Chapter 18
Gene Expression

Gene Expression and Metabolic Pathways
A gene is being "expressed" or "activated" when a protein is being made.

Some are expressed for a time and then turned off to conserve cell resources.

Controls of Metabolic Pathways
1. Regulation of enzymes already present
   - in response to fluctuations of needed substances
2. Regulation of production of enzymes not present
   - at transcription level

Prokaryotic Gene Expression
"Players in Gene Expression"

Operon: cluster of related genes with on/off switch
(promoter, operator, genes)

- Promoter: control sequence, site RNA polymerase attaches and replication starts
- Operator: DNA sequence between promoter and enzyme genes, acts as on/off switch for genes, controls access of RNA polymerase
- Genes: code for related enzymes in a pathway
- Inducer: molecule that initiates gene expression, must be present
- Repressor: protein binds to operator to block RNA polymerase

Gene Regulation
Negative vs Positive Control of Operons

Genes are not continually transcribing mRNA. Production must be regulated.

Negative control: operons are switched off by active form of repressor protein
Ex: trp operon, lac operon

Positive control: regulatory protein interacts directly with genome to turn on transcription
Ex: cAMP & CAP

Negative Control
Regulatory gene: produces repressor protein that binds to operator (blocks RNA polymerase access)

Negative Control: Operon Types
Repressible Operon: default mode is ON
Induceable Operon: default mode is OFF
Negative Control: Operon Types

Repressible Operon
- Normally ON
- Anabolic (builds organic mols.)
- Organic molecule product acts as corepressor (binds to repressor to activate it)
- Activated operon is turned OFF
  Ex: trp operon (tyrptophan production)

Inducible Operon
- Normally OFF
- Catabolic (breaks down food for energy)
- Repressor is active → inducer binds to and inactivates repressor
- Activated operon is turned ON
  Ex: lac operon (lactase production)

Positive Control

CAMP (cyclic AMP) and CRP (cAMP receptor protein)
- Low glucose conditions cause the buildup of cyclic AMP (cAMP).
- cAMP binds to a regulatory protein CRP.
  - activates the protein which binds to promoter of the operon.
  - allows RNA polymerase to bind and begin transcription.
- As glucose levels rise, cAMP levels lower, thus decreasing rate of transcription

Eukaryotic Gene Expression

Differential Gene Expression
- Different cell types (with identical genomes) turn on different genes to carry out specific functions
- Regulation of the expression is essential for cell specialization in multicellular organisms.
- Typical human cell: only 20% of genes expressed at any given time
- Expression is regulated at different stages
Eukaryotic Gene Expression

Eukaryotes have a greater variety of control mechanisms for gene expression. Each stage is a potential control point to turn on/off, accelerate or slow down expression.

Regulation Mechanisms
1. Chromatin structure
2. Transcription initiation
3. Post-transcriptional regulation
4. Post-translation regulation

Regulation of Chromatin Structure

- Chromatin remodeling (alteration of chromatin structure) makes region of DNA more or less able to be transcribed.
- Initiation of transcription requires that nucleosomes become less compact so available to RNA polymerase.
- Regulates gene expression:
  - genes within highly condensed chromatin usually not expressed
  - location of nucleosomes on gene’s promoter affects transcription
  - modifications to histone proteins influence gene expression
- DNA sequence is not changed, only condensation of chromatin.

DNA methylation, cont.

- X Chromosome inactivation:
  - Females XX should produce 2x more protein than males XY
  - One X chromosome is heavily methylated and inactivated
  - known as heterochromatin (barr body)
  - Maternal or paternal X may be inactivated (chance) so genes not expressed
  - Ex: calico cats

Regulation of Chromatin Structure

Histone Acetylation
- Acetyl groups (-COCH₃) added to histone tails
  - chromatin is opened up
  - promotes transcription

DNA Methylation
- Methyl groups (-CH₃) groups added to DNA cytosines
  - condenses chromatin/represses transcription
  - genes tend to be inactivated (silenced)
  - methylation patterns are stable and inherited with cell divisions

Epigenetic Inheritance
- Phenotypic trait variations that result from external or environmental factors that switch genes on and off and affect how cells express genes (methylation of DNA)
- Epigenetic changes modify the activation of certain genes, but not the genetic code sequence of DNA.
- Reversible (by de-methylation of DNA)
  - Ex: identical twin differences
    - Methylation patterns same when young
    - Patterns are different by age 50
    - Expression of genes is different
    - Alterations in methylation seen in some diseases, mental disorders and cancers

Chromatin

- Chromatin: less tightly coiled DNA/protein complex that makes up chromosomes
  - composed of DNA and nucleosomes which are folded over and over again so it can fit into nucleus
    - DNA double helix is 1000x longer than chromosome
- Nucleosome: basic unit, complex of coiled bead like structures composed of 8 histone proteins and DNA
2. Regulation of Transcription Initiation

- Specific transcription factors (activators or repressors) bind to control elements (non-coding sections of DNA with particular nucleotide sequences) to complete transcription complex.
- Allows RNA polymerase to attach to promoter.
- Regulate gene expression in different cell types:
  - Activators: increase transcription
  - Enhancers: short (50-1500 bp) region of DNA
  - Repressors: decrease transcription

Regulation of Transcription Initiation

1. Activator proteins bind to distal control elements (enhancer).
2. Bound activators brought closer to promoter.
3. Activators bound to other proteins and RNA polymerase to make active transcription initiation complex on promoter.

3. Post Transcriptional Regulation

Post Transcriptional Regulation

- mRNA Degradation:
  - Eukaryotic mRNA can survive for hours, days or weeks and be transcribed more than once.

Initiation of Translation:
- Can be blocked by regulatory proteins and prevent attachment of ribosomes.

Regulation of Transcription Initiation

Combinational Control of Gene Activation/Cell Type Specific

Appropriate activator proteins must be present at precise times in development to activate transcription in different cell types.

- Both liver and lens cells have genes for making albumin and crystallin.
- Liver cells → albumin: have activator proteins for albumin only.
- Lens cells → crystallin: have activator proteins for crystallin only.

RNA Processing:

- Alternative RNA splicing:
  - Different RNA molecules are made from same primary transcript.
  - Regulatory proteins specific to cell type control intron-exon choices.
  - More than 90% of human genes undergo alternative splicing.

mRNA Degradation:

Initiation of Translation:

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Post Transcriptional Regulation

Non-Coding RNAs Effect on Transcription

Only ~1.5% of DNA is coding (exons) in most organisms. "Junk DNA" found to have functions in expression of genes.

- MicroRNAs (miRNAs)
- Small interfering RNAs (siRNAs)

- Bind to complementary mRNAs
- Degrade or block translation
- Some now found to remodel chromatin affecting gene expression

4. Post Translation Regulation

Protein Processing and Degradation

- Length of time a protein functions is regulated by selective degradation.
  - ex: cyclins - short lived
- Small protein ubiquitin attached to protein
- Proteosomes recognize and degrade ubiquitin attached proteins
  - mutations in some cells during cell cycle make them impervious to proteosome degradation - can lead to cancer

Summary of Eukaryotic Gene Expression

Expression is Regulated at Many Stages

Chromatin modification

- Genes in highly compacted chromatin are generally not transcribed.
- Histone acetylation seems to loosen chromatin structure, enhancing transcription.
- DNA methylation generally represses transcription.

Transcription

- Regulation of transcription initiation: DNA control elements in enhancers bind specific transcription factors.
- Bending of the DNA enables activators to contact proteins at the promoter, initiating transcription.
- Coordinate regulation: Enhancer for liver-specific genes
- Enhancer for lens-specific genes

RNA processing

- Alternative RNA splicing

Translation

- mrRNA or siRNA can block the translation of specific mRNAs.

Protein processing and degradation

- Initiation of translation can be controlled via regulation of initiation factors.
- Protein processing and degradation by proteosomes are subject to regulation.

Summary of Eukaryotic Gene Expression

Non-coding RNAs play roles in expression

- Small or large noncoding RNAs can promote the formation of heterochromatin in certain regions, blocking transcription.

mRNA degradation

- mRNA or siRNA can target specific mRNAs for destruction.

Gene Expression during Development

During zygote development:

- Cell division: large number of identical cells thru mitosis
- Cell differentiation: development of different cells with specialized functions, due to differential gene expression
- Morphogenesis: development of form in an organism
Cytoplasmic Determinants

Most cells have the same genome, yet develop differently.

**Determination:**
Irreversible series of events that lead to cell differentiation

Cell attains its determined fate

As cells differentiate, they become more different in structure and function

- Cytoplasmic determinants: maternal substances in the egg, influence course of early development
  - Maternal substances in cytoplasm distributed unevenly
  - Nuclei of new cells exposed to different maternal substances
  - Regulates gene expression during development

**Induction:**
Cells triggered to differentiate

Neighboring embryonic cells release molecules that signal (induce) nearby cells to change their gene expression

- Involves cell surface receptors and signaling pathway proteins
- Expression of genes for tissue specific proteins
- mRNA for these proteins
- Transcription - main regulatory point

Ex: growth factors

**Pattern Formation:** Setting Up the Body Plan

Key Events in Drosophila Development

- Egg develops within ovariole follicle
- Nurse cell
- Unfertilized egg
- Diploid nurse cells
- Fertilization
- Laying of egg
- Embryonic development
- Segmented embryo
- Body segments
- D.1 mm
- Larval stage
- Hatching
- Development from egg to larva

**Body Development Genes**

- **Maternal effect gene (egg polarity gene):** if mutated will cause defective axes in embryo
  - embryonic lethals - causes embryonic death
  - bicoid: “two tailed” - morphogen that determines head structures

- **Morphogens:** substances that establish an embryo’s axes and other features of its form

Pattern formation: spatial organization of tissues and organs in their proper locations

Positional information: molecular clues that control pattern formation

- Provided by cytoplasmic determinants and inductive signals

Example: Determination and Differentiation of Muscle Cells

- Signals from other cells lead to activation of master regulatory gene
- Cell makes MyoD protein (transcription factor) acts as an activator
- Cell becomes a myoblast - committed to becoming a skeletal muscle cell

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Role of Apoptosis in Embryonic Development
programmed cell death

- Most embryonic cells are produced in excess
- Used to sculpt organs and tissues
- Development of body structures
- DNA and organelles get chopped up
- Blebbing occurs: multilobed
- Blebs engulfed by scavenger cells

Homeotic Genes

Homeotic genes: regulatory genes that determine where certain anatomical structures will develop in an organism during morphogenesis

- master control genes for development of body organization
- regulate gene expression by turning genes on and off
- increases or decreases cell division rates in areas of developing organism
- every cell in an organism carries, within its DNA, all of the information necessary to build the entire organism

Homeobox Sequences

- DNA sequences of many genes that control body pattern contain a common stretch of about 180 nucleotides within its sequence
  - homeobox: specific common DNA sequence, codes for proteins that regulate patterns of development
    - only a portion of each gene
  - homeotic genes: contain the homeobox
    - ex: if the words below were homeotic genes, the capital letters would represent the homeobox
togeTHer
THeoretical
goTHering
baTHer

Cancer

Cells lose ability to control their cell cycle and divide uncontrollably

- Due to abnormal regulation of genes that affect the cell cycle

Genetics of Cancer

Proto-oncogene: codes for proteins that stimulate normal cell growth and division

Oncogene: "cancer genes" - mutated proto-oncogene
uncontrolled cell proliferation
cancer causing gene

Tumor: abnormal proliferation of cell that results from uncontrolled, abnormal cell division

Benign: non cancerous, cells stay within the mass

Malignant: uncontrolled dividing cells invade and destroy healthy tissues in body

Metastasis: spread of cancer cells beyond original site
Tumor suppressor gene: codes for proteins that prevents uncontrolled cell growth and division
- we have two copies (both must be mutated)
- mutation causes suppressor expression not to work leading to uncontrolled growth

P53 gene (tumor suppressor)
- Guardian angel of the cell
- halts cell cycle for DNA repair
- activates apoptosis
- turns on DNA repair
- 50-60% of cancers
  - Ex: BRCA 1 and 2 breast cancer- 50-60% mutated

Cancer is result of ~ six changes in DNA
- at least one oncogene and loss of several tumor suppressor genes
- mutations accumulate as we age leading increase in cancer
- Predisposition colorectal cancer: 60% APC gene mutated

Causes of Cancer
Exposure to the following:
- Radiation
- Viruses
- Chemicals