Chapter 18:
Regulation of Gene Expression

1. Gene Regulation in Bacteria
2. Gene Regulation in Eukaryotes
3. Gene Regulation in Development
4. Gene Regulation & Cancer
Gene regulation refers to all aspects of controlling the levels and/or activities of specific gene products.

- the gene product is either a protein or an RNA molecule
- regulation can occur at any stage of gene expression which involves
  - accessibility of the gene itself (chromatin structure)
  - transcription & translation (if gene encodes protein)
  - modification of the gene product
Transcription Factors

Transcription factors are proteins that either help activate or inhibit transcription.

Many transcription factors bind to specific DNA sequences in the regulatory regions of genes.

- DNA-binding transcription factors have a DNA-binding domain and one or more activation domains that mediate effects on transcription.
1. Gene Regulation in Bacteria

Chapter Reading – pp. 361-364
Bacterial Gene Regulation

Gene regulation in bacteria is generally accomplished at the levels of transcription and post-translational modification of protein activity.

Bacterial genes are commonly organized in multi-gene structures called operons:

- multiple gene coding regions organized in sequence under control of a single promoter
- genes in the operon are part of same metabolic pathway
- operons are typically inducible or repressible
Regulation of Tryptophan Production

- enzymes involved in tryptophan synthesis are part of a single operon

- regulation involves transcription & post-translational modification (feedback inhibition)

(a) Regulation of enzyme activity
(b) Regulation of enzyme production
The *trp* Operon

- *trp* repressor is *inactive* unless bound to tryptophan
- low tryptophan = ON
- high tryptophan = OFF

**repressible** operon
The *lac* Operon

- *lac* repressor is *active* unless bound to allolactose
- low allolactose = OFF
- high allolactose = ON

(a) Lactose absent, repressor active, operon off

(b) Lactose present, repressor inactive, operon on
...more on the *lac* Operon

When ON the *lac* operon is on “low” by default

If glucose (preferred sugar) is unavailable, *lac* operon is “turned up” due to CAP activation

- cAMP is produced if glucose is low
- cAMP binds and activates CAP
- active CAP binds CAP site increasing Tx

(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized
2. Gene Regulation in Eukaryotes

Chapter Reading – pp. 365-376
Overview of Eukaryotic Gene Regulation

Eukaryotic genes generally have the following:

- a single coding region consisting of exons & introns
- a single promoter
- multiple proximal and distal control sequences
  - distal control sequences can be 1000s of base pairs away

Eukaryotic gene regulation is dependent on chromatin structure in addition all stages between transcription initiation and the production of a functional gene product.
Stages of Gene Regulation

Chromatin structure*
  - controls access to genes

Transcription
  - key stage of gene regulation

RNA processing*
  - splicing of the RNA transcript

RNA stability

Translation of mRNA

Post-translation modifications

*relevant to eukaryotes only
Chromatin Structure

Chromatin structure is regulated through modifications of either the DNA itself or the histone proteins associated with the DNA:

**DNA modifications**
- addition of methyl (CH$_3$) groups to cytosines
- results in more compact, less accessible chromatin
- responsible for **X-inactivation**, **genomic imprinting**

**Histone modifications**
- addition of acetyl groups (“opens” chromatin)
- addition of CH$_3$ (“closed”) or PO$_4$ (“open”) groups
Histones & Chromatin Structure

DNA is wrapped around histone cores in structures called **nucleosomes**.

- tails of histone proteins in nucleosomes can have acetyl, methyl or phosphate groups added to induce a more “open” or “closed” chromatin structure

(a) Histone tails protrude outward from a nucleosome

(b) Acetylation of histone tails promotes loose chromatin structure that permits transcription
**Proximal & Distal Regulation**

- **Enhancer (distal control elements)**
- **Proximal control elements**
- **Transcription start site**
- **Poly-A signal sequence**
- **Transcription termination region**

Distal elements interact with promoter due to bending of DNA.

- Control elements bind specific transcription factors
- Can be located near the promoter (proximal) or very far from the promoter (distal)

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Current Model of Eukaryotic Transcription Initiation

Involves specific transcription factors as well as general transcription factors and other proteins involved in all transcription initiation.
Differential Gene Expression

Different genes are expressed in different cell types due to:

• differences in transcription factors

• differences in chromatin structure

(a) Liver cell
(b) Lens cell
Regulatory roles of non-coding RNA

Spliceosomes

- contain snRNA molecules that direct the process of splicing introns from primary RNA transcripts

MicroRNAs (miRNA)

- complex with specific proteins to facilitate destruction of specific mRNA molecules that contain sequences complementary to miRNA sequence
- target chromatin modification to the centromeres of chromosomes resulting in highly condensed heterochromatin in the centromeres
- protection from infection by RNA viruses
Alternative Splicing of RNA

DNA

Troponin T gene

Primary RNA transcript

RNA splicing

mRNA

or
(a) Primary miRNA transcript

miRNA Production

(b) Generation and function of miRNAs
Protein Degradation

- proteins to be degraded in cells (e.g., cyclins) are “tagged” with a small protein called ubiquitin
- ubiquitinated proteins are directed to proteosomes which then degrade them

![Diagram of Protein Degradation](image)
Chromatin modification
- Genes in highly compacted chromatin are generally not transcribed.
- Histone acetylation seems to loosen chromatin structure, enhancing transcription.
- DNA methylation generally reduces transcription.

Transcription
- Regulation of transcription initiation: DNA control elements in enhancers bind specific transcription factors.
- Coordinate regulation: Enhancer for liver-specific genes
  Enhancer for lens-specific genes

Bending of the DNA enables activators to contact proteins at the promoter, initiating transcription.

RNA processing
- Alternative RNA splicing:
  Primary RNA transcript
  mRNA or

Initiation of translation can be controlled via regulation of initiation factors.

Translation
- Protein processing and degradation by proteasomes are subject to regulation.

mRNA degradation
- Each mRNA has a characteristic life span, determined in part by sequences in the 5’ and 3’ UTRs.

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3. Gene Regulation in Development

Chapter Reading – pp. 376-382
Embryonic Development

“From fertilization to fully developed organism.”

Involves regulation of maternal and embryonic gene expression:

- maternal genes involved in packaging the egg during oogenesis (egg production)
- embryonic genes control development after fertilization

Mutations in either maternal or embryonic genes can result in developmental defects
Key Events in Animal Development

Oogenesis

- egg production in the ovary results in essential gene regulatory factors (RNA, protein) being packaged very specifically and unevenly in the developing egg

Fertilization

- triggers translation of maternal mRNA and rapid series of mitotic nuclear divisions (cleavage)

Gastrulation & Induction

- cell rearrangement and cell-cell signaling resulting in the differentiation of cells and formation of distinct body structures
(a) Cytoplasmic determinants in the egg

Egg is packaged unevenly with regulatory factors that are then partitioned into different cells after fertilization.

(b) Induction by nearby cells

Cell-cell communication also induces changes in gene expression.

Unfertilized egg

Molecules of two different cytoplasmic determinants

Early embryo (32 cells)

NUCLEUS

Signal transduction pathway

Signal receptor

Signaling molecule (inducer)
Early Drosophila Development

- maternal genes determine body axes and early pattern formation
- embryonic genes eventually take over and determine subsequent morphogenesis

(b) Development from egg to larva
**Bicoid Determines Anterior End**

The mutant phenotype named “Bicoid” results in larva with 2 posteriors and no anterior (NO head!).

- **due to a mutation in the maternal Bicoid gene**
- **Bicoid mRNA is deposited in the anterior end of all eggs during oogenesis**
- **Bicoid activates anterior gene expression after fertilization**
Localization of Bicoid Protein, mRNA

RESULTS

Bicoid mRNA in mature unfertilized egg

Bicoid is a morphogen of maternal origin

Fertilization, translation of bicoid mRNA

Bicoid mRNA in mature unfertilized egg

Bicoid mRNA is expressed into protein after fertilization

This results in a Bicoid “morphogen gradient”

Bicoid protein in early embryo

Bicoid protein in early embryo
Nucleus

Embryonic precursor cell

Myoblast (determined)

Part of a muscle fiber (fully differentiated cell)

Master regulatory gene *myoD*

MyoD protein (transcription factor)

Other muscle-specific genes

OFF

Myosin, other muscle proteins, and cell cycle–blocking proteins

Specification of vertebrate muscle tissue
4. Gene Regulation & Cancer

Chapter Reading – pp. 383-388
Oncogenes are genes with a role in cell cycle progression that have undergone a mutation that contributes to cancer formation (normal version is called a proto-oncogene).

- generally due to dominant “gain-of-function” mutations
- mutations are of 3 general types:
  1) translocation of the gene
  2) amplification (duplication) of the gene
  3) mutations in the coding or regulatory regions of the gene
Proto-oncogene

DNA

Translocation or transposition: gene moved to new locus, under new controls

Gene amplification: multiple copies of the gene

Point mutation: within a control element within the gene

New promoter

Normal growth-stimulating protein in excess

Normal growth-stimulating protein in excess

Normal growth-stimulating protein in excess

Hyperactive or degradation-resistant protein

• mutations that result in excessive expression or function can contribute to cancer

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Growth factor

Receptor

G protein

Protein kinases (phosphorylation cascade)

NUCLEUS

Transcription factor (activator)

DNA

Gene expression

Protein that stimulates the cell cycle

(a) Cell cycle–stimulating pathway

Ras is a G protein that is a proto-oncogene.

Gain-of-function Ras mutations can trigger “signal-independent” activation of cell cycle.

Hyperactive Ras protein (product of oncogene) issues signals on its own.
Tumor Suppressor Genes encode gene products that inhibit cell cycle progression.

Mutations in tumor suppressor genes are typically recessive “loss-of-function” mutations.

- typically requires 2 mutant alleles (recessive)
- loss of functional gene product leads to defect in:
  - inhibiting cell cycle progression
  - triggering apoptosis
  - activating DNA repair
(b) Cell cycle–inhibiting pathway

1. DNA damage in genome
2. Protein kinases
3. Active form of p53

UV light

DNA damage

Active form of p53

Protein that inhibits the cell cycle

MUTATION
Defective or missing transcription factor, such as p53, cannot activate transcription.

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Cancer Requires Multiple Mutations

The “multi-step” or “multi-hit” hypothesis.

**EFFECTS OF MUTATIONS**

1. **Loss of tumor-suppressor gene** *APC* (or other)
   - Colon wall
   - Normal colon epithelial cells

2. **Activation of ras oncogene**
   - Small benign growth (polyp)

3. **Loss of tumor-suppressor gene** *DCC*
   - Larger benign growth (adenoma)

4. **Loss of tumor-suppressor gene** *p53*
   - Malignant tumor (carcinoma)

5. **Additional mutations**

**Protein overexpressed**
- Cell cycle overstimulated
- Increased cell division

**Protein absent**
- Cell cycle not inhibited

(c) Effects of mutations
Key Terms for Chapter 18

- nucleosome, euchromatin, heterochromatin
- operon, repressor, operator, repressible, inducible
- control elements, distal, proximal, enhancer
- general vs specific transcription factors
- mediator proteins, DNA bending protein
- miRNA, alternative RNA splicing, Dicer, hairpin
- oogenesis, cytoplasmic determinants, induction
- morphogenesis, morphogen, morphogen gradient
- oncogene, proto-oncogene, tumor suppressor gene
- gain-of-function, loss-of-function mutations