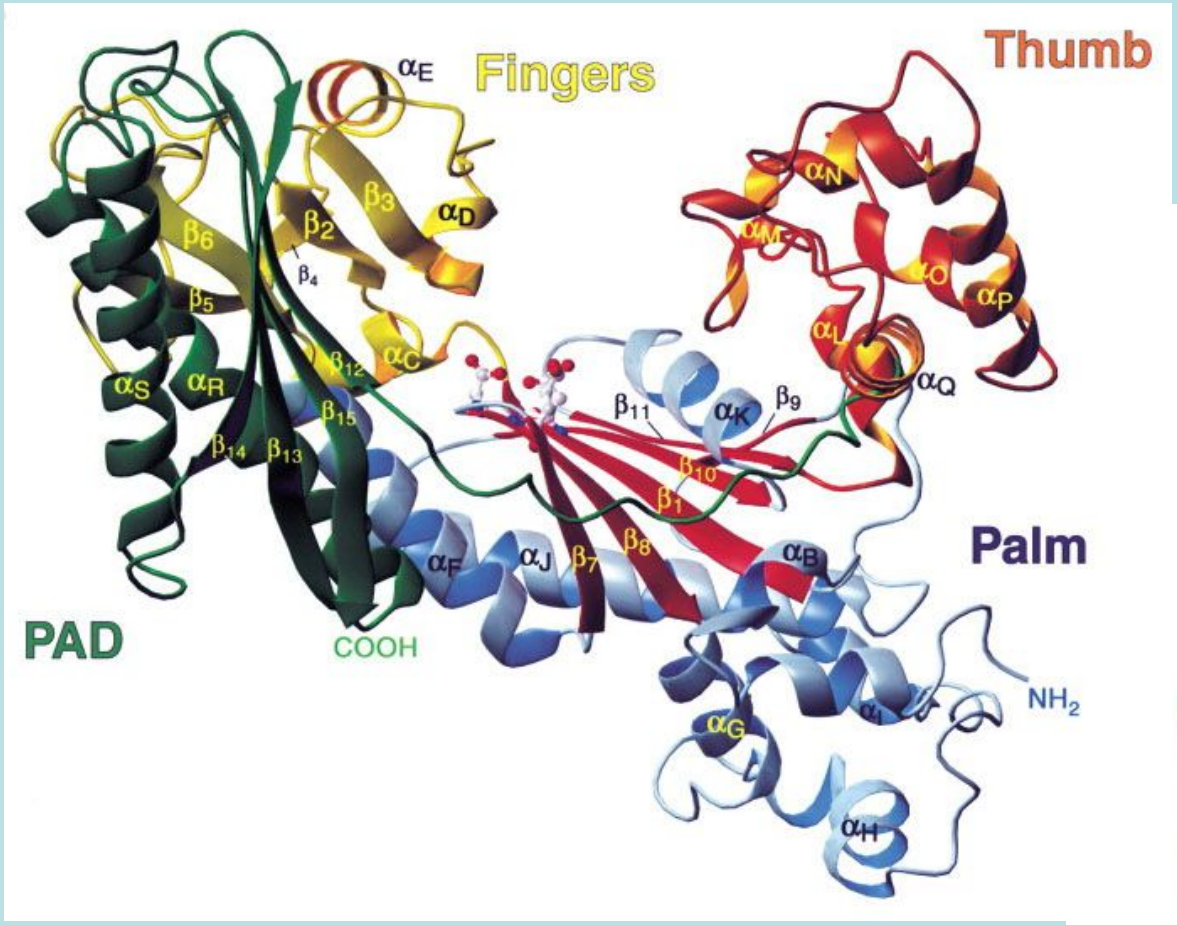
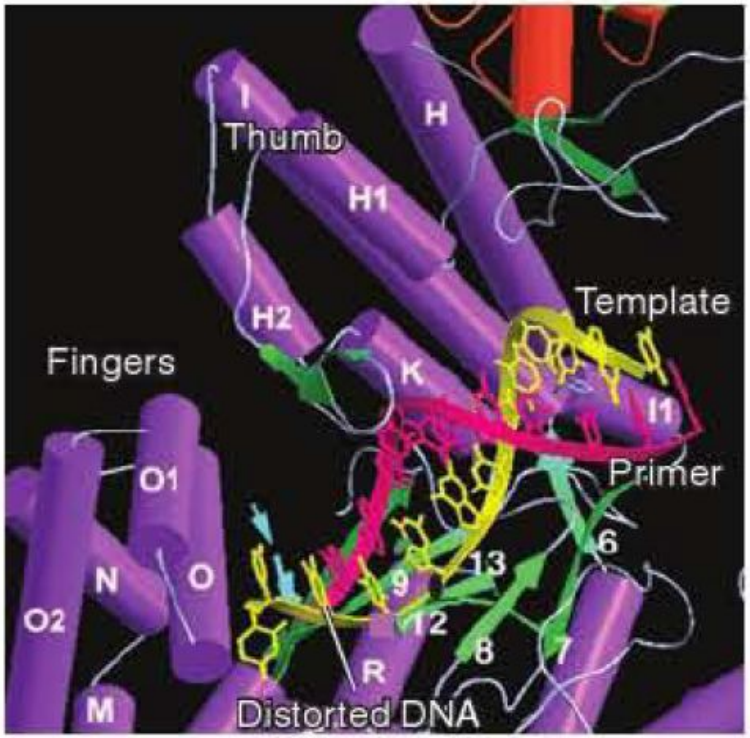
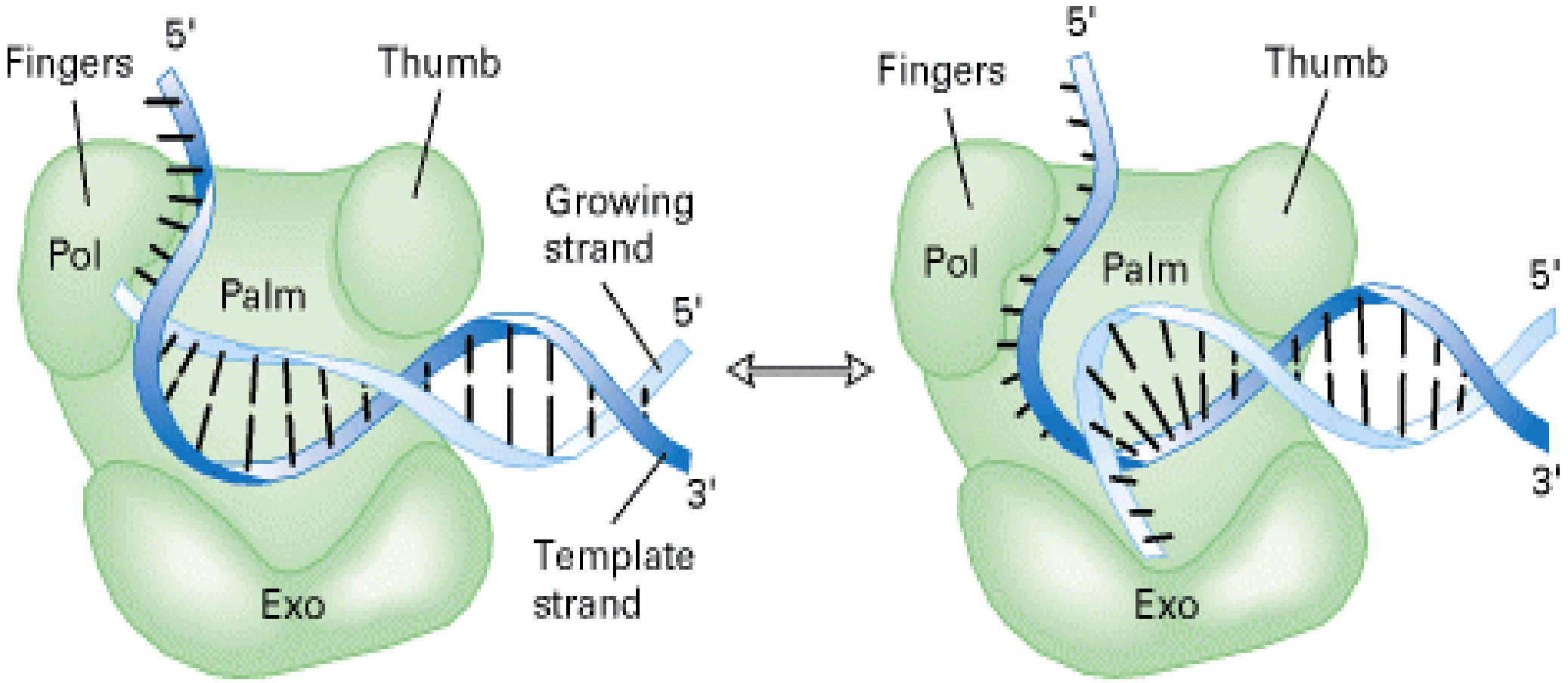




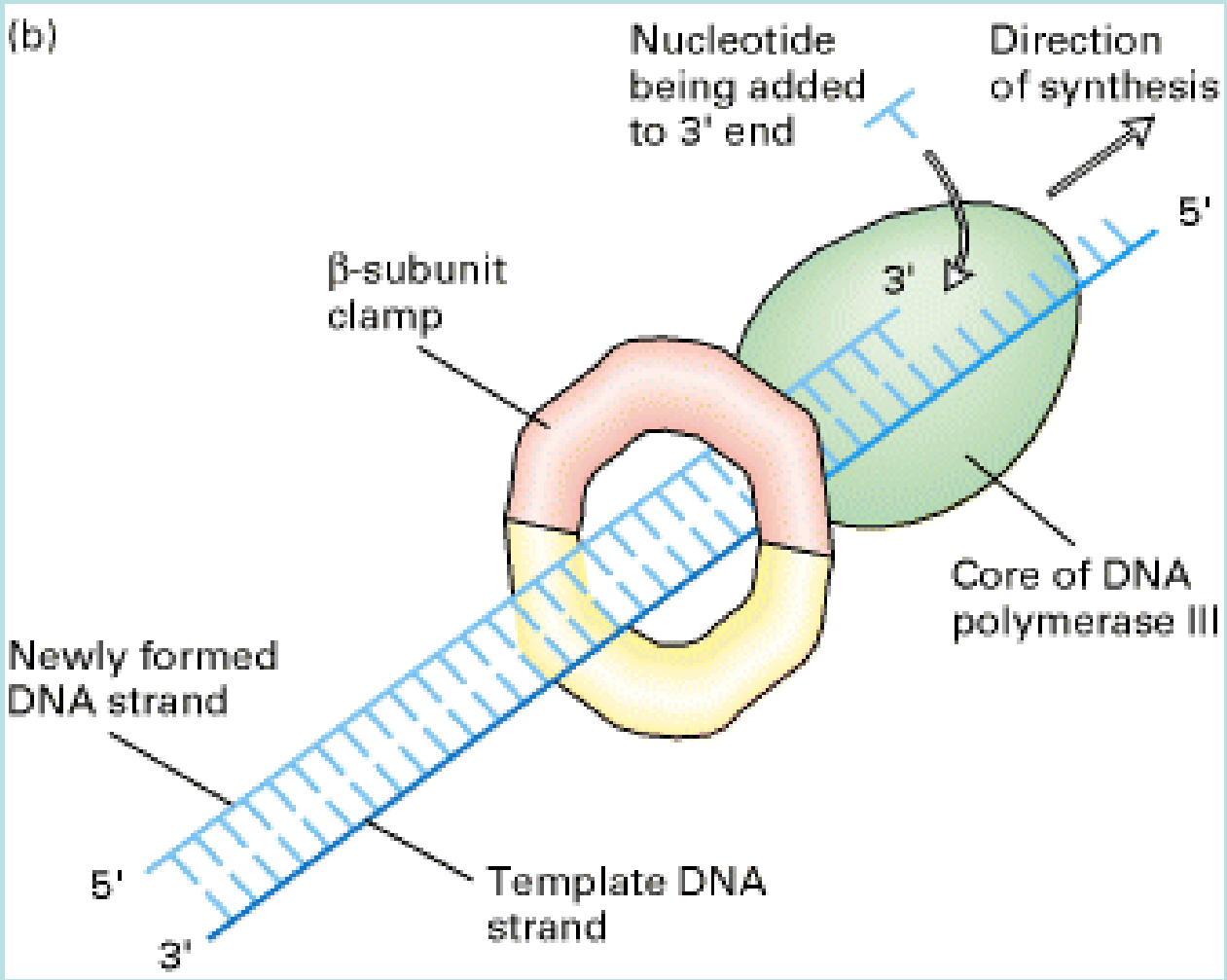
DNA polymerase enzyme core

FIGURE 13.9 The crystal structure of phage T7 DNA polymerase shows that the template strand takes a sharp turn that exposes it to the incoming nucleotide. Photo courtesy of Charles Richardson and Thomas Ellenberger, Washington University School of Medicine.


Fingers
5'
Thumb
Pol
Palm
Growing strand
5'
3'
Template strand
Exo
Fingers
5'
Thumb
Pol
Palm
5'
3'
Exo

E. coli DNA polymerase III

Heteromultimer composed of more than 10 different subunits
"Processing" enzyme → up to 10^5 nucleotide streches



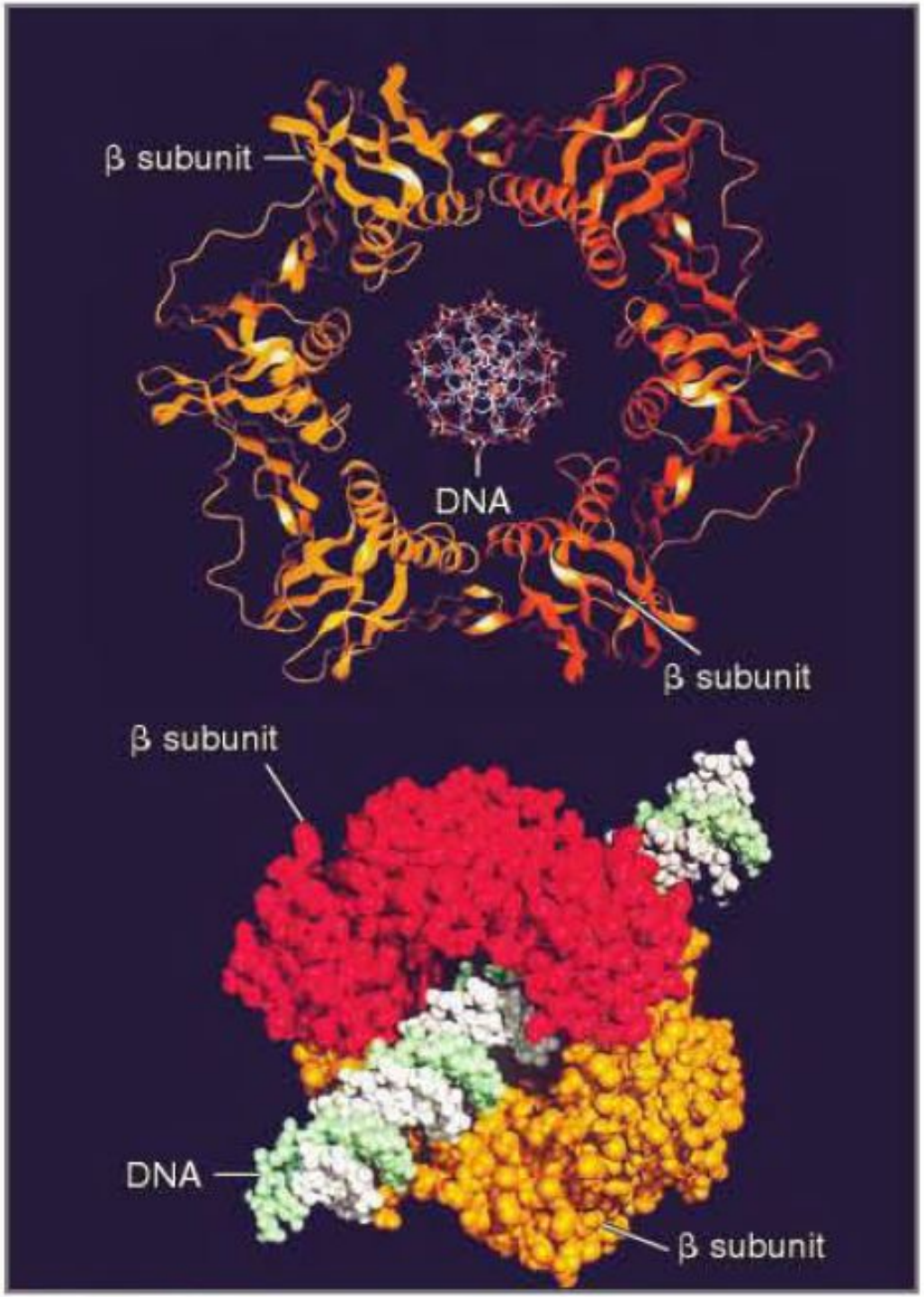


FIGURE 13.17 The subunit of DNA polymerase III holoenzyme consists of a head-to-tail dimer (the two subunits are shown in red and orange) that forms a ring completely surrounding a DNA duplex (shown in the center). Reprinted from *Cell*, vol. 69, X. P. Kong, et al., Three-dimensional structure of the β . . . , pp. 425–437. Copyright 1992, with permission from Elsevier [http://www.sciencedirect.com/science/journal/00928674]. Photo courtesy of John Kuriyan, University of California, Berkeley.

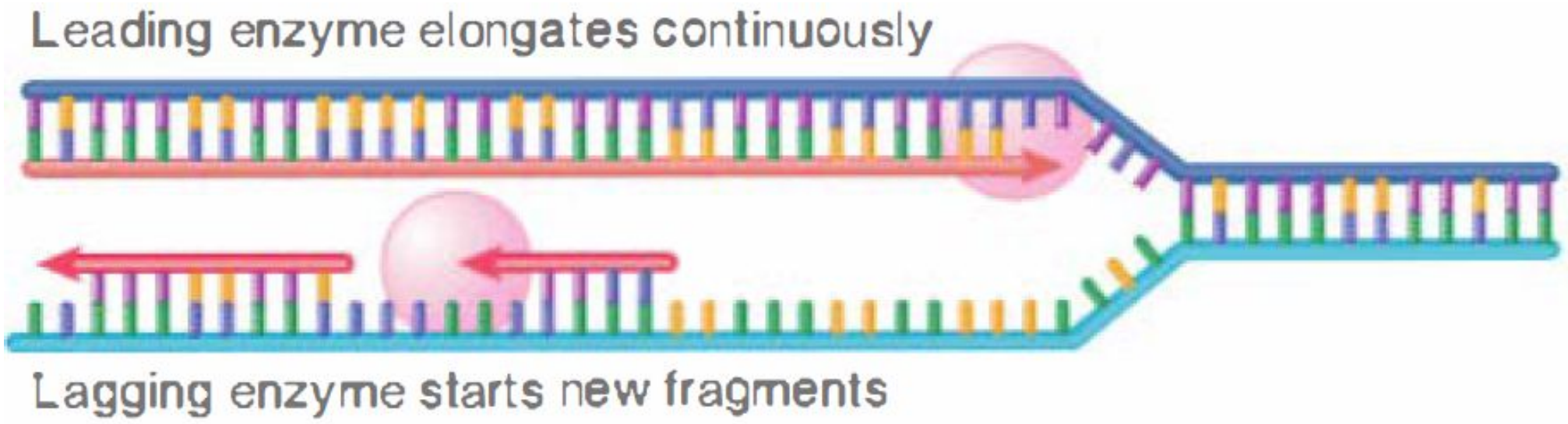
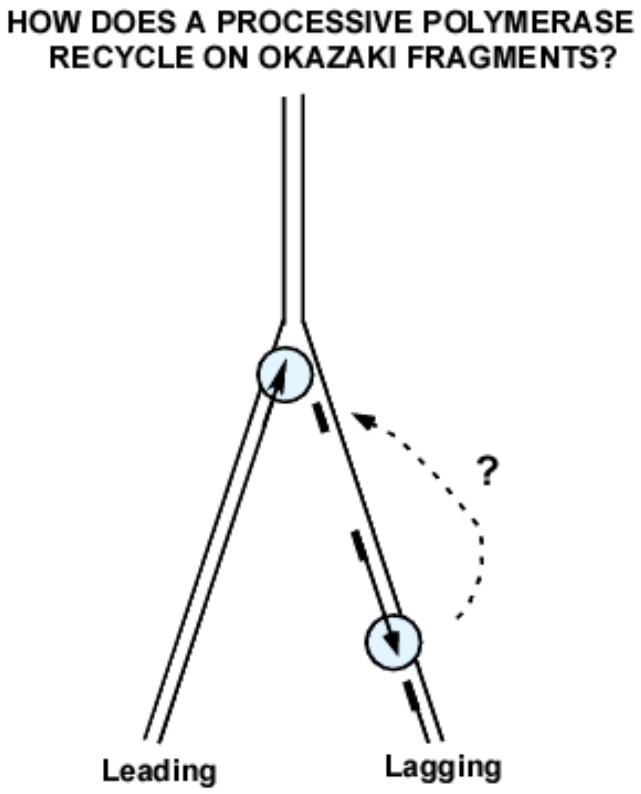
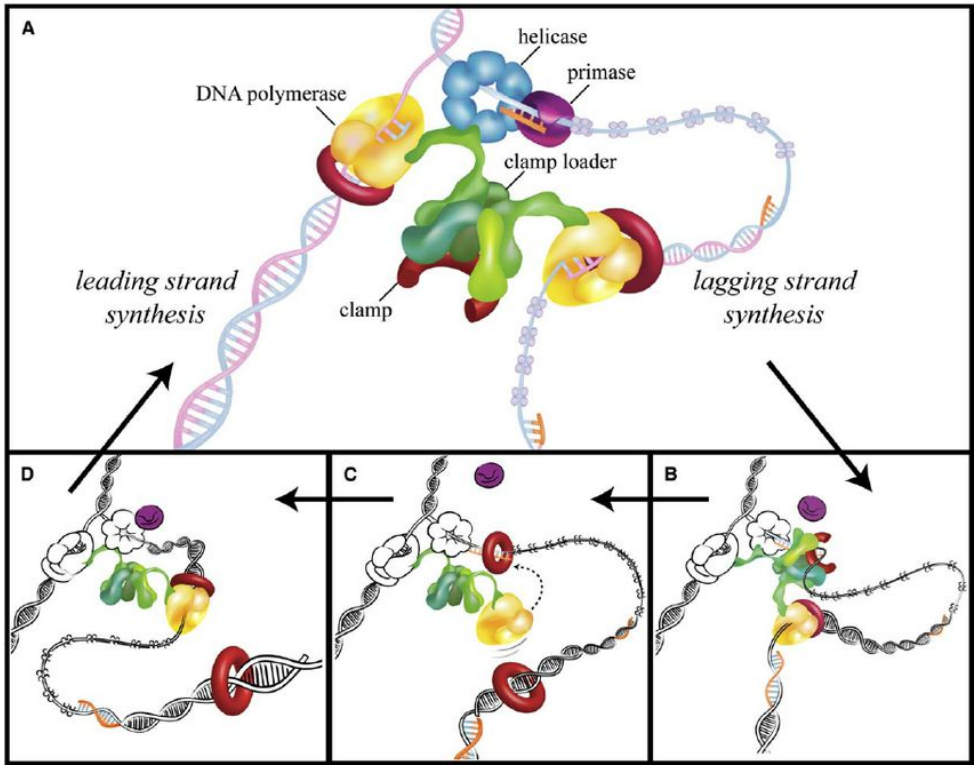


FIGURE 13.15 A replication complex contains separate catalytic units for synthesizing the leading and lagging strands.



DNA polymerase III (blue) is processive because it binds very tight to DNA. But on the lagging strand, it must rapidly recycle to numerous Okazaki fragments. How does Pol III come off a completed Okazaki fragment, so that it can start a new Okazaki fragment?

Presumably Pol III needs a release factor to pry it off DNA when it finishes, enabling Pol III to recycle to another primed DNA molecule.
Pol III core stays bound to beta clamp and DNA during synthesis, but upon finishing DNA, the Pol III core ejects off the DNA and the beta clamp.



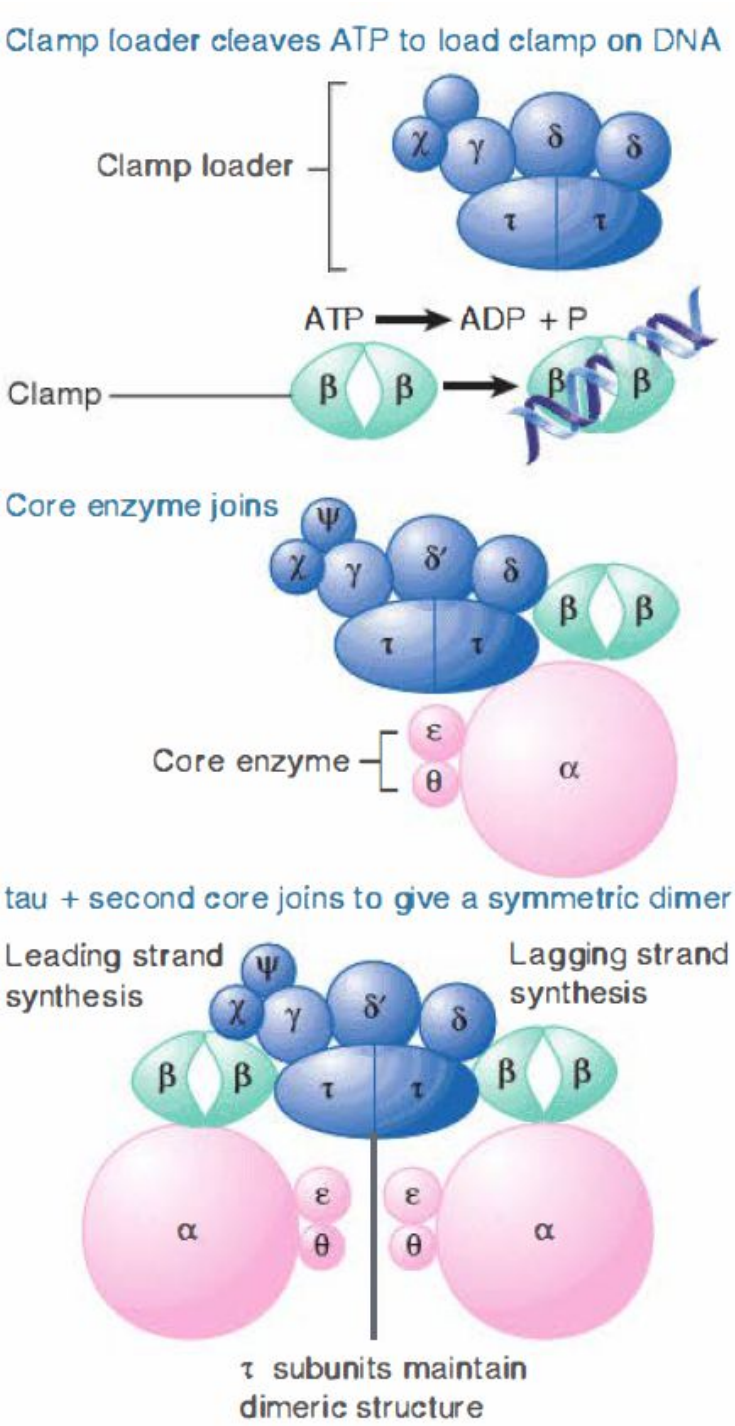
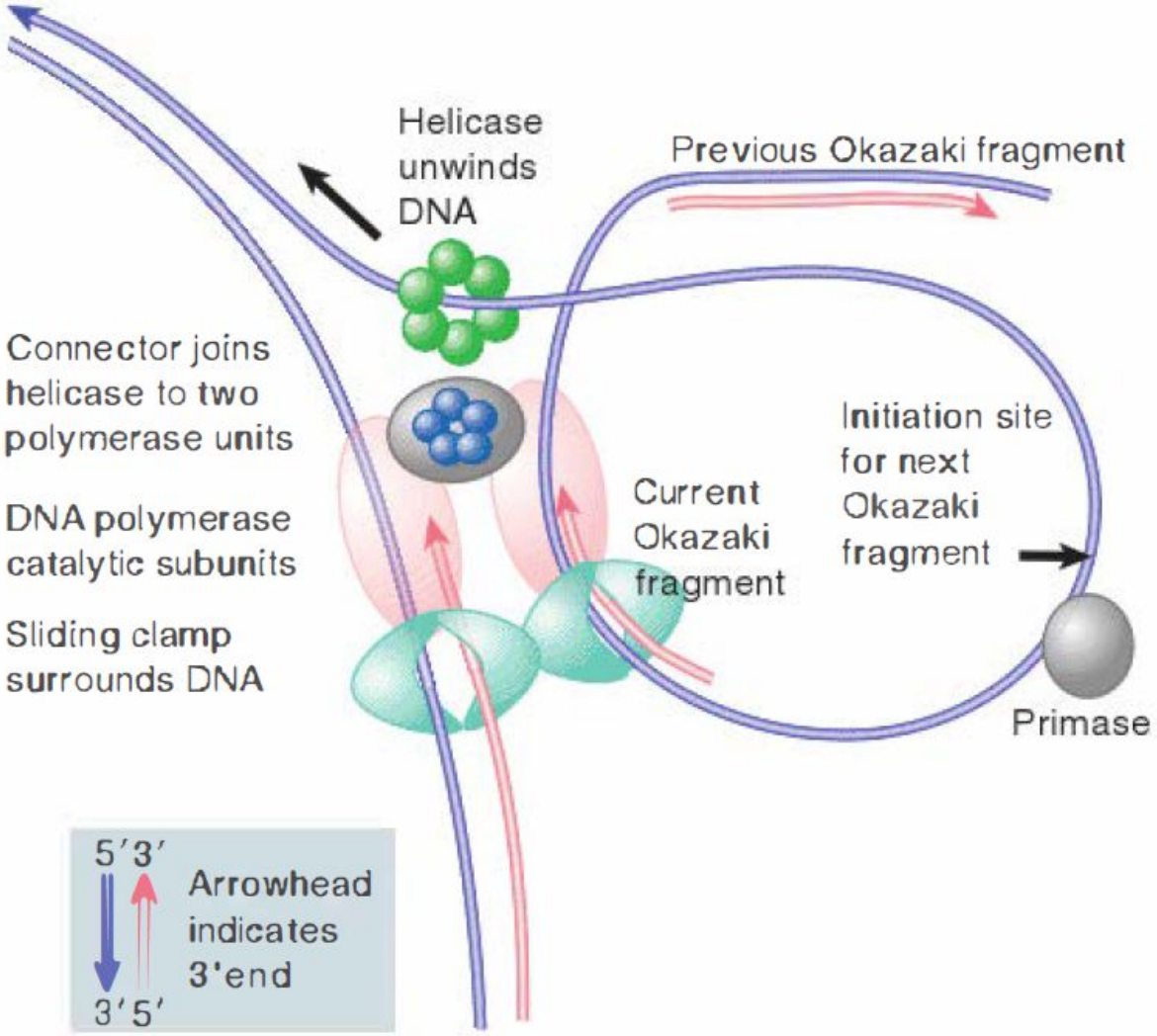


FIGURE 13.16 DNA polymerase III holoenzyme assembles in stages, generating an enzyme complex that synthesizes the DNA of both new strands.

FIGURE 13.18 The helicase creating the replication fork is connected to two DNA polymerase catalytic subunits, each of which is held onto DNA by a sliding clamp. The polymerase that synthesizes the leading strand moves continuously. The polymerase that synthesizes the lagging strand dissociates at the end of an Okazaki fragment and then reassociates with a primer in the single-stranded template loop to synthesize the next fragment.



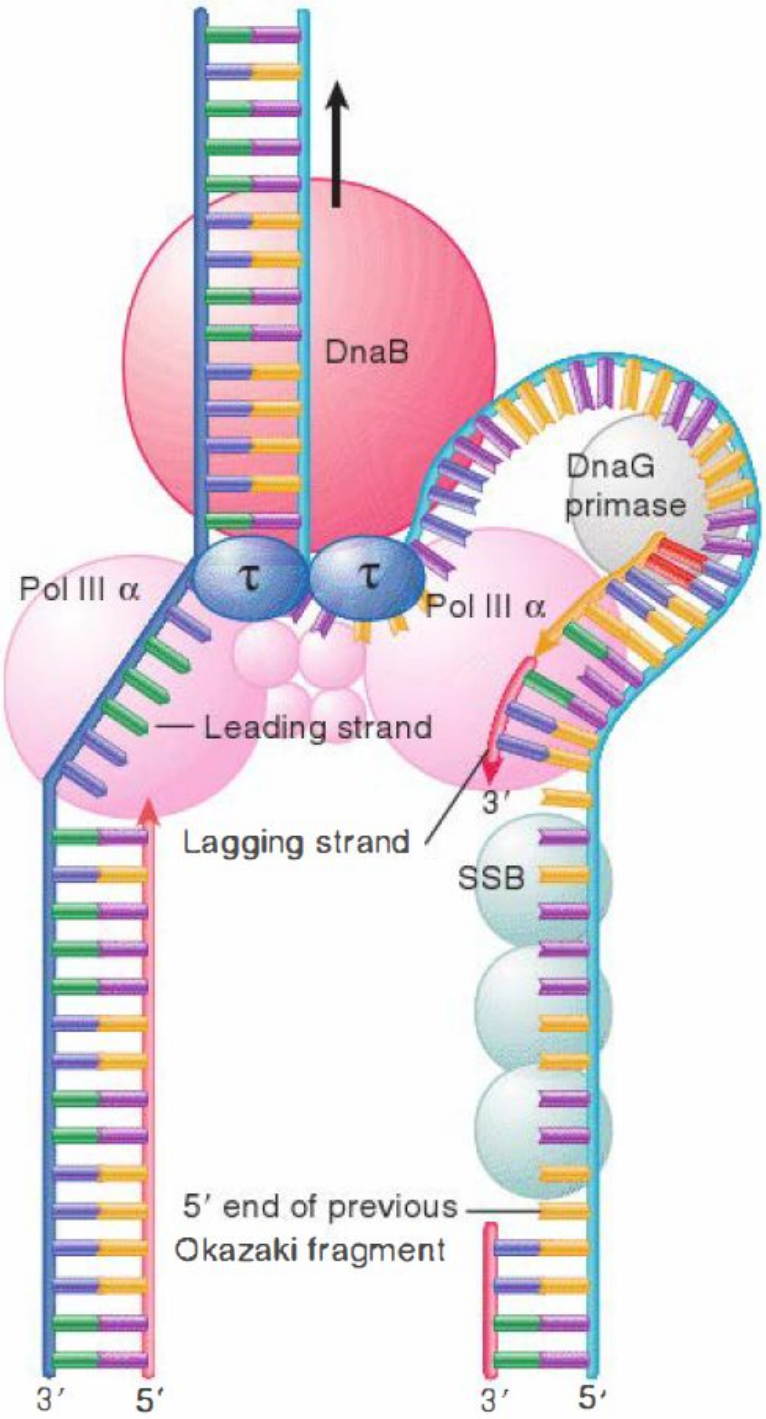
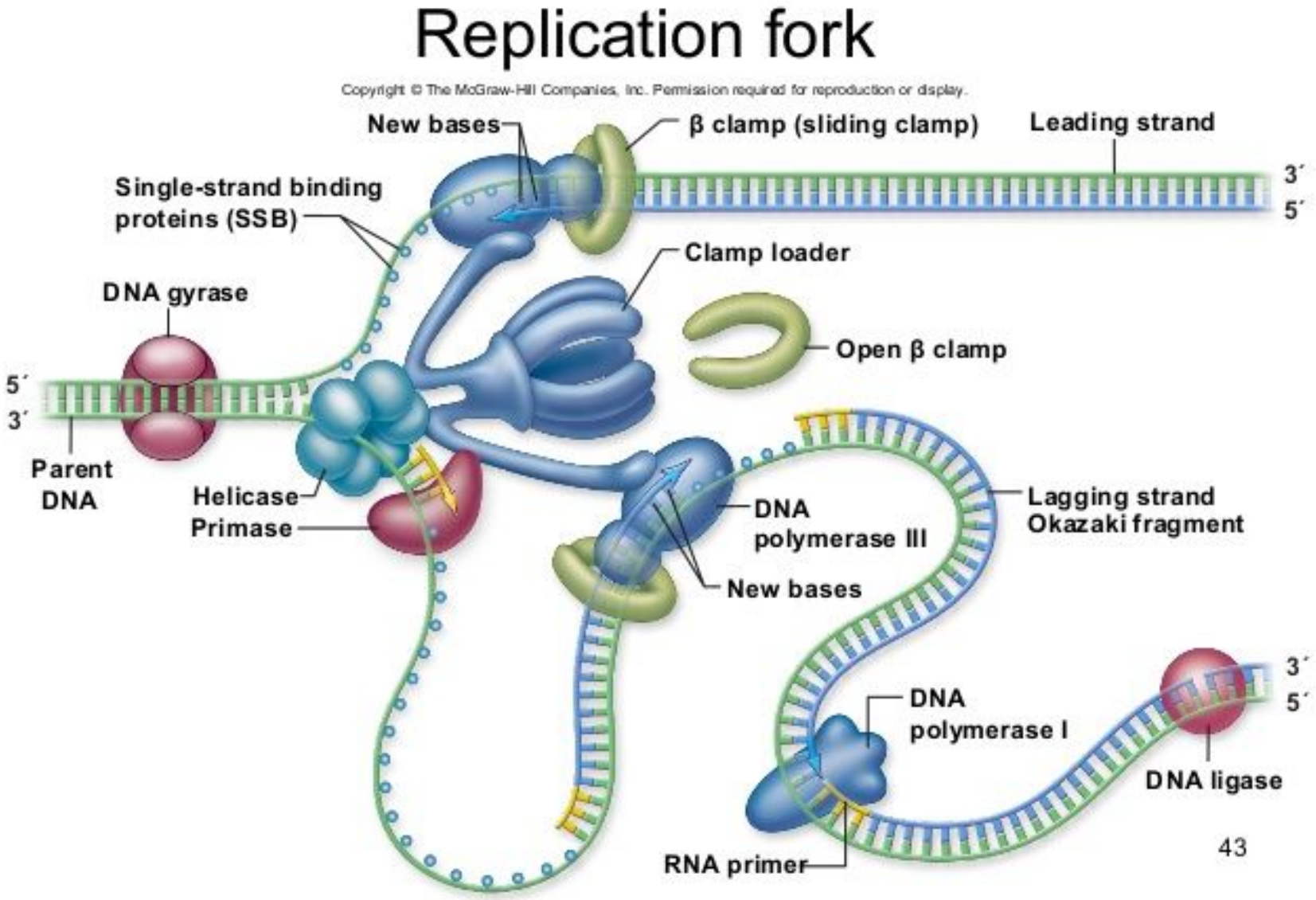


FIGURE 13.19 Each catalytic core of Pol III synthesizes a daughter strand. DnaB is responsible for forward movement at the replication fork.

Leading and lagging strands synthesis are coordinated



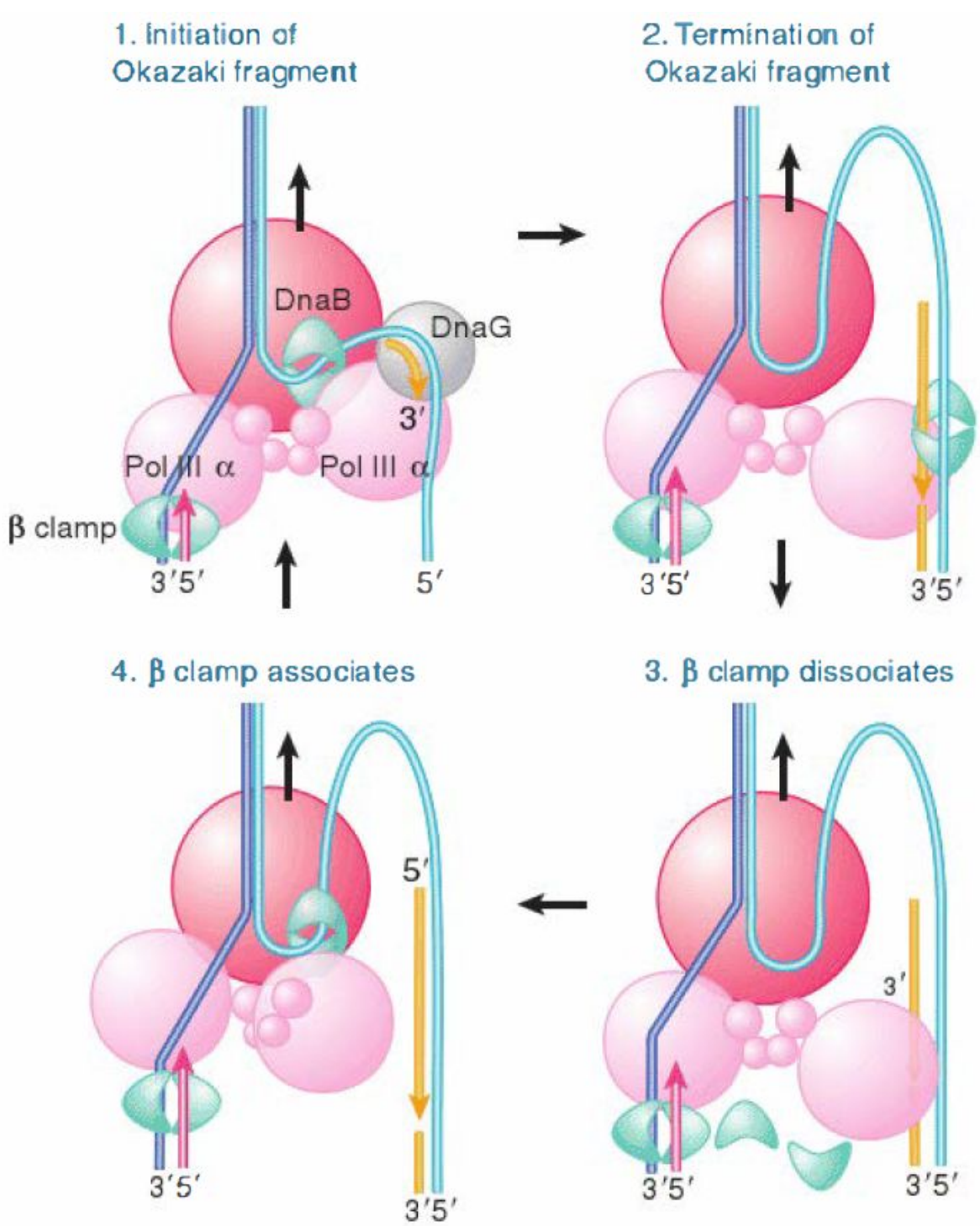


FIGURE 13.20 Core polymerase and the clamp dissociate at completion of Okazaki fragment synthesis and reassociate at the beginning.

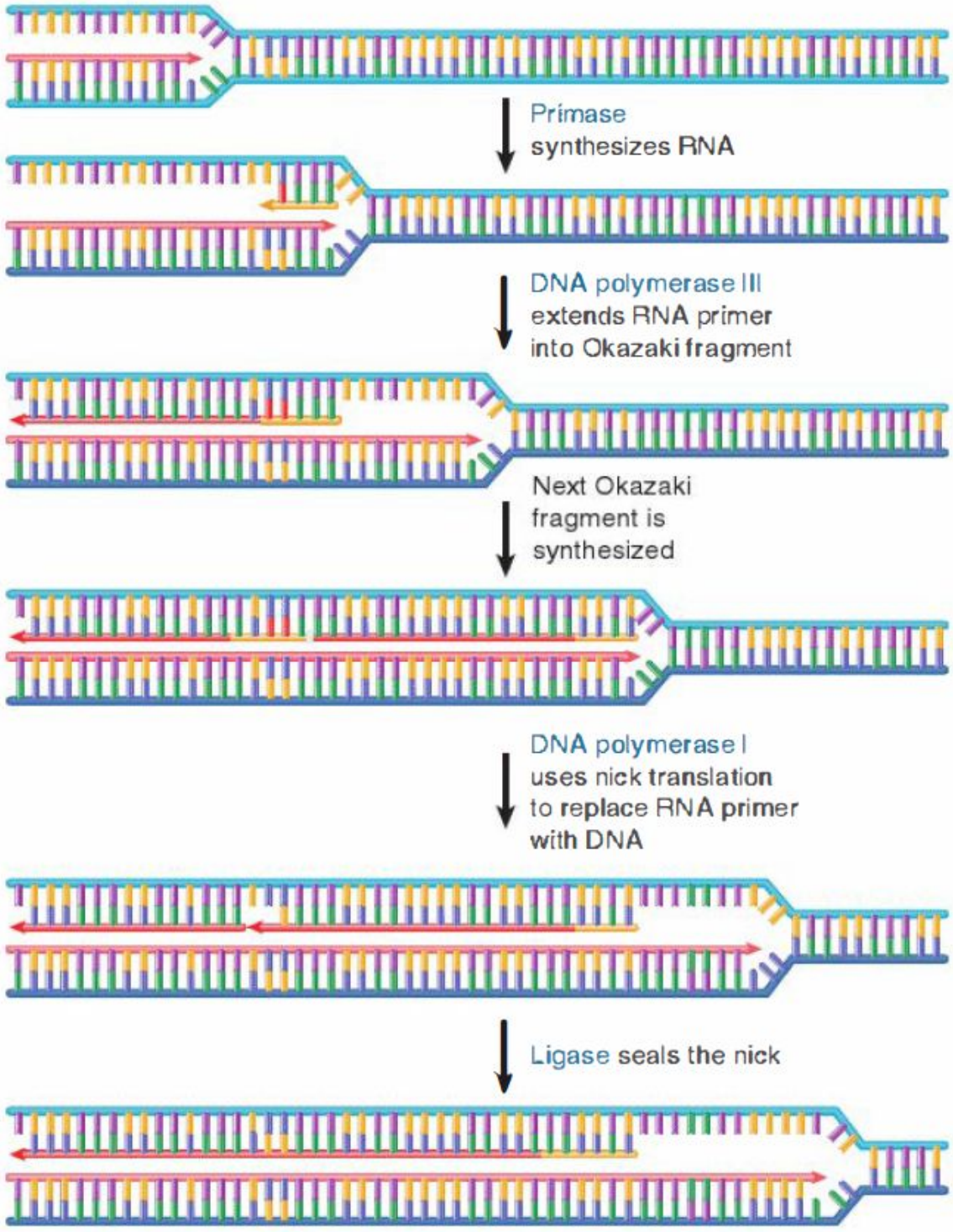
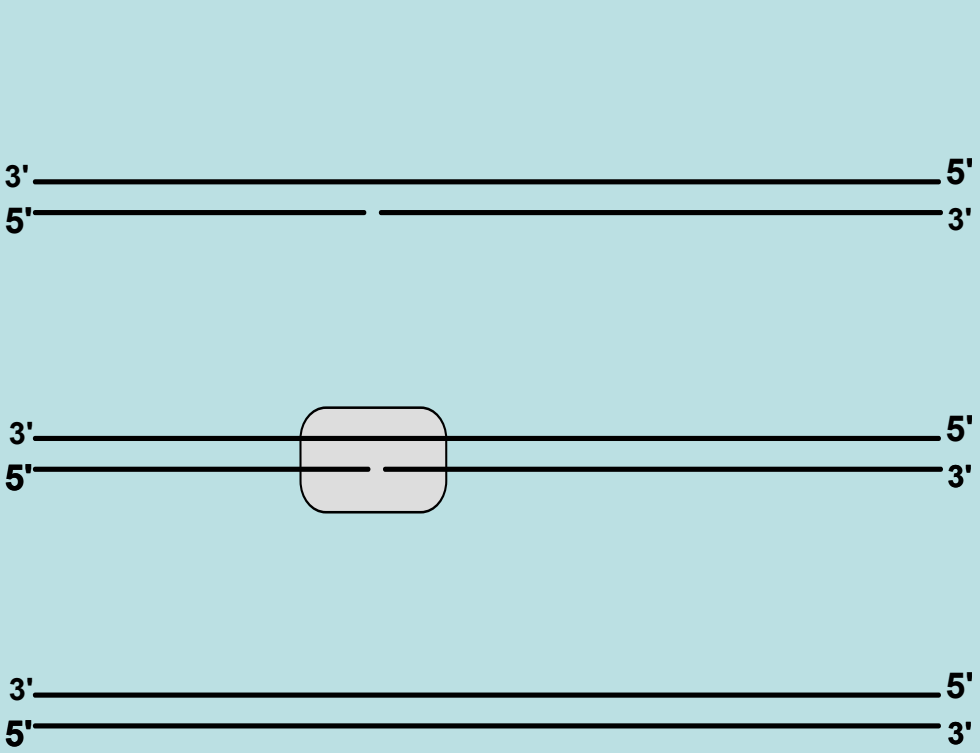
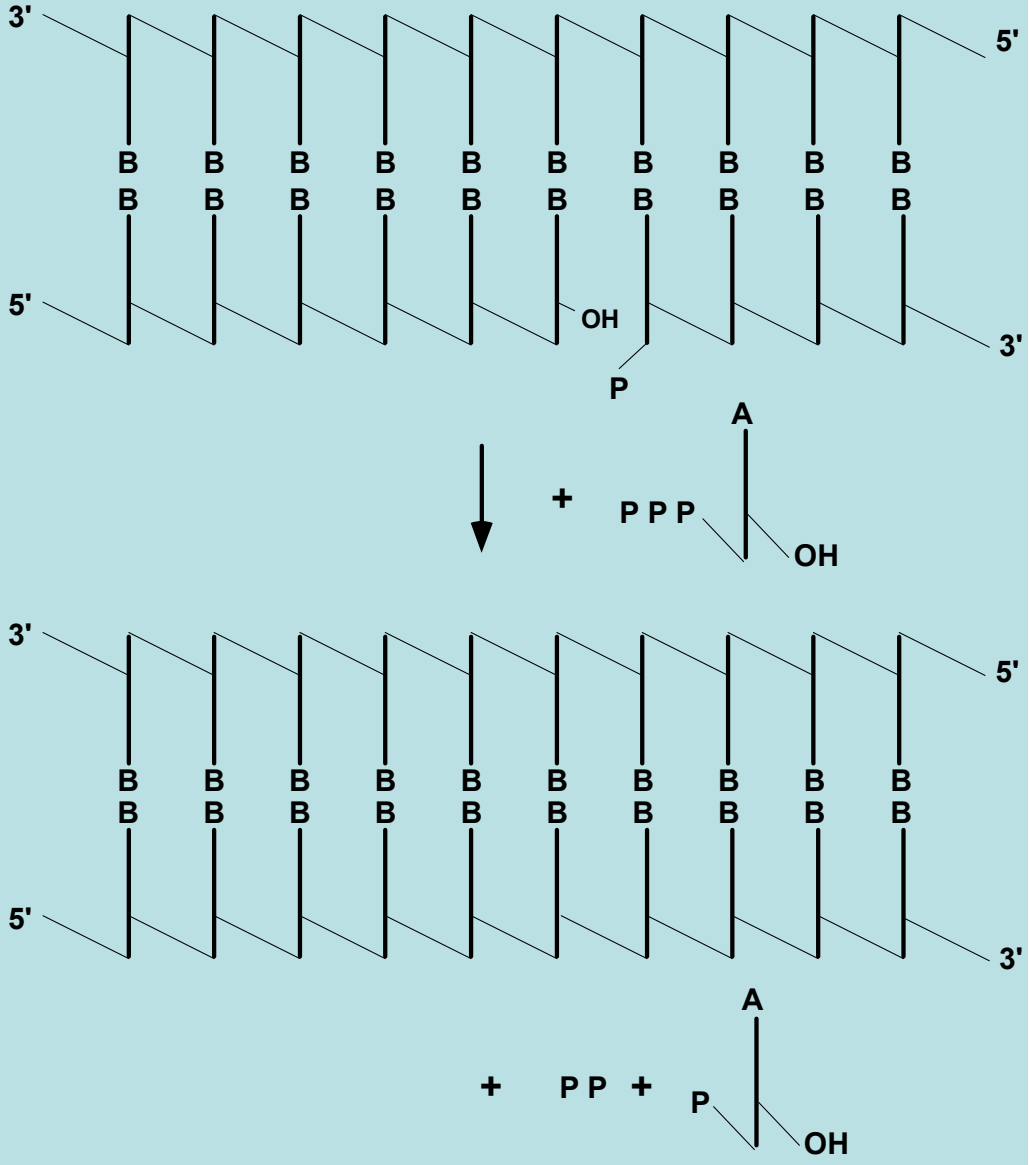


FIGURE 13.21 Synthesis of Okazaki fragments require priming, extension, removal of RNA primer, gap filling, and nick ligation.

DNA ligase activity





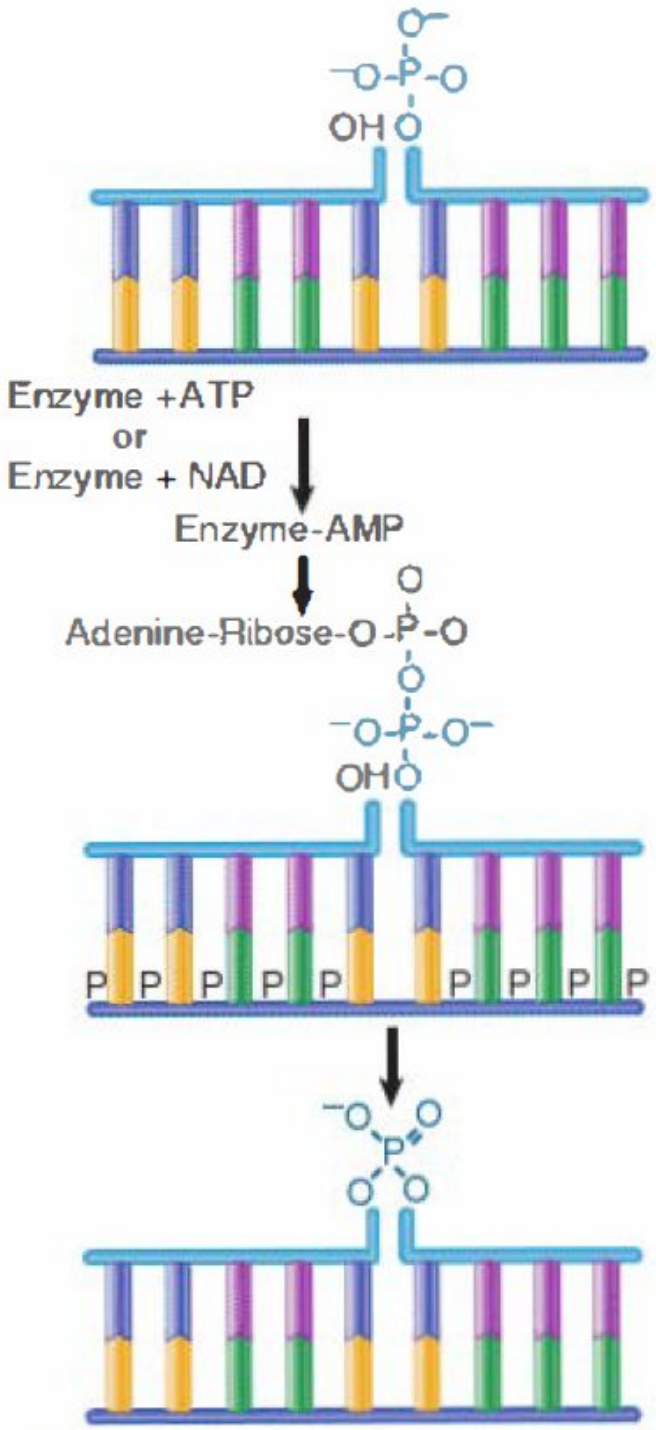
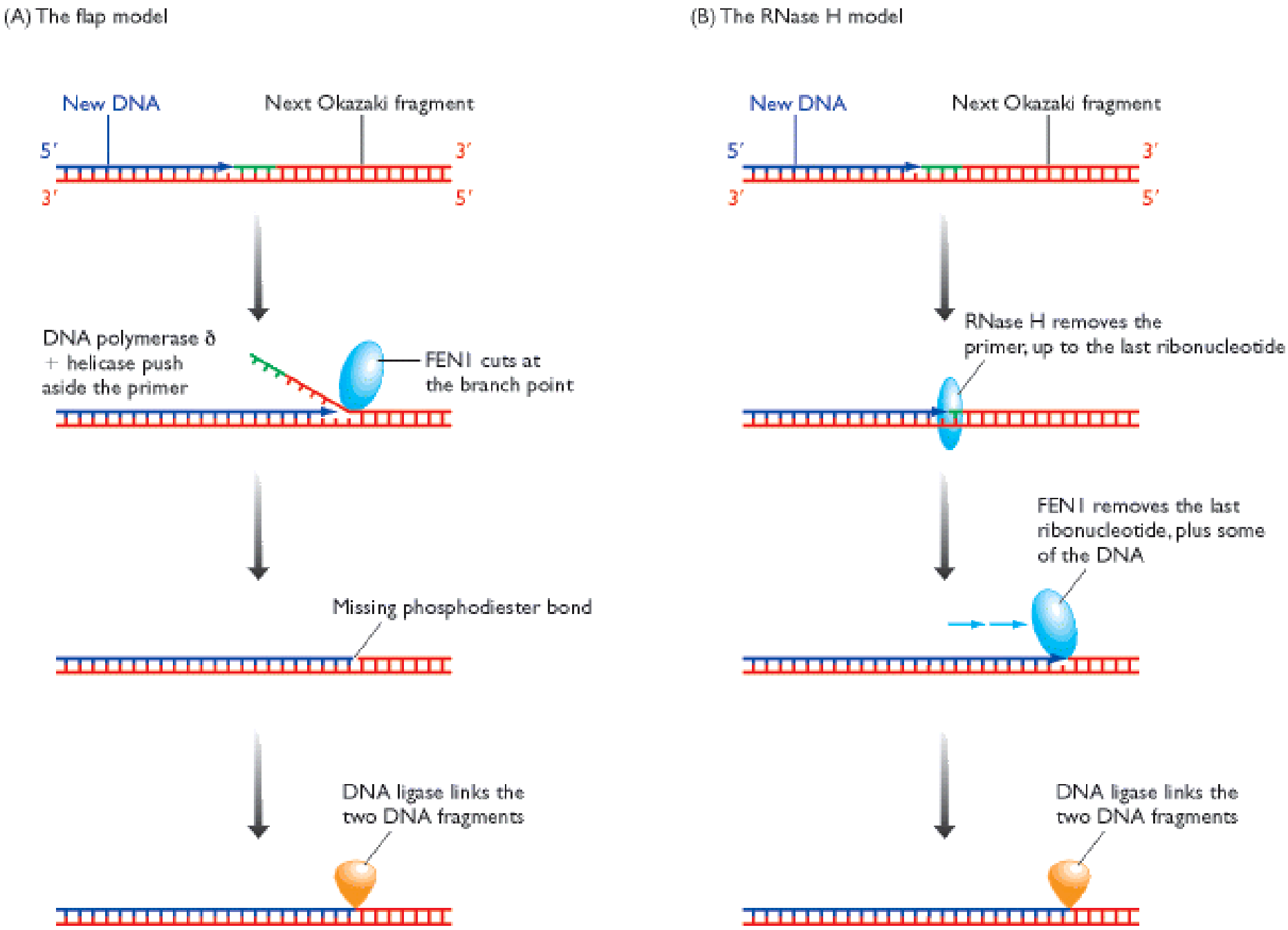


FIGURE 13.23 DNA ligase seals nicks between adjacent nucleotides by employing an enzyme–AMP intermediate.

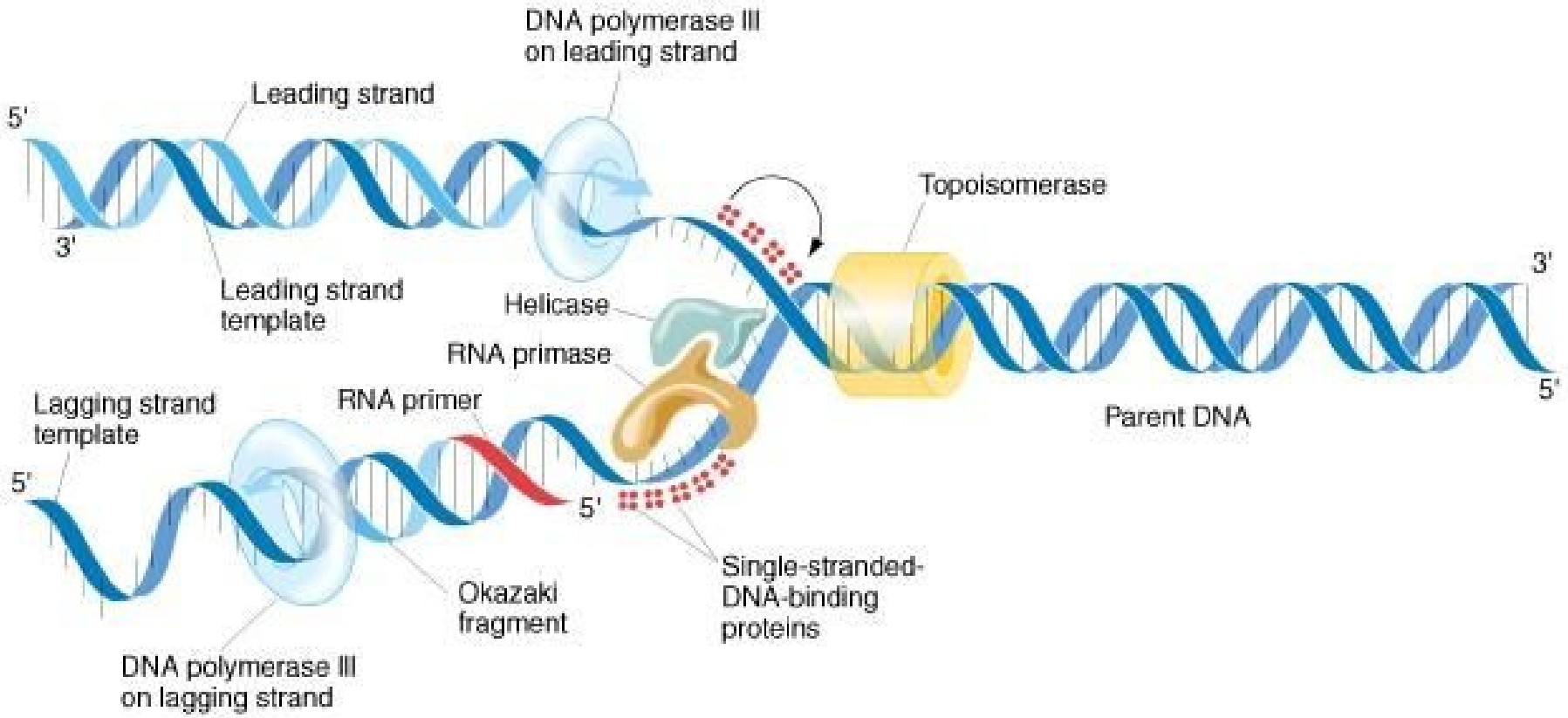
DNA polymerase	Function	Structure
	High fidelity replicases	
α	Nuclear replication	350 kD tetramer
δ	Lagging strand	250 kD tetramer
ϵ	Leading strand	350 kD tetramer
γ	Mitochondrial replication	200 kD dimer
	High-fidelity repair	
β	Base excision repair	39 kD monomer
	Low-fidelity repair	
ζ	Base damage bypass	heteromer
η	Thymine dimer bypass	monomer
ι	Required in meiosis	monomer
κ	Deletion and base substitution	monomer

FIGURE 13.24 Eukaryotic cells have many DNA polymerases. The replication enzymes operate with high fidelity. Except for the β enzyme, the repair enzymes all have low fidelity. Replication enzymes have large stiuctures, with separate subunits for different activities. Repair enzymes have much simpler structures.

In Eukaryotes...



Role of the helicase DnaB during replication of DNA in E. coli



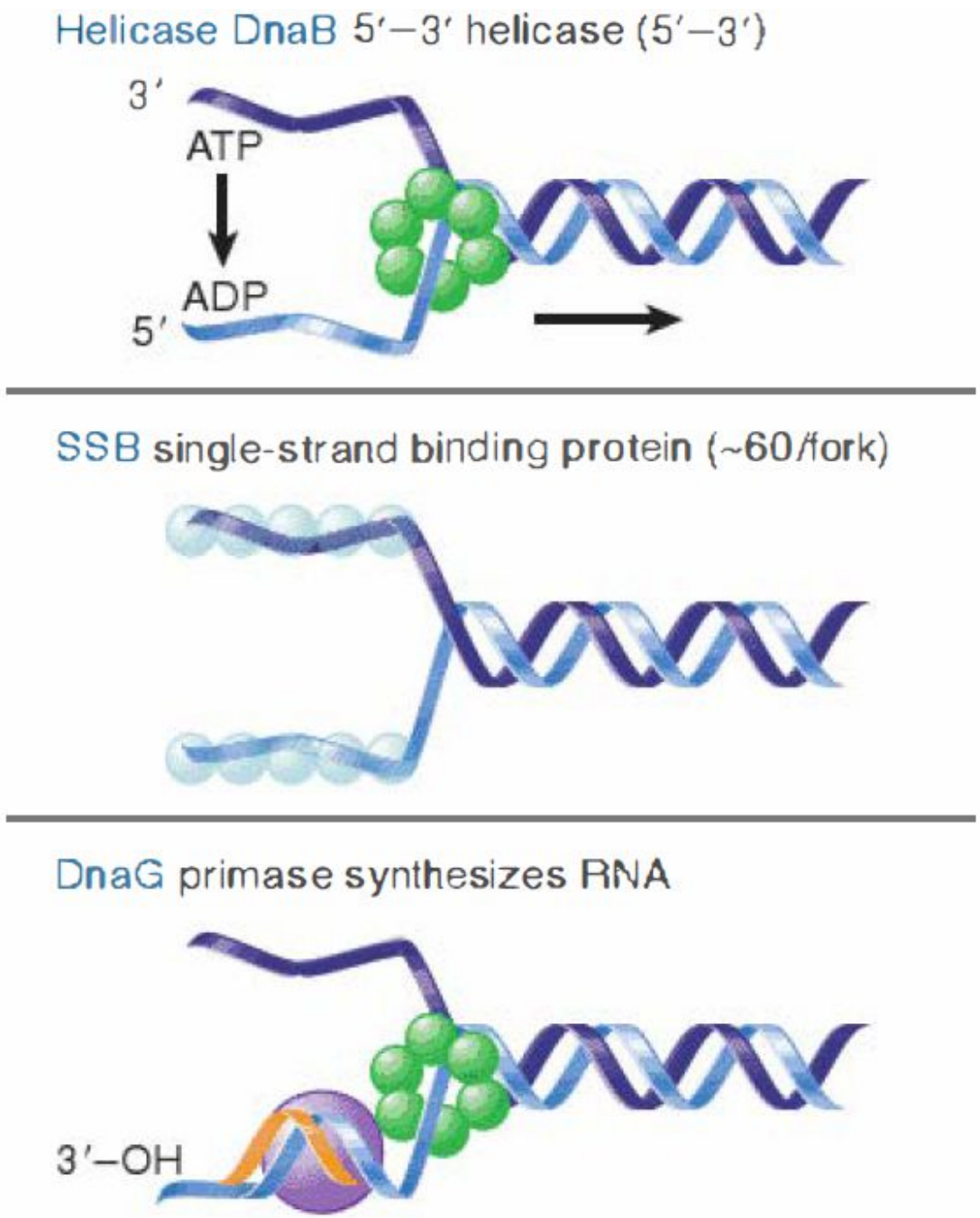
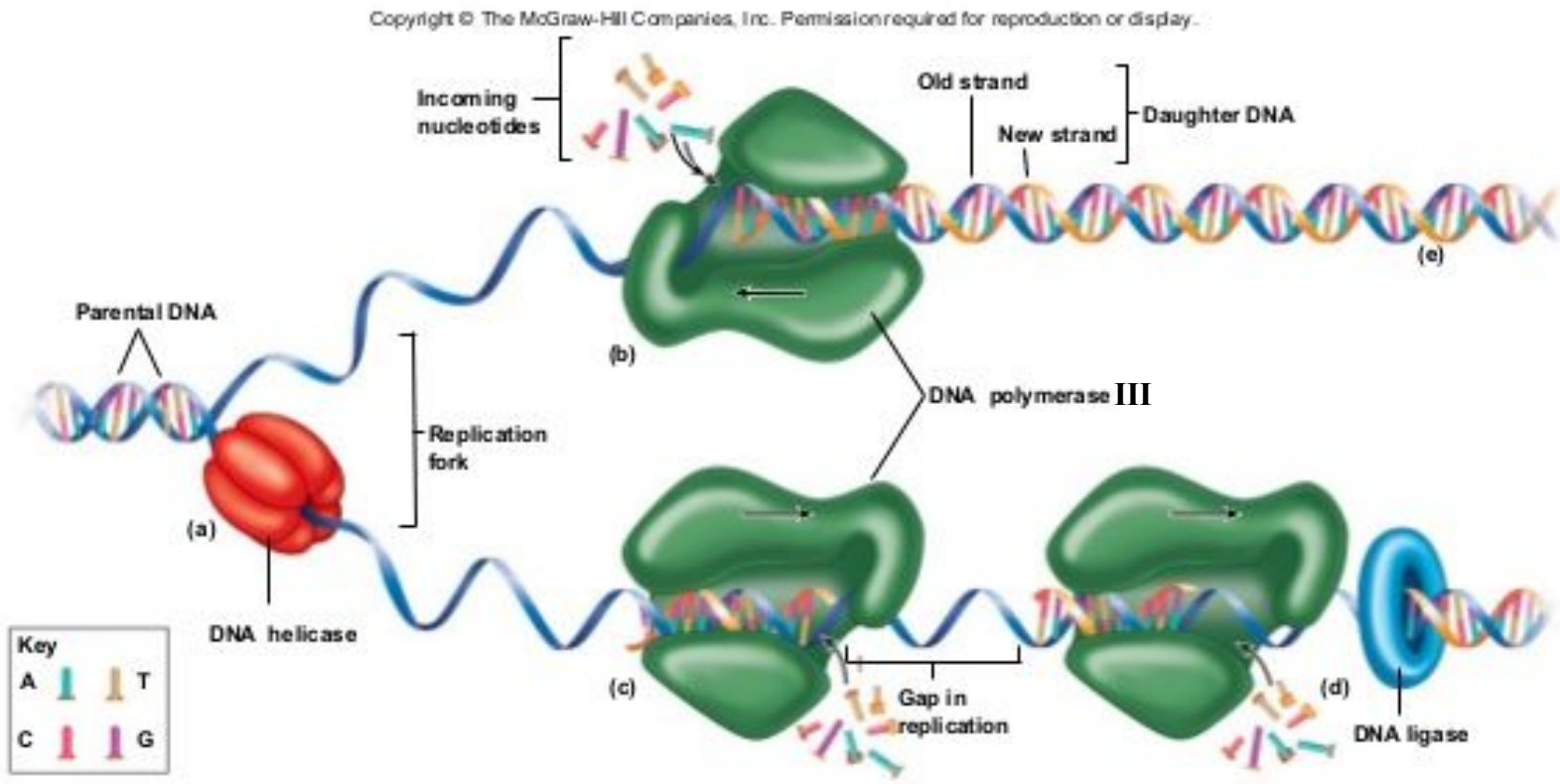
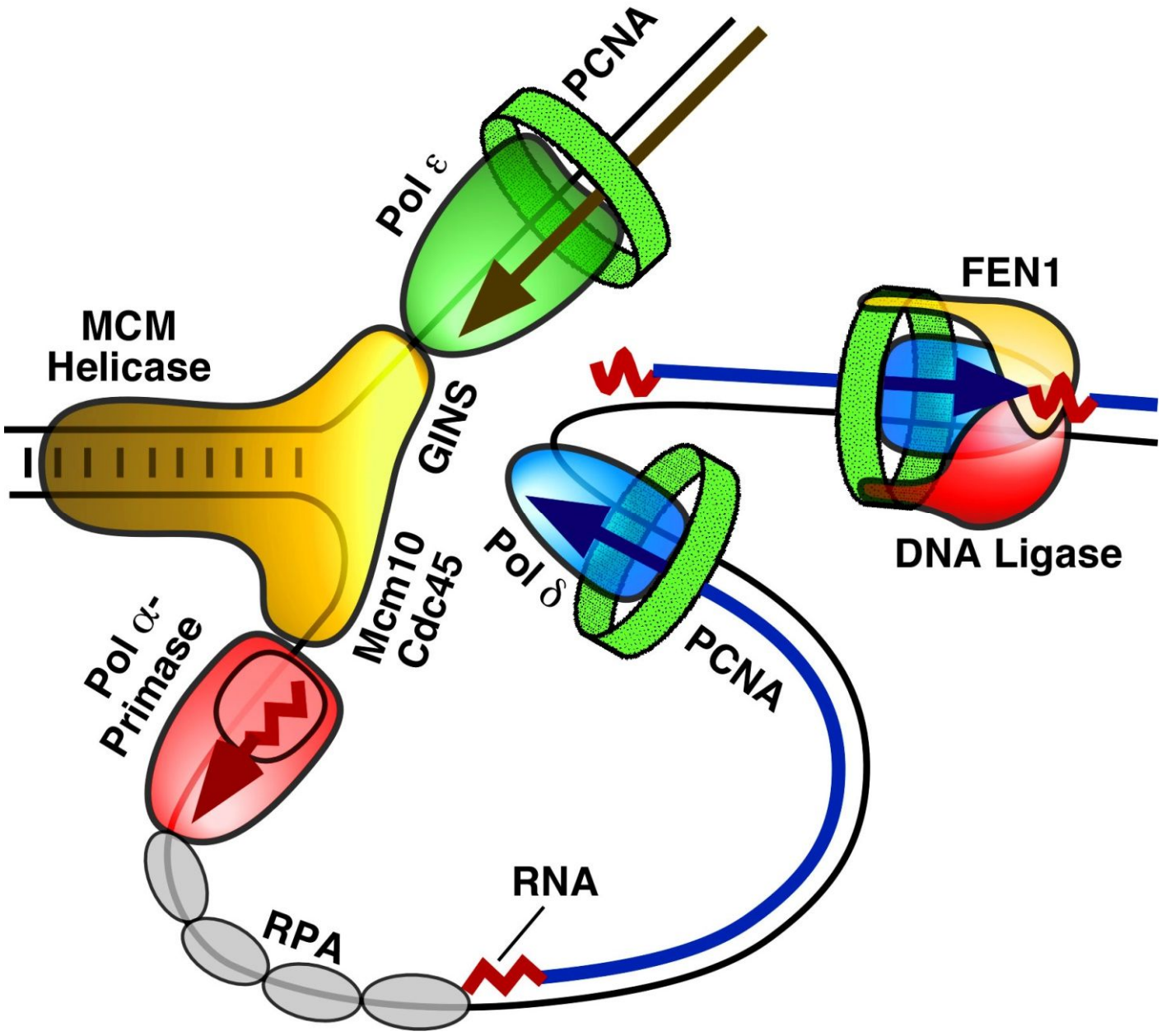


FIGURE 13.14 Initiation requires several enzymatic activities, including helicases, single-strand binding proteins, and synthesis of the primer.

Prokaryotes



Eukaryotes


PCNA
Pol ε
MCM
Helicase
GINS
FEN1
DNA Ligase
Pol δ
PCNA
Pol α-
Primase
Mcm10
Cdc45
RNA
RPA

The E. coli origin of replication *oriC*

FIGURE 12.8 The minimal origin is defined by the distance between the outside members of the 13-mer and 9-mer repeats.

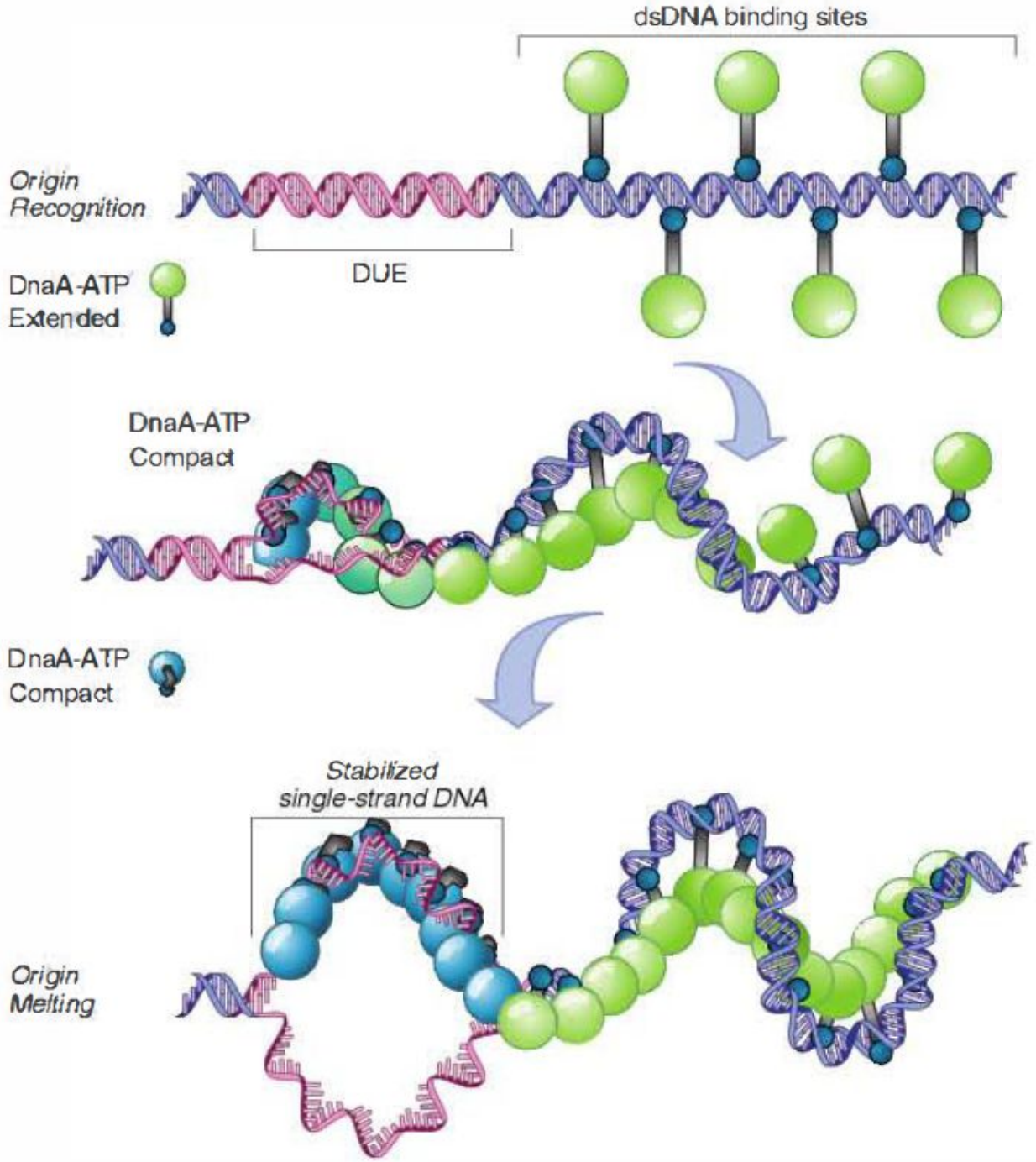
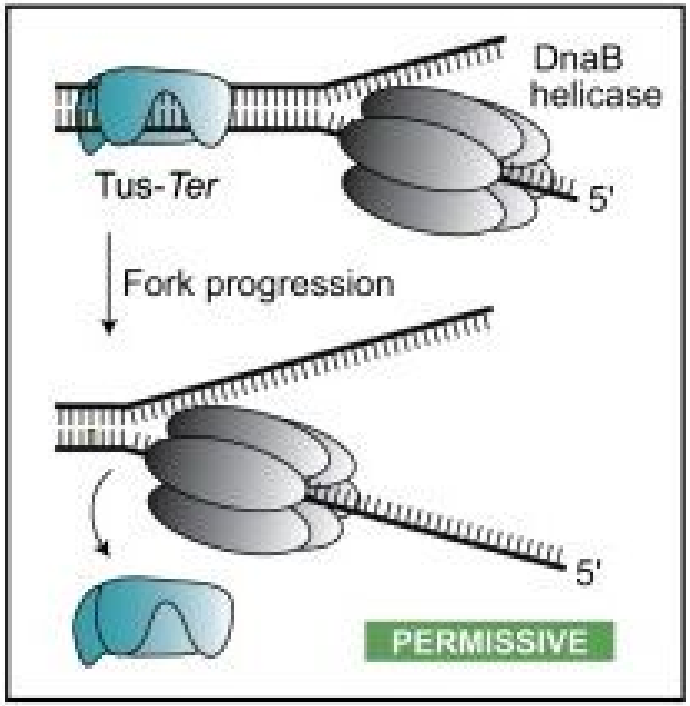
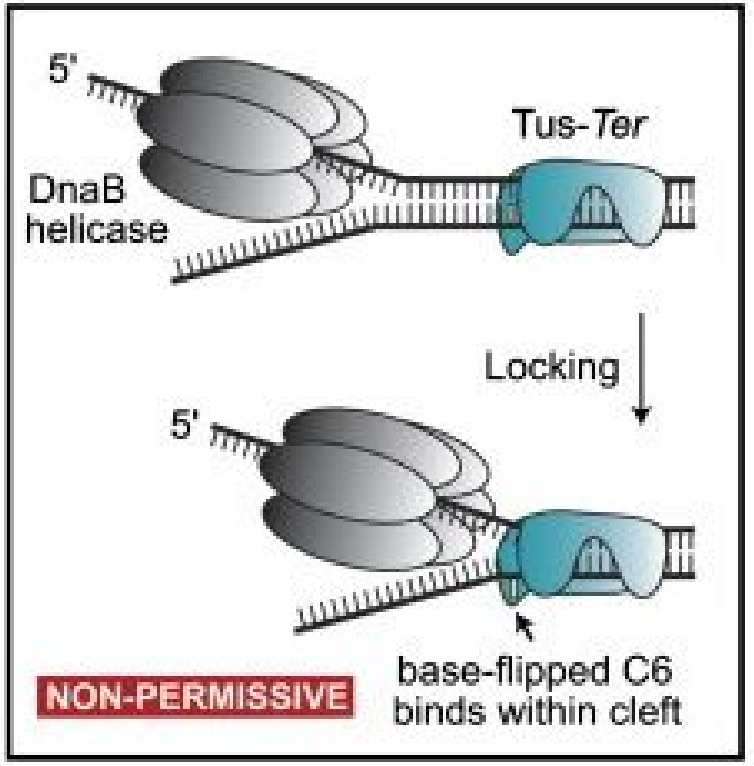


FIGURE 12.9 A two-state assembly model during initiation. DnaA-ATP monomers in an extended state associate with the high-affinity 13-mer sequences. DnaA-ATP transitions to a compact state as the 9-mer region begins to melt, stabilizing the single-stranded DNA. Adapted from *Journal of Biological Chemistry*, vol 285, Karl E. Duderstadt, et al., Origin Remodeling and Opening in Bacteria..., pp. 28229-28239. © 2010 The American Society for Biochemistry and Molecular Biology.


DnaB
helicase
Tus-*Ter*
5'
Fork progression
5'
PERMISSIVE


5'
Tus-*Ter*
DnaB
helicase
Locking
5'
NON-PERMISSIVE
base-flipped C6
binds within cleft

Function	*E. coli*	Eukaryote	Phage T4
Helicase	DnaB	MCM complex	41
Loading helicase/primase	DnaC	cdc6	59
Single-strand maintenance	SSB	RPA	32
Priming	DnaG	Polα/primase	61
Sliding clamp	β	PCNA	45
Clamp loading (ATPase)	γδi complex	RFC	44/62
Catalysis	*Pol III core*	Polδ + Polε	43
Holoenzyme dimerization	τ	?	43
RNA removal	*Pol I*	FEN1	43
Ligation	*Ligase*	Ligase 1	T4 ligase

FIGURE 13.25 Similar functions are required at all replication forks.

Proteins necessary for eukaryotic DNA replication

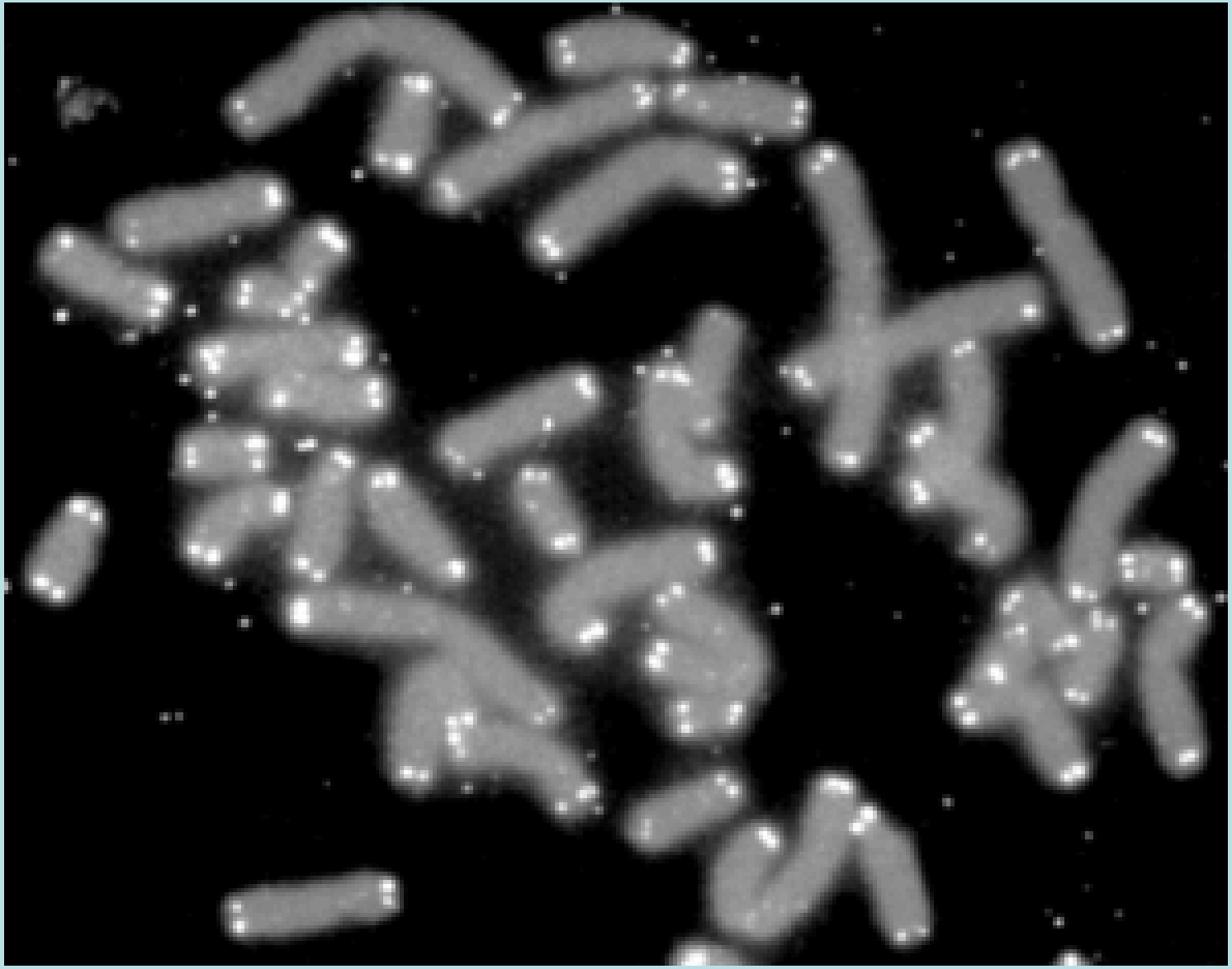
Protein	Function
DNA polymerase α	Priming
DNA polymerase δ	DNA synthesis
PCNA	Processivity
Replication factor C	Elongation
Replication factor A	SSB protein
Topoisomerase I e II	Strands separation
MF1 (FEN I)	RNA removal
DNA ligase	Nick ligation

Telomeres structure and function

Tandemly repeated sequences in telomeres

ORGANISM	DNA ORIGINE	5'-3‘ REPEATED UNIT
Tetrahymena, Paramecium (Holotrichia ciliates)	macronucleus	CCCCAA
Stylonichia, Oxytricha (Hipotrichia ciliates)	macronucleus	CCCCAAAA
Trypanosome, Leishmania (Flagellates)	minichromosomes	CCCTA
Physarum, Dictyostelium (Mixamoebae)	rDNA	CCCTA
Saccharomyces	chromosomes	CCcACAcaca
Arabidopsis	chromosomes	CCCTAAA
Homo sapiens	chromosomes	CCCTAA

Telomeres are located at the end of chromosomes



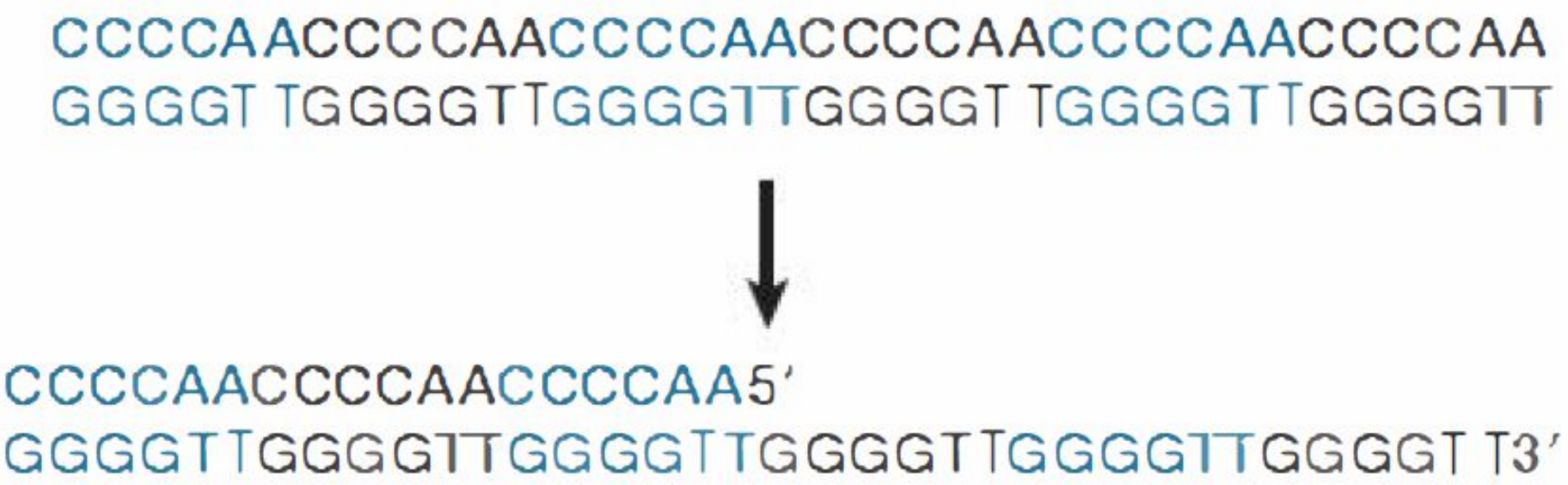
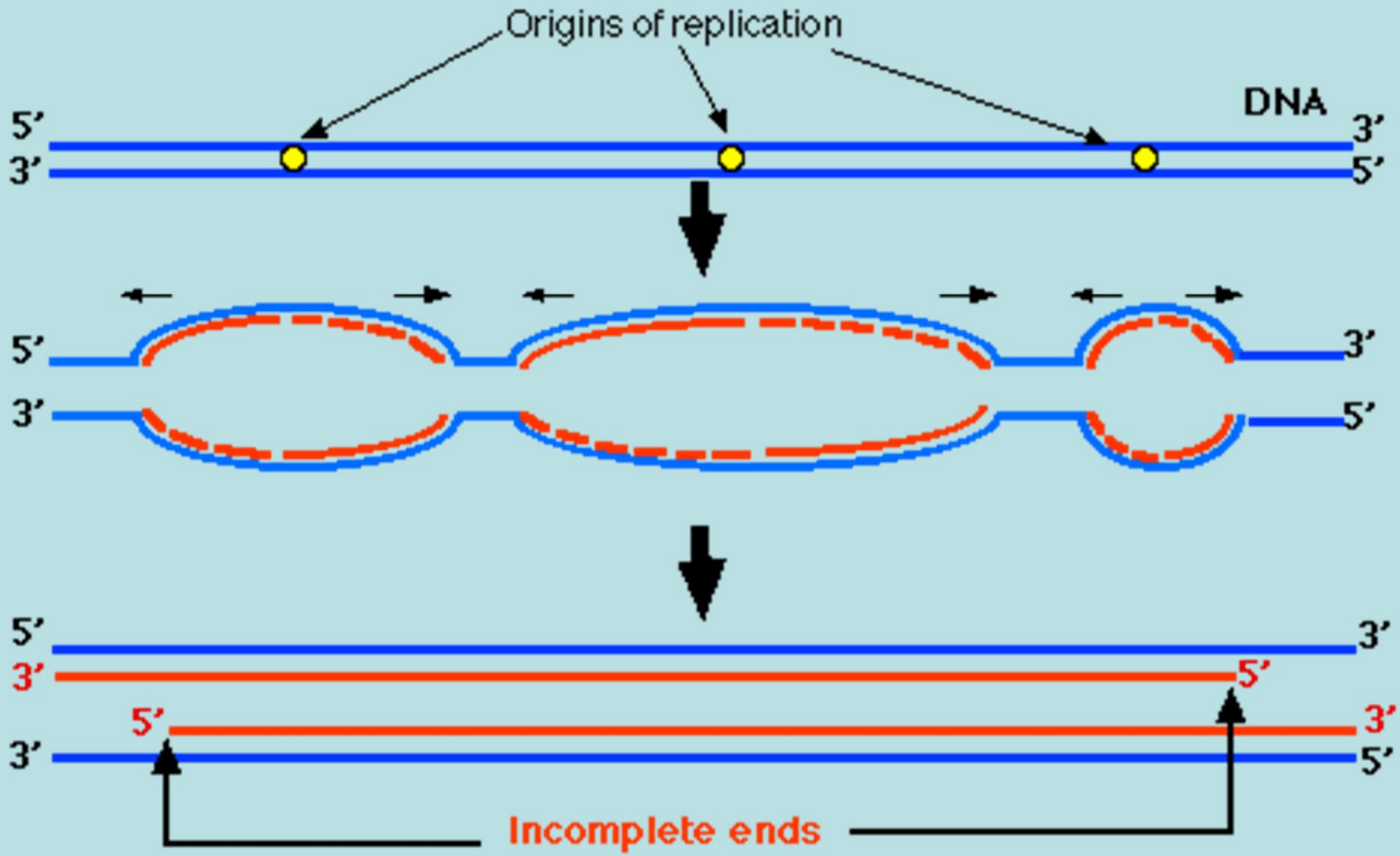


FIGURE 9.28 A typical telomere has a simple repeating structure with a G-T–rich strand that extends beyond the C-A–rich strand. The G-tail is generated by a limited degradation of the C-A–rich strand.

Telomeres shortening during DNA replication



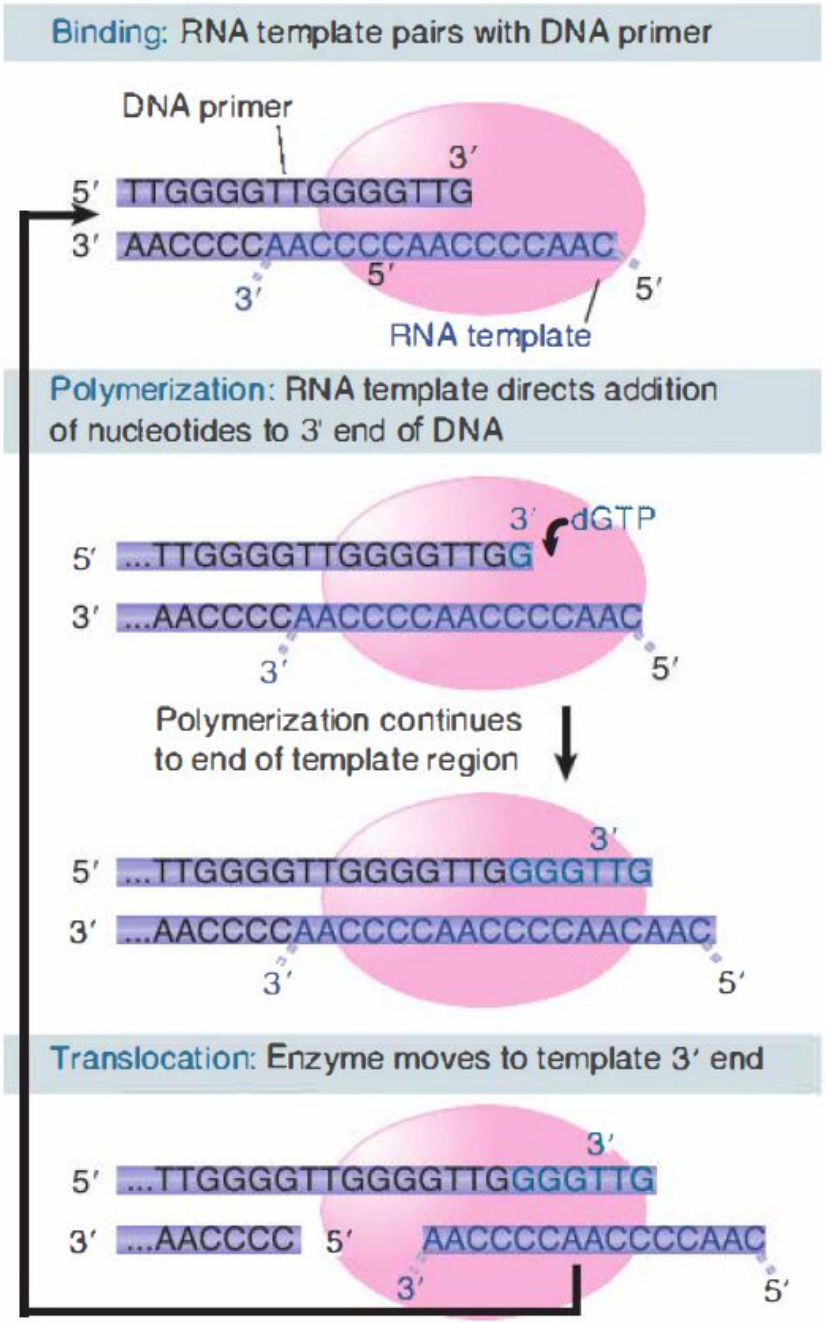
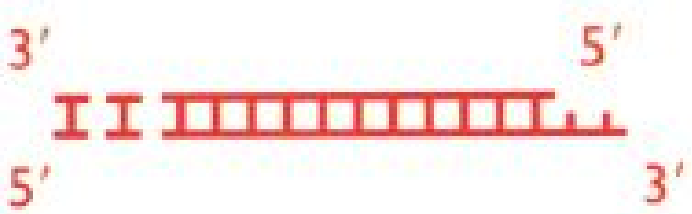
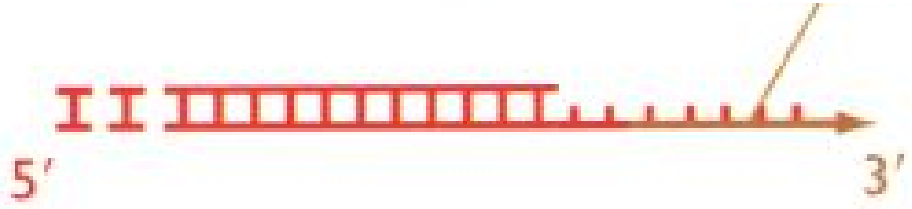


FIGURE 9.33 Telomerase positions itself by base pairing between the RNA template and the protruding single-stranded DNA primer. It adds G and T bases, one at a time to the primer, as directed by the template. The cycle starts again when one repeating unit has been added.



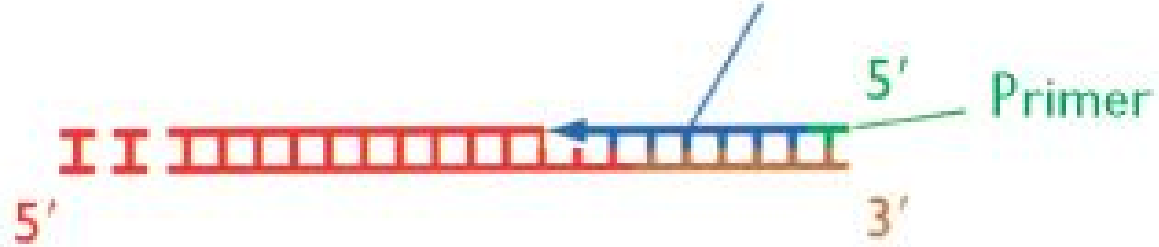
Telomerase enzyme extends the C -A-rich strand

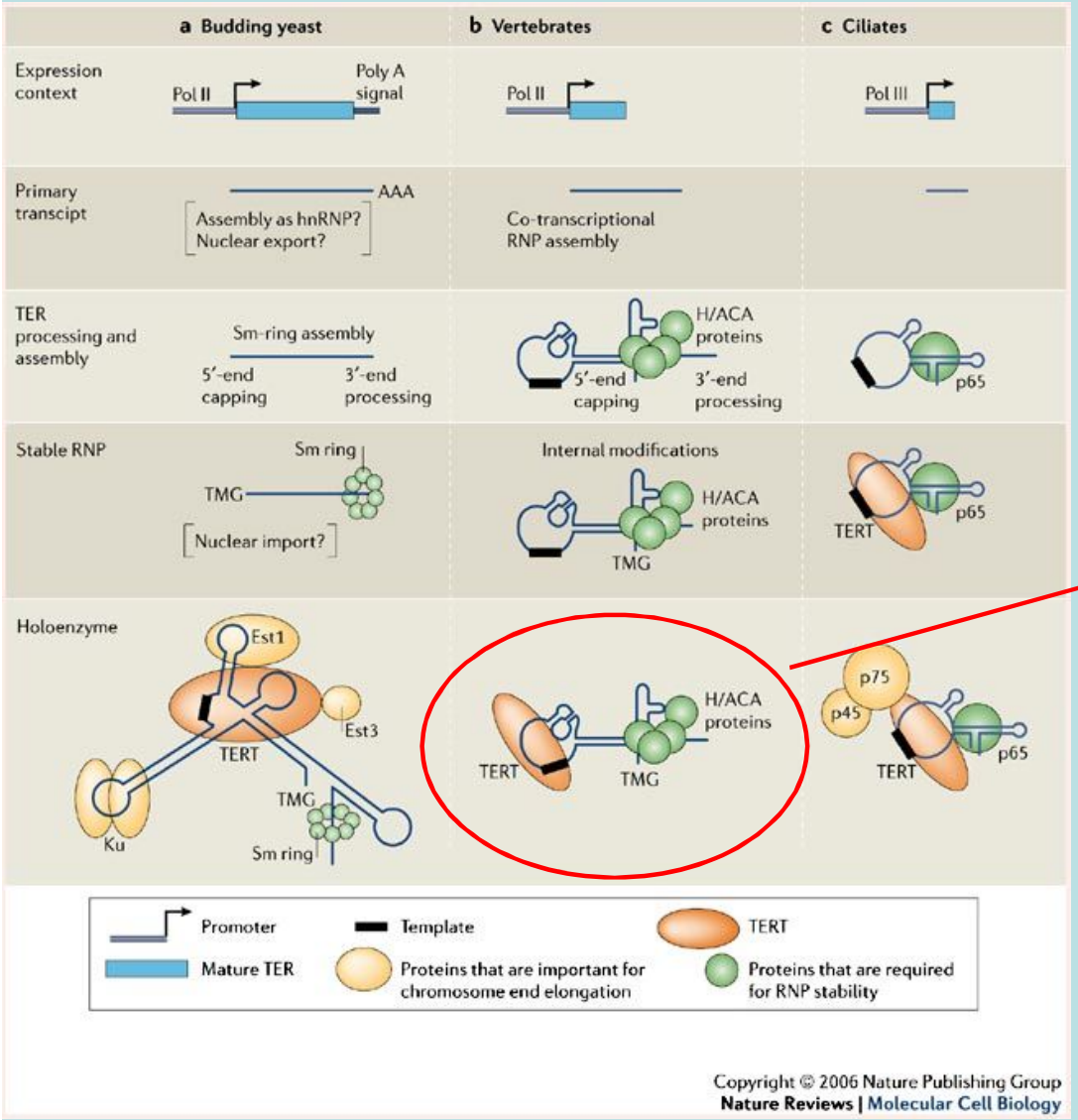
New DNA strand



When the new DNA strand is long enough, a new Okazaki fragment can be primed

Okazaki fragment




a Budding yeast
b Vertebrates
c Ciliates
Expression context
Pol II
Poly A signal
Pol II
Pol III
Primary transcipt
AAA
Assembly as hnRNP?
Nuclear export?
Co-transcriptional RNP assembly
TER processing and assembly
Sm-ring assembly
5′-end capping
3′-end processing
H/ACA proteins
5′-end capping
3′-end processing
p65
Stable RNP
Sm ring
TMG
Nuclear import?
Internal modifications
H/ACA proteins
TMG
TERT
p65
Holoenzyme
Est1
Est3
TERT
TMG
Ku
Sm ring
H/ACA proteins
TERT
TMG
p75
p45
TERT
p65
Promoter
Template
TERT
Mature TER
Proteins that are important for chromosome end elongation
Proteins that are required for RNP stability
Copyright © 2006 Nature Publishing Group
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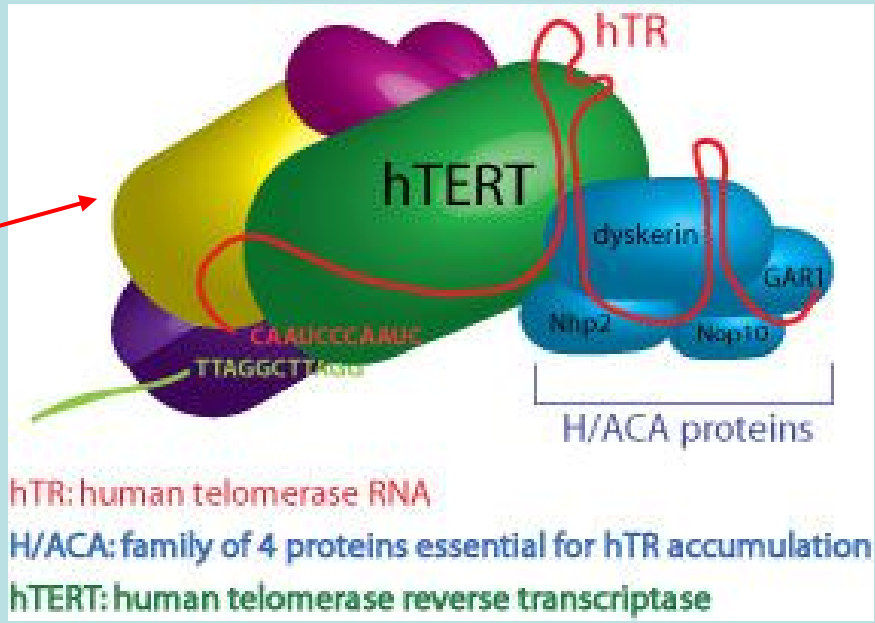
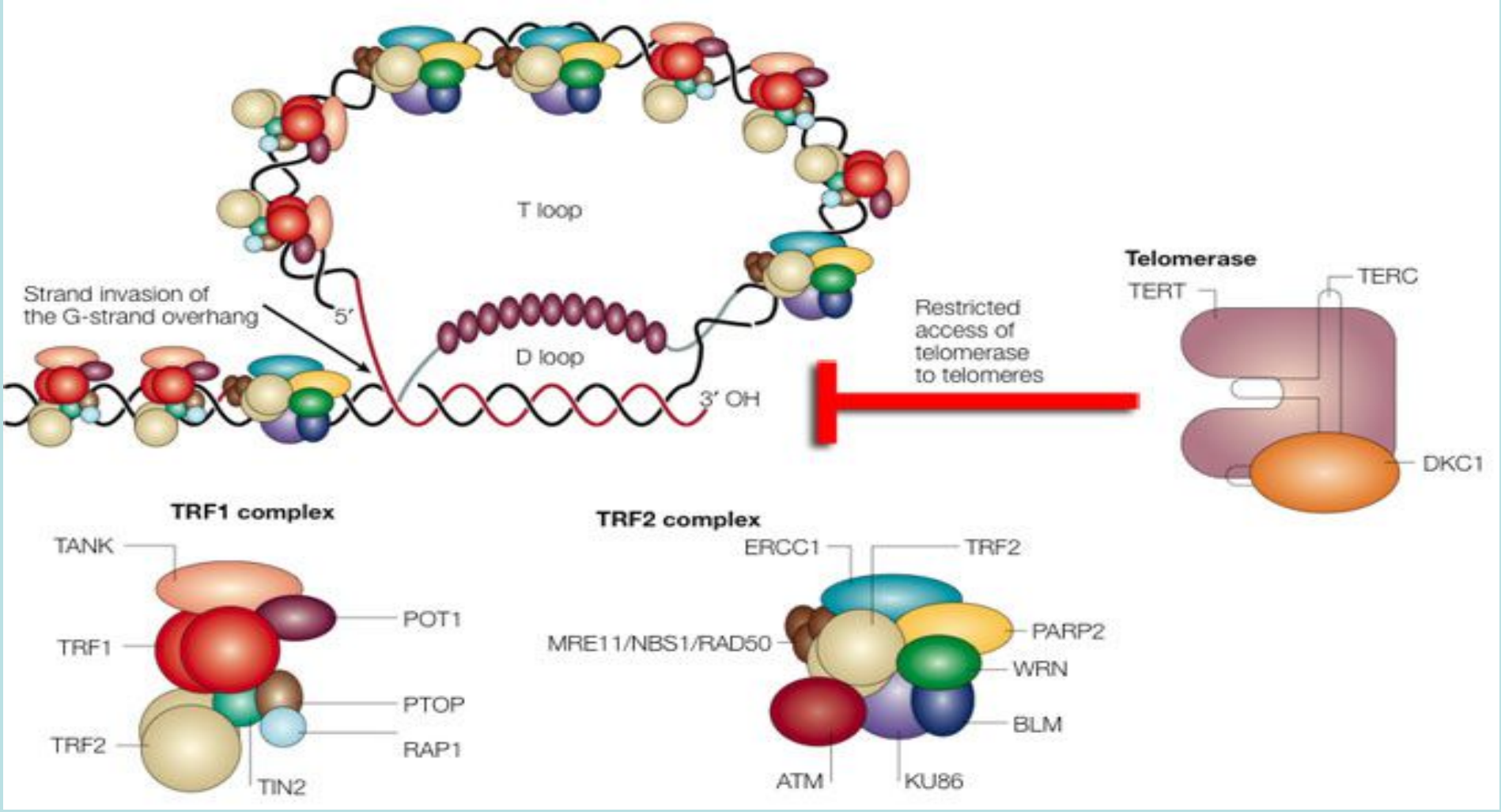
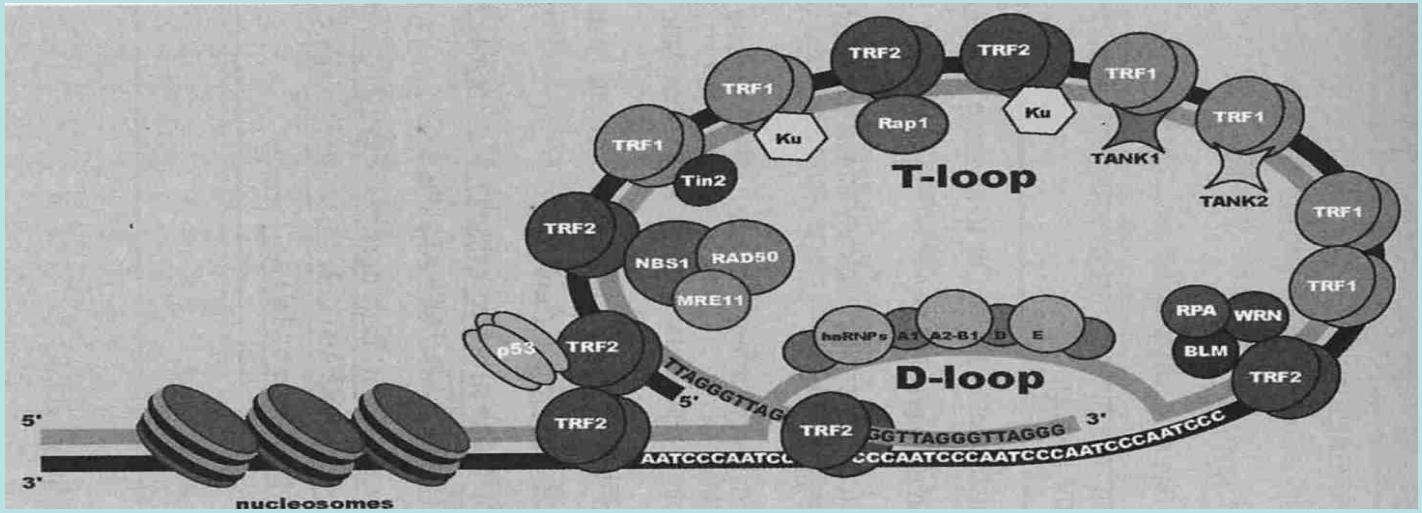

hTR
hTERT
dyskerin
GAR1
Nhp2
Nop10
H/ACA proteins
hTR: human telomerase RNA
H/ACA: family of 4 proteins essential for hTR accumulation
hTERT: human telomerase reverse transcriptase


T loop
Strand invasion of
the G-strand overhang
5′
D loop
3′ OH
Restricted
access of
telomerase
to telomeres
Telomerase
TERT
TERC
DKC1
TRF1 complex
TANK
POT1
TRF1
PTOP
TRF2
RAP1
TIN2
TRF2 complex
ERCC1
TRF2
PARP2
MRE11/NBS1/RAD50
WRN
BLM
ATM
KU86


TRF2
TRF2
TRF1
TRF1
Rap1
Ku
Ku
TRF1
TRF1
TANK1
TANK2
Tin2
T-loop
TRF1
TRF2
NBS1
RAD50
MRE11
TRF1
RPA
WRN
hnRNPs
A1
A2-B1
D
E
BLM
p53
TRF2
TRF2
D-loop
TTAGGGTTAG
5'
TRF2
TRF2
GGTTAGGGTTAGGG
3'
5'
AATCCCAATC
CCAATCCCAATCCCAATCCCAATCCC
3'
nucleosomes

Telomerase activity

- Telomerase synthesizes the individual repeats that are added to the chromosome ends, but does not itself control the number of repeats.
- Other proteins are involved in determining the length of the telomere binding the telomerase, and may influence the length of the telomere by controlling the access of telomerase to its substrate.
- Telomerase is expressed in actively dividing cells and is not expressed in quiescent cells.
- Telomerase activity is low in somatic cells: loss of telomeres results in senescence.
- In Tumor cells there is an high expression and activity of telomerase → it can be a targhet for anticancer drugs.