CHAPTER 12 OUTLINE

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12.1 THE REGULATION OF GENE EXPRESSION IS CRITICAL FOR ALL ORGANISMS

• Genes and Regulatory Elements

• Levels of Gene Regulation

GENES AND REGULATORY ELEMENTS

• Structural genes: encoding proteins
• Regulatory genes: encoding products that interact with other sequences and affect the transcription and translation of these sequences
• Regulatory elements: DNA sequences that are not transcribed but play a role in regulating other nucleotide sequences

The case of bacterial stress induced ‘sex’ and gene regulation

• S. pneumoniae regulates the ability to exchange genetic material by regulating the competency genes (Remember Griffith’s experiment?)
• Competence stimulating protein (CSP) regulates the competency genes

GENES AND REGULATORY ELEMENTS

• Constitutive expression: continuously expressed under normal cellular conditions
• Positive control: stimulate gene expression
• Negative control: inhibit gene expression
LEVELS OF GENE REGULATION

- Four points where genes can be regulated
  - through the alteration of DNA or chromatin structure
  - at the level of transcription
  - mRNA processing
  - regulation of RNA stability
  - translation control
  - posttranslational modification

CONCEPT CHECK 2

Why is transcription a particularly important level of gene regulation in both bacteria and eukaryotes?

12.2 GENE REGULATION IN BACTERIAL CELLS

- Operon Structure
- Negative and Positive Control: Inducible and Repressible Operons
  - The lac Operon of E. coli
  - Mutations in lac
  - Positive Control and Catabolite Repression
  - The trp Operon of E. coli

12.2 OPERON STRUCTURE

- Operon: promoter + additional sequences that control transcription (operator) + structure genes
- Regulator gene: DNA sequence encoding products that affect the operon function, but are not part of the operon

NEGATIVE AND POSITIVE CONTROL; INDUCIBLE AND REPRESSIBLE OPERONS

- Inducible operons: Transcription is usually off and needs to be turned on.
  - Repressible operons: Transcription is normally on and needs to be turned off.

NEGATIVE AND POSITIVE CONTROL; INDUCIBLE

- Negative inducible operons: The control at the operator site is negative. Molecule binding is to the operator, inhibiting transcription. Such operons are usually off and need to be turned on, so the transcription is inducible.
  - Inducer: small molecule that turns on the transcription
NEGATIVE AND POSITIVE CONTROL; REPRESSIBLE OPERONS

- **Negative repressible operons**: The control at the operator site is negative and needs to be turned off.
- **Corepressor**: a small molecule that binds to the repressor and makes it incapable of binding to the operator to turn off transcription.

NEGATIVE AND POSITIVE CONTROL; INDUCIBLE AND REPRESSIBLE OPERONS

- **Positive inducible operon**
- **Positive repressible operon**

THE LAC OPERON OF ESCHERICHIA COLI

- **A negative inducible operon**
- **Lactose metabolism**
- **Regulation of the lac operon**
- **Inducer**: allolactose
  - **lacI**: repressor encoding gene
  - **lacP**: operon promoter
  - **lacO**: operon operator

THE LAC OPERON OF ESCHERICHIA COLI

- **Structural genes**
  - **lacZ**: encoding β-galactosidases
  - **lacY**: encoding permease
  - **lacA**: encoding transacetylase
  - **The repression of the lac operon never completely shuts down transcription.**

MUTATIONS IN LAC

- **Partial diploid**: full bacterial chromosome + an extra piece of DNA on F plasmid
- **Structural-gene mutations**: affect the structure of the enzymes, but not the regulations of their synthesis
  - **lacZ**/lacY⁻ / lacZ⁻/lacY⁺ produce fully functional β-galactosidase and permease.
**Mutations in Lac**

- **Regulator gene mutations**: lacI<sup>-</sup> leads to constitutive transcription of three structure genes.
  - lacI<sup>-</sup> is dominant over lacI<sup>+</sup> and is trans acting. A single copy of lacI<sup>-</sup> brings about normal regulation of lac operon.
  - lacI<sup>-</sup> lacZ<sup>-</sup> / lacI<sup>-</sup> lacZ<sup>+</sup> produce fully functional β-galactosidase.

- **Operator mutations**: lacO:<br>  - lacO<sup>C</sup> = constitutive<br>  - lacO<sup>C</sup> is dominant over lacO<sup>+</sup>, which is cis acting.<br>  - lacI<sup>+</sup> lacO<sup>C</sup> lacZ<sup>-</sup> / lacI<sup>+</sup> lacO<sup>+</sup> lacZ<sup>-</sup> produce fully functional β-galactosidase constitutively.

- **Promoter mutations**
  - lacP<sup>-</sup>: cis acting<br>  - lacI<sup>-</sup> lacP<sup>-</sup> lacZ<sup>-</sup> / lacI<sup>+</sup> lacP<sup>-</sup> lacZ<sup>-</sup> fails to produce functional β-galactosidase.

**Positive Control and Catabolite Repression**

- **Catabolite repression**: using glucose when available, and repressing the metabolite of other sugars.
  - The positive effect is activated by catabolite activator protein (CAP). cAMP is bound to CAP, together CAP–cAMP complex binds to a site slightly upstream from the lac gene promoter.

- **cAMP**—adenosine-3',5'-cyclic monophosphate
  - The concentration of cAMP is inversely proportional to the level of available glucose.

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**The Trp Operon of Escherichia Coli**

- **A negative repressible operon**
  - Five structural genes<br>  - trpE, trpD, trpC, trpB, and trpA—five enzymes together convert chorismate to tryptophane.
12.3 GENE REGULATION IN EUKARYOTIC CELLS TAKES PLACE AT MULTIPLE LEVELS

- Chromatin remodeling
  - Chromatin-remodeling complexes: bind directly to DNA sites and reposition nucleosomes
- Histone modification
  - Addition of methyl groups to the histone protein tails
  - Addition of acetyl groups to histone proteins

12.3 GENE REGULATION IN EUKARYOTIC CELLS TAKES PLACE AT MULTIPLE LEVELS

- Acetylation of histones controls flowering in Arabidopsis
  - Flowering locus C (FLC) gene
  - Flowering locus D (FLD) gene

12.3 GENE REGULATION IN EUKARYOTIC CELLS TAKES PLACE AT MULTIPLE LEVELS

- DNA Methylation
  - DNA methylation of cytosine bases adjacent to guanine nucleotides (CpG)–CpG islands

12.3 GENE REGULATION IN EUKARYOTIC CELLS TAKES PLACE AT MULTIPLE LEVELS

- Transcriptional activators and repressors
  - Bind to *silencers*
  - Enhancers and Insulators
  - Enhancer: DNA sequence stimulating transcription from a distance away from promoter
  - Insulator: DNA sequence that blocks or insulates the effect of enhancers
12.3 GENE REGULATION IN EUKARYOTIC CELLS TAKES PLACE AT MULTIPLE LEVELS

- Coordinated gene regulation
- Response elements: common regulatory elements upstream of the start sites of a collective group of genes in response to a common environmental stimulus

12.3 GENE REGULATION IN EUKARYOTIC CELLS TAKES PLACE AT MULTIPLE LEVELS

- Gene regulation through RNA splicing
  - Alternative splicing in the T-antigen gene
  - Alternative splicing in Drosophila sexual development
12.3 Gene Regulation in Eukaryotic Cells Takes Place at Multiple Levels

- The Degradation of RNA
  - 5' cap removal
  - Shortening of the poly(A) tail
  - Degradation of 5' UTR, coding sequence, and 3' UTR

- Small interfering RNAs and microRNAs
  - Dicer
  - RISC: RNA-induced silencing complex
  - RNA cleavage: RISC containing an siRNA, pair with mRNA molecules and cleavage to the mRNA.
  - Inhibition of translation
  - Transcriptional silencing: altering chromatin structure.

12.4 Epigenetic Effects Influence Gene Expression

- Epigenetic Effects
  - Changes induced by maternal behavior
  - Effects caused by prenatal exposure
  - Effects in monozygotic twins

- Molecular Mechanisms of Epigenetic Changes
  - DNA methylation is maintained from generation to generation