Lecture 18

In the preceding examples of bacterial gene regulation, we have used known regulatory mechanisms to see how mutations in different elements of the system would behave in dominance tests and cis/trans tests. However, one is often trying to learn about a new operon and is therefore faced with the problem of deducing mechanism from the behavior of mutants.

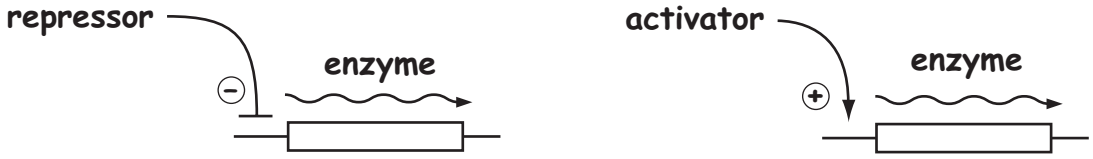
The steps to analyzing a new operon are as follows:

1) Isolate mutants that affect regulation. These could be either constitutive or uninducible. The most common regulatory mutations are recessive loss of function mutants in trans-acting factors. This is because there are usually many more ways to disrupt the function a gene than there are ways to make a dominant mutation. Promoter, operator, and initiator sites are usually much shorter than genes encoding proteins and these sites present much smaller targets for mutation.

2) Check to see whether the mutation is recessive and trans-acting (most will be).

If the mutation is constitutive then it is likely in the gene for a repressor.

If the mutation is uninducible then it is likely in the gene for an activator.



Although loss of function mutations in genes for repressors or activators are generally the most common type of regulatory mutation, the table below will help you to interpret mutations in sites or more complicated mutations in proteins.

Type of Mutation	Phenotype	Dominant/Recessive	Cis/Trans-acting
repressor^{-}	constitutive	recessive	trans-acting
activator^{-}	uninducible	recessive	trans-acting
operator^{-}	constitutive	dominant	cis-acting
promoter^{-}	uninducible	recessive	cis-acting
repressor^{-d} or activator^{s}	constitutive	dominant	trans-acting
repressor^{s} or activator^{-d}	uninducible	dominant	trans-acting

Regulatory Pathways

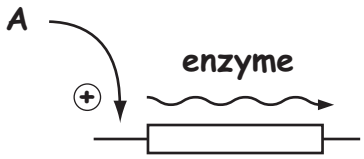
So far we have been considering simple regulatory systems with either a single repressor (Lac) or a single activator (Mal). Often genes are regulated by a more complicated set of regulatory steps, which together can be thought of as a **regulatory pathway**. Although there are good methods that can be used to determine the order of steps in a regulatory pathway (as will be discussed shortly), it is usually difficult at first to tell whether a given component identified by mutation is acting directly on the DNA of the regulated gene or whether it is acting at a step upstream in a regulatory pathway. For example, it will often be the case that a recessive trans-acting mutation that causes constitutive expression is not an actual repressor protein, but a protein acting upstream in a regulatory pathway in such a way that the net effect of this proteins is to cause repression of gene function. The best way to represent this situation is to call the gene product a **negative regulator** and to reserve the term repressor for cases in which we know that the protein actually shuts off transcription directly by binding to an operator site. Similarly, the best way to represent a gene defined by a recessive, trans-acting mutation that causes uninducible expression as a **positive activator** until more specific information can be obtained about whether or not the gene product directly activates transcription. The diagrams to be used are shown below.

An important note about interpreting such diagrams is that the arrow or blocking symbol do not necessarily imply direct physical interaction simply that the negative regulator or positive activator have a net negative or positive effect, respectively, on gene expression

Ordering gene functions in a regulatory pathway

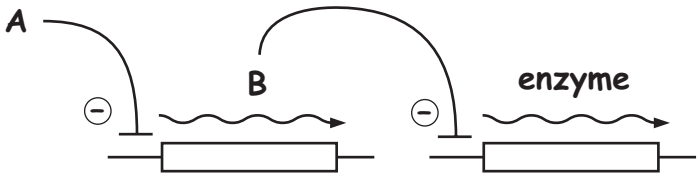
Imagine that we are studying the regulation of an enzyme and we find a recessive, trans-acting mutation in gene **A,** that gives uninducible enzyme expression. The simplest interpretation is that gene A is a positive activator of the enzyme:

Model 1



Now, say that we find a recessive, trans-acting mutation in gene **B** that gives constitutive enzyme expression. The following model takes into account the behavior of mutations in **A** and **B**:

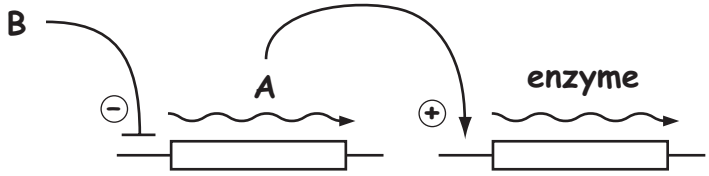
Model 2



The idea is that the gene for the enzyme is negatively regulated by gene **B** which in turn is negatively regulated by gene **A**. The net outcome is still a positive effect of gene **A** on enzyme expression. To distinguish the two models we will need more mutations.

However, we can also modify Model 1 as shown below to fit the new data.

Model 1 (revised)



The best way to distinguish the two possible models is to test the phenotype of a double mutant. In one case the **A^- B^-** double mutant is predicted to be uninducible and in the other case it is predicted to be constitutive.

	Model 1	Model 2
A^- B^-	uninducible	constitutive

This experiment represents a powerful form of genetic analysis known as an *epistasis* test. In the example above, if the double mutant were constitutive we would say that the mutation **B^-** is epistatic to **A^-**. Such a test allows us to determine the order in which different functions in a regulatory pathway act. If the double mutant in the example were constitutive, we would deduce that gene **B** functions after gene **A** in the regulatory pathway. To perform an epistasis test, it is necessary that the different mutations under examination produce opposite phenotypic consequences. When the double mutant is constructed, its phenotype will be that of the function that acts later in the pathway.

Epistasis tests are of very general utility. If the requirement that two mutations have opposite phenotypes is met, almost any type of hierarchical relationship between elements in a regulatory pathway can be worked out. For example, the **$LacO^c$** mutation is in a site, not a gene, but it is still possible to perform an epistasis between **$LacO^c$** and **$LacI^s$** since these mutations satisfy the basic requirement for an epistasis test. One mutation is uninducible while the other is constitutive for **Lac** gene expression. When the actual double mutant, **$LacO^c$ $LacI^s$**, is evaluated it is constitutive (this makes sense given what we know about the **Lac** operon since a defective operator site that prevents repressor binding should allow constitutive expression regardless of the form of the repressor protein). Formally, this result shows that a mutation in **LacO** is epistatic to a mutation in **LacI**. Even if we did not know the details of **Lac** operon regulation before hand, this epistasis test would allow us to deduce that the operator functions at a later step than the repressor.

Stable regulatory circuits

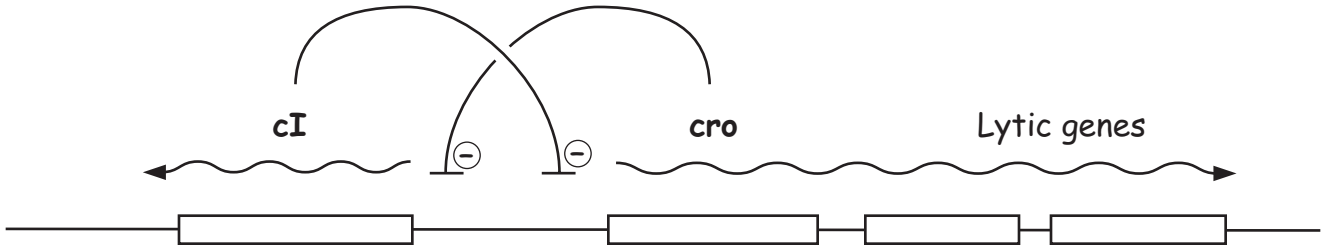
We have been considering enzymes that are regulated in response to the availability of nutrients. There is another general type of regulation whereby genes can be held in stable on or off states. In development of multicellular organisms all cells (except for the germ cells and cells of the immune system) have the same genotype yet cells in different tissues express different sets of genes. Cell-type specification is in part a program of gene transcription that is established by extracellular signals. In most cases, after the cell type has been specified the cells do not readily change back when the signals are removed. This general behavior of cells in development implies the existence of stable regulatory states for gene control.

The best understood case of a stable switch is the lysis vs. lysogeny decision made by phage λ. When phage λ infects cells there are two different developmental fates of the phage.

1) In the lytic program the phage: replicates DNA, make heads, tails, packages DNA, and lyses host cells.

2) In the lysogenic program the phage: integrates DNA and shuts down phage genes. The resulting quiescent phage integrated into the genome is known as a lysogen

The decision between these two options must be made in a committed way so the proper functions act in concert. The switch in the case of phage λ hinges on the activity of two repressor genes **cI** and **cro**. The **cI** and **cro** genes have mutually antagonistic regulatory interactions that can be diagramed as follows:



After an initial unstable period immediately after infection, either **cro** expression or **cI** expression will dominate.

Mode 1: High **cro** expression blocks **cI** expression. In this state, all of the genes for lytic growth are made and the phage enters the lytic program.

Mode 2: High **cI** expression blocks **cro** expression. In this state, none of the genes except for **cI** are expressed. This produces a stable lysogen.

In gene regulation, as in good circuit design, stability is achieved by feedback. The result is a bi-stable switch that is similar to a "flip-flop", one of the basic elements of digital electronic circuits.

Other genes participate in the initial period to bias the decision to one mode or the other. These genes act so that the lytic mode is favored when *E. coli* is growing well and there are few phage per infected cell, whereas the lysogenic mode is favored when cells are growing poorly and there are many phage per infected cell.