Replication

زیست شناسی مولکولی

منابع:

بیوشیمی لنینجر،

Molecular Cell Biology, Lodish et al.

همانند سازی نیمه حفاظتی

Meselson-Stahl experiment

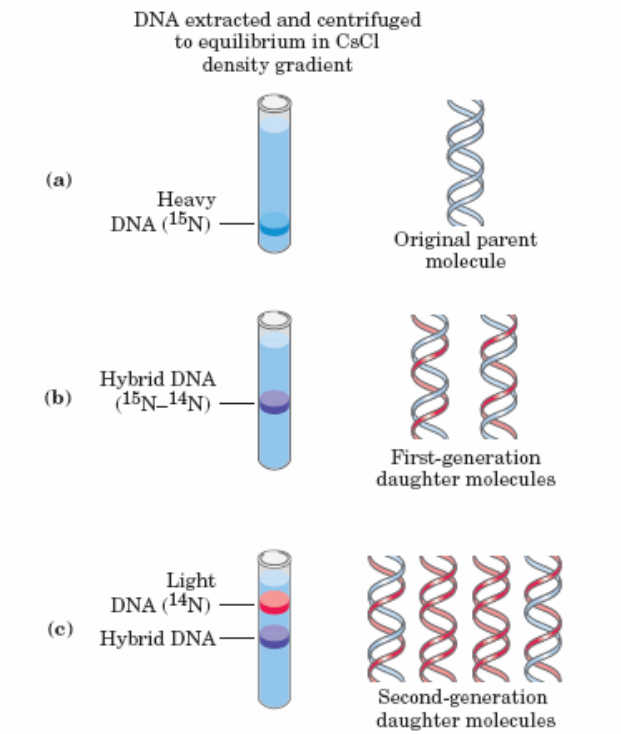
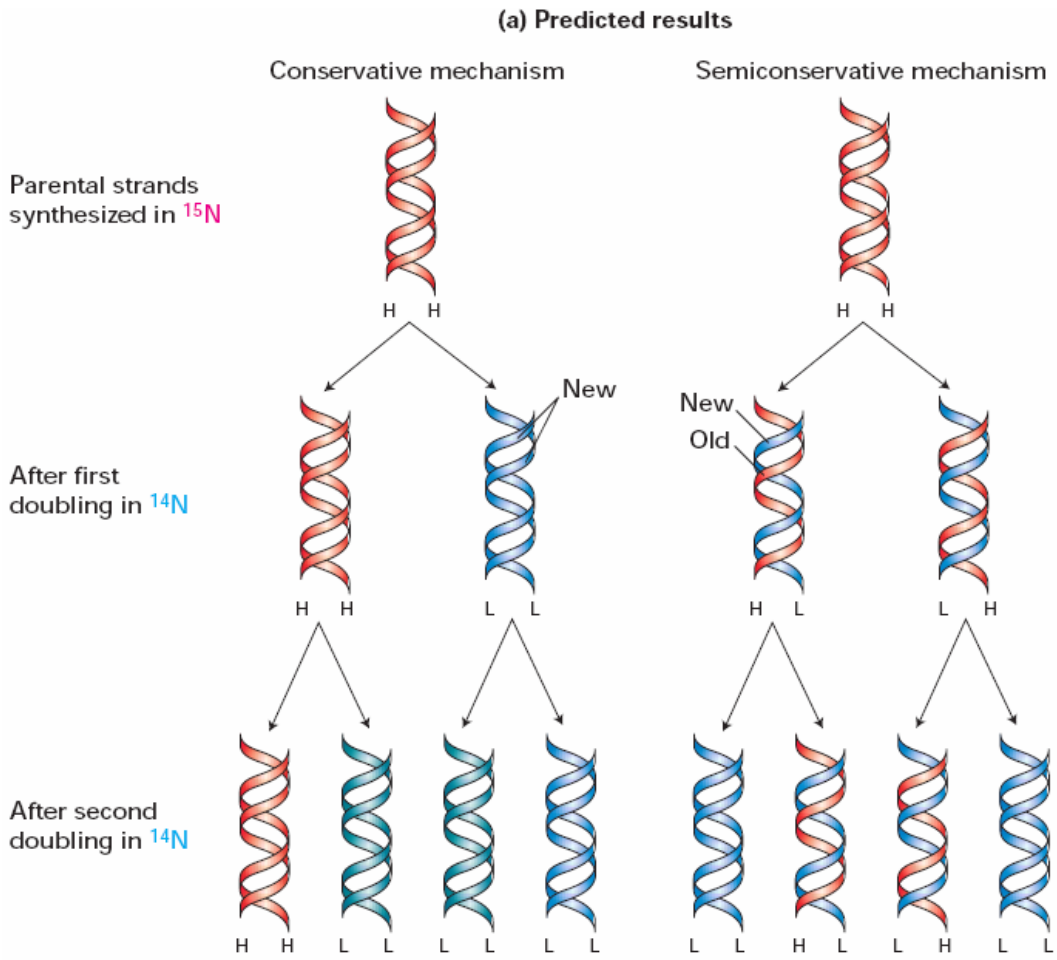


FIGURE 25–2 The Meselson-Stahl experiment. (a) Cells were grown for many generations in a medium containing only heavy nitrogen, ^{15}N, so that all the nitrogen in their DNA was ^{15}N, as shown by a single band (blue) when centrifuged in a CsCl density gradient. (b) Once the cells had been transferred to a medium containing only light nitrogen, ^{14}N, cellular DNA isolated after one generation equilibrated at a higher position in the density gradient (purple band). (c) Continuation of replication for a second generation yielded two hybrid DNAs and two light DNAs (red), confirming semiconservative replication.



همانند سازی از یک مبدا و دو طرفه

- Ori (245bp)
- θ replication

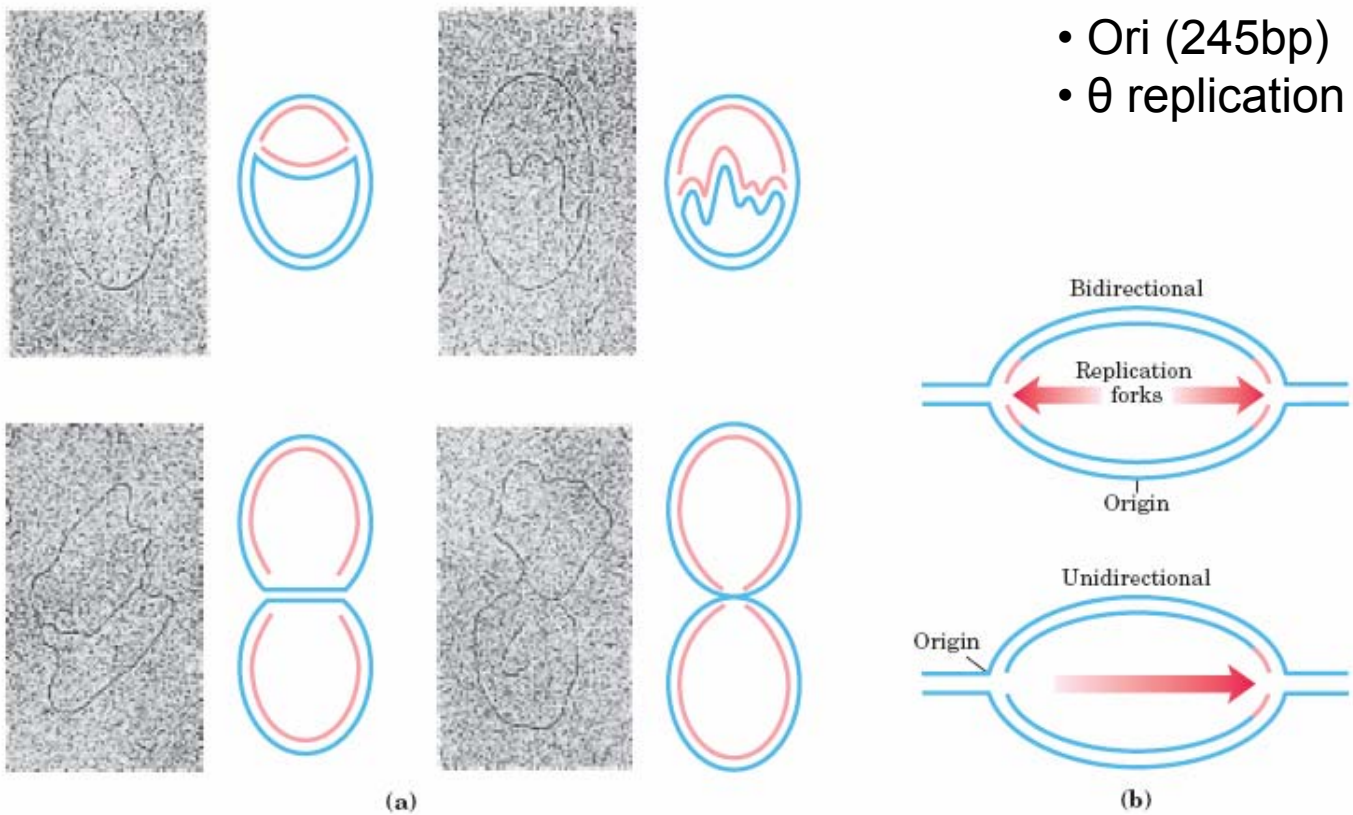
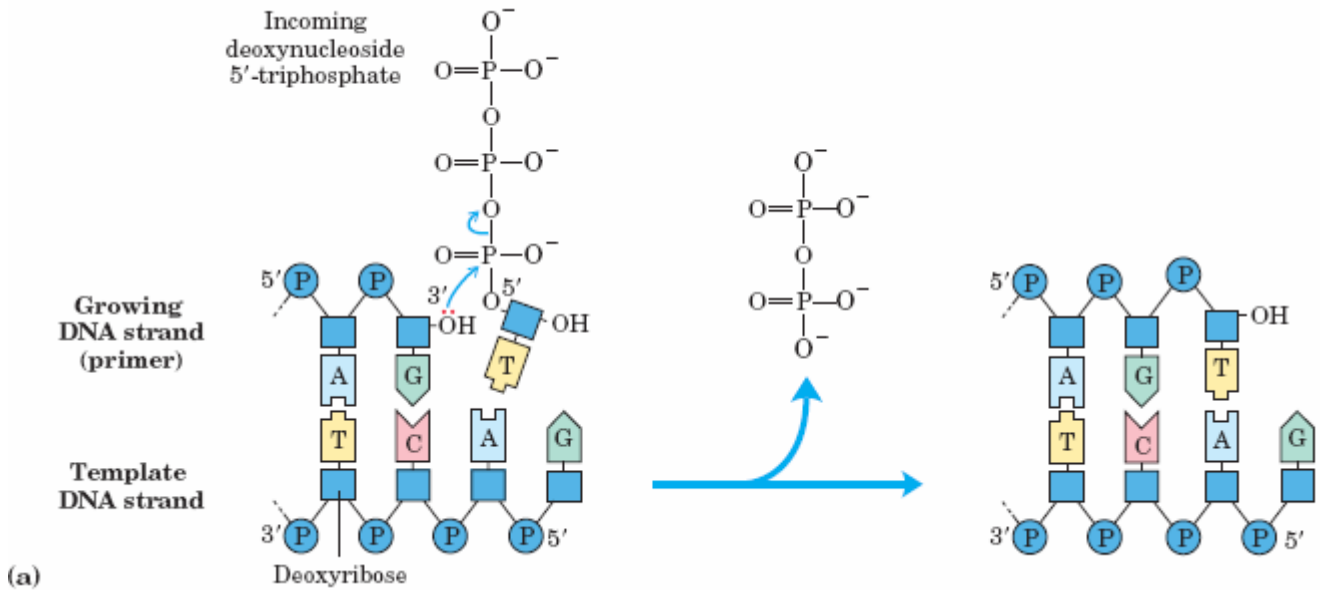


FIGURE 25–3 Visualization of bidirectional DNA replication. Replication of a circular chromosome produces a structure resembling the Greek letter theta (θ). **(a)** Labeling with tritium (^{3}H) shows that both strands are replicated at the same time (new strands shown in red). The electron micrographs illustrate the replication of a circular *E. coli* plasmid as visualized by autoradiography. **(b)** Addition of ^{3}H for a short period just before the reaction is stopped allows a distinction to be made between unidirectional and bidirectional replication, by determining whether label (red) is found at one or both replication forks in autoradiograms. This technique has revealed bidirectional replication in *E. coli, Bacillus subtilis,* and other bacteria.

سنتز DNA

Go to the animation 12-1

- جفت شدن بازها
- آزاد شدن پیروفسفات



MECHANISM FIGURE 25–5 **Elongation of a DNA chain.** **(a)** DNA polymerase I activity requires a single unpaired strand to act as template and a primer strand to provide a free hydroxyl group at the 3′ end, to which a new nucleotide unit is added. Each incoming nucleotide is selected in part by base pairing to the appropriate nucleotide in the template strand. The reaction product has a new free 3′ hydroxyl, allowing the addition of another nucleotide. **(b)** The catalytic mechanism

تصحیح خطا (proofreading)

- 3’->5’ exonuclease
- یک خطا به ازاء هر $۱۰^{۱۰}$– $۱۰^{۹}$

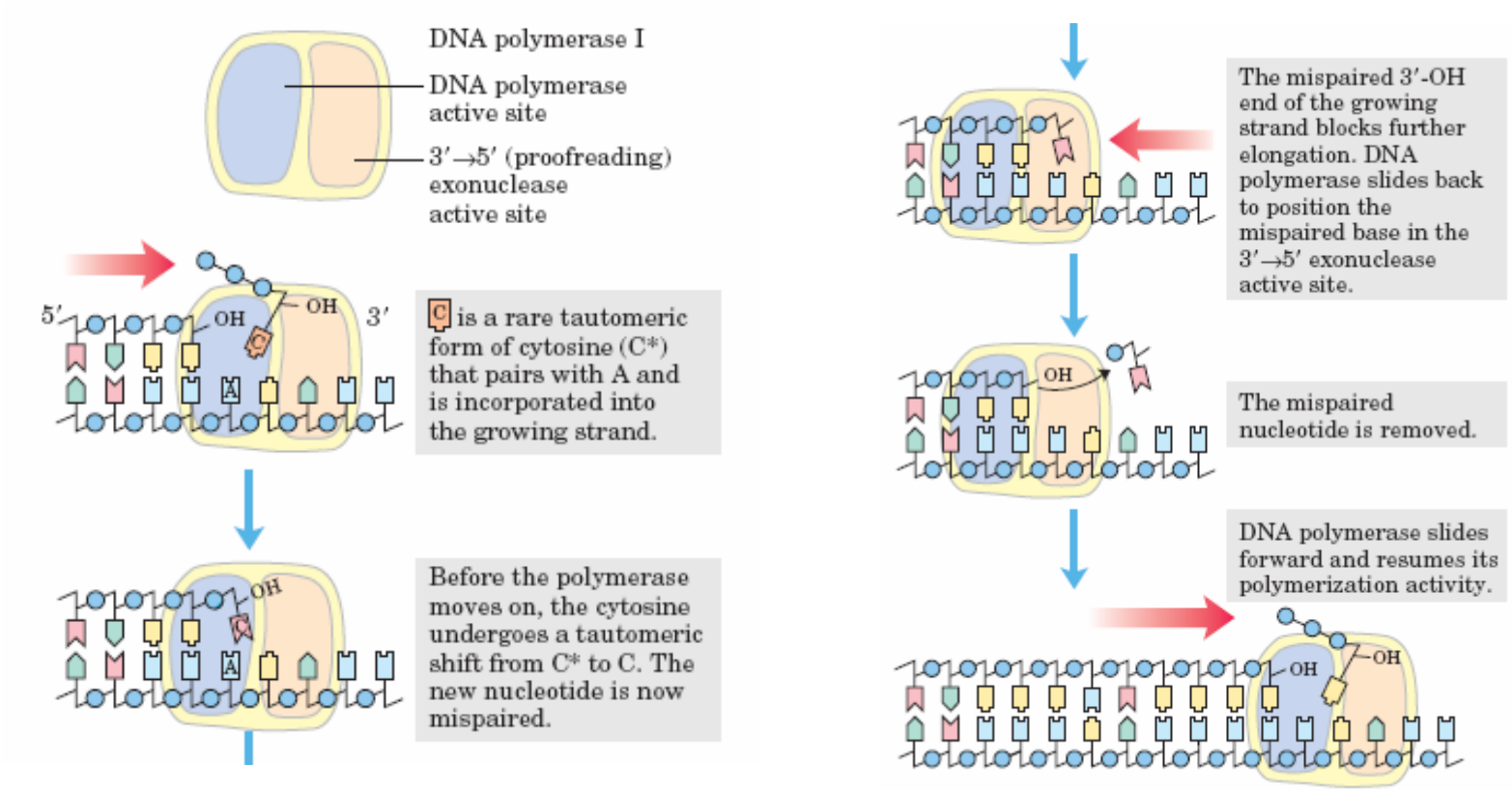


FIGURE 25–7 An example of error correction by the 3′→5′ exonuclease activity of DNA polymerase I. Structural analysis has located the exonuclease activity ahead of the polymerase activity as the enzyme is oriented in its movement along the DNA. A mismatched base (here, a C–A mismatch) impedes translocation of DNA polymerase I to the next site. Sliding backward, the enzyme corrects the mistake

DNA پلیمراز های باکتریایی

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 β, γ, δ, δ', χ, and ψ 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	
α	2	129,900	*polC* (*dnaE*)	Polymerization activity	Core polymerase
ε	2	27,500	*dnaQ* (*mutD*)	3′→5′ Proofreading exonuclease	
θ	2	8,600	*holE*		
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	
δ	1	38,700	*holA*	Clamp opener	
δ'	1	36,900	*holB*	Clamp loader	
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 Box 27–1) that leads to premature translational termination.

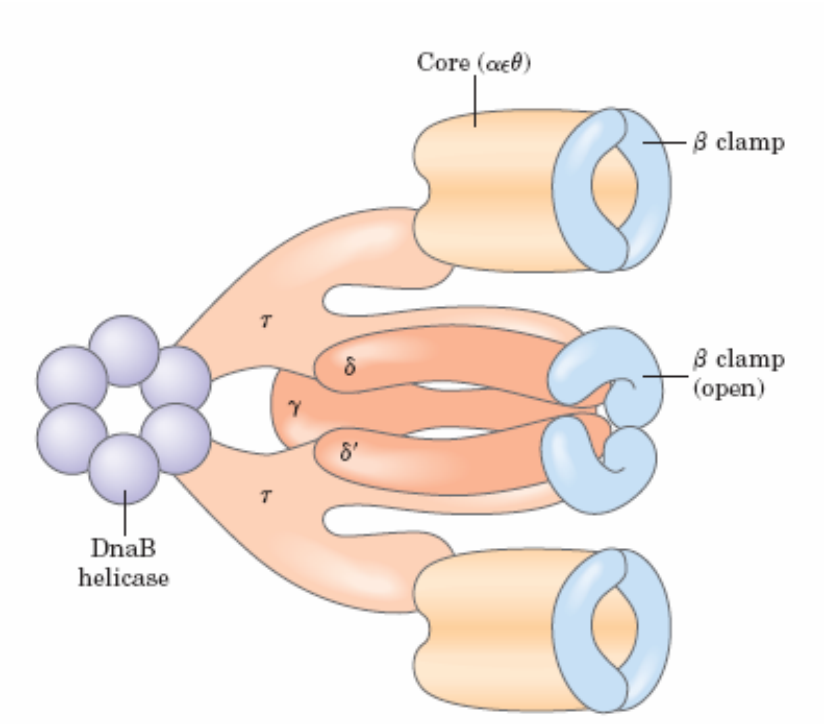
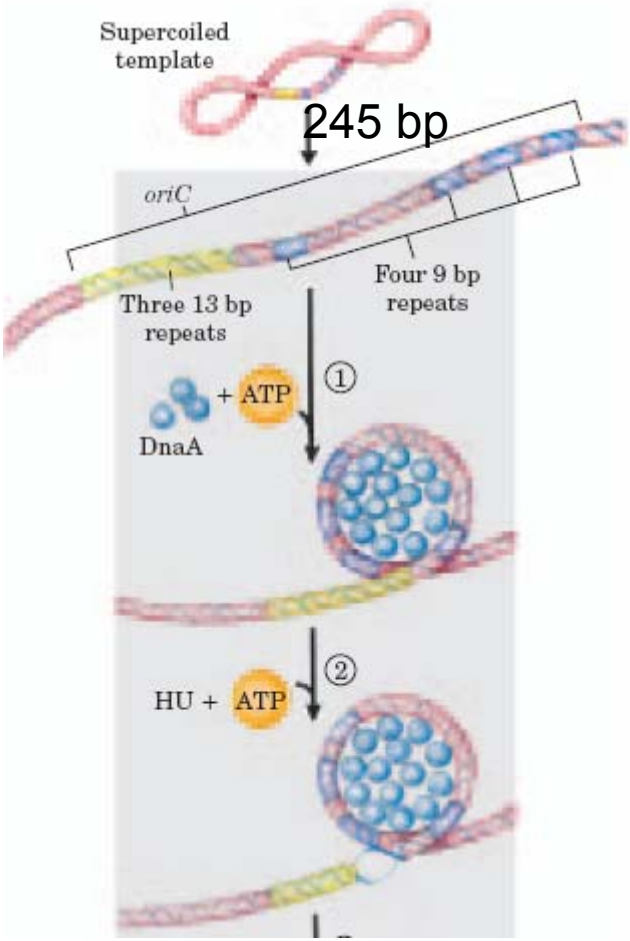


FIGURE 25–10 DNA polymerase III. **(a)** Architecture of bacterial DNA polymerase III. Two core domains, composed of subunits α, ε, and θ, are linked by a five-subunit γ complex (also known as the clamp-loading complex) with the composition $\tau_2\gamma\delta\delta'$. The γ and τ subunits are encoded by the same gene. The γ subunit is a shortened version of τ; the τ subunit thus contains a domain identical to γ, along with an additional segment that interacts with the core polymerase. The other two subunits of DNA polymerase III*, χ and ψ (not shown), also bind to the γ complex. Two β clamps interact with the two-core subassembly, each clamp a dimer of the β subunit. The complex interacts with the DnaB helicase through the τ subunit. **(b)** Two β subunits of *E. coli* polymerase III form a circular clamp that surrounds the DNA. The clamp slides along the DNA molecule, increasing the processivity of the polymerase III holoenzyme to greater than 500,000 by preventing its dissociation from the DNA. The end-on view shows the two β subunits as gray and light-blue ribbon structures surrounding a space-filling model of DNA. In the side view, surface contour models of the β subunits (gray) surround a stick representation of a DNA double helix (light and dark blue) (derived from PDB ID 2POL).

DnaA

- تترامر
- شناسایی OriC
- ۲۰ تا ۳۰ عدد
- جلب پروتئین های دیگر



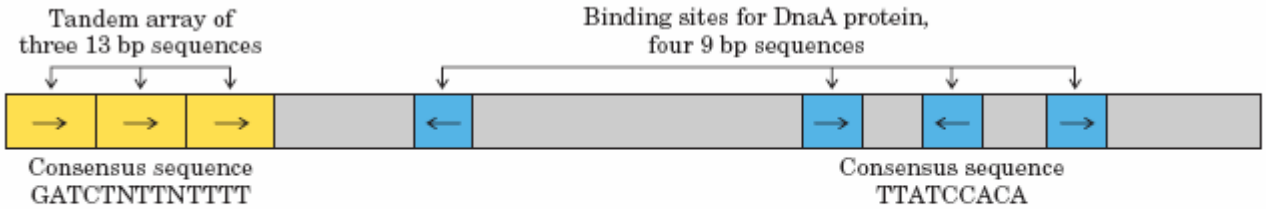
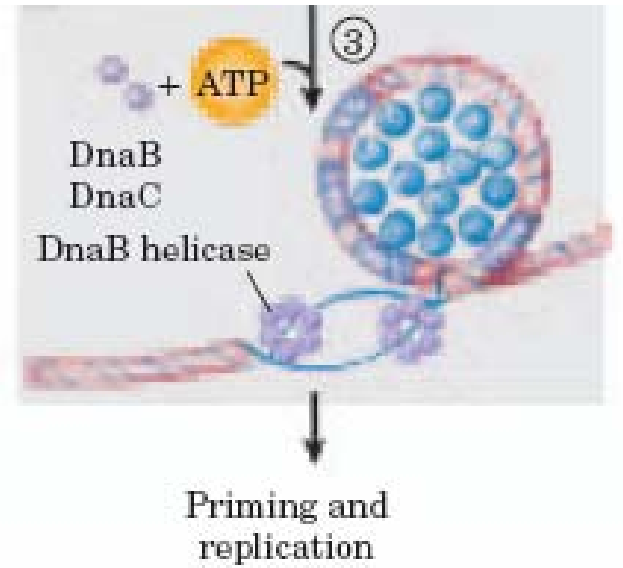


FIGURE 25–11 **Arrangement of sequences in the *E. coli* replication origin, *oriC*.** Although the repeated sequences (shaded in color) are not identical, certain nucleotides are particularly common in each position, forming a consensus sequence. In positions where there is no consensus, N represents any of the four nucleotides. The arrows indicate the orientations of the nucleotide sequences.

DnaB & DnaC

- Dna B= هلیکاز، هگزامر
- Dna C= به کمک Dna B ناحیه Ori را باز می کند، مونومر



SSB

- Single Stranded Binding Protein
- تترامر
- ۳۲ نوکلئوتید را می پوشاند

پرایموزوم

- پرایماز (Dna G) و پروتئین های n و n’ و n” و i

پروتئین های شروع همانند سازی

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.

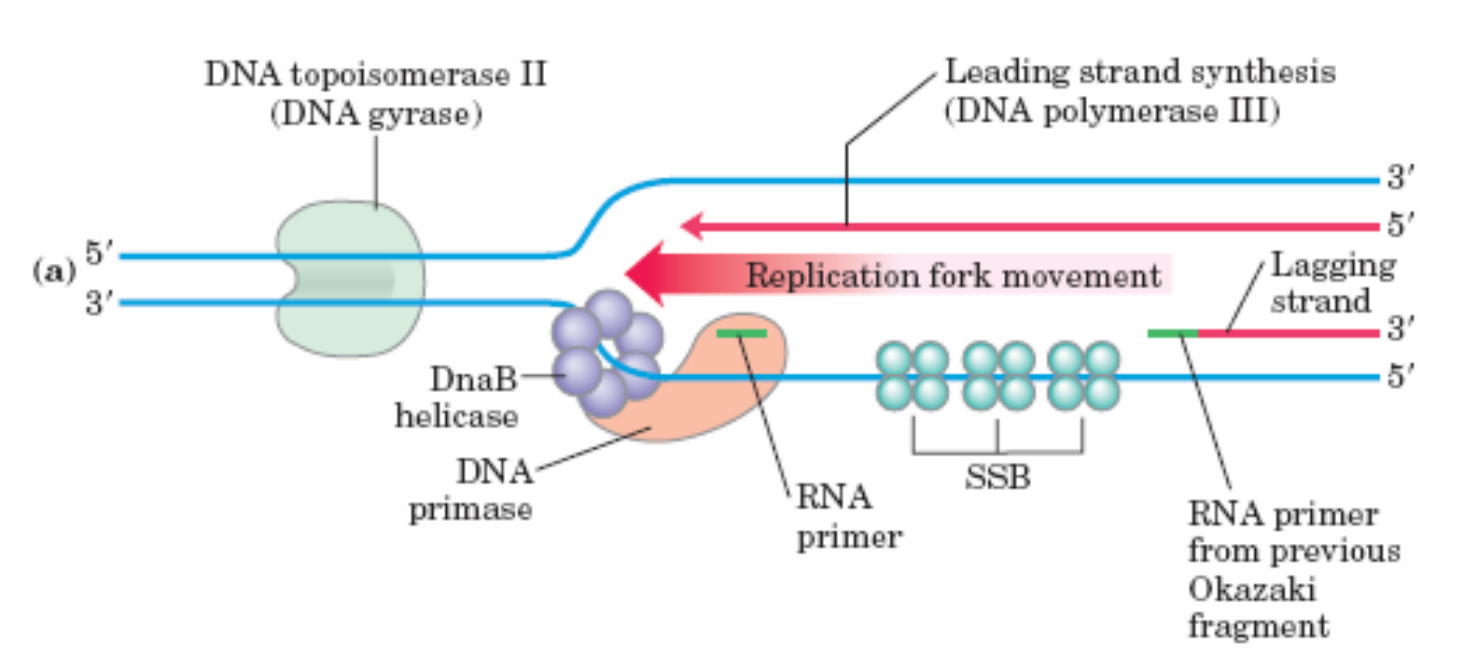
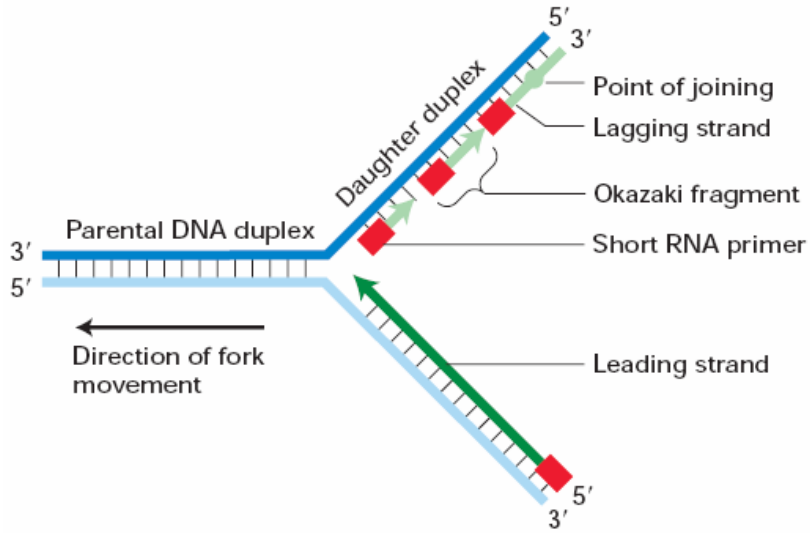


TABLE 25–4 Proteins at the *E. coli* Replication Fork

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.

چنگال همانند سازی



▲ FIGURE 4-33 Schematic diagram of leading-strand and lagging-strand DNA synthesis at a replication fork. Nucleotides are added by a DNA polymerase to each growing daughter strand in the 5′⟶3′ direction (indicated by arrowheads). The leading strand is synthesized continuously from a single RNA primer (red) at its 5′ end. The lagging strand is synthesized discontinuously from multiple RNA primers that are formed periodically as each new region of the parental duplex is unwound. Elongation of these primers initially produces Okazaki fragments. As each growing fragment approaches the previous primer, the primer is removed and the fragments are ligated. Repetition of this process eventually results in synthesis of the entire lagging strand.

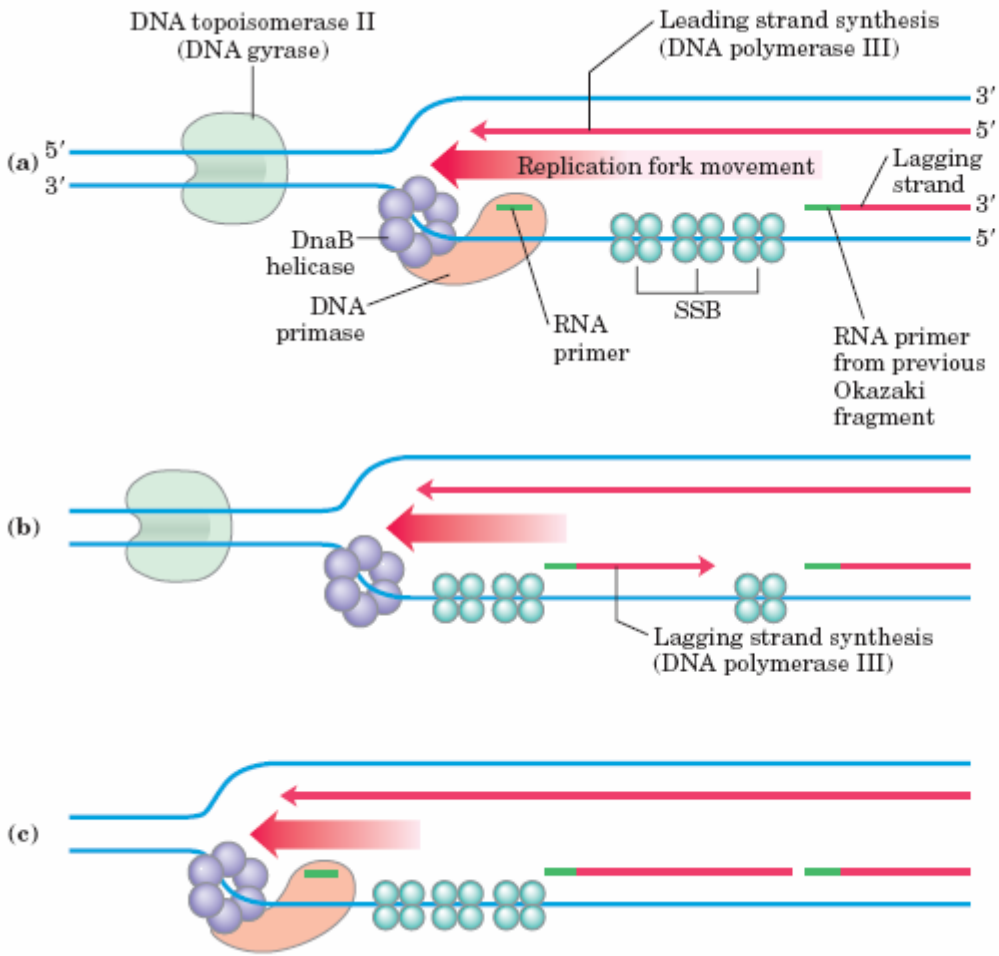
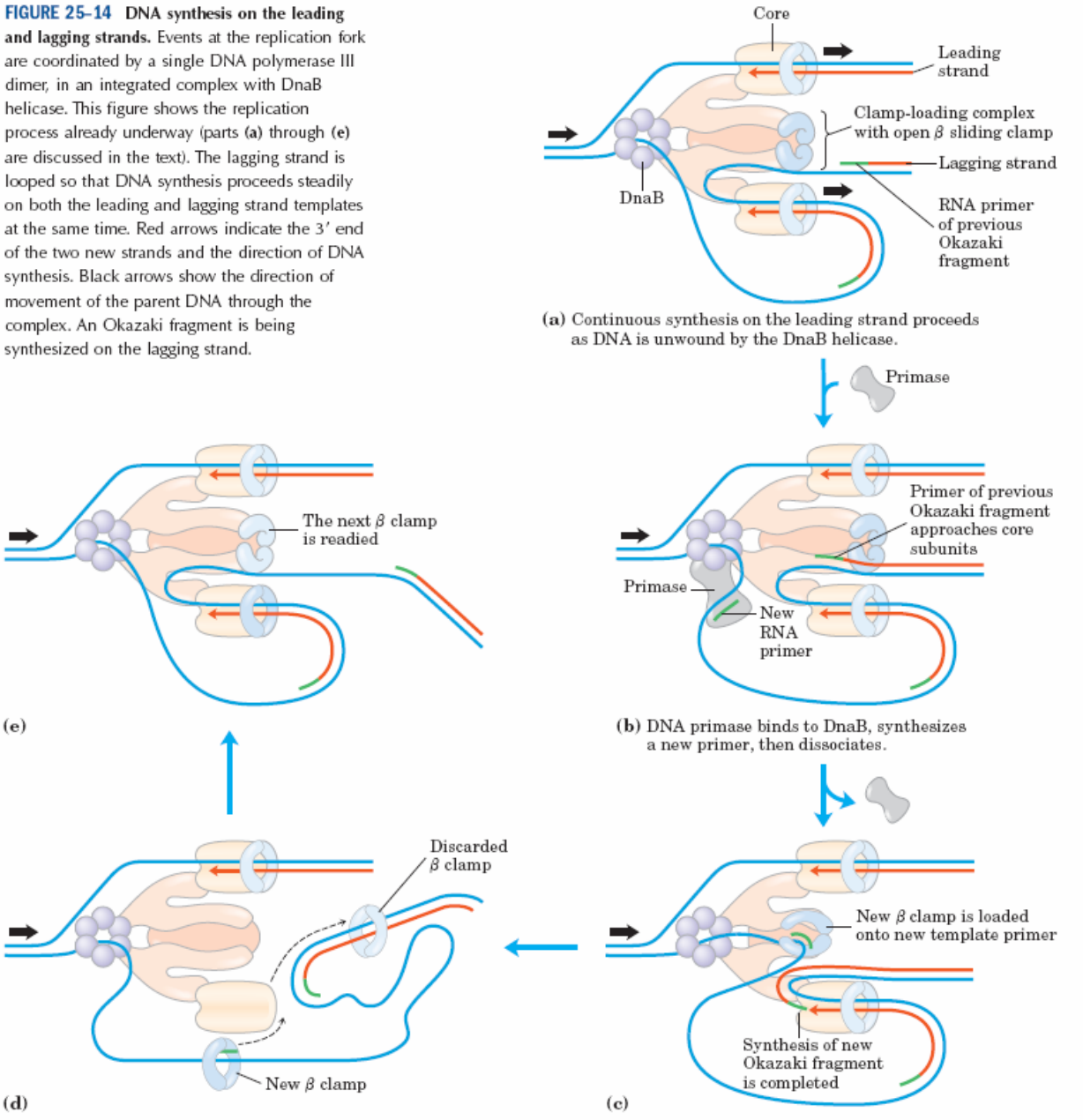


FIGURE 25–13 Synthesis of Okazaki fragments. **(a)** At intervals, primase synthesizes an RNA primer for a new Okazaki fragment. Note that if we consider the two template strands as lying side by side, lagging strand synthesis formally proceeds in the opposite direction from fork movement. **(b)** Each primer is extended by DNA polymerase III. **(c)** DNA synthesis continues until the fragment extends as far as the primer of the previously added Okazaki fragment. A new primer is synthesized near the replication fork to begin the process again.

FIGURE 25–14 DNA synthesis on the leading and lagging strands. Events at the replication fork are coordinated by a single DNA polymerase III dimer, in an integrated complex with DnaB helicase. This figure shows the replication process already underway (parts **(a)** through **(e)** are discussed in the text). The lagging strand is looped so that DNA synthesis proceeds steadily on both the leading and lagging strand templates at the same time. Red arrows indicate the 3′ end of the two new strands and the direction of DNA synthesis. Black arrows show the direction of movement of the parent DNA through the complex. An Okazaki fragment is being synthesized on the lagging strand.



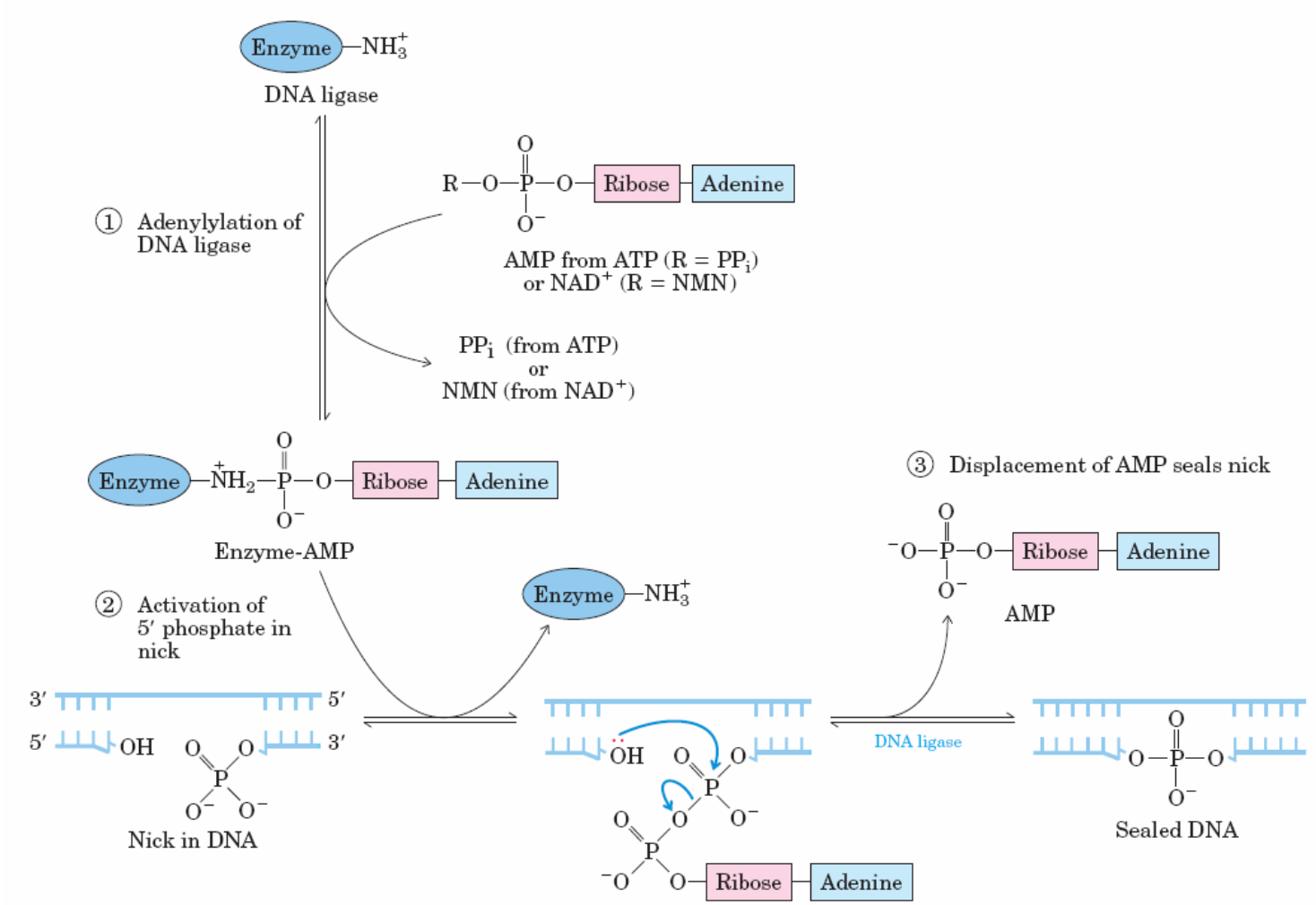
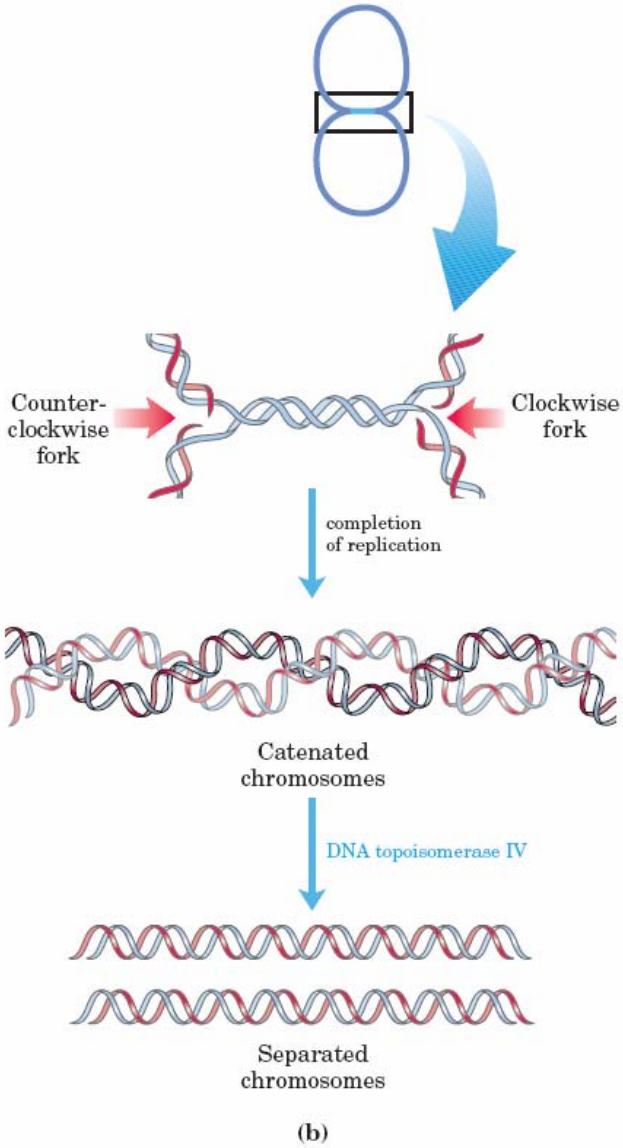


FIGURE 25-16 **Mechanism of the DNA ligase reaction.** In each of the three steps, one phosphodiester bond is formed at the expense of another. Steps ① and ② lead to activation of the 5′ phosphate in the nick. An AMP group is transferred first to a Lys residue on the enzyme and then to the 5′ phosphate in the nick. In step ③, the 3′-hydroxyl group attacks this phosphate and displaces AMP, producing a phosphodiester bond to seal the nick. In the *E. coli* DNA ligase reaction, AMP is derived from NAD^+. The DNA ligases isolated from a number of viral and eukaryotic sources use ATP rather than NAD^+, and they release pyrophosphate rather than nicotinamide mononucleotide (NMN) in step ①.



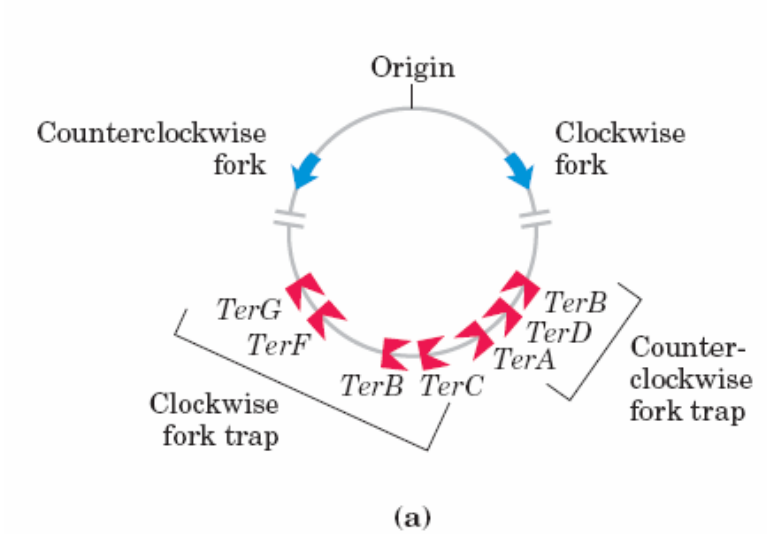
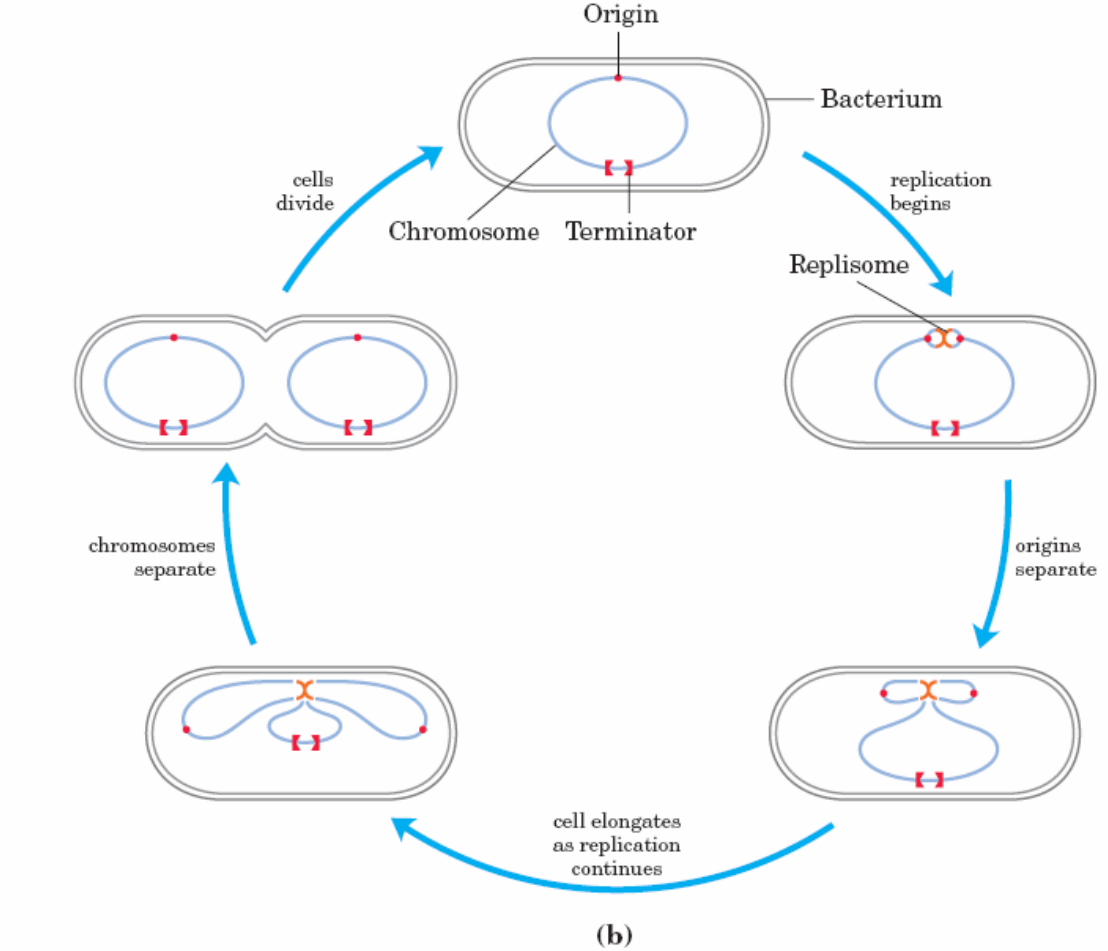


FIGURE 25–17 **Termination of chromosome replication in *E. coli*.** (**a**) The Ter sequences are positioned on the chromosome in two clusters with opposite orientations. (**b**) Replication of the DNA separating the opposing replication forks leaves the completed chromosomes joined as catenanes, or topologically interlinked circles. The circles are not covalently linked, but because they are interwound and each is covalently closed, they cannot be separated—except by the action of topoisomerases. In *E. coli*, a type II topoisomerase known as DNA topoisomerase IV plays the primary role in the separation of catenated chromosomes, transiently breaking both DNA strands of one chromosome and allowing the other chromosome to pass through the break.


Origin
Bacterium
Chromosome
Terminator
replication begins
Replisome
origins separate
cell elongates as replication continues
chromosomes separate
cells divide

(b)