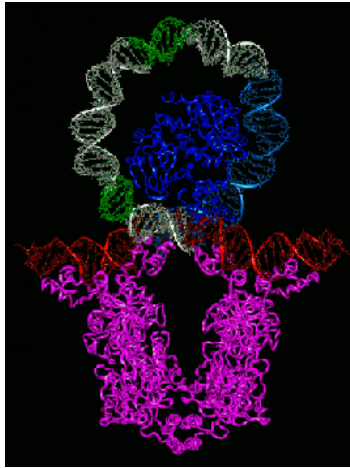


Chapter 13: Regulation of Gene Transcription



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Prokaryotic Gene Regulation

[The Logic and Basics of Prokaryotic Gene Regulation](#)

Logic

- Bacteria can utilize different types of carbon sources
- Different sets of enzymes are required to metabolize each types of sugars
- Bacteria synthesize different enzymes depending on the available resource in the environment

How?

- turn on genes required to synthesize appropriate enzymes
- mechanism to shut down genes not needed

Basics of gene regulation in Prokaryotes:

Sequence specific DNA-protein interactions near the transcription start site regulate transcription

The *lac* Operon:

[Structure and Regulatory Components](#)

- consists of a series of genes (**P**, **O**, **Z**, **Y** and **A**).
- used to regulate lactose metabolism in prokaryotes

Two environmental conditions must be met before activation:

1. lactose must be present
2. glucose must be absent

[Mutational Analysis](#)

Techniques

Enzymatic assays for β -galactosidase and permease in two states:

- **uninduced** state
 - assesses whether the genetic circuitry for repression is present
- **induced** state
 - shows whether the genetic circuitry necessary to overcome repression is functioning normally

Results of Genetic Analysis

On production of β -galactosidase and permease:

- Inactive structural genes for β -galactosidase and permease are recessive
- Mutations in the repressor and operator cause misregulation of the *lac* operon structural genes
- O^c mutation makes the operator nonfunctional
- O^c mutations are constitutive
- Operators are cis-acting, or restricted to the *lac* structural genes on the same chromosome

On effects of constitutive I^- mutations

- In I^- , the DNA binding site of repressor is mutated so no functional operator-binding repressor protein is made
- I^- mutants are constitutive
- I^+ is dominant to I^-
- I^+ is trans acting

Genetic Evidence for Allostery

lac repressor has to inhibit transcription at *lac* operon in absence of inducer.

Must also permit transcription when inducer is present.

This is accomplished through the allosteric site.

I^s mutations cause repression even in the presence of an inducer.

Promoter and Target DNA Sites

Promoter:

- Located between I and O elements
- Initiation site for transcription
- RNA polymerase binds two sites of promoter (P)
- Promoter mutations affect transcription of whole operon

Target sites:

- * Lac repressor binding-site and CAP-cAMP binding-sites very different
- * Both have rotational twofold symmetry

Summary

CAP-cAMP

- * Binding bends DNA; helps RNA Polymerase binding to promoter
- * Has protein-protein contacts with RNA Polymerase alpha subunit
- * Required for RNA Polymerase binding to *lac* promoter
- * Activation or positive control

lac Repressor

- * Glucose breakdown product inhibits high cAMP levels that form CAP-cAMP complex
- * Lactose must bind to repressor protein to remove it from operator
- * Inducer-repressor control an example of negative control

lac Operon Animation

The Arabinose Operon

Genes: **araB**, **araA**, and **araD**.

Encode metabolic enzymes that break down arabinose.

Transcription starts at the initiator (**araI**).

araC codes the activator protein that activates transcription of arabinose operon.

Additional activation events are mediated by **CAP-cAMP**.

In the presence of arabinose:

- CAP-cAMP complex and araC-arabinose complex bind to initiator region
- RNA polymerase binds to the promoter
- transcription begins

In the absence of arabinose:

- **araC** protein assumes a different conformation
 - acts as a repressor
 - binds to **araI** and a second operator region **araO**
 - forms a loop
 - this loop prevents transcription
-

Eukaryotic Gene Regulation

Transcriptional Regulation

Coordination of gene expression in eukaryotes is much more complex than in prokaryotes

Cis-Acting Sequences in transcriptional regulation

Three classes of cis-acting elements that serve as targets for trans-acting factor:

1. Core promoter-located near the transcription start site
2. Promoter-proximal elements- located close to the core promoter
3. Distance independent cis-acting elements

Trans-acting regulatory proteins and transcription regulation:

Bind to core promoter and promoter-proximal elements

Help RNA polymerase II to initiate transcription

Distance independent trans-acting regulatory proteins:

Proteins that bind to distance-independent cis-acting elements

Tissue-specific regulation of transcription:

Enhancer can function in tissue-specific manner

Activator may be limited to some types of cells

Repressor binds to silencer element located close to the enhancer

Regulatory Elements and Mutations

Finding the DNA Sequences (Enhancers):

Transgenic construct:

- Recombine DNA molecules
- Find where regulatory elements are
- Determine how and what it controls

Regulatory elements can be exploited:

- * Genes of interest identified by transgenic reporter genes
- * Reporter-gene-construct transcription unit contains a weak promoter
- * Expression of the reporter protein in tissues indicates enhancers
- * Enhancer can be identified by using smaller and smaller pieces of the original DNA

Regulatory Elements and Dominant Mutations

- * Help to understand gain-of-function dominant mutations

Transcription Factors

Sequence-specific DNA-binding proteins

Bind to cis-regulatory elements

Directly or indirectly affect initiation of transcription

Can act as activators or repressors

Common features of transcription factors

Separate functional domains:

1. DNA binding domain
2. Main core of the protein

DNA-binding proteins have various structural motifs

Examples: Helix-turn-helix
Zinc-finger protein

Amplified effect of transcription factors

Multiple transcription factor binding sites are often found adjacent to one another in an enhancer

Cooperative binding of certain TFs to these target sites lead to superadditive effect on transcription

Epigenetic Inheritance

Heritable modifications in gene function not due to changes in DNA base sequence

- * A piece of chromosome can be labelled as different
- * Depends on ancestry or presence of other genes in genome

Paramutation: genetic activity of a normal allele is heritably reduced because it is heterozygous with a paramutagenic allele

Parental imprinting: the activity of a gene depends on whether it's inherited from the father or the mother

Dosage compensation: gene product amounts from two X copies in females must be made equal to products of one X copy in males

Endocrine Regulation

- * Master regulator coordinating changes in transcription of cells
- * Lipid-soluble hormones pass through plasma membrane
- * Bind/regulate specific transcription factors in nucleus

Genomics

- * Eukaryotic genomes contain tens of thousands of genes
- * Only ~5% of human genome codes for proteins
- * Instruction manual of genes: enhancers and regulatory elements

How are these elements identified?

- * Comparative Genomics and Expression Studies
- * Measure Binding of Transcription Factors