Control of Gene Expression

Gene Regulation Is Necessary?

By switching genes off when they are not needed, cells can prevent resources from being wasted. There should be natural selection favoring the ability to switch genes on and off.

Complex multicellular organisms are produced by cells that switch genes on and off during development.

A typical human cell normally expresses about 3% to 5% of its genes at any given time.

Cancer results from genes that do not turn off properly. Cancer cells have lost their ability to regulate mitosis, resulting in uncontrolled cell division.

Prokaryotes

Much of our understanding of gene control comes from studies of prokaryotes.

Prokaryotes have two levels of gene control. Transcriptional mechanisms control the synthesis of mRNA and translational mechanisms control the synthesis of protein after mRNA has been produced.

Operons

Operons are groups of genes that function to produce proteins needed by the cell. There are two different kinds of genes in operons:

*Structural genes* code for proteins needed for the normal operation of the cell. For example, they may be proteins needed for the breakdown of sugars. The structural genes are grouped together and a single mRNA molecule is produced during their transcription.

*Regulator genes* code for proteins that regulate other genes.

Operons have not been found in eukaryotes

The lac operon

Lactose is a sugar found in milk. If lactose is present, *E. coli* (the common intestinal bacterium) needs to produce the necessary enzymes to digest it. Three different enzymes are needed.

In the diagrams below, genes A, B, and C represent the genes whose products are necessary to digest lactose. In the normal condition, the genes do not function because a *repressor protein* is
active and bound to the DNA preventing transcription. When the repressor protein is bound to the DNA, RNA polymerase cannot bind to the DNA. The protein must be removed before the genes can be transcribed.

Below: Allolactose, an isomer of lactose, binds with the repressor protein inactivating it.
The repressor protein is produced by a regulator gene. The region of DNA where the repressor protein binds is the operator site. The promoter site is a region of DNA where RNA polymerase can bind. The entire unit (promoter, operator, and genes) is an operon.

The operator acts like a switch that can turn several genes on or off at the same time.

The lac operon is an example of an inducible operon because the structural genes are normally inactive. They are activated when lactose is present.

**The trp Operon**

Repressible operons are the opposite of inducible operons. Transcription occurs continuously and the repressor protein must be activated to stop transcription.

Tryptophan is an amino acid needed by *E. coli* and the genes that code for proteins that produce tryptophan are continuously transcribed as shown below.

If tryptophan is present in the environment, however, *E. coli* does not need to synthesize it and the tryptophan-synthesizing genes should be turned off. This occurs when tryptophan binds with the repressor protein, activating it. Unlike the repressor discussed with the lac operon, this repressor will
not bind to the DNA unless it is activated by binding with tryptophan. Tryptophan is therefore a corepressor.

The trp operon is an example of a repressible operon because the structural genes are active and are inactivated when tryptophan is present.

<table>
<thead>
<tr>
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<th>Structural Genes</th>
<th>Repressor</th>
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<tbody>
<tr>
<td>Inducible Operons</td>
<td>Inactive</td>
<td>Active (inhibits)</td>
</tr>
<tr>
<td>Repressible Operons</td>
<td>Active</td>
<td>Inactive (inhibits when activated)</td>
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**Negative Feedback Inhibition**

In addition to genetic regulation, tryptophan can inhibit the first enzyme in the synthesis pathway. This is an example of feedback inhibition. The presence of high levels of tryptophan inhibits the activity of the enzyme as shown in the biosynthesis pathway below.

**Negative and Positive Control**

The trp and lac operons discussed above are examples of negative control because a repressor blocks transcription. In one case (lac operon) the repressor is active and prevents transcription. In the other case (trp) the repressor is inactive and must be activated to prevent transcription.
### Control of Gene Expression

<table>
<thead>
<tr>
<th>Control Type</th>
<th>Structural Genes</th>
<th>Repressor or Regulator</th>
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<tr>
<td><strong>Negative Control</strong>&lt;br&gt;(an active repressor inhibits transcription)</td>
<td>Inducible Operons</td>
<td>Inactive</td>
</tr>
<tr>
<td></td>
<td>Repressible Operons</td>
<td>Active</td>
</tr>
<tr>
<td><strong>Positive Control</strong>&lt;br&gt;(an active regulator promotes transcription)</td>
<td>Inactive</td>
<td>Inactive (promotes when activated)</td>
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Positive control mechanisms require the presence of an activator protein before RNA polymerase will attach. The activator protein itself must be bound to an inducer molecule before it attaches to mRNA.

![Diagram of gene control mechanisms](image)

- **Inducer**: activates the gene
- **Activator**: binds to the activator binding site
- **RNA polymerase**: transcribes the gene
- **DNA**: genetic material
- **Promoter**: binding site for RNA polymerase
- **Gene**: coding sequence

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faculty.clintoncc.suny.edu/faculty/michael.gregory/files/bio 101/Bio 101 Lectures/Gene Regulation/gene.htm
Genes which code for enzymes necessary for the digestion of maltose are regulated by this mechanism. Maltose acts as the inducer, binding to an activator and then to mRNA. The activator bound to mRNA stimulates the binding of RNA polymerase.

**The Arabinose Operon**

The arabinose operon uses both positive and negative control.

The operon contains three different genes (ara A, ara B, and ara D) that code for three enzymes needed to convert arabinose to a usable form. A fourth gene codes for a protein (ara C) that acts to regulate the structural genes.

The regulator protein (ara C) is needed for transcription of the three structural genes (ara A, ara B, and ara D). It binds to its own structural gene preventing its own transcription, thus autoregulating its own level. When the level of ara C is low, transcription occurs and more ara C is synthesized.
Ara C also binds to other sites within the operon, inhibiting transcription of the three structural genes. The genes therefore are normally not active.

When arabinose is present, it binds to ara C causing it to change shape. The new shape promotes the attachment of RNA polymerase to the DNA, thus allowing transcription to occur.

**The Lac Operon also has Positive Control**

In addition to the negative control mechanism described earlier in this chapter, the lac operon is also regulated by a positive control mechanism.
E. coli prefer glucose when both glucose and lactose are present. When allolactose binds to and inhibits the repressor, the genes that code for enzymes needed to digest lactose are transcribed but only at a low level. Much of the cell's energy comes from glucose instead of lactose.

When the level of glucose is low, the level of cAMP increases. Cyclic AMP activates a molecule (CAP), which then binds to the lac promoter and enhances the rate of transcription.

**Promoters and Sigma Factors**

The nucleotide sequence of promoters is similar but not identical. The more similar the sequence is to a consensus sequence, the more likely that RNA polymerase will attach and produce mRNA from the associated genes.

Part of the RNA polymerase enzyme that recognizes the promoter is called the sigma factor. After transcription begins, this unit dissociates from the enzyme.

Different sigma factors recognize different promoters and thus, the availability of sigma factors can regulate the transcription of genes associated with these promoters.

The availability of sigma factors can be used to regulate sets of genes. For example, a group of genes whose product is rarely needed might have a different promoter sequence than other genes and thus require different sigma factors. These genes would only be transcribed when the correct sigma factor became available.

**Example of Translational Control in Prokaryotes: Antisense RNA**

Normally, mRNA is synthesized off of the template (antisense) strand of DNA. Antisense RNA is synthesized from the noncoding (sense) strand of DNA. The two mRNA molecules bond together, inactivating the mRNA.
This mechanism appears to be universal among bacteria. It has not been shown to be a normal means in eukaryotes.

Antisense RNA can be injected into eukaryotic cells as a control mechanism.

**Types of control in Eukaryotes**

Gene expression in eukaryotes is controlled by a variety of mechanisms that range from those that prevent transcription to those that prevent expression after the protein has been produced. The diagram below shows five kinds of general mechanisms that can be used.

**Transcriptional** - These mechanisms prevent transcription.

**Posttranscriptional** - These mechanisms control or regulate mRNA after it has been produced.

**Translational** - These mechanisms prevent translation. They often involve protein factors needed for translation.

**Posttranslational** - These mechanisms act after the protein has been produced.

**Transcriptional**

These mechanisms prevent mRNA from being synthesized.

**Heterochromatin and Euchromatin**

Heterochromatin is tightly wound DNA and visible during interphase. It is inactive because DNA cannot be transcribed while it is tightly wound.

Euchromatin is not tightly wound. It is active.
Acetylation/deacetylation of DNA

Condensation of DNA involves coiling around proteins called histones. Acetylation is when acetyl groups (-COCH$_3$) are attached to lysines in the histone tails. This reduces condensation and promotes transcription because the transcription machinery has better access to the DNA.

The addition of methyl groups (-CH$_3$) also promotes condensation.

The addition of a phosphate group to an amino acid that is next to a methylated amino acid results in less condensation.

Example: Lampbrush Chromosomes

The meiotic cells (oocytes) of some amphibians (frogs, toads, salamanders) have chromosomes that appear bristled. The bristles are loops of unwound DNA with many RNA polymerase and mRNA molecules. They are called lampbrush chromosomes because they resemble the brushes used to clean oil lamps.

Example: Puffs on Polytene Chromosomes

Polytene chromosomes are seen in the salivary glands of fruit fly larvae and the larvae of some other insects.

They have about 1000 chromatids; produced by DNA replication without mitosis. When viewed under a microscope, the many chromatids look like a giant chromosome. The large number of chromatids allows the cell to produce more mRNA and therefore more gene product (protein).

Developmental stages in the larva are associated with the appearance of chromosomal puffs. These are where DNA is unwound and actively being transcribed. As development proceeds, some puffs disappear and others appear indicating that some genes become inactive while others become active.

DNA Methylation and Epigenic Inheritance

The DNA can also be methylated (usually cytosines). Inactive DNA such as the Barr body (inactive X chromosome in females) is usually more methylated.

Methylated DNA usually remains methylated after cell division. This allows clusters of cells to develop into tissues that have the same genes inactivated.

Epigenic inheritance is the inheritance of traits that are not due to change in the DNA sequence.

Transcription Factors

RNA polymerase requires the presence of general transcription factors before transcription can begin. Interactions between the transcription factors, RNA polymerase, and the promotor allow the polymerase to move along the gene and begin transcription.

Higher levels of transcription occur with the presence of specific transcription factors as described.
The activators shown below are specific transcription factors. They function by binding to a region of the DNA called the *enhancer*. The enhancer may be located at a distance from the gene. Bending proteins assist in bending the DNA so that the enhancer is near the promoter. Activators, general transcription factors, and other proteins attach, forming an initiation complex. Transcription begins when the initiation complex is formed.

Hundreds of different transcription factors have been discovered; each recognizes and binds with a specific nucleotide sequence in the DNA. A specific combination of transcription factors is necessary to activate a gene.

Transcription factors are regulated by signals produced from other molecules. For example, hormones activate transcription factors and thus enable transcription. Hormones therefore activate certain genes.

**Posttranscriptional Control**

These mechanisms control or regulate mRNA after it has been produced.

**Alternative RNA Splicing**

This can produce variations in the mRNA produced. Different mRNA may have different introns removed.

The removal of introns enables a gene to code for more than one different protein. An average human gene is thought to code for 3 different proteins.

For example, experiments using radioactive labeling show that calcitonin produced by the hypothalamus is different from that produced by the thyroid. In each case, the same gene produces the protein.
Speed of Transport of mRNA Through the Nuclear Pores

Evidence suggests that this time may vary.

Longevity of mRNA

Messenger RNA can last a long time. For example, mammalian red blood cells eject their nucleus but continue to synthesize hemoglobin for several months. This indicates that mRNA is available to produce the protein even though the DNA is gone.

Ribonucleases

Ribonucleases are enzymes that destroy mRNA.

Messenger RNA has noncoding nucleotides at either end of the molecule. These segments contain information about the number of times mRNA is transcribed before being destroyed by ribonucleases.

Hormones stabilize certain mRNA transcripts.

Example

Prolactin is a hormone that promotes milk production because it affects the length of time the mRNA for casein (a major milk protein) is available.

Ribonucleases destroy the mRNA.

Prolactin is a hormone that prevents destruction of the mRNA.
Translational Control

These mechanisms prevent the synthesis of protein. They often involve protein factors needed for translation.

Preventing Ribosomes From Attaching

Proteins that bind to specific sequences in the mRNA and prevent ribosomes from attaching can prevent translation of certain mRNA molecules.

Initiation Factors

Initiation factors are proteins that enable ribosomes to attach to mRNA. These factors can be produced when certain proteins are needed. For example, the eggs of many organisms contain mRNA that is not needed until after fertilization. At this time, an initiation factor is activated.

Posttranslational Control

These mechanisms act after the protein has been produced.

Protein Activation

Some proteins are not active when they are first formed. They must undergo modification such as folding, enzymatic cleavage, or bond formation.

Example: Proinsulin is a precursor to the hormone insulin. It must be cleaved into 2 polypeptide chains and then some amino acids must be removed to form insulin.

Many proteins are activated by adding phosphate groups. They can be inactivated by removing phosphate groups. For example, kinases activate by adding phosphate groups and phosphodiesterase inactivates by removing the phosphate groups.

Feedback Control

Some enzymes in a metabolic pathway may be negatively inhibited by products of the pathway.

Click here for more details on feedback control.

Modification of DNA

Gene Amplification
In *Drosophila* (fruit flies), the chorion (eggshell) gene is copied many times in certain cells of the oviduct. These cells make large quantities of the protein needed to surround the egg. In other cells of the body, there is only one copy of this gene.

**The Immunoglobin Genes**

Immunoglobins (antibodies) are proteins that are used to defend the body against foreign invaders. They are able to do this because they have a shape that matches a shape found on the invader, allowing it to become attached. Particles that have antibodies attached are quickly destroyed by other cells in the immune system.

*Our bodies contain millions of different antibodies, each produced by a type of white blood cell called a lymphocyte. A single lymphocyte can produce only one specific kind of antibody, thus, there are millions of different kinds of lymphocytes.*

The genes that code for these antibodies differ from one lymphocyte to the next because when the lymphocytes are produced, different regions of the DNA are deleted so that each lymphocyte has a somewhat different version of the genes involved.

**Transposons**

Transposons are segments of DNA that are capable of moving to another location, either on the same chromosome or on a different one. If a transposon inserts itself within another gene, it can prevent the gene from expressing itself. Sometimes the transposon carries a gene which can become activated if it becomes inserted downstream from an active promoter.