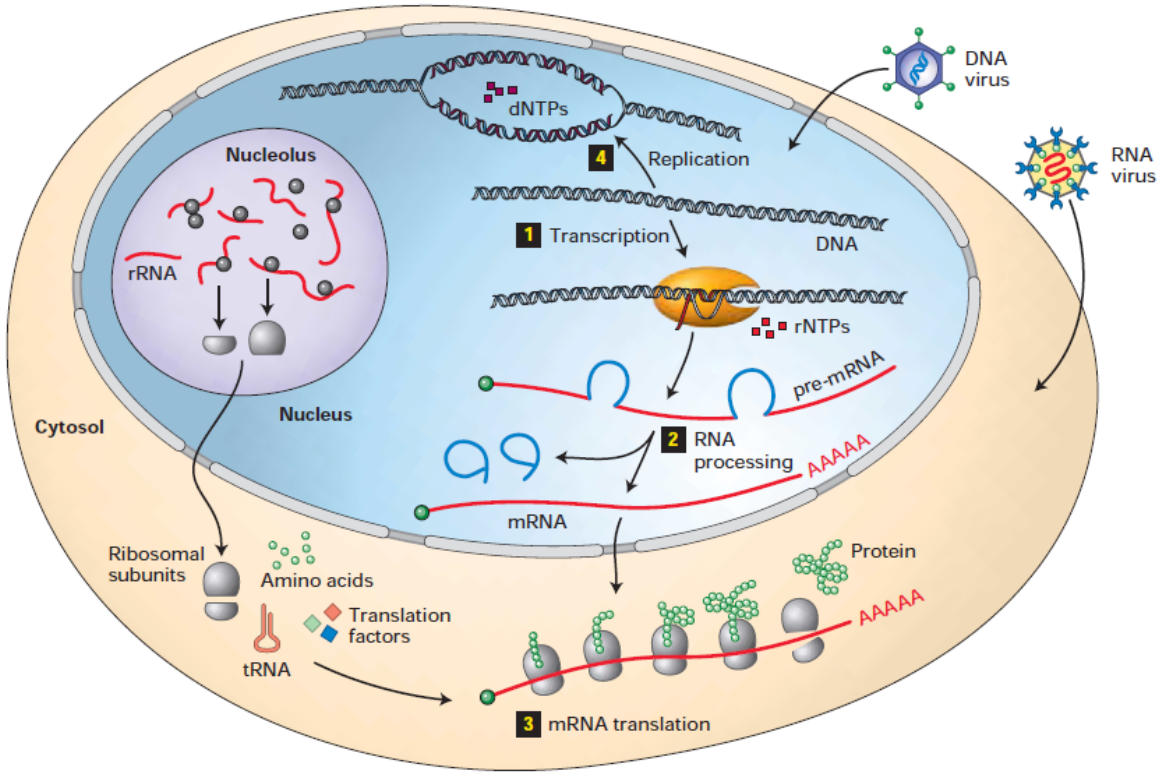
Transcription

Overview of four basic molecular genetic processes



▲ **FIGURE 4-1 Overview of four basic molecular genetic processes.** In this chapter we cover the three processes that lead to production of proteins (1–3) and the process for replicating DNA (4). Because viruses utilize host-cell machinery, they have been important models for studying these processes. During transcription of a protein-coding gene by RNA polymerase (1), the four-base DNA code specifying the amino acid sequence of a protein is copied into a precursor messenger RNA (pre-mRNA) by the polymerization of ribonucleoside triphosphate monomers (rNTPs). Removal of extraneous sequences and other modifications to the pre-mRNA (2), collectively known as *RNA processing*, produce a functional mRNA, which is transported to the cytoplasm. During translation (3), the four-base code of the mRNA is decoded into the 20–amino acid "language" of proteins. Ribosomes, the macromolecular machines that translate the mRNA code, are composed of two subunits assembled in the nucleolus from ribosomal RNAs (rRNAs) and multiple proteins (*left*). After transport to the cytoplasm, ribosomal subunits associate with an mRNA and carry out protein synthesis with the help of transfer RNAs (tRNAs) and various translation factors. During DNA replication (4), which occurs only in cells preparing to divide, deoxyribonucleoside triphosphate monomers (dNTPs) are polymerized to yield two identical copies of each chromosomal DNA molecule. Each daughter cell receives one of the identical copies.

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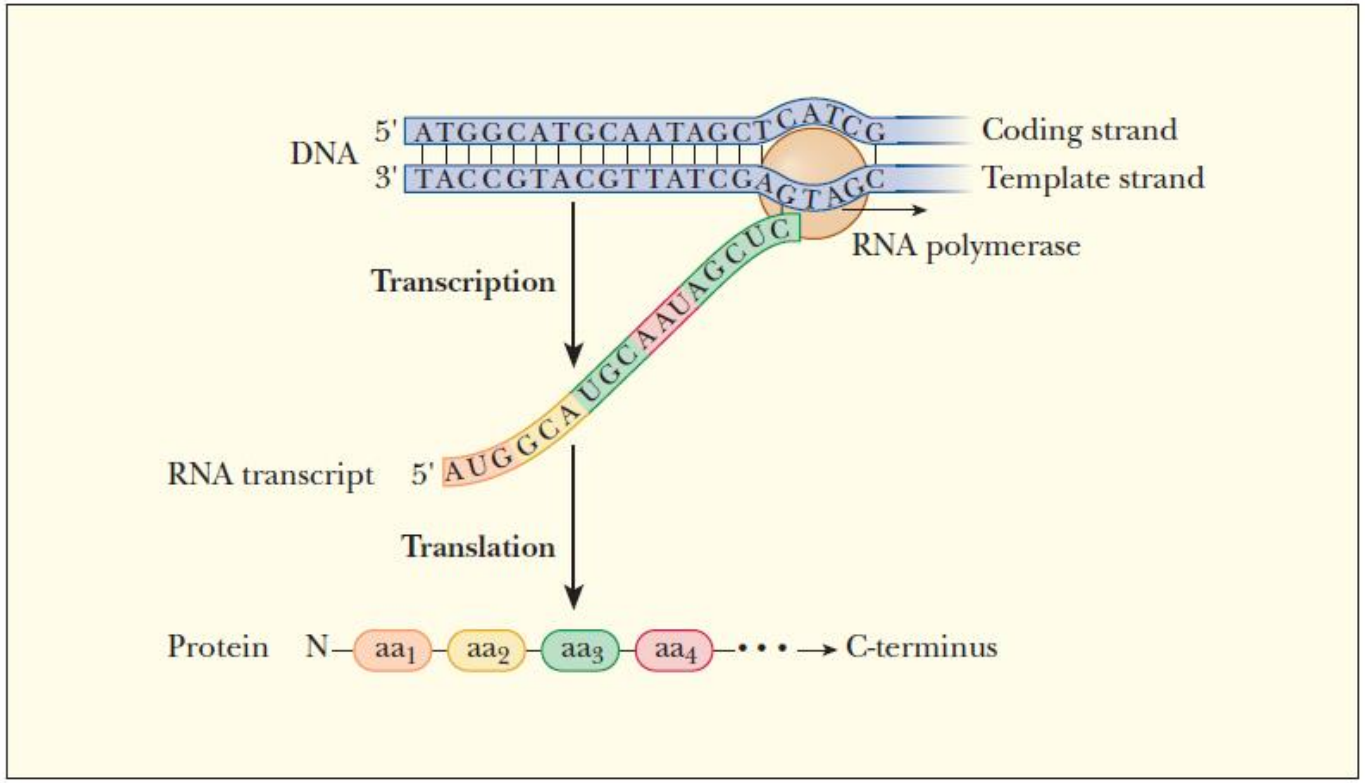
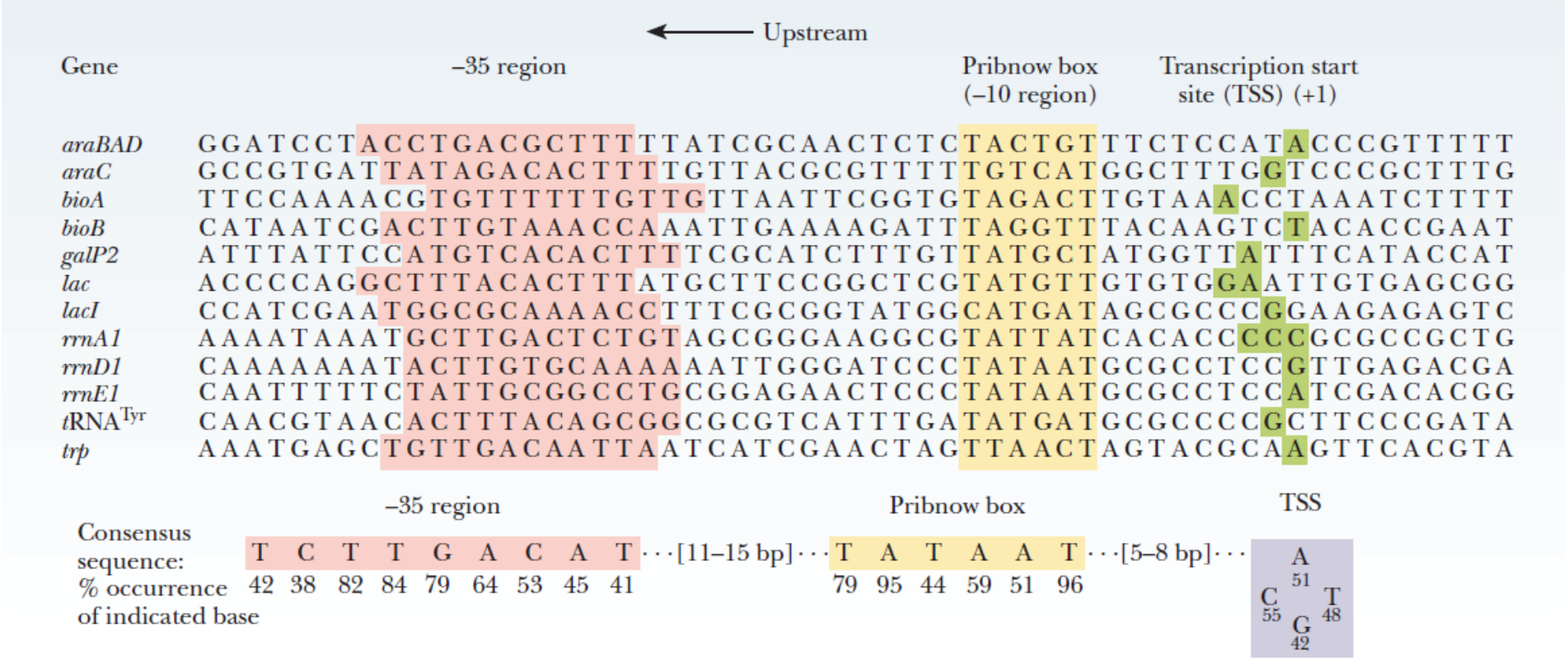


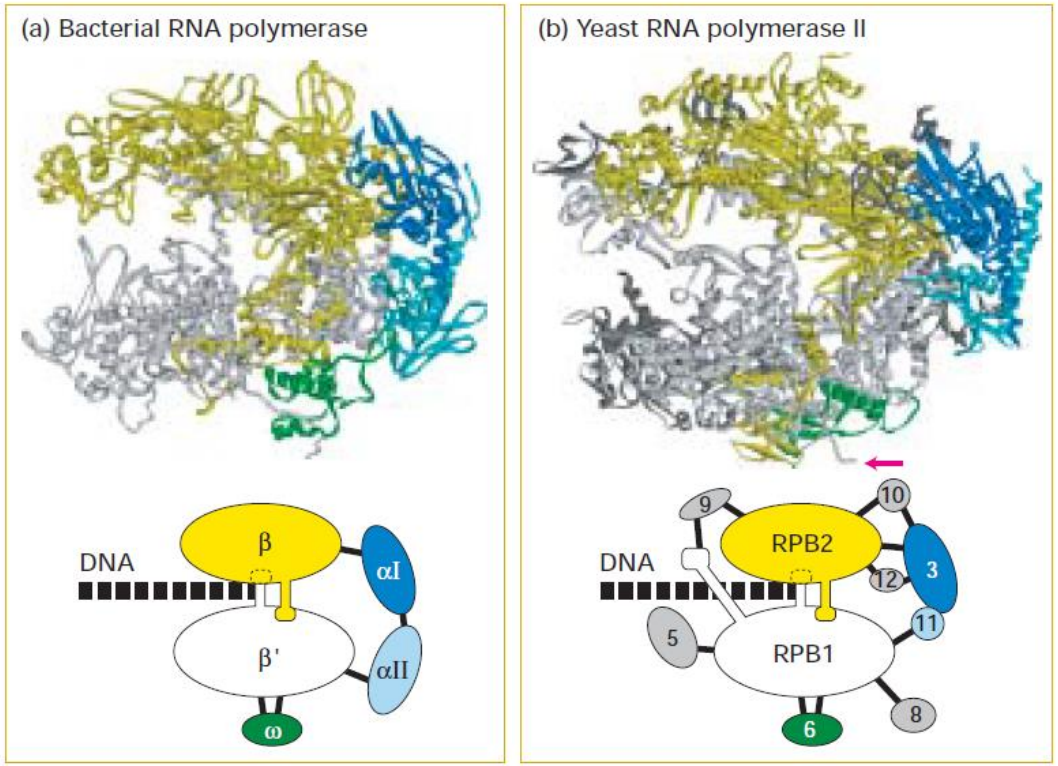
■ **FIGURE 11.1 The basics of transcription.** RNA polymerase uses the template strand of DNA to make an RNA transcript that has the same sequence as the nontemplate DNA strand, with the exception that T is replaced by U. If this RNA is mRNA, it can later be translated to protein.

Promoter Structure



■ **FIGURE 11.2** **Sequences of representative promoters from *E. coli*.** By convention, these are given as the sequence that would be found on the coding strand going from left to right as the 5' to 3' direction. The numbers below the consensus sequences indicate the percentage of the time that a certain position is occupied by the indicated nucleotide.

RNA Polymerase



▲ FIGURE 11-5 Comparison of three-dimensional structures of bacterial and eukaryotic RNA polymerases. These C_α trace models are based on x-ray crystallographic analysis of RNA polymerase from the bacterium *T. aquaticus* and RNA polymerase II from *S. cerevisiae*. (a) The five subunits of the bacterial enzyme are distinguished by color. Only the N-terminal domains of the α subunits are included in this model. (b) Ten of the twelve subunits constituting yeast RNA polymerase II are shown in this model. Subunits that are similar in conformation to those in the bacterial enzyme are shown in the same colors. The C-terminal domain of the large subunit RPB1 was not observed in the crystal structure, but it is known to extend from the position marked with a red arrow. (RPB is the abbreviation for "*R*NA *p*olymerase *B*," which is an alternative way of referring to RNA polymerase II.) [Part (a) based on crystal structures from G. Zhang et al.,1999, *Cell* **98**:811. Part (b) from P. Cramer et al., 2001, *Science* **292**:1863.]

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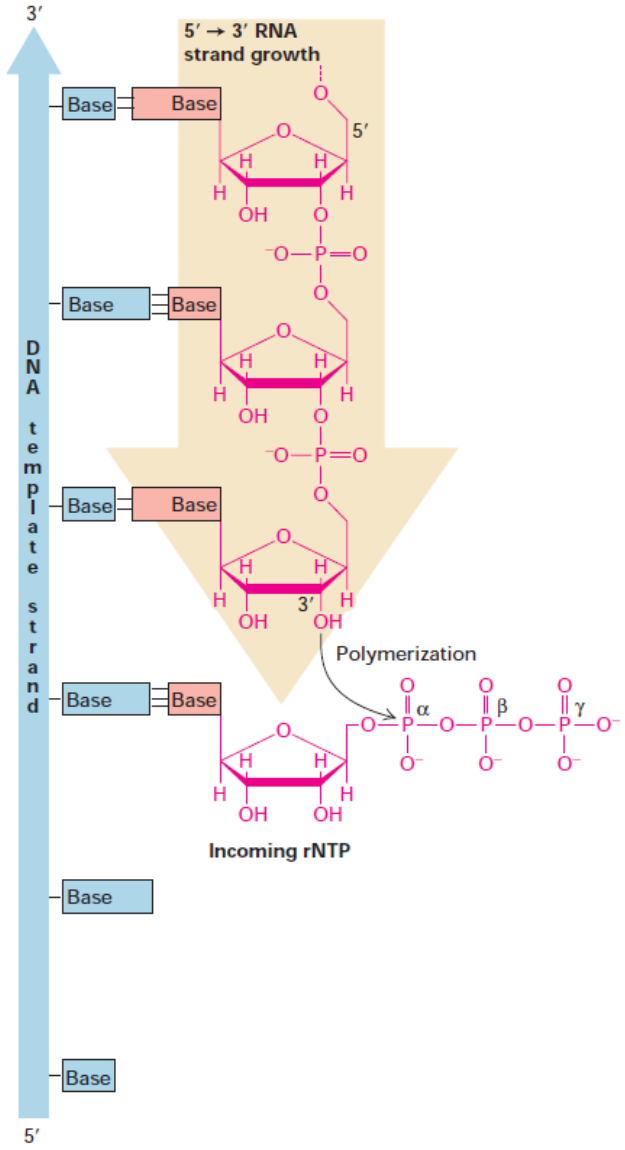
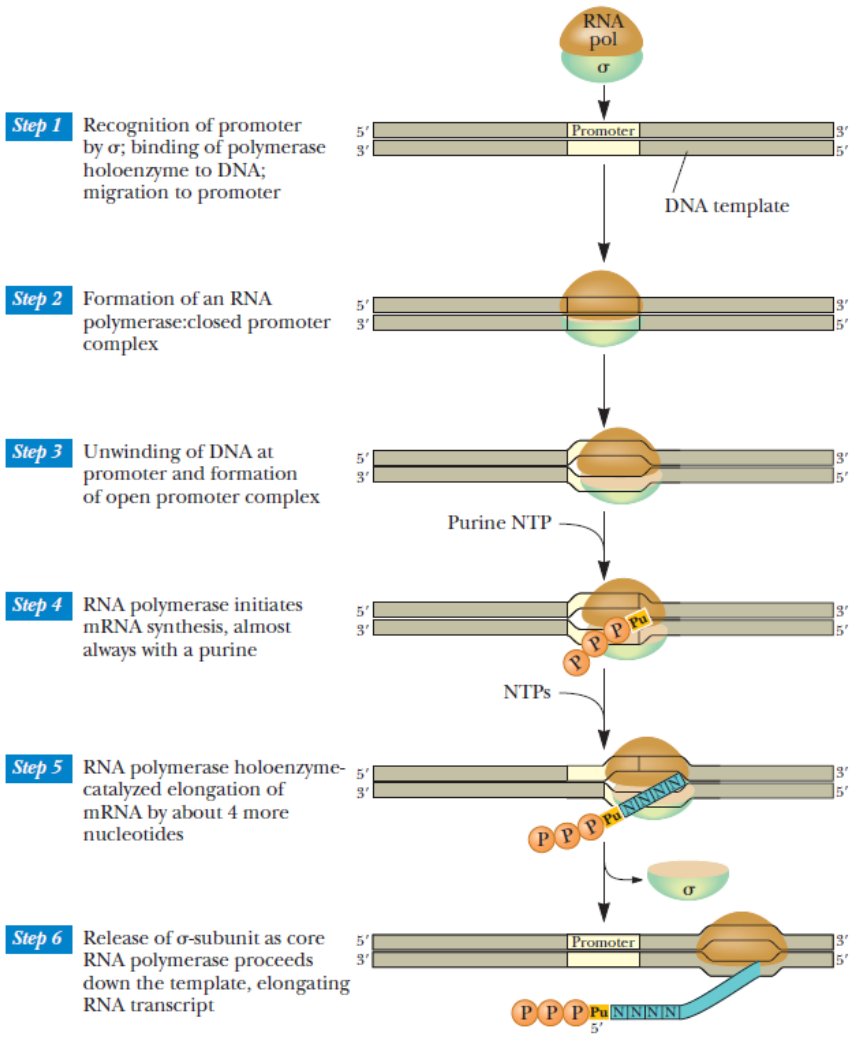


Polymerization of ribonucleotides by RNA polymerase during transcription. The ribonucleotide to be added at the 3 end of a growing RNA strand is specified by base pairing between the next base in the template DNA strand and the complementary incoming ribonucleoside triphosphate (rNTP). A phosphodiester bond is formed when RNA polymerase catalyzes a reaction between the 3 O of the growing strand and the phosphate of a correctly base-paired rNTP. RNA strands always are synthesized in the 5n3 direction and are opposite in polarity to their template DNA strands.

Transcription in prokaryotes



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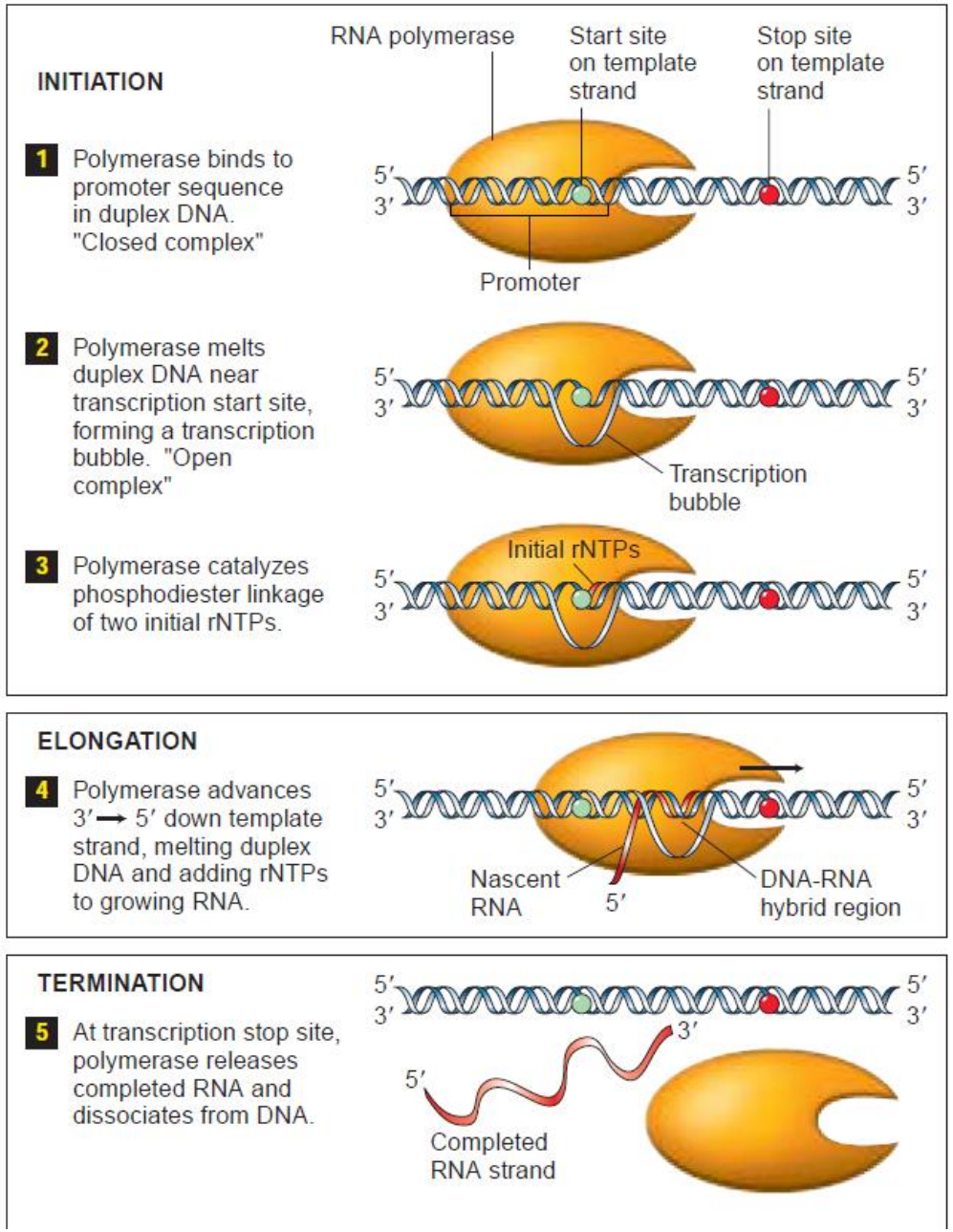
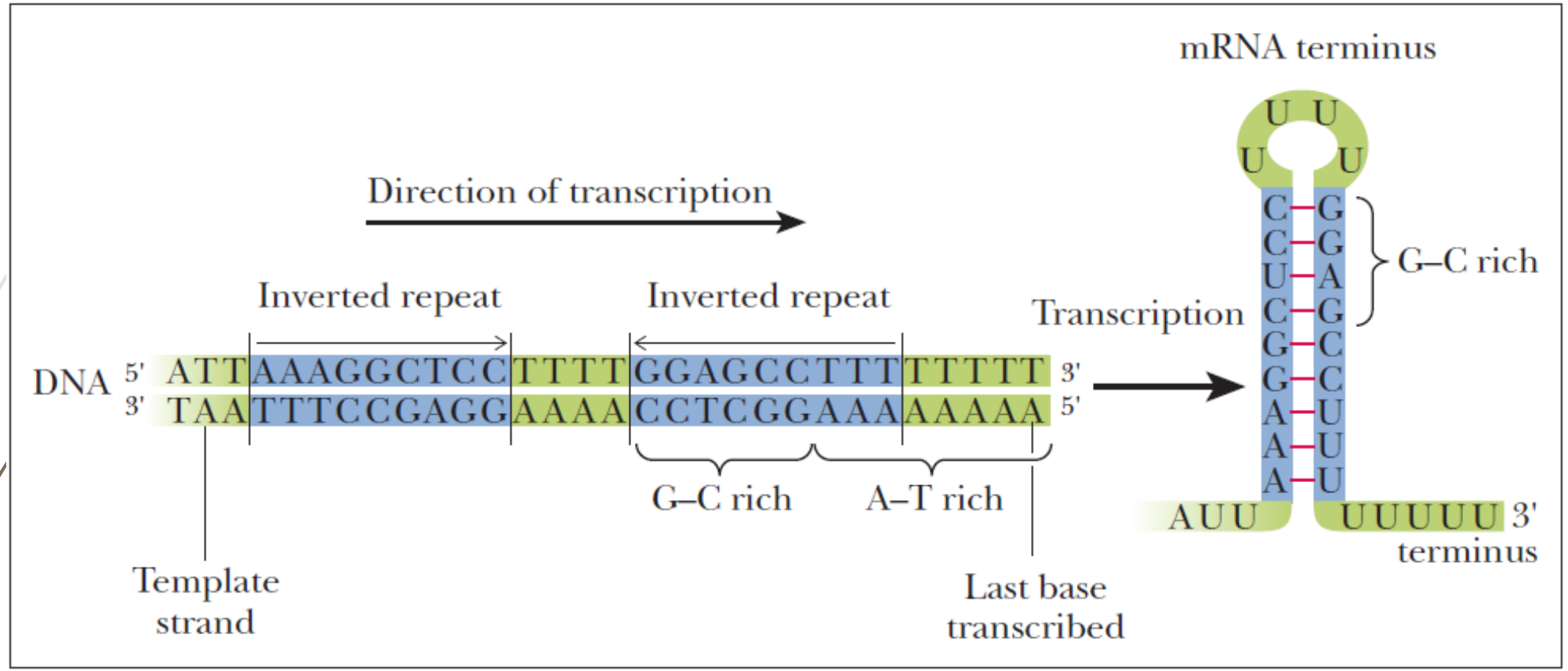
■ ACTIVE FIGURE 11.3 **Sequence of events in the initiation and elongation phases of transcription as it occurs in prokaryotes.** Nucleotides in this region are numbered with reference to the base at the transcription start site, which is designated +1. **Sign in at www.thomsonedu.com/login to explore an interactive version of this figure.**

Transcription



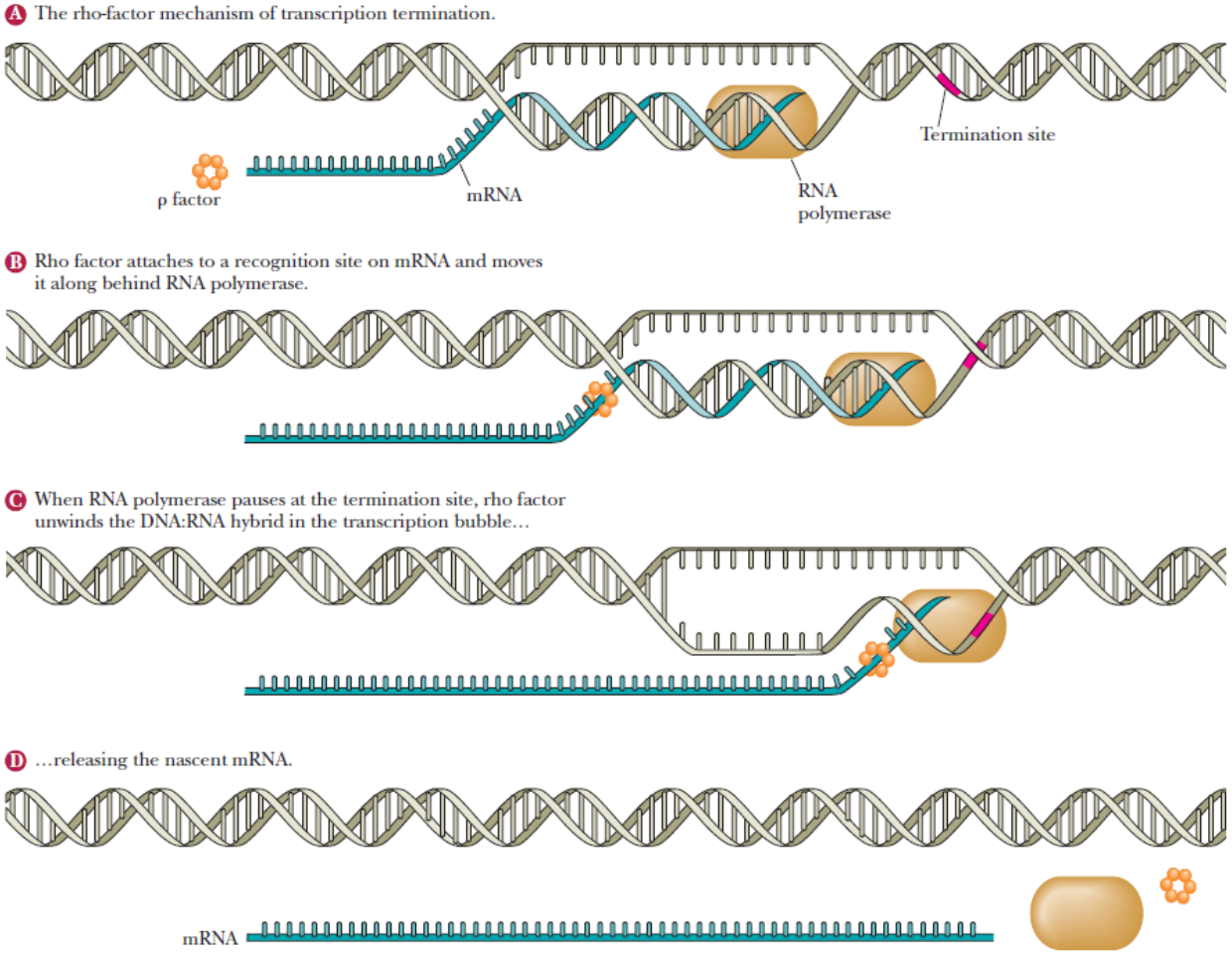
Three stages in transcription. During initiation of transcription, RNA polymerase forms a transcription bubble and begins polymerization of ribonucleotides (rNTPs) at the start site, which is located within the promoter region. Once a DNA region has been transcribed, the separated strands reassociate into a double helix, displacing the nascent RNA except at its 3 end. The 5’ end of the RNA strand exits the RNA polymerase through a channel in the enzyme. Termination occurs when the polymerase encounters a specific termination sequence (stop site). See the text for details.

Rho-Independent termination



Inverted repeats terminate transcription. Inverted repeats in the DNA sequence being transcribed can lead to an mRNA molecule that forms a hairpin loop. This is often used to terminate transcription.

Rho-dependent termination



■ **FIGURE 11.7 The rho-factor mechanism of transcription termination.** Rho (ρ) factor (a) attaches to a recognition site on mRNA and (b) moves along it behind RNA polymerase. (c) When RNA polymerase pauses at the termination site, rho factor unwinds the DNA:RNA hybrid in the transcription bubble, releasing the nascent mRNA (d).

Table 11.2

Yeast RNA Polymerase II Subunits

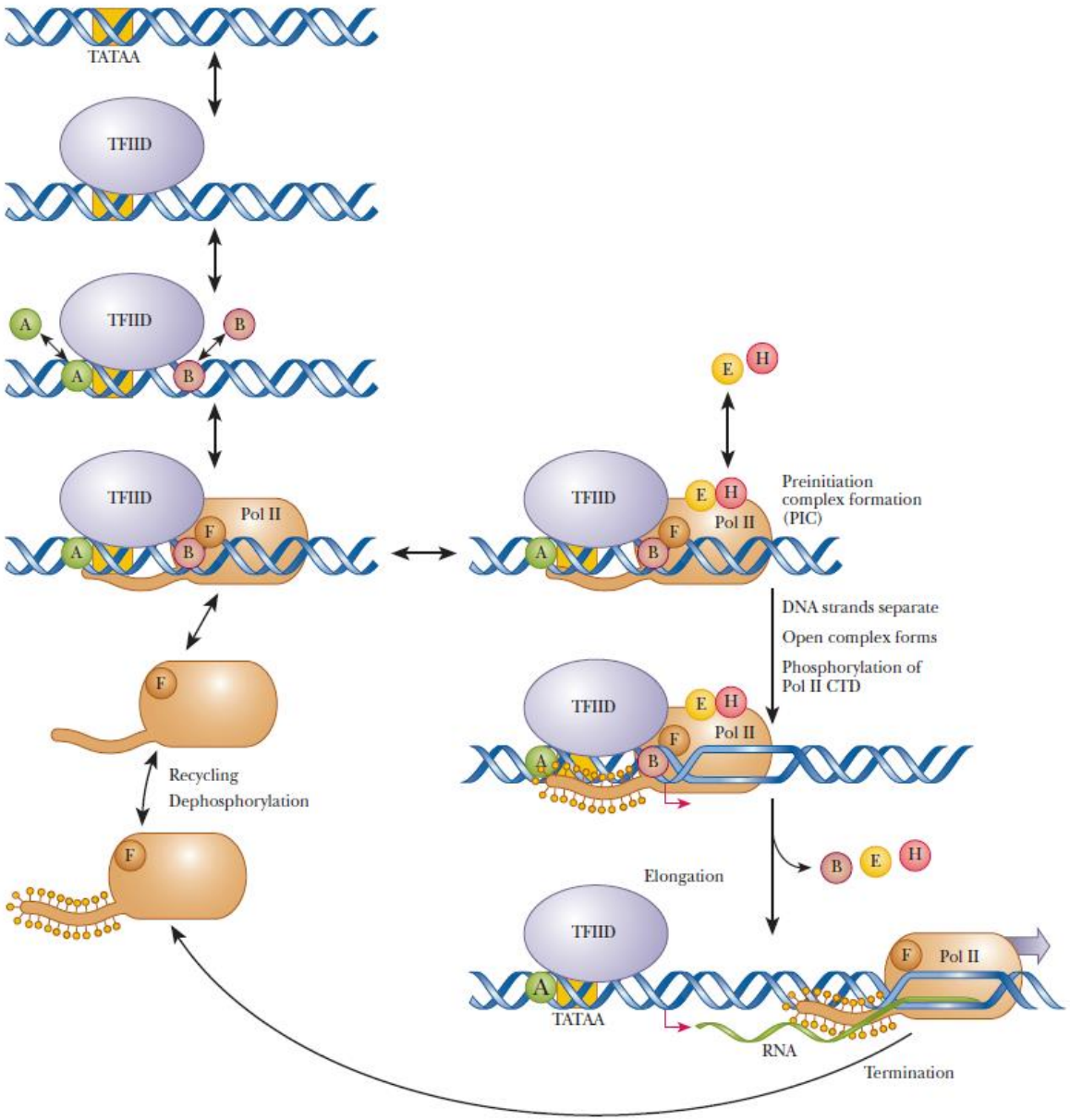
Subunit	Size (kDa)	Features	E. coli Homologue
RPB1	191.6	Phosphorylation site	β'
RPB2	138.8	NTP binding site	β
RPB3	35.3	Core assembly	α
RPB4	25.4	Promoter recognition	σ
RPB5	25.1	In Pol I, II, and III	
RPB6	17.9	In Pol I, II, and III	
RPB7	19.1	Unique to Pol II	
RPB8	16.5	In Pol I, II, and III	
RPB9	14.3		
RPB10	8.3	In Pol I, II, and III	
RPB11	13.6		
RPB12	7.7	In Pol I, II, and III	

Table 11.3

General Transcription Initiation Factors

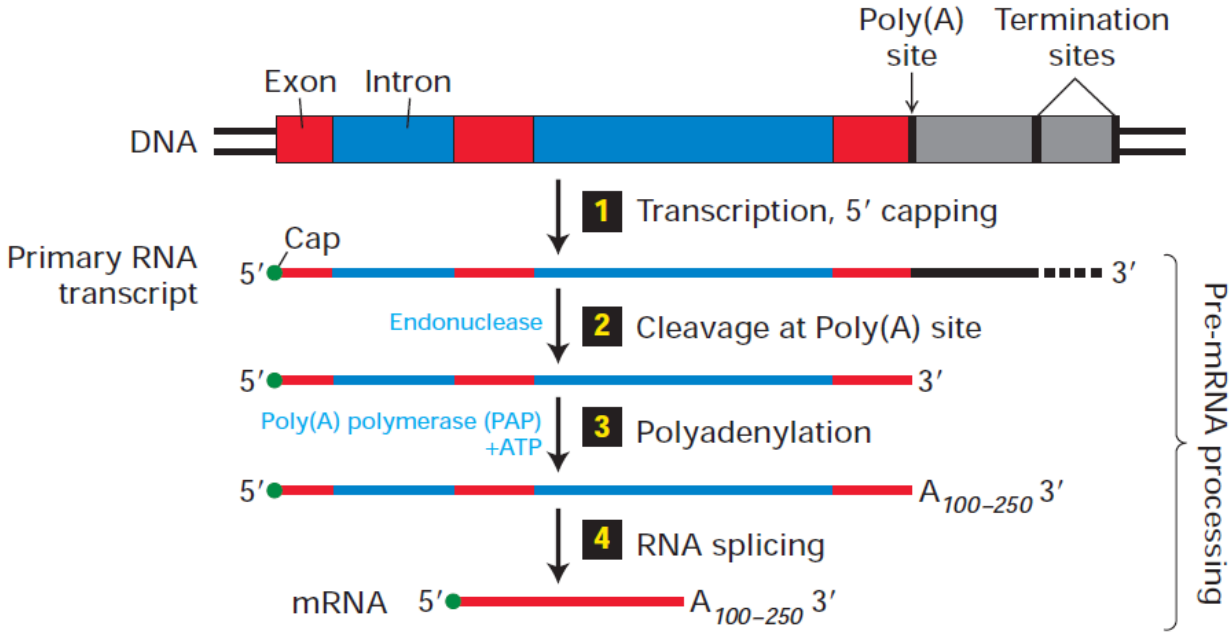
Factor	Subunits	Size (kDa)	Function
TFIID-TBP	1	27	TATA box recognition, positioning of TATA box DNA around TFIIB and Pol II
TFIID-TAF_{II}s	14	15–250	Core promoter recognition (non-TATA elements), positive and negative regulation
TFIIA	3	12, 19, 35	Stabilization of TBP binding; stabilization of TAF–DNA binding
TFIIB	1	38	Recruitment of Pol II and TFIIF; start-site recognition for Pol II
TFIIF	3	156 total	Promoter targeting of Pol II
TFIIE	2	92 total	TFIIH recruitment; modulation of TFIIH helicase ATPase, and kinase activities; promoter melting
TFIIH	9	525 total	Promoter melting; promoter clearance via phosphorylation of CTD

Transcription in Eukaryotes



TFIID (which contains the TATA-box binding protein, TBP) binds to the TATA box. TFIIA and TFIIB then bind, followed by recruitment of RNA polymerase II and TFIIF. TFIIH and TFIIE then bind to form the preinitiation complex (PIC). Kinases phosphorylate the C-terminal domain of Pol II, leading to the open complex in which the DNA strands are separated. RNA is produced during elongation as Pol II and TFIIF leave the promoter and the other general transcription factors behind. Pol II dissociates during the termination phase, and the CTD is dephosphorylated. Pol II/TFIIF is then recycled to bind to another promoter.

mRNA processing in eukaryotes



▲ **FIGURE 12-2 Overview of mRNA processing in eukaryotes.** Shortly after RNA polymerase II initiates transcription at the first nucleotide of the first exon of a gene, the 5′ end of the nascent RNA is capped with 7-methylguanylate (step 1). Transcription by RNA polymerase II terminates at any one of multiple termination sites downstream from the poly(A) site, which is located at the 3′ end of the final exon. After the primary transcript is cleaved at the poly(A) site (step 2), a string of adenosine (A) residues is added (step 3). The poly(A) tail contains ≈250 A residues in mammals, ≈150 in insects, and ≈100 in yeasts. For short primary transcripts with few introns, splicing (step 4) usually follows cleavage and polyadenylation, as shown. For large genes with multiple introns, introns often are spliced out of the nascent RNA during its transcription, i.e., before transcription of the gene is complete. Note that the 5′ cap and sequence adjacent to the poly(A) tail are retained in mature mRNAs.