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PRINCIPLES OF BIOCHEMISTRY

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CHAPTER 26

RNA Metabolism

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Transcription

The synthesis of RNA molecules using DNA strands as the templates so that the genetic information can be transferred from DNA to RNA.

Similarity between replication and transcription

- **Both processes use DNA as the template.**
- **Phosphodiester bonds are formed in both cases.**
- **Both synthesis directions are from 5′ to 3′.**

Differences between replication and transcription

	replication	transcription
template	Both strands	single strand
substrate	dNTP	NTP
primer	yes	no
Enzyme	DNA polymerase	RNA polymerase
product	dsDNA	ssRNA
base pair	A-T, G-C	A-U, G-C

Template

- The whole genome of DNA needs to be replicated, but only small portion of genome is transcribed in response to the development requirement, physiological need and environmental changes.
- DNA regions that can be transcribed into RNA are called structural genes or Transcription unit.
- The template strand is the strand from which the RNA is actually transcribed. It is also termed as antisense strand.
- The coding strand is the strand whose base sequence specifies the amino acid sequence of the encoded protein. Therefore, it is also called as sense strand.
-

5'------ G C A G T A C A T G T C------ 3' coding strand

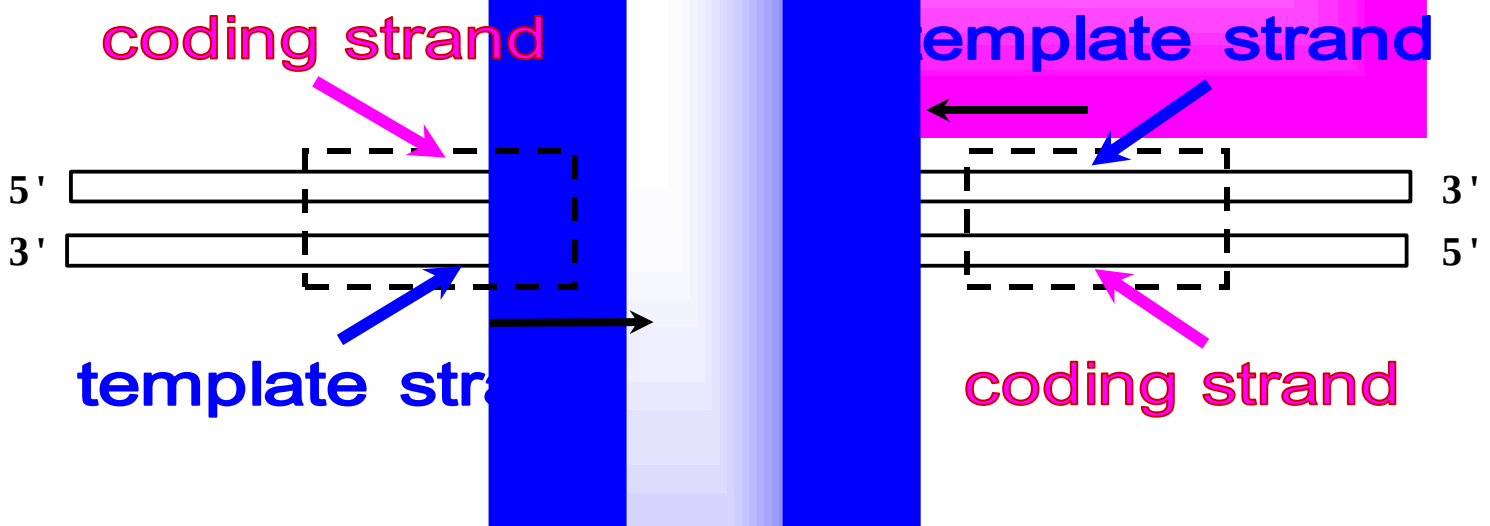
3'------ C G T C A T G T A C A G------ 5' template strand

transcriptio

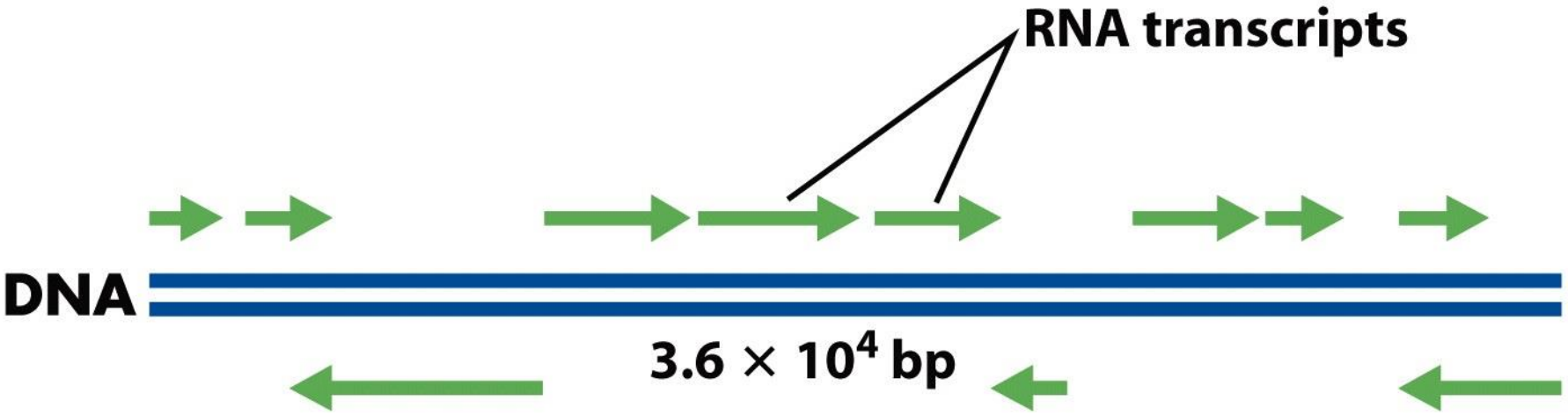
5'------ G C A G U A C A U G U C------ 3' NA

Asymmetric transcription

- Only the template strand is used for the transcription, but the coding strand is not.
- Both strands ca es.
- The transcript strands is opp
- This feature is etric transcription.



Organization of coding information in the adenovirus genome



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(5′) C G C T A T A G C G T T T (3′)	DNA nontemplate (coding) strand
(3′) G C G A T A T C G C A A A (5′)	DNA template strand
(5′) C G C U A U A G C G U U U (3′)	RNA transcript

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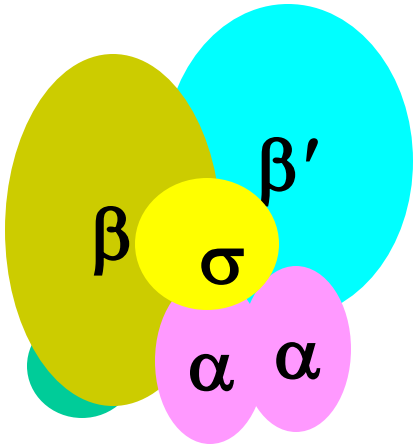
§ 1.2 RNA Polymerase

- **The enzyme responsible for the RNA synthesis is DNA-dependent RNA polymerase.**
  - **The prokaryotic RNA polymerase is a multiple-subunit protein of ~480kD.**
  - **Eukaryotic systems have three kinds of RNA polymerases, each of which is a multiple-subunit protein and responsible for transcription of different RNAs.**

Holoenzyme

The holoenzyme of RNA-pol in *E.coli* consists of 5 different subunits: $\alpha_2\ \beta\ \beta'\ \omega\sigma$.

holoenzyme



subunit	MW	function
α	36512	Determine the DNA to be transcribed
β	150618	Catalyze polymerization
β′	155613	Bind & open DNA template
σ	70263	Recognize the promoter for synthesis initiation

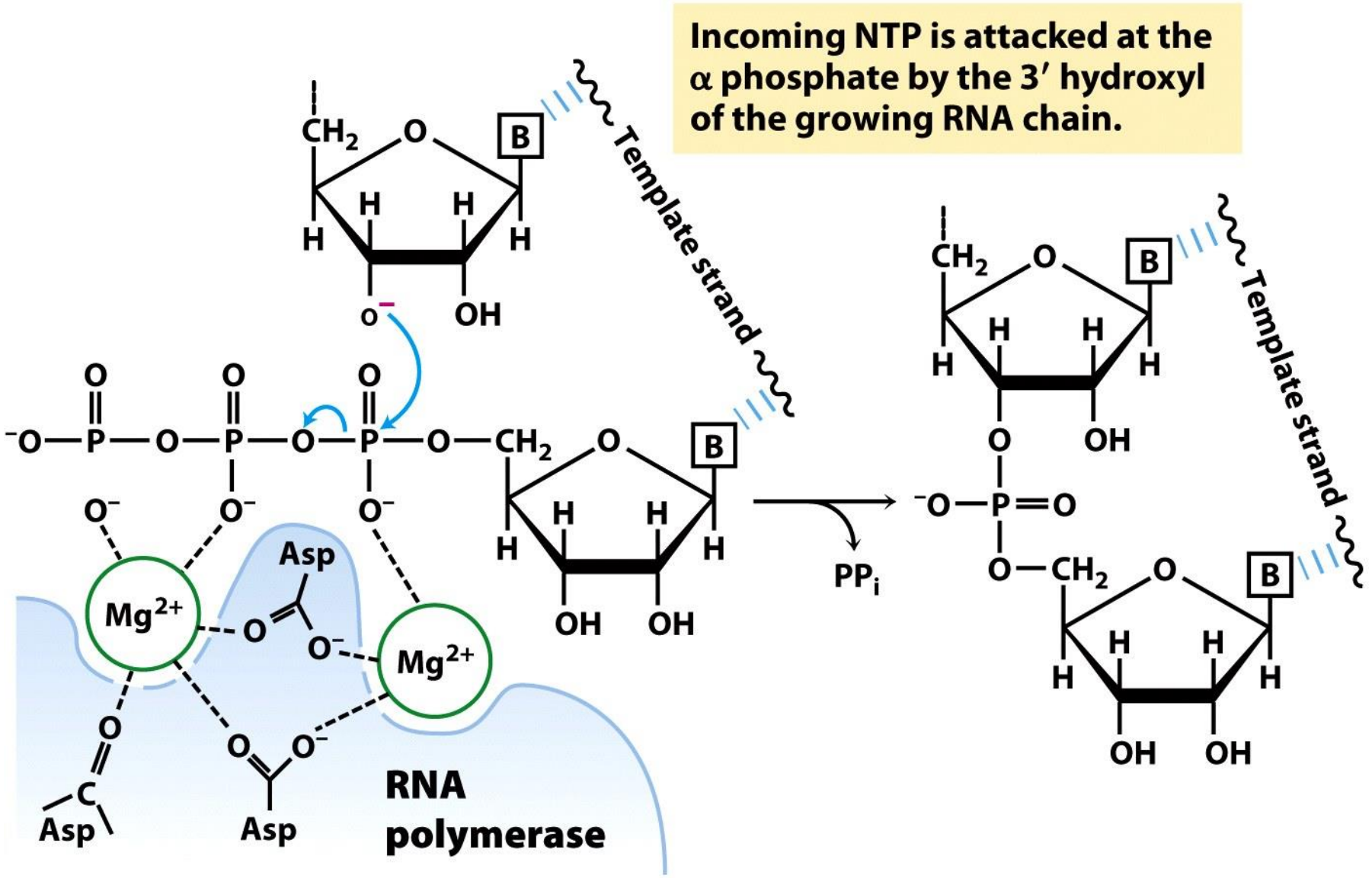


Figure 26-1b
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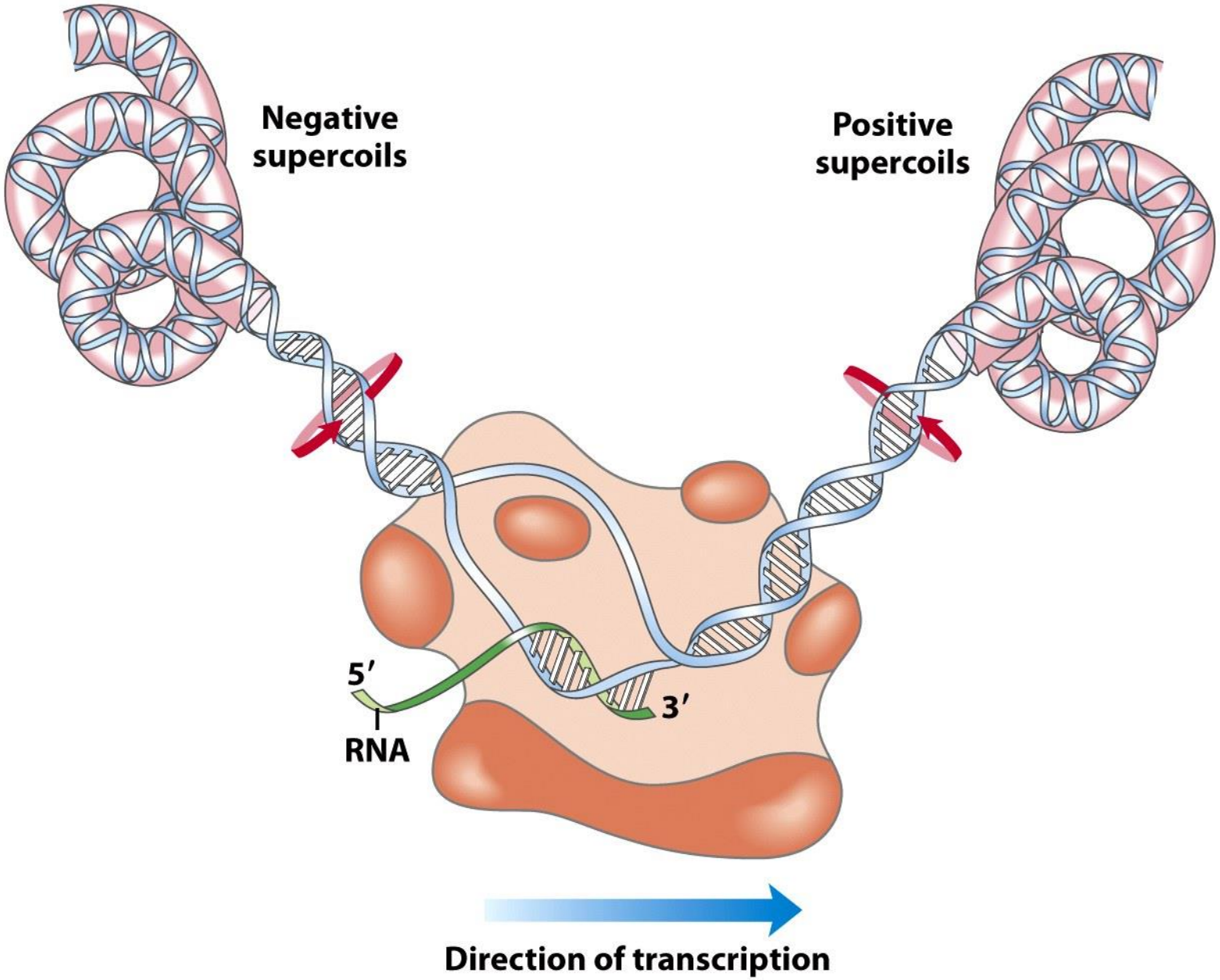

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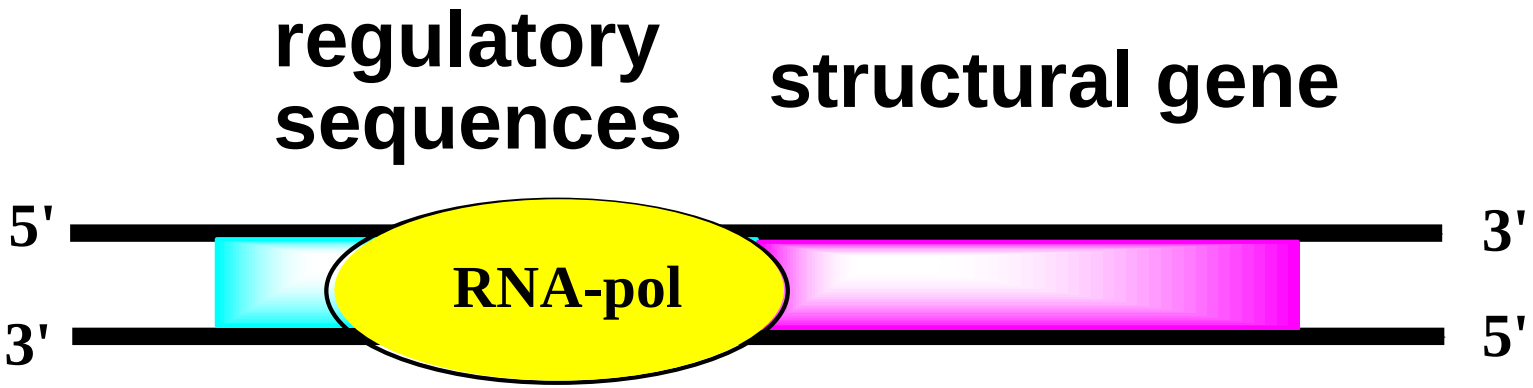
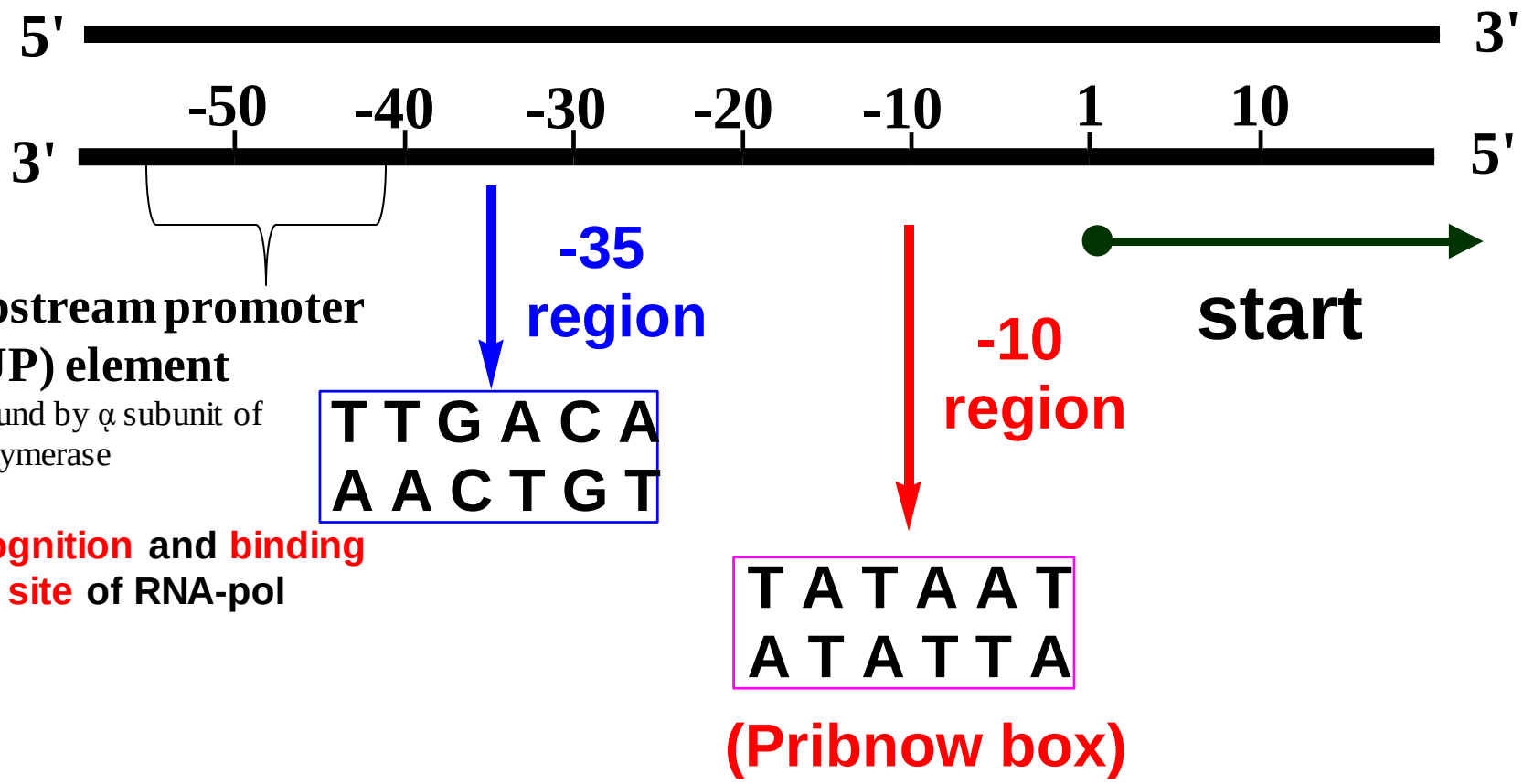
§ 1.3 Recognition of Origins

- **Each transcriptable region is called operon.**
- **One operon includes several structural genes and upstream regulatory sequences (or regulatory regions).**
- **The promoter is the DNA sequence that RNA-pol can bind. It is the key point for the transcription control.**
- **The -10 region of TATAAT is the region at which a stable complex of DNA and RNA-pol is formed.**

Promoter

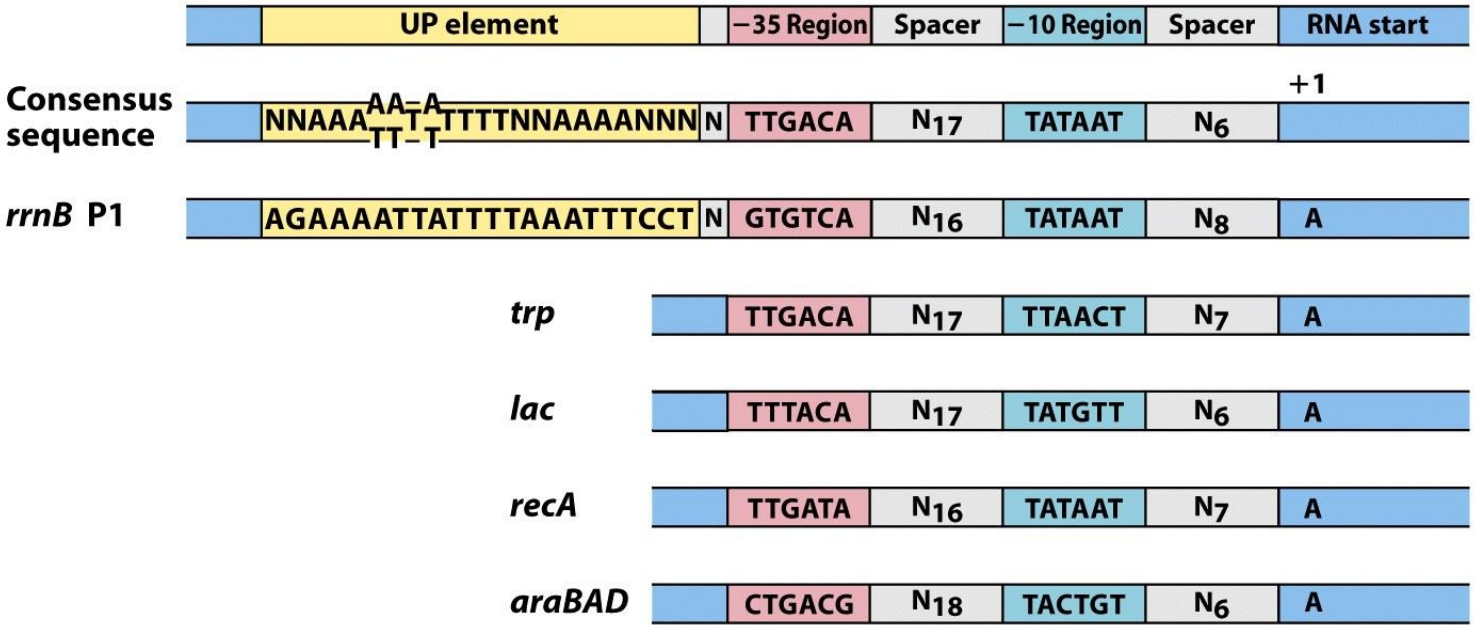


Prokaryotic promoter



Consensus sequence

- The sequences vary from one promoter to the next, but comparisons of many promoters reveal similarities, particularly in the –10 and –35 regions.
- The sequence element UP, not present in all E. coli promoters, generally occurring in the region between –40 and –60, strongly stimulate transcription at the promoters that contain them.
- The consensus sequence for E. coli promoters recognized by σ70 is shown second from the top. Spacer regions contain slightly variable numbers of nucleotides (N).



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Section 2

Transcription Process

General concepts

- **Three phases: initiation, elongation, and termination.**
- **The prokaryotic RNA-pol can bind to the DNA template directly in the transcription process.**
- **The eukaryotic RNA-pol requires co-factors to bind to the DNA template together in the transcription process.**

§ 2.1 Transcription of Prokaryotes

- **Initiation phase: RNA-pol recognizes the promoter and starts the transcription.**
- **Elongation phase: the RNA strand is continuously growing.**
- **Termination phase: the RNA-pol stops synthesis and the nascent RNA is separated from the DNA template.**

a. Initiation

- **No primer is needed for RNA synthesis.**
- **RNA-pol recognizes the TTGACA region, and slides to the TATAAT region, then opens the DNA duplex.**
- **The unwound region is about 17±1 bp.**

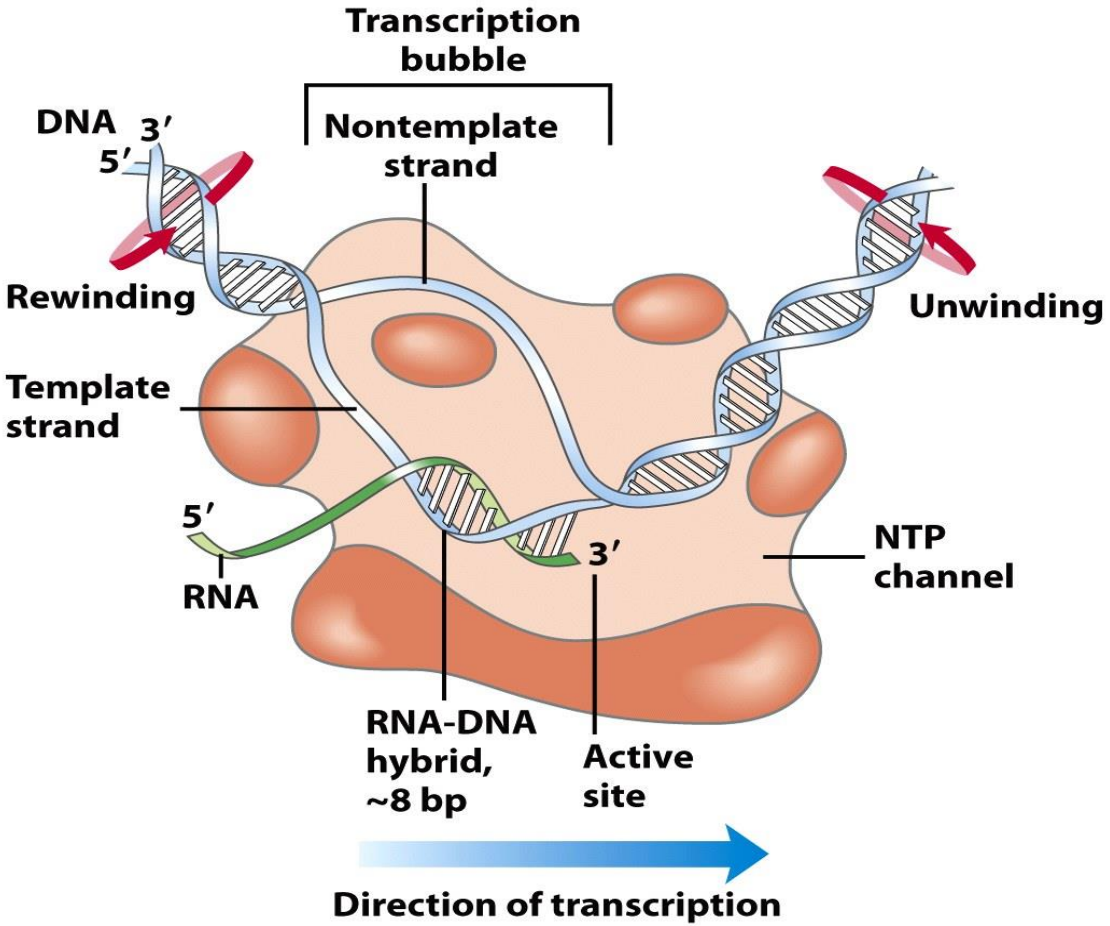

Figure 26-1a
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- **The first nucleotide on RNA transcript is always purine triphosphate. GTP is more often than ATP.**
- **The pppGpN-OH structure remains on the RNA transcript until the RNA synthesis is completed.**
- **The three molecules form a transcription initiation complex.**

RNA pol ($\alpha_2\beta\beta'\sigma$) - DNA - pppGpN- OH 3′

- Transcription is initiated within the complex
- The σ (sigma) subunit falls off once the first 3′,5′ phosphodiester bond is formed.
- Leading to a conformational change that converts the complex to the elongation form followed by movement of the transcription complex away from the promoter (promoter clearance)

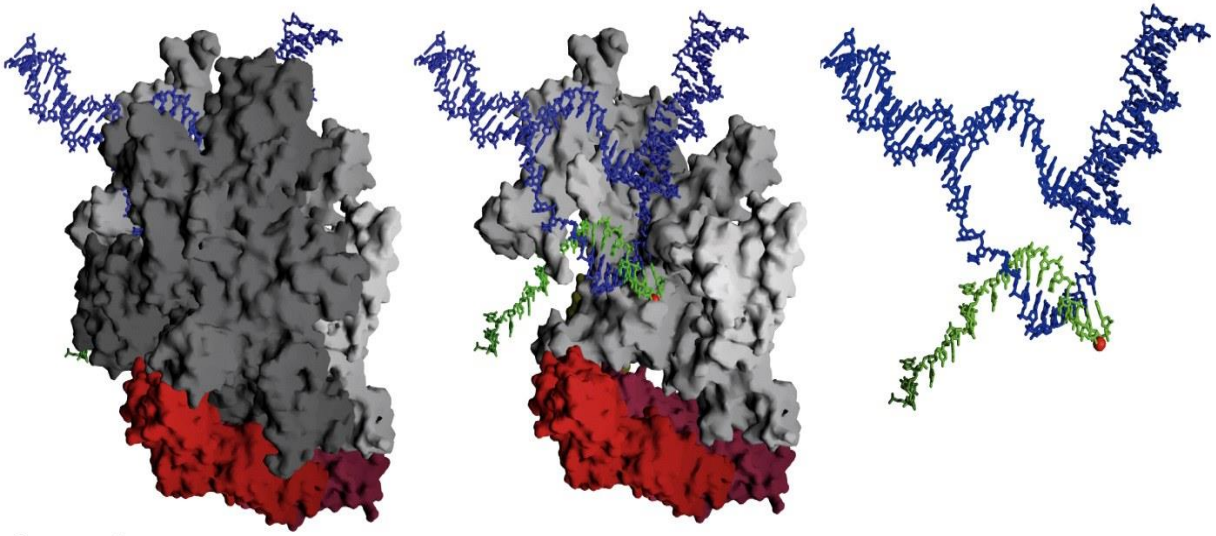

Figure 26-6b
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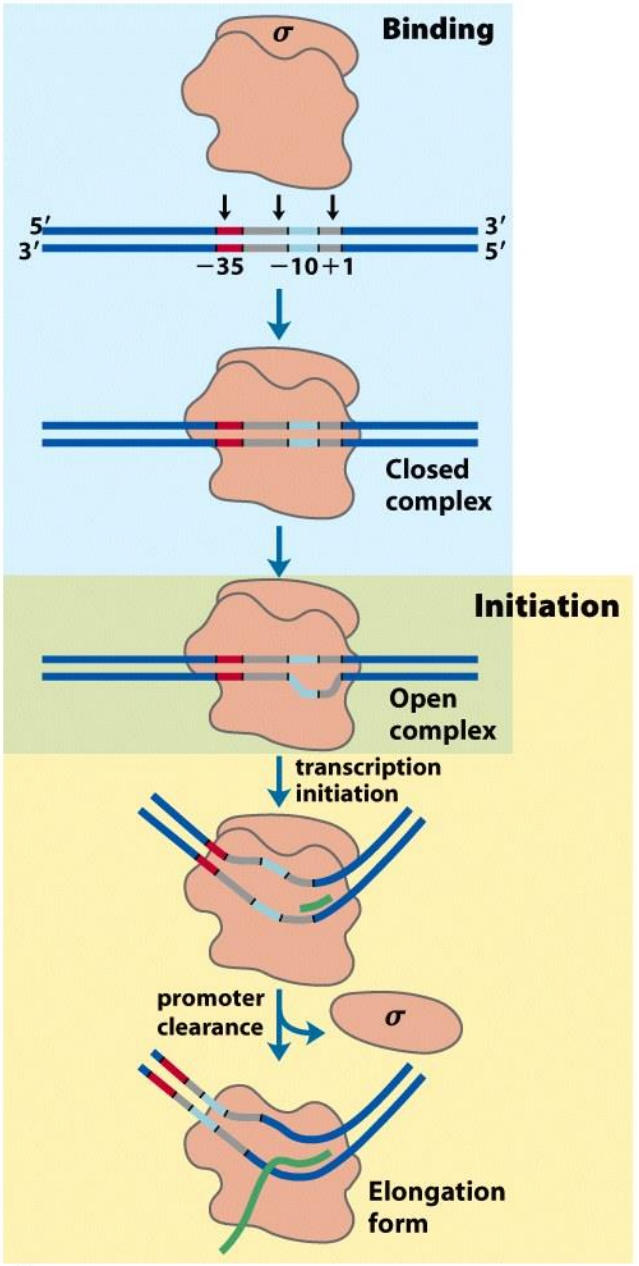

Figure 26-6a
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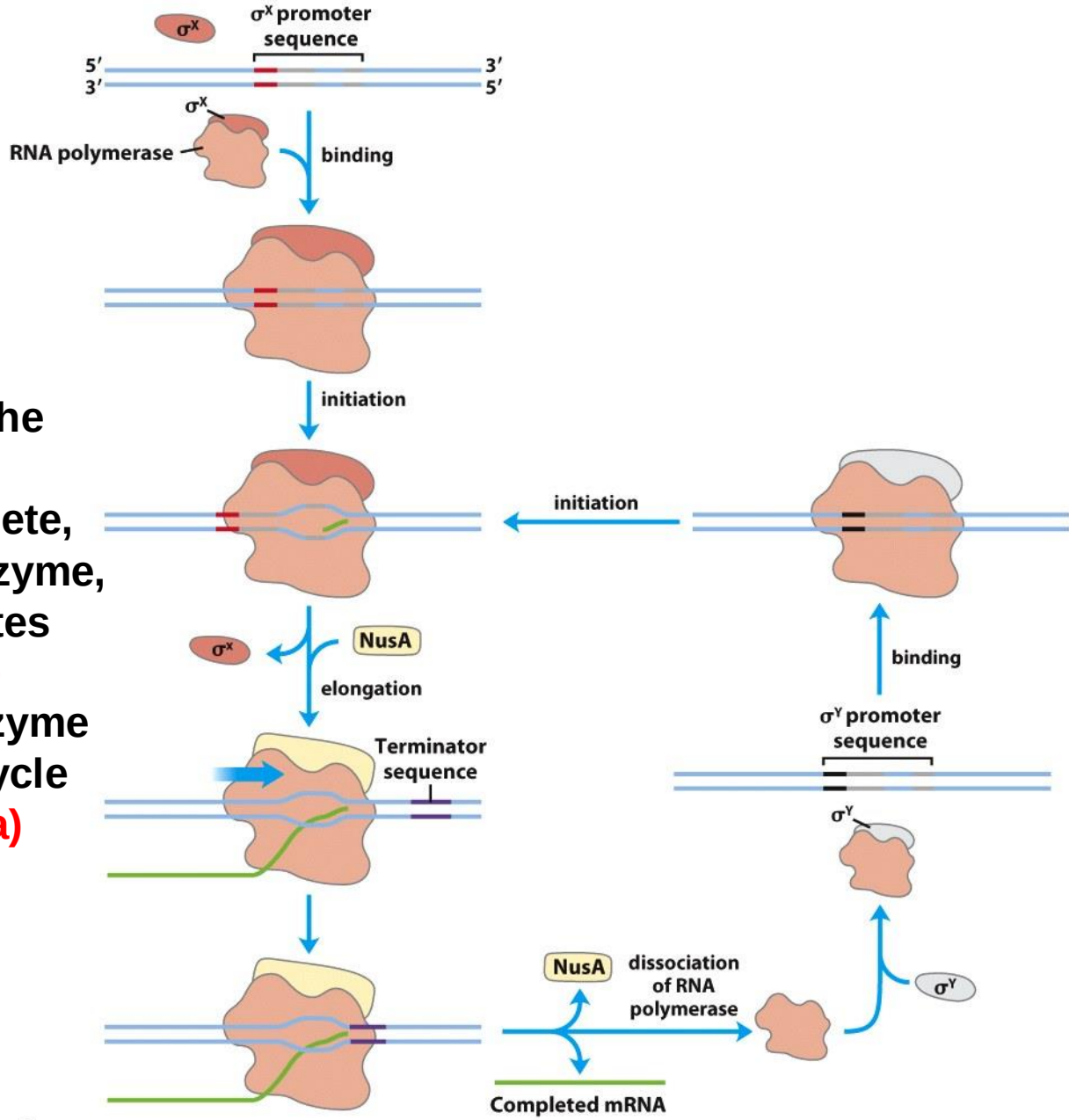
- The **core enzyme** moves along the DNA template to enter the elongation phase.

- The protein NusA binds to the elongating RNA polymerase.
- Once transcription is complete, NusA dissociates from the enzyme, the RNA polymerase dissociates from the DNA, and a σ (sigma) factor can now bind to the enzyme to initiate transcription, in a cycle sometimes called the σ (sigma) cycle.



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Promoter Clearance and Elongation

- Occurs after 4- 10 nt are added
- First rnt becomes unpaired from antisense (template) strand. ∴ DNA strands re-anneal
- Polymerase loses sigma, sigma recycled
  - Result "Closed hand" surrounds DNA
- NusA binds to core polymerase
- As each nt added to 3', another is melted from 5', allowing DNA to re-anneal.
- RNA pol/NusA complex stays on until termination. Rate=20-50nt/second.

b. Elongation

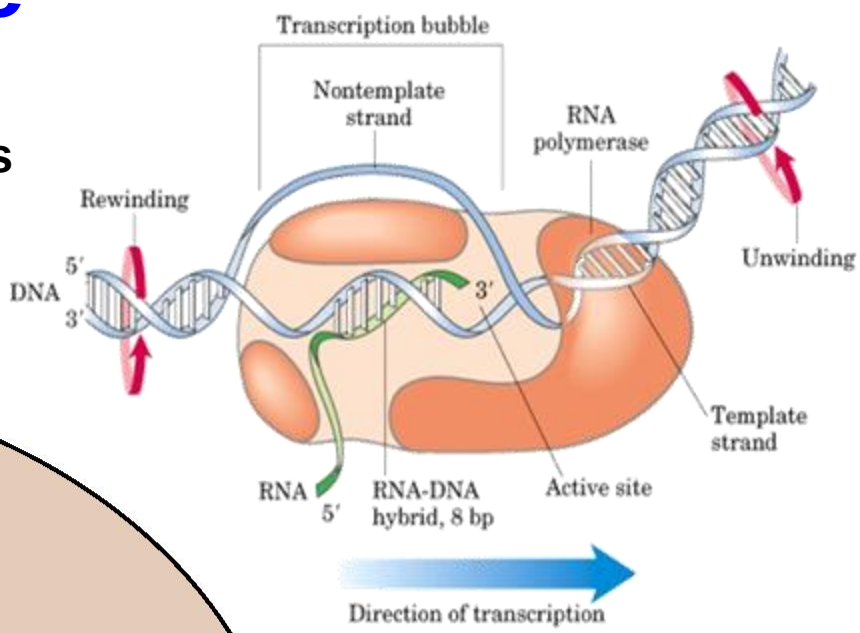
$(NMP)_n + NTP \rightarrow (NMP)_{n+1} + PPi$

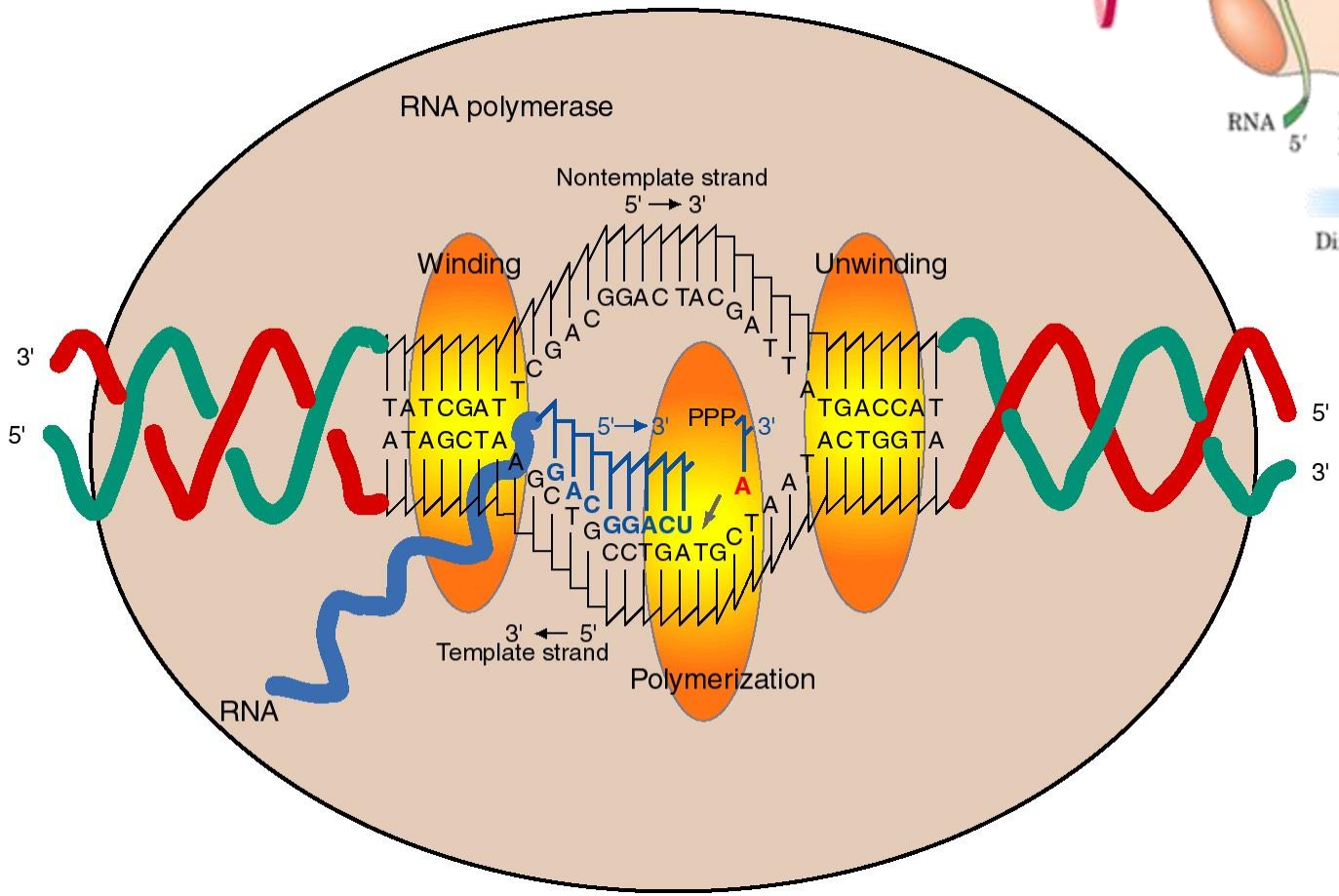
RNA strand — substrate — elongated RNA strand

- The release of the σ subunit causes the conformational change of the core enzyme. The core enzyme slides on the DNA template toward the 3′ end.
- Free NTPs are added sequentially to the 3′ -OH of the nascent RNA strand.

Transcription bubble

The 3′ segment of the nascent RNA hybridizes with the DNA template, and its 5′ end extends out the transcription bubble as the synthesis is processing.



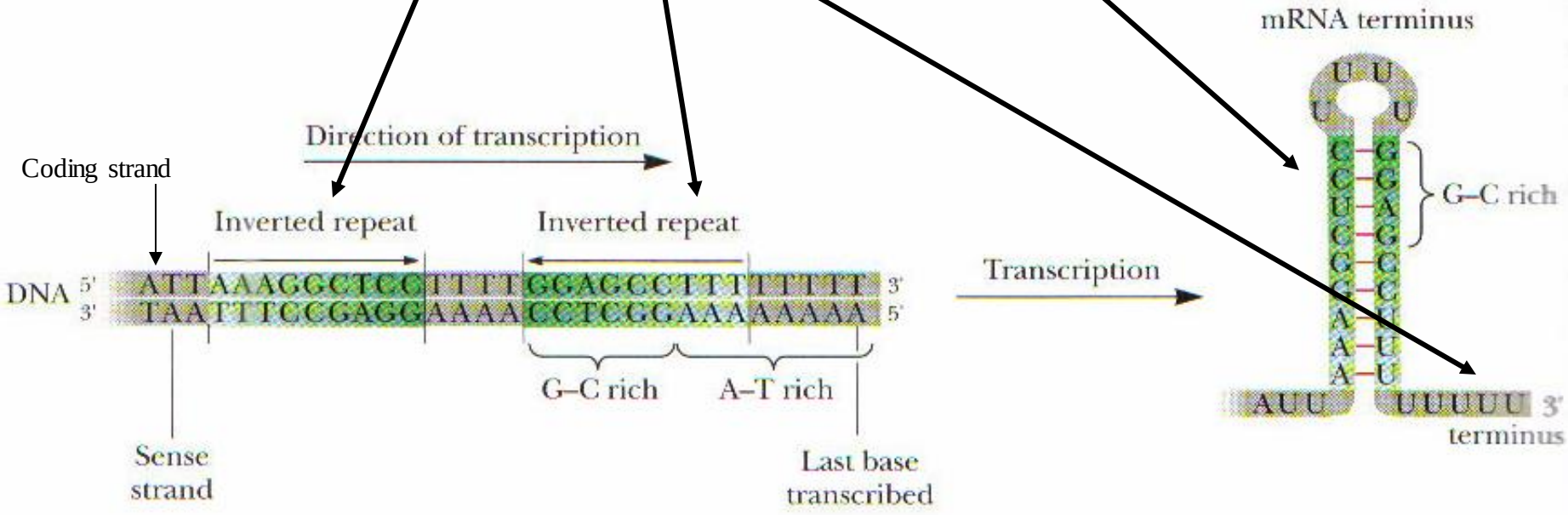


Termination

- Occurs at specific sites on template strand, called Terminators
- Two types of termination
  - Intrinsic terminators
  - Rho (ρ) dependent treminators
- Sequences required for termination are in transcript
- Variation in efficiencies.

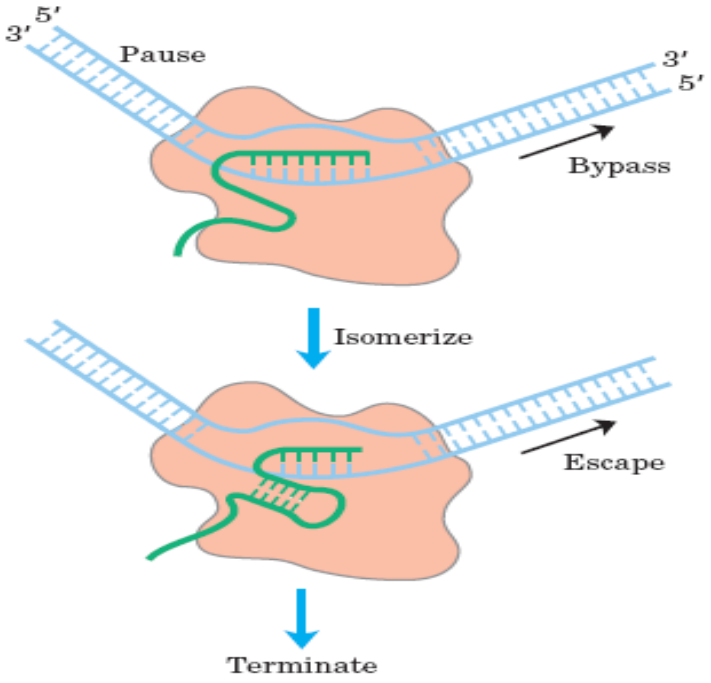
Intrinsic Terminators

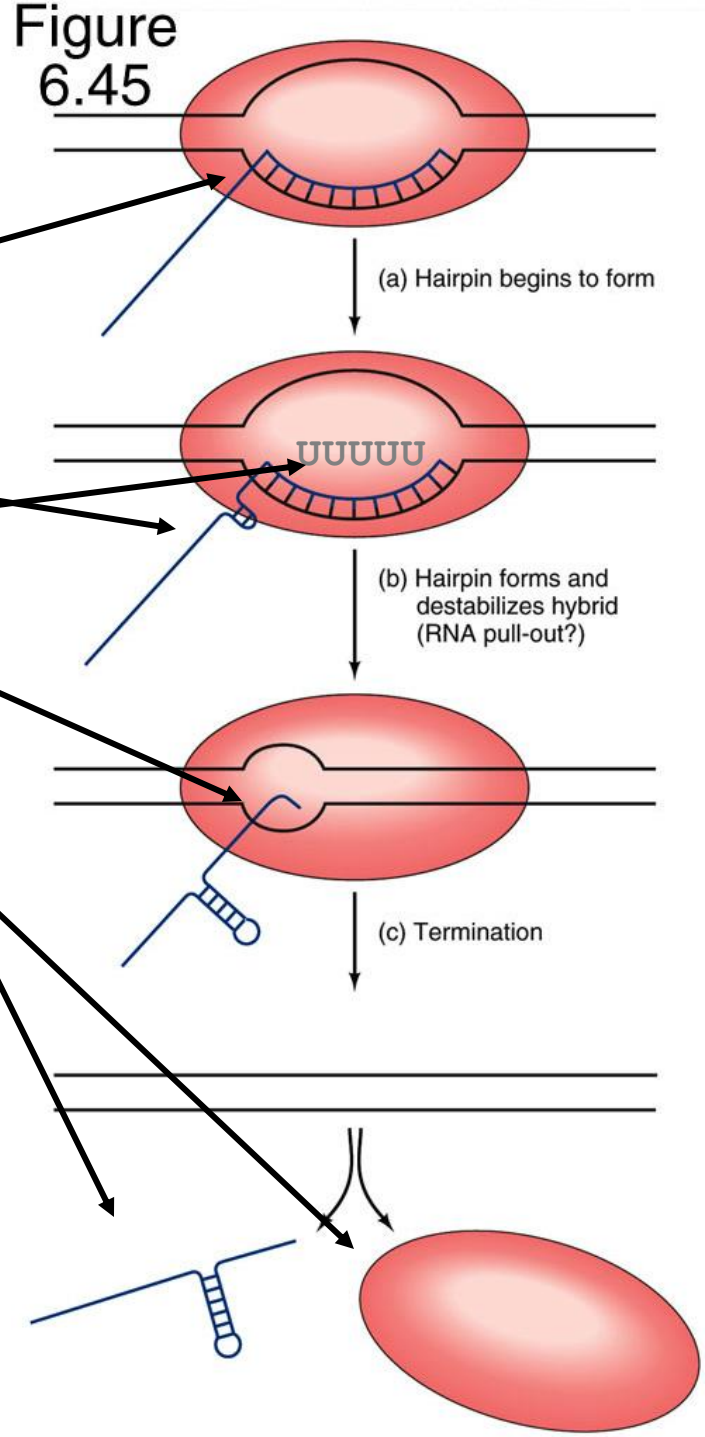
- DNA template contains inverted repeats (G-C rich)
  - ∴ Can form hairpins
- 6 to 8 A sequence on the DNA template that codes for U
- Consequences of poly-U:poly-A stretch?



Intrinsic Termination

- RNA pol passes over inverted repeats
- Hairpins begin to form in the transcript
- Poly-U:poly-A stretch melts
- RNA pol and transcript fall off





ρ-independent termination

Have two distinguishing features.

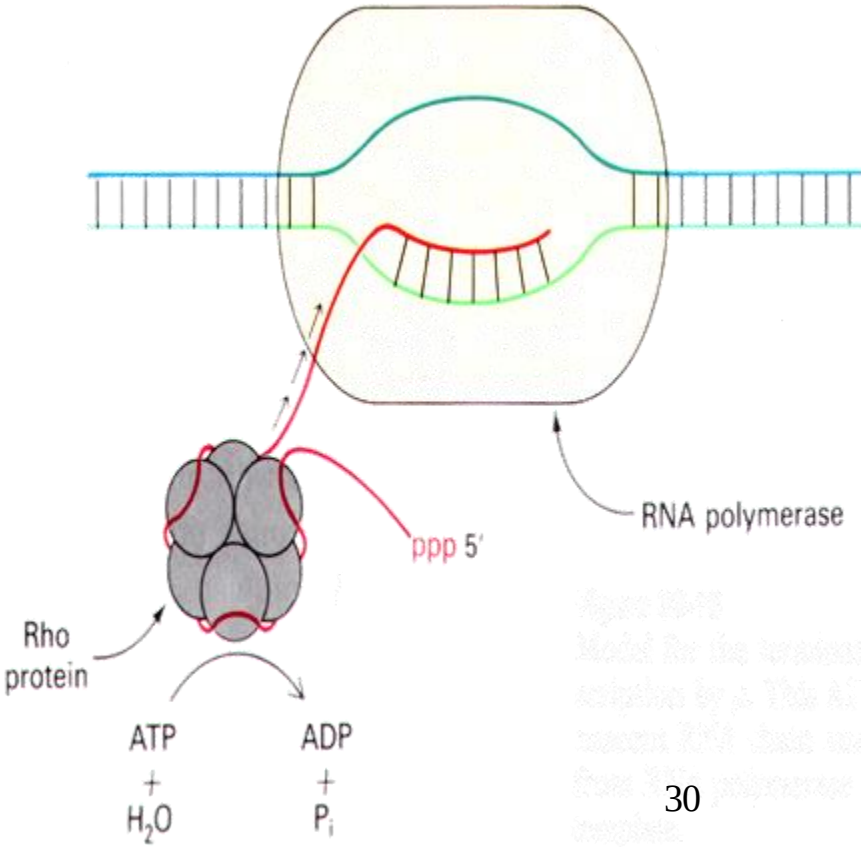
- **The first is a region that produces an RNA transcript with *self complementary sequences*, permitting the formation of a hairpin structure**
- **The second feature is a highly conserved string of three A residues in the template strand that are transcribed into U residues near the 3 end of the hairpin.**

This structural transformation disrupts several A=U base pairs in the RNA-DNA hybrid segment and may disrupt important interactions between RNA and the RNA polymerase, facilitating dissociation of the transcript.

The termination function of ρ factor

The ρ factor, is a hexameric protein, having ATP-dependent helicase activity.

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.
Here it contributes to release of the RNA transcript. The protein has an ATP-dependent RNA-DNA helicase activity that promotes translocation of the protein along the RNA, and ATP is hydrolyzed by *protein during the termination process.*

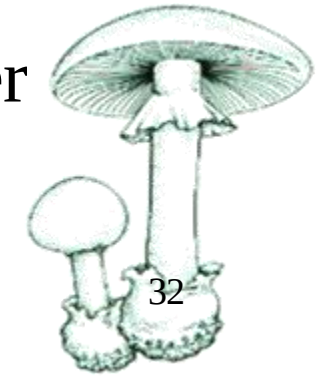


Transcription of Eukaryotes

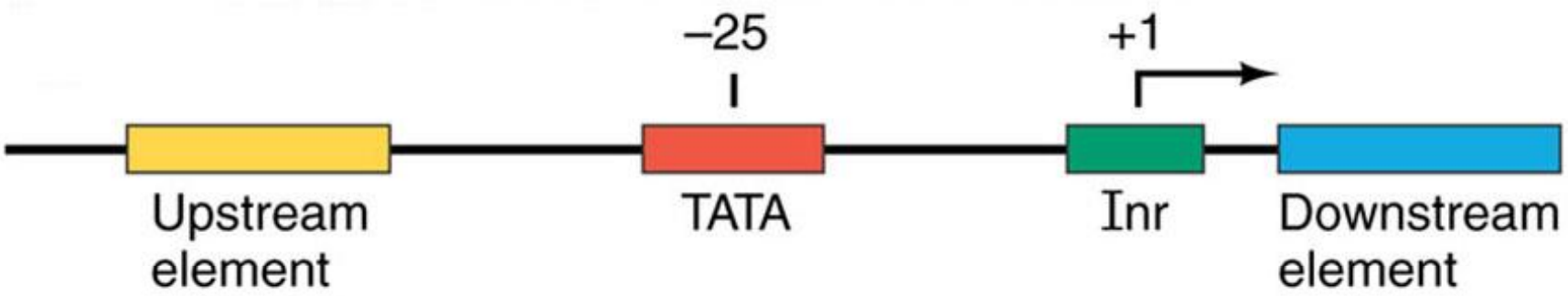
RNA-polymerase of eukaryotes

RNA-pol	I	II	III
products	rRNA	mRNA	tRNA
promoters	Vary greatly specie to specie	Recognize thousands of promoter	Well defined
Sensitivity to Amanitin	No	high	moderate

- Each polymerase recognizes a distinct promoter



Amanitin Toxin is a specific inhibitor of RNA-pol II and III.



- Two parts
  - Core promoter
  - Upstream element
- Core promoter
  - TATA box at ~-30 bases
  - Initiator—on the transcription start site
  - Downstream element-further downstream
- Many natural promoters lack recognizable versions of one or more of these sequences

Some genes transcribed by RNA pol II lack the TATA box. Ex: Housekeeping genes (expressed constitutively).

Transcription Factors for eukaryotic transcription

RNA-pol does not bind the promoter directly.

RNA-pol II associates with six transcription factors, TFII A - TFII H.

TABLE 26–2 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	
pTEFb	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	

*The function of all elongation factors is to suppress the pausing or arrest of transcription by the Pol II—TFIIF complex.

†Name derived from eleven-nineteen *l*ysine-rich *l*eukemia. The gene for ELL is the site of chromosomal recombination events frequently associated with acute myeloid leukemia.

Table 26-2

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Transcription at RNA polymerase II promoters.

- The sequential assembly of TBP (often with TFIIA), TFIIB, TFIIF plus Pol II, TFIIE, and TFIIH results in a closed complex.
- Within the complex, the DNA is unwound at the Inr region by the helicase activity of TFIIH and perhaps of TFIIE, creating an open complex.
- The carboxyl-terminal domain of the largest Pol II subunit is phosphorylated by TFIIH, and the polymerase then escapes the promoter and begins transcription.
- Elongation is accompanied by the release of many transcription factors and is also enhanced by elongation factors.
- After termination, Pol II is released, dephosphorylated, and recycled.

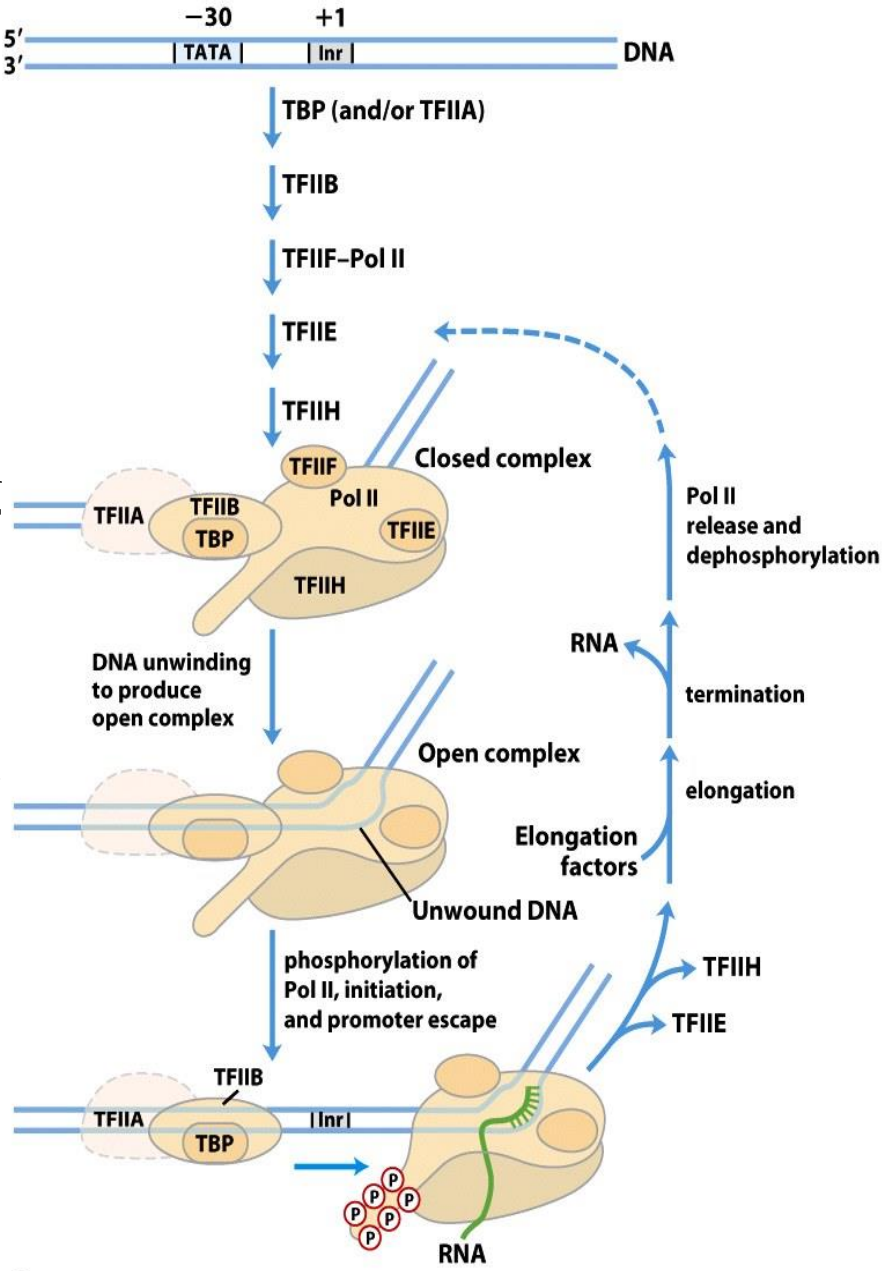

Figure 26-10a
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b. Elongation

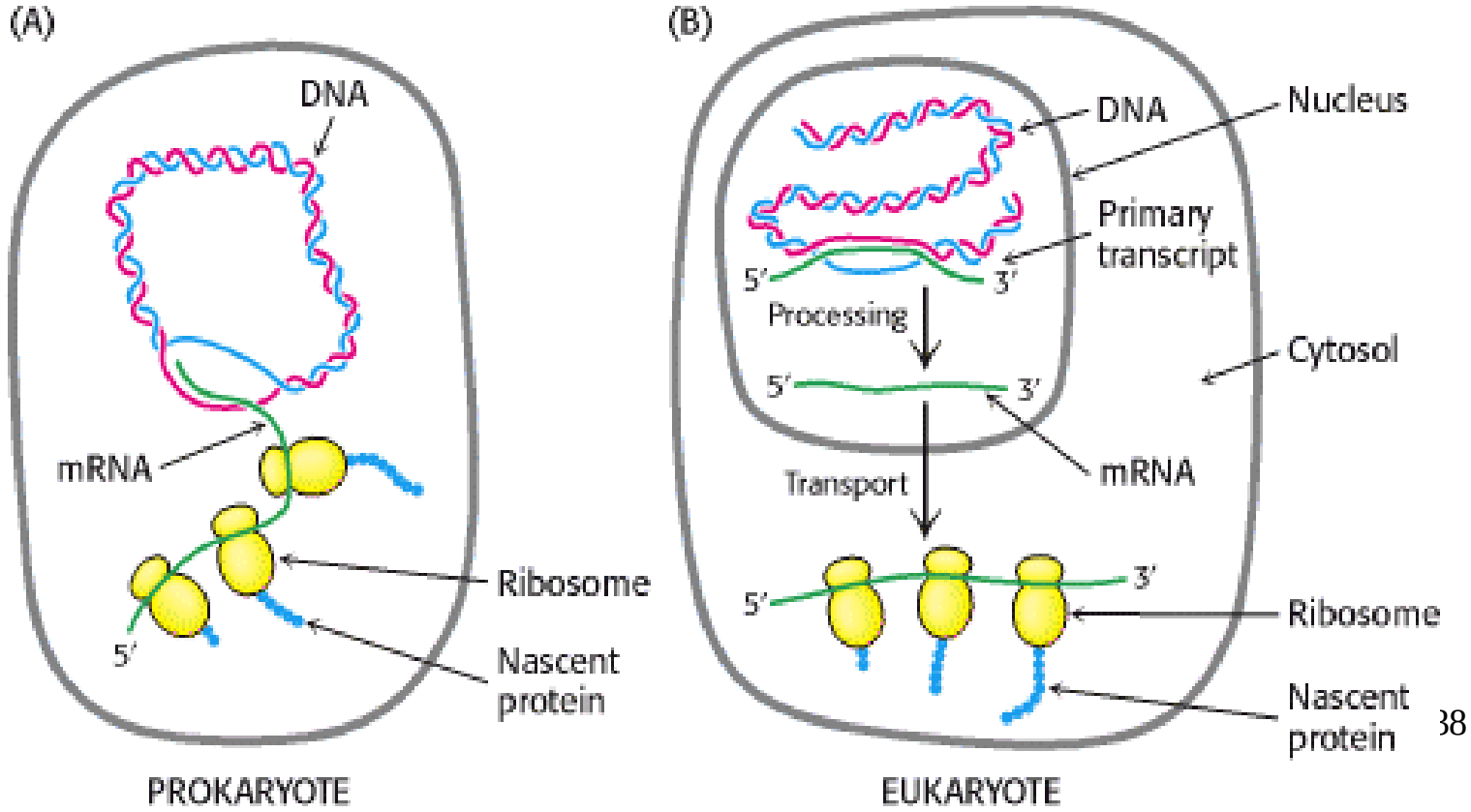
- **The elongation is similar to that of prokaryotes.**
- **The transcription and translation do not take place simultaneously since they are separated by nuclear membrane.**

c. Termination

- **The termination sequence is AATAAA followed by GT repeats.**
- **The termination is closely related to the post-transcriptional modification.**

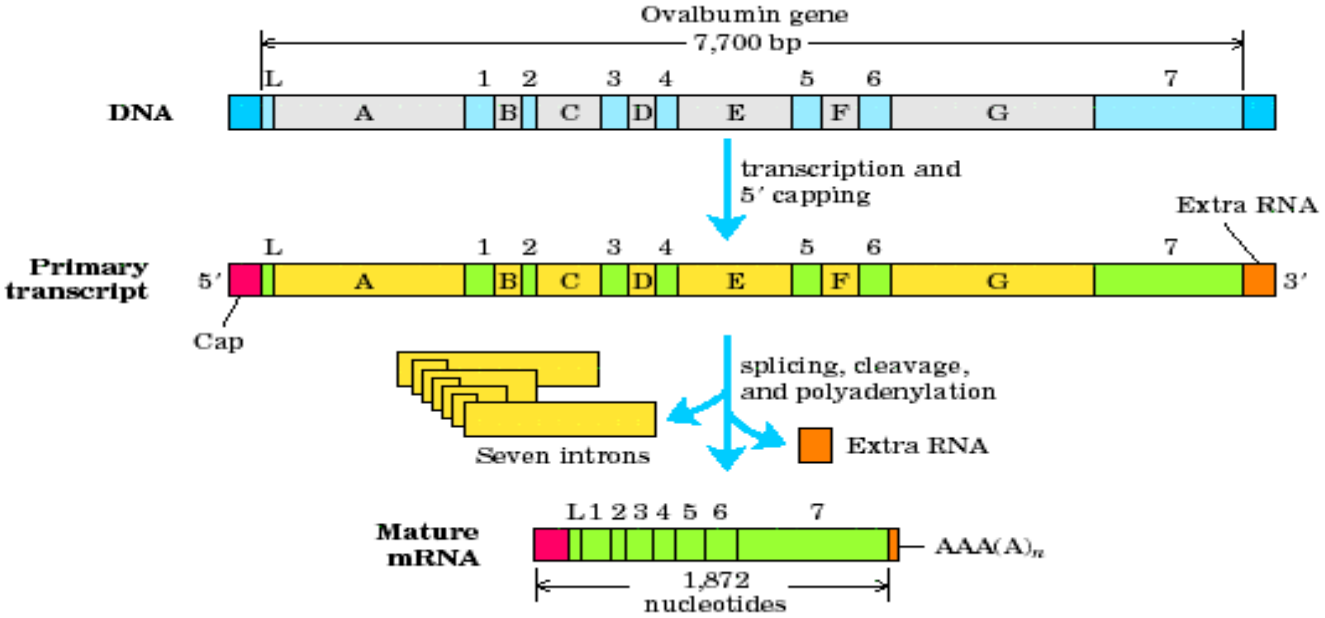
Post-Transcriptional Modification

- **The nascent RNA, also known as primary transcript, needs to be modified to become functional tRNAs, rRNAs, and mRNAs.**
- **The modification is critical to eukaryotic systems.**



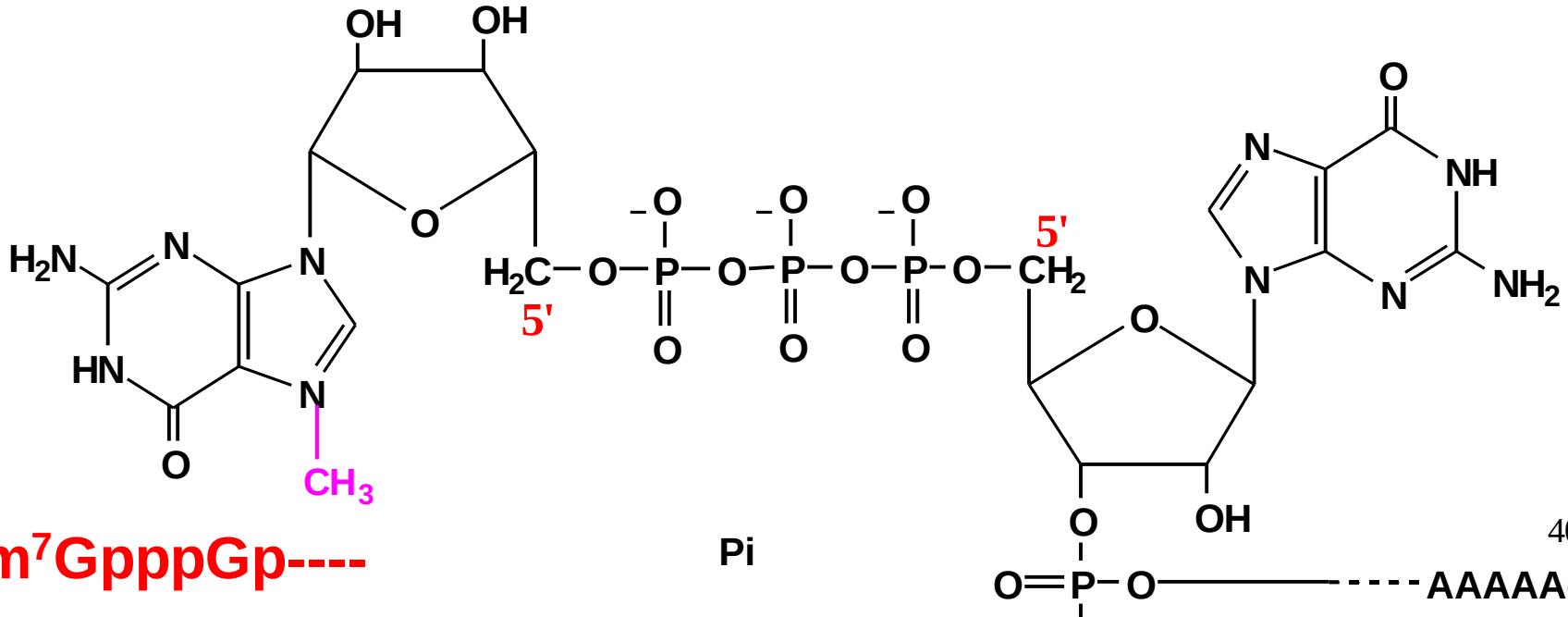
§ 3.1 Modification of Nascent RNA

- **Primary transcripts of mRNA are larger than matured mRNA by many folds.**
- **Modification includes**
  - **Capping at the 5′- end**
  - **Tailing at the 3′- end**
  - **mRNA splicing**



Capping

- Addition of 7-methylguanosine linked to the 5 terminal residue of the mRNA through an unusual ***5,5-triphosphate linkage***
- The 5′- cap structure is found on primary RNA too. ⇒ The capping process occurs in nuclei.
- The cap structure of mRNA will be recognized by the cap-binding protein required for translation.
- The capping occurs prior to the splicing.



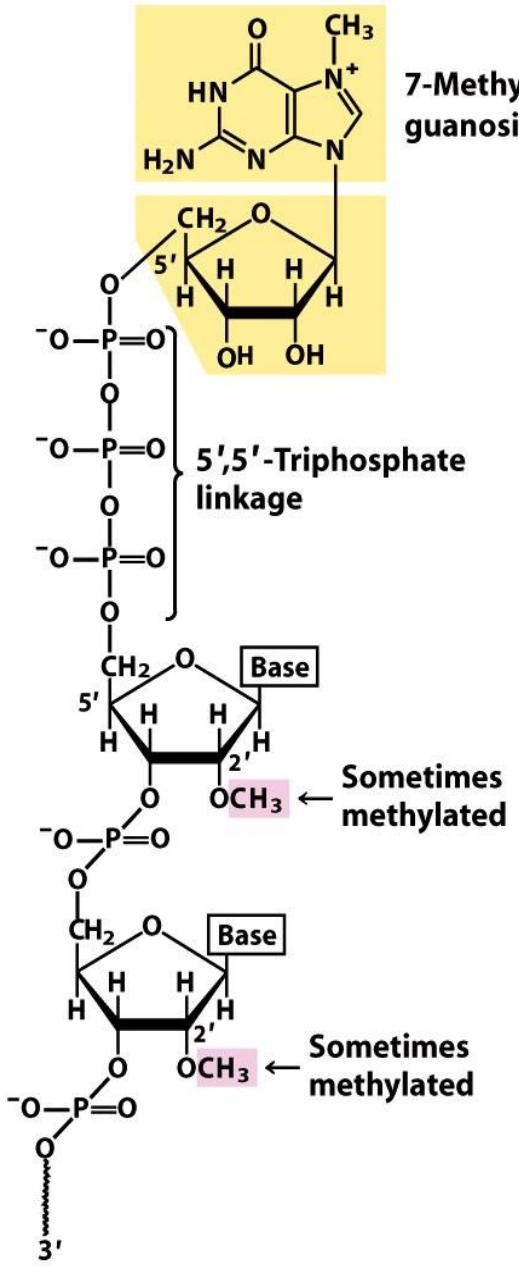


Figure 26-13a
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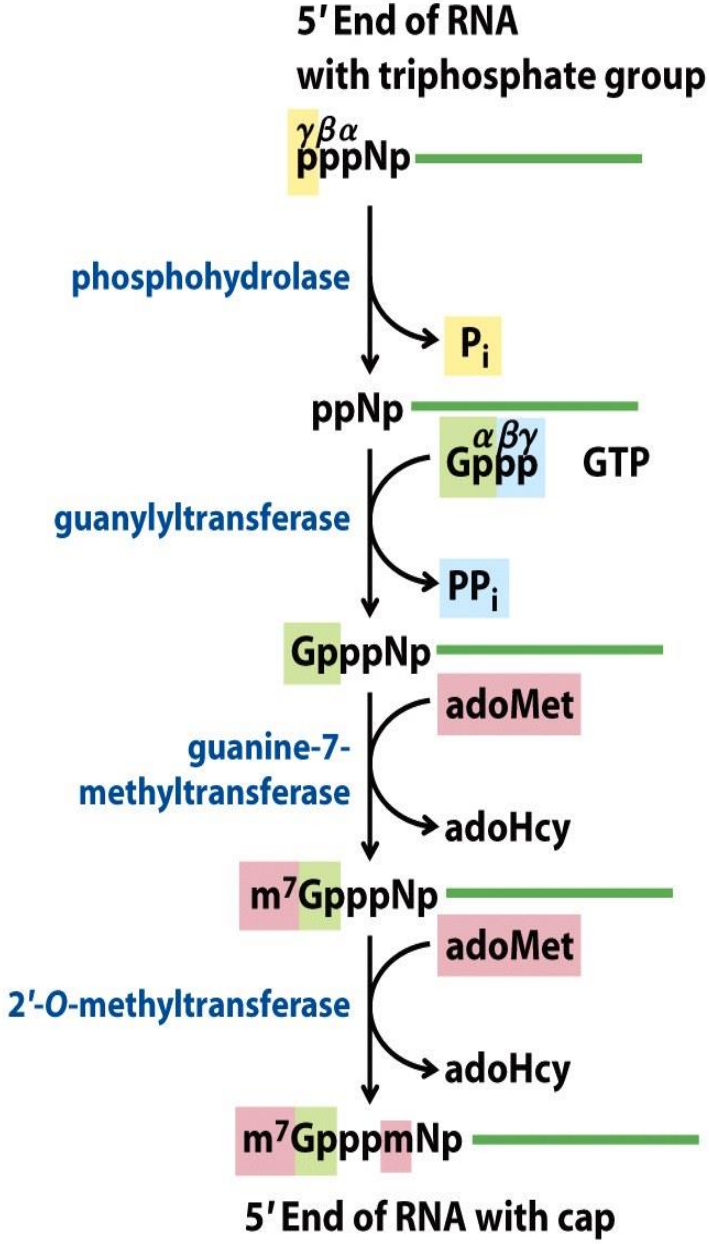

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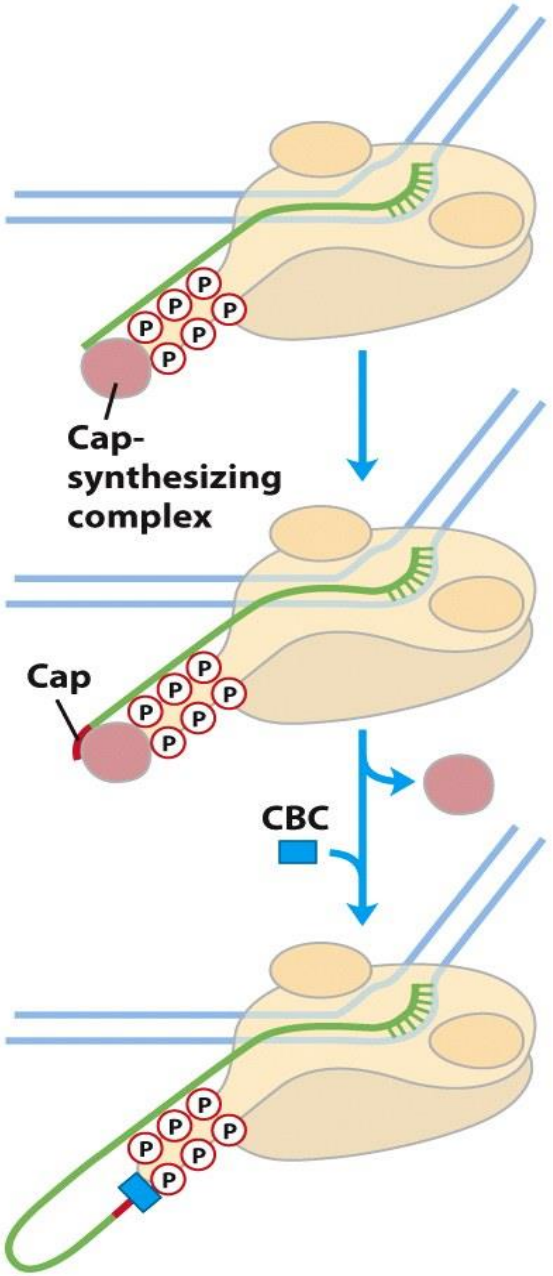

Figure 26-13c
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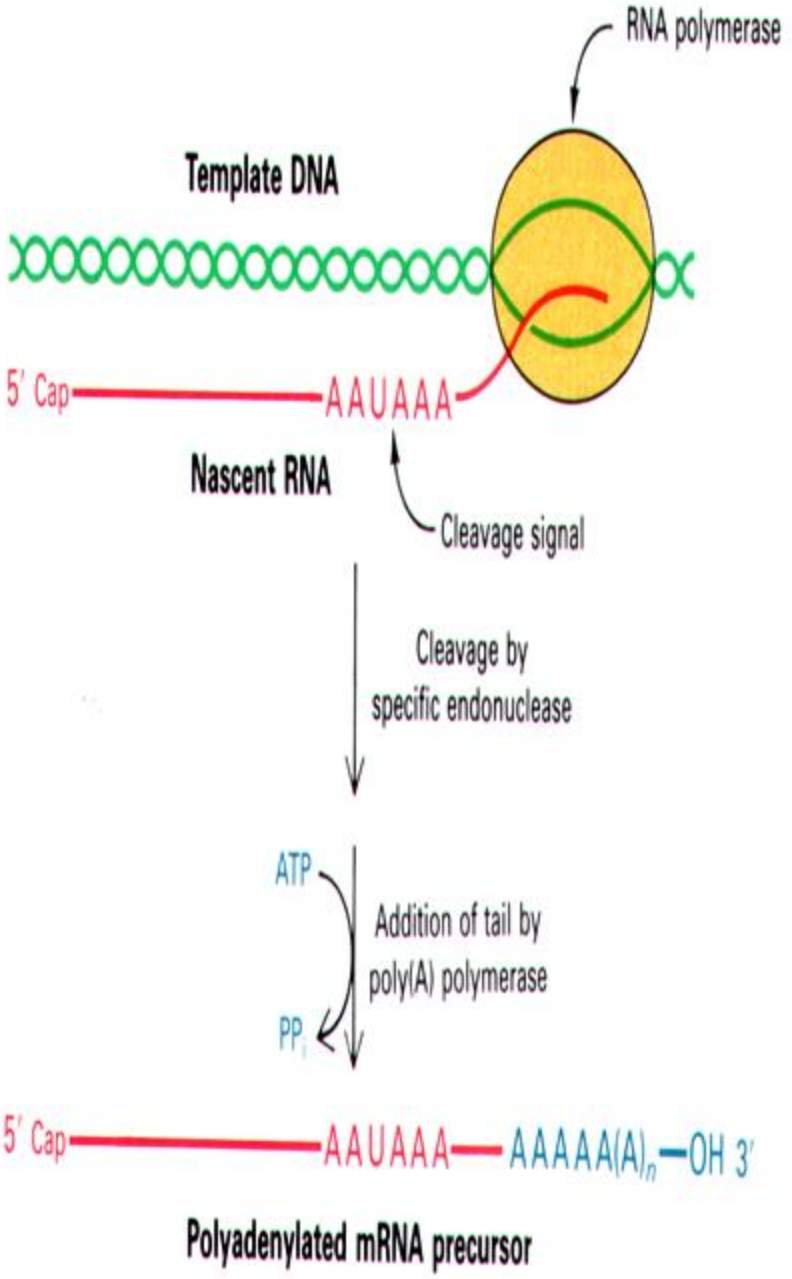
b. Poly-A tailing at 3′

- There is no poly(dT) sequence on the DNA template. ⇒ The tailing process dose not depend on the template.
- The tailing process occurs prior to the splicing.
- The tailing process takes place in the nuclei.



Addition of the poly(A) tail to the primary RNA transcript

- Synthesize **poly(A) tail** beyond the segment of the transcript containing the cleavage signal sequences including (5'AAUAAA).
- The cleavage signal sequence is bound by an enzyme complex that includes an endonuclease, polyadenylate polymerase and several other multi-subunit proteins involved in sequence recognition and stimulation of cleavage and regulation of the length of the poly(A)tail.
- The RNA is cleaved by the endonuclease at a point 10 to 30 nucleotides downstream to the sequence A AUAAA.
- The polyadenylate polymerase synthesize poly(A) tail 80 to 250 nucleotide long beginning.

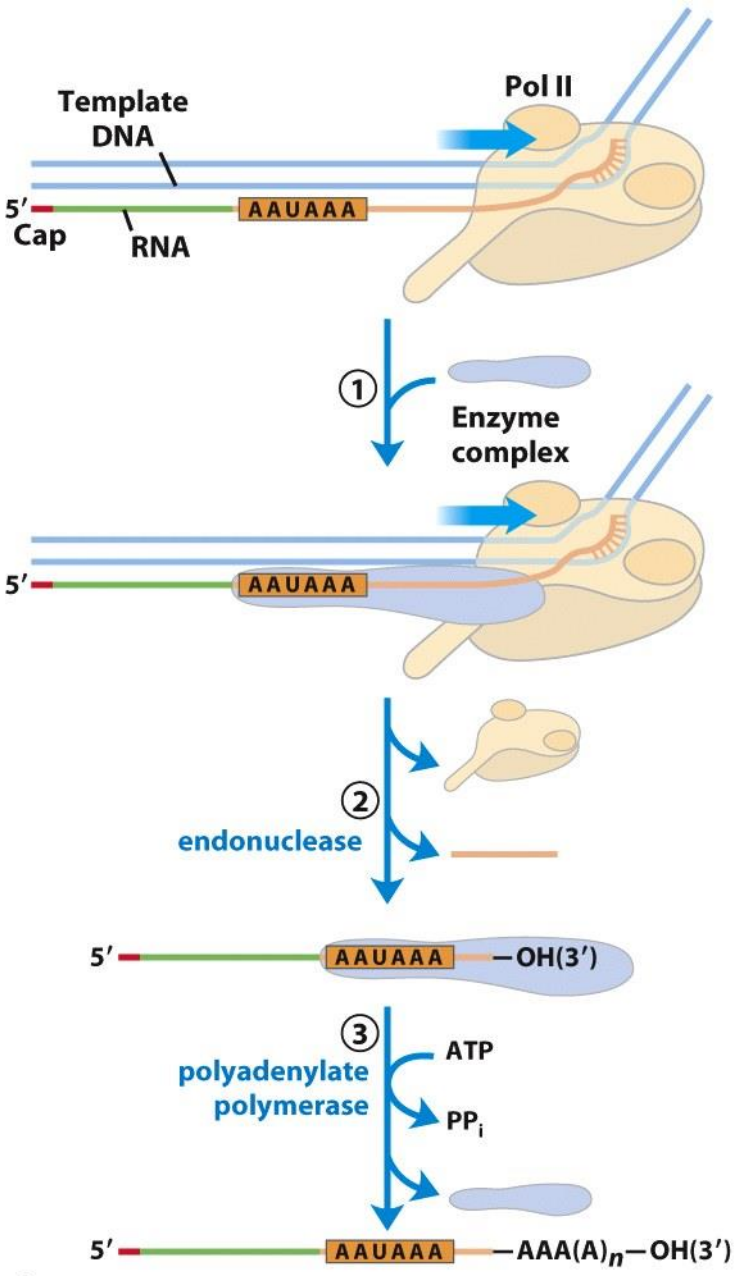


Figure 26-18
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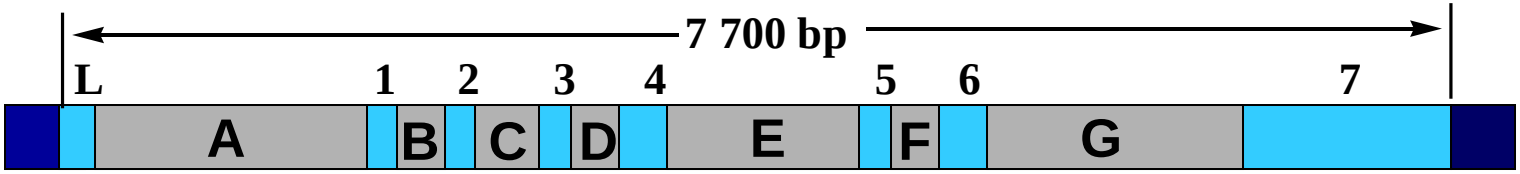
The structural genes are composed of EXONS (coding) and INTRONS (non-coding) regions that are alternatively separated.



A~G no-coding region 1~7 coding region

44

Exon and intron

Exons are the coding sequences that appear on split genes and primary transcripts, and will be expressed to matured mRNA.

Introns are the non-coding sequences that are transcripted into primary mRNAs, and will be cleaved out in the later splicing process.

- **The matured mRNAs are much shorter than the DNA templates.**

RNA splicing is the process by which introns, regions of RNA that do not code for protein, are removed from the pre-mRNA and the remaining exons conneted to re-form a single continuous molecule.

Types:

- ***Spliceosomal Splicing*****:** Their removal occurs within and is catalyzed by a large protein complex called a **spliceosome.**

- ***Self-splicing:*** *no protein enzymes are involved.*
  - *Group I*
  - *Group II*
- **tRNA splicing:**

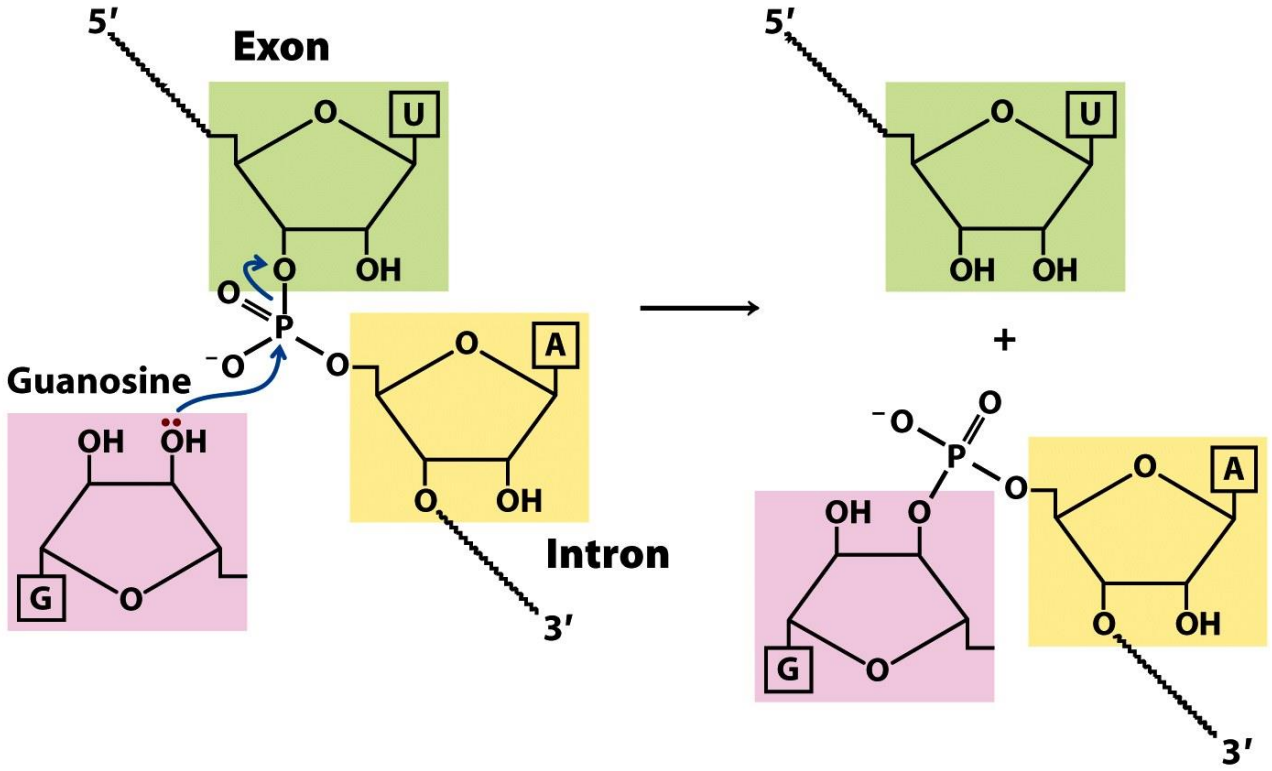


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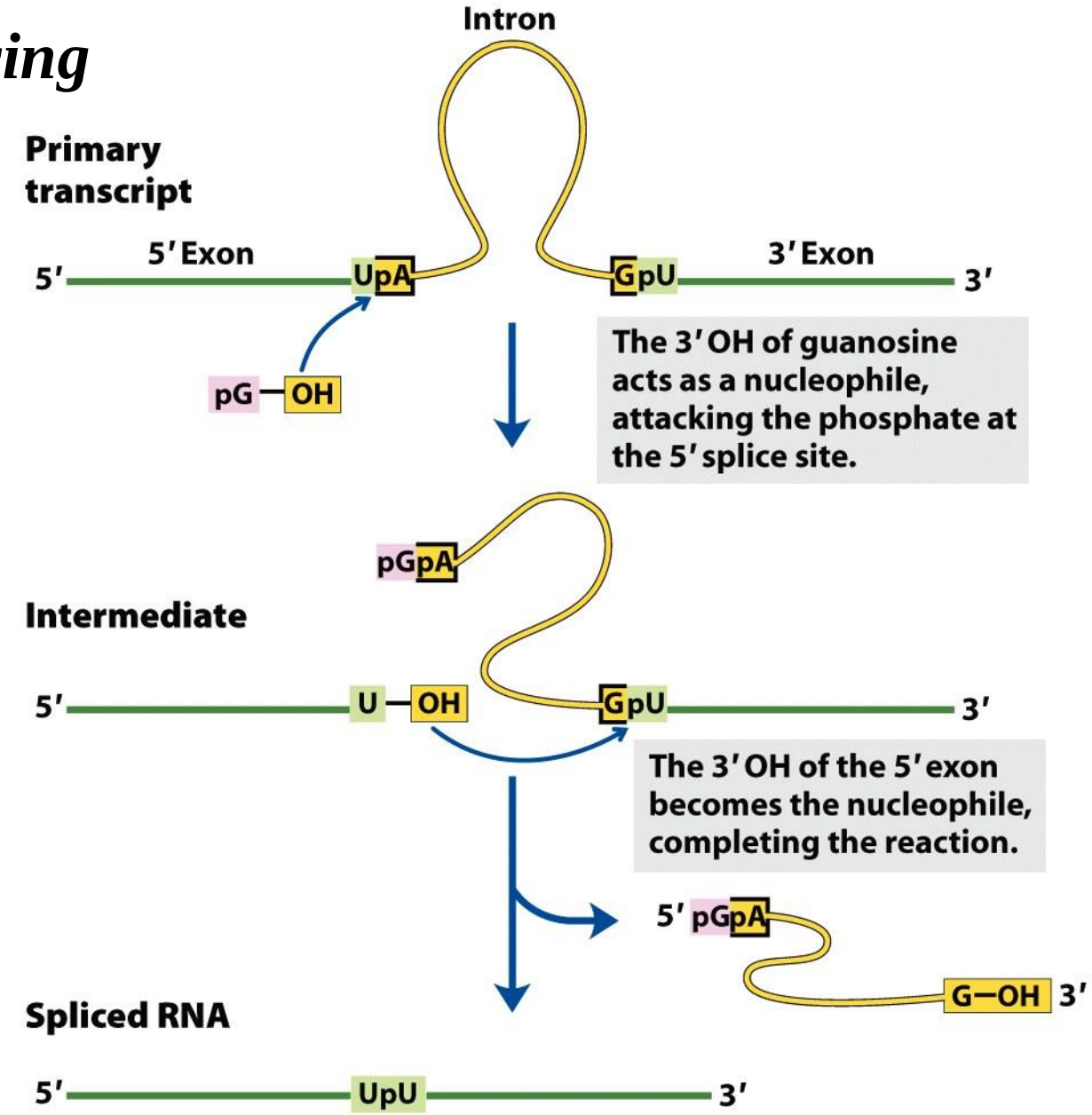
FIGURE 26–14 Transesterification reaction. Shown here is the first step in the two-step splicing of group I introns. In this example, the 3′ OH of a guanosine molecule acts as nucleophile, attacking the phosphodiester linkage between U and A residues at an exon-intron junction of an mRNA molecule (see Fig. 26–15).

Group I Self-splicing

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.



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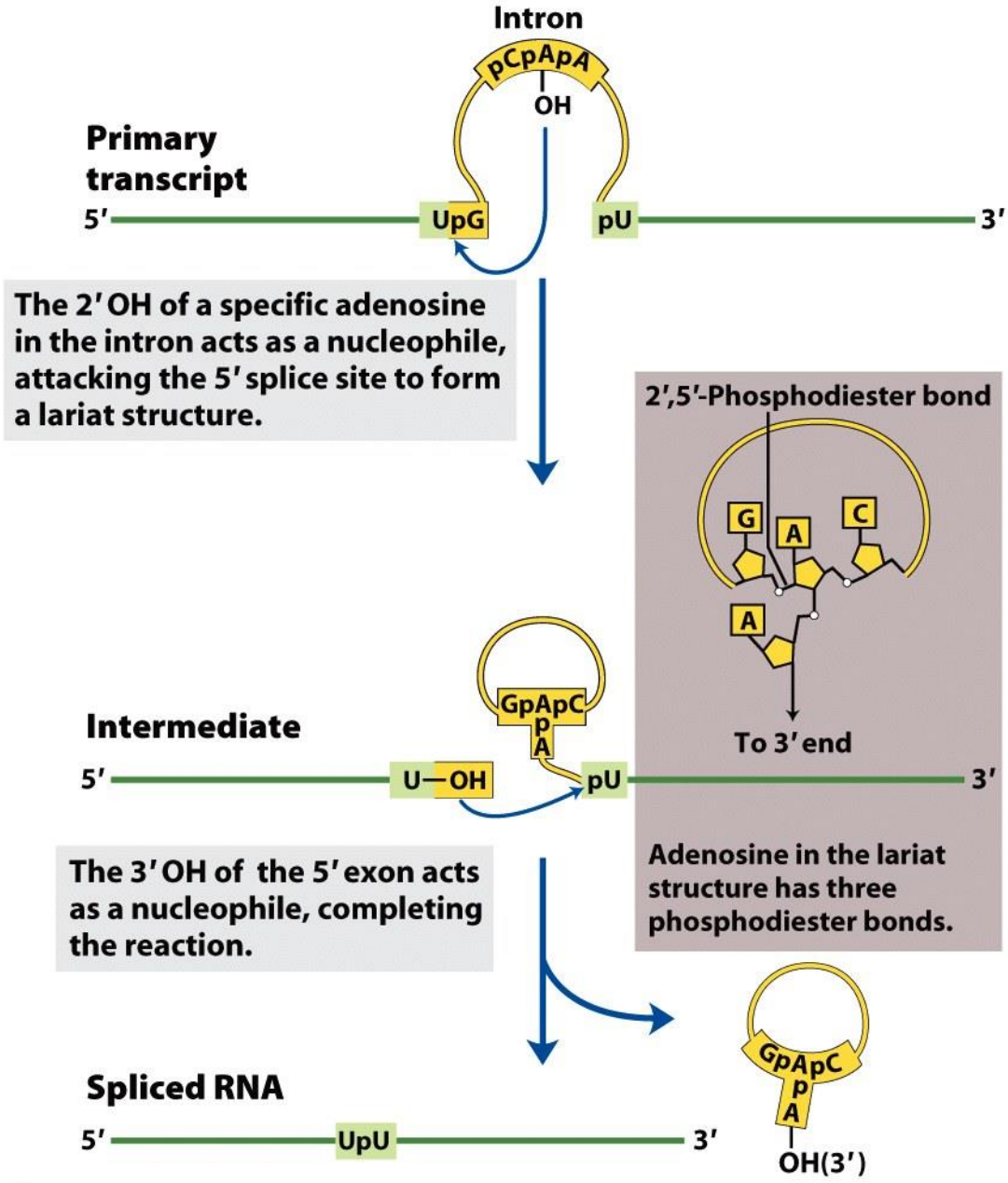
Group II Self-splicing



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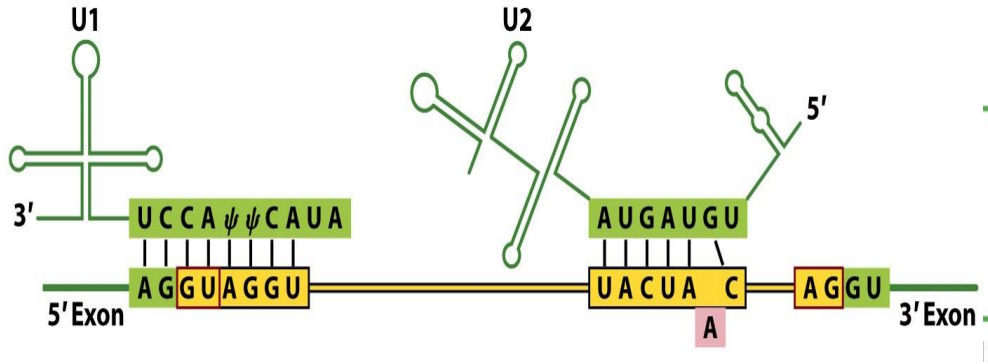
Splicing mechanism of group II introns. The chemistry is similar to that of group I intron splicing, except for the identity of the nucleophile in the first step and formation of a lariat like intermediate, in which one branch is a 2′,5′-phosphodiester bond.

Spliceosomal Splicing

Within the spliceosome, the introns undergo splicing by the lariat-forming mechanism

The spliceosome is made up of specialized RNA-protein complexes, *small nuclear ribonucleoproteins* (snRNPs, often pronounced "snurps").



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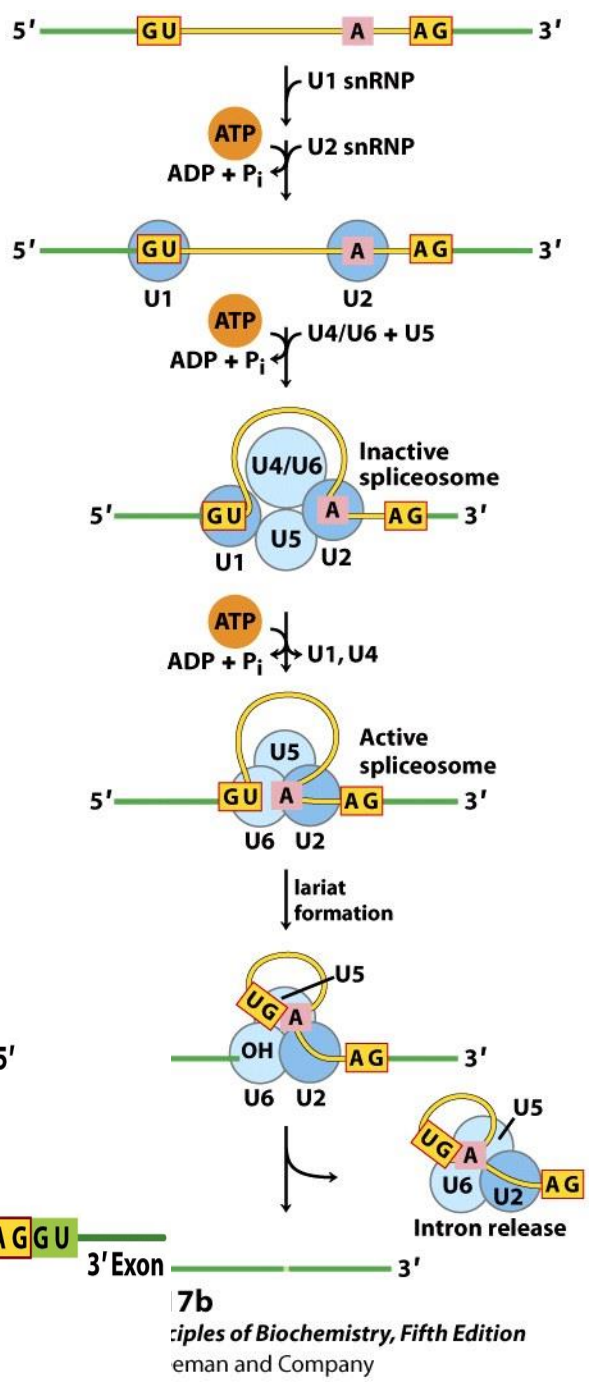

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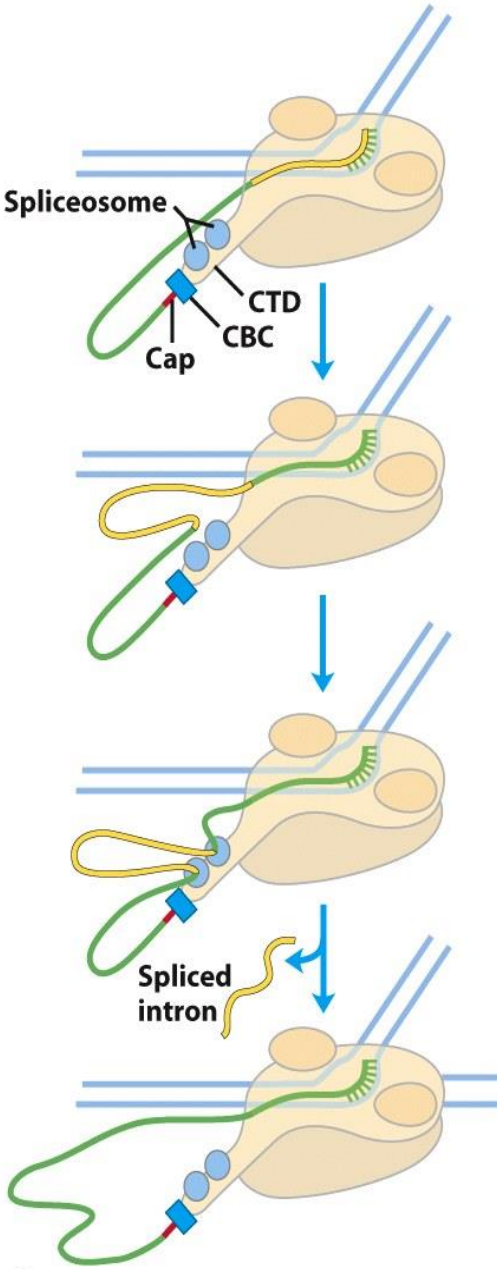

Figure 26-17c
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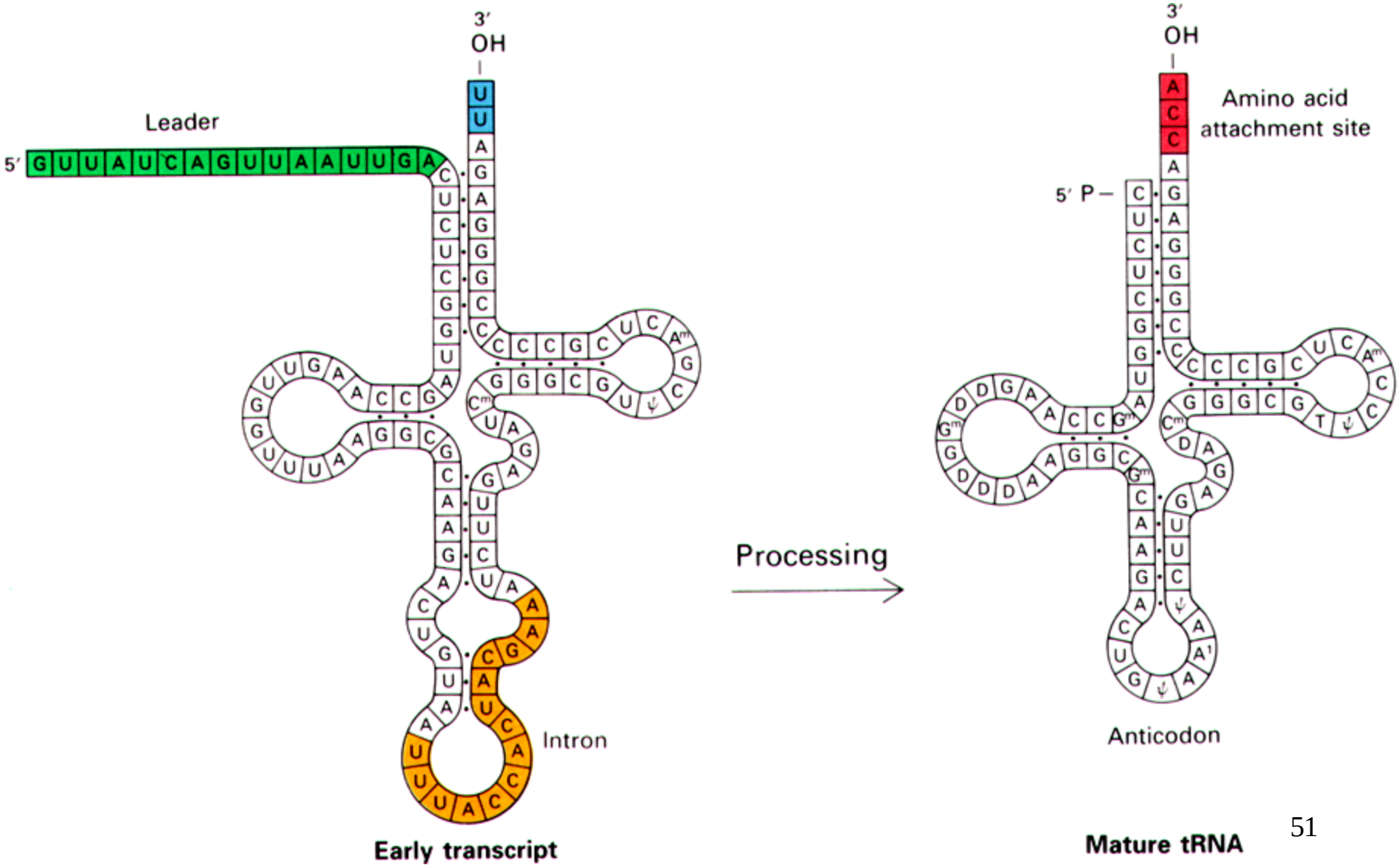
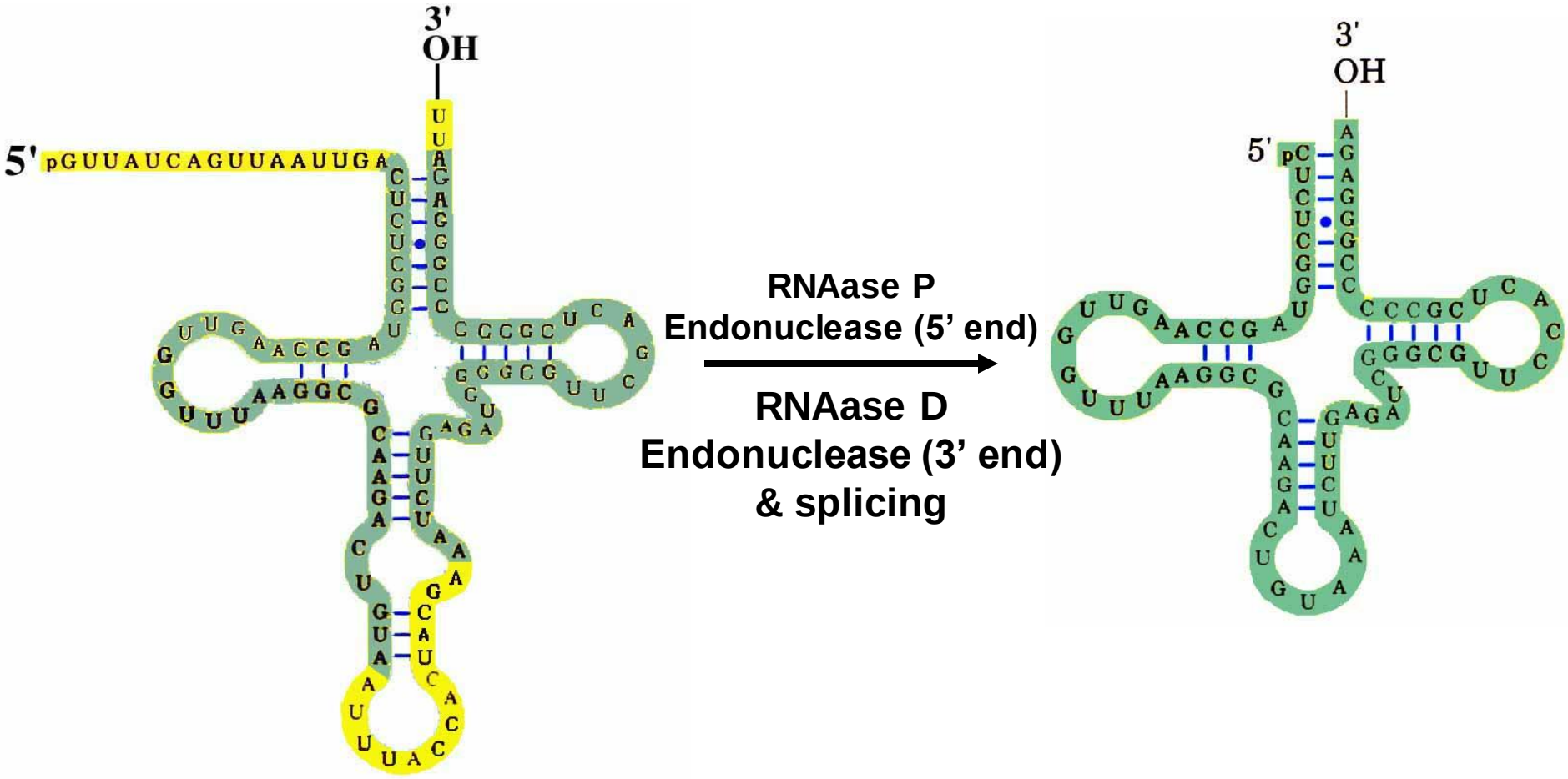
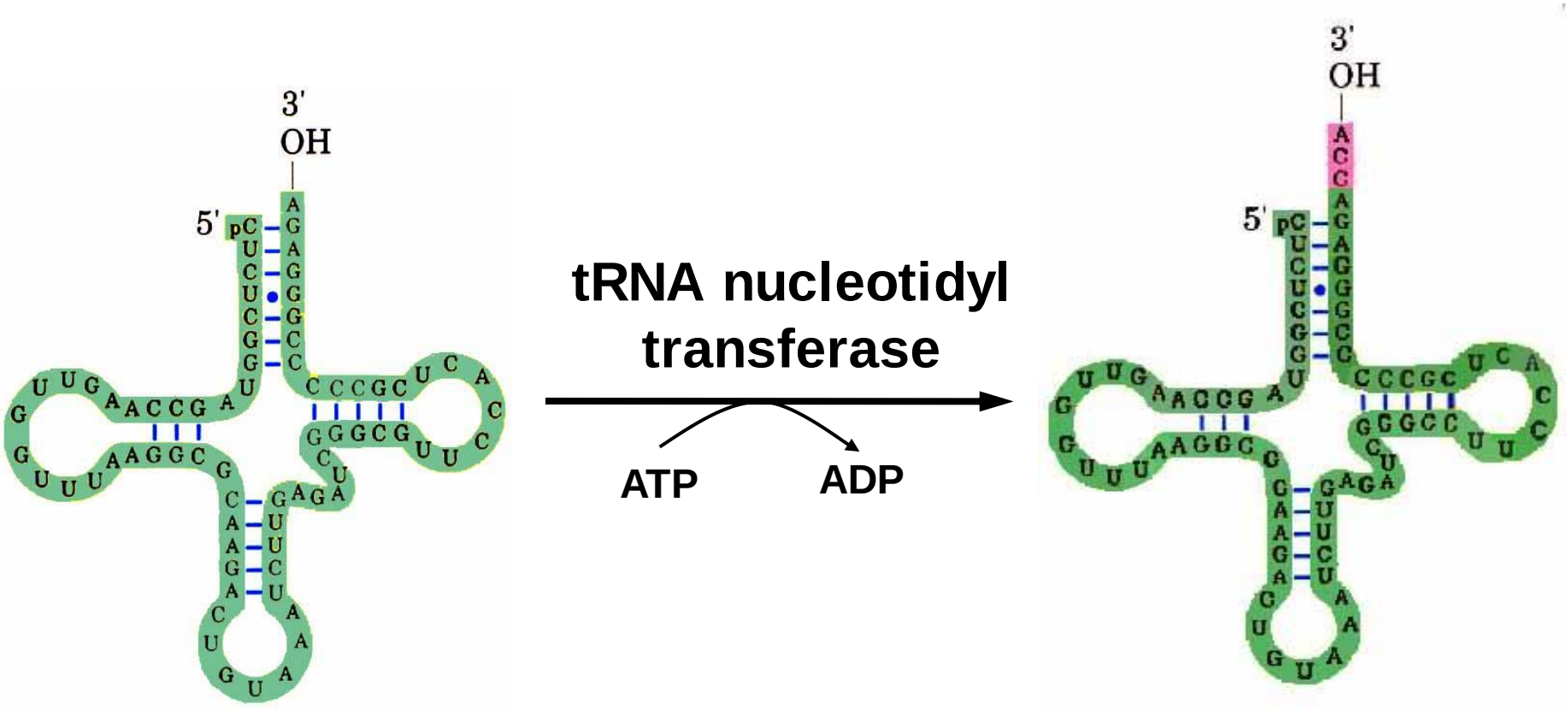
Modification of tRNA



tRNA Splicing

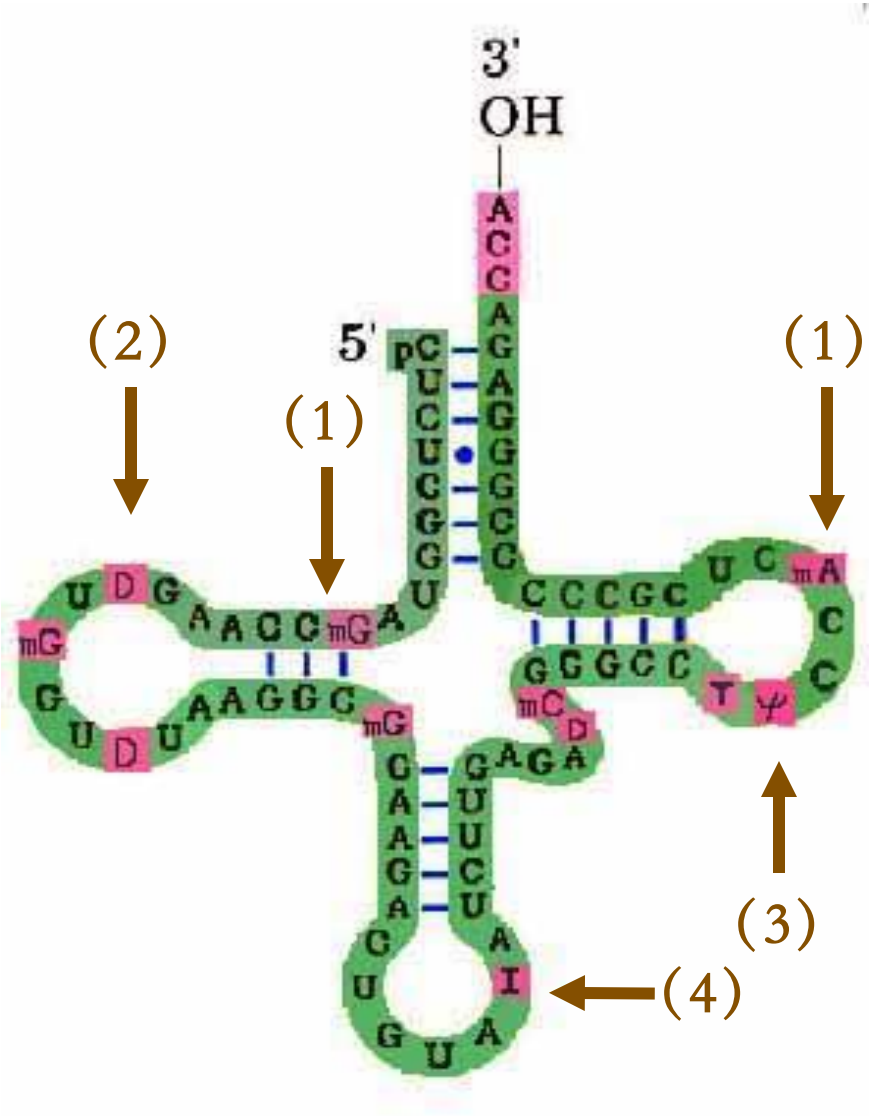


Addition of CCA-OH

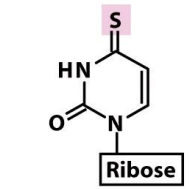


Trinucleotide CCA(3) to which an amino acid is attached is during protein synthesis is absent in some bacterial and all eukaryotic tRNAs and is added during processing

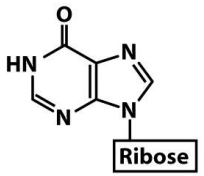
Base modification



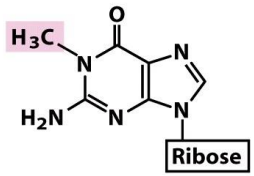
1. Methylation A→mA, G→mG
2. Reduction U→DHU
3. Transversion U→ψ
4. Deamination A→I



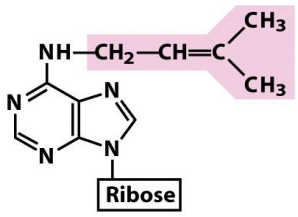
4-Thiouridine (S^4U)



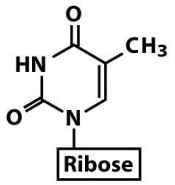
Inosine (I)



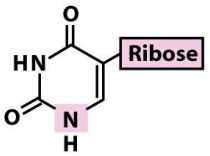
1-Methylguanosine (m^1G)



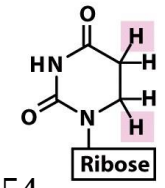
N^6-Isopentenyladenosine (i^6A)



Ribothymidine (T)



Pseudouridine (ψ)



Dihydrouridine (D)

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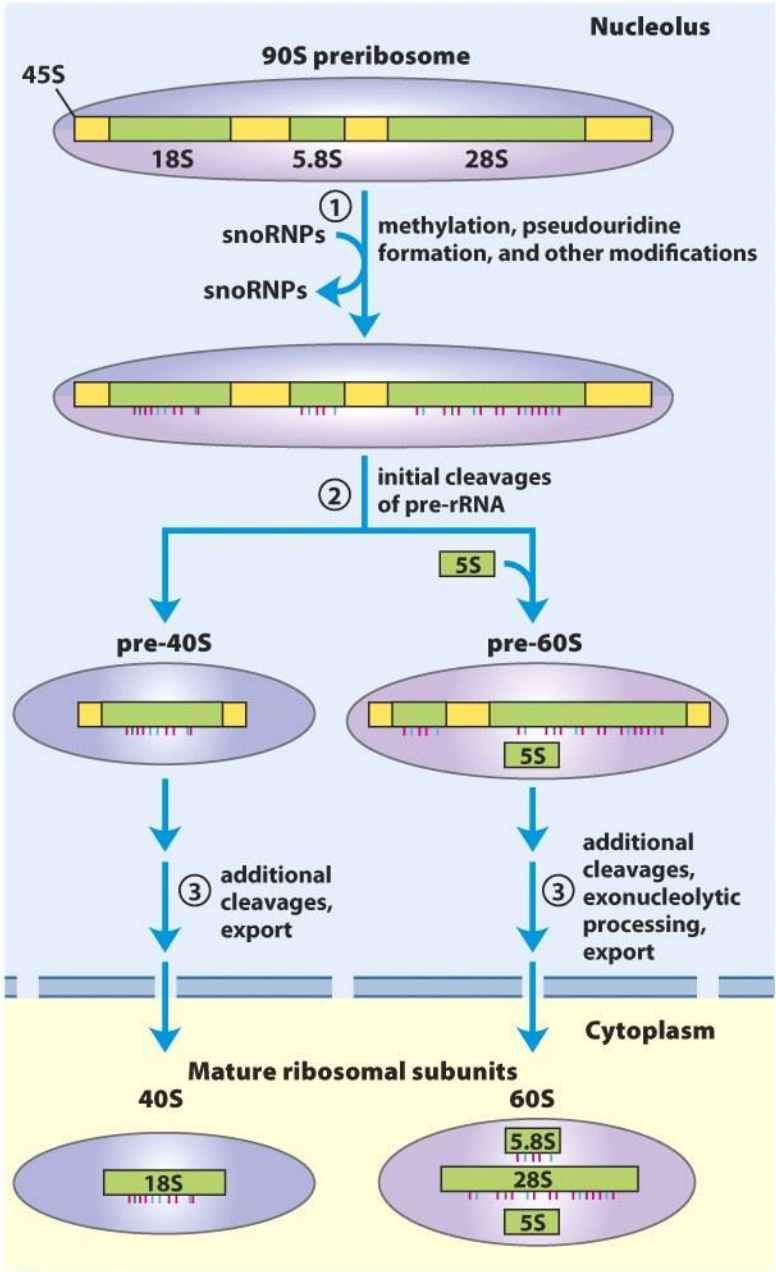
Modification of rRNA

- In eukaryotes, 45S transcript in nucleus is the precursor of 3 kinds of rRNAs.
- The matured rRNA will be assembled with ribosomal proteins to form ribosomes that are exported to cytosolic space.



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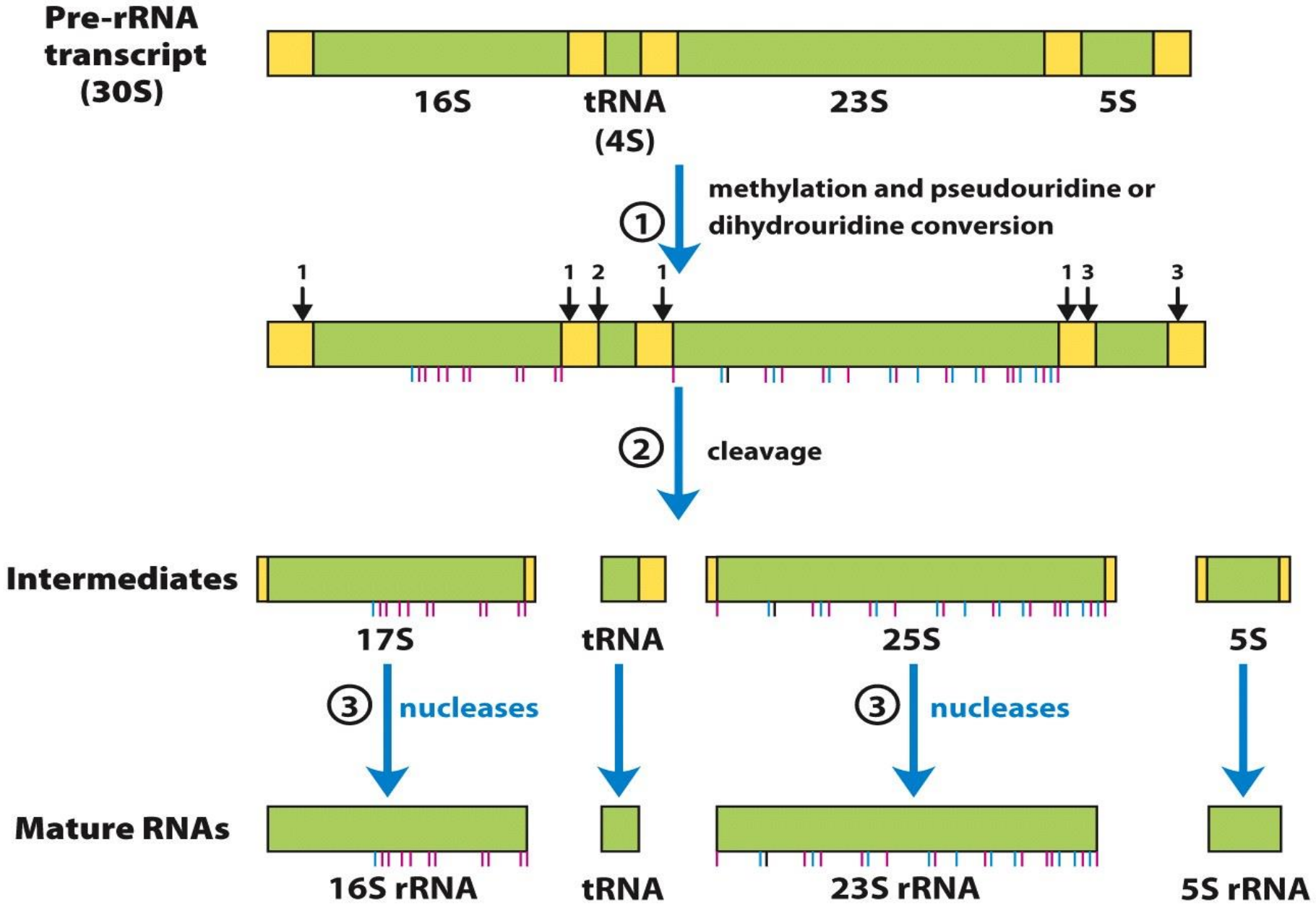

Figure 26-24
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