Lecture 21

Having seen how to delineate all of the components of a bacterial regulatory system through the isolation and analysis of mutants we now have all of the genetic tools needed to work out the mechanism of a new regulatory pathway.

The steps to analyzing a new bacterial regulatory pathway are as follows:

1) Establish a reporter system that allows regulation to be easily studied.

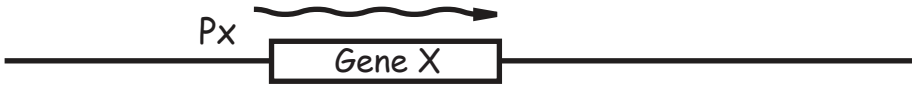
2) Define physiological regulators (inducers or inhibitors) to establish regulation of wild type cells.

3) Isolate mutants that affect regulation (uninducible or constitutive), and delineate major regulatory factors by focusing on trans-acting loss of function mutations.

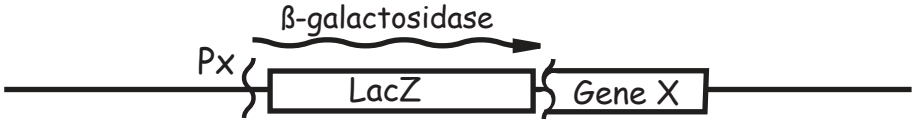
4) Determine the order of function by constructing double mutants for epistasis tests.

1) Reporter genes:

Often one is faced with the problem of studying regulation of a gene whose product is difficult to assay. In such cases it is usally easiest to fuse the regulated promoter under examination to athe coding sequence for an enzyme that is easily assayed such as LacZ. In this case LacZ is called a reporter gene because its expression "reports" the level of expression form the regulated promoter. For example, say we wished to study regulation of Gene X.



The idea would then be to use recombinant DNA methods to insert the LacZ coding sequence next to the promoter for Gene X (Px).



After the endogenous LacZ gene is inactivated, the assay of ß-galactosidase can then be used to ascertain when Px active for transcription.

2) Establish physiological conditions for regulation.

Once an appropriate reporter has been set up the physiological conditions for regulation need to be established. The tow examples we have seen are regulated Lac and Mal genes that are needed for the utilization of lactose and maltose respectively. These regulated genes are **activated** by their respective substrates because the regulated utilization genes are only needed when their substrate is present. Another general type of regulation is for pathways responsible for the biosynthesis of compounds such as the amino acid tryptophan. In such cases the biosynthetic genes are turned off when the product such as tryptophan is present. Tryptophan would be considered an **inhibitor** of the genes for tryptophan synthesis.

We can now begin to diagram the regulatory pathway as follows:

An important note about such diagrams is that the symbol for negative regulation (bar) or positive activation (arrow) do not imply direct physical interaction simply that the activator or inhibitor have a **net** positive or negative effect, respectively, on gene expression.

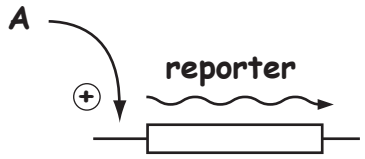
3) Isolate regulatory mutations.

Mutants that affect regulation can 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.

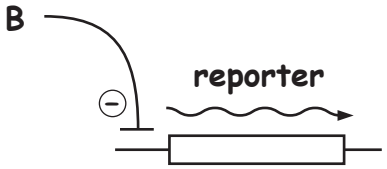
To delineate the elements of a regulatory pathway initially mutations in **trans-acting** factors are evaluated. The easiest way to show a mutation is trans-acting is by a linkage test to show that it is not tightly linked to the reporter. In cases of close linkage (such as the LacI gene) a cis/trans test will be needed to show that a mutation exerts its effect in cis or in trans.

To deduce the function of a trans acting factor it is necessary to know the phenotype of a loss-of-function (lof) mutation. A mutation can be assumed to be loss of function i) if it is recessive, ii) if it is a nonsense mutation (ie can be suppresed by a nonsense suppressor), or iii) if it was caused by a transposon insertion.

If we found a trans-acting lof mutation in gene A, that gives uninducible reporter expression the simplest interpretation is that gene A is a positive activator of the reporter and would be diagrammed as follows:



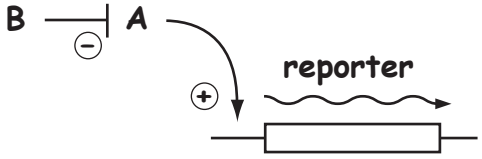
Alternatively if we found a trans-acting lof mutation in gene B, that gives constitutive reporter expression the simplest interpretation is that gene B is a negative regulator of the reporter and would be diagrammed as follows:



4) Ordering multiple gene functions in a regulatory pathway

If we consider a reporter gene that is regulated by regulatory genes A and B as described above, there are two possible orders of action of the products of gene A and gene B.

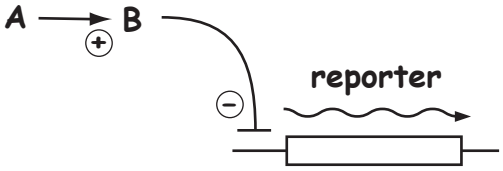
Model 1:



The idea is that the reporter is activated by the product of gene A which in turn is negatively regulated by gene B. The net outcomes are still a positive effect of gene A on enzyme expression and a negative effect of gene B on enzyme expression.

However, we can propose a different model (Model 2 as shown below) that fits the data equally well but that has the oposite order of action of the the A and B genes.

Model 2:



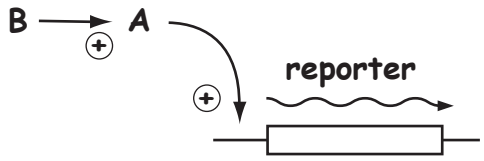
The best way to distinguish the two possible models is to test the phenotype of a double mutant. In one case the A^- B^- double mutant is predicted to be uninducible and in the other case it is predicted to be constitutive.

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

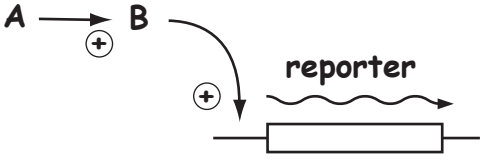
This experiment represents a powerful form of genetic analysis known as an **epistasis test**. In the example above, if the double mutant were constitutive we would say that the mutation B^- is **epistatic** to A^-. Such a test allows us to determine the order in which different functions in a regulatory 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.

Importantly, epistasis tests can be done using mutants that are either dominant or recessive so long as they have opposite phenotypes. Consider a new example in which we have trans-acting lof mutations in two different genes A and B that are both uninducible. Accordingly the two possible regulatory models would be:

Model 1



Model 2



As it stands these models cant be resolved by examining an A^- B^- is double mutant because both mutants have the same phenotype. Now imagine that a dominant trans-acting constitutive mutant is isolated. Further genetic analysis shows that this mutation is very tightly linked to A^- and therefore is likely to be an allele of gene A that has a super activator property (ie is constitutively active). This dominant constitutive mutation in gene A can now be combined with a B^- mutant. If the double mutant is constitutive then Model 1 fits the data, whereas if the double mutant is uninducible then Model 2 fits the data.