

411-2 2008

Kendall- idea that this lecture is like a microcosm of the whole course

**Nomenclature:** Lac<sup>-</sup> phenotype vs *lacZ* or *lacY* genotype- phenotype is what's observed; genotype is what's hidden unless do DNA sequencing and must be inferred- both mutants fail to grow on lactose

**Basic idea of this lecture-** put yourselves in mindset of people who didn't yet understand how lactose utilized. Forget all the fancy book-learning!! How would you address the problem experimentally?

**Background-Physiology** -before there were any mutants- three "properties" associated with lactose uptake were known

1. First function - break open cells, add lactose- can show using chemical analytic means that gets converted to galactose and glucose (=beta-galactosidase)
2. second function (=lactose permease)  
-lactose taken into cells - mix lactose with cells and show concentrated in them
3. Third function  
-more lactose cleavage activity and lactose transport faster in cells grown on lactose than on glucose (= "induction")

**Questions answered genetically:** (know answers, but that's not what's important here- rather where answer came from- increased level of sophistication requires knowing something about how secure information is)

1. **How many genes are required for lactose utilization?**
2. **What is the mechanistic "logic" of induction? (i.e., activation of a positive factor or inhibition of a negative factor by lactose?)**

1. How many genes are required for lactose utilization? what do they do? (Association between genes and physiology completely obscure when this question was addressed)

### **Genetic Analysis (steps)**

**A. Isolate hundreds of Lac<sup>-</sup> mutants (Lac<sup>-</sup><sub>1</sub>, Lac<sup>-</sup><sub>2</sub>, ...). Mutants specifically defective in growth on lactose. How isolated?**

- On OH- show replica-printing and indicator medium used to isolate

- B. How many genes affected by Lac<sup>-</sup> mutations?**  
- Answer using Complementation analysis

General form of test:  $lac^{-1}/lac^{-2}$  = grows on lactose or not? How to construct cell with two *lac* regions? (Using F'*lac* factors.)

Explain what boxes in table mean (growth or not on lactose minimal)  
Dominant or recessive? Recessive- what expect if mutation reduces or eliminates activity

So two groups of mutations- defines two genes. But what do genes code for?

Functional assignment required assays:

One set of mutants (*lacZ*<sup>-</sup>) were beta-galactosidase<sup>-</sup>, transport<sup>+</sup> = **beta-galactosidase gene**

Second set of mutants (*lacY*<sup>-</sup>) were beta-galactosidase<sup>+</sup> transport<sup>-</sup> = **lactose permease gene**

>What if there were two genes needed for beta-galactosidase - e.g., two different subunits? Extra complementation group lacking beta-galactosidase activity

Go back and draw *lac*<sup>-6</sup> row- what is? (deletion, promoter-down mutation, super-repressor, unlinked)- write down!

Draw in fifth column in table above with  $Flac^{+}$  and no growth for *lac*<sup>-6</sup>- what does that rule out of above possibilities?

Mutation maps elsewhere (not on F'*lac*) or dominant *lac* region mutation (*lacI* super-repressor)

## 2. What is mechanistic “logic” of *lac* induction? (i.e., is there a positively- or negatively-acting regulatory factor?)

### A. Alternative models: Negative vs positive- OH

Experiment to distinguish depended on the properties of a special class of mutants called constitutive mutants- these express *lac* whether or not inducer present

**OH:** Negative regulation – predicts most common class would eliminate repressor

Positive regulation: predicts that most common class would permanently activate activator

## **OH-Steps in analysis**

### **1. Isolate constitutives**

### **2. Test if constitutive expression dominant or recessive to regulated expression**

#### **1. Isolation of constitutive mutants:**

OH Different galactosides

First-distinguish action as beta-galactosidase substrate and action as inducer of *lac* operon- first mention IPTG, then complementary galactoside = PG. Can use galactose from PG as C source

Could be used to isolate mutants which express *lac* operon all the time- constitutive mutants- how might these galactosides be used to isolate?  
-use PG as carbon source

constitutives- most common classes based on models above- if negative-loss of repressor; if positive, always-on activator. (loss of repressor activity or constitutive activation of activator- cell thinks lactose is always there)

### **2. Test if constitutive expression dominant or recessive to regulated expression**

Need cell with two copies of *lac* region, one constitutive and one regulated, to see which property dominates. How would you do this? F'*lac* factor to make partial diploid. But F's not discovered yet, so more complicated alternative way

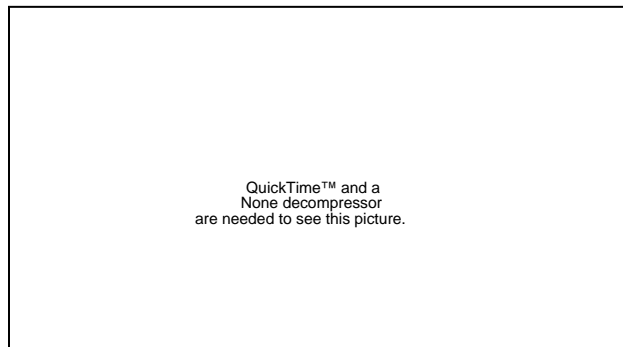
strS doesn't get transferred significantly, but doesn't matter anyway

Hfr  $lacI^+ Z^+ / str^S$  with  $F^- lacI^- lacZ^- str^R$   
Cells:



Mix, add streptomycin to prevent protein synthesis in Hfr donor strain, measure beta galactosidase activity in culture (recipients)

Draw out predictions on board



OH- Show results- no inducer and get a little beta-galactosidase synthesis and then it stops-refer back to predictions

Why is there initial increase? Takes time for repressor to be made  
Why have inducer added? Control to tell what level of activity is if all lacZ in recipient is expressed and activated

so repressed state dominant to constitutive state- idea is that DNA gets transferred and expressed (beta-galactosidase) because no repressor; but repressor is made and stops so get plateau. So this shows that induction

involves release of a negative factor (lac repressor). What would have expected if induction had been due to activation of a factor? Wouldn't have turned off at 3 hrs.

OH of model- negative

>Predicted that repressor would bind to DNA and block transcription- makes prediction that should be possible to isolate mutations making binding site nonfunctional.

Operator constitutive mutants- got using diploid *lac+ / lac+* strains- requiring growth on PG- explain in more detail- draw out haploid vs diploid cells and expectations

-For 2007, need to draw out two types of constitutives and stress dominance of operator types

