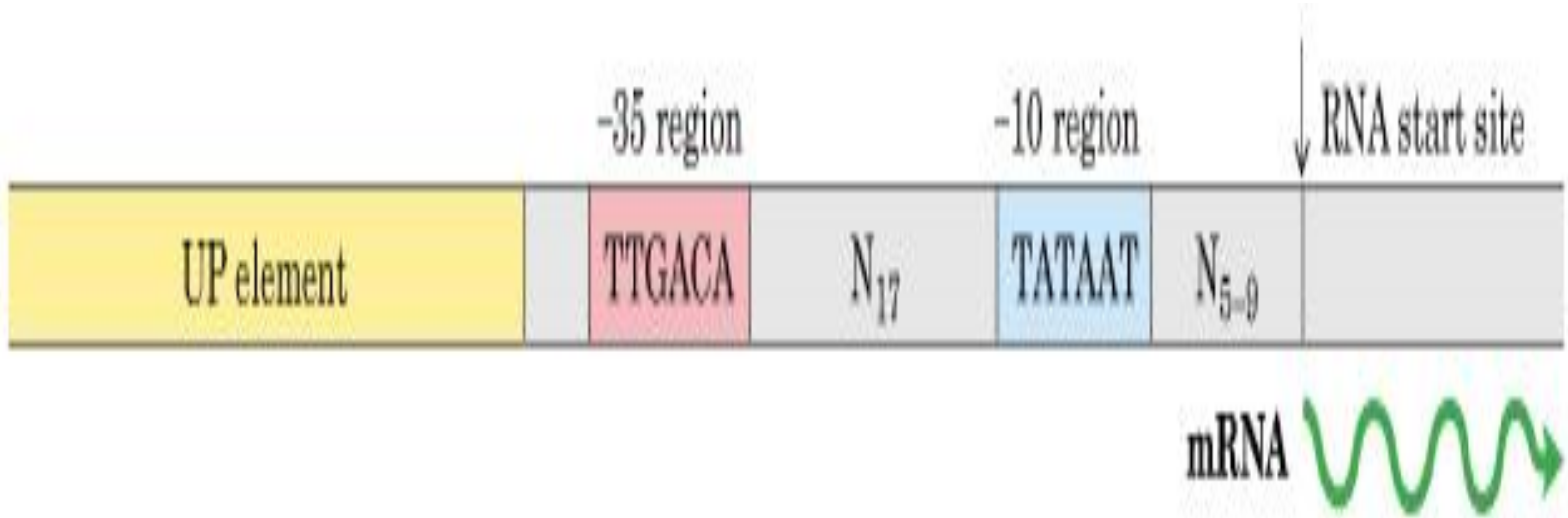
A promoter, an operator and regulatory proteins.

Principles of Gene Regulation:

1) RNA polymerase binds to DNA at promoters



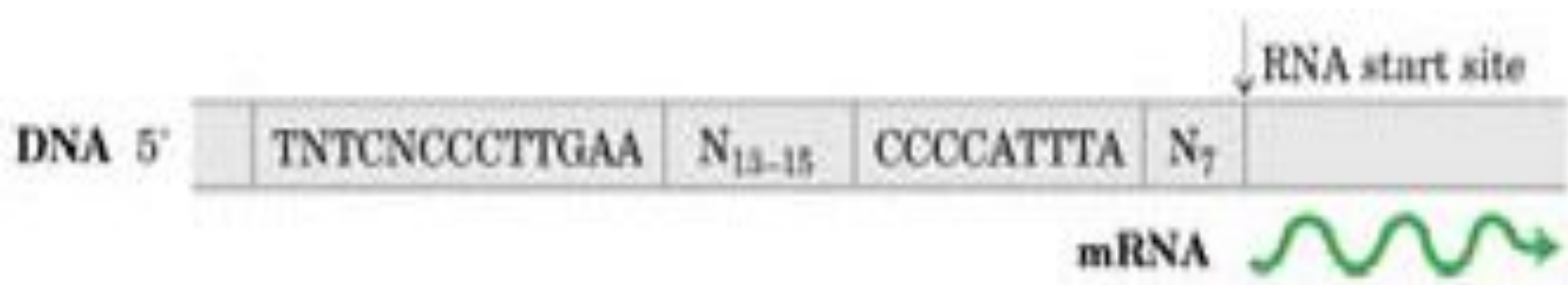
2) Transcription initiation is regulated by proteins that bind to or near promoters.

Repression of a repressible gene:(*i.e., negative regulation)* repressors (vs. activators) bind to operators of DNA.

Repressor is regulated by an effector, usually a small molecule or a protein, that binds and causes a conformational change.

Activator binds to DNA sites called enhancer to enhance the RNA polymerase activity. (*i.e., positive regulation)*

Induction of an inducible gene, *e.g., heat-shock genes.*



OPERON in gene regulation of prokaryotes:

Definition: **a few genes that are controlled collectively by one promoter**

Its structure: **Each Operon is consisted of few structural genes(cistrons) and**

some cis-acting element such as promoter (P) and operator (O).

Its regulation: **There are one or more regulatory gene outside of the Operon that produce trans-acting factors such as repressor or activators.**

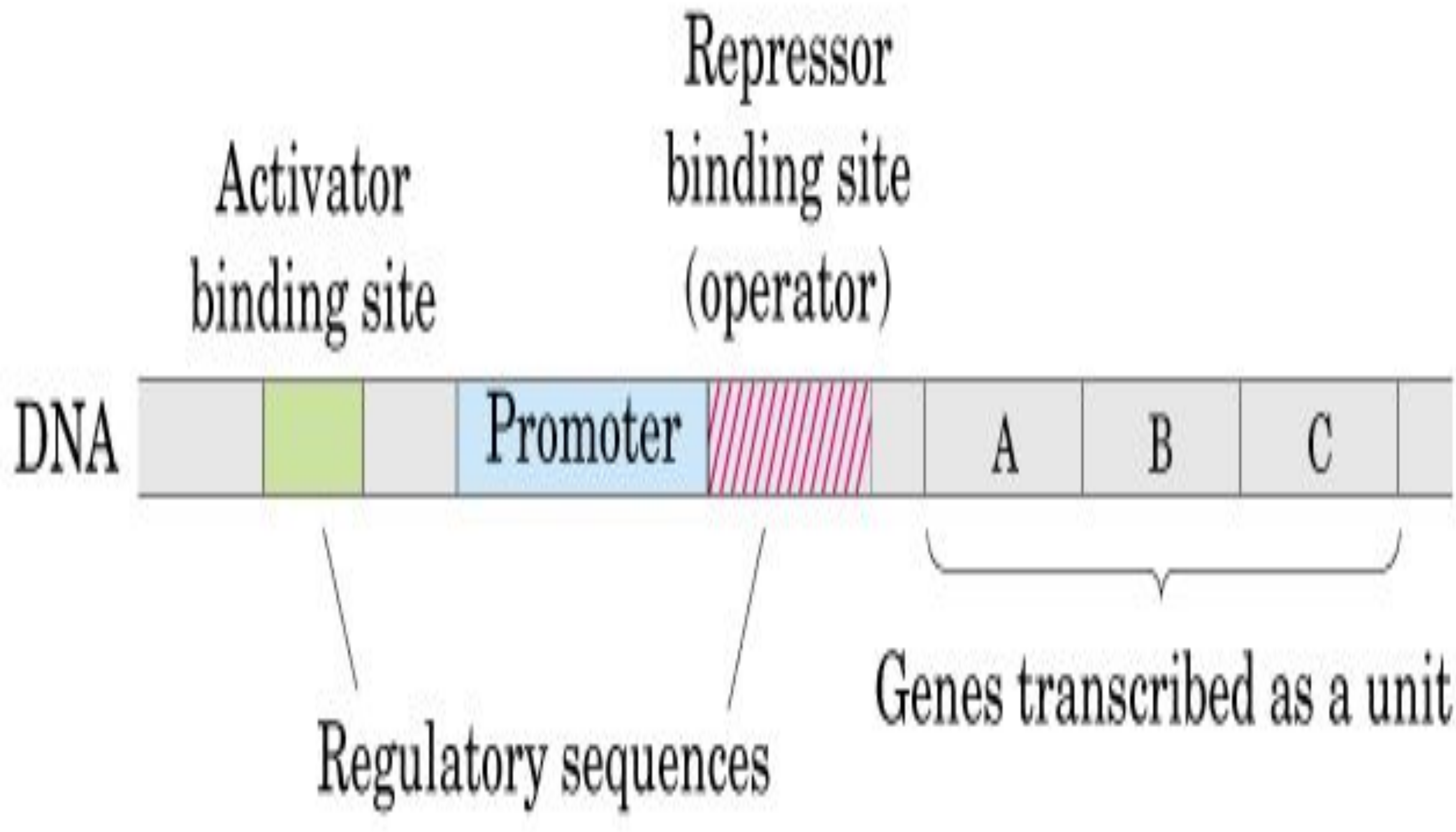
Classification:

1- Catabolic (inducible) such as Lac OPERON

2- Anabolic (repressible) such as ara OPERON

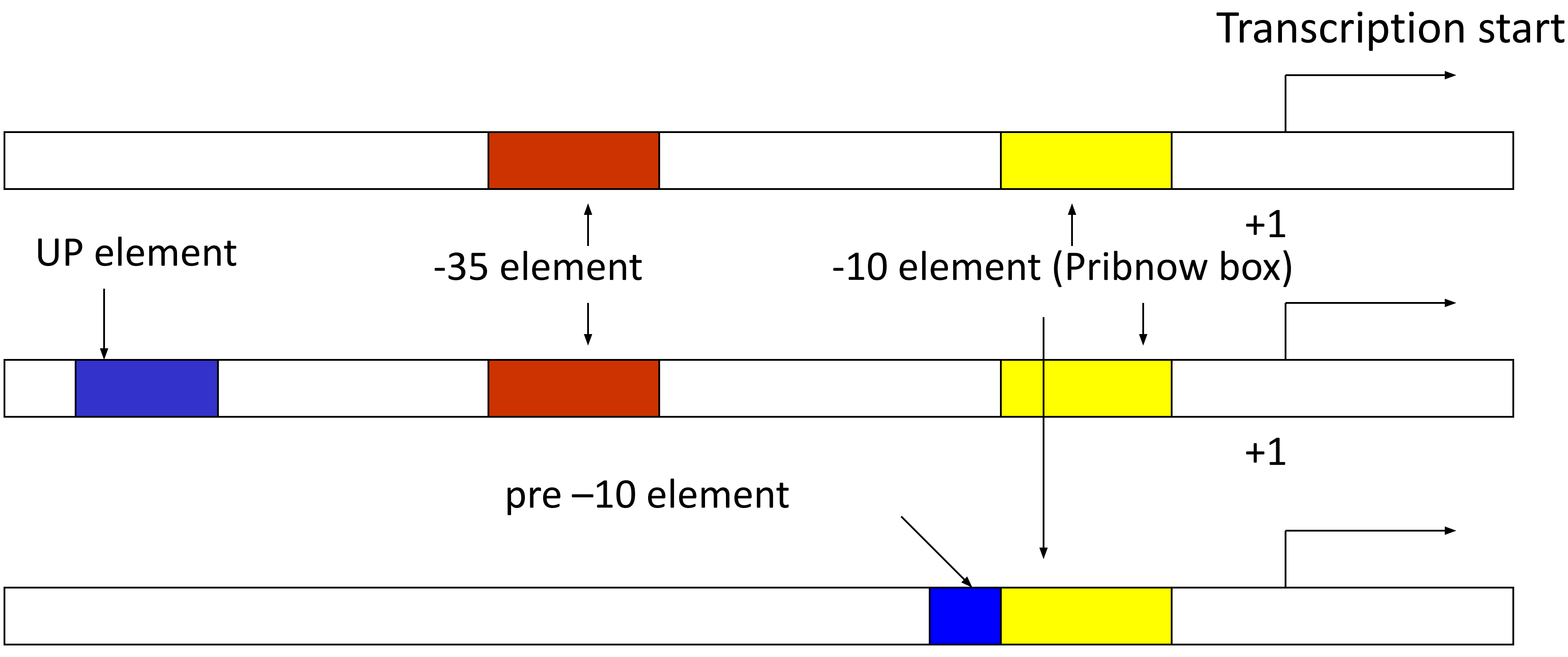
3- Other types

Operon:



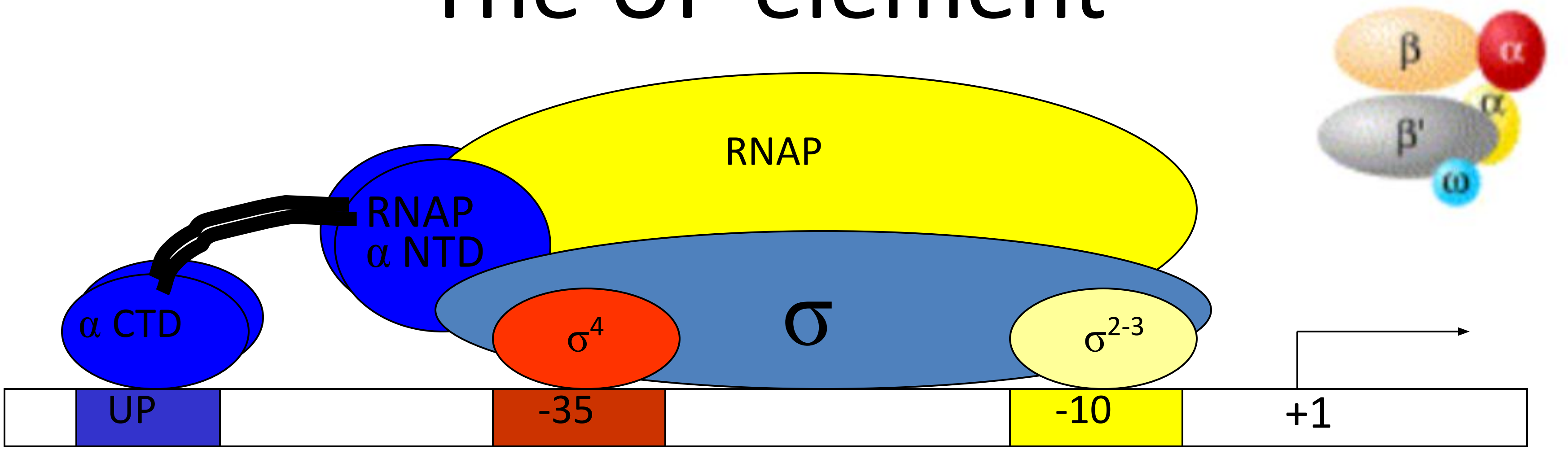
Repressor
binding site
(operator)
Activator
binding site
DNA
Promoter
A
B
C
Regulatory sequences
Genes transcribed as a unit

Bacterial promoters



- Most bacterial promoters have –35 and –10 elements
- Some have UP element
- Some lack –35 element, but have extended –10 region

The UP element



- UP element is an AT rich motif present in some strong (e.g. rRNA) promoters
- UP element interacts directly with C-terminal domain of RNA polymerase α subunits

Constitutive and inducible promoters

- Certain genes are transcribed at all times and circumstances

-Examples – tRNAs, rRNAs, ribosomal proteins, RNA polymerase

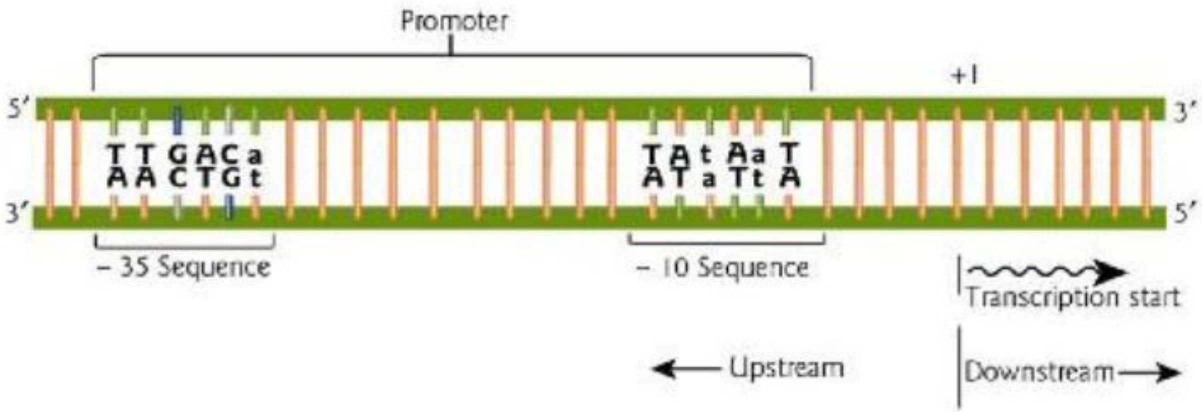
-Promoters of those genes are called constitutive

- Most genes, however, need to be transcribed only under certain circumstances or periods in cell life cycles

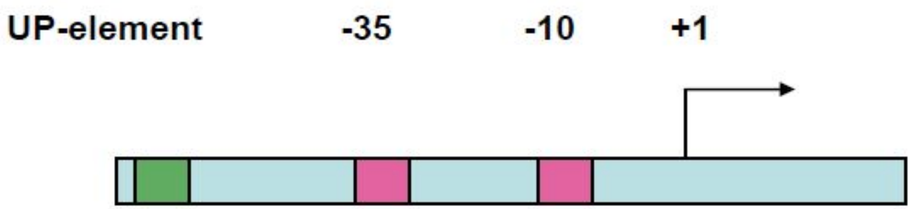
-The promoters of those genes are called inducible and they are subject to up- and down- regulation

Basal Promoter Elements

Promoter: The combination of DNA sequence elements required for the recruitment of RNA polymerase



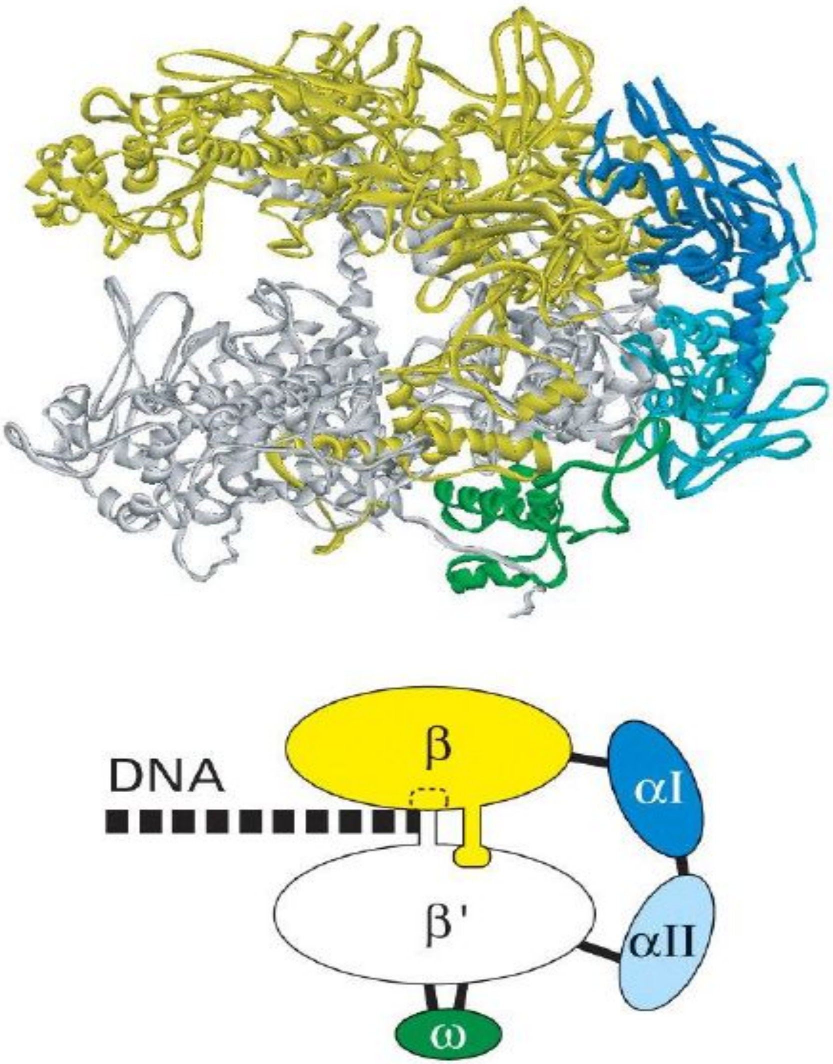
-35 and -10 elements are recognized by sigma factor



UP element:
AT rich element that interacts with C-terminal domain of the alpha subunit of RNA polymerase

Structure of RNA polymerase

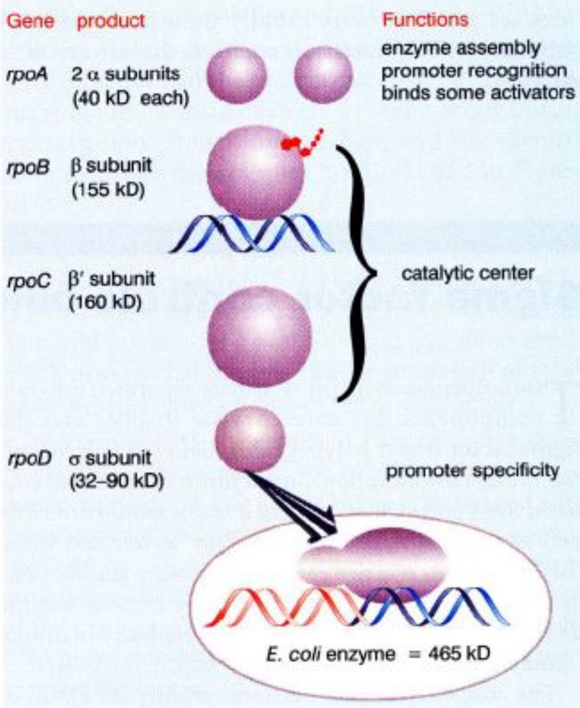
(a) Bacterial RNA polymerase

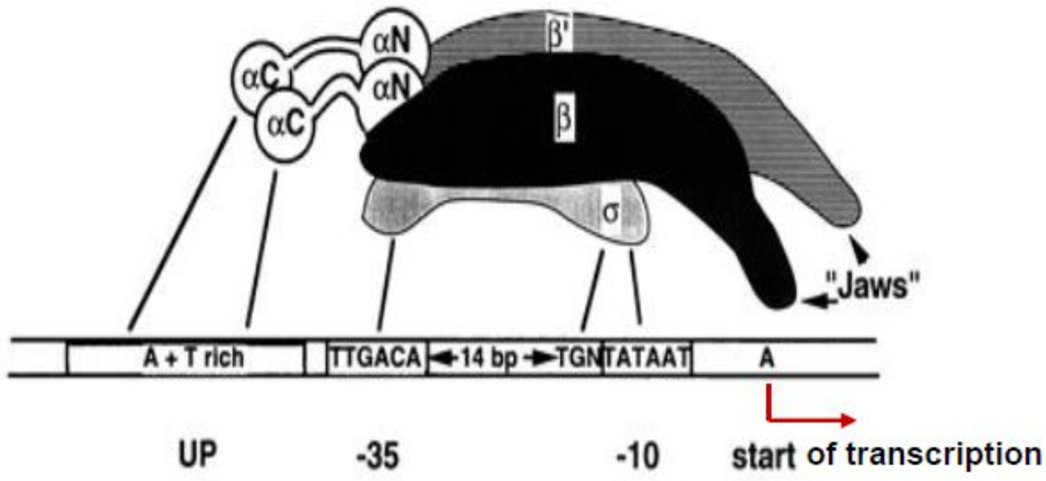


The core RNA polymerase (alpha, beta)
associate with the sigma factor (mostly sigma 70)to generate the RNA polymerase holoenzyme. The sigma factor Is required for recruiting the RNA polymerase to the promoter. The active site contains a magnesium ion that is required for the catalytic activity

RNA Polymerase Subunits and Promoter Recognition

Figure 9.9 Eubacterial RNA polymerases have four types of subunit; α, β, and β′ have rather constant sizes in different bacterial species, but σ varies more widely.





(TGNTATAAT = extended -10)

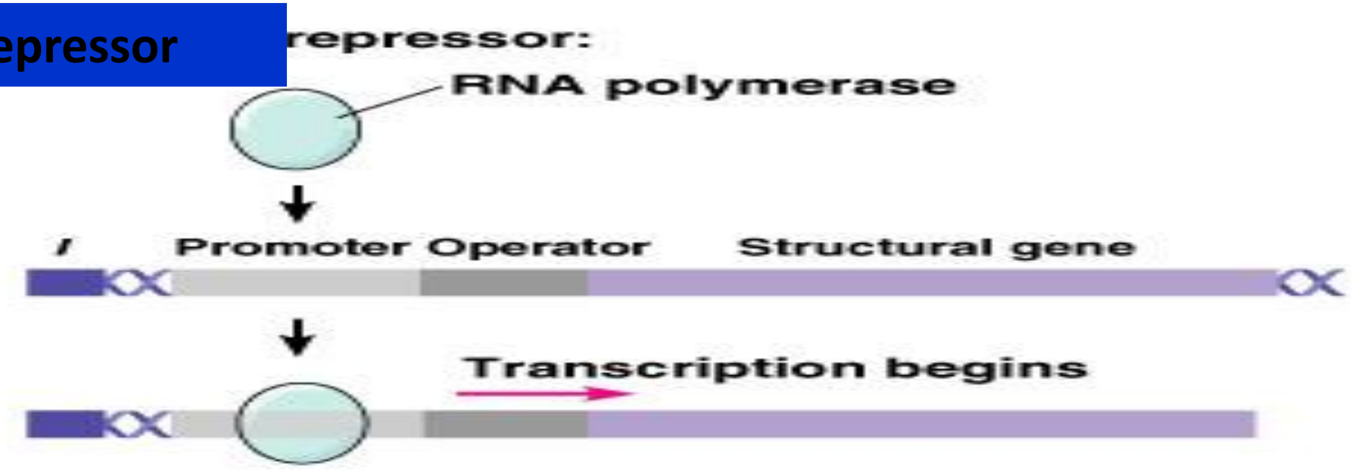
The sigma subunit interacts with the -10 and -35 region, the alpha subunits contact the AT-rich UP element

Figure 9.19 E. coli sigma factors recognize promoters with different consensus sequences. (Numbers in the name of a factor indicate its mass.)

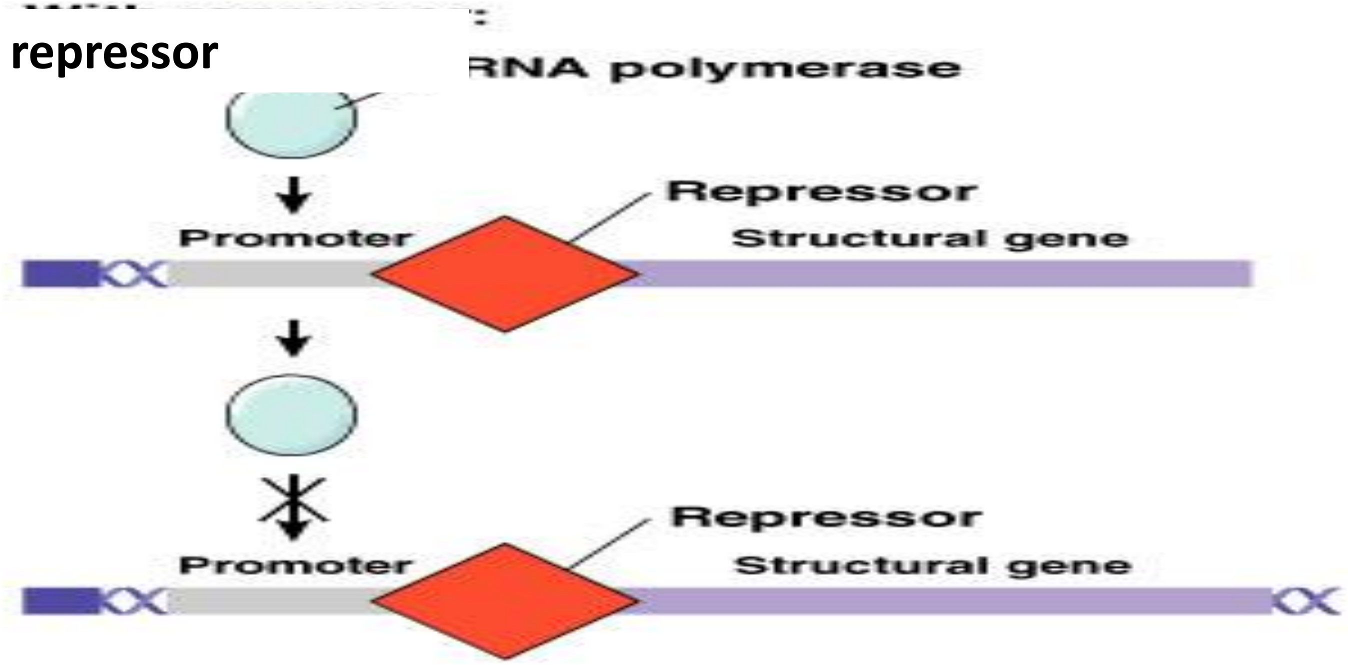
Gene	Factor	Use	–35 Sequence	Separation	–10 Sequence
rpoD	σ^{70}	general	TTGACA	16–18 bp	TATAAT
rpoH	σ^{32}	heat shock	CCCTTGAA	13–15 bp	CCCGATNT
rpoE	σ^{E}	heat shock	not known	not known	not known
rpoN	σ^{54}	nitrogen	CTGGNA	6 bp	TTGCA
fliA	σ^{F}	flagellar	CTAAA	15 bp	GCCGATAA

The activity of an Operon in the presence or the absence of repressor:

No repressor



With repressor



Gene Activation at a distance

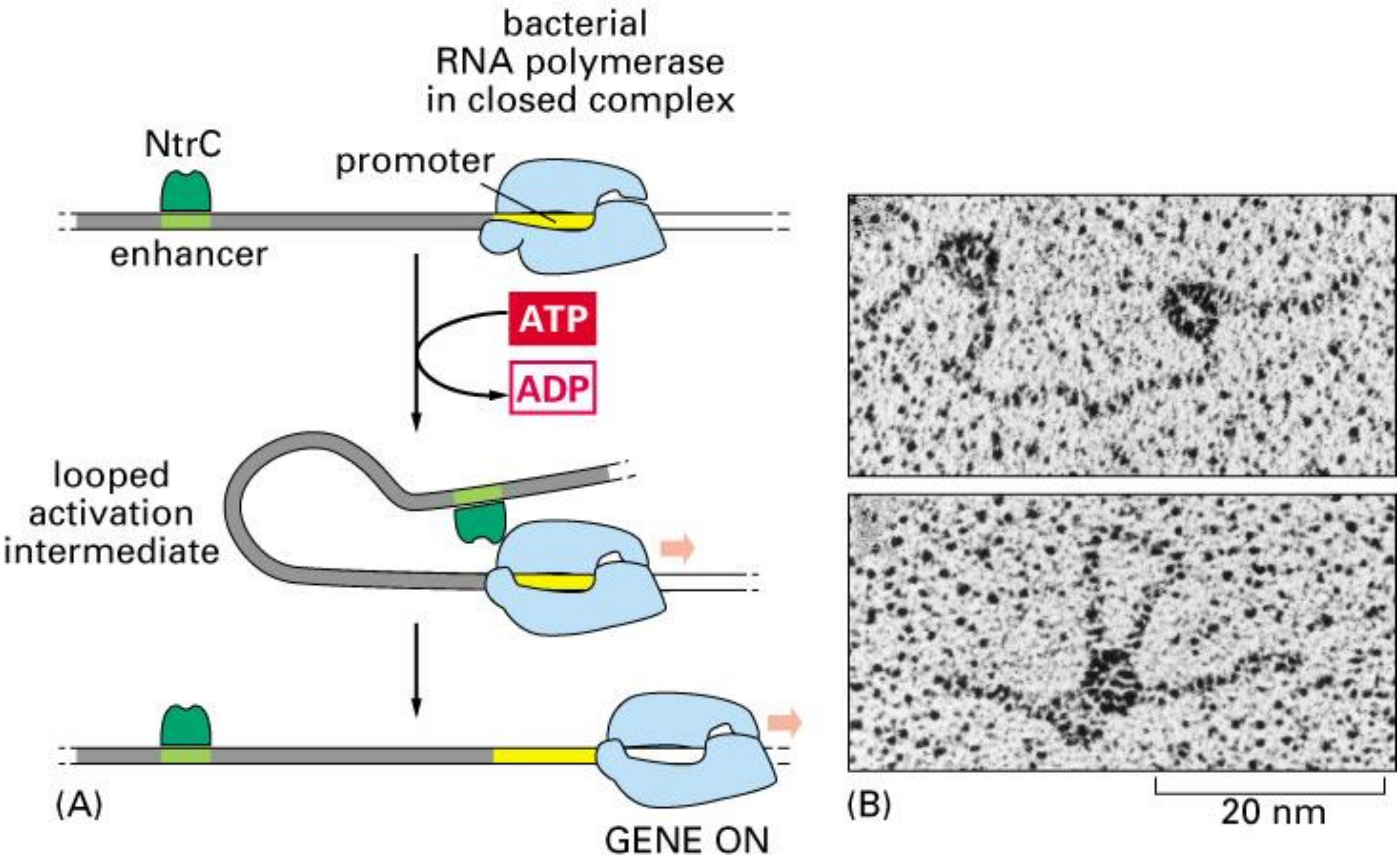


Figure 7–40. Molecular Biology of the Cell, 4th Edition.

Regulation of an eucaryotic gene

TFs are similar, gene regulatory proteins could be very different for different gene regulations

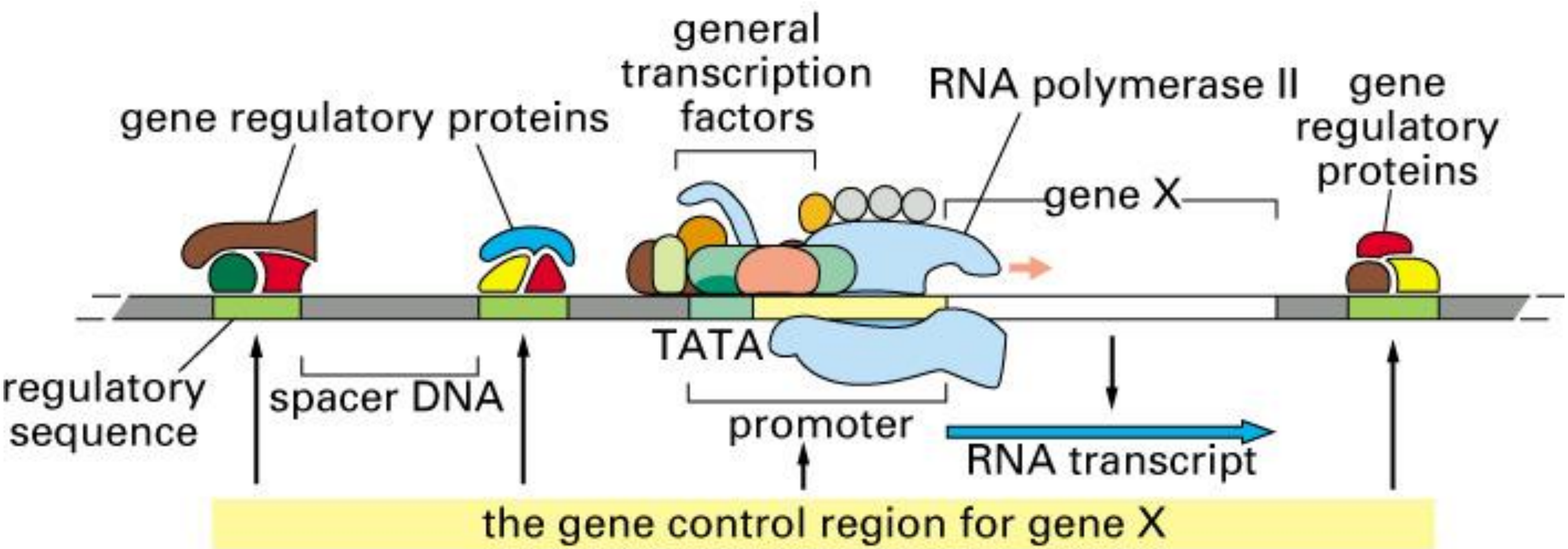


Figure 7–41. Molecular Biology of the Cell, 4th Edition.

Functional Domain of gene activation protein

1. Activation domain and 2. DNA binding domain

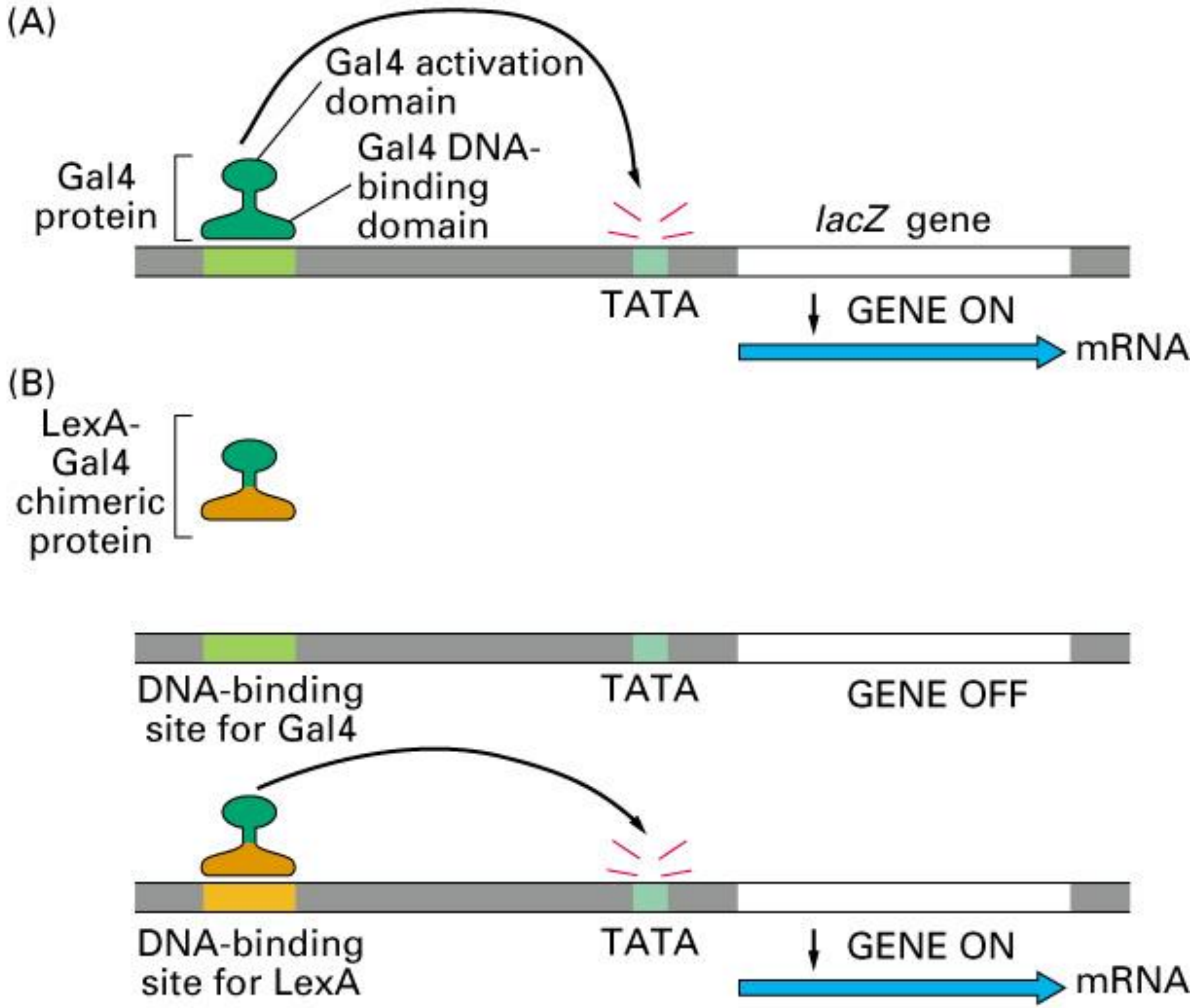


Figure 7–42. Molecular Biology of the Cell, 4th Edition.

Gene Activation by the recruitment of RNA polymerase II holoenzyme

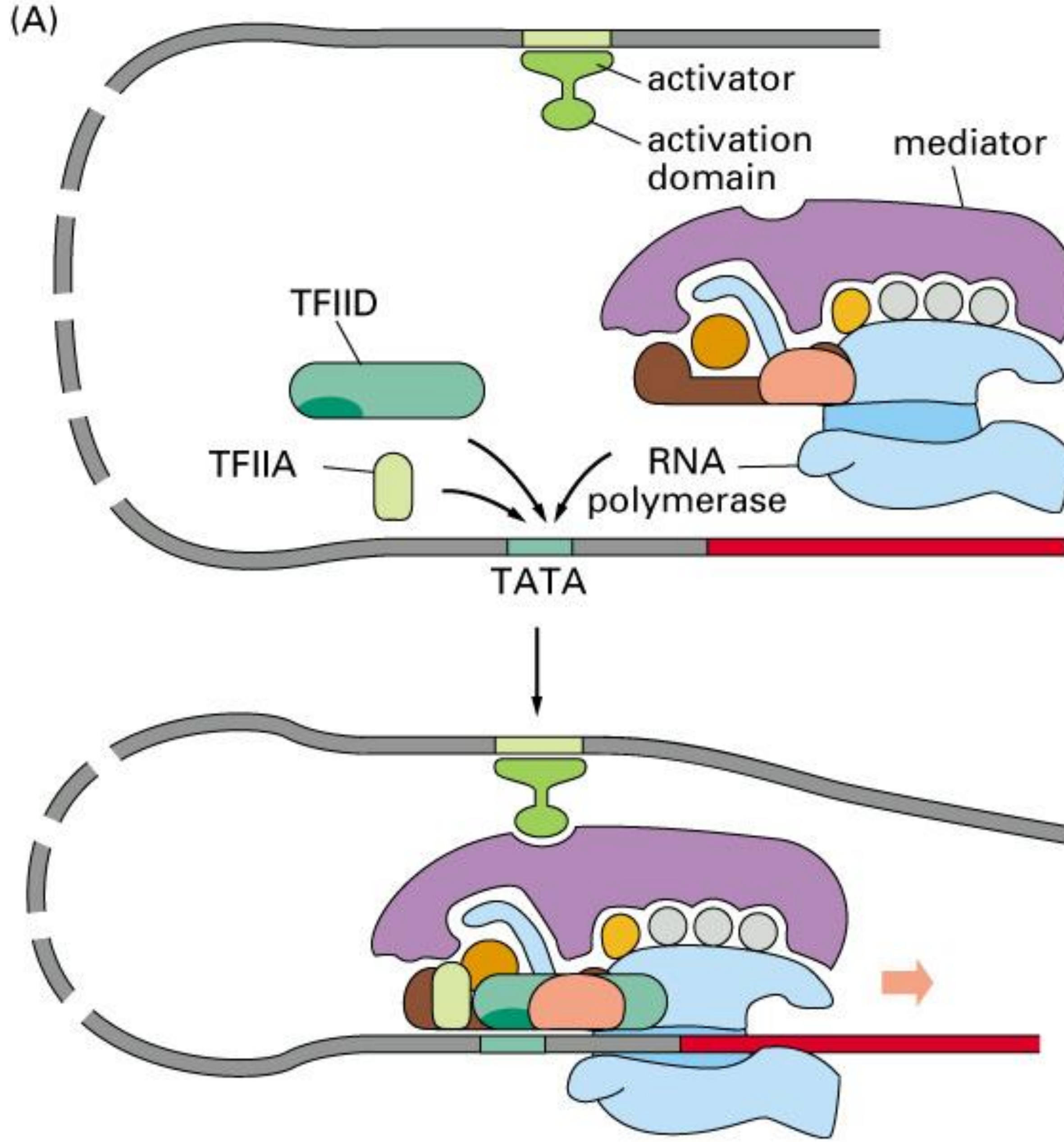


Figure 7–43 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Gene engineering revealed the function of gene activation protein

Directly fuse the mediator protein to enhancer binding domain, omitting activator domain, similar enhancement is observed

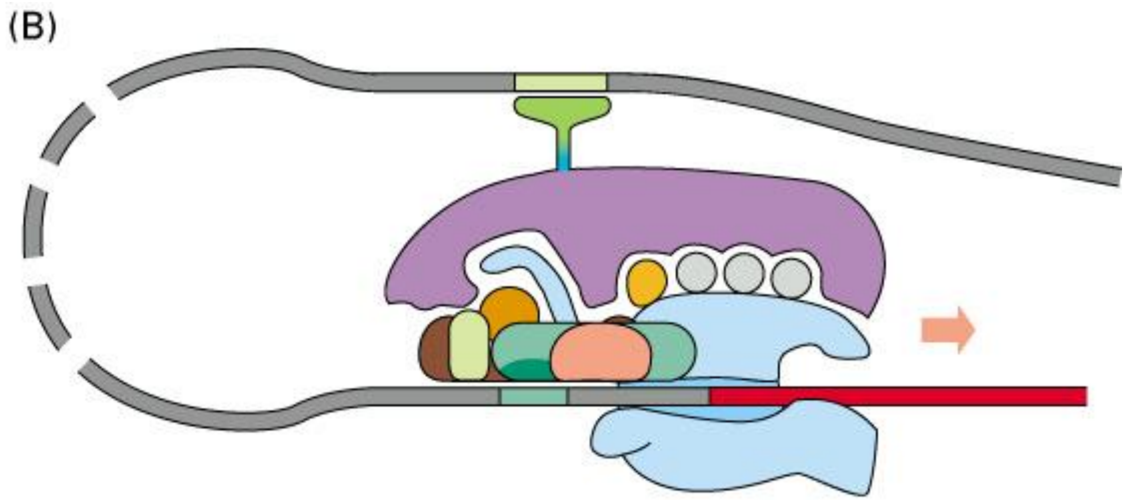


Figure 7–43 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Gene regulatory proteins help the recruitment and assembly of transcription machinery

(General model)

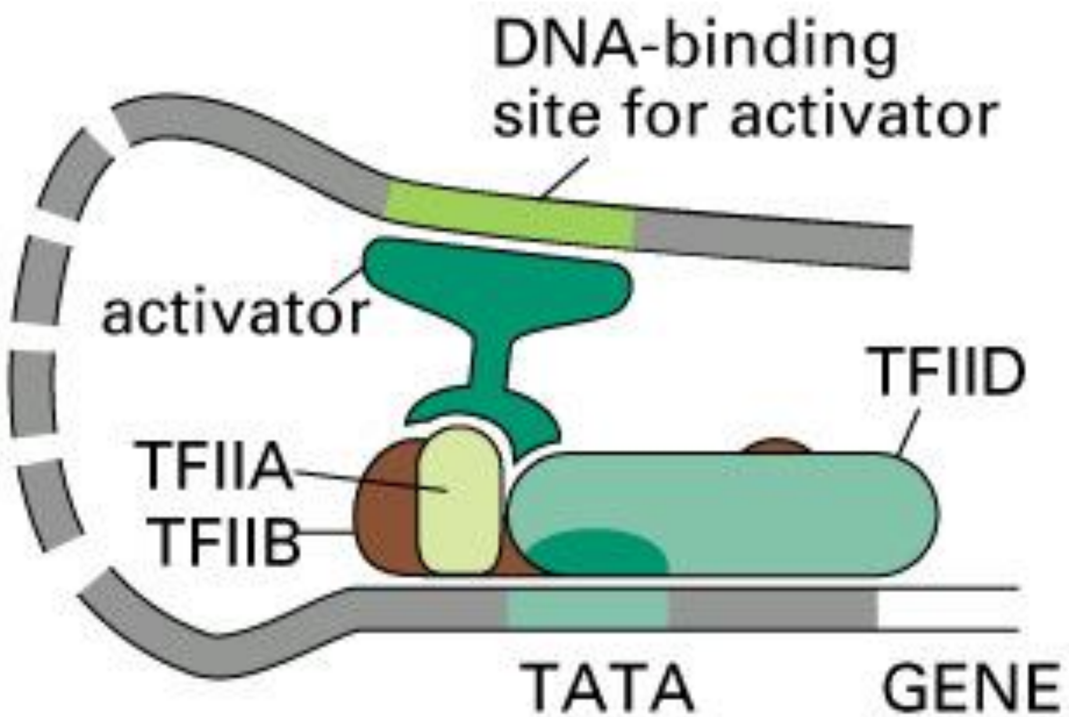


Figure 7–44. Molecular Biology of the Cell, 4th Edition.

The Roles of Transcription Factors

- To initiate transcription, eukaryotic RNA polymerase requires the assistance of proteins called transcription factors
- General transcription factors are essential for the transcription of all protein-coding genes
- In eukaryotes, high levels of transcription of particular genes depend on control elements interacting with specific transcription factors

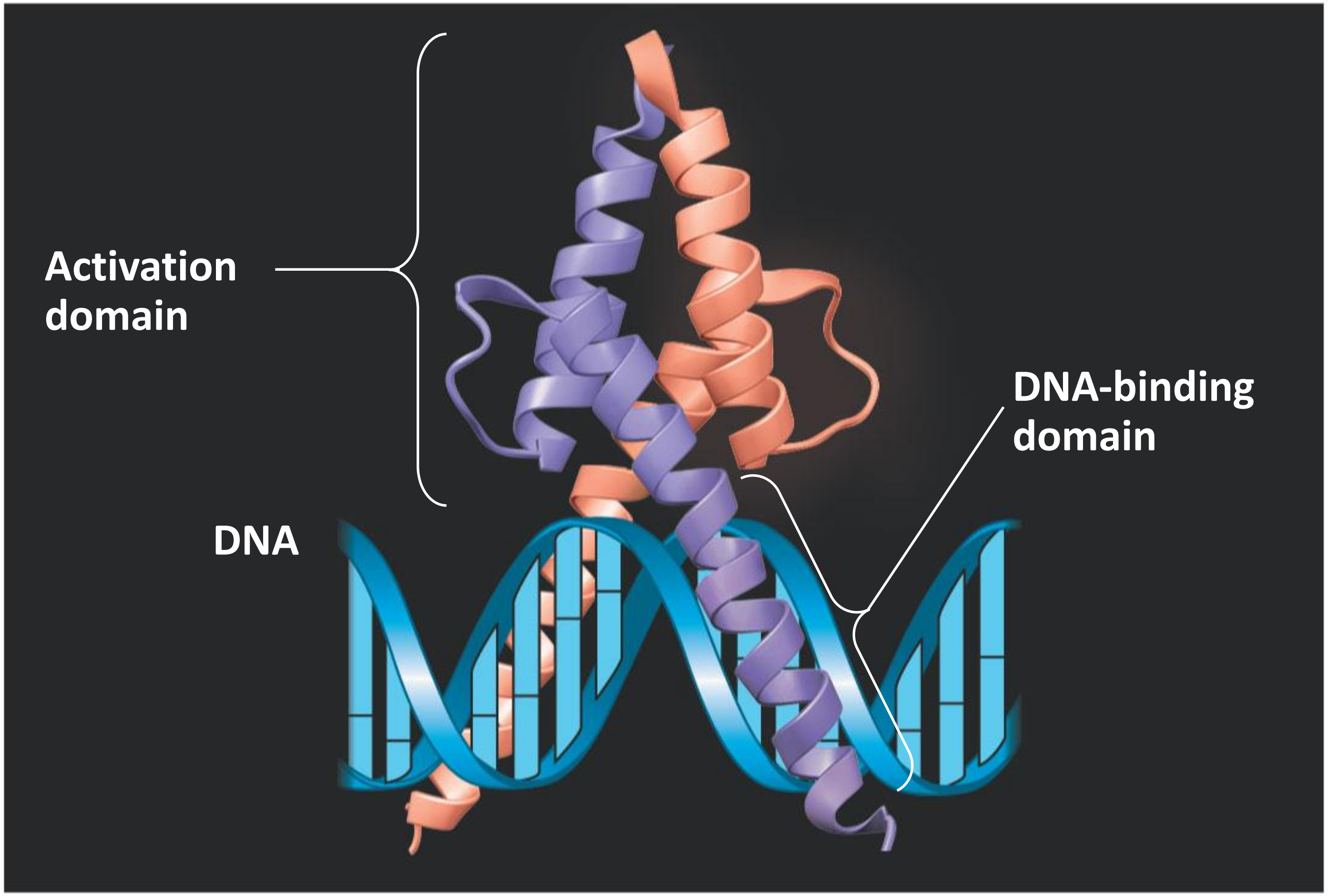
Enhancers and Specific Transcription Factors

- Proximal control elements are located close to the promoter
- Distal control elements, groupings of which are called **enhancers**, may be far away from a gene or even located in an intron

- An activator is a protein that binds to an enhancer and stimulates transcription of a gene
- Activators have two domains, one that binds DNA and a second that activates transcription
- Bound activators facilitate a sequence of protein-protein interactions that result in transcription of a given gene

PLAY Animation: Initiation of Transcription

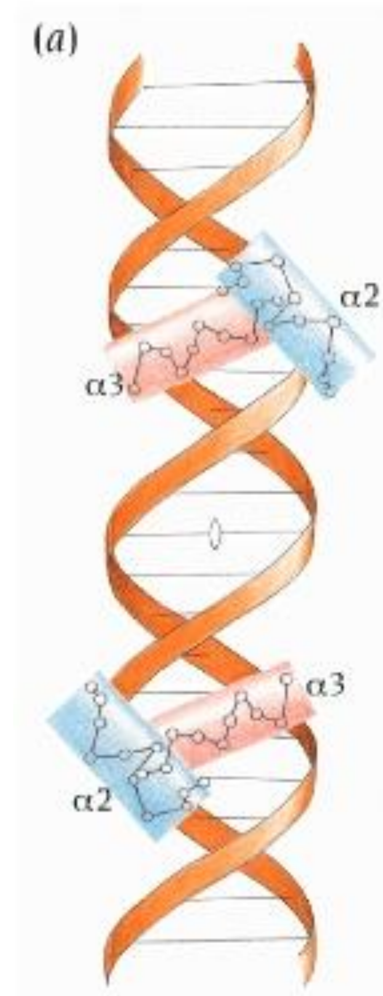
Figure 18.9



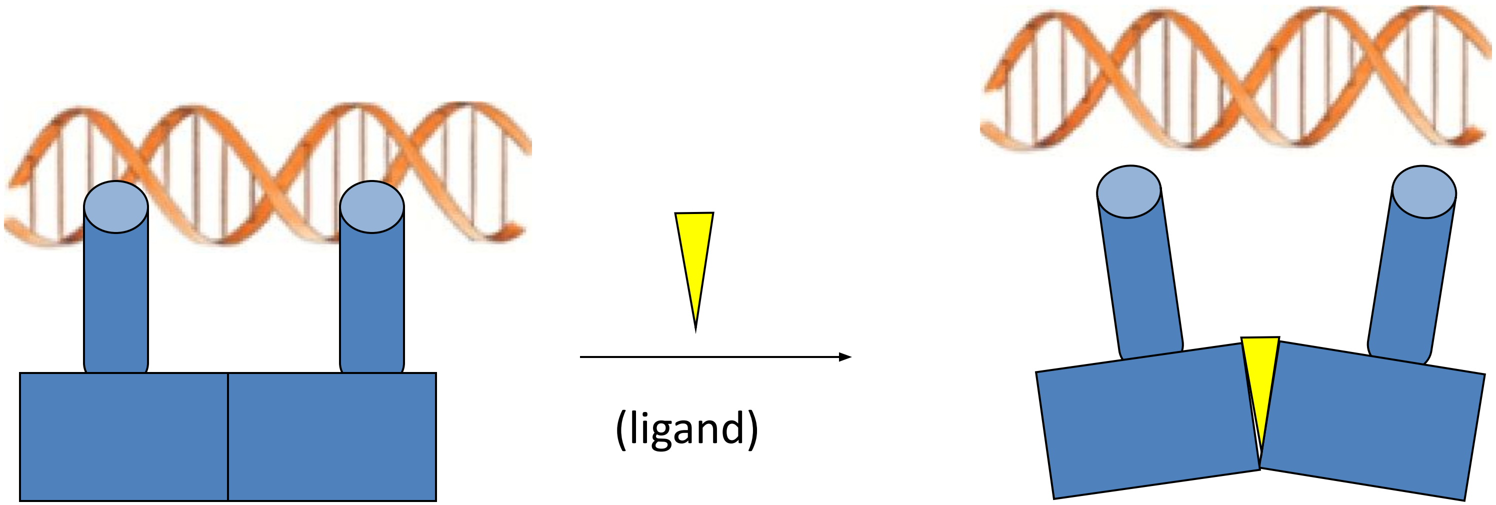
Helix-turn-helix DNA binding motif

- Helix-turn-helix motif is the most common DNA-binding motif in prokaryotes, present in many transcription repressors and activators
- One of the helices, DNA recognition helix, gets inserted in the major groove of DNA
- Helix-turn-helix proteins are often dimeric, with two recognition helices recognizing two adjacent DNA sequences
- Why dimeric?

1) Dimer binds to DNA stronger than monomer
2) By changing the relative positions of monomers, the dimer activity can be easily turned on and off



A common principle to activate or inactivate dimeric helix-turn-helix proteins



- Ligand changes the position of DNA binding a helices, so they do not bind DNA any more
- Or the opposite – ligand changes the position of helices, so they do bind to DNA