Chapter 18

Overview: Conducting the Genetic Orchestra

Prokaryotes and eukaryotes alter gene expression in response to their changing environment

- In multicellular eukaryotes, gene expression regulates development and is responsible for differences in cell types
- RNA molecules play many roles in regulating gene expression in eukaryotes

Concept 18.1: Bacteria often respond to environmental change by regulating transcription

- Natural selection has favored bacteria that produce only the products needed by that cell
- A cell can regulate the production of enzymes by feedback inhibition or by gene regulation
- Gene expression in bacteria is controlled by the operon model

Operons: The Basic Concept

- A cluster of functionally related genes can be under coordinated control by a single "on-off switch"
- The regulatory "switch" is a segment of DNA called an **operator** usually positioned within the promoter
- An **operon** is the entire stretch of DNA that includes the operator, the promoter, and the genes that they control
- The operon can be switched off by a protein **repressor**
- The repressor prevents gene transcription by binding to the operator and blocking RNA polymerase
- The repressor is the product of a separate **regulatory gene**
- The repressor can be in an active or inactive form, depending on the presence of other molecules
- A **corepressor** is a molecule that cooperates with a repressor protein to switch an operon off
- For example, *E. coli* can synthesize the amino acid tryptophan
- By default the *trp* operon is on and the genes for tryptophan synthesis are transcribed
- When tryptophan is present, it binds to the *trp* repressor protein, which turns the operon off
- The repressor is active only in the presence of its corepressor tryptophan; thus the *trp* operon is turned off (repressed) if tryptophan levels are high

Repressible and Inducible Operons: Two Types of Negative Gene Regulation

- A repressible operon is one that is usually on; binding of a repressor to the operator shuts off transcription
- The *trp* operon is a repressible operon
- An inducible operon is one that is usually off; a molecule called an inducer inactivates the repressor and turns on transcription
- The *lac* operon is an inducible operon and contains genes that code for enzymes used in the hydrolysis and metabolism of lactose
- By itself, the *lac* repressor is active and switches the *lac* operon off
- A molecule called an **inducer** inactivates the repressor to turn the *lac* operon on

- nducible enzymes usually function in catabolic pathways; their synthesis is induced by a chemical signal
- Repressible enzymes usually function in anabolic pathways; their synthesis is repressed by high levels of the end product
- Regulation of the *trp* and *lac* operons involves negative control of genes because operons are switched off by the active form of the repressor

Positive Gene Regulation

- Some operons are also subject to positive control through a stimulatory protein, such as catabolite activator protein (CAP), an **activator** of transcription
- When glucose (a preferred food source of *E. coli*) is scarce, CAP is activated by binding with **cyclic AMP (cAMP)**
- Activated CAP attaches to the promoter of the *lac* operon and increases the affinity of RNA polymerase, thus accelerating transcription
- When glucose levels increase, CAP detaches from the *lac* operon, and transcription returns to a normal rate
- CAP helps regulate other operons that encode enzymes used in catabolic pathways

Concept 18.2: Eukaryotic gene expression is regulated at many stages

- All organisms must regulate which genes are expressed at any given time
- In multicellular organisms regulation of gene expression is essential for cell specialization

Differential Gene Expression

- Almost all the cells in an organism are genetically identical
- Differences between cell types result from **differential gene expression**, the expression of different genes by cells with the same genome
- Abnormalities in gene expression can lead to diseases including cancer
- Gene expression is regulated at many stages

Regulation of Chromatin Structure

- Genes within highly packed heterochromatin are usually not expressed
- Chemical modifications to histones and DNA of chromatin influence both chromatin structure and gene expression

Histone Modifications

- In **histone acetylation**, acetyl groups are attached to positively charged lysines in histone tails
- This loosens chromatin structure, thereby promoting the initiation of transcription
- The addition of methyl groups (methylation) can condense chromatin; the addition of phosphate groups (phosphorylation) next to a methylated amino acid can loosen chromatin
- The *histone code hypothesis* proposes that specific combinations of modifications, as well as the order in which they occur, help determine chromatin configuration and influence transcription

DNA Methylation

- **DNA methylation**, the addition of methyl groups to certain bases in DNA, is associated with reduced transcription in some species
- DNA methylation can cause long-term inactivation of genes in cellular

differentiation
- In genomic imprinting, methylation regulates expression of either the maternal or paternal alleles of certain genes at the start of development

Epigenetic Inheritance
- Although the chromatin modifications just discussed do not alter DNA sequence, they may be passed to future generations of cells
- The inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called **epigenetic inheritance**

Regulation of Transcription Initiation
- Chromatin-modifying enzymes provide initial control of gene expression by making a region of DNA either more or less able to bind the transcription machinery

Organization of a Typical Eukaryotic Gene
- Associated with most eukaryotic genes are multiple **control elements**, segments of noncoding DNA that serve as binding sites for transcription factors that help regulate transcription
- Control elements and the transcription factors they bind are critical to the precise regulation of gene expression in different cell types

The Roles of Transcription Factors
- To initiate transcription, eukaryotic RNA polymerase requires the assistance of proteins called transcription factors
- General transcription factors are essential for the transcription of all protein-coding genes
- In eukaryotes, high levels of transcription of particular genes depend on control elements interacting with specific transcription factors
- Proximal control elements are located close to the promoter
- Distal control elements, groupings of which are called **enhancers**, may be far away from a gene or even located in an intron
- An activator is a protein that binds to an enhancer and stimulates transcription of a gene
- Activators have two domains, one that binds DNA and a second that activates transcription
- Bound activators facilitate a sequence of protein-protein interactions that result in transcription of a given gene
- Some transcription factors function as repressors, inhibiting expression of a particular gene by a variety of methods
- Some activators and repressors act indirectly by influencing chromatin structure to promote or silence transcription
- A particular combination of control elements can activate transcription only when the appropriate activator proteins are present

Coordinately Controlled Genes in Eukaryotes
- Unlike the genes of a prokaryotic operon, each of the co-expressed eukaryotic genes has a promoter and control elements
- These genes can be scattered over different chromosomes, but each has the same combination of control elements
- Copies of the activators recognize specific control elements and promote simultaneous transcription of the genes

Nuclear Architecture and Gene Expression

- Loops of chromatin extend from individual chromosomes into specific sites in the nucleus
- Loops from different chromosomes may congregate at particular sites, some of which are rich in transcription factors and RNA polymerases
- These may be areas specialized for a common function

Mechanisms of Post-Transcriptional Regulation

- Transcription alone does not account for gene expression
- Regulatory mechanisms can operate at various stages after transcription
- Such mechanisms allow a cell to fine-tune gene expression rapidly in response to environmental changes

RNA Processing

- In **alternative RNA splicing**, different mRNA molecules are produced from the same primary transcript, depending on which RNA segments are treated as exons and which as introns

mRNA Degradation

- The life span of mRNA molecules in the cytoplasm is a key to determining protein synthesis
- Eukaryotic mRNA is more long lived than prokaryotic mRNA
- Nucleotide sequences that influence the lifespan of mRNA in eukaryotes reside in the untranslated region (UTR) at the 3′ end of the molecule

Initiation of Translation

- The initiation of translation of selected mRNAs can be blocked by regulatory proteins that bind to sequences or structures of the mRNA
- Alternatively, translation of all mRNAs in a cell may be regulated simultaneously
- For example, translation initiation factors are simultaneously activated in an egg following fertilization

Protein Processing and Degradation

- After translation, various types of protein processing, including cleavage and the addition of chemical groups, are subject to control
- **Proteasomes** are giant protein complexes that bind protein molecules and degrade them

Concept 18.3: Noncoding RNAs play multiple roles in controlling gene expression

- Only a small fraction of DNA codes for proteins, and a very small fraction of the non-protein-coding DNA consists of genes for RNA such as rRNA and tRNA
- A significant amount of the genome may be transcribed into noncoding RNAs (ncRNAs)
- Noncoding RNAs regulate gene expression at two points: mRNA translation and chromatin configuration

Effects on mRNAs by MicroRNAs and Small Interfering RNAs

- **MicroRNAs** (**miRNAs**) are small single-stranded RNA molecules that can bind to mRNA
- These can degrade mRNA or block its translation

- The phenomenon of inhibition of gene expression by RNA molecules is called **RNA interference (RNAi)**
- RNAi is caused by **small interfering RNAs (siRNAs)**
- siRNAs and miRNAs are similar but form from different RNA precursors

Chromatin Remodeling and Effects on Transcription by ncRNAs

- In some yeasts siRNAs play a role in heterochromatin formation and can block large regions of the chromosome
- Small ncRNAs called piwi-associated RNAs (piRNAs) induce heterochromatin, blocking the expression of parasitic DNA elements in the genome, known as transposons
- RNA-based mechanisms may also block transcription of single genes

The Evolutionary Significance of Small ncRNAs

- Small ncRNAs can regulate gene expression at multiple steps
- An increase in the number of miRNAs in a species may have allowed morphological complexity to increase over evolutionary time
- siRNAs may have evolved first, followed by miRNAs and later piRNAs

Concept 18.4: A program of differential gene expression leads to the different cell types in a multicellular organism

- During embryonic development, a fertilized egg gives rise to many different cell types
- Cell types are organized successively into tissues, organs, organ systems, and the whole organism
- Gene expression orchestrates the developmental programs of animals

A Genetic Program for Embryonic Development

- The transformation from zygote to adult results from cell division, cell differentiation, and morphogenesis
- **Cell differentiation** is the process by which cells become specialized in structure and function
- The physical processes that give an organism its shape constitute **morphogenesis**
- Differential gene expression results from genes being regulated differently in each cell type
- Materials in the egg can set up gene regulation that is carried out as cells divide

Cytoplasmic Determinants and Inductive Signals

- An egg's cytoplasm contains RNA, proteins, and other substances that are distributed unevenly in the unfertilized egg
- **Cytoplasmic determinants** are maternal substances in the egg that influence early development
- As the zygote divides by mitosis, cells contain different cytoplasmic determinants, which lead to different gene expression
- The other important source of developmental information is the environment around the cell, especially signals from nearby embryonic cells
- In the process called **induction**, signal molecules from embryonic cells cause transcriptional changes in nearby target cells
- Thus, interactions between cells induce differentiation of specialized cell types

Sequential Regulation of Gene Expression During Cellular Differentiation

- **Determination** commits a cell to its final fate
- Determination precedes differentiation
- Cell differentiation is marked by the production of tissue-specific proteins
- Myoblasts produce muscle-specific proteins and form skeletal muscle cells
- *MyoD* is one of several "master regulatory genes" that produce proteins that commit the cell to becoming skeletal muscle
- The MyoD protein is a transcription factor that binds to enhancers of various target genes

Pattern Formation: Setting Up the Body Plan

- **Pattern formation** is the development of a spatial organization of tissues and organs
- In animals, pattern formation begins with the establishment of the major axes
- **Positional information**, the molecular cues that control pattern formation, tells a cell its location relative to the body axes and to neighboring cells
- Pattern formation has been extensively studied in the fruit fly *Drosophila melanogaster*
- Combining anatomical, genetic, and biochemical approaches, researchers have discovered developmental principles common to many other species, including humans

***The Life Cycle of* Drosophila**

- In *Drosophila*, cytoplasmic determinants in the unfertilized egg determine the axes before fertilization
- After fertilization, the embryo develops into a segmented larva with three larval stages

***Genetic Analysis of Early Development:* Scientific Inquiry**

- Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric Wieschaus won a Nobel Prize in 1995 for decoding pattern formation in *Drosophila*
- Lewis discovered the **homeotic genes**, which control pattern formation in late embryo, larva, and adult stages
- Nüsslein-Volhard and Wieschaus studied segment formation
- They created mutants, conducted breeding experiments, and looked for corresponding genes
- Many of the identified mutations were **embryonic lethals,** causing death during embryogenesis
- They found 120 genes essential for normal segmentation

Axis Establishment

- **Maternal effect genes** encode for cytoplasmic determinants that initially establish the axes of the body of *Drosophila*
- These maternal effect genes are also called **egg-polarity genes** because they control orientation of the egg and consequently the fly
- One maternal effect gene, the ***bicoid*** gene, affects the front half of the body
- An embryo whose mother has no functional *bicoid* gene lacks the front half of its body and has duplicate posterior structures at both ends
- This phenotype suggests that the product of the mother's *bicoid* gene is concentrated at the future anterior end

- This hypothesis is an example of the morphogen gradient hypothesis, in which gradients of substances called **morphogens** establish an embryo's axes and other features
- The *bicoid* research is important for three reasons
  - It identified a specific protein required for some early steps in pattern formation
  - It increased understanding of the mother's role in embryo development
  - It demonstrated a key developmental principle that a gradient of molecules can determine polarity and position in the embryo

Concept 18.5: Cancer results from genetic changes that affect cell cycle control

- The gene regulation systems that go wrong during cancer are the very same systems involved in embryonic development

Types of Genes Associated with Cancer

- Cancer can be caused by mutations to genes that regulate cell growth and division
- Tumor viruses can cause cancer in animals including humans

- **Oncogenes** are cancer-causing genes
- **Proto-oncogenes** are the corresponding normal cellular genes that are responsible for normal cell growth and division
- Conversion of a proto-oncogene to an oncogene can lead to abnormal stimulation of the cell cycle
- Proto-oncogenes can be converted to oncogenes by movement of DNA within the genome: if it ends up near an active promoter, transcription may increase
  - Amplification of a proto-oncogene: increases the number of copies of the gene
  - Point mutations in the proto-oncogene or its control elements: cause an increase in gene expression

Tumor-Suppressor Genes

- **Tumor-suppressor genes** help prevent uncontrolled cell growth
- Mutations that decrease protein products of tumor-suppressor genes may contribute to cancer onset
- Tumor-suppressor proteins
  - Repair damaged DNA
  - Control cell adhesion
  - Inhibit the cell cycle in the cell-signaling pathway

Interference with Normal Cell-Signaling Pathways

- Mutations in the *ras* proto-oncogene and *p53* tumor-suppressor gene are common in human cancers
- Mutations in the ***ras* gene** can lead to production of a hyperactive Ras protein and increased cell division
- Suppression of the cell cycle can be important in the case of damage to a cell's DNA; *p53* prevents a cell from passing on mutations due to DNA damage
- Mutations in the ***p53* gene** prevent suppression of the cell cycle

The Multistep Model of Cancer Development

- Multiple mutations are generally needed for full-fledged cancer; thus the incidence

increases with age
- At the DNA level, a cancerous cell is usually characterized by at least one active oncogene and the mutation of several tumor-suppressor genes

Inherited Predisposition and Other Factors Contributing to Cancer

- Individuals can inherit oncogenes or mutant alleles of tumor-suppressor genes
- Inherited mutations in the tumor-suppressor gene *adenomatous polyposis coli* are common in individuals with colorectal cancer
- Mutations in the *BRCA1* or *BRCA2* gene are found in at least half of inherited breast cancers, and tests using DNA sequencing can detect these mutations