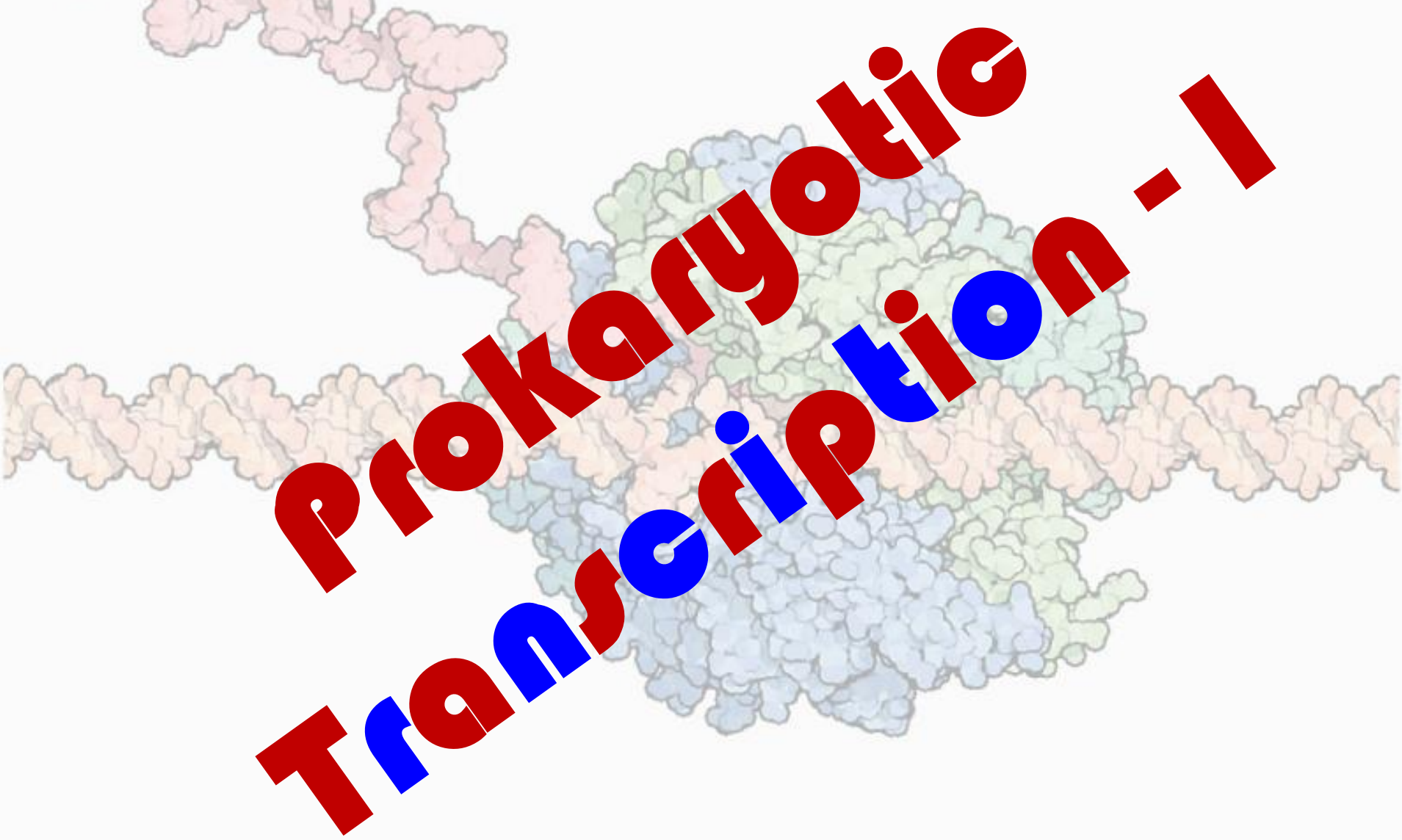
SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 205: Fundamentals of Molecular Biology (Unit II)

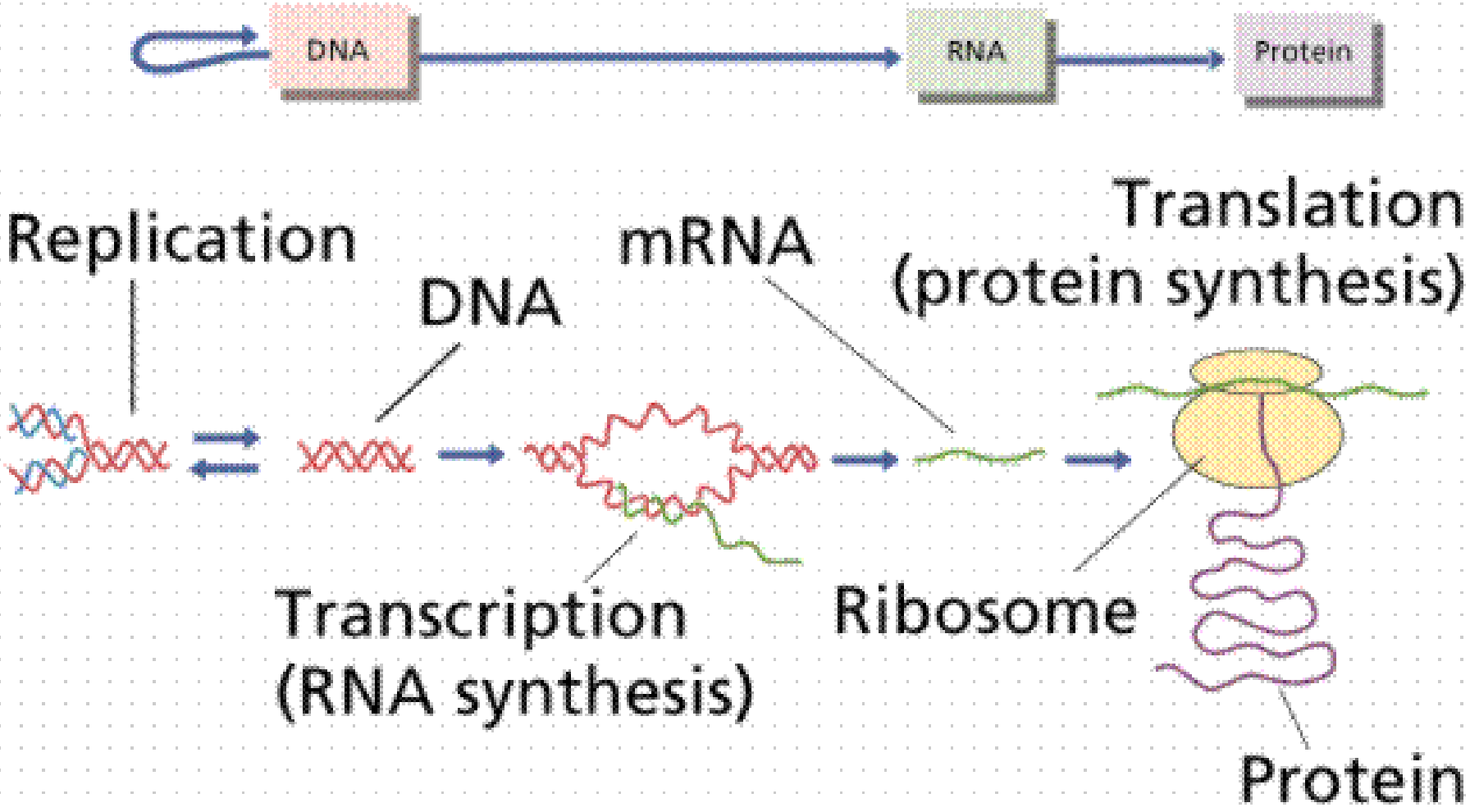


Prokaryotic Transcription - I

- **The information for making proteins is stored in DNA.**

- **There is a process (transcription and translation) by which DNA is converted to protein.**

- **By understanding this process and how it is regulated we can make predictions and models of cells.**


DNA
RNA
Protein
Replication
DNA
mRNA
Translation
(protein synthesis)
Transcription
(RNA synthesis)
Ribosome
Protein

Gene Expression

Gene expression refers to a process by which information(s) encoded in DNA structure is read out into a gene product (that may be a RNA or a protein).

Gene Expression

- **Gene differs from other forms of genetic information because they are expressed.**

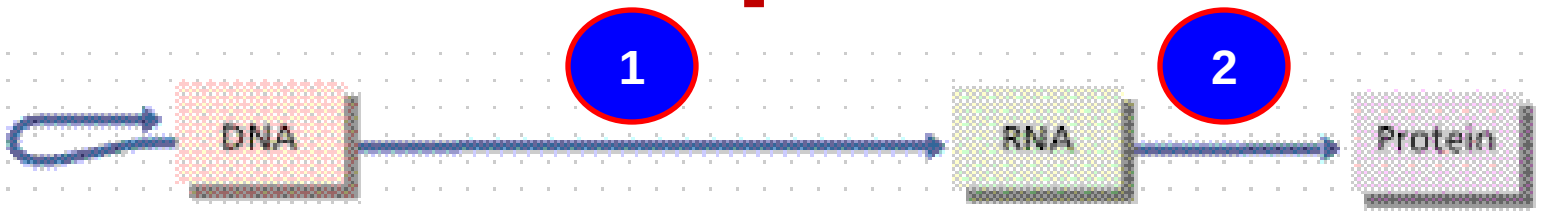
- **Gene expression involves taking the information from the gene and using it to generate a gene product which may be RNA or protein.**

- **In its simplest form, gene expression can be summarized by the Central Dogma of molecular biology, which states that genetic information flows unidirectionally from DNA through RNA to proteins.**

- **Only Gene found over the genome possess the capability to get expressed.**
- **Rest part of the genome plays more or less some role in regulation.**

Gene expression involves taking the information from the gene and using it to generate a gene product (RNA or protein).

Gene Expression



There are 4 major events that occur during the process of gene expression

- **Transcription - major**
  - **RNA processing (for fine tuning)**
- **Translation - major**
  - **Protein processing (for fine tuning)**

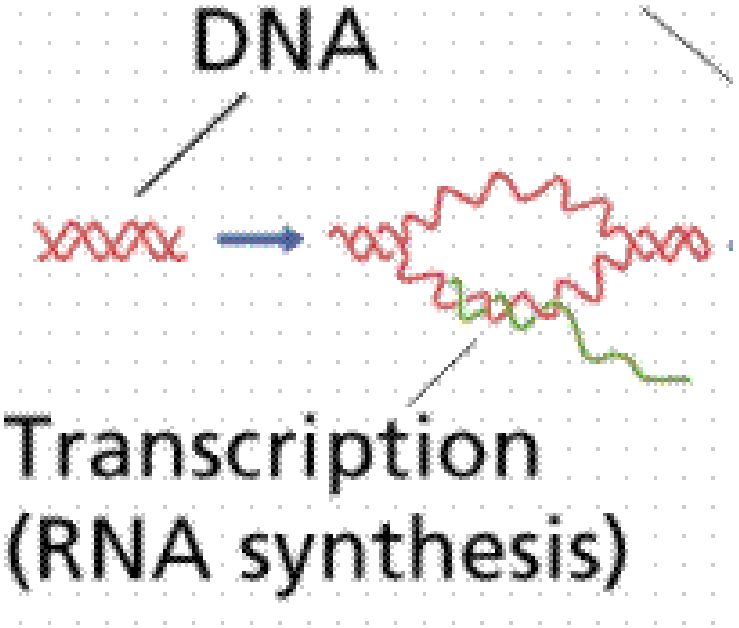
WHY THE PROCESS IS NAMED AS TRANSCTIPTION?

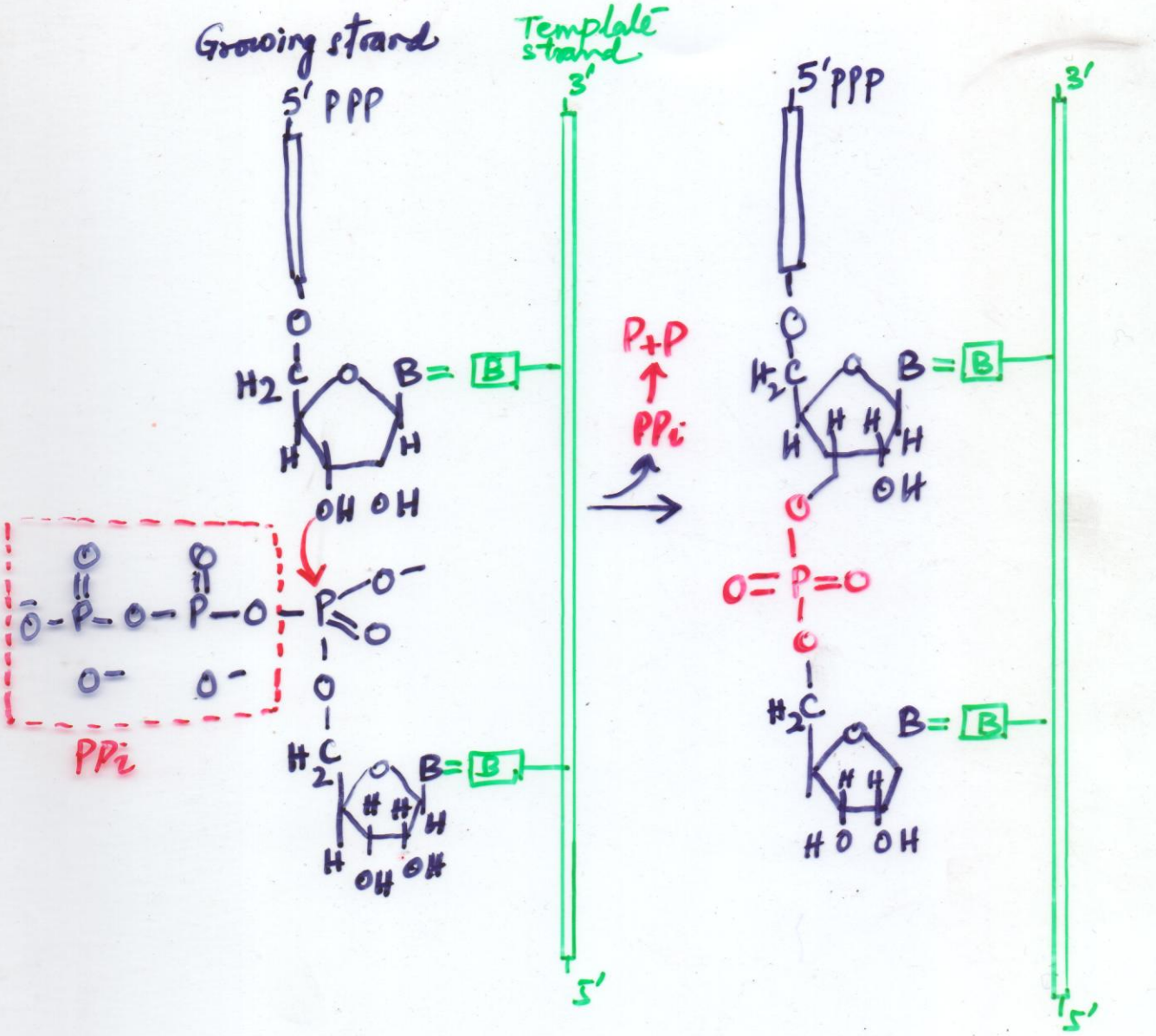
प्रतिलेखन

1.किसी आलेख, पत्र या पुस्तक आदि से कोई चीज़ ज्यों का त्यों उतारने की क्रिया या भाव

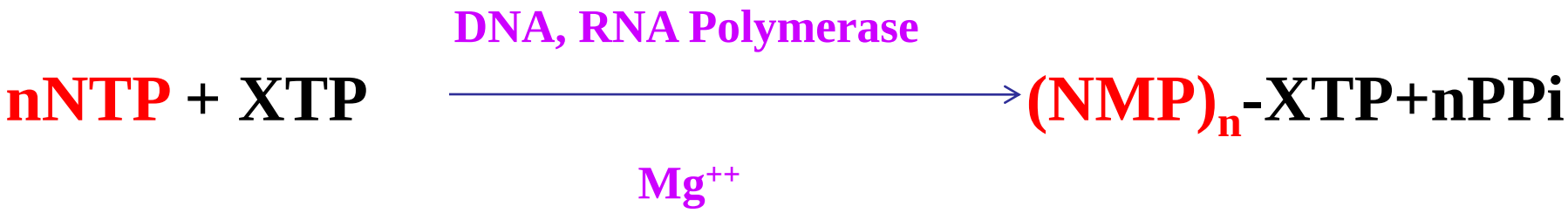
2. भाषण या संकेत लिपि में अंकित तथ्यों या टिप्पणियों के आधार पर पढ़ने योग्य लिखित प्रति तैयार करना; (ट्रांसक्रिप्शन)।

BASIC FEATURES





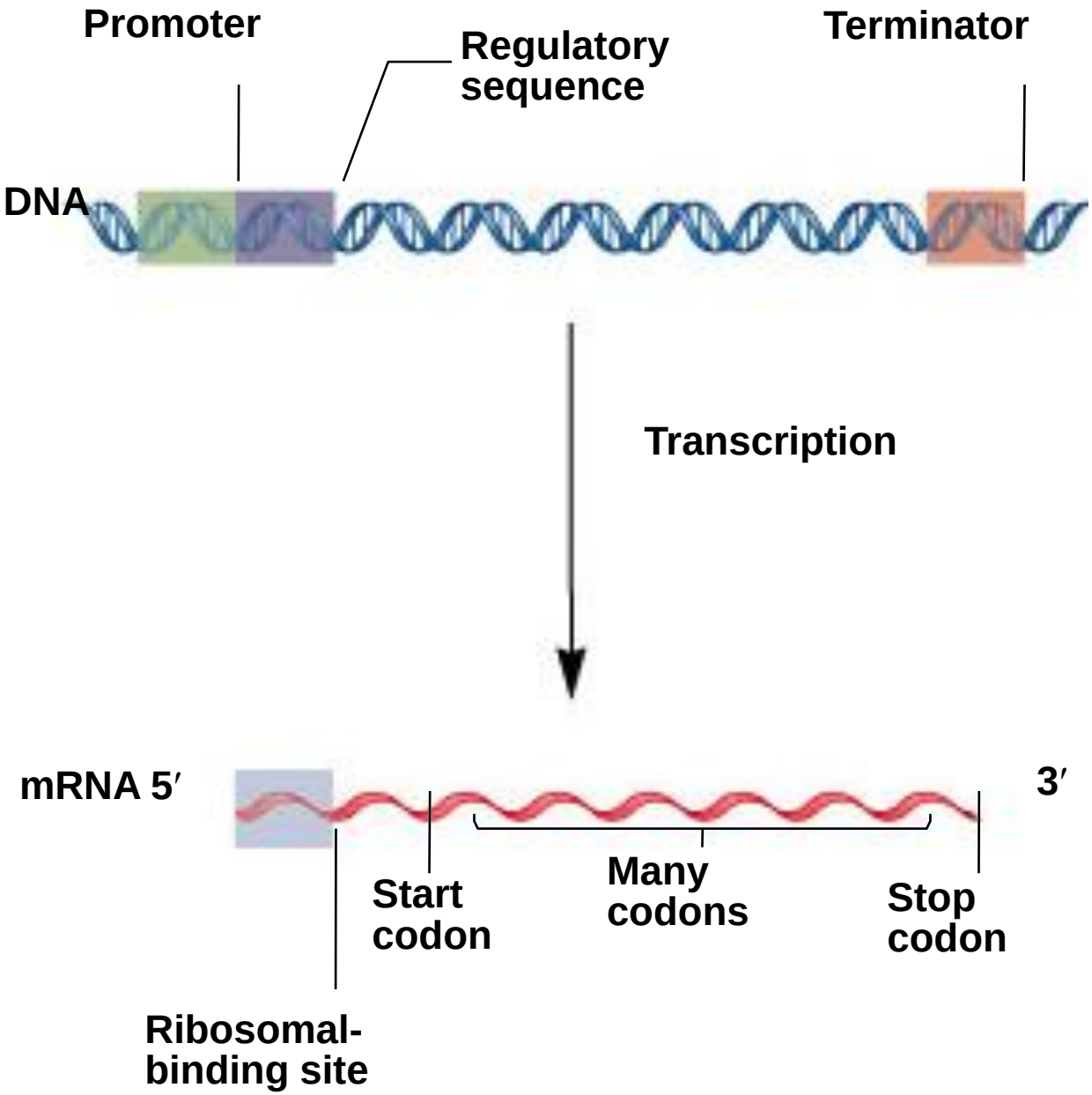
Mechanism of Chain Elongation reaction catalyzed by RNA polymerase.


DNA, RNA Polymerase
nNTP + XTP
(NMP)n-XTP+nPPi
Mg++

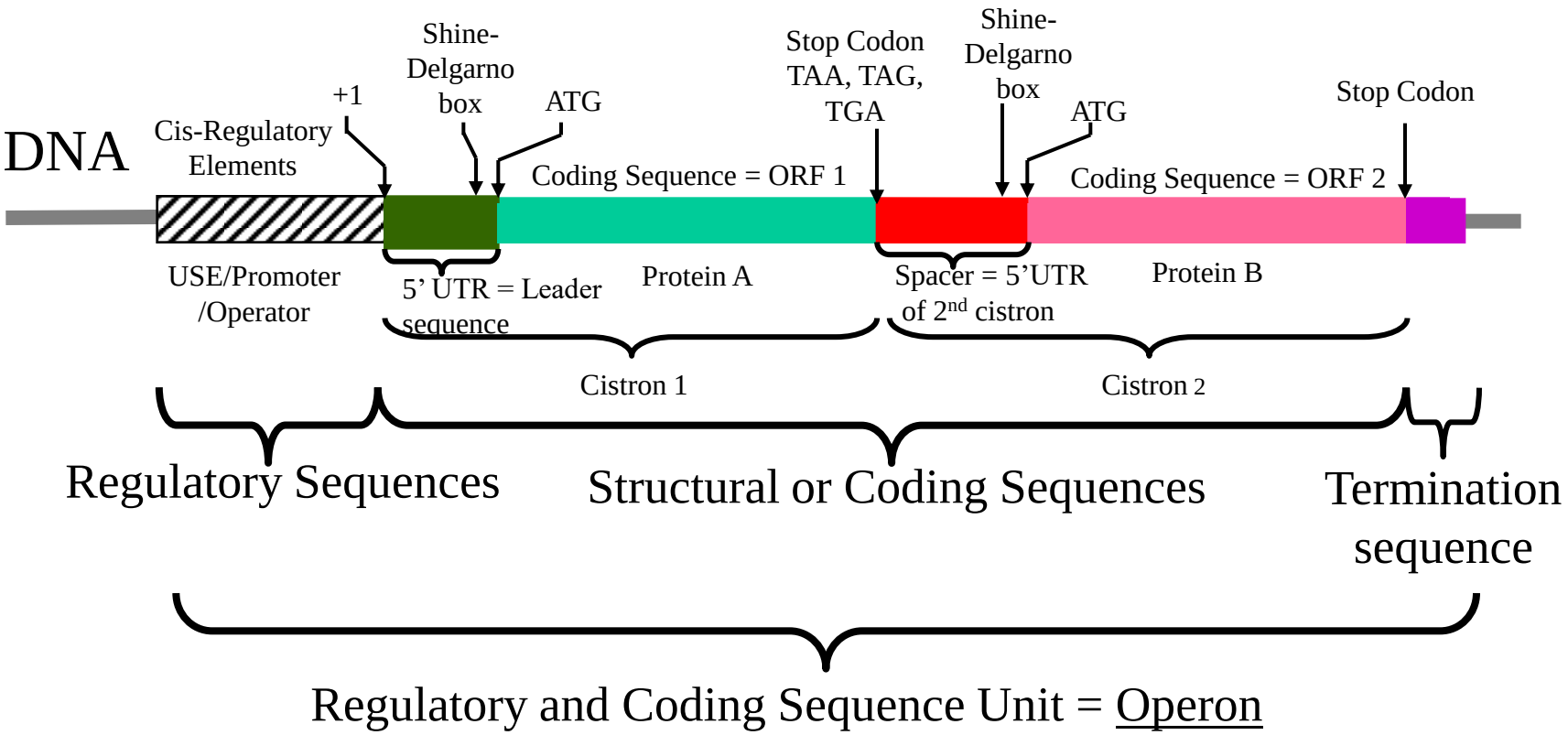
Gene

Gene = Transcription Unit + Regulatory Sequences

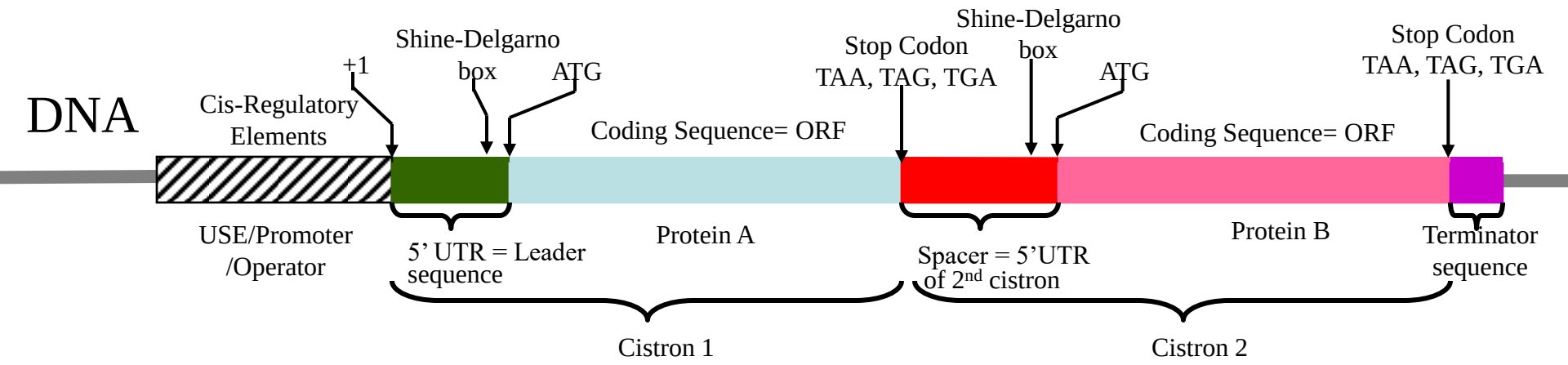
A Gene is a Transcription Unit



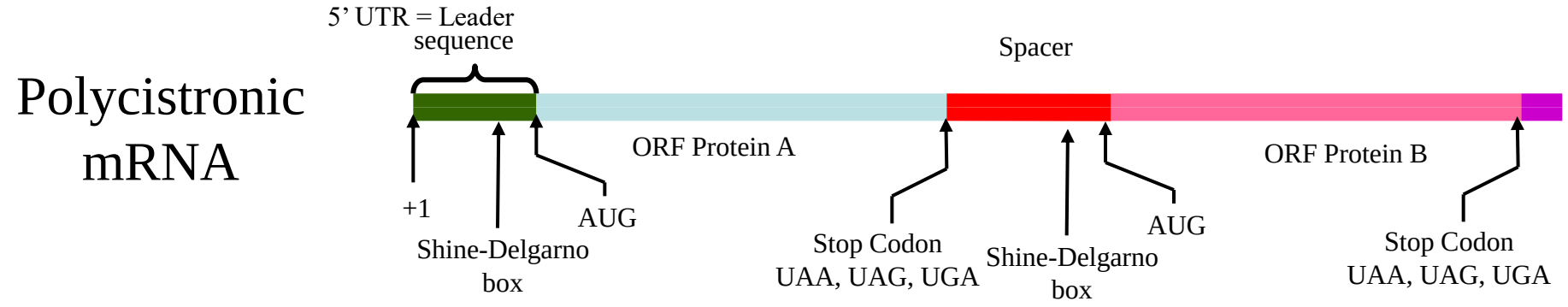
Prokaryotic Gene Structure



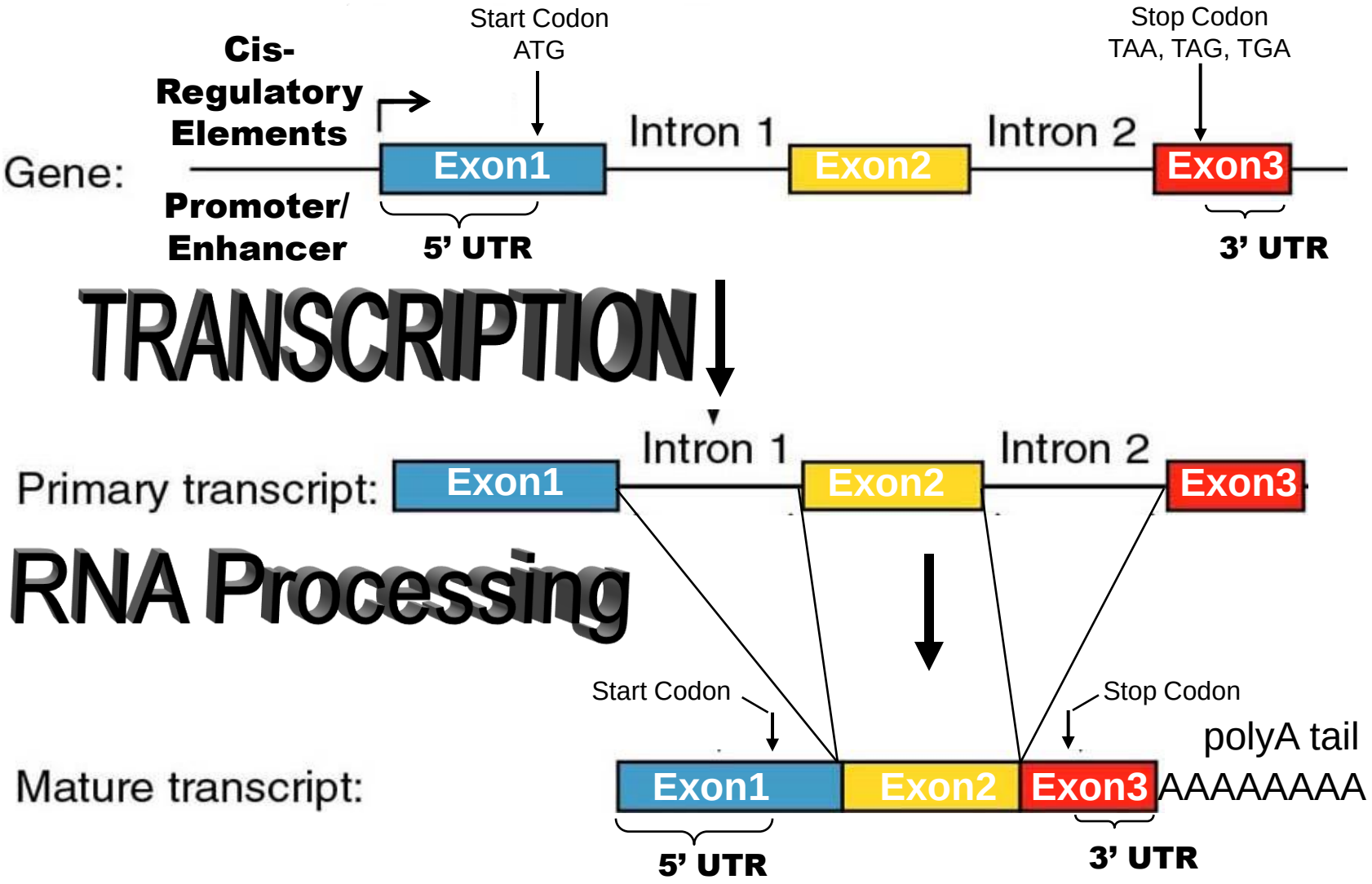
Prokaryotic Gene Structure



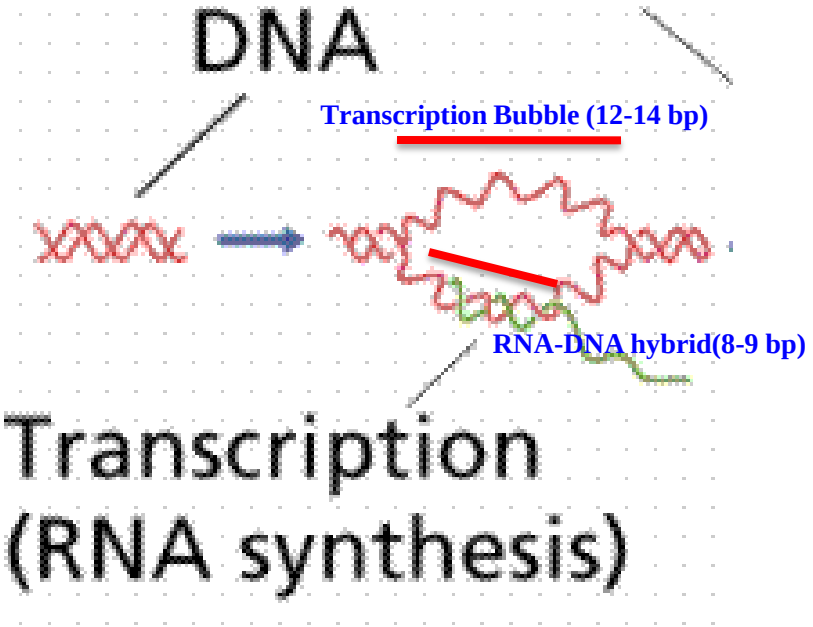
TRANSCRIPTION



Eukaryotic Gene Structure

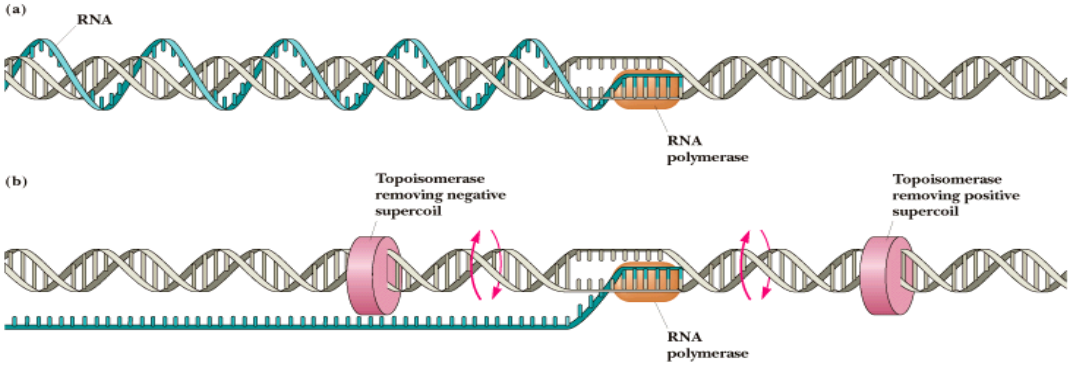


STEPS OF TRANSCRIPTION



STEPS

1. **Binding of RNA Polymerase to DNA template to a specific site (Promoter)**
2. **Initiation of RNA synthesis**
3. **RNA chain elongation**
4. **Chain termination & release from the machinery**



Remember!!!!!!!

Transcription unit extends from the promoter to the terminator.

A transcription unit may encode more than one gene.

RNA Polymerases

Bacterial RNA Polymerases

RNA Polymerases

Only one polymerase in bacteria (transcribes all types of bacterial RNA)

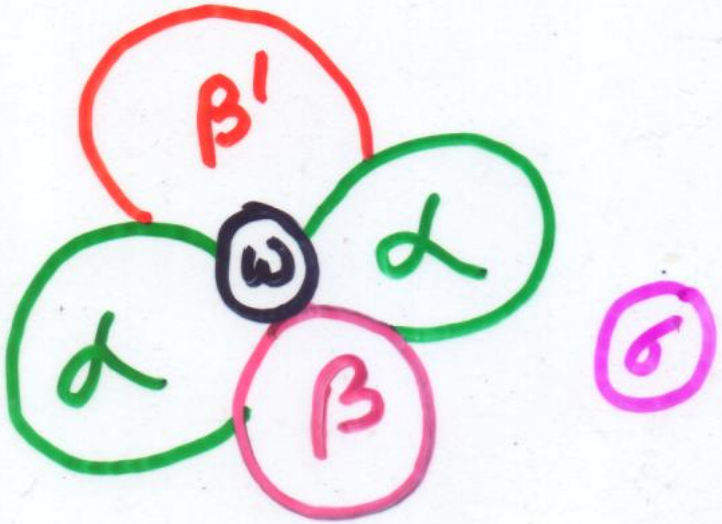
Three types in eukaryotes

RNAP I – transcribes 28S, 18S an 5.8S rRNA

RNAP II – transcribes mRNA & snRNA

RNAP III – transcribes tRNA & 5S rRNA

Bacterial RNA Polymerase



~90 × 95 × 160 Å

$\alpha_2 \beta \beta' \sigma^{70} \rightleftharpoons \alpha_2 \beta \beta' + \sigma^{70}$

Holoenzyme (~460 kD)

Core Polymerase (~400 kD)

Sigma-70 (70kD)

Subunit/protein	Gene Name	Map position	MW (kd)	# of Enzyme	Function	Properties
β'	rpoC	90	155	1	DNA Binding	Basic
β	rpoB	90	151	1	Active site (nucleotide Binding)	Acidic
α	rpoA	73	36.5	2	Enzyme assembly; interacts with some regulatory proteins	"
ω	rpoZ	?	10	1	? Enzyme assembly	"
σ^{70}	rpoD	67	70	1	Promoter recognition and initiation	"
σ^{54}	ntrA	70	54	1	"	"
σ^{32}	htpR	76	32	1	"	"

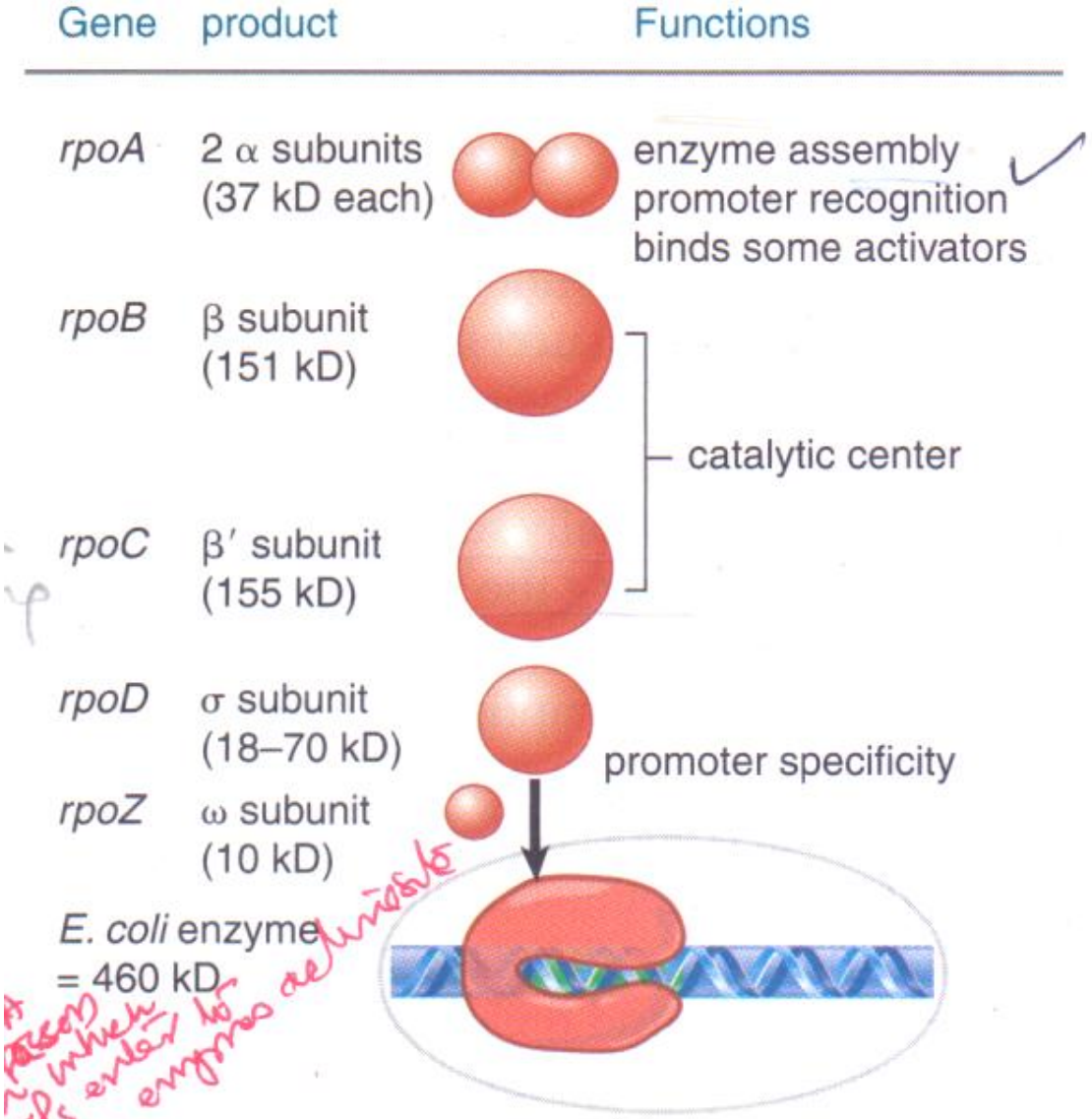
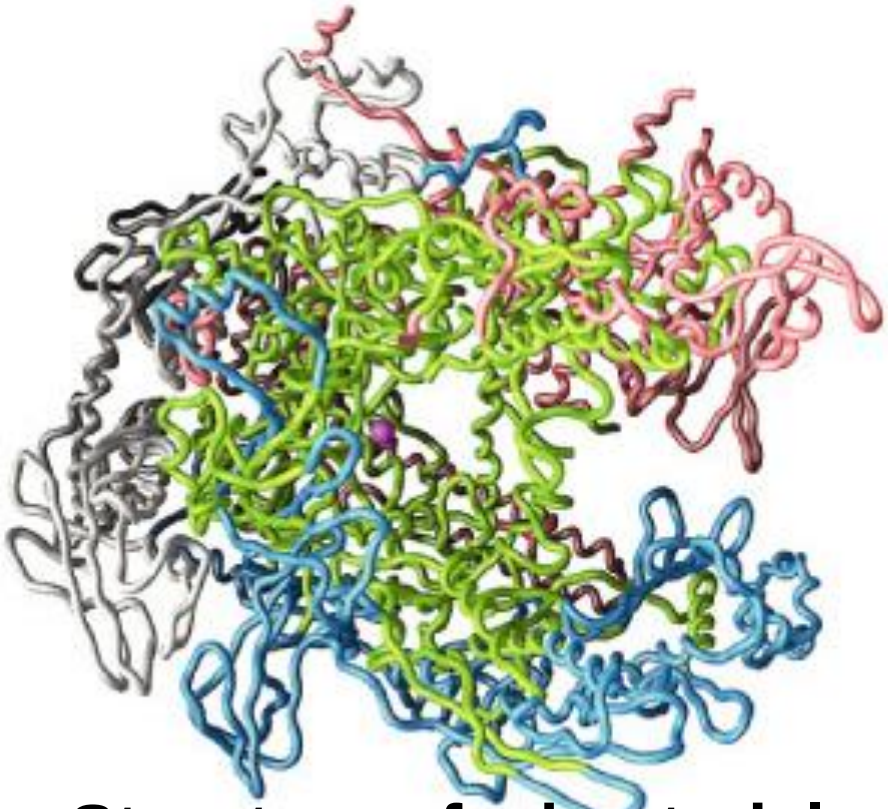


FIGURE 19.7 Eubacterial RNA polymerases have five types of subunits: α, β, β′ and ω have rather constant sizes in different bacterial species, but σ varies more widely.

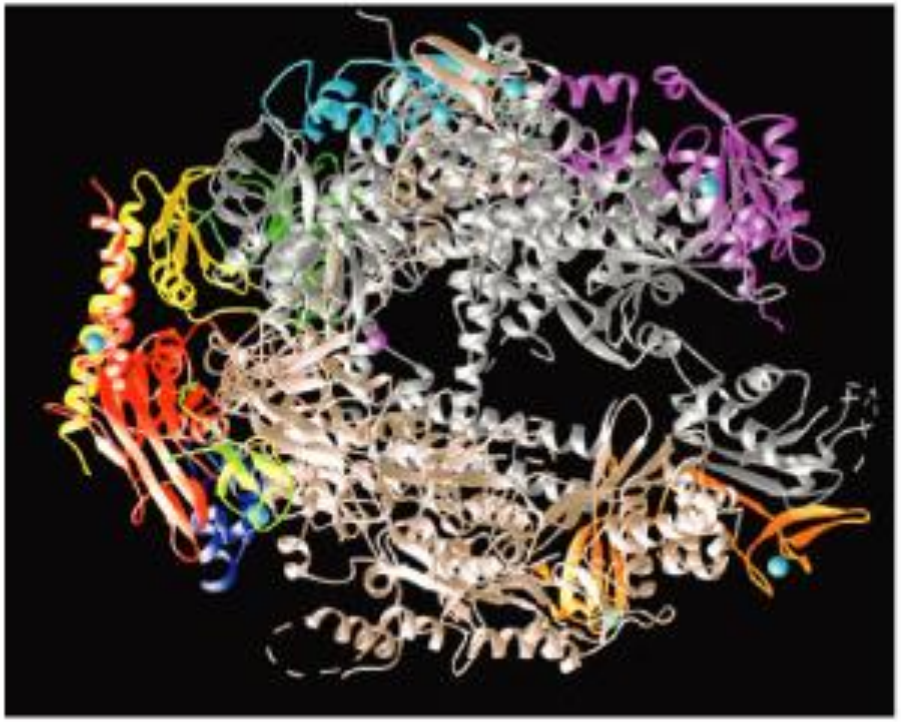
RNA Polymerases

- Differences between eukaryotes & prokaryotes
- Prokaryotes
  - 1 enzyme with 4 subunits
    - 2 α's, 1 β, & 1 β'
    - actual polymerase function
  - Sigma factors (σ)
    - recognize & bind promoter DNA sequence
- Eukaryotes
  - 3 separate holoenzymes – each has ~12 subunits
    - RNA Pol I – 28S, 18S, 5.8S rRNA
    - RNA Pol II – mRNA, snRNA
    - RNA Pol III – tRNA, 5S rRNA
  - 3 sets of basal transcription factors
    - recognize promoter DNA sequences

RNA Polymerases



Structure of a bacterial RNA polymerase



Structure of a eukaryotic RNA polymerase II

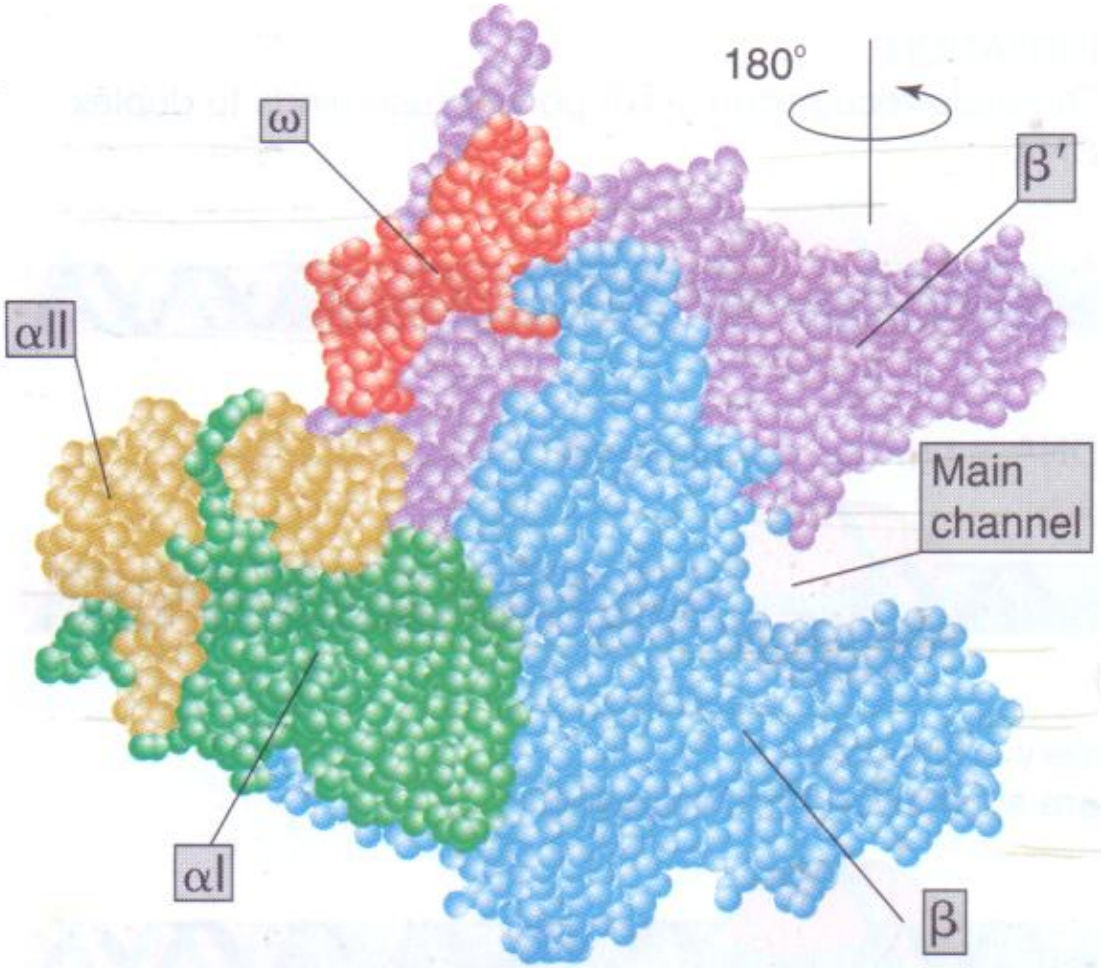
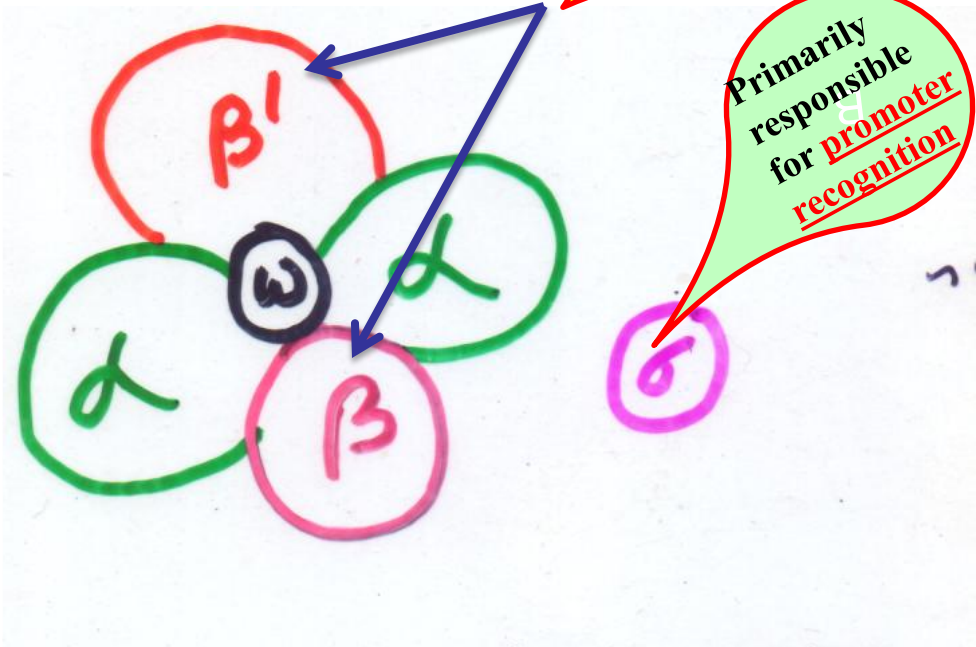


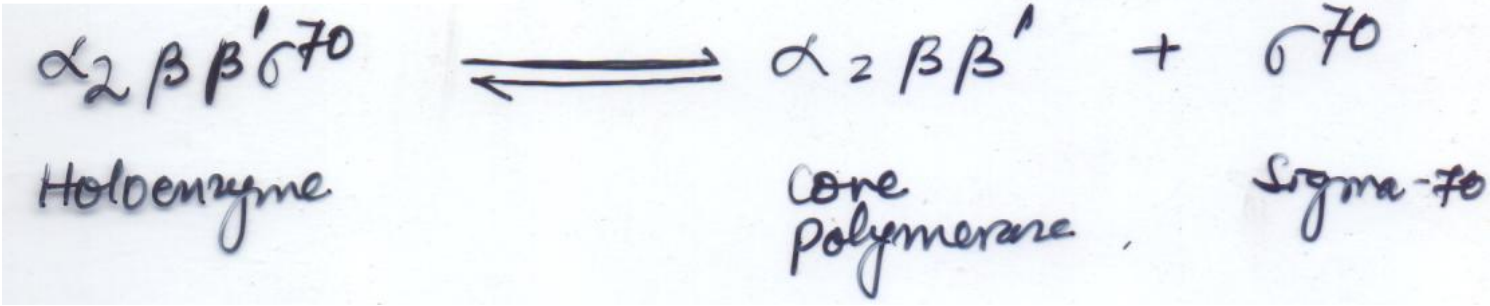
FIGURE 19.8 The upstream face of the core RNA polymerase, illustrating the 'crabclaw' shape of the enzyme. β (cyan) and β′ subunit (pink) of RNA polymerase have a channel for the DNA template. α I is shown in green and α II in yellow, ω is red. Adapted from K. M. Geszvain and R. Landick (ed. N. P. Higgins). *The Bacterial Chromosome*. American Society for Microbiology, 2004.

1. **Main Channel, through which DNA passes**
2. **Secondary Channel, through which rNTPs enter to enzyme on their path to the active site**
3. **Exit Channel, through which nascent RNA leaves enzyme**

Bacterial RNA Polymerase

1. **~13,000 RNA polymerase molecules are present per *E. coli* cell.**
2. **Although the precise number varies with the growth conditions.**
3. **Not all RNA polymerases are actually engaged in transcription at any given time.**
4. **Almost all are bound either specifically or non-specifically to DNA.**
5. **A very little fraction remain in cytosol.**





(~460 kD) **(~400 kD)** **(70kD)**

SIGMA (σ) FACTOR

Dual Functions:

1. **Reduces the affinity of holoenzyme for non-specific DNA & increases its affinity for specific DNA (promoter)**
2. **In accurate initiation**

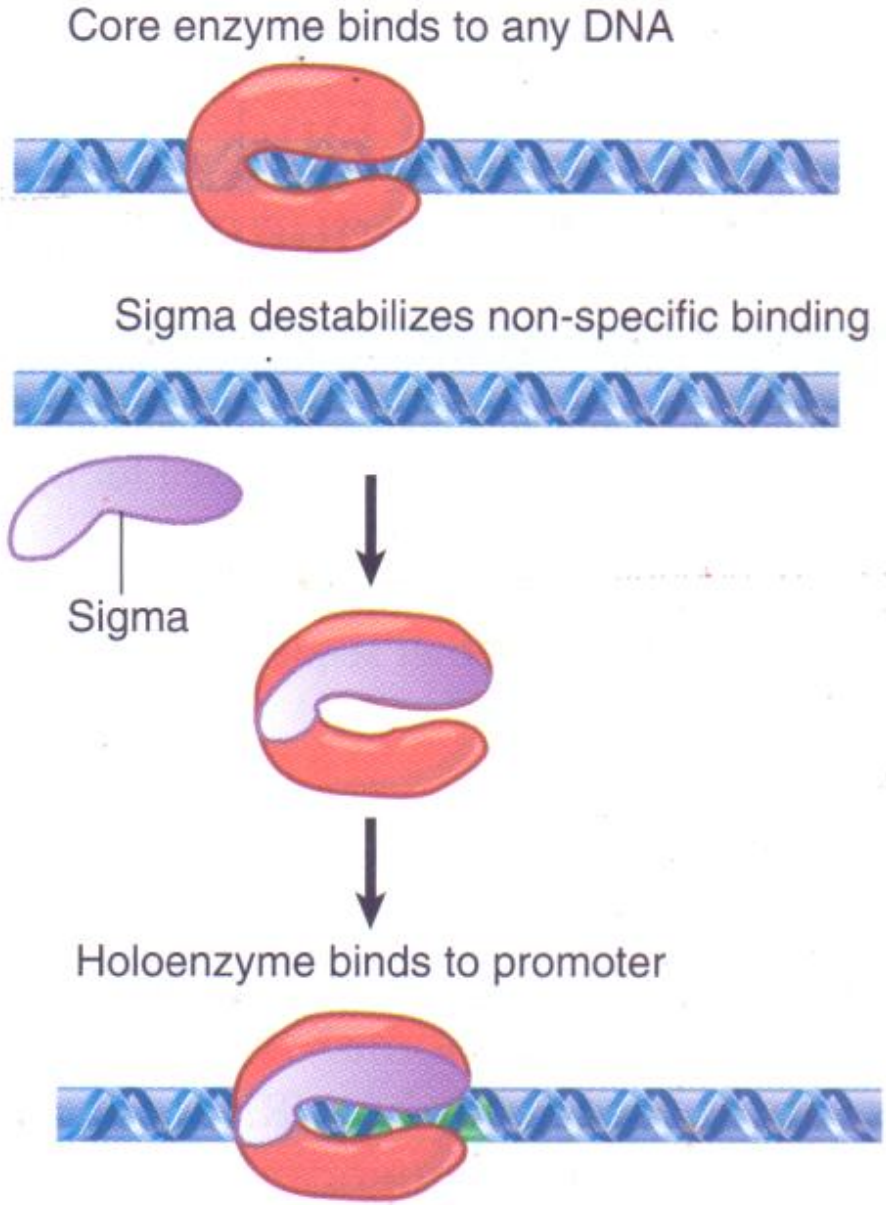


FIGURE 19.10 Core enzyme binds indiscriminately to any DNA. Sigma factor reduces the affinity for sequence-independent binding and confers specificity for promoters.

SIGMA (σ) FACTORS

1. A sigma factor is a protein needed only for initiation of RNA synthesis.
2. Sigma factor changes the DNA binding properties of RNA polymerase so that its affinity for general DNA is reduced and its affinity for promoters is increased.
3. It is a bacterial transcription initiation factor that enables specific binding of RNA polymerase to gene promoters.
4. The specific sigma factor used to initiate transcription of a given gene will vary. It depends on following two factors:
   a) On gene itself
   b) On the environmental signals needed to initiate transcription of that gene.
4. Every molecule of RNA polymerase holoenzyme contains exactly one sigma factors .
5. The number of sigma factors varies between bacterial species.

#	Species	Types of sigma factors
1	*E. coli*	07
2	*B. subtilis*	18
3	*Streptomyces coelicolor*	60

FUNCTIONS OF SIGMA (σ) FACTOR

1. **There is a wide variation in the rate at which the holoenzyme binds to different promoter sequences.**
2. **This is an important parameter in determining 'promoter strength (the efficiency of an individual promoter in initiating transcription).**
3. **Frequency of initiation varies (from ~1/sec to <1/30 min.)**
4. **σ factor is usually released when the RNA chain reaches less than ~10 nt in length, leaving the core enzyme responsible for elongation.**

The eubacterial transcription cycle

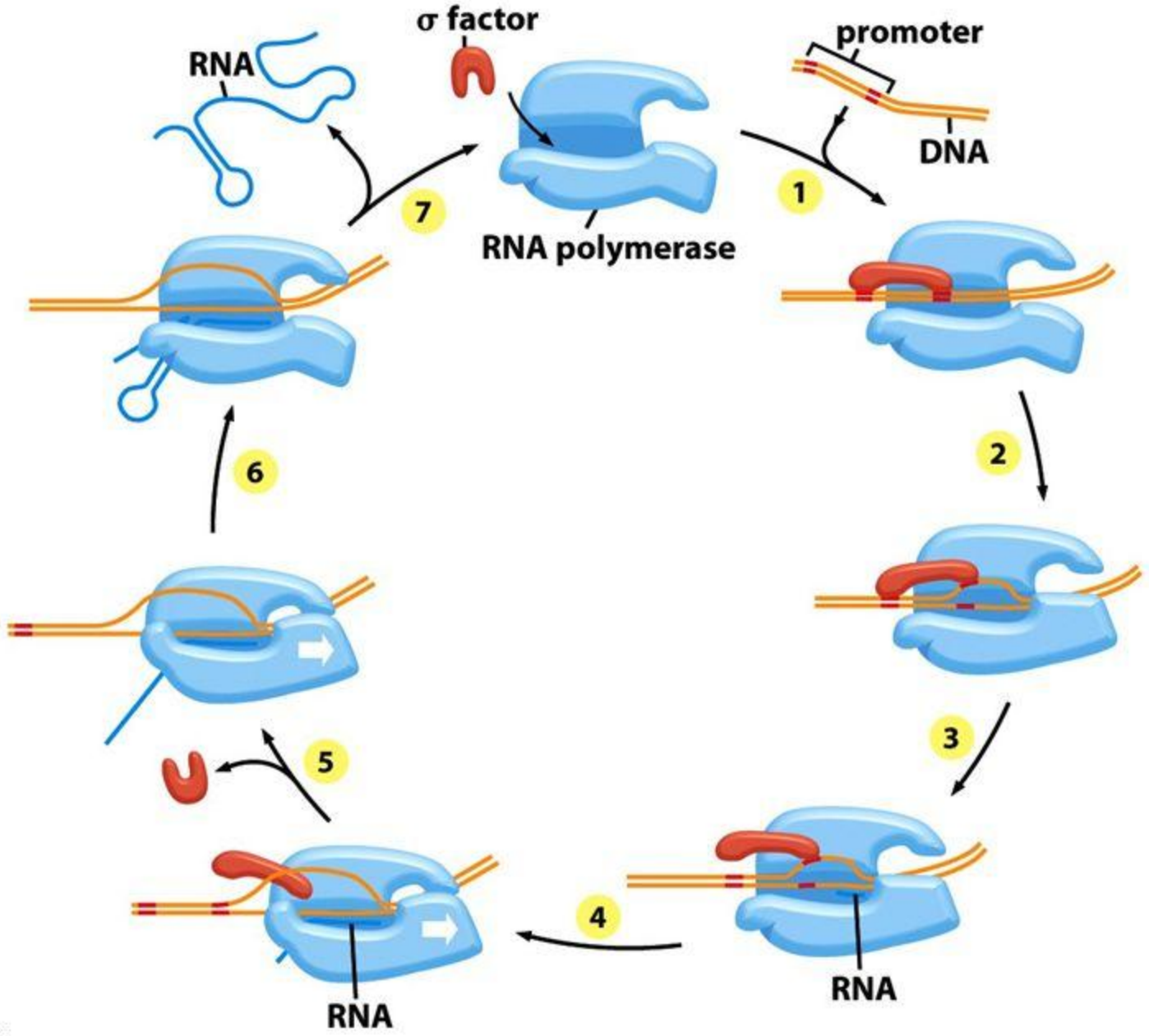
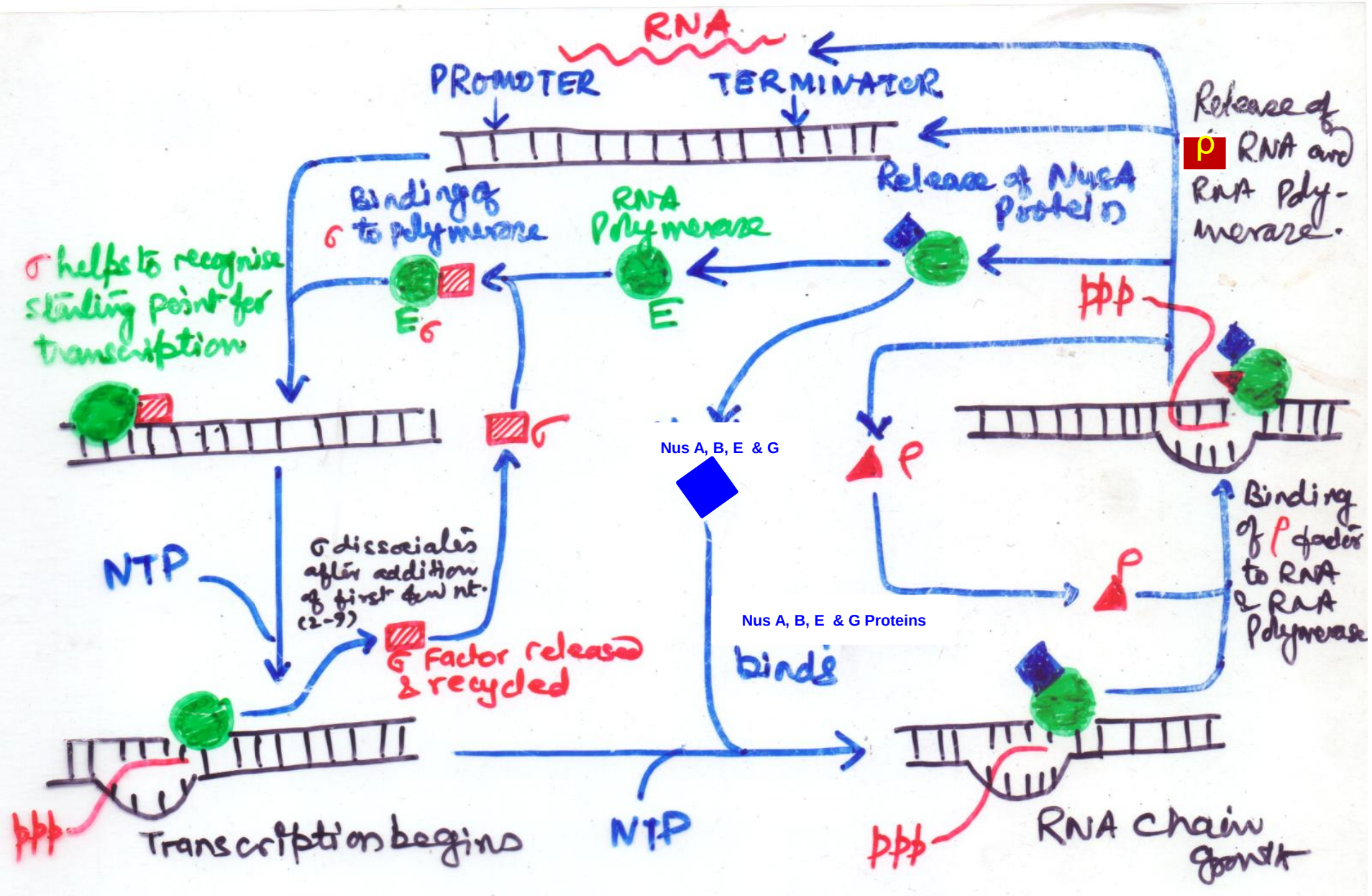


Figure 6-11 *Molec*

SIGMA (σ) CYCLE

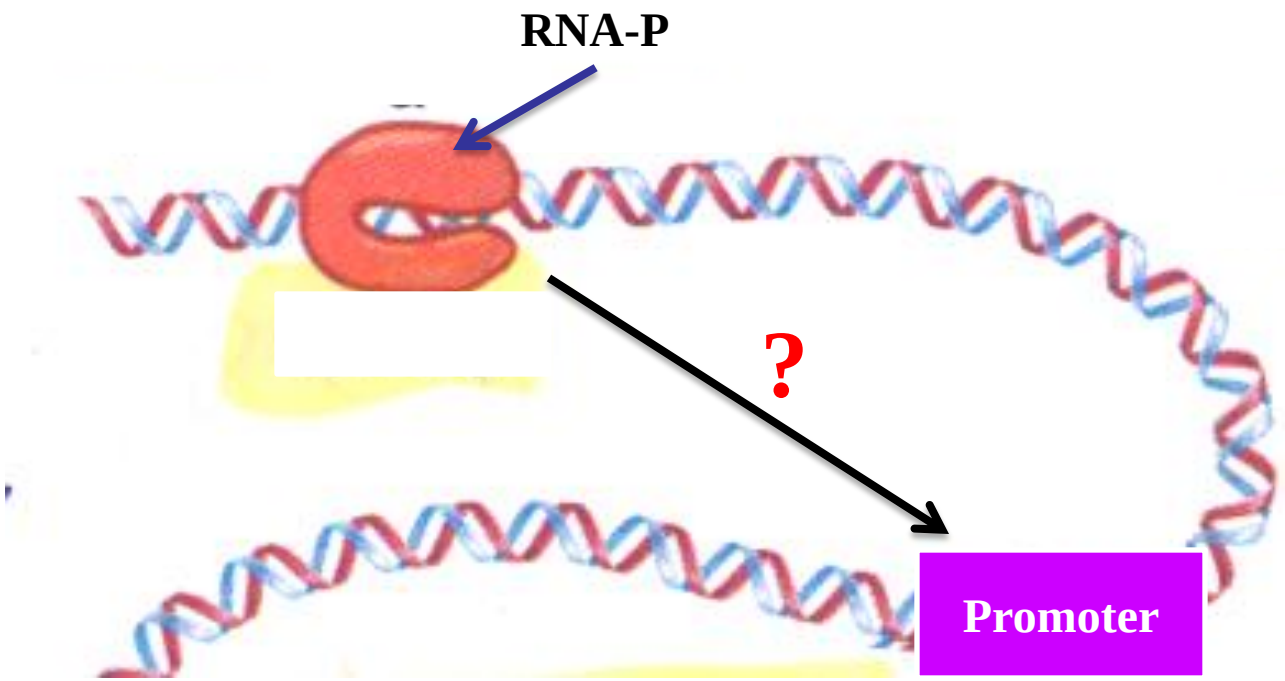


How does RNA Polymerase

finds

a Promoter Sequence on DNA?

(*E. coli* genome size is ~4 x 10^6 bp, where ~ 2,000 promoters are found).



Ans.: RNA Polymerase finds a Promoter Sequence by three mechanisms?

a) By Sliding Mechanism
b) By Intersegment Transfer Mechanism
c) By Intrasegment Transfer Mechanism
[intra domain Association or dissociation (hopping)]

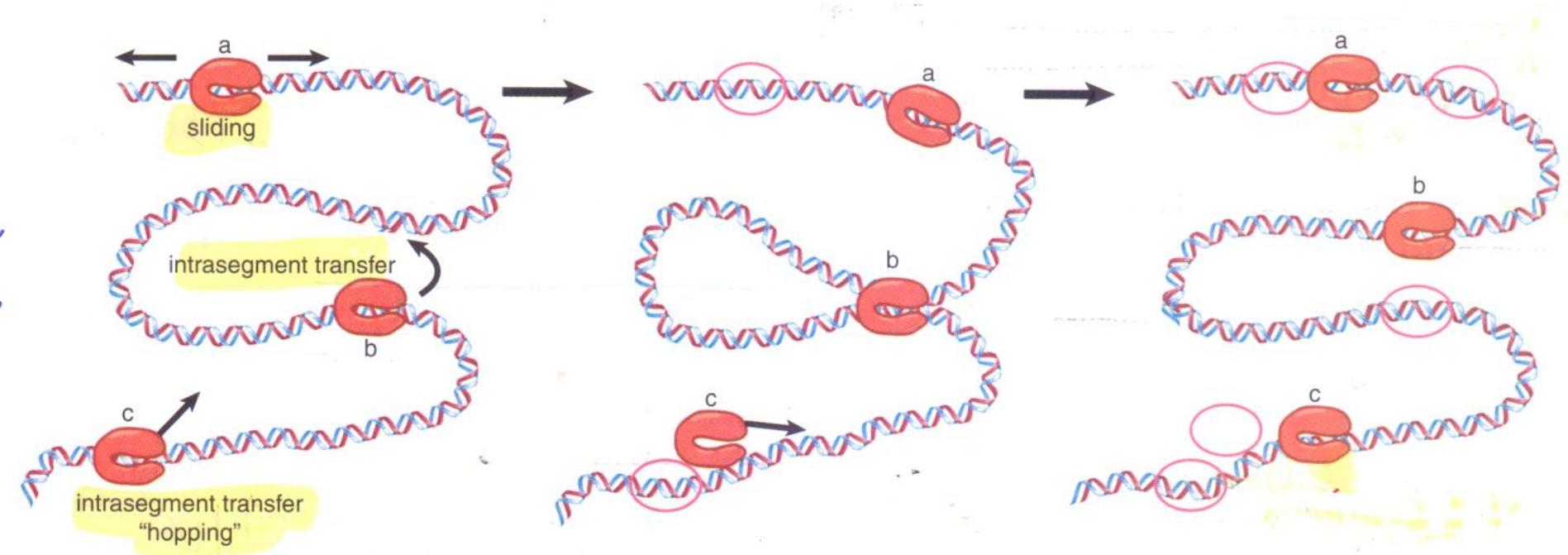
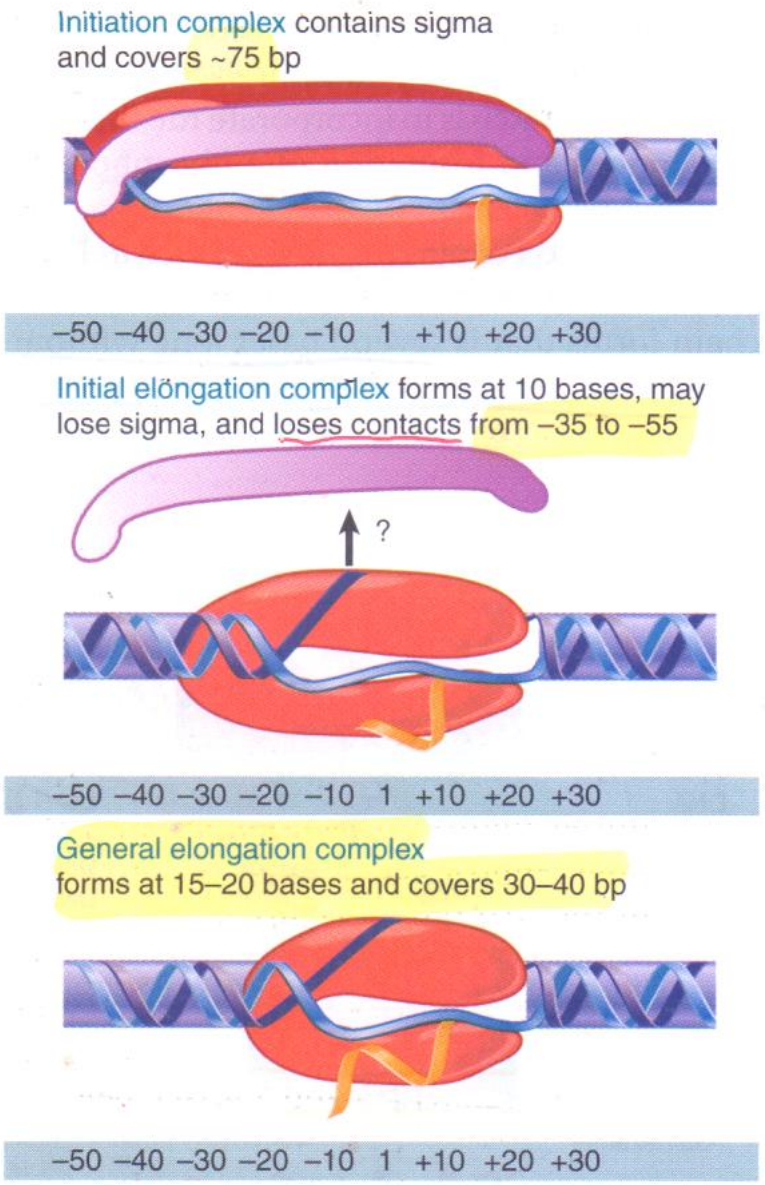


FIGURE 19.11 Proposed mechanisms for how RNA polymerase finds a promoter. (a) sliding (b) intersegment transport (c) intradomain association and dissociation or hopping. Adapted from C. Bustamante, et al., *J. Biol. Chem.* 274 (1999): 166665–166668.

Holoenzyme goes through transitions in the process of recognizing and escaping from promoter



~55 bp

~30-40 bp

FIGURE 19.13 RNA polymerase initially contacts the region from –55 to +20. When sigma dissociates, the core enzyme contracts to –30; when the enzyme moves a few base pairs, it becomes more compactly organized into the general elongation complex.

Binding of RNA-P to Promoter

Description	length (bp) covered	Regions on DNA covered
1. Closed binary complex (ds DNA opens from −11 to +3 region when RNAP binds to Promoter DNA)	55 bp	−55 to +1 position
2. open complex (Initialized complex) (10 nts are added)	75 bp	−55 to +20 position no movement of enzyme
Sigma is dissociated → 3. Initial elongation complex	55 bp	−35 to +20 position
4. General elongation complex	30-40 bp	−20 to +20 position

Enzyme actually succeed in escaping the promoter after addition of 20 nts.