DNA replication, DNA repair, and DNA recombination

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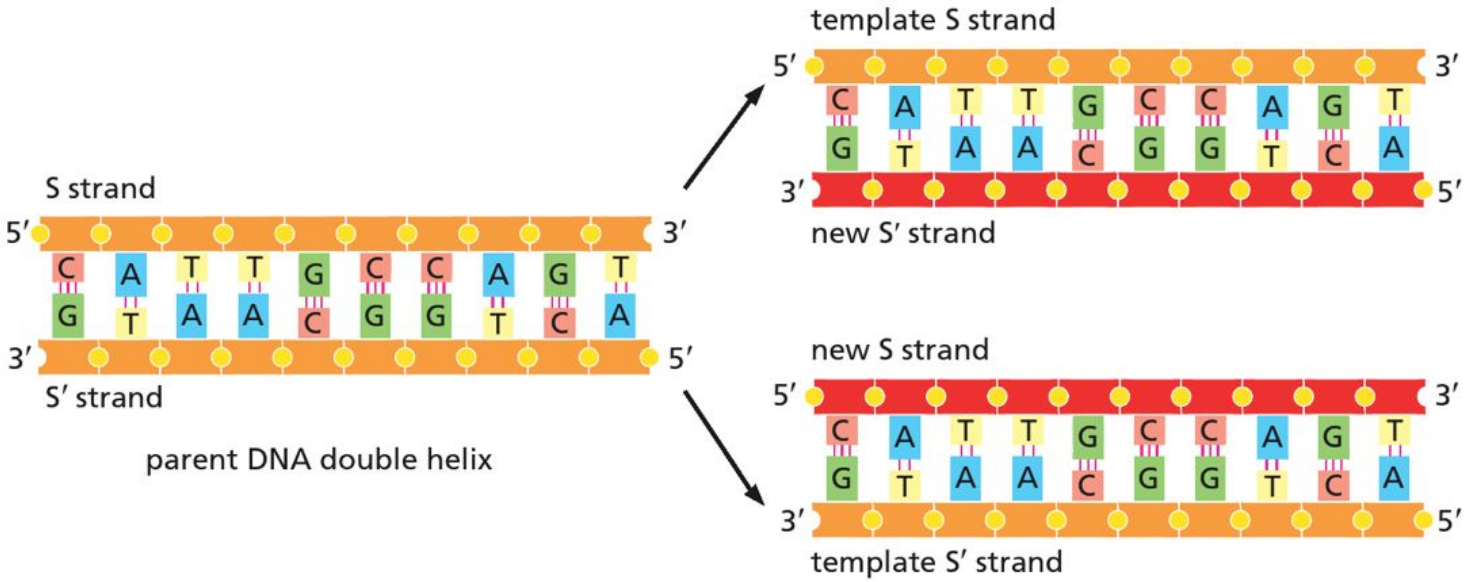
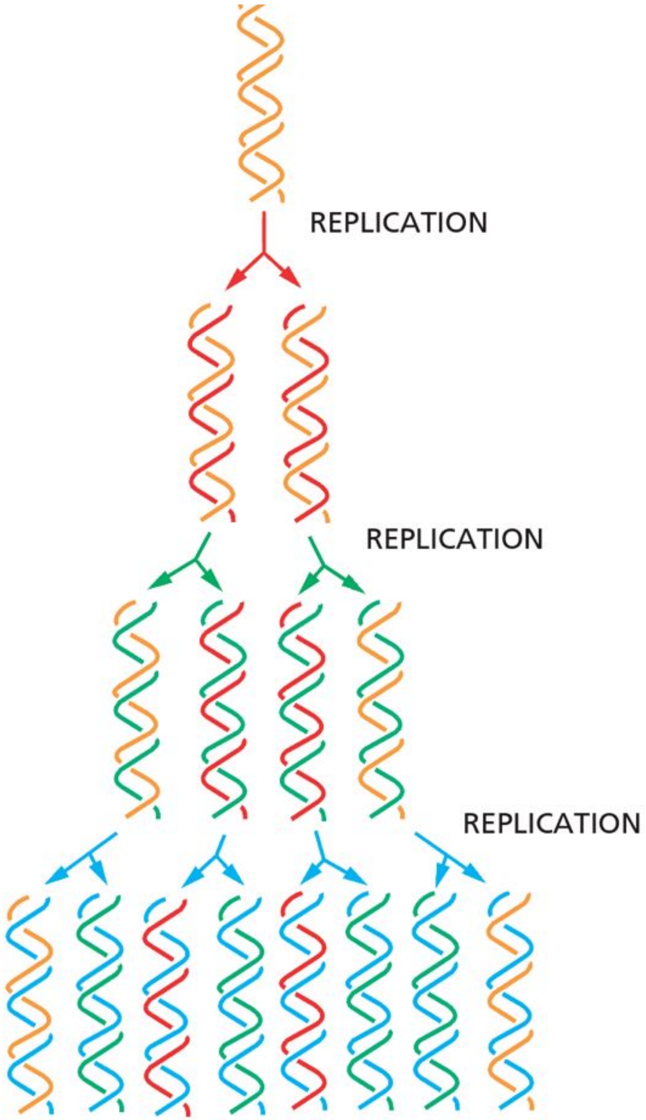
DNA replication


S strand
5′ C A T T G C C A G T 3′
3′ G T A A C G G T C A 5′
S′ strand
parent DNA double helix
template S strand
5′ C A T T G C C A G T 3′
3′ G T A A C G G T C A 5′
new S′ strand
new S strand
5′ C A T T G C C A G T 3′
3′ G T A A C G G T C A 5′
template S′ strand



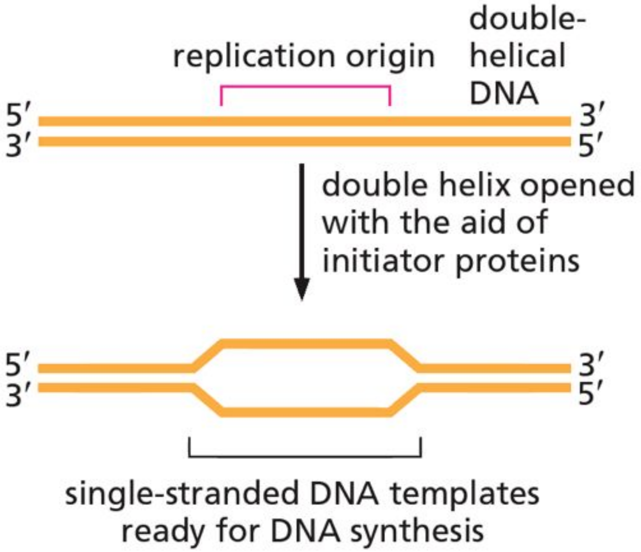


Figure 6–4 A DNA double helix is opened at replication origins. DNA sequences at replication origins are recognized by initiator proteins (not shown), which locally pry apart the two strands of the double helix. The exposed single strands can then serve as templates for copying the DNA.

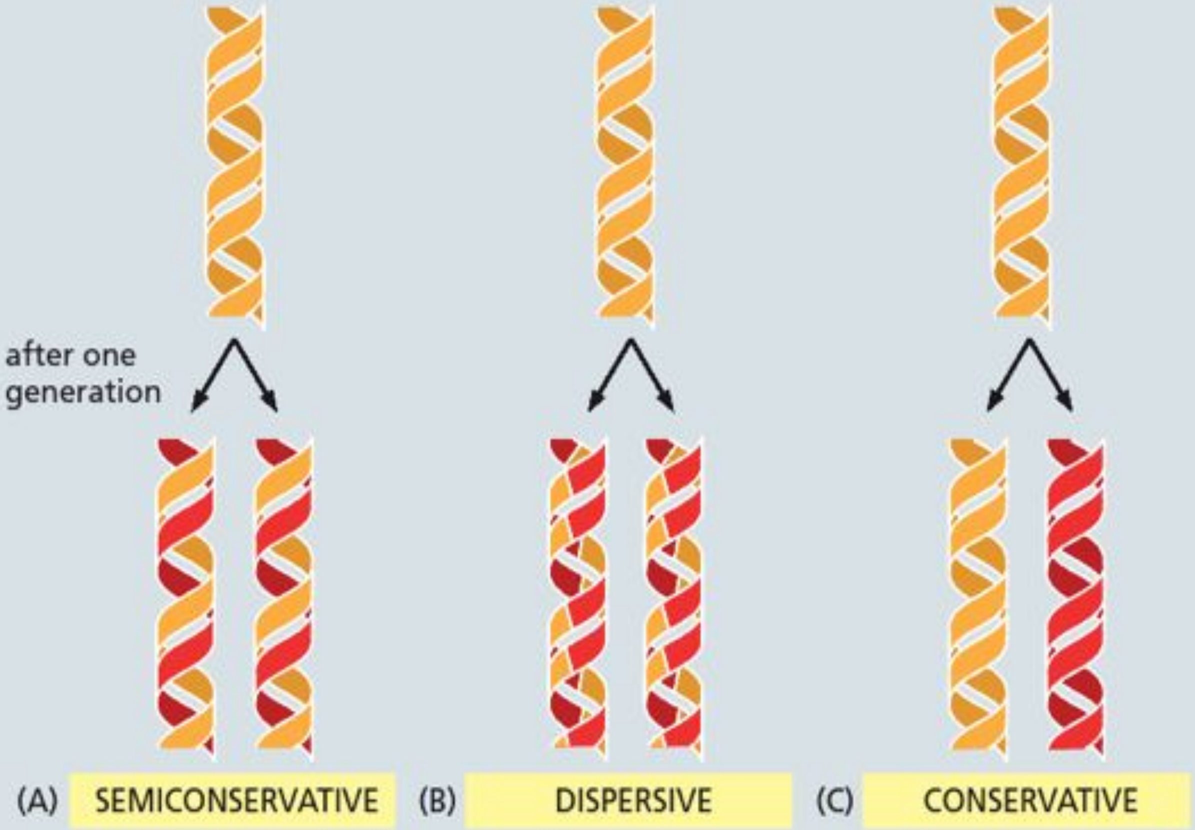


Figure 6–5 Three models for DNA replication make different predictions. (A) In the semiconservative model, each parent strand serves as a template for the synthesis of a new daughter strand. The first round of replication would produce two hybrid molecules, each containing one strand from the original parent in addition to one newly synthesized strand. A subsequent round of replication would yield two hybrid molecules and two molecules that contain none of the original parent DNA (see Figure 6–3). (B) In the dispersive model, each generation of daughter DNA will contain a mixture of DNA from the parent strands and the newly synthesized DNA. (C) In the conservative model, the parent molecule remains intact after being copied. In this case, the first round of replication would yield the original parent double helix and an entirely new double helix. For each model, parent DNA molecules are shown in *orange*; newly replicated DNA is *red*. Note that only a very small segment of DNA is shown for each model.

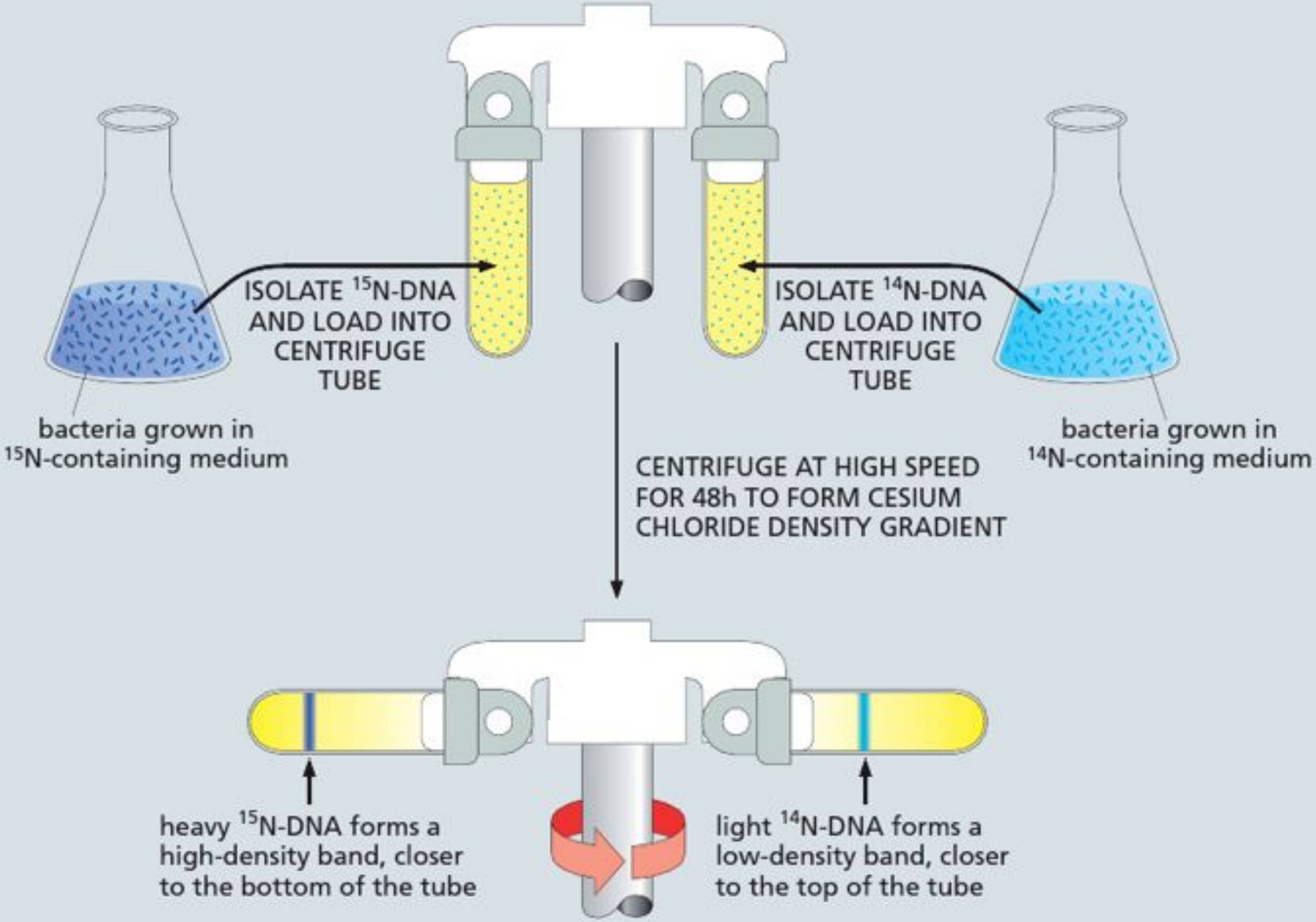
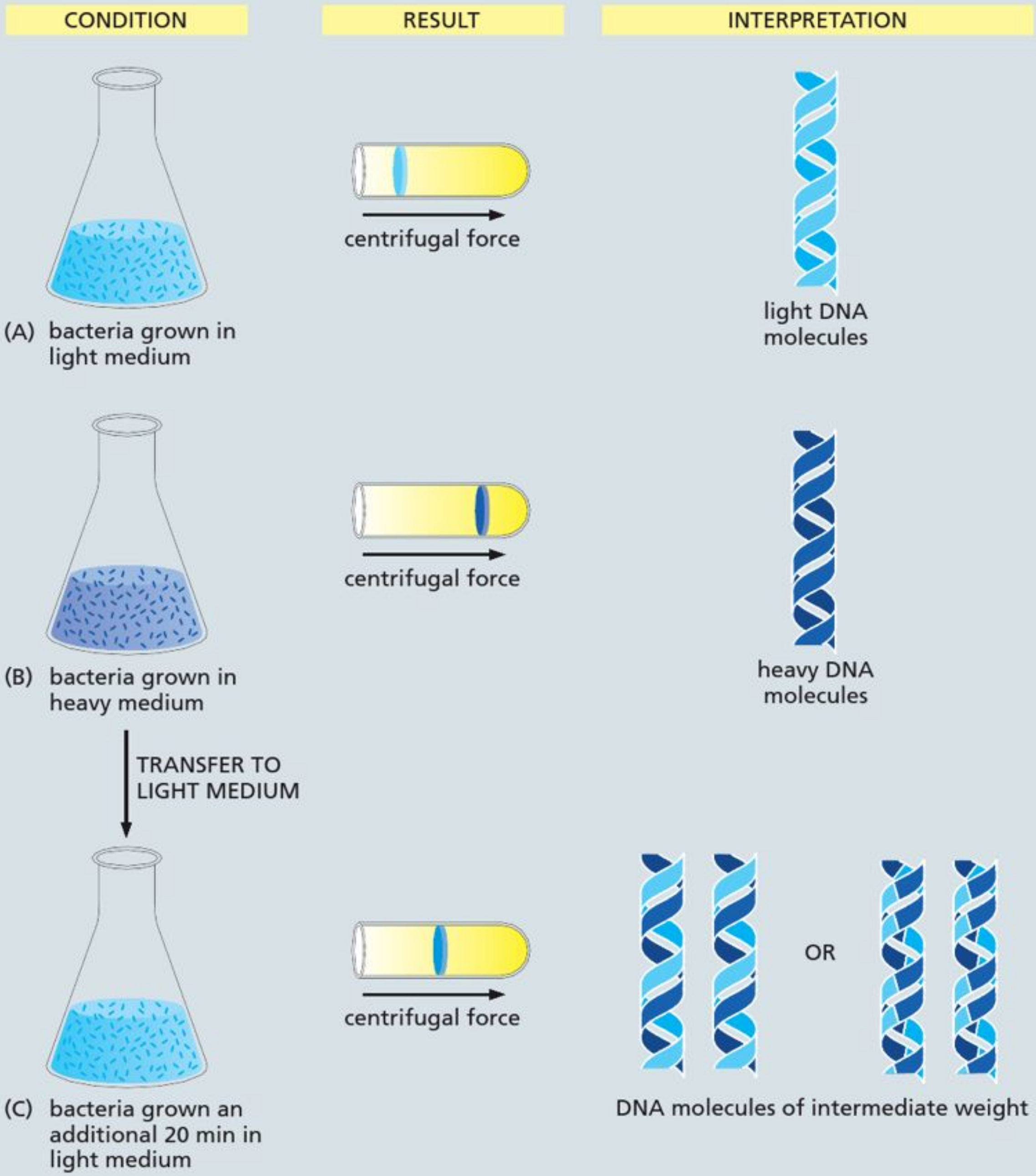


Figure 6–6 Centrifugation in a cesium chloride gradient allows the separation of heavy and light DNA. Bacteria are grown for several generations in a medium containing either ^{15}N (the heavy isotope) or ^{14}N (the light isotope) to label their DNA. The cells are then broken open, and the DNA is loaded into an ultracentrifuge tube containing a cesium chloride salt solution. These tubes are centrifuged at high speed for two days to allow the DNA to collect in a region where its density matches that of the salt surrounding it. The heavy and light DNA molecules collect in different positions in the tube.


CONDITION
RESULT
INTERPRETATION
centrifugal force
light DNA molecules
(A) bacteria grown in light medium
centrifugal force
heavy DNA molecules
(B) bacteria grown in heavy medium
TRANSFER TO LIGHT MEDIUM
centrifugal force
OR
(C) bacteria grown an additional 20 min in light medium
DNA molecules of intermediate weight

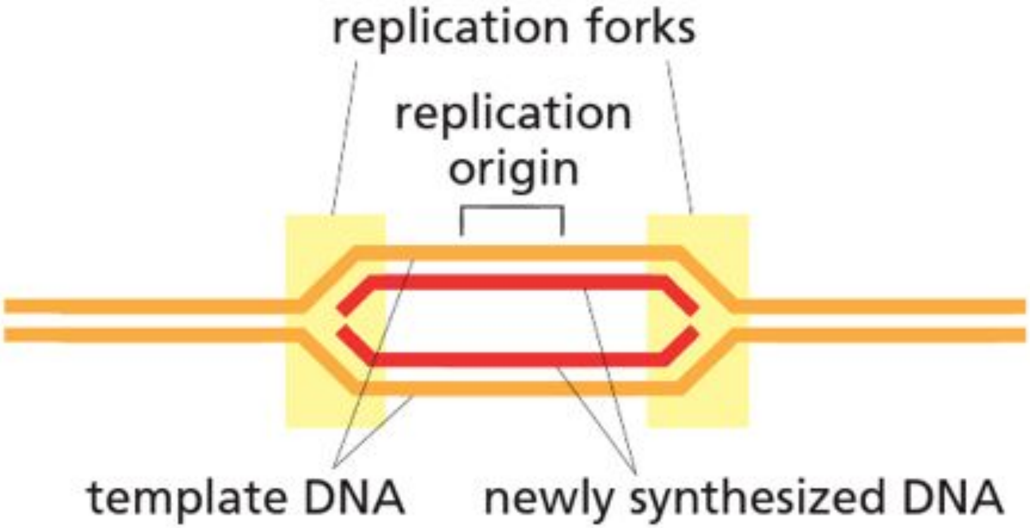
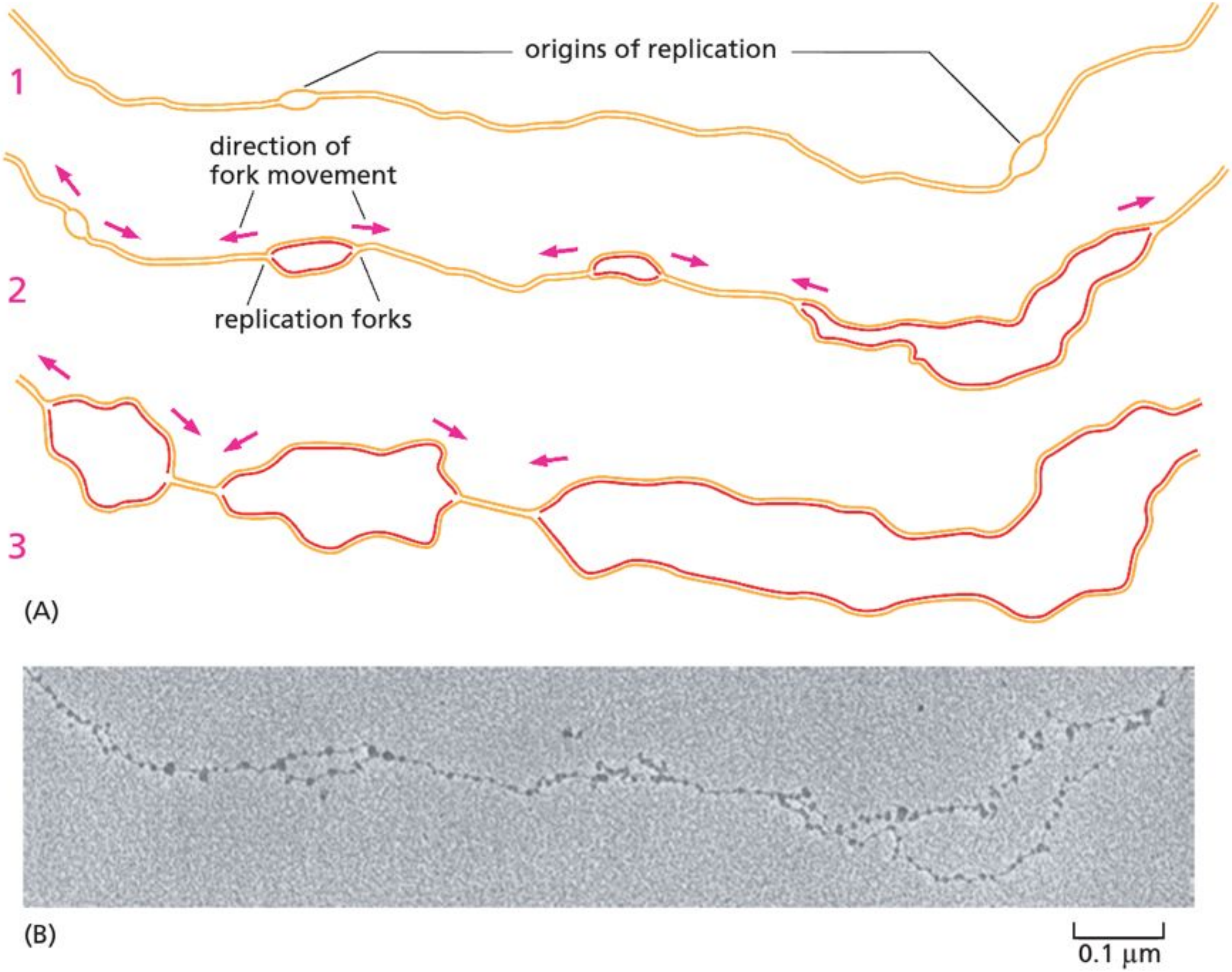


Figure 6–8 DNA synthesis occurs at Y-shaped junctions called replication forks. Two replication forks are formed at each replication origin.


origins of replication
1
direction of fork movement
2
replication forks
3
(A)
(B)
0.1 µm

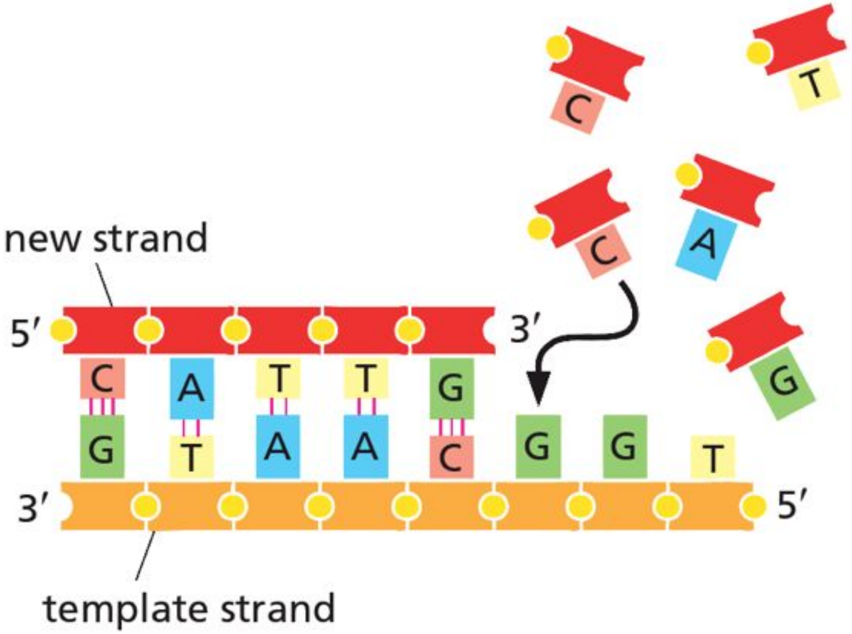


Figure 6–10 A new DNA strand is synthesized in the 5′–to–3′ direction. At each step, the appropriate incoming nucleotide is selected by forming base pairs with the next nucleotide in the template strand: A with T, T with A, C with G, and G with C. Each is added to the 3′ end of the growing new strand, as indicated.

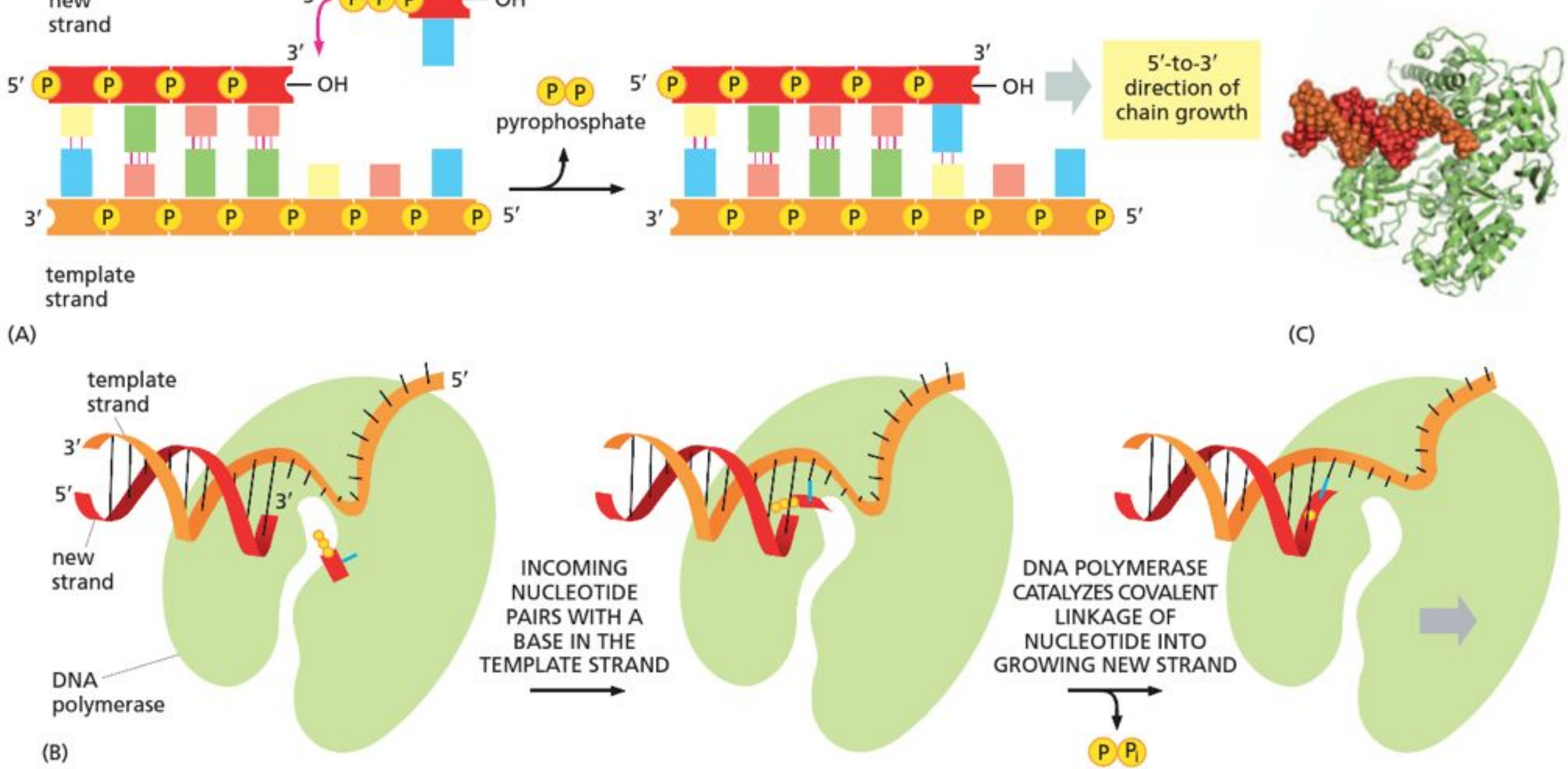


Figure 6–11 DNA polymerase adds a deoxyribonucleotide to the 3′ end of a growing DNA chain. (A) Nucleotides enter the reaction as deoxyribonucleoside triphosphates. This incoming nucleotide forms a base pair with its partner in the template strand. It is then linked to the free 3′ hydroxyl on the growing DNA strand. The new DNA strand is therefore synthesized in the 5′-to-3′ direction. Breakage of a high-energy phosphate bond in the incoming nucleoside triphosphate—accompanied by the release of pyrophosphate—provides the energy for the polymerization reaction. (B) The reaction is catalyzed by the enzyme DNA polymerase (*light green*). The polymerase guides the incoming nucleotide to the template strand and positions it such that its 5′ terminal phosphate will be able to react with the 3′-hydroxyl group on the newly synthesized strand. The *gray arrow* indicates the direction of polymerase movement. (C) Structure of DNA polymerase, as determined by X-ray crystallography, which shows the positioning of the DNA double helix. The template strand is the longer of the two DNA strands (Movie 6.1).

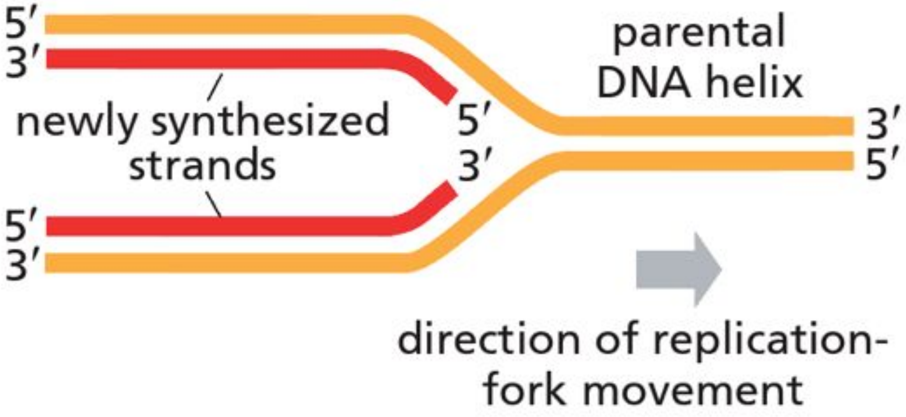
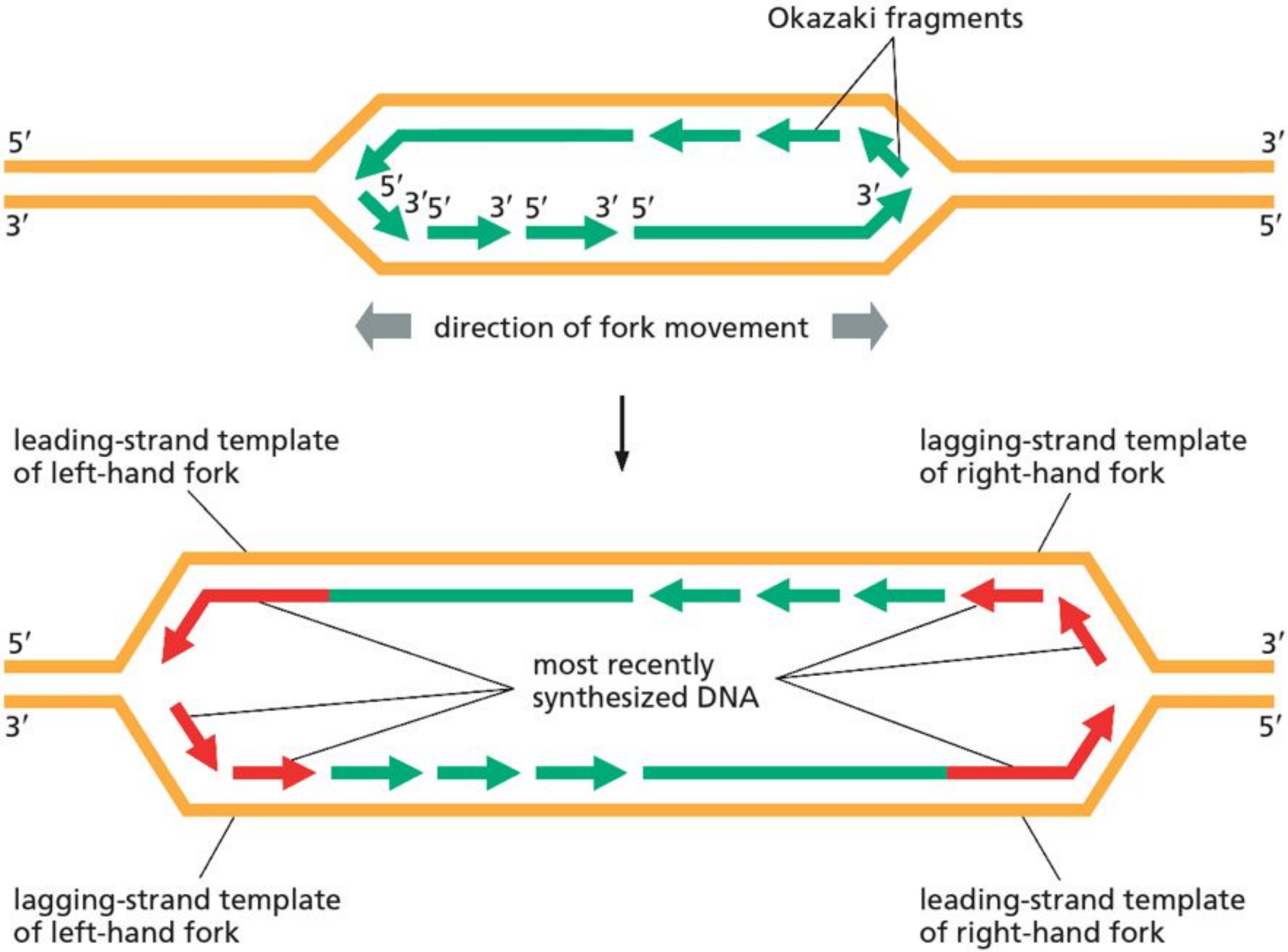


Figure 6–12 At a replication fork, the two newly synthesized DNA strands are of opposite polarities. This is because the two template strands are oriented in opposite directions.


Okazaki fragments
5′
3′
3′
5′
5′
3′
5′
3′
5′
3′
5′
3′
direction of fork movement
leading-strand template of left-hand fork
lagging-strand template of right-hand fork
5′
3′
3′
5′
most recently synthesized DNA
lagging-strand template of left-hand fork
leading-strand template of right-hand fork

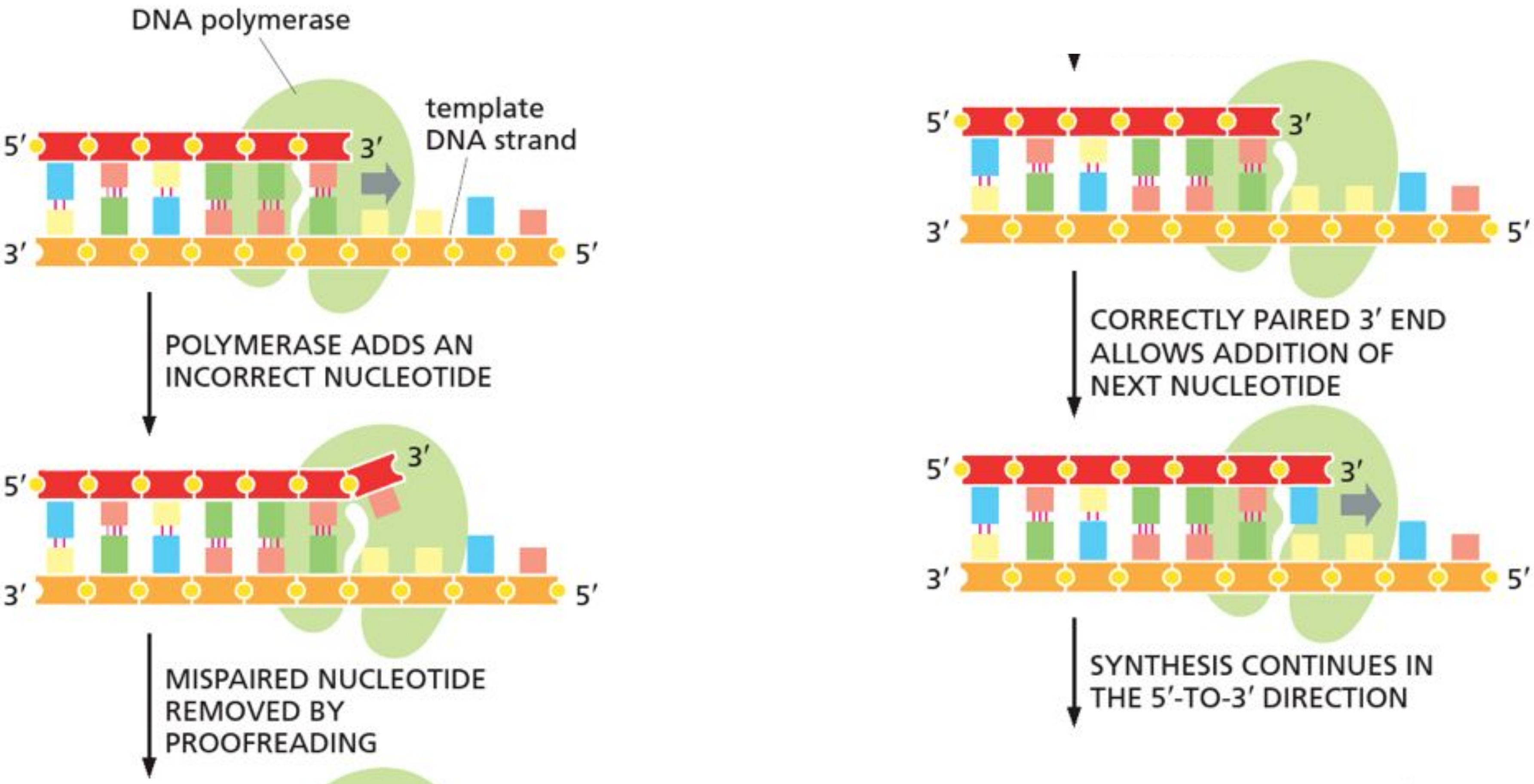
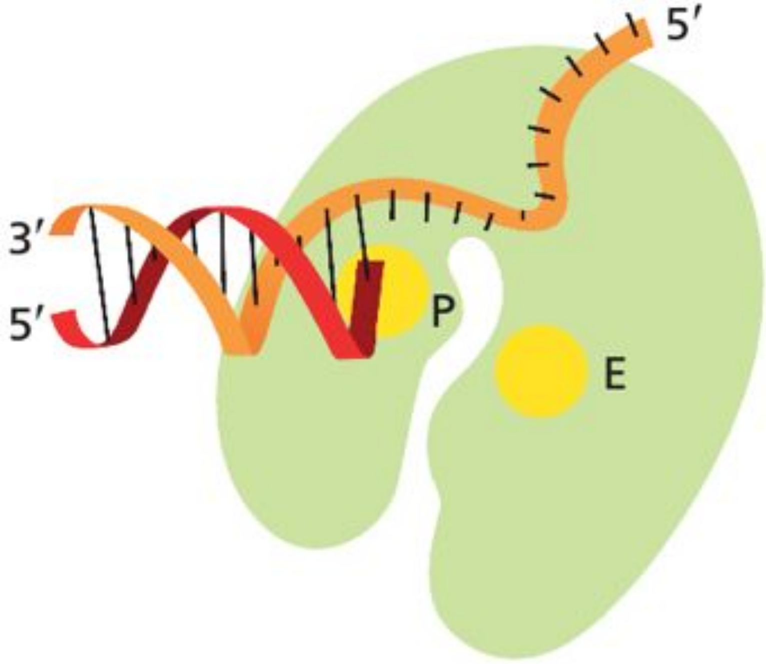
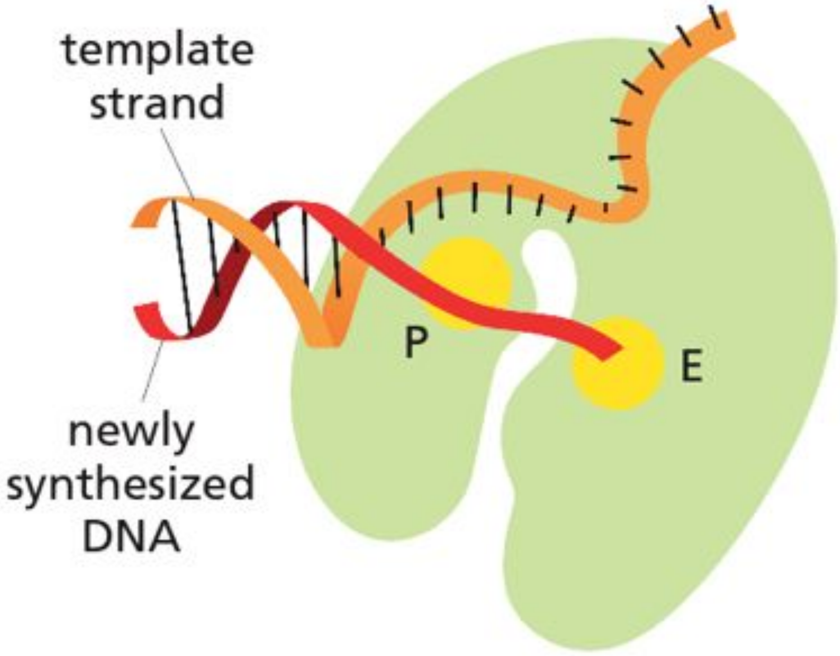


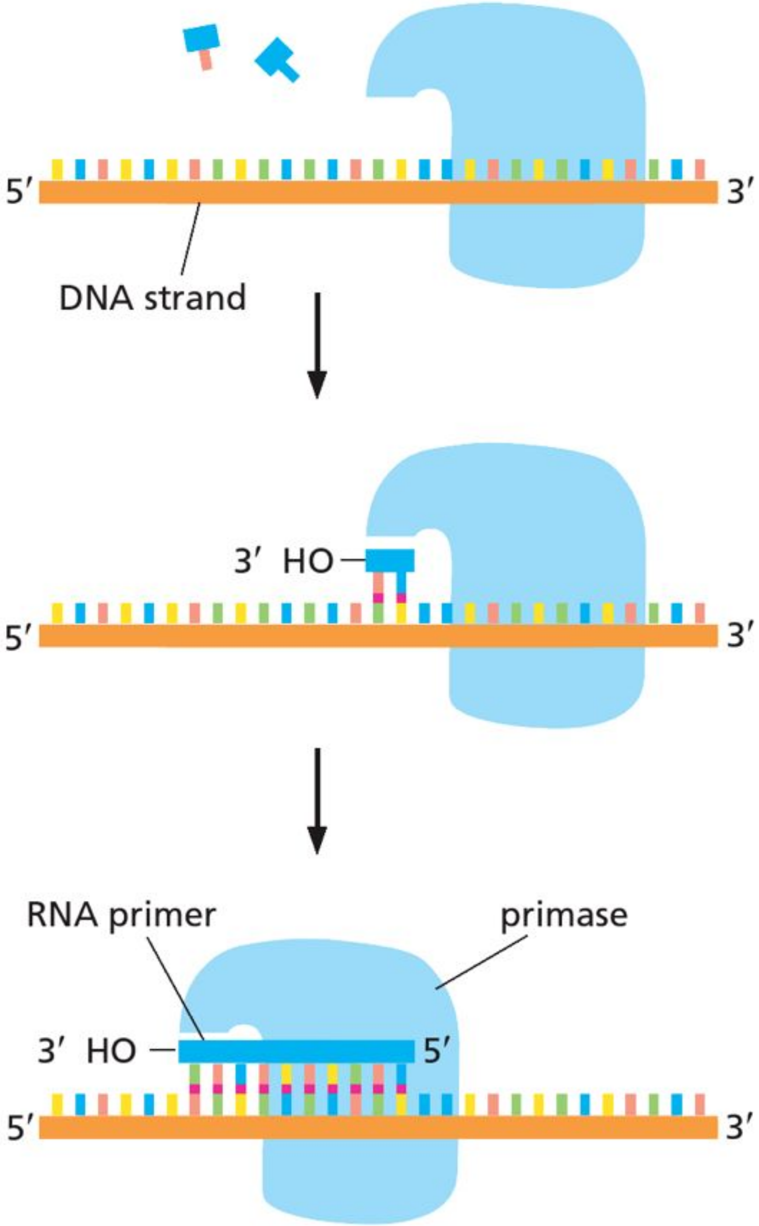
Figure 6–14 During DNA synthesis, DNA polymerase proofreads its own work. If an incorrect nucleotide is added to a growing strand, the DNA polymerase cleaves it from the strand and replaces it with the correct nucleotide before continuing.

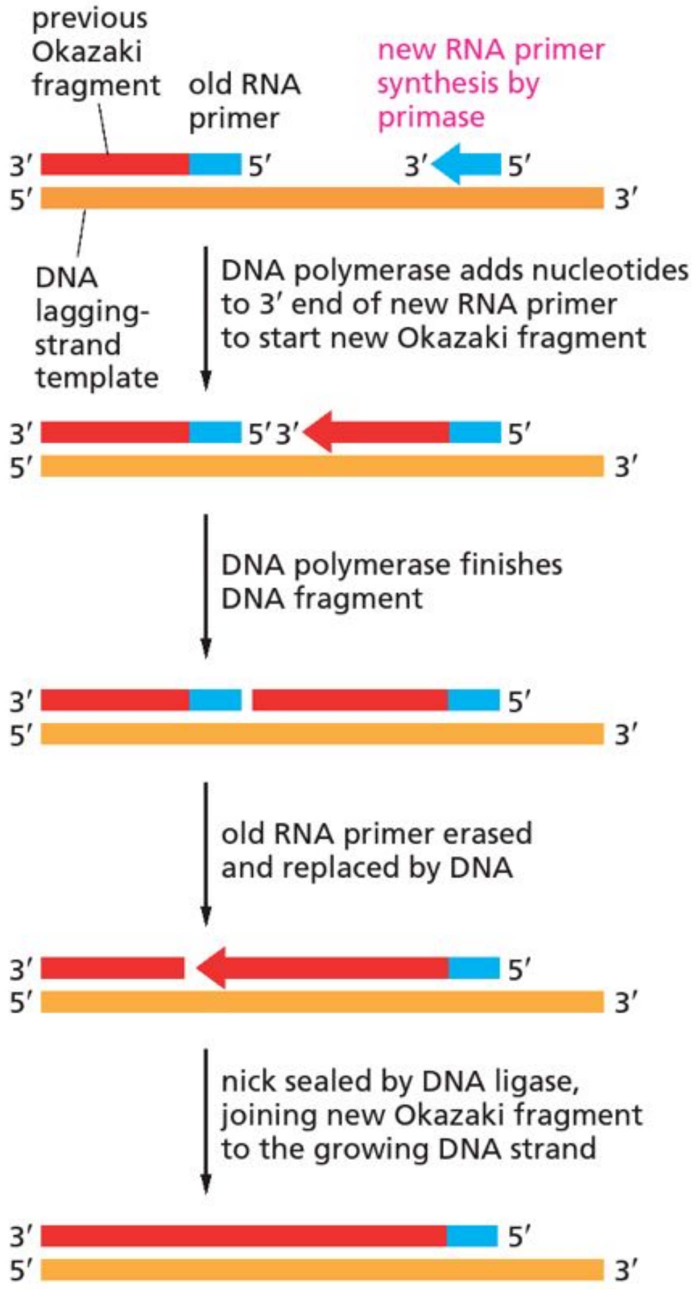


POLYMERIZING



EDITING


5′
3′
DNA strand
3′ HO
5′
3′
RNA primer
primase
3′ HO
5′
5′
3′

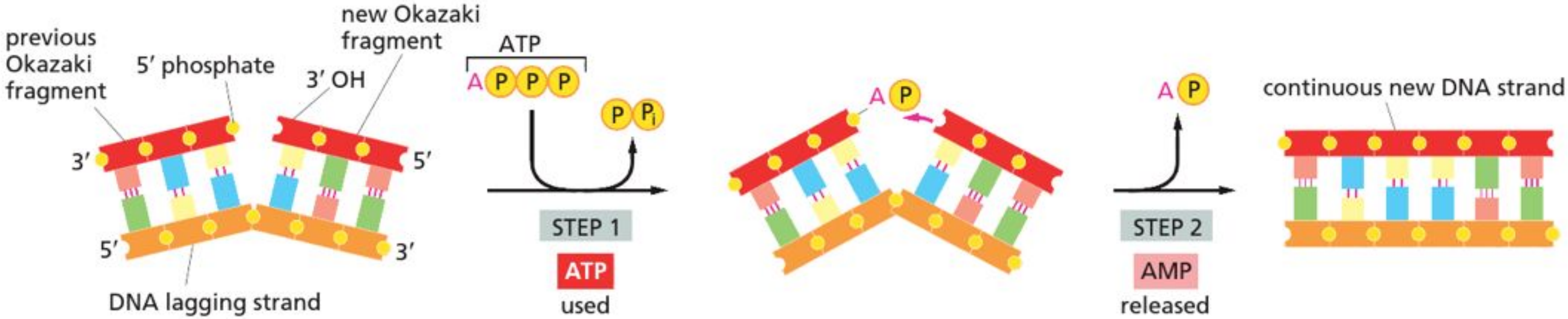


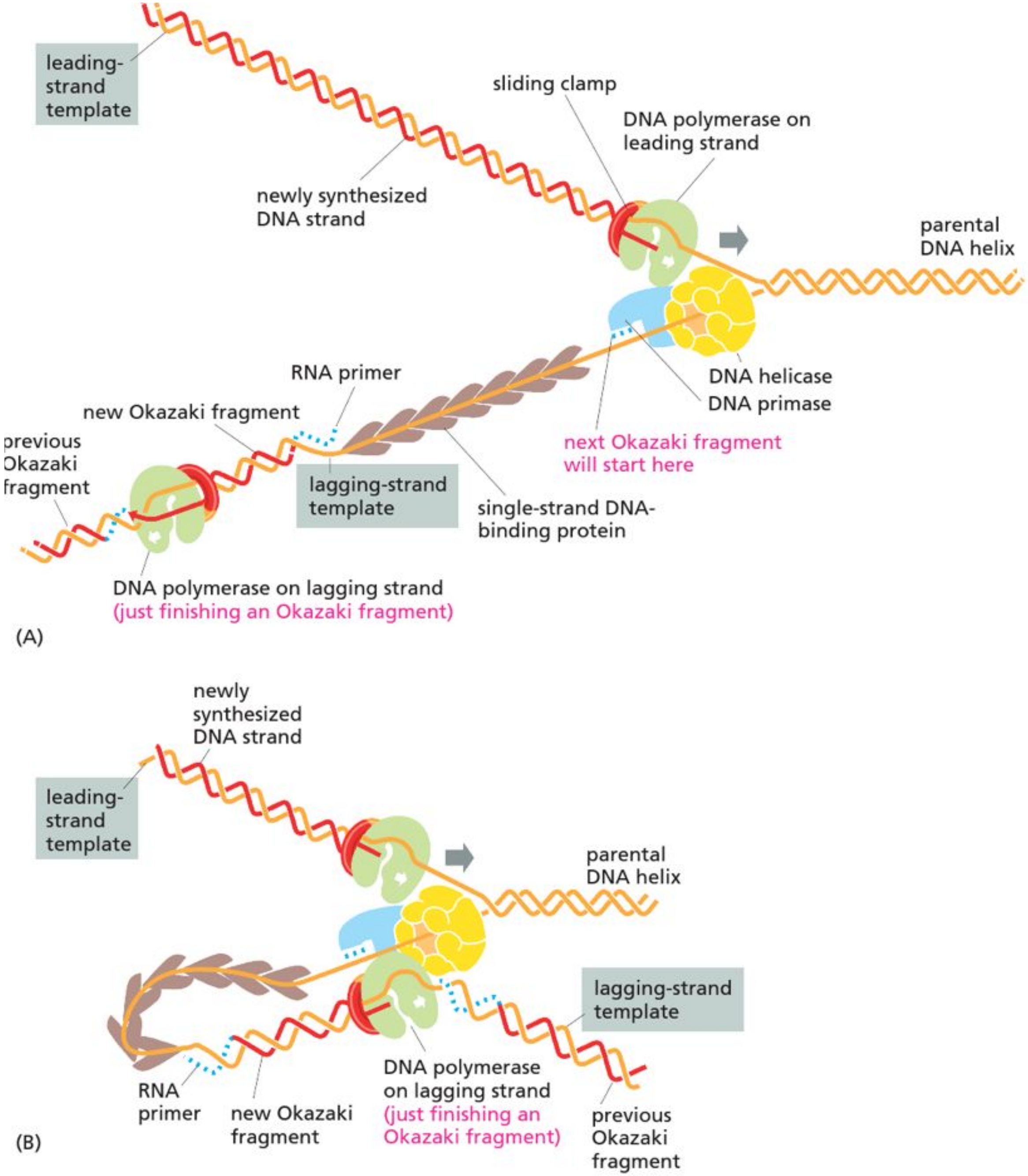
DNA polymerase adds nucleotides to 3′ end of new RNA primer to start new Okazaki fragment

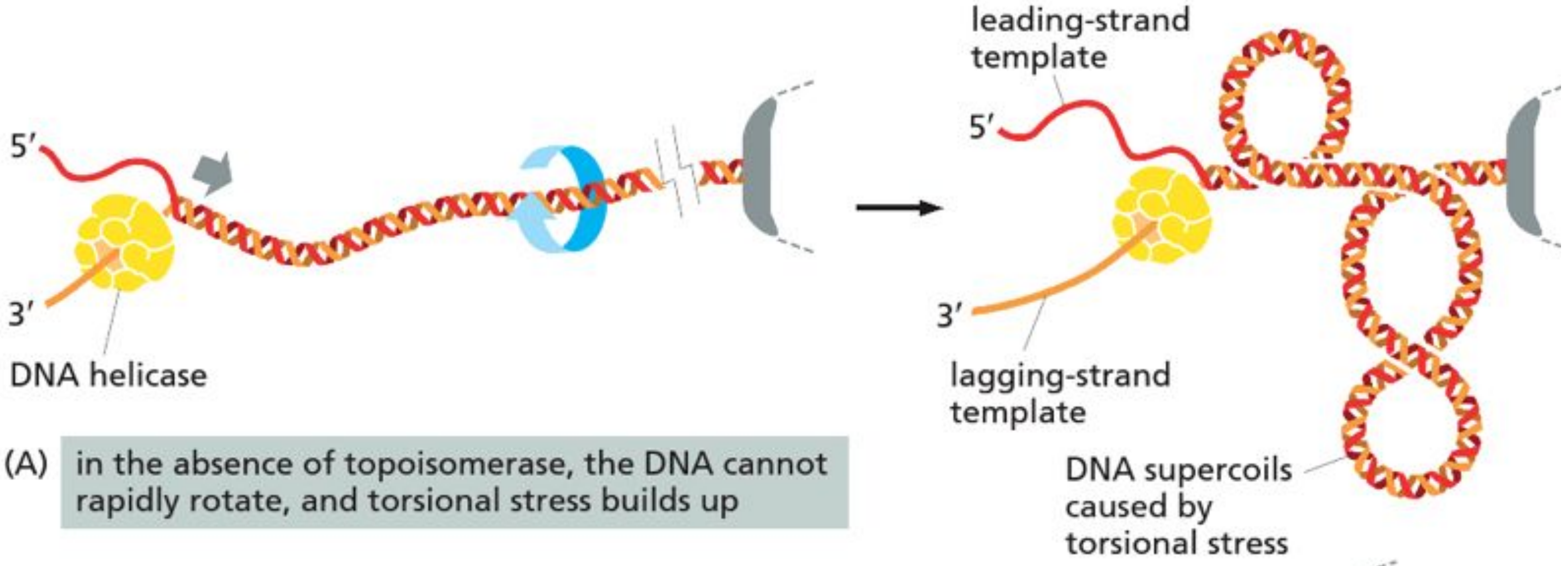
DNA polymerase finishes DNA fragment

old RNA primer erased and replaced by DNA

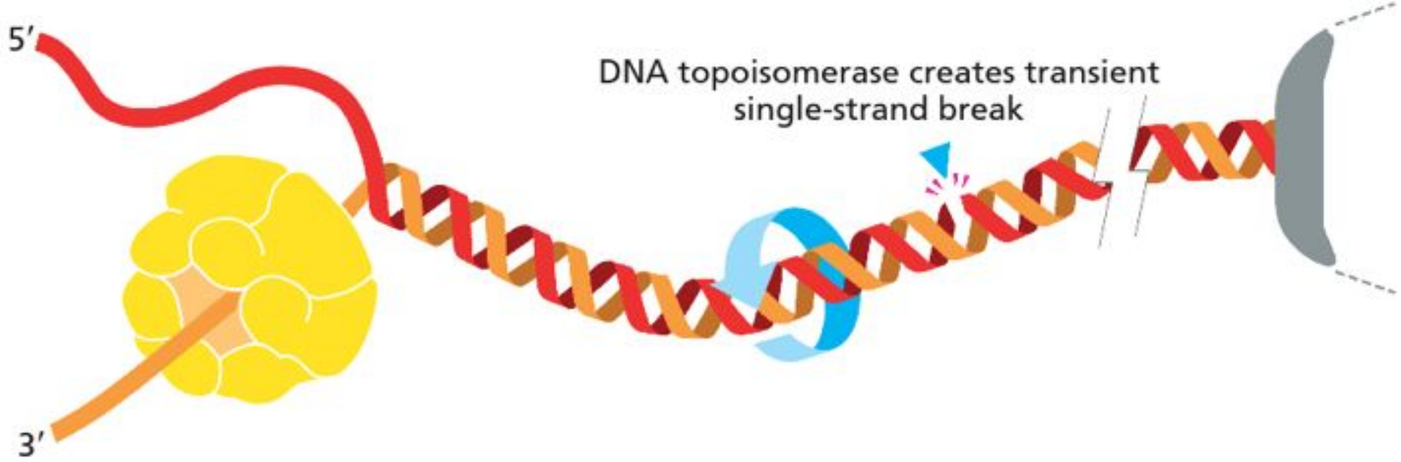
nick sealed by DNA ligase, joining new Okazaki fragment to the growing DNA strand

previous Okazaki fragment
5′ phosphate
new Okazaki fragment
3′ OH
3′
5′
5′
3′
DNA lagging strand
ATP
A P P P
P Pi
STEP 1
ATP
used
A P
A P
STEP 2
AMP
released
continuous new DNA strand


leading-strand template
newly synthesized DNA strand
sliding clamp
DNA polymerase on leading strand
parental DNA helix
DNA helicase
DNA primase
RNA primer
new Okazaki fragment
previous Okazaki fragment
lagging-strand template
single-strand DNA-binding protein
next Okazaki fragment will start here
DNA polymerase on lagging strand
(just finishing an Okazaki fragment)
(A)
newly synthesized DNA strand
leading-strand template
parental DNA helix
lagging-strand template
RNA primer
new Okazaki fragment
DNA polymerase on lagging strand
(just finishing an Okazaki fragment)
previous Okazaki fragment
(B)



(A) in the absence of topoisomerase, the DNA cannot rapidly rotate, and torsional stress builds up



(B) free rotation of double helix about phosphodiester bond relieves torsional stress ahead of helicase, after which single-strand break is sealed

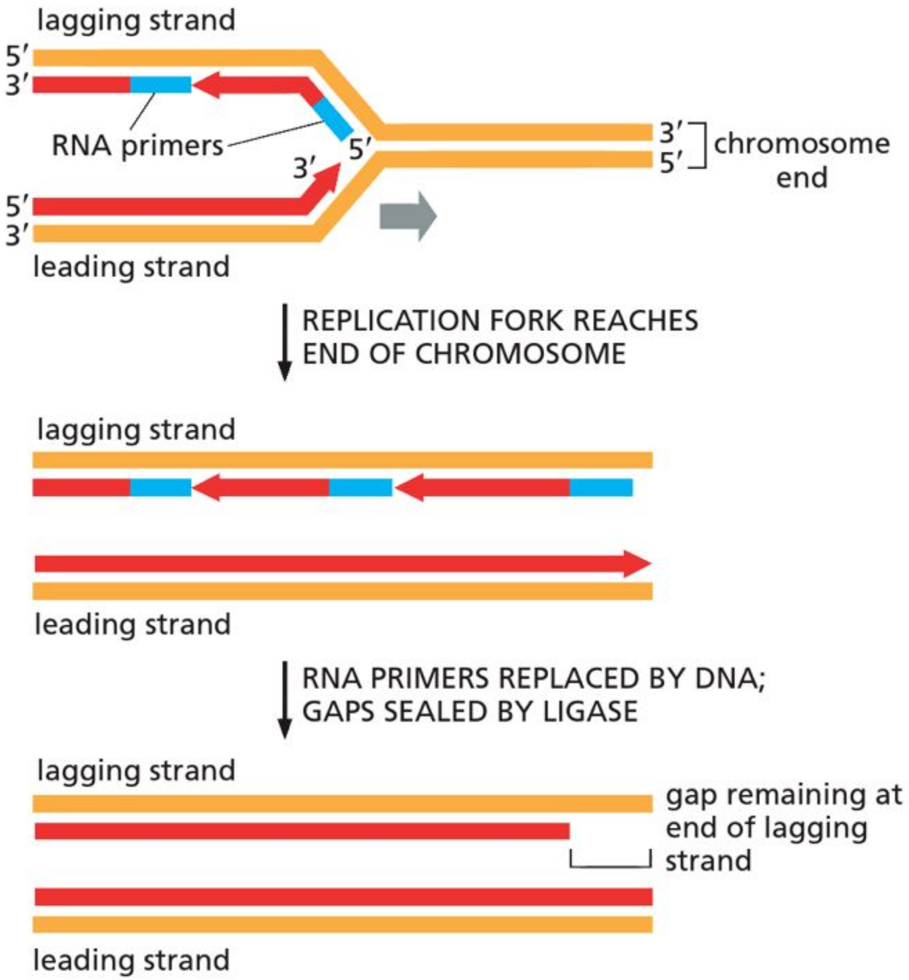


Figure 6–21 Without a special mechanism to replicate the ends of linear chromosomes, DNA would be lost during each round of cell division. DNA synthesis begins at origins of replication and continues until the replication machinery reaches the ends of the chromosome. The leading strand is reproduced in its entirety. But the ends of the lagging strand can't be completed, because once the final RNA primer has been removed there is no way to replace it with DNA. These gaps at the ends of the lagging strand must be filled in by a special mechanism to keep the chromosome ends from shrinking with each cell division.

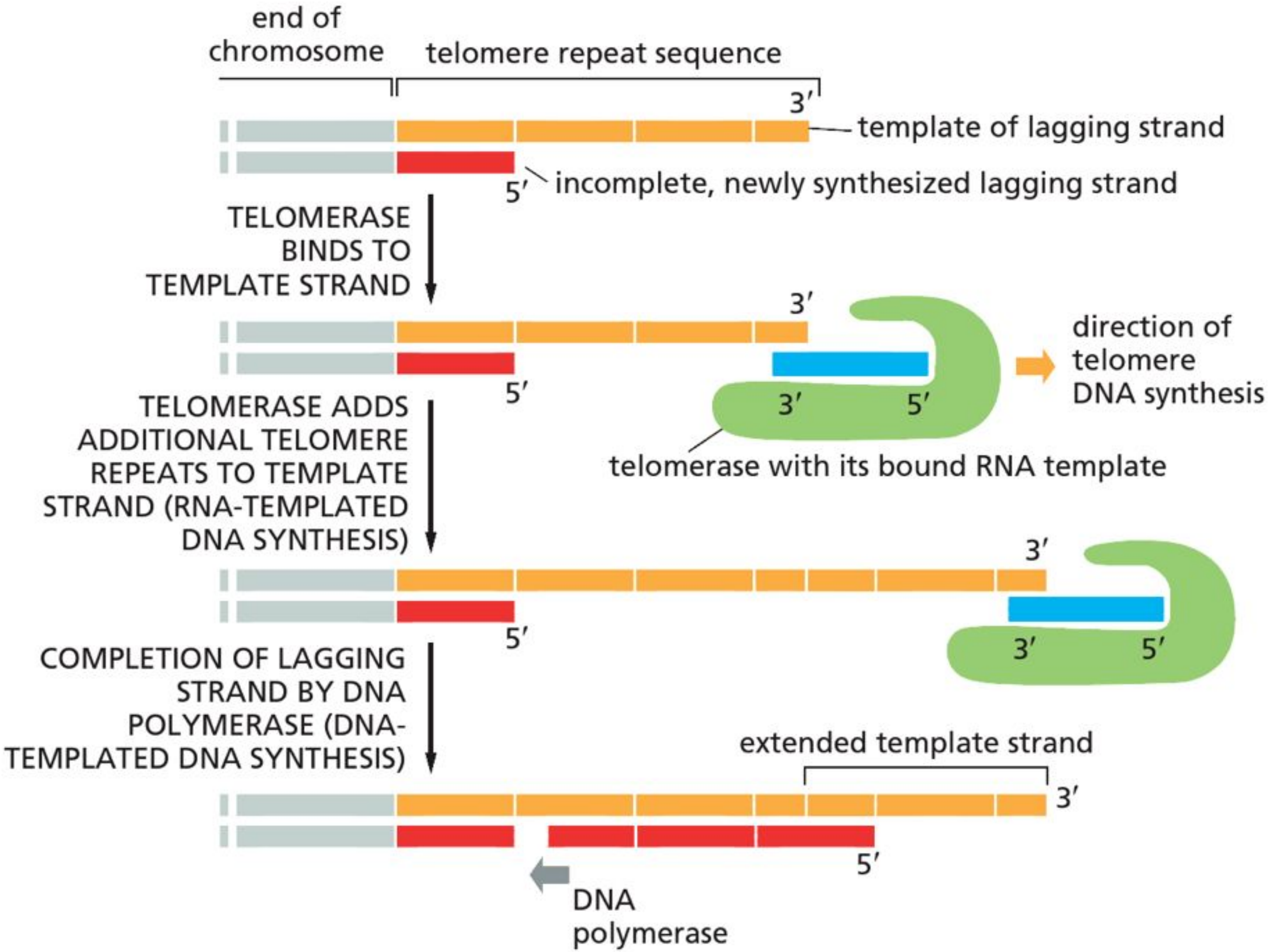
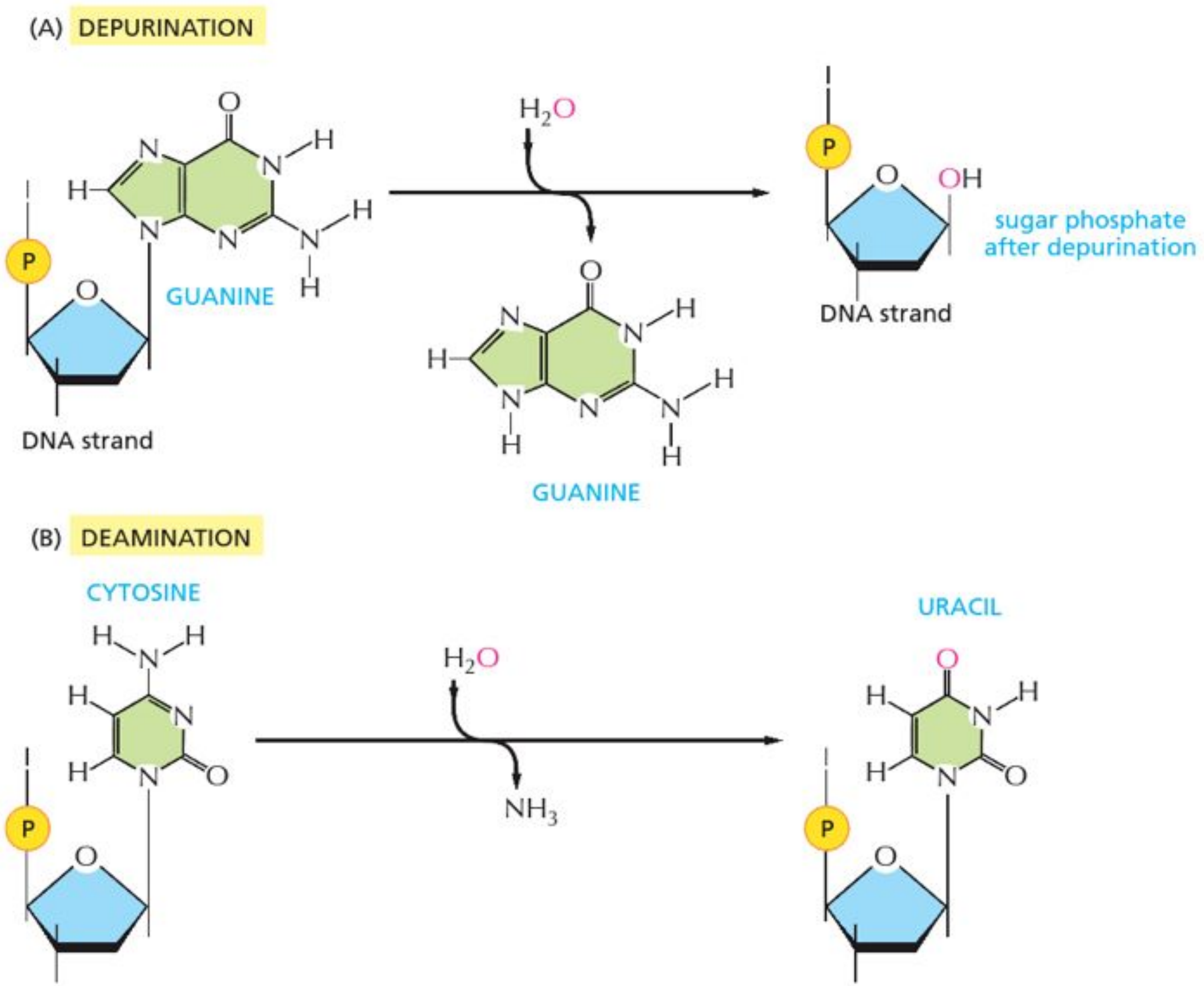
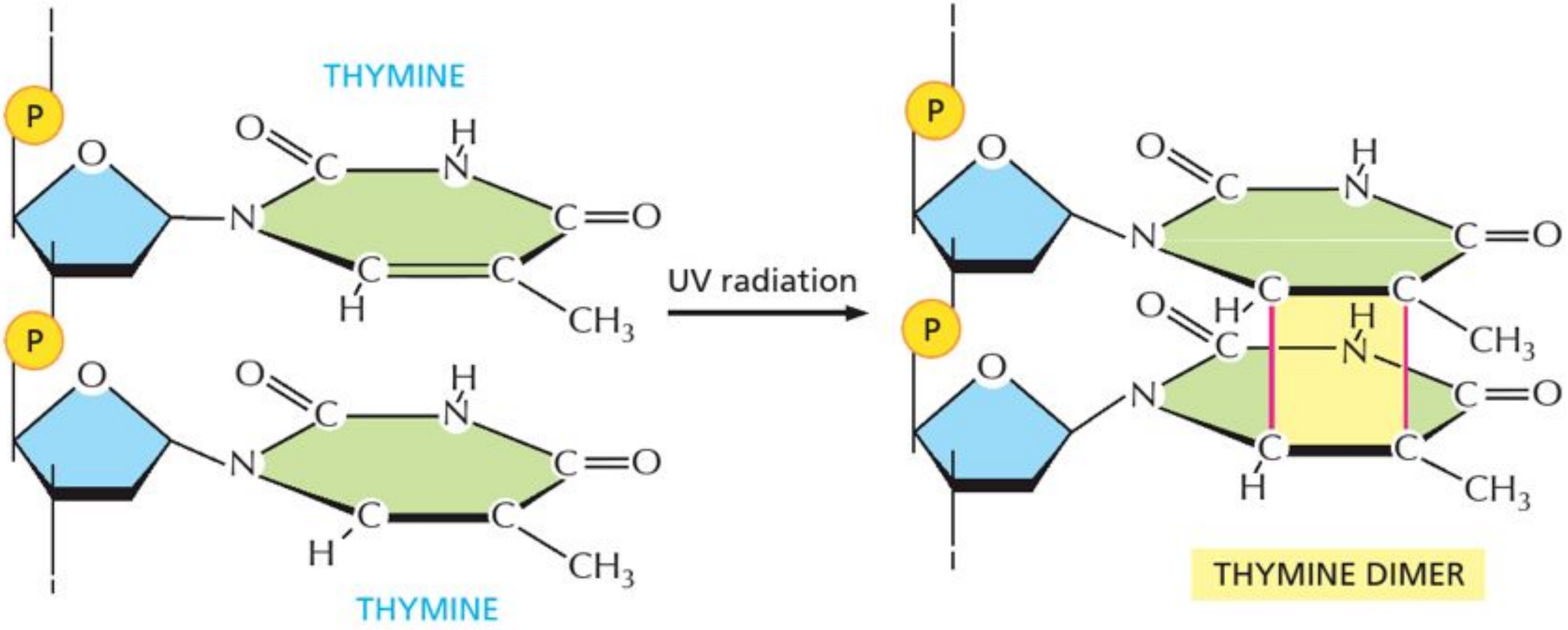
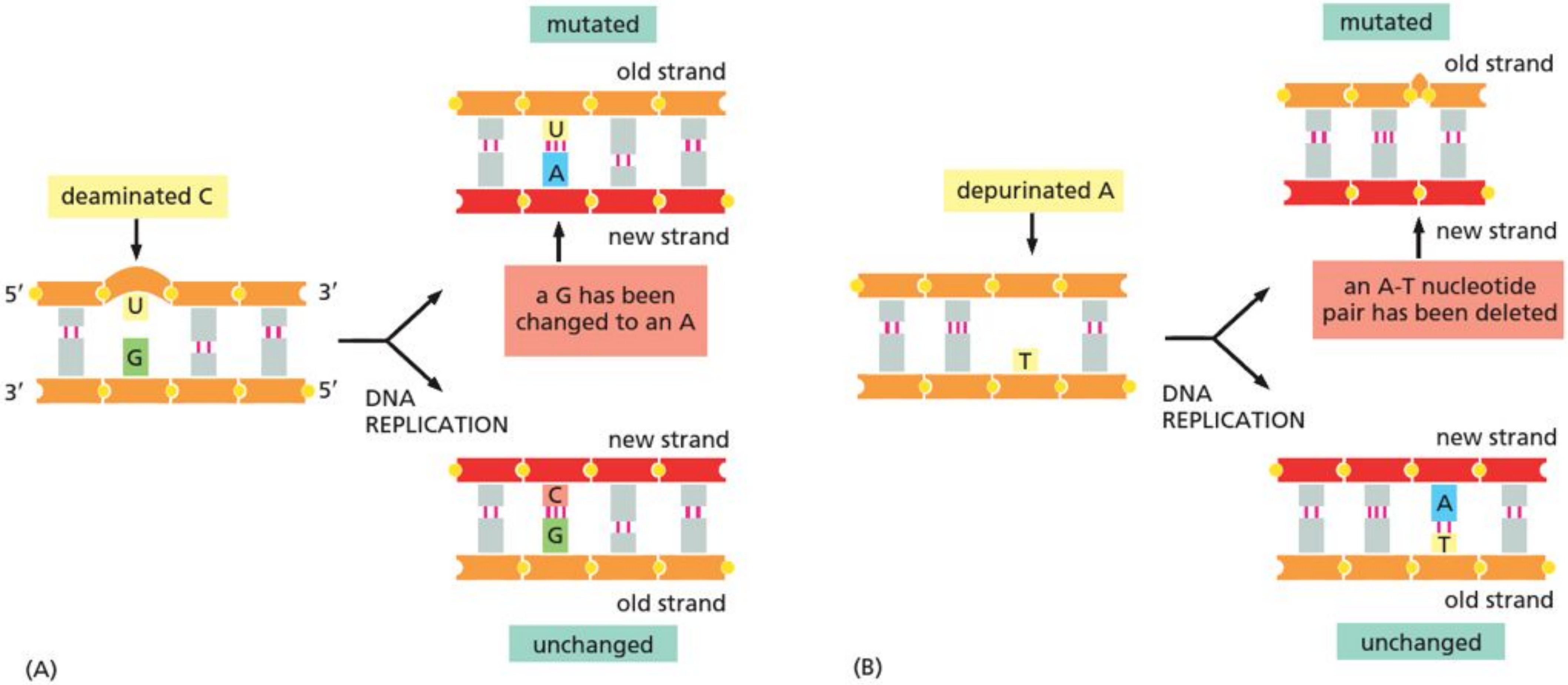
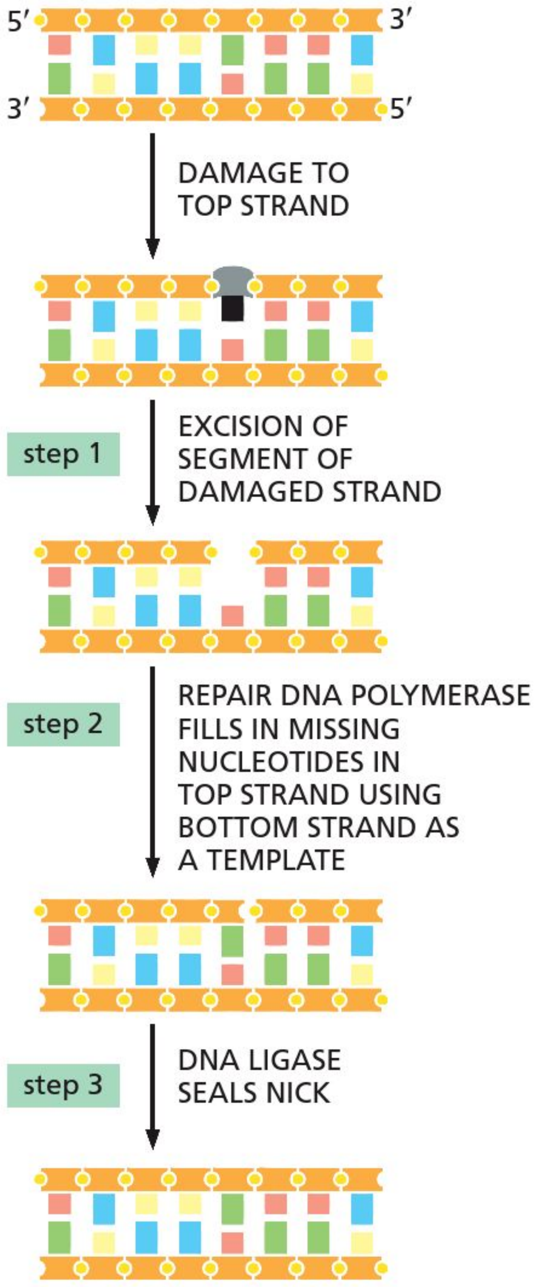

end of chromosome
telomere repeat sequence
3′
template of lagging strand
5′
incomplete, newly synthesized lagging strand
TELOMERASE BINDS TO TEMPLATE STRAND
3′
5′
3′
5′
direction of telomere DNA synthesis
telomerase with its bound RNA template
TELOMERASE ADDS ADDITIONAL TELOMERE REPEATS TO TEMPLATE STRAND (RNA-TEMPLATED DNA SYNTHESIS)
3′
5′
3′
5′
COMPLETION OF LAGGING STRAND BY DNA POLYMERASE (DNA-TEMPLATED DNA SYNTHESIS)
extended template strand
3′
5′
DNA polymerase

Figure 6–23 Depurination and deamination are the most frequent chemical reactions known to create serious DNA damage in cells. (A) Depurination can remove guanine (or adenine) from DNA. (B) The major type of deamination reaction converts cytosine to an altered DNA base, uracil; however, deamination can also occur on other bases as well. Both depurination and deamination take place on double-helical DNA, and neither break the phosphodiester backbone.




THYMINE
P
P
UV radiation
THYMINE
THYMINE DIMER


deaminated C
5′
3′
U
G
3′
5′
DNA
REPLICATION
mutated
old strand
U
A
new strand
a G has been changed to an A
new strand
C
G
old strand
unchanged
(A)
depurinated A
T
DNA
REPLICATION
mutated
old strand
new strand
an A-T nucleotide pair has been deleted
new strand
A
T
old strand
unchanged
(B)



DAMAGE TO TOP STRAND

step 1

EXCISION OF SEGMENT OF DAMAGED STRAND

step 2

REPAIR DNA POLYMERASE FILLS IN MISSING NUCLEOTIDES IN TOP STRAND USING BOTTOM STRAND AS A TEMPLATE

step 3

DNA LIGASE SEALS NICK

NET RESULT: REPAIRED DNA

TABLE 6–1 ERROR RATES

US Postal Service on-time delivery of local first-class mail	13 late deliveries per 100 parcels
Airline luggage system	1 lost bag per 150
A professional typist typing at 120 words per minute	1 mistake per 250 characters
Driving a car in the United States	1 death per 10^4 people per year
DNA replication (without proofreading)	1 mistake per 10^5 nucleotides copied
DNA replication (with proofreading; without mismatch repair)	1 mistake per 10^7 nucleotides copied
DNA replication (with mismatch repair)	1 mistake per 10^9 nucleotides copied

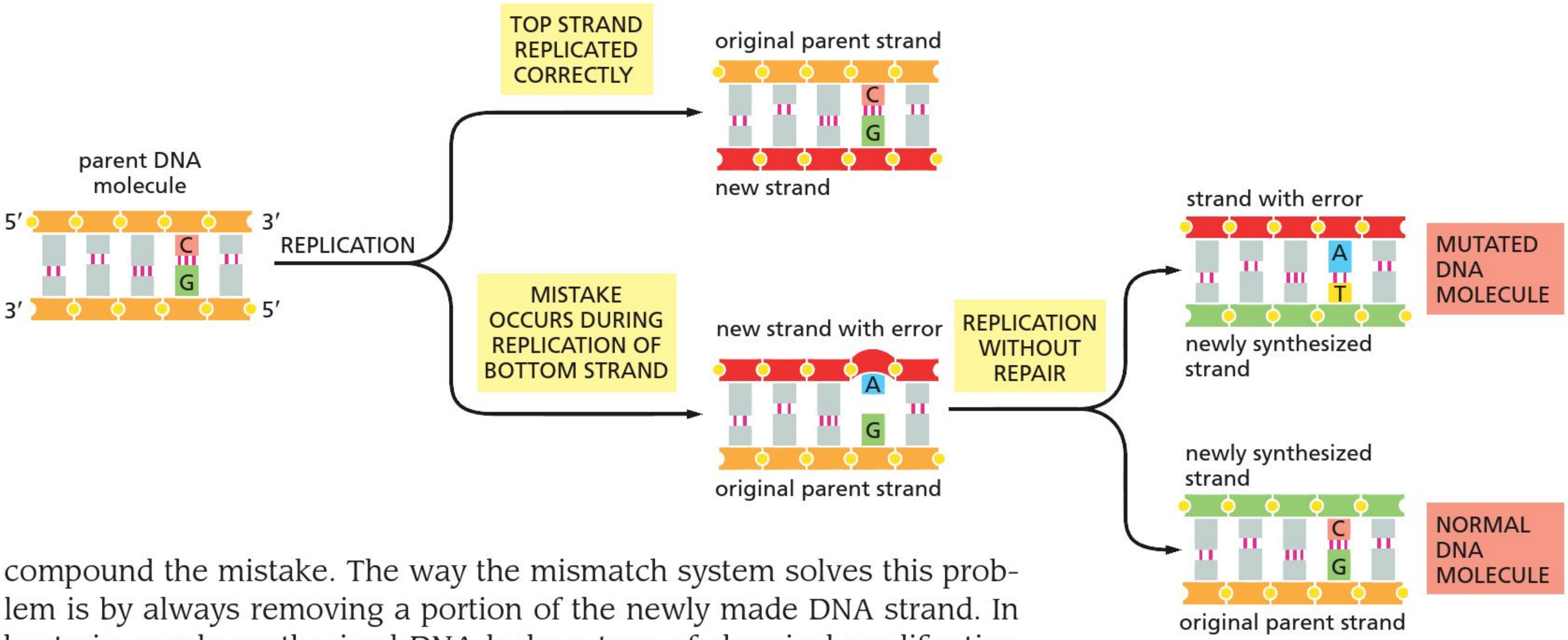


Figure 6–27 Errors made during DNA replication must be corrected to avoid mutations. If uncorrected, a mismatch will lead to a permanent mutation in one of the two DNA molecules produced by the next round of DNA replication.

compound the mistake. The way the mismatch system solves this problem is by always removing a portion of the newly made DNA strand. In bacteria, newly synthesized DNA lacks a type of chemical modification that is present on the preexisting parent DNA. Other cells use other strategies for distinguishing their parent DNA from a newly replicated strand.

Mismatch repair plays an important role in preventing cancer. An inherited predisposition to certain cancers (especially some types of colon cancer) is caused by mutations in genes that encode mismatch repair

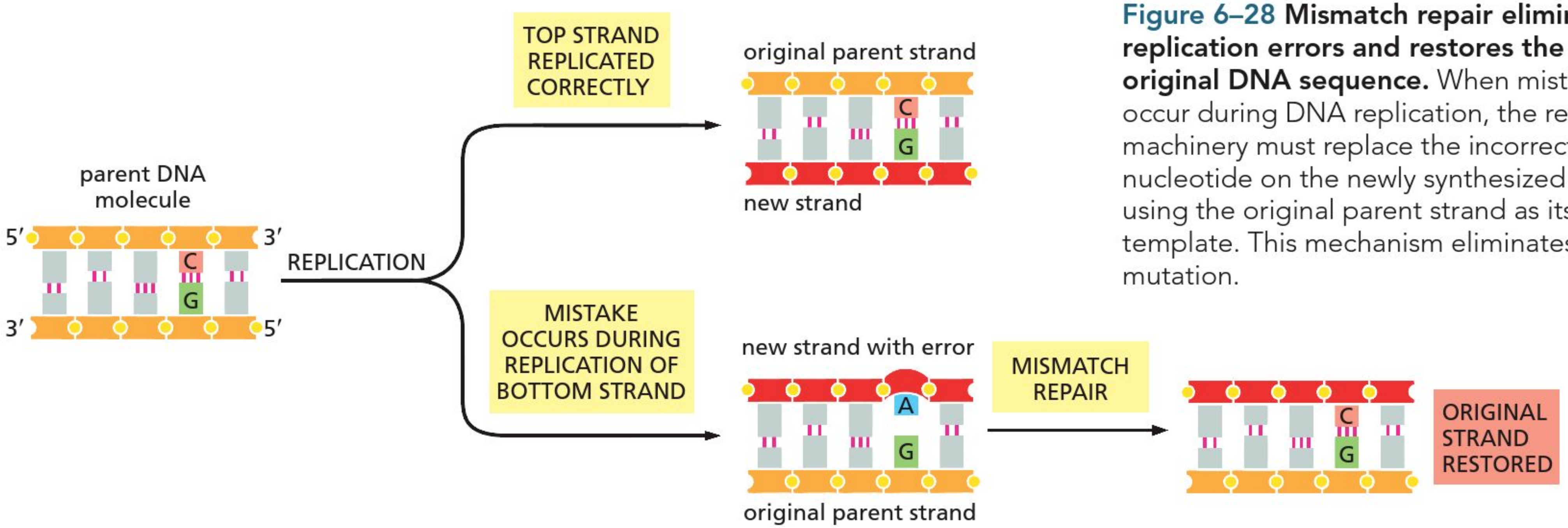
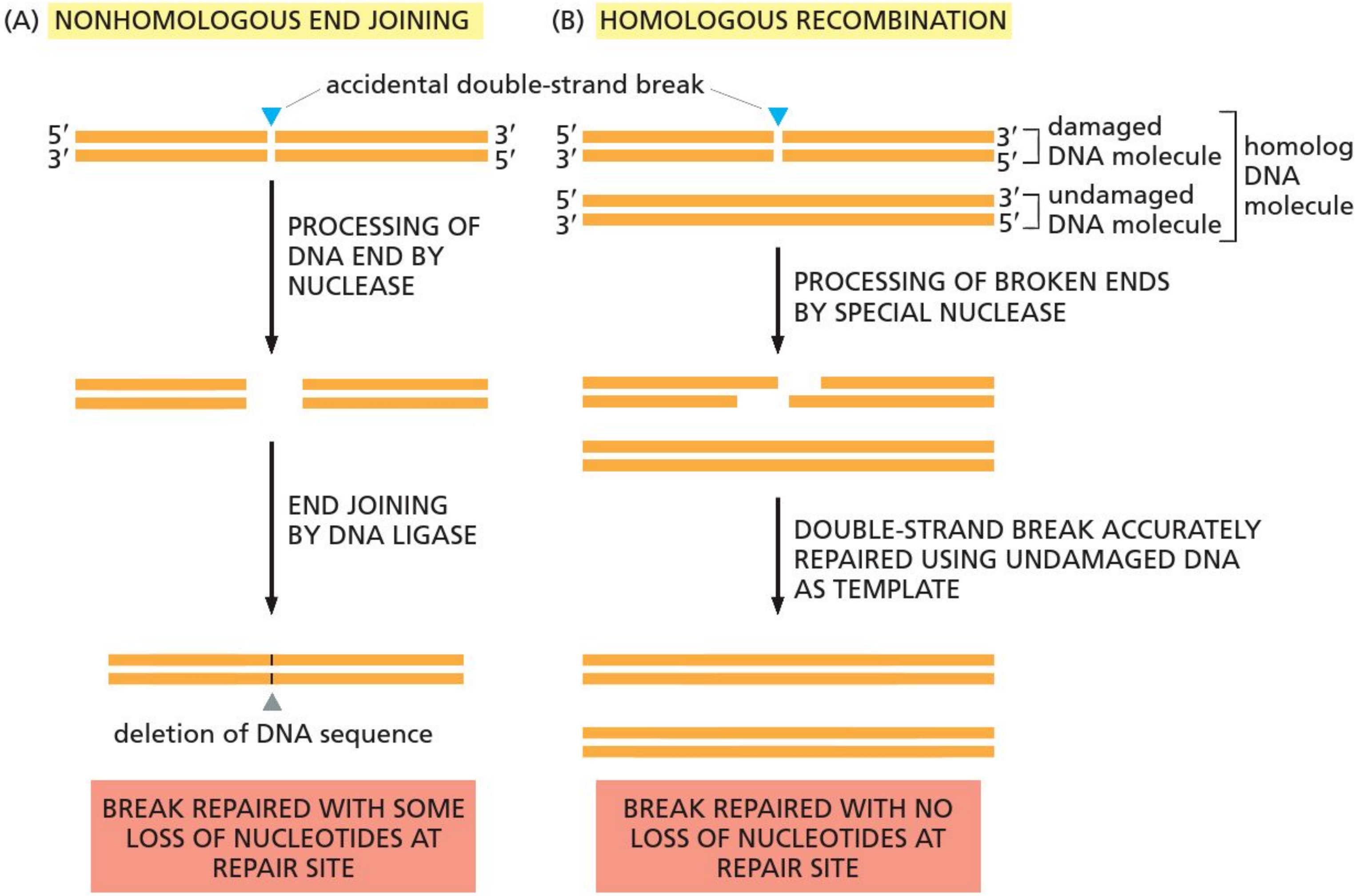
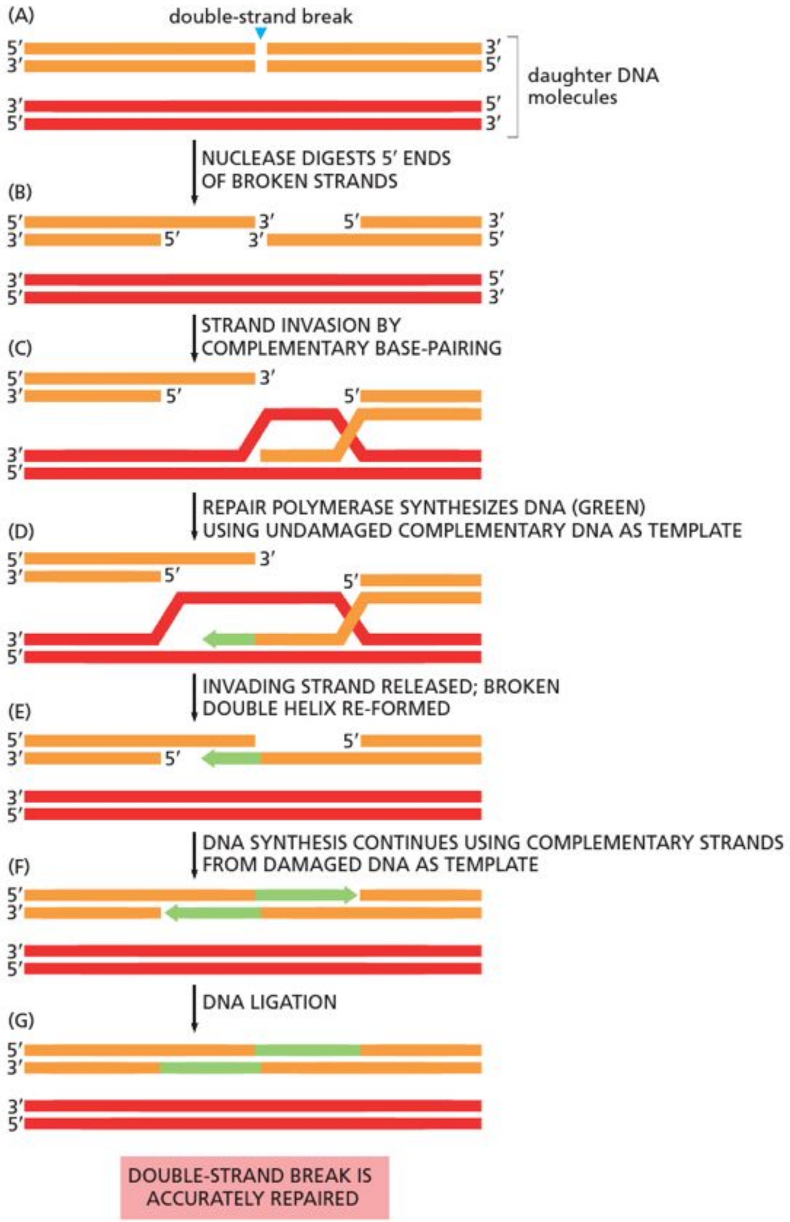


Figure 6–28 Mismatch repair eliminates replication errors and restores the original DNA sequence. When mistakes occur during DNA replication, the repair machinery must replace the incorrect nucleotide on the newly synthesized strand, using the original parent strand as its template. This mechanism eliminates the mutation.

Figure 6–29 Cells can repair double-strand breaks in one of two ways. (A) In nonhomologous end joining, the break is first "cleaned" by a nuclease that chews back the broken ends to produce flush ends. The flush ends are then stitched together by a DNA ligase. Some nucleotides are lost in the repair process, as indicated by the black lines in the repaired DNA. (B) If a double-strand break occurs in one of two daughter DNA double helices after DNA replication has occurred, but before the daughter chromosomes have been separated, the undamaged double helix can be readily used as a template to repair the damaged double helix by homologous recombination. This is a more involved process than non-homologous end joining, but it accurately restores the original DNA sequence at the site of the break. The detailed mechanism is presented in Figure 6–30.



double-strand break. Such lesions are particularly dangerous, becau



NUCLEASE DIGESTS 5′ ENDS OF BROKEN STRANDS

STRAND INVASION BY COMPLEMENTARY BASE-PAIRING

REPAIR POLYMERASE SYNTHESIZES DNA (GREEN) USING UNDAMAGED COMPLEMENTARY DNA AS TEMPLATE

INVADING STRAND RELEASED; BROKEN DOUBLE HELIX RE-FORMED

DNA SYNTHESIS CONTINUES USING COMPLEMENTARY STRANDS FROM DAMAGED DNA AS TEMPLATE

DNA LIGATION

DOUBLE-STRAND BREAK IS ACCURATELY REPAIRED