DNA Replication

Tong-Jin Zhao

School of Life Sciences, Xiamen University

Outlines

Part I. General Features of DNA Replication

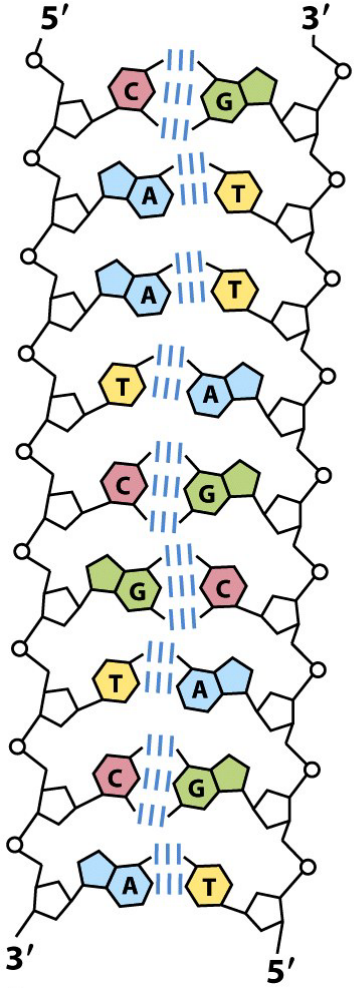
Part II. Machinery Required for DNA Replication

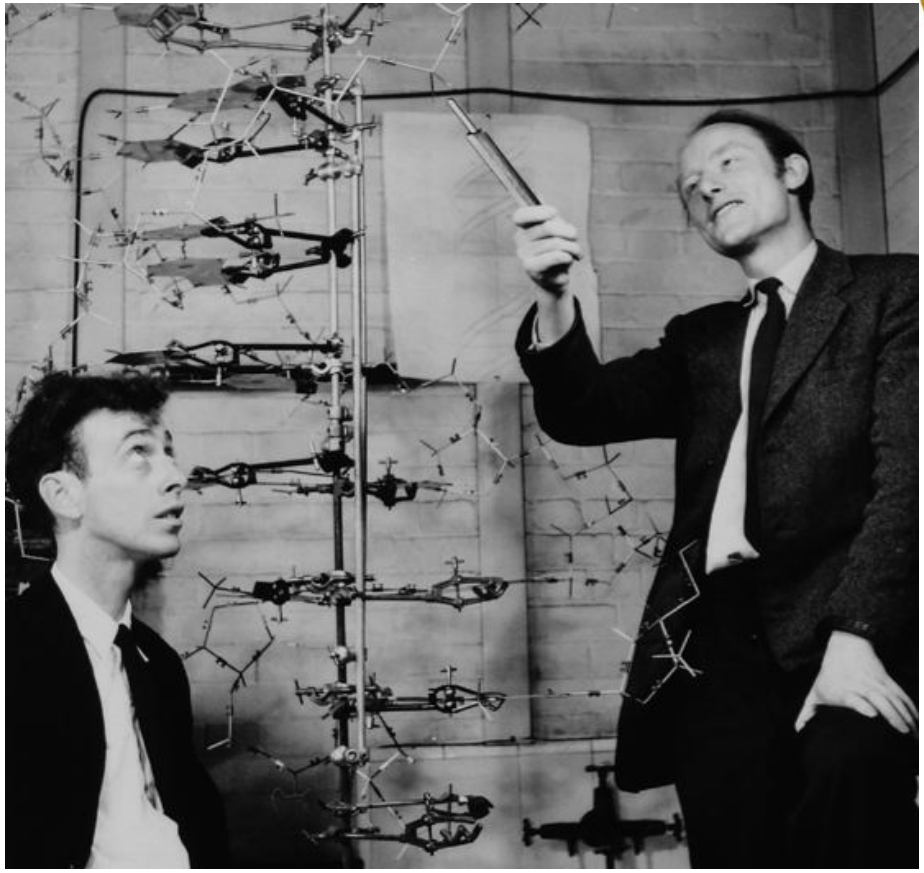
Part III. DNA Replication Proceeds in Stages

Part I. General Features of DNA Replication

Part I

How Does a Cell Produce Two Identical Copies of the Original DNA?



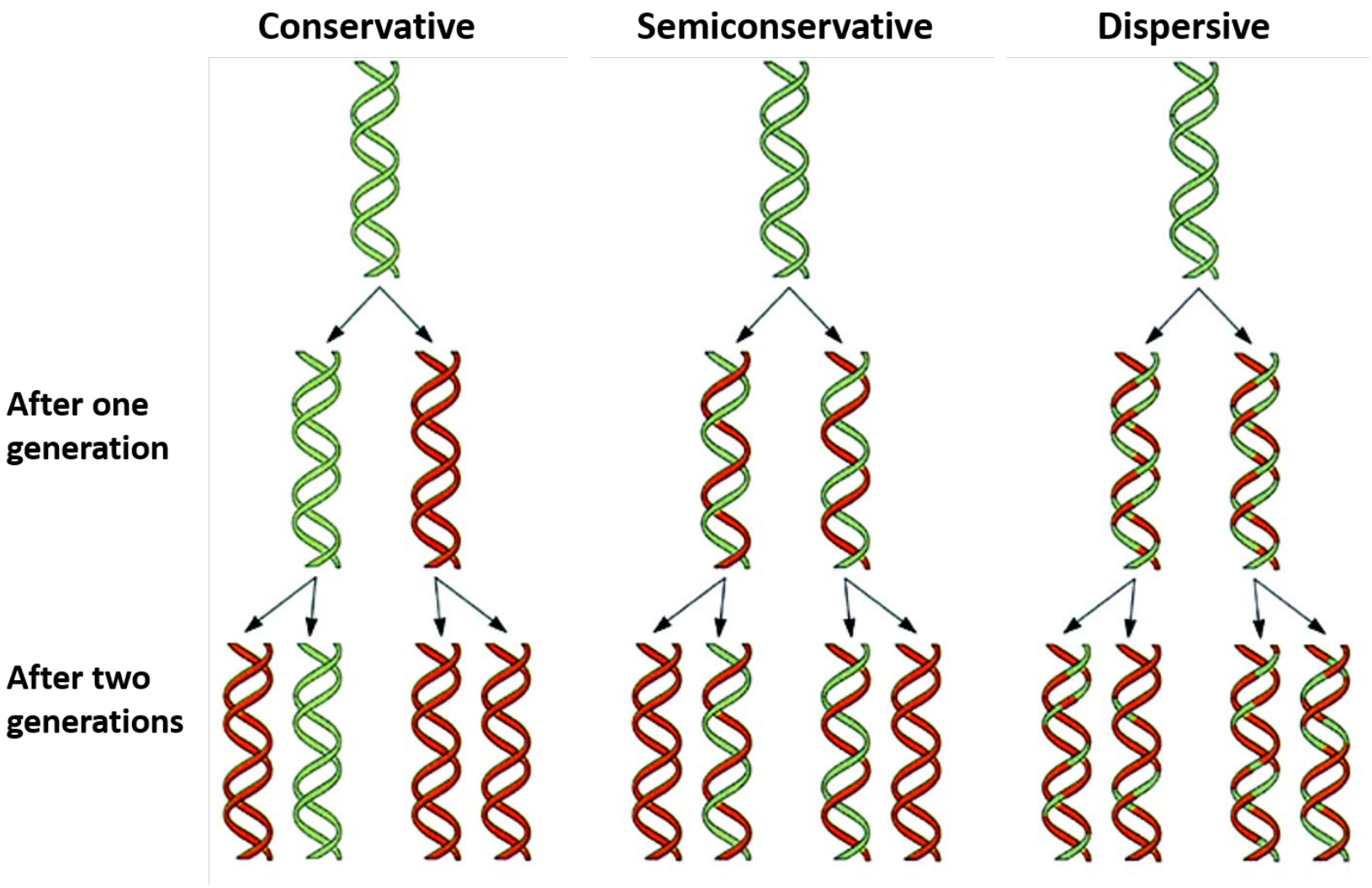


Watson & Crick, 1953

Antiparallel and complementary

Part I

Three Potential Models of DNA Replication



Which model is correct?

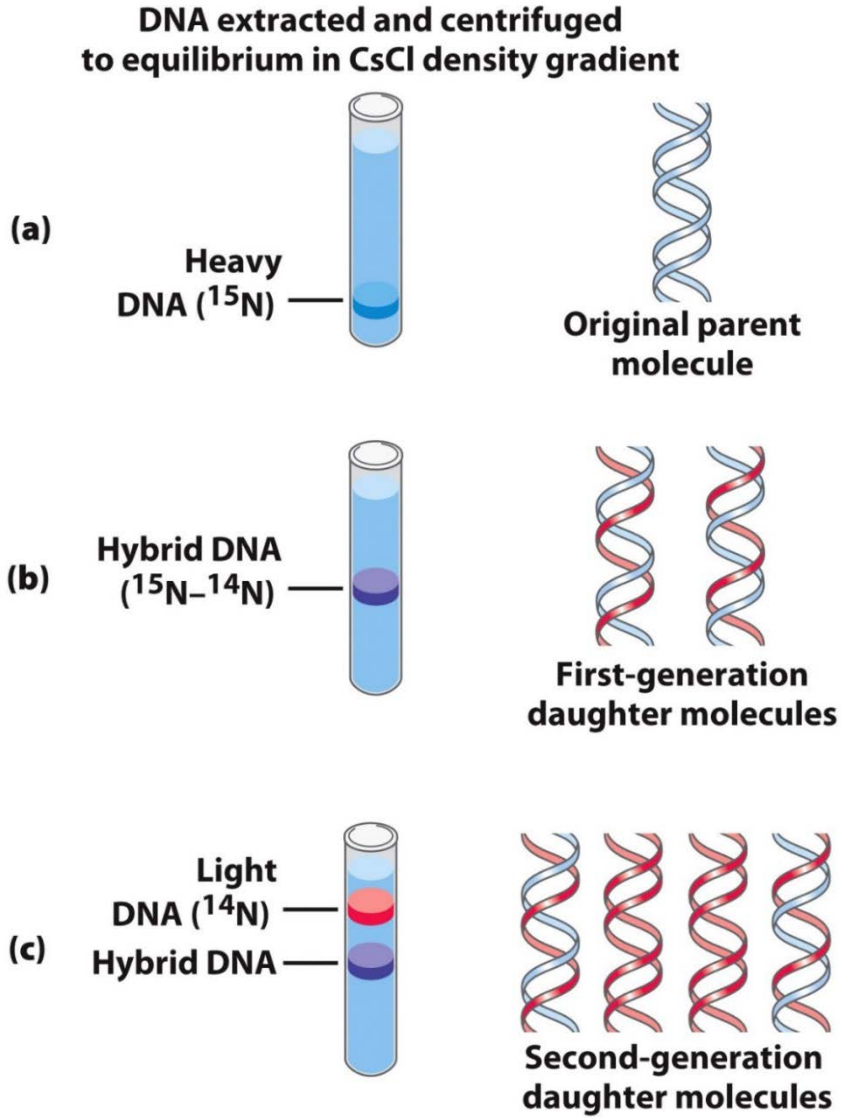
Part I

The Meselson-Stahl Experiment

Starting DNA: Heavy (N^{15}) /Heavy

1st generation: All Heavy/Light (N^{14})

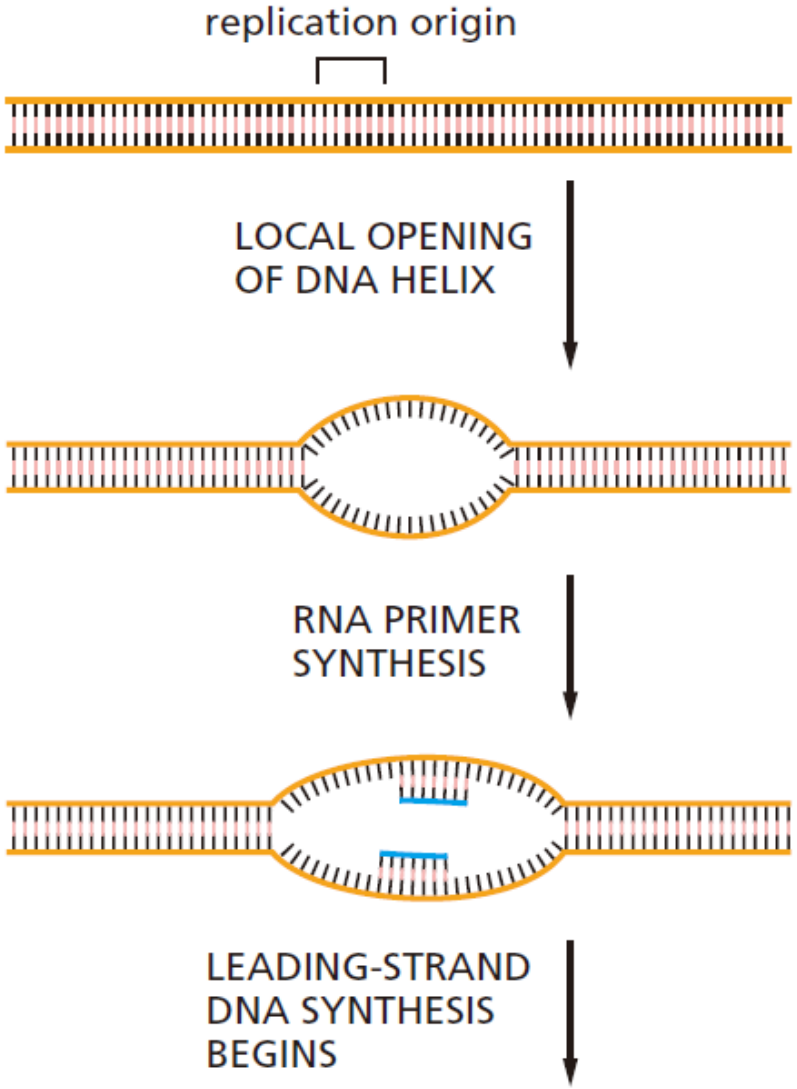
2nd generation: 2 Heavy/Light
2 Light/Light

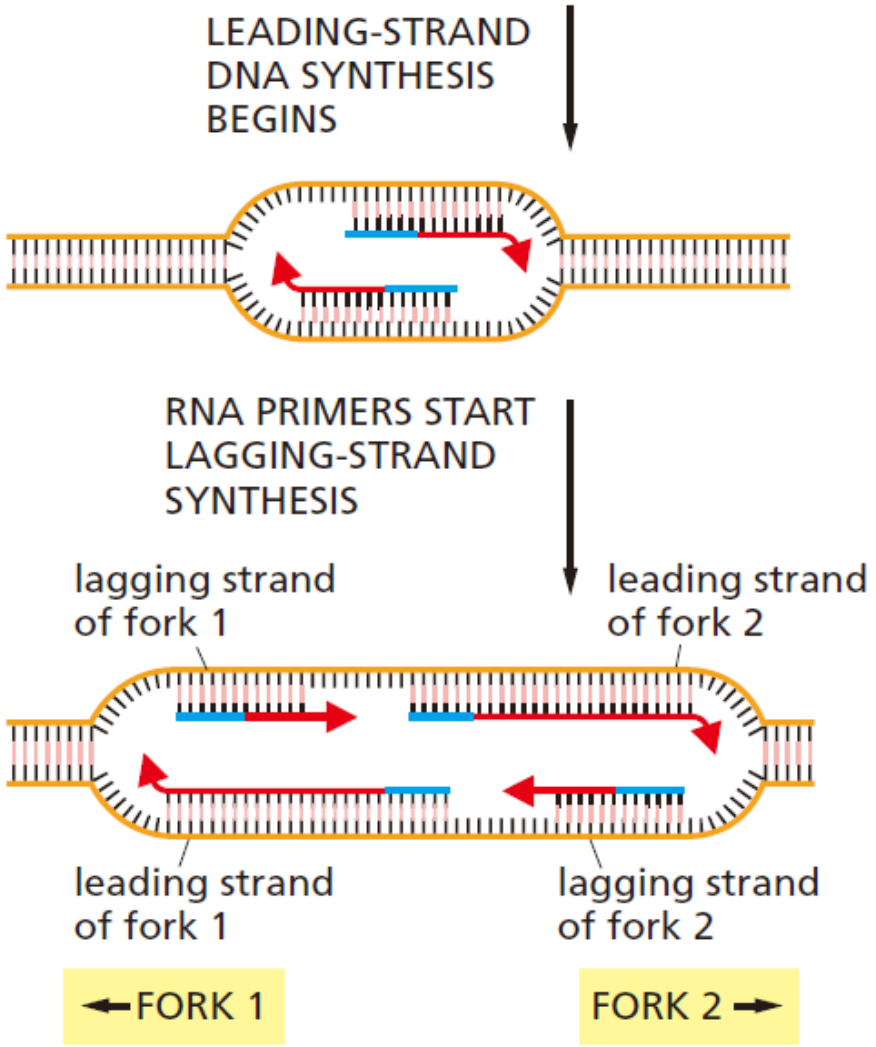


1. Replication Is Semiconservative

Part I

2. Replication Begins at an Origin and Is Bidirectional

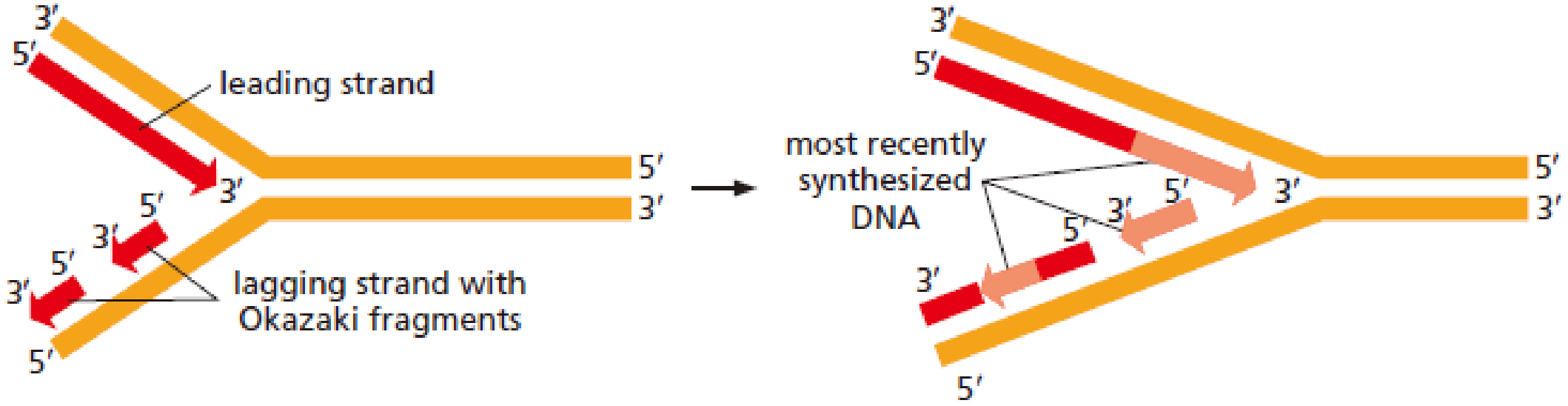




Replication forks

Part I

3. DNA Synthesis Proceeds in a 5'-to-3' Direction and Is Semidiscontinuous



Replication Fork

Okazaki Fragments: named after Reiji Okazaki

Leading strand: continuous, proceeds in the same direction as the replication fork movement

Lagging strand: discontinuous, proceeds in the opposite direction to the replication fork movement

Part I

General Features of DNA Replication

1. Replication is semiconservative

2. Replication begins at an origin and is bidirectional

3. DNA synthesis proceeds in a 5’-to-3’ direction and is semidiscontinuous

Part II. Machinery Required for DNA Replication

Part II

DNA replication Requires Many Enzymes and Protein Factors

DNA helicase

DNA gyrase (Topoisomerase II)

Single-strand DNA binding protein (SSB)

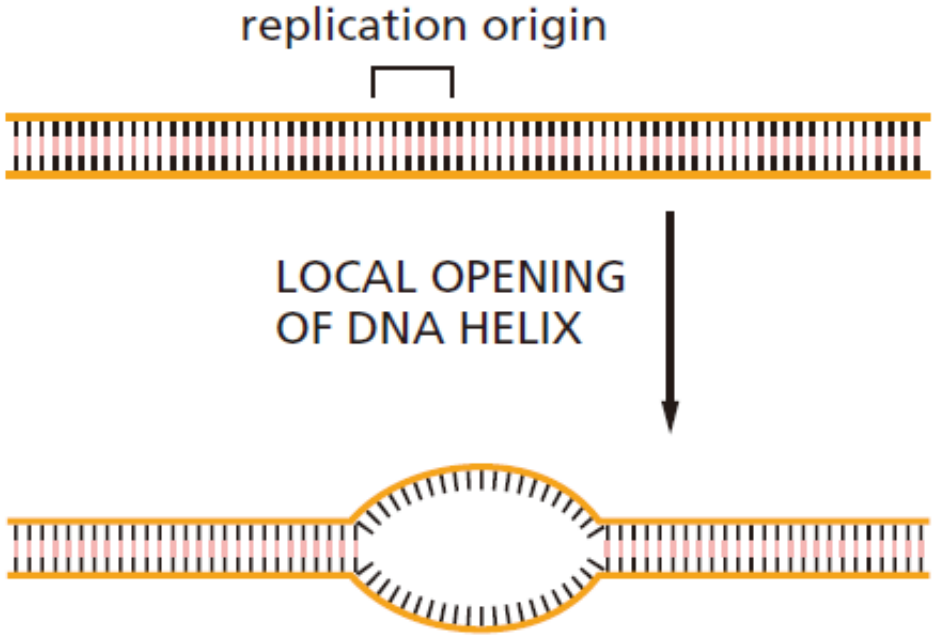
DNA Primase

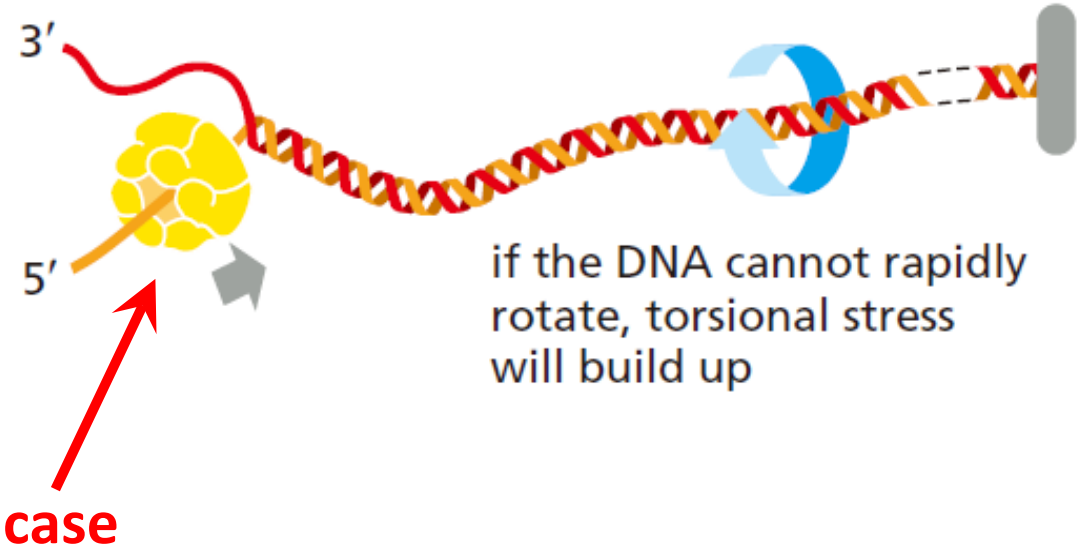
DNA polymerases

DNA ligase

Part II

DNA Helicase

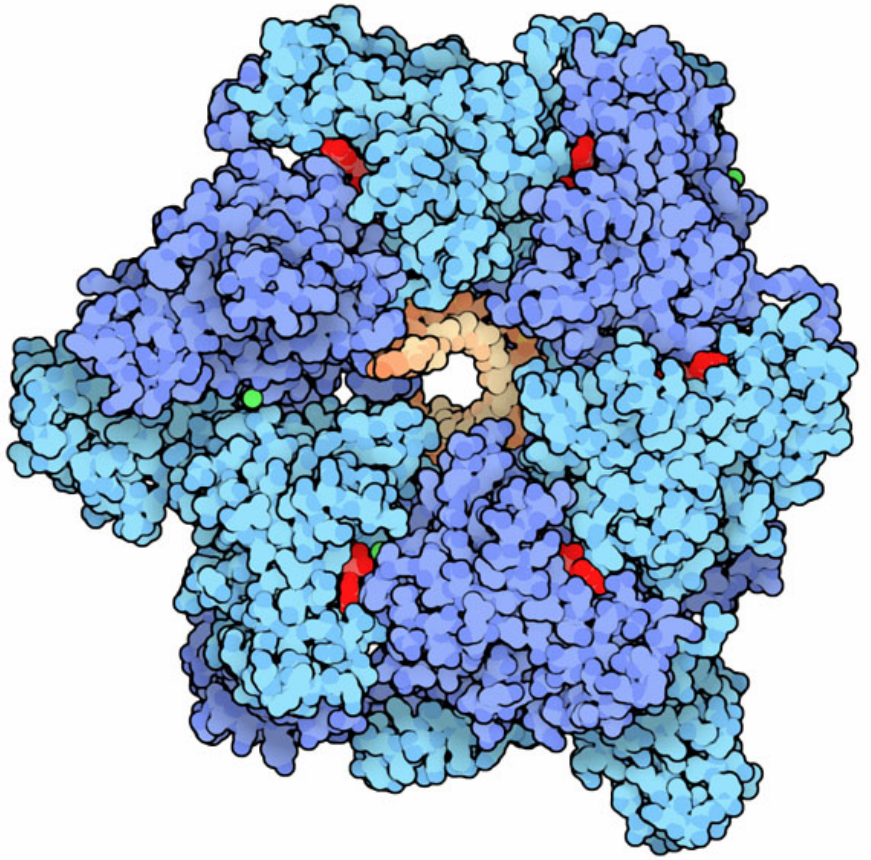




DNA Helicase

Helicase: unwinds DNA duplex
use 2 ATP to separate each base pair

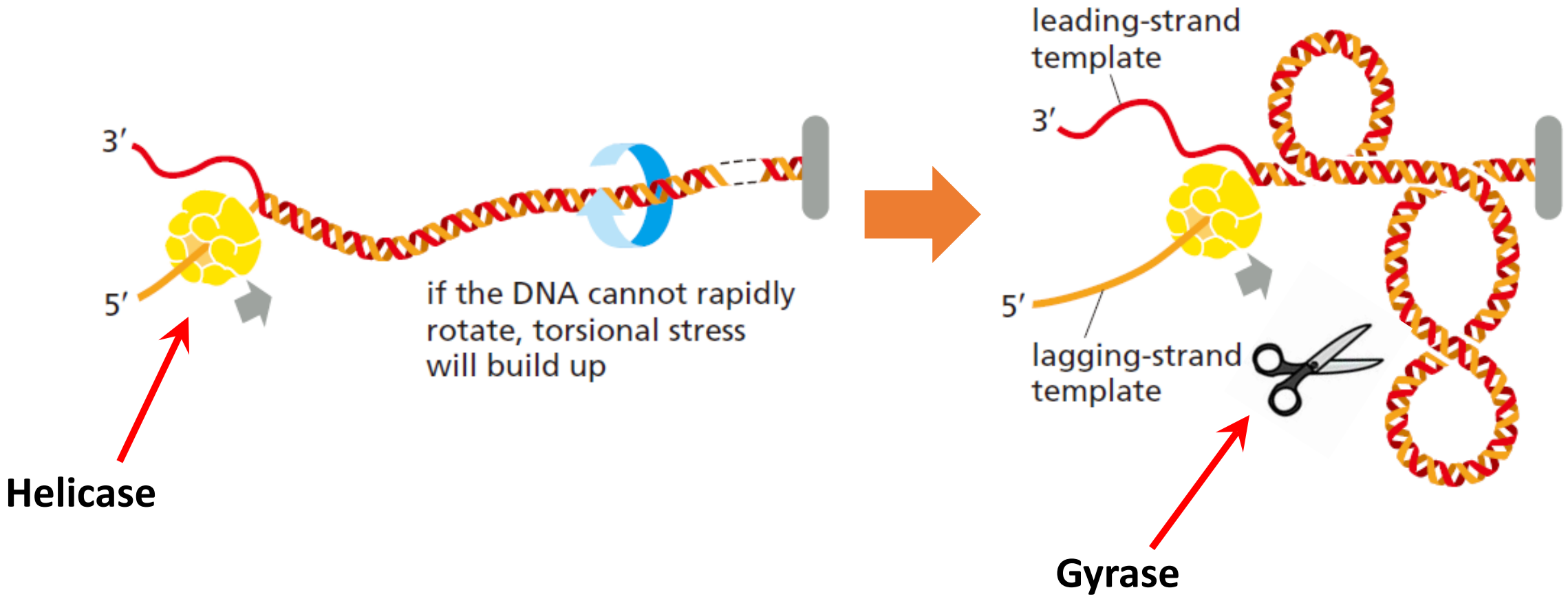
DnaB in E. coli. moves 5'→3' .
Slides on the lagging template strand.



Hexameric ring

Part II

Gyrase (Topoisomerase II)



Helicase

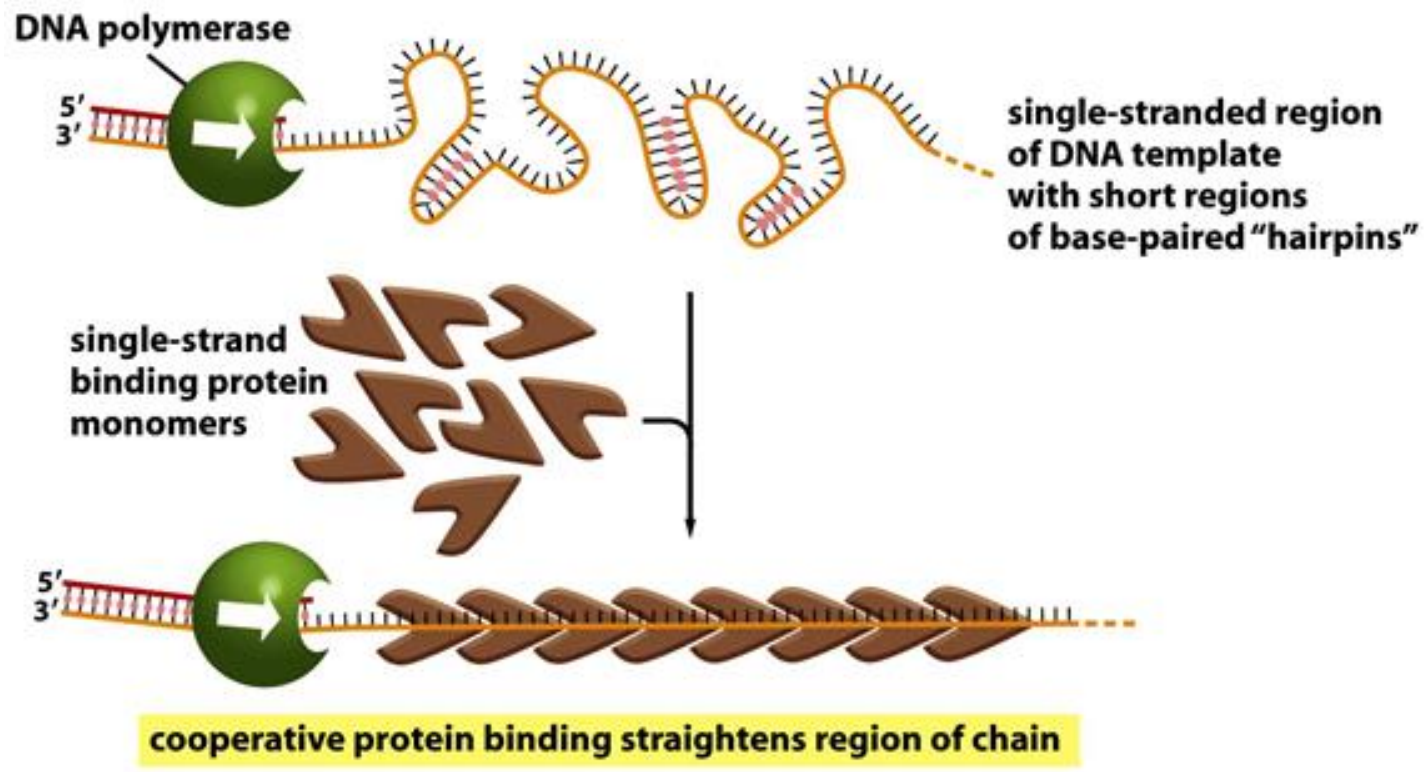
Gyrase

Cut double-strand DNA

Introduce -2 supercoils into DNA per reaction

Part II

Single-Strand DNA Binding (SSB) Protein

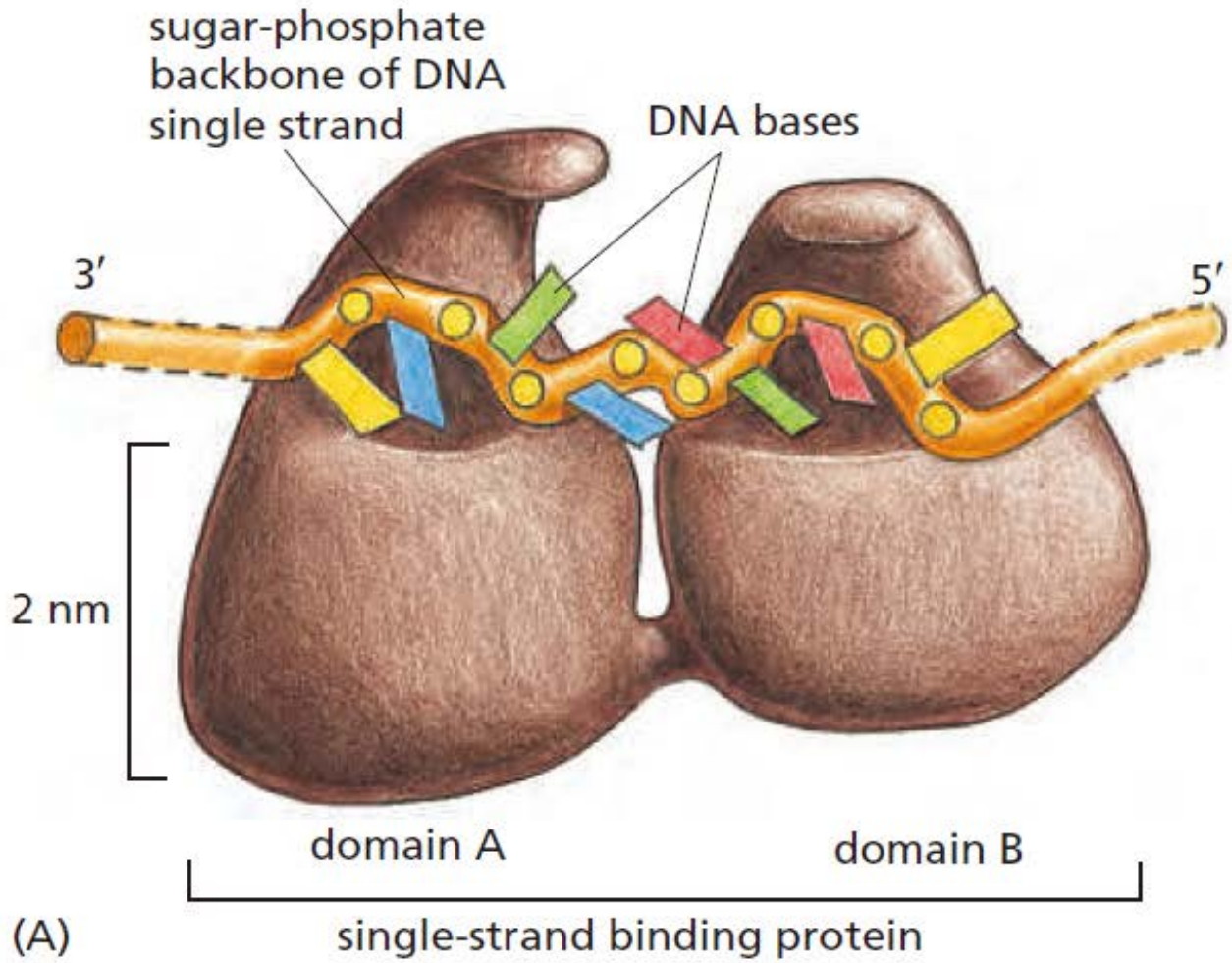


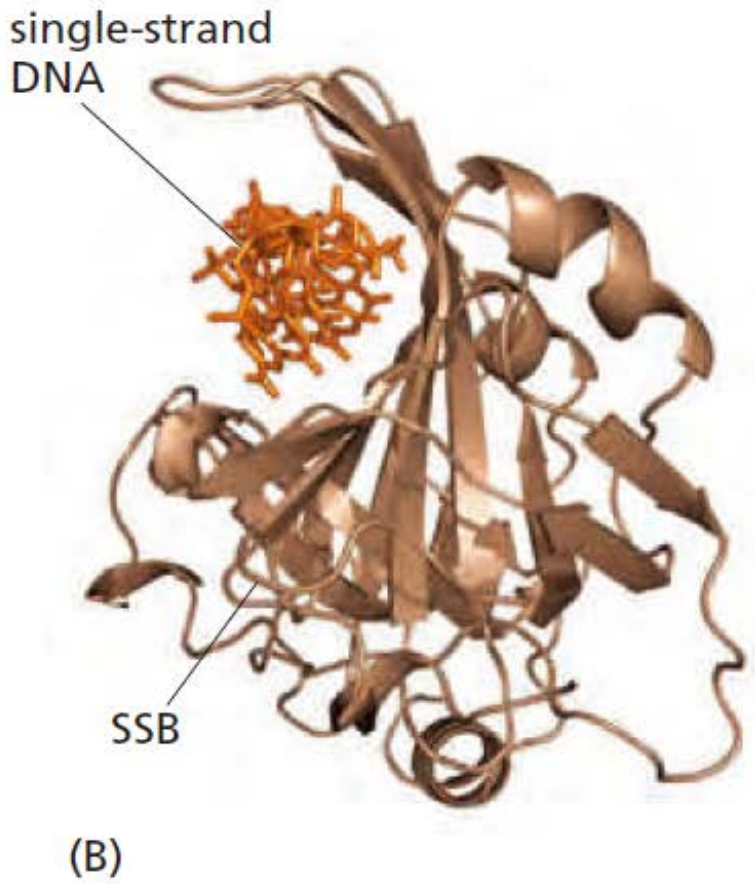
Also called helix-destabilizing proteins

SSB proteins bind tightly and cooperatively to exposed single-strand DNA without covering the bases, which therefore remain available as template.

Part II

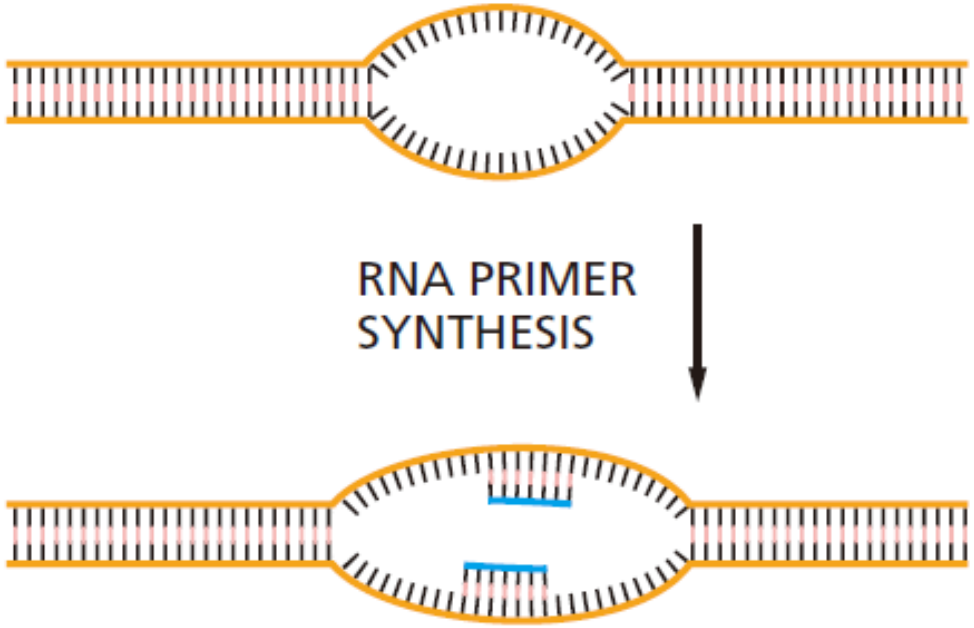
Human Single-Strand Binding Protein Bound to DNA





Part II

DNA Primase



- DNA synthesis starts from a primer
- Primase: RNA polymerase,
  make RNA primers

Part II

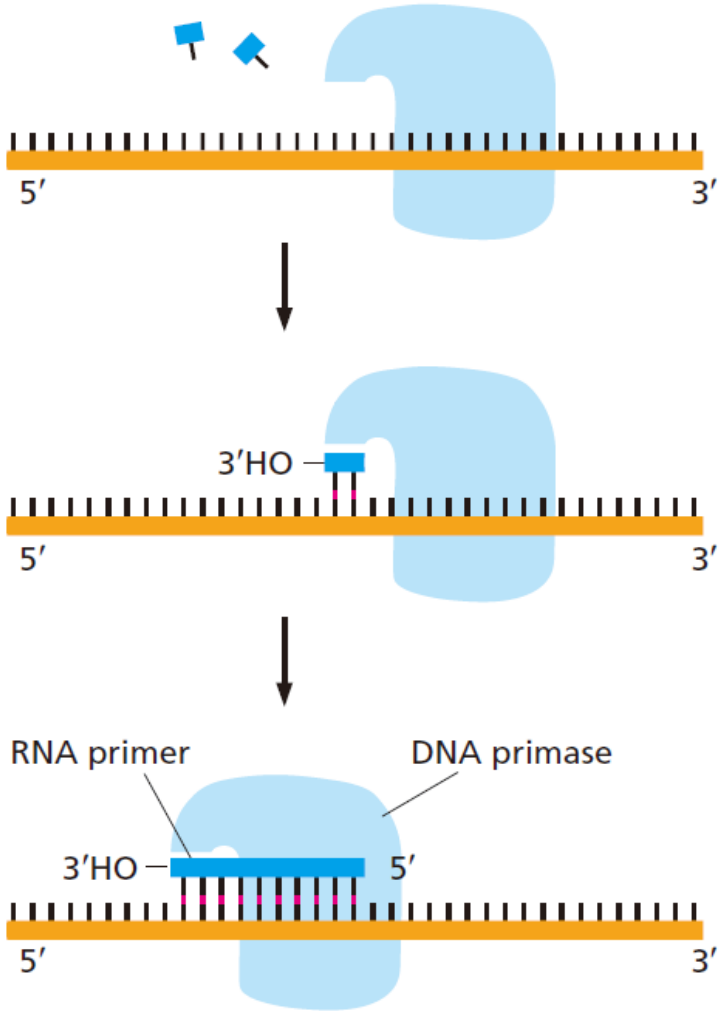
DNA Primase

DNA polymerase: can continue but not initiate synthesis

Primase: synthesizes ~10 nt RNA in eukaryotes
at intervals of 100-200 nt on the lagging strand

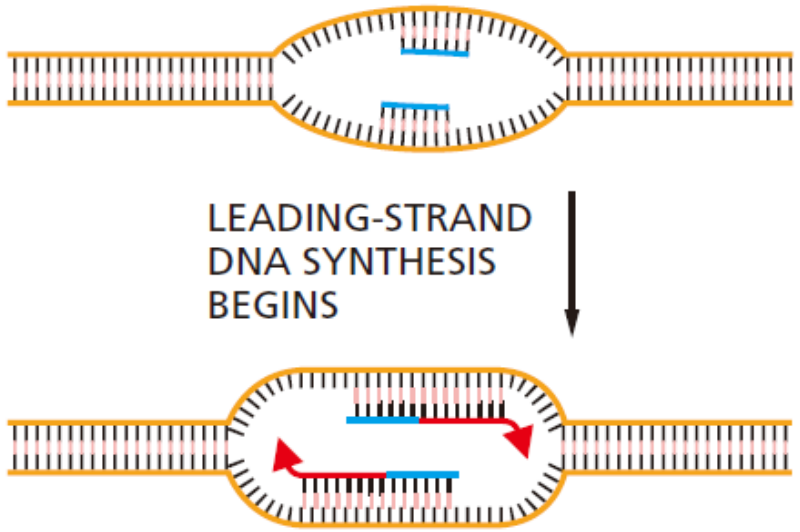
RNA primers are ultimately removed and replaced with DNA,

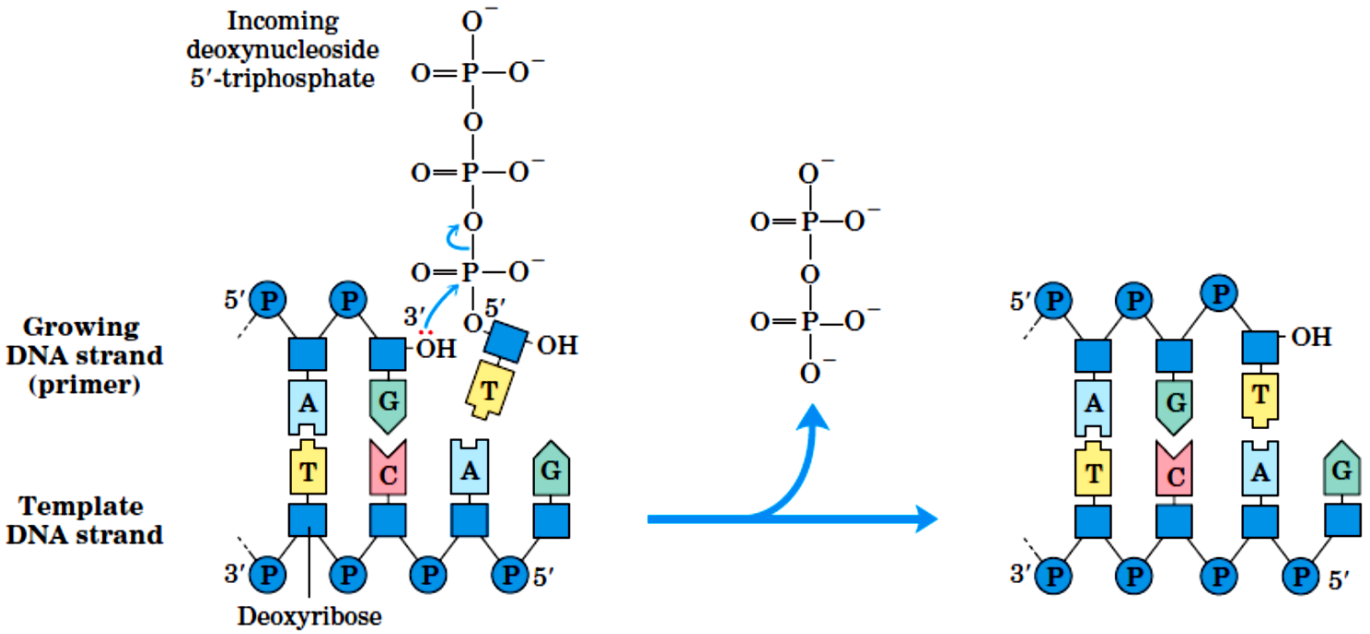
which is catalyzed by DNA polymerase I in *E. Coli.*



Part II

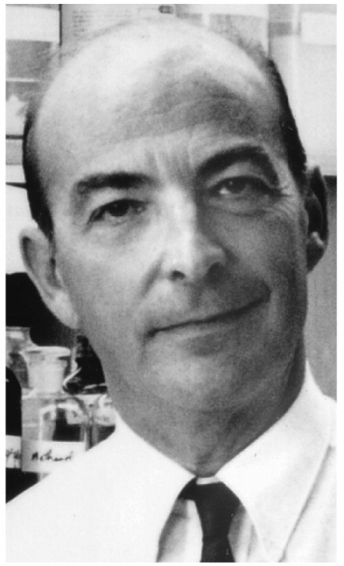
DNA Polymerase



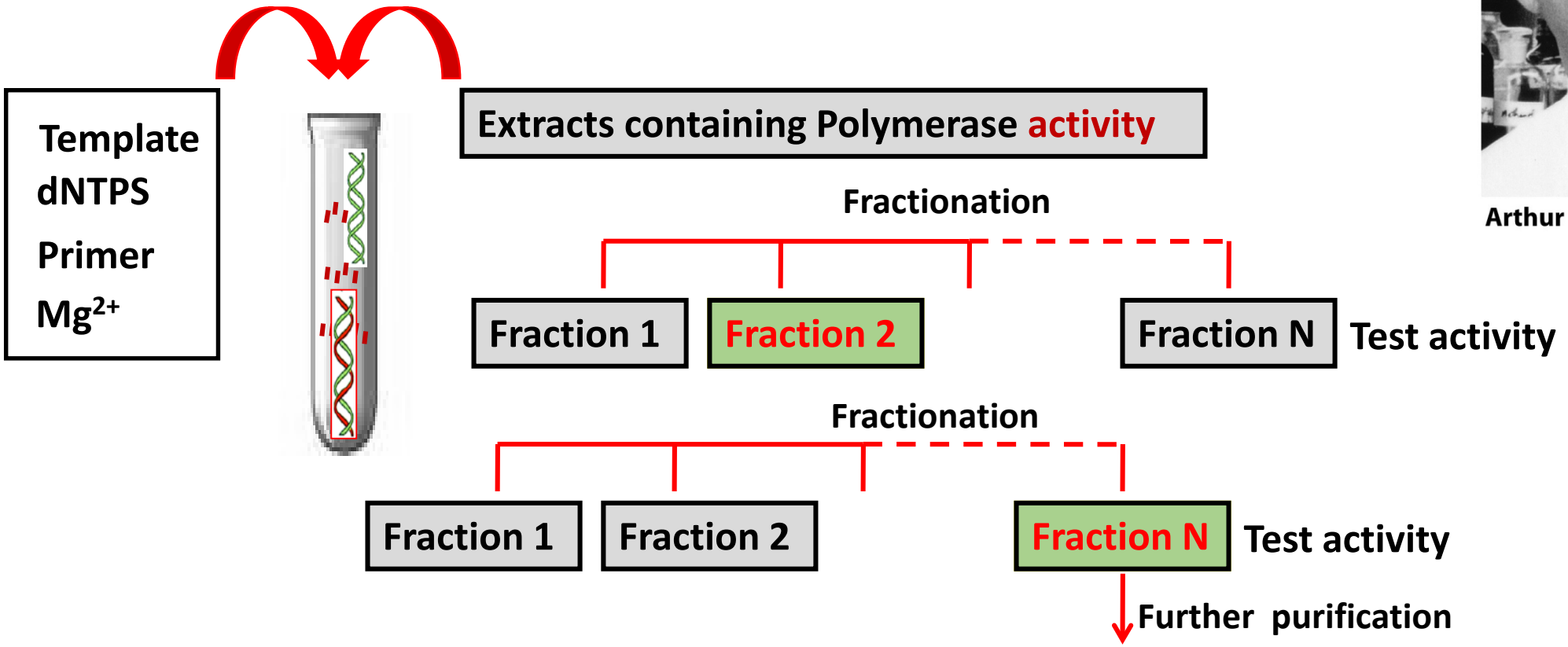


Part II

Discovery of DNA Polymerase



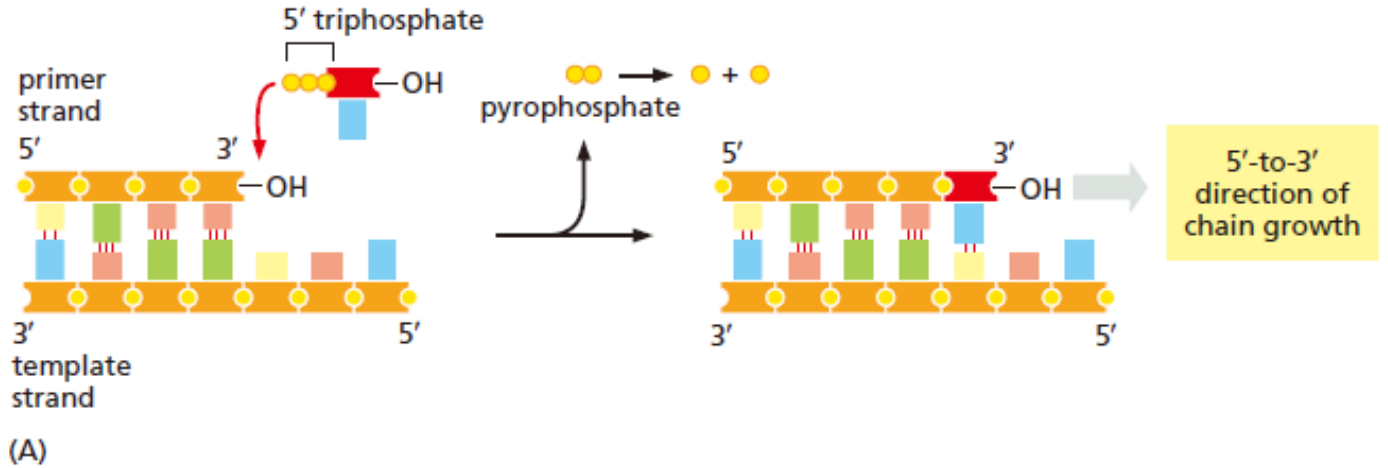
Arthur Kornberg, 1918–2007



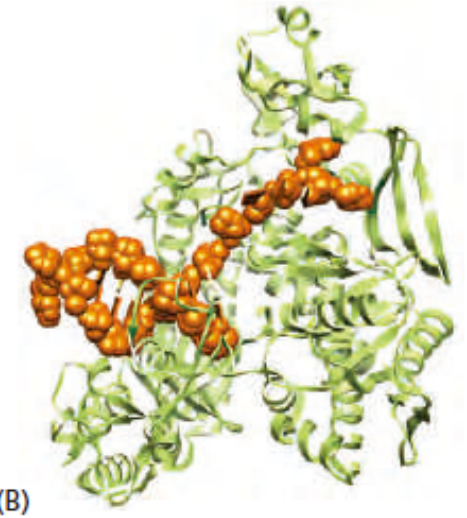
Purification scheme of *E. Coli* DNA polymerase I

Part II

DNA Synthesis Catalyzed by DNA Polymerase



(A)



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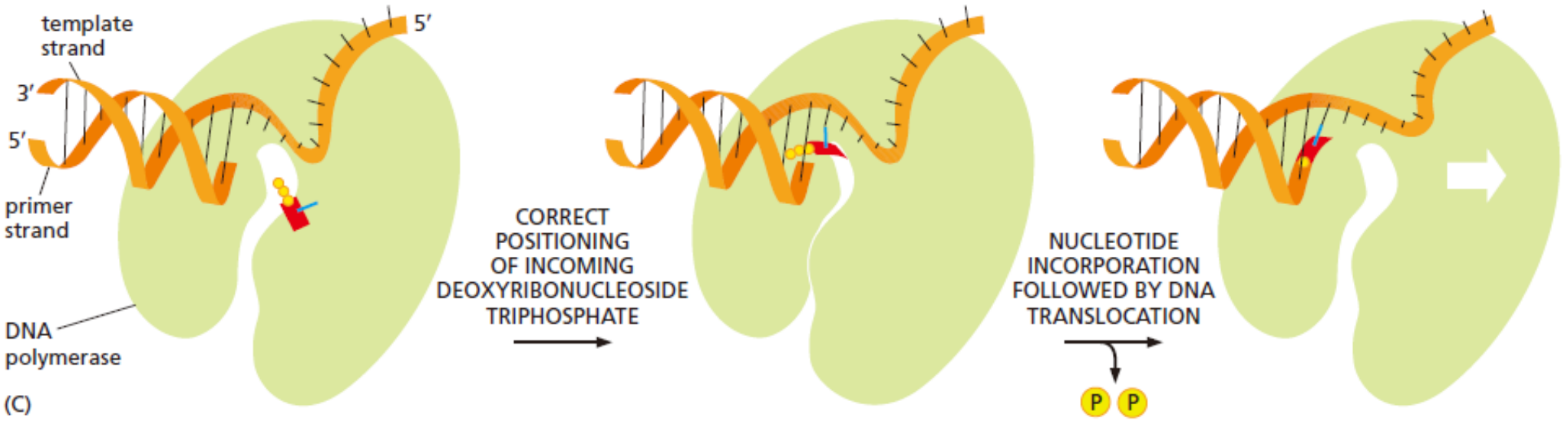
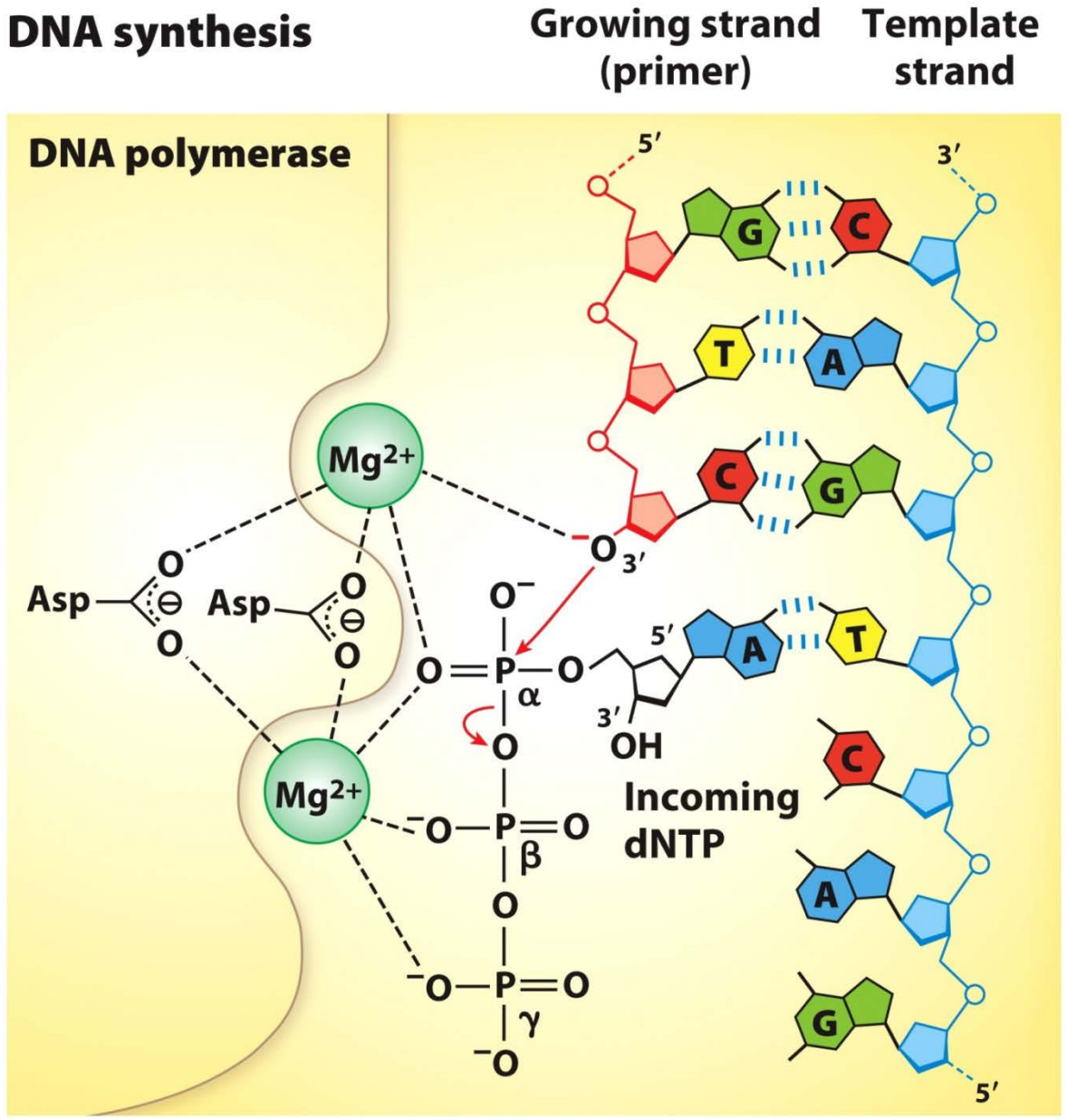


(C)

Part II

Mechanism of DNA synthesis by DNA polymerase



Incoming dNTP is attacked at the α phosphate by the 3′ hydroxyl of the growing DNA chain.

Mg^{2+} is required for the catalytic activity of DNA polymerase.

Replication Is Very Accurate

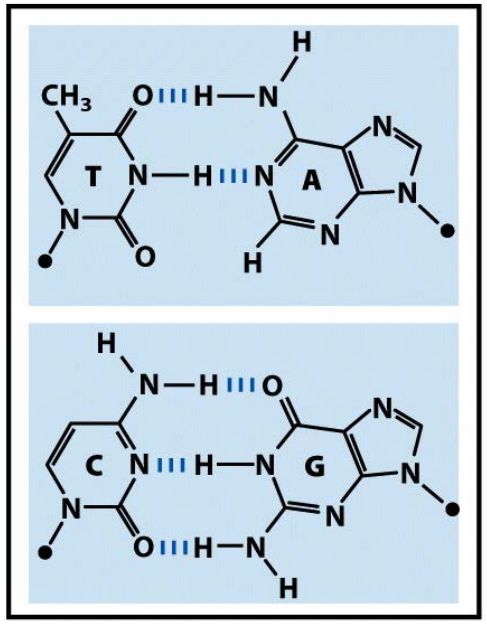
High fidelity: One mistake every 10^9 to 10^{10} nucleotides added in E. Coli (4.6×10^6).

Nucleases

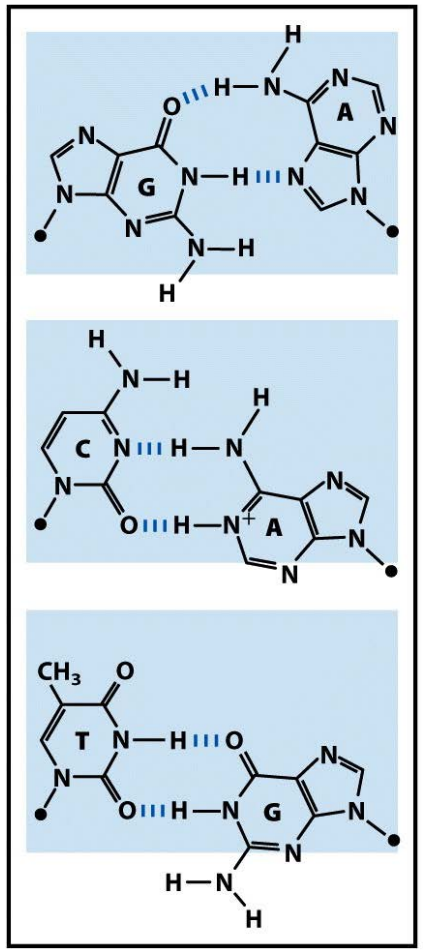
- **Exonucleases: Degrade nucleic acids from one end of the molecule, 5'-to-3' or 3'-to-5'**
- **Endonucleases: Degrade at specific internal sites**

Part II

Contribution of Base-Pair Geometry to the Fidelity of DNA Replication



Correct



Incorrect

Exonucleolytic Proofreading by DNA Polymerase

First Step of Proofreading:

Just before a new nucleotide is covalently added

Higher binding affinity for correct nucleotide

Conformation change occurs more readily with correct nucleotide

Second, Exonucleolytic Proofreading:

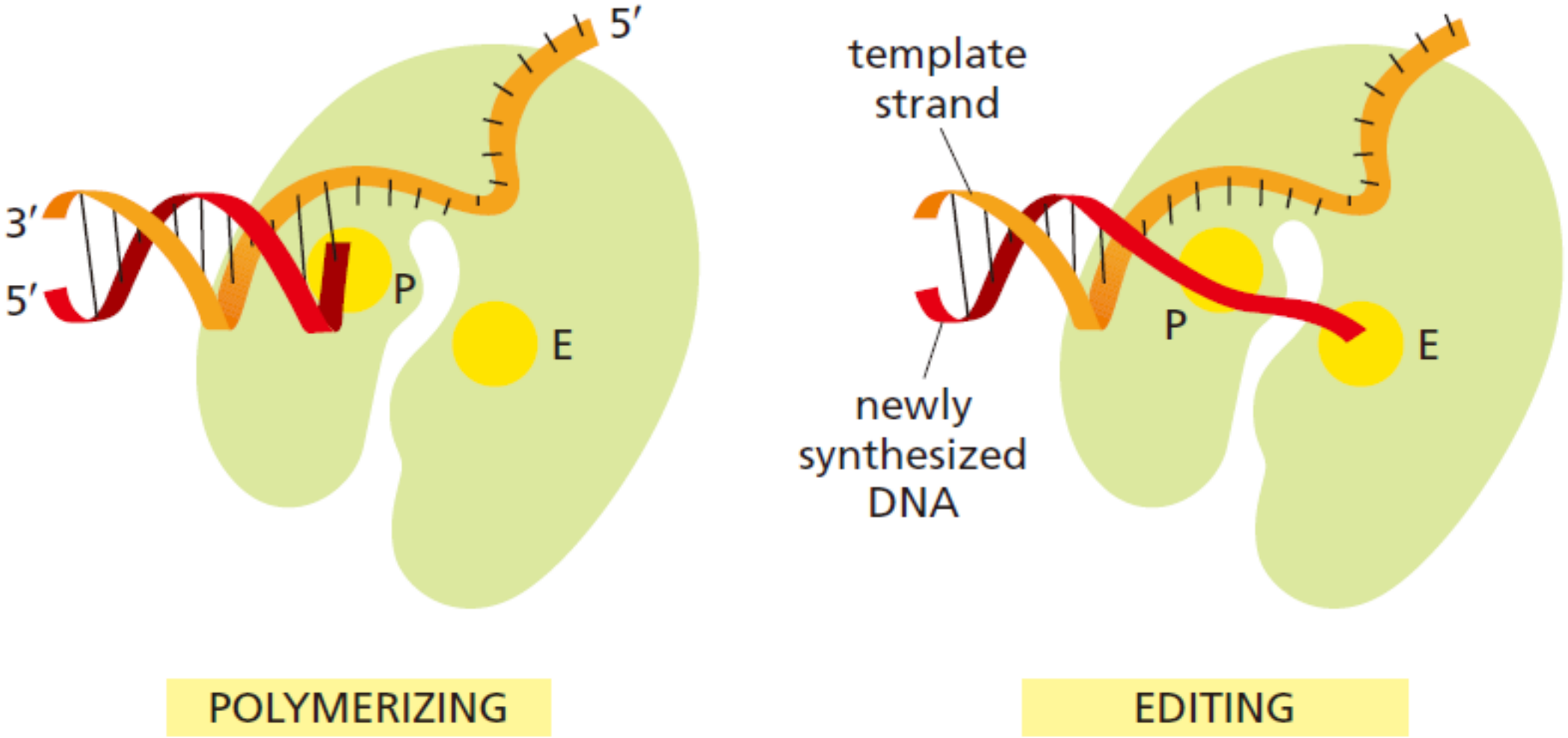
3’-5’ proofreading exonuclease activity

Third, Strand-Directed Mismatch Repair

Will talk about in *DNA Repair*.

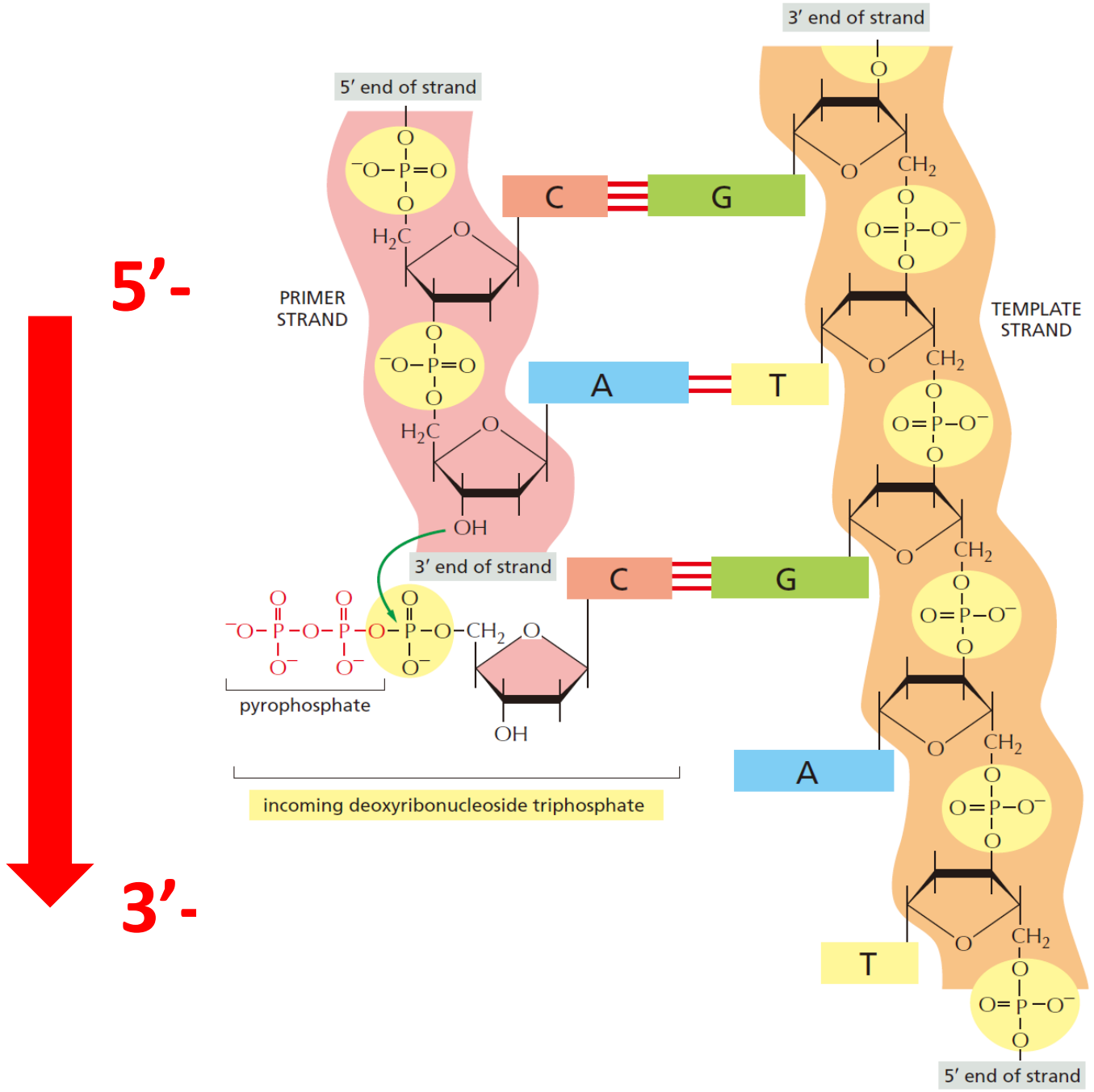
Part II

Editing by DNA Polymerase



Part II

Only DNA Replication in the 5’-to-3’ Direction Allows Efficient Correcting



Part II

TABLE 5–1 The Three Steps That Give Rise to High-Fidelity DNA Synthesis

Replication step	Errors per nucleotide added
5′ ⟶ 3′ polymerization	1 in 10^5
3′ ⟶ 5′ exonucleolytic proofreading	1 in 10^2
Strand-directed mismatch repair	1 in 10^3
Combined	1 in 10^{10}

The third step, strand-directed mismatch repair, is described later in this chapter. For the polymerization step, "errors per nucleotide added" describes the probability that an incorrect nucleotide will be added to the growing chain. For the other two steps, "errors per nucleotide added" describes the probability that an error will not be corrected. Each step therefore reduces the chance of a final error by the factor shown.

Part II

TABLE 25–1 Comparison of Three 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

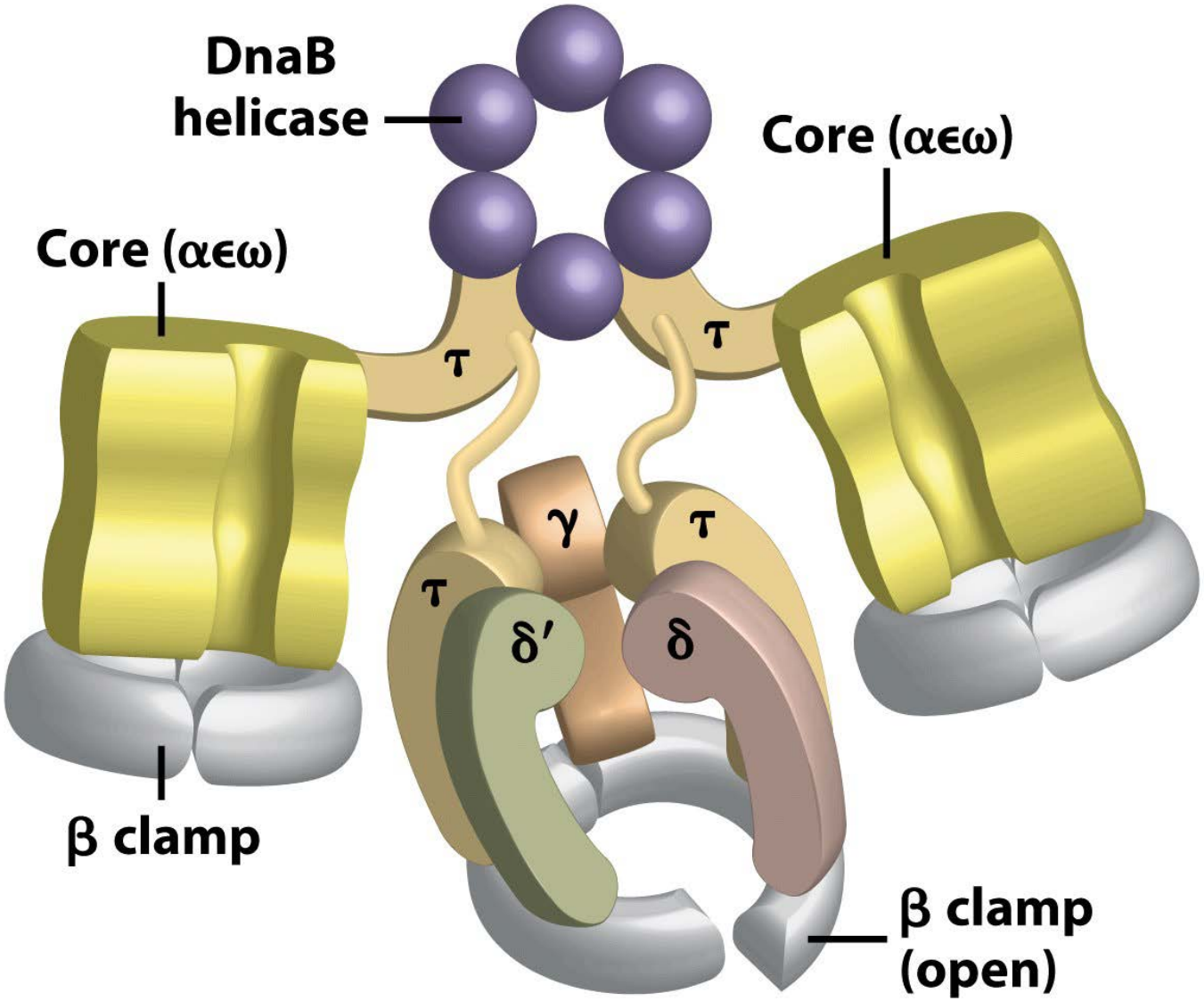
***E. Coli* has at least five DNA polymerases.**

Klenow fragment: 5'-to-3' exonuclease activity removed from DNA polymerase I.

DNA polymerase III is the principal replication enzyme in *E. Coli*.

Part II

Architecture of Bacterial DNA polymerase III



Part II

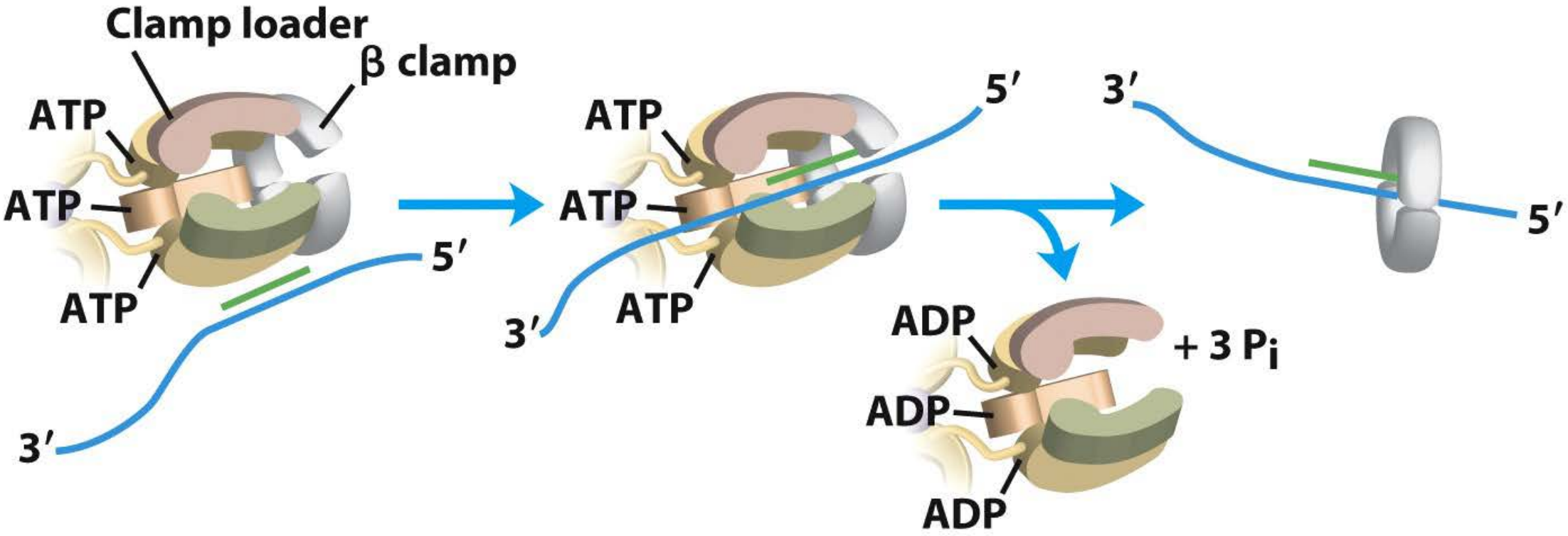
TABLE 25–2 Subunits of DNA Polymerase III of *E. coli*

Subunit	Number of subunits per holoenzyme	M_r of subunit	Gene	Function of subunit	
α	2	129,900	*polC* (*dnaE*)	Polymerization activity	Core polymerase
ε	2	27,500	*dnaQ* (*mutD*)	3′→5′ Proofreading exonuclease	Core polymerase
θ	2	8,600	*holE*	Stabilization of ε subunit	Core polymerase
τ	2	71,100	*dnaX*	Stable template binding; core enzyme dimerization	Clamp-loading (γ) complex that loads β subunits on lagging strand at each Okazaki fragment
γ	1	47,500	*dnaX**	Clamp loader	Clamp-loading (γ) complex that loads β subunits on lagging strand at each Okazaki fragment
δ	1	38,700	*holA*	Clamp opener	Clamp-loading (γ) complex that loads β subunits on lagging strand at each Okazaki fragment
δ'	1	36,900	*holB*	Clamp loader	Clamp-loading (γ) complex that loads β subunits on lagging strand at each Okazaki fragment
χ	1	16,600	*holC*	Interaction with SSB	
ψ	1	15,200	*holD*	Interaction with γ and χ	
β	4	40,600	*dnaN*	DNA clamp required for optimal processivity	

*The γ subunit is encoded by a portion of the gene for the τ subunit, such that the amino-terminal 66% of the τ subunit has the same amino acid sequence as the γ subunit. The γ subunit is generated by a translational frameshifting mechanism (see p. •••) that leads to premature translational termination.

Part II

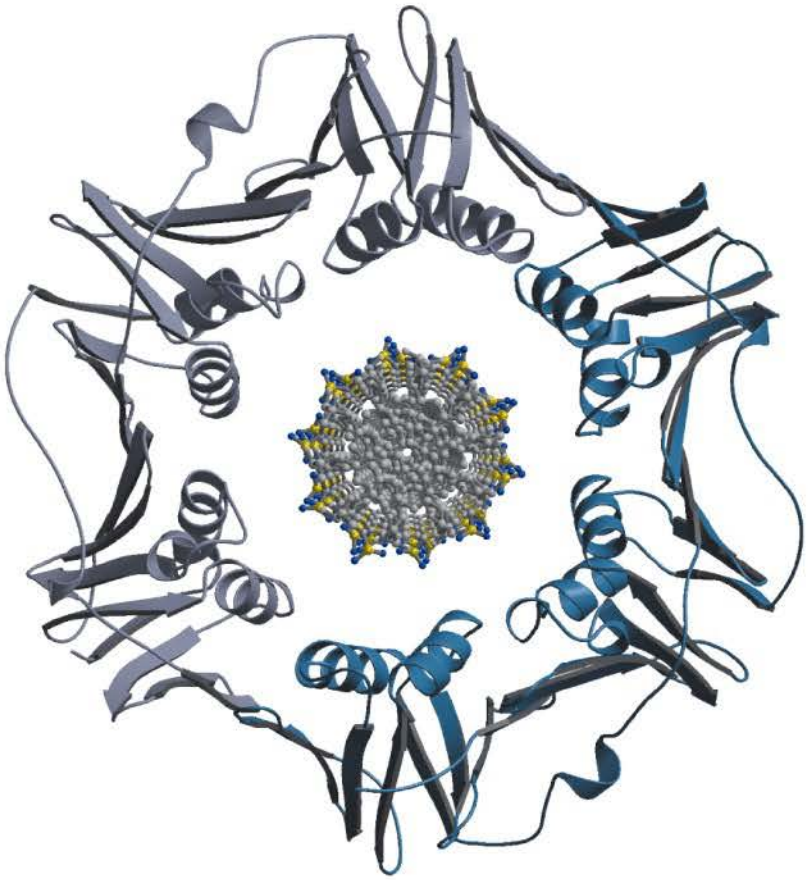
Sliding Clamp and Clamp Loader



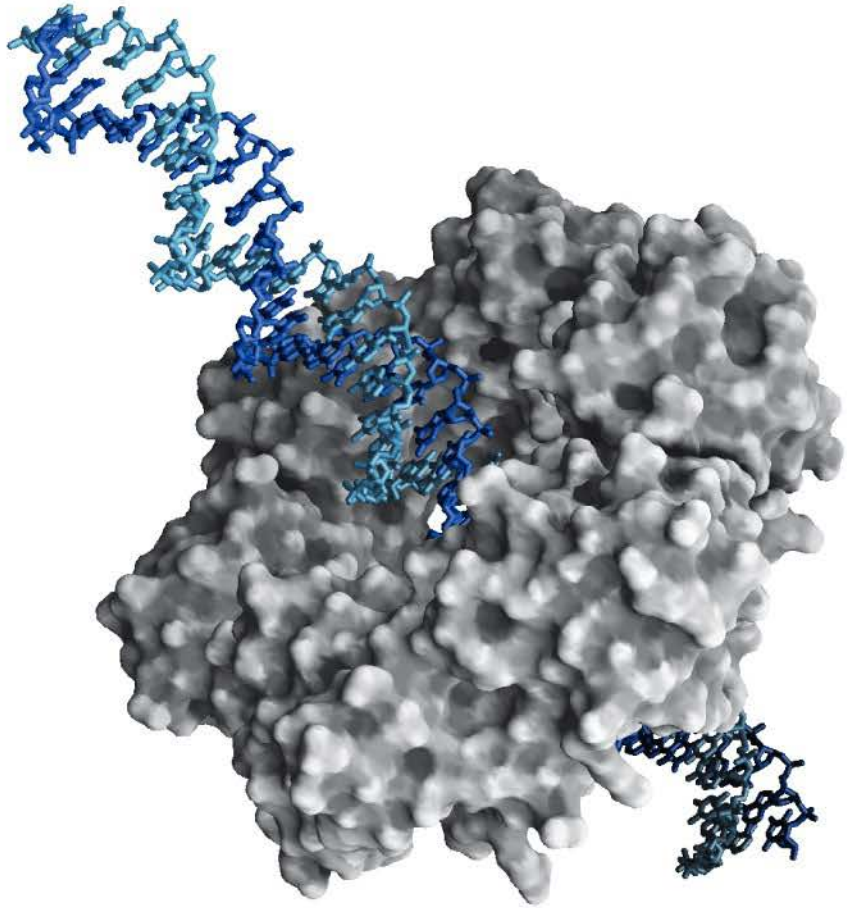
A sliding ring holds a moving DNA polymerase onto the DNA.

Part II

Two β Subunits Form a Circular Clamp



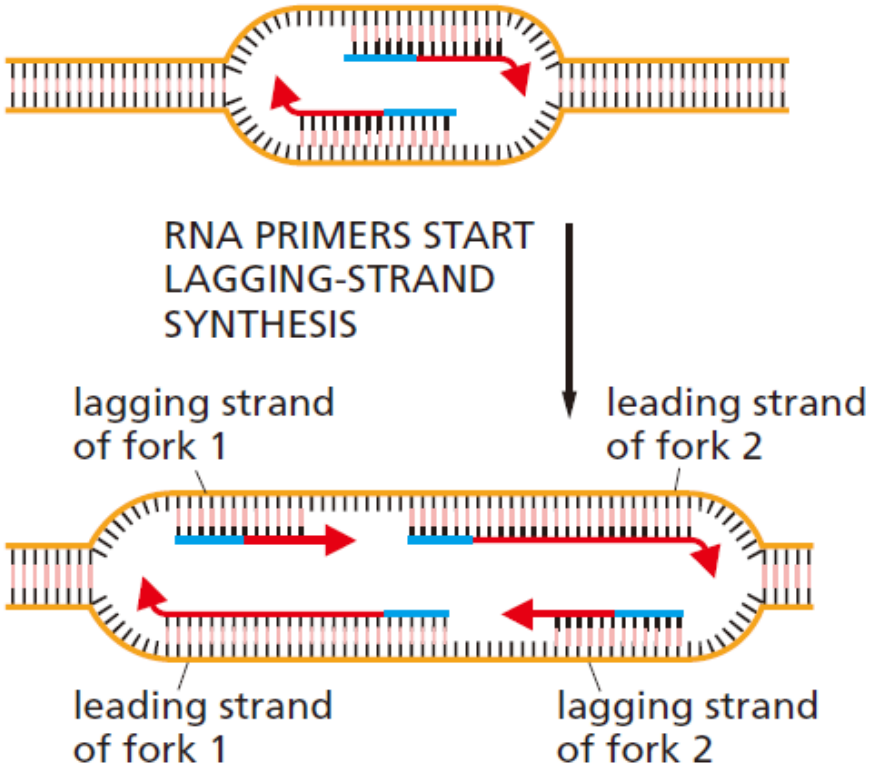
End view

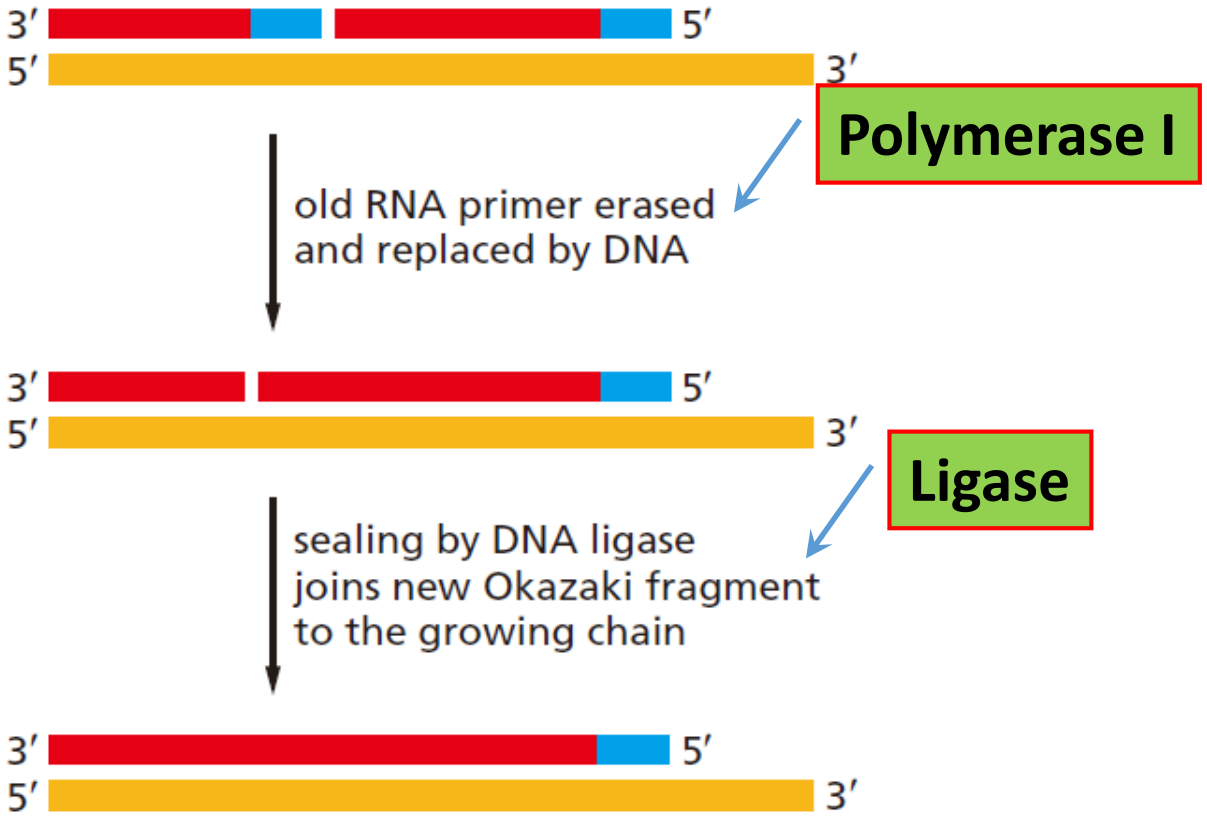


Side view

Part II

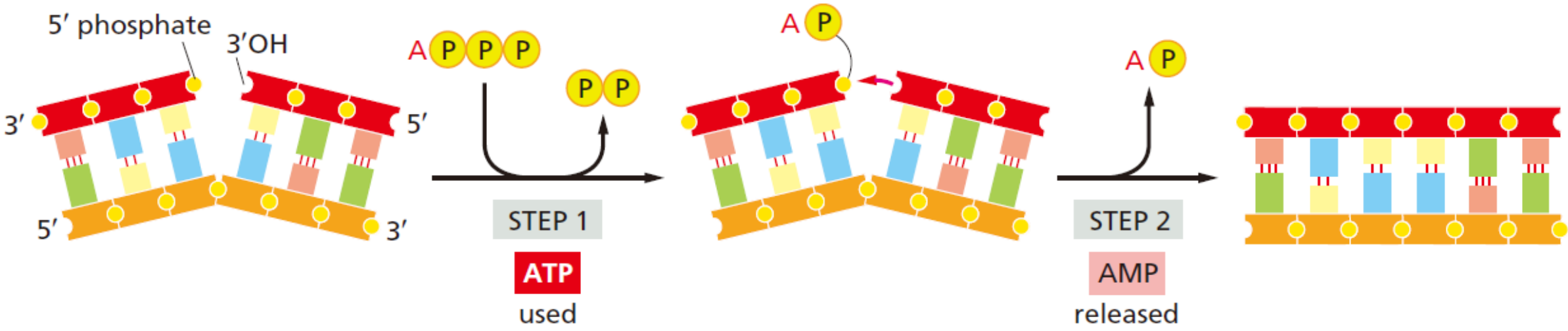
DNA Polymerase I and Ligase





Part II

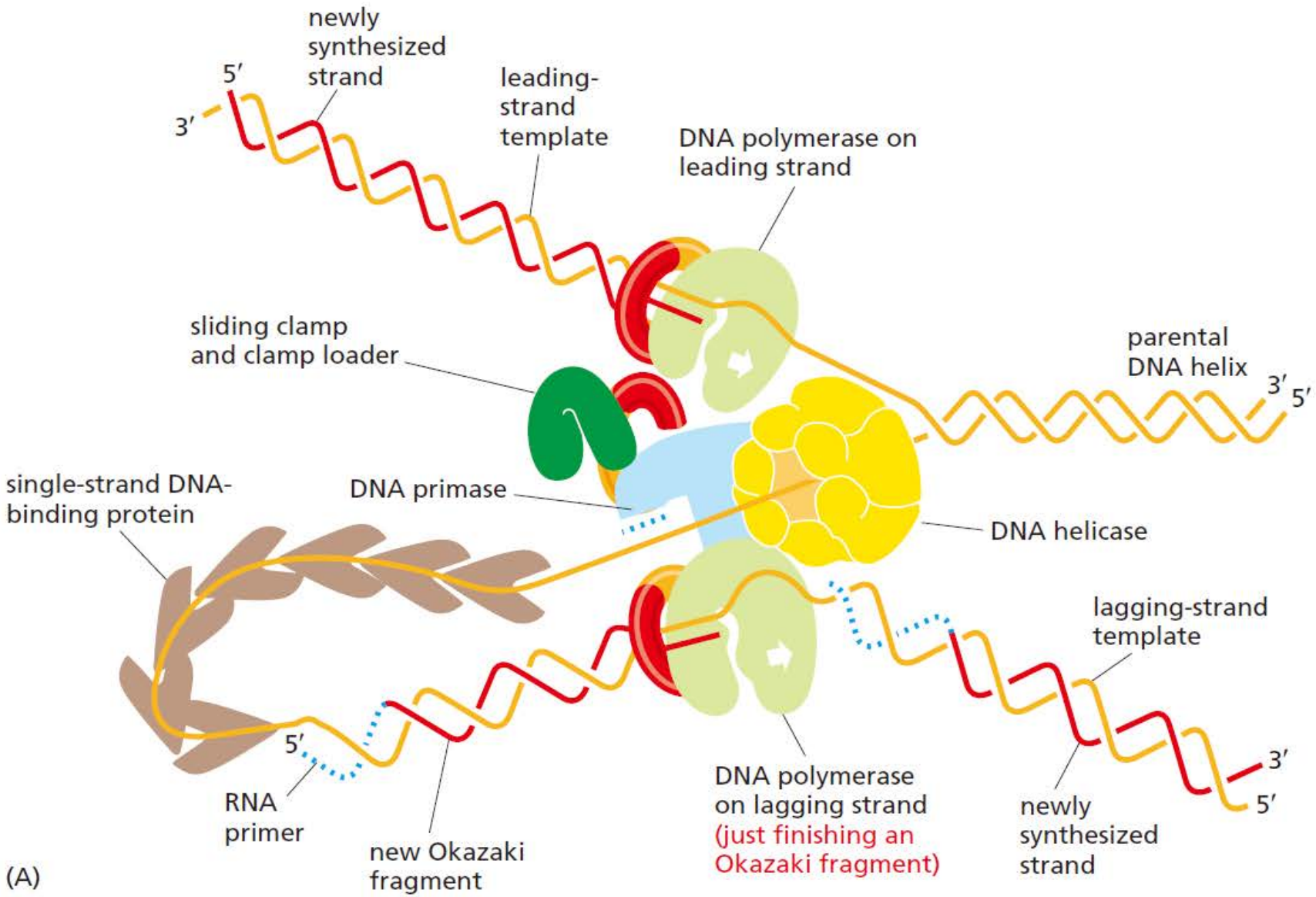
DNA Ligase



DNA ligase joins the 3’-OH of the new DNA fragment to the 5’-phosphate of the previous Okazaki fragment.

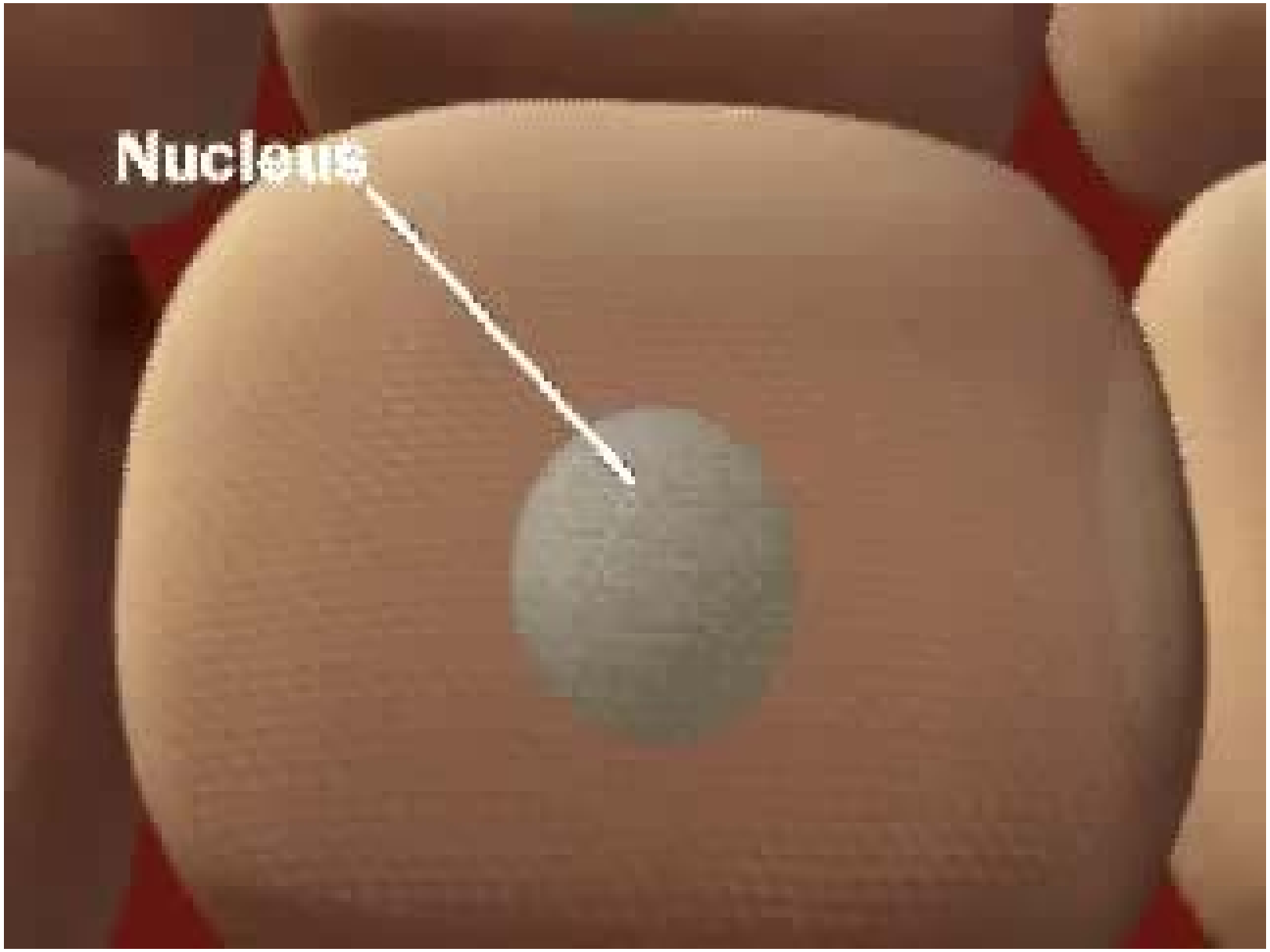
Part II

Replisome at A Replication Fork



Part II

DNA Replication in a Quick View



www.dnatube.com/video/335/Animated-DNA-Replication

Part II. Machinery of DNA Replication

What proteins are involved?

What confers the high fidelity in DNA replication?

What are the components in a PCR reaction?

Part III. DNA Replication Proceeds in Stages

Part III

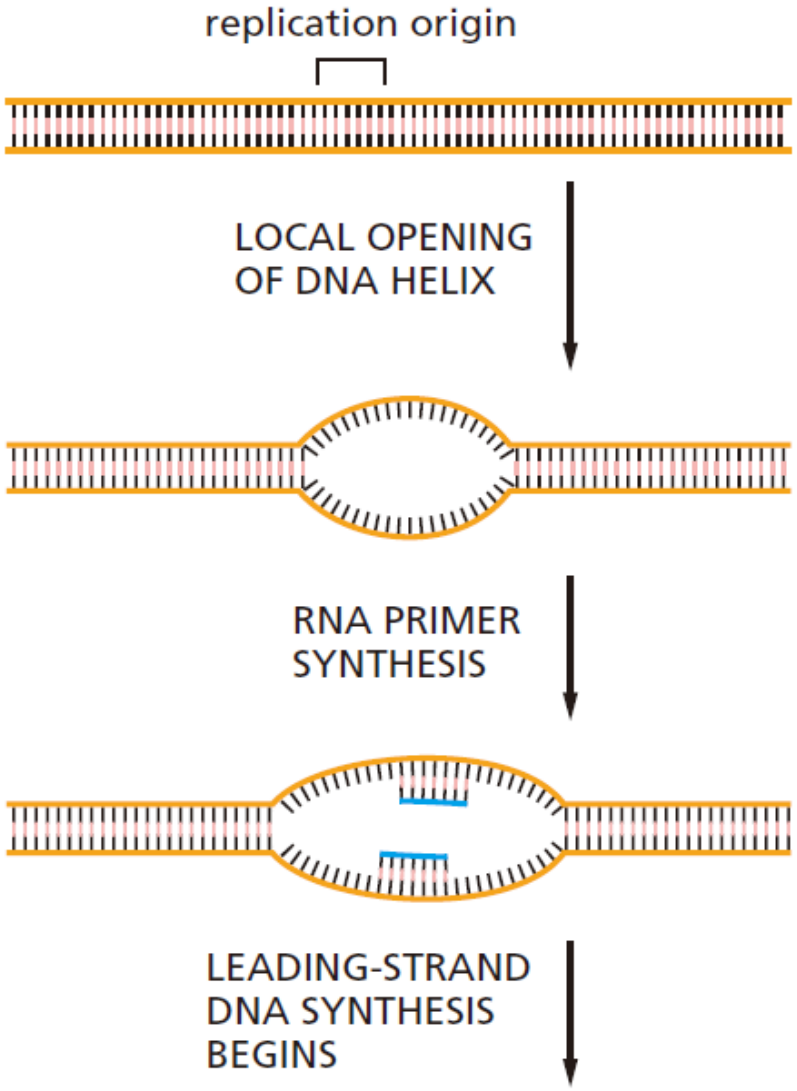
The Initiation and Completion of DNA Replication in Chromosomes

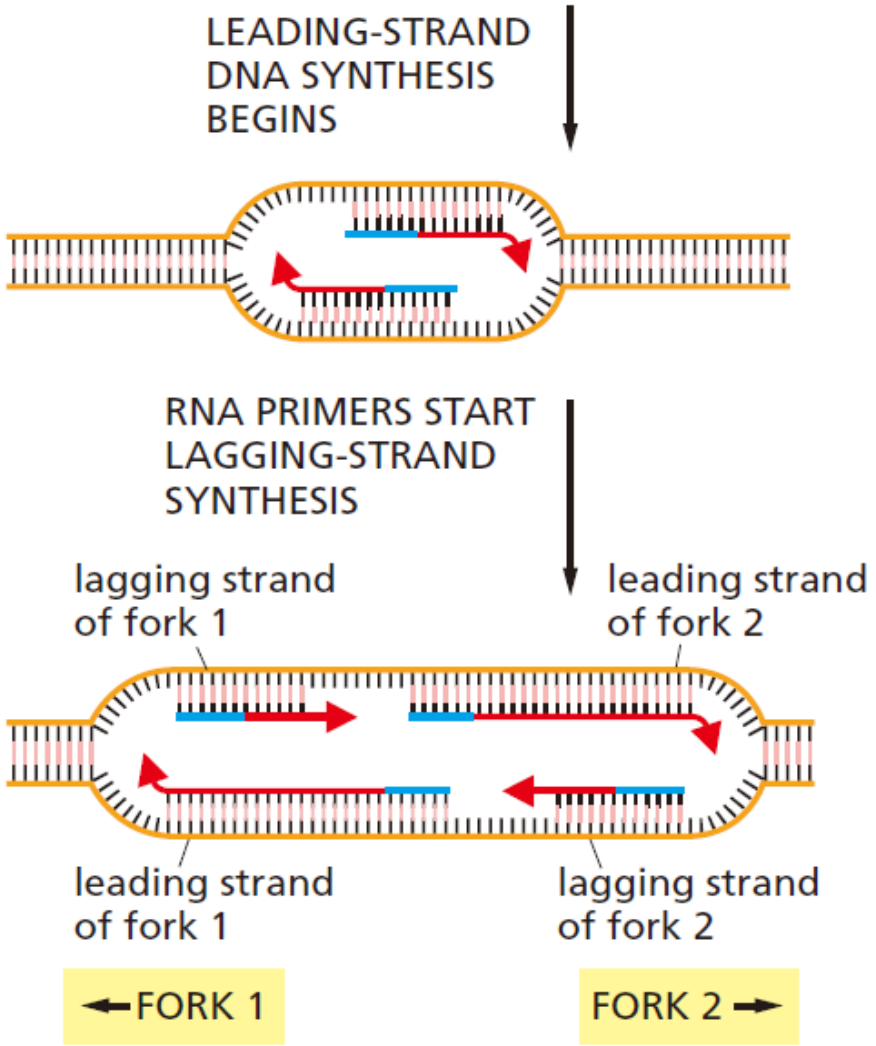
- Initiation
- Elongation
- Termination

Part III

Initiation

DNA Synthesis Begins at Replication Origins





DNA Replication Origin

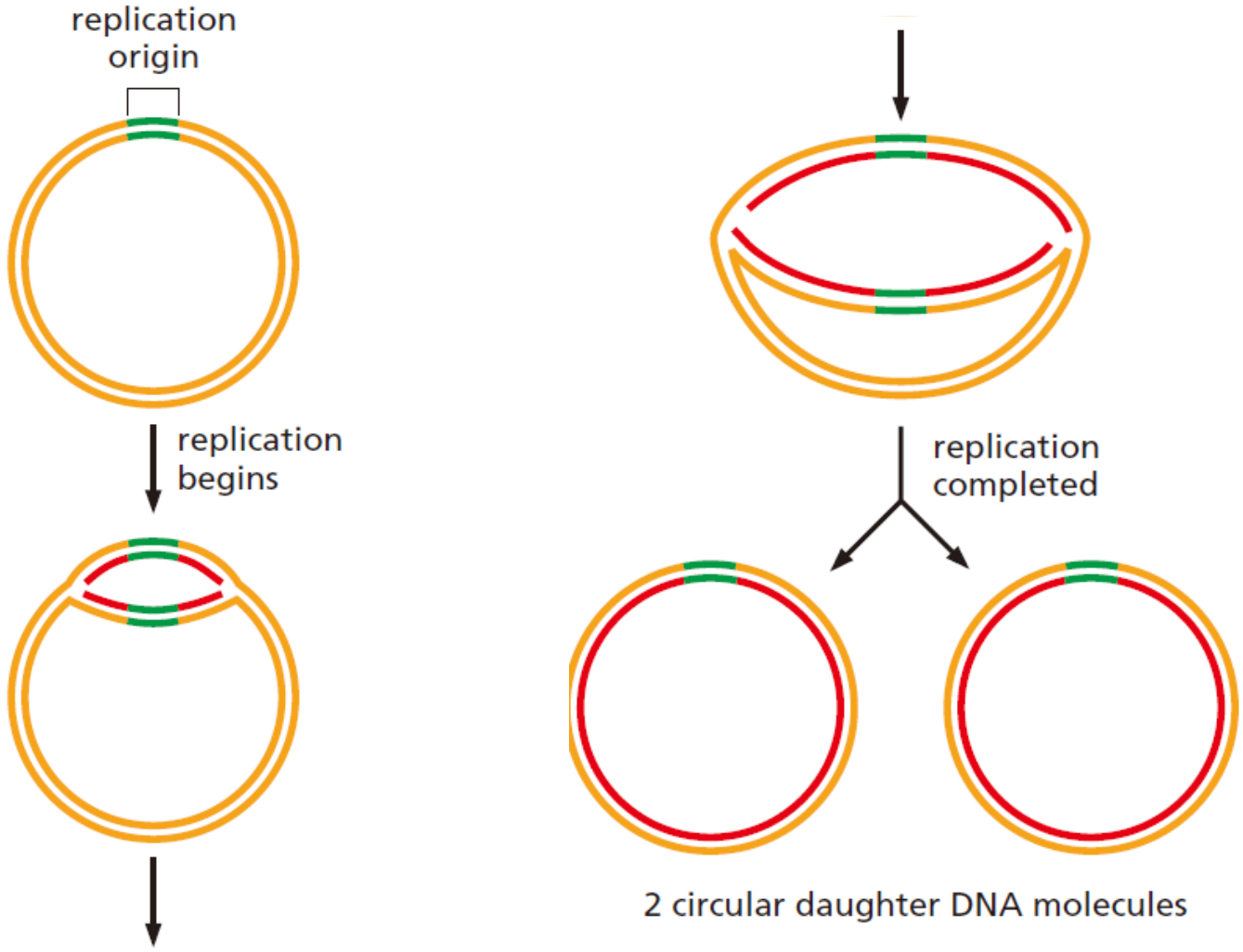
Replication origin: The position at which the DNA helix is first open

Contains short sequences that attract initiator proteins and stretches of DNA that are especially easy to open

Typically regions of DNA enriched in A-T base pairs

Part III

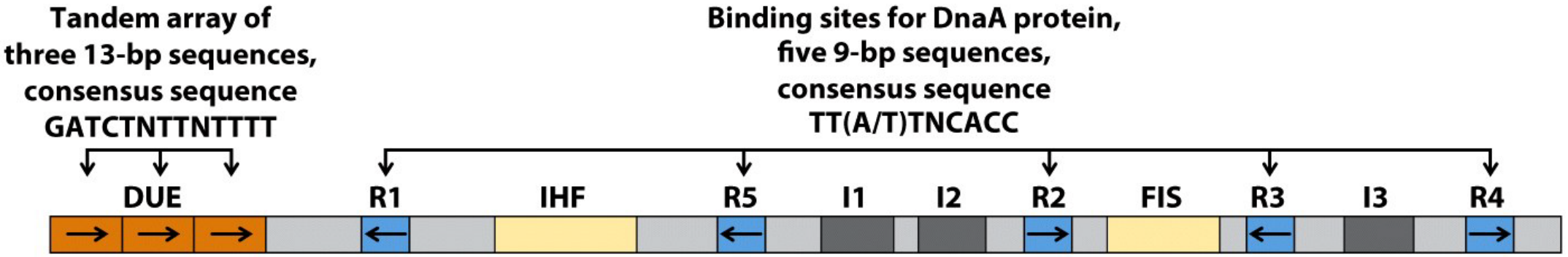
Bacterial Chromosomes Typically Have a Single Origin of DNA Replication



E. Coli **genome contains a single circular DNA molecule of 4.6×10^6 nucleotide pairs.**

Part III

Arrangement of Sequences in the E. Coli Replication Origin, *oriC*

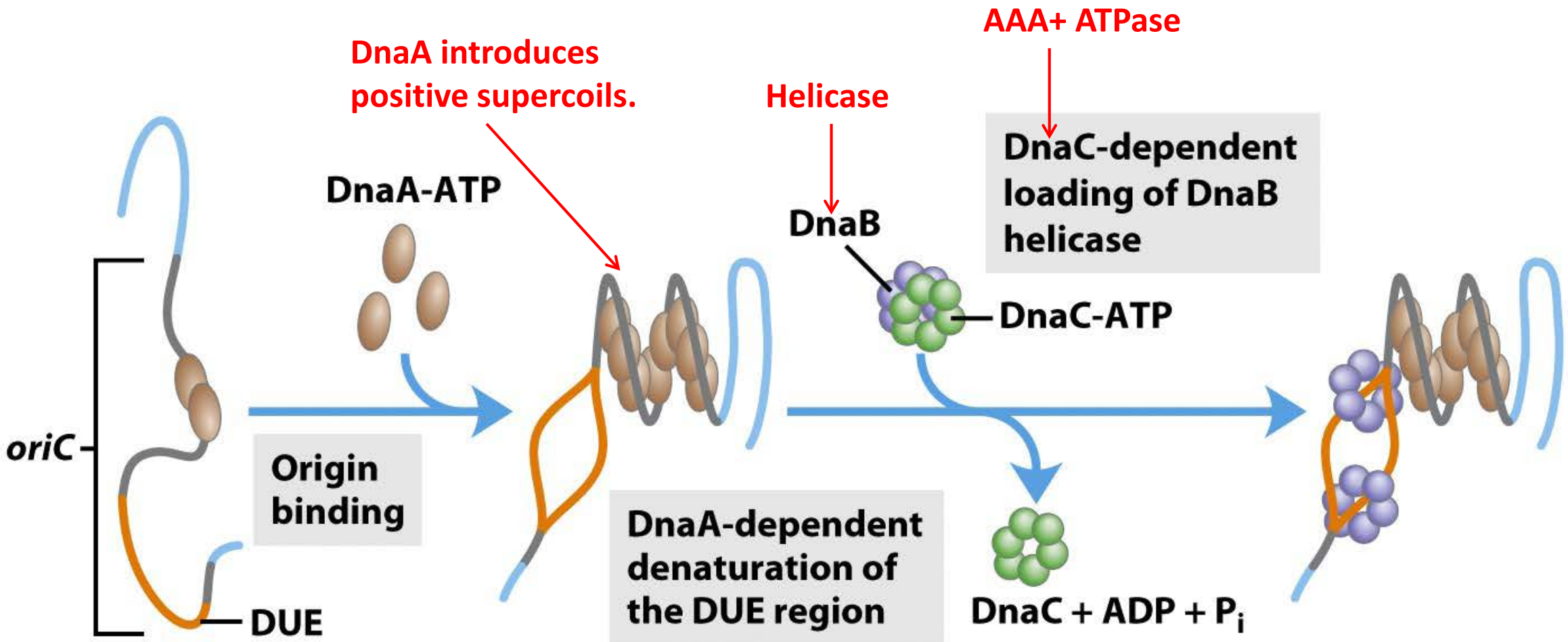


DUE: DNA Unwinding Element
R Sites: five repeats of 9 bp sequence, binding sites of DnaA
I Sites: Additional binding sites of DnaA
IHF: Integration host factor
FIS: Factor for inversion stimulation

DnaA: AAA+ ATPase, forms oligomers (8 molecules)
active in ATP-bound form and inactive in ADP-bound form
Has higher affinity in R sites than I sites
Binds R sites equally well in ATP- or ADP- bound form
Binds I sites with only ATP-bound form

Part III

Model for Initiation of Replication in *E. Coli* Origin



Loading of DnaB helicase is the key step in replication initiation.
All other proteins in the replication forks are linked directly or indirectly to DnaB.

Part III

Proteins Required to Initiate Replication

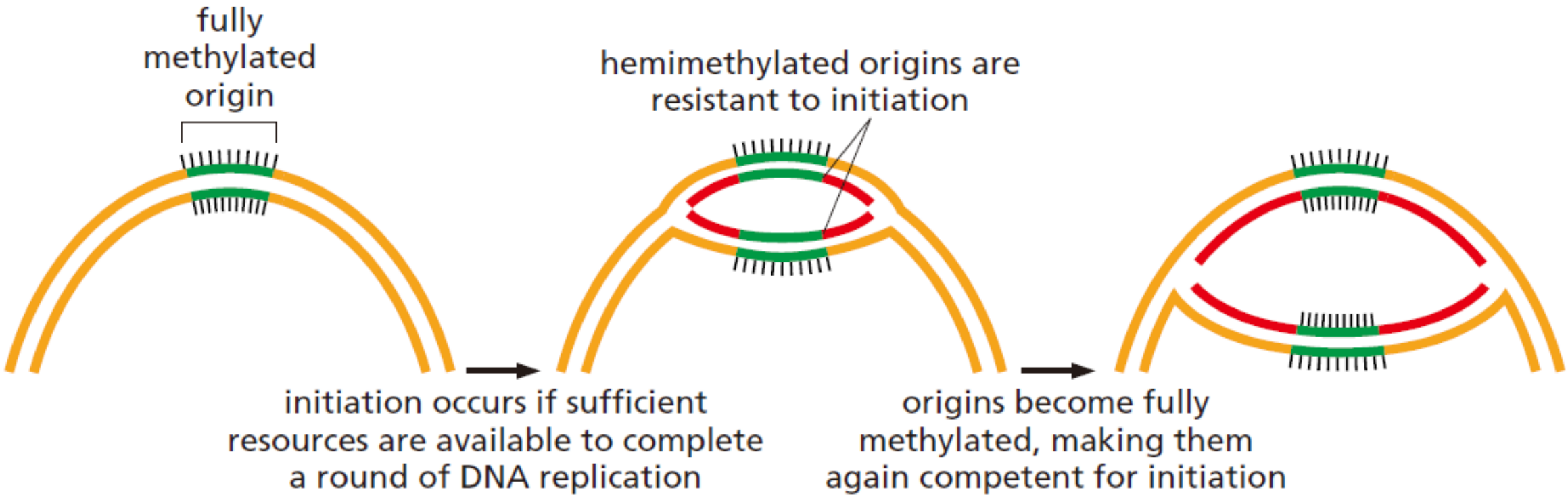
TABLE 25–3 Proteins Required to Initiate Replication at the *E. coli* Origin

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	174,000	6*	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
FIS	22,500	2*	DNA-binding protein; stimulates initiation
IHF	22,000	2	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
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.

Part III

Methylation of the *E. Coli* Replication Origin Creates a Refractory Period for DNA Initiation



Part III

Initiation Is the Only Phase of DNA Replication That Is Regulated

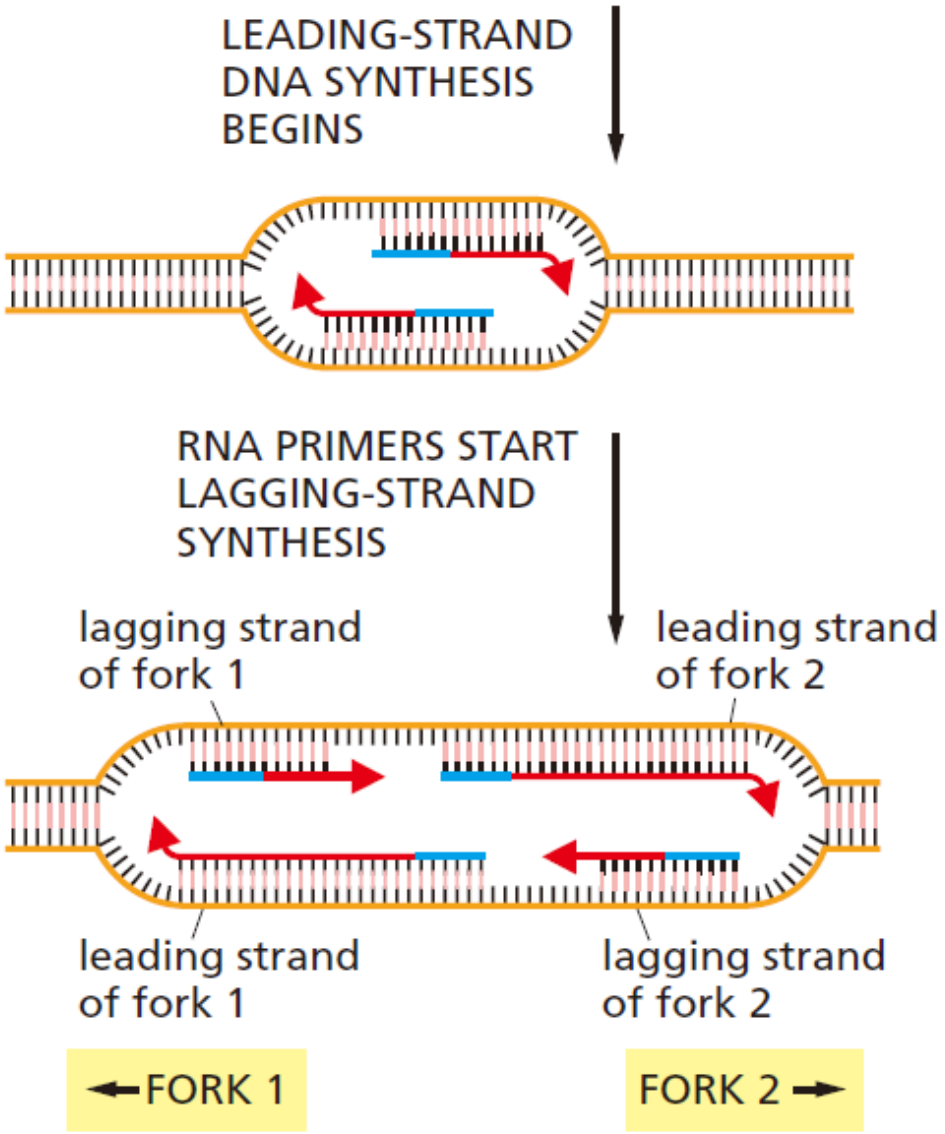
- Initiation occurs only when sufficient nutrients are available
  - Initiator proteins in the ATP-bound state
- Only one round of replication for each cell division
- Refractory period: after replication is initiated, the initiator protein is inactivated by hydrolysis of its bound ATP mole
- Refractory period is caused by a delay in the methylation of newly incorporated A nucleotides in the origin (GATC)
- Initiation cannot occur until the A's are methylated and the initiator protein is restored to ATP-bound state

Part III

Elongation

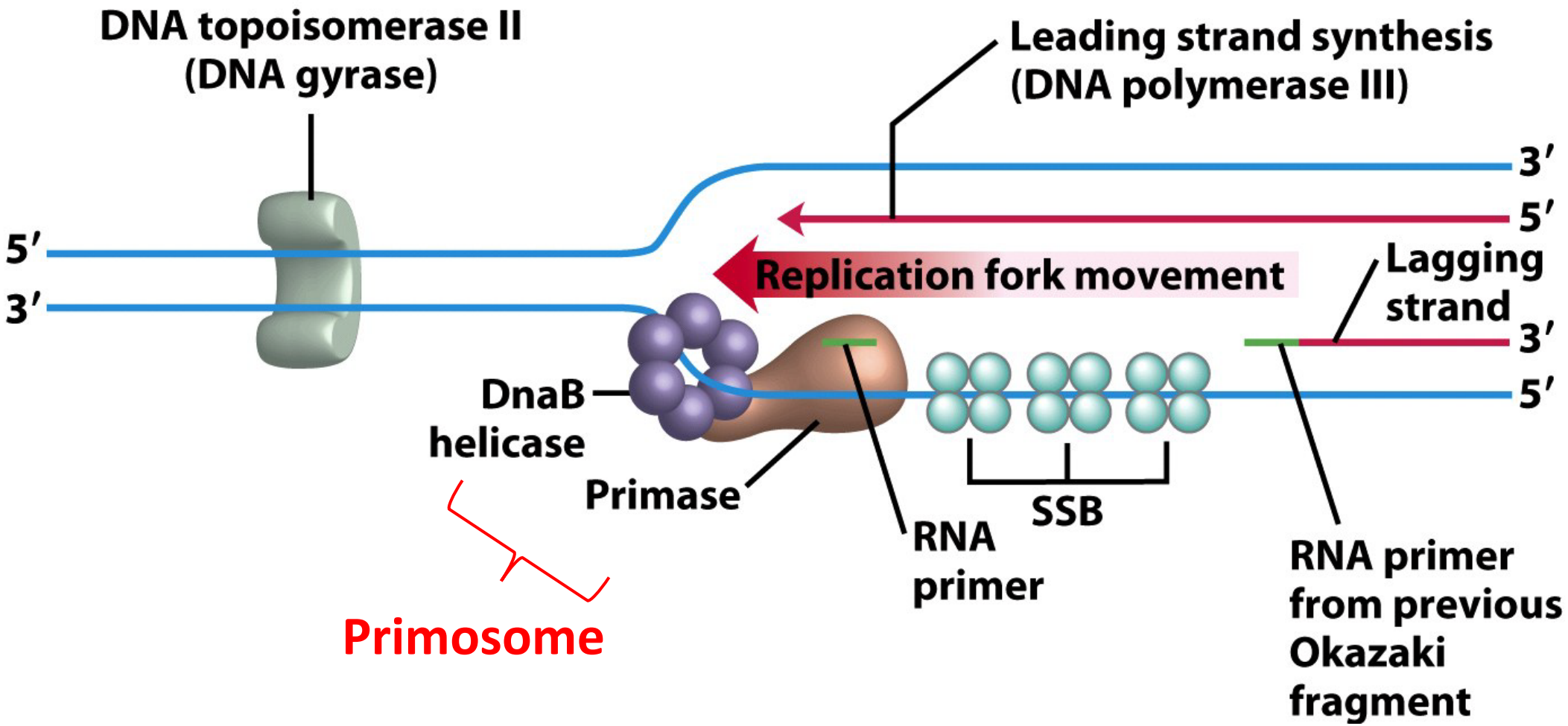
Leading strand synthesis
Lagging strand synthesis

Helicase: DnaB
Primase: DnaG
DNA Polymerase III



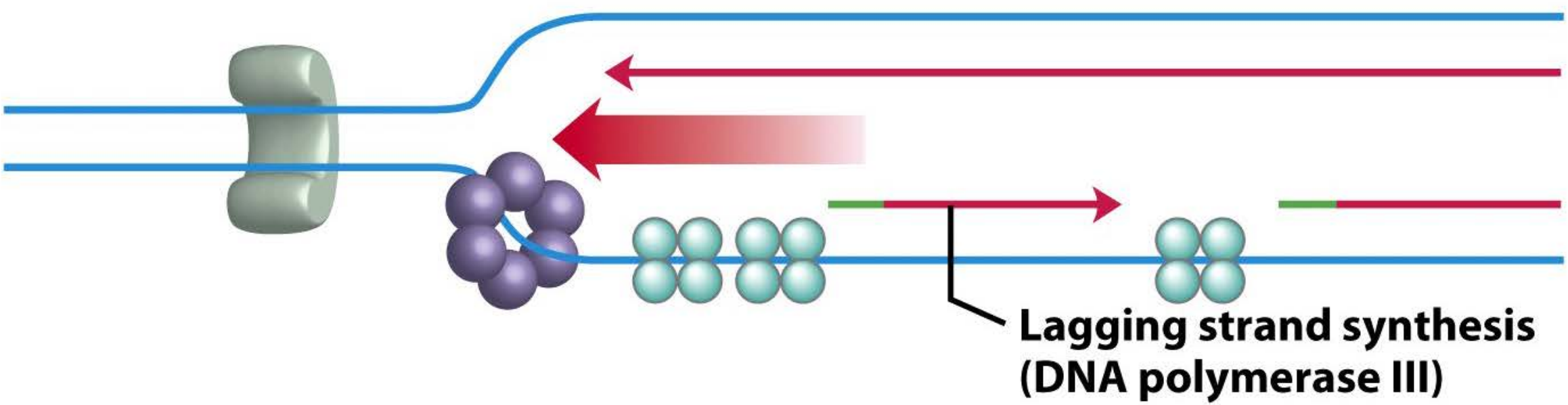
Part III

Lagging Strand Synthesis



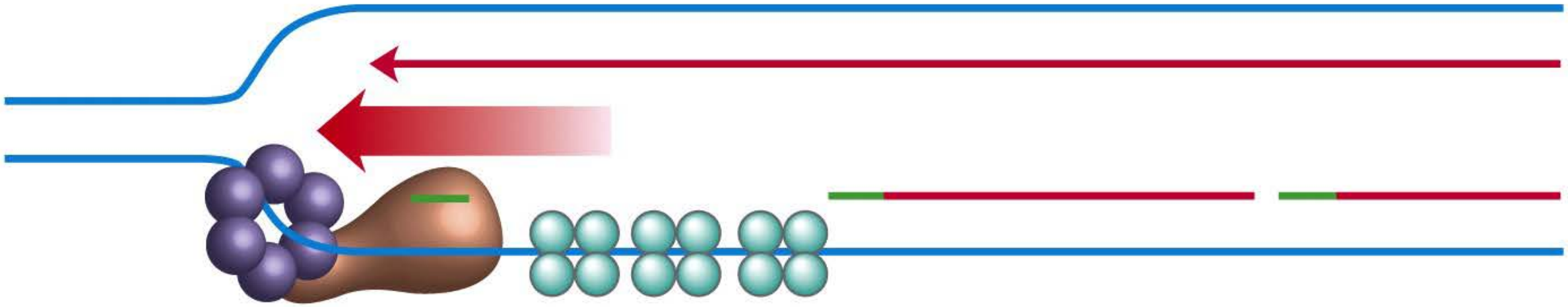
Part III

Lagging Strand Synthesis



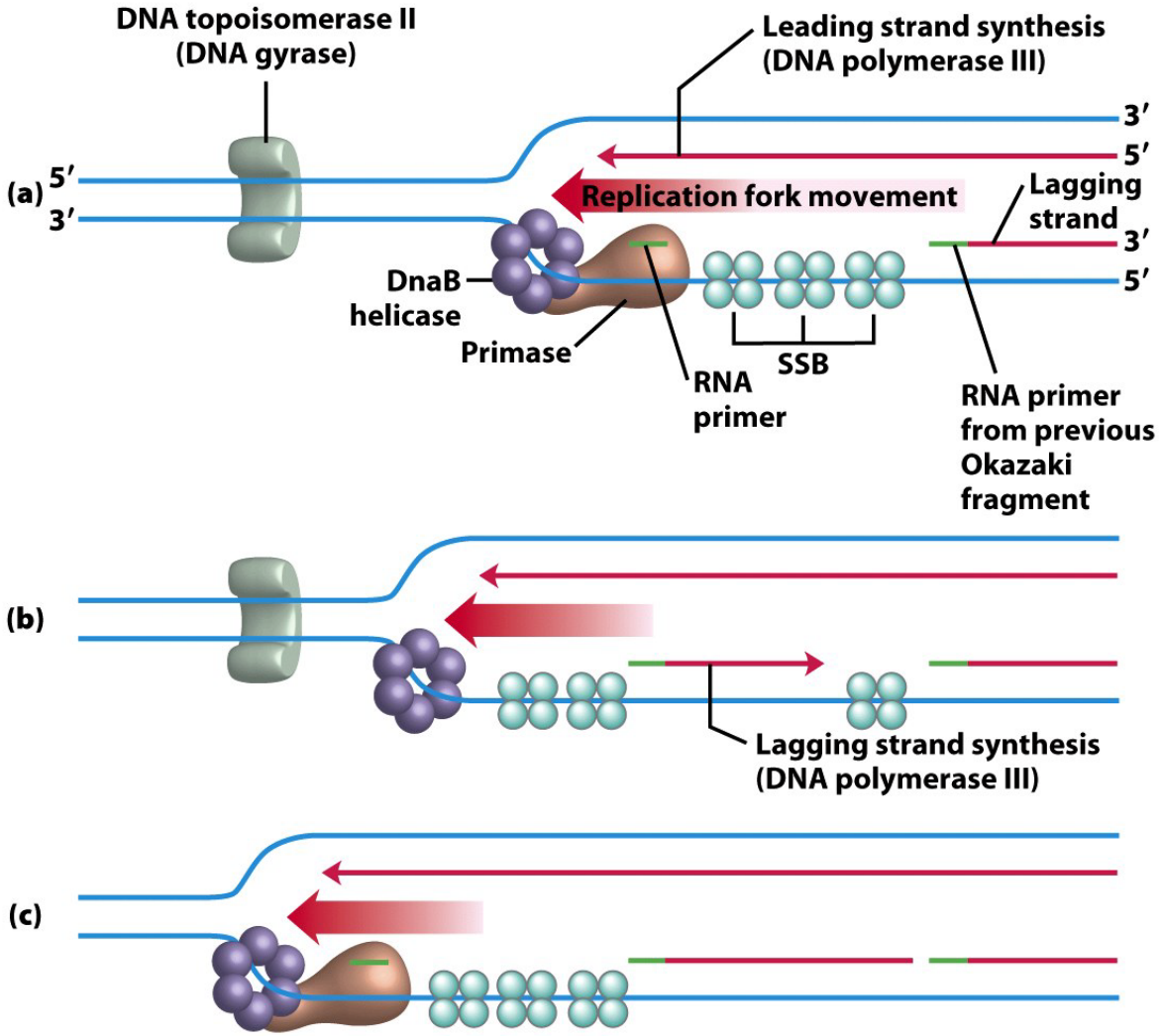
Part III

Lagging Strand Synthesis

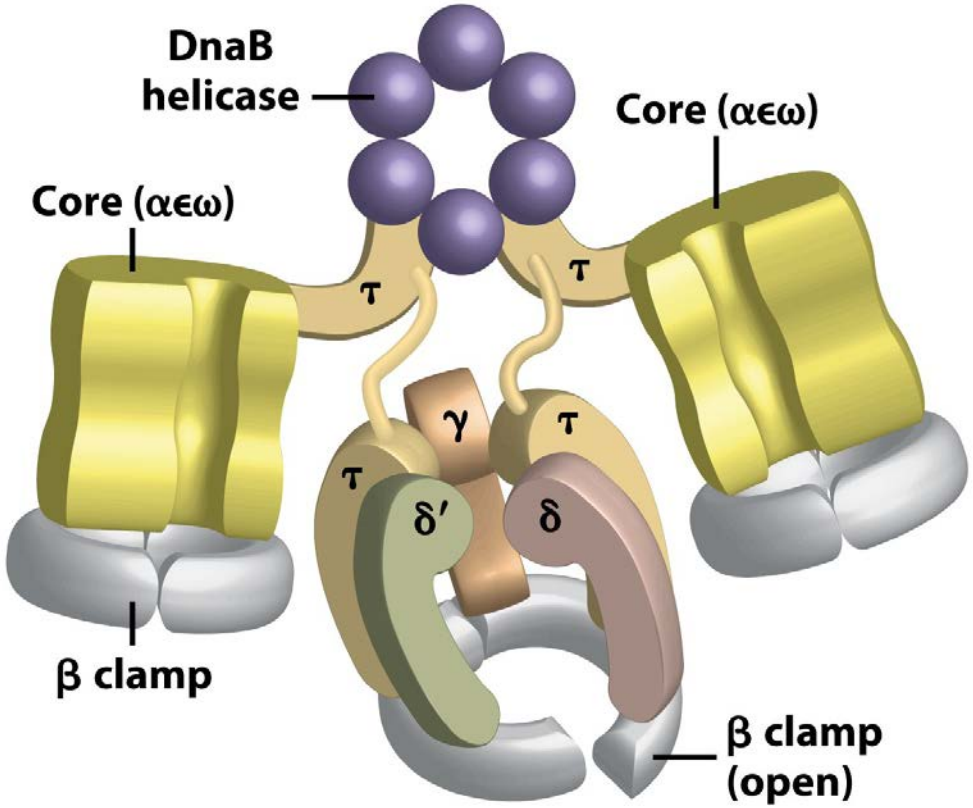


Part III

Lagging Strand Synthesis



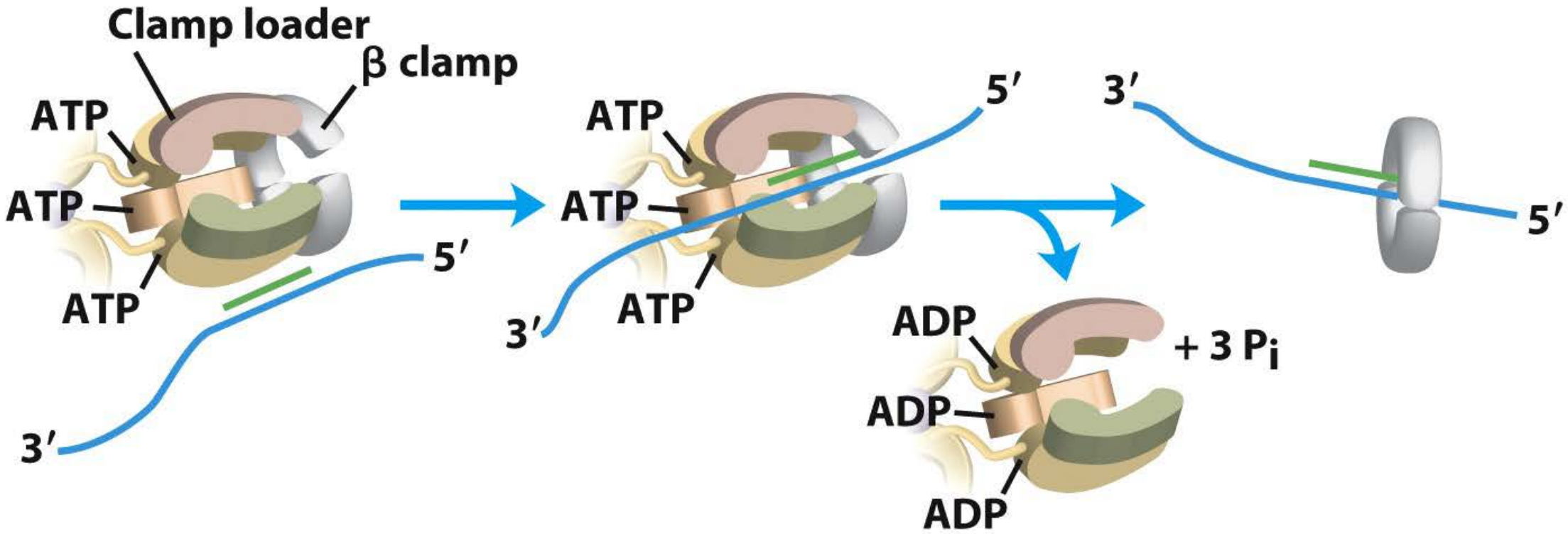
DNA Synthesis on the lagging Strand Is Quite Complex



Coordination of leading and lagging strand by a single symmetric DNA polymerase III.

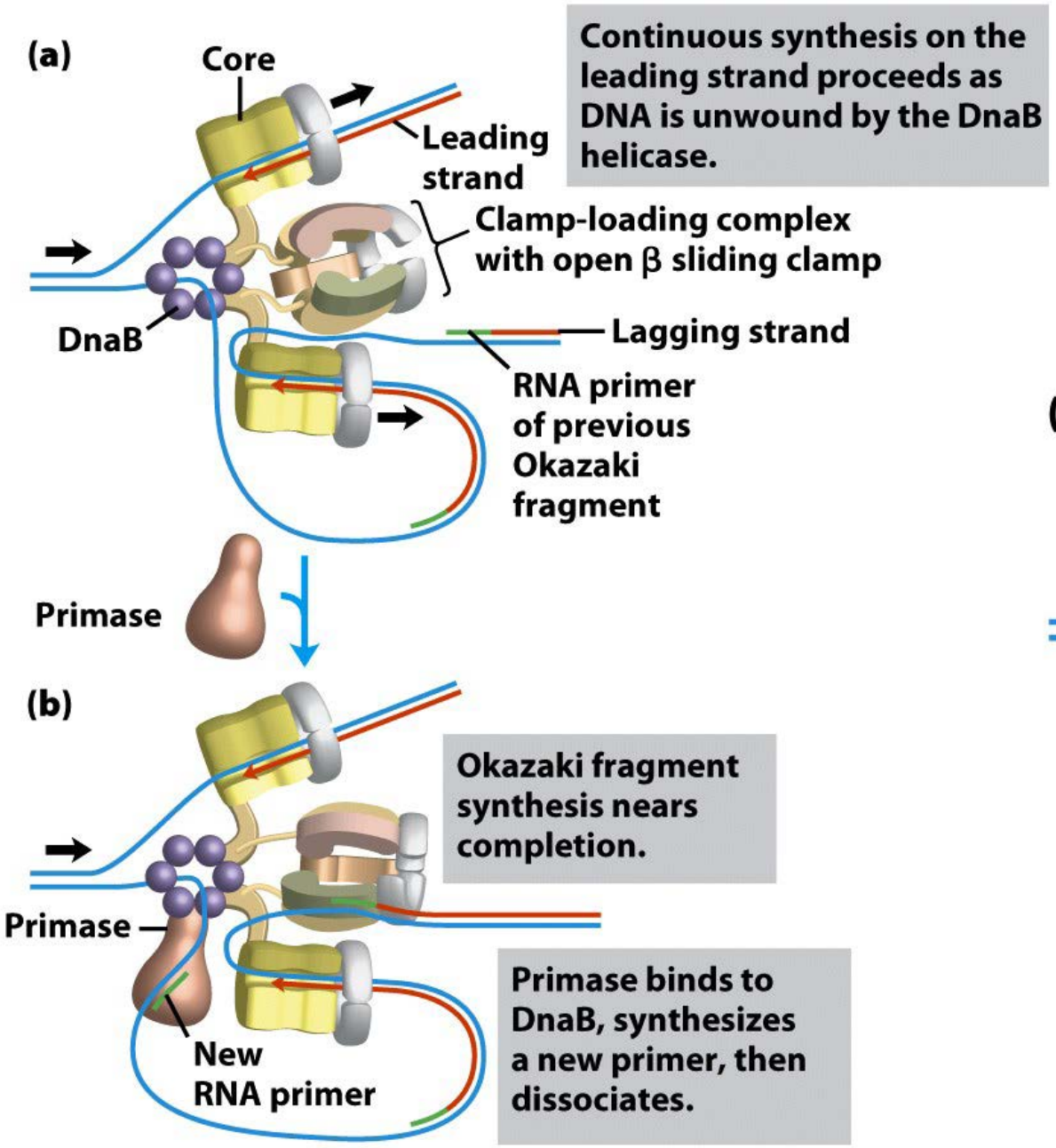
Part III

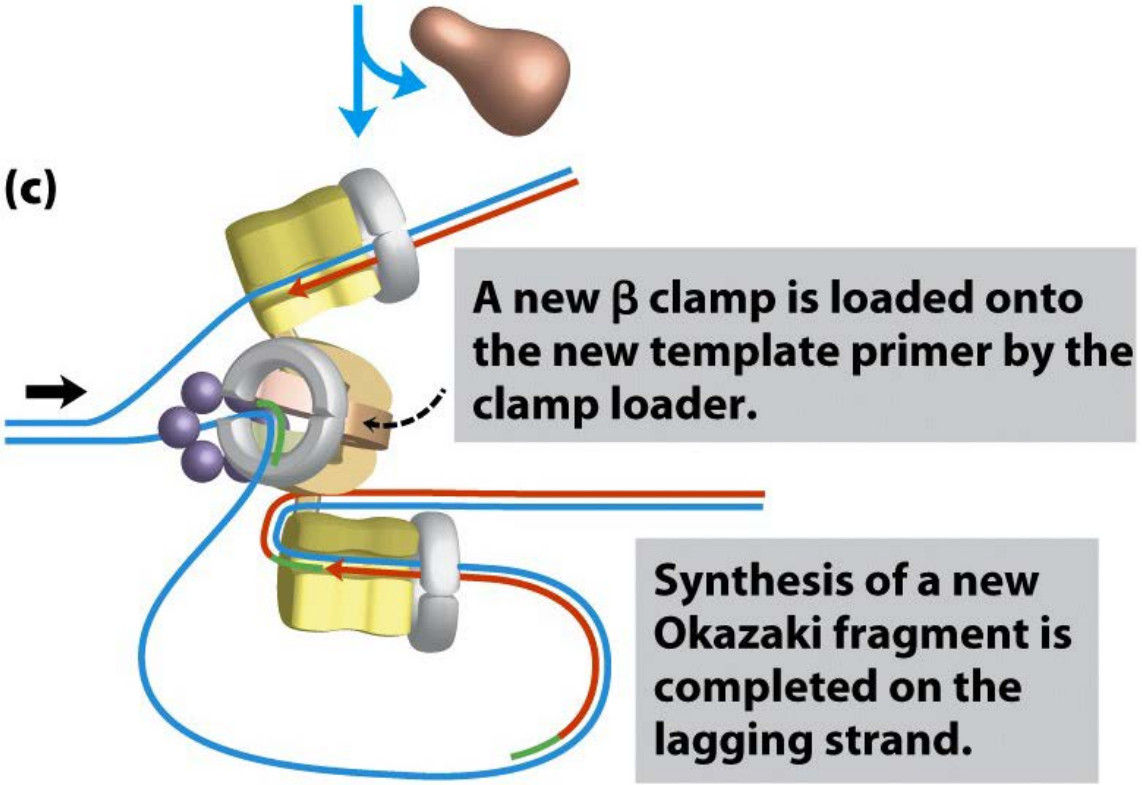
Sliding Clamp and Clamp Loader



Part III

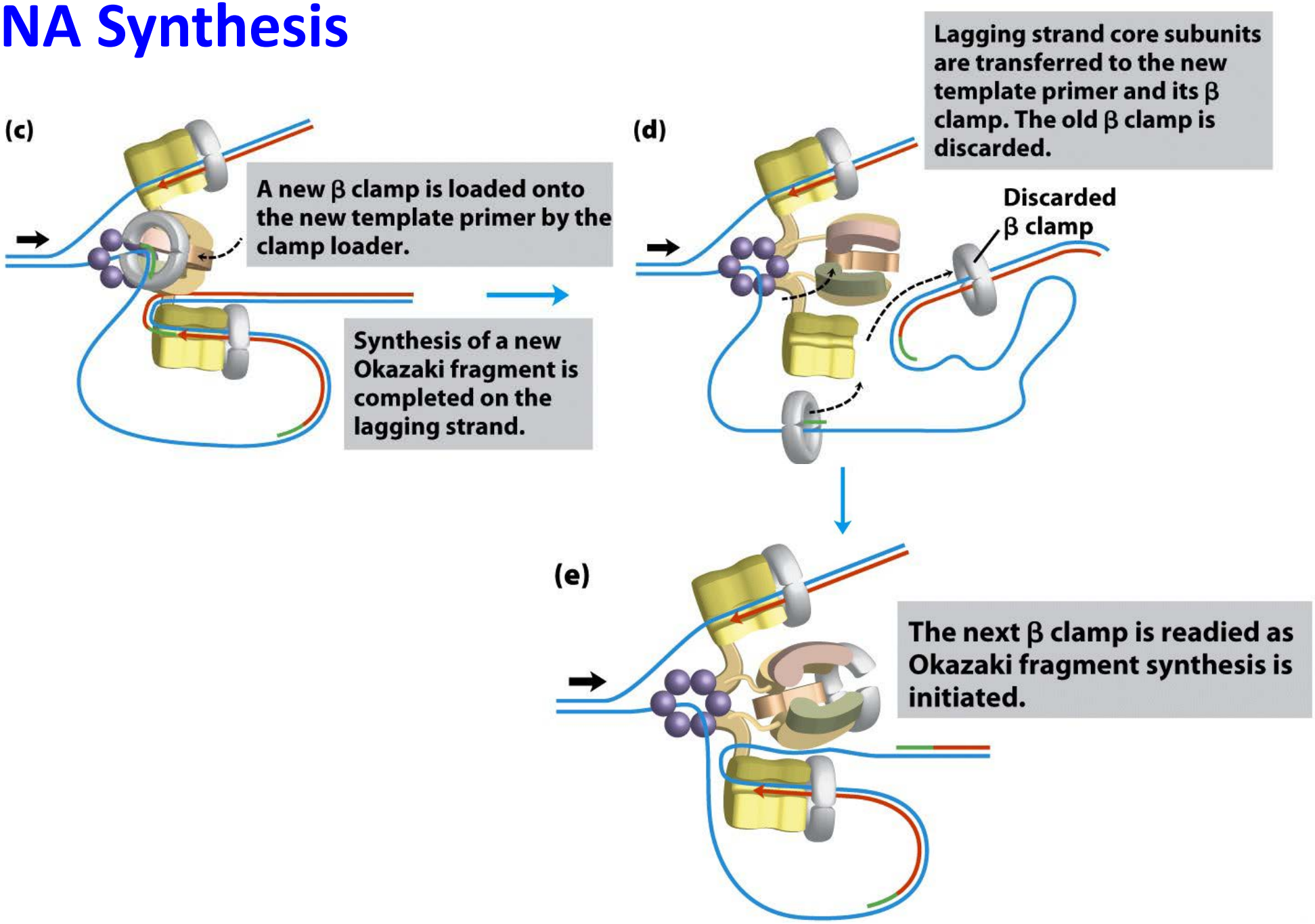
DNA Synthesis





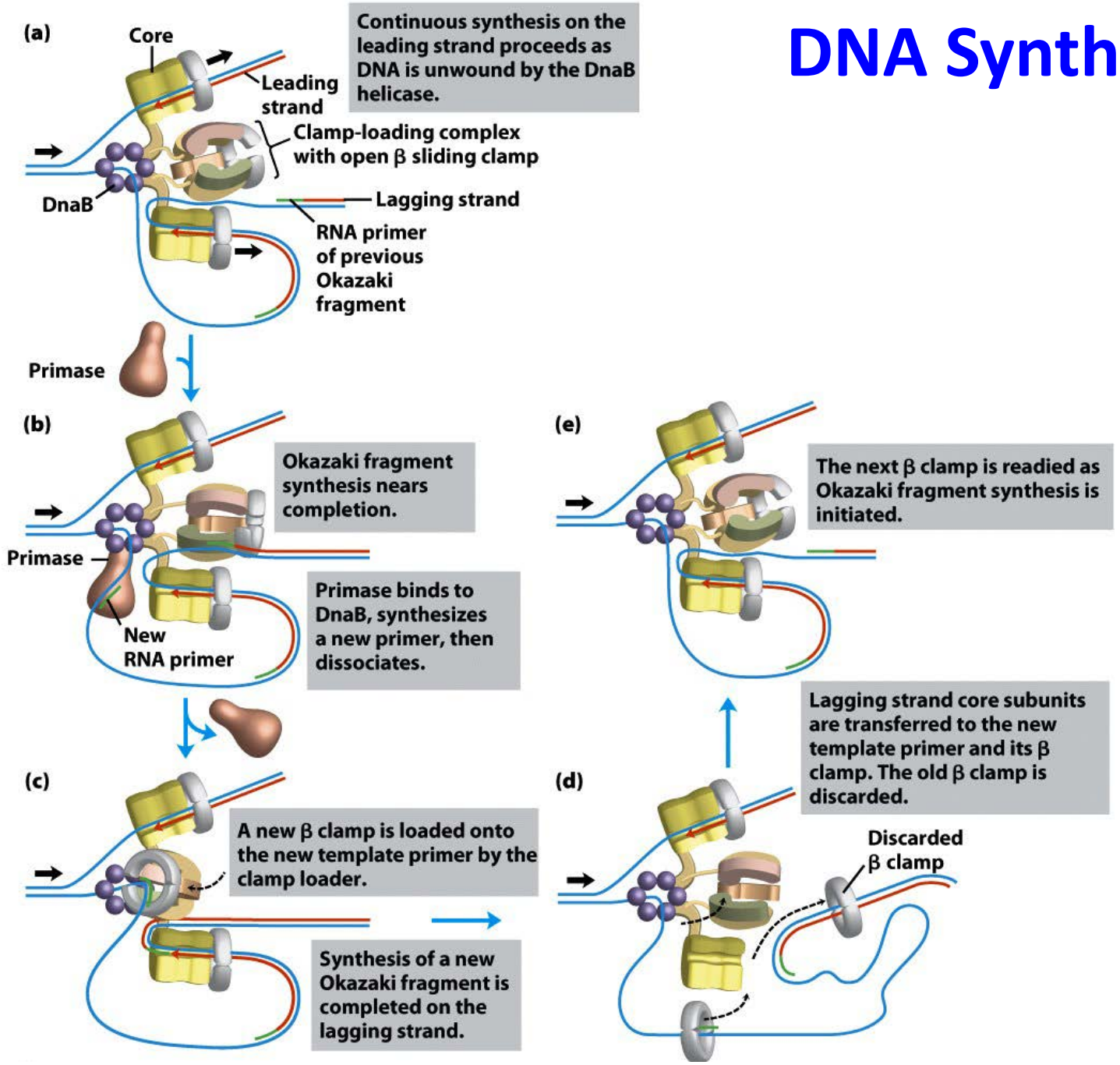
Part III

DNA Synthesis



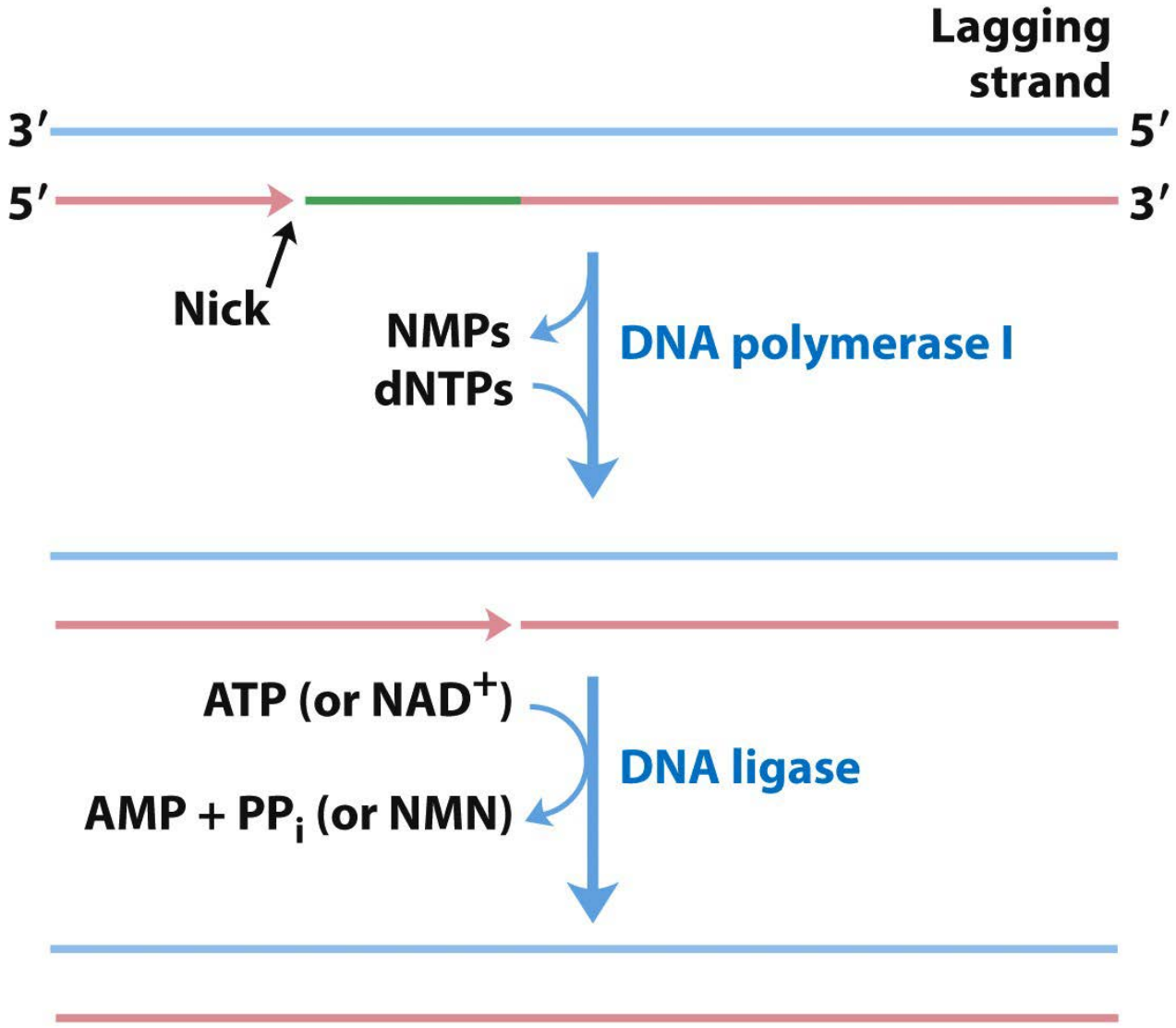
Part III

DNA Synthesis



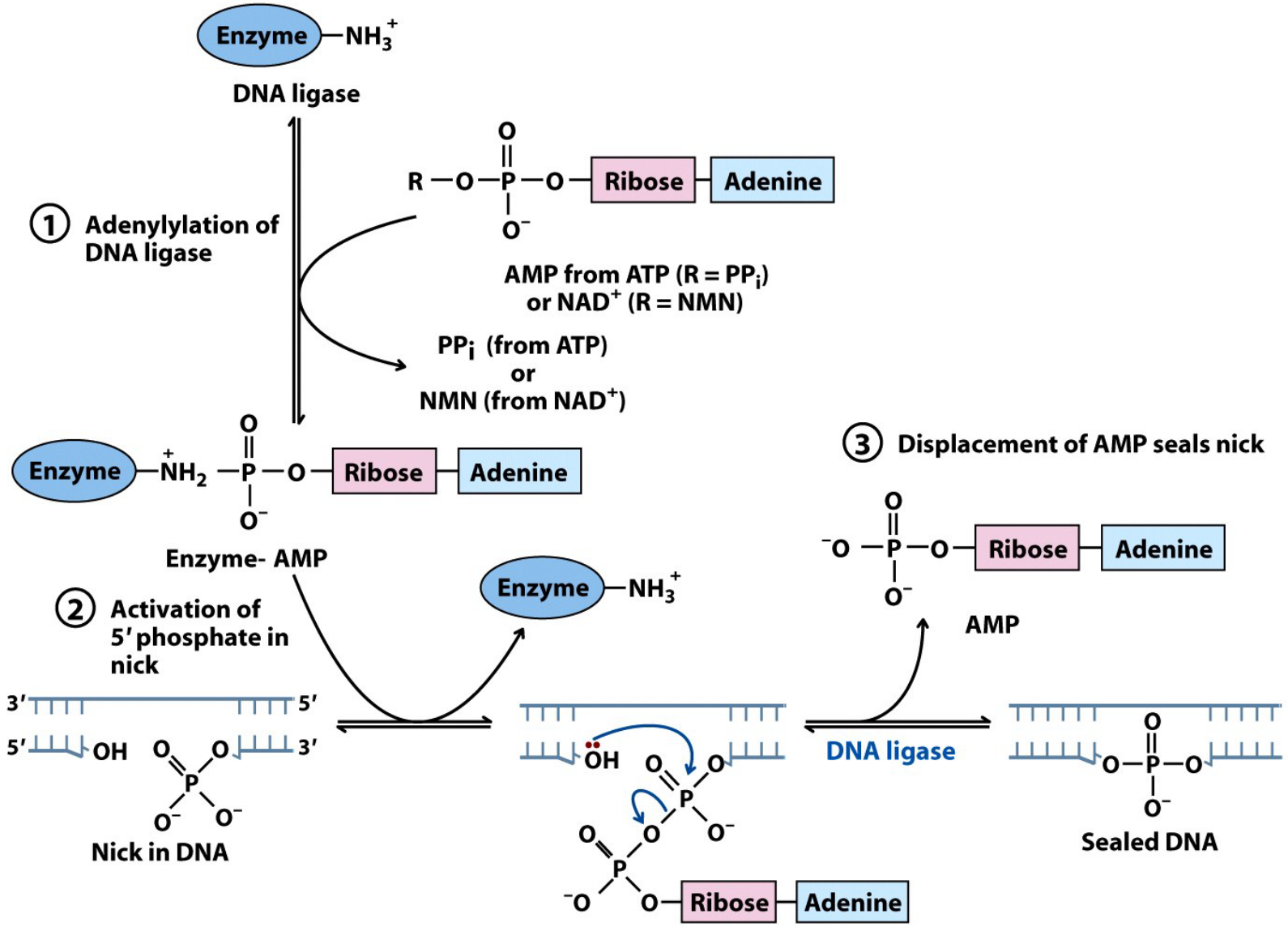
Part III

Final Steps in the Synthesis of Lagging Strand Segments



Part III

Mechanism of DNA Ligase Reaction



Part III

Replisome

TABLE 25–4 Proteins of the *E. coli* Replisome

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

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

Part III

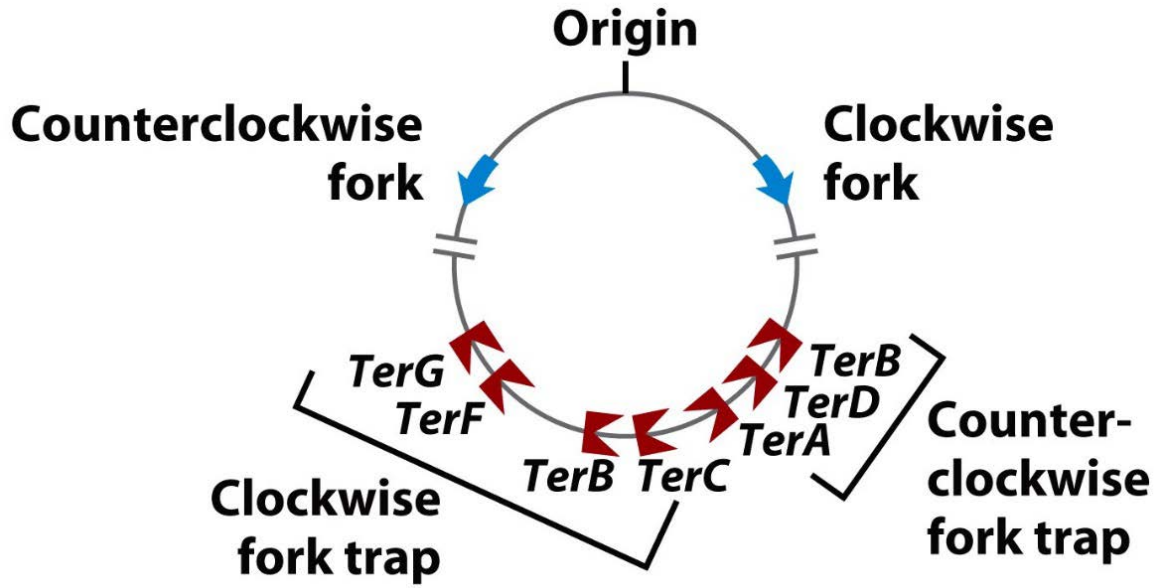
Termination

Eventually two replication forks meet at a terminal region containing multiple copies of a 20 bp sequence called Ter.

Ter sequences are arranged to create a trap that a replication fork can enter but cannot leave.

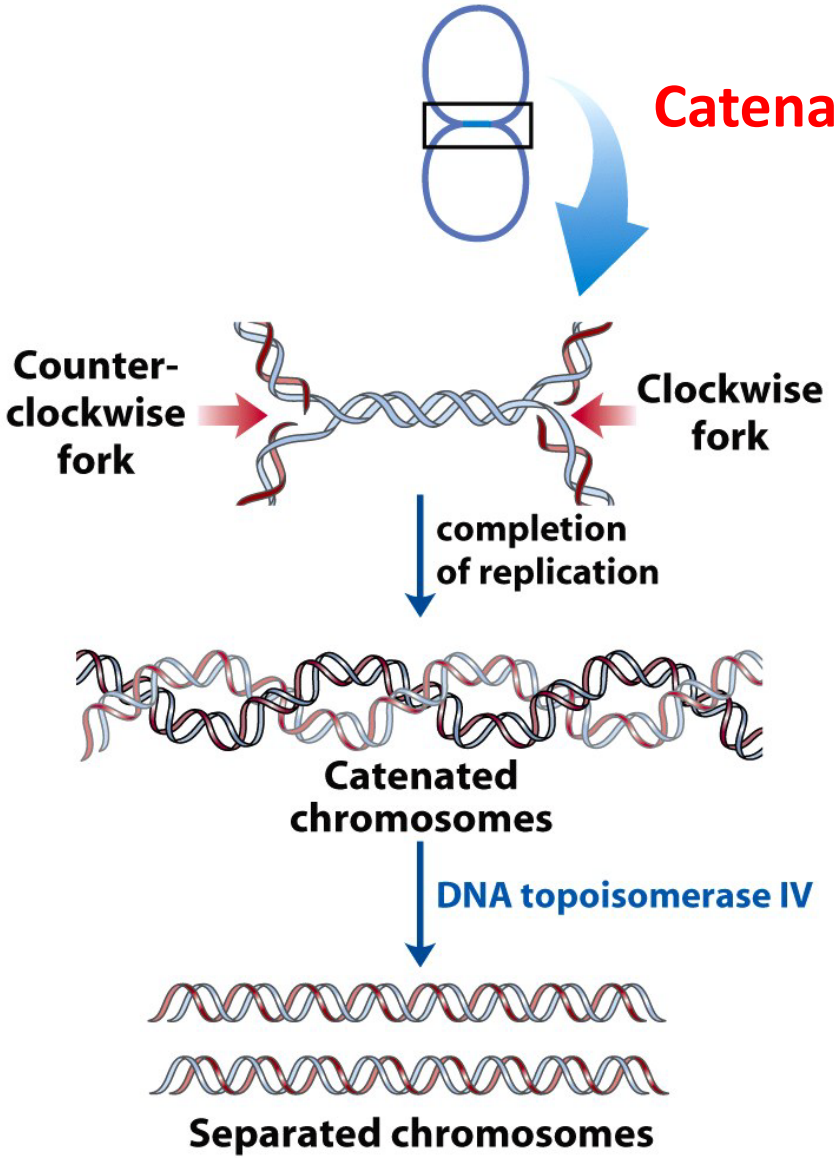
Ter sequences function as binding sites for Tus (terminus utilization substance).

Only one Tus-Ter complex functions per replication cycle (The complex first encountered by either replication fork).



Part III

Role of Topoisomerases in Replication Termination

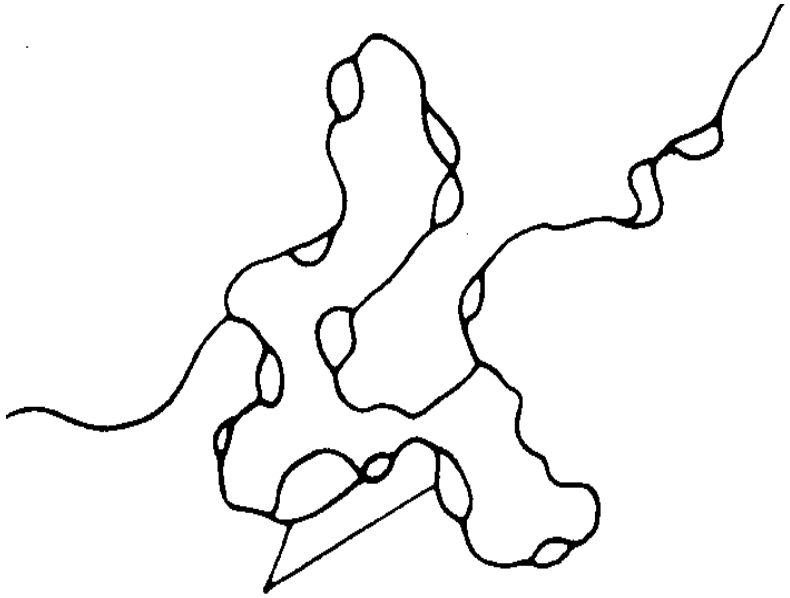


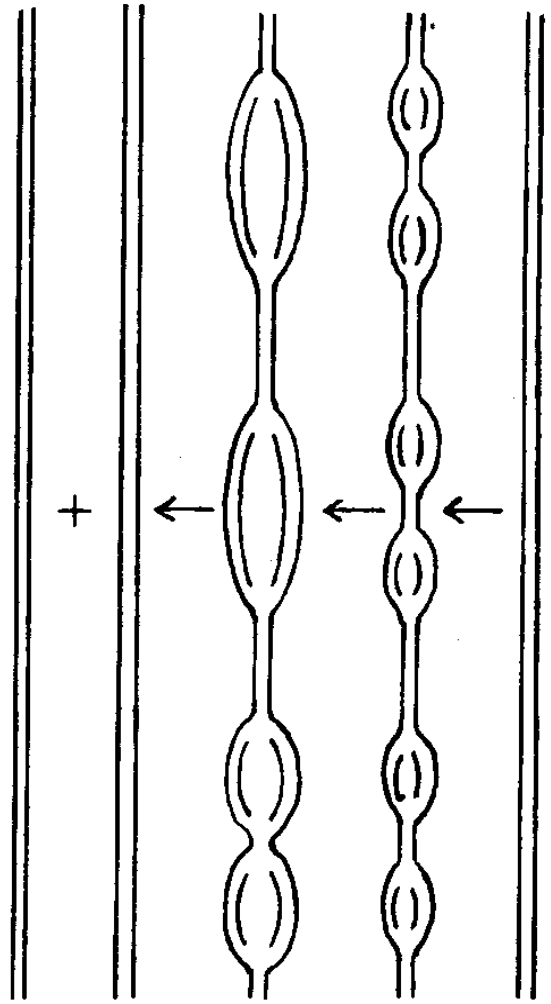
What about DNA Replication in Eukaryotes?

Both Similar and More Complex

Part III

Eukaryotic Chromosomes Contain Multiple Origins of Replication



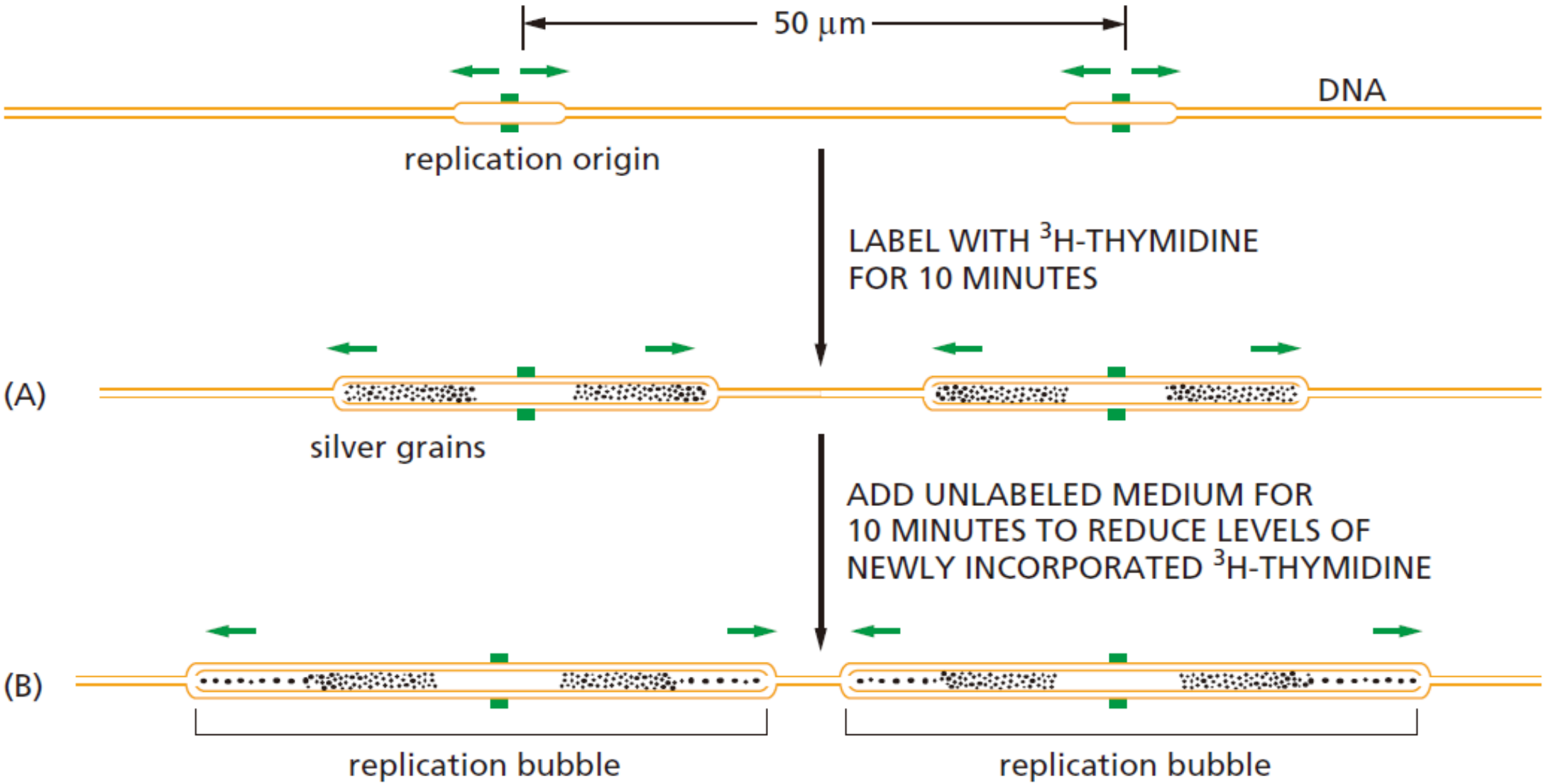


In yeast, defined replication origins are called autonomously replicating sequences (ARS), or replicators. They are less defined in higher eukaryotes.

About 400 replicators are distributed among the 16 chromosomes of the haploid yeast genome.

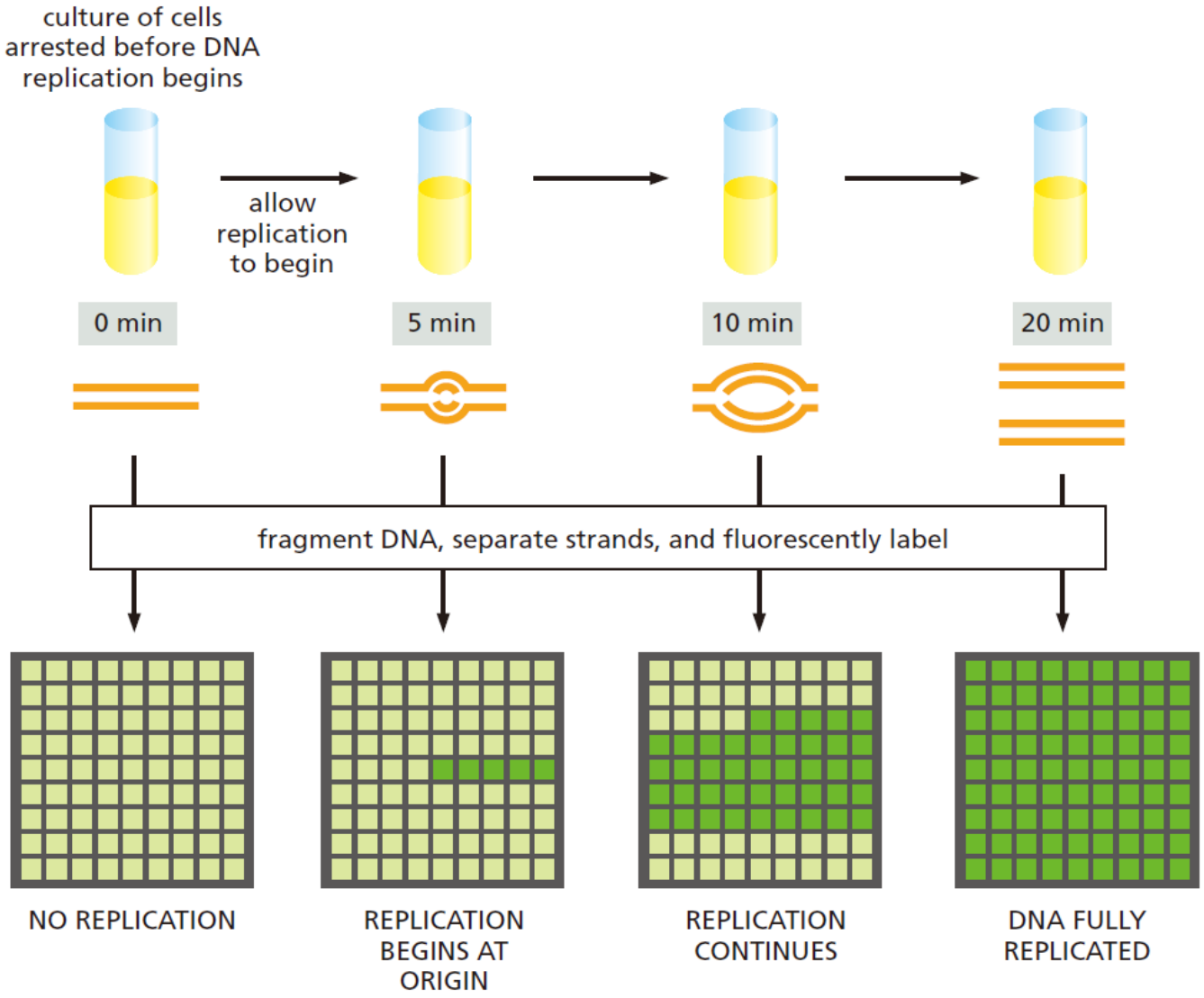
Part III

Replication Fork on Eukaryotic Chromosomes



Part III

Microarray to Monitor Formation and Progress of Replication Forks



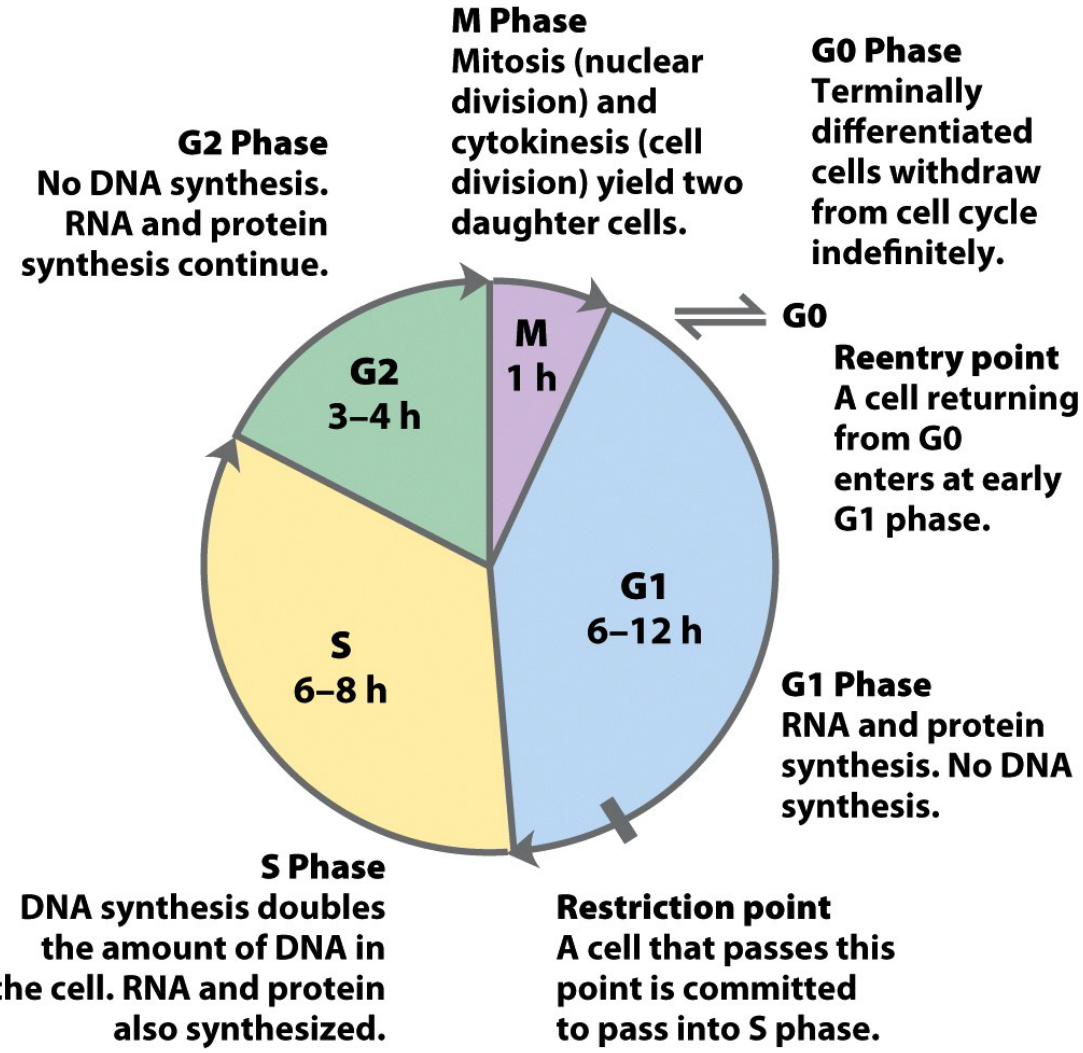
Part III

In Eukaryotes, DNA Replication Takes Place During Only One Part of the Cell Cycle

When growing rapidly, bacteria replicate their DNA nearly continuously.

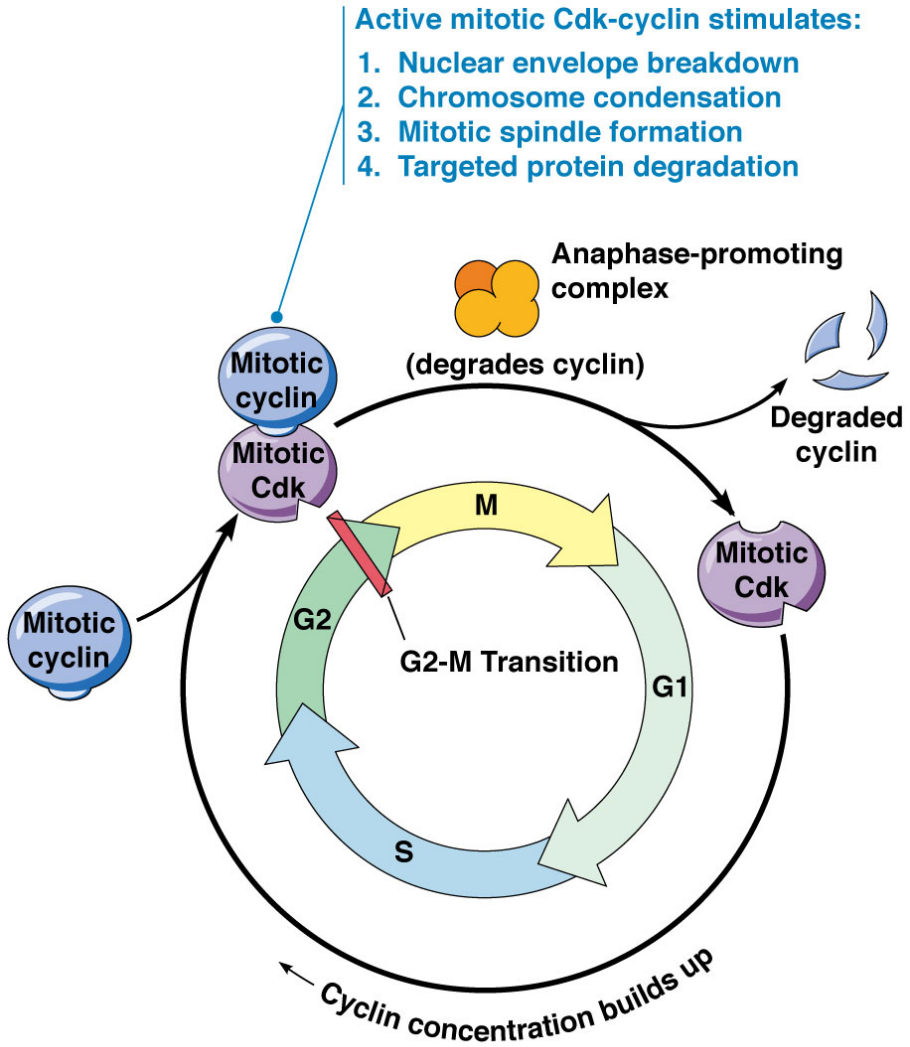
In contrast, DNA replication in eukaryotes occurs in DNA-synthesis phase, or S phase.

Different regions on the same chromosome replicate at distinct time in S phase.



Part III

Regulation of Eukaryotic Cell Cycles Involves Cyclins and Cyclin-Dependent Kinases



Cyclins are rapidly degraded by ubiquitin-dependent proteolysis at the end of M-phase, and the absence of cyclins allows the establishment of pre-replicative complexes (Pre-RCs).

Rapid growing cells: at the end of M phase

Slow-growing cells: at the end of G1

Licensing

Part III

Assembly of a Pre-replicative Complex at a Eukaryotic Replication Origin

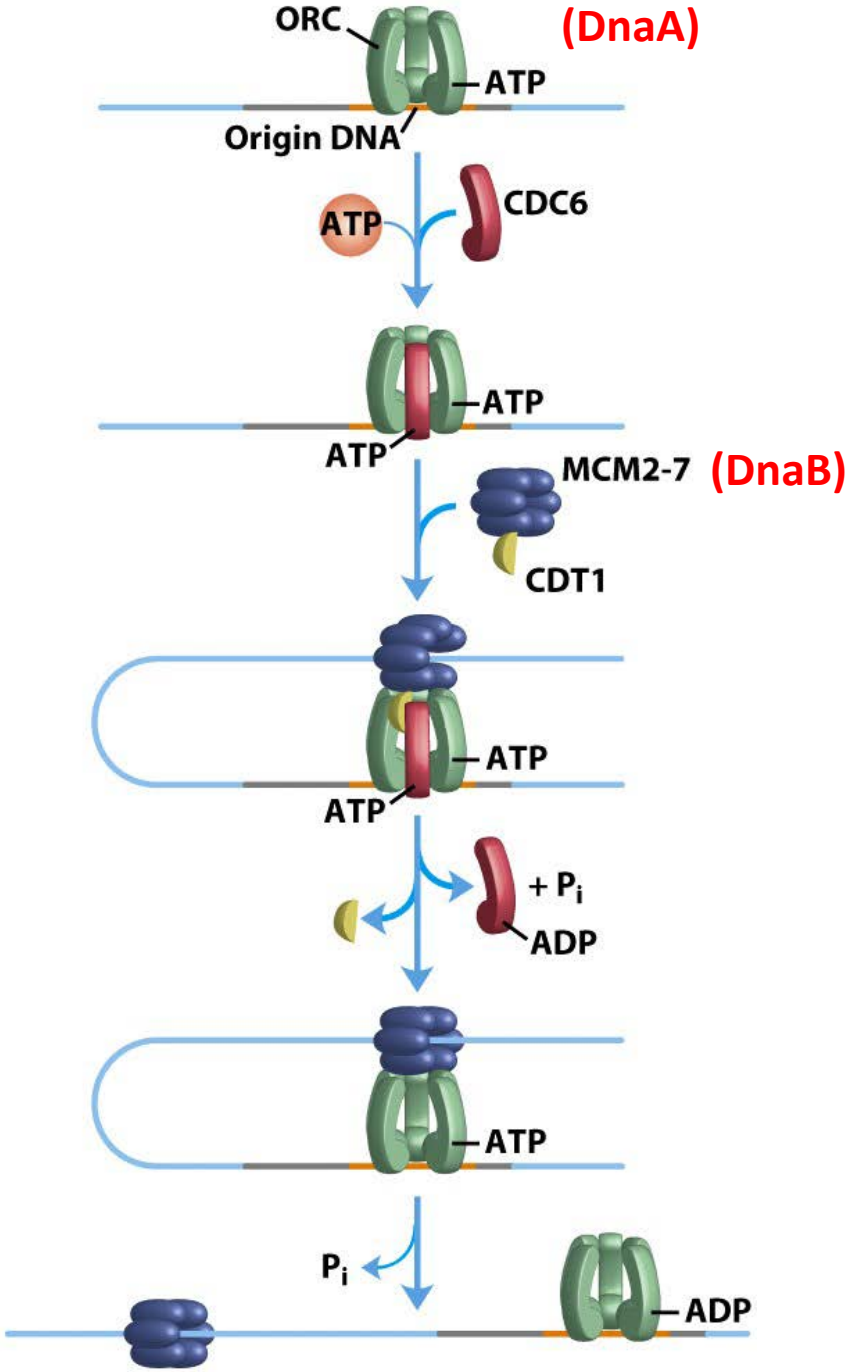
ORC: Origin Recognition Complex, AAA+ ATPase

CDC6: Cell division cycle 6, AAA+ ATPase

MCM: Minichromosome maintenance proteins
DNA Helicase, hexameric protein

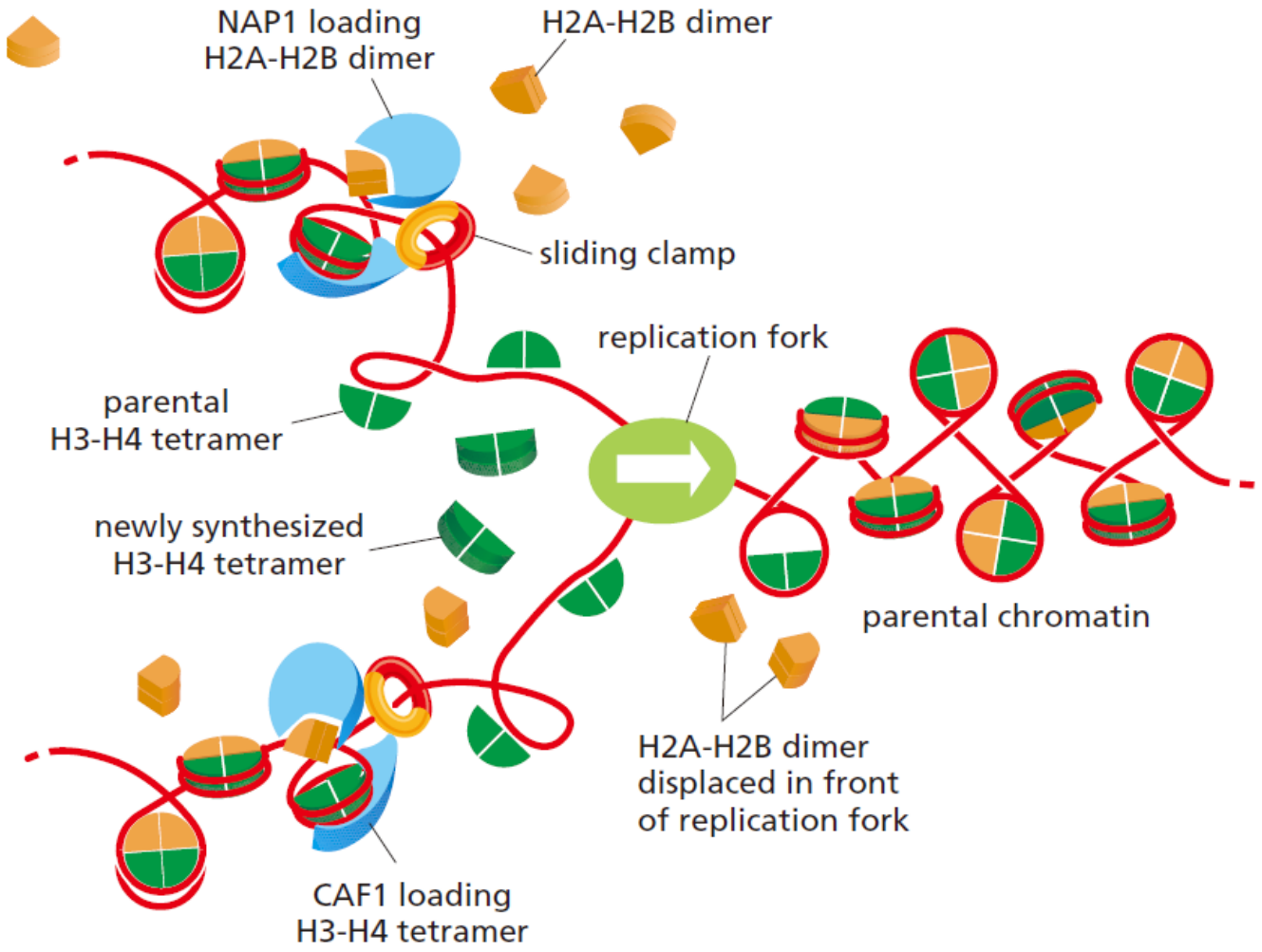
CDT1: CDC10-dependent transcript 1

Phosphorylation plays an essential role in initiation of DNA replication.



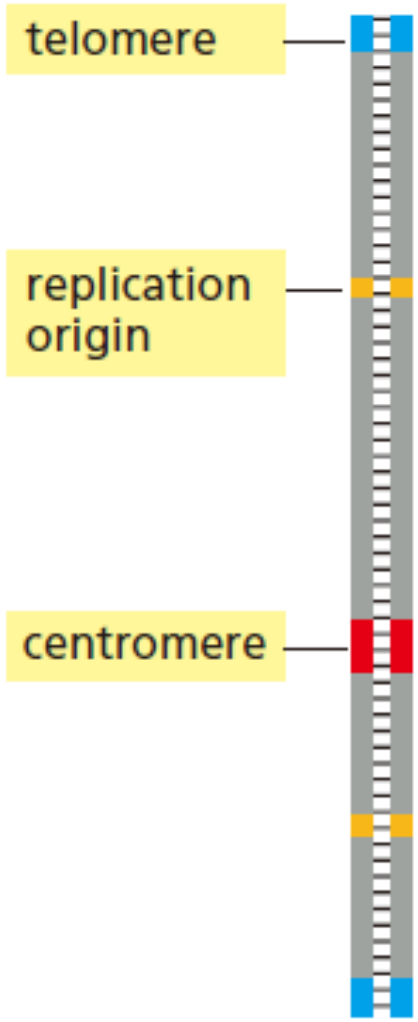
Part III

New Nucleosomes Are Assembled Behind the Replication Fork



Part III

Telomerase Replicates the Ends of Chromosomes



Bacteria: Circular chromosome
Eukaryote: Linear chromosome

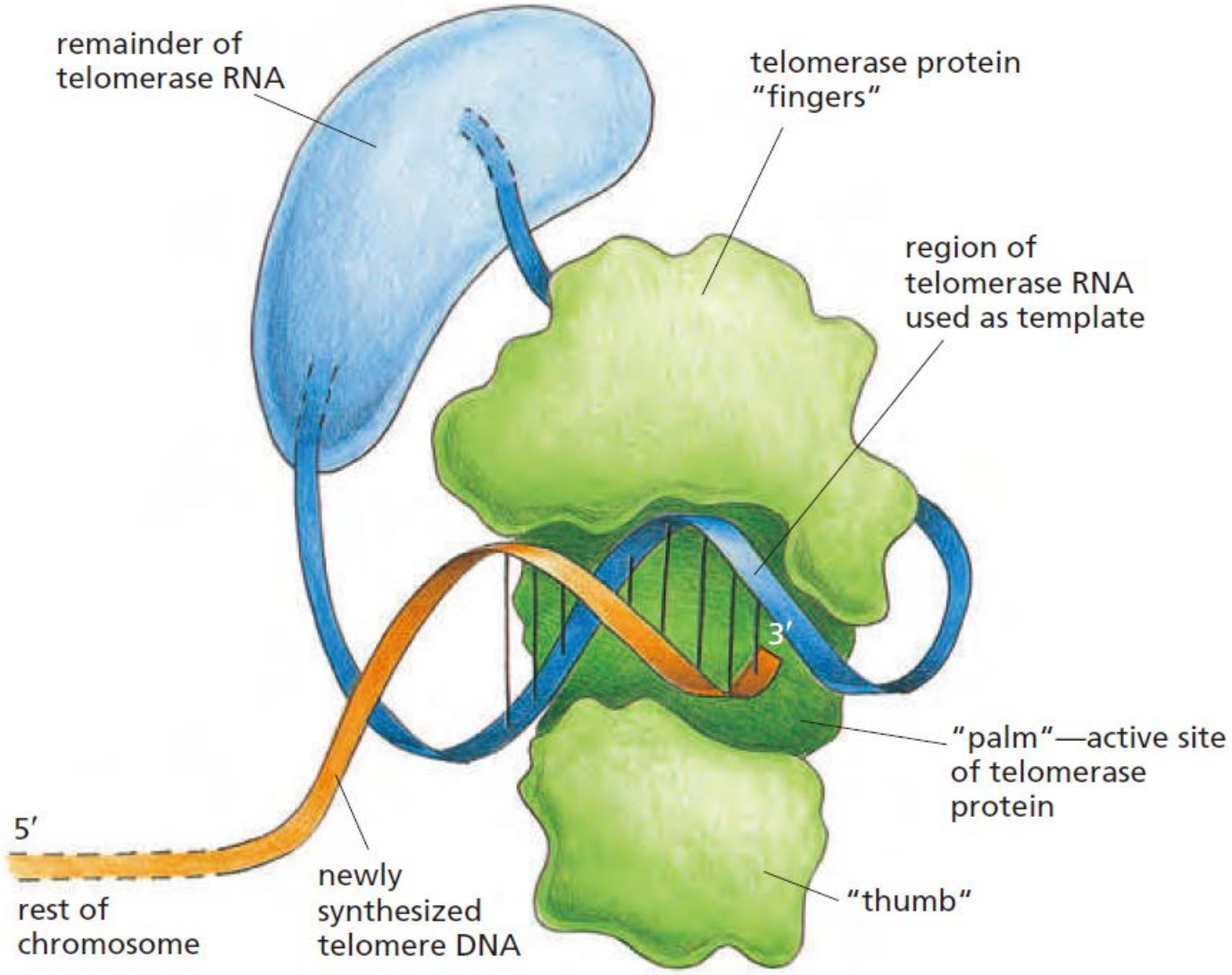
End-Replication Problem:
The final RNA primer synthesized on the lagging strand template cannot be replaced by DNA.

Telomeres:
Telomeres contain many tandem repeats of a short sequence.
TxGy repeats, repeated roughly about a thousand times at each telomere.

Telomerase:
Enzyme to replenish these sequences each time a cell divides.
Telomerase is a large protein-RNA complex.

Part III

Telomerase Is a Specialized Reverse Transcriptase



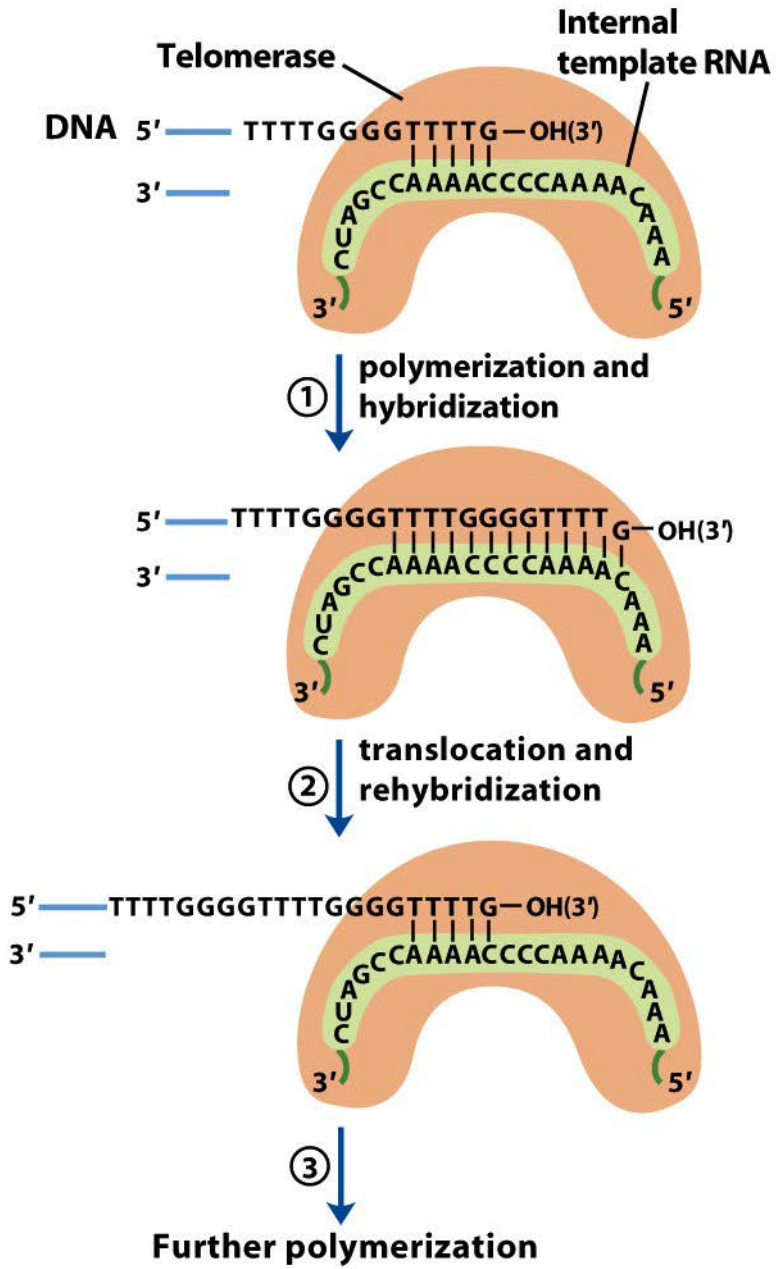
Consists of RNA and Protein components

RNA is about 150 nt long, about 1.5 copies of the appropriate CyAx repeat.

Serves as template for DNA synthesis.

Part III

Telomere Replication

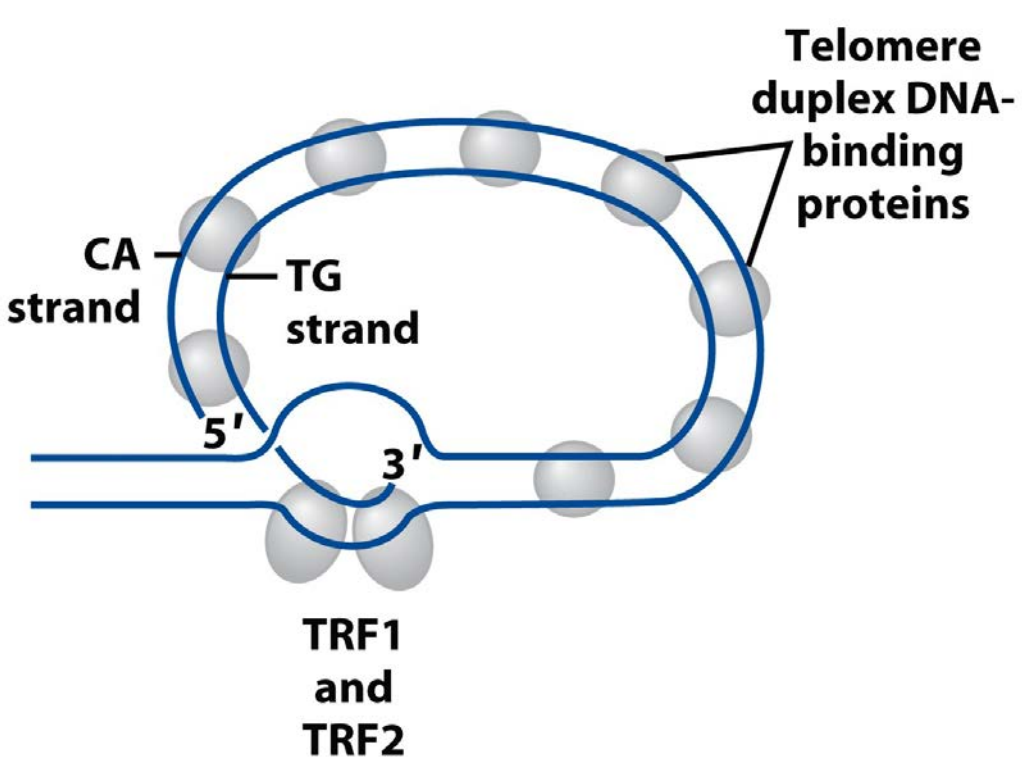


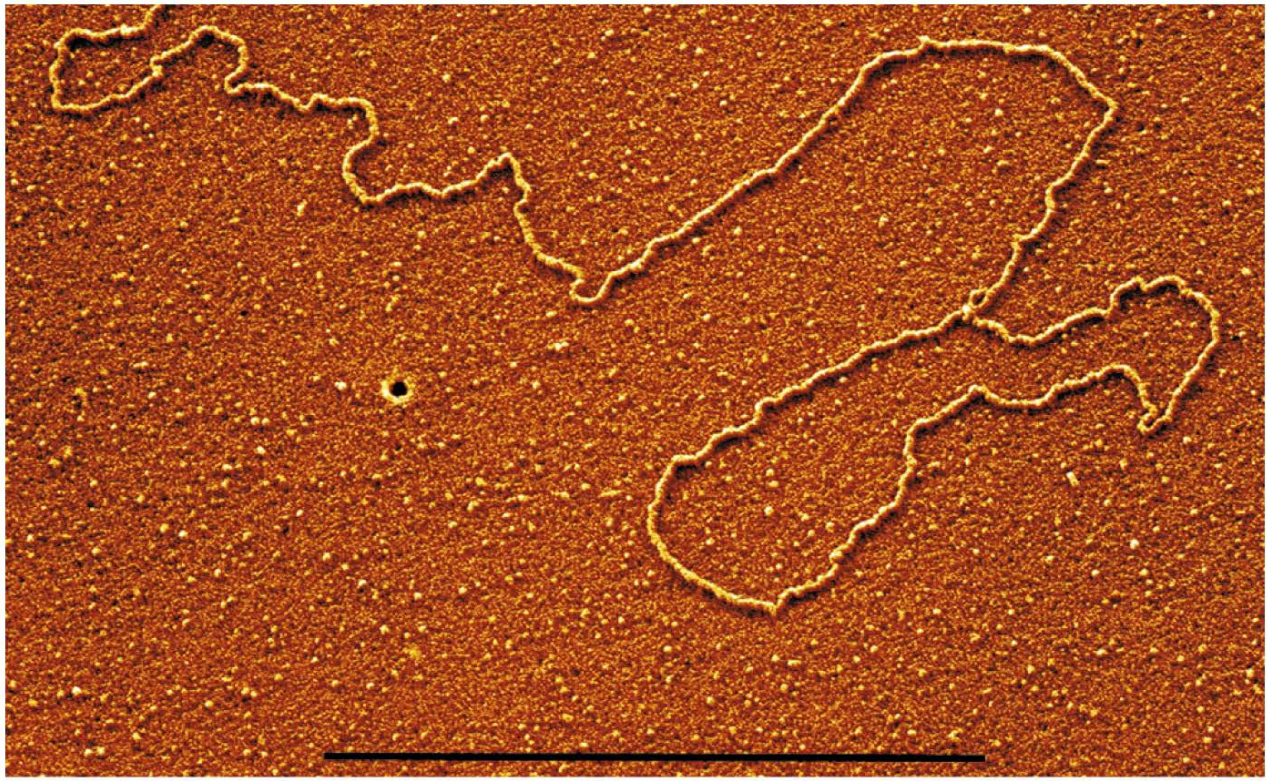
What happens with the protruding 3’-end?

by DNA polymerase

Part III

A T-loop at the End of a mammalian Chromosome

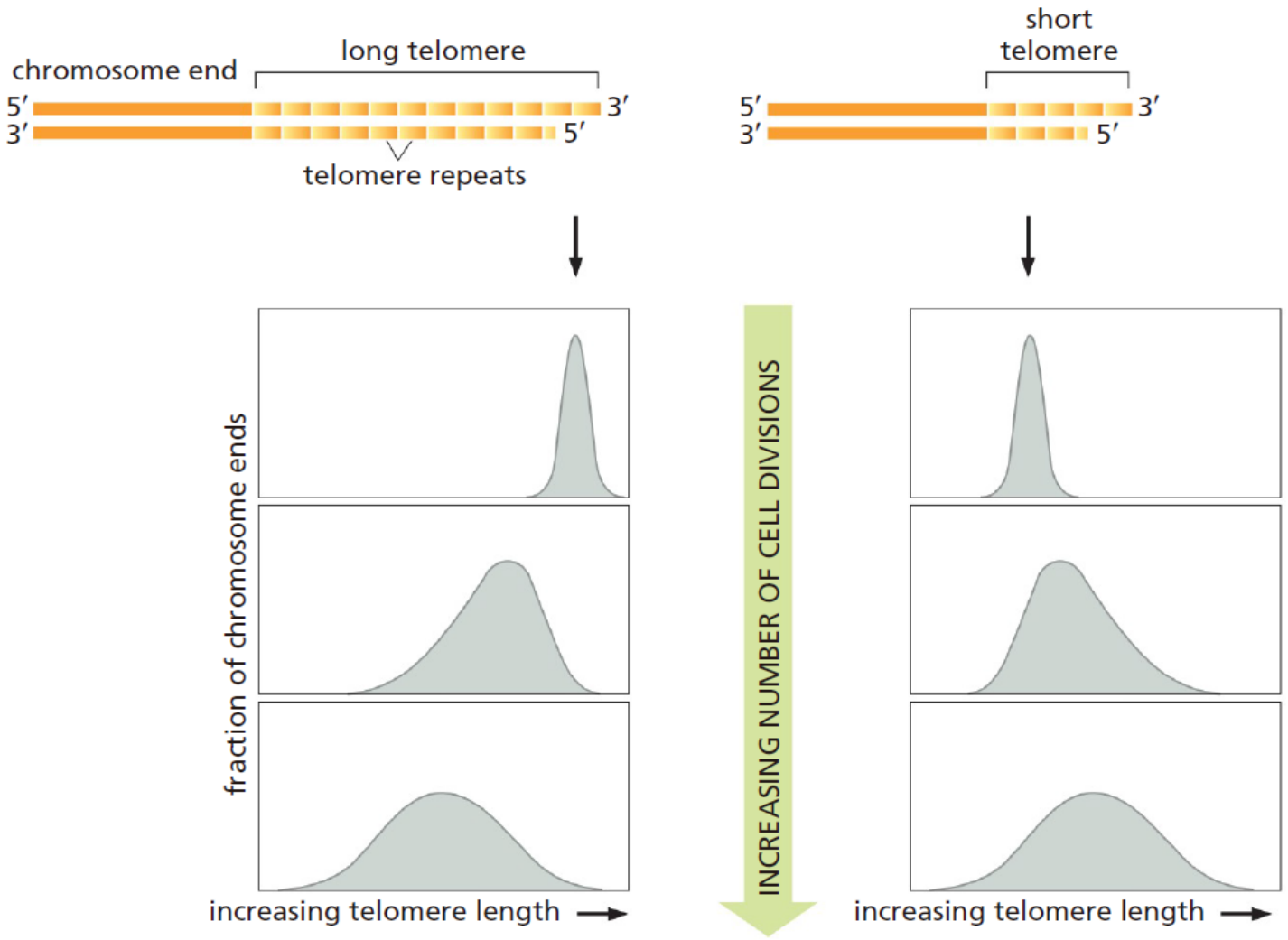




The protruding end, in combination with the TxGy repeats in telomeres, attracts a group of proteins that form a protective chromosome cap known as shelterin.

Part III

Telomere Length Is Regulated by Cells and Organisms



Cells have homeostatic mechanism that maintain the length of telomere within a limited range.

The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

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Elizabeth H. Blackburn

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Carol W. Greider

Photo: Jussi Puikkonen

Jack W. Szostak

Part III

Replication Proceeds in Stages

What is a replisome?

Initiation

Refractory period

Elongation

Termination

Telomere replication