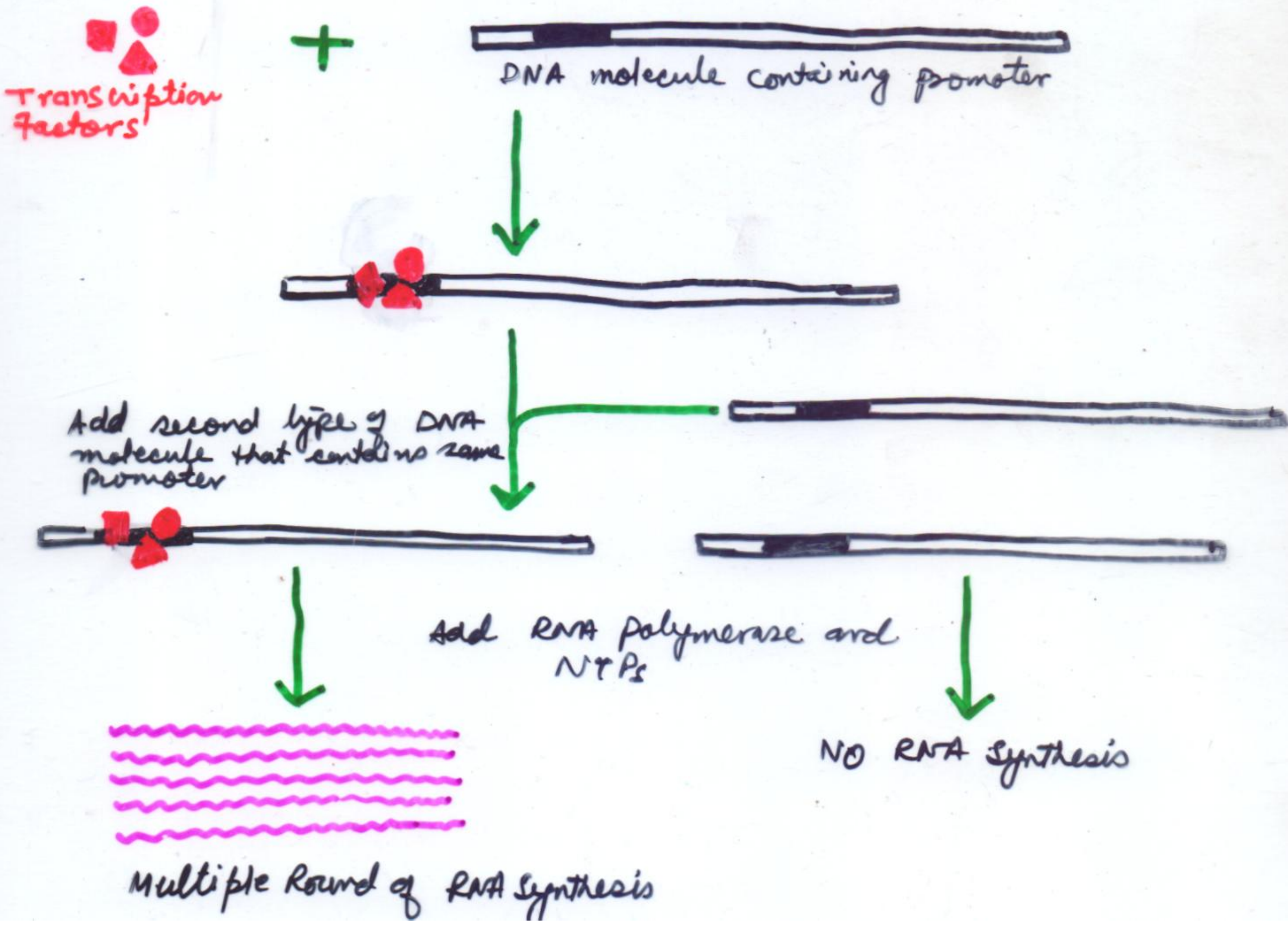
SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 205: Fundamentals of Molecular Biology (Unit III)

Eukaryotic Transcription - I

Transcription in Eukaryotes

1. **It has been experimentally shown that a DNA molecule complexed with the appropriate factors is used repeatedly for RNA synthesis, whereas a second DNA molecule that is otherwise identical is ignored.**
2. **Any protein that is needed for the initiation of transcription, but which is not itself the part of RNA polymerase is defined as transcription factor.**
3. **Many transcription factors act by recognizing cis acting sites that are classified as comprising parts of promoters and enhancers.**
4. **A factor may recognize another factor or may recognize RNA polymerase or possibly may be incorporated into an initiation complex only in the presence of several other proteins.**


Transcription factors
+
DNA molecule containing promoter
Add second type of DNA molecule that contains same promoter
Add RNA polymerase and NTPs
Multiple Round of RNA Synthesis
NO RNA Synthesis

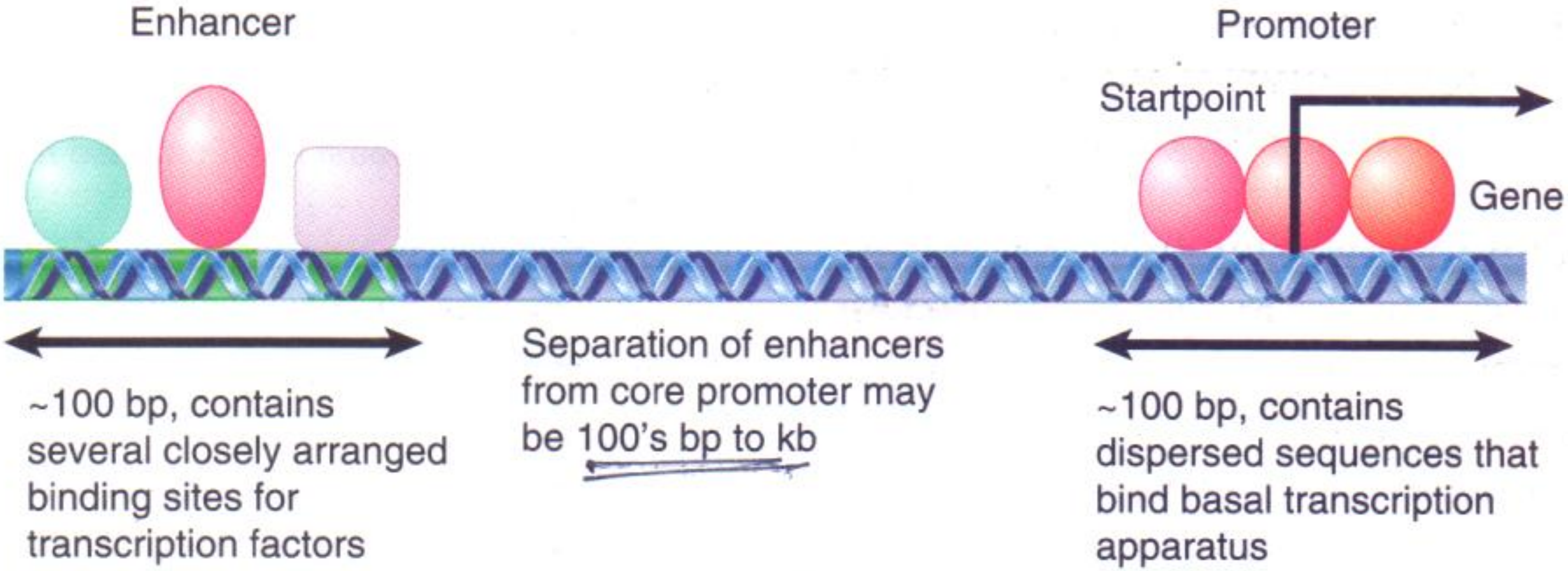
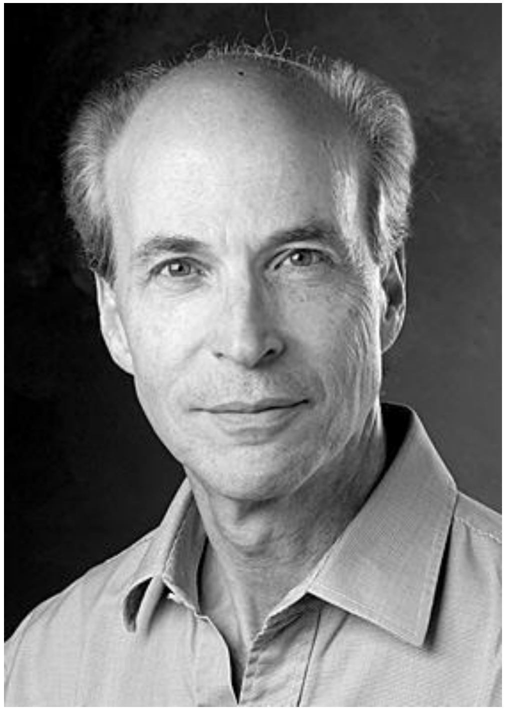


FIGURE 20.1 A typical gene transcribed by RNA polymerase II has a promoter that extends upstream from the site where transcription is initiated. The promoter contains several short (~10 bp) sequence elements that bind transcription factors, dispersed over ~100 bp. An enhancer containing a more closely packed array of elements that also bind transcription factors may be located several hundred bp to several kb distant. (DNA may be coiled or otherwise rearranged so that transcription factors at the promoter and at the enhancer interact to form a large protein complex.)

Nobel Prize for Chemistry in 2006



Roger D. Kornberg
Born: 24 April 1947, St. Louis, MO, USA
Field: Biochemistry, Structural chemistry
Prize share: 1/1

- Kornberg discovered mediator complex in eukaryotic transcriptional initiation.
- In fact Kornberg crated an actual picture of how transcription work at molecular level.

Work: “for his studies of the molecular basis of eukaryotic transcription”

Eukaryotic RNA Polymerases

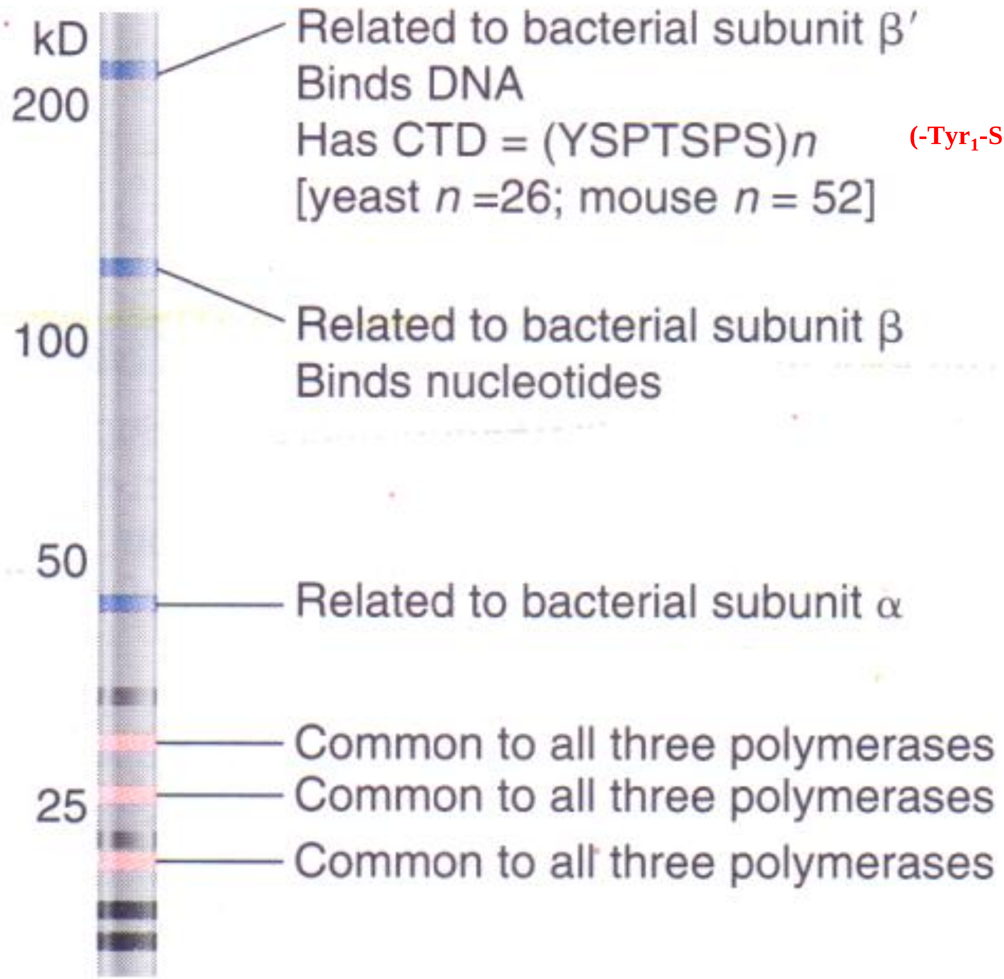
Name	Location	Product
RNA Polymerase I (Pol I, Pol A)	nucleolus	larger ribosomal RNA (rRNA) (28S, 18S, 5.8S)
RNA Polymerase II (Pol II, Pol B)	nucleus	messenger RNA (mRNA), most small nuclear RNAs (snRNAs), small interfering RNA (siRNAs) and micro RNA (miRNA).
RNA Polymerase III (Pol III, Pol C)	nucleus (and possibly the nucleolus-nucleoplasm interface)	transfer RNA (tRNA), other small RNAs (including the small 5S ribosomal RNA (5s rRNA), snRNA U6,signal recognition particle RNA (SRP RNA) and other stable short RNAs

Eukaryotic RNA Polymerases consist of many subunits

1. RNA polymerase I synthesizes rRNA in the nucleolus
2. RNA polymerase II synthesizes mRNA in the nucleoplasm
3. RNA polymerase III synthesizes small RNAs in the nucleoplasm
4. All eukaryotic RNA polymerases have ~8-14 subunits (~12 subunits) and are complexes of ~500 kD

#	Polymerases	Subunits
1	I	14
2	II	12
3	III	17

5. Some subunits are common to all three RNA polymerases
6. The largest subunit in RNA polymerase II has a CTD (carboxyl terminal domain) consisting of multiple repeats of heptamer.

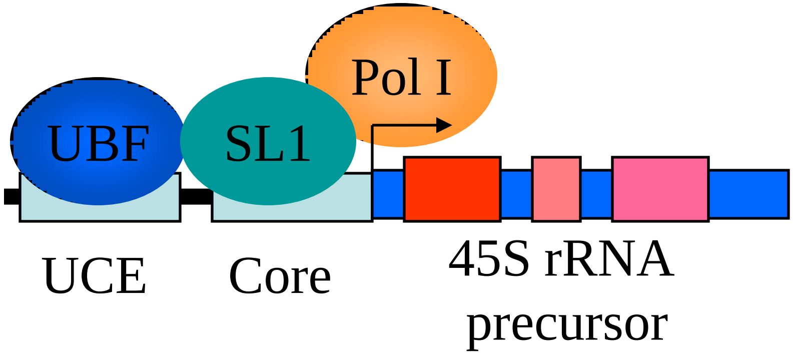


$(-Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7-)n$

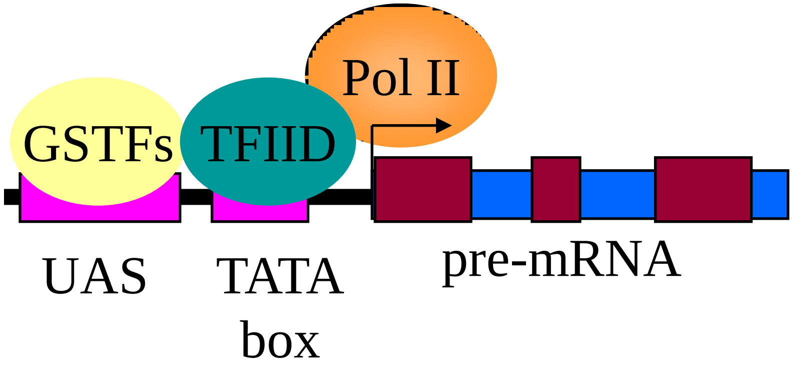
FIGURE 20.2 Some subunits are common to all classes of eukaryotic RNA polymerases and some are related to bacterial RNA polymerase. This drawing is a simulation of purified yeast RNA polymerase II run on an SDS gel to separate the subunits by size.

Eukaryotic Promoters

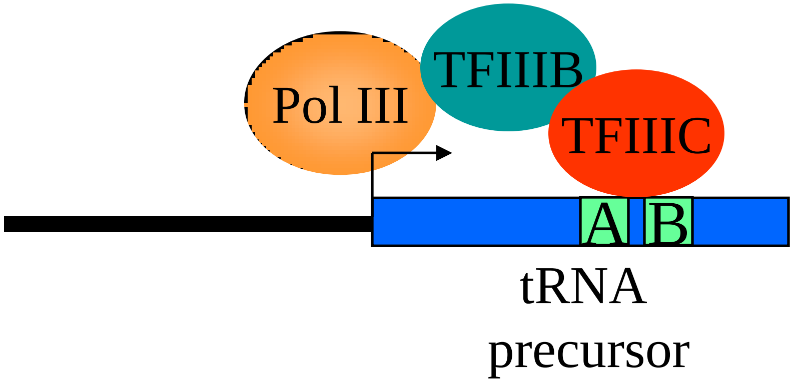
- RNA Pol I
  - rRNA precursor



- RNA Pol II
  - mRNAs,
    U6 snRNA



- RNA Pol III
  - tRNA, 5S rRNA,
    U1-U5 snRNAs



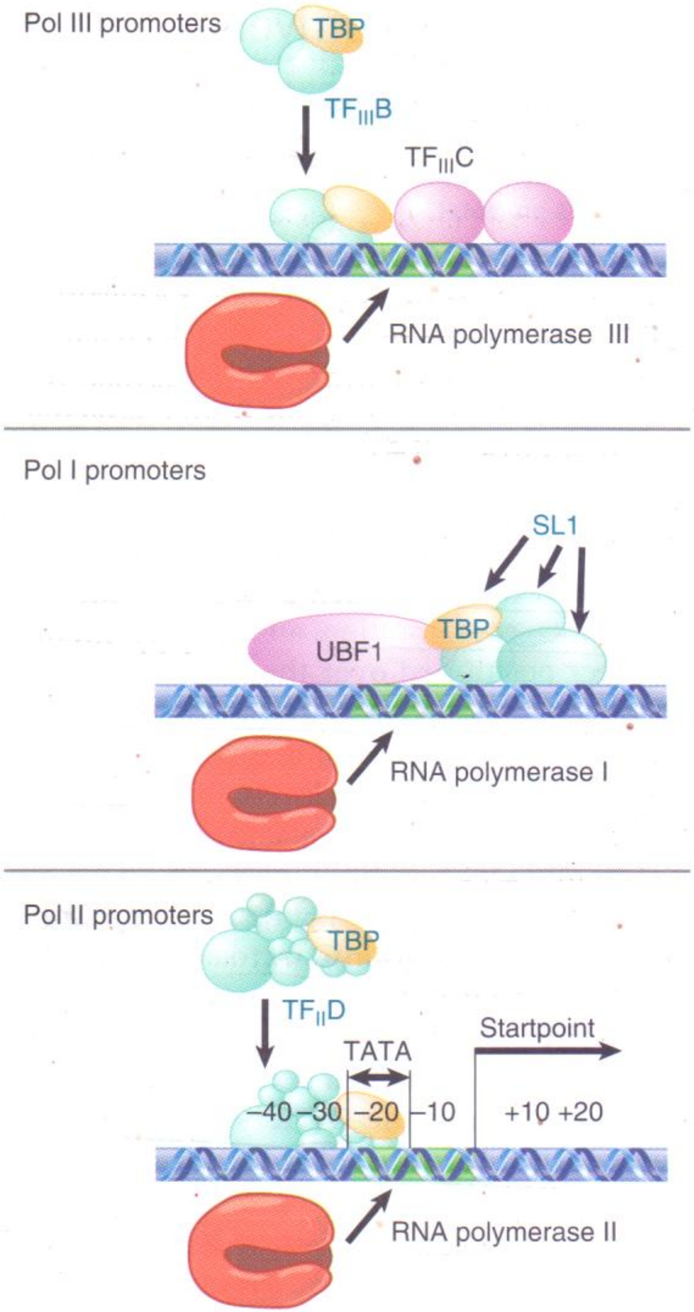


FIGURE 20.8 RNA polymerases are positioned at all promoters by a factor that contains TBP.

Initiation of Transcription

by

Eukaryotic RNA Polymerase I, II and III

RNA Polymerase I

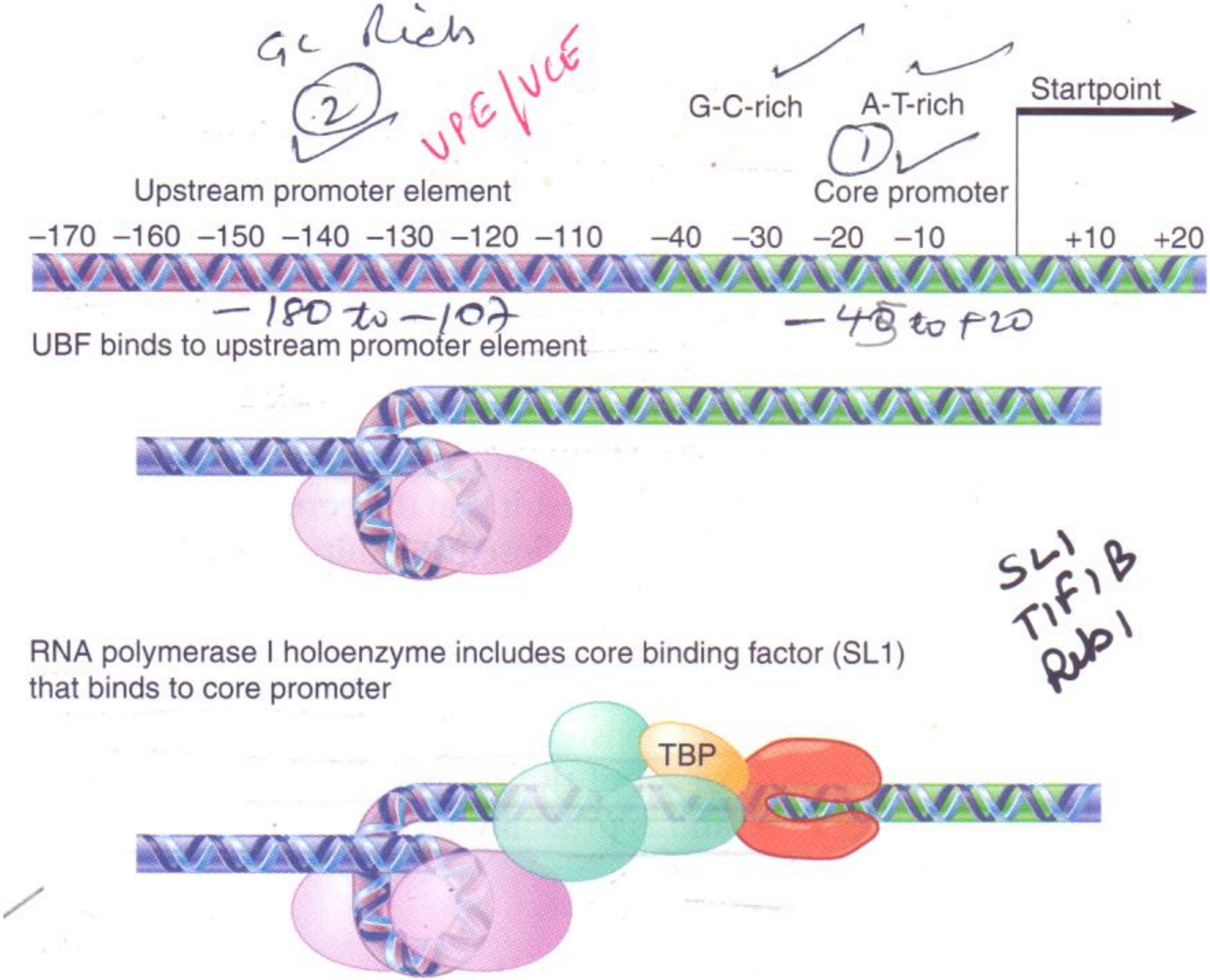
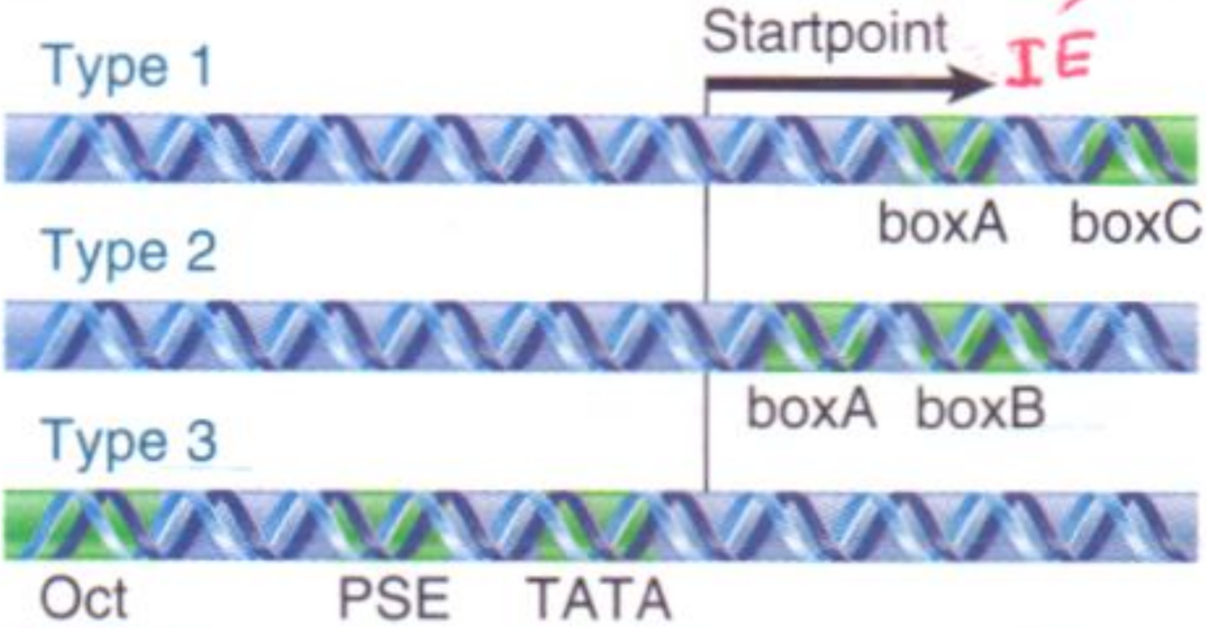


FIGURE 20.3 Transcription units for RNA polymerase I have a core promoter separated by ~70 bp from the upstream promoter element. UBF binding to the UPE increases the ability of core-binding factor to bind to the core promoter. Core-binding factor (SL1) positions RNA polymerase I at the startpoint.

RNA Polymerase III

PSE = proximal sequence element

5S ICR = box A - IE - box C



IE — Intermediate Element

5S rRNA
~~tRNA~~

tRNA

Sn RNA

FIGURE 20.4 Promoters for RNA polymerase III may consist of bipartite sequences downstream of the startpoint, with *boxA* separated from either *boxC* or *boxB*, or they may consist of separated sequences upstream of the startpoint (Oct, PSE, TATA).

type 2

tRNA

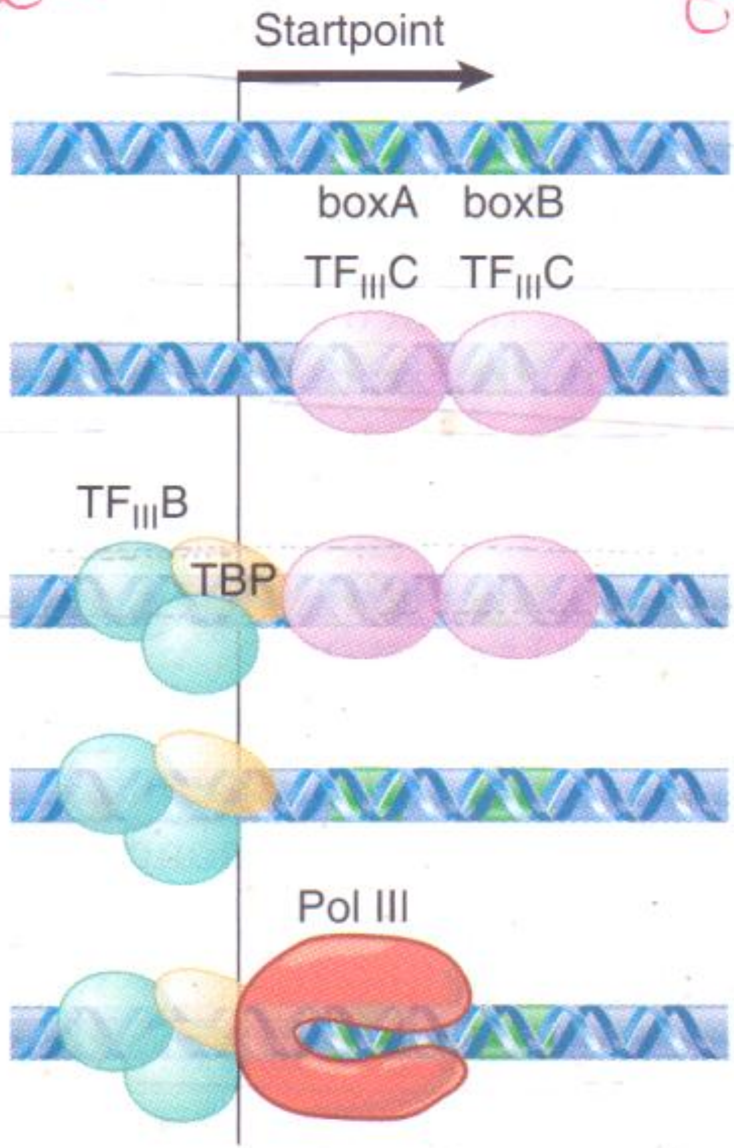


FIGURE 20.5 Internal type 2 pol III promoters use binding of $TF_{III}C$ to *boxA* and *boxB* sequences to recruit the positioning factor $TF_{III}B$, which recruits RNA polymerase III.

type 1

5S rRNA

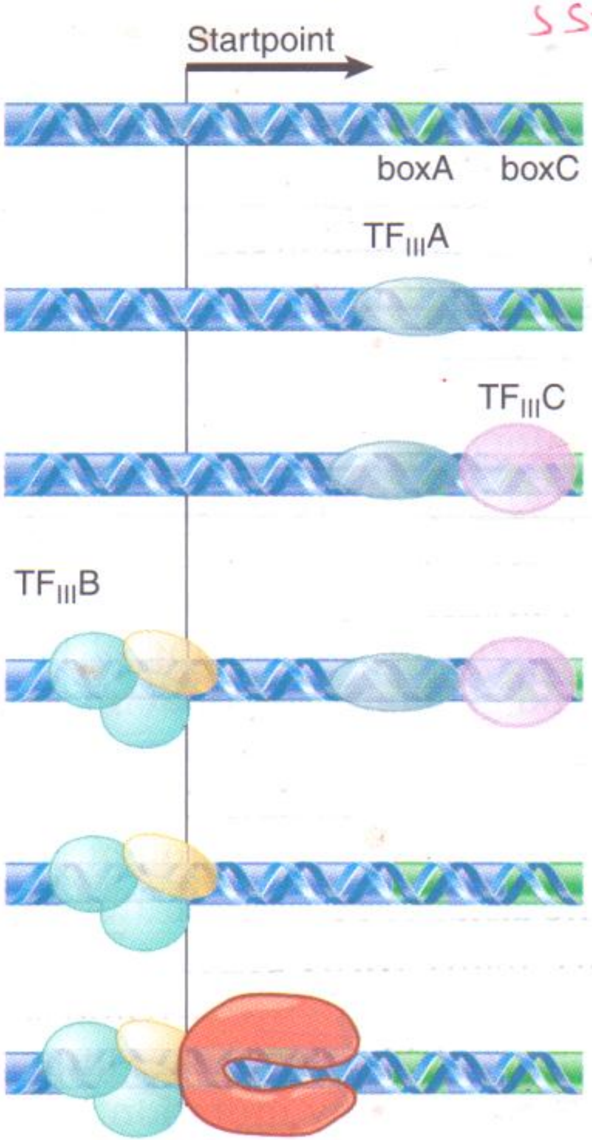
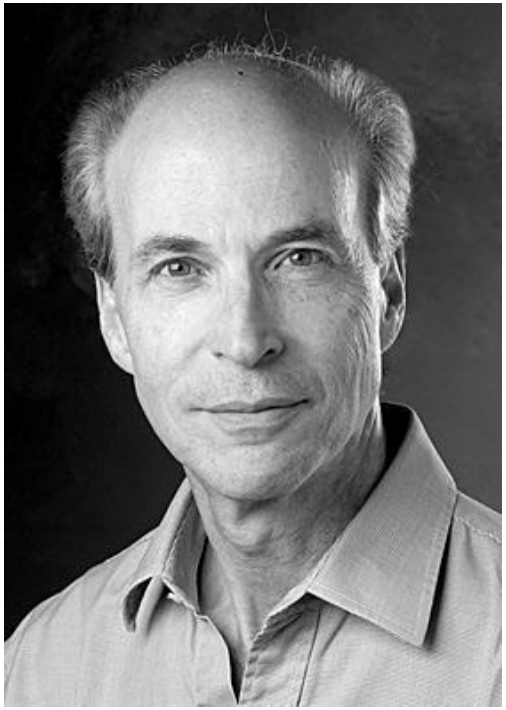


FIGURE 20.6 Internal type 1 pol III promoters use the assembly factors TF$_{III}$A and TF$_{III}$C, at *boxA* and *boxC*, to recruit the positioning factor TF$_{III}$B, which recruits RNA polymerase III.

RNA Polymerase II

Nobel Prize for Chemistry in 2006



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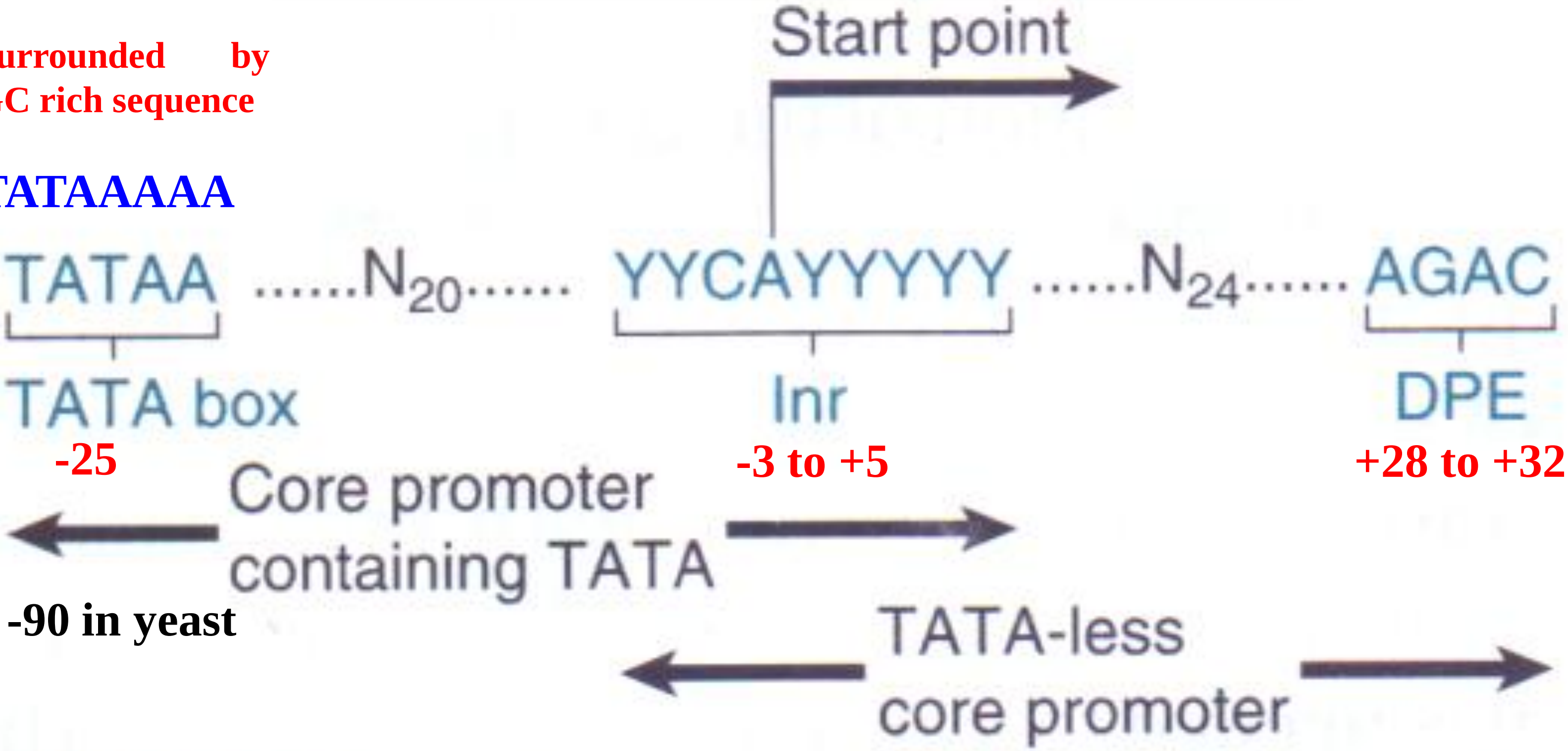
PROMOTER ORGANIZATION

(for RNA Pol II)

Pol II Core Promoter Elements

Surrounded by GC rich sequence

TATAAAAA



-25

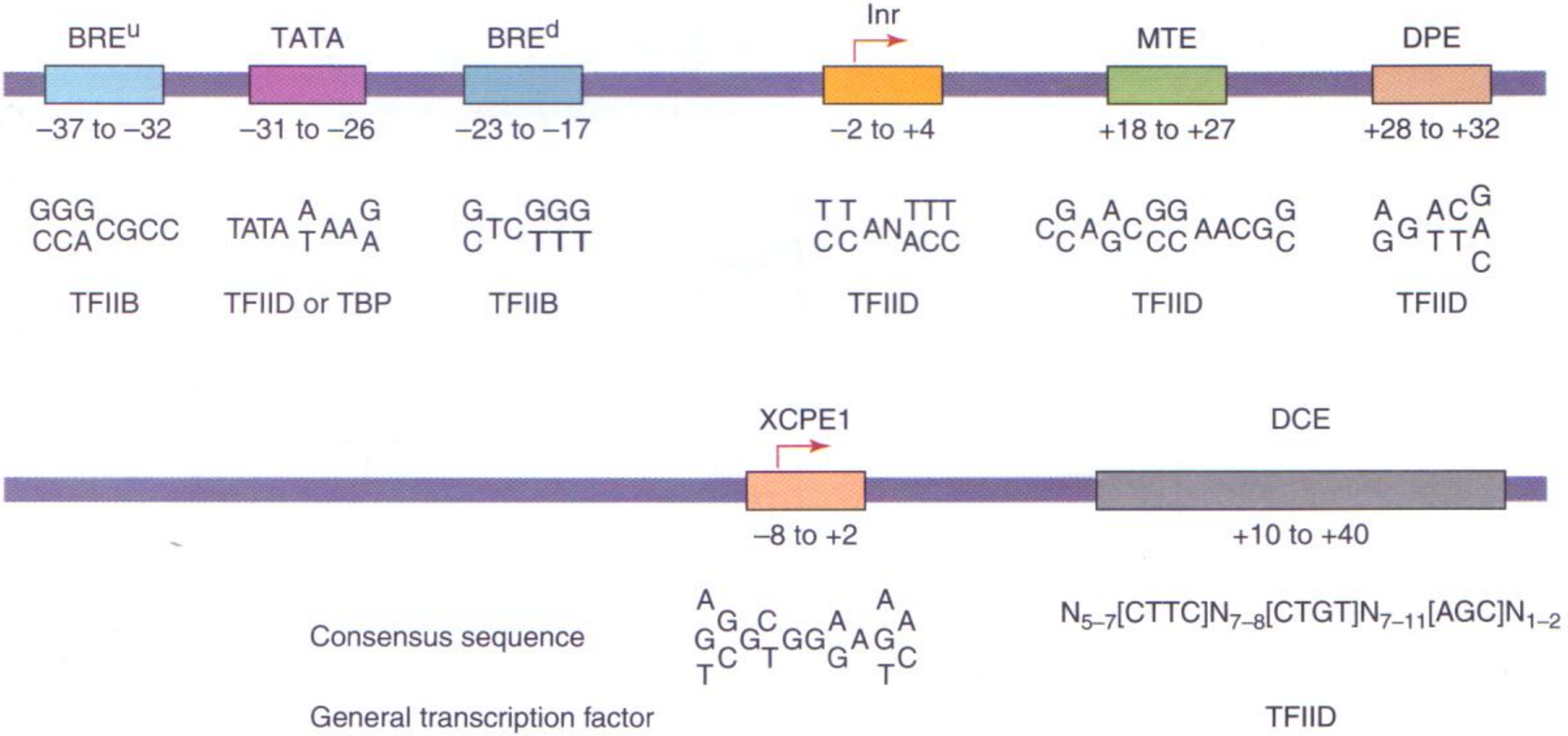
-3 to +5

+28 to +32

-90 in yeast

FIGURE 20.7 A minimal pol II promoter may have a TATA box ~25 bp upstream of the Inr. The TATA box has the consensus sequence of TATAA. The Inr has pyrimidines (Y) surrounding the CA at the start point. The DPE is downstream of the start point. The sequence shows the coding strand. **(Core Promoter Elements = TATA box+ Inr or Inr+DPE)**

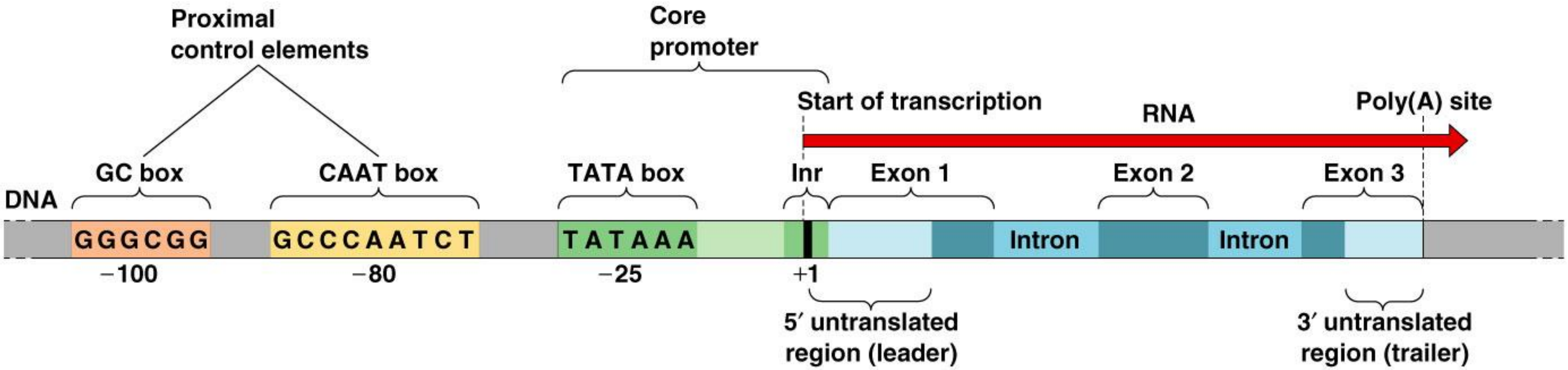
(Other combinations with minor elements also exist)



DPE – **Downstream Core Promoter Element**
MTE – **Motif Ten Element**
DCE – **Downstream Core Element**
BRE – **TFIIB Recognition Element**
XCPE1 – **X Core Promoter Element**

Anatomy of a Typical Eukaryotic Gene, with Its Core Promoter and Proximal Control Region

-----control elements

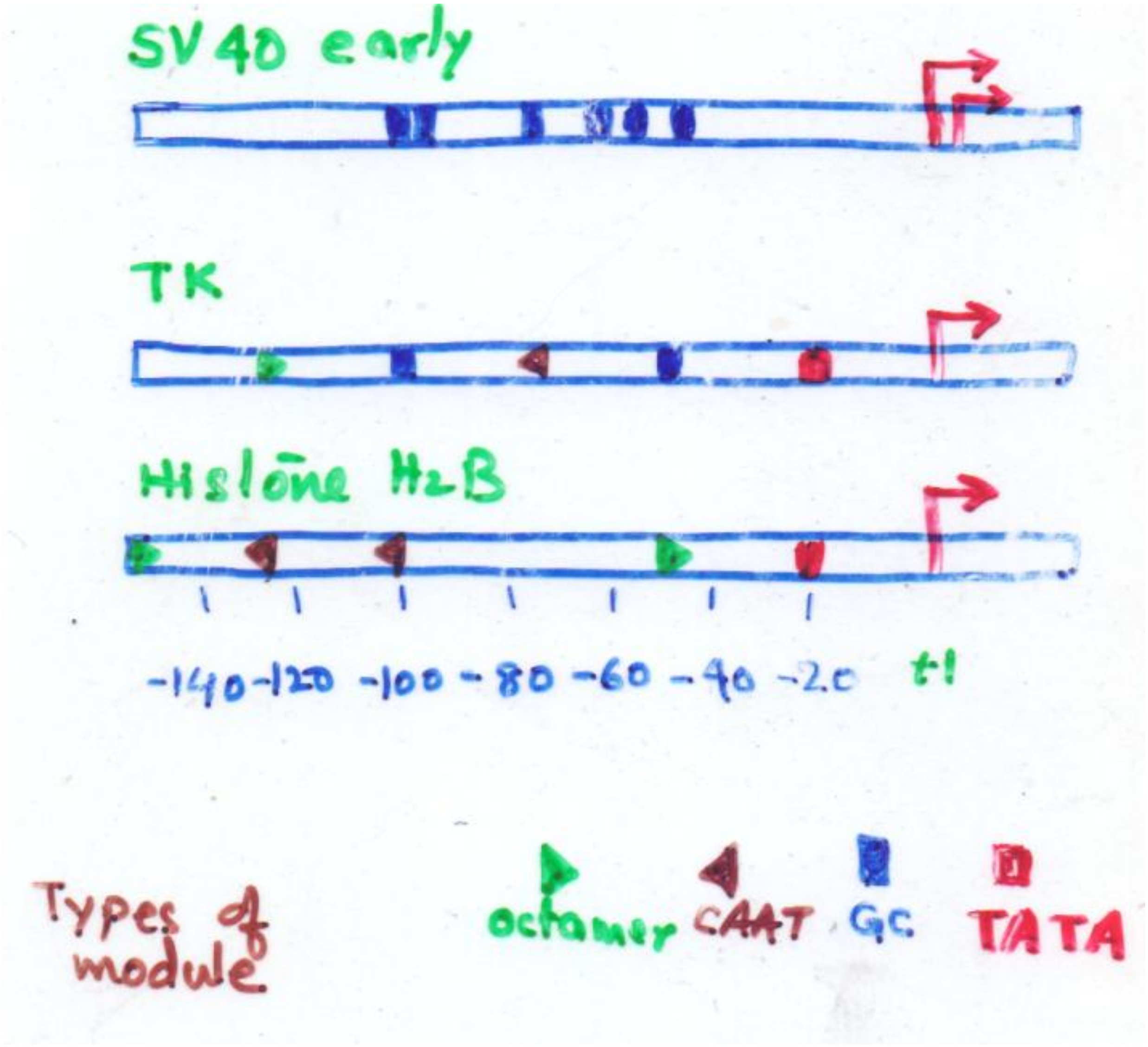


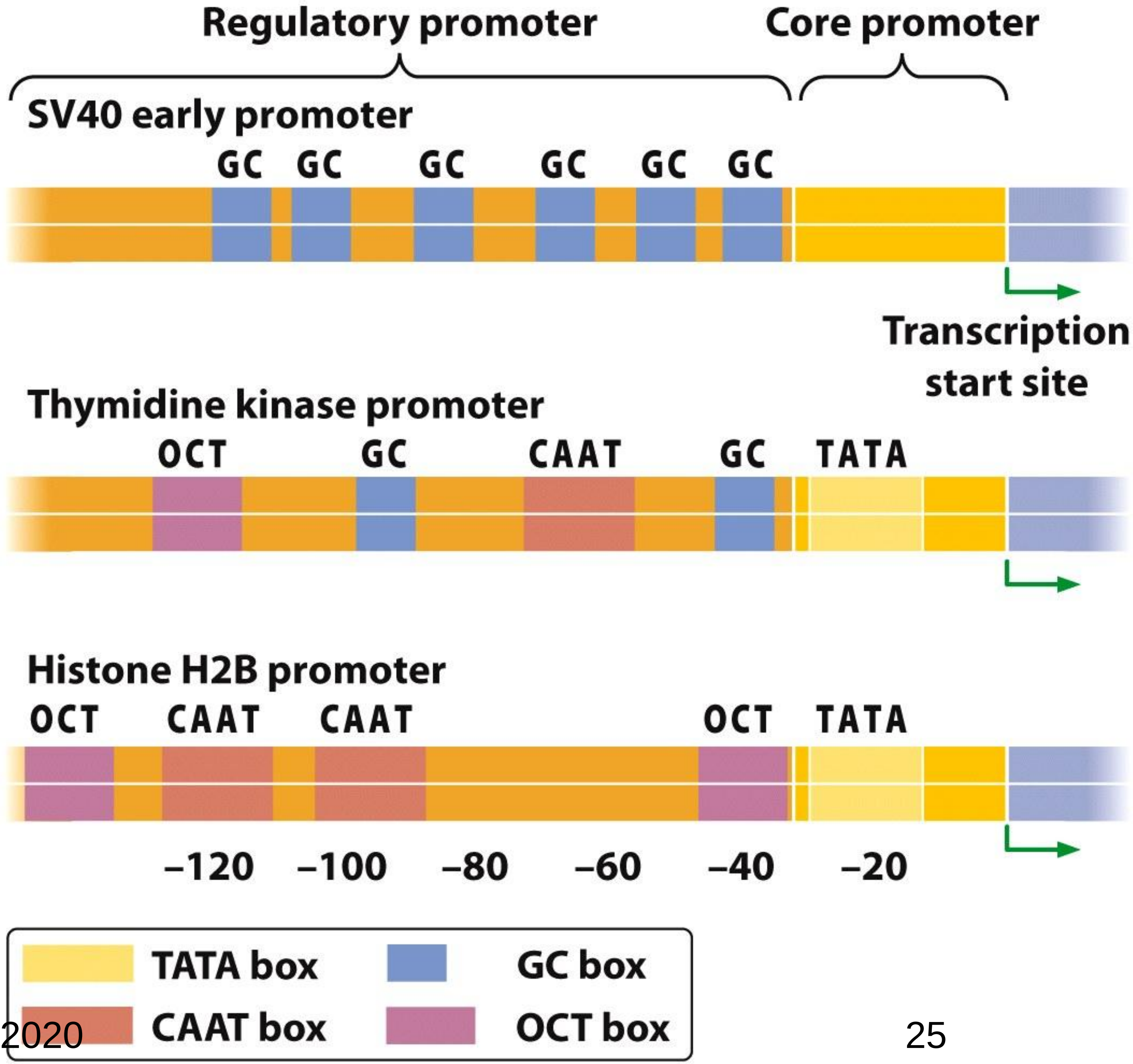
Enhancer

Silencer

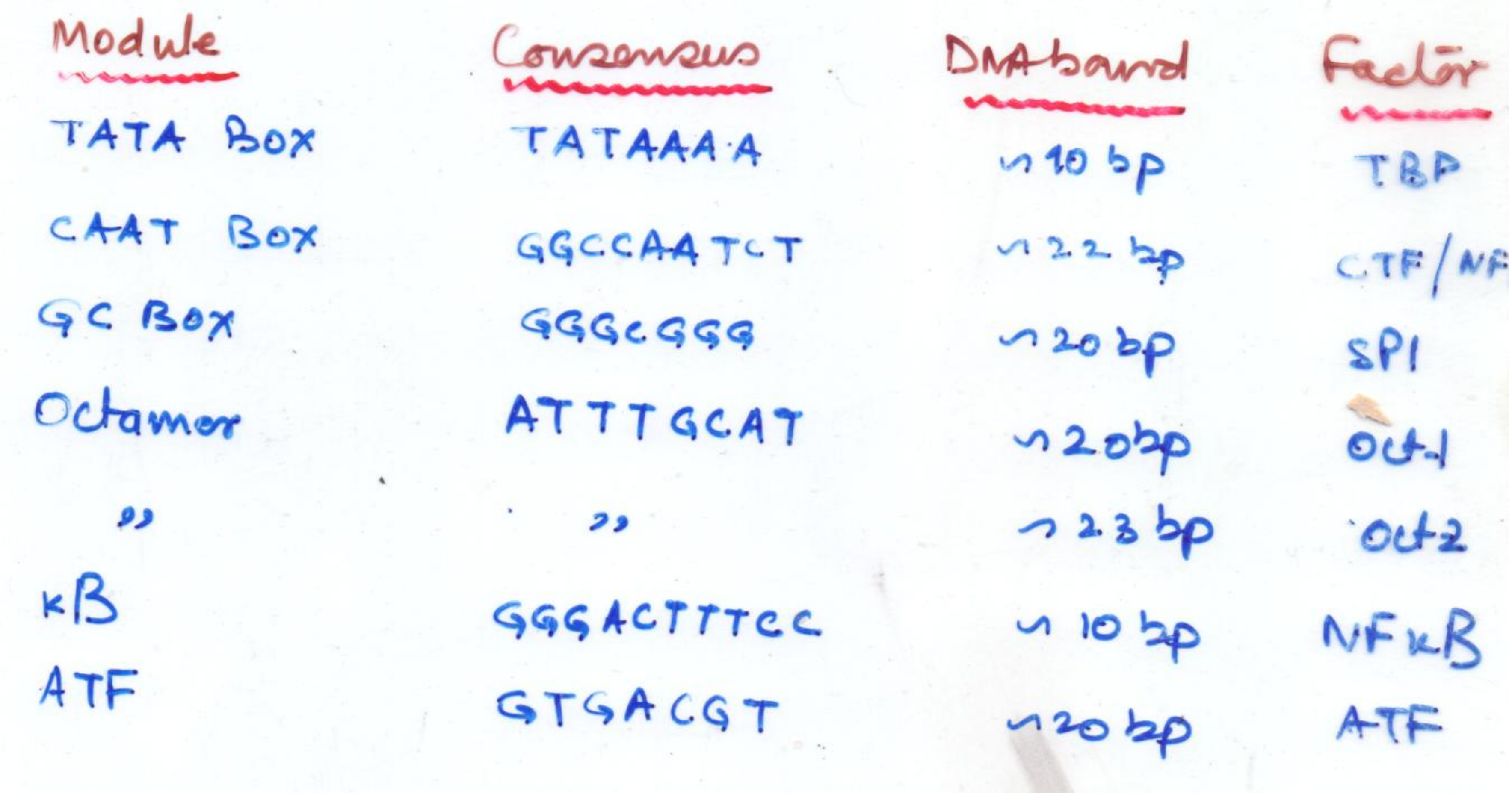
Monocistronic

Promoters contain different combinations of TATA boxes, CAAT boxes and other elements

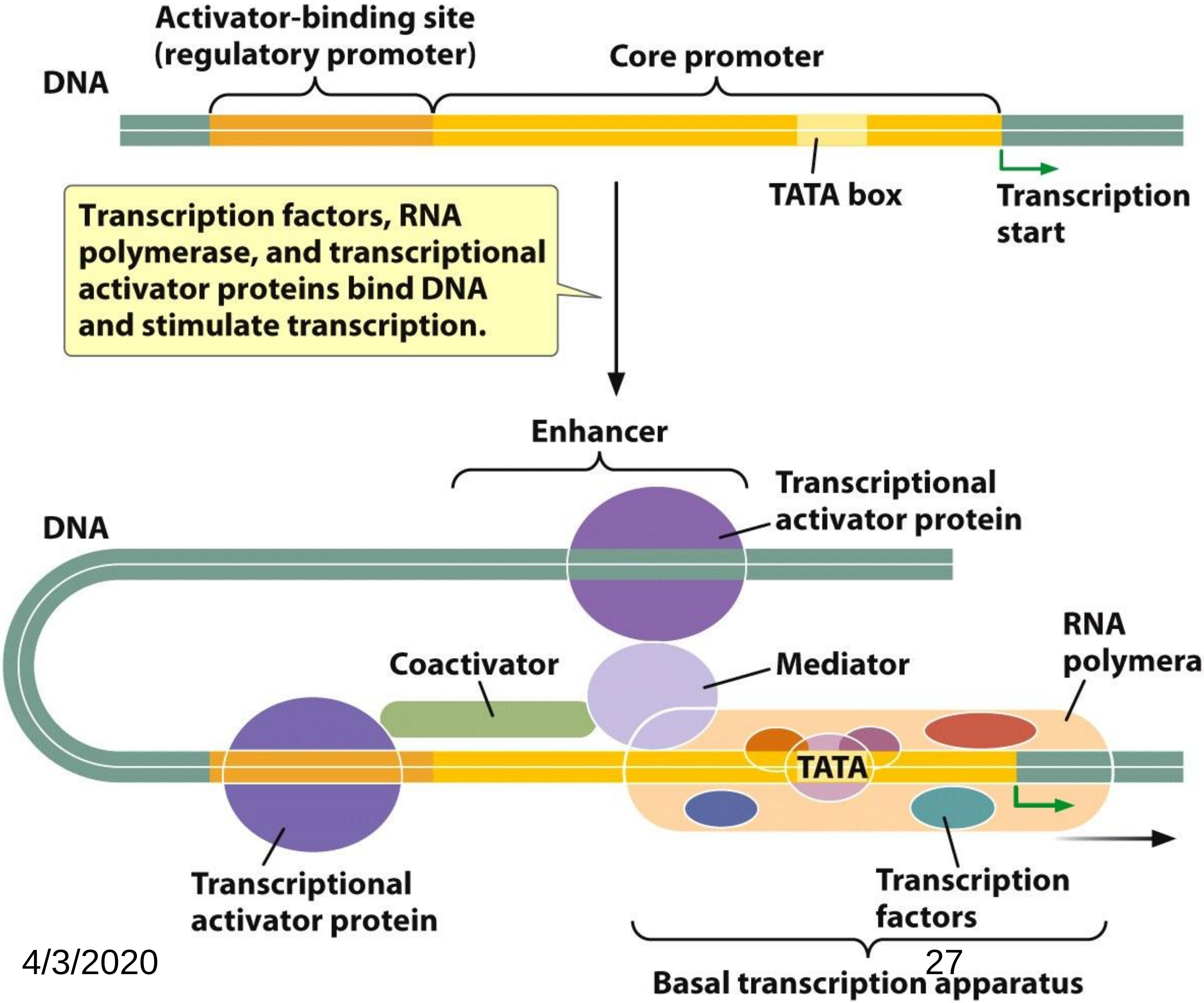



Regulatory promoter
Core promoter
SV40 early promoter
GC
GC
GC
GC
GC
GC
Transcription start site
Thymidine kinase promoter
OCT
GC
CAAT
GC
TATA
Histone H2B promoter
OCT
CAAT
CAAT
OCT
TATA
−120
−100
−80
−60
−40
−20
TATA box
GC box
CAAT box
OCT box

Upstream transcription factors bind to sequence elements that are common to mammalian RNA Pol II promoter



Module	Consensus	DNA band	Factor
TATA Box	TATAAAA	∼10 bp	TBP
CAAT Box	GGCCAATCT	∼22 bp	CTF/NF
GC Box	GGGCGGG	∼20 bp	SP1
Octamer	ATTTGCAT	∼20 bp	Oct-1
"	"	∼23 bp	Oct-2
κB	GGGACTTTCC	∼10 bp	NFκB
ATF	GTGACGT	∼20 bp	ATF



Transcription factors, RNA polymerase, and transcriptional activator proteins bind DNA and stimulate transcription.

Points to be Remembered…………

1. Binding of activators at these sites may influence the formation of the initiation of complexes at any one of several stages.
2. Promoters are organized on a principle of 'Mix and Match'.
3. A variety of elements can contribute to promoter function, but none is essential for all promoters.