Biol 213 Genetics: Wednesday, October 25, 2000
Lac Operon (Part I)

Inspirational Message

The last exam was a draining experience for many of you, and if it failed to give you the exhilaration that comes from looking back on what you have accomplished, let me try to do that here. Most exams that you've studied hard for, you go in and say, Hey, I know that... Hey, that's easy now! And so you feel good. We're going for bigger game, to give you the opportunity to learn how to go into a situation where you DON'T know that, one that will NEVER be easy, and still you find your way to a solution.

I hope that you can step back from the exam, if not now then sometime, and say, Hey, I'm not sure I know how to handle hairless mice, but several weeks ago this problem might have had me shredding the wallpaper and now, right or wrong, at least I can stay with the problem and give some reasonably intelligent answer. Note also that some of the problems came straight out of the scientific literature. This is not trivial stuff!

Exhortation

Two responses on Exit Questionnaires by graduating seniors:

- *My research at UR has… probably had the biggest influence on my desire to pursue grad education.*
- *I did not think that I had time for research because I was concentrating on my coursework in hopes of improving my grades.*

Believe it or not, this is the best possible time for you to join a research laboratory. The earlier you begin, the deeper the experience and the more it augments your classroom understanding. Even though research takes time, it also makes time by making sense out of class material. Those who do research are not taking a hit on grades -- generally just the opposite. In any event, nothing is more impressive to graduate/professional schools than independent research. GPAs are predictors of success. Successful research IS success. If you don't know how to get involved, try talking to students who've been there and looking through faculty web pages. Or talk to one of us.

Outline

I. OVERVIEW OF UNIT III: Regulation of Gene Expression and Development

II. DIAUXIC GROWTH
- A. The phenomenon
- B. Possible explanations of diauxic growth

III. DISSECTION OF LAC REGULATION (pp.394-403)
- A. Biochemical route: Behavior of different galactosides
- B. Genetic route: Behavior of different lac mutants
- C. The nature of the *lacI* gene

IV. THE *LAC* OPERON

I. OVERVIEW OF UNIT III: Regulation of Gene Expression and Development

It's nine weeks into the semester. You know the secret of life -- DNA. You know how to read its code. In principle, though not yet in fact, you can predict from the sequence of amino acids what function the protein will have and even change that function to suit your wishes. You can make quantitative predictions as to how traits are inherited.

But don't feel smug: you still don't know how even the simplest living organism is formed.

Upon reflection, you should not be surprised. Suppose I could read every thought in your head, every thought you ever thought, even every thought you haven't thought yet. Everything you were capable of thinking. Would that tell me who you are? Not at all. If every possible thought went through your mind at once, there would be chaos, and you are not chaos.

What's missing is the regulation of your thoughts -- what relationships there are between what is around you and what is called to mind, how one thought connects to another. And that's what's missing from our understanding of genetics at this point: regulation.

At any given moment only a fraction of the genes an organism possesses are expressed as protein, and if they all turned on at the same time... certain death. You have genes that are turned on to protect you when you are overheated, when you are exposed to heavy metals, genes that are expressed only during early embryogenesis, and so forth. To understand how genes determines the form and function of an organism, we must understand not only what genes are but what regulates their expression, and that is the subject of the next three weeks.

The first week we will focus on the *lac* operon of *E. coli*, amongst the first genes whose regulation was understood. Understanding one set of regulation strategies thoroughly will give us the tools to view regulation in general. The second week, we'll talk about gene regulation in eukaryotes and note a family resemblance with how it's done in *E. coli*. The third week we'll look at a few well studied examples of how gene regulation is underlies the development of whole organisms.

II. DIAUXIC GROWTH (NOT IN TEXT)

II.A. The phenomenon

Each section of this course has had one or two heroes. Watson and Crick come to mind for the first section, Mendel for the second. The hero of this section, at least the beginning must be Jacques Monod. You no doubt had heard of the first three. Monod may be less familiar to many of you. Unlike Watson, who knew that he wanted to find the secret of life, Monod's early career seems rather rootless. He spent some time at Cal Tech in Morgan's lab nominally studying Drosophila genetics but in reality spending his creative energies conducting a local chamber orchestra (he was an outstanding

musician). Returning home to Paris, he worked on growth and nutrition of protozoa and finally switched his attention to bacteria.

His given project was to understand the role sugars play in determining the growth rate of bacteria. If this does not sound like the secret of life to you, it probably seemed initially not much more interesting to Monod. It is what he did with the subject that commands our attention.

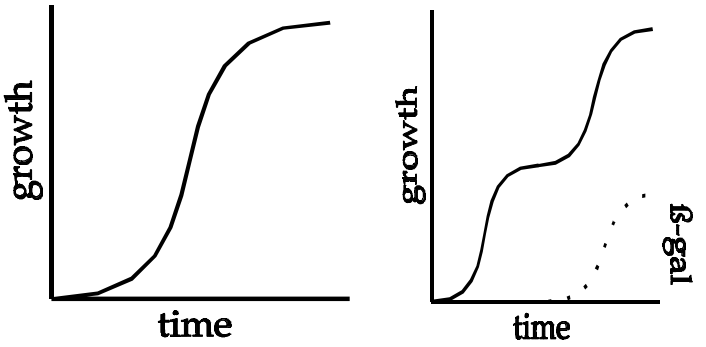


Fig. 1. Growth of *E. coli* on sugars. (**A**) Typical curve for exponential growth on one sugar. Growth saturates when the sugar is exhausted. (**B**) Diauxic growth (solid line) on glucose + lactose (1:1). Glucose is consumed first and then, after a lag period, lactose. ß-galactosidase activity (dotted line) appears concomitantly with lactose consumption.

His initial experiments gave no surprises: give food to bacteria and they grew pretty much as you might expect (Fig. 1A). They grew until they had exhausted the sugar, then they stopped. Certainly the rate of growth depended on the sugar added. Some sugars, like glucose, supported the maximal rate of growth, while other sugars were not as effective.

Once he had gone through all of the obvious sugars, what could he do for encores? Well, there are the combinations: what's the rate of growth if you mix two different sugars, and so forth. Gripping stuff. Actually, the story became more interesting than you might think, because this is the type of results Monod got when he tried to grow the bacteria on a 1:1 mixture of the sugars glucose and lactose (Fig. 1B).

This type of growth is called diauxic for its two growth periods (*auxein* means to grow). What experiments could you do to get a handle on what's going on? Varying the ratio of glucose to lactose clarified the situation: the bacteria appeared to eat all its preferred food, glucose, pause for thought, and then eat all the lactose.

SQ1. Draw the growth curve expected from a bacterial culture growing on glucose + lactose in the ratio of 1:3.

II.B. Possible explanations of diauxic growth

How could all of this be explained? This was the age of biochemistry, in which most of the metabolic pathways we're familiar with were elucidated, so it was reasonable to suppose the phenomenon was attributable to differences in enzyme activity. Indeed, when the ability to break down lactose was measured (ß-galactosidase activity), it was found only during the second stage of growth. It must be noted that enzyme activity is the capacity for the enzyme-catalyzed reaction to take place, whether or not that capacity is actually utilized (imagine a factory that may or may not be using its full potential).

SQ2. Which of the following can explain the appearance of ß-galactosidase activity only during certain phases of growth on glucose + lactose?

a. Glucose prevents the appearance of ß-galactosidase activity
b. Lactose induces the appearance of ß-galactosidase activity
c. ß-galactosidase is always present but its activity is evident only in the latter stage of growth

Monod spent much of the rest of his scientific life explaining how the presence of sugars influenced the enzyme activity. It became evident that there were two effects:

1. Lactose is required for the appearance of ß-galactosidase activity
2. Glucose prevents the appearance of activity, despite the presence of lactose

To explain these effects, Monod initially put forth an explanation that was typical of his age, one excited about the newly appreciated power of proteins, particularly antibodies. It was thought that antibodies got their remarkable specificities by wrapping themselves around the molecules they bound to, like making a mold of a key. Similarly, Monod postulated that there exists a universal preenzyme that wraps itself around glucose when glucose is present, and in that form is able to break down glucose. When glucose is consumed, the enzyme reforms itself by wrapping itself around lactose and adapts its activity so that it can break down lactose but not glucose.

It is important to see that there are two functions envisioned in this model for lactose: (1) inducer of enzyme activity and (2) substrate for the enzymatic reaction. In Monod's model, the two functions were achieved in the same way, by lactose interacting with a single protein.

SQ3. Against this once popular view of the biochemical world, reconsider Beadle and Tatum's experiments with Neurospora. If there existed a universal precursor to enzymes, encoded by a gene, what phenotype would their mutants have exhibited?

III. DISSECTION OF *LAC* REGULATION

III.A. Biochemical route: Behavior of different galactosides

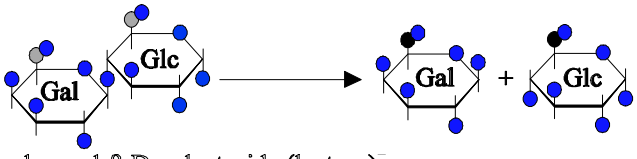
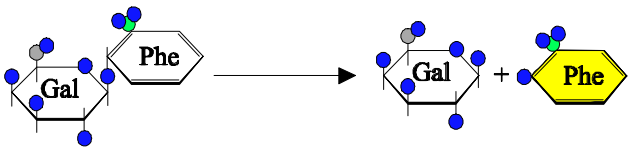
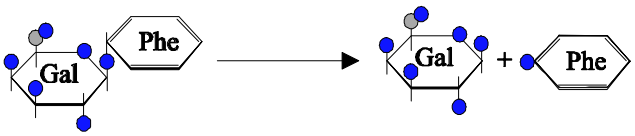
It turns out Monod's view was wrong. We now know, of course, that the function of an enzyme is determined by the amino acid sequence encoded by its gene. Proteins are not generic putty that learns its shape and function by the chemicals it acts on. Nonetheless, small molecules like lactose exert enormous influence on levels within the cell, and they do so by binding to protein.

In the case of lactose metabolism, this insight was gained by separating the two functions of lactose: lactose the inducer and lactose the substrate for metabolism. To see the basis for this insight, it is necessary to learn a bit about what lactose is (see Fig. 18.2). Lactose is a disaccharide -- two sugars -- and the enzyme that metabolizes it splits lactose into its component sugars, glucose and galactose. The enzyme is called ß-galactosidase, because it acts on ß-galactosides, compounds in which something is

attached to galactose by a ß-linkage. An alpha-linkage would be one in which the substituent is attached on the other side of the sugar.

It was found that the enzyme ß-galactosidase acts on other ß-galactosides besides lactose (Table 1). For example, phenylgalactoside (PGal) is also cleaved by ß-galactosidase. In contrast, isopropyl thiogalactoside (IPTG) is not cleaved by the enzyme even though it is a powerful inducer of ß-galactosidase activity. Clearly, the two functions of lactose -- inducer and substrate -- are distinct, two separate properties possessed by a single molecule.

Table 1: ß-galactosides and their properties

ß-galactoside[a]	Reacts with ß-galactosidase to produce[a]	Induces *lac* operon	Use
 glucosyl-ß-D-galactoside (lactose)		Yes	Natural substrate
 isopropyl-ß-D-thiogalactoside (IPTG)		Yes	Induces operon
 o-nitrophenyl-ß-D-galactoside (ONPG)		?	Assay for ß-galactosidase
 phenyl-ß-D-galactoside (Pgal)		No	Selects for *lacI*$^-$

[a]Blue, red, green, and gray spheres represent oxygen, sulfer, nitrogen, and carbon atoms, respectively. Gal represents galactose, Glc represents glucose, and Phe represents phenyl.

SQ4. IPTG is an inducer but not a substrate of ß-galactosidase. PGal is a substrate but not an inducer.

a. Can *E. coli* grow on IPTG as a sole carbon source?

b. Can *E. coli* grow on PGal as a sole carbon source?

c. Can *E. coli* grow on IPTG + PGal as sole carbon sources?

SQ5. IPTG can enter cells without the aid of membrane protein, while lactose requires the aid of the membrane protein Lac permease. From the structures of the two molecules, speculate on why this might be so.

III.B. Genetic route: Behavior of different *lac* mutants

The development of bacterial genetic techniques led to the ultimate resolution of how ß-galactosidase was induced. As is often the case, breakthroughs came when critical mutants were found, in this case, those defective in lactose metabolism or regulation (Table 2).

Table 2: Phenotypes of *lac* mutants

		ß-galactosidase activity		
Genotype	**Defective Product**	**No addition**	**+ lactose**	**+ IPTG**
wild type	---	-	+	+
z^- (*lacZ*)	ß-galactosidase	-	-	-
y^- (*lacY*)	Lac permease	-	-	+
a^- (*lacA*)	Lac transacetylase	-	+	+
i^- (*lacI*)	Lac repressor	+	+	+

The wild type strain, of course, has low ß-galactosidase activity unless lactose is added. If IPTG is added -- remember that this is the compound able to induce but not serve as a substrate -- the result is the same as adding lactose. A search for mutant strains unable to use lactose turned up two main classes of mutations:

lacZ mutations resulted in the loss of ß-galactosidase activity under all circumstances. We now know that *lacZ* is the gene for ß-galactosidase, so mutating the gene affects the protein.

lacY mutations has no ß-galactosidase activity even when lactose is present. We now know that *lacY* encodes a membrane protein that allows the hydrophilic sugar lactose to enter the cell. The protein is called Lac permease, because it helps lactose permeate the membrane. Lactose cannot induce increased ß-galactosidase production unless it can enter the cell. IPTG, on the other hand, because of the hydrophobic isopropyl group, can swim through the hydrophobic membrane directly and thus reach the induction mechanism.

Monod searched for other enzyme activities that act on ß-galactosides and is induced by lactose. He found the enzyme transacetylase, encoded by *lacA*. The biological function of transacetylase remains obscure.

SQ6. Predict whether the strains listed below will be able to grow under the given conditions

Strain	Sugar(s) added	Growth
wild type	lactose	+
lacZ	lactose	
lacY	lactose	
wild type	Pgal	
wild type	PGal + IPTG	
lacY	lactose + IPTG	

A critically important mutation was found by a technique that exploits the characteristic inability of PGal to support growth in wild type *E. coli*. Recall that PGal can get into the cell through Lac permease and it can be metabolized through ß-galactosidase. Its problem is that it can't induce the production of the enzyme. However, both Monod and Lederberg found mutants of *E. coli* that can grow on sugars like PGal. This phenotype was due to mutations in a gene, *lacI*. This gene does not encode a protein directly involved in the metabolism of lactose but rather regulates the expression of required genes.

SQ7. *E. coli* normally cannot use PGal as the sole carbon source, because it cannot induce ß-galactosidase, the enzyme that metabolizes it. Explain why selecting for mutants of *E. coli* that can use PGal gives you mutants defective in *lacI*.

III.C. The nature of the *lacI* gene

The *lacI* gene was clearly remarkable. It was the first example of a gene whose primary purpose appeared to be controlling when other genes were turned on and off. As such, it pointed to the answer to the question we started with: How does a cell turn on the right fraction of its genes at the right time. One of the first questions addressed by Monod was whether *lacI* behaved as if it encoded a protein. This may seem surprising to you -- what else would it encode? -- but we'll see in a couple of days that there are other possibilities.

The question of *lacI*'s nature was addressed through a simple complementation test, illustrated on pp.401-403. If *lacI* encoded a protein, then the wildtype allele, $lacI^+$ should be able to complement a mutant $lacI^-$ allele that failed to make protein. A complementation test is easily done with *Drosophila*, but it is not obvious how to do such a test with haploid bacteria that don't have sex. Monod overcame this obstacle by employing a plasmid, **F'*lac***, that had been found to carry the *lac* genes, including *lacI*. Since plasmids replicate independently from the bacterial chromosome, moving **F'*lac*** into a strain of *E. coli* with defective $lacI^-$ would create a partial diploid: the resulting strain would contain two copies of the *lac* genes owing to the extra copy carried by **F'*lac***. We will revisit this kind of experiment time and again, and it will tell us a lot about the nature of gene regulation.

Follow the panels on pp.401-402 marked "Conceptual Level". Two strains of *E. coli* were mixed together. One strain, called the donor, carried **F'*lac*** (called simply **F'** in the text) in addition to chromosomal DNA. The second strain, called the recipient, carried only chromosomal DNA. The strain is *lacI*$^-$, so ß-galactosidase is present even in the absence of inducer. When the two strains were mixed together, a copy of **F'*lac*** passed from the donor to the recipient, a process called conjugation. Plasmids encode proteins that make such DNA transfer possible, resulting in the rapid spread of plasmids through a bacterial population (the primary cause of the explosive increase in antibiotic-resistant bacteria). Note that *lacI* and the other *lac* genes are represented simply by one letter: *i*, *z*, etc.

SQ8. There are a couple of major problems of scale in the figure shown on p.401. What should be the size of chromosomal DNA relative to plasmid DNA? What should be the size of the several *lac* genes shown relative to the total size of the chromosome?

The right hand panels on p.402 illustrate the four conditions tested:

1. *lacI*$^-$ recipient in the presence of inducer (lactose)
   lacZ is active -(ß-galactosidase is made)
2. *lacI*$^-$ recipient in the absence of inducer
   lacZ is still active, as a result of the *lacI*$^-$ mutation
3. *lacI*$^-$ recipient PLUS **F'*lac*** in the presence of inducer (lactose)
   lacZ is still active (introduction of *lacI*$^+$ makes no difference when inducer is present)
4. *lacI*$^-$ recipient PLUS **F'*lac*** in the absence of inducer
   lacZ is inactive -(wild type *lacI*$^+$ shuts down expression)

This last result is the critical one. It shows that the phenotype of *lacI*$^+$ is dominant over the phenotype of *lacI*$^-$, as you would expect if *lacI* encodes a protein.

The bottom right panels of p.402 illustrate the method by which ß-galactosidase activity is measured. Cells from each of the four conditions described above were broken, releasing their contents, including any ß-galactosidase they may contain. *o*-Nitrophenyl galactoside (ONPG) was added to the resulting suspensions. This analog of lactose (see Table 1) turns yellow when cleaved by ß-galactosidase. The amount of yellow color over a set period of time, then, is a measure of how much ß-galactosidase is present in a suspension. You will be using this very method next week in lab.

SQ9. Suppose that the cross had been reversed: F'*lac* carried *lacI*$^-$ and the chromosome of the recipient strain carried *lacI*$^+$. Which of the four conditions would have shown ß-galactosidase activity?

IV. THE *LAC* OPERON

The remarkable thing about *lacI* mutants is that not only was ß-galactosidase activity constitutive, i.e. present despite the absence of inducer, so was transacetylase

activity. In fact, transacetylase and ß-galactosidase responded similarly to a wide variety of different conditions. Monod and Francois Jacob rationalized these findings by postulating that *lacZ* and *lacA* were regulated coordinately by a repressor encoded by *lacI* .

Fig. 15-4 provides a cartoon of how the two genes (and *lacY*) are related. When lactose is present, the three genes *lacZ*, *lacY*, and *lacA* are transcribed as a single piece of mRNA. Ribosomes bind to the mRNA at three different places and make three different proteins: ß-galactosidase, permease, and transacetylase. Jacob and Monod termed genes that are coregulated in this way operons.

SQ10. Suppose three genes comprise an operon. Which of the following are true?

a. Each gene has its own start codon
b. Each gene encodes a protein different from the other genes
c. Ribosomes bind to each gene separately
d. RNA polymerase binds to each gene separately
e. Each gene is replicated separately

The protein encoded by *lacI*, called the Lac repressor, is critical to this scheme. The Lac repressor binds to DNA at the start of the operon and prevents transcription of the entire operon. In this way it coordinately regulates expression of the operon as a whole. The Lac repressor explains the effects of lactose and other inducers, because it binds inducers and when so occupied, it is unable to bind to DNA and thus unable to block transcription. Therefore, inducers permit the transcription of the genes of the operon and the expression of the proteins encoded by those genes.

SQ11. Considering the definition of an operon -- genes that are coordinately regulated -- is *lacI* part of the *lac* operon?

SQ12. What phenotype would you predict for a *lacI* mutant that is able to bind to DNA normally but is not able to bind allolactose? (see Table 2 for examples and Fig. 15-4 for inspiration)

SQ13. With what you now know about the *lac* operon, explain the observation that some galactosides induce ß-galactosidase but are not substrates for the enzyme, while others are good substrates but don't induce the enzyme.

SQ14. With what you now know about the *lac* operon, explain Monod's initial observations on diauxic growth in the presence of glucose + lactose (Fig. 2).