Chapter 22
Lecture Outline

See separate PowerPoint slides for all figures and tables pre-inserted into PowerPoint without notes.
DNA Biology and Technology
Points to ponder

• What are three functions of DNA?
• Review DNA and RNA structure.
• What are the three major types of RNA and their functions?
• Compare and contrast the structure and function of DNA and RNA.
• How is DNA replicated?
• Describe transcription and translation in detail.
• Describe the genetic code.
• Review protein structure and function.
• What are the four levels of regulating gene expression?
Points to ponder

• What did we learn from the Human Genome Project, and where do we go from here?
• What is *ex vivo* and *in vivo* gene therapy?
• Define biotechnology, transgenic organisms, genetic engineering, and recombinant DNA.
• What are some uses of transgenic bacteria, plants, and animals?
What does DNA do?

1. It replicates to be passed on to the next generation.
2. DNA stores information.
3. It undergoes mutations to provide genetic diversity.
DNA structure: A review

- It is a double-stranded helix.
- DNA is composed of repeating nucleotides (made of a pentose sugar, phosphate, and a nitrogenous base).
- Sugar and phosphate make up the backbone, while the bases make up the “rungs” of the ladder.
- Bases have complementary pairing: cytosine (C) pairs with guanine (G), and adenine (A) pairs with thymine (T).
Figure 22.1 The structure of DNA.
How is DNA replicated?

• The two strands unwind as the H bonds are broken.
• Complementary nucleotides are added to each strand by DNA polymerase.
• Each new double-stranded helix is made of one new strand and one old strand (semiconservative replication).
• The sequence of bases makes each individual unique.
DNA replication

Figure 22.2  Semiconservative replication.
RNA structure and function

- It is single-stranded.
- RNA is composed of repeating nucleotides.
- Sugar-phosphate is the backbone.
- Bases are A, C, G, and uracil (U).
Three types of RNA

- **Messenger RNA** (mRNA) carries genetic information from DNA to the ribosomes.
- **Ribosomal RNA** (rRNA) joins with proteins to form ribosomes.
- **Transfer RNA** (tRNA) transfers amino acids to a ribosome where they are added to a forming protein.
RNA structure

Figure 22.4 The structure of RNA.

Base is uracil instead of thymine
Types of RNA

Figure 22.9 The roles of all three forms of RNA in translation.

a. An mRNA is threaded between ribosomal subunits and a polypeptide extends to the side.

b. A ribosome has two binding sites where codons bind to anticodons. A tRNA bearing a polypeptide is at the P site. A new tRNA amino acid is approaching the A site.

c. A tRNA amino acid is coming to the ribosome. Upon arrival, its anticodon, CUG, will bind to its codon, GAC.
Comparing DNA and RNA

• Similarities
  – They are nucleic acids.
  – They are made of nucleotides.
  – The have sugar-phosphate backbones.
  – They are found in the nucleus.

• Differences
  – DNA is double-stranded while RNA is single-stranded.
  – DNA has T while RNA has U.
  – RNA is also found in the cytoplasm as well as the nucleus while DNA is not.
Proteins: A review

- Proteins are composed of subunits called amino acids.

- The sequence of amino acids determines the shape of the protein.

- They are synthesized at the ribosomes.

- Proteins are important for diverse functions in the body including hormones, enzymes, and transport.

- They can denature, causing a loss of function.
22.2 Gene Expression

2 steps of gene expression

1. Transcription – DNA is read to make a mRNA in the nucleus

2. Translation – mRNA is read to make a protein in the cytoplasm

Figure 22.5 Summary of gene expression.
The genetic code

- It is made of four kinds of bases.
- Bases act as a code for amino acids used in translation.
- Every three bases of the mRNA is called a codon; a typical codon specifies a particular amino acid in translation.

**Figure 22.6** The genetic code.
1. Transcription

- mRNA is made from a DNA template.
- mRNA is processed before leaving the nucleus.
- mRNA moves to the ribosomes to be read.

Figure 22.7 Transcription of DNA into mRNA.
Modifications of mRNA

- One end of the RNA is capped.
- Introns are removed.
- A poly-A tail is added.
22.2 Gene Expression

Processing of mRNA after transcription

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Figure 22.8 mRNA processing.
2. Translation

3 steps

1. **Initiation**: mRNA binds to the small ribosomal subunit and causes the two ribosomal units to associate

2. **Elongation**: polypeptide lengthens
   - tRNA picks up an amino acid.
   - tRNA has an anticodon that is complementary to the codon on the mRNA.
   - tRNA anticodon binds to the codon and drops off an amino acid to the growing polypeptide.

3. **Termination**: a stop codon on the mRNA causes the ribosome to fall off the mRNA
22.2 Gene Expression

**Visualizing the 3 steps of translation**

![Diagram of translation steps](image)

**Figure 22.10** Formation of the polypeptide during translation.

- **a. Initiation**: A small ribosomal subunit binds to mRNA; an initiator tRNA pairs with the mRNA start codon AUG.

- **b. Elongation**: A tRNA–amino acid approaches the ribosome and binds at the A site. Two tRNAs can be at a ribosome at one time; the anticodons are paired to the codons. Peptide bond formation attaches the peptide chain to the newly arrived amino acid. The ribosome moves forward; the "empty" tRNA exits from the E site; the next amino acid–tRNA complex is approaching the ribosome.

- **c. Termination**: The large ribosomal subunit completes the ribosome. Initiator tRNA occupies the P site. The A site is ready for the next tRNA. The ribosome comes to a stop codon on the mRNA. A release factor binds to the site. The release factor hydrolyzes the bond between the last tRNA at the P site and the polypeptide, releasing them. The ribosomal subunits dissociate.
22.2 Gene Expression

Overview of transcription and translation

**TRANSCRIPTION**

1. DNA in nucleus serves as a template for mRNA.
2. mRNA is processed before leaving the nucleus.

**Translation**

3. mRNA moves into cytoplasm and becomes associated with ribosomes.

4. tRNAs with anticodons carry amino acids to mRNA.
5. Anticodon–codon complementary base pairing occurs.
6. Polypeptide synthesis takes place one amino acid at a time.

**Figure 22.12** Summary of transcription and translation.
Regulation of gene expression

5 levels

1. **Pretranscriptional control** (nucleus)
   - e.g., chromatin density and DNA accessibility

2. **Transcriptional control** (nucleus)
   - e.g., transcription factors

3. **Posttranscriptional control** (nucleus)
   - e.g., mRNA processing

4. **Translational control** (cytoplasm)
   - e.g., differential ability of mRNA to bind ribosomes

5. **Posttranslational control** (cytoplasm)
   - e.g., changes to the protein to make it functional
Regulation of gene expression

Figure 22.13 Control of gene expression in eukaryotic cells.
DNA Technology

1. Gene cloning through recombinant DNA
2. Polymerase chain reaction (PCR)
3. DNA fingerprinting
4. Biotechnology products from bacteria, plants, and animals
DNA Technology terms

- **Genetic engineering** – altering DNA in bacteria, viruses, plants, and animal cells through recombinant DNA technology

- **Recombinant DNA** – contains DNA from two or more different sources

- **Transgenic organisms** – organisms that have a foreign gene inserted into them

- **Biotechnology** – using natural biological systems to create a product or to achieve an end desired by humans
DNA Sequencing

• The order of nucleotides in a DNA sequence is determined

• 1970s: performed manually using dye-terminator substances

• Now performed using dyes attached to nucleotides, with a laser and computerized machine to determine sequence
Automated DNA sequencer and an electropherogram

Figure 22.14  Automated DNA sequencer and an electropherogram.
Polymerase chain reaction (PCR)

- Polymerase chain reaction is used to clone small pieces of DNA.
- It is important for amplifying DNA for analysis such as in DNA fingerprinting.
Polymerase chain reaction (PCR)

1. Sample is first heated to denature DNA.
2. DNA is cooled to a lower temperature to allow annealing of primers.
3. DNA is heated to 72°C, the optimal temperature for Taq DNA polymerase to extend primers.

**Figure 22.15** The polymerase chain reaction.
DNA fingerprinting

- Fragments are separated by their charge/size ratios.
- It results in a distinctive pattern for each individual.
- It is often used for paternity testing, or to identify an individual at a crime scene or unknown body remains.

Figure 22.16 PCR and electrophoresis used for DNA fingerprinting.
Gene cloning

- **Recombinant DNA** – contains DNA from two or more different sources that allows genes to be cloned.

- Bacteria used to clone the human insulin gene
  - **Restriction enzyme** is used to cut the vector (plasmid) and the human DNA with the insulin gene.
  - **DNA ligase** seals together the insulin gene and the plasmid.
  - Bacterial cells take up plasmid, the gene is copied, and the product can be made.
Visualizing gene cloning

**Figure 22.17** Cloning of a human gene.

1. **Restriction enzyme cleaves DNA.**
2. **DNA ligase seals the insulin gene into the plasmid.**
3. **Host cell takes up recombinant plasmid.**
4a. **Gene cloning occurs.**
4b. **Bacteria produce a product.**

**Bacteria produce a product.**

<table>
<thead>
<tr>
<th>human DNA</th>
<th>plasmid (vector)</th>
<th>bacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>human cell</td>
<td>insulin gene</td>
<td>DNA ligase seals the insulin gene into the plasmid.</td>
</tr>
</tbody>
</table>

**Restriction enzyme**

**DNA ligase**

**plasmid**

**bacterium**

**human cell**

**insulin gene**

**recombinant DNA**

**Host cell takes up recombinant plasmid.**
Biotechnology products: Transgenic organisms

• Important uses
  – Production of:
    • Insulin
    • Human growth hormone (HGH)
    • Clotting factor VIII
    • Tissue plasminogen activator (t-PA)
    • Hepatitis B vaccine
  – Naturally-occurring oil-degrading bacteria can be made more effective through genetic engineering
Biotechnology products: Transgenic organisms

Figure 22.18 Transgenic organisms.
Biotechnology products: Transgenic plants

- Important uses
  - Produce human proteins in their seeds such as hormones, clotting factors, and antibodies
  - Plants resistant to herbicides
  - Plants resistant to insects
  - Plants resistant to frost
Genetically engineered plants

- Corn, soybean, and cotton plants are commonly genetically altered.

- In 2011, 94% of the soybeans and 80% of the corn planted in the United States had been genetically engineered.

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Figure 22.19 Genetically engineered plants.

a. Herbicide-resistant soybean plants
b. Nonresistant potato plant
(c) Pest-resistant potato plant (all): Courtesy Monsanto
Biotechnology products: Transgenic plants

<table>
<thead>
<tr>
<th>Transgenic Crops of the Future</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Improved Agricultural Traits</strong></td>
</tr>
<tr>
<td>Disease-protected</td>
</tr>
<tr>
<td>Herbicide-resistant</td>
</tr>
<tr>
<td>Salt-tolerant</td>
</tr>
<tr>
<td>Drought-tolerant</td>
</tr>
<tr>
<td>Cold-tolerant</td>
</tr>
<tr>
<td>Improved yield</td>
</tr>
<tr>
<td>Modified wood pulp</td>
</tr>
<tr>
<td><strong>Improved Food Quality Traits</strong></td>
</tr>
<tr>
<td>Fatty acid/oil content</td>
</tr>
<tr>
<td>Protein/starch content</td>
</tr>
<tr>
<td>Amino acid content</td>
</tr>
</tbody>
</table>

Figure 22.20 Genetically engineered plants for desirable traits.
Biotechnology products: Transgenic animals

• Gene is inserted into the egg that when fertilized will develop into a transgenic animal

• Current uses
  – Gene pharming: production of pharmaceuticals in the milk of farm animals
  – Larger animals: includes fish, cows, pigs, rabbits, and sheep
  – Mouse models: the use of mice for various gene studies
  – **Xenotransplantation**: pigs can express human proteins on their organs making it easier to transplant them into humans
Production of a transgenic animal

Figure 22.21 Production of a transgenic animal.
What did we learn from the Human Genome Project (HGP)?

- The human genome consists of about 3.4 billion bases and 23,000 genes.
- The human genome was sequenced in 2003.
- There are many polymorphisms, or small regions of DNA that vary among individuals identified.
- Genome size is not correlated with the number of genes or complexity of the organisms.
What is the next step in the HGP?

- **Functional genomics**
  - It helps us understand how the 23,000 genes function.
  - Genes make up less than 2% of the entire human genome.

- **Comparative genomics**
  - It helps understand how species have evolved.
  - Comparing genomes may help identify base sequences that cause human illness.
  - It helps in our understanding of gene regulation.
Figure 22.22  Functional and comparative genomics between chimpanzees and humans.
New endeavors

- **Proteomics** – the study of the structure, function, and interactions of cell proteins
  - This can be difficult to study because
    - protein concentrations differ greatly between cells.
    - protein location and concentration interactions differ from minute to minute.
    - understanding proteins may lead to the discovery of better drugs.

- **Bioinformatics** – the application of computer technologies to study the genome
How can we modify a person’s genome?

- **Gene therapy** – insertion of genetic material into human cells to treat a disorder
  - In *ex vivo therapy*, cells are removed from the body for treatment, and then reintroduced back into the body.
  - In *in vivo therapy*, the vector is introduced directly into the body.

- Gene therapy has been most successful in treating cancer.
1. Remove bone marrow stem cells.

2. Use retroviruses to bring the normal gene into the bone marrow stem cells.

3. Viral recombinant DNA carries normal gene into genome.

4. Return genetically engineered cells to patient.

Gene therapy

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Figure 22.23 Gene therapy.