

UNIT 2: INFORMATION TRANSFER AND MOBILE GENETIC ELEMENTS

Introduction:

A cell's DNA contains all the information needed for the cell's growth and division into two similar cells. In active cells some of the genetic information is used to generate "working" molecules, such as the enzymes that catalyze the reactions leading to accumulation of cell material. We have already learned that most enzymes are proteins, and now we shall see how the information in the cell's DNA is used to make its proteins--including DNA polymerase, the enzyme that helps to replicate the DNA itself.

In the replication of DNA, the entire molecule is copied exactly. A cell's DNA typically codes for a much larger diversity of proteins than those that are regularly used by the cell, so synthesis of proteins following the instructions contained in DNA is selective. For instance, bacteria grown on one type of food source will usually not contain the enzymes for using an alternative food source that is not present. Consider also that DNA in the nucleus of any mammalian cell contains the information for making the oxygen carrier protein hemoglobin, although hemoglobin is normally made only in cells destined to become red blood cells.

Transfer of the information contained in DNA into functional proteins is indirect, involving the production of a "middle-molecule," or messenger ribonucleic acid (mRNA). This messenger molecule in turn must interact with a complex organelle, the ribosome, made up of proteins and ribonucleic acid, before amino acids can be correctly assembled into a functional polypeptide. Furthermore, translation requires adaptor molecules, tRNAs, to carry activated amino acids to the correct position on the ribosome-mRNA complex.

In Units 3 and 4 last semester we focused on aspects of metabolism in which ATP was synthesized. In this unit we will confront some of the primary processes in which ATP is utilized. Macromolecules, which make up most of the dry matter of cells, are built up from monomer subunits. These monomers are assembled into the cell's DNA, RNA, and proteins by way of condensation reactions requiring an energy input in the form of ATP. By far the greatest part of the ATP used in synthesizing macromolecules--about 70% of the total--goes into making protein. This is partly because proteins are costly to make in terms of ATP used/gram of protein produced, and also because proteins make up the greatest part of the cell's dry weight.

Readings: Chapter 17
Chapter 19 (pages 381-385)
Chapter 27 (pages 561-563)
Chapter 21 (page 435)
Chapter 20
Chapter 38 (pages 812-819)

To Do This Unit:

1. Read through the objectives.
2. Read the text assignments, focusing on the material covered in the objectives.
3. Examine carefully the demonstration materials online and in the Study Center.

4. Go back over the text and demonstration materials and write out answers to the objectives.
5. Take an examination on this unit. You will be given a sequence of DNA with the direction of transcription, and asked to replicate it and to transcribe and translate it, using the information presented in this unit and the last.

KEY CONCEPTS AND OBJECTIVES:

After you have studied the material in this unit you should understand the following concepts and you should be able to carry out the objectives for each.

The sequence of bases in DNA determines the sequence in which amino acids will be linked in protein synthesis.

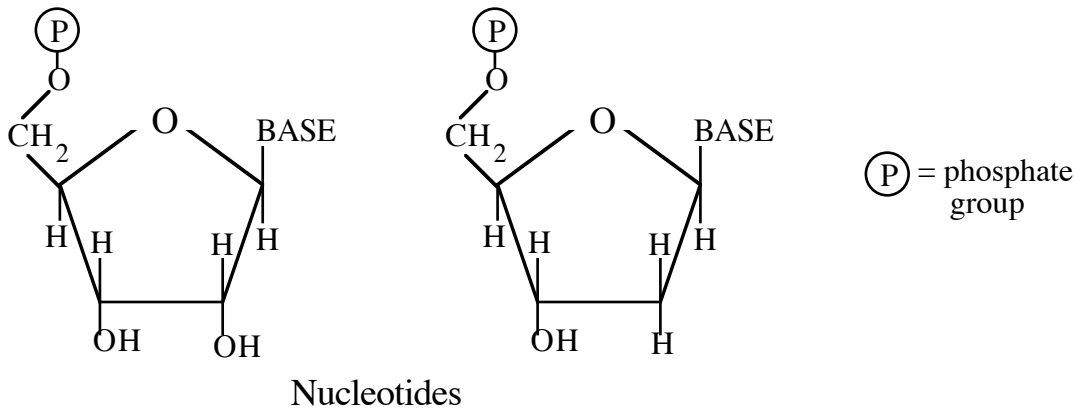


1. Using Fig. 17.2 (p. 327) explain the "one gene-one polypeptide hypothesis."

During transcription, a faithful copy of the information in DNA is transformed into mRNA.



2. a) In the diagrams below, label the 5 carbons in each sugar, indicate which sugar would be found in DNA and which in RNA, and explain the structural difference between the two sugars. (See Fig. 5.27, p. 87.)



b) List three differences between DNA and RNA.

c) Which nucleotides are found in DNA and which in RNA? Which are purines and which are pyrimidines? (See Fig. 5.27, p. 87 and p. 88.)

In DNA, the strand that is being transcribed into mRNA is called the template strand and the non-template strand is referred to as the complementary strand. (Note that transcription of the template produces an RNA version of the complementary strand).

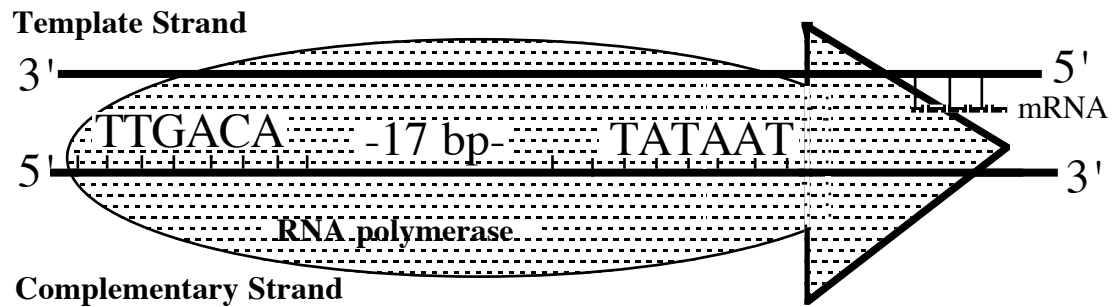
The site at which RNA polymerase binds is called a promoter. The promoter sequence specifies which strand is to be transcribed and orients the RNA polymerase in the right direction.

3. The following information on transcription in bacterial cells is given to help you understand how the RNA polymerase complex "knows" where to bind on the DNA and how it is oriented to move along the DNA in a particular direction.

(1) In bacteria, the **promoter** sequence consists of two DNA sequences, each six nucleotides long, that are separated from one another by 17 other base pairs (bp):

5' ---TTGACA ---(17 bp)--- TATAAT---> 3'

(2) RNA polymerase recognizes and binds to the promoter on the **complementary strand**. The promoter is read in the 5' to 3' direction.



(3) Once bound to the complementary strand, the RNA polymerase is oriented to move along the DNA in one direction (direction of the arrowhead, in this case, left to right) and will move "downstream," toward the 3' end of the complementary strand.

(4) *The RNA polymerase will transcribe the other (**template**) strand (the 3' to 5' strand--in this case, the top strand) because nucleic acid synthesis can only occur in the 5' to 3' direction. In other words, the polymerase complex recognizes the promoter on the 5' to 3' complementary strand, but must transcribe the 3' to 5' template strand in order to synthesize 5' to 3'.*

REMEMBER:

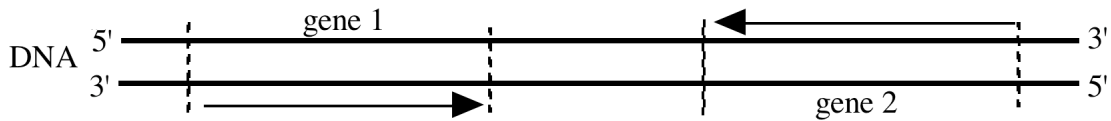
- ❶ RNA polymerase recognizes and binds to the promoter on the complementary strand.
- ❷ The template strand = the strand to be transcribed.
- ❸ Transcription always goes 3' → 5' on template strand (because mRNA synthesis is 5' → 3').
- ❹ The mRNA synthesized has the same sequence and polarity as the complementary strand (with U instead of T).

(5) In bacteria, the **start signal** for transcription on the complementary strand is 5' CAT 3'. What would it be on the template strand? _____

a) Locate the promoter sequence, and determine the direction of transcription and which strand will be transcribed in the following sequence of bacterial DNA. Circle the start signal on the complementary strand.

5' GTCCACAGATGTAGGCATTATA . . 17 . bp . . TGTC AACGATGGCCTGCATGA 3'
3' CAGGTGTCTACATCCGTAATAT . . 17 . bp . . ACAGTTGCTACCGGACGTACT 5'

b) Note: The polymerase may use different strands for different genes; *it is the promoter sequences that determine the direction of transcription and the strand used.*



Arrows represent direction of transcription.

c) The sequence of nucleotides constituting a promoter is not the same for all promoters--rather, each promoter's nucleotide sequence is a variation on a theme (theme = consensus sequence). Given a group of related sequences, derive a consensus sequence from them. Example:

5 ' TTGACG
 5 ' TAGACA
 5 ' CTTACA
 5 ' TTGAAA
 5 ' TTGACC
 5 ' TTGTCA

Consensus sequence: 5 ' TTGACA

What do you think determines whether a given promoter is strong or weak (i.e., whether initiation of transcription occurs at that promoter with high or low frequency)? Why is this important? In thinking about this question, consider that *E. coli* has only a single RNA polymerase that transcribes all genes. Would it be advantageous for an organism to transcribe all of its genes at the same frequency? Why or why not? See **demo** for additional information.

d) Using information in the demo, explain how the RNA polymerase knows where on the DNA to stop transcription in bacteria?

In eukaryotes, the information in most genes is split into coding and non-coding sequences. The mRNA is modified after transcription.

4. Describe the process of transcription in **eukaryotic cells** using Figs. 17.3 (p. 329), 17.7 (p. 332), and 17.8 (p. 333). Then describe the mRNA processing that occurs in eukaryotic cells (see Fig. 17.9, p. 334). In doing so, identify and define the following. Are the same processes found in prokaryotes as well?
- binding of transcription factors
 - TATA box
 - sites where transcription is initiated¹
 - sites where transcription is terminated (What is the signal in eukaryotes?)
 - the primary transcript (or pre-mRNA molecule)
 - the exons and introns; Which are excised?
 - the end of the primary transcript that is capped (5' vs. 3')
 - the end of the primary transcript that carries a poly A tail (5' vs. 3')
 - the mechanism by which editing occurs (Figs. 17.10-17.11, p. 335). (Note: you are not responsible for the details of the editing process; strive for an overall understanding of the process.)

5. a) In your written exam, you will be given a sequence of DNA, together with the polarity of the chains (5' vs. 3') and the direction of transcription (arrow). For example:

—————Direction of transcription—————→

5' - ATCAGGAGGTCTACGGAATGTTCCCACTGACATGATCAACCACGT - 3'
 3' - TAGTCCTCCAGATGCCTTACAAGGGTGACTGTACTAGTTGGTGCA - 5'

*

From this information, you are to determine which strand is being transcribed (i.e., top or bottom) and, starting at the end of the correct strand, write the sequence of nucleotides in the mRNA that would be transcribed from this DNA. Indicate the 5' and 3' ends of the transcript. (Note: On your test we will not put the promoter sequence in; assume that the polymerase has bound and begun transcription.)

¹Prokaryotes have but one type of RNA polymerase but transcription in eukaryotes is more complex and requires three kinds of RNA polymerase. Like the promoter sequences in bacteria, eukaryotic promoters have two recognition sites, one between 40 and 110 bases upstream of the start site for transcription, and a second is a TATA sequence on the complementary strand about 25 bases upstream of the start site. (The TATA box was discovered by Professor Michael Goldberg here at Cornell.)

- b) What is the start signal (i.e., initiation codon) for **translation** in both prokaryotes and eukaryotes? Using the sequence of mRNA that you transcribed, find the proper start signal for translation, and, using the genetic code on page 330, write the sequence of amino acids that might be translated from this mRNA.
- c) What are the termination signals for translation? (See Fig. 17.5, p. 330.)
6. a) In Fig. 17.14b (p. 338) point out the amino acid attachment site and the anticodon. Using Fig. 17.5 (p. 330), determine the codon for tryptophan (trp) and give the anticodon. (Label the 3' and 5' ends!)
- b) What are ribosomes composed of? Using Fig. 17.16 (p. 339), explain the process of translation in bacteria. The **demo** model will clarify the process (see objective 6d).
- c) Using Fig. 17.17 (p. 340), describe the initiation of translation.
- d) Using the **demo**, cardboard models of ribosomes, mRNA, tRNA, and amino acids, and the information provided with the model, describe the individual steps in polypeptide synthesis including:
- attachment of amino acids to tRNAs to form activated amino acids (Fig. 17.15, p. 338). What compound provides the energy to attach the amino acids?
 - formation of ribosome-mRNA complex and initiation.
 - binding of charged tRNAs to P and A sites. What is the initiation codon for translation?
 - formation of peptide bond.
 - What happens to the met (or fmet) in most proteins?

e) Use Fig. 17.18 (p. 341) to describe the elongation cycle.

f) Use Fig. 17.19 (p. 342) to show how translation is terminated.

g) Where does protein synthesis take place in eukaryotic cells? What special roles do signal recognition particles (SRPs) and endoplasmic reticulum, play in protein synthesis?

7. a) Using Fig. 17.3 (p. 329), 17.24 (p. 347), and 17.25 (p. 348) compare the processes of transcription and translation in prokaryotes and eukaryotes. Completing the following chart may help you to summarize the differences.

	Prokaryotes	Eukaryotes
Are transcription factors required? (Y/N)		
Does transcription proceed past the termination signal? (Y/N)		
Is the mRNA processed to remove introns? (Y/N)		
Is the transcript capped? (Y/N)		
Does the transcript receive a poly-A tail? (Y/N)		
Can transcription and translation take place at same time? (Y/N)		
Are the ribosomes large or small?		

b) Summary objective: Describe the flow of information from the gene to the synthesis of a protein in (1) prokaryotic cells and (2) eukaryotic cells.

A gene determines primary structure of a protein, and primary structure determines protein conformation.

8. a) Find the asterisk in the sequence of nucleotides shown in Objective 5a. What is the consequence (in terms of the polypeptide that would be translated from this mRNA) if adenine were substituted for the guanine at the asterisk and the GC base pair were changed to an AT base pair? (Hint: Check the codons in Fig. 17.5, p. 330.)
- b) What is the consequence (in terms of the polypeptide that would be translated from this mRNA) of making the same base substitution in the next GC pair to the right after the asterisk?
- c) What is the consequence (in terms of the polypeptide that would be translated from this mRNA) of deleting the GC base pair at the asterisk? Of deleting the GC base pair and the next two base pairs? Which of these changes (mutations) would have the most detrimental effect on the structure and function of the polypeptide in question? Why are mutations in which one or two nucleotides are inserted or deleted called frameshift mutations?
- d) Give the difference between missense mutations and nonsense mutations. Which is generally the more serious?
- e) What are some ways that such mutations might be caused?

- b) Using diagrams such as Figs. 27.12-27.13 (pp. 562-563), describe the process of conjugation in bacteria. What is the difference between a F^- , F^+ , and Hfr cell?
12. a) Explain what transposons are and indicate whether they are characteristic of prokaryotes or eukaryotes, or both. (Note: Barbara McClintock, who discovered transposons, received her Ph.D. at Cornell and did much of her work here.)
- b) Explain how transpositions are fundamentally different from other mechanisms of genetic shuffling. How does "cut-and-paste" transposition differ from "copy-and-paste" transposition?
- c) What would be the result (in terms of the protein that would be synthesized) if a transposon inserts itself into the middle of a functional gene? ...its promoter?
13. a) Explain what is meant by "cloning" genes, and give reasons why scientists are interested in cloning genes.
- b) Using Fig. 20.3 (p. 398), explain how large molecules of DNA can be broken down into smaller pieces of DNA that can be manipulated in the laboratory. What are the characteristics of a restriction site? What is the importance of "sticky ends?"

- c) Using Fig. 20.4 (p. 399), describe the procedure by which eukaryotic DNA can be inserted into plasmids (vectors). What is the role of ligase?
- d) Explain how the cloning vectors can be taken up into bacterial cells. How can the cells that successfully incorporated new DNA be separated from the millions of cells that received no new DNA?
- e) How can the host cell (or cells) that received the gene of interest be isolated from the vast majority of cells in the culture medium? (See Fig. 20.7, p. 402.)
- f) Describe three problems in getting eukaryotic genes expressed in prokaryotic cells and explain two procedures that have been used to surmount these problems.
- g) Explain the different advantages of cDNA approaches and those using genomic DNA. See p. 401 and the **demo**.
14. Describe the PCR procedure and give one practical application of PCR. See **demo** for additional information.
15. a) Explain how gel electrophoresis works (see Fig. 20.9) and, using Fig. 20.11 (p. 407), explain the procedure of Southern blotting.
- b) What are RFLPs (see page 417) and how can they be used?

16. a) Explain how the following techniques can be used to help map the human genome: nucleic acid hybridization, genome analysis (analyzing DNA sequences, studying gene expression, and determining gene function), linkage mapping, physical mapping, and DNA sequencing. (analyzing DNA sequences, studying gene expression, and determining gene function). Note: You need only understand the general principles, not the details.
- b) Give an example of how DNA technology can be used for (1) diagnosis of disease in humans; (2) human gene therapy (What are some of the problems faced?); and (3) producing pharmaceutical products.
17. a) Explain how DNA technology can be used in the process of DNA profiling (DNA fingerprinting) and explain what STRs are and why they are useful.
- b) Give one example of how DNA technology can be used in environmental work or agriculture. How has DNA technology been used to make products for animal husbandry? What are transgenic organisms?
- c) Give reasons why plants have proven to be easier to engineer than animals (see also page 814). Using Figs. 20.25 (p. 421) explain how foreign genes can be inserted into plants using the Ti plasmid? What are some of the uses of this technology in crop plants?
- d) Describe how stem cells can be used to treat human diseases.

Below are summary questions relating to important concepts in this unit. The TA may use these questions in his or her oral test or you may see one of them as an essay question on the final exam. Take a few moments now to formulate your answers.

Describe the DNA molecule and relate the concept of "genes" to its structure. Explain how genetic material is replicated and how genes control cellular functioning. Include some detail regarding the mechanism of protein synthesis.

Describe the steps involved in recombinant DNA technology, and explain how this technology could be used for "DNA fingerprinting."

Unit 2 Evaluation: PLEASE FILL OUT THIS EVALUATION SHEET AFTER YOU HAVE COMPLETED THE UNIT, AND PLACE IT IN THE WOODEN BOX IN THE STUDY CENTER. THESE FORMS WILL PROVIDE FEEDBACK NECESSARY FOR IMPROVING THE UNITS.

How many hours did you spend studying for this unit? _____

Do you consider the time you spent studying for this unit to be:

A. too little? B. about right? C. excessive?

Was the majority of the material in this unit:

A. new to you? B. somewhat familiar? C. very familiar?

How many times have you taken this unit test? _____

How effective were the objectives in organizing your study?

A. ineffective B. effective C. very effective

Did the objectives clearly indicate what you were expected to know about the unit material?

A. Yes B. No

What objectives did you have the most problems with? _____

Was the demo helpful in learning the unit material?

A. not at all B. moderately C. very helpful; essential

The amount of information in this unit is:

A. too little B. about right C. excessive

The level of difficulty of this unit is:

A. too easy B. about right C. too hard

How crowded was the Study Center when you took your test?

A. not crowded B. crowded, but OK C. very crowded; a long wait

Indicate day of the week and time of day that you took this exam: _____

Did you need any special help from TAs in learning this unit material?

A. Yes B. No

If you needed help from a staff member, was he/she willing and able to provide the help you needed?

A. unwilling/unable B. satisfactory C. very helpful

Name of TA who tested you on this unit: _____

Did you feel the TA who tested you was well prepared?

A. poorly prepared B. satisfactorily prepared C. very well prepared

Do you feel the TA who tested you gave you a fair and impartial test?

A. Yes B. No

Any comments or suggestions?