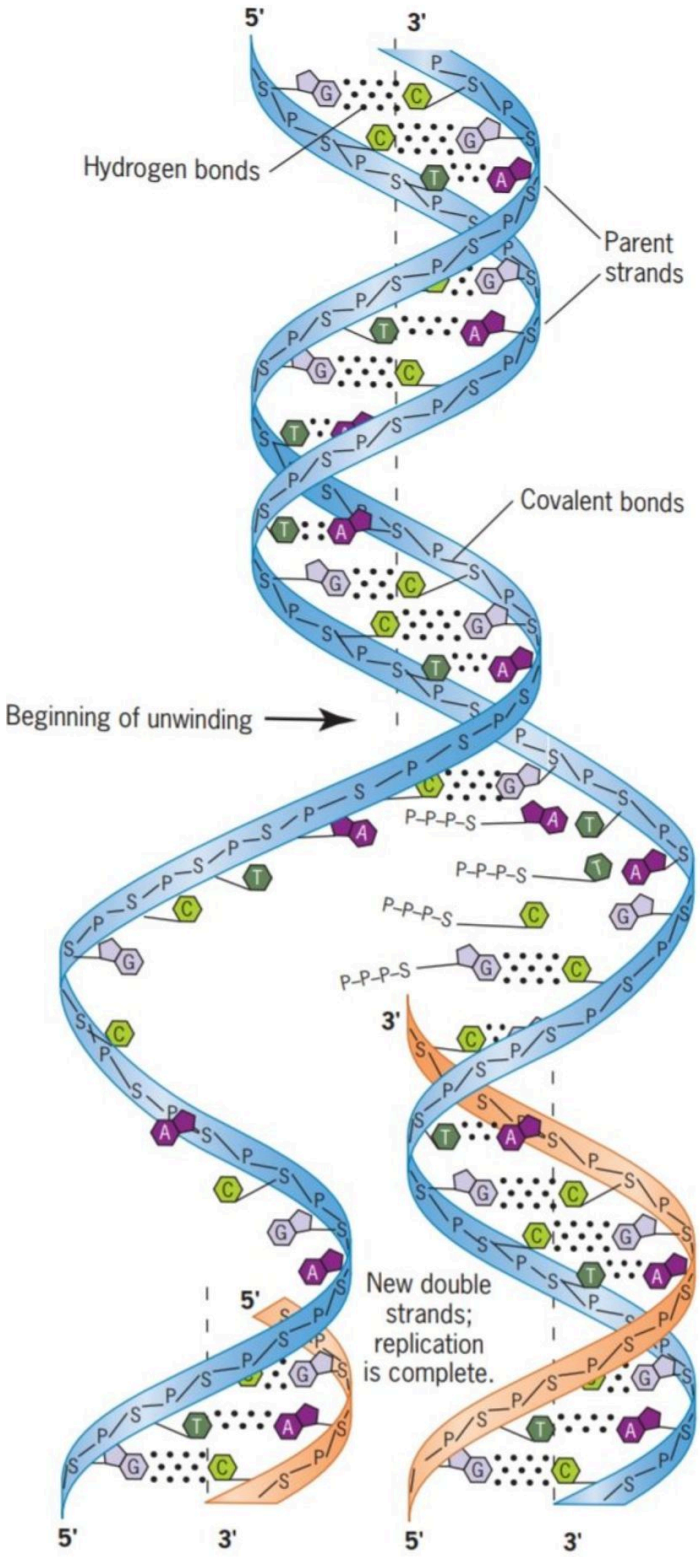
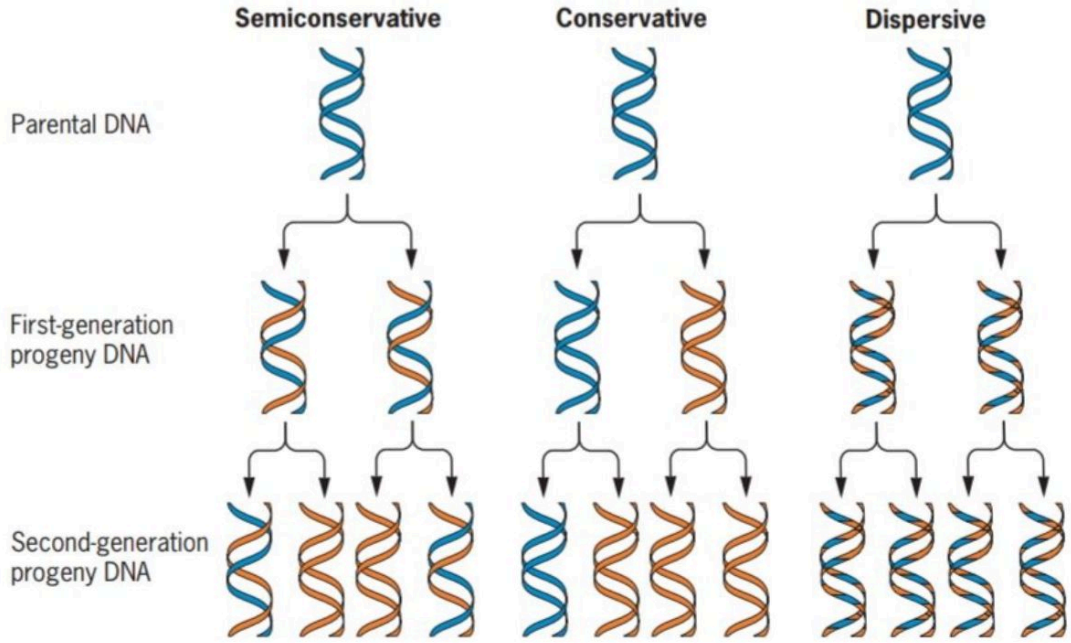
Seven pairs of identical twins. Although the twins were given no instructions regarding how to pose for the photograph, note the similar posture, hand placement, and facial expressions of both members of each pair of twins.

Four pairs of twins with their mothers at the Iowa State Fair.



■ **FIGURE 10.1** Semiconservative DNA replication. Watson and Crick first proposed this mechanism of DNA replication based on complementary base-pairing between the two strands of the double helix. Note that each of the parental strands is conserved and serves as a template for the synthesis of a new complementary strand; that is, the base sequence in each progeny strand is determined by the hydrogen-bonding potentials of the bases in the parental strand.

romosomes

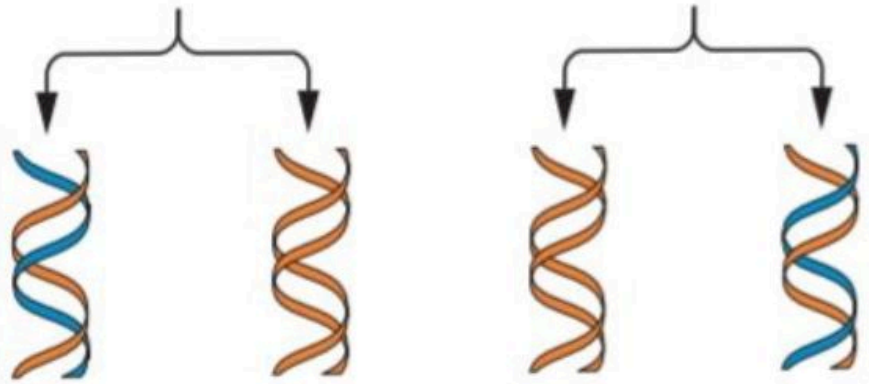


■ **FIGURE 10.2** The three possible modes of DNA replication: (1) semiconservative, in which each strand of the parental double helix is conserved and directs the synthesis of a new complementary progeny strand; (2) conservative, in which the parental double helix is conserved and directs the synthesis of a new progeny double helix; and (3) dispersive, in which segments of each parental strand are conserved and direct the synthesis of new complementary strand segments that are subsequently joined to produce new progeny strands.

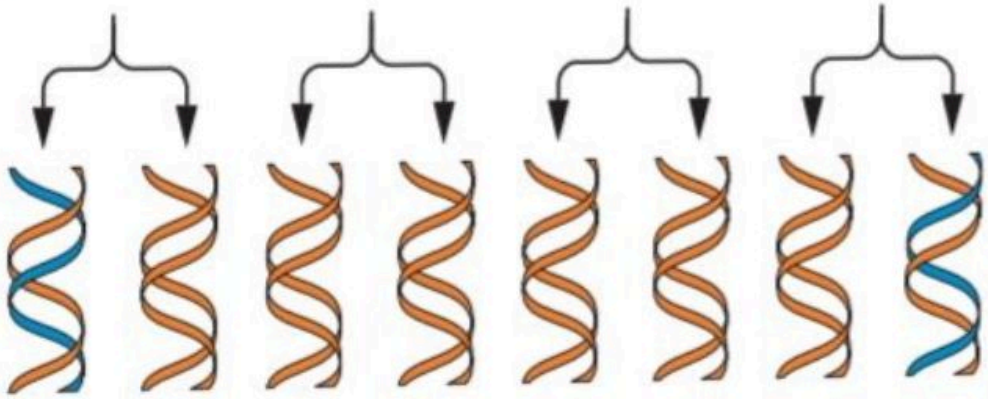
STEP 1 *E.coli* cells are grown on ^{15}N for several generations.

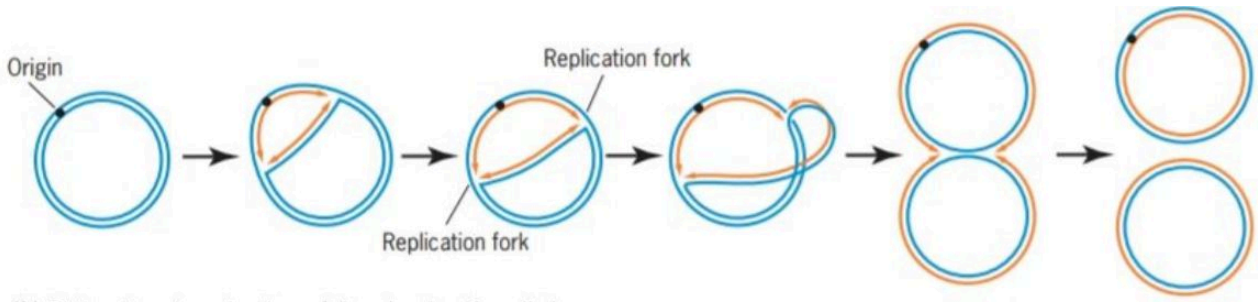
STEP 3 Cells are then transferred to medium containing ^{14}N for one generation.

STEP 5 For two generations.



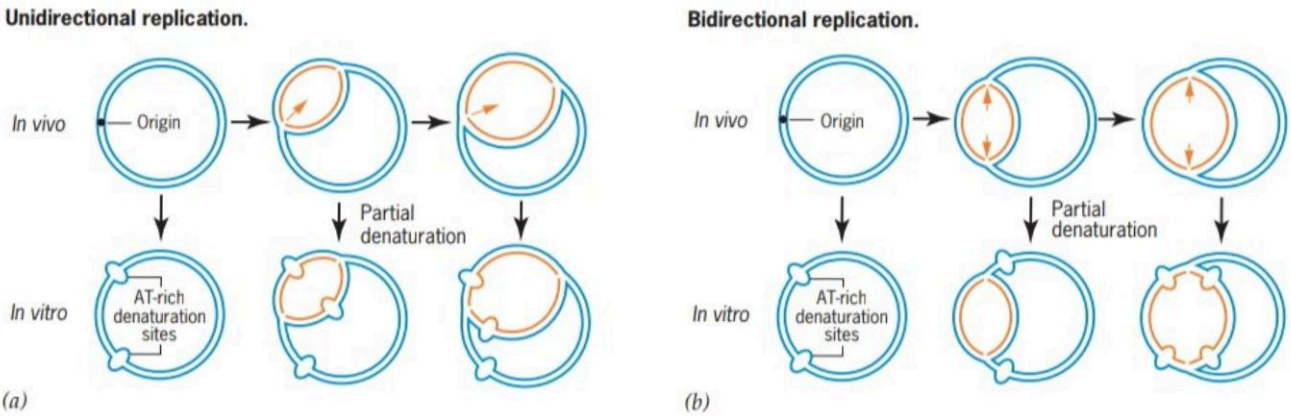
STEP 7 For three generations.





(b) Bidirectional replication of the circular *E. coli* chromosome.

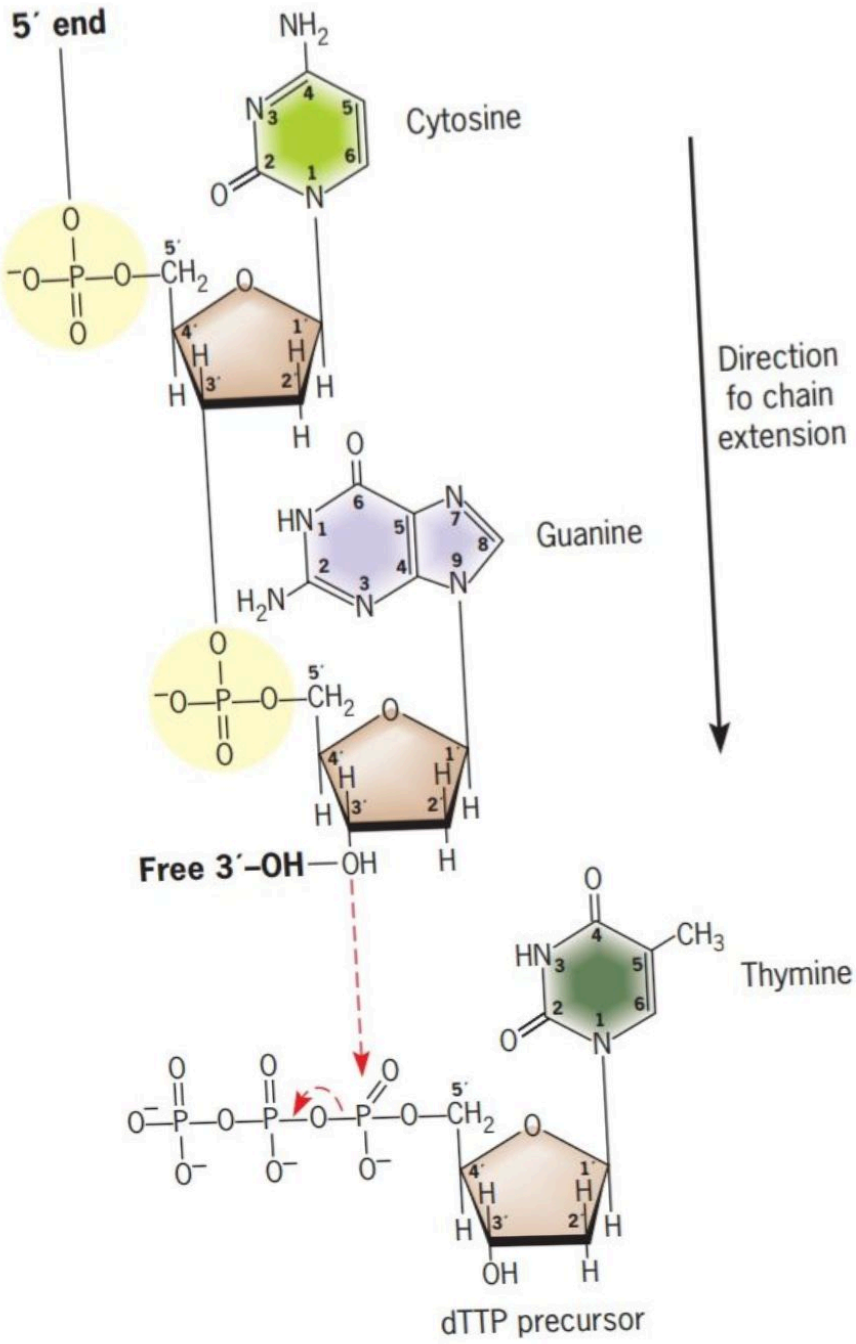
■ **FIGURE 10.7** Visualization of the replication of the *E. coli* chromosome by autoradiography. (*a*) One of Cairns's autoradiographs of a θ-shaped replicating chromosome from a cell that had been grown for two generations in the presence of ^{3}H-thymidine, with his interpretative diagram shown at the upper left. Radioactive strands of DNA are shown as solid lines and nonradioactive strands as dashed lines. Loops A and B have completed a second replication in ^{3}H-thymidine; section C remains to be replicated the second time. (*b*) A diagram showing how Cairns's results are explained by bidirectional replication of the *E. coli* chromosome initiated at a unique origin of replication.



■ **FIGURE 10.10** Rationale of the denaturation mapping procedure used by Schnös and Inman to distinguish between (*a*) unidirectional and (*b*) bidirectional modes of chromosome replication.

KEY POINTS

- *DNA replicates by a semiconservative mechanism: as the two complementary strands of a parental double helix unwind and separate, each serves as a template for the synthesis of a new complementary strand.*
- *The hydrogen-bonding potentials of the bases in the template strands specify complementary base sequences in the nascent DNA strands.*
- *Replication is initiated at unique origins and usually proceeds bidirectionally from each origin.*



■ **FIGURE 10.12** Mechanism of action of DNA polymerases: covalent extension of a DNA primer strand in the 5′ → 3′ direction. The existing chain terminates at the 3′ end with the nucleotide deoxyguanylate (deoxyguanosine-5′-phosphate). The diagram shows the DNA polymerase-catalyzed addition of deoxythymidine monophosphate (from the precursor deoxythymidine triphosphate, dTTP) to the 3′ end of the chain with the release of pyrophosphate (P_2O_7).

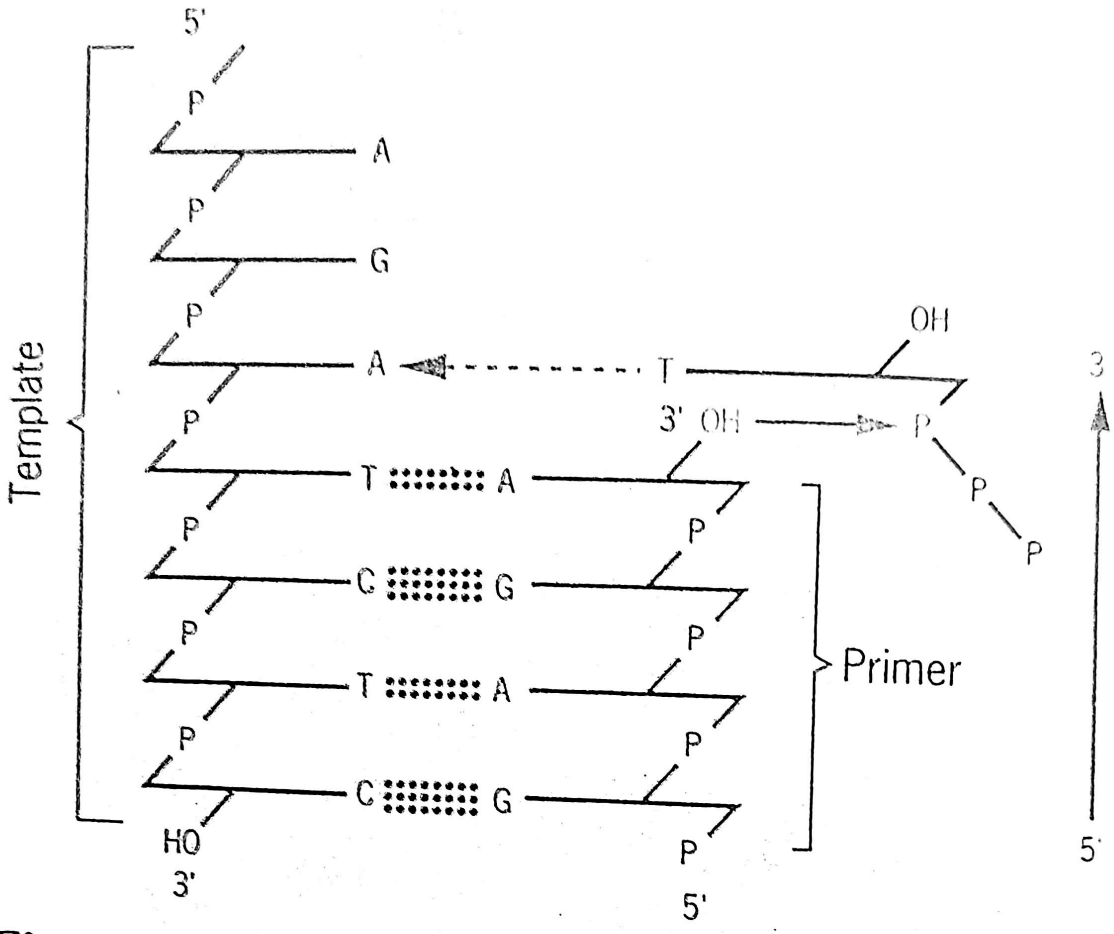
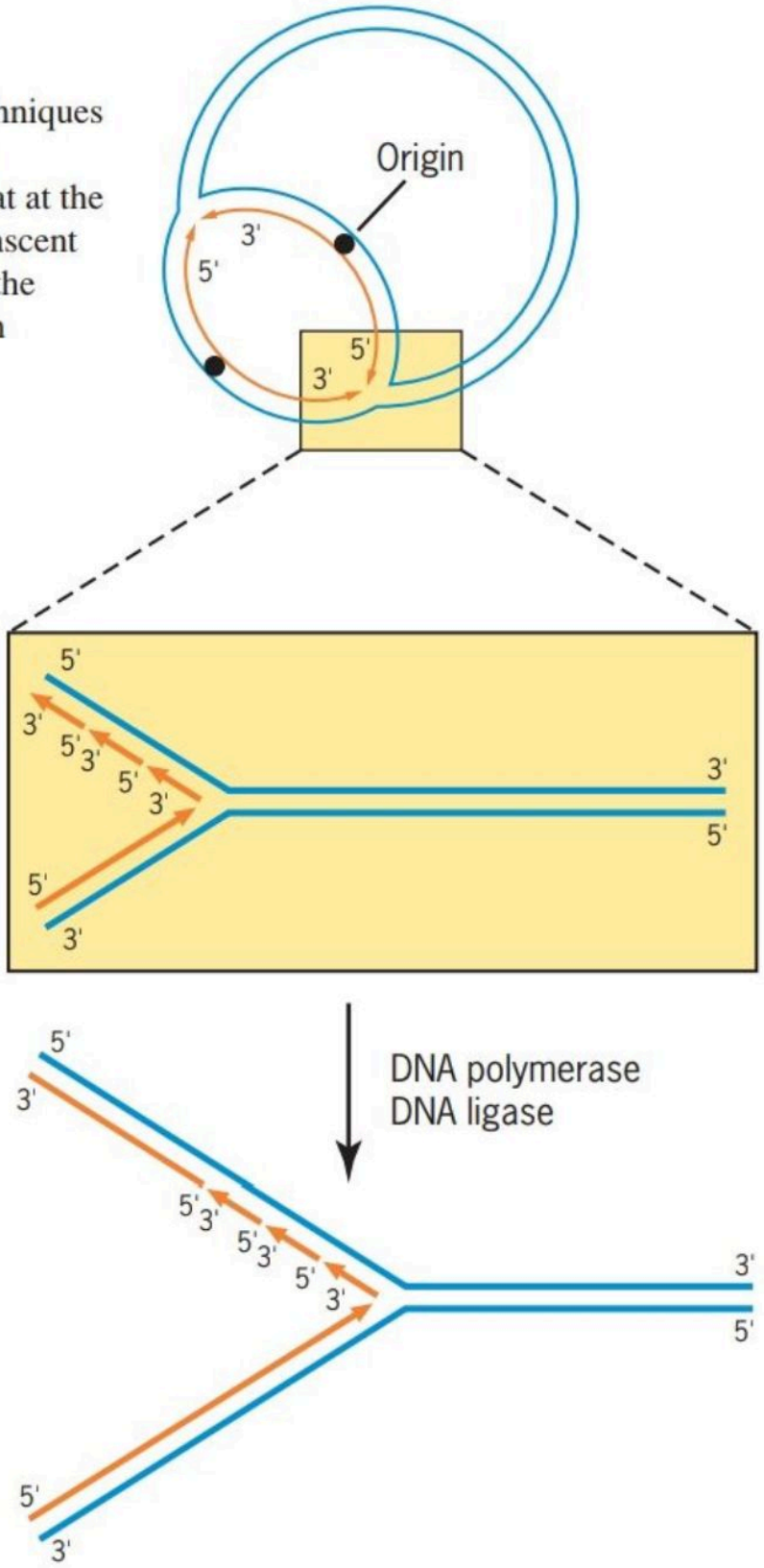


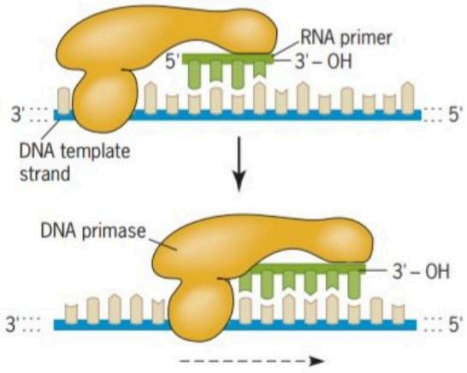
Figure 11.11 Template and primer requirements of DNA polymerases. All DNA polymerases require a primer strand (shown on the right) with a free 3′-hydroxyl. The primer strand is covalently extended by the addition of nucleotides (such as dTMP, derived from the incoming precursor dTTP shown). In addition, DNA polymerases require a template strand (shown on the left), which determines the base sequence of the strand being synthesized. The new strand will be complementary to the template strand.

Relatively low-resolution techniques such as autoradiography and electron microscopy show that at the macromolecular level both nascent DNA chains are extended in the same overall direction at each replication fork.

(a)



(b) High-resolution biochemical techniques such as pulse-labeling and density-gradient analysis show that replication of the lagging strand is discontinuous—short fragments are synthesized in the 5'→ 3' direction and subsequently joined by DNA ligase.

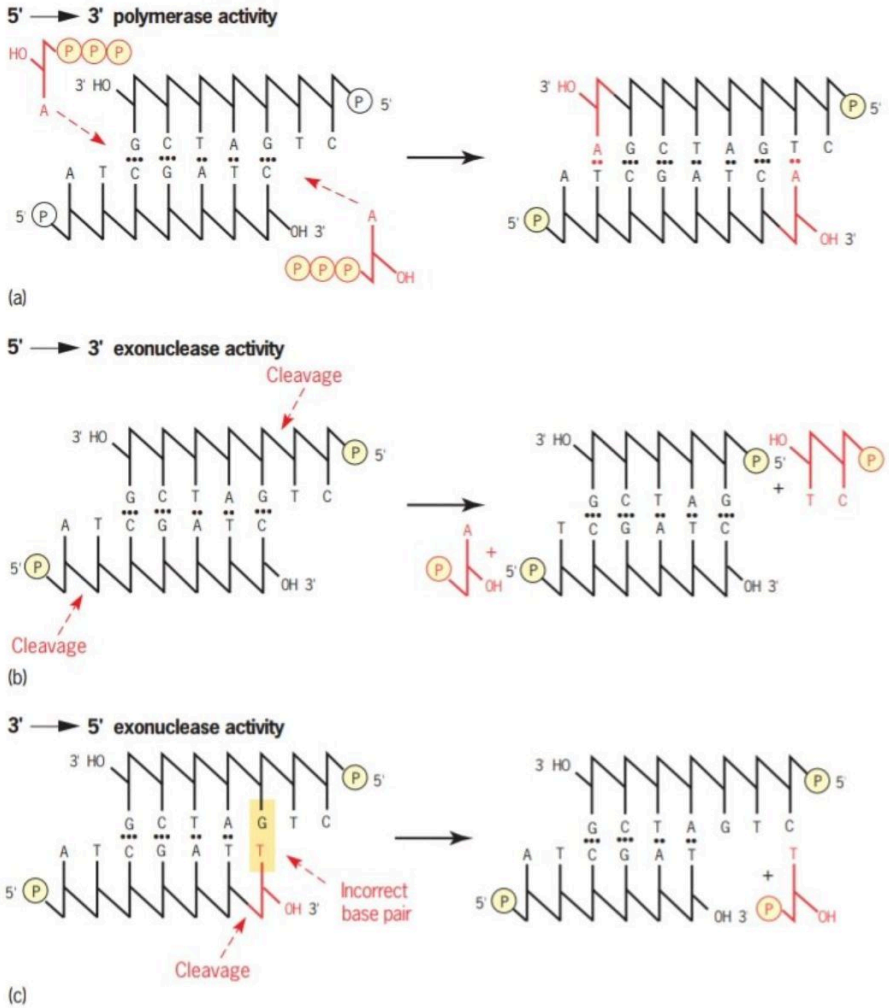


■ **FIGURE 10.16** The initiation of DNA strands with RNA primers. The enzyme DNA primase catalyzes the synthesis of short (10 to 60 nucleotides long) RNA strands that are complementary to the template strands.

new RNA chains at specific sites on the DNA. When th
is formed in which the nascent RNA is hydrogen bonde
DNA polymerases are capable of extending either DN
free 3′-OH, scientists began testing the idea that DNA
using RNA primers. Their results proved that this idea

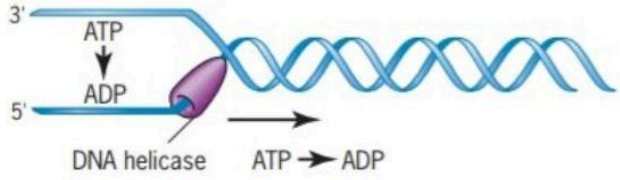
Subsequent research demonstrated that each ne
a short **RNA primer** synthesized by **DNA primase** (■ Fi
primase is the product of the *dnaG* gene. In prokar
10 to 60 nucleotides long, whereas in eukaryotes
10 nucleotides long. The RNA primers provide t
covalent extension of polynucleotide chains by DN
enzyme that catalyzes the semiconservative replicatio
merase called **DNA polymerase III** (see the section Mu
Proofreading). DNA polymerase III catalyzes the ad
to RNA primers, either continuously on the leading
the synthesis of Okazaki fragments on the lagging str
minates an Okazaki fragment when it bumps into the
Okazaki fragment.

The RNA primers subsequently are excised an
This step is accomplished by DNA polymerase I in *E.*



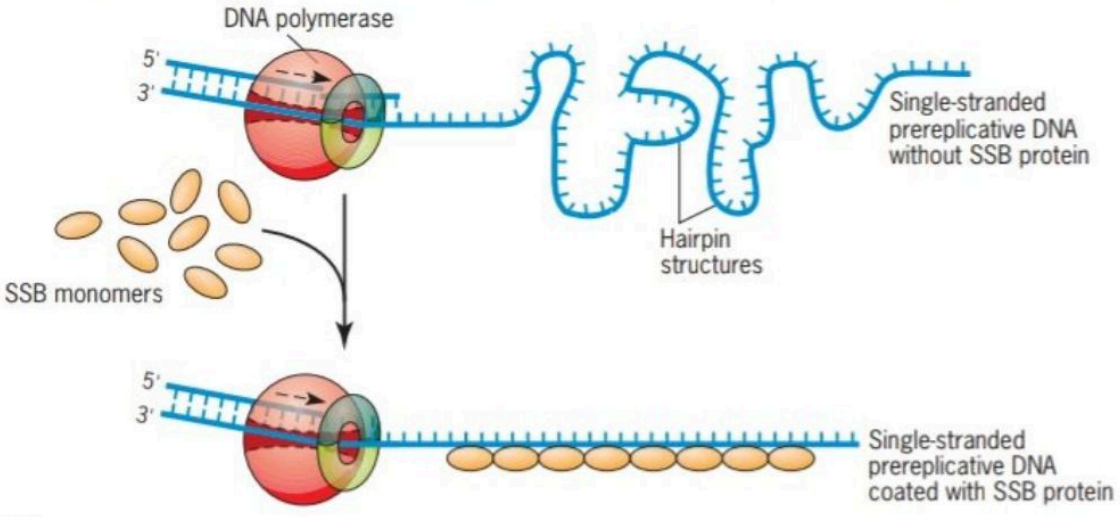
■ **FIGURE 10.17** The three activities of DNA polymerase I in *E. coli*. The DNA molecules are shown here using flattened "stick" diagrams with one complementary strand on the top and the other on the bottom. "Stick" diagrams nicely emphasize the opposite chemical polarity (5′ → 3′ and 3′ → 5′) of the complementary strands. As is discussed in the text, all three activities—(*a*) 5′ → 3′ polymerase activity, (*b*) 5′ → 3′ exonuclease activity, and (*c*) 3′ → 5′ exonuclease activity—play important roles in *E. coli* cells.

DNA helicase catalyzes the unwinding of the parental double helix.



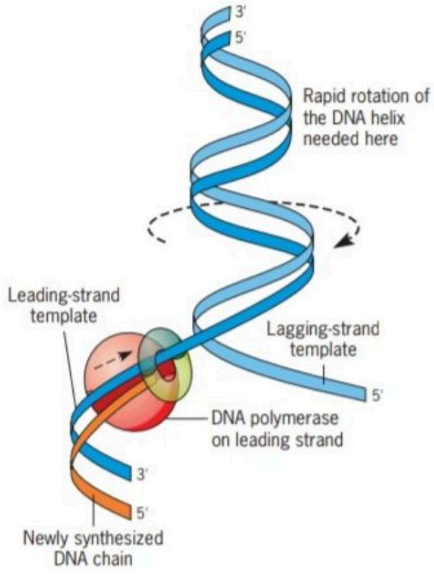
(a)

Single-strand DNA-binding (SSB) protein keeps the unwound strands in an extended form for replication.



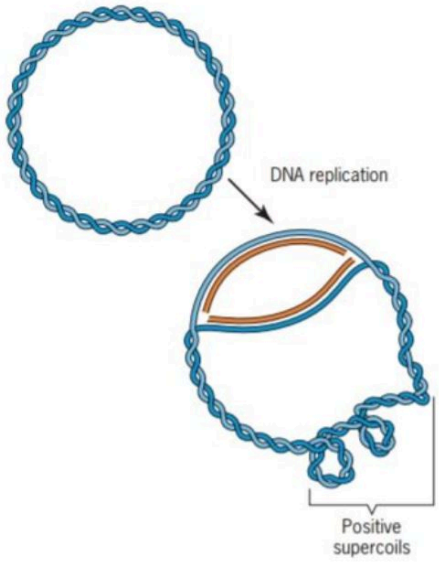
(b)

To unwind the template strands in *E. coli*, the DNA helix in front of the replication fork must spin at 3000 rpm.



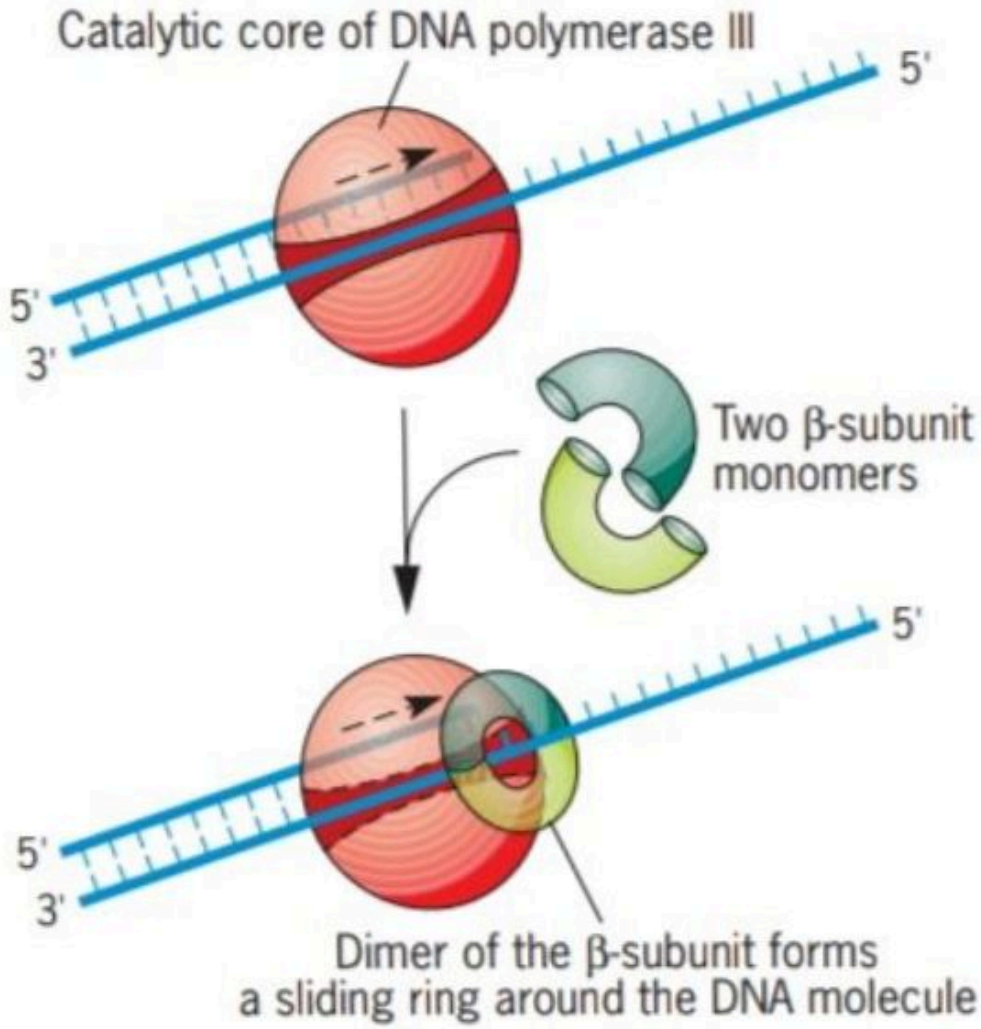
(a)

Without a swivel or axis of rotation, the unwinding process would produce positive supercoils in front of the replication forks.



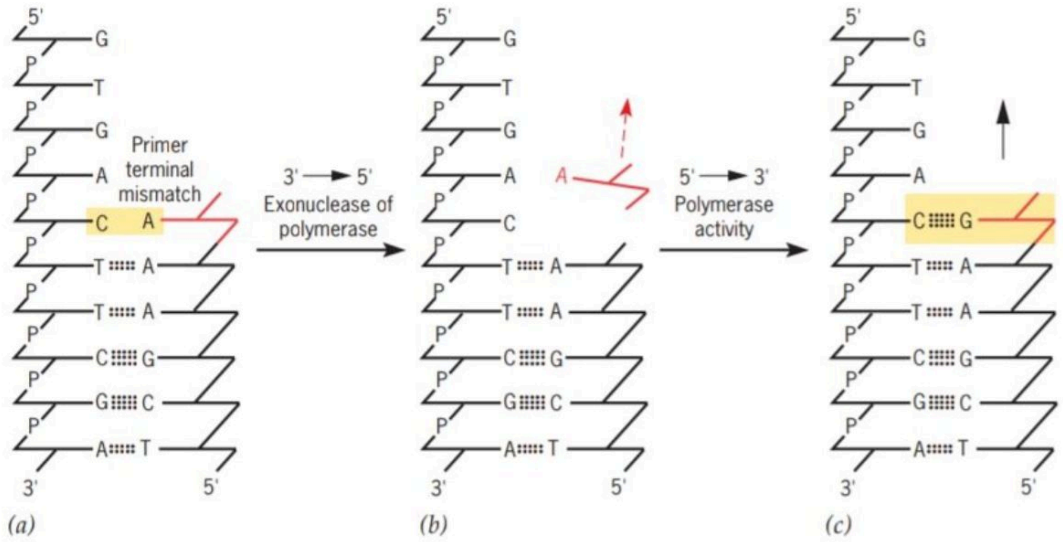
(b)

■ **FIGURE 10.20** A swivel or axis of rotation is required during the replication of circular molecules of DNA like those in the *E. coli* or phage λ chromosomes. (*a*) During replication, the DNA in front of a replication fork must spin to allow the strands to be unwound by the helicase. (*b*) In the absence of an axis of rotation, unwinding will result in the production of positive supercoils in the DNA in front of a replication fork.

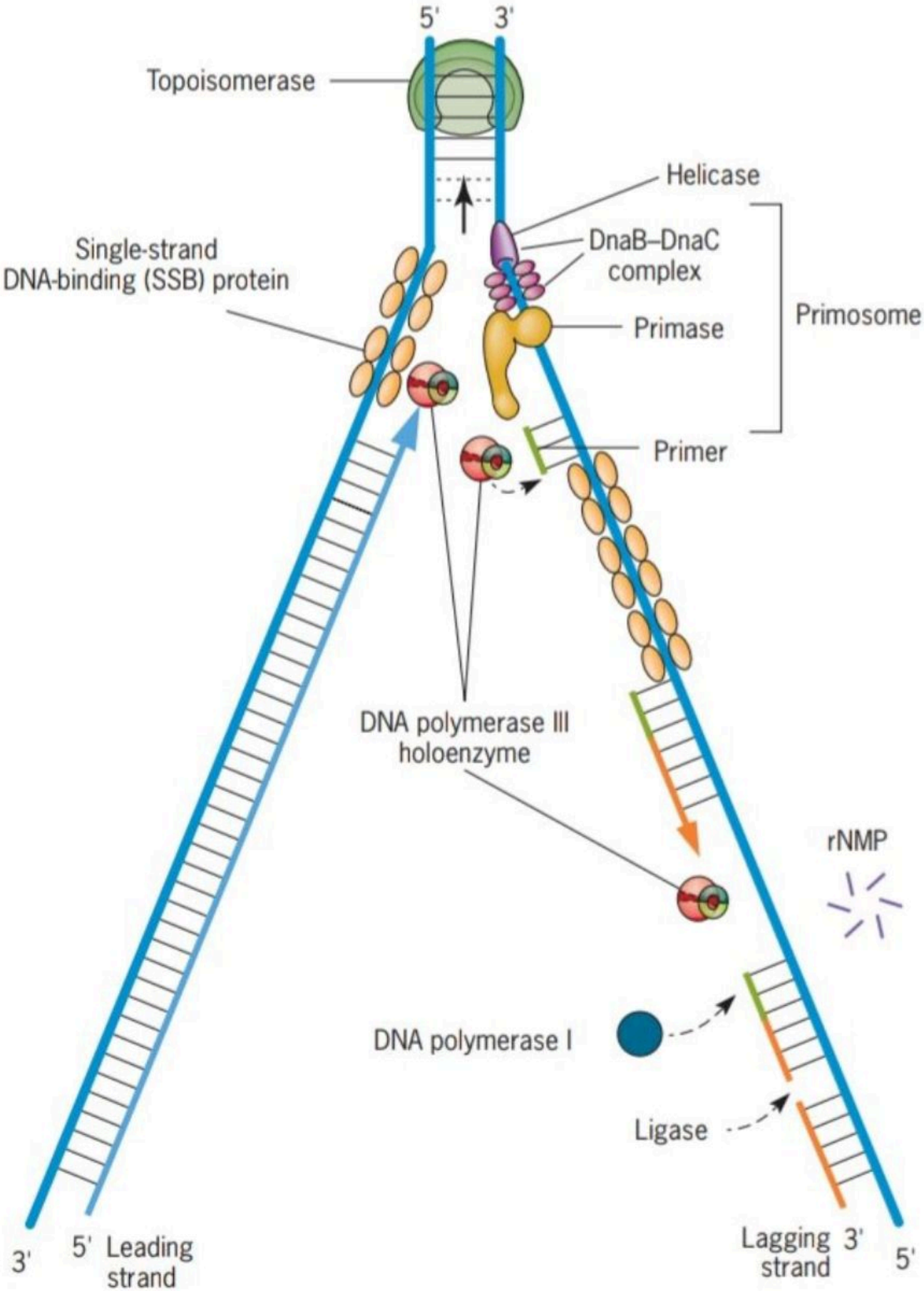


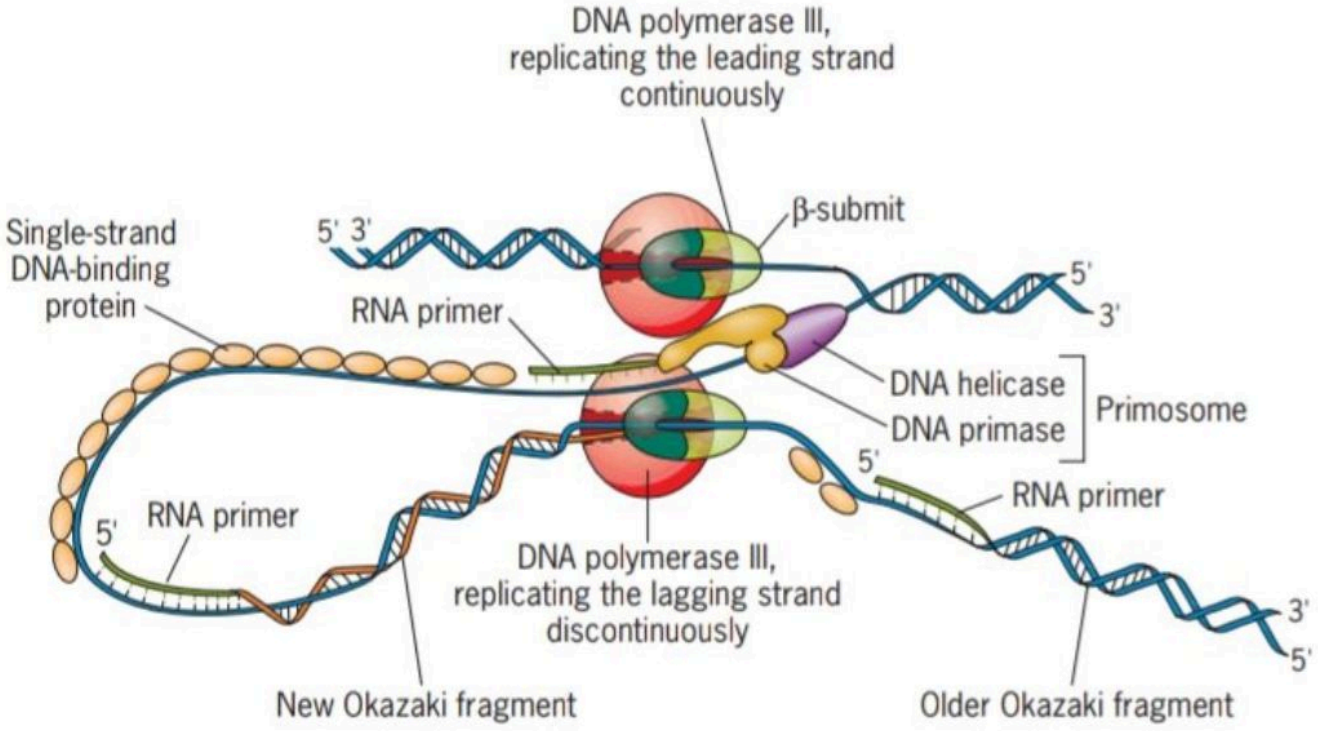
(b)

■ **FIGURE 10.25** Space-filling model (*a*) and diagram (*b*) showing how two β-subunits (light and dark green) of DNA polymerase III clamp the enzyme to the DNA molecule (blue).



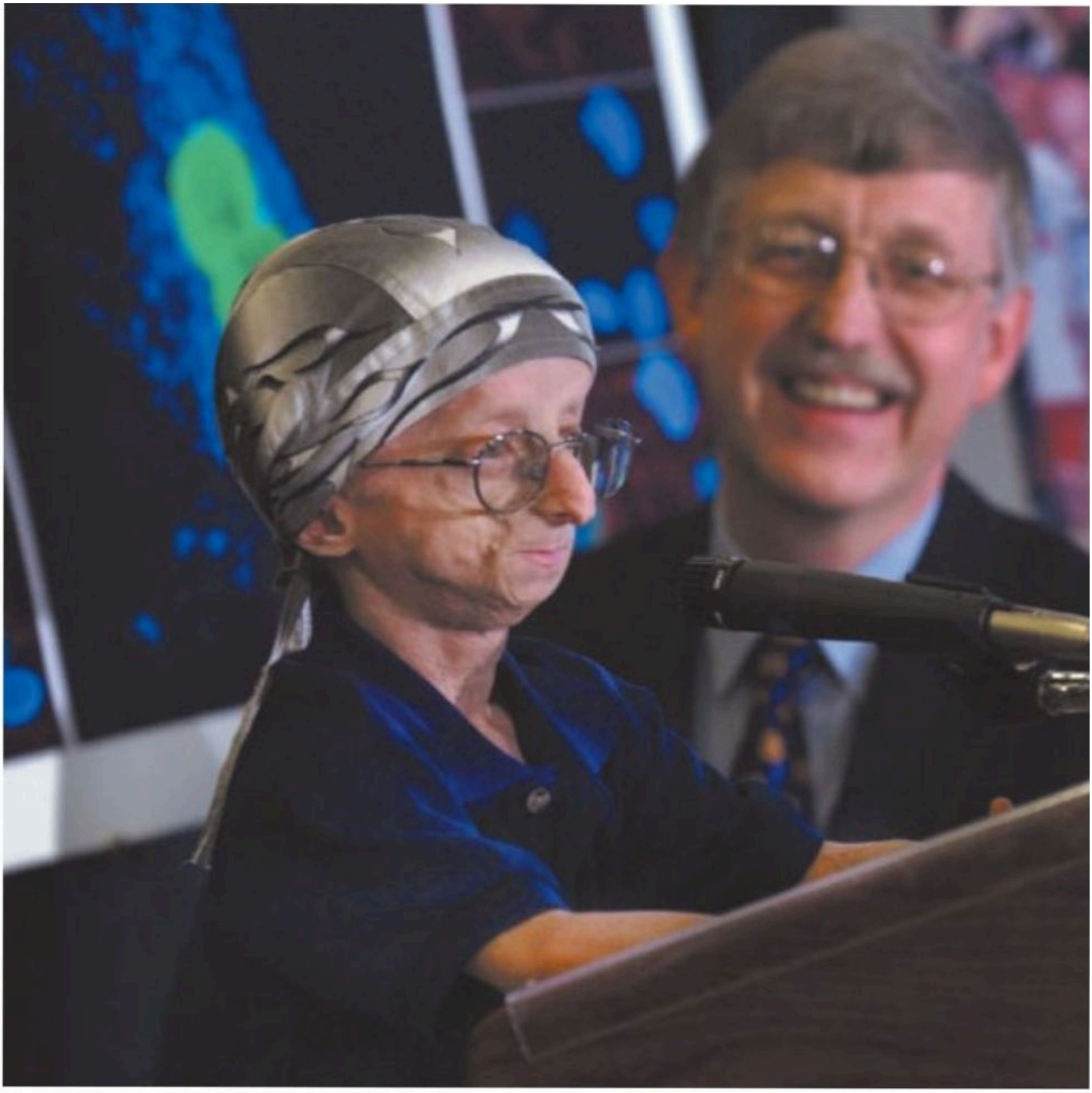
■ **FIGURE 10.27** Proofreading by the 3′ → 5′ exonuclease activity of DNA polymerases during DNA replication. As introduced in Figure 10.17, the DNA molecules are shown as "stick" diagrams. If DNA polymerase is presented with a template and primer containing a 3′ primer terminal mismatch (*a*), it will not catalyze covalent extension (polymerization). Instead, the 3′→ 5′ exonuclease activity, an integral part of many DNA polymerases, will cleave off the mismatched terminal nucleotide (*b*). Then, presented with a correctly base-paired primer terminus, DNA polymerase will catalyze 5′ → 3′ covalent extension of the primer strand (*c*).


5'
3'
Topoisomerase
Helicase
DnaB–DnaC complex
Primosome
Single-strand DNA-binding (SSB) protein
Primase
Primer
DNA polymerase III holoenzyme
rNMP
DNA polymerase I
Ligase
3'
5' Leading strand
Lagging strand 3'
5'

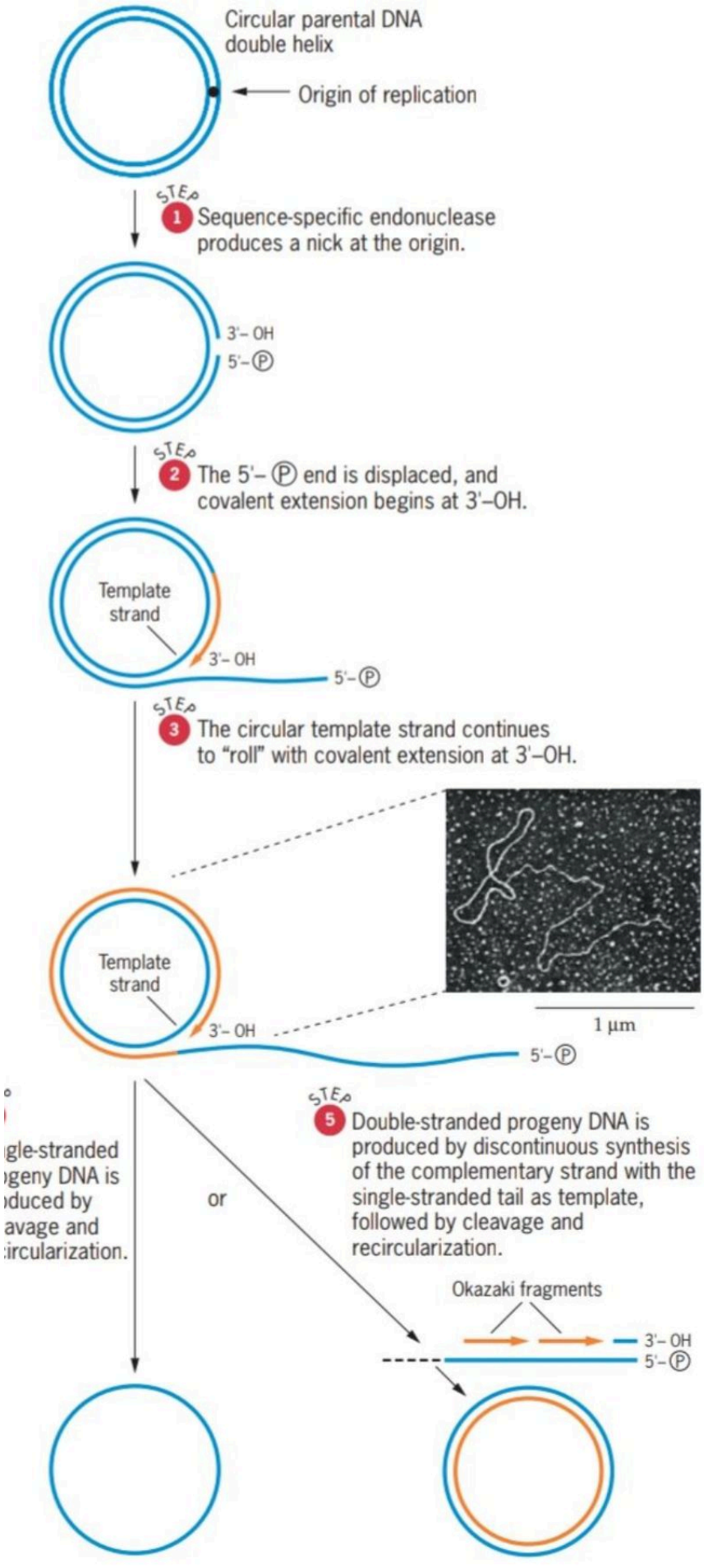

DNA polymerase III,
replicating the leading strand
continuously
β-submit
5' 3'
Single-strand
DNA-binding
protein
RNA primer
5'
3'
DNA helicase
DNA primase
Primosome
5'
RNA primer
5'
RNA primer
DNA polymerase III,
replicating the lagging strand
discontinuously
3'
5'
New Okazaki fragment
Older Okazaki fragment

KEY POINTS

- *DNA replication is complex, requiring the participation of a large number of proteins.*
- *DNA synthesis is continuous on the progeny strand that is being extended in the overall 5′→ 3′ direction, but is discontinuous on the strand growing in the overall 3′→ 5′ direction.*
- *New DNA chains are initiated by short RNA primers synthesized by DNA primase.*
- *DNA synthesis is catalyzed by enzymes called DNA polymerases.*
- *All DNA polymerases require a primer strand, which is extended, and a template strand, which is copied.*
- *All DNA polymerases have an absolute requirement for a free 3′-OH on the primer strand, and all DNA synthesis occurs in the 5′ to 3′ direction.*
- *The 3′→ 5′ exonuclease activities of DNA polymerases proofread nascent strands as they are synthesized, removing any mispaired nucleotides at the 3′ termini of primer strands.*
- *The enzymes and DNA-binding proteins involved in replication assemble into a replisome at each replication fork and act in concert as the fork moves along the parental DNA molecule.*



■ **FIGURE 10.35** John Tacket, 15, of Bay City, Michigan, speaks about his illness, progeria, during a news conference called in Washington, April 16, 2003, to announce the discovery of the gene that causes this rare, fatal genetic condition, characterized by the appearance of accelerated aging. To Tacket's right is Dr. Francis S. Collins, director of the National Institutes of Health.


Circular parental DNA double helix
Origin of replication
STEP 1
Sequence-specific endonuclease produces a nick at the origin.
3'– OH
5'–Ⓟ
STEP 2
The 5'–Ⓟ end is displaced, and covalent extension begins at 3'–OH.
Template strand
3'– OH
5'–Ⓟ
STEP 3
The circular template strand continues to "roll" with covalent extension at 3'–OH.
Template strand
3'– OH
5'–Ⓟ
1 µm
gle-stranded
geny DNA is
duced by
avage and
ircularization.
or
STEP 5
Double-stranded progeny DNA is produced by discontinuous synthesis of the complementary strand with the single-stranded tail as template, followed by cleavage and recircularization.
Okazaki fragments
3'– OH
5'–Ⓟ