Transcription

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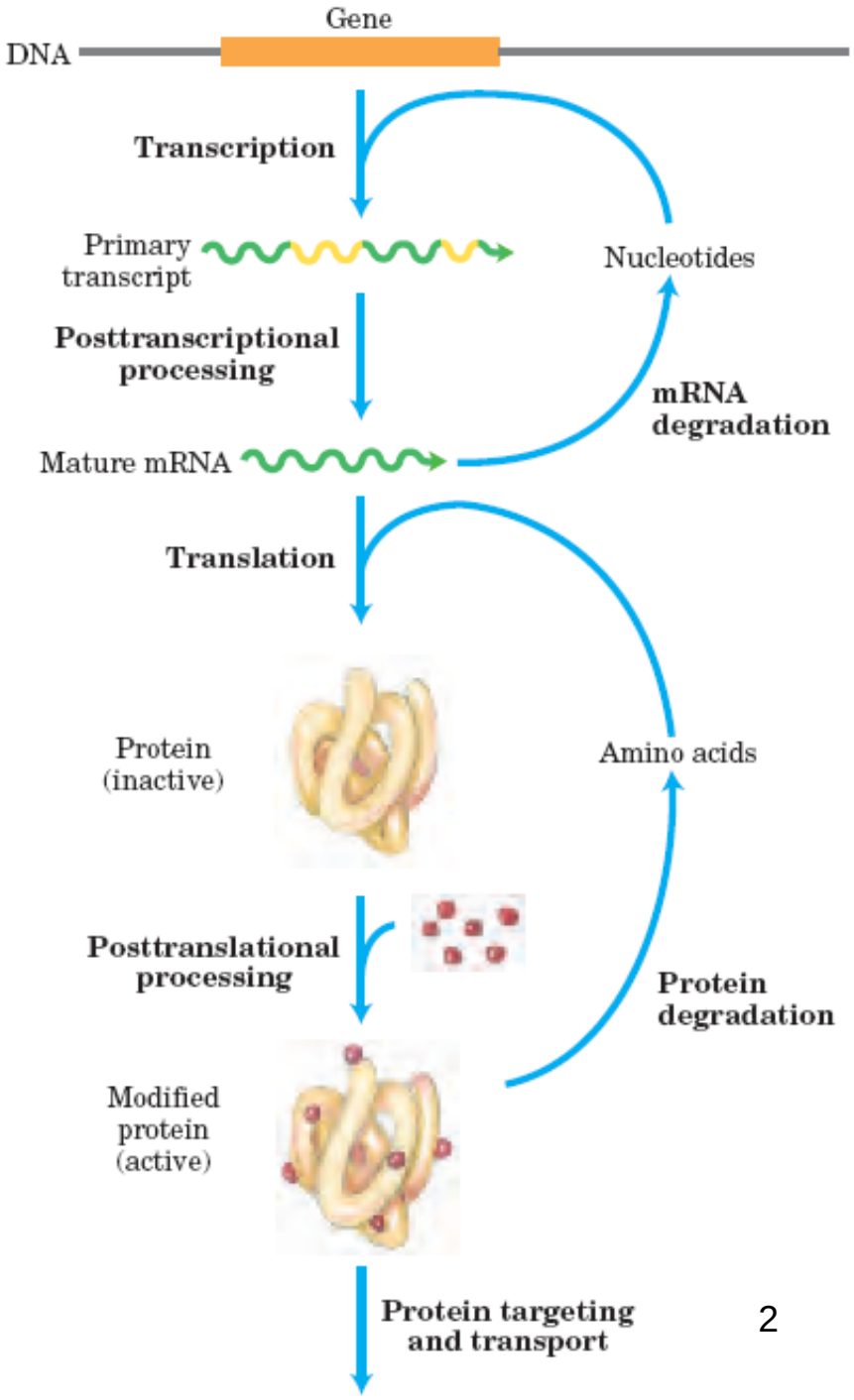
REGULATION OF GENE EXPRESSION

1. Synthesis of the primary RNA transcript (transcription)
2. Posttranscriptional modification of mRNA
3. Messenger RNA degradation
4. Protein synthesis (translation)
5. Posttranslational modification of proteins
6. Protein targeting and transport
7. Protein degradation

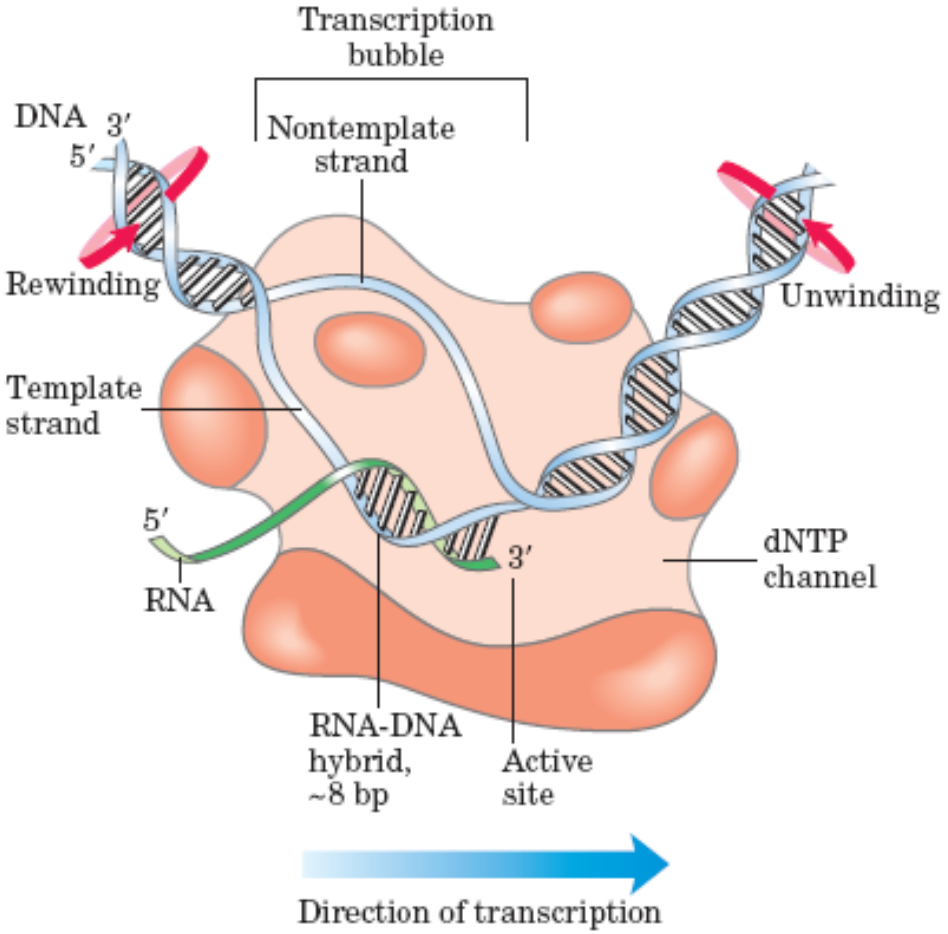


RNA

- **Messenger RNAs (mRNAs) encode the amino acid sequence of** one or more polypeptides specified by a gene or set of genes.
- **Transfer RNAs (tRNAs) read the information** encoded in the mRNA and transfer the appropriate amino acid to a growing polypeptide chain during protein synthesis.
- **Ribosomal RNAs (rRNAs) are constituents** of ribosomes, the intricate cellular machines that synthesize proteins.

RNA Is Synthesized by RNA Polymerases

1. **DNA duplex must unwind (±*17 bp* unwound) → a transcription "bubble."**
2. **The 8 bp RNA-DNA hybrid.**
3. **Elongation of a transcript by *E. coli RNA* polymerase proceeds at a rate of 50 to 90 nucleotides/s.**



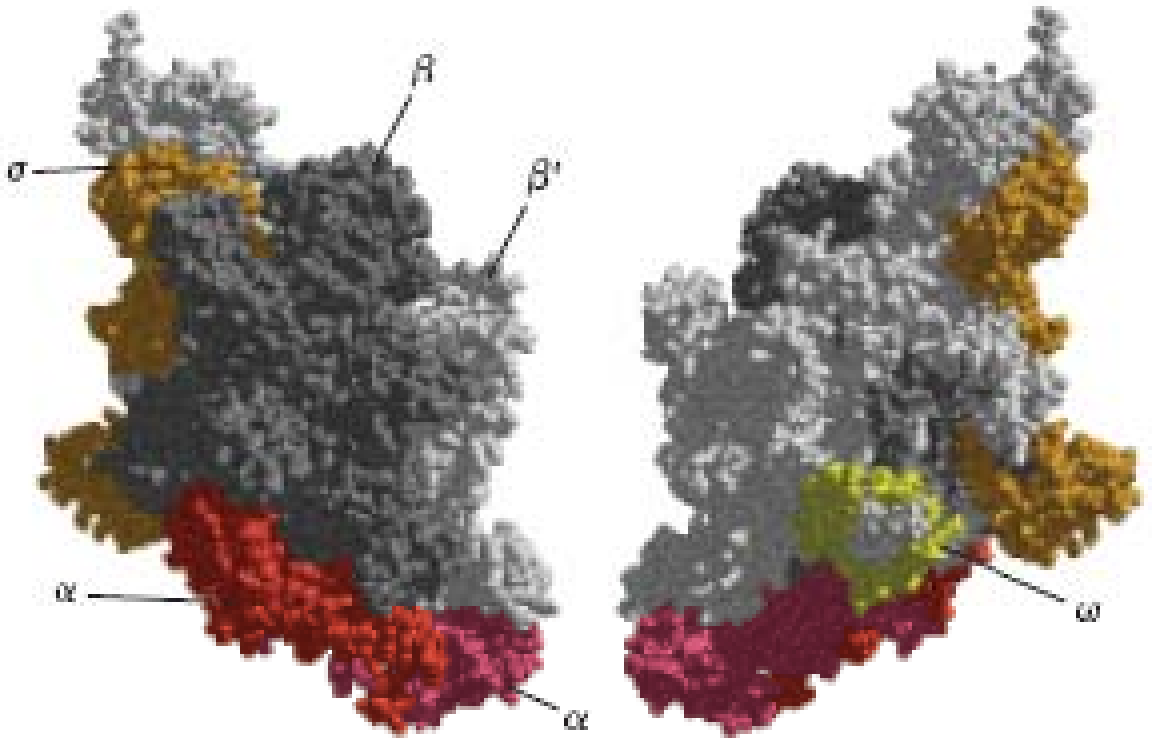
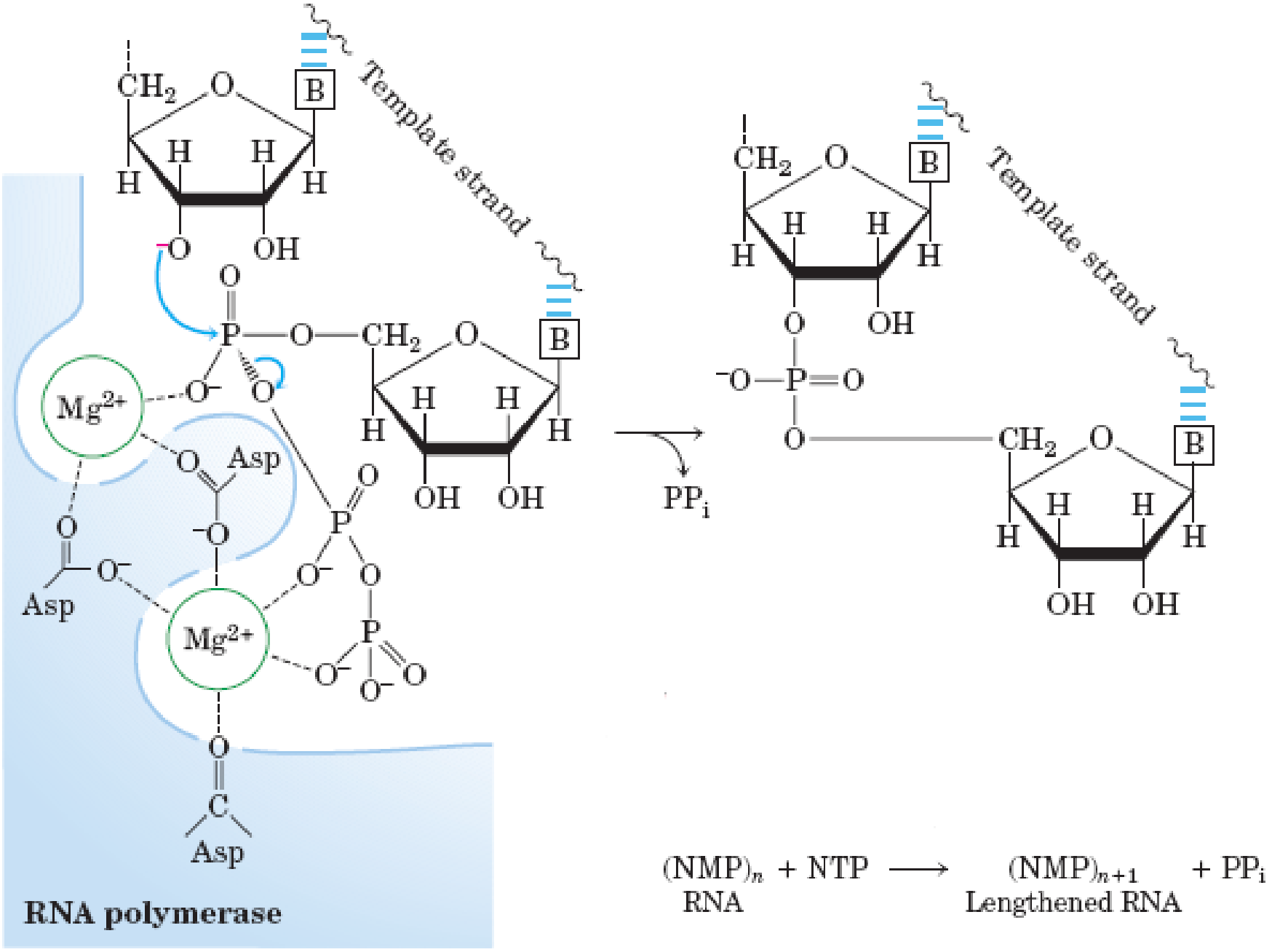


FIGURE 26–4 Structure of the RNA polymerase holoenzyme of the bacterium *Thermus aquaticus*. (Derived from PDB ID 1IW7.) The overall structure of this enzyme is very similar to that of the *E. coli* RNA polymerase; no DNA or RNA is shown here. The β subunit is in gray, the β′ subunit is white; the two α subunits are different shades of red; the ω subunit is yellow; the σ subunit is orange. The image on the left is oriented as in Figure 26–6. When the structure is rotated 180° about the *y* axis (right) the small ω subunit is visible.



$$(NMP)_n + NTP \longrightarrow (NMP)_{n+1} + PP_i$$

RNA — Lengthened RNA

DNA template

RNA polymerase is most active when bound to a double-stranded DNA. Only one of the two DNA strands serves as a template.

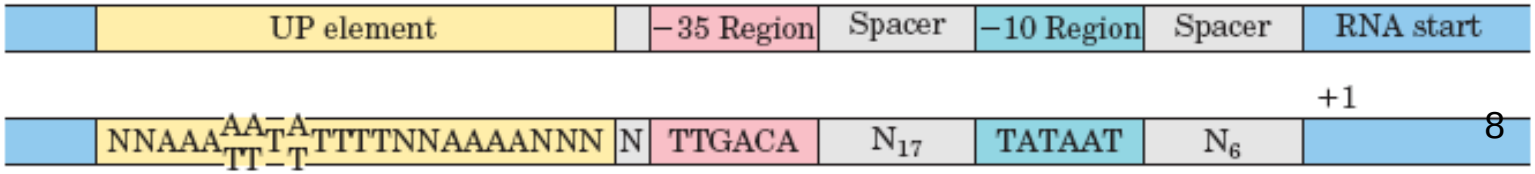
(5′) CGCTATAGCGTTT (3′)	DNA nontemplate (coding) strand
(3′) GCGATATCGCAAA (5′)	DNA template strand
(5′) CGCUAUAGCGUUU (3′)	RNA transcript

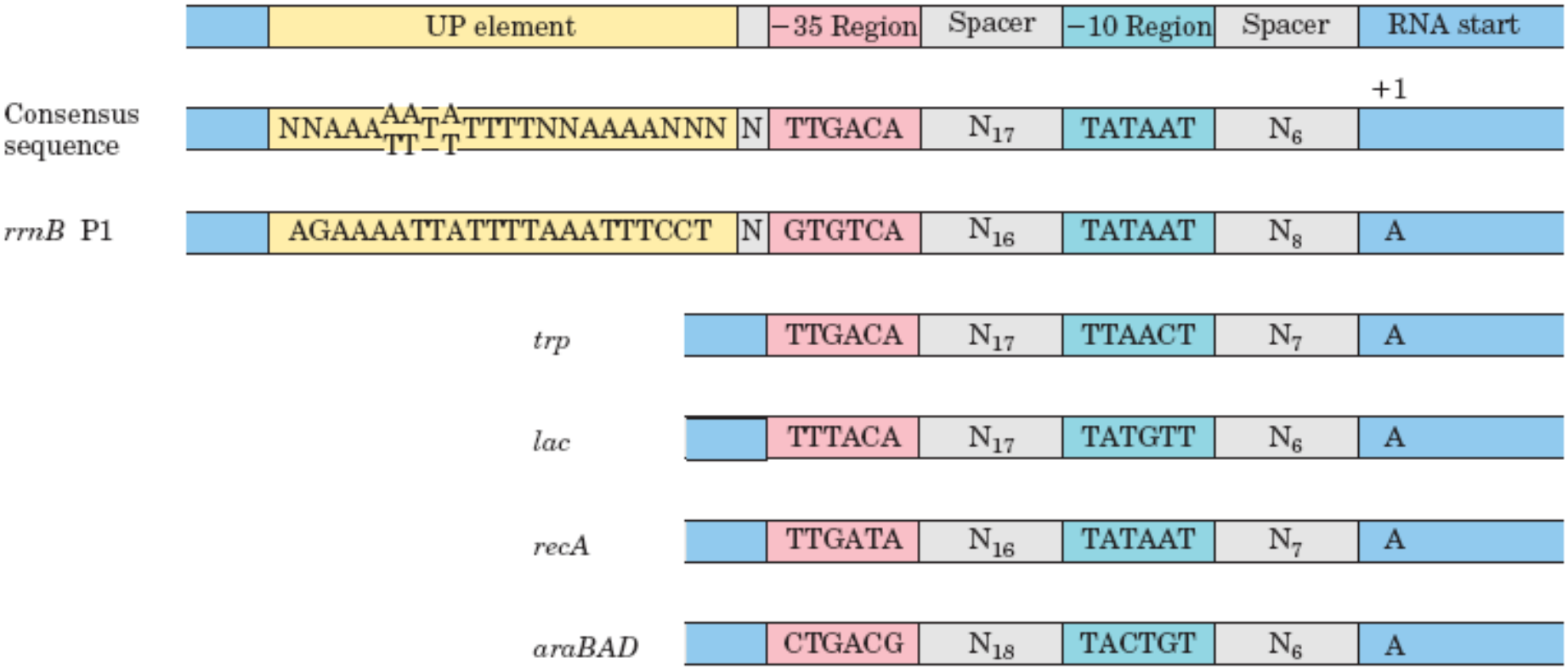
RNA Synthesis Begins at Promoters

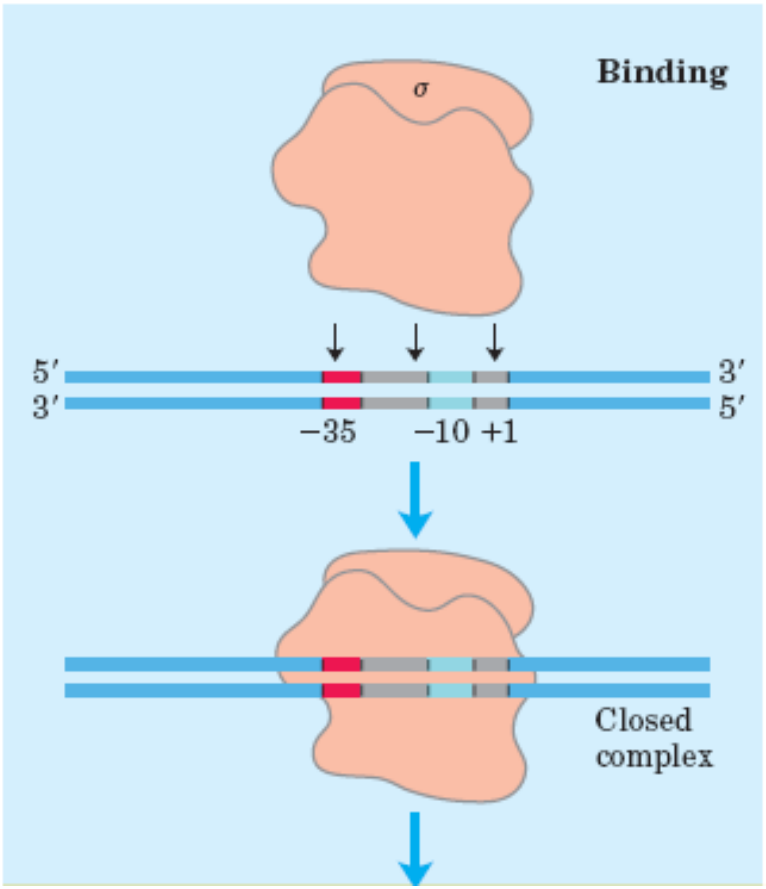
- an RNA polymerase binds to specific sequences in the DNA → Promoters
- The promoter region → -70 and + 30
- **consensus sequence :** -10 and -35 → interaction sites for the σ70 subunit

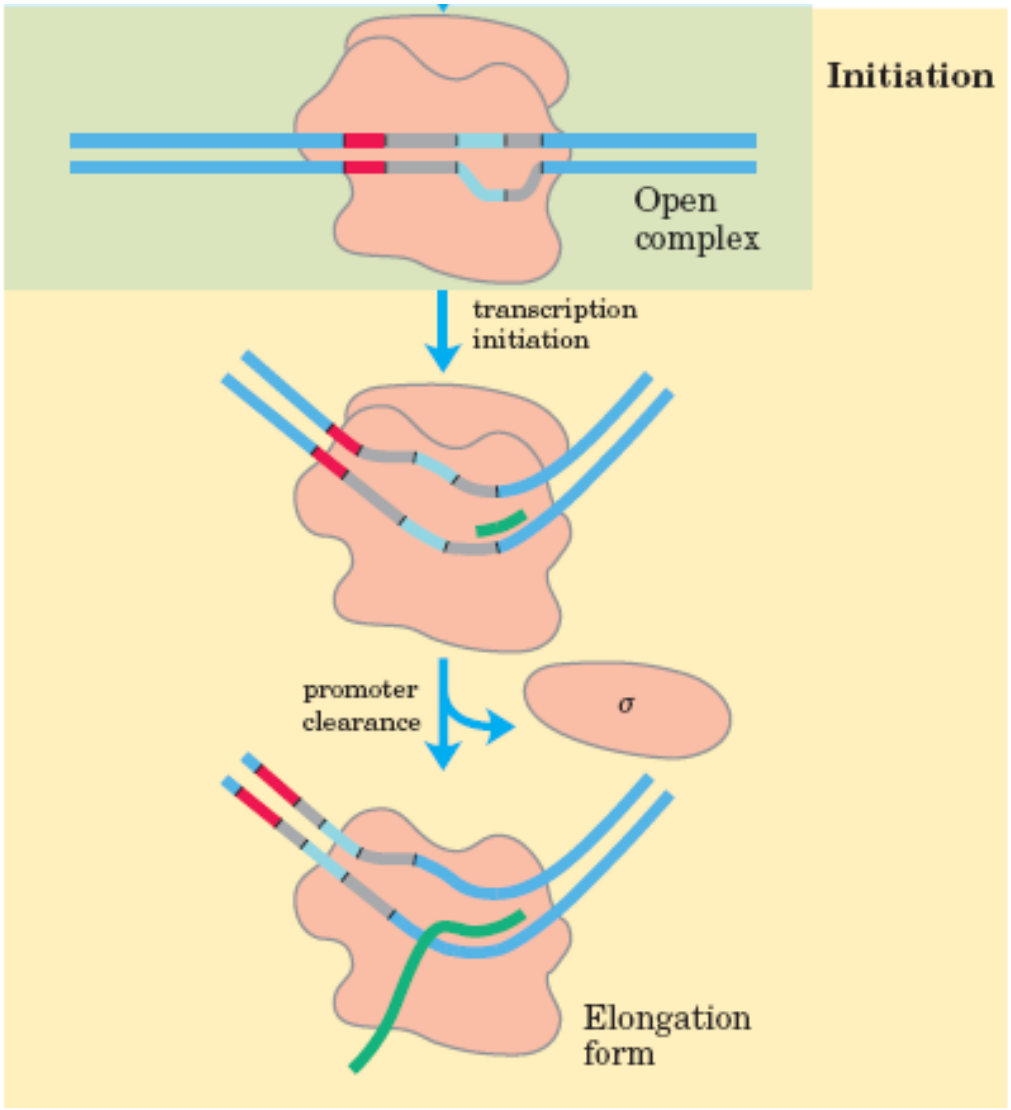
  -10 region is (5')TATAAT(3')

  -35 region is (5')TTGACA(3')
- AT-rich recognition element UP (upstream promoter) element -40 and -60 → is bound by the α *subunit of RNA polymerase.*
- → the efficiency of RNA polymerase binding and transcription initiation.




UP element
−35 Region
Spacer
−10 Region
Spacer
RNA start
+1
Consensus sequence
NNAAA AA T A TTTTNNAAAAANNN
TT T
N
TTGACA
N17
TATAAT
N6
rrnB P1
AGAAAATTATTTTAAATTTCCT
N
GTGTCA
N16
TATAAT
N8
A
trp
TTGACA
N17
TTAACT
N7
A
lac
TTTACA
N17
TATGTT
N6
A
recA
TTGATA
N16
TATAAT
N7
A
araBAD
CTGACG
N18
TACTGT
N6
A





Transcription initiation and elongation by *E. coli RNA* polymerase.

Specific Sequences Signal Termination of RNA Synthesis

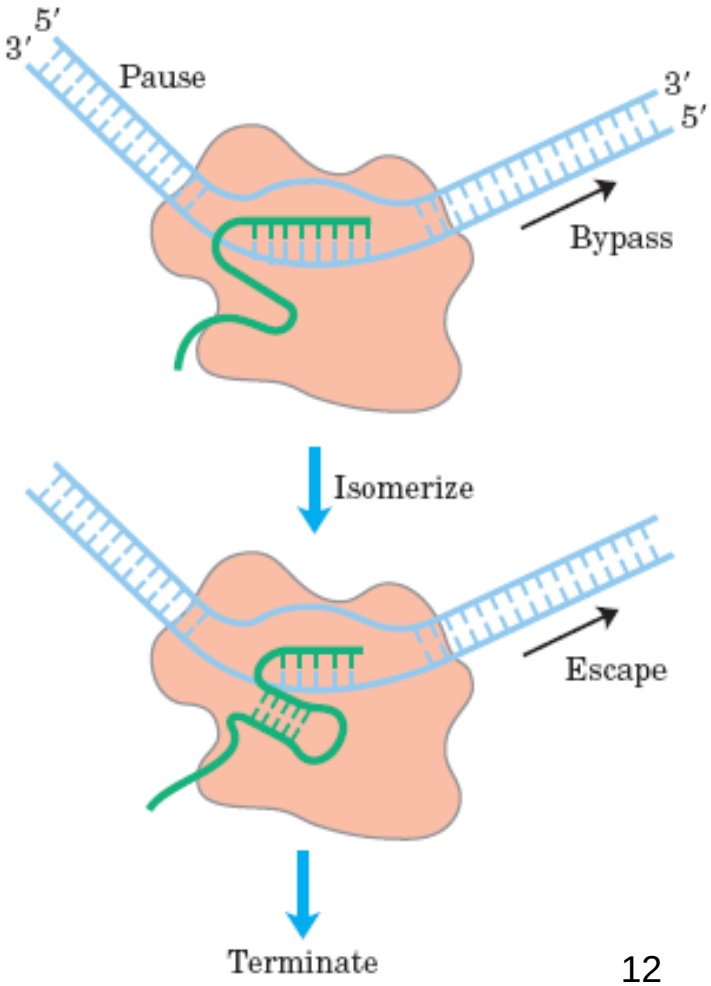
termination signals:

1. protein factor ρ *(rho)*
2. ρ *-independent.*

- protein factor ρ *(rho)* → a CA-rich sequence called a *rut (rho utilization)* element.
- The *protein associates with the RNA at specific* binding sites and migrates in the 5→3 direction until it reaches the transcription complex that is paused at a termination site.
- The ρ *protein has an ATP-dependent* RNA-DNA helicase activity that promotes translocation of the protein along the RNA, and ATP is hydrolyzed by ρ *protein during the termination process.* → release of the RNA transcript.

ρ *-independent*

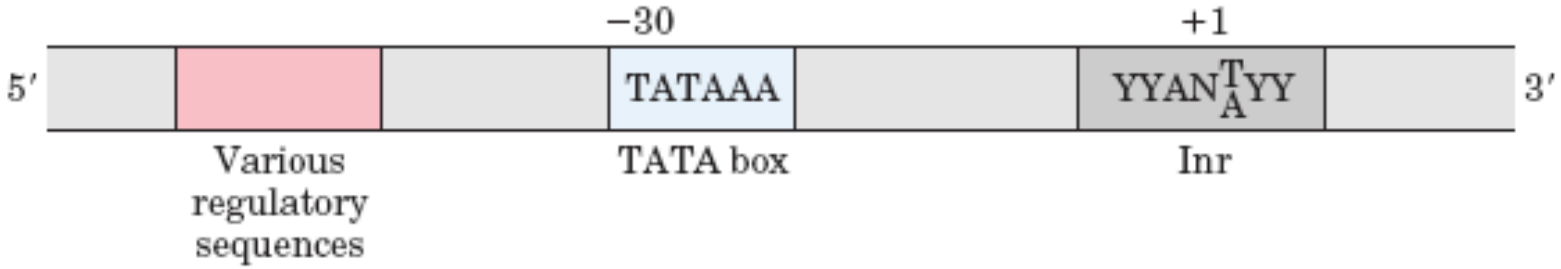
1. A region that produces an RNA transcript with self-complementary sequences, permitting the formation of a **hairpin structure** centered **15 to 20 nucleotides** before the projected end of the RNA strand.
2. A highly conserved string of three A residues in the template strand that are transcribed into U residues near the 3 end of the hairpin.



Eukaryotic Cells Have Three Kinds of Nuclear RNA Polymerases

- RNA polymerase I (Pol I) → the synthesis of only one type of RNA, a transcript called preribosomal RNA (or pre-rRNA)
- RNA polymerase II (Pol II) → synthesis of mRNAs and some specialized RNAs.
- RNA polymerase III (Pol III) → makes tRNAs, the 5S rRNA, and some other small specialized RNAs

RNA Polymerase II



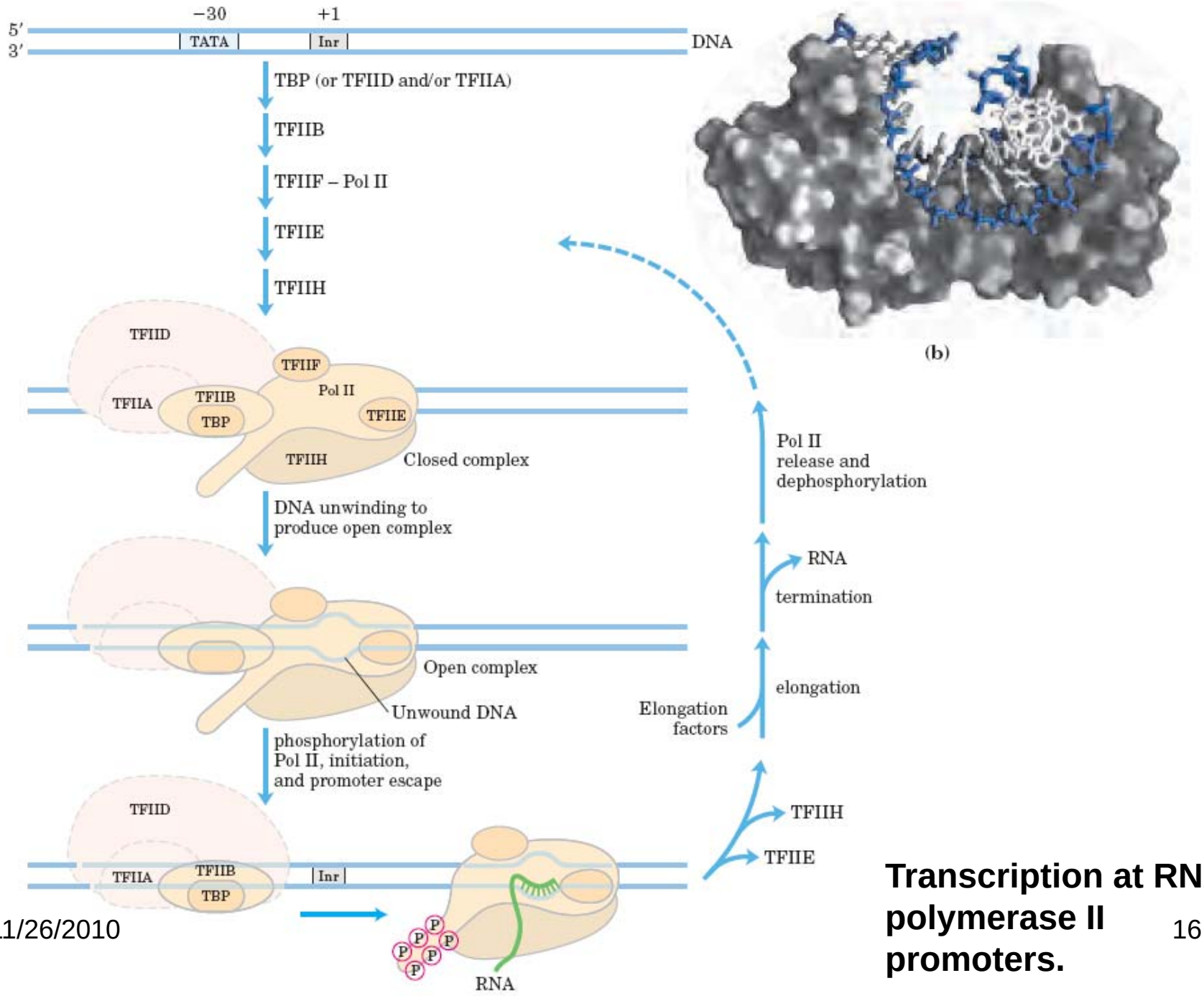
- The TATA box is the major assembly point for the proteins of the preinitiation complexes of Pol II.
- The DNA is unwound at the initiator sequence (Inr), and the transcription start site is usually within or very near this sequence.

RNA Polymerase II Requires Many Other Protein Factors for Its Activity

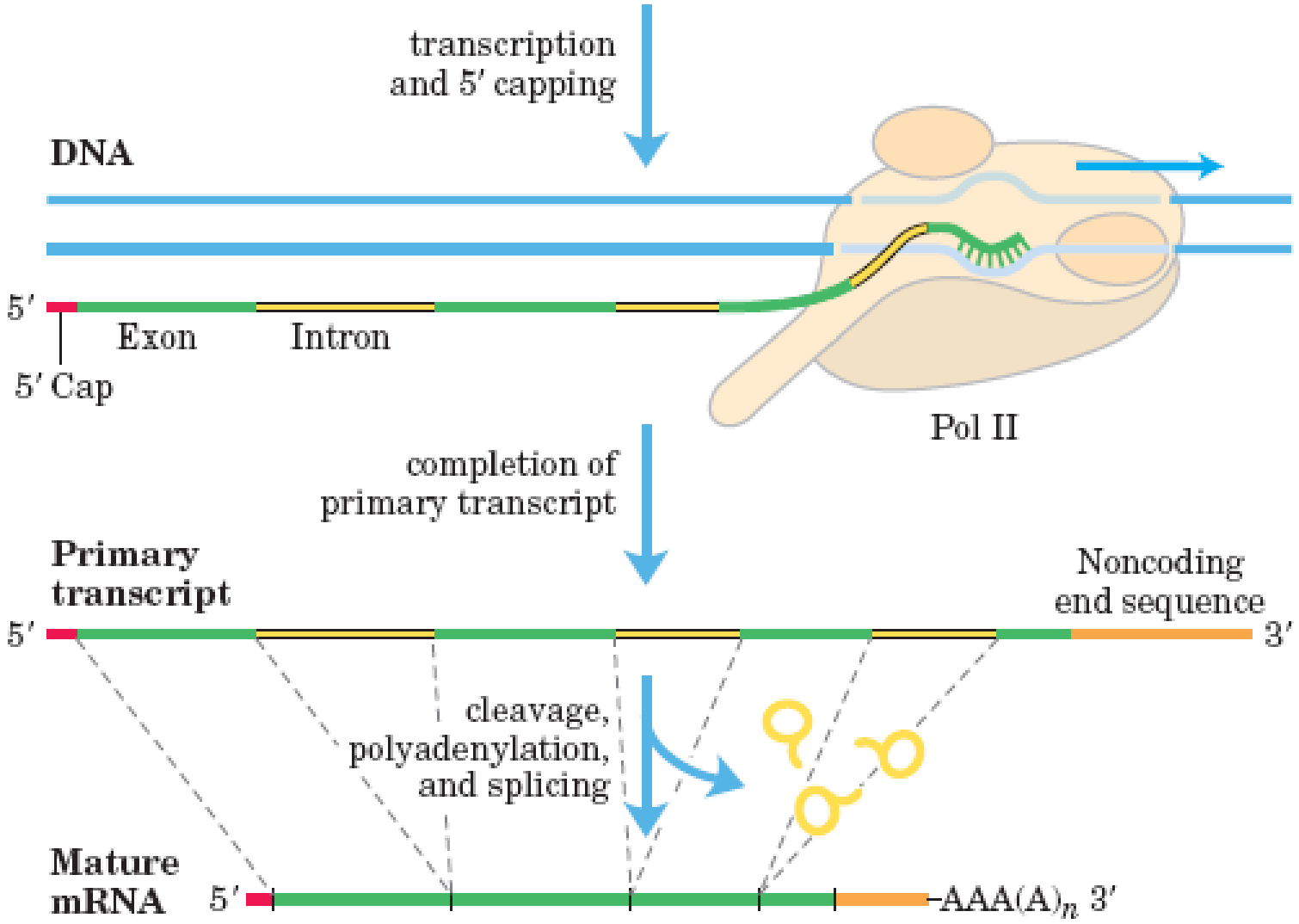
- RNA polymerase II requires an array of other proteins, called **transcription factors, in order to form** the active transcription complex.

TABLE 26–1 Proteins Required for Initiation of Transcription at the RNA Polymerase II (Pol II) Promoters of Eukaryotes

Transcription protein	*Number of subunits*	*Subunit(s) M_r*	*Function(s)*
Initiation			
Pol II	12	10,000–220,000	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	38,000	Specifically recognizes the TATA box
TFIIA	3	12,000, 19,000, 35,000	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	35,000	Binds to TBP; recruits Pol II–TFIIF complex
TFIIE	2	34,000, 57,000	Recruits TFIIH; has ATPase and helicase activities
TFIIF	2	30,000, 74,000	Binds tightly to Pol II; binds to TFIIB and prevents binding of Pol II to nonspecific DNA sequences
TFIIH	12	35,000–89,000	Unwinds DNA at promoter (helicase activity); phosphorylates Pol II (within the CTD); recruits nucleotide-excision repair proteins
Elongation[*]			
ELL[†]	1	80,000	
p-TEFb	2	43,000, 124,000	Phosphorylates Pol II (within the CTD)
SII (TFIIS)	1	38,000	
Elongin (SIII)	3	15,000, 18,000, 110,000	

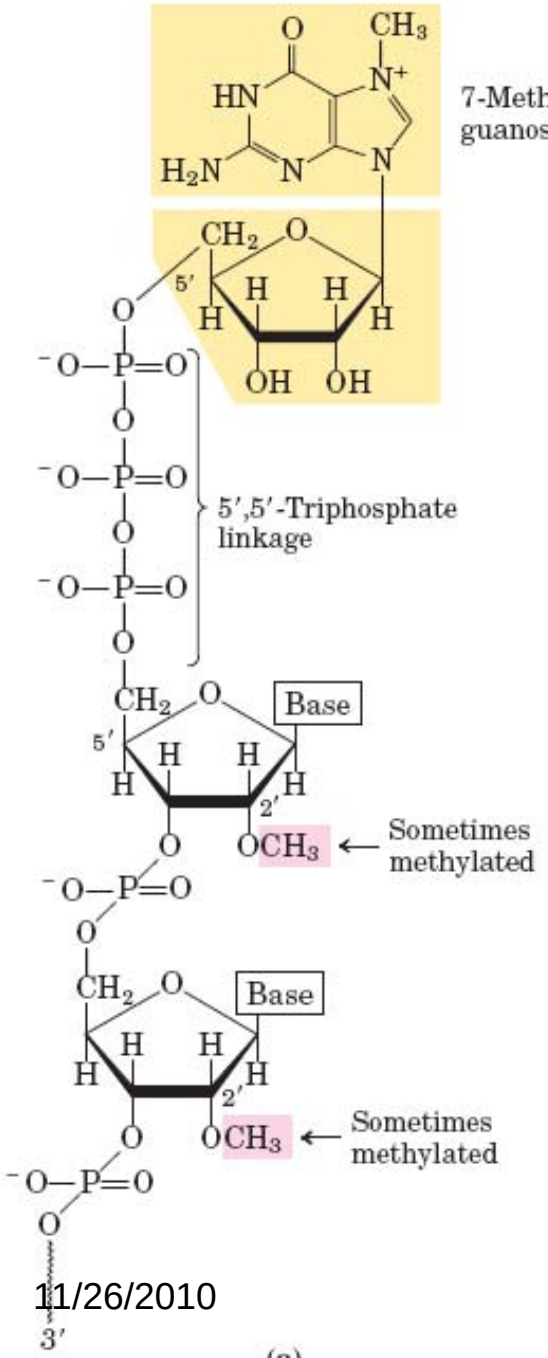


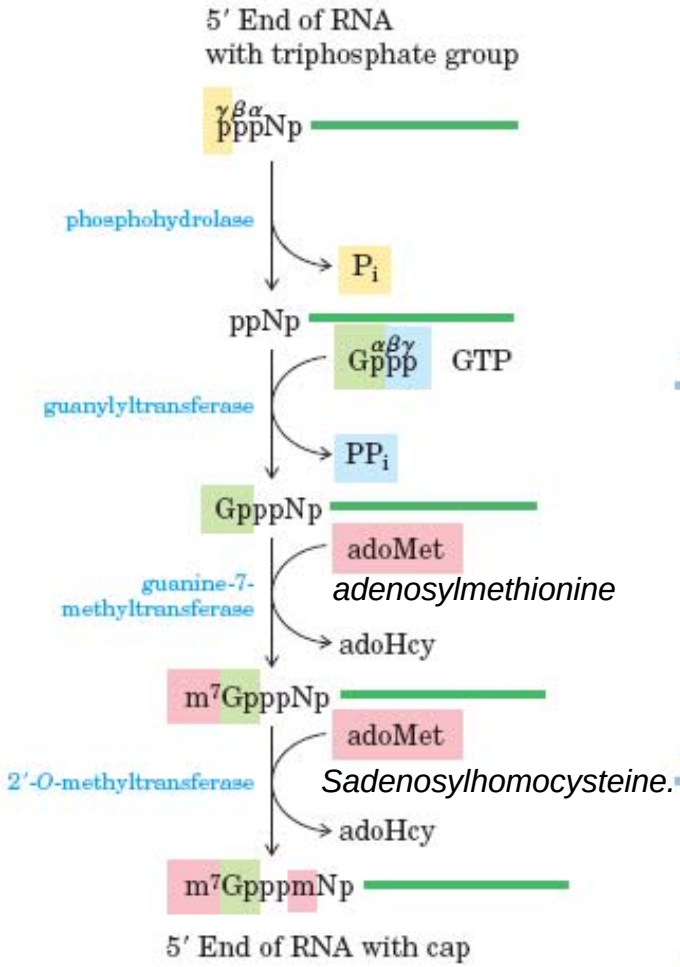
Transcription at RNA polymerase II promoters.


transcription
and 5′ capping
DNA
5′
Exon
Intron
5′ Cap
Pol II
completion of
primary transcript
Primary
transcript
Noncoding
end sequence
5′
3′
cleavage,
polyadenylation,
and splicing
Mature
mRNA
5′
AAA(A)n 3′

The 5’ cap of mRNA.

- The 5 cap helps protect mRNA from ribonucleases.
- The cap also binds to a specific capbinding complex of proteins and participates in binding of the mRNA to the ribosome to initiate translation
- The 5 cap is formed by condensation of a molecule of GTP with the triphosphate at the 5 end of the transcript.
- The guanine is subsequently methylated at N-7
- additional methyl groups are often added at the 2’ hydroxyls of the first and second nucleotides adjacent to the cap → The methyl groups are derived from *S-adenosylmethionine*

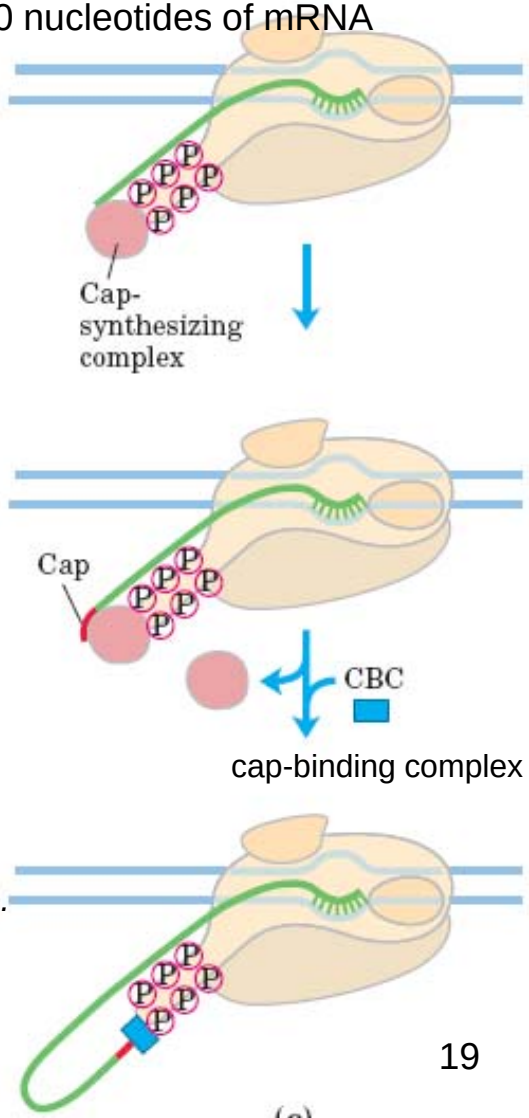




20 to 30 nucleotides of mRNA

adenosylmethionine

Sadenosylhomocysteine.



INTRONS

- Group I introns are found in some nuclear, mitochondrial, and chloroplast genes coding for rRNAs, mRNAs, and tRNAs.
- Group II introns are generally found in the primary transcripts of mitochondrial or chloroplast mRNAs in fungi, algae, and plants

Group 1

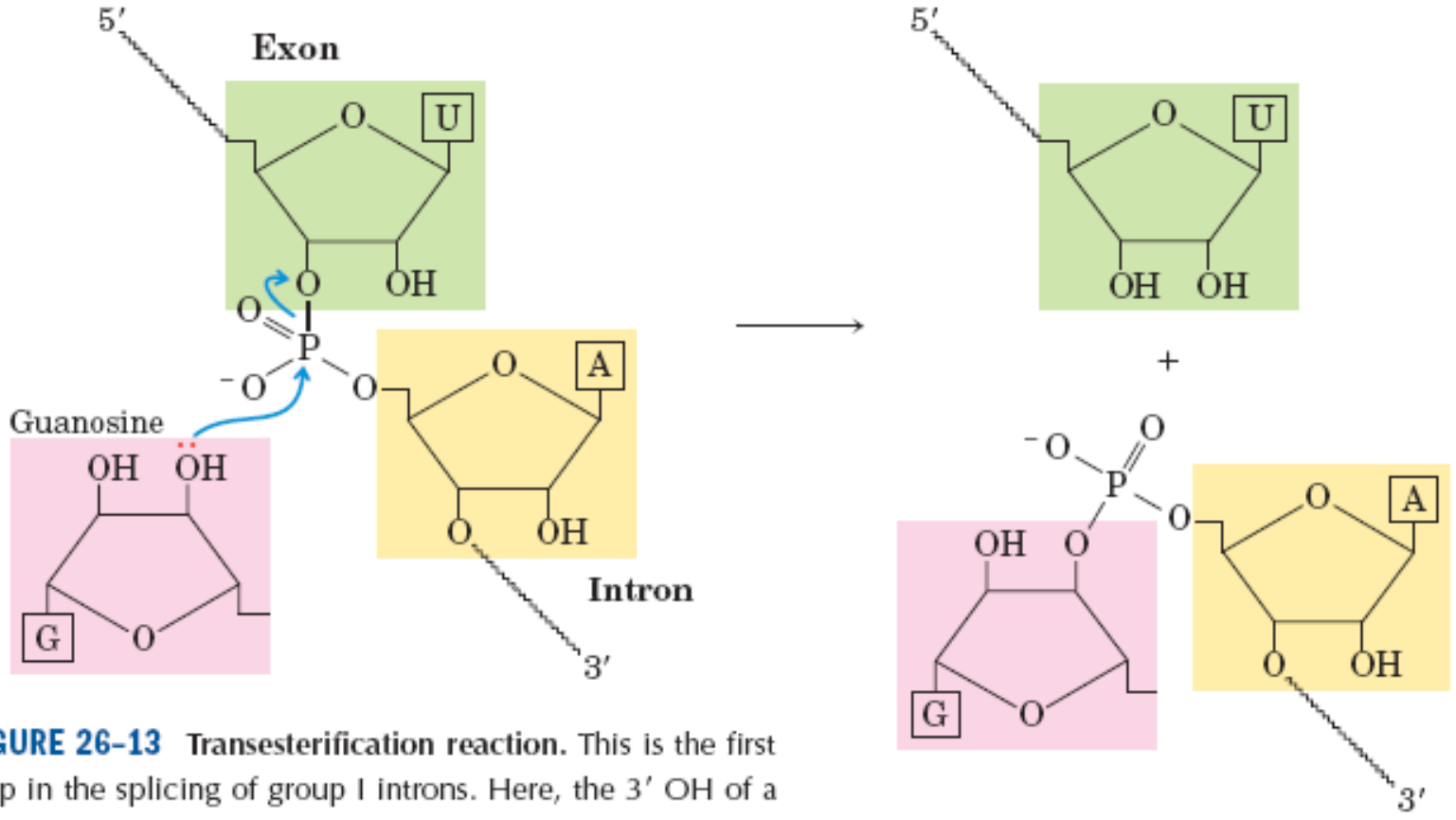


FIGURE 26–13 **Transesterification reaction.** This is the first step in the splicing of group I introns. Here, the 3′ OH of a guanosine molecule acts as nucleophile.

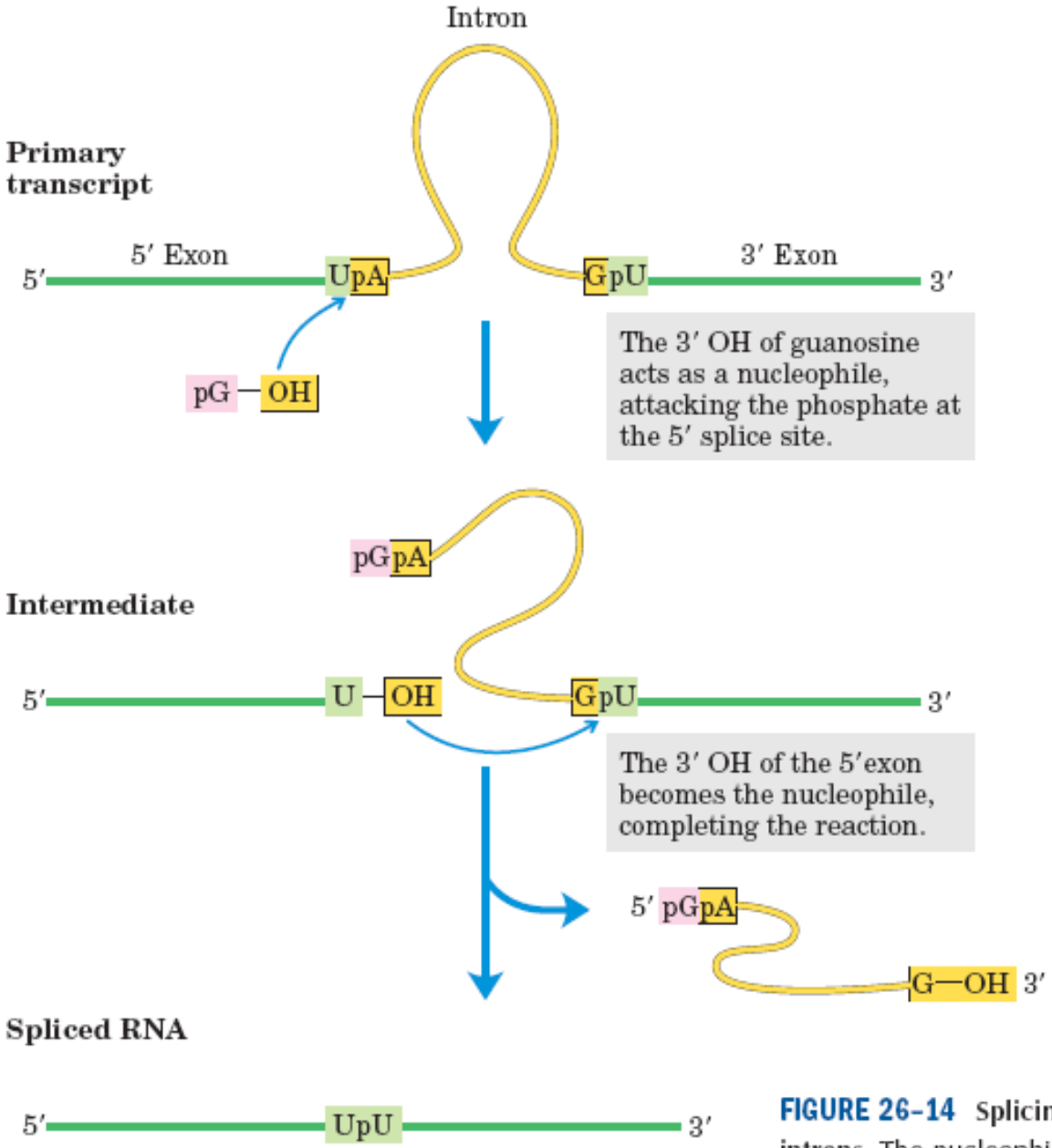


FIGURE 26–14 Splicing mechanism of group I introns. The nucleophile in the first step may be guanosine, GMP, GDP, or GTP. The spliced intron is eventually degraded.

Group 2

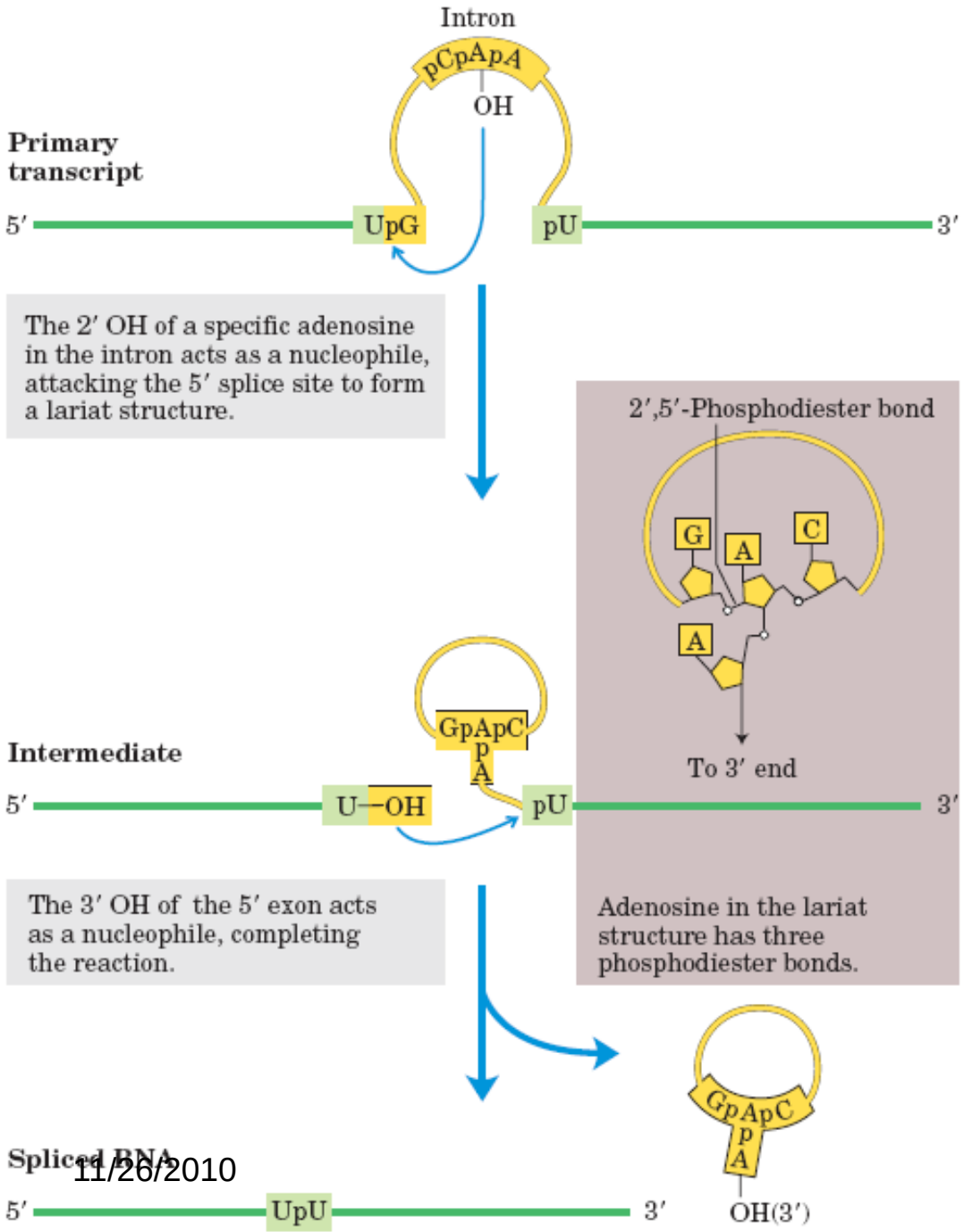
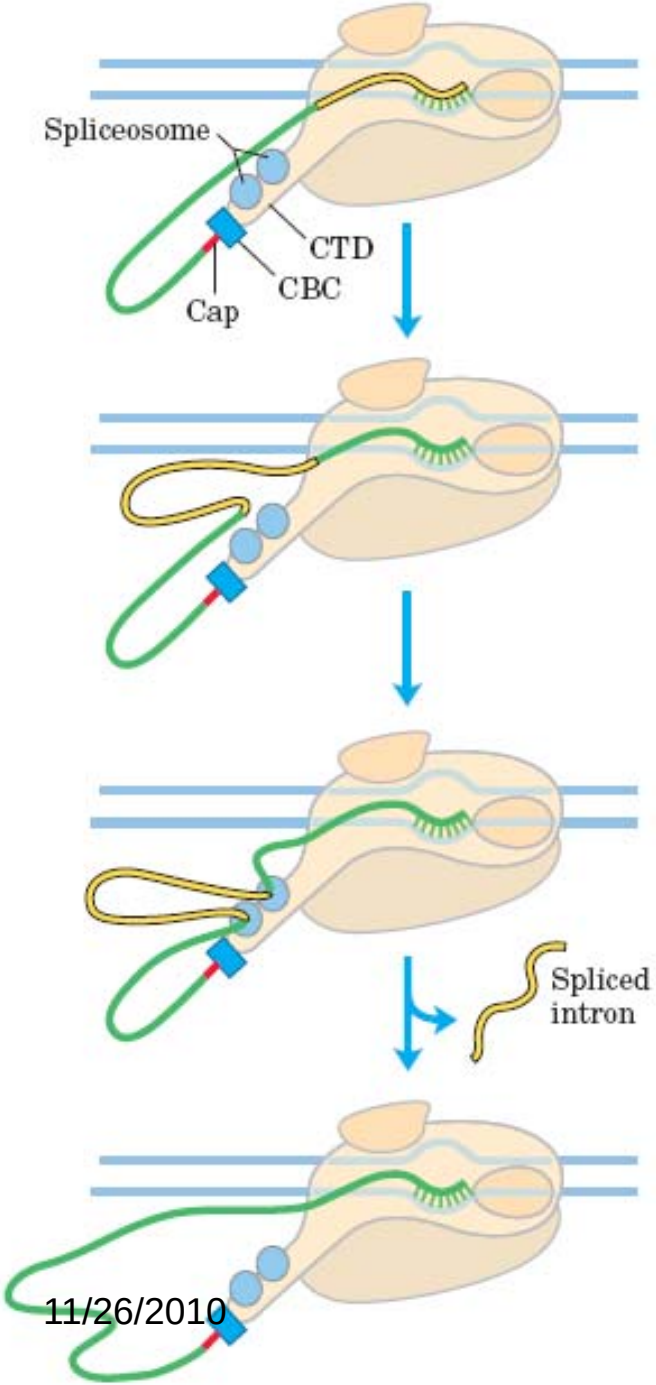


FIGURE 26–15 Splicing mechanism of group II introns. The chemistry is similar to that of group I intron splicing, except for the identity of the nucleophile in the first step and formation of a lariatlike intermediate, in which one branch is a 2′,5′-phosphodiester bond.

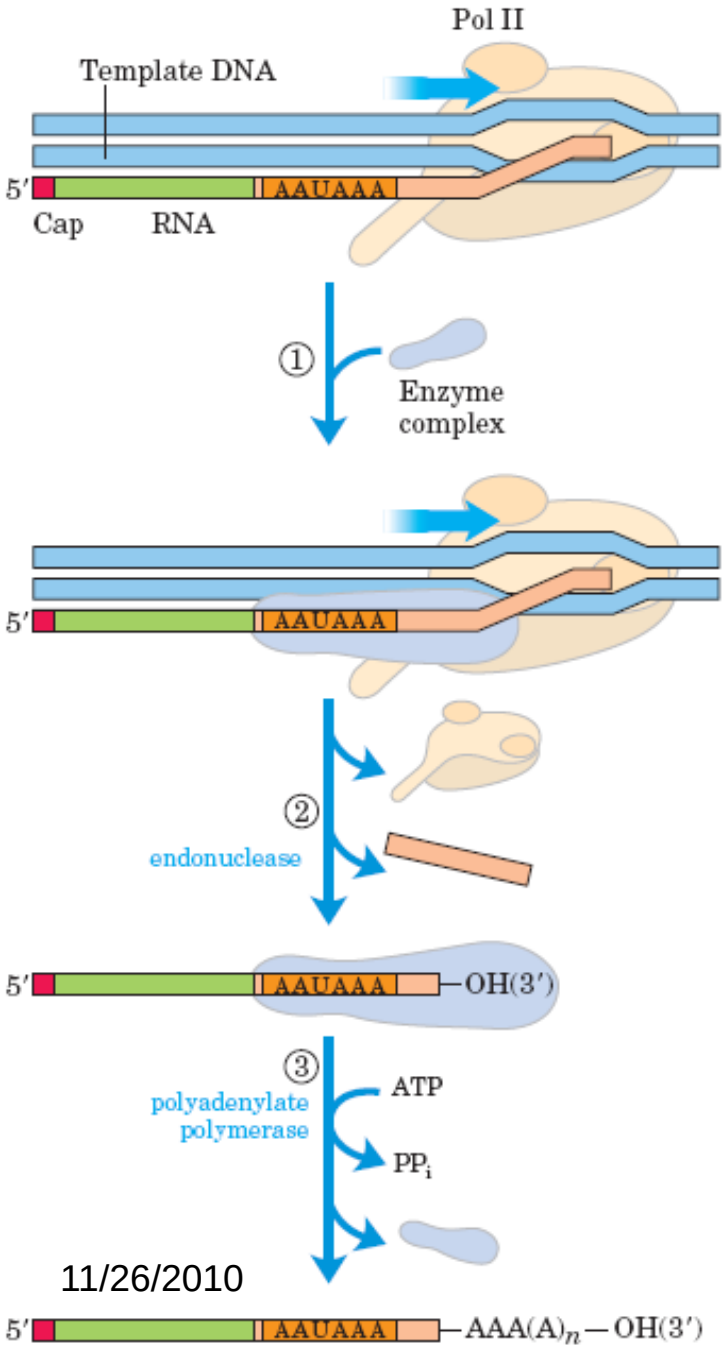


The fourth class of introns, found in certain tRNAs

- requires ATP and an endonuclease.
- The splicing endonuclease cleaves the phosphodiester bonds at both ends of the intron
- the two exons are joined by a mechanism similar to the DNA ligase reaction

Poly(A) tail

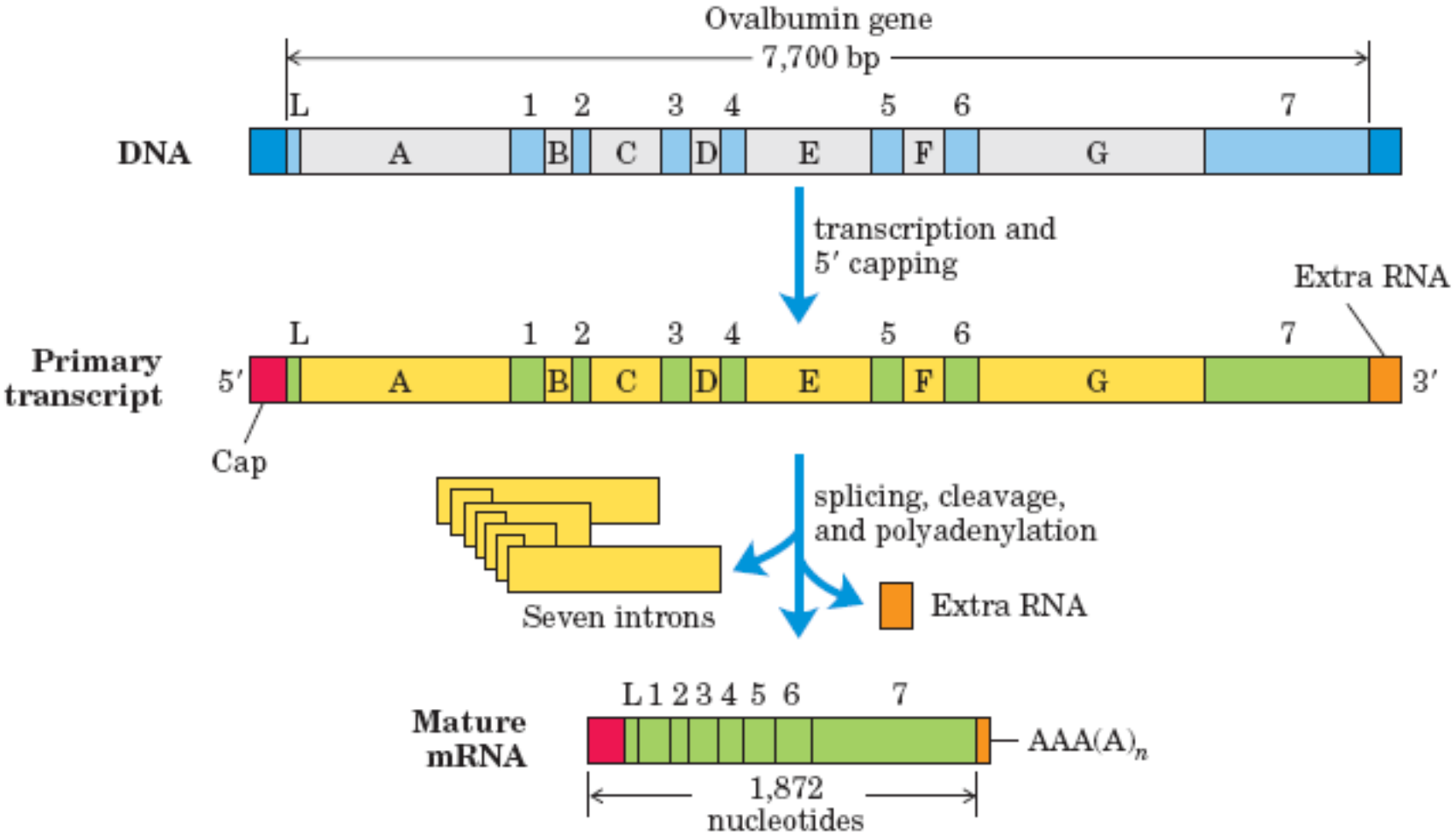
- At their 3 end, most eukaryotic mRNAs have a string of 80 to 250 A residues, making up the **poly(A) tail.**
- This tail serves as a binding site for one or more specific proteins.
- The poly(A) tail and its associated proteins probably help protect mRNA from enzymatic destruction.

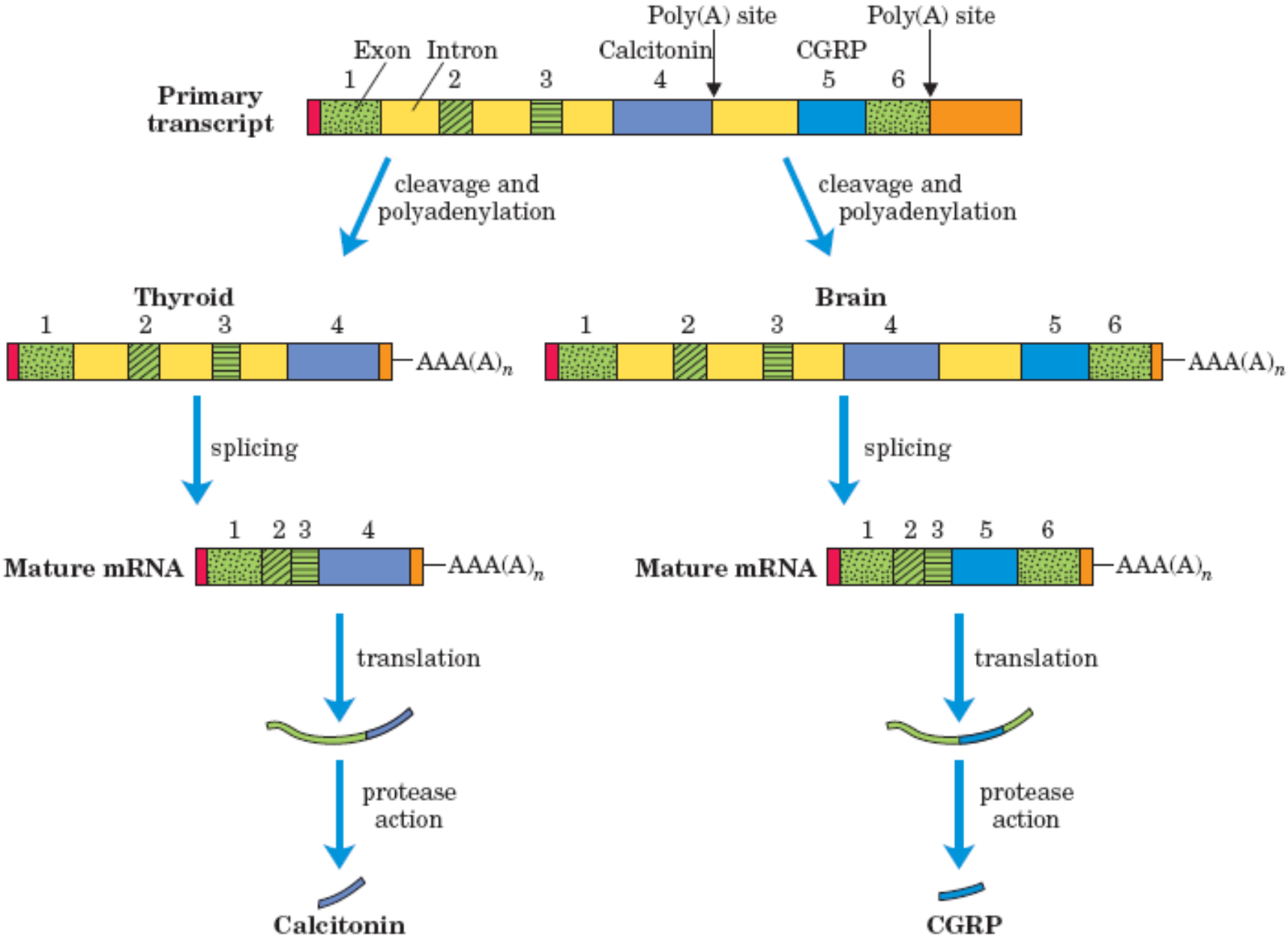


$$\text{RNA} + n\text{ATP} \longrightarrow \text{RNA–(AMP)}_n + n\text{PP}_i$$

FIGURE 26–17 **Addition of the poly(A) tail to the primary RNA transcript of eukaryotes.** Pol II synthesizes RNA beyond the segment of the transcript containing the cleavage signal sequences, including the highly conserved upstream sequence (5′)AAUAAA. (1) The cleavage signal sequence is bound by an enzyme complex that includes an endonuclease, a polyadenylate polymerase, and several other multisubunit proteins involved in sequence recognition, stimulation of cleavage, and regulation of the length of the poly(A) tail. (2) The RNA is cleaved by the endonuclease at a point 10 to 30 nucleotides 3′ to (downstream of) the sequence AAUAAA. (3) The polyadenylate polymerase synthesizes a poly(A) tail 80 to 250 nucleotides long beginning at the cleavage site.

The processing of a eukaryotic mRNA





calcitonin-gene-related peptide