

Laboratory 3.

Molecular Genetics

I. The Basics of DNA Structure

DNA molecules are composed of small building blocks called nucleotides:

- A. Each DNA nucleotide is composed of three smaller molecules bonded together: one nitrogen base, one phosphate and one five-carbon sugar (deoxyribose).
- B. Four different types of nucleotides are needed to build a DNA molecule. Each of these four nucleotides has a different nitrogen base: adenine, cytosine, guanine or thymine.
- C. DNA consists of a structure analogous to a twisted ladder: The two sides of the ladder are made of alternating sugar and phosphate molecules.
- D. Each sugar molecule is attached to one nitrogen base. The two strands of DNA are attached by bonds between the nitrogen bases on each side of the ladder. Each nucleotide base only bonds with one specific partner. The combination of two bases is called a base pair.

Adenine **always** pairs with Thymine **A-T**
 Guanine **always** bonds with Cytosine **G-C**

- E. Fill in the blanks on the incomplete DNA molecule in Fig. 3-1. Use the Symbol P for phosphate, S for sugar, and A, C, G, or T for the appropriate nitrogen bases.

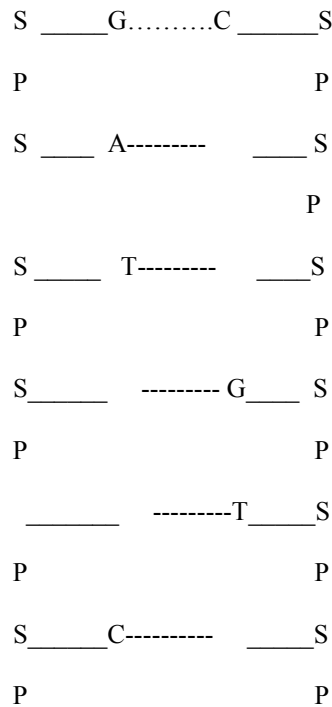


Fig. 3-1

Fill in the blanks on the incomplete DNA molecule in Figure 3-1. Use the symbol “P” for phosphate, “S” for sugar, and “A”, “T”, “G”, or “C” for the appropriate nitrogen bases.

Questions:

1. On Fig. 3-1 draw a box around one complete **nucleotide**.
2. How many nucleotides are shown in the DNA molecule I Fig. 3-1? _____
3. How many different types of nucleotides were used to construct the DNA molecule in Fig. 3-1?
4. _____
Hand in Fig. 3-1 and answers to the questions above to your TF prior to leaving the laboratory today. Make sure your name is on the page.

II. Building a Model of DNA

- A. Work in pairs. Get a DNA model kit.
- B. Construct the support stand for the model by assembling one gray tube (about 8 inches long), three green tubes (about 2 inches long) and one black connector with four prongs. Set the stand aside (you will need it later).
- C. Separate the parts of the DNA model according to the description in Table 3-1 (below)
- D. Using your knowledge of DNA structure, assemble the DNA model.
- E. Place the model onto the stand by inserting the vertical tube on the stand through the holes in the stand.

Place the model onto the stand by inserting the vertical tube on the stand through the holes in the white base-to-base connectors.

Twist the model as you slide it onto the stand. Stop twisting when the entire molecule fits onto the stand.

The twisted shape of the DNA molecule is known as a double helix.

Table 3-1

PARTS OF THE DNA MODEL

<u>Parts of the DNA molecule</u>	<u>Description of Model Part</u>
Deoxyribose	black, three prongs
Phosphate	red, two prongs
Sugar-to-phosphate connectors	yellow tube
Adenine (A) base	blue tube
Thymine (T) base	red tube
Guanine (G) base	green tube
Cytosine (C) base	gray tube
Base-to-base connectors	white, two prongs

Questions:

1. How many nucleotides are present in your DNA model? _____

2. What is the base-pairing rule?

3. Why would it be appropriate to call a DNA molecule a “polynucleotide”?

4. Assume that the model you just built is an exact representation of your DNA code. Would you use the same bases to construct your lab partner’s DNA? _____

Would you assemble the bases in the same order to make a model of your lab partner’s DNA? Explain.

Each gene has the instruction to make a single polypeptide chain. These instructions are part of the genetic code. Polypeptide chains are the structural units of proteins. A polypeptide chain is made of many amino acids bonded together.

The key to the genetic code is the sequence of nitrogen bases along one side of the DNA molecule. To construct a protein you must know the order of the bases. The code is written in three letter “words”. Each of these words (or triplets) tells the cell which amino acid should come next when building a protein.

For proteins to function correctly, the amino acids must be assembled in the correct order.

5. How many triplets are present along one side of your DNA model ? _____

6. How many amino acids will be present in the protein made from your model? _____

Hand in answers to the questions above to your TF prior to leaving the laboratory today. Make sure your name is on the page. Don’t take the model apart yet.

III. Protein Synthesis

When a specific protein is required by the body, regions of the double helix unwind, so that a cell gains access to the genes that contain the coded information to make that protein. Protein synthesis has two steps: transcription (reading the message from the DNA) takes place in the nucleus and translation (translating it into protein) occurs in the cytoplasm. Both steps require molecules of RNA (ribonucleic acid).

Although the nucleus contains instructions for protein synthesis, the machinery to make proteins is located in the cytoplasm. The coded information is transferred from the nucleus to the cytoplasm during transcription.

Transcription

1. During transcription, DNA bases are copied to form a single strand of RNA, called messenger RNA (mRNA). As with the DNA, mRNA is divided into coded three letter words. In mRNA these 3 letter “words” are called codons.
2. The base pairing rule is used to form messenger RNA with one exception. RNA molecules do not have the nitrogen base thymine. They have uracil in its place.

Base pairing to form mRNA

DNA BASE

C
G
T
A

mRNA Bases

G
C
A
U

3. The coded information to make a protein appears along one side of the double helix. Practice transcription by filling in the correct messenger RNA codons in Table 3-2

Table 3-2

TRANSCRIPTION OF mRNA

DNA triplets CGC ATA GAC TTT CTT ACT TAG CAT AAA

mRNA codons

4. How many amino acids would be in this protein? _____
5. Which of the five types of nitrogen bases is not found in mRNA? _____

TRANSLATION

1. A Cell needs amino acids to construct proteins. The amino acids are carried to the ribosomes by another type of RNA molecule, called transfer RNA (tRNA). A tRNA has two functional ends. One end picks up amino acids in the cytoplasm (See Fig 3-2)
2. The other end is called the anticodon. It contains three nitrogen bases that can form a base pair with a matching codon in the messenger RNA.

3. Each type of tRNA can carry only one type of amino acid. There are enough different types of tRNA molecules to carry all the different types of amino acids needed to make your body's proteins.
4. Where do the tRNA molecules take the amino acids? They take them to ribosomes, organelles in the cytoplasm where proteins are "manufactured". Ribosomes are made of proteins plus a third type of RNA called ribosomal RNA.
5. Ribosomes read messenger RNA codons and accept amino acids brought by tRNA molecules. Ribosomes help bond amino acids together in the order specified by the messenger RNA codons to construct the polypeptide chain.

Summary of Protein Synthesis

- DNA contains the instructions to make polypeptide chains. A region of the DNA double helix unwinds. Coded instructions to make a protein are exposed.
- This information is carried to the cytoplasm by mRNA molecules (transcription).
- Amino acids in the cytoplasm are used to build polypeptides.
- Transfer RNA molecules pick up the amino acids and transport them to ribosomes, the locations where proteins are made.
- Ribosomes bond amino acids together according to the instructions in the genetic code.

Hand in Table 3-2 and answers to the questions above to your TF prior to leaving the laboratory today. Make sure your name is on the page.

IV. Building a Real Protein

Imagine the following situation: you are about to give birth. The brain produces the hormone oxytocin (a small protein), which causes uterine muscles to contract for childbirth. Following birth, this same hormone causes muscles in the mammary glands to contract, releasing milk to nurse the baby.

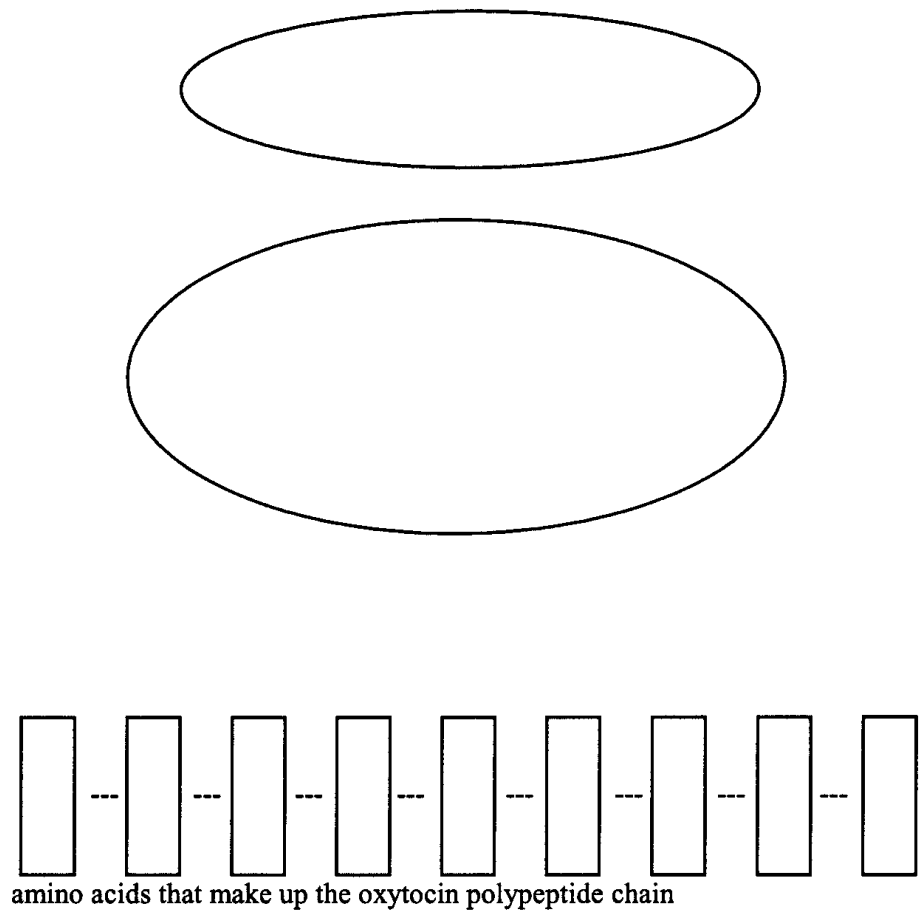
Imagine that this is your first baby. How does the brain know how to make oxytocin if it has never been required before? This information is stored in your DNA "reference library".

1. Using the steps outlined in III (above) build the protein oxytocin. Oxytocin is one of the smallest proteins with only nine amino acids connected into a single polypeptide chain.
2. Transcribe the DNA triplets that code for oxytocin below into mRNA codons and add them to the appropriate spaces below.












DNA triplets ACG ATG TAT GTT TTG ACG GCA GAC CCC
 mRNA codons _____

3. The messenger RNA must detach from the DNA and leave the nucleus for translation to occur. Cut out the strip of mRNA and move it to the ribosome in the ribosome figure (looks like a big acorn) Fig. 3-2. Notice that the ribosome has two parts, separated by a groove. The mRNA should be placed along the groove between the upper and lower sections.

Figure 3.2



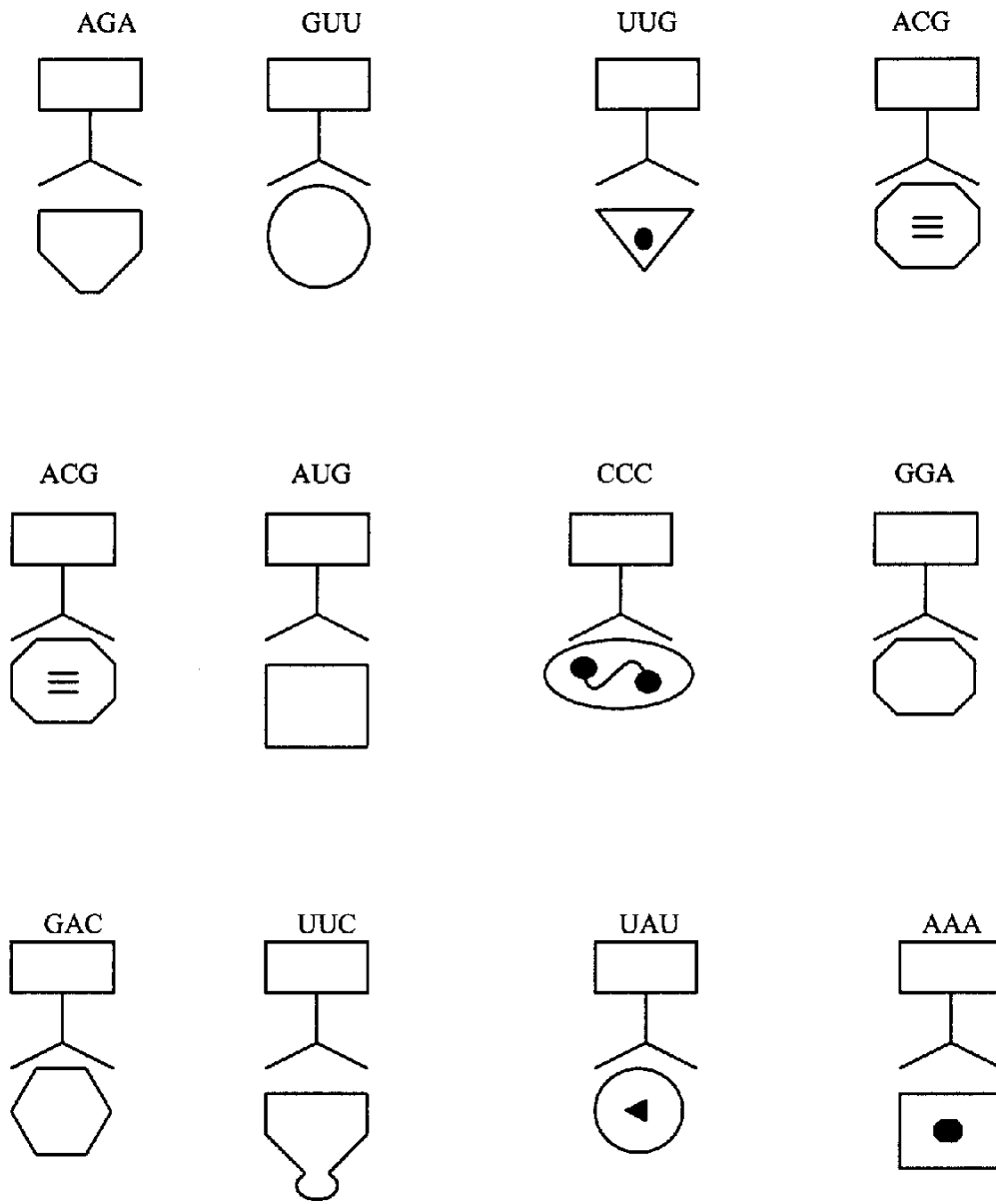
Refer to Table 3-3 to determine the name of each amino acid in your oxytocin polypeptide chain.

Table 3.3 Key to identifying Amino Acids	
SHAPE	NAME OF AMINO ACID
	histidine
	glutamine
	asparagine
	cysteine
	tyrosine
	glycine
	proline
	leucine
	alanine
	isoleucine
	serine

4. Each tRNA molecule in Fig. 3-3 is ready to deliver an amino acid to the ribosome. Where should each tRNA molecule deliver its amino acid?
- Cut out the tRNA molecules.
 - Place each tRNA molecule under the correct mRNA codon.
 - Suggestion: you will be able to match each tRNA with its codon by following the base pairing rule.

Each box at the bottom of Fig. 3-3 represents one amino acid in the chain. Write the names of the amino acids in the order in each box in Fig 3-3.

Figure 3.3



Questions:

1. If the second amino acid in your polypeptide chain was valine, would the protein still be oxytocin? Explain your answer.
2. Circle one answer: The DNA triplet AAA would be transcribed into the mRNA codon TTT/UUU.
3. Put the following steps of protein synthesis in the correct order.

- _____ tRNA molecules pick up amino acids
- _____ mRNA transcribed
- _____ DNA double helix unwinds
- _____ mRNA binds to ribosome
- _____ ribosome bonds amino acids together
- _____ tRNA anticodon links with mRNA codon
- _____ mRNA leaves nucleus
- _____ polypeptide chain completed

Hand in your answers to the questions above to your TF prior to leaving the laboratory today. Make sure your name is on the page.