

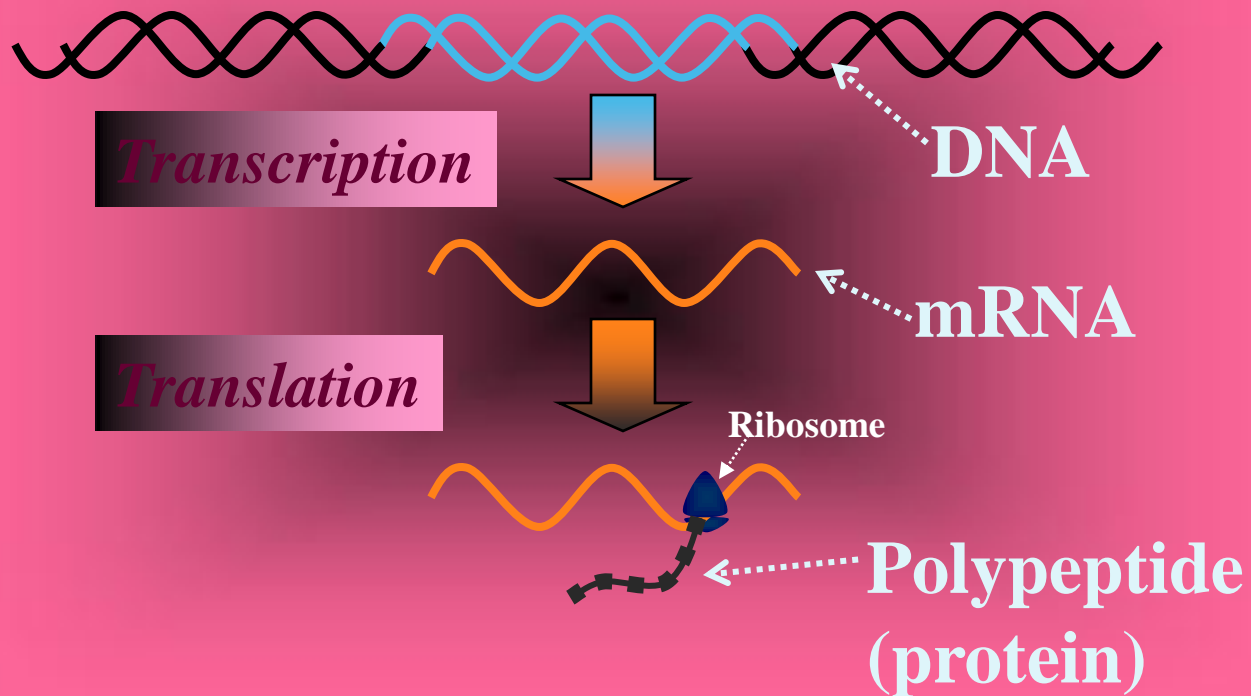
Transcription

**in Prokaryotes &
Eukaryotes**

Introduction

The Central Dogma of Molecular Biology

Cell

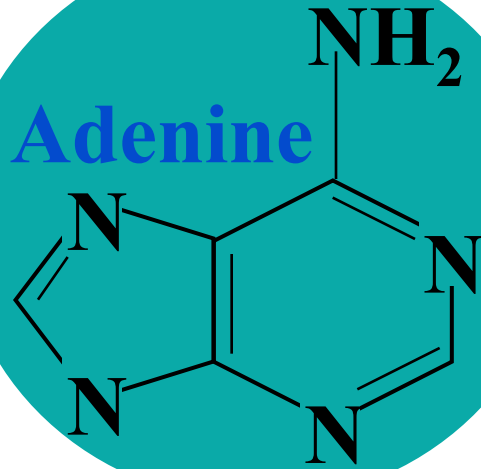


MAJOR CLASSES OF RNA

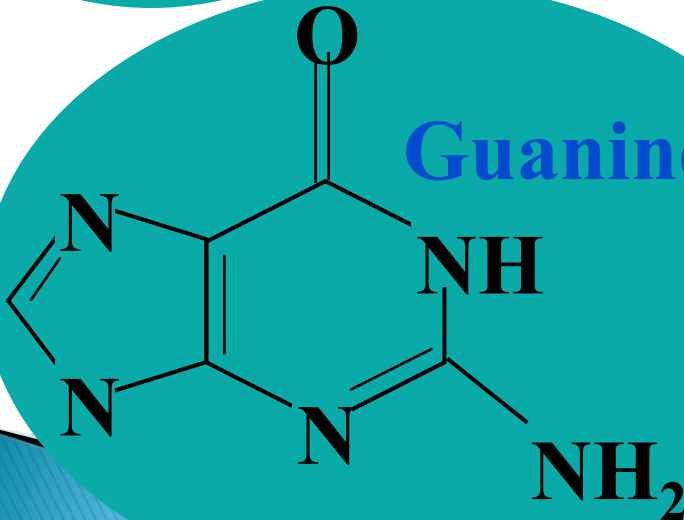
- ▶ rRNA (Ribosomal RNA) 80% V.Stable.
- ▶ mRNA (messenger RNA) 2–5% Unstable.
- ▶ tRNA (Transfer RNA) 15% V.Stable.
- ▶ snRNA (Small nuclear RNA) 1% V.Stable.
- ▶ miRNA (Micro RNA) 1% Stable.

Two Families of Bases

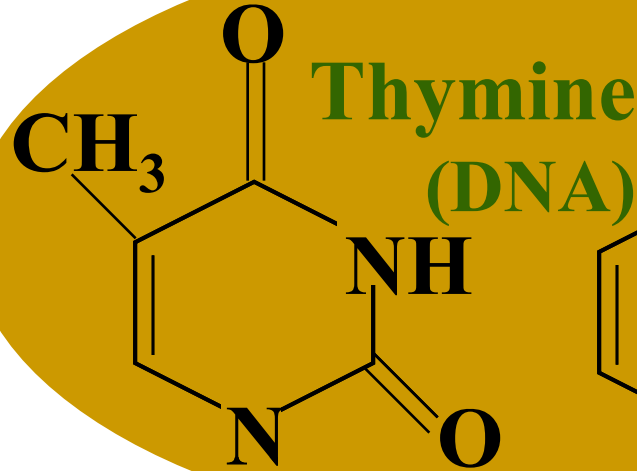
Purines



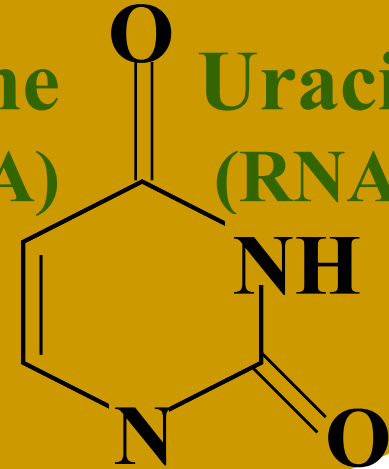
Guanine



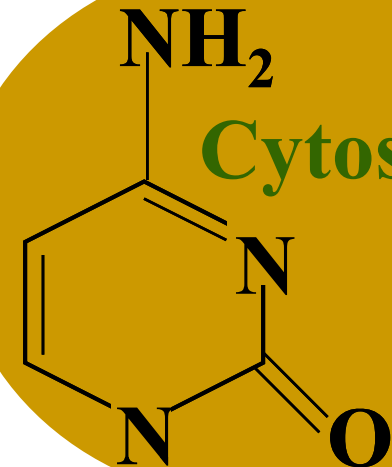
Pyrimidines



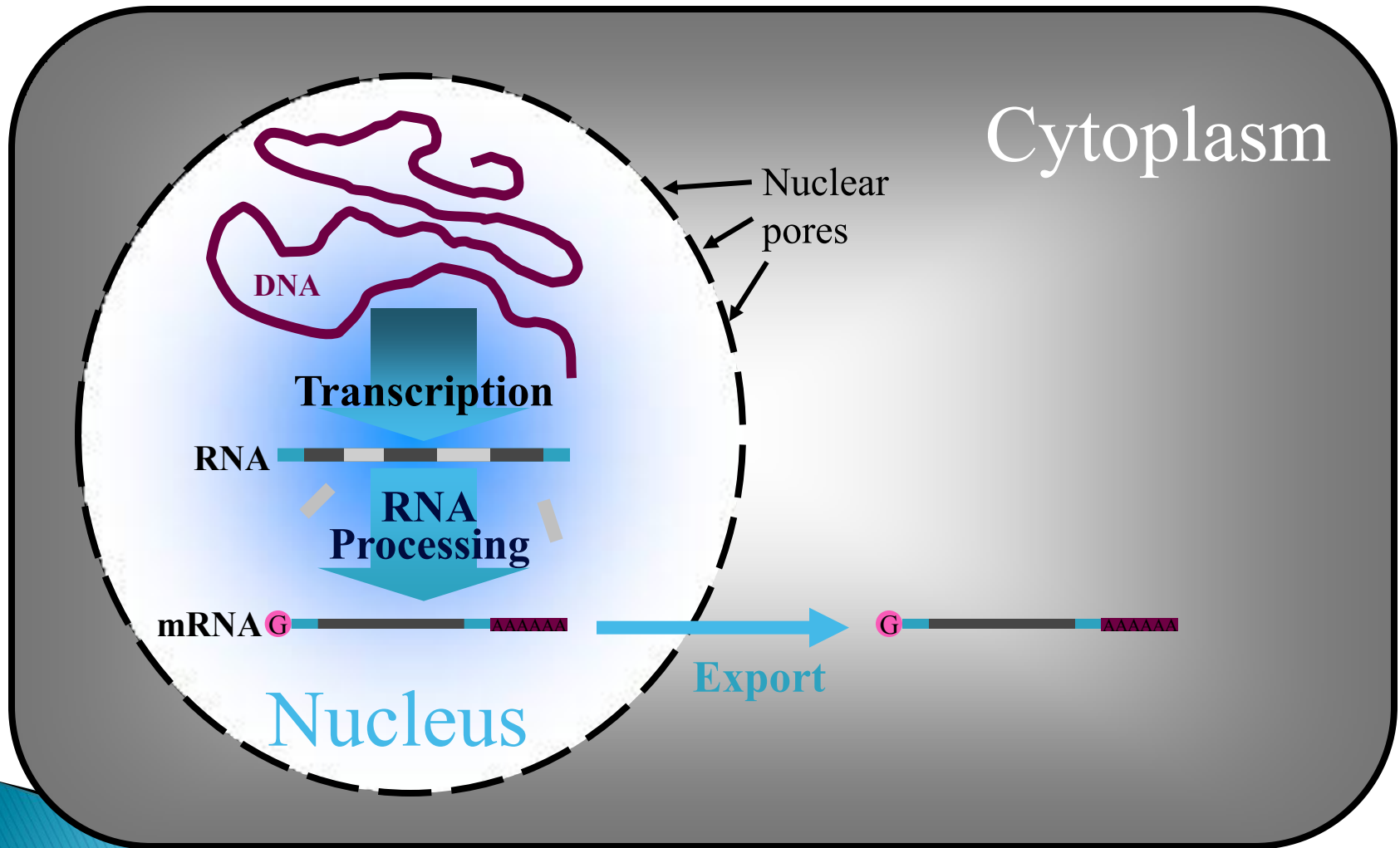
Uracil (RNA)



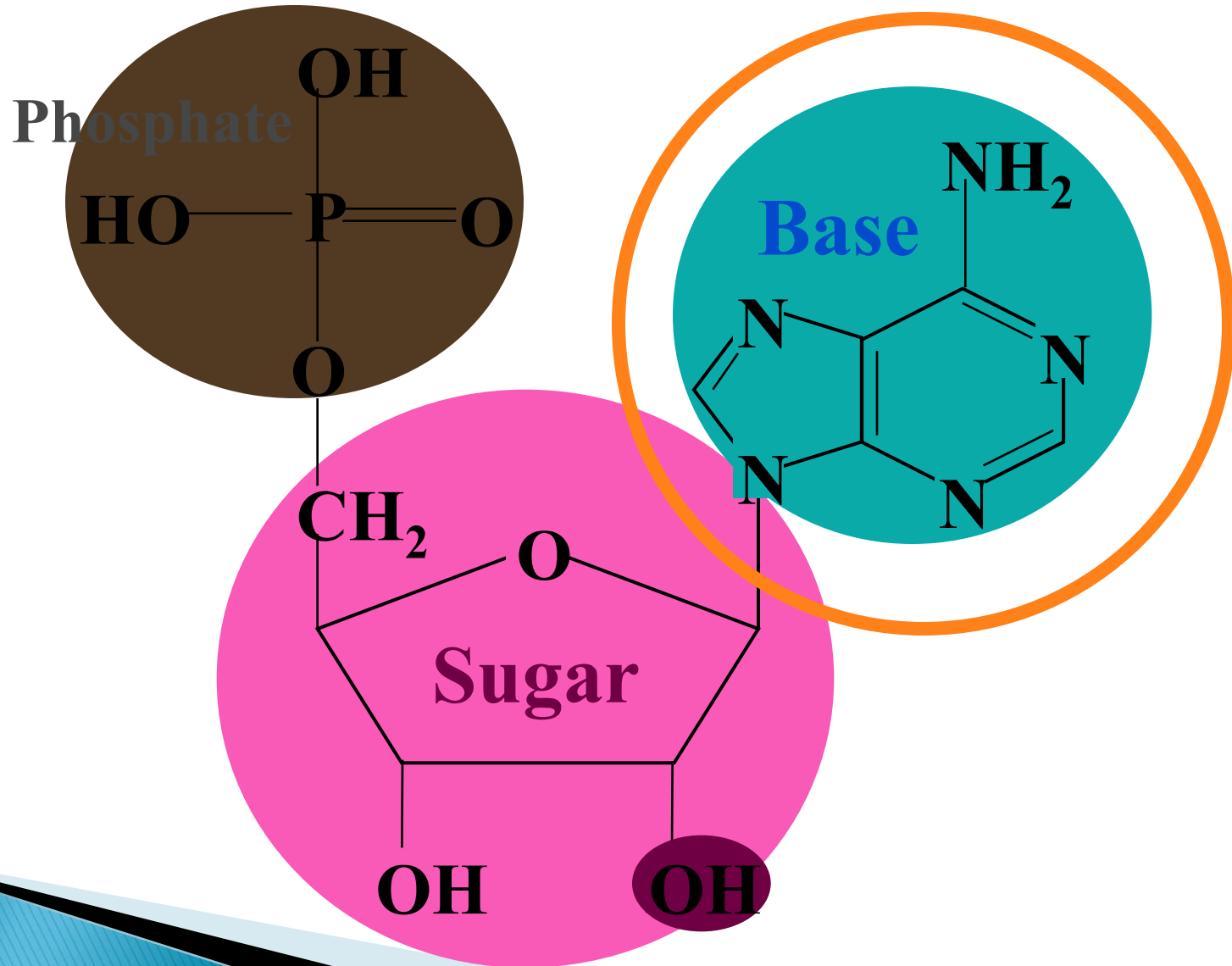
Cytosine



Eukaryotic Transcription



A Nucleotide



Transcription

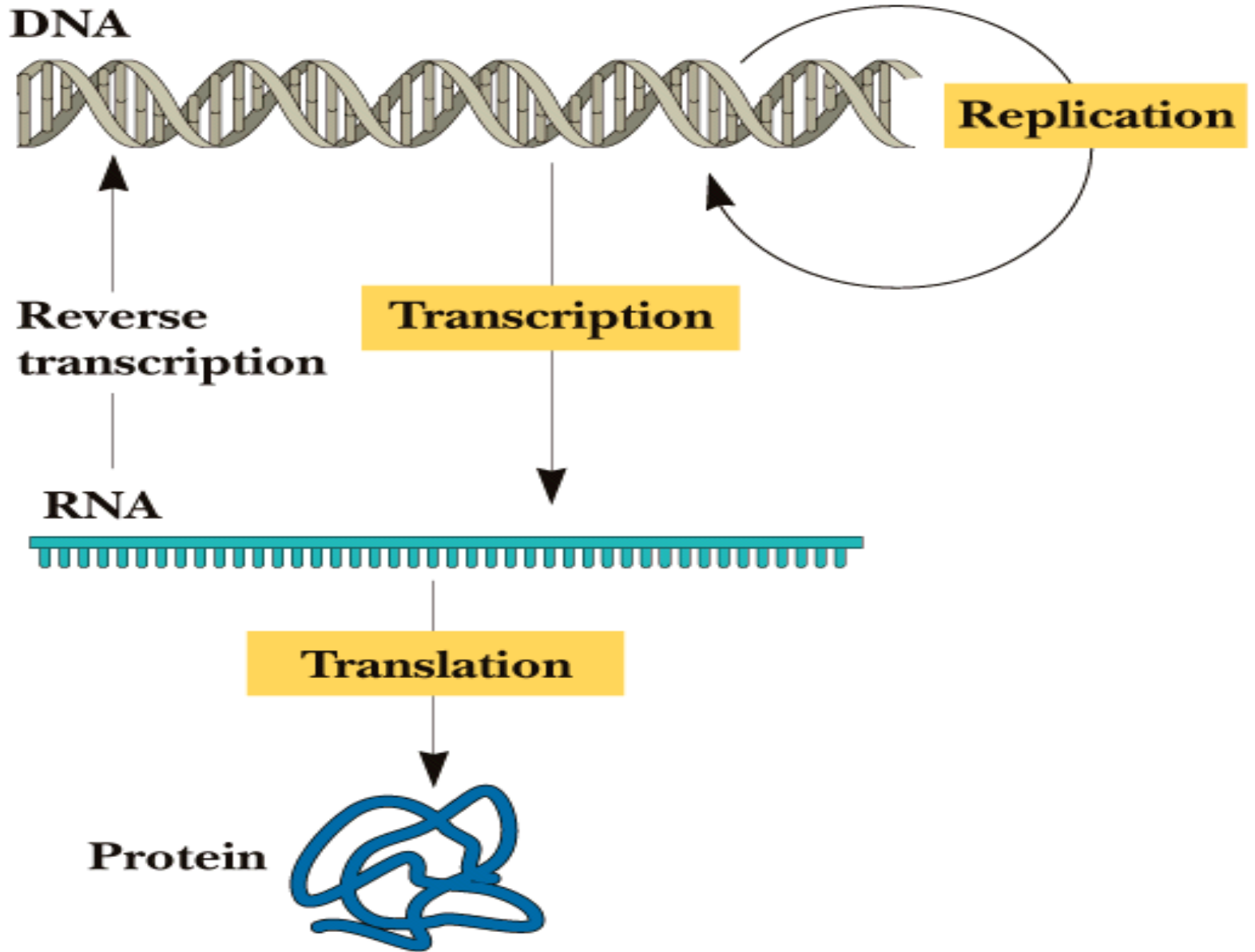
The synthesis of RNA molecules using DNA strands as the templates so that the genetic information can be transferred from DNA to RNA.

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	double 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, T-A, G-C



- ▶ **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**.**

§ 1.1 Template

The template strand is the strand from which the RNA is actually transcribed. It is also termed as **antisense strand**.

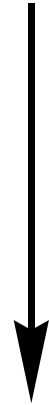
The coding strand is the strand whose base sequence specifies the amino acid sequence of the encoded protein. Therefore, it is also called as **sense strand**.

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



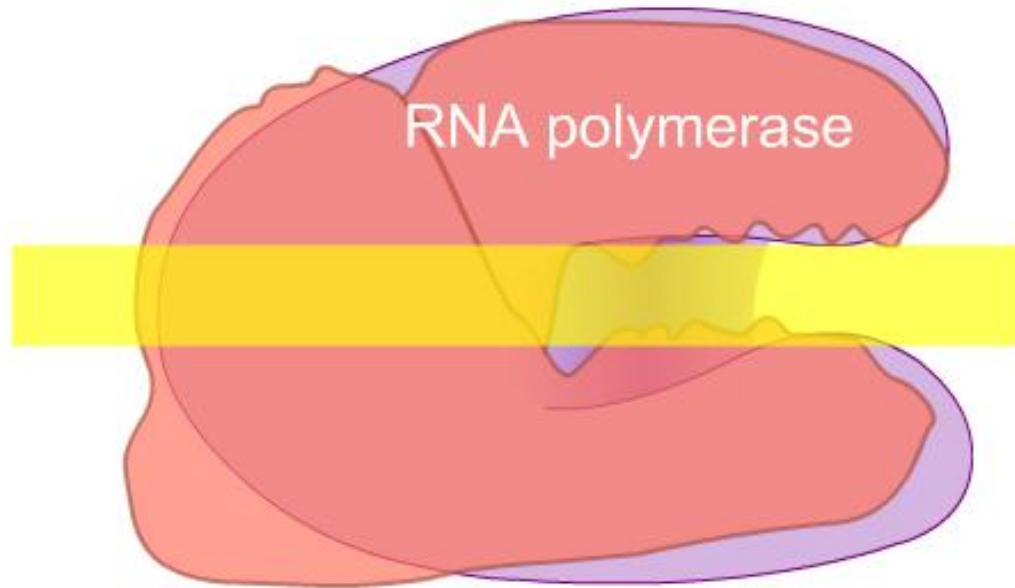
transcription

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

RNA

§ 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.**

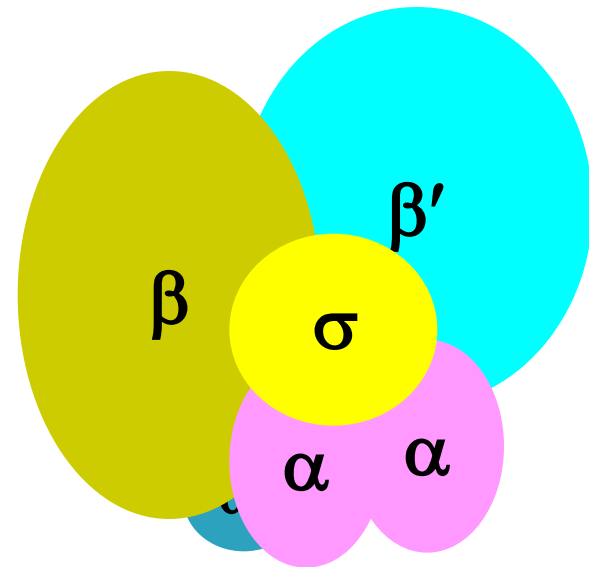


The shape of each enzyme resembles a crab claw.

Holoenzyme

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

holoenzyme



RNA-pol of *E. Coli*

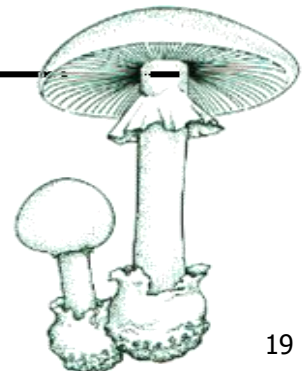
subunit	MW	function
α	36512	Determine the DNA to be transcribed
β	150618	Catalyze polymerization
β'	155613	Bind & open DNA template
σ	70263	Recognize the promoter for synthesis initiation

- ▶ **Rifampicin, a therapeutic drug for tuberculosis treatment, can bind specifically to the β subunit of RNA-pol, and inhibit the RNA synthesis.**
- ▶ **RNA-pol of other prokaryotic systems is similar to that of *E. coli* in structure and functions.**

RNA-pol of eukaryotes

RNA-pol	I	II	III
products	45S rRNA	hnRNA	5S rRNA tRNA snRNA
Sensitivity to Amanitin	No	high	moderate

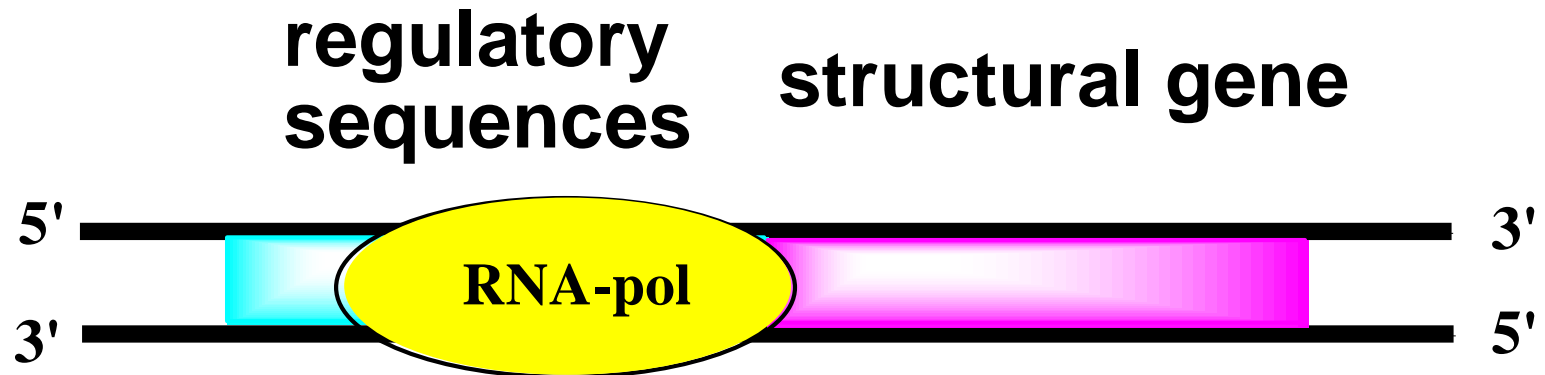
Amanitin is a specific inhibitor of RNA-pol.



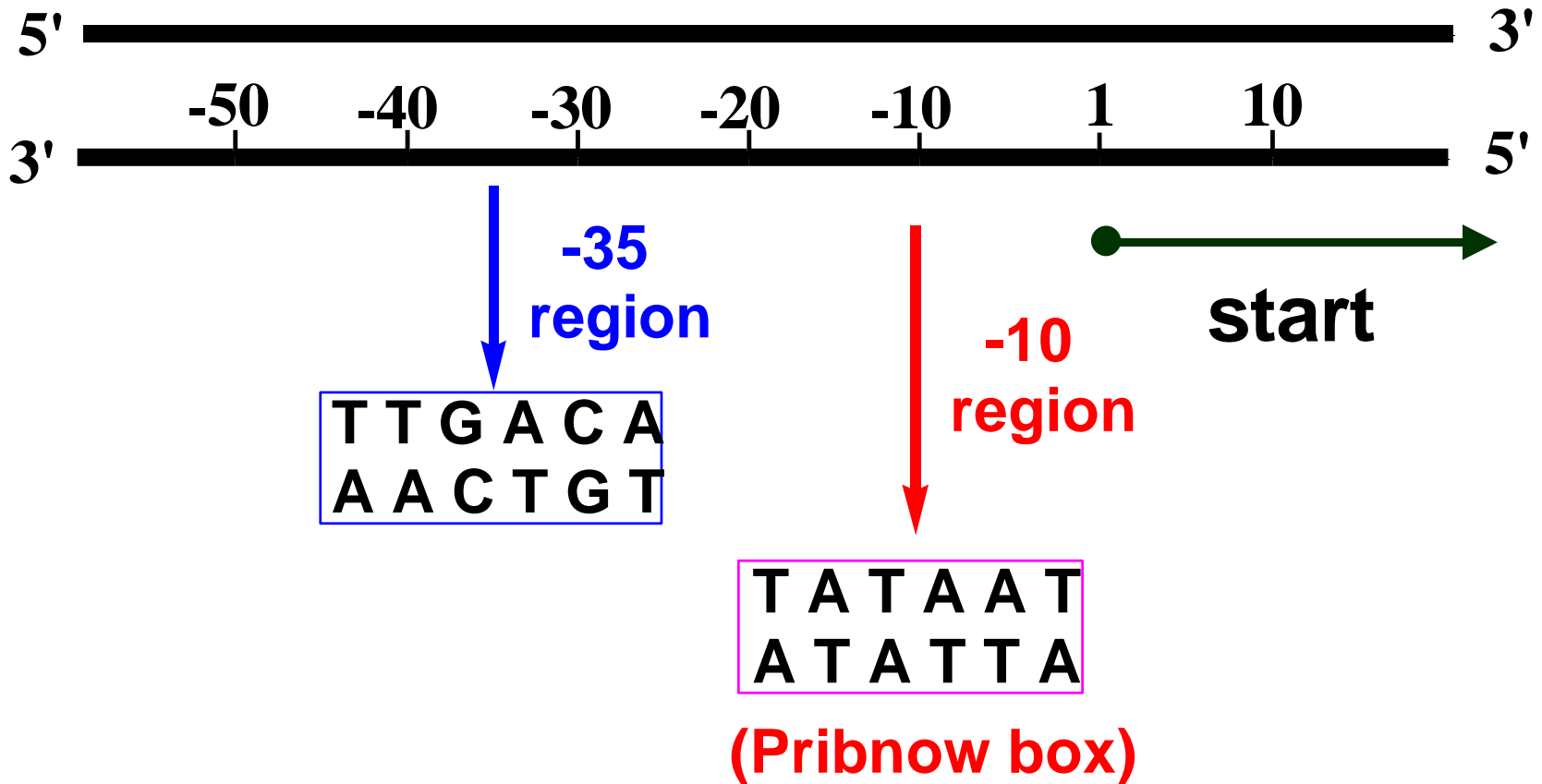
§ 1.3 Recognition of Origins

- ▶ **Each transcribable 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.**

Promoter

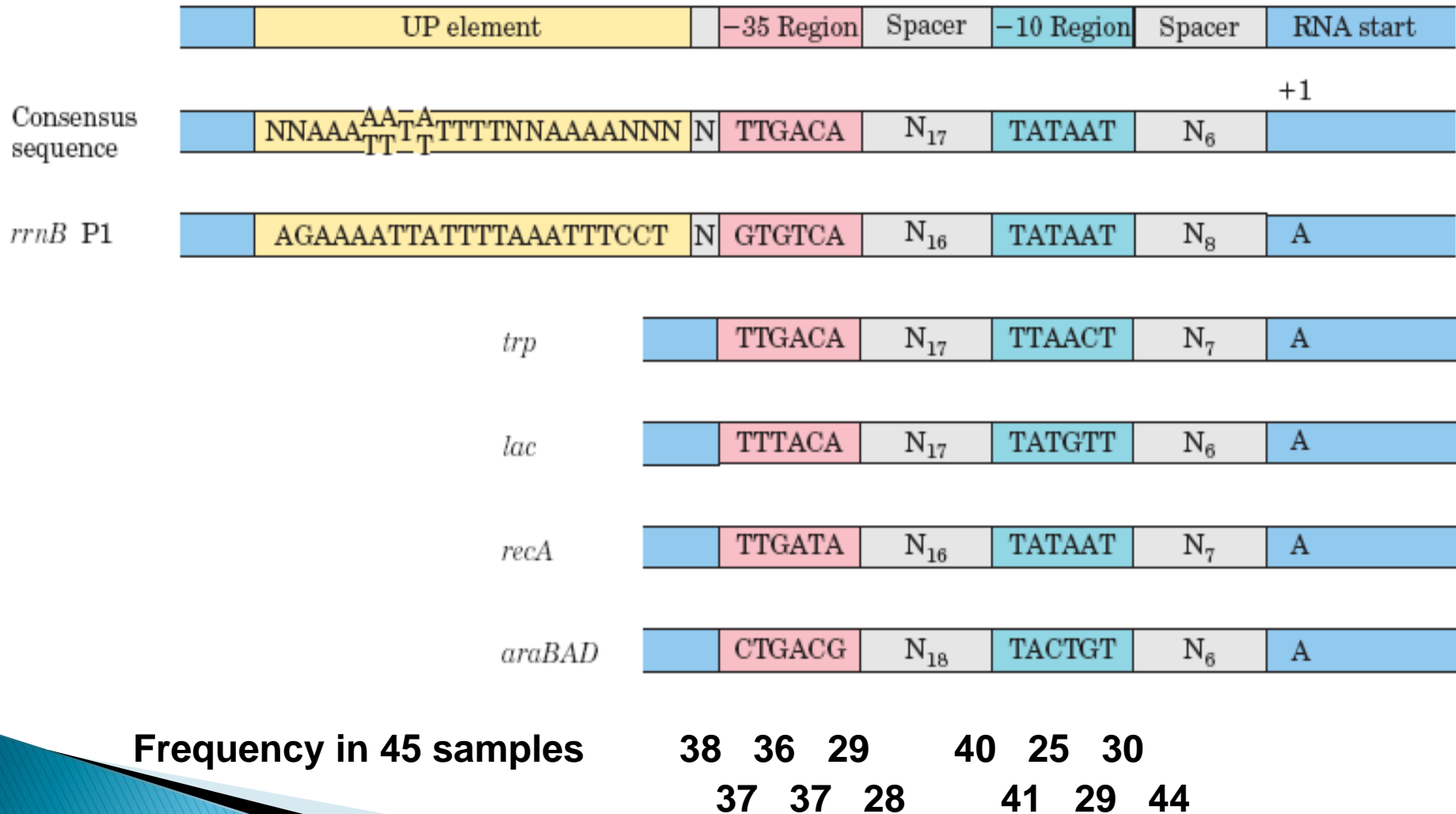


Prokaryotic promoter



Consensus sequence

Consensus Sequence



- ▶ **The -35 region of TTGACA sequence is the recognition site and the binding site of RNA-pol.**
- ▶ **The -10 region of TATAAT is the region at which a stable complex of DNA and RNA-pol is formed.**

Transcription Process

General concepts

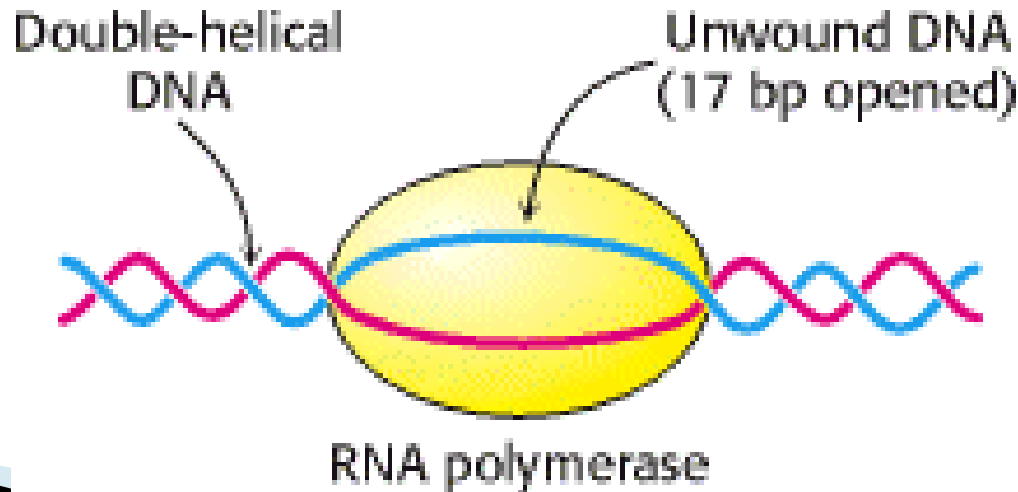
- ▶ **Three phases: initiation, elongation, and termination.**
- ▶ **The prokaryotic RNA-pol can bind to the DNA template directly in the transcription process.**
- ▶ **The eukaryotic RNA-pol requires co-factors to bind to the DNA template together in the transcription process.**

§ 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.**

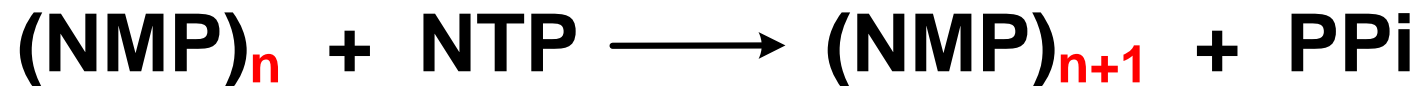
a. Initiation

- ▶ 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.



b. Elongation

- 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.



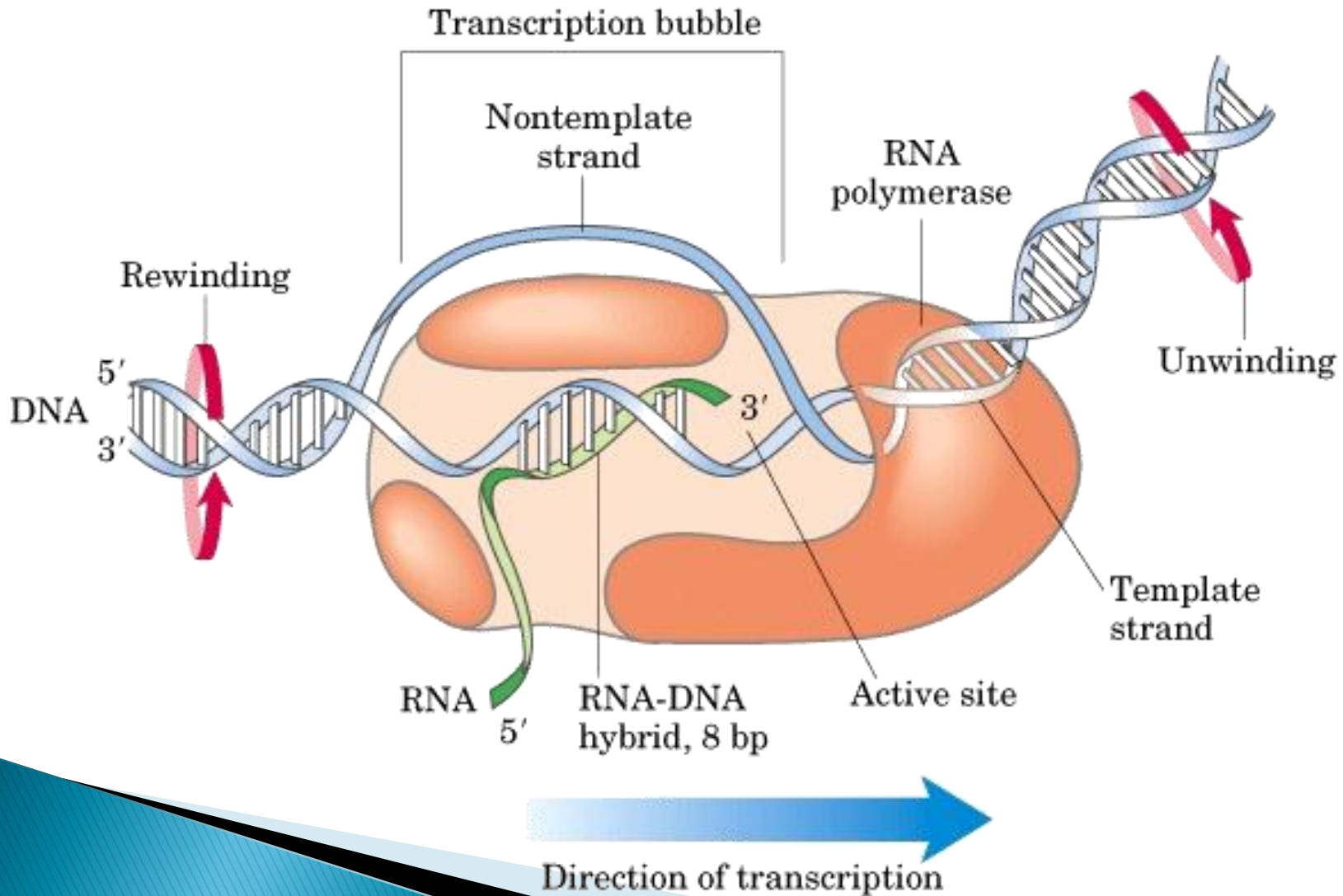
RNA strand

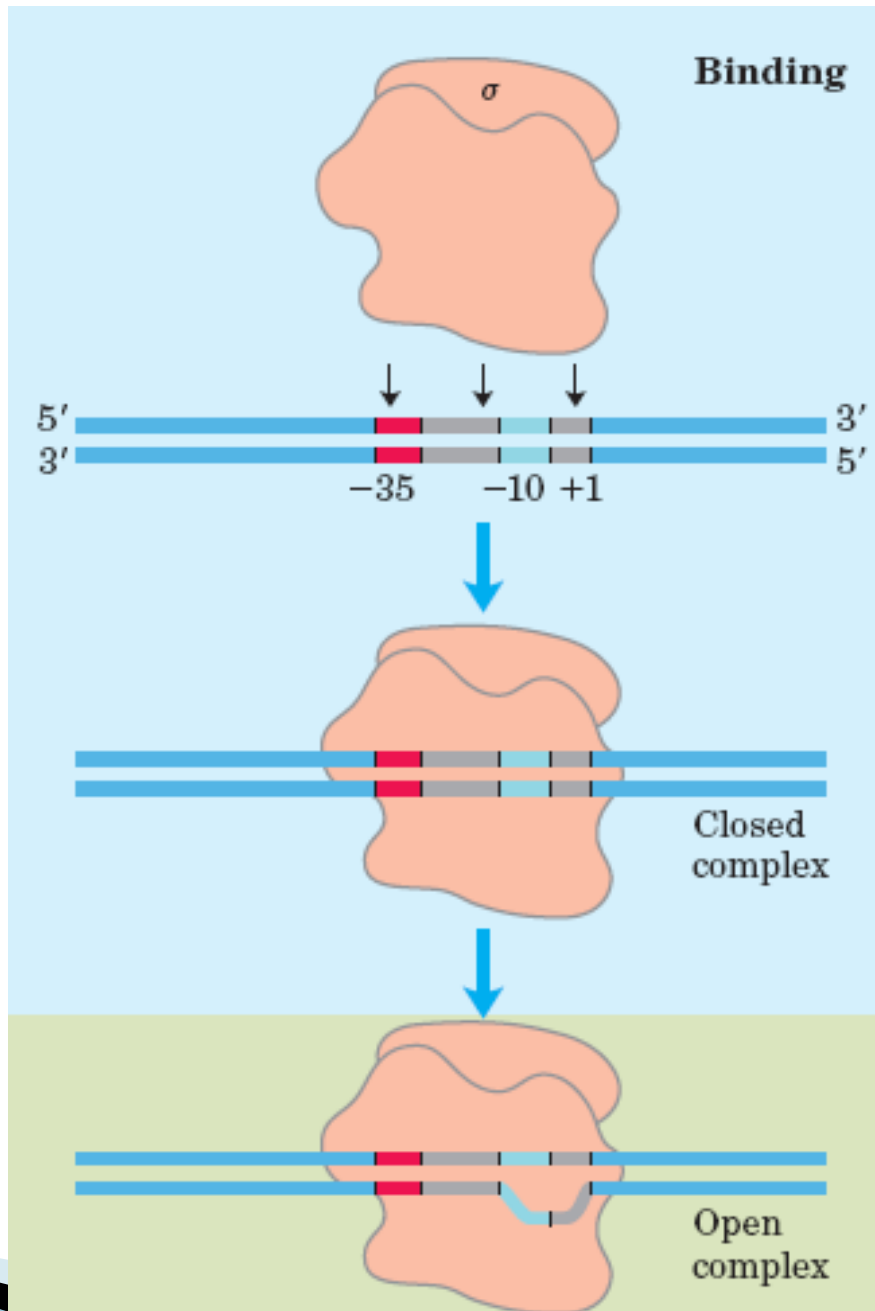
substrate

elongated
RNA strand

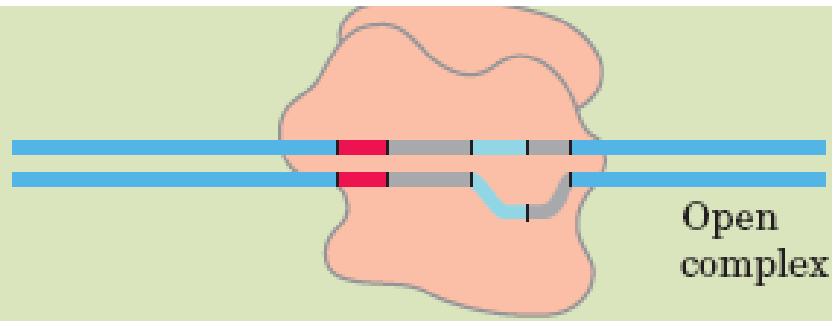
- RNA-pol, DNA segment of ~40nt and the nascent RNA form a complex called the **transcription bubble**.
- The 3' segment of the nascent RNA hybridizes with the DNA template, and its 5' end extends out the transcription bubble as the synthesis is processing.

Transcription bubble

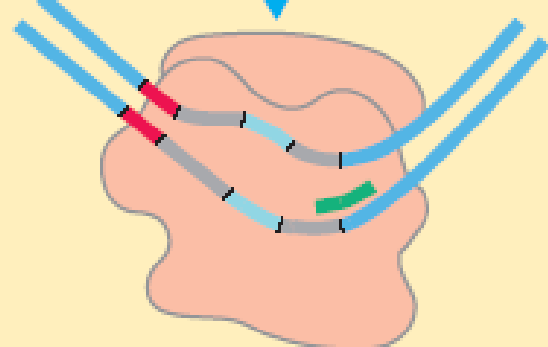




Initiation

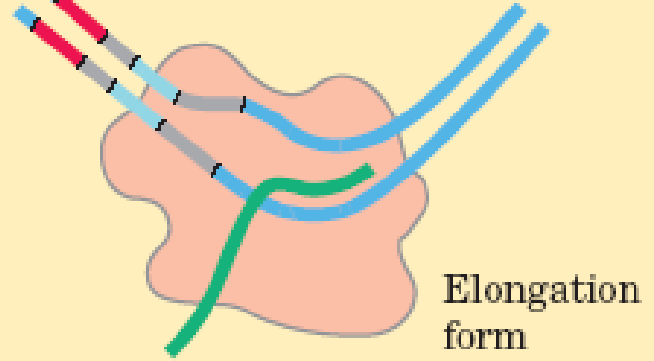


transcription initiation



promoter clearance

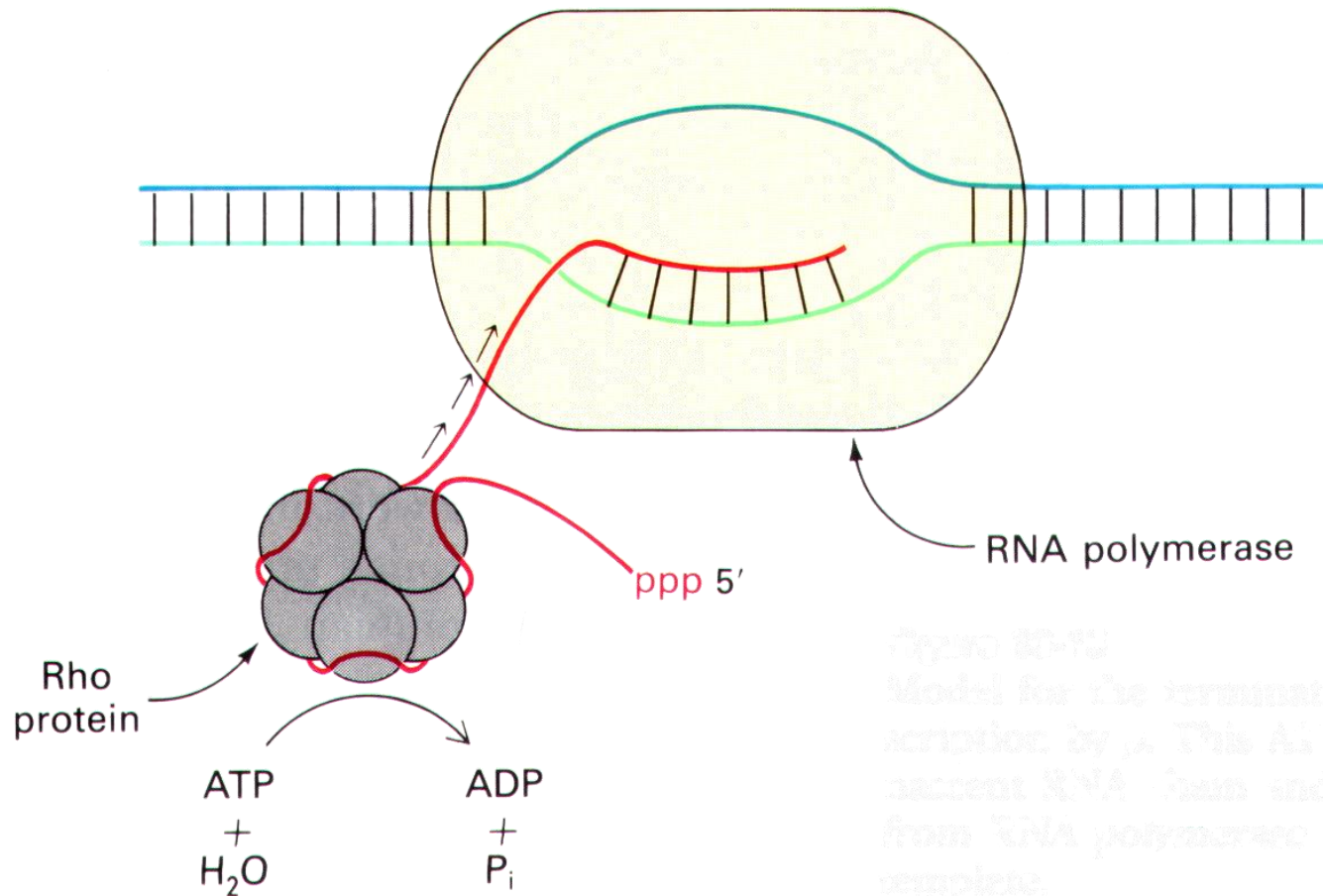
σ



c. Termination

- ▶ **The RNA-pol stops moving on the DNA template. The RNA transcript falls off from the transcription complex.**
- ▶ **The termination occurs in either ρ - dependent or ρ -independent manner.**

The termination function of ρ factor



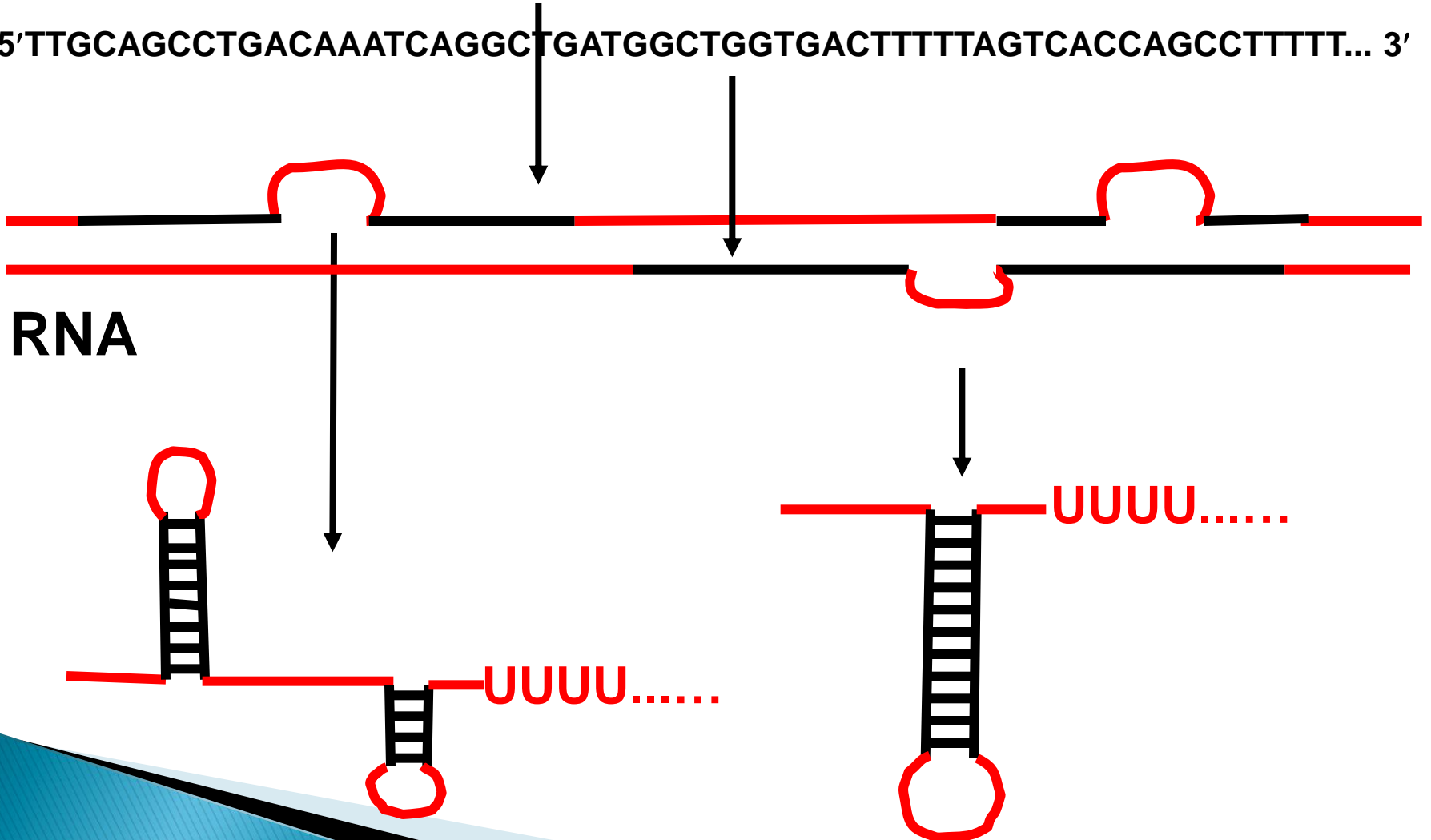
The ρ factor, a hexamer, is a **ATPase** and a **helicase**.

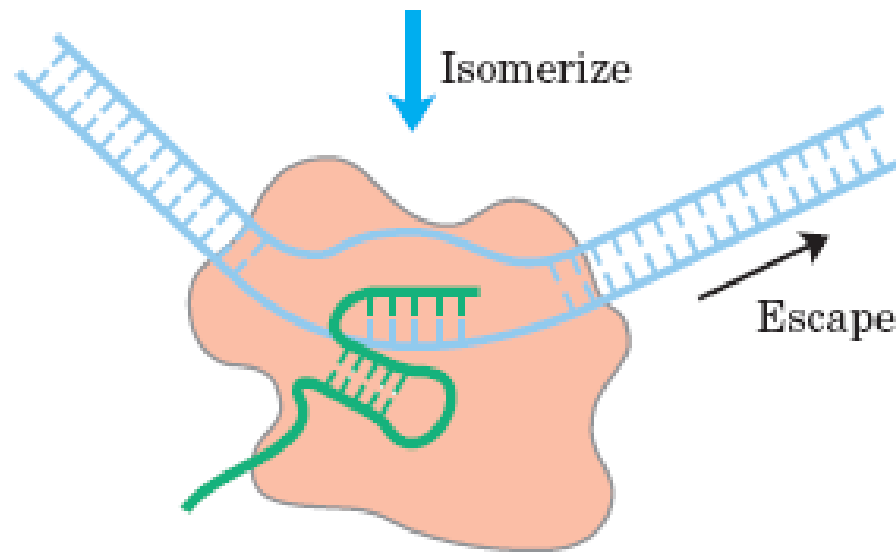
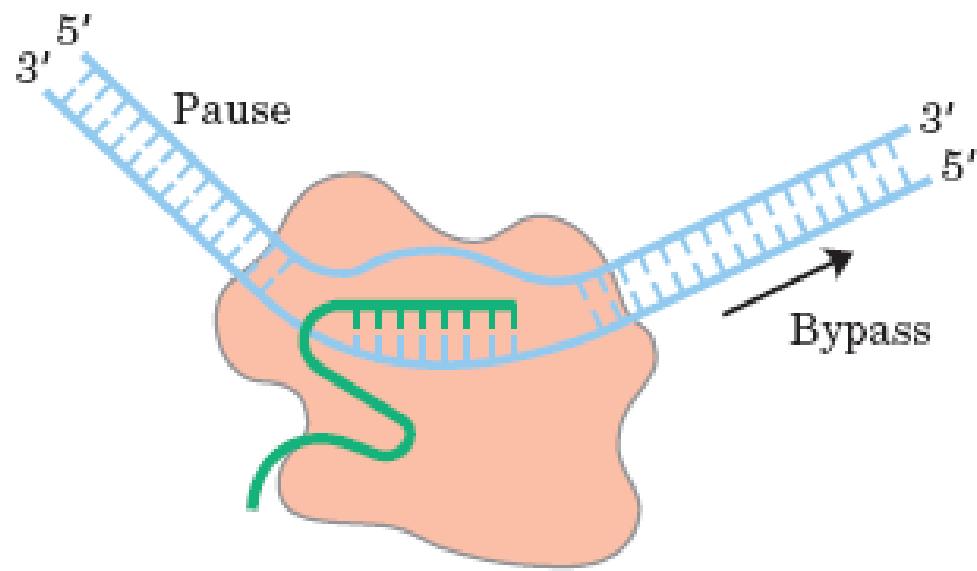
ρ -independent termination

- **The termination signal is a stretch of 30-40 nucleotides on the RNA transcript, consisting of many GC followed by a series of U.**
- **The sequence specificity of this nascent RNA transcript will form particular stem-loop structures to terminate the transcription.**

rplL protein

DNA
5'TTGCAGCCTGACAAATCAGGCTGATGGCTGGTGACTTTTTAGGCACCAGCCTTTTT... 3'
5'TTGCAGCCTGACAAATCAGGCTGATGGCTGGTGACTTTTTAGTCACCAGCCTTTTT... 3'





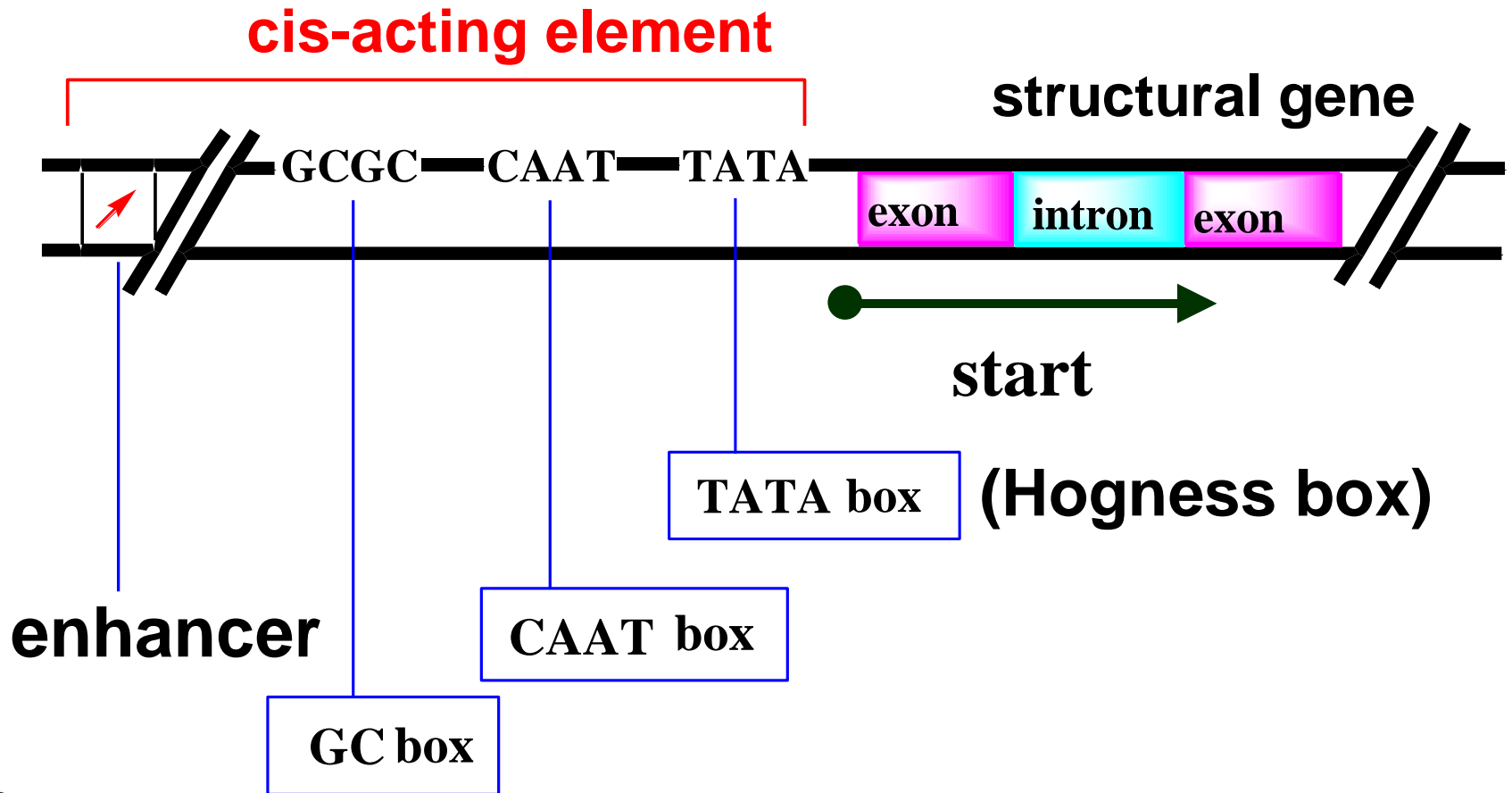
Terminate

§ 2.2 Transcription in Eukaryotes

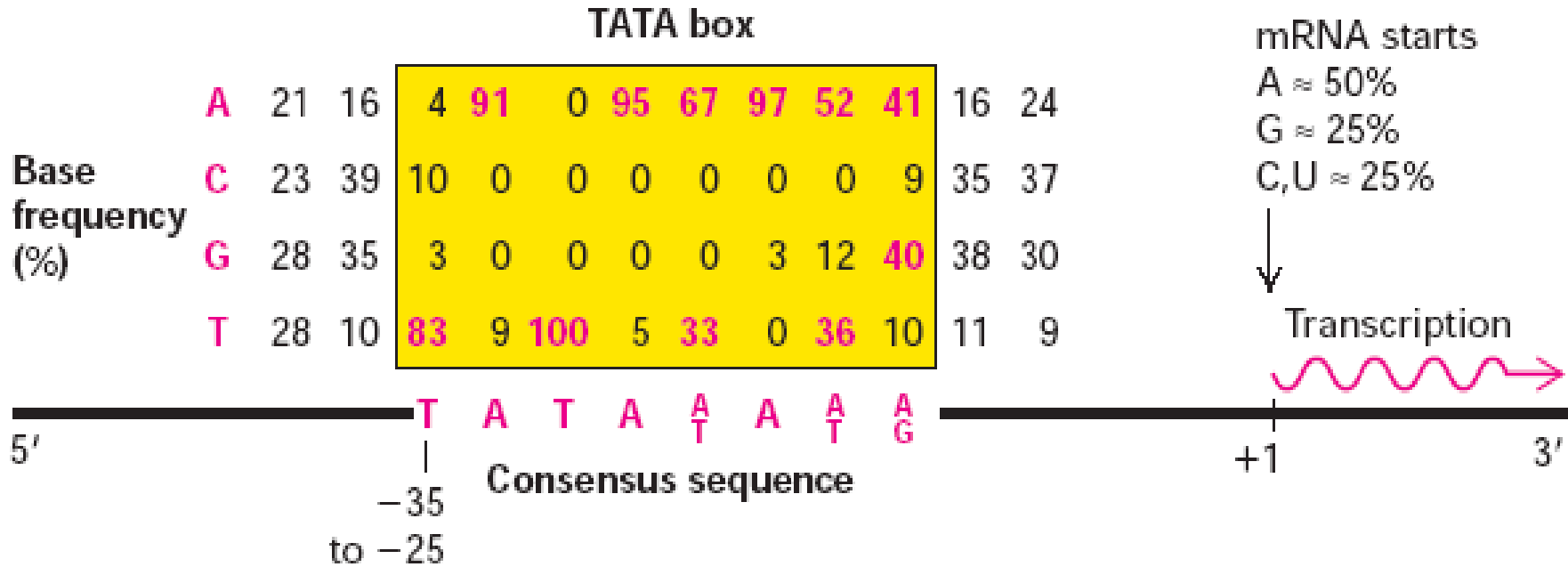
a. Initiation

- ▶ **Transcription initiation needs promoter and upstream regulatory regions.**
- ▶ **The cis-acting elements are the specific sequences on the DNA template that regulate the transcription of one or more genes.**

Cis-acting element



TATA box



Transcription factors

- ▶ **RNA-pol does not bind the promoter directly.**
- ▶ **RNA-pol II associates with six transcription factors, TFII A - TFII H.**
- ▶ **The trans-acting factors are the proteins that recognize and bind directly or indirectly cis-acting elements and regulate its activity.**

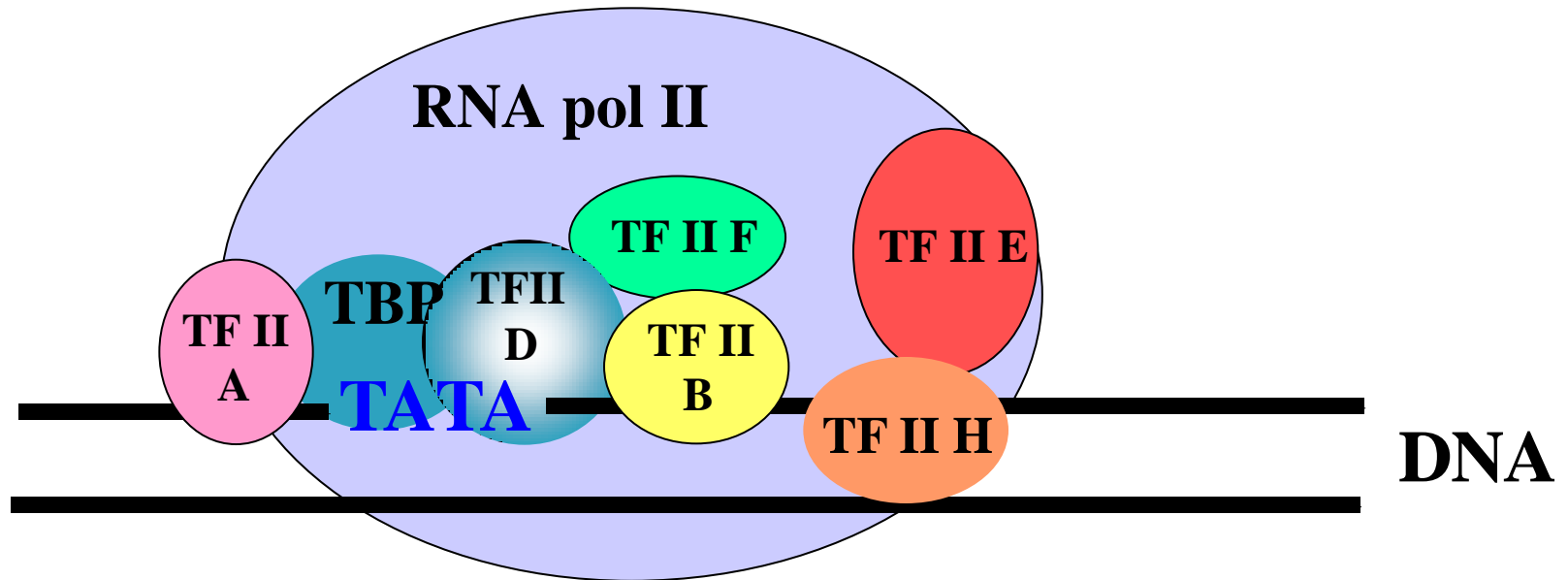
TF for eukaryotic transcription

<i>Transcription protein</i>	<i>Number of subunits</i>	<i>Function(s)</i>
Initiation		
Pol II	12	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	Specifically recognizes the TATA box
TFIIA	3	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	Binds to TBP; recruits Pol II–TFIIF complex
TFIIE	2	Recruits TFIIH; has ATPase and helicase activities
TFIIF	2	Binds tightly to Pol II; binds to TFIIB and prevents binding of Pol II to nonspecific DNA sequences
TFIIH	12	Unwinds DNA at promoter (helicase activity); phosphorylates Pol II (within the CTD); recruits nucleotide-excision repair proteins
Elongation*		
ELL [†]	1	
p-TEFb	2	Phosphorylates Pol II (within the CTD)
SII (TFIIS)	1	
Elongin (SIII)	3	

Pre-initiation complex (PIC)

- ▶ **TBP of TFII D binds TATA**
- ▶ **TFII A and TFII B bind TFII D**
- ▶ **TFII F-RNA-pol complex binds TFII B**
- ▶ **TFII F and TFII E open the dsDNA (helicase and ATPase)**
- ▶ **TFII H: completion of PIC**

Pre-initiation complex (PIC)



Phosphorylation of RNA-pol

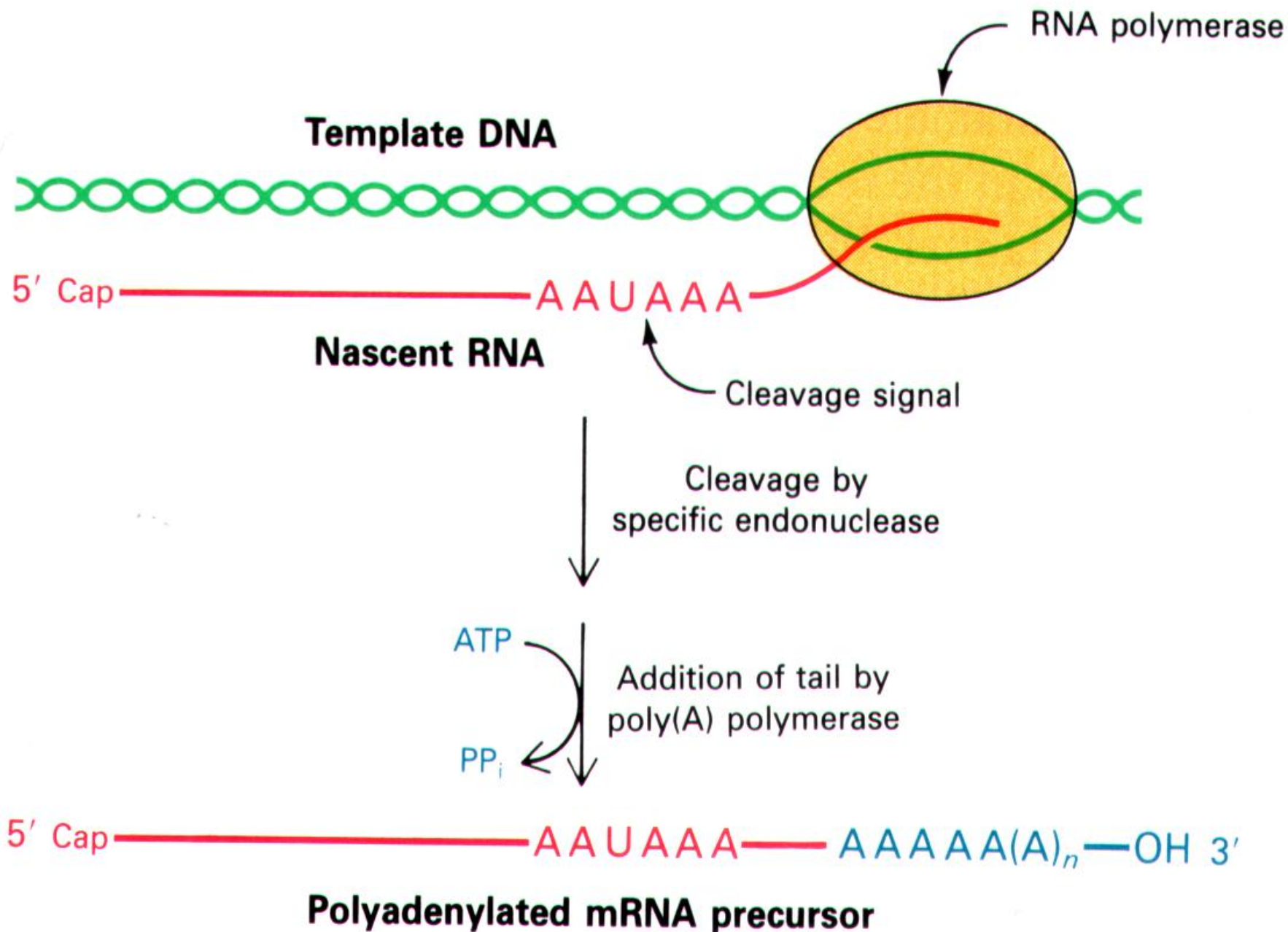
- ▶ **TF II H is of protein kinase activity to phosphorylate CTD of RNA-pol. (CTD is the C-terminal domain of RNA-pol)**
- ▶ **Only the p-RNA-pol can move toward the downstream, starting the elongation phase.**
- ▶ **Most of the TFs fall off from PIC during the elongation phase.**

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.**



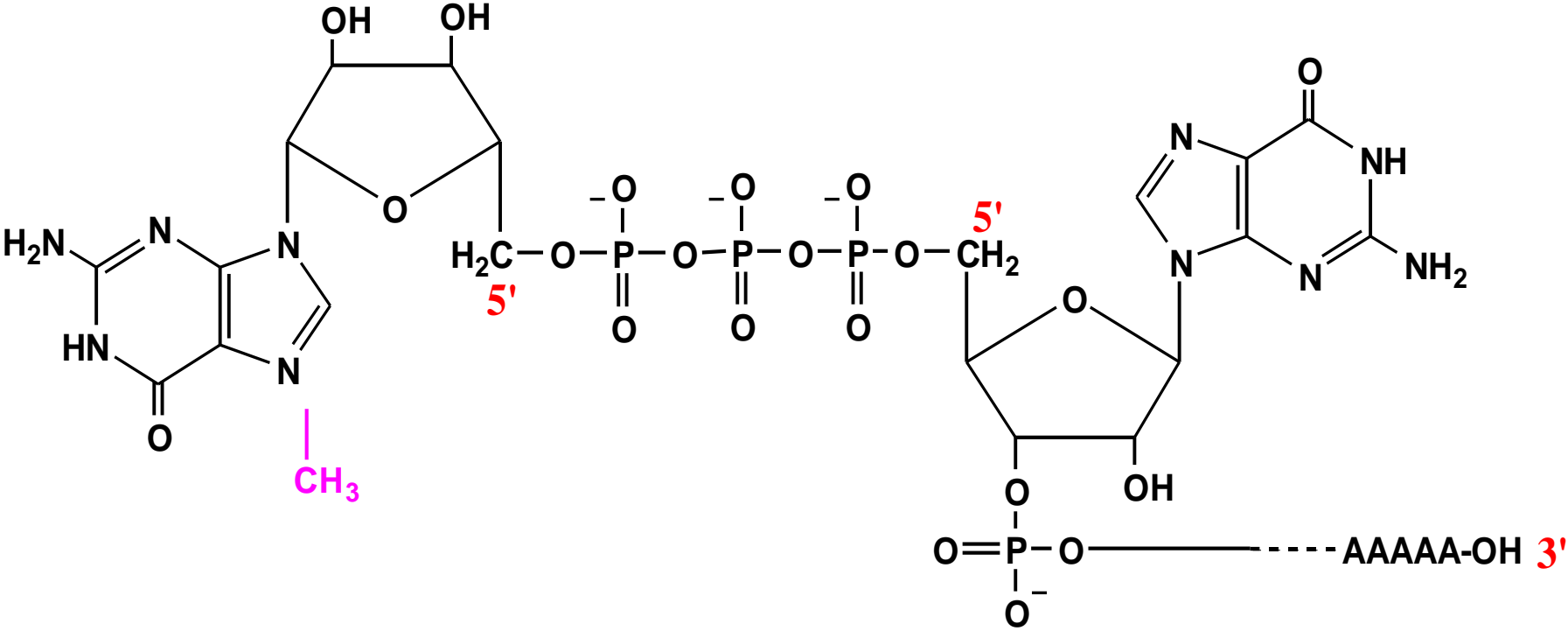
Post-Transcriptional Modification

- ▶ **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 hnRNA

- ▶ **Primary transcripts of mRNA are called as heteronuclear RNA (hnRNA).**
- ▶ **hnRNA are larger than matured mRNA by many folds.**
- ▶ **Modification includes**
 - **Capping at the 5'- end**
 - **Tailing at the 3'- end**
 - **mRNA splicing**
 - **RNA edition**

a. Capping at the 5'- end

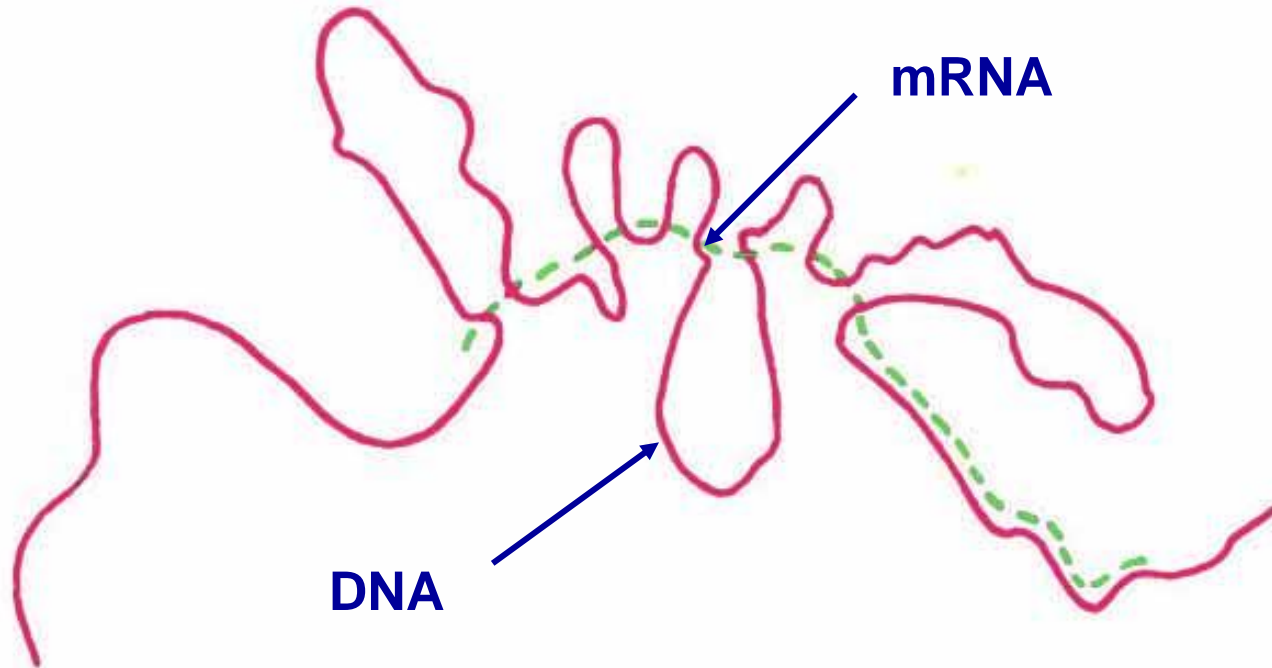


m⁷GpppGp----

b. Poly-A tailing at 3' - end

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

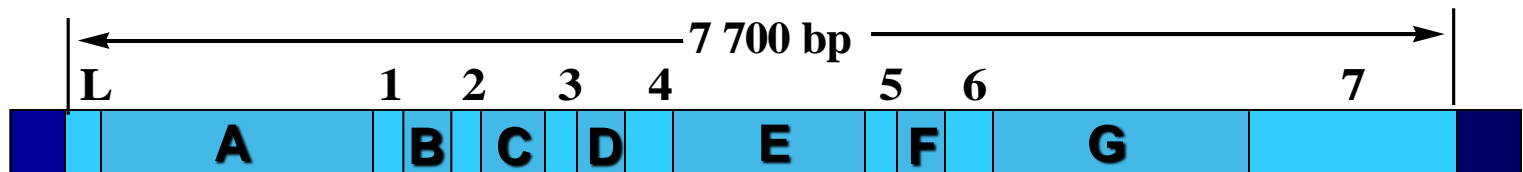
c. mRNA splicing



The matured mRNAs are much shorter than the DNA templates.

Split gene

The structural genes are composed of coding and non-coding regions that are alternatively separated.



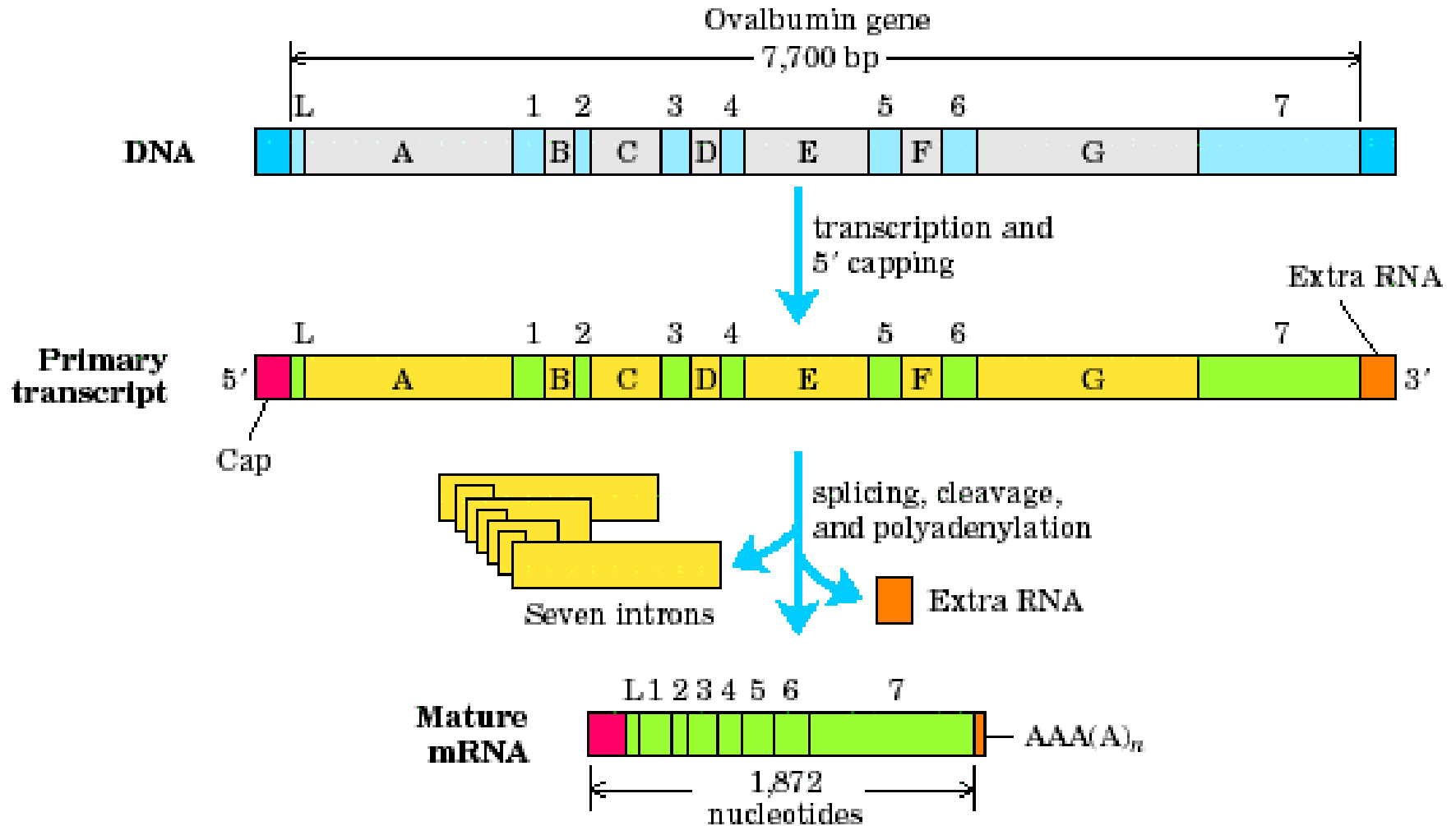
A~G no-coding region **1~7 coding region**

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 transcribed into primary mRNAs, and will be cleaved out in the later splicing process.

mRNA splicing



d. mRNA editing

- ▶ **Taking place at the transcription level**
- ▶ **One gene responsible for more than one proteins**
- ▶ **Significance: gene sequences, after post-transcriptional modification, can be multiple purpose differentiation.**