

Molecular Biology

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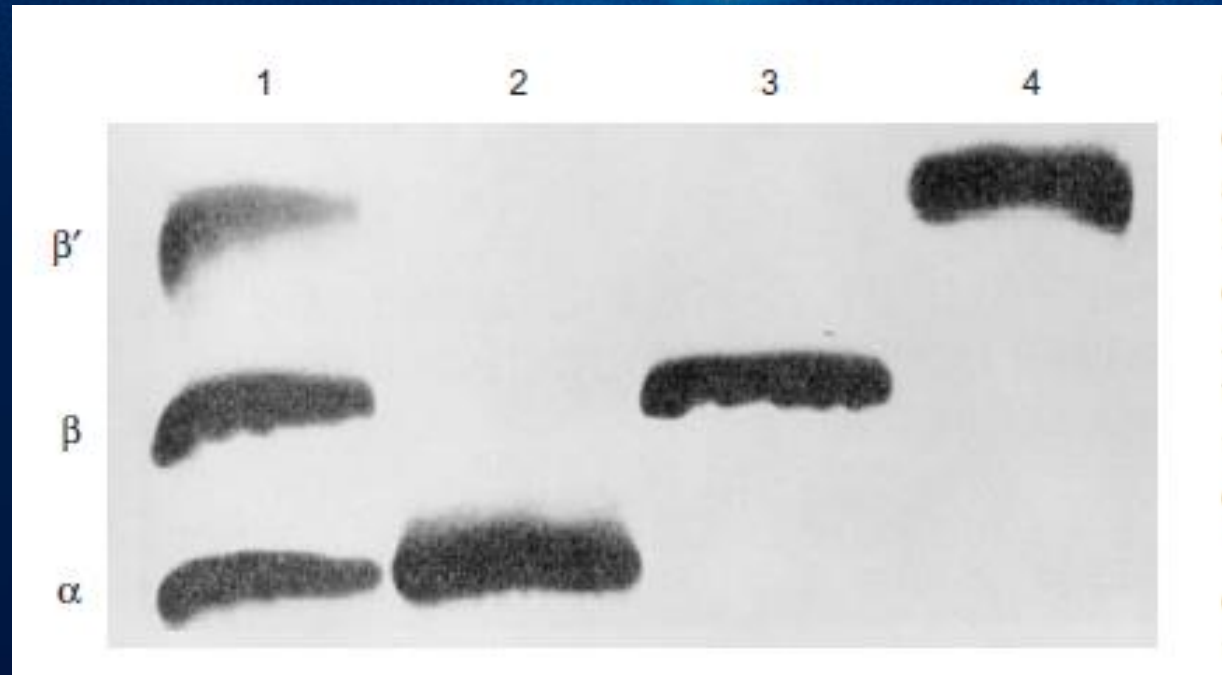
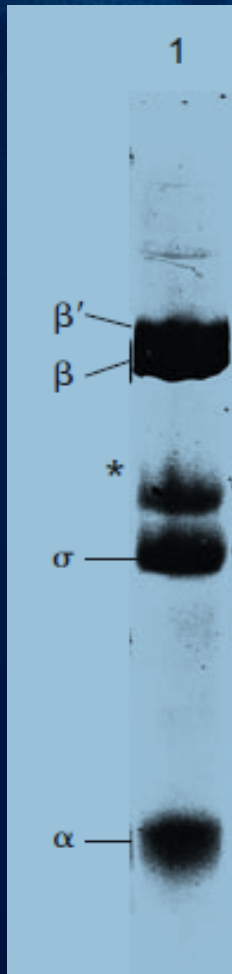
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Elongation:

- the **core polymerase** contains the RNA synthesizing machinery, so the core is the central player in elongation.

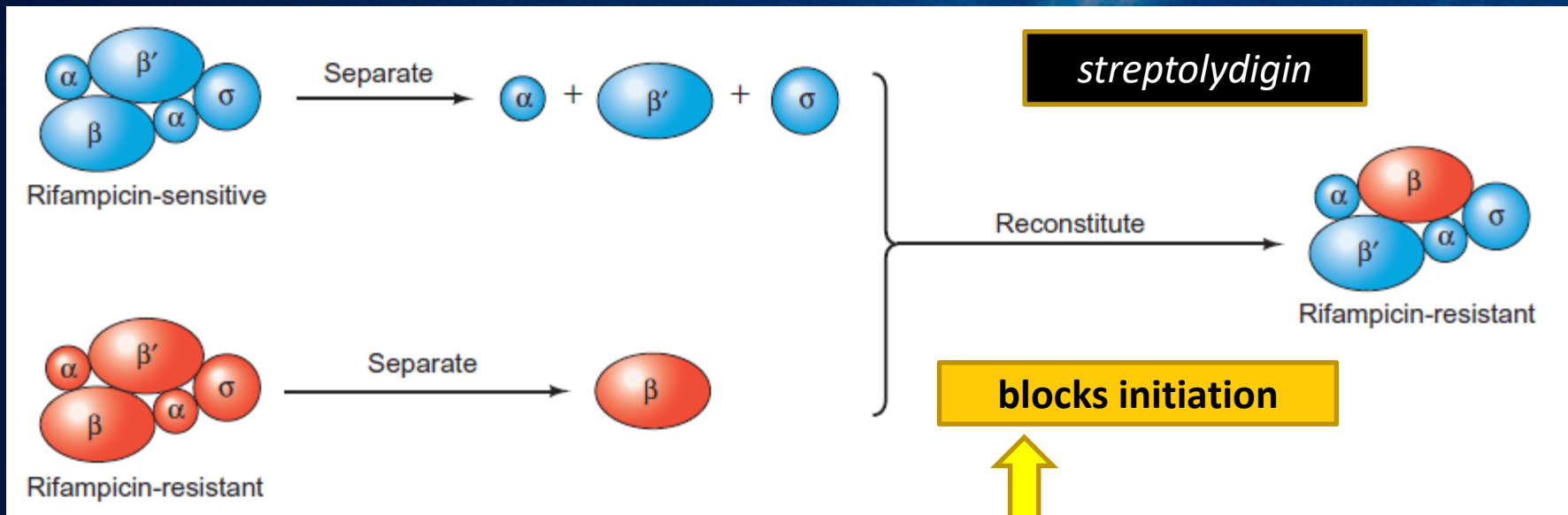
σ'  Core enzyme

The Role of β in Phosphodiester Bond Formation



The Role of β in Phosphodiester Bond Formation

- Separation- reconstitution

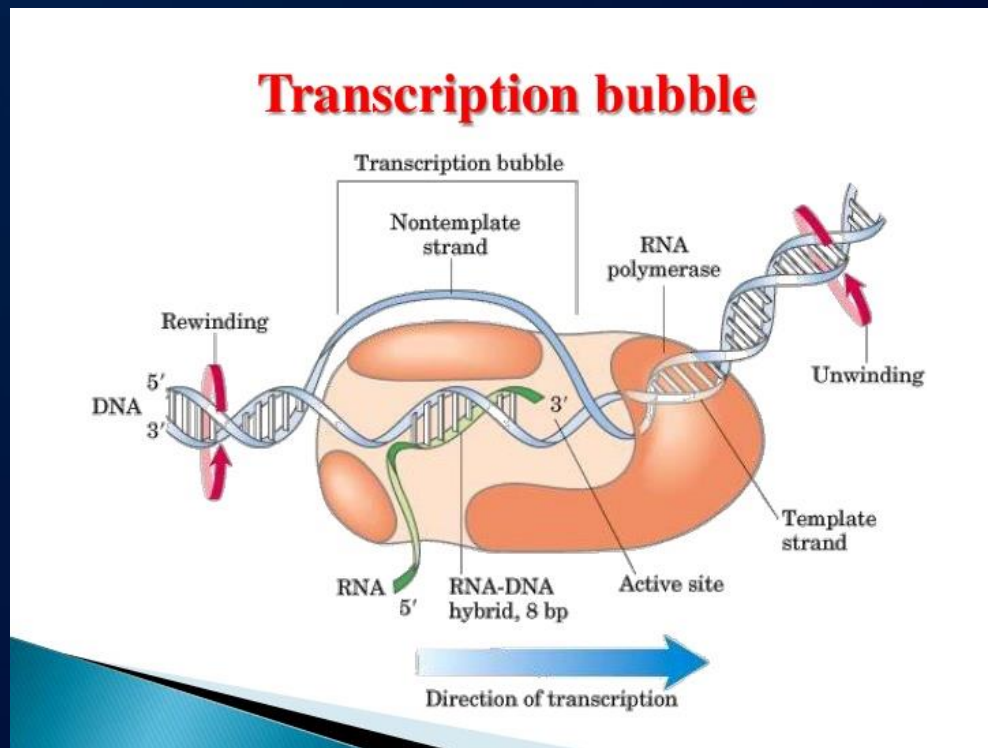


β -subunit is obviously the determinant of **rifampicin** sensitivity or resistance

Streptolydigin: blocks RNA chain elongation

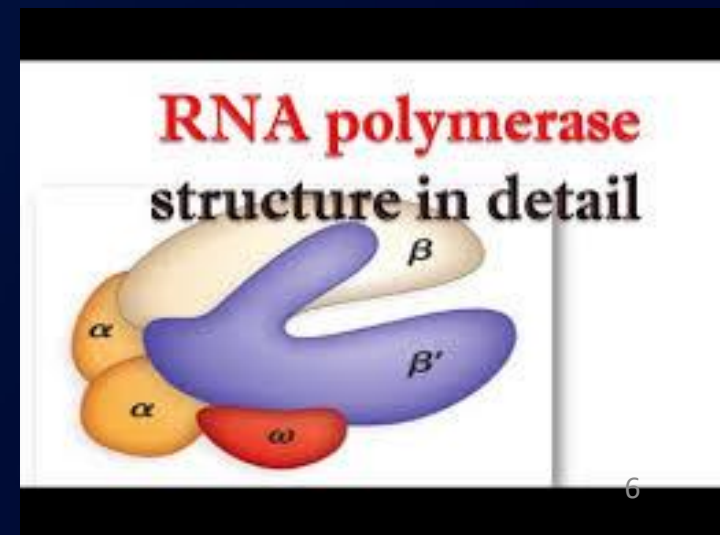
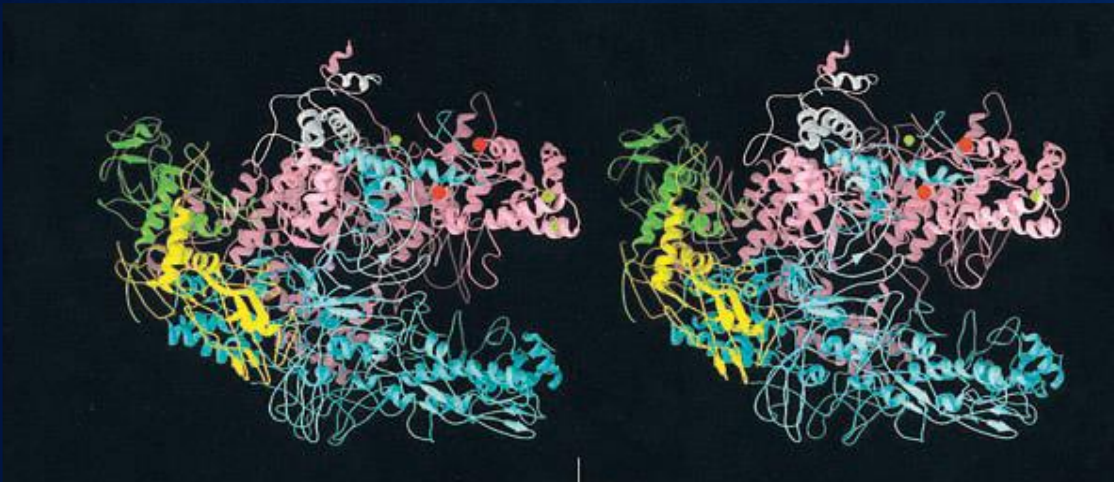
The RNA–DNA Hybrid

- The RNA–DNA hybrid within the *E. coli* elongation complex extends from position **-1** to position **-8 or -9** with respect to the 3′-end of the nascent RNA.



Structure of the Core Polymerase

- an open crab claw



□ enzyme contains a channel, about 27 Å wide

□ Mg²⁺ : (NADFDGD) occurs in the β' –subunit (aspartate)

□ the three Asp residues and a Mg²⁺ ion are at the catalytic center of the enzyme.

□ a rifampicin-binding site in the part of the β –subunit.

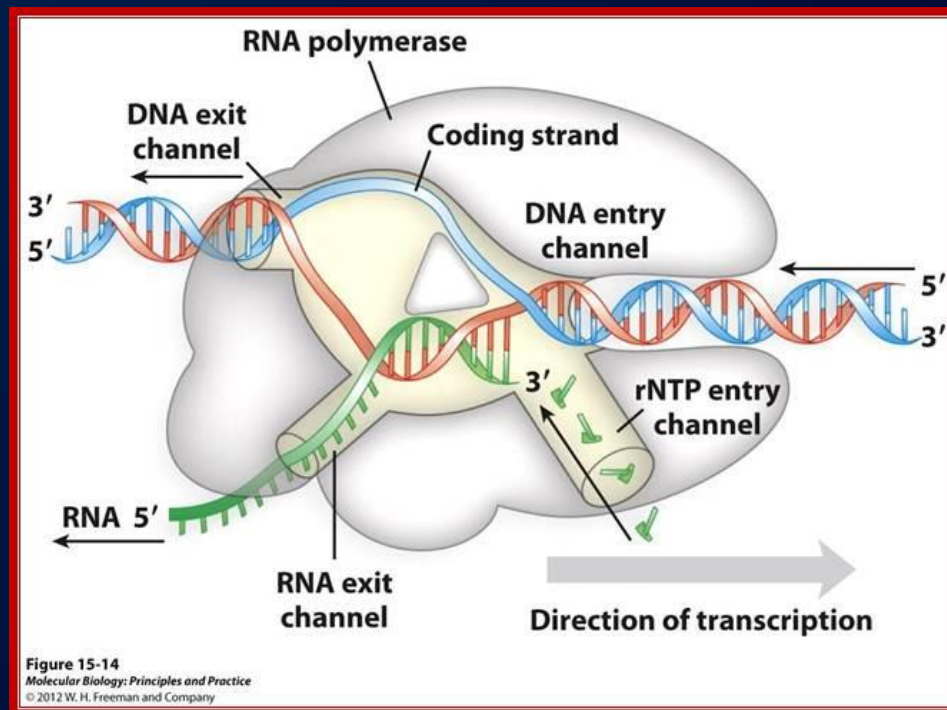


prevents growth of a short RNA



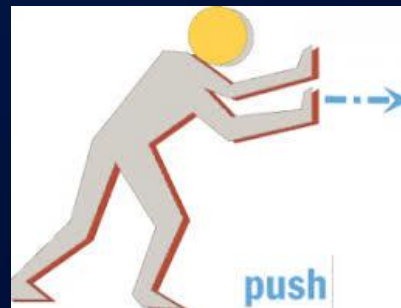
Structure of the Elongation Complex

14 nt double-stranded DNA + 9 bp of RNA–DNA hybrid + 7 nt of RNA product in the RNA exit channel.

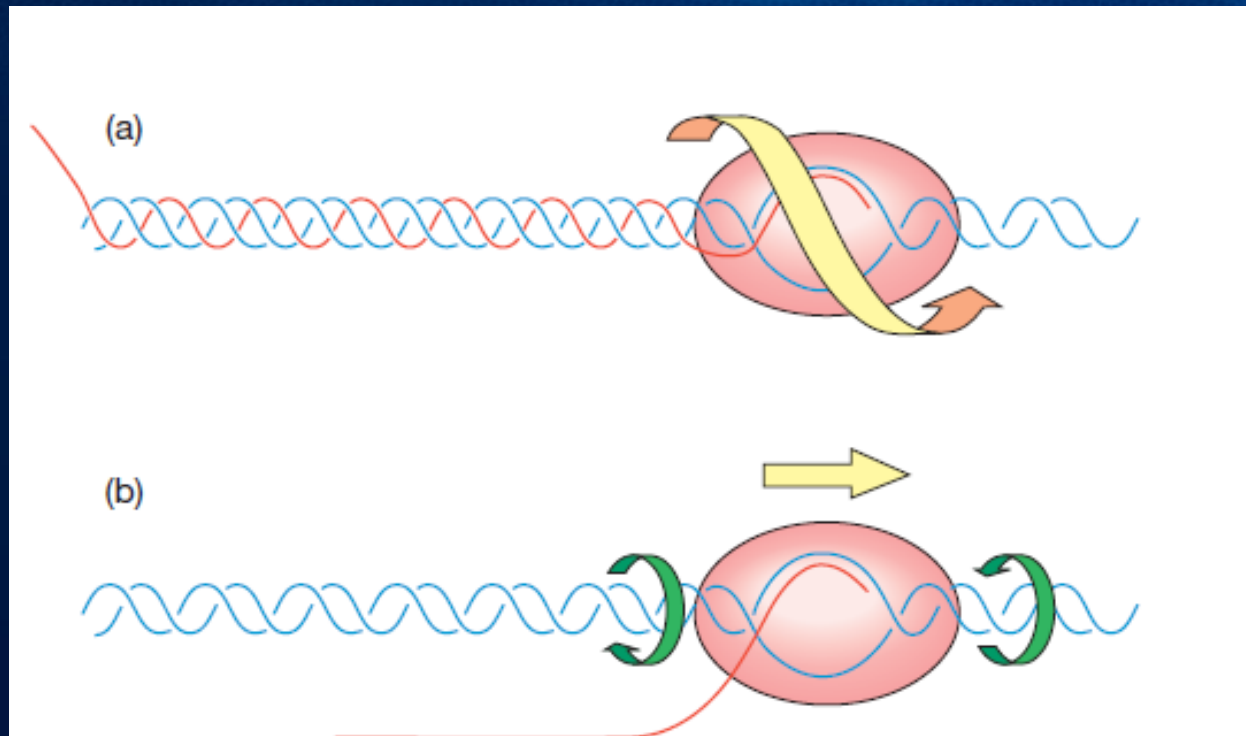


□ a valine residue in the β' subunit inserts into the minor groove of the downstream DNA.

- the downstream DNA is double-stranded up to and including the +2 base pair (so only one nucleotide at a time can bind specifically to the complex).



Topology of Elongation





Pausing and Proofreading

- Pauses significantly slow the overall rate of transcription.
- Pausing is physiologically important for at least two reasons:

- 1- it allows translation
- 2- a step in termination



- Sometimes the polymerase even backtracks by reversing.

proofread



Pause

proteins known as **GreA** and **GreB** stimulate an inherent RNase activity of the polymerase

Termination of Transcription

1- intrinsic terminators

- function with the RNA polymerase by itself without help from other proteins.

1- an inverted repeat = hairpin
(G- C)



2- followed immediately by a **T-rich** region in the **non template strand** of the gene

5'-TACGAAGTTCGTA-3'

•
3'-ATGCTTCAAGCAT-5'

Typical prokaryotic terminator

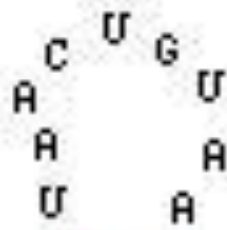
5' ATCTTA **GCCGCC** TAACTGTAA **GGCGGG** CTTTTT 3'
3' TAGAAT **CGGGCC** ATTGACATT **CCGCCCG** AAAAAA 5'

DNA



5' AUCUUA **GCCGCC** UAAACUGUAA **GGCGGG** CUUUUU 3'

mRNA

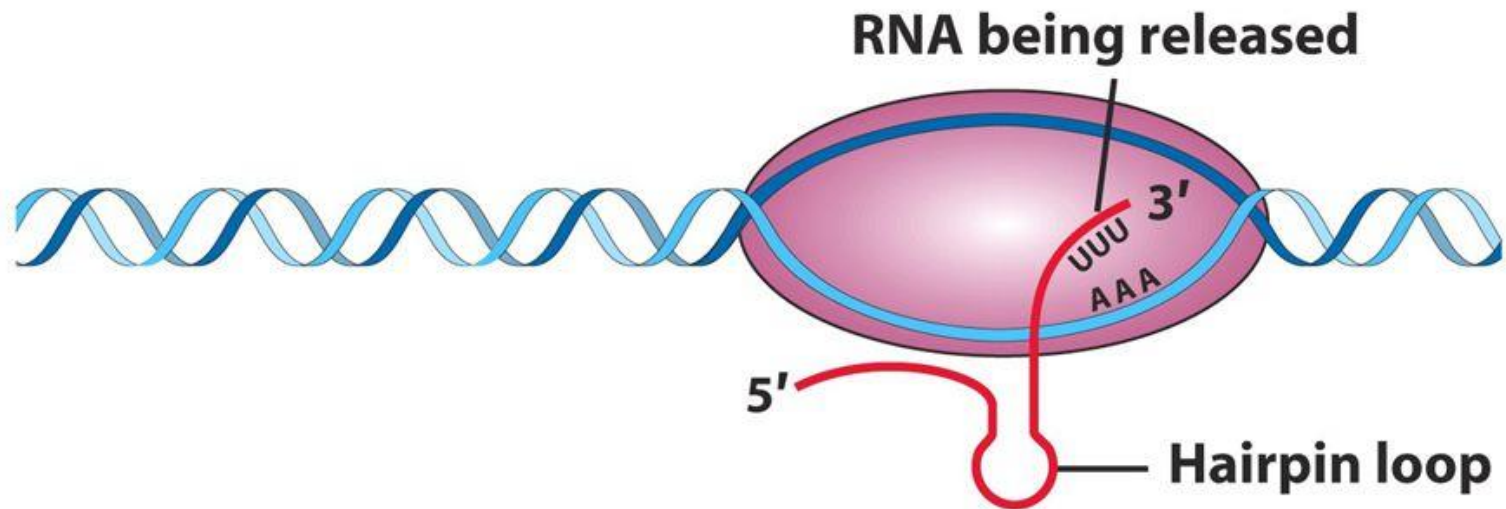


hairpin loop
in mRNA

5' AUCUUA **G** CUUUUU 3'

Termination

Termination: intrinsic mechanism

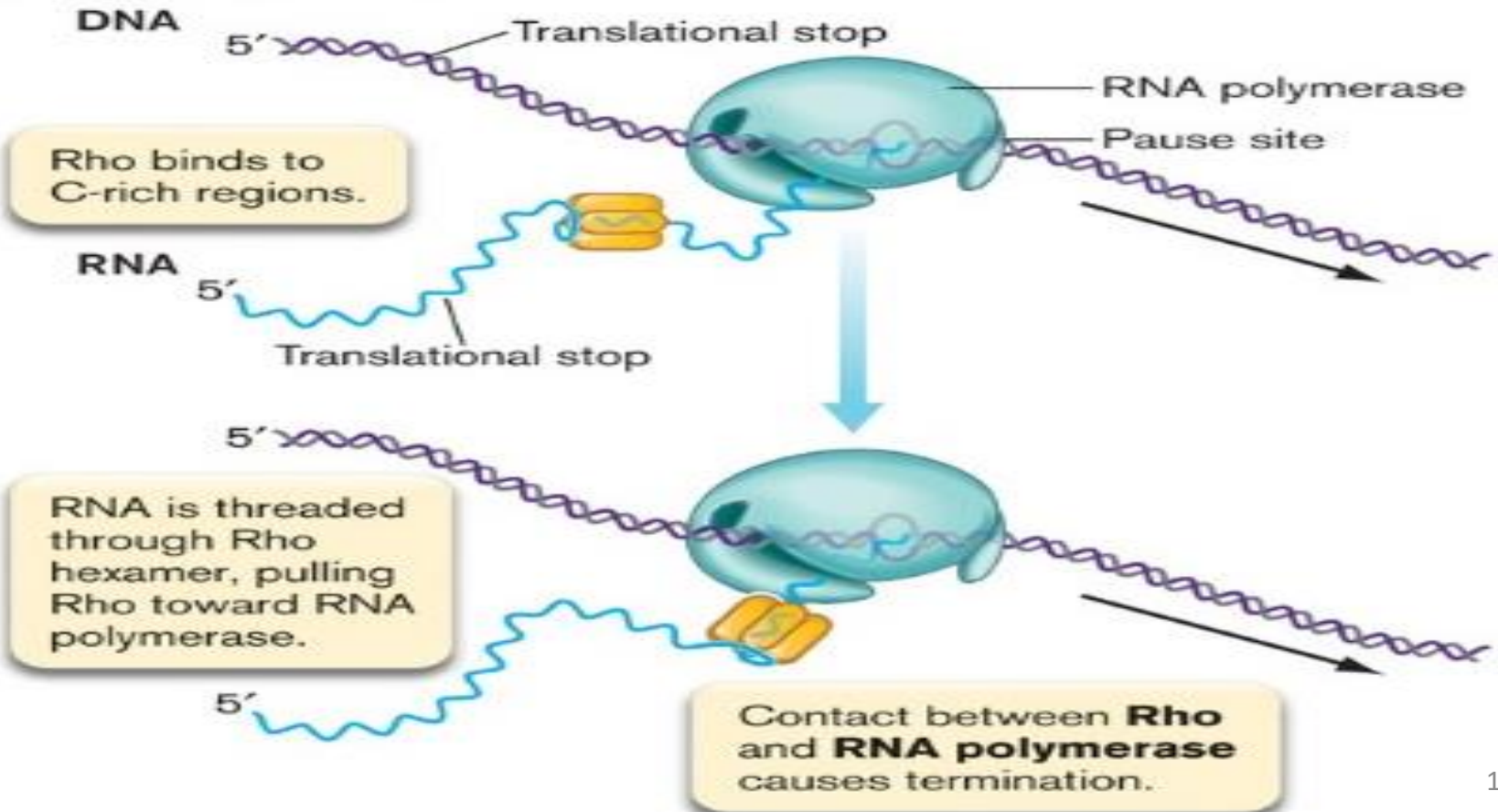


2- Rho-Dependent Termination

rho utilization site (*rut*)

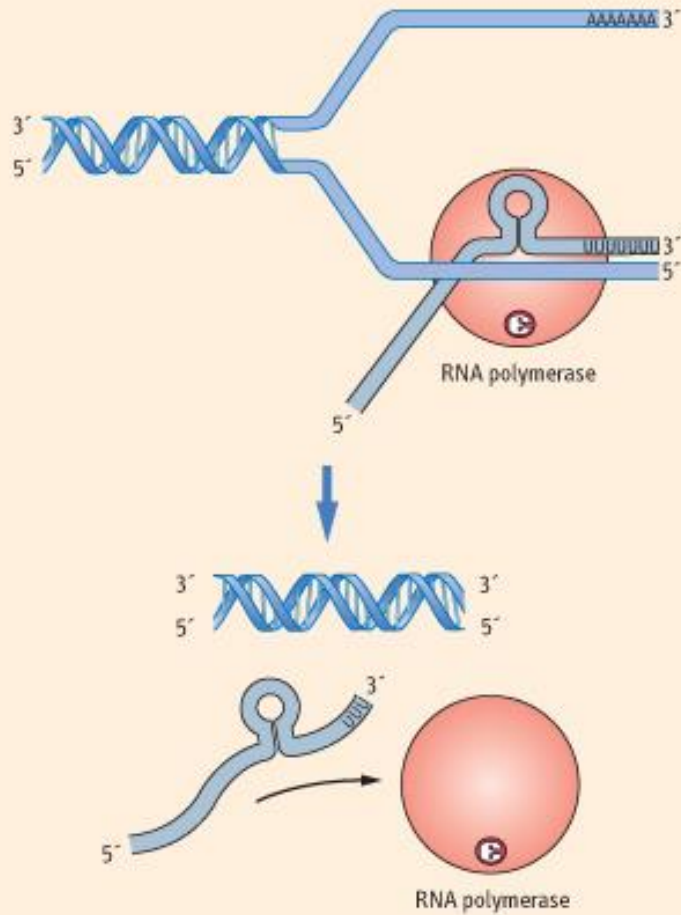
rho has RNA–DNA helicase activity

A. Rho-dependent termination

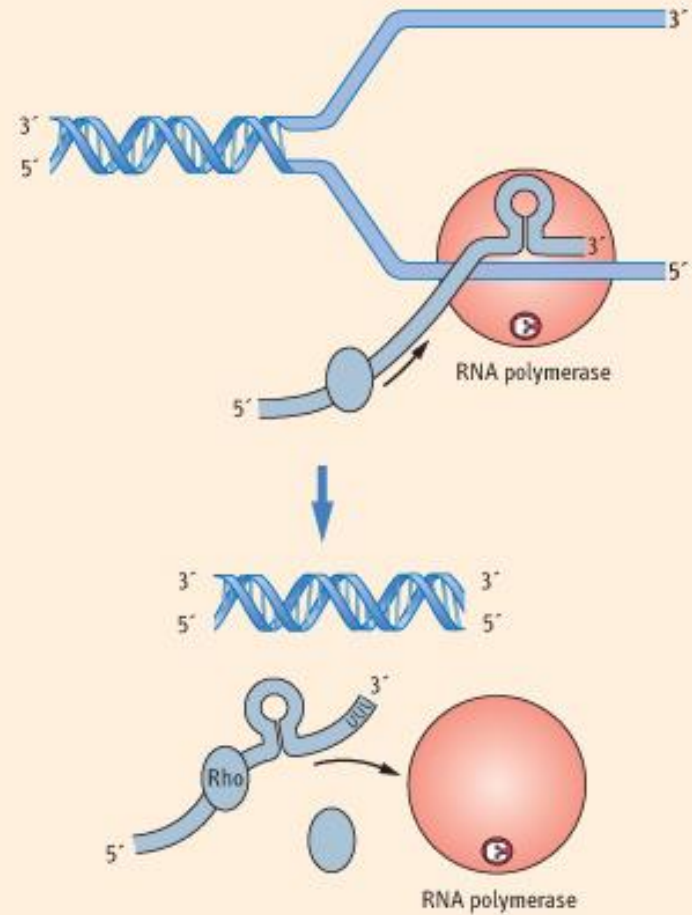


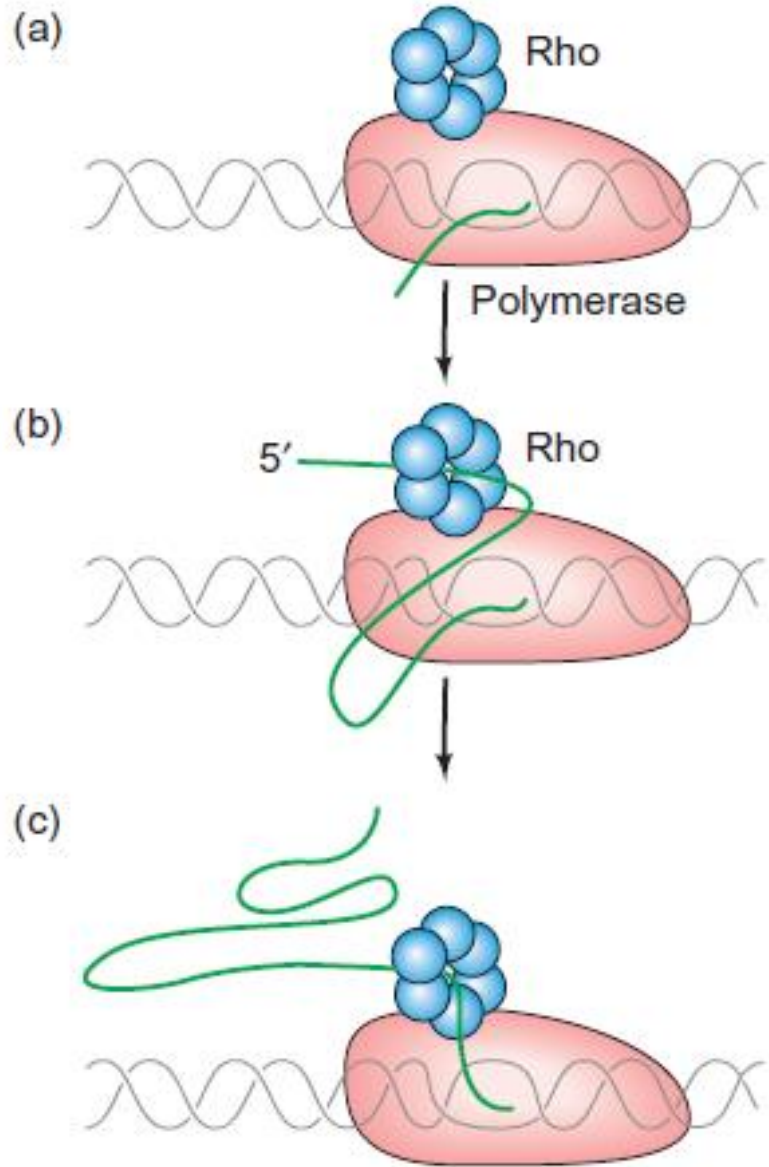
Transcription termination in prokaryotes

rho-independent termination

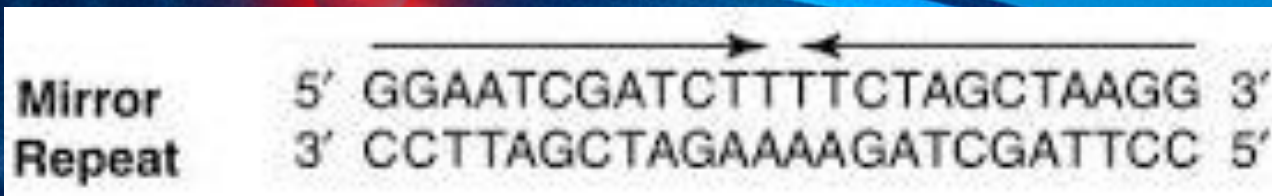


rho-dependent termination



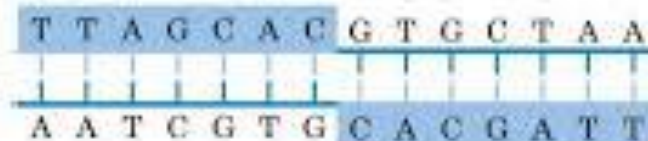


Mirror repeat



Palindromic (inverted repeat)

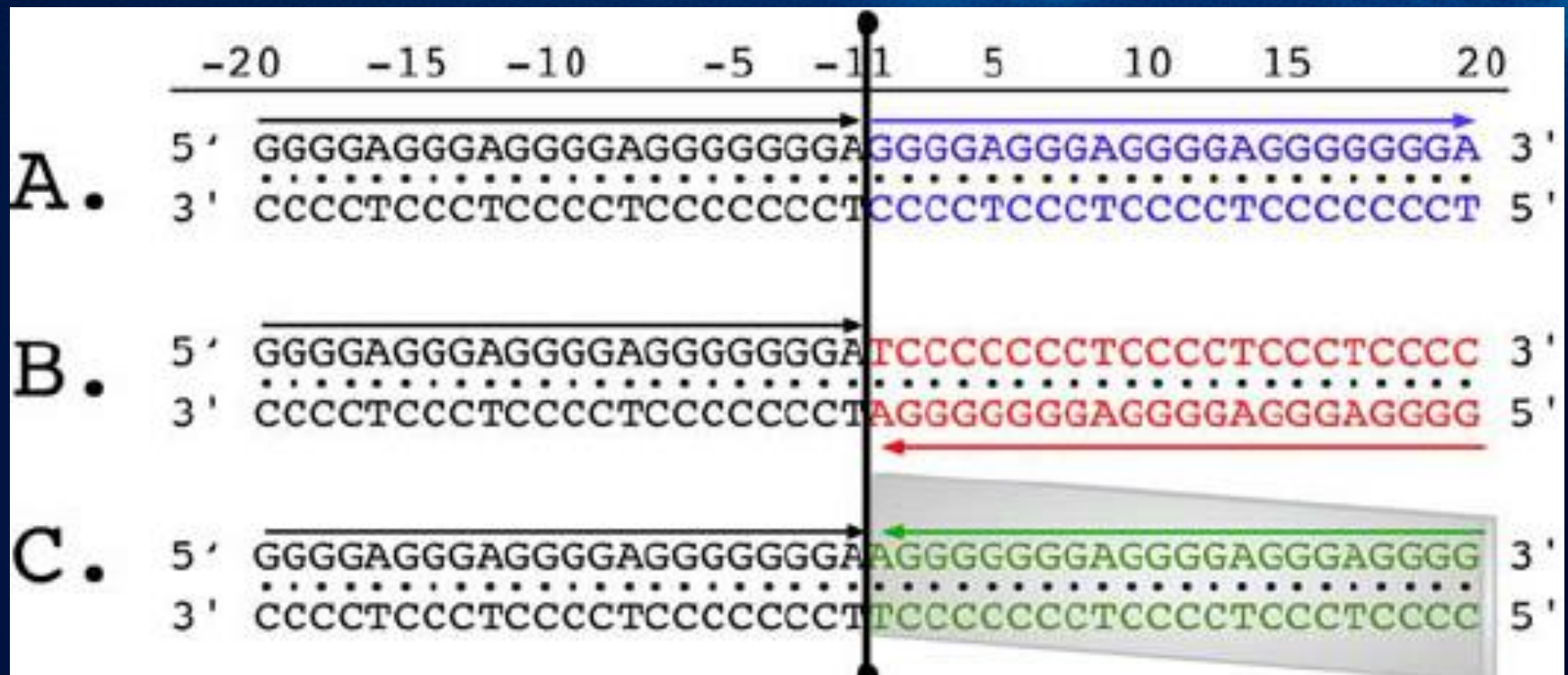
Palindrome



Mirror repeat



Direct repeat





**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.

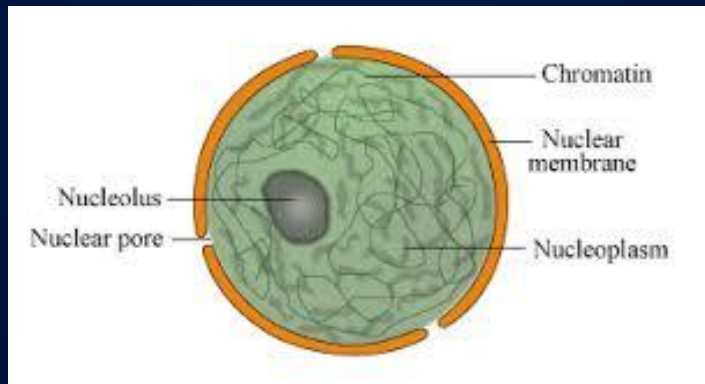
2- unusually repetitive

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

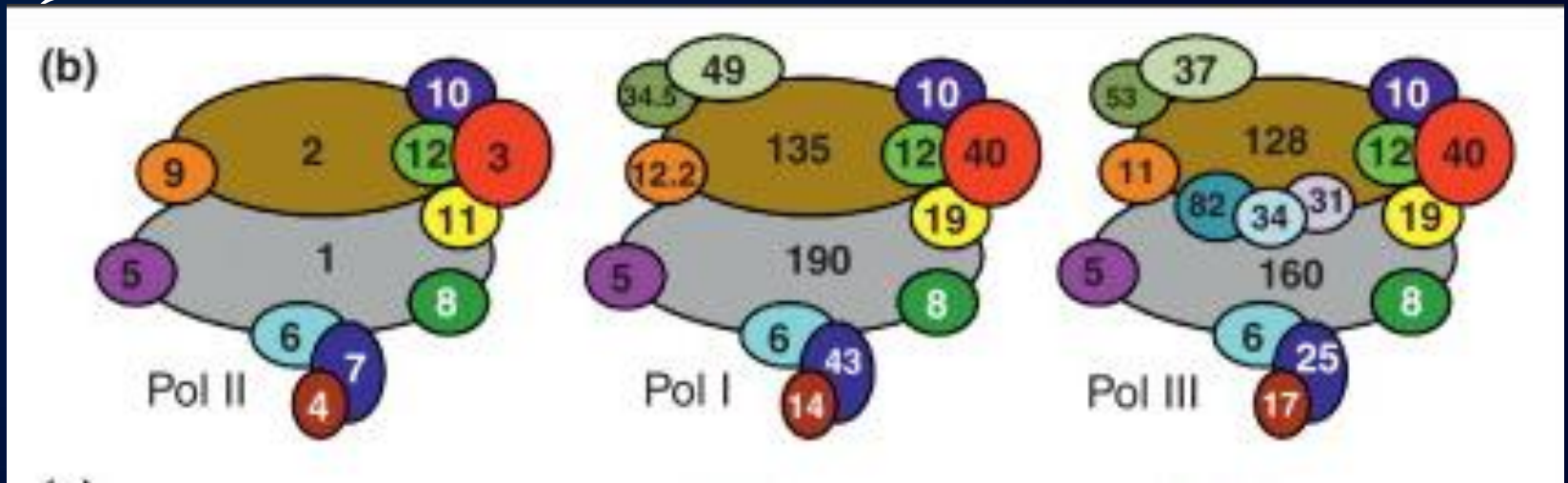
heterogeneous nuclear RNA (hnRNA)
microRNAs (miRNAs)
small nuclear RNAs (snRNAs)

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; β' ortholog
hRPB2	<i>RPB2</i>	139	Contains active site; involved in start site selection, elongation rate; β ortholog
hRPB3	<i>RPB3</i>	35	May function with Rpb1 1 as ortholog of the α 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 α 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

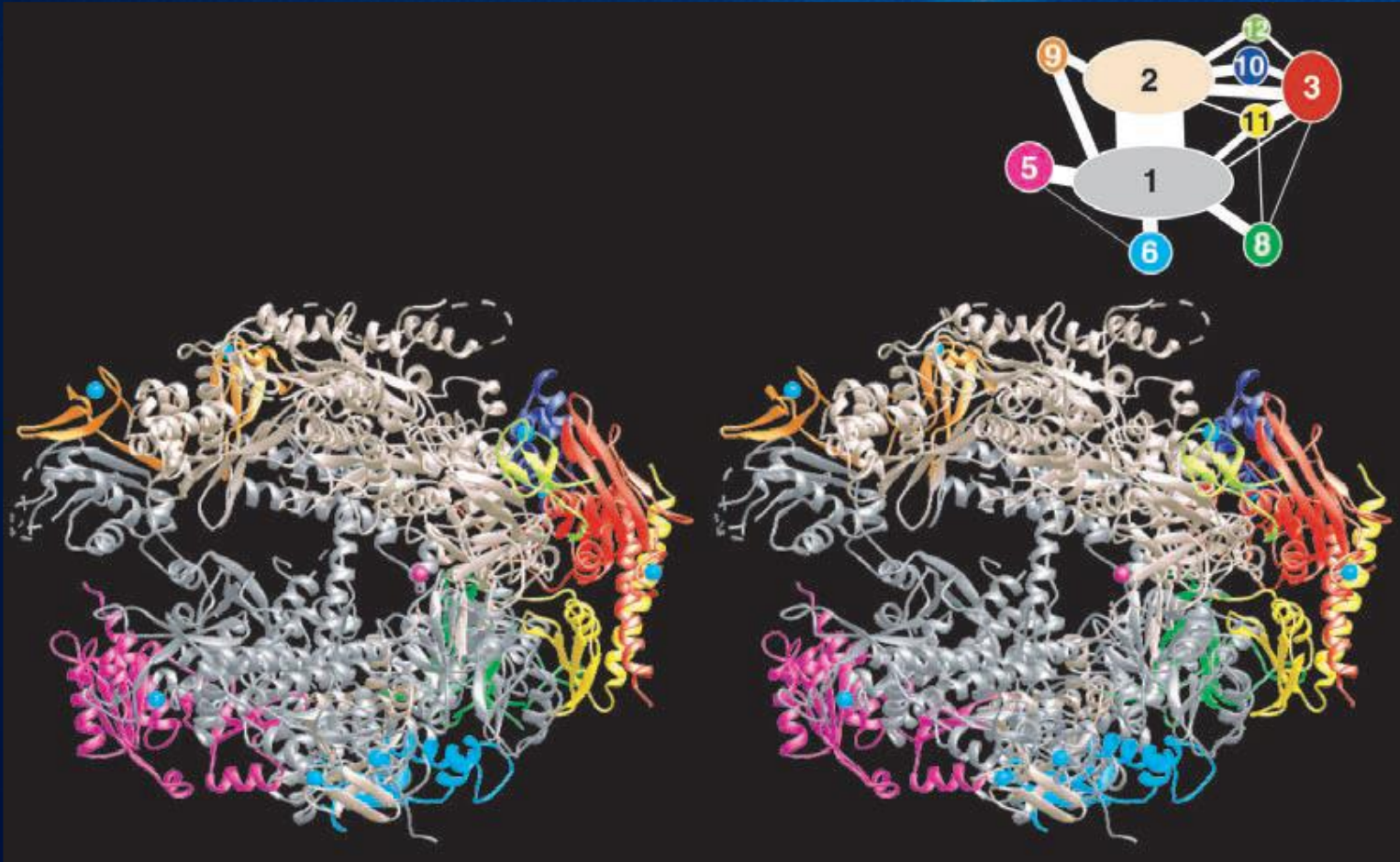
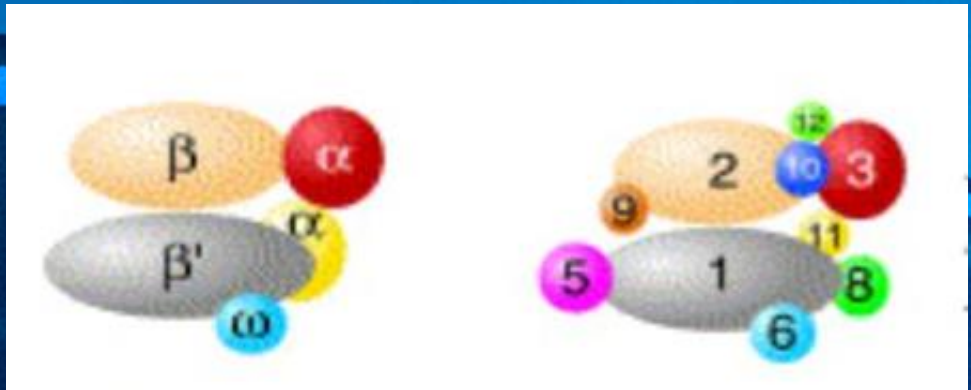
□ **IIo** : serines 2, 5, and sometimes 7 in the heptad are found to be phosphorylated in the IIo subunit.

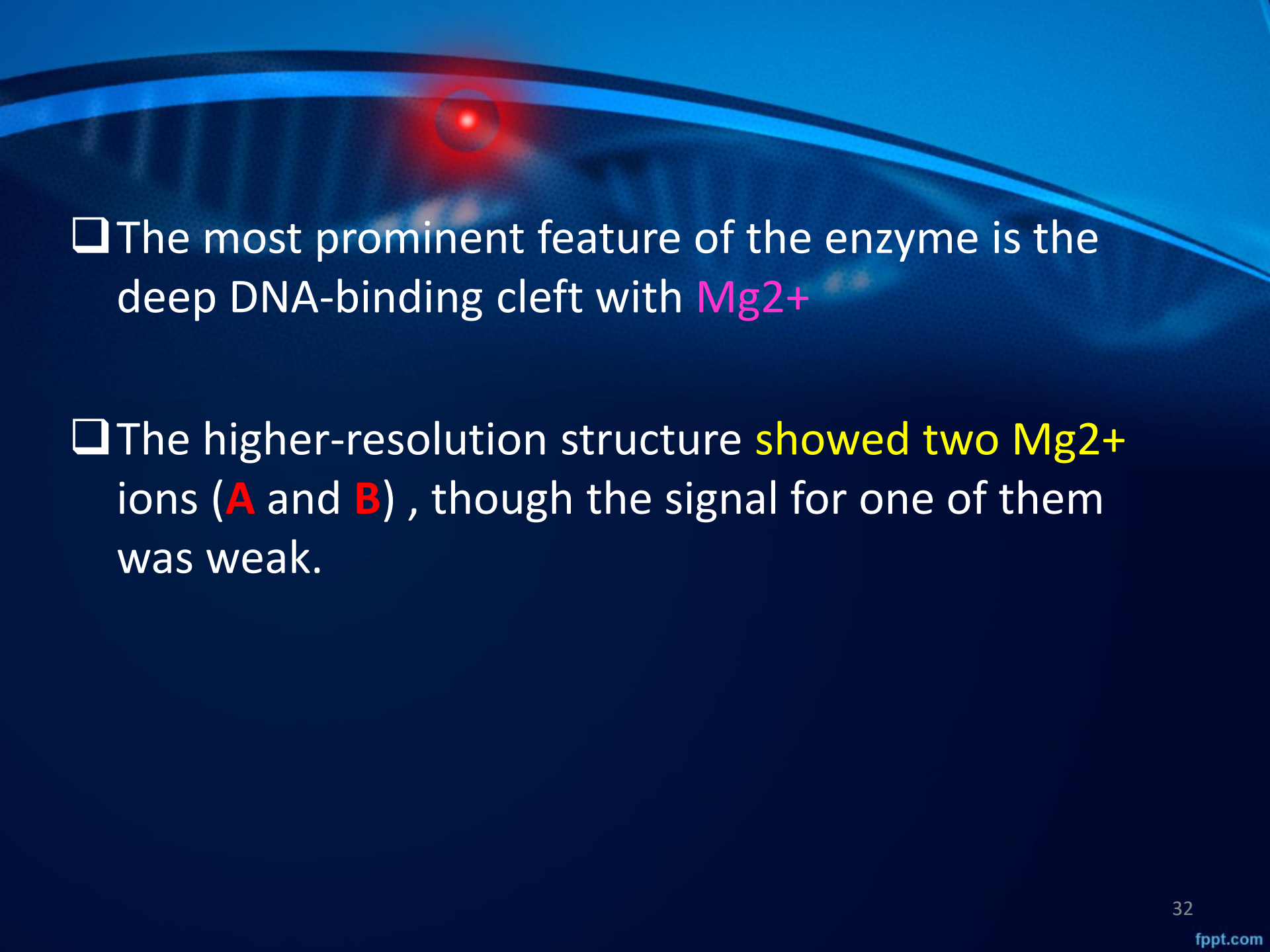


IIA (the unphosphorylated form of the enzyme) is the species that initially **binds to the promoter**.

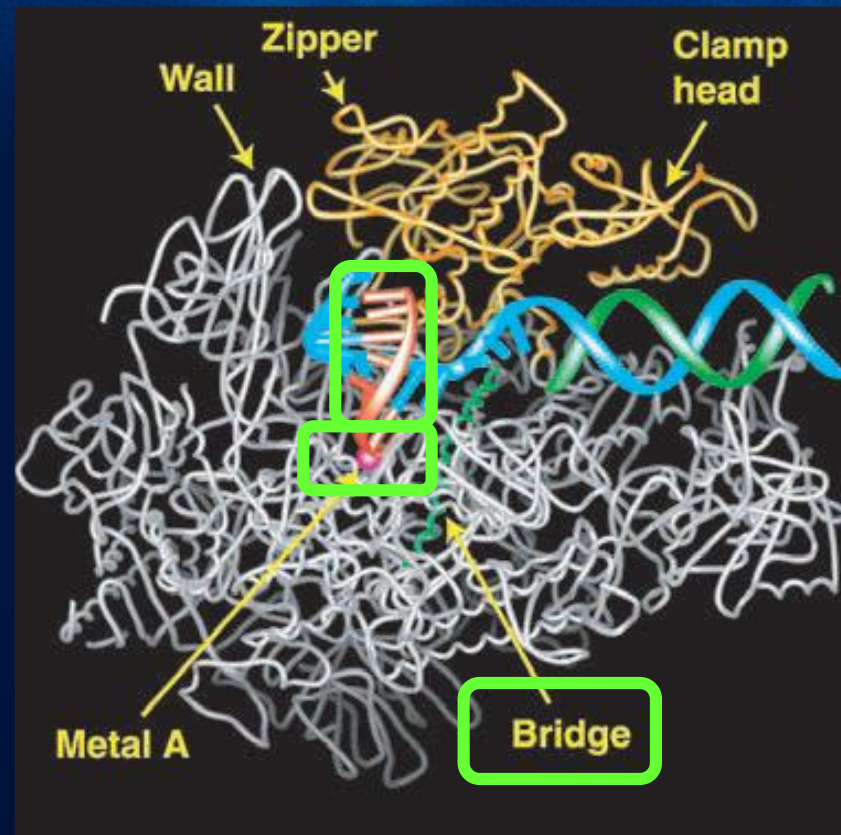
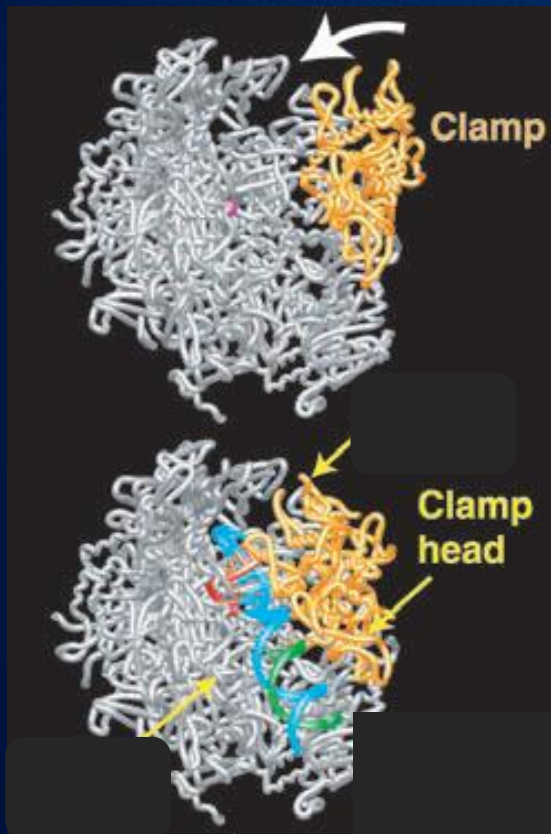
IIO (with its CTD phosphorylated) is the species that carries out **elongation**.

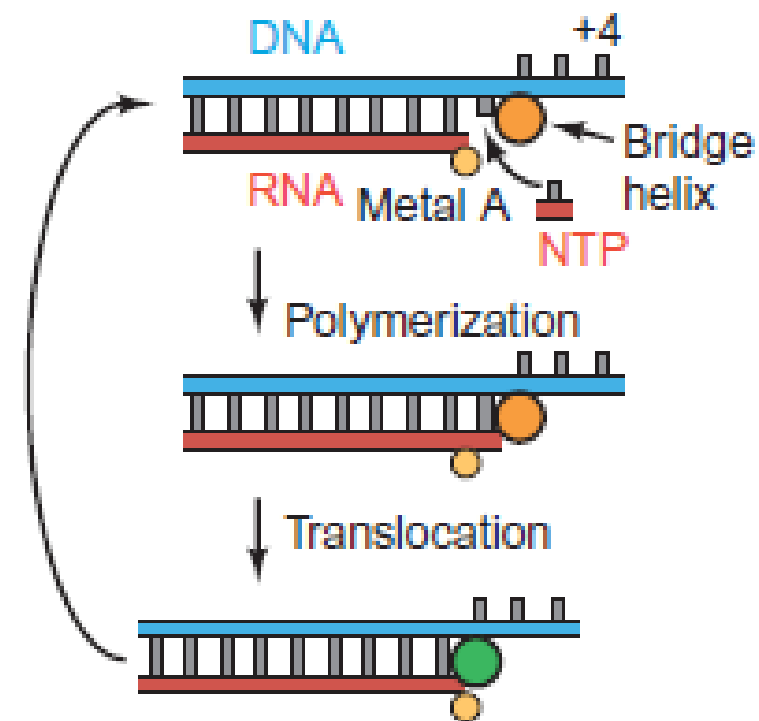
Thus, **phosphorylation** of the CTD appears to accompany the transition from **initiation to elongation**.



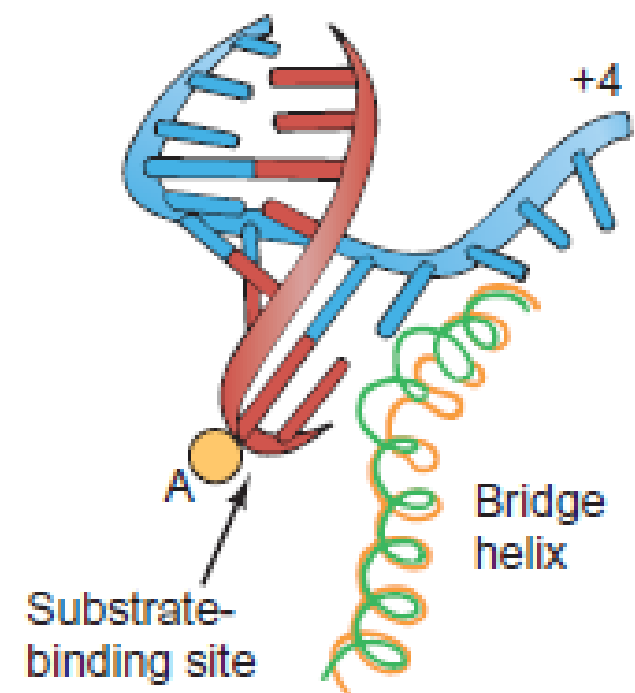
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- The most prominent feature of the enzyme is the deep DNA-binding cleft with Mg^{2+}
 - The higher-resolution structure showed two Mg^{2+} ions (**A** and **B**), though the signal for one of them was weak.

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





(a)



(b)