

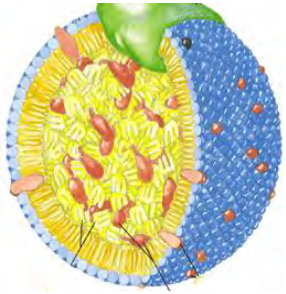


# BIOCHEMISTRY REVIEW

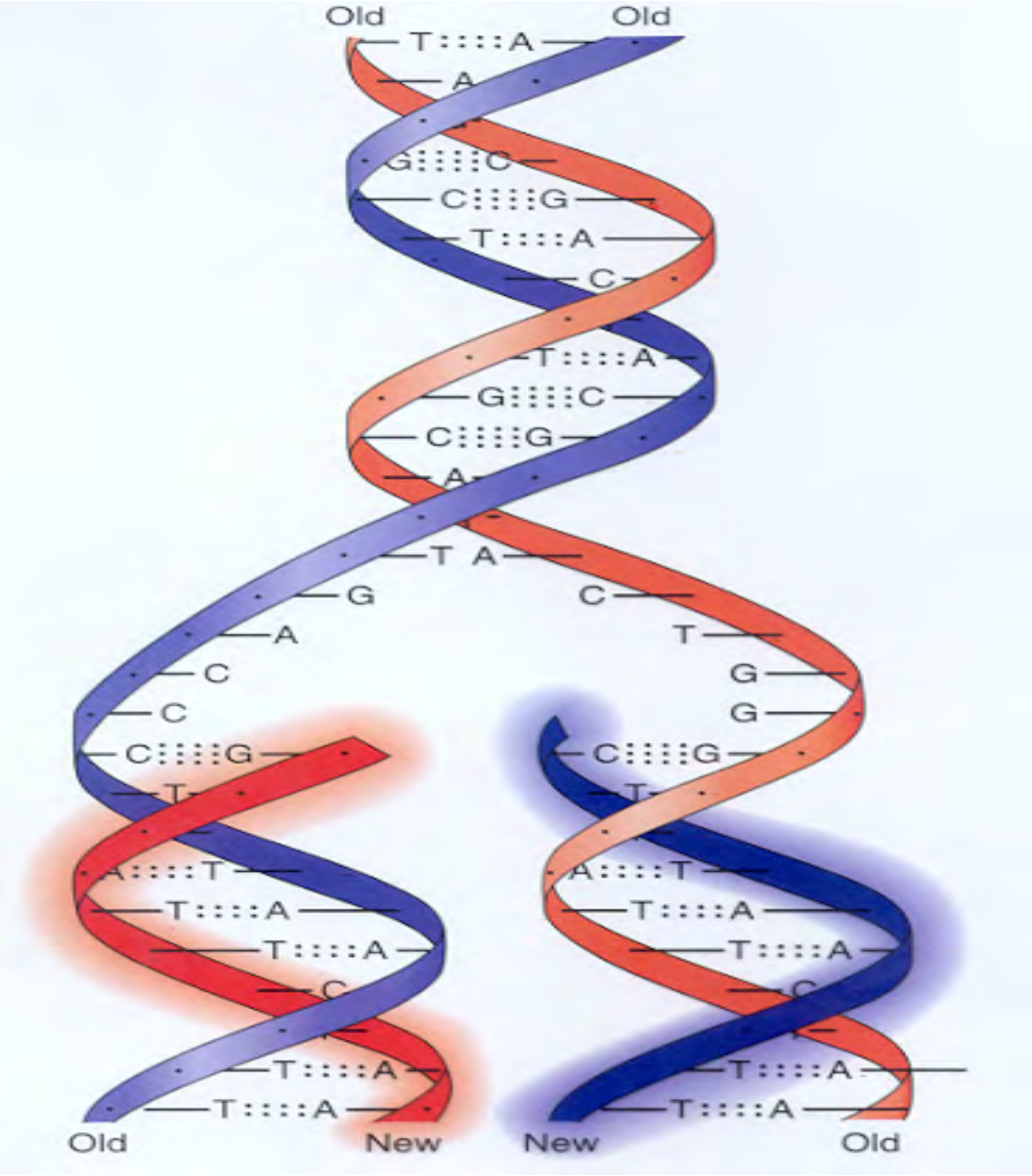
## Overview of Biomolecules

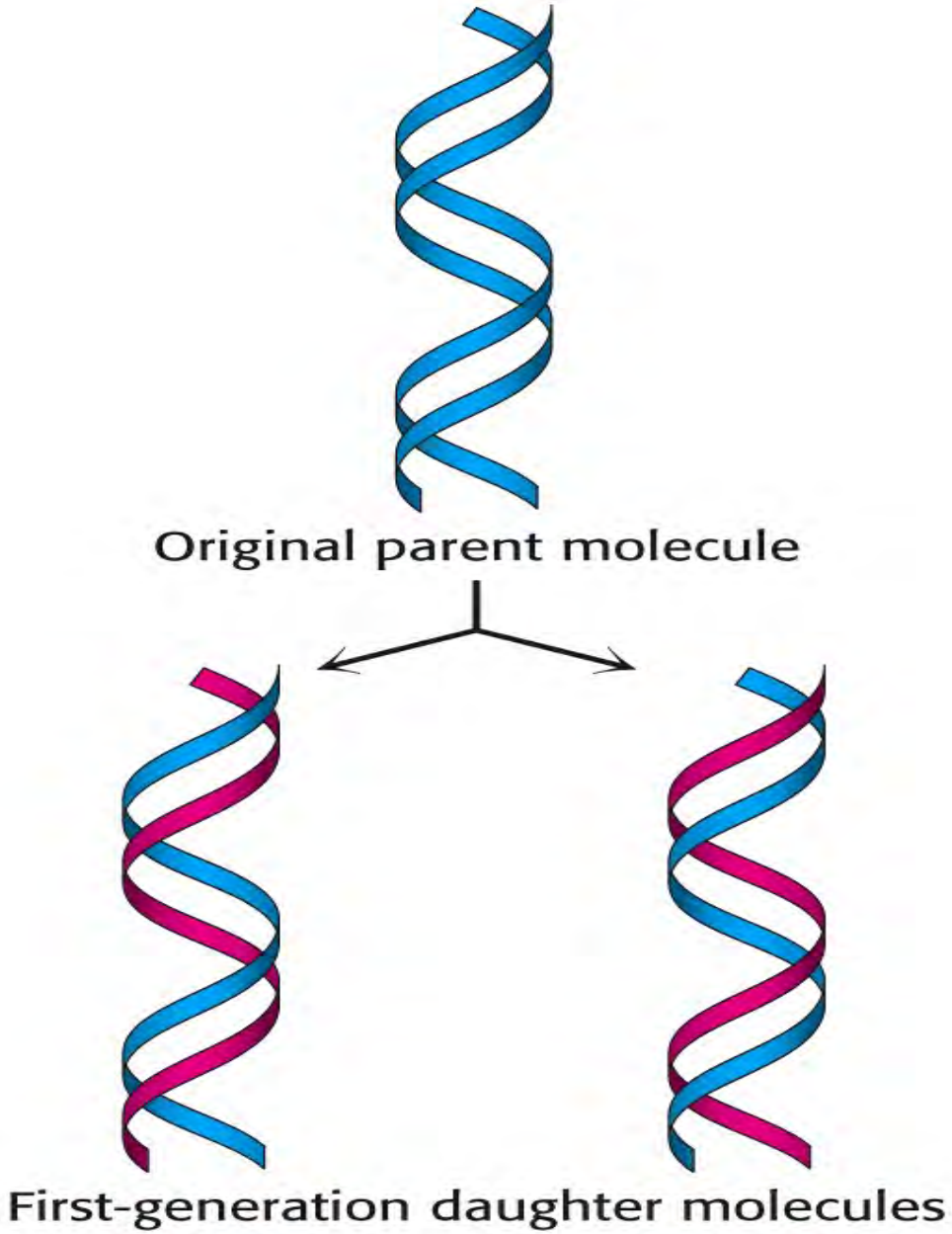
### Chapter 11

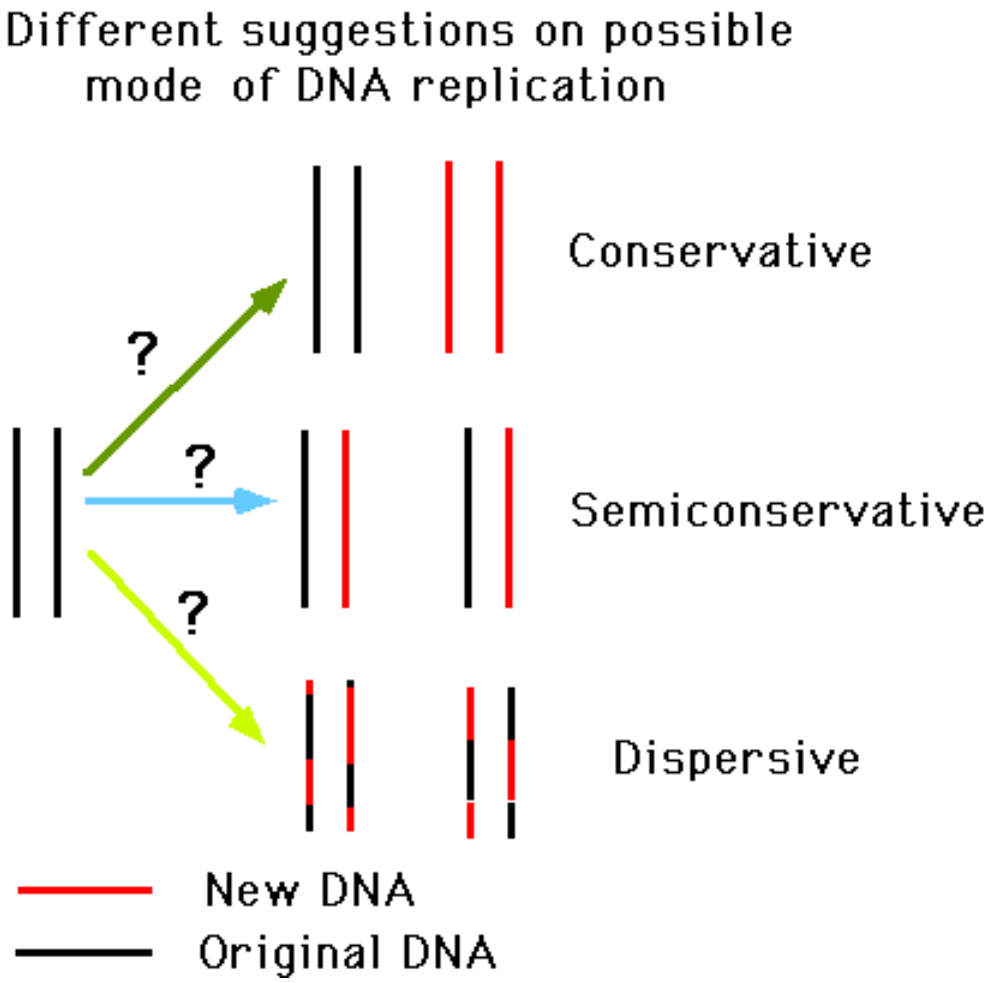
### DNA Replication

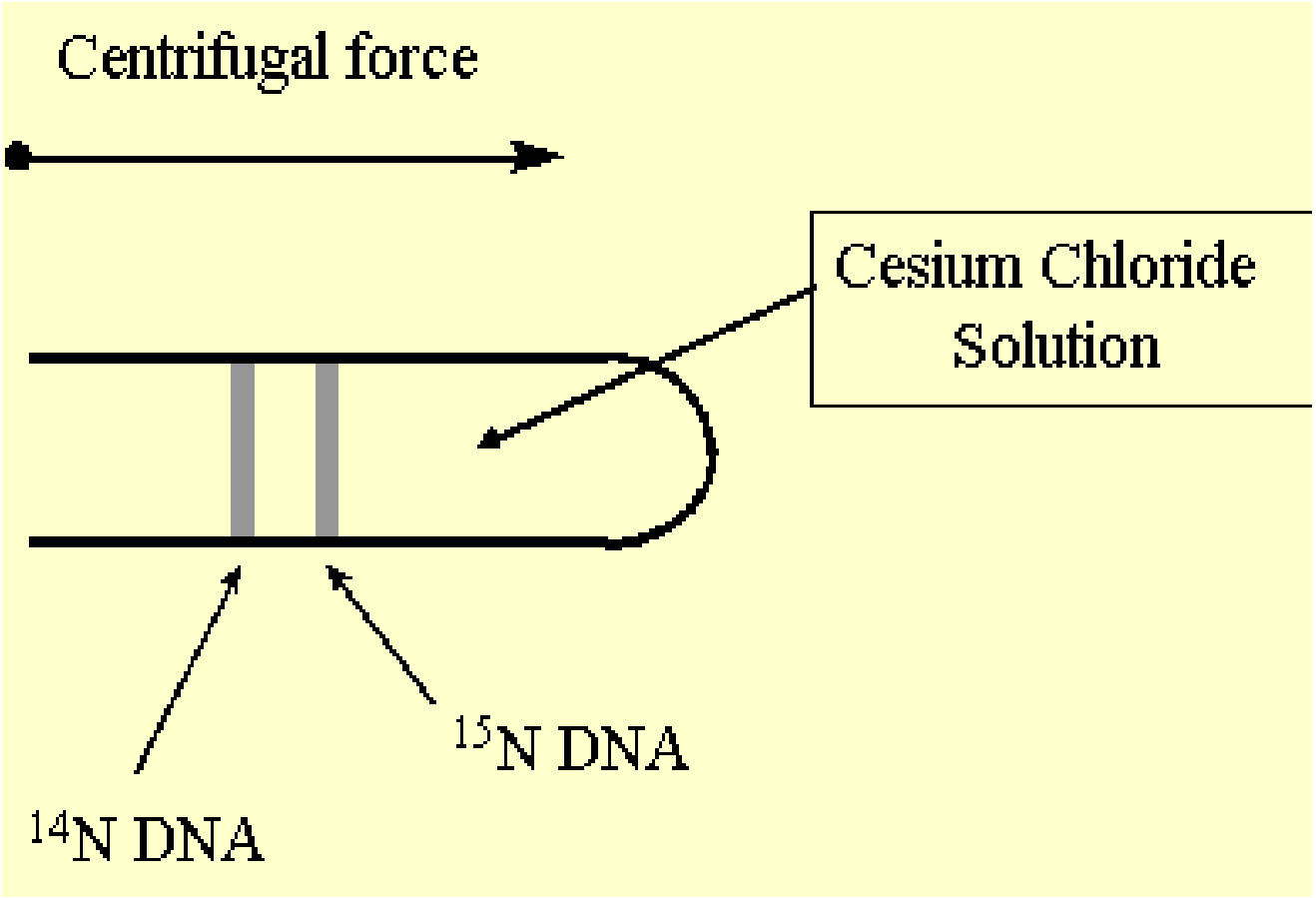


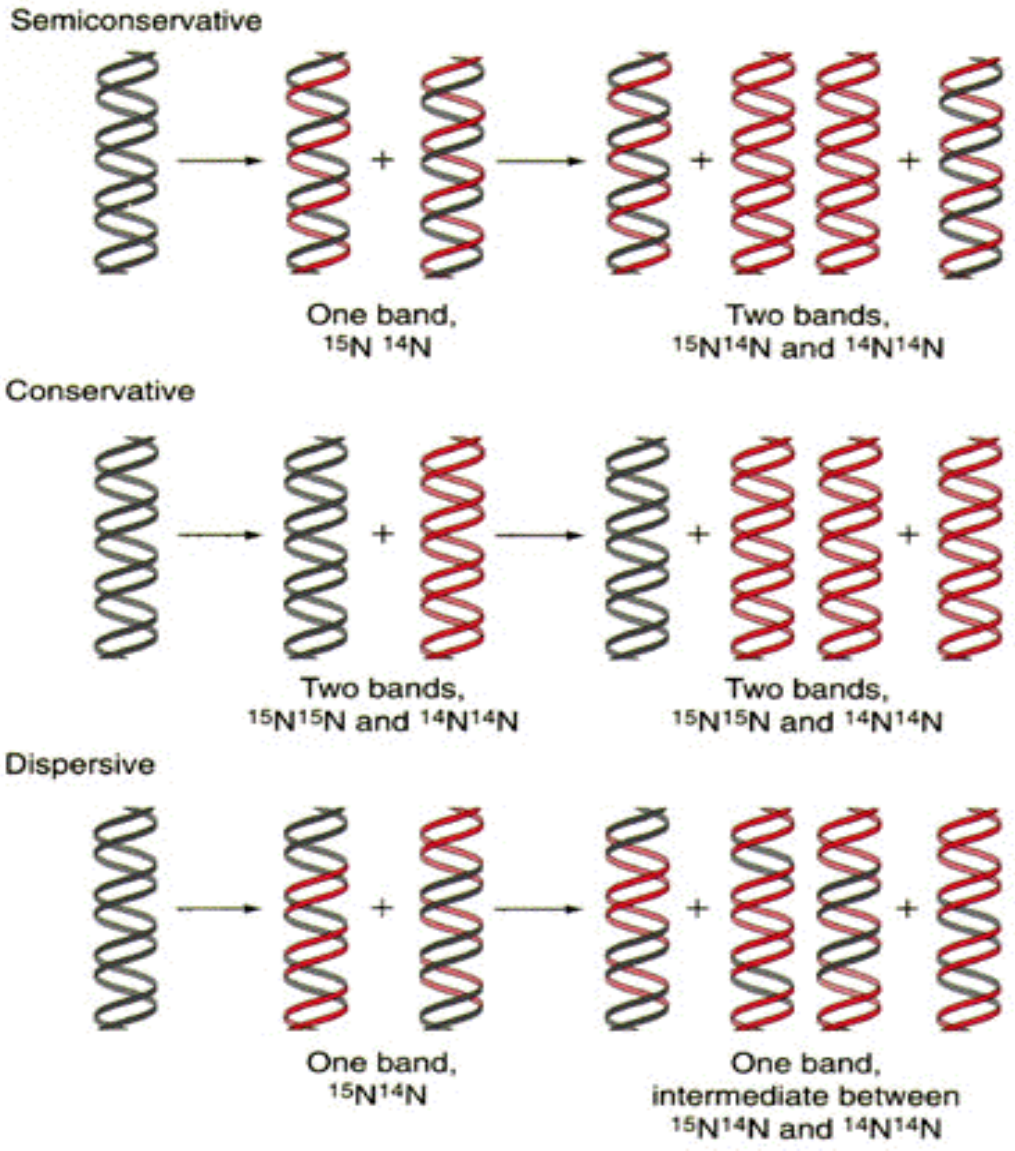


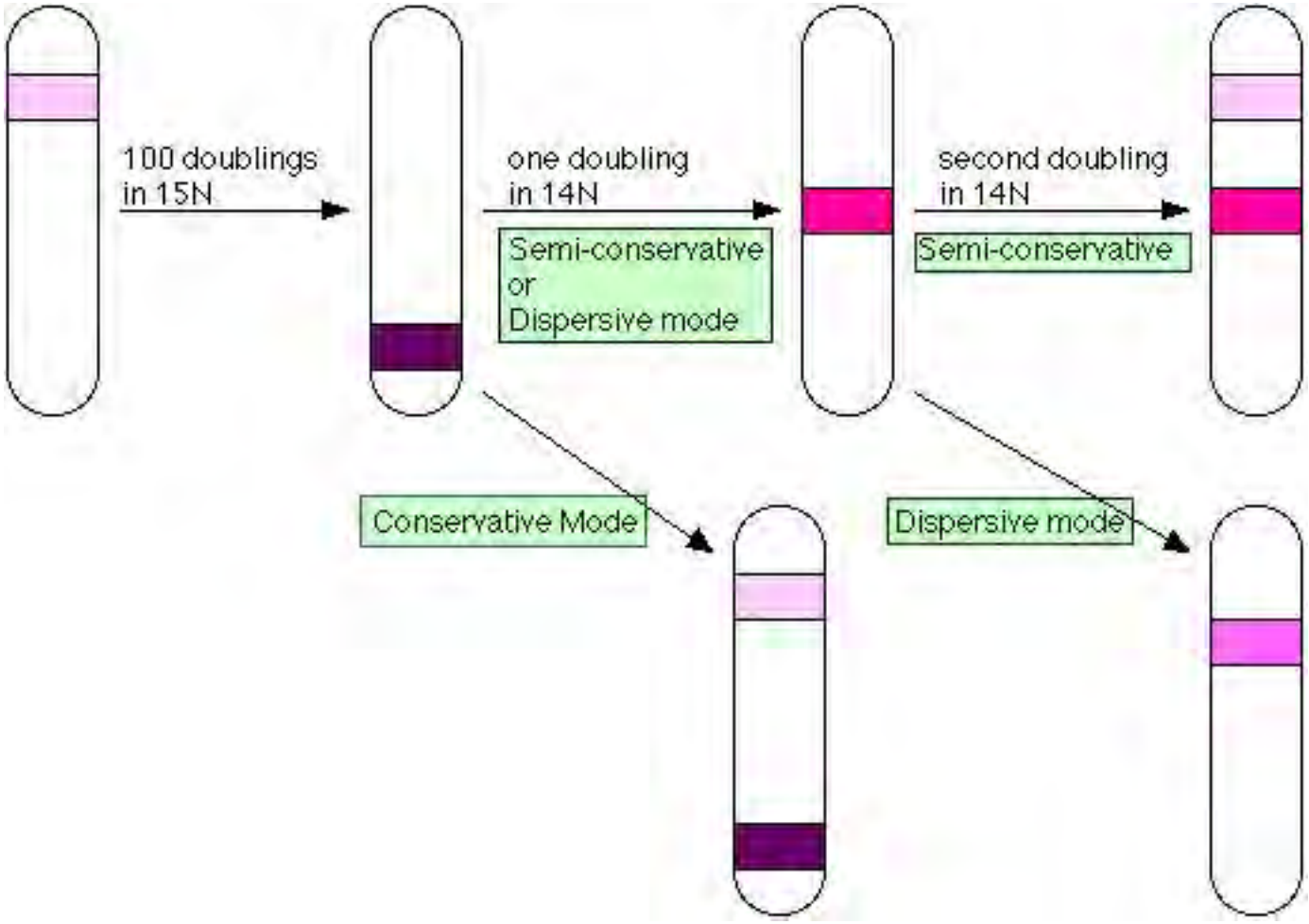

Old
Old
T::::A
A
G::::C
C::::G
T::::A
C
T::::A
G::::C
C::::G
A
T A
G
A
C
C
C::::G
T
A::::T
T::::A
T::::A
C
T::::A
T::::A
Old
New
C
T
G
G
C::::G
T
A::::T
T::::A
T::::A
C
T::::A
T::::A
New
Old

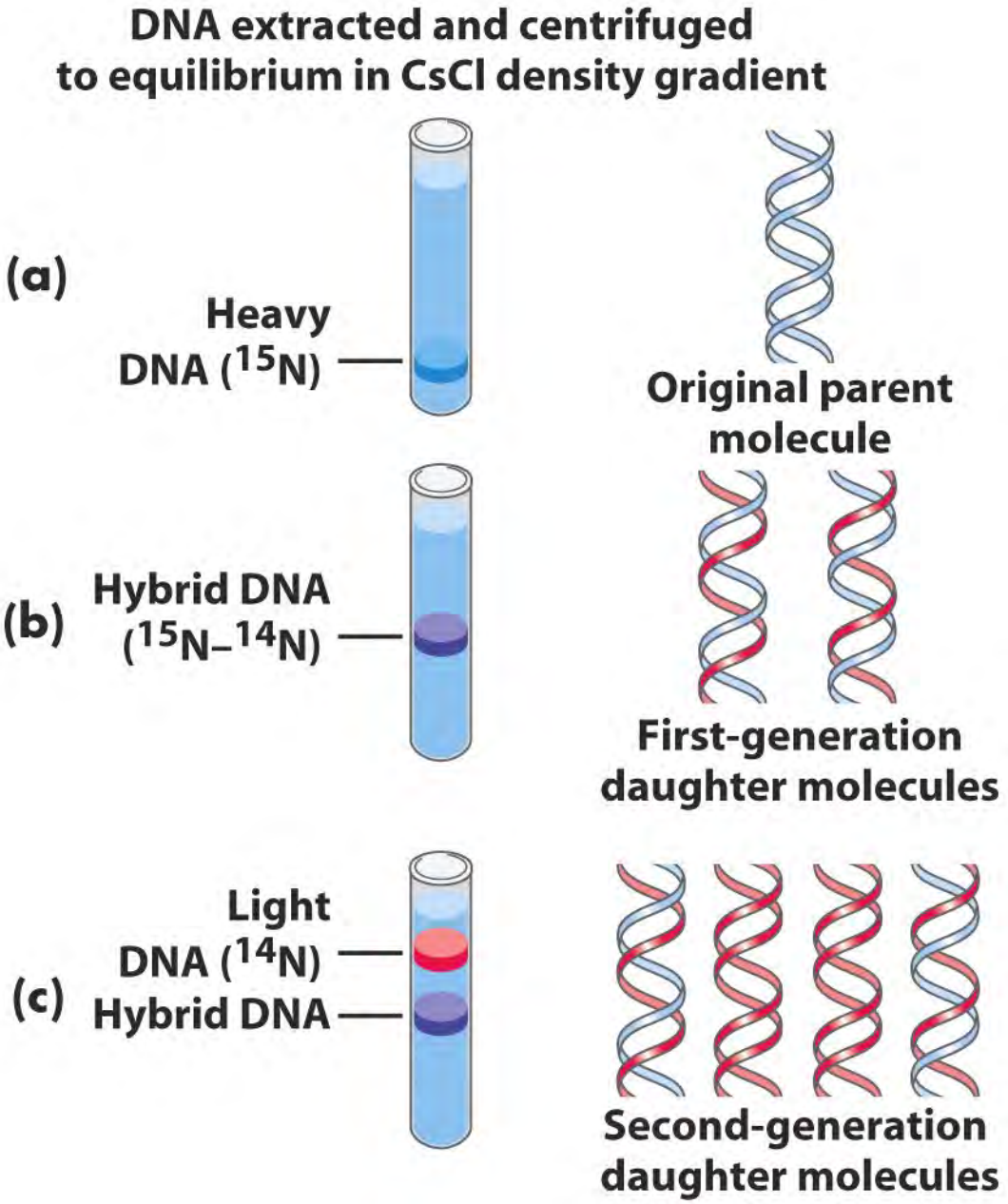

Original parent molecule
First-generation daughter molecules

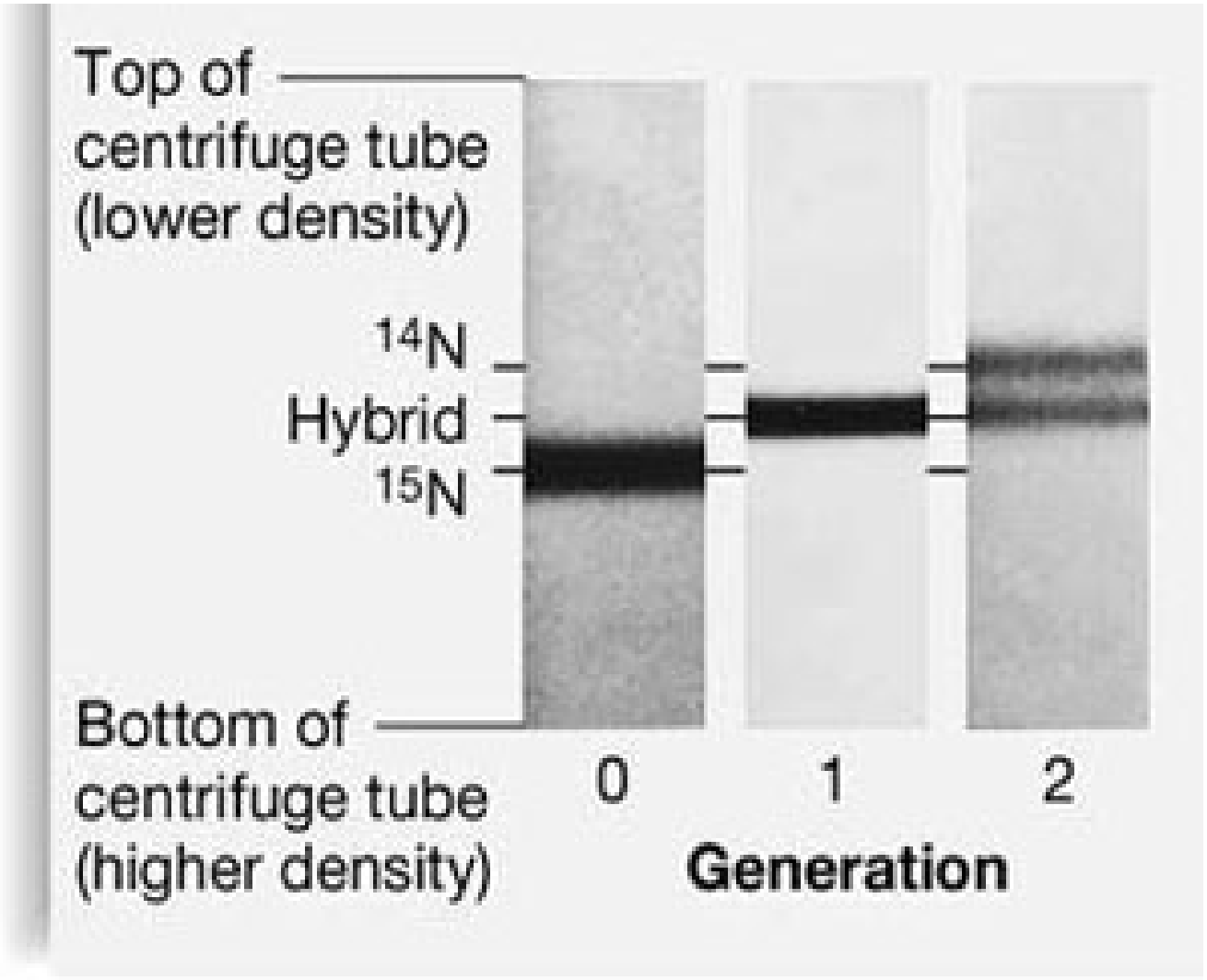

Different suggestions on possible mode of DNA replication
Conservative
Semiconservative
Dispersive
?
?
?
New DNA
Original DNA


Centrifugal force
Cesium Chloride Solution
$^{15}$N DNA
$^{14}$N DNA


Semiconservative
One band,
15N 14N
Two bands,
15N14N and 14N14N
Conservative
Two bands,
15N15N and 14N14N
Two bands,
15N15N and 14N14N
Dispersive
One band,
15N14N
One band,
intermediate between
15N14N and 14N14N


100 doublings
in 15N
one doubling
in 14N
Semi-conservative
or
Dispersive mode
second doubling
in 14N
Semi-conservative
Conservative Mode
Dispersive mode


DNA extracted and centrifuged
to equilibrium in CsCl density gradient
(a)
Heavy
DNA ($^{15}$N)
Original parent
molecule
(b)
Hybrid DNA
($^{15}$N–$^{14}$N)
First-generation
daughter molecules
(c)
Light
DNA ($^{14}$N)
Hybrid DNA
Second-generation
daughter molecules


Top of centrifuge tube (lower density)
$^{14}N$
Hybrid
$^{15}N$
Bottom of centrifuge tube (higher density)
0
1
2
Generation



# Are You Getting It??



**Which characteristics will be part of semi-conservative replication?** ***(multiple answers)***

a) **The two old DNA strands will separate.**
b) **The two new DNA strands form a double-helix.**
c) **Each DNA strand has a mixture of old DNA and new DNA.**
d) **Each new DNA strand is complementary to an old strand.**
e) **The old DNA strands are conserved while the new strands are dispersed.**



# Are You Getting It??



*Answer*

**Which characteristics will be part of semi-conservative replication?**

***a)*** ***The two old DNA strands will separate.***

***b)*** **The two new DNA strands form a double-helix.**

***c)*** **Each DNA strand has a mixture of old DNA and new DNA.**

***d)*** ***Each new DNA strand is complementary to an old strand.***

***e)*** **The old DNA strands are conserved while the new strands are dispersed.**



# Are You Getting It??



**Which types of DNA will band with hybrid density in CsCl density gradients after one generation when old DNA contains $^{15}N$ while new DNA contains $^{14}N$? *(multiple answers)***

a) A double-stranded DNA molecule made by conservative replication.
b) A double-stranded DNA molecule made by semi-conservative replication.
c) A double-stranded DNA molecule made by dispersive replication.



# Are You Getting It??



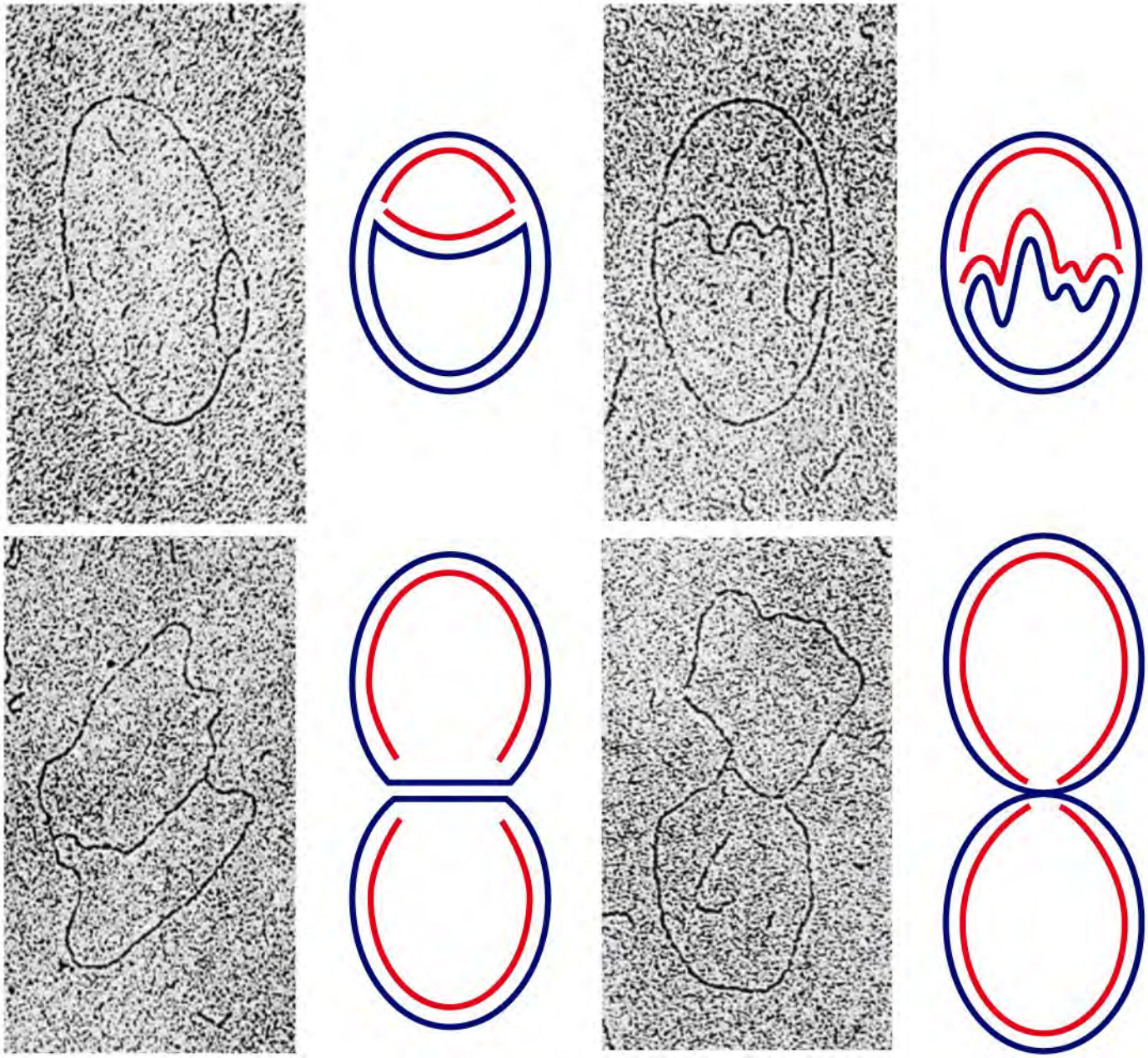
## *Answer*

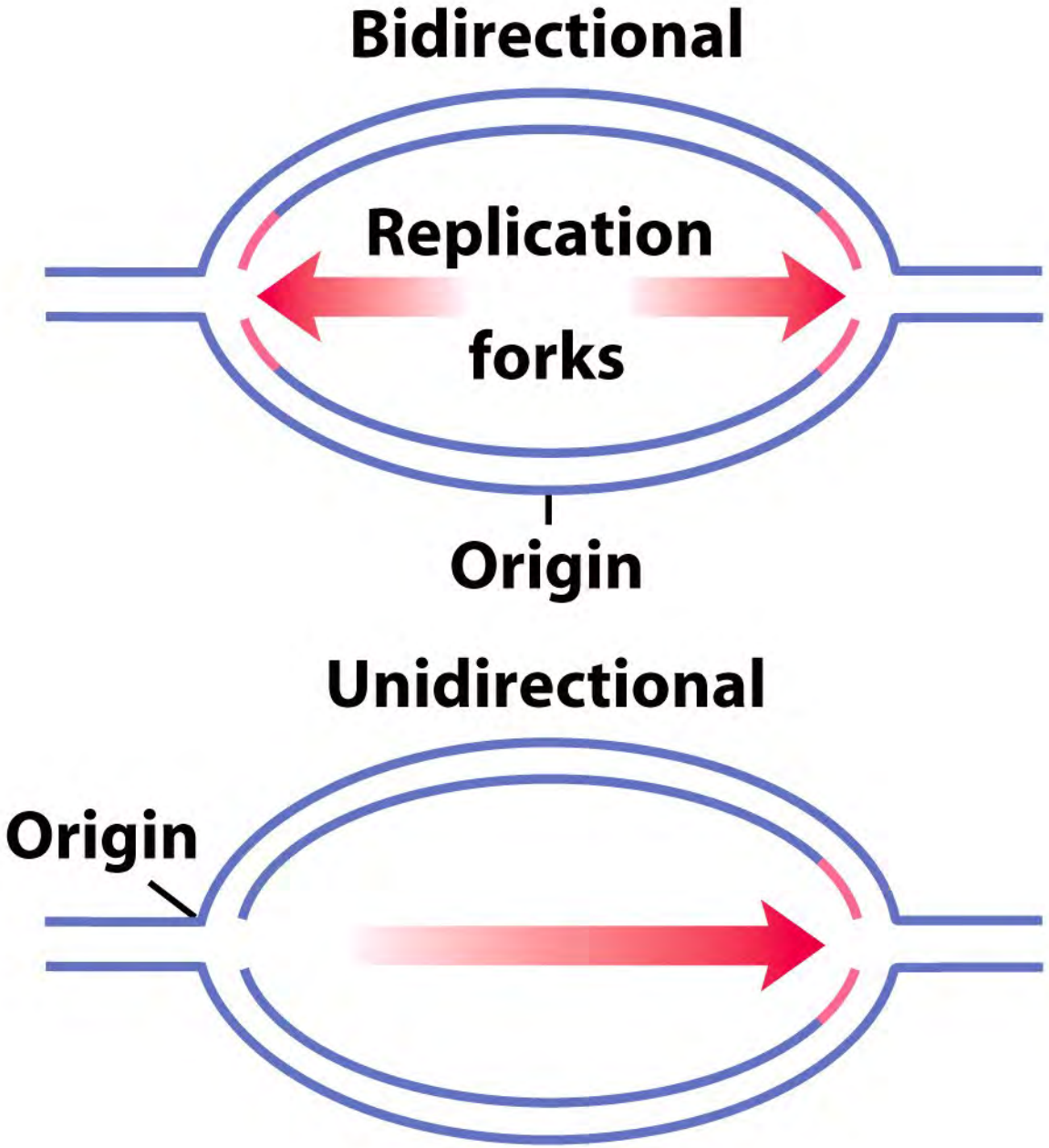
**Which types of DNA will band with hybrid density in CsCl density gradients after one generation when old DNA contains $^{15}N$ while new DNA contains $^{14}N$?**

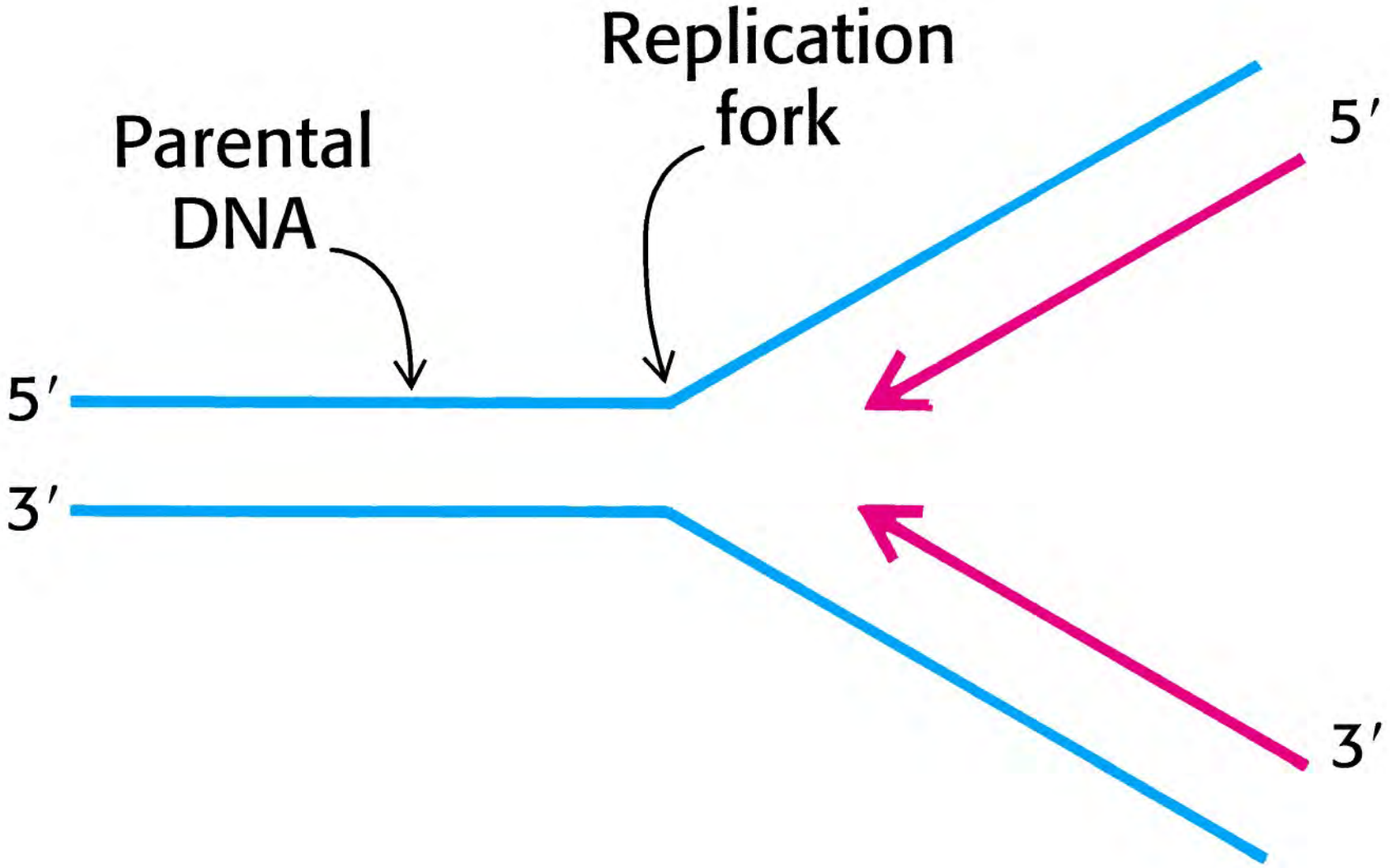
a) **A double-stranded DNA molecule made by conservative replication.**

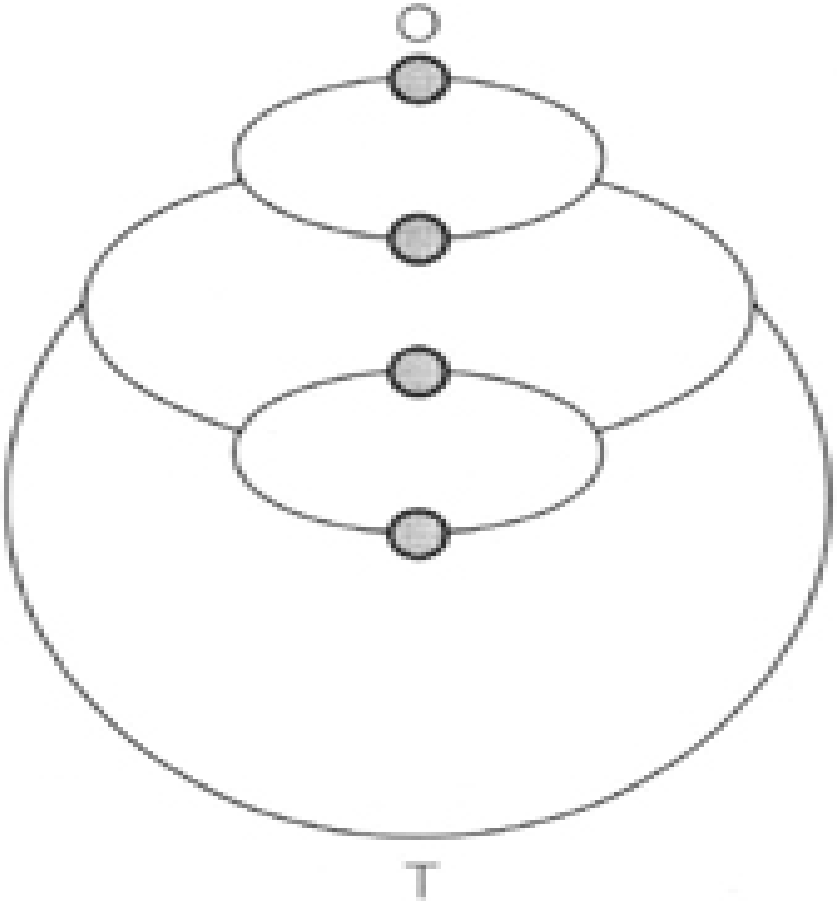
b) ***A double-stranded DNA molecule made by semi-conservative replication.***

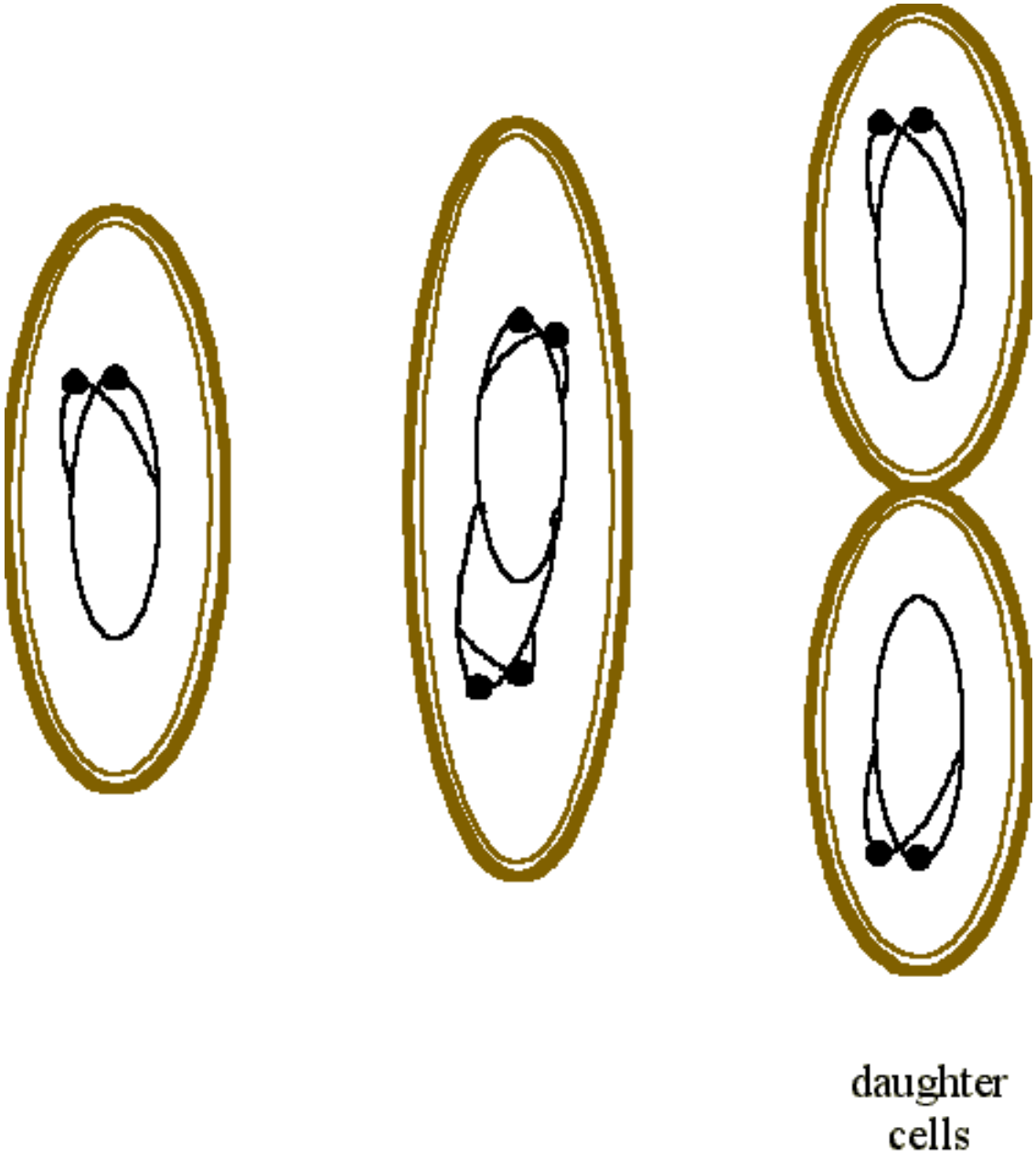
c) ***A double-stranded DNA molecule made by dispersive replication.***




Bidirectional
Replication
forks
Origin
Unidirectional
Origin


Replication fork
Parental DNA
5′
5′
3′
3′


O
T



daughter cells



# Are You Getting It??



**Which properties can be found in a replicating E. coli DNA molecule?** ***(multiple answers)***

a) Replication always starts at the origin.
b) Replication is normally unidirectional.
c) A replicating molecule can have two forks.
d) The rate of replication is constant at 37° C.
e) Only one round of replication can occur at any time.



# Are You Getting It??



*Answer*

**Which properties can be found in a replicating E. coli DNA molecule?**

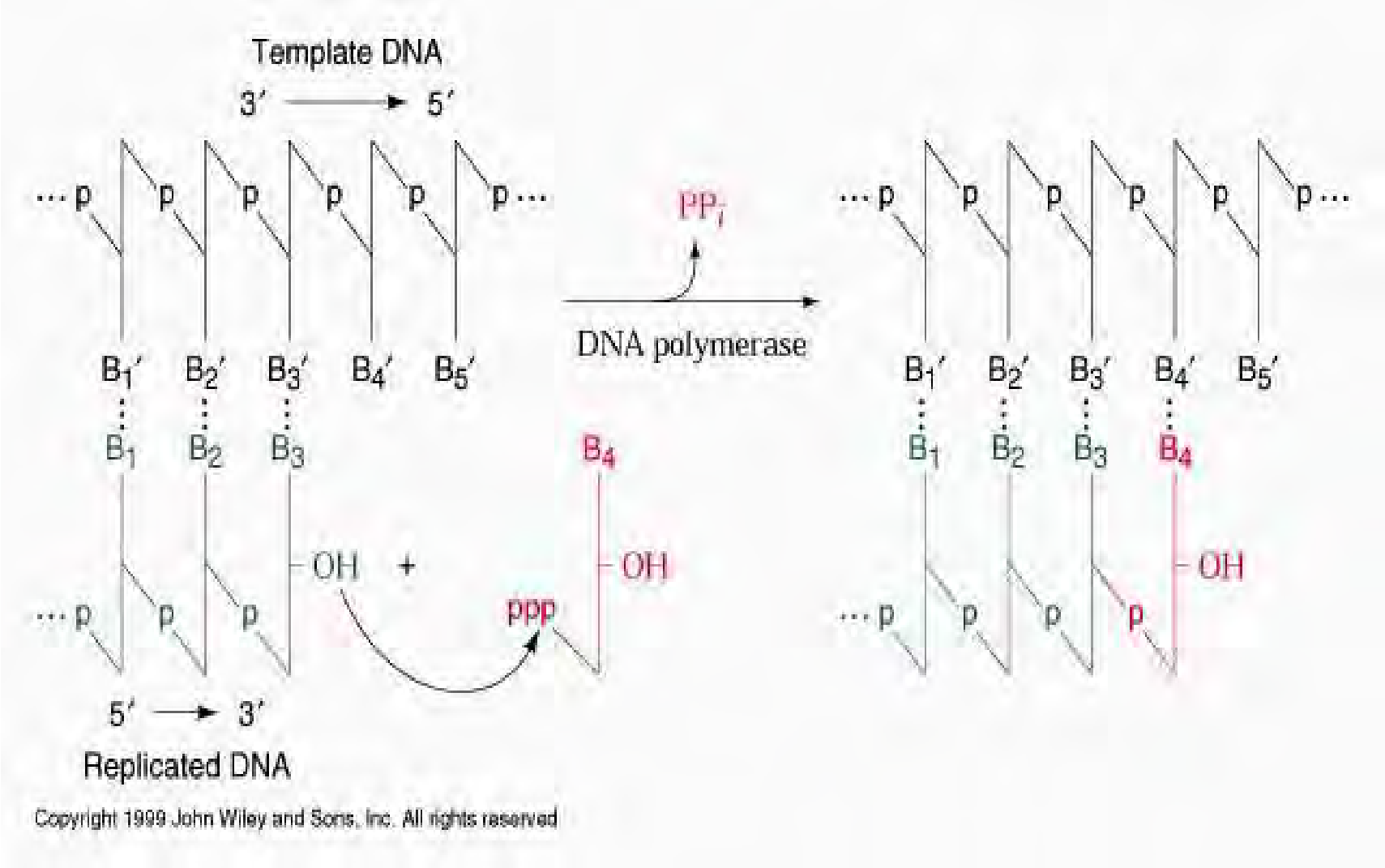
***a)*** ***Replication always starts at the origin.***

***b)*** **Replication is normally unidirectional.**

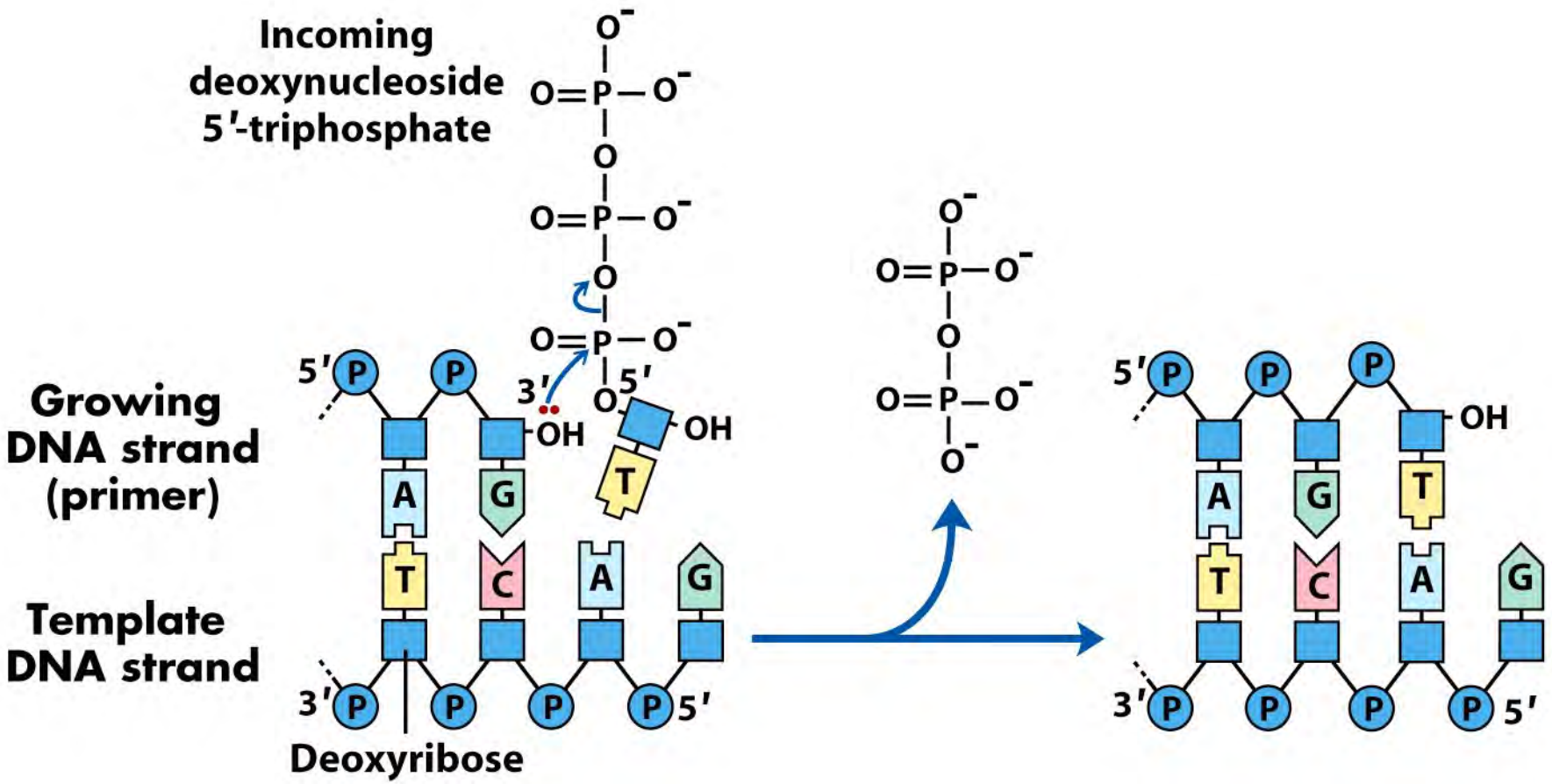
***c)*** ***A replicating molecule can have two forks.***

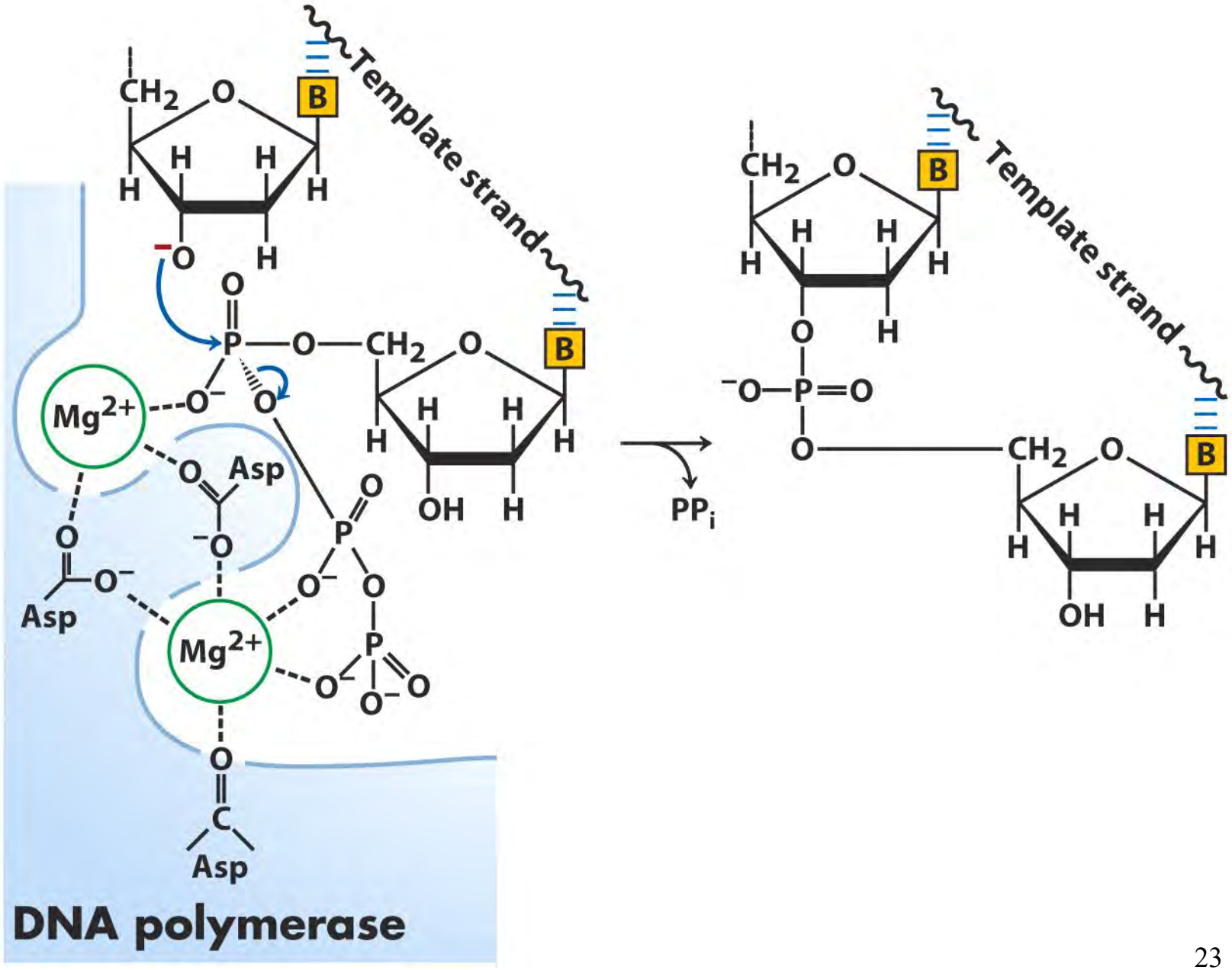
***d)*** ***The rate of replication is constant at 37$^{o}$ C.***

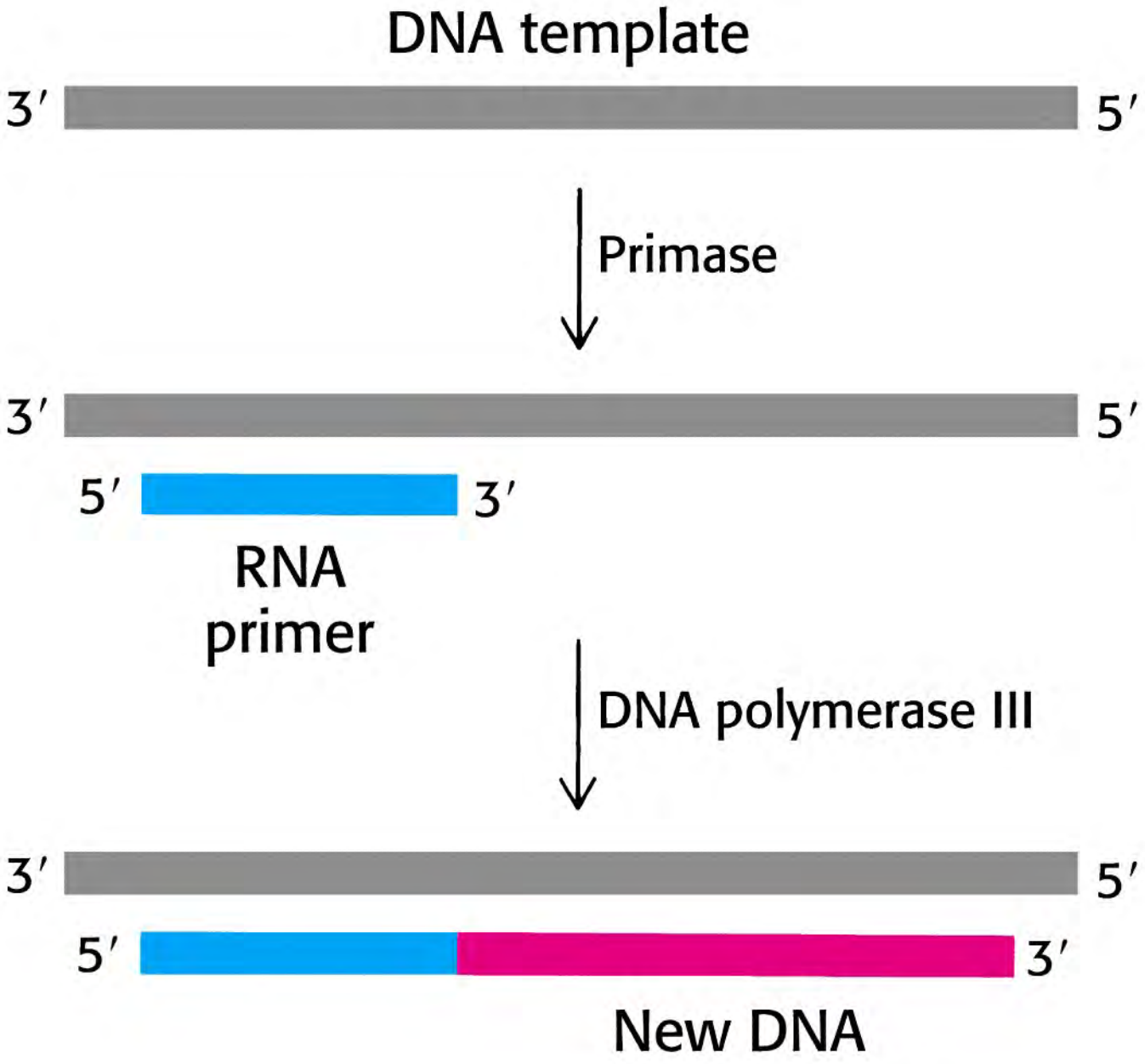
***e)*** **Only one round of replication can occur at any time.**



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Incoming
deoxynucleoside
5′-triphosphate
Growing
DNA strand
(primer)
Template
DNA strand
Deoxyribose
5′
3′
OH
A
G
T
C
A
G
T
C
A
G
T
OH
3′
5′


Template strand
B
CH2
O
H
OH
Mg2+
Asp
PPi
DNA polymerase


DNA template
3′
5′
Primase
3′
5′
5′
3′
RNA
primer
DNA polymerase III
3′
5′
5′
3′
New DNA



# Are You Getting It??



**Which components are necessary for DNA replication?**
***(multiple answers)***

a) a DNA template
b) a complementary primer
c) deoxyribonucleoside triphosphates
d) base-pairing
e) release of pyrophosphate
f) formation of phosphodiester bonds



# Are You Getting It??



## *Answer*

**Which components are necessary for DNA replication?**

*a) a DNA template*
*b) a complementary primer*
*c) deoxyribonucleoside triphosphates*
*d) base-pairing*
*e) release of pyrophosphate*
*f) formation of phosphodiester bonds*

## TABLE 25–1 Comparison of DNA Polymerases of *E. coli*

| | DNA polymerase | | |
|---|---|---|---|
| | I | II | III |
| Structural gene* | *polA* | *polB* | *polC* (*dnaE*) |
| Subunits (number of different types) | 1 | 7 | ≥10 |
| $M_r$ | 103,000 | 88,000† | 791,500 |
| 3′→5′ Exonuclease (proofreading) | Yes | Yes | Yes |
| 5′→3′ Exonuclease | Yes | No | No |
| Polymerization rate (nucleotides/s) | 16–20 | 40 | 250–1,000 |
| Processivity (nucleotides added before polymerase dissociates) | 3–200 | 1,500 | ≥500,000 |

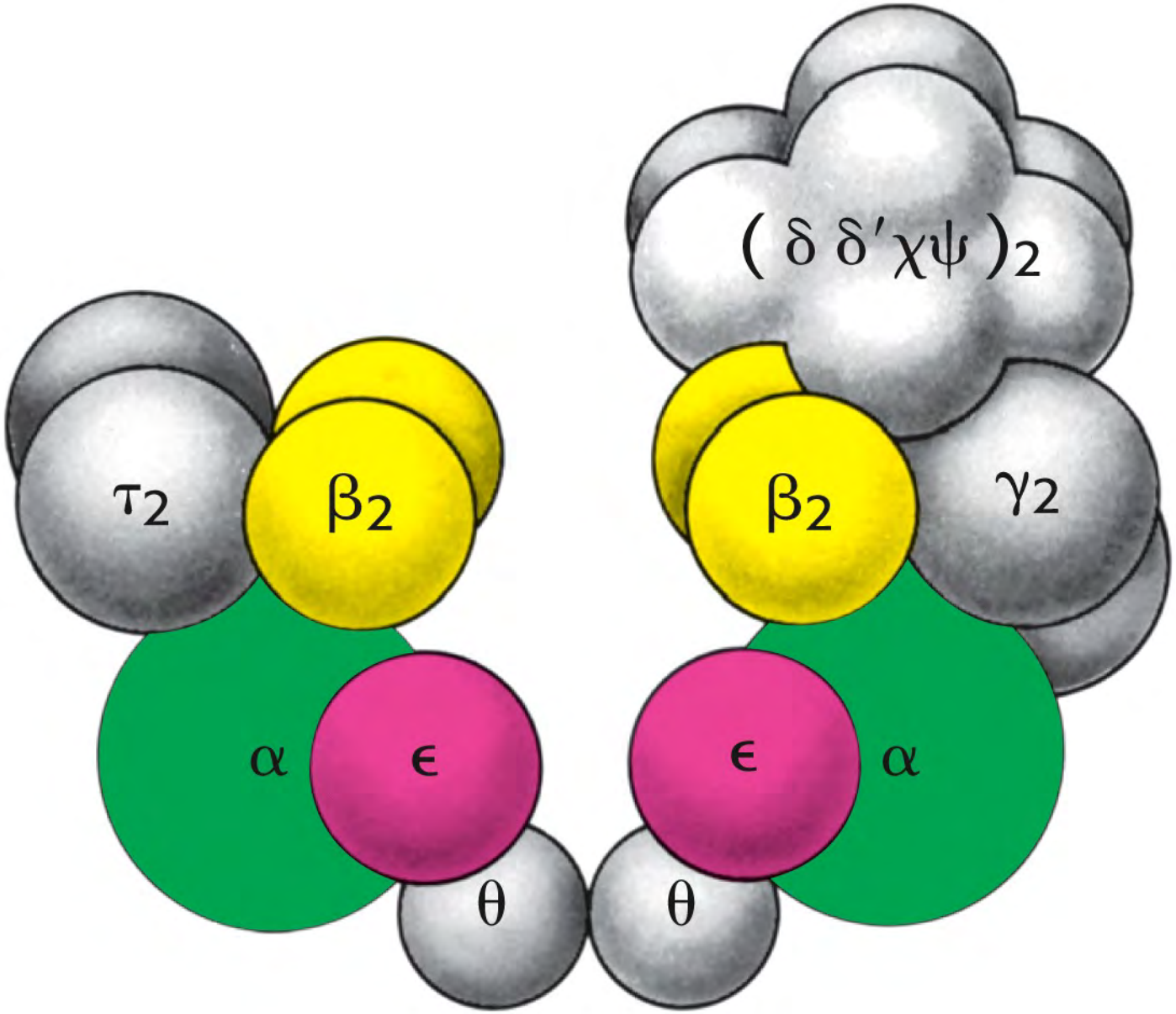
*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dnaE* is an earlier designation for the gene now referred to as *polC*.

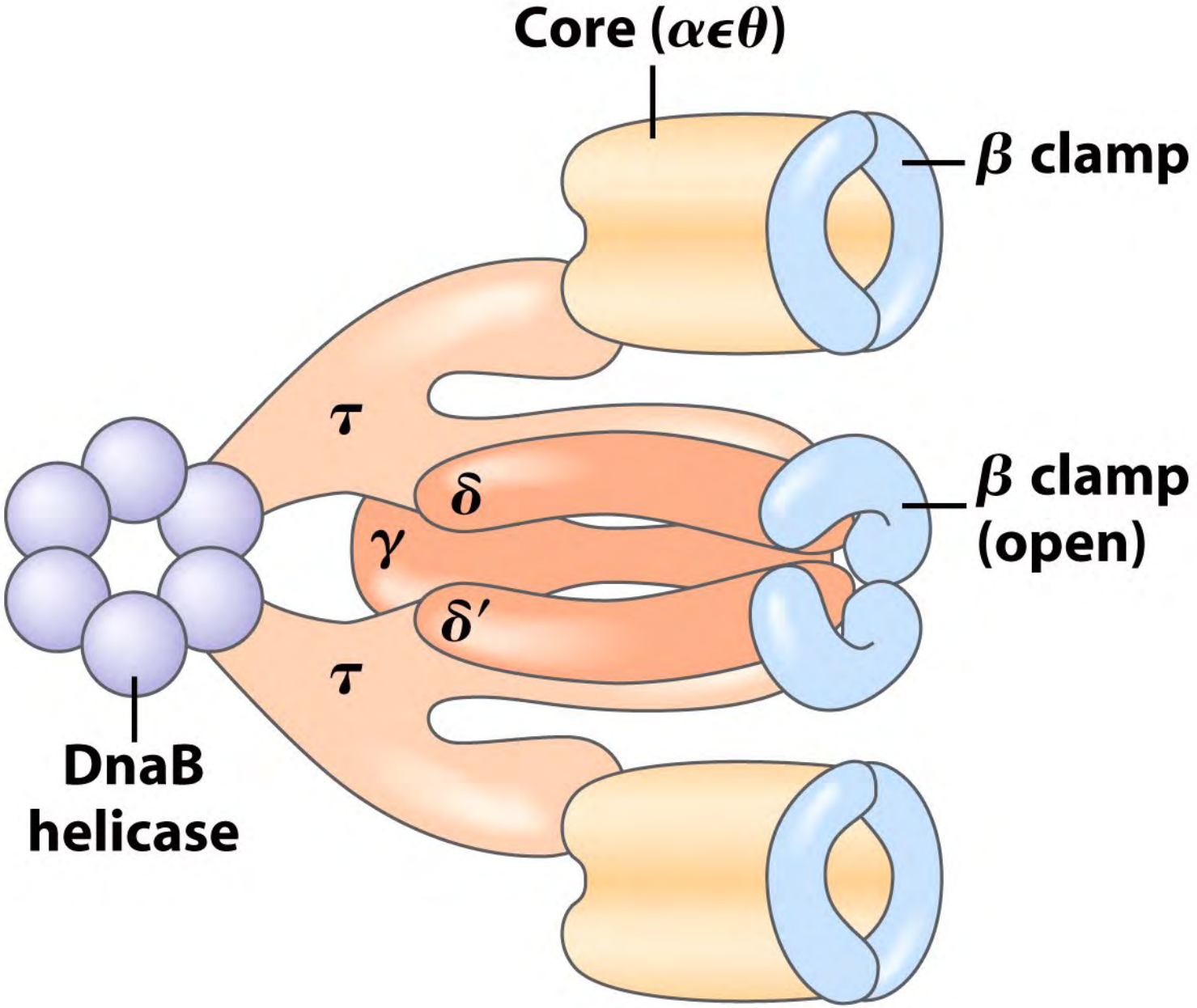
†Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the $\beta$, $\gamma$, $\delta$, $\delta'$, $\chi$, and $\psi$ subunits (see Table 25–2).

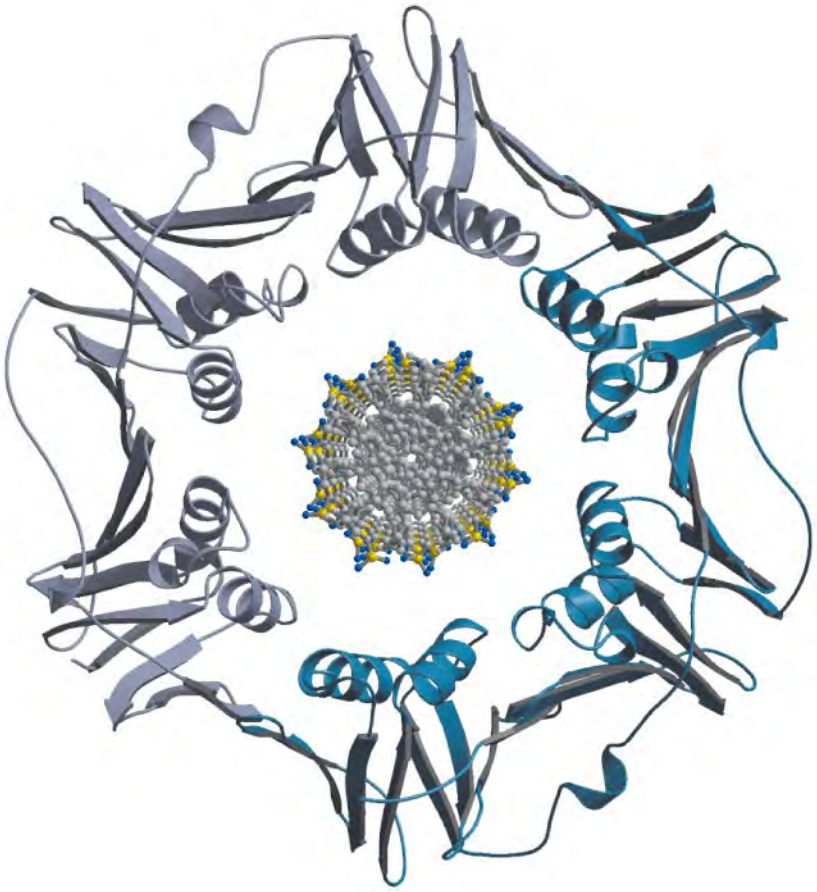
**TABLE 25–2** Subunits of DNA Polymerase III of *E. coli*

| Subunit | Number of subunits per holoenzyme | $M_r$ of subunit | Gene | Function of subunit | |
|---|---|---|---|---|---|
| $\alpha$ | 2 | 129,900 | *polC* (*dnaE*) | Polymerization activity | Core polymerase |
| $\varepsilon$ | 2 | 27,500 | *dnaQ* (*mutD*) | 3′→5′ Proofreading exonuclease | |
| $\theta$ | 2 | 8,600 | *holE* | | |
| $\tau$ | 2 | 71,100 | *dnaX* | Stable template binding; core enzyme dimerization | Clamp-loading ($\gamma$) complex that loads $\beta$ subunits on lagging strand at each Okazaki fragment |
| $\gamma$ | 1 | 47,500 | *dnaX** | Clamp loader | |
| $\delta$ | 1 | 38,700 | *holA* | Clamp opener | |
| $\delta'$ | 1 | 36,900 | *holB* | Clamp loader | |
| $\chi$ | 1 | 16,600 | *holC* | Interaction with SSB | |
| $\psi$ | 1 | 15,200 | *holD* | Interaction with $\gamma$ and $\chi$ | |
| $\beta$ | 4 | 40,600 | *dnaN* | DNA clamp required for optimal processivity | |

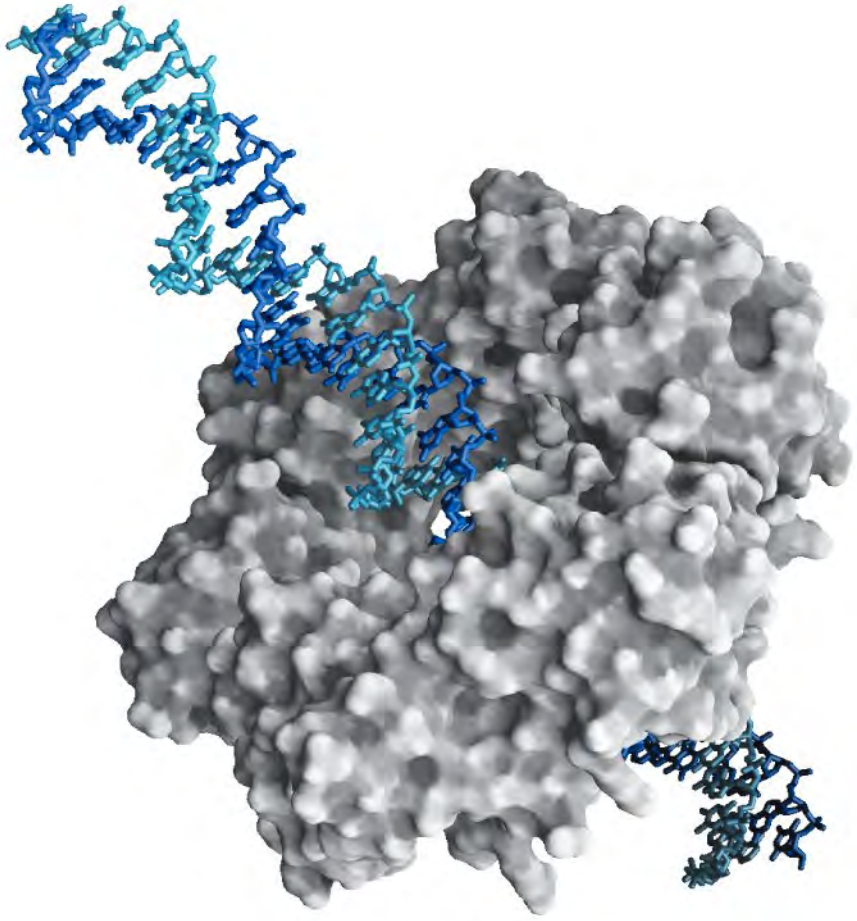
*The $\gamma$ subunit is encoded by a portion of the gene for the $\tau$ subunit, such that the amino-terminal 66% of the $\tau$ subunit has the same amino acid sequence as the $\gamma$ subunit. The $\gamma$ subunit is generated by a translational frameshifting mechanism (see Box 27–1) that leads to premature translational termination.


( δ δ′χψ )2
τ2
β2
β2
γ2
α
ϵ
ϵ
α
θ
θ


Core (αϵθ)
β clamp
τ
δ
γ
δ′
τ
β clamp
(open)
DnaB
helicase



End view



Side view



# Are You Getting It??



**Which property is shared by E. coli DNA polymerases I, II, and III?**

a) They all contain multiple subunits.
b) They all have similar molecular weights.
c) They all function in replication.
d) They all add nucleotides to the 3'- end.
e) They all polymerize at the same rate.
f) They all have the same processivity.



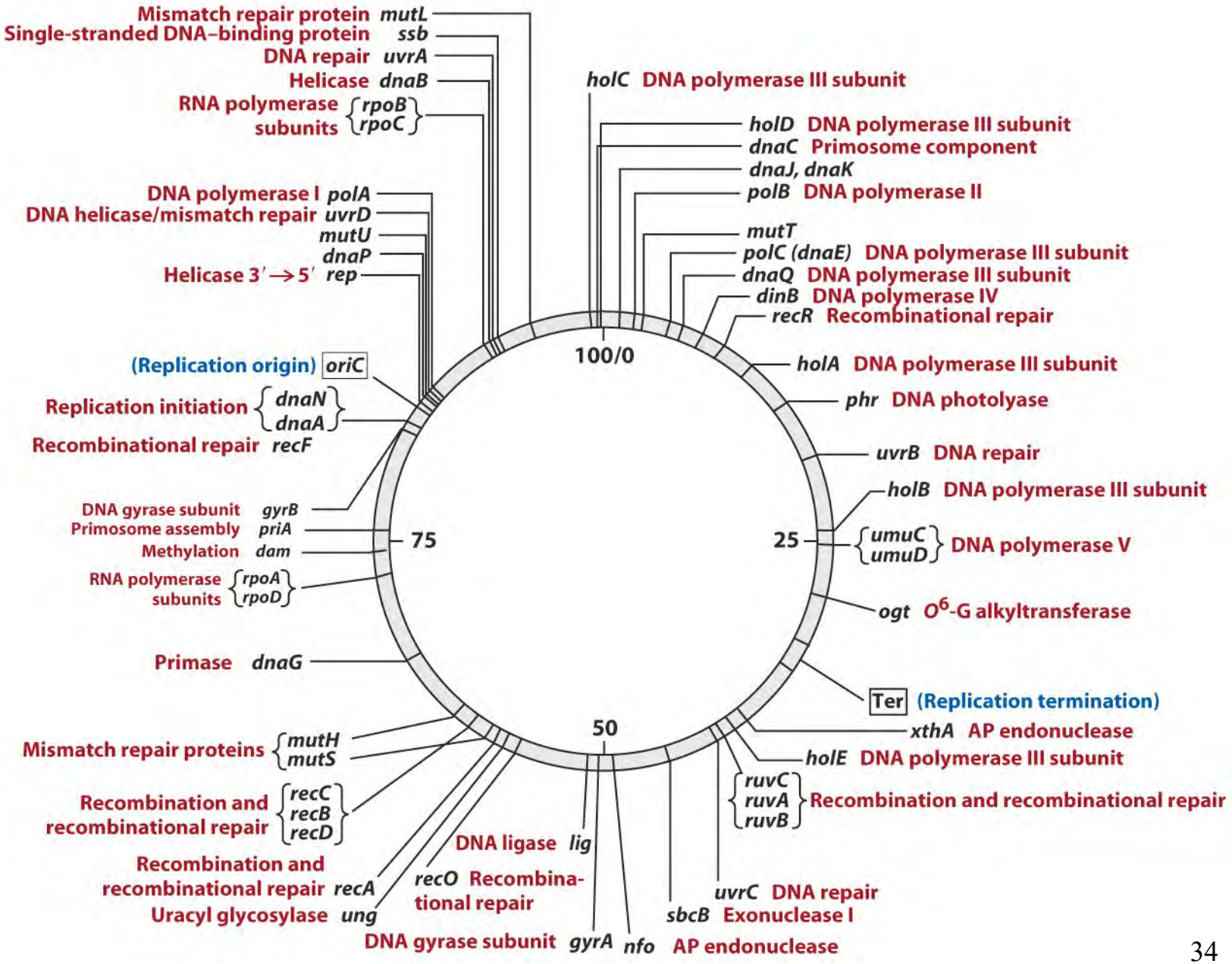
# Are You Getting It??

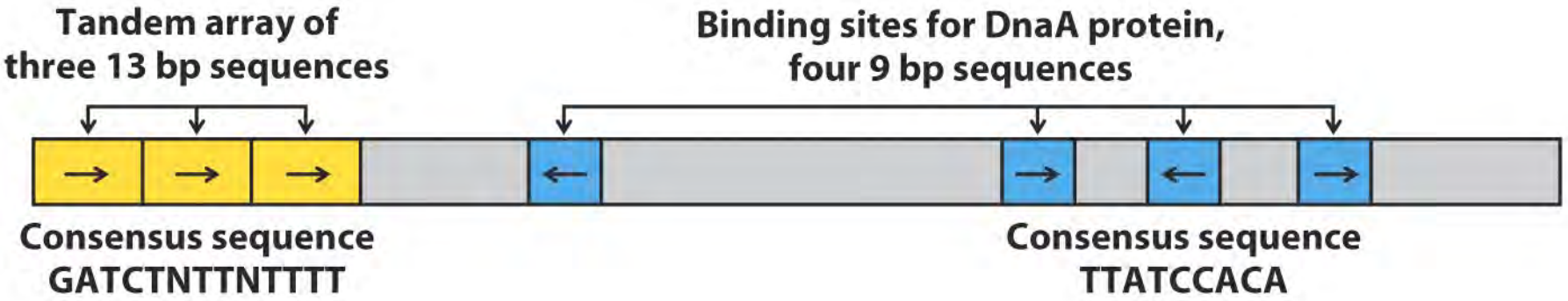


*Answer*

**Which property is shared by E. coli DNA polymerases I, II, and III?**

a) **They all contain multiple subunits.**
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c) **They all function in replication.**
d) ***They all add nucleotides to the 3'- end.***
e) **They all polymerize at the same rate.**
f) **They all have the same processivity.**


Mismatch repair protein mutL
Single-stranded DNA–binding protein ssb
DNA repair uvrA
Helicase dnaB
RNA polymerase subunits rpoB rpoC
DNA polymerase I polA
DNA helicase/mismatch repair uvrD
mutU
dnaP
Helicase 3′→5′ rep
(Replication origin) oriC
Replication initiation dnaN dnaA
Recombinational repair recF
DNA gyrase subunit gyrB
Primosome assembly priA
Methylation dam
RNA polymerase subunits rpoA rpoD
Primase dnaG
Mismatch repair proteins mutH mutS
Recombination and recombinational repair recC recB recD
Recombination and recombinational repair recA
Uracyl glycosylase ung
DNA gyrase subunit gyrA
recO Recombinational repair
DNA ligase lig
nfo AP endonuclease
sbcB Exonuclease I
uvrC DNA repair
ruvC ruvA ruvB Recombination and recombinational repair
holE DNA polymerase III subunit
xthA AP endonuclease
Ter (Replication termination)
ogt O6-G alkyltransferase
umuC umuD DNA polymerase V
holB DNA polymerase III subunit
uvrB DNA repair
phr DNA photolyase
holA DNA polymerase III subunit
recR Recombinational repair
dinB DNA polymerase IV
dnaQ DNA polymerase III subunit
polC (dnaE) DNA polymerase III subunit
mutT
polB DNA polymerase II
dnaJ, dnaK
dnaC Primosome component
holD DNA polymerase III subunit
holC DNA polymerase III subunit
100/0
25
50
75


Tandem array of
three 13 bp sequences
Binding sites for DnaA protein,
four 9 bp sequences
Consensus sequence
GATCTNTTNTTTT
Consensus sequence
TTATCCACA

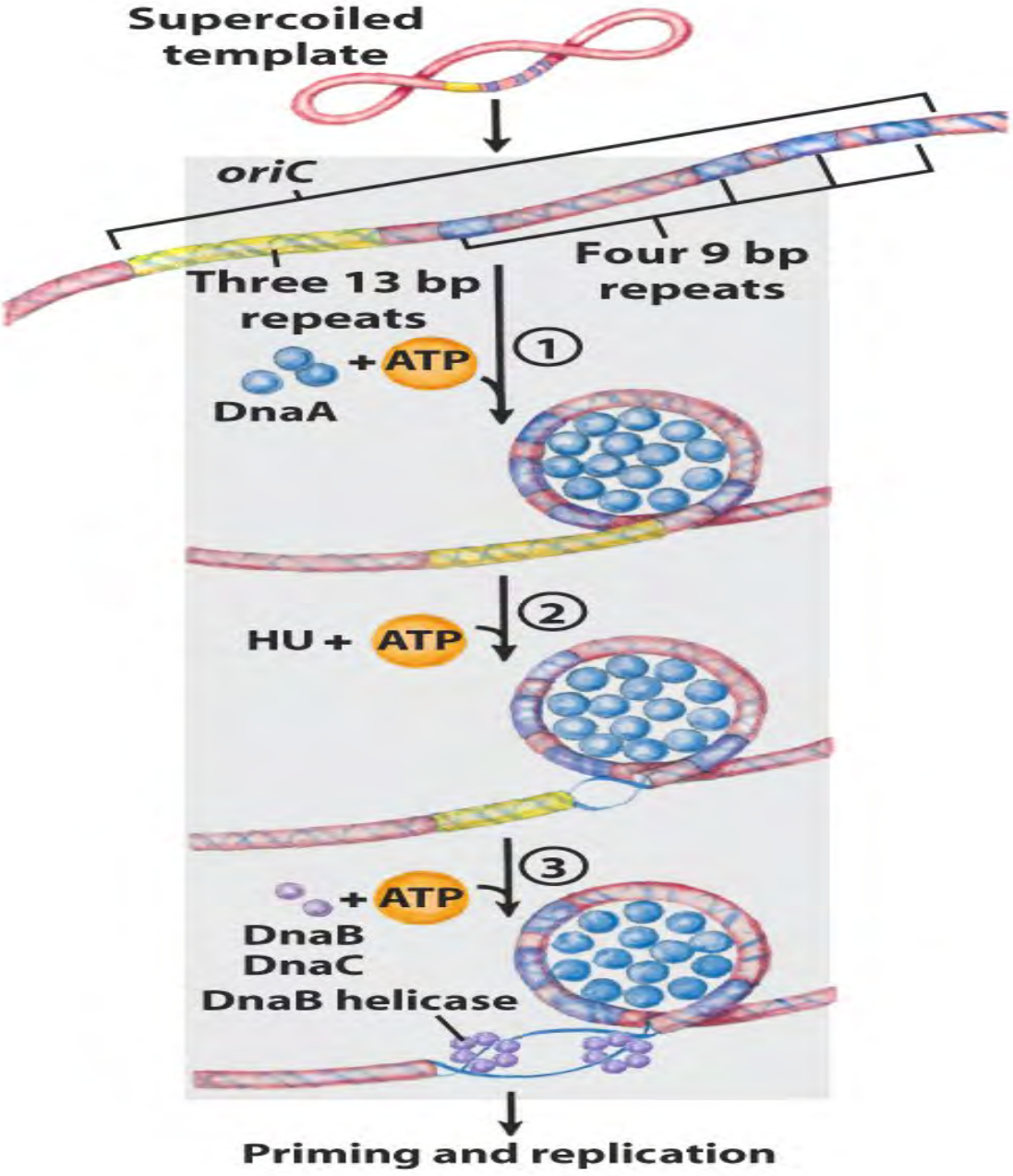

Supercoiled template
oriC
Three 13 bp repeats
Four 9 bp repeats
+ ATP
DnaA
1
HU + ATP
2
+ ATP
3
DnaB
DnaC
DnaB helicase
Priming and replication

**TABLE 25–3** Proteins Required to Initiate Replication at the *E. coli* Origin

| *Protein* | $M_r$ | *Number of subunits* | *Function* |
|---|---|---|---|
| DnaA protein | 52,000 | 1 | Recognizes ori sequence; opens duplex at specific sites in origin |
| DnaB protein (helicase) | 300,000 | 6* | Unwinds DNA |
| DnaC protein | 29,000 | 1 | Required for DnaB binding at origin |
| HU | 19,000 | 2 | Histonelike protein; DNA-binding protein; stimulates initiation |
| Primase (DnaG protein) | 60,000 | 1 | Synthesizes RNA primers |
| Single-stranded DNA–binding protein (SSB) | 75,600 | 4* | Binds single-stranded DNA |
| RNA polymerase | 454,000 | 5 | Facilitates DnaA activity |
| DNA gyrase (DNA topoisomerase II) | 400,000 | 4 | Relieves torsional strain generated by DNA unwinding |
| Dam methylase | 32,000 | 1 | Methylates (5′)GATC sequences at *oriC* |

*Subunits in these cases are identical.



# Are You Getting It??



**Which events occur during initiation of replication in E. coli?**
***(multiple answers)***

a) DNA is denatured with the help of proteins.
b) All replication proteins are needed at the origin.
c) DNA polymerase binds to repeated sequences.
d) The frequency of DNA replication is controlled.
e) The origin is degraded as replication starts.



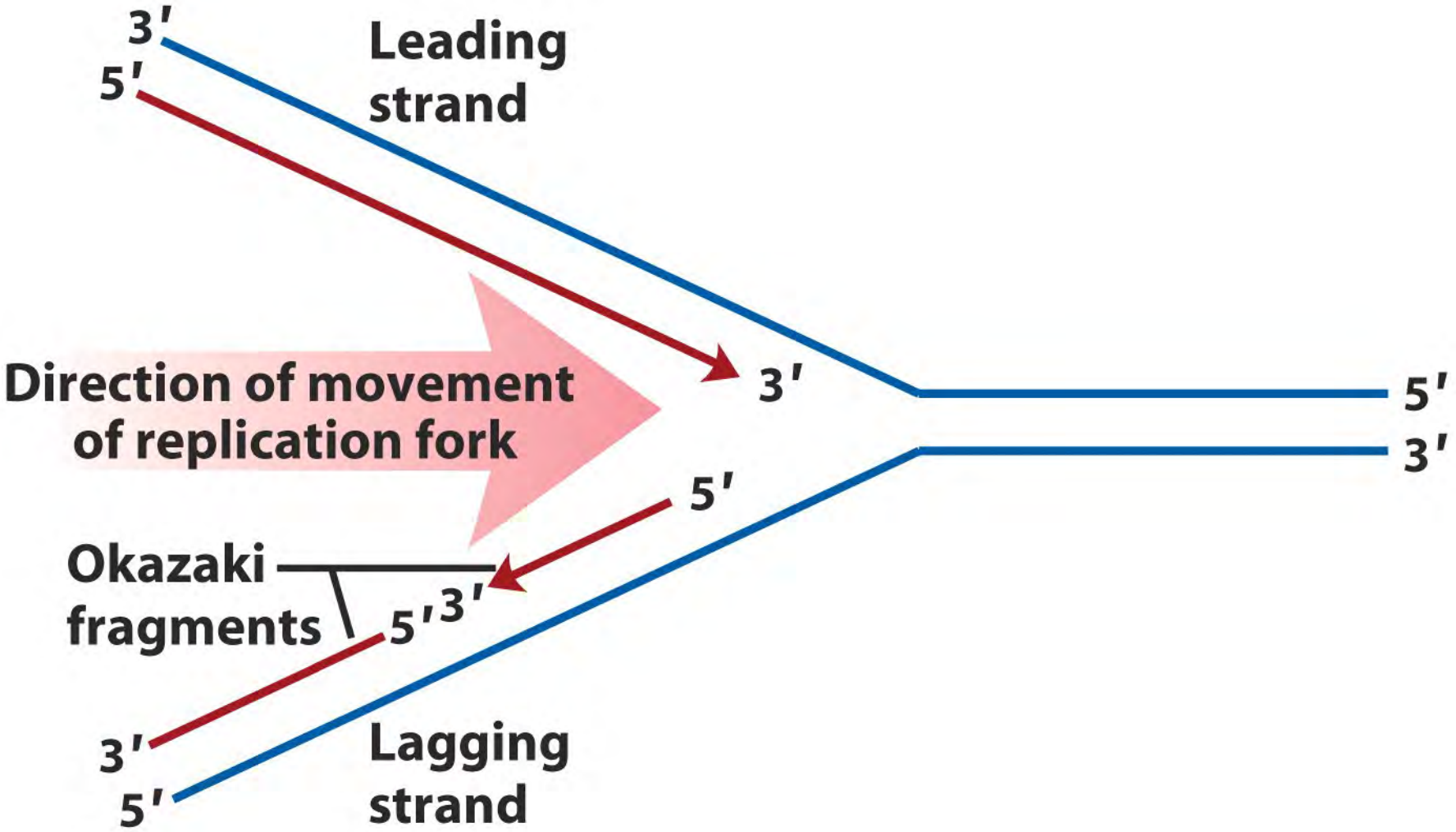
# Are You Getting It??

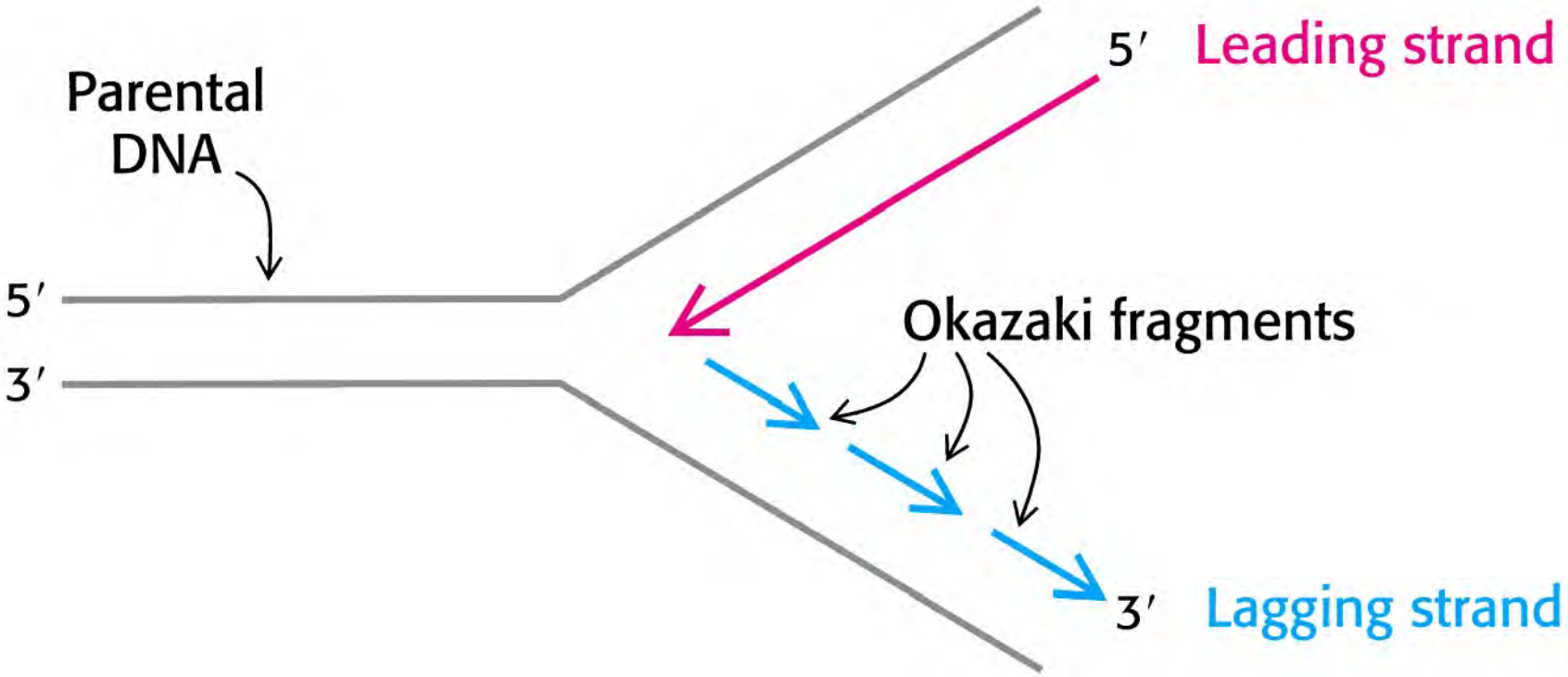


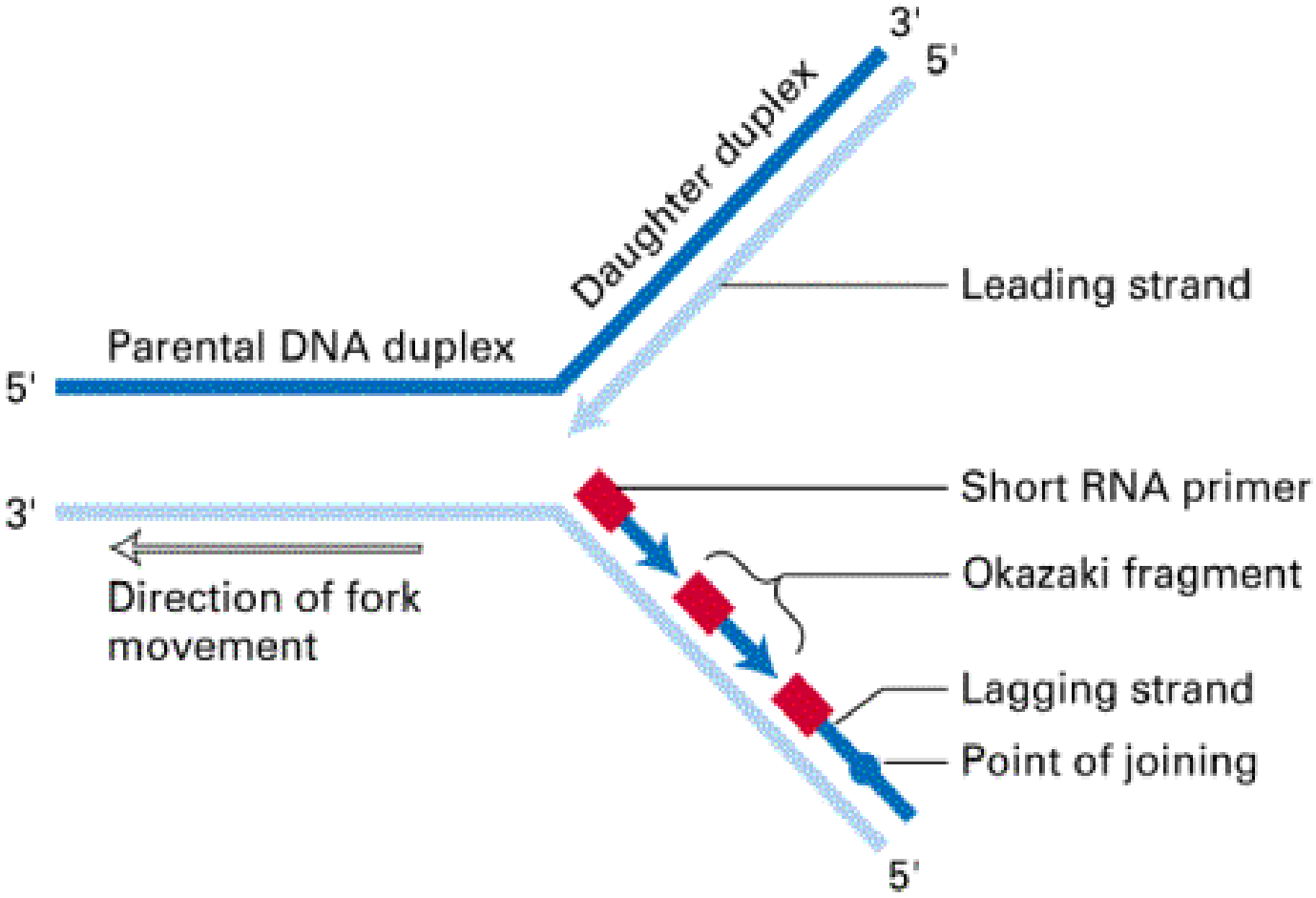
*Answer*

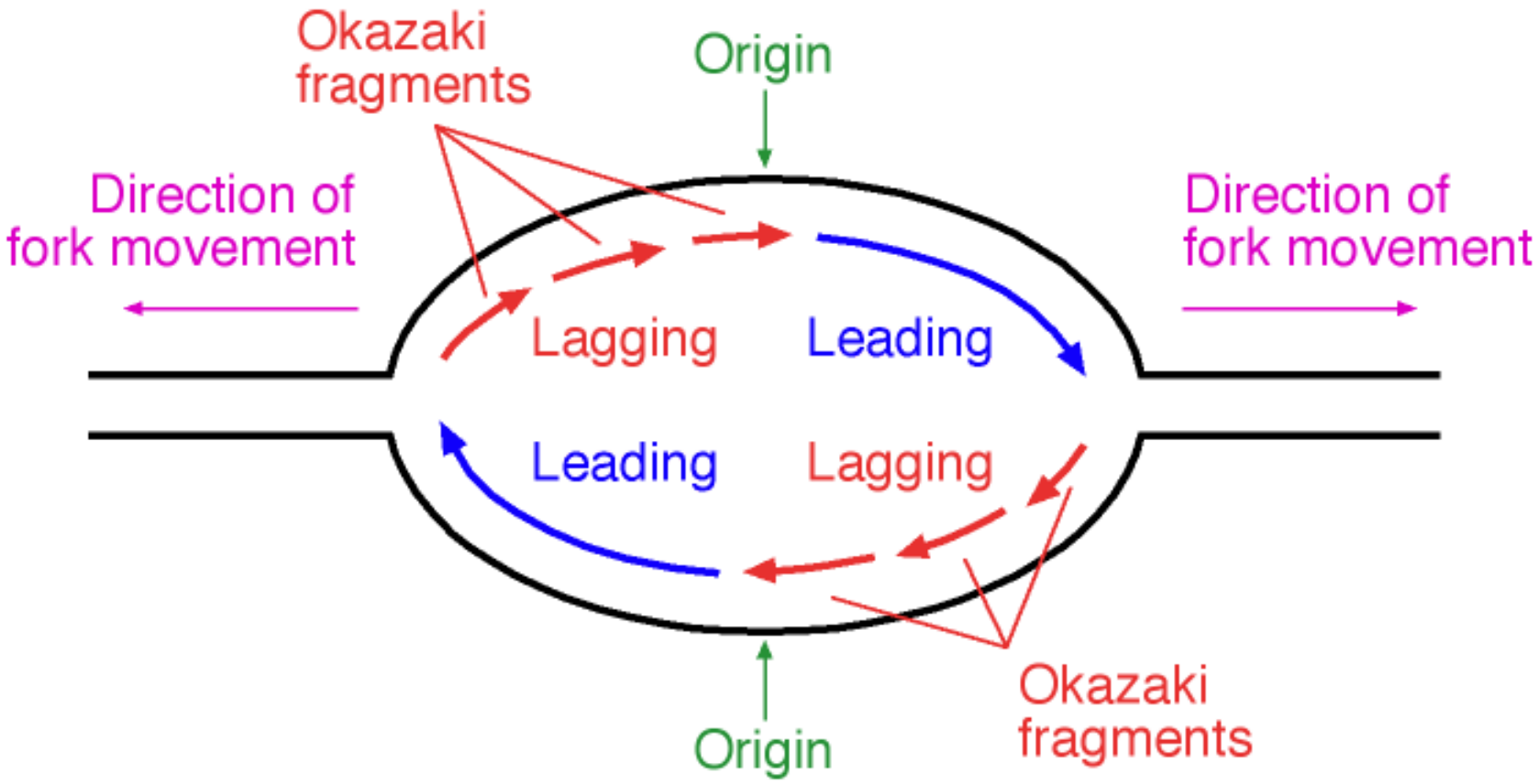
**Which events occur during initiation of replication in E. coli?**

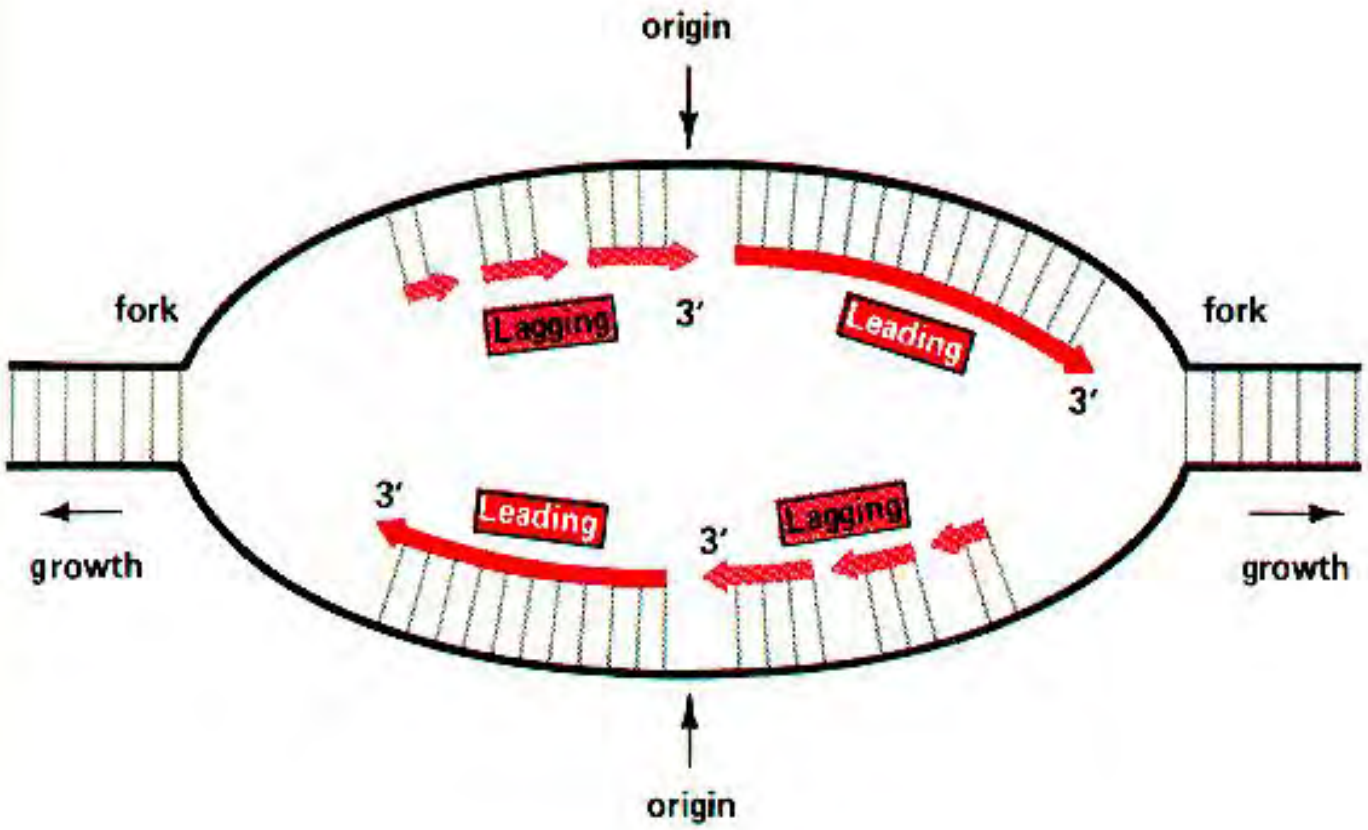
***a)*** ***DNA is denatured with the help of proteins.***
***b)*** **All replication proteins are needed at the origin.**
***c)*** **DNA polymerase binds to repeated sequences.**
***d)*** ***The frequency of DNA replication is controlled.***
***e)*** **The origin is degraded as replication starts.**

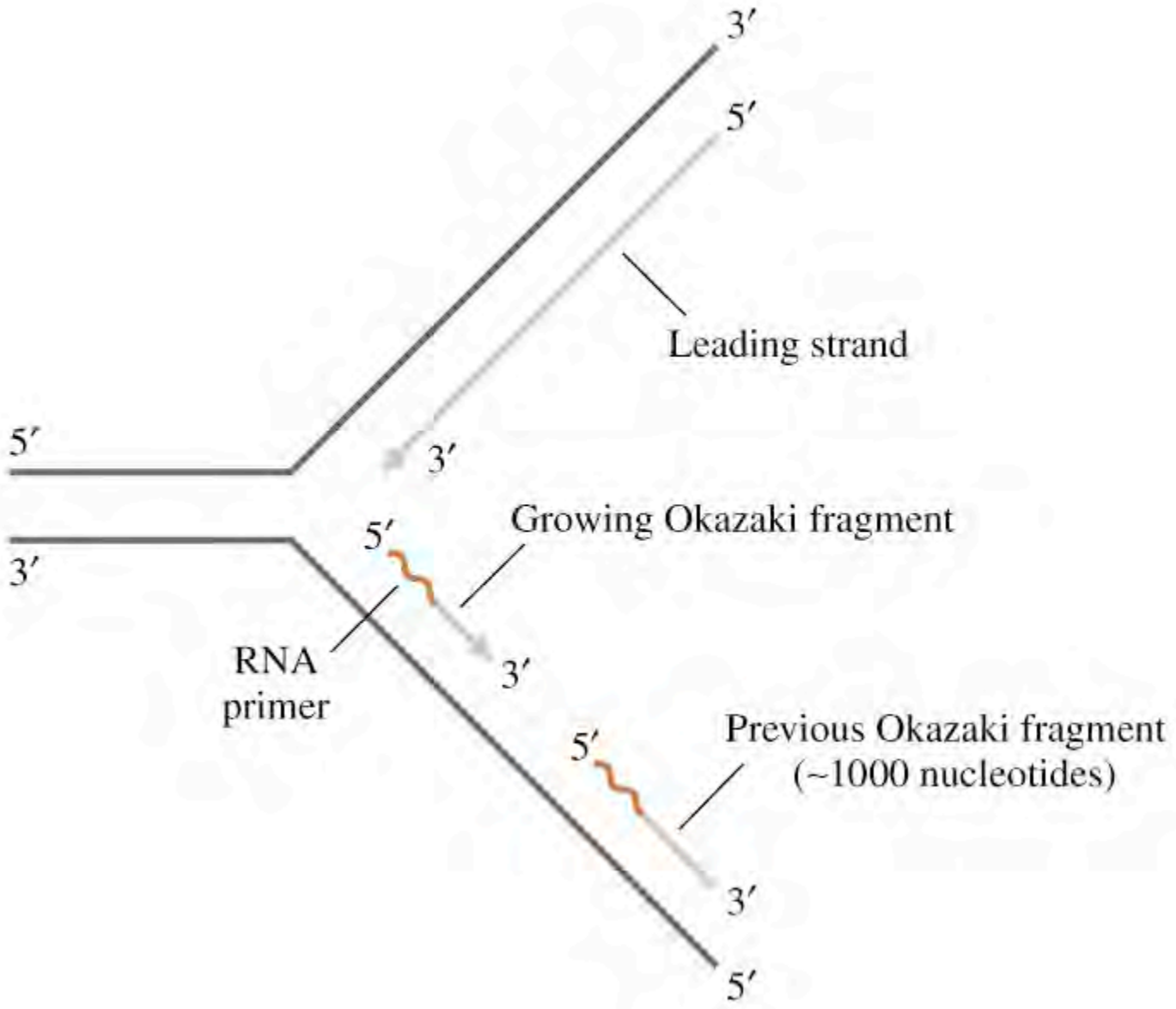

3′
5′
Leading strand
Direction of movement of replication fork
3′
5′
3′
5′
Okazaki fragments
5′
3′
3′
5′
Lagging strand


Parental DNA
5′
3′
5′
Leading strand
Okazaki fragments
3′
Lagging strand


3'
5'
Daughter duplex
Leading strand
Parental DNA duplex
5'
3'
Short RNA primer
Okazaki fragment
Direction of fork
movement
Lagging strand
Point of joining
5'


Okazaki fragments
Origin
Direction of fork movement
Direction of fork movement
Lagging
Leading
Leading
Lagging
Origin
Okazaki fragments


origin
fork
Lagging
3′
Leading
fork
3′
3′
Leading
3′
Lagging
growth
growth
origin


3′
5′
Leading strand
5′
3′
5′
Growing Okazaki fragment
3′
RNA
primer
3′
5′
Previous Okazaki fragment
(~1000 nucleotides)
3′
5′



# Are You Getting It??



**Which characteristics are found in an E. coli replication fork?**
***(multiple answers)***

a) There are two leading strands.
b) There are two lagging strands.
c) All DNA is made with Okazaki pieces.
d) All Okazaki pieces start with an RNA primer.
e) Replication is semi-discontinuous.
f) An Okazaki pieces has ~1000 nucleotides.



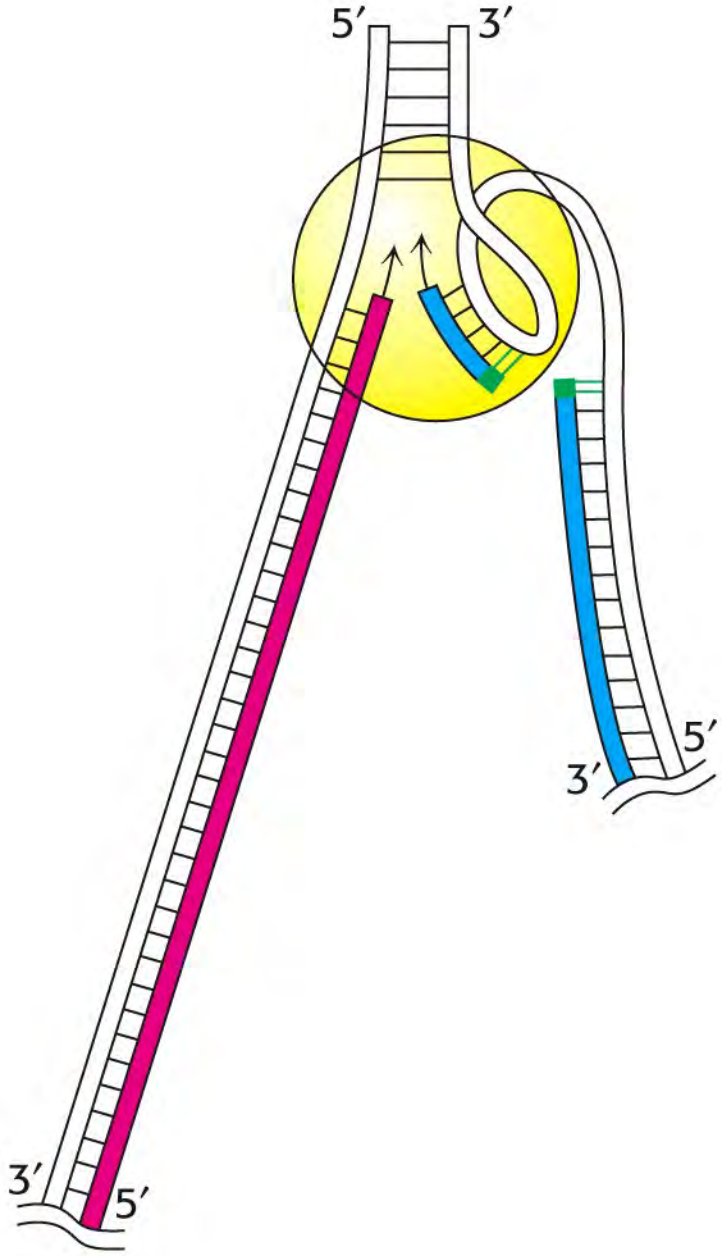
# Are You Getting It??

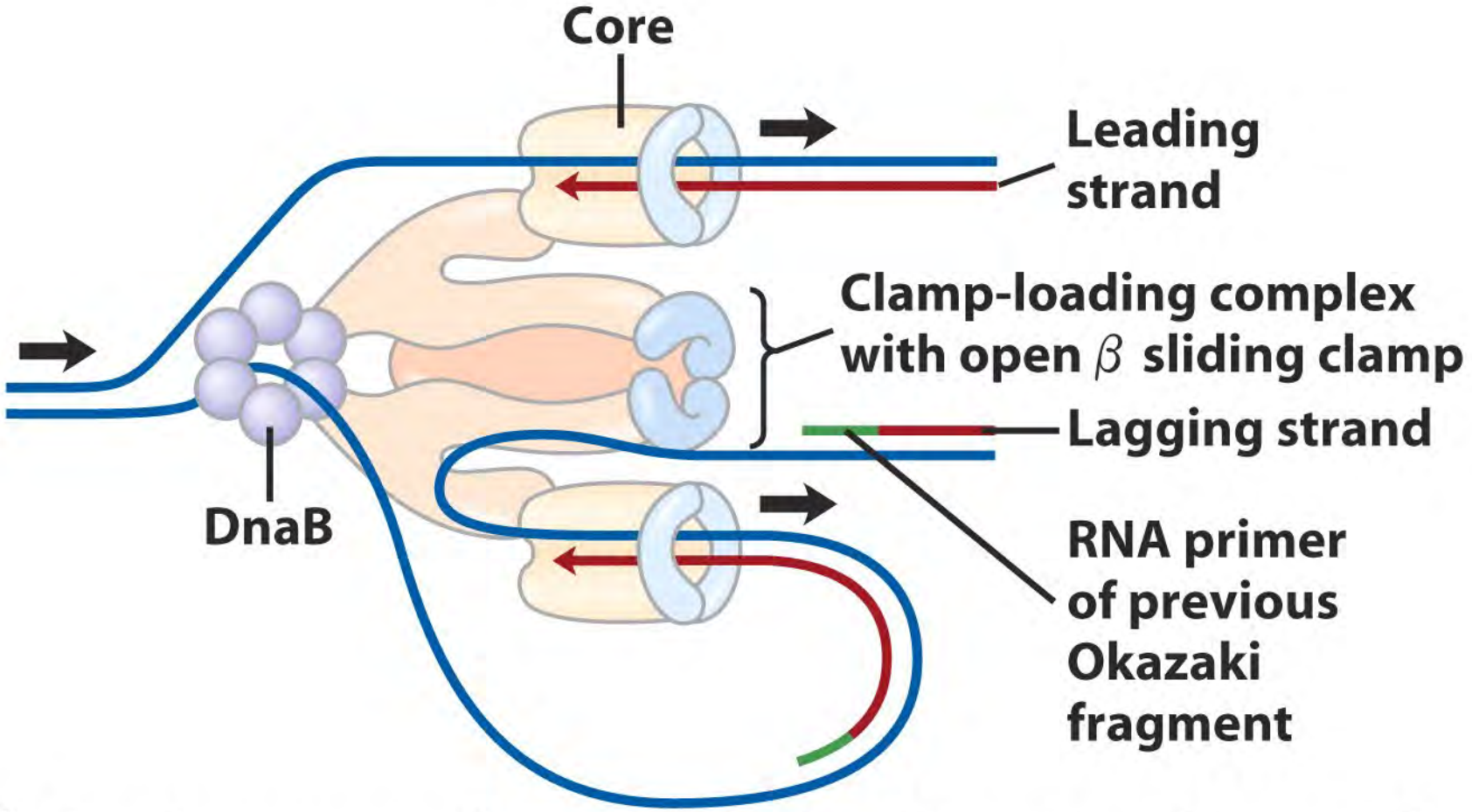


*Answer*

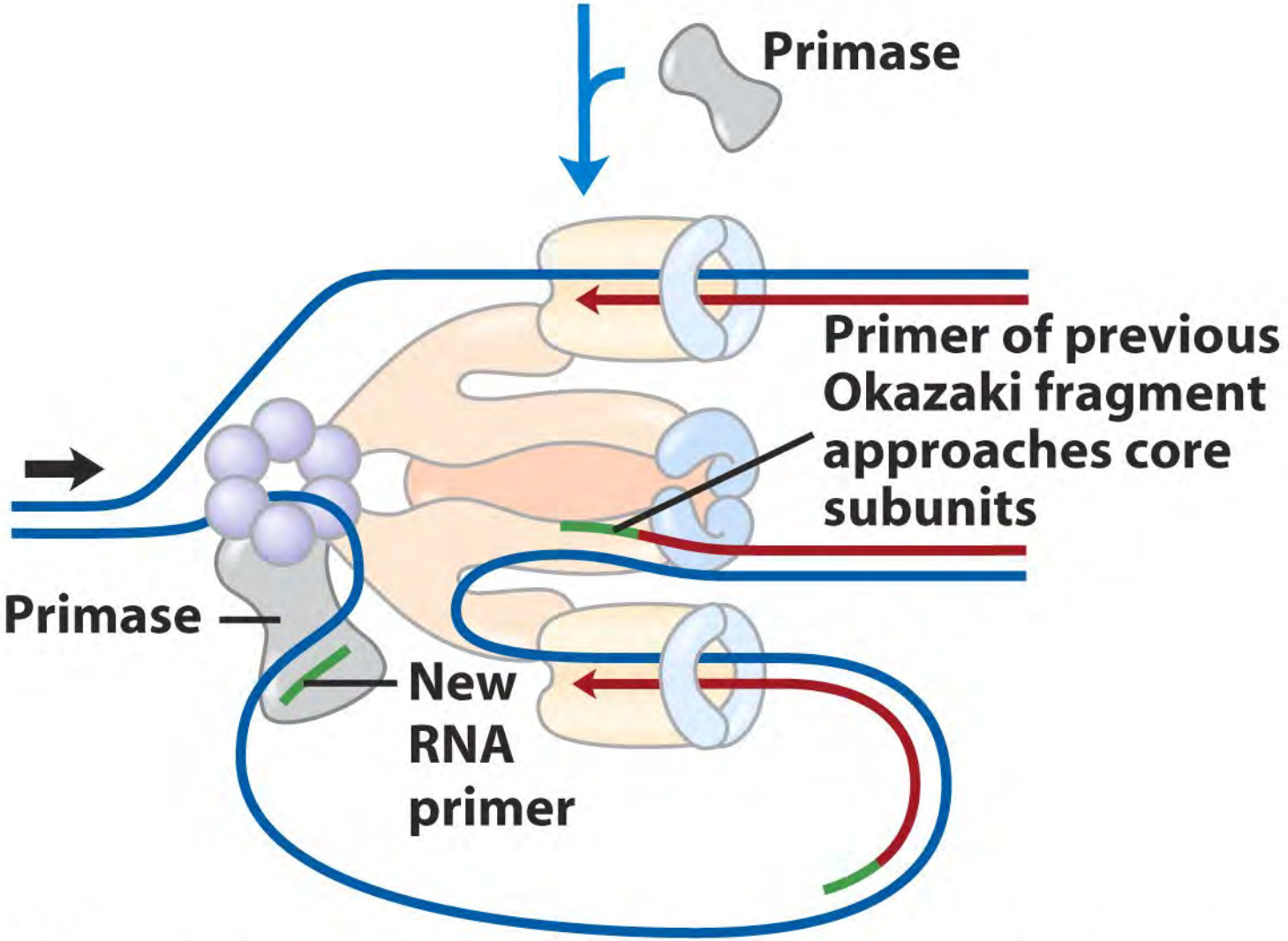
**Which characteristics are found in an E. coli replication fork?**

a) There are two leading strands.
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d) *All Okazaki pieces start with an RNA primer.*
e) *Replication is semi-discontinuous.*
f) *An Okazaki pieces has ~1000 nucleotides.*

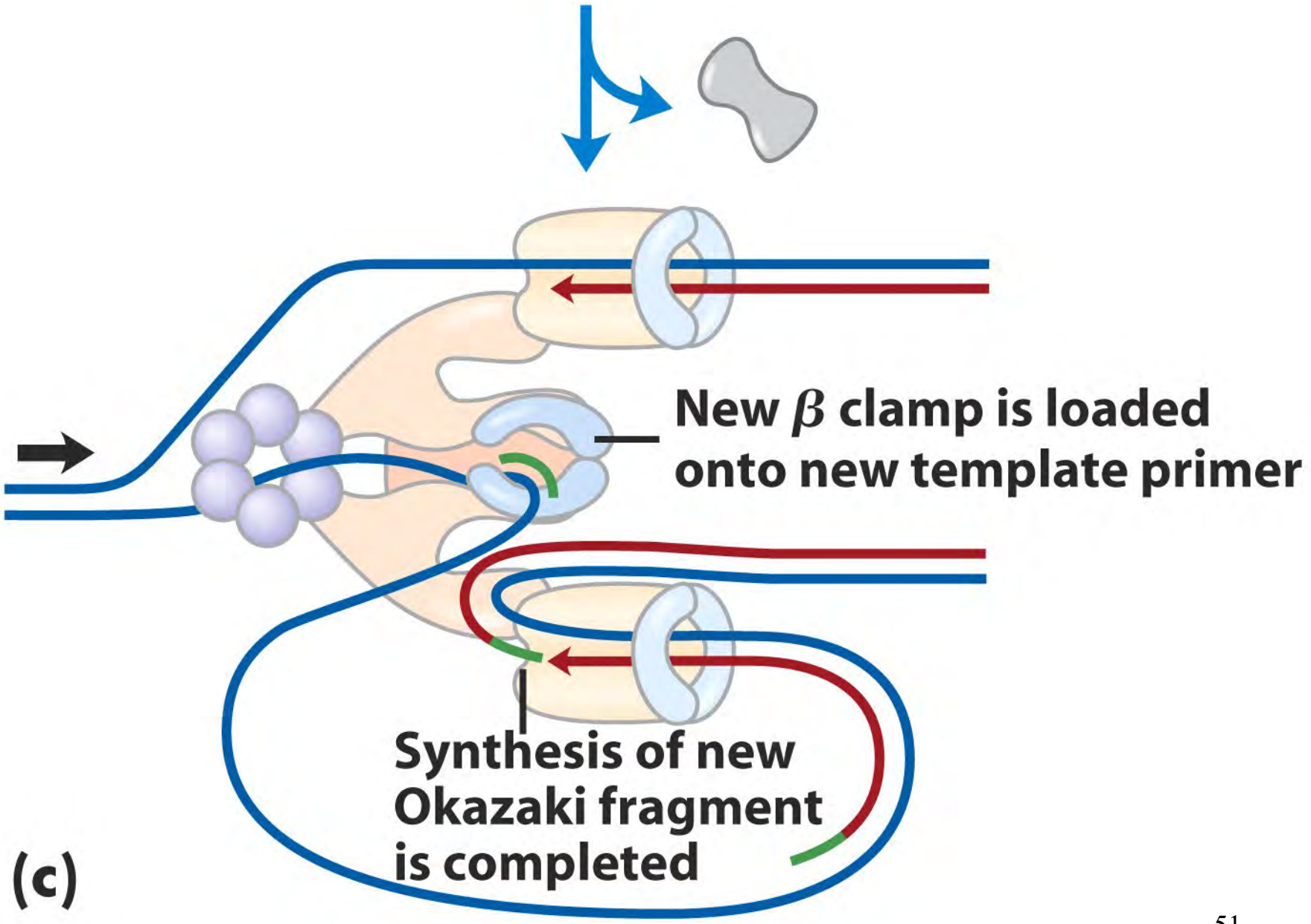

5′
3′
3′
5′
3′
5′

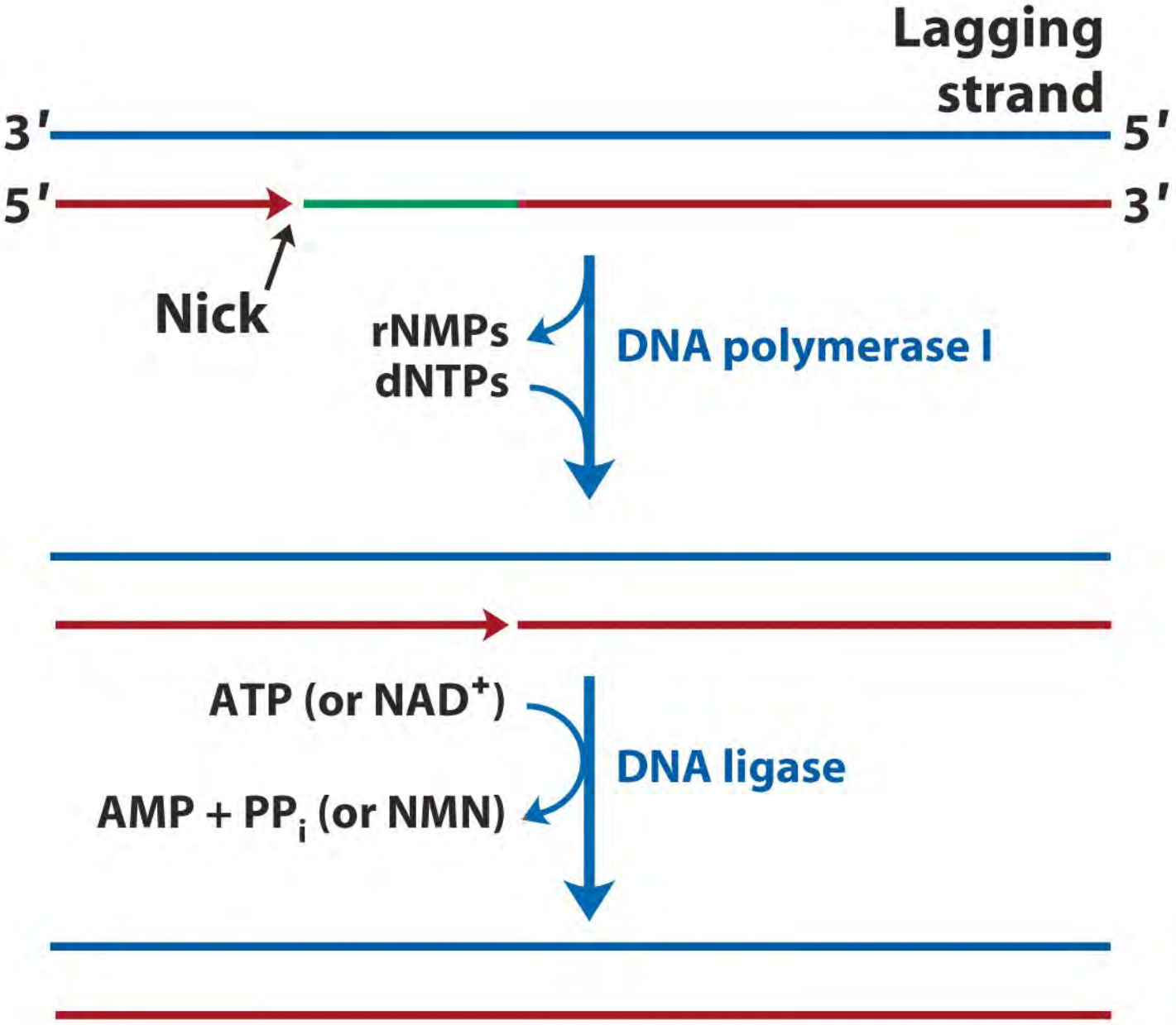


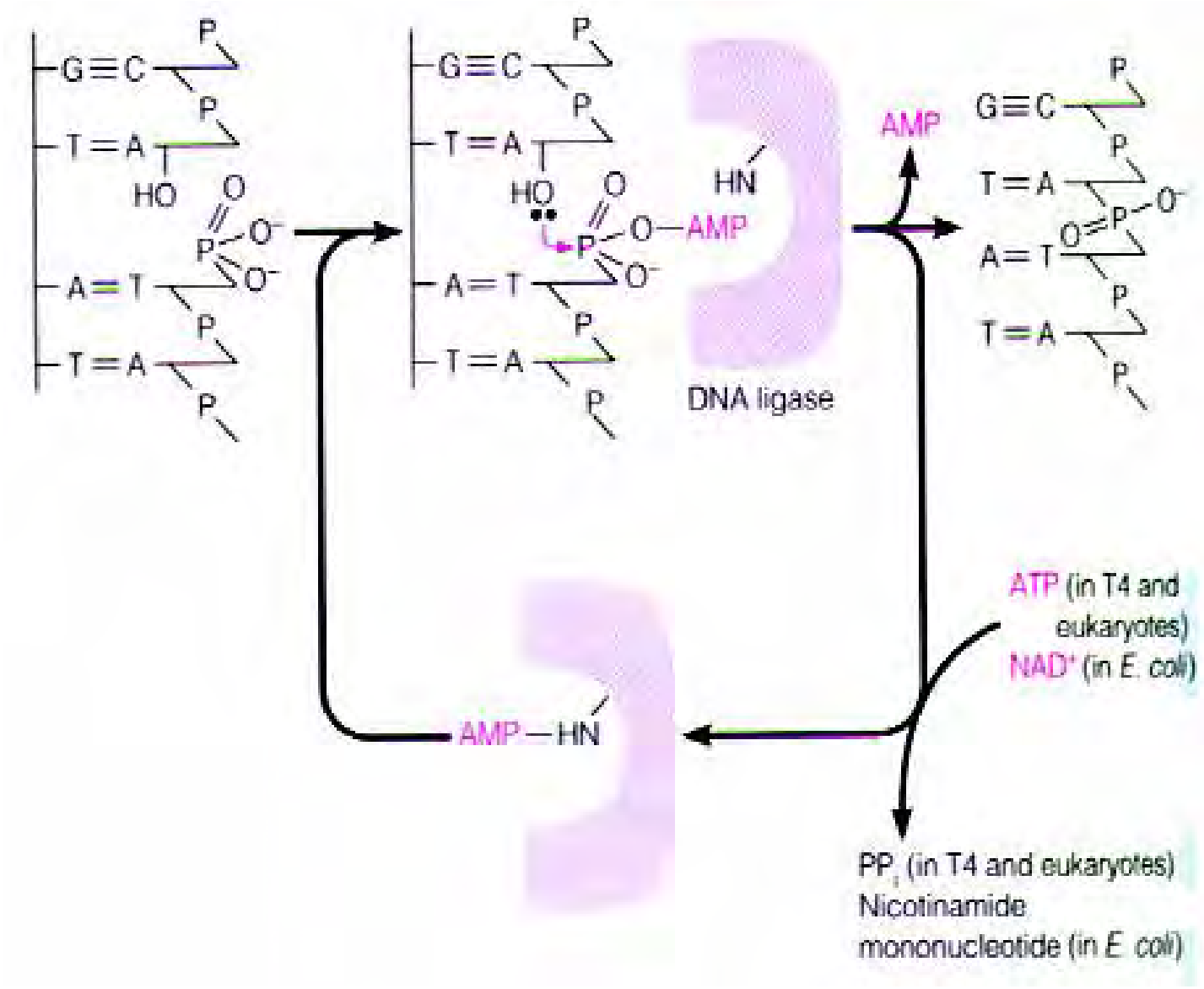

(a) Continuous synthesis on the leading strand proceeds as DNA is unwound by the DnaB helicase.

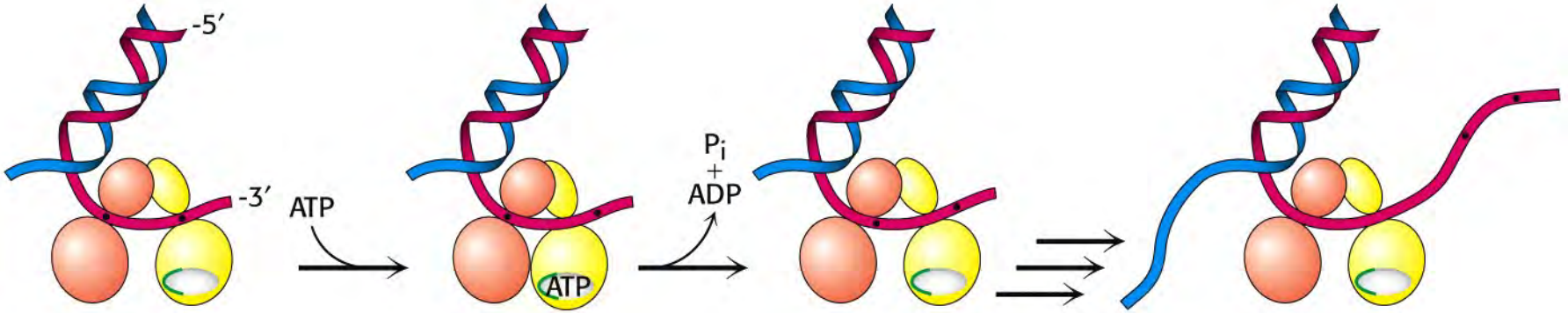



(b) DNA primase binds to DnaB, synthesizes a new primer, then dissociates.

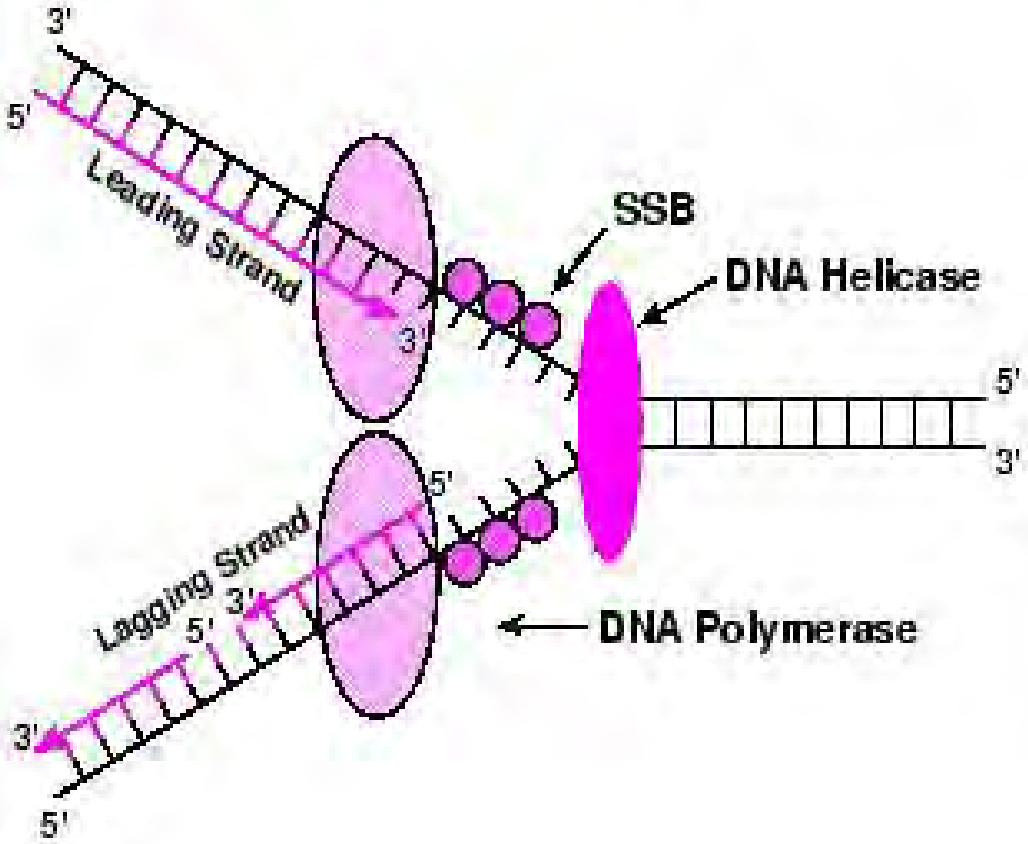

New β clamp is loaded
onto new template primer
Synthesis of new
Okazaki fragment
is completed
(c)

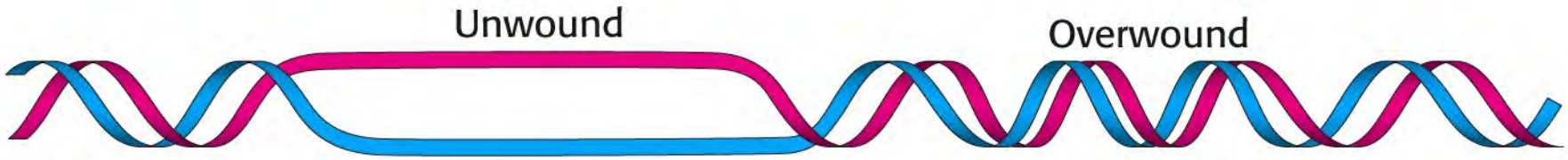

Lagging strand
3′
5′
5′
3′
Nick
rNMPs
dNTPs
DNA polymerase I
ATP (or NAD+)
AMP + PPi (or NMN)
DNA ligase

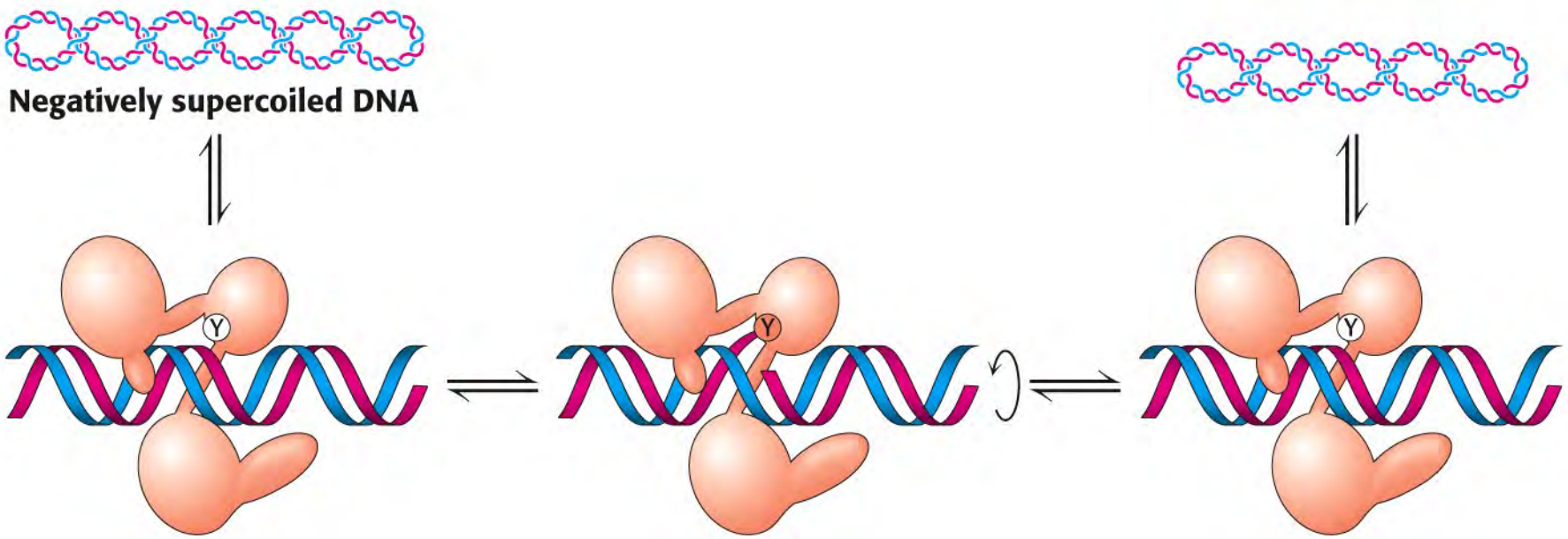

G≡C
T=A
A=T
T=A
HO
HN
DNA ligase
AMP
ATP (in T4 and eukaryotes)
NAD+ (in E. coli)
AMP—HN
PPi (in T4 and eukaryotes)
Nicotinamide mononucleotide (in E. coli)


-5′
-3′
ATP
ATP
Pi
+
ADP

## 5.10 STRUCTURE OF A REPLICATION FORK




Unwound
Overwound


Negatively supercoiled DNA
Y
Y
Y



# Are You Getting It??



**Match the replication protein with its function.**

| | |
|---|---|
| a) helicase | i) seals nicks |
| b) pol I | ii) adjusts supercoiling |
| c) ligase | iii) maintains denatured DNA |
| d) topoisomerase | iv) removes primers |
| e) SSB | v) unwinds the double-helix |



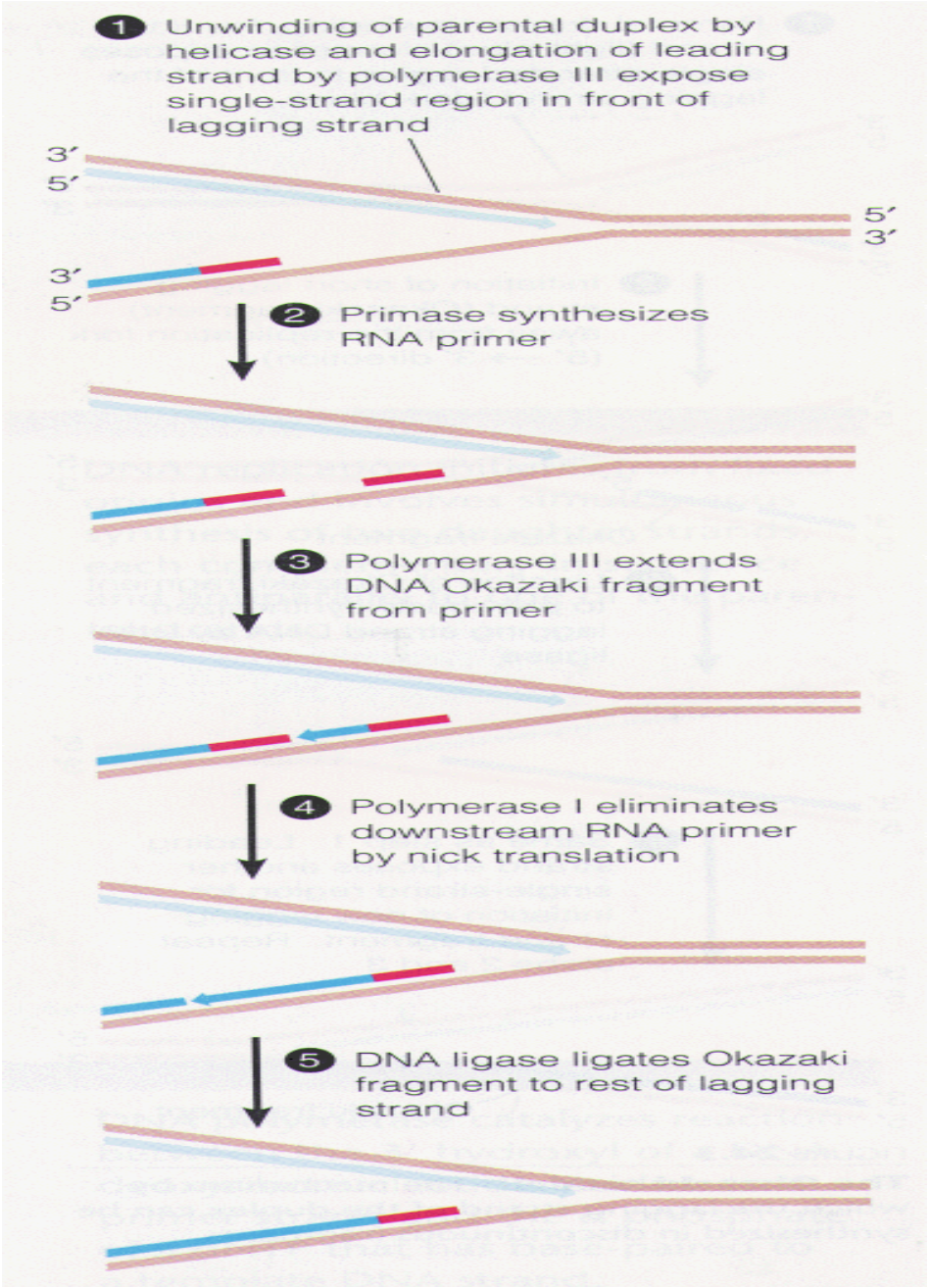
# Are You Getting It??

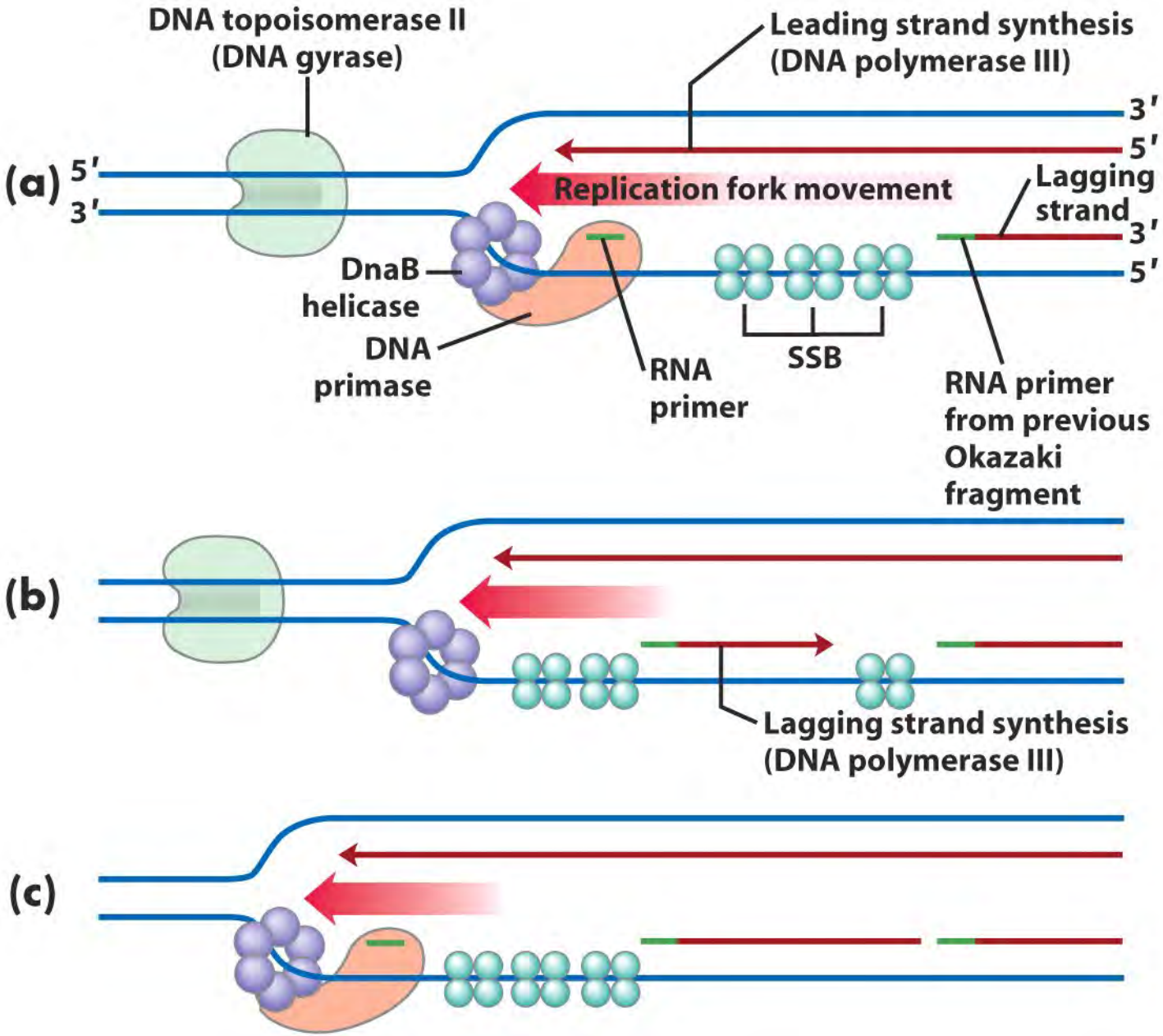


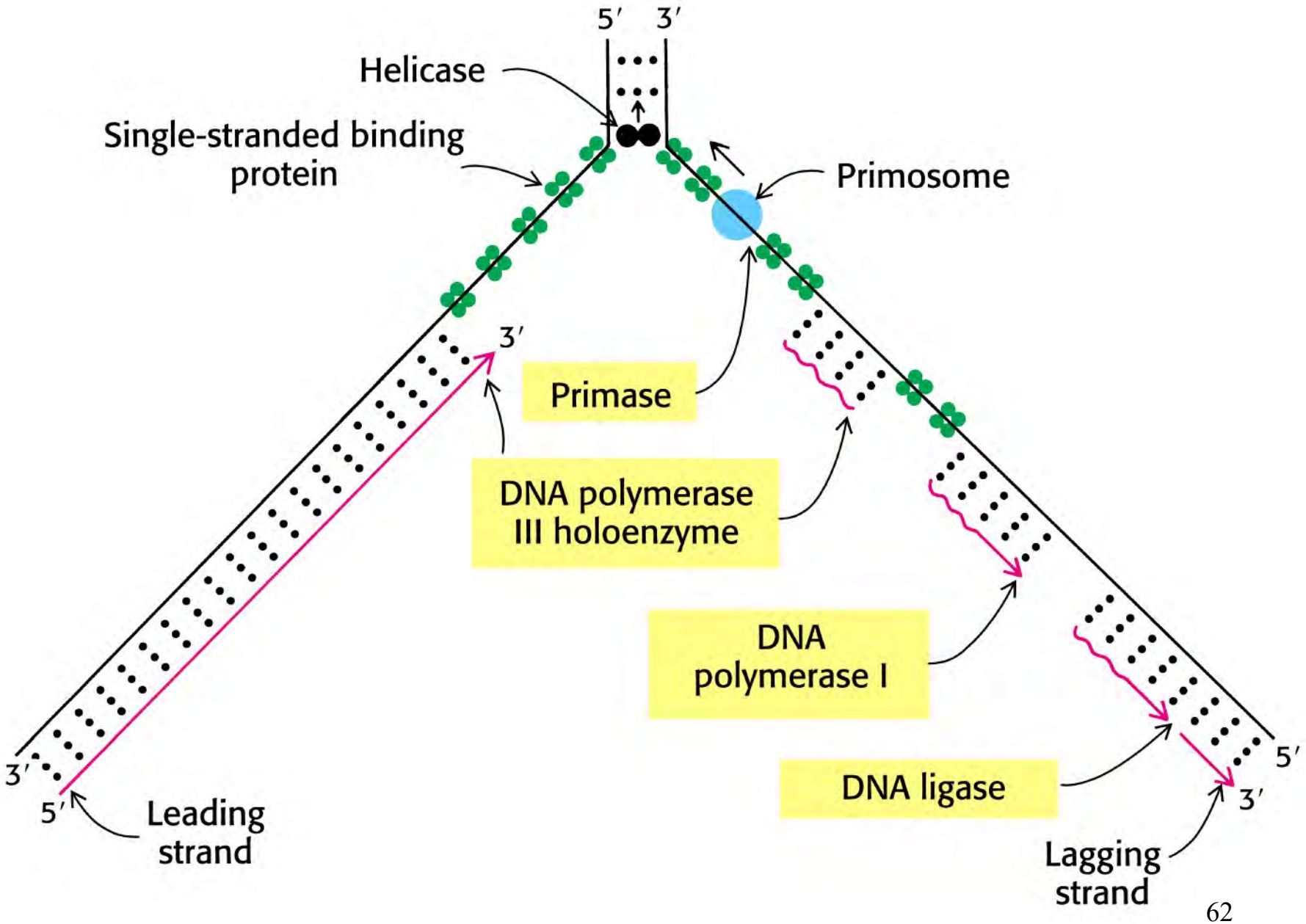
*Answer*

**Match the replication protein with its function.**

a) helicase unwinds the double-helix
b) pol I removes primers
c) ligase seals nicks
d) topoisomerase adjusts supercoiling
e) SSB maintains denatured DNA


1 Unwinding of parental duplex by helicase and elongation of leading strand by polymerase III expose single-strand region in front of lagging strand
3′
5′
5′
3′
3′
5′
2 Primase synthesizes RNA primer
3 Polymerase III extends DNA Okazaki fragment from primer
4 Polymerase I eliminates downstream RNA primer by nick translation
5 DNA ligase ligates Okazaki fragment to rest of lagging strand


DNA topoisomerase II
(DNA gyrase)
Leading strand synthesis
(DNA polymerase III)
3′
5′
(a) 5′
3′
Replication fork movement
Lagging
strand
3′
5′
DnaB
helicase
DNA
primase
RNA
primer
SSB
RNA primer
from previous
Okazaki
fragment
(b)
Lagging strand synthesis
(DNA polymerase III)
(c)


5′
3′
Helicase
Single-stranded binding protein
Primosome
3′
Primase
DNA polymerase III holoenzyme
DNA polymerase I
DNA ligase
3′
5′
5′
Leading strand
Lagging strand

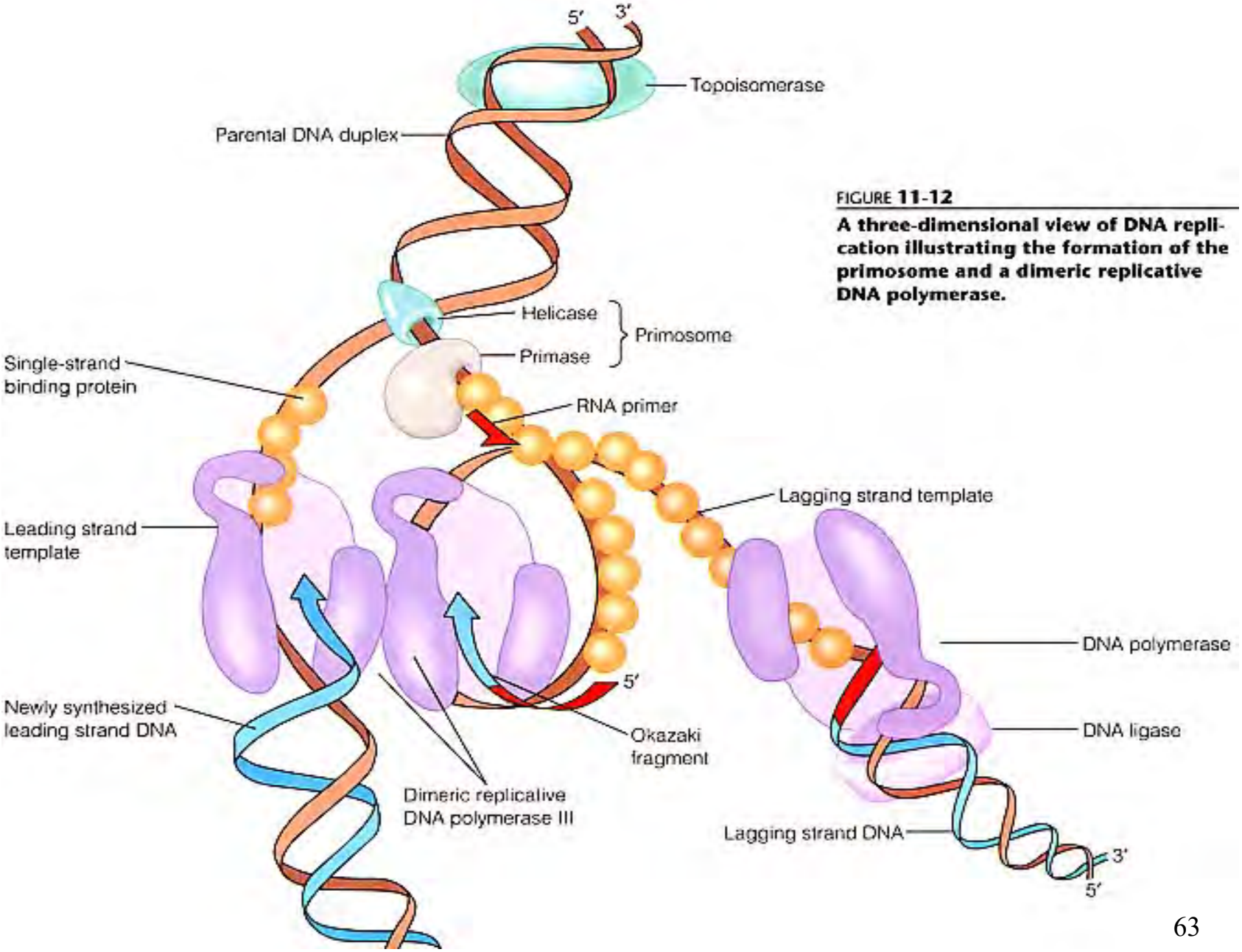



FIGURE **11-12**

**A three-dimensional view of DNA replication illustrating the formation of the primosome and a dimeric replicative DNA polymerase.**

**TABLE 25–4** **Proteins at the *E. coli* Replication Fork**

| *Protein* | $M_r$ | *Number of subunits* | *Function* |
|---|---|---|---|
| SSB | 75,600 | 4 | Binding to single-stranded DNA |
| DnaB protein (helicase) | 300,000 | 6 | DNA unwinding; primosome constituent |
| Primase (DnaG protein) | 60,000 | 1 | RNA primer synthesis; primosome constituent |
| DNA polymerase III | 791,500 | 17 | New strand elongation |
| DNA polymerase I | 103,000 | 1 | Filling of gaps; excision of primers |
| DNA ligase | 74,000 | 1 | Ligation |
| DNA gyrase (DNA topoisomerase II) | 400,000 | 4 | Supercoiling |

Modified from Kornberg, A. (1982) *Supplement to DNA Replication,* Table S11–2, W. H. Freeman and Company, New York.



# Are You Getting It??



**Which replication proteins are needed for elongation of the leading strand or the lagging strand?**

a) pol I
b) pol III
c) Dna A
d) Dna B
e) primase
f) ligase
g) gyrase



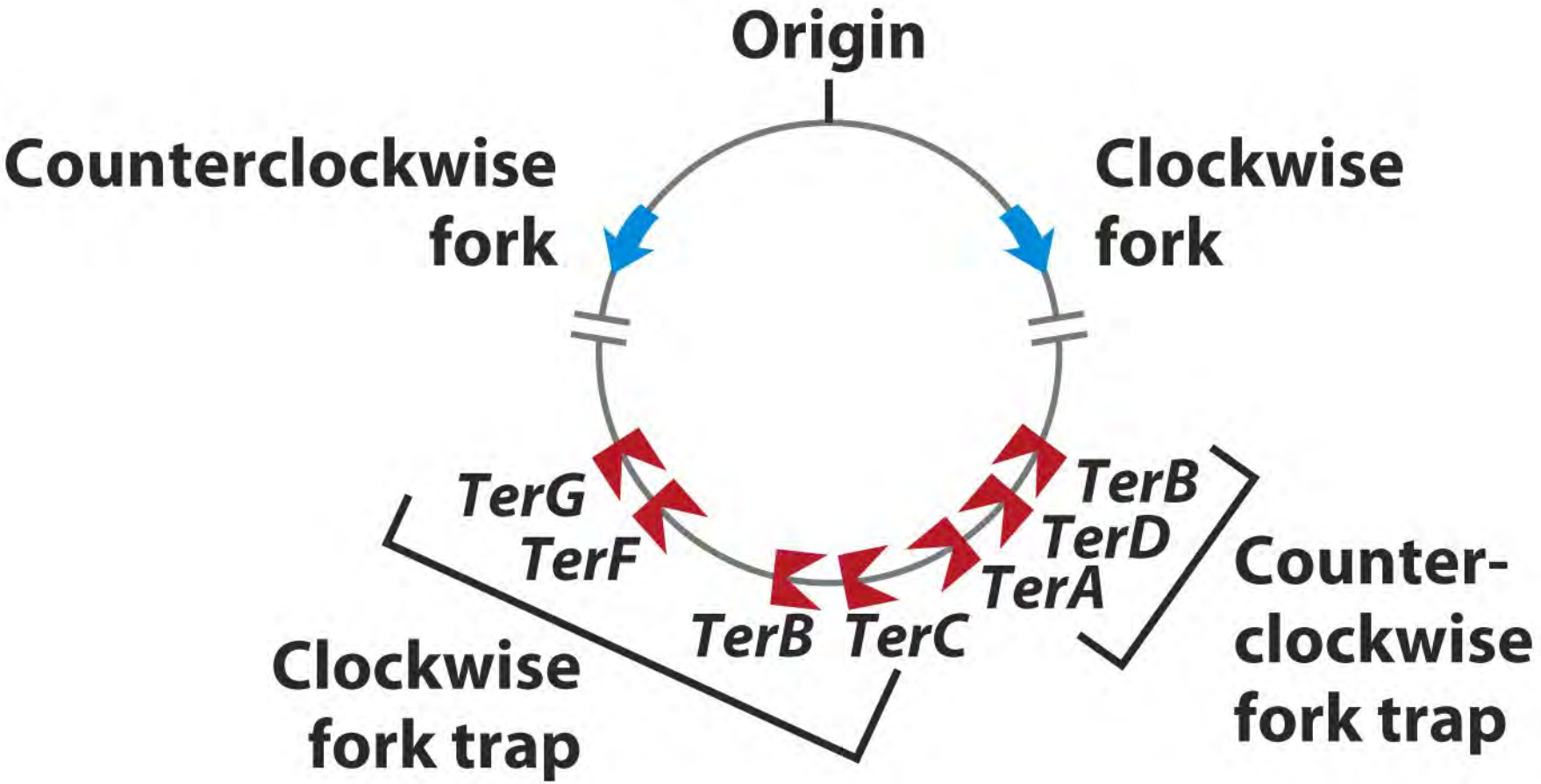
# Are You Getting It??

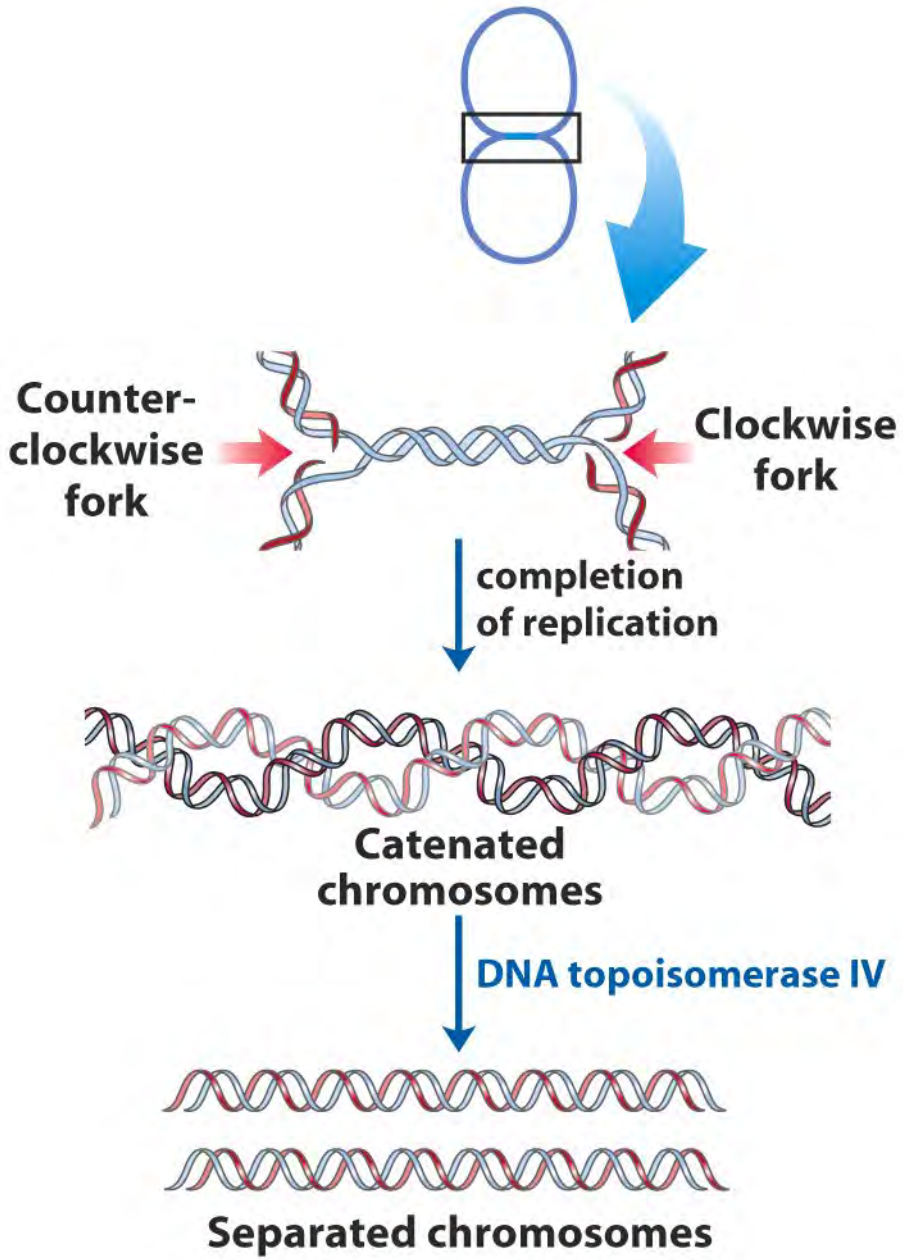


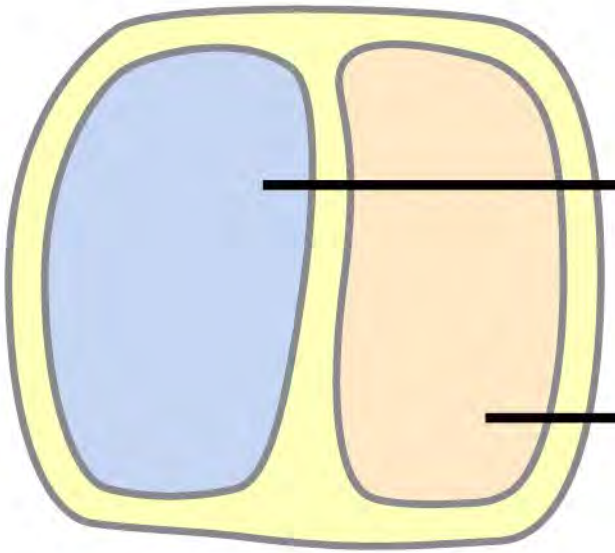
***Answer***

**Which replication proteins are needed for elongation of the leading strand or the lagging strand?**

a) pol I *lagging*
b) pol III *both*
c) Dna A *neither*
d) Dna B *both*
e) primase *lagging*
f) ligase *lagging*
g) gyrase *both*


Origin
Counterclockwise fork
Clockwise fork
TerG
TerF
TerB
TerD
TerA
TerB
TerC
Clockwise fork trap
Counter-clockwise fork trap

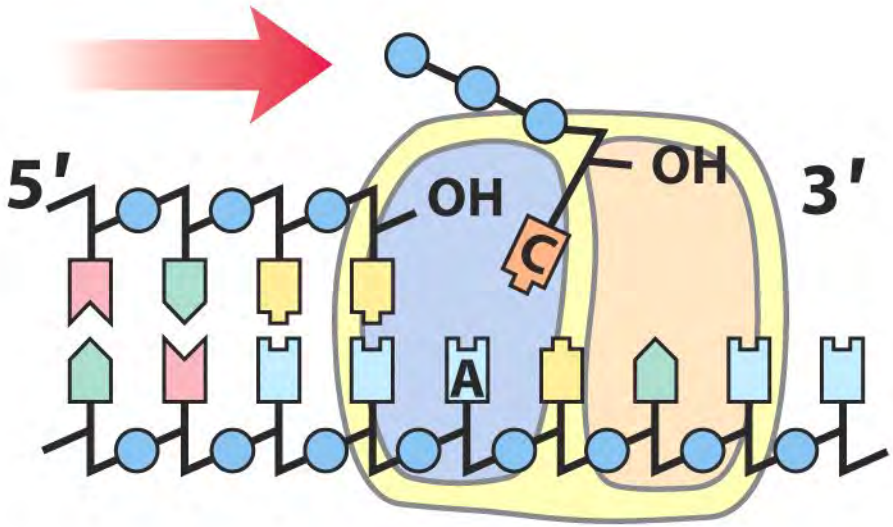

Counter-
clockwise
fork
Clockwise
fork
completion
of replication
Catenated
chromosomes
DNA topoisomerase IV
Separated chromosomes



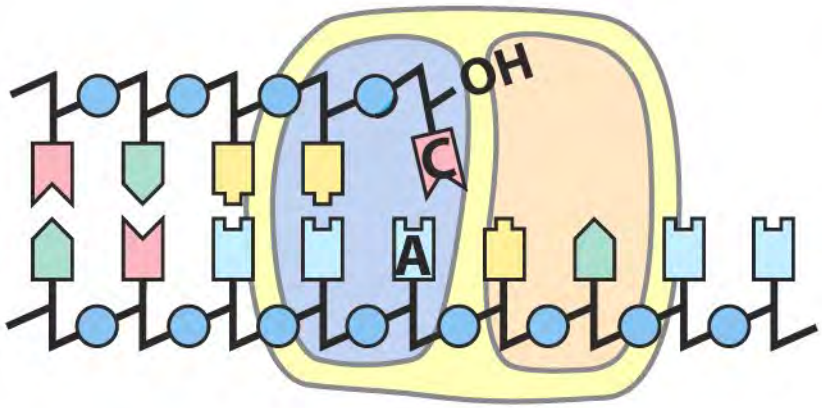
DNA polymerase I

DNA polymerase active site

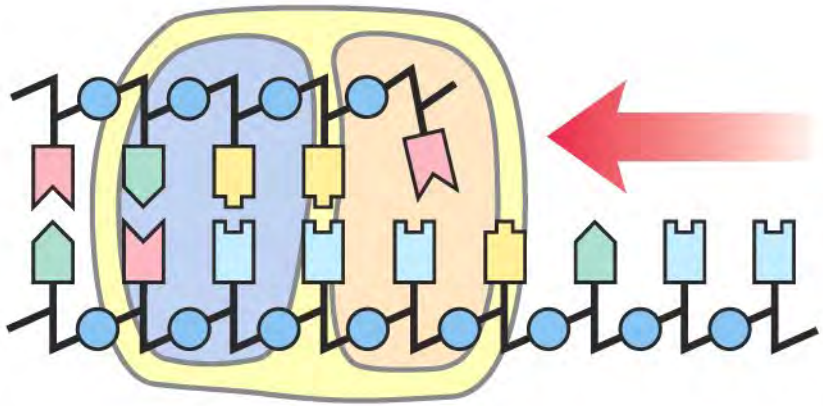
3′→5′ (proofreading) exonuclease active site



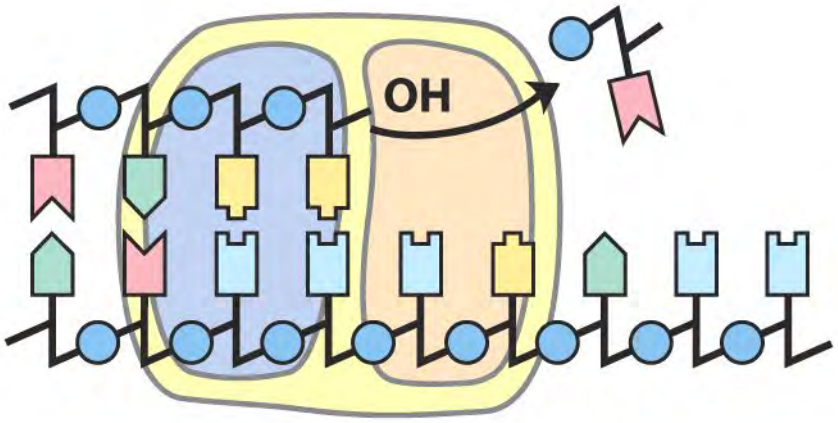

C is a rare tautomeric form of cytosine (C*) that pairs with A and is incorporated into the growing strand.




**Before the polymerase moves on, the cytosine undergoes a tautomeric shift from C* to C. The new nucleotide is now mispaired.**

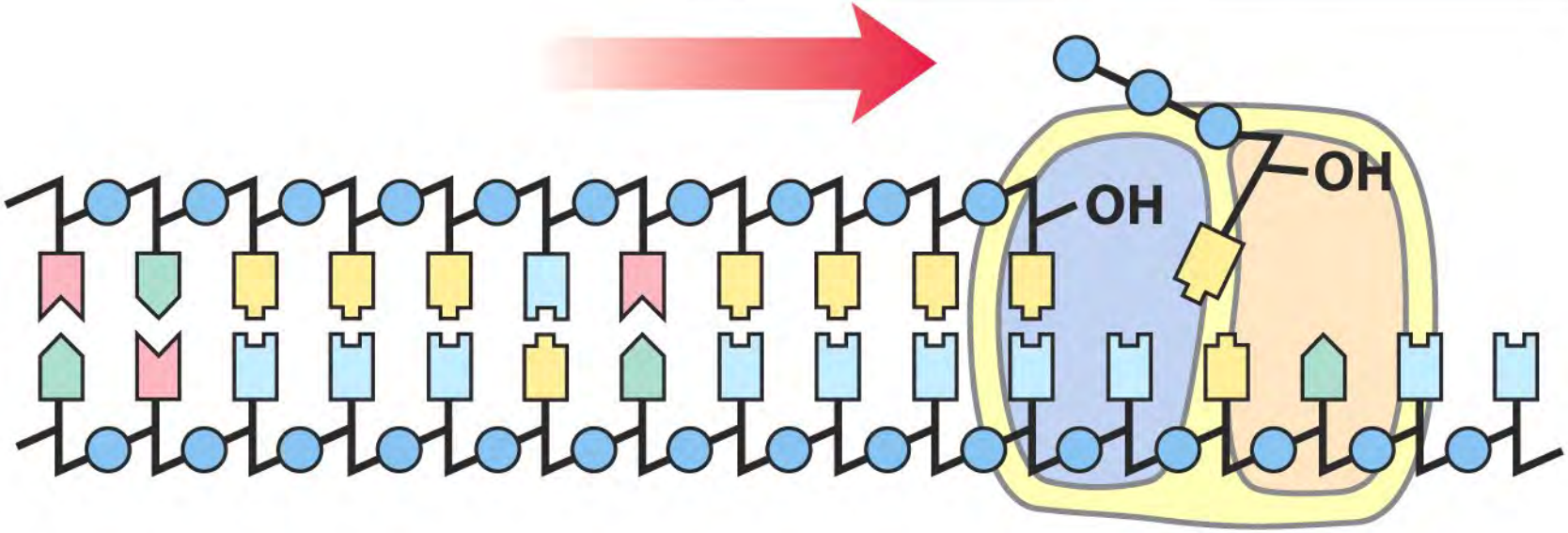


The mispaired 3′-OH end of the growing strand blocks further elongation. DNA polymerase slides back to position the mispaired base in the 3′→5′ exonuclease active site.

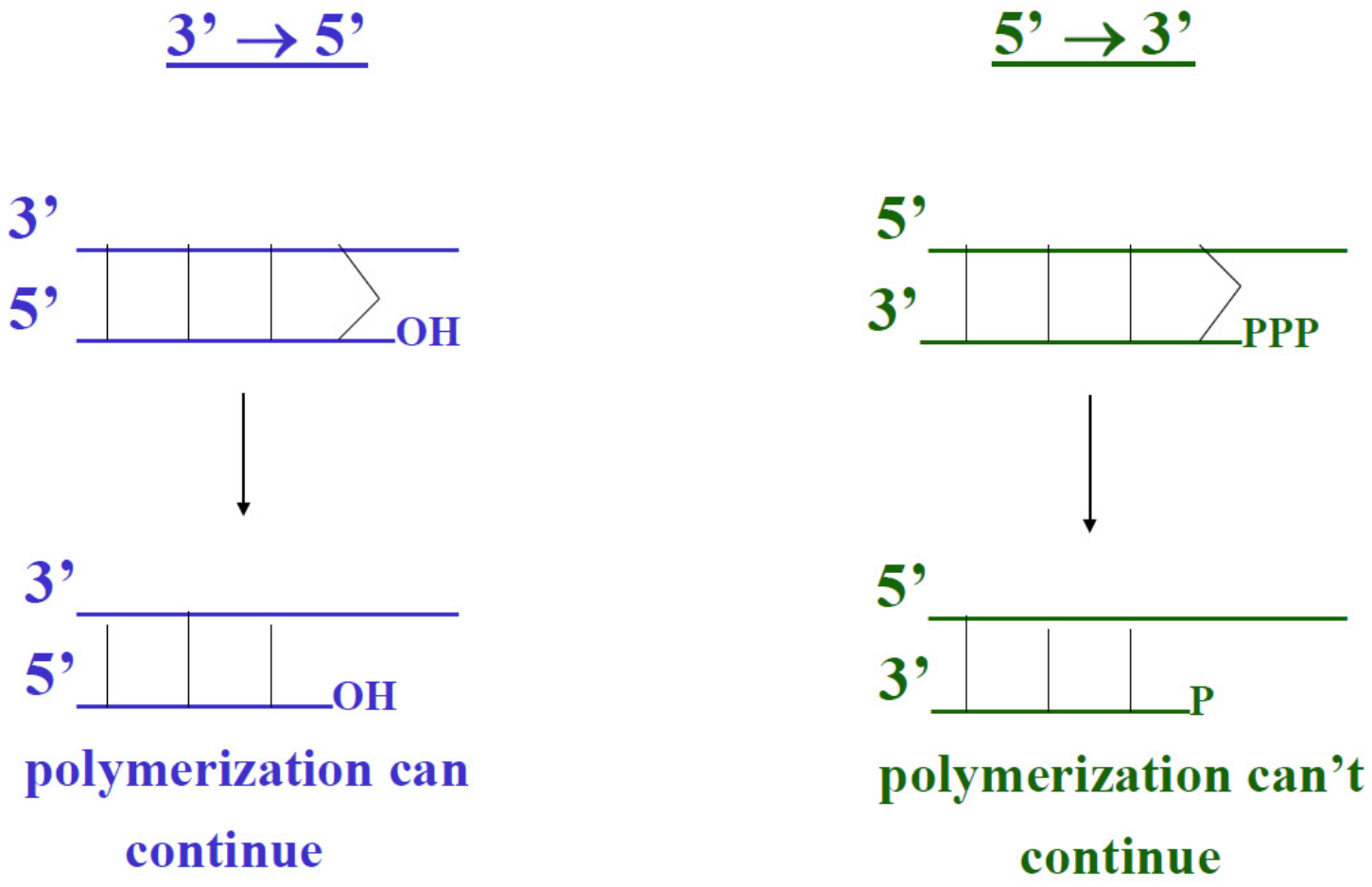


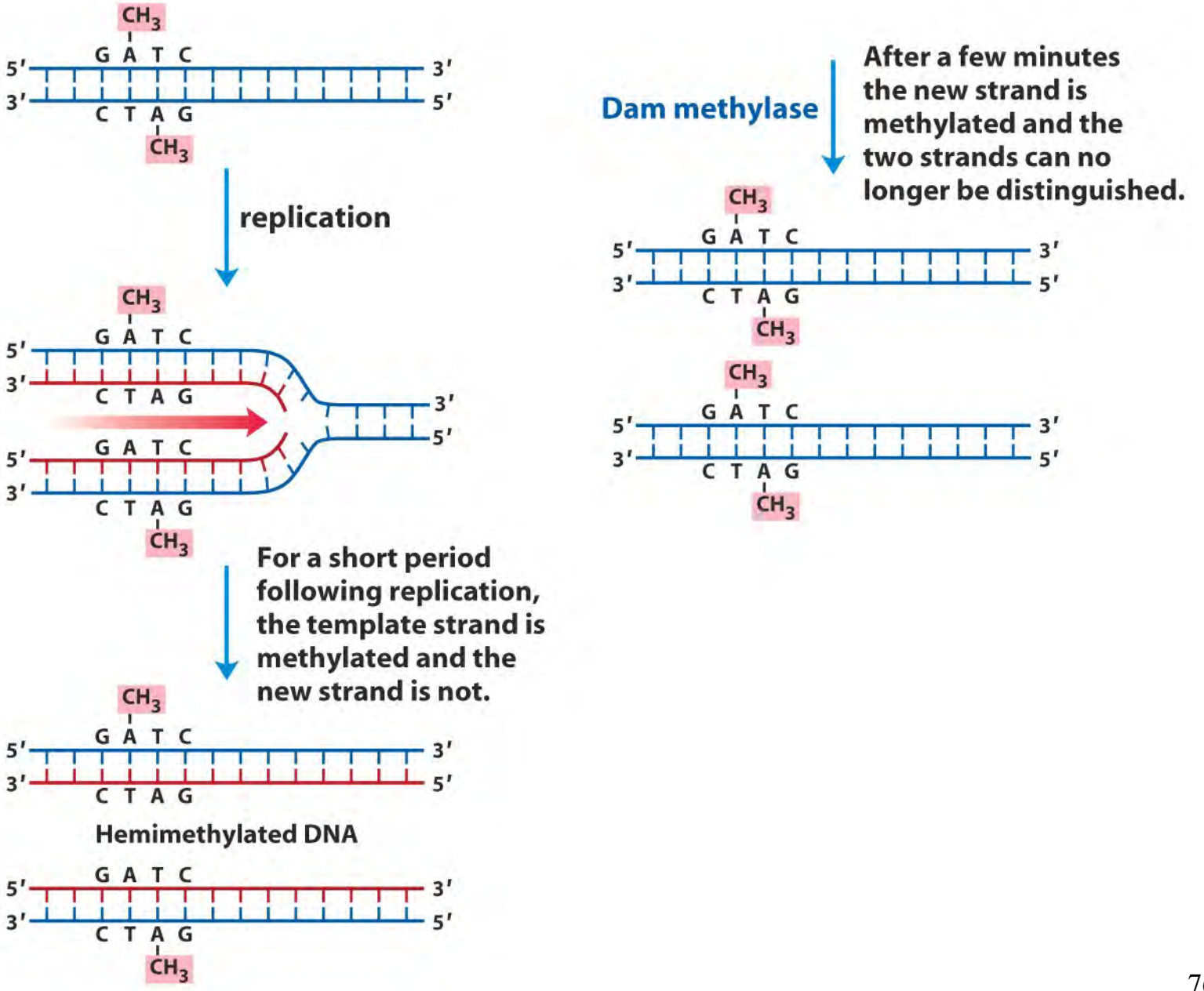

**The mispaired nucleotide is removed.**

**DNA polymerase slides forward and resumes its polymerization activity.**



# PROOF-READING




CH3
G A T C
5′
3′
3′
5′
C T A G
CH3
replication
CH3
G A T C
5′
3′
C T A G
3′
5′
G A T C
5′
3′
C T A G
CH3
For a short period following replication, the template strand is methylated and the new strand is not.
CH3
G A T C
5′
3′
3′
5′
C T A G
Hemimethylated DNA
G A T C
5′
3′
3′
5′
C T A G
CH3
Dam methylase
After a few minutes the new strand is methylated and the two strands can no longer be distinguished.
CH3
G A T C
5′
3′
3′
5′
C T A G
CH3
CH3
G A T C
5′
3′
3′
5′
C T A G
CH3



# Are You Getting It??



**Which are properties of the proof-reading activity of E. coli DNA polymerases?** ***(multiple answers)***

a) It is present in both pol I and pol III.
b) It works on the lagging strand but not the leading strand.
c) It is a 3'→ 5' exonuclease activity.
d) It recognizes a mismatched base.
e) It works only on methylated DNA.
f) It works on primers.
g) It breaks a phosphodiester bond.



# Are You Getting It??



*Answer*

**Which are properties of the proof-reading activity of E. coli DNA polymerases?**

***a)*** ***It is present in both pol I and pol III.***

***b)*** **It works on the lagging strand but not the leading strand.**

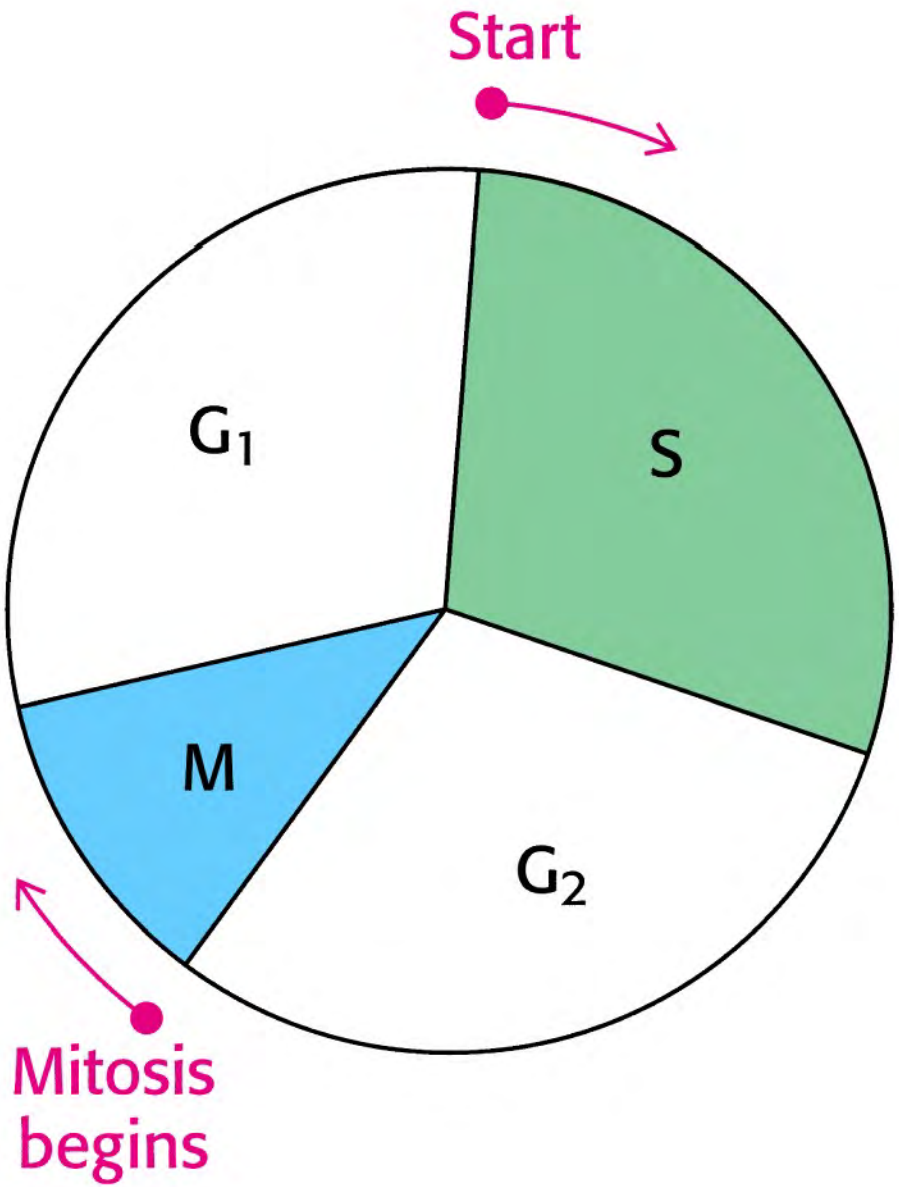
***c)*** ***It is a 3'→ 5' exonuclease activity.***

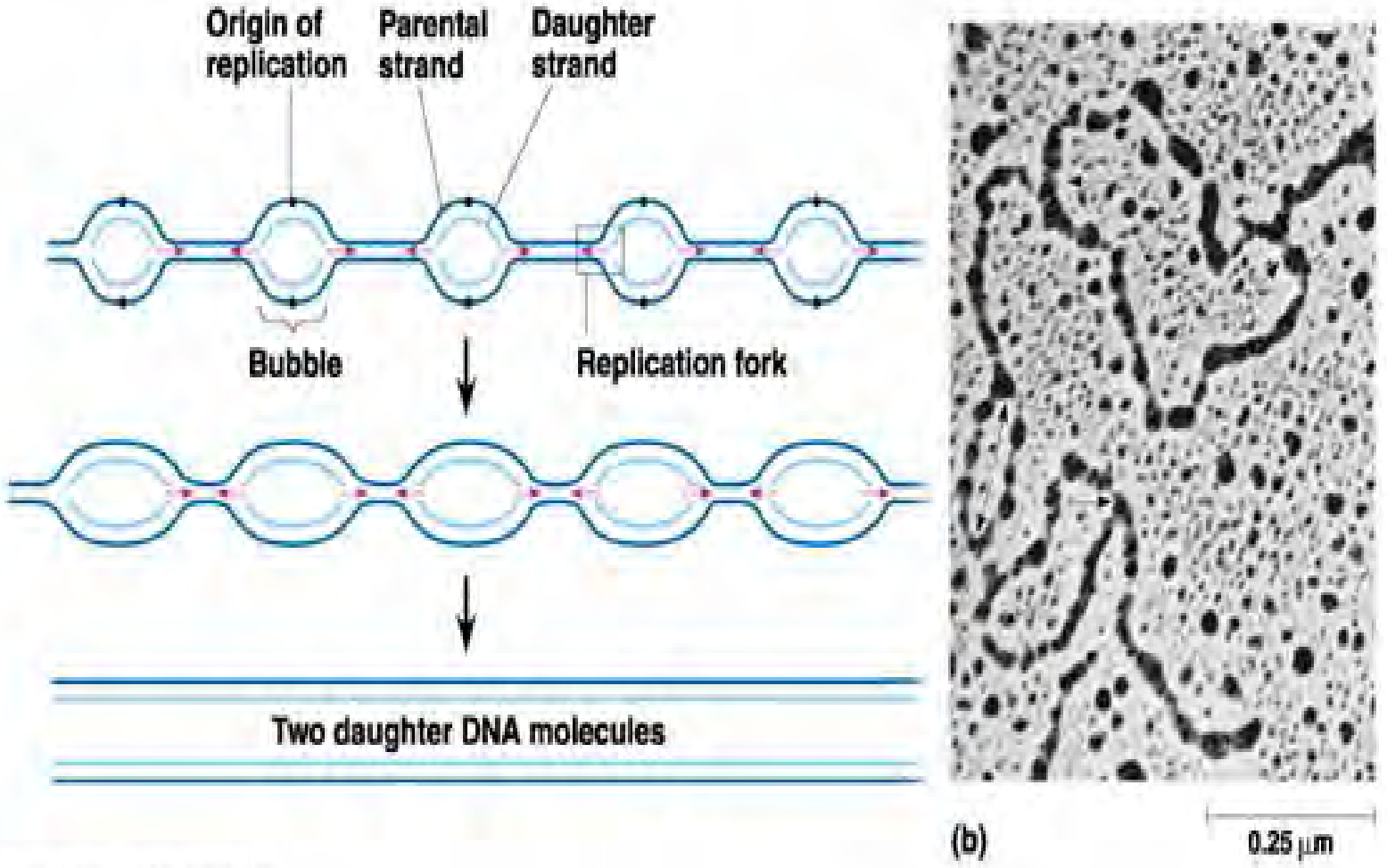
***d)*** ***It recognizes a mismatched base.***

***e)*** **It works only on methylated DNA.**

***f)*** **It works on primers.**

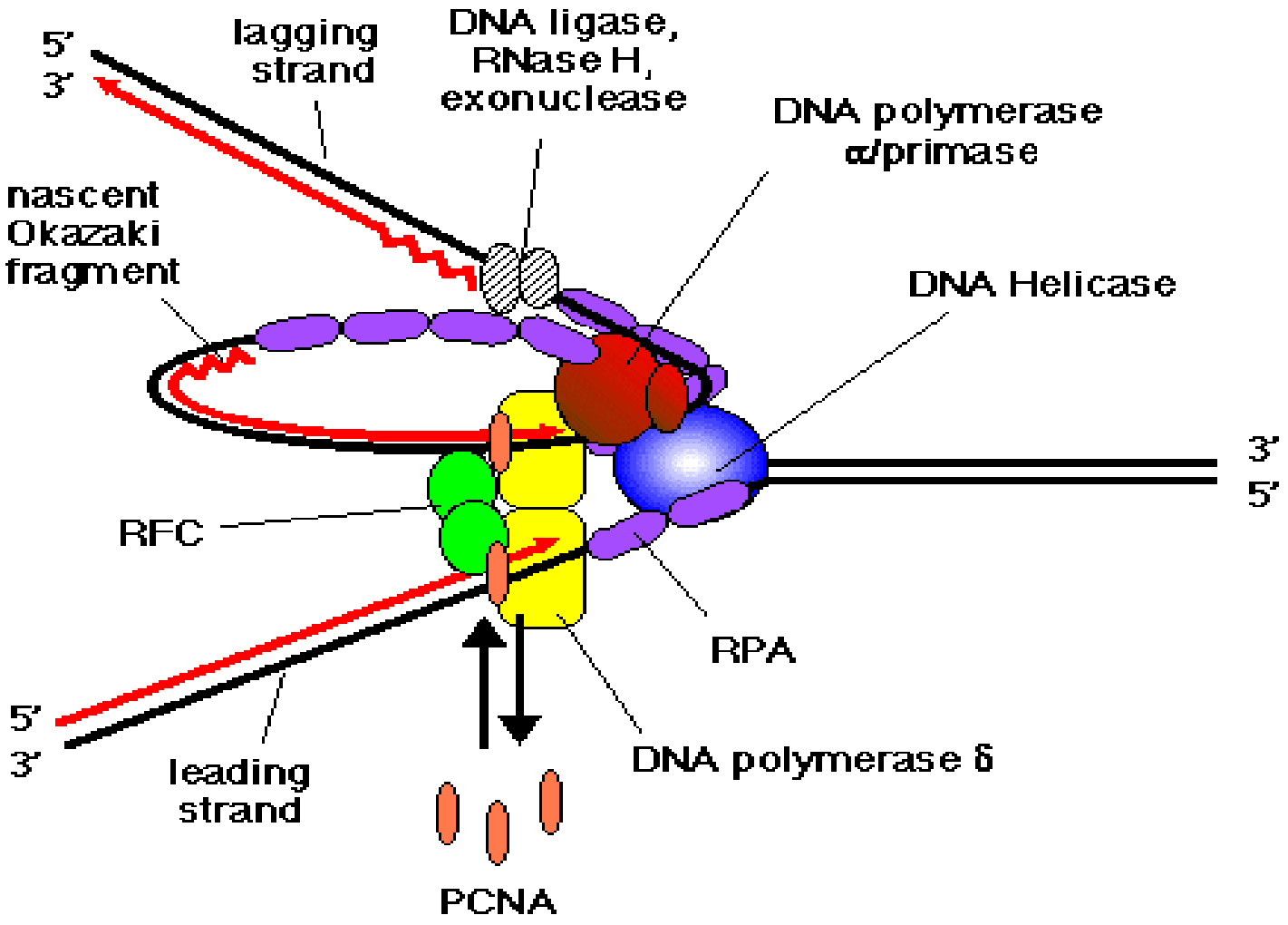
***g)*** ***It breaks a phosphodiester bond.***

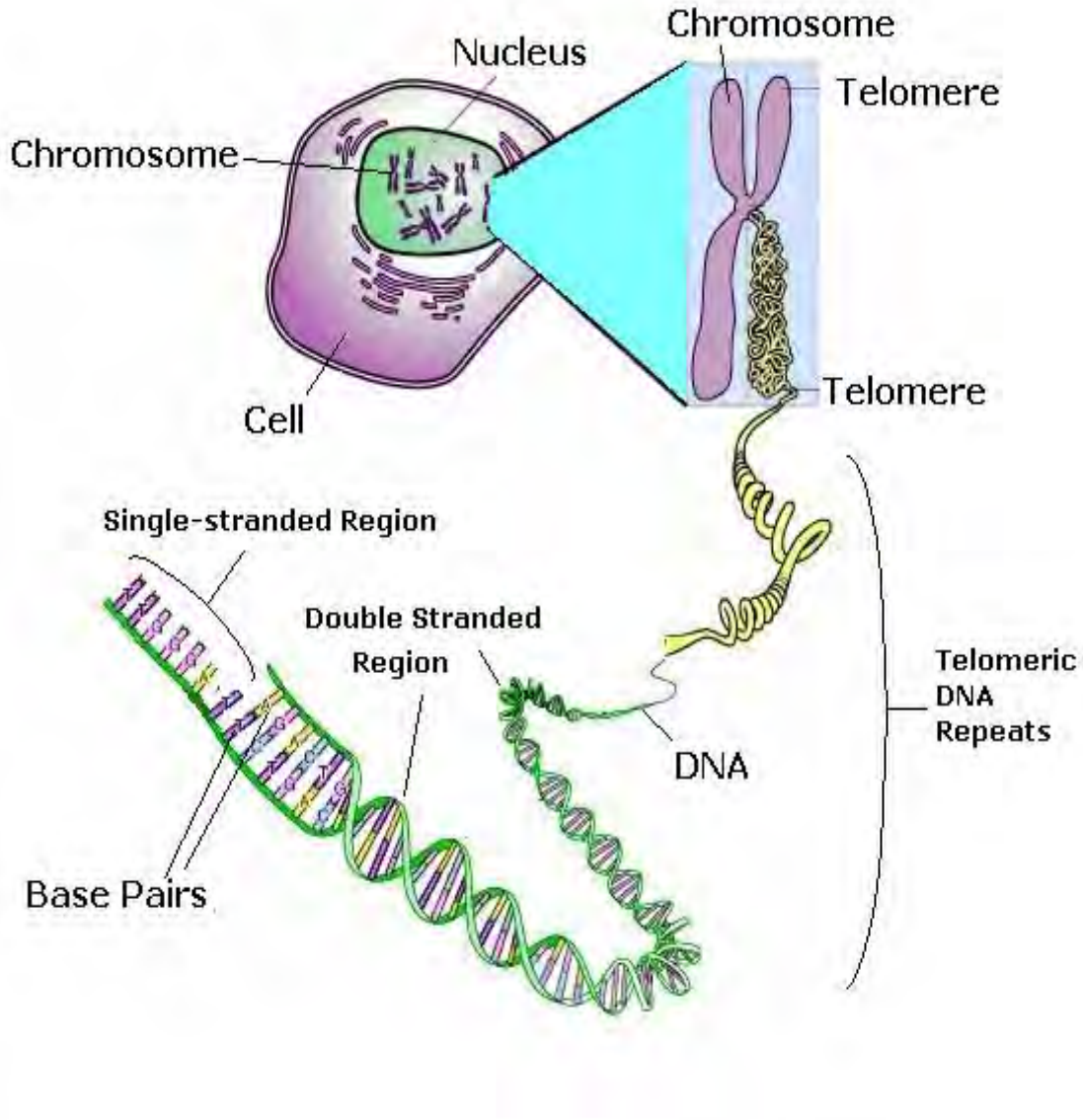

Start
G1
S
M
G2
Mitosis begins

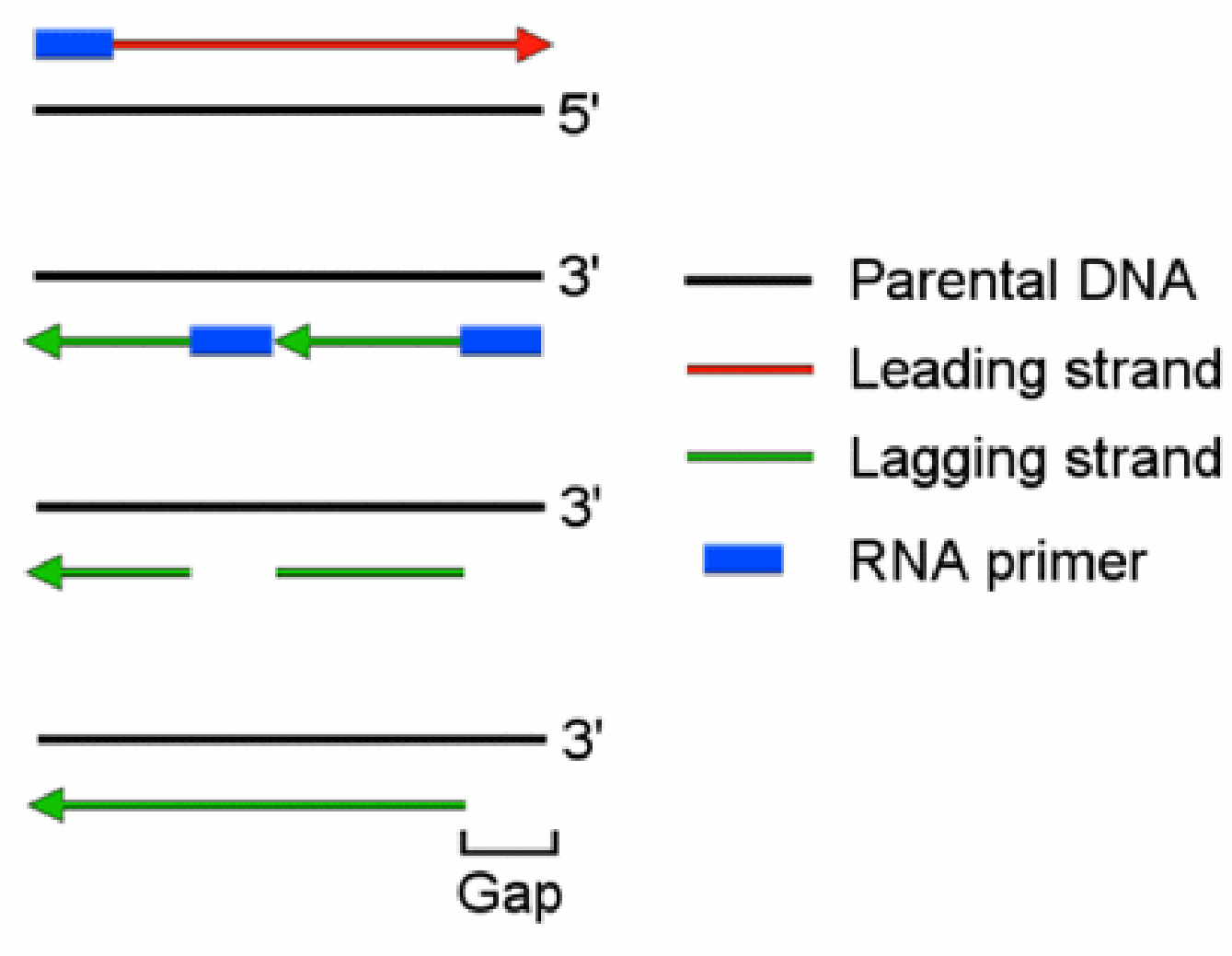


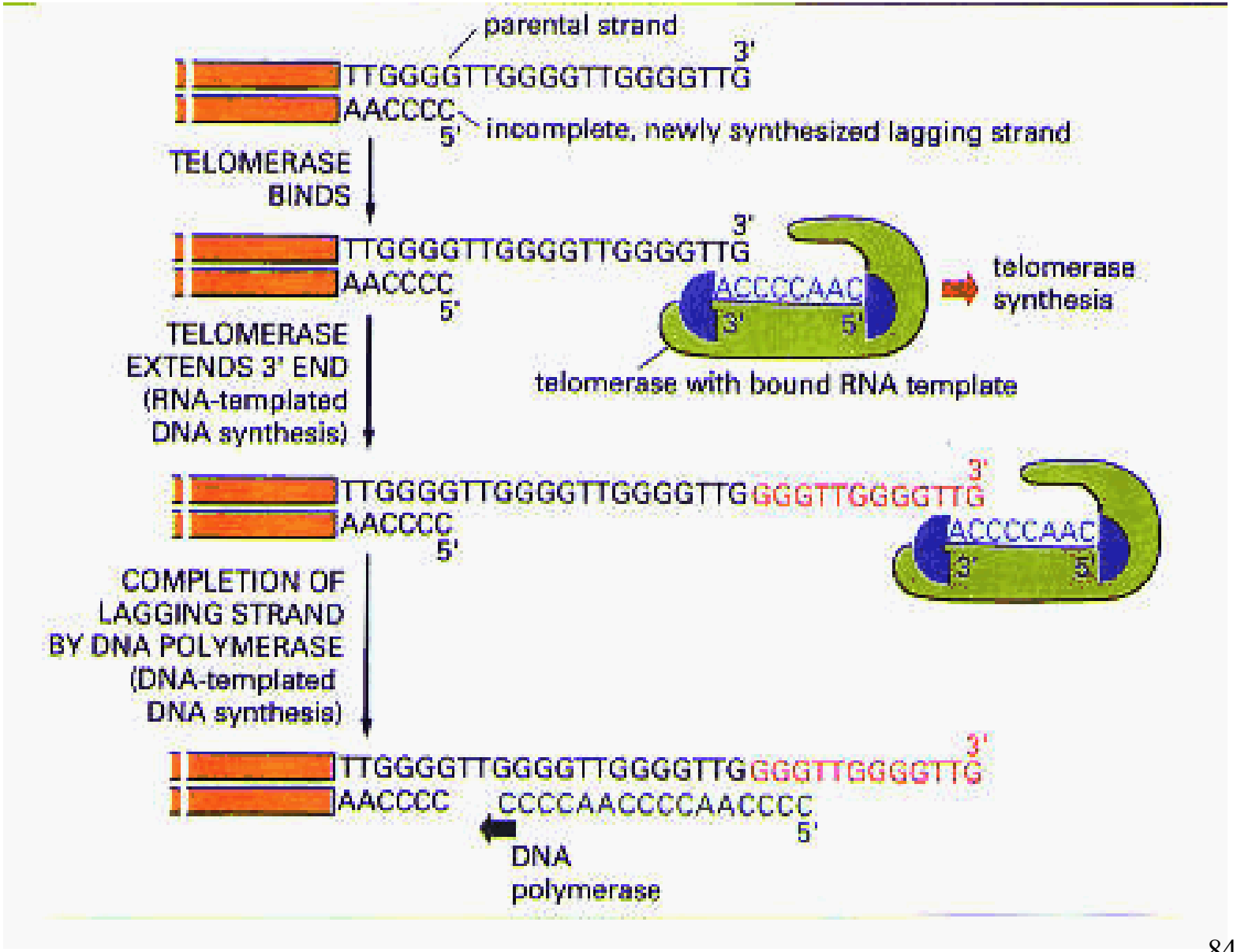

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# Model of a eukaryotic replication fork




Chromosome
Nucleus
Telomere
Chromosome
Telomere
Cell
Single-stranded Region
Double Stranded
Region
Telomeric
DNA
Repeats
DNA
Base Pairs


5'
3'
3'
3'
Gap
Parental DNA
Leading strand
Lagging strand
RNA primer


parental strand
3'
TTGGGGTTGGGGTTGGGGTTG
AACCCC
5'
incomplete, newly synthesized lagging strand
TELOMERASE BINDS
3'
TTGGGGTTGGGGTTGGGGTTG
AACCCC
5'
ACCCCAAC
3'
5'
telomerase synthesis
telomerase with bound RNA template
TELOMERASE EXTENDS 3' END (RNA-templated DNA synthesis)
3'
TTGGGGTTGGGGTTGGGGTTGGGGTTGGGGTTG
AACCCC
5'
ACCCCAAC
3'
5'
COMPLETION OF LAGGING STRAND BY DNA POLYMERASE (DNA-templated DNA synthesis)
3'
TTGGGGTTGGGGTTGGGGTTGGGGTTGGGGTTG
AACCCC
CCCCAACCCCAACCCC
5'
DNA polymerase



# Are You Getting It??



**Which aspects of DNA replication are found in prokaryotes or eukaryotes?**

a) multiple origins
b) multiple Okazaki pieces
c) RNA primers
d) bidirectional replication
e) telomerase
f) proof-reading
g) S phase



# Are You Getting It??



*Answer*

**Which aspects of DNA replication are found in prokaryotes or eukaryotes?**

a) multiple origins *eukaryotes*
b) multiple Okazaki pieces *both*
c) RNA primers *both*
d) bidirectional replication *both*
e) telomerase *eukaryotes*
f) proof-reading *both*
g) S phase *eukaryotes*