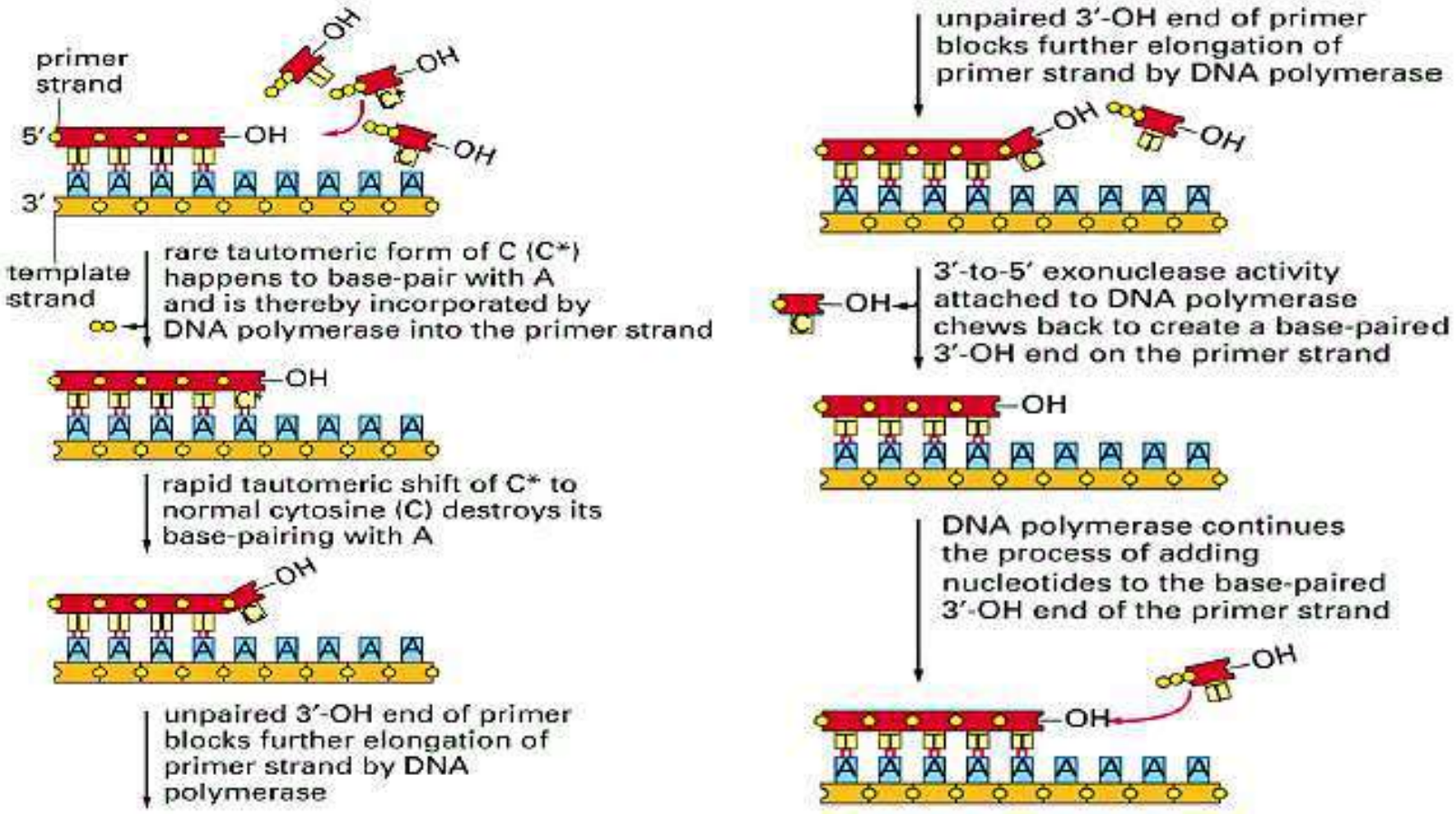
Figure 5–10. Molecular Biology of the Cell, 4th Edition.

This results in a very low error rate of 1 in 1 billion nucleotides

Elongation

Error correction

- 3’ to 5’ exonuclease activity corrects errors



Elongation

Sealing the nick of Okazaki fragments

- DNA ligase seals the nicks between Okazaki fragments
- It requires close and free 3'-OH and 5'-P and proper base-pairing
- NAD^+ required in prokaryotes

ATP required in eukaryotes

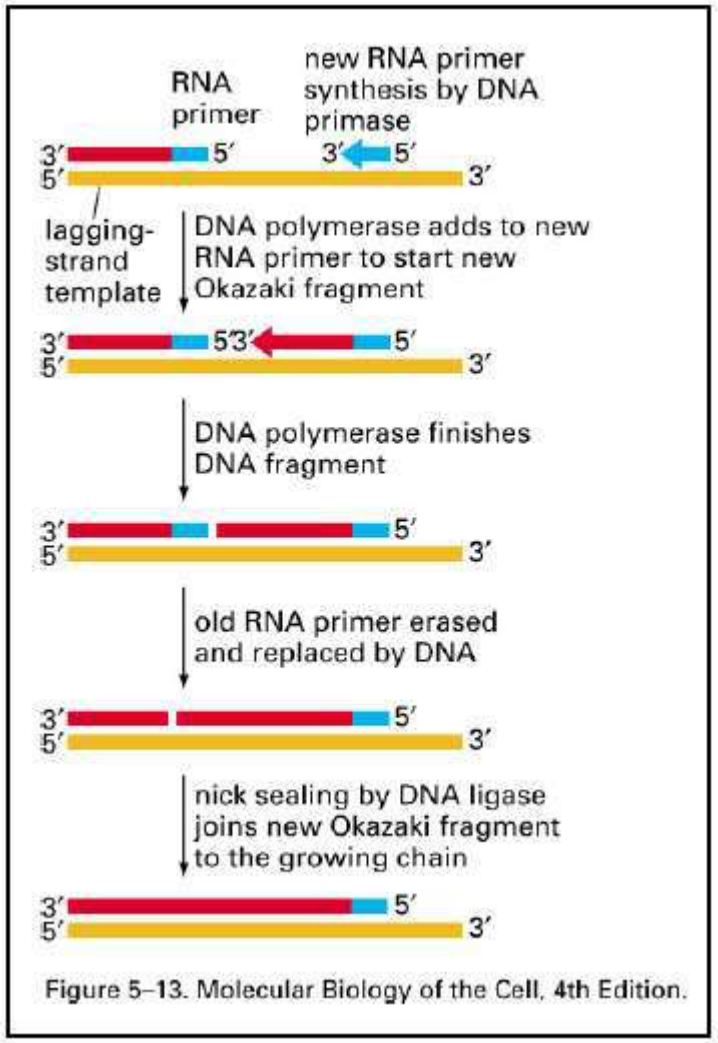
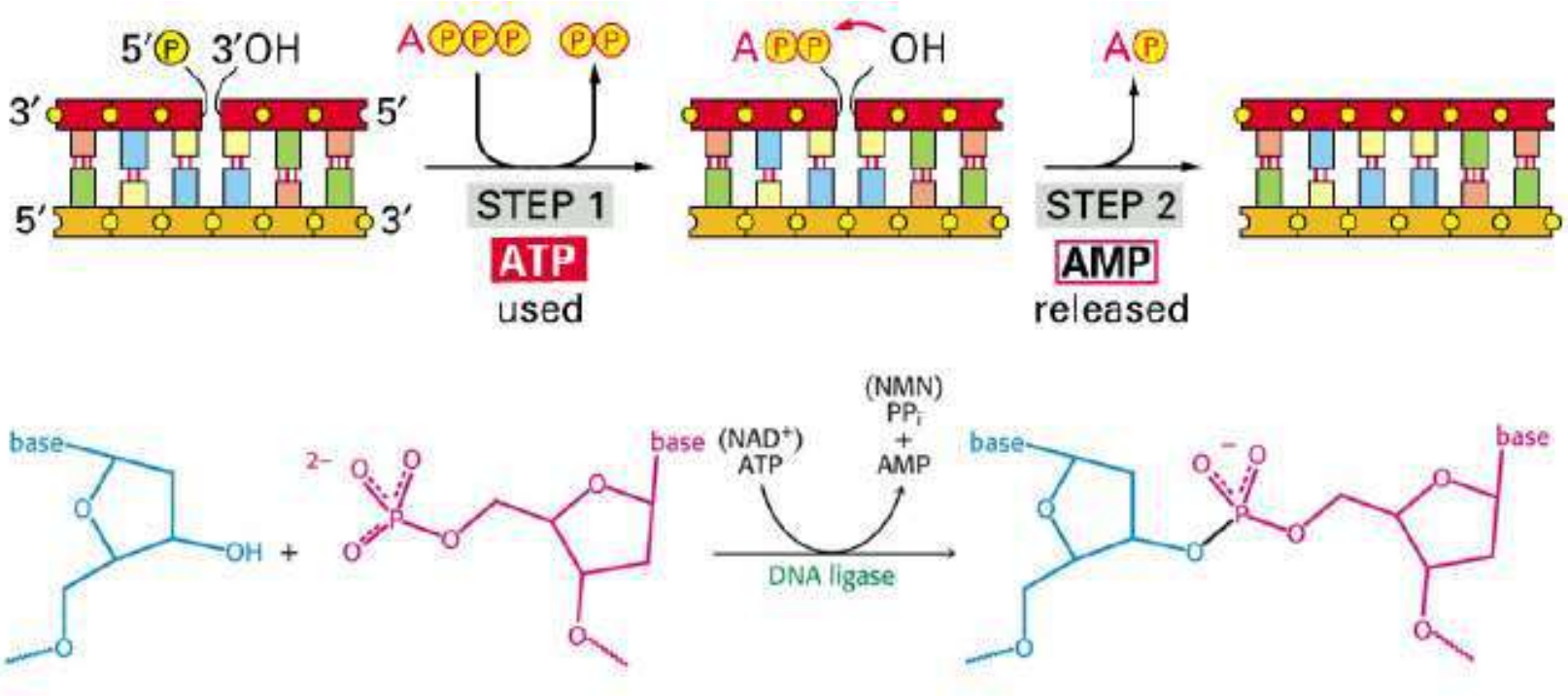


Figure 5–13. Molecular Biology of the Cell, 4th Edition.

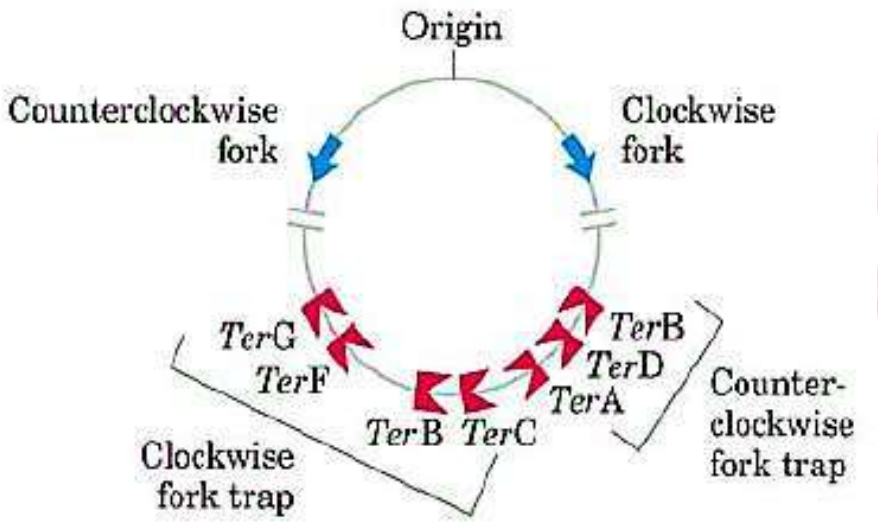
Elongation

Sealing the nick of Okazaki fragments – Nick sealing by DNA ligase



Termination

Termination of replication takes place at the termination site in the prokaryotic DNA



Terminus **U**tilization **S**ubstance **(Tus)**

- ***DNA replication terminates when replication forks reach specific 'termination sites', i.e. replication forks meet each other on the opposite end of the parental circular DNA.***
- ***The two replication forks are synchronized by 10-23 bp Ter sequences that bind Tus proteins***
- ***Tus proteins can only be displaced by replisomes coming from one direction***

- ***Tus protein binds to terminator sequences (Ter sequence) and acts as a counter-helicase when it comes in contact with an advancing helicase. The bound Tus protein effectively halts DNA polymerase movement.***

Enzymes and Proteins of Prokaryotic DNA Replication

Enzyme/protein	Specific function
DNA pol I	Exonuclease activity removes RNA primer and replaces with newly synthesized DNA
DNA pol II	Repair function
DNA pol III	Main enzyme that adds nucleotides in the 5'-3' direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Topoisomerase	Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA
Single-strand binding proteins (SSB)	Binds to single-stranded DNA to avoid DNA rewinding back.

Steps of the DNA replication in prokaryotes

- DNA unwinds at the origin of replication.
- Helicase opens up the DNA-forming replication forks; these are extended bidirectionally.
- Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
- Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling.
- Primase synthesizes RNA primers complementary to the DNA strand.
- DNA polymerase starts adding nucleotides to the 3'-OH end of the primer.
- Elongation of both the lagging and the leading strand continues.
- RNA primers are removed by exonuclease activity.
- Gaps are filled by DNA pol by adding dNTPs.
- The gap between the two DNA fragments is sealed by DNA ligase, which helps in the formation of phosphodiester bonds.

Steps of the DNA replication in prokaryotes

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