

### Results on Exams

Exam #	mean	std dev	range
1	55.2	12.2	62
2	62.1	13.1	61
3	57.6	13.7	61

### Gene Regulation

Positive  
Negative

### Regulation of protein *activity* vs. *amount*

- Regulate protein activity
  - Allostery
  - Covalent modification
  - Sequestration
- Regulate the amount of protein
  - Gene transcription
  - RNA processing
  - RNA turnover
  - mRNA translation
  - Protein processing, assembly, turnover

### Operons

- An operon is a cluster of **coordinately regulated** genes. It contains:
  - Structural genes:** encode enzymes
  - Regulatory genes:** encode repressors or activators of expression
  - Regulatory sites:** e.g. promoters, operators

### Positive vs negative control

	Regulatory protein is <u>present</u>	Example of regulatory <u>protein</u>	Mutate regulatory gene to lose <u>function</u>
Positive control	Operon ON	Activator	Operon OFF
Negative control	Operon OFF	Repressor	Operon ON

### Catabolic vs. biosynthetic operons

Operon encodes	Absence of	Effect	Presence of	Effect
Catabolic enzymes	Substrate	Repressed	Substrate	Induced (derepressed)
Biosynthetic enzymes	Product	Induced	Product	Repressed

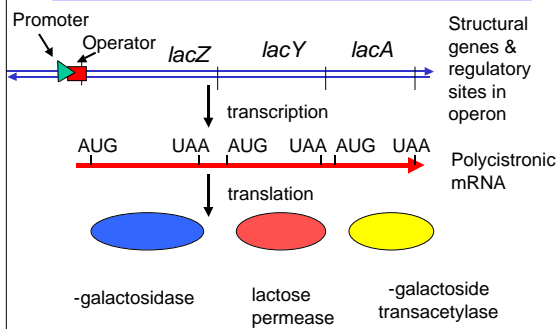
## Inducible vs. repressible operons

Defined by response of operon to a metabolite (small molecule).

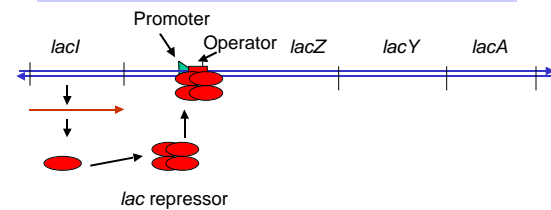
Type of operon	Presence of	Effect	Metabolite	Operon	Examples
Inducible	metabolite	ON	lactose	<i>lac</i>	
Repressible	metabolite	OFF	Trp	<i>trp</i>	

## Negative control of the *lac* operon

### Induced (derepressed) *lac* operon

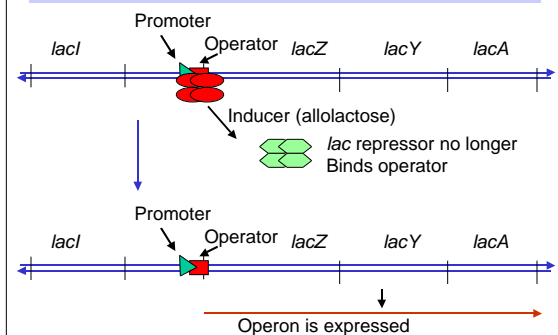


### Repressed *lac* operon



**Repressor** binds to the **operator** in the absence of the **inducer** (a metabolite of lactose), and blocks transcription of the *lac* operon.

### Induction of the *lac* operon by derepression



### Inducers of the *lac* operon

- Lactose, the substrate for the operon, is converted to its isomer allolactose.
- Allolactose is the natural inducer.
- A **gratuitous inducer** induces the operon but is not metabolized itself.
  - e.g. isopropylthiogalactoside= IPTG

### Regulatory mutations in the *lacI* gene

Genotype	<i>(lac Z)</i> -galactosidase		<i>(lac A)</i> transacetylase		Conclusion
	-IPTG	+IPTG	-IPTG	+IPTG	
<i>I<sup>-</sup>Z<sup>+</sup>A<sup>+</sup></i>	<0.1	100	<1	100	Inducible
<i>I<sup>+</sup>Z<sup>-</sup>A<sup>+</sup></i>	<0.1	<0.1	<1	100	
<i>I<sup>-</sup>Z<sup>+</sup>A<sup>+</sup></i>	100	100	100	100	Constitutive
<i>I<sup>-</sup>Z<sup>-</sup>A<sup>+</sup> / F<sup>+</sup>I<sup>-</sup>Z<sup>+</sup>A<sup>+</sup></i>	<0.1	100	<1	200	<i>I<sup>-</sup> &gt; I<sup>-</sup></i> in trans
<i>I<sup>S</sup>Z<sup>+</sup>A<sup>+</sup></i>	<0.1	<1	<1	<1	Noninducible
<i>I<sup>S</sup>Z<sup>+</sup>A<sup>+</sup> / F<sup>+</sup>I<sup>+</sup>Z<sup>+</sup>A<sup>+</sup></i>	<0.1	1	<1	1	<i>I<sup>S</sup> &gt; I<sup>+</sup></i> in trans

- The *lacI* gene encodes a *trans*-acting factor (protein) needed for repression.
- Most *lacI<sup>-</sup>* mutants are constitutive.
- The *lacI<sup>S</sup>* allele is noninducible.

### Regulatory mutations in the operator

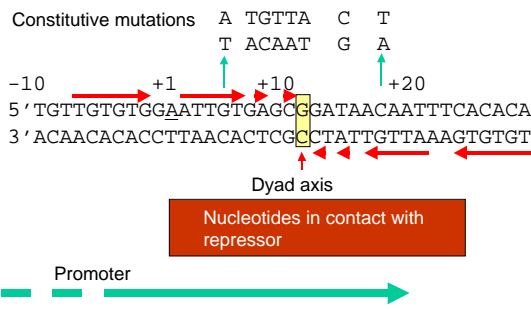
Genotype	-galactosidase		Conclusion
	-IPTG	+IPTG	
<i>I<sup>+</sup>o<sup>+</sup>Z<sup>+</sup></i>	<0.1	100	Inducible
<i>I<sup>+</sup>o<sup>c</sup>Z<sup>+</sup></i>	100	100	Constitutive
<i>I<sup>+</sup>o<sup>c</sup>Z<sup>+</sup> / F<sup>+</sup>I<sup>+</sup>o<sup>+</sup>Z<sup>-</sup></i>	100	100	Constitutive
<i>I<sup>+</sup>o<sup>c</sup>Z<sup>-</sup> / F<sup>+</sup>I<sup>+</sup>o<sup>+</sup>Z<sup>+</sup></i>	<0.1	100	Inducible

Loss-of-function alleles of the operator confer a constitutive phenotype on the operon. They are called *o<sup>c</sup>*.

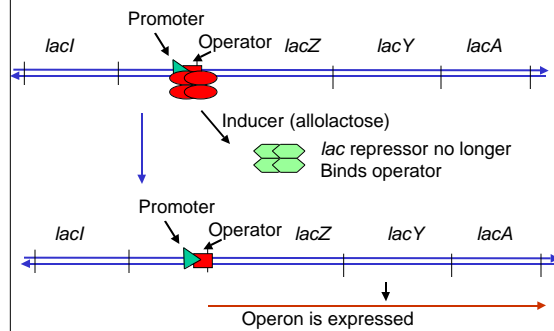
The operator acts in *cis*, i.e. it affects the allele to which it is linked.

The allele of the operator that is in *cis* to the active reporter gene is the dominant allele. The operator shows *cis* - dominance.

### Interactions between operator and repressor



### *lac* repressor



### Picky eater?



### Positive control: "catabolite repression"

- Glucose is the preferred carbon source for *E. coli*.
- Glucose causes repression of operons whose products catalyze the metabolism of other carbon sources, e.g. *lac* operon and lactose.
- This is called **catabolite repression**.
- In the absence of glucose, operons needed for metabolism of other carbon sources are induced.



### Problem 15.5

Consider a hypothetical regulatory scheme in which citrulline induces the production of urea cycle enzymes. **Four genes** (*citA*, *citB*, *citC*, *citD*) affecting the activity or regulation of the enzymes were analyzed by assaying the wild-type and mutant strains for **argininosuccinate lyase** activity and arginase activity in the **absence (-cit) or presence (+cit) of citrulline**. In the following table, wild-type alleles of the genes are indicated by a + under the letter of the *cit* gene and mutant alleles are indicated by a - under the letter. The activities of the enzymes are given in units such that 1 = the uninduced wild-type activity, 100 = the induced activity of a wild-type gene, and 0 = no measurable activity. **In the diploid analysis, one copy of each variant of the operon is present in each cell.**

### 15.5: Haploid analysis

Strain Number	genes				lyase activity		arginase act.	
	A	B	C	D	- cit	+ cit	- cit	+ cit
Haploid:	A	B	C	D				
1	+	+	+	+	1	100	1	100
2	-	+	+	+	100	100	100	100
3	+	-	+	+	0	0	1	100
4	+	+	-	+	100	100	100	100
5	+	+	+	-	1	100	0	0

Strain 1 (wt) : operon is inducible by citrulline.

Strains 2 & 4: Mutation in A and C make the operon constitutive.

Strains 3 & 5: Genes B and D encode enzymes.

### 15.5: Diploid analysis

Strain Number	genes								lyase activity		arginase act.		
	A	B	C	D	A	B	C	D	- cit	+ cit	- cit	+ cit	
Diploid:	A	B	C	D	A	B	C	D					
6	+	+	+	-	/	+	-	+	+	1	100	1	100
7	-	+	+	+	/	+	-	+	+	1	100	2	200
8	+	+	-	+	/	+	-	+	-	100	100	100	100
9	+	-	-	+	/	+	+	+	-	1	100	100	100

Strain 6: B- complements D-; the genes encode enzymes.

Strain 7: B- complements A-, so A encodes a *trans*-acting regulatory factor. A+ > A-

Strain 8: B- does NOT complement C-. *citC* shows *cis*-dominance, and thus is a regulatory site on the DNA.

### Regulatory scheme for 15.5

- Gene *citB* encodes argininosuccinate lyase.
- Gene *citD* encodes arginase.
- Gene *citA* encodes a diffusible, regulatory molecule, such as a repressor.
- "Gene" *citC* is a site on DNA at which the repressive effect of CitA is exerted. e.g. the operator at which CitA repressor binds.
- In the presence of the substrate citrulline, the repressor no longer binds the operator, and the operon is induced.