

Figure 9.1 Overview:
the function of RNA
polymerase is to copy
one strand of duplex
DNA into RNA.

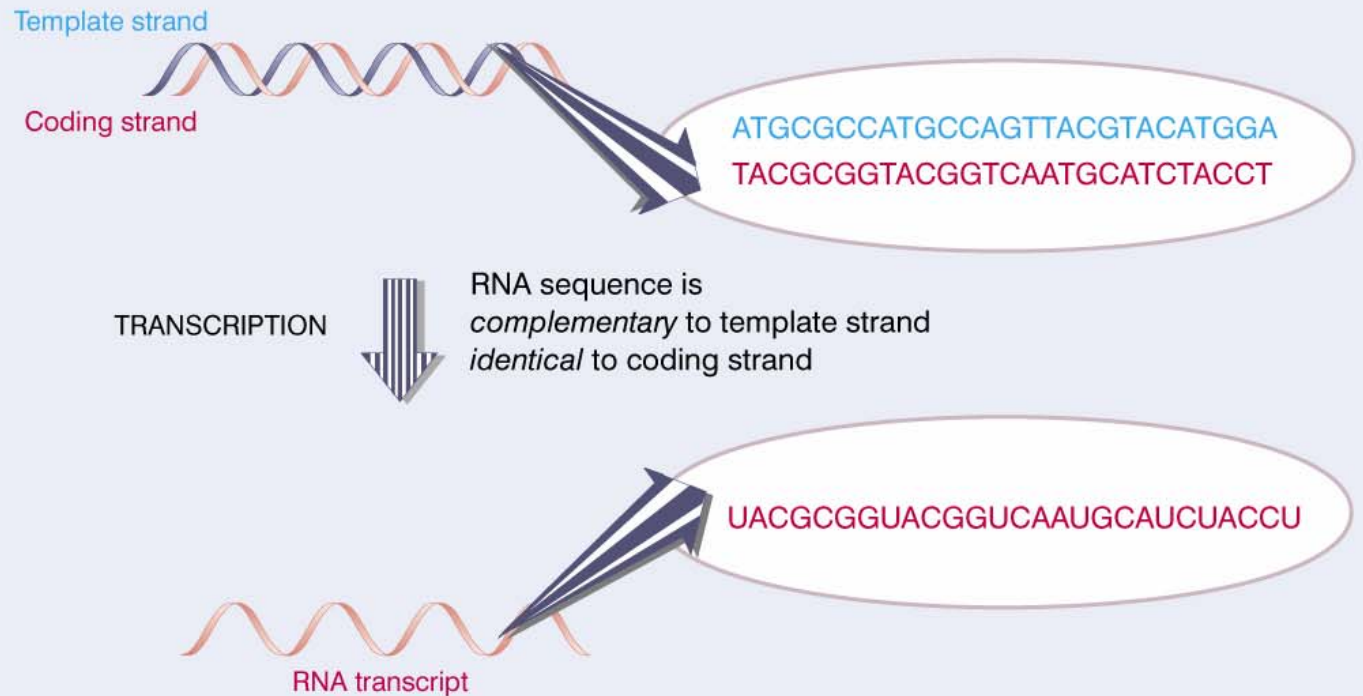
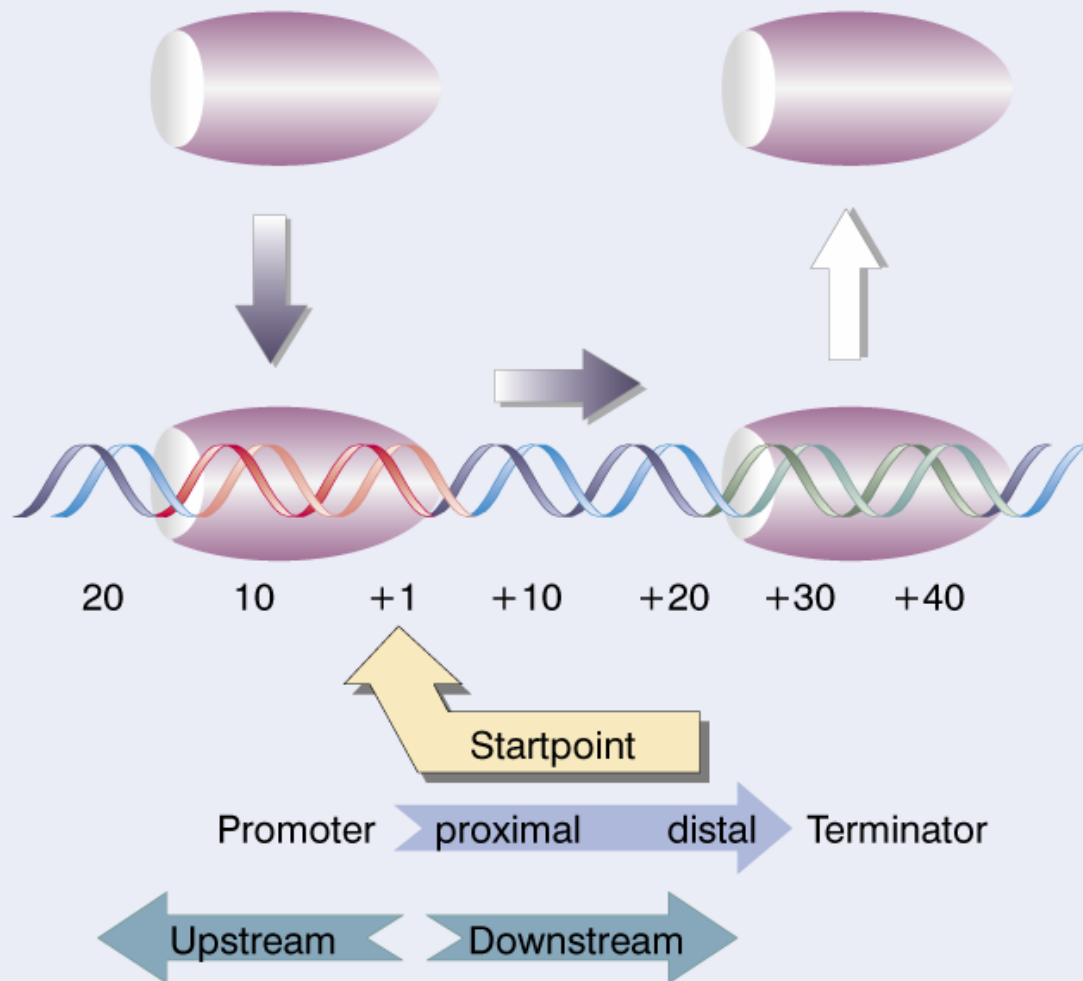


Figure 9.2 Overview: a transcription unit is a sequence of DNA transcribed into a single RNA, starting at the promoter and ending at the terminator.



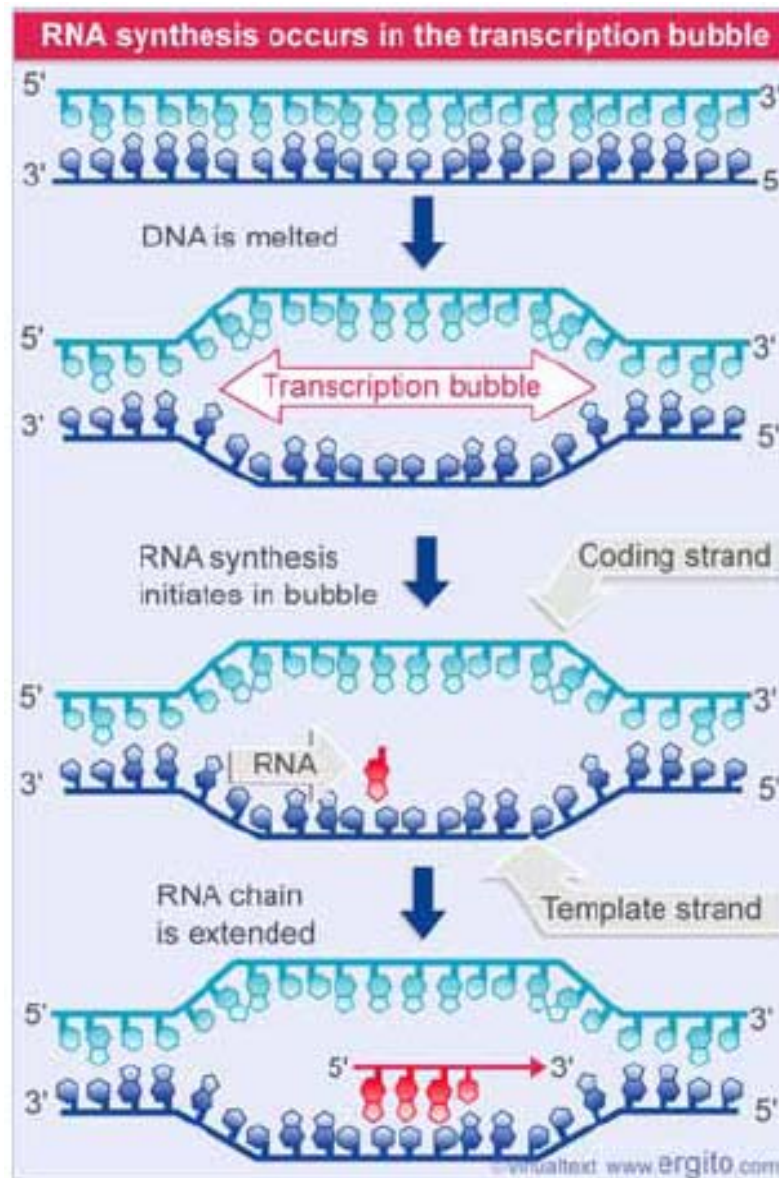


Figure 9.3 DNA strands separate to form a transcription bubble. RNA is synthesized by complementary base pairing with one of the DNA strands.

Figure 9.3 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 reforms behind it, displacing the RNA in the form of a single polynucleotide chain.

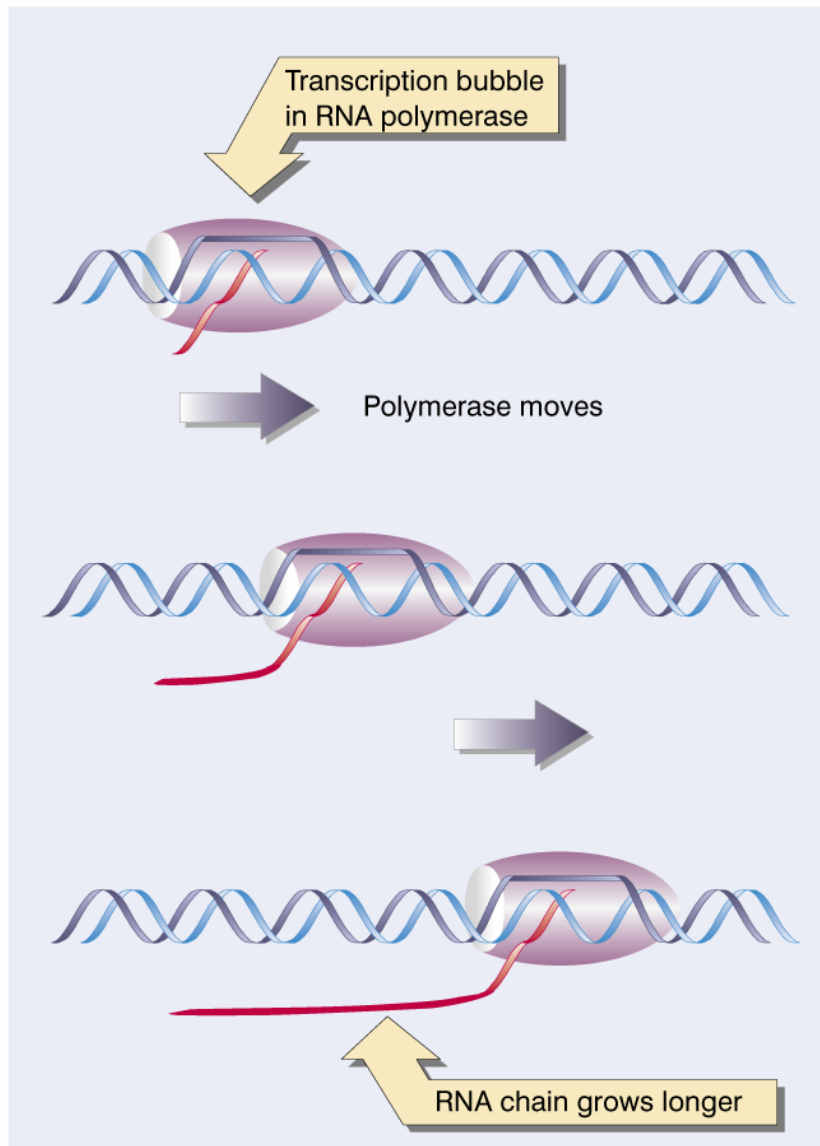


Figure 9.4 During transcription, the bubble is maintained within bacterial RNA polymerase, which unwinds and rewinds DNA, maintains the conditions of the partner and template DNA strands, and synthesizes RNA.

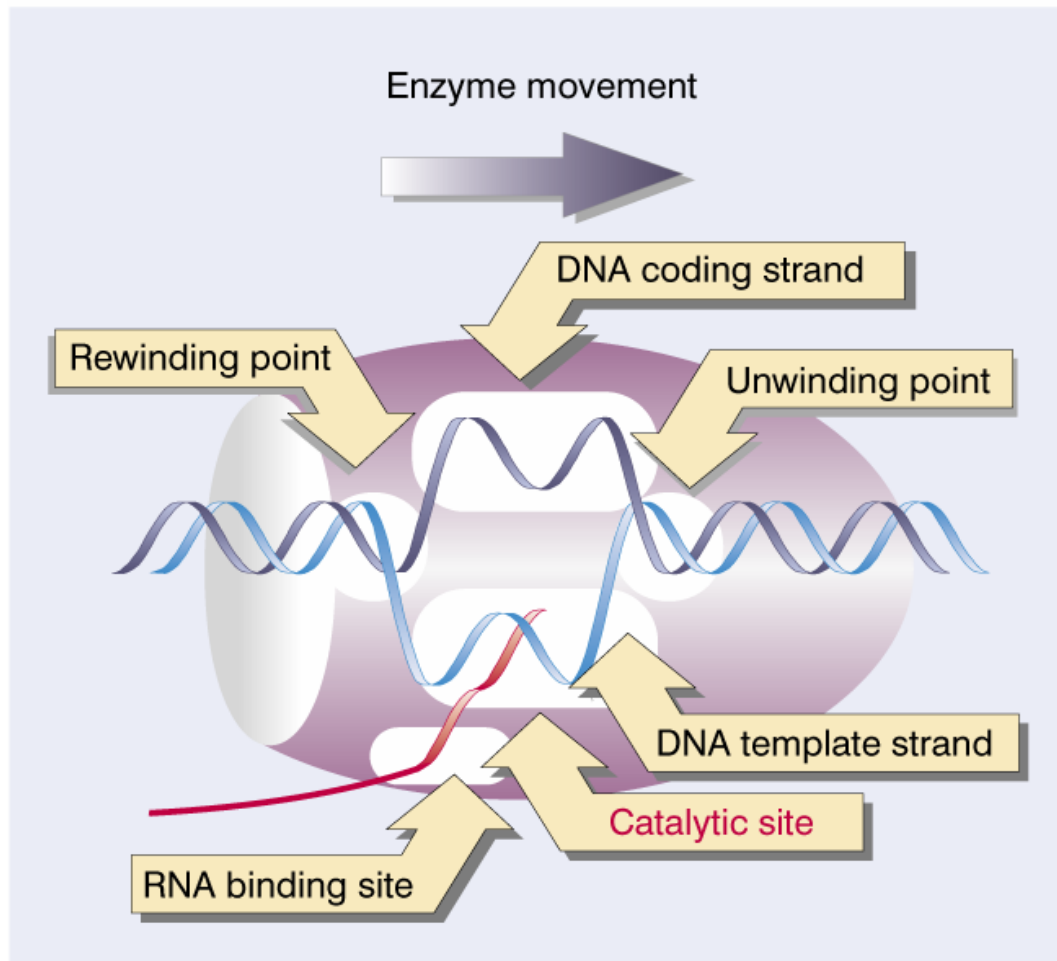


Figure 9.6 Phosphodiester bond formation involves a hydrophilic attack by the 3'-OH group of the last nucleotide of the chain on the 5' triphosphate of the incoming nucleotide, with release of pyrophosphate.

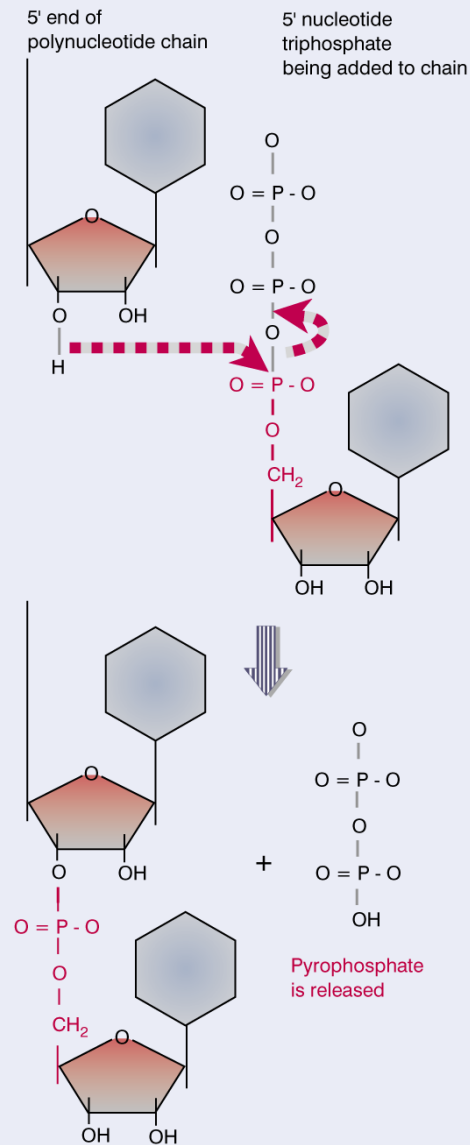


Figure 9.8 Transcription has four stages, which involve different types of interaction between RNA polymerase and DNA. The enzyme binds to the promoter and melts DNA, remains stationary during initiation, moves along the template during elongation, and dissociates at termination.

Template recognition: RNA polymerase binds to duplex DNA



DNA is unwound at promoter



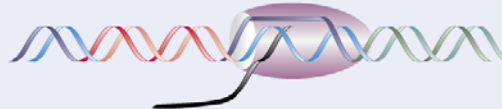
Initiation: Chains of 2–9 bases are synthesized and released



Elongation: RNA polymerase synthesizes RNA



Unwound region moves with RNA polymerase



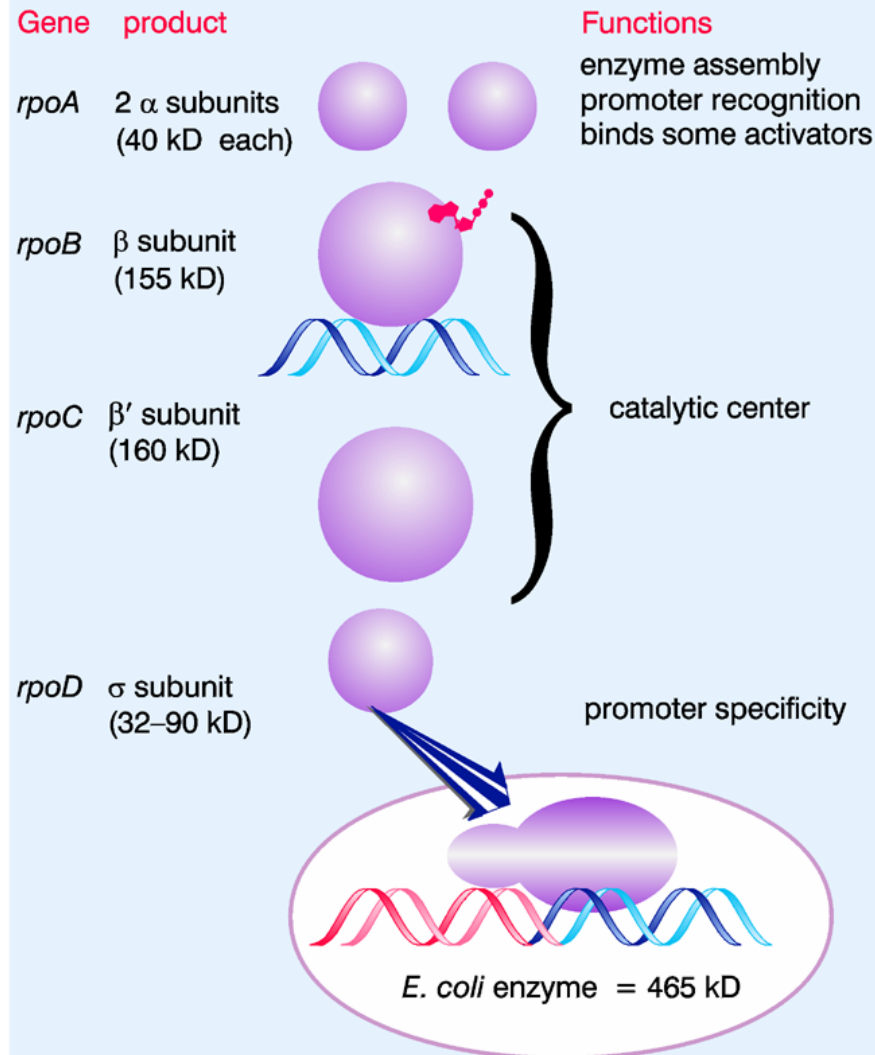
RNA polymerase reaches end of gene



Termination: RNA polymerase and RNA are released



Figure 9.9 Eubacterial RNA polymerases have four types of subunit; α , β , and β' have rather constant sizes in different bacterial species, but σ varies more widely.



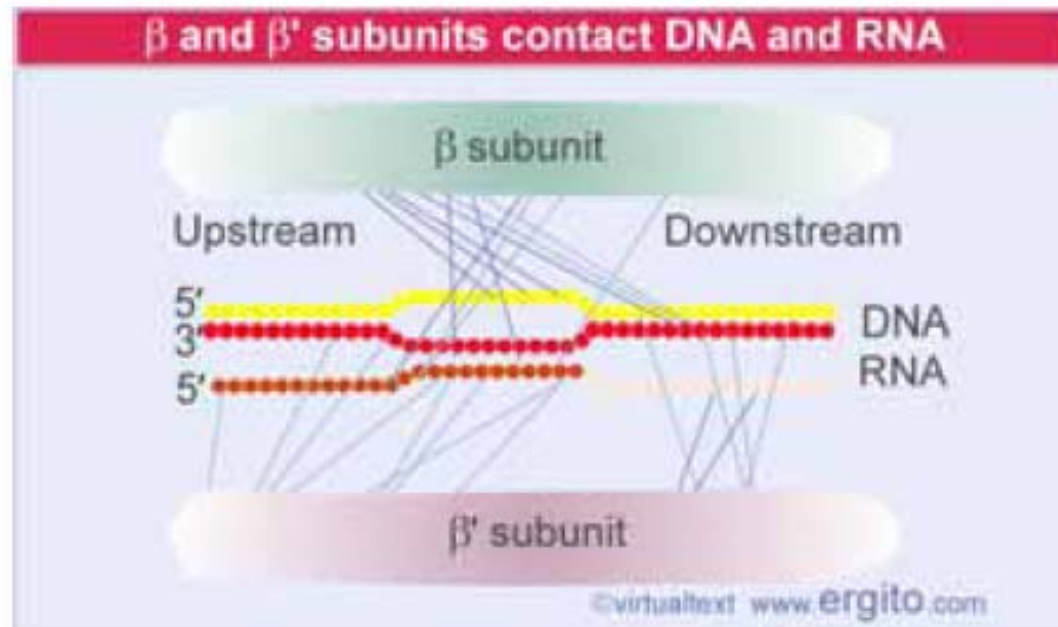


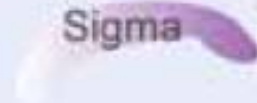
Figure 9.17 Both the template and coding strands of DNA are contacted by the β and β' subunits largely in the region of the transcription bubble and downstream. The RNA is contacted mostly in the transcription bubble. (Usually there is no downstream RNA, and contacts with RNA downstream occur only in the special case when the enzyme backtracks.) (Based

Sigma factor controls specificity

Core enzyme binds to any DNA



Sigma destabilizes binding



Holoenzyme binds to promoter



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Figure 9.18 Core enzyme binds indiscriminately to any DNA. Sigma factor reduces the affinity for sequence-independent binding, and confers specificity for promoters.

Figure 9.10 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.

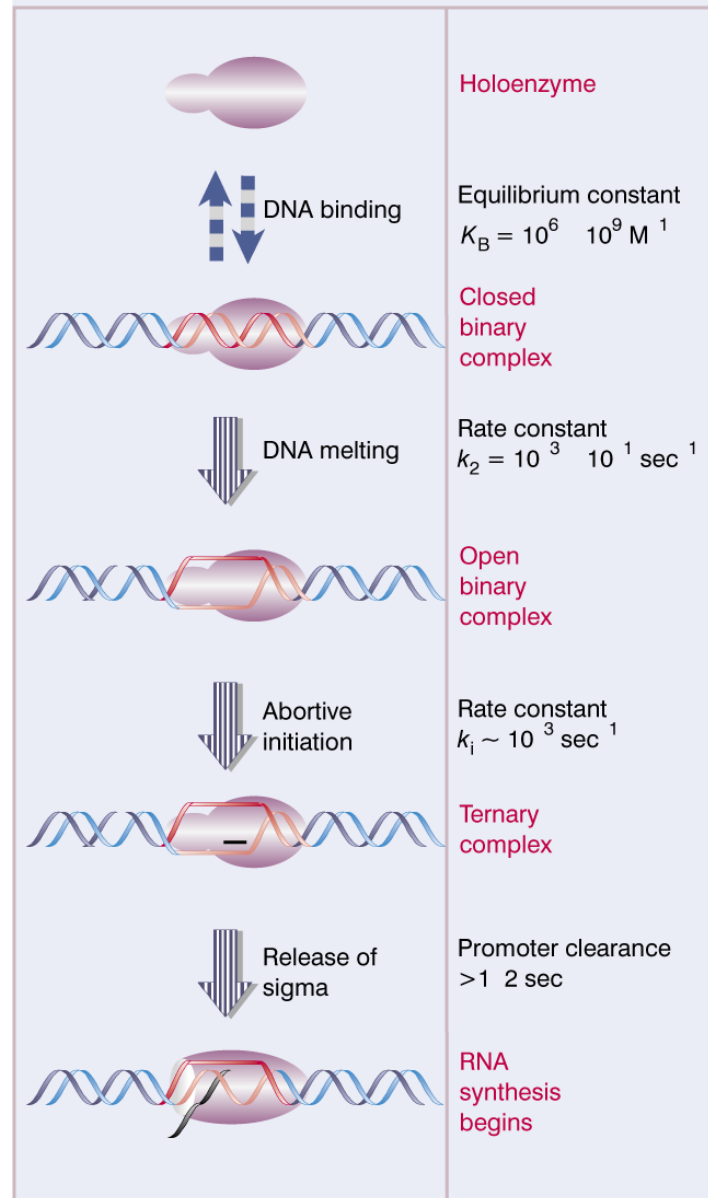
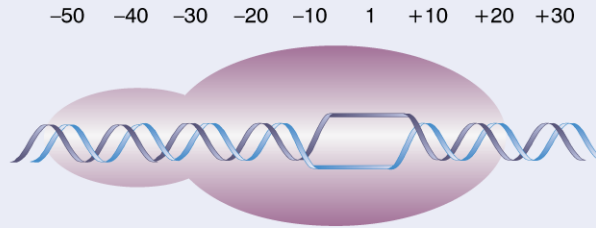
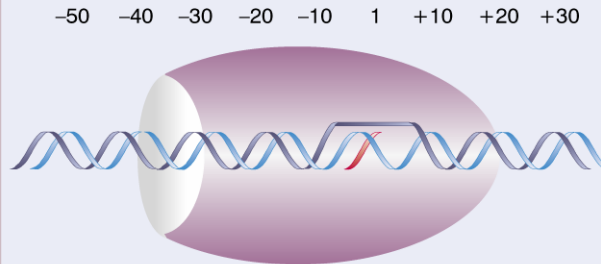


Figure 9.11 RNA polymerase initially contacts the region from -55 to +20. When sigma dissociates, the core enzyme contracts to -30; when the enzyme moves a few base pairs, it becomes more compactly organized into the general elongation complex.

Initiation complex contains sigma and covers 75–80 bp



Initial elongation complex forms at 10 bases, loses sigma, and loses contacts in -35 to -55 region



General elongation complex forms at 15–20 bases and covers 30–40 bp

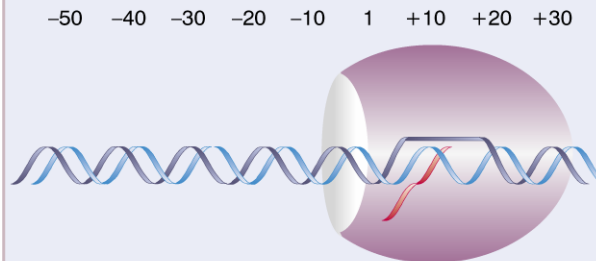


Figure 9.12 Core enzyme and holoenzyme are distributed on DNA, and very little RNA polymerase is free.

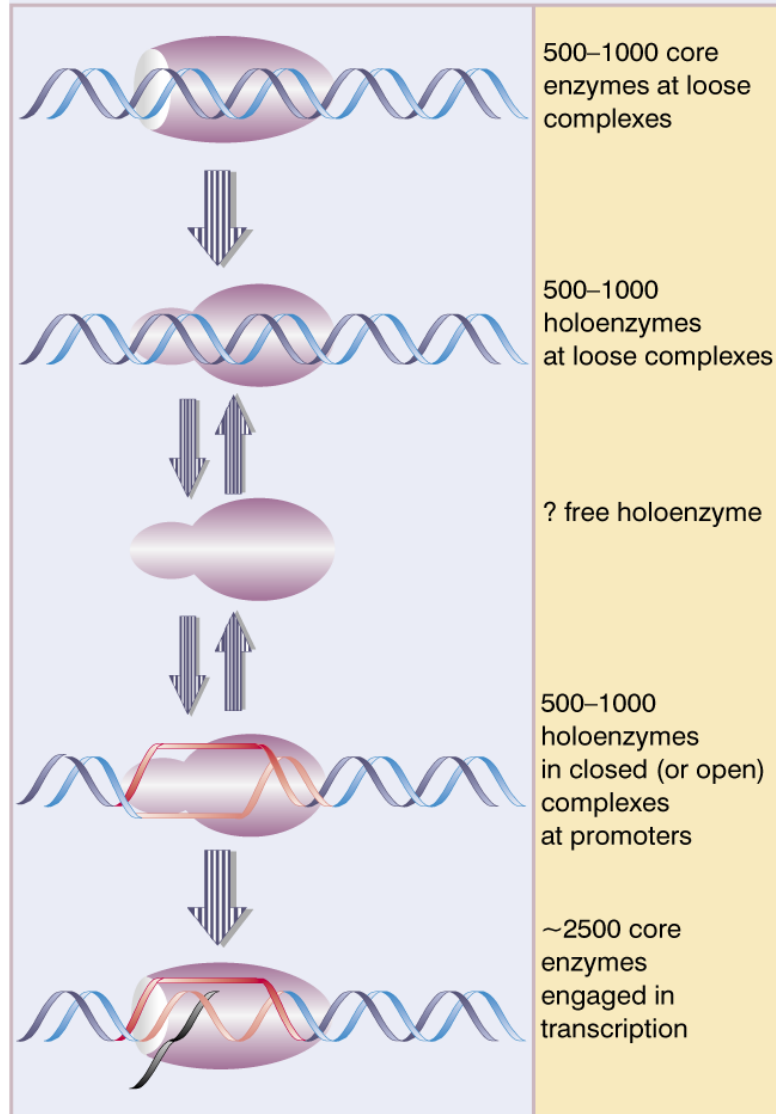


Figure 9.13 How does RNA polymerase find target promoters so rapidly on DNA?

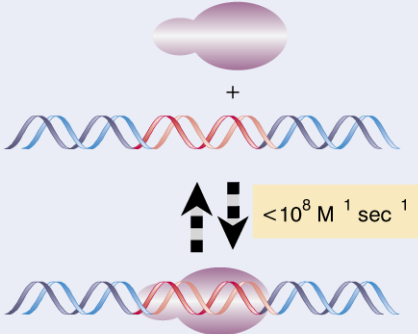
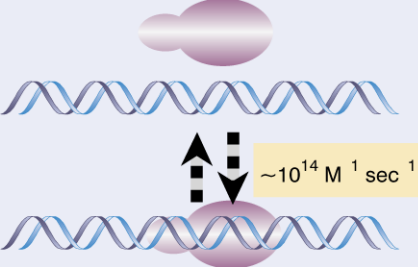
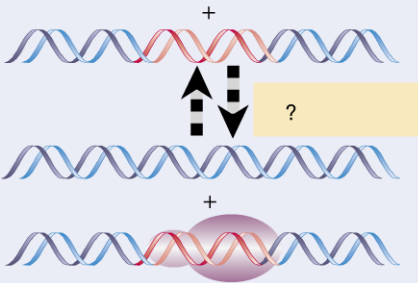
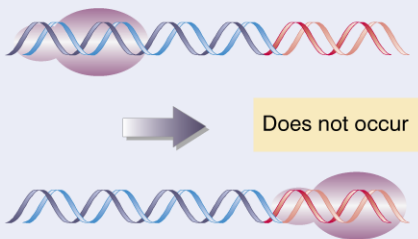
Model	Reaction
Random diffusion to target	
Random diffusion to any DNA	
followed by Random displacement between DNA	
Sliding along DNA	

Figure 9.14 Sigma factor and core enzyme recycle at different points in transcription.

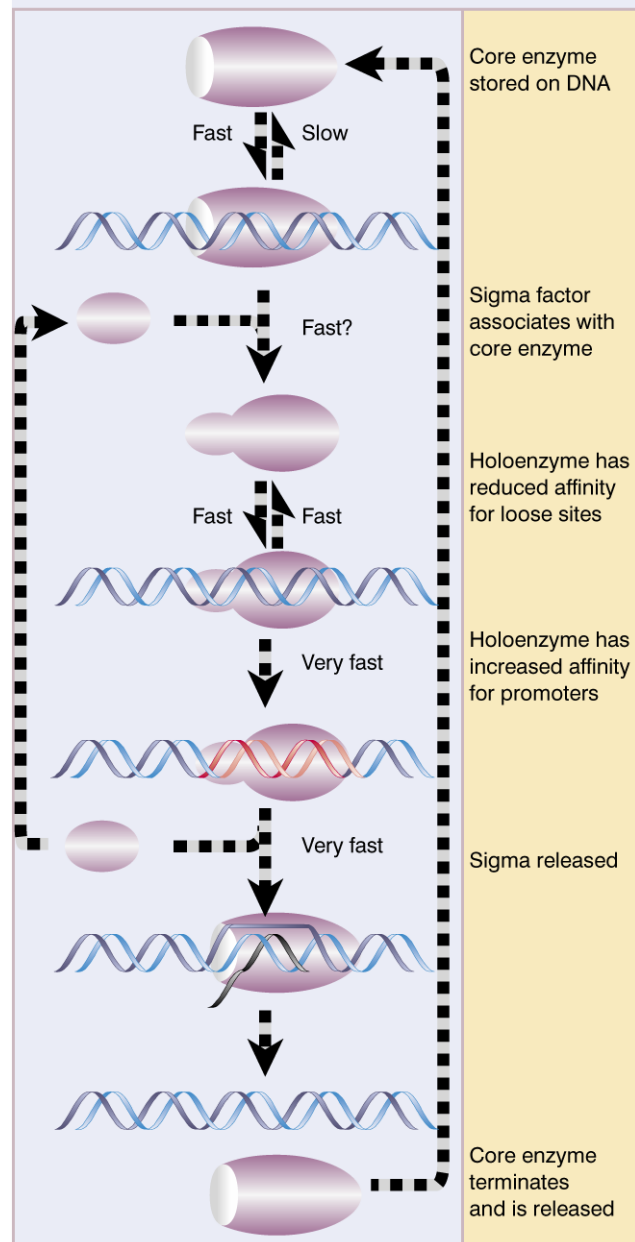
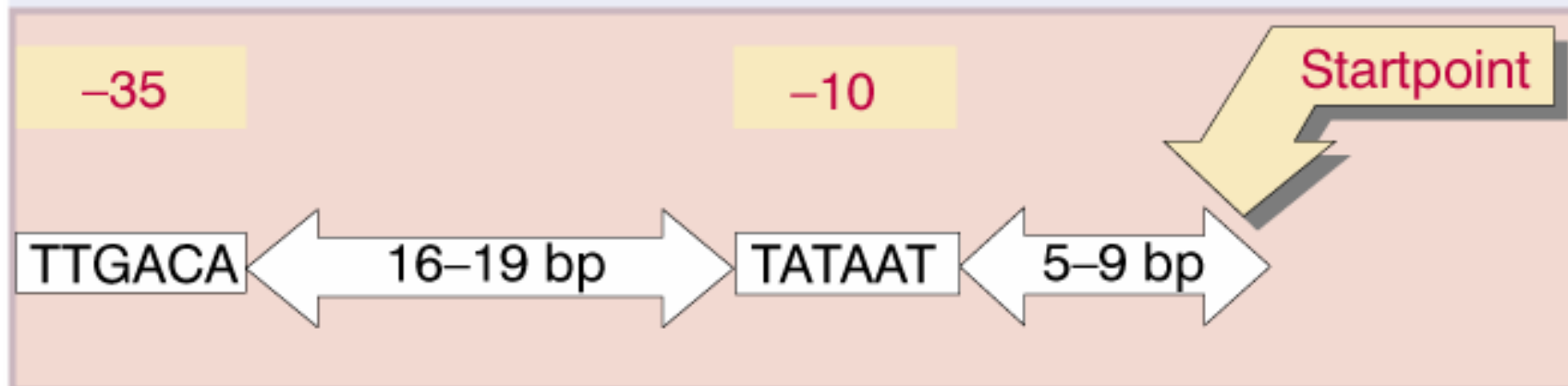


Figure 9.15 A typical promoter has three components, consisting of consensus sequences at -35 and -10, and the startpoint.



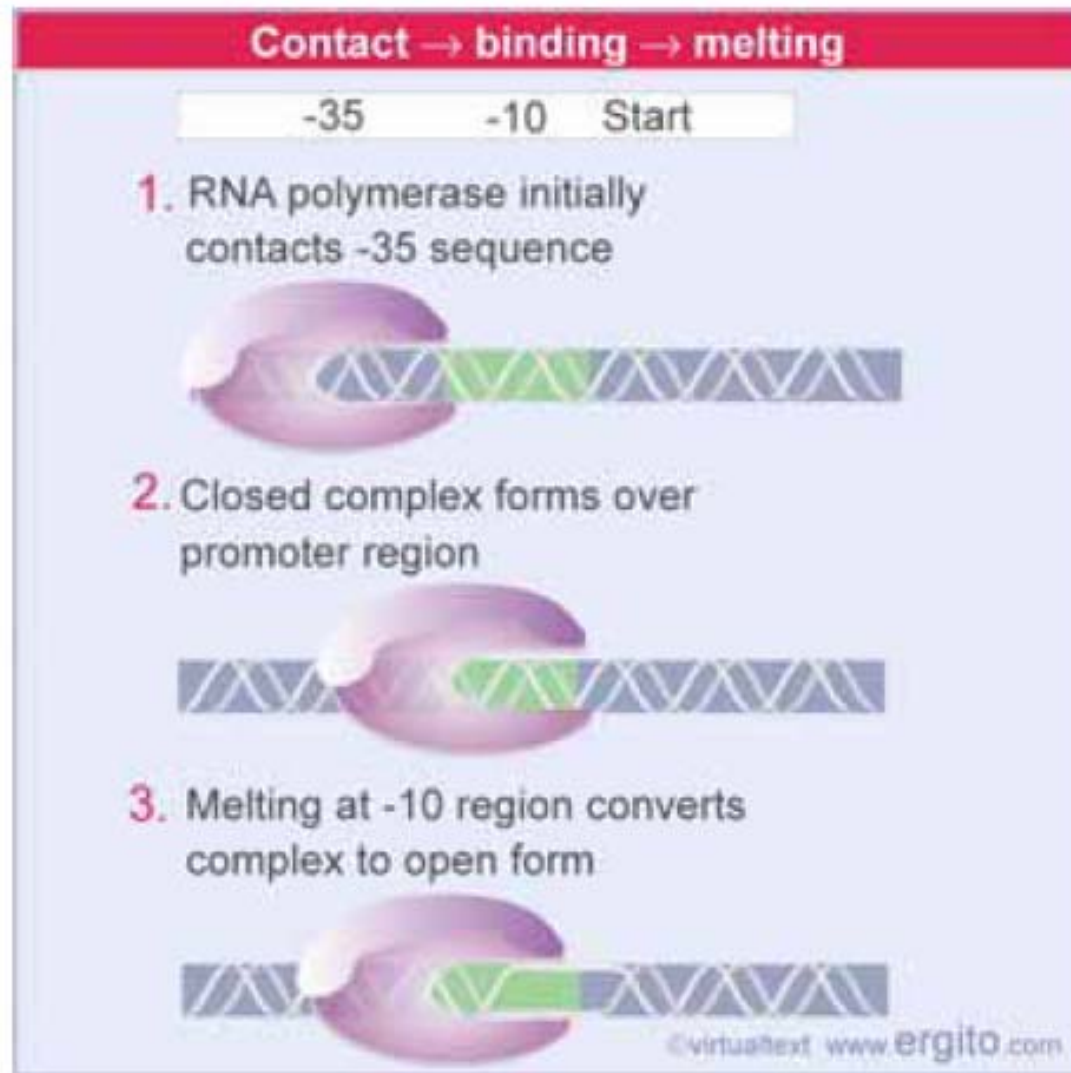


Figure 9.27 The -35 sequence is used for initial recognition, and the -10 sequence is used for the melting reaction that converts a closed complex to an open complex.

Figure 9.16 Footprinting identifies DNA-binding sites for proteins by their protection against nicking.

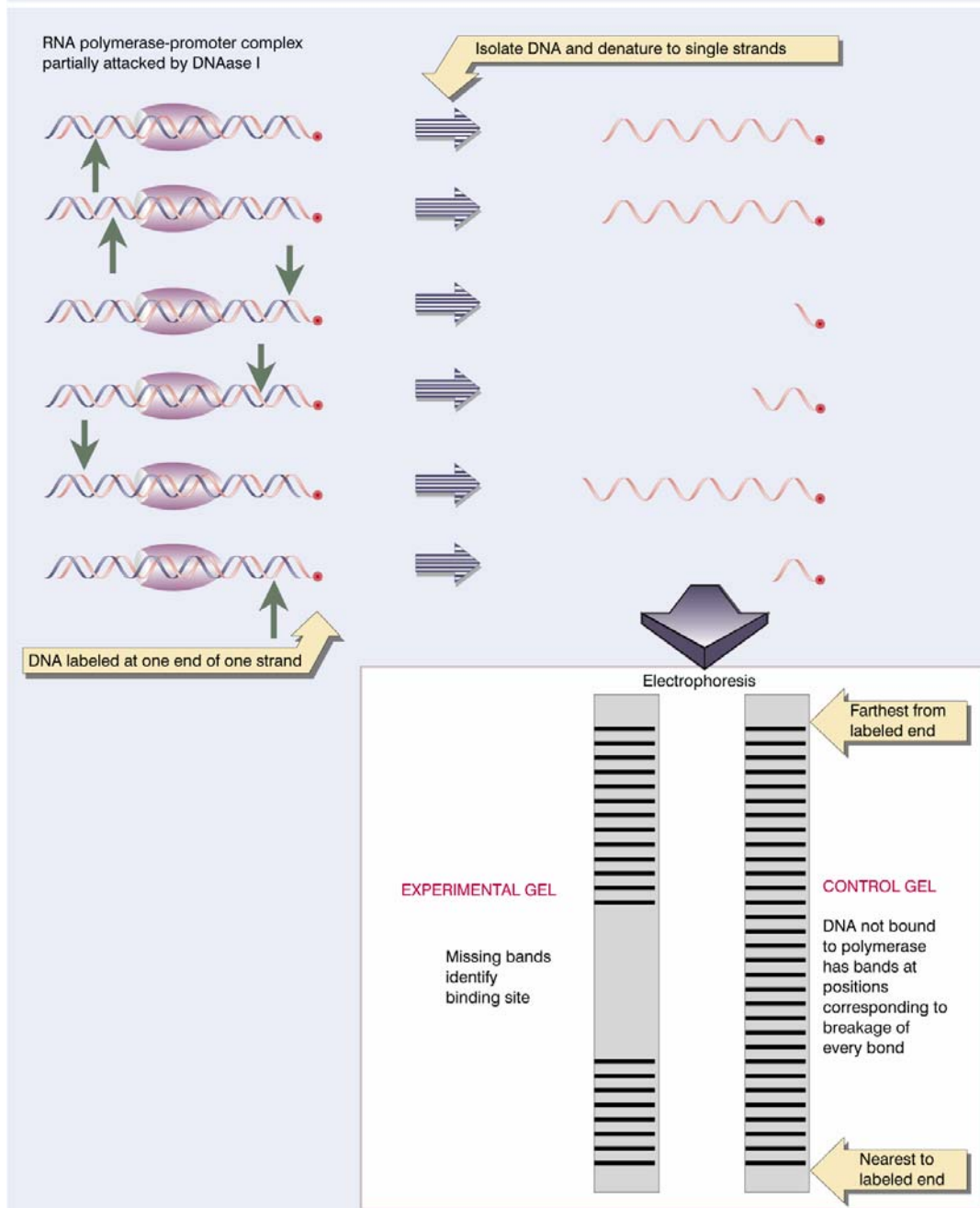


Figure 9.17 One face of the promoter contains the contact points for RNA.

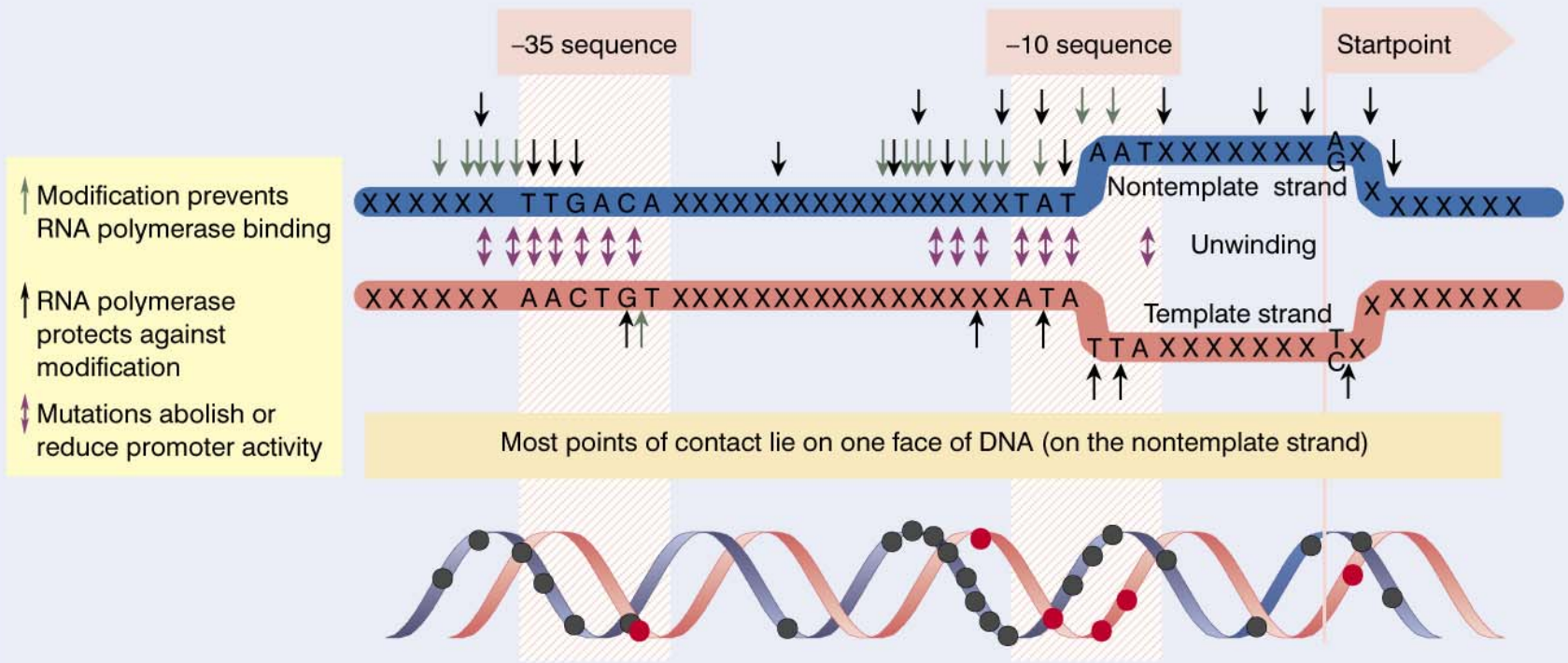
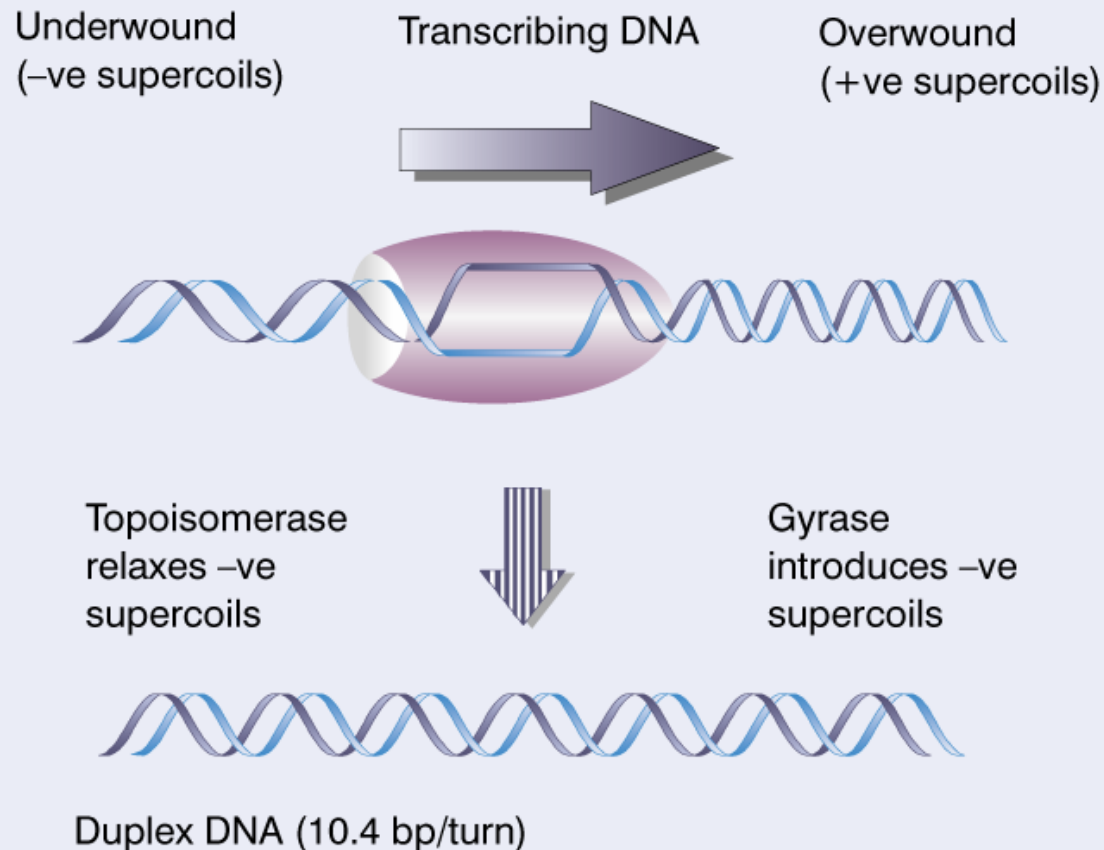


Figure 9.18 Transcription may generate more tightly wound (positively supercoiled) DNA ahead of RNA polymerase, while the DNA behind becomes less tightly wound (negatively supercoiled).



Sigma controls promoter recognition

Holoenzyme with σ^{70} recognizes one set of promoters



Substitution of sigma factor causes enzyme to recognize a different set of promoters



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E. coli has several sigma factors

Gene	Factor	Use
<i>rpoD</i>	σ^{70}	general
<i>rpoS</i>	σ^S	stress
<i>rpoH</i>	σ^{32}	heat shock
<i>rpoE</i>	σ^E	heat shock
<i>rpoN</i>	σ^{54}	nitrogen
<i>fliA</i>	σ^{28} (σ^F)	flagellar

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Figure 9.32 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 9.31 The sigma factor associated with core enzyme determines the set of promoters where transcription is initiated.

Figure 9.19 *E. coli* sigma factors recognize promoters with different consensus sequences. (Numbers in the name of a factor indicate its mass.)

Gene	Factor	Use	-35 Sequence	Separation	-10 Sequence
<i>rpoD</i>	σ^{70}	general	TTGACA	16–18 bp	TATAAT
<i>rpoH</i>	σ^{32}	heat shock	CCCTTGAA	13–15 bp	CCCGATNT
<i>rpoE</i>	σ^E	heat shock	not known	not known	not known
<i>rpoN</i>	σ^{54}	nitrogen	CTGGNA	6 bp	TTGCA
<i>fliA</i>	σ^F	flagellar	CTAAA	15 bp	GCCGATAA

Figure 9.20 A map of the *E. coli* σ^{70} factor identifies conserved regions. Regions 2.1 and 2.2 contact core polymerase, 2.3 is required for melting, and 2.4 and 4.2 contact the -10 and -35 promoter elements. The N-terminal region prevents 2.4 and 4.2 from binding to DNA in the absence of core enzyme.

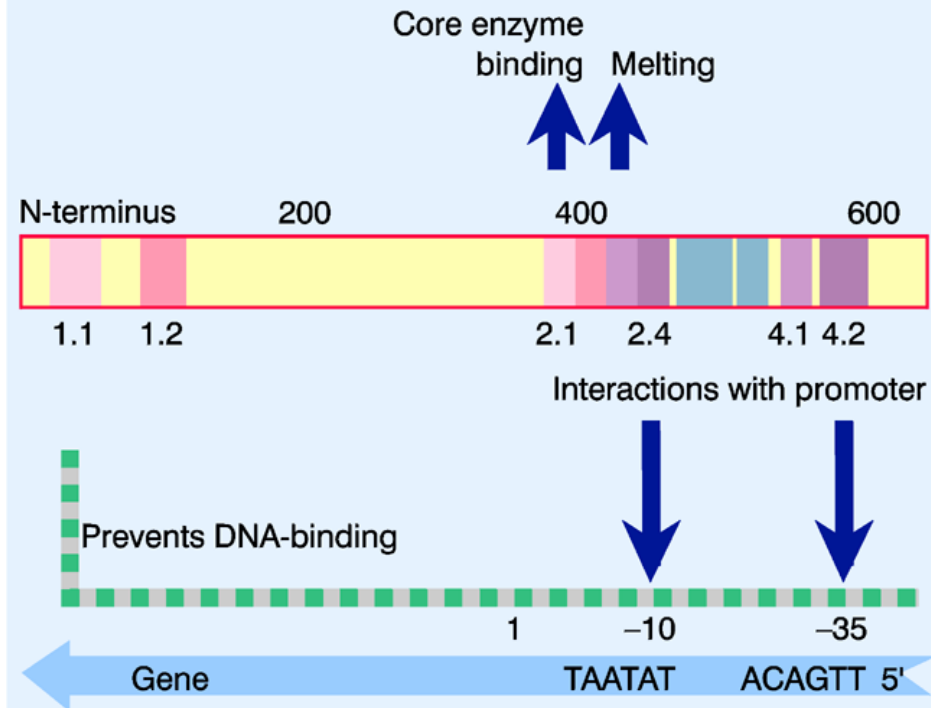


Figure 9.22

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

