Regulation of Gene expression (II)

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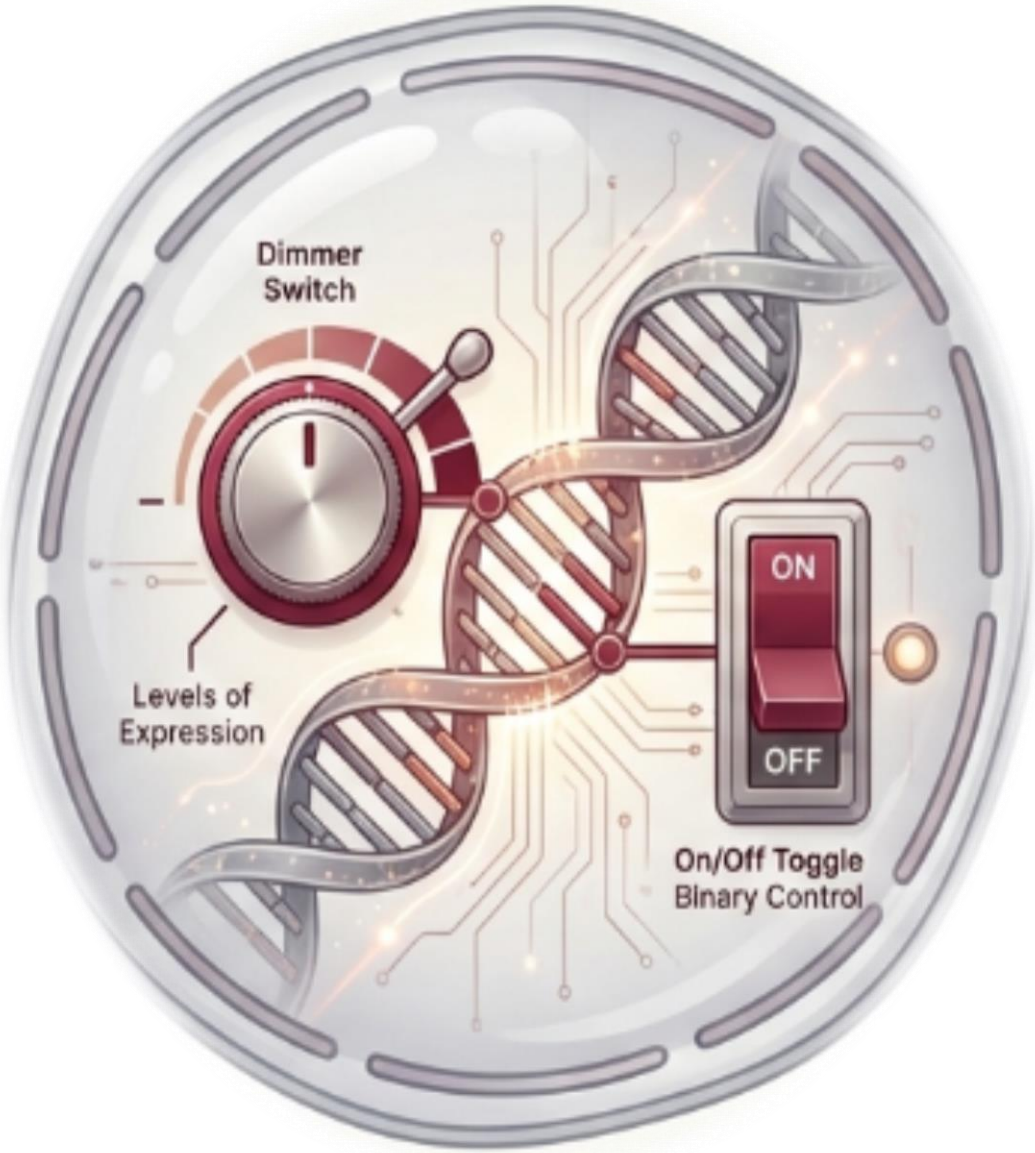


Every cell holds the same manual. Regulation decides which pages are read.

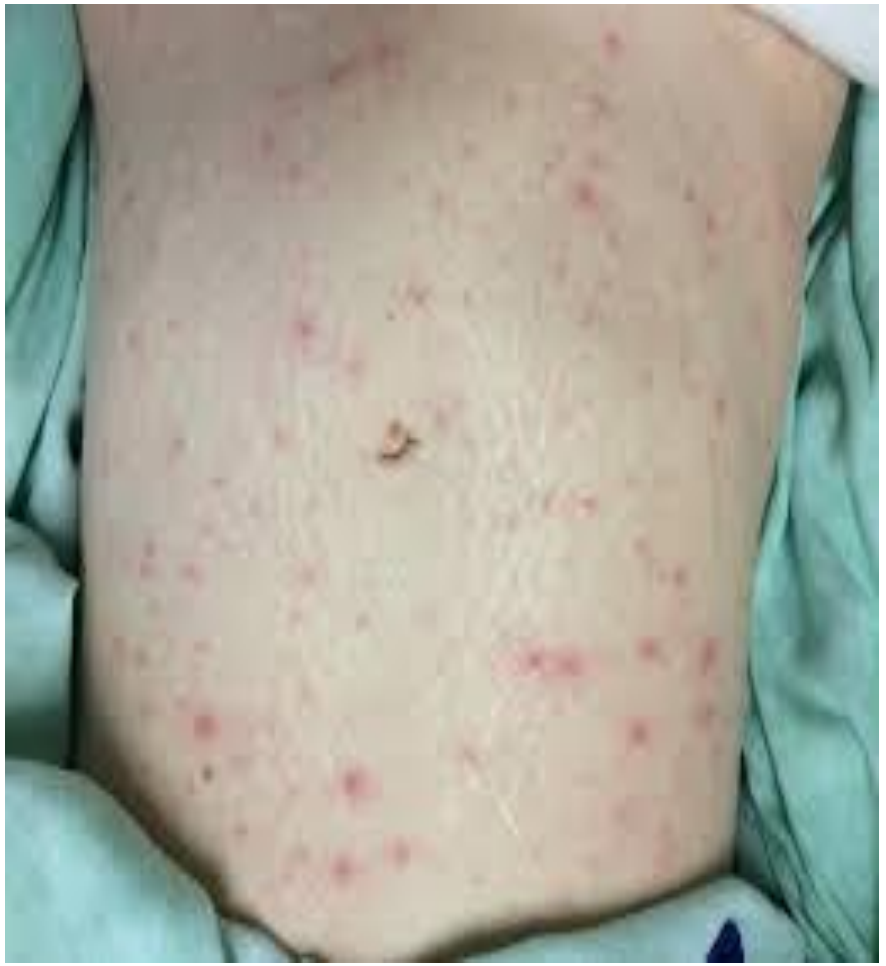
Learning outcomes

By the end of lecture, students should be able to:

- *Recognize different levels of regulation of gene expression in Eukaryotes.*
- *Discuss different mechanisms for regulation of gene expression*
- *Correlate gene expression regulation with clinical conditions*

Case scenario

Mosad a 48-year-old male presented to the hospital with severe fatigue, bone ache, tiny red spots in skin and easy bleeding. The patient had been diagnosed with acute leukemia. He had been taking methotrexate but the patient develops resistance to the drug.

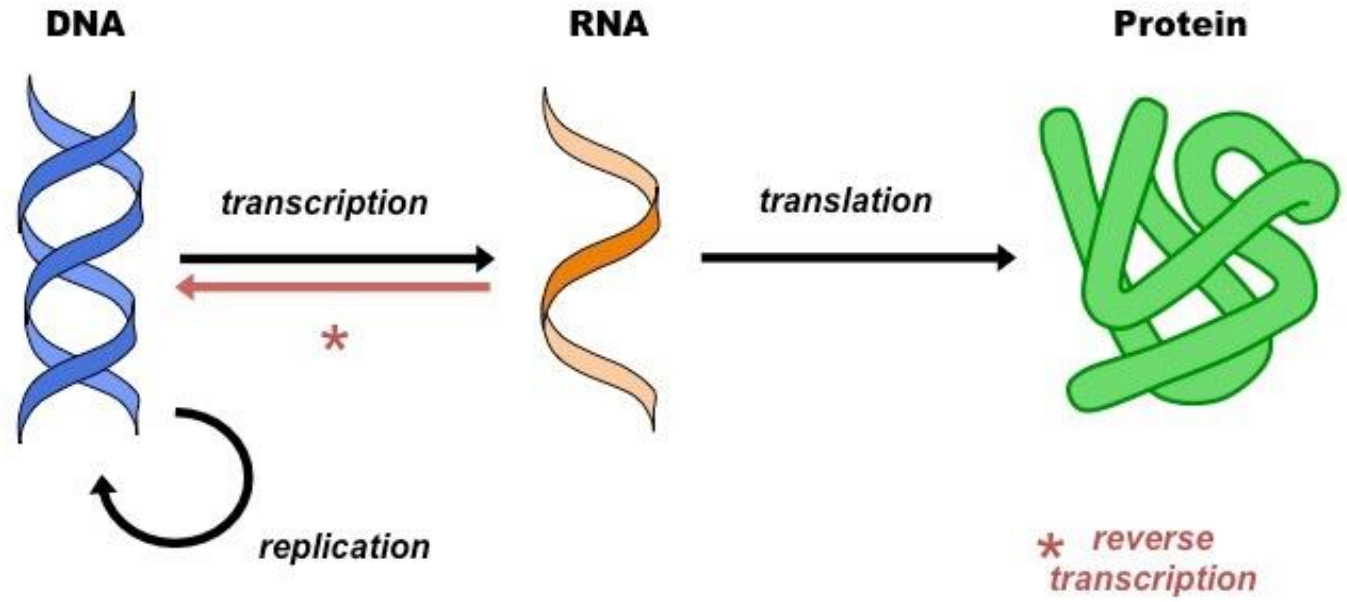


1- Explain mechanism of action of methotrexate ?

2- Explain mechanism of methotrexate resistance ?

Regulation of gene expression

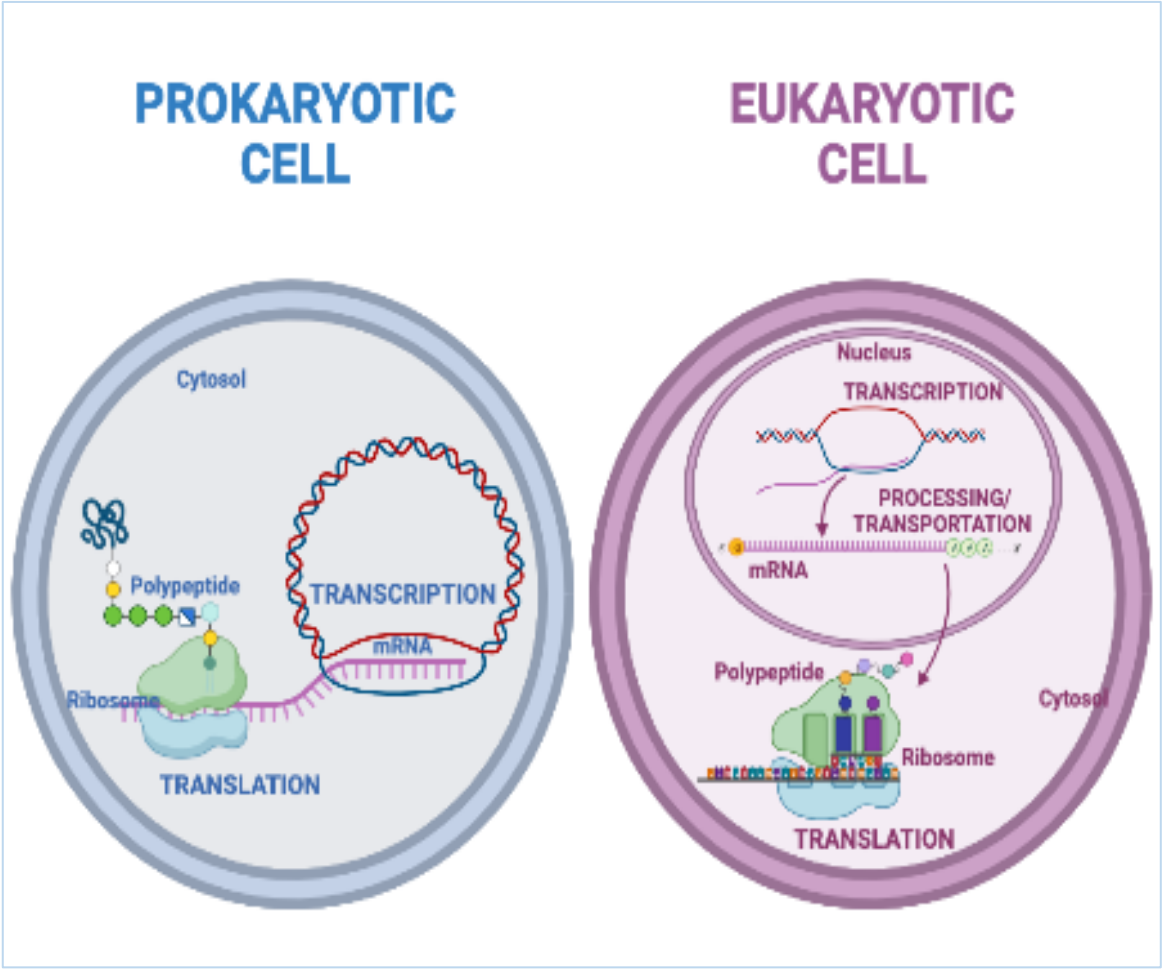
- It includes various mechanisms used to increase or decrease the production of specific gene products (protein or RNA) .



Regulation in Eukaryotes

- Complexity: More complex than prokaryotes due to compartmentalization (nucleus vs. cytoplasm)

- Levels of Control: Regulation occurs at multiple stages: chromatin structure, transcription, post-transcription, translation, and post-translation



Regulation of gene expression in Eukaryotes

It occurs at several levels:

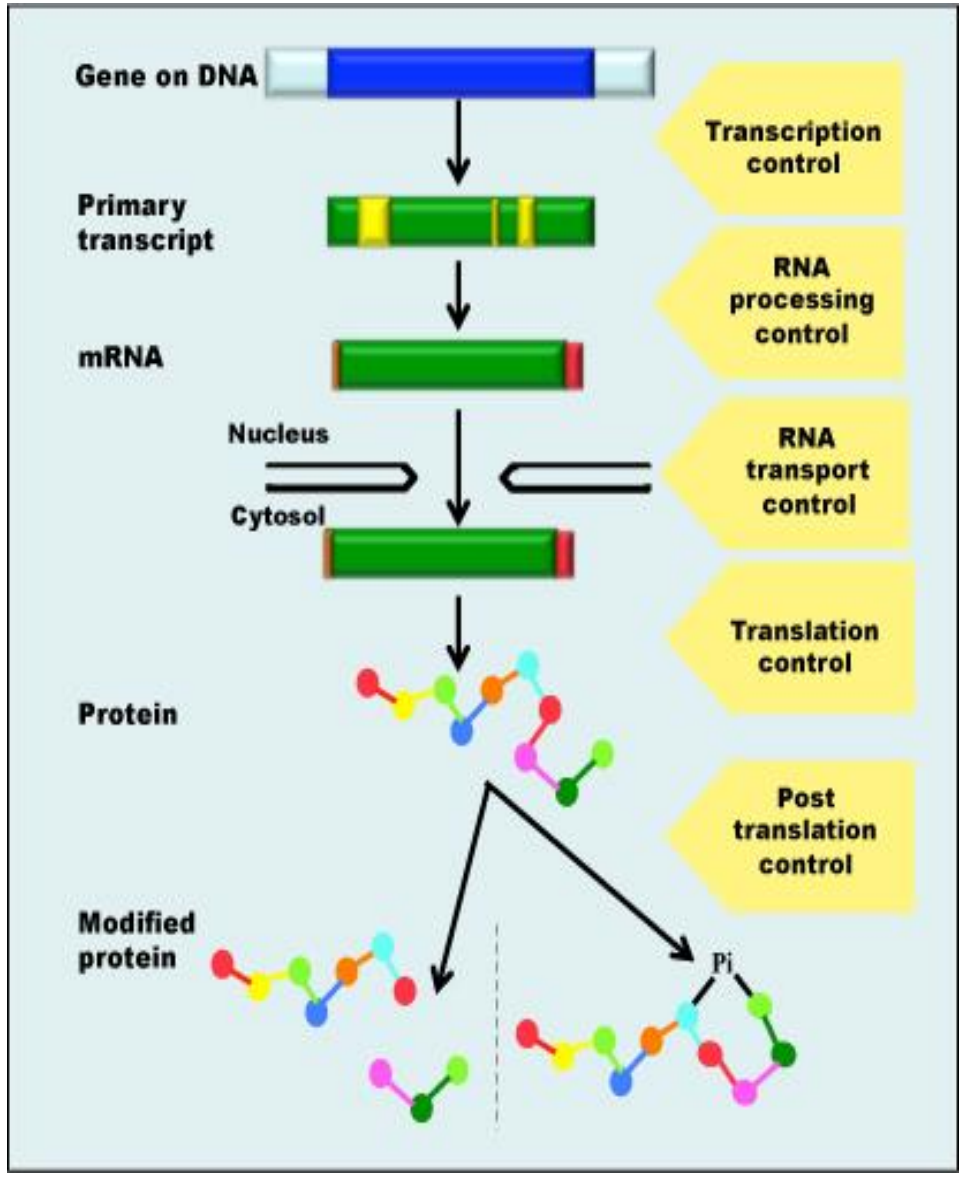
A. **Control at DNA level**

B. **Control at transcription level**

C. **Control at post-transcriptional level**

D. **Control at translational level**

E. **Control at post-translational level**



1-At the level of DNA

1- Gene loss .

2-Gene amplification

3-Gene rearrangement .

1-At the level of DNA

A- Gene loss: if genes are deleted or partially deleted from cells → no proteins produced

Ex: gene loss during RBCs development

Red Blood Cells

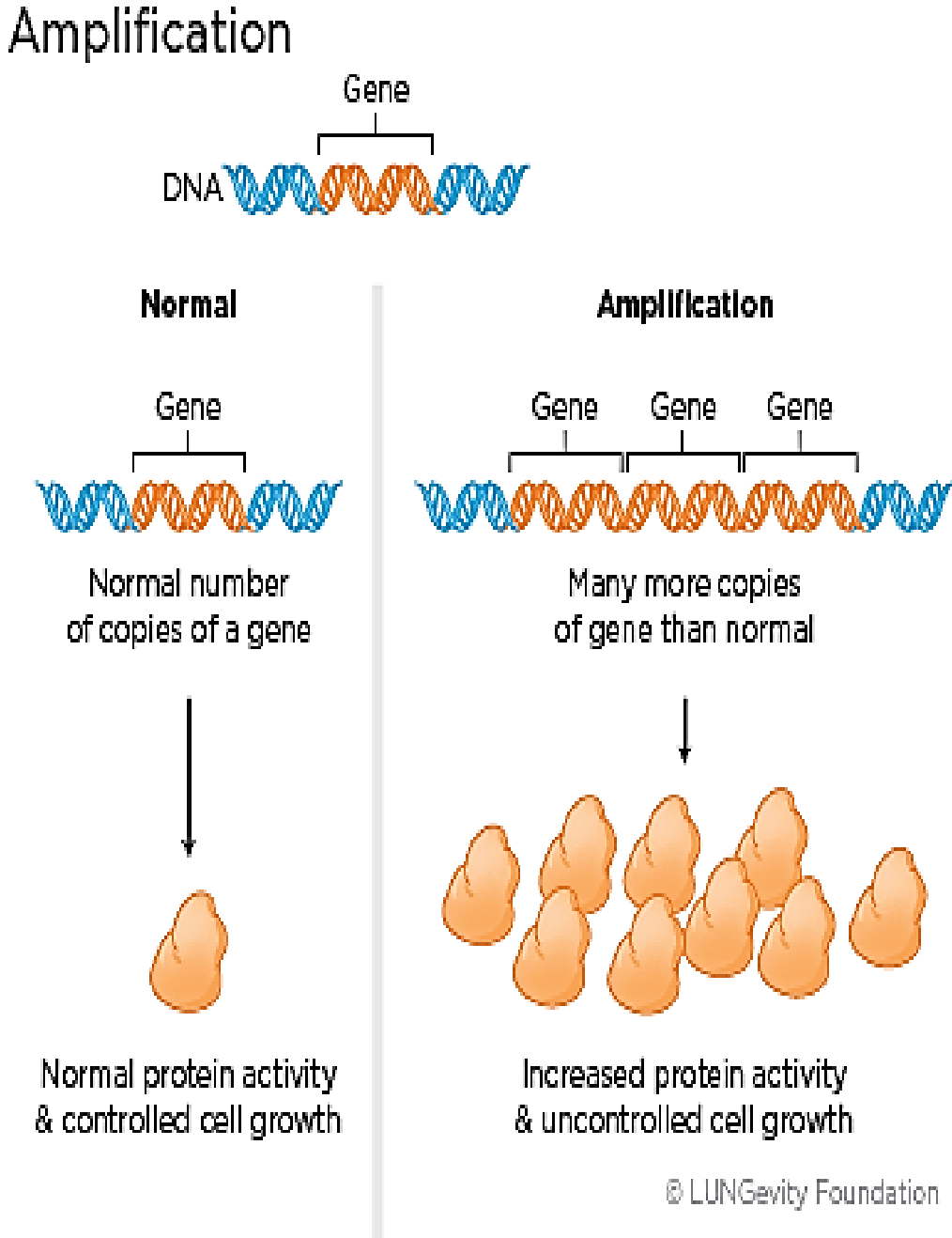
1-At the level of DNA

B- Gene amplification:

Increase number of gene copies with increase production of protein encoded by the amplifying genes

Ex :

Malignant cells develop resistance to methotrexate by increasing number of genes for di-hydrofolate reductase which is the target for methotrexate .



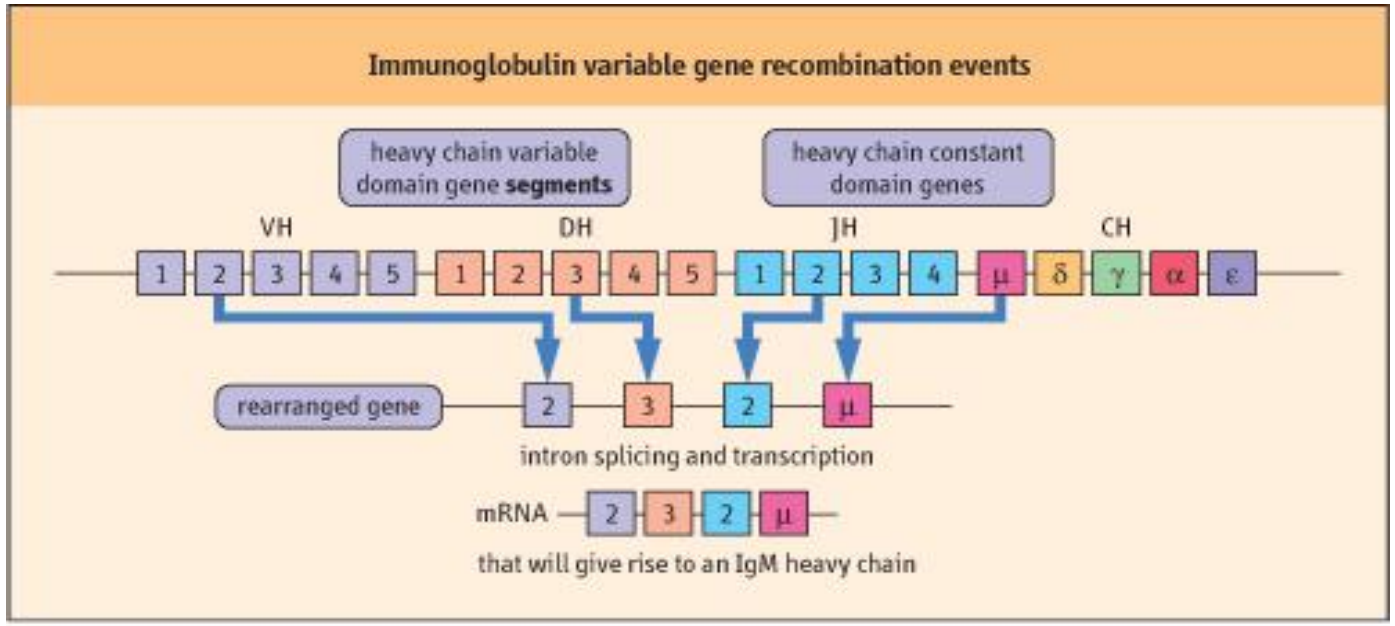
Clinical application

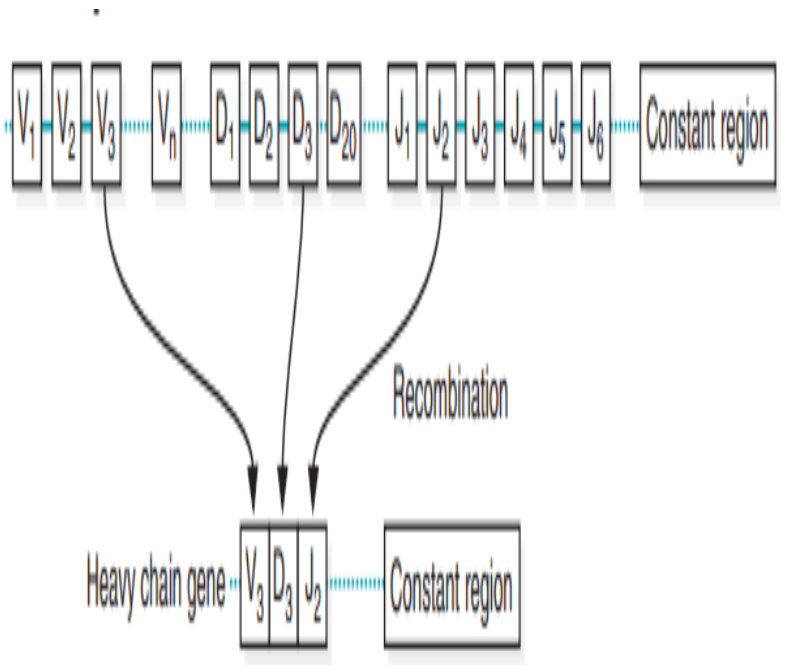
- Methotrexate is an anticancer drug which inhibits di-hydrofolate reductase.
- With long term therapy, cancer cells develop resistance to this drug by increasing the number of genes for di-hydrofolate reductase by gene amplification
- Oncogenes may also get amplified in many cancer cells

1-At the level of DNA

C-Gene re-arrangement : segment of DNA move from one site to another , associating with each other in different ways different proteins are produced

Ex: Antibody gene rearrangement in B-lymphocytes .





At the level of transcription

1-Chromatin remodeling :

A- Chromatin condensation .

B- Chromatin acetylation .

C- DNA methylation

2-DNA regulatory regions :

A- Enhancer .

B- Silencer .

At the level of transcription

1-Chromatin remodeling

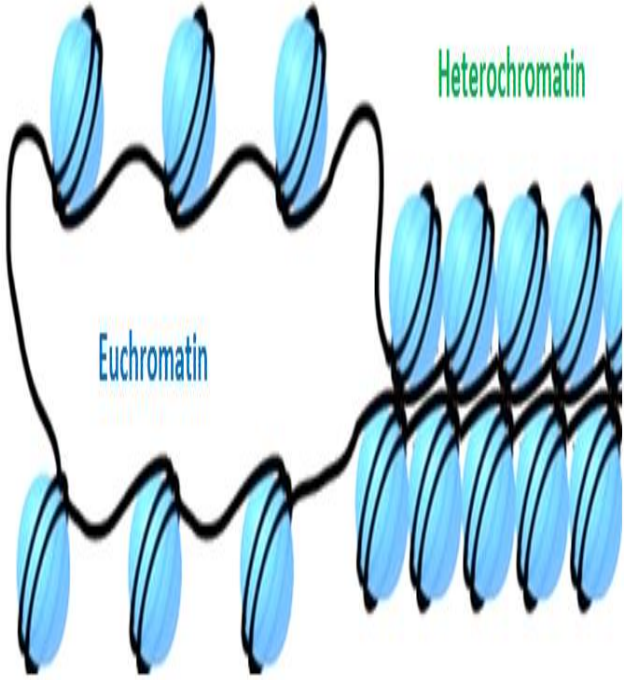
A- Chromatin condensation:

Affects the ability of RNA polymerase to reaches specific genes to activate transcription.

2 forms:

1-Heterochromatin : transcriptionally **inactive**

2-Euchromatin : transcriptionally **active**



At the level of transcription

1-Chromatin Remodeling

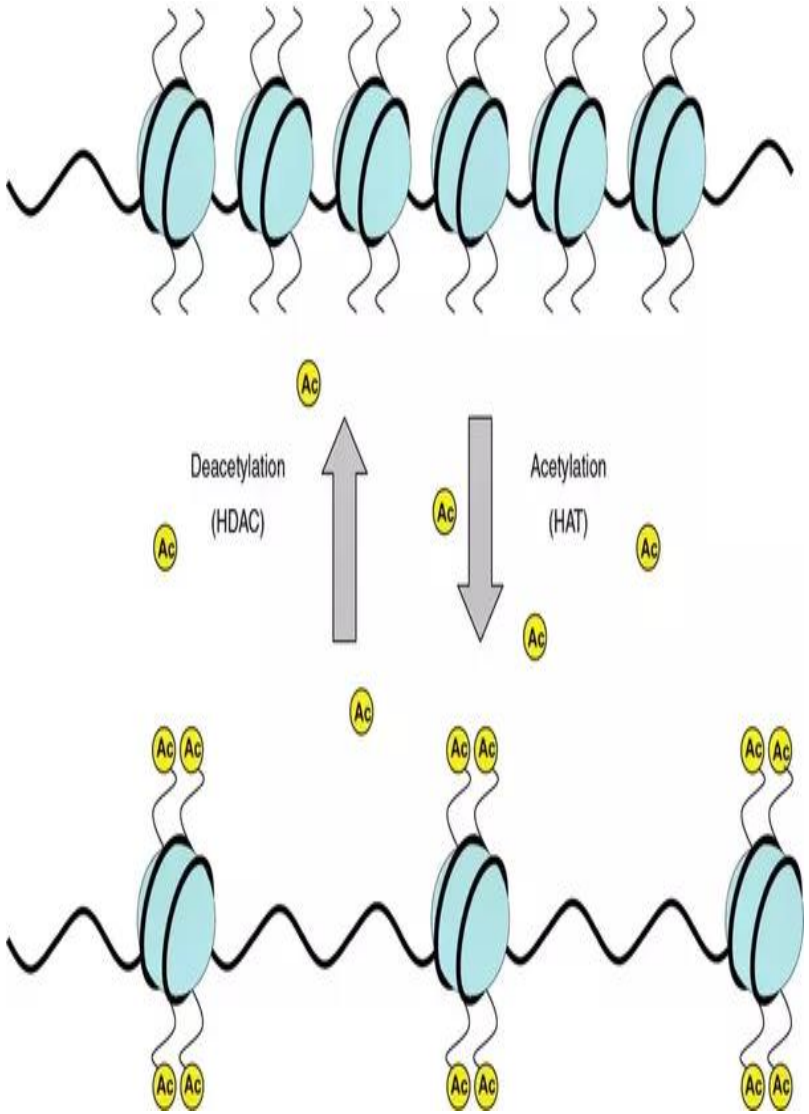
B- Chromatin acetylation (Dynamic and Reversible)

1- Acetylation of lysine residues of histone protein removes the + ve charge of histone , reducing affinity between histones and DNA leads to repulsion of histones .

2-This makes RNA polymerase reaches the genes

and **enhance transcription**

3-It is catalyzed by **histone acetyl-transferase** (HATs) , histone deacetylation by **histone deacetylase**



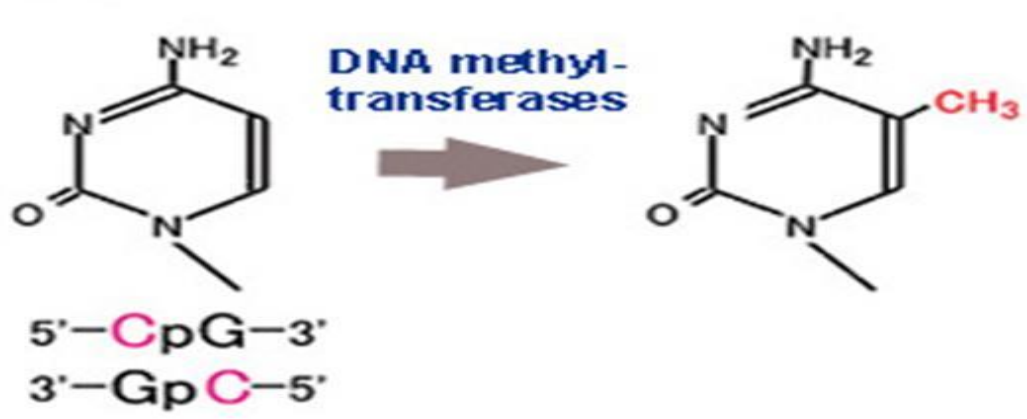
At the level of transcription

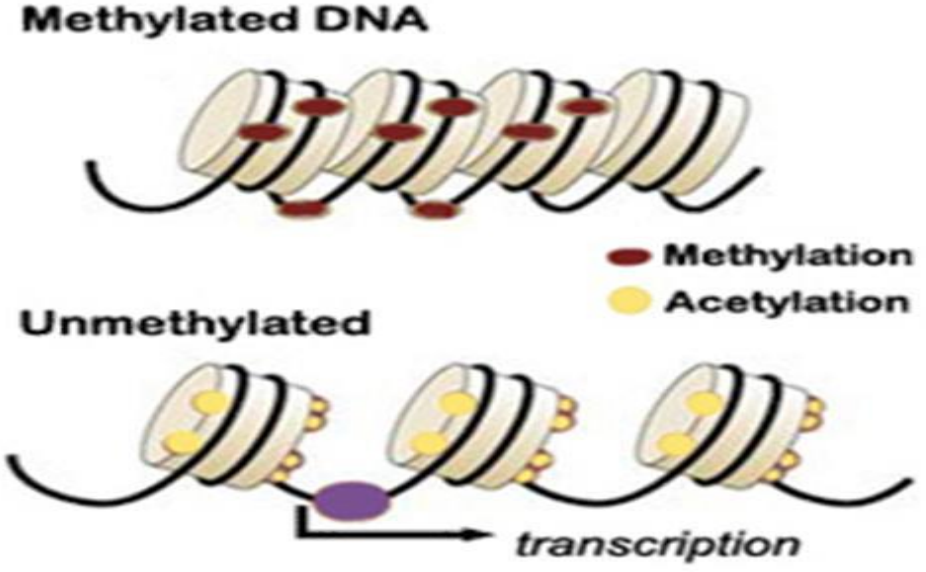
1-Chromatin Remodeling

C- DNA methylation

-DNA methylation is catalyzed by DNA methyltransferases (DNMTs)

-Methylation of cytosine bases in DNA **inhibit transcription** , maintains genes turn off .

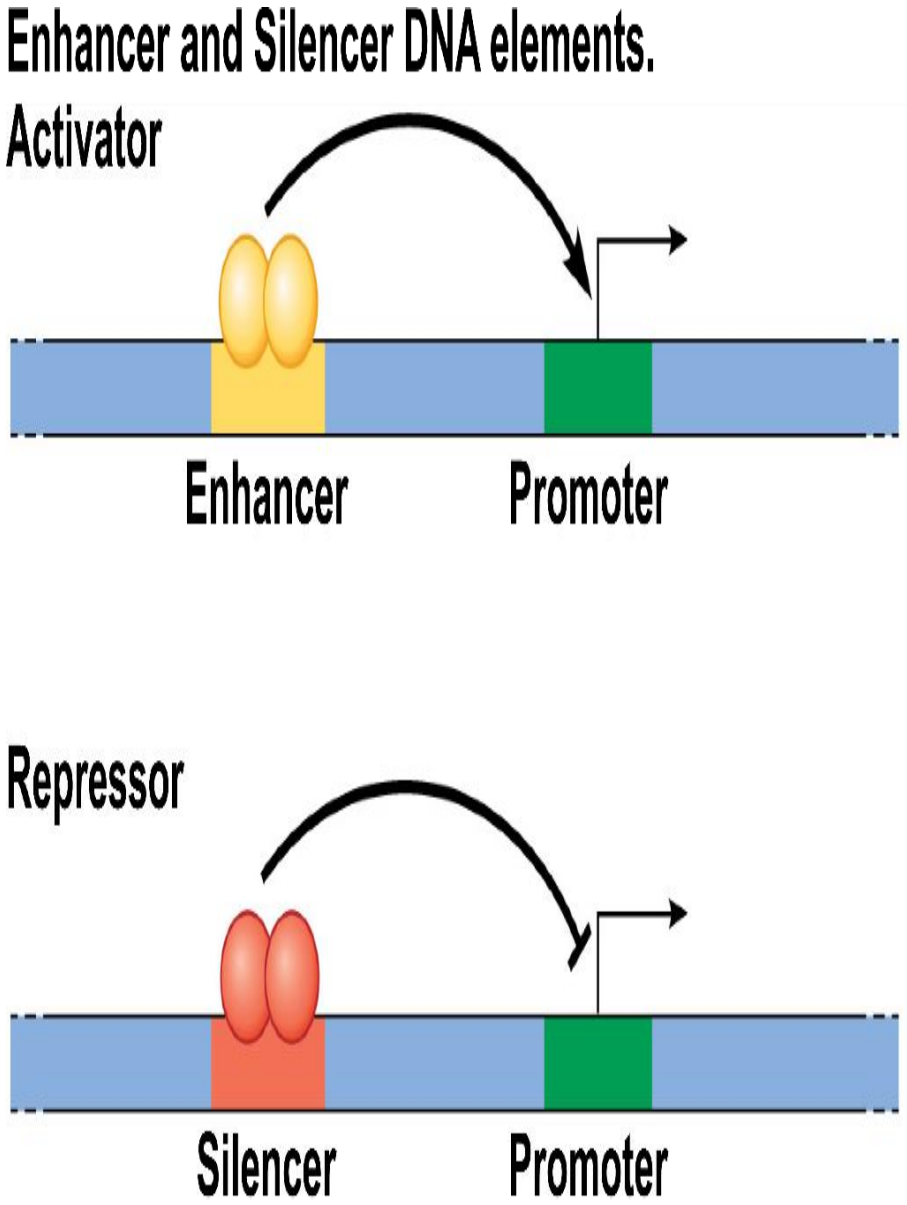




At the level of transcription

2-DNA regulatory regions

1 Enhancer	2 Silencer
DNA sequence which activates transcription	DNA sequence which inhibit transcription
They are recognized by specific enhancer binding proteins (TF-activator)	They are recognized by proteins (repressors)
-It facilitates binding of RNA polymerase to promoter	repressors prevent RNA polymerase from binding to promoter .



3-post-trancriptional regulation

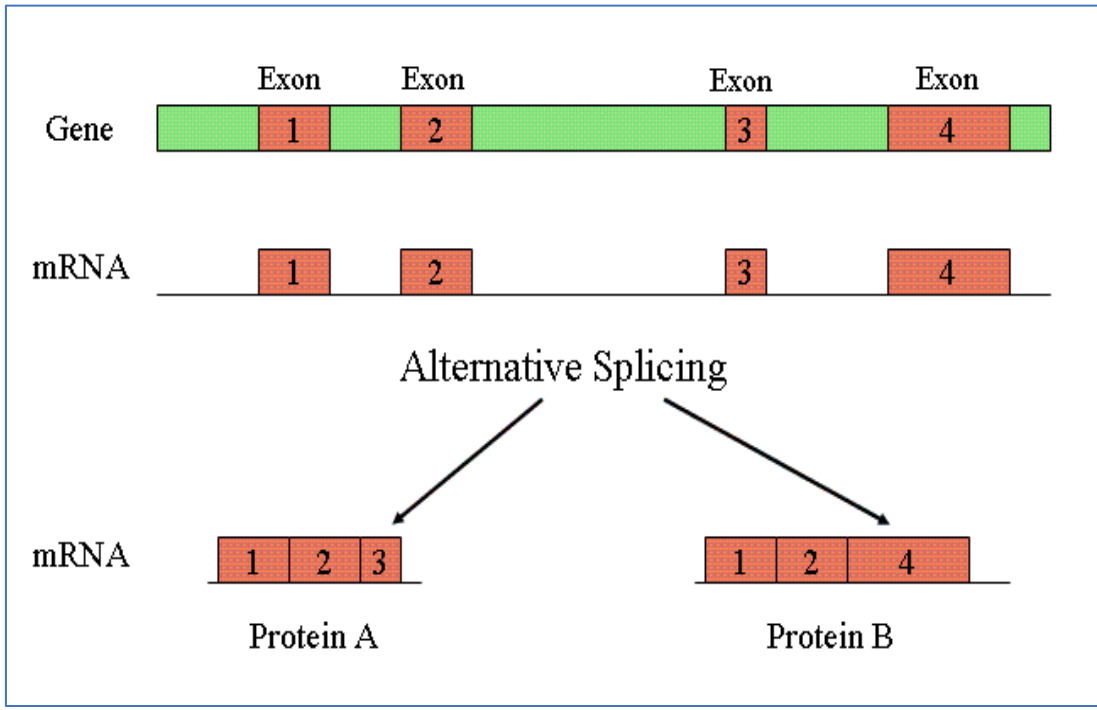
1- Alternative splicing.

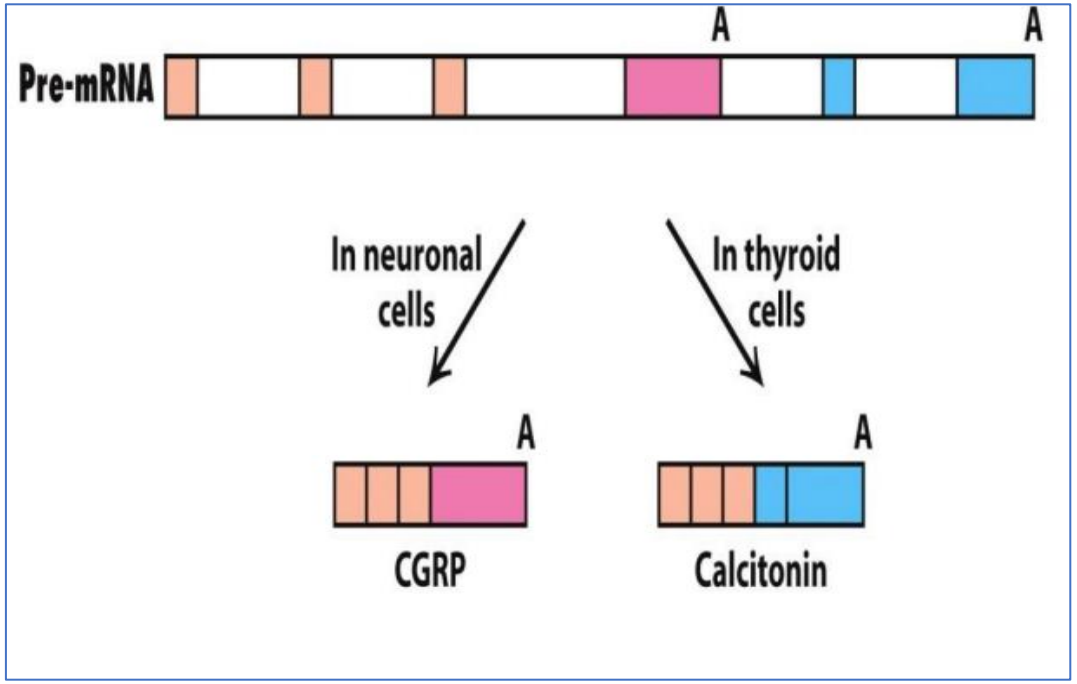
2-RNA stability .

3-post-trancriptional regulation

1-Alternative (differential) splicing

different patterns of splicing leads to production of different types of mature m.RNA from 1ry m.RNA in different types of cells. **Ex:** calcitonin gene





3-post-trancriptional regulation

2-RNA stability

m.RNA half-life is variable from few minutes to hours

Long half life m.RNA	Short half life m.RNA
code for proteins with long duration of action	code for proteins with short duration of action
as β-globin gene m.RNA (half life= 10 hrs)	as growth factor gene m.RNA (half life ≤ 1 hr)

3-post-trancriptional regulation

Factors affecting m.RNA stability

1- **Length of poly (A) tail** : longer poly (A) tail , has longer half life.

2-5` UTR : its deletion prolong half life.

3-Interaction with different proteins : some protect m.RNA from nucleases and other promote nuclease attack

4-At the level of translation

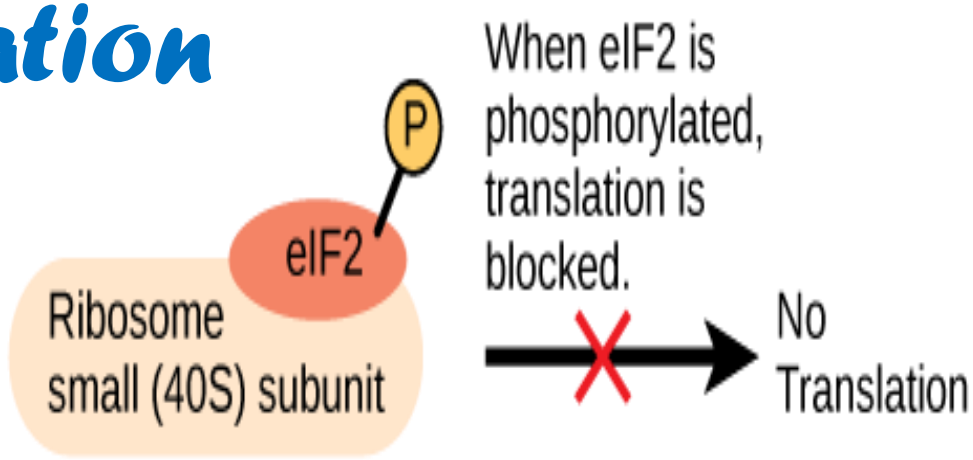
- Translation initiation

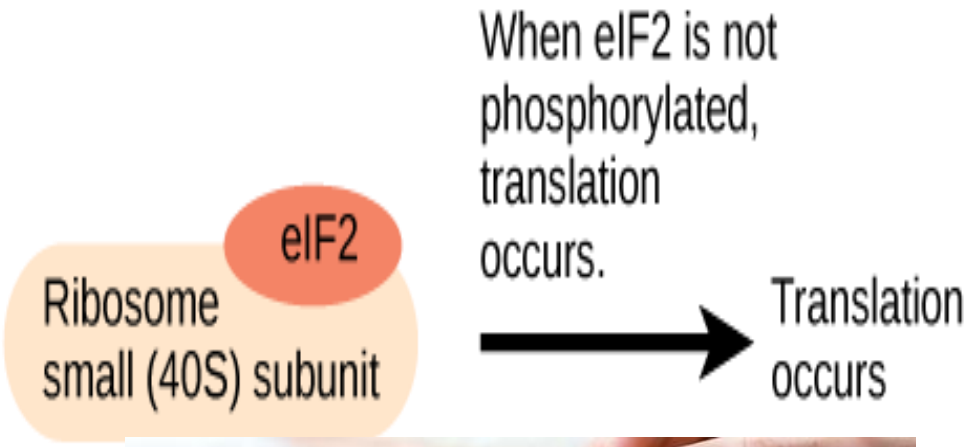
-by control activity of initiation factors (If-2)

EX :

-Heme: ↑ globin synthesis by preventing phosphorylation of IF-2.

-Interferon (anti-viral drug) stimulates IF-2 phosphorylation so inhibit translation leads to decrease viral protein synthesis.





- Translation elongation

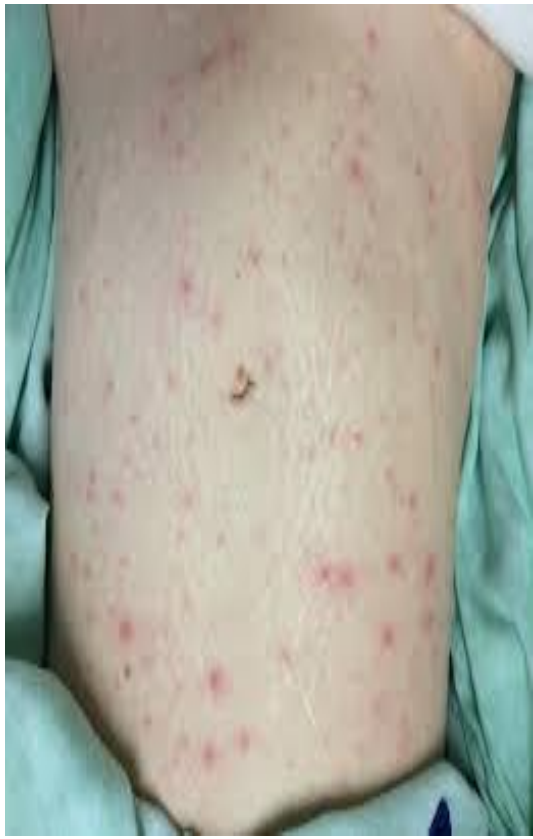
- Diphtheria toxin inhibits EF-2 leads to inhibition of protein synthesis → cell death .

SUMMARY

Level	Mechanism		Example
DNA level	Gene loss		Mature RBCs have no nuclei .
	Gene rearrangement		Antibody gene in β lymphocytes
	Gene amplification		DHFA reductase gene in cancer cells
Transcriptional level	Chromatin remodeling	Chromatin condensation	Loose euchromatin is active , Tight heterochromatin is less active .
		Chromatin acetylation	Repulsion of histone , activates transcription .
		DNA methylation	Inhibits gene transcription (maintains turn off)
	DNA regulatory regions	Enhancer	Binds to activator to activate gene transcription
		Silencer	Binds to repressor to inhibits gene transcription
Post-transcriptional	Alternative splicing		Different types of m.RNA (calcitonin gene)
	m.RNA stability		Different half lives of m.RNA
Translation level	Initiation		Interferon inhibits IF-2 but heme activates it
	Elongation		Diphtheria toxin inhibits EF-2

Case scenario

Male patient, 48-year-old presented to the hospital with severe fatigue, bone ache, tiny red spots in skin and easy bleeding . The patient had been diagnosed with acute leukemia . He had been taking methotrexate, but the patient develops resistance to the drug.



1- Explain mechanism of action of methotrexate ?

Inhibits dihydrofolate reductase leads to decrease in thymine synthesis which leads to decrease in DNA synthesis, cell division .

2- Explain mechanism of methotrexate resistance ?

Amplification of dihydrofolate reductase gene, the target for methotrexate

MCQ

1-Enhancer elements :

a-are sites on DNA where RNA polymerase binds

b-they are DNA sequence that are recognized by transcription factors

c-its presence decrease the transcription activity

d-needs co-repressor to act

MCQ

2-Amplification of DHFA reductase gene develop drug resistance to :

a-folic acid

b-5-Flurouracil

c-trimethoprim

d-methotrexate

MCQ

3-Histone acetylation results in :

a-inhibition of gene expression

b-enhances process of transcription

c-makes DNA more compact

d-makes histones more +ve charge

e-denaturation of histones

best wishes