Eukaryotic Transcription

Eukaryotic RNA polymerases

Enzyme	Localization	Product	Activity	Sensibility to α-amanitin
RNA polymerase I	nucleolus	ribosomal RNA	50-70 %	non sensible
RNA polymerase II	nucleoplasm	nuclear RNA	20-40 %	sensible
RNA polymerase III	nucleoplasm	tRNA, 5S RNA	~10%	species-specific

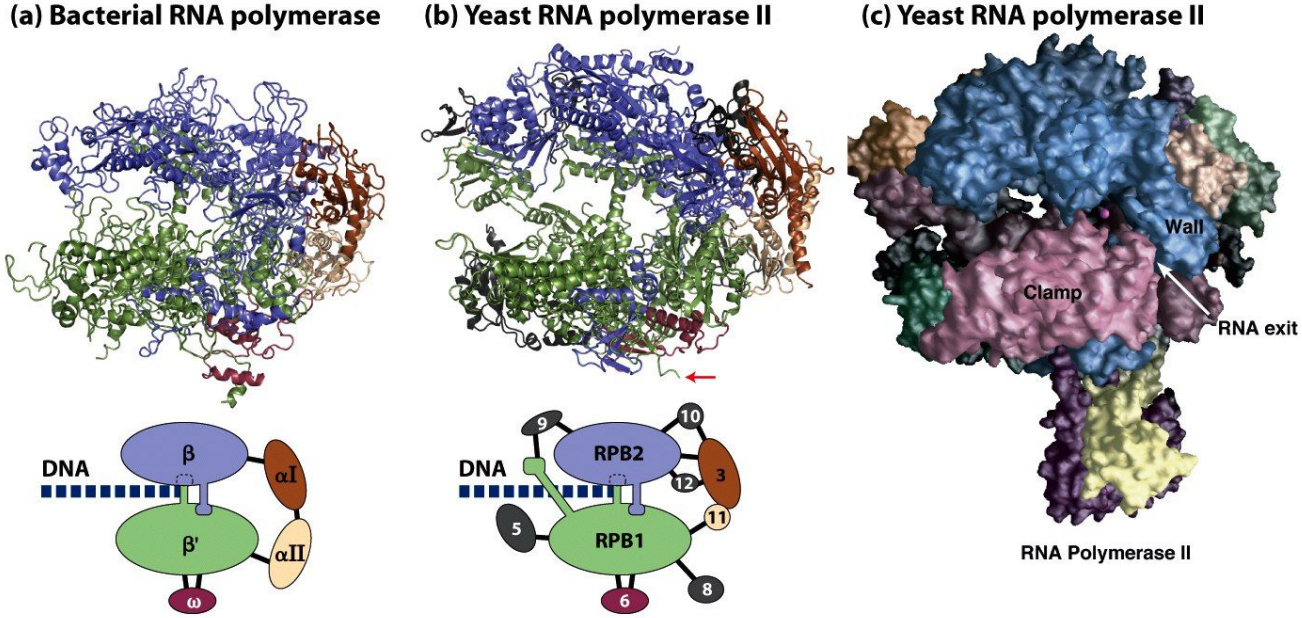


Figure 7-9
Molecular Cell Biology, Sixth Edition

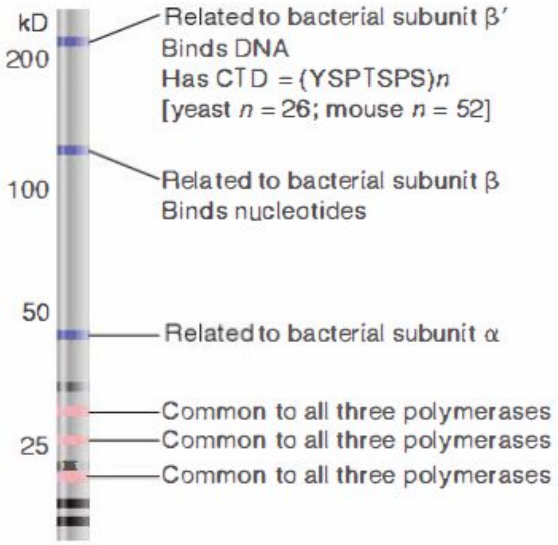


FIGURE 20.2 Some subunits are common to all classes of eukaryotic RNA polymerases and some are related to bacterial RNA polymerase. This drawing is a simulation of purified yeast RNA polymerase II run on an SDS gel to separate the subunits by size.

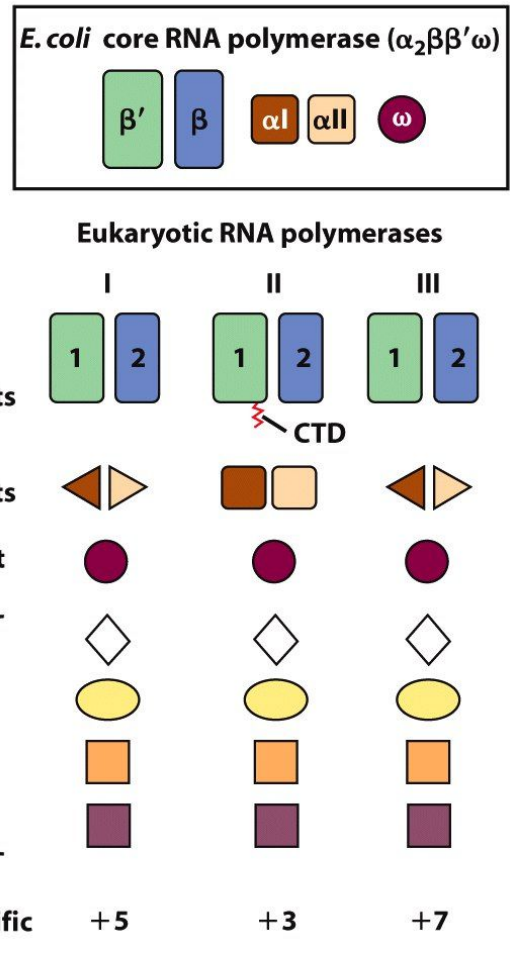


Figure 7-10
Molecular Cell Biology, Sixth Edition

The promoter borders can be determined by progressive deletion analysis

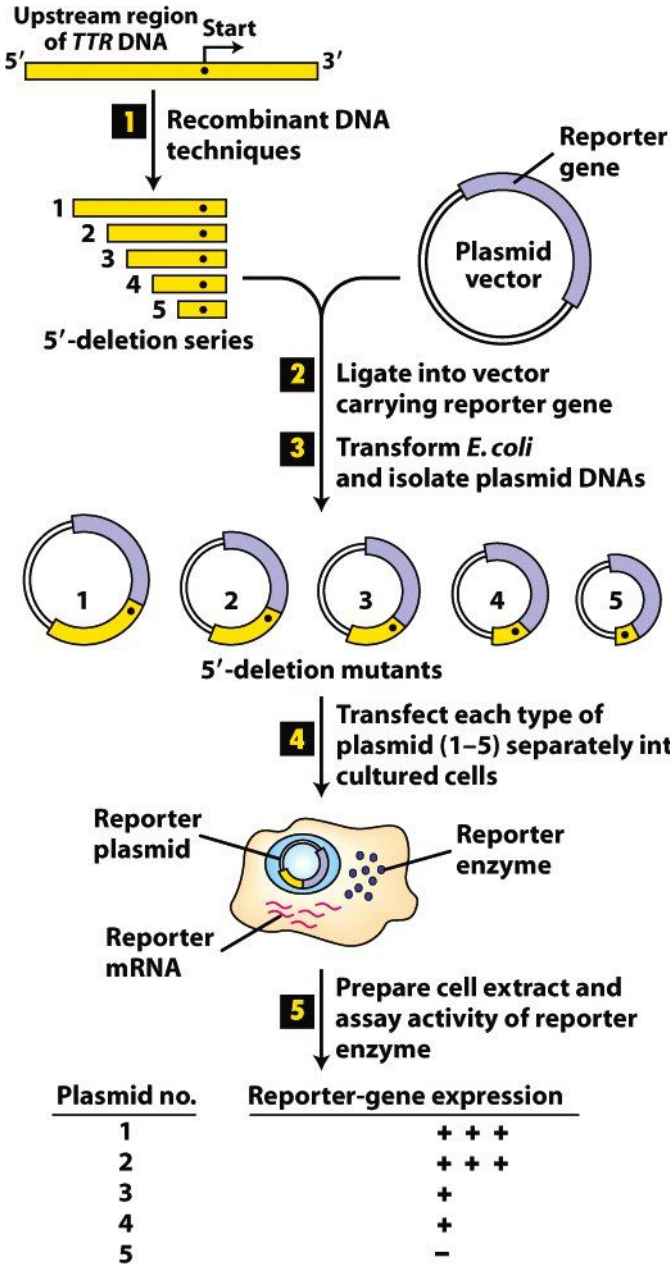


Figure 7-13
Molecular Cell Biology, Sixth Edition

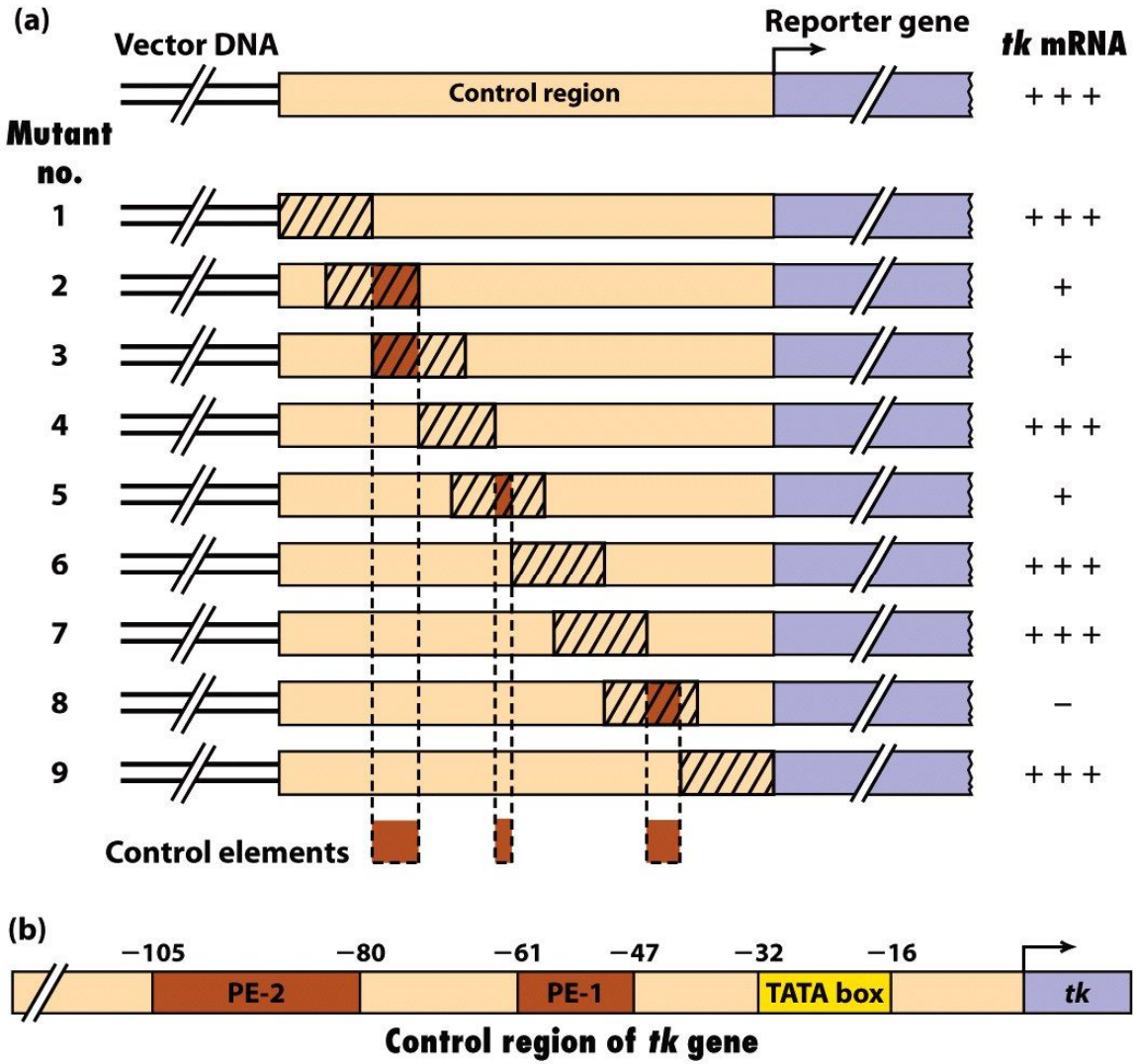


Figure 7-14
Molecular Cell Biology, Sixth Edition

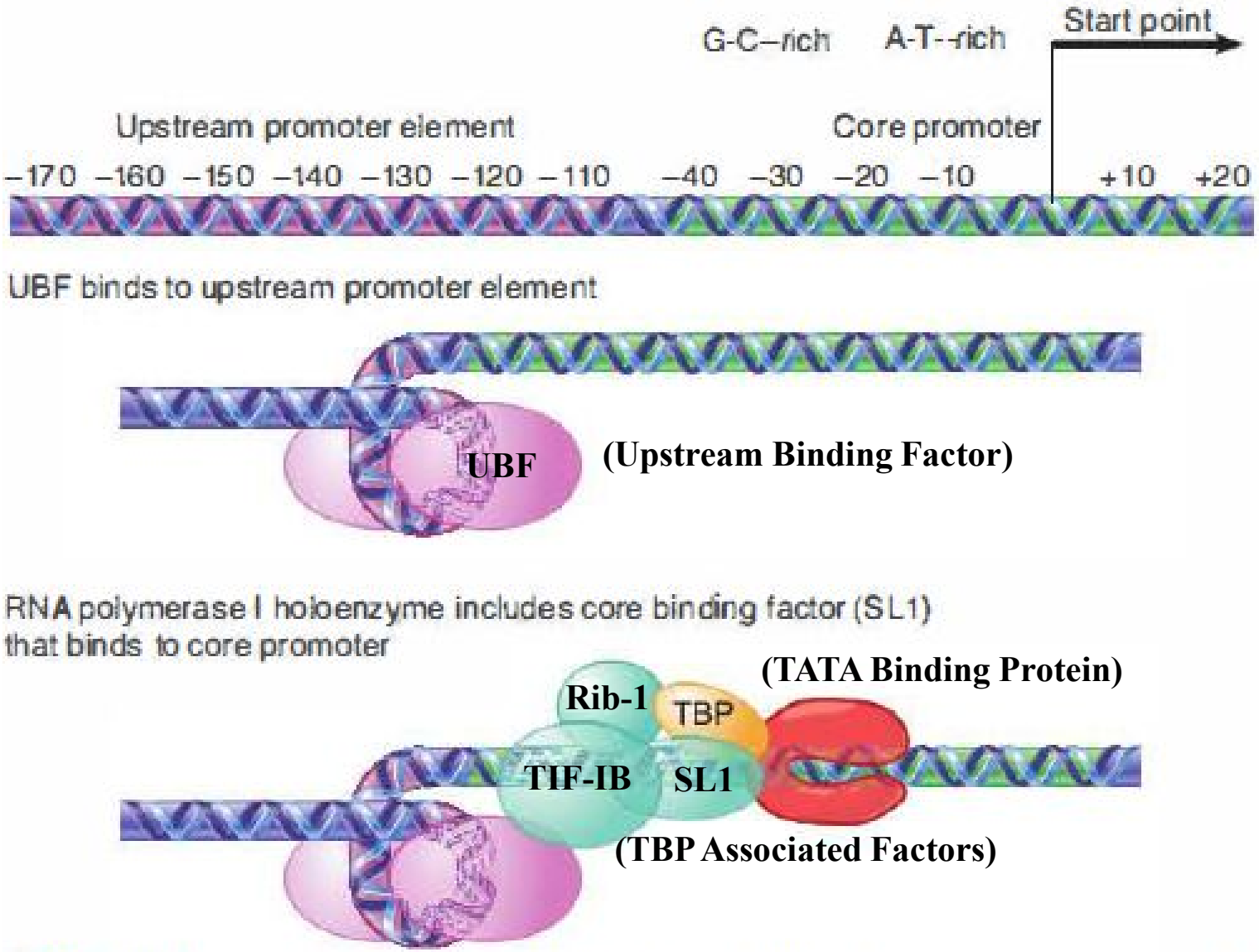
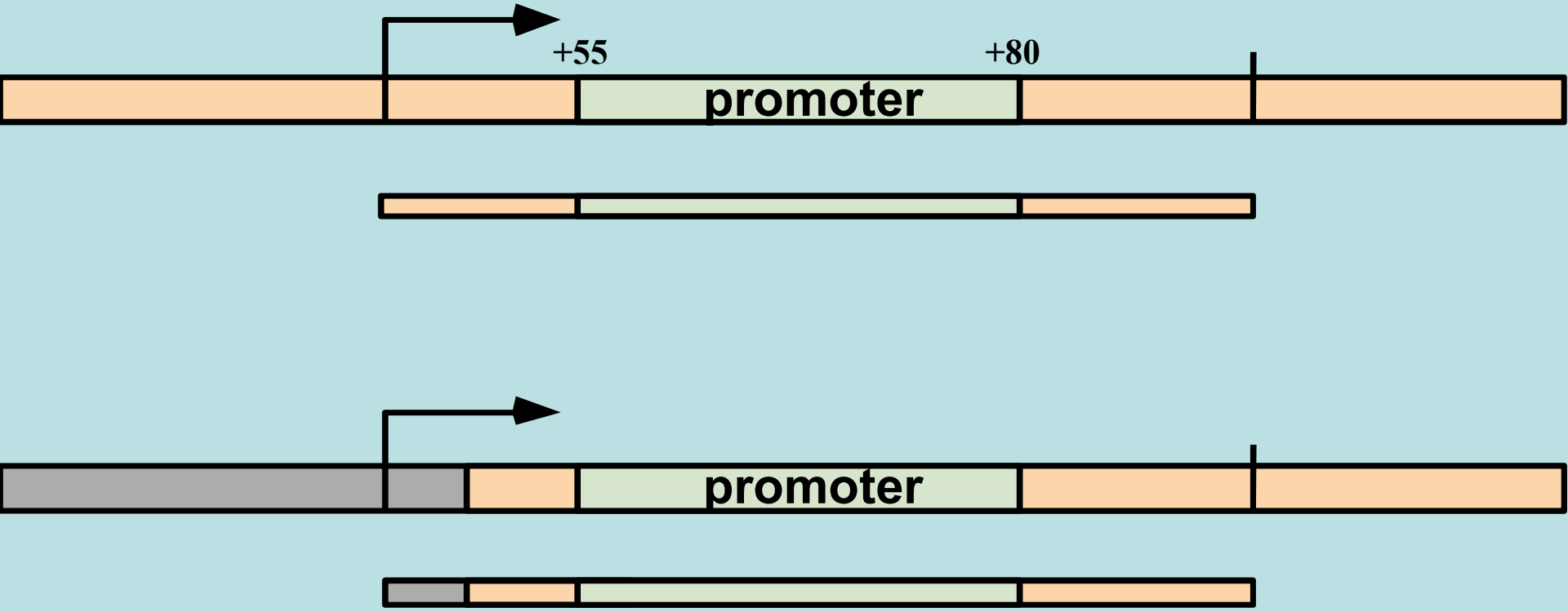


FIGURE 20.3 Transcription units for RNA polymerase I have a core promoter separated by ~70 bp from the upstream promoter element. UBF binding to the UPE increases the ability of core-binding factor to bind to the core promoter. Core-binding factor (SL1) positions RNA polymerase I at the start point.

The deletion analysis shows that the promoter for Pol III of the 5S RNA gene is internal

the start point is at a fixed distance (55 bp) upstream the promoter



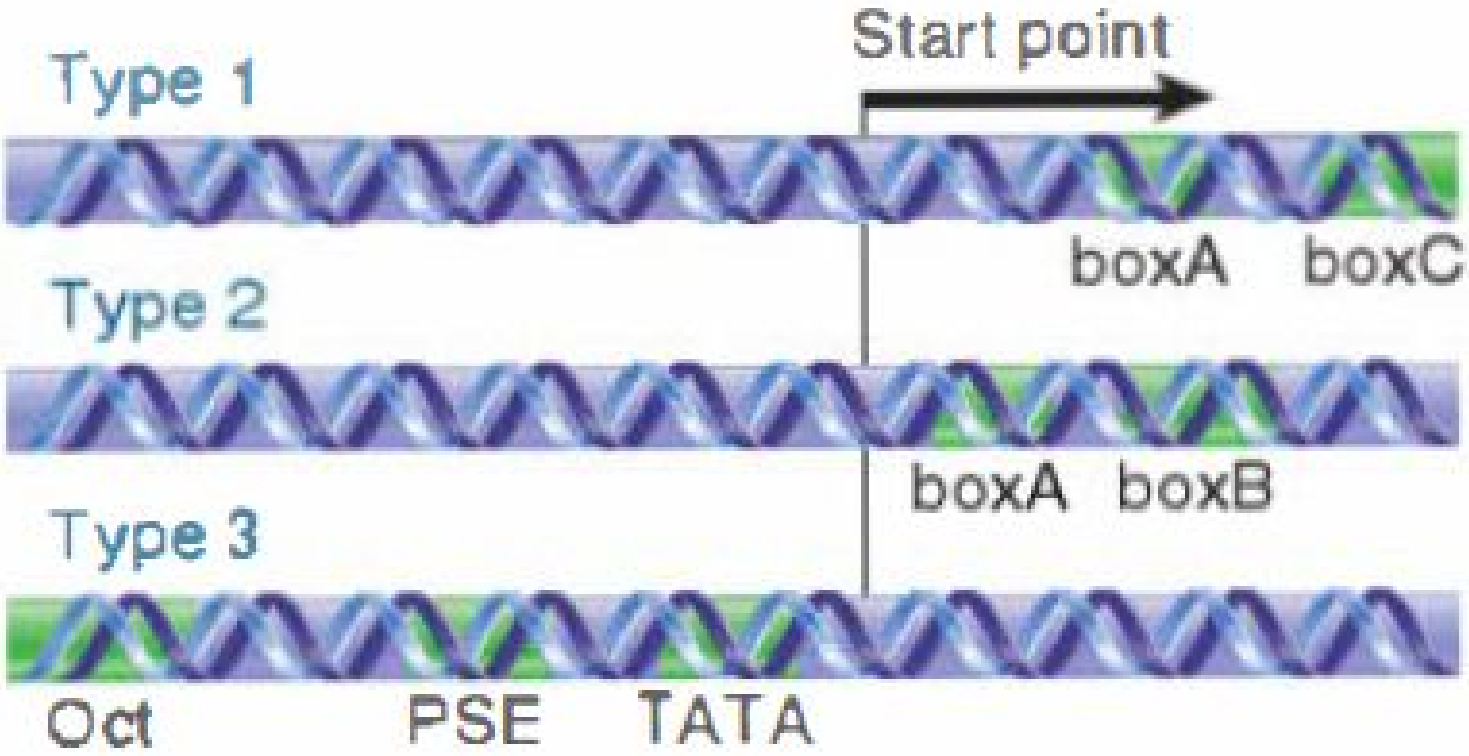


FIGURE 20.4 Promoters for RNA polymerase III may consist of bipartite sequences downstream of the start point, with *boxA* separated from either *boxC* or *boxB*, or they may consist of separated sequences upstream of the start point (Oct, PSE, TATA).

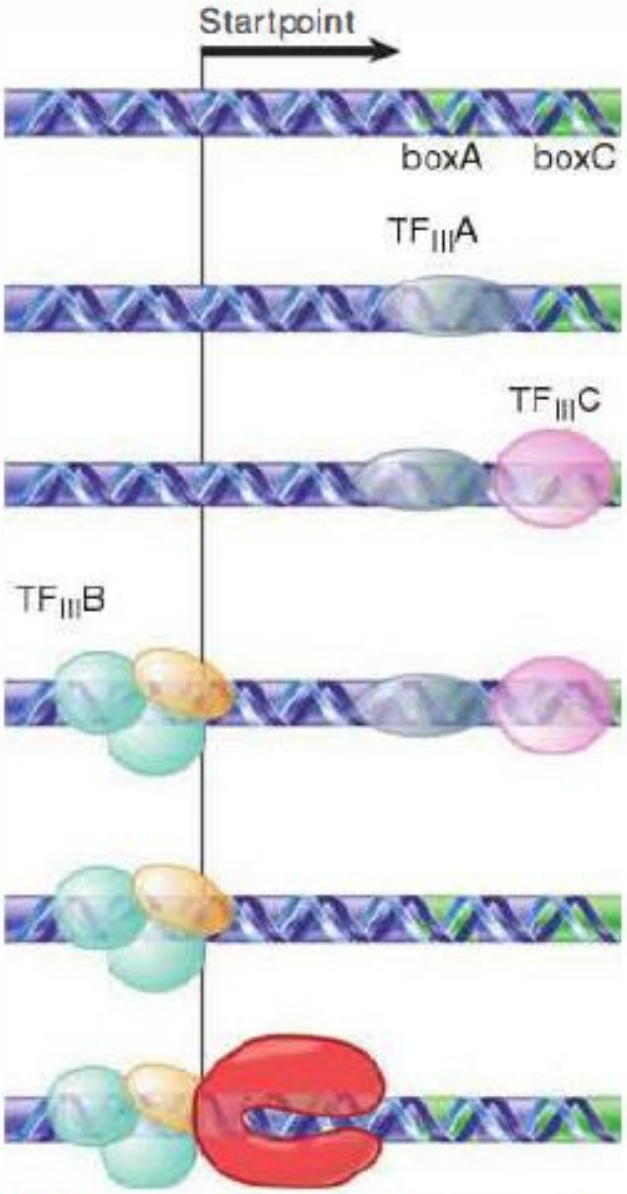


FIGURE 20.6 Internal type 1 pol III promoters use the assembly factors $TF_{III}A$ and $TF_{III}C$, at *boxA* and *boxC*, to recruit the positioning factor $TF_{III}B$, which recruits RNA polymerase III.

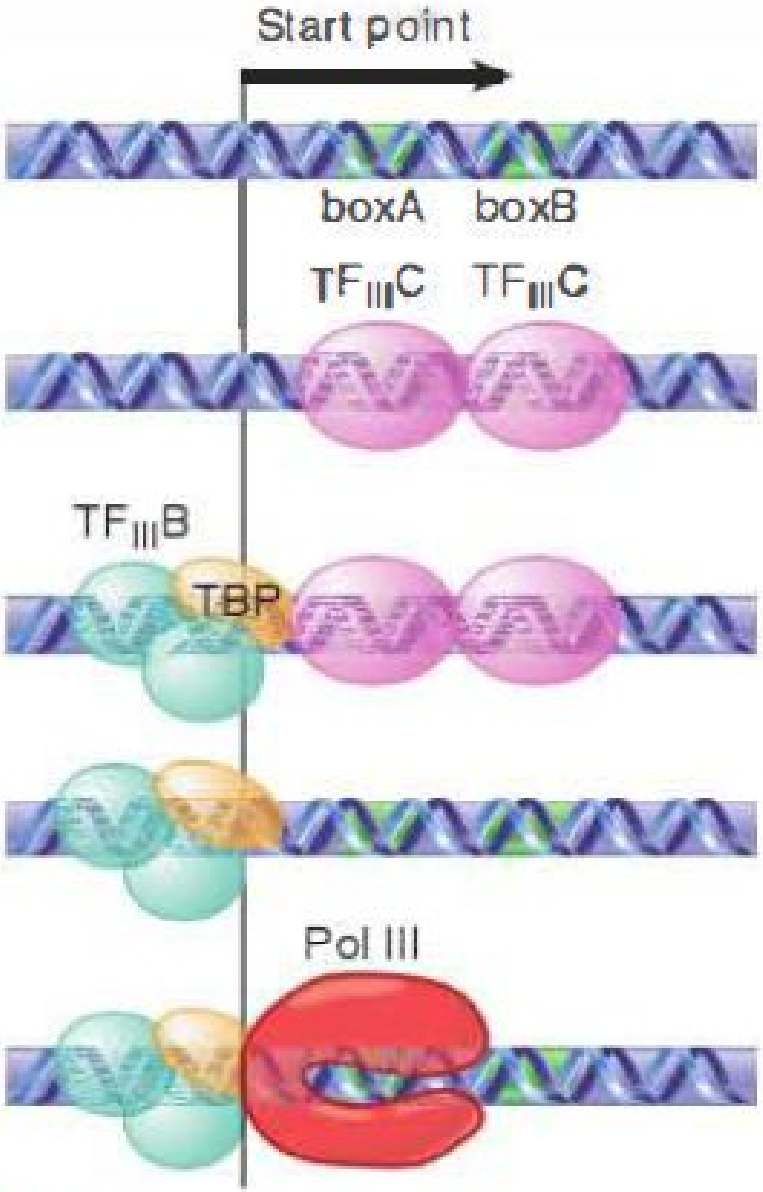


FIGURE 20.5 Internal type 2 pol III promoters use binding of $TF_{III}C$ to *boxA* and *boxB* sequences to recruit the positioning factor $TF_{III}B$, which recruits RNA polymerase III.

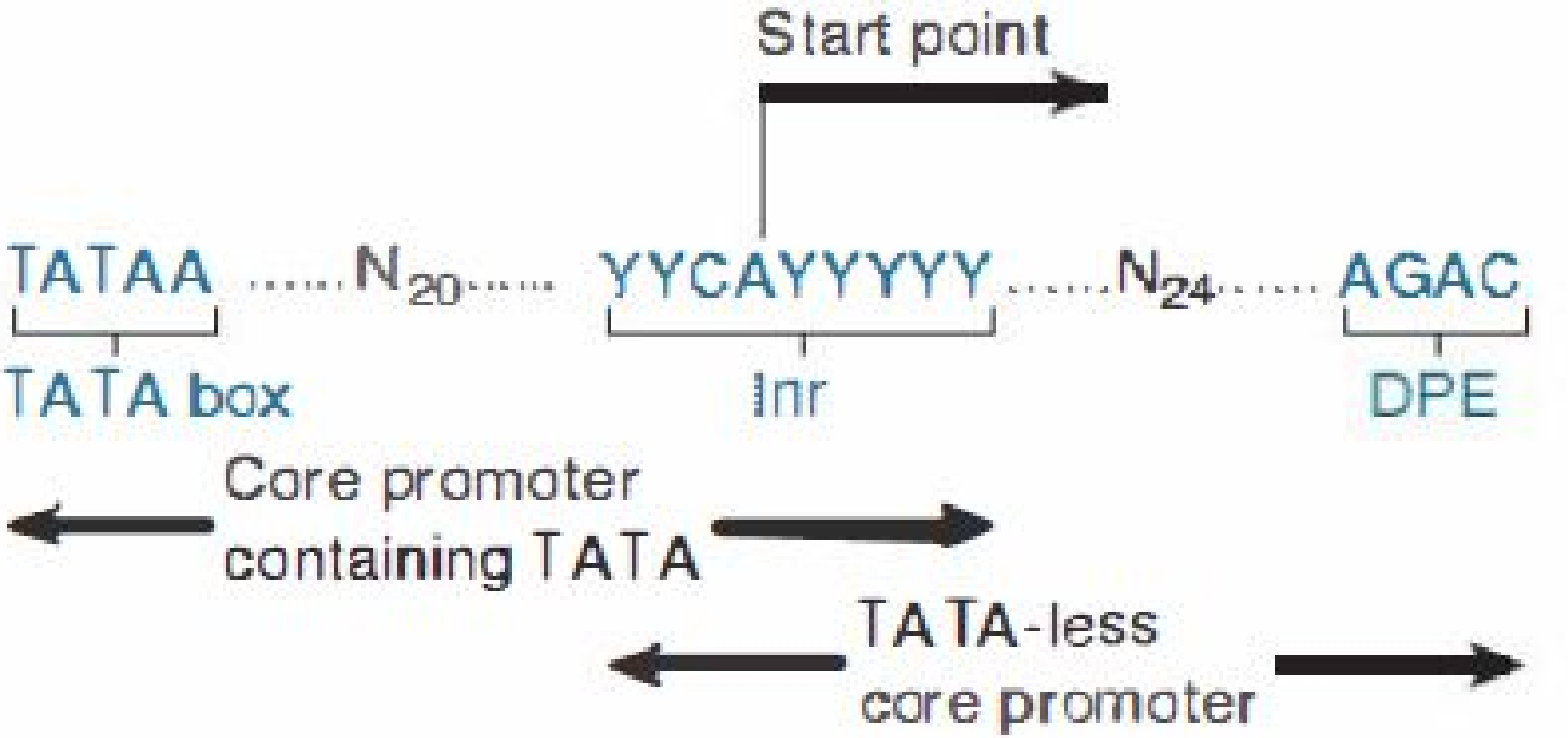


FIGURE 20.7 A minimal pol II promoter may have a TATA box ~25 bp upstream of the Inr. The TATA box has the consensus sequence of TATAA. The Inr has pyrimidines (Y) surrounding the CA at the start point. The DPE is downstream of the start point. The sequence shows the coding strand.

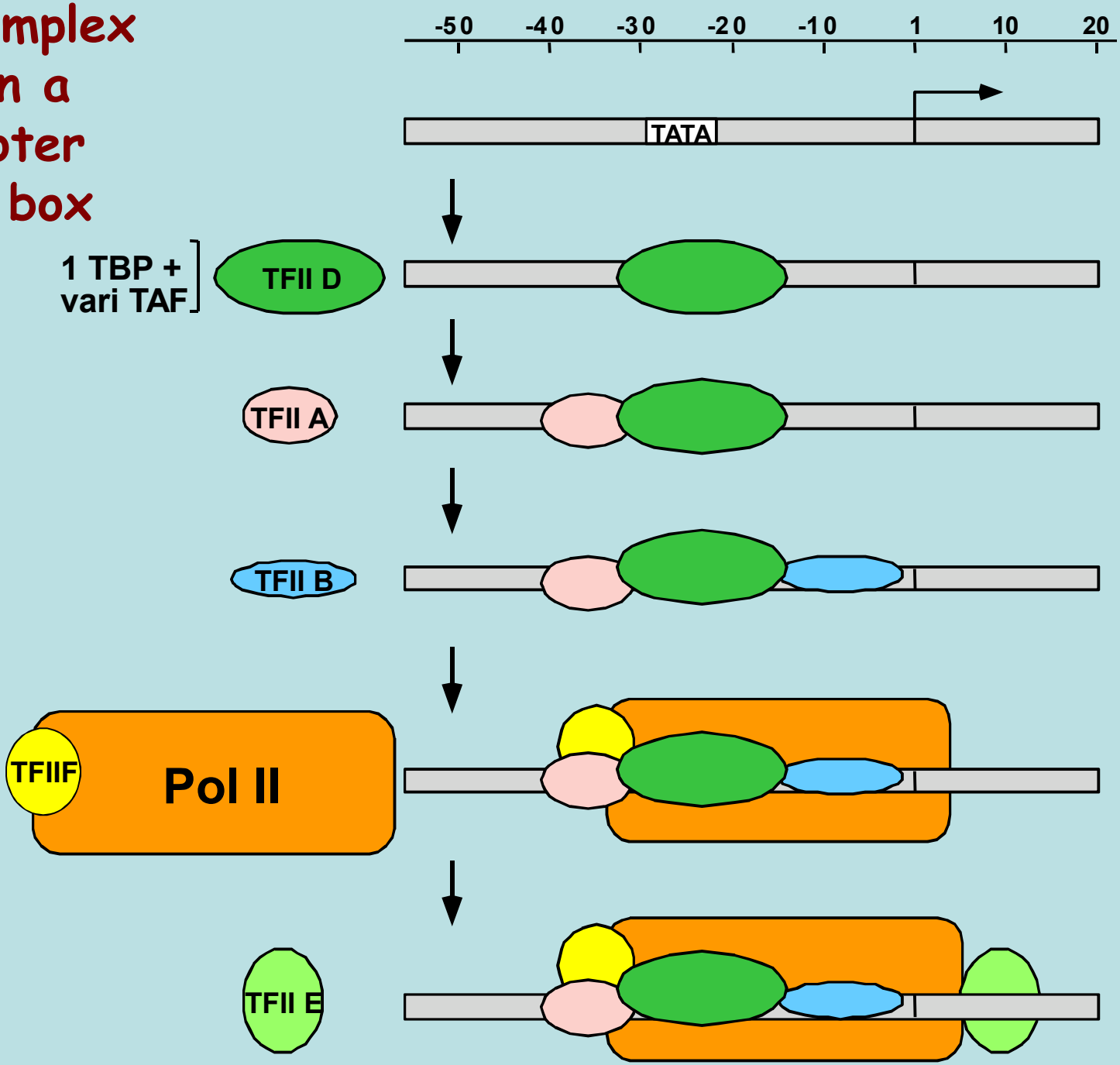
EUKARYOTIC TRANSCRIPTION FACTORS
(trans-acting factors)

Can be divided in:

- General transcription factors

- Activators/inactivators

Initiation complex assembly don a Pol II promoter With TATA box



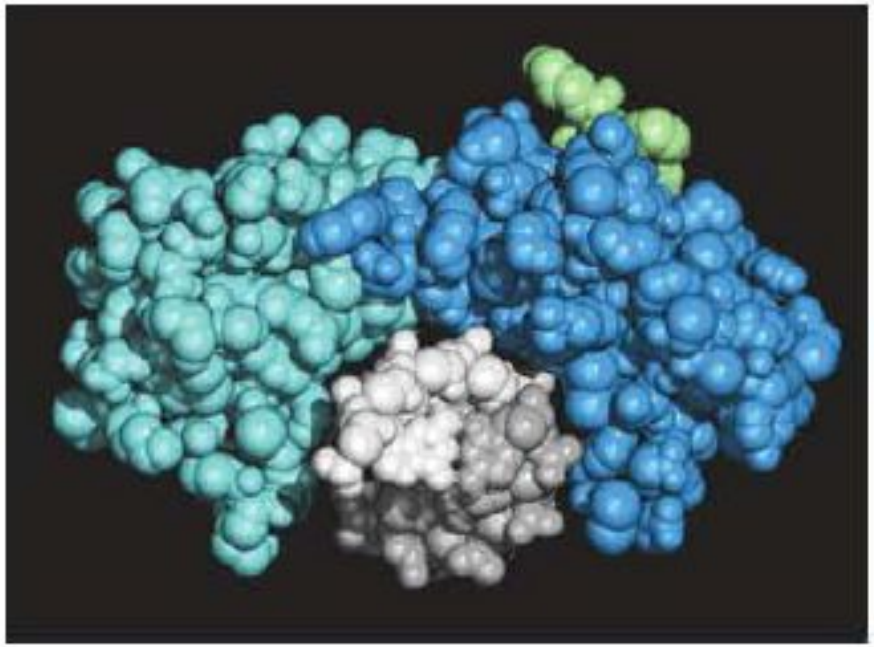


FIGURE 20.9 A view in cross-section shows that TBP surrounds DNA from the side of the narrow groove. TBP consists of two related (40% identical) conserved domains, which are shown in light and dark blue. The N-terminal region varies extensively and is shown in green. The two strands of the DNA double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

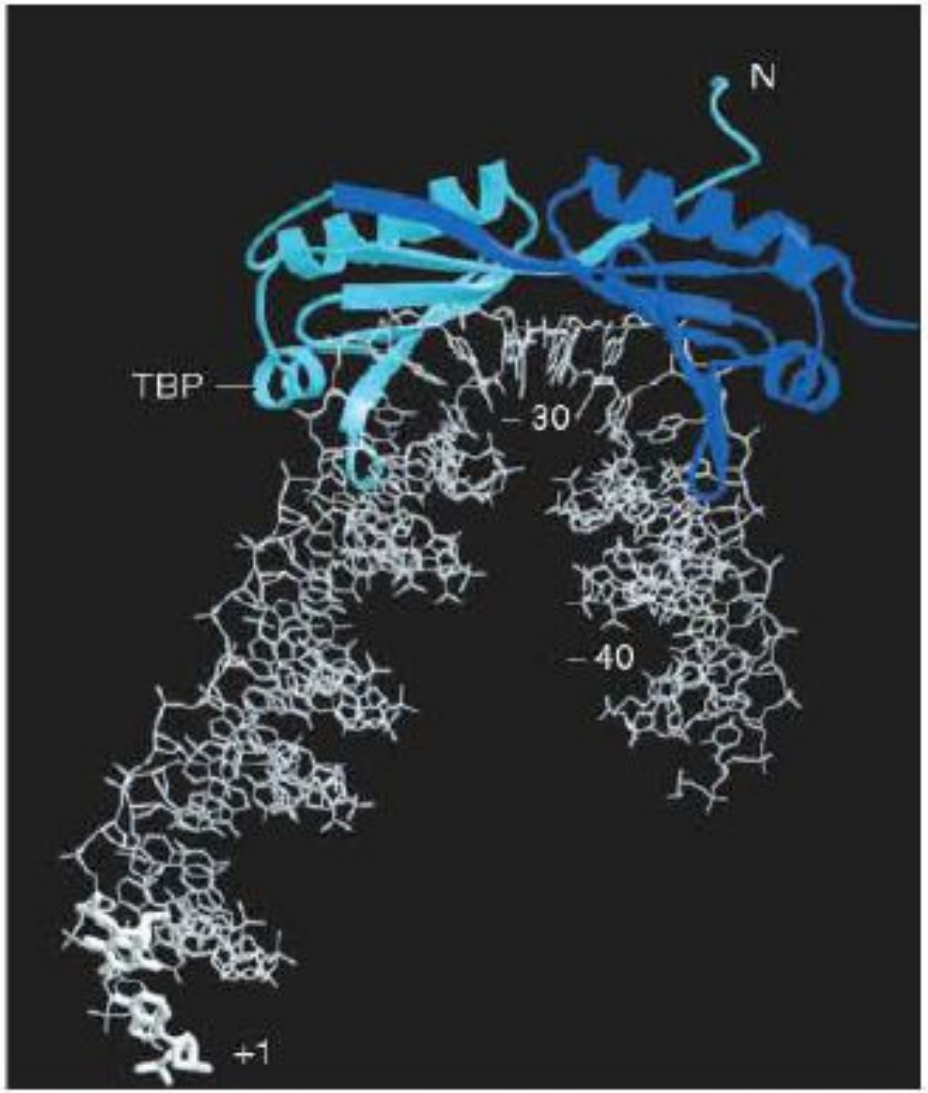


FIGURE 20.10 The cocrystal structure of TBP with DNA from −40 to the start point shows a bend at the TATA box that widens the narrow groove where TBP binds. Photo courtesy of Stephen K. Burley.

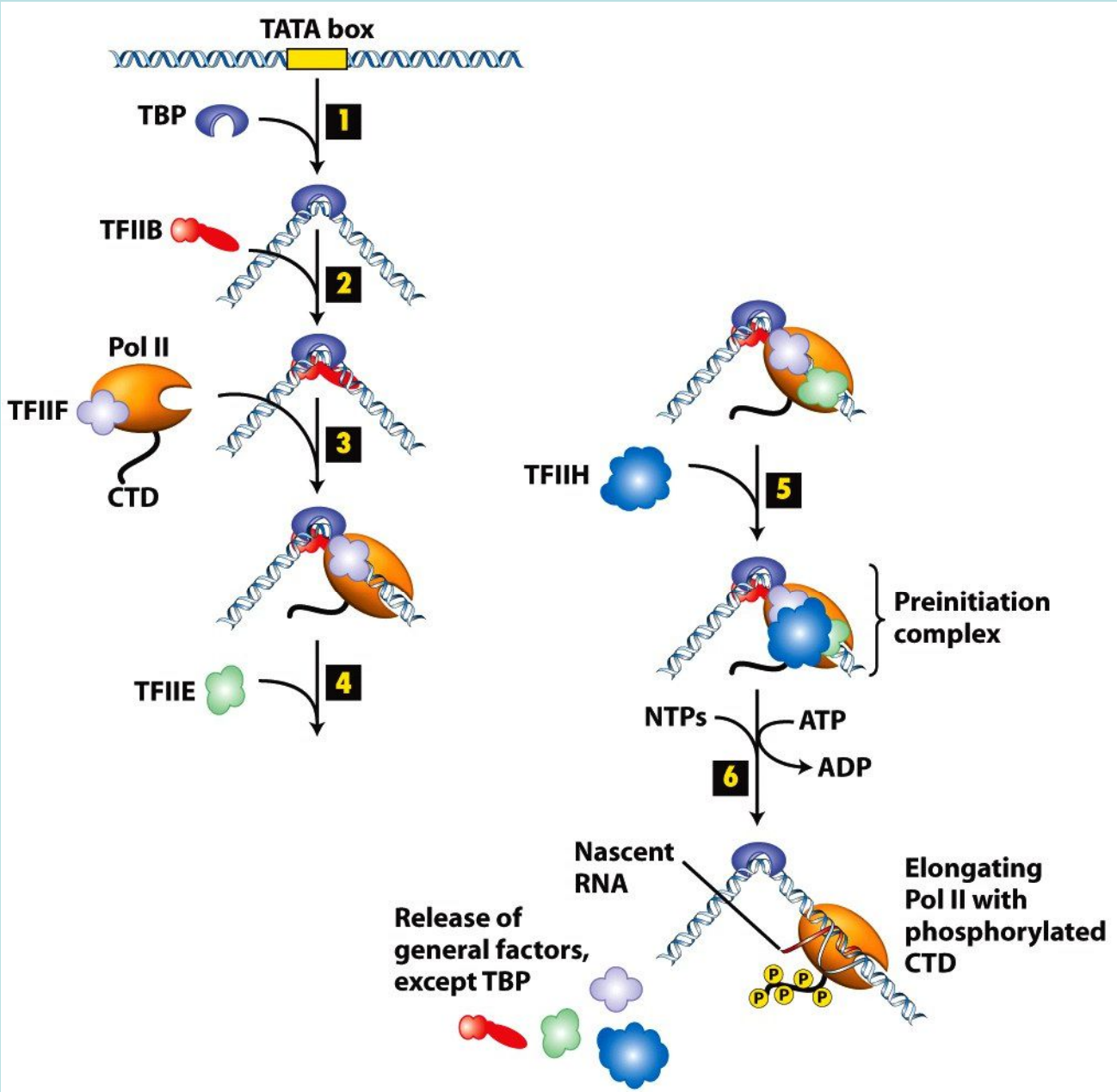


Figure 7-31
Molecular Cell Biology, Sixth Edition

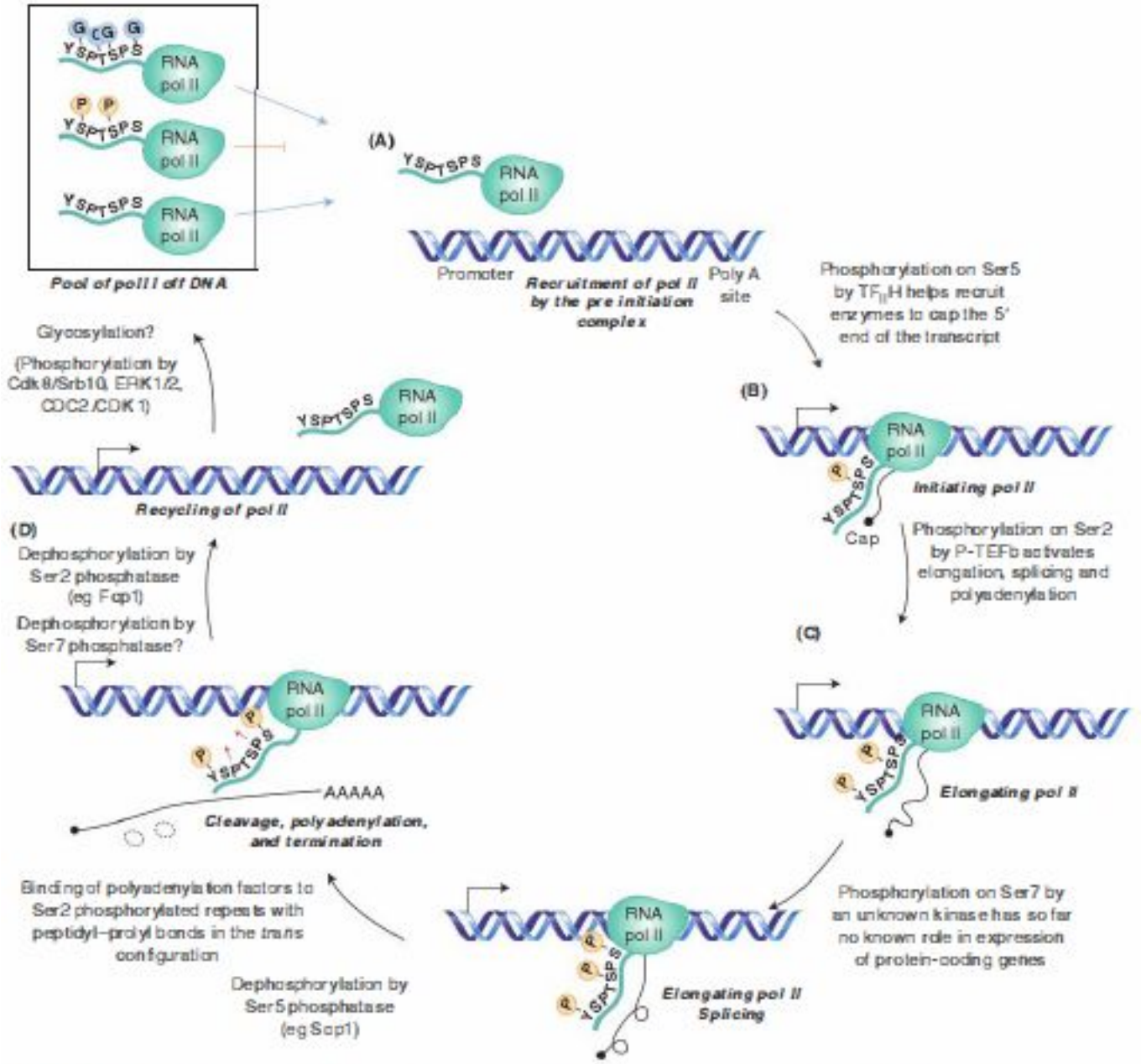
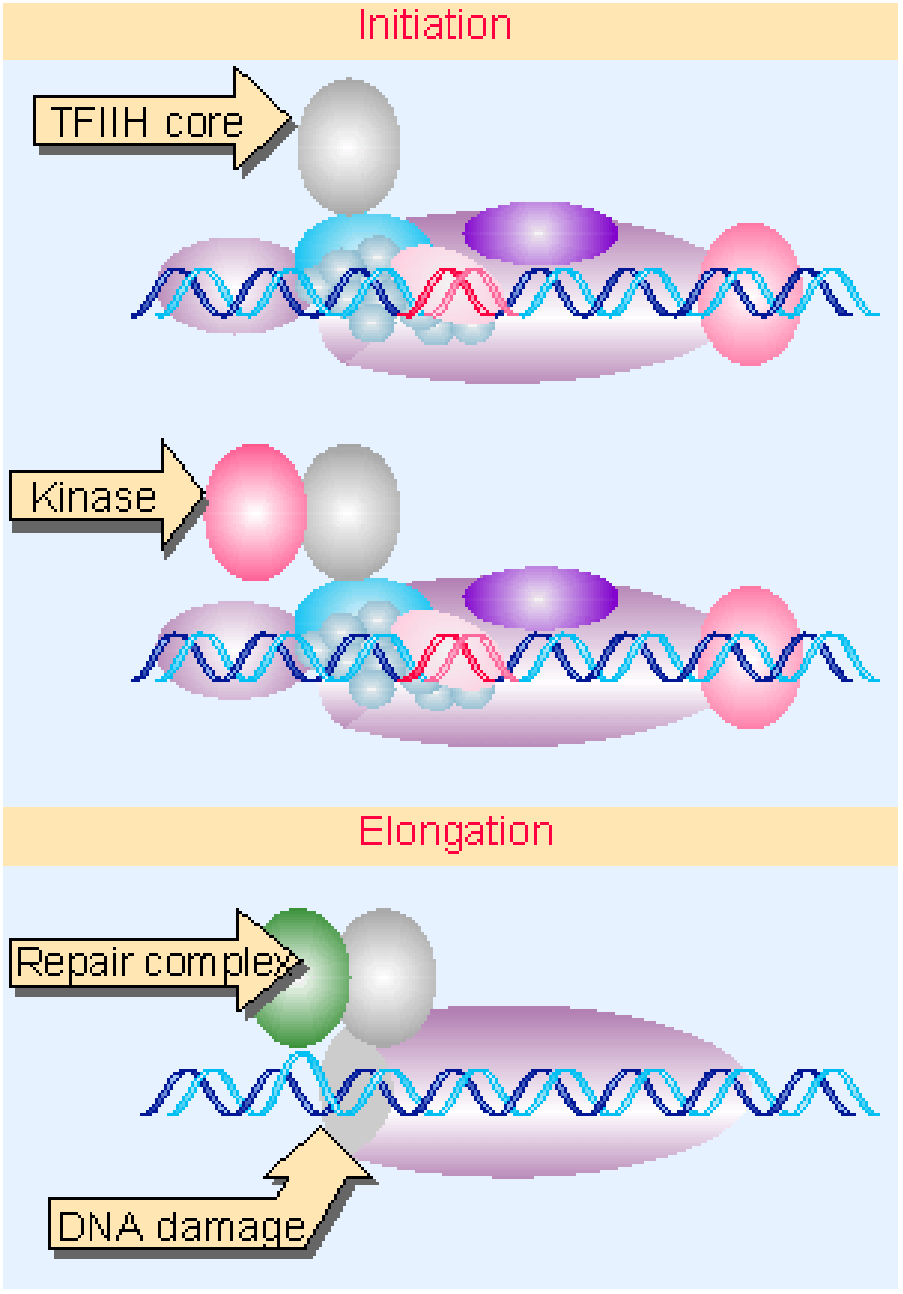


FIGURE 20.14 Modification of the RNA polymerase II CTD heptapeptide during transcription. The CTD of RNA polymerase II when it enters the preinitiation complex is unphosphorylated. Phosphorylation of Ser residues serves as binding sites for both mRNA processing enzymes and kinases that catalyze further phosphorylation as described in the figure. Reprinted from *Trends Genet.*, vol. 24, S. Egloff and S. Murphy, Cracking the RNA polymerase II CTD code, pp. 280–288. Copyright 2008, with permission from Elsevier [http://www.sciencedirect.com/science/journal/01689525].



TFIIH: multiple independent enzymatic activities:

- Helicases of both polarities → double helix unwind
- Kinase activity
- ATPase

Involved in repair of damage to DNA during transcription

Some TFIIH subunit recognize RNA polymerase stopped on a damaged region and activate one of the repairing processes

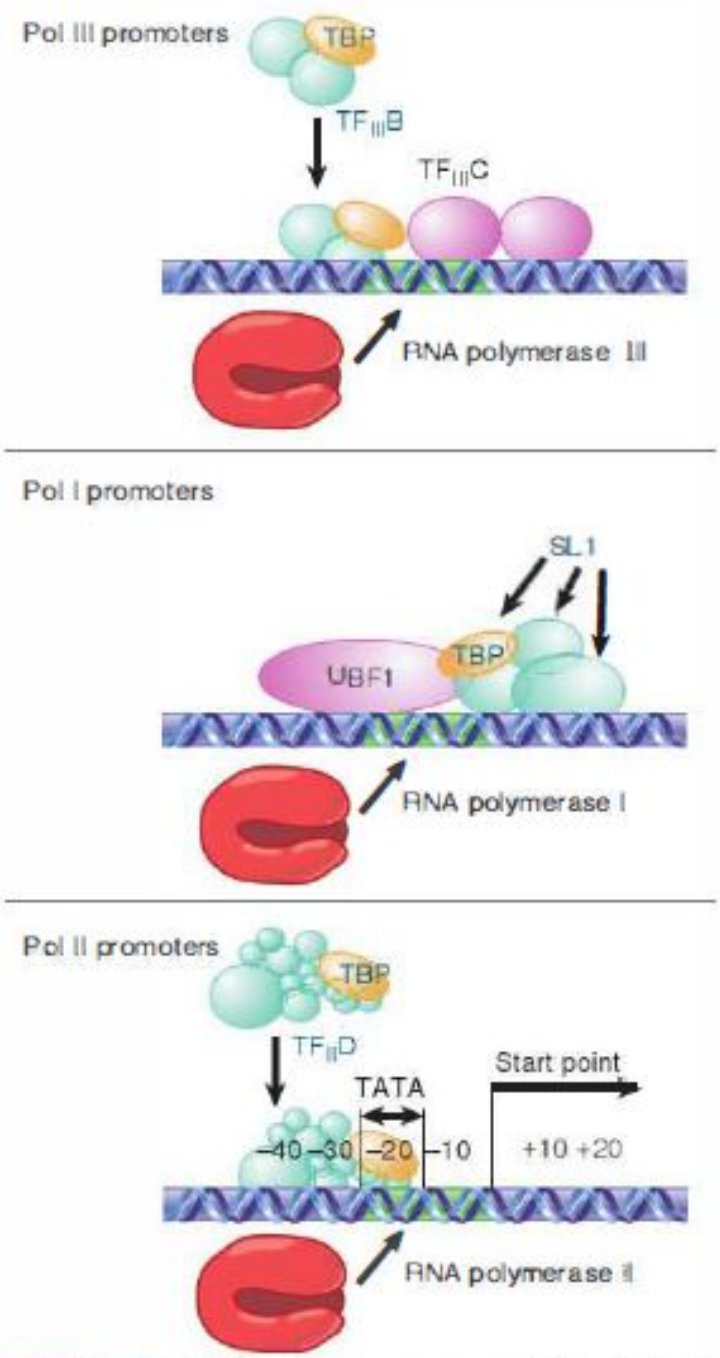
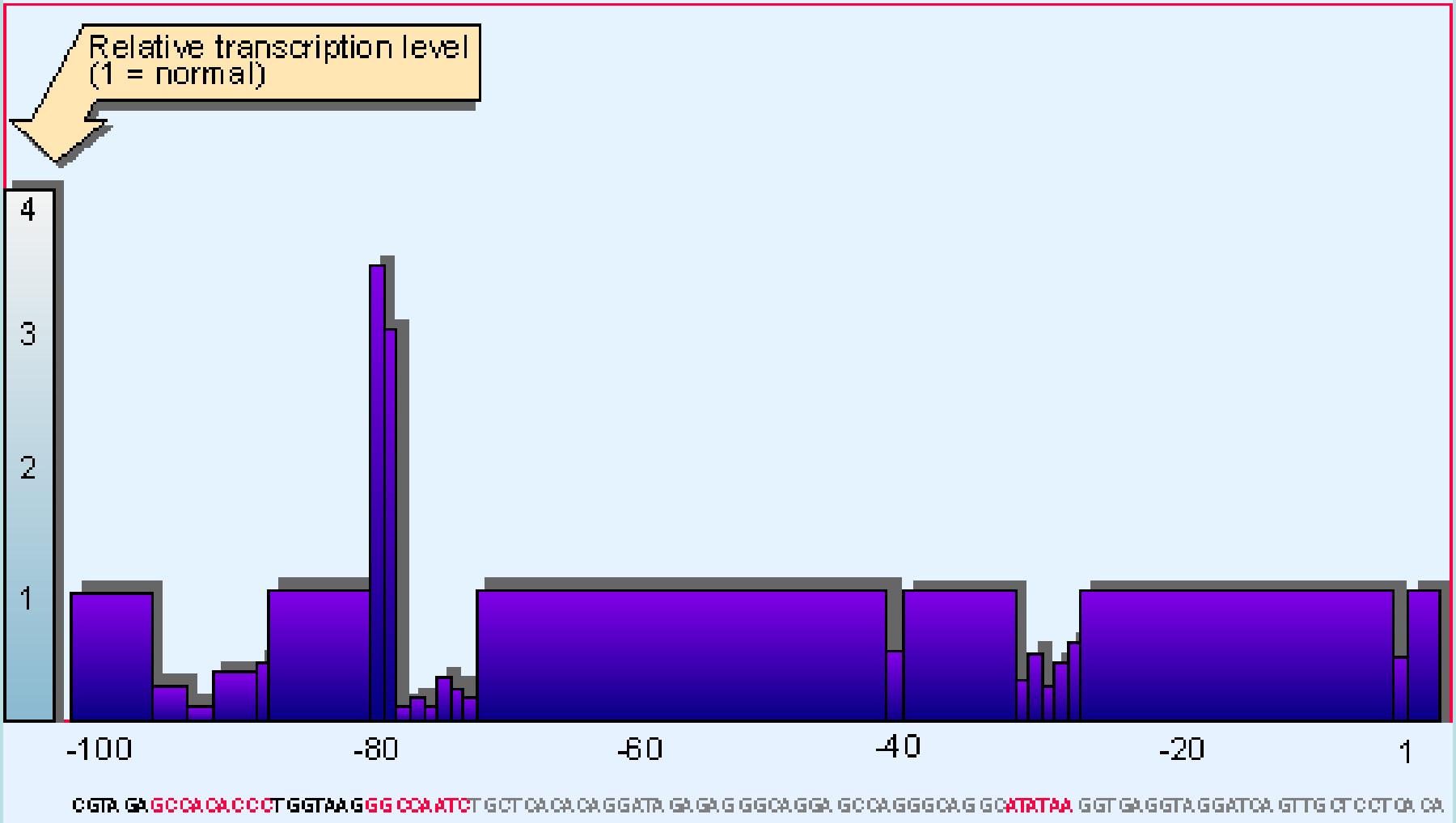
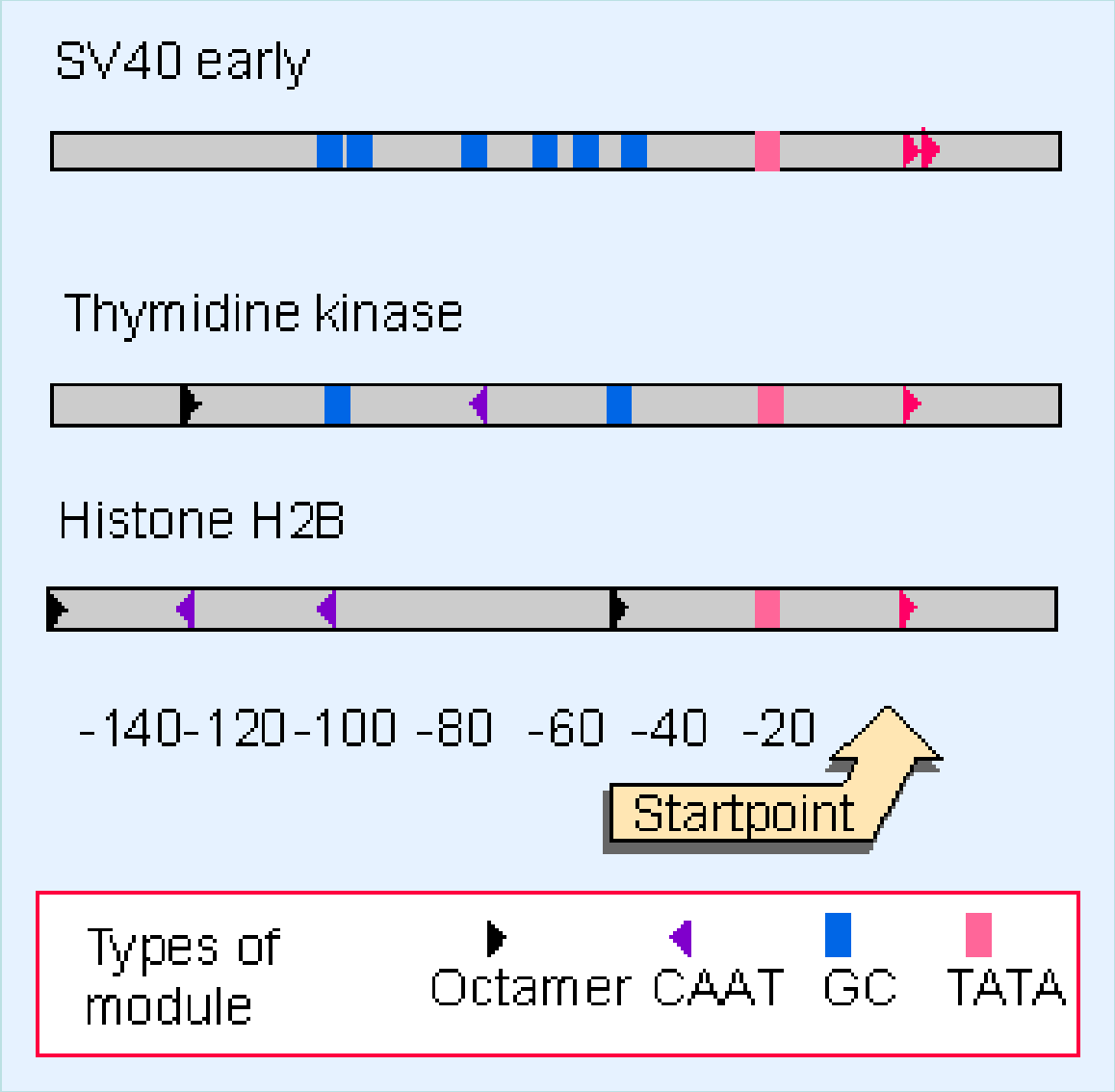


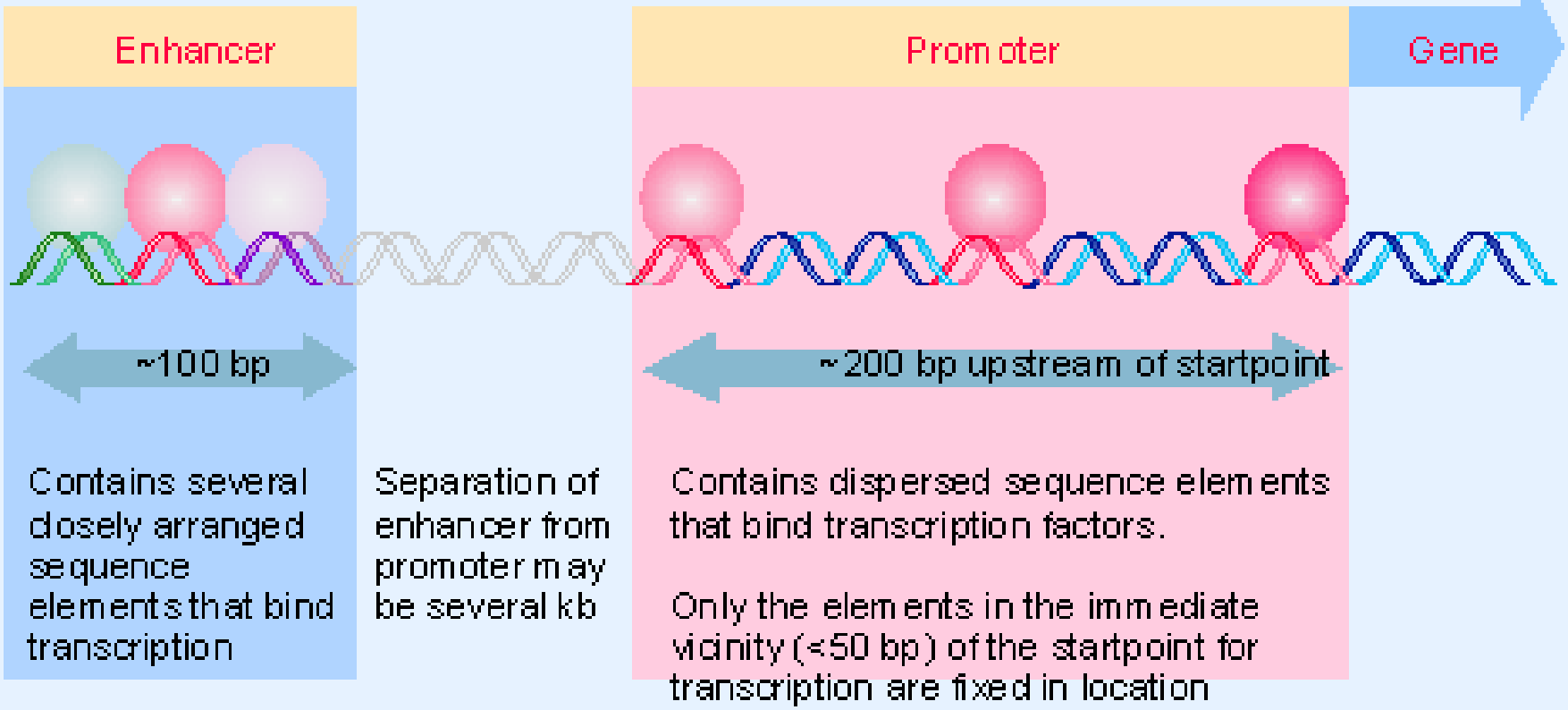
FIGURE 20.8 RNA polymerases are positioned at all promoters by a factor that contains TBP.



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



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



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.

Enhancer → cis-acting element

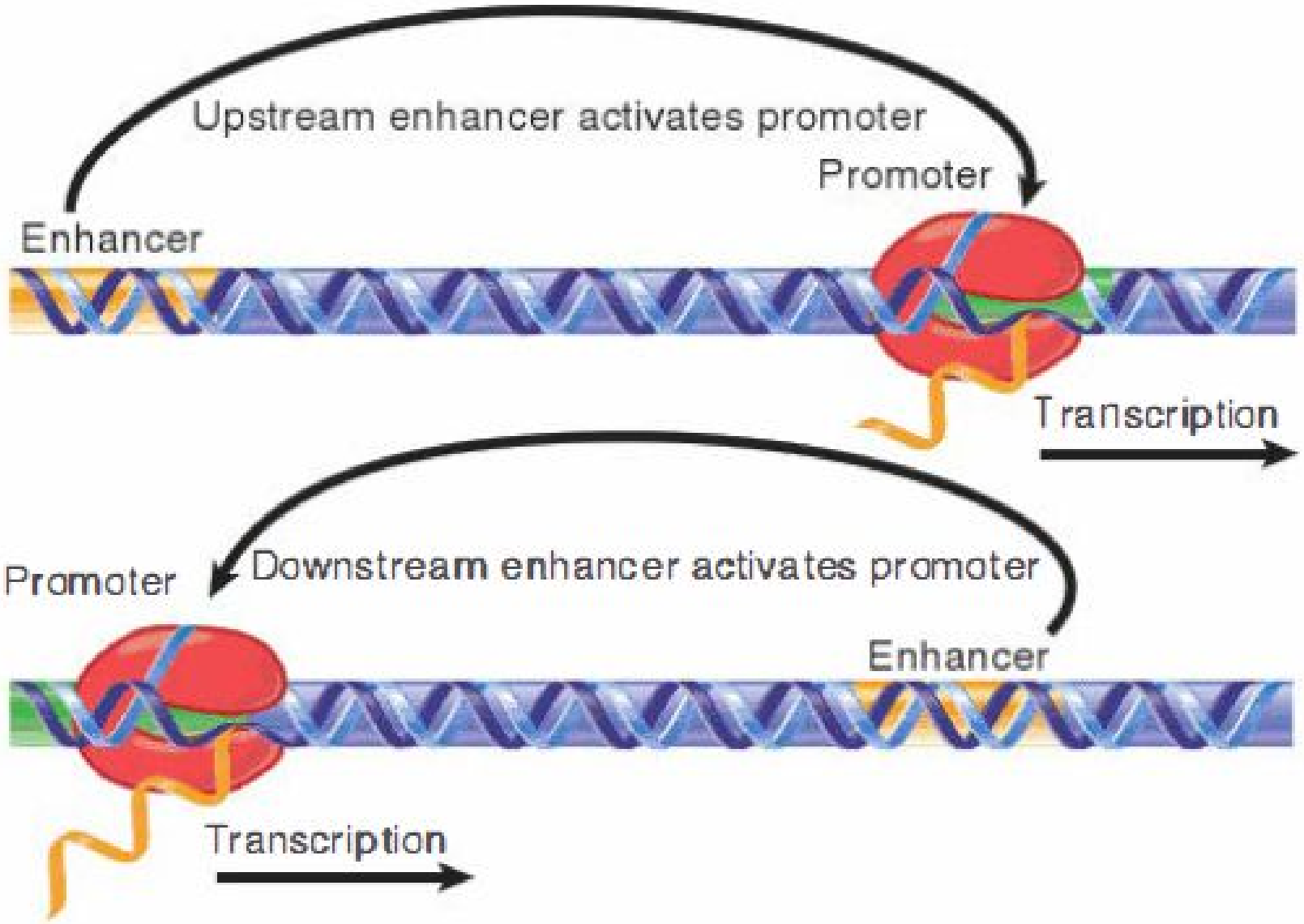
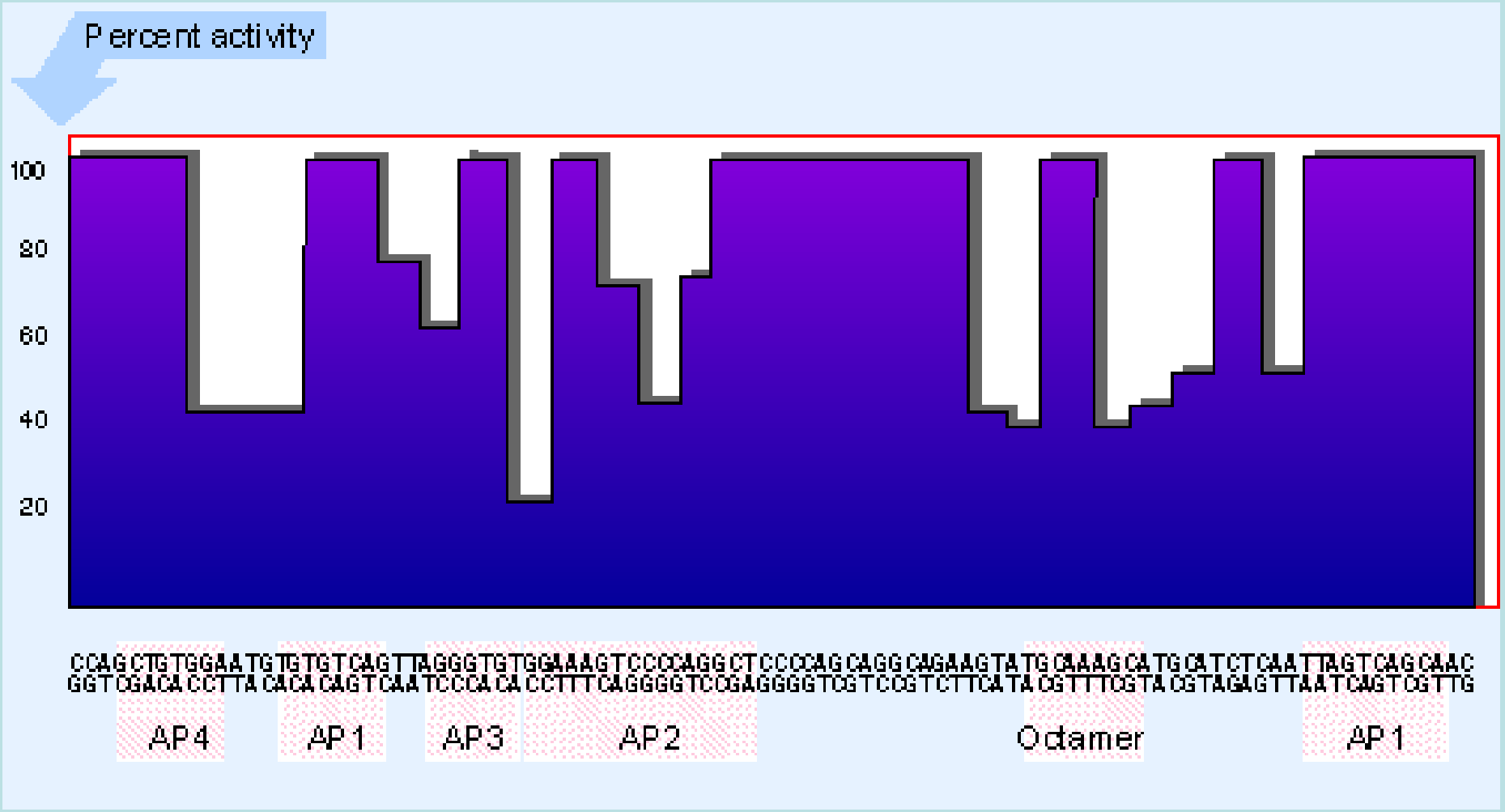


FIGURE 20.15 An enhancer can activate a promoter from upstream or downstream locations, and its sequence can be inverted relative to the promoter.



An enhancer contains several structural motifs. The histogram plots the effect of all mutations that reduce enhancer function to <75% of wild type. Binding sites for proteins are indicated below the histogram.