بســـم الله الرحمـــن الرحيـــم

NO NEED TO REFER TO THE SLIDES.

Do we need all the non coding regions of the DNA?

Two weeks ago, they discovered that the genome of a plant is very small (recall that plant genome is much larger than human genome). All the genes were present but most of the non coding regions were not. They concluded that we don`t need all these regions.

Synthetic cells: do bacteria need all its genes? No.

A scientist known as John Craig Venter, (the founder of Celera Genomics, The Institute for Genomic Research) one of the first to sequence the human genome and the first to create a cell with a synthetic genome, synthesized a synthetic bacterial DNA with the important genes only (not all the genes) and introduced it into a bacterial cell of different species after removing its own DNA. Few weeks later, a colony appeared on the Petri dish. Which means that the new bacterial cell was able to express the synthetic DNA that was introduced.

These bacterial cells can be used in disposing plastic wastes. How? By knowing the gene that can modify plastic and introducing it into bacteria. We can also synthesize (NOT create) bacterial cells that have the ability to dispose nuclear wastes.

Later, Venter was able to modify algal genes producing algae with specific functions.

mRNA transport:

Transport of mRNA from the nucleus to the cytoplasm, where it is translated into protein, is highly selective and is associated to correct RNA processing.

Defective eukaryotic mRNA molecules like interrupted RNA, mRNA with inaccurate splicing, and so on, are not transported outside the nucleus and will be degraded immediately in the nucleus.

This is a system to make sure that the protein that is expressed is functional.

Degradation of mRNAs:

The vast majority of mRNAs in a bacterial cell are very unstable, having a half-life of about 3 minutes.

The mRNAs in eukaryotic cells are more stable (up to 10 hours; average of 30 minutes).

Exonucleases are responsible for degradation.

Regulation of mRNA stability:

Iron-responsive elements (IREs):

In human cells, there are regions of mRNA called iron responsive elements (IREs).

These regions are contained within the mRNA sequences that code for certain proteins that regulate the levels of iron.

- Examples on proteins that regulate iron levels:

Ferritin (a protein that stores iron in liver), Transferrin (a protein that transports iron in blood and binds to the transferrin receptor on cell surfaces), transferrin receptor (helps in taking iron from blood to cells), ferroportin, and DMT1(divalent metal transporter 1).

Iron responsive element binding protein (IRE-BP) or (IRP) binds to these mRNA sequences influencing protein expression .

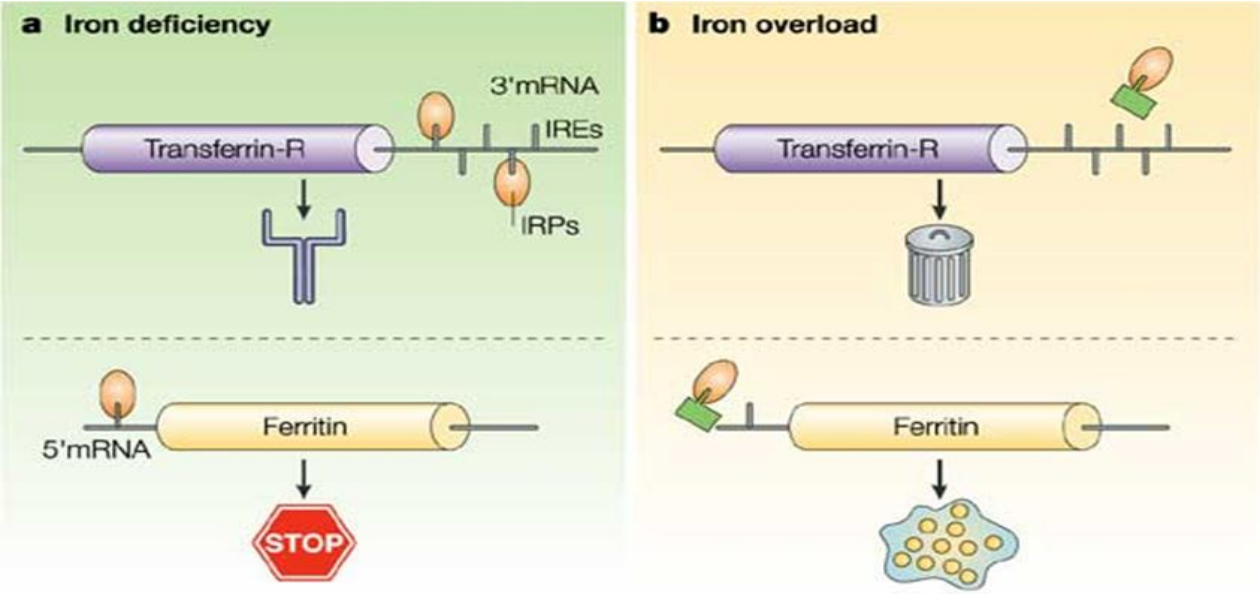
Ferritin and Transferrin:

If there is iron deficiency, then there is no need for ferritin so iron is released from ferritin into the blood and you need transferrin receptors on cell surfaces so that they can take in the iron.

If there is iron overload, iron will be stored into ferritin (each ferritin molecule can store 4000 molecules of iron) and there is no need for transferrin receptor.

So we have counter regulation of ferritin and transferrin and regulation of both proteins must be connected with each other.

Effect on expression:



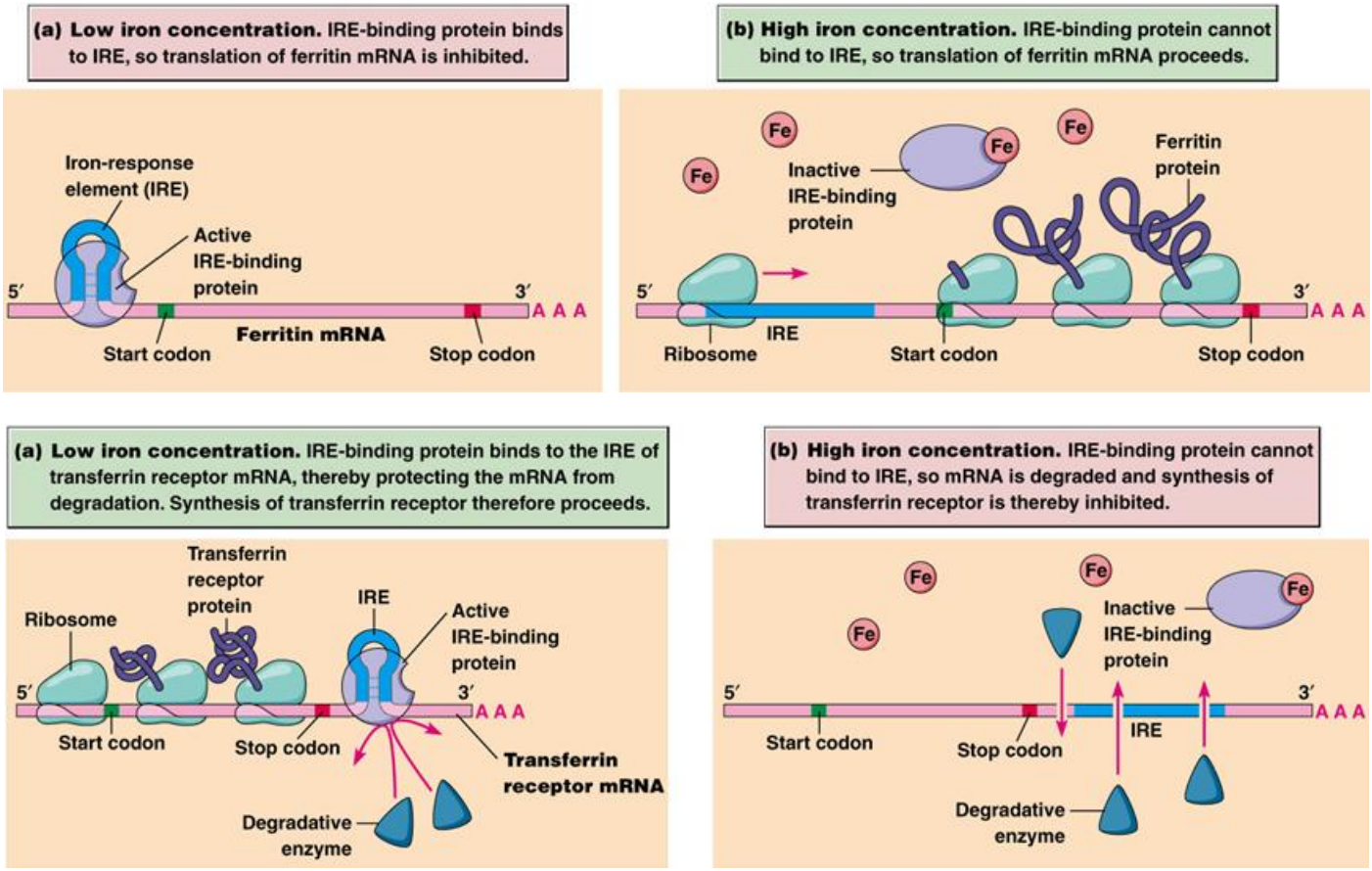
a) If there is iron deficiency:

what happens is that after the mRNA for the transferrin receptor is expressed, Iron Responsive Element binding Protein (IRE-BP) or (IRP) binds to the Iron Responsive Element (IRE) in the 3` region of the mRNA. This binding result in stabilizing the mRNA. So the half-life is now longer and we have continuous expression of the transferrin receptor to take in the iron into the cells. At the same time, the same protein (IRP) binds to the IRE in the 5` region of the ferritin mRNA blocking its transcription, (there is no need for iron storage). So, the amount of ferritin protein goes down and the amount of transferrin receptor protein goes up.

b) If there is iron overload:

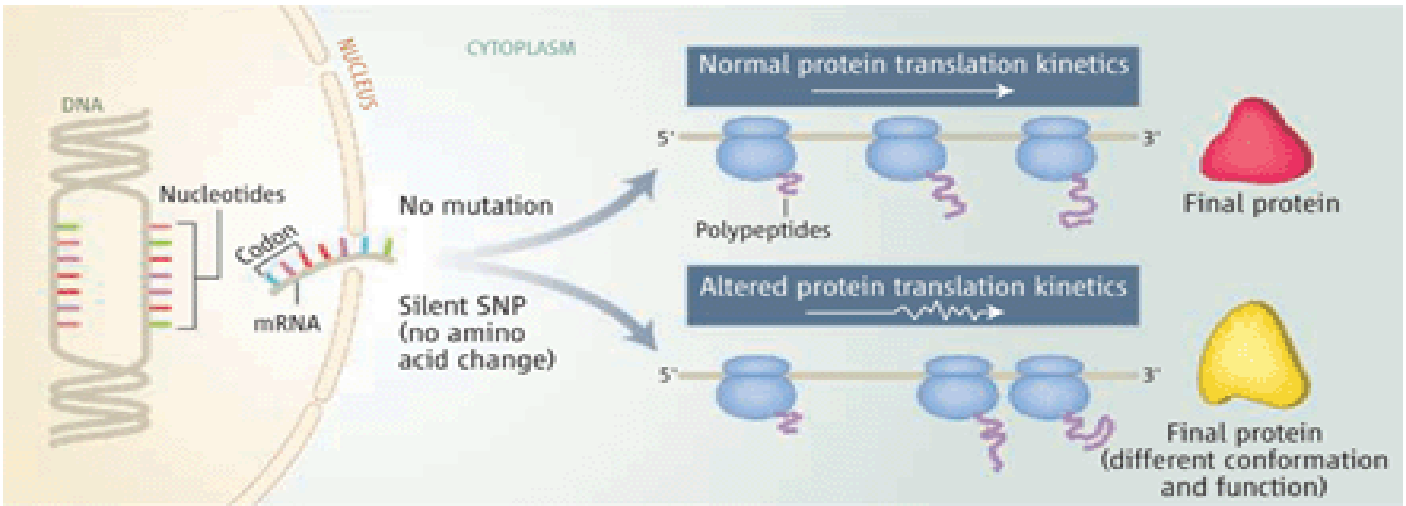
In this case, iron binds to the IRP preventing it from binding to the IRE in the 3` region of the mRNA of transferrin receptor resulting in the degradation of the mRNA so its half-life is now short and there is no expression of transferrin receptor anymore (an opposite effect is seen on the stability of transferrin mRNA). At the same time, iron binds to the IRP preventing it from binding to the IRE in 5` region of the ferritin mRNA allowing for translation from it which result in increasing the amount of ferritin in liver cells so that iron can now be stored. Therefore, the iron itself causes the cell to produce more iron storage molecules (ferritin).

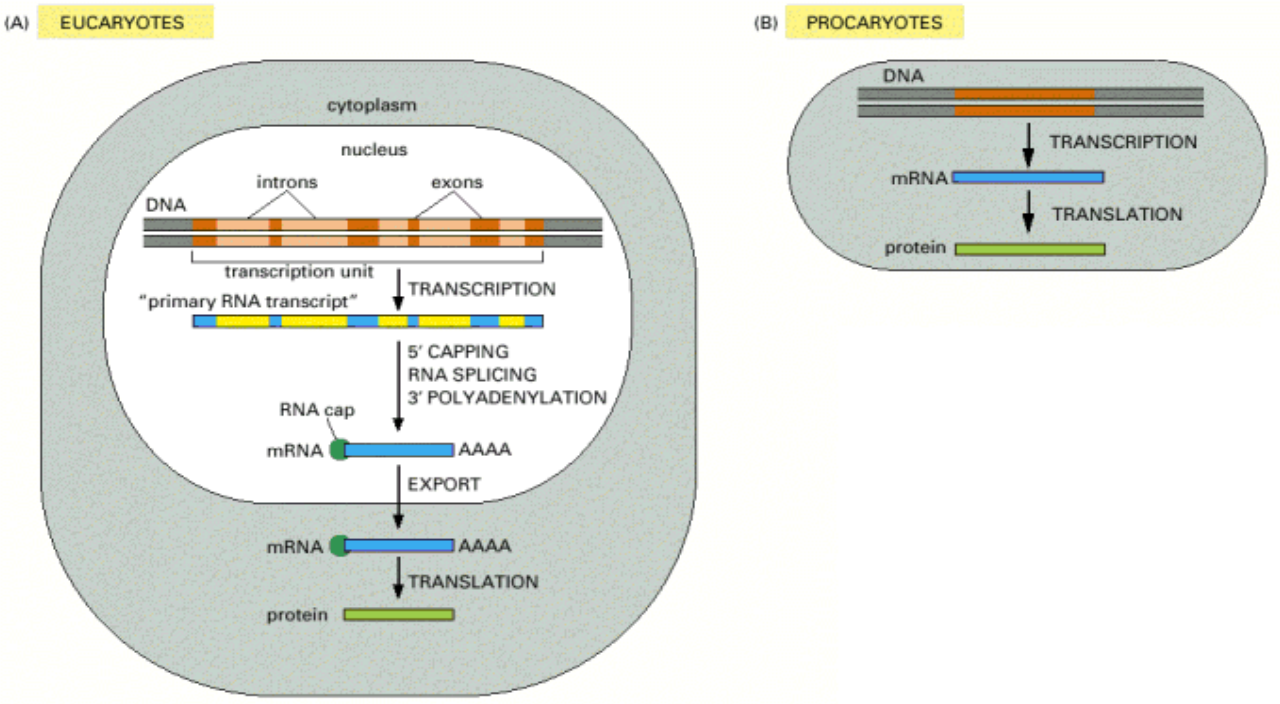
This is called **posttranscriptional regulation** (after transcription and before translation): Regulating the amount of proteins based on the amount of mRNA.



Role of SNPs in mRNA stability:

This is a very new discovery. They found the connection between SNPs and mRNA stability. What they found basically that in some cases in some cells the protein is expressed but it's not functional. They looked for mutations in these cells and they couldn`t find any mutation. But they found a SNP (single nucleotide polymorphism) and this SNP does not alter the amino acid sequence of the protein so we call it a silent mutation. They found that in a normal protein when it`s functional there is a certain kinetic for translation for protein synthesis and this SNP changes the kinetic of translation (the speed of translation) (making it faster or slower) resulting in the formation of a non-functional protein with a different conformation.



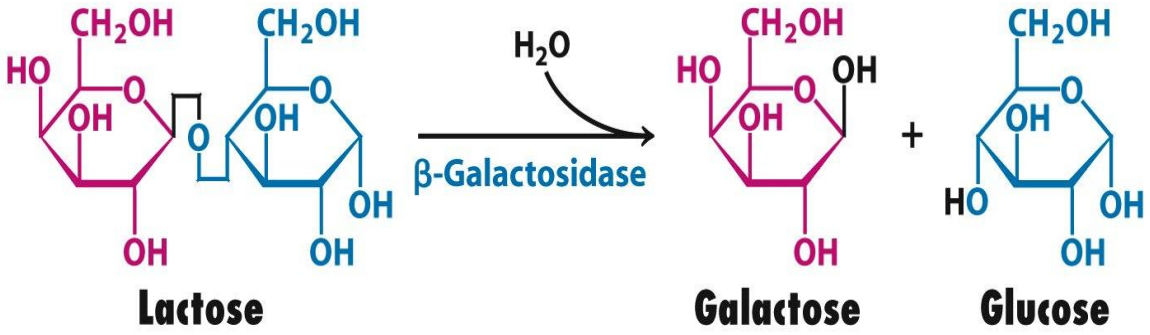


Transcription-regulation:

Regulation of transcription in prokaryotes:

Metabolism of lactose in bacteria:

In the 1950s, pioneering experiments were carried out by François Jacob and Jacques Monod who studied regulation of gene transcription in E. coli by analyzing the expression of enzymes involved in the metabolism of lactose.



Bacteria can metabolize glucose and lactose. When lactose is metabolized, its metabolized by an enzyme called β-Galactosidase into galactose and glucose. If there is glucose only, bacteria would metabolize it and if there is glucose and lactose they would metabolize glucose (easier to metabolize) and if there is lactose only, bacteria would then metabolize lactose.

What is an operon? A cluster of bacterial genes transcribed from one promoter producing a polycistronic mRNA (single mRNA that produces different polypeptides).

Components of the lac operon:

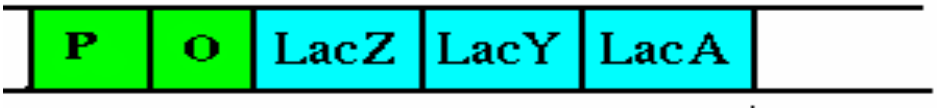
Lactose induces the synthesis of enzymes involved in its own metabolism including:

- β-Galactosidase (LacZ): catalyzes the cleavage of lactose.

- Lactose permease (LacY): transports lactose into the cell.

- A transacetylase (LacA): acetylates β-galactosides.

These structural genes are located in one operon known as the **lac operon**.



The operator:

The DNA region that regulates gene expression (transcription) is called a promoter. Usually this region is localized right before the start site of transcription (so the operator is not transcribed).

It includes the RNA polymerase binding site.

The promoter also includes a region known as the operator region that also regulates transcription.

The i protein (lac repressor):

Transcription of the lac operon is also controlled by a protein expressed by different gene (the *i* gene) that has its own promoter.

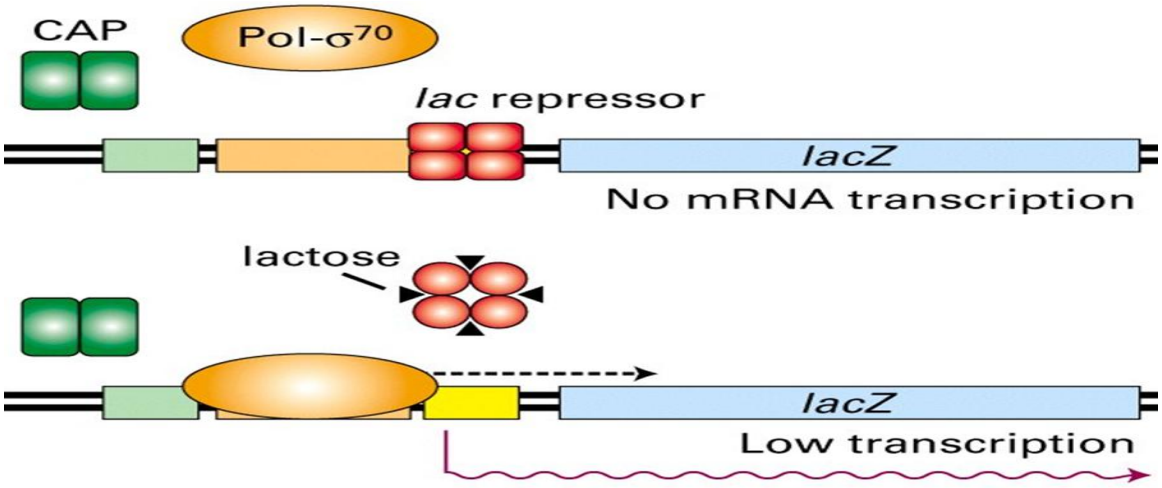
The *i* protein (lac repressor) blocks transcription by binding to the operator preventing the RNA polymerase from biding to the promoter.

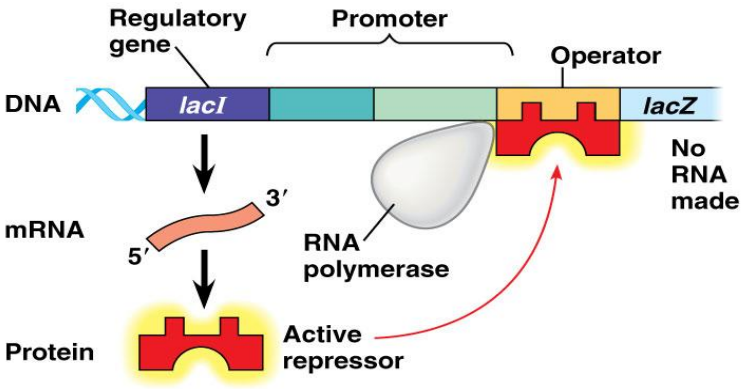
Regulation by lactose (positive):

(Lac operon is an "*inducible operon*". Meaning that it`s usually off but can be induced by an inducer [which is allolactose: an isomer of lactose formed in small amounts from lactose that enters the cell]).

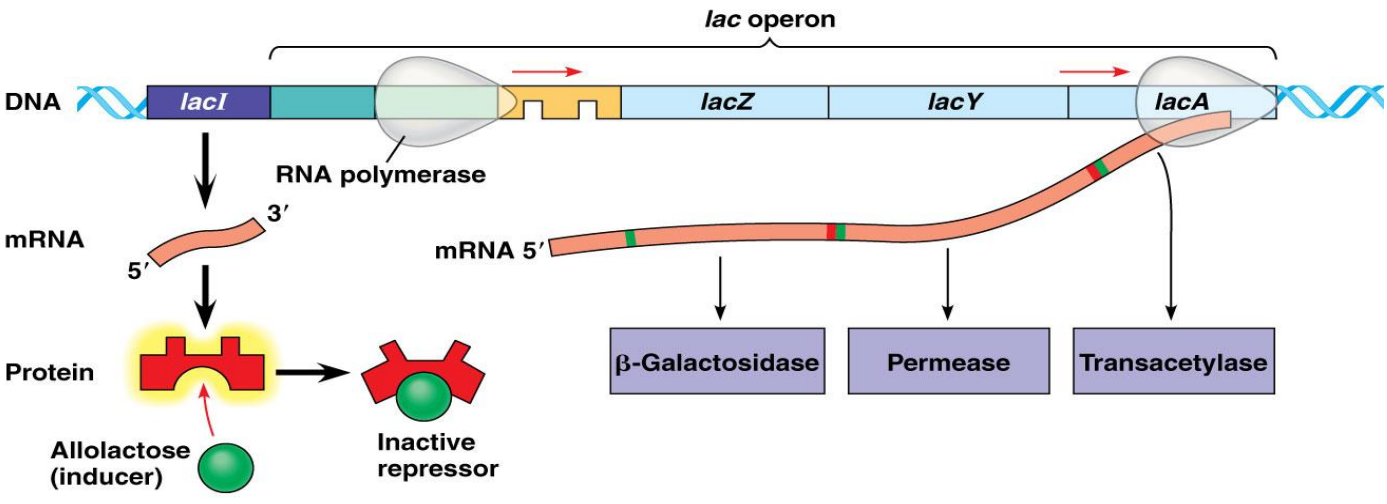
The addition of lactose leads to the induction of the operon because some lactose molecules bind to the lac repressor, thereby preventing it from binding to the operator DNA. Allowing the RNA polymerase to bind to the promoter and stimulate transcription.

This is known as positive regulation (there is expression of lac operon).





(a) Lactose absent, repressor active, operon off



(b) Lactose present, repressor inactive, operon on

Cis vs. trans regulatory elements:

Regulatory sequences like the **operator and promoter** are called ***cis***-acting control elements, because they regulate the transcription of genes on the same DNA molecule.

Proteins like the **repressor (the i protein)** are called ***trans****-acting factors* because they can regulate the transcription of genes located on other chromosomes within the cell.

Effect of mutations on the LacI gene: mutants of *i* are either constitutive (always on) or noninducible (always off).

Constitutive i mutants: like mutations that prevent lac repressor (i protein) from binding to the operator (i is always bound to lactose and the operator is always free, so expression of operon is always induced).

Noninducible I mutants: the repressor binds to the operator very tightly even in the presence of lactose.

Regulation by glucose (negative):

Glucose is preferentially utilized by bacterial cells.

If *E. coli* are grown in medium containing both glucose and lactose, the *expression of lac* operon is not really high even in the presence of the normal inducer (lactose) because glucose acts as a negative regulatory molecule.

This is known as negative regulation because the presence of a molecule reduces the expression of that gene.

How does glucose repress the expression of the lac operon?

Low glucose activates the enzyme adenylyl cyclase, which converts ATP to cAMP.

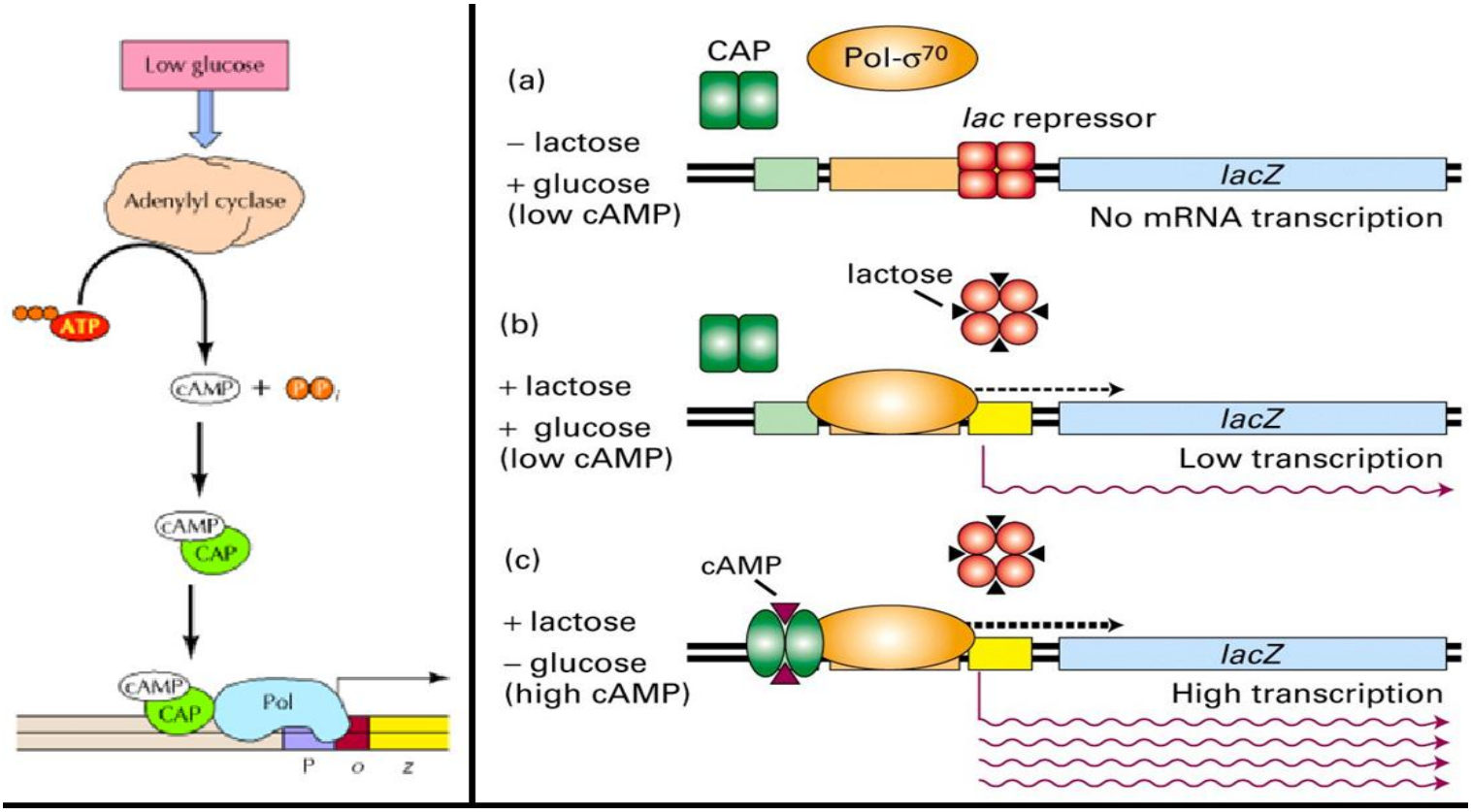
cAMP then binds to catabolite activator protein (CAP).

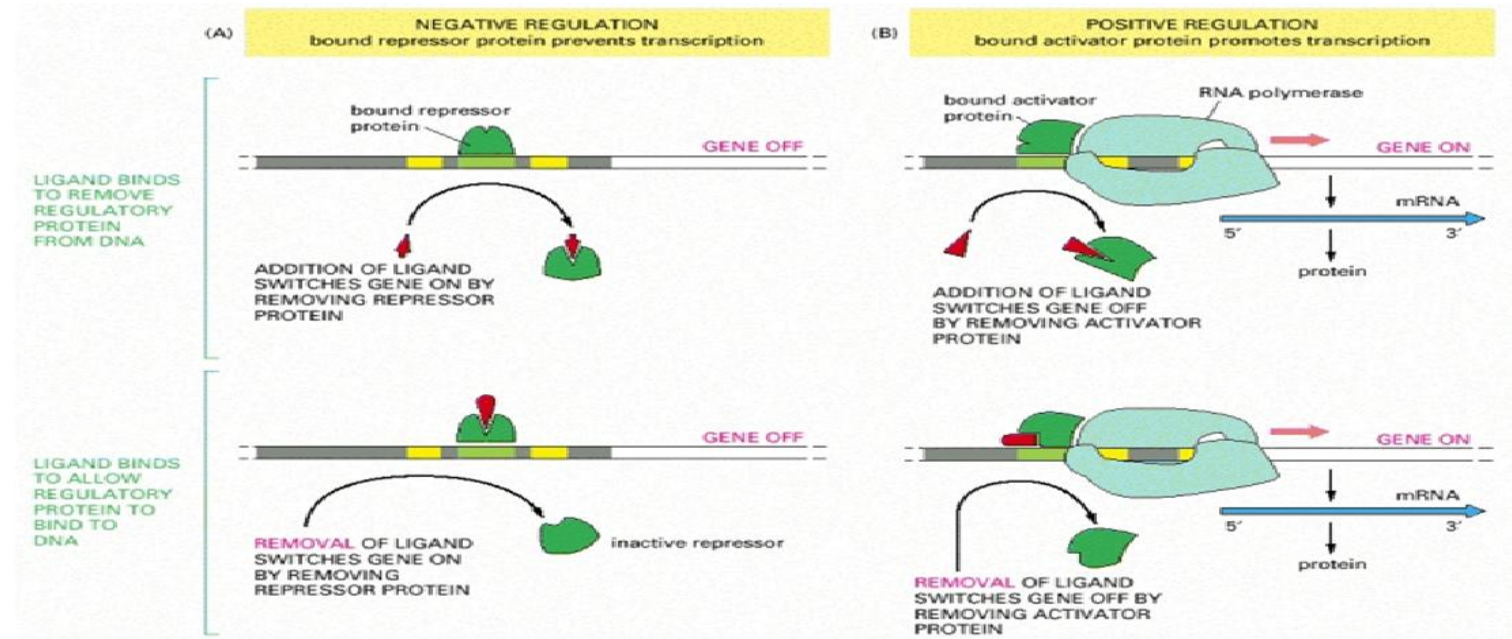
cAMP stimulates the binding of CAP to DNA upstream of the promoter.

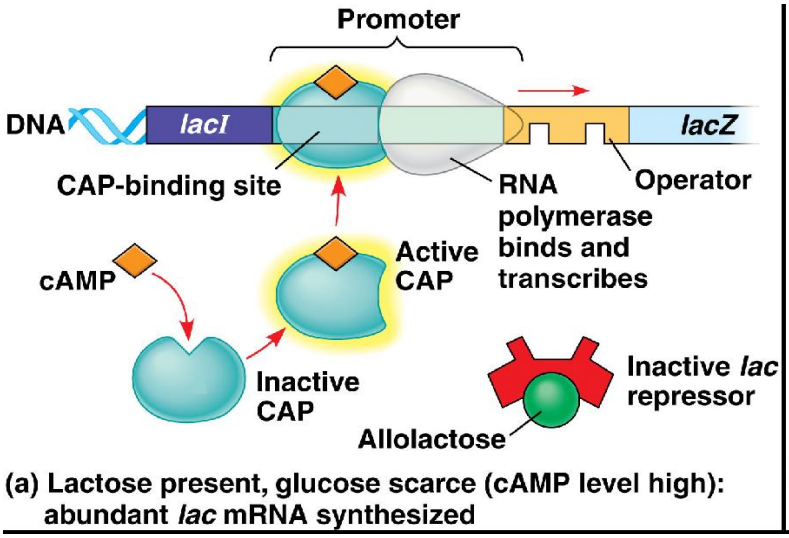
CAP then interacts with the RNA polymerase, facilitating the binding of polymerase to the promoter and activating transcription.

If we have only lactose, this means that the lac operon must be expressed by binding to the lac repressor and allowing transcription. In this case, the activity of the RNA polymerase is not really high. Because RNA polymerase needs another molecule to increase its activity. This molecule is CAP. When CAP is bound to the upstream to the promoter region because of low glucose levels, this CAP can now interact with the RNA polymerase and it pushes it to transcribe the lac operon.

If we have high glucose, this would inactivates the adenylyl cyclase making it unable to produce cAMP. So CAP is not bound to cAMP and it won`t bind to the DNA. In this case, the RNA polymerase is bound but it's not very active.







Regulation of transcription in eukaryotes:

Regulatory mechanisms:

Although the control of gene expression is far more complex in eukaryotes than in bacteria, the same basic principles apply.

Transcription in eukaryotic cells is controlled by:

- Cis-acting DNA sequences.

- Transcriptional regulatory proteins.

- Repressor proteins.

- Modification of DNA and its packaging into chromatin. (DNA can be modified by modifying the nucleotides (by methylation) or by modifying the histones).

Regulatory sequences (promoters and enhancers):

As already discussed, transcription in bacteria is regulated by the binding of proteins to cis-acting sequences (e.g., the lac operator).

Similar cis-acting sequences regulate the expression of eukaryotic genes:

- Promoters

- Enhancers

General components of promoters:

Genes transcribed by RNA polymerase II have four elements:

1- TATA box (also in bacteria).

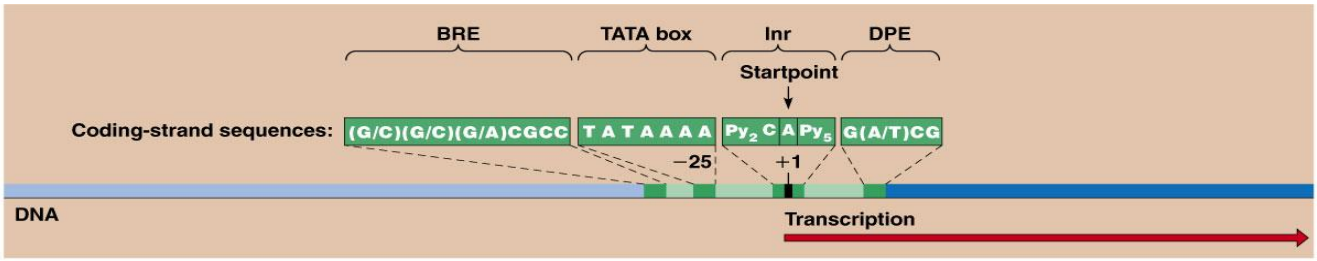
2- G-C rich element (BRE, B Recognition Element) (also in bacteria).

Another elements that found downstream to these two sequences:

3- Inr sequence (transcriptional initiator).

4- DPE (downstream promoter element)

- TATA box and Inr sequence are considered as the two core promoter elements.



(b) Core promoter elements for RNA polymerase II

Enhancers:

Many genes in mammalian cells are controlled by **cis-acting regulatory sequences** called enhancers.

Usually, these are located farther away from the transcription start site.

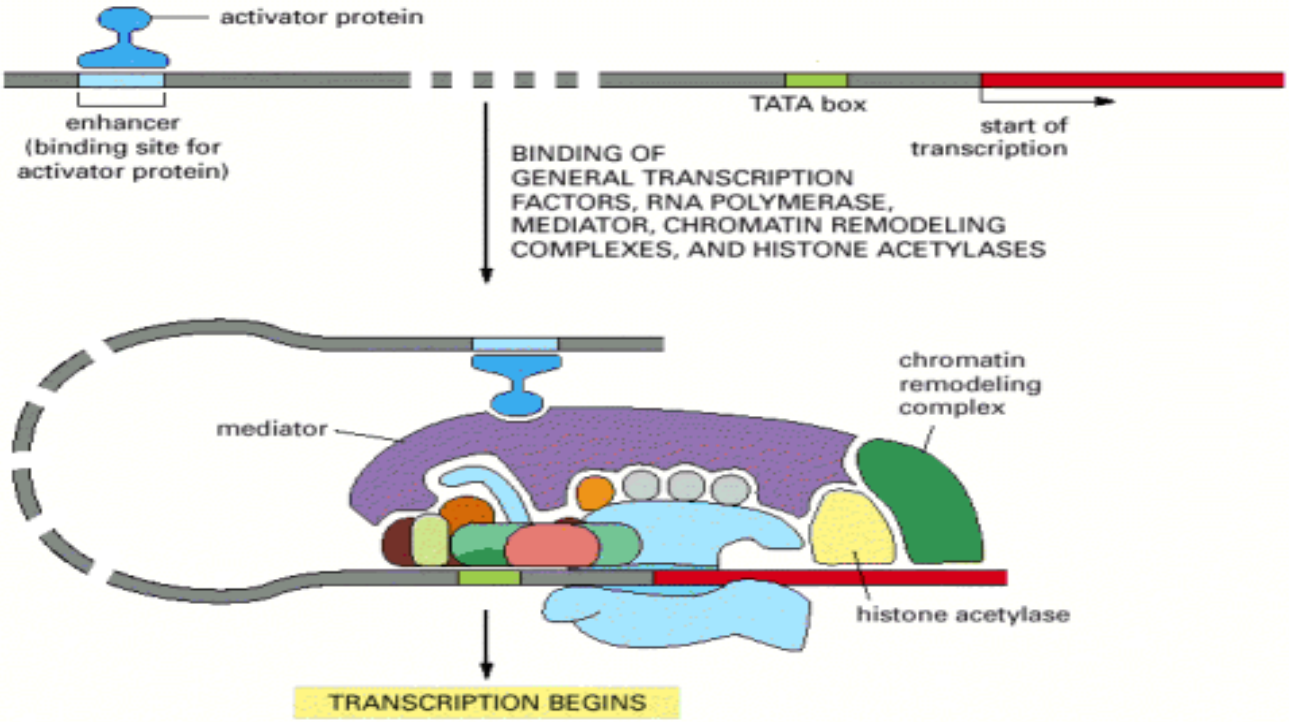
Enhancers, like promoters, function by binding proteins (transcription factors) that then regulate RNA polymerase.

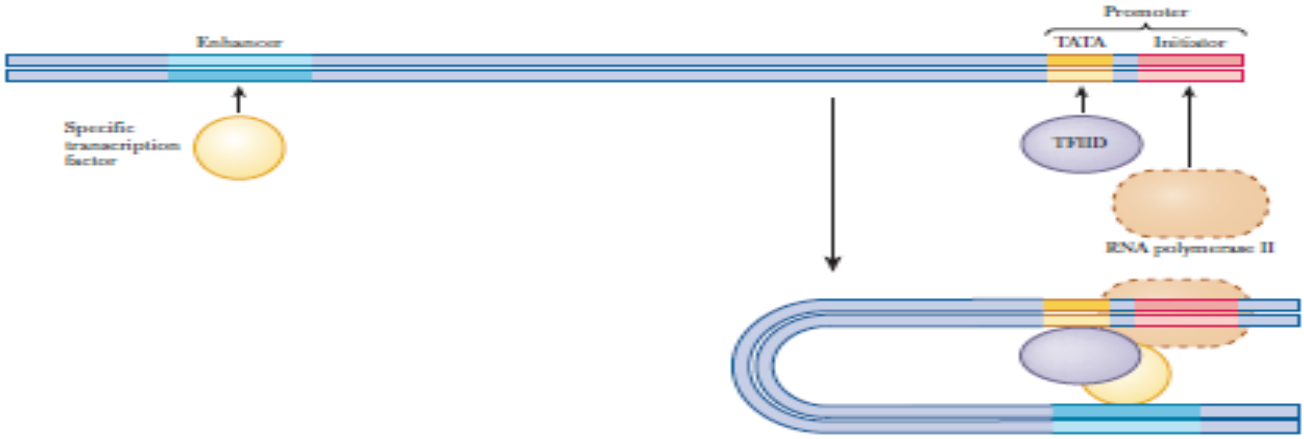
They have no common consensus sequences.

Mechanism of enhancer-dependent regulation:

They can stimulate transcription when placed either upstream or downstream or even within the gene, in either a forward or backward orientation (we can flip it and it would still be functional).

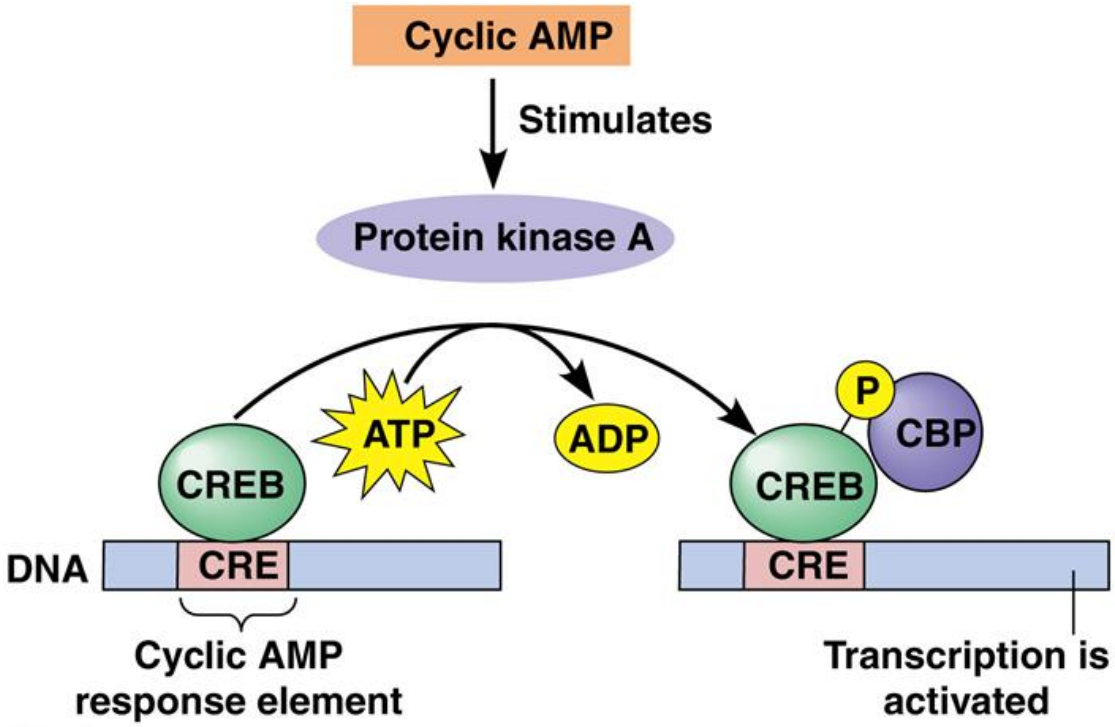
This is possible because of DNA looping (recall that DNA is flexible), which allows a transcription factor bound to a distant enhancer to interact with RNA polymerase or general transcription factors at the promoter and activates them.


activator protein
enhancer
(binding site for
activator protein)
TATA box
start of
transcription
BINDING OF
GENERAL TRANSCRIPTION
FACTORS, RNA POLYMERASE,
MEDIATOR, CHROMATIN REMODELING
COMPLEXES, AND HISTONE ACETYLASES
chromatin
remodeling
complex
mediator
histone acetylase
TRANSCRIPTION BEGINS

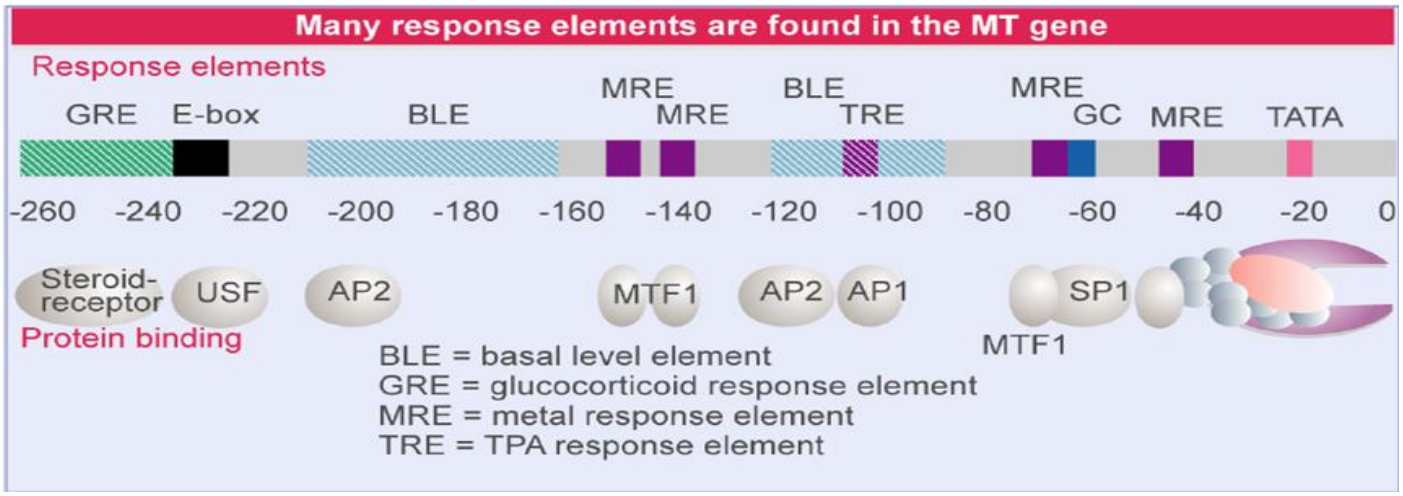

Promoter
Enhancer
TATA
Initiator
Specific
transcription
factor
TFIID
RNA polymerase II

Two examples of enhancers:

1- cAMP response element (CRE): (meaning that this element is regulated by the presence of cAMP). CRE can bind to a protein known as cAMP response element binding protein (CREB). CREB can bind to CBP (CREB binding Protein). CREB is always bound to CRE but it's not active. It needs the CBP to be active. CBP is only bound to CREB when CREB is phophorylated by Protein Kinase A (PKA) which is activated by cAMP.



2- Metallothionein (MT): is a protein that can sequester (isolate) metals. So, protecting cells from harmful metals. They found that there are multiple enhancers upstream of the metallothionein (e.g. MRE: metal response element, GRE:glucocorticoid response element [meaning that metallothionein gene can be activated by high levels of metals and glucocorticoids]).



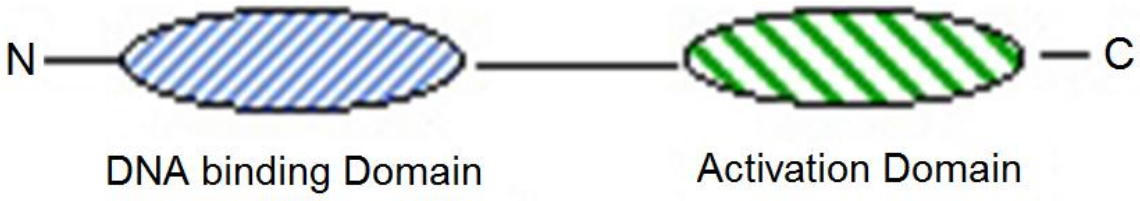
Transcriptional regulatory proteins:

These proteins (e.g. BREB) consist of two domains:

1- One region of the protein specifically binds DNA (DNA-binding domain)

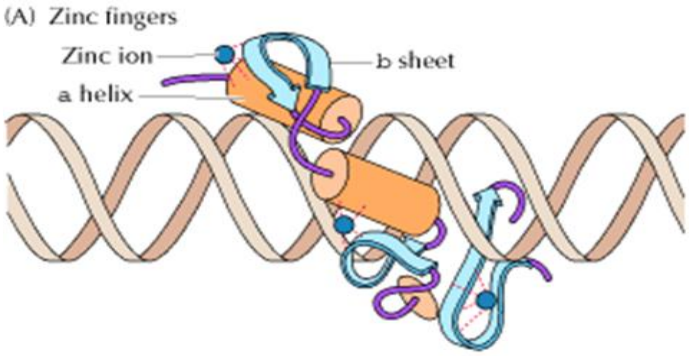
2- the other activates transcription by interacting with other components of the transcriptional machinery (regulatory or activation domain).

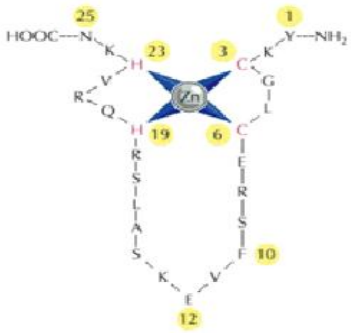
Both activities are ***independent*** and can be separated from each other (meaning that if we linked the activation domain of the CREB to the DNA-binding domain of the metallothionein, the protein will be activated by high levels of cAMP and when its activated it would bind to the metallothionein regulating genes).

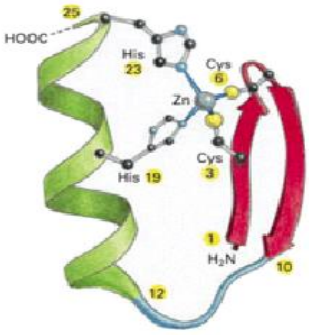


DNA-binding domains:

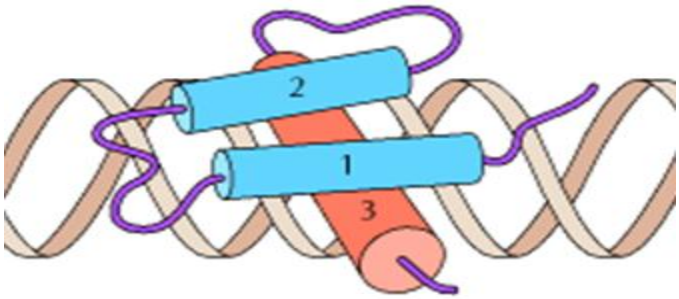
1- Zinc finger domains: contain repeats of cysteine and histidine residues that bind zinc ions and fold into looped structures ("fingers") that bind DNA (in Steroid receptors).



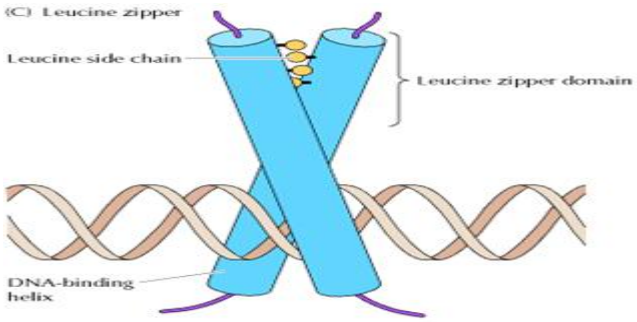




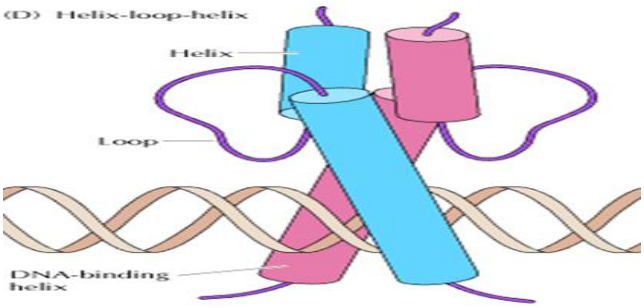
2- Helix-turn-helix motif: constructed from two α helices connected by a short extended chain of amino acids, which constitutes the "turn".



3- Leucine zipper: The leucine zipper is rich with lucine contains four or five leucine residues used for interaction with other proteins (e.g. CREB).



4- Helix-loop-helix: Two domains are each formed by two helical regions separated by a loop.



The activation domains: These activation domains are thought to stimulate transcription by interacting with general transcription factors, such as TFIIB or TFIID, thereby facilitating the assembly of a transcription complex on the promoter.

- Acidic domains.
- Glutamine-rich domains.
- Proline-rich domains.

Eukaryotic Repressors: bind to specific DNA sequences and inhibit transcription.

Repressors may have:

- Both DNA-binding and protein-binding domains (bind to DNA and to proteins that can suppress transcription).
- DNA-binding domains, but not protein-interaction domains. (bind to the DNA and prevent the activating proteins from binding to the DNA. The same mechanism of competitive inhibitors).
- Protein-interacting domains, but not DNA-binding domains (sequestering 'isolating' the proteins that activate transcription and prevent them from binding to the DNA).

عبد الرحمن محمد الريالات