Genetics & molecular biology

◉ Sheet ○ Slide

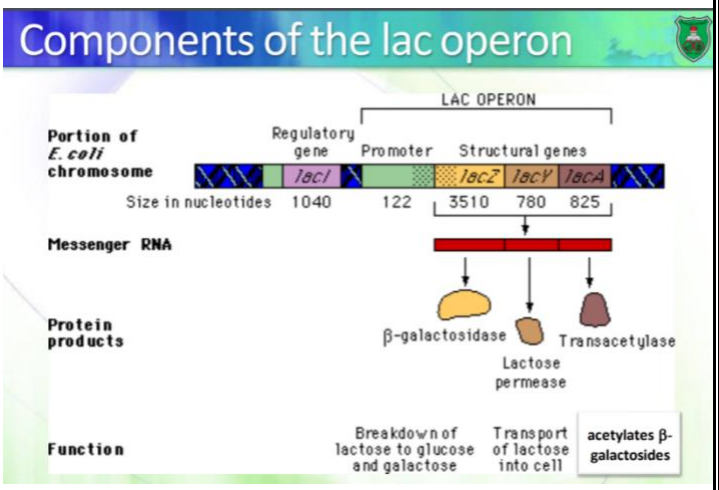
Number:	-11
Done by:	-Farah Azizi
Corrected by:	-Faisal Nimri
Doctor:	-Mamoun Ahram

<u>This sheet will include the mechanism of transcription /activation of transcription in both prokaryotes and eukaryotes</u>

- We start with prokaryotes transcription regulation because its simpler than the transcription regulation in eukaryotic system.
- In the 1950s, pioneering experiments were carried out by François Jacob and Jacques Monod who studied regulation of gene transcription in E. coli by analysing the expression of enzymes involved in the metabolism of lactose, at the end of their experiment they deduced the whole mechanism of lac operon.
- Hence, in prokaryote transcription regulation we will specifically be concerned with the lac operon.

<u>Extra note: Transcription can be regulated at the stages of both initiation and elongation, but most transcriptional regulation in bacteria operates at the level of initiation.</u>

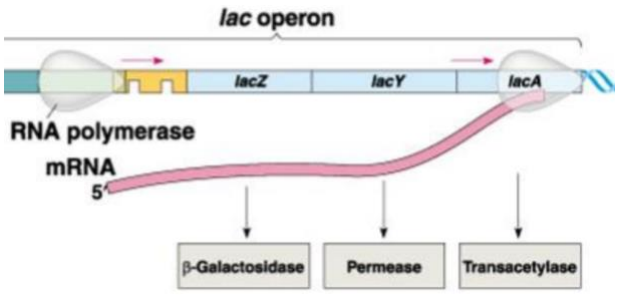
<u>Operon</u> **is a genetic unit containing a cluster of genes under the control of a single promoter producing a polycistronic MRNA** (each region of the MRNA can produce a different protein with a totally different function but the functions collectively work in the same metabolic pathway)



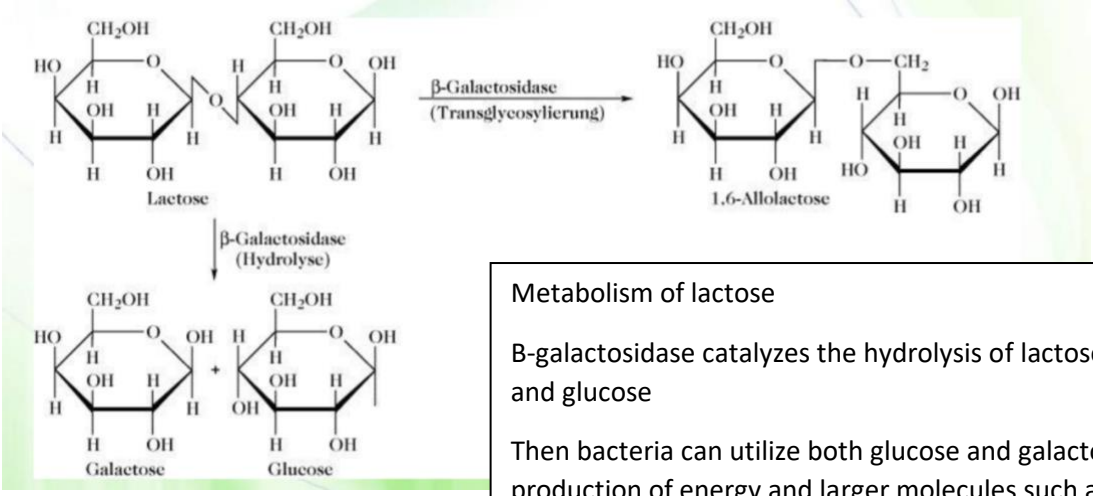
The lac operon is the operon responsible for producing the proteins involved in the metabolism of lactose.

Lac operon encodes for three proteins

- β-galactosidase: catalyzes the cleavage of lactose.
- lactose permease: transports lactose into the cell.
- transacetylase: acetylates β-galactosides.



What happens in metabolism of lactose (disaccharide made of glucose +galactose)?



Metabolism of lactose

B-galactosidase catalyzes the hydrolysis of lactose to galactose and glucose

Then bacteria can utilize both glucose and galactose for production of energy and larger molecules such as lipopolysaccharide

Some lactose is isomerized by the same enzyme in to an isomer (1,6-allolactose) instead of (1,6 instead of 1,4 glyosidic bond)

<u>Prokaryotic cells have two main ways for regulating this operon:</u>

1. Positive regulation
2. Negative regulation

1. Regulation by lactose (positive):

Operator is located downstream of RNA polymerase binding site, (between promoter and transcription start site), so the operator is technique in the promoter region

- Within the Lac operon there is a region called the operator region, a regulatory element *<u>(element referring to a DNA sequence)</u>* which is the binding site of a protein called the lac repressor.
- when this repressor binds to the operator, it will prevent RNA polymerase from binding to the promoter, thus it will inhibit transcription.
- This repressor is encoded for by a separate gene from the Lac gene called LacI gene (a separate monocistronic gene that has its own promoter).

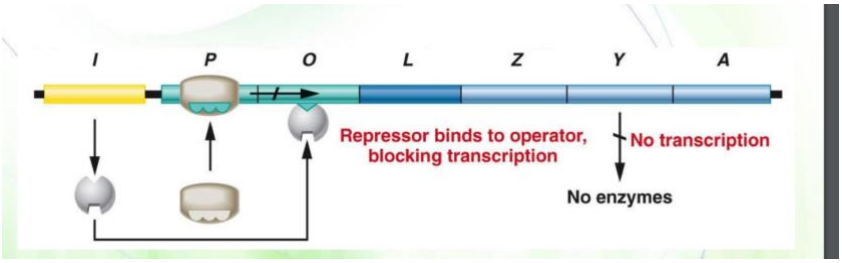
How does Regulation take place?

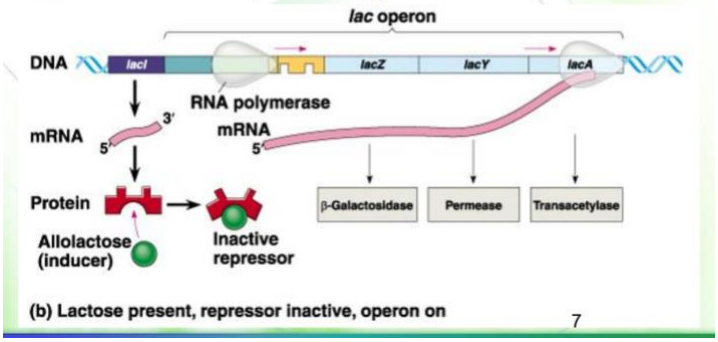
This repressor can also bind to allolactose, this causes a change in the structure of repressor which can no longer bind to operator and do its repressive action

So when lactose level in the cell is low, the repressor will be bound to the operator and no lactose metabolism proteins will be transcribed, and if lactose level is high (isomerized to allolactose), it will bind to the repressor leaving the operator free, RNA polymerase can bind to promoter and start transcription of lac gene.

Why is it called positive regulation?

Because lactose positively regulates the transcription of lac operon (more lactose more transcription)





Cis vs. trans regulatory elements

Cis acting elements:

Regulatory sequences located on a specific location in a gene if they were moved to any other location, they won't be functional because

they affect the expression of only genes linked on the same DNA molecule; examples: operator in lac gene, enhancers

Trans acting elements

Regulatory elements that can regulate genes distant from the gene they are transcribed from (they can do their function regardless of the location)

Proteins like the repressor are called transacting factors because they can affect the expression of genes located on other chromosomes within the cell. They are produced from trans-acting elements, example LacI gene.

Next

To genetically understand the function of any gene we induce different mutations and observe their effect, and that's what the 2 French scientists did with the lac operon

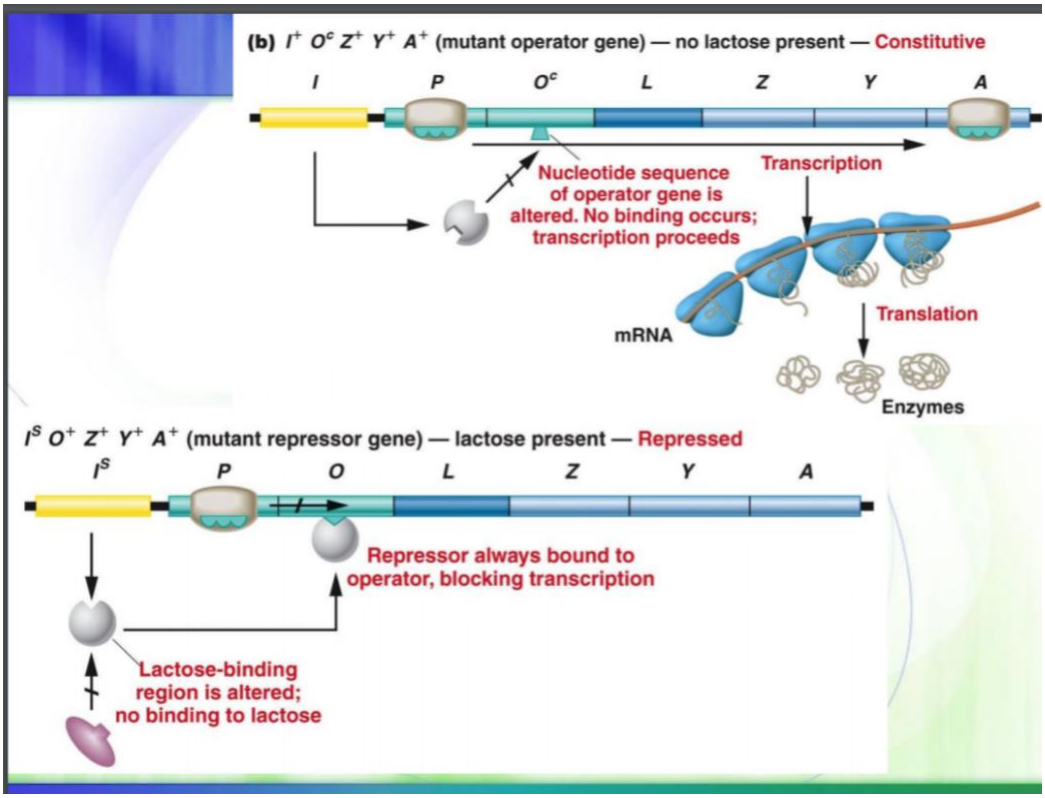
Effect of different mutations

1. **Some mutations result in constitutive expression**

- (always on).
- Even if you put no lactose at all lac operon will always be on
- Examples:
- deletion of operator, thus lac repressor cannot bind to operator anymore
- deletion of gene responsible of repressor production (lacI)

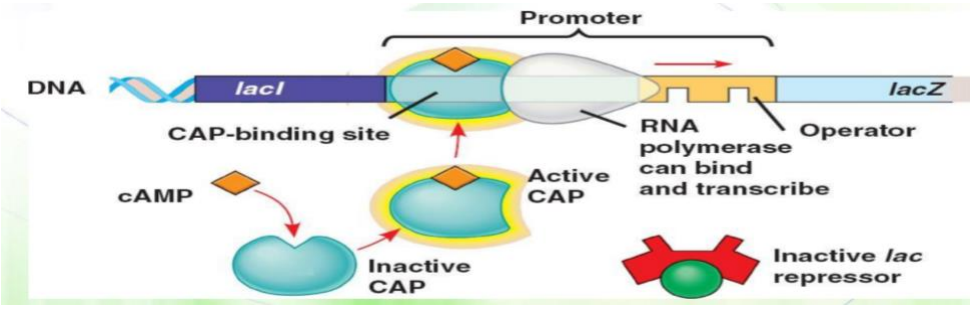
2. **Other mutations cause non-inducible or repressed**

- expression (always off).
- Even if you put a tonne of lactose there will be no expression of the lac operon
- Examples:
- Conformational change in Lac repressor, allolactose cannot bind to it and repressor will still be bound operator
- Mutation in promoter thus RNA polymerase cannot bind to the promoter



Now onto Negative regulation

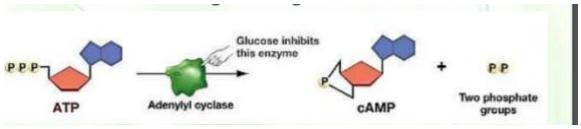
- It is mediated by a protein called, *catabolite activating protein* (CAP).
- CAP binds cAMP
- CAP can then bind to the DNA upstream to the promoter stabilizing the interaction of the RNA polymerase to the DNA inducing/activating transcription even further.
- This is needed Because the interaction of RNA polymerase when it binds the promoter to open up the DNA to form the open promoter complex is a weak interaction and can dissociate, resulting in low efficiency of transcription.
- Hence function of CAP is to make transcription more efficient



The Second type of regulation of lac operon that we mentioned before

2. **Regulation by glucose (negative)**

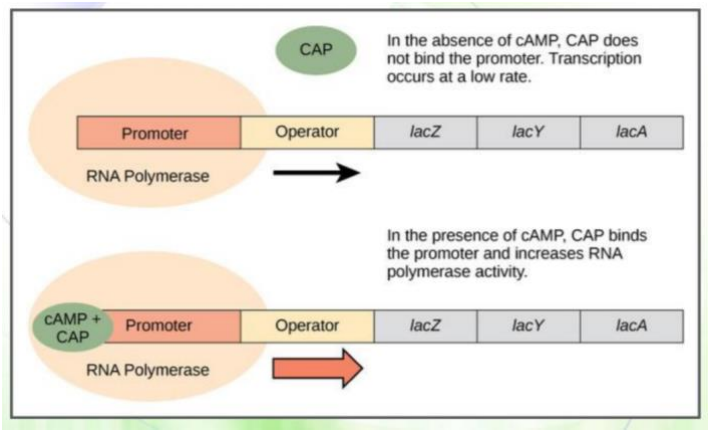
- Glucose negatively regulates expression of lac operon how?
- The ability of CAP to bind to the promoter is influenced by how much cAMP is in the cell



- cAMP is produced by adenylyl cyclase enzyme (ATP→ cAMP), adenylyl cyclase is inhibited by high level of glucose.

Why is this needed?

Because Glucose is preferentially utilized by bacterial cells over lactose so it's important for lac operon to be repressed even in the presence of the normal inducer (lactose).



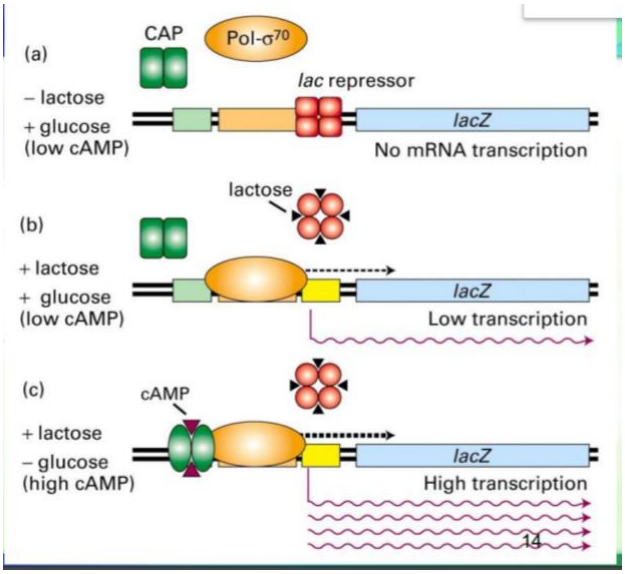
Four possible scenarios:

1***. No glucose, no lactose***: there will be no expression of lac operon because the repressor will be bound to the operator as there is no lactose.

2. ***Elevated level of glucose but no lactose***: there will be also no Expression of lac operon because of the same reason

3***. Elevated level of lactose with no glucose***: very strong expression of lac operon because the repressor is not bound to the operator, and CAP has high amount of cAMP.

4. ***High levels of both:*** there will be expression of the Lac operon, but it won't be as strong as previous one because CAP is not active.



Regulation of transcription in Eukaryotes

- 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:**

1. *Cis-acting elements*

Examples: Promoters, proximal promoter elements, silencers and enhancers

2. *Transcriptional regulatory proteins*

Examples: Activators and Repressors

3. *Chromatin remodelling* (not found in prokaryotes)
4. *Noncoding RNA molecules*

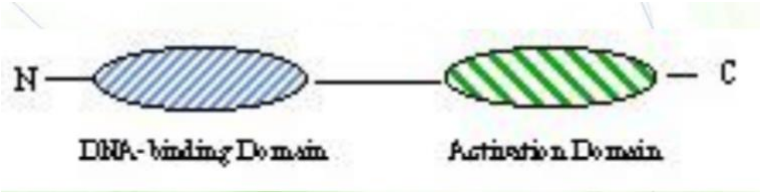
Transcriptional regulatory proteins

These proteins consist of different domains (at least two domains):

- A DNA-binding domain
- A regulatory or activation domain that interacts with transcriptional proteins

Both domain activities are independent and can be separated from each other.

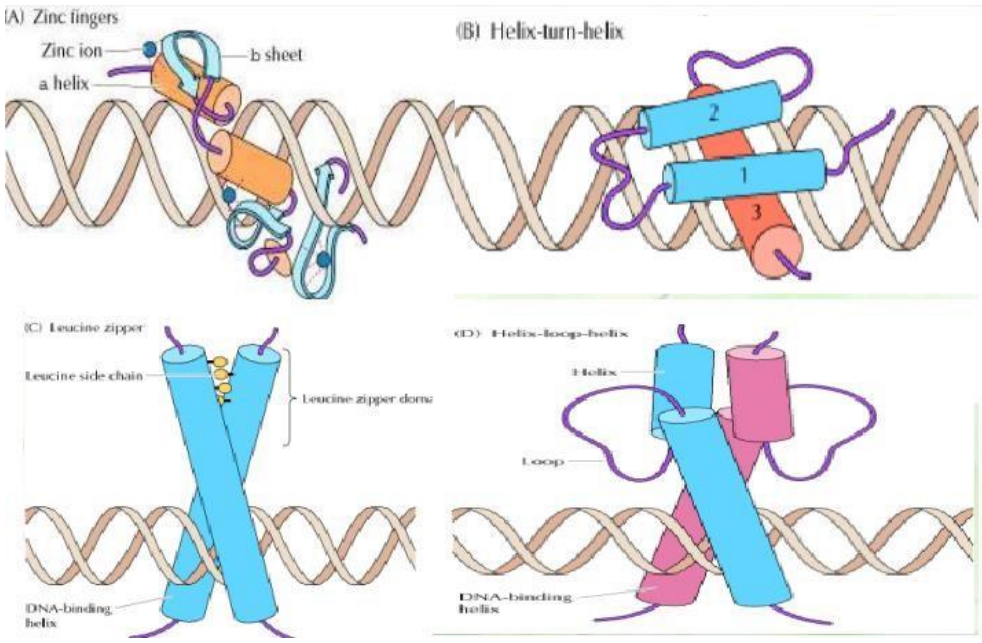
Domain: a super secondary structure, made of multiple secondary structures (alpha helices, beta strands….), domains can fold independently from the rest of protein so if a domain is isolated from the rest of the protein it maintains structure and function.



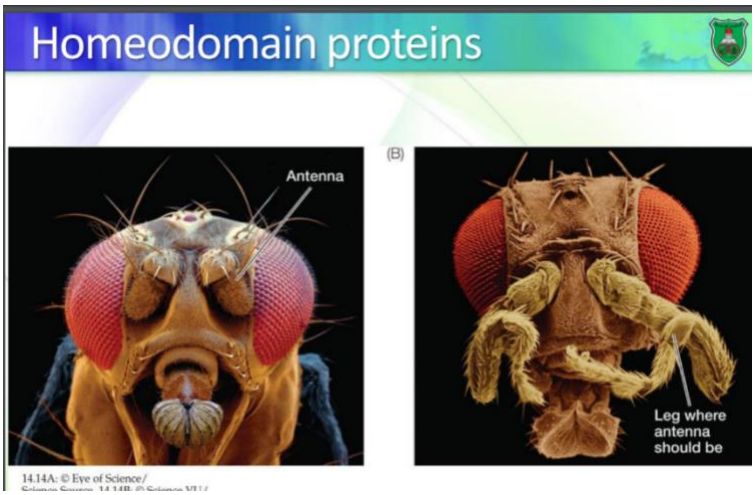
Here in this transcriptional regulatory protein if we cut the link between the two domains each of them can still do its function separately

DNA-binding domains

1. Zinc finger domains (Steroid receptors)
2. Helix-turn-helix motif (homeodomain proteins)
3. Leucine zipper (CREB)
4. Helix-loop-helix



Zinc finger domain is one of the most common domains, zinc is part of the alpha helix and stabilizes it, (the two alpha helices are like two fingers) that fit around DNA, interact with it and activate transcription



Homeodomain proteins are transcription factors that are responsible for development and differentiation so when these proteins where discovered, to study their function they studied their expression and they noticed that a certain protein gets specifically expressed in lower part of larva, what they did:

Allowed expression of this certain protein in the head,

Result: legs came out of the head

Conclusion: these proteins are responsible for

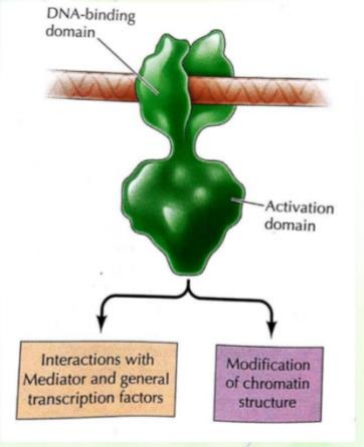
differentiation of cells into legs

note that homeodomain proteins are encoded by homeobox genes

The activation domains

Activation domains stimulate transcription in two ways

1. interacting with mediators (like RNA polymerase), general transcription factors, facilitating the assembly of a transcription complex on the promoter..
2. modifying the chromatin, or DNA by methylation



Example of transcription factors

1. **Steroid hormone receptors**

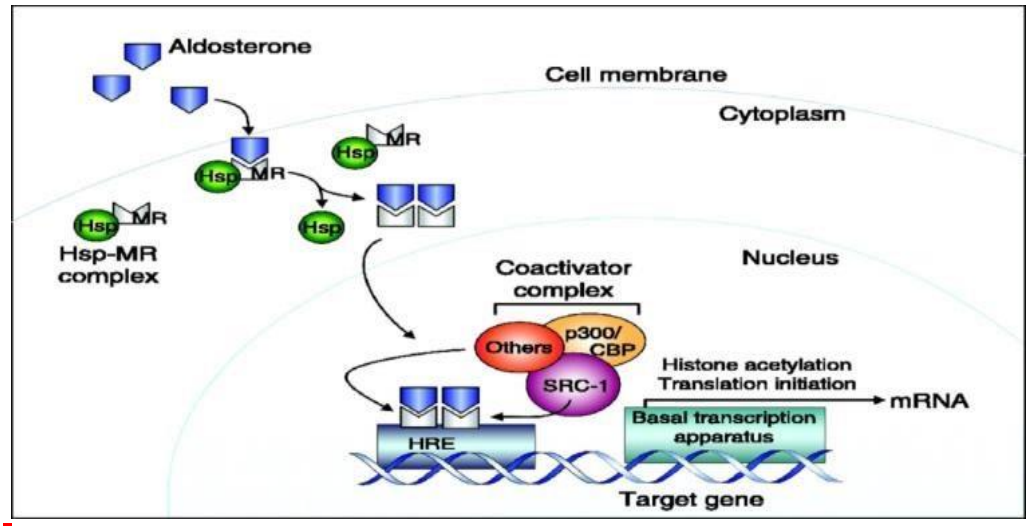
- Cytosolic or nuclear receptors that bind steroid hormones (ligand)
- These receptors bind steroid hormones at ligand binding domain, then translocate into the nucleus where they bind specific DNA sequences called hormone response element via their DNA-

binding domain, and recruit and bind transcriptional regulatory proteins using their activation domain.

- steroid receptors have 3 domains, DNA binding domain (zinc finger), ligand binding domain and activation domain
- Steroids (like oestrogen, androgen, aldosterone ....)are small lipophilic molecules (no transporters are needed) so they diffuse via plasma membrane to bind to their cytoplasmic (or Nuclear) receptors which contain zinc finger as DNA binding domain , the binding will induce the dimerization of their receptors and once they dimerize they will be translocated to the nucleus in order to bind to a specific sequence (known as HRE(Hormone Response Element)), the binding will recruit transcriptional regulatory proteins inducing or inhibiting the transcription of certain genes depending on which proteins they recruit or interact with (the genes which will be affected they are known as steroids regulated genes)

- HRE IS the proximal promoter element that is present in more than one gene so when the hormone enters and binds to HRE it binds to all the genes that have it so regulation of all these genes happens at the same time as they have the same regulatory element, the HRE

Proximal promoter element here is equivalent to operon in prokaryotes



since domains are independent of each other and each domain contains a part of a gene, they can create a transcription factor that has domains of different reception, for example

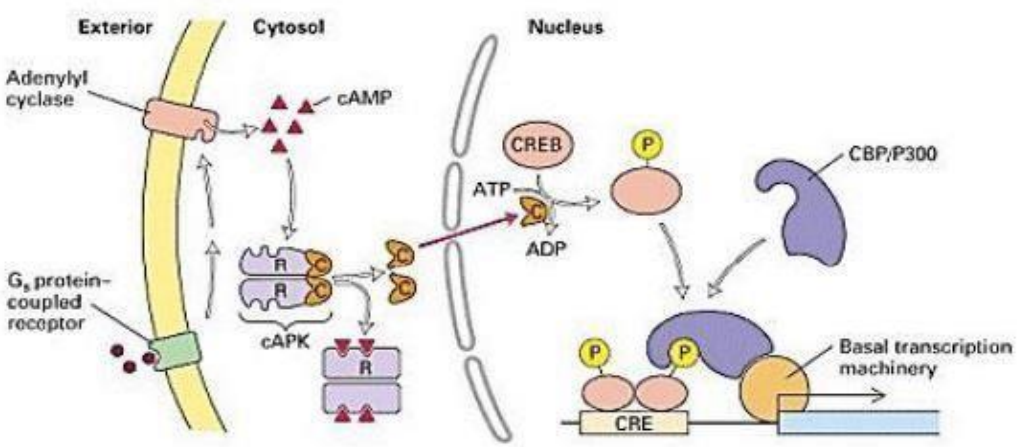
- Ligand binding domain of androgen receptor
- Activation binding domain of progesterone receptor
- DNA binding domain of estrogen receptor

how will this new genetically engineered protein function?

the ligand androgen will bind ligand binding domain, bind to estrogenic response element, progesterone activating domain sequesters progesterone transcription mediators.

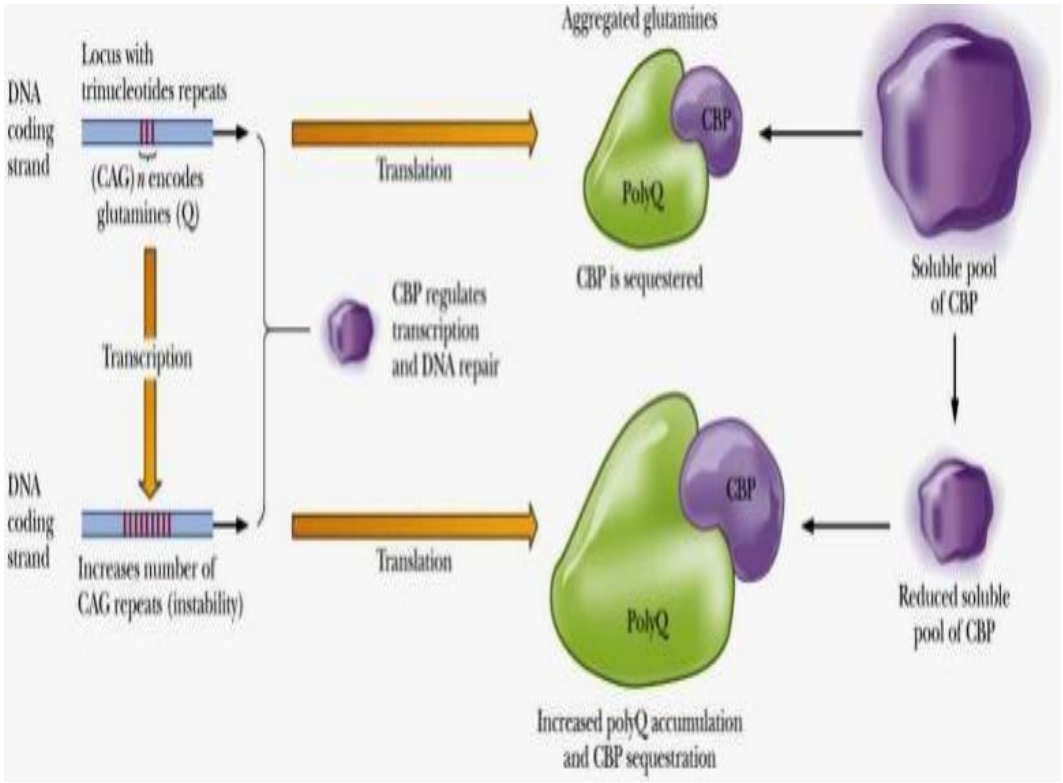
2. <u>cAMP-response element (CRE) binding protein</u>

- Many ligands (like epinephrine) bind to cell surface receptor (G protein coupled receptors) inducing the activation of adenylyl cyclase converting ATP into cAMP (remember c-AMP is widely used in cell signalling since it is small, versatile molecule that is regulated very efficiently meaning levels of it increase quickly and decrease quickly),
- then c-AMP will activate PKA (protein kinase A) which will phosphorylate proteins activating or inhibiting them One example of the proteins that will be phosphorylated by PKA is CREB protein (cAMP response element binding protein),
- ***<u>CREB</u>***: a protein that binds to certain elements on the DNA called cAMP response element
- once the CREB are phosphorylated they will dimerize and attract another protein called CBP (CREB binding protein) and bind to it
- This new dimer will bind to to CRE (cAMP Response Element), and once the binding between the CRE and CREB dimer occurs, this will facilitate the interaction with either mediators (general transcription factors) or RNA polymerase



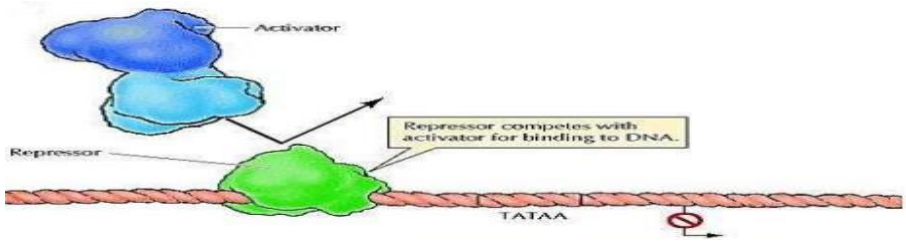
Huntington's disease

- a neurodegenerative disease, symptoms usually appear around 30s and early 40's, person starts to lose control and cognition, causes paralysis and It is fatal.
- This syndrome is caused by a mutation in the gene coding for transcription protein known as huntingtin.
- Huntington protein: it is a transcription factor that can interact with CRB (CREB binding protein) by binding to it.
- Normally in this gene there is a CAG repeat (20-50 repeats, number influenced by variation), CAG encodes for glutamine so since there is more than one CAG repeat the sequence it encodes for is called polyglutamine= poly Q.
- The mutation is an increase in the number of a trinucleotide repeat of CAG, so increase in polyglutamine size
- (for example, instead of 50 repeats you'll have 500 repeats)
- When the size of the Huntington transcription factor increases it will work like a sponge, the polyglutamine product sequesters CREB-binding protein (CBP), making less of it available for molecular processes of regulation inside the cell, an example of such affected processes is transcription and DNA repair genes expression
- The loss of the DNA repair gene expression leads to the inability to repair DNA damage and cells eventually die.

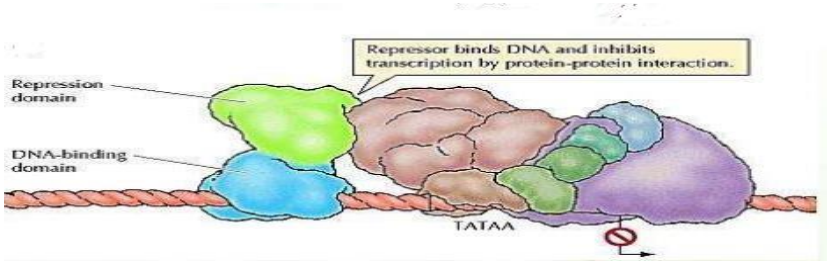


Eukaryotic Repressors (*Of two types)*

1. Repressors that only have a DNA binding domain (but not a protein interacting domains) bind to specific DNA sequences and prevent activators from binding inhibit transcription competitively



2. Repressors that have both DNA-binding and protein-binding domains (repressor binding domain /repression domain) that can interact with different factors that suppress expression or bind to RNA polymerase and general transcription factors tightly and inhibit them thus inhibiting transcription in these cells.


Repressor binds DNA and inhibits transcription by protein-protein interaction.
Repression domain
DNA-binding domain
TATAA