CHAPTER 15 REGULATION OF GENE ACTIVITY AND GENE MUTATIONS

Chapter Outline

15.1 Prokaryotic Regulation

A. The Operon Model
   1. Bacteria do not require the same enzymes all the time; they produce just those enzymes needed at the moment.
   2. In 1961, French microbiologists Francois Jacob and Jacques Monod proposed the operon model to explain regulation of gene expression in prokaryotes; they received a Nobel prize for this.
      a. In the operon model, several genes code for an enzyme in the same metabolic pathway and are located in a sequence on a chromosome; expression of structural genes is controlled by the same regulatory genes.
      b. An operon is the structural and regulatory genes that function as a single unit; it includes the following:
         1) A **regulator gene** located outside the operon codes for a repressor protein molecule that controls whether the operon is active or not.
         2) A **promotor** is the sequence of DNA where RNA polymerase attaches when a gene is transcribed.
         3) An **operator** is a short sequence of DNA where an active repressor binds, preventing RNA polymerase from attaching to the promotor and transcription therefore does not occur.
         4) **Structural genes** are one to several genes coding for enzymes of a metabolic pathway that are transcribed as a unit.

B. The *trp* Operon
   1. Jacob and Monod found some operons in *E. coli* usually exist in the on rather than the off condition.
   2. This prokaryotic cell (*E. coli*) produces five enzymes as part of the metabolic pathway to synthesize the amino acid tryptophan.
   3. If tryptophan is already present in medium, these enzymes are not needed and the operon is turned off by the following method.
      a. In the *trp* operon, the regulator codes for a repressor that usually is unable to attach to the operator.
      b. The repressor has a binding site for tryptophan (if tryptophan is present, it binds to the repressor).
      c. This changes the shape of the repressor that now binds to the operator.
   4. The entire unit is called a **repressible operon**; tryptophan is the **corepressor**.
   5. Repressible operons are involved in anabolic pathways that synthesize substances needed by cells.

C. The *lac* Operon
   1. If *E. coli* is denied glucose and given lactose instead, it makes three enzymes to metabolize lactose.
   2. These three enzymes are encoded by three genes.
      a. One gene codes for β-galactosidase that breaks lactose to glucose and galactose.
      b. A second gene codes for a permease that facilitates entry of lactose into the cell.
      c. A third gene codes for enzyme transacetylase, which is an accessory in lactose metabolism.
   3. The three genes are adjacent on a chromosome and under control of one promoter and one operator.
   4. The regulator gene codes for a *lac* operon repressor protein that binds to the operator and prevents transcription of the three genes.
   5. When *E. coli* is switched to medium containing an allolactose, this lactose binds to the repressor and the repressor undergoes a change in shape that prevents it from binding to the operator.
   6. Because the repressor is unable to bind to the operator, the promoter is able to bind to RNA polymerase, which carries out transcription and produces the three enzymes.

7. An **inducer** is any substance (lactose in the case of the *lac* operon) that can bind to a particular repressor protein, preventing the repressor from binding to a particular operator, consequently permitting RNA polymerase to bind to the promoter and causing transcription of structural genes.

D. Further Control of the *lac* Operon
   1. Since *E. coli* prefers to break down glucose, how does *E. coli* know how to turn on when glucose is absent?
   2. When glucose is absent, cyclic AMP (cAMP) accumulates; cAMP has only one phosphate group and attaches to ribose at two locations.
      a. CAP is a catabolite activator protein (CAP) in the cytoplasm.
      b. When cAMP binds to CAP, the complex attaches to a CAP binding site next to the *lac* promoter.
      c. When CAP binds to DNA, DNA bends, exposing the promoter to RNA polymerase.
      d. Only then does RNA polymerase bind to the promoter; this allows expression of the lac operon structural genes.
   3. When glucose is present, there is little cAMP in the cell.
      a. CAP is inactive and the lactose operon does not function maximally.
      b. CAP affects other operons when glucose is absent.
      c. This encourages metabolism of lactose and provides a backup system for when glucose is absent.
   4. Negative Versus Positive Control
      a. Active repressors shut down the activity of an operon; they are negative control.
      b. CAP is example of positive control; when the molecule is active, it promotes the activity of the operon.
      c. Use of both positive and negative controls allows cell to fine-tune its control of metabolism.
      d. If both glucose and lactose are present, the cell preferentially metabolizes glucose.

15.2 Eukaryotic Regulation

A. Expression of Genes
   1. Different cells in the human body turn on different genes that code for different protein products.
   2. Eukaryotes have a four levels of regulatory mechanisms to control gene expression; two in the nucleus and two in the cytoplasm.
   3. There levels of control modify the amount of gene product.
      a. **Transcriptional control** in nucleus determines which structural genes are transcribed and rate of transcription; it includes organization of chromatin and transcription factors initiating transcription.
      b. **Posttranscriptional control** occurs in nucleus after DNA is transcribed and preliminary mRNA forms.
         1) This may involve differential processing of preliminary mRNA before it leaves the nucleus.
         2) The speed that mature mRNA leaves nucleus affects ultimate amount of gene product.
      c. **Translational control** occurs in cytoplasm after mRNA leaves nucleus but before there is a protein product.
         1) The life expectancy of mRNA molecules can vary, as well as their ability to bind ribosomes.
         2) Some mRNAs may need additional changes before they are translated at all.
      d. **Posttranslational control** takes place in the cytoplasm after protein synthesis.
         1) Polypeptide products may undergo additional changes before they are biologically functional.
         2) A functional enzyme is subject to feedback control; binding of an end product can change the shape of an enzyme so it no longer carries out its reaction.

D. Transcriptional Control
   1. Organization of Chromatin
      a. During interphase, some chromatin is highly compact, darkly stained, and genetically inactive **heterochromatin**.
      b. The rest is diffuse lightly colored **euchromatin** thought to be genetically active.
      c. Barr Bodies
         1) Since human males have only one X chromosome, it might be supposed that they produce half the gene product of a female with two X chromosomes.
         2) However, females have a darkly staining Barr body that is condensed at the side of the nucleus that is the inactive chromatin of the second X chromosome.
         3) Which X chromosome is condensed is determined by chance.

4) Body of heterozygous females is mosaic; half her cells express alleles on one X chromosome and half of her cells express the alleles on the other X chromosome.
5) Female gonads do not show Barr bodies; X chromosomes are both needed in development.
6) Only one active X chromosome in female zygote means that a lower gene product is normal.
7) Other examples of this mosaic effect include: ocular albinism, Duchenne muscular dystrophy, and female calico cat coat color.

d. **Euchromatin** activity is related to the extent nucleosomes are coiled and condensed.
1) A nucleosome is a bead-like unit made of a segment of DNA wound around complex of histone proteins.
2) Nucleosomes contain five primary histones: H1, H2A, H2B, H3 and H4.
3) When DNA is transcribed, activators called remodeling proteins are able to push aside the histone portion so transcription can begin.

e. Lampbrush chromosomes in egg cells of vertebrates present many loops for mRNA synthesis.
f. Polytene chromosome puffs in larval insects are made of many duplicated sister chromatids.
g. Use of radioactive uridine label for RNA shows DNA is being actively transcribed at puffs.
h. Gene amplification is replication of a gene so there are many copies; *Xenopus* frog germ cells increase nucleoli and therefore copies of rRNA genes by 1,000-fold.

2. Transcription Factors
a. Transcription is controlled by DNA-binding proteins called transcription factors; operons have not been found in eukaryotic cells.
b. Each cell contains different transcription factors; different combinations regulate activity of gene.
c. A group of transcription factors binds to a promoter adjacent to a gene; then the complex attracts and binds RNA polymerase but transcription may still not begin.
d. As well as DNA sequences, enhancers are involved in controlling transcription in eukaryotes.
1) Enhancers are regions where factors that help regulate transcription of the gene can bind.
2) Enhancers can be quite a distance from the promoter.
3) A hairpin loop in the DNA brings the factor attached to an enhancer into contact with transcription factors and RNA polymerase at promotor; this enables transcription to begin.
e. Transcription factors are always present in cell and most likely they have to be activated in some way (e.g., regulatory pathways involving kinases or phosphatases) before they bind to DNA.

C. Posttranscriptional Control
1. Posttranscriptional control begins once there is an mRNA transcript.
2. Messenger RNA molecules are processed before they leave the nucleus and enter the cytoplasm.
3. Differential excision of introns and splicing of mRNA can vary the type of mRNA that leaves nucleus.
a. The hypothalamus and thyroid glands produce calcitonin but the mRNA that leaves the nucleus is not same in both types of cells.
b. Radioactive labeling shows they vary because of a difference in mRNA splicing.
c. Evidence of different patterns of mRNA splicing is found in cells that produce neurotransmitters, muscle regulatory proteins, and antibodies.
4. Speed of transport of mRNA from nucleus into cytoplasm affects the amount of gene product realized per unit of time.
5. There is difference in the length of time it takes various mRNA molecules to pass through nuclear pores.

D. Translational Control
1. Masking of mRNA
a. Frog eggs contain mRNA “masked messengers” that are not translated until fertilization occurs.
a. When fertilization occurs, they unmask and there is rapid gene product synthesis.
2. Life of mRNA
a. The longer an active mRNA molecule remains in the cytoplasm, the more product is produced.
b. Mature mammal red blood cells eject their nucleus but synthesize hemoglobin for several months; the mRNAs must persist during this time.
c. Ribonucleases are enzymes associated with ribosomes that degrade mRNA.
d. Mature mRNA has non-coding segments at 3' cap and 5' poly-A tail ends; differences in these segments influence how long the mRNA avoids being degraded.

3. Influence of Hormones
    a. Prolactin promotes milk production by affecting the length of time mRNA persists and is translated.
    b. Estrogen interferes with action of ribonuclease to prolong vitellin production in amphibian cells.

E. Posttranslational Control
    1. Degradation of the Protein Product
        a. Some proteins are not active after synthesis; the polypeptide product has to undergo additional changes before it is biologically functional.
        b. Bovine proinsulin is inactive when first produced; a single long polypeptide folds into a three-dimensional structure, a sequence of 30 amino acids is removed from the middle, and the two polypeptide chains are bonded together by disulfide bonds resulting in an active protein.
    2. Degradation of a Protein
        a. Many proteins are short-lived in cells and degraded or destroyed so they are no longer active.
        b. Giant protein complexes call **proteasomes** carry out this task. .
        c. One example is cyclins that control the cell cycle; they are only temporarily present..

15.3 Genetic Mutations

A. A genetic mutation is a permanent change in the sequence of bases in DNA; mutations range from no effect to total inactivity.

B. Effect of Mutations on Protein Activity
    1. **Point mutations** change a single nucleotide and therefore change a single specific codon.
        a. They range in effect depending on the particular codon change.
        b. Changes to codons that have same effect have no effect; UAU to UAC both code tyrosine.
        c. A change from UAC to UAG (a stop codon) results in a shorter protein, and a change from UAC to CAC incorporates histidine instead of tyrosine.
        d. Sickle cell disease results from a single base change in DNA where the α chain of hemoglobin contains valine instead of glutamate at one location and the resulting distorted hemoglobin causes blood cells to clog vessels and die sooner.
    2. Frameshift Mutations
        a. Reading frame depends on the sequence of codons from the starting point: THE CAT ATE THE RAT.
        b. If C is deleted, the reading frame is shifted: THE ATA TET HER AT.
        c. Frameshift mutations occur when one or more nucleotides are inserted or deleted from DNA.
        d. The result of a frameshift mutation is a new sequence of codons and nonfunctional proteins.
    3. Nonfunctional Proteins
        a. A single nonfunctioning protein can cause dramatic effects.
        b. PKU results when a person cannot convert phenylalanine and it builds up in the system.
        c. A faulty code for an enzyme in the same pathway results in an albino individual.
        d. The human transposon *Alu* is responsible for hemophilia when it places a premature stop codon in the gene for clotting factor IX.
        e. Cystic fibrosis is due to inheriting a faulty code for a chloride transport protein in plasma membrane.
        f. Androgen insensitivity is due to a faulty receptor for male sex hormones; body cells cannot respond to testosterone and develop as a female although all of the body cells are XY.

C. Carcinogenesis
    1. Researchers have identified many proto-oncogenes whose mutation to an oncogene cause increased growth and lead to a tumor.
    2. The *ras* family of genes are the most common oncogenes implicated in human cancers.
    3. Alteration of one nucleotide pair converts a normal functioning *ras* proto-oncogene to an oncogene.
    4. A major tumor-suppressor gene *p53* is more frequently mutated in human cancers than any other known gene.
        a. The *p53* protein acts as a transcription factor to turn on the expression of genes whose products are cell cycle inhibitors.
        b. The *p53* can also stimulate apoptosis, programmed cell death.

D. Cause of Mutations
    1. Some mutations are spontaneous, others are due to environmental mutagens.
    2. Mutations due to replication errors are very rare

3. DNA polymerase constantly monitors, proofreads a new strand against the old, and repairs any irregularities, reducing mistakes to one out of every one billion nucleotide pairs replicated.
4. **Environmental mutagens** are environmental substances that increase the chances of mutation.
    a. Common mutagens are radiation and organic chemicals.
    b. Cancer is a genetic disorder caused by a failure in the regulation of gene activity.
    c. Carcinogens are mutagens that increase the chances of cancer.
    d. X rays and gamma rays are ionizing radiation that creates free radicals, ionized atoms with unpaired electrons.
    e. Ultraviolet (UV) radiation is easily absorbed by pyrimidines in DNA.
        1) Where two thymine molecules are near each other, UV may bond them together as thymine dimers.
        2) Usually dimers are removed from damaged DNA by special enzymes called repair enzymes.
    f. Lack of repair enzymes produces xeroderma pigmentosum with a higher incidence of skin cancer.
    g. Some organic chemicals act directly on DNA.
        1) 5-bromouracil pairs with thymine but rearranges to a form that pairs with cytosine at the next DNA replication: an A—T pair becomes a G—C pair.
        2) Chemicals may add hydrocarbon groups or remove amino groups from DNA bases.
        3) Tobacco smoke contains a number of chemical carcinogens.
5. Transposons
    a. Transposons are specific DNA sequences that can move within and between chromosomes.
    b. Such “jumping genes” were first detected in corn and are now recognized in bacteria, fruit flies, and other organisms.
    c. Charcot-Marie-Tooth disease is a rare human disorder where muscles and nerves of legs and feet wither away; caused by a transposon also found in fruit flies.