Biology 13A Lab #2: Focus on DNA

Lab #2 Table of Contents:

Expected Learning Outcomes

At the end of this lab, you will be able to

- describe the structure of DNA;
- extract DNA from plant and animal tissues;
- explain the differences between DNA and RNA;
- explain how DNA codes for proteins; and
- analyze how comparison of DNA sequences may be used to determine relationships among organisms.

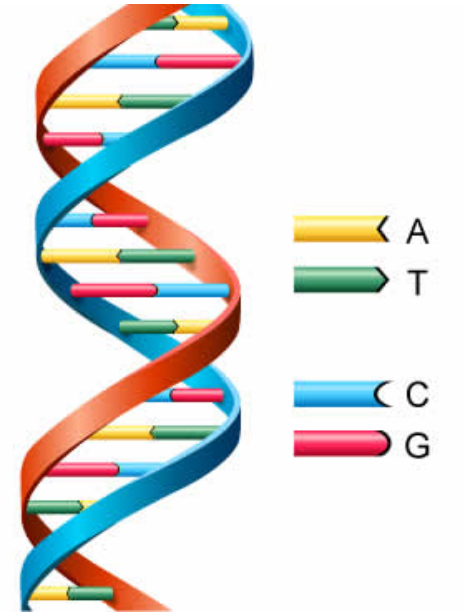


Figure 2.1 DNA Double Helix

Introduction

The genetic, hereditary material, **DNA**, is the language of life. DNA determines whether an individual will be a dog or a human, and it underlies physical and behavioral characteristics. It is found in the nucleus of each cell, coiled in long package-like structures called chromosomes. Uncoiled there would be about 6 feet of DNA in each cell in your body. However, it is a very thin thread, only a few nanometers wide, and is impossible for us to see directly.

You have probably heard of the scientists who first described DNA in **1953** – James Watson and Francis Crick, with the help of X-ray diffraction images taken by Rosalind Franklin. The discovery of the structure of DNA revolutionized the study of biology. During the 1950's and 60's scientists discovered that chromosomes are composed of **genes**, sections of DNA that code for a particular **protein**. Further, researchers figured out how the message contained in DNA is used by cells to build proteins. Proteins are necessary for life because they control every cellular process and they provide building blocks for our tissues and organs—you are literally built on a scaffolding of proteins such as collagen.

In the 55 years since the description of the double helix, there have been astounding discoveries concerning the functions of DNA. In the 21st century, the **human genome** (the library of human genes) has been mapped. Human genome research has revealed that there are 30,000 to 40,000 genes. These are inheritable instructions for how to build a human being. In addition, the genomes of many other species have also been mapped, providing powerful tools for comparative studies. The information is being used to better understand health and disease, including the relative roles of heredity and environment in the development of complex diseases such as diabetes and cancer. In the future, the study of DNA and proteins may provide the ability to tailor drugs specifically so that individuals can prevent and fight disease more successfully.

Check Your Understanding: Answer the following questions based on your reading of the introduction.

1. Why do we say that DNA is the "language of life?"
a. Because DNA is found in the nucleus of most cells. b. Genes are sections of DNA that code for proteins. c. DNA codes for proteins, which control all cellular processes and underlie all biological structures.

2. Which scientists described the structure of DNA? What was the year?

3. Which is false?
a. The human genome has largely been mapped. b. There are 80,000 to 90,000 genes in the human genome. c. Description of genes and the proteins they code for provides a tool for tailoring drugs to specific individuals and diseases. d. Mapping of genomes of humans and other organisms allows scientists to compare living creatures at the molecular level.

Activity 1: DNA and RNA Structure

DNA
On the DNA figure below, choose a dark color for the title.
In 1953, James Watson and Francis Crick established that the shape of DNA is a **double helix** which is like a twisted ladder. The sides of the ladder are made of alternating sugar and **phosphate** molecules. The sugar is **deoxyribose**.

Color all the phosphates a single color (one is labeled with a "p"). Color all the deoxyriboses another color (one is labeled with a "D").

The rungs of the ladder are pairs of 4 types of **nitrogenous bases**. The bases are known by their coded letters **A, G, T, C**. These bases always bond in a certain way. Adenine (A) will only bond to thymine (T). Guanine (G) will only bond with cytosine (C). This is known as the "Base-Pair Rule". The bases can occur in any order along a strand of DNA. The order of the bases is the **genetic code** that contains instructions for building proteins. For example, ATGCACATA would code for a different protein than AATTACGGA. A strand of DNA contains millions of bases. (For simplicity, the image only contains a few.)

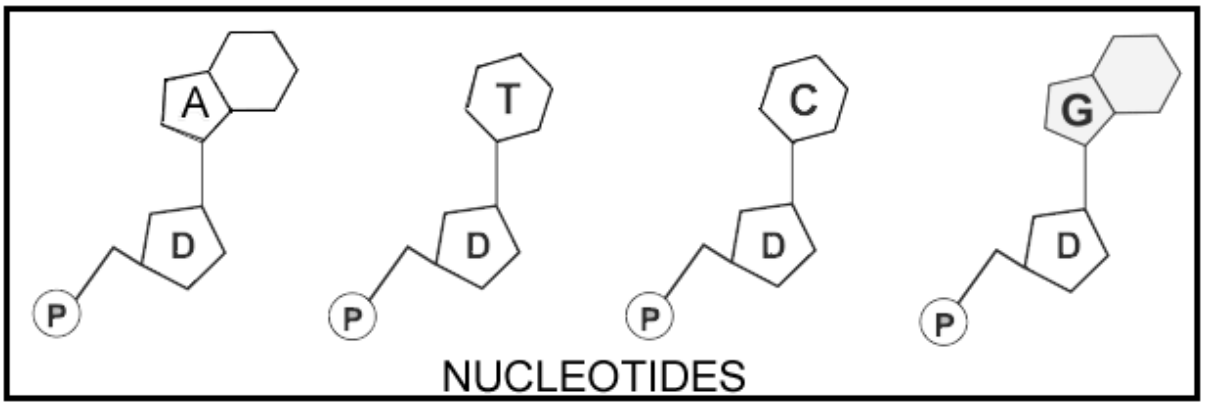
Choose 4 different colors and color the thymines a single color, the adenines another color, and so on for the cytosines and guanines. Note that that the bases attach to the sides of the ladder at the sugars and not the phosphate.

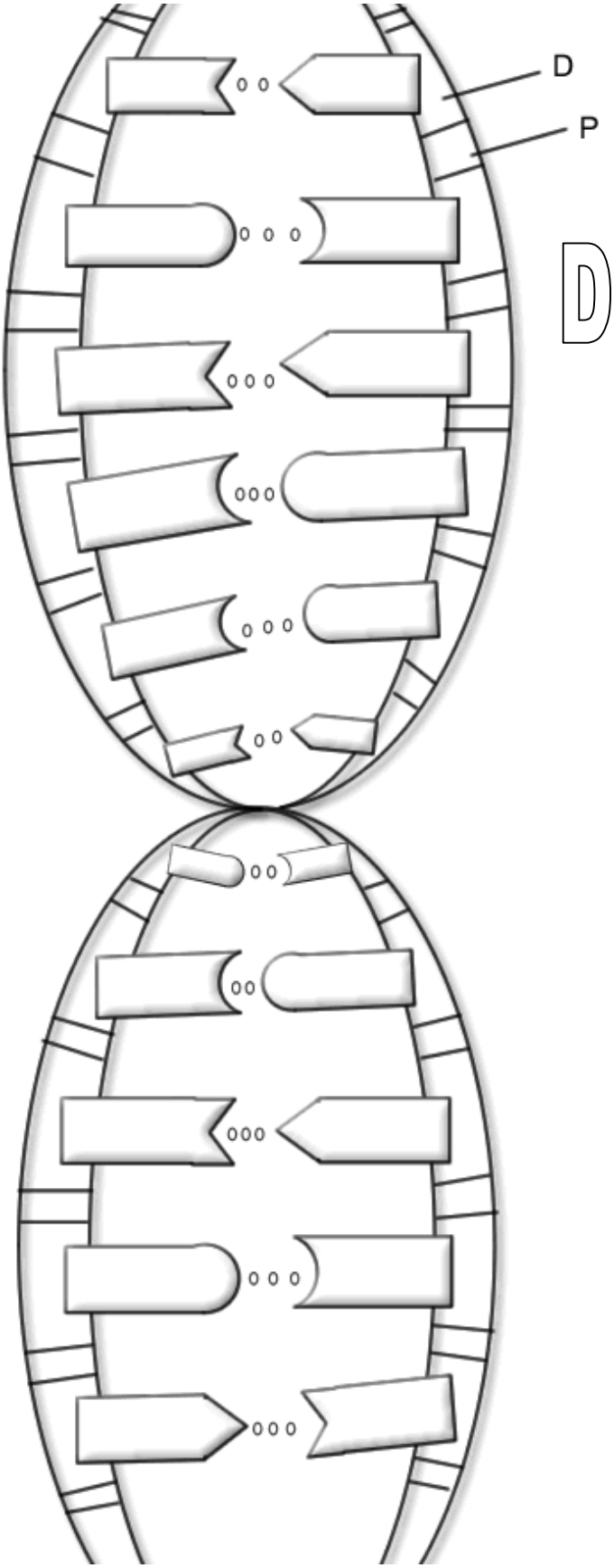
The two sides of the DNA ladder are held together loosely by **hydrogen bonds**. The DNA can actually "unzip" when it needs to **replicate**, make a copy of itself. DNA needs to copy itself when a cell divides so that the new cells each contain a copy of the DNA. Without these instructions, the new cells wouldn't have the correct information.

The hydrogen bonds are represented by small circles. Color the hydrogen bonds.

The DNA helix is actually made of repeating units called **nucleotides**. Each nucleotide consists of three molecules: a sugar (**deoxyribose**), a **phosphate**, which links the sugars together, and then one of the four bases. Two of the bases are **purines** - adenine and guanine. The **pyrimidines** are thymine and cytosine. Note that the pyrimidines are single ringed and the purines are double ringed.

Color the nucleotides using the same colors that you used in the double helix.





DNA Double Helix

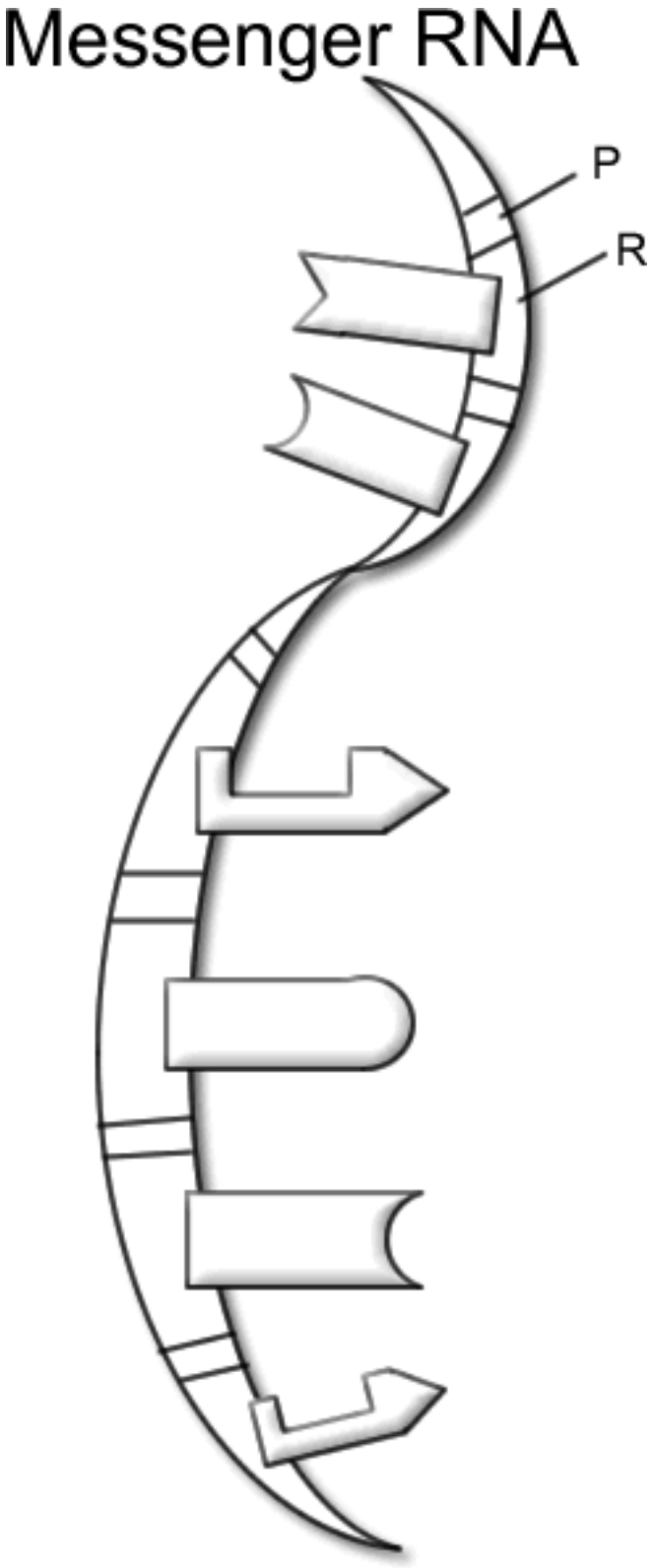
RNA

We know the nucleus controls the cell's activities through the DNA, but how? The sequence of bases on the DNA determines which protein is to be made and the proteins determine which cellular activity will be performed. The DNA is contained in the nucleus which is why it is the "control center" of the cell. The DNA is too big to go through the nuclear pores and, anyway, the message in the gene has to be used over and over to make the same protein, just as one uses a familiar recipe in a cookbook to make a favorite cookie over and over. So a chemical is used to read and copy the DNA in the nucleus. That chemical is **messenger RNA**. The messenger RNA (**mRNA**) is small enough to go through the nuclear pores. It takes the "message" of the DNA to the **ribosomes**. The information in the DNA is like a recipe that calls for certain ingredients—the ingredients are **amino acids**, the building blocks for proteins. The job of the messenger RNA is to carry the message from the DNA to the cytoplasm, so that the required amino acids can be assembled in the correct order to build a functioning protein.

Messenger RNA is similar to DNA, except that it is a single strand and it has no thymine. Instead of thymine, mRNA contains the base **uracil**. In addition to that difference, mRNA has the sugar **ribose** instead of deoxyribose. RNA stands for **Ribonucleic Acid**.

Color the mRNA as you did the DNA, except choose different colors for the ribose and uracil.

Fill out the worksheet that follows. Please work in pairs.



* Information and images are modified from K. Miller and J. Levine <www.biologycorner.com/worksheets.php>.

1. Write out the full name for DNA.
_____

2. What is a gene? _____

3. Where in the cell are chromosomes located?
_____

4. DNA can be found in what two organelles?
_____

5. What three scientists established the structure of DNA?
_____

6. What is the shape of DNA? _____

7. What are the sides of the DNA ladder made of?
_____

8. What are the "rungs" of the DNA ladder made of?
_____

9. What sugar is found in DNA? _____ In RNA?
_____

10. How do the bases bond together? A bonds with _____ G bonds with _____

11. The two purines in DNA are
_____.

12. DNA is made of repeating units called
_____

13. Why is RNA necessary to act as a messenger? Why can't the code be taken directly from the DNA?

14. Where in the cell are proteins made?

15. How do some cells become brain cells and others become skin cells, when the DNA in ALL the cells is exactly the same. In other words, if the instructions are exactly the same, how does one cell become a brain cell and another a skin cell?

16. Why is DNA called the "Blueprint of Life"?

Activity 2: DNA Extraction

All living cells contain DNA. The DNA in a cell is about 100,000 times as long as the cell itself, yet only takes up only about 10% of the cell's volume. The DNA molecules are folded and packed into chromosomes in the nucleus of a cell. Before DNA can be studied, it must be extracted from cells. Today, we will extract DNA from plant and animal cells using onion and beef thymus tissues.

There are two to three basic steps in DNA extraction. The cell must be lysed (broken open) to release the nucleus. Plant cells have a cell wall in addition to the cell membrane. The cell wall is composed of cellulose, a polysaccharide composed of chains of glucose molecules. It forms a tough barrier that must be broken down to allow the contents of the cell to get out. The onions are mashed in a blender to break the cellulose molecules.

Both plant and animal cells have a plasma (cell) membrane composed primarily of lipids that must also be broken. The nucleus (if present) must also be opened to release the DNA. Detergents break down the lipid membranes that enclose the entire cell and the nucleus in a manner similar to washing a greasy pan. Once the DNA is released into solution, it must be precipitated in cold ethanol alcohol. Precipitation is the act of making molecules stick together. In the presence of ethanol, DNA is no longer soluble and falls out of the solution so that we can isolate and look at it.

The stringy, slimy material that we end up with is similar to the raw material that researchers use in studies of DNA.

Procedure for Onion DNA Extraction*

1. Cut an inch square out of the center of 3 medium onions. Chop and place in a blender.
2. Add 100 ml of detergent/salt solution.
3. Blend on high 30 sec-1 minute.
4. Strain the mixture into a beaker using cheesecloth.
5. Add 20-30 ml meat tenderizer and stir to mix.
6. Place 6 ml filtrate in a test tube.
7. Pour 6 ml ice cold ethanol carefully down the side of the tube to form a layer.
8. Let the mixture sit undisturbed 2-3 minutes until bubbling stops.
9. The DNA will float in the alcohol. Swirl a glass stirring rod at the interface of the two layers to see the small threads of DNA.

*Modified from: "Isolation of DNA from Onion" Ellen Averill

Procedure for Thymus DNA Extraction*

1. Cut out a chunk of 1 inch square thymus and place in the blender.
2. Add 100 -150 ml buffer solution and 10 ml detergent solution to the blender.
3. Blend for 1 minute or until the mixture is smooth.
4. Pour the mixture into a beaker.
5. Transfer 1 ml of the mixture to a centrifuge tube.
6. Add 2 ml of salt solution, cap, and shake for 2 minutes.
7. Centrifuge for 7 minutes in a balanced centrifuge.
8. Carefully remove the tube from the centrifuge and note the two layers: the upper layer - liquid (supernatant) is what has the DNA in it.
9. Pipet or carefully pour the liquid into a clean test tube.
10. Pour 5 ml ice cold ethanol carefully down the side of the tube to form a layer.
11. Let the mixture sit undisturbed for a minute or two.
12. The DNA will float in the alcohol. The DNA of the thymus will be long threads that easily spool.

*Modified from:
"Thymus DNA Extractions" by Lana Hays

DNA EXTRACTION SUMMARY CHART

QUESTIONS	ONION	THYMUS
What are the cell characteristics?		
What lyses the cell nucleus?		
What precipitates the DNA?		
Describe amount of DNA		
Description of DNA		

Activity 3: DNA Function

DNA contains instructions for making proteins. Usually thousands of DNA bases are required to provide the information for one protein. Below are two partial sequences of DNA bases (shown for only one strand of DNA). Sequence 1 is from a human and sequence 2 is from a cow. In both humans and cows, this sequence is part of a set of instructions for controlling a physiological function. In this case, the sequence is part of the gene to make the protein **insulin**. Insulin is a hormone that is essential for regulating blood sugar. After a meal, insulin is released into the blood and acts as a signal for cells to transport glucose (sugar) into themselves. The effect is to decrease levels of blood sugar.

Diabetes results if an individual does not produce adequate insulin and loses the ability to regulate blood sugar.

Cows and humans diverged from a common ancestor approximately 90 million years ago, so it is to be expected that they would have accumulated differences (mutations) in their DNA. Somewhat surprisingly, comparative studies of DNA show that DNA sequences are conserved across diverse species; for example, humans, yeast, and fruit flies share sequences of DNA in genes that control such fundamental features of life as cell division.

All mammals share great similarity in numerous genes for proteins such as hormones like insulin but over millions of years, mutations accumulate. One would expect that more closely related species would have fewer differences in their DNA and, indeed, researchers have found that this is the case. Humans share about 99% of our DNA with chimpanzees. The cow genome has not been completely sequenced but we share 60% of our DNA with fruit flies, so we can safely predict that much of our DNA is shared with the cow.

Check Your Understanding: Answer the following questions based on your reading of the introduction to this section.

1. What is represented by the sequence of letters in the box below?

2. What is the function of insulin?

3. Which would you expect to be most similar?
   a. chimpanzee and human DNA sequences for insulin
   b. cow and human DNA sequences for insulin
   c. cow and chimpanzee sequences for insulin

4. Explain your choice in #3. Why would two species have similar DNA sequences for a specific protein?

Instructions:

1. Using the DNA sequence given below, make a complimentary RNA strand from both the human and the cow. Write the RNA directly below the DNA strand (remember to substitute U's for T's in RNA).

2. Use the genetic code table (below) to determine what amino acids are assembled to make the insulin protein in both the cow and the human. Write your amino acid chain directly below the RNA sequence.

Sequence 1 - Human
C C A T A G C A C G T T A C A A C G T G A A G G T A A

RNA :

Amino Acids:

Sequence 2 - Cow

C C G T A G C A T G T T A C A A C G C G A A G G C A C

RNA:

Amino Acids:

Analysis

1. Comparing the human gene to the cow gene, how many of the **codons** are exactly the same? _____

2. How many of the amino acids in the sequence are exactly the same? _____

3. Could two humans (or two cows) have some differences in their DNA sequences for insulin, yet still make the exact same insulin proteins? Explain.

4. Find ALL of the codons that can code for the amino acid **leucine** and list them.

5. Diabetes is a disease characterized by the inability to break down sugars. Often a person with diabetes has a defective DNA sequence that codes for the making of the insulin protein.

Suppose a person has a mutation in their DNA and the first triplet for the insulin gene reads T A T. The normal gene reads T A G. What amino acid does the mutant DNA and the normal DNA code for and will the person with this mutation be diabetic?

6. Another mutation changes the insulin gene to read T C T (instead of the normal T A G). Will this person be diabetic? Explain.

7. DNA sequences are often used to determine relationships between organisms. DNA sequences that code for a particularly gene can vary, though organisms that are closely related will have very similar sequences. This table shows the amino acid sequences of 4 organisms.

Human: C C A T A G C A C C T A	Chimpanzee: C C A T A A C A C C T A
Pig: C C A T G T A A A C G A	Cricket: C C T A A A G G G A C G

Based on these sequences, which two organisms are most closely related?
_____

8. An unknown organism is found in the forest and the gene is sequenced as follows:

Unknown: C C A T G G A A T C G A

What kind of an animal do you think this is? _____

* Information and images are modified from K. Miller and J. Levine <www.biologycorner.com/worksheets.php>.

THE GENETIC CODE

20 Amino acids and their corresponding DNA codons.

Amino Acid	DNA codons
Isoleucine	ATT, ATC, ATA
Leucine	CTT, CTC, CTA, CTG, TTA, TTG
Valine	GTT, GTC, GTA, GTG
Phenylalanine	TTT, TTC
Methionine	ATG
Cysteine	TGT, TGC
Alanine	GCT, GCC, GCA, GCG
Glycine	GGT, GGC, GGA, GGG
Proline	CCT, CCC, CCA, CCG
Threonine	ACT, ACC, ACA, ACG
Serine	TCT, TCC, TCA, TCG, AGT, AGC
Tyrosine	TAT, TAC
Tryptophan	TGG
Glutamine	CAA, CAG
Asparagine	AAT, AAC
Histidine	CAT, CAC
Glutamic acid	GAA, GAG
Aspartic acid	GAT, GAC
Lysine	AAA, AAG
Arginine	CGT, CGC, CGA, CGG, AGA, AGG
Stop codons	TAA, TAG, TGA

Source: www.cbs.dtu.dk/courses/27619/**codon**.html

One Last Thing. . .

Next week we will be examining tissues (histology). Today we need to make jello with pasta so that we can visualize perspectives on viewing tissues under the microscope. Trust me, it will be useful!