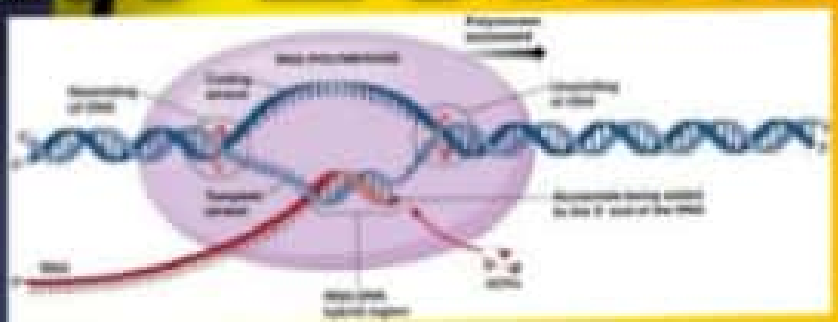
TRANSCRIPTION IN EUKARYOTES



INTRODUCTION

- By the fall of 1953, the working hypothesis was adopted that the chromosomal DNA functions as template for RNA molecule .

- The RNA molecule subsequently moves to the cytoplasm, where they determine the arrangement of amino acid within the proteins.

In 1956, F. Crick referred to this pathway as central dogma .

This pathway is :

TRANSCRIPTION

- It is the synthesis of an RNA molecule from a DNA template.
- All cellular RNAs are synthesized from the DNA templates through this process.
- DNA regions that can be transcribed into RNA are called **structural genes.**
- 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 also called as **sense strand.**
- Only the template strand is used for the transcription, but the coding strand is not.
- only a small portion of DNA is transcribed in response to the development requirement, physiological need and environmental changes.

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, G-C

RNA POLYMERASE

- An enzyme that catalyzes RNA synthesis.
- It does not need a primer, rather it can initiate transcription de novo.
- It performs the same reaction in all cells, from bacteria to humans.
- Bacteria have only a single RNA polymerase.
- Eukaryotes have 3 RNA polymerases i.e. RNA Pol I, II & III.
- Pol II is the most studied of these enzymes, and is responsible for transcribing all protein-encoding genes.
- Pol I & Pol III are responsible for transcribing specialized, Rna-encoding genes.
- The shape of RNA Polymerase resembles a **crab claw.**

STEPS OF TRANSCRIPTION

Transcription by RNA Polymerase proceeds through a series of well-defined steps which are grouped into 3 phases :

- Initiation
- Elongation &
- Termination

EUKARYOTIC TRANSCRIPTION

- Transcription in eukaryotes is undertaken by different RNA polymerases.
- Eukaryotes have 3 polymerases : Pol I, II & III.
- Several initiation factors are required for efficient & promoter-specific initiation in eukaryotes, and are called as **general transcription factors (GTFs).**
- In vitro, the GTFs is required, together with Pol II, to initiate transcription on a DNA template.
- Sometimes the GTFs are not sufficient to promote significant expression. Rather, the additional factors are required such as **mediator complex, DNA binding regulatory proteins** and **chromatin modifying enzymes.**

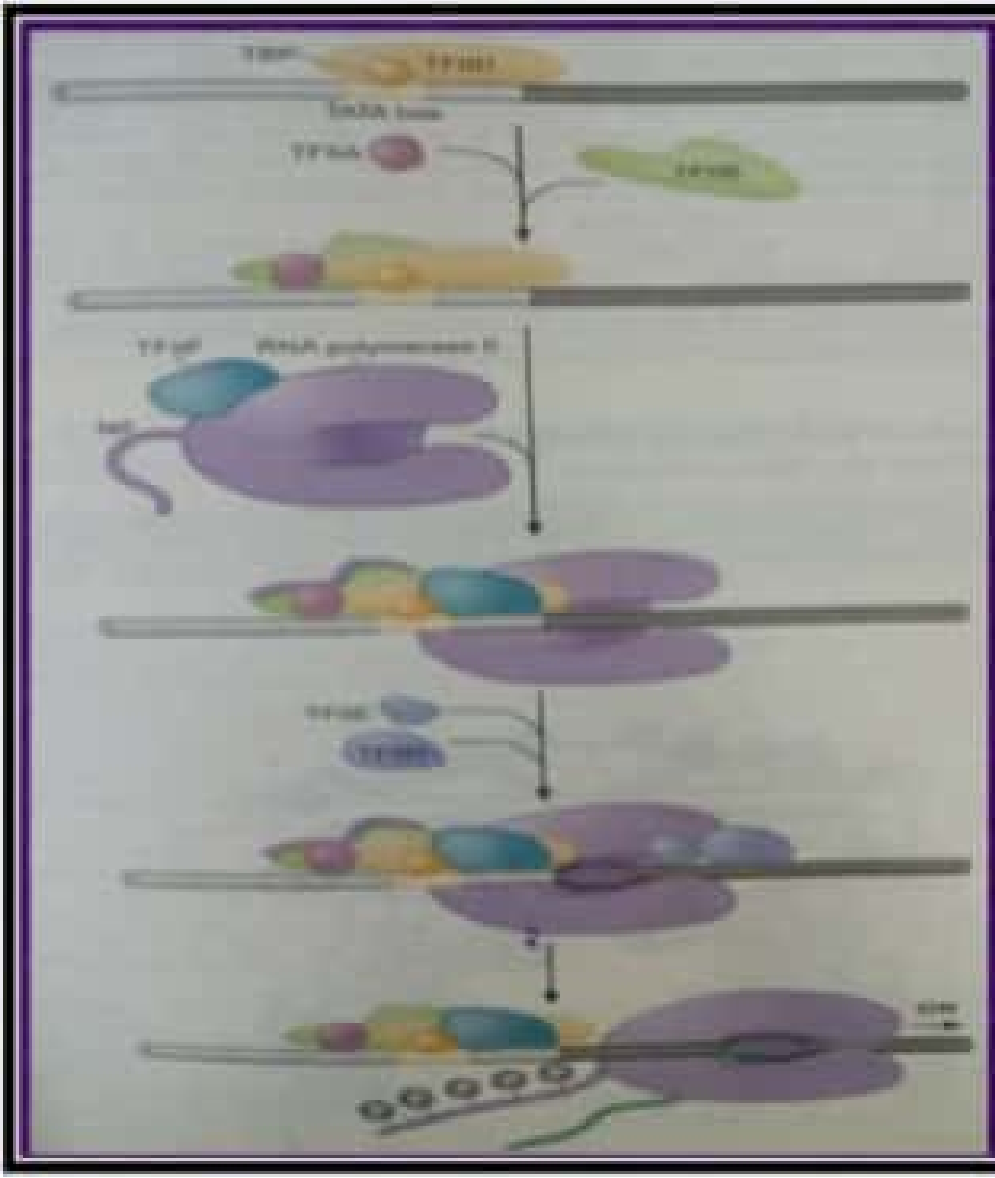
CORE PROMOTER :

- It refers to the minimal set of sequence elements required for accurate transcription initiation by Pol II.
- A core promoter is about 40 nucleotides long, extending either upstream or downstream of the transcription start site.
- Relative to the transcription start site, there are 4 elements found in Pol II core promoter.
- These are the TFIIB recognition element (BRE), the TATA element, the initiator (Inr) & the downstream promoter elements (DPE).
- Promoter includes only 2 or 3 of these 4 elements.

PRE INITIATION COMPLEX FORMATION

- The GTFs help polymerase bind to the promoter and melt DNA.
- The complete set of GTFs & polymerase bound together at the promoter and poised for initiation, is called as **pre-initiation complex**.
- Many Pol II promoters contains **TATA elements**, where pre-initiation complex formation begins.
- The TATA elements recognized by GTFs called **TFIID**.
- The components of TFIID that binds to the TATA DNA sequence is called **TBP**.
- The other subunit is **TAFs** that control the DNA binding activity of TBP.

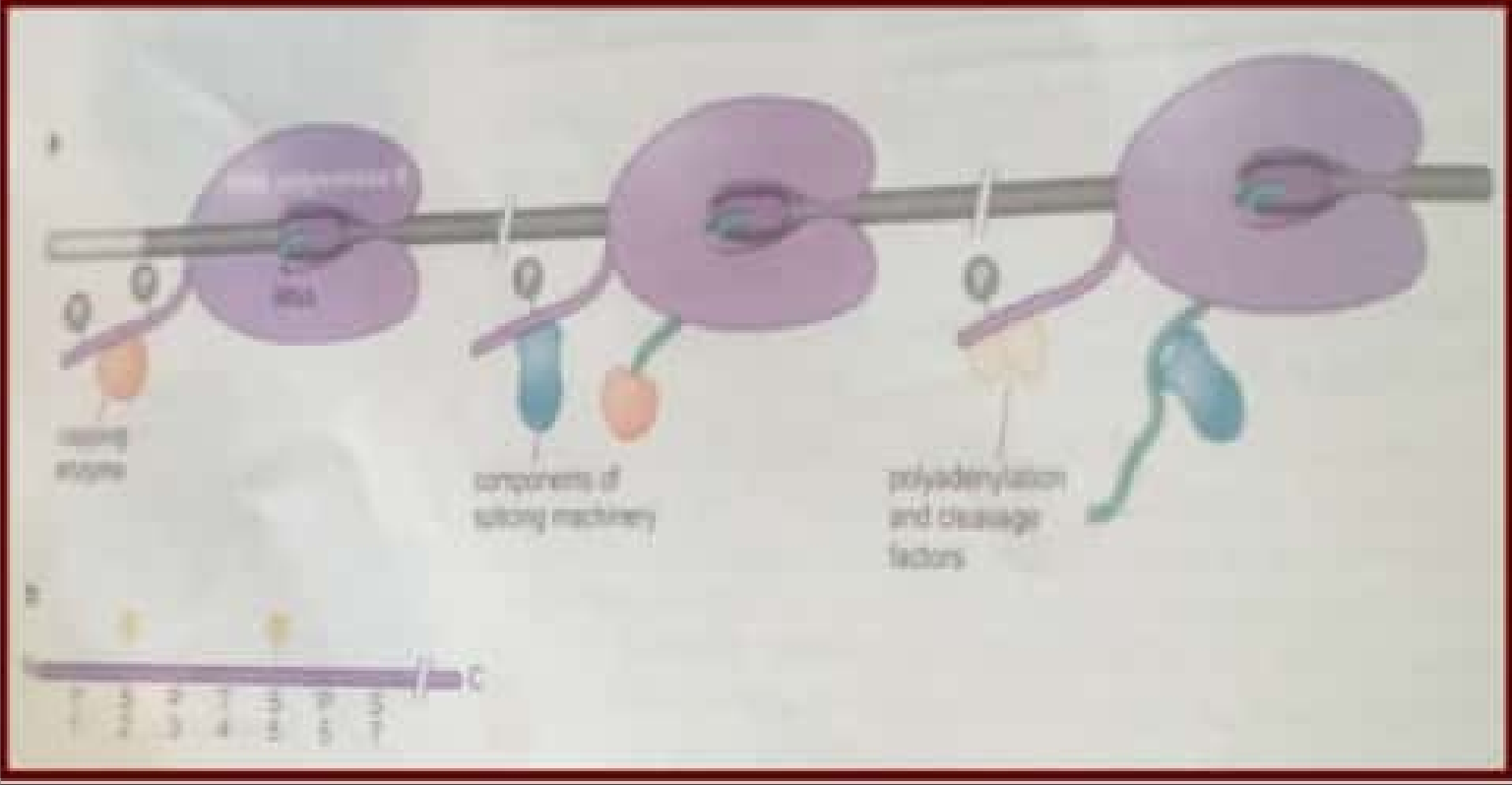
- The resulting TBP-DNA complex provide a platform for attachment of other GTFs & polymerase.
- The factors TFIIA & TFIIB bind to this complex.
- After that TFIIF together with polymerase also bind to the complex.
- At last, the two factors TFIIE & TFIIH bind to upstream of Pol. II resulting in the formation of pre-initiation complex.
- Formation of this complex containing these all components is followed by promoter melting.
- Promoter melting in eukaryotes requires hydrolysis of ATP and is mediated by TFIIH.
- The large subunit of Pol. II has a C-terminal domain (CTD), which extends as a 'tail'.
- The CTD contains a series of repeats of heptapeptide sequence: Tyr-Ser-Pro-Thr-Ser-Pro-Ser.



ELONGATION

- Once polymerase has initiated transcription, it shifts into the elongation phase.
- Elongation requires another set of factors, such as **TFIIS & hSPT5, known as elongation factors.**
- This factors stimulate elongation and also required for RNA processing.
- These factors also favor the phosphorylated form of CTD. The phosphorylation of CTD leads to an exchange of initiation factors with elongation factors.
- Various proteins are thought to stimulate elongation by Pol II.
- The **protein P-TEFb** stimulates elongation in 3 separate steps.
- This protein bound to Pol II and phosphorylates the serine residue at position 2 of the CTD repeats .

- This P-TEFb also activates another protein, called hSPT5 which is an elongation factor.
- At last, this P-TEFb activates one another elongation factor called TAT-SF1.

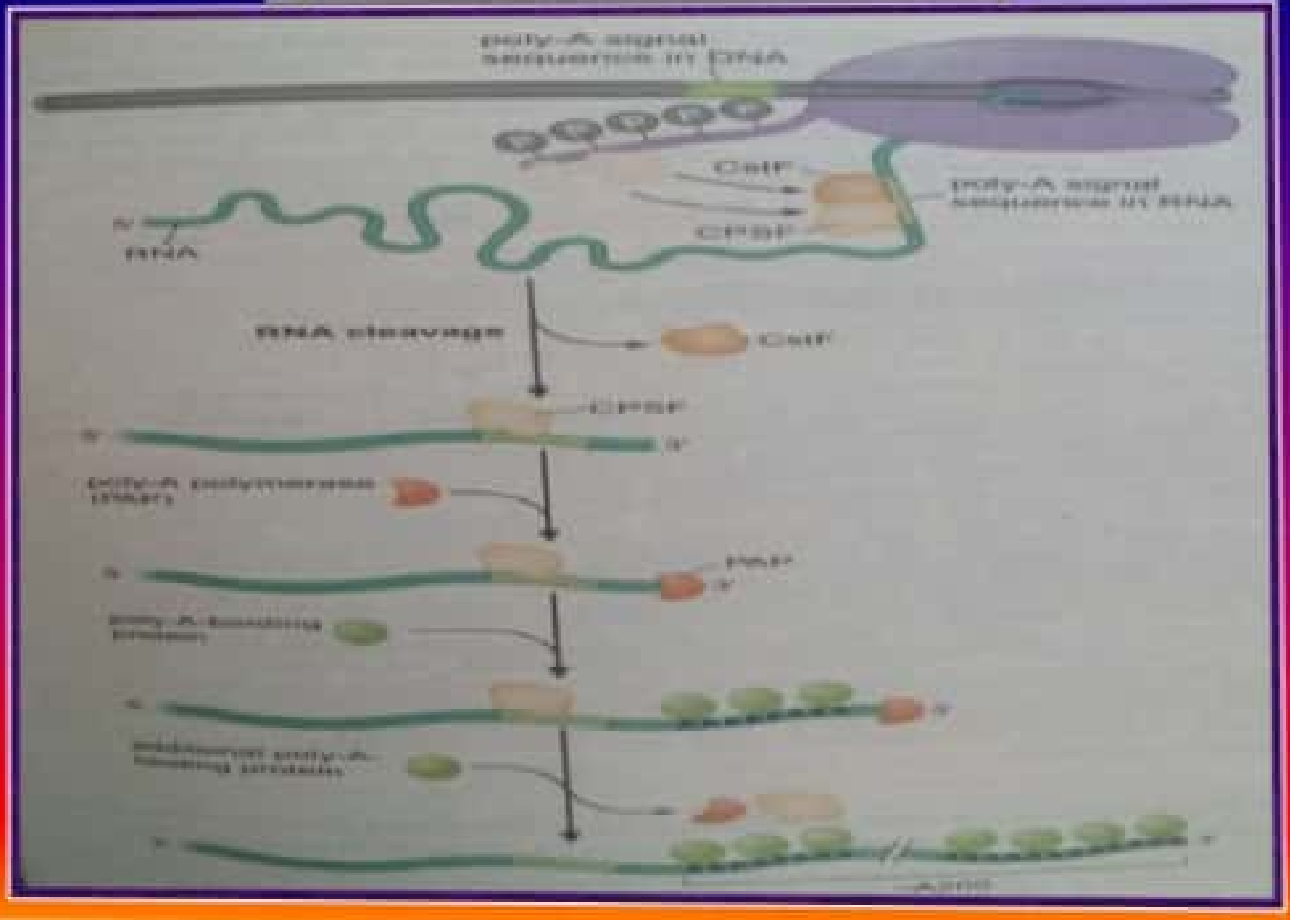


POLYADENYLATION & TERMINATION

- Once the elongation is completed, it proceeds through the RNA processing events i.e. polyadenylation and termination.
- Polyadenylation occurs at the 3' end of the mRNA which is linked with the termination of transcription.
- The polymerase CTD tail is involved in recruiting the enzymes necessary for polyadenylation.
- Two protein complexes are carried by the CTD of polymerase called, CPSf (cleavage & polyadenylation specificity factor) & CstF (cleavage stimulation factor).
- The sequences which one transcribed into RNA, trigger transfer of these factors to the RNA , are called poly-A signals.
- Once CPSF & CstF bound to the RNA, it results in RNA cleavage and then polyadenylation.

- After cleavage of RNA, polyadenylation is mediated by an enzyme called poly-A polymerase (PAP) followed by the addition of poly-A binding protein.
- This protein along with the enzyme uses ATP as precursor and adds the nucleotides, using the same chemistry as RNA polymerase.
- Before termination, the RNA molecule become very long due to addition of several nucleotides.
- The polymerase along with CPSF & PAP then dissociates from the template, releasing the new RNA, which is degraded without ever leaving the nucleus.
- This involves the termination of RNA, i.e. the mature mRNA is released from polymerase and then transported from the nucleus.

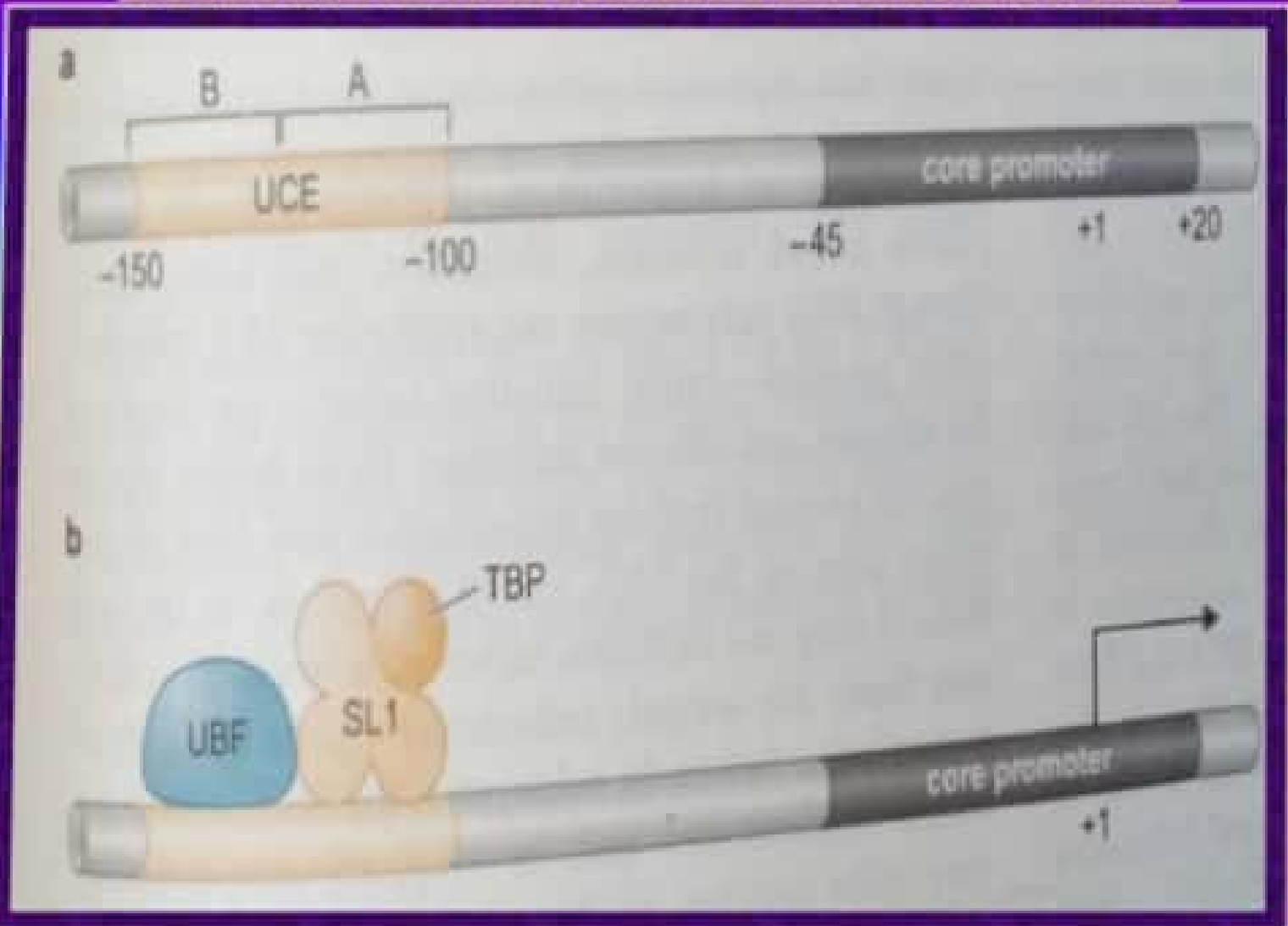
POLYADENYLATION & TERMINATION

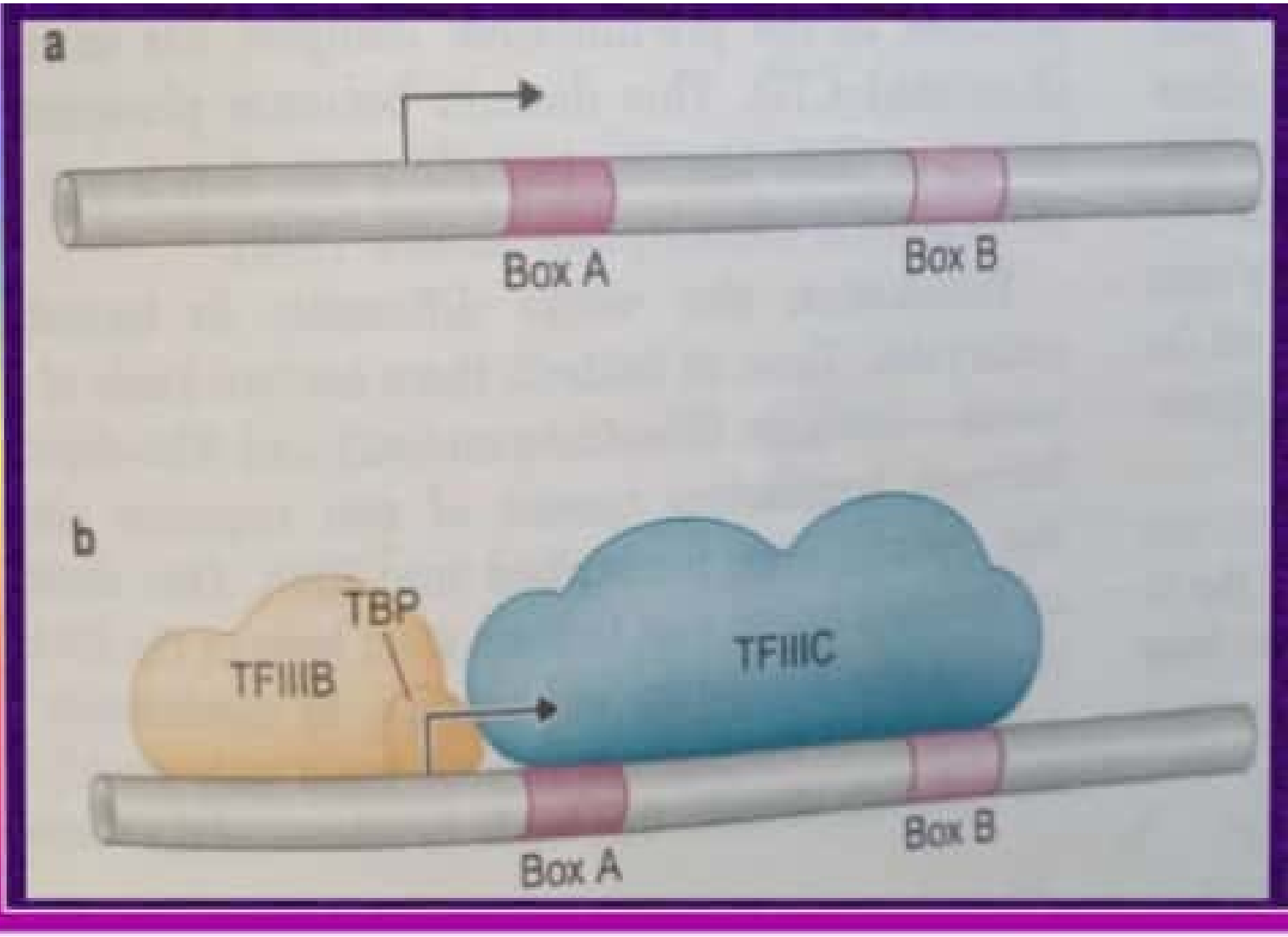


RNA POLYMERASE I

- This enzyme is related to Pol. II, but they initiate transcription from distinct promoters and transcribed distinct genes.
- Pol I is required for the expression of only one gene that encoding the rRNA precursor.
- The promotor of rRNA genes comprises 2 parts : core elements & UCE (upstream control element).
- In addition to Pol. I, initiation requires two other factors, called SL1 & UBF.
- SL1 comprises TBP & three TAFs specific for transcription.
- These complex bound to the UCE in the presence of UBF and stimulates transcription fromcore promoter by recruiting Pol. I.

TRANSCRIPTION INITIATION BY RNA POLYMERASE I





TRANSCRIPTION INITIATION BY RNA POLYMERASE III