

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

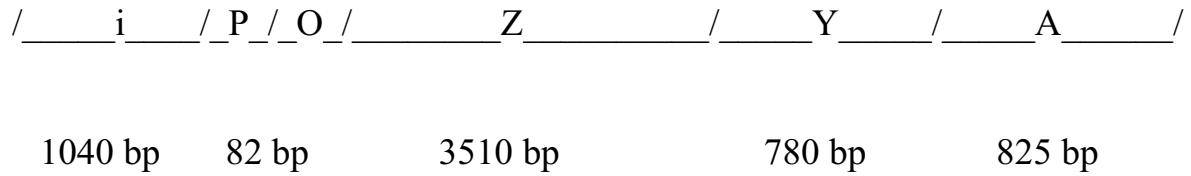
Rapid movement if trp abundant (plenty of tRNA-trp)

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