Prokaryotic Transcription

HHMI

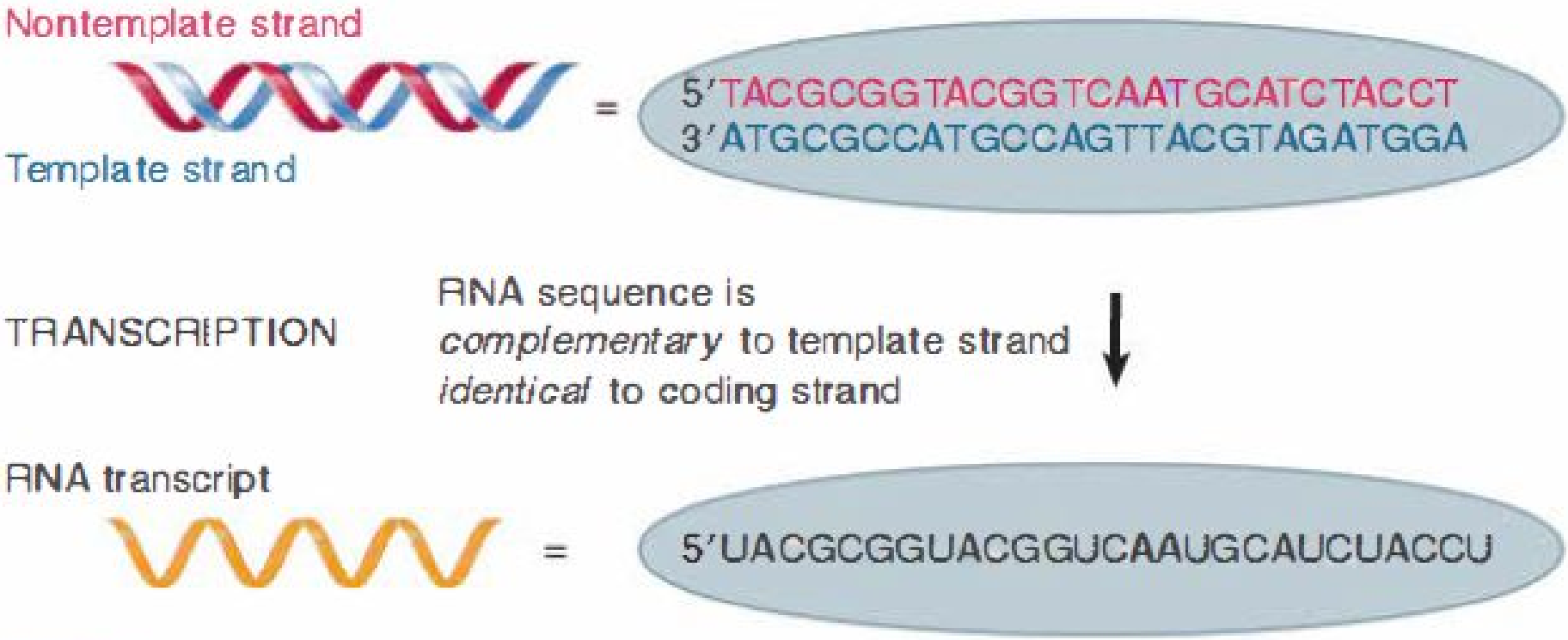

FIGURE 19.1 The function of RNA polymerase is to copy one strand of duplex DNA into RNA.

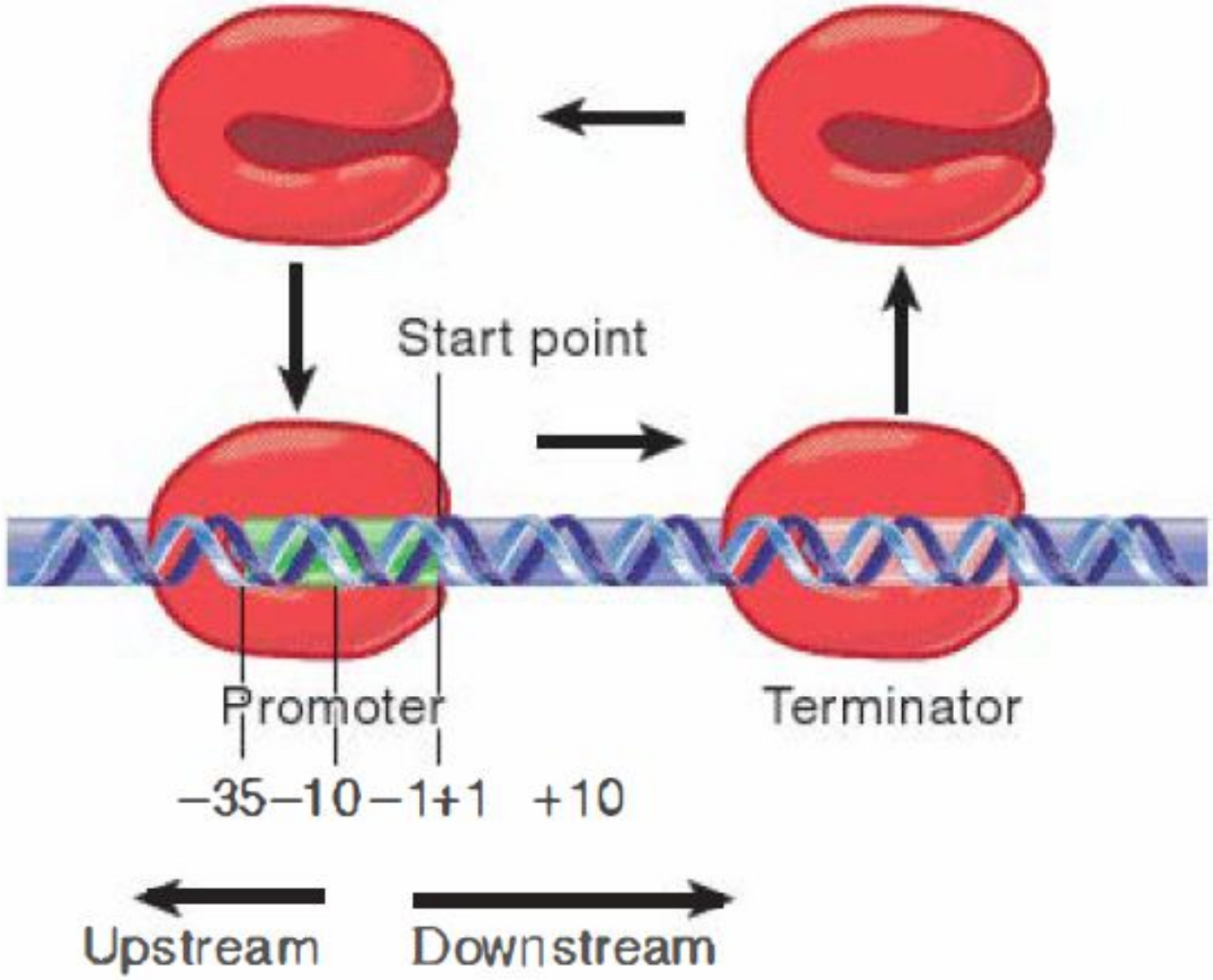

FIGURE 19.2 A transcription unit is a sequence of DNA transcribed into a single RNA, starting at the promoter and ending at the terminator.

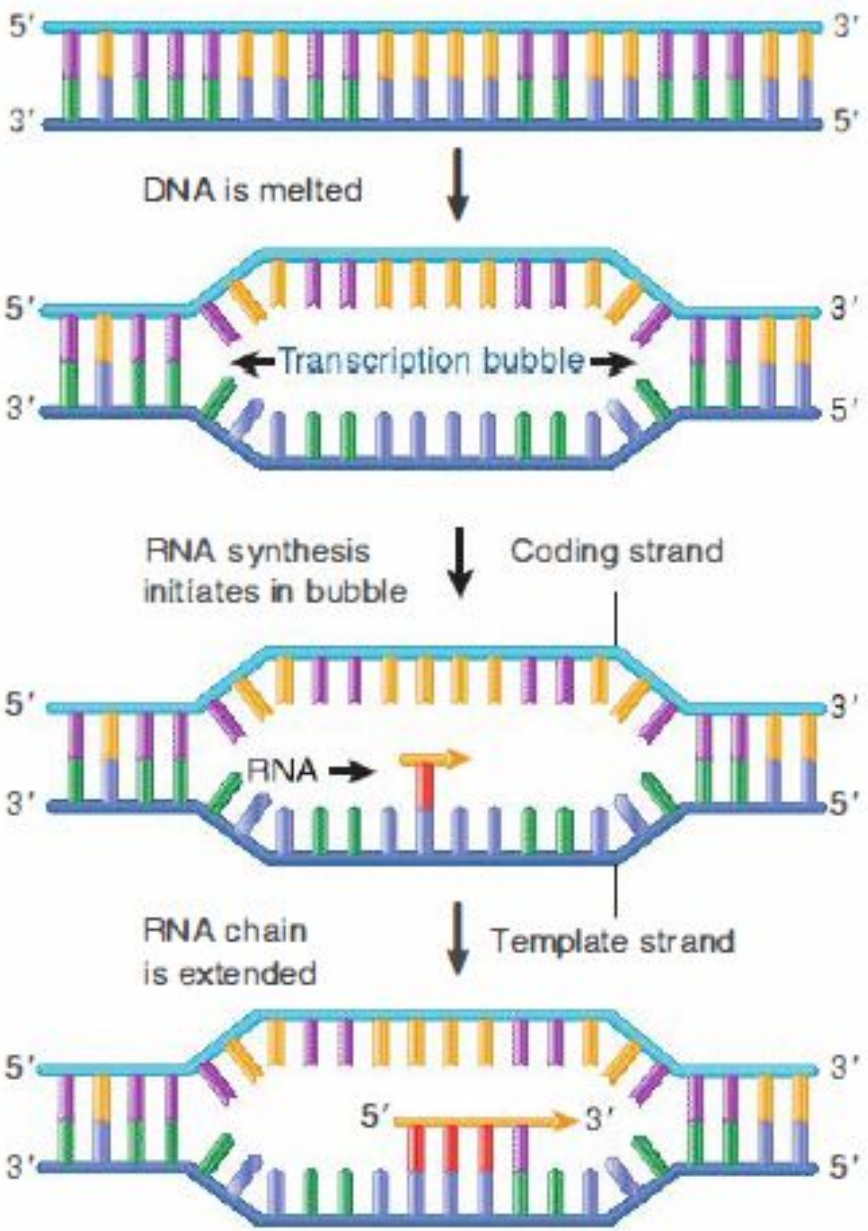

FIGURE 19.3 DNA strands separate to form a transcription bubble. RNA is synthesized by complementary base pairing with one of the DNA strands.

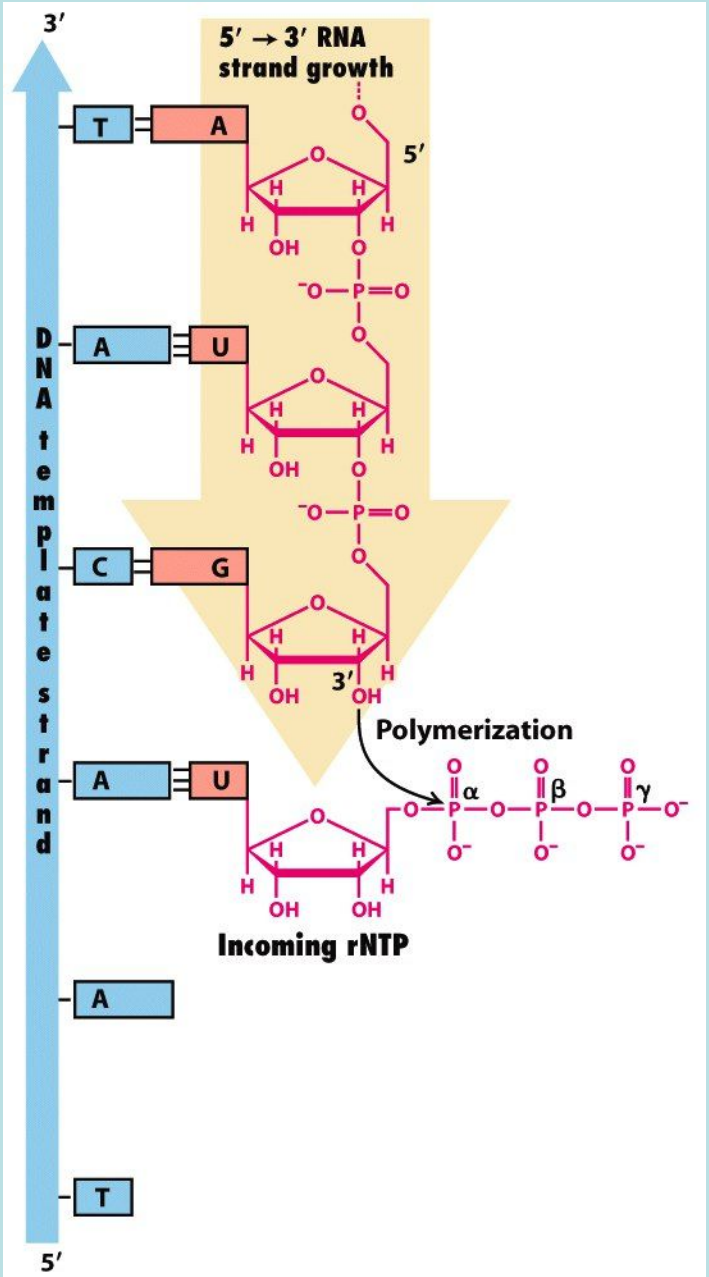

Figure 4-10a
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Reaction rate for the bacterial RNA polymerase: ~ 40-50 nuc/sec at 37˚C (15 amino acids/sec)

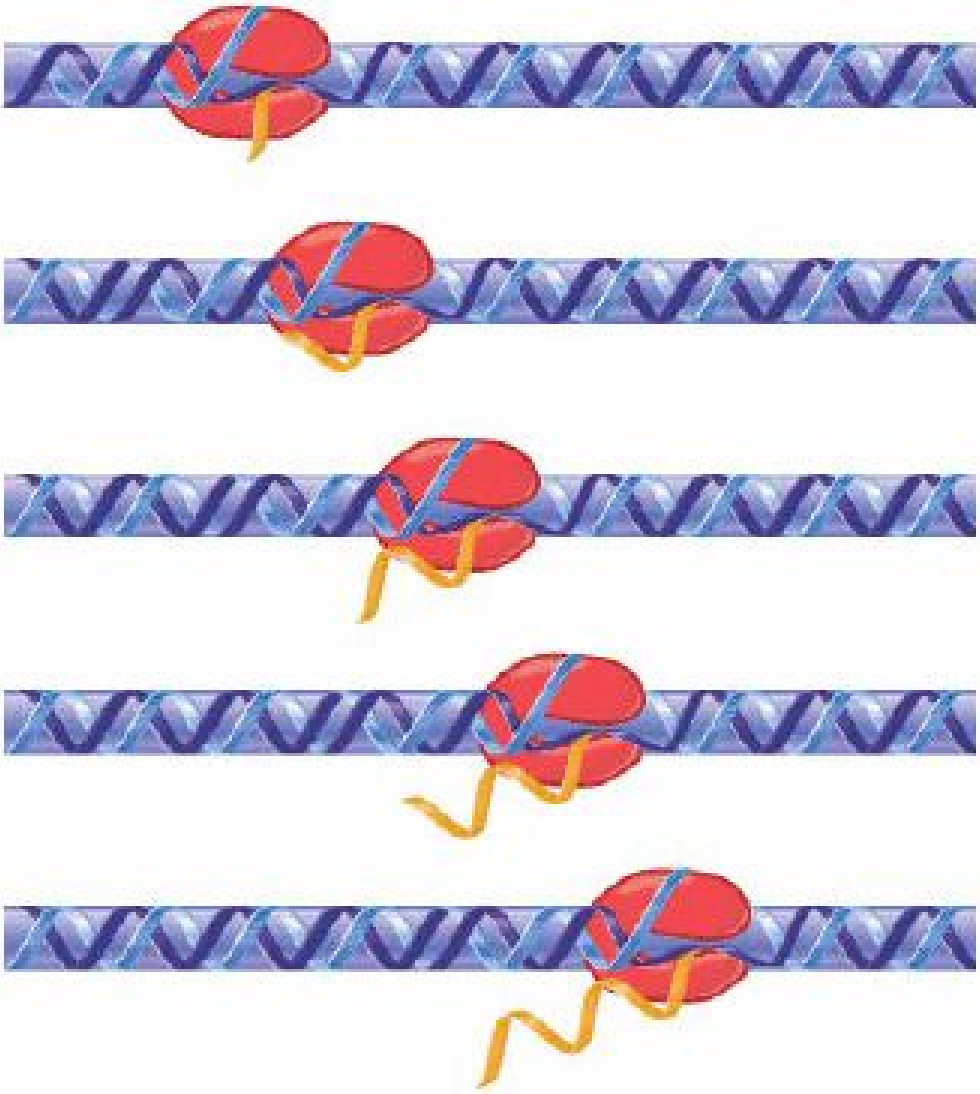

FIGURE 19.4 Transcription takes place in a bubble, in which RNA is synthesized by base pairing with one strand of DNA in the transiently unwound region. As the bubble progresses, the DNA duplex re-forms behind it, displacing the RNA in the form of a single polynucleotide chain.

The length of the transcription bubble is 12 to 14 bp, but the length of the RNA-DNA hybrid within the bubble is only 8 to 9 bp.

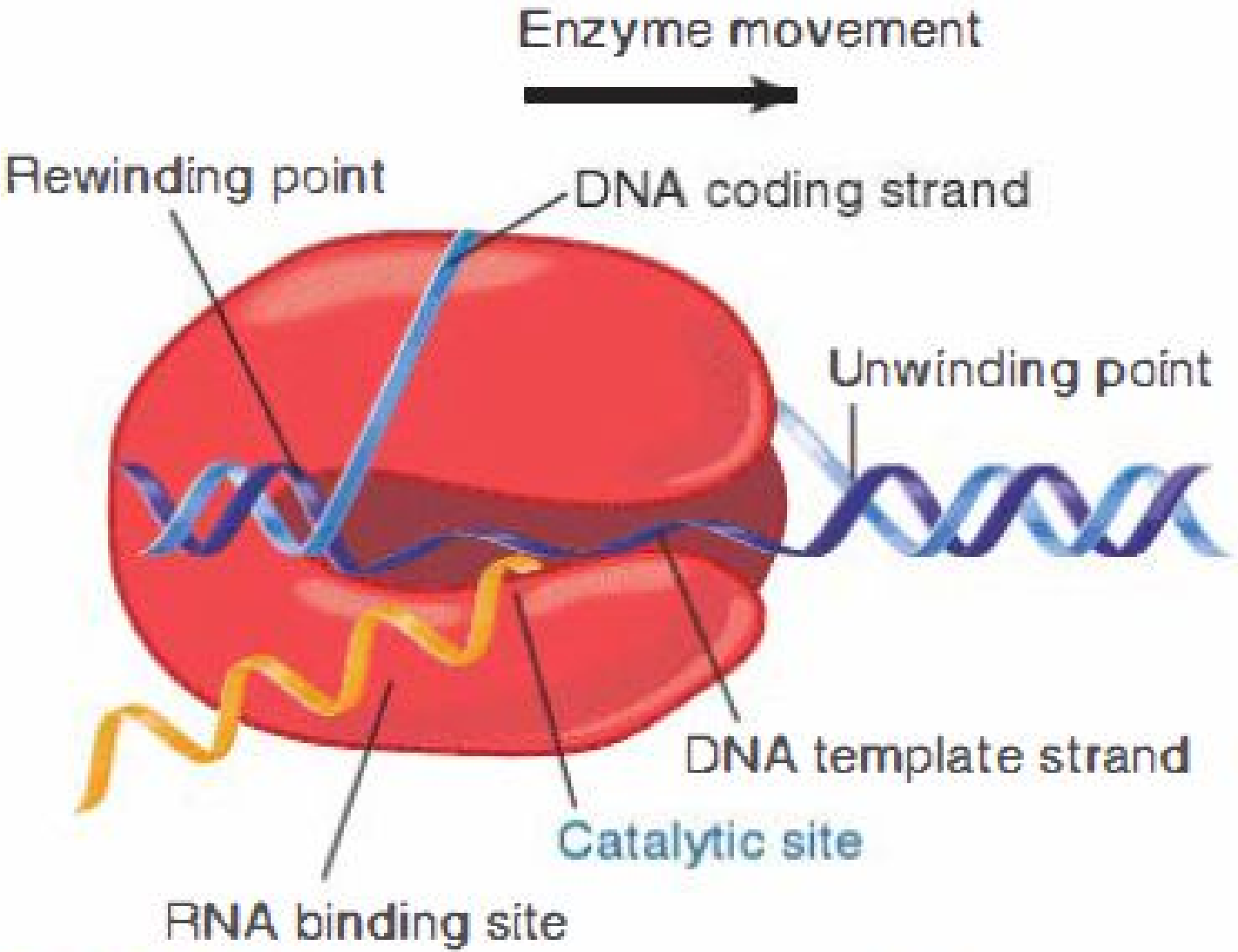

FIGURE 19.5 During transcription, the bubble is maintained within bacterial RNA polymerase, which unwinds and rewinds DNA and synthesizes RNA.

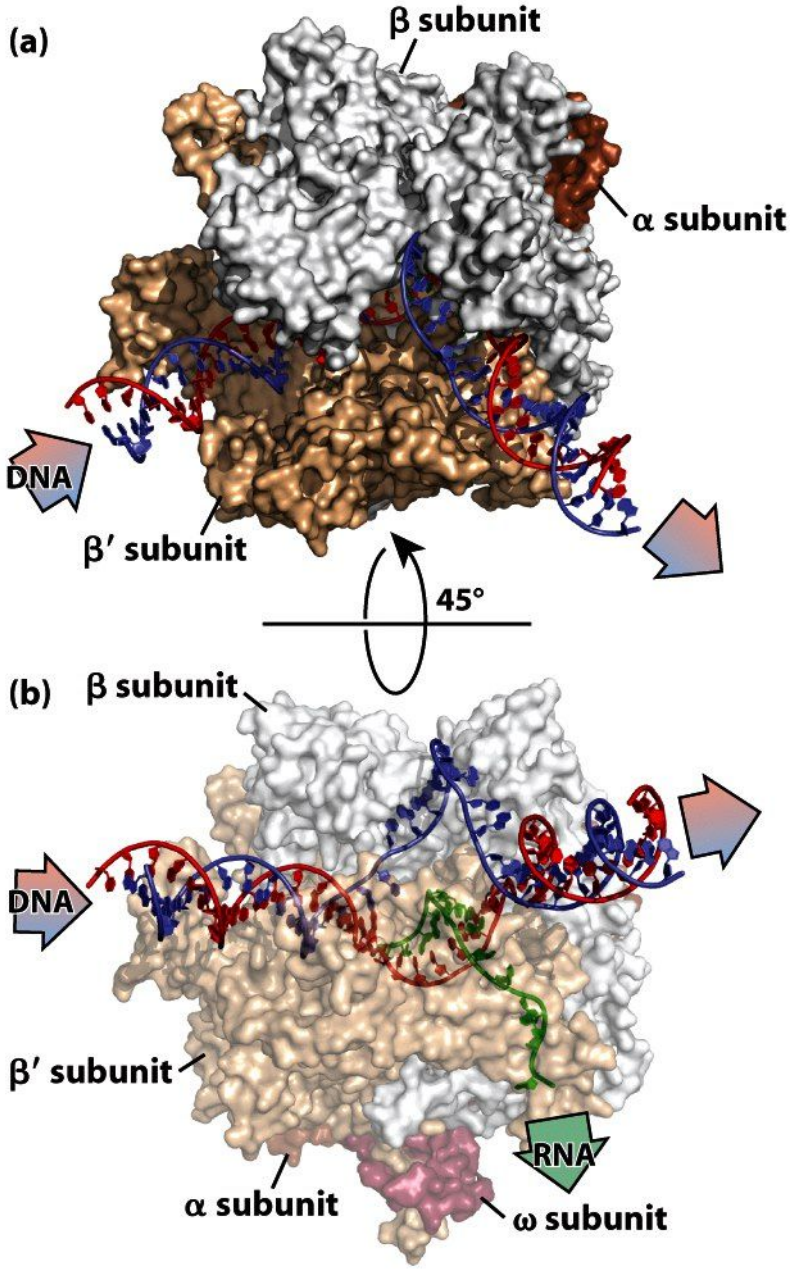

Figure 4-12
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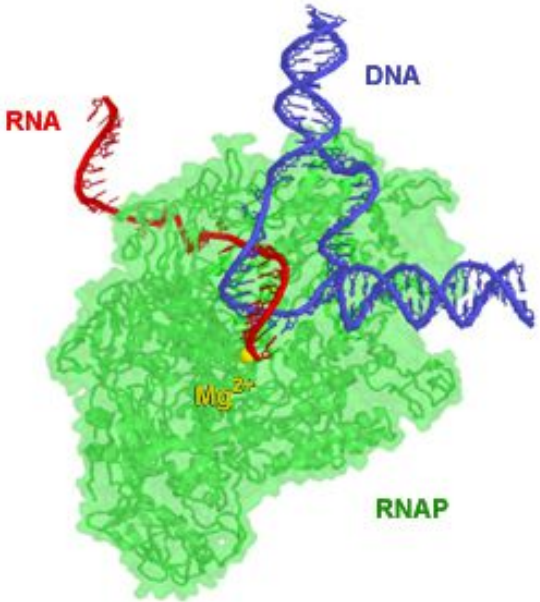

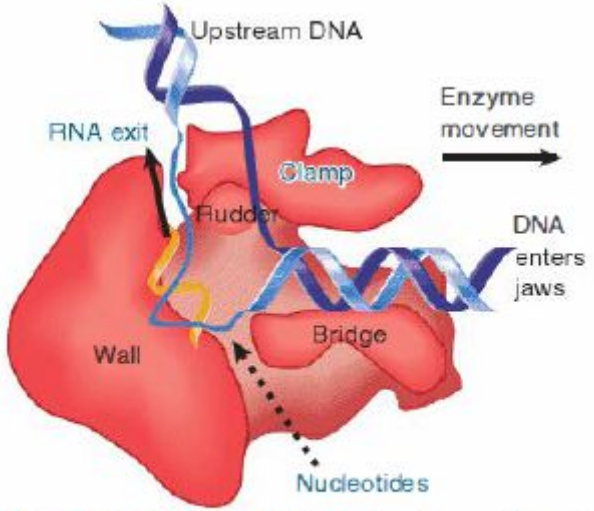

FIGURE 19.26 DNA is forced to make a turn at the active site by a wall of protein. Nucleotides may enter the active site through a pore in the protein.

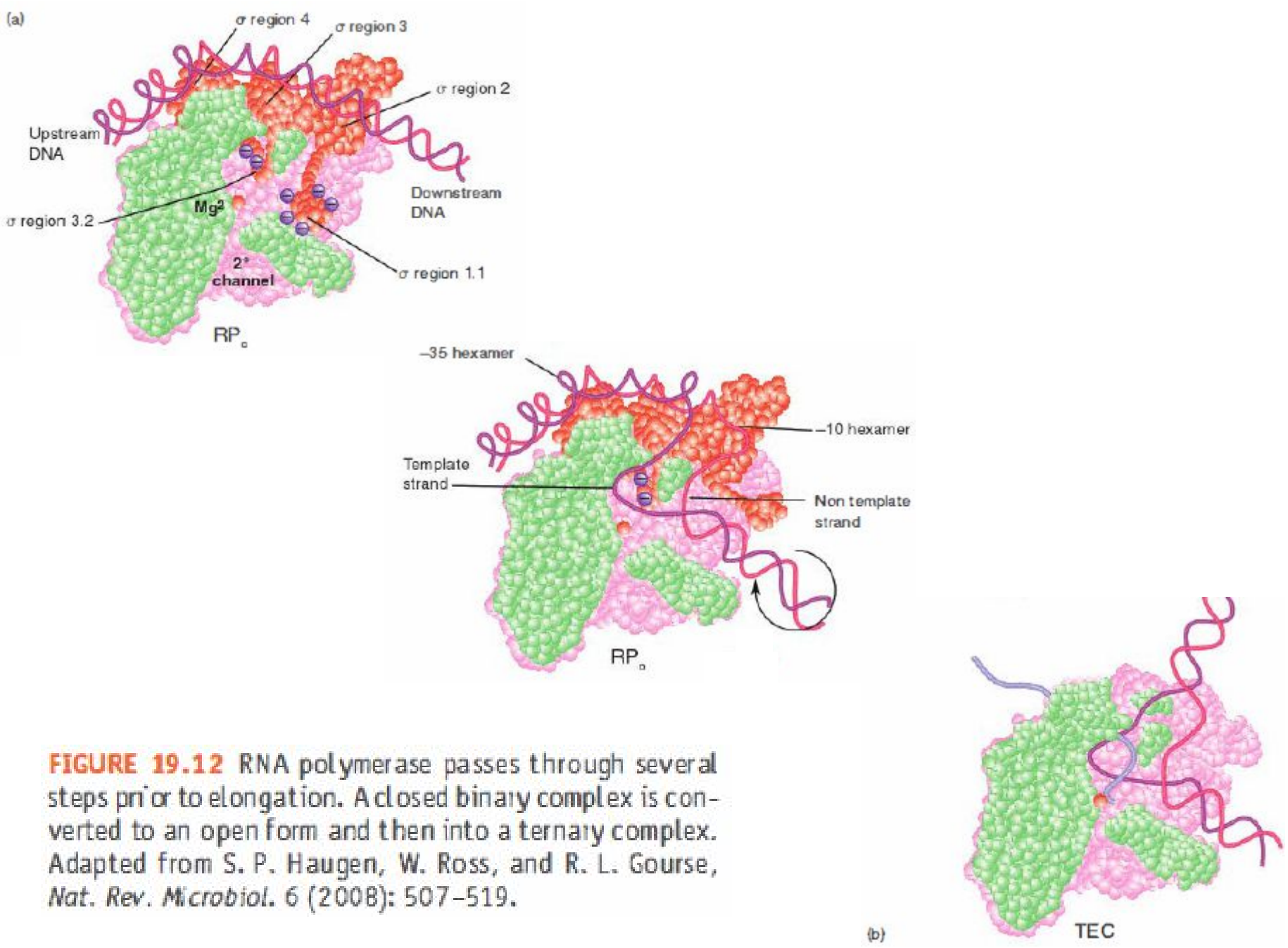

FIGURE 19.12 RNA polymerase passes through several steps prior to elongation. A closed binary complex is converted to an open form and then into a ternary complex. Adapted from S. P. Haugen, W. Ross, and R. L. Gourse, *Nat. Rev. Microbiol.* 6 (2008): 507–519.

E. coli RNA polymerase

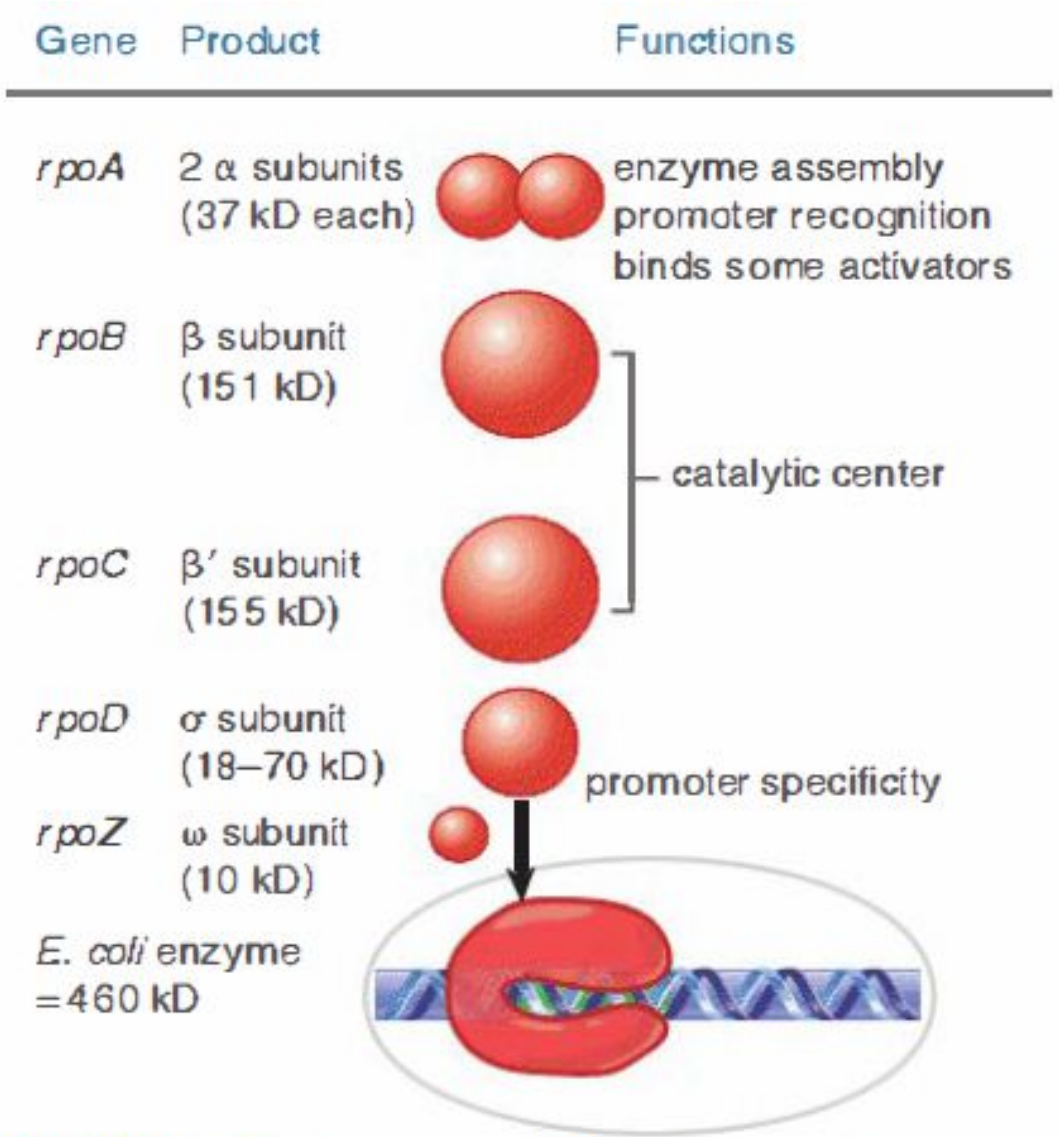

FIGURE 19.7 Eubacterial RNA polymerases have five types of subunits: α, β, β′, and ω have rather constant sizes in different bacterial species, but σ varies more widely.

Nucleus

$\alpha_2\beta\beta'\sigma\omega$

Holoenzyme

Transcription can be divided into four phases:

1. Promoter recognition
2. Transcription start
3. Elongation
4. Termination

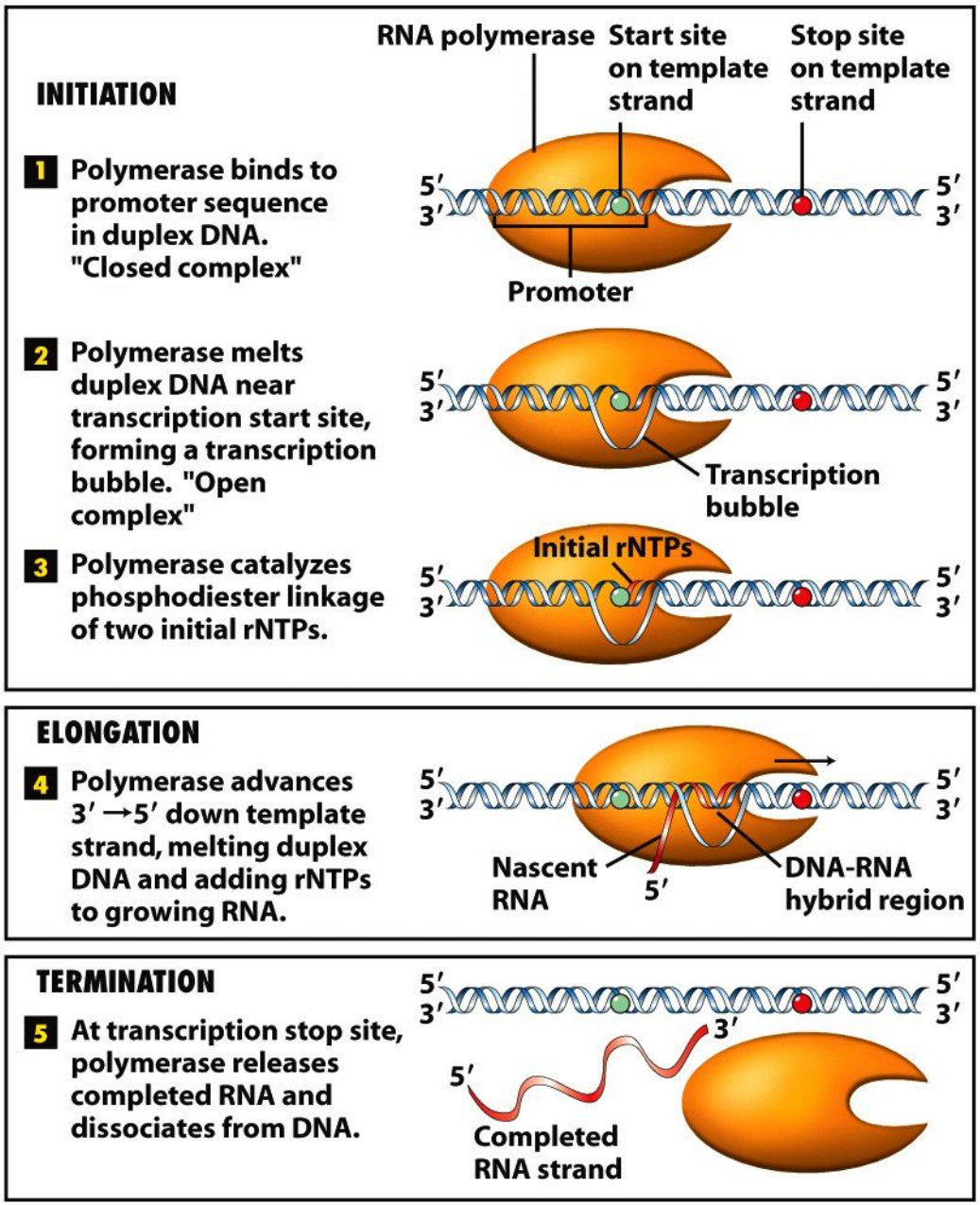

Figure 4-11
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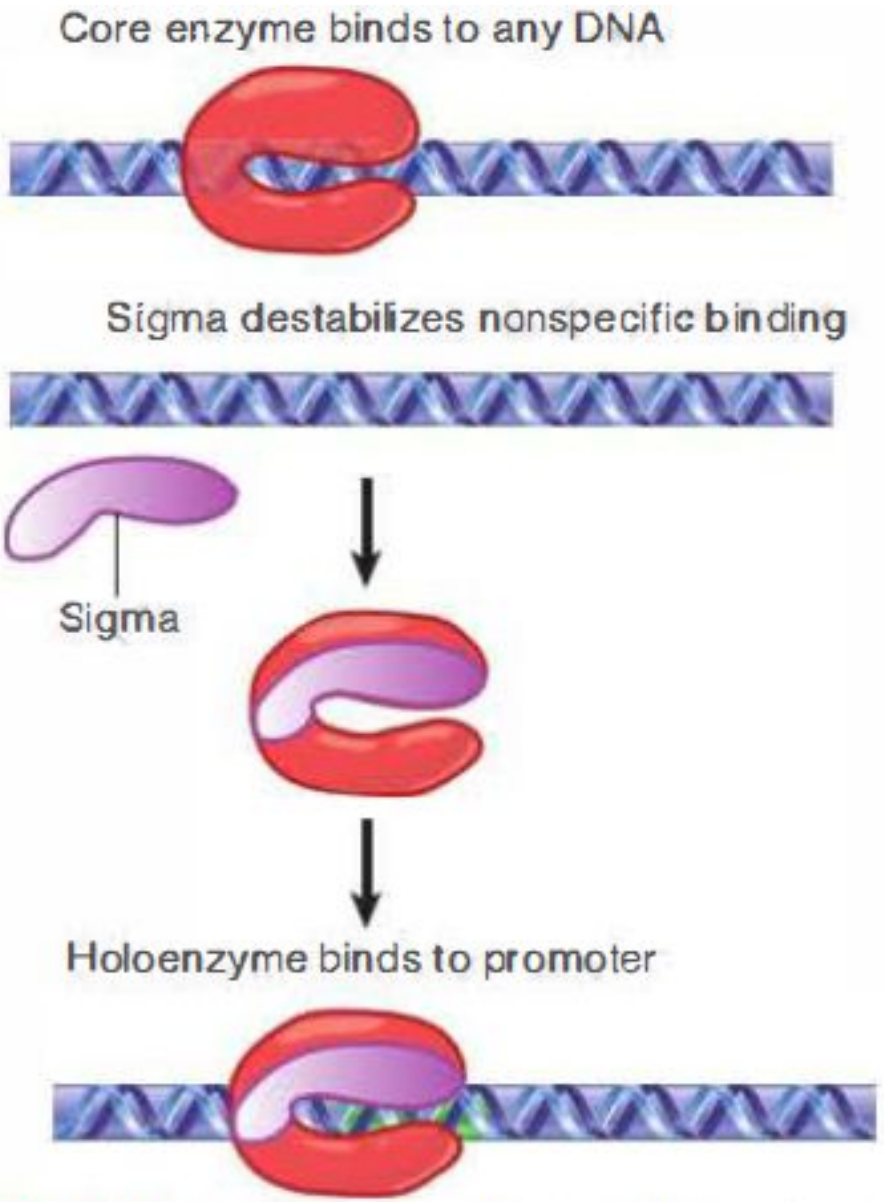


FIGURE 19.10 Core enzyme binds indiscriminately to any DNA. Sigma factor reduces the affinity for sequence-independent binding and confers specificity for promoters.

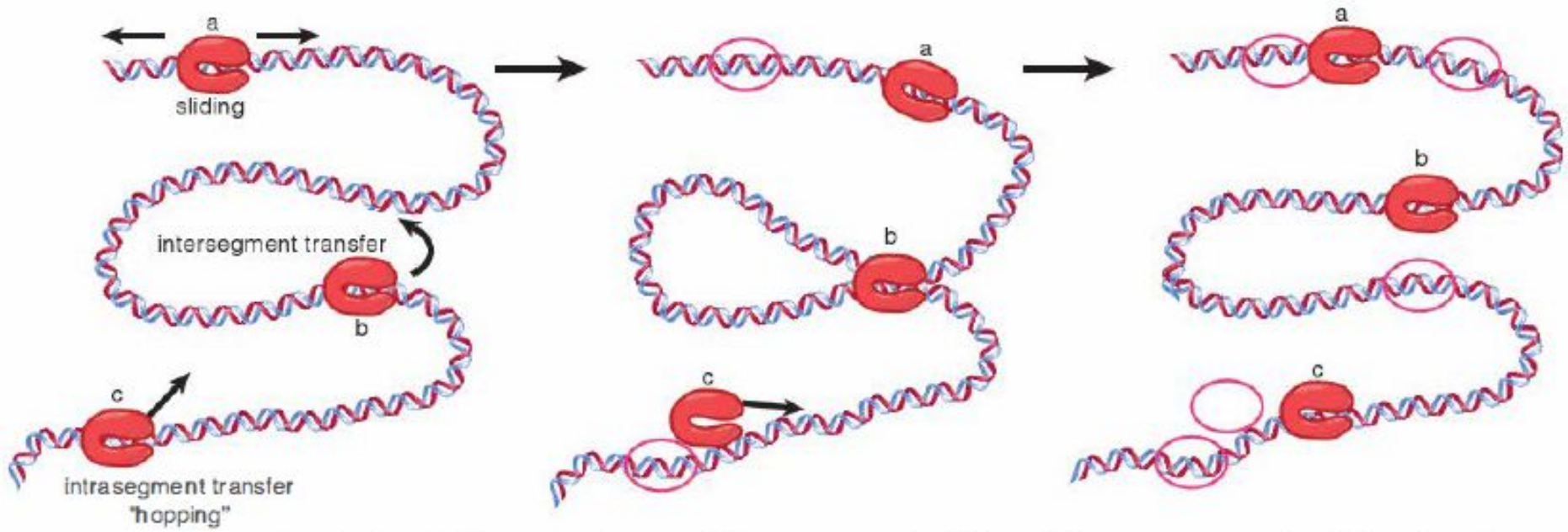


FIGURE 19.11 Proposed mechanisms for how RNA polymerase finds a promoter: (a) sliding, (b) intersegment transfer, (c) intradomain association and dissociation or hopping. Adapted from C. Bustamante, et al., *J. Biol. Chem.* 274 (1999): 16665–16668.

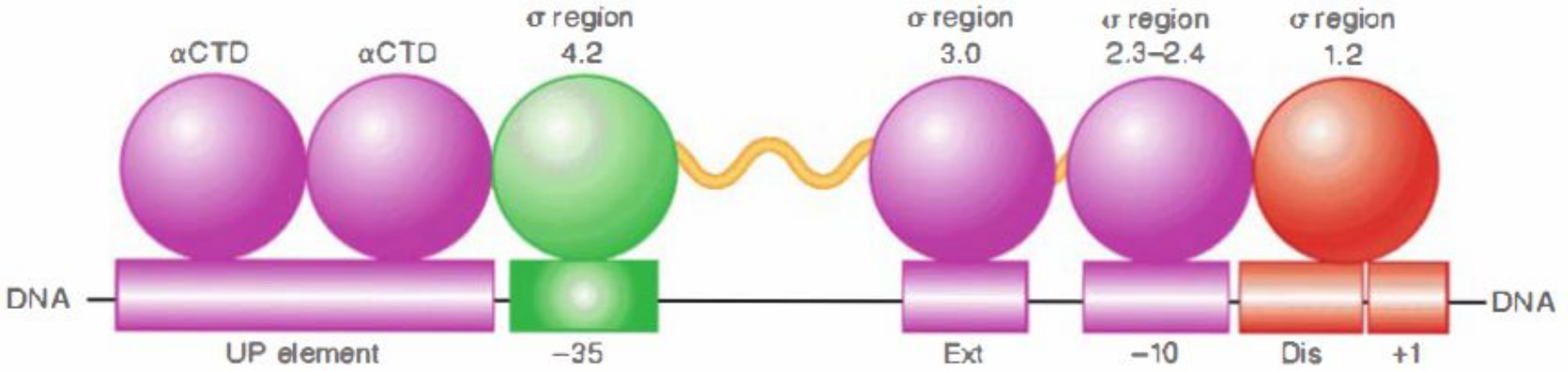


FIGURE 19.14 DNA elements and RNA polymerase modules that contribute to promoter recognition by sigma factor. Adapted from S. P. Haugen, W. Ross, and R. L. Gourse, *Nat. Rev. Microbiol.* 6 (2008): 507–519.

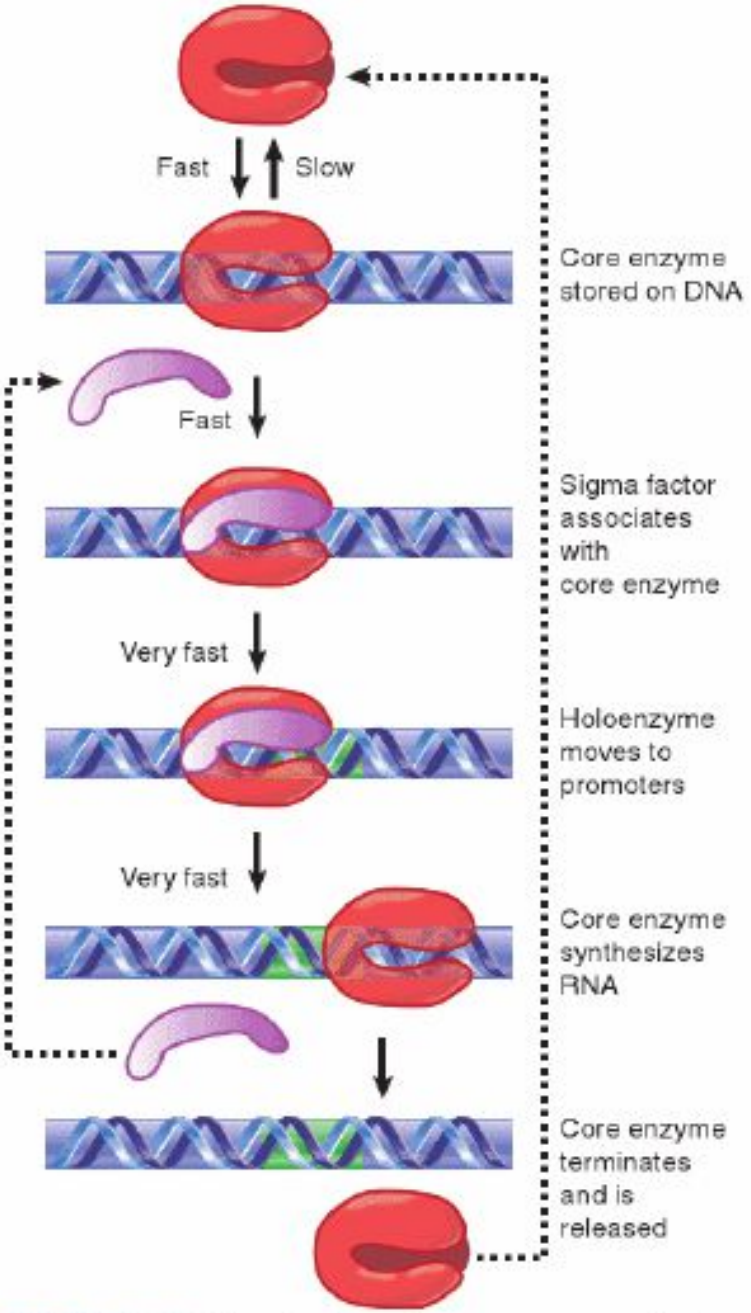
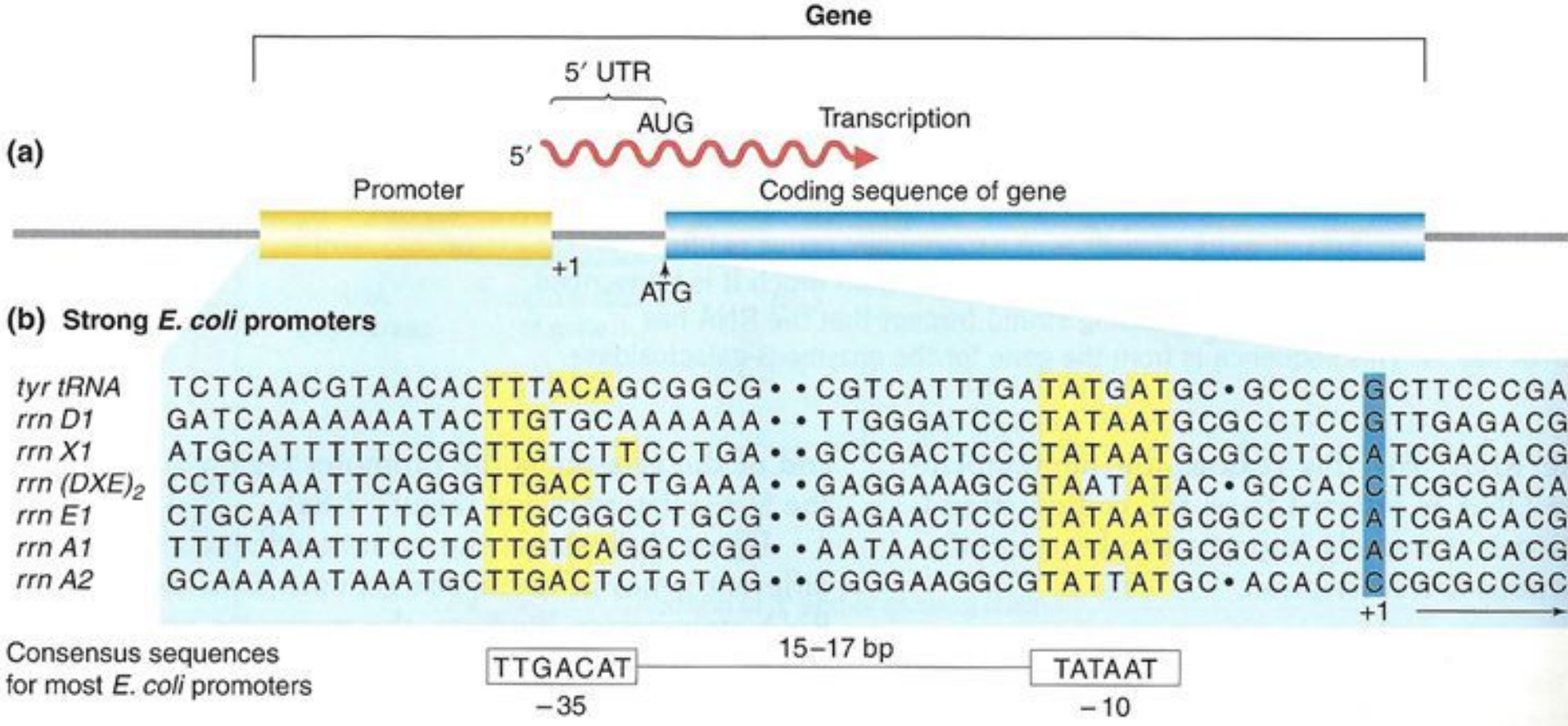


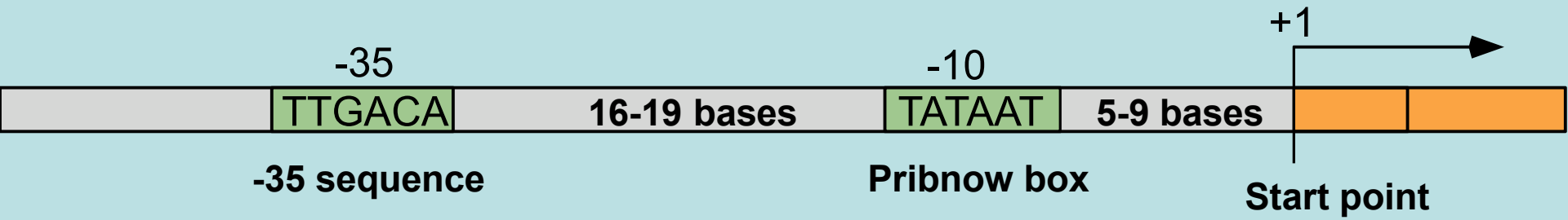
FIGURE 19.24 Sigma factor and core enzyme recycle at different points in transcription.

Prokaryotic promoters

- A promoter is defined by the presence of short consensus sequences at specific locations.
- The promoter consensus sequences usually consist of a purine at the start point, a hexamer with a sequence close to TAT AAT centered at ~-10, and another hexamer with a sequence similar to TTGACA centered at ~-35.


Gene
5′ UTR
AUG
Transcription
(a)
5′
Promoter
Coding sequence of gene
+1
ATG
(b) Strong E. coli promoters
tyr tRNA TCTCAACGTAACACTTTACAGCGGCG••CGTCATTTGATATGATGC•GCCCCGCTTCCCGA
rrn D1 GATCAAAAAAATACTTGTGCAAAAAA••TTGGGATCCCTATAATGCGCCTCCGTTGAGACG
rrn X1 ATGCATTTTTCCGCTTGTCTTCCTGA••GCCGACTCCCTATAATGCGCCTCCATCGACACG
rrn (DXE)2 CCTGAAATTCAGGGTTGACTCTGAAA••GAGGAAAGCGTAATATAC•GCCACCTCGCGACA
rrn E1 CTGCAATTTTTCTATTGCGGCCTGCG••GAGAACTCCCTATAATGCGCCTCCATCGACACG
rrn A1 TTTTAAATTTCCTCTTGTCAGGCCGG••AATAACTCCCTATAATGCGCCACCACTGACACG
rrn A2 GCAAAAATAAATGCTTGACTCTGTAG••CGGGAAGGCGTATTATGC•ACACCCCGCGCCGC
+1
Consensus sequences for most E. coli promoters
TTGACAT
15–17 bp
TATAAT
−35
−10

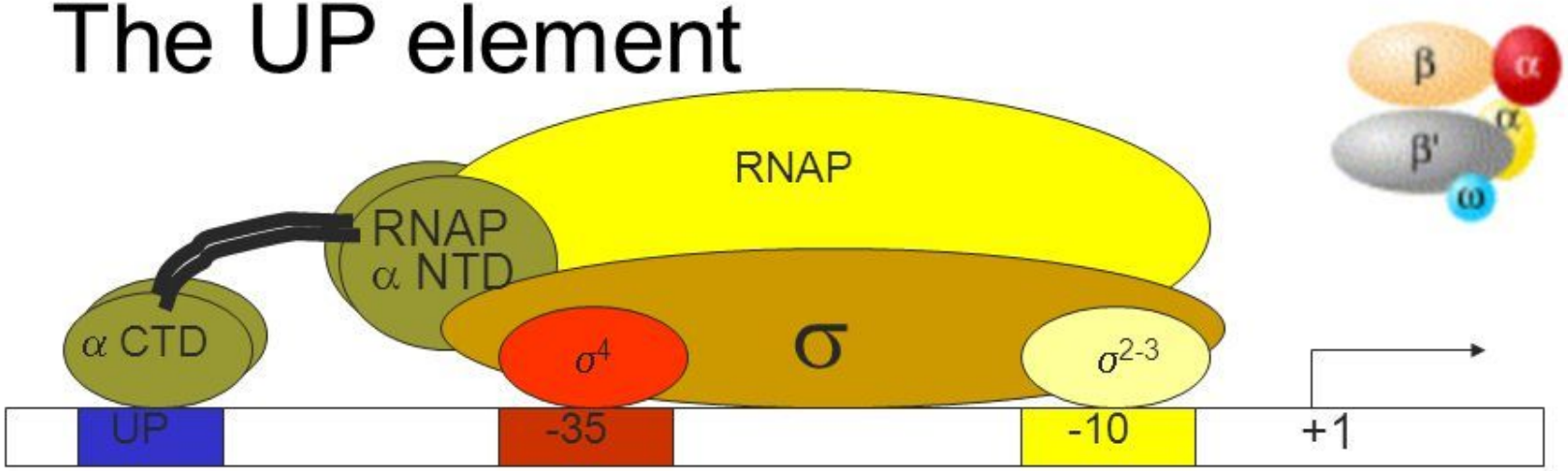
A classic *E.coli promoter* is made by three elements: the -35 and -10 consensus sequences, and the start point



Consensus Sequence -10 $T_{80}A_{95}T_{45}A_{60}A_{50}T_{96}$ 5-9 bp **A/G**

Consensus Sequence -35 $T_{82}T_{84}G_{78}A_{65}C_{54}A_{45}$ 16-19 bp **-10**

The UP element



- UP element is an AT rich motif present in some strong (e.g. rRNA) promoters
- UP element interacts directly with C-terminal domain of RNA polymerase α subunits

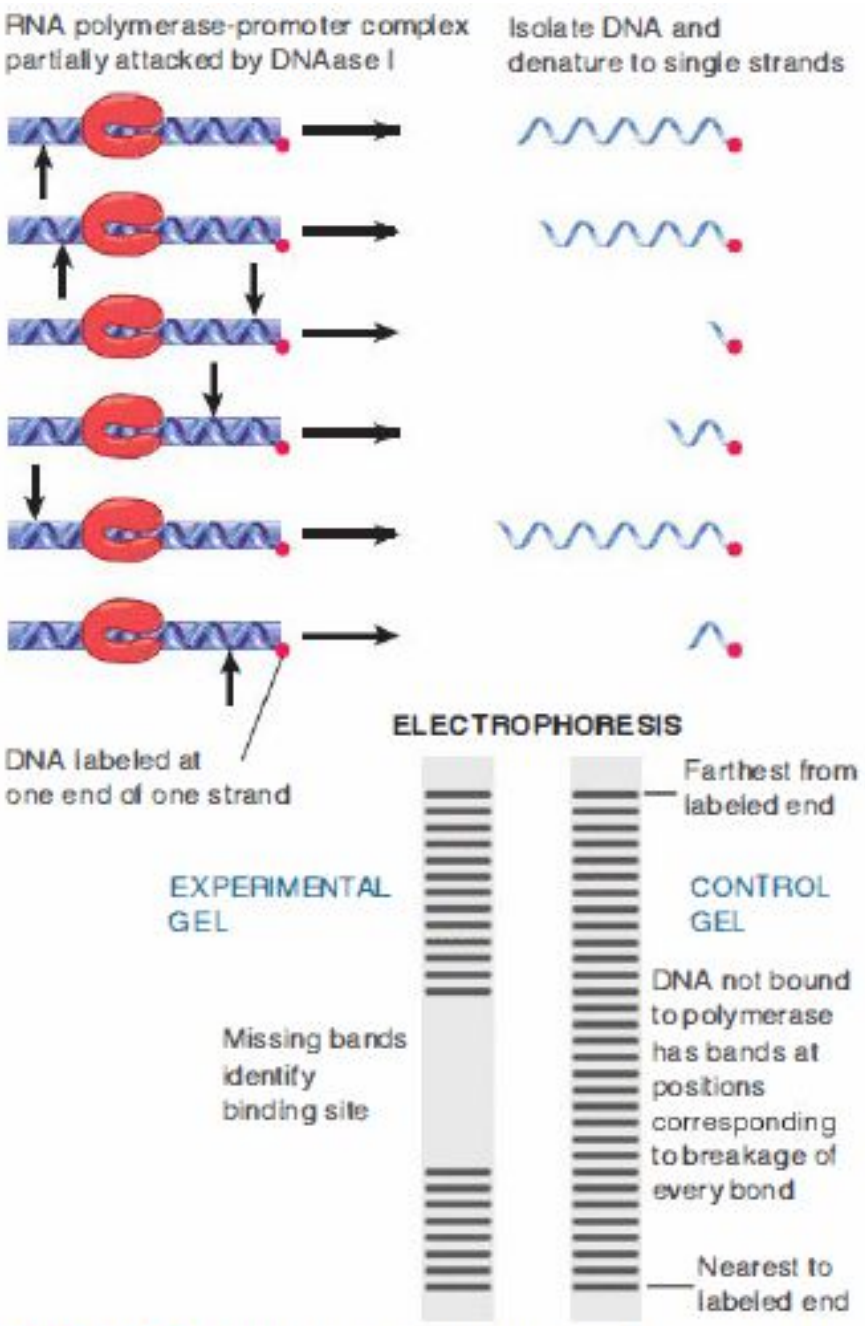


FIGURE 19.21 Footprinting identifies DNA-binding sites for proteins by their protection against nicking.

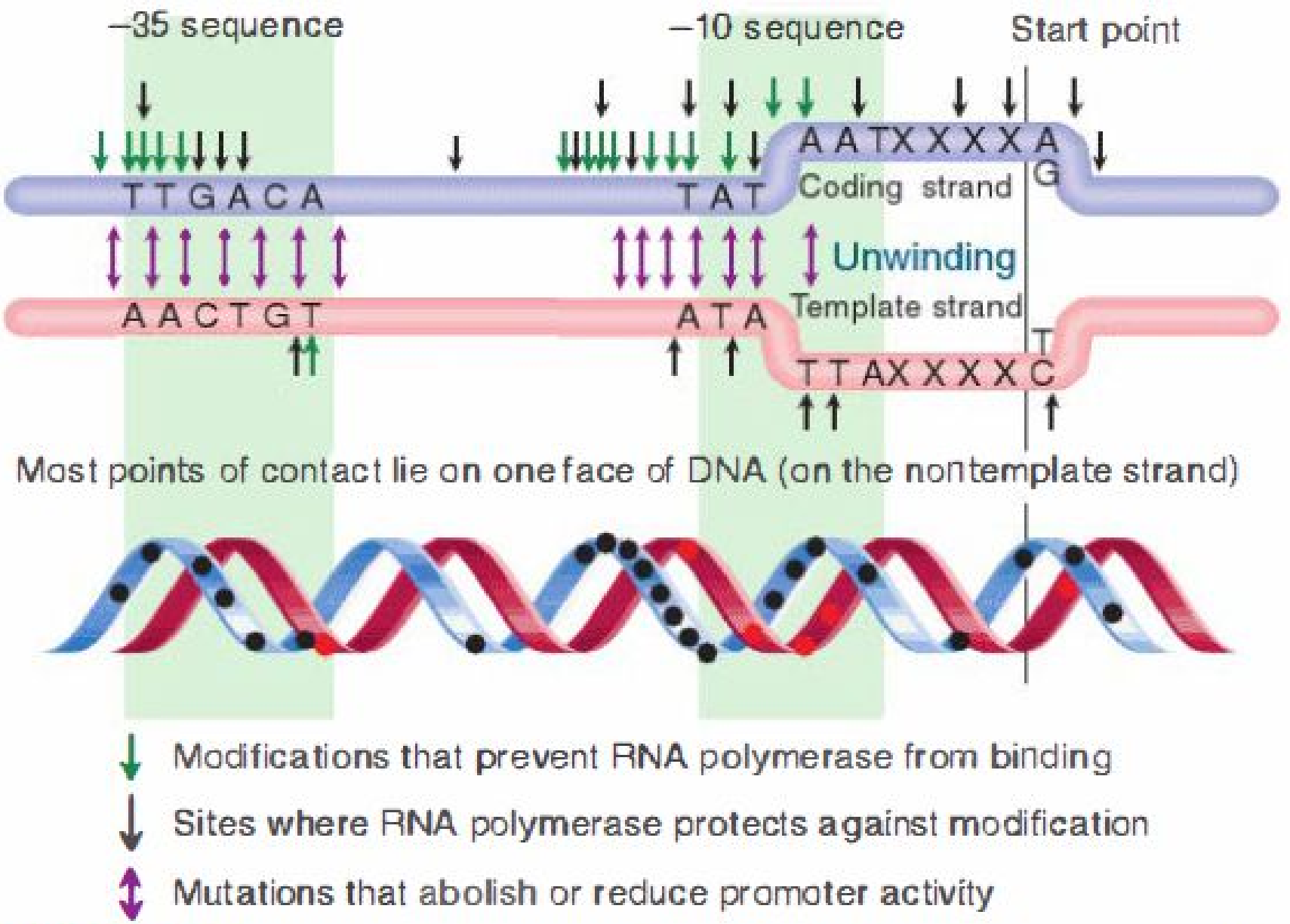


FIGURE 19.22 One face of the promoter contains the contact points for RNA.

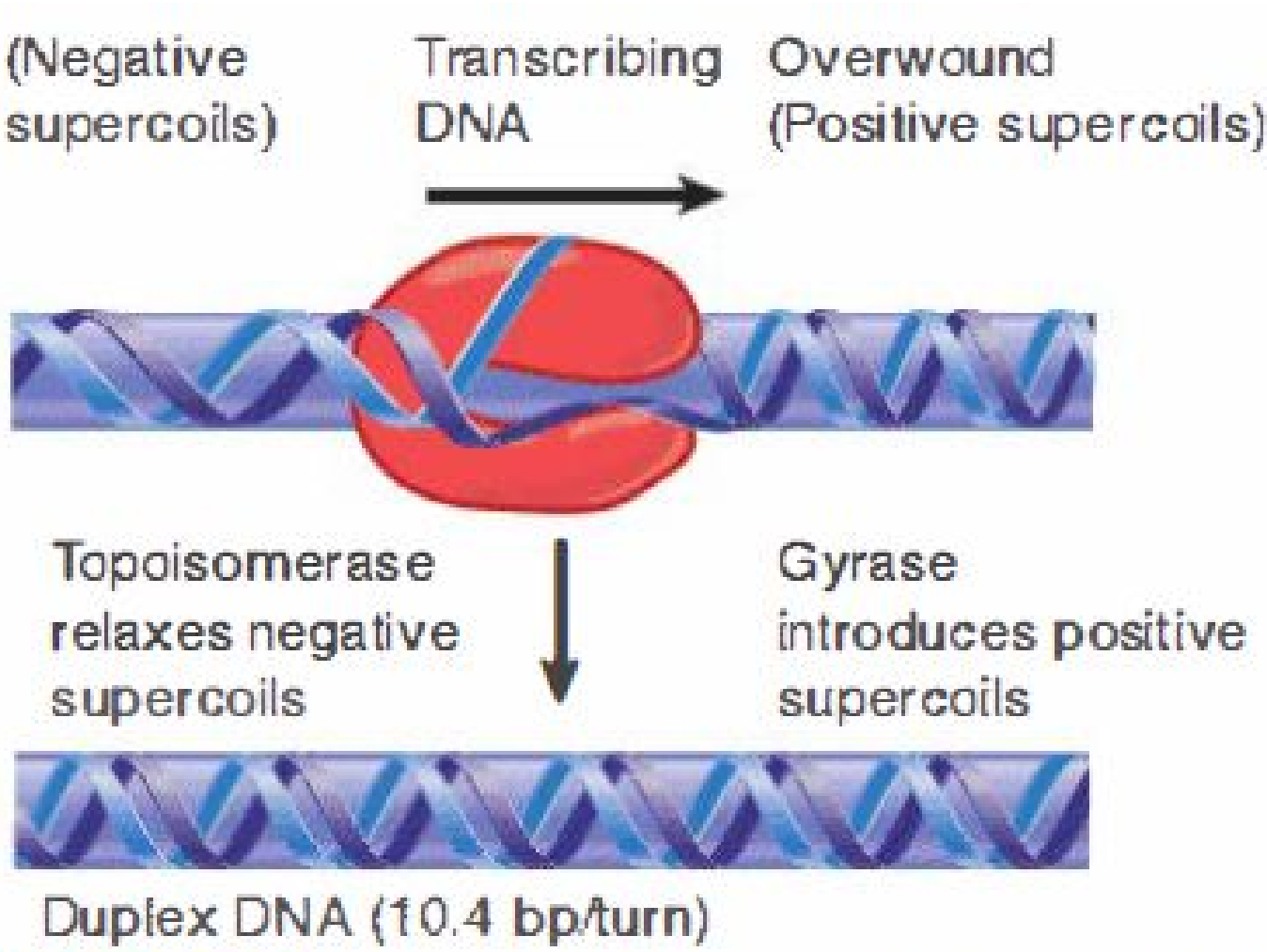


FIGURE 19.34 Transcription generates more tightly wound (positively supercoiled) DNA ahead of RNA polymerase, while the DNA behind becomes less tightly wound (negatively supercoiled).

E.Coli Sigma Factors

Gene	Factor	Use
rpoD	σ^{70}	most required functions
rpoS	σ^{S}	stationary phase/some stress responses
rpoH	σ^{32}	heat shock
rpoE	σ^{E}	periplasmic/extracellular proteins
rpoN	σ^{54}	nitrogen assimilation
rpoF	σ^{F}	flagellar synthesis/chemotaxis
fecI	σ^{fecI}	iron metabolism/transport

FIGURE 19.37 In addition to σ^{70}, *E. coli* has several sigma factors that are induced by particular environmental conditions. (A number in the name of a factor indicates its mass.)

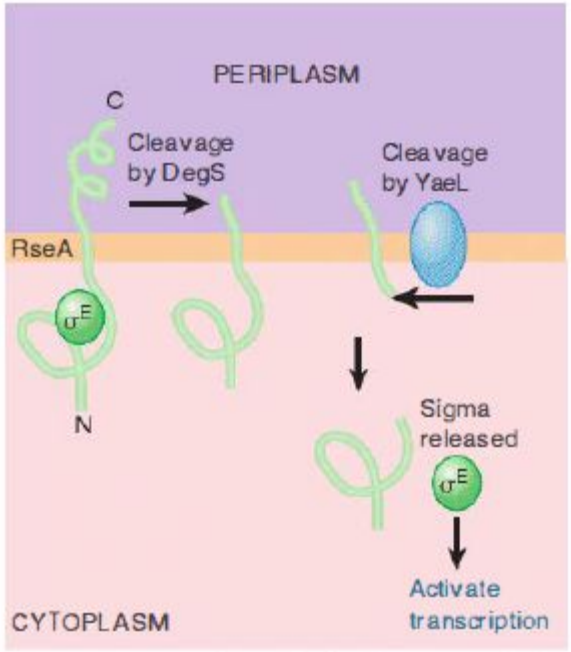


FIGURE 19.38 RseA is synthesized as a protein in the inner membrane. Its cytoplasmic domain binds the σ^E factor. RseA is cleaved sequentially in the periplasmic space and then in the cytoplasm. The cytoplasmic cleavage releases σ^E.

TABLE 7-1 Sigma Factors of *E. Coli*

SIGMA FACTOR	PROMOTERS RECOGNIZED	PROMOTER CONSENSUS −35 REGION	Separation	PROMOTER CONSENSUS −10 REGION
σ^{70}	Housekeeping genes, most genes in exponentially replicating cells	TTGACA	16-18 bp	TATAAT
σ^{S}	Stationary-phase genes and general stress response	TTGACA		TATAAT
σ^{32}	Induced by unfolded proteins in the cytoplasm; genes encoding chaperones that refold unfolded proteins and protease systems leading to the degradation of unfolded proteins in the cytoplasm	TCTCNCCCTTGAA	13-15 bp	CCCCATNTA
σ^{E}	Activated by unfolded proteins in the periplasmic space and cell membrane; genes encoding proteins that restore integrity to the cellular envelope	GAACTT		TCTGA
σ^{F}	Genes involved in flagellum assembly	CTAAA	15 bp	CCGATAT
FecI	Genes required for iron uptake	TTGGAAA		GTAATG
		−24 REGION		−12 REGION
σ^{54}	Genes for nitrogen metabolism and other functions	CTGGNA	6 bp	TTGCA

SOURCES: C. A. Gross, M. Lonetto, and R. Losick, 1992, in *Transcriptional Regulation*, S. L. McKnight and K. R. Yamamoto, eds., Cold Spring Harbor Laboratory Press; D. N. Arnosti and M. J. Chamberlin, 1989, *Proc. Nat'l. Acad. Sci. USA* **86**:830; K. Tanaka et al., 1993, *Proc. Nat'l. Acad. Sci., USA* **90**:3511; C. Dartigalongue et al., 2001, *J. Biol. Chem.* **276**:20866; A. Angerer and V. Braun, 1998, *Arch. Microbiol.* **169**:483.

Table 7-1
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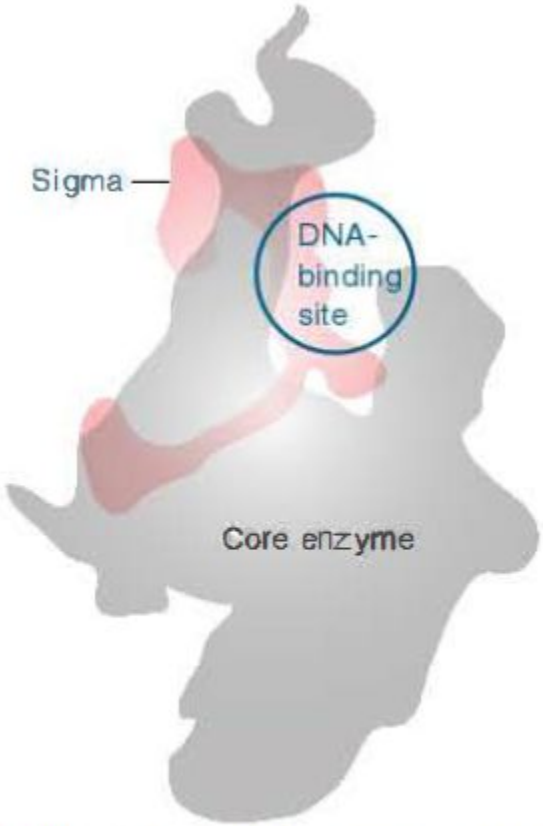

FIGURE 19.19 Sigma factor has an elongated structure that extends along the surface of the core subunits when the holoenzyme is formed.

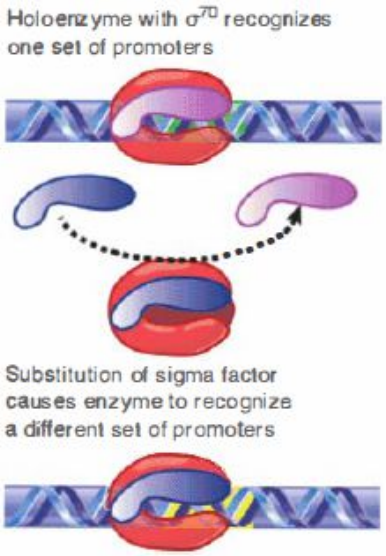

FIGURE 19.36 The sigma factor associated with core enzyme determines the set of promoters at which transcription is initiated.

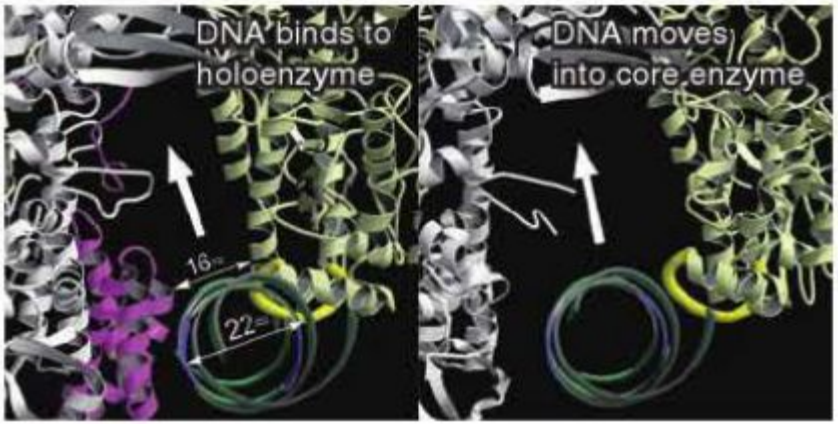

FIGURE 19.20 DNA initially contacts sigma factor (pink) and core enzyme (gray). It moves deeper into the core enzyme to make contacts at the −10 sequence. When sigma is released, the width of the passage containing DNA increases. Reprinted by permission from Macmillan Publishers Ltd: Nature, D. G. Vassylyev, et al., vol. 417, pp. 712–719, copyright 2002. Photo courtesy of Shigeyuki Yokoyama, The University of Tokyo.

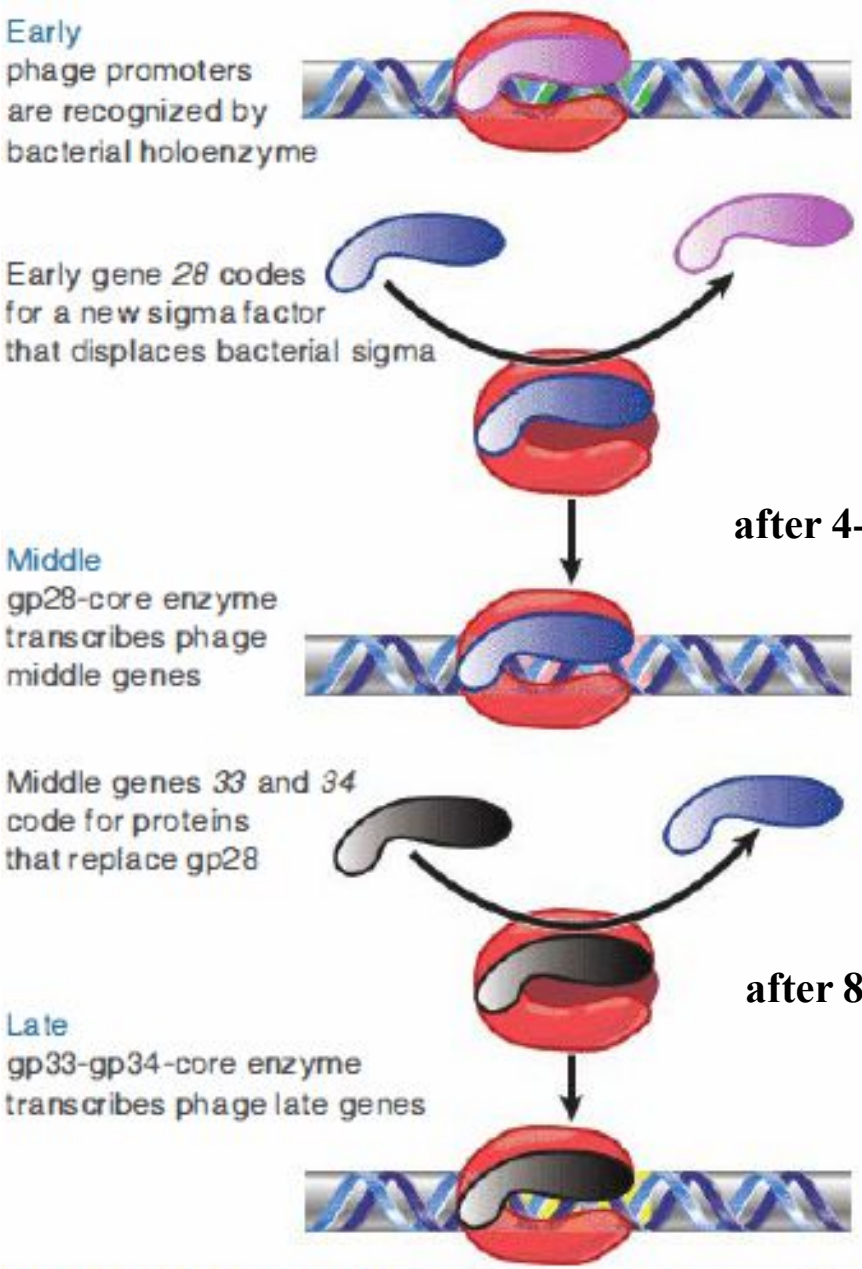

FIGURE 19.39 Transcription of phage SPO1 genes is controlled by two successive substitutions of the sigma factor that change the initiation specificity.

B. subtilis infection by SPO1 phage

after 4-5 min

after 8-12 min