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

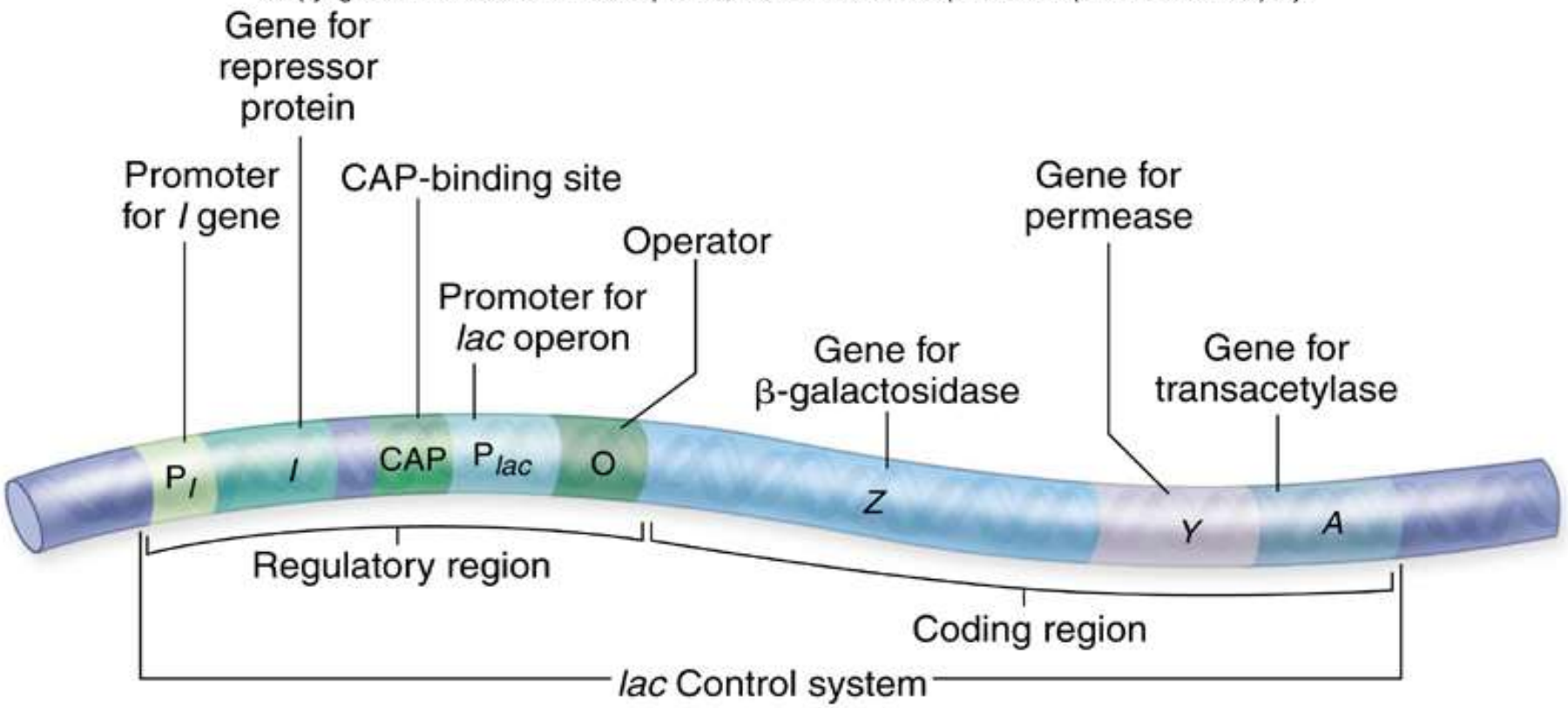
Regulation of gene expression in prokaryotes

- 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).

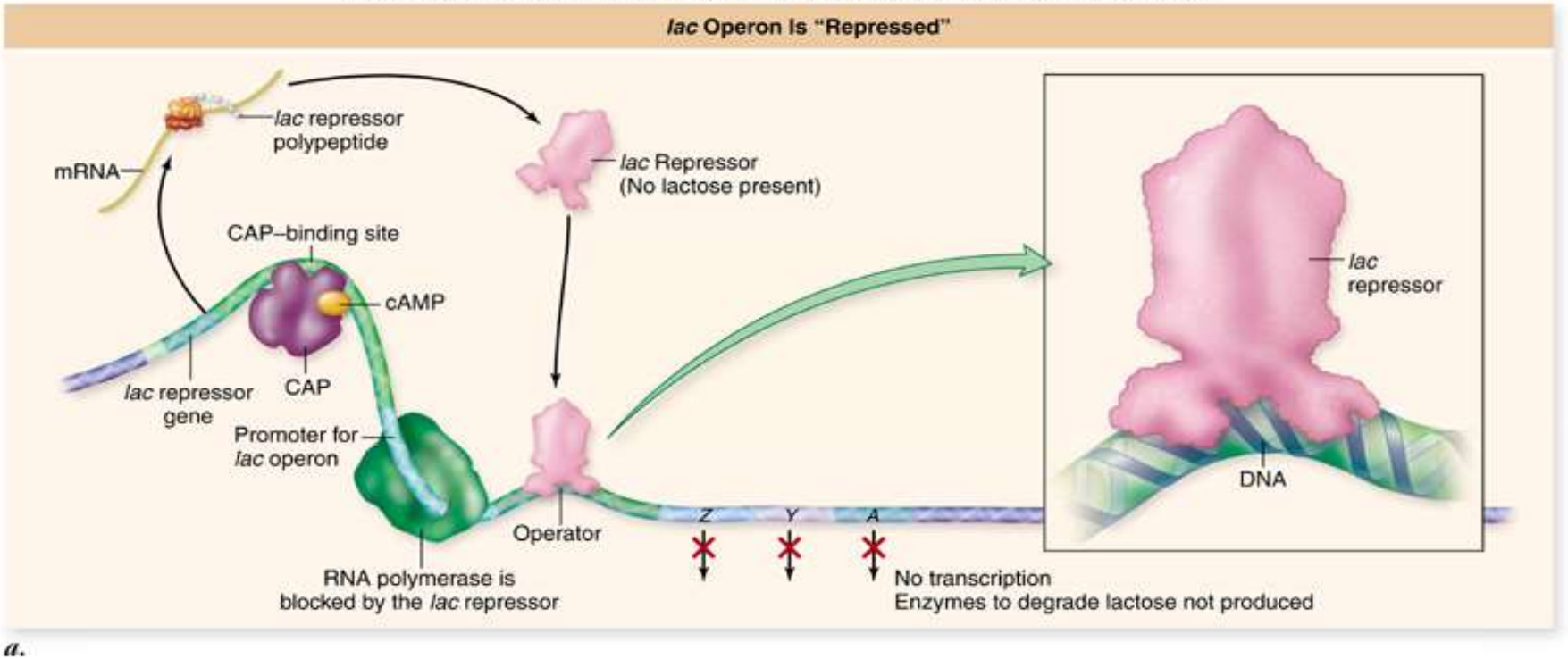
- Prokaryotic cells often respond to their environment by changes in gene expression.
- Genes involved in the same metabolic pathway are organized in **operons**.

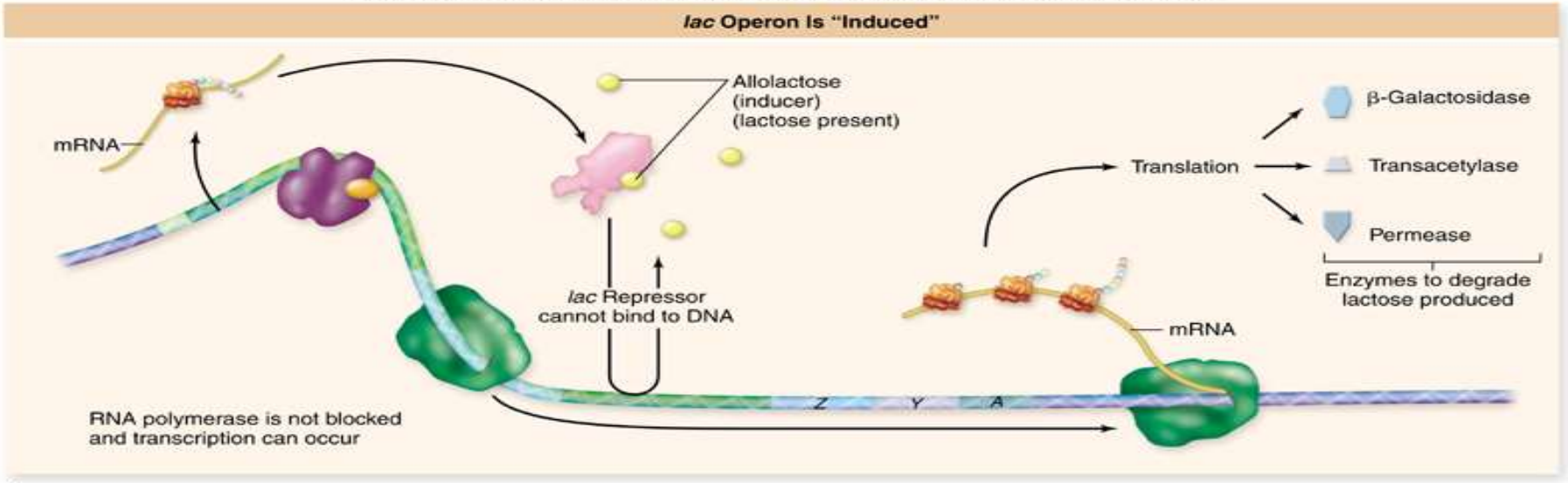
- Some operons are induced when the metabolic pathway is needed.
- Some operons are repressed when the metabolic pathway is no longer needed.

- 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



- 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





b.

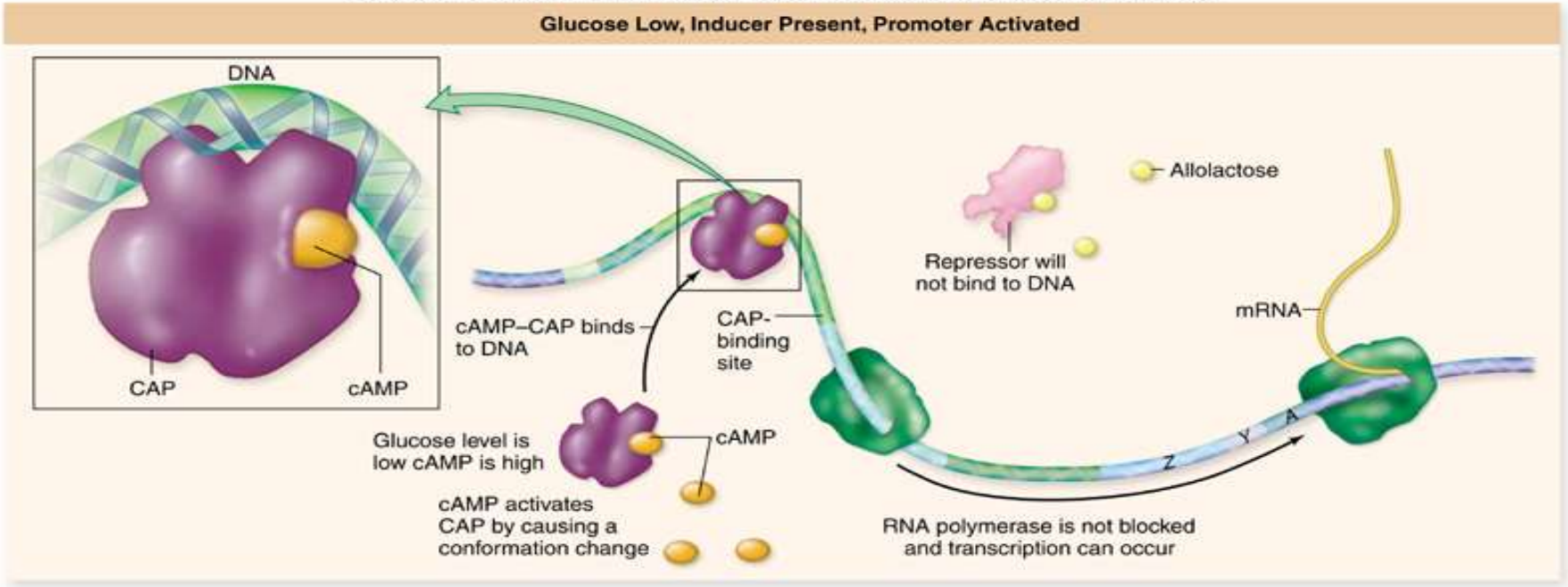
- In the presence of both **glucose** and **lactose**, bacterial cells prefer to use **glucose**.
- Glucose prevents induction of the ***lac* operon**.

  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 → low **cAMP** → **no induction**

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



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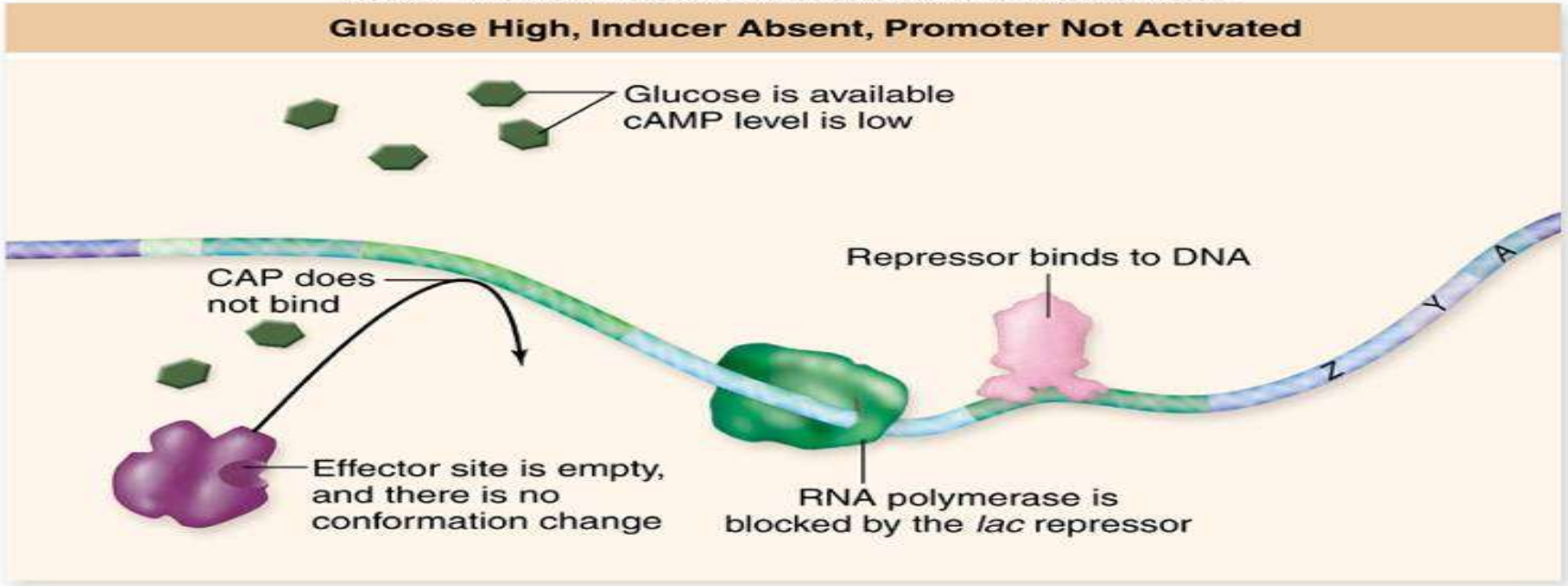


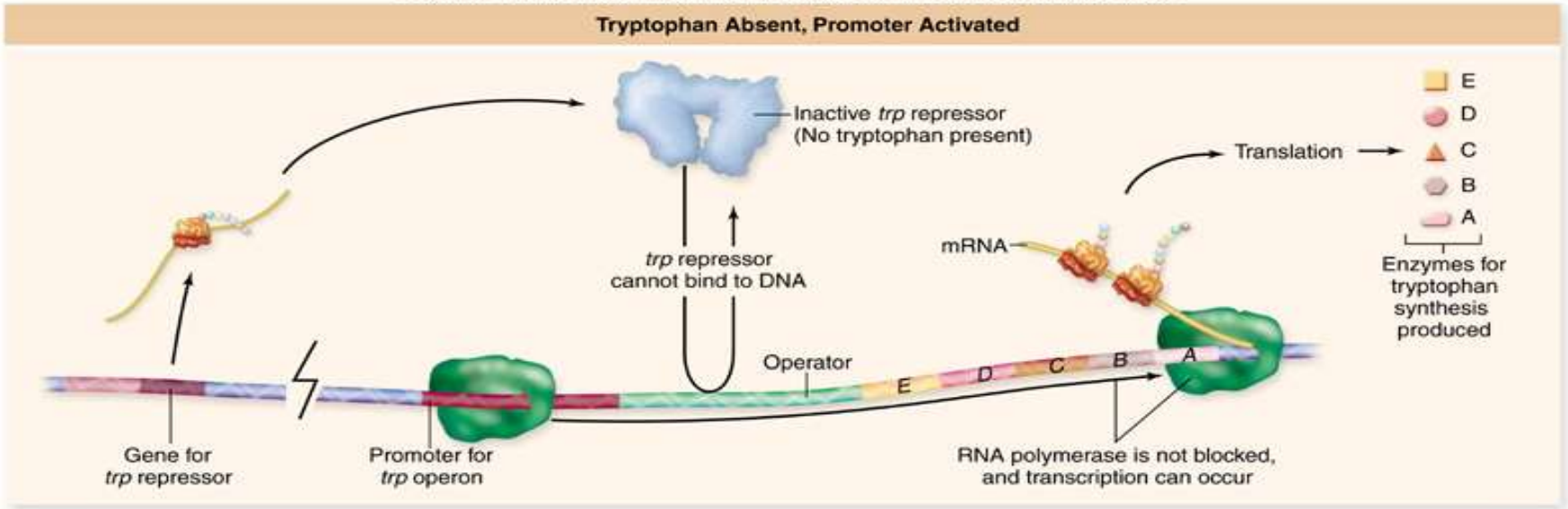
b.

- 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



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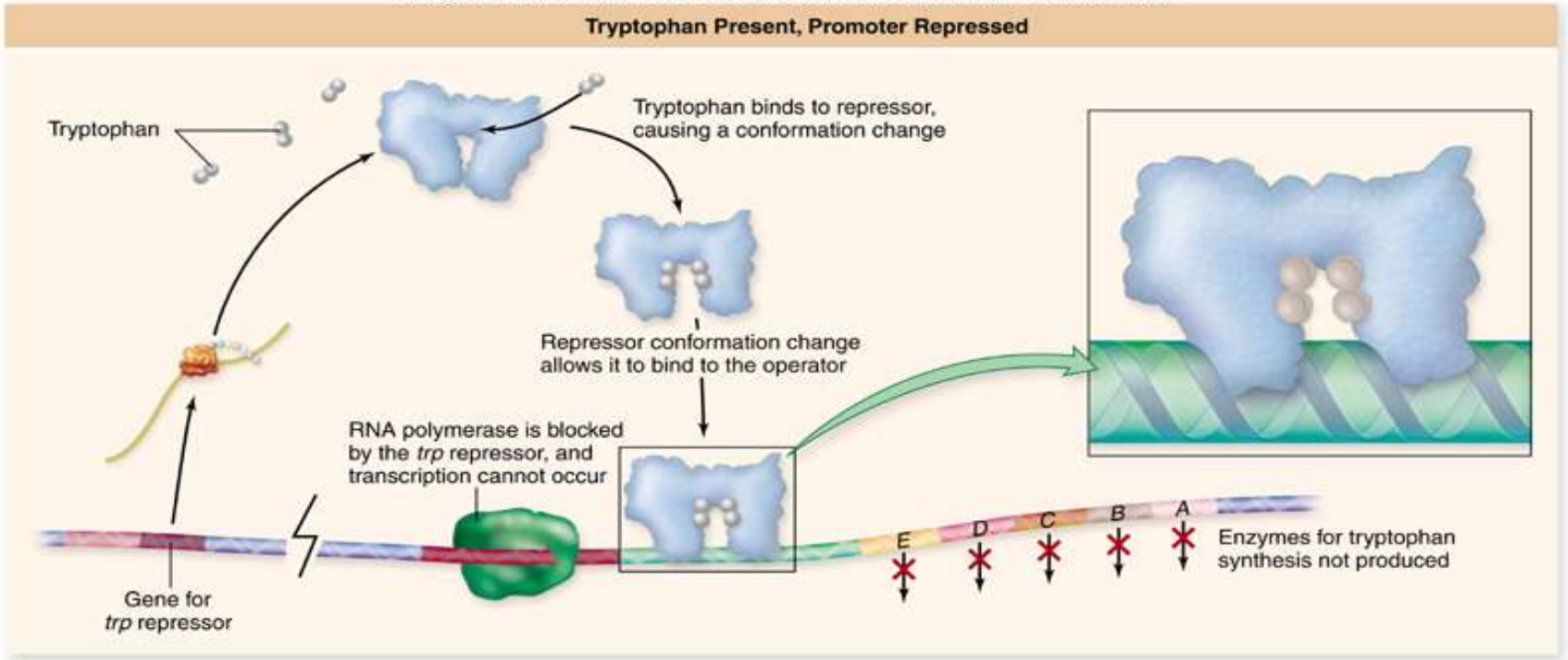


b.

Regulation of gene expression in eukaryotes

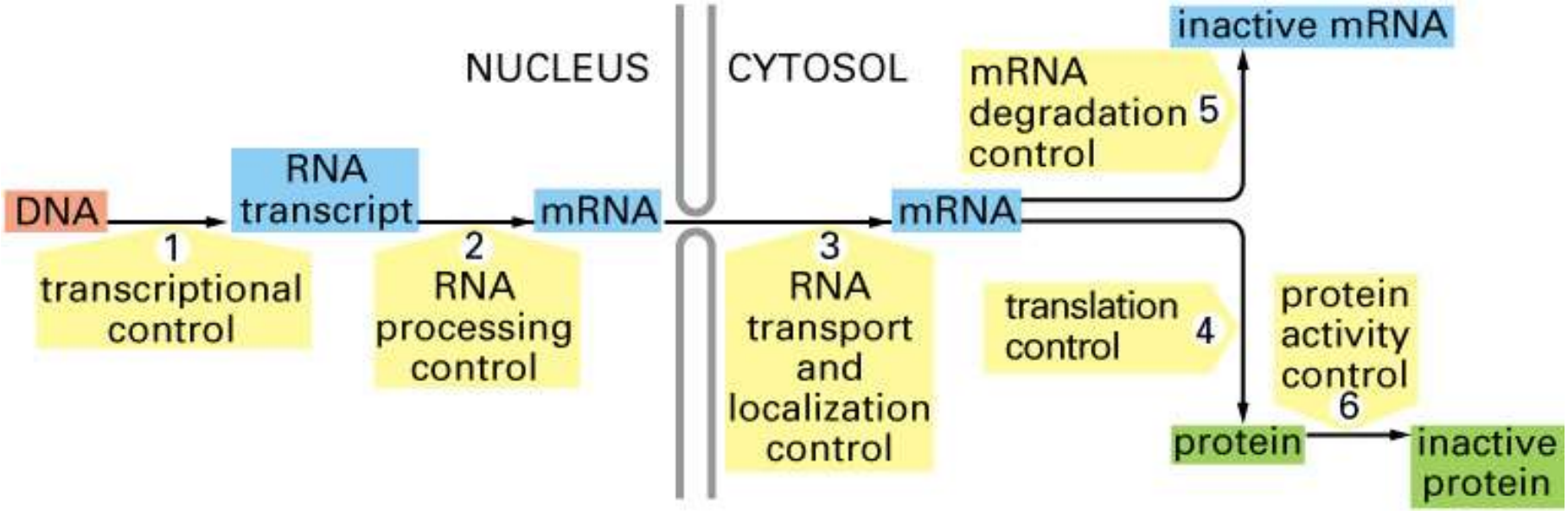
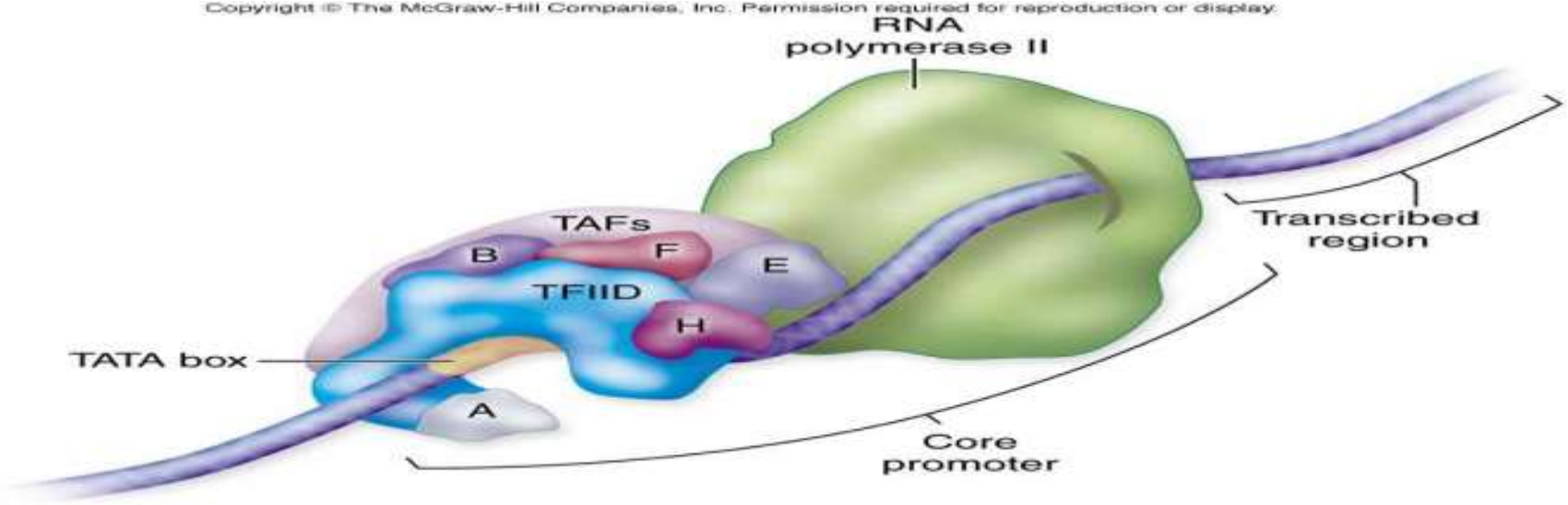


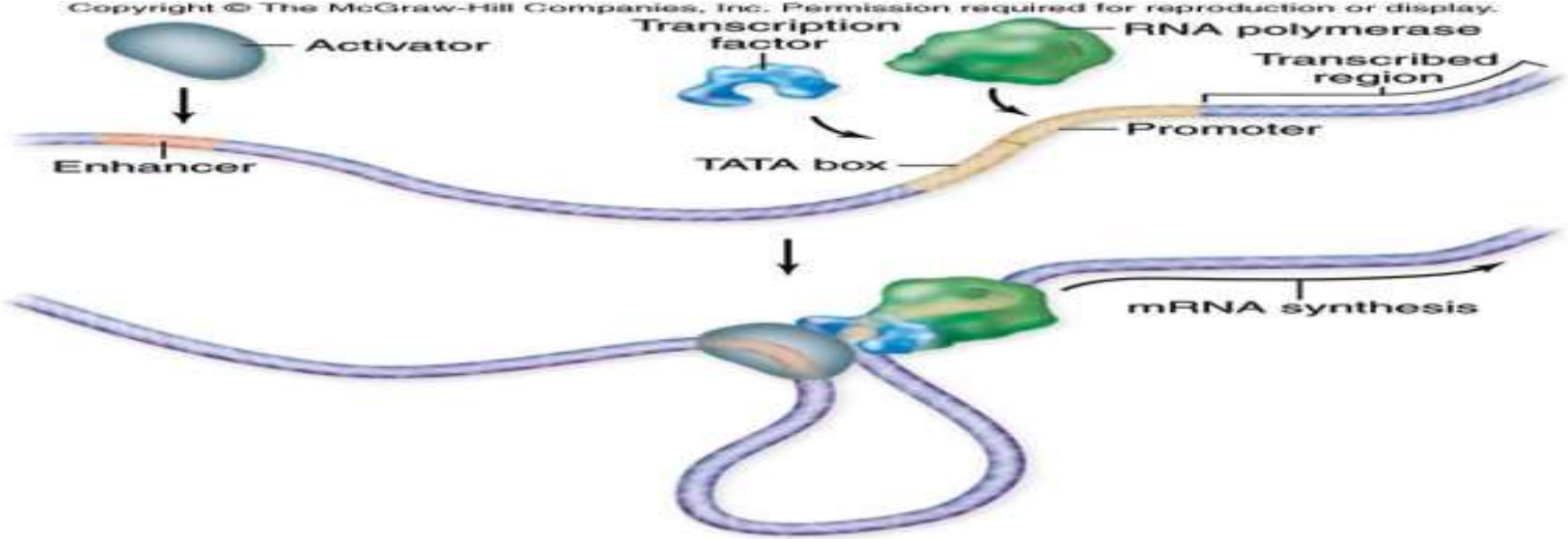
Figure 7–5. Molecular Biology of the Cell, 4th Edition.

- 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

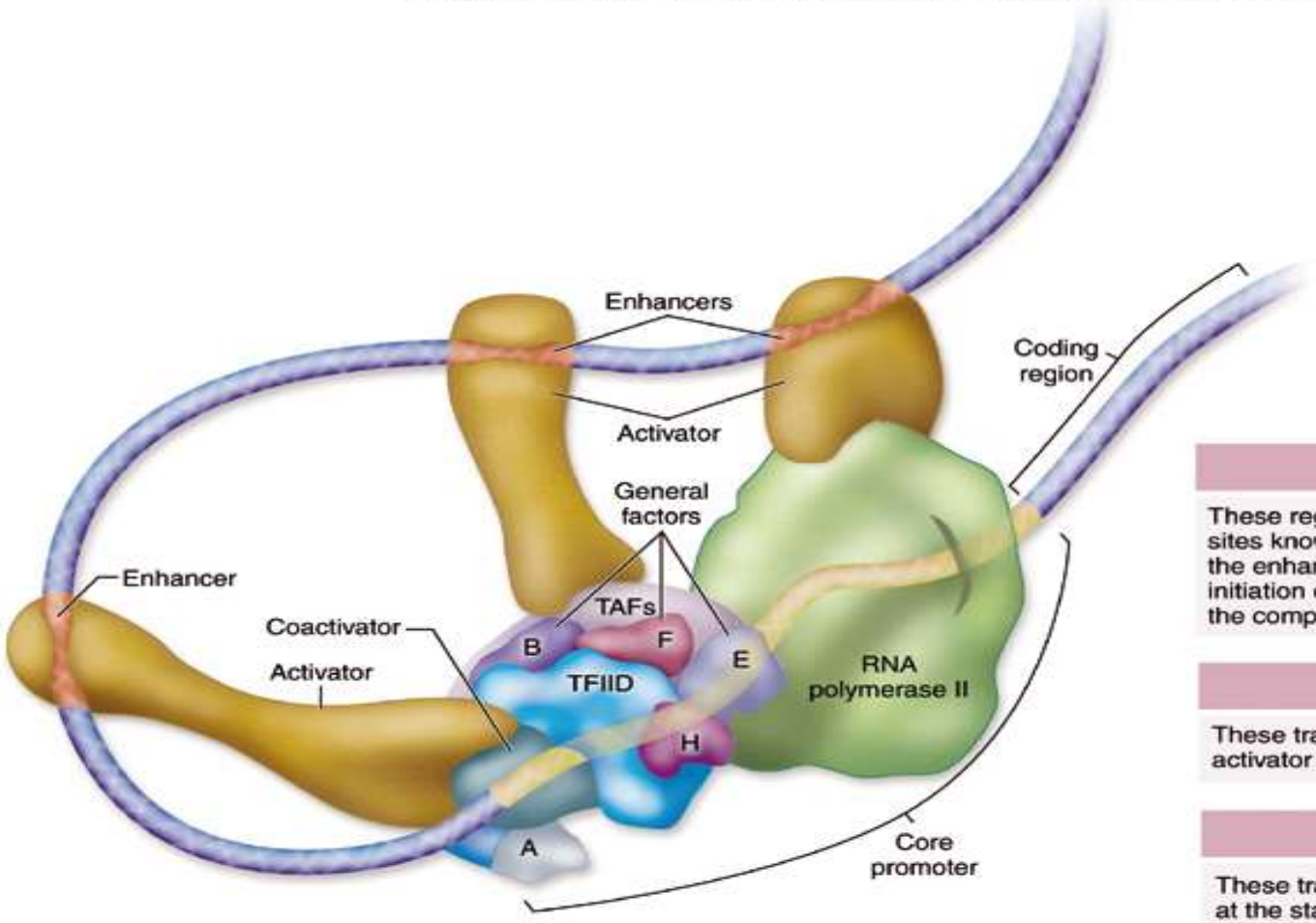
- General transcription factors bind to the promoter region of the gene.
- RNA polymerase II then binds to the promoter to begin transcription at the start site (+1).

- Enhancers are DNA sequences to which specific transcription factors (activators) bind to increase the rate of transcription.





- **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



Activators

These regulatory proteins bind to DNA at distant sites known as enhancers. When DNA folds so that the enhancer is brought into proximity with the initiation complex, the activator proteins interact with the complex to increase the rate of transcription.

Coactivators

These transcription factors transmit signals from activator proteins to the general factors.

General Factors

These transcription factors position RNA polymerase at the start of a protein-coding sequence and then release the polymerase to initiate transcription.

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

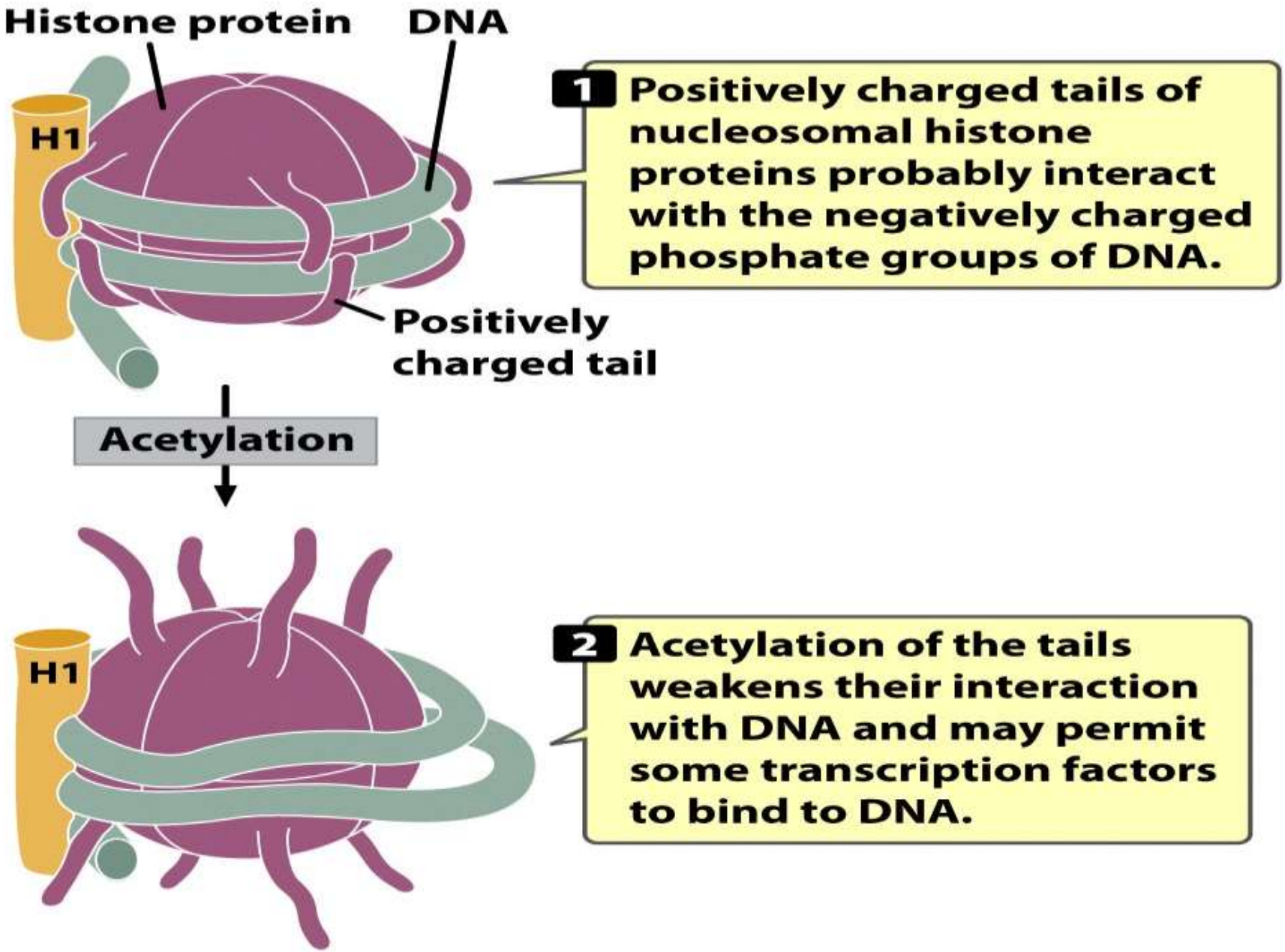


Figure 17-2
Genetics: A Conceptual Approach, Third Edition

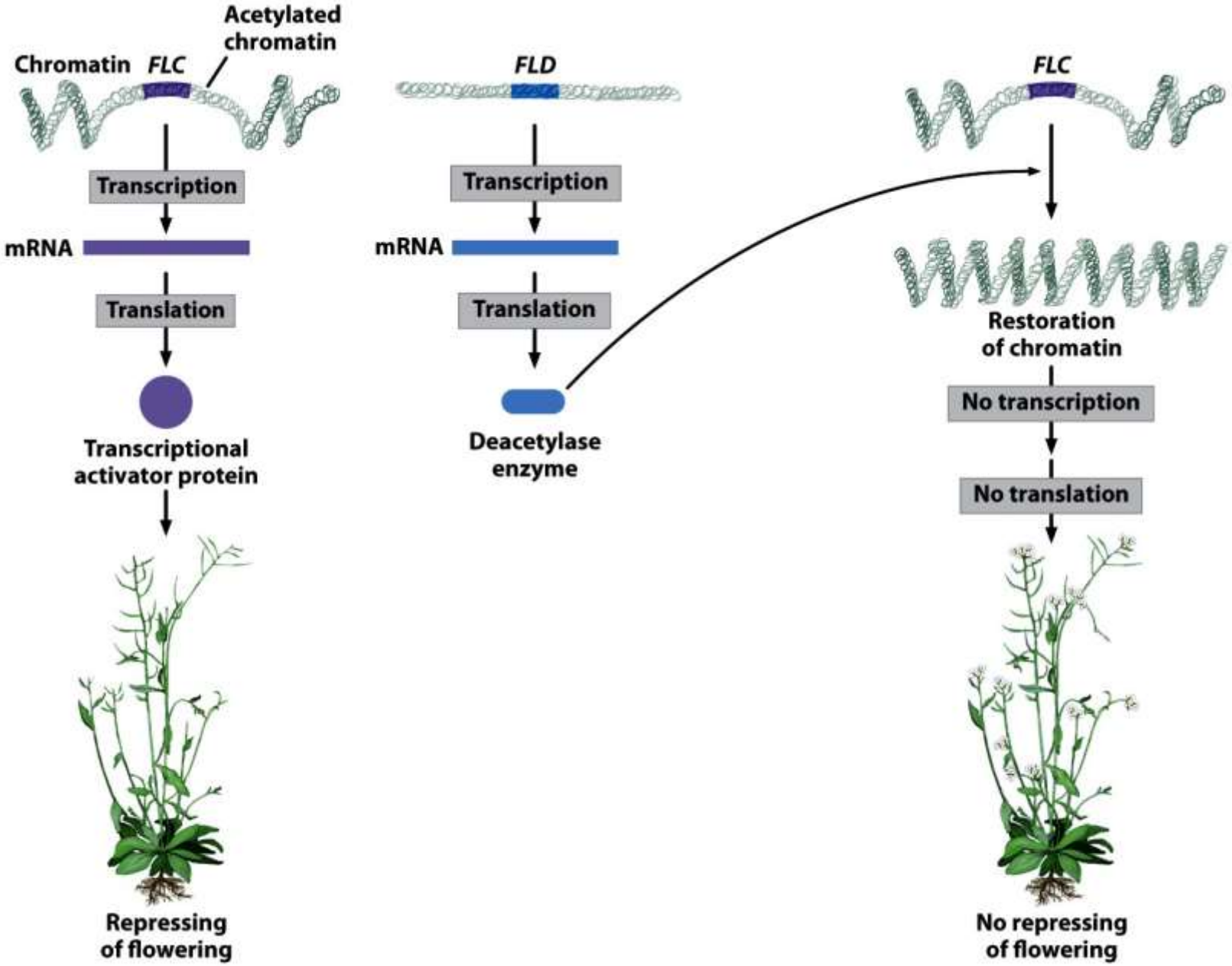


Figure 17-3
Genetics: A Conceptual Approach, Third Edition

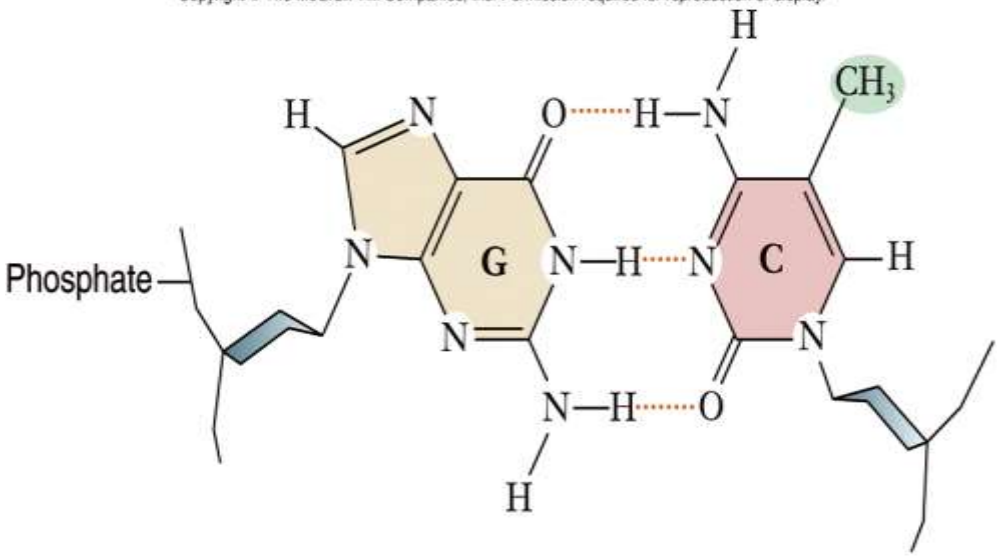
Methylation

- 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- <u>RNA editing</u> 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.

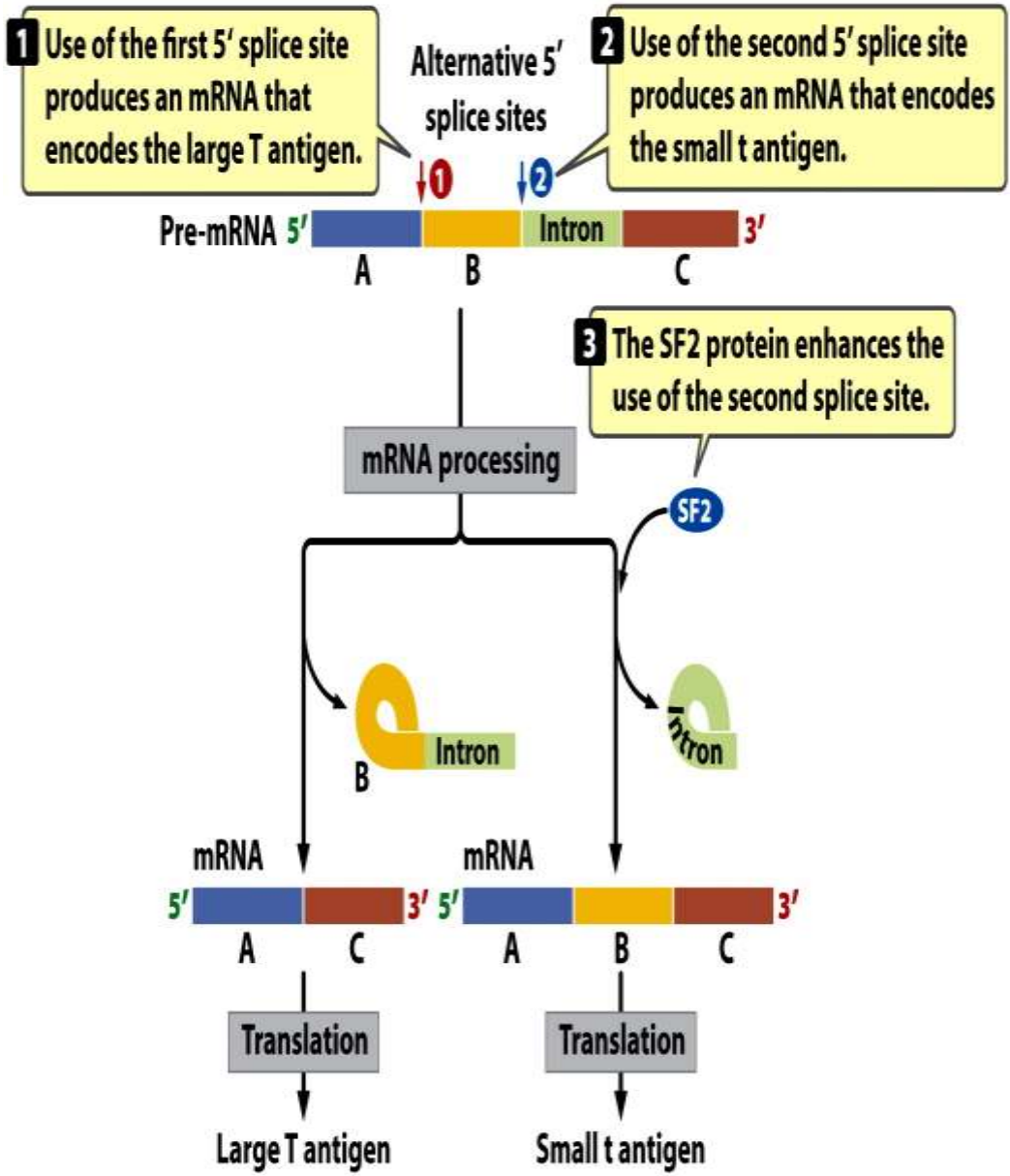


Figure 17-9
Genetics: A Conceptual Approach, Third Edition

- 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

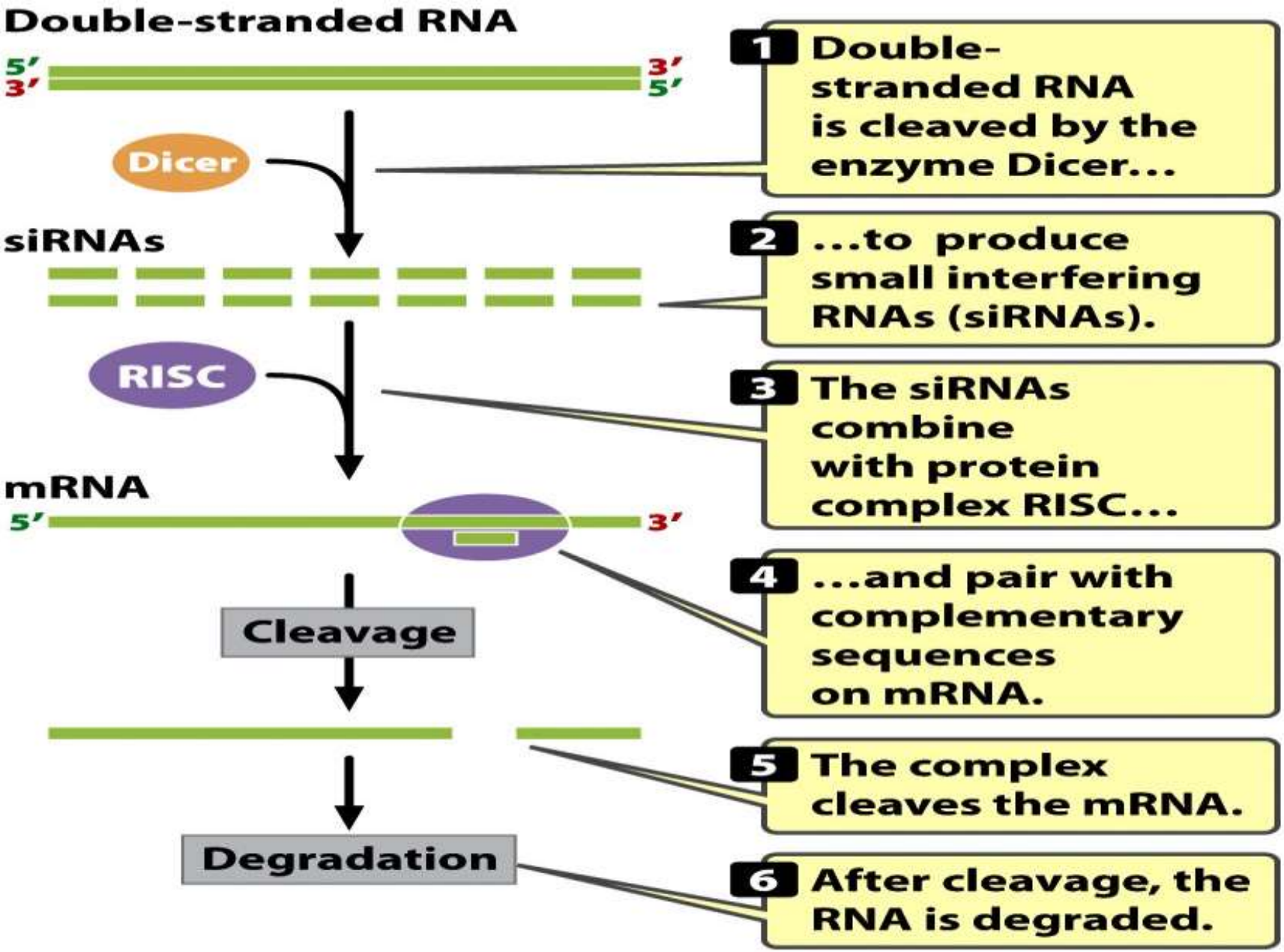


Figure 17-12a
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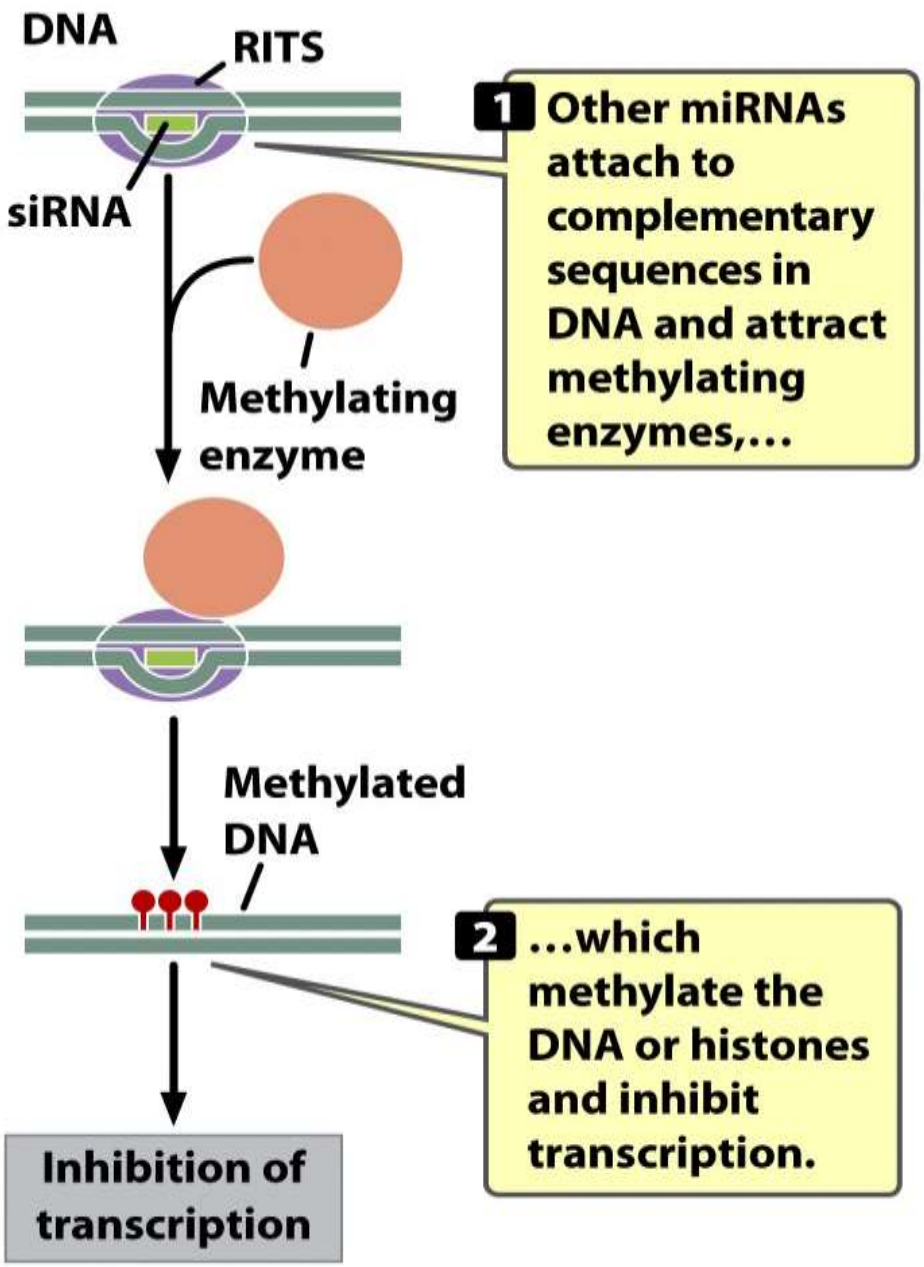

Figure 17-12c
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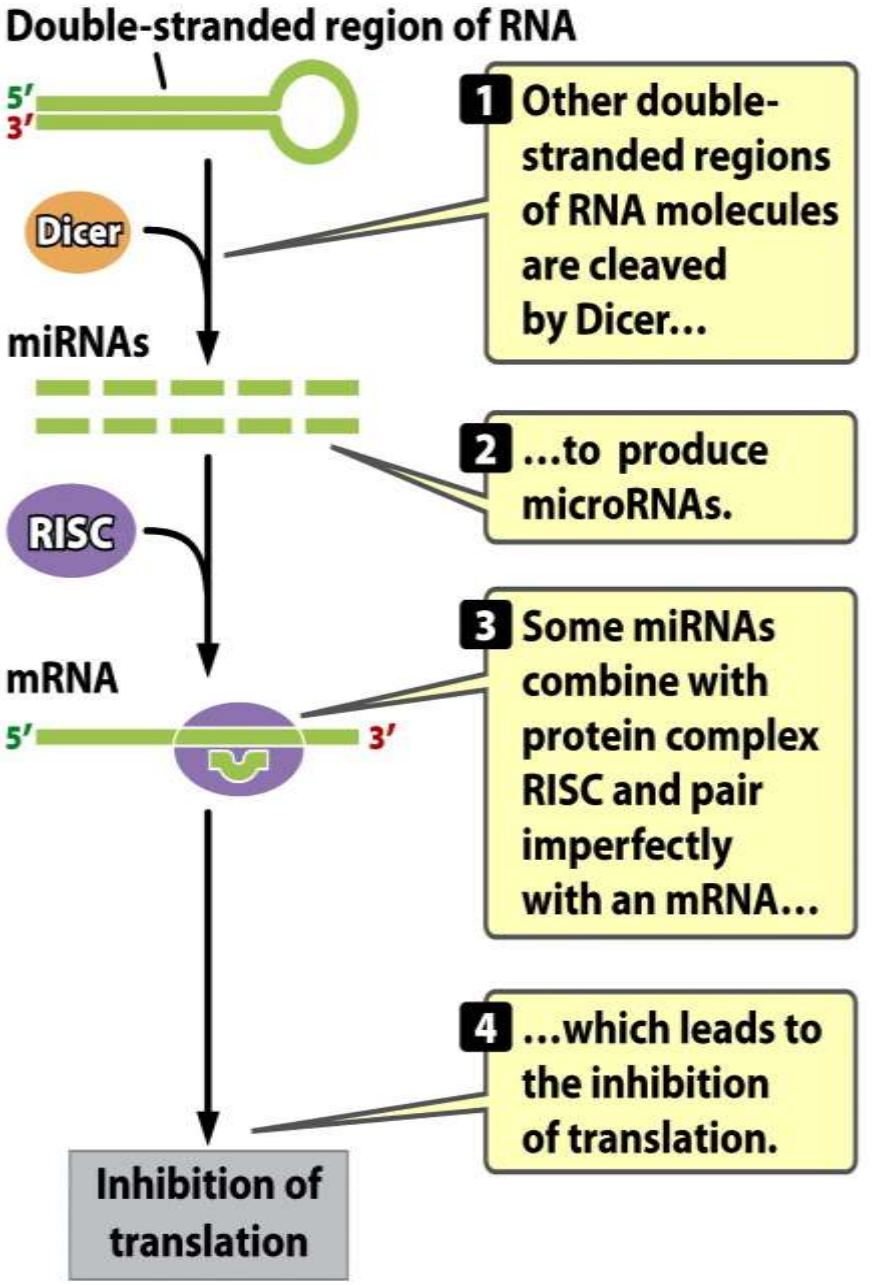

Figure 17-12b
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The Fate of Proteins after translation

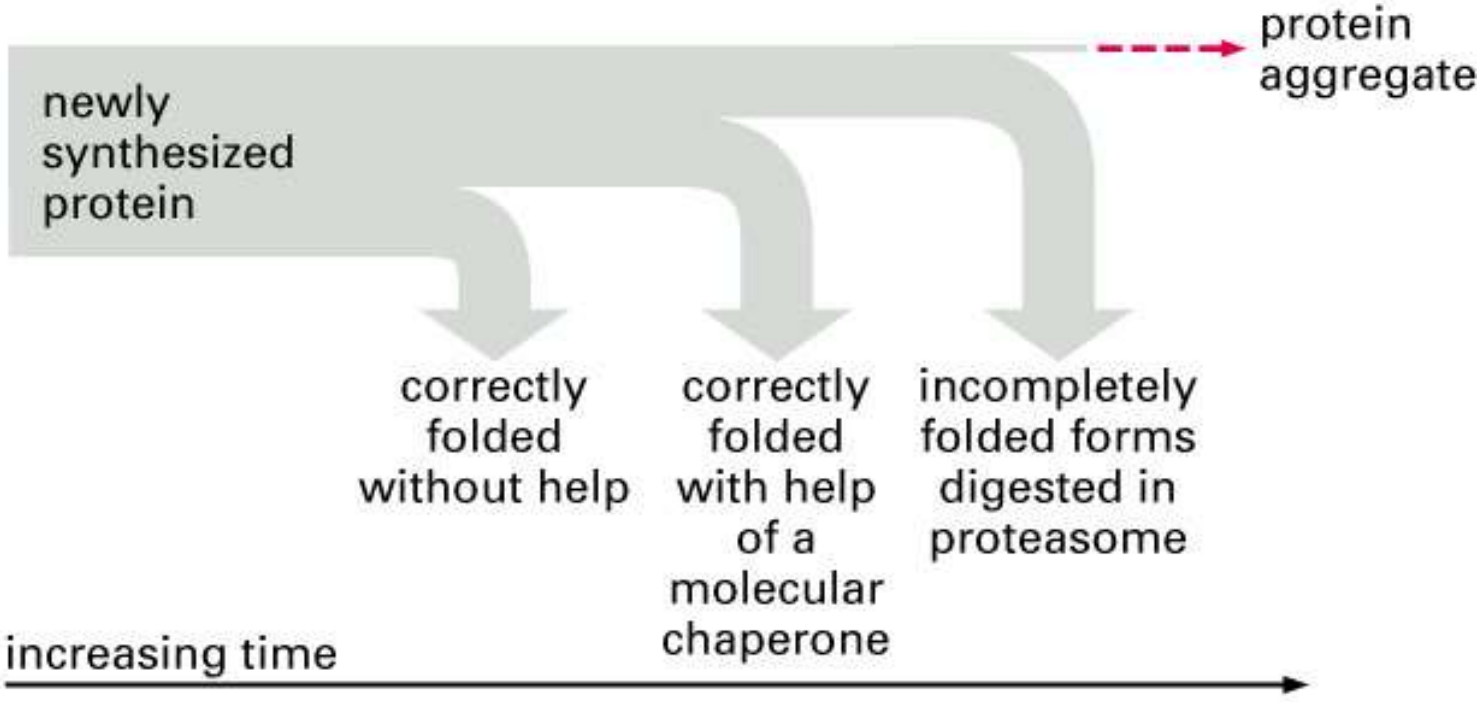
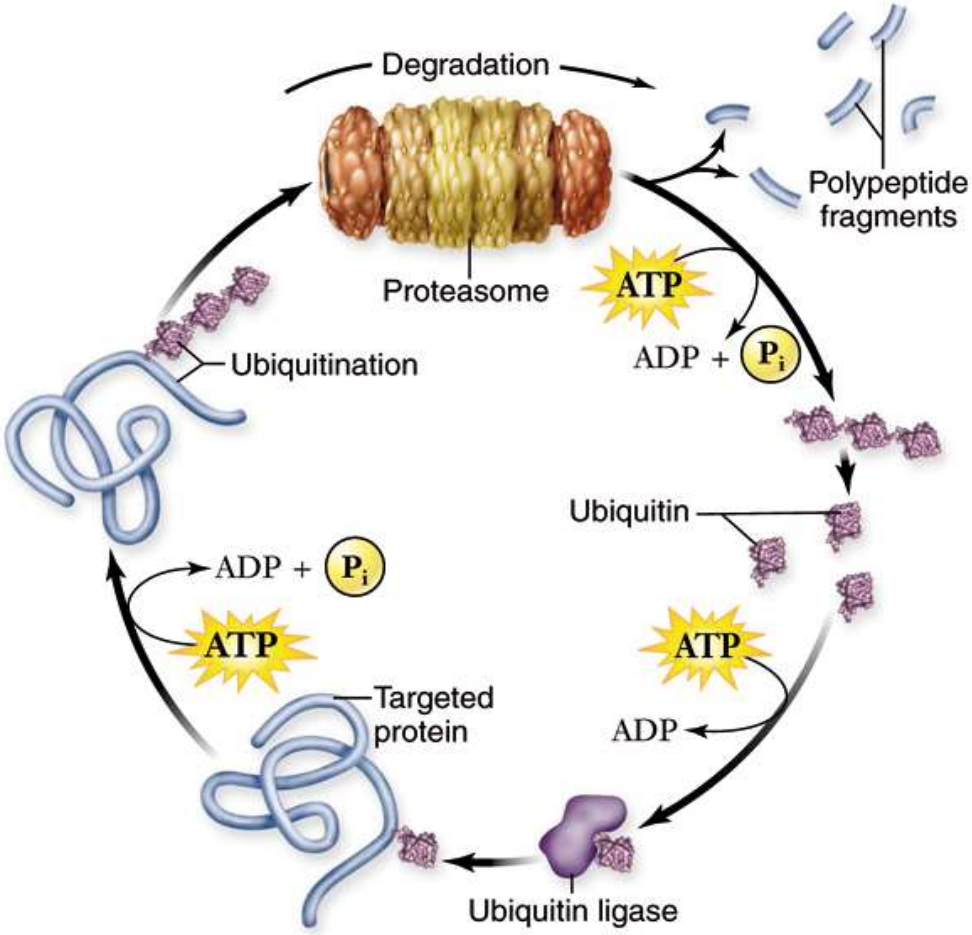
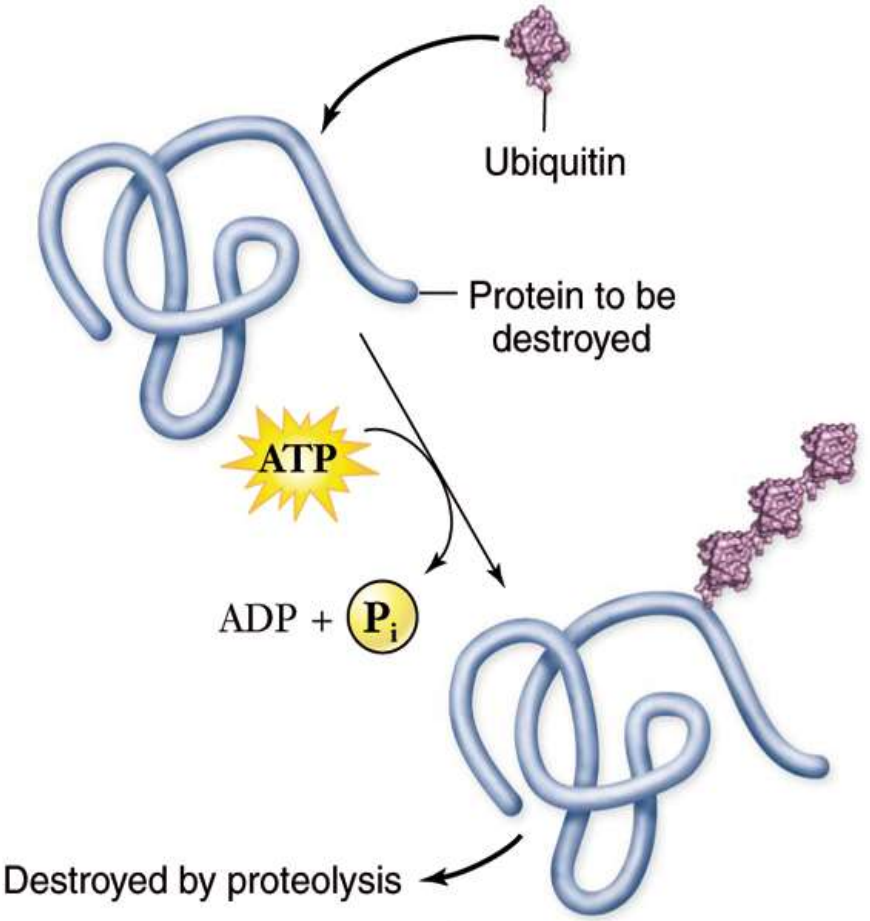


Figure 6–85. Molecular Biology of the Cell, 4th Edition.

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**.





E1: ubiquitin activating enzyme; E2/3: ubiquitin ligase

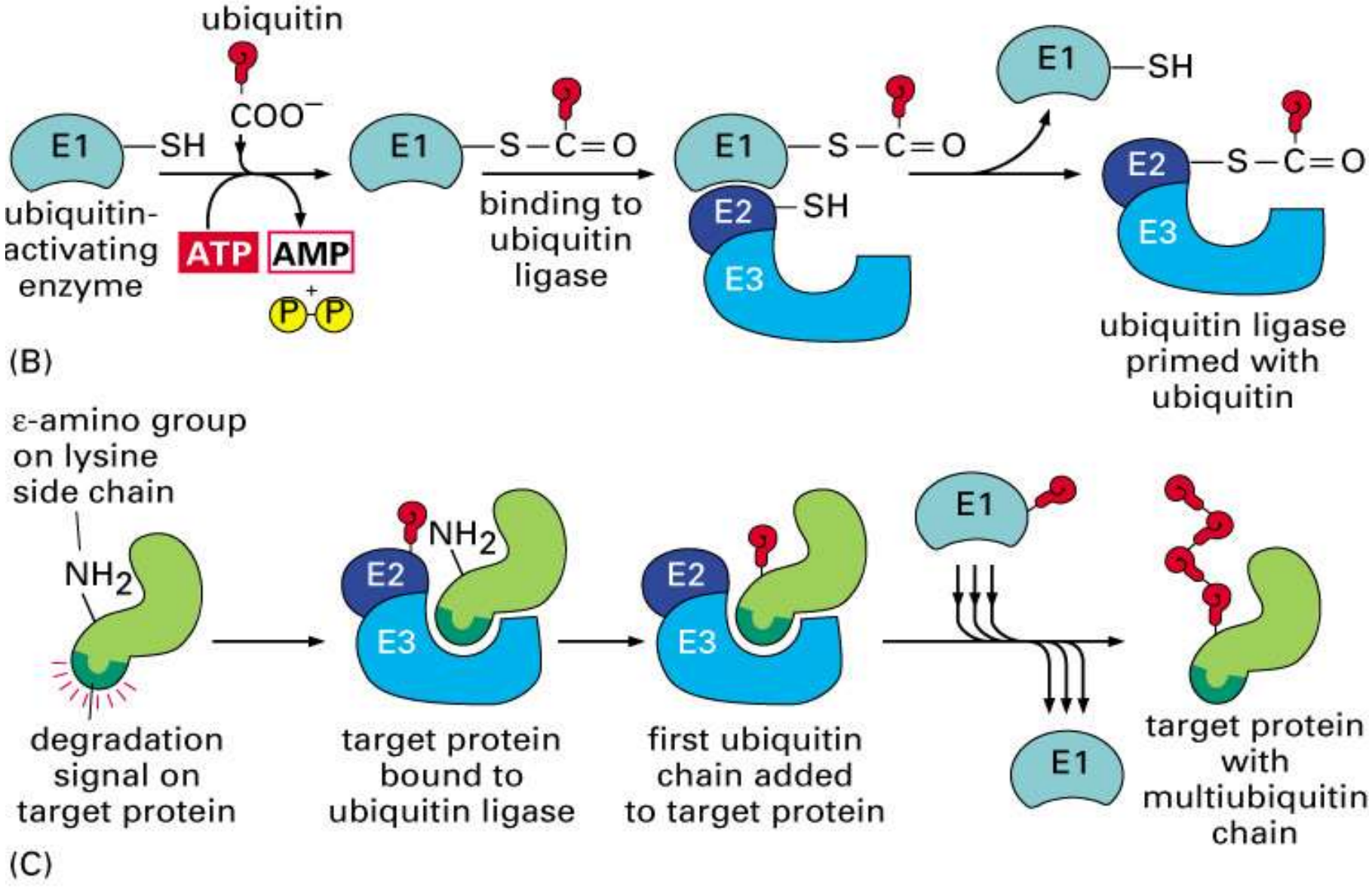


Figure 6–87 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

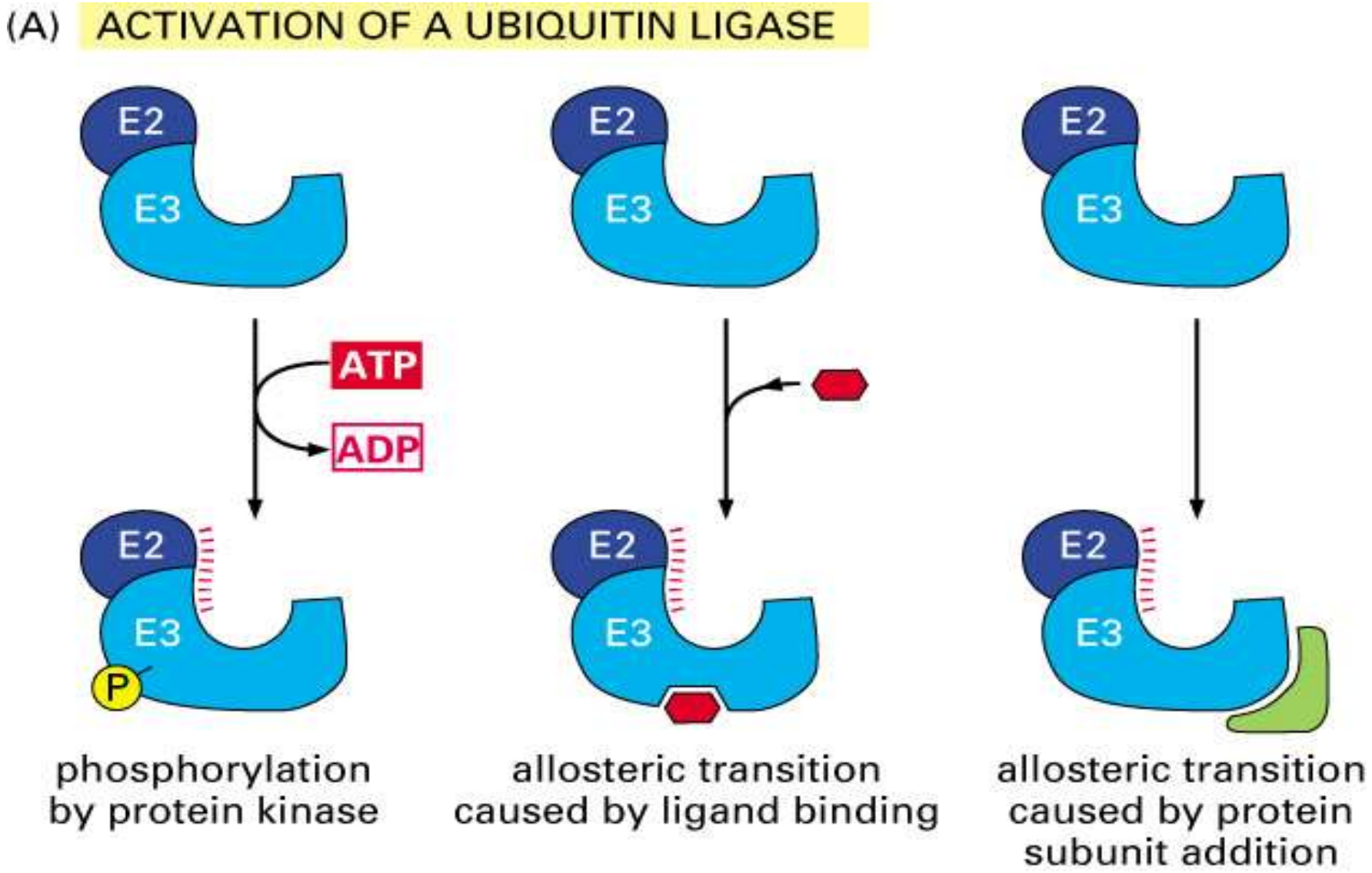


Figure 6–88 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

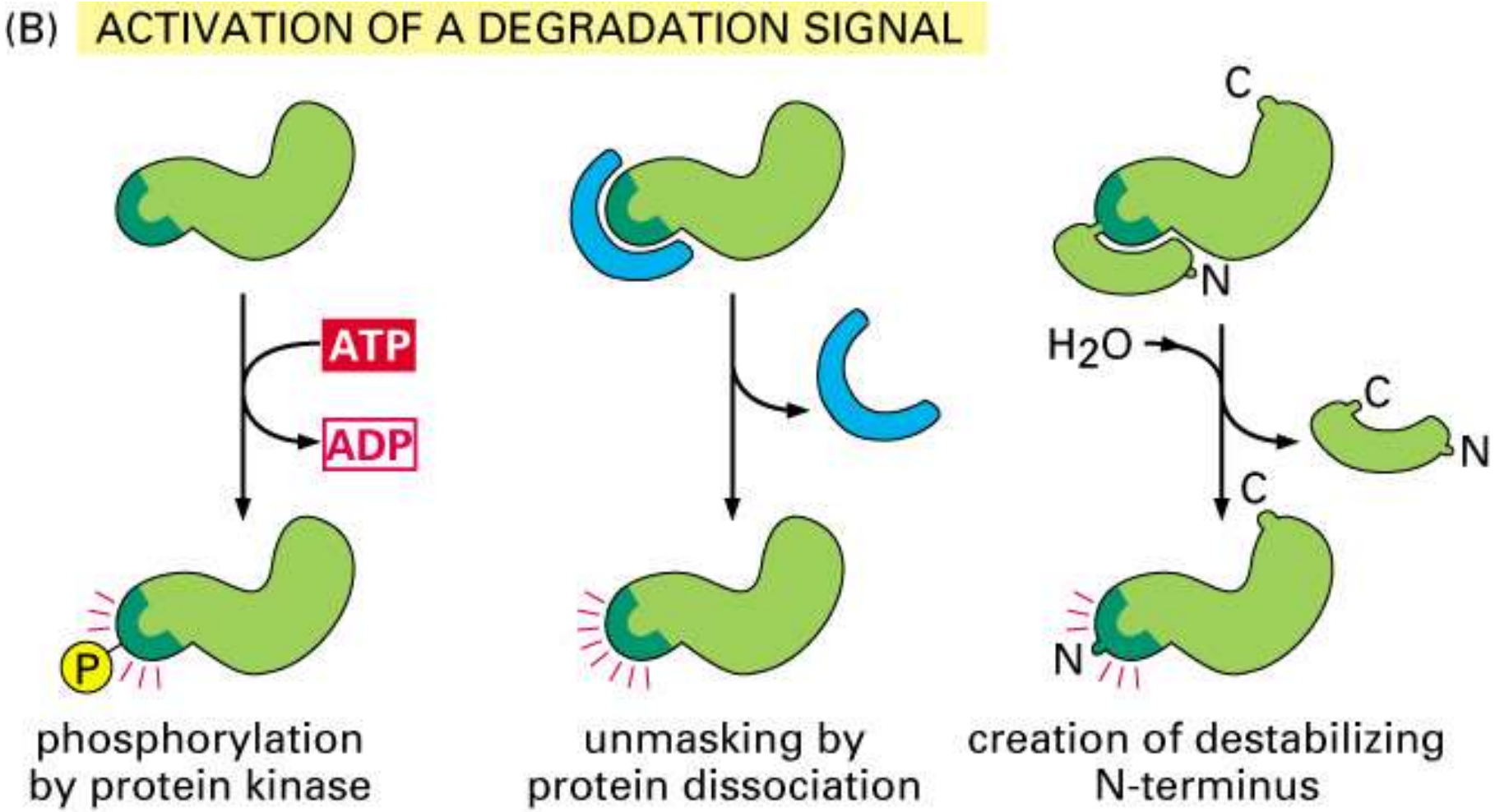


Figure 6–88 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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.