

# Molecular Biology

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**Eukaryotic  
RNA Polymerases**

## Multiple Forms of Eukaryotic RNA Polymerase

- ribosomal RNA genes
- the rest of the nuclear genes

1- They have a **different base composition** from that of other nuclear genes.

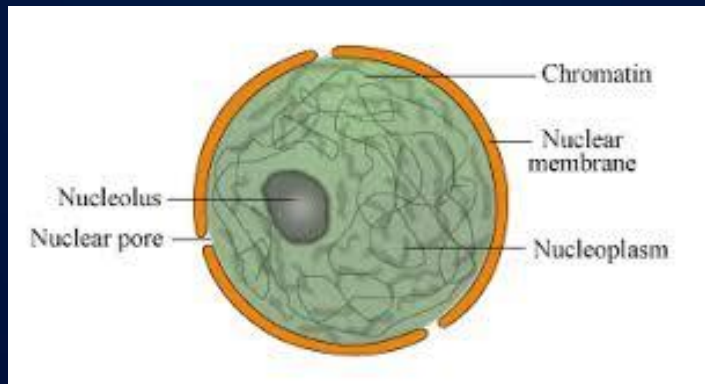
2- unusually repetitive

3-They are found in a **nucleolus**

# Separation of the Three Nuclear Polymerases

Eukaryotic nuclei contain three RNA polymerases:

- I. RNA polymerase I → nucleolus : transcribes the rRNA genes
- II. RNA polymerase II
- III. RNA polymerase III



# The Roles of the Three RNA Polymerases

RNA Polymerase	Cellular RNAs Synthesized	Mature RNA (Vertebrate)
I	Large rRNA precursor	28S, 18S, and 5.8S rRNAs
II	hnRNAs snRNAs miRNA precursors	mRNAs snRNAs miRNAs
III	5S rRNA precursor tRNA precursors	5S rRNA tRNAs

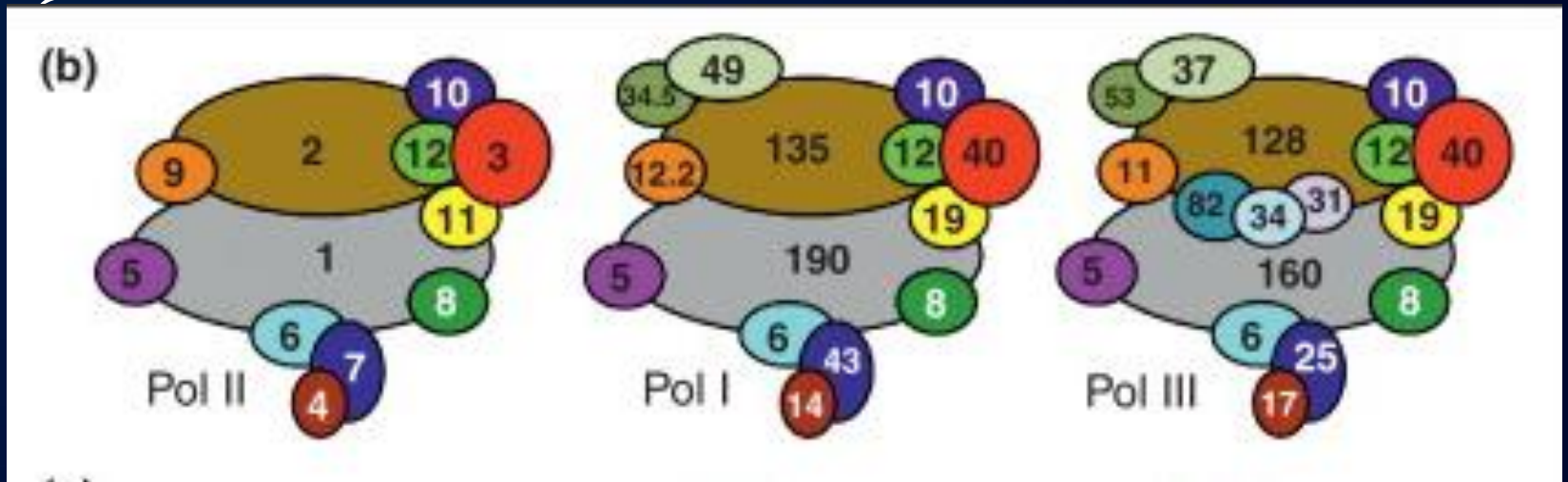
heterogeneous nuclear RNA (hnRNA)  
microRNAs (miRNAs)  
small nuclear RNAs (snRNAs)

additional RNA polymerases in **flowering plants**:

**RNA polymerase IV** and **RNA polymerase V** : silences genes.

## RNA Polymerase Subunit Structures:

- I. RNA polymerase I (A) : 14 subunits
- II. RNA polymerase II (B) : 12 subunits
- III. RNA polymerase III (C) : 17 subunits



Subunit	Yeast Gene	Yeast Protein (kD)	Features
hRPB1	<i>RPB1</i>	192	Contains CTD; binds DNA; involved in start site selection; $\beta'$ ortholog
hRPB2	<i>RPB2</i>	139	Contains active site; involved in start site selection, elongation rate; $\beta$ ortholog
hRPB3	<i>RPB3</i>	35	May function with Rpb1 1 as ortholog of the $\alpha$ dimer of prokaryotic RNA polymerase
hRPB4	<i>RPB4</i>	25	Subcomplex with Rpb7; involved in stress response
hRPB5 *	<i>RPB5</i>	25	Shared with Pol I, II, III; target for transcriptional activators
hRPB6 *	<i>RPB6</i>	18	Shared with Pol I, II, III; functions in assembly and stability
hRPB7	<i>RPB7</i>	19	Forms subcomplex with Rpb4 that preferentially binds during stationary phase
hRPB8 *	<i>RPB8</i>	17	Shared with Pol I, II, III; has oligonucleotide/oligosaccharide-binding domain
hRPB9	<i>RPB9</i>	14	Contains zinc ribbon motif that may be involved in elongation: functions in start site selection
hRPB10 *	<i>RPB10</i>	8	Shared with Pol I, II, III
hRPB11	<i>RPB11</i>	14	May function with Rpb3 as ortholog of the $\alpha$ dimer of prokaryotic RNA polymerase
hRPB12 *	<i>RPB12</i>	8	Shared with Pol I, II, III

How do the structures of polymerases I and III compare with this polymerase II structure?

## Core Subunits

- These three polypeptides, Rpb1, Rpb2, and Rpb3, are all absolutely required for enzyme activity.
- *E. coli* B'-subunit binds DNA, and so does Rpb1.
- *E. coli* B-subunit at the active site of the enzyme = Rpb2



## Heterogeneity of the Rpb1 Subunit

□ **IIa** : a repeating string of seven amino acids (a *heptad*) = **CTD (carboxyl-terminal domain)**

Tyr-Ser-Pro-Thr-Ser-Pro-Ser = heptad

□ **IIb** : lacks CTD

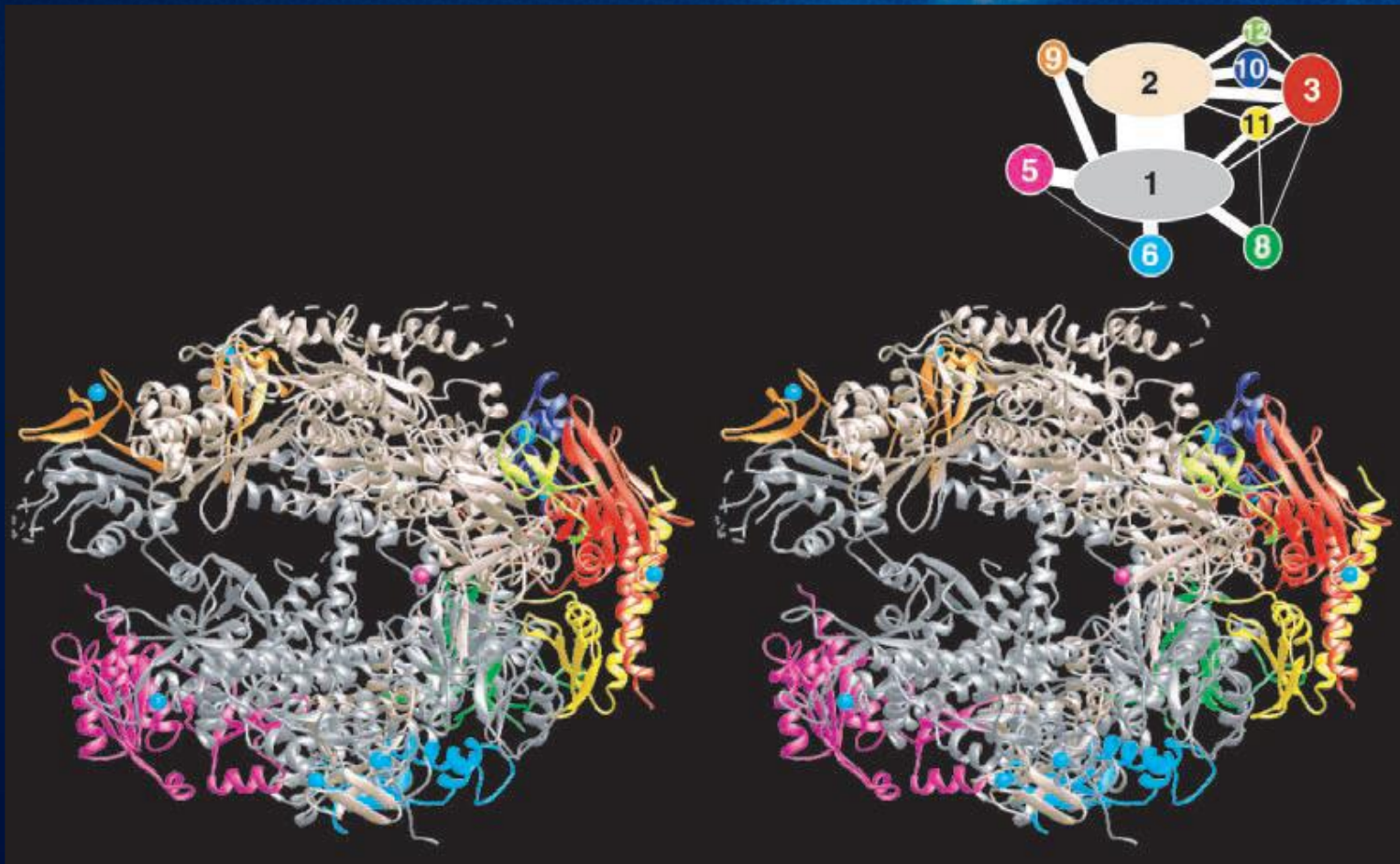
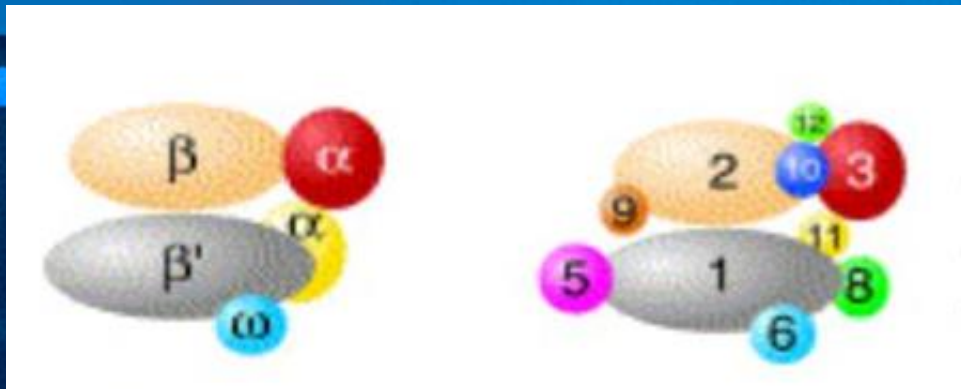
□ **IIo** : serines 2, 5, and sometimes 7 in the heptad are found to be phosphorylated in the IIo subunit.

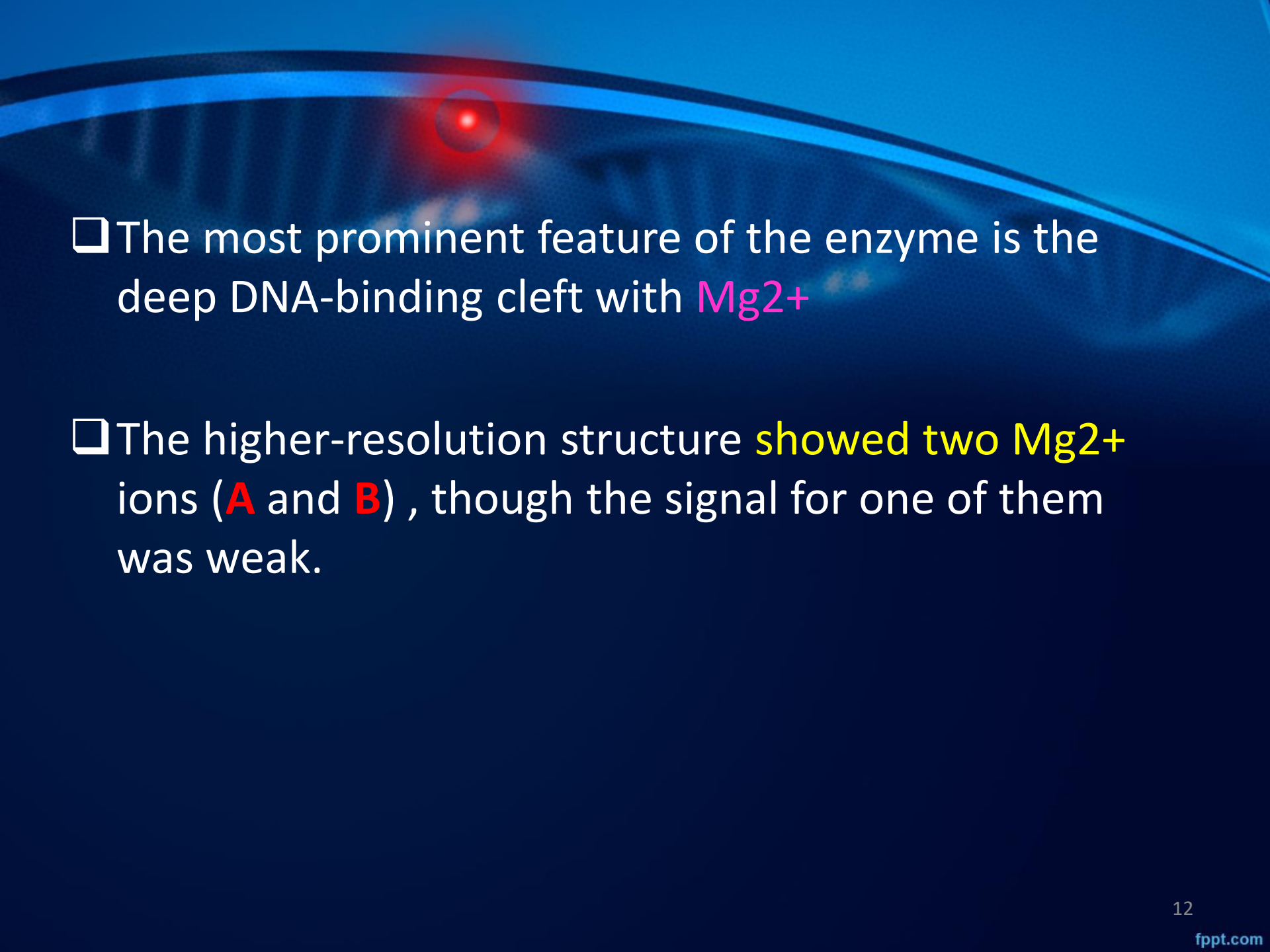


**IIA** (the unphosphorylated form of the enzyme) is the species that initially **binds to the promoter**.

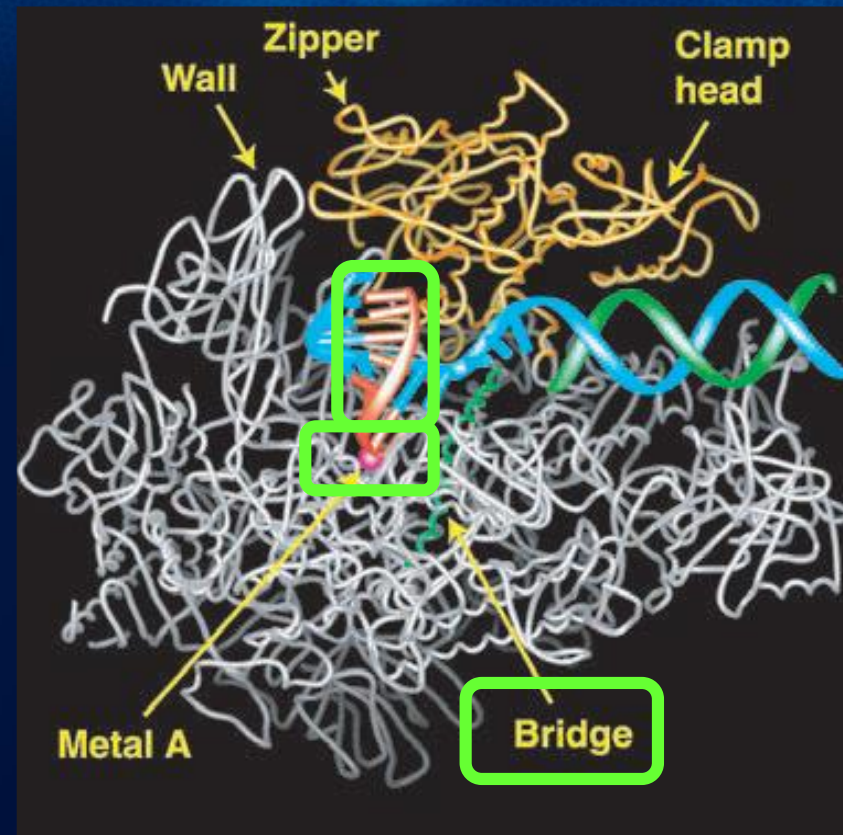
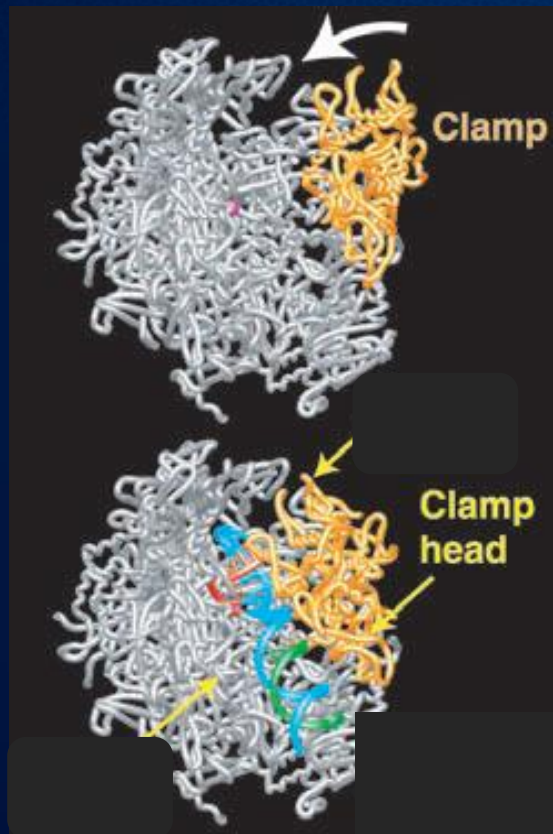
**IIO** (with its CTD phosphorylated) is the species that carries out **elongation**.

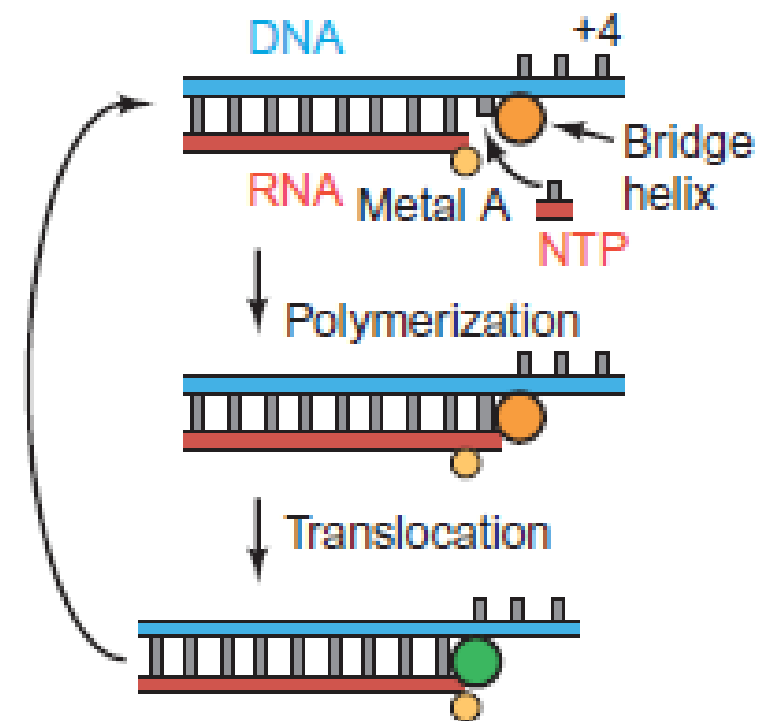
Thus, **phosphorylation** of the CTD appears to accompany the transition from **initiation to elongation**.



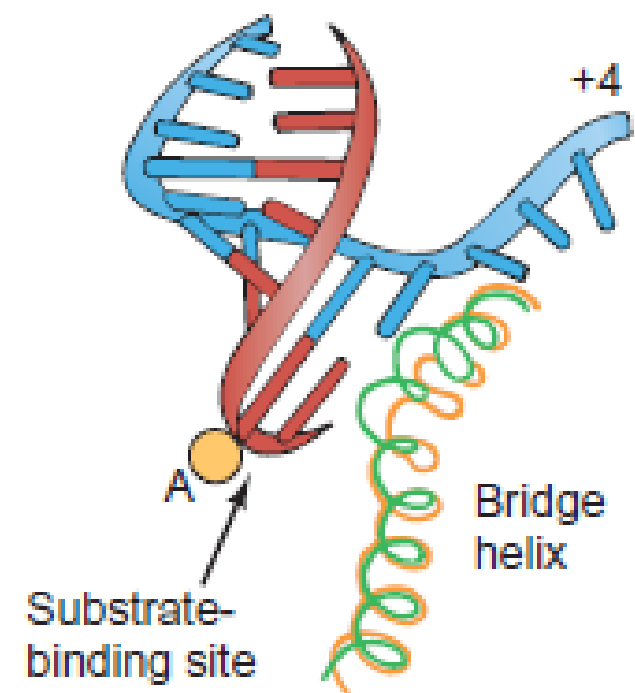
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- The most prominent feature of the enzyme is the deep DNA-binding cleft with  $Mg^{2+}$
  - The higher-resolution structure showed two  $Mg^{2+}$  ions (**A** and **B**), though the signal for one of them was weak.

# Three-Dimensional Structure of RNA Polymerase II in an Elongation Complex





(a)



(b)

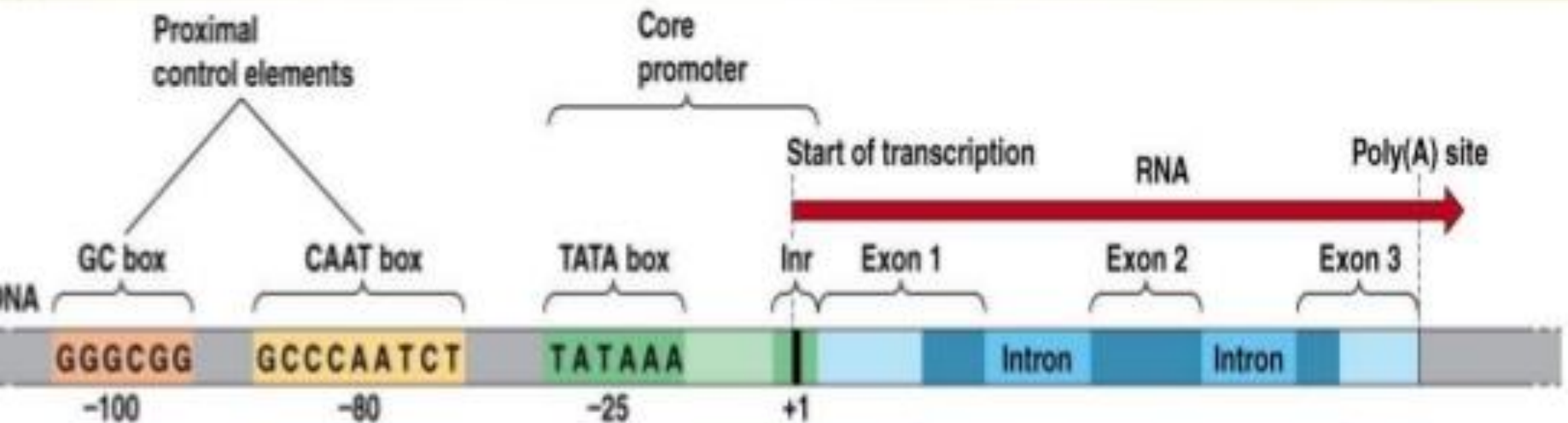


# Promoters

## Class II Promoters

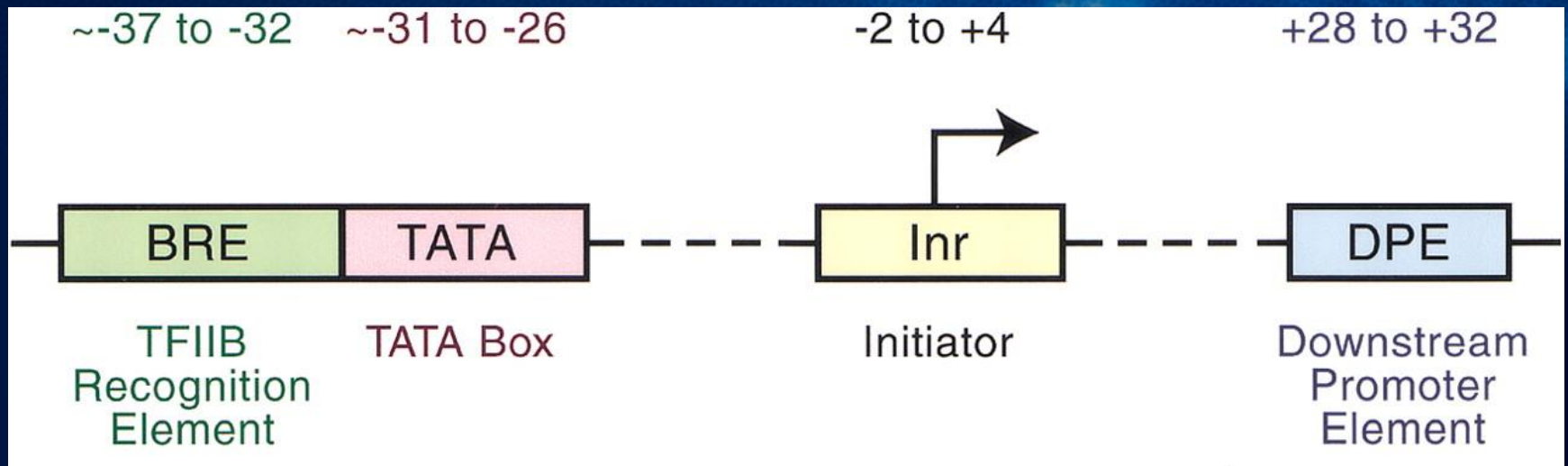
- core promoter
- proximal promoter (upstream promoter elements)

UPE





## core promoter

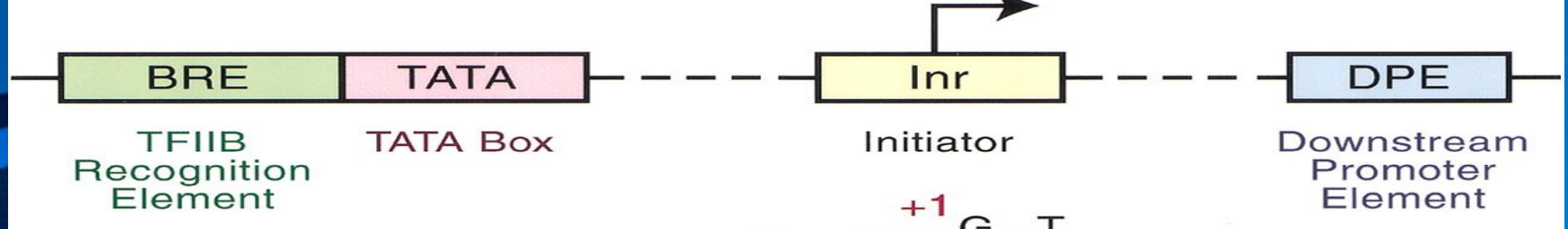


## TATA BOX (TATAAA)

- ❑ The **last A** of this sequence usually lies **25 to 30 bp upstream** of the transcription start site in higher eukaryotes.
- ❑ close similarity between the eukaryotic TATA box and the **prokaryotic -10 box**.
- ✓ TATA-less promoters

## What is the function of the TATA box?

- Some class II promoters require the TATA box for **function**, but others need it only to **position the transcription start site**.
- **preinitiation complex** = a collection of **transcription factors** and **RNA polymerase**.
- The first protein to bind is **TFIID**, including the **TATA-box-binding protein (TBP)**, which then attracts the other factors.



## Initiators

- conserved sequences around their transcription start sites that are required for optimal transcription.

## DPE

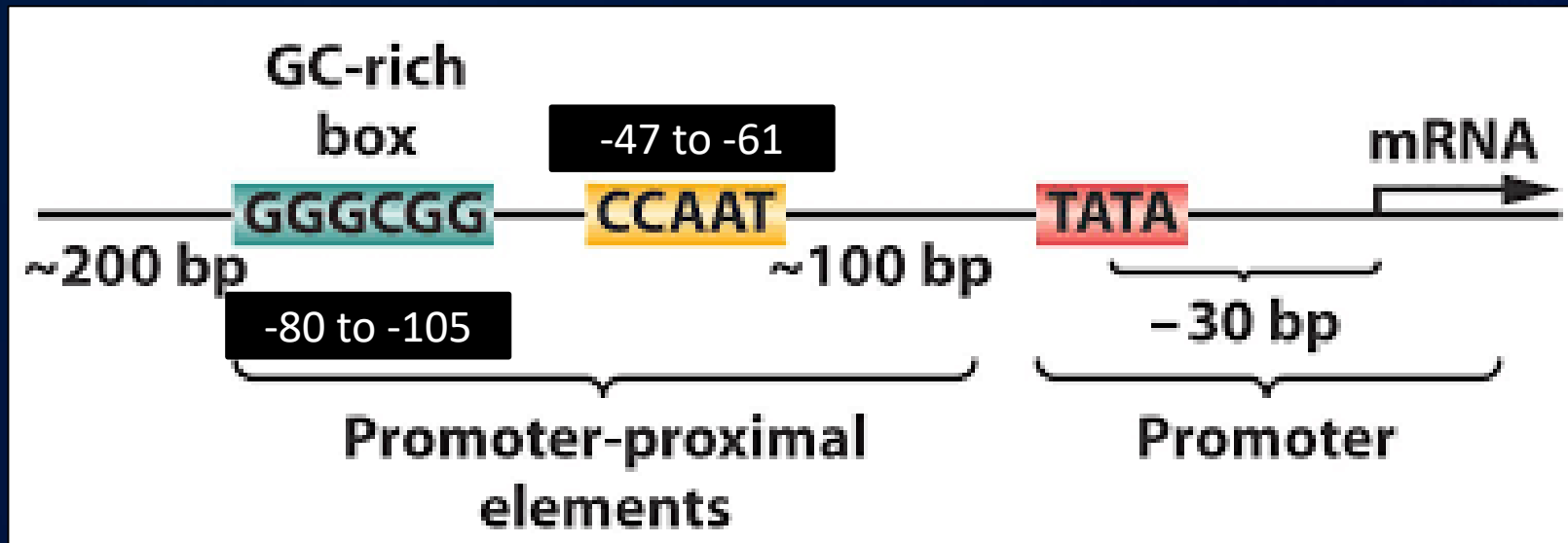
- In fact, **TATA-less promoters** tend to have DPEs, at least in *Drosophila*.

## BRE

- Another important **general transcription factor** is **TFIIB**, which binds to the promoter along with TFIID to form a **preinitiation complex** that is competent to begin transcription.

## Proximal Promoter Elements:

- mutations in these elements (GC-rich box) significantly decreased promoter activity.



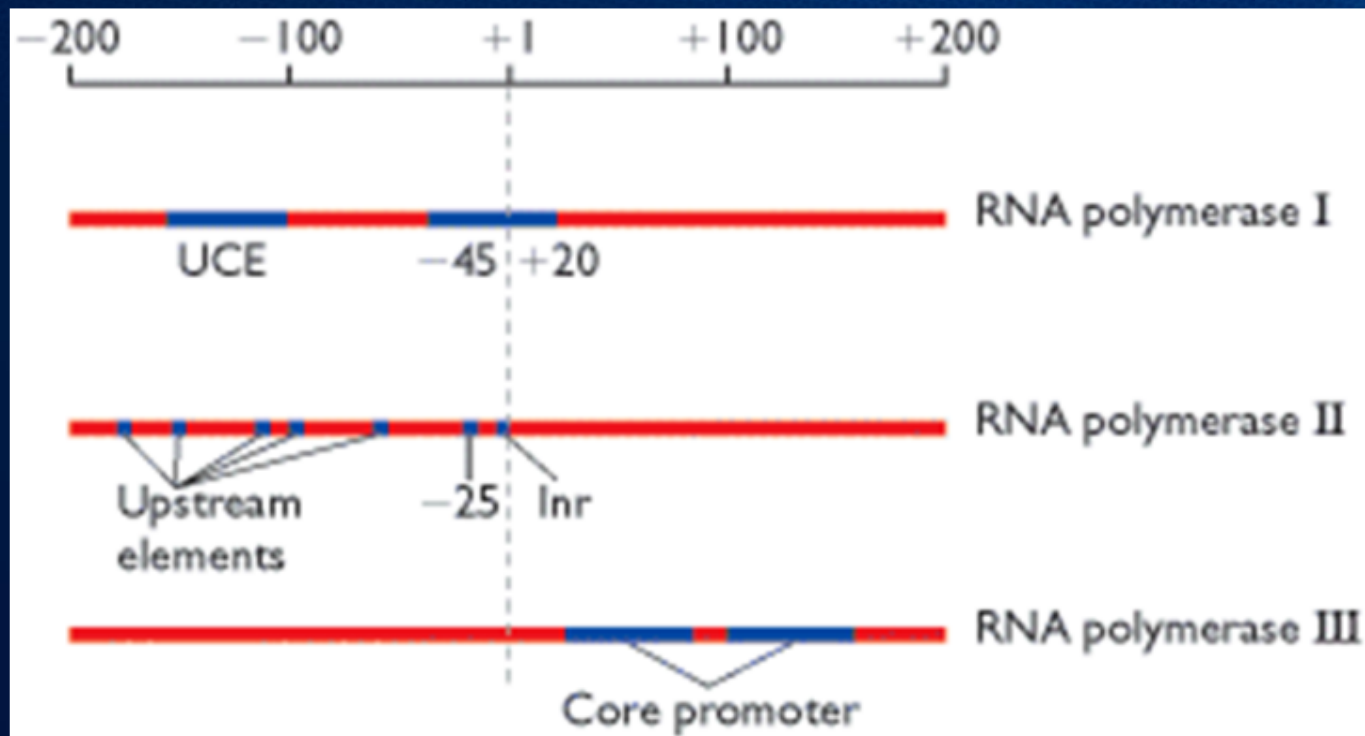
- **GC boxes** bind the transcription factor **Sp1**, while **CCAAT** boxes bind **CTF (CCAAT-binding transcription factor)**

## Class I Promoters

- Each copy of **rRNA** virtually the same as the others, and they all have the same promoter sequence.
- It consists of two elements, a **core element** surrounding the **transcription start site**, and an **upstream promoter element (UPE)** about 100 bp farther upstream.



# Class III Promoters



# Class III Promoters

