Genetics Lecture Notes

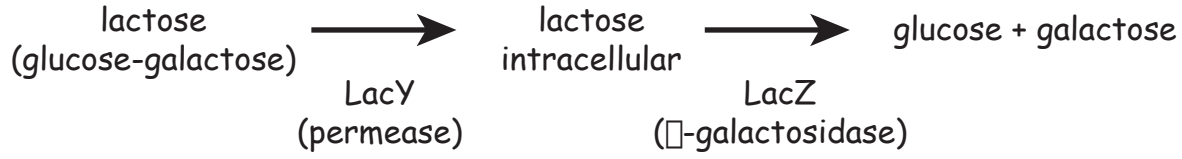
7.03 2005

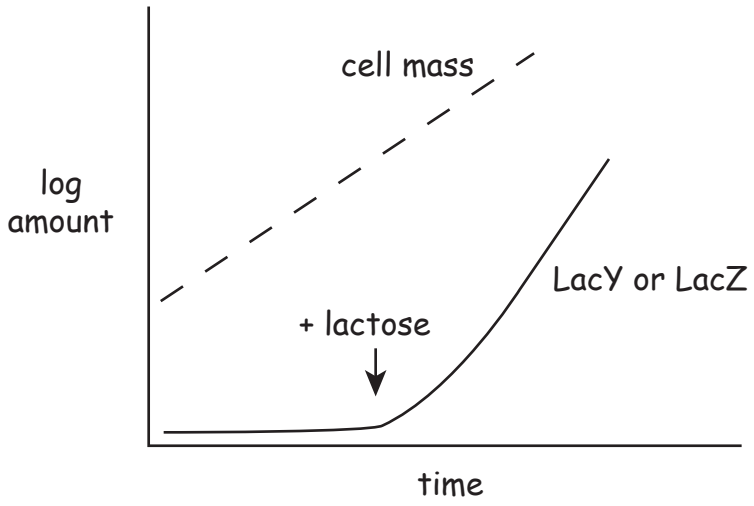
Lectures 17 – 19

Lecture 17

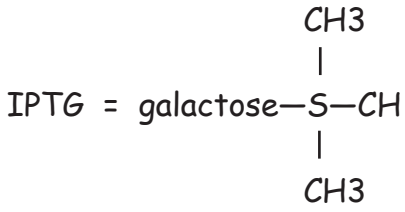
Gene Regulation

We are now going to look at ways that genetics can be used to study gene regulation. The issue is how cells adjust the expression of genes in response to different environmental conditions. The principles of gene regulation were first worked out by Jacob and Monod studying the E. coli genes required for cells to use the sugar lactose as a nutrient.



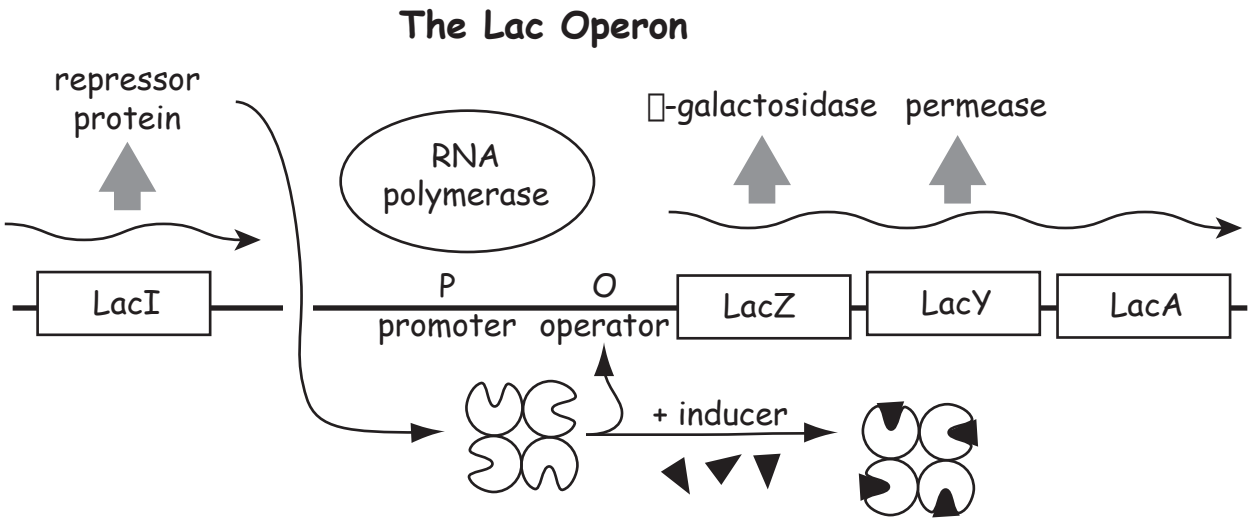


The logic of the Lac operon is that the proteins required to use lactose are only made when their substrate (lactose) is available. This prevents wasteful expression of enzymes when their substrates are not available.
At first, scientists noted that lactose is both an inducer and substrate for the enzymes of the Lac operon and they therefore concluded that lactose was somehow acting as a template for the formation of the enzyme. Then compounds were discovered that could act as inducers but were not themselves substrates for the Lac enzymes. The classic example of such a "gratuitous inducer" is IPTG, which is an effective inducer of LacZ expression but isn't hydrolyzed by ß-galactosidase.



The existence of compounds such as IPTG shows that recognition of the inducer is a separate molecular event from lactose breakdown.

The next major finding was the discovery of LacI⁻ mutants. LacI⁻ mutants are constitutive, meaning that they always express ß-galactosidase at high levels regardless of whether there is an inducer present or not. LacI⁻ mutants have apparently lost a component of the machinery the cell uses to turn off ß-galactosidase expression.
The regulatory system turns out to be quite simple and by isolation of mutants and simple genetic tests Jacob and Monod were able to figure out the following scheme:



The idea is that the inducer has a net positive effect on expression because the inducer is a negative regulator of the repressor, which is itself a negative regulator of the gene for ß-galactosidase.

We will now consider how regulatory mutants can be analyzed genetically. We will use as examples different mutations in the Lac system but the genetic tests are very general and can be applied to most regulatory systems.

Dominance test

	ß-galactosidase		
	-IPTG	+IPTG	Interpretation
$I^+ Z^+$	-	+	
$I^- Z^+$	+	+	I^- is constitutive
$I^- Z^+ / F' I^+ Z^+$	-	+	I^- is recessive
$I^+ Z^-$	-	-	Z^- is uninducible
$I^+ Z^- / F' I^+ Z^+$	-	+	Z^- is recessive
$I^+ Z^- / F' I^- Z^+$	-	+	I^- and Z^- mutations complement (the mutations are in different genes)

A second type of constitutive mutant inactivates the operator site and is known as a $LacO^c$ mutation. $LacO^c$ mutations are dominant as revealed in tests of the appropriate merodiploids:

	-IPTG	+IPTG	Interpretation
$O^c\ Z^+$	+	+	O^c is constitutive
$O^c\ Z^+ / F'\ O^+\ Z^+$	+	+	O^c is dominant

You might think that on the basis of a dominance test we could tell whether we have a $LacO^c$ or a $LacI^-$ mutation. However, life is not so simple, because it is possible to find $LacI^-$ mutations that are dominant. Such mutations are known as $LacI^{-d}$. They are dominant because the repressor protein is a tetramer and $LacI^{-d}$ mutant subunits can combine with normal subunits and interfere with their function.

	-IPTG	+IPTG	Interpretation
$I^{-d}\ Z^+$	+	+	I^{-d} is constitutive
$I^{-d}\ Z^+ / F'\ I^+\ Z^+$	+	+	I^{-d} is dominant

We will now consider a new genetic test that will let us distinguish $LacO^c$ (operator constitutive) from $LacI^{-d}$ (dominant repressor negative) mutations.

Cis/trans test

	-IPTG	+IPTG	Interpretation
$I^+\ O^+\ Z^+$	-	+	
$I^{-d}\ Z^+ / F'\ I^+\ Z^-$ (cis)	+	+	I^{-d} is dominant in cis or in trans with Z^+; Therefore we say it is "trans-acting".
$I^{-d}\ Z^- / F'\ I^+\ Z^+$ (trans)	+	+	
$O^c\ Z^+ / F'\ O^+\ Z^-$ (cis)	+	+	O^c is dominant only in cis with Z^+; Therefore we say it is "cis-acting".
$O^c\ Z^- / F'\ O^+\ Z^+$ (trans)	-	+	

If a mutation is cis-acting we take this as evidence that the mutation affects a site on DNA like an operator. If a mutation is trans-acting we take this as evidence that the mutation affects a diffusible gene product such as a repressor.

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Until now we have been considering mutations that lead to constitutive synthesis of ß-galactosidase. It is also possible to get mutations that are uninducible. For example, a mutation in the promoter ($LacP^-$) is uninducible.

	-IPTG	+IPTG	Interpretation
$P^- Z^+$	-	-	P^- is uninducible
$P^- Z^+ / F' P^+ Z^+$	-	+	P^- is recessive
$*P^- Z^+ / F' P^+ Z^-$	-	-	P^- is cis-acting
$P^- Z^- / F' P^+ Z^+$	-	+	

*Note that this experiment can also be viewed as a complementation test that shows that $LacP^-$ and $LacZ^-$ are mutations in the same gene. This fits with our primary definition of a gene as the DNA segment needed to make a protein, since the promoter is certainly needed for protein expression.

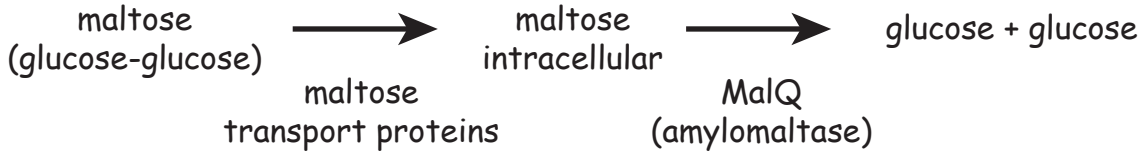
Promoter mutants in Lac operon can be distinguished from simple $LacZ^-$ mutations since promoter mutations affect the LacY and LacA genes as well.

I^S designates a "super repressor" which binds to the operator DNA but won't bind inducer.

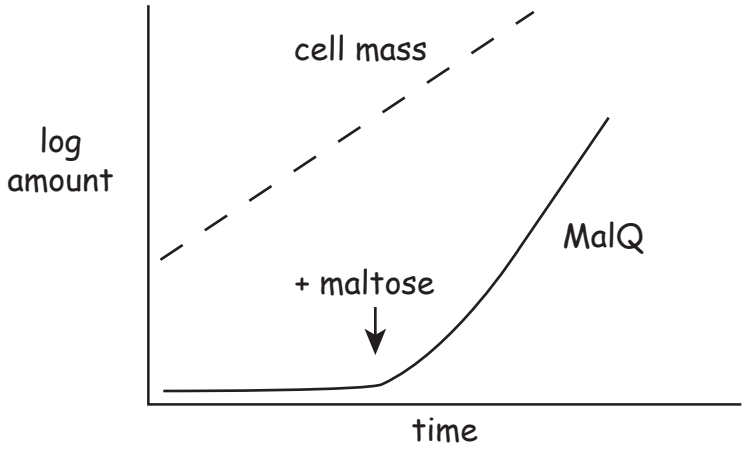
	-IPTG	+IPTG	Interpretation
$I^S Z^+$	-	-	I^S is uninducible
$I^S Z^+ / F' I^+ Z^+$	-	-	I^S is dominant

Positive regulation.

Now we will consider how a different E. coli operon is regulated. The Mal operon encodes several genes necessary to take up and degrade maltose; a disaccharide composed of two glucose residues.



Much like the Lac operon, the products of the Mal operon are induced when maltose is added to cells. Thus, maltose acts as an inducer.

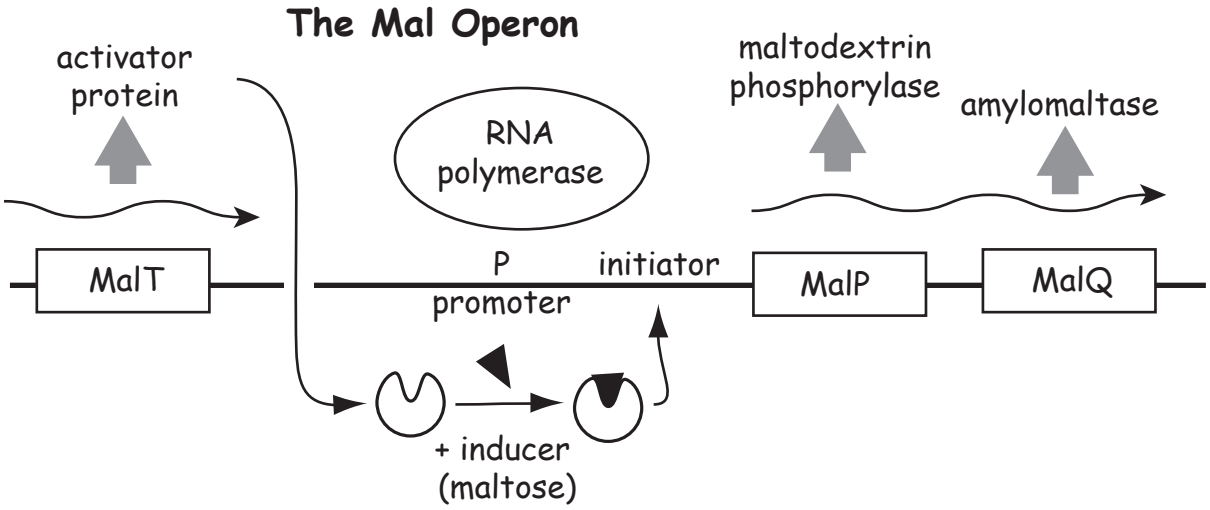


When mutants that affect the regulation of the Mal operon were isolated, the most common type consisted of uninducible mutations in a gene known as MalT. We can apply dominance tests and cis-trans tests to MalT mutations with the following results:

	maltase activity		
	-maltose	+maltose	Interpretation
Mal^+	-	+	Maltose induces Mal operon
$MalT^-$	-	-	$MalT^-$ is uninducible
$MalT^-$ / F′ $MalT^+$	-	+	$MalT^-$ is recessive
$MalT^-$ MalQ+ / F′ $MalT^+$ $MalQ^-$	-	+	MalT is trans-acting
$MalT^-$ $MalQ^-$ / F′ $MalT^+$ $MalQ^+$	-	+	

From this table it looks as if the $MalT^-$ trait is not expressed either in cis or in trans. Because $MalT^-$ is recessive, it makes more sense to consider the properties of the dominant $MalT^+$ allele in the cis/trans test. Viewed in this way, the $MalT^+$ trait is expressed in both cis and trans and therefore MalT is considered to be trans-acting.

This behavior is different from any of the Lac mutations that we have discussed. The interpretation is that MalT encodes a diffusible gene product (not a site on DNA) that is required for activation of transcription of the Mal operon. This type of gene is usually called an activator. As shown in the diagram below, maltose binds to the MalT activator protein causing a conformational change in MalT allowing it to bind near to the promoter and to stimulate transcription. Note that the genes required for maltose uptake are located in an operon elsewhere on the chromosome, but these genes are also regulated by MalT.



This model requires a site, called the initiator, which is where the activator binds near the promoter to activate transcription. If you think about how mutations in an initiator site should behave in dominance and cis/trans tests, you will see why in practice it is difficult to distinguish initiator site mutations from promoter mutations.

It is also possible to isolate "super activator" mutants that will bind to the initiator site and activate transcription regardless of whether the inducer maltose is present. Such alleles of the MalT gene are called $MalT^c$ and their properties are given below.

	-maltose	+maltose	Interpretation
$MalT^c$	+	+	$MalT^c$ is constitutive
$MalT^c$ / F' $MalT^+$	+	+	$MalT^c$ is dominant
$MalT^c$ $MalQ^+$ / F'$MalT^+$ $MalQ^-$	+	+	$MalT^c$ is trans-acting
$MalT^c$ $MalQ^-$ / F'$MalT^+$ $MalQ^+$	+	+	

For a multimeric activator it should also be possible to isolate activator^{-d} mutants that will interfere with the binding of wild-type subunits to the initiator site. Actually $MalT^{-d}$ mutants have not been isolated, because MalT is a monomer.

Lecture 19

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.

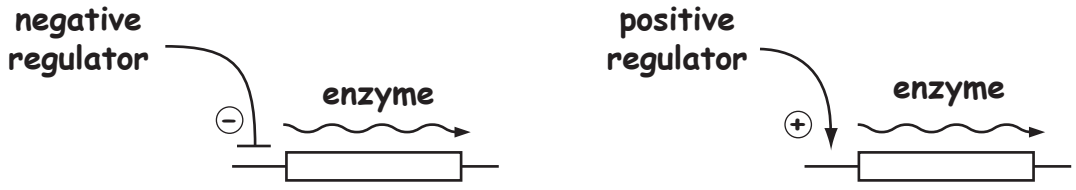
Although loss of function mutations in genes for repressors or activators are generally the most common type of regulatory mutation, the table below will help you to interpret mutations in sites or more complicated mutations in proteins.

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 is as a positive regulator 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.

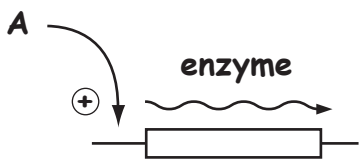


An important note about interpreting such diagrams is that the arrow or blocking symbol does not necessarily indicate a direct physical interaction, but simply implies that the negative regulator or positive regulator have a net negative or positive effect, respectively, on gene expression.

Ordering gene functions in a regulatory pathway

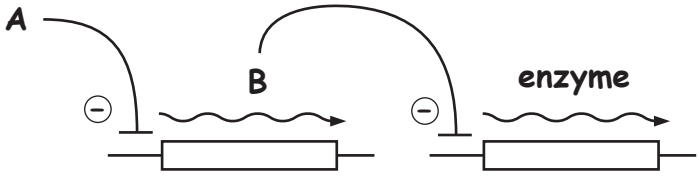
Imagine that we are studying the regulation of an enzyme and we find a recessive, trans-acting mutation in gene A, that gives uninducible enzyme expression. The simplest interpretation is that gene A is a positive regulator of the enzyme:

Initial Model 1



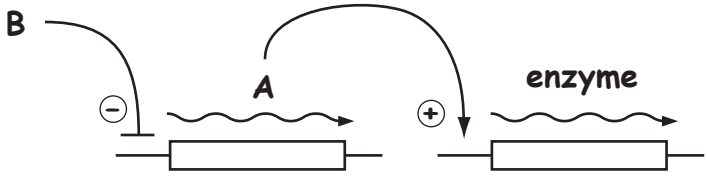
Now, say that we find a recessive, trans-acting mutation in gene B that gives constitutive enzyme expression. The following model takes into account the behavior of mutations in A and B:

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

Revised Model 1



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 (revised)	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, $LacO^c$ $LacI^c$, 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

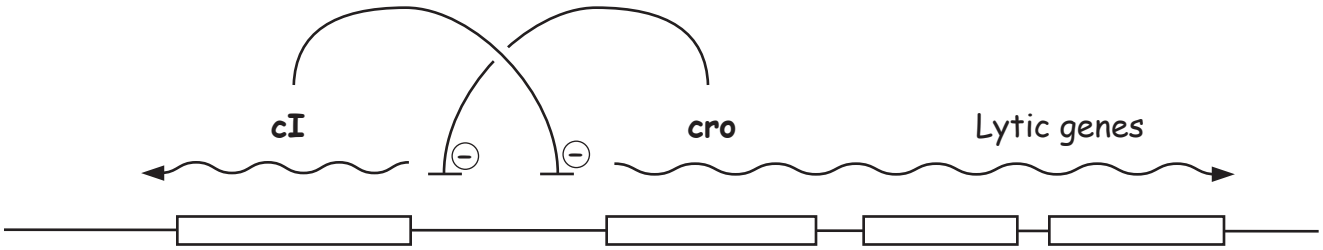
We have been considering enzymes that are regulated in response to the availability of nutrients. There is another general type of regulation whereby genes can be held in stable on or off states. In development of multicellular organisms all cells (except for the germ cells and cells of the immune system) 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 □. When phage □ 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 the lytic or lysogenic options must be made in a committed way so the proper functions act in concert. The switch in the case of phage 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.