BHARATHIDASAN UNIVERSITY
Tiruchirappalli- 620024
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Programme: M.Sc., Biochemistry

Course Title : Biochemistry of Signal Transduction
Course Code : BC203CR

Unit-I
Regulation of transcription and translation

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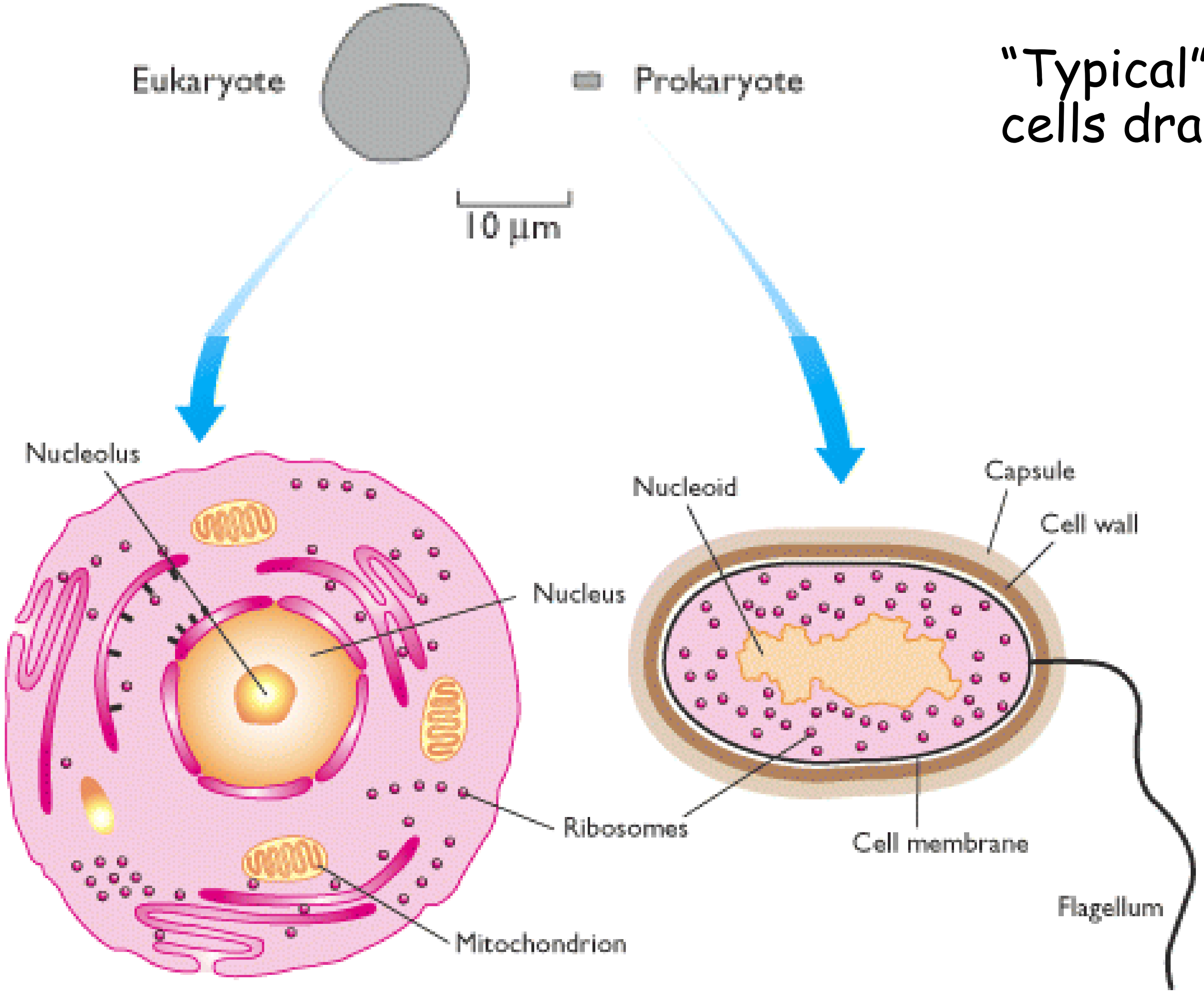
Professor

Regulation of gene transcription in prokaryotes and eukaryotes

Eukaryotes vs Prokaryotes



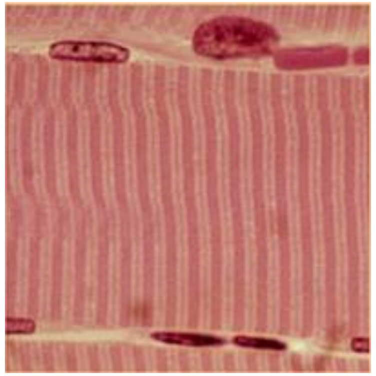
“Typical” human & bacterial cells drawn to scale.

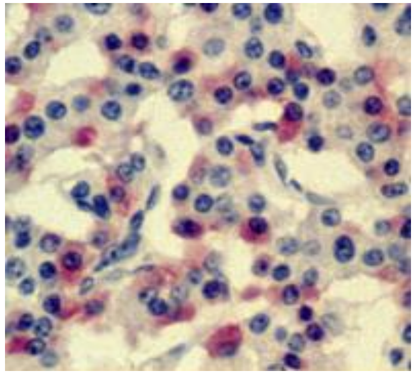
Eukaryotic cells are characterized by membrane-bound compartments, which are absent in prokaryotes.

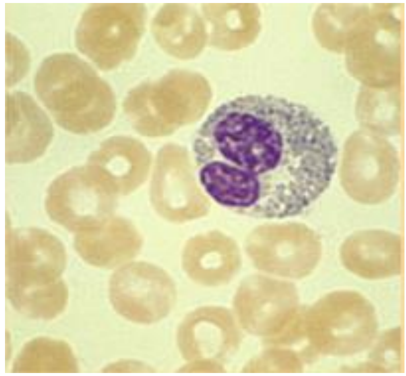
INTRODUCTION

- ❑ DNA, the chemical vehicle heredity, is composed of **functional units**, namely **genes**.
- ➢ The term **genome** refers to the total genetic information contained in a cell.
- ➢ The bacteria E.coli contains about 4,400 genes present on a single chromosome.
- ➢ The **genome of humans** is more complex, with 23 pairs of (diploid) chromosome containing 6 billion (6×10^9) base pairs of DNA, with an estimated **30,000 – 40,000 genes.**

GENE EXPRESSION IN DIFFERENT CELLS







Genes for	Muscle cell	Pancreatic cells alpha cells	 beta cells	Blood cells WBCs	 RBCs
Glycolysis	ON	ON	ON	ON	ON
Insulin	OFF	OFF	ON	OFF	OFF
Glucagon	OFF	ON	OFF	OFF	OFF
Hemoglobin	OFF	OFF	OFF	OFF	ON

GENE EXPRESSION & REGULATION

❑ Organisms adapt to environmental changes by altering gene expression.

❑ The regulation of gene expression of genes is necessary for the:

- growth
- development
- differentiation &
- very existence of the organism.

- ❑ The process of alteration of gene expression has been studied in details in prokaryotes.
  - ➢ It generally involves the interaction of specific binding proteins with various regions of DNA in the immediate vicinity of the transcription site and this produces either a positive or negative effect on transcription.
- ❑ In eukaryotes, studies are not extensive and not well understood.
  - ➢ Cells in eukaryotes utilise the same basic principle but uses other mechanisms to regulate transcription.

Types of Gene Expression:

- ❑ Mainly 2 types of gene expression & regulation:-
  - a. Positive regulation &
  - b. Negative regulation.

a. Positive regulation:-

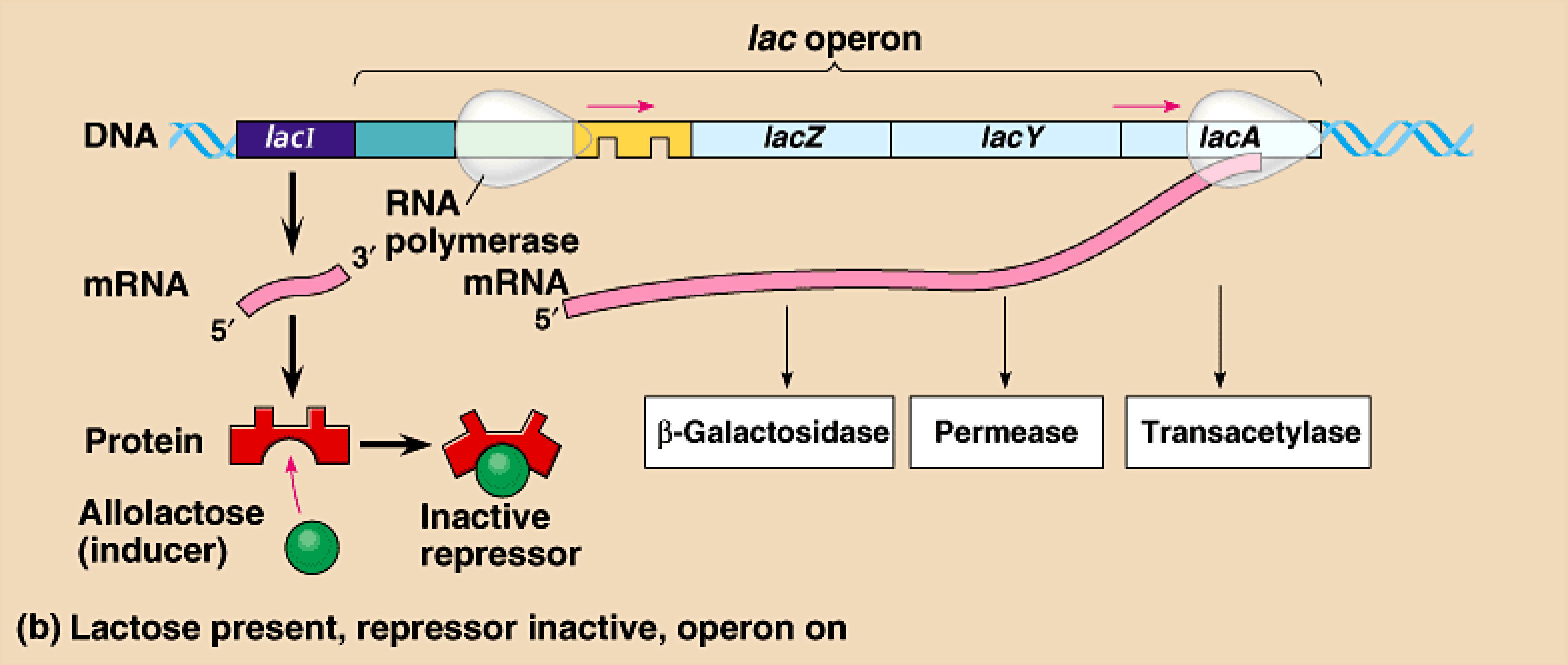
- ✓ When the expression of genetic information is quantitatively increased by the presence of specific regulatory element, it is called as positive regulation.
- ✓ The element or molecule mediating positive regulation is called positive regulator.

NOTE: A double negative has the effect of acting as a positive. An effector that inhibits the function of a negative regulatory appears to bring about a positive regulation.

Lactose operon

What happens when lactose is present?

Need to make lactose-digesting enzymes



(b) Lactose present, repressor inactive, operon on

Lactose is allosteric regulator of repressor protein

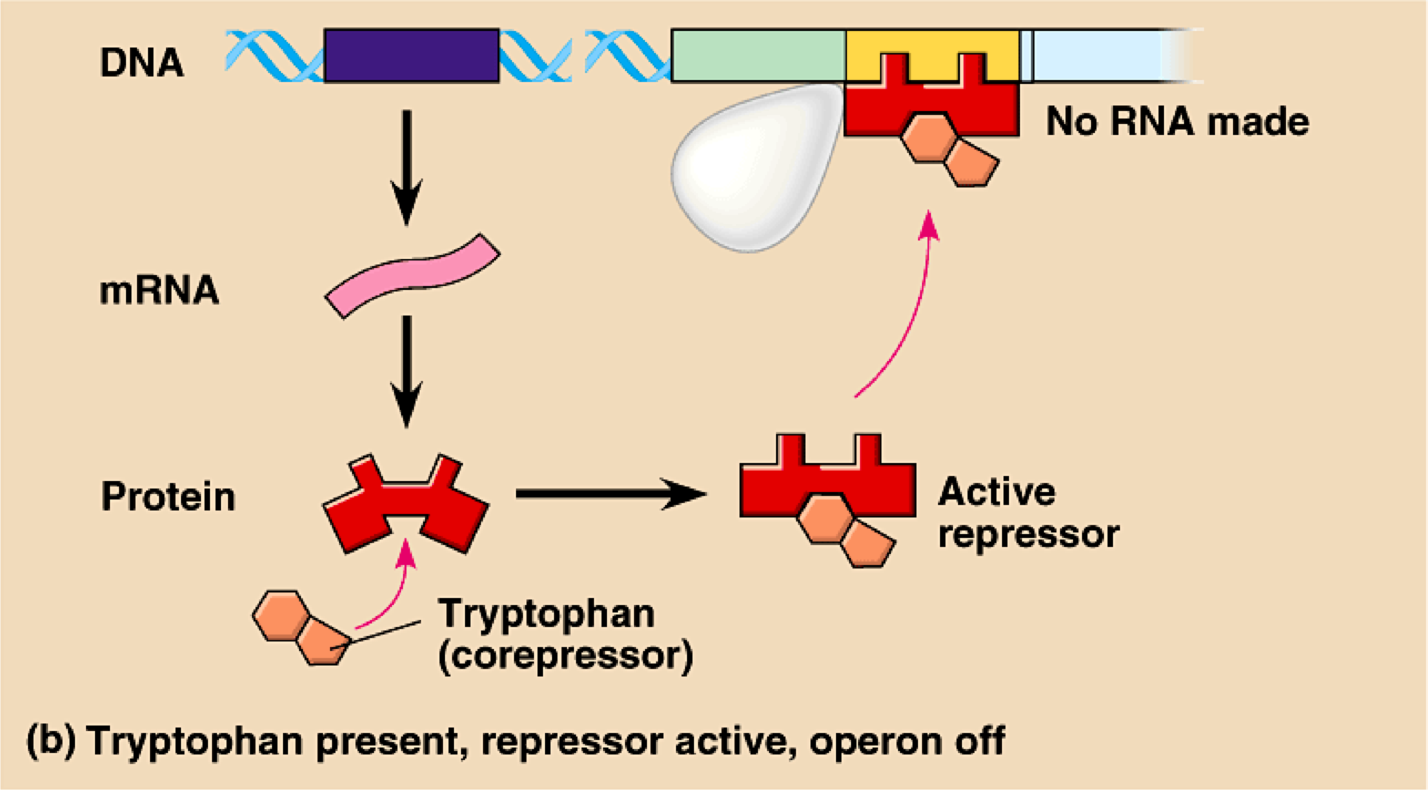
b. Negative regulation:-

- ✓ When the expression of genetic information is quantitatively decreased by the presence of specific regulatory element, it is called as negative regulation.
- ✓ The element or molecule mediating positive regulation is called negative regulator.

Tryptophan operon

What happens when tryptophan is present?

Don't need to make tryptophan-building enzymes



(b) Tryptophan present, repressor active, operon off

Tryptophan is allosteric regulator of repressor protein

Type of genes: based on the functions

- ❑Two types-

1. Inducible genes &
2. Constitutive genes.

1. Inducible genes:-

- ✓ The expression of the inducible gene increased in response to an inducer.
- ✓ Inducers are small molecules.
- ✓ Some proteins produced by E.coli, e.g. β-galactosidase are said to be inducible because they are only produced in significant amounts when a specific inducer "Lactose" is present.
- ✓ Tryptophan pyrrolase of liver is induced by tryptophan.

2. Constitutive genes:

- ✓ The constitutive genes are expressed at more or less constant rate in almost all the cells and they are not subjected to regulation.
- ✓ The products of these genes are required all the time in cells.
- ✓ E.g. Enzymes of citric acid cycle.

One Cistron -One Subunit Concept

- ✓ Earlier hypothesis proposed that one gene produces one enzyme or protein and "one gene-one enzyme" concept was introduced.
- ✓ It is now know that some enzymes and protein molecules are composed of two or more non-identical subunits, which cannot be explained by "one gene-one enzyme" theory & so it is not valid.
- ✓ The "cistron" is now considered as the genetic unit coding for the structure of the subunit of an enzyme or protein molecule, acting as the smallest unit of genetic expression.
- ✓ Hence, the "one gene-one enzyme" idea might be more accurately regarded as "one cistron-one subunit concept".

Regulation Gene Expression in Prokaryotes

- **Simple**
- **Understood by operon mechanism**
- **Inducible – lac operon**
- **Repressible – trp operon**

Gene Expression in Prokaryotes

Operon: The concept of operon was introduced by Jacob and Monod in 1961.

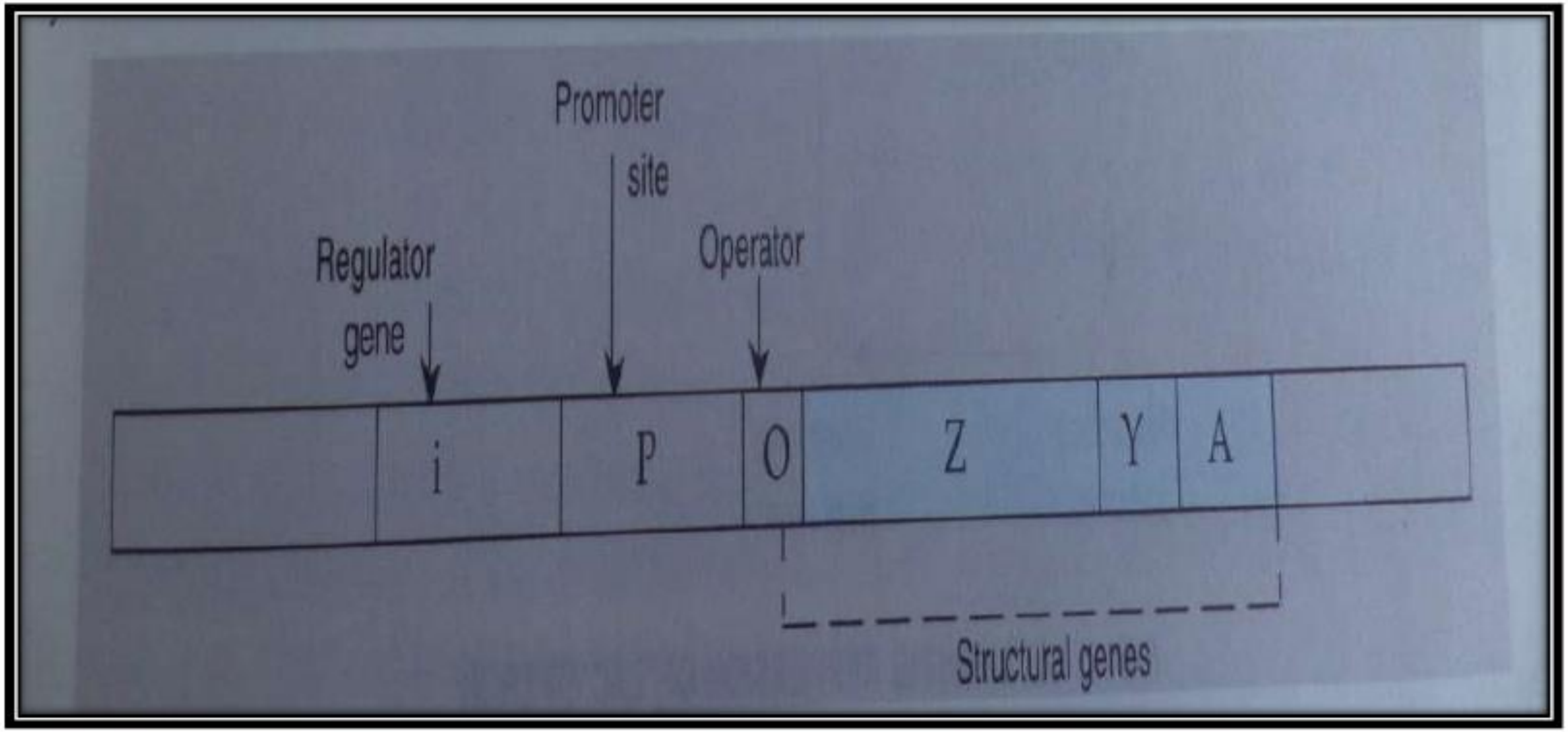
✓ Operon is defined as a segment of a DNA strand consisting of:

- Structure genes: A cluster of several structural genes, which carries the codons which can be translated into proteins.
- Operator genes: One operator gene which has an overall control over the process of translation.

- Regulator gene: A third gene called regulator gene is located sometimes at a distance from the operator gene on the same DNA strand.

✓ Regulator gene transcribe m-RNA which synthesizes “repressor protein” molecules which regulate the transcription.

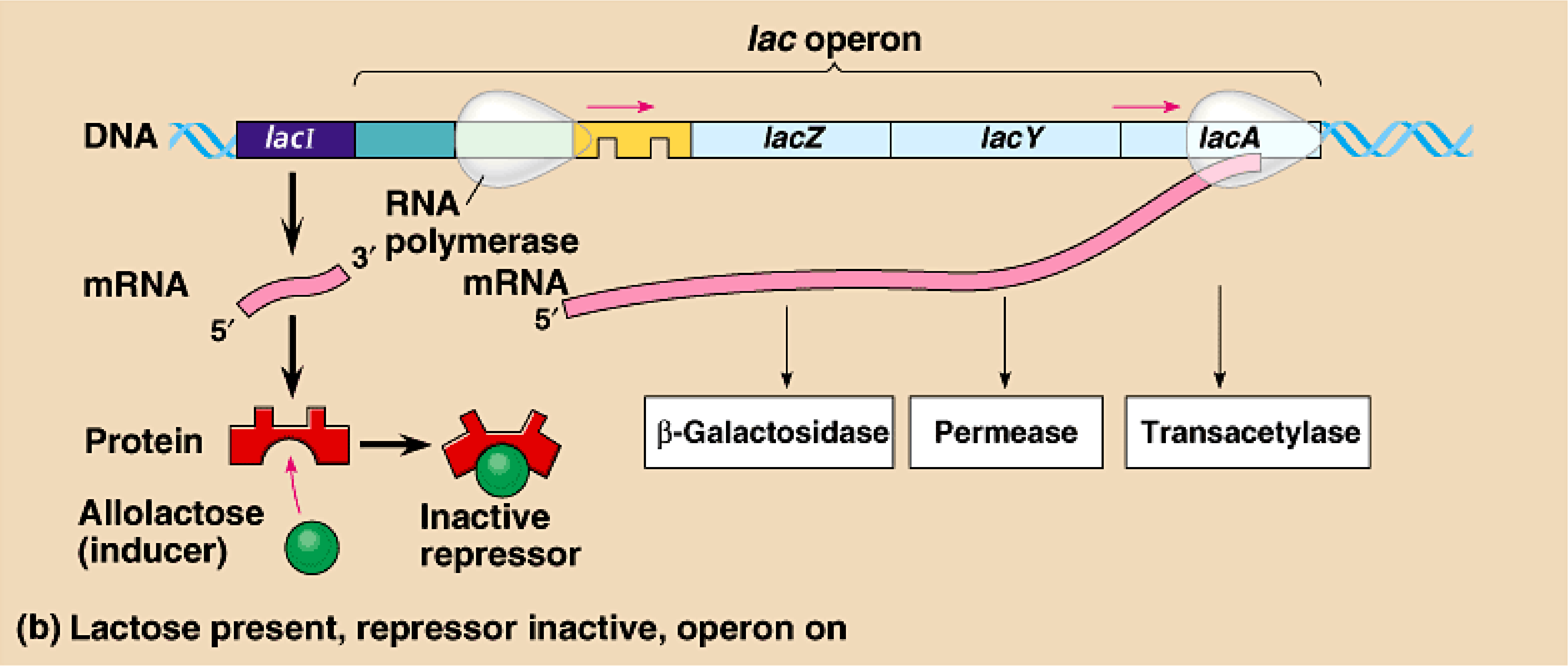
- P site (promoter site): is situated between operator gene & regulator gene.


Promoter site
Regulator gene
Operator
i
P
O
Z
Y
A
Structural genes

Lactose operon

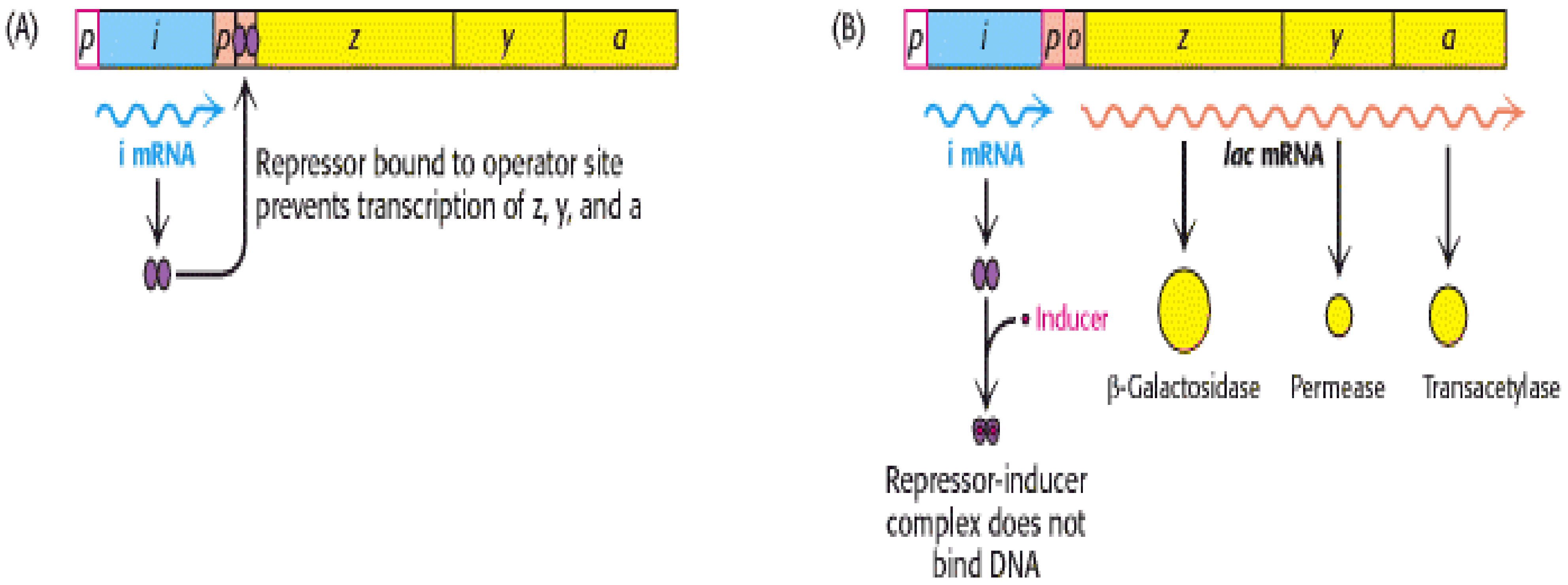
What happens when lactose is present?

Need to make lactose-digesting enzymes

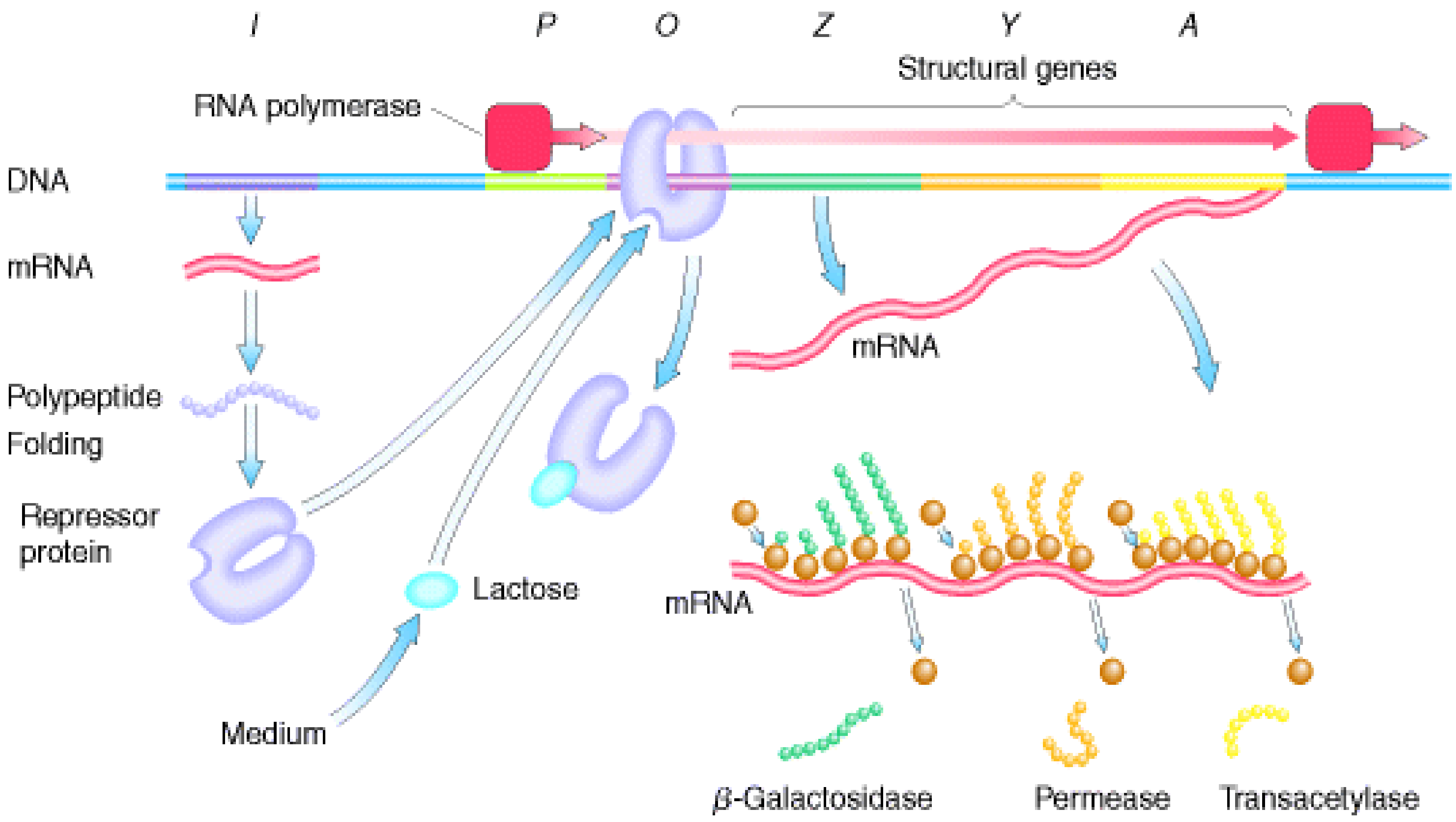


(b) Lactose present, repressor inactive, operon on

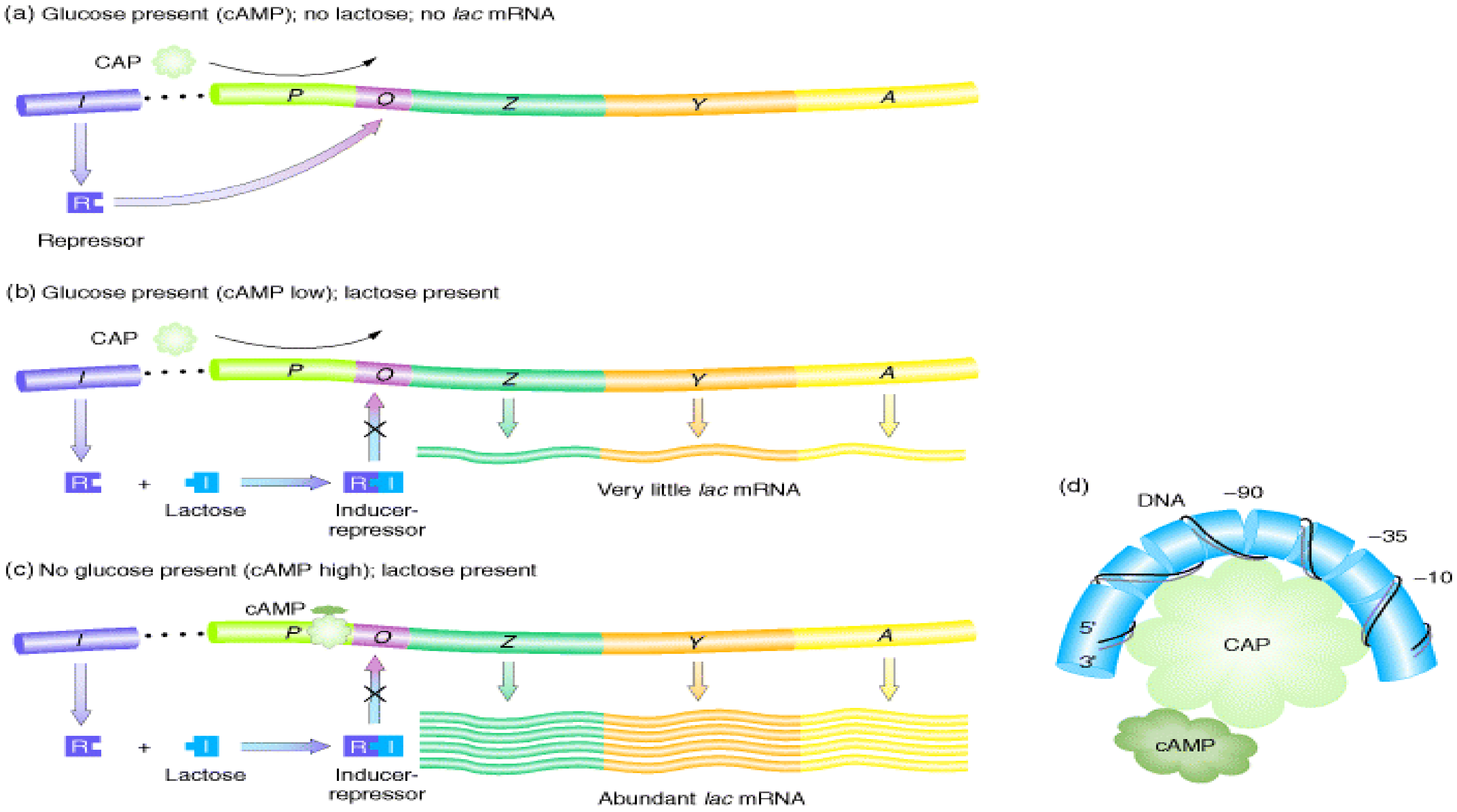
Lactose is allosteric regulator of repressor protein



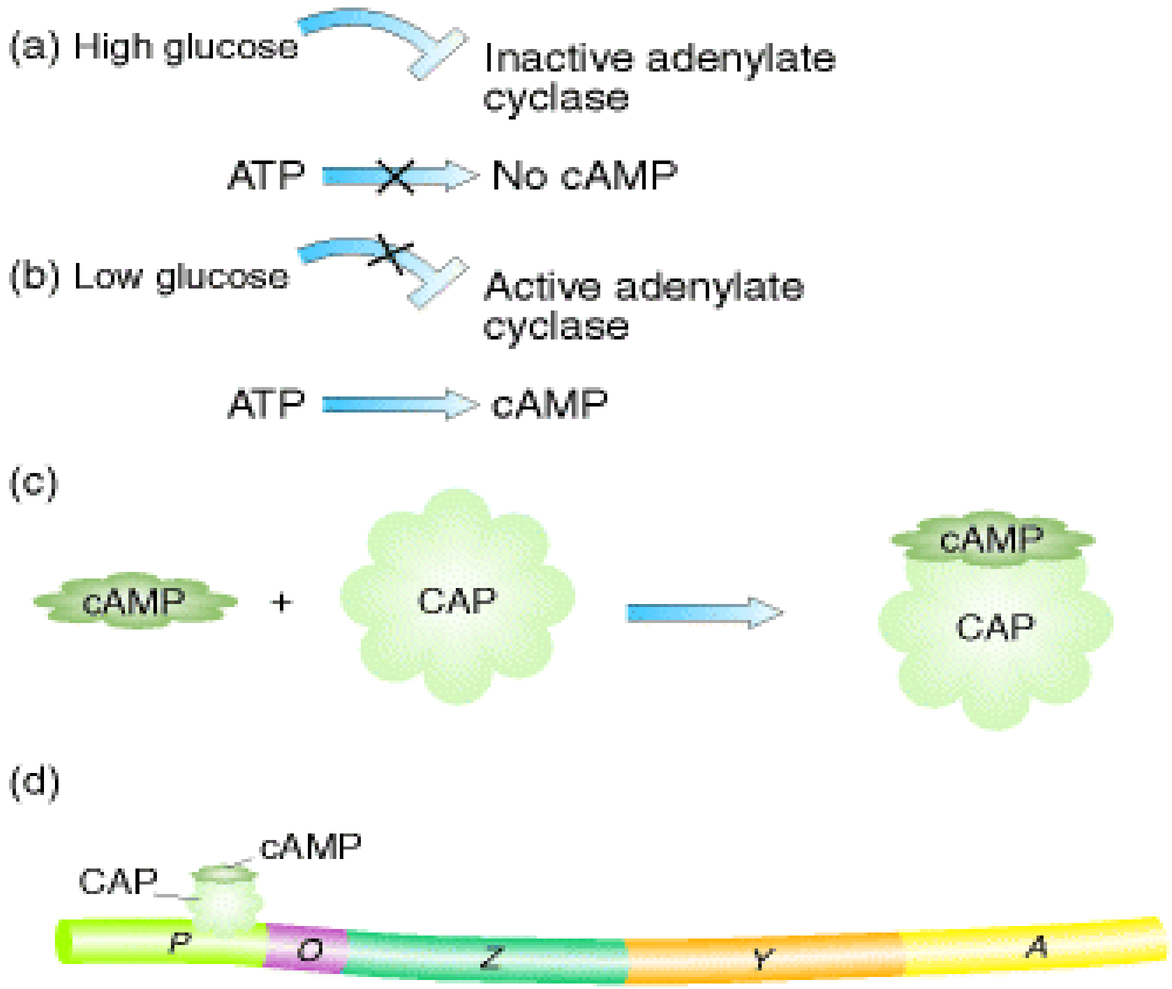
Induction of the *LAC* Operon. (A) In the absence of lactose, the *lac* repressor binds DNA and represses transcription from the *lac* operon. (B) Allolactose or another inducer binds to the *lac* repressor, leading to its dissociation from DNA and to the production of *lac* mRNA.



Regulation of the *lac* operon. The *I* gene continually makes repressor. The repressor binds to the *O* (operator) region, blocking the RNA polymerase bound to *P* (the promoter region) from transcribing the adjacent structural genes. When lactose is present, it binds to the repressor and changes its shape so that the repressor no longer binds to *O*. The RNA polymerase is then able to transcribe the *Z, Y,* and *A* structural genes, so the three enzymes are produced.



Negative and positive control of the *lac* operon by the Lac repressor and catabolite activator protein (CAP), respectively. (a) In the absence of lactose to serve as an inducer, the Lac repressor is able to bind the operator; regardless of the levels of cAMP and the presence of CAP, mRNA production is repressed. (b) With lactose present to bind the repressor, the repressor is unable to bind the operator; however, only small amounts of mRNA are produced because the presence of glucose keeps the levels of cAMP low, and thus the cAMP–CAP complex does not form and bind the promoter. (c) With the repressor inactivated by lactose and with high levels of cAMP present (owing to the absence of glucose), cAMP binds CAP. The cAMP–CAP complex is then able to bind the promoter; the *lac* operon is thus activated, and large amounts of mRNA are produced. (d) When CAP binds the promoter, it creates a bend greater than 90° in the DNA. Apparently, RNA polymerase binds more effectively when the promoter is in this bent configuration. (e) CAP bound to its DNA recognition site. This part is derived from the structural analysis of the CAP–DNA complex

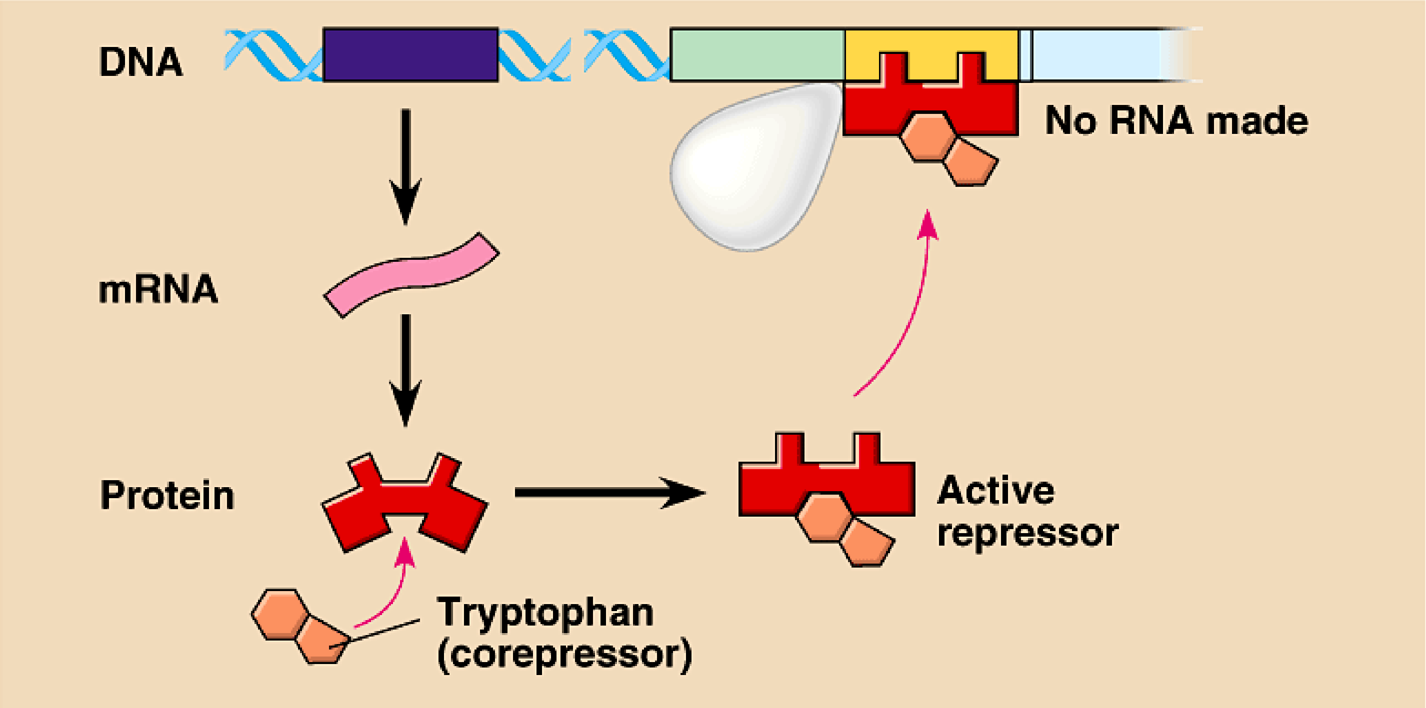


Catabolite control of the *lac* operon. The operon is inducible by lactose to the maximal levels when cAMP and CAP form a complex. (a) Under conditions of high glucose, a glucose breakdown product inhibits the enzyme adenylate cyclase, preventing the conversion of ATP into cAMP. (b) Under conditions of low glucose, there is no breakdown product, and therefore adenylate cyclase is active and cAMP is formed. (c) When cAMP is present, it acts as an allosteric effector, complexing with CAP. (d) The cAMP–CAP complex acts as an activator of *lac* operon transcription by binding to a region within the *lac* promoter. (CAP = catabolite activator protein; cAMP = cyclic adenosine monophosphate.)

Tryptophan operon

What happens when tryptophan is present?

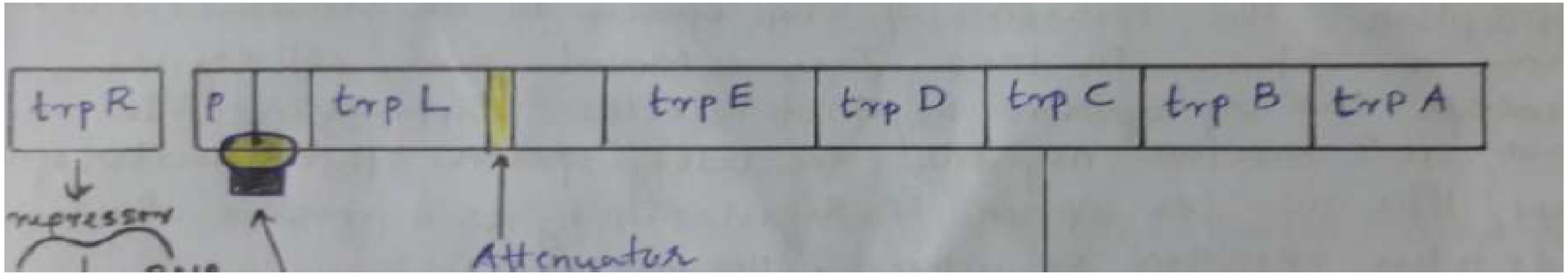
Don't need to make tryptophan-building enzymes



(b) Tryptophan present, repressor active, operon off

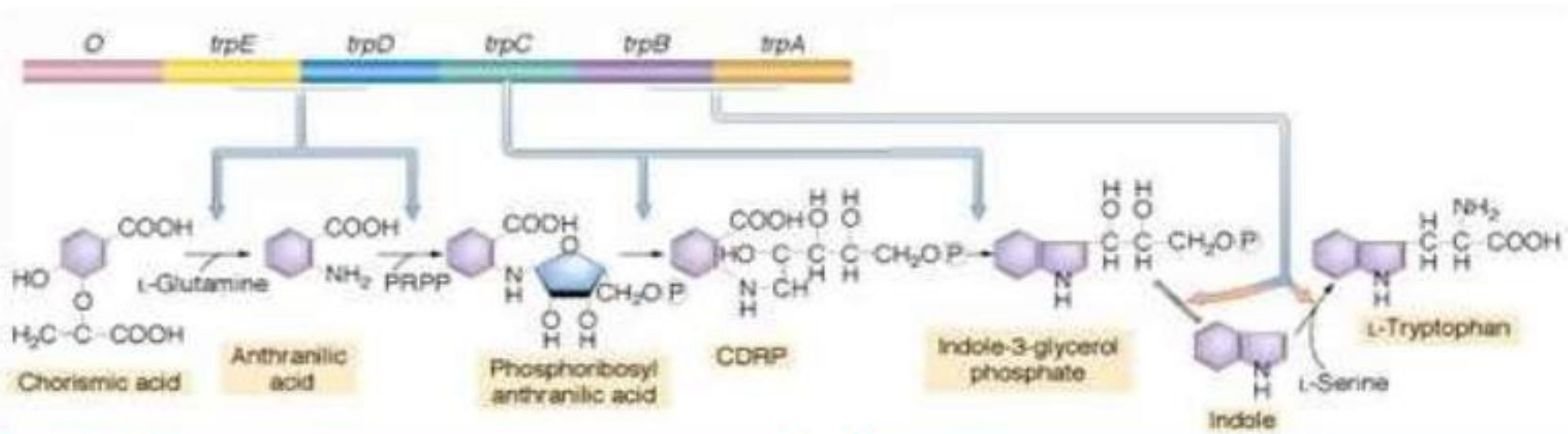
Tryptophan is allosteric regulator of repressor protein

trp OPERON - structure



trp Operon Gene	Gene Function
P/O	**Promoter; operator sequence is found in the promoter**
trp L	**Leader sequence; attenuator (A) sequence is found in the leader**
trp E	**Gene for anthranilate synthetase subunit1**
trp D	**Gene for anthranilate synthetase subunit2**
trp C	**Gene for glycerolphosphate synthetase**
trp B	**Gene for tryptophan synthetase subunit1**
trp A	**Gene for tryptophan synthetase subunit**

1



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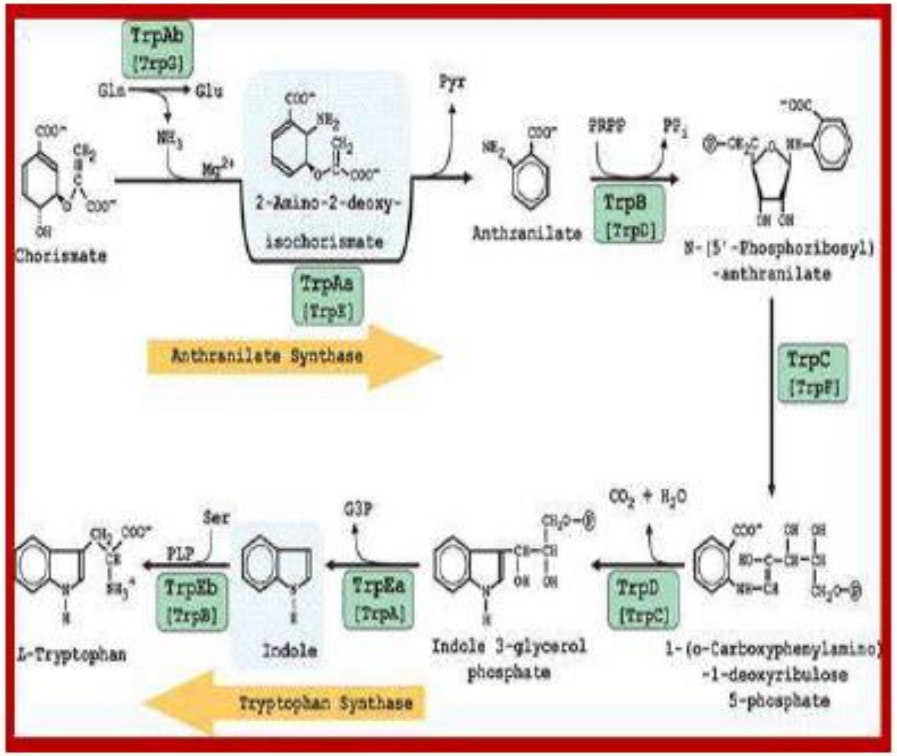

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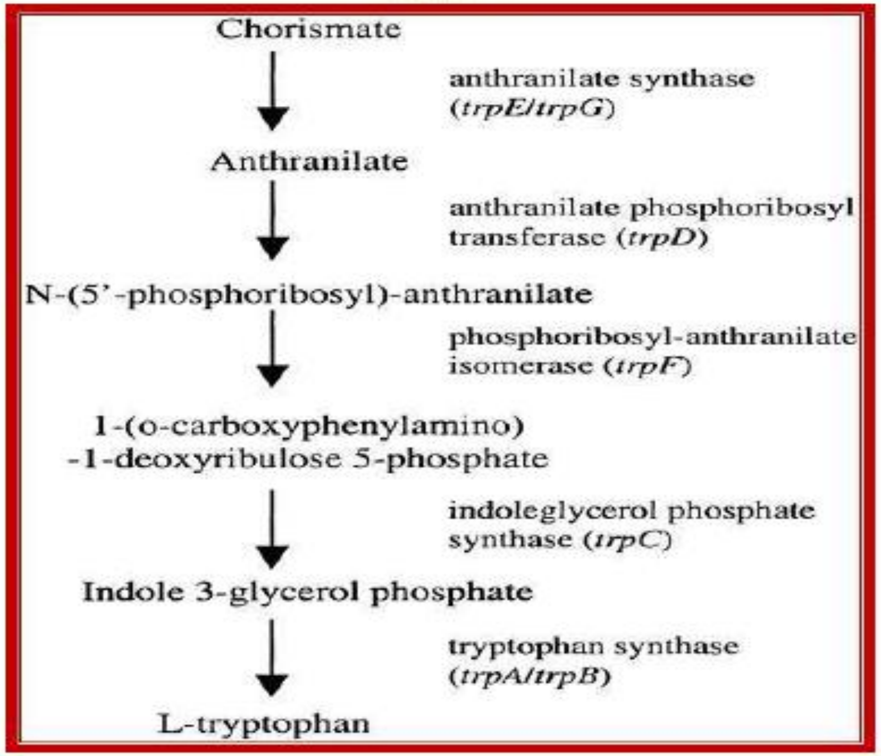


Chorismate

anthranilate synthase (*trpE/trpG*)

Anthranilate

anthranilate phosphoribosyl transferase (*trpD*)

N-(5'-phosphoribosyl)-anthranilate

phosphoribosyl-anthranilate isomerase (*trpF*)

1-(o-carboxyphenylamino) -1-deoxyribulose 5-phosphate

indoleglycerol phosphate synthase (*trpC*)

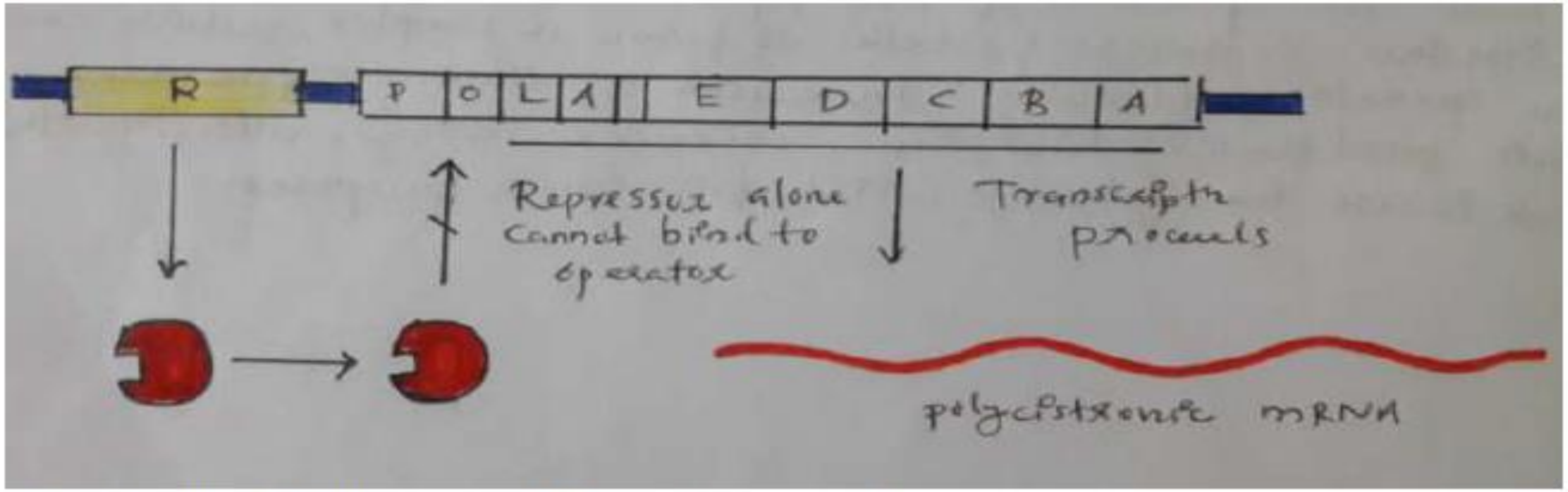
Indole 3-glycerol phosphate

tryptophan synthase (*trpA/trpB*)

L-tryptophan

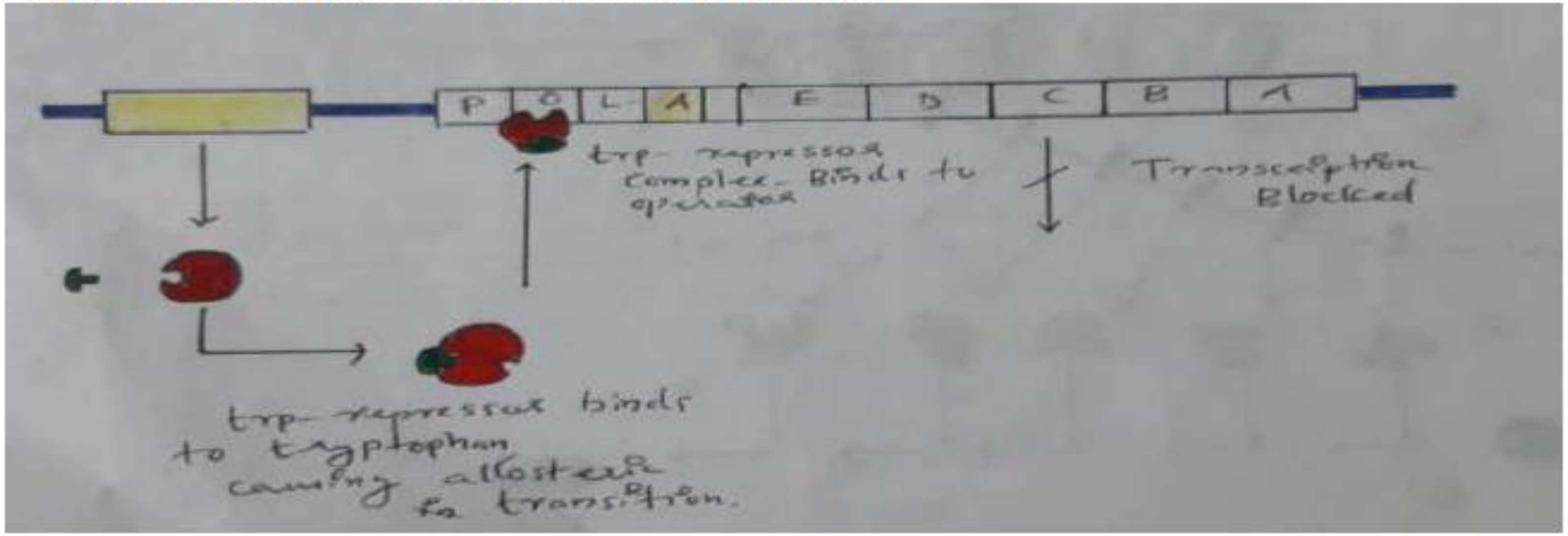
Repression

1



tryptophan is absent in the *environment*

2

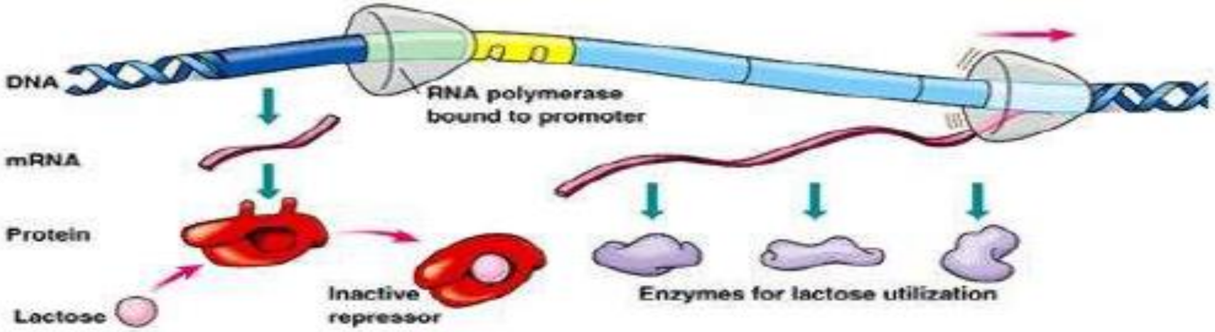


tryptophan is present in the *environment*

Regulation – trp operon

The **trp operon** is controlled by both

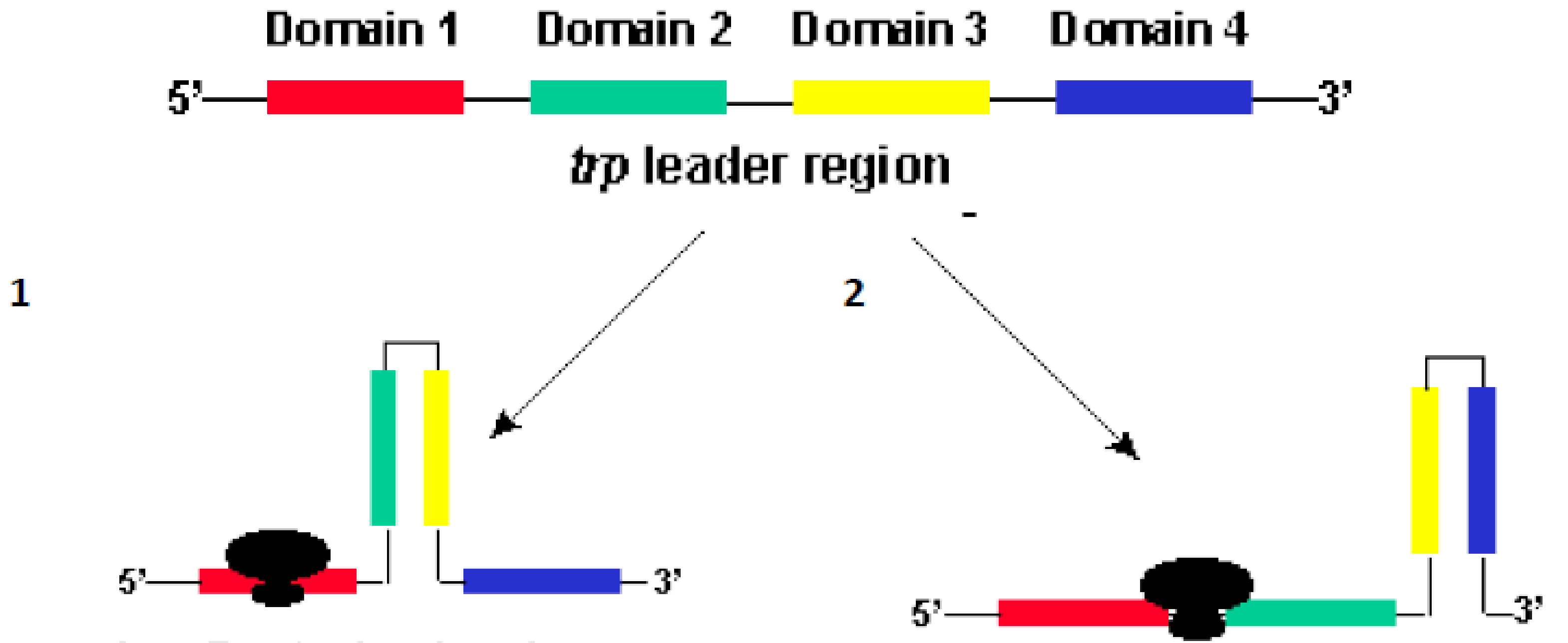
** repressor protein binding to the operator region*



**translation-induced transcriptional attenuation*

Attenuation

Transcriptional attenuation is a regulatory mechanism that causes premature termination of **transcription** under certain conditions



Low Tryptophan Levels

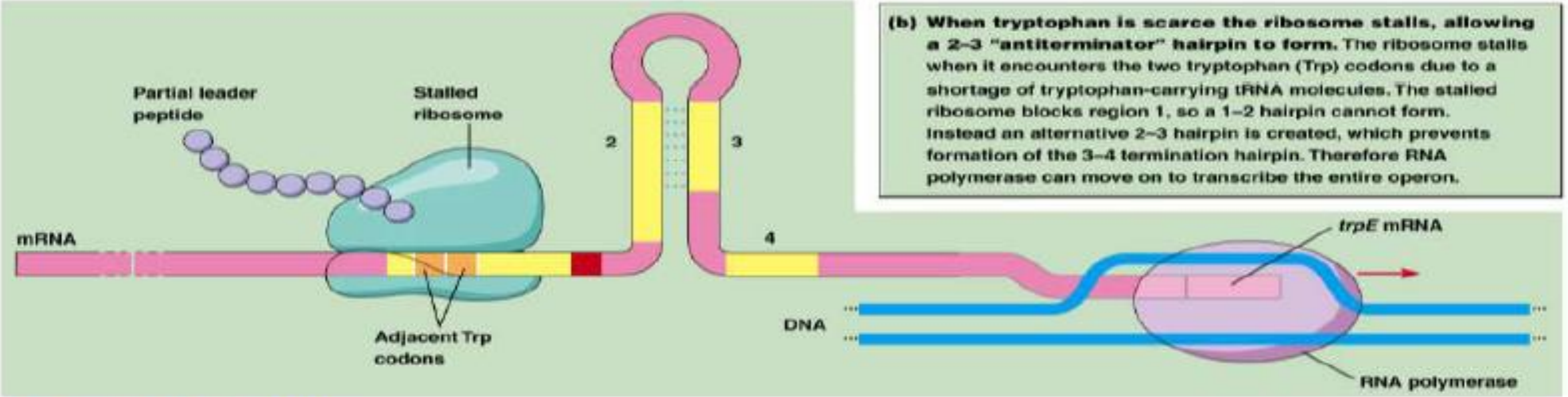
-Slow translation of Domain 1 peptide
-Domain 2-3 pairing occurs
-Normal full gene transcription

High Tryptophan Levels

-Fast translation of domain 1 peptide
-Domain 2 blocked by ribosome
-Domain 3-4 pairing occurs
-Attentuation of transcription occurs
-Only 10% of normal mRNAs made

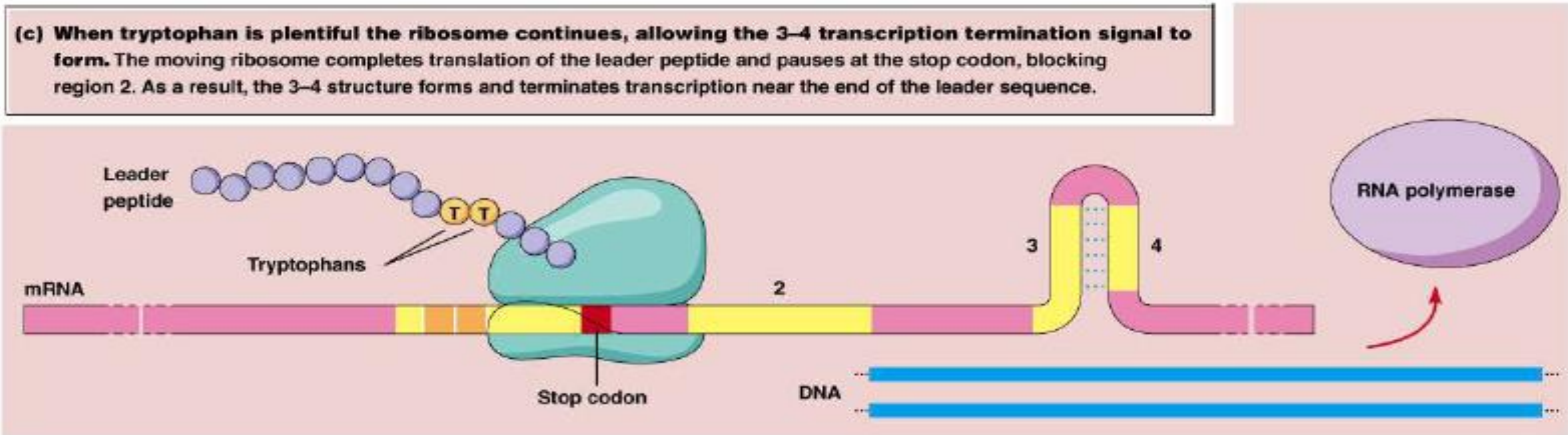
Attenuation loops

anti-termination hairpin



(b) When tryptophan is scarce the ribosome stalls, allowing a 2–3 "antiterminator" hairpin to form. The ribosome stalls when it encounters the two tryptophan (Trp) codons due to a shortage of tryptophan-carrying tRNA molecules. The stalled ribosome blocks region 1, so a 1–2 hairpin cannot form. Instead an alternative 2–3 hairpin is created, which prevents formation of the 3–4 termination hairpin. Therefore RNA polymerase can move on to transcribe the entire operon.

termination hairpin



(c) When tryptophan is plentiful the ribosome continues, allowing the 3–4 transcription termination signal to form. The moving ribosome completes translation of the leader peptide and pauses at the stop codon, blocking region 2. As a result, the 3–4 structure forms and terminates transcription near the end of the leader sequence.

REGULATION OF GENE EXPRESSION IN EUKARYOTES

Gene Regulation Can Take Place at Many Levels

- Chromosome
- Transcription
- Processing of transcripts
- Translation

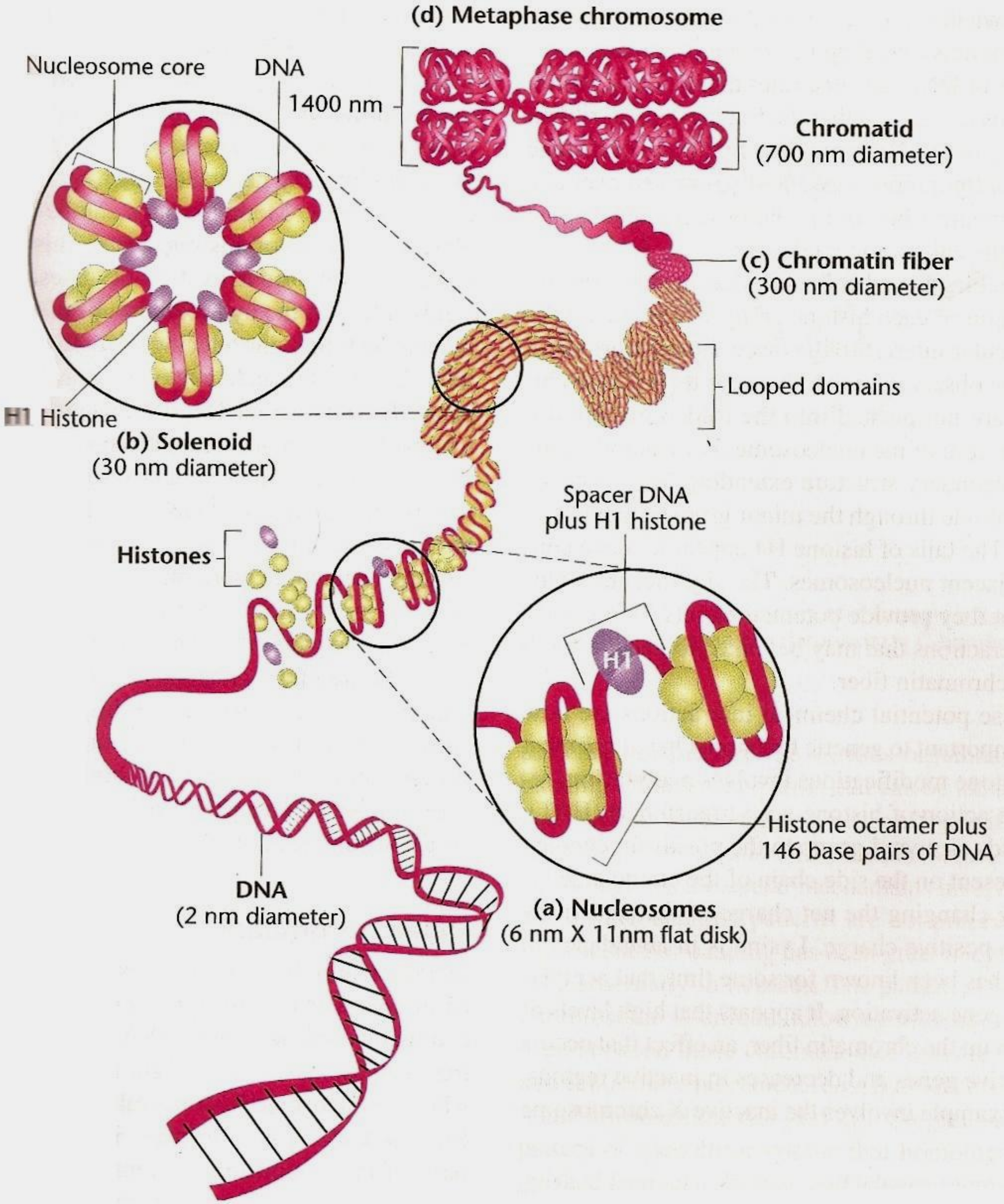
GENE REGULATION AT THE CHROMOSOMAL LEVEL

Two Types of Chromatin

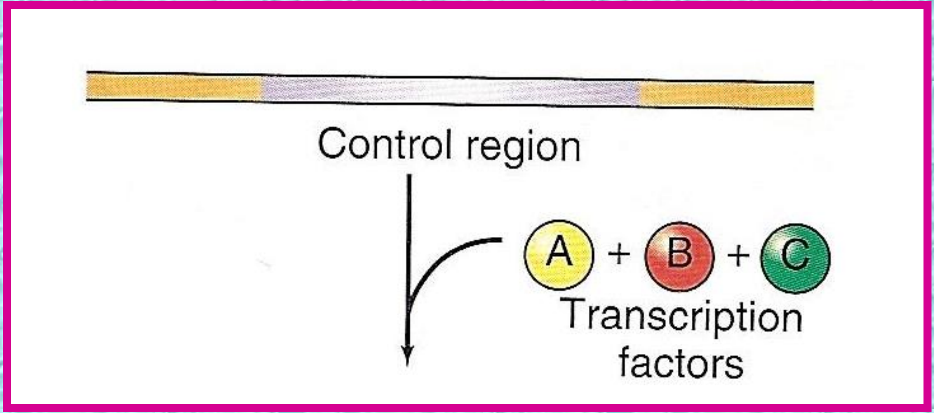
- **HETEROCHROMATIN**
  - **Trancriptionally inactive**
  - **tightly condensed**

- **EUCHROMATIN**
  - **Transcriptionally active**
  - **Relatively relaxed**

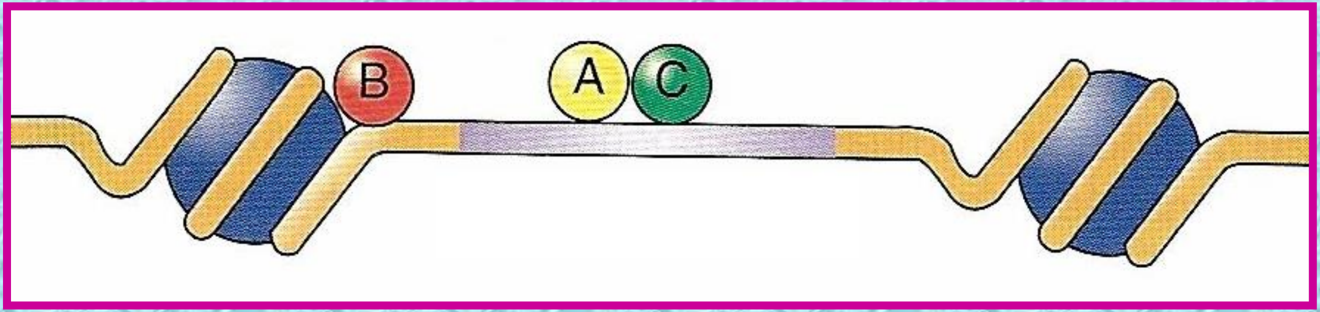
CHROMATIN STRUCTURE



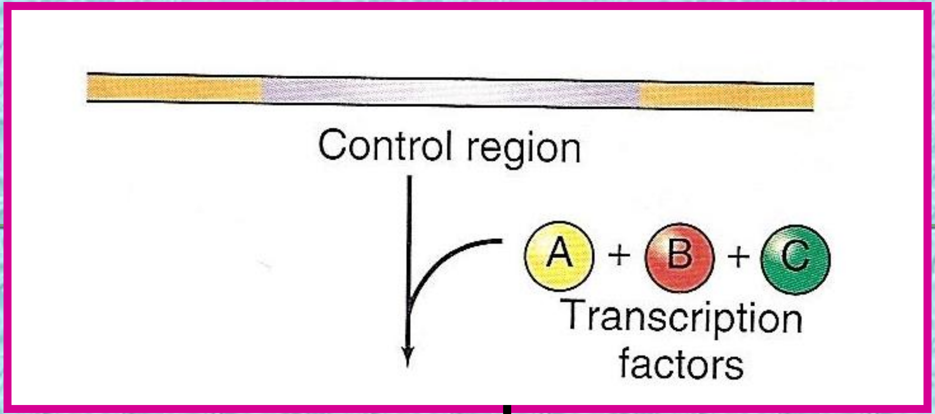
EFFECT OF CHROMATIN STRUCTURE ON TRANSCRIPTION



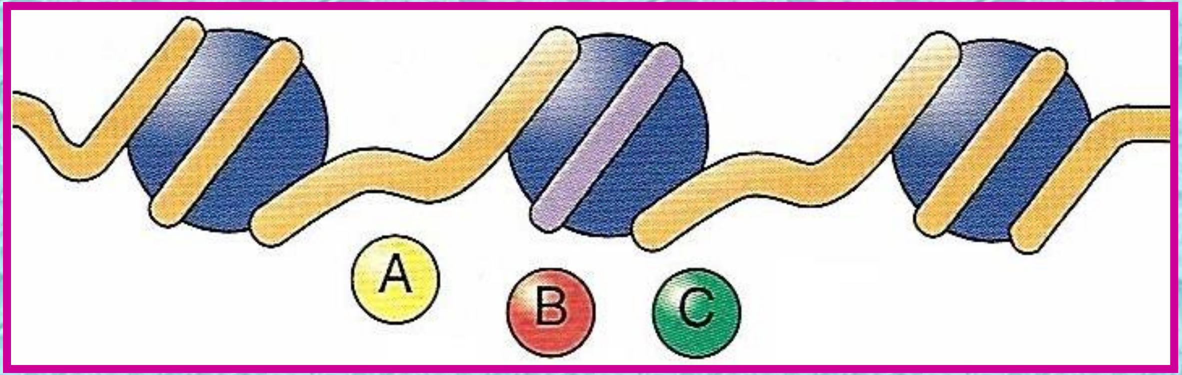
Control region outside nucleosome



GENE IS ACTIVE

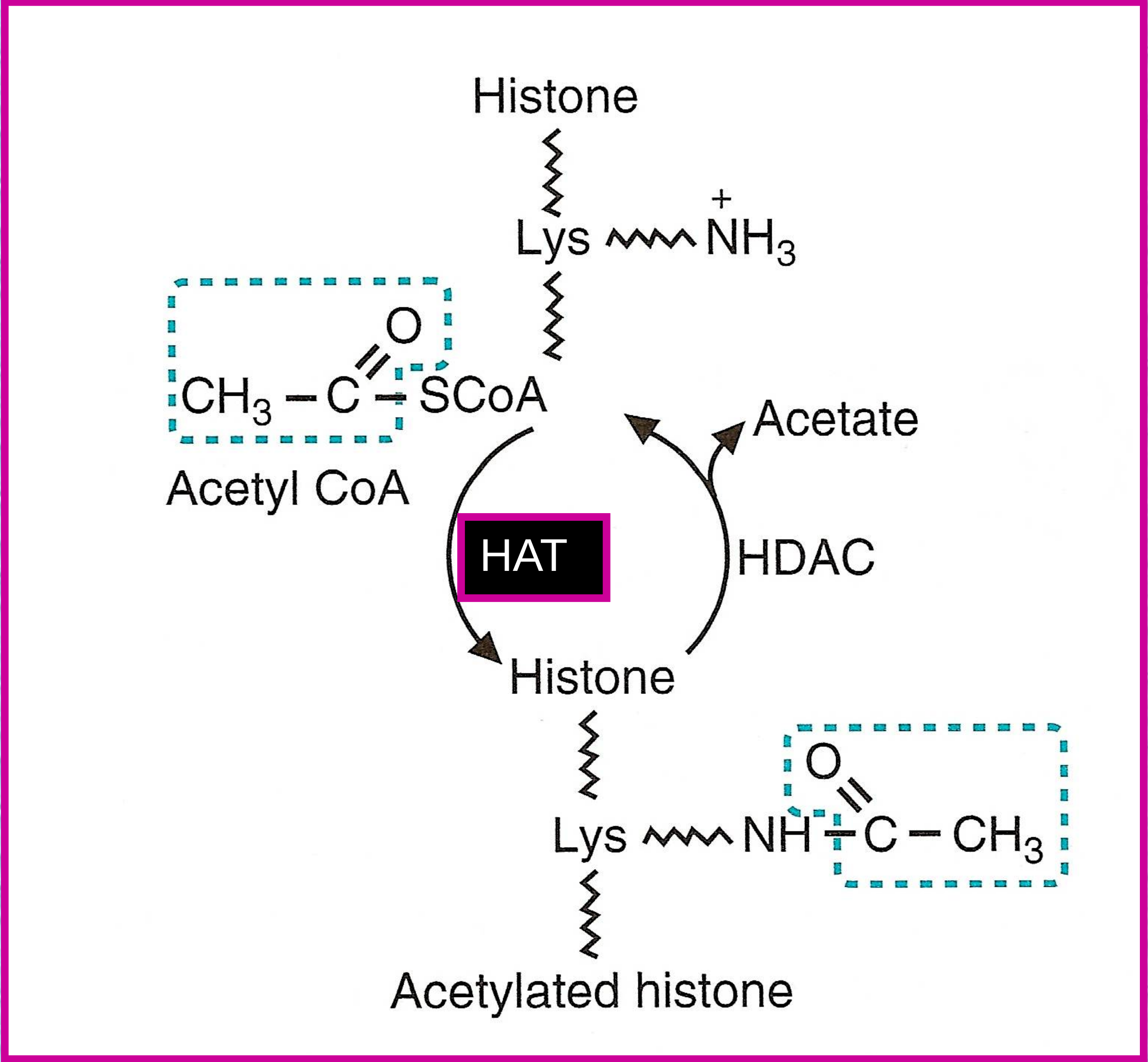


Control region within nucleosome

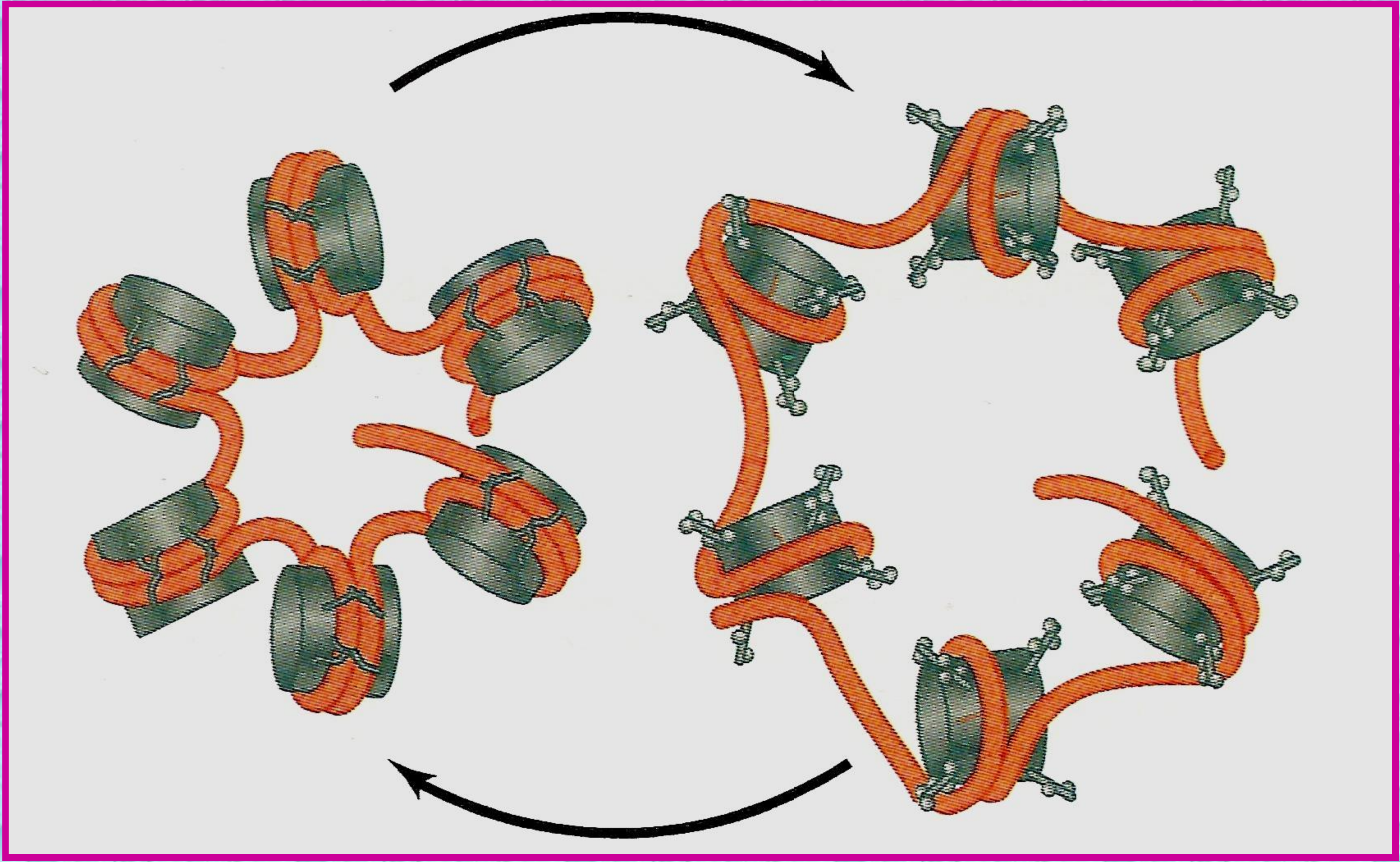


GENE IS INACTIVE

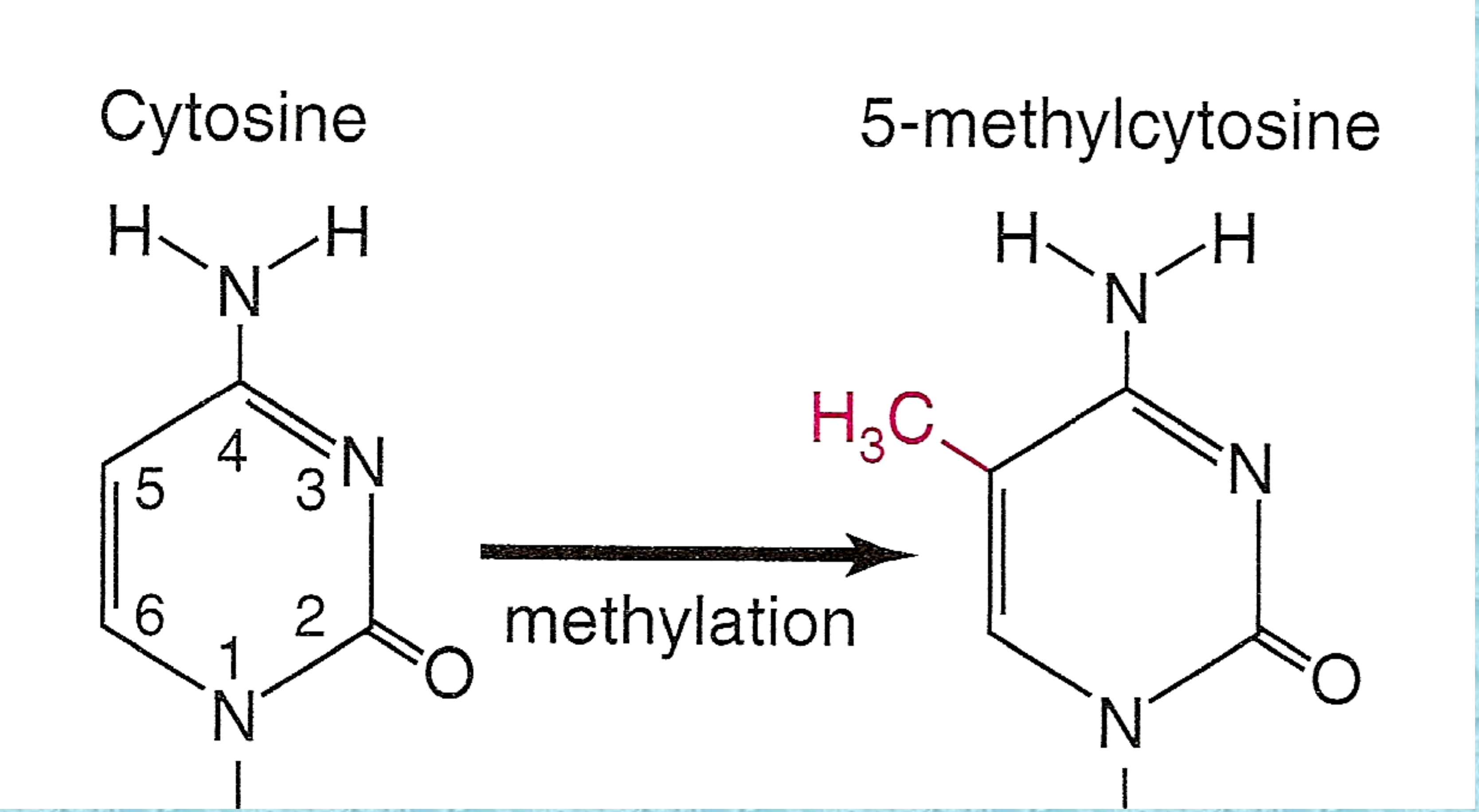
Histone Acetylation

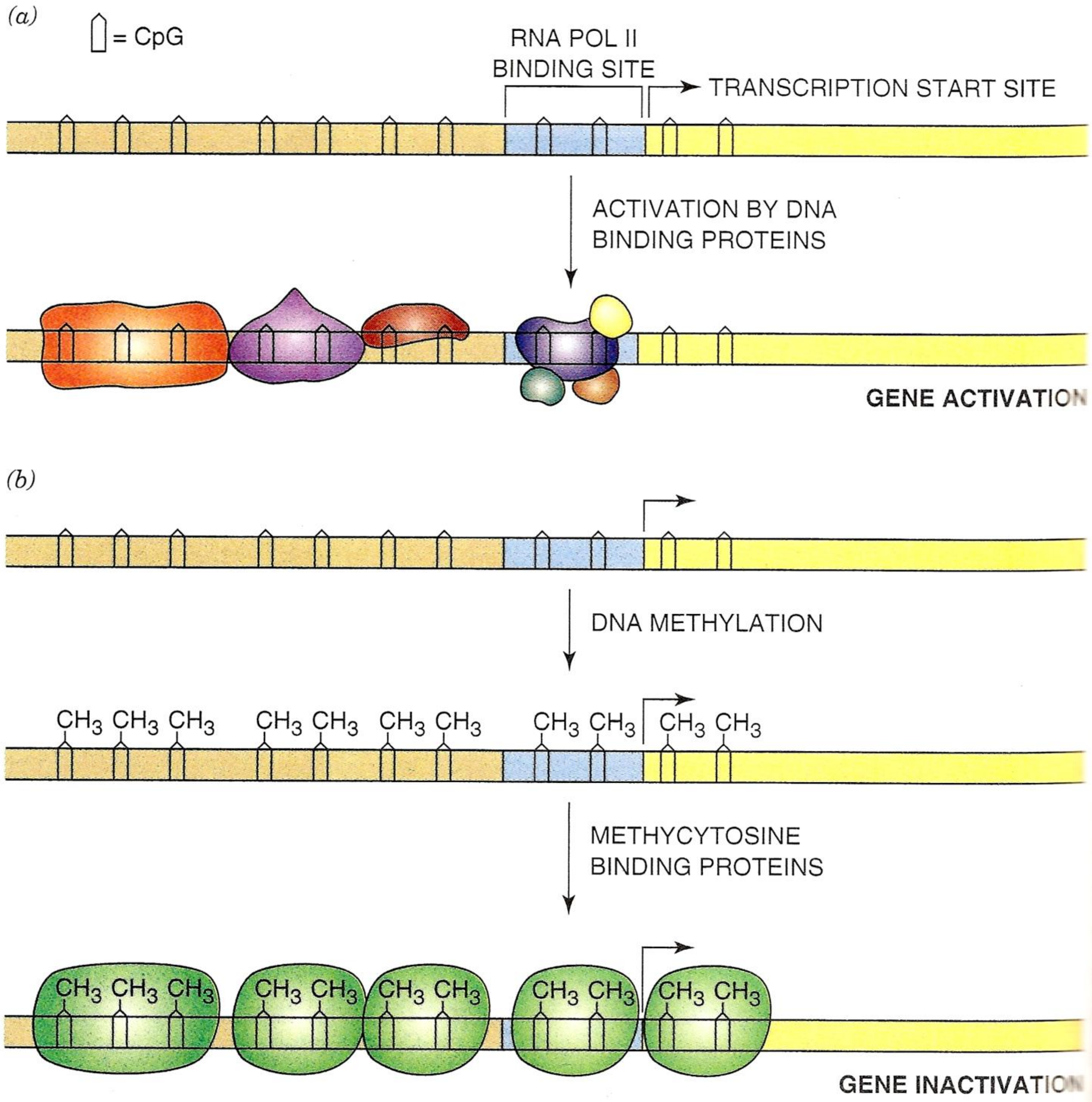


Histone Acetylation



DNA Methylation




(a)
= CpG
RNA POL II
BINDING SITE
TRANSCRIPTION START SITE
ACTIVATION BY DNA
BINDING PROTEINS
GENE ACTIVATION
(b)
DNA METHYLATION
CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3
METHYCYTOSINE
BINDING PROTEINS
CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3 CH_3
GENE INACTIVATION

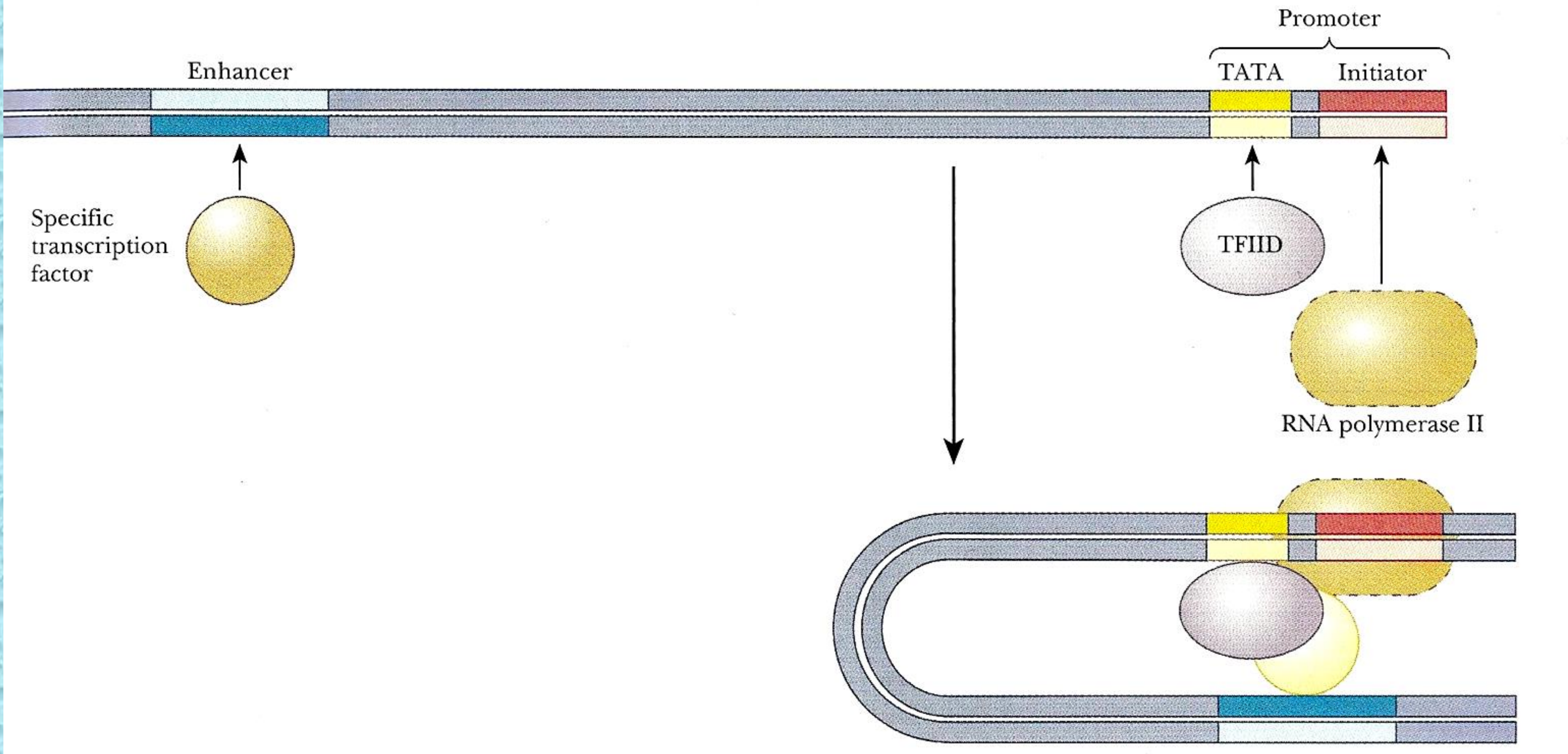
REGULATION AT THE LEVEL OF TRANSCRIPTION

- **GTFs(General transcription factors) only produce a basal level of transcription i.e. very low**
- **Gene-specific factors (activators) are further required to regulate the activity of gene expression**

Enhancers/Silencers

- Upstream or downstream
- Close to the promoter or thousands of base pairs away
- On either of the two strands of DNA
- Act through intermediary or gene specific transcription factors proteins
- Enhancers activate transcription
- Silencers deactivate transcription

Enhancers/Silencers



Response Elements

- Enhancers contain response elements that are responsive to certain metabolic factors

- Cyclic AMP response element (CRE)
- Glucocorticoid response element (GRE)
- Heat shock element (HSE)

- REs bind transcription factors produced under certain cell conditions to activate several related genes

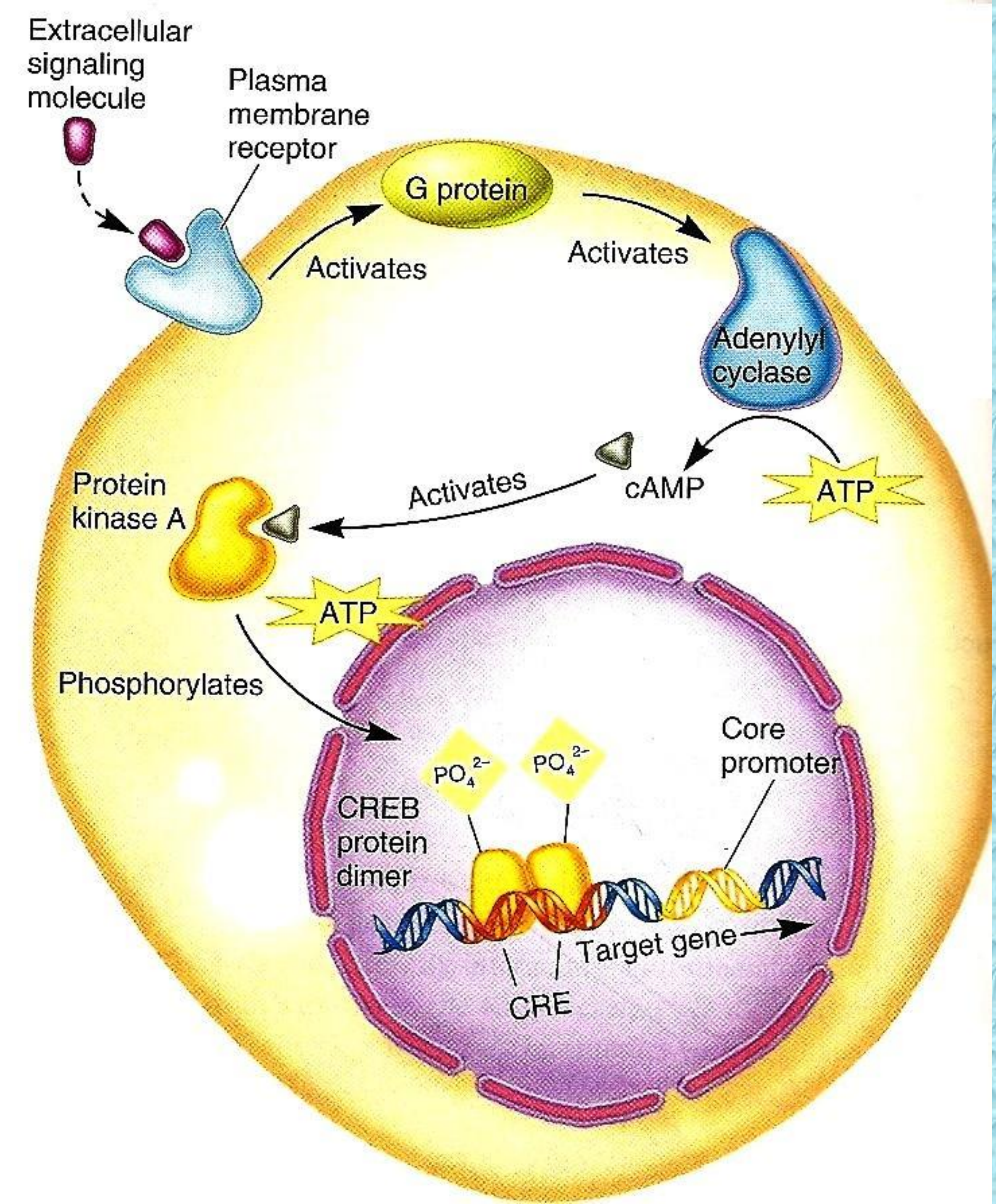
cAMP Response Element (CRE)

5’- TGACGTCA -3’
3’- ACTGCAGT -5’

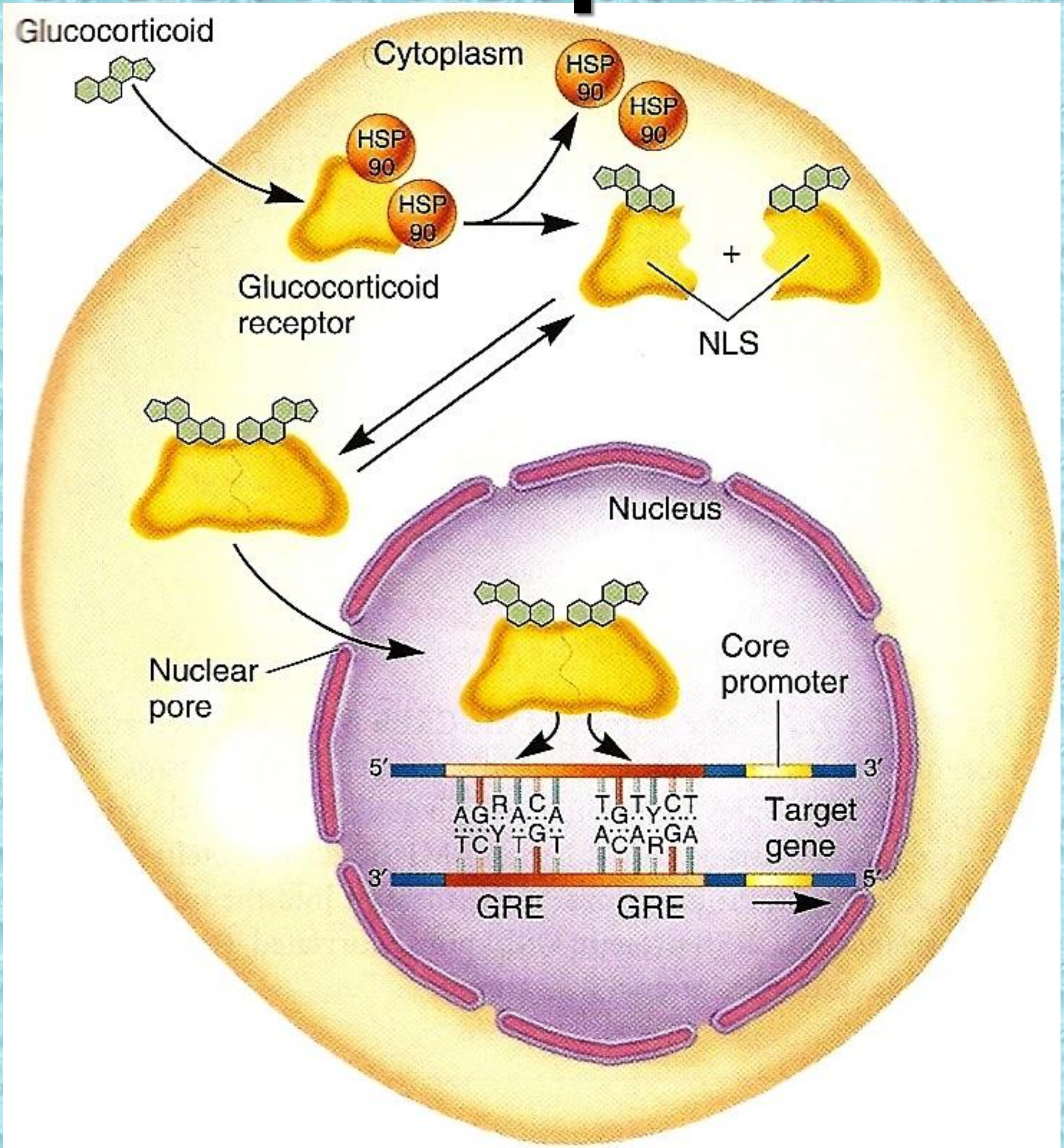
CREB – the most important protein that you have never heard of

- Implicated in
  - Cell proliferation
  - Cell differentiation
  - Spermatogenesis
  - Release of somatostatin (inhibitor growth hormone)
  - Development of T lymphocytes
  - Metabolism of the pineal gland
  - Adaptation to physical stress
  - Transcription of metabolic enzymes
  - Critical in learning and long term memory

Activation of CREB

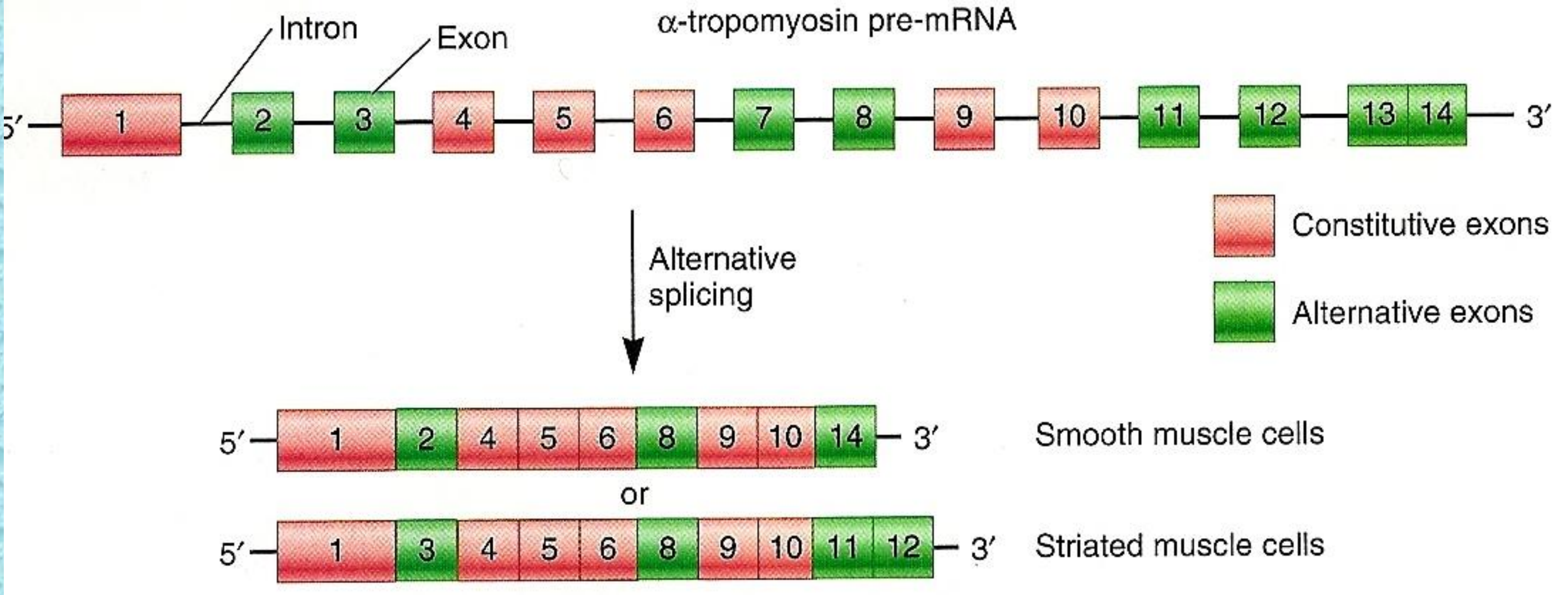


Glucocorticoid Response Element

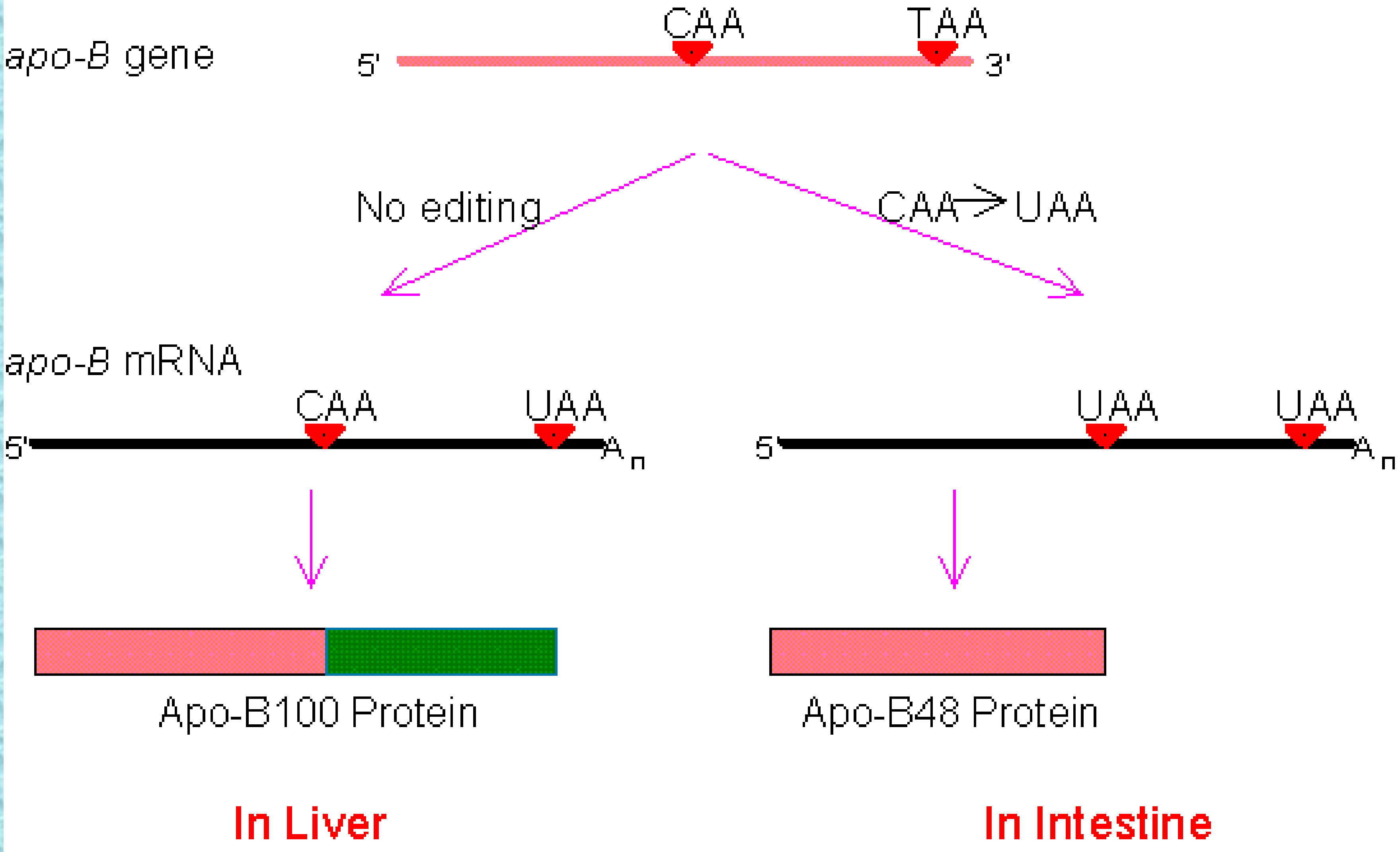


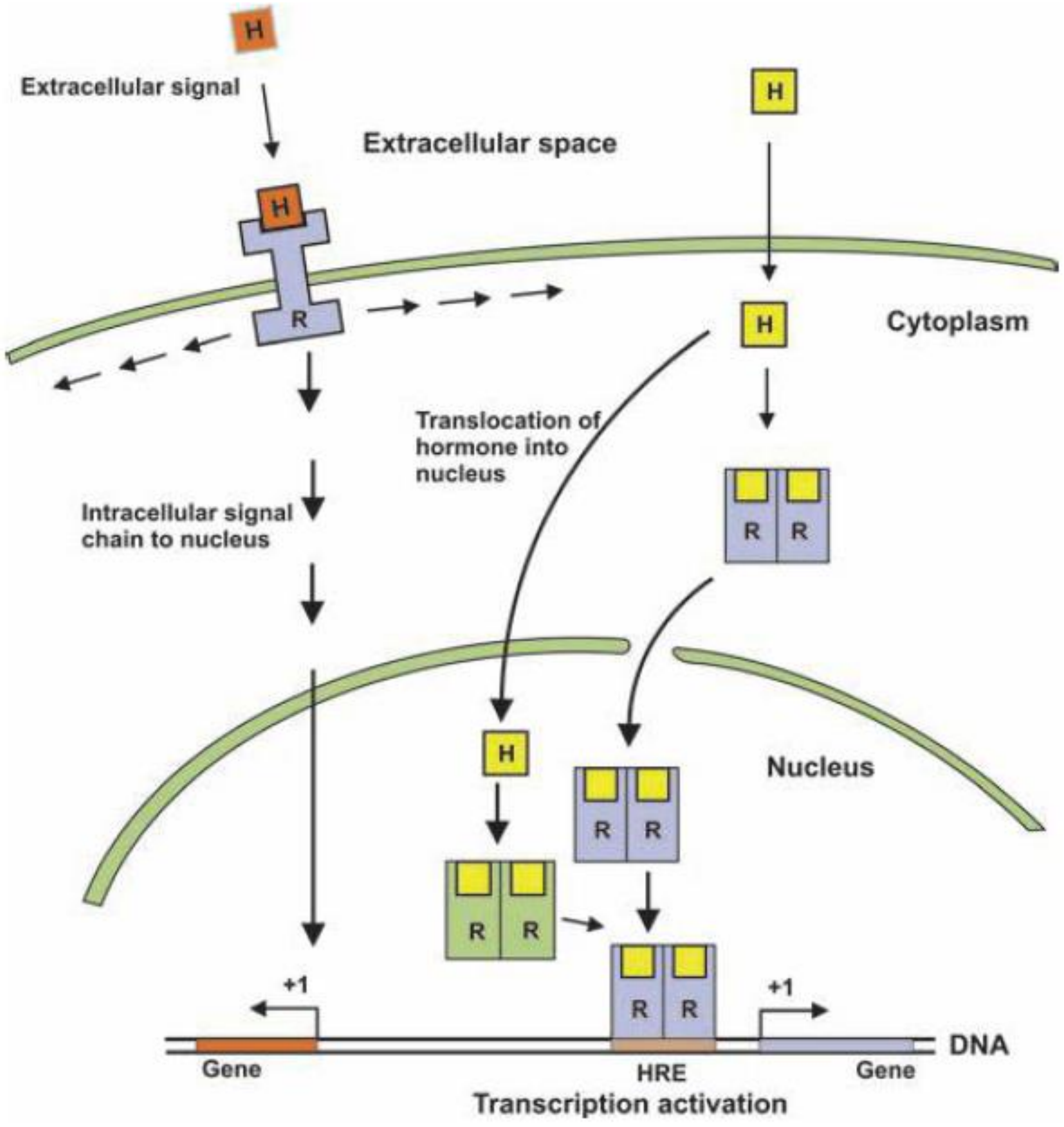
GENE REGULATION BY PROCESSING OF TRANSCRIPTS

Alternative Splicing



RNA EDITING


apo-B gene
5'
CAA
TAA
3'
No editing
CAA → UAA
apo-B mRNA
5'
CAA
UAA
A n
5'
UAA
UAA
A n
Apo-B100 Protein
Apo-B48 Protein
In Liver
In Intestine


H
Extracellular signal
Extracellular space
H
H
R
Cytoplasm
Translocation of hormone into nucleus
Intracellular signal chain to nucleus
R R
Nucleus
H
R R
R R
R R
+1
+1
DNA
Gene
HRE
Gene
Transcription activation

DNA binding motifs in pro-and eukaryotes

- Certain DNA binding proteins having specific motifs bind certain region of DNA to influence the rate of transcription.

- The specificity involved in the control of transcription requires that regulatory proteins bind with high affinity to the correct region of DNA.

- Three unique motifs—the **helix-turn-helix,** the **zinc finger,** and the **leucine zipper**—account for many of these specific protein-DNA interactions.

- The motifs found in these proteins are unique; their presence in a protein of unknown function suggests that the protein may bind to DNA.

- The protein-DNA interactions are maintained by hydrogen bonds and van der Waals forces.

Helix-turn-helix (HTH) motifs: This motif uses a recognition helix that is embedded in the large groove of the DNA. Additional α-helices stabilize the arrangement of the recognition helix. Transcription factors of the forkhead (FH) and homeodomain class belong to the HTH superclass.

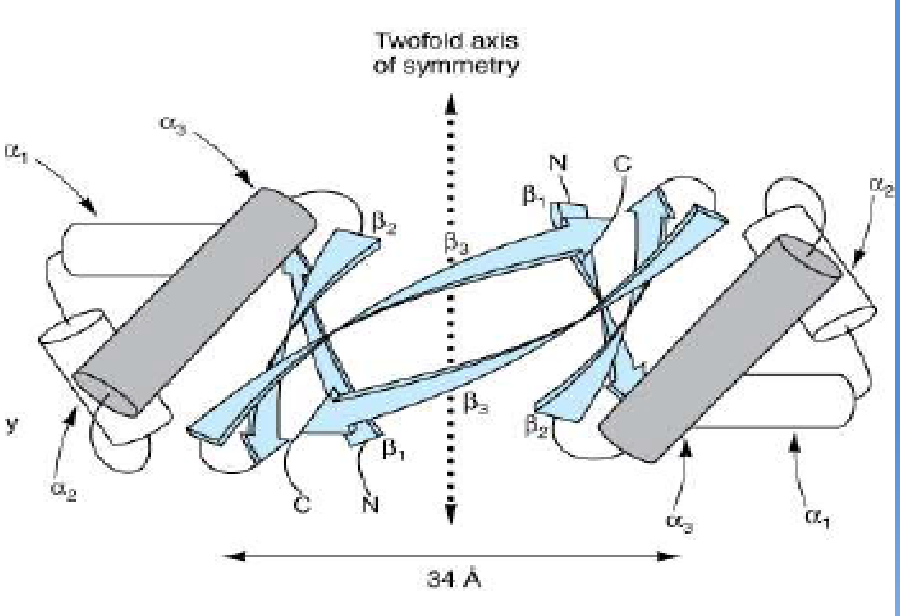
Zn^{2+}-binding motifs: These motifs contain Zn^{2+} complexed by four ligating Cys and/or His residues. Based on the stoichiometry of the complex, zinc fingers of the type Zinc-Cys_2His_2, Zinc-Cys_4 and $Zinc_2$-Cys_6 can be distinguished. The zinc-binding motifs play, above all, a structuring role by ensuring that a recognition helix is correctly oriented and stabilized and they may be involved in dimerization. The nuclear receptors are well-studied examples of transcription factors containing Zn-motifs (see Chapter 6).

Basic motifs: This group of binding motifs displays as a characteristic structural element an extended bundle of two α-helices that are wound around each other in the form of a "coiled-coil." At their end is a basic region which mediates the DNA binding. The basic region is often unstructured in the absence of DNA, and adopts a distinct structure only upon binding to the recognition element. This motif is used mainly for dimerization of transcription factor. Subclasses are distinguished by the presence of basic leucine zipper or helix-loop-helix motif. Important examples are the Myc/Max transcription factors (Section 16.2.3)

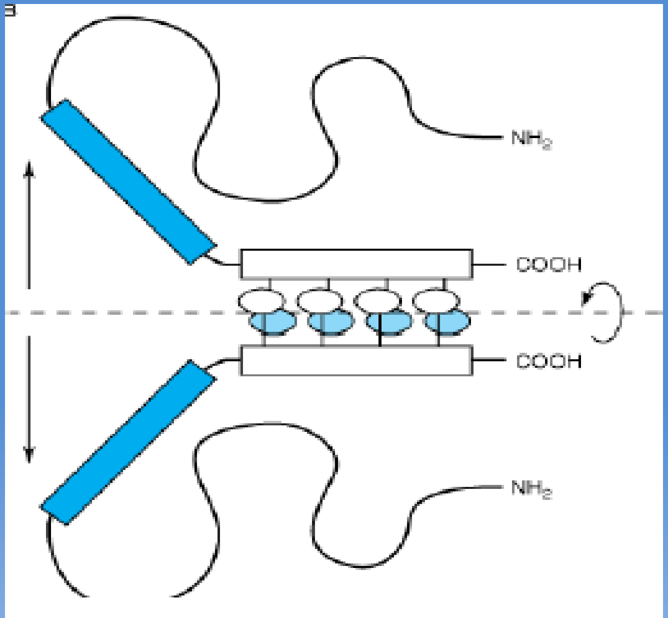
β-scaffold: Proteins belonging to this class use β-sheets to contact the DNA. A well-characterized example is the transcription factor NFκB.

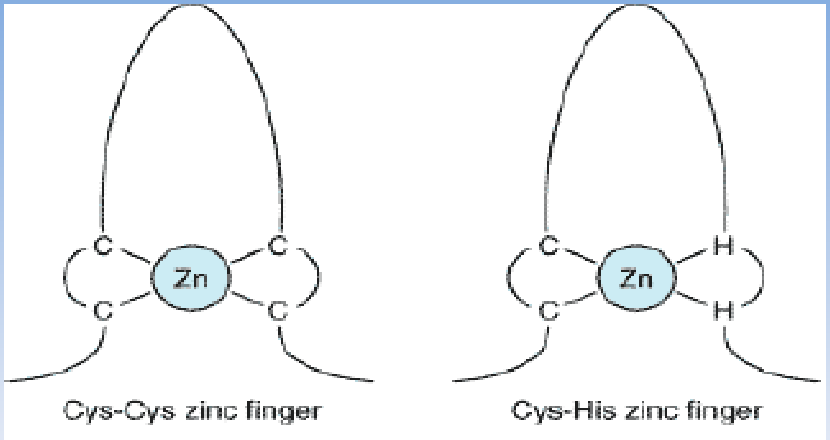
Three unique motifs of DNA binding proteins

Helix –turn- helix



Leucine zipper





Cys-Cys zinc finger

Cys-His zinc finger

Zinc finger

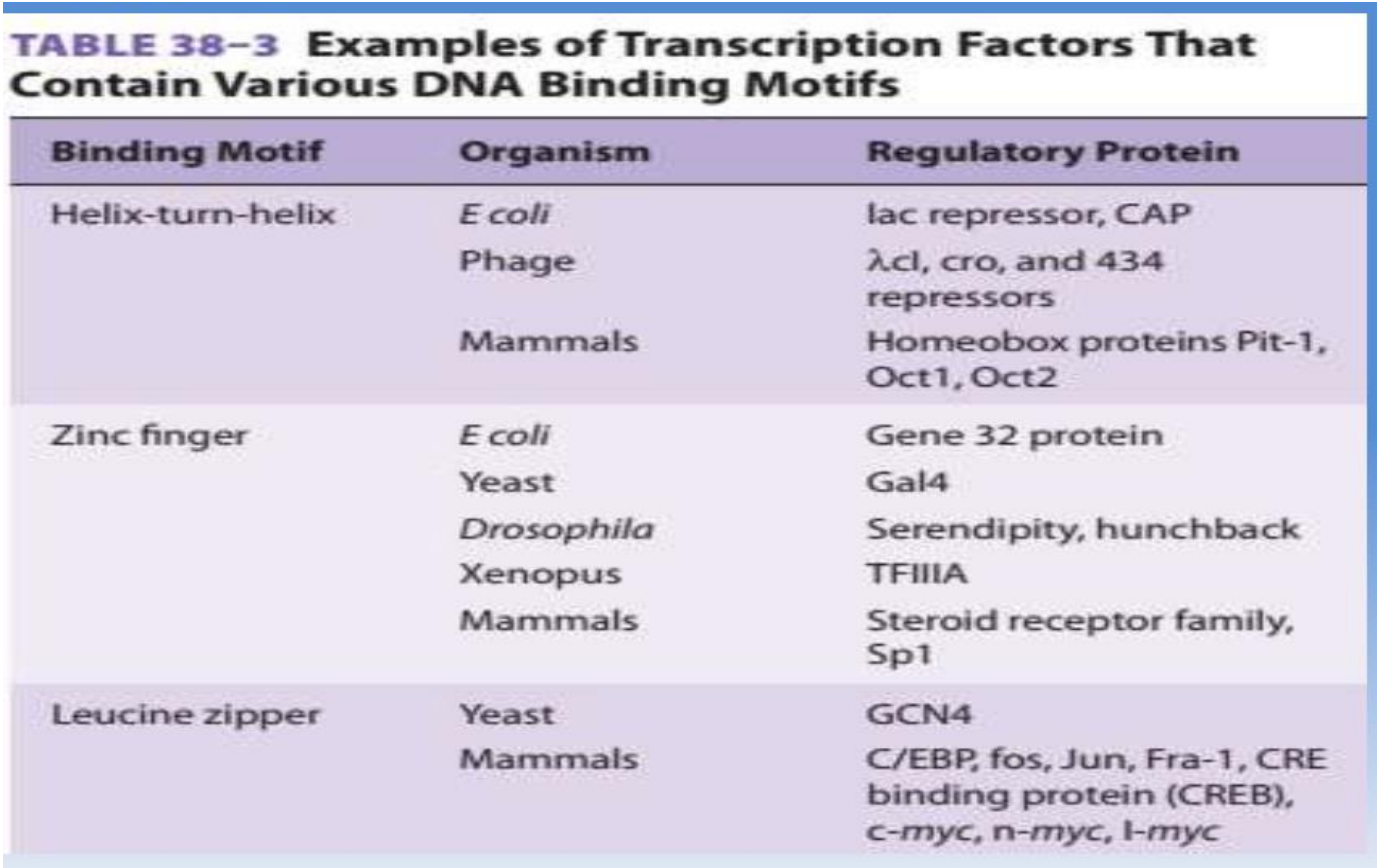
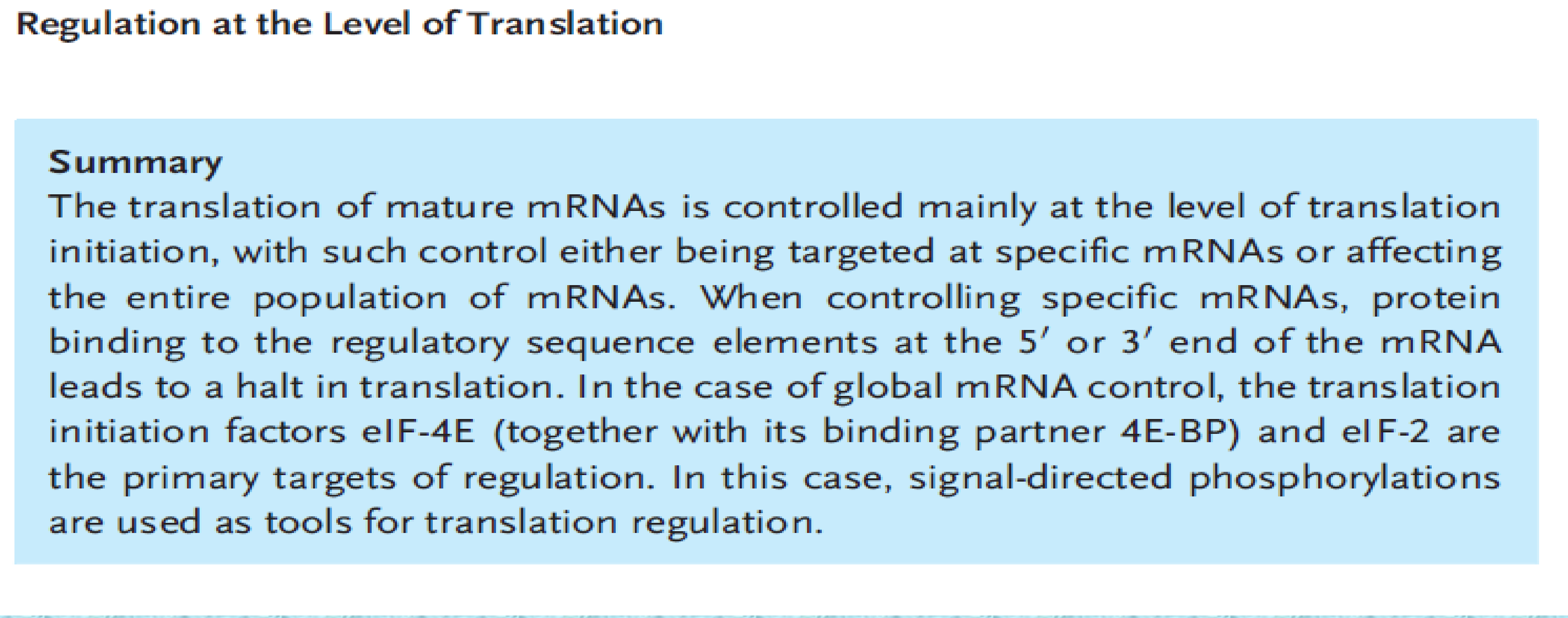


TABLE 38–3 Examples of Transcription Factors That Contain Various DNA Binding Motifs

Binding Motif	Organism	Regulatory Protein
Helix-turn-helix	*E coli*	lac repressor, CAP
	Phage	λcl, cro, and 434 repressors
	Mammals	Homeobox proteins Pit-1, Oct1, Oct2
Zinc finger	*E coli*	Gene 32 protein
	Yeast	Gal4
	Drosophila	Serendipity, hunchback
	Xenopus	TFIIIA
	Mammals	Steroid receptor family, Sp1
Leucine zipper	Yeast	GCN4
	Mammals	C/EBP, fos, Jun, Fra-1, CRE binding protein (CREB), c-*myc*, n-*myc*, l-*myc*

REGULATION AT THE LEVEL OF TRANSLATION

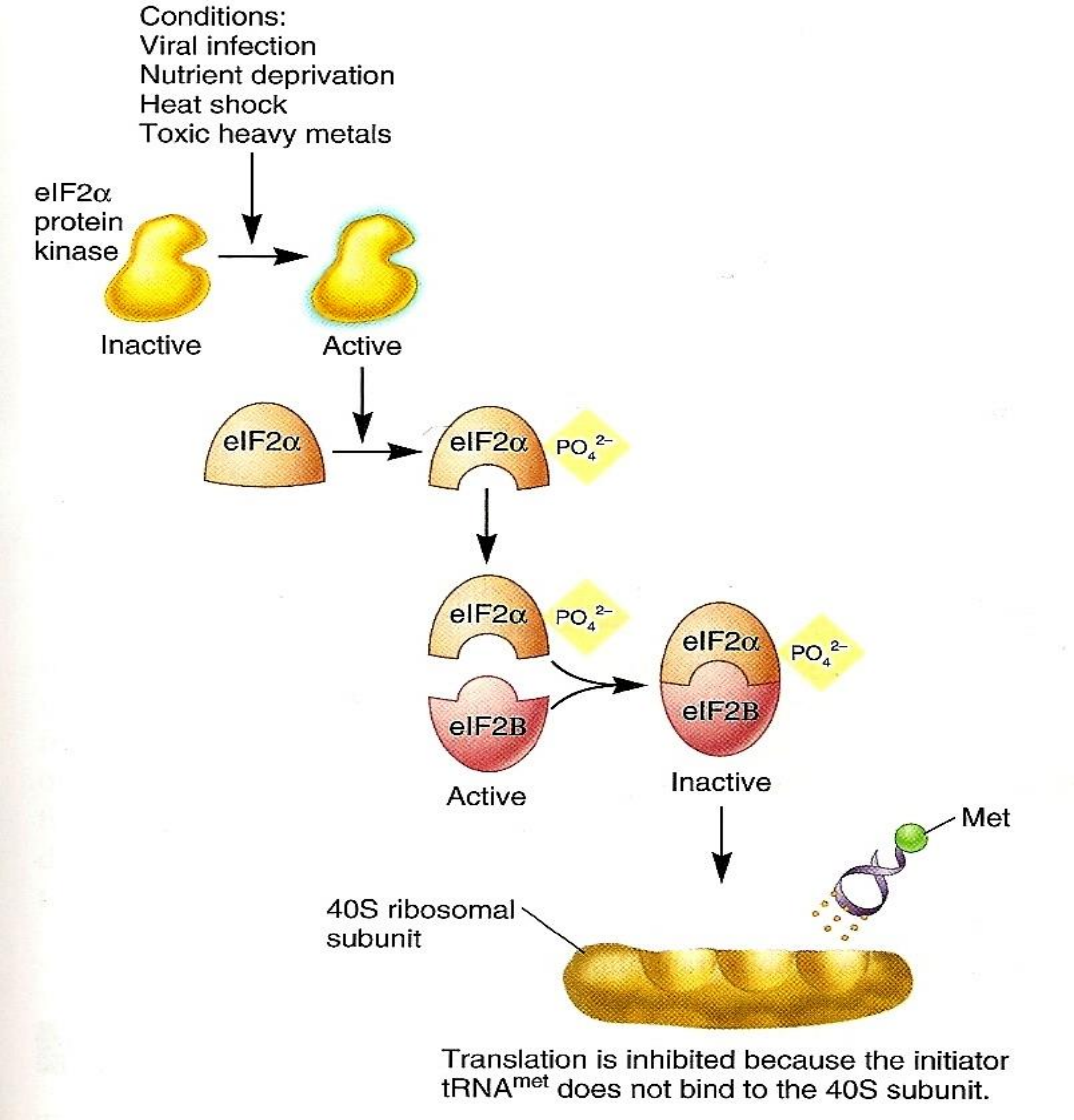


Regulation at the Level of Translation

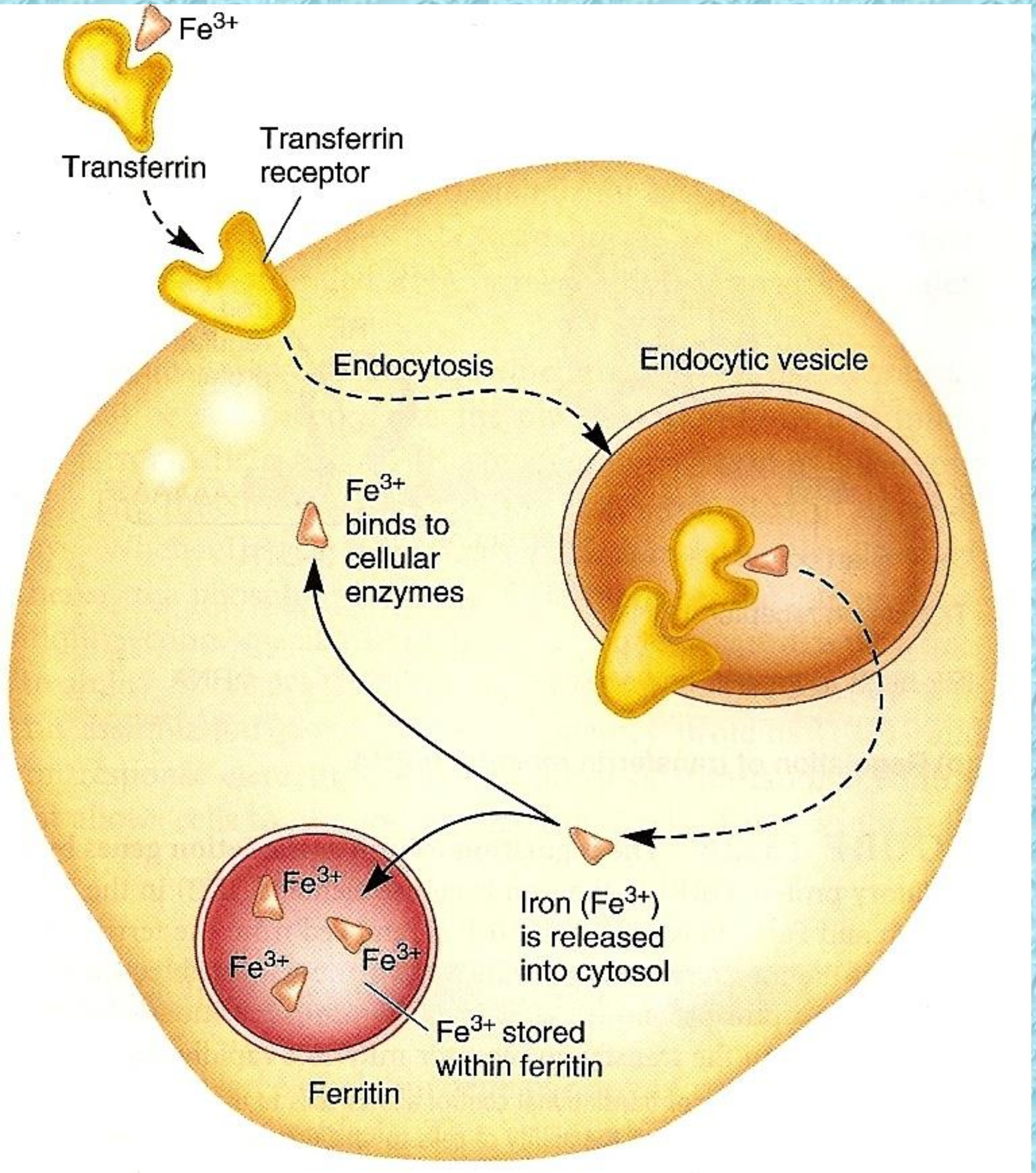
Summary

The translation of mature mRNAs is controlled mainly at the level of translation initiation, with such control either being targeted at specific mRNAs or affecting the entire population of mRNAs. When controlling specific mRNAs, protein binding to the regulatory sequence elements at the 5′ or 3′ end of the mRNA leads to a halt in translation. In the case of global mRNA control, the translation initiation factors eIF-4E (together with its binding partner 4E-BP) and eIF-2 are the primary targets of regulation. In this case, signal-directed phosphorylations are used as tools for translation regulation.

Phosphorylation

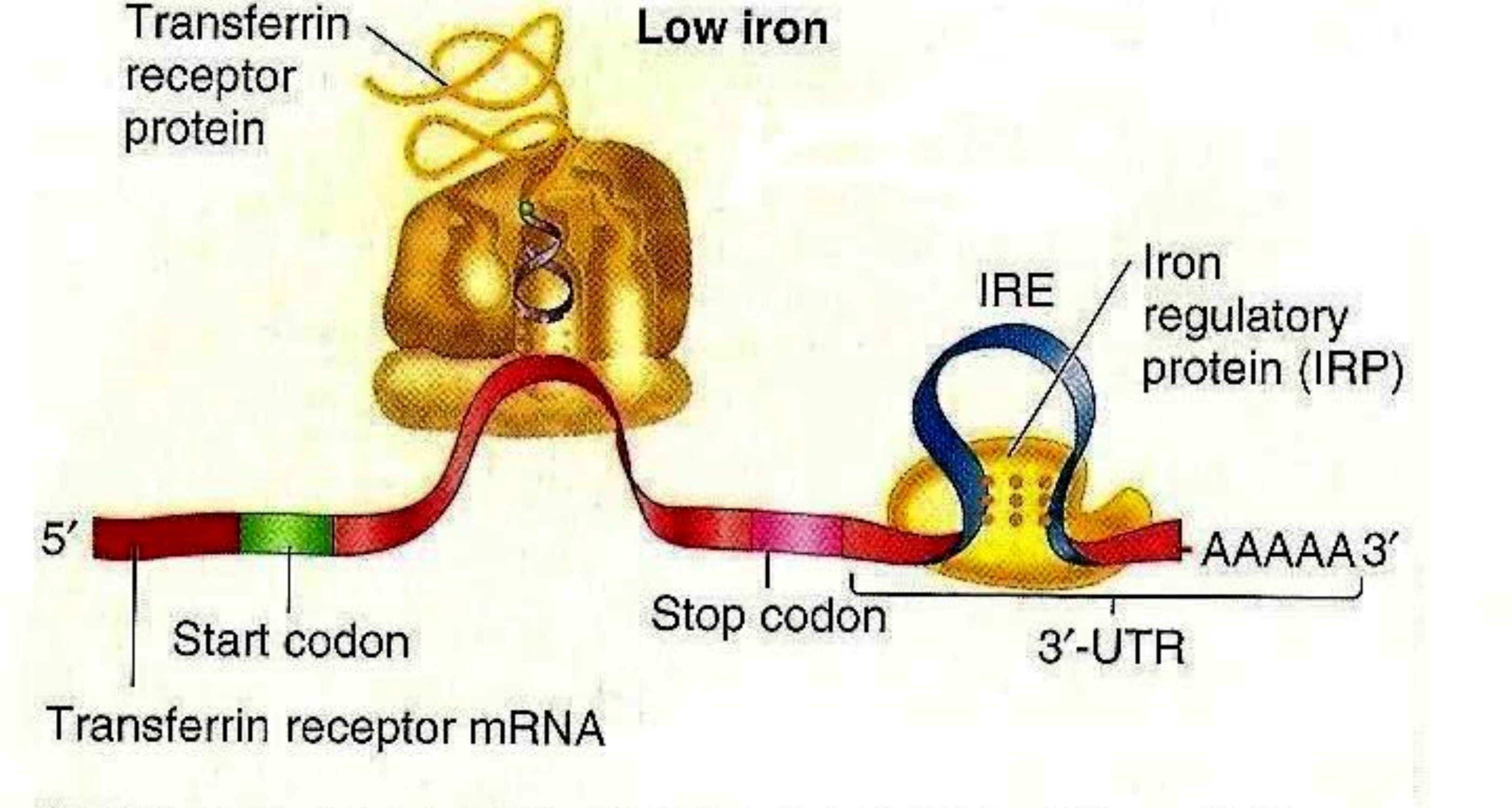


Translation is inhibited because the initiator $tRNA^{met}$ does not bind to the 40S subunit.



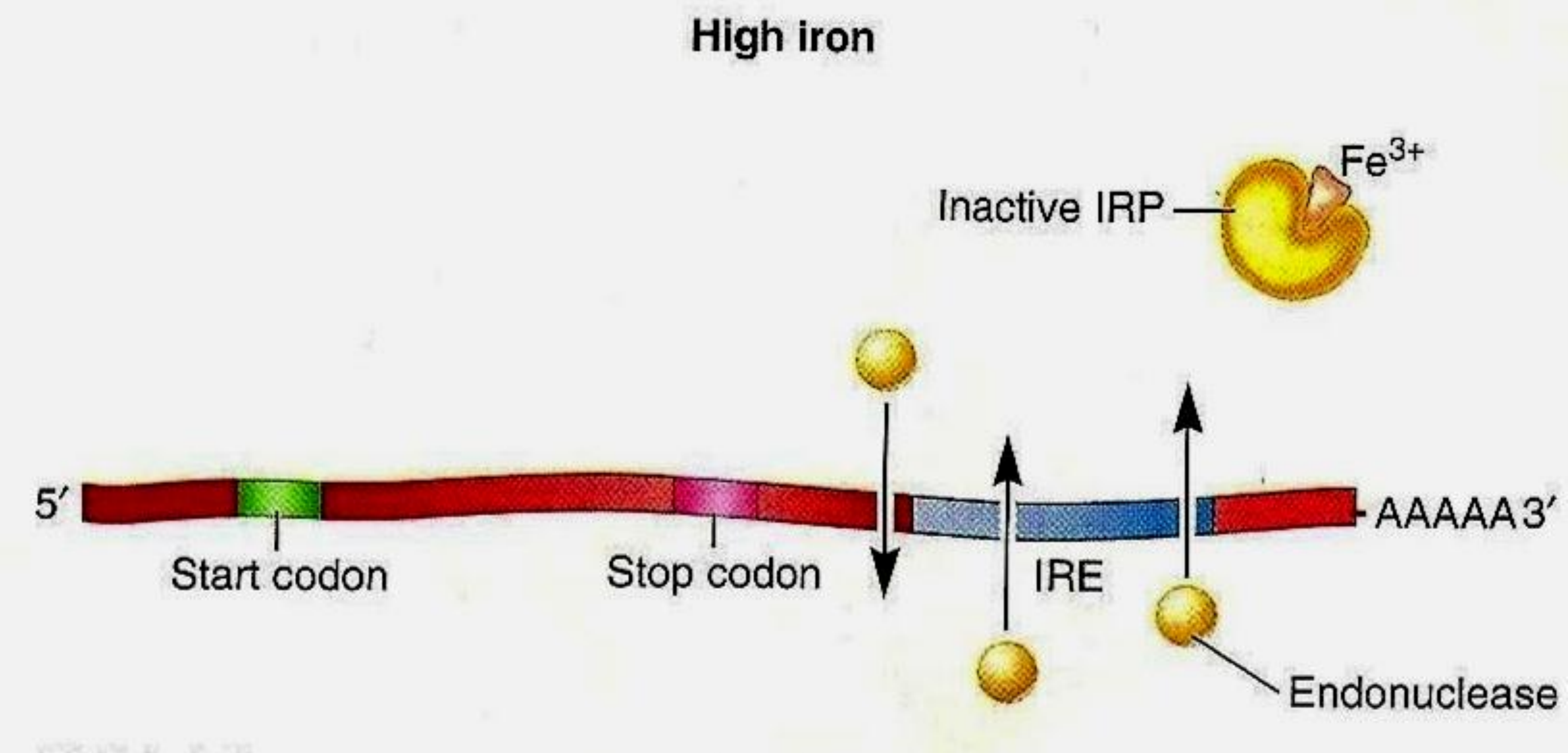
mRNA Stability

Regulation of Transferrin Expression



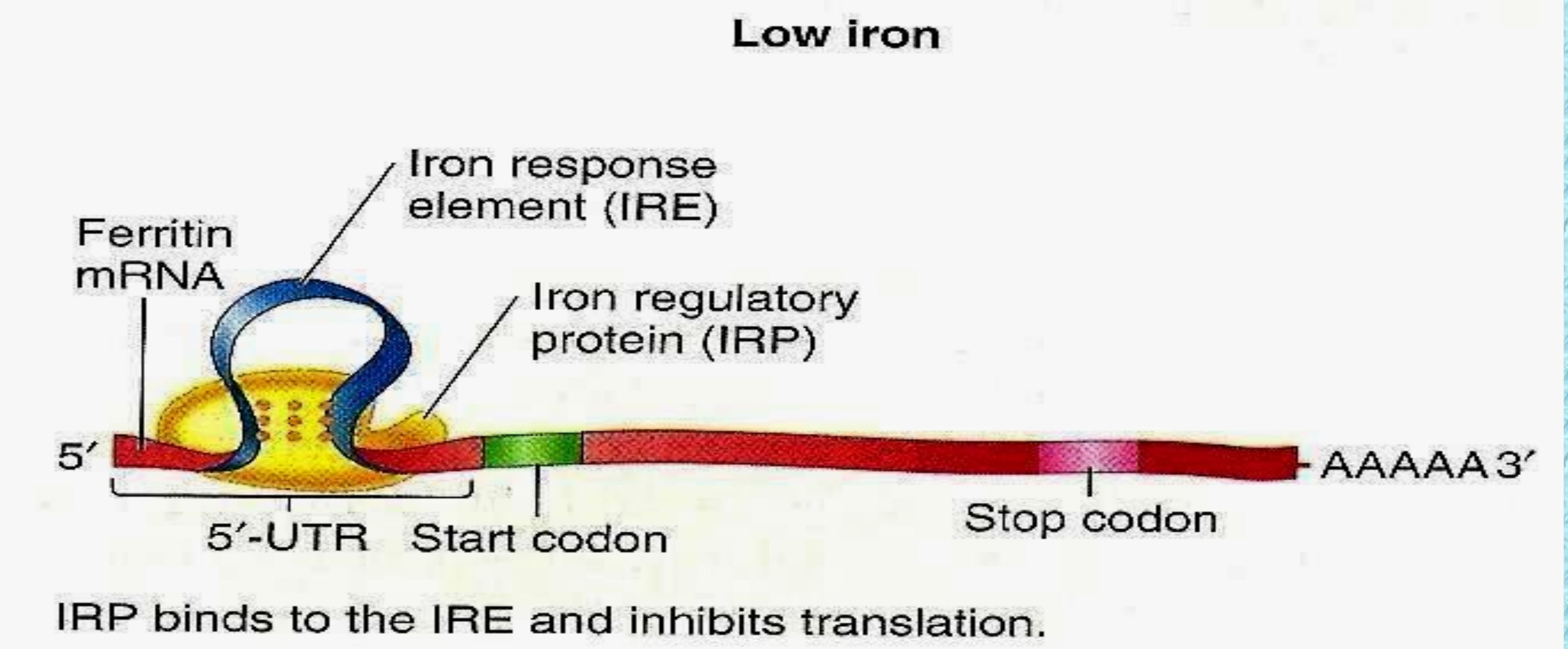
IRP binds to the IRE and enhances the stability of the mRNA.

Regulation of Transferrin Expression



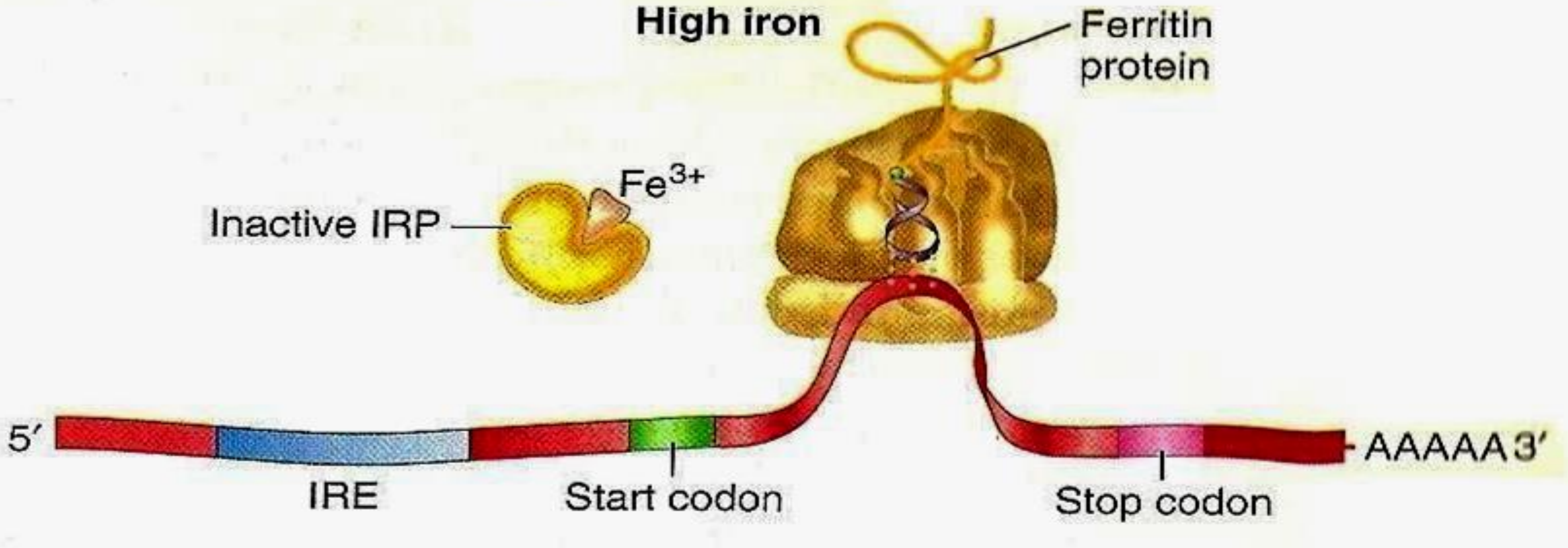
IRP binds iron and is released from the IRE; the mRNA is degraded.

Regulation of ferritin expression



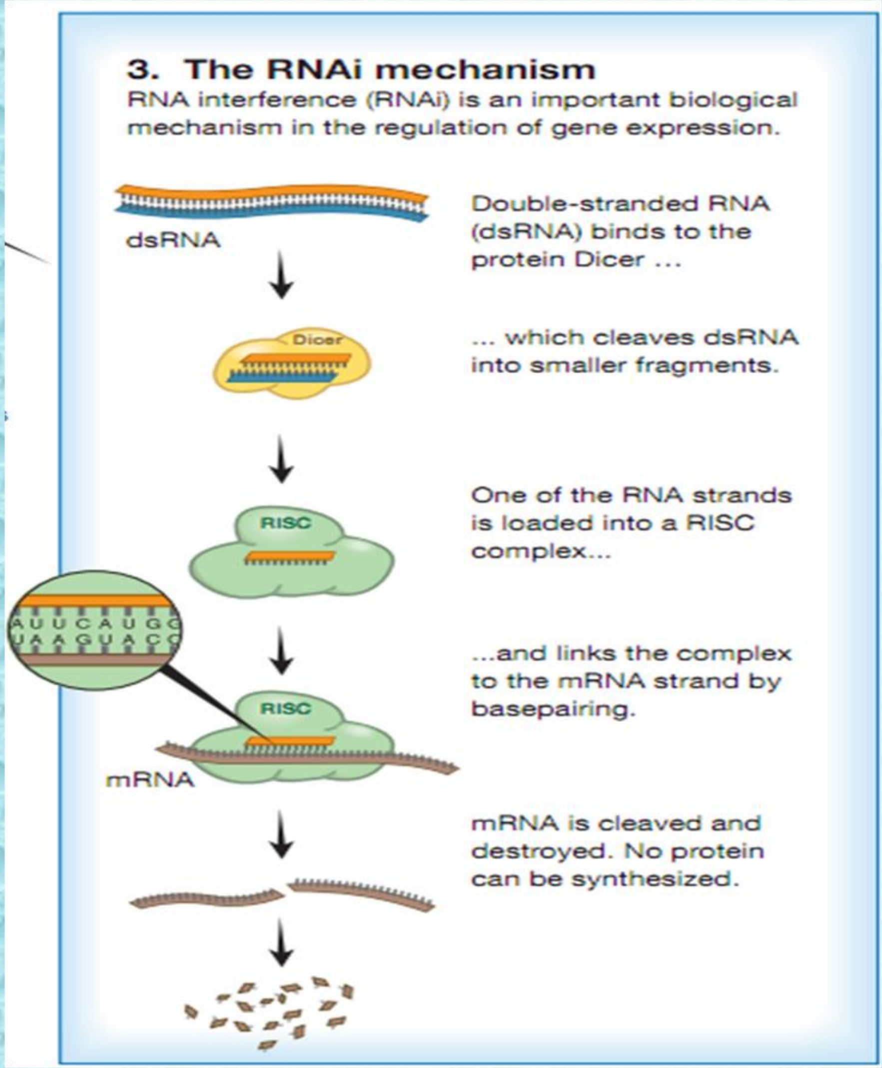
IRP binds to the IRE and inhibits translation.

Regulation of ferritin expression



IRP binds iron and is released from the IRE; translation proceeds.

RNA Interference



3. The RNAi mechanism

RNA interference (RNAi) is an important biological mechanism in the regulation of gene expression.

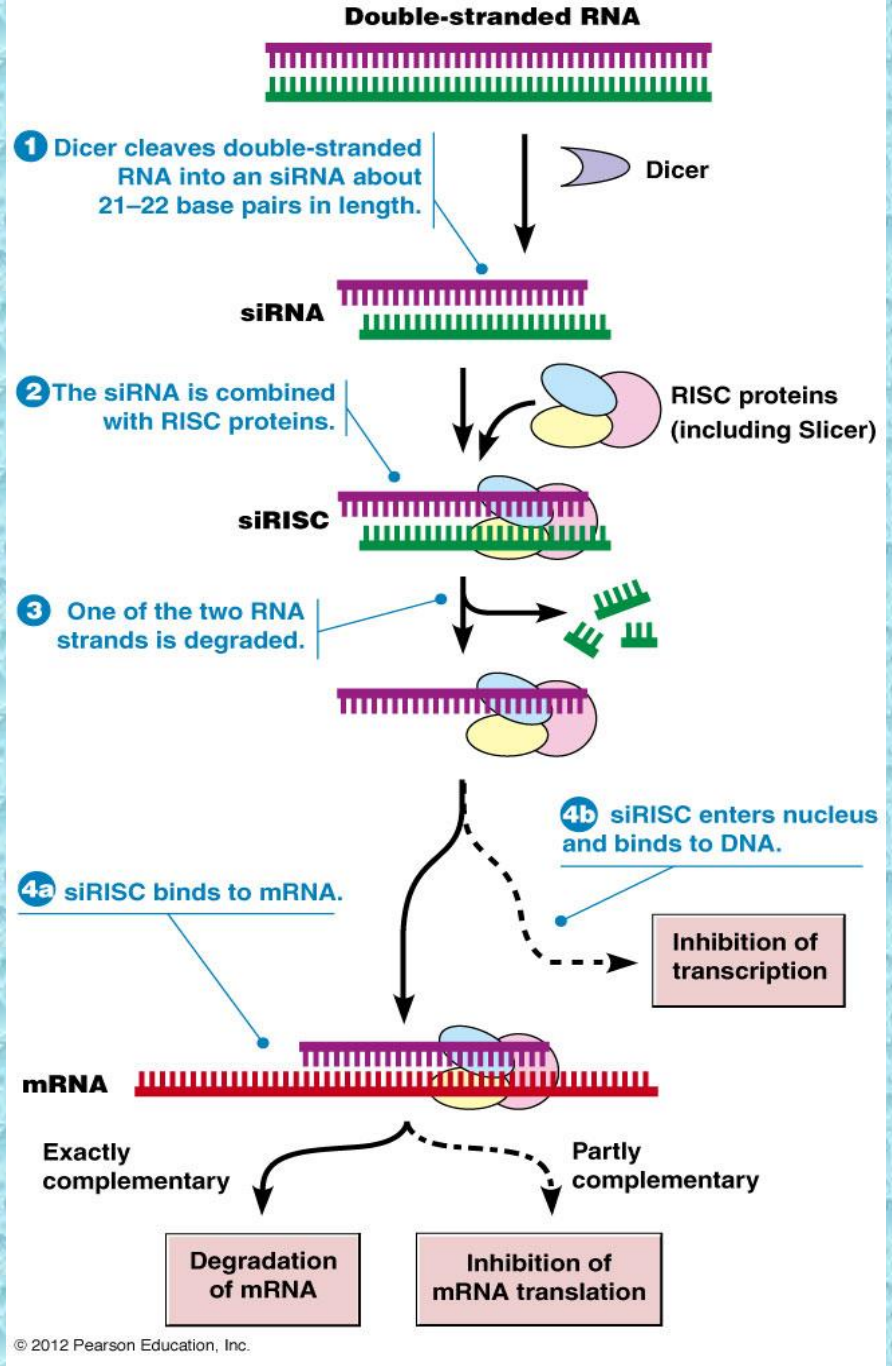
Double-stranded RNA (dsRNA) binds to the protein Dicer …

… which cleaves dsRNA into smaller fragments.

One of the RNA strands is loaded into a RISC complex…

…and links the complex to the mRNA strand by basepairing.

mRNA is cleaved and destroyed. No protein can be synthesized.



① Dicer cleaves double-stranded RNA into an siRNA about 21–22 base pairs in length.

② The siRNA is combined with RISC proteins.

③ One of the two RNA strands is degraded.

4a siRISC binds to mRNA.

4b siRISC enters nucleus and binds to DNA.