Eukaryotic & Prokaryotic Transcription

RNA polymerases

RNA Polymerases

A. *E. coli* RNA polymerase

1. core enzyme = ββ'(α)2 has catalytic activity but cannot recognize start site of transcription

~500,000 daltons

dimensions: 100 X 100 X 160 angstroms

requires Mg2+ for activity

b' binds 2 Zn atoms

2. holoenzyme = core enzyme + sigma factor (s)

carries out four functions:

(i) template binding

(ii) RNA chain initiation

(iii) chain elongation

(iv) chain termination

Why would RNA polymerase in eukaryotes need to be different than in prokaryotes?

Don’t they carry out the same basic process.

Genome Size Comparison

- Prokaryotes are generally in the ~10^6 bp size range – see Genome Sizes
- Eukaryotes are more in the ~10^9 bp size range
- Larger genome means it requires more specificity.
- Also the diversity of function – organelles, different cell type, and so on.

RNA Polymerases

B. Eukaryotic RNA polymerases (RNAP)

1. 3 nuclear RNA polymerases

a. RNAP I- transcribes rRNA genes

b. RNAP II - transcribes mRNA genes

c. RNAP III - transcribes tRNA, 5S rRNA, and other small RNA genes

d. have 10-17 different subunits, large multisubunit complexes are functionally similar to E. coli RNA polymerase

e. cannot bind to their respective promoters alone, but requires transcription factor for promoter specific recruitment

TABLE 31-2. RNA Polymerase Subunits[a]

S. cerevisiae RNAP I (14 subunits)	*S. cerevisiae* RNAP II (12 subunits)	*S. cerevisiae* RNAP III (15 subunits)	*E. coli* RNAP Core (5 subunits)	Class[b]
Rpa1 (A190)	Rbp1 (B220)	Rpc1 (C160)	β′	Core
Rpa2 (A135)	Rbp2 (B150)	Rpc2 (C128)	β	Core
Rpc5 (AC40)	Rpb3 (B44.5)	Rpc5 (AC40)	α	Core
Rpc9 (AC19)	Rpb11 (B13.6)	Rpc9 (AC19)	α	Core
Rbp6 (ABC23)	Rbp6 (ABC23)	Rpb6 (ABC23)	ω	Core/common
Rpb5 (ABC27)	Rpb5 (ABC27)	Rpb5 (ABC27)		Common
Rpb8 (ABC14.4)	Rpb8 (ABC14.4)	Rpb8 (ABC14.4)		Common
Rbp10 (ABC10β)	Rpb10 (ABC10β)	Rpb10 (ABC10β)		Common
Rbp12 (ABC10α)	Rpb12 (ABC10α)	Rpb12 (ABC10α)		Common
Rpa9 (A12.2)	Rpb9 (B12.6)	Rpc12 (C11)		
Rpa8 (A14)[c]	Rpb4 (B32)	—		
Rpa4 (A43)[c]	Rpb7 (B16)	Rpc11 (C25)		
+2 others[d]		+4 others[d]		

[a]Homologous subunits occupy the same row. In the alternative subunit names in parentheses, the letter(s) indicates the RNAPs in which the subunit is a component (A, B, and C for RNAPs I, II, and III) and the numbers indicate its approximate molecular mass in kD.

[b]Core: sequence partially homologous in all RNAPs; common: shared by all eukaryotic RNAPs.

[c]Potential homologs of Rbp4 and Rbp7.

[d]Rpa3 (A49) and Rpa5 (A34.5) in RNAP I and Rpc3 (C74), Rpc4 (C53), Rpc6 (C34), and Rpc8 (C31) in RNAP III.

Source: Mainly Cramer, P., *Curr. Opin. Struct. Biol.* **12,** 89 (2002).

RNA Polymerases

2. organelle specific RNA polymerases

   more prokaryotic-like

   1. chloroplast
   2. mitochondria

Of the 3 nuclear RNA polymerases which would be the more specialized?

Which would require more diversity of action?

RNA Polymerases

3. RNAP II

a. core subunits - have sequence similarity to the core subunits of

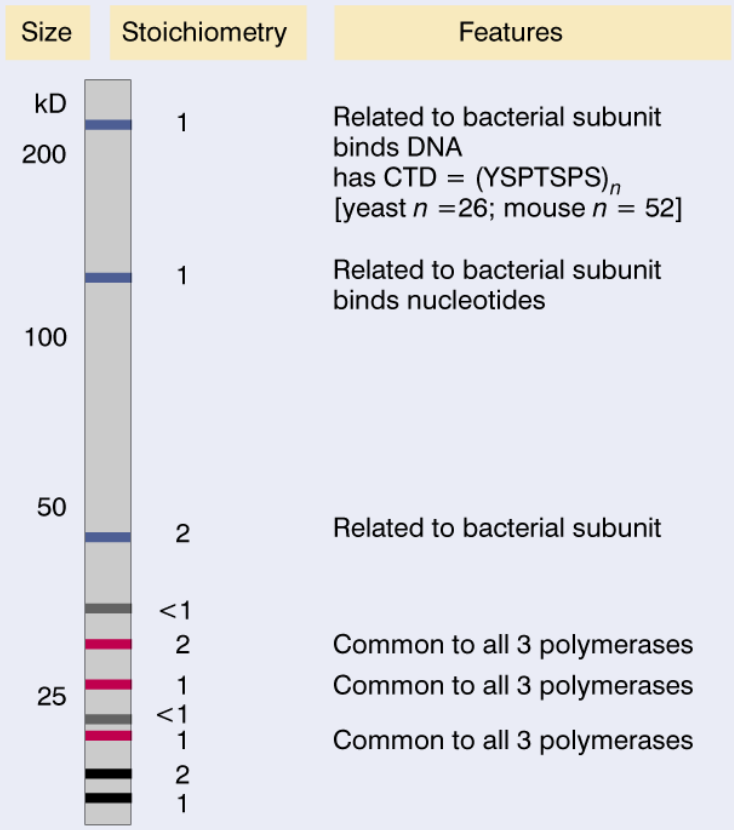
E. coli core RNA polymerase or subunits of other eukaryotic RNA polymerases

b. shared or common subunits

same subunits found in RNAP III and II or in RNAP I and RNAP II

c. unique subunits - no similar homologs found anywhere else

Figure 20.2 Eukaryotic RNA polymerase II has >10 subunits.



RNA Polymerases

3. RNAP II

d. CTD or C-terminal domain of the largest subunit

i. a heptapeptide repeat - 52X humans and 26X yeast

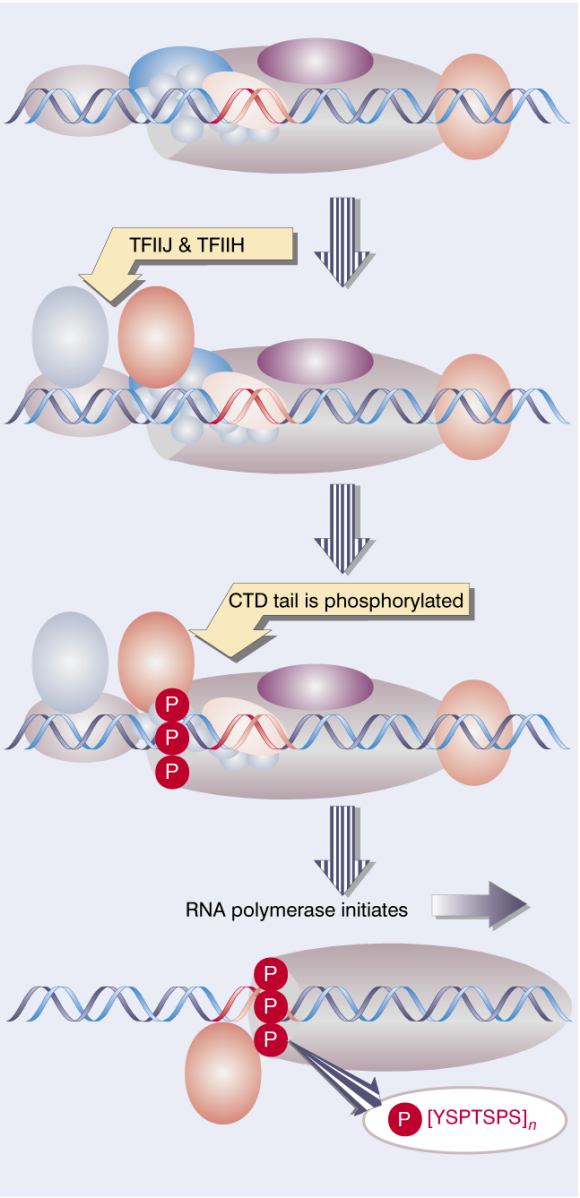
ii. can be highly phosphorylated at Ser and Thr

iii. three subforms of RNAP II: IIo, IIa, and IIb

e. cycling of phosphorylated and dephosphorylated forms of RNAP II associated with different stages in transcription

f. CTD is also required to recruit proteins for capping of 5'-end of mRNA, as well as for splicing and polyadenylation of the 3'end of mRNA

Figure 20.13 Phosphorylation of the CTD by the kinase activity of TFIIH may be needed to release RNA polymerase to start transcription.



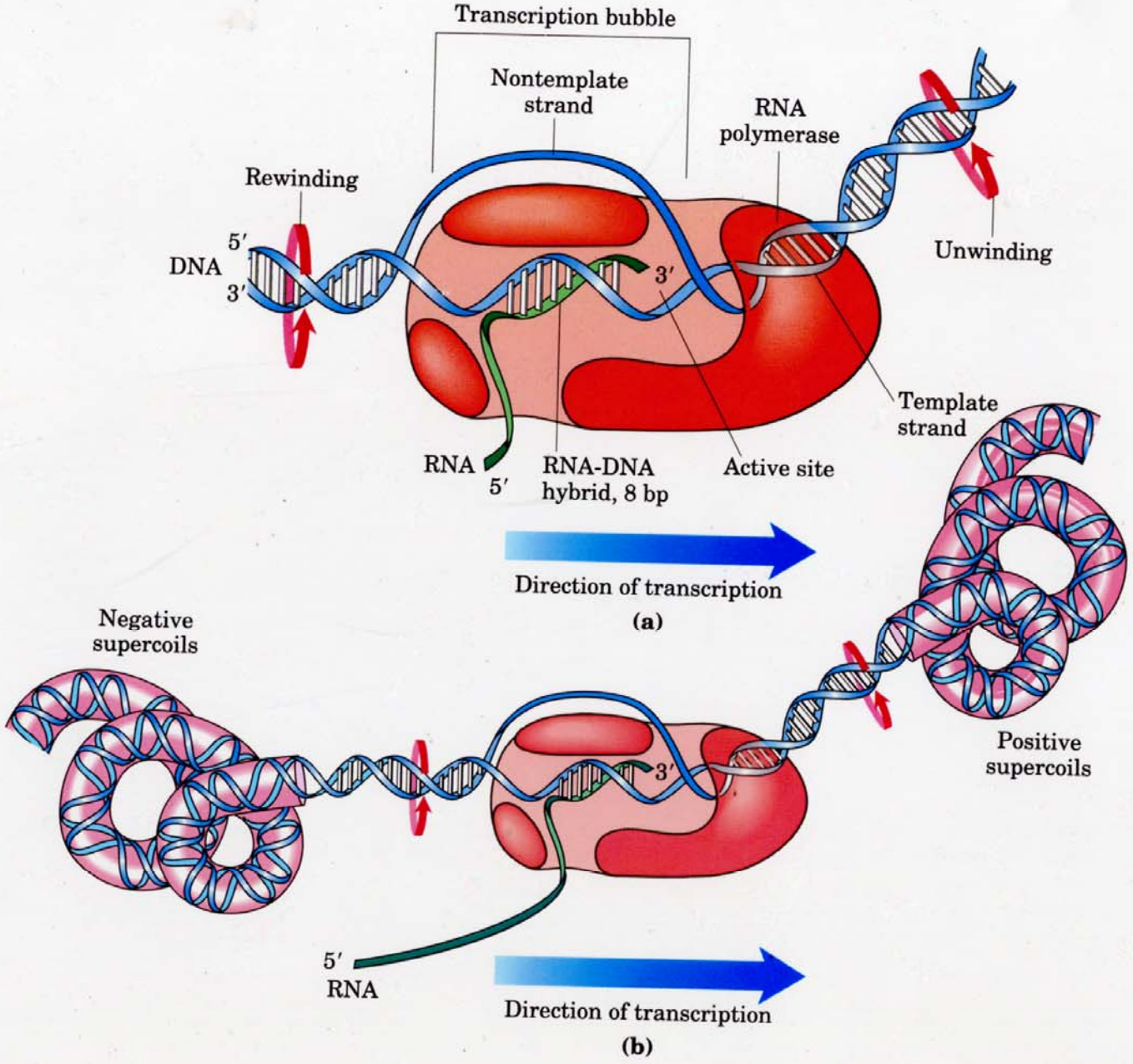
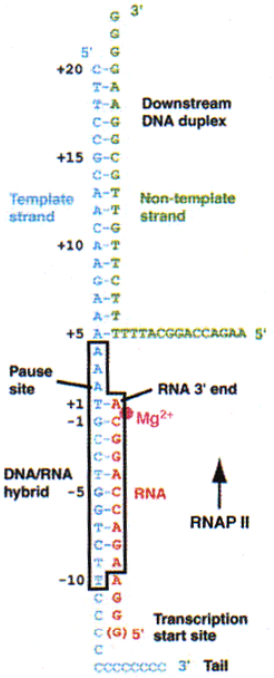
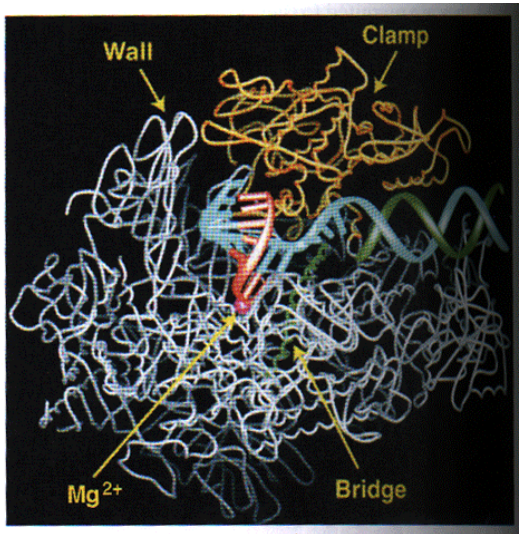

Transcription bubble
Nontemplate strand
RNA polymerase
Rewinding
5′
DNA
3′
3′
Unwinding
Template strand
RNA
5′
RNA-DNA hybrid, 8 bp
Active site
Direction of transcription
(a)
Negative supercoils
Positive supercoils
3′
5′
RNA
Direction of transcription
(b)

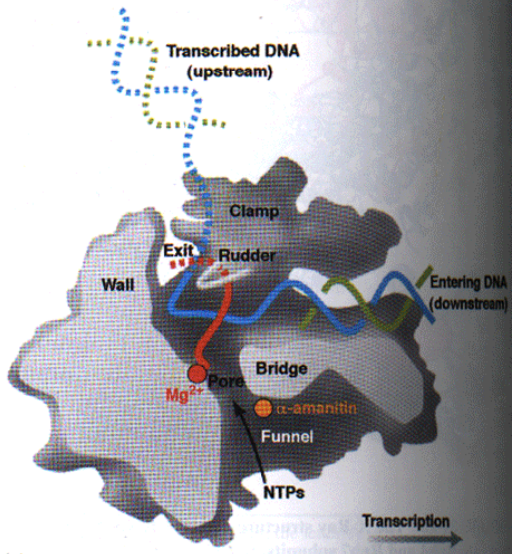
figure 26-1



(a)



(b)



(c)

FIGURE 31-21 X-Ray structure of an RNAP II elongation complex. (*a*) The RNA · DNA complex in the structure with the template DNA cyan, the nontemplate DNA green, and the newly synthesized RNA red. The magenta dot marked Mg^{2+} represents the strongly bound active site metal ion. The black box encloses those portions of the complex that are clearly visible in the structure; the double-stranded portion of the DNA marked "Downstream DNA duplex" is poorly ordered, and the remaining portions of the complex are disordered. (*b*) View of the transcribing complex from the bottom of Fig. 31-20*a* in which portions of Rpb2 that form the near side of the cleft have been removed to expose the bound RNA · DNA complex. The protein is represented by its backbone in which the clamp, which is closed over the downstream DNA duplex, is yellow, the bridge helix is green, and the remaining portions of the protein are gray. The DNA and RNA are colored as in Part *a* with their well-ordered portions drawn in ladder form and their less ordered portions drawn in backbone form. The active site Mg^{2+} ion is represented by a magenta sphere. (*c*) Cutaway schematic diagram of the transcribing complex in Part *b* in which the cut surfaces of the protein are light gray, its remaining surfaces are darker gray, and several of its functionally important structural features are labeled. The DNA, RNA, and active site Mg^{2+} ion are colored as in Part *a* with portions of the DNA and RNA that are not visible in the X-ray structure represented by dashed lines. The α-amanitin binding site is marked by an orange dot. [Modified from diagrams by Roger Kornberg, Stanford University. PDBid 1I6H.]

Where does the specificity come in for transcription?

How does RNA polymerase II know where to begin?

Promoter Structure

B. mRNA genes transcribed by RNAP II

1. TATA box element - located between -30 and -20 bps
2. Initiator region or In: centered on the start site of transcription
3. DPE: downstream promoter element
4. Response elements (RE)
   a. upstream of the TATA box
   b. many different kinds - help respond to signals
   c. multiple RE present - synergy

Figure 20.1 *Overview:* 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 >200 bp. An enhancer containing a more closely packed array of elements that also bind transcription factors may be located 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.)

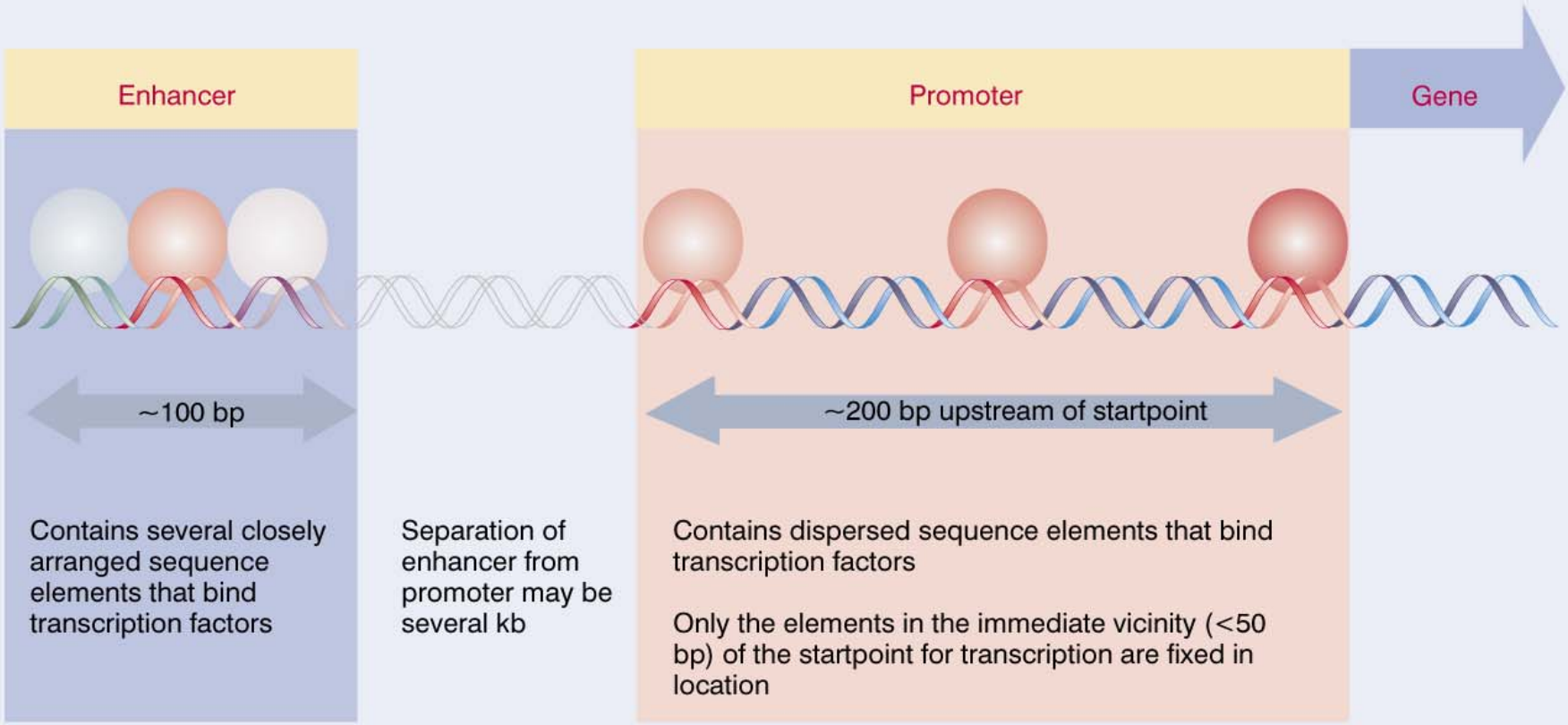


Figure 20.16 Saturation mutagenesis of the upstream region of the -globin promoter identifies three short regions (centered at -30, -75, and -90) that are needed to initiate transcription. These correspond to the TATA, CAAT, and GC boxes.

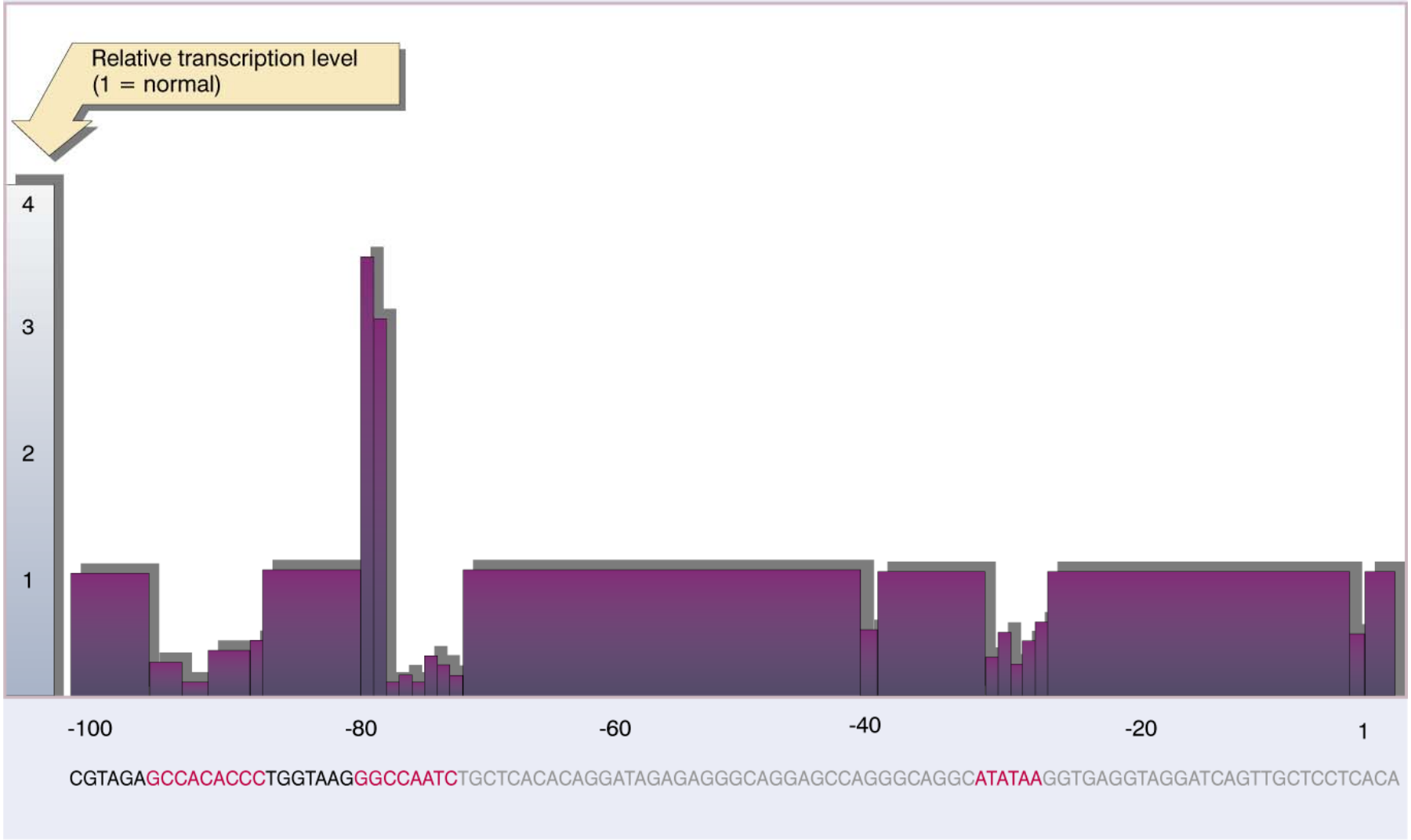
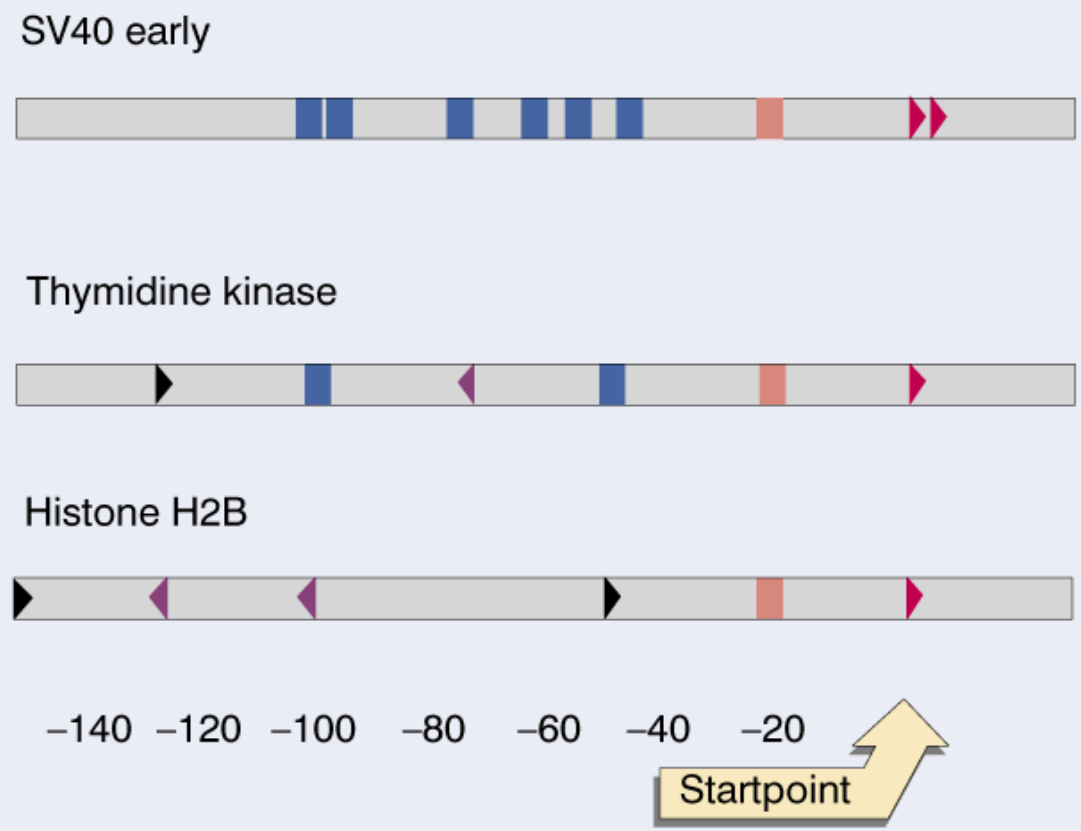


Figure 20.17 Promoters contain different combinations of TATA boxes, CAAT boxes, GC boxes, and other elements.



How is transcription of particular genes get turned on in response to external stimuli such as stress (heat, starvation, and so on), hormones and other small molecule effectors?

Figure 21.7 Glucocorticoids regulate gene transcription by causing their receptor to bind to an enhancer whose action is needed for promoter function.

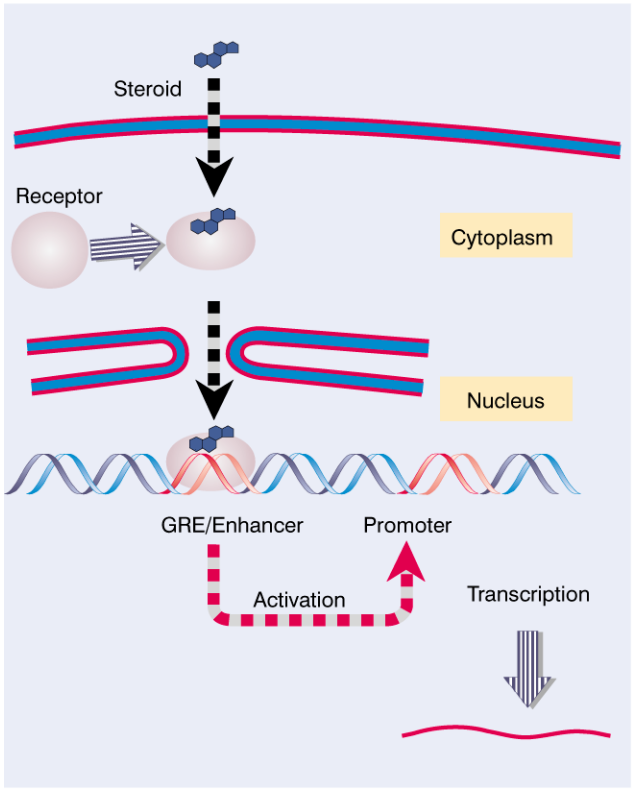
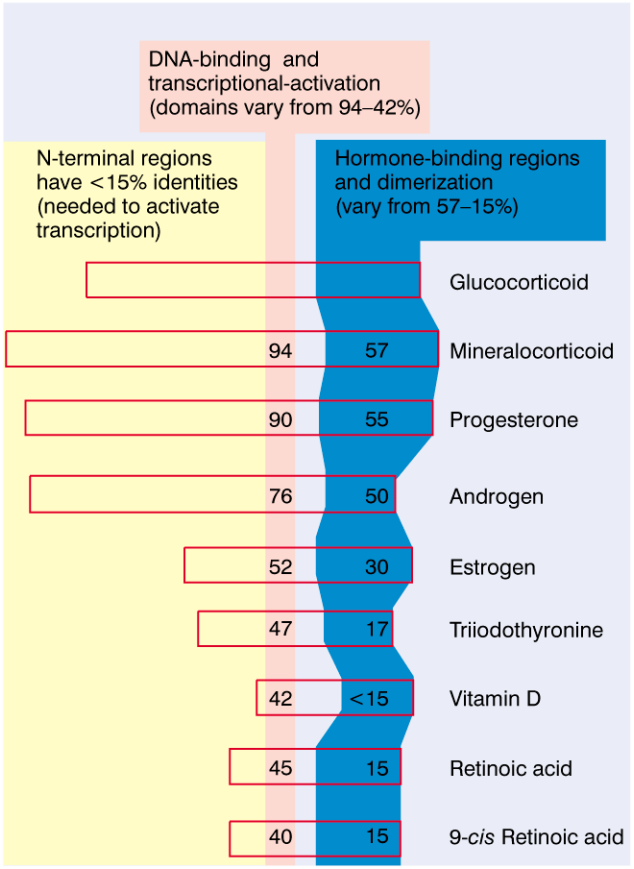


Figure 21.8 Receptors for many steroid and thyroid hormones have a similar organization, with an individual N-terminal region, conserved DNA-binding region, and a C-terminal hormone-binding region.



Promoter Structure

B. mRNA genes transcribed by RNAP II

5. enhancers
    a. can be located at great distances (>1000 bps) from start site of transcription either from the 5' or 3' end of gene
    b. stimulates transcription (~100 times)
    c. orientation independent

Promoter Structure

B. mRNA genes transcribed by RNAP II

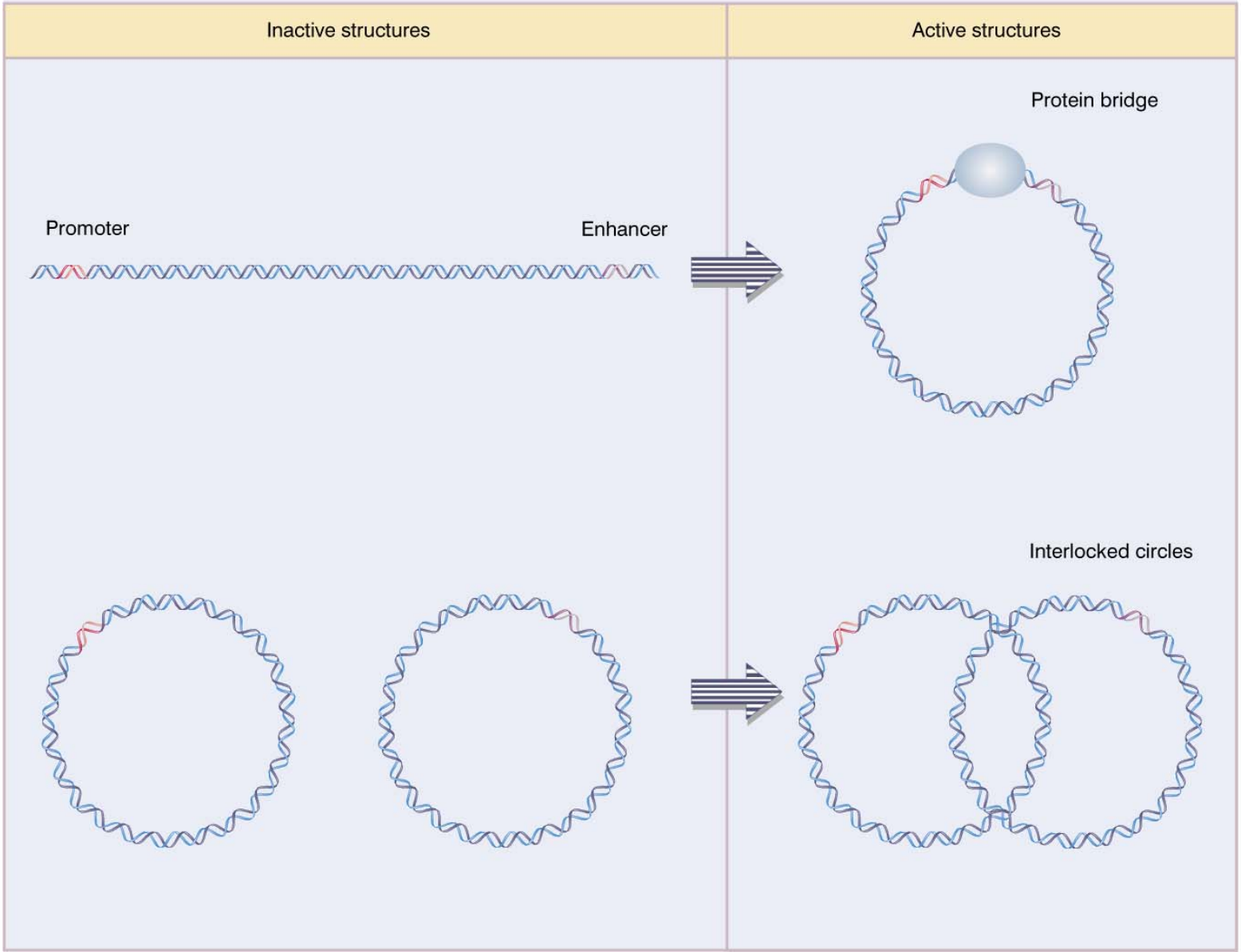
5. enhancers

d. two models of how enhancers might work

i. entry point of RNAP II by preventing nucleosomes from binding or an altered DNA conformation that promotes RNAP II recognition

ii. transcription factors bound to enhancer will stimulate binding of RNAP II to promoter regions closer to the start site of transcription

Figure 20.20 An enhancer may function by bringing proteins into the vicinity of the promoter. An enhancer does not act on a promoter at the opposite end of a long linear DNA, but becomes effective when the DNA is joined into a circle by a protein bridge. An enhancer and promoter on separate circular DNAs do not interact, but can interact when the two molecules are catenated.



How different are enhancers from promoter regions?

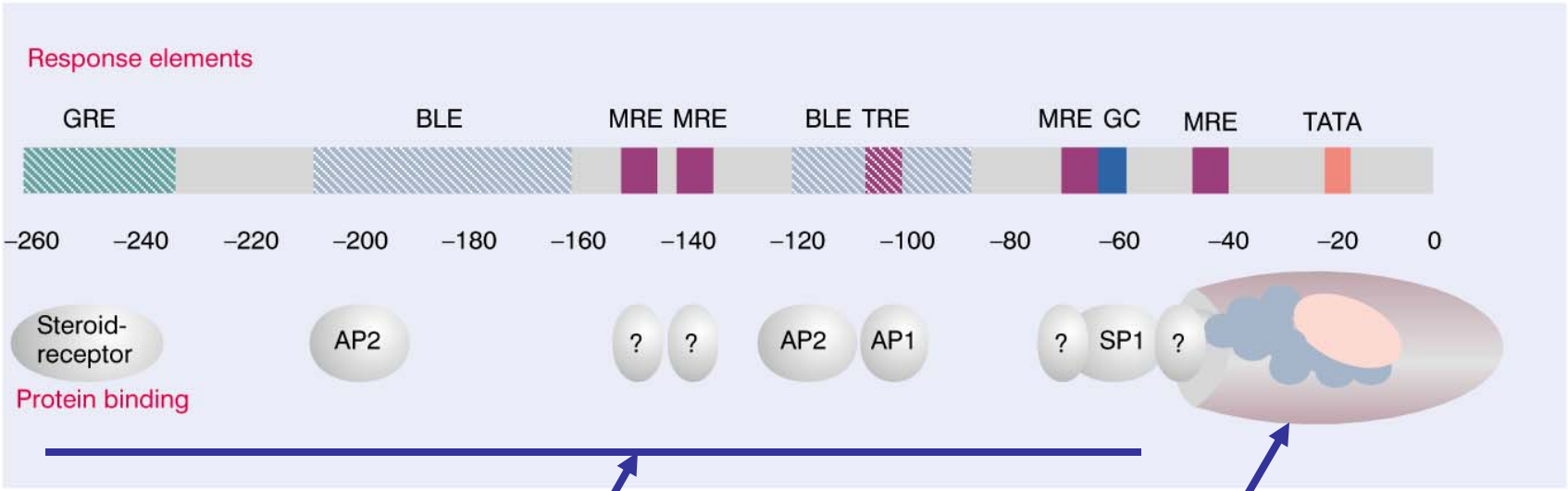
Are different DNA elements used in each?

Transcription Factors

General versus promoter specific transcription factors.

Factors that are required for all mRNA genes and others that are required for only a small subset of genes

Figure 21.1 The regulatory region of a human metallothionein gene contains regulator elements in both its promoter and enhancer. The promoter has elements for metal induction; an enhancer has an element for response to glucocorticoid. Promoter elements are shown above the map, and proteins that bind them are indicated below.

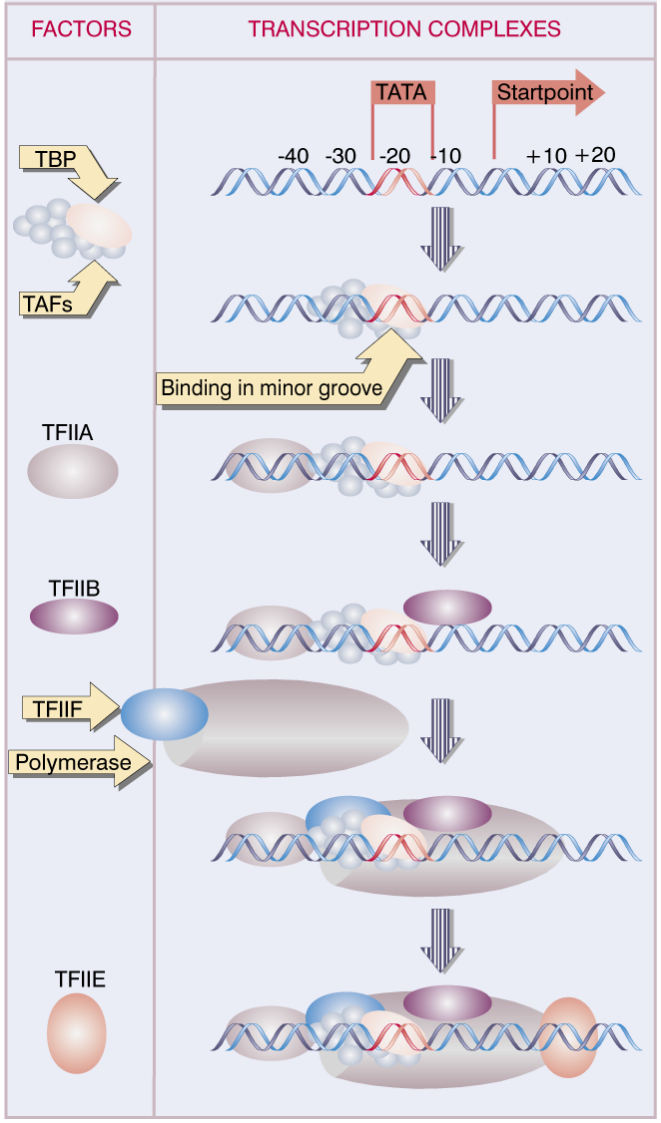


General Transcription Factors

Promoter Specific Transcription Factors

How does RNA polymerase II find its correct binding site and is there such a thing as a sigma factor in this case?

Figure 20.11 An initiation complex assembles at promoters for RNA polymerase II by an ordered sequence of association with transcription factors.



Regulation of Transcription

I. Basal vs. activated transcription for mRNA genes

A. General transcription factor (TF) vs. promoter-specific

1. general TFs are required by all mRNA genes

a. an absolute requirement

b. transcription can occur alone with these factors and by definition the basal level of transcription

2. promoter-specific TFs are different for each gene

3. the promoter-specific TFs are required for maximal level of transcription or for activated transcription (induction)

General transcription factors

A. TFIID = TBP +TAFs

1. TBP binds the minor groove of the TATA box and kinks DNA
2. TAFs interact with the Inr region
3. makes contact with TFIIB and TFIIA
4. have up to 14 different TAF proteins bound to TBP
5. only TBP is required for basal transcription

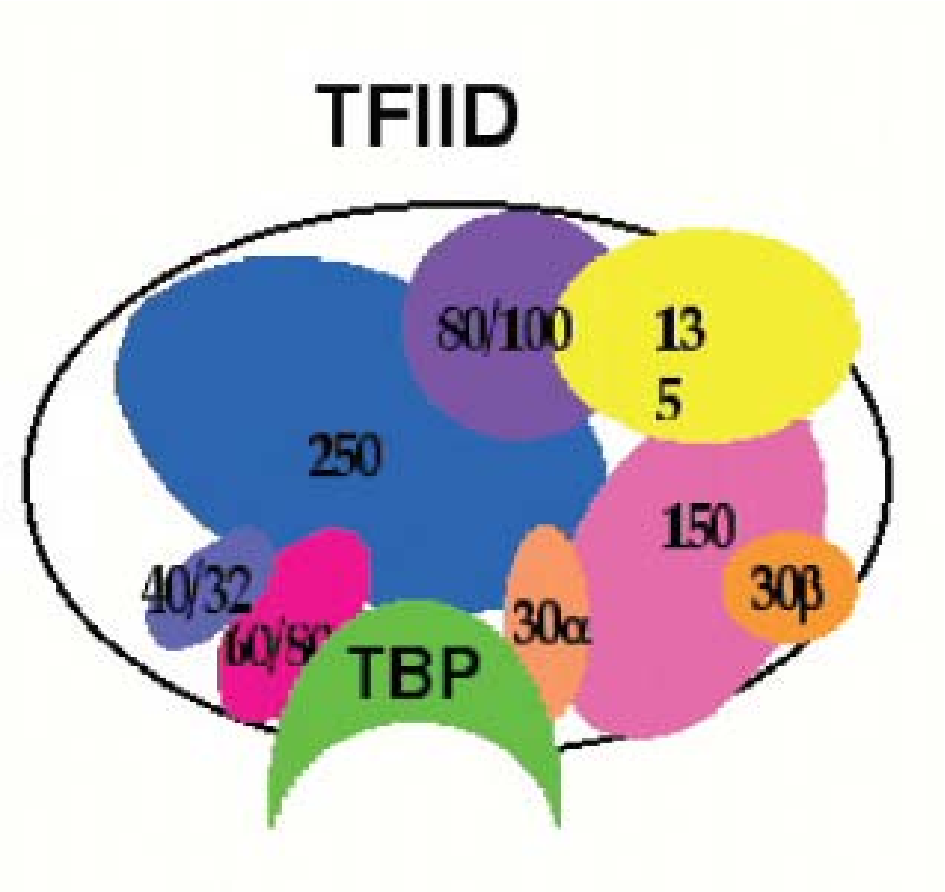

TFIID
80/100
13
5
250
150
40/32
30β
30α
TBP

Figure 20.9 A view in cross-section shows that TBP surrounds DNA from the side of the narrow groove. TBP consists of two related (40% identical) conserved domains, which are shown in light and dark blue. The N-terminal region varies extensively and is shown in green. The two strands of the DNA double helix are in light and dark grey. Photograph kindly provided by Stephen Burley.

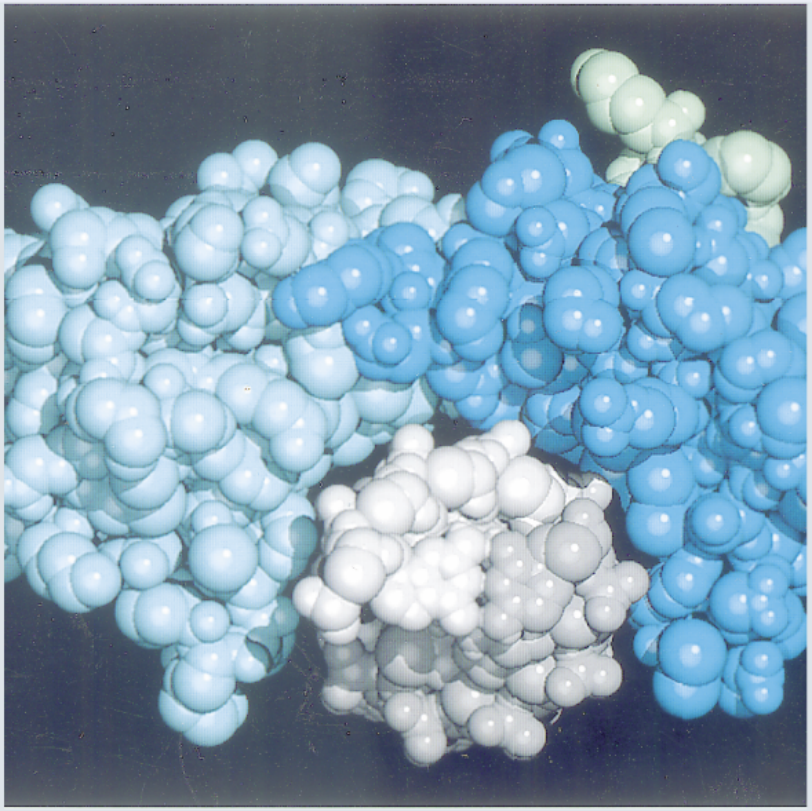


Figure 20.10 The cocrystal structure of TBP with DNA from -40 to the startpoint shows a bend at the TATA box that widens the narrow groove where TBP binds. Photograph kindly provided by Stephen Burley.

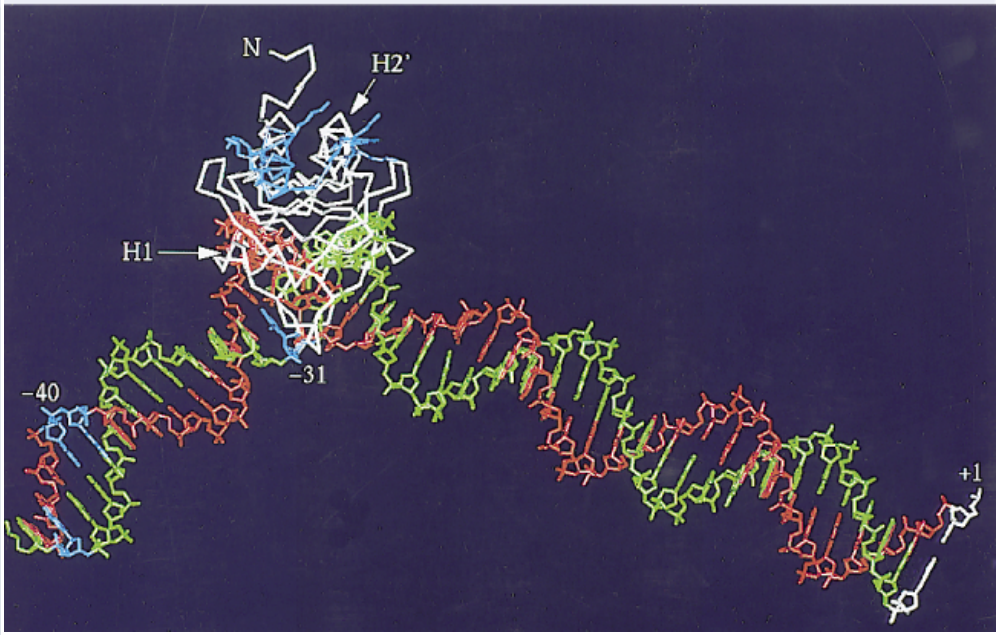
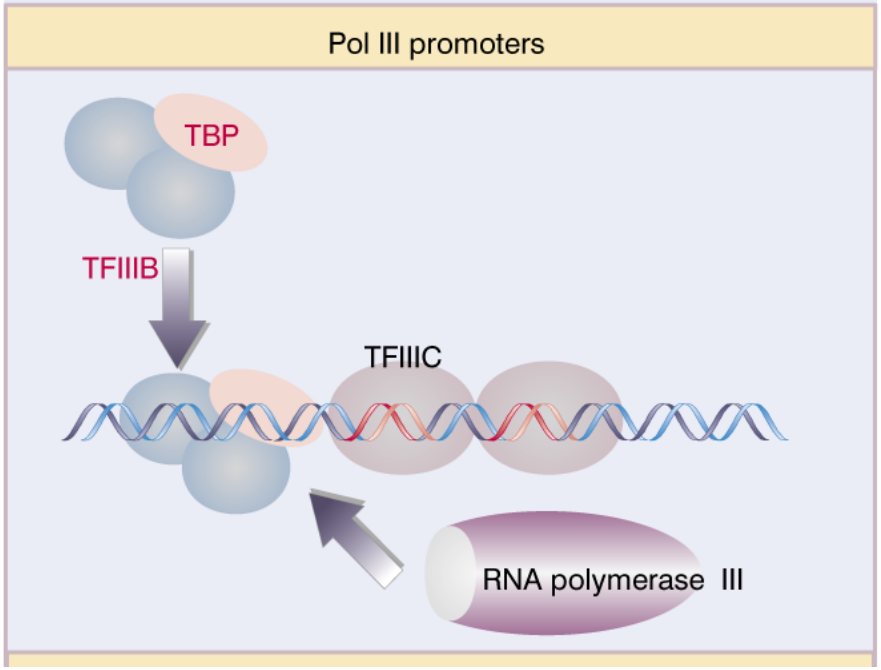
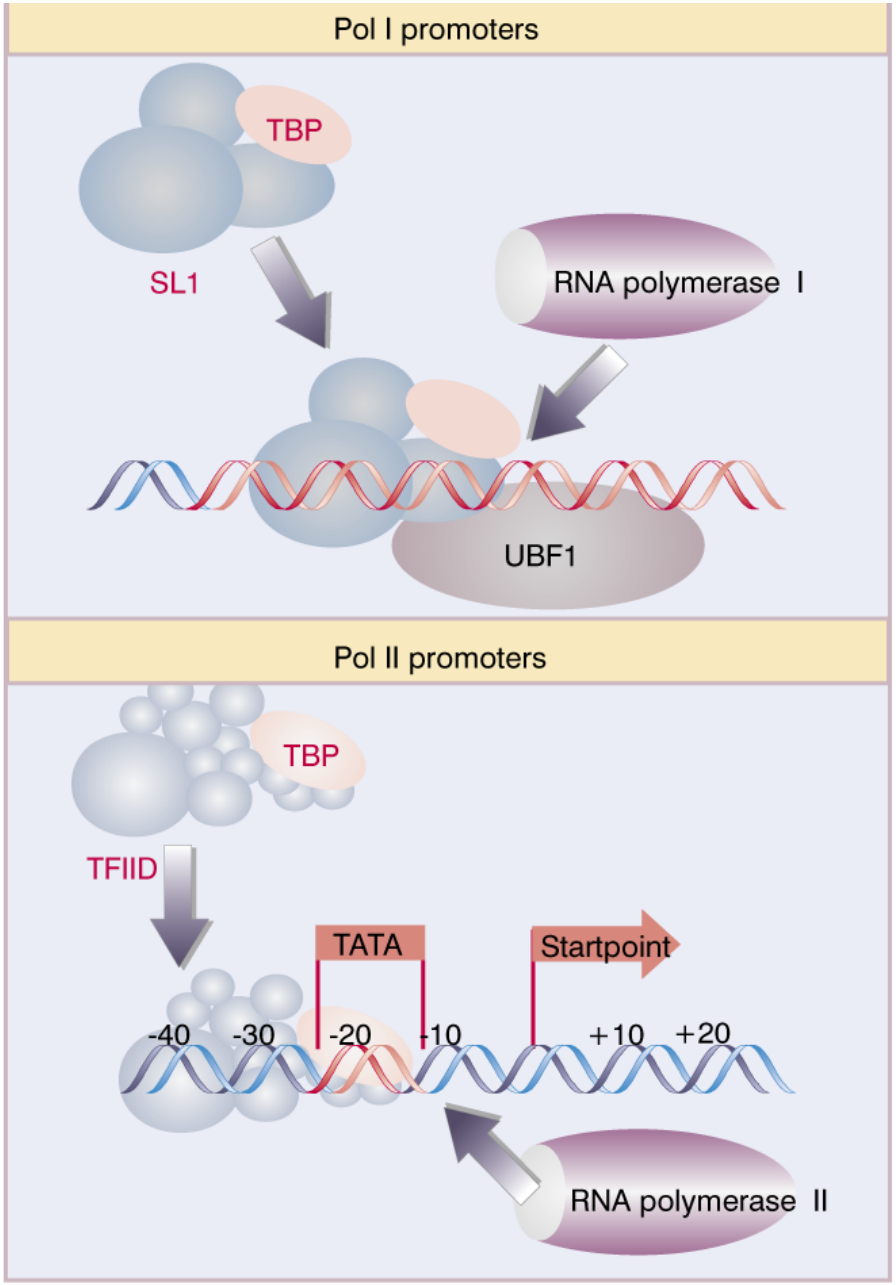
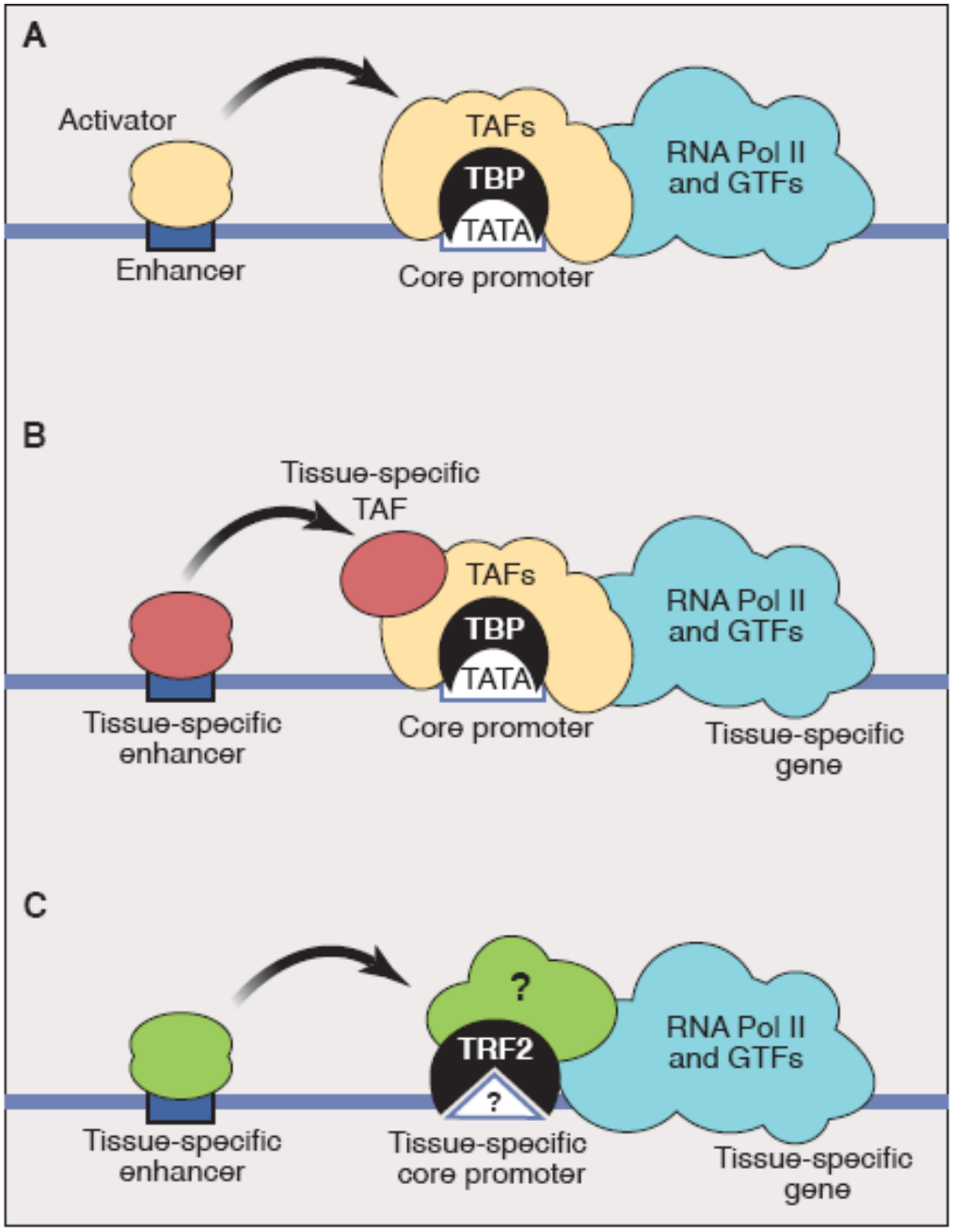


Figure 20.8 RNA polymerases are positioned at all promoters by a factor that contains TBP.






A
Activator
Enhancer
TAFs
TBP
TATA
Core promoter
RNA Pol II
and GTFs
B
Tissue-specific
TAF
Tissue-specific
enhancer
TAFs
TBP
TATA
Core promoter
RNA Pol II
and GTFs
Tissue-specific
gene
C
Tissue-specific
enhancer
?
TRF2
?
Tissue-specific
core promoter
RNA Pol II
and GTFs
Tissue-specific
gene

General transcription factors

B. TFIIA

1. helps stabilize TBP-DNA interactions
2. required for activation and to counteract repression
3. not essential in a highly purified system

C. TFIIB

1. is a single polypeptide
2. involved in start site selection
3. homologous to a subunit of TFIIIB
4. interacts directly with TBP

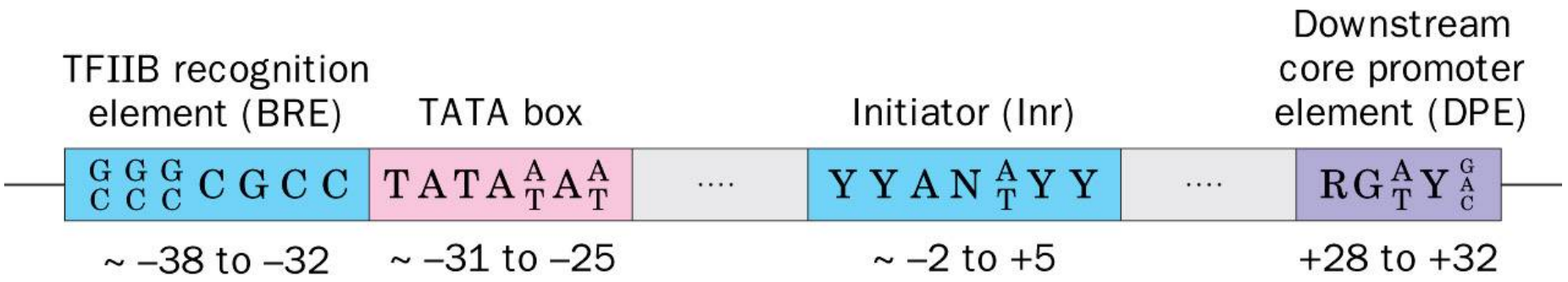
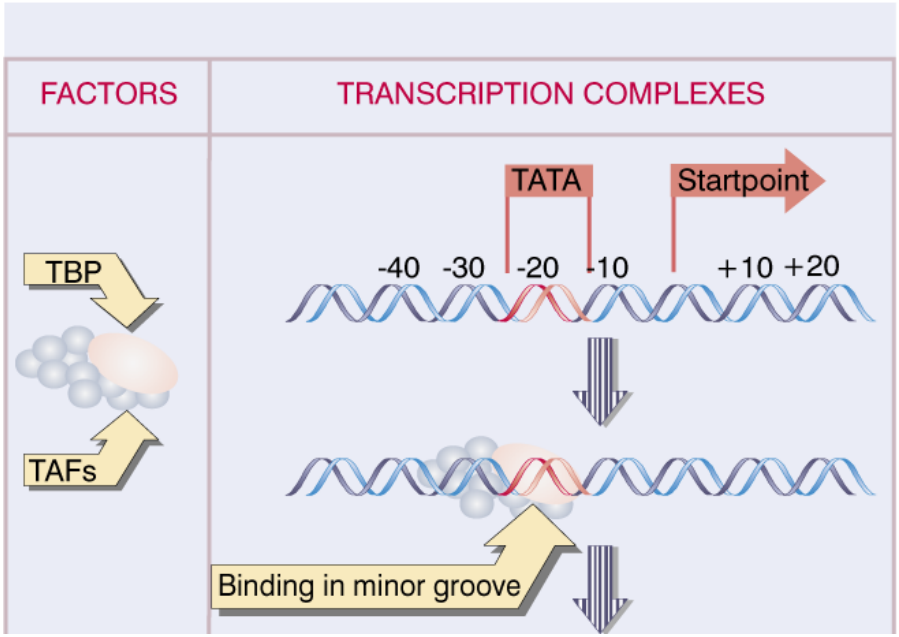

TFIIB recognition element (BRE)
TATA box
Initiator (Inr)
Downstream core promoter element (DPE)
G/C G/C G/C C G C C
T A T A A/T A A/T
....
Y Y A N A/T Y Y
....
R G A/T Y G/A/C
~ –38 to –32
~ –31 to –25
~ –2 to +5
+28 to +32

Figure 20.11 An initiation complex assembles at promoters for RNA polymerase II by an ordered sequence of association with transcription factors.



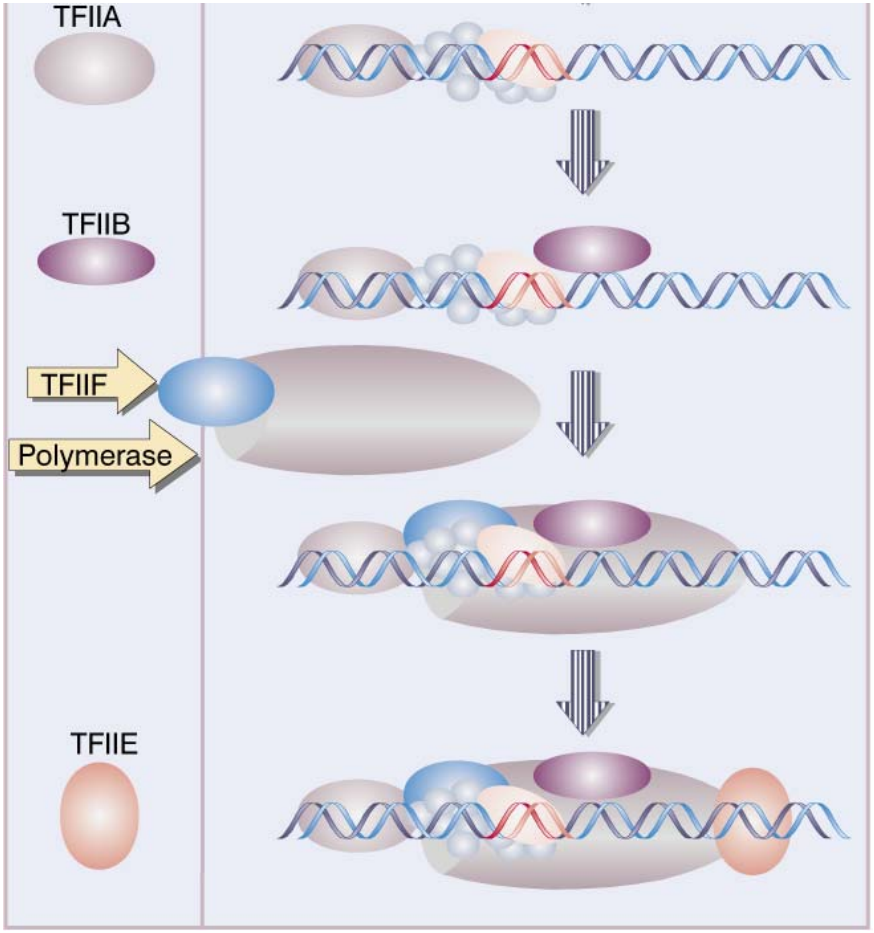
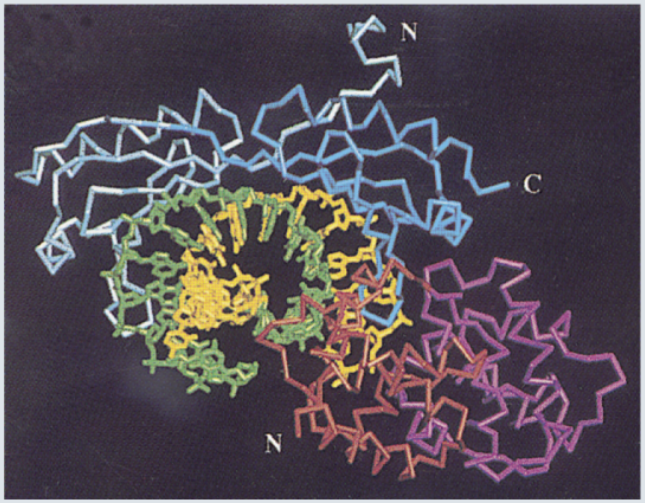
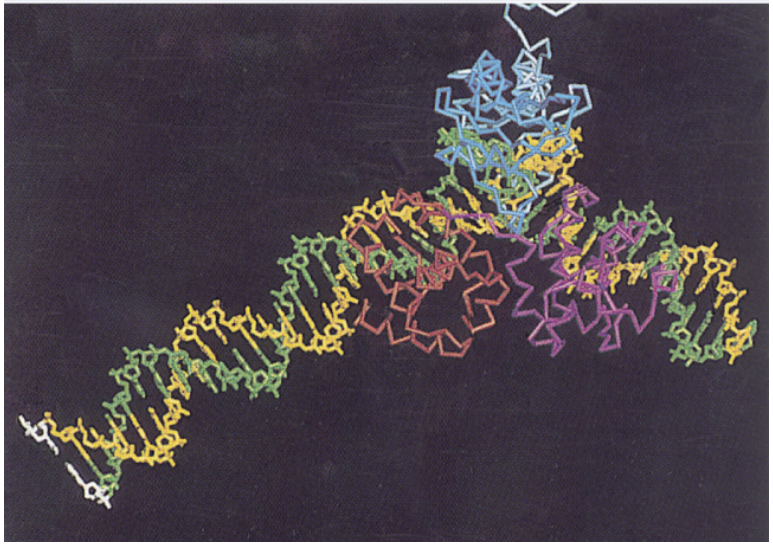


Figure 20.12 Two views of the ternary complex of TFIIB-TBP-DNA show that TFIIB binds along the bent face of DNA. The two strands of DNA are green and yellow, TBP is blue, and TFIIB is red and purple. Photograph kindly provided by Stephen Burley.





General transcription factors

D. TFIIF

1. originally identified as a RNAP II associated protein (RAP)
2. has sigma like activity

enhances RNAP II binding to promoter DNA

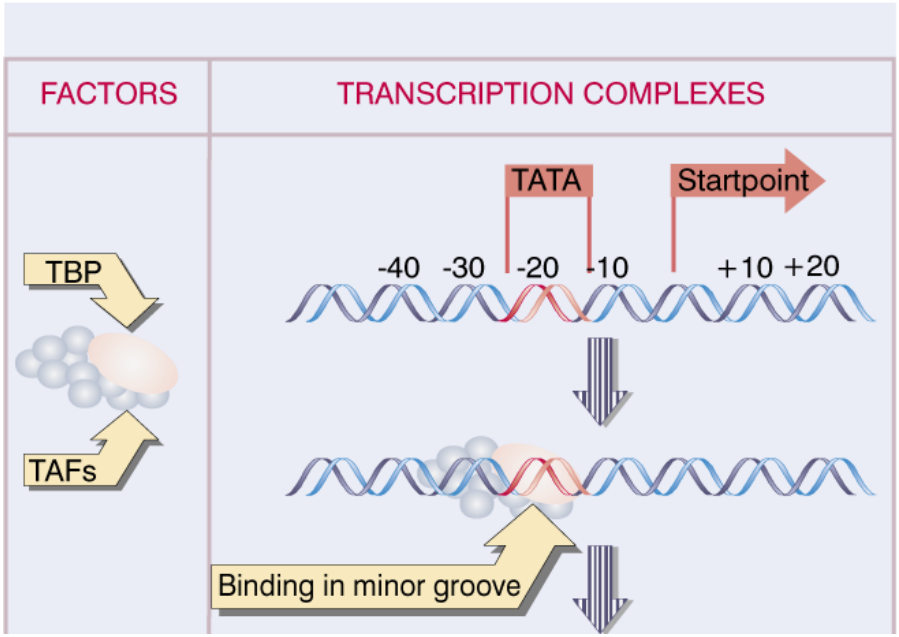
and reduces its nonspecific binding to DNA

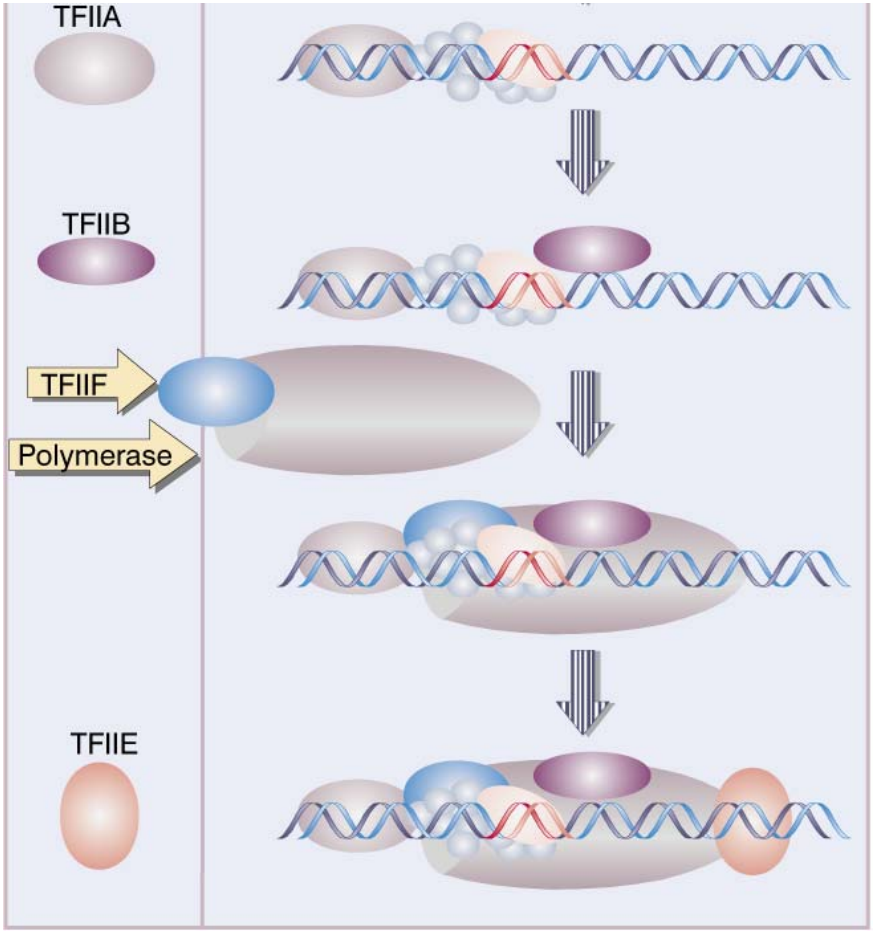
3. also important for promoter clearance

E. TFIIE

1. heterodimer (34 & 56 kD)
2. can be shown to bind RNAPII directly
3. required for recruitment of TFIIH

Figure 20.11 An initiation complex assembles at promoters for RNA polymerase II by an ordered sequence of association with transcription factors.





General transcription factors

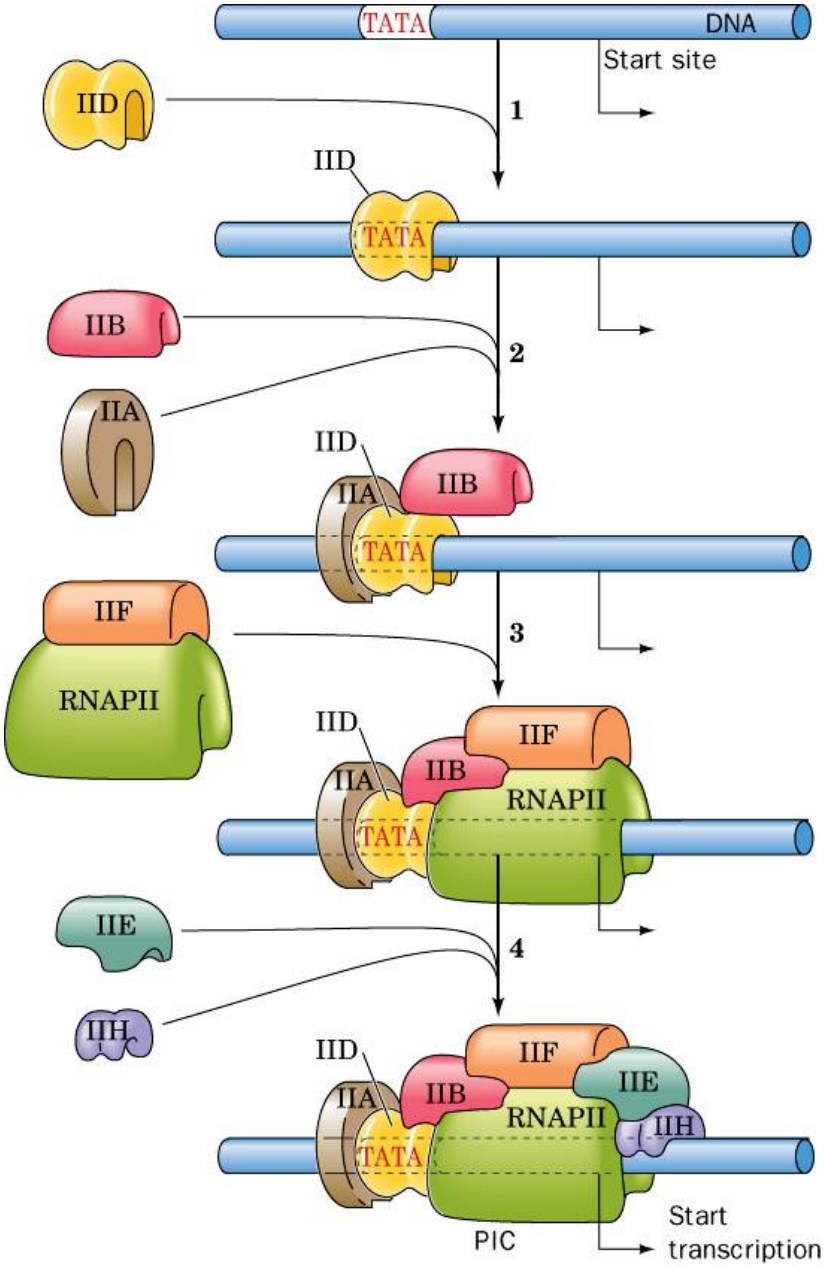
F. TFIIH

1. has both 3'-5' and 5'-3 helicase activity which requires ATP hydrolysis
2. the 3'-5' helicase activity is essential for promoter opening
   a. there is an ATP requirement for promoter opening
   b. can circumvent by using supercoiled DNA or premelted DNA eliminates the need for TFIIH
3. the Cdk7 subunit is a CTD kinase and is regulated by another subunit of IIH called cyclin H
   a. this complex is a CAK (CTD kinase activating kinase)
   b. can be dissociated from the core TFIIH complex
   c. not required for formation of the first phosphodiester bond formation
4. TFIIH is also involved in nucleotide excision repair of DNA
   a. actively transcribed DNA is more readily repaired
   b. interaction with TFIIE modulates the ATPase, helicase, and kinase activity of TFIIH

Table 1. TFIIH Composition and Subunit Function

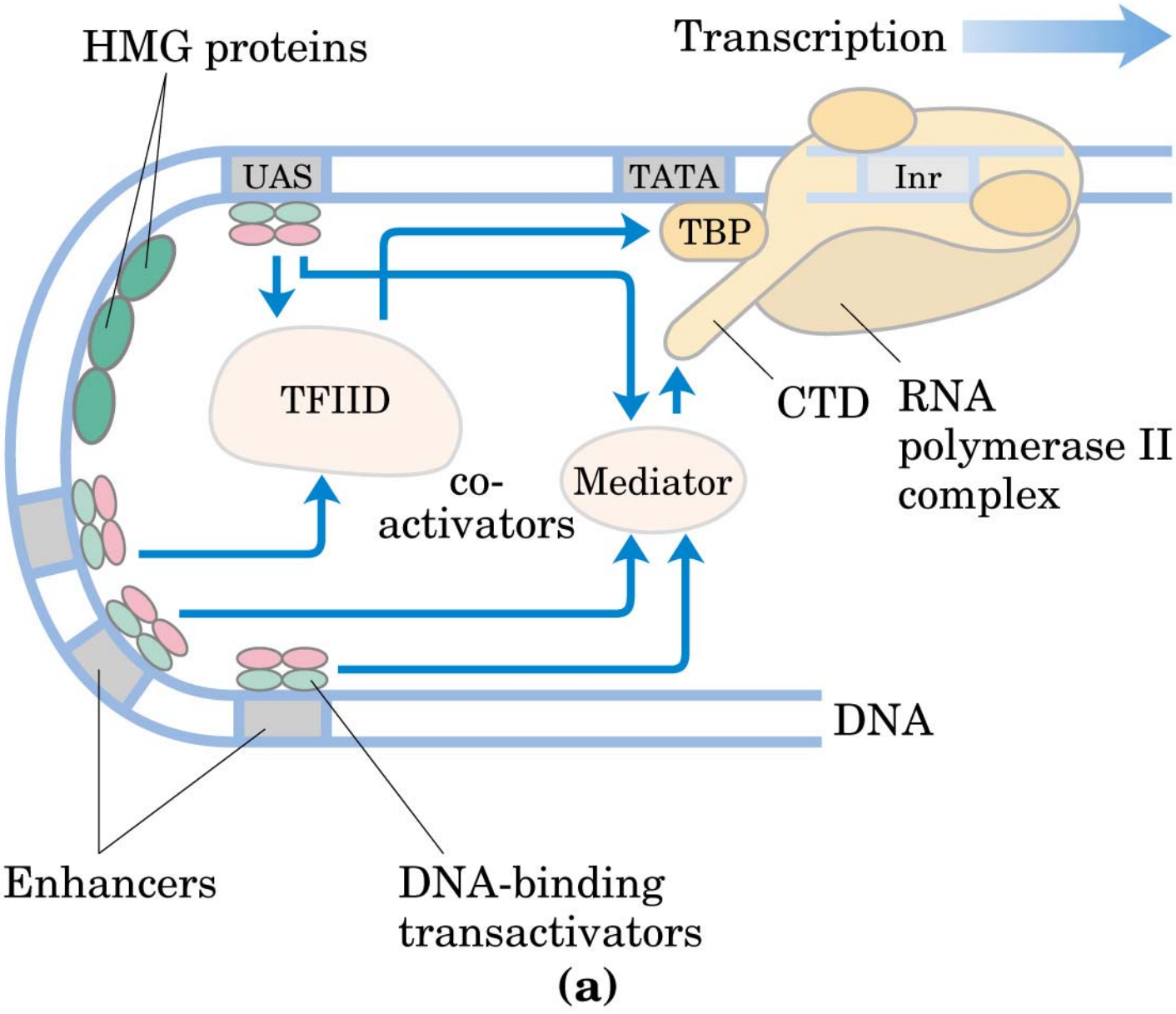
Human	m.w. (kD)	Function	*Saccharomyces cerevisiae*
XPB/ERCC3	89	3′-5′ helicase ATPase: essential for promoter opening	Rad25/Ssl2
XPD/ERCC2	80	5′-3′ helicase APTase: CAK anchoring subunit	Rad3
p62	62	n.d.	Tfb1
p52	52	WD domains; interaction with other proteins?	Tfb2
p44	44	DNA binding and stimulation of XPD helicase activity	Ssl1
p34	34	DNA binding?	Tfb4
Cdk7 (CAK)	40	CTD kinase	Kin28 (TFIIK)
Cyclin H (CAK)	34	regulation of Cdk7	Ccl1 (TFIIK)
MAT1 (CAK)	32	ring finger; kinase stimulation	

(XPB, XPD) Xeroderma pigmentosum group B and D; (CTD kinase) carboxy-terminal domain kinase; (CAK) CDK-activating kinase


TATA
DNA
Start site
IID
1
IID
TATA
IIB
2
IIA
IID
IIA
IIB
TATA
IIF
RNAPII
3
IID
IIF
IIA
IIB
RNAPII
TATA
IIE
4
IIH
IID
IIF
IIA
IIB
IIE
RNAPII
IIH
TATA
PIC
Start
transcription

Summary time

How does the activity of RNA polymerase II get modulated?


HMG proteins
Transcription
UAS
TATA
Inr
TBP
TFIID
CTD
RNA polymerase II complex
co-activators
Mediator
DNA
Enhancers
DNA-binding transactivators

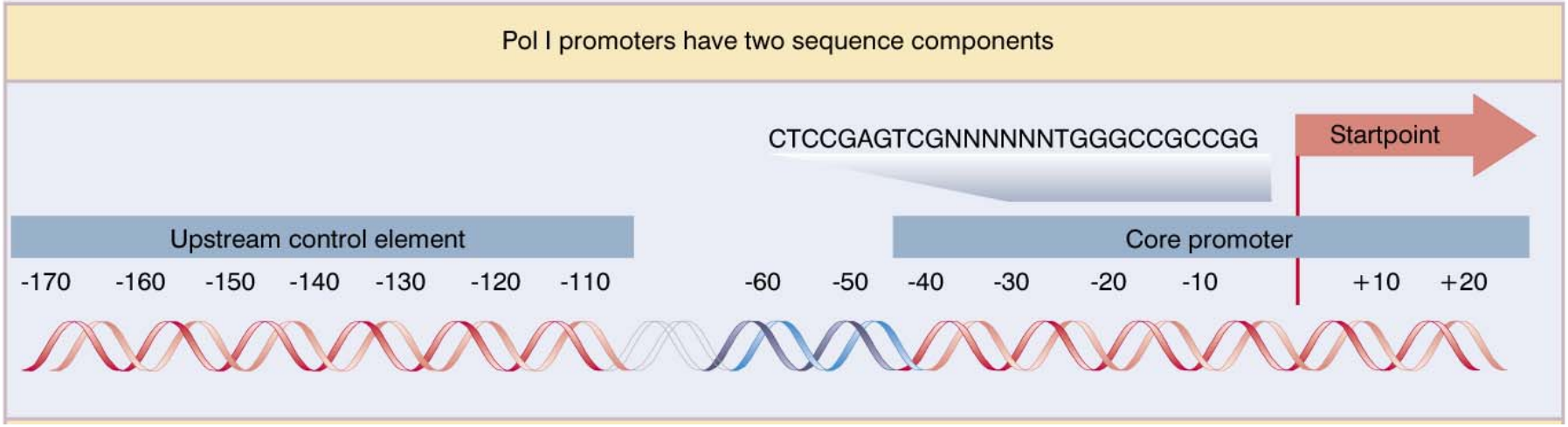
(a)

Promoter Structure

A. rRNA genes - transcribed by RNAP I

1. Core promoter element (-45 to +20) alone is sufficient for transcription
2. Upstream control element (-180 to -107)
   required for efficient transcription
3. transcription factor UBF binds to both promoter elements
4. which then helps recruit a second transcription factor called SL1
   a. contains 4 subunits
   b. one of the subunits is TBP
   c. referred to as specificity factor
      - species specific

Figure 20.4 Transcription units for RNA polymerase I have a core promoter separated by ~70 bp from the upstream control element. UBF1 binds to both regions, after which SL1 can bind. RNA polymerase I then binds to the core promoter. The nature of the interaction between the factors bound at the upstream control element and those at the core promoter is not known.



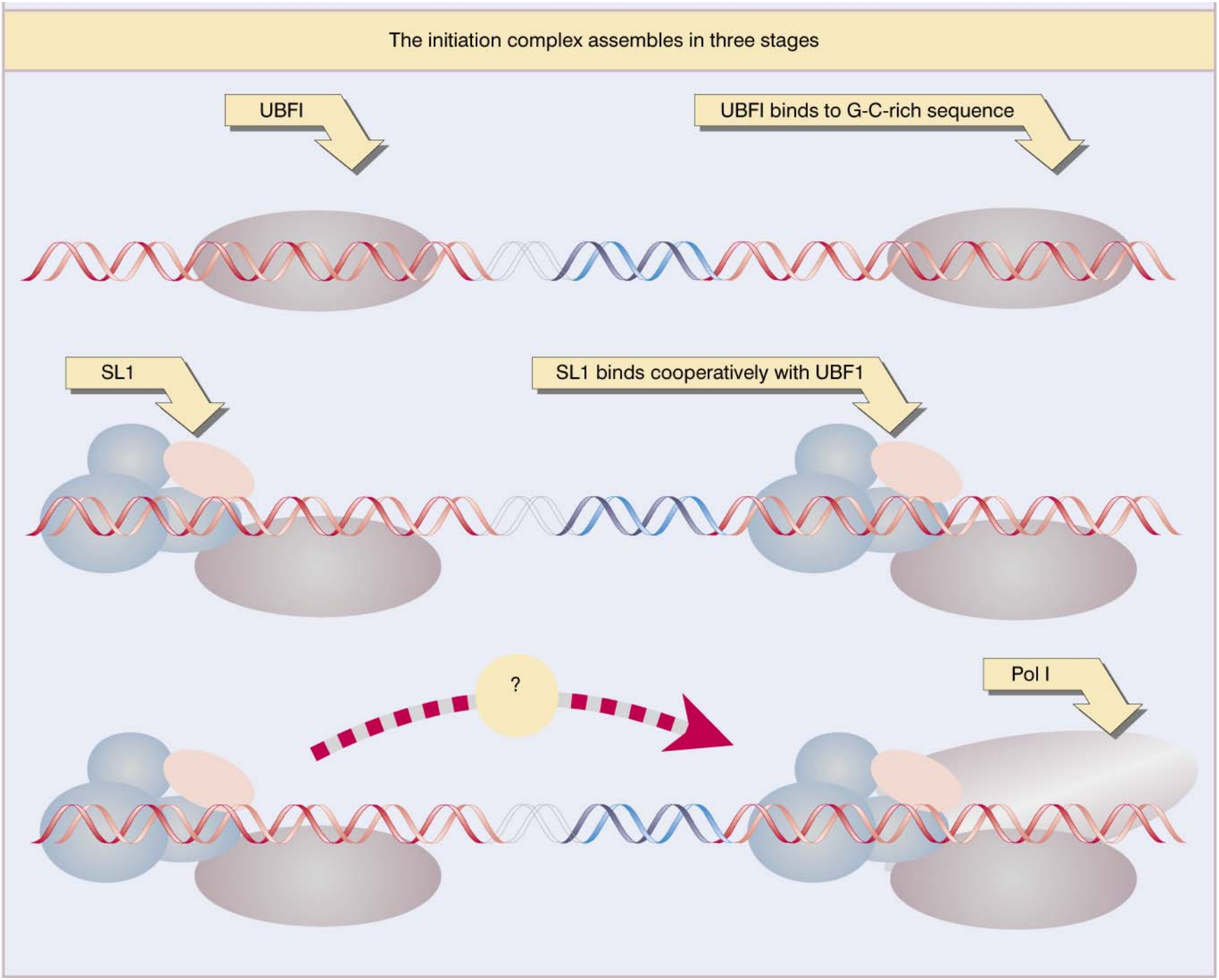
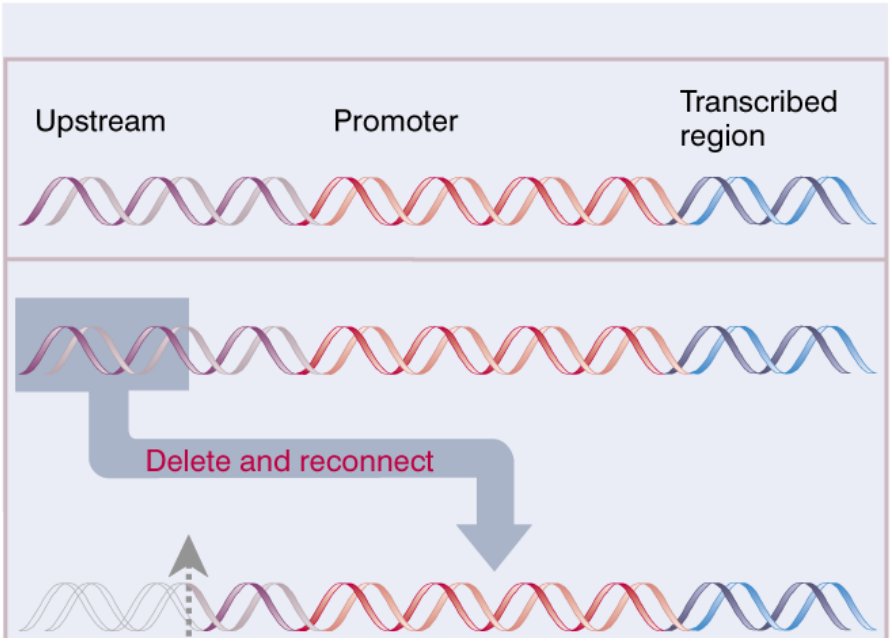

The initiation complex assembles in three stages
UBFI
UBFI binds to G-C-rich sequence
SL1
SL1 binds cooperatively with UBF1
?
Pol I

Figure 20.3 Promoter boundaries can be determined by making deletions that progressively remove more material from one side. When one deletion fails to prevent RNA synthesis but the next stops transcription, the boundary of the promoter must lie between them.



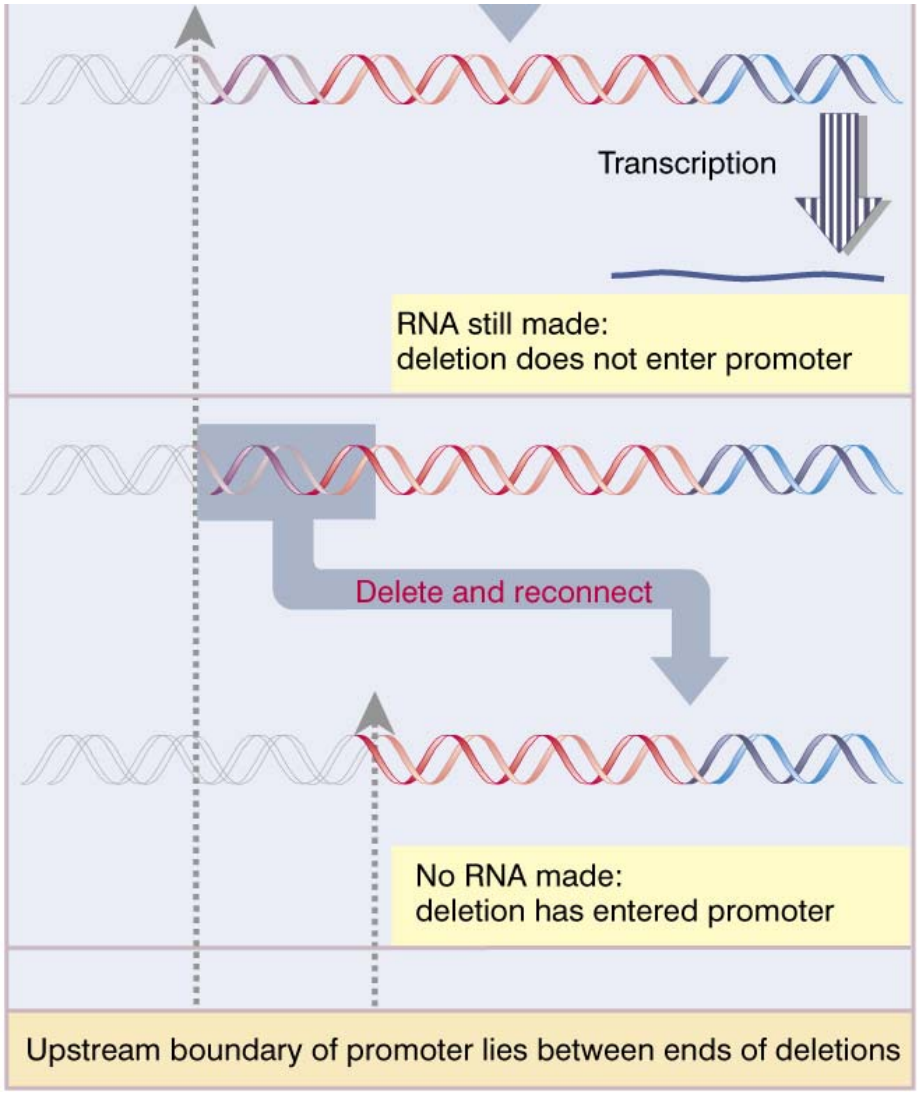
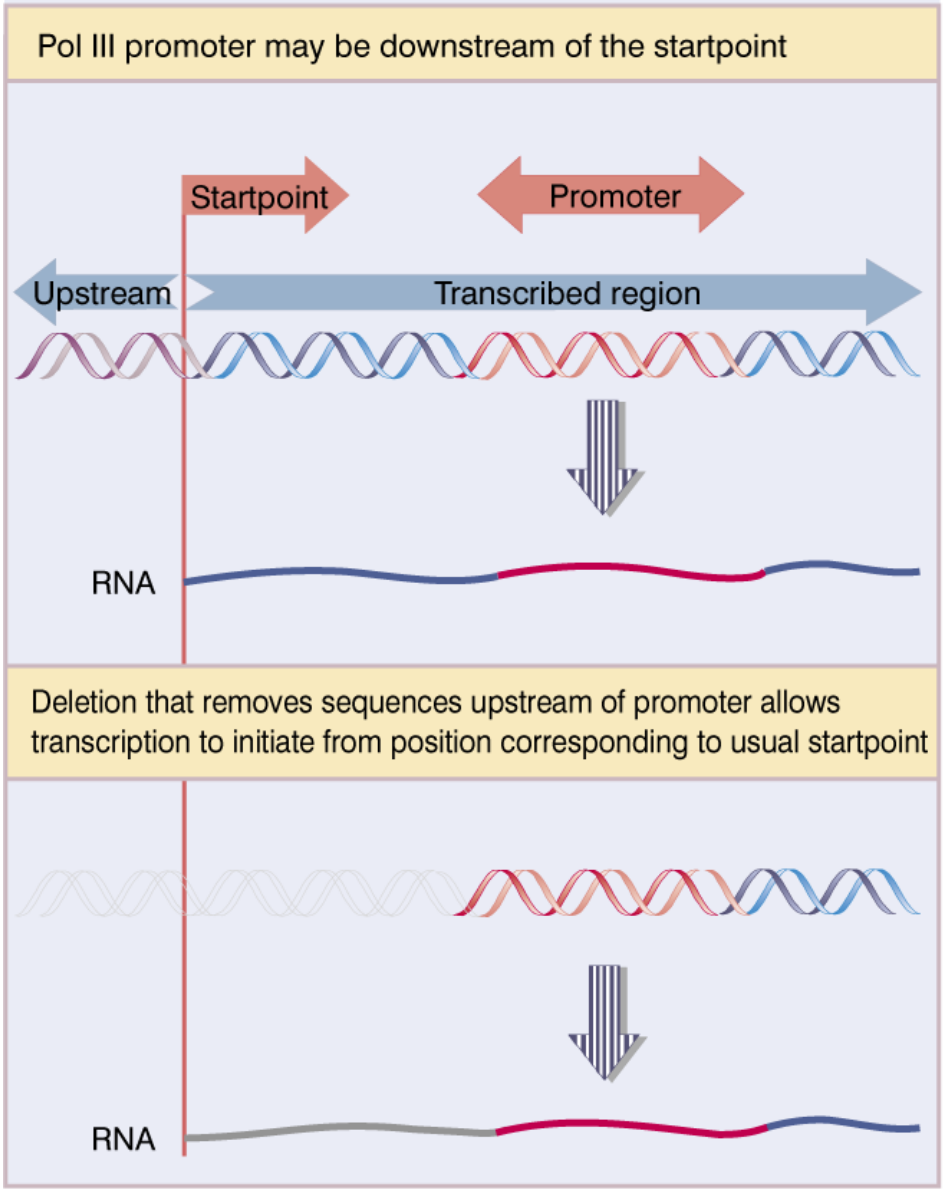
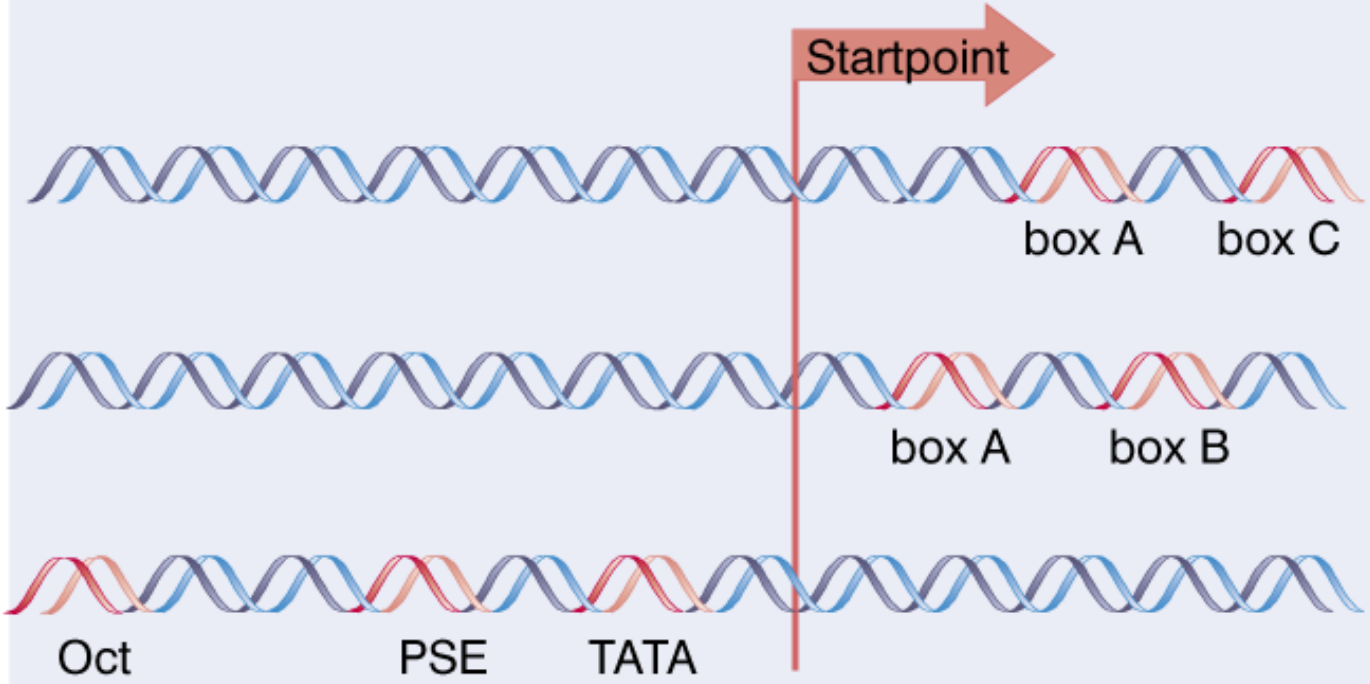


Figure 20.5 Deletion analysis shows that the promoter for 5S RNA genes is internal; initiation occurs a fixed distance (~55 bp) upstream of the promoter.



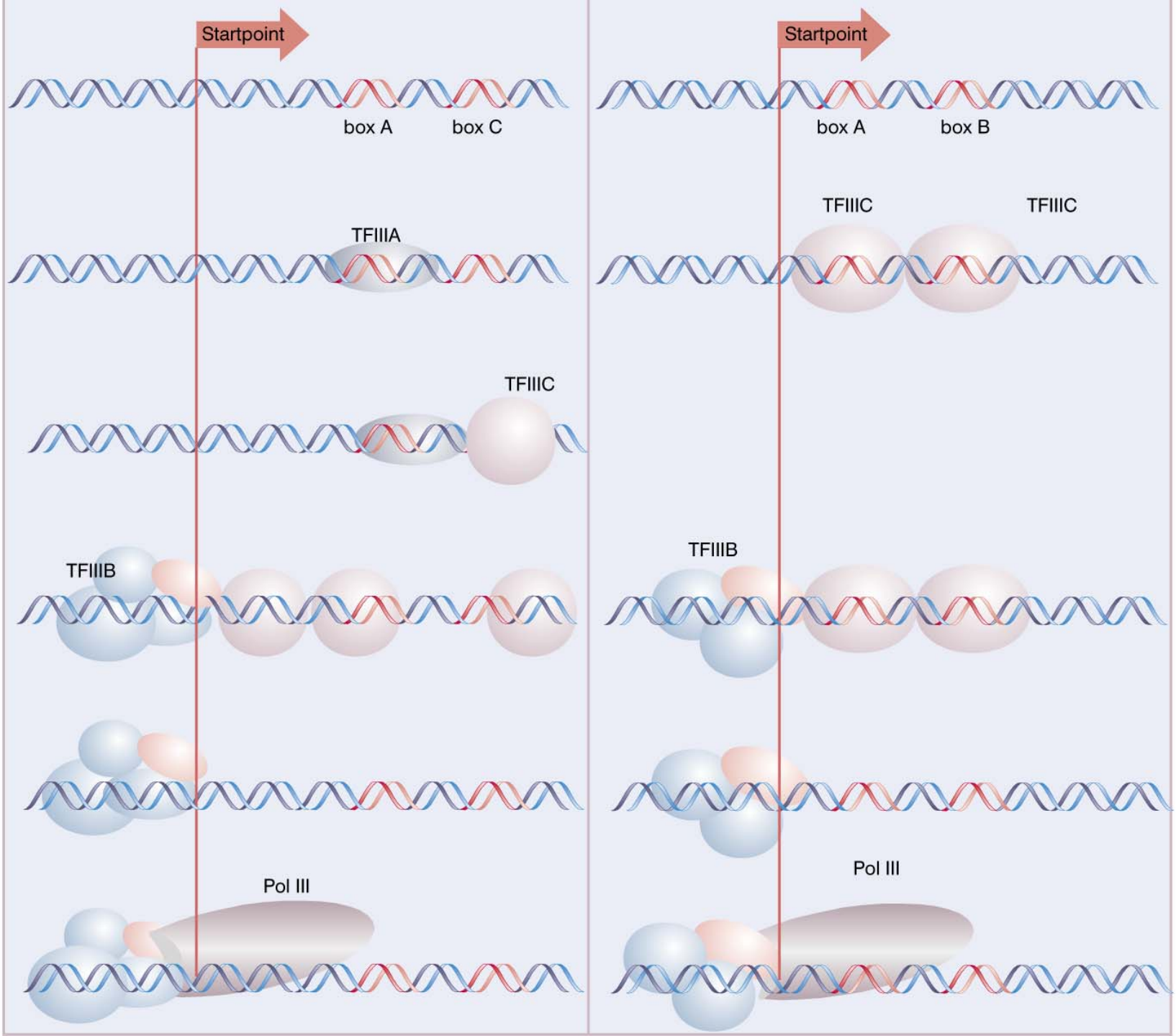
How are RNA polymerase I promoter and transcription factor requirements fundamentally different from RNA polymerase II?

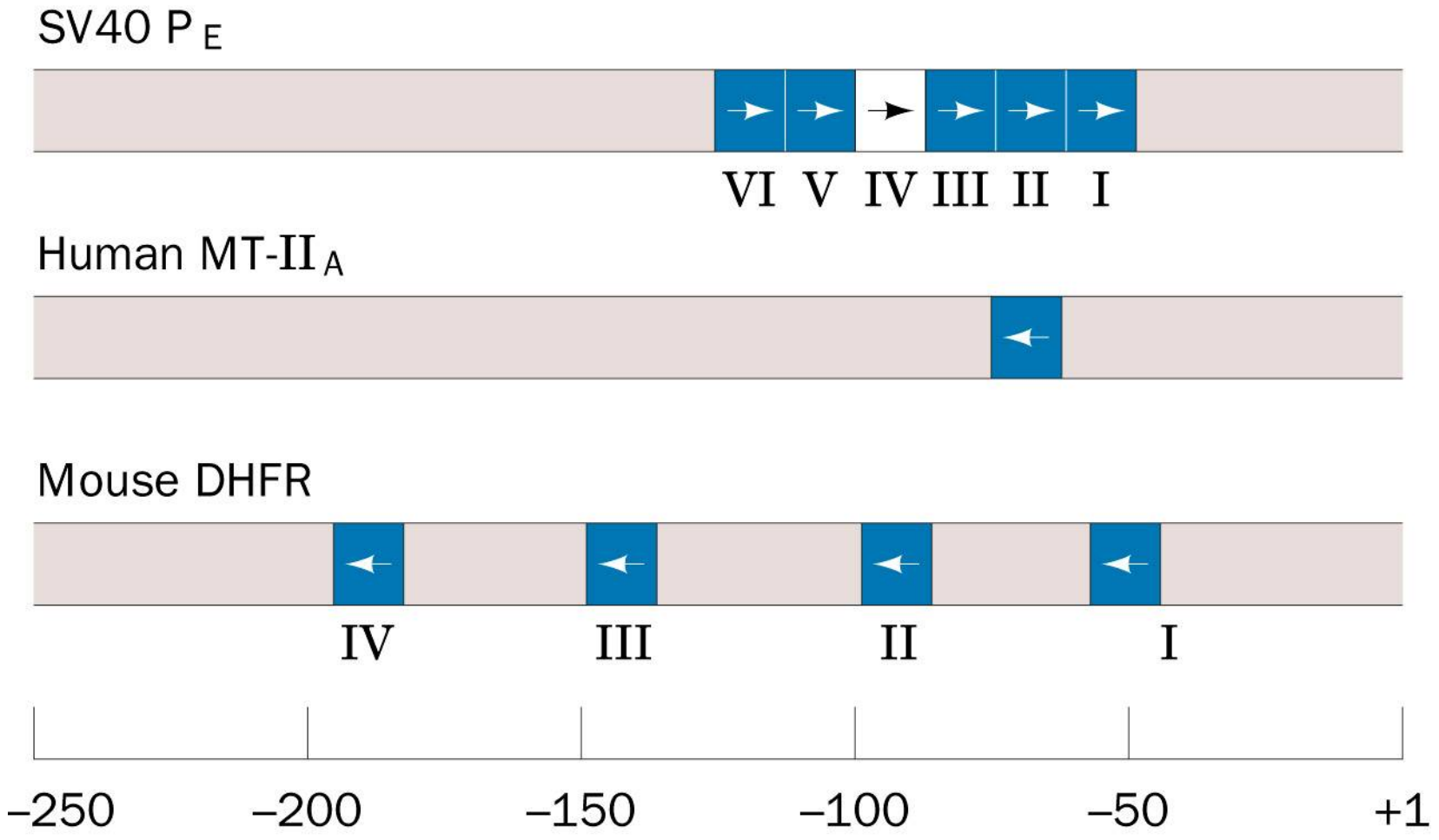
Figure 20.6 Promoters for RNA polymerase III may consist of bipartite sequences downstream of the startpoint, with boxA separated from either boxC or boxB. Or they may consist of separated sequences upstream of the startpoint (Oct, PSE, TATA).



Promoter Structure

B. genes transcribed by RNAP III
   1. tRNA genes
      a. box A: crucial for start site selection
      b. box B
      c. spacing between the two is variable
      d. TFIIIC is bound to both box A and B
      e. recruits TFIIIB to DNA upstream of start site of transcription
   2. 5S rRNA gene
      a. box A
      b. box C is bound by TFIIIA
      c. TFIIIA helps recruit TFIIIC and in turn TFIIIB
   3. U6 snRNA gene
      a. TATA box
      b. PSE: proximal stimulatory element, centered near bp -50
      c. DSE: distal stimulatory element


Startpoint
box A
box C
TFIIIA
TFIIIC
TFIIIB
Pol III
Startpoint
box A
box B
TFIIIC
TFIIIC
TFIIIB
Pol III


SV40 P E
VI V IV III II I
Human MT-II A
Mouse DHFR
IV III II I
–250 –200 –150 –100 –50 +1