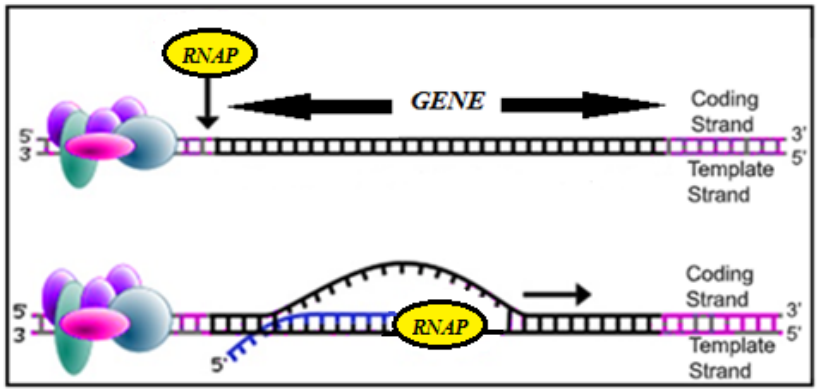
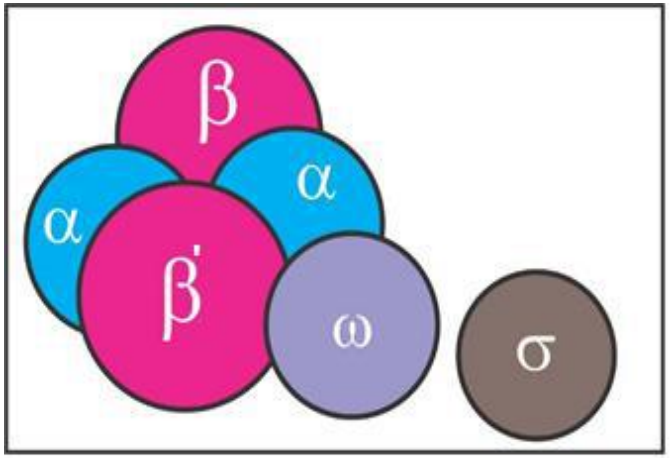
# TRANSCRIPTION

**Unit 3**
**Paper – Molecular Biology**
**B.Sc. (H) Microbiology, III Sem**

# Coding and Template strand during transcription in prokaryotic cell



# STRUCTURE




RNA polymerase: Core Enzyme + Sigma factor = Holoenzyme

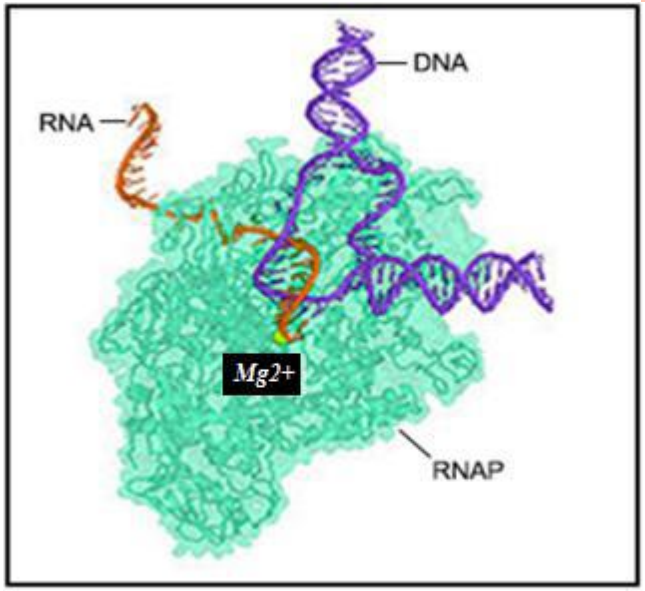
5 subunits

Core enzyme

2 α subunits, β, β'

σ---helps in recognition

Holoenzyme= Core + sigma

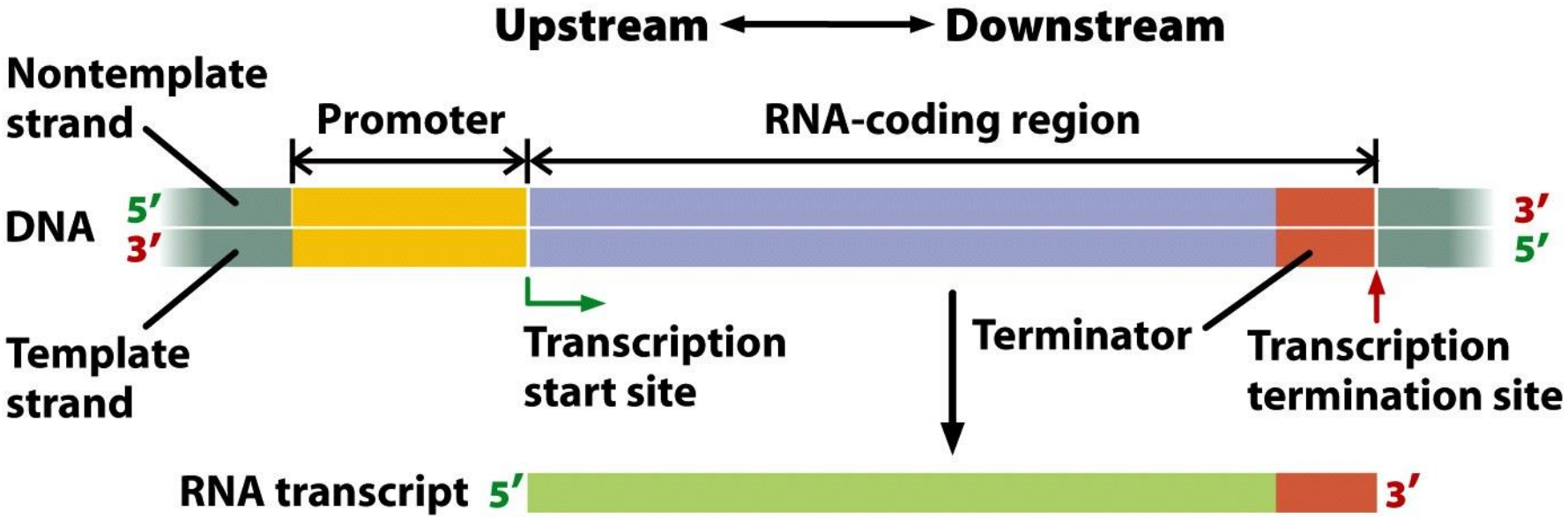



RNA Polymerase from *Thermus aquaticus* pictured during elongation.

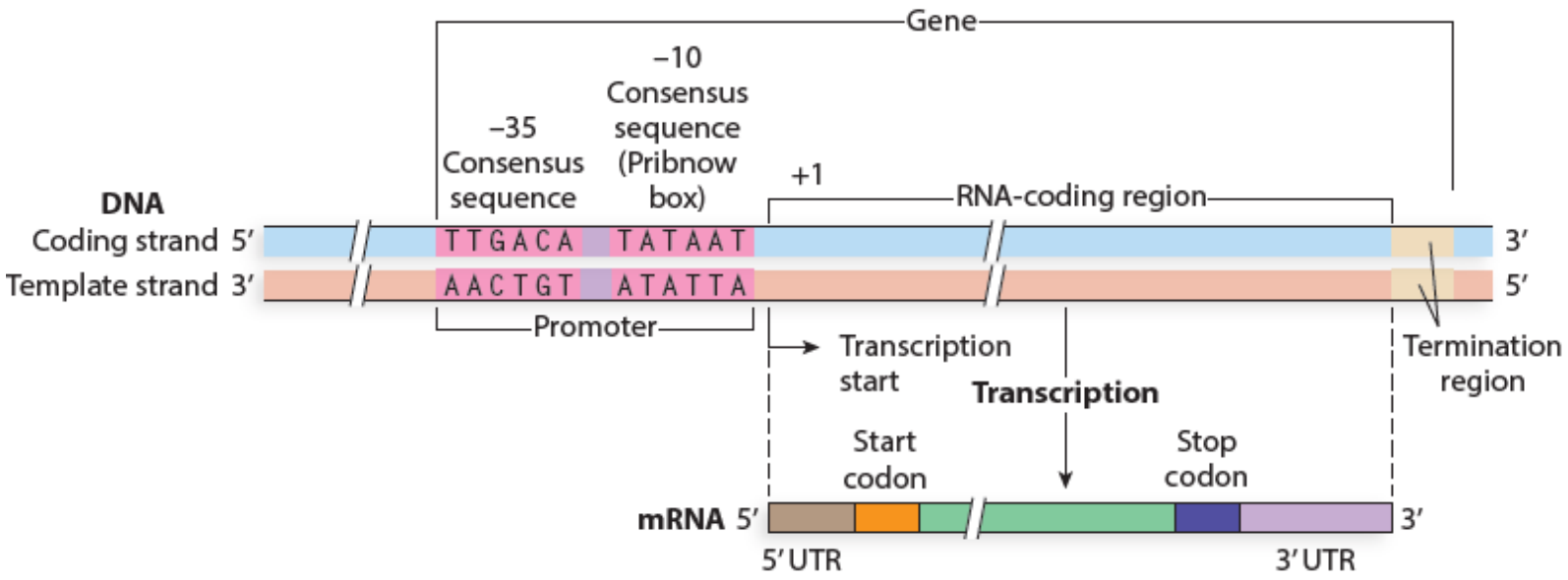
$Mg^{2+}$ at the active site

# RNA Polymerase features

- Starts at a promoter sequence, ends at termination signal
- Proceeds in 5’ to 3’ direction
- Forms a temporary DNA:RNA hybrid
- Has complete processivity


Upstream
Downstream
Nontemplate strand
Promoter
RNA-coding region
DNA
5′
3′
3′
5′
Template strand
Transcription start site
Terminator
Transcription termination site
RNA transcript
5′
3′

# Bacterial promoter structure




**Two promoter consensus sequences—the Pribnow box at -10 and the -35 sequence—are essential promoter regulatory elements.**

# Consensus sequences

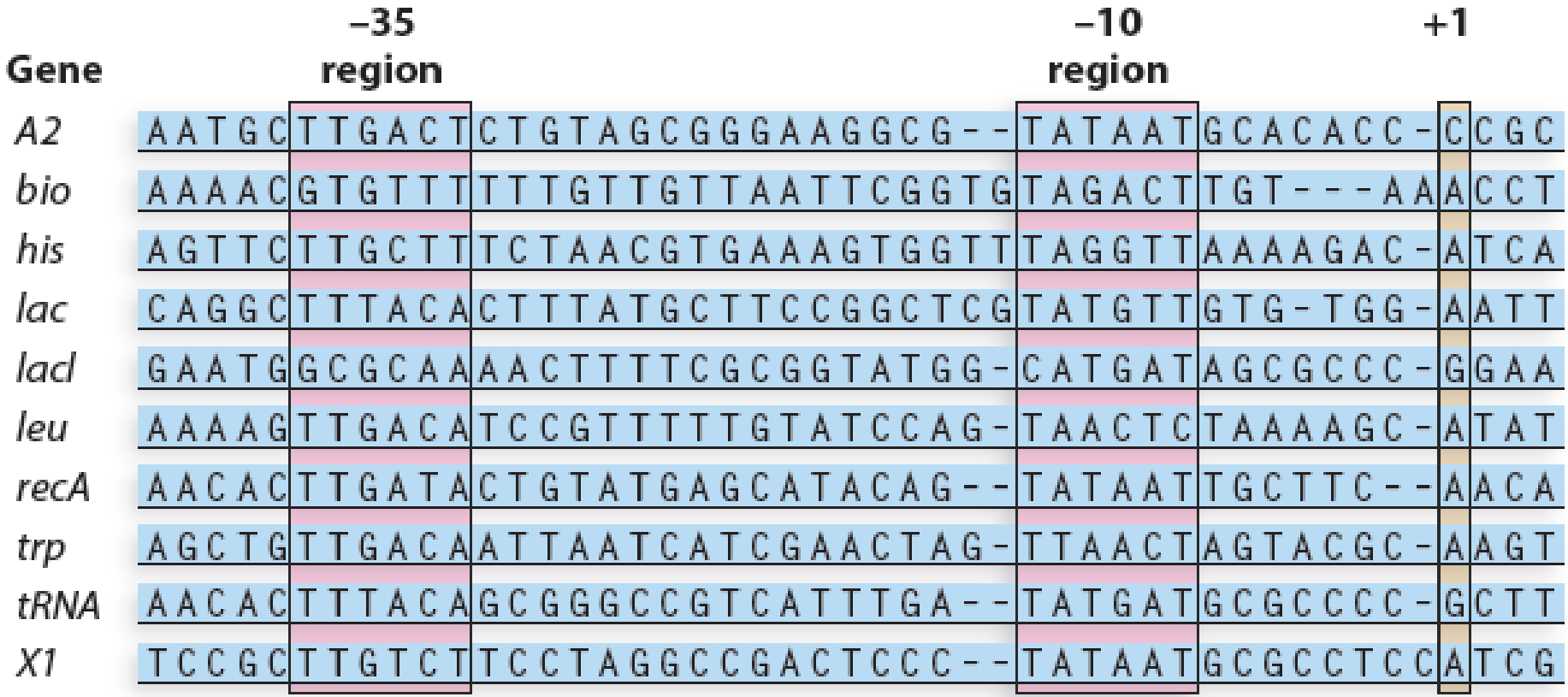
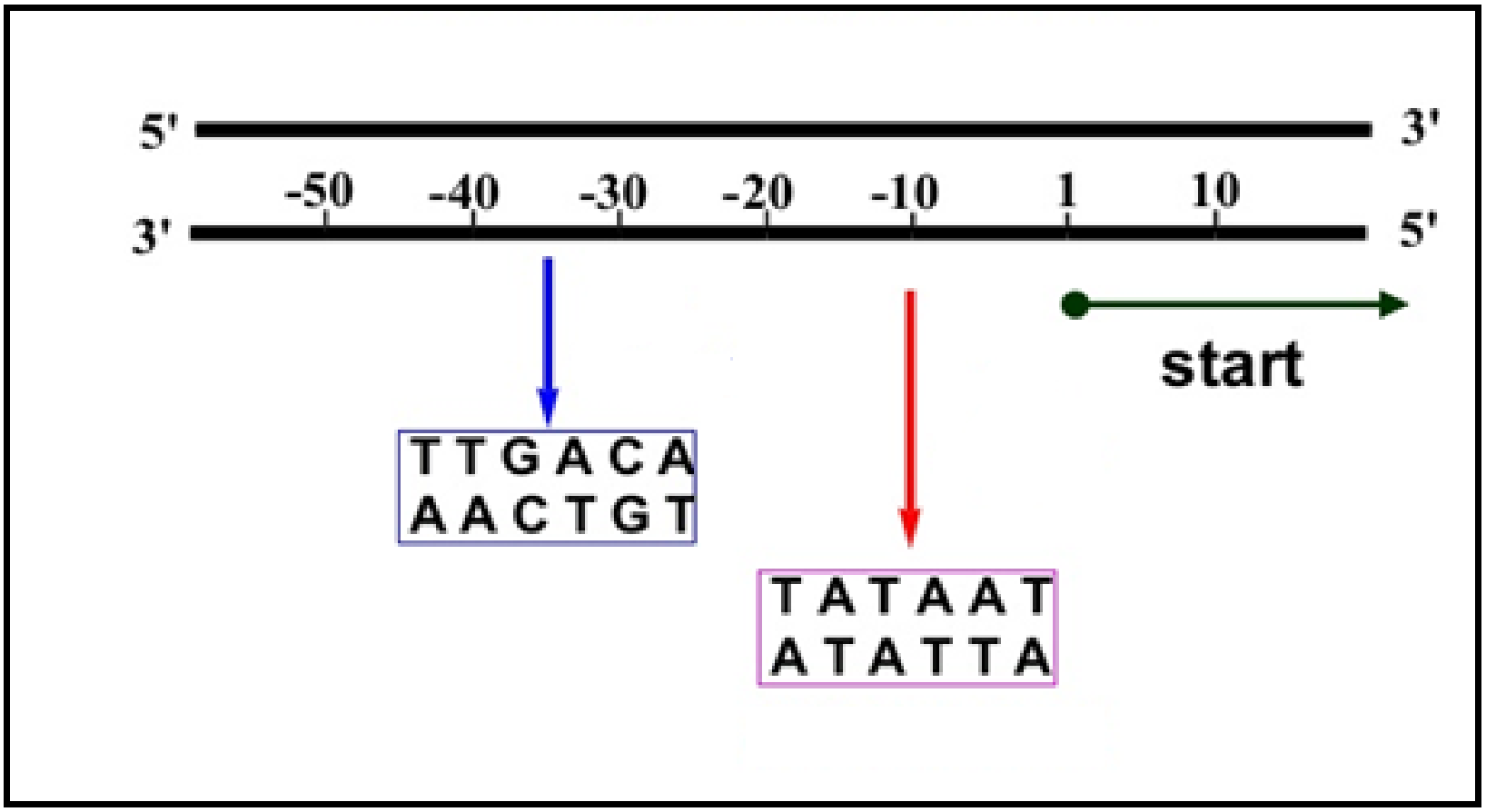


**Table 8.2** *Escherichia coli* RNA Polymerase Sigma Subunits

| Subunit | Molecular Weight (Daltons) | Consensus Sequence | | Function |
|---|---|---|---|---|
| | | −35 | −10 | |
| $\sigma^{28}$ | 28 | TAAA | GCCGATAA | Flagellar synthesis and chemotaxis |
| $\sigma^{32}$ | 32 | CTTGAA | CCCCATTA | Heat shock genes |
| $\sigma^{54}$ | 54 | CTGGPyAPyPu | TTGCA | Nitrogen metabolism |
| $\sigma^{70}$ | 70 | TTGACA | TATAAT | Housekeeping genes |

# Prokaryotic Promoter Sequence



# *Interesting Facts*

- *In 1959, **Severo Ochoa** won the Nobel Prize in Physiology/Medicine. Ochoa developed a process for synthesizing RNA in vitro using polynucleotide phosphorylase.*
- ***In 2006, Roger D. Kornberg** won the Nobel Prize in Chemistry. Kornberg was awarded for his studies of the molecular basis of eukaryotic transcription.*

# Stages of Transcription

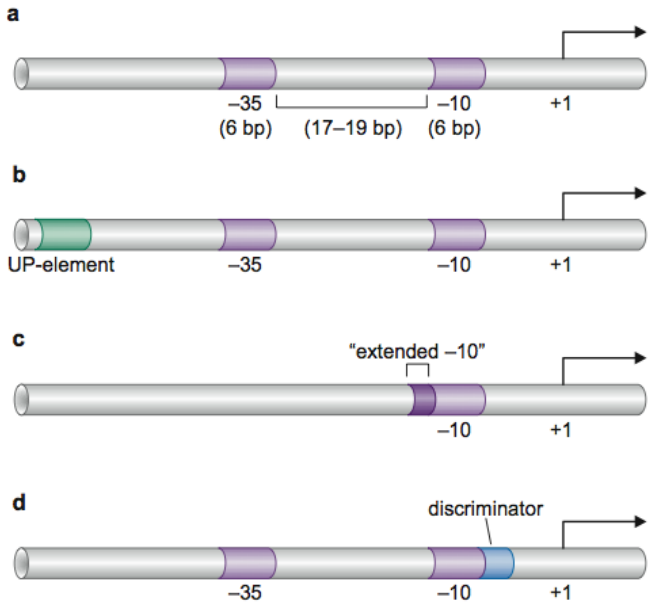
1. Template recognition
   - RNA pol binds to DNA
   - DNA unwound (a melted region of approximately 14 base pairs, called the *transcription bubble)*
2. Initiation
3. Elongation
   - RNA pol moves and synthesizes RNA
   - Unwound region moves
4. Termination
   - RNA pol reaches end
   - RNA pol and RNA released
   - DNA duplex reforms

# Transcription Initiation

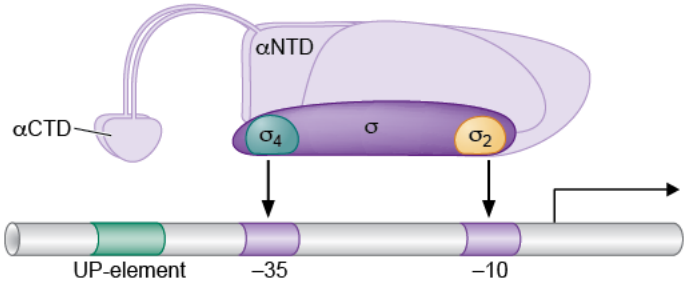
- Steps
  - Formation of **closed promoter (binary) complex**
  - Formation of **open promoter complex**
  - **Ternary complex** (RNA, DNA, and enzyme), abortive initiation
  - **Promoter clearance** (elongation ternary complex)!!! mechanism
    - First ribonucleotide becomes unpaired
    - Polymerase loses sigma
    - NusA binds
  - Ribonucleotides added to 3' end

# Bacterial promoters

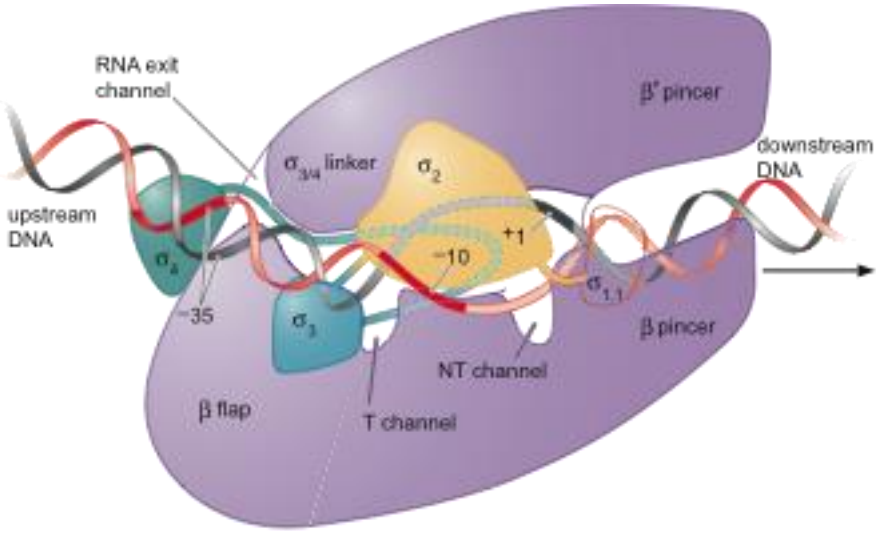
There are several flavors of promoters

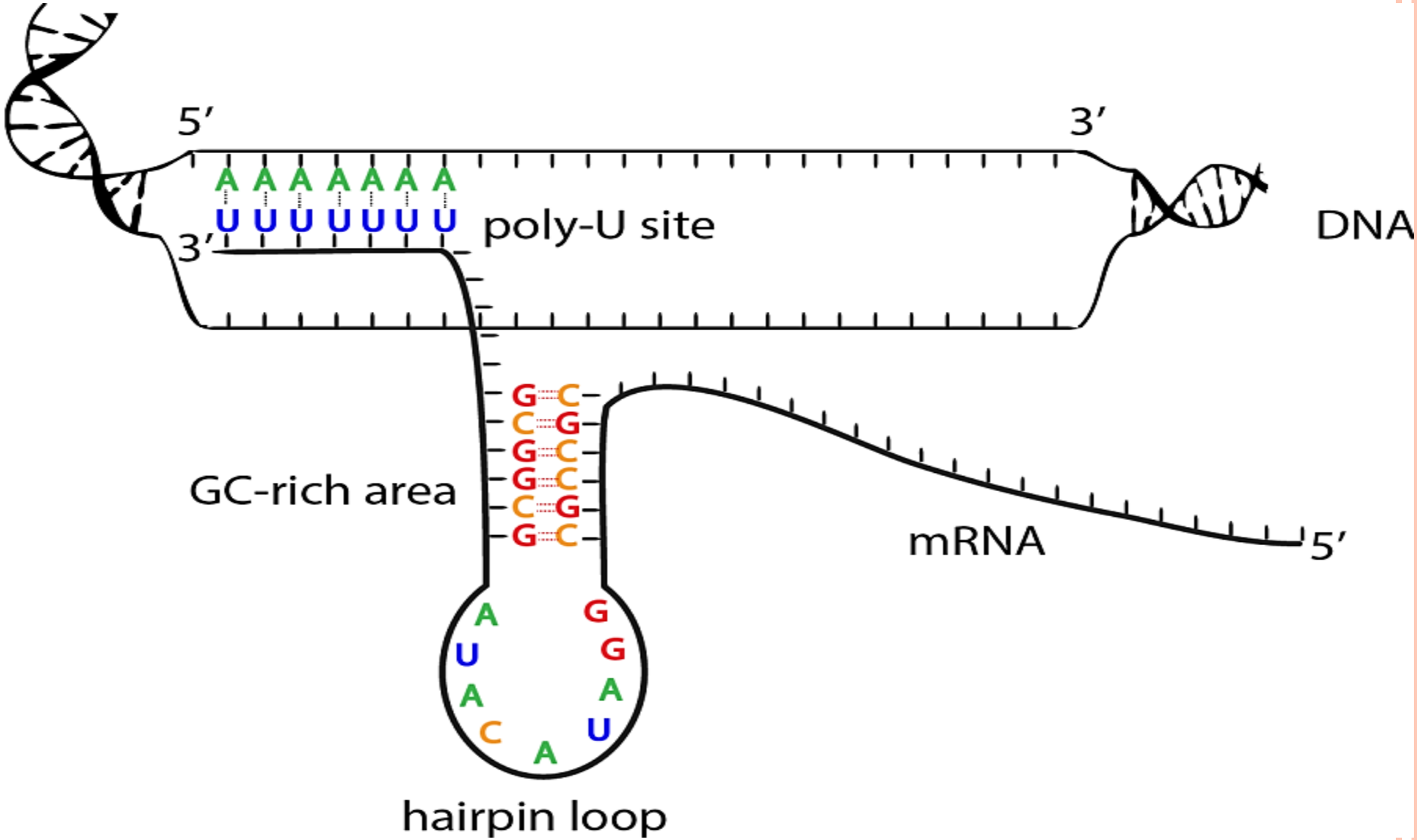



σ and α recruit RNAP to promoter DNA

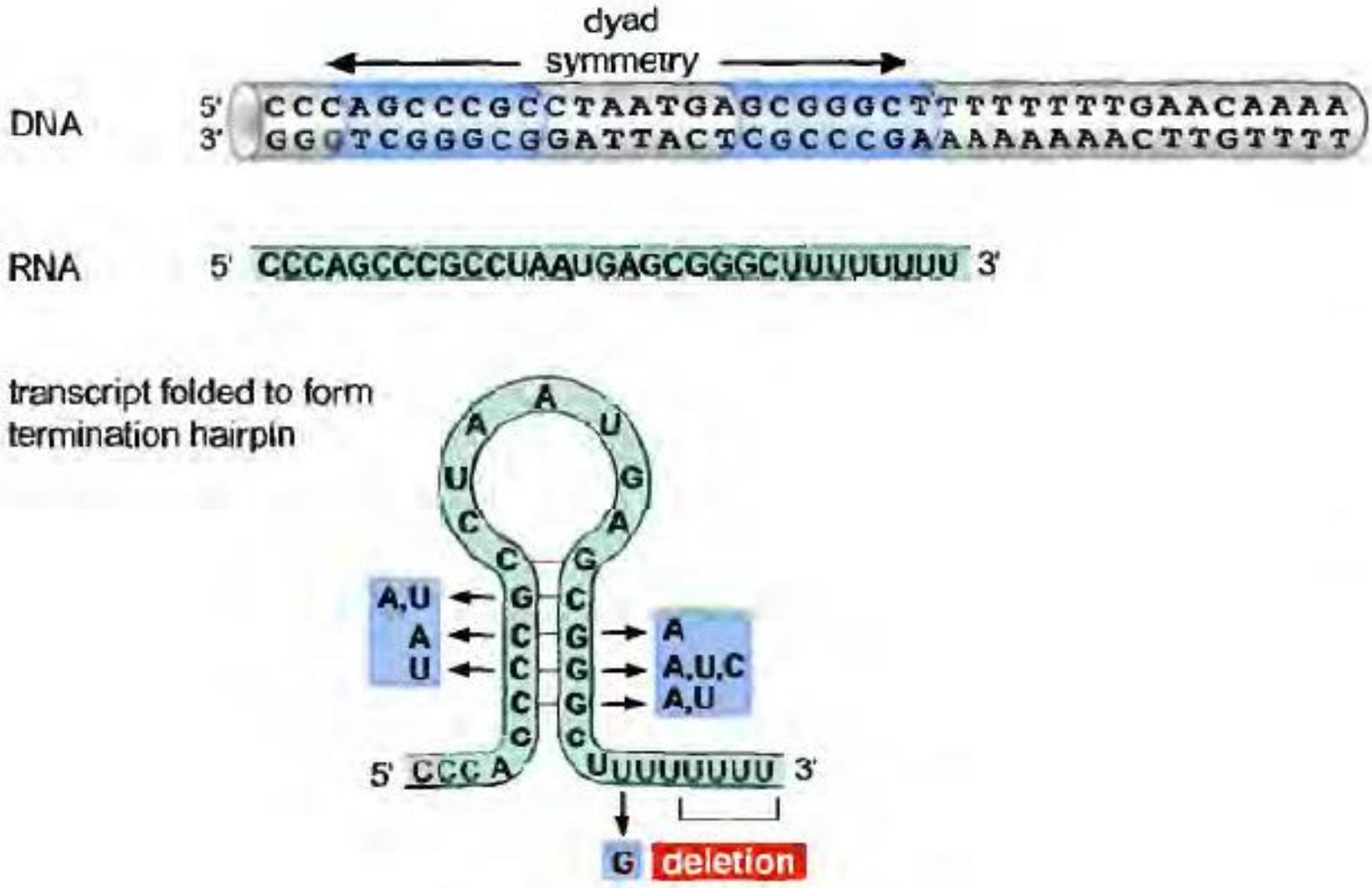


# SIGMA IS POSITIONED FOR DNA RECOGNITION

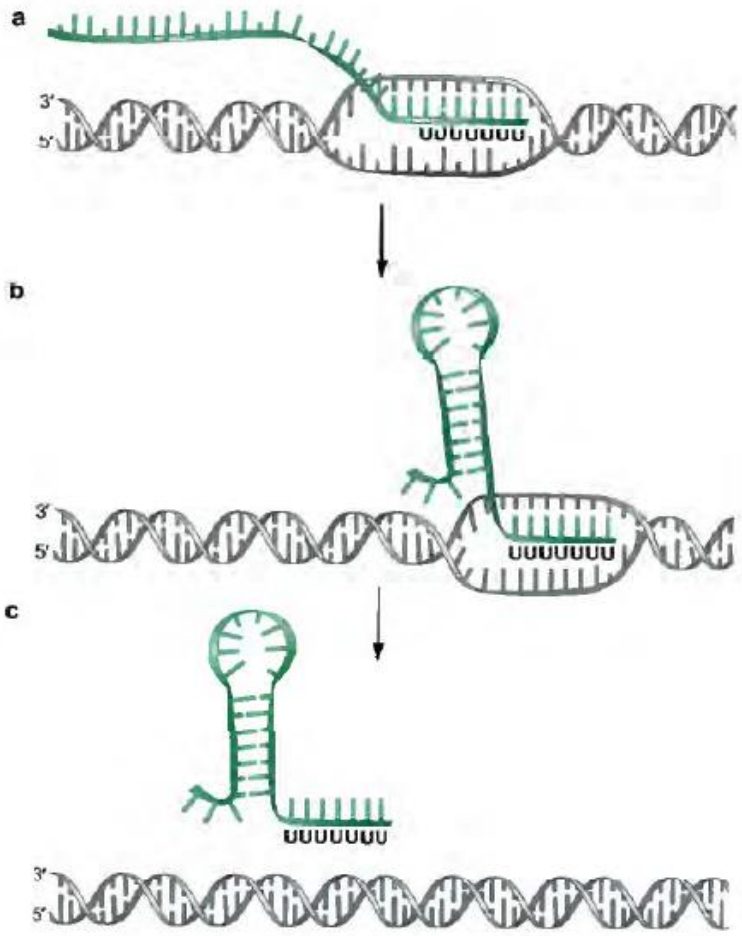



5′
3′
A A A A A A A
U U U U U U U
3′
poly-U site
DNA
G C
C G
G C
G C
C G
G C
GC-rich area
mRNA
5′
A
U
A
C
A
G
G
A
U
hairpin loop

# Sequence of Rho independent terminator



# Rho independent terminator

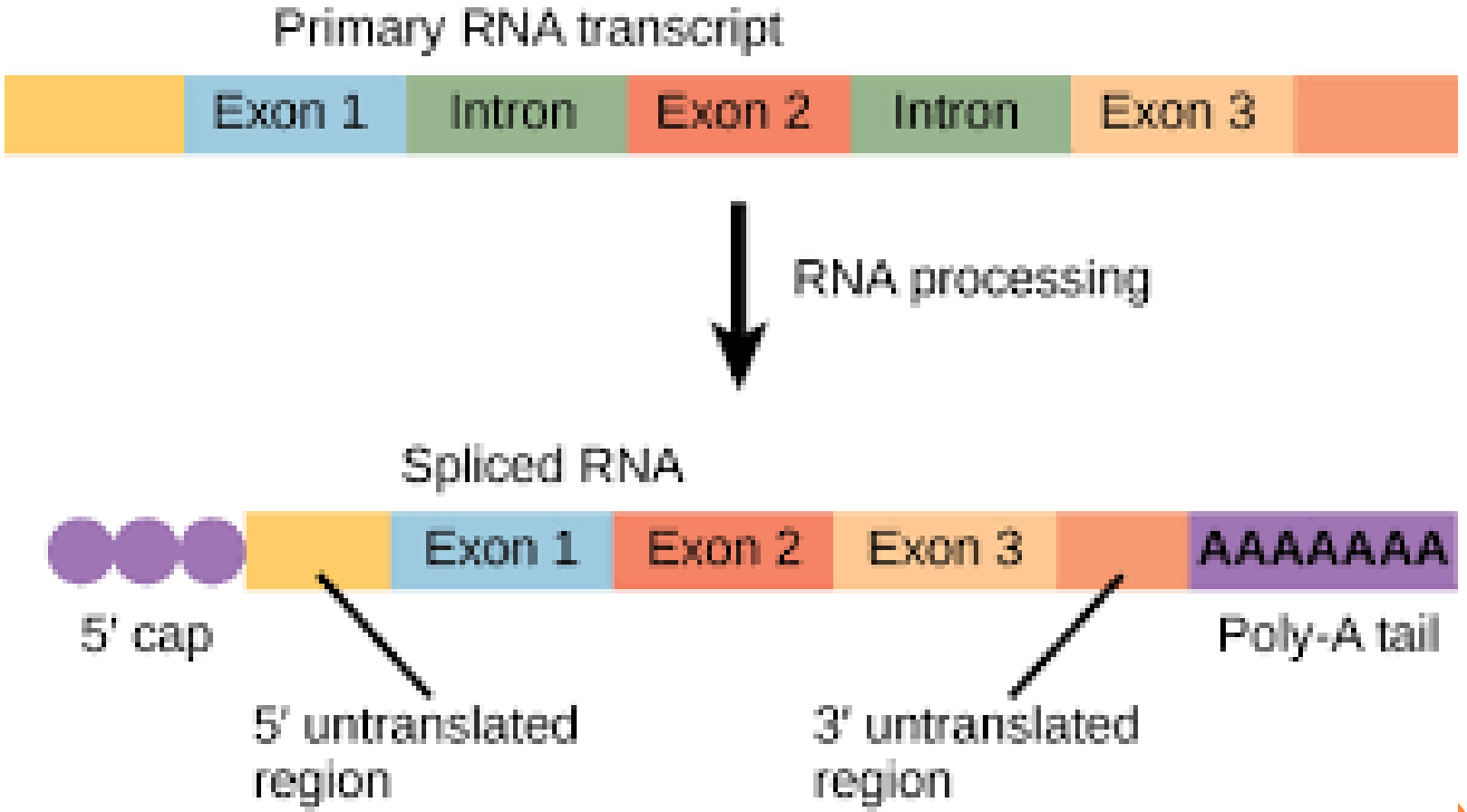


# Eukaryotic Transcription

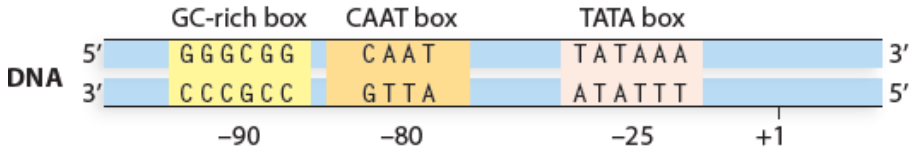
- Regulation very complex
- Three different polymerases
- Distinguished by α-amanitin  sensitivity
  - Pol I—rRNA, least sensitive
  - Pol II– mRNA, most sensitive
  - Pol III– tRNA and 5R RNA moderately sensitive
- Each polymerase recognizes a distinct promoter

# Eukaryotic RNA Polymerases

| RNA Pol. | Location | Products | α-Amanitin Sensitivity | Promoter |
|---|---|---|---|---|
| **I** | **Nucleolus** | **Large rRNAs (28S, 18S, 5.8S)** | **Insensitive** | **bipartite promoter** |
| **II** | **Nucleus** | **Pre-mRNA, some snRNAs** | **Highly sensitive** | **Upstream** |
| **III** | **Nucleus** | **tRNA, small rRNA (5S), snRNA** | **Intermediate sensitivity** | **Internal promoter and terminator** |

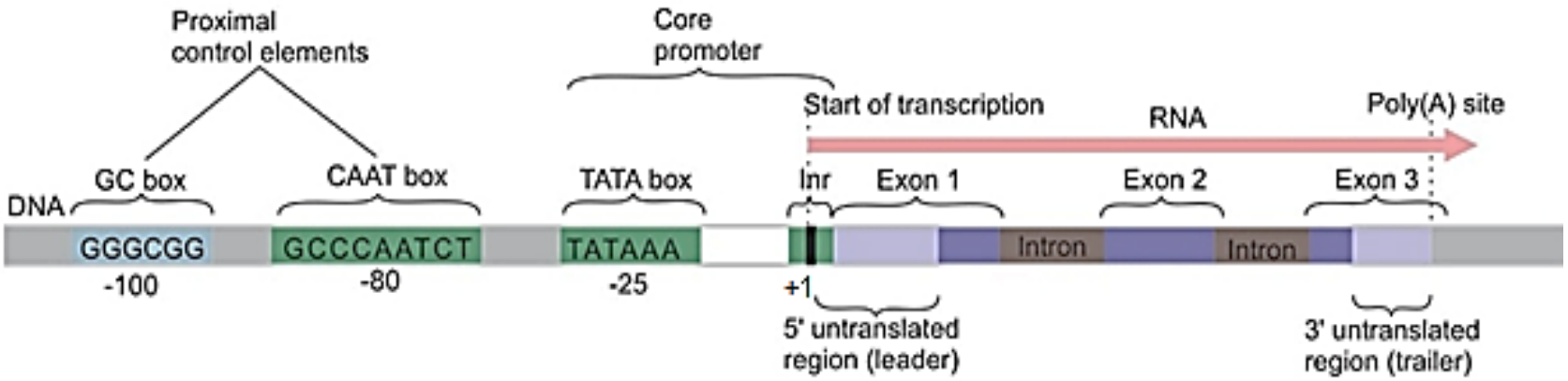

Primary RNA transcript
Exon 1
Intron
Exon 2
Intron
Exon 3
RNA processing
Spliced RNA
Exon 1
Exon 2
Exon 3
AAAAAAA
5' cap
Poly-A tail
5' untranslated region
3' untranslated region

# Eukaryotic promoter




Three eukaryotic promoter consensus sequence elements. The TATA box and the CAAT box are common; the presence of the upstream GC-rich box is more variable.

# Eukaryotic promoter



# Promoter elements