

Chapter for today: Chap. XV**Major points for the day:**

1. Special issues for gene expression in eukaryotes
2. Alternative modes of gene regulation

Reprise

Last time we considered the ways in which genes can be regulated.

- All genes are not expressed equally in all tissue or at all times
- Many genes are controlled at the level of RNA synthesis. The example I gave last time was the *lacZ* gene of the common bacterium *Escherichia coli*.
- Jacob and Monod used genetics to study how bacteria regulated the expression of the genes for digestion of the sugar lactose, which is found in milk. They developed an operon model that called for negative regulation of the process of mRNA synthesis.
- The negative regulation is caused by a protein called the *lac* repressor, which binds to a site in the operon called the operator. Binding the repressor to the operator blocks the ability of RNA polymerase to transcribe the *lac* genes.
- Expression of the *lac* genes can be induced by addition of lactose, which binds to the repressor causing it to dissociate from the operator, and allowing expression of the *lac* genes.
- We also discussed positive control in which a protein promotes binding of RNA polymerase to a weak promoter (one that interacts with polymerase poorly) and so increases the production of mRNA

How do we know the *lac* system works this way?

Through analysis of mutations we can assign a function to the two regulatory elements of the system, the *lacI* gene for the repressor and the *lac* operator, its binding site.

- A mutation in the *lacI* gene can make a defective repressor. Such a mutant is called a *lacI* mutation.
- The mutant gene expresses a defective form of the repressor protein, which can not bind to the operator site
- As a result, the *lac* genes are expressed all the time

We can ask what happens if we give the bacterium two copies of the *lac* genes.

- Both of the operons can be expressed, and if there is no functional form of the repressor then both are expressed all the time.
- Adding one copy of a normal *lacI* gene restores expression of the functional repressor protein, which then turns off transcription of both *lac* operons

What would happen if we had a mutation of the *lac* operator instead?

- A mutant *lac* operator will not allow repressor to bind the DNA, so that the *lac* genes are expressed all the time.
- If there are two copies of the *lac* genes, one with a good operator and one with a bad operator then a functional *lac* repressor will repress only the genes that are next to a good operator.

The repressor is said to act “in trans” meaning it can affect genes that are not physically connected to it. The operator works “in cis” meaning it will only affect genes that are physically linked to it. These genetic experiments, done in the 1960s confirmed the operon model of Jacob and Monod even before we knew the exact nature of the repressor or the operator.

Different styles of regulation of gene expression in bacteria

A typical bacterial gene is part of an operon, a group of genes that are transcribed together on a single mRNA molecule. This evolved to allow coordinate regulation of the genes

- The *lac* operon, encodes three proteins all involved in the metabolism of the sugar lactose. Addition of lactose to a cell causes repression by the *lac* repressor to be released, allowing expression of all three enzymes in a 1:1:1 ratio on the single mRNA.
- As another example, the genes required for the synthesis of an amino acid, tryptophan, are all encoded in a single operon, the *trp* operon. When a bacterium finds itself without sufficient tryptophan, transcription of the operon is turned on, resulting in expression of all of the enzymes necessary for the construction of the amino acid.

Already, we have two types of control. In the *lac* system the presence of a small molecule that needs to be broken down to be used as a source of carbon induces the expression of the proteins needed for that degradation. In the *his* system the absence of a small molecule that is needed to build proteins (not used as carbon source) stimulates expression of the genes necessary to produce it.

So, genes can respond either to the presence or absence of small molecules, but in both cases by causing greater transcription of relevant genes.

Global control

In both of these cases, a change in environmental conditions results in a change in the expression of a single operon. This is not the only way that gene control can operate. For instance, there are systems in which a change in environmental conditions calls for the expression of a large number of genes located in several operons. This is called global control.

- As an example of global control, most cells respond to a brief exposure to heat by turning on the expression of a large number of heat shock response genes
- In bacteria, these genes can be located in multiple operons, so heat shock must turn on the expression of multiple transcription units

Eukaryotes have a different form of genome organization

Eukaryotes do not have operons. Instead, each mRNA includes only a single expressed gene. Because of that it is not possible to effect control of multiple genes by regulating the activity of a single promoter. That means that eukaryotes use a form of control that resembles global control in bacteria.

- As an example, a eukaryote like the common baker's yeast can grow on medium containing glucose, using it as carbon source. In the absence of glucose yeast can grow on other sugars.
- When the related sugar galactose is present yeast induce the expression of three genes necessary to convert galactose to glucose. Like the *lac* case, the GAL genes are induced about 1000-fold when the yeast change from glucose to galactose.
- Each of the three genes is encoded onto a different mRNA, each carrying one gene. Galactose induction activates the promoters corresponding to each of these genes independently.
- Control is positive, similar to the CAP protein system in bacteria. RNA polymerase can't interact with the promoters of the GAL genes without help. A protein binds to the region near the promoters and in the presence of galactose it stimulates RNA polymerase to bind and begin synthesis of the GAL mRNAs

This style of control is the norm in eukaryotes with multiple separately encoded genes being turned on by a single regulatory protein that helps RNA polymerase initiate synthesis of all the regulated mRNA. This is, however, not the only form of gene control in eukaryotes.

Control of processes other than transcription

Genetic control can be exerted during any step of the central dogma, and beyond. Up to now we have concentrated on regulating the process of transcription initiation, but biological systems use a wide diversity of mechanisms to modulate gene expression.

- **DNA rearrangement:** the structure of DNA in chromosomes can be changed in cells to create new arrangement of information. An example is the immune system in which genes specifying proteins called antibodies rearrange create a huge variety of different protein structures.
- **RNA processing:** the arrangement of information in DNA doesn't always directly correspond to the structure in RNAs. We've already talked about the fact that eukaryotic mRNAs are spliced to remove extra sequences called introns. Splicing can be used to create sequence diversity as well.
- **Translational control:** sometimes the amount of protein expressed from an mRNA can be regulated. This is termed translational control. In some cases an mRNA can be completely silenced in a process akin to the negative control we saw in the *lac* operon. This is a common mode of control in early development when a developing embryo has to depend on previously synthesized mRNAs, controlling the pattern and timing of their translation.
- **Post-translational modification:** the function of proteins is not always encoded specifically in their primary structure. Some proteins need to be chemically altered either by covalent modification or cleavage. One of the important aspects of post-translational modification is paradoxically the fact that proteins need to be selectively destroyed as part of their role in the cell.
- **Whole chromosomes can be inactivated:** an important issue in mammalian species is the fact that males and females have a different number of X chromosomes. Since many genes are encoded on the X that have nothing to do with gender the difference can be a problem, with males having only 50% as much of these genes as females. In mammals, the solution to this issue is for females to randomly inactivate one of their X chromosomes.

Genome rearrangement

We used to believe that the genome was unchangeable. Every cell was thought to have exactly the same array of genes on identical chromosomes. This turns out to be wrong, though DNA rearrangements remain the exceptions to the rule.

An example is the immune system. Our bodies express proteins called antibodies that are designed to find and destroy foreign material in the body (e.g. viruses and bacteria).

- A cell that produces antibodies, called lymphocytes, makes exactly one type of antibody, but each cell makes a slightly different antibody.
- We need to have millions of different kinds of antibodies since many different types of foreign agents might infect the body.
- It would be impossible to encode all of these antibodies (remember that humans only have around 30,000 genes in all!)
- The solution is to encode many interchangeable parts that can be combined in a huge number ways. The combinatorial nature of gene rearrangements of

antibody genes generates much of the variety needed to achieve the needed diversity in structure.

- These are permanent events. The modification occurs only in cells that are dedicated to production of antibodies. In particular, this kind of rearrangement can not happen in the cells that develop into eggs and sperm, the germ cells.

Post-transcriptional processing

I've already spoken about the fact that alternative splicing can regulate genes. Some genes encodes hundreds of proteins by alternative splicing, each with a slightly different function to perform.

A second type of post-transcriptional processing is simply that enzymes called ribonucleases specifically destroy mRNAs. The amount of protein produced from a gene depends directly on the amount of time the mRNA is available to be translated. Some proteins are needed in very small amounts while others are needed in massive quantities.

Translational control

Another way that the amount of protein is produced is regulated is by the amount of protein produced from each mRNA molecule.

Remember that proteins like *lac* repressor can block access by RNA polymerase to genes and shut of their transcription. Similarly, some proteins can block ribosomes from binding to mRNAs, shutting of their translation.

Why would cells want to do that?

- A good example is during early development when the embryo is rapidly growing, to quickly for it to make all of the factors it needs to build more cells.
- To make this rapid growth possible, the mother fills the egg with mRNAs encoding all of the proteins that will be needed during development
- But the proteins are not all expressed all the time, or in all of the cells of the embryo. This is why many of the mRNAs are subject to translational control. They are only allowed to be translated in the cells and at the time that is appropriate.

Post-translational modification

In many cases the level of expression or activity of that protein depends on events which occur after translation is completed

- Many exported proteins must be extensively modified before they are fully functional. Proteins being exported from the cell must pass through specialized organelles that decorate them. For example, the cell recognizes added polysaccharides as molecular tags identifying the location to which the proteins should be sent.

X-Inactivation

One of the most interesting examples of global genetic control is the phenomenon of X-inactivation

- Because males carry only a single copy of the X chromosome while females have two, the level of expression of all of the genes on the X would be double in females in the absence of some compensation
- The effect of doubling the expression of genes can be drastic
- Remember that the presence of extra chromosomes can be very deleterious, if not lethal (e.g., Down's Syndrome)

It is critical to adjust for the difference in DNA copy number in the two sexes

The way that this is dealt with in humans is by inactivation of one of the X chromosomes in females

- This process was discovered by Mary Lyon, who showed that a morphological marker of female cells, the **Barr body** was in fact an inactivated X chromosome
- The chromosome is condensed (as in mitosis) even during interphase, making it impossible to express RNA from any of its genes
- This condensation occurs randomly early in development, but is inherited by all the cells derived from the cell in which it occurs
- As a result, women's bodies are a composite (or **mosaic**) of cells with distinct X chromosomes being expressed