

يا رب يا مسهل

Gene Expression Regulation

The Enhancers or Silencers: non promoter DNA sequence which bind to transcription factor

to activate or to inhibit

G.E.R
two form

- positive ⇒ Stimulation, increase rate (enhancers, activator) ماتوب
- Negative ⇒ repressor, blocking, reduction (inhibition) دلتة

Regulatory protein → bind to the either major or minor groove.

- 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

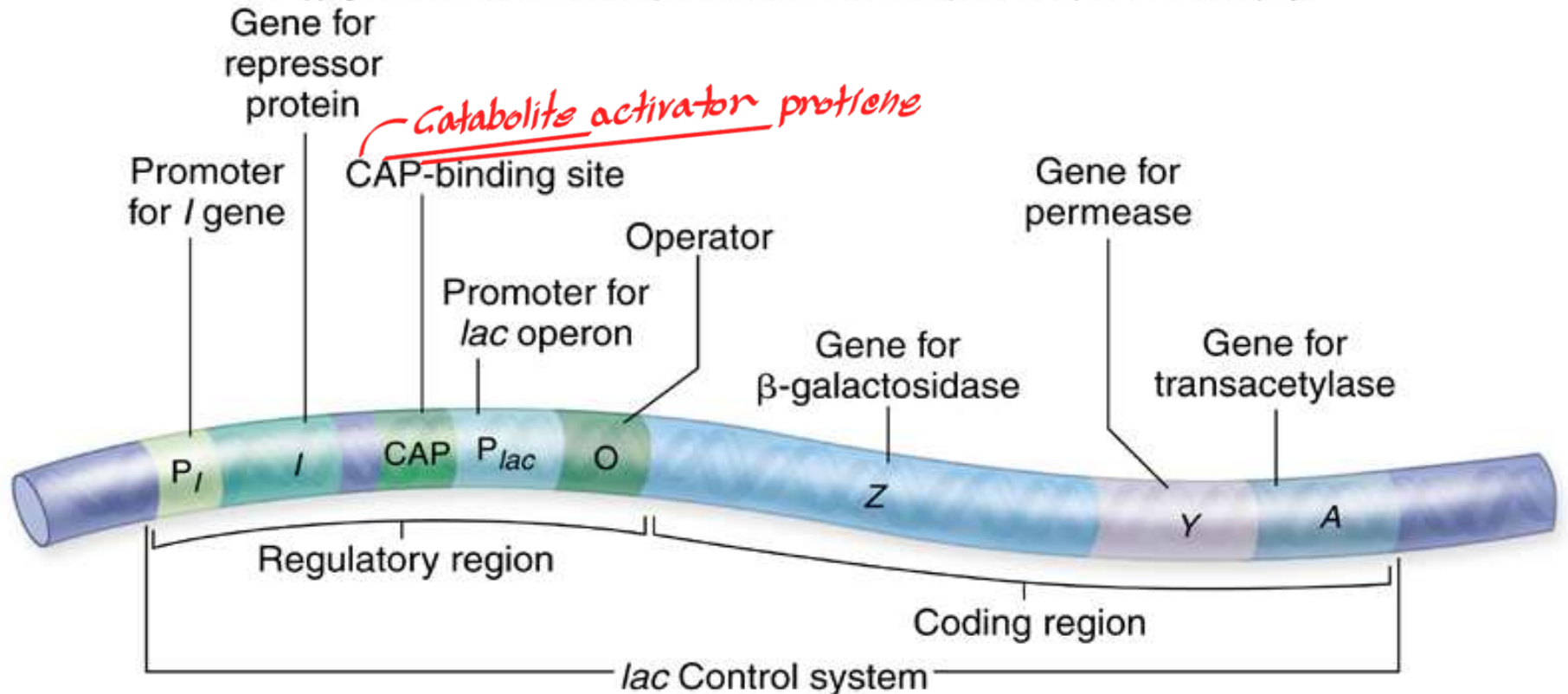
- Control of transcription initiation can be:
- Positive control – increases transcription when activators bind to DNA
- Negative control – reduces transcription when repressors bind to DNA regulatory regions (operators).

- 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) *3 gene \approx 3 Enzyme*
 - 1- **lac Z**: encodes β -galactosidase which cleaves lactose into glucose and galactose *\Rightarrow cleave glycosidic bond*
 - 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

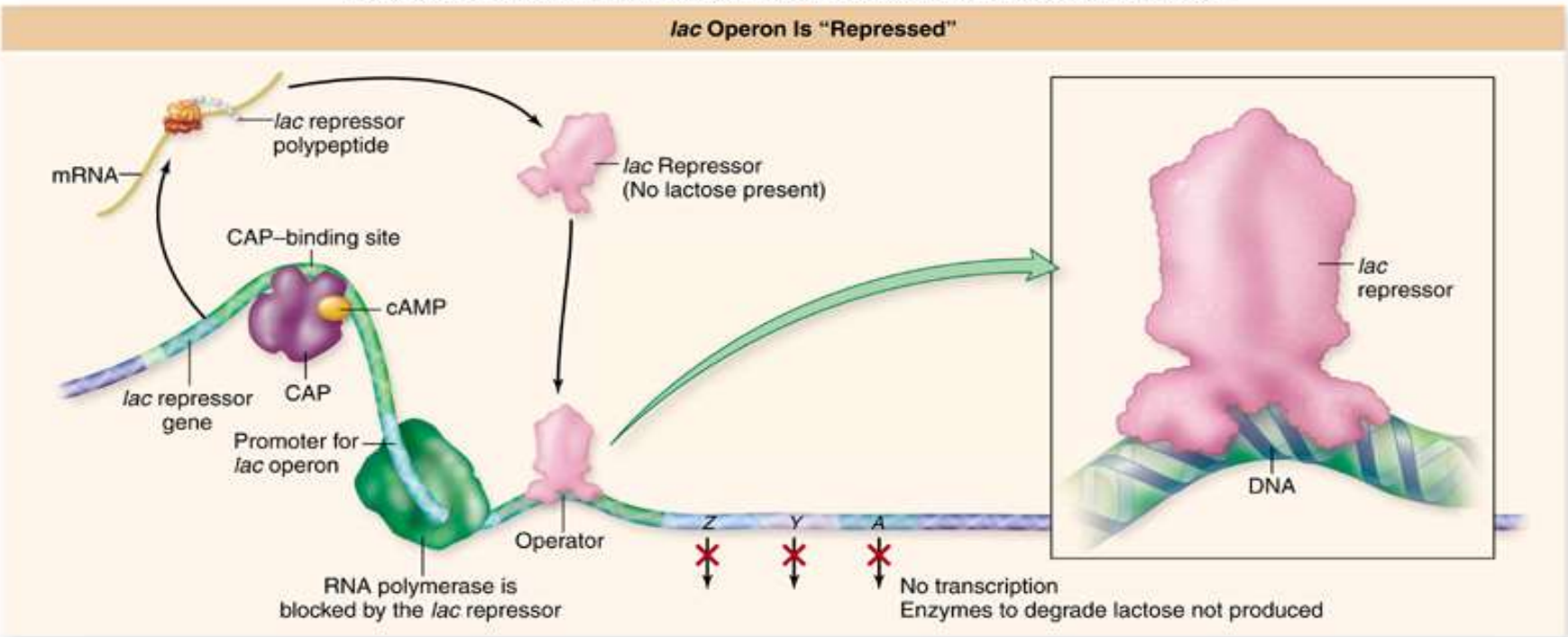
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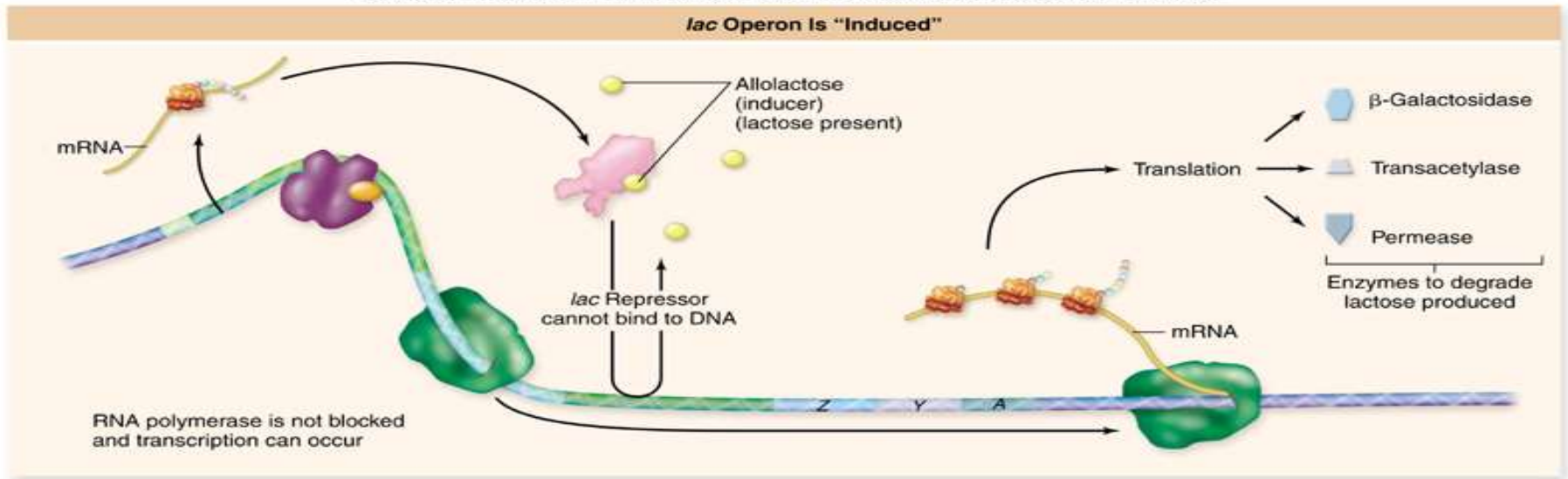


- Regulatory regions of the operon include the CAP (Catabolite activator protein) binding site, promoter, and the operator. *الجزيء من RNA Polymerase*
- The *lac* operon is **negatively regulated** by a repressor protein:
 - *lac* repressor binds to the operator to block transcription *إلي يتعرف على الـ operator*
 - In the presence of lactose, an inducer molecule binds to the repressor protein *علشانه بيخلص*
 - Repressor can no longer bind to operator *النسخ هو Sigma factor*
 - 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**.

إيمنع ال repressor من إنها تتركب بال operator
 لأنها بعد conformational changing

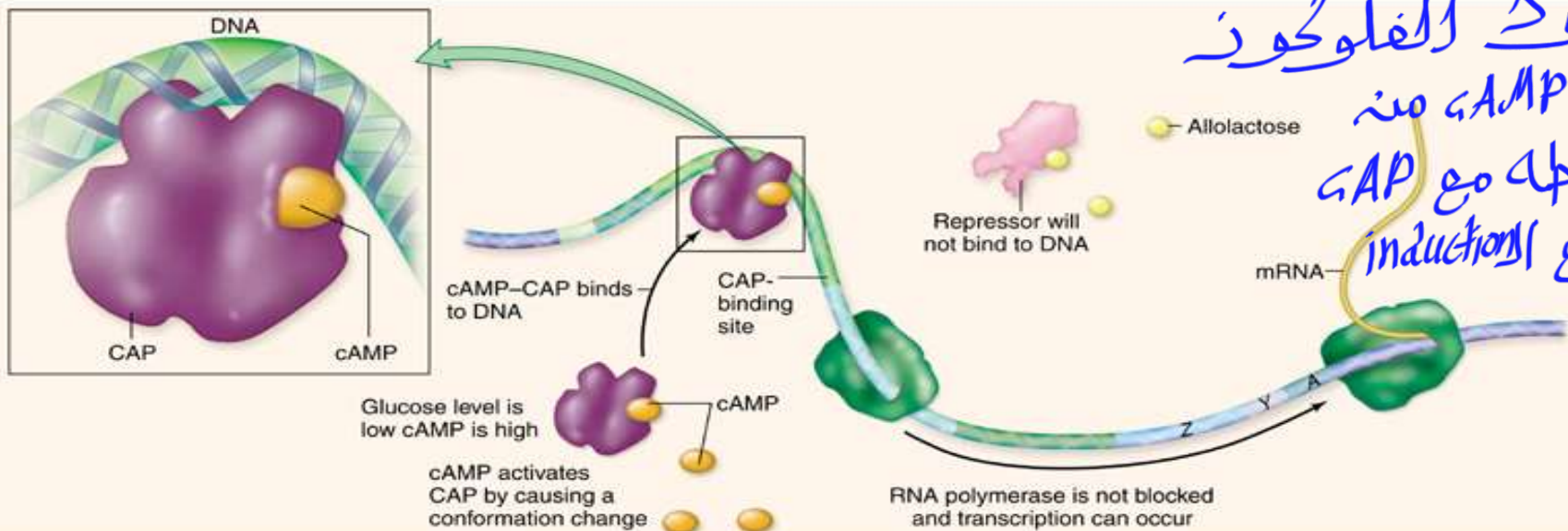
- Glucose prevents induction of the **lac operon**.
 binding of **CAP – cAMP** complex to the CAP binding site is required for induction of the **lac operon**

Repressor proteins
 ← اللاكتوز وان
 هو (L-lactose)
 من (D-lactose)

high glucose levels cause low **cAMP** levels
 high glucose → low **cAMP** → no induction

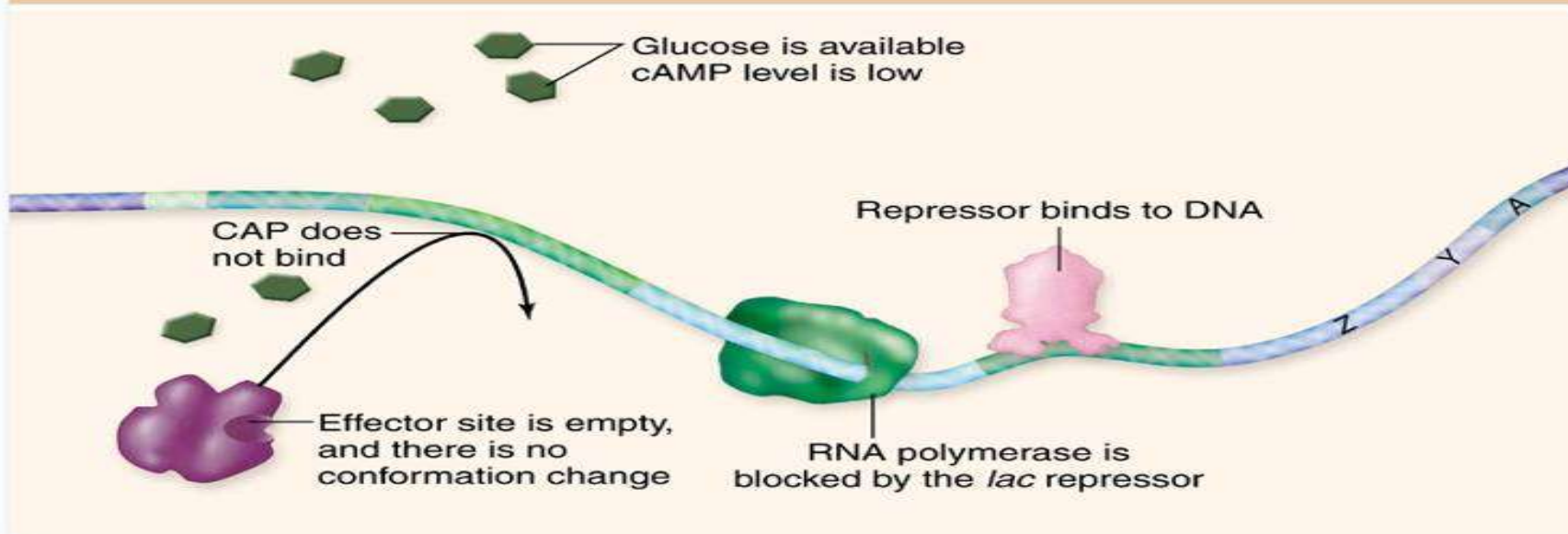
Summary: **Lac operon** is active only in time, when the activator **CAP-cAMP** complex is attached to promotor (no glucose) and when is not present repressor on operator (**lactose present**).

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**.



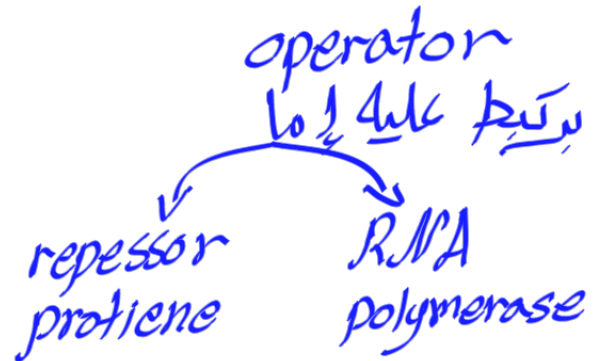
- The operon is not expressed when the cell contains sufficient amounts of tryptophan.

- The operon is expressed when levels of tryptophan are low.

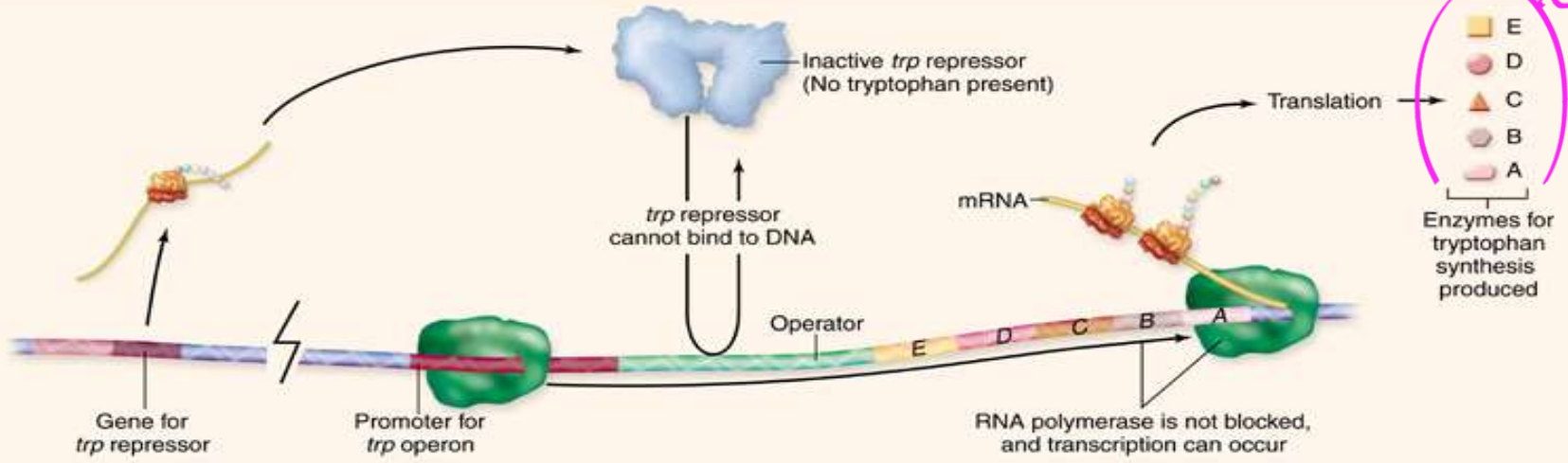
- The *trp* operon is **negatively** regulated by the *trp* repressor protein

- *trp* repressor binds to the operator to block transcription
- Binding of repressor to the operator requires a **corepressor** which is tryptophan → **Feedback Inhibitions**
- Low levels of tryptophan prevent the repressor from binding to the operator

بما صنع الله إذا كان في Tryptophan
 كثير، راجح يحبس مُستطاد لا repressor
 علسانه يرتبط في الـ operators ويحبس إنتاج نفسه بنفسه



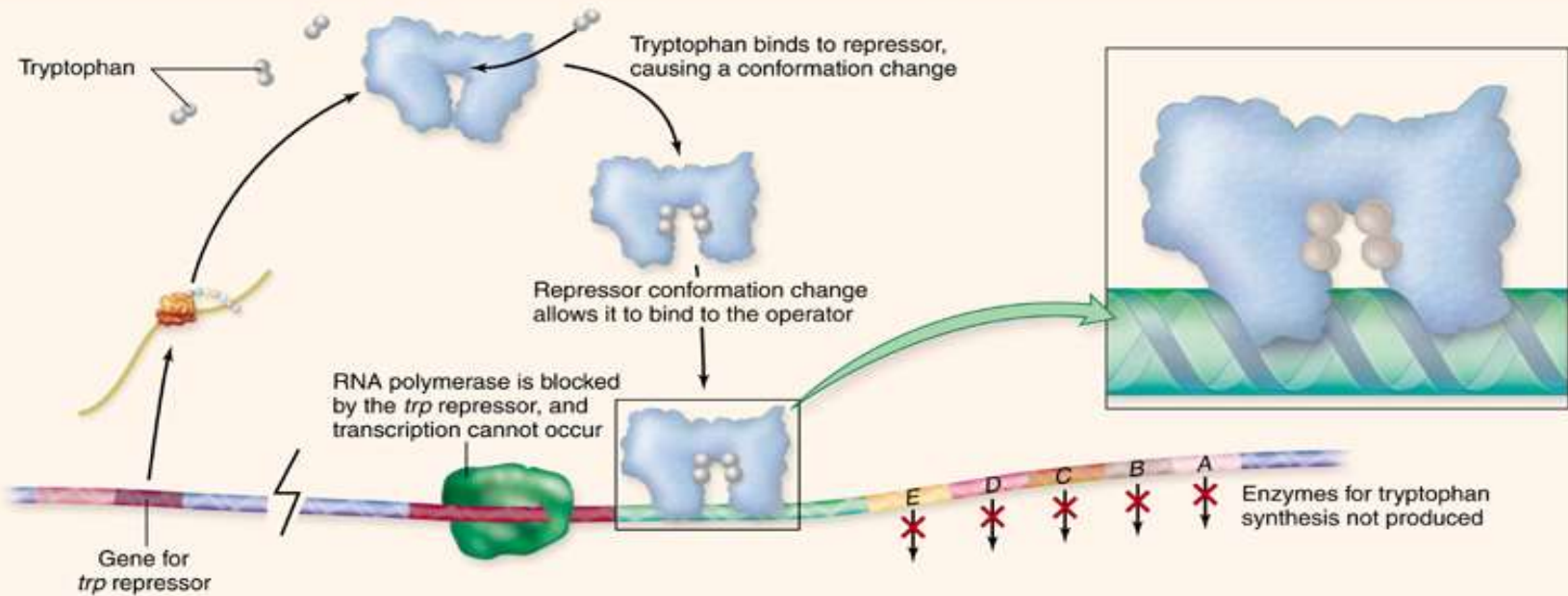
Tryptophan Absent, Promoter Activated



a.

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Tryptophan Present, Promoter Repressed



b.

Regulation of gene expression in eukaryotes

Post transcription modification » Processing of RNA » Translation Controller

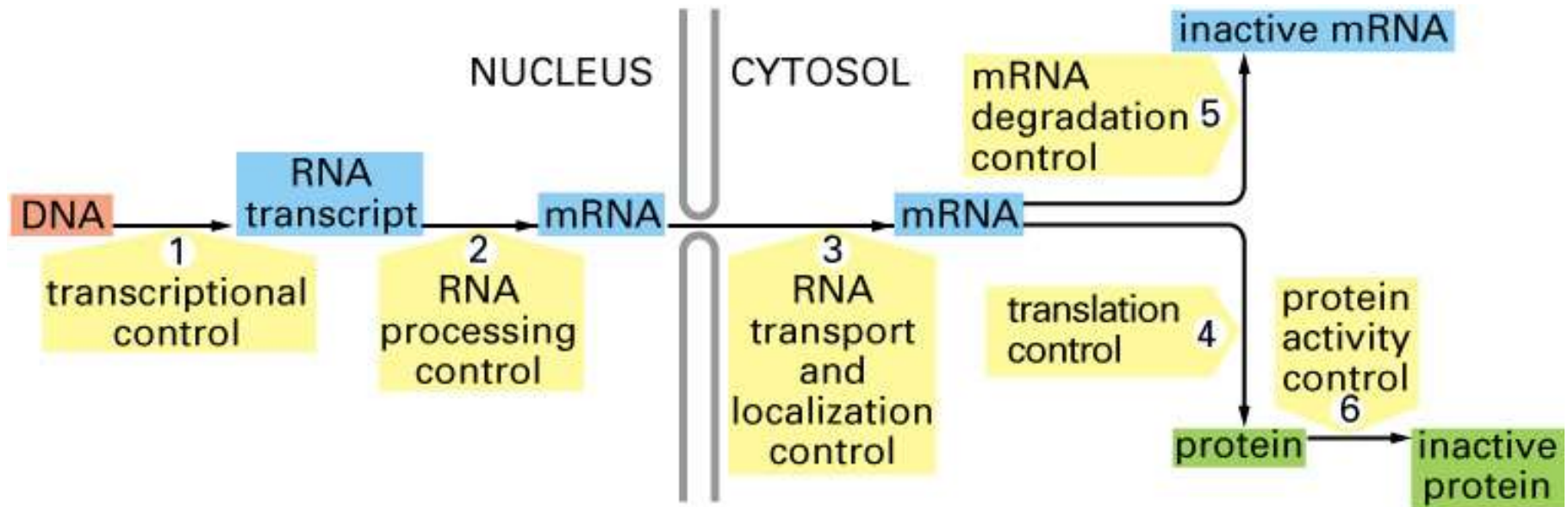


Figure 7-5. Molecular Biology of the Cell, 4th Edition.

- Controlling the expression of eukaryotic genes requires **transcription factors**.

7 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

حكمة الوظائف تبعاً لاحتياجات الخلية

- 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).

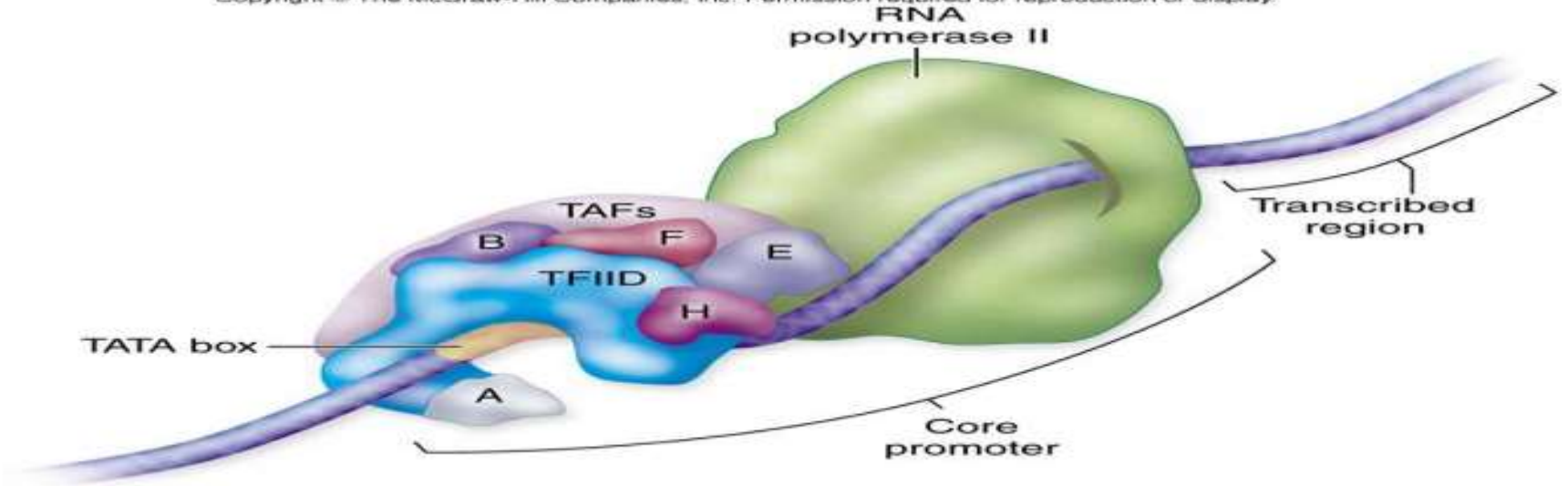
bind to TATA Box and other promoter region

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

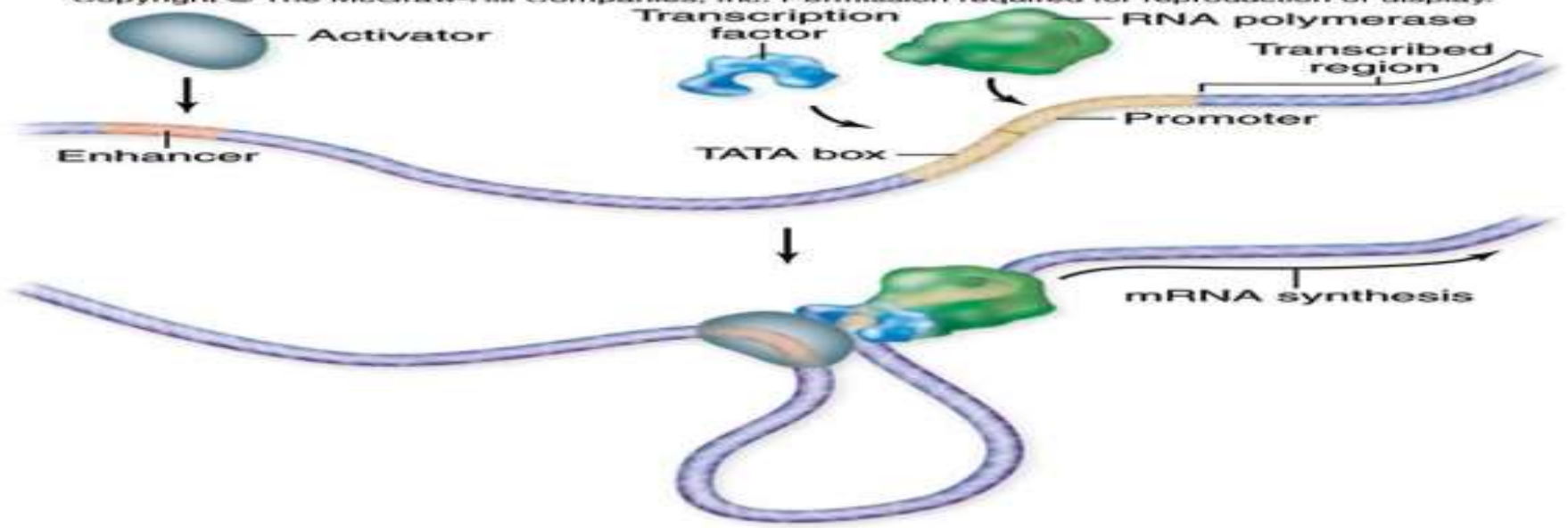
In certain cells *also to a particular Genes*

كل ما زاد عدد الفاكورز بتزيد القدرة على التحكم والتنظيم

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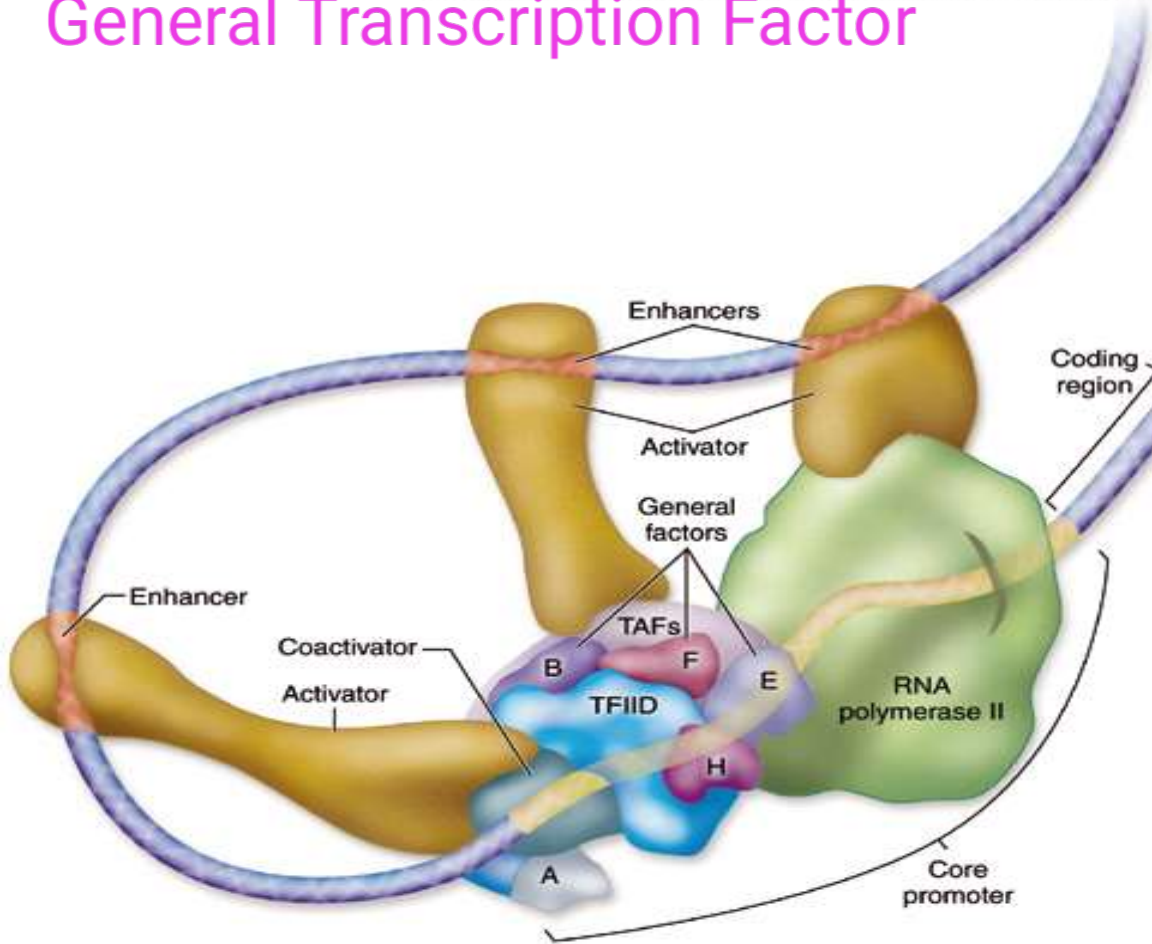


- **Coactivators** and **mediators** are also required for the function of transcription factors.

- Coactivators and mediators bind to transcription factors and bind to other parts of the transcription apparatus

Function: Is to transmit the signals from activator to the General Transcription Factor

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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

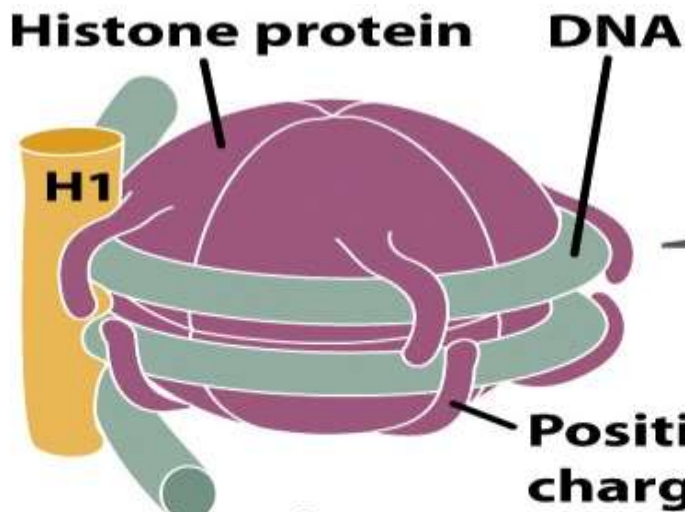
- Addition of methyl groups to the histone protein tails **Methylation**
- Addition of acetyl groups to histone proteins **Acetylation**

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
If the unwrapped happen then they will be successfully Accessible for 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



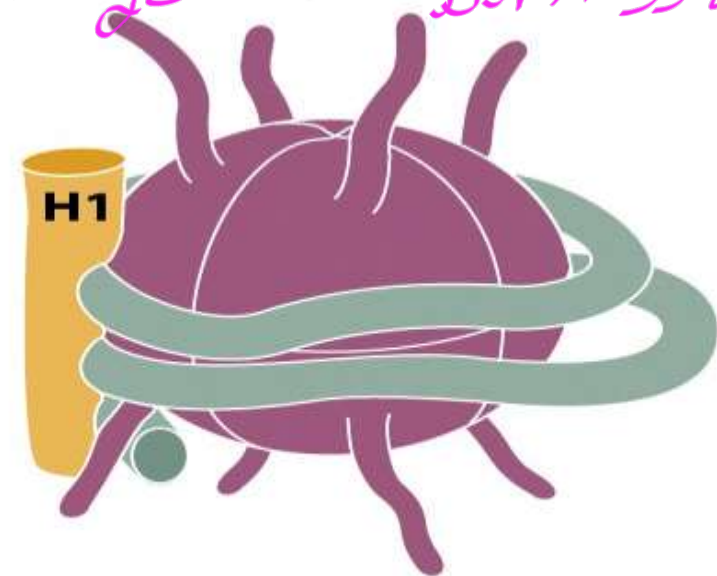
1 Positively charged tails of nucleosomal histone proteins probably interact with the negatively charged phosphate groups of DNA.

بالا سلباً بترتیب موجبة

Acetylation

Change (-)

الأستيل مع اللستون، يبدل منه ال DNA
ويعيد ترتيبه



2 Acetylation of the tails weakens their interaction with DNA and may permit some transcription factors to bind to DNA.

Figure 17-2
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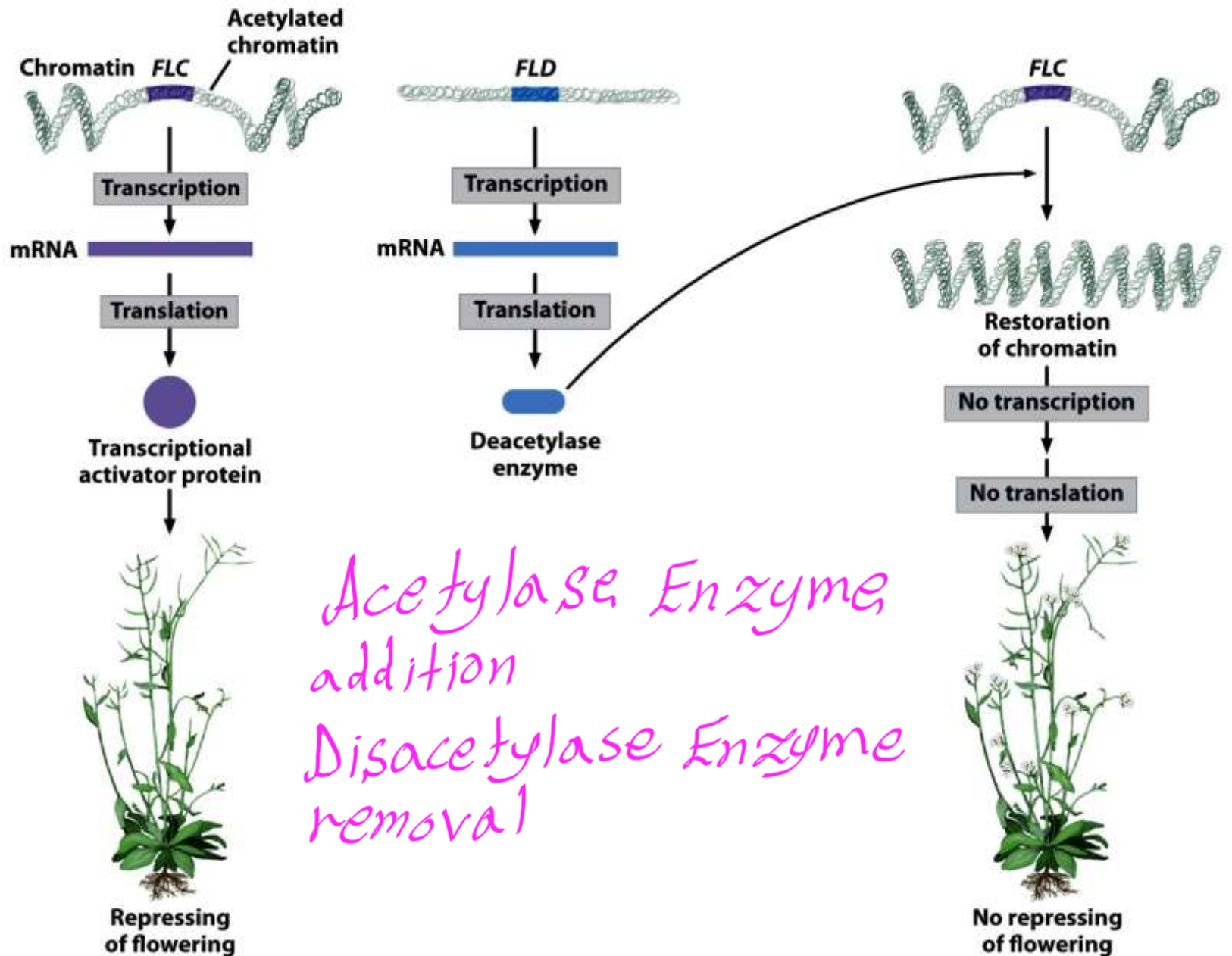


Figure 17-3
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Cytosine JJ 26

Methylation

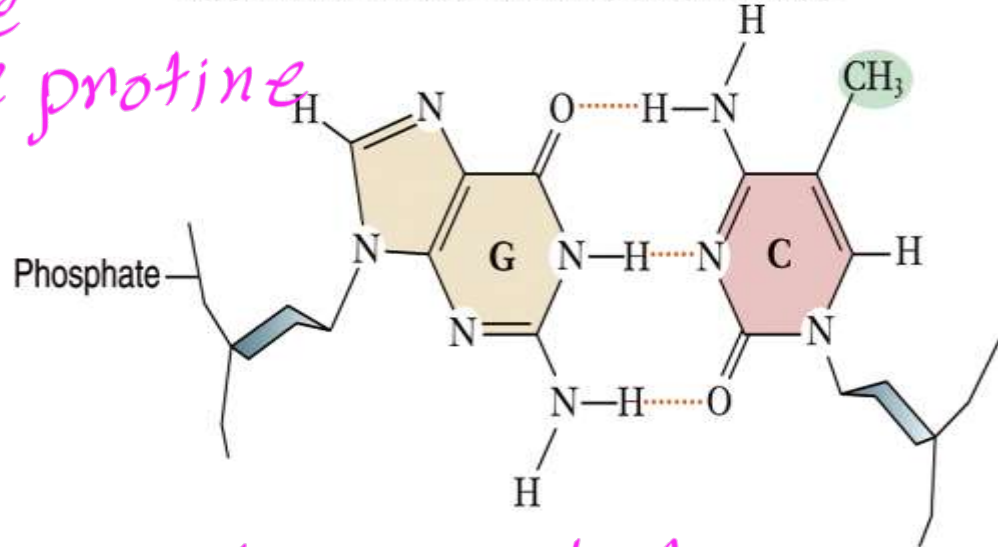
- Addition of methyl group to DNA or histone proteins is associated with the control of gene expression.
- Clusters of methylated cytosine nucleotides bind to a protein that prevents activators from binding to DNA.
- Methylated histone proteins are associated with inactive regions of chromatin.

Methylation in the both of DNA cytosine and the histone protine

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

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Methylation to histone => highly inactive region of DNA.

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

Cleavage => sn-RNA *وهذا ينتج الـ sn-RNA*

A- RNA interference involves the use of small RNA molecules

*ذكر كلة وظيفة
فانه يكفي الملاحظة (5)*

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

Endoribonucleas with two activities
→ RNAse
→ Helicase

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

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

B- Alternative splicing *→ can regulate 50% of total number of Gene*

- 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.

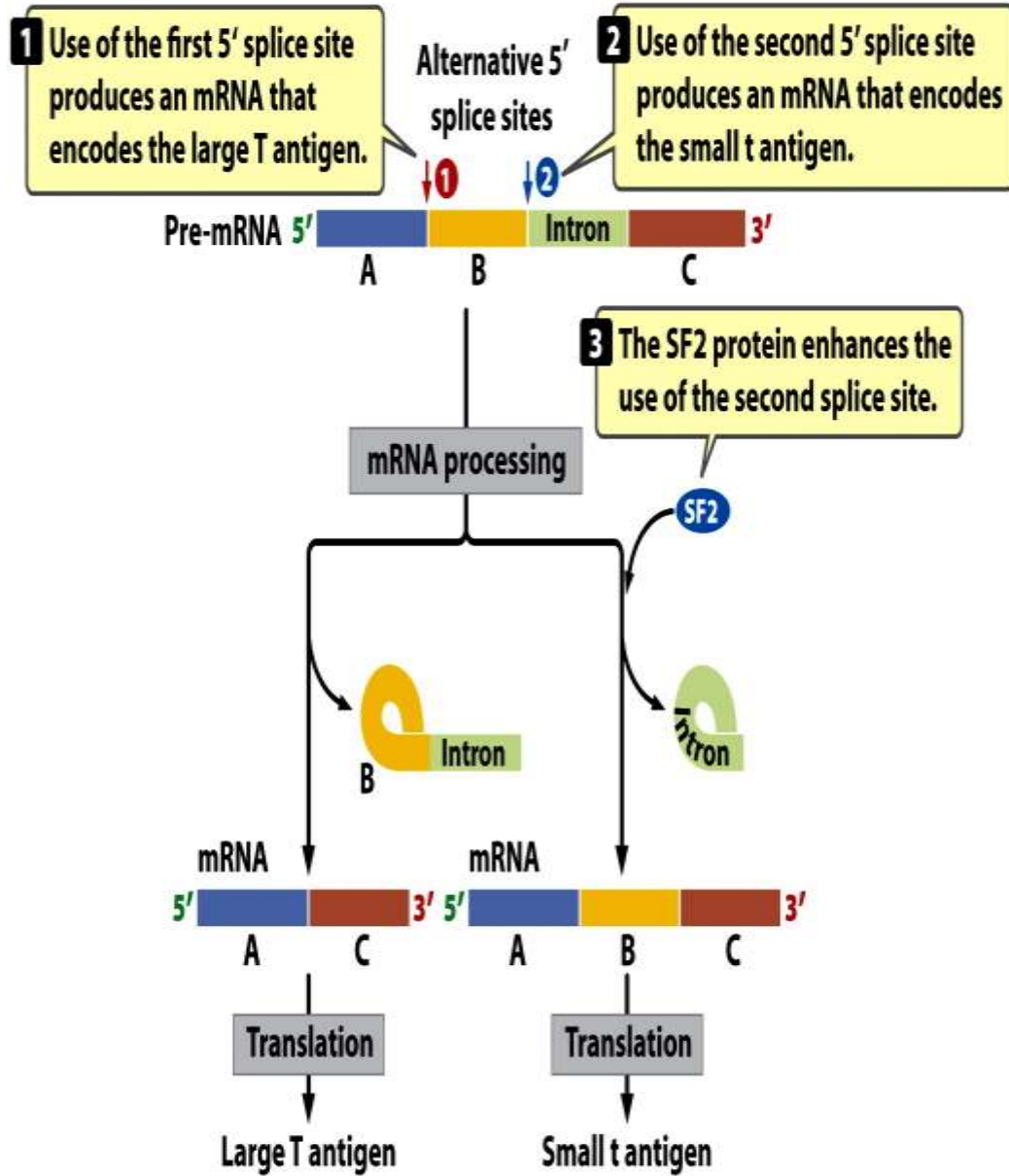


Figure 17-9
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- For example:
 - Apolipoprotein B exists in 2 isoforms
 - One isoform is produced by editing the mRNA to create a stop codon instead of glutamine codon (Apo B48 in intestine) and (Apo B 100 in liver)
 - This RNA editing is tissue-specific
- 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.

إذا كانه كودون وقفه بدلوا
 أما إذا كانه غلوتامين فيكله ويعطى

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

RNA Induced Silencing Complex: contain RNA interfering and pair of mRNA and cleavage to mRNA
↑ work along the small interference RNA to inhibit the translation

Double-stranded RNA

5' 3' 3' 5'

Dicer

siRNAs

RISC

mRNA

5' 3'

Cleavage

Degradation

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

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

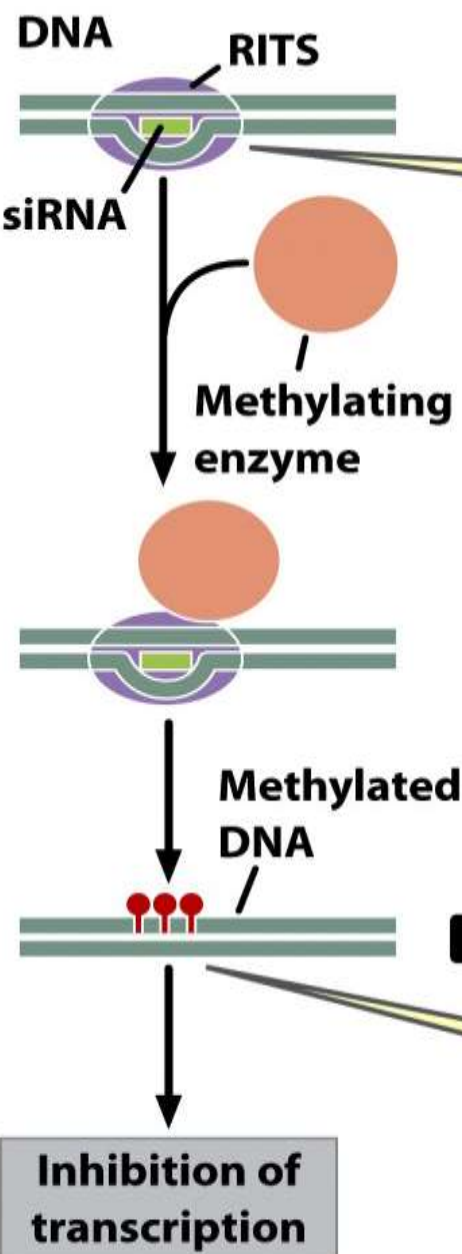
3 The siRNAs combine with protein complex RISC...

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

5 The complex cleaves the mRNA.

6 After cleavage, the RNA is degraded.

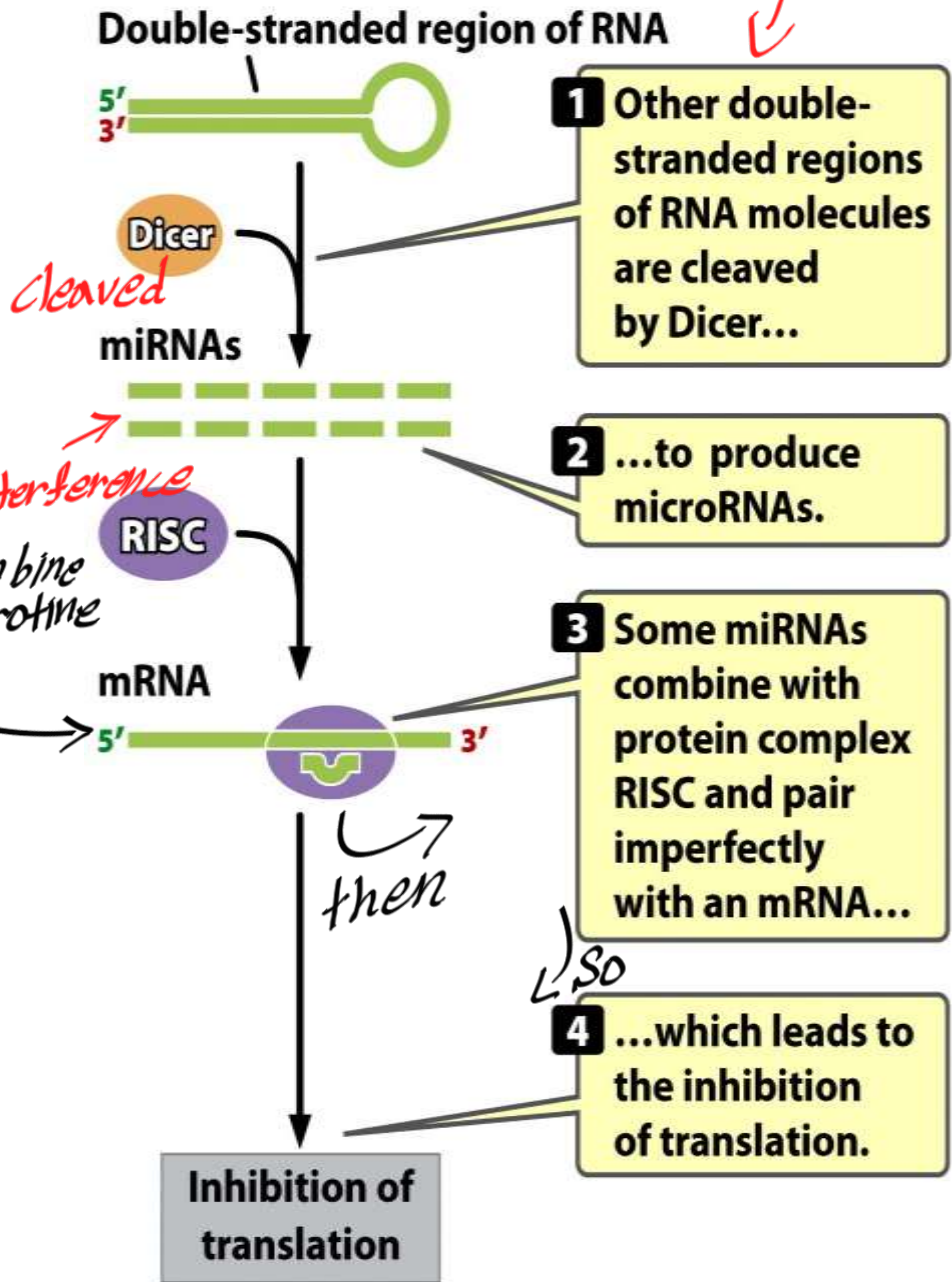
links to siRNA



1 Other miRNAs attach to complementary sequences in DNA and attract methylating enzymes,...

2 ...which methylate the DNA or histones and inhibit transcription.

Figure 17-12c
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1 Other double-stranded regions of RNA molecules are cleaved by Dicer...

2 ...to produce microRNAs.

3 Some miRNAs combine with protein complex RISC and pair imperfectly with an mRNA...

4 ...which leads to the inhibition of translation.

small interference then combine with a protine

then

so

Figure 17-12b
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The Fate of Proteins after translation

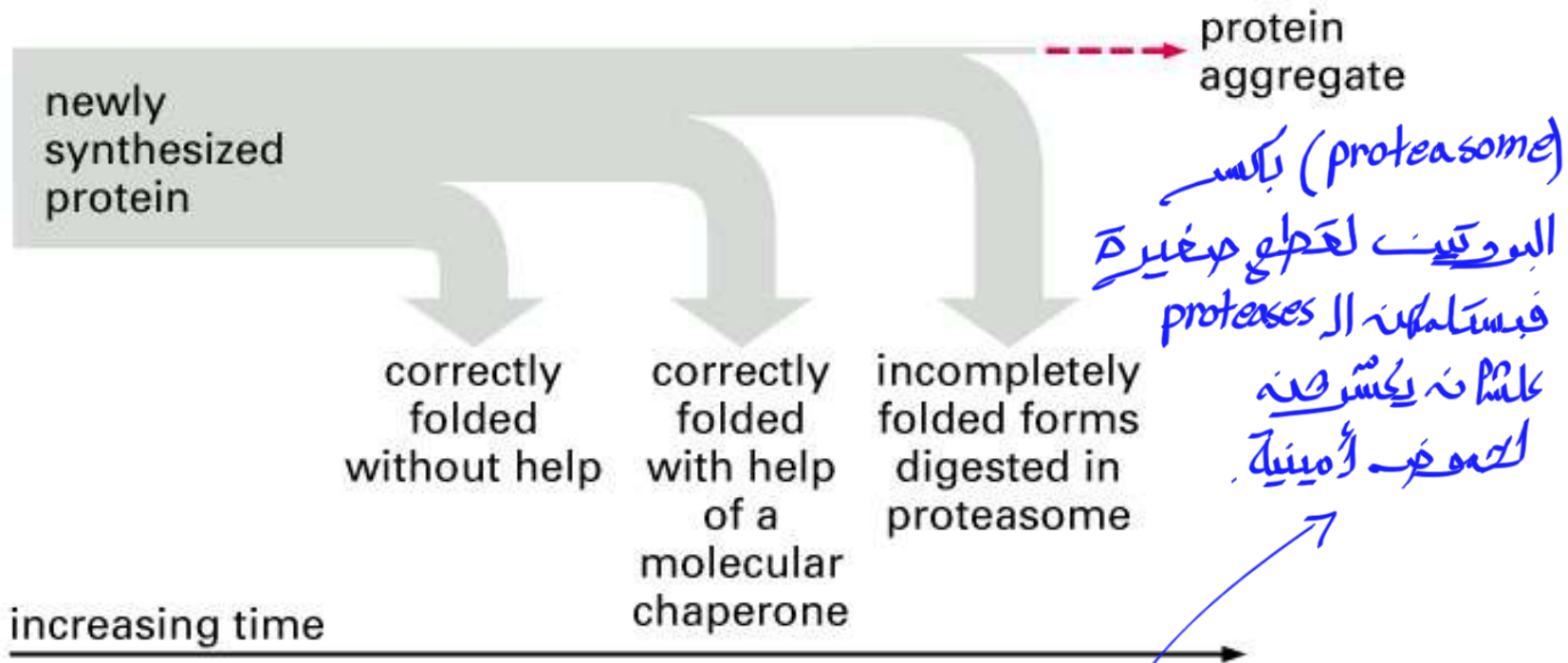


Figure 6-85. Molecular Biology of the Cell, 4th Edition.

Protein Degradation

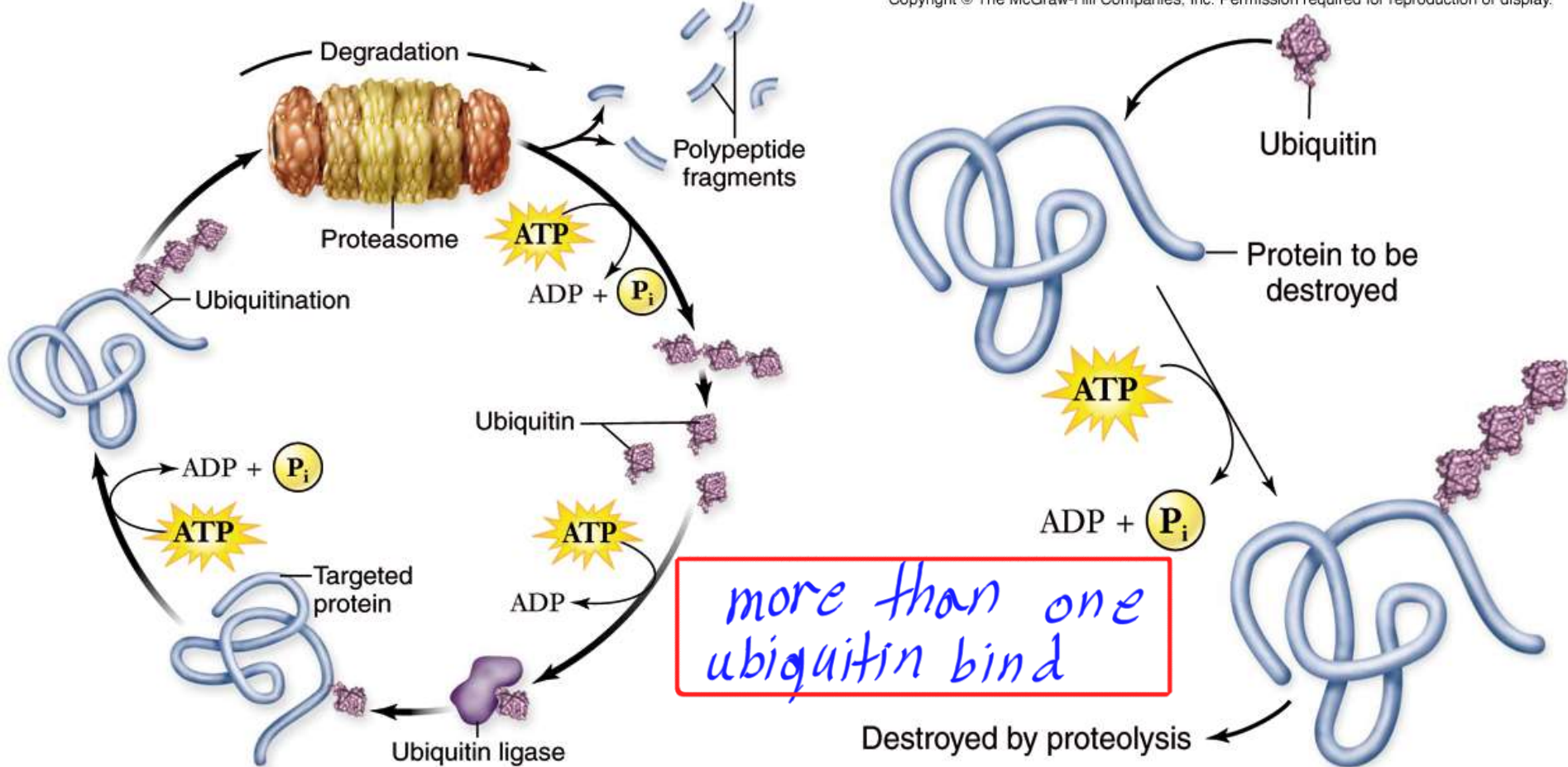
- Proteins are produced and degraded continually in the cell.
- Proteins to be degraded are tagged with **ubiquitin**. *M.W. J. ADA*
- Degradation of proteins marked with ubiquitin occurs at the **proteasome**.

1) Excess protein
2) Incorrectly folded protein

هذه العملية بتسمى لـ:

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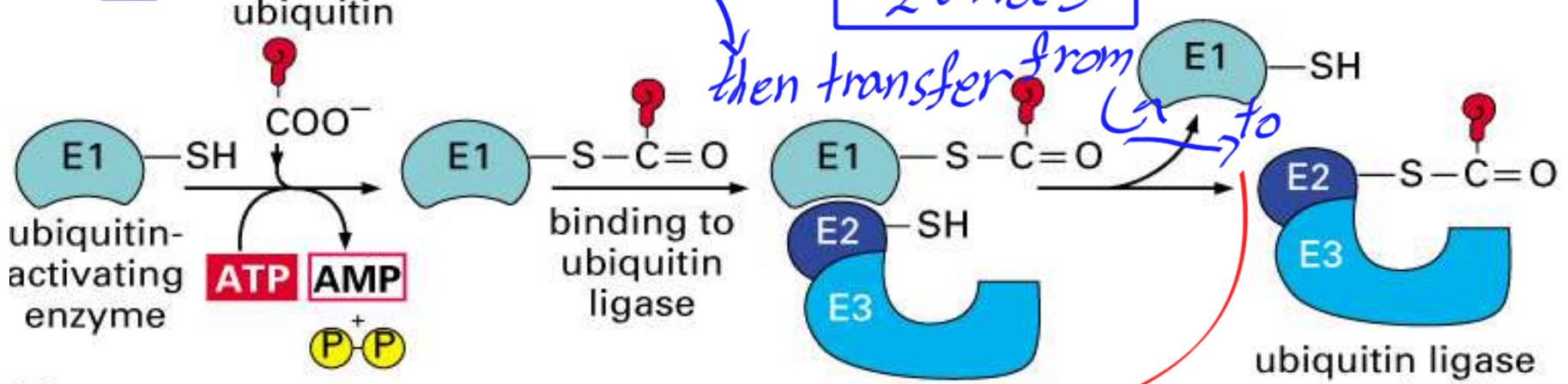
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E1: ubiquitin activating enzyme; E2/3: ubiquitin ligase

bind of [SH] in cysteine of E₁ to ubiquitin

2 and 3



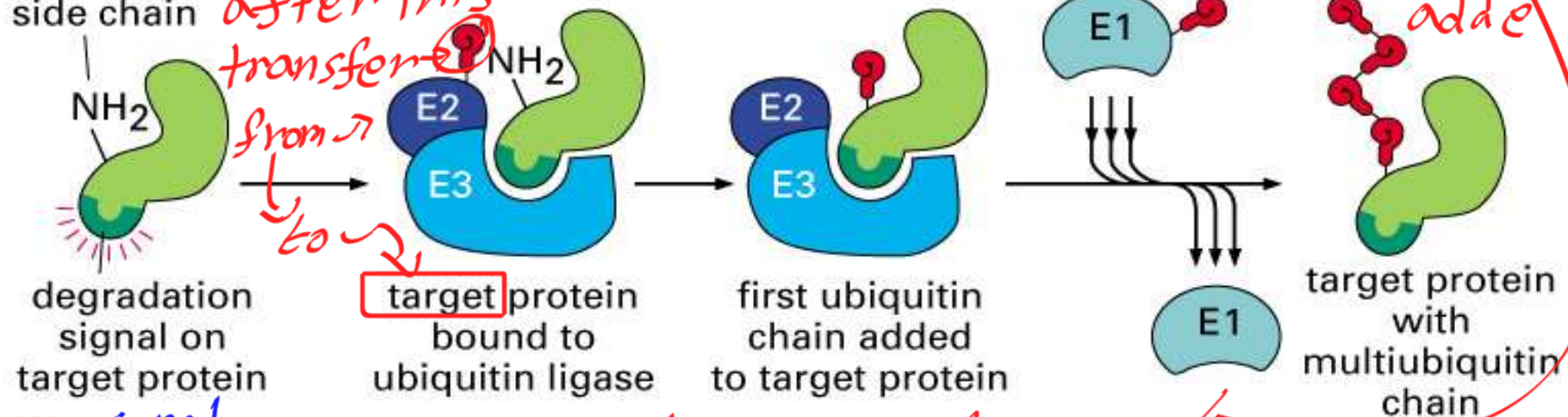
then transfer from E1 to E2

(B)

ε-amino group on lysine side chain

after this transfer from E2 to

many ubiquitin added



(C) *signal through lysin*

because of polyubiquitination

(A) ACTIVATION OF A UBIQUITIN LIGASE

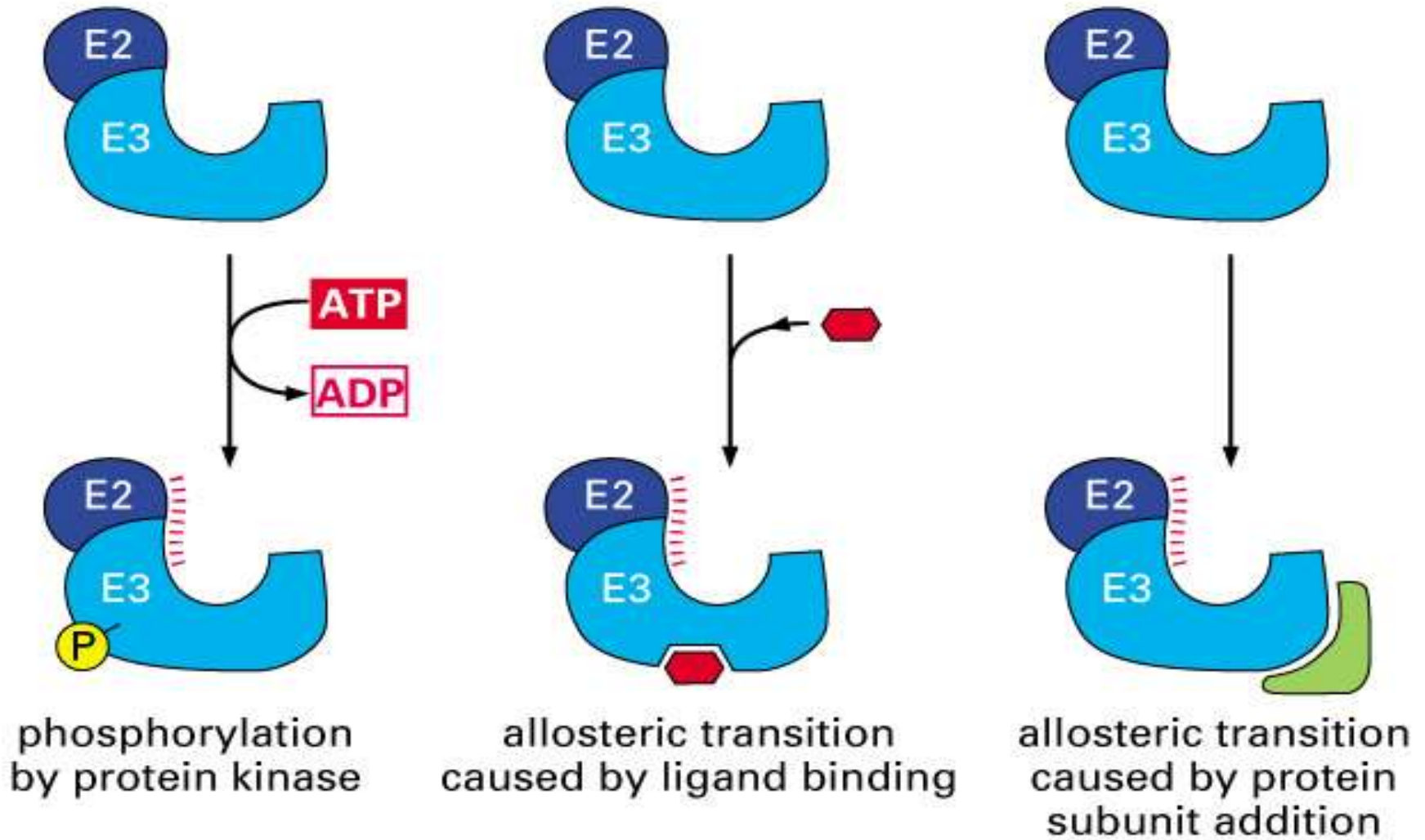


Figure 6–88 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

(B) ACTIVATION OF A DEGRADATION SIGNAL

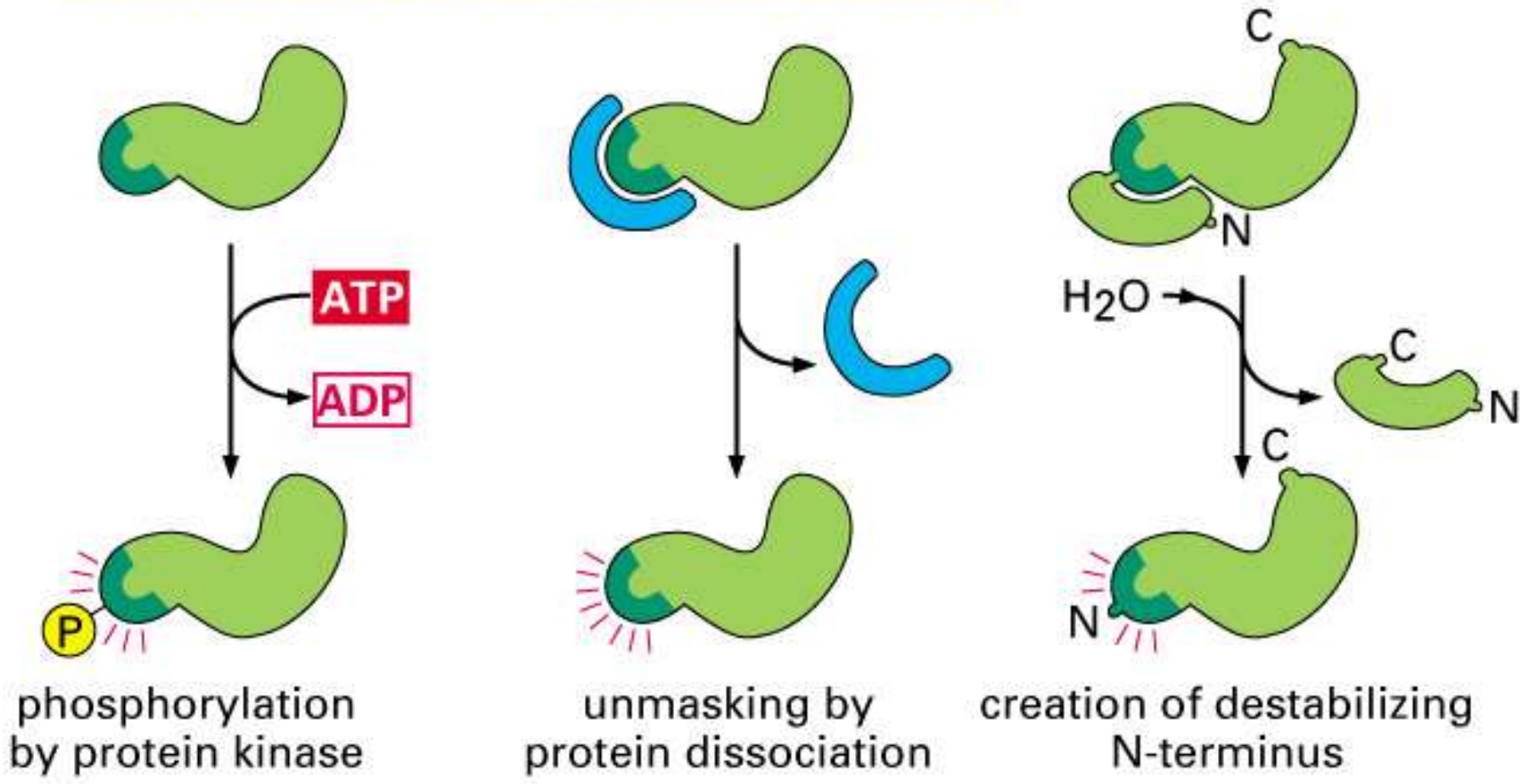


Figure 6-88 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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.