REPLICATION

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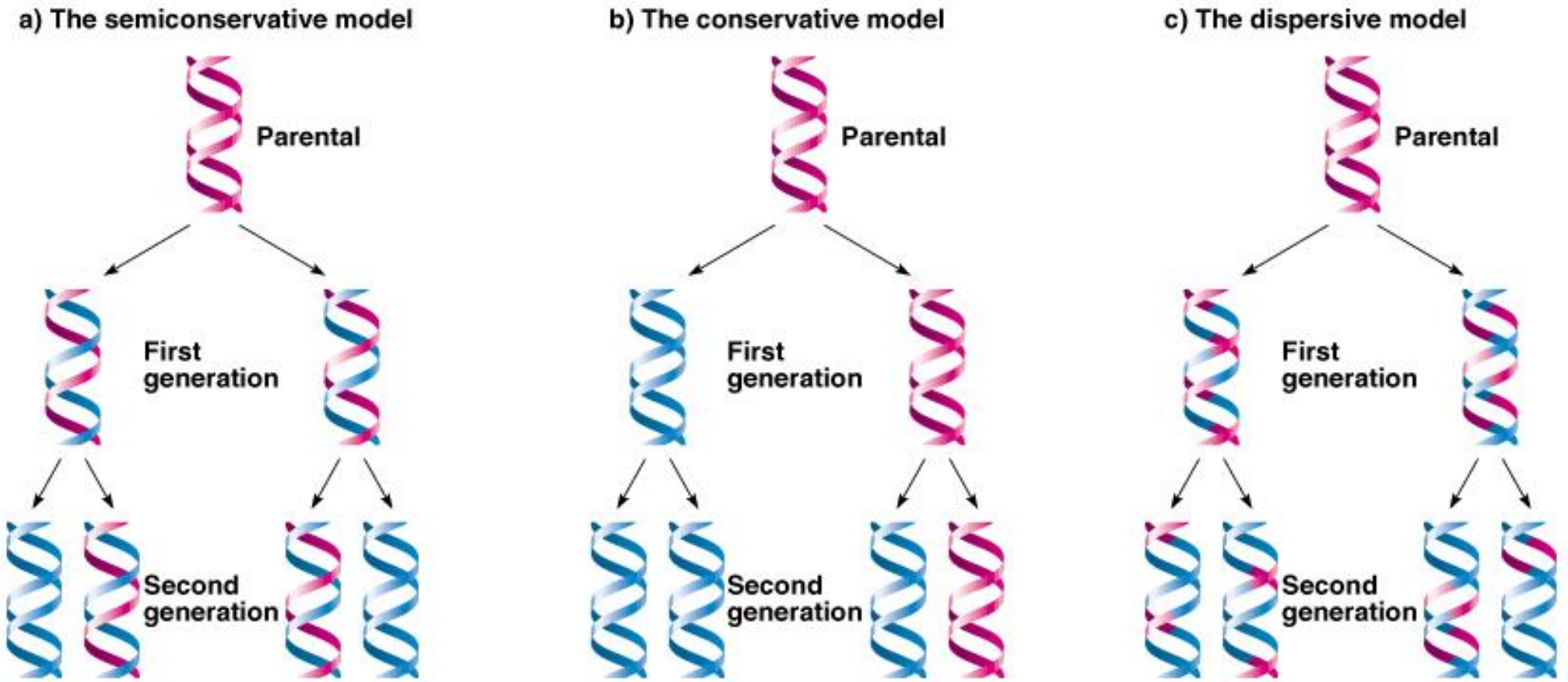
DEPARTMENT OF BOTANY

PANIHATI MAHAVIDYALAYA



1. Watson and Crick DNA model implies a mechanism for replication:
    a. Unwind the DNA molecule.
    b. Separate the two strands.
    c. Make a complementary copy for each strand.

Fig. 3.2 The Meselson-Stahl experiment, which showed that DNA replicates semiconservatively

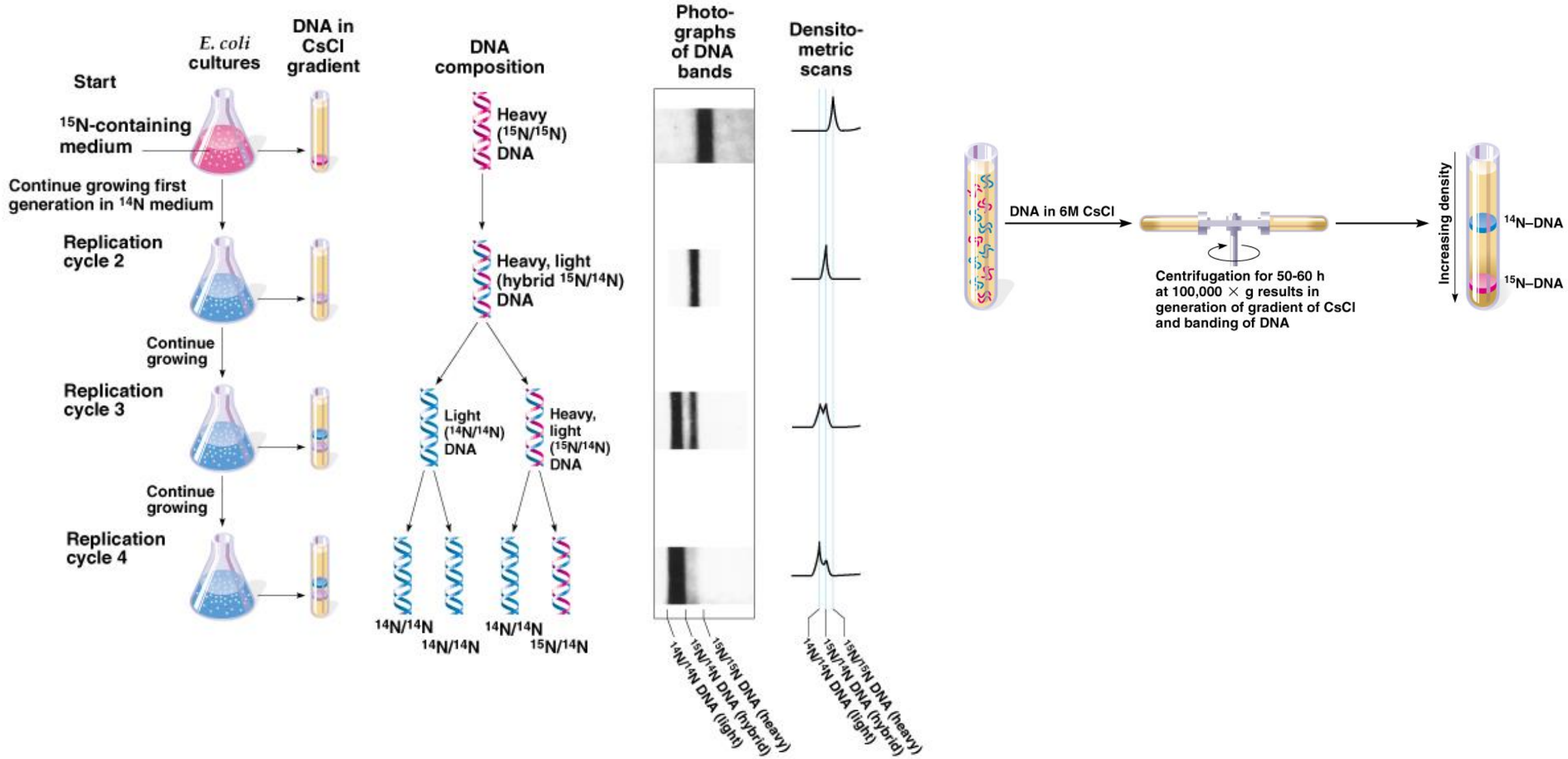
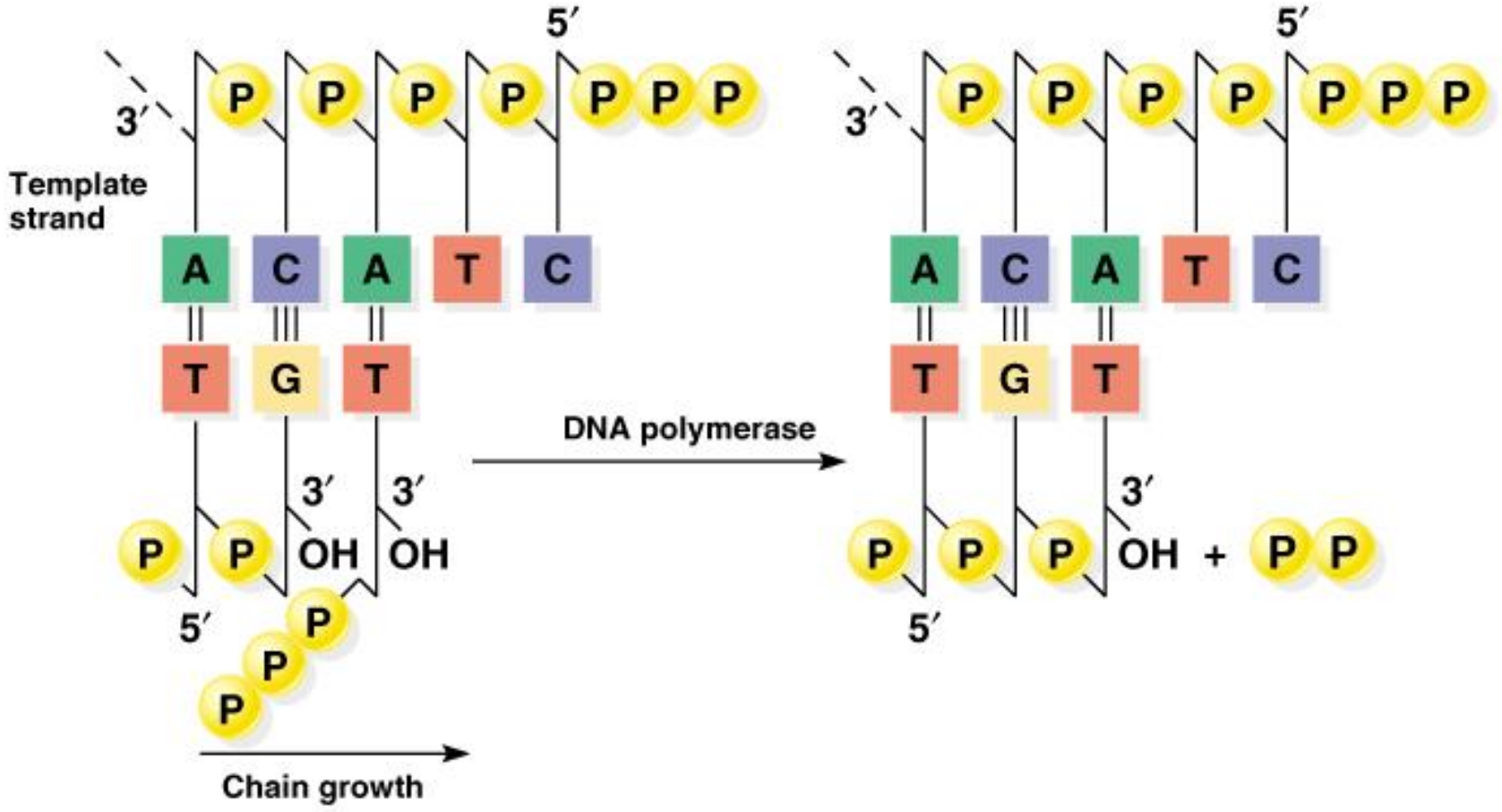
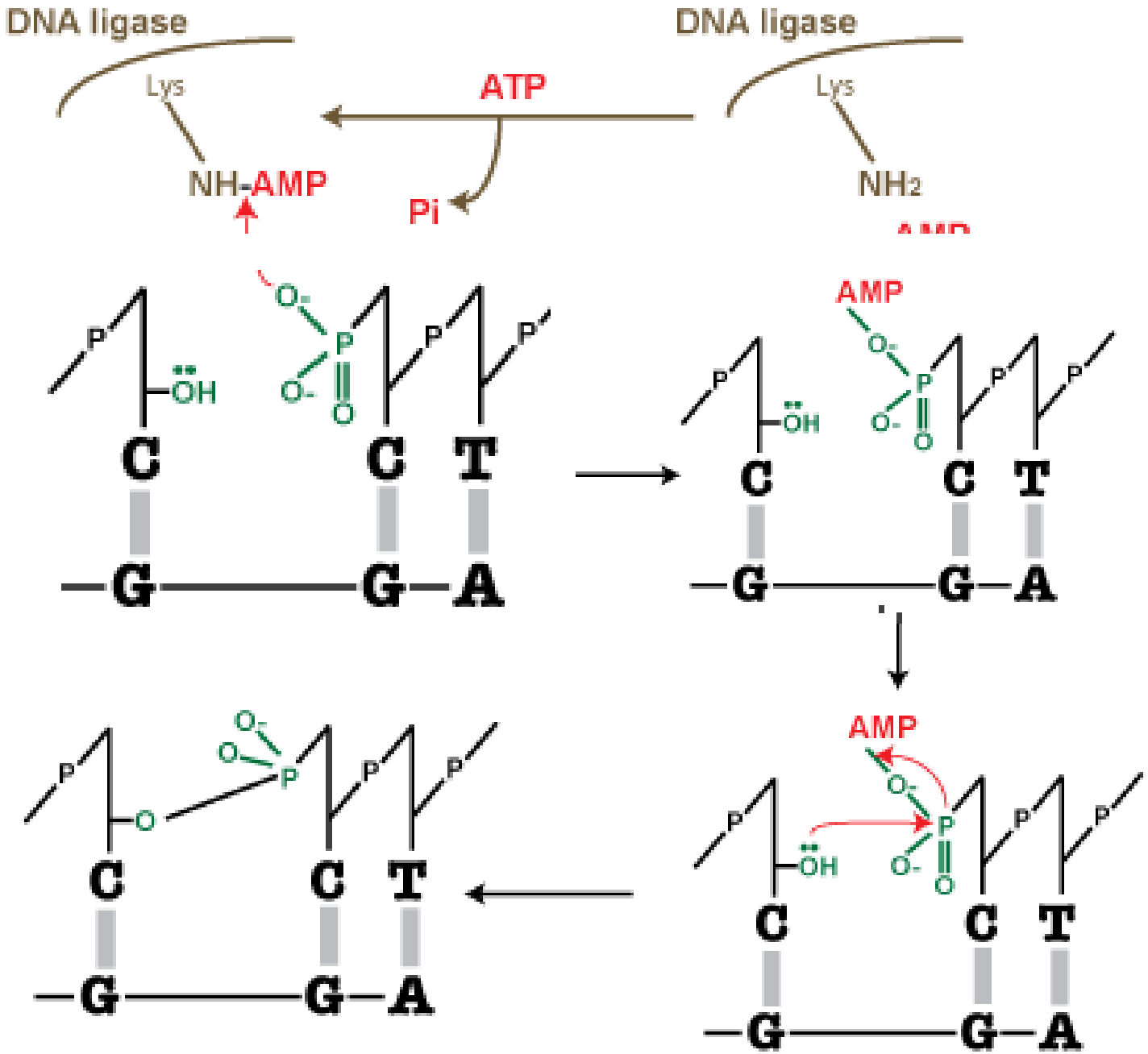


Fig. 3.4b DNA chain elongation catalyzed by DNA polymerase

DNA polymerization



b) Shorthand notation



Polymerization properties

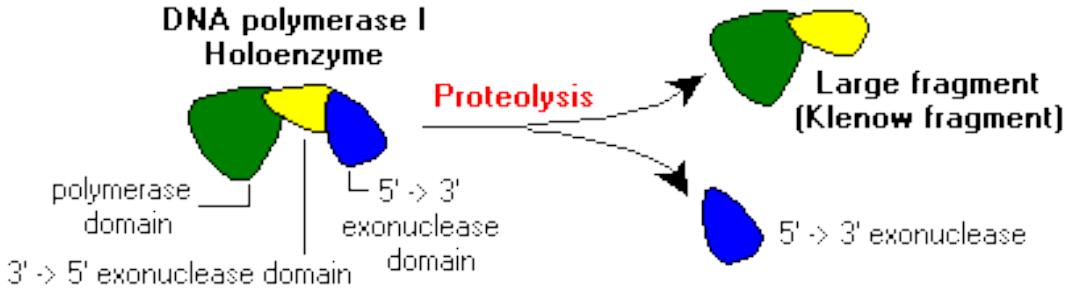
- Polymerization requires at least 2 phosphate group

DNA polymerase

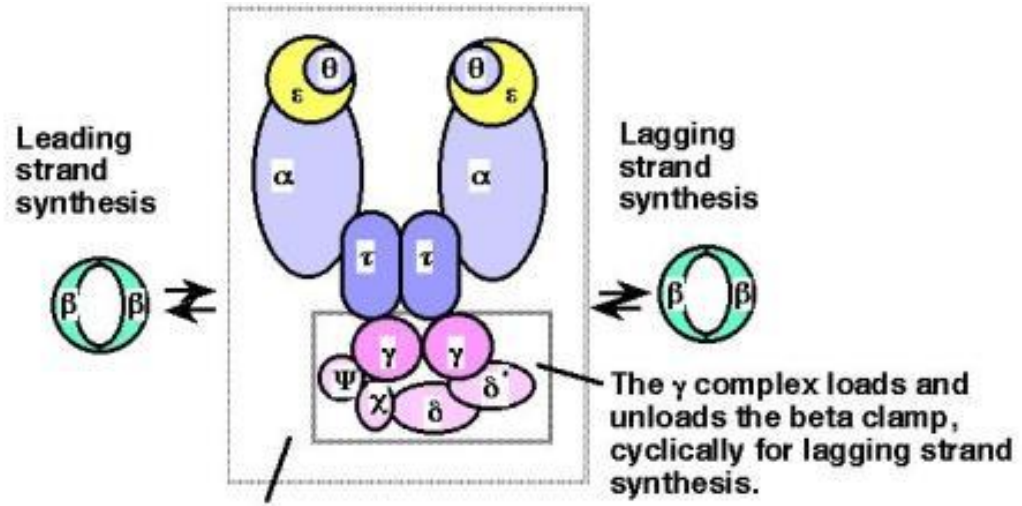
E coli	Mammalian	Function
I	Alpha	Gap filling and synthesis of lagging strand
II	Epsilon	DNA proofreading and repair
	βeta	DNA repair
	Gamma	Mitochondrial DNA synthesis
III	delta	Processive , Leading strand synthesis

	DNA pol I	DNA pol II	DNA pol III
Polymerization Rate	Low	Low	High
Processivity	Low	Low	High
Proof reading	3'-5' and 5'-3' Exonuclease activities	3'-5' Exonuclease activity	3'-5' Exonuclease activity
Primer removal	Best	Nil	Nil
Strand synthesis	Lagging strand	No role	Both strands
DNA repair	Active	Active	No role

Klenow Fragment



DNA polymerase Structure



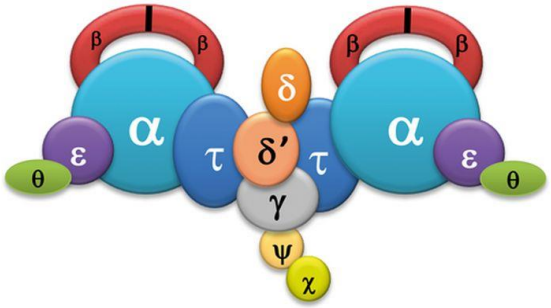
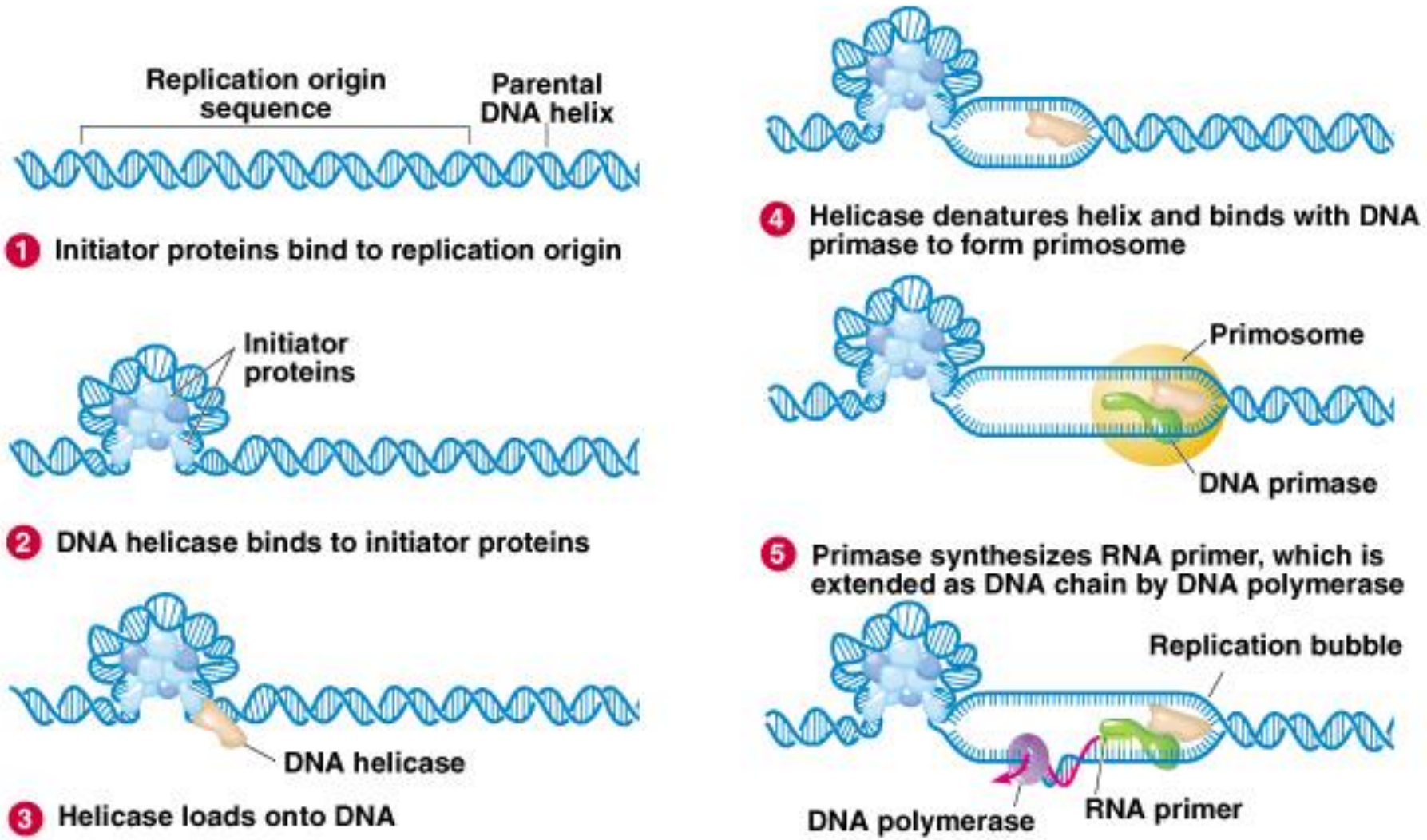
	Pol I	Pol II	Pol III	Pol IV	Pol V
DNA polymerase family	A	B	C	Y	Y
Activity	5'-3' polymerase 3'-5' exonuclease 5'-3' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase	5'-3' polymerase
	PolA	PolB		DinB	UmuC UmuD' UmuD'
Number of molecules/ cell					
– SOS	400	50 - 75	10 - 20	150 - 250	< 15
+ SOS	400	350 - 1000	10 - 20	1200 - 2500	200
Biological functions in the cell	DNA replication, Okazaki fragment maturation, DNA repair	DNA replication (backup DNA polymerase), DNA repair, TLS	DNA replication DNA repair	TLS	TLS

Fig. 3.5 Model for the formation of a replication bubble at a replication origin in *E. coli* and the initiation of the new DNA strand

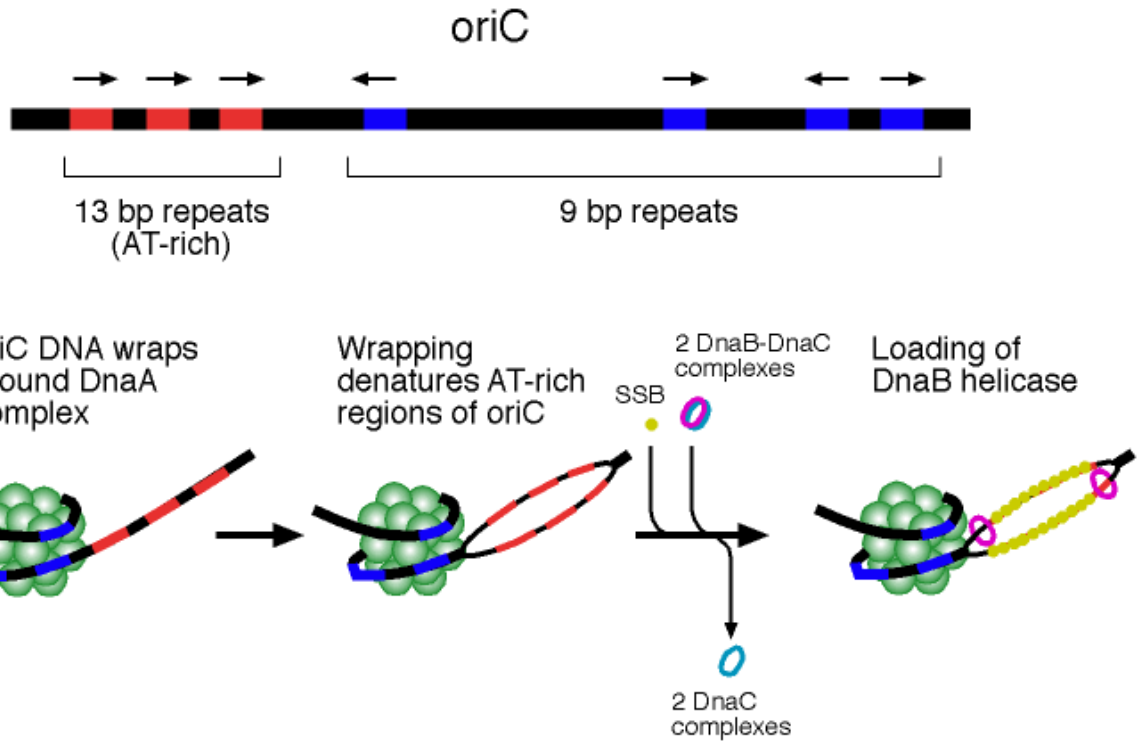


Replication initiation

Initiates reverse coiling

-Melts the DNA

-AT rich region facilitates the process



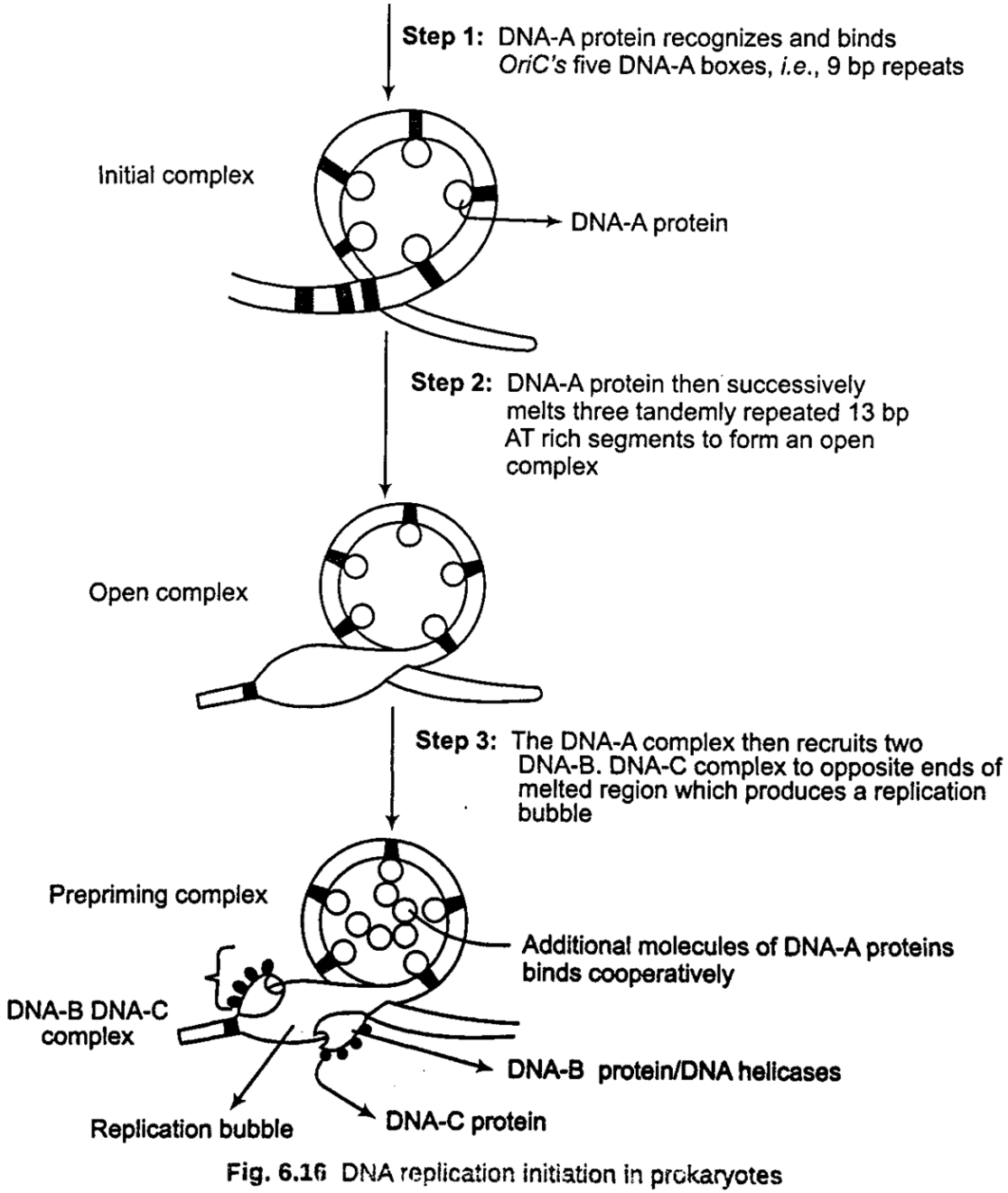


Fig. 6.16 DNA replication initiation in prokaryotes

Fig. 3.6a, b Model for the events occurring around a single replication fork of the *E. coli* chromosome

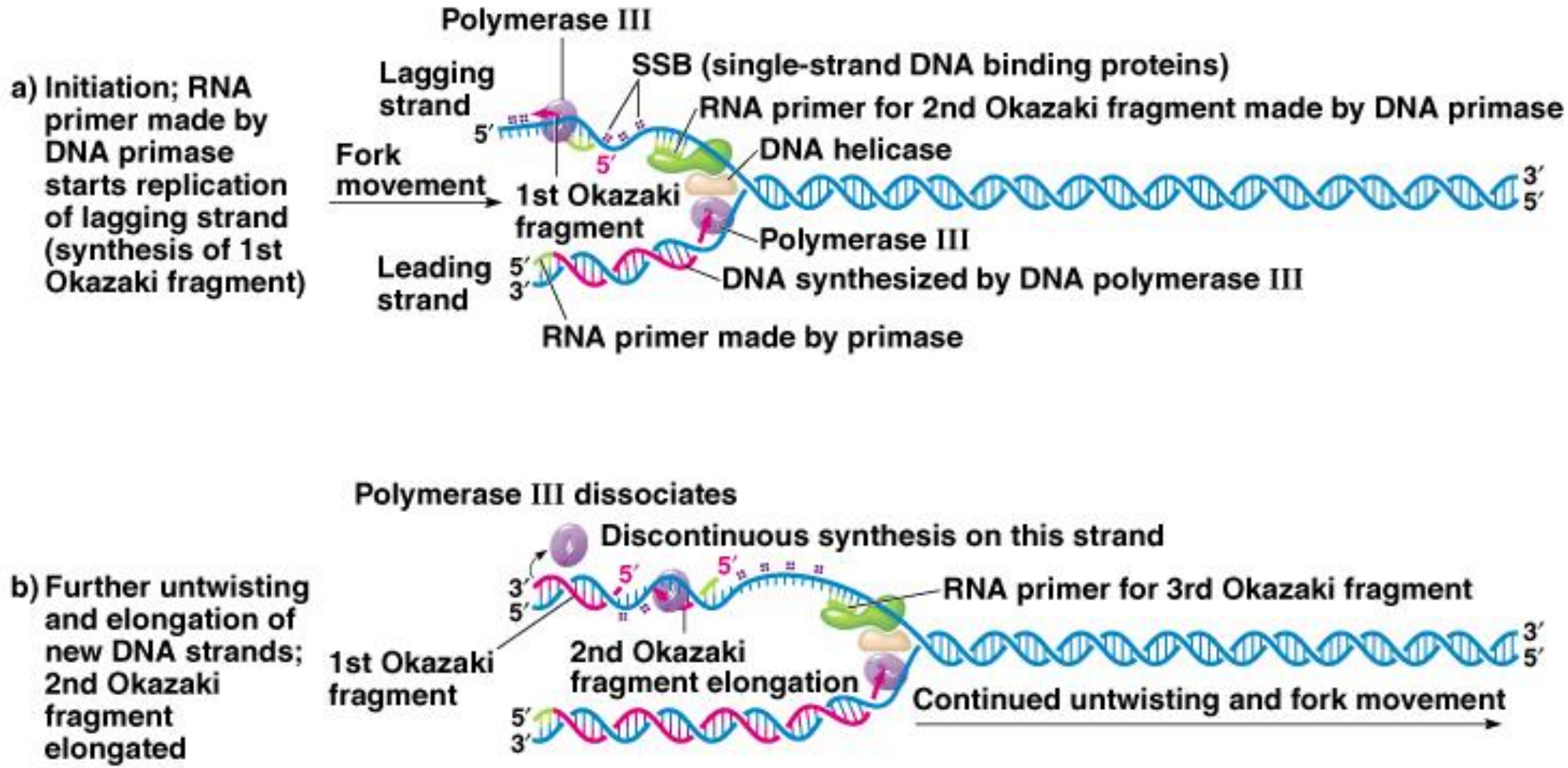


Fig. 3.6c-e Model for the events occurring around a single replication fork of the *E. coli* chromosome

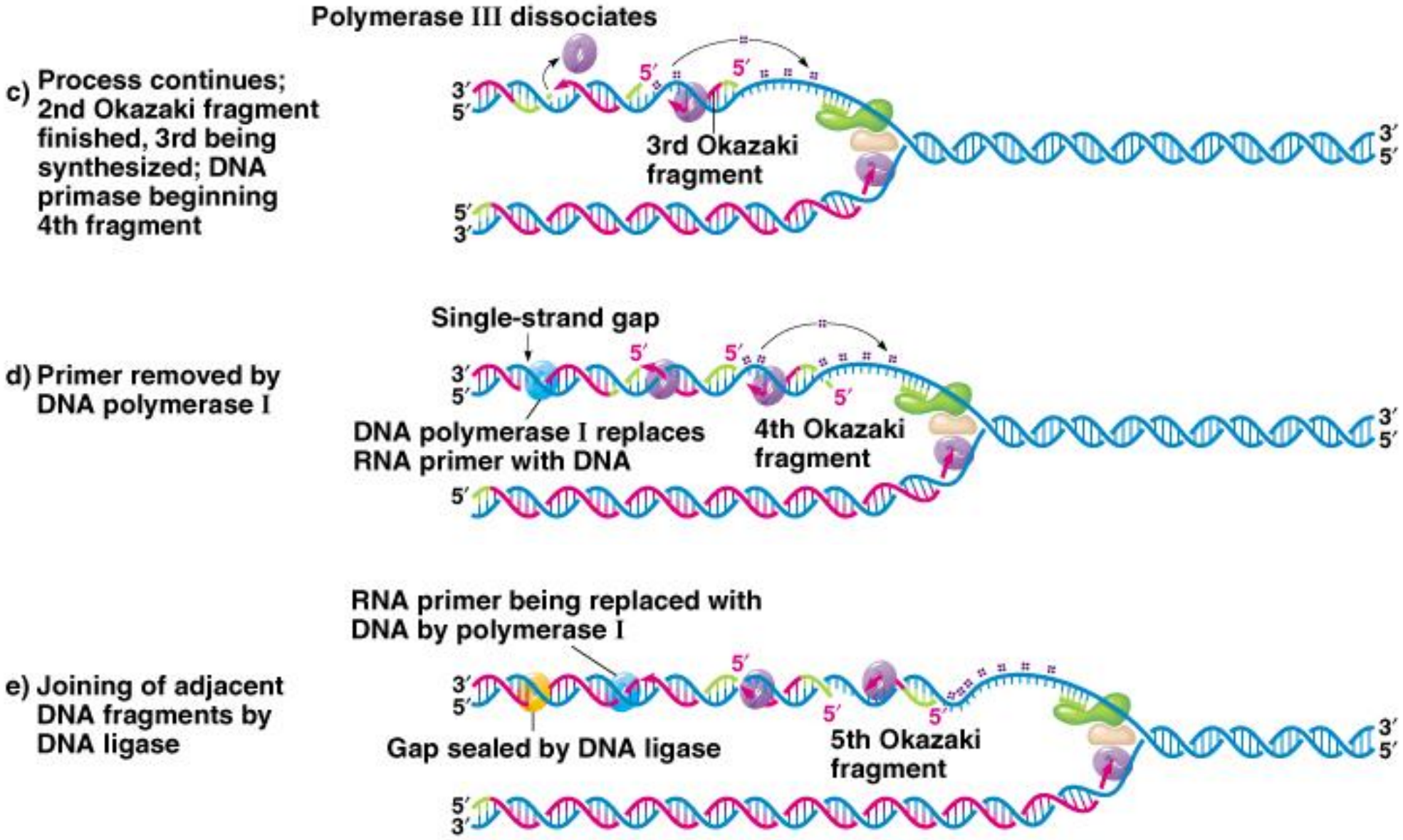
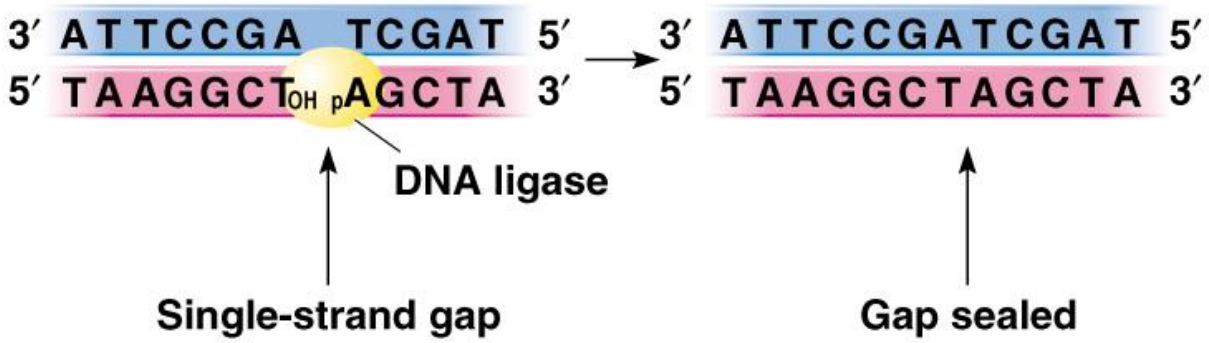


Fig. 3.7 Action of DNA ligase in sealing the gap between adjacent DNA fragments to form a longer, covalently continuous chain

DNA ligase



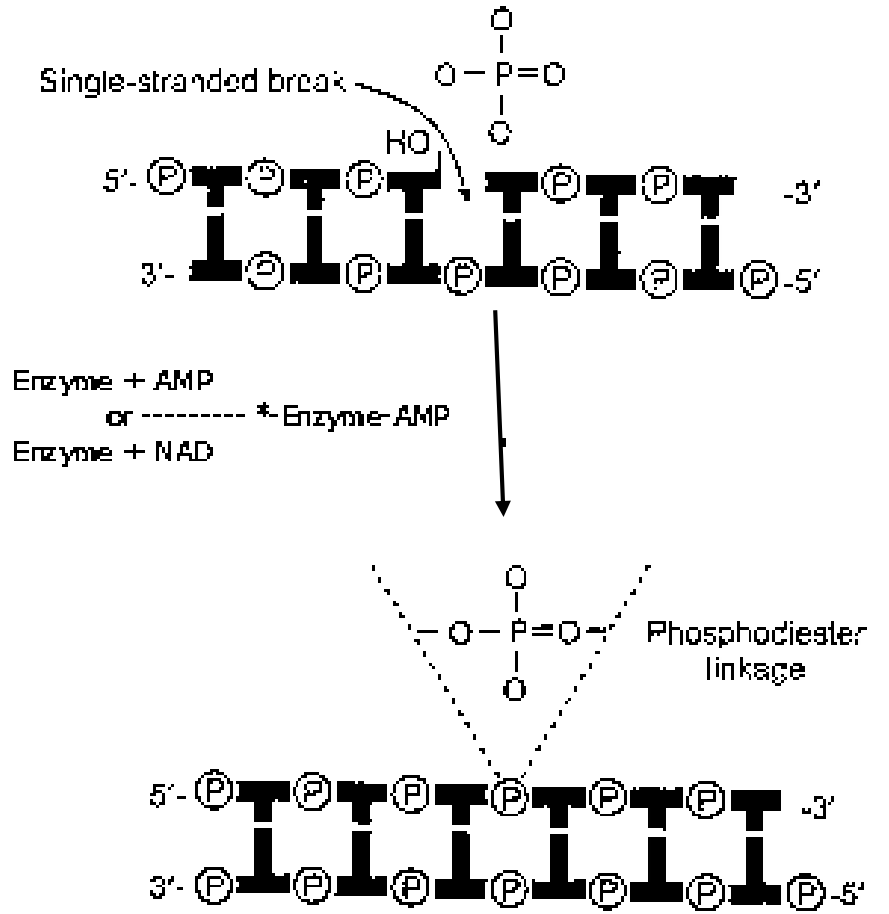
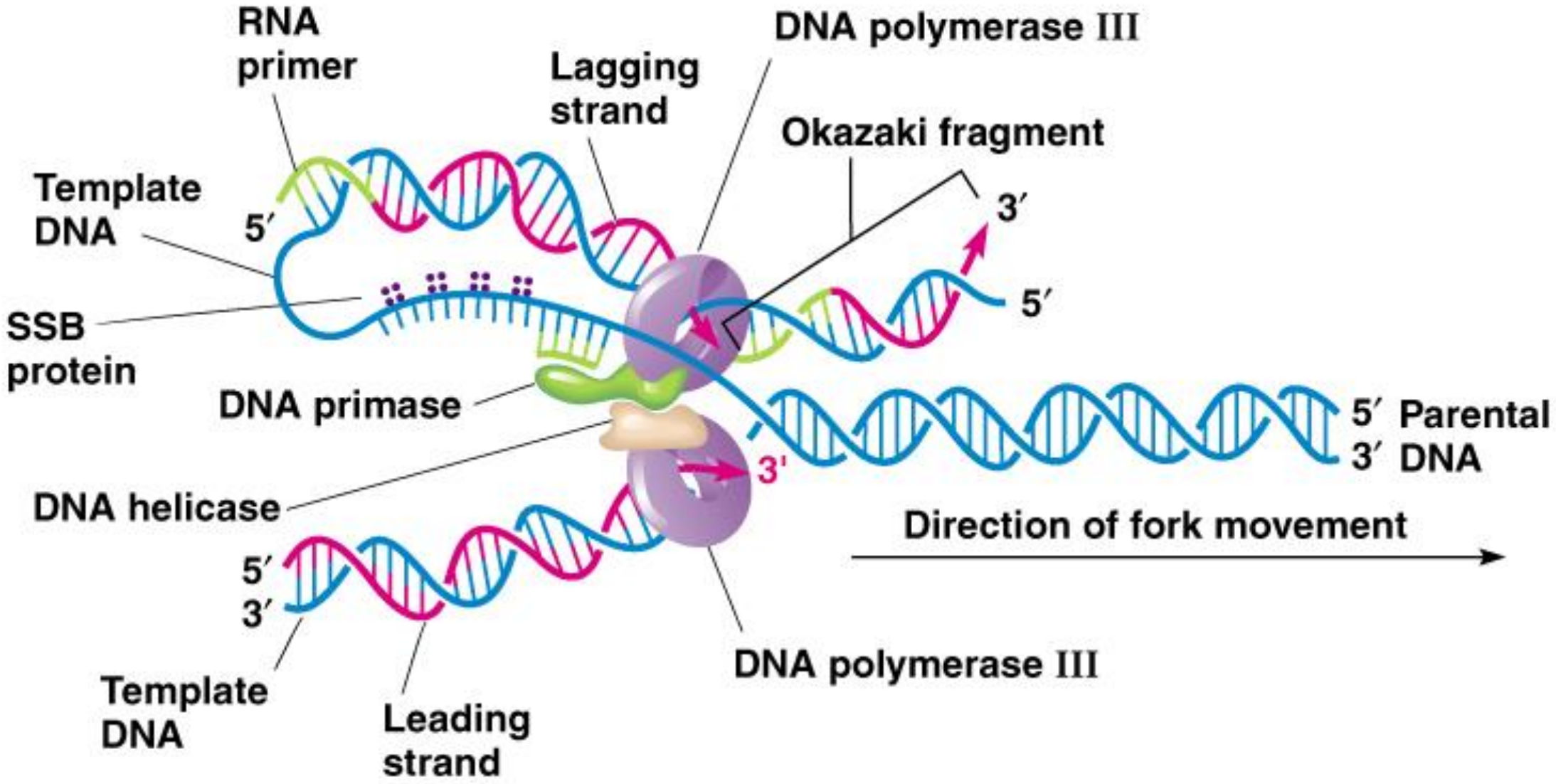


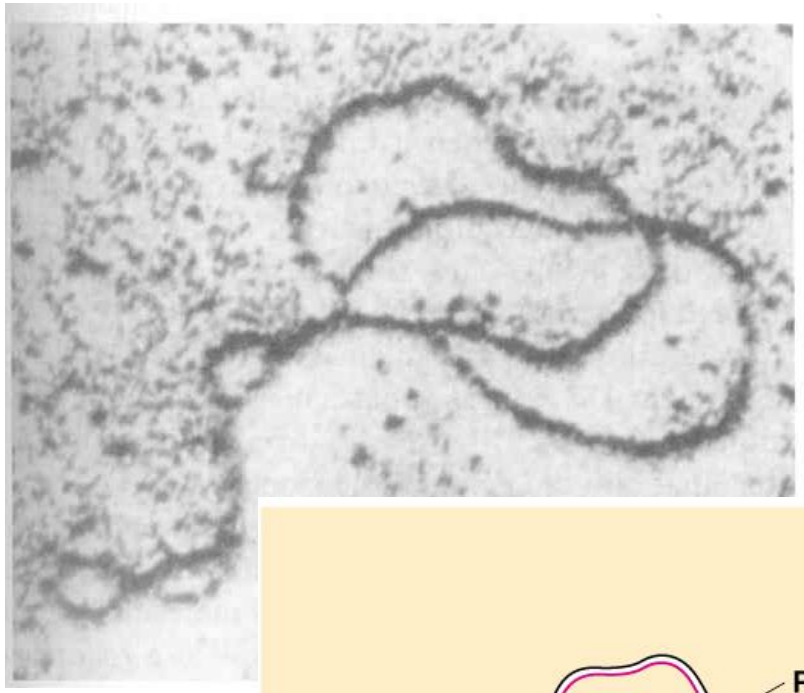
Fig. 6.14 DNA ligase catalyzes the covalent closure of nicks in DNA. The energy required to form the ester linkage is provided by either adenosine triphosphate (ATP) or nicotinamide-adenine dinucleotide (NAD), depending on the series

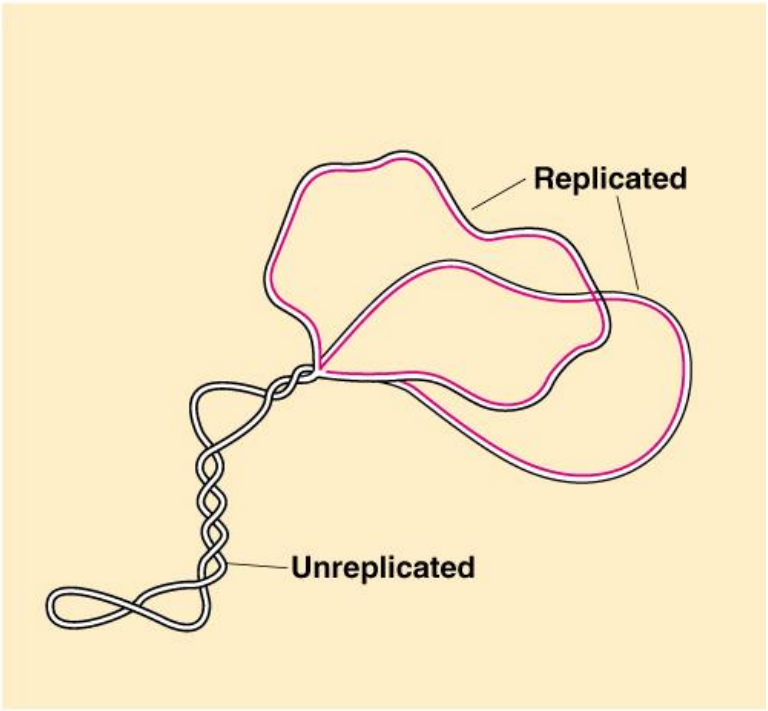
Fig. 3.8 Model for the "replication machine," or replisome, the complex of key replication proteins, with the DNA at the replication fork



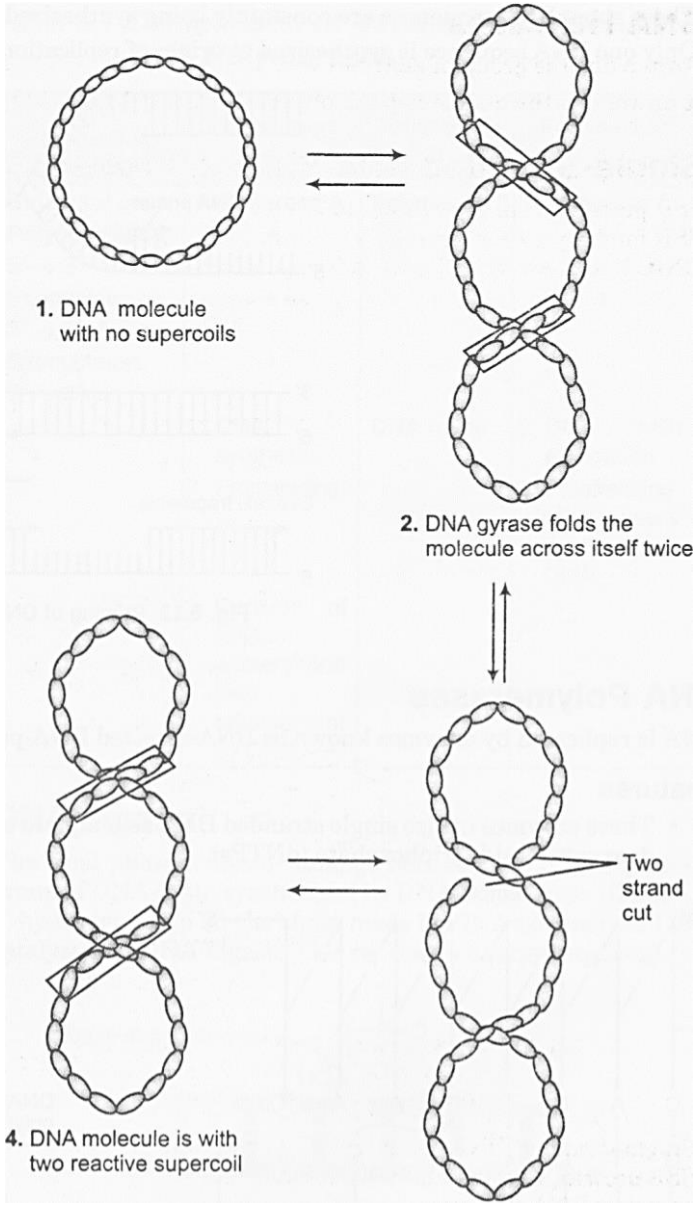
Replication of circular DNA and the supercoiling problem

1. Some circular chromosomes (e.g., *E. coli*) are circular throughout replication, creating a theta-like (θ) shape. As the strands separate on one side of the circle, positive supercoils form elsewhere in the molecule. Replication fork moves about 500 nt/ second, so at 10 bp/turn, replication fork rotates at 3,000 rpm.
2. Topoisomerases relieve the supercoils, allowing the DNA strands to continue separating as the replication forks advance.





Role of Topoisomerase



Type II DNA Topoisomerases

- Binds tightly to DNA double helix and makes transient breaks in both strands.
- The enzyme then causes a second stretch of the DNA double helix to pass through the break and finally, reseals the breaks.
- DNA gyrase, a type II topoisomerase found in *E.coli* has the unusual property of being able to introduce negative supercoils into relaxed circular DNA using energy from the hydrolysis of ATP

Type I DNA topoisomerases

- **Reversibly cut a single stranded of double helix**
- **They have both nuclease (strand cutting) and ligase (strand sealing) activity.**
- **They do not require ATP**

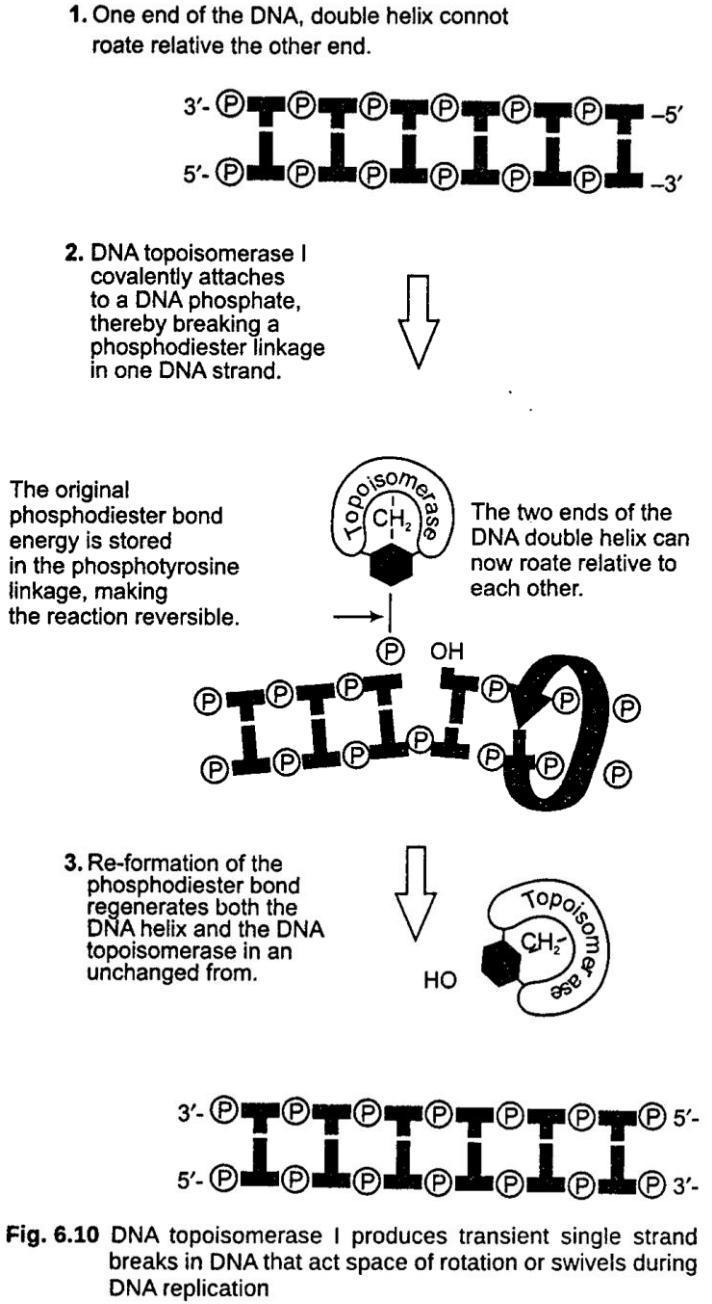
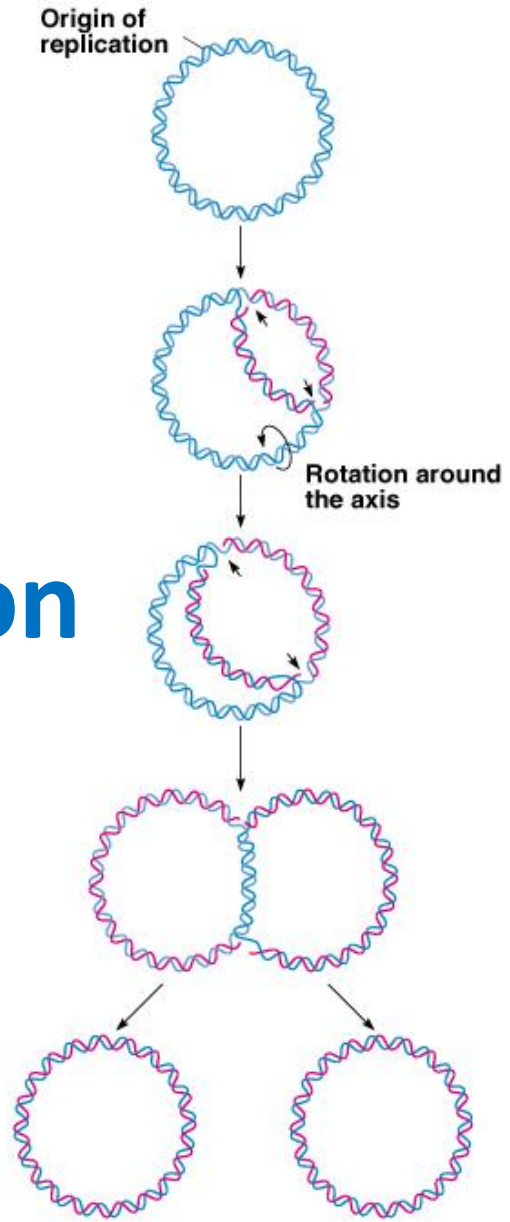


Fig. 6.10 DNA topoisomerase I produces transient single strand breaks in DNA that act space of rotation or swivels during DNA replication

Fig. 3.9 Bidirectional replication of circular DNA molecules



Theta replication

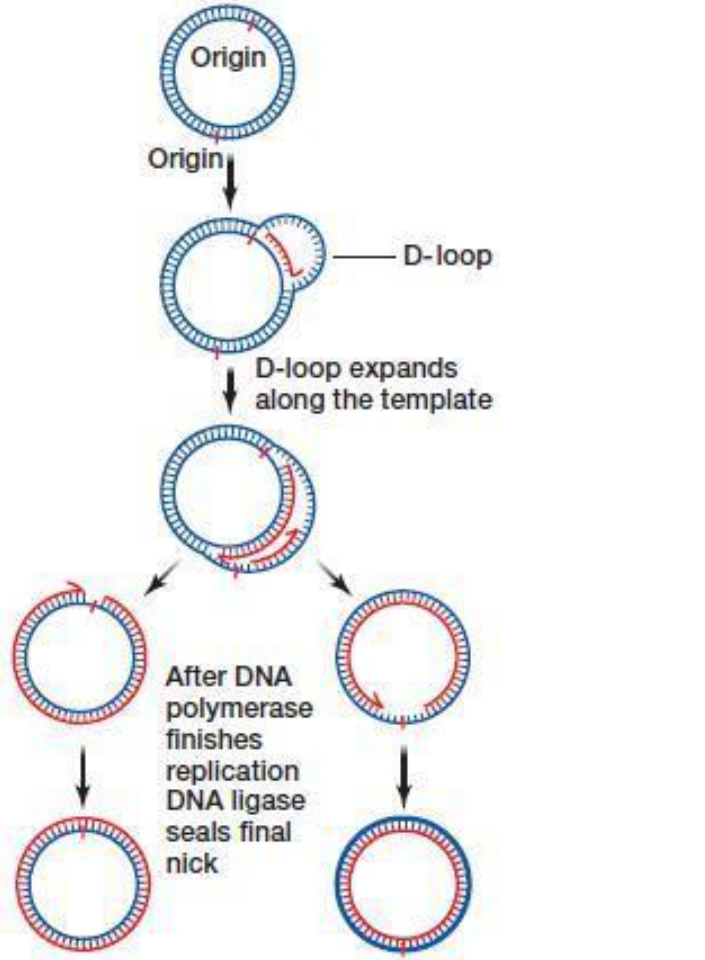


figure 9.28 D-loops form during mitochondrial and chloroplast DNA replication because the origins of replication are at different places on the two strands of the double helix. This results in unidirectional leading-strand synthesis from both origins.

Fig. 3.10 The replication process of double-stranded circular DNA molecules through the rolling circle mechanism

Rolling circle Replication

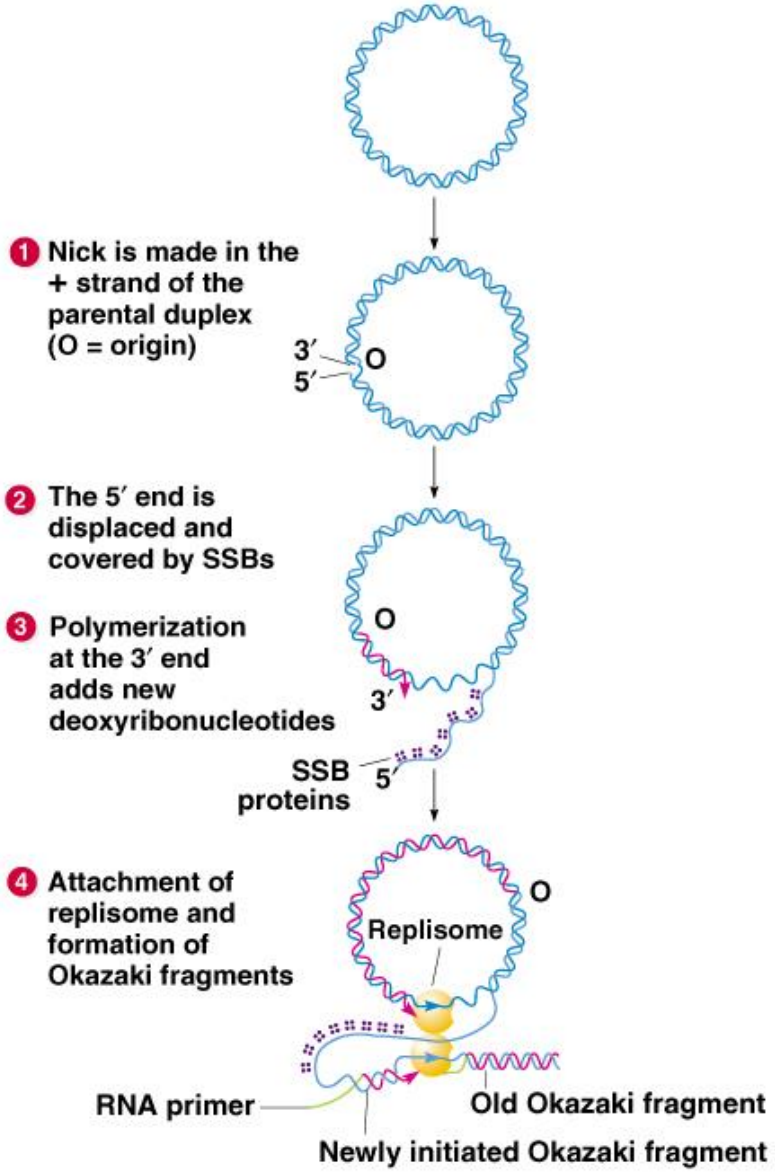
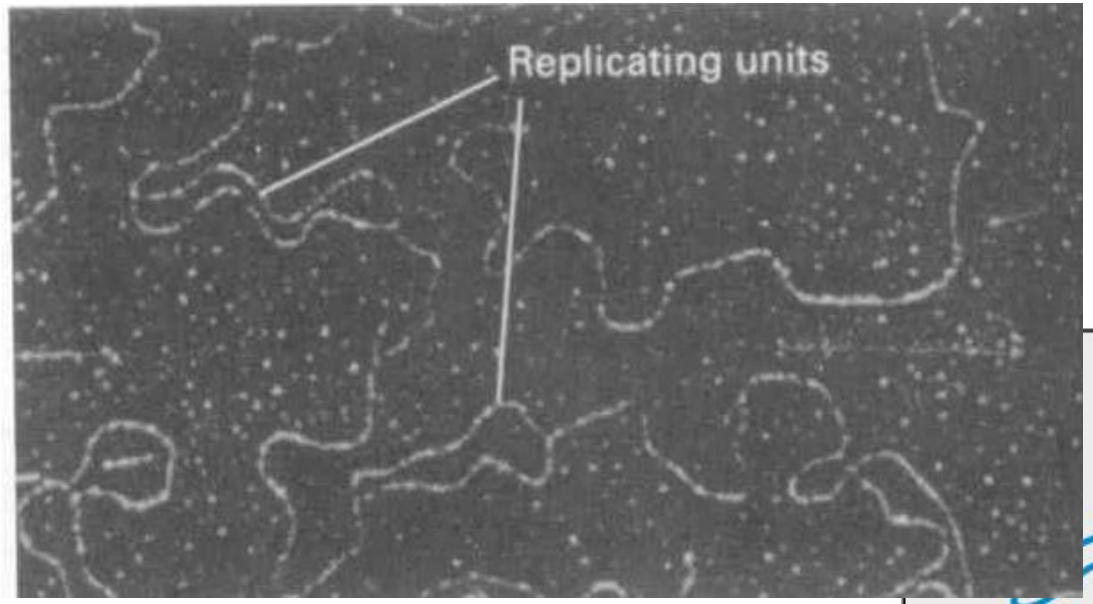
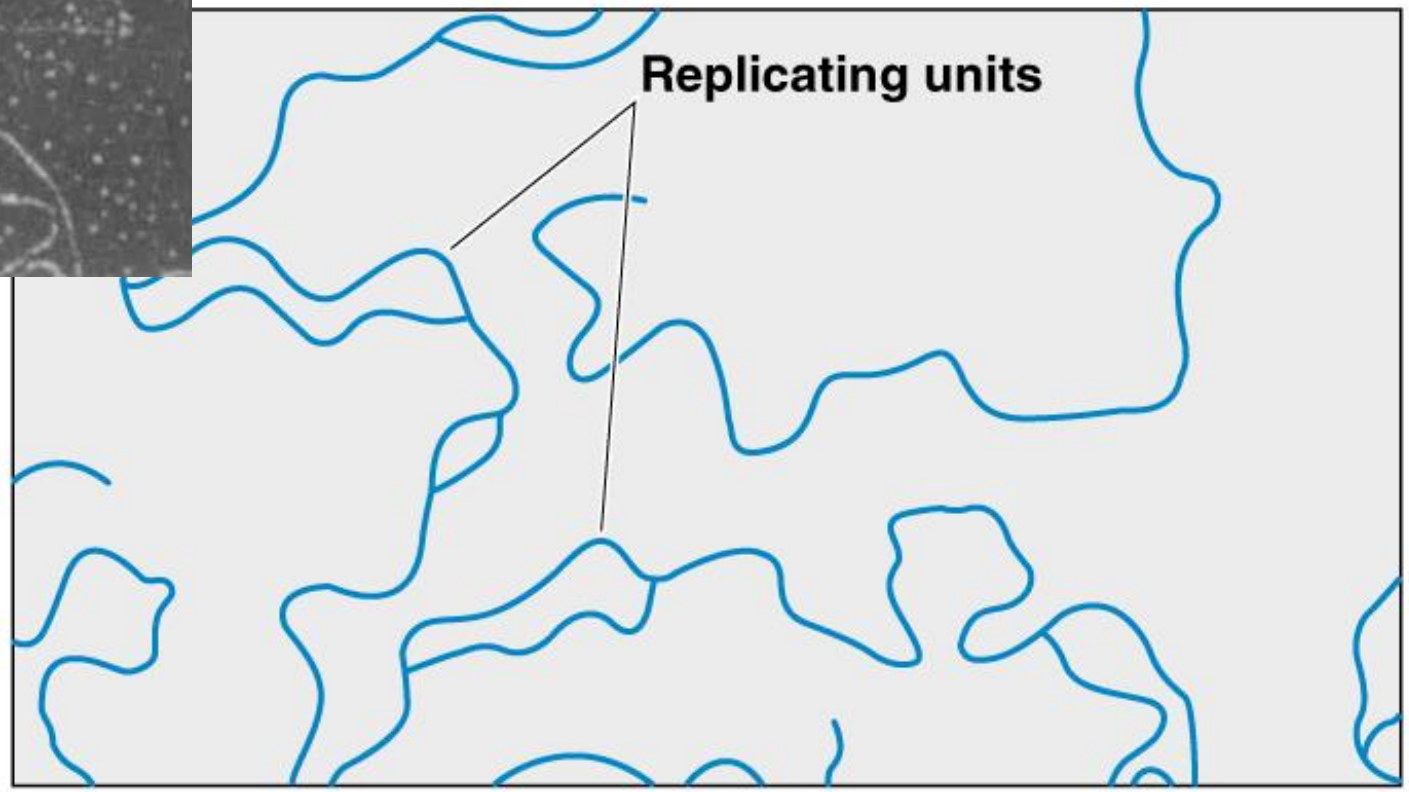
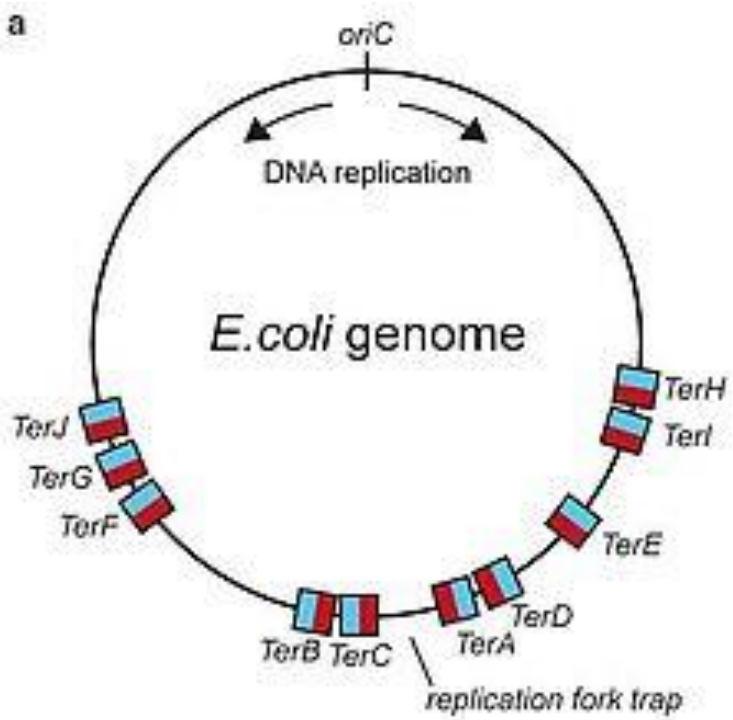


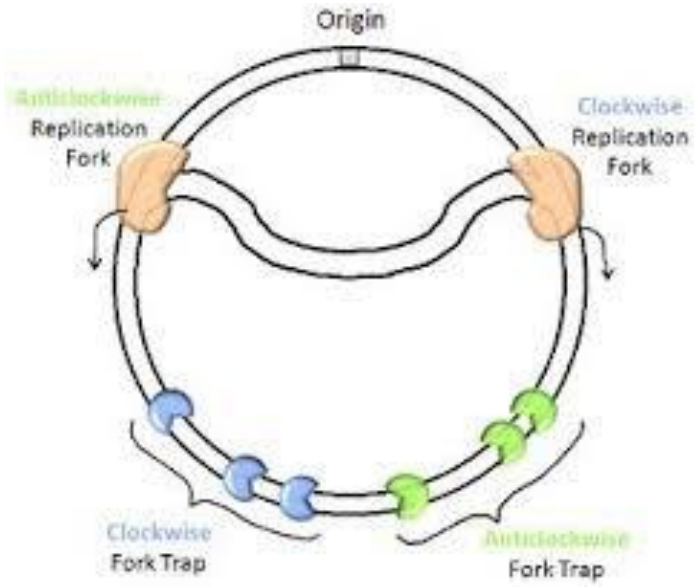
Fig. 3.12 Replicating DNA of *Drosophila melanogaster*

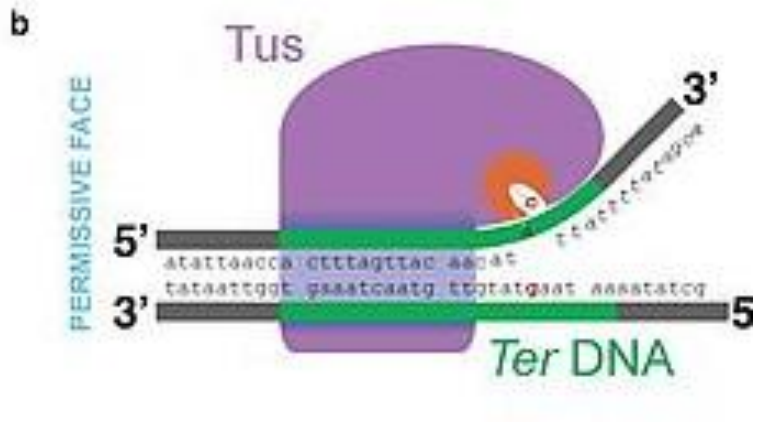


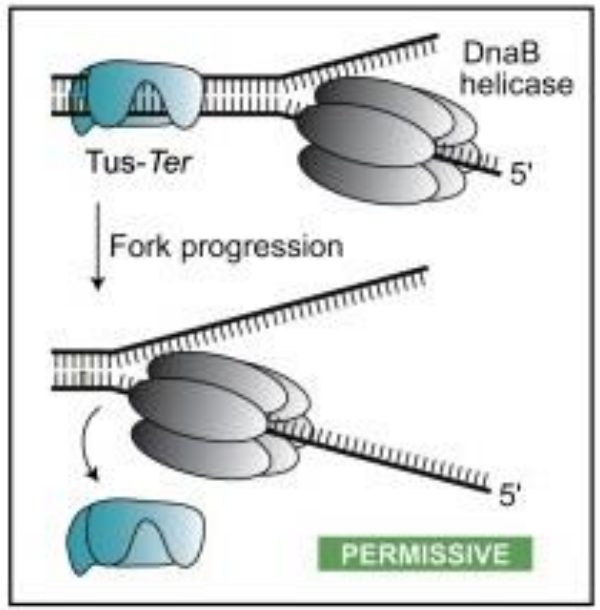


Replication termination in *E.coli*









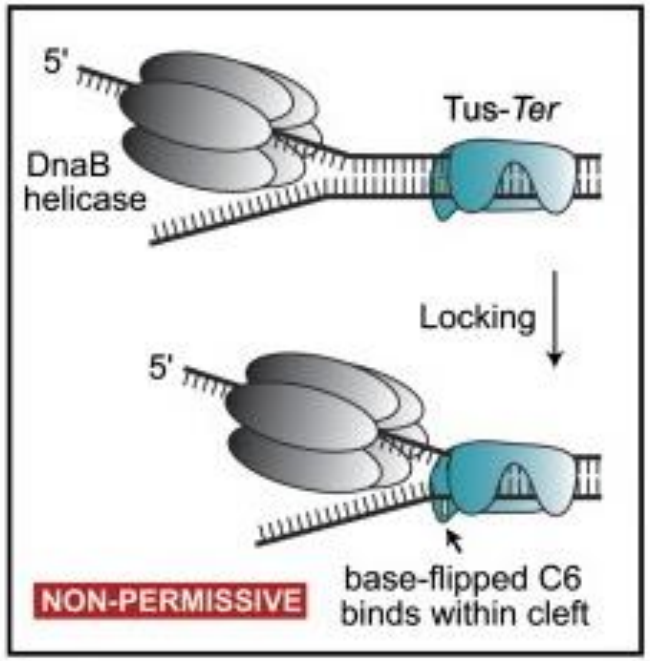
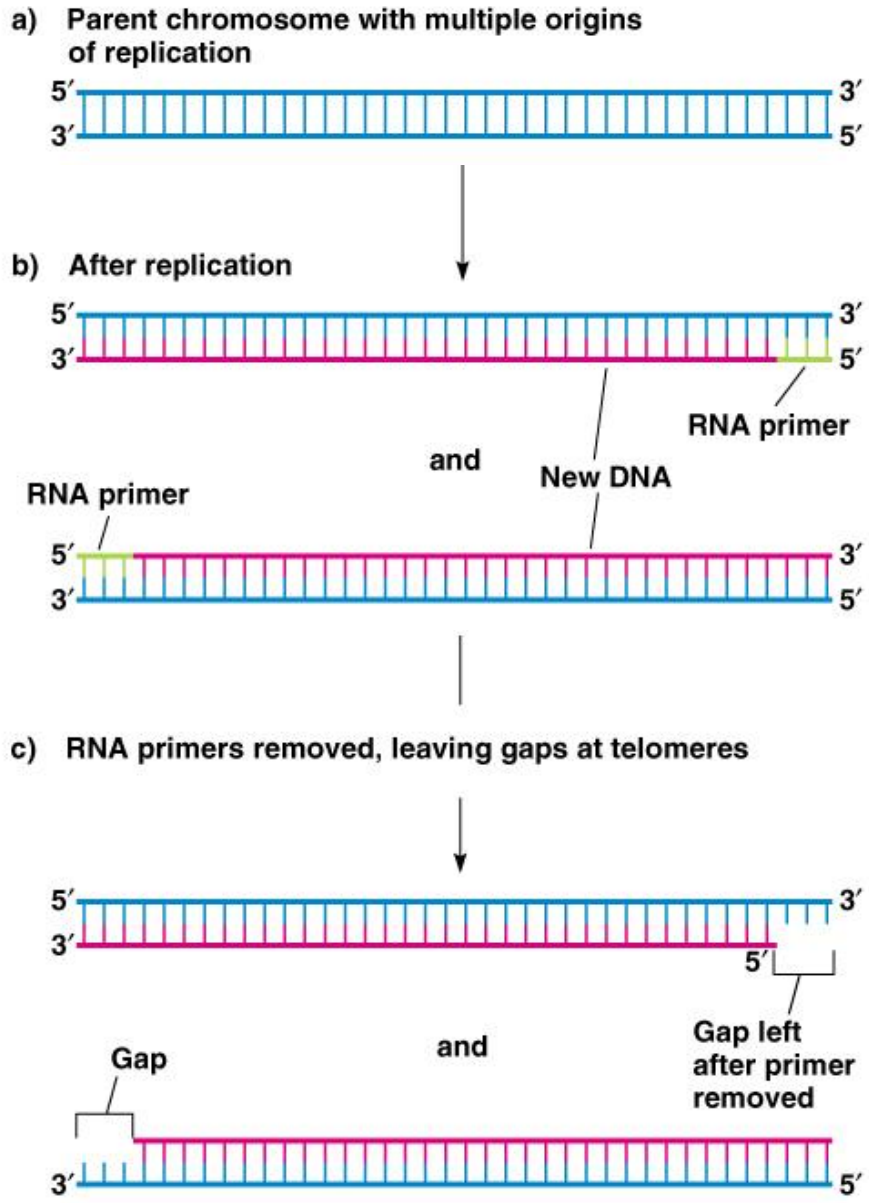
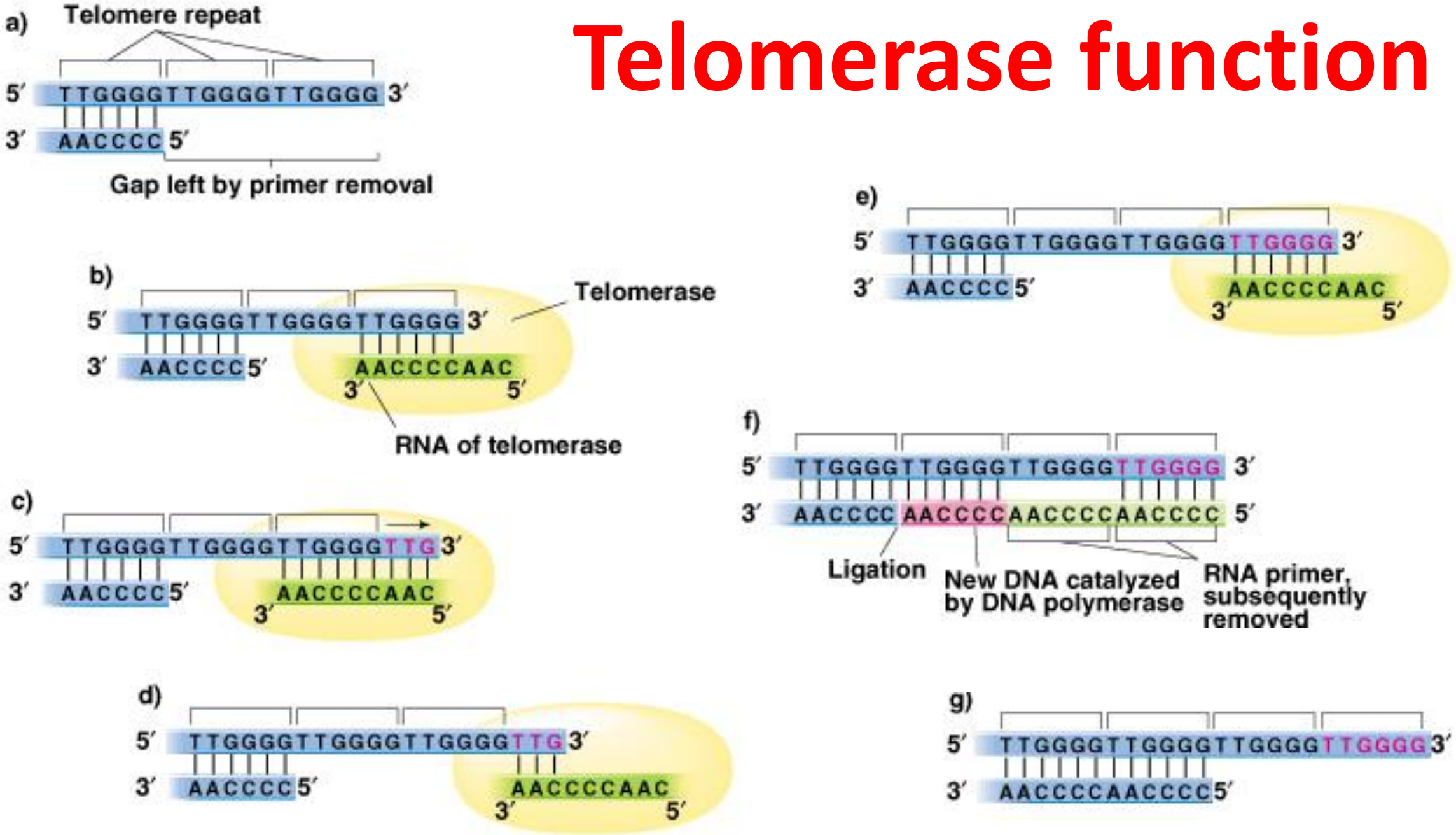


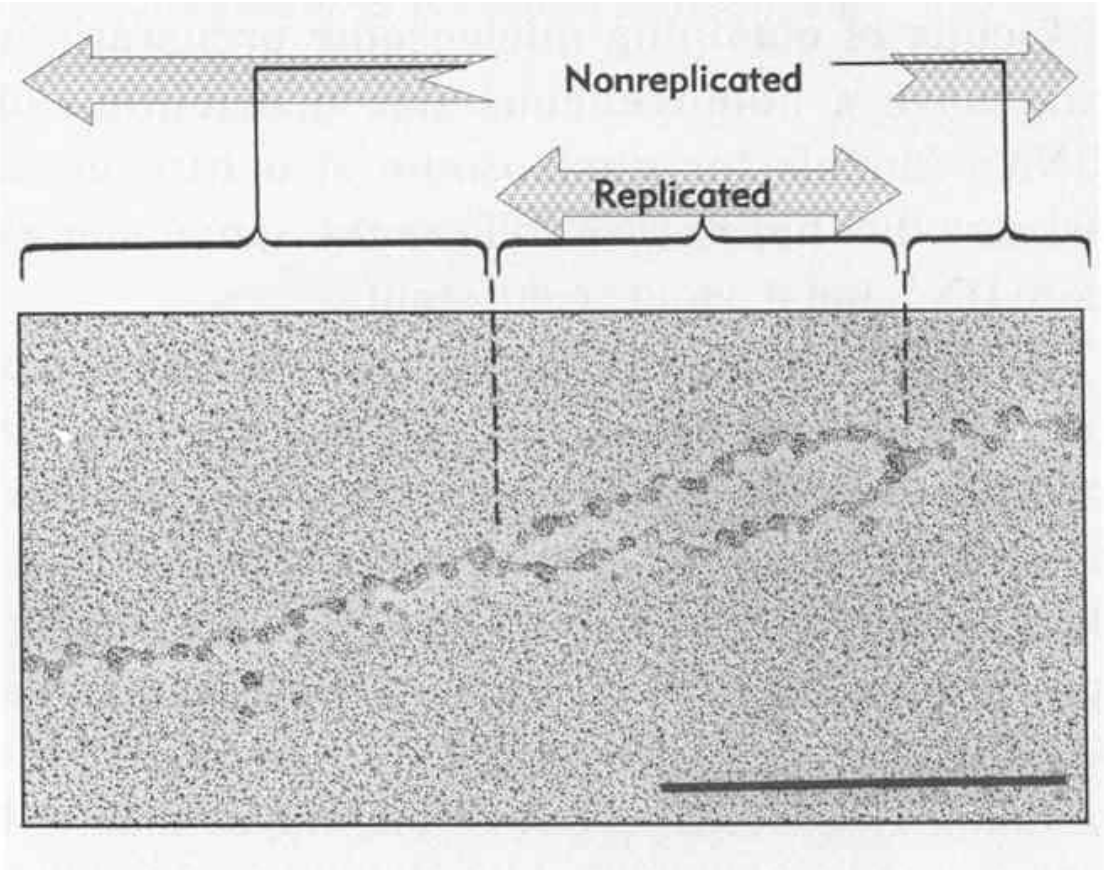
Fig. 3.14 The problem of replicating completely a linear chromosome in eukaryotes

End replication problem

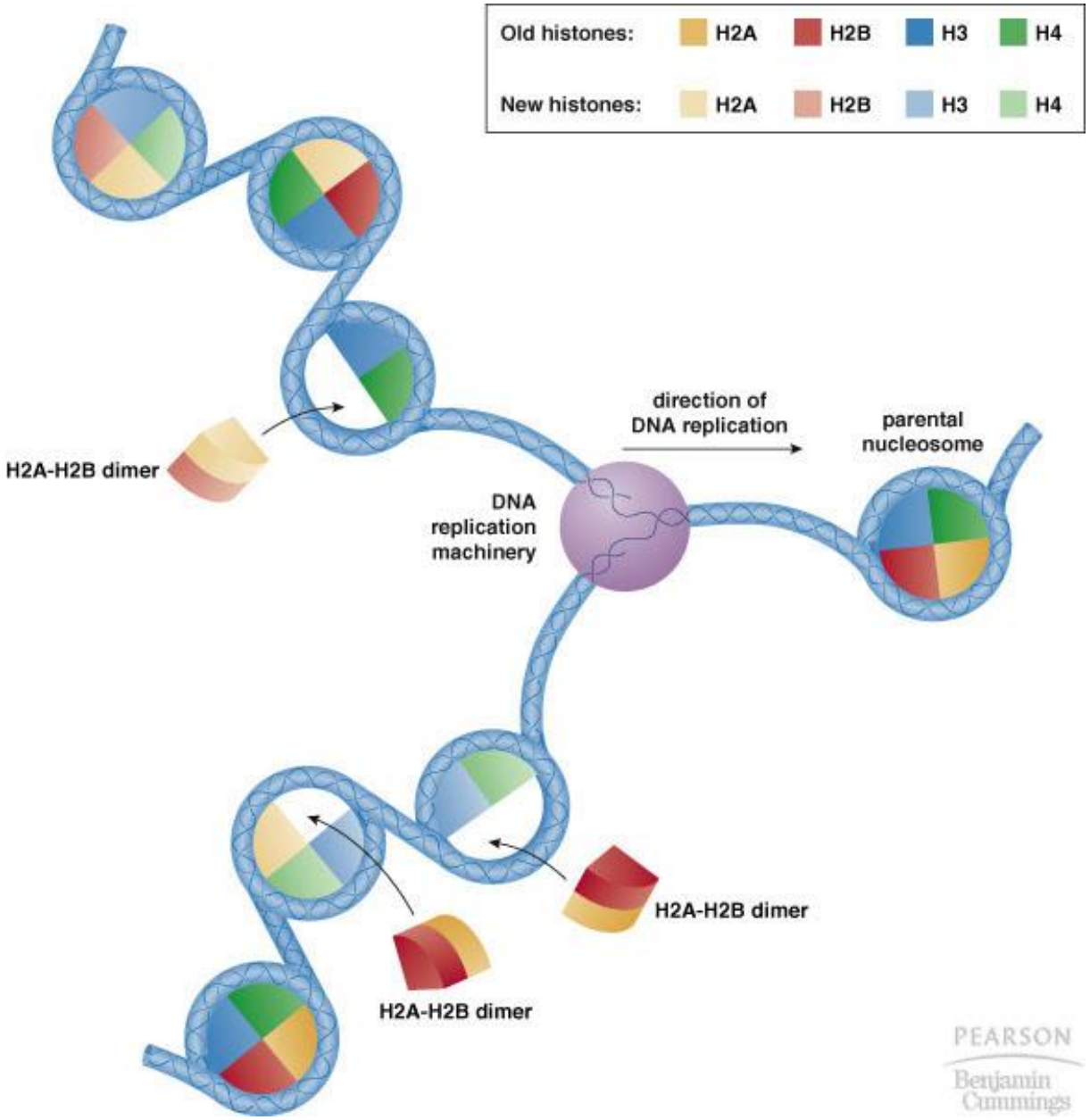


Telomerase function





Recycling of histones



Remember this! – Part 1

Table 16.1 Bacterial DNA replication proteins and their functions

Protein	Function for Leading and Lagging Strands	
Helicase	Unwinds parental double helix at replication forks	
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template	
Topoisomerase	Corrects "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands	
	Function for Leading Strand	**Function for Lagging Strand**
Primase	Synthesizes a single RNA primer at the 5′ end of the leading strand	Synthesizes an RNA primer at the 5′ end of each Okazaki fragment
DNA pol III	Continuously synthesizes the leading strand, adding on to the primer	Elongates each Okazaki fragment, adding on to its primer
DNA pol I	Removes primer from the 5′ end of leading strand and replaces it with DNA, adding on to the adjacent 3′ end	Removes the primer from the 5′ end of each fragment and replaces it with DNA, adding on to the 3′ end of the adjacent fragment
DNA Ligase	Joins the 3′ end of the DNA that replaces the primer to the rest of the leading strand	Joins the Okazaki fragments

Remember this!- part 2

DNA Replication in Prokaryotes and Eukaryotes	
Prokaryotes	**Eukaryotes**
Five polymerases (I, II, III, IV, V)	Five polymerases ($\alpha, \beta, \gamma, \delta, \varepsilon$)
Functions of polymerase:	Functions of polymerase:
I is involved in synthesis, proofreading, repair, and removal of RNA primers	α: a polymerizing enzyme
II is also a repair enzyme	β: a repair enzyme
III is main polymerizing enzyme	γ: mitochondrial DNA synthesis
IV, V are repair enzymes under unusual conditions	δ: main polymerizing enzyme
	ε: function unknown
Polymerase are also exonucleases	Not all polymerases are exonucleases
One origin of replication	Several origins of replication
Okazaki fragments 1000-2000 residues long	Okazaki fragments 150-200 residues long
No proteins complexed to DNA	Histones complexed to DNA

Acknowledgement

- I am grateful to Professor Srirupa Mukherjee, Principal of Panihati Mahavidyalaya for providing necessary facilities and advice and for preparation of this seminae lecture.

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THANK YOU