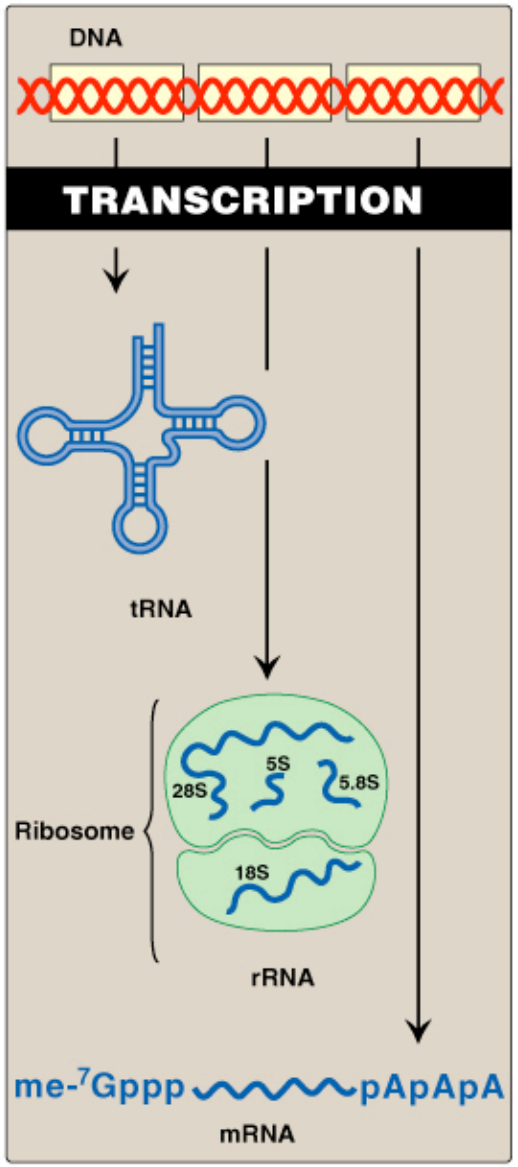
RNA Transcription and Processing

lecturer
J R Rodriguez Medina, Ph.D.

Gene expression by transcription



Genome

Transcriptome

Figure 30.1
Expression of genetic information by transcription. [Note: RNAs shown are eukaryotic.] me-^{7}Gppp = 7-methylguanosine triphosphate "cap," described on p. 414. AAA = poly-A tail, described on p. 414.

Ribosomal RNAs

23S and 28S rRNAs are ribozymes - catalyze the peptidyl transfer reaction

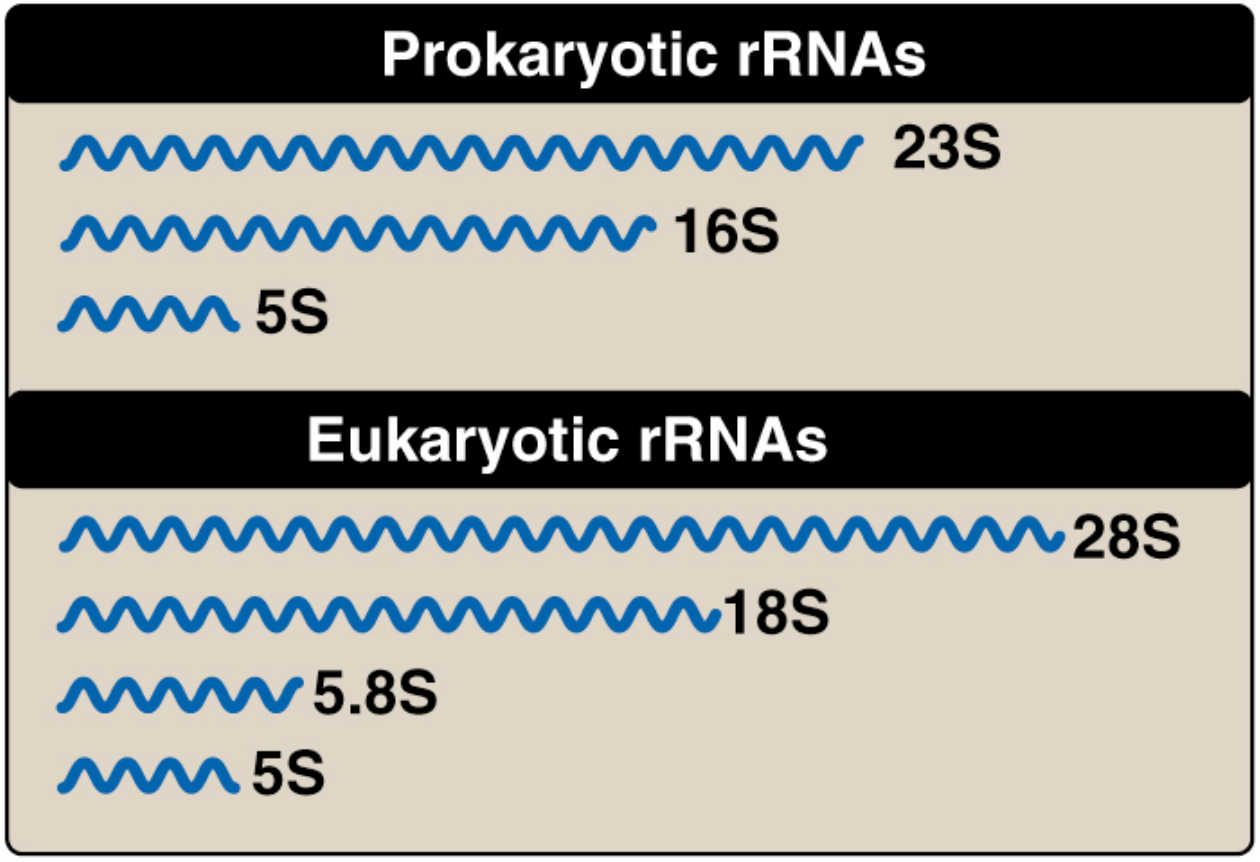


Figure 30.2
Prokaryotic and eukaryotic rRNAs.

tRNA structure

tRNAs function as adaptor molecules

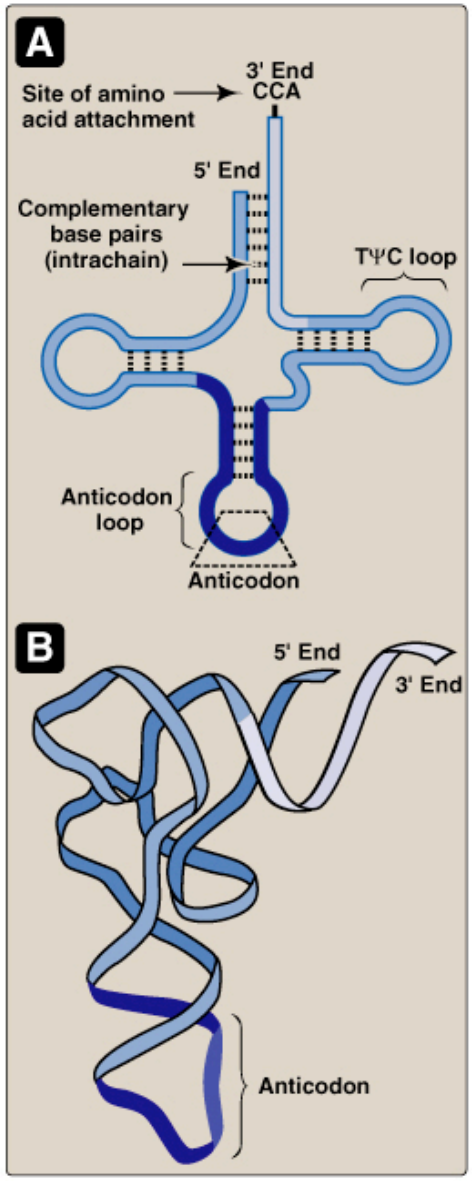


Figure 30.3

A. Characteristic tRNA structure.
B. Folded tRNA structure found in cells.

Eukaryotic mRNA Structure

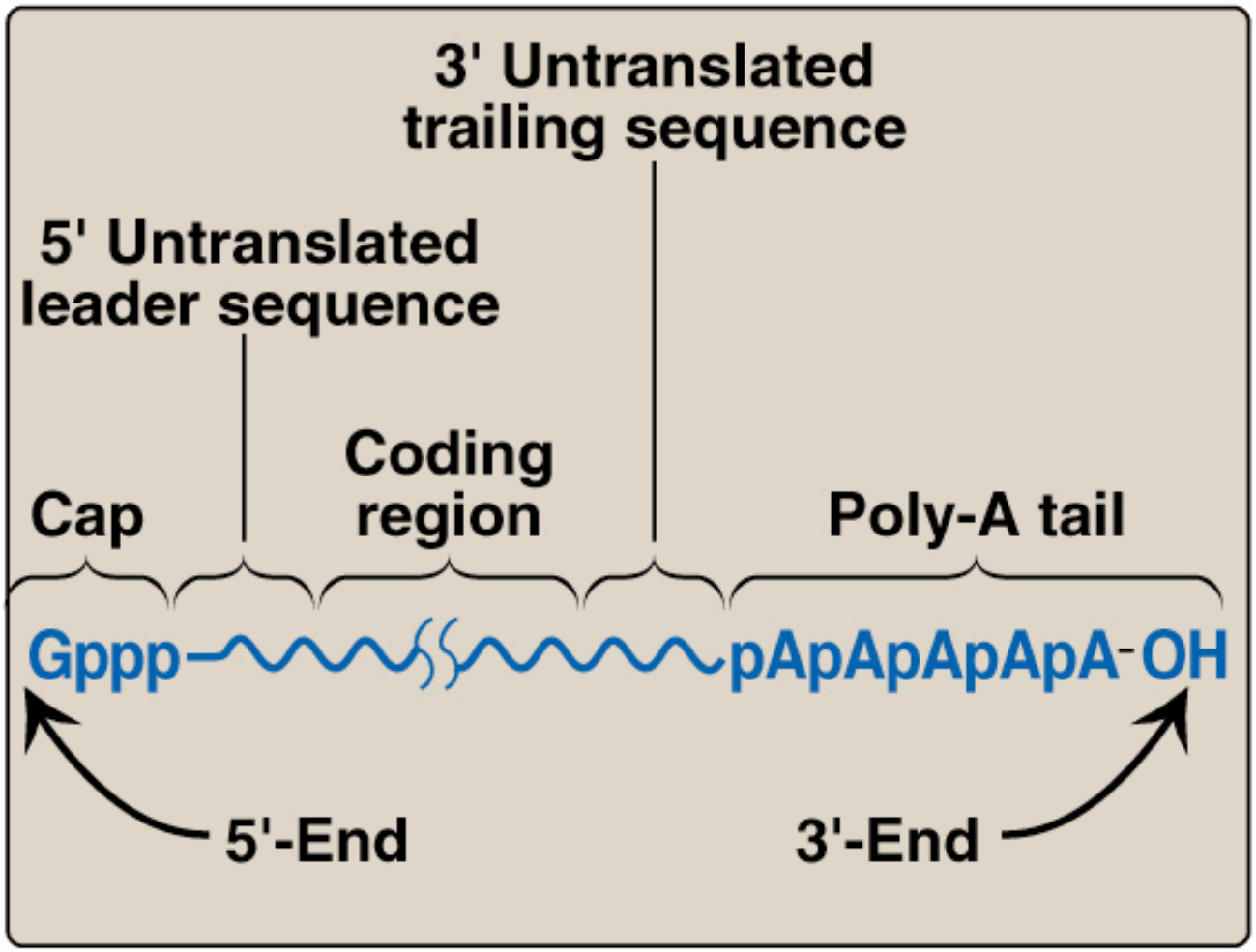


Figure 30.4
Structure of eukaryotic messenger RNA.

Three major types of RNA:

- Ribosomal RNA (rRNA)-about 80% of total
  processed by endonuclease cleavage, some modified bases. Modified in both prokaryotes and eukaryotes.

- Transfer RNA (tRNA)-about 15% of total
  Spliced, 5' and 3' ends trimmed, CCA sequence added at the 3' end, and bases are modified. Modified in both prokaryotes and eukaryotes.

- Messenger RNA (mRNA)-about 5% of total
  Spliced, capped at 5' end and polyA tail added at 3' end, only in eukaryotic cells. None of these modifications occur on prokaryote mRNAs.

RNA Polymerases

A single RNA polymerase in prokaryotes

multiple sigma factors

Three different nuclear RNA polymerases in eukaryotes

Pol I - rRNA (28S, 18S, 5.8S)
Pol II - mRNA and most snRNAs
Pol III- tRNA, 5S rRNA, some small nuclear RNAs

Eukaryotic RNA Pol II has a high sensitivity to the poisonous toxin α-Amanitin that inhibits mRNA synthesis.

Unlike DNA polymerases, RNA polymerases do not require a primer and do not have proofreading activity.

Prokaryotic RNA Polymerase

2α-assembly

1β'-template binding (interacts with TATA box and -35 sequence)

1β -5'->3' polymerase activity

σ - sigma factor, provides promoter specificity to core enzyme

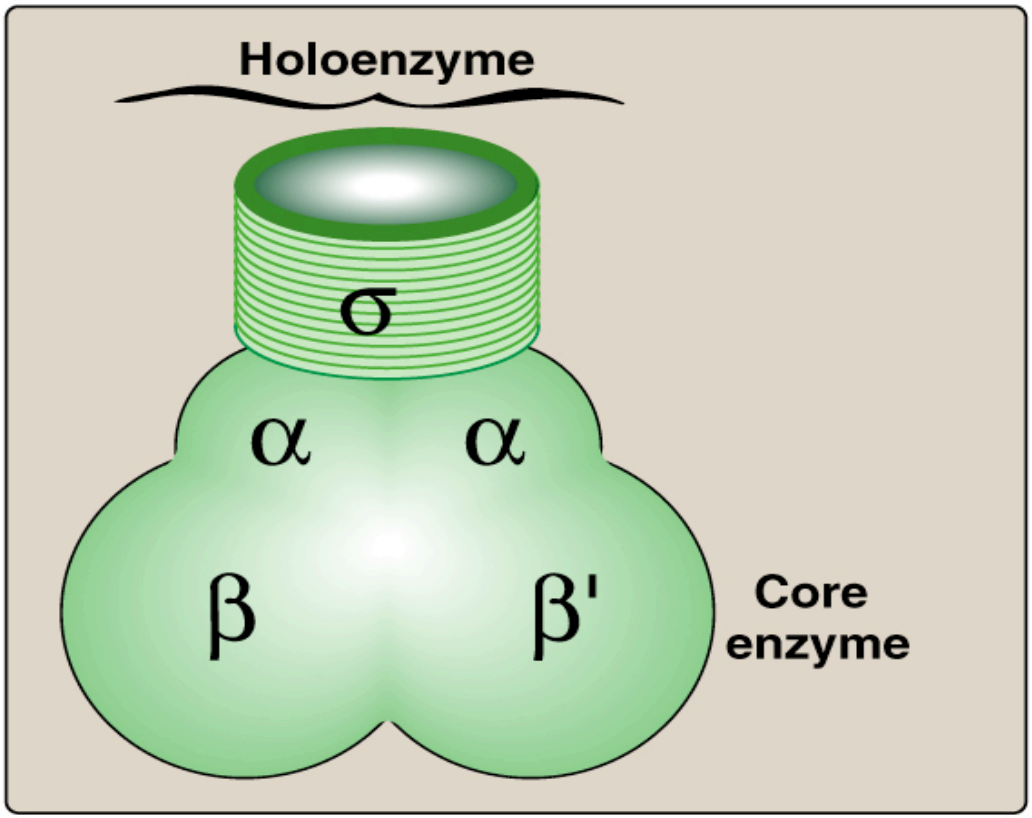
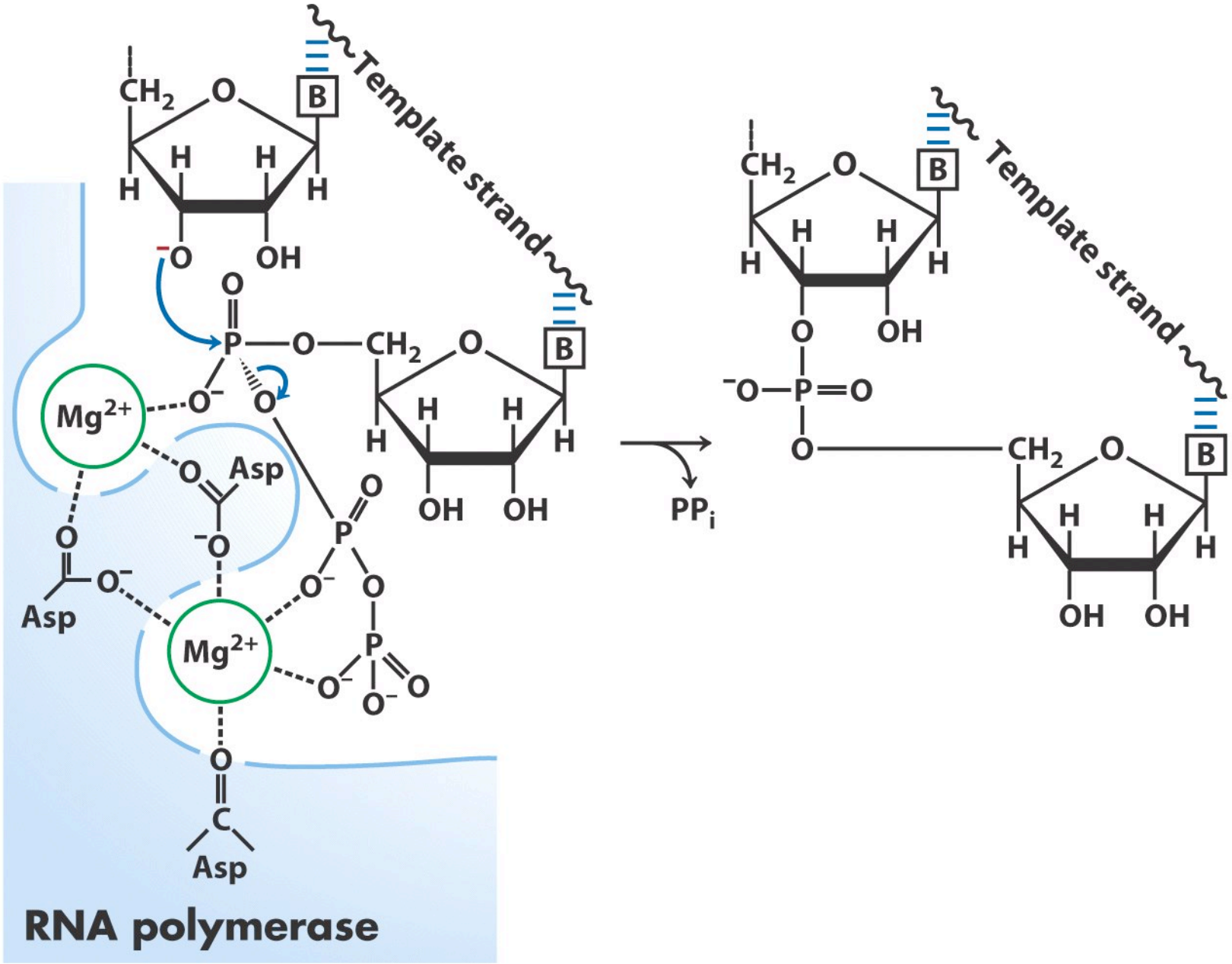


Figure 30.6
Prokaryotic *RNA polymerase.*

Transcription of RNA refers to reactions catalyzed by a DNA-dependent RNA polymerase.

$$\text{DNA template} + n(\text{NTP}) \rightarrow \text{pppN}(\text{pN})_{n-1} + (n-1)\text{PP}_i + \text{DNA template}$$

Transcription Reaction Mechanism



DNA-RNA complementarity during transcription
Like DNA replication, RNA synthesis proceeds in the 5'->3' direction

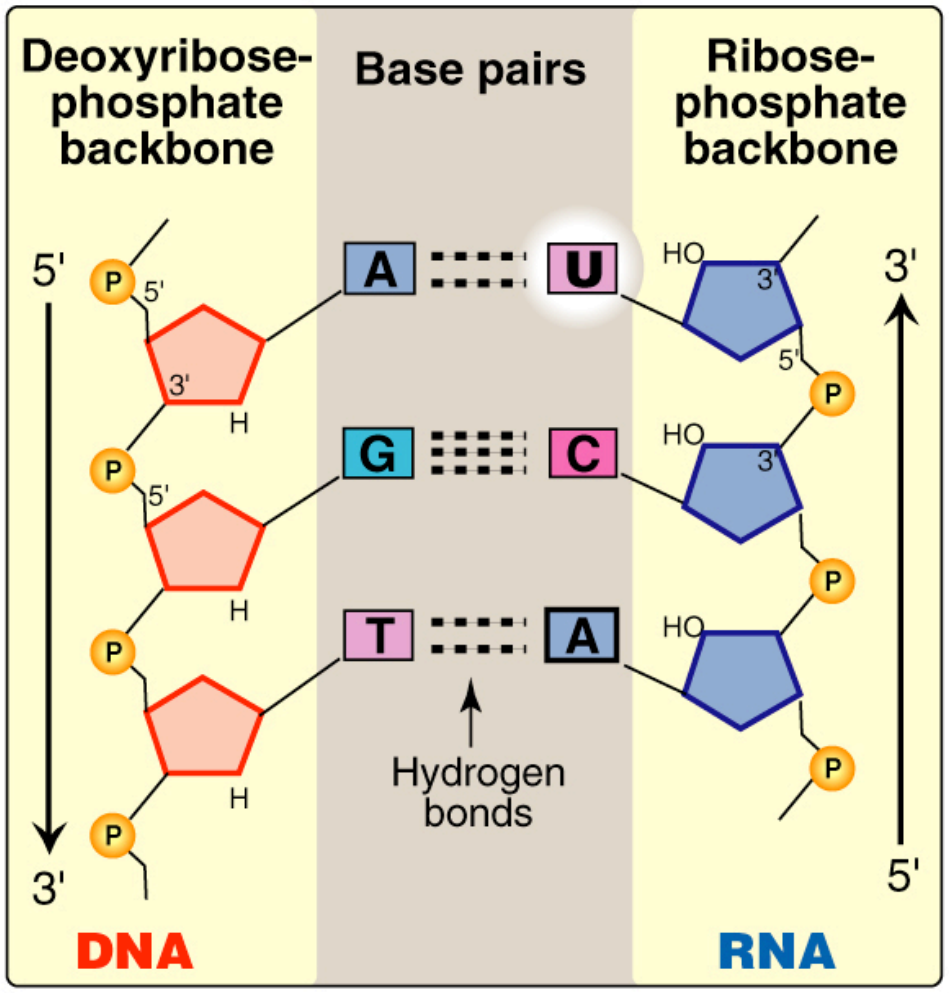


Figure 30.5
Antiparallel, complementary base pairs between DNA and RNA.

A promoter is a region on the DNA that contains nucleotide sequences recognized by RNA polymerase as the start-site for transcription. It determines the specificity of transcription.

Structure of the prokaryotic promoter

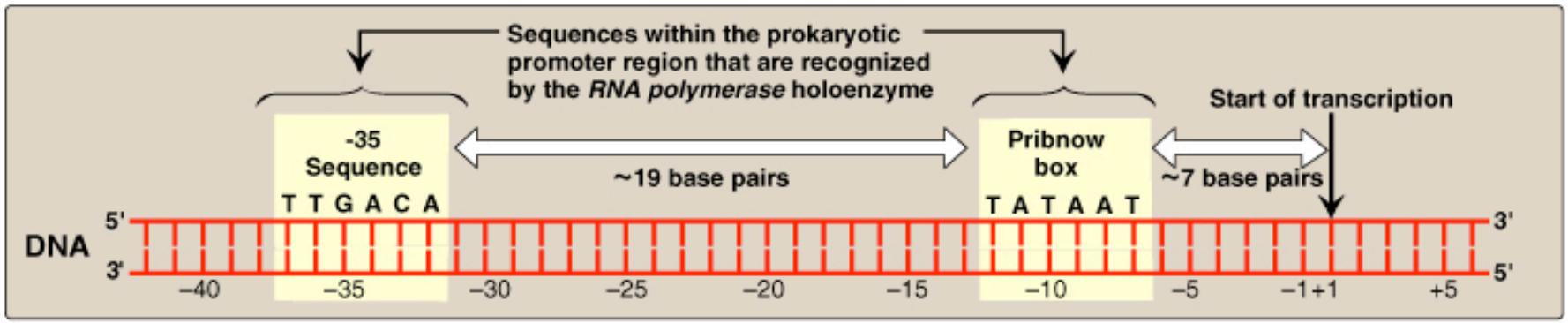


Figure 30.7
Structure of the prokaryotic promoter region.

Local unwinding caused by RNA polymerase during transcription generates supercoils that can be relaxed by DNA topoisomerases.

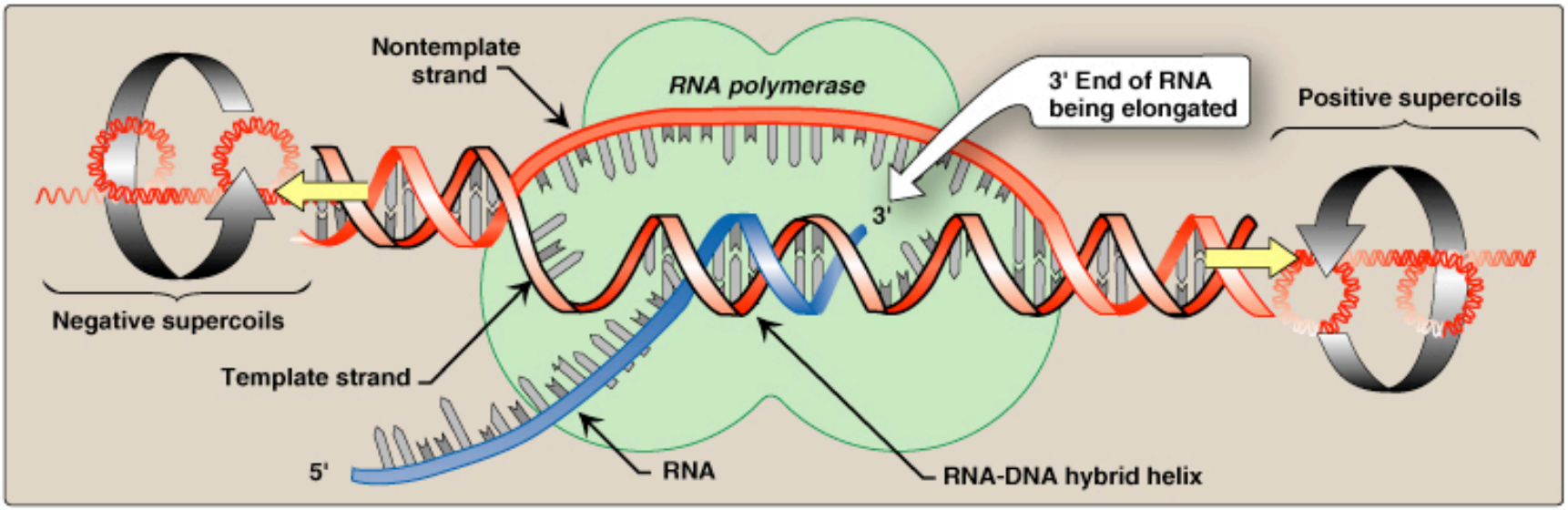


Figure 30.8
Local unwinding of DNA caused by *RNA polymerase*.

Early events in prokaryoric transcription

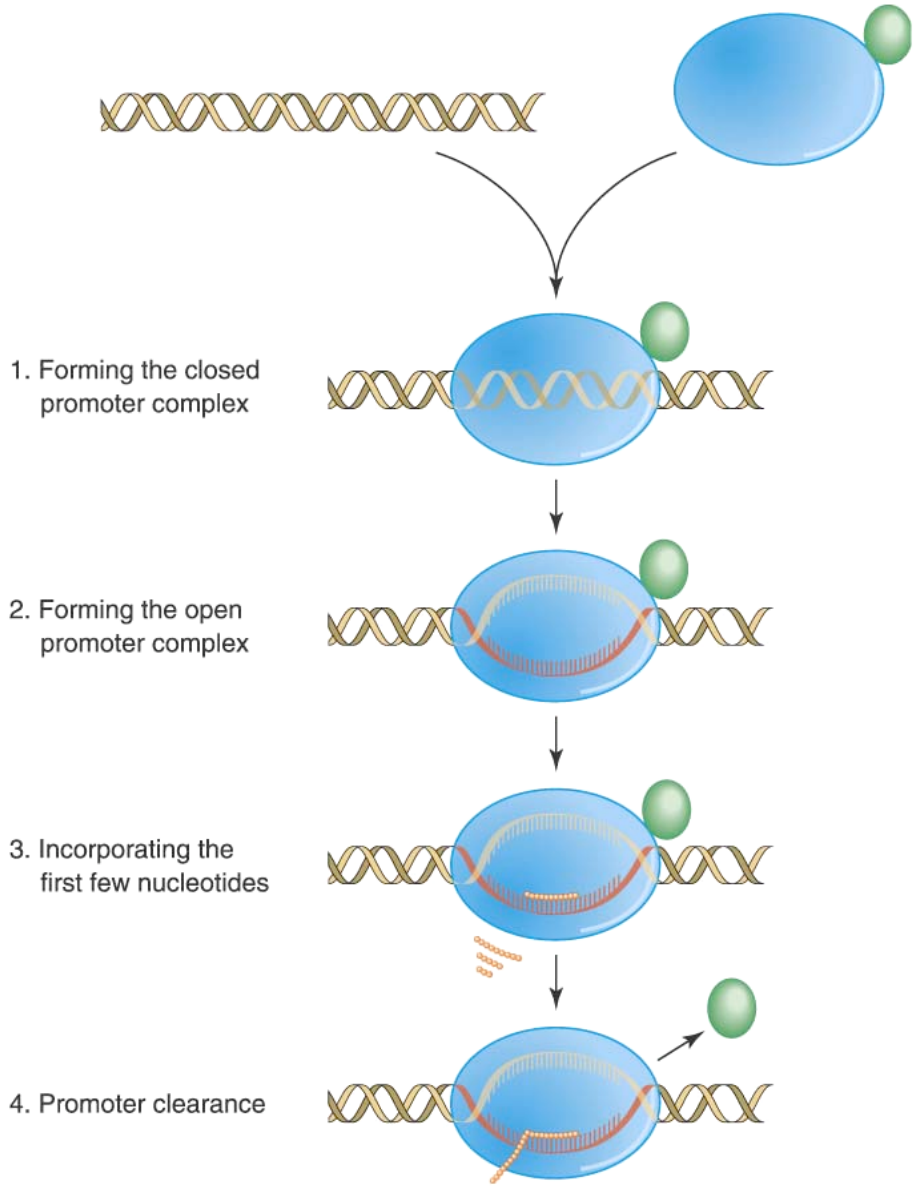


Figure 5.2 Early events in prokaryotic transcription.

Simultaneous transcription of a gene by multiple RNA polymerases can be visualized by electron microscopy.

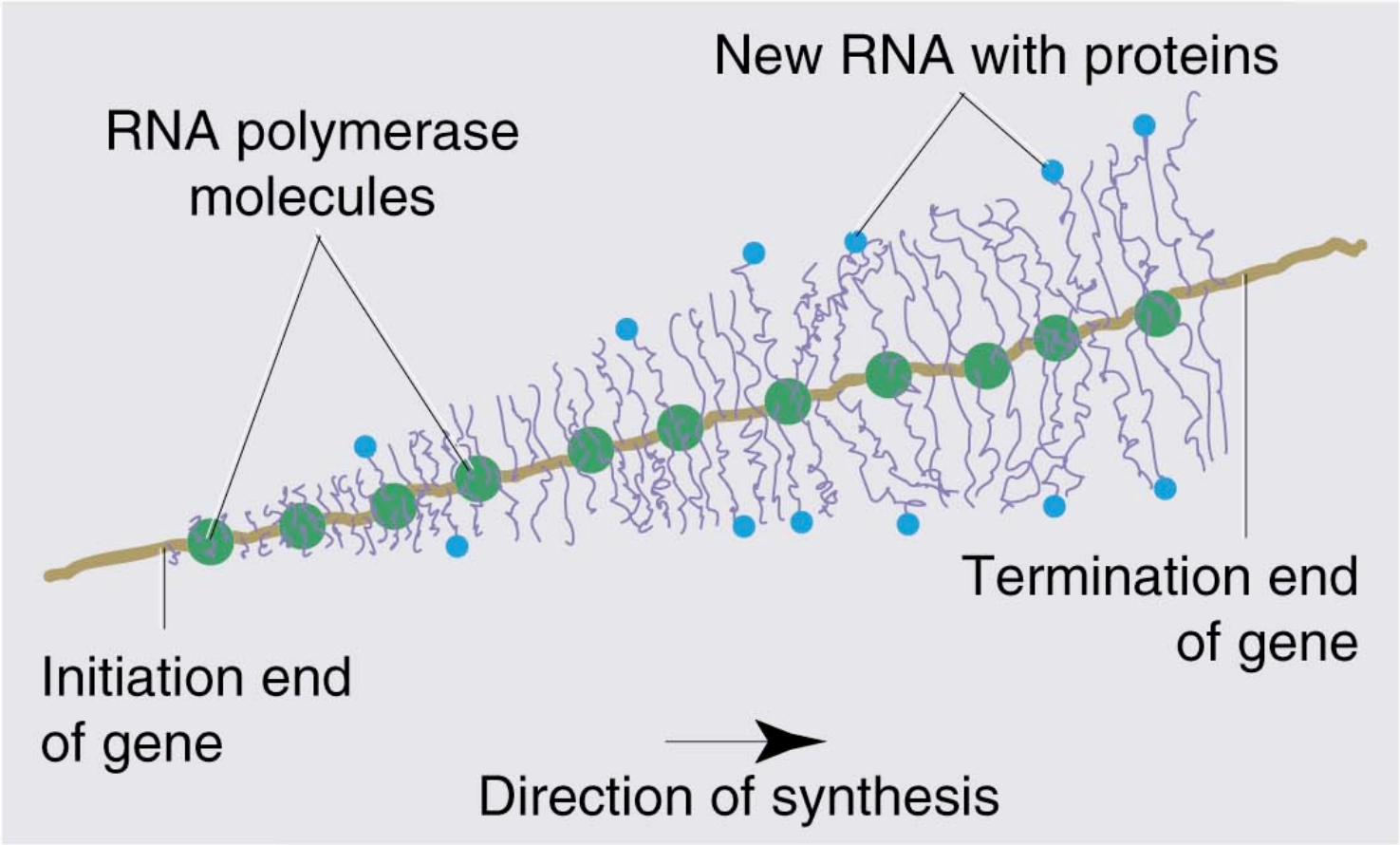


Figure 5.3. Simultaneous transcription of a gene by many RNA polymerases, depicting increasing length of nascent RNA molecules. Courtesy of Dr. O. L. Miller, University of Virginia. Reproduced with permission from Miller, O. L. and Beatty, B. R. *J . Cell Physiol.* 74:225, 1969.

Rho-dependent termination, is an enzymatic mechanism that employs a hexomeric ATP dependent RNA-DNA helicase.
Binds a Rho recognition site near the ‘3 end of the nascent RNA.
Migrates to the RNAP at these specific sites
Separates the mRNA transcript from the DNA template.

Rho-independent termination mechanism is non-enzymatic

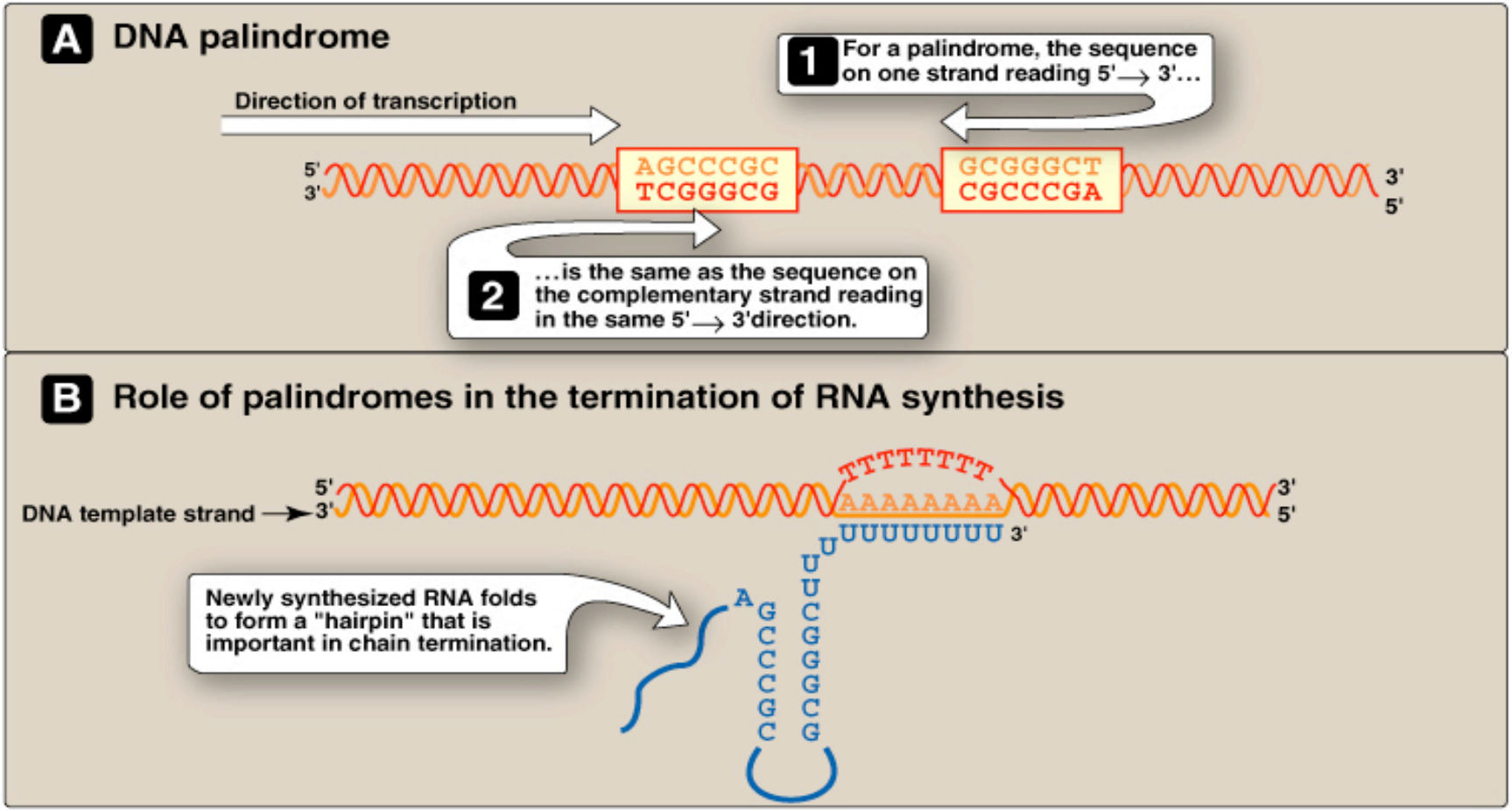
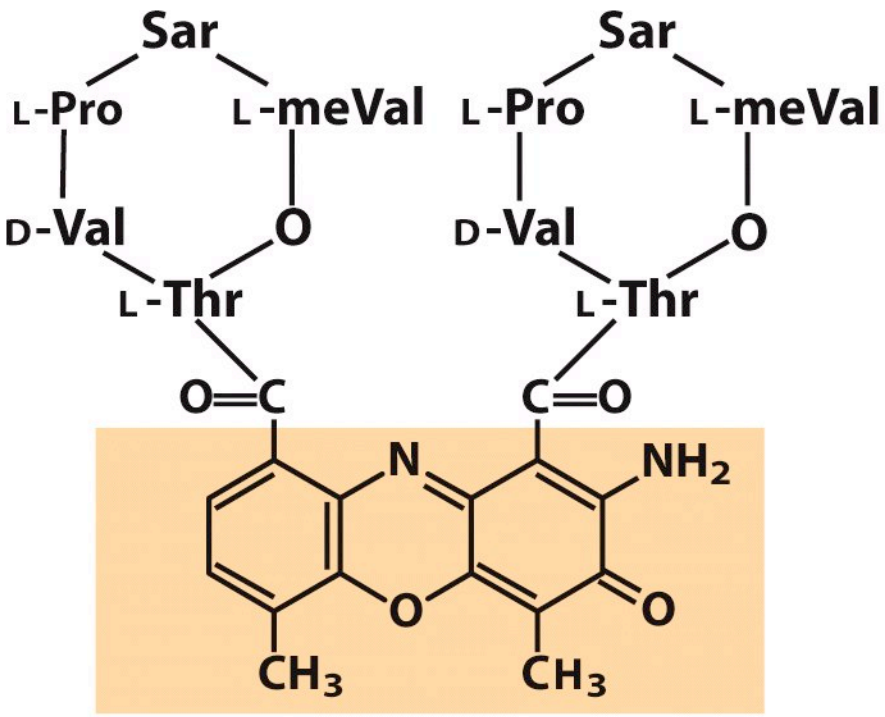
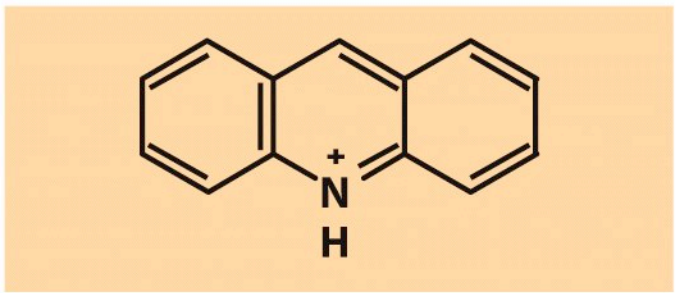


Figure 30.9
Rho-independent termination of transcription. A. An example of a palindrome in double-stranded DNA. B. A transcribed DNA palindrome codes for RNA that can form a hairpin turn.

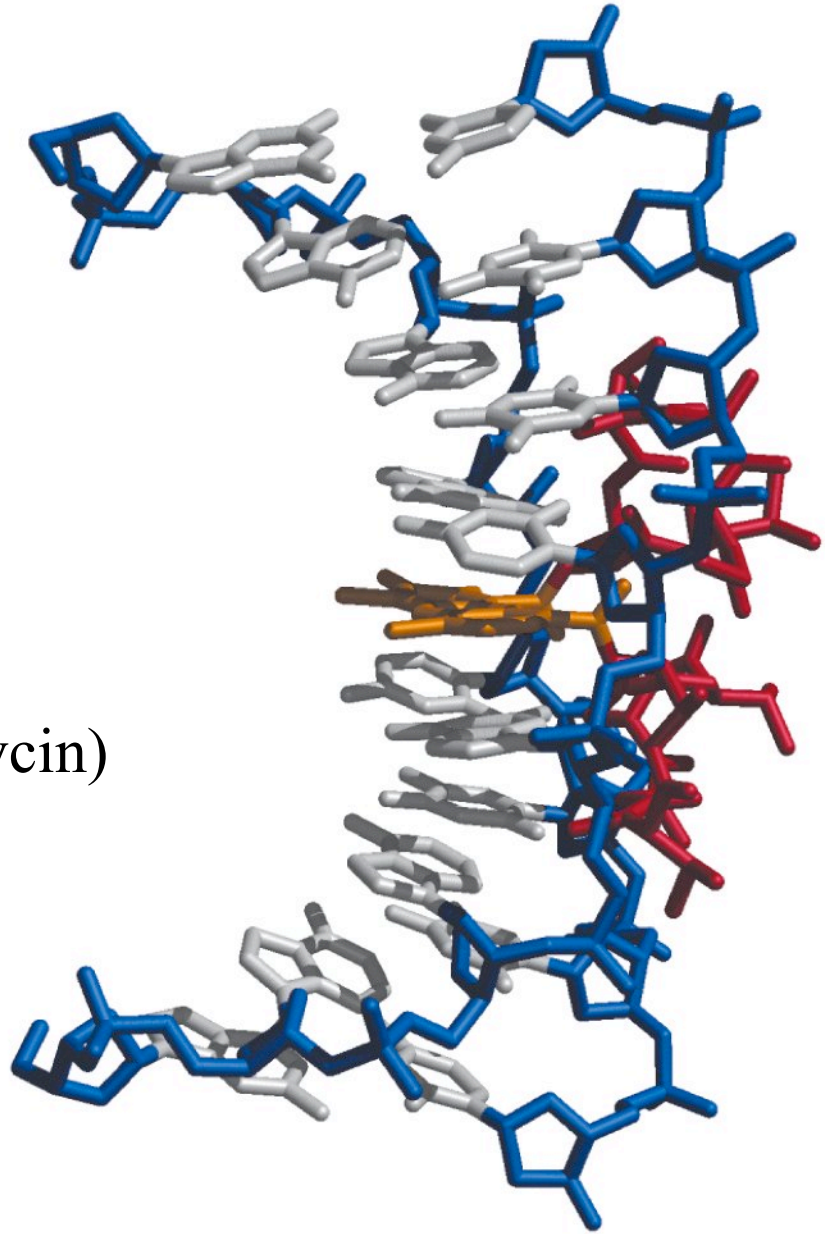
DNA intercalating antibiotic agents act as transcription inhibitors



Actinomycin D (Dactinomycin)



Acridine



Inactivation of bacterial RNA polymerase by Rifampin

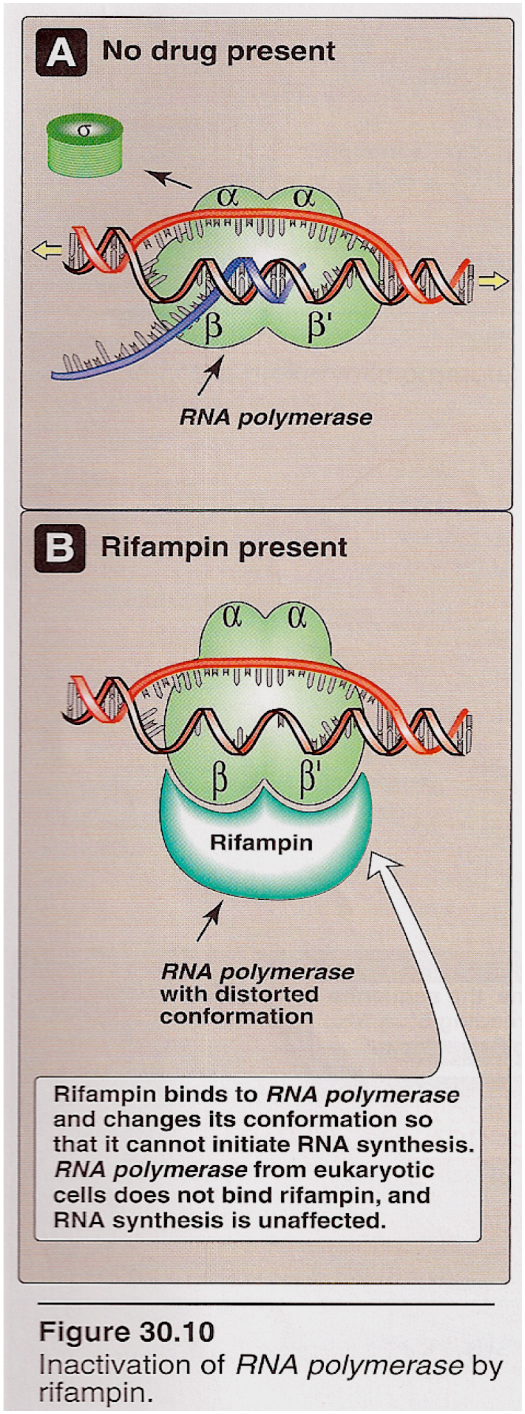


Figure 30.10
Inactivation of *RNA polymerase* by rifampin.

From Lippincott's Illustrated Reviews
Biochemistry, 3rd edition

The lactose operon of E. coli bacteria.

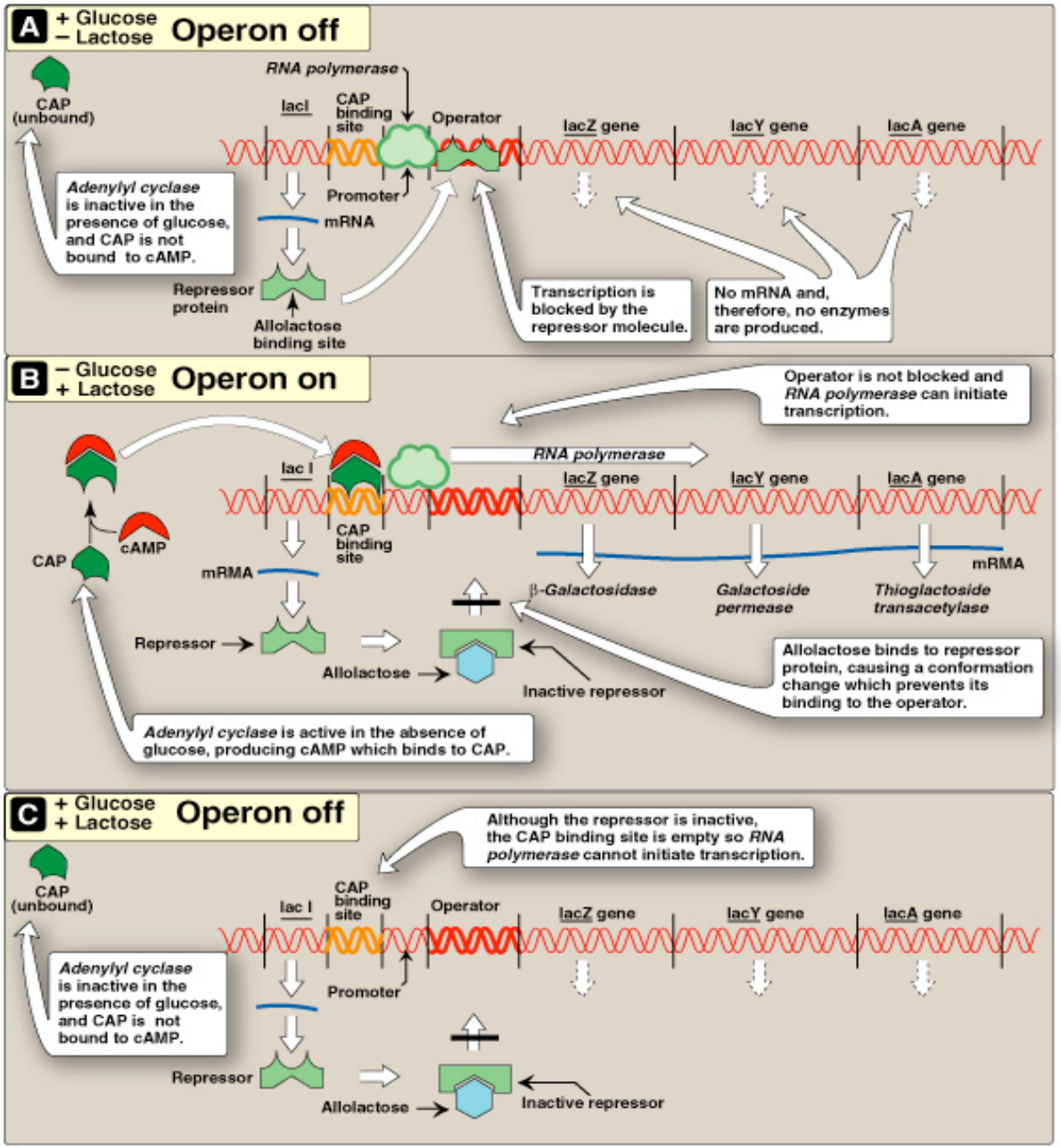


Figure 30.11
The lactose operon of E. coli.

Eukaryotic promoter structure
Contain DNA sequences recognized by proteins (transcription factors)
DNA sequences are “cis-acting elements”

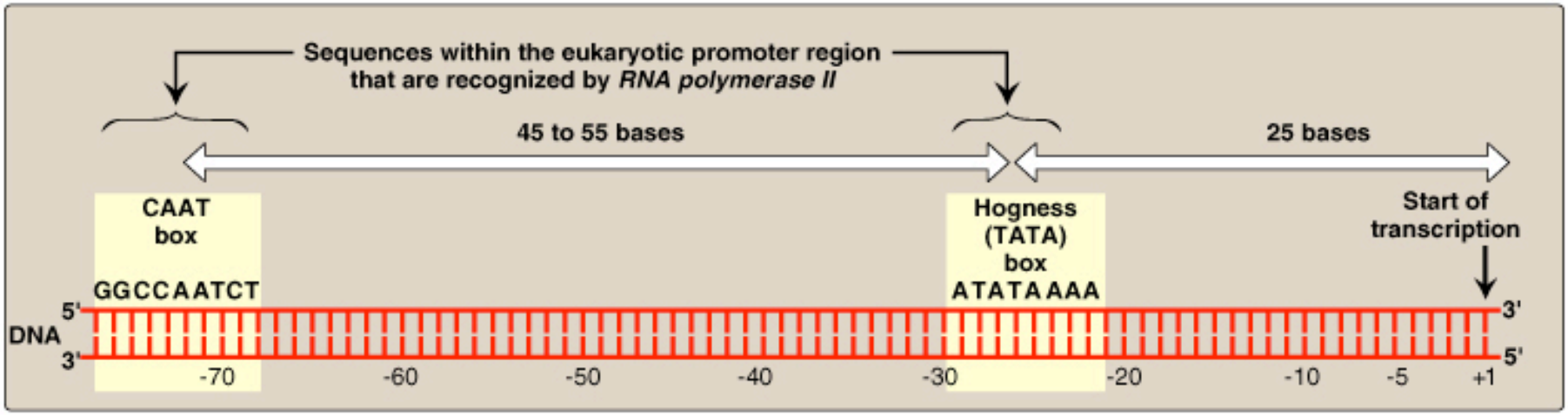


Figure 30.12
Eukaryotic gene promoter concensus sequences.

Eukaryotic **General Transcription Factors**(TFs)

TFs are proteins acting as "trans-acting factors"

TFIID-binds to TATA box

TFIIF-brings RNAP to the promoter

TFIIH- opens ds DNA and phosphorylates CTD

Co-activators-TFs that bind to other proteins such as HATs

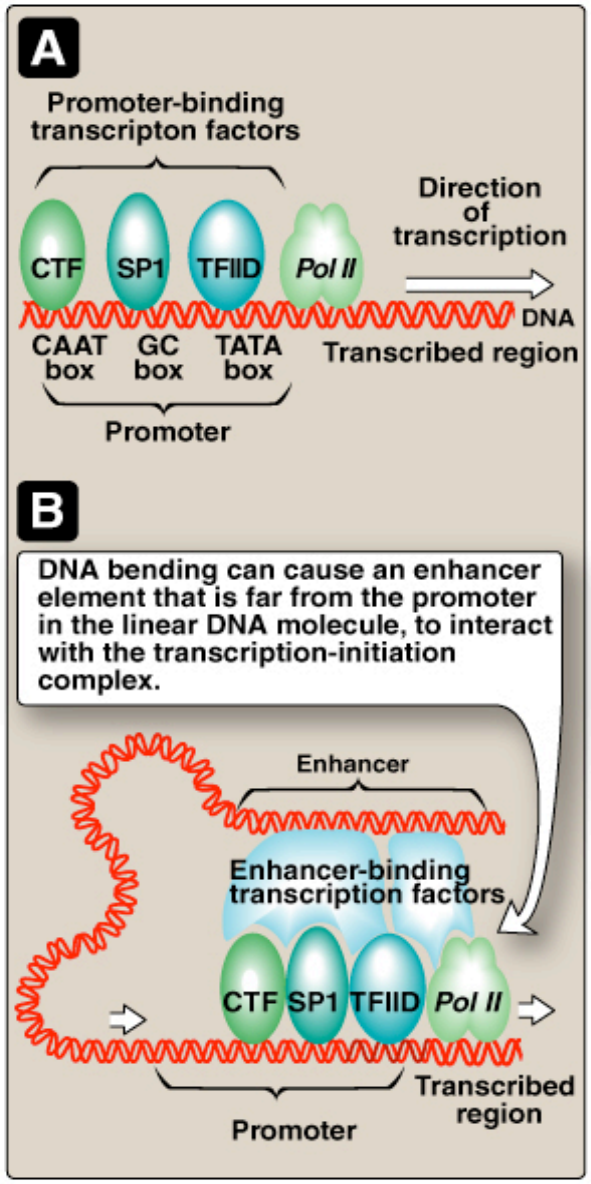


Figure 30.13

A. Eukaryotic general transcription factors bound to the promoter. CTF, SP1, and TFIID are general transcription factors. B. Enhancer stimulation of *RNA polymerase II*.

Enhancers

Contain response elements in their DNA sequence that bind TFs to activate gene transcription

Have similar characteristics to "Silencers" except that these repress gene transcription

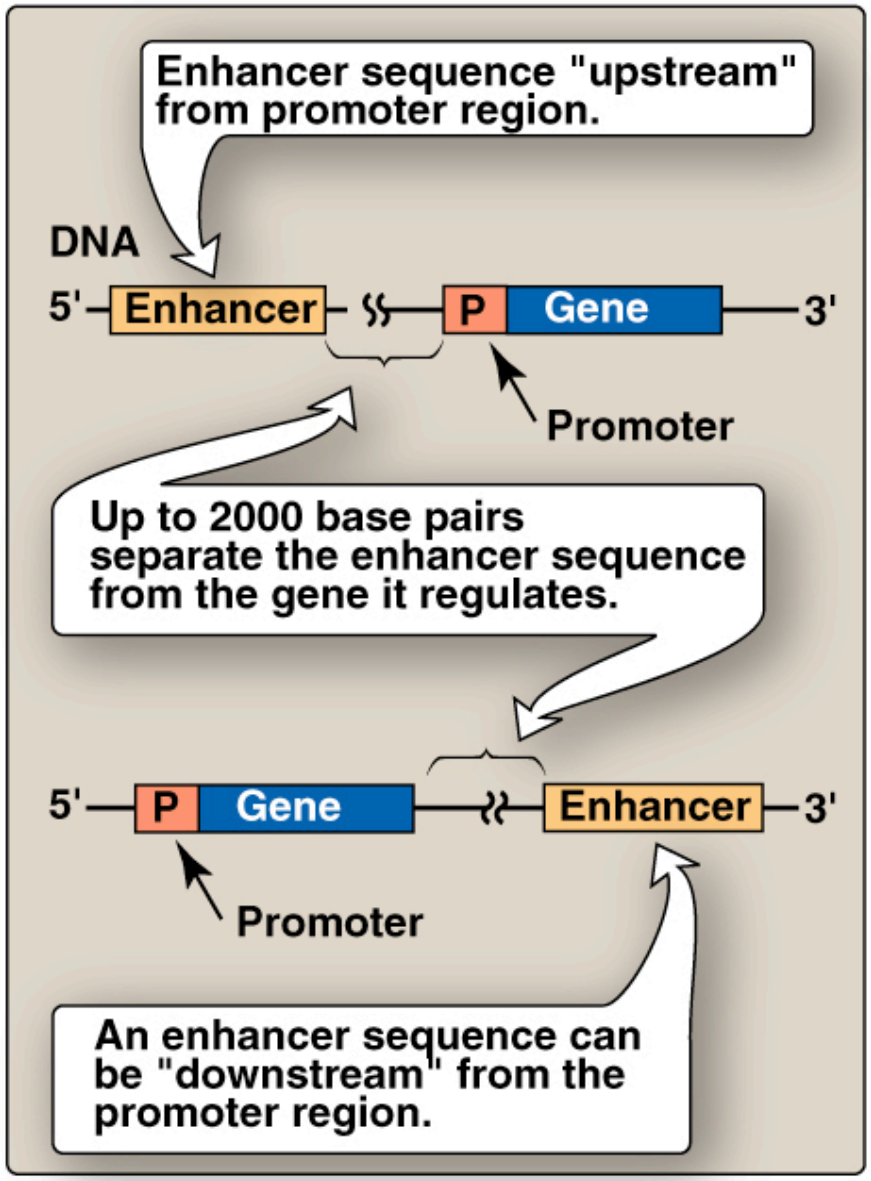


Figure 30.14
Some possible locations of enhancer sequences.

Chromatin Remodeling-Some general rules

Repressed chromatin DNA is hypermethylated (heterochromatin)
5-methyl cytosine in 5'CpG3' islands most common

Active chromatin DNA (euchromatin) is demethylated
Histone acetylation coincides with demethylation of DNA for gene activation

The Histone Code is a series of post-translational modifications on histones proposed to regulate gene activation

- phosphorylation
- **acetylation (regulated by HATs, HDACs)**
- methylation
- mono-ubiquitination

Post-transcriptional modifications of RNA

Eukaryotic rRNA processing steps

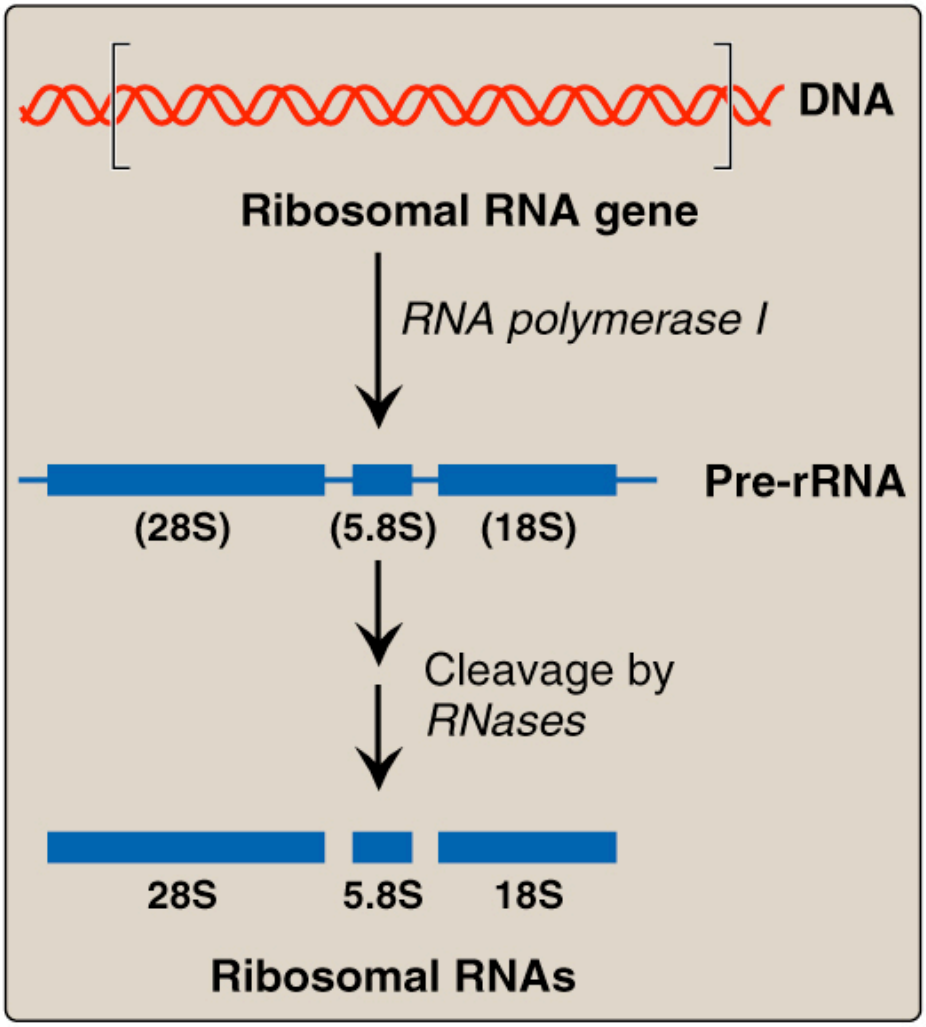


Figure 30.15
Posttranscriptional processing of eukaryotic ribosomal RNA by ribonucleases.

Primary transcript

Processing is carried out by Ribonucleases, Exonucleases, snoRNAs participate in base and sugar modifications

General tRNA processing steps

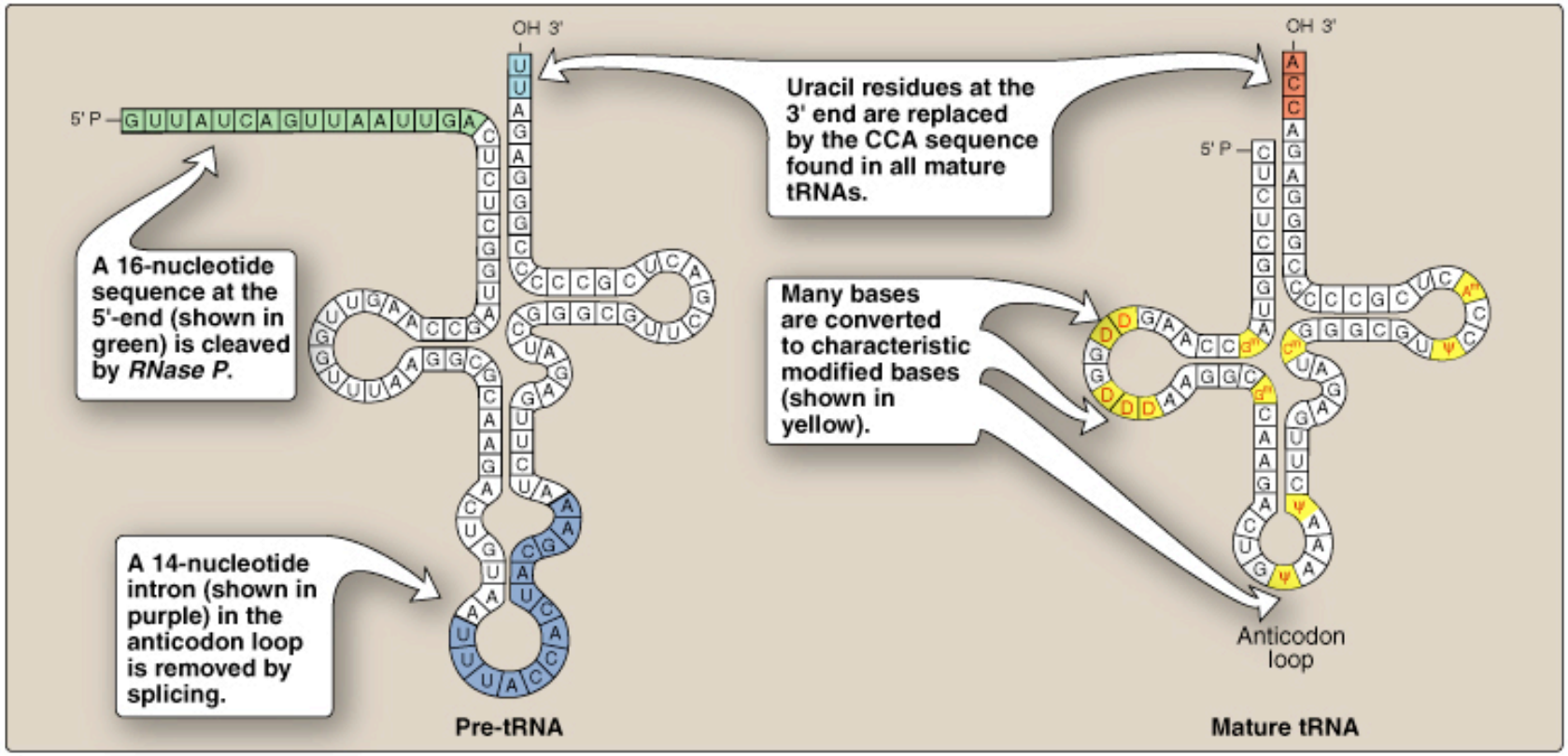


Figure 30.16

A. Primary tRNA transcript. B. Functional tRNA after posttranscriptional modification. Modified bases include D (dihydrouridine), ψ (pseudouridine), and ^{m}, which means that the base has been methylated.

Eukaryotic mRNA processing steps

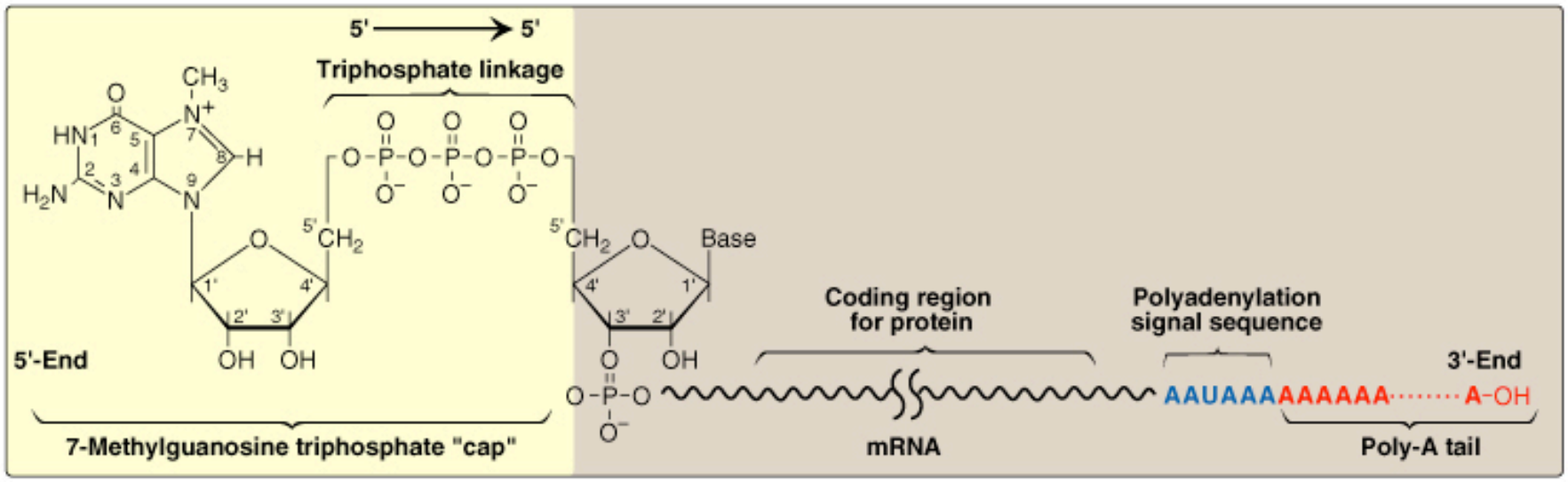
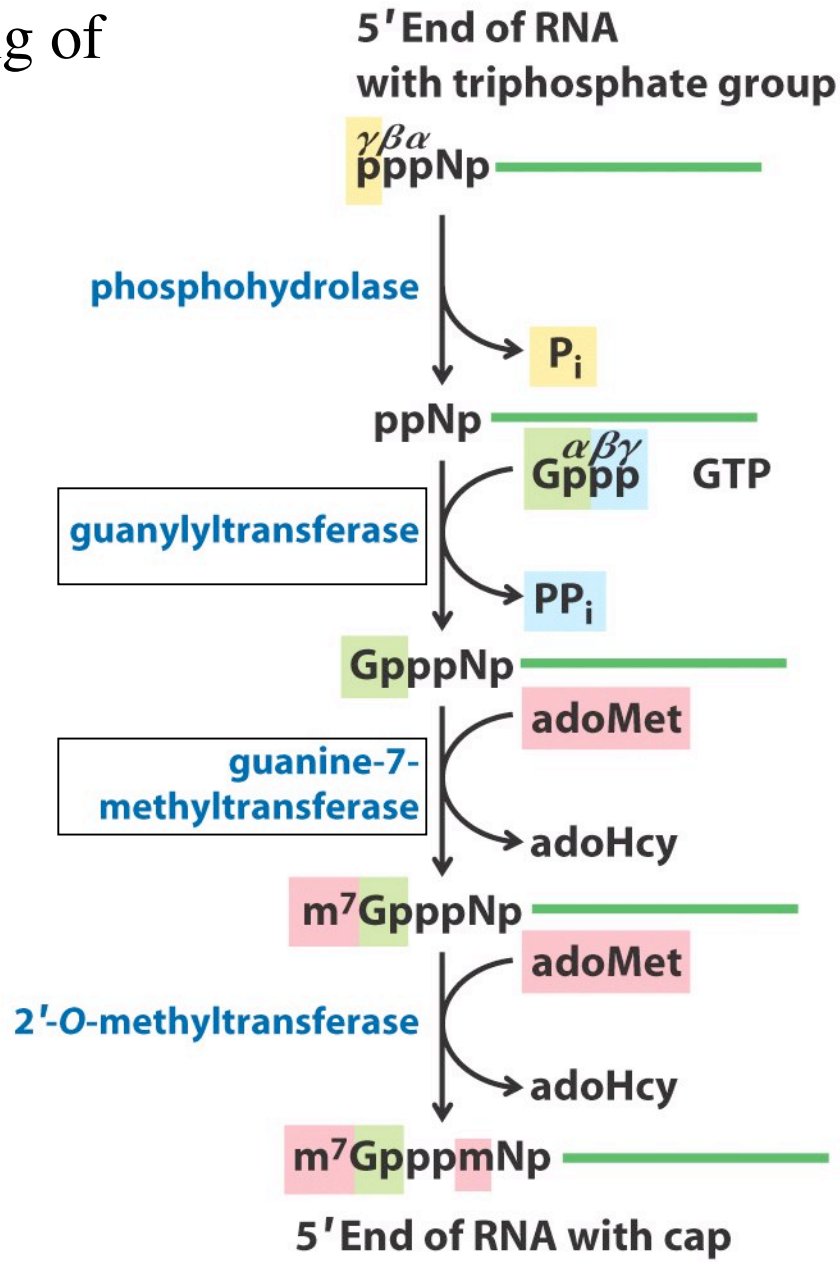


Figure 30.17
Posttranscriptional modification of mRNA showing the 7-methylguanosine cap and poly-A tail.

Steps for capping of mRNAs



Polyadenylation requires 3'-end cleavage by a endonuclease and poly-A addition by *polyadenylate polymerase***.**

(exception: Histone mRNAs are not polyadenylated)

- **Cleavage of primary transcript approximately 10-30 nucleotides downstream of the AAUAAA polyA-addition concensus sequence**
- **Addition of up to 200 adenylate residues by polyA pol.**
- **Association of polyA-binding protein (PABP) at polyA tail**
- **PolyA stabilizes mRNA**

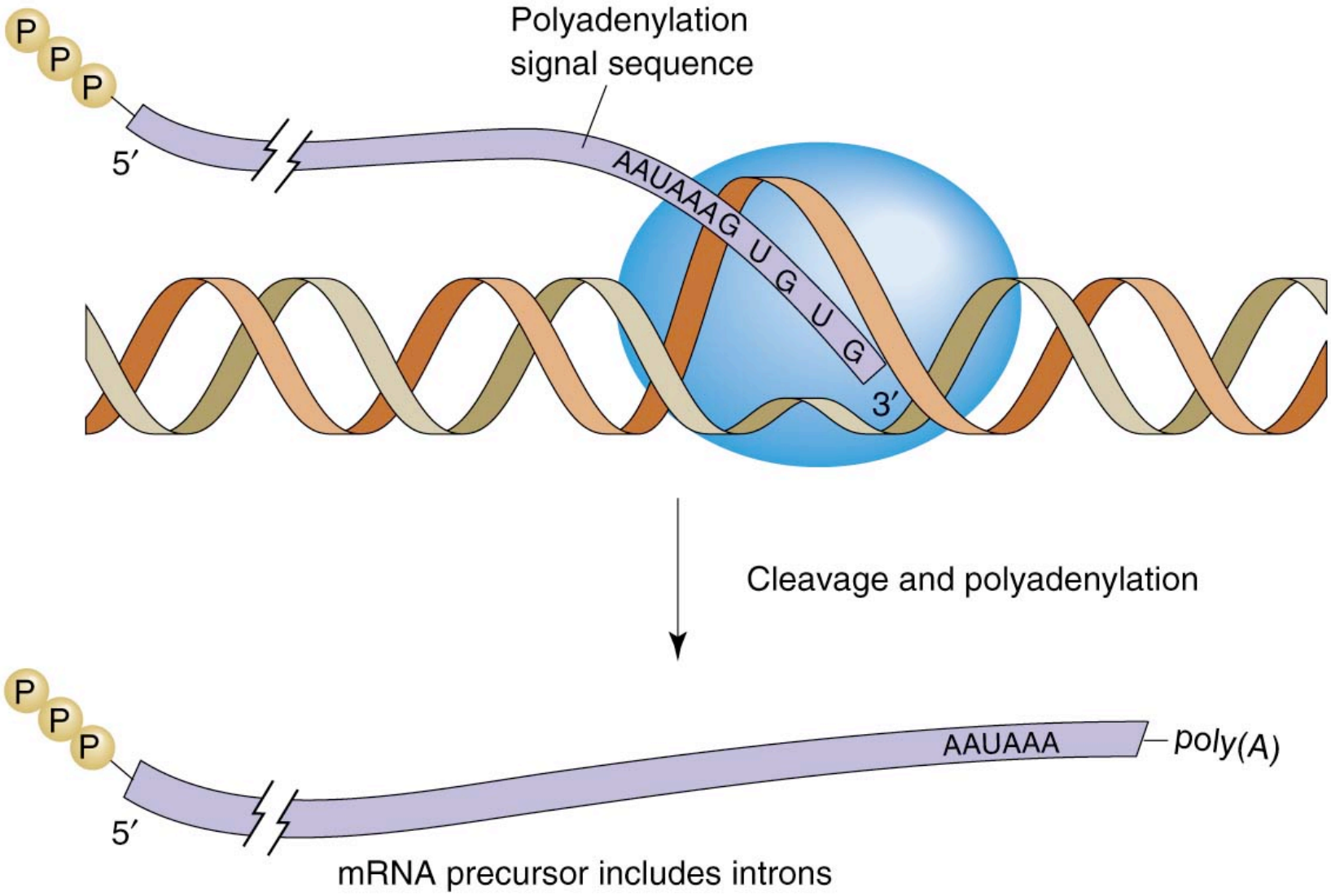


Figure 5.15. Cleavage and polyadenylation of eukaryotic mRNA precursors. Adapted from Proudfoot, N. J. *Trends Biochem. Sci.* 14:105, 1989.

Eukaryotic mRNA splicing

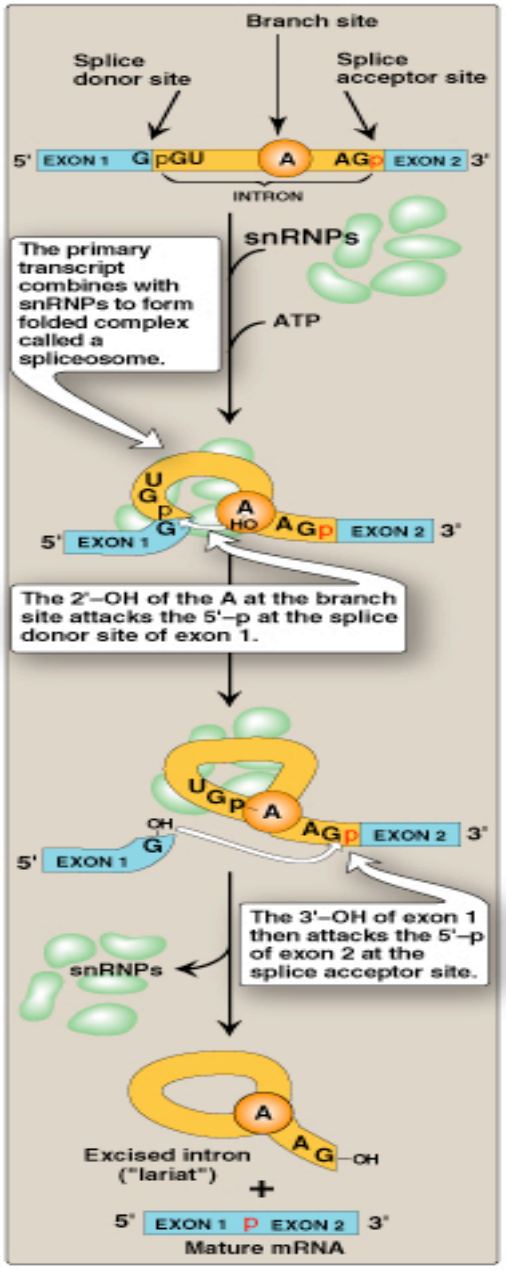
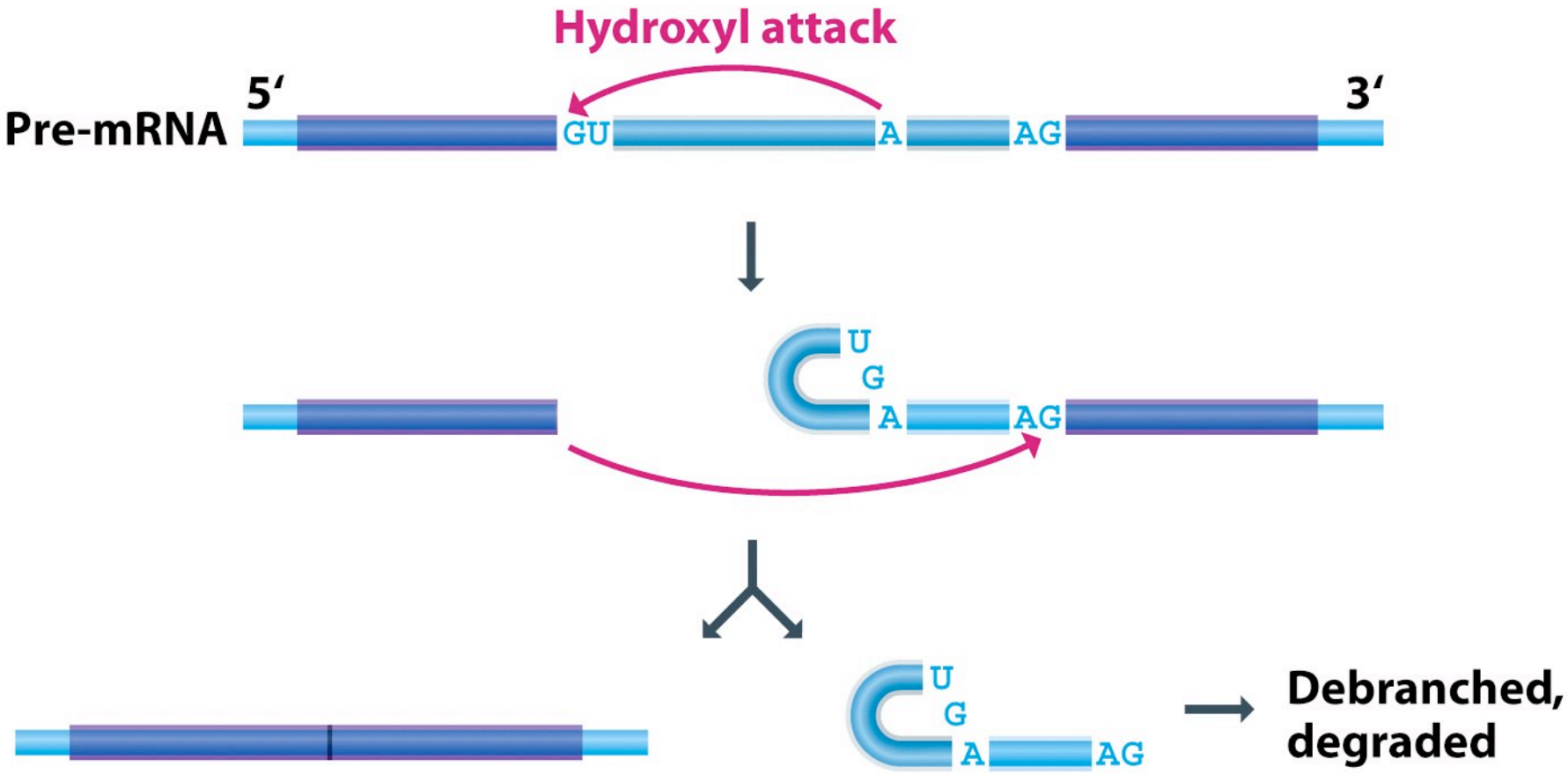
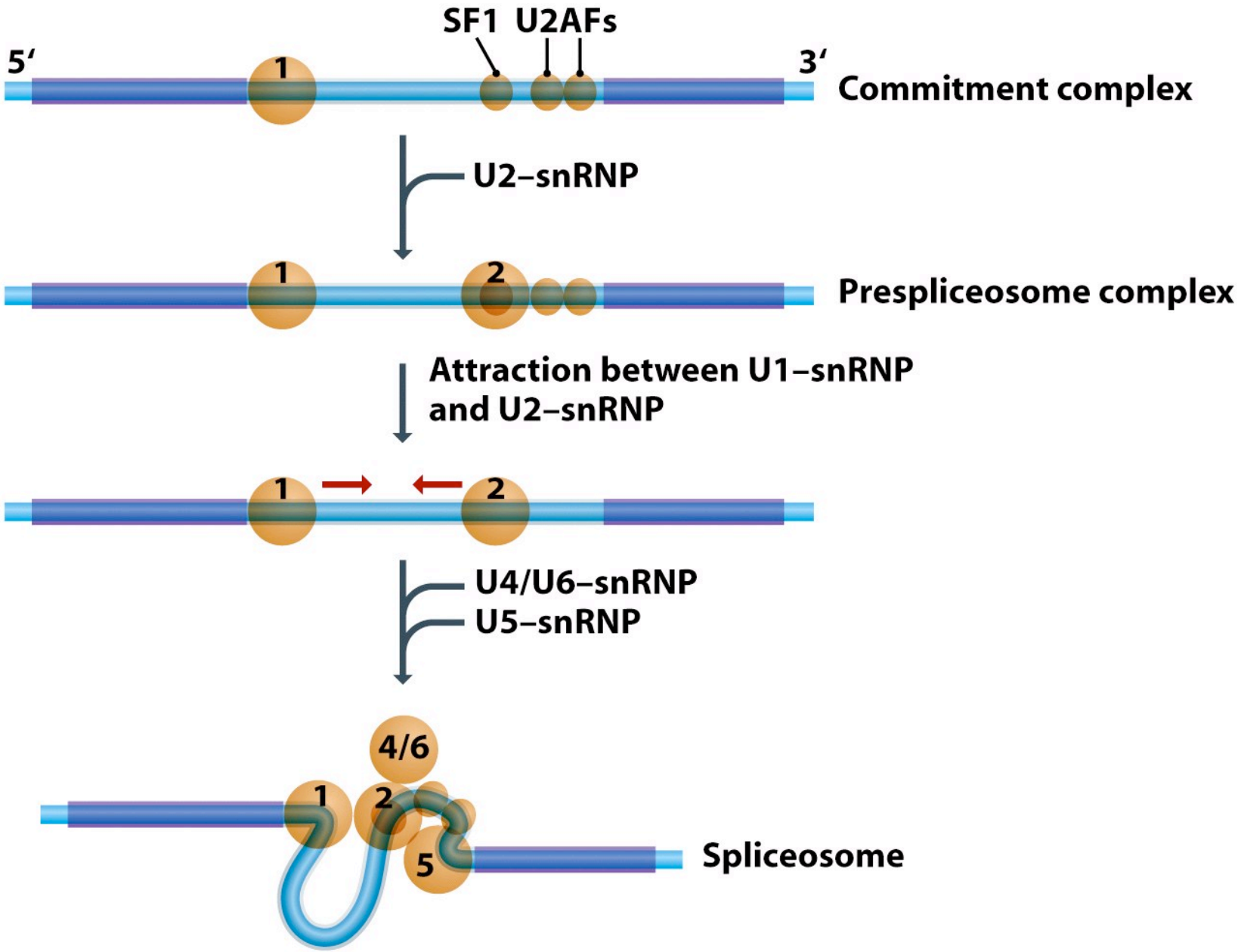


Figure 30.18
Removal of introns. snRNP = small nuclear ribonucleoprotein particle.



Splicing of GU-AG introns

Figure 12.27 *Genomes 3* (© Garland Science 2007)



The role of snRNPs and associated proteins during splicing

Figure 12.30 *Genomes 3* (© Garland Science 2007)

Alternative splicing patterns for eukaryotic mRNAs

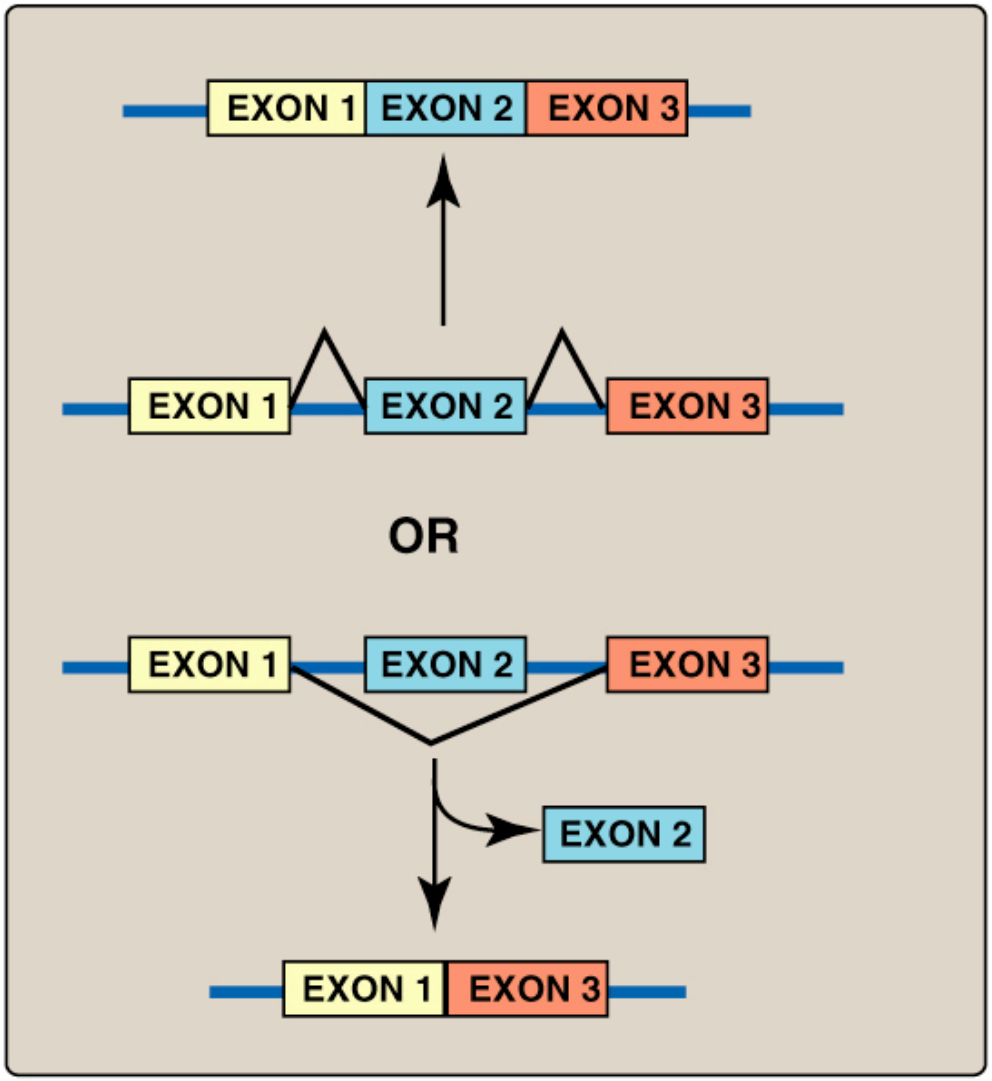
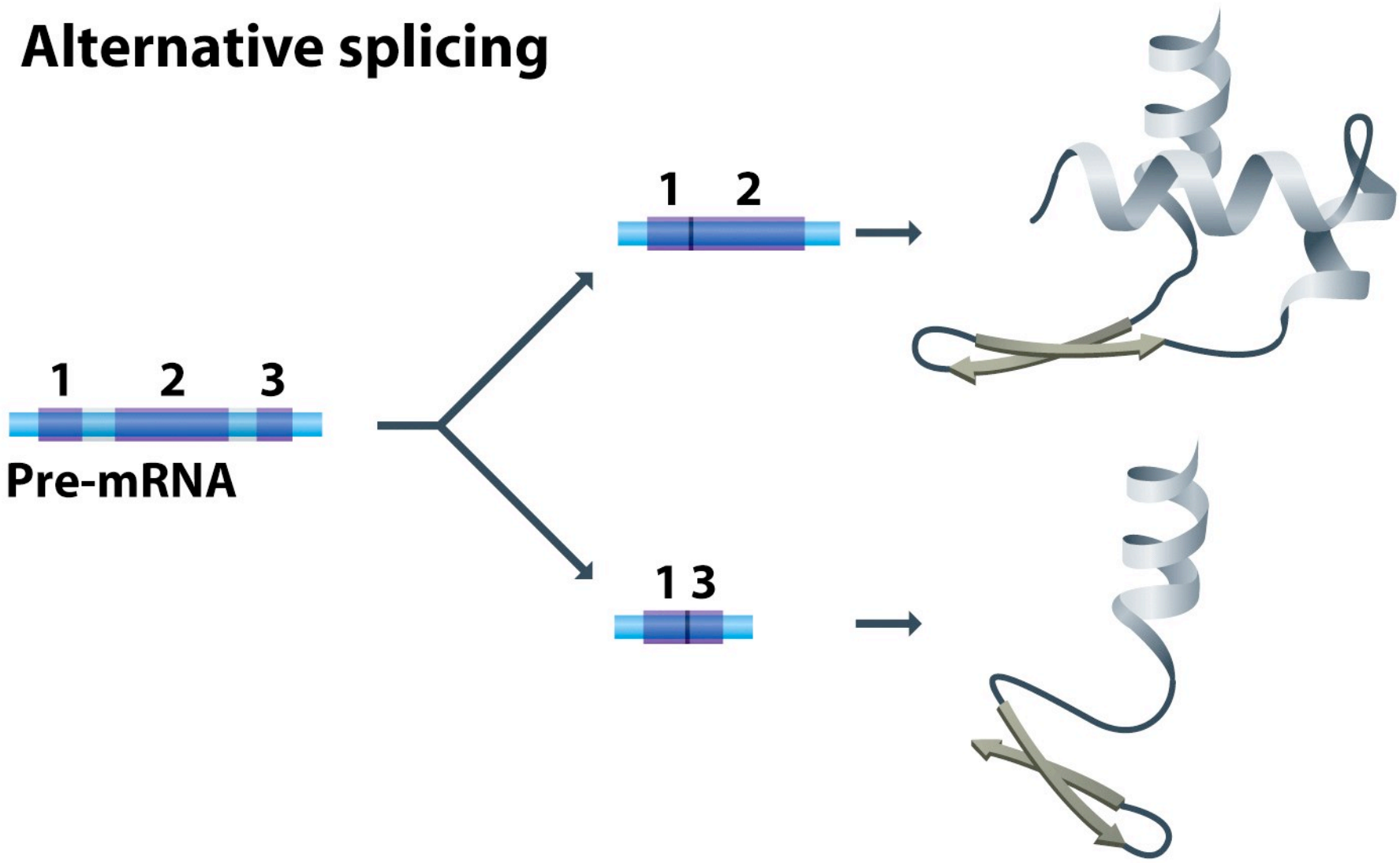


Figure 30.19
Alternative splicing patterns in eukaryotic mRNA.



Single versus alternative splicing pathways

Figure 12.32b *Genomes 3* (© Garland Science 2007)

RNA interference

siRNA, short interfering RNA
miRNA, micro-RNA-protein
RISC, RNA-induced silencing complex

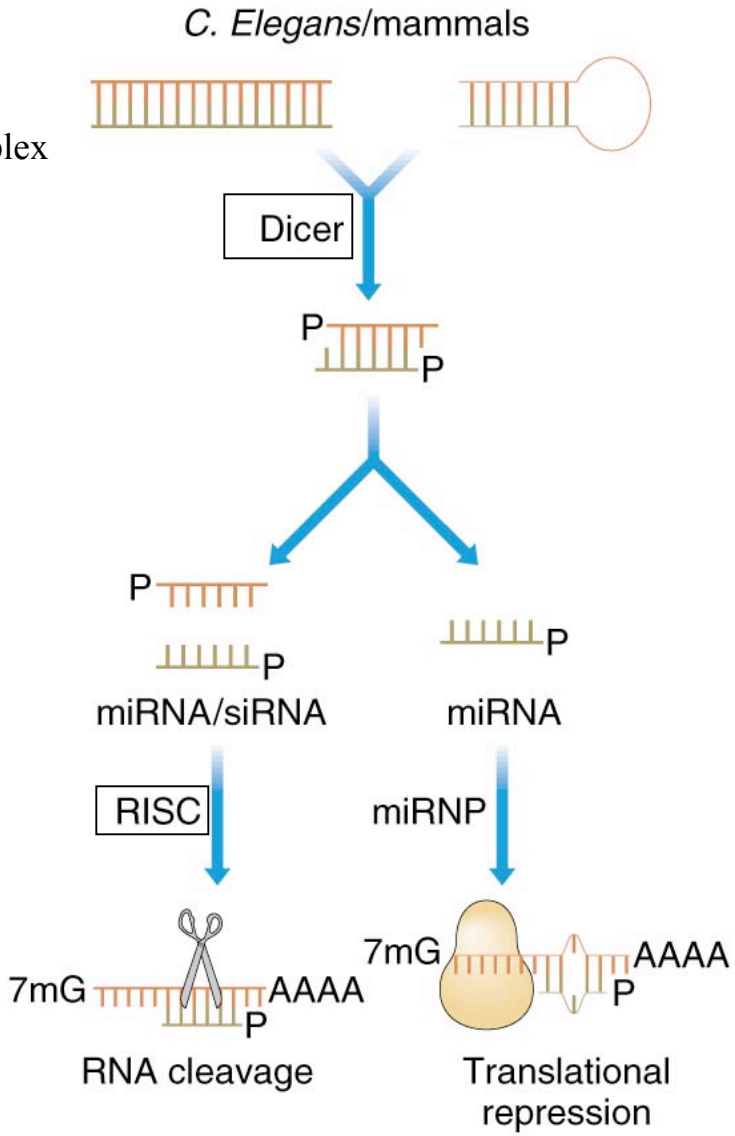
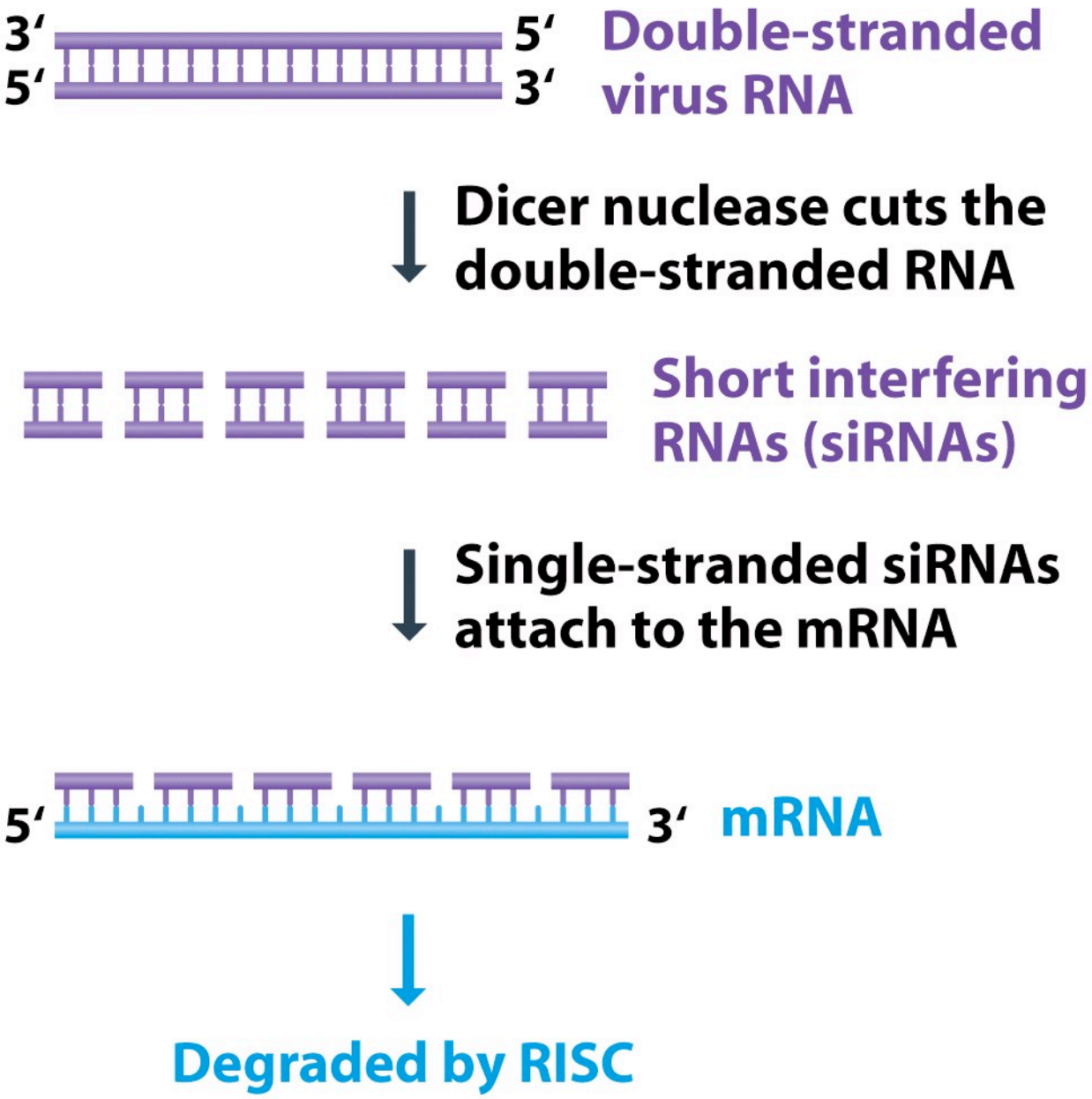
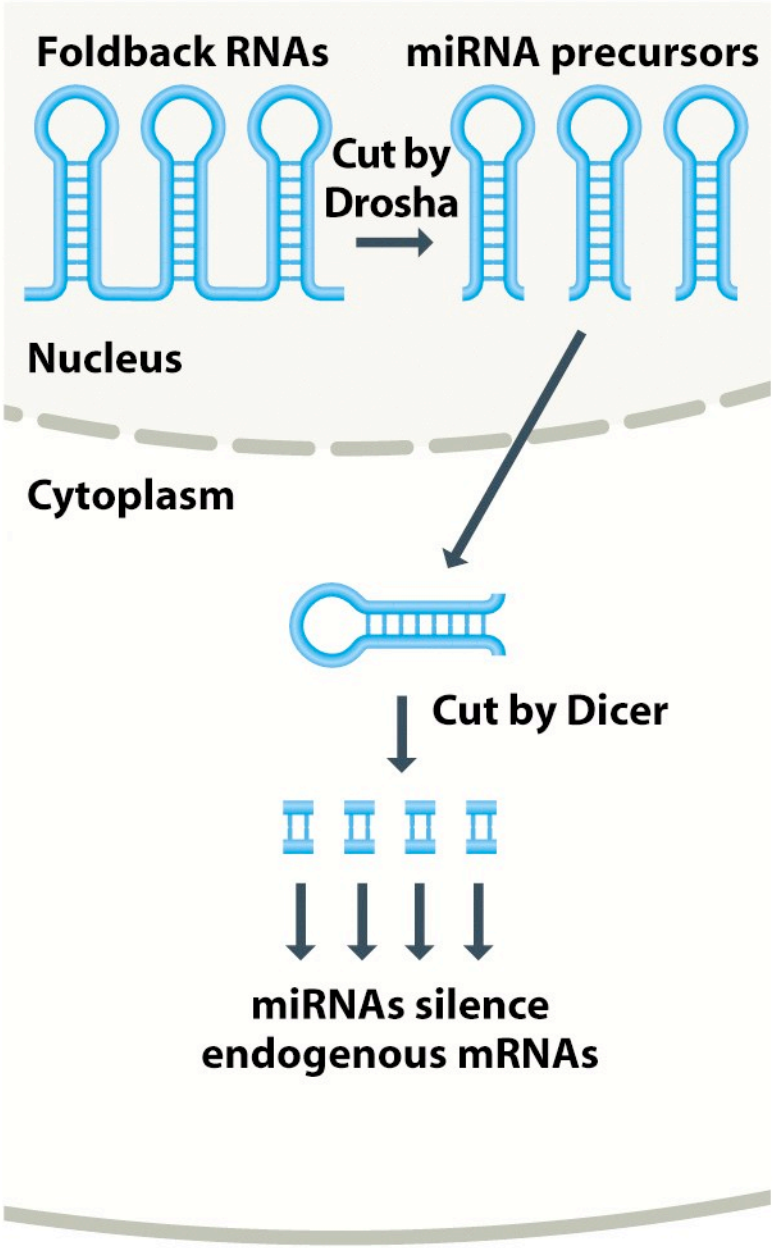


Figure 5.18. Effects of small inhibitory RNAs on eukaryotic mRNA metabolism. Redrawn from Meister, G. and Tuschl, T. Mechanisms of gene silencing by double-stranded RNA. *Nature* 431:343, 2004.



The RNA interference pathway

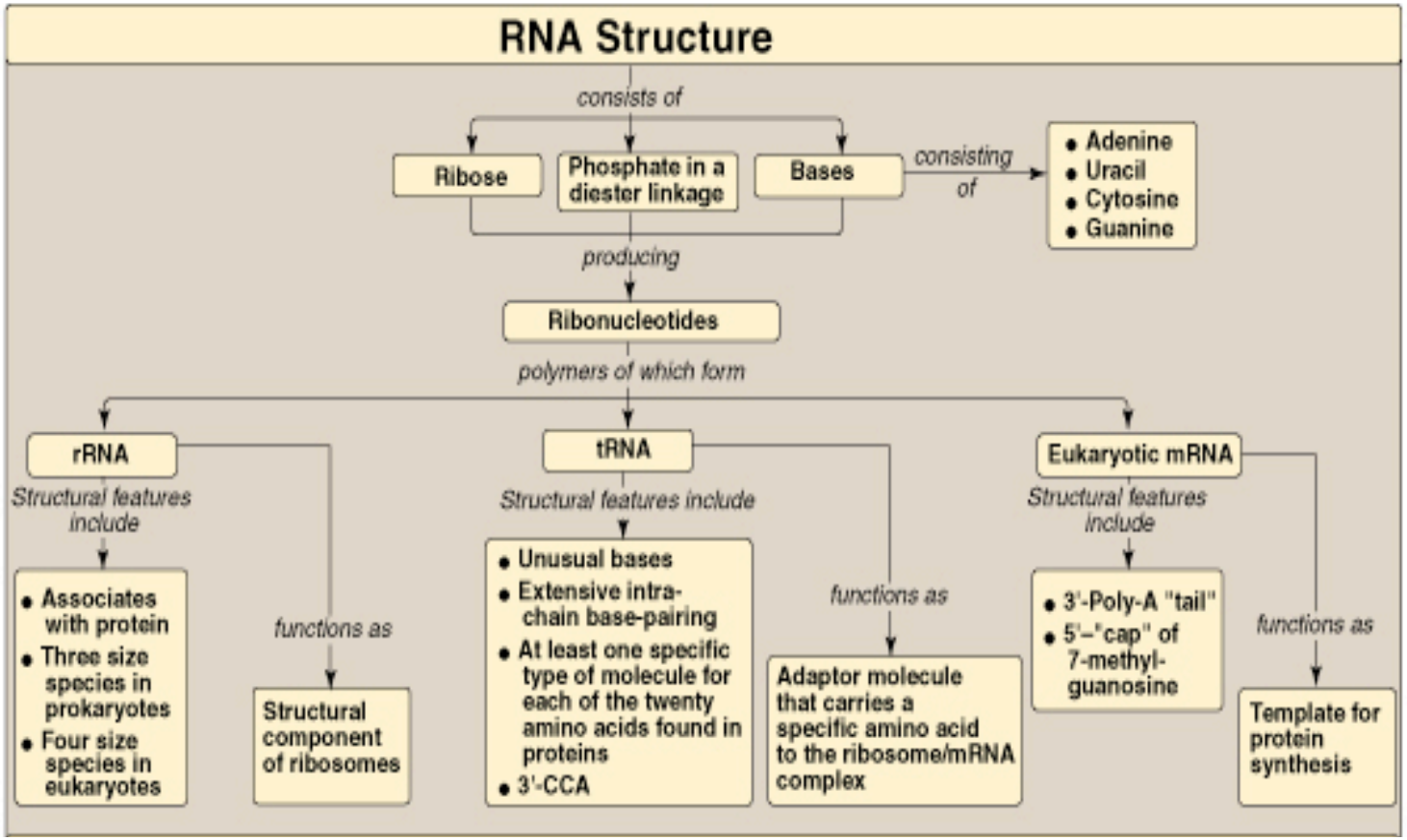
Figure 12.46 *Genomes 3* (© Garland Science 2007)



The microRNA interference pathway

Figure 12.48 *Genomes 3* (© Garland Science 2007)

Review slide



Review slide

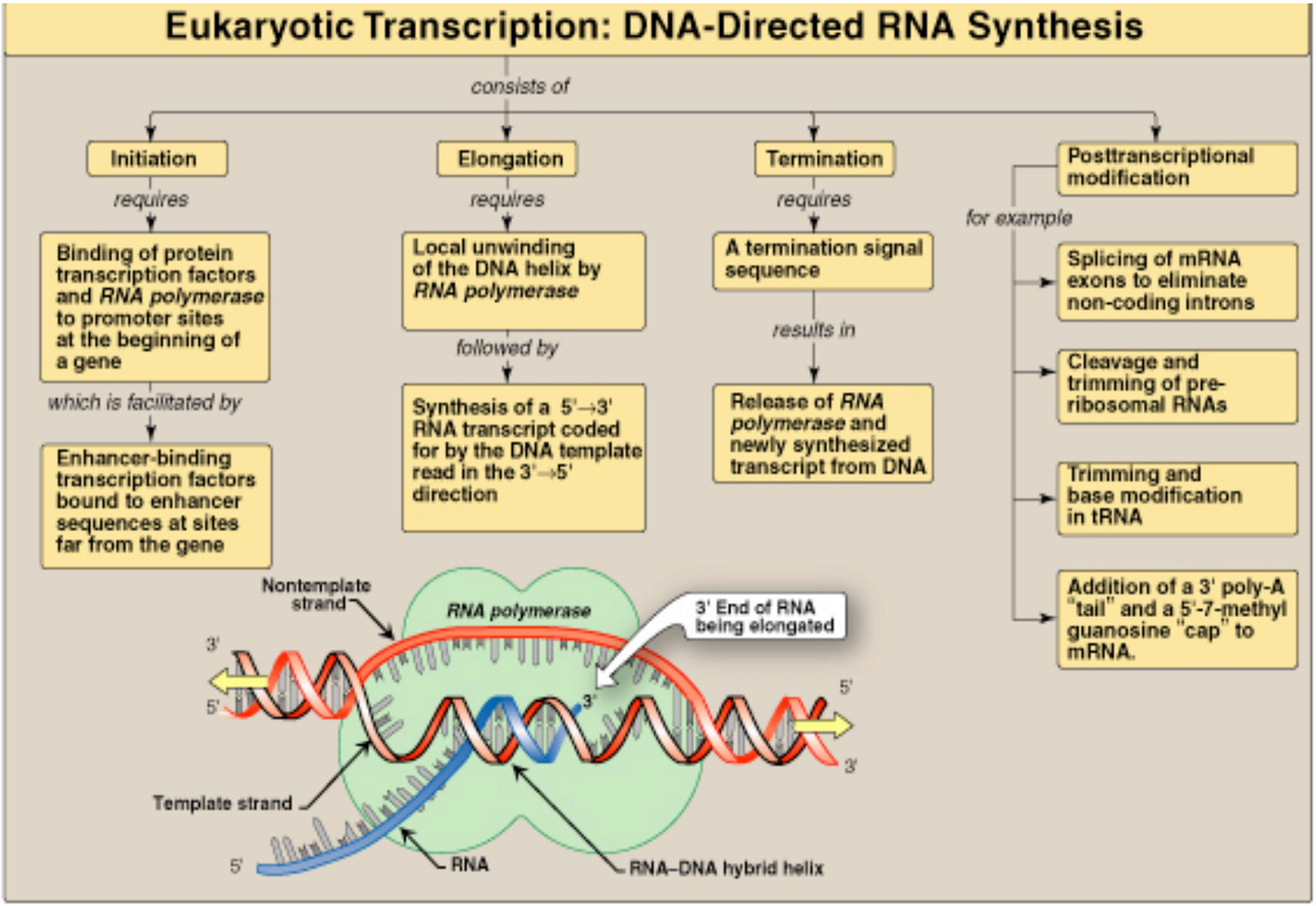


Figure 30.20

Key concept map for RNA structure and synthesis.

Pop Quiz:

1-Define transcription_____

2- The RNA Polymerase(s) most sensitive α-amanitin _____

3- The RNA Polymerase(s) most inhibited by Actinomycin D_____

4- Sigma factor is necessary for what process in transcription? _____

5-Rho factor is required for what process in transcription?_____

6-Answer "P" for prokaryotic and "E" for eukaryotic:

a-mRNA 5'-end has a phosphate group _____
b-mRNA contains poly-A tail _____
c-mRNA is spliced _____

7- Define "siRNA"_____

8- A sequenced required for 3' end polyadenylation of mRNAs_____

9-What is the RNA transcript derived from the DNA template GATCTAC ? _____

Study Questions

Choose the ONE correct answer.

30.1 A 1-year-old male with chronic anemia is found to have β-thalassemia. Genetic analysis shows that one of his β-globin genes has a mutation that creates a new splice acceptor site 19 nucleotides upstream of the normal splice acceptor site of the first intron. Which of the following best describes the new mRNA molecule that can be produced from this mutant gene?

A. Exon 1 will be too short.
B. Exon 1 will be too long.
C. Exon 2 will be too short.
D. Exon 2 will be too long.
E. Exon 2 will be missing.

Correct answer = D. Because the mutation creates an additional splice acceptor site (the 3'-end) upstream of the normal acceptor site of intron 1, the 19 nucleotides that are usually found at the 3'-end of the excised intron 1 lariat can remain behind as part of exon 2. Exon 2 can, therefore, have these extra 19 nucleotides at its 5'-end. The presence of these extra nucleotides in the coding region of the mutant mRNA molecule will prevent the ribosome from translating the message into a normal β-globin protein molecule. Those mRNA for which the normal splice site is used to remove the first intron will be normal, and their translation will produce normal β-globin protein.

30.2 The base sequence of the strand of DNA used as the template for transcription is GATCTAC. What is the base sequence of the RNA product? (All sequences are written 5'→ 3' according to standard convention.)

A. CTAGATG.
B. GTAGATC.
C. GAUCUAC.
D. CUAGAUG.
E. GUAGAUC.

Correct answer = E. The RNA product has a sequence that is complementary to the template strand and identical to the coding strand of DNA. Uracil (U) is found in RNA in place of the thymine (T) in DNA. Thus, the DNA template 5'-GATCTAC-3' would produce the RNA product 3'-CUAGAUG-5' or, written correctly in the standard direction, 5'-GUAGAUC-3'.

30.3 A 4-year-old child who becomes easily tired and has trouble walking is diagnosed with Duchenne muscular dystrophy, an X-linked recessive disorder. Genetic analysis shows that the patient's gene for the muscle protein dystrophin contains a mutation in its promoter region. Of the choices listed, which would be the most likely effect of this mutation?

A. Initiation of dystrophin transcription will be defective.
B. Termination of dystrophin transcription will be defective.
C. Capping of dystrophin mRNA will be defective.
D. Splicing of dystrophin mRNA will be defective.
E. Tailing of dystrophin mRNA will be defective.

Correct answer = A. Mutations in the promoter typically prevent formation of the RNA polymerase II transcription complex, resulting in a decrease in the initiation of mRNA synthesis. A deficiency of dystrophin mRNA will result in a deficiency in the production of the dystrophin protein. Capping, splicing and tailing defects are not a consequence of promoter mutations. They can, however, result in mRNA with decreased stability (capping and tailing defects), or a mRNA in which too many or too few introns have been removed (splicing defects)

30.4 A mutation to this sequence in eukaryotic mRNA will affect the process by which the 3'-end poly-A tail is added to the mRNA.

A. CAAT
B. CCA
C. GGGGCG
D. AAUAAA
E. TATAAA

Correct answer = D. An endonuclease cleaves mRNA just downstream of this polyadenylation signal, creating a new 3'-end to which the *pol A polymerase* adds the poly-A tail using ATP as the substrate in a template-independent process. CAAT, GGGGCGT, and TATAAA are sequences found in promoters for *RNA polymerase II*. CCA is added to the 3'-end of tRNA by *nucleotidyl transferase.*