Genetics
& Cell biology

◉ Sheet ○ Slides

Number

11

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Regulation of mRNA stability (continued)
Slide #5 (60-69)

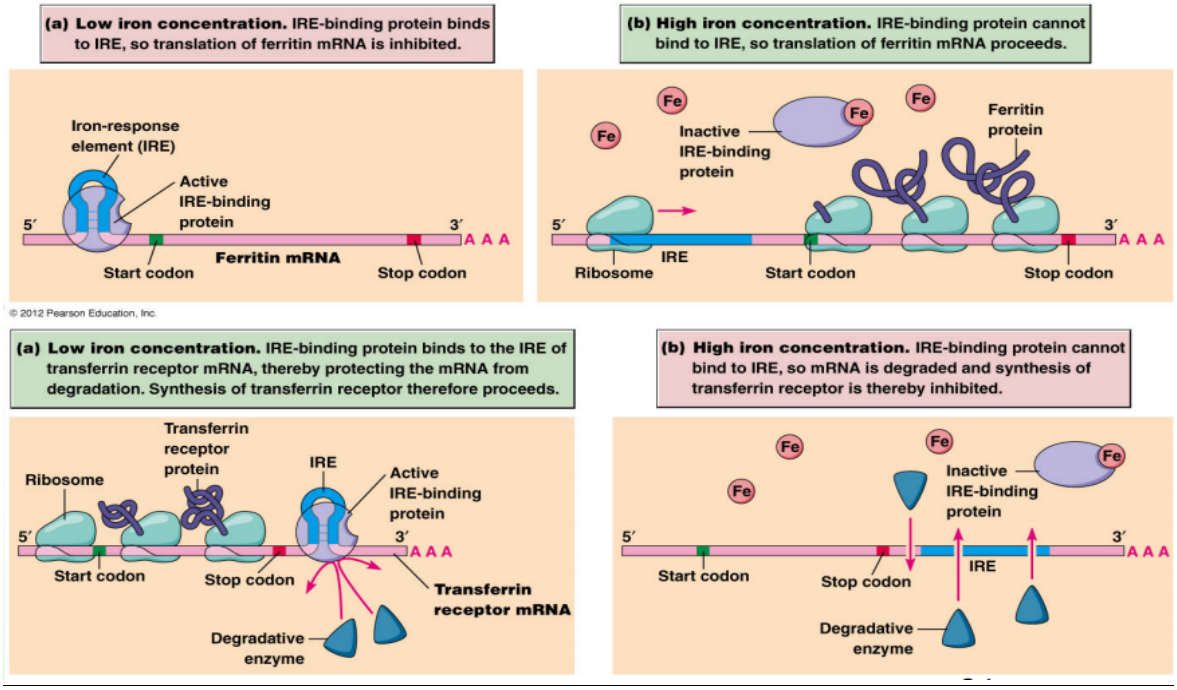
There are certain genes regulated at the same level (regulated all together). An example clarifying this concept is enzymes; a certain enzyme is inhibited while another enzyme catalyzing an opposite pathway is activated (one is inhibited, the other is activated). The same concept applies for the regulation of gene expression.
The expression of certain genes can be regulated by stabilizing or destabilizing their mRNA all at the same time.

Iron-Responsive Elements (IRE)

IREs are regions contained within the mRNA sequences that code for certain proteins that regulate the levels of iron (Ferritin, transferrin receptor, ferroportin, and DMT1). It is a binding site for a protein named IRE-Binding Protein, whose binding influences protein expression. If it binds, it stabilizes the mRNA, which results in more protein, blocking the synthesis of another protein of an opposite pathway at the same time (absorption into cells vs. inhibition of storage).

Effect on expression

***** When **iron is abundant**, it binds to IRE-BP, disabling the binding of IR-BP to ferritin mRNA. This prevents the degradation of the mRNA molecules allowing the production of more ferritin protein. Therefore, the iron itself causes the cell to produce more iron storage molecules. I.e. cells do not need transferrin receptor, so it becomes unstable and is degraded. However, this iron needs to be stored for emergencies, so ferritin is increased.
***** On the other hand, at **low iron levels**, the IRE-BP will bind to the ferritin mRNA and, thus, the mRNA will be destabilized, making less ferritin protein.
***** An opposite effect is seen on the stability of transferrin receptor mRNA.
***** They are regulated **reciprocally.** Reciprocal regulation means opposite regulation; one is up-regulated; the other is down-regulated.



Immunoglobulins (Antibodies)

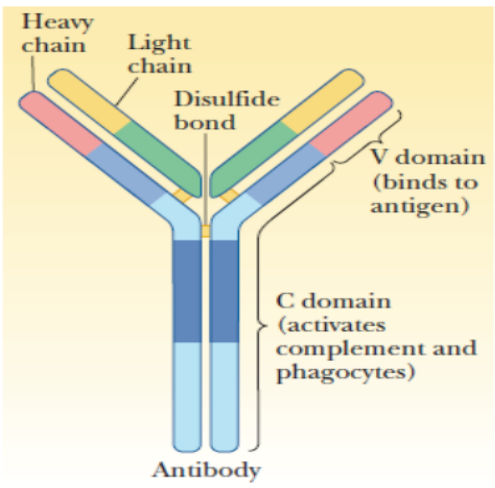
They are proteins produced by B-cells.

Structure

Two identical heavy chains and two identical light chains held together by disulfide bonds. Each of these chains (heavy and light) contains constant and variable regions. The variable regions are diverse and are responsible for recognition of antigens (foreign bodies).

There are a huge number of antigens, so in order to detect and recognize those antigens, the same number of antibodies needs to be present. However, it is impossible to have billions of genes, for each one of these immunoglobulins.

Our immune system has the ability to produce about (10^{10}-10^{11}) different antibodies. So how is this diversity generated?



There is a **mechanism** for producing a huge number of antibodies:

1. Gene rearrangement of the light chain

***** There are two types of immunoglobulin light chain constant regions: κ and λ**.**

***** Each light chain is a product of at least 3 genes:

a. Variable (VL) gene: 250 genes

b. Joining region (J) gene: 4 genes

c. Constant region (CL) gene: 1 gene

***** The possible combinations are ~2000 (250 x 4 × 2) unique light chains

➡ each one of the 250 variable genes has the possibility of combining with any of the 4 joining regions and any of the 2 constant regions. So we end up with the possibility of having 2000 different light chains.

2. Gene rearrangement of the heavy chain

***** Heavy chains have 5 constant regions, each with a specific function (γ, α, μ, ϵ, δ); thus named IGG, IGA, IGM, IGE, IGD respectively

***** Heavy chain is a product of at least 4 genes:

a. Variable region (VH) gene: 500 genes

b. Diversity region (D) gene: 12 genes

c. Joining region (J) gene: 4 genes

d. Constant region (CH) gene: 1 gene

***** The possible combinations are ~24000 (500 x 4 x 12) unique heavy chains

➡ each one of the 500 variable exons (genes) has the possibility of combining with any of the 4 different joining regions and any of the 12 different diversity regions. So we end up with the possibility of having 24000 different heavy chains.

Therefore

2000 *light chains* **X 24,000** *heavy chains* **= ~ 5 × 10^7** *different immunoglobulin molecules*. However, this is still not enough, so additional mechanisms are needed for more diversity.

Additional Mechanisms

1. Imprecise joining of immunoglobulin gene segments, resulting in the formation of ~10^5 different light chains and ~ 2×10^6 heavy chains, which can then combine to form more than 10^{11} distinct antibodies. (I.e. increased possibility of having different sequences of proteins)

2. Addition or deletion of nucleotides during recombination itself, which also increases the diversity of the sequence of the gene.

3. Somatic hypermutation, which results in the introduction of frequent mutations into the antigen-binding variable regions of both heavy-chain and light-chain genes.
During replication, some errors occur and they could be repaired. But in B-cells these errors are not repaired. This error in replication is induced by an enzyme known as **Activation-Induced Deaminase (AID)** which removes the amino group from the cytidine base in DNA converting it to uridine (C►U). This error is not repaired, and even if it is, U would be converted into T (Repair or lack of repair results in creation of single base substitutions.)
So when replication takes place, the rate of error is increased, this also increases the diversity of variable regions.

AID is called so because it is stimulated by the activation of B-cells. B-cells present in the bone marrow or lymph nodes are stored and are waiting for an antigen to interact with. When the B-cell recognizes the antigen, it gets stimulated and proliferates, activating AID, which then starts converting C to U in the DNA increasing the rate of error during proliferation of B-cells. We end up making an unlimited number of B-cells; each one of them has an antibody that can specifically recognize the antigen it first interacted with.

(Mins 00:00 - 14:15)

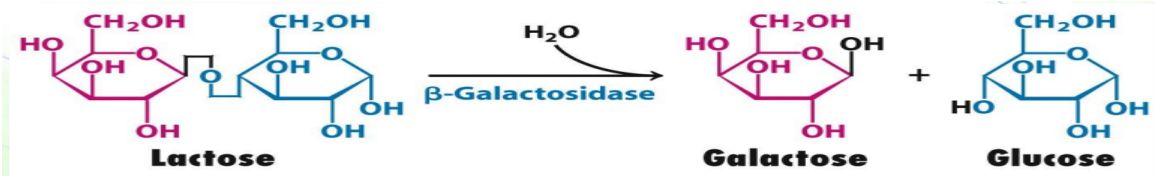
Regulation of Transcription
Slide #6 (1-20)

Regulation of Transcription in Prokaryotes

The Lac Operon

We have previously talked about **operons** defining them in bacteria as a cistron or a genetic unit that produces ONE mRNA, different parts of which can produce different polypeptides, each one of those polypeptides carrying out a different function but participating in the same pathway. (A cluster of genes transcribed from one promoter producing a polycistronic mRNA.)

The lac operon is a cistron, or a genetic unit that produces one mRNA responsible for producing **3** different proteins, all of which are involved in the metabolism of lactose. Lactose is cleaved by the enzyme β-Galactosidase into galactose and glucose. Glucose is then used by bacterial cells as a source of energy. Ultimately, the bacterial cell makes use of lactose by converting it into glucose which is then used as an energy source.

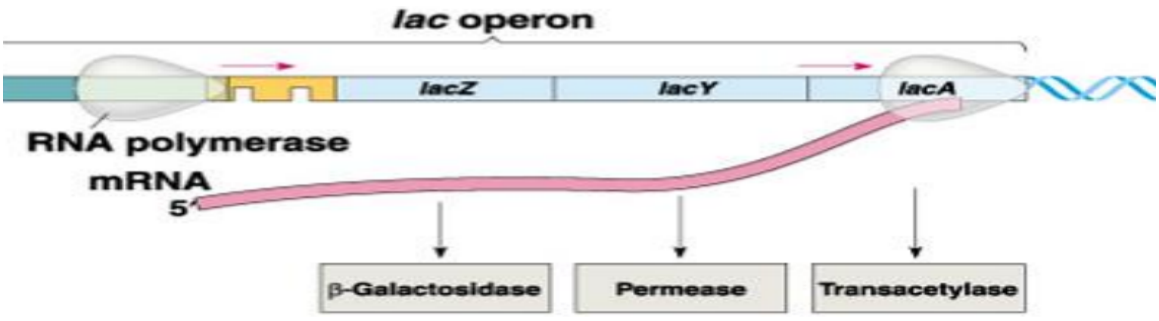


What are those **3** proteins?

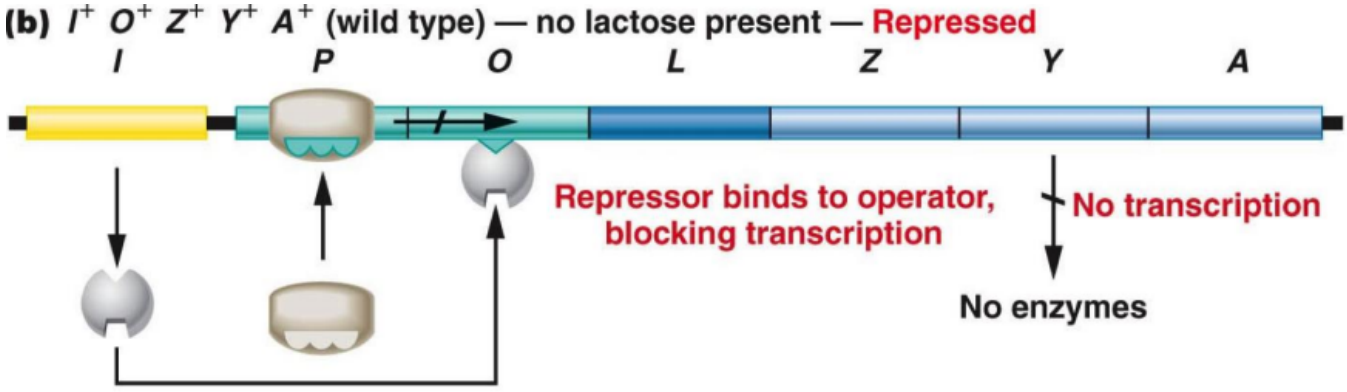
1. B-Galactosidase (lacZ gene): catalyzes the cleavage of lactose
2. Permease (lacY gene): transports lactose into the cell
3. Transacetylase (lacA gene): acetylates β-galactosides (could be as a preparation for metabolism)

When one mRNA is transcribed each of the parts (Z, Y, A) is responsible for the formation of β-galactosidase, permease, and transacylase respectively.

These 3 genes are located in **one** operon known as the lac operon.



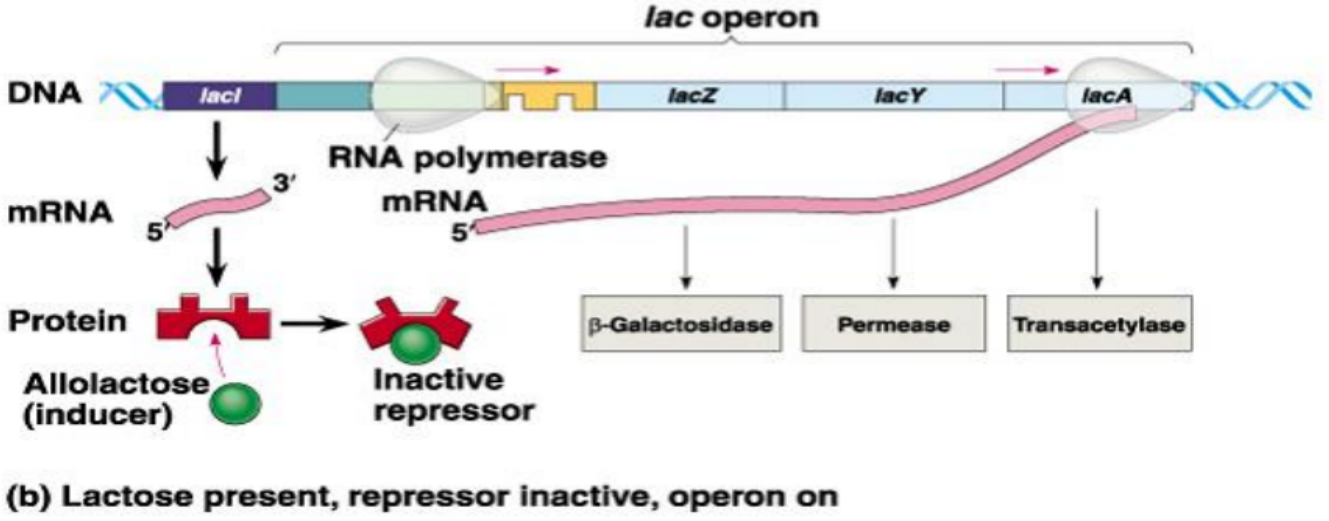
The operon also contains a **promoter (P)** which is the binding site for RNA polymerase.
***** The promoter region includes the operator region, which is a binding site of a protein called the lac repressor or I repressor which comes from a different gene called I gene. The I protein binds the operator and blocks transcription of the lac operon by preventing the RNA polymerase from unwinding the promoter.
*Note: The repressor is specific to the lac operon; it is not present in all genes.



***** Wild type means normal type. Geneticists used this term while studying the fruit fly *Drosophila.*

Regulation by Lactose (Positive)

***In the absence of lactose:** No need to transcribe this unit of genes. The operator functions as the binding site for the lac repressor.
***In the presence of lactose:** Lactose binds to the repressor preventing it from binding the operator. So RNA polymerase can then transcribe the genes (β-galactosidase, permease, and transacylase)
this process is known as Induction.



*Student's **Q:** Permease is needed for the entry of the lactose into the cell; wouldn't the absence of lactose in the first place block the production of permease?
A: It's not "all or none." Despite the absence of lactose, there must be little amounts of permease in the cell, which allow the entry of the first lactose OR lactose could enter using other transporters or sensors on the cell surface, or any other mechanism etc.

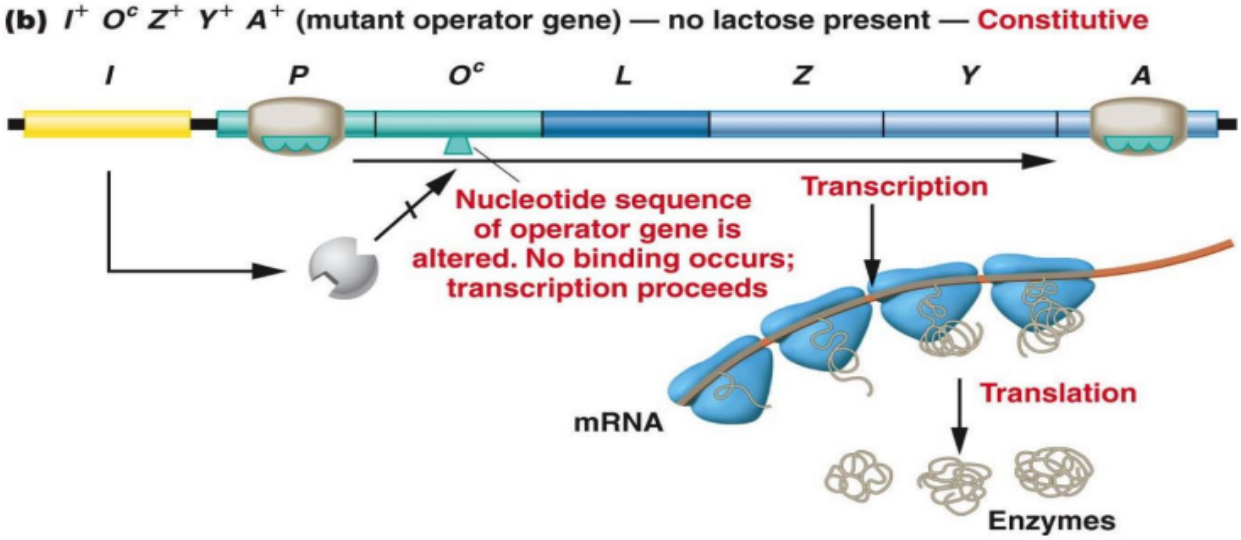
Cis and Trans Regulatory Elements/Factors

Cis: At the same level. Present on the same strand of DNA (e.g. Promoter and Operator). I.e. if the promoter and the operator were removed from a chromosome to a plasmid there would not be transcription or regulation. So these regulatory elements must be present on the same chromosome that carries the lac operon.

Trans: At different levels. Could be on another chromosome (e.g. Lac Repressor). If the lac repressor gene with its promoter was removed and placed on a plasmid, it will still perform its function of regulation (synthesis and inhibition).

Effects of mutations

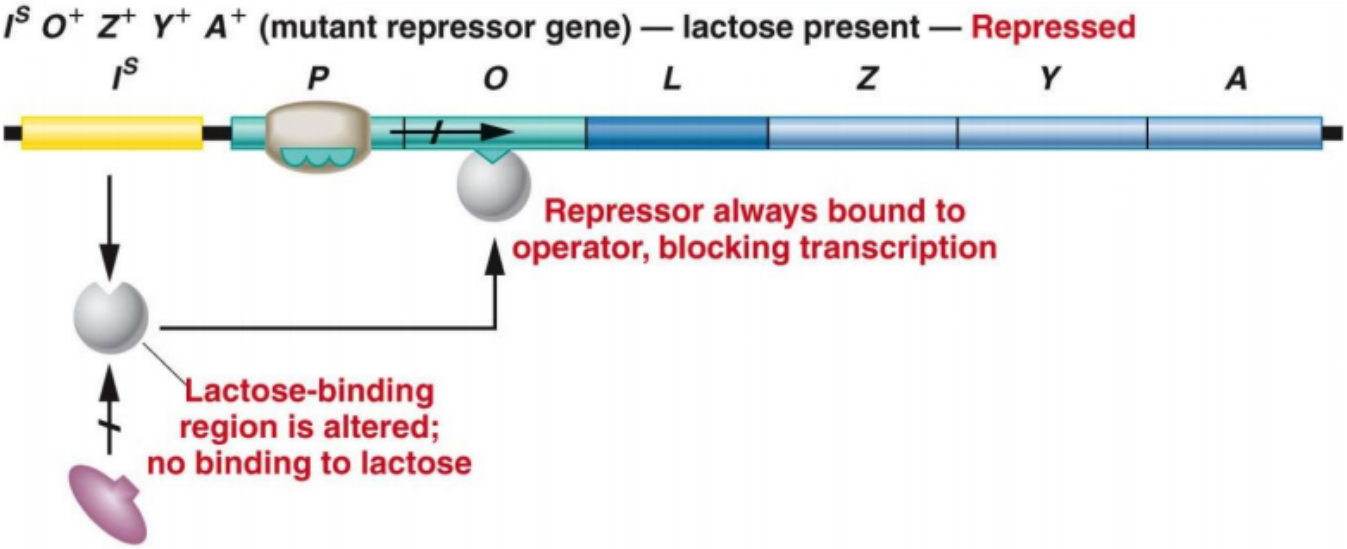
In order to determine the importance of each gene, scientists start presenting mutations on the genes (promoter gene, operator gene etc.…)
1. Mutations affecting O result in constitutive expression (always on) since these mutations prevent I from binding to the operator.



2. Mutations of I are either constitutive or noninducible (always off).

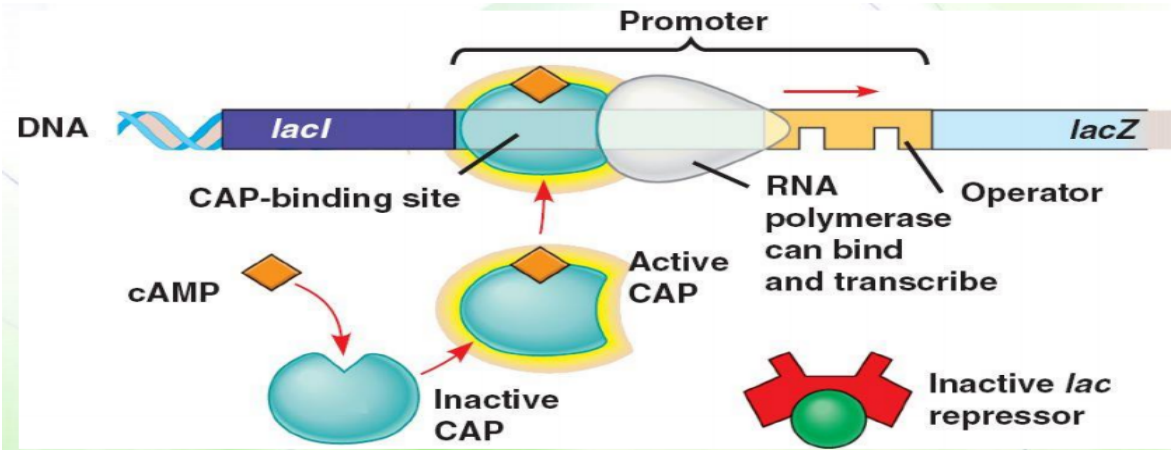
- In constitutive I mutants, I always binds lactose, so expression of the operon is always induced.
- In noninducible I mutants, the repressor binds to the operator very tightly even in the presence of lactose.

3. Mutations of lactose binding site on repressor, lactose cannot bind operator



(Mins 14:15 – 26:15)

Another protein that regulates lac operon itself is called Catabolite Activator Protein CAP. If RNA polymerase binds promoter while operator is empty transcription takes place. However, RNA is not totally active; it needs a push to start moving (CAP). CAP is regulated by cAMP which binds to CAP activating it, then CAP binds to a region that precedes (before) the promotes, it then touches the RNA polymerase and pushes it. So CAP activates RNA polymerase which in turn expresses lac operon massively.



Regulation by Glucose (Negative)

cAMP is regulated by glucose. If both glucose and lactose are available in a bacterial cell, glucose is more preferable. So when glucose is available, lac operon should not be expressed as much. Glucose is consumed first until it runs out, only then is lac operon expressed.

Glucose is a negative regulator. The presence of glucose is responsible for inhibition/reduction of expressing lac operon. How is this?

Adenylyl cyclase, which converts ATP to cAMP, is regulated by glucose. The binding of glucose to adenylate cyclase inhibits it.

High glucose

↓

Inhibited adenylyl cyclase

↓

No conversion of ATP to cAMP

↓

Low amounts of cAMP

↓

CAP is not active

↓

No binding to DNA

↓

No activation of RNA polymerase

Transcription is fairly low
(Present but low)

No glucose

↓

Activated adenylyl cyclase

↓

Conversion of ATP to cAMP

↓

High amounts of cAMP

↓

CAP is activated

↓

Binding of CAP to DNA

↓

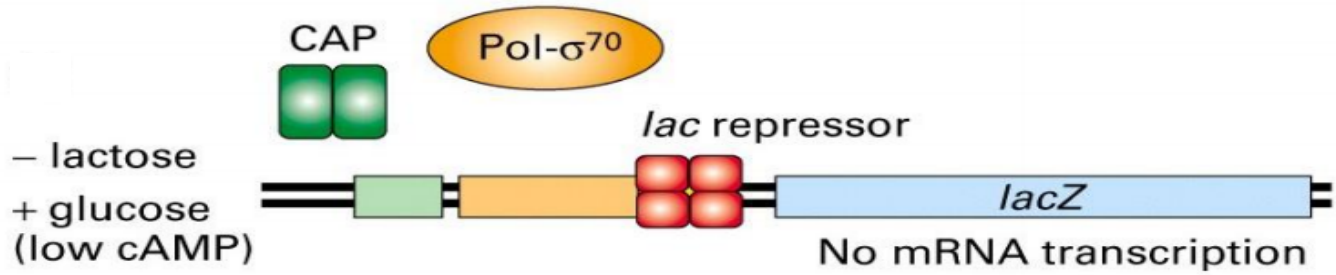
RNA polymerase is activated

Expression of lac operon

There are four cases regarding the presence or absence of each, glucose and lactose in the cell:

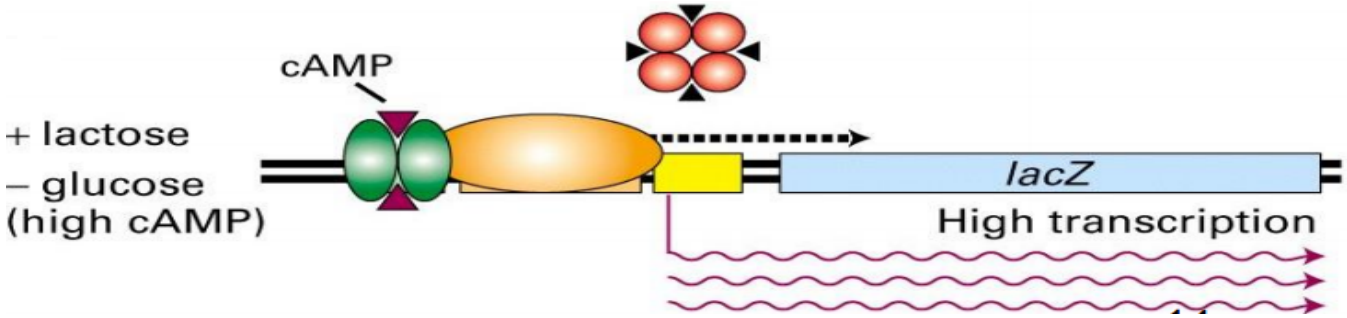
<u>Case 1:</u> Glucose is present, Lactose is absent.

Adenylyl cyclase is inactive, low amounts of cAMP, CAP is inactive, RNA polymerase is not so active, it is bound to the promoter but with no movement because there is no lactose. Lac repressor is not inhibited. No transcription.



<u>Case 2:</u> Glucose is absent, Lactose is present.

Adenylyl cyclase is active, high amounts of cAMP, CAP is active, lactose binds repressor inhibiting it, no binding of repressor to operator, and RNA polymerase is active. So, High transcription.

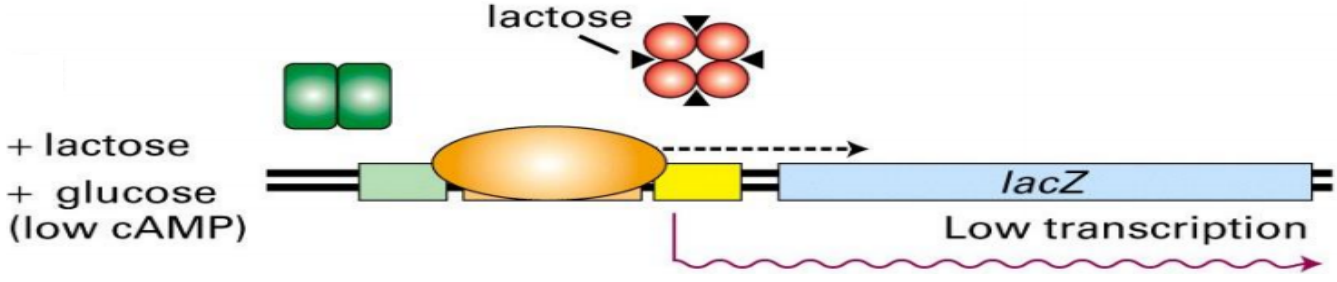


<u>Case 3:</u> Glucose and Lactose Both Absent

Lac repressor is bound to operator. RNA polymerase is bound to promoter but inactive. No transcription.

<u>Case 4:</u> Glucose and Lactose Both Present

Lac repressor is not bound to operator. RNA polymerase can start transcription but it is not so active because it needs CAP, CAP needs cAMP but there is no cAMP because of the glucose. The cell consumes glucose first. So, Low transcription.



(Mins 26:15 – 32:18)

Regulation of Transcription in Eukaryotes

The mechanism in eukaryotes is more complex but with the same basic principles. Transcription in eukaryotic cells is controlled by:

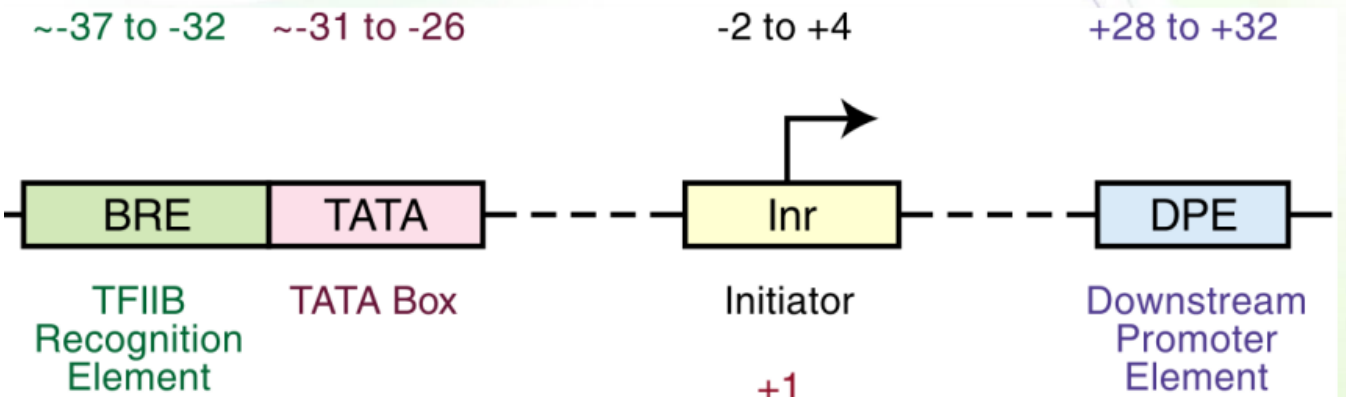
1. Cis-acting DNA sequences
   -Promoters and enhancers
2. Transcriptional regulatory proteins
3. Repressor proteins
4. Modification of DNA and its packaging into chromatin (very important role)

Promoters

General components of promoters:

1. An upstream element (BRE) that is binding site of TFIIB.
2. The TATA box, which is binding site of TFIID. A bit far from the start site of transcription. Genes containing TATA box in eukaryotic cells constitute around 20%, the rest of the genes have other types of promoter genes.
3. The initiator element (Inr), which surrounds the +1 site (first nucleotide to be read).
4. Multiple downstream elements.

*Note: In prokaryotes, the promoter is present before the start site while in eukaryotes, multiple regions compose the promoter.



Enhancers

Cis-acting regulatory sequences present throughout the genome, which are binding sites for gene-specific transcription factors that regulate RNA polymerase II. They could be far from promoters, beside them, or to the middle of the genome in introns.

*Advantage: They work regardless of their location or orientation because of a characteristic of the DNA called looping (the DNA is so flexible it can loop). So when the regulatory proteins bind to the enhancer, the DNA can loop around and these

regulatory proteins can touch the complex… activating it. Wherever these proteins are located, the DNA can still reach the complex.

Mediators

Mediate interaction between regulatory proteins on the enhancer with the complex bound on the promoter.

Cohesion

The formation of DNA loops is facilitated by a protein called cohesion. Stabilizes the structure of DNA looping allowing the interaction to be strong and stable.

(Mins 32:18 – 39:35)

Methods for studying promoter regions/enhancers and defining important/unimportant regions:

Gene Transfer Assay (Reporter Gene)

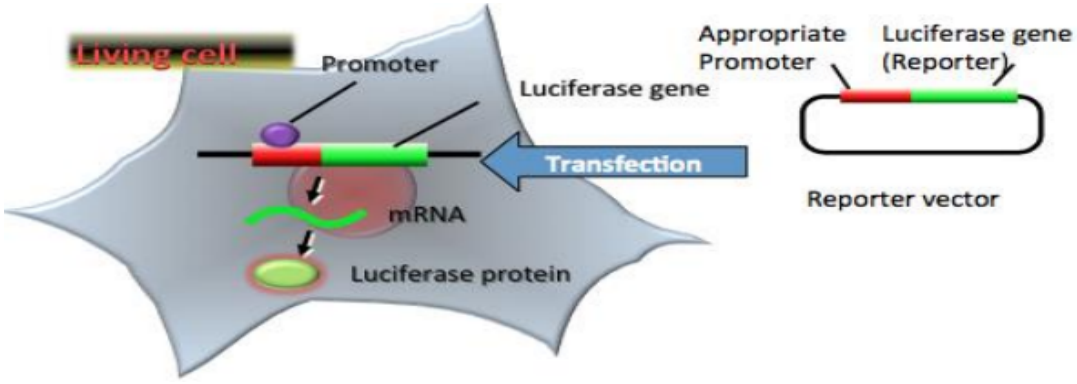
* A DNA sequence suspected to be a regulatory sequence is placed upstream of a reporter gene such as luciferase in a plasmid.

***** Reporter gene is a gene that gives a signal. E.g. Luciferase enzyme present in the firefly. Luciferin, which is cleaved from luciferase, gives signal (fluorescence). Luciferase gene is placed on a plasmid, preceded by the promoter region/enhancer of the gene whose regulatory sequences are to be studied. Then the plasmid is inserted into the eukaryotic cell in a process called **Transfection.**

***** If the promoter/enhancer region was functional (RNA polymerase or regulatory proteins can bind) the gene will become active. So expression of luciferase will take place, manifested in fluorescence.

***** High fluorescence indicates high expression, low fluorescence indicates low expression, and no fluorescence indicates no expression (no signal/no promoter).

* Luciferase is not expressed in our cells. But if it is expressed in those cells, it will emit a fluorescent signal, and the amount of fluorescence can be measured.



Example 1: Luciferin is inserted in tumor cells into the body of mice, and then the tumor is allowed to develop. Imaging of the mouse shows fluorescent tumor cells along with their metastasis.

*Luciferin is an indicator of the activity of the gene.

Example 2 (important for the exam, not in the slides):

Regulatory region (promoter). Starting with a certain number of nucleotides, we started deleting/removing bits then measuring luciferin. At a certain length: high expression. When further nucleotides were removed, the expression remained unaffected. This indicates that the region removed is of no importance. Further removal stopped the transcription, indicating the binding site for activator (removed enhancer part). This last region is the important region.

(Mins 39:35 – 49:09)

*Please refer to the figures in the slides for better understanding.

The End

Best of Luck!