BI 8 LECTURE 12

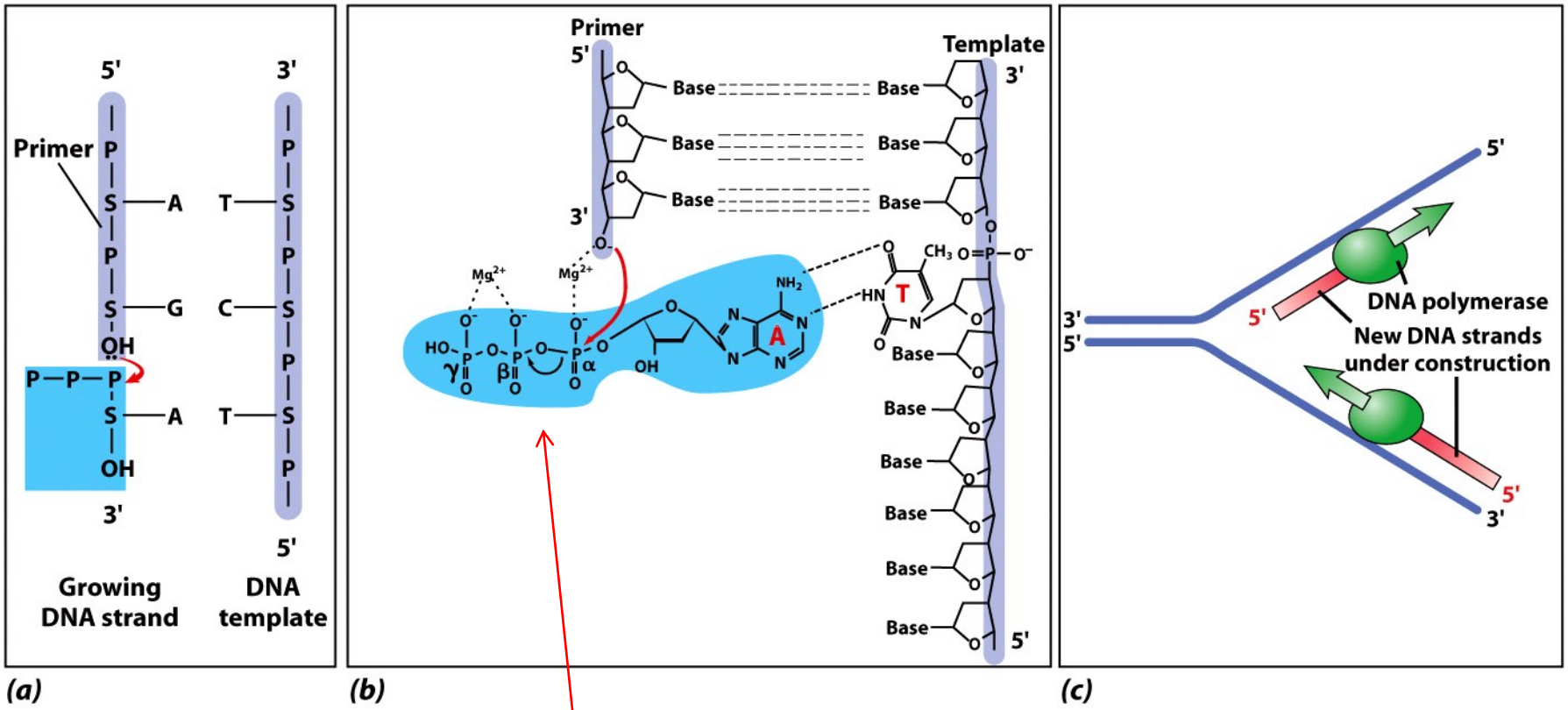
PROTECTING THE FAMILY JEWELS: DNA REPLICATION

Ellen Rothenberg

11 February 2016

Reading for this week: Alberts et al. Ch. 5

Basic outline of DNA polymerization: like RNA polymerization except with dNTP subunits



New phosphodiester bond is "paid for" by liberating PP_i… which is then hydrolyzed to 2 P_i, making reaction energetically favorable

Genomic DNA replication is semi-conservative… one parental strand stays in replicated duplex of each daughter dsDNA
(in bacteria and eukaryotes, but *not* in all viruses)

Chemically identical but isotopically distinct DNA molecules can be separated by density…
HH, HL, and LL all distinct
on CsCl density gradients

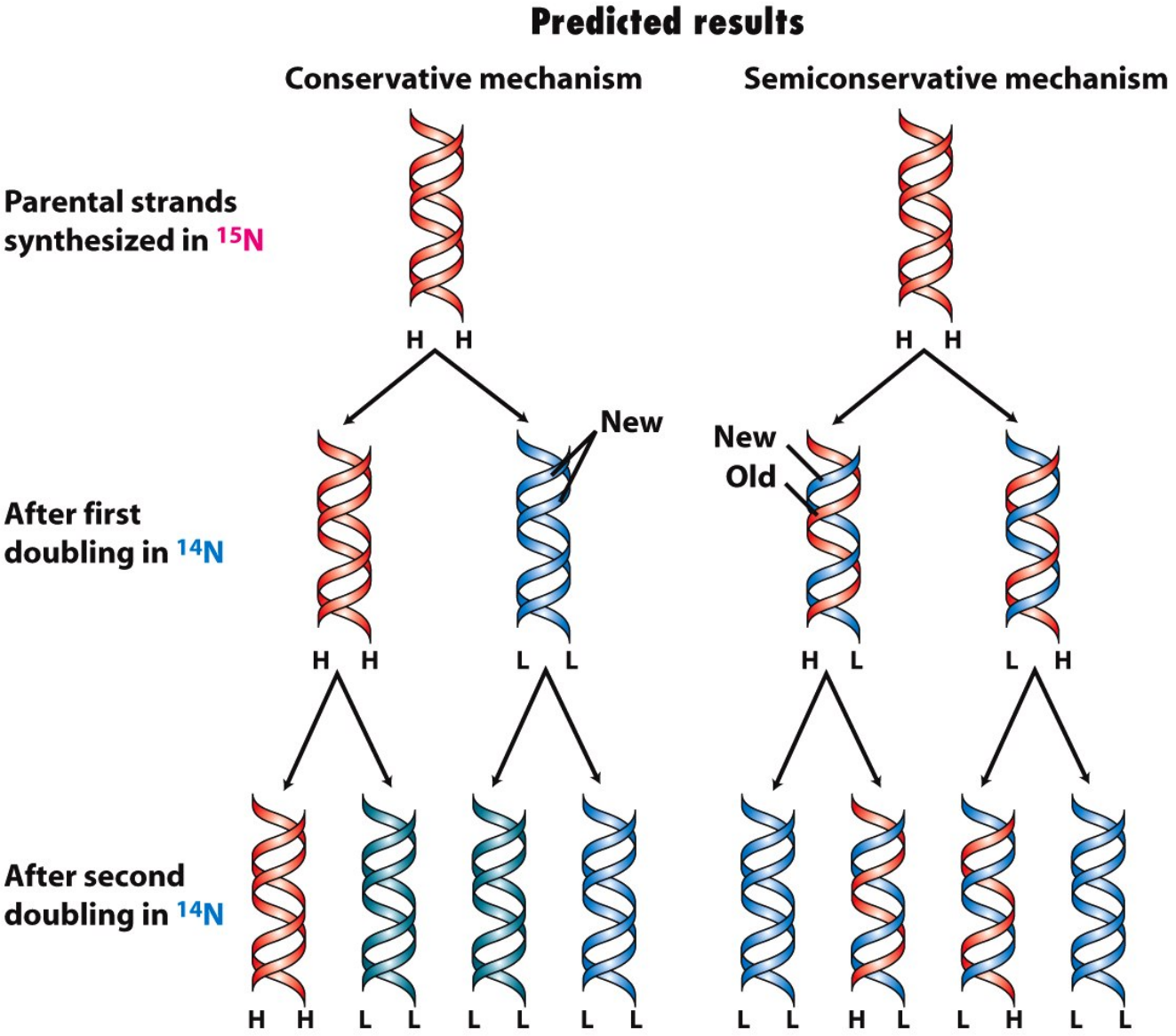
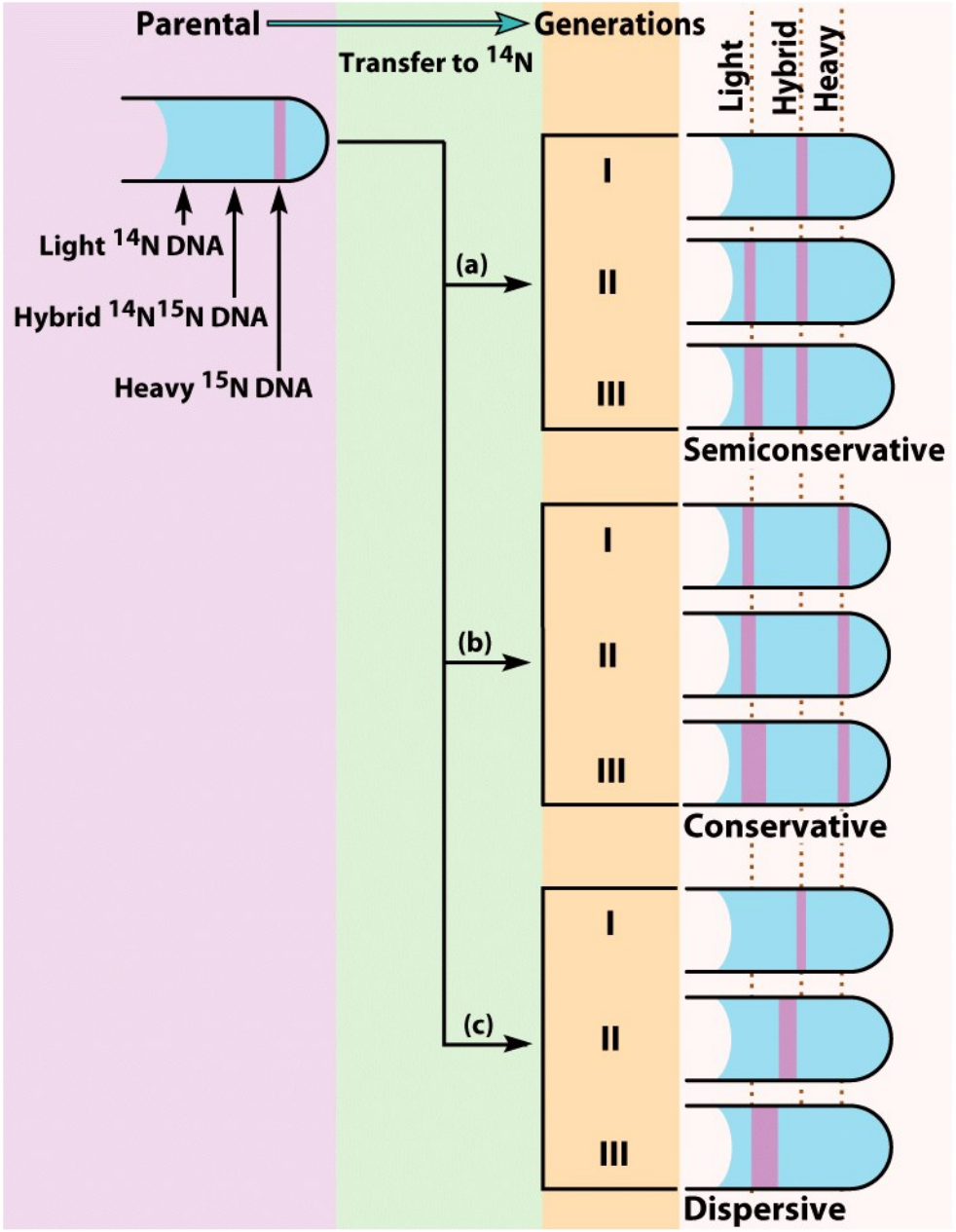


Figure 4-29a
Molecular Cell Biology, Sixth Edition

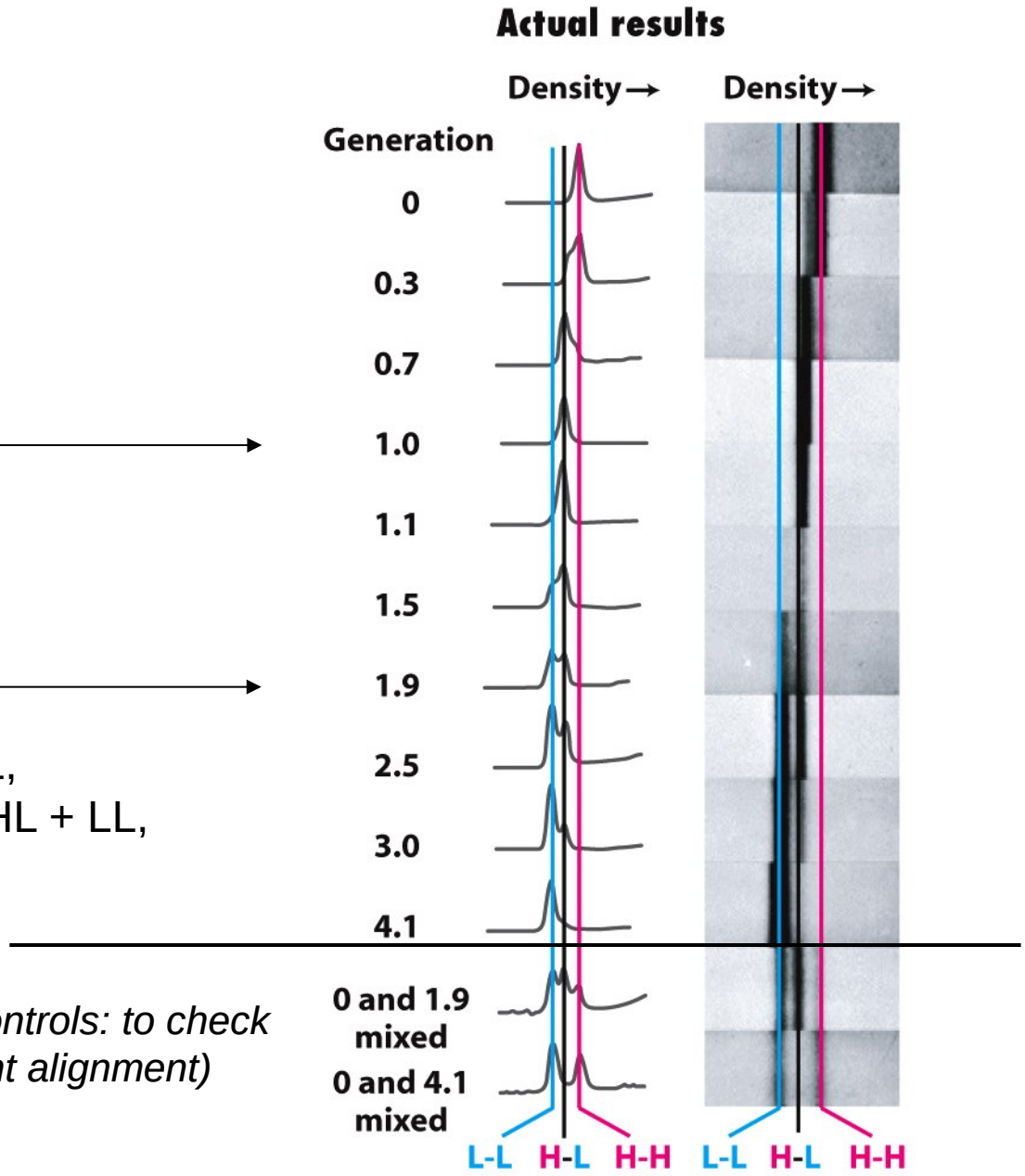
Equilibrium CsCl density gradient centrifugation allows individual DNA molecules to sediment/float to their own densities in the heavy salt gradient

Only a duplex of one “all-light” strand and one “all heavy” strand will band tightly at intermediate density



The Meselson-Stahl experiment (done here at Caltech) proved basic nature of DNA replication

HH + light nucleotides → all HL,
then HL + light nucleotides → HL + LL,
etc.



(Controls: to check right alignment)

Figure 4-29b
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DNA synthesis is extremely high fidelity… one error per 10^9 vs. one error per 10^4 for RNA transcription and RNA-dependent viral RNA synthesis

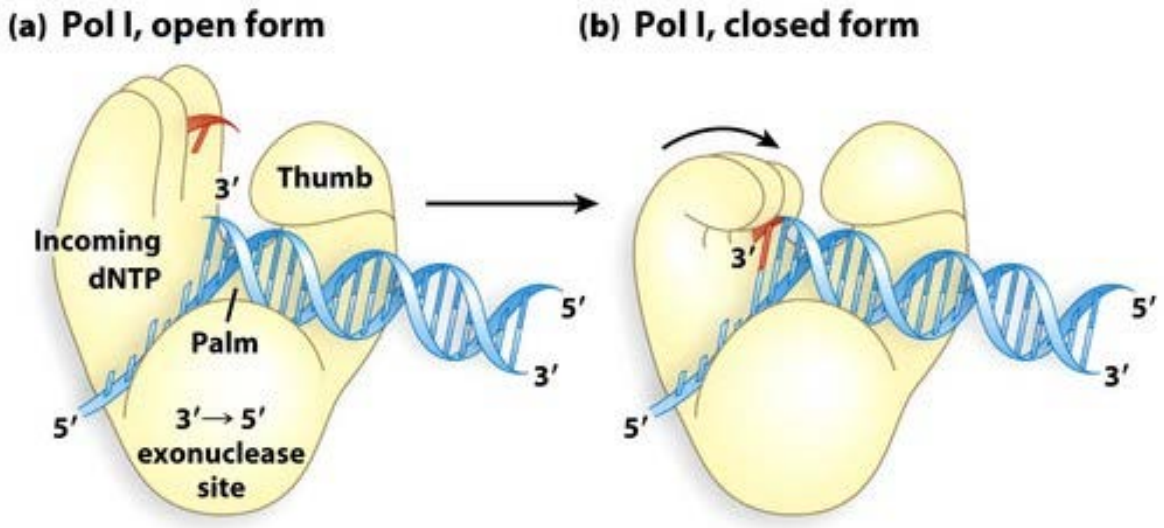
Table 5–1 The Three Steps That Give Rise to High-Fidelity DNA Synthesis

REPLICATION STEP	ERRORS PER NUCLEOTIDE
5′ → 3′ polymerization	1 in 10^5
3′ → 5′ exonucleolytic proofreading	1 in 10^2
Strand-directed mismatch repair	1 in 10^2
Combined	1 in 10^9

The third step, strand-directed mismatch repair, is described later in this chapter.

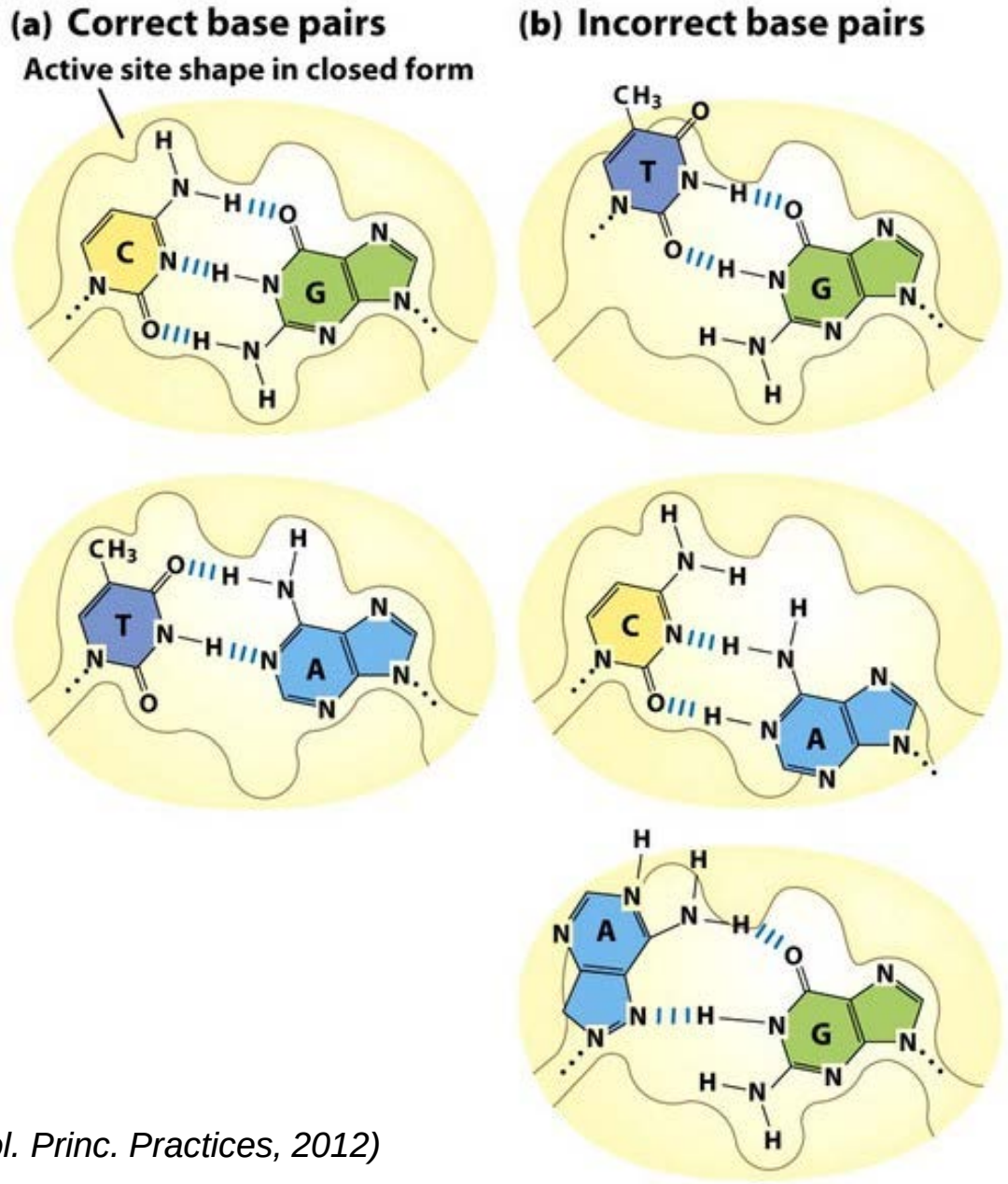
Table 5-1 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA polymerase forms a “hand” that clasps perfect duplex tightly before adding new dNTP



(Cox, Doudna, O’Donnell, Molec. Biol. Princ. Practices, 2012)

Within the polymerase, mispaired bases are detected by poor fit → stall elongation



(Cox, Doudna, O'Donnell, Molec. Biol. Princ. Practices, 2012)

Within the polymerase, 3'→5' exonuclease site is there just behind the point of new dNTP addition to undo errors

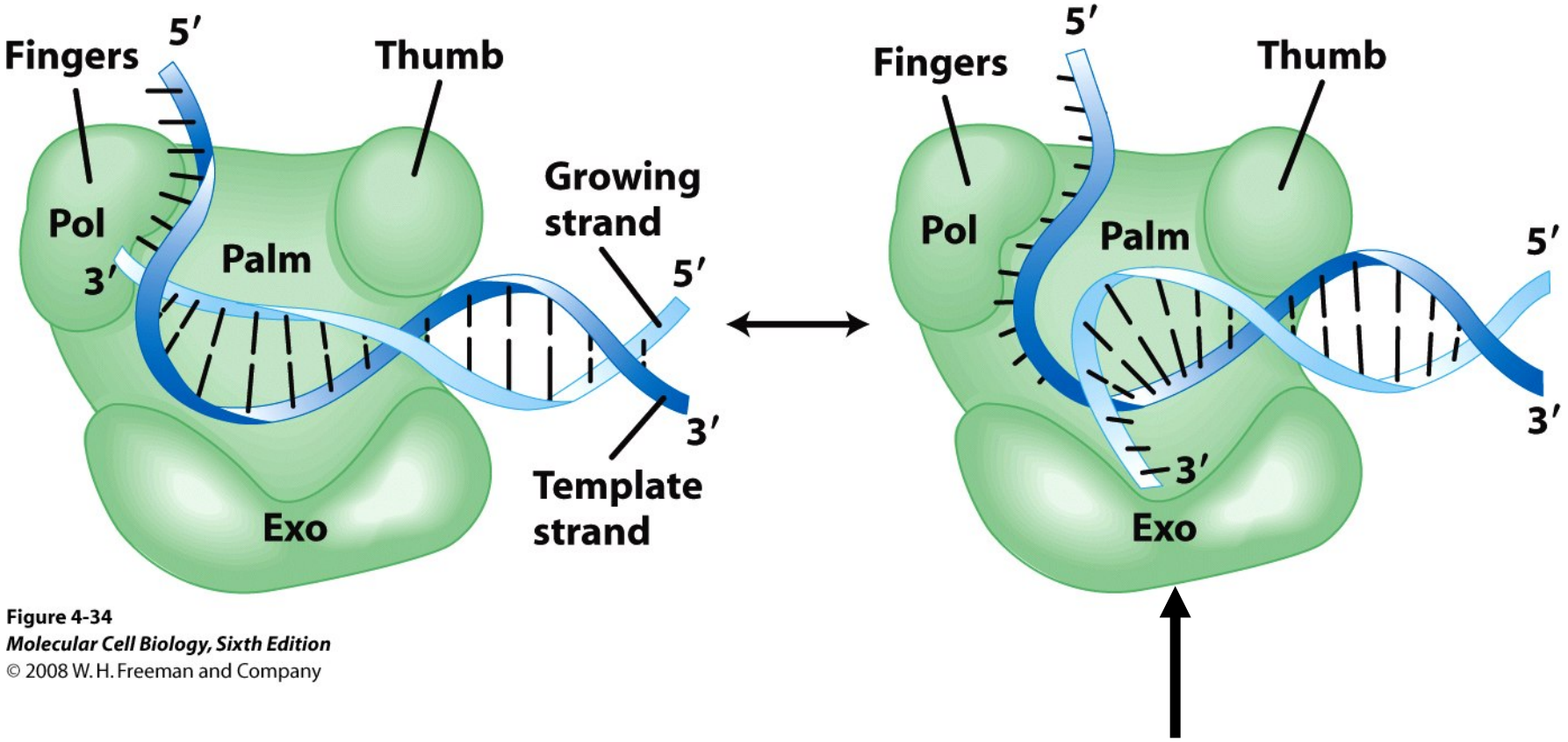


Figure 4-34
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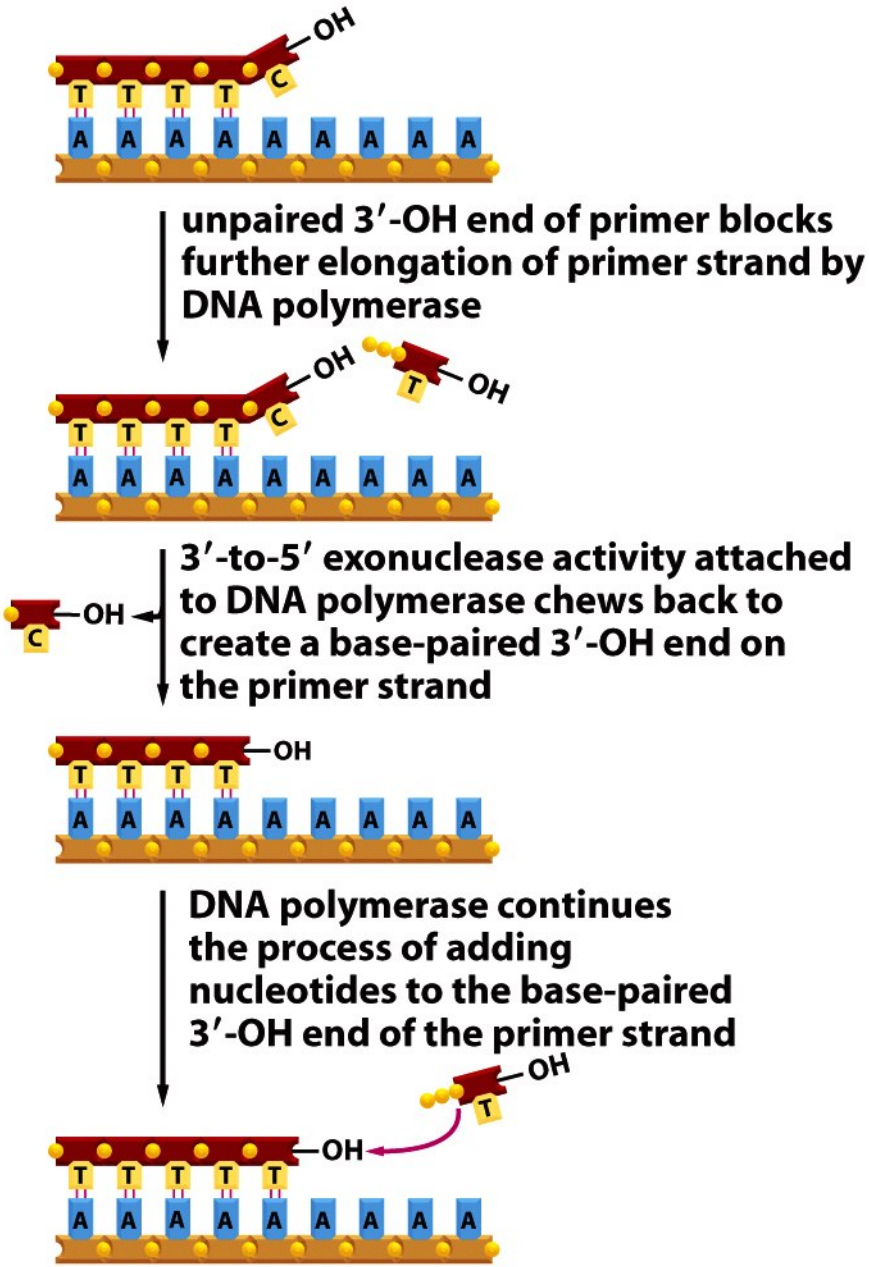


Exonuclease destroys unpaired polymer from the 3’ end back to the last fully base-paired region

Figure 5-8 (part 2 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

Proofreading is energetically possible because DNA strands are polymerized 5’ to 3’ with nucleotides phosphorylated on their 5’ ends…

so that each new deoxynucleotide to be tried out brings in its own triphosphate “entrance fee”

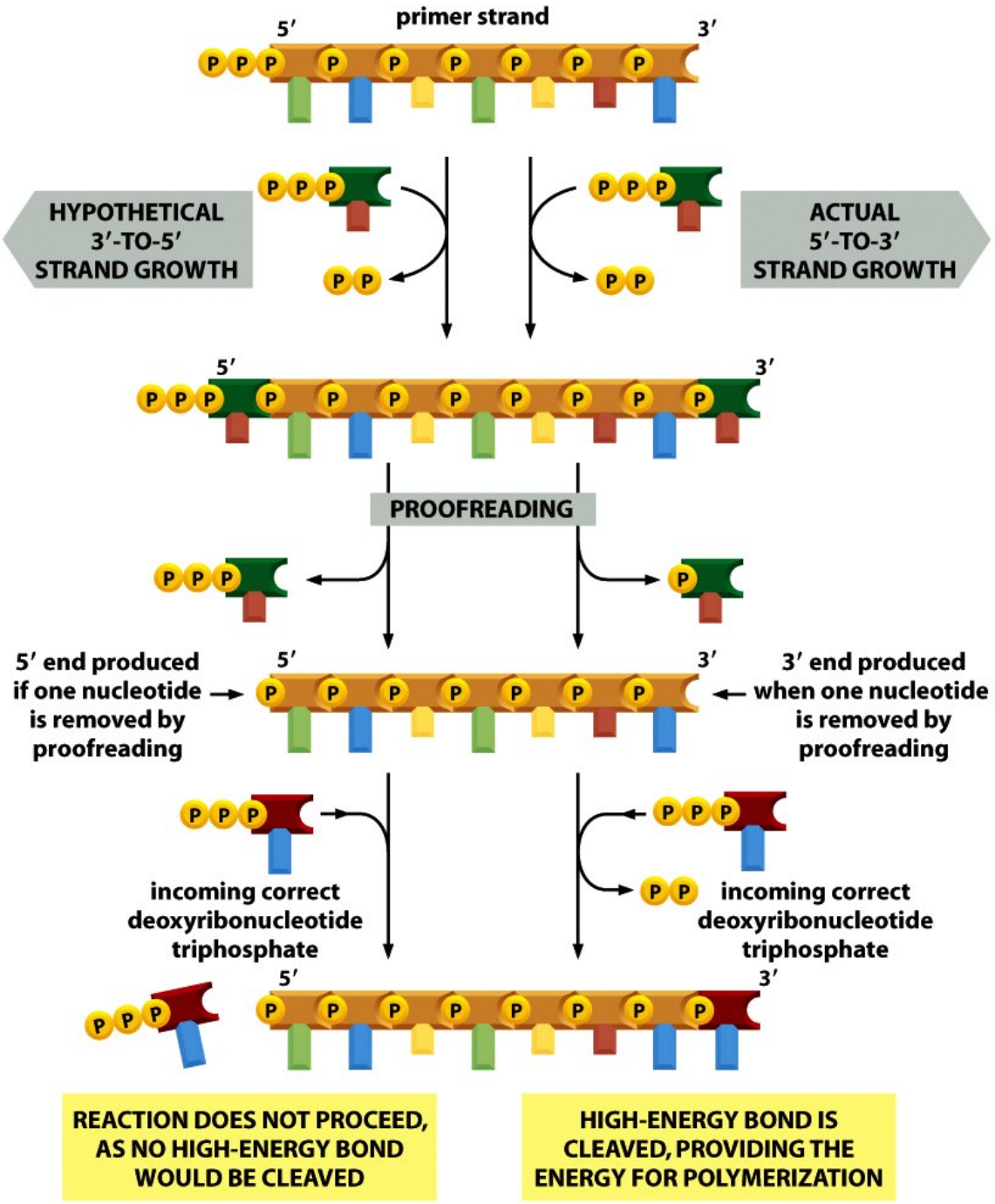


Figure 5-10 *Molecular Biology of the Cell* (© Garland Science 2008)

Defining origins of DNA replication: *All* DNA synthesis starts from primers… and DNA replication begins with synthesis of RNA primers

"DNA primase" makes short RNA primers to start DNA replication… in prokaryotes and eukaryotes

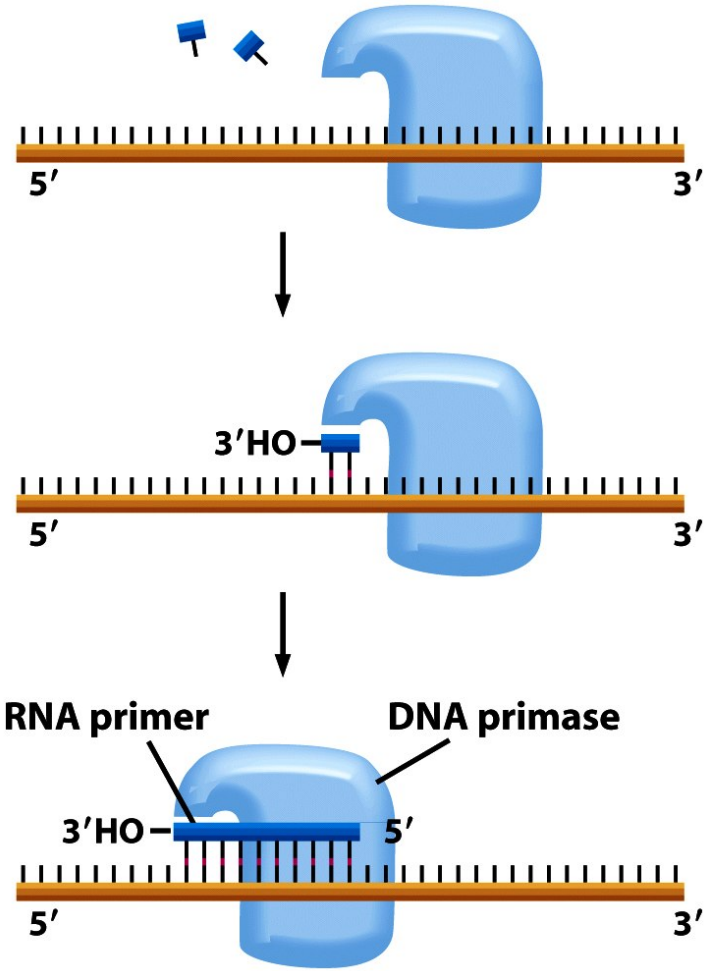


Figure 5-11 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA replication from origins starts bidirectionally from RNA primers that are locally synthesized “on demand”:

note distinction between leading and lagging strands

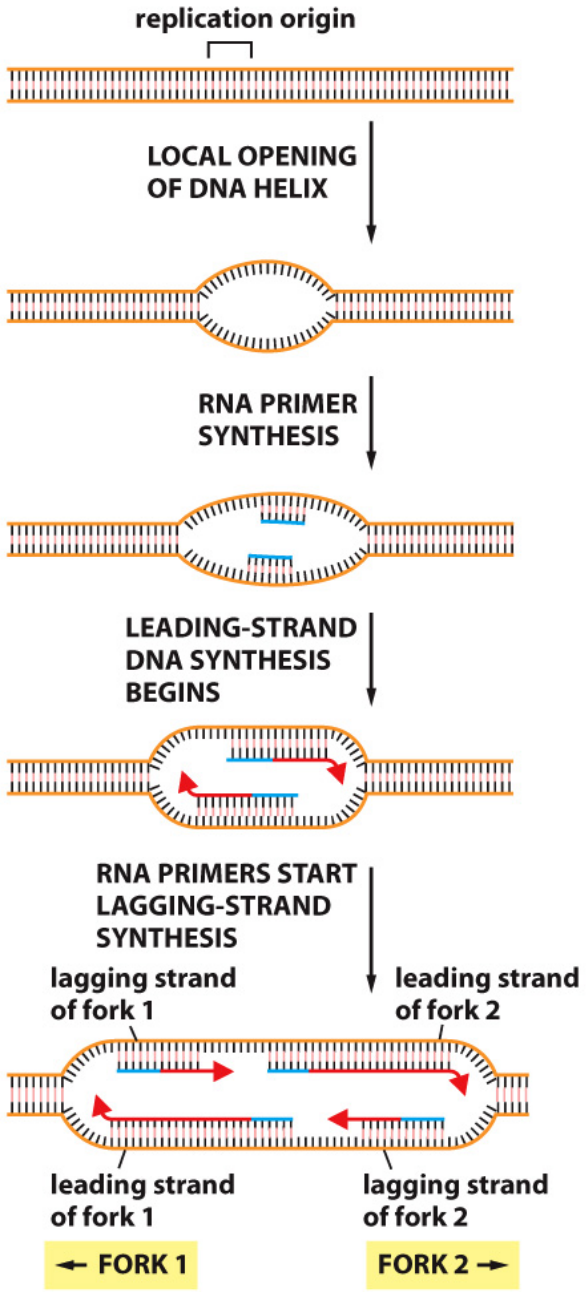


Figure 5-23 Molecular Biology of the Cell 6e (© Garland Science 2015)

Bacterial chromosome: circular, one origin

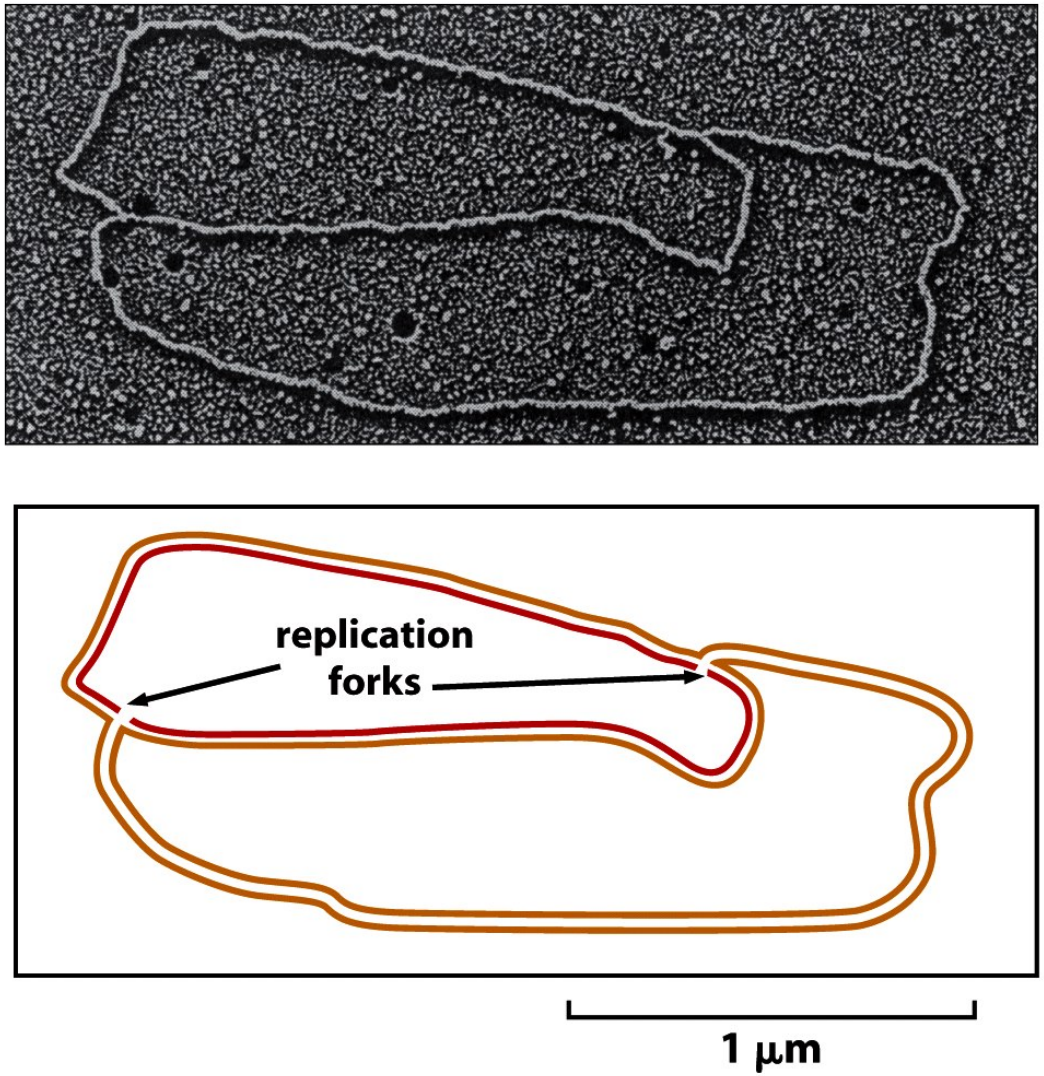


Figure 5-6 *Molecular Biology of the Cell* (© Garland Science 2008)

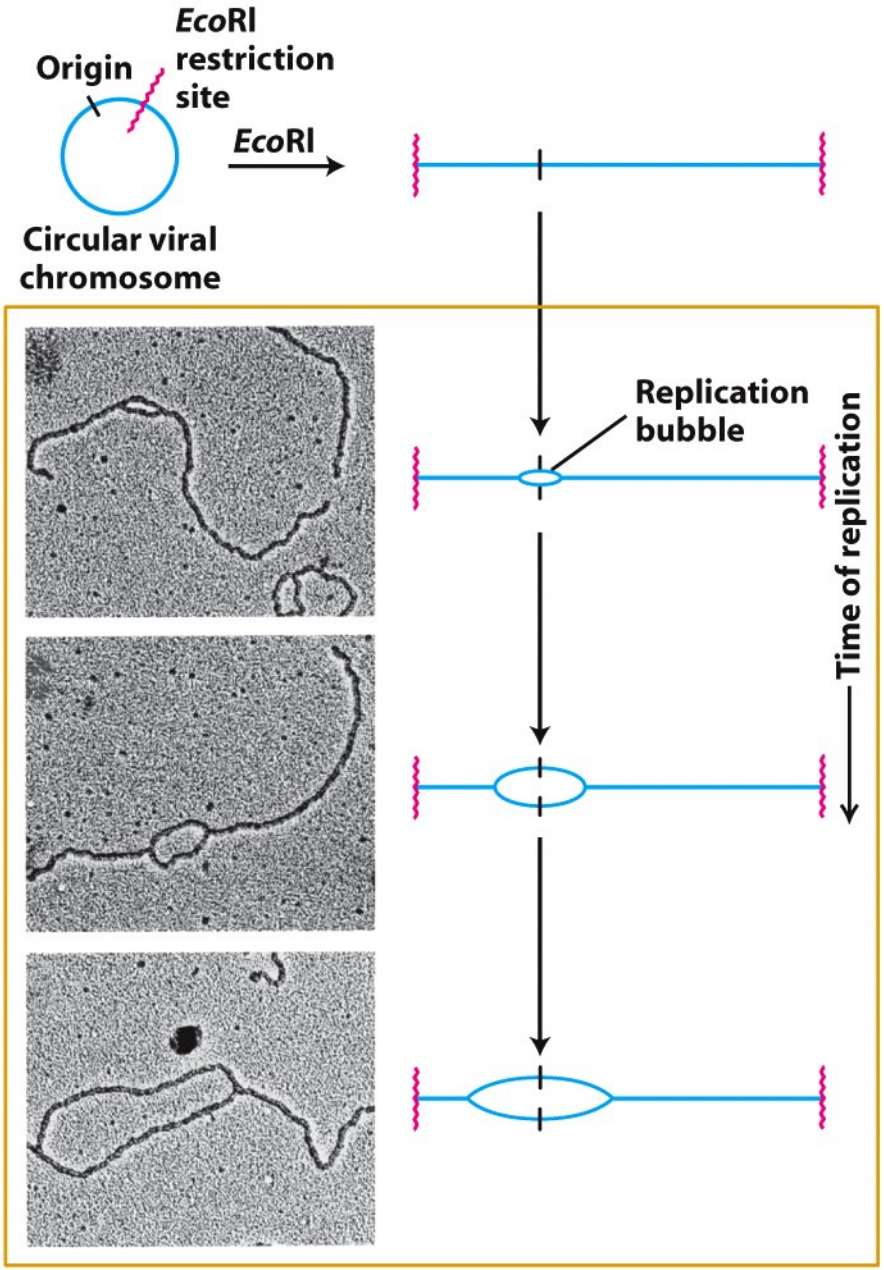
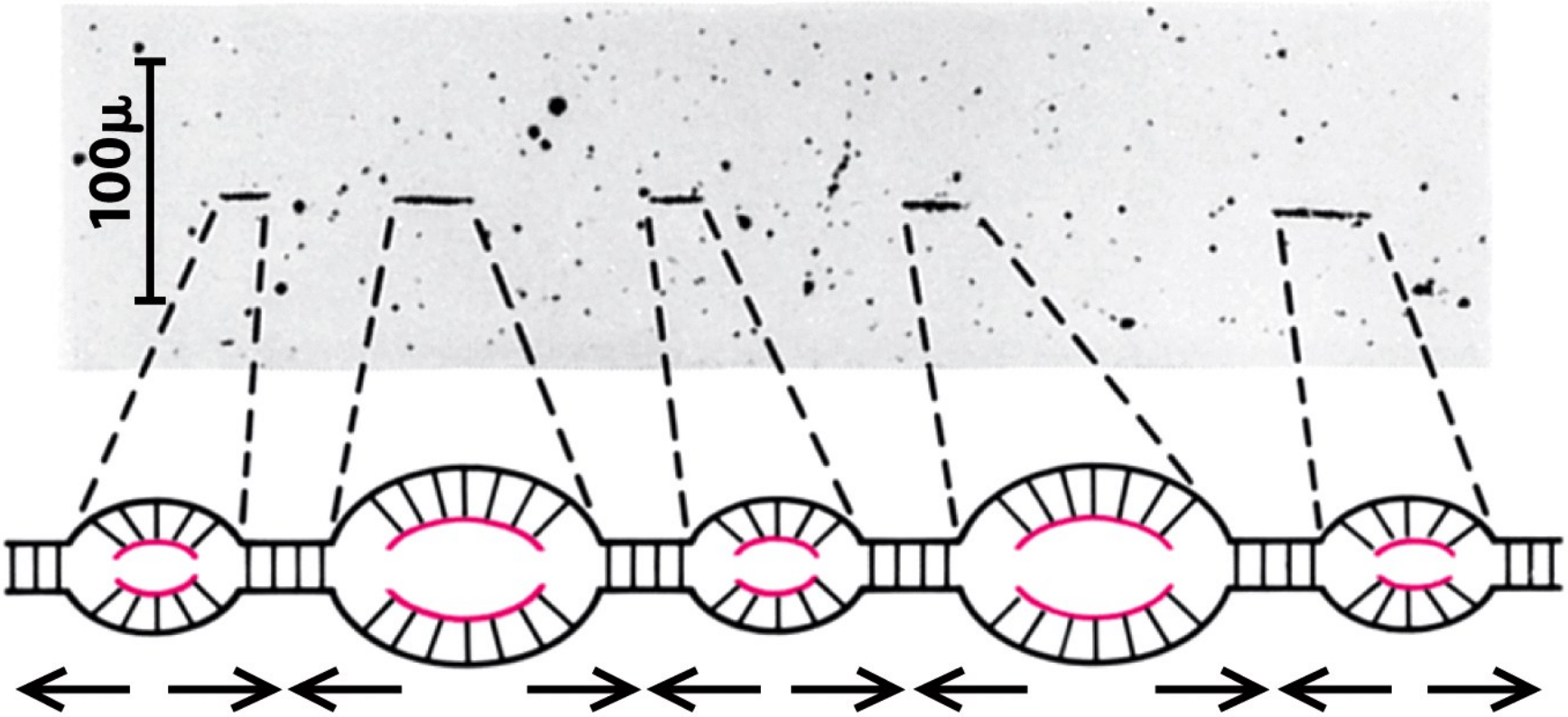


Figure 4-32
Molecular Cell Biology, Sixth Edition

Bidirectional outward movement of replication forks from origin as extent of replicated region expands

Eukaryotic chromosome: linear, multiple origins



Replication goes “only” 50 nt/sec… a long cell cycle if you replicated 3 x 10^9 bp (haploid genome) from only one origin per each of 23 chromosomes!
… in fact, ~1 origin per 30-250 kb

In situ autoradiography of newly synthesized eukaryotic DNA also shows bidirectional replication from origins

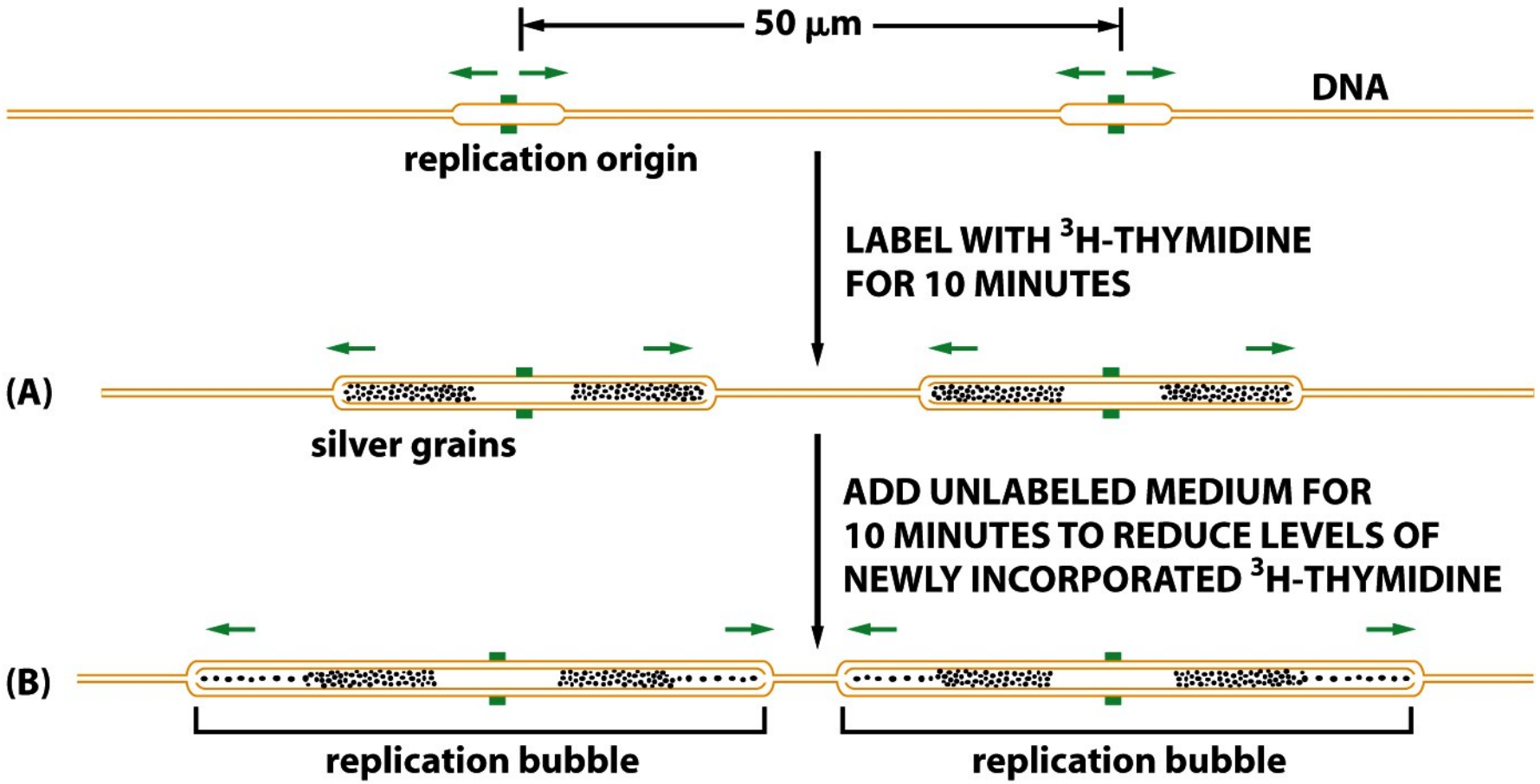
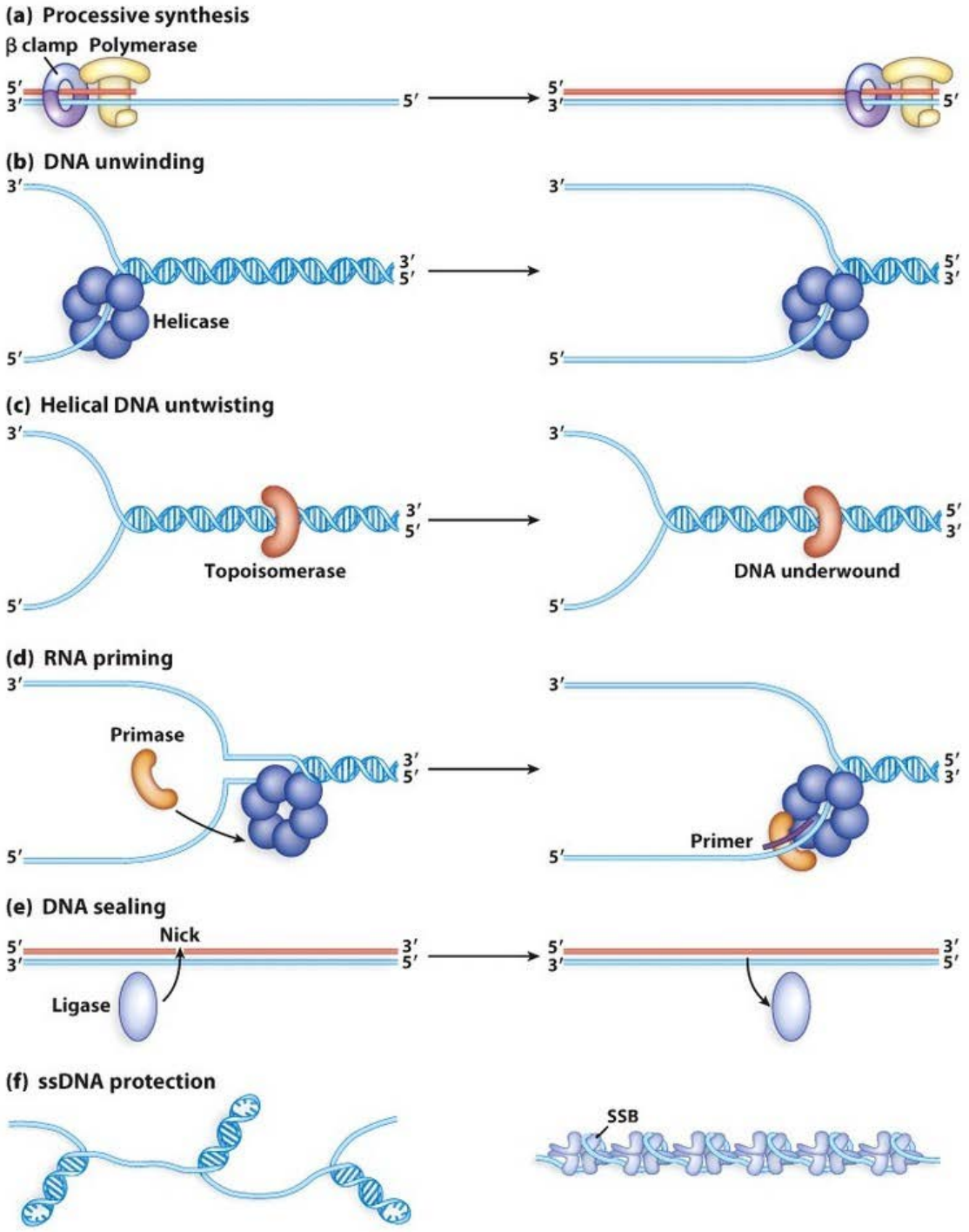


Figure 5-29 *Molecular Biology of the Cell* (© Garland Science 2008)

Many enzymatic activities are needed at each DNA replication fork!



(Cox, Doudna, O'Donnell, Molec. Biol. Princ. Practices, 2012)

Problem of the lagging strand: how do you prime replication in opposite direction from the fork?

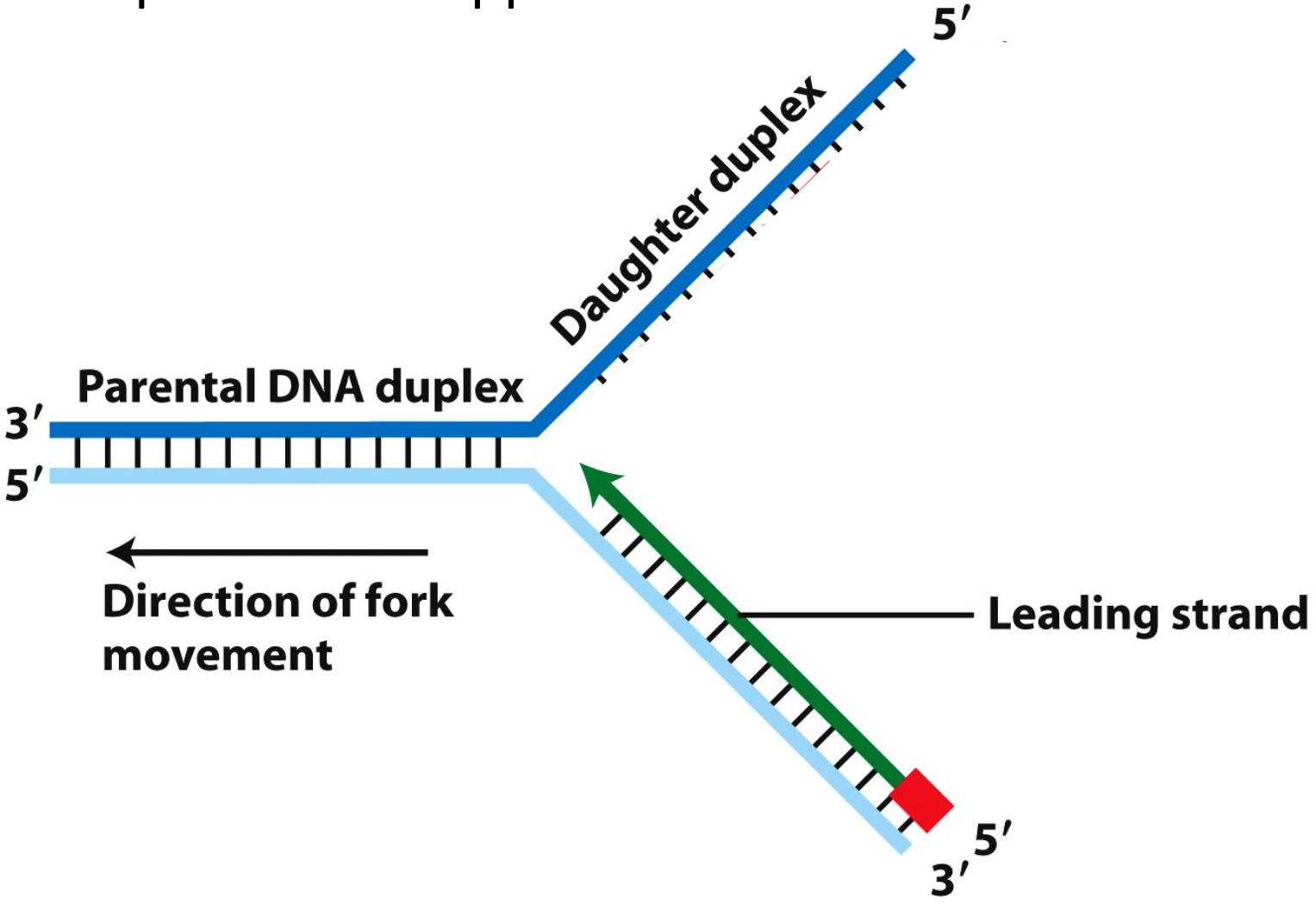


Figure 4-30
Molecular Cell Biology, Sixth Edition

Individual “Okazaki fragments” are individually primed with their own RNA primers

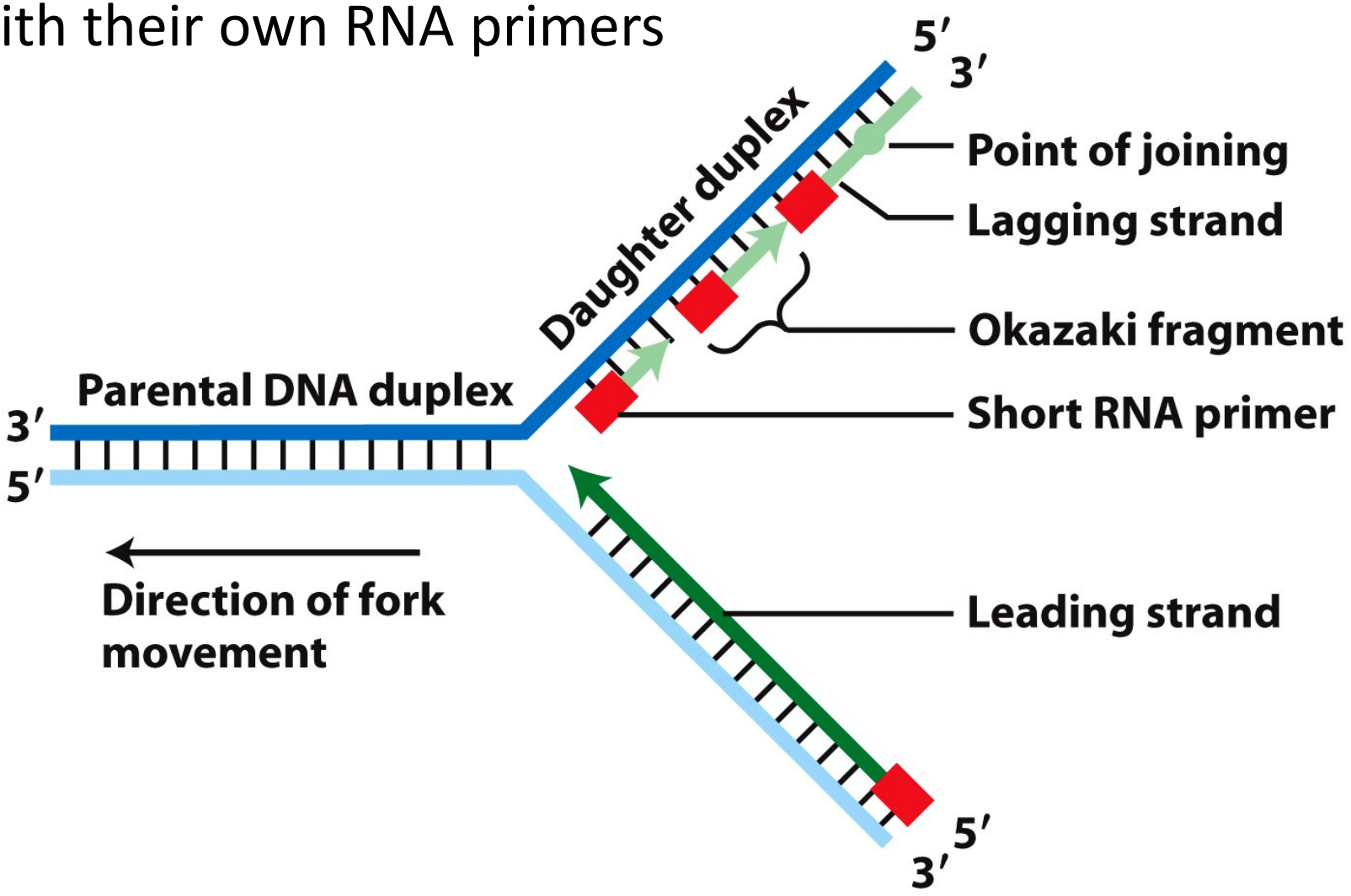
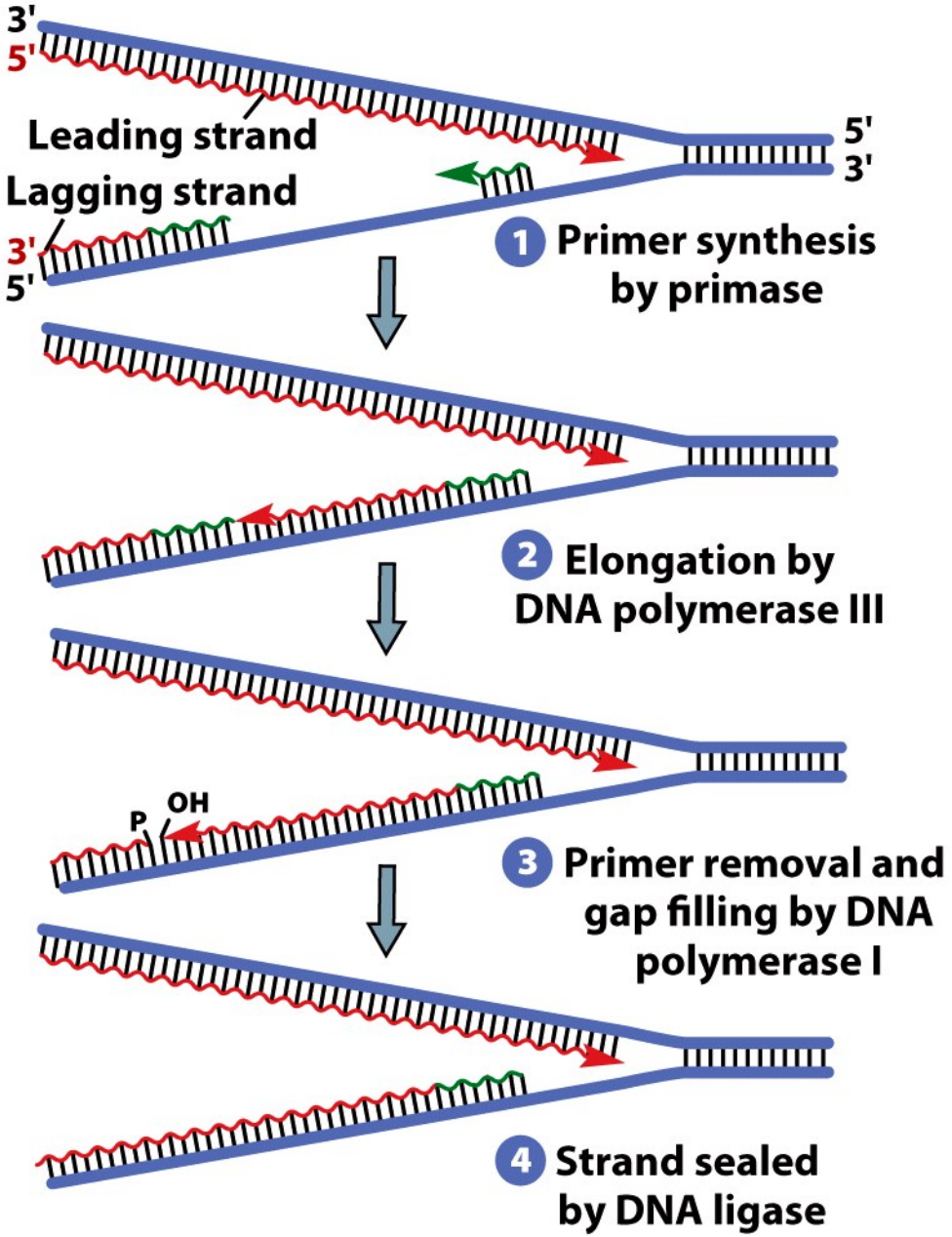
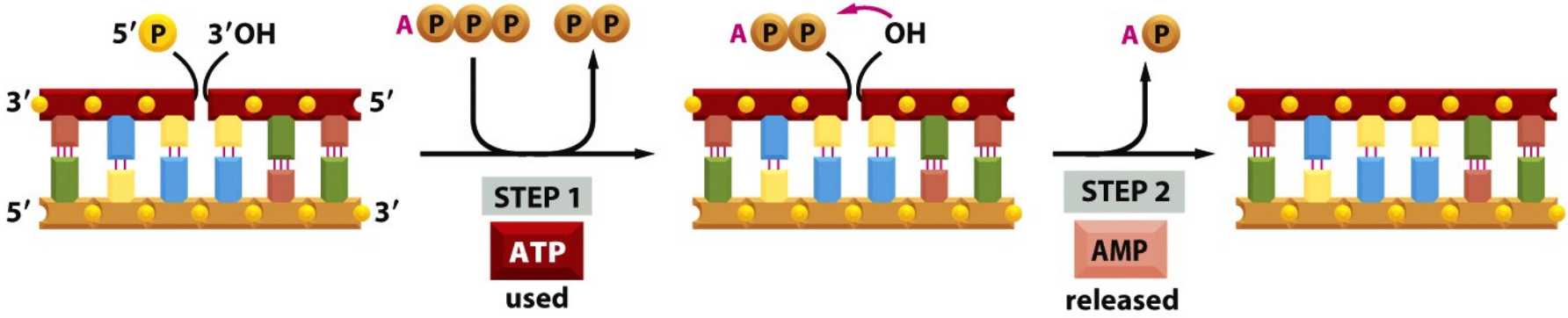


Figure 4-30
Molecular Cell Biology, Sixth Edition

Creation of a continuous duplex from Okazaki fragments requires 5’→ 3’ “editing out” of the RNA primers by a separate DNA polymerase, once strands collide, and nick sealing by ligase



The DNA ligase reaction can use ATP to seal nicks in the DNA



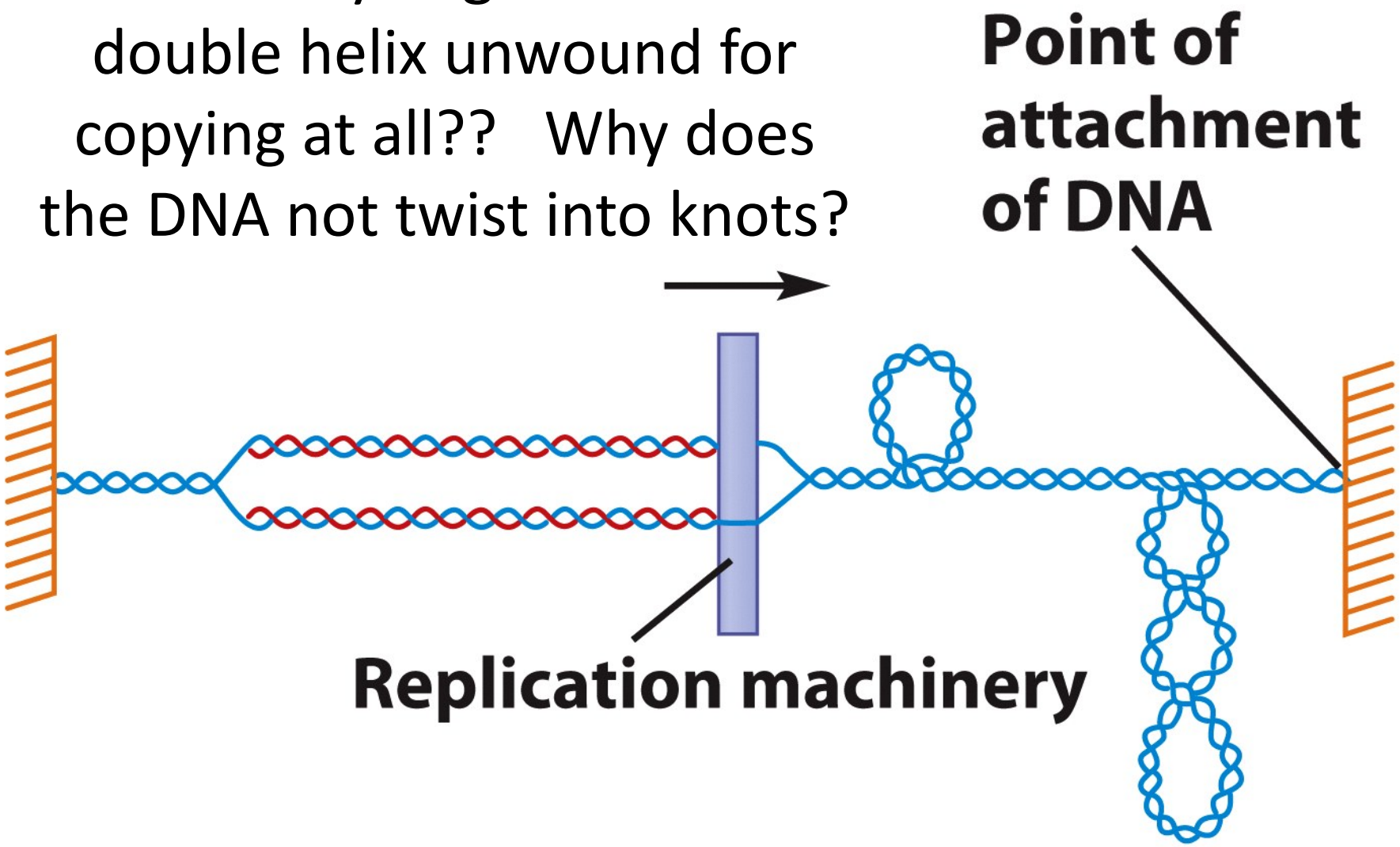
"Nick" = all base pairs are present, but one phosphodiester bond is missing

Eligible nick: the nick leaves a 5'-phosphate one one side and a 3'-OH on the other

ATP hydrolysis "activates" the 5'-OPO_3^{2-} at the break by AMP addition and creates favorable leaving group for 3'-OH attack

Figure 5-13 *Molecular Biology of the Cell* (© Garland Science 2008)

How can you get the DNA double helix unwound for copying at all?? Why does the DNA not twist into knots?



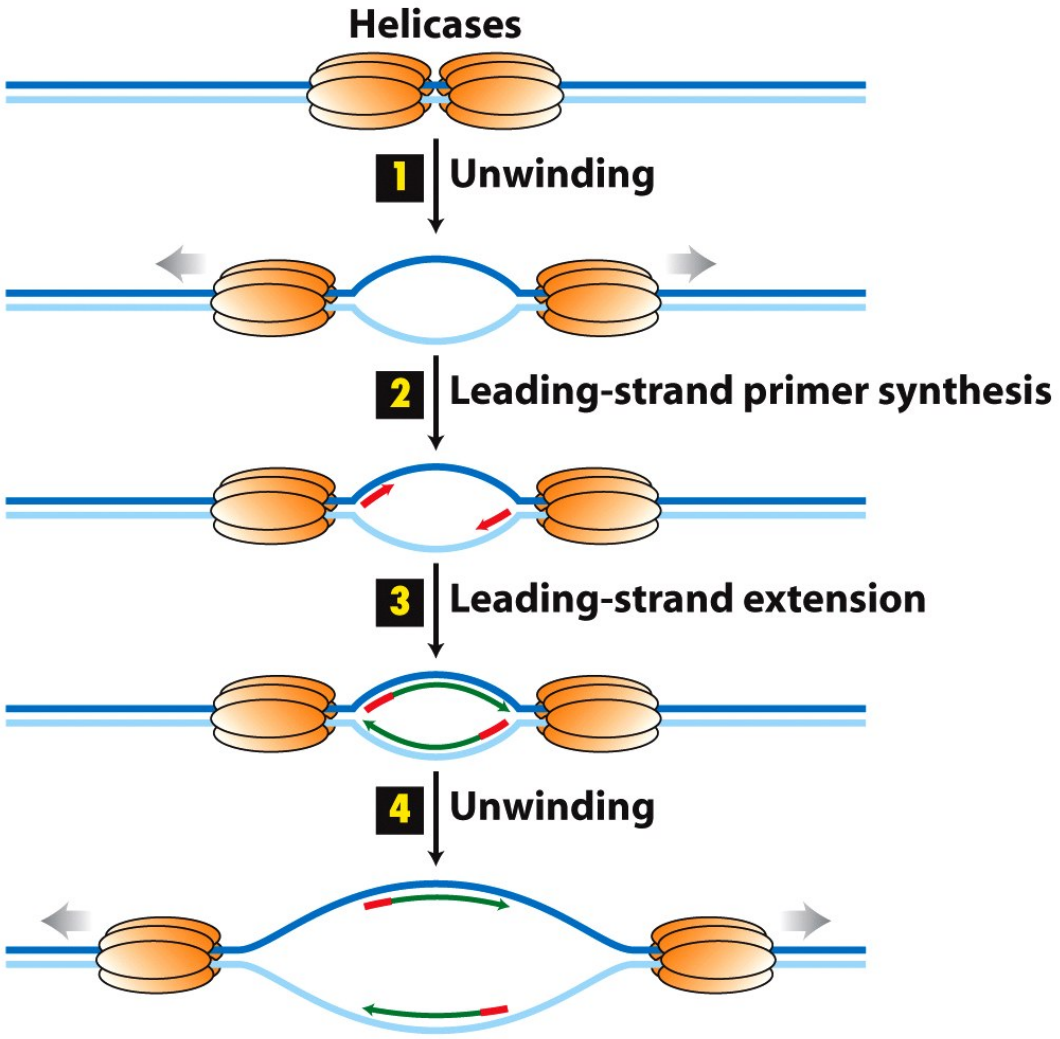
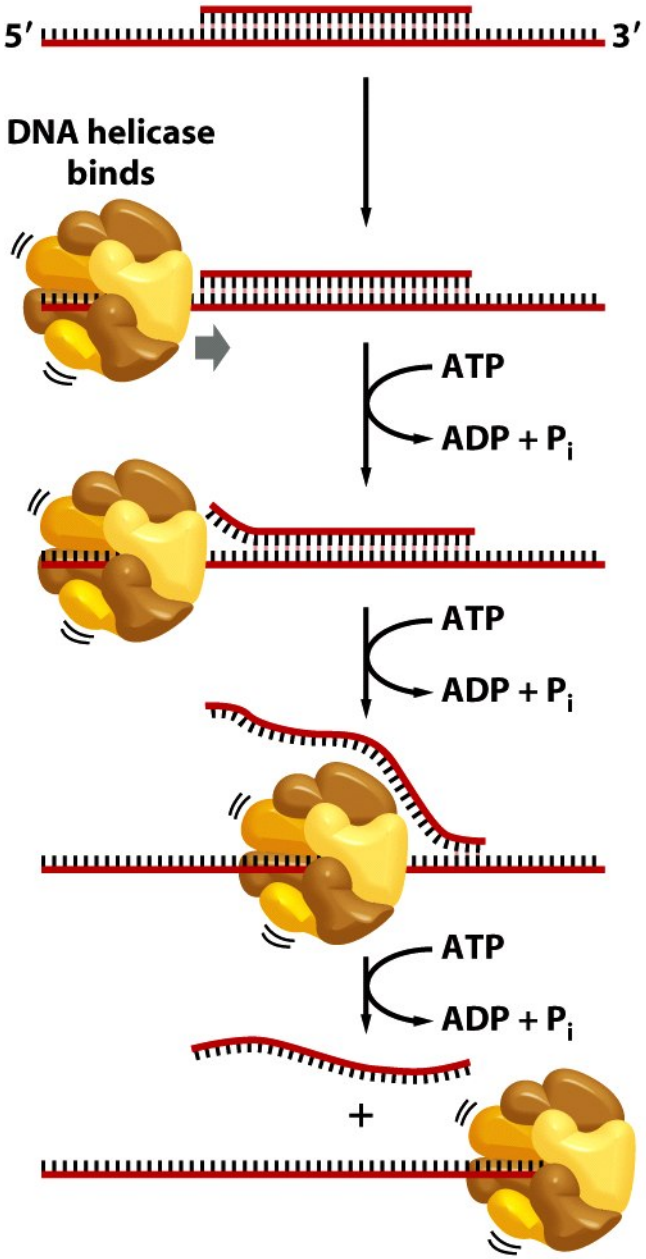


Figure 4-33 part 1
Molecular Cell Biology, Sixth Edition

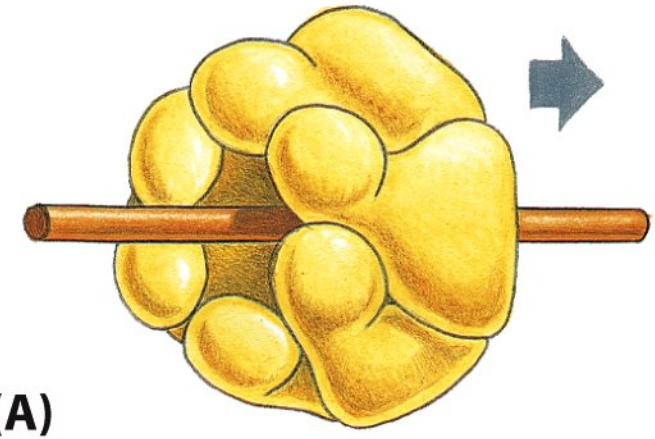
One part of the answer: Helicase can unwind DNA ahead of polymerase



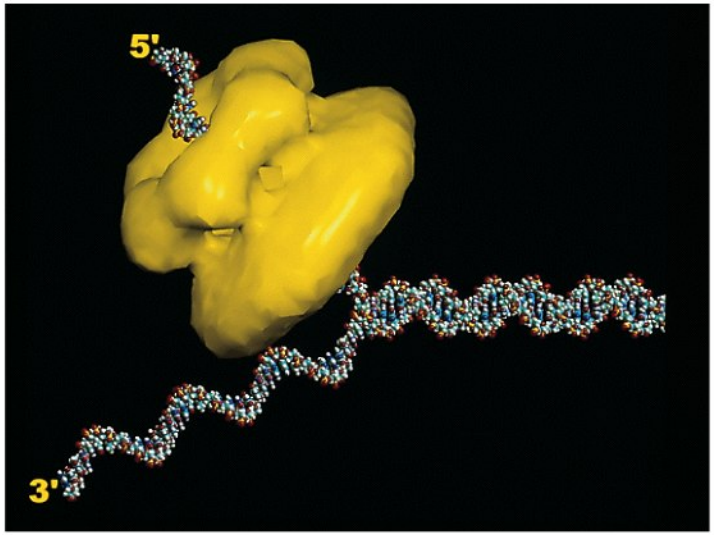
DNA helicase binds ssDNA, denatures DNA "ahead" of it processively by hydrolyzing ATP

Figure 5-14 *Molecular Biology of the Cell* (© Garland Science 2008)

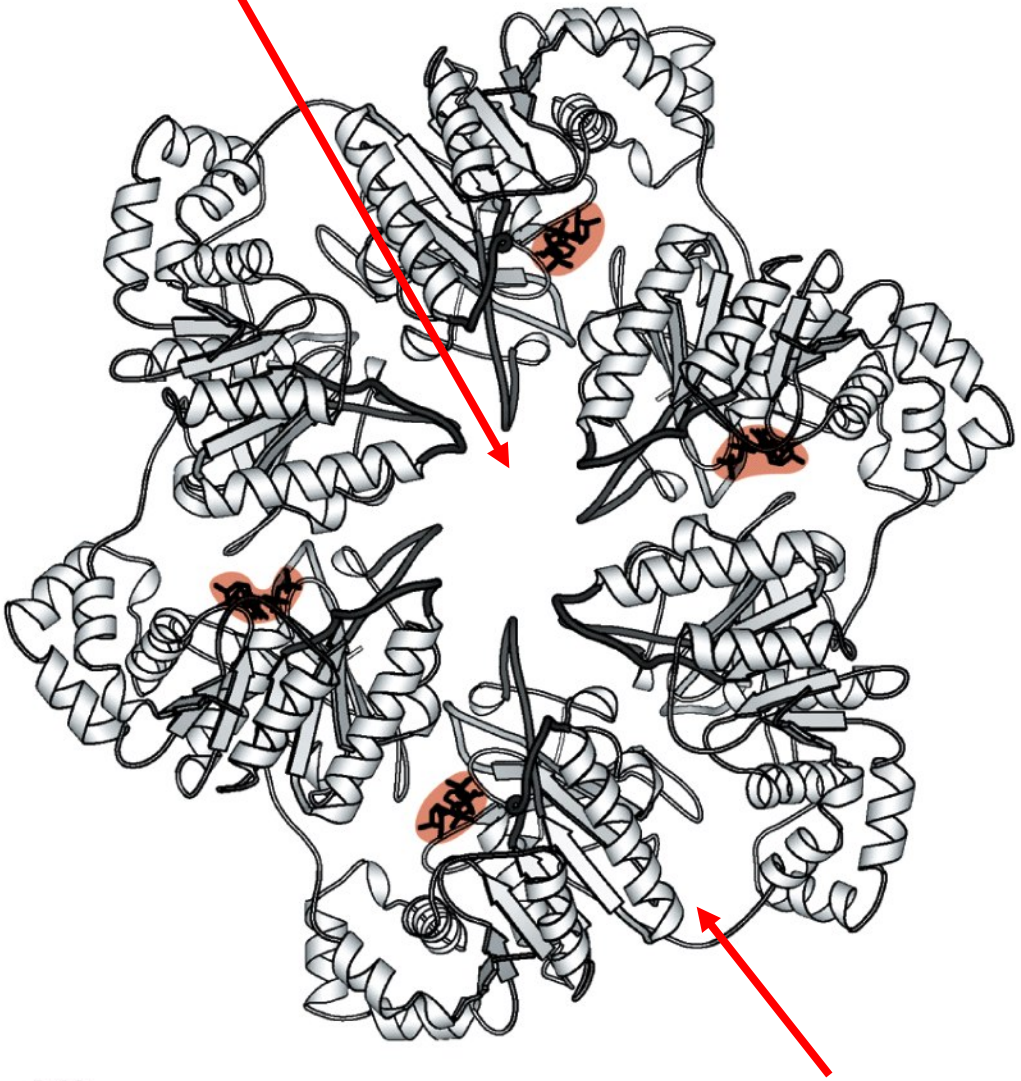
Diameter of helicase central channel is big enough only for ssDNA, not dsDNA



(A)



(B)

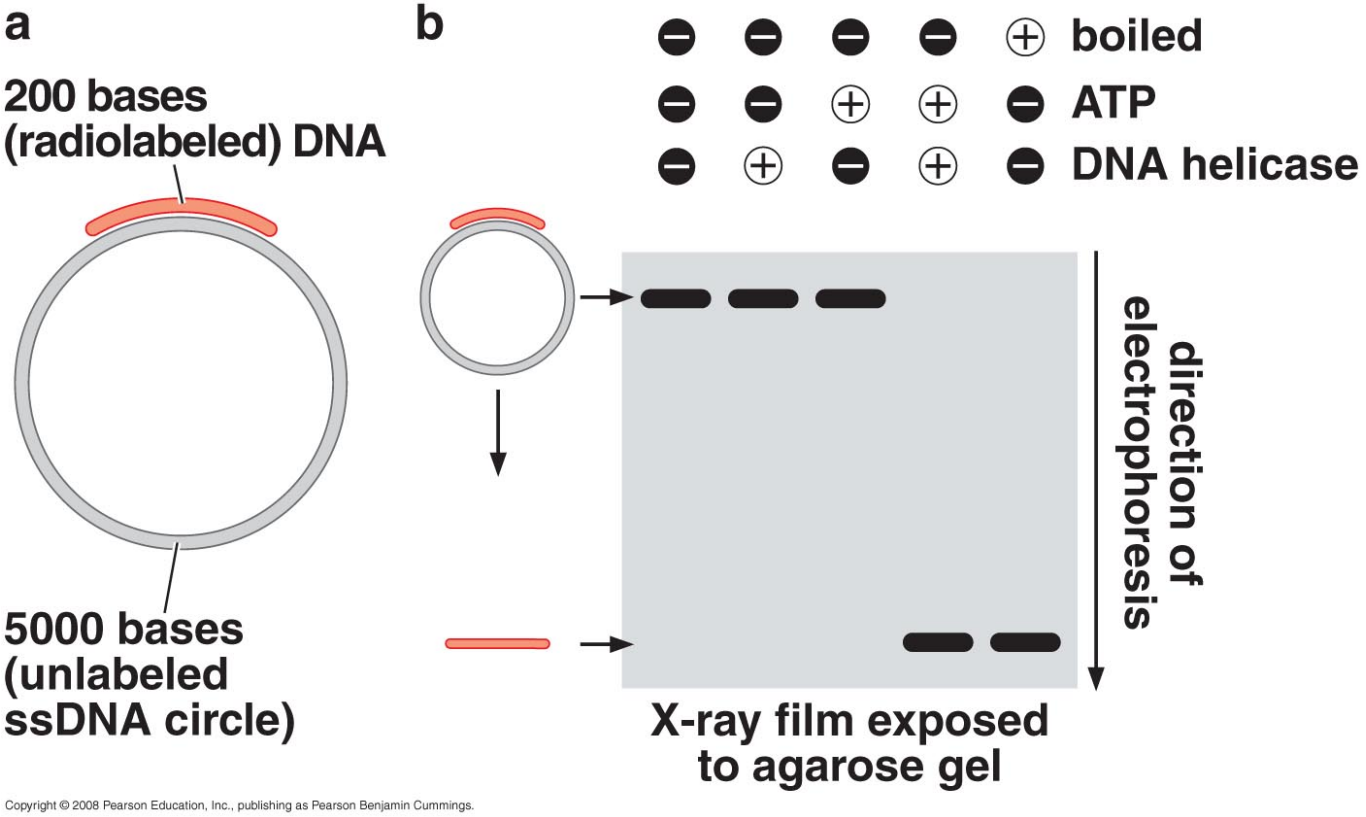


(C)

"hand over hand" torquing along DNA by six blade-like "hands"

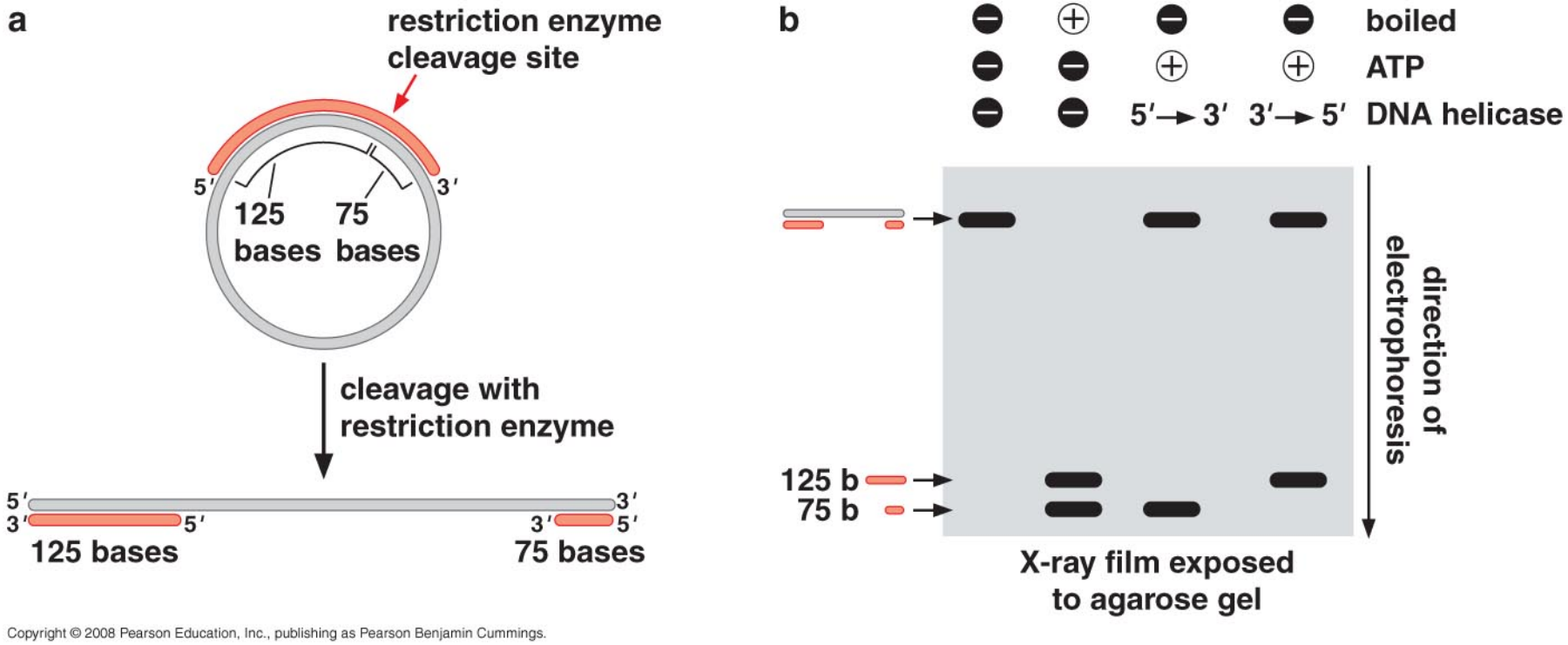
Figure 5-15 *Molecular Biology of the Cell* (© Garland Science 2008)

Biochemical assay for helicase activity: release of ssDNA probe from larger duplex



Different helicases *process* along DNA from 5'→3' or from 3' → 5'…
Helicase on lagging strand slides 5'→3' to pry open replication fork

How you can measure the direction that a helicase goes along the DNA to unwind duplex



Key fact: in vitro, without replication loading complex, helicases can only start on ssDNA…
so tell which direction it went from a gap, by making ssDNA "loading region" internal with asymmetrical duplexes around it, then adding helicase

Single-stranded DNA opened up by helicase needs to be protected from self-hybridization

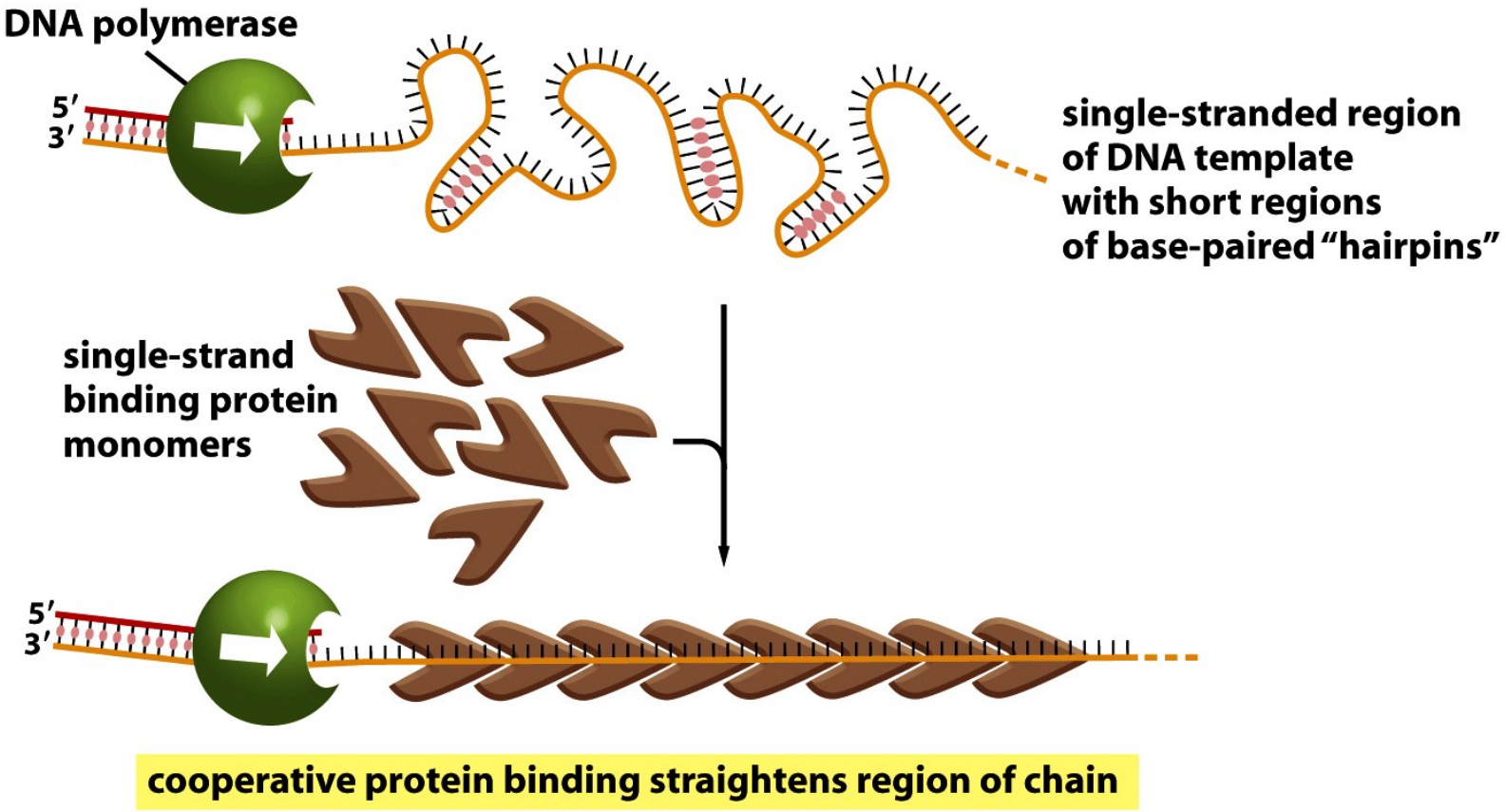
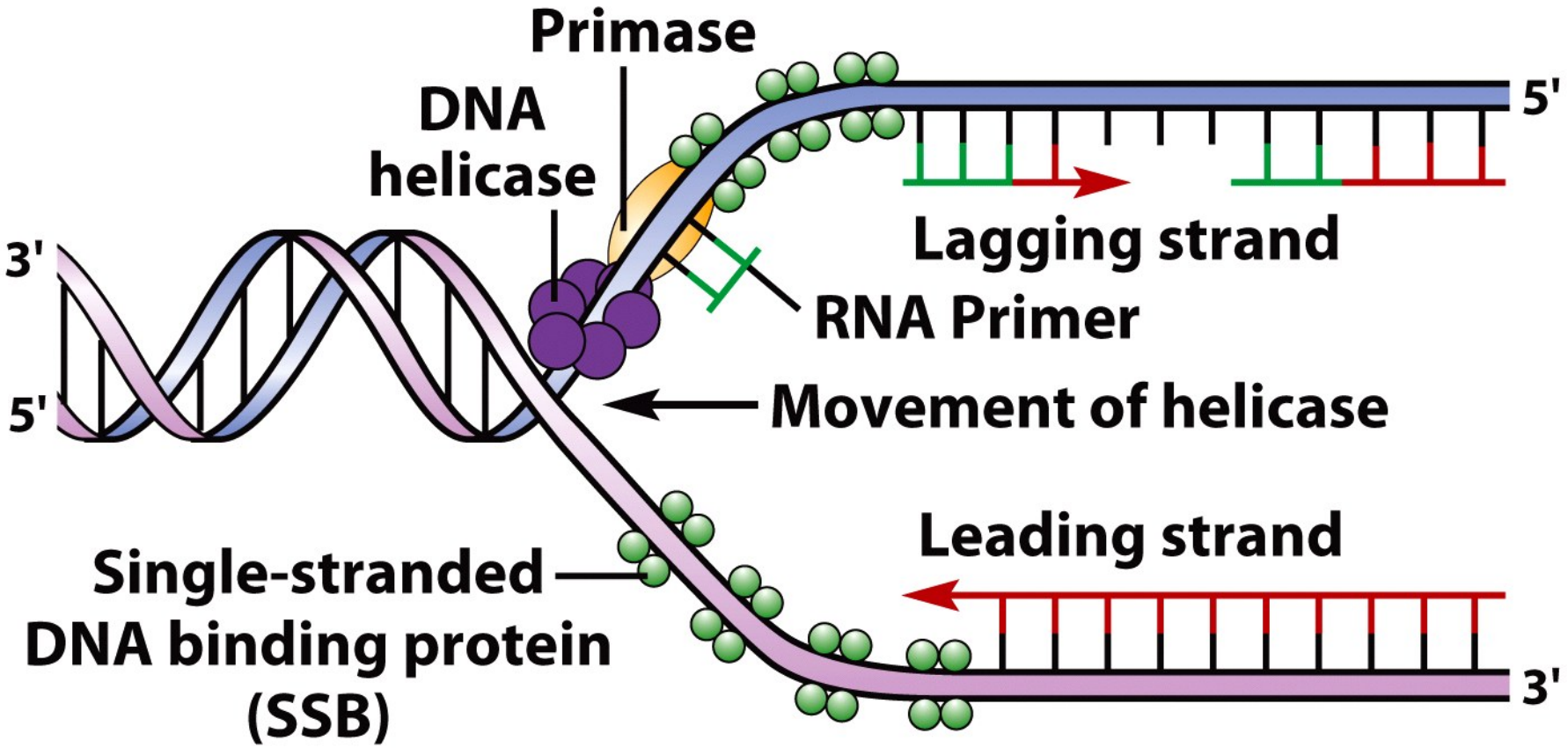


Figure 5-16 *Molecular Biology of the Cell* (© Garland Science 2008)



Processivity is crucial to prevent DNA synthesis from becoming unbalanced or aborted

DNA polymerase action is kept "processive" along continuous ssDNA stretches by mounting on a "sliding clamp"

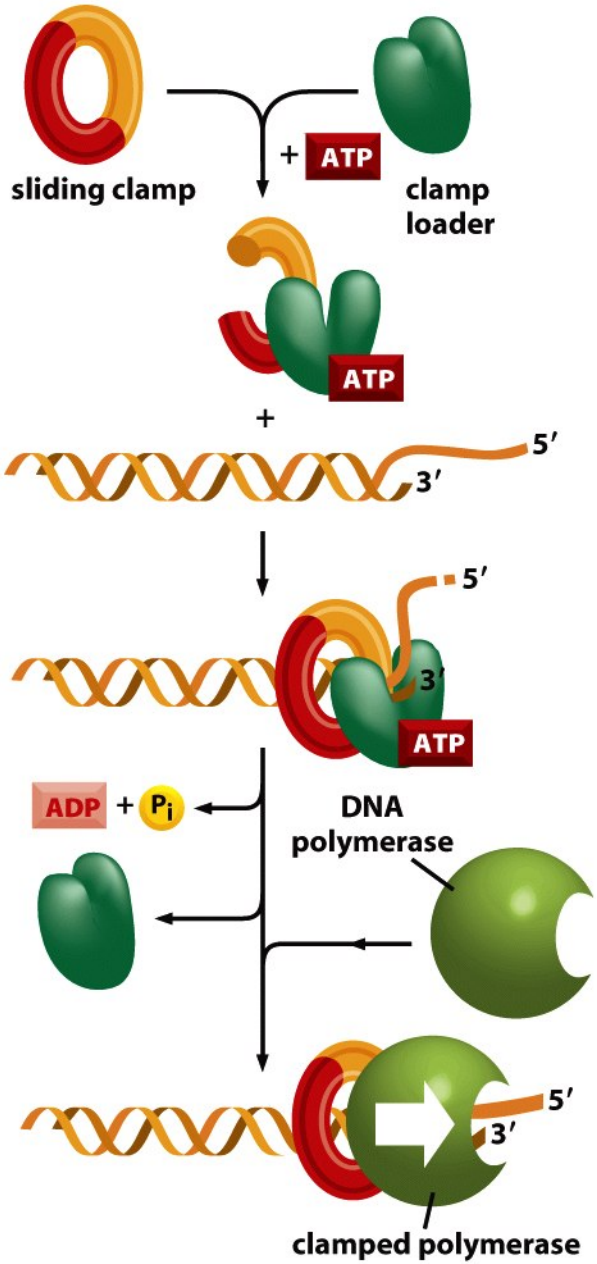


Figure 5-18c *Molecular Biology of the Cell* (© Garland Science 2008)

This is also called the “trombone model”: first proposed by Bruce Alberts

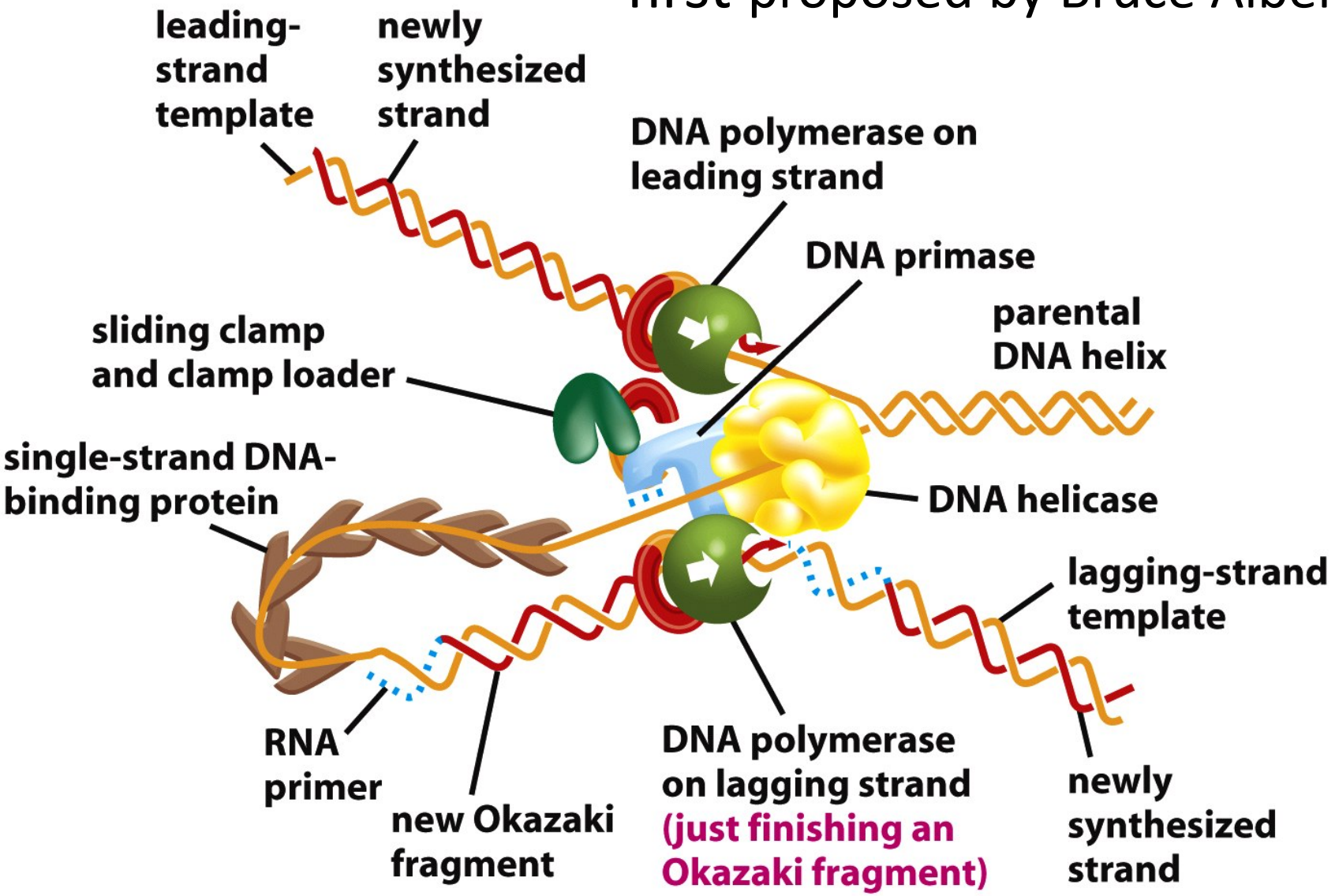
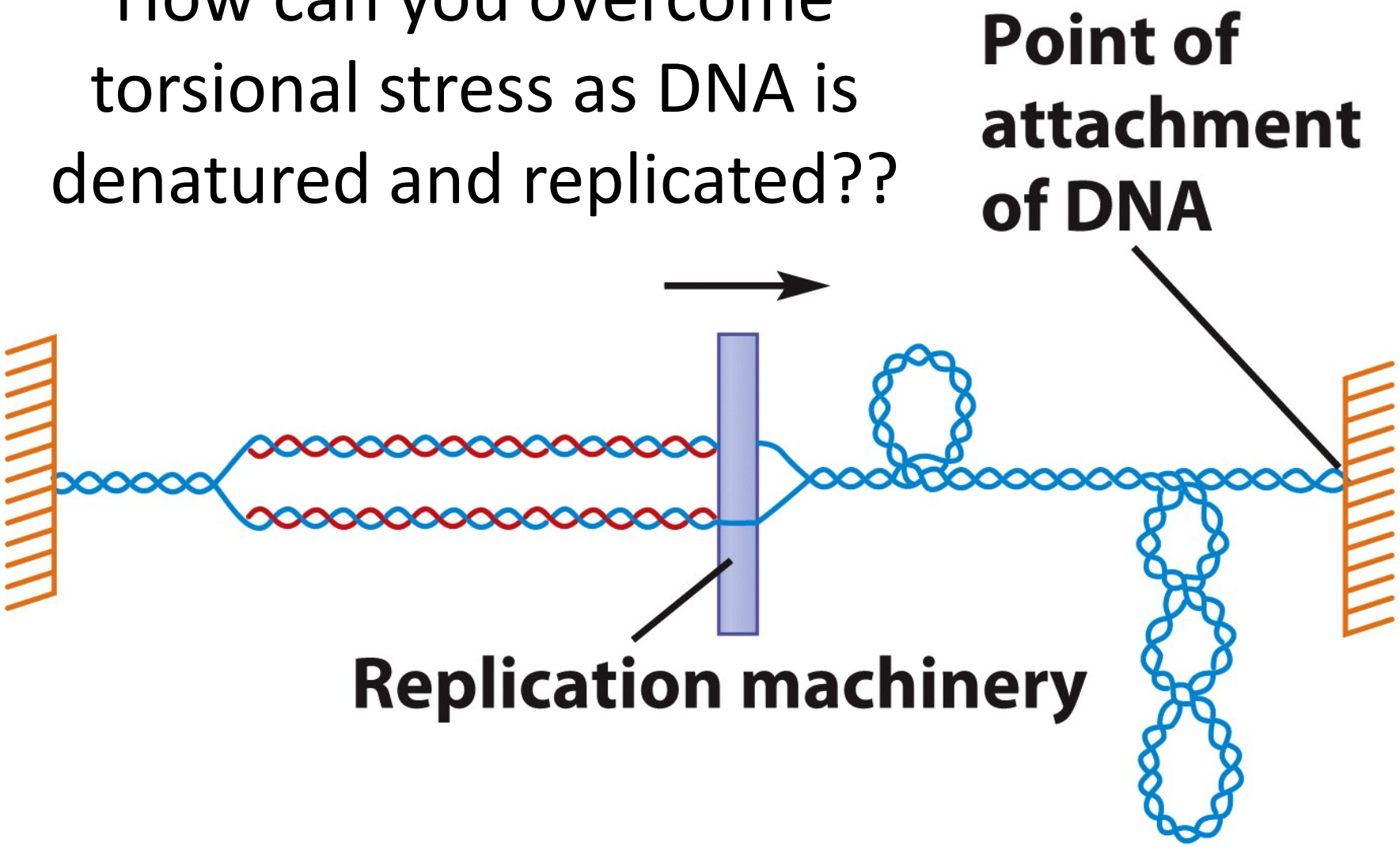
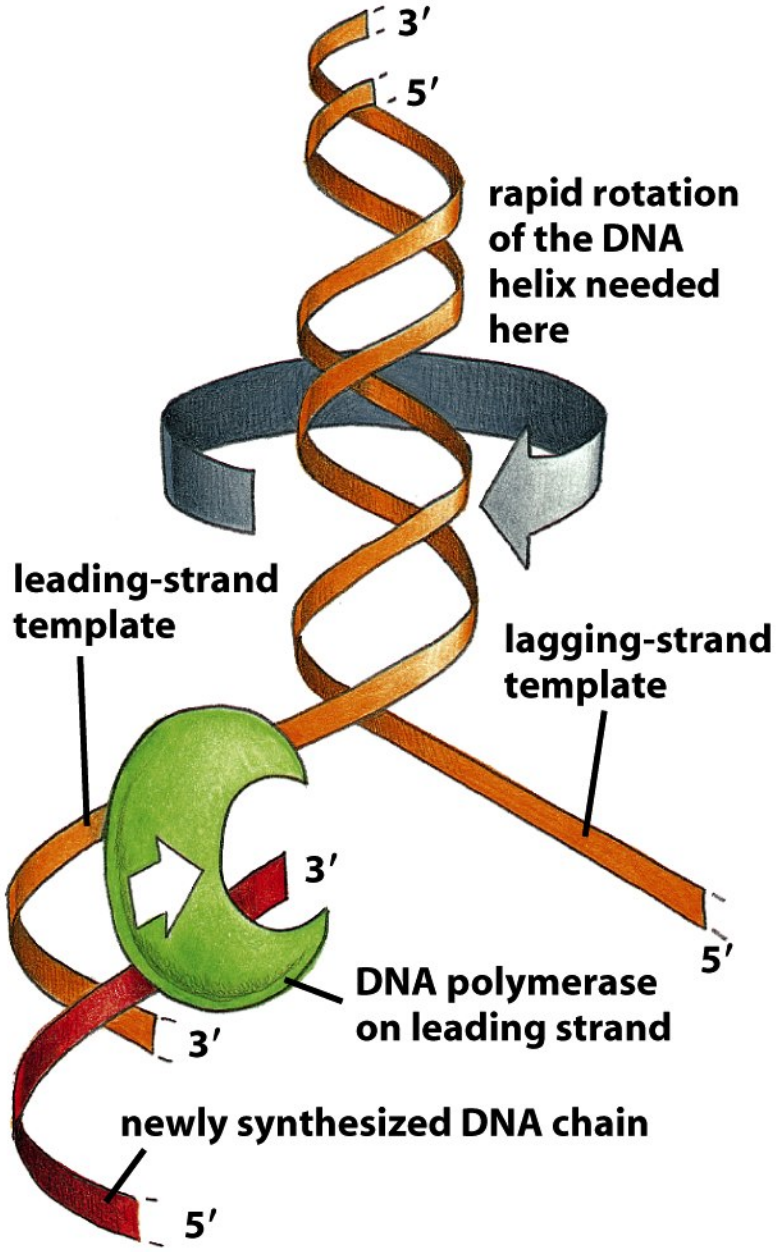


Figure 5-19a *Molecular Biology of the Cell* (© Garland Science 2008)

How can you overcome torsional stress as DNA is denatured and replicated??



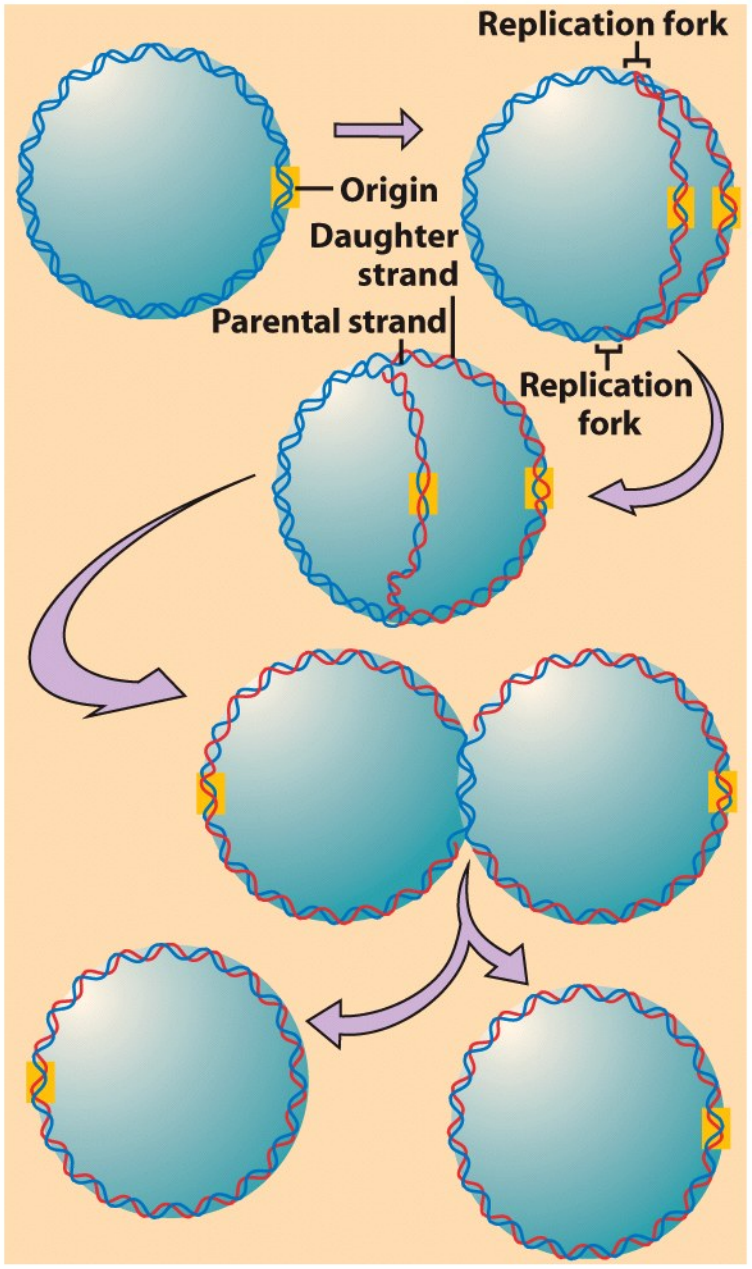


The price of denaturation is also a DNA twist relaxation enzyme: topoisomerase

Quick single-strand nicking lets DNA spin to let out stress, then reconnection

“type I topoisomerases”

Figure 5-21 *Molecular Biology of the Cell* (© Garland Science 2008)



Topoisomerases also exist that make brief dsDNA breaks to untwist daughter circular DNAs after replication

“type II topoisomerases”

Important for later: eukaryotic DNA is packaged with histones, and new histones are quickly loaded on new DNA

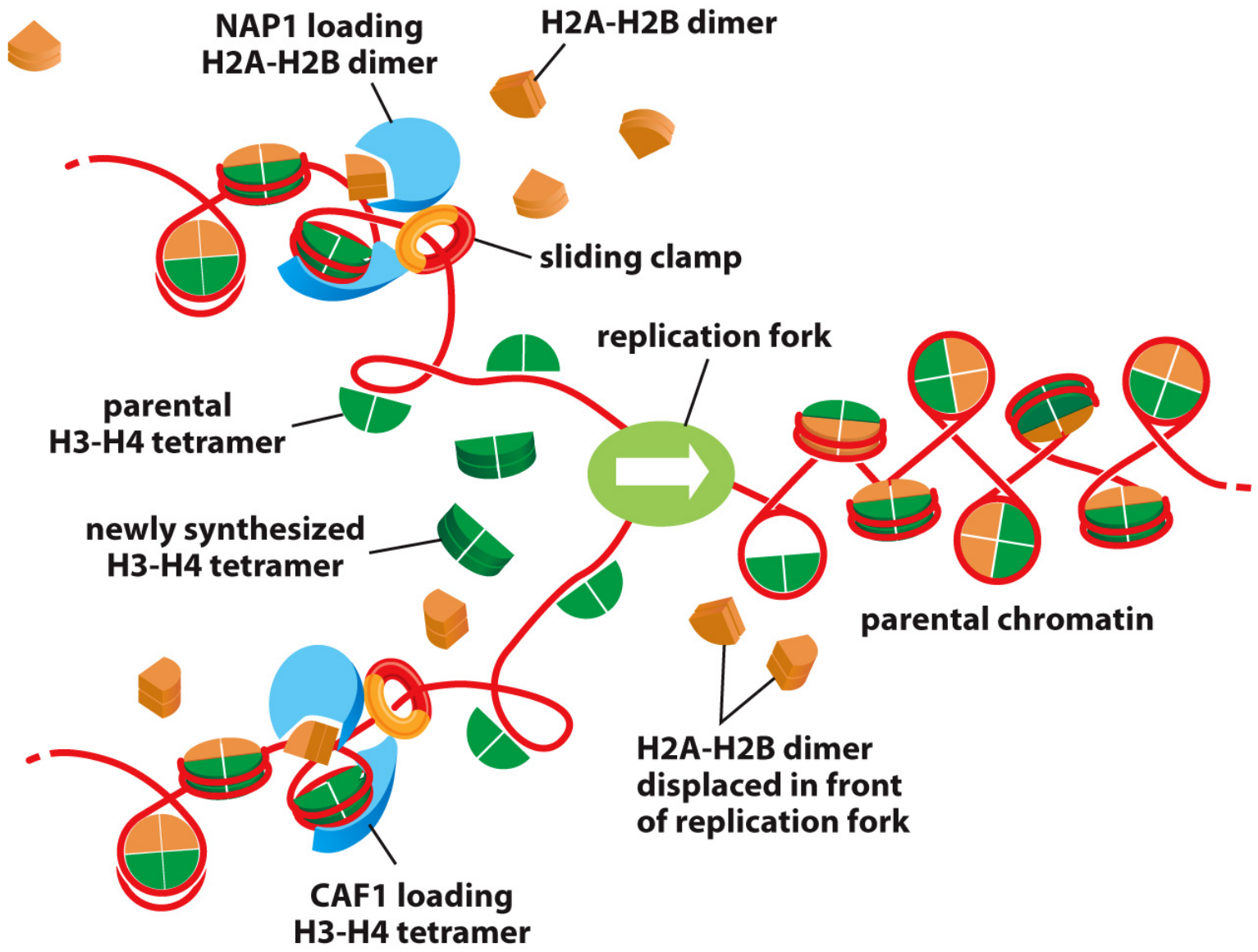


Figure 5-32 Molecular Biology of the Cell 6e (© Garland Science 2015)