

Molecular Biology—Gene Regulation

A. Gene regulation basics

1. What is a gene?

A gene is a functional unit of heredity. It is a segment of DNA that contains all of the information to encode the sequence of a product and to regulate its expression. A gene encodes when, where, and what polypeptide or functional RNA should be made.

2. What is a protein?

A protein is a polypeptide product of a gene that performs a particular function in the cell.

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?

Although all cells in a given organism can trace their lineage to the original fertilized egg cell, cell division does not have to produce cells that behave in identical ways. Two cells that result from one division, and have the same genetic material can have different morphology and behavior due to differentiation— expressing a different set of genes to perform a different function in the organism. It is important to understand that differentiation usually involves the selective reading of a genome rather than a change in the sequence of the genome.

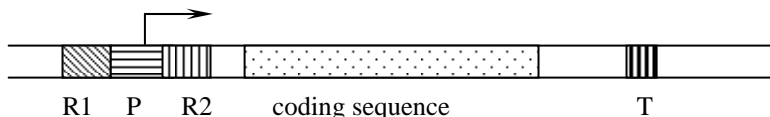
4. Why is there a need for gene regulation? In general terms, how is it accomplished?

Not all genes need to be expressed at all times. Therefore, gene products are regulated in their timing and abundance. In general terms, gene expression is regulated at the level of production of the mRNA transcript. If no mRNA for a particular polypeptide is produced, no polypeptide is produced. If mRNA is allowed to be produced, then that mRNA can be translated into the polypeptide encoded by the gene.

Levels of protein can also be regulated by abundance and stability of the mRNA transcript or by post-translational modifications.

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

A gene that encodes a protein consists of a regulatory region, a promoter, a start codon, a stop codon, the intervening polypeptide encoding sequence and a transcriptional terminator.



In the figure above, R1 and R2 are regulatory regions (enhancers are typically in front of the promoter, while the repressors are typically between the promoter and the coding sequence. Either may overlap with the coding sequence); P is the promoter; and T is the transcription terminator. R regions bind proteins that regulate the transcription of the gene. Transcription starts at the promoter and ends at the transcription terminator. The coding sequence starts with the start codon (ATG—Met), and ends with one of the three possible stop codons (TAA, TAG, TGA).

6. What regulates the expression of a gene?

RNA Polymerase and regulator proteins interact with regulatory regions by binding to either promote or prevent transcription.

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

Cis-acting elements are sequences of DNA. Trans-acting elements are gene products (and small molecules).

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?

Since trans-acting elements are gene products that diffuse freely through the area where regulation is taking place, recessive defects in trans-acting elements can be repaired by introducing another copy of the gene encoding the element into the cell.

Since cis-acting elements are sequences of DNA, defects in cis-acting elements cannot be repaired by simply introducing another copy of the element into the cell (e.g. RNA polymerase binds the promoter and begins transcription only of physically connected DNA). Depending on the trait imparted by the defect, sometimes the defect in a cis-acting element can be repaired by introducing an entire copy of a gene into the cell (e.g. functional promoter in front of a functional copy of the coding sequence would restore the cell to wild-type trait, but a functional copy of a gene would not restore a cell where the defect was in the operator—the copy with the “broken” operator will keep being transcribed regardless of the presence of a wild-type copy.).

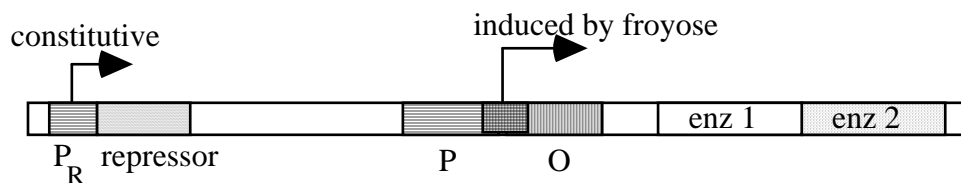
9. What proteins will be affected by mutations in the trans-acting elements? Cis-acting elements?

Mutations in trans-acting elements will affect the expression of all genes whose transcription is regulated by the trans-acting element. Mutations in cis-acting elements will affect only the protein encoded by the gene where the cis-acting element is located.

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:
- constitutive (constant) expression of enzymes 1 and 2?
this would result from either
 - the mutation in the operator, such that the repressor can not bind, or
 - a mutation in the repressor gene, such that the resulting repressor protein can not bind the operator, or
 - in the promoter for the repressor gene, such that no repressor protein is produced.
 - no expression of enzymes 1 and 2?
this would result from a mutation in the promoter for the enzymes 1 and 2.
 - expression of enzyme 1, but not 2 upon addition of froyose?
this would result from a mutation in the coding sequence of enzyme 2.
 - expression of enzyme 2, but not 1 upon addition of froyose?
this would result from a mutation in the coding sequence of enzyme 1.

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:

Genotype	- froyose		+ froyose		Mutation
	Enz 1	Enz 2	Enz 1	Enz 2	
wild-type	low	low	high	high	
A ⁻	low	low	low	low	<i>promoter for enzymes 1, 2</i>
B ⁻	high	high	high	high	<i>repressor or operator</i>
C ⁻	low	low	low	low	<i>promoter for enzymes 1, 2</i>
D ⁻	low	low	low	high	<i>enzyme 1 coding sequence</i>
E ⁻	low	low	high	low	<i>enzyme 2 coding sequence</i>
F ⁻	high	high	high	high	<i>repressor or operator</i>
G ⁻	low	low	high	low	<i>enzyme 2 coding sequence</i>

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?
This would result from a gain of function mutation in the coding sequence of the repressor gene, such that repressor protein can not release the operator, even in the presence of the de-repression signal.
5. For a loss of function mutations, what experiment can you perform to distinguish between the mutations in the operator and in the repressor?
Since one is a cis-acting element, and one is a trans-acting element, we could introduce a wild-type copy of the operon into each of the mutants F⁻ and B⁻, and see which one, if either, is rescued by the addition.

You then construct the following diploids:

	Genotype	- froyose		+ froyose	
		Enz 1	Enz 2	Enz 1	Enz 2
1	$\frac{D^+ E^-}{D^- E^+}$	low	low	high	high
2	$\frac{E^+ G^-}{E^- G^+}$	low	low	high	low
3	$\frac{A^- D^+ E^+}{A^+ D^- E^-}$	low	low	low	low
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.

Diploid strain 1: D and E strains have mutations in different genes, therefore you see complementation in the diploid and a return to wild-type control.

Diploid strain 2: E and G strains have mutations in the same gene, enzyme 2 does not get produced (is always low), enzyme 1 shows appropriate control.

Diploid strain 3: strain A has a mutation in the promoter. Because promoters act in cis, a wild-type promoter upstream of mutant copies of the genes for enzyme 1 and 2 cannot restore normal control.

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

Strains D and E have mutations in different genes, and therefore in absence of other mutations, if one good copy of each is present, the cell exhibits wild-type phenotype. Thus, this is a good background to distinguish a repressor mutation from an operator mutation.

If the mutation is in the operator (which acts in cis), you cannot restore normal control with a wild-type operator upstream of mutant copies of the genes for enzyme 1 and 2.

If the mutation is in the repressor (which acts in trans), you can restore normal control with a wild-type repressor upstream of mutant copies of the genes for enzyme 1 and 2.

*Therefore, the F strain has a mutation in the repressor
the B strain has a mutation in the operator*