

Gene Expression Regulation

- Controlling gene expression is often accomplished by controlling transcription initiation.
- **Regulatory proteins** bind to DNA to either (block or stimulate) transcription, depending on how they interact with RNA polymerase.
- Prokaryotic organisms regulate gene expression in response to their environment.
- Eukaryotic cells regulate gene expression to maintain **homeostasis** in the organism.

Regulatory Proteins

- The regulatory proteins are binding to specific DNA sequences to regulate gene expression.
- They gain access to the bases of DNA at the **major groove** by possessing **DNA-binding motifs**
- DNA-binding motifs are **regions of regulatory proteins which bind to DNA**
 - 1- Helix-turn-helix motif
 - 2- Homeodomain motif
 - 3- Zinc finger motif
 - 4- Leucine zipper motif

Regulation of gene expression in prokaryotes

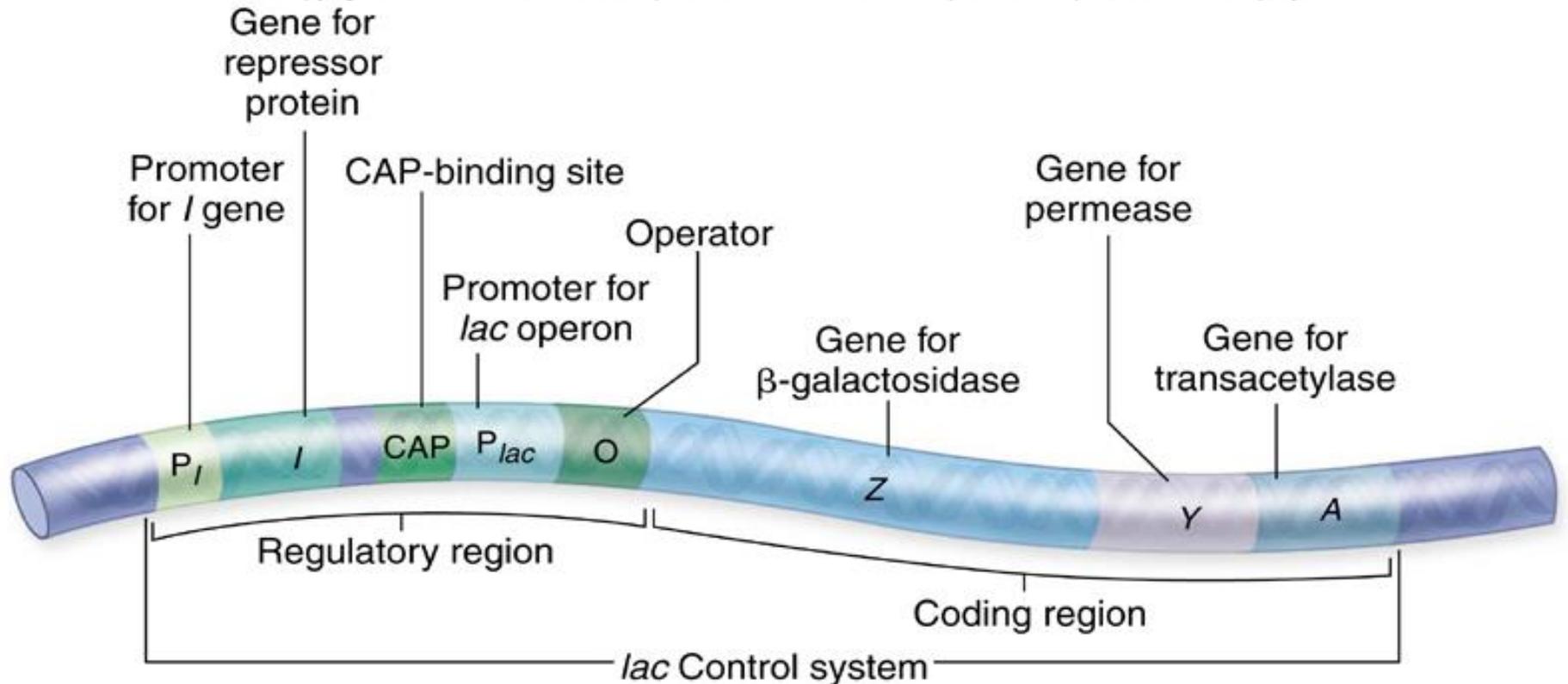
- Control of transcription initiation can be:
- Positive control – increases transcription when activators bind to DNA
- Negative control – reduces transcription when repressors bind to DNA regulatory regions (operators).

- Prokaryotic cells often respond to their environment by changes in gene expression.
- Genes involved in the same metabolic pathway are organized in **operons**.

- Some operons are induced when the metabolic pathway is needed.
- Some operons are repressed when the metabolic pathway is no longer needed.

- The ***lac*** operon contains genes for the use of **lactose** as an energy source. (it includes genes encoding for three enzymes)
 - 1- *lac Z*: encodes β -galactosidase which cleaves lactose into glucose and galactose
 - 2- *lac Y*: encodes lactose permease to transport lactose into the cell
 - 3- *lac A*: encodes galactoside O- acetyltransferase which plays a role in cell detoxification

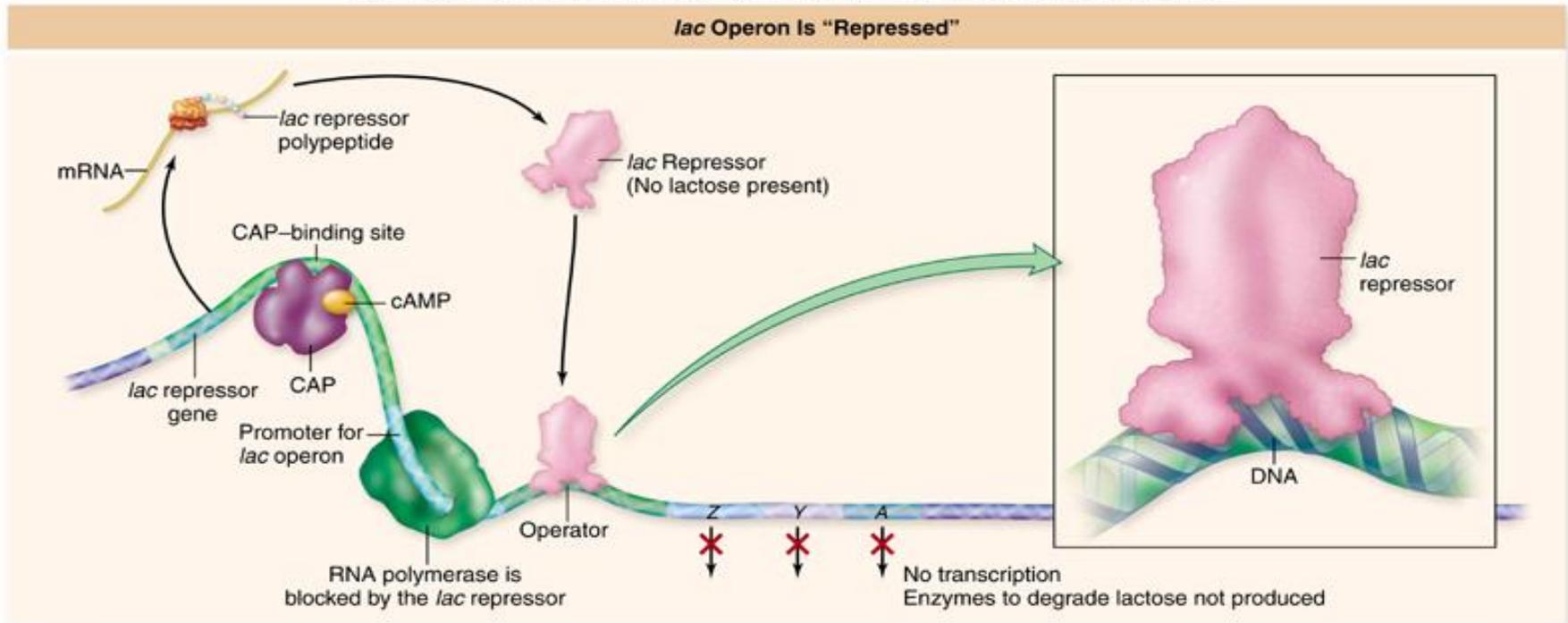
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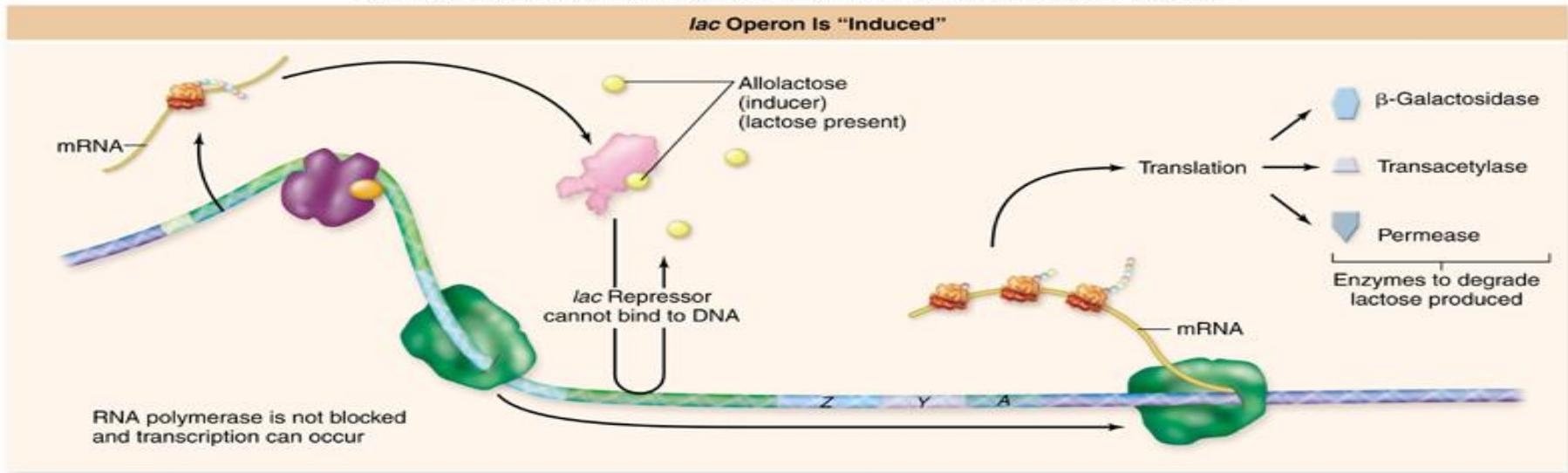


- Regulatory regions of the operon include the CAP (Catabolite activator protein) binding site, promoter, and the operator.
- The *lac* operon is **negatively regulated** by a repressor protein:
 - *lac* repressor binds to the operator to block transcription
 - In the presence of lactose, an inducer molecule binds to the repressor protein
 - Repressor can no longer bind to operator
 - Transcription proceeds

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lac Operon Is "Repressed"



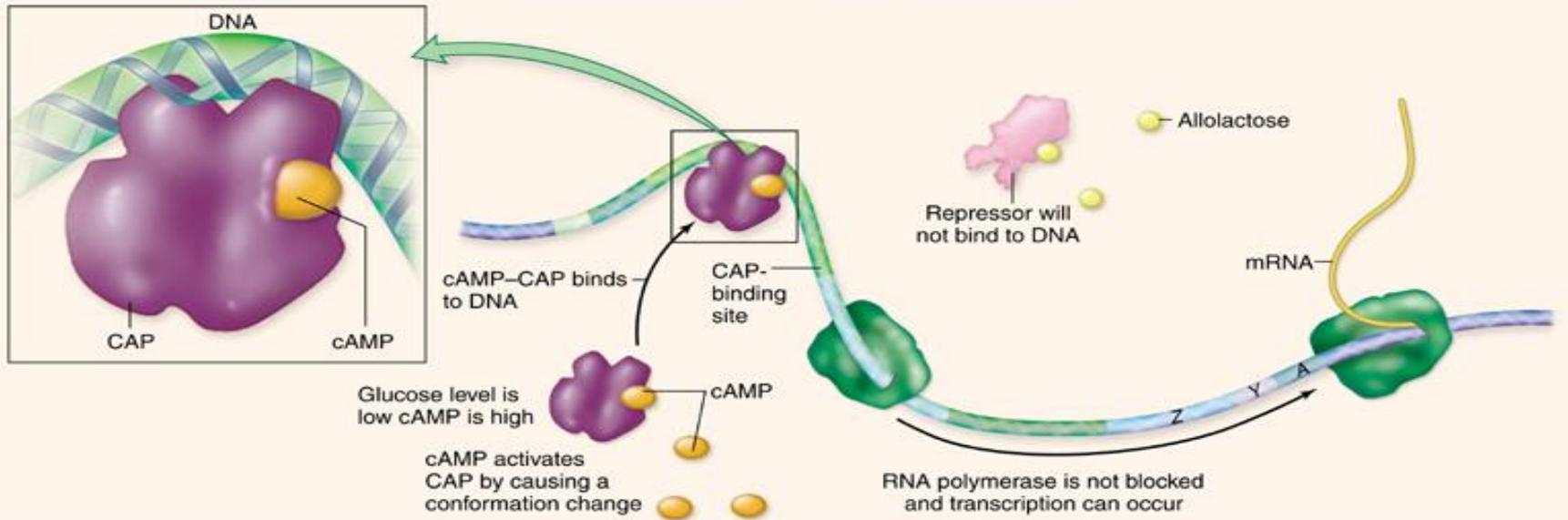


b.

- In the presence of both **glucose** and **lactose**, bacterial cells prefer to use **glucose**.
- Glucose prevents induction of the *lac* operon.
 - binding of **CAP – cAMP complex** to the CAP binding site is required for induction of the *lac* operon
 - high glucose levels cause low **cAMP** levels
 - high glucose \rightarrow low **cAMP** \rightarrow **no induction**

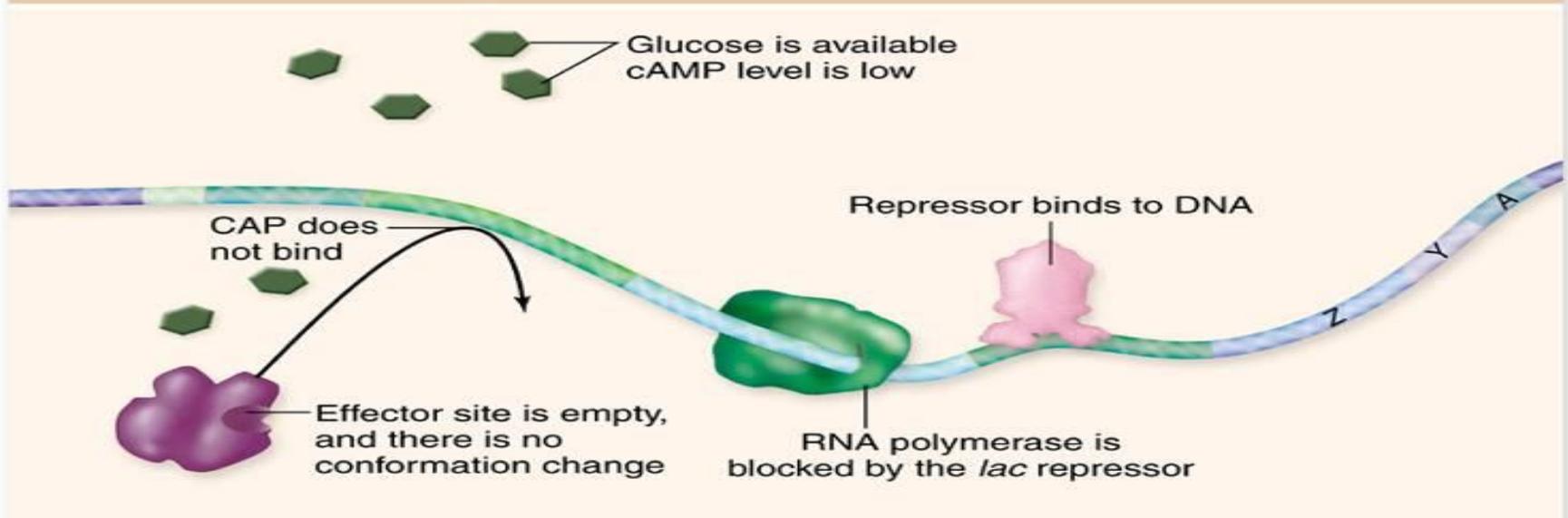
Summary: *Lac* operon is active only in time, when the activator **CAP-cAMP** complex is attached to promoter (**no glucose**) and when is not present repressor on operator (**lactose present**).

Glucose Low, Inducer Present, Promoter Activated



a.

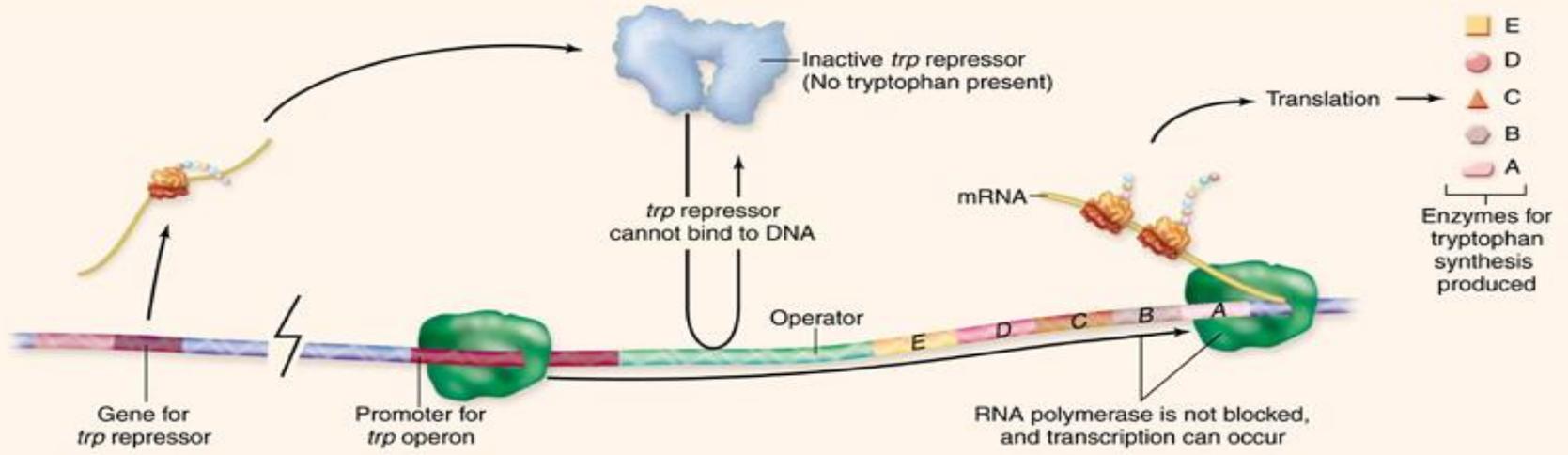
Glucose High, Inducer Absent, Promoter Not Activated



b.

- The ***trp* operon** encodes genes for the **biosynthesis of tryptophan**.
- The operon is not expressed when the cell contains sufficient amounts of tryptophan.
- The operon is expressed when levels of tryptophan are low.
- The ***trp* operon** is **negatively** regulated by the ***trp* repressor protein**
 - ***trp* repressor** binds to the operator to block transcription
 - Binding of repressor to the operator requires a **corepressor** which is tryptophan
 - Low levels of tryptophan prevent the repressor from binding to the operator

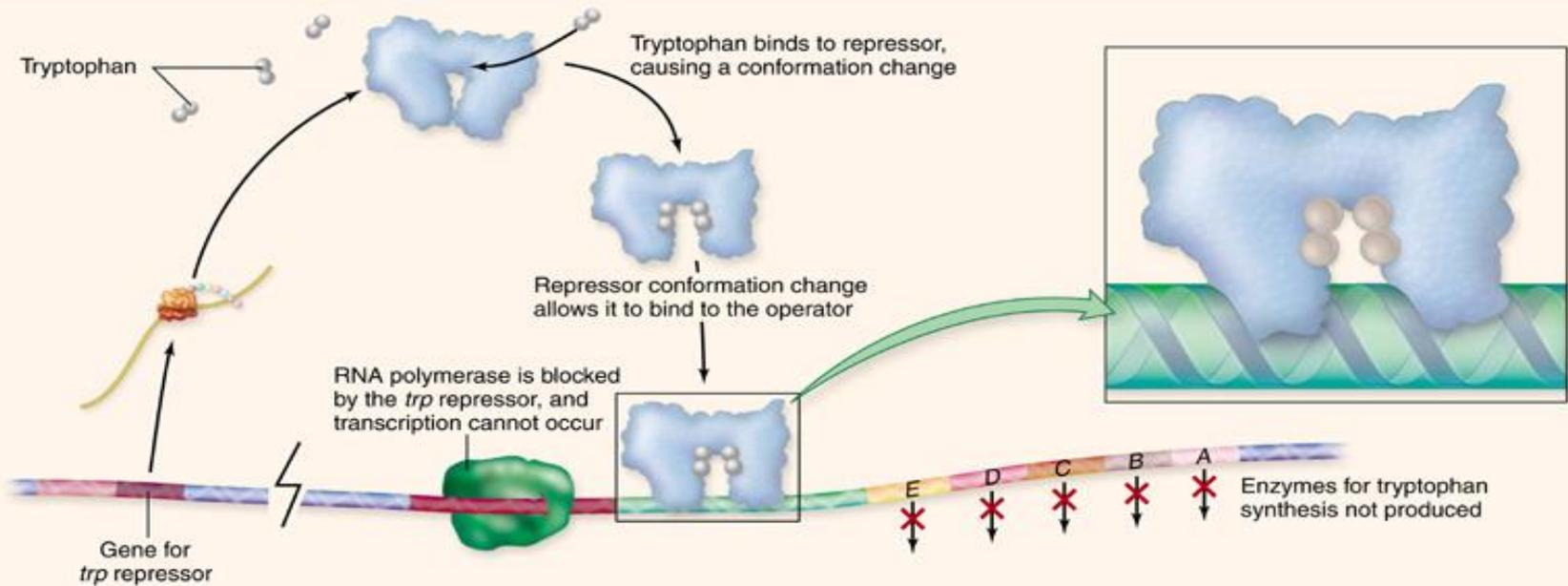
Tryptophan Absent, Promoter Activated



a.

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Tryptophan Present, Promoter Repressed



b.

Regulation of gene expression in eukaryotes

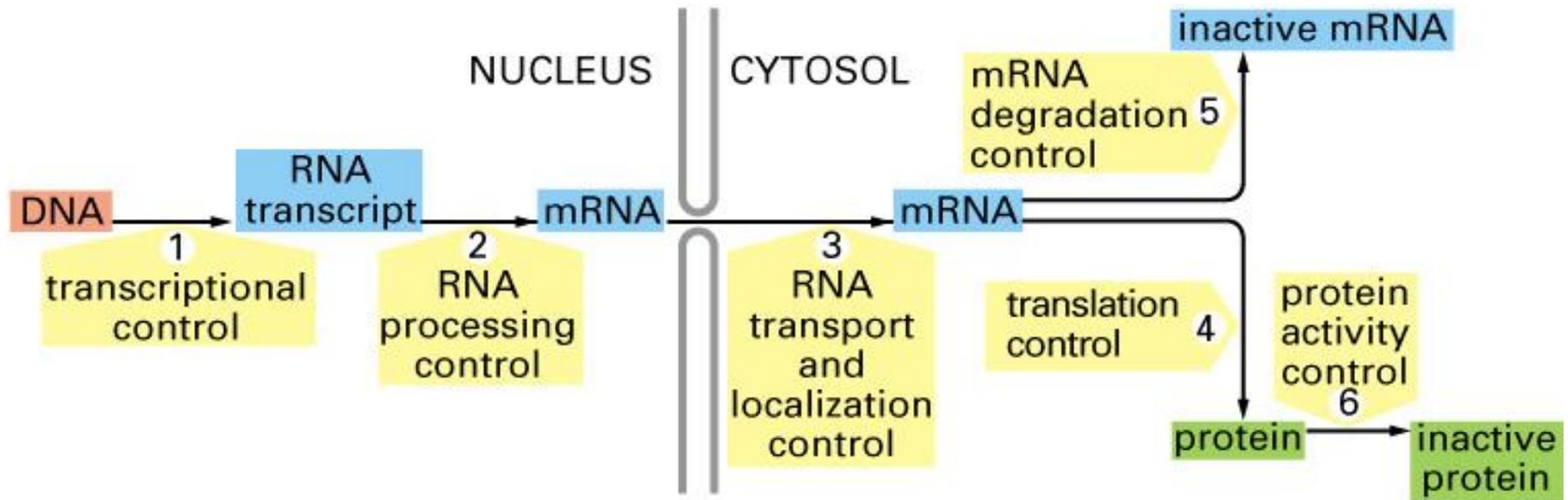
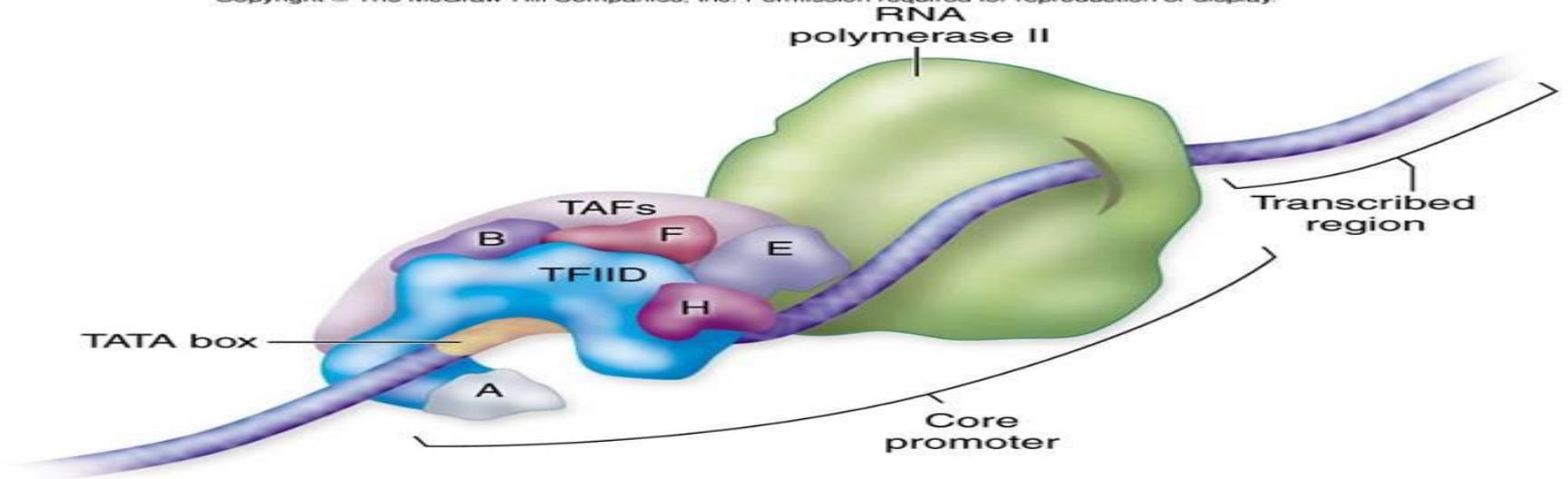


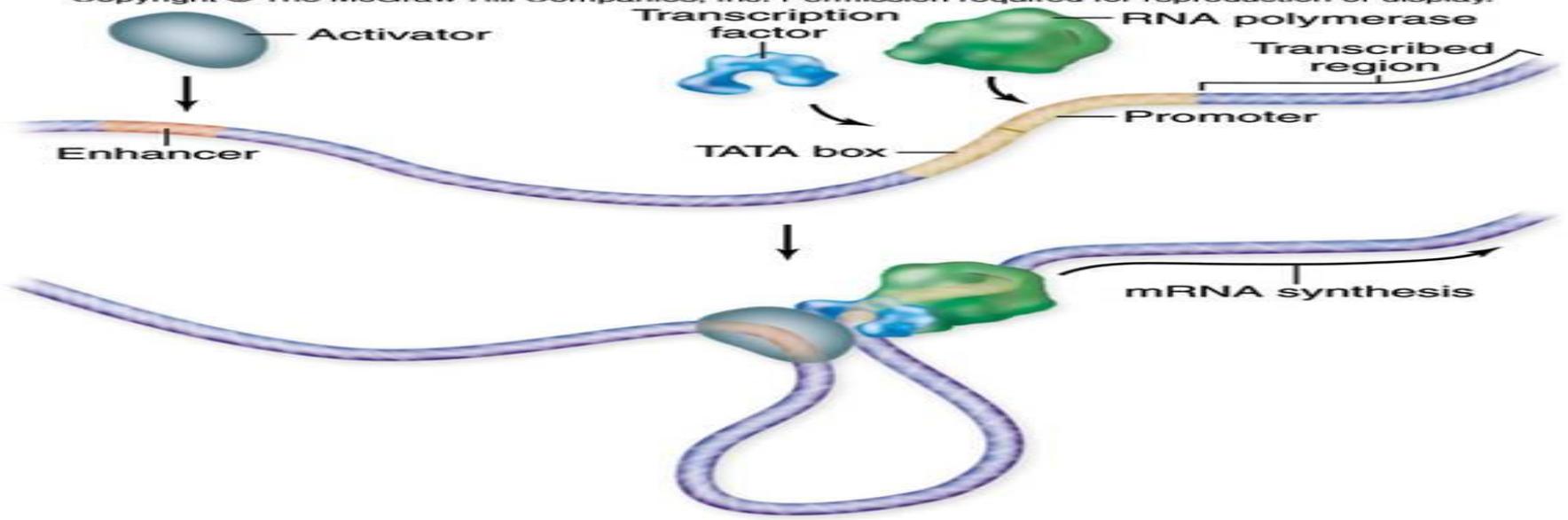
Figure 7-5. Molecular Biology of the Cell, 4th Edition.

- Controlling the expression of eukaryotic genes requires **transcription factors**.
 - **General transcription factors** are required for transcription initiation (for proper binding of RNA polymerase to the DNA).
 - **Specific transcription factors** increase transcription in certain cells or in response to signals
- General transcription factors bind to the promoter region of the gene.
- RNA polymerase II then binds to the promoter to begin transcription at the start site (+1).
- Enhancers are DNA sequences to which specific transcription factors (activators) bind to increase the rate of transcription.

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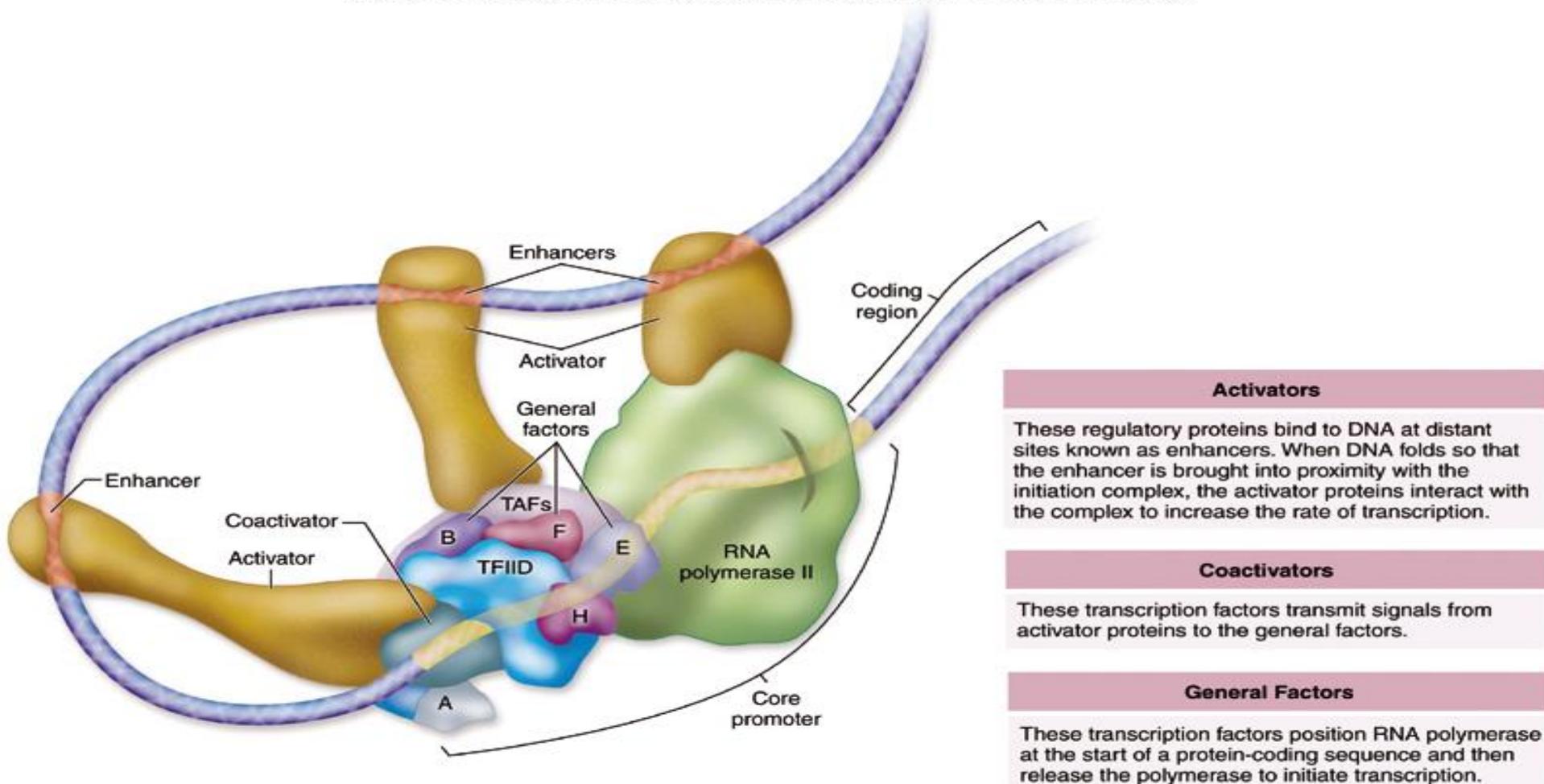


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- **Coactivators** and **mediators** are also required for the function of transcription factors.
 - Coactivators and mediators bind to transcription factors and bind to other parts of the transcription apparatus

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Eukaryotic Chromosome Structure

- Eukaryotic DNA is packaged into chromatin which is directly related to the control of gene expression.
- Chromatin structure begins with the organization of the DNA into nucleosomes.
- Nucleosomes may block RNA polymerase II from gaining access to promoters.

The effects of chromatin structure changes on genes expression

1- DNase I hypersensitivity

DNase I hypersensitive sites: more open chromatin configuration site, upstream of the transcription start site

2- Histone modification

- Addition of methyl groups to the histone protein tails
- Addition of acetyl groups to histone proteins

Chromatin Structure

- In eukaryotes, the DNA is organized into nucleosomes: about 200 bp of DNA wrapped around a protein core.
- The protein core consists of 8 histone proteins
- Histones are basic (i.e. alkaline): they contain positively charged amino acids that bind to the negative charges on the DNA (backbone phosphate groups).
- DNA tightly wrapped around histones is inaccessible to RNA polymerase
- Thus, one important event in preparing a gene for transcription is “chromatin remodeling”: sliding the nucleosomes along the DNA to expose the promoter region.

Histone Acetylation

- A second event needed for transcription affects large regions of the chromosome instead of individual genes.
- DNA is normally tightly wrapped around the histones and is inaccessible to transcription factors.
- The structure can be loosened by acetylating the histones.
- Acetyl groups are added to lysines, which removes their positive charge.
- The binding of the DNA to the histones is lessened, and the DNA structure opens up, allowing access to transcription factors.
- Conversely, deacetylation tightens the chromatin structure, preventing transcription throughout that region of the chromosome

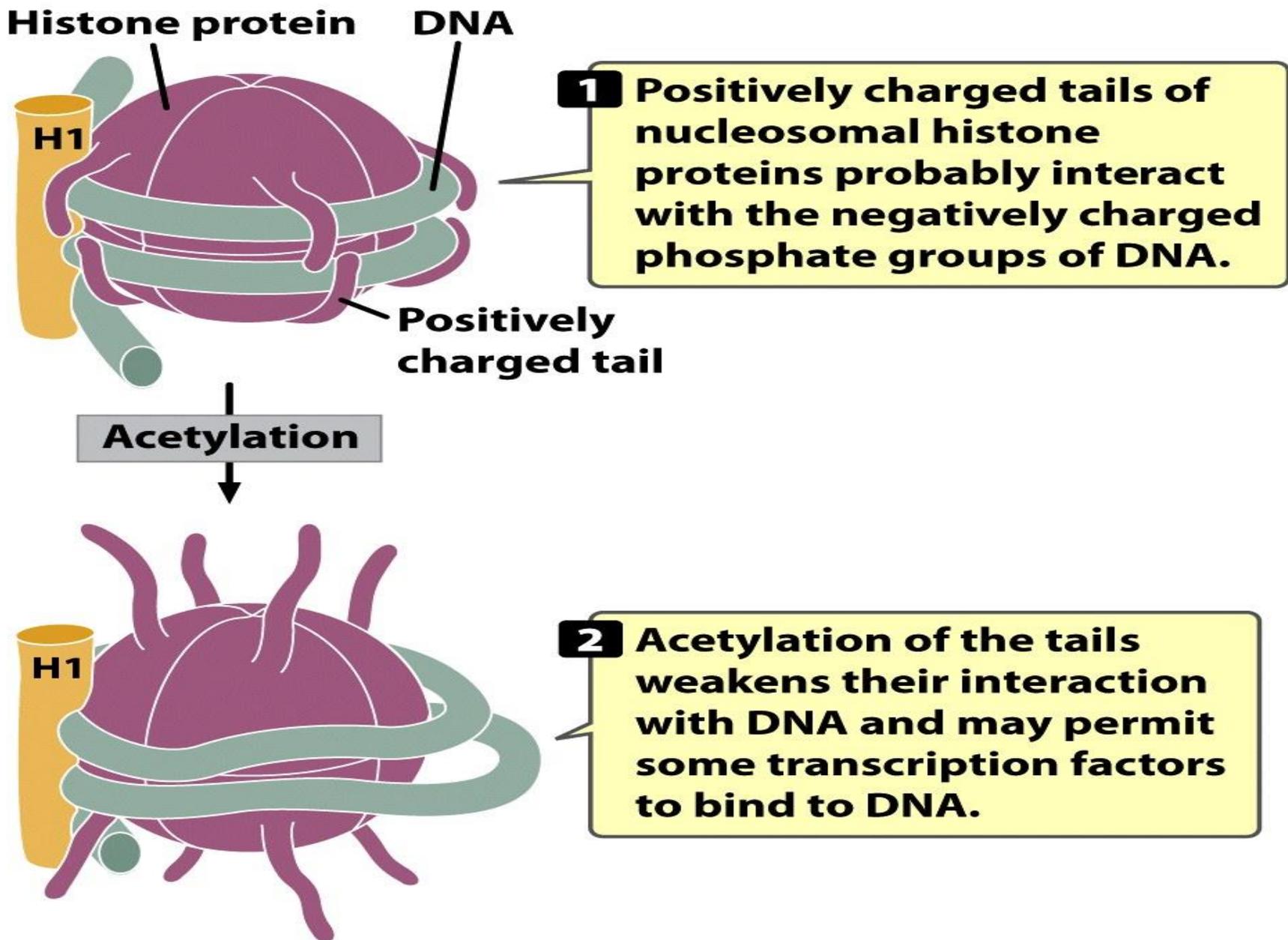


Figure 17-2
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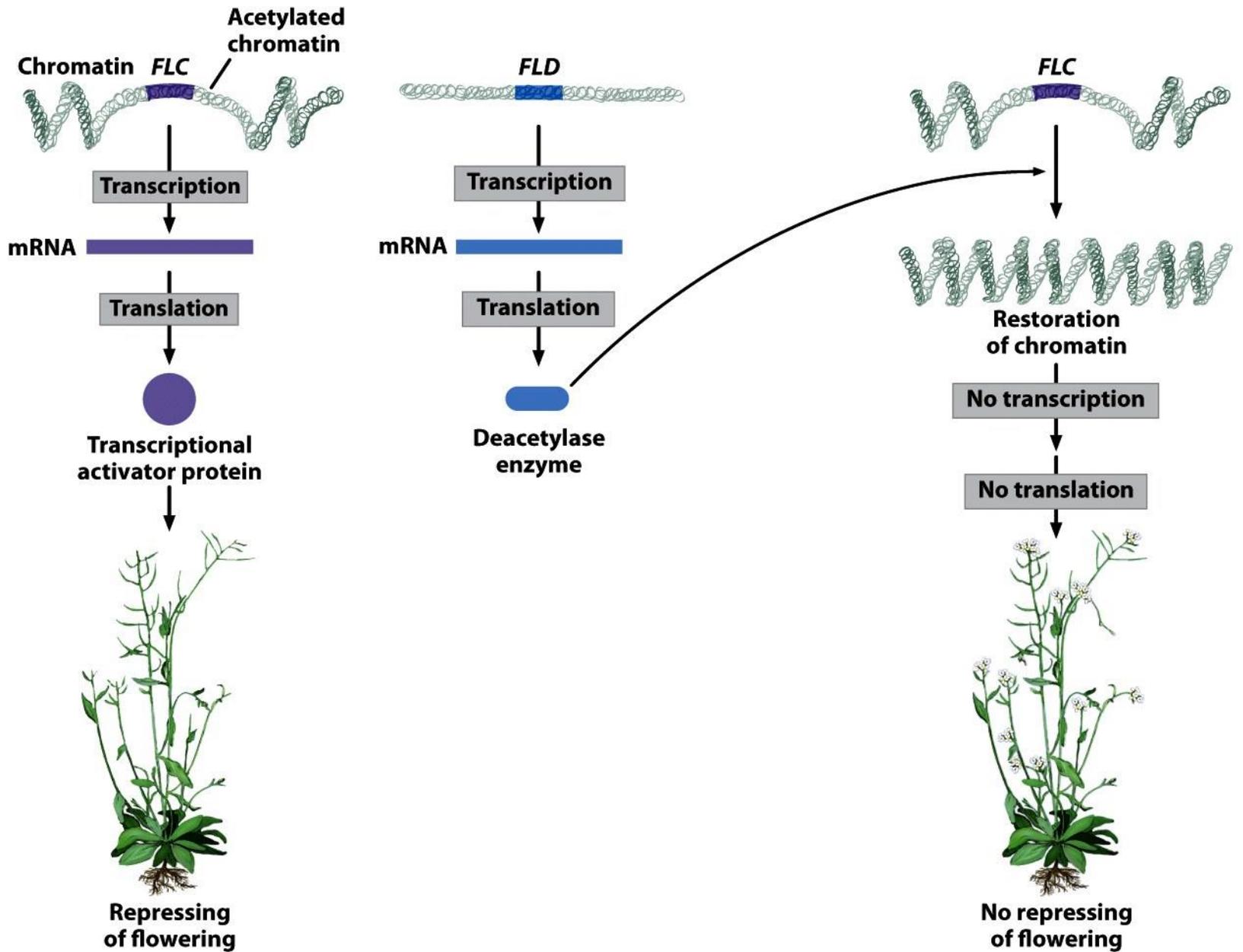


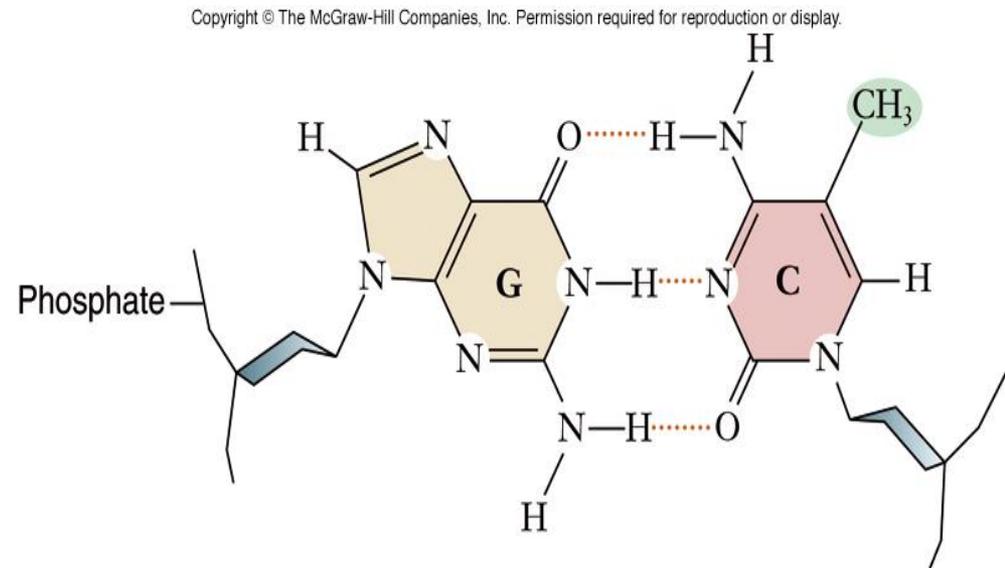
Figure 17-3
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Methylation

- Addition of methyl group to DNA or histone proteins is associated with the control of gene expression.
- Clusters of methylated cytosine nucleotides bind to a protein that prevents activators from binding to DNA.
- Methylated histone proteins are associated with inactive regions of chromatin.

Chromatin remodeling

- Chromatin-remodeling complexes: bind directly to DNA sites and reposition nucleosomes
- DNA methylation of cytosine bases adjacent to guanine nucleotides (CpG)–CpG islands



Posttranscriptional Regulation

- Control of gene expression usually involves the control of transcription initiation.

- But gene expression can be controlled after transcription, with mechanisms such as:

A- RNA interference

B- alternative splicing

C- RNA editing

D- mRNA degradation

A- **RNA interference** involves the use of small RNA molecules

- The enzyme **Dicer** chops double stranded RNA into small pieces of RNA

- **Micro-RNAs** bind to complementary RNA to prevent translation

- **Small interfering RNAs** degrade particular mRNAs before translation

B- **Alternative splicing**

- Introns are spliced out of pre-mRNAs to produce the mature mRNA that is translated.

- The spliceosome recognizes different splice sites in different tissue types.

- The mature mRNAs in each tissue possess different exons, resulting in different polypeptide products from the same gene.

C- RNA editing creates mature mRNA that are not truly encoded by the genome.

- Editing in the coding region to produce isoforms of the same protein and pre- and post-coding region affecting pre-mRNA splicing, mRNA transport to cytosol or RNA translation effectiveness.

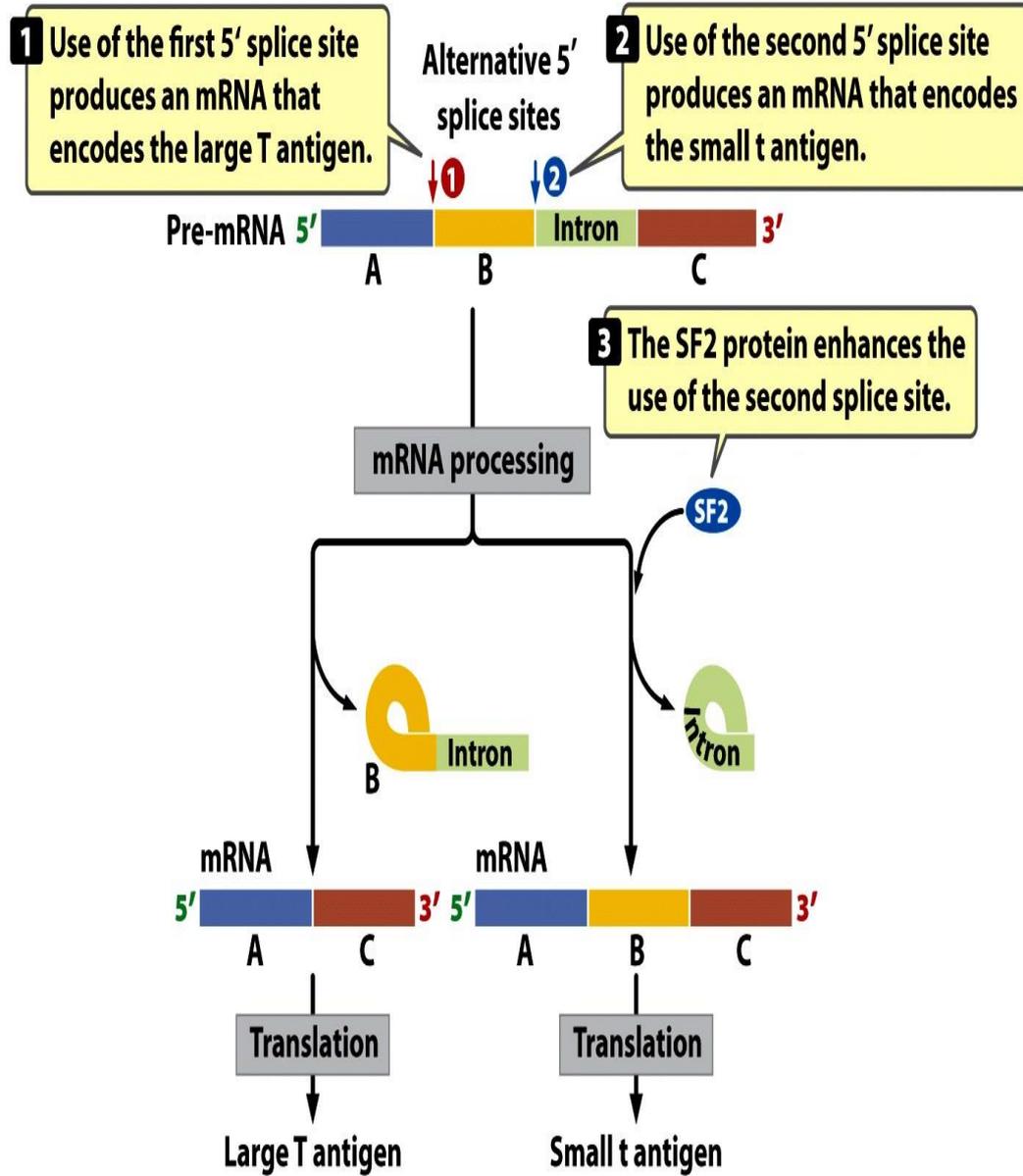


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- For example:
 - Apolipoprotein B exists in 2 isoforms
 - One isoform is produced by editing the mRNA to create a stop codon instead of glutamine codon (Apo B48 in intestine) and (Apo B 100 in liver)
 - This RNA editing is tissue-specific
- Mature mRNA molecules have various half-lives depending on the gene and the location (tissue) of expression and in turn it is affecting the amount of the polypeptide produced.

D- Control of RNA Transport and intracellular Localization

- The control of transporting nuclear mRNAs to cytosol and their
- localization to specific cellular compartment which is affected by the post-transcriptional processing of hn-RNA
- The intracellular localization is under effect of elements (localization elements) specified by cis-acting elements (mostly found in 3'UTR)
- Localization elements are recognized by trans-acting factors (RNA-binding proteins)

E- RNA processing and degradation can regulate some genes

- The degradation of RNA

- 5'-cap removal
- Shortening of the poly (A) tail
- Degradation of 5' UTR, coding sequence, and 3' UTR

Mechanisms of Gene regulation by RNA interference RNA cleavage:

- RISC containing an siRNA, pair with mRNA molecules and cleavage to the mRNA
- Inhibition of translation
- Transcriptional silencing: altering chromatin structure
- Silencer-independent degradation of mRNA

Double-stranded RNA

5' 3' 3' 5'



Dicer

1 Double-stranded RNA is cleaved by the enzyme Dicer...

siRNAs

2 ...to produce small interfering RNAs (siRNAs).

RISC

3 The siRNAs combine with protein complex RISC...

mRNA

4 ...and pair with complementary sequences on mRNA.

Cleavage

5 The complex cleaves the mRNA.

Degradation

6 After cleavage, the RNA is degraded.

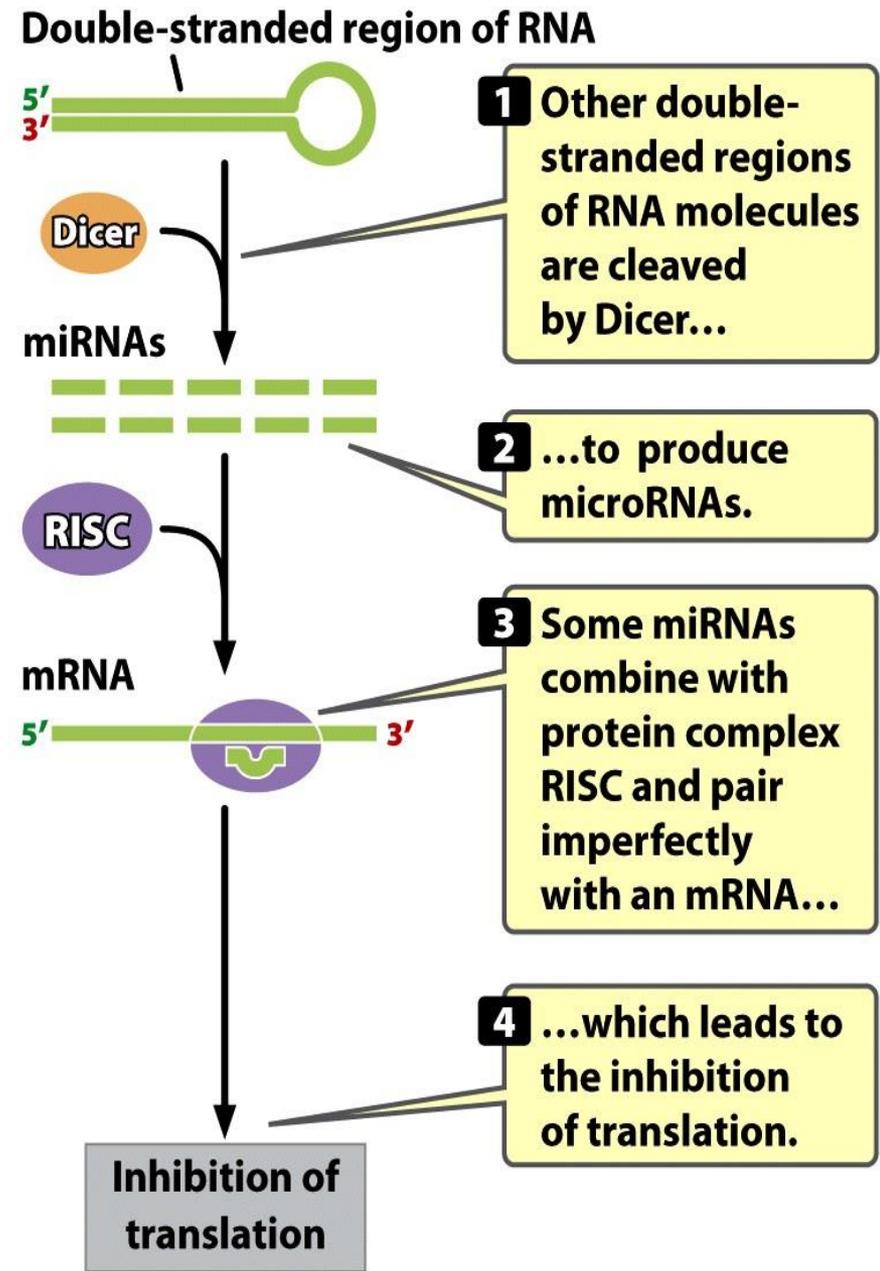
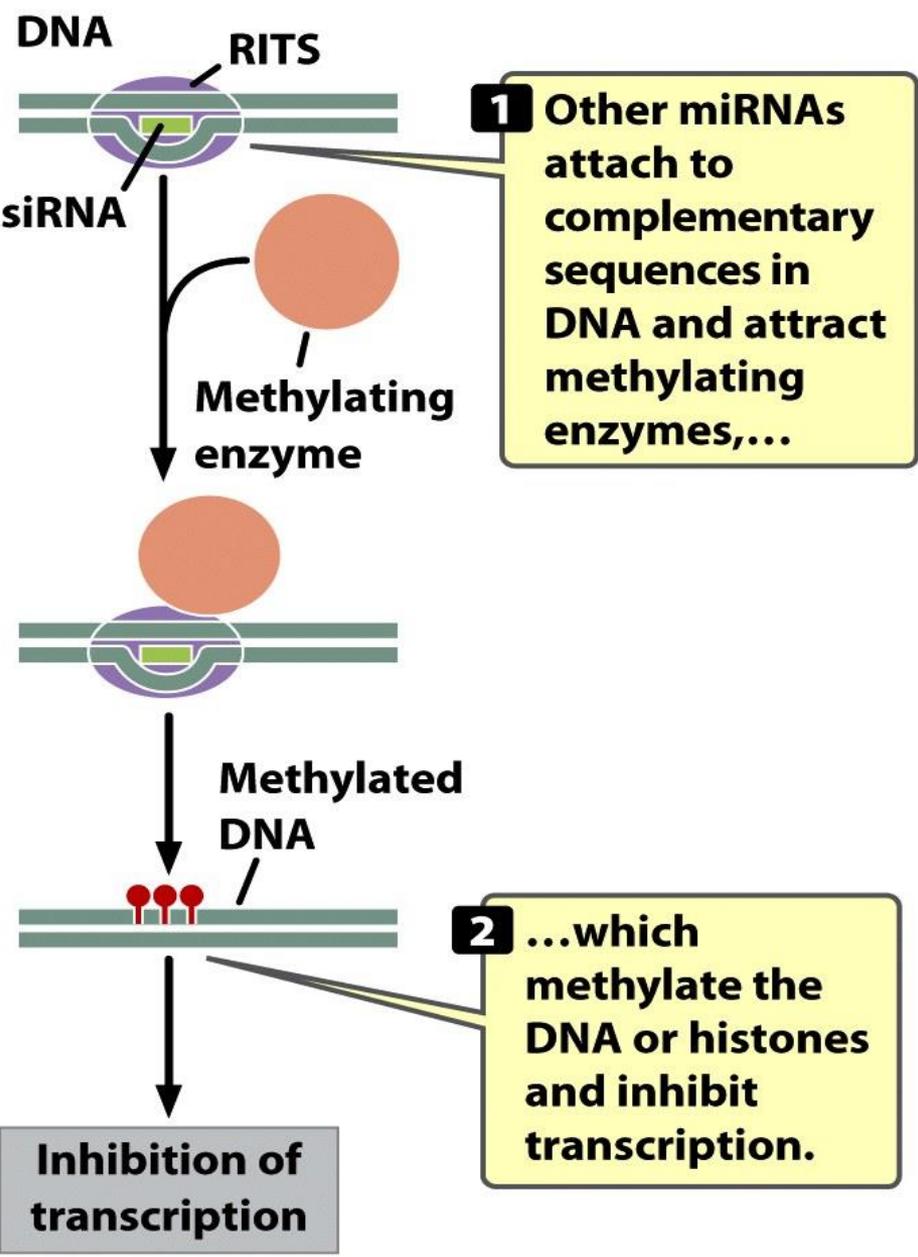


Figure 17-12c
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Figure 17-12b
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The Fate of Proteins after translation

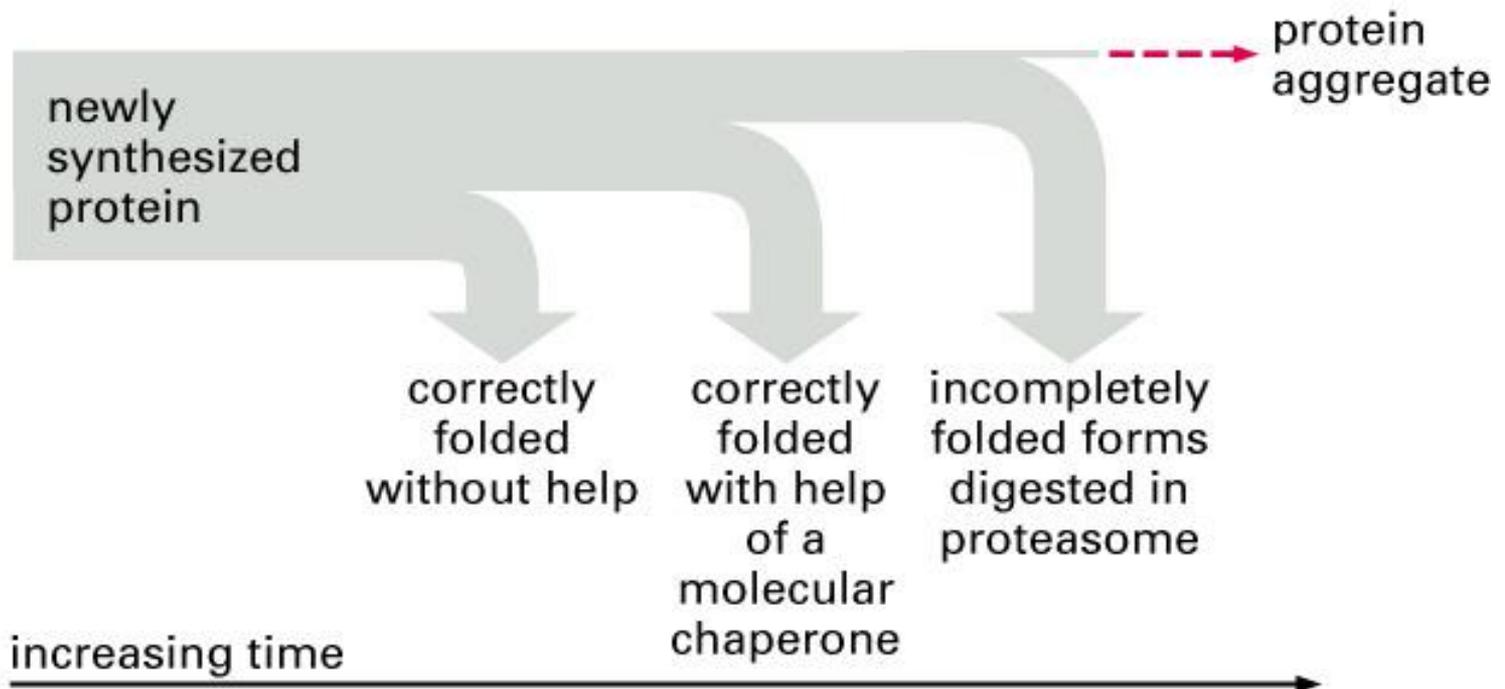
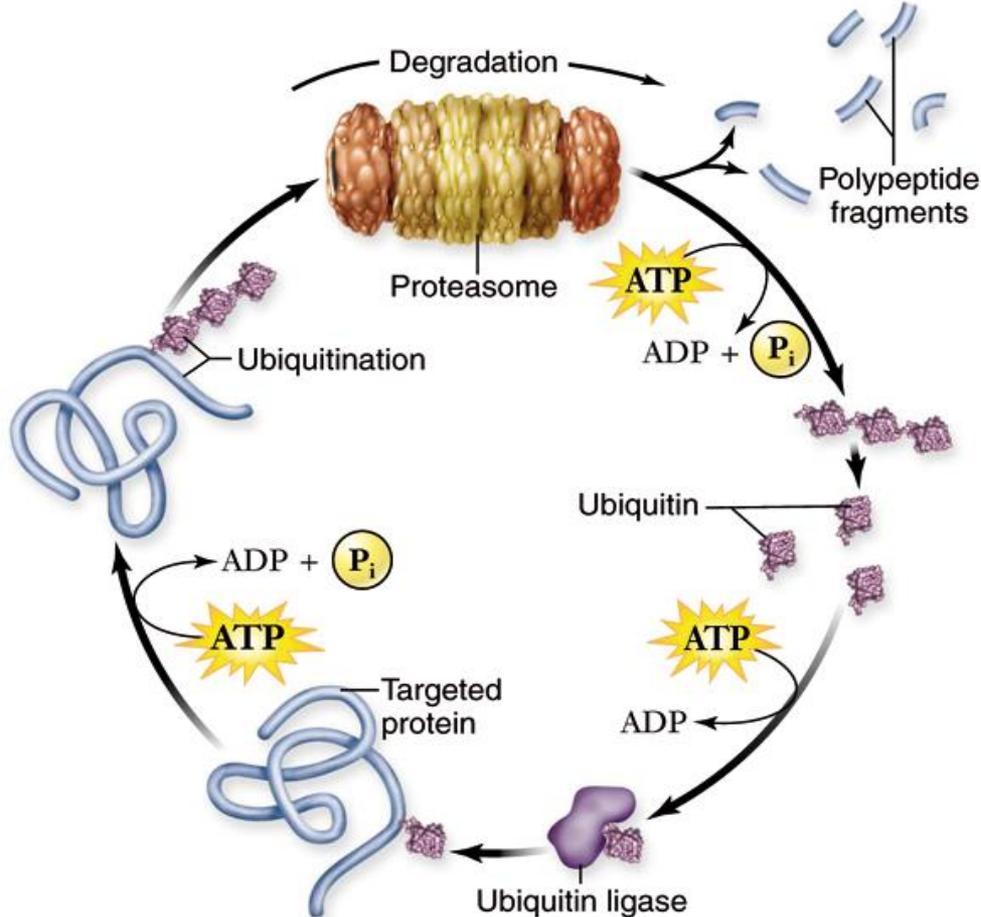


Figure 6–85. Molecular Biology of the Cell, 4th Edition.

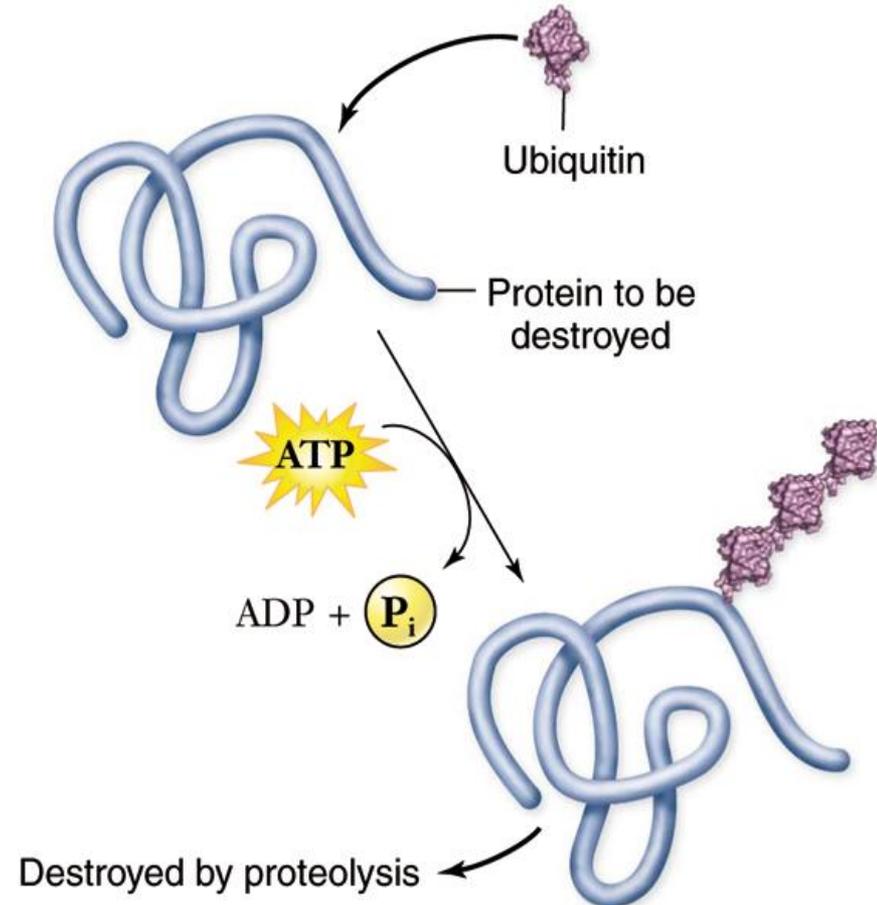
Protein Degradation

- Proteins are produced and degraded continually in the cell.
- Proteins to be degraded are tagged with **ubiquitin**.
- Degradation of proteins marked with ubiquitin occurs at the **proteasome**.

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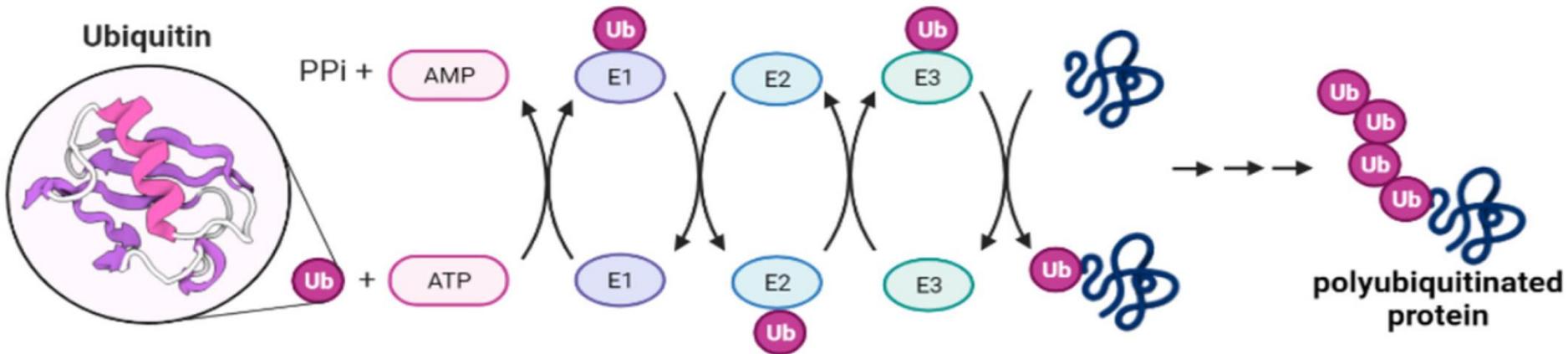
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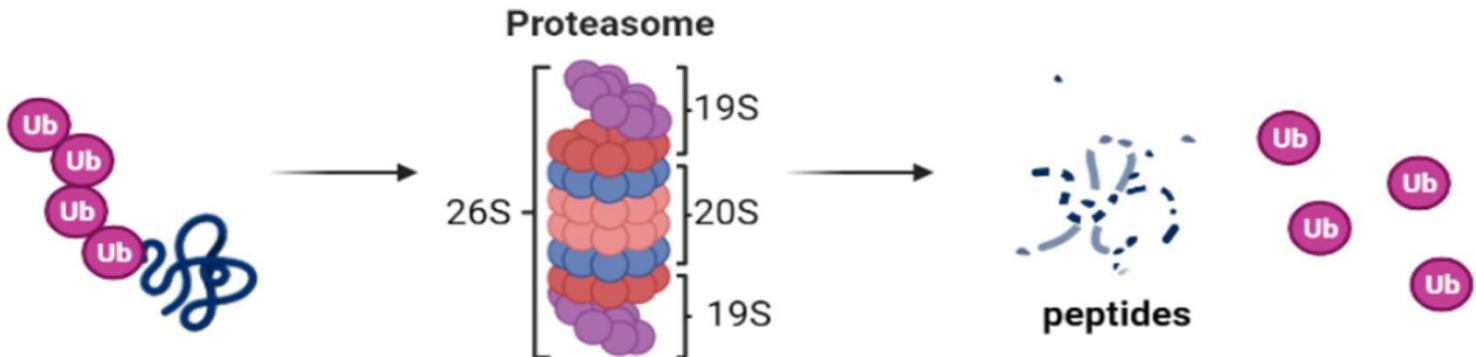
Enzymes of protein ubiquitination pathway

- Ubiquitin-activating enzyme (E1),
- Ubiquitin-conjugating enzyme (E2),
- Ubiquitin-protein ligase (E3).

a Protein Ubiquitination Pathway



b Polyubiquitinated Protein Degradation



Prokaryotic vs. Eukaryotic

- Bacterial genetics are different.
- Prokaryote genes are grouped in operons.
- Prokaryotes have one type of RNA polymerase for all types of RNA
- mRNA is not modified
- The existence of introns in prokaryotes is extremely rare.
- To initiate transcription in bacteria, sigma factors bind tRNA polymerases. RNA polymerases/ sigma factors complex can then bind to promoter prior to the gene coding region.
- In prokaryotes, the newly synthesized mRNA is polycistronic (polygenic) (code for more than one polypeptide chain).
- In prokaryotes, transcription of a gene and translation of the resulting mRNA occur simultaneously, so many polysomes are found associated with an active gene.