Transcription and Translation in Eukaryotes

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- Many of the basic features of gene transcription are very similar in bacterial and eukaryotic species.
- In general, gene transcription in eukaryotes is more complex than that of their bacterial counterparts.
- Eukaryotes have multiple RNA polymerases (3) that are structurally similar to the bacterial enzyme.
- All three RNA polymerases are structurally very similar to each other and are composed of many subunits.

Table 15-3 Eukaryotic RNA Polymerases and Promoter Elements

Polymerase	RNA Products	Promoter Elements
Pol I	18S, 25S, and 5.8S rRNAs	UCE, core sequence
Pol II	mRNA, microRNAs, some noncoding RNAs	BRE, TATA box, Inr, DPE
Pol III	tRNA	Box A, Box B
	5S rRNA	Box A, Box C
	7SL RNA	TATA box

Table 15-3
Molecular Biology: Principles and Practice

Core Promoter and Regulatory Elements

- For transcription to occur at an appropriate rate, eukaryotic genes have two features: **a core promoter** and **regulatory elements**.
- The **core promoter** is a relatively short DNA sequence that is necessary for transcription to take place.
- It typically consists of a TATAAA sequence called the **TATA box** and the transcriptional start site, where transcription begins.

- **Regulatory elements** are short DNA sequences that affect the ability of RNA polymerase to recognize the core promoter and begin the process of transcription.
- These elements are recognized by **transcription factors (TF)**—proteins that influence the rate of transcription.

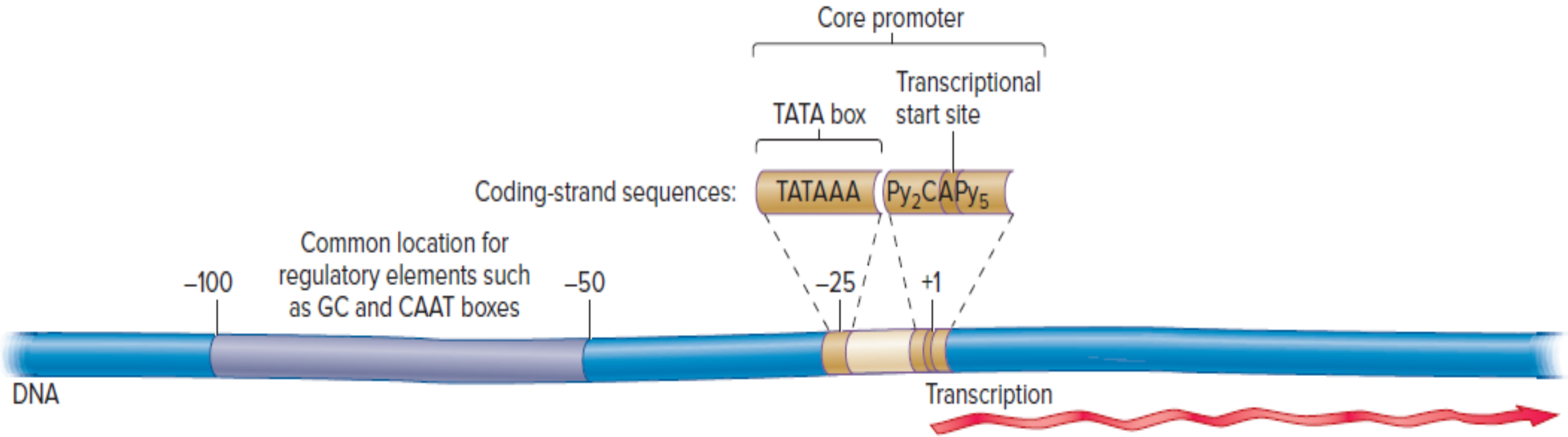
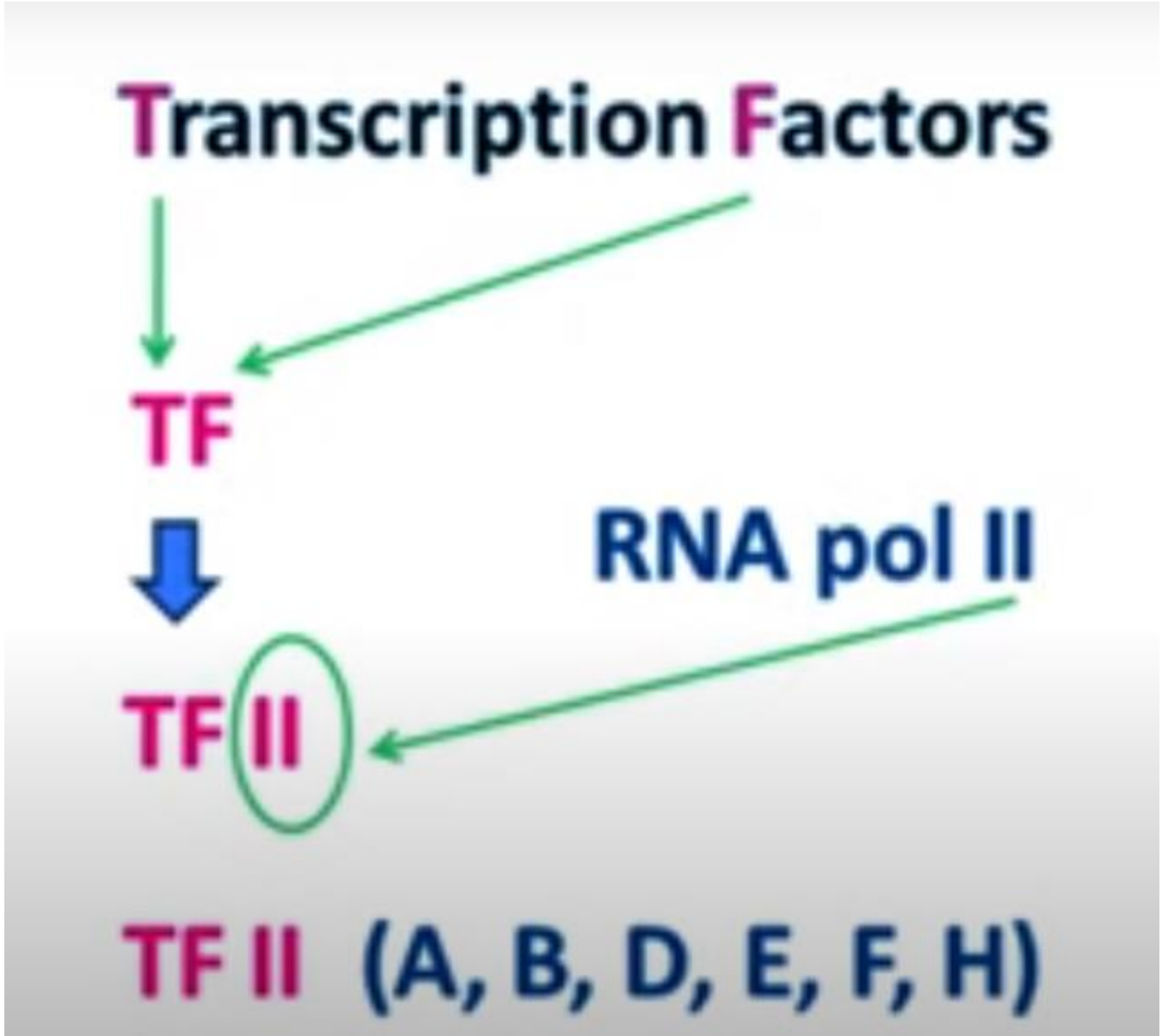


FIGURE 12.13 A common pattern for the promoter of protein-encoding genes recognized by RNA polymerase II. The start site usually occurs at adenine (A); two pyrimidines (Py: cytosine or thymine) and a cytosine (C) are to the left of this adenine, and five pyrimidines (Py) are to the right. A TATA box is approximately 25 bp upstream from the start site. However, the sequences that constitute eukaryotic promoters are quite diverse, and not all protein-encoding genes have a TATA box. Regulatory elements, such as GC or CAAT boxes, vary in their locations but are often found in the –50 to –100 region. The core promoters for RNA polymerase I and III are quite different. A single upstream regulatory element is involved in the binding of RNA polymerase I to its promoter, whereas two regulatory elements, called A and B boxes, facilitate the binding of RNA polymerase III.


Transcription Factors
TF
RNA pol II
TF II
TF II (A, B, D, E, F, H)

Transcription of Eukaryotic Protein-Encoding Genes Is Initiated When RNA Polymerase II and General Transcription Factors Bind to a Promoter Sequence

TABLE 12.1

Proteins Needed for Transcription via the Core Promoter of Eukaryotic Protein-Encoding Genes

RNA polymerase II: The enzyme that catalyzes the linkage of nucleotides in the 5′ to 3′ direction, using DNA as a template. Most eukaryotic RNA polymerase II proteins are composed of 12 subunits. The two largest subunits are structurally similar to the β and β′ subunits found in *E. coli* RNA polymerase.

General transcription factors:

TFIID: Composed of TATA-binding protein (TBP) and other TBP-associated factors (TAFs). Recognizes the TATA box of eukaryotic protein-encoding gene promoters.

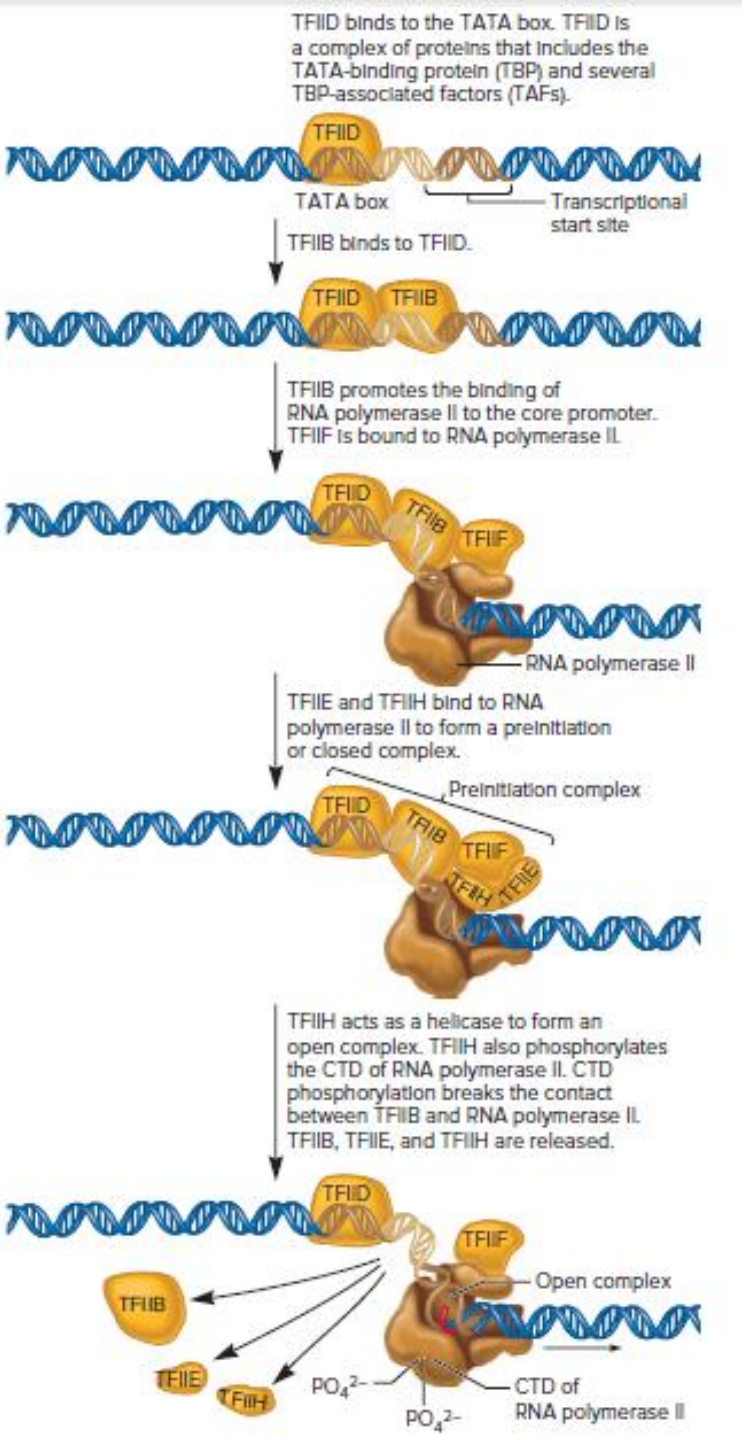
TFIIB: Binds to TFIID and then enables RNA polymerase II to bind to the core promoter. Also promotes TFIIF binding.

TFIIF: Binds to RNA polymerase II and plays a role in its ability to bind to TFIIB and the core promoter. Also plays a role in the ability of TFIIE and TFIIH to bind to RNA polymerase II.

TFIIE: Plays a role in the formation or the maintenance (or both) of the open complex. It may exert its effects by facilitating the binding of TFIIH to RNA polymerase II and regulating the activity of TFIIH.

TFIIH: A multisubunit protein that has multiple roles. First, certain subunits act as helicases and promote the formation of the open complex. Other subunits phosphorylate the carboxyl terminal domain (CTD) of RNA polymerase II, which releases its interaction with TFIIB, thereby allowing RNA polymerase II to proceed to the elongation phase.

Transcription of Eukaryotic Protein-Encoding Genes Is Initiated When RNA Polymerase II and General Transcription Factors Bind to a Promoter Sequence



https://www.youtube.com/watch?v=EMDuf_kBJcs

Transcriptional Termination Occurs After the 3′ End of the Transcript Is Cleaved Near the Polyadenylation Signal Sequence

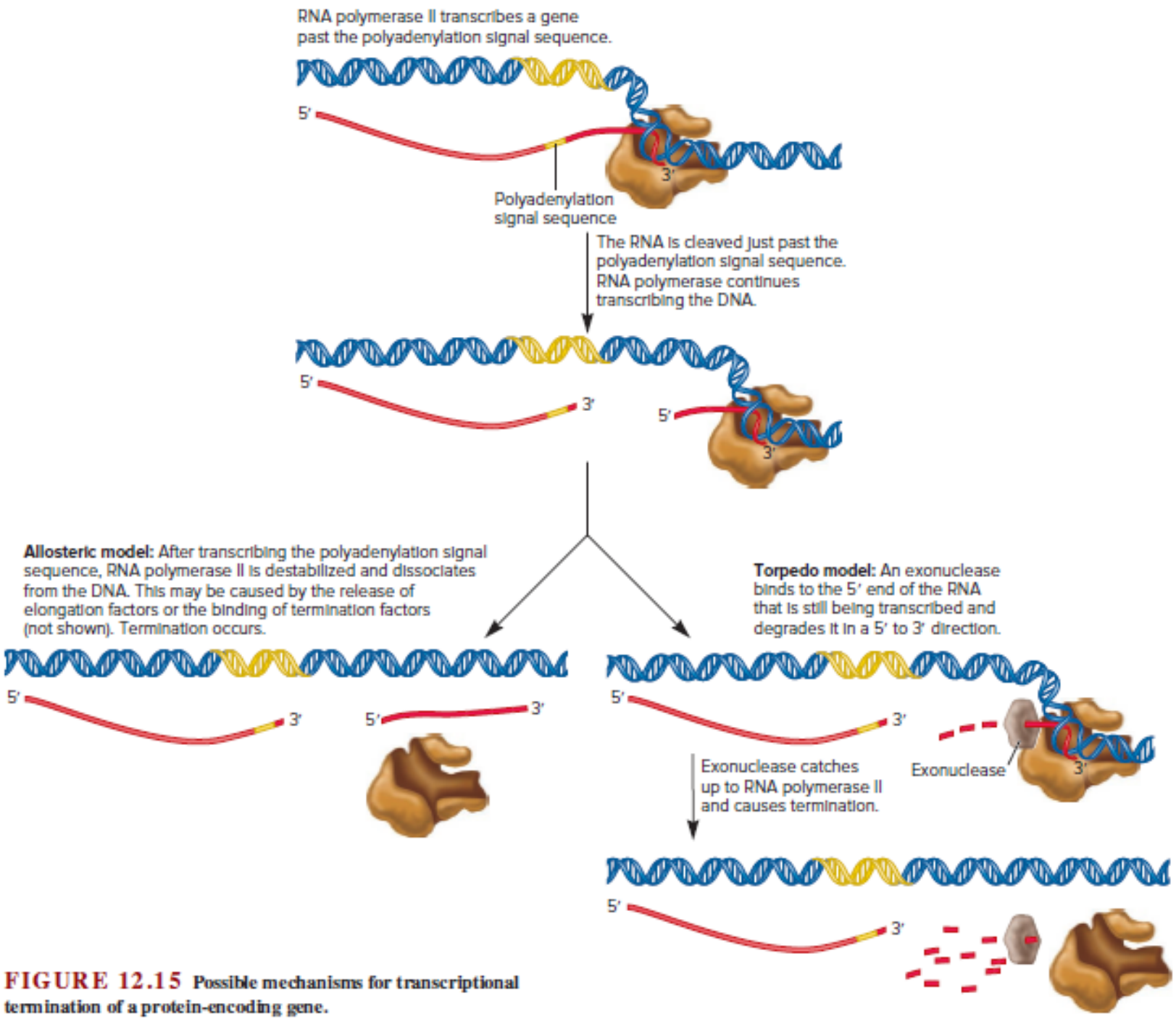
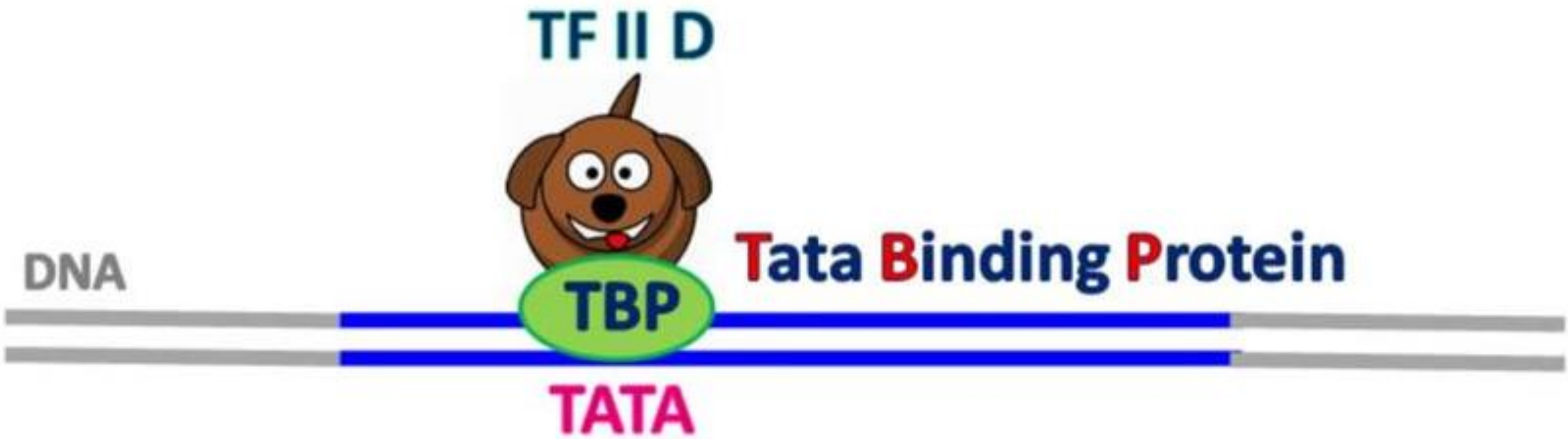


FIGURE 12.15 Possible mechanisms for transcriptional termination of a protein-encoding gene.

Eukaryotic transcription



Mnemonics

TF II D

The dog eats the Apple TF II A

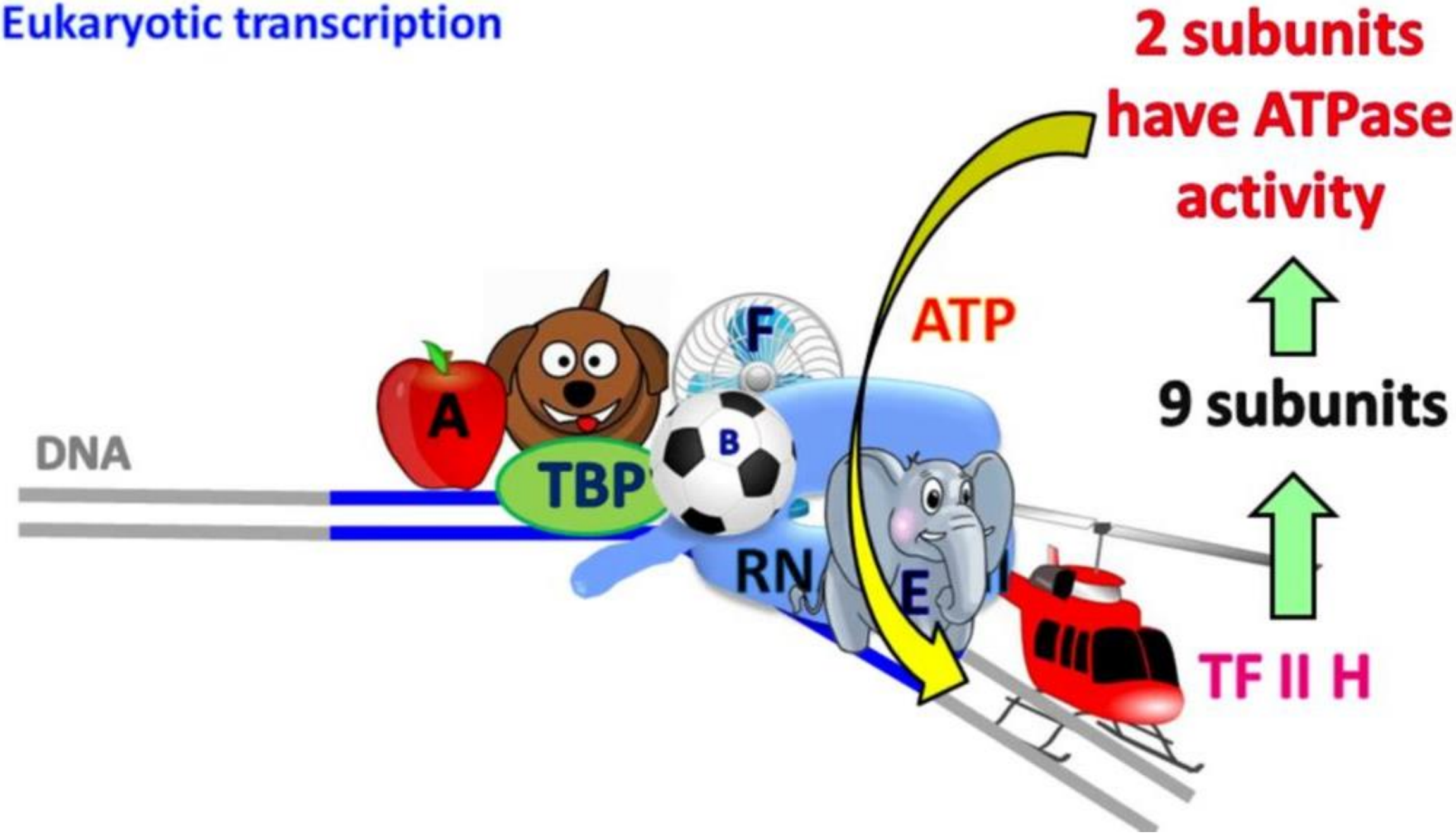
And plays with the foot-Ball TF II B

He gets tired and sits in front of the Fan TF II F

When he sees the Elephant TF II E

He runs away in the Helicopter TF II H

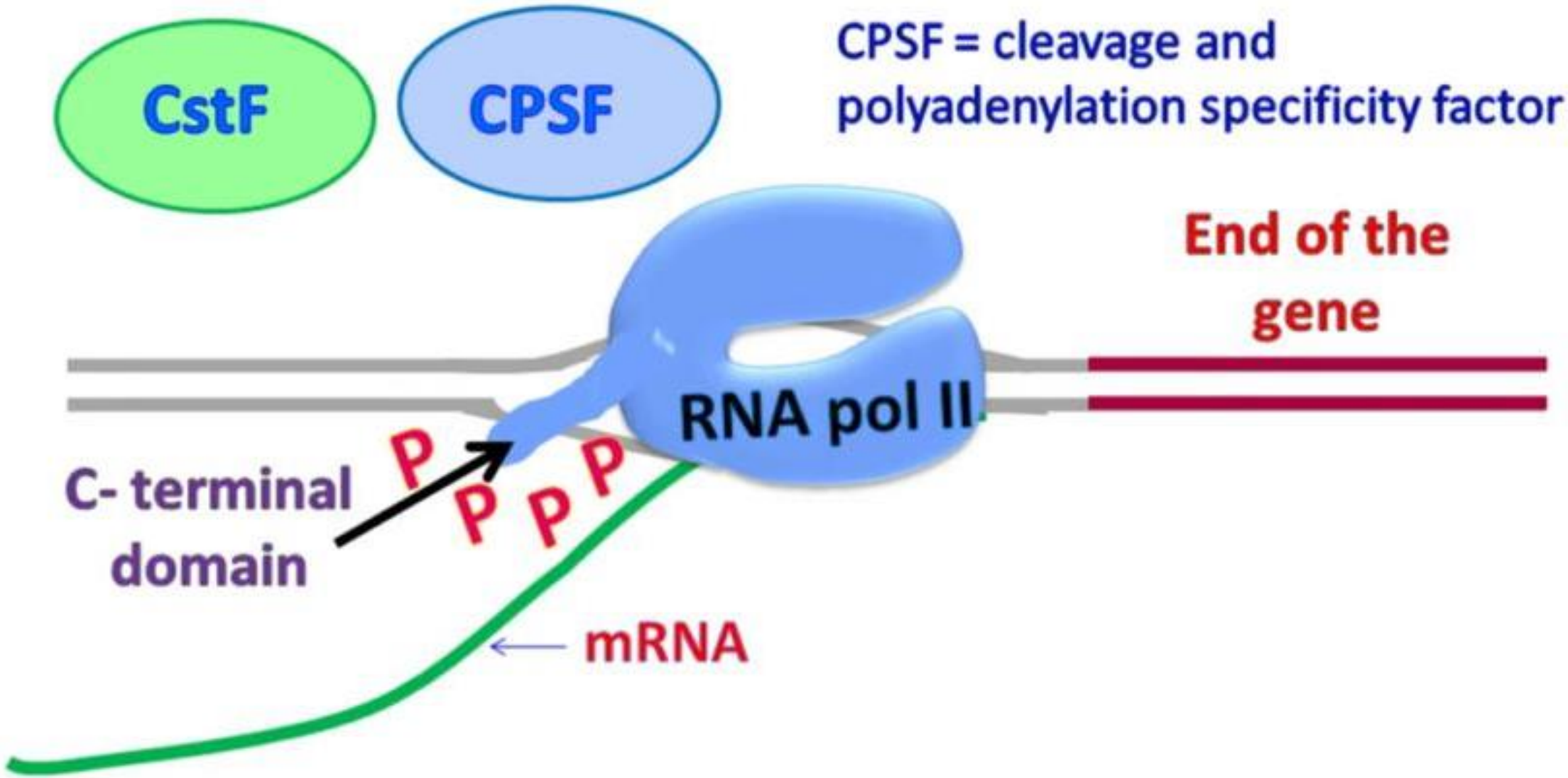
Eukaryotic transcription

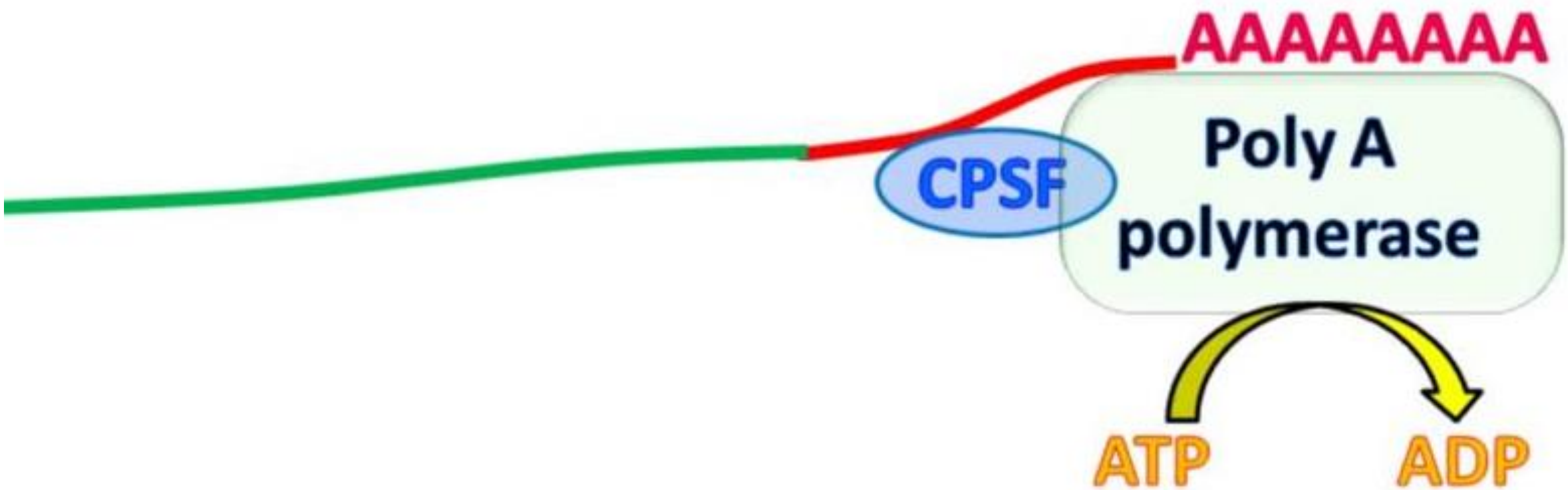


Eukaryotic transcription

CstF = cleavage stimulation factor

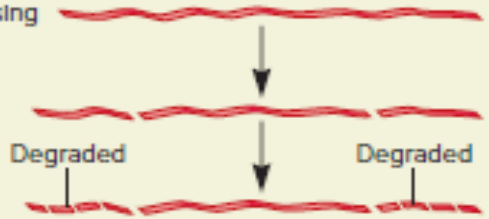
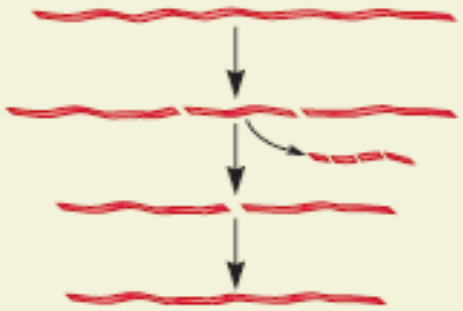
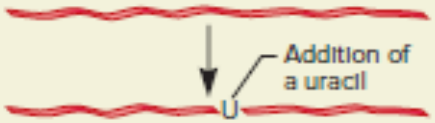
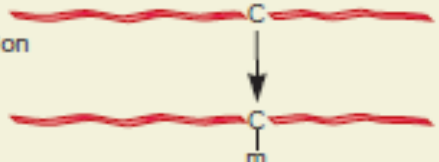
CPSF = cleavage and polyadenylation specificity factor

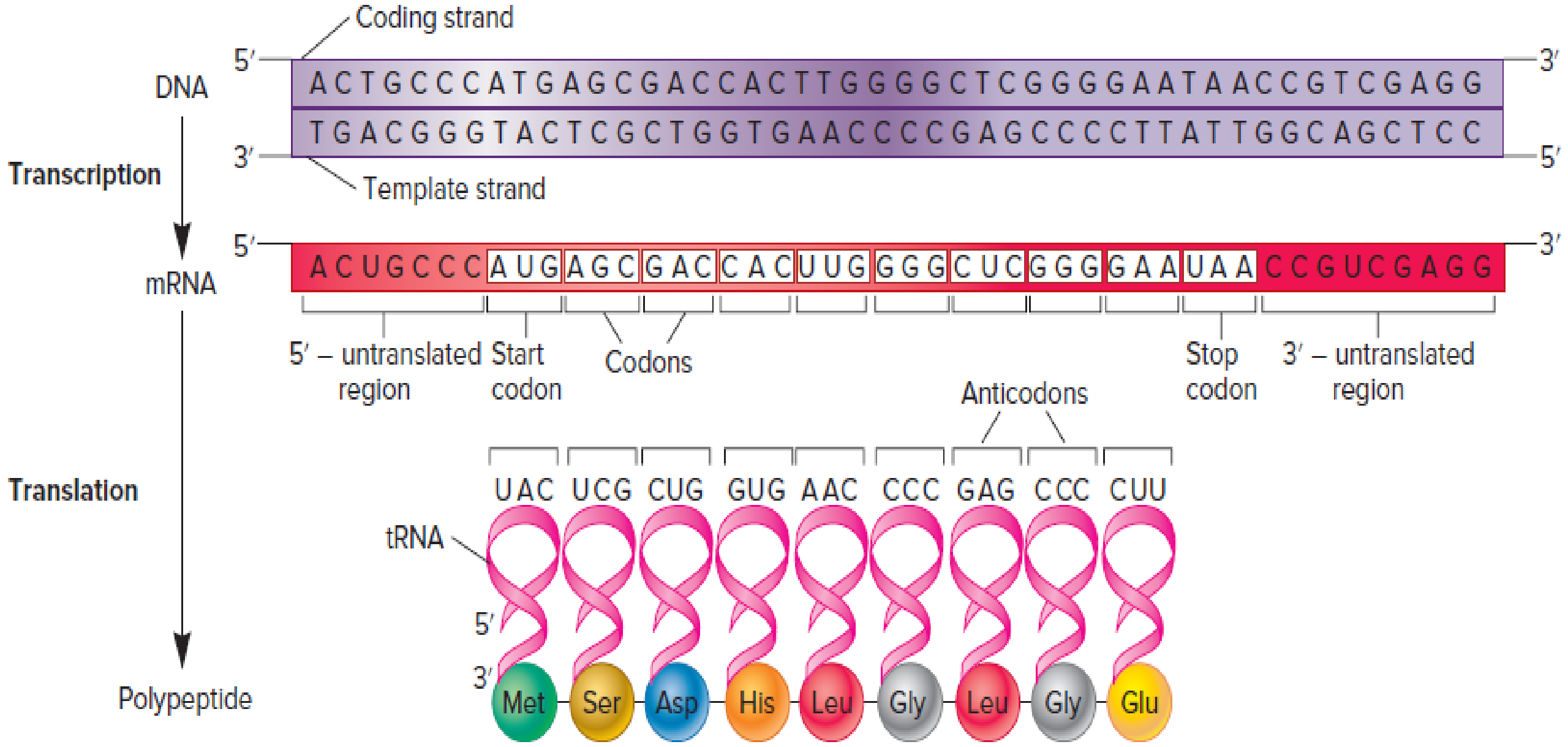



AAAAAAAA
CPSF
Poly A
polymerase
ATP
ADP

RNA MODIFICATION

Modifications That May Occur to RNAs

Modification	Description	Occurrence
Processing 	The cleavage of a large RNA transcript into smaller pieces. One or more of the smaller pieces becomes a functional RNA molecule.	Processing occurs in both prokaryotic and eukaryotic rRNAs and tRNAs.
Splicing 	Splicing involves both cleavage and joining of RNA molecules. The RNA is cleaved at two sites, which allows an internal segment of RNA, known as an intron, to be removed. After the intron is removed, the two ends of the RNA molecules are joined together.	Splicing is common among eukaryotic pre-mRNAs, and it also occurs occasionally in rRNAs, tRNAs, and a few bacterial RNAs.
5′ capping 	The attachment of a 7-methylguanosine cap (m^7G) to the 5′ end of mRNA. The cap plays a role in the splicing of introns, the exit of mRNA from the nucleus, and the binding of mRNA to the ribosome.	Capping occurs on eukaryotic mRNAs.
3′ polyA tailing 	The attachment of a string of adenine-containing nucleotides to the 3′ end of mRNA at a site where the mRNA is cleaved (see upward-pointing arrow). It is important for RNA stability and translation in eukaryotes.	Occurs on eukaryotic mRNAs and occasionally occurs on bacterial RNAs.
RNA editing 	The change of the base sequence of an RNA after it has been transcribed.	Occurs occasionally in eukaryotic RNAs.
Base modification 	The covalent modification of a base within an RNA molecule.	As discussed in Chapter 13, base modification commonly occurs in tRNA molecules found in both prokaryotes and eukaryotes. C—m indicates that cytosine has undergone methylation.


Coding strand
5′
3′
DNA
A C T G C C C A T G A G C G A C C A C T T G G G G C T C G G G G A A T A A C C G T C G A G G
T G A C G G G T A C T C G C T G G T G A A C C C C G A G C C C C T T A T T G G C A G C T C C
3′
5′
Transcription
Template strand
5′
3′
mRNA
A C U G C C C A U G A G C G A C C A C U U G G G G C U C G G G G A A U A A C C G U C G A G G
5′ – untranslated region
Start codon
Codons
Stop codon
3′ – untranslated region
Anticodons
Translation
U A C U C G C U G G U G A A C C C C G A G C C C C U U
tRNA
5′
3′
Polypeptide
Met
Ser
Asp
His
Leu
Gly
Leu
Gly
Glu

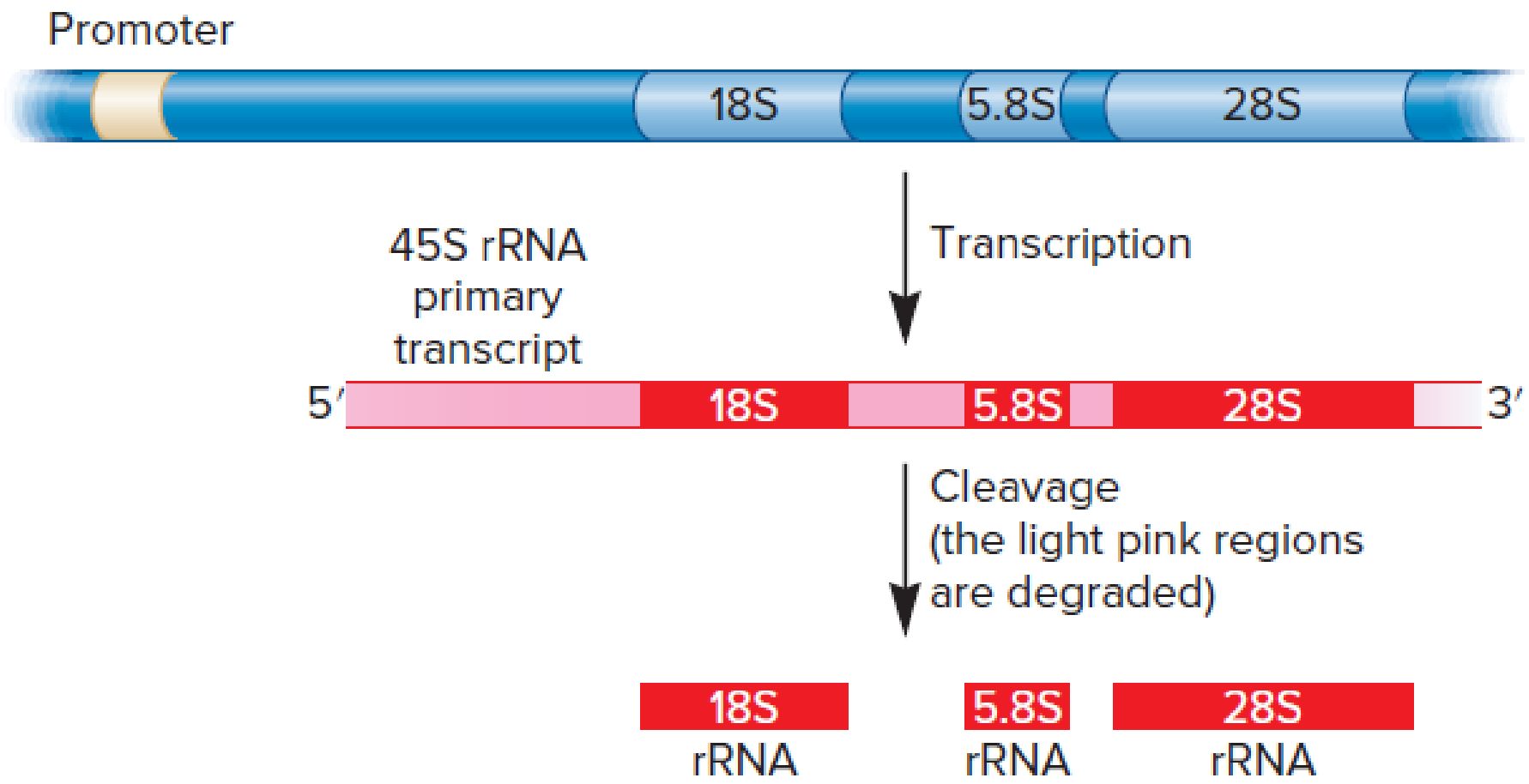
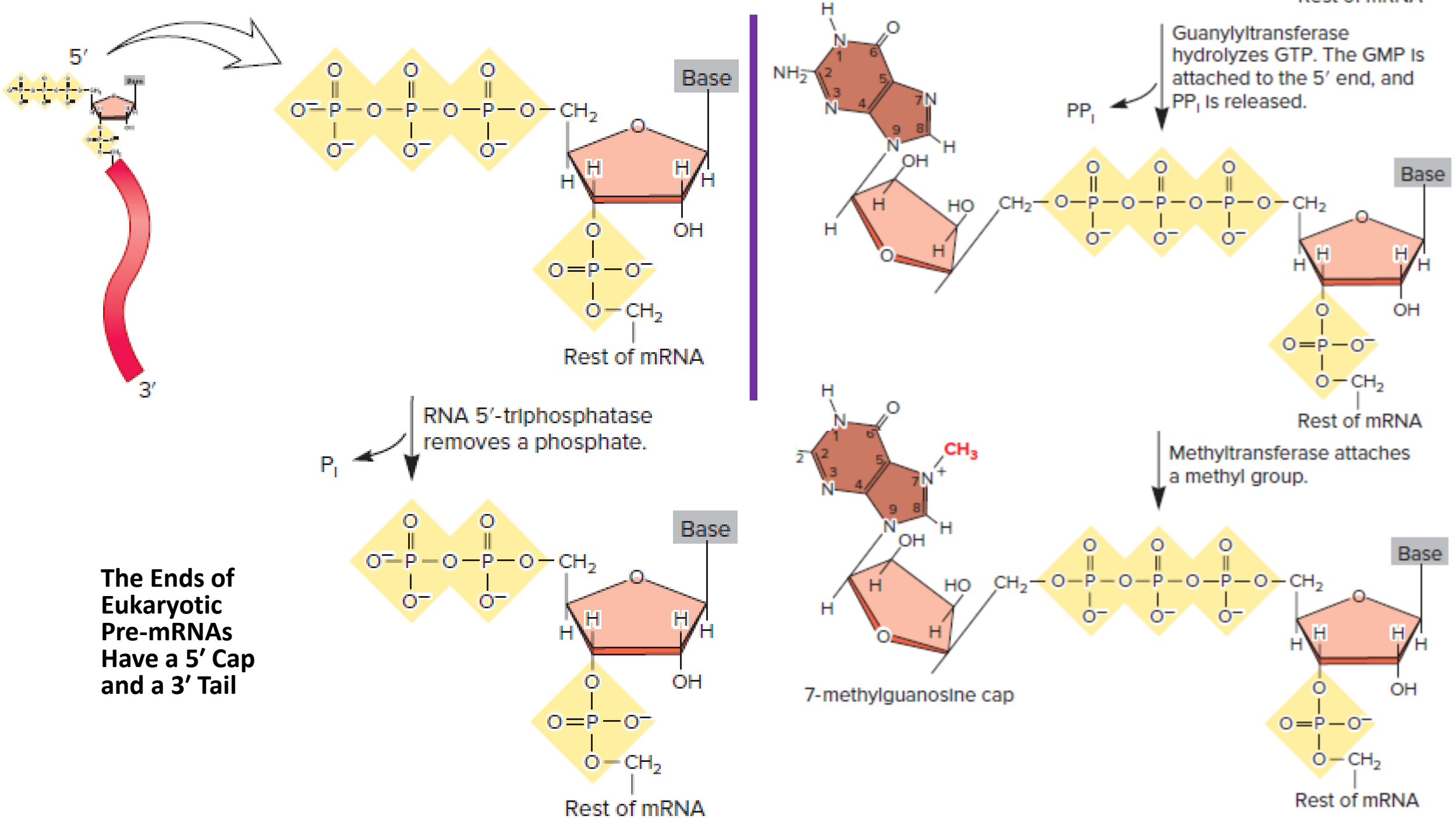


FIGURE 12.16 The processing of ribosomal RNA in eukaryotes. The large ribosomal RNA gene is transcribed into a long 45S rRNA primary transcript. This transcript is cleaved to produce 18S, 5.8S, and 28S rRNA molecules, which become associated with protein subunits in the ribosome. This processing occurs within the nucleolus of the cell.



The Ends of Eukaryotic Pre-mRNAs Have a 5′ Cap and a 3′ Tail

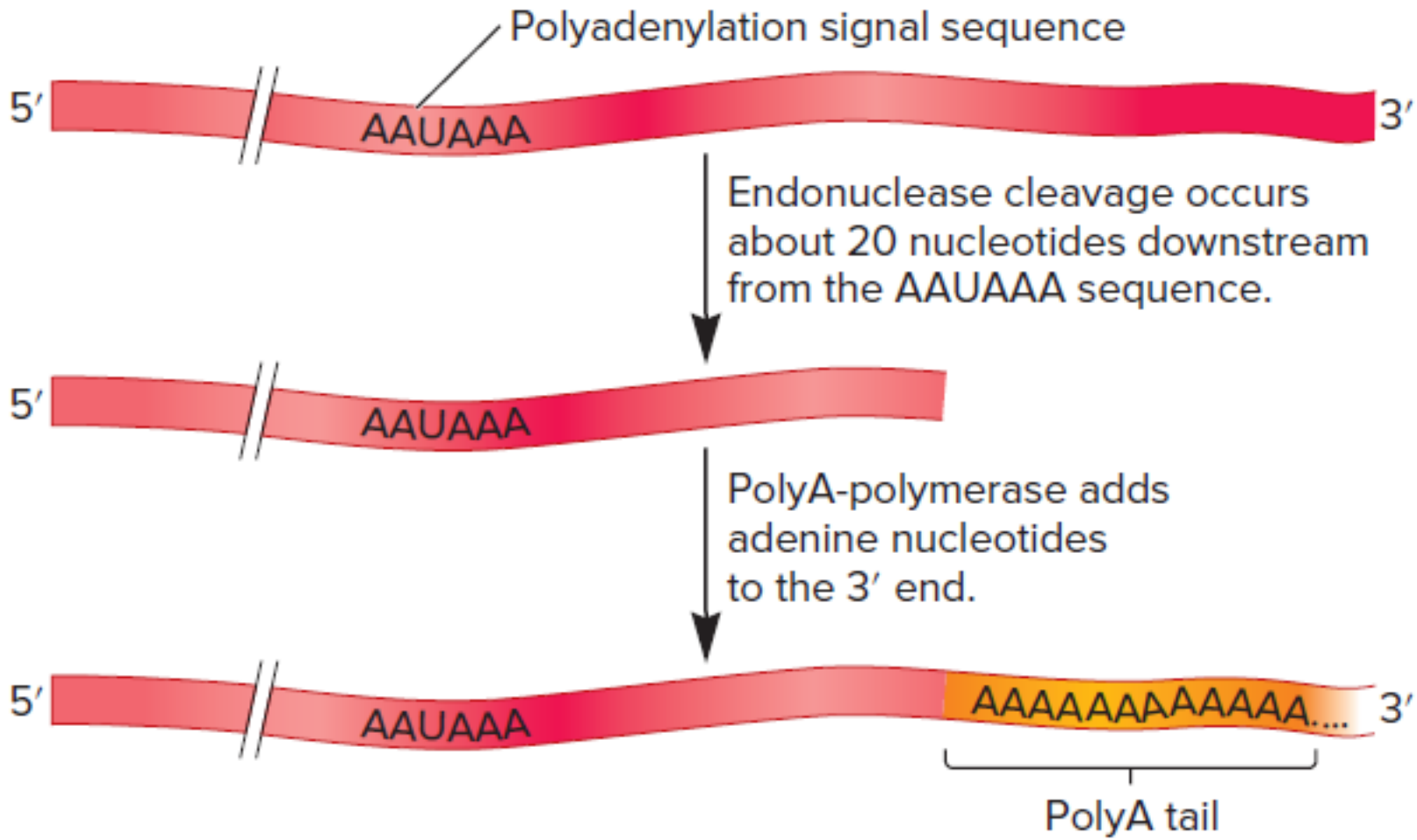
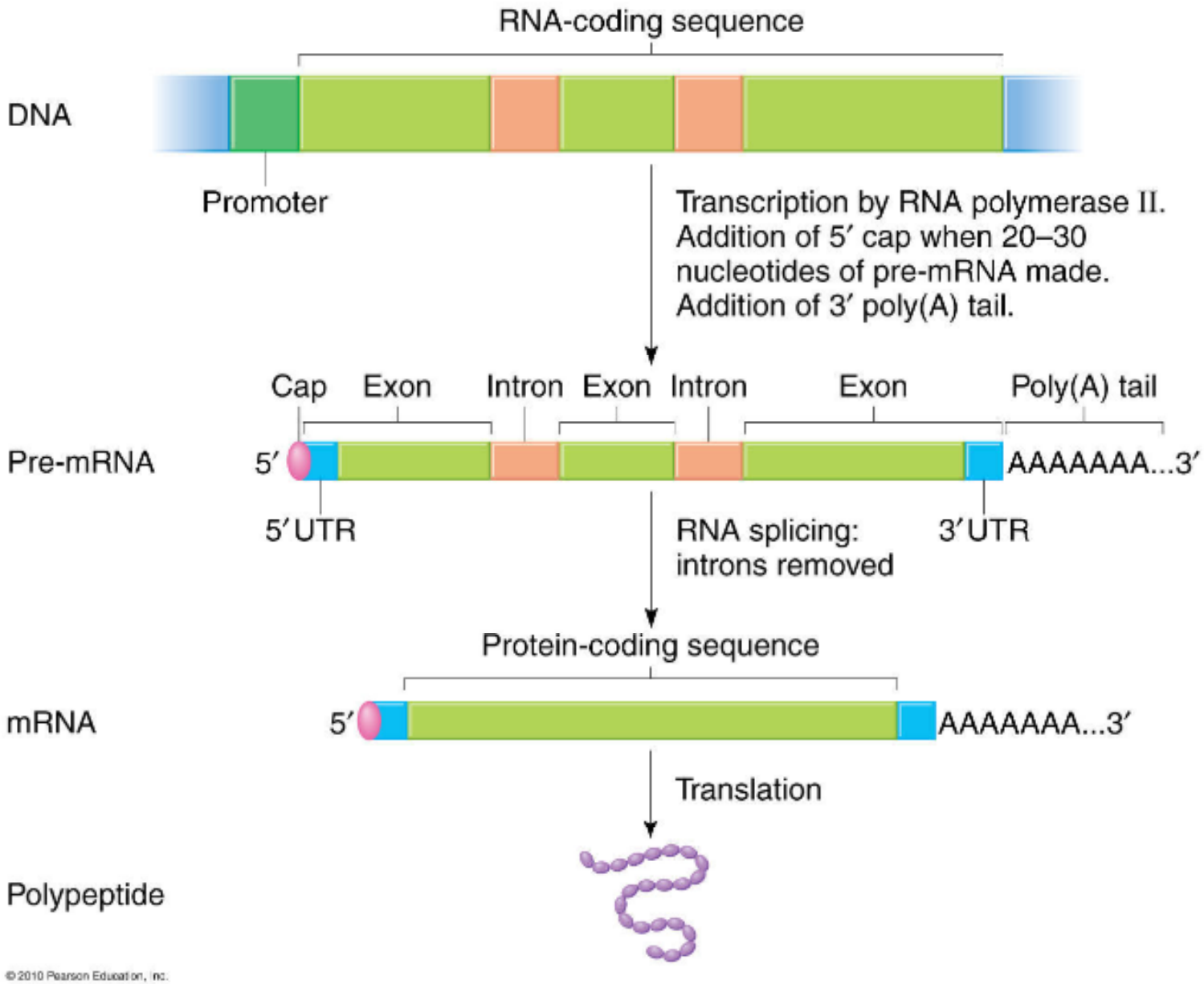
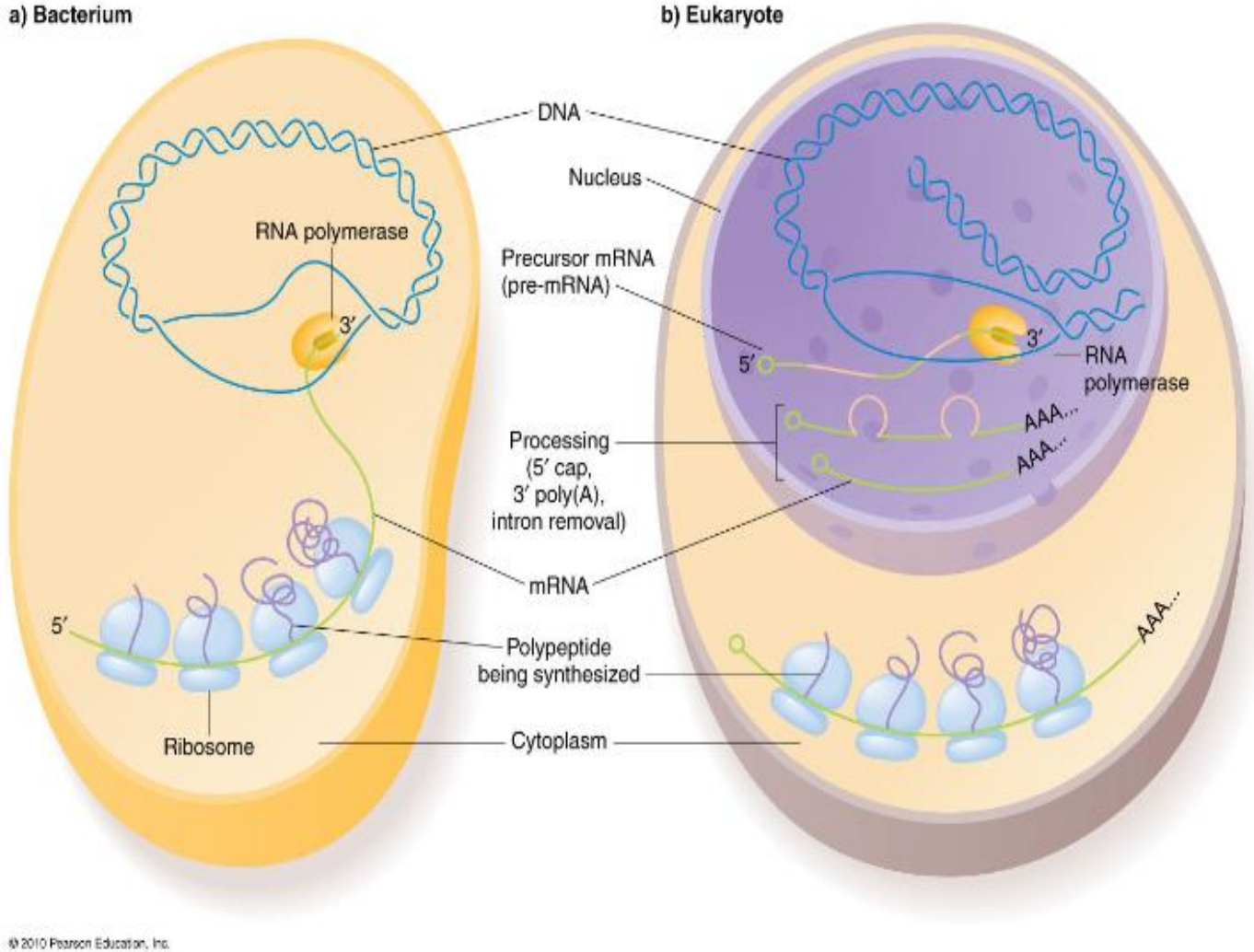


FIGURE 12.24 Attachment of a polyA tail. First, an endonuclease cuts the RNA at a location that is 11–30 nucleotides after the AAUAAA polyadenylation sequence, making the RNA shorter at its 3′ end. Adenine-containing nucleotides are then attached, one at a time, to the 3′ end by the enzyme polyA-polymerase.



Post-transcriptional processing of RNA in Eukaryotes

[An important note on terminology: Splicing of Exons & Introns]



The Central Dogma in prokaryotic *versus* eukaryotic cells

In **prokaryotes** (organisms without a nuclear membrane), **DNA** undergoes **replication** and **transcription** and **RNA** undergoes **translation** in an undivided compartment. All three processes can occur simultaneously.

In **eukaryotes** (organisms with a **nuclear membrane**), **DNA** undergoes **replication** and **transcription** in the **nucleus**, and **proteins** are made in the **cytoplasm. RNA** must therefore travel across the **nuclear membrane** before it undergoes **translation**. This means that **transcription** and **translation** are *physically separated*. The primary transcript, **heterogeneous nuclear RNA** (**hnRNA**), undergoes extensive **post-transcriptional processing** to make a **messenger RNA** (**mRNA**)molecule that can pass through the nuclear membrane.

The terms '***exon***' and '***intron***' refer to **expressed** and **intervening DNA sequences**, respectively. However, as in the above diagram, they are sometimes used to refer to the corresponding sequences in **hnRNA** that are retained or removed, respectively, from the final **mRNA** product. This is technically incorrect: these should properly be called *intron and exon* ***transcripts***. This means of course that the sequences of the **DNA** *exons* in the *sense strand* are identical to the corresponding **mRNA** *exon transcripts*, except for substitution of **U** for **T**. Thus the corresponding amino acid sequences can be either 'read' directly from the **DNA** sense strand, or 'translated' from the **mRNA**.

Similarly, '***splicing***' involves the **joining** of two things, for example the ends of two ropes. (Don't confuse '*splicing*' with '*splitting*'). In molecular biology, **splicing** refers to the process of **joining exon transcripts**, during which intron transcripts are removed, which process is often referred to as '***splicing out***'