Recitation Section 9
March 7-8, 2005

Molecular Biology—Gene Regulation

A. Gene regulation basics

1. What is a gene?

2. What is a protein?

3. How is it possible that your liver cell and your skin cell can have identical DNA content, and yet look different and perform different functions?

4. Why is there a need for gene regulation? In general terms, how is it accomplished?

5. What does a gene consist of? What is the role of each element?

6. What regulates the expression of a gene?

7. What are the cis- and trans-acting elements?

8. Can a deficiency in a trans-acting element be overcome by the addition of another copy of the gene to a cell? What about a cis-acting element? Why?

9. What proteins will be affected by mutations in the trans-acting elements? Cis-acting elements?

B. Gene regulation—an example

The imaginary bacterium *E. fictionalis* requires two enzymes for the metabolism of the imaginary sugar froyose. Enzyme 1 converts froyose to sweetose; enzyme 2 converts sweetose to glucose. Both enzymes are synthesized from a single mRNA and are induced in response to froyose; the operon is known to be regulated by repression.

1. Diagram the froyose metabolism operon.

2. A **loss of function** mutation (i.e. a mutation that inactivates the component encoded by the portion of DNA where the mutation occurs) in what element(s) of the operon would lead to each of the following characteristics:
    a. constitutive (constant) expression of enzymes 1 and 2?

    b. no expression of enzymes 1 and 2?

    c. expression of enzyme 1, but not 2 upon addition of froyose?

    d. expression of enzyme 2, but not 1 upon addition of froyose?

You have isolated several mutants (A through G) that are altered in their metabolism of froyose. In the following charts, $^{+}$ indicates wild-type sequence; $^{-}$ indicates a mutant allele. You have an assay for the level of enzymes 1 and 2. The results with haploid strains are shown below:

	- froyose		**+ froyose**		
Genotype	**Enz 1**	**Enz 2**	**Enz 1**	**Enz 2**	**Mutation**
wild-type	low	low	high	high	
A^{-}	low	low	low	low	
B^{-}	high	high	high	high	
C^{-}	low	low	low	low	
D^{-}	low	low	low	high	
E^{-}	low	low	high	low	
F^{-}	high	high	high	high	
G^{-}	low	low	high	low	

3. In the table above, identify the likely location of each of the mutations.

4. A **gain of function** mutation (i.e. a mutation that enhances the function of the component encoded by the portion of DNA where the mutation occurs) in what element(s) of the operon would lead to no expression of enzymes 1 and 2?

5. For a loss of function mutations, what experiment can you perform to distinguish between the mutations in the operator and in the repressor?

You then construct the following diploids:

			- froyose			+ froyose	
	Genotype		**Enz 1**	**Enz 2**		**Enz 1**	**Enz 2**
1	$\frac{D^+ \ E^-}{D^- \ E^+}$						
2	$\frac{E^+ \ G^-}{E^- \ G^+}$						
3	$\frac{A^- \ D^+ \ E^+}{A^+ \ D^- \ E^-}$						
4	$\frac{B^- \ D^+ \ E^+}{B^+ \ D^- \ E^-}$		high	high		high	high
5	$\frac{F^- \ D^+ \ E^+}{F^+ \ D^- \ E^-}$		low	low		high	high

6. For strains 1-3, fill in the table above with the expected values for enzymes 1 and 2 in the absence and presence of froyose. Explain your choices.

7. Explain the phenotypes listed in the table for strains 4 and 5. Which mutation(s) are in the repressor? The operator?