CHAPTER 14 GENE ACTIVITY: HOW GENES WORK

Chapter Outline

14.1 The Function of Genes

A. Investigators Recognize Gene Activity
   1. English physician Sir Archibald Garrod introduced phrase *inborn error of metabolism.*
      a. Garrod proposed that inherited defects could be caused by the lack of a particular enzyme.
      b. Knowing that enzymes are proteins, Garrod suggested a link between genes and proteins in the early 1900s.

B. Genes Specify Enzymes
   1. In 1940, George Beadle and Edward Tatum X-rayed spores of red bread mold, *Neurospora crassa.*
   2. They discovered some resulting cultures lacked a particular enzyme for growth on medium.
   3. They found that a single gene was mutated, which resulted in the lack of a single enzyme.
   4. They proposed the **one gene–one enzyme hypothesis**: one gene specifies the synthesis of one enzyme.

C. Genes Specify a Polypeptide
   1. Linus Pauling and Harvey Itano compared hemoglobin in red blood cells of persons with sickle-cell disease and normal individuals.
   2. They discovered that the chemical properties of chain of sickle-cell hemoglobin differed from normal hemoglobin by using electrophoresis to separate molecules by weight and charge.
   3. Vernon Ingram showed the biochemical change to chain of sickle-cell hemoglobin is due to the substitution of a nonpolar amino acid valine for the negatively charged amino acid glutamate.
   4. Pauling and Itano formulated the **one gene–one polypeptide hypothesis:** each gene specifies one polypeptide of a protein, a molecule that may contain one or more different polypeptides.

D. From DNA to RNA to Protein
   1. Genetics treats a gene as any of the particles of inheritance on a chromosome.
   2. To a molecular geneticist, a gene is a sequence of DNA nucleotide bases that codes for a product.
   3. DNA is restricted to nucleus; protein synthesis occurs at ribosomes in the cytoplasm.
   4. Ribonucleic acid (RNA) is found in both regions and was likely intermediary in protein synthesis.

E. Types of RNA
   1. Like DNA, RNA is a polymer of nucleotides.
   2. Unlike DNA, RNA is single-stranded (not a double helix), contains the sugar ribose, and contains the base uracil instead of thymine.
   3. There are three major classes of RNA.
      a. Messenger RNA (mRNA) takes a message from DNA in nucleus to ribosomes in cytoplasm.
      b. Ribosomal RNA (rRNA) and proteins make up ribosomes where proteins are synthesized.
      c. Transfer RNA (tRNA) transfers a particular amino acid to a ribosome.

F. The Required Steps
   1. DNA undergoes transcription to mRNA, which is translated to a protein.
   2. DNA is a template for RNA formation during transcription.
   3. **Transcription** is the first step in gene expression; it is the process whereby a DNA strand serves as a template for the formation of mRNA.
   4. During **translation**, an mRNA transcript directs the sequence of amino acids in a polypeptide.

14.2 The Genetic Code

A. Sequence of Bases in DNA
   1. The **central dogma** of molecular biology states that the sequence of nucleotides in DNA specifies the order of amino acids in a polypeptide.
   2. The **genetic code** is a **triplet code** comprised of 64 three-base code words (codons).
   3. A codon consists of 3 nucleotide bases of DNA.
   4. Four nucleotides based on 3-unit codons allows up to 64 different amino acids to the specified.

B. Finding the Genetic Code
   1. In 1961, Marshall Nirenberg and J. Heinrich Matthei found that an enzyme that could be used to construct synthetic RNA in a cell-free system; they showed the codon UUU coded for phenylalanine.
   2. By translating just three nucleotides at a time, they assigned an amino acid to each of the RNA codons,

and discovered important properties of the genetic code.
3. The code is **degenerate**: there are 64 triplets to code for 20 naturally occurring amino acids and this robustness protects against potentially harmful mutations.
4. The genetic code is **unambiguous**; each triplet codon has only one meaning.
5. The code has **start and stop signals**: there is one start codon and three stop codons.

C. The Code Is Universal
1. The few exceptions to universality of the genetic code suggests the code dates back to the very first organisms and that all organisms are related.
2. Once the code was established, changes would be very disruptive.

14.3 The First Step: Transcription

A. Transcription
1. Transcription is the first step required for gene expression and takes place in the nucleus of eukaryotic cells.
2. mRNA formation usually leads to a polypeptide gene product; however, tRNA and rRNA are also transcribed from DNA templates and are products themselves.
3. Enzymes called **RNA polymerases** are involved in transcription.

B. Messenger RNA is Formed
1. Next, a segment of the DNA helix unwinds and unzips.
2. As RNA polymerase moves along the *template* strand of the DNA, complementary RNA nucleotides pair with DNA nucleotides of the strand.
3. Transcription begins when RNA polymerase attaches to a promoter on DNA.
4. **RNA polymerase** is an enzyme that speeds formation of RNA from a DNA template.
5. A **promoter** is region of DNA region defines the start of the gene, the direction of transcription, and the strand copied.
6. RNA polymerase joins the RNA nucleotides together in the 5' —> 3' direction.
7. Transcription begins when RNA polymerase attaches to a region of DNA called a **promoter**; a promoter defines the start of a gene, the direction of transcription, and the strand transcribed.
8. The RNA/DNA association is not as stable as DNA helix; therefore, only the newest portion of the RNA molecule associated with RNA polymerase is bound to DNA; the rest dangles off to side.
9. Elongation of mRNA continues until RNA polymerase comes to a DNA **terminator**.
10. The terminator causes RNA polymerase to stop transcribing DNA and to release mRNA transcript.
11. RNA polymerase molecules work to produce mRNA from same DNA molecule at same time.
12. Cells produce thousands of copies of same mRNA molecule and many copies of coded protein in a shorter period of time than if a single copy of DNA were used to direct protein synthesis.

C. Messenger RNA is Processed
1. In eukaryotes, newly formed primary mRNA transcript is processed before leaving the nucleus.
2. **Primary mRNA transcript** is the immediate product of transcription; it contains exons and introns.
3. The ends of the mRNA molecule are altered: a cap is put on the 5' end and a poly-A tail is put on the 3' end.
   a. The “cap” is a modified guanine (G) that tells a ribosome where to attach to begin translation.
   b. The “poly-A tail” consists of a 150–200 adenine (A) nucleotide chain that facilitates transport of mRNA out of the nucleus and inhibits degradation of mRNA by hydrolytic enzymes.
4. Portions of the primary mRNA transcript, called introns, are removed.
   a. An **exon** is a portion of DNA code in primary mRNA transcript eventually expressed as result of polypeptide synthesis.
   b. An **intron** is a non-coding segment of DNA removed by spliceosomes before the mRNA leaves nucleus.
5. **Spliceosomes** are a complex that contains several kinds of ribonucleoproteins.
   a. Spliceosomes cut the primary mRNA transcript and then rejoin adjacent exons.
   b. Spliceosomes may allow the same DNA to be divided differently and produce different products.

6. The role of introns is being investigated; introns may divide a gene into regions that can be joined in different combinations for different products: the thyroid and pituitary glands process same primary mRNA transcript that produces different products.
7. Investigators have found that the simpler the eukaryote, the less likely that introns will be present.
8. An intron has been discovered in the gene for a tRNA molecule in the cyanobacterium *Anabaena*; this particular intron is "self-splicing" (it has capability of splicing itself out of an RNA transcript).
9. **Ribozymes** are RNAs with an enzymatic function restricted to cleaving RNA at specific locations.
    a. RNA could have served as both genetic material and as first enzymes in early life forms.
    b. This hypothesis eliminates dilemma of which came first, DNA or protein; RNA came first.

14.4 The Second Step: Translation

A. Translation
1. Translation takes place in cytoplasm of eukaryotic cells.
2. Translation is the second step by which gene expression leads to protein synthesis.
3. One language (nucleic acids) is translated into another language (protein).

B. The Role of Transfer RNA
1. **Transfer RNA (tRNA)** molecules transfer amino acids to the ribosomes.
2. tRNA is a single-stranded ribonucleic acid that doubles back on itself to create regions where complementary bases are hydrogen-bonded to one another.
3. At the 3' end it binds to amino acid; at other end it has an **anticodon** that binds to mRNA codon; an **anticodon** is group of nucleotides on tRNA that is complementary to the codon on mRNA.
4. There is at least one tRNA molecule for each of the 20 amino acids found in proteins.
5. There are fewer tRNAs than codons because some tRNAs pair with more than one codon; if an anticodon contains a U in the third position, it will pair with either an A or G–this is called the wobble effect.
6. The **tRNA synthetases** are amino acid-activating enzymes that recognize which amino acid should join which tRNA molecule, and then catalyze ATP-requiring reactions joining them.
7. Amino acid–tRNA complex forms, then travels in the cytoplasm to a ribosome for protein synthesis.

C. The Role of Ribosomal RNA
1. Ribosomal RNA (rRNA) is produced from a DNA template in the nucleolus of nucleus.
2. The rRNA is packaged with a variety of proteins into ribosomal subunits, one larger than the other.
3. Subunits move separately through nuclear envelope pores into the cytoplasm where they combine when translation begins.
4. Ribosomes can float free in cytosol or attach to endoplasmic reticulum.
5. Prokaryotic cells contain about 10,000 ribosomes; eukaryotic cells contain many times more.
6. Ribosomes have a binding site for mRNA and binding sites for two transfer RNA (tRNA) molecules.
7. They facilitate complementary base pairing between tRNA anti-codons and mRNA codons; one protein is an enzyme that joins amino acids together by means of a peptide bond.
8. A ribosome moves down the mRNA molecule, new tRNAs arrive, the amino acids join, and a polypeptide forms.
9. Translation terminates once the polypeptide is formed; the ribosome then dissociates into its two subunits.
10. **Polyribosomes** are clusters of several ribosomes synthesizing the same protein.
11. To get from a polypeptide to a function protein requires correct bending and twisting; chaperone molecules make sure that the final protein develops the correct shape.
12. Some proteins contain more than one polypeptide; they must be joined to achieve the final three-dimensional shape.

D. Translation Requires Three Steps
1. During translation, mRNA codons base-pair with tRNA anti-codons carrying specific amino acids.
2. Codon order determines the order of tRNA molecules and the sequence of amino acids in polypeptides.
3. Protein synthesis involves chain initiation, chain elongation, and chain termination.
4. Enzymes are required for all three steps; energy is needed for the first two steps.
5. Chain Initiation
    a. A small ribosomal subunit attaches to mRNA in the vicinity of the **start codon**: a base triplet (AUG).
    b. First or initiator tRNA pairs with this codon; then the large ribosomal subunit joins to the small subunit.

c. Each ribosome contains three binding sites: the **P** (for peptide) **site**, the **A** (for amino acid) **site**, and the **E** (for exit) **site**.
d. The initiator tRNA binds to the P site although it carries one amino acid, methionine.
e. The A site is for next tRNA carrying the next amino acid.
f. The E site is for discharged tRNAs.
g. Initiation factor proteins are required to bring the necessary translation components (the small ribosomal subunit, mRNA, initiator tRNA, and large ribosomal subunit) together.

6. Chain Elongation
   a. tRNA with attached polypeptide is at the P site and a tRNA—amino acid complex is just arriving at the A site.
   b. Proteins called **elongation factors** facilitate complementary base pairing between the tRNA anticodon and the mRNA codon.
   c. The polypeptide is transferred and attached by a peptide bond to the newly arrived amino acid.
   d. This reaction is catalyzed by a ribozyme, which is part of the larger subunit.
   e. The tRNA molecule in the P site leaves.
   f. **Translocation** occurs with mRNA, along with peptide-bearing tRNA, moves to the P site and spent tRNA moves from the P site to the E site.
   g. As the ribosome moves forward three nucleotides, there is a new codon now located at the empty A site.
   h. The complete cycle is rapidly repeated, about 15 times per second in *Escherichia coli*.
7. Chain Termination
   a. Termination of polypeptide synthesis occurs at a stop codon that does not code for amino acid.
   b. The polypeptide is enzymatically cleaved from the last tRNA by a release factor.
   c. The tRNA and polypeptide leave the ribosome, which dissociates into its two subunits.
8. Definition of a Gene and a Genetic Mutation
   a. Originally a gene was defined as a locus on the chromosome; this allowed us to solve genetic problems.
   b. The one gene—one polypeptide concept connected inborn errors of metabolism with a sequence of DNA bases.
   c. A gene could also be defined as a sequence of DNA bases coding for a single polypeptide or a single RNA.
   d. These concepts can allow us to define a mutation as a permanent change in the sequence of DNA bases.

E. Protein Synthesis and the Eukaryotic Cell
   1. If a polypeptide is to enter the rough ER, the signal peptide is recognized by signal-recognition particles (SRP) which bring it to a receptor protein in the ER membrane.
   2. After the polypeptide enters the lumen of the ER, it is folded and further processed by addition of sugars, phosphates, or lipids.
   3. Transport vesicles carry the proteins between organelles and to the plasma membrane.