Promoters, Activators and Repressors

Unit V

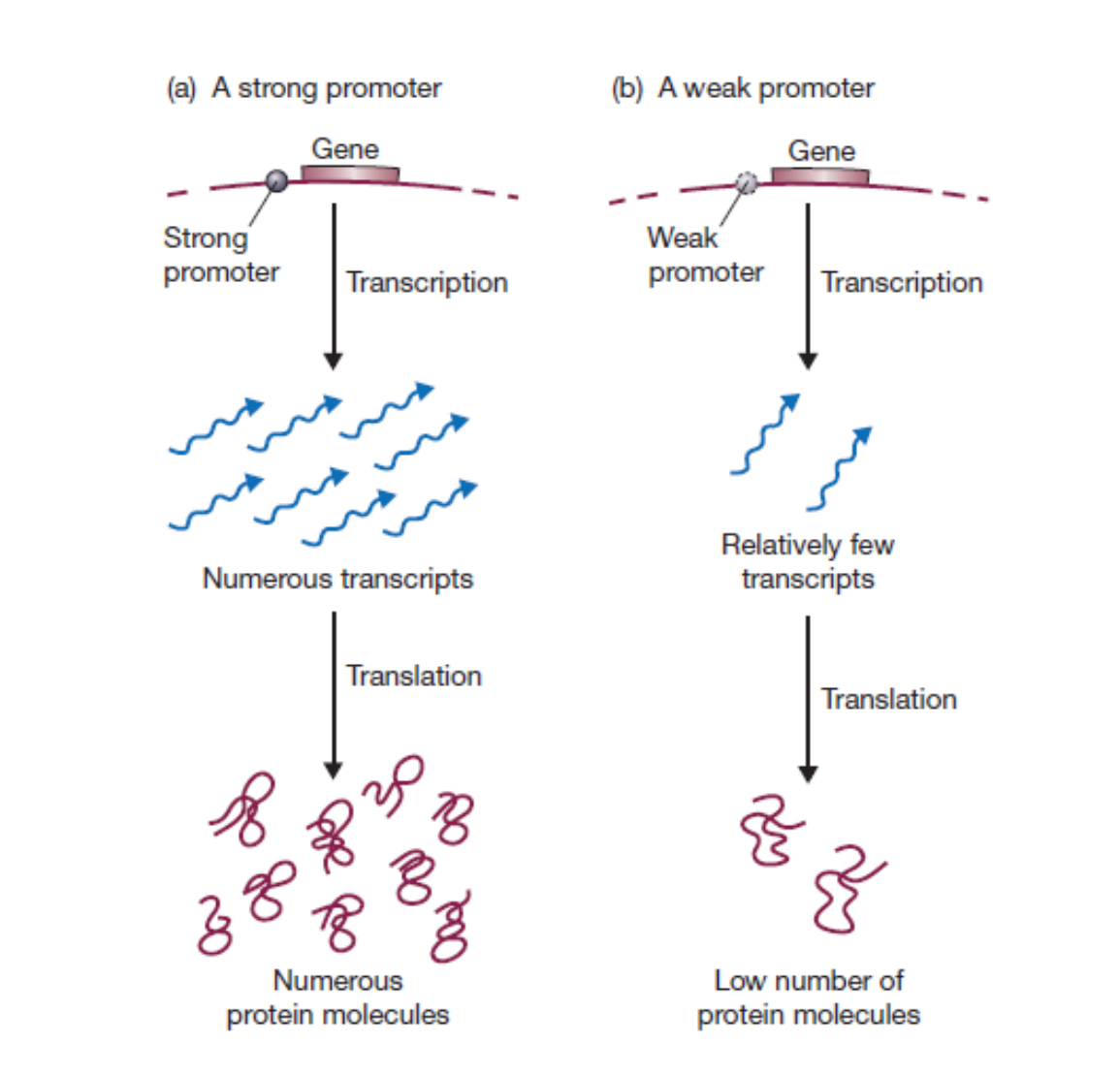
Promoter

- Promoter sequences are DNA sequences that define where transcription of a gene by RNA polymerase begins. Promoter sequences are typically located directly upstream or at the 5' end of the transcription initiation site. RNA polymerase and the necessary transcription factors bind to the promoter sequence and initiate transcription. Promoter sequences define the direction of transcription and indicate which DNA strand will be transcribed; this strand is known as the sense strand.
- Many eukaryotic genes have a conserved promoter sequence called the TATA box, located 25 to 35 base pairs upstream of the transcription start site. Transcription factors bind to the TATA box and initiate the formation of the RNA polymerase transcription complex, which promotes transcription.

Activators and Repressors

- There are two different types of gene regulation: positive and negative. Activators (and sometimes inducers) instigate positive regulation, and repressors instigate negative regulation. When an activator or inducer binds to an operon, the transcription process either increases in rate or is allowed to continue. When a repressor binds to an operon, the transcription process is slowed or halted.
- Activators determine the frequency of transcription.
- Activators work by making protein-protein contacts with basal factors.
- Activators may work via coactivators.
- Repression is achieved by affecting chromatin structure or by binding to and masking activators.

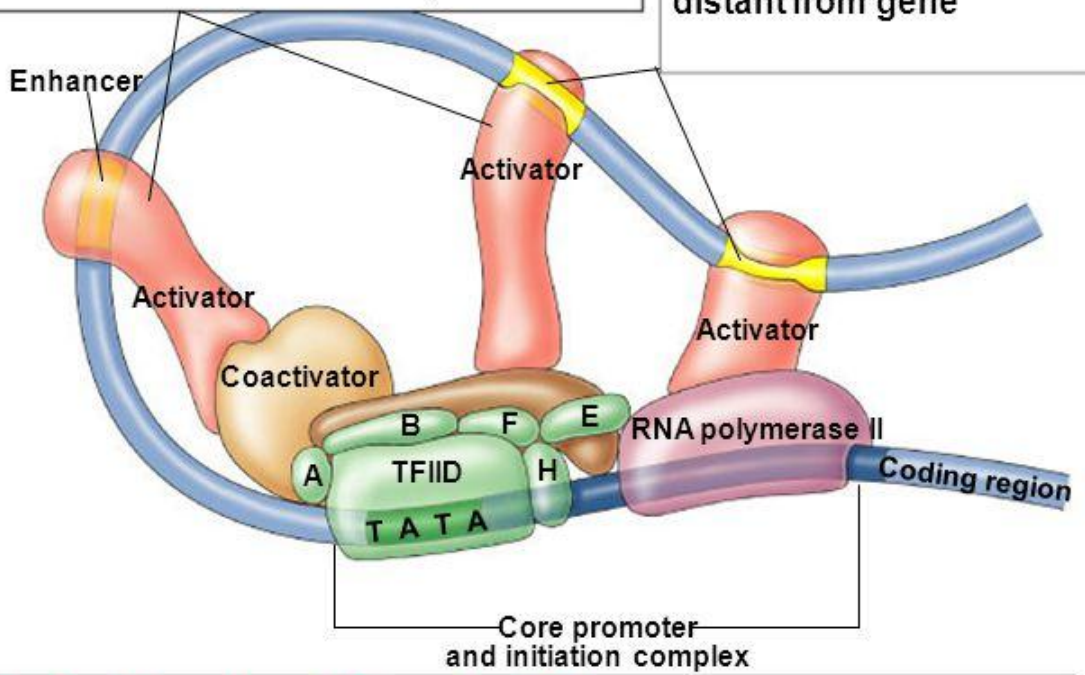
Promoter


(a) A strong promoter
Gene
Strong promoter
Transcription
Numerous transcripts
Translation
Numerous protein molecules
(b) A weak promoter
Gene
Weak promoter
Transcription
Relatively few transcripts
Translation
Low number of protein molecules

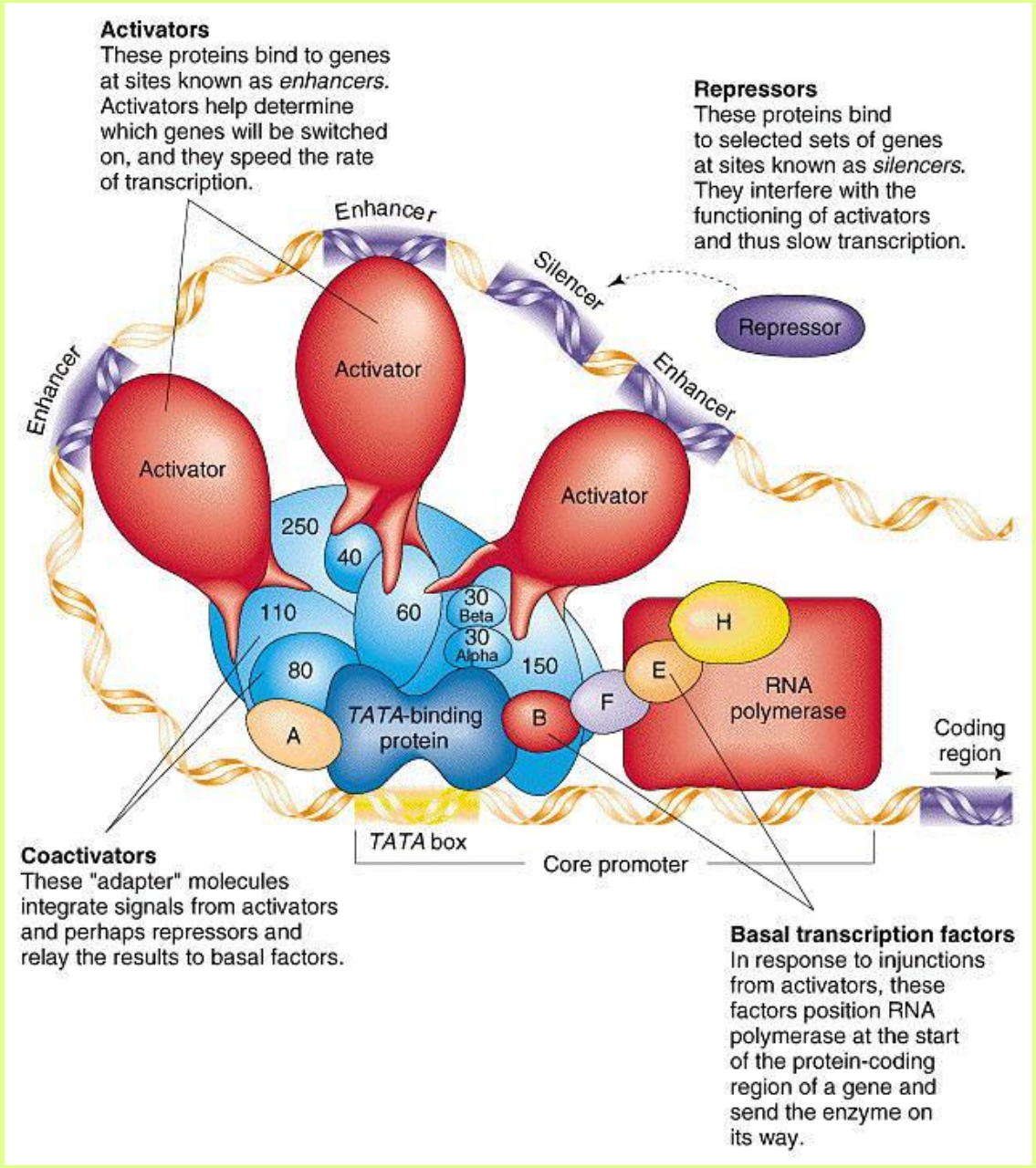
Transcription complex

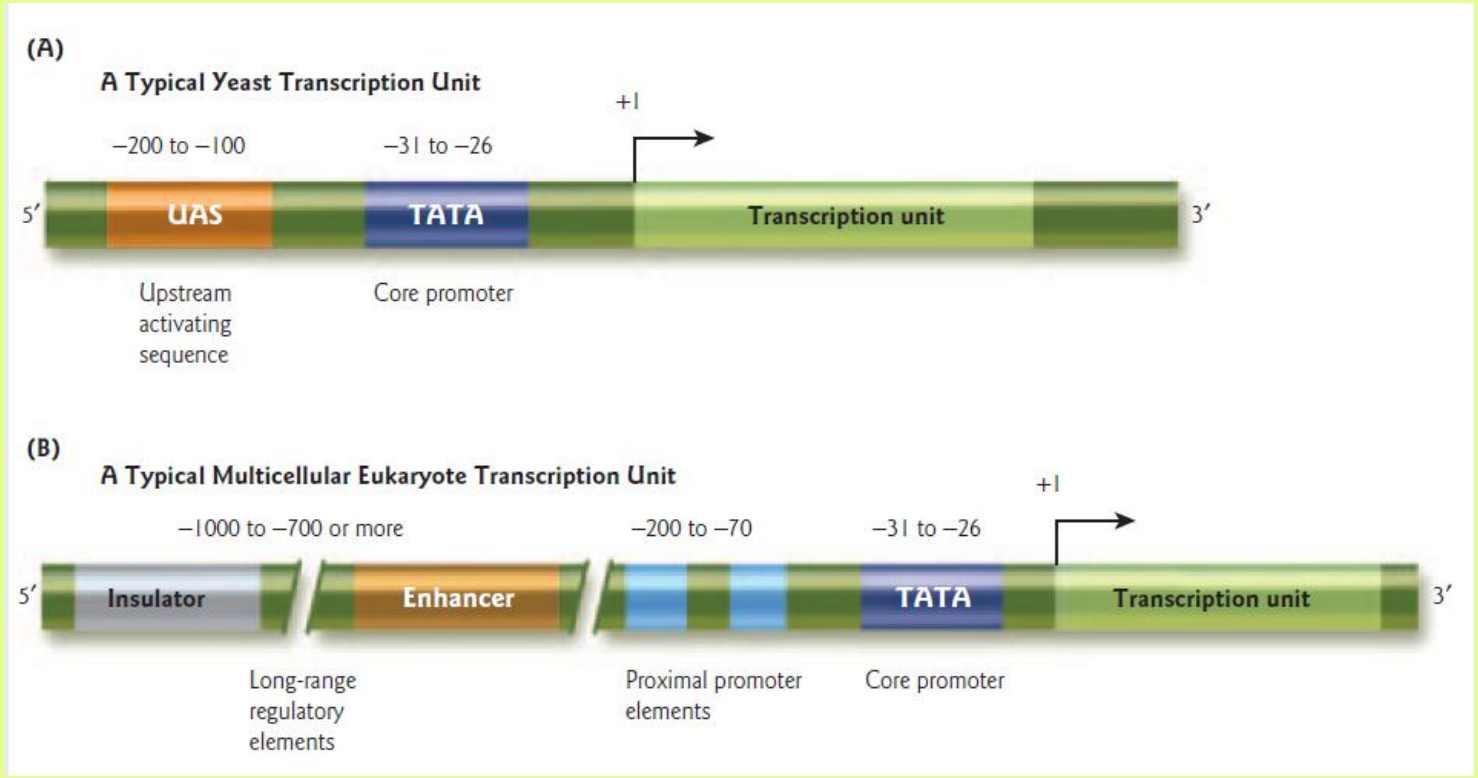
Activator Proteins
- regulatory proteins bind to DNA at distant enhancer sites
- increase the rate of transcription

Enhancer Sites
regulatory sites on DNA distant from gene

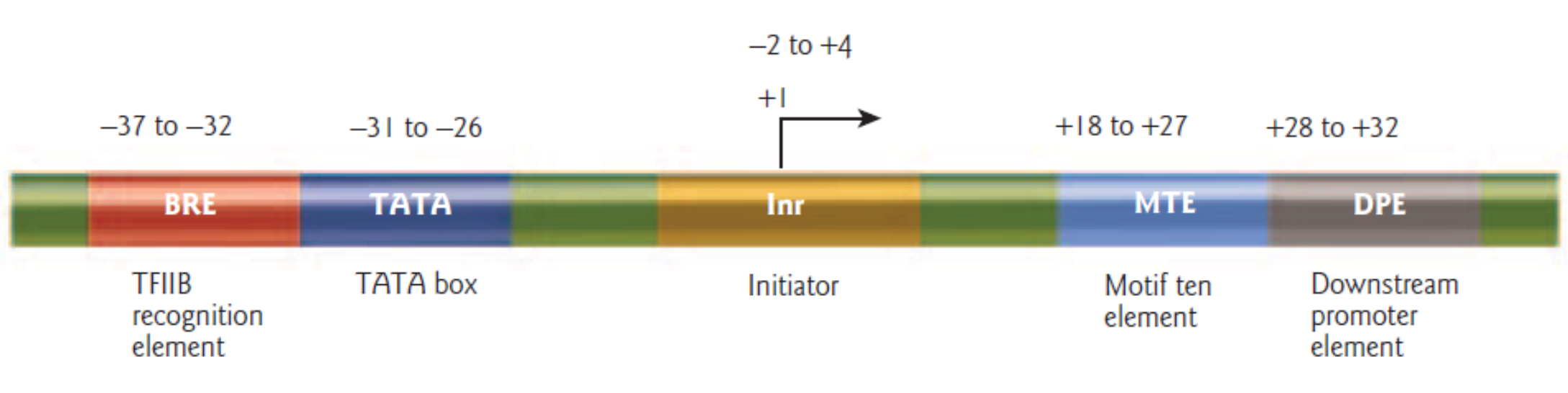


Initiation Complex at Promoter Site binding site of RNA polymerase


Activators
These proteins bind to genes at sites known as *enhancers*. Activators help determine which genes will be switched on, and they speed the rate of transcription.
Repressors
These proteins bind to selected sets of genes at sites known as *silencers*. They interfere with the functioning of activators and thus slow transcription.
Enhancer
Silencer
Enhancer
Enhancer
Repressor
Activator
Activator
Activator
250
40
110
60
30 Beta
30 Alpha
150
80
A
TATA-binding protein
B
F
E
H
RNA polymerase
Coding region
TATA box
Core promoter
Coactivators
These "adapter" molecules integrate signals from activators and perhaps repressors and relay the results to basal factors.
Basal transcription factors
In response to injunctions from activators, these factors position RNA polymerase at the start of the protein-coding region of a gene and send the enzyme on its way.



Comparison of a simple and complex RNA pol II transcription unit. (A) A typical yeast (unicellular eukaryote) transcription unit. The start of transcription (+1) of the protein-coding gene (transcription unit) is indicated by an arrow. (B) A typical multicellular eukaryote transcription unit with clusters of proximal promoter elements and long-range regulatory elements located upstream from the core promoter (TATA). There is variation in whether a particular element is present or absent, the number of distinct elements, their orientation relative to the transcriptional start site, and the distance between them. Although the figure is drawn as a straight line, the binding of transcription factors to each other draws the regulatory DNA sequences into a loop.



RNA pol II core promoter motifs. Sequence elements that can contribute to basal transcription from the core promoter. A particular core promoter may contain some, all, or none of these motifs. The locations of the TFIIB recognition element (BRE), TATA box (TATA), initiator (Inr), motif ten element (MTE), and downstreampromoter element (DPE) motifs are indicated relative to the start of transcription (+1).

Promoter Regions

- There are three main portions that make up a promoter: core promoter, proximal promoter, and distal promoter. Below describes the specifics of these regions in eukaryotic cells
- **Core Promoter**
- The core promoter region is located most proximal to the start codon and contains the RNA polymerase binding site, TATA box, and transcription start site (TSS). RNA polymerase will bind to this core promoter region stably and transcription of the template strand can initiate. The TATA box is a DNA sequence (5'-TATAAA-3') within the core promoter region where general transcription factor proteins and histones can bind. Histones are proteins found in eukaryotic cells that package DNA into nucleosomes. Histone binding prevents the initiation of transcription whereas transcription factors promote the initiation of transcription. The most 3' portion (closest to the gene's start codon) of the core promoter is the TSS which is where transcription actually begins. Only eukaryotes and archaea, however, contain this TATA box. Most prokaryotes contain a sequence thought to be functionally equivalent called the Pribnow box which usually consists of the six nucleotides, TATAAT.
- **Proximal Promoter**
- Further upstream from the core promoter you will find the proximal promoter which contains many primary regulatory elements. The proximal promoter is found approximately 250 base pairs upstream from the TSS and it is the site where general transcription factors bind.
- **Distal Promoter**
- The final portion of the promoter region is called the distal promoter which is upstream of the proximal promoter. The distal promoter also contains transcription factor binding sites, but mostly contains regulatory elements.

Eukaryotic promoter elements.

Promoter	Position	Transcription factor	Consensus sequence
Upstream core promoter elements			
TFIIB recognition element (BRE)	−37 to −32	TFIIB	(G/C)(G/C)(G/A)CGCC
TATA box	−31 to −26	TBP	TATA(A/T)AA(G/A)
Initiator (Inr)	−2 to +4	TAF1 ($TAF_{II}250$) TAF2 ($TAF_{II}150$)	$PyPyA_{+1}N(T/A)PyPy$
Downstream core promoter elements			
Motif ten element (MTE)	+18 to +27	TFIID	C(G/A)A(A/G)C(G/C) (C/A/G)AACG(G/C)
Downstream promoter element (DPE)	+28 to +32	TAF9 ($TAF_{II}40$) TAF6 ($TAF_{II}60$)	(A/G)G(A/T)(C/T)(G/A/C)
Proximal promoter elements			
CAAT box	−200 to −70	CBF, NF1, C/EBP	CCAAT
GC box	−200 to −70	Sp1	GGGCGG

Most, but not all, CAAT and GC boxes are located between −200 and −70.

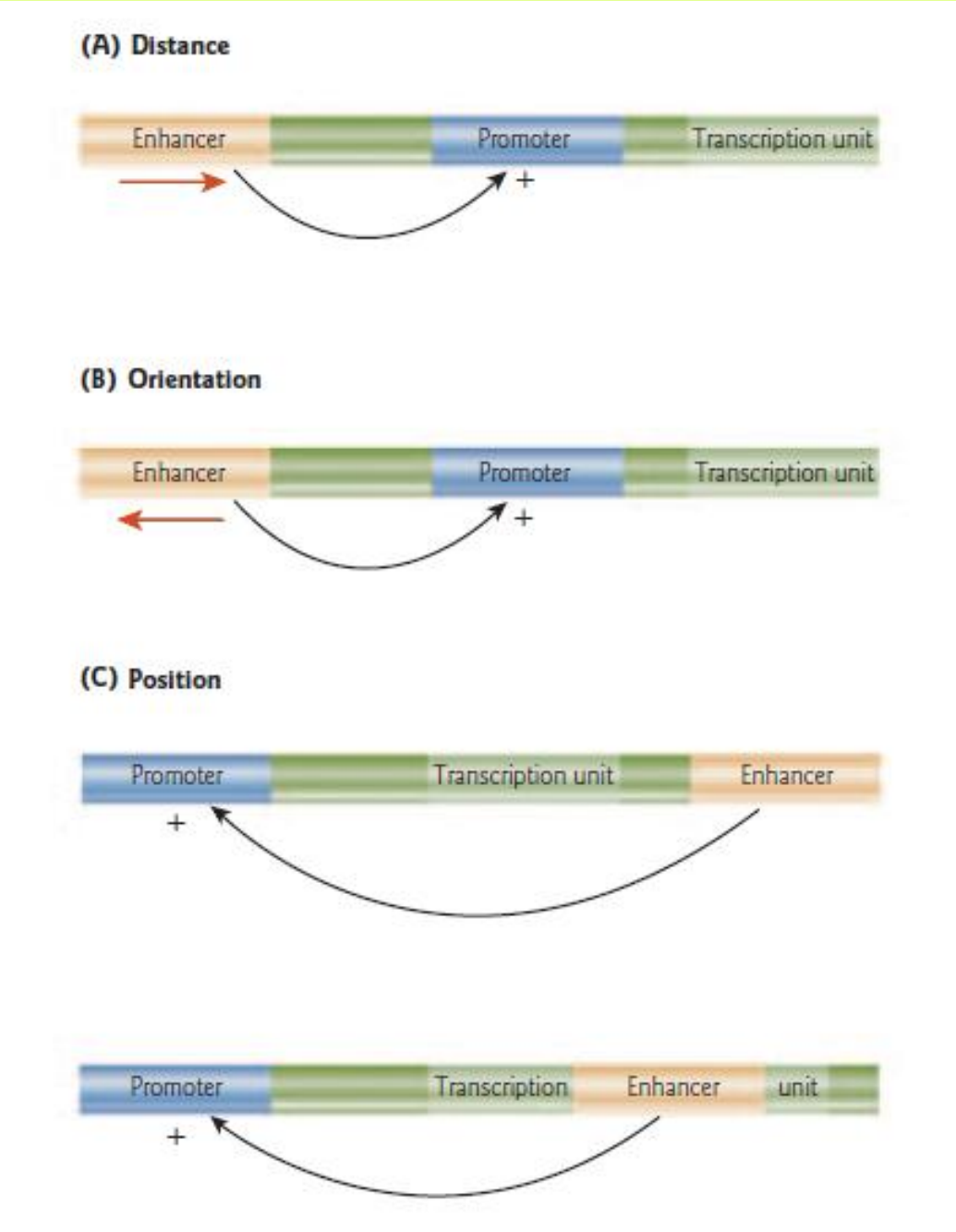
CBF, CAAT-binding protein; C/EBP, CAAT/enhancer-binding protein; N, any (A, T, C, or G); Py, pyrimidine (C or T).

Types of Promoters

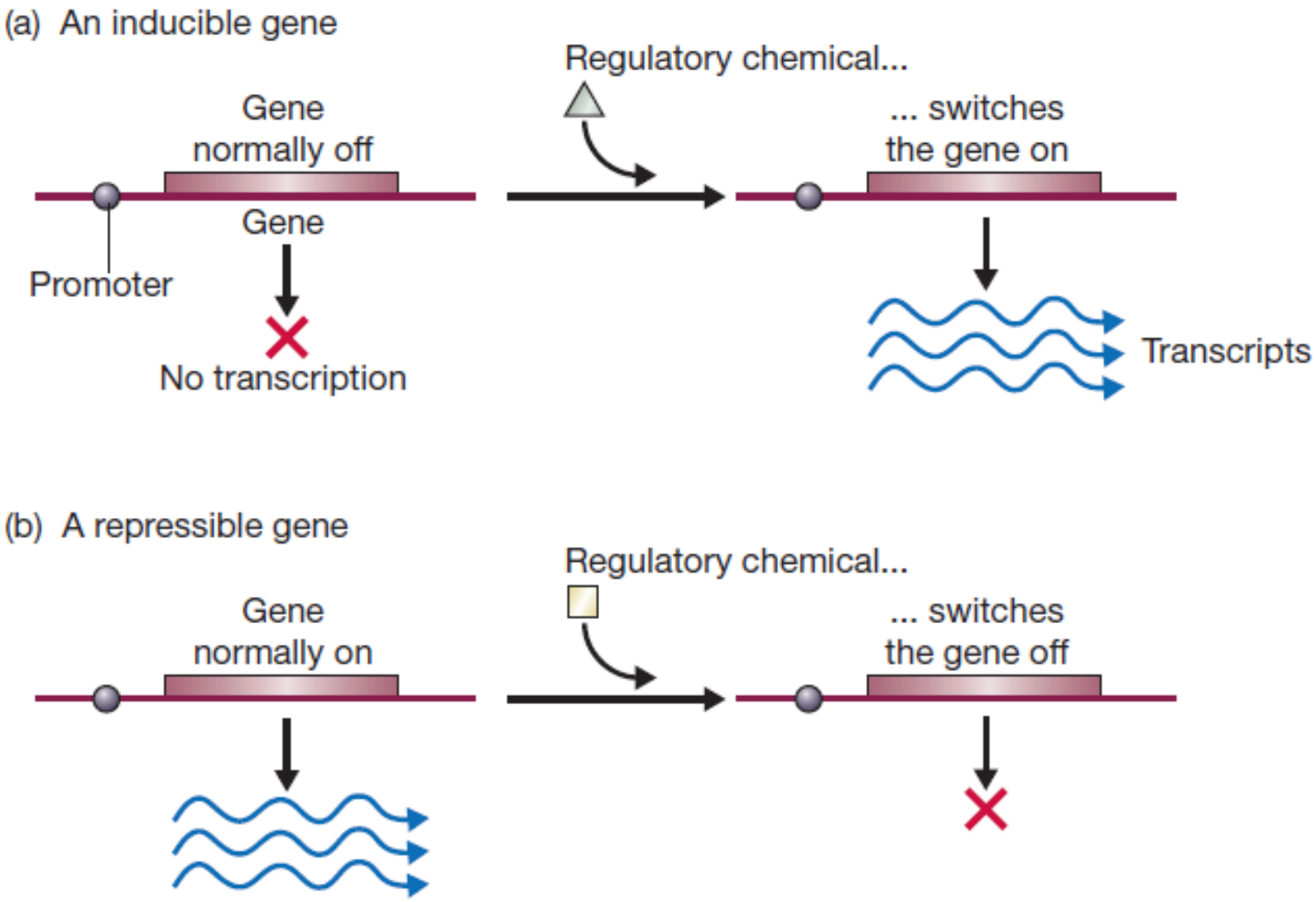
- Constitutive promoters facilitate expression of the gene in all tissues regardless of the surrounding environment and development stage of the organism. Such promoters can turn on the gene in every living cell of the organism, all the time, throughout the organism's lifetime. These promoters can often be utilized across species. Examples of constitutive promoters that are commonly used for plants include Cauliflower mosaic virus (CaMV) 35S, opine promoters, plant ubiquitin (Ubi), rice actin 1 (Act-1) and maize alcohol dehydrogenase 1 (Adh-1). CaMV 35S is the most commonly used constitutive promoter for high levels of gene expression in dicot plants. Maize Ubi and rice Act-1 are the currently the most commonly used constitutive promoters for monocots.
- Tissue-specific or development-stage-specific promoters facilitate expression of a gene in specific tissue(s) or at certain stages of development while leaving the rest of the organism unmodified. In the case of plants, such promoters might specifically influence expression of genes in the roots, fruits, or seeds, or during the vegetative, flowering, or seed-setting stage. If the developer wants a gene of interest to be expressed in more than one tissue type for example the root, anthers and egg sac, then multiple tissue-specific promoters may have to be included in the gene construct.
- Effective gene expression in specific plant parts or development stages often has been observed when promoters from closely related species are used. There are many promoters in this category because they have different tissue and developmental specificities. An example of a tissue-specific promoter is the phosphoenolpyruvate (PEP) carboxylase promoter which induces gene expression only in cells that are actively involved in photosynthesis. In plant genetic engineering, this promoter is used for traits desired in the shoot, leaves and sometimes the stem. Expression of genes controlled by this promoter is reduced later in the growing season as the plant approaches senescence.
- Inducible promoters are activated by exogenous (i.e., external) factors. Exogenous factors may be abiotic such as heat, water, salinity, chemical, or biotic like pathogen or insect attack. Promoters that react to abiotic factors are the most commonly used in plant genetic engineering because these can easily be manipulated. Such promoters respond to chemical compounds such as antibiotics, herbicides or changes in temperature or light. Inducible promoters can also be tissue or development stage specific.
- Promoters can be derived directly from naturally occurring genes, or may be synthesized to combine regulatory sequences from different promoter regions. The promoters interact with other regulatory sequences (enhancers or silencers) and regulatory proteins (transcription factors) to influence the amount of gene transcription/expression.

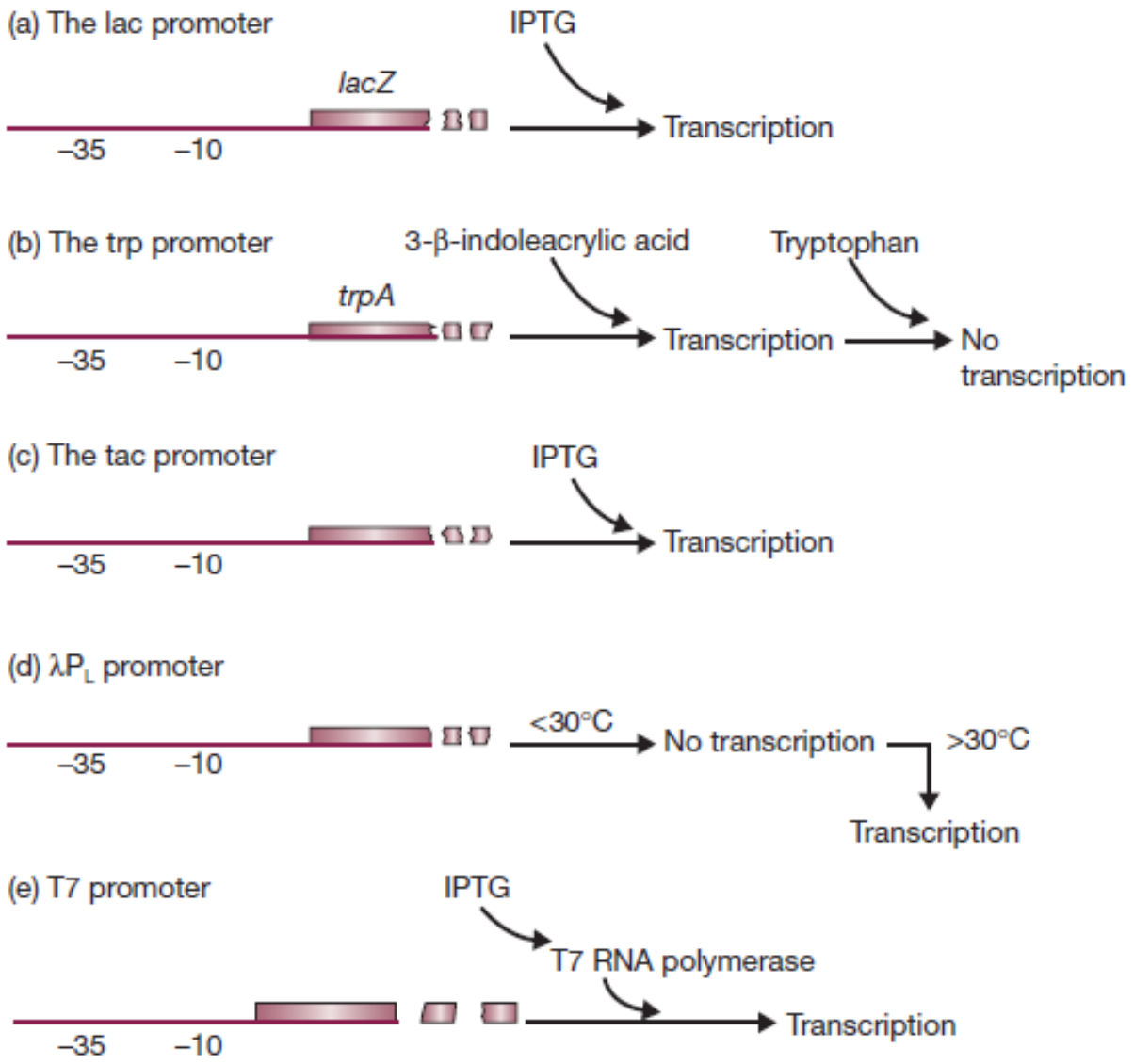
Common Eukaryotic Promoters Used in Research

Promoter	Expression	Description
PGK	Constitutive	Mammalian **promoter** from phospholycerate kinase gene
TRE	Inducible	Tetracycline response element **promoter**
U6	Constitutive	Human U6 nuclear **promoter** for small RNA expression
UAS	Specific	Drosophila **promoter** containing Gal4 binding sites

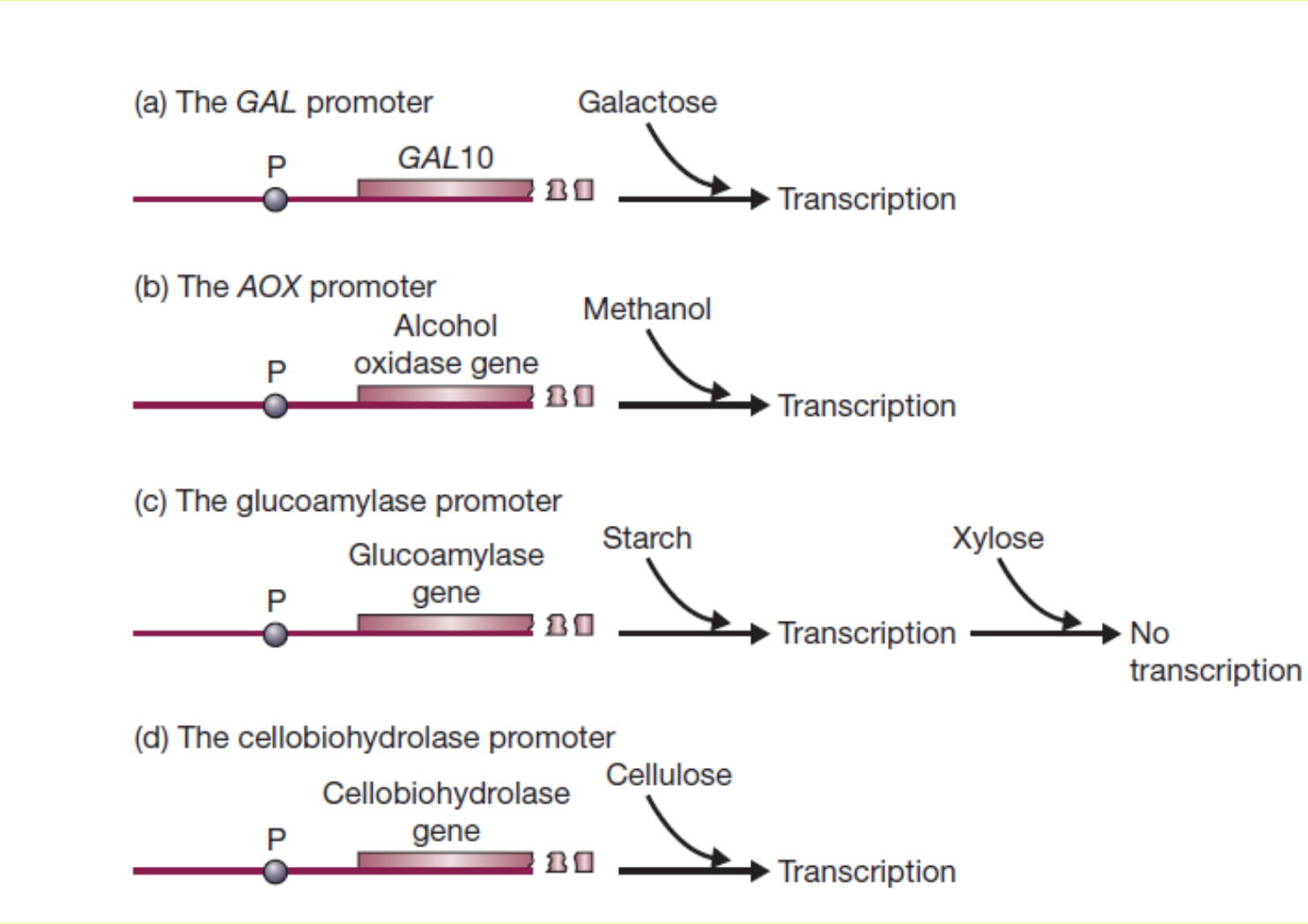


Three key characteristics of an enhancer element. An enhancer element can activate a promoter at a distance (A), in either orientation (B) or when positioned upstream, downstream, or within a transcription unit (C).

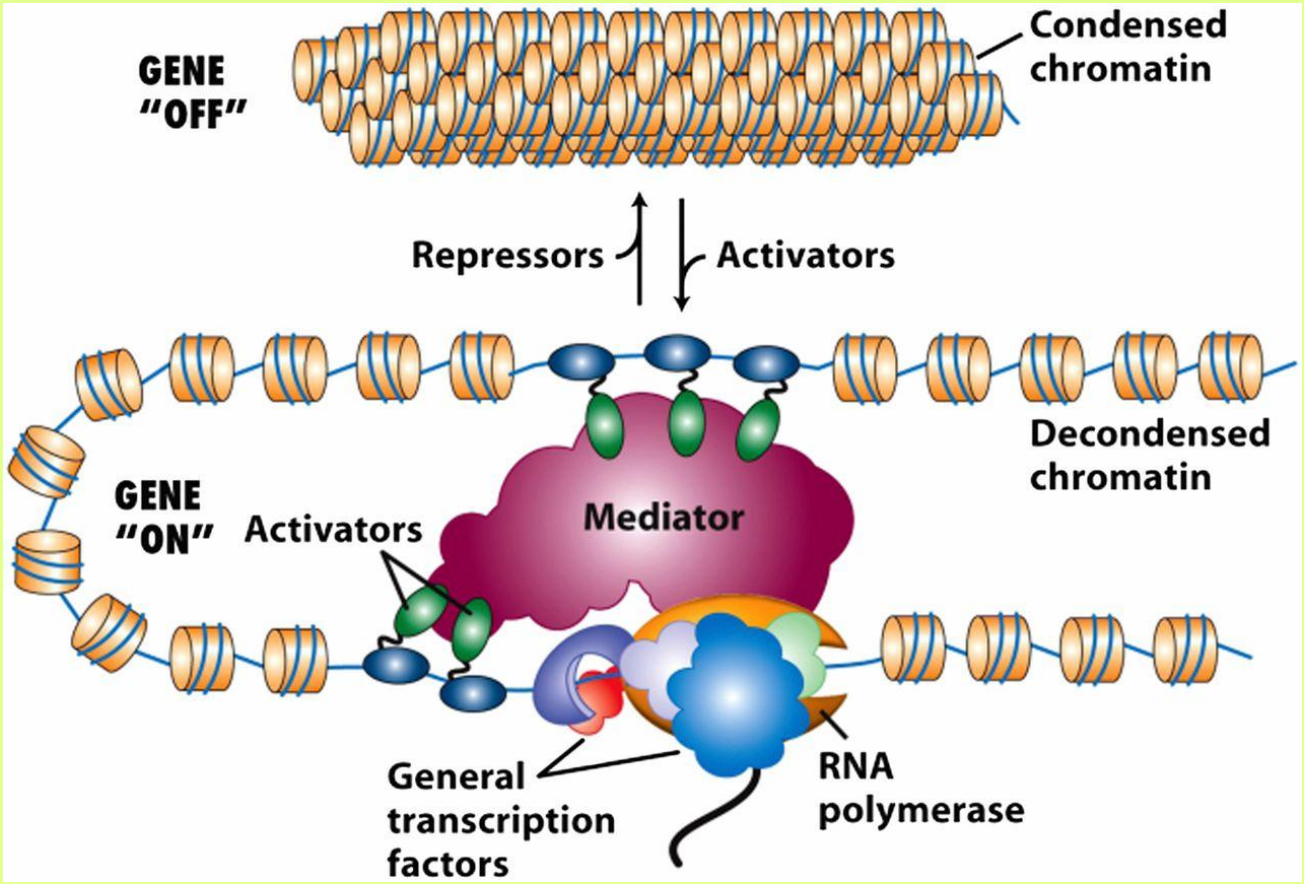

(a) An inducible gene
Regulatory chemical...
Gene
normally off
... switches
the gene on
Gene
Promoter
No transcription
Transcripts
(b) A repressible gene
Regulatory chemical...
Gene
normally on
... switches
the gene off


(a) The lac promoter
IPTG
lacZ
Transcription
–35
–10
(b) The trp promoter
3-β-indoleacrylic acid
Tryptophan
trpA
Transcription
No transcription
–35
–10
(c) The tac promoter
IPTG
Transcription
–35
–10
(d) λP$_L$ promoter
<30°C
No transcription
>30°C
Transcription
–35
–10
(e) T7 promoter
IPTG
T7 RNA polymerase
Transcription
–35
–10

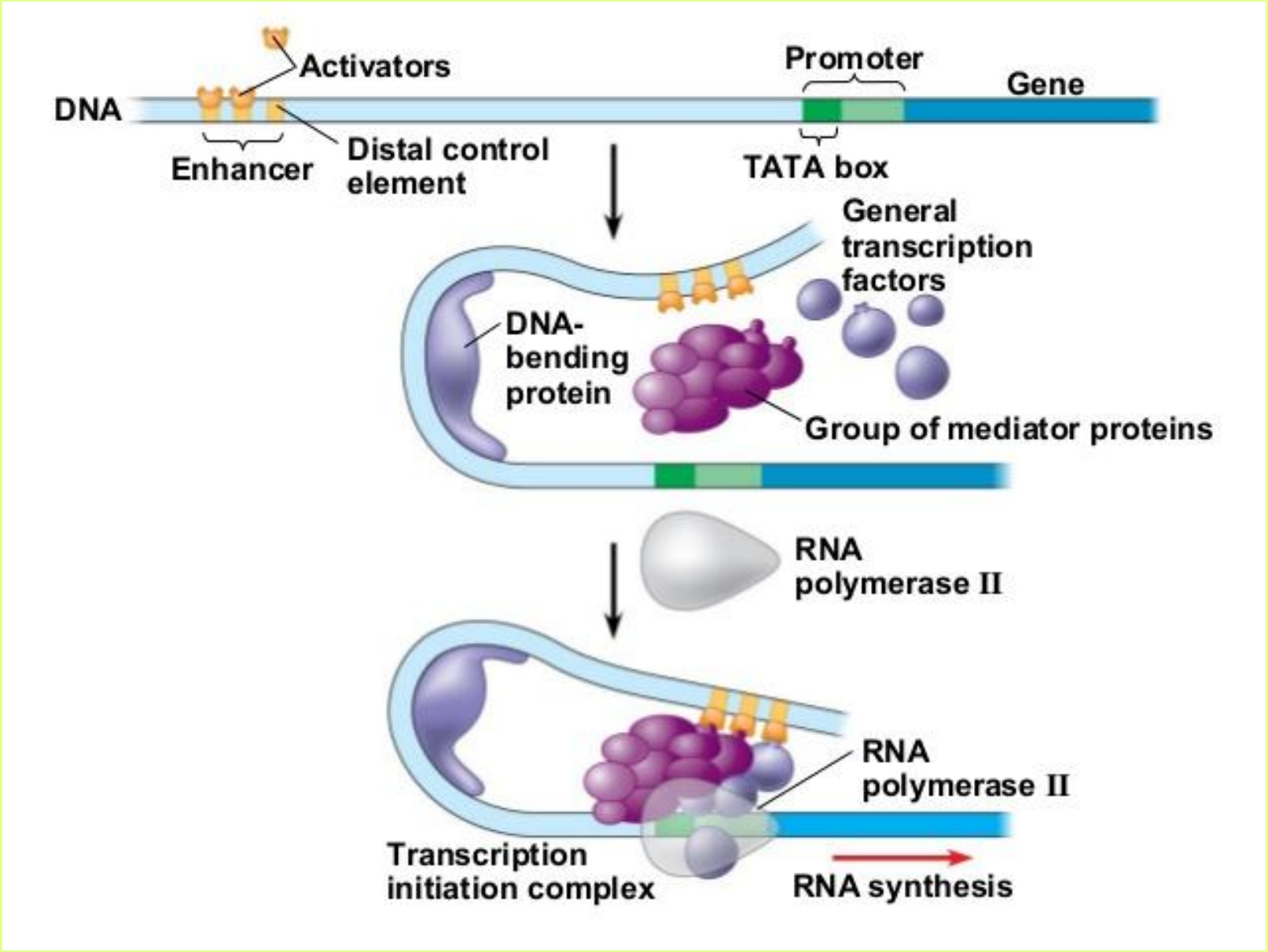
Four promoters frequently used in expression vectors for microbial eukaryotes.



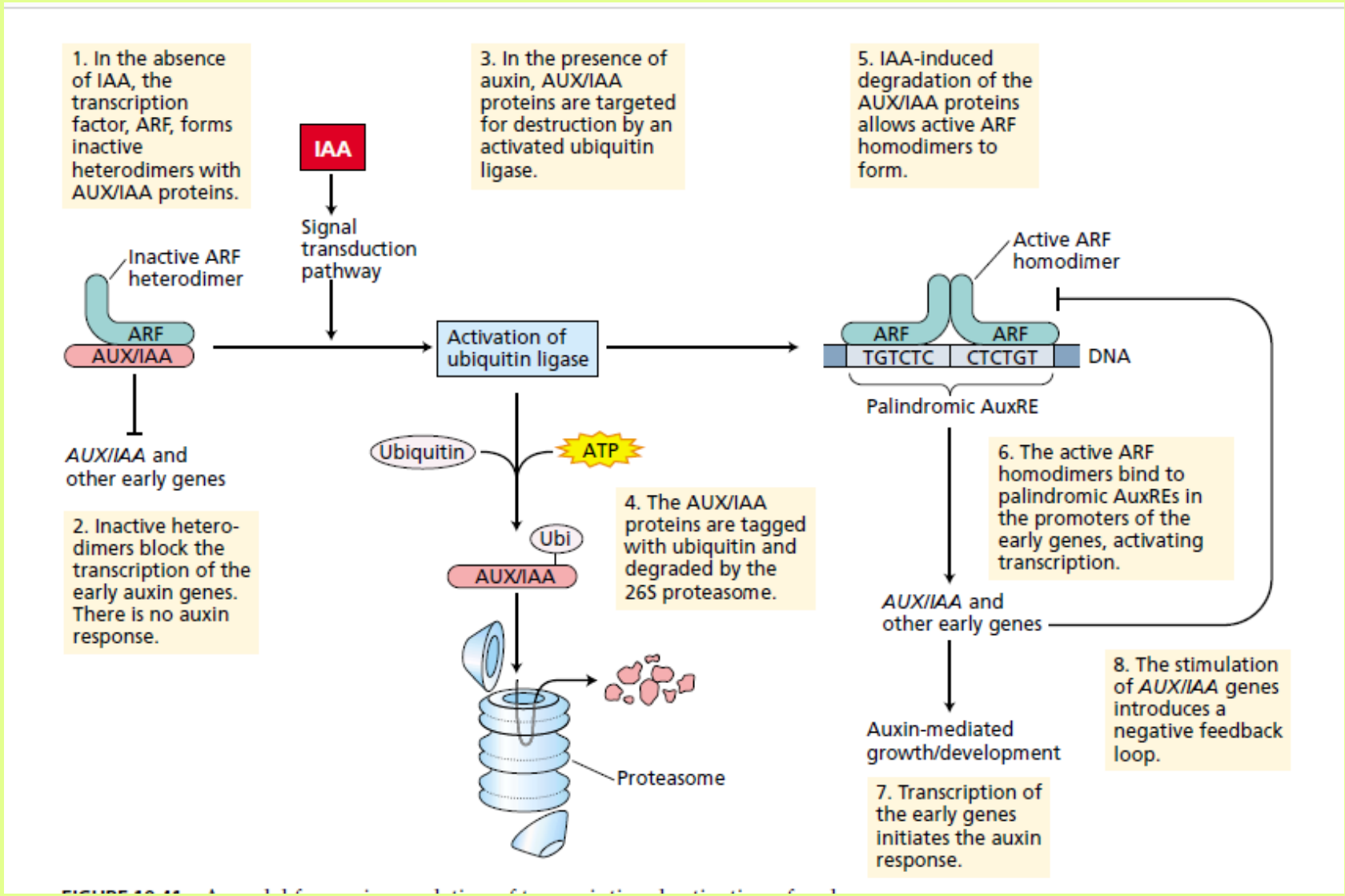
Overview of eukaryotic transcription control



Activator proteins bind to specific DNA control elements in chromatin and interact with multiprotein co-activator machines, such as mediator, to decondense chromatin and assemble RNA polymerase and general transcription factors on promoters. Inactive genes are assembled into regions of condensed chromatin that inhibit RNA polymerase and their associated general transcription factors (GTFs) from interacting with promoters. Alternatively, repressor proteins bind to other control elements to inhibit initiation by RNA polymerase and interact with multiprotein co-repressor complexes to condense chromatin.


Activators
Promoter
Gene
DNA
Enhancer
Distal control element
TATA box
General transcription factors
DNA-bending protein
Group of mediator proteins
RNA polymerase II
RNA polymerase II
Transcription initiation complex
RNA synthesis

A model for auxin regulation of transcriptional activation of early response genes by auxin. (After Gray et al. 2001.)



1. GA_1 from the embryo first binds to a cell surface receptor.

2. The cell surface GA receptor complex interacts with a heterotrimeric G-protein, initiating two separate signal transduction chains.

3. A calcium-independent pathway, involving cGMP, results in the activation of a signaling intermediate.

4. The activated signaling intermediate binds to DELLA repressor proteins in the nucleus.

5. The DELLA repressors are degraded when bound to the GA signal.

6. The inactivation of the DELLA repressors allows the expression of the *MYB* gene, as well as other genes, to proceed through transcription, processing, and translation.

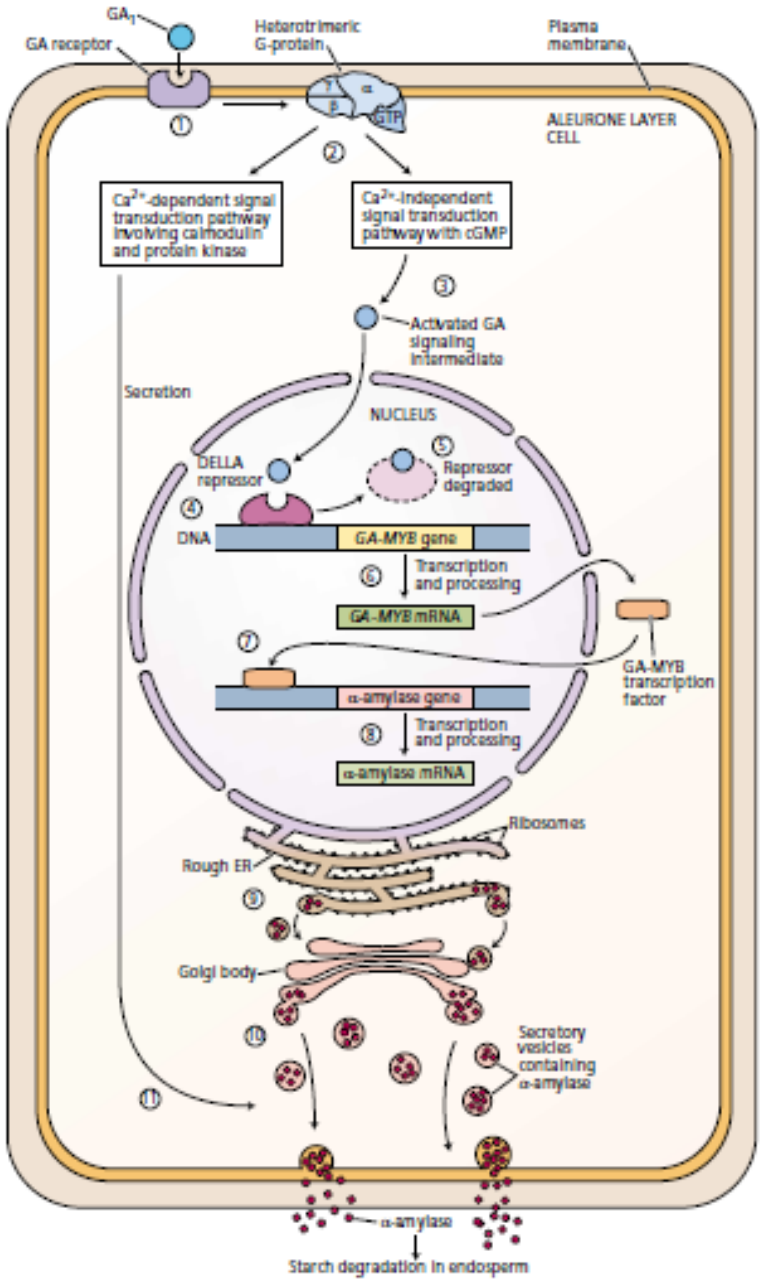
7. The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for α-amylase and other hydrolytic enzymes.

8. Transcription of α-amylase and other hydrolytic genes is activated.

9. α-Amylase and other hydrolases are synthesized on the rough ER.

10. Proteins are secreted via the Golgi.

11. The secretory pathway requires GA stimulation via a calcium–calmodulin-dependent signal transduction pathway.



References

- https://www.addgene.org/mol-bio-reference/promoters/
- Lewin's Genes XI
- Molecular Cell Biology, Lodish et al., 6th Edition
- http://nepad-abne.net/biotechnology/process-of-developing-genetically-modified-gm-crops/commonly-used-promoters/