

## Regulation of Gene Expression

*In any given cell at particular moment in time:*

*Some genes ON: Active in transcription*  
*Some genes OFF: Not synthesizing RNA*

*What switches genes ON and OFF?*

**Operons:** *Models for regulation of gene expression in bacteria*

*Small groups of adjacent genes*  
*Coordinately regulated*  
*Switched ON and OFF together*

**Lactose Operon of *E. coli*** *(Jacob and Monod)*

*Contains 3 genes involved in lactose utilization*  
*Lactose = Disaccharide (Glucose – Galactose)*  
*Lactose ---> Glucose + Galactose*  
*Catalyzed by B-galactosidase (Inducible enzyme)*  
*Enzyme levels increase with [lactose] in medium*

*Lactose: Inducer (switches on B-galactosidase gene)*

*Also induces synthesis of 2 other enzymes*  
*B-galactosidase permease*  
*Thiogalactosidase transacetylase*

*Genes for these 3 enzymes are:*

*Physically adjacent and coordinately regulated*  
*Transcribed into a single polycistronic mRNA*  
*Organized into unit called “lac” operon*

*When lactose is absent:*

*Repressor binds to operator*  
*No transcription (operon OFF)*

*When lactose is present:*

*Inducer binds to repressor*  
*Removes repressor from operator*  
*Allows transcription (operon ON)*

*Jacob and Monod model of gene regulation – Nobel Prize*

*Model based on analysis of mutants with altered response to lactose.*

***Mutations in Structural Genes (z, y, a)***

*Missense: 1 amino acid change in 1 gene product*

*Nonsense: Polar mutations; alter > 1 gene product*

***Mutations in Operator (o)***

*o(c) Operator constitutive mutants  
Altered nucleotide sequence in operator region  
Mutant operator fails to recognize repressor  
Operon continuously ON*

***Mutations in Repressor Gene (i)***

*i(-) Repressor protein cannot bind to operator  
Operon continuously ON*

*i(s) Repressor protein cannot bind to inducer  
Operon continuously OFF*

***Mutations in Promoter Region (p)***

*p(-) Altered nucleotide sequence in promoter  
Mutant promoter fails to bind RNA polymerase  
Operon continuously OFF*

*Key Concept - Repressor gene encodes diffusible protein*

*Demonstrated through use of F' partial diploids  
Combine 2 copies of lac operon in single cell  
One on chromosome; another on F' factor*

*Discovered that i(+) transdominant to i(-)*

*Dominant: i(+)/i(-) resembles i(+)*

*Trans: 2 genes not adjacent*

*Also found that i(s) transdominant to i(+)*

*Promoter/operator mutations have cis-dominant effect*

*Disrupt transcription of linked genes only  
Do not code for diffusible protein*

*Example:  $i(+)$  $p(-)$  $o(+)$  $z(+)$  $y(-)$  /  $i(-)$  $p(+)$  $o(+)$  $z(-)$  $y(+)$*

*In presence of lactose:*

*Normal “z” gene product? NO  
Normal “y” gene product? YES*

### ***Lactose Operon Exhibits Catabolite Repression***

*Cells grown on lactose + glucose:*

*Operon OFF until glucose gone  
Switch involves cAMP molecule*

*Mechanism:*

*Glucose catabolite lowers [cAMP]  
Lac operon promoter – 2 binding sites  
RNA polymerase and CAP/cAMP  
Low [cAMP] --> Low [CAP/cAMP]  
Reduces efficiency of RNA polymerase binding*

### ***Recent advances in understanding of Lac operon:***

*Entire operon cloned and sequenced  
Repressor protein purified; structure characterized  
Repressor / operator interaction examined in detail*

*Lac operon remains a model for understanding DNA-protein interactions  
in regulation of gene expression*

### ***Tryptophan Operon of E. coli:***

*Tryptophan produced by bacteria, plants, fungi  
5 genes in tryptophan operon  
Code for enzymes involved in tryptophan biosynthesis*

*Repressible operon:*

*Tryptophan present: Operon OFF  
Tryptophan absent: Operon ON*

*Regulatory gene (r) codes for aporepressor protein*  
*Aporepressor + co-repressor = functional repressor*  
*Co-repressor = tryptophan*  
*Repressor functional only when tryptophan present*

*Attenuation - Second mechanism for turning operon OFF*

*High [trp]: Efficient binding of repressor to operator*  
*Blocks initiation of transcription*  
*Attenuation not involved*

*Mod [trp]: Inefficient binding of repressor*  
*Some transcripts being produced*  
*Transcripts terminate at attenuator*

*Low [trp]: Attenuation relaxed*  
*Transcripts continue through attenuator*

### ***How Does Attenuation Work?***

*5' end of RNA transcript contains short ORF*  
*Several UGG (trp) codons present in this ORF*  
*Region of transcript has alternate folding patterns*

*Folding pattern depends on rate of ribosome movement through portion of transcript containing trp codons*

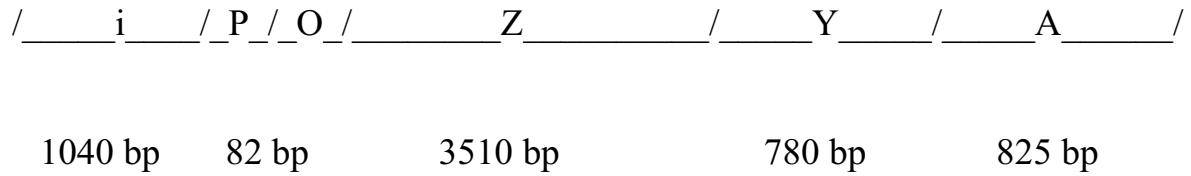
*Rapid movement if trp abundant (plenty of tRNA-trp)*

*Result: Folding pattern destabilizes RNA/DNA*  
*Transcript falls off DNA template*  
*Transcription terminates at attenuator*

*Slow movement if trp scarce (low levels of tRNA-trp)*

*Result: Folding pattern stabilizes RNA/DNA*  
*Transcription continues through attenuator*  
*Entire operon is transcribed*

## Lactose Absent



Z,Y,A: Genes encoding proteins required for lactose utilization

O: Operator; DNA binding site of repressor protein

P: Promoter; DNA binding site of RNA polymerase

i: Gene encoding repressor protein

Repressor protein

Inducer (Lactose)

## Lactose Present:



High [Tryptophan]

/    r    / // /    P    /    O    /    L    /    A    /            Genes E D C B A            /

E,D,C,B,A Genes encoding proteins required for tryptophan biosynthesis

P,O: Promoter, Operator

r: Regulatory gene encoding aporepressor protein

L: Leader sequence

A: Attenuator site

Aporepressor protein

Co-repressor (Tryptophan)

Low [Tryptophan]

/    r    / // /    P    /    O    /    L    /    A    /            Genes E D C B A            /

Intermediate [Tryptophan]

/    r    / // /    P    /    O    /    L    /    A    /            Genes E D C B A            /



Intermediate [Tryptophan] – Attenuation Occurs

/    P    /    O    /    L    /    A    /    Genes    →

Ribosome does NOT stall at UGG codons (tRNA-trp available)

This ALLOWS formation of destabilizing ds RNA hairpin (3+4)

RNA polymerase and RNA:DNA heteroduplex DESTABILIZED at attenuator

Transcription TERMINATES PREMATURELY

## Gene Regulation in Eukaryotes

### 1. *Very Few Operons:*

Evidence: Mapping Studies  
*Genome Projects*

Example: Biotin auxotrophs of *Arabidopsis*

*bio1* and *bio2* mutants - embryo defectives  
Rescued in culture by different precursors  
Genes encode different biosynthetic enzymes  
Mutations map to different chromosomes  
Results confirmed by genome sequencing

How do eukaryotes coordinate gene expression?

### 2. *[Protein] in Cell Regulated at Different Levels*

DNA amplification (rare)  
Transcription (promoter; transcription factors)  
RNA processing, transport, and stability  
Efficiency of translation  
Protein targeting and stability

### 3. *Transcription Factors Important- Regulate Dispersed Genes in a Coordinated Manner*

Final targets of signal transduction pathways  
Play critical role in growth and development  
Loss of gene function results in striking defects

## Mutant Analysis of Transcriptional Regulation in Eukaryotes:

### *Example #1: Drosophila Homeotic Mutants*

Mutant phenotype: Misplaced body parts in adult fly

Explanation: Change in cell identity during metamorphosis  
Imaginal disc cells receive incorrect instructions  
Normal gene function: regulate disc cell identity  
Homeodomain DNA binding proteins  
Similar proteins regulate body form in mammals

*Example #2: Drosophila eyeless Mutant*

- Mutant phenotype: Loss of eye tissues
- Normal function: Promote differentiation of eye tissue
- Ectopic expression: Introduce extra copy of wild-type gene  
Activate this gene throughout the fly  
Result: Differentiation of eye tissues in multiple locations
- Conclusion: Gene product (transcription factor) necessary and sufficient for eye tissue differentiation

*Example #3: Arabidopsis leafy cotyledon Mutant*

- Phenotype: Cotyledons partially transformed into leaves
- Normal Function: Promote cotyledon identity during seed development
- Ectopic Expression: Enhanced production of embryos in vegetative parts of the plant