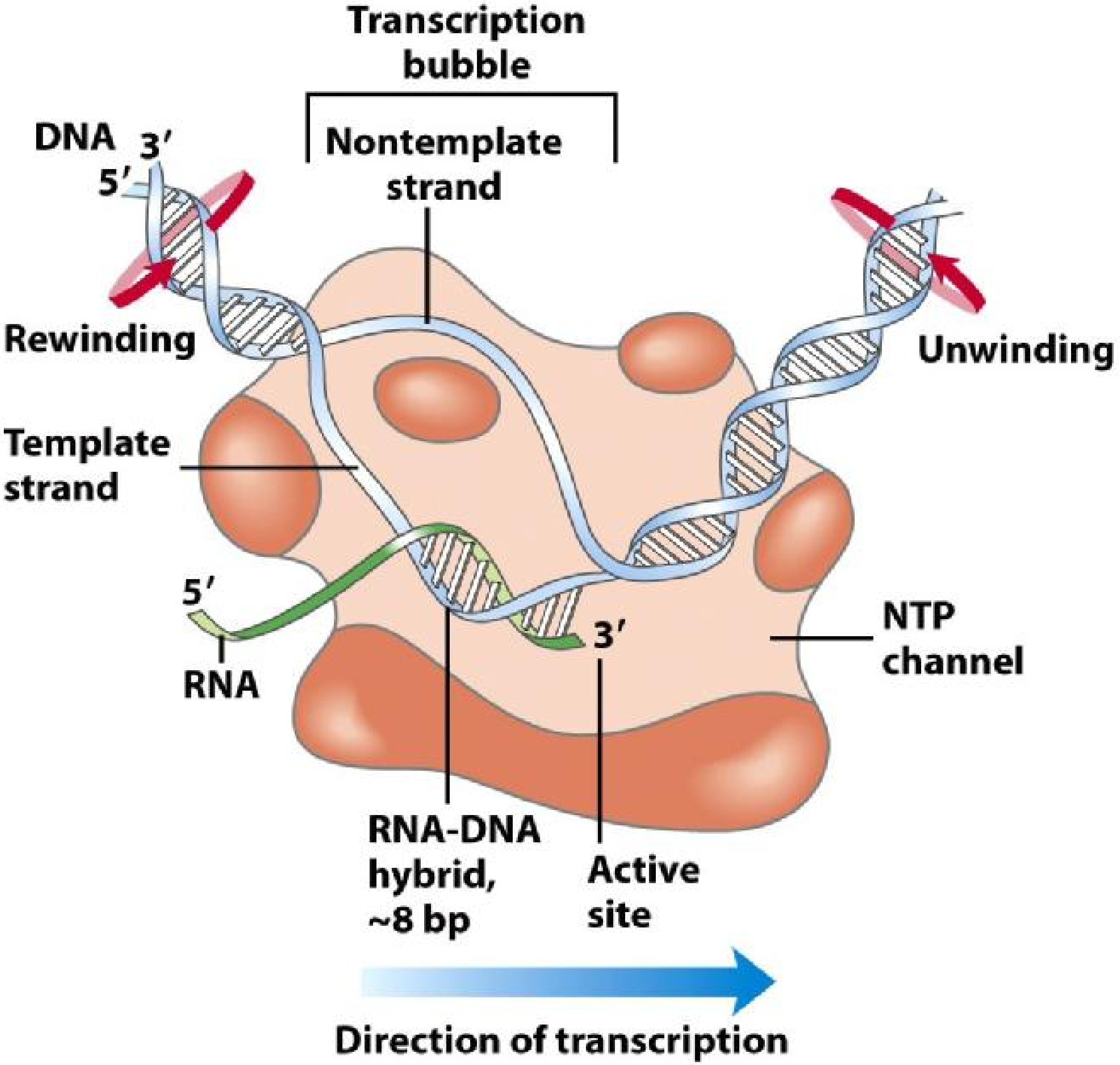

Figure 26-1a
Lehninger Principles of Biochemistry, Fifth Edition

Incoming NTP is attacked at the α phosphate by the 3′ hydroxyl of the growing RNA chain.

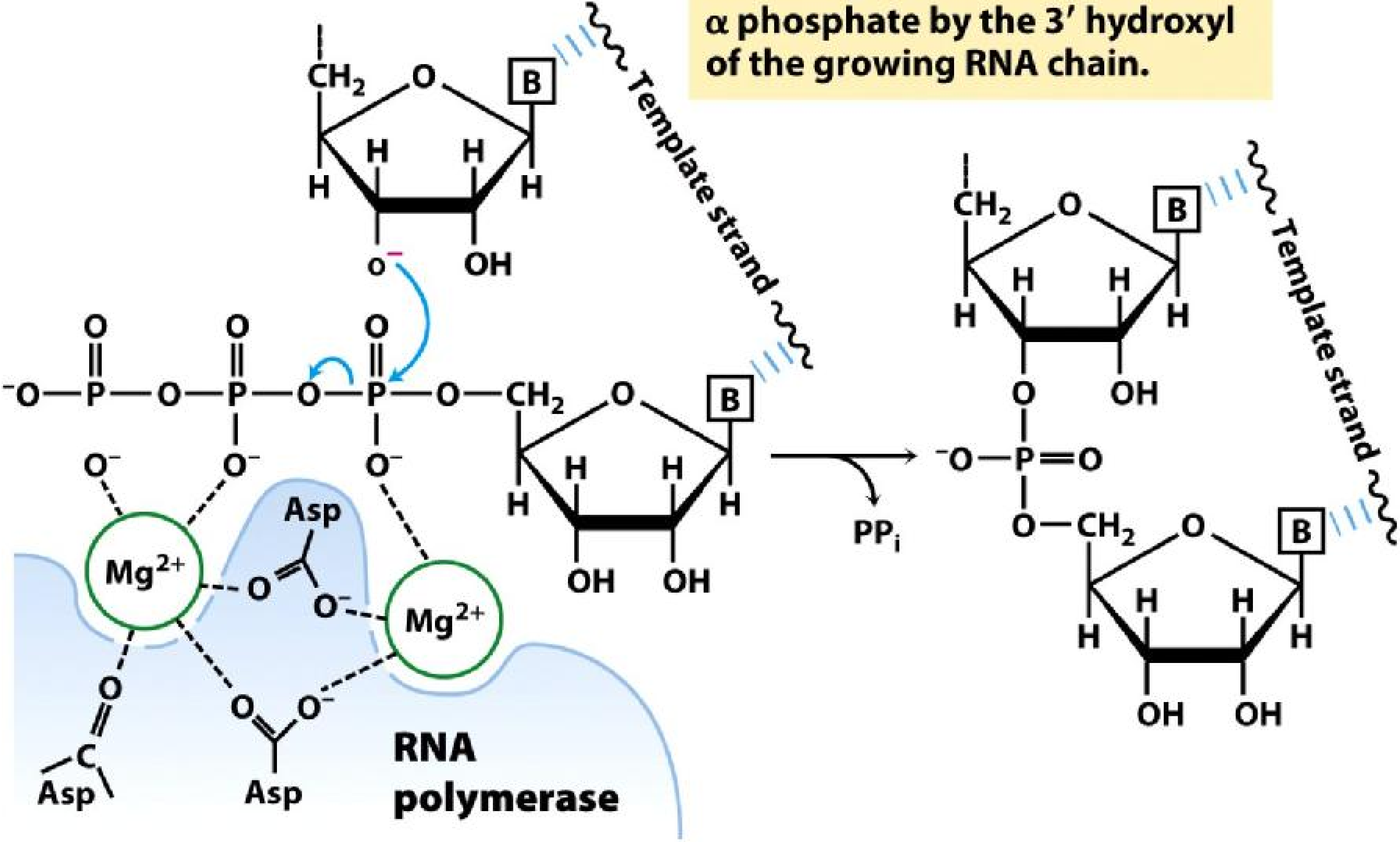

Figure 26-1b

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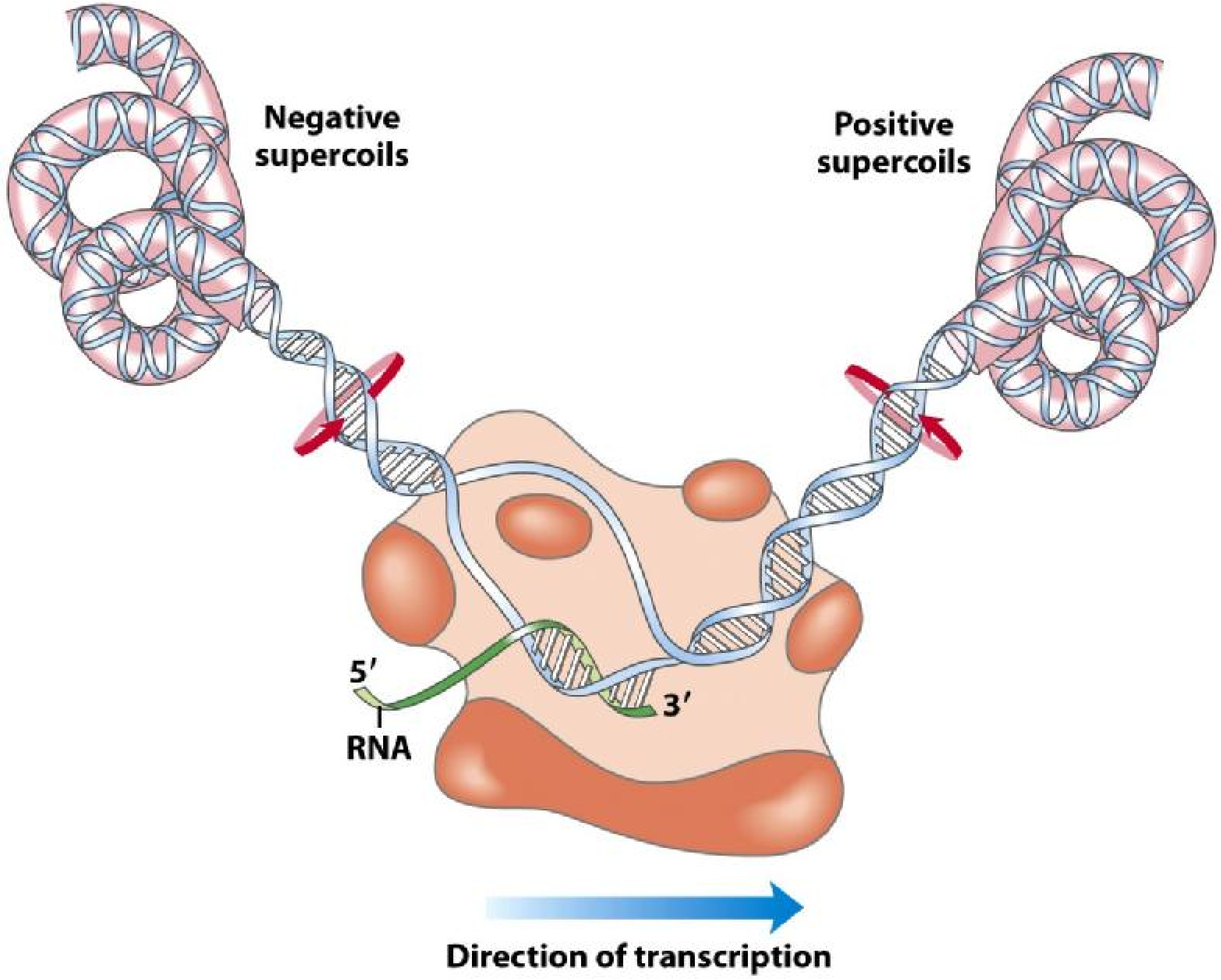

Figure 26-1c
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(5′) C G C T A T A G C G T T T (3′)	**DNA nontemplate (coding) strand**
(3′) G C G A T A T C G C A A A (5′)	**DNA template strand**
(5′) C G C U A U A G C G U U U (3′)	**RNA transcript**

Figure 26-2
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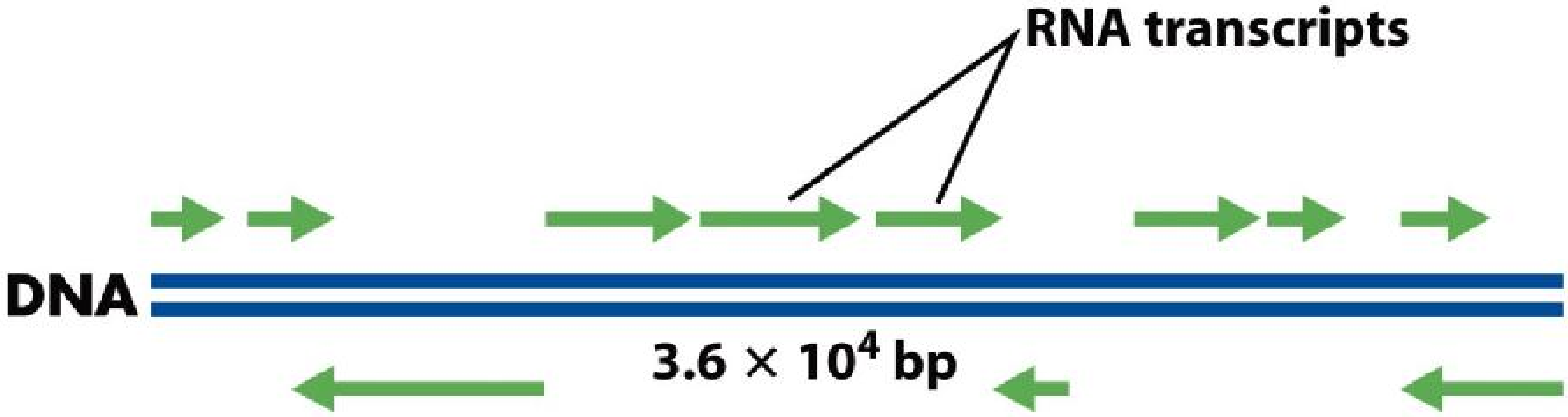

Figure 26-3

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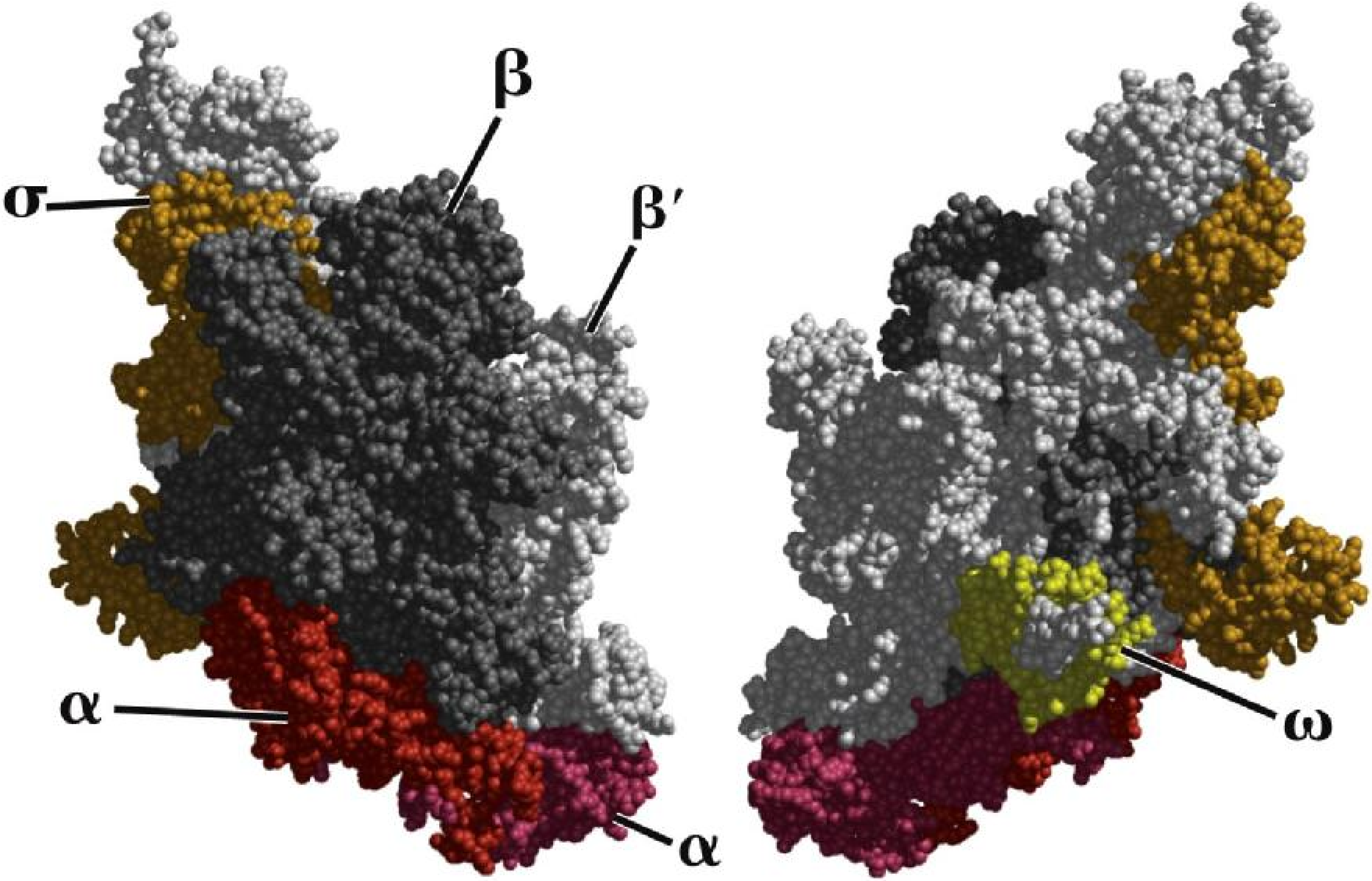

Figure 26-4

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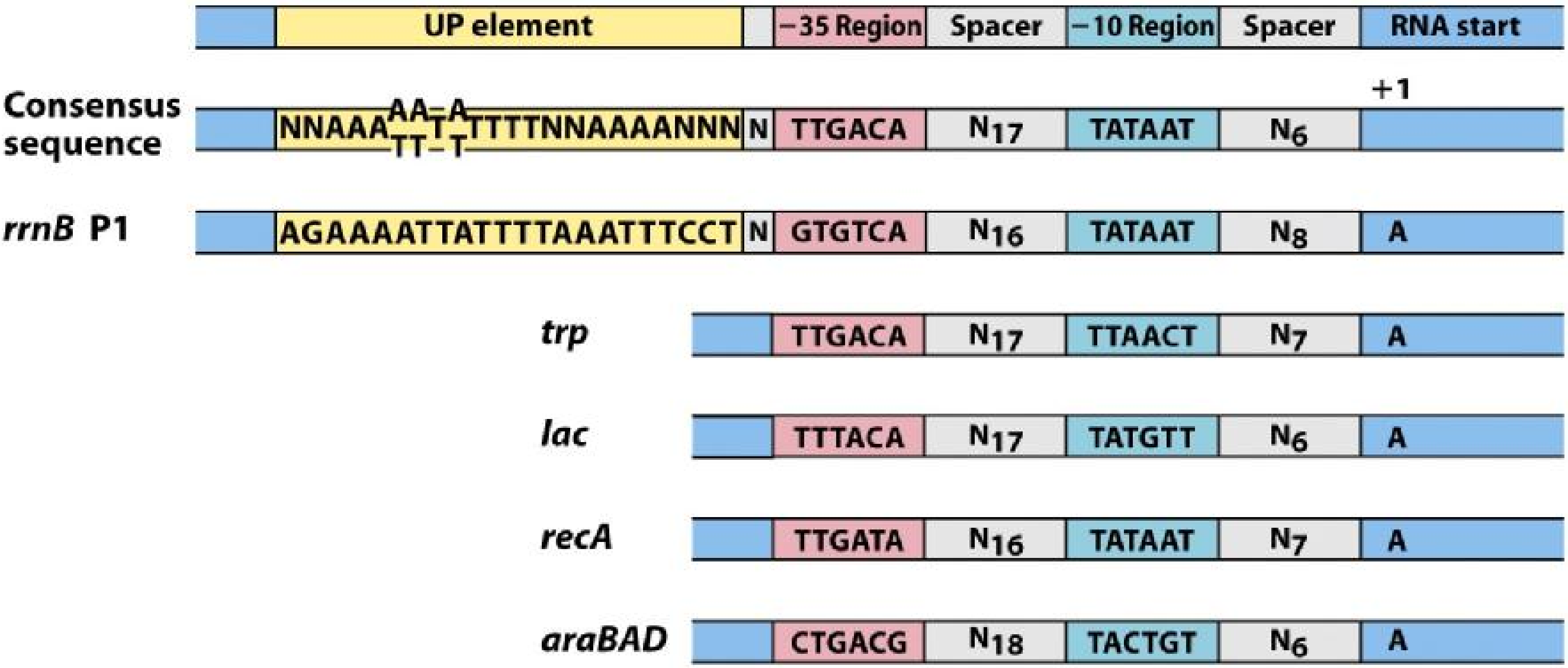

	UP element		−35 Region	Spacer	−10 Region	Spacer	RNA start
Consensus sequence	NNAAA$\overset{AA}{\underset{TT}{}}$–T–$\overset{A}{\underset{T}{}}$TTTTNNAAAANNN	N	TTGACA	N$_{17}$	TATAAT	N$_{6}$	+1
rrnB P1	AGAAAATTATTTTAAATTTCCT	N	GTGTCA	N$_{16}$	TATAAT	N$_{8}$	A
trp			TTGACA	N$_{17}$	TTAACT	N$_{7}$	A
lac			TTTACA	N$_{17}$	TATGTT	N$_{6}$	A
recA			TTGATA	N$_{16}$	TATAAT	N$_{7}$	A
araBAD			CTGACG	N$_{18}$	TACTGT	N$_{6}$	A

Figure 26-5

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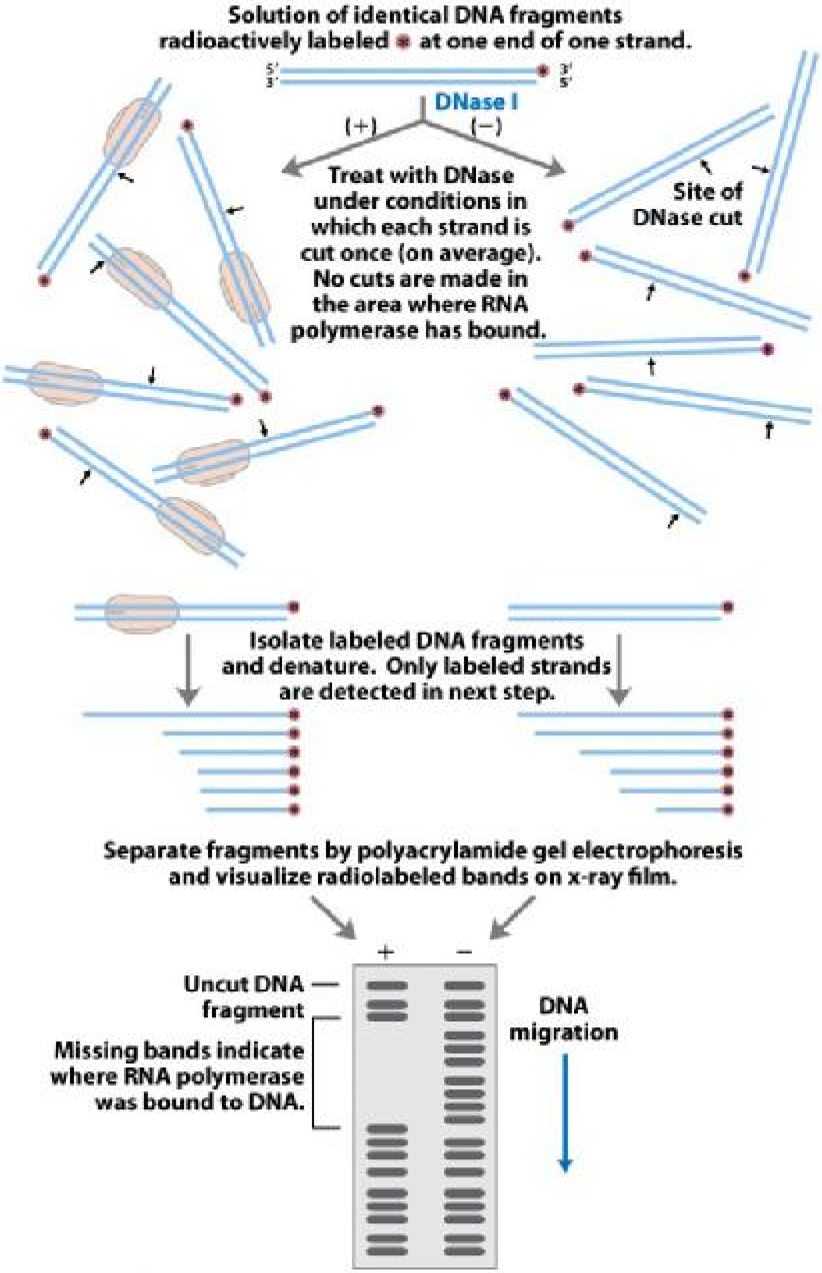

Box 26-1 figure 1

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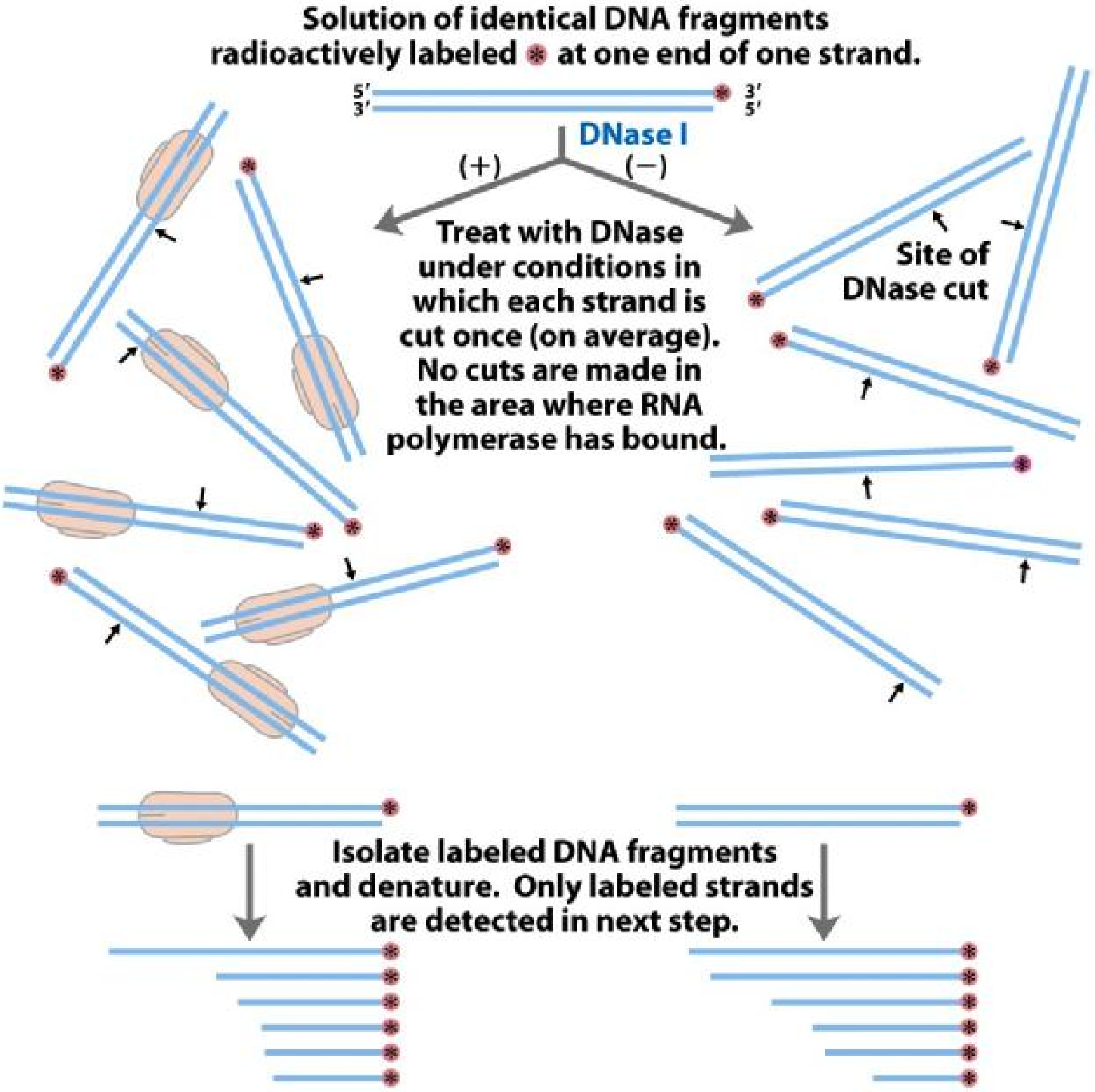

Solution of identical DNA fragments radioactively labeled ✱ at one end of one strand.

Treat with DNase under conditions in which each strand is cut once (on average). No cuts are made in the area where RNA polymerase has bound.

Isolate labeled DNA fragments and denature. Only labeled strands are detected in next step.

Box 26-1 figure 1 part 1

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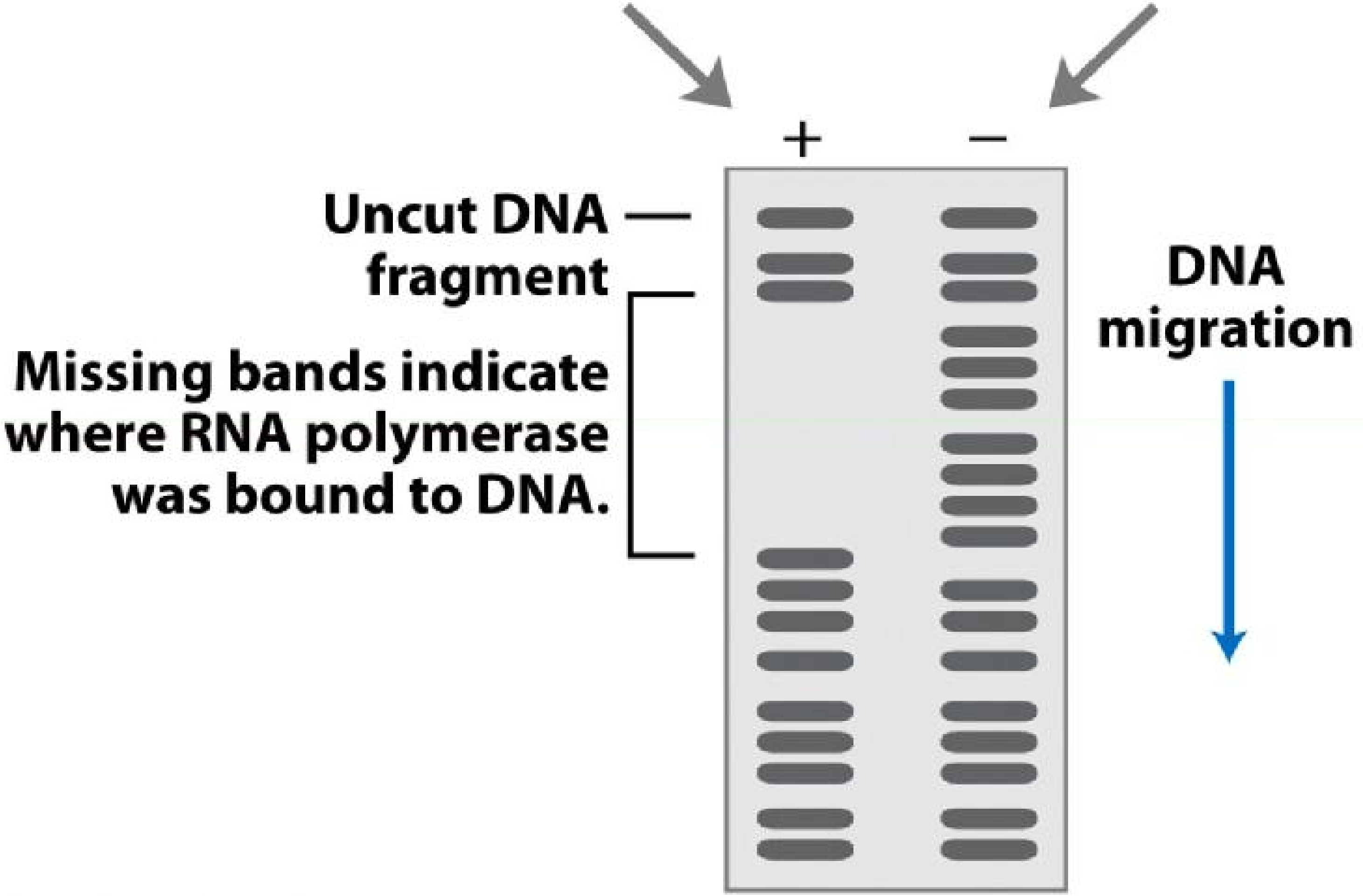

Separate fragments by polyacrylamide gel electrophoresis and visualize radiolabeled bands on x-ray film.

Uncut DNA fragment

Missing bands indicate where RNA polymerase was bound to DNA.

DNA migration

Box 26-1 figure 1 part 2

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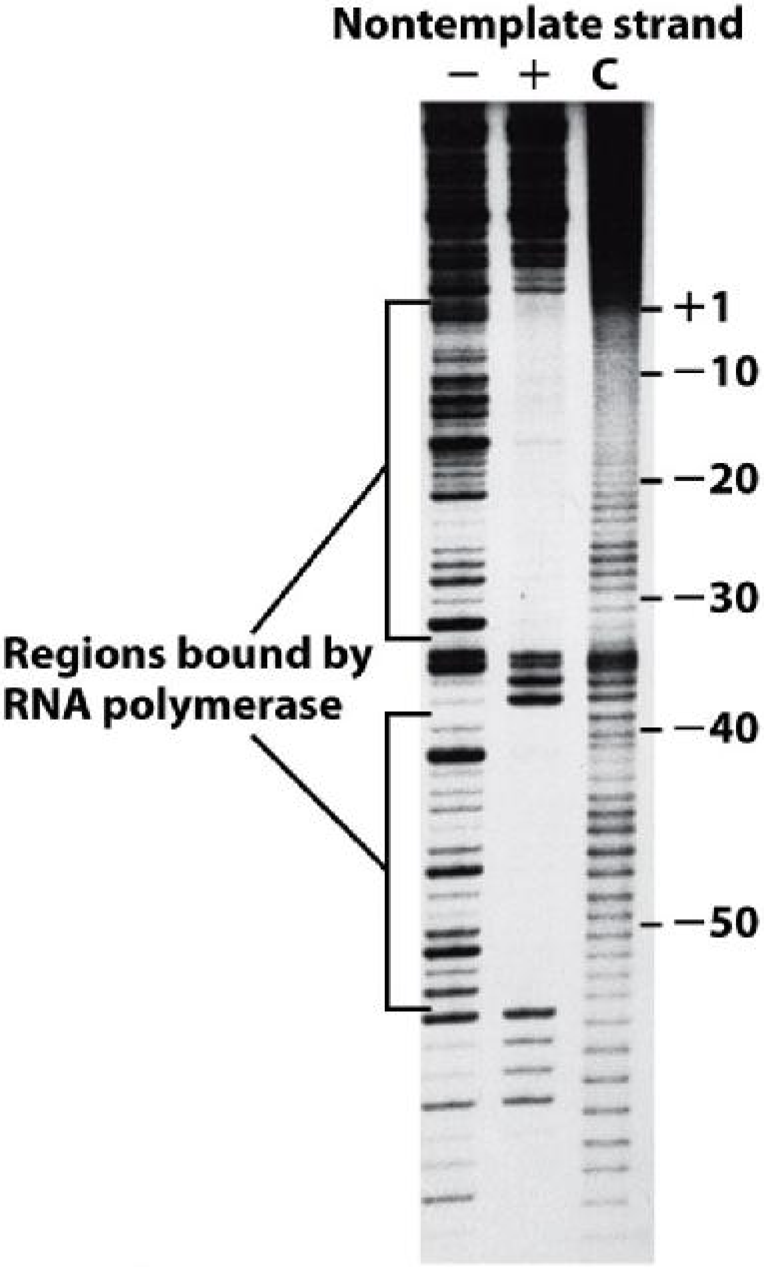

Box 26-1 figure 2
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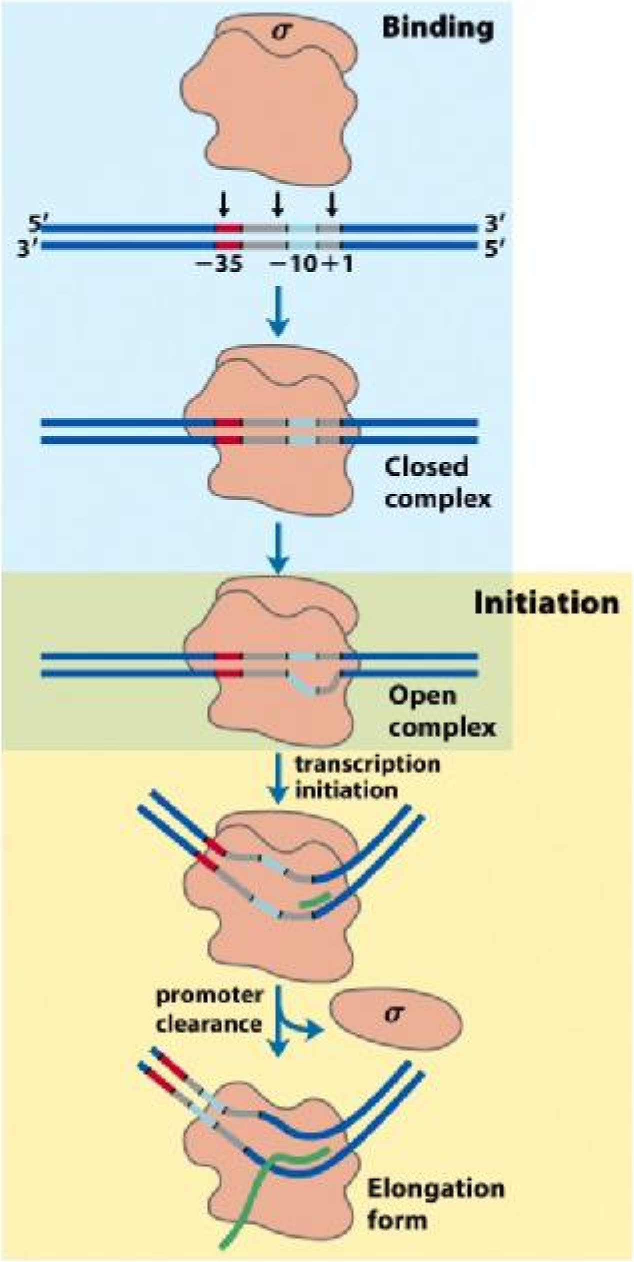

Figure 26-6a

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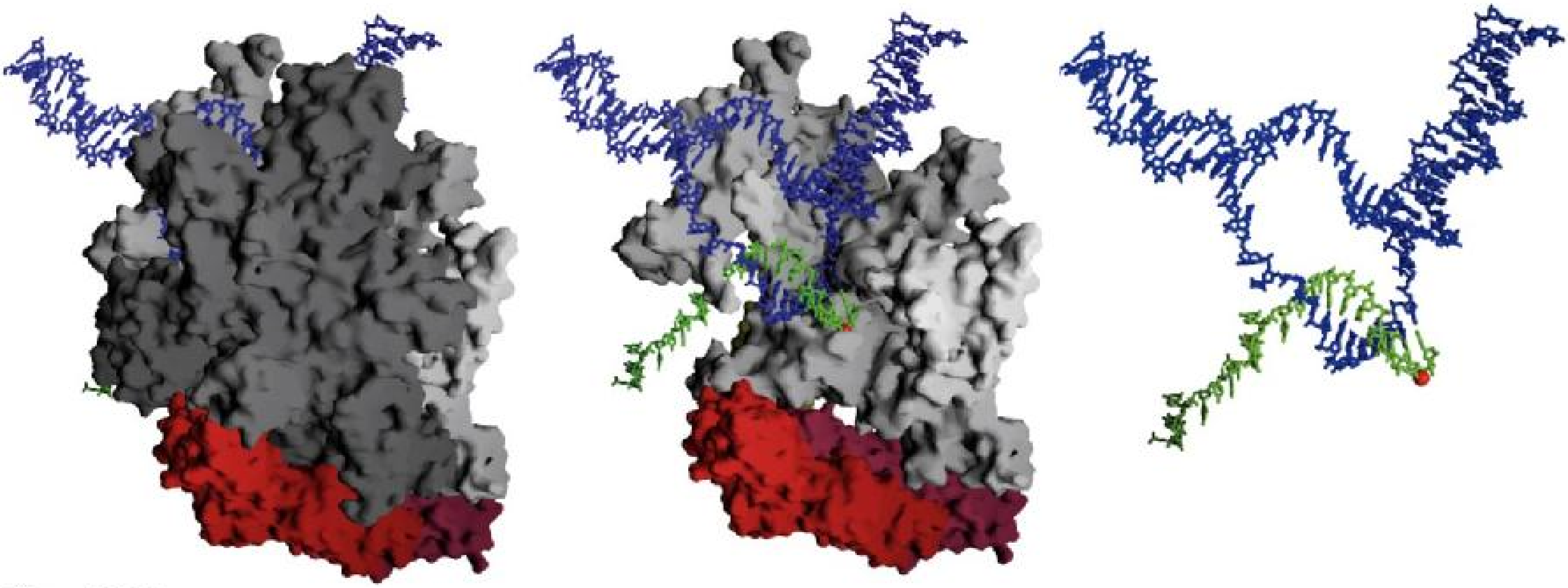

Figure 26-6b

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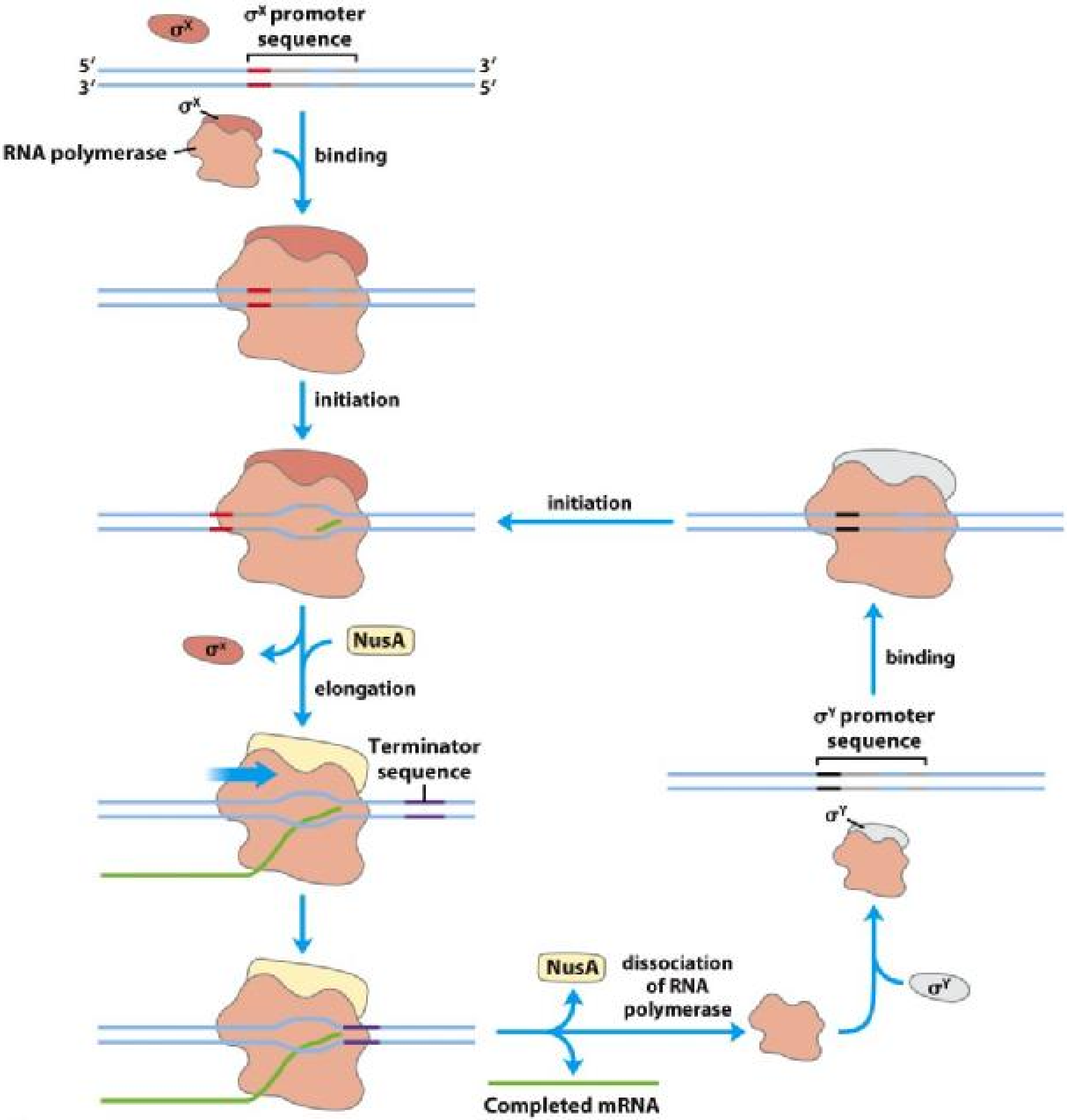

Figure 26-7

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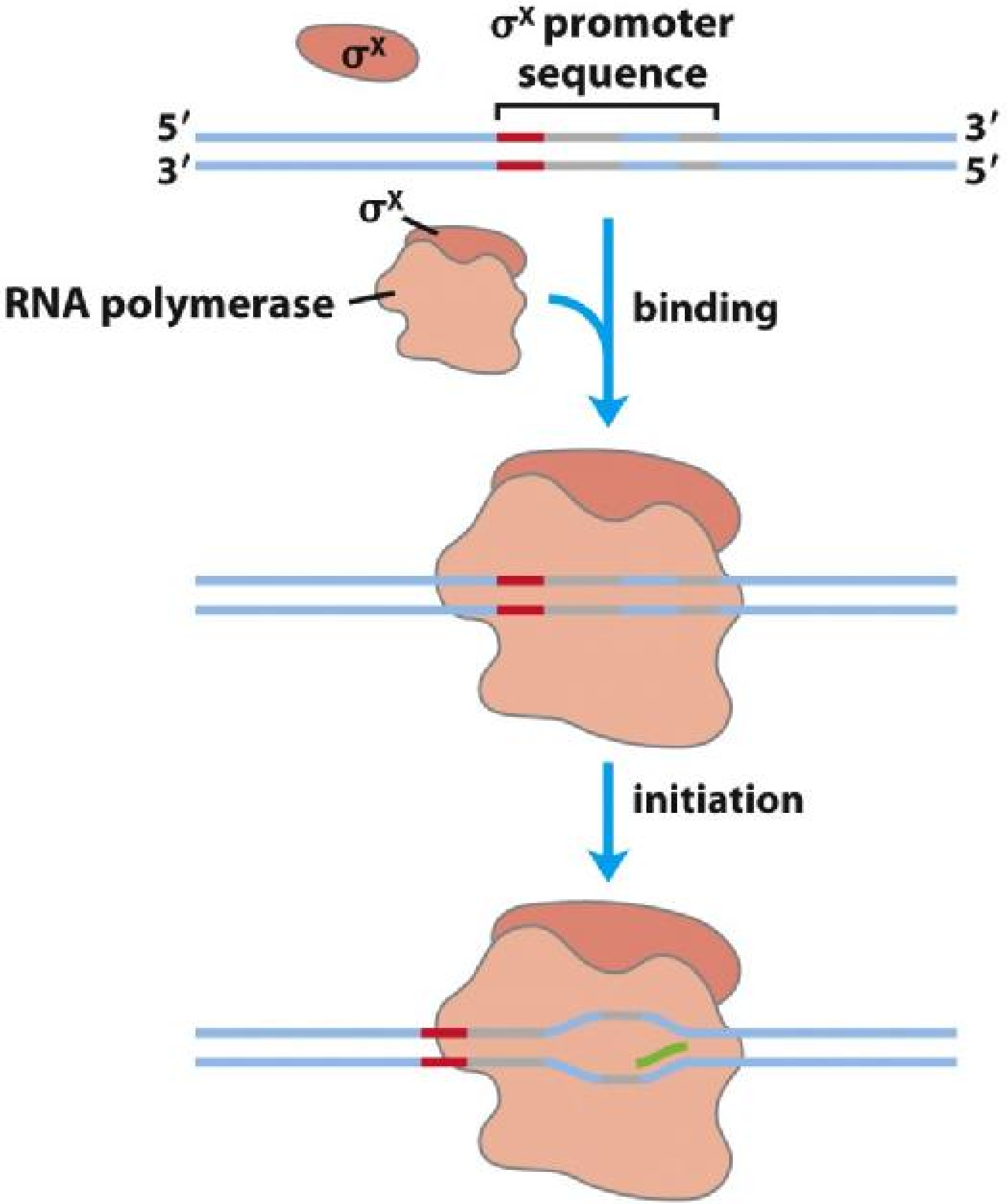

Figure 26-7 part 1
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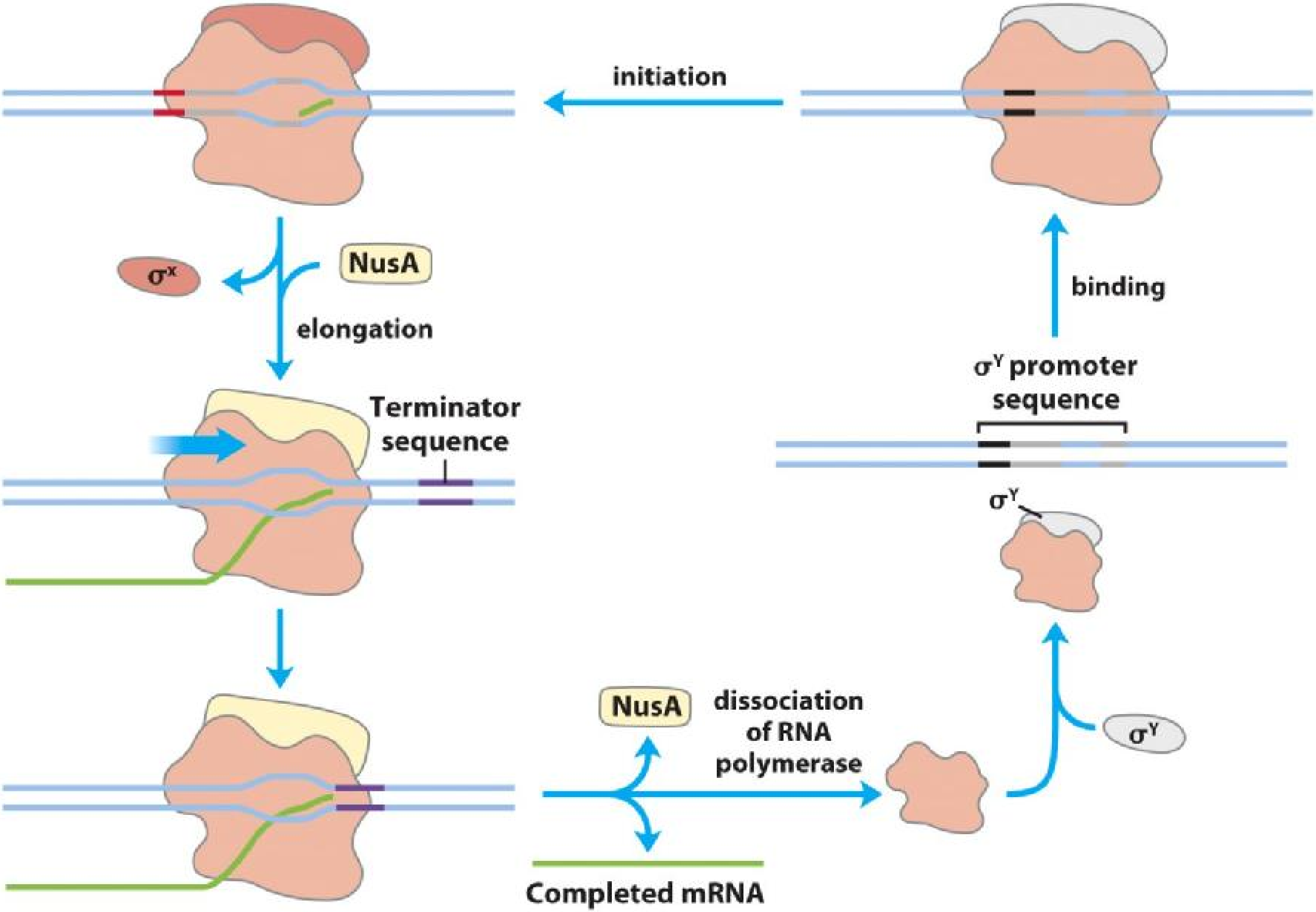

Figure 26-7 part 2

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TABLE 26–1 The Seven Subunits of *Escherichia coli*

σ subunit	K_d (nM)	Molecules/cell*	Holoenzyme ratio (%)*	Function
s^{70}	0.26	700	78	Housekeeping
s^{54}	0.30	110	8	Modulation of cellular nitrogen levels
s^{38}	4.26	<1	0	Stationary phase genes
s^{32}	1.24	<10	0	Heat shock genes
s^{28}	0.74	370	14	Flagella and chemotaxis genes
s^{24}	2.43	<10	0	Extracytoplasmic functions; some heat shock functions
s^{18}	1.73	<1	0	Extracytoplasmic functions, including ferric citrate transport

Source: Adapted from Maeda, H., Fujita, N., & Ishihama, A. (2000) *Nucleic. Acids Res.* **28**, 3500.

Note: σ factors are widely distributed in bacteria; the number varies from a single σ factor in *Mycoplasma genitalium* to 63 distinct σ factors in *Streptomyces coelicolor.*

*Approximate number of each σ subunit per cell and the fraction of RNA polymerase holoenzyme complexed with each σ subunit during exponential growth. The numbers change as growth conditions change. The fraction of RNA polymerase complexed with each σ subunit reflects both the amount of the particular subunit and its affinity for the enzyme.

Table 26-1

Lehninger Principles of Biochemistry, Fifth Edition

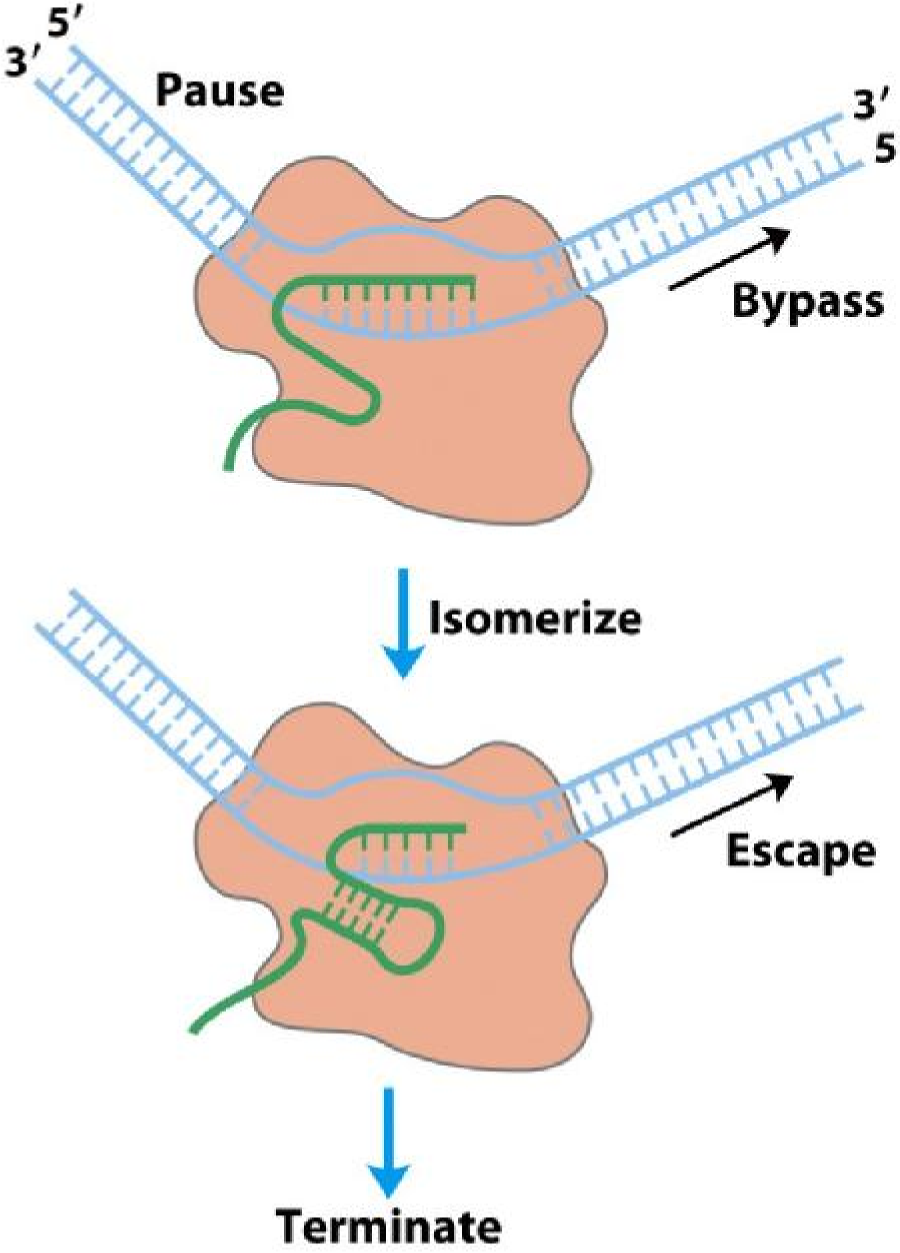

Figure 26-8

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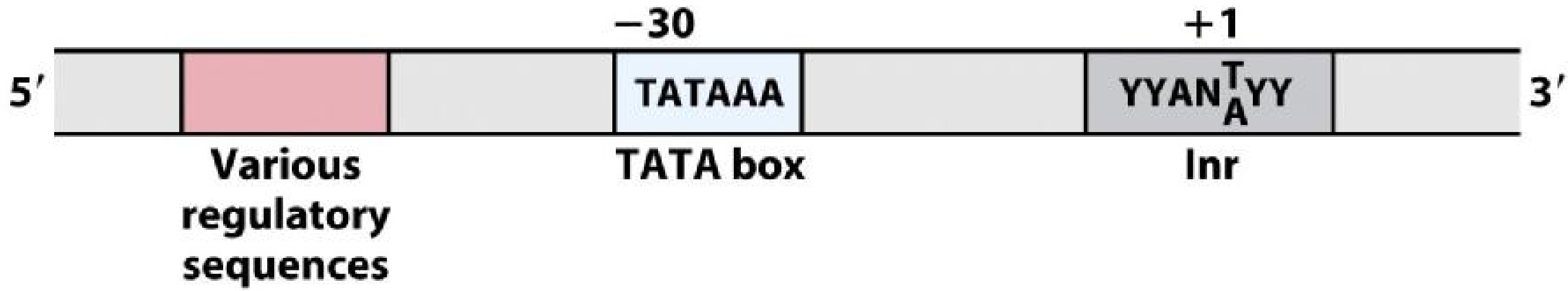

Figure 26-9
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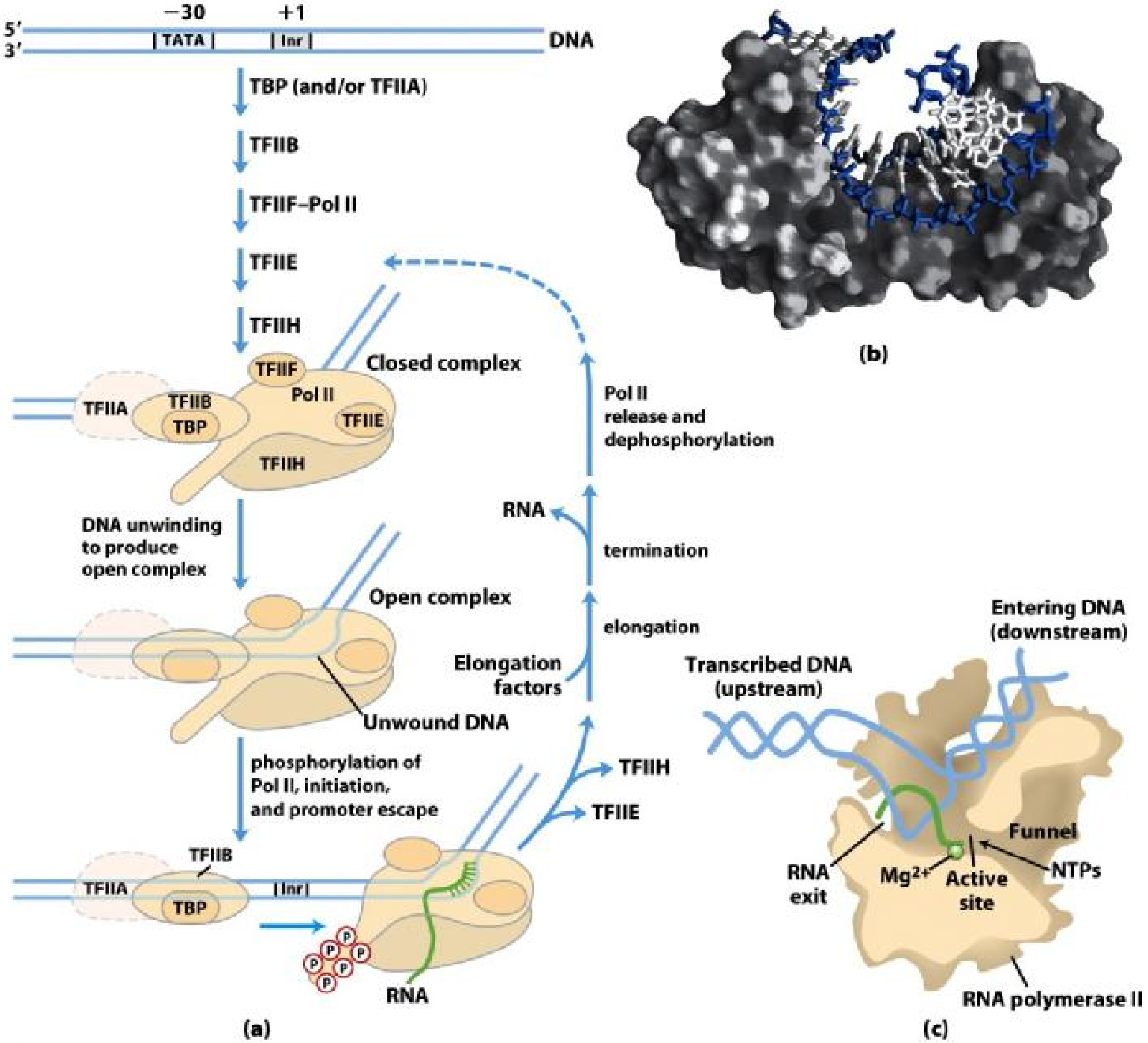

Figure 26-10

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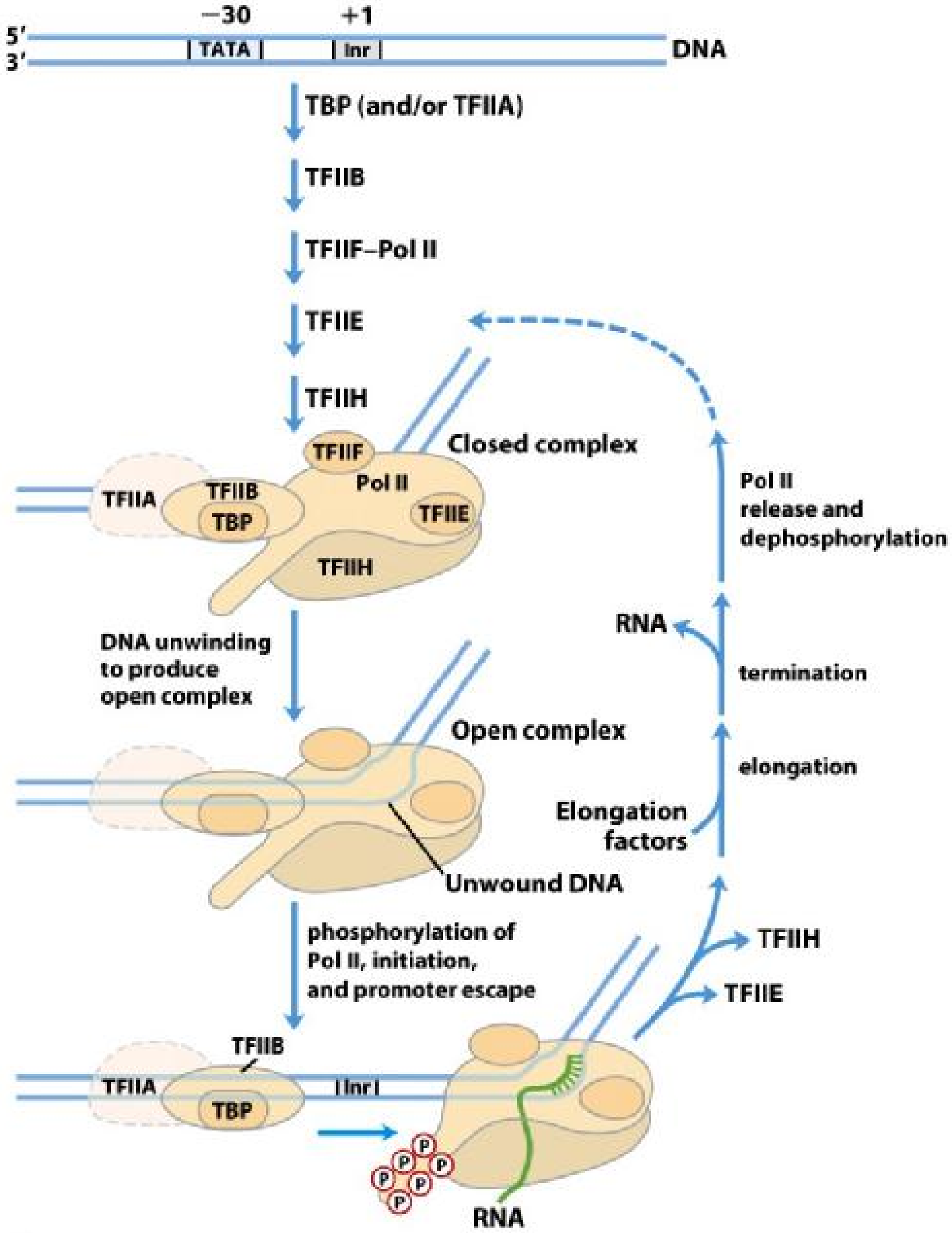

Figure 26-10a

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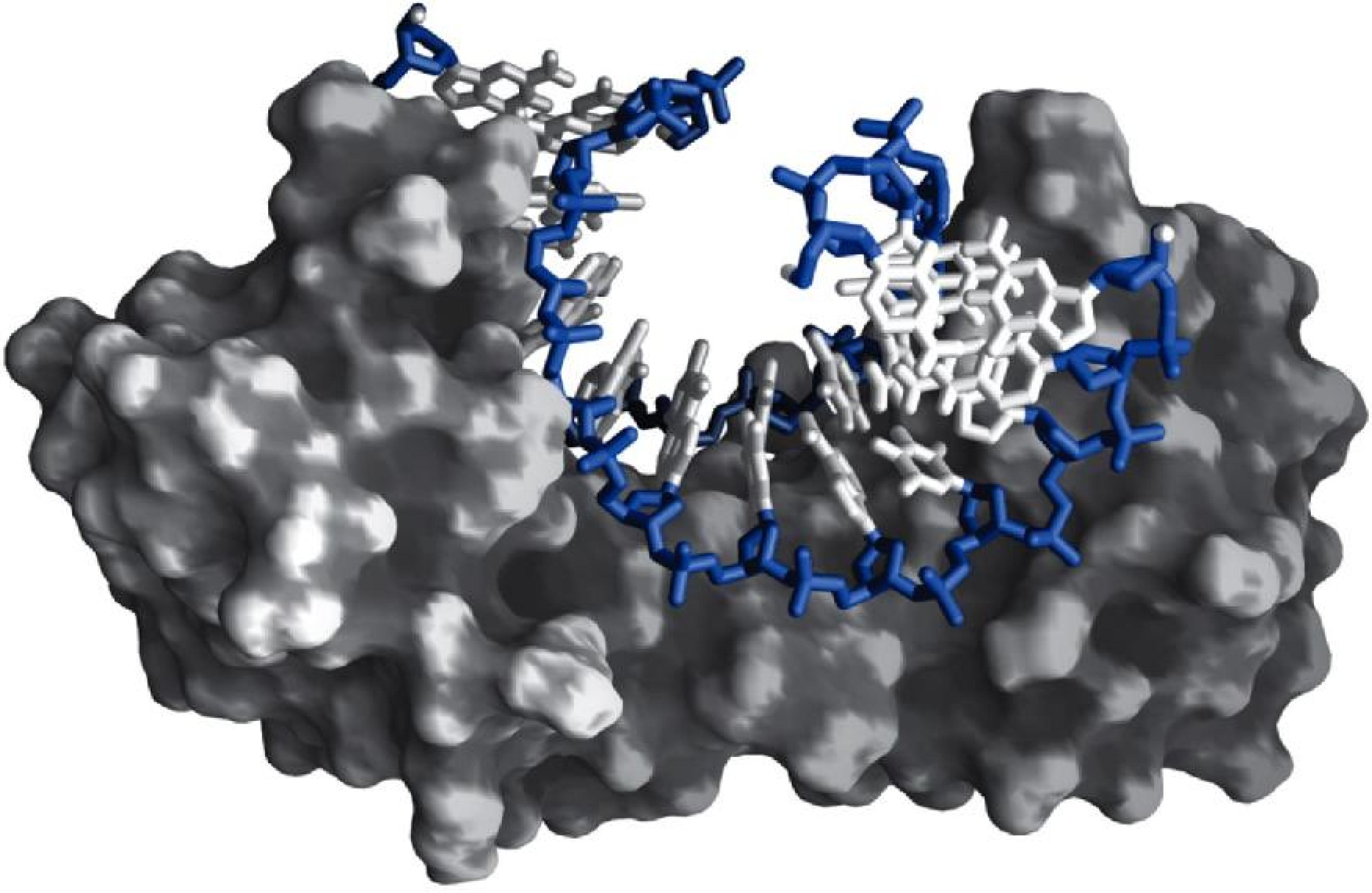

Figure 26-10b

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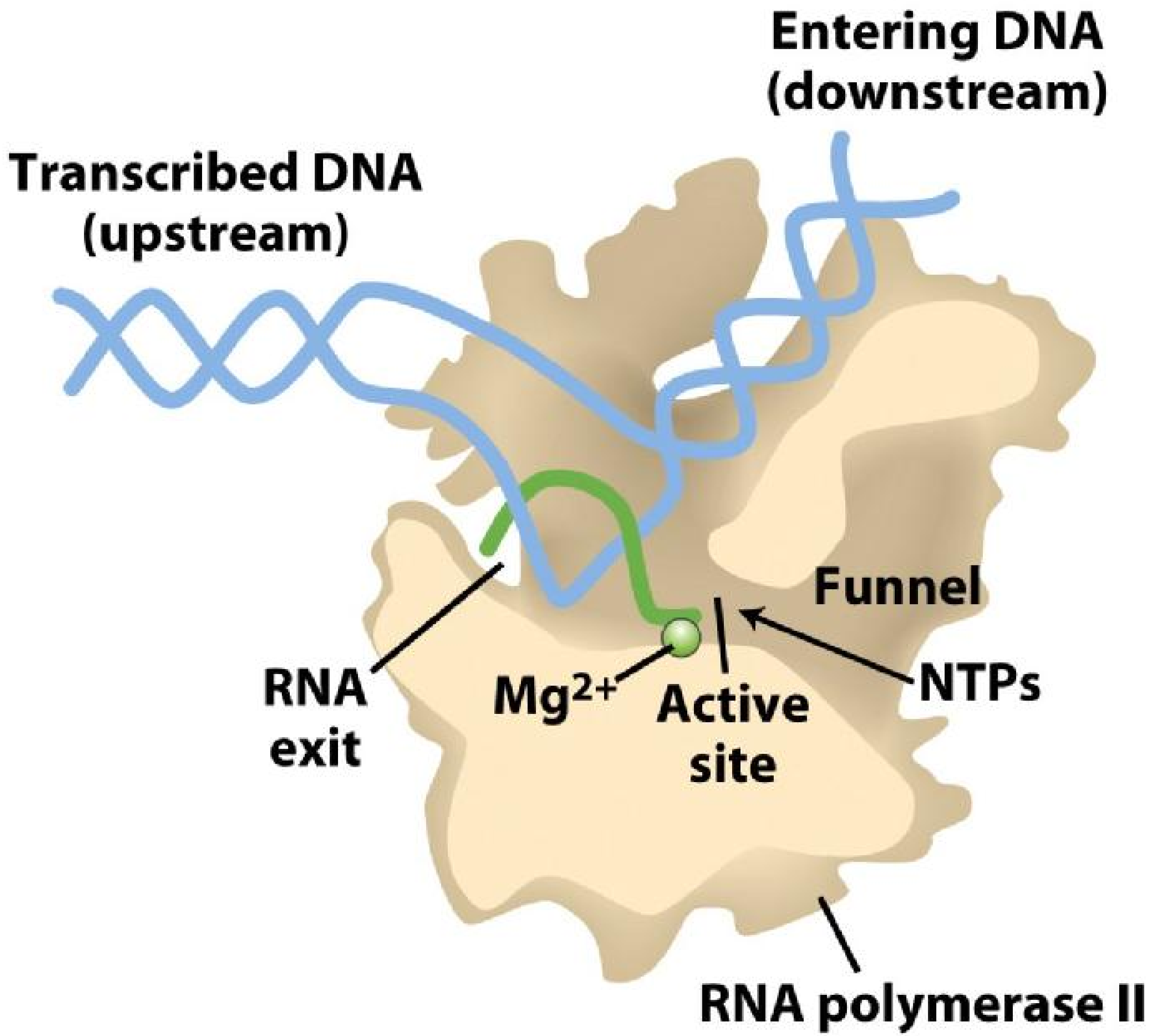

Figure 26-10c

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TABLE 26–2 Proteins Required for Initiation of Transcription at the RNA Polymerase II (Pol II) Promoters of Eukaryotes

Transcription protein	Number of subunits	Subunit(s) M_r	Function(s)
Initiation			
Pol II	12	10,000–220,000	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	38,000	Specifically recognizes the TATA box
TFIIA	3	12,000, 19,000, 35,000	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	35,000	Binds to TBP; recruits Pol II–TFIIF complex
TFIIE	2	34,000, 57,000	Recruits TFIIH; has ATPase and helicase activities
TFIIF	2	30,000, 74,000	Binds tightly to Pol II; binds to TFIIB and prevents binding of Pol II to nonspecific DNA sequences
TFIIH	12	35,000–89,000	Unwinds DNA at promoter (helicase activity); phosphorylates Pol II (within the CTD); recruits nucleotide-excision repair proteins
Elongation[*]			
ELL[†]	1	80,000	
pTEFb	2	43,000, 124,000	Phosphorylates Pol II (within the CTD)
SII (TFIIS)	1	38,000	
Elongin (SIII)	3	15,000, 18,000, 110,000	

[*] The function of all elongation factors is to suppress the pausing or arrest of transcription by the Pol II—TFIIF complex.

[†] Name derived from eleven-nineteen *l*ysine-rich *l*eukemia. The gene for ELL is the site of chromosomal recombination events frequently associated with acute myeloid leukemia.

Table 26-2

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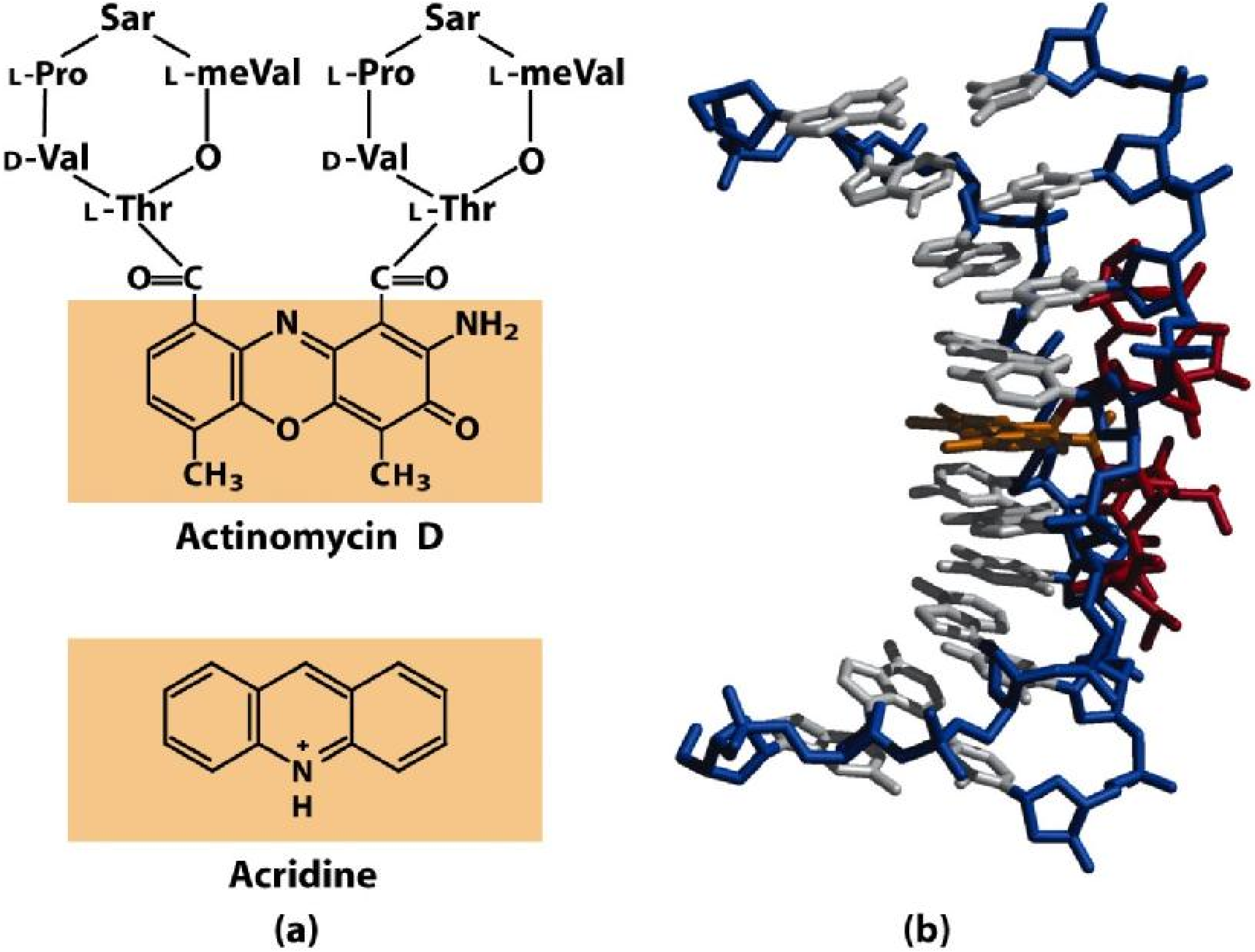

Figure 26-11

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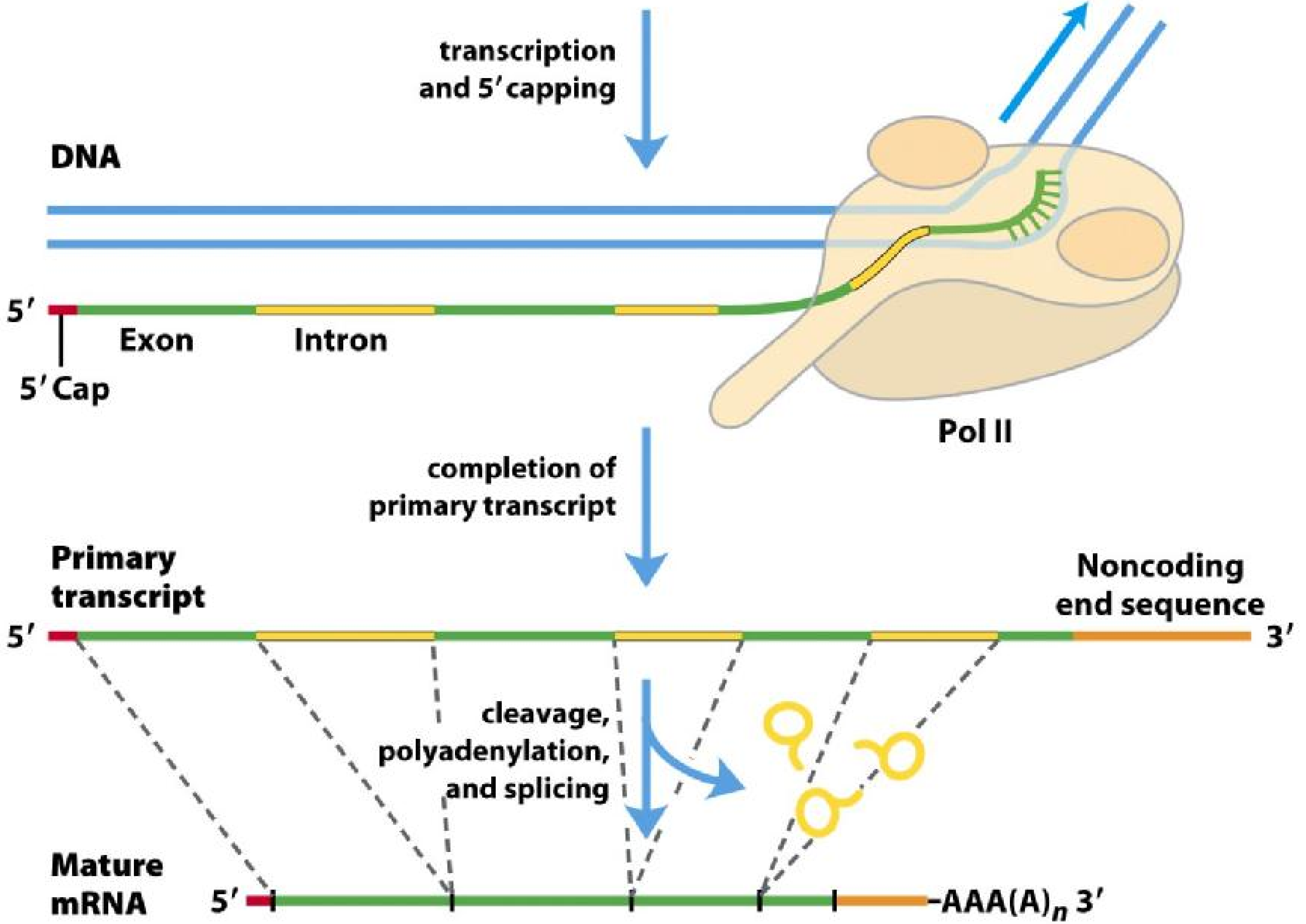

Figure 26-12

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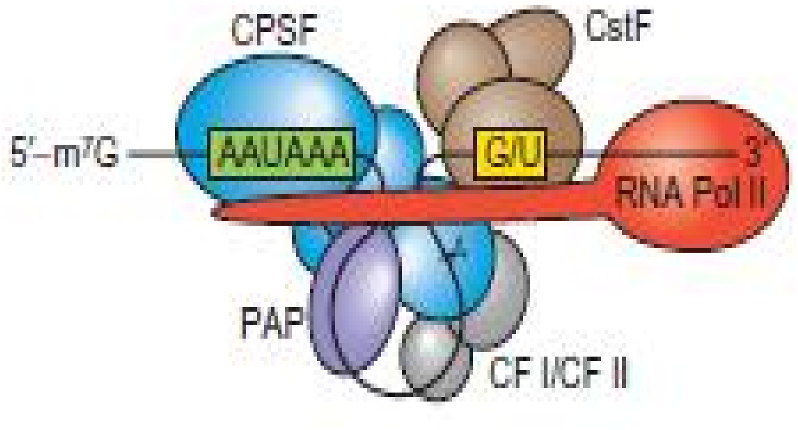

A model for the precleavage complex. This partly hypothetical model shows the apparent positions of all the proteins presumed to be involved in cleavage, with respect to the two parts of the polyadenylation signal (green and yellow). The scissors symbol denotes the active site of CPSF-73.

CPSF: Cleavage and Polyadenylation Specificity Factor (4 subunits)
CstF: Cleavage Stimulatory Factor (3-subunits)
PAP: Poly (A) polymerase
CF: Cleavage Factor

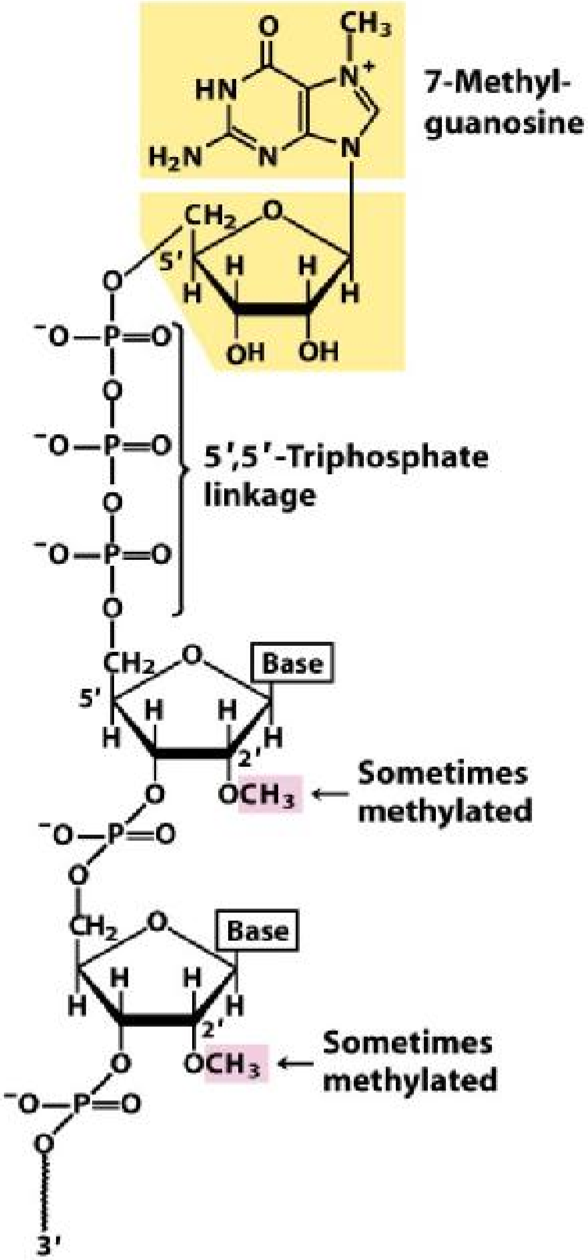

Figure 26-13a

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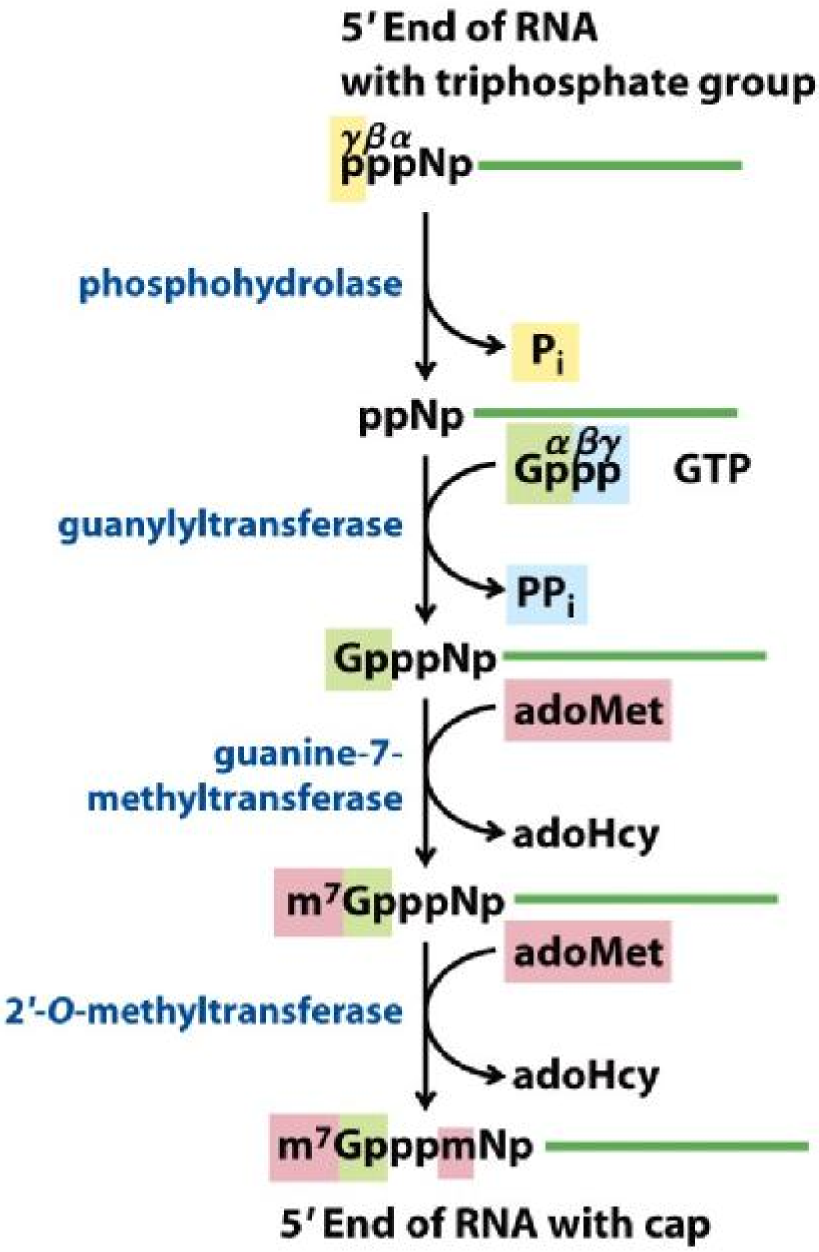

Figure 26-13b

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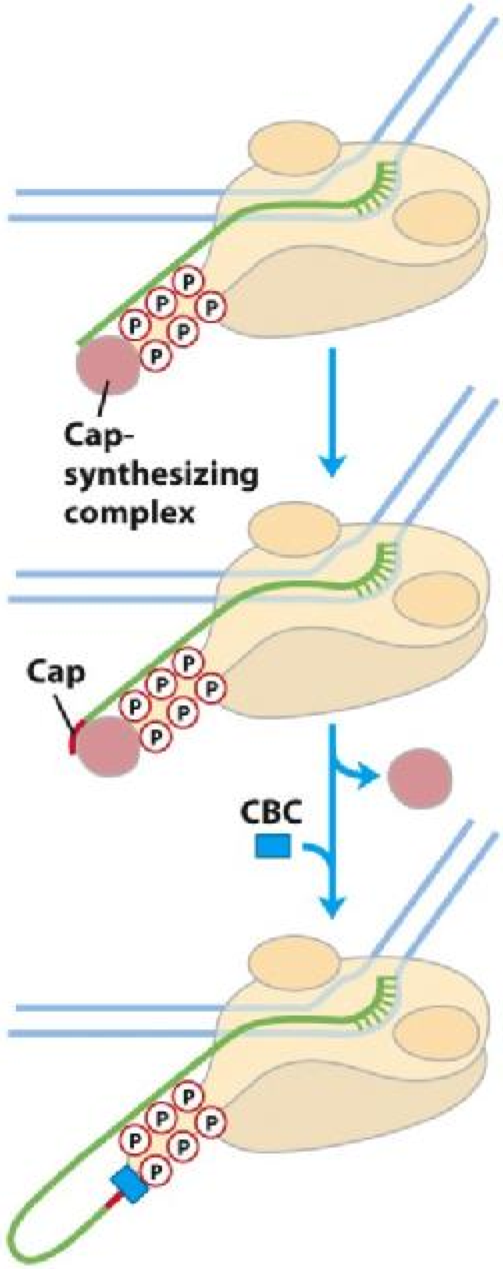

Figure 26-13c

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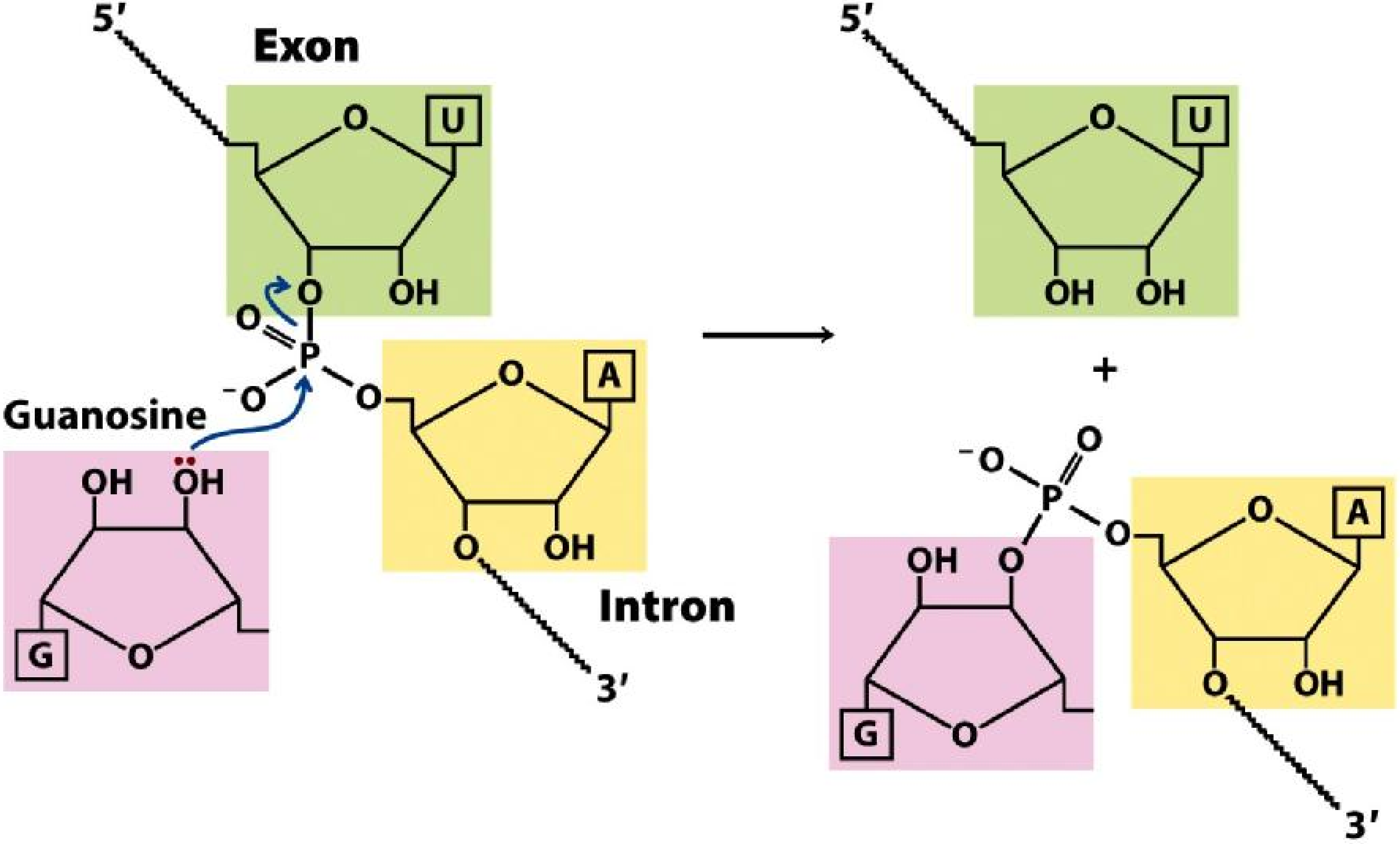

Figure 26-14
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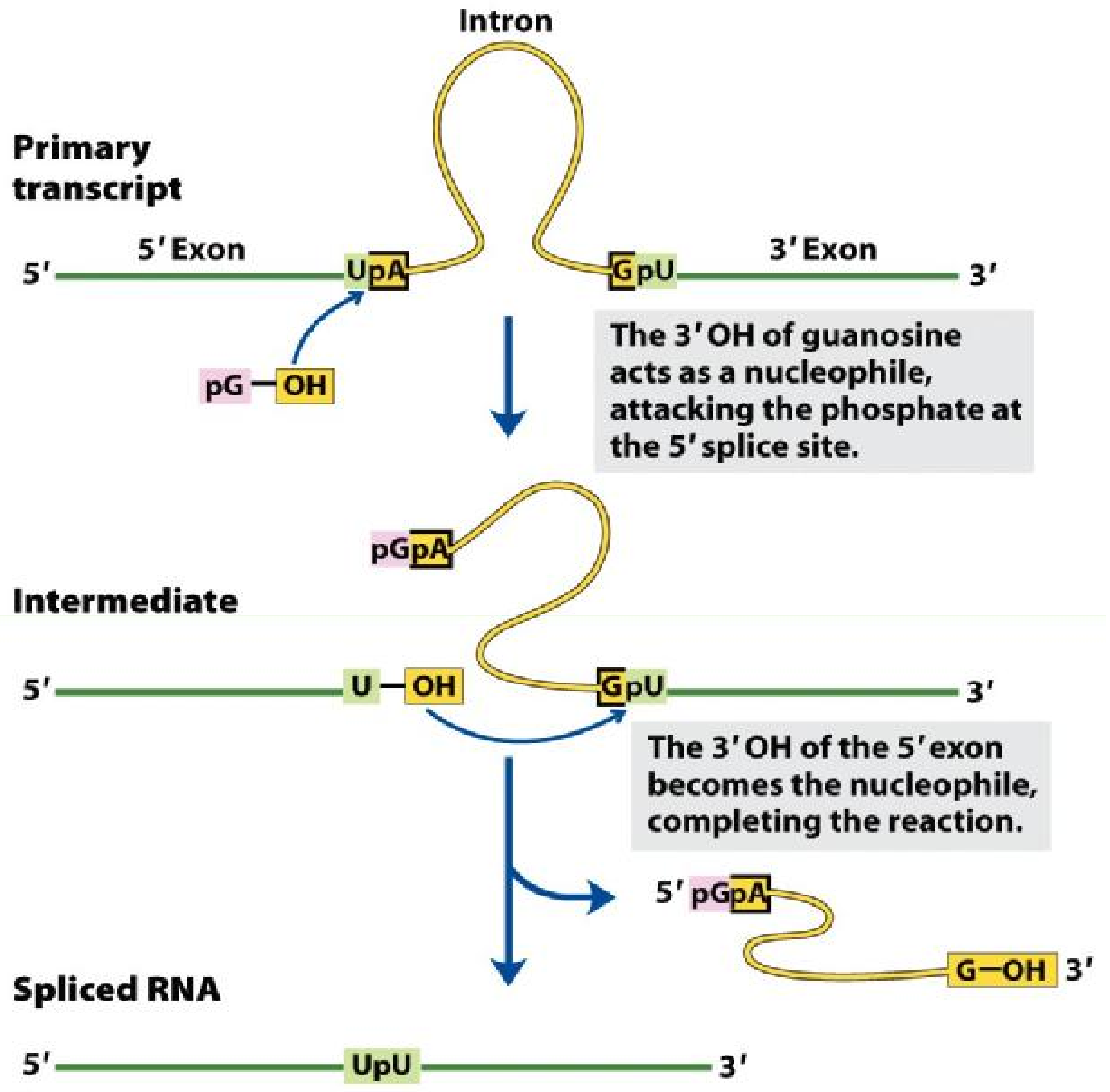

The 3′OH of guanosine acts as a nucleophile, attacking the phosphate at the 5′ splice site.

The 3′OH of the 5′exon becomes the nucleophile, completing the reaction.

Figure 26-15
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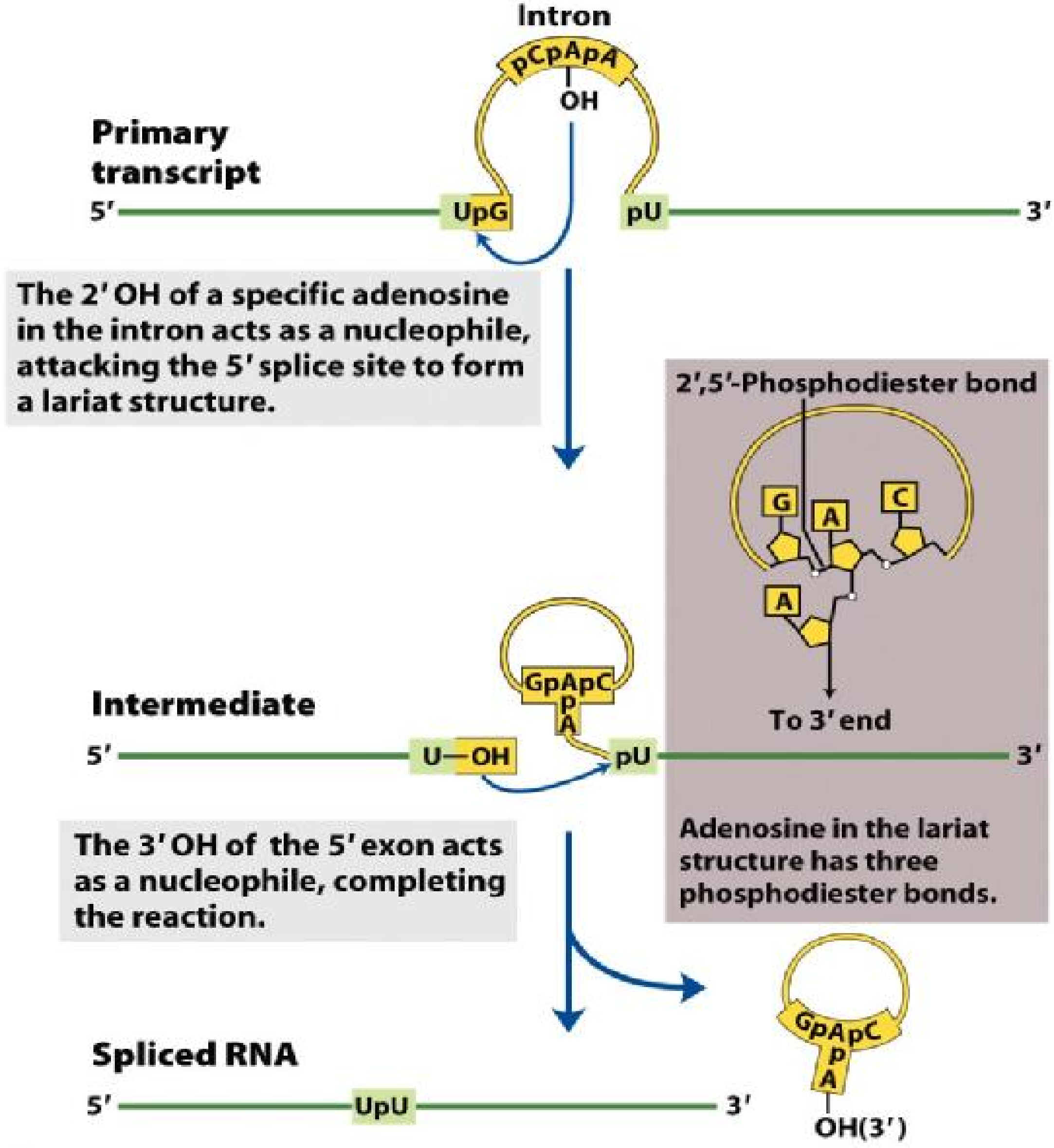
Splicing mechanism of group I introns



The 2′ OH of a specific adenosine in the intron acts as a nucleophile, attacking the 5′ splice site to form a lariat structure.

Adenosine in the lariat structure has three phosphodiester bonds.

The 3′ OH of the 5′ exon acts as a nucleophile, completing the reaction.

Figure 26-16
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Splicing mechanism of group II introns

Thomas Cech

Unnumbered 26 p1037

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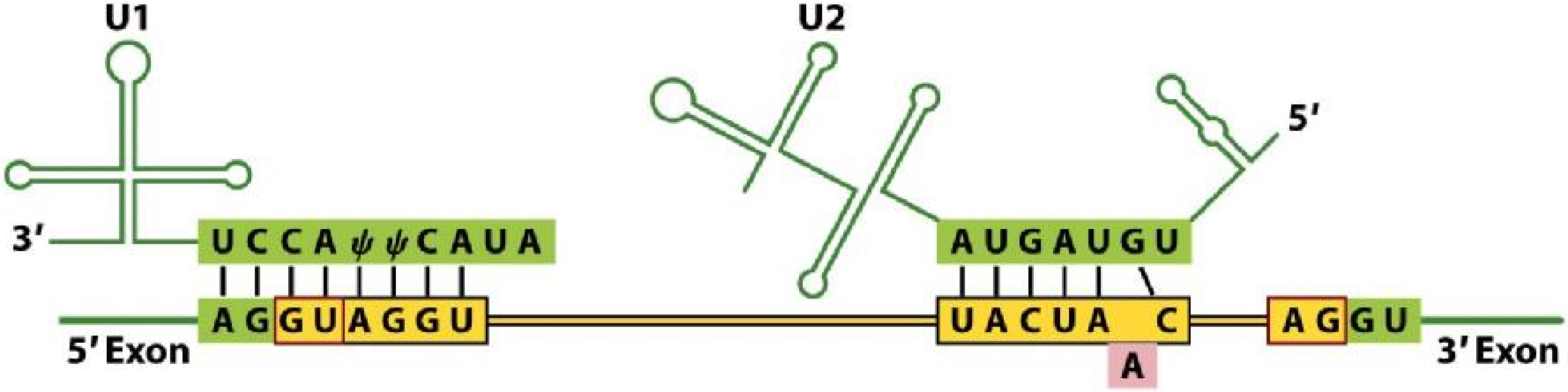

Figure 26-17a
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Splicing mechanism in mRNA primary transcripts

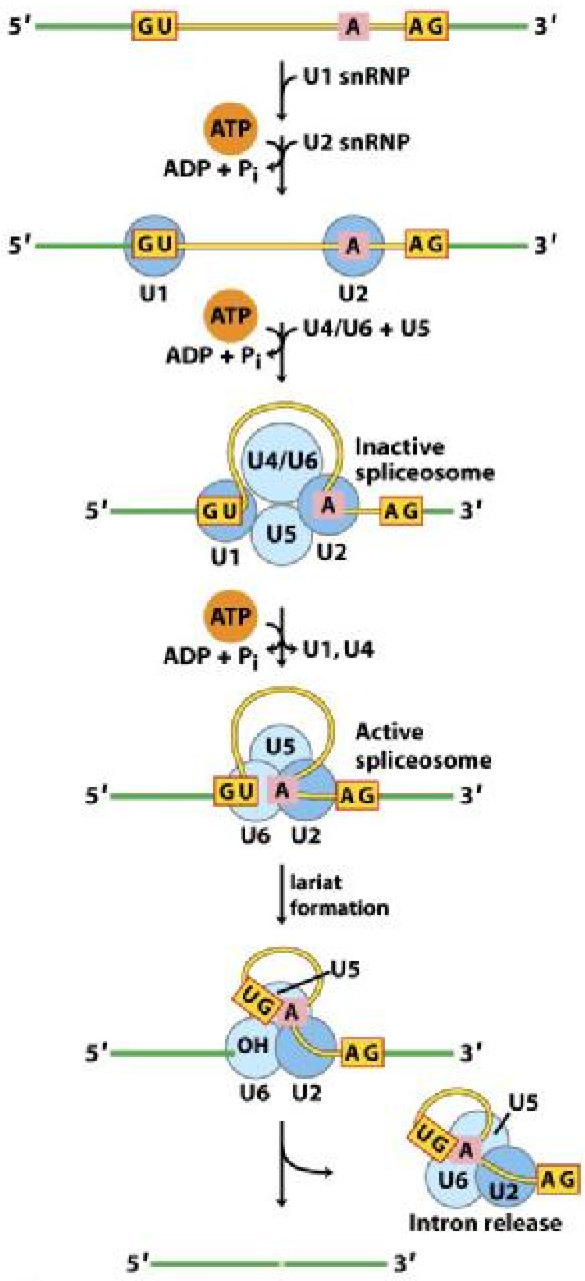

Figure 26-17b

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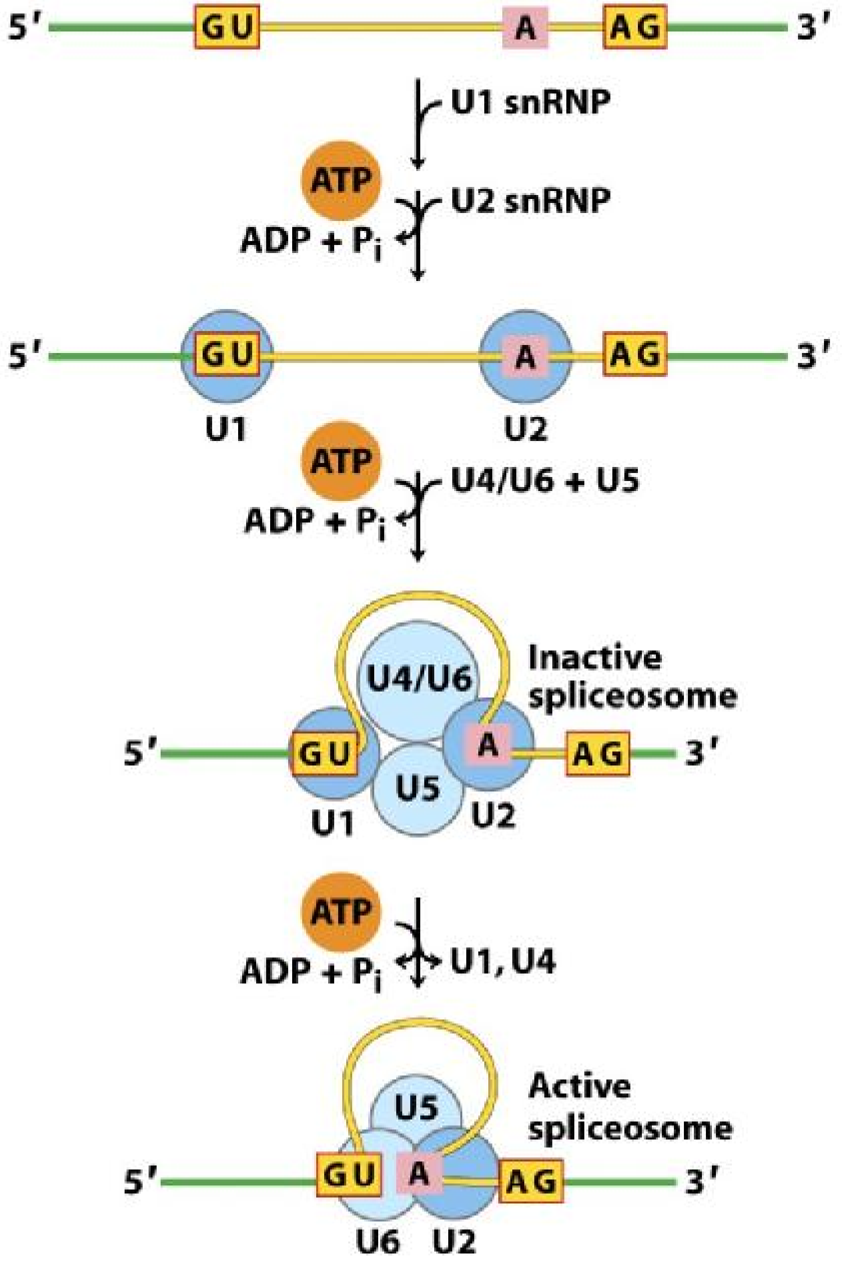

Figure 26-17b part 1

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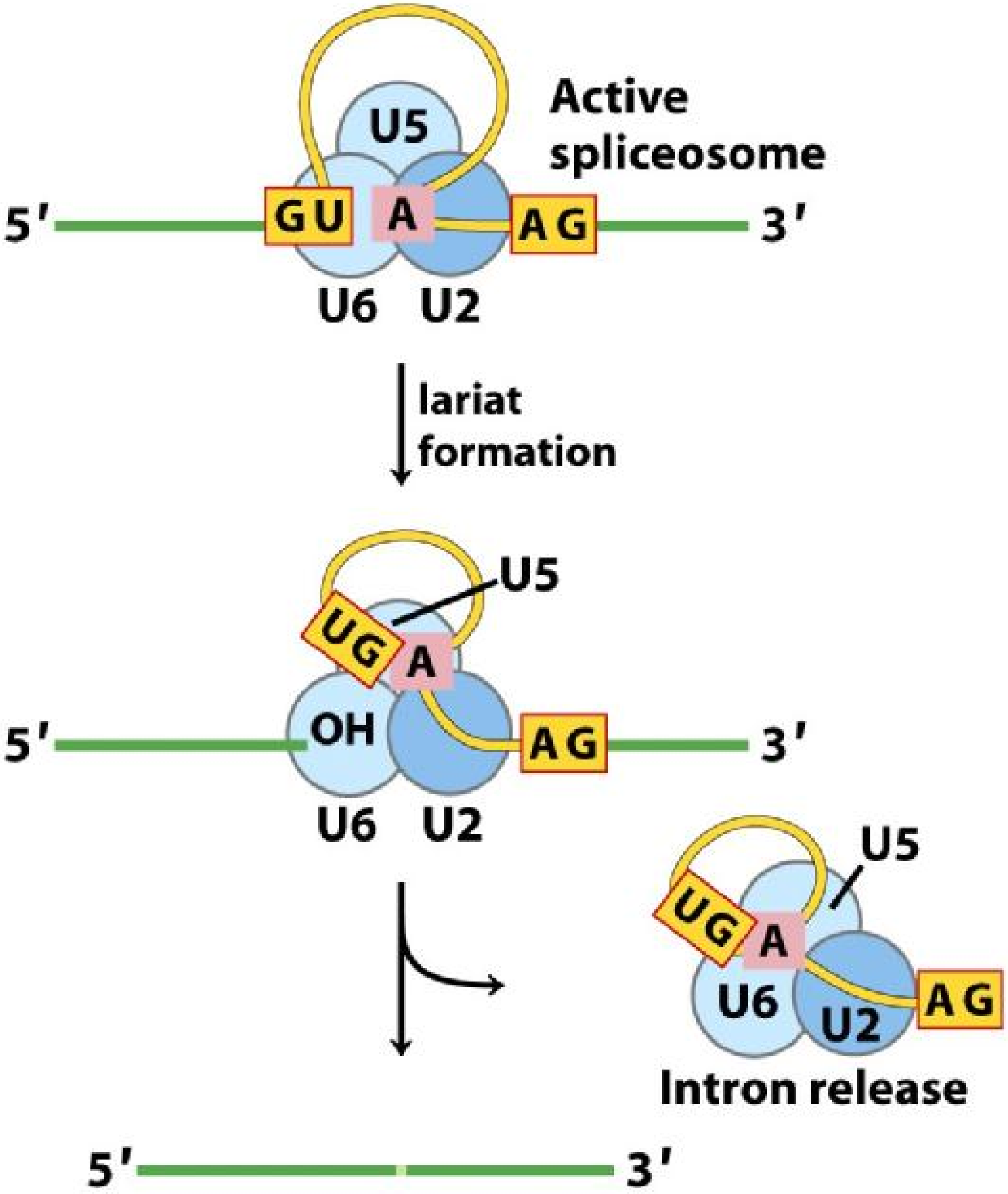

Figure 26-17b part 2

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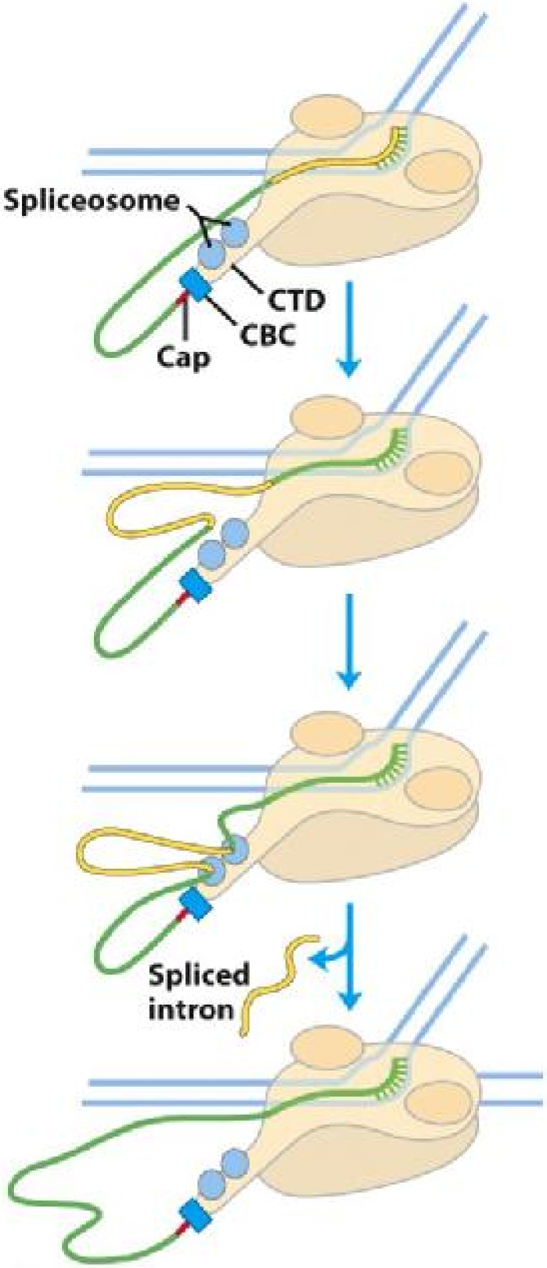

Figure 26-17c

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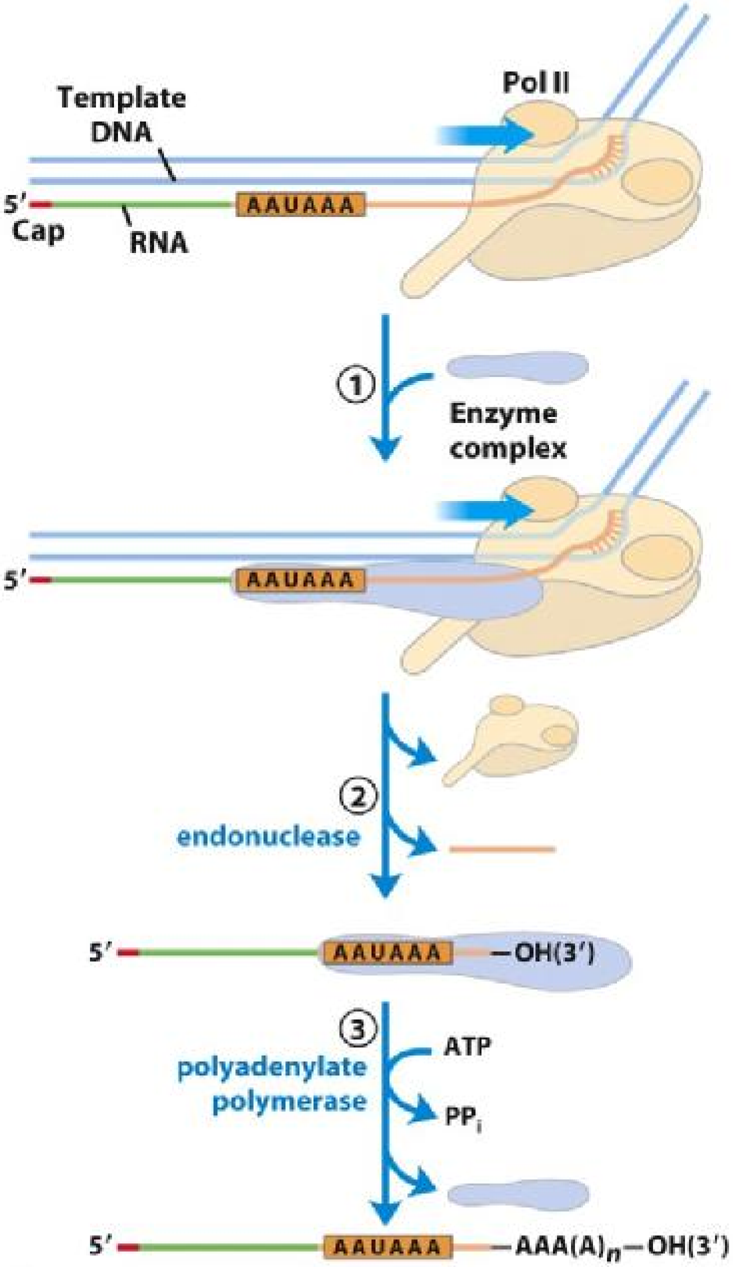

Figure 26-18

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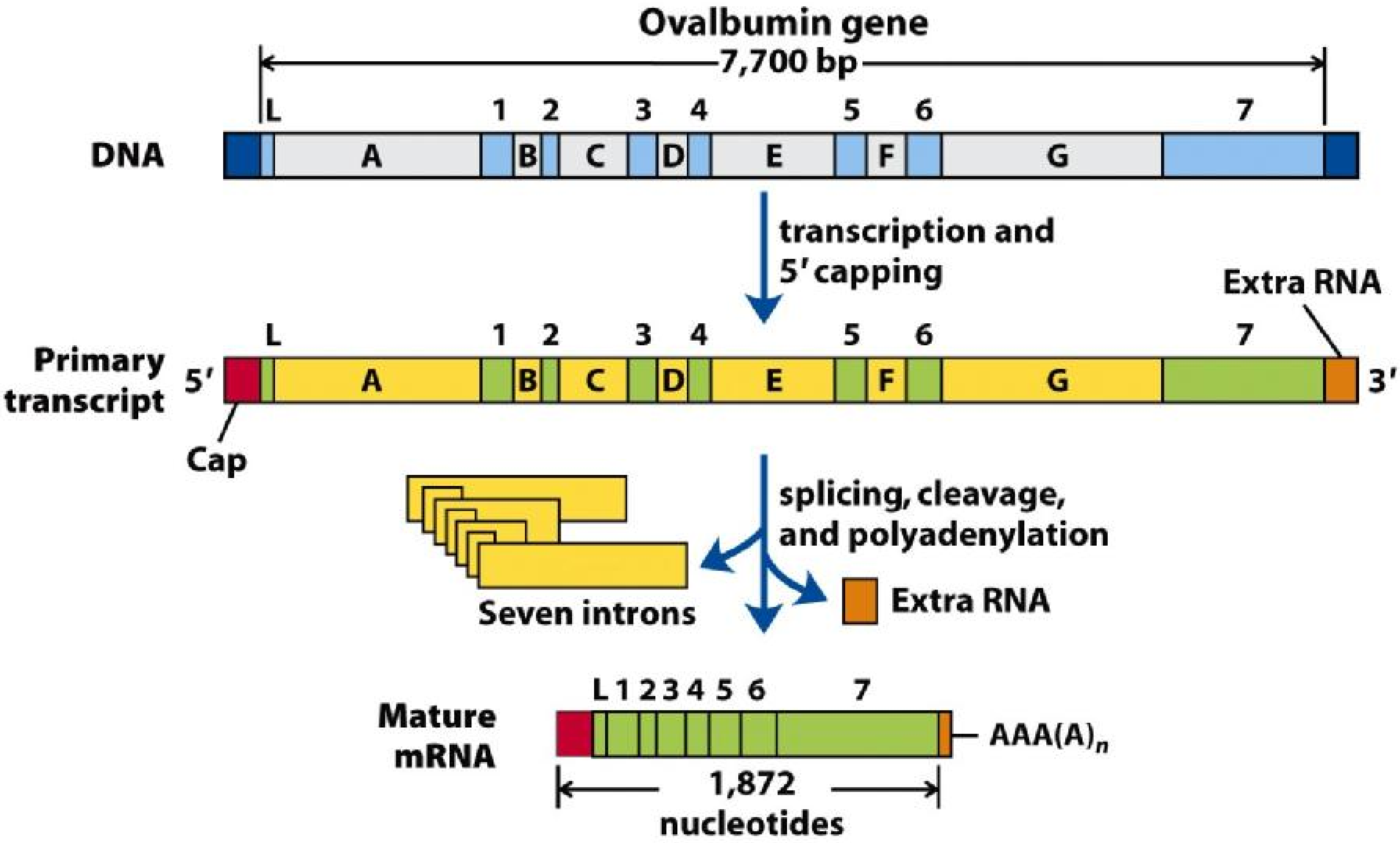

Figure 26-19

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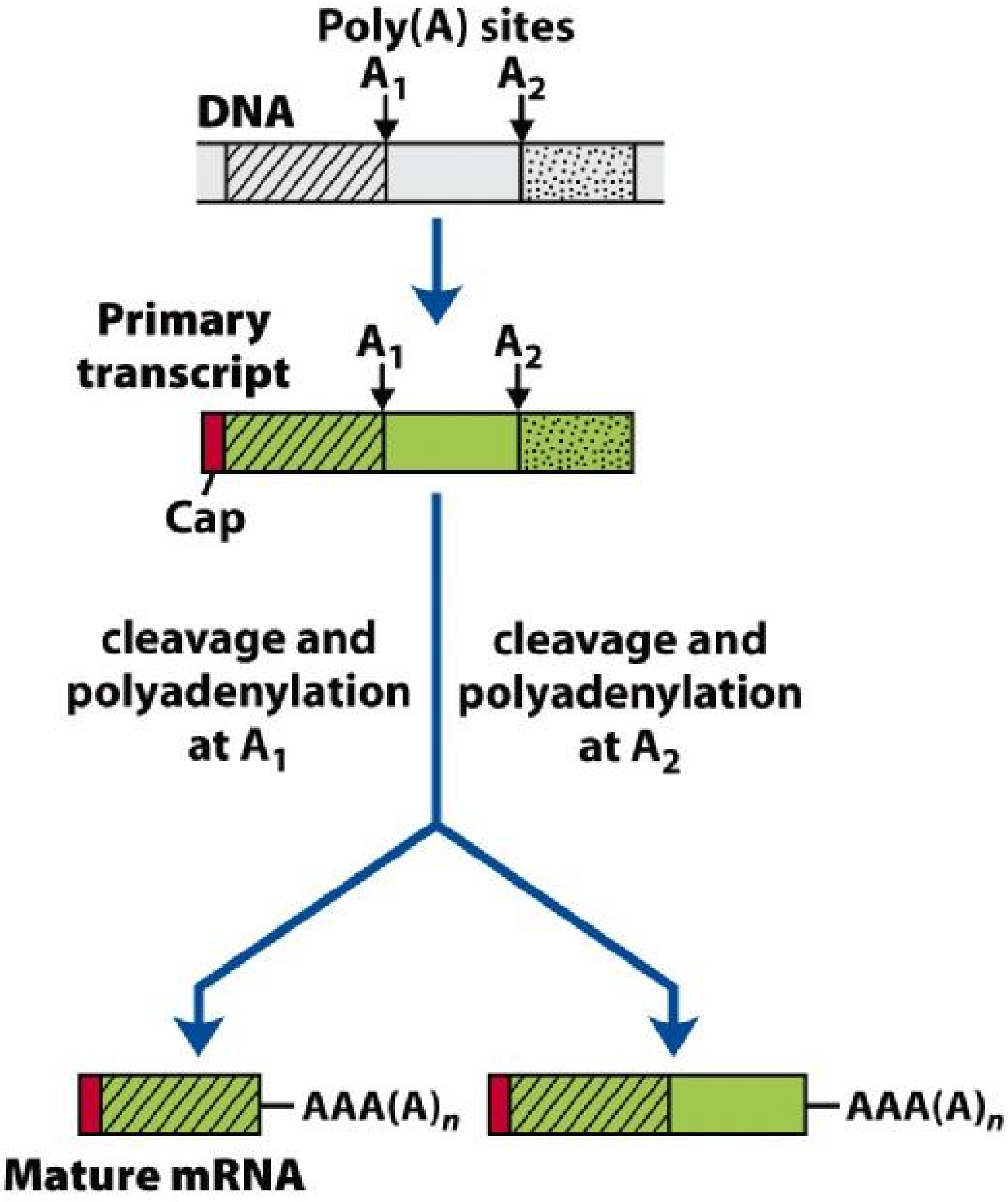

Figure 26-20a

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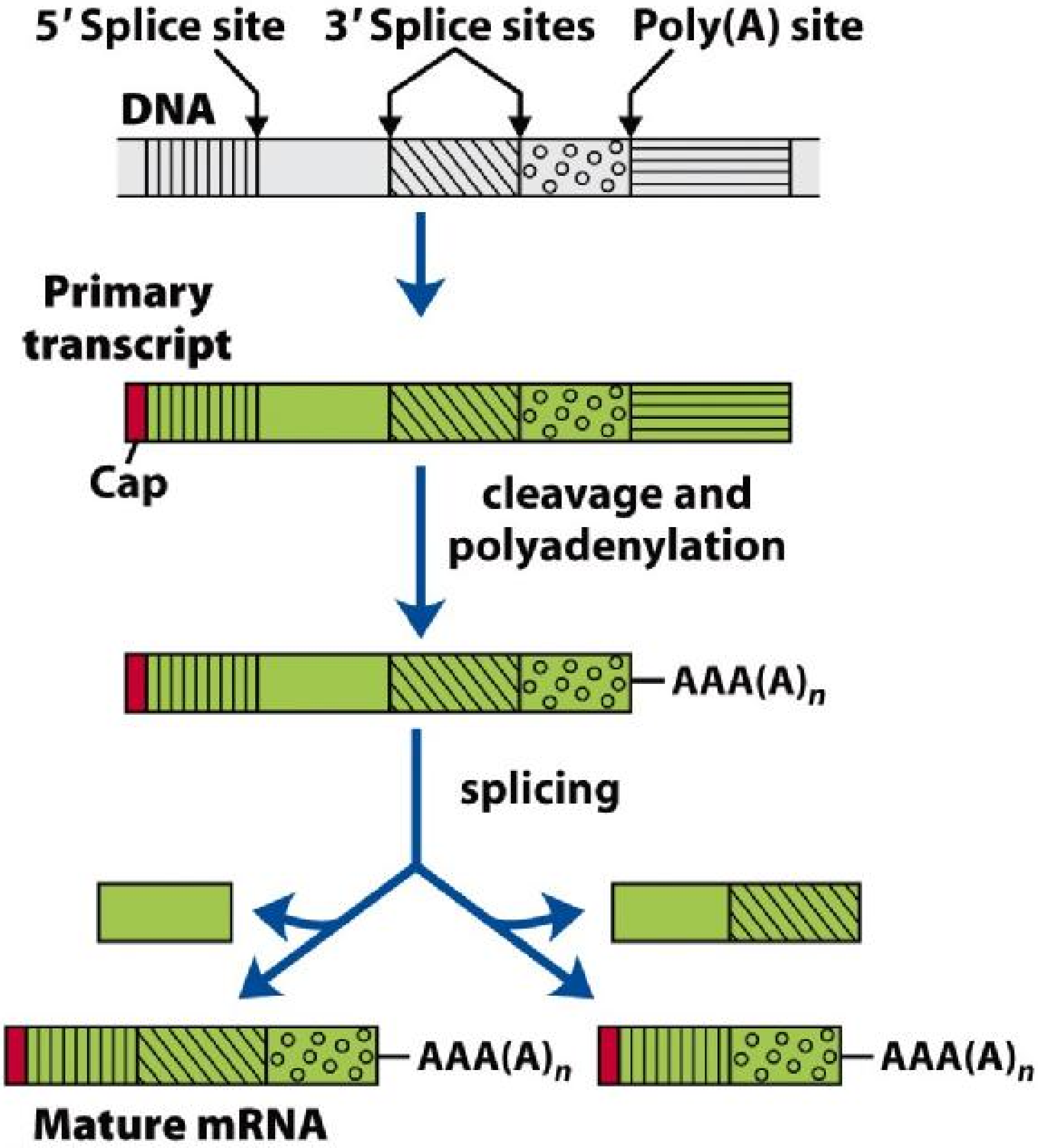

Figure 26-20b

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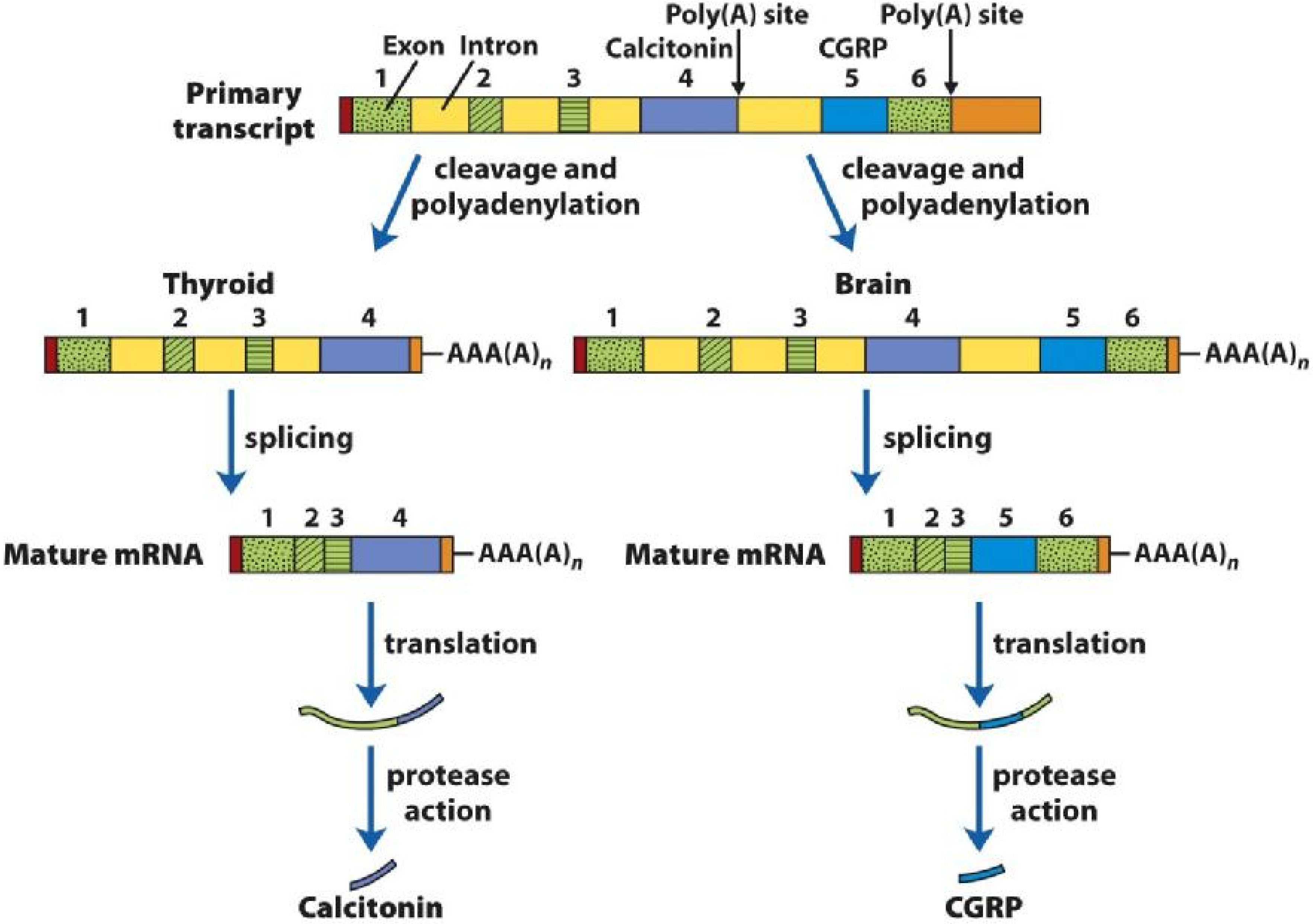

Figure 26-21

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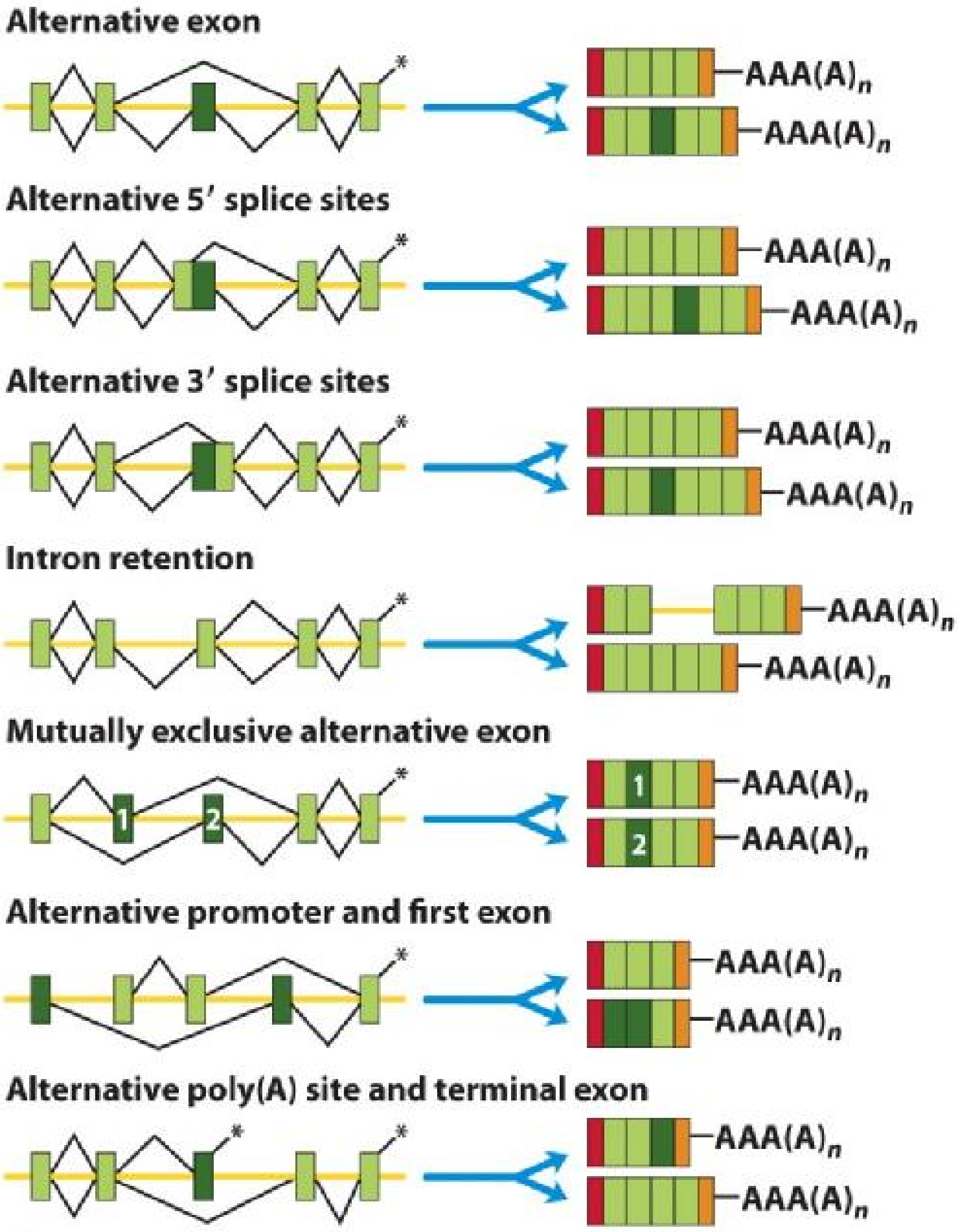

Figure 26-22
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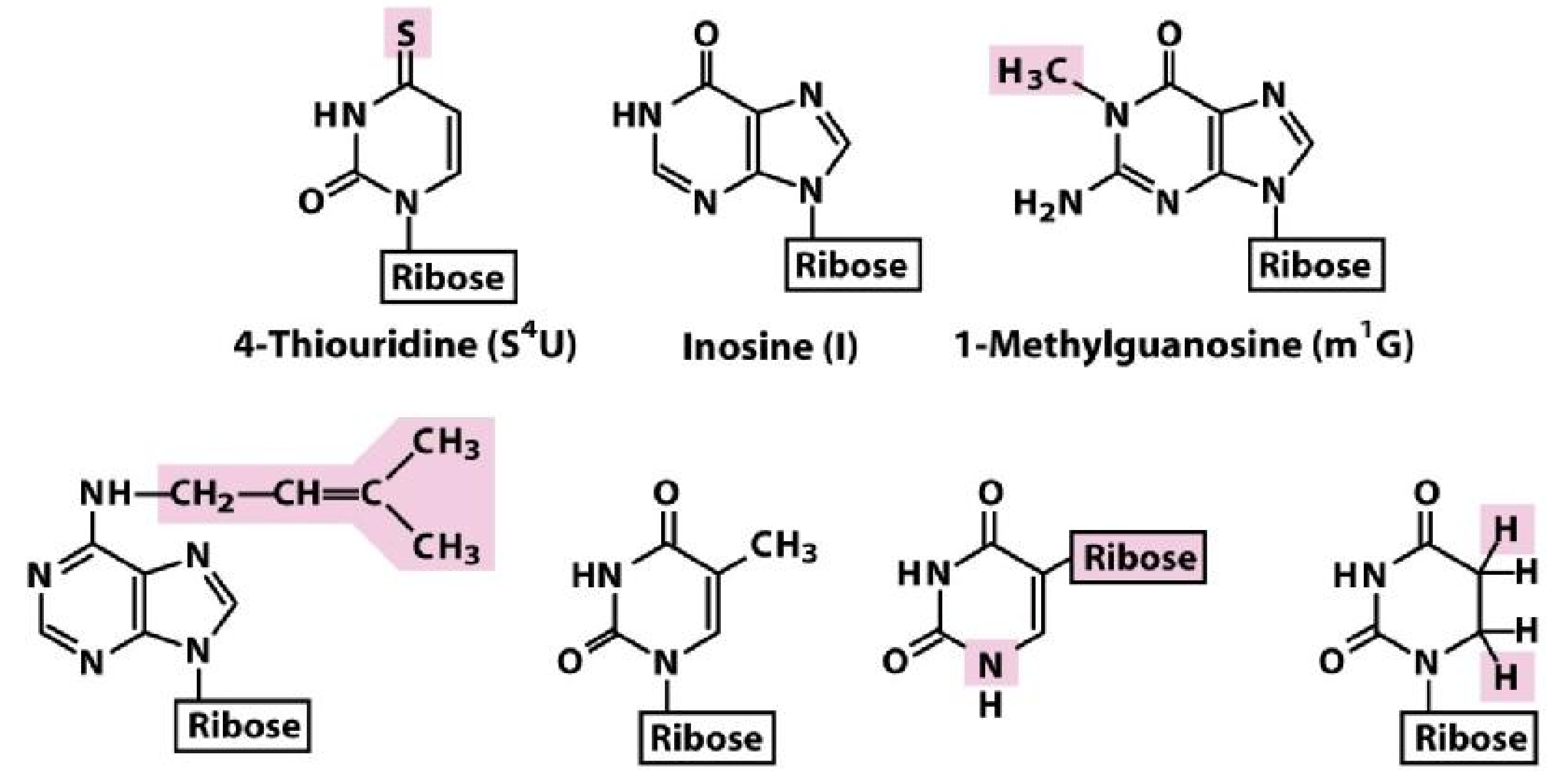

4-Thiouridine (S^4U) Inosine (I) 1-Methylguanosine (m^1G)

N^6-Isopentenyladenosine (i^6A) Ribothymidine (T) Pseudouridine (ψ) Dihydrouridine (D)

Figure 26-23

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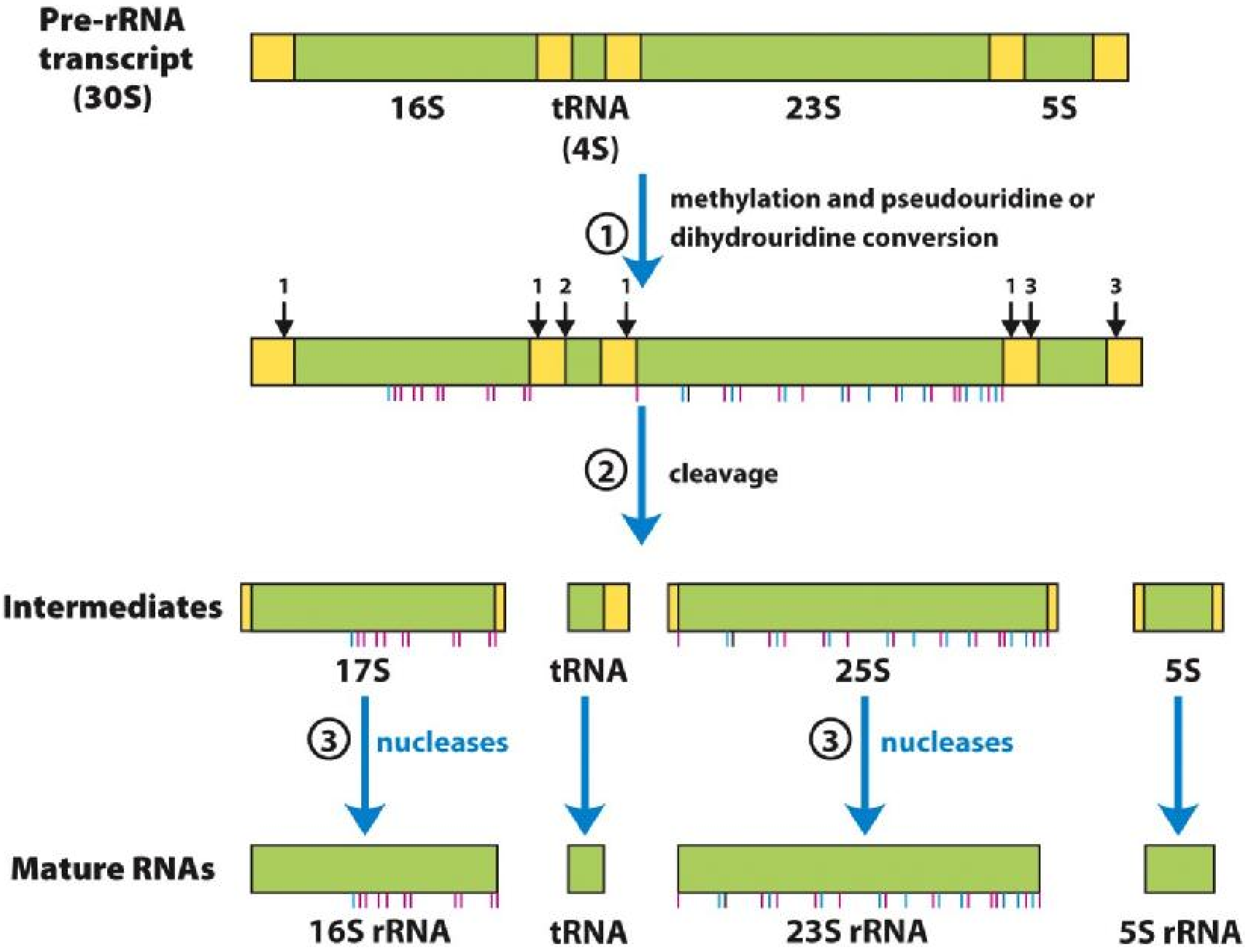

Figure 26-24

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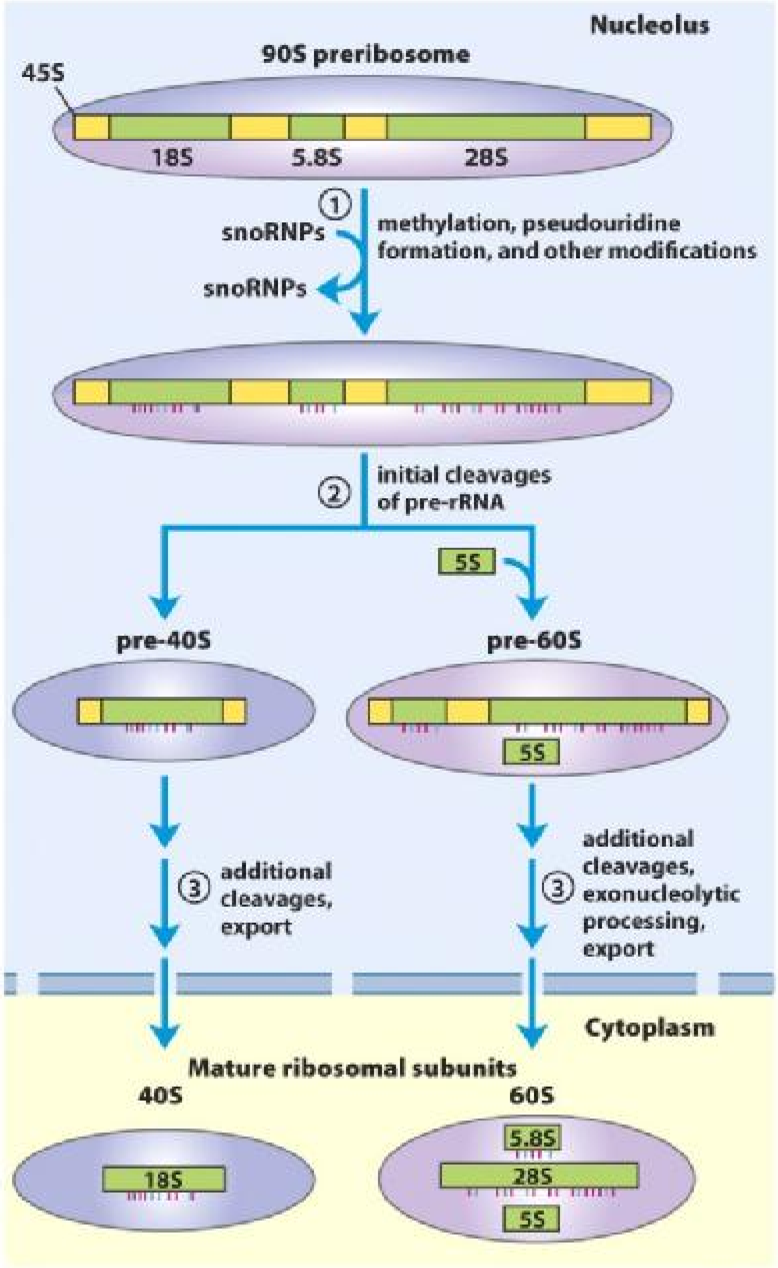

Figure 26-25

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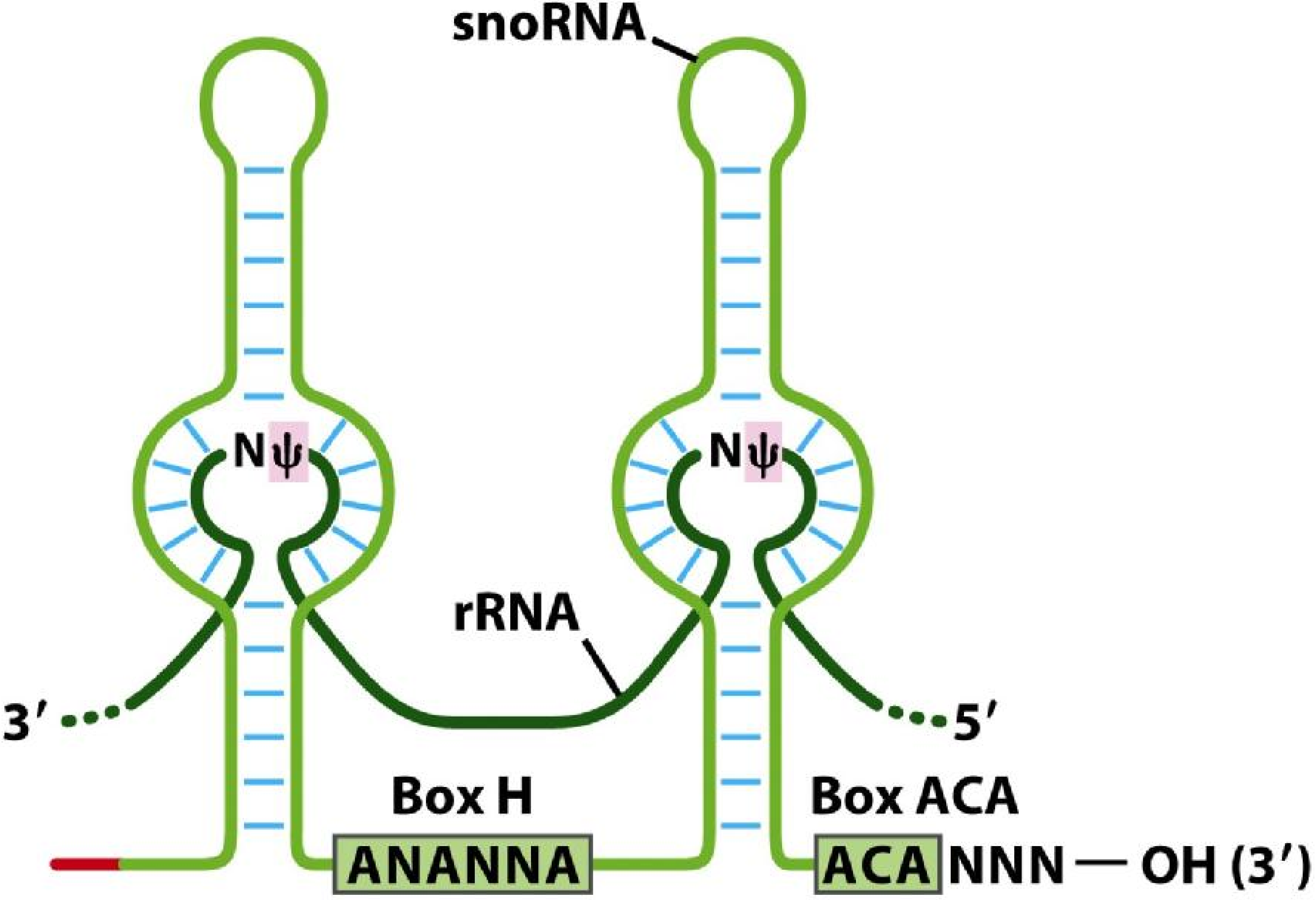

Figure 26-26b

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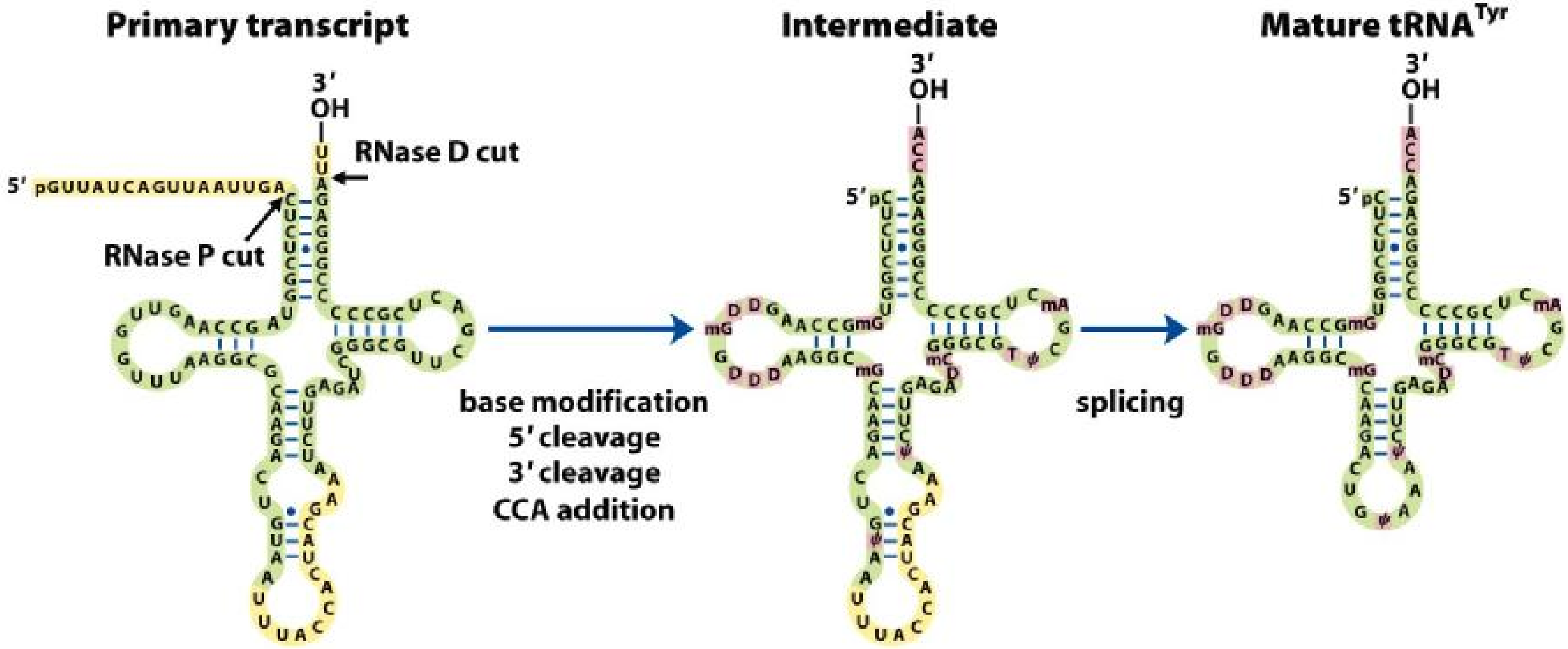

Figure 26-27
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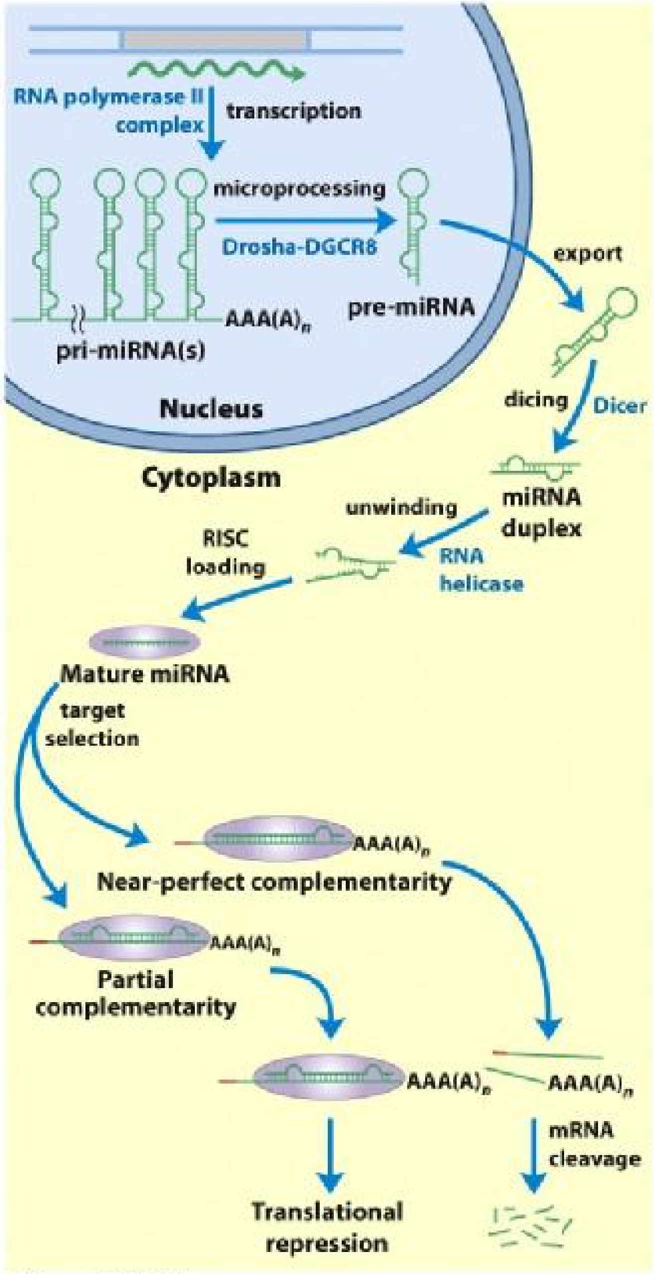

Figure 26-28
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RNA-induced silencing complex (RISC)

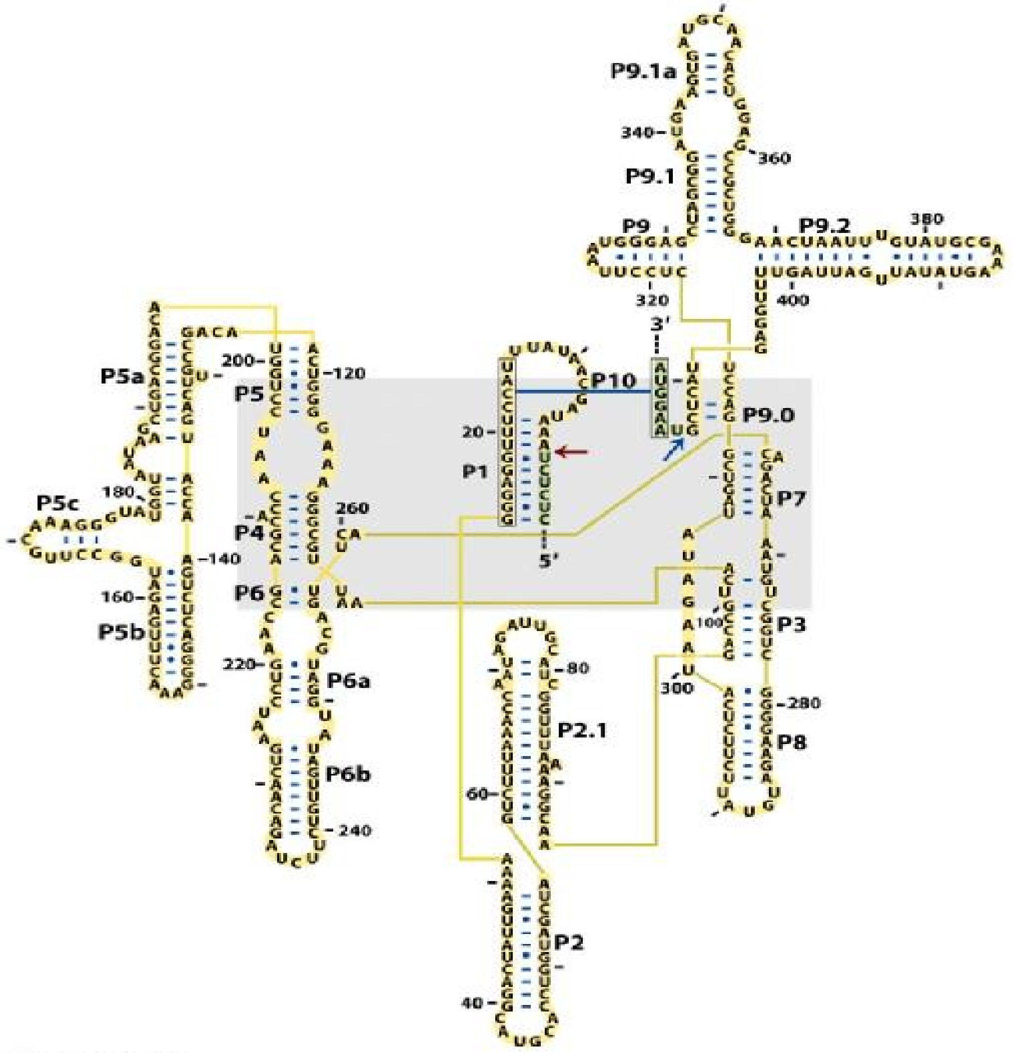

Figure 26-30
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Secondary structure of the self-splicing rRNA intron of *Tetrahymena.*

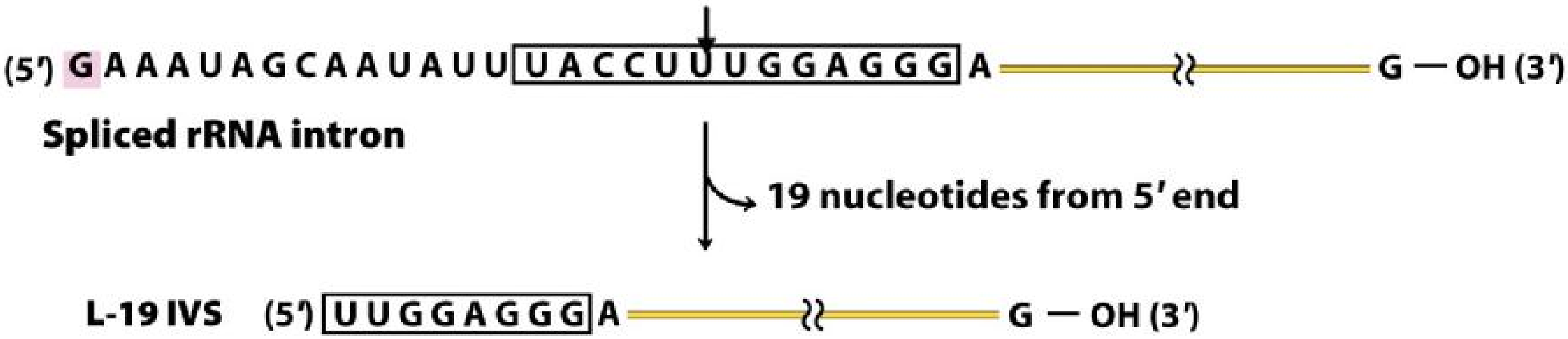

Figure 26-31a
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In vitro catalytic activity of L-19 IVS. (a) L-19 IVS is generated by the autocatalytic removal of 19 nucleotides from the 5′ end of the spliced *Tetrahymena* intron. The cleavage site is indicated by the arrow in the internal guide sequence (boxed). The G residue (shaded pink) added in the first step of the splicing reaction is part of the removed sequence. A portion of the internal guide sequence remains at the 5′ end of L-19 IVS.

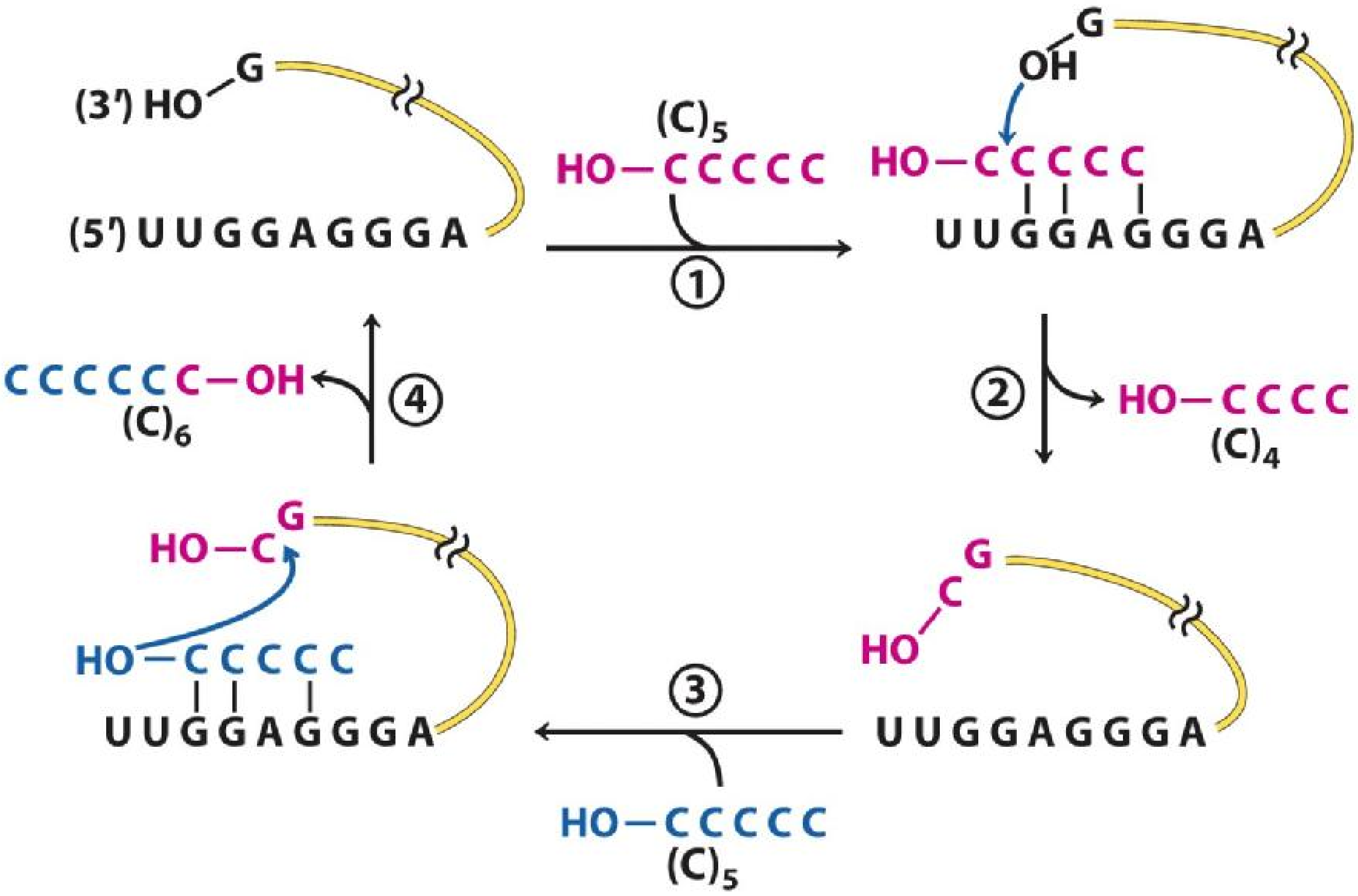

Figure 26-31b
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In vitro catalytic activity of L-19 IVS

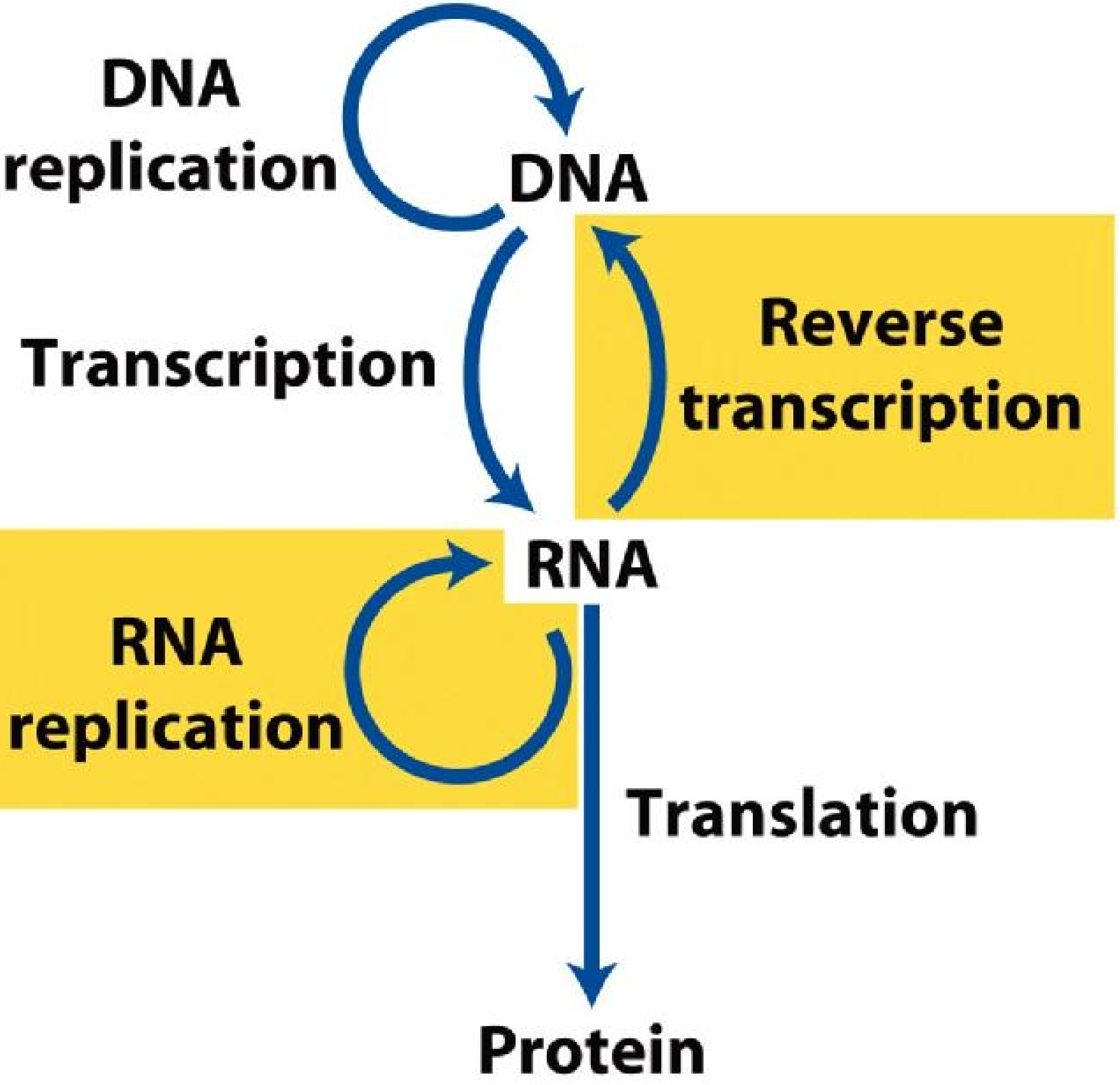

Figure 26-32
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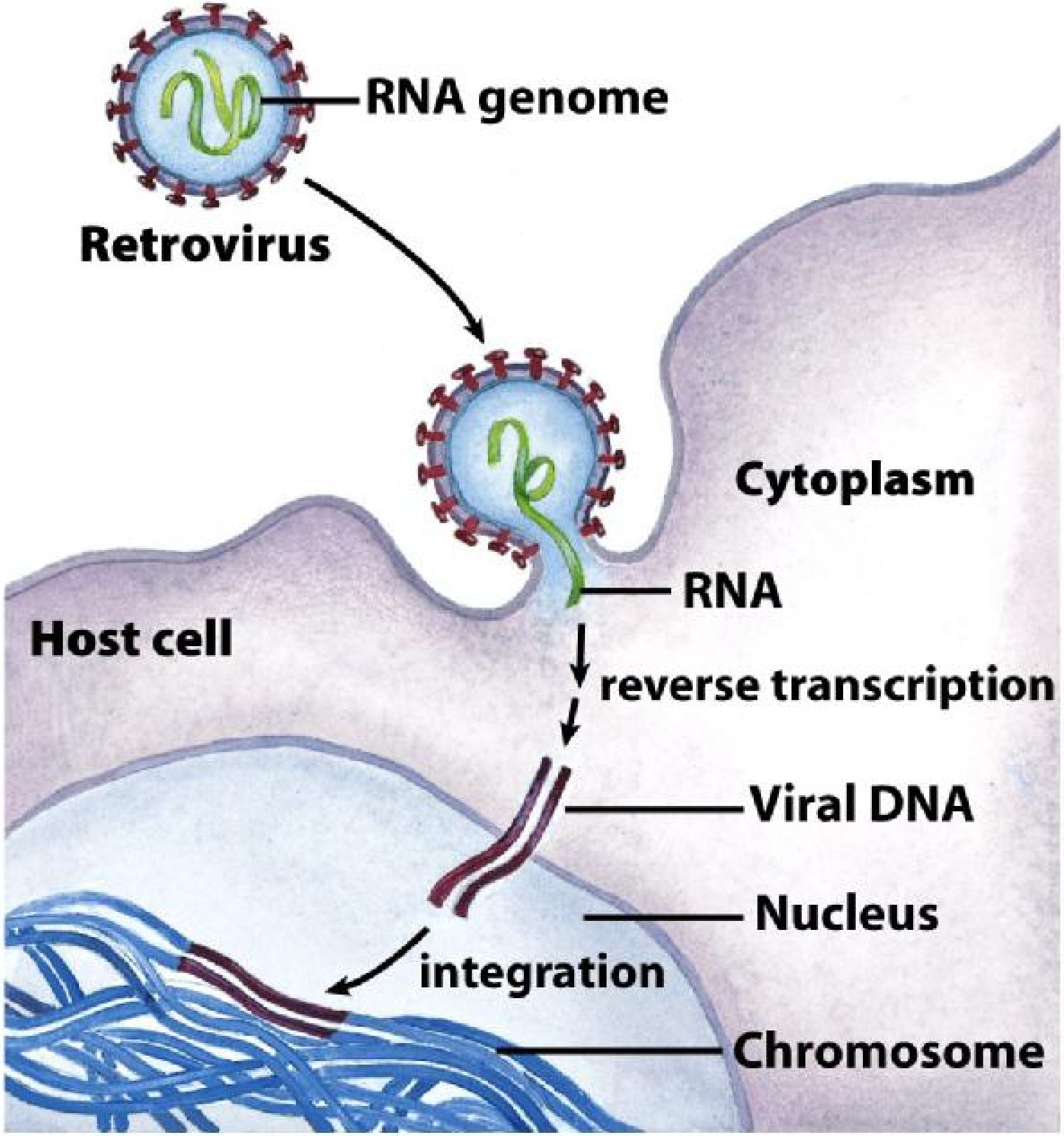

Figure 26-33
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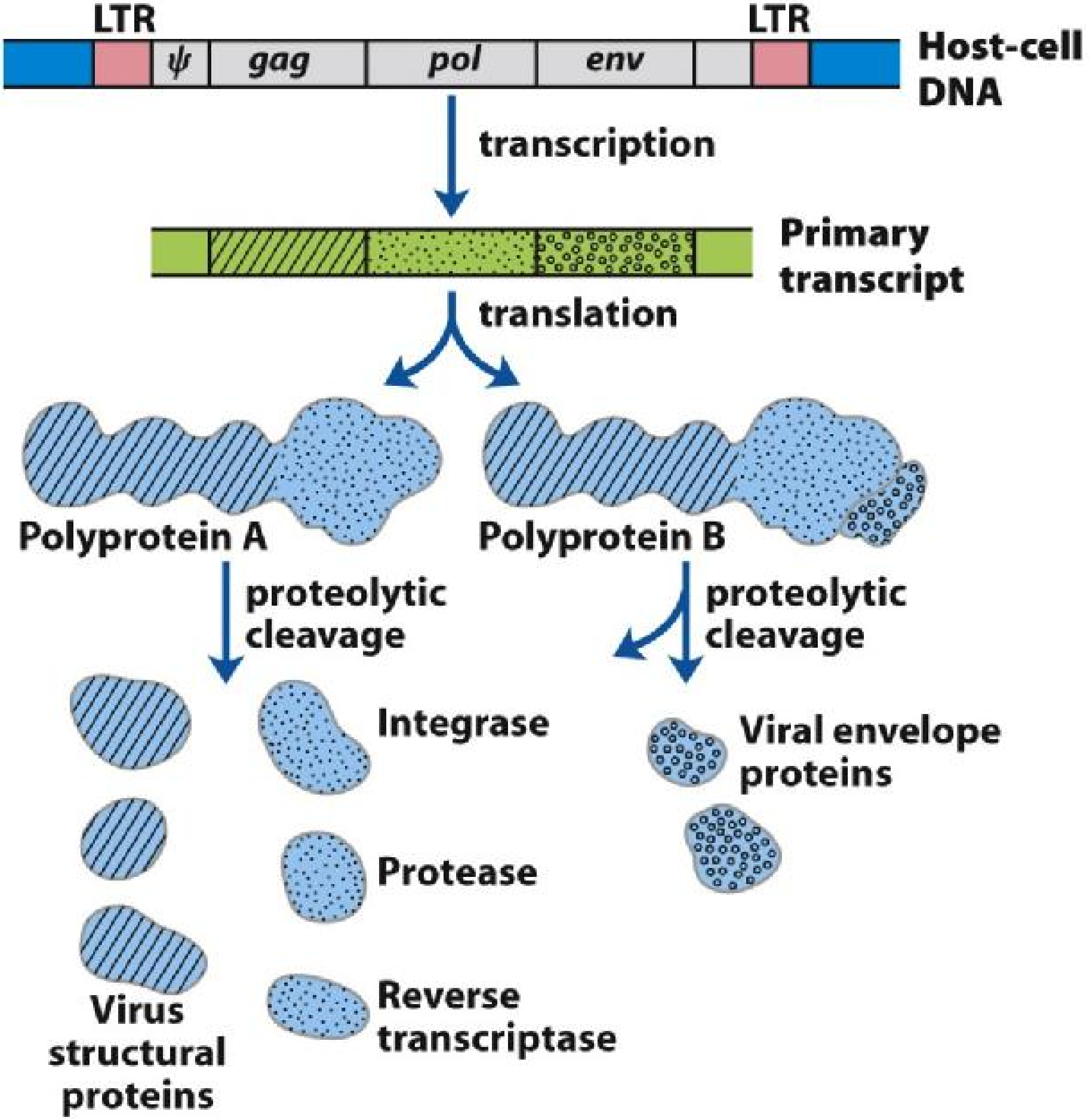

Figure 26-34
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Figure 26-35

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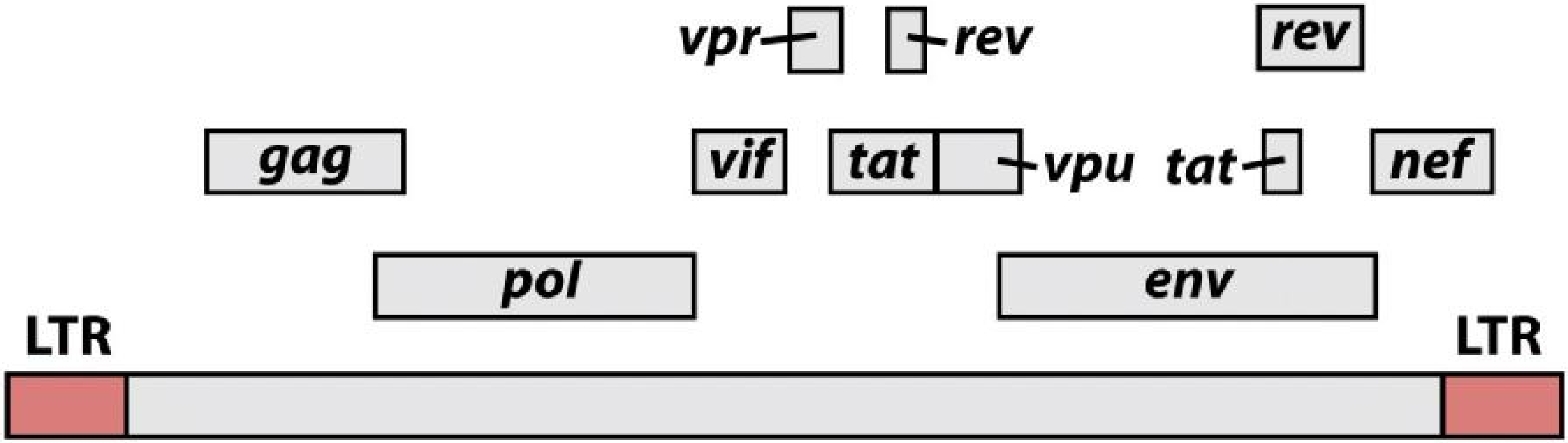

Figure 26-36

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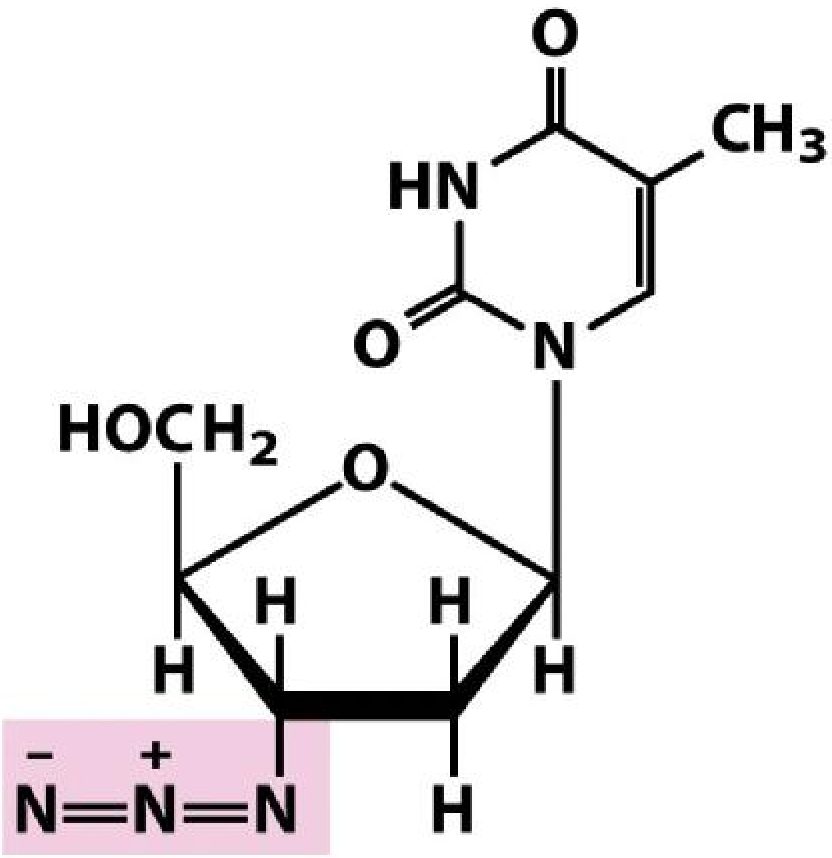
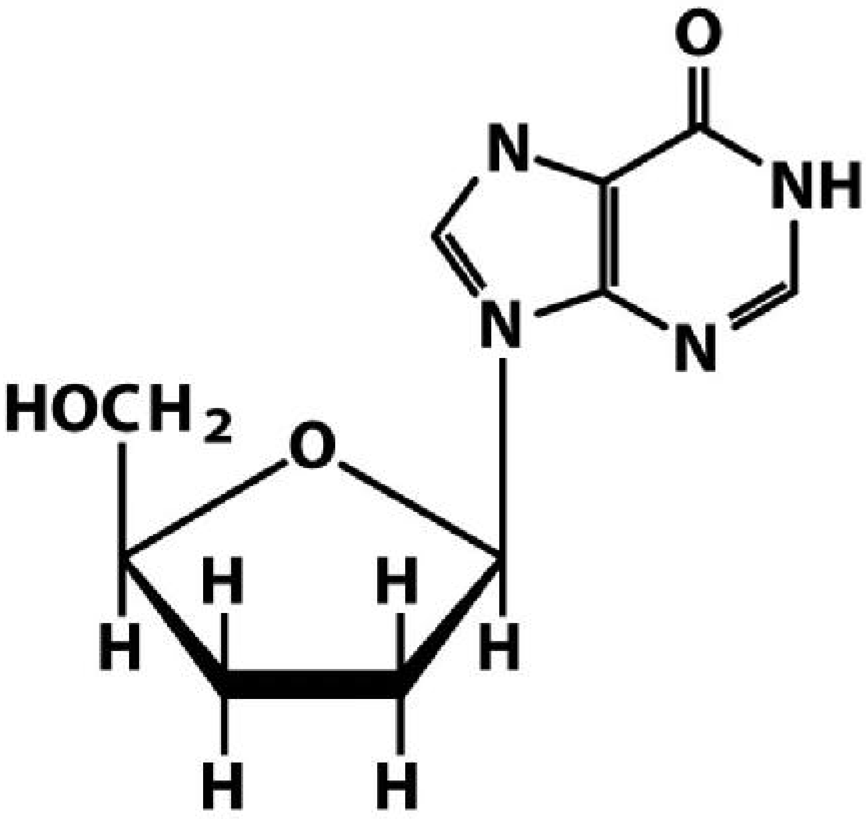


3′-Azido-2′,3′-dideoxy-thymidine (AZT)



2′,3′-Dideoxyinosine (DDI)

Box 26-2

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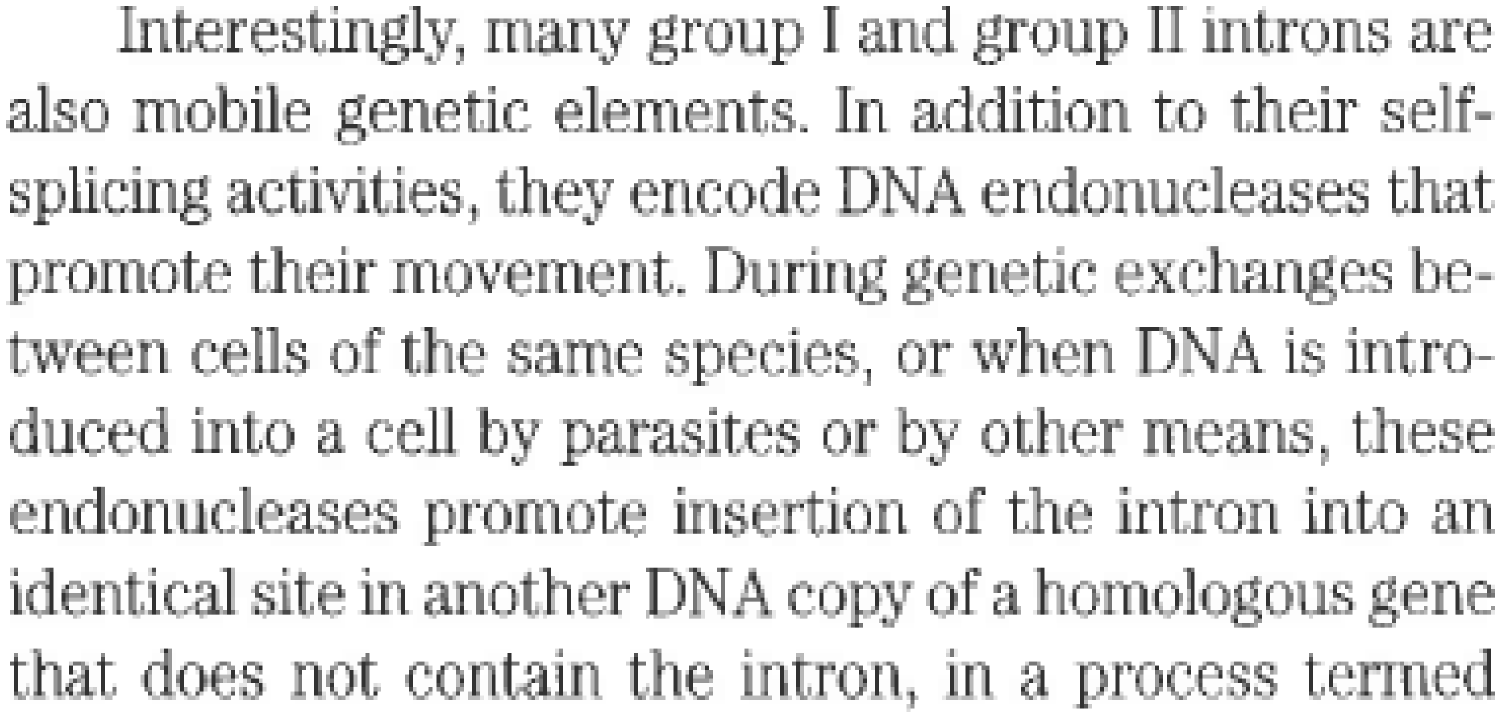
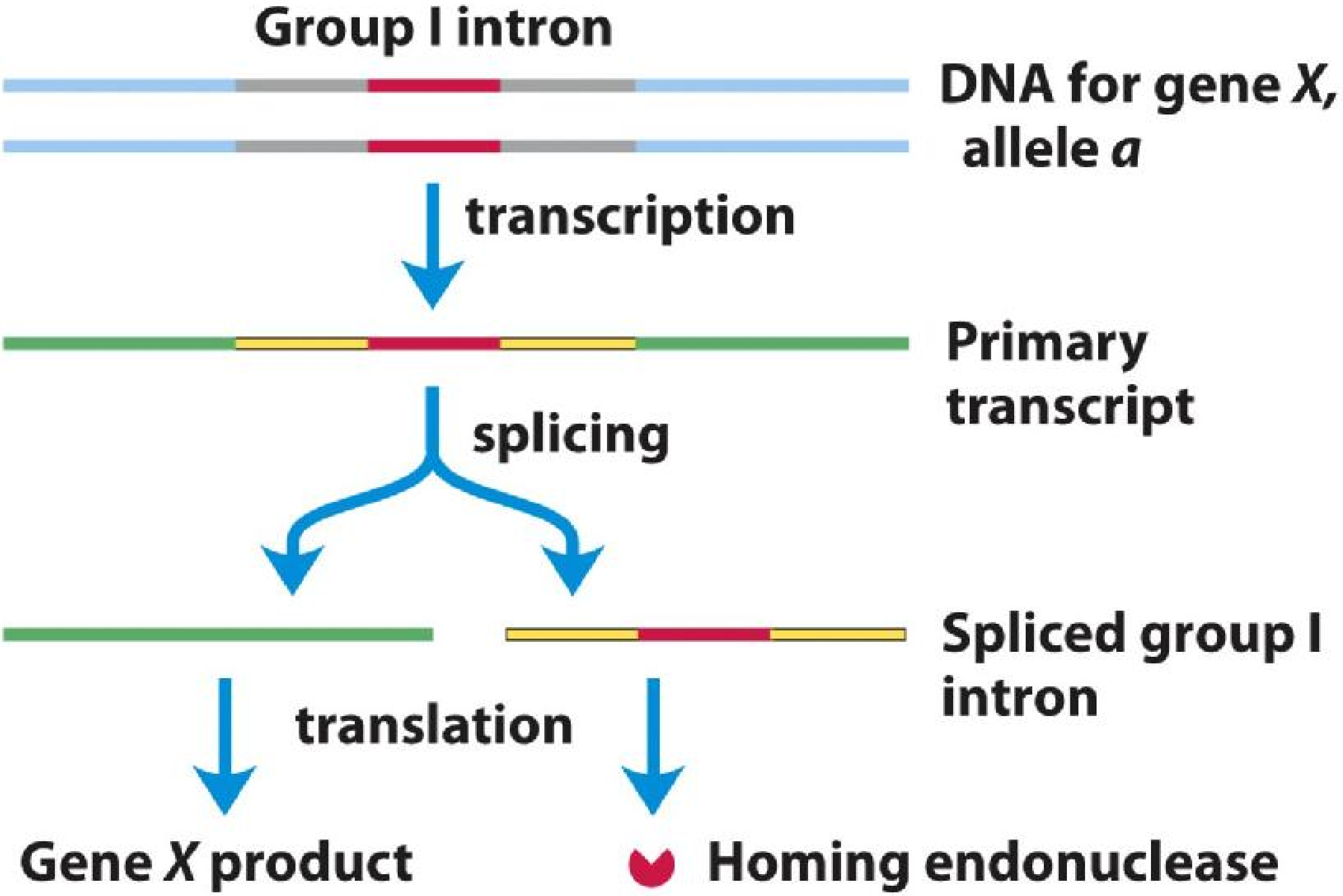


Interestingly, many group I and group II introns are also mobile genetic elements. In addition to their self-splicing activities, they encode DNA endonucleases that promote their movement. During genetic exchanges between cells of the same species, or when DNA is introduced into a cell by parasites or by other means, these endonucleases promote insertion of the intron into an identical site in another DNA copy of a homologous gene that does not contain the intron, in a process termed

HOMING

Production of homing endonuclease



Group I intron

DNA for gene *X*,
allele *a*

transcription

Primary
transcript

splicing

Spliced group I
intron

translation

Gene *X* product

Homing endonuclease

Figure 26-38a
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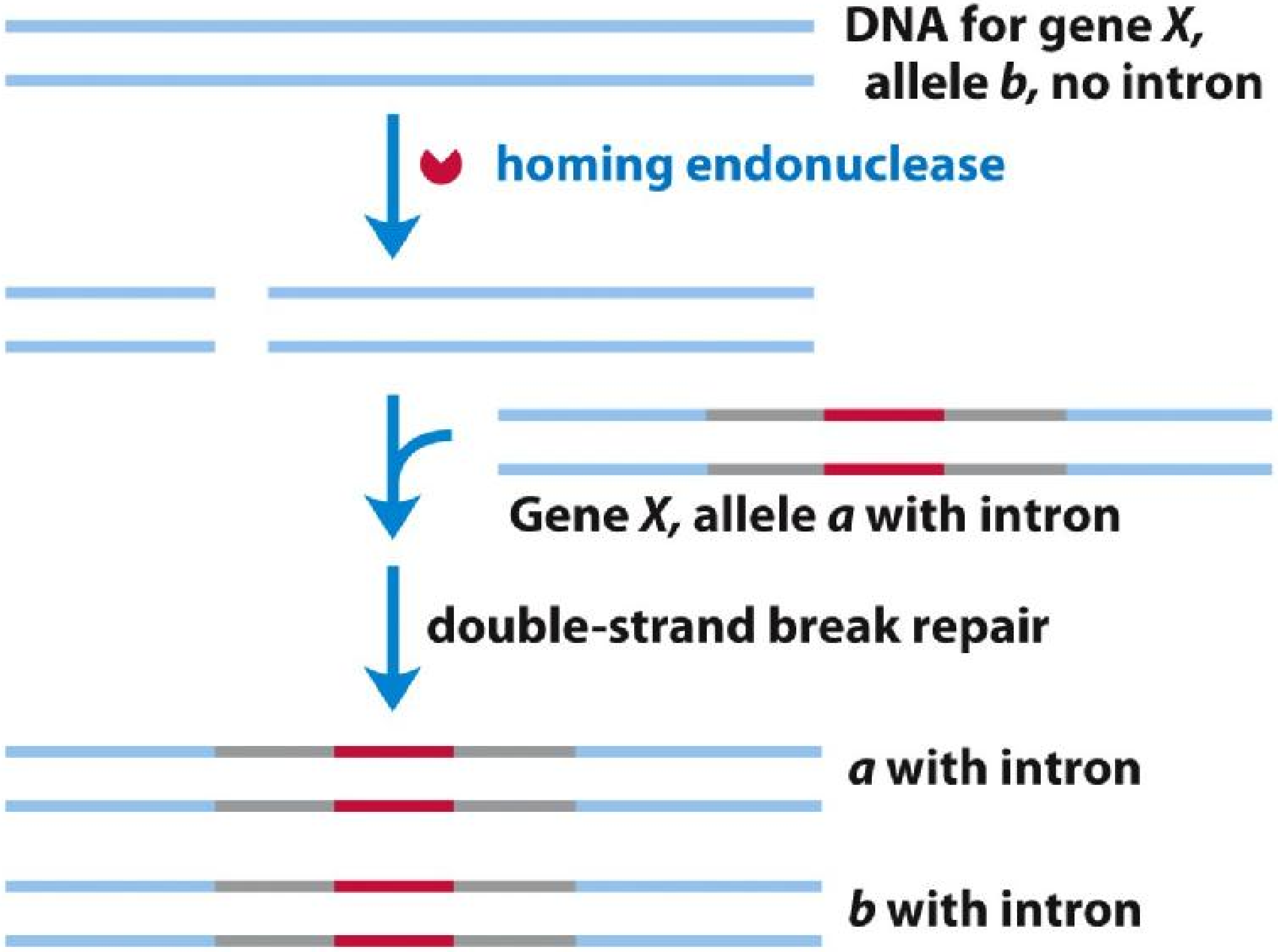
Homing



DNA for gene *X*, allele *b*, no intron

homing endonuclease

Gene *X*, allele *a* with intron

double-strand break repair

a with intron

b with intron

Figure 26-38b
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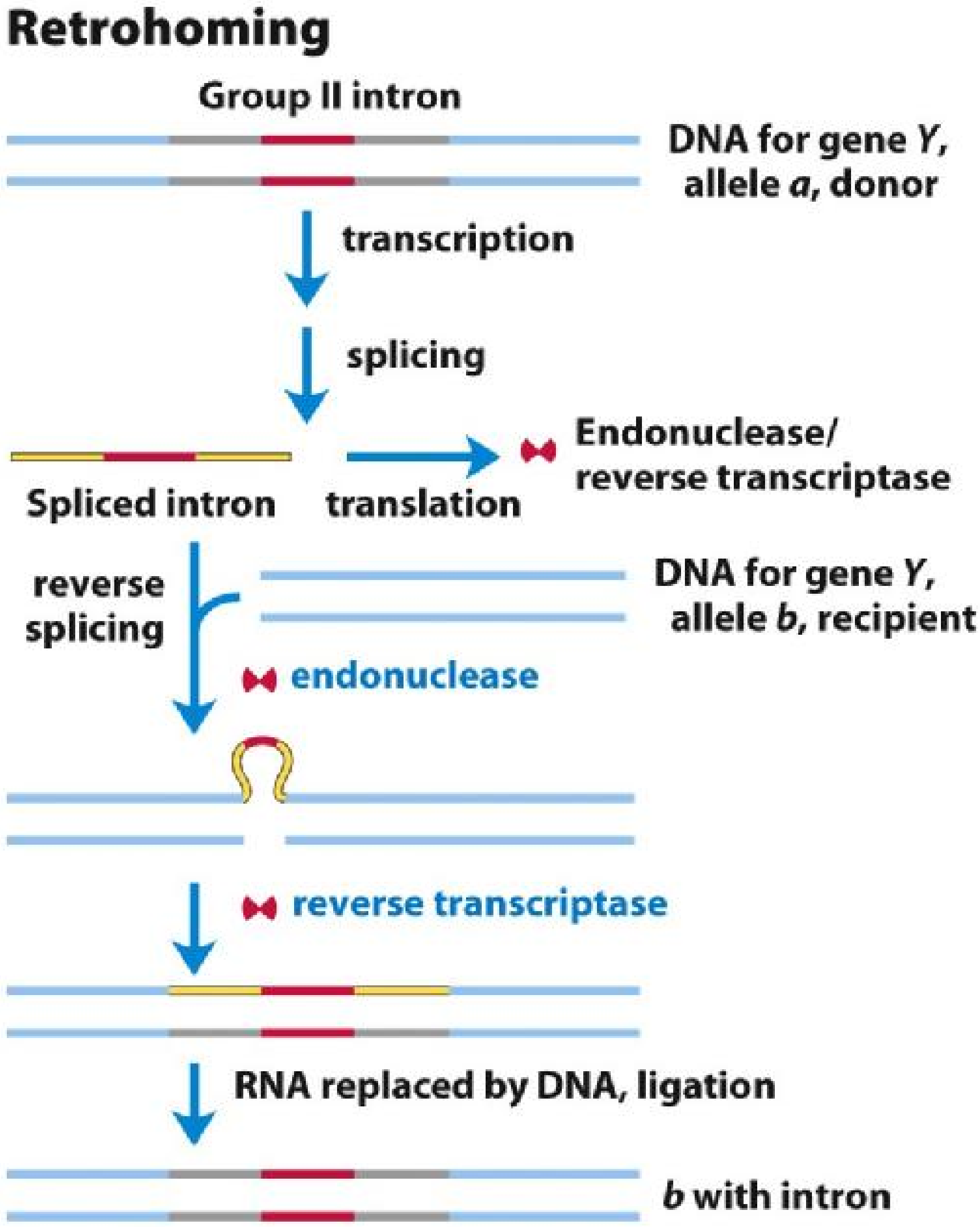

Figure 26-38c

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Cellular mrRNAs are degraded at different rates

The concentration of any molecule depends on two factors: its rate of synthesis and its rate of degradation.

The rates of degradation vary greatly for mRNAs
from different eukaryotic genes.
For a gene product that is needed only briefly, the half-life of its mRNA may be only minutes or even seconds.
Gene products needed constantly by the cell may have mRNAs that are stable over many cell generations.
The average half-life of the mRNAs of a vertebrate cell is about 3 hours, with the pool of each type of nRNA turning over about
10 times per cell generation.
The half-life of bacterial mRNAs is much shorter-only about 1.5 min-perhaps because of regulatory requirements

Messenger RNA is degraded by ribonucleases present in all cells

In *E. coli* the process begins with one or several cuts by an endoribonuclease followed b y 3‘ to 5'degradation by exoribonucleases.

In lower eukaryotes, the major pathway involves first shortening the poly(A) tail, then decapping the 5' end and degrading the mRNA in the 5‘ to 3' direction.

A 3‘ to 5' degradative pathway also exists and may be the major path in **higher eukaryotes**.

All eukaryotes have a complex of up to 10 conserved 3‘ to 5' exoribonucleases called the **exosome**.

Polynucleotide phosphorylase makes random RNA-like polymers

It is not template-dependent as RNA polymerases are

$$(\mathrm{NMP})_n + \mathrm{NDP} \rightleftharpoons \underset{\substack{\text{Lengthened}\\ \text{polynucleotide}}}{(\mathrm{NMP})_{n+1}} + \mathrm{P}_i$$