DNA—The Genetic Material: Replication, Transcription, and Translation

LABORATORY 17

OVERVIEW

Exact, yet variable and mutable—these are the characteristics of our genetic material, **DNA (deoxyribonucleic acid).** DNA is composed of subunits called **nucleotides** which bond together to form long polynucleotide strands. When nucleotides in one strand pair specifically (by hydrogen bonding) with nucleotides in a second strand, a double-stranded molecule—the DNA helix—is formed.

Each DNA nucleotide consists of a sugar (deoxyribose), a phosphate, and a nitrogenous base. An enormous amount of information is encoded in DNA using only four nitrogenous bases (adenine, guanine, cytosine, and thymine) in DNA nucleotides. Variability in DNA results from the arrangement (or sequence) of nucleotide bases along the polynucleotide strands. This sequence is transmitted faithfully, as exact copies, through DNA synthesis (**replication**) at each cell division, and from generation to generation, in all organisms. Occasionally, however, changes (**mutations**) occur in the nucleotide sequence; these are the basis of evolution.

According to the "central dogma of molecular biology," DNA does not act directly, but rather codes for the synthesis of **RNA (ribonucleic acid)** molecules in a process called **transcription.** These RNA "messages" are decoded in the process of protein synthesis (**translation**). Thus DNA regulates cell activity and determines the phenotype of organisms by determining the type of proteins produced by the cell (Figure 17-I).

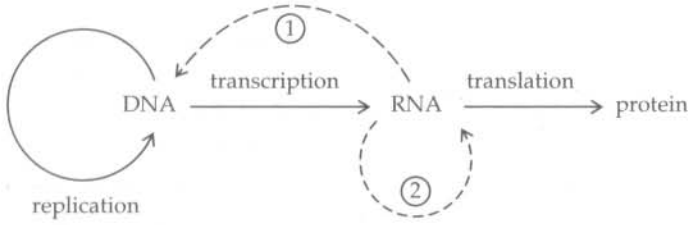


Figure 17-I *The "central dogma of molecular biology" states that DNA is transcribed to form messenger RNA which can the be translated into protein. In this way, information stored in DNA is encoded in RNA and then is decoded to form a protein product. However, it is now known that exceptions to the central dogma exist: certain RNA viruses ① can produce DNA by reverse transcription (RNA → DNA) and ② can also replicate their RNA molecules (RNA → RNA).*

STUDENT PREPARATION

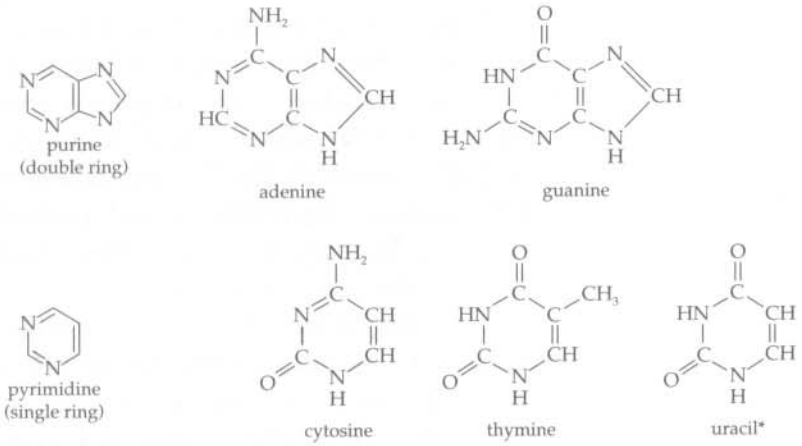
Cut out the "nucleotides" on both the green and blue sheets of paper distributed by your instructor. Keep these in two separate envelopes. Also, cut out the yellow "amino acids." Bring these materials to the laboratory with you.

Familiarize yourself with the processes of replication, transcription, and translation by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency.

✔ EXERCISE A Replication

The DNA molecule is composed of two strands of nucleotides (polynucleotides) hydrogen-bonded together and twisted to form double-stranded DNA. The double-stranded DNA helix is regular, linear, and stable because small nucleotide bases called **pyrimidines** always pair specifically with larger nucleotide bases called **purines.** Thymine (T) and cytosine (C) are the pyrimidines and adenine (A) and guanine (G) are the corresponding purines. Adenine always pairs with thymine, forming two hydrogen bonds (A=T), and cytosine always bonds with guanine, forming three hydrogen bonds (G≡C) (Figure 17A-1).

Figure 17A-1 *The purine and pyrimidine bases present in the nucleotides of DNA and RNA.*



*Replaces thymine in RNA molecules; the CH_3 group present in thymine is absent in uracil.

DNA polynucleotide strands have beginnings and ends, just like sentences. At one end of each strand is a nucleotide bearing a phosphate group that is linked to carbon number 5 (5′ carbon) of the sugar deoxyribose. This is called the 5′ end. At the other end of the chain, a hydroxyl (—OH) group extends from carbon number 3 (3′ carbon) of the deoxyribose in the last nucleotide. This end is called the 3′ end. Similarly, within the DNA molecule, each bond between two adjacent nucleotides in the polynucleotide strand is formed between the 3′ hydroxyl of one nucleotide and the 5′ phosphate of the next. These bonds are called 3′ → 5′ phosphodiester bonds (Figure 17A-2).

One polynucleotide chain in the double-stranded molecule always runs in a 5′ ⟶ 3′ direction while the other runs in a 3′ → 5′ direction: the chains are said to be arranged **antiparallel** to each other (Figure 17A-3). You may not think this is important, but before you read a book you must know which direction to read. Before DNA can work, it too must know its directions.

Replication of DNA is **semiconservative.** (*Semi-*, like *hemi-*, means half.) It is possible to break the hydrogen bonds of double-stranded helical DNA molecules, separating the two polynucleotide strands. Because the base pairing is specific (A=T and G≡C), each single strand can then serve as a **template** or

Figure 17A-2 *Nucleotides are added to a DNA chain, one at a time, by attaching the 5′ phosphate of an incoming nucleotide to the 3′ hydroxyl of the last nucleotide in the lengthening DNA chain. A phosphodiester bond is formed and inorganic pyrophosphate (PP_i) is released. The overall direction of synthesis is 5′ → 3′ for the new polynucleotide strand.*

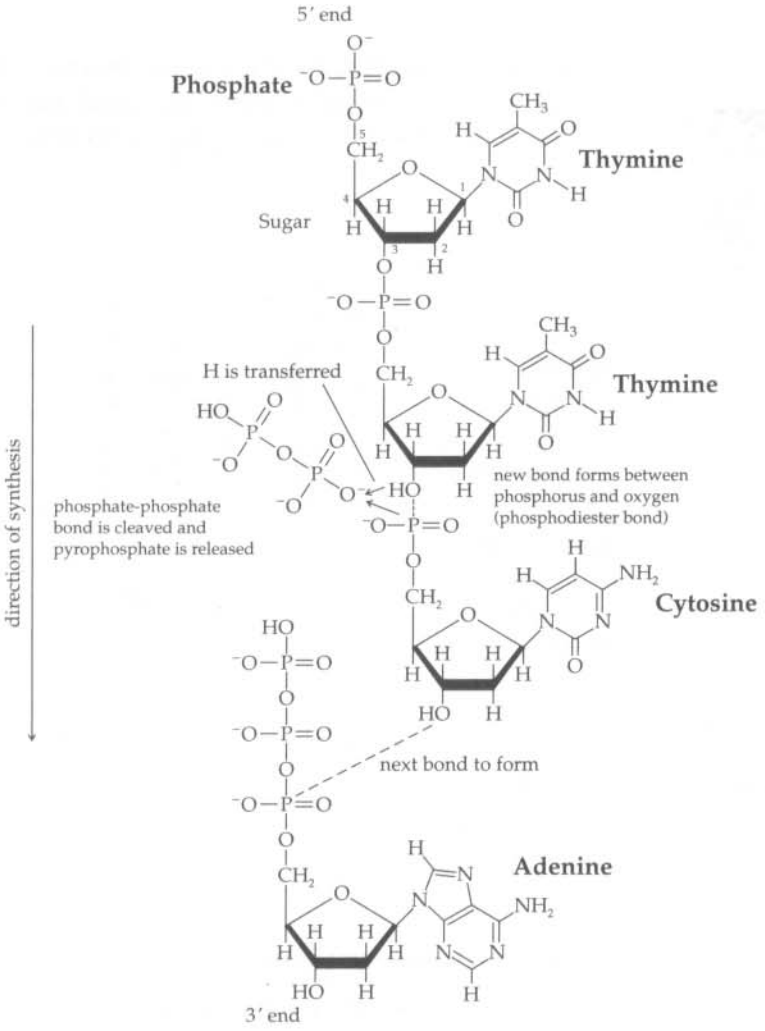
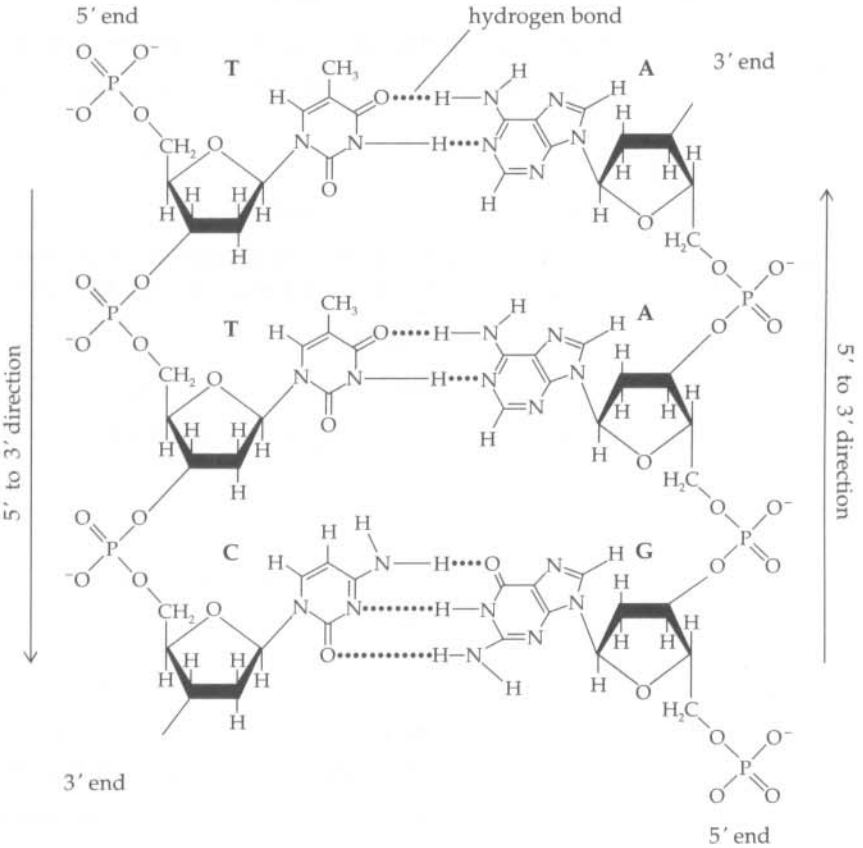


Figure 17A-3 *Polynucleotide strands in the double-stranded helical DNA molecule run antiparallel to one another. Dotted lines represent hydrogen bonds.*



pattern for the formation of a **complementary strand.** Two new double-stranded DNA molecules are produced—each composed of an old polynucleotide strand and a newly synthesized polynucleotide strand. In other words, each of the "daughter" DNA molecules is half new and half old (Figure 17A-4).

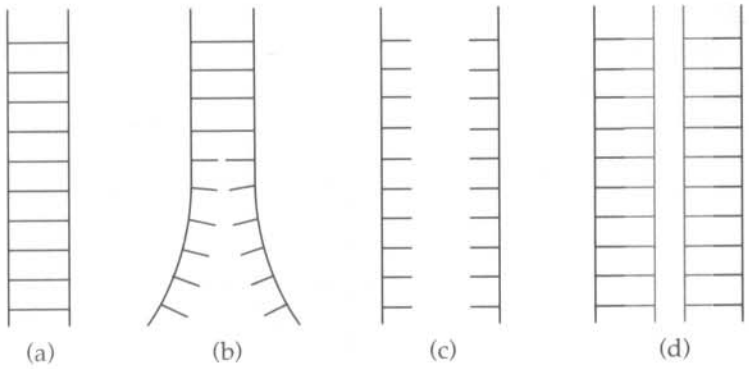


Figure 17A-4 *DNA replication is semiconservative. (a) Nucleotides in DNA are specifically paired and held together by hydrogen bonds. (b) During replication, the hydrogen bonds break and (c) the two original halves of the DNA molecule can serve as templates for the synthesis of complementary strands made from new nucleotides. (d) The two "daughter" molecules of DNA are duplicates of the original DNA. One polynucleotide strand of each molecule consists of nucleotides from the original DNA molecule; the other strand is composed of newly synthesized DNA.*

Objectives

- ☐ Describe the process of semiconservative replication.
- ☐ Explain how the structure of the DNA molecule (including hydrogen bonding and base-pairing specificity) makes semiconservative replication possible.
- ☐ Describe how semiconservative replication duplicates the parent DNA molecule.
- ☐ Describe the structure of a double-stranded DNA molecule and explain the nature of its antiparallel-stranded structure.

Procedure

A model kit has been prepared to help you understand the basic mechanisms of replication, transcription, and translation. Check to see that your kit includes the following materials:

1 DNA molecule (white)

1 sheet of deoxyribonucleotides (blue): dA, dG, dT, dC (*d* indicates *deoxy*ribonucleotide)

1 sheet of ribonucleotides (green): A, G, U, C

1 sheet of amino acids (yellow)

1 ribosome (black)

4 aminoacyl-tRNA synthetase enzymes (green)

4 tRNA molecules (blue)

1 ATP molecule (orange)

1. Begin with the white DNA molecule. Cut out the two strips and paste them together as indicated on the strips. Label the 5′ and 3′ ends of each of the two strands.
2. Use scissors to cut the hydrogen bonds and separate the two nucleotide chains labeled I and II.

3. Using the blue nucleotides, semiconservatively replicate the DNA. Line up the blue nucleotides in the proper order along each of the original DNA strands (Figure 17A-5a).

   a. Which four bases are present in the nucleotides used to synthesize DNA?

   _____

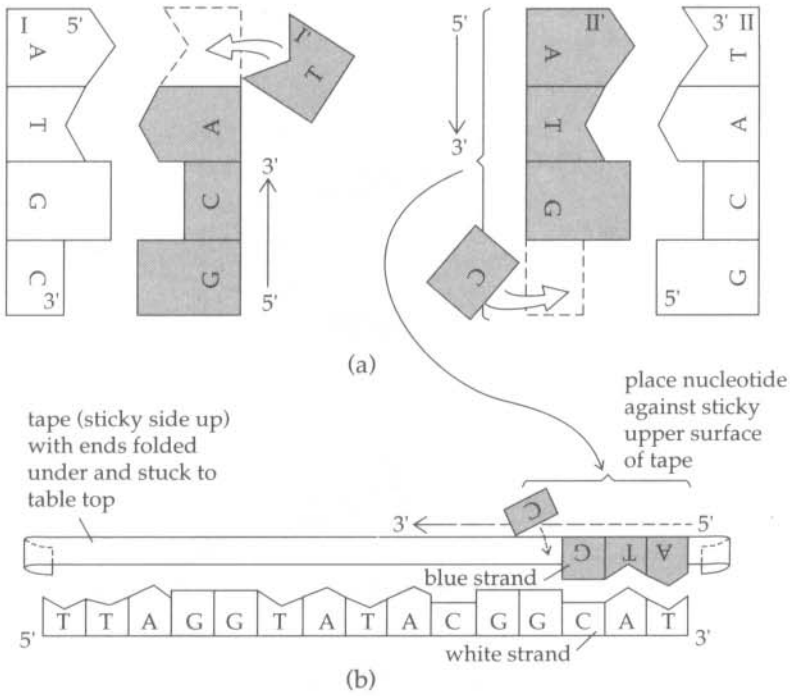


Figure 17A-5 *Replicating a DNA molecule. (a) Blue nucleotides (shown here in shading) complementary to those in the white DNA are lined up properly. (b) Nucleotides are taped to a piece of transparent tape to simulate polymerization of a polynucleotide strand in a 5′ → 3′ direction.*

4. Obtain a piece of transparent adhesive tape. Stick one end to the laboratory bench, then turn the tape over, keeping its sticky side up; turn the other end under and stick it to the bench (Figure 17A-5b). Attach each new nucleotide, letter side up, to the sticky side of the tape, aligning the straight bottom edge of each nucleotide along the straight edge of the tape so that most of the nucleotide covers the tape. Each blue nucleotide should correspond to its complementary nucleotide in the white strand (Figure 17A-5b). Be sure to synthesize the new DNA strand in the proper direction—starting at the 5′ end of each *new* strand (opposite the 3′ end of the original white DNA strand), you should add one nucleotide at a time until you reach the 3′ end of the new chain. This will be opposite the 5′ end of the original white DNA strand. Use the same method to tape together the nucleotides of the other new strand.

   This process of bonding one nucleotide to the next within a lengthening or "growing" strand is called **polymerization** and is accomplished by an enzyme, **DNA polymerase.** Do *not* tape the blue and white strands to each other.

5. Indicate which new strand was made using strand I DNA as a template and which was made using strand II DNA as a template by writing I′ and II′ on them (Figure 17A-5a). Mark the 5′ and 3′ ends of each newly synthesized strand.

   b. What types of bonds are made between nucleotides within the new strand?

   _____

   For the purposes of making a model, you have kept the blue and white DNA strands separated. However, during replication, nucleotides in the original strand are bonded to complementary nucleotides in the new strand as it is being synthesized.

   c. What types of bonds join the template and complementary polynucleotide strands together?

   _____

d. Compare the new strands (blue) with the old strands (white). To which white strand is the blue strand I′ identical? _____ *Complementary?* _____

6. Save these molecules to show your laboratory assistant, who will be coming around to check your work.

Note that the piece of DNA you have been working with is fairly short and represents only a small part of a replication "bubble" within a longer DNA molecule that is being replicated *bidirectionally* (Figure 17A-6a).

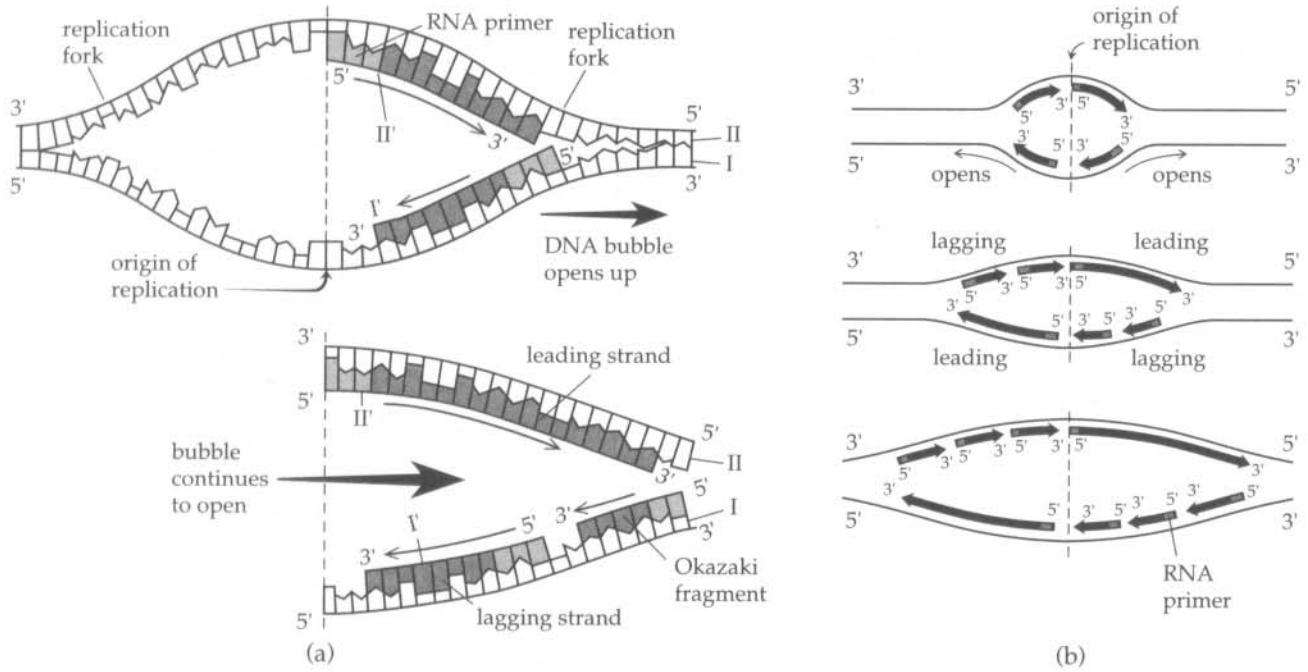


Figure 17A-6 *As a DNA replication bubble opens up bidirectionally, one strand of DNA at each replication fork can be used as a template for continuous synthesis of complementary DNA in a 5′ → 3′ direction, while the other DNA strand serves as a template for the discontinuous synthesis of a complement in short 5′ → 3′ segments that are eventually linked together.*

(a) In this replication fork, strand II serves as the complement for continuous synthesis of II′ in a 5′ → 3′ direction, corresponding to the direction in which the bubble is expanding. This strand is called the "leading strand." On the other side, however, the complement to strand I is being made discontinuously. Short I′ pieces are synthesized in a 5′ → 3′ direction that is opposite to that of bubble expansion. These pieces (called Okazaki fragments) will eventually be linked together by the enzyme DNA ligase. This new strand, synthesized more slowly and in small pieces, is called the "lagging strand." Small RNA primers are necessary to start the synthesis of both leading and lagging strands.

(b) Note that leading and lagging strands reverse in "top-bottom" orientation at opposite ends of the replication bubble because of the directions in which the opposite ends of the bubble are opening.

EXERCISE B Transcription and Translation

DNA directs the synthesis of proteins through the processes of **transcription** (DNA → RNA) and **translation** (RNA → protein). Replication, however, does *not* necessarily precede transcription and translation.

Although the genetic material, the DNA, ultimately codes for all the RNA and protein made by a cell, DNA is not *directly* involved in protein synthesis, or translation. DNA functions as a reference library from which books do not circulate. There is always an intermediate step between DNA and protein: the **messenger RNA (mRNA).**

For each particular protein to be synthesized, the DNA nucleotide sequence is first transcribed into mRNA. In eukaryotes, where a nuclear membrane separates the DNA from the cytoplasm, the mRNA is modified and then moves from the nucleus to the cytoplasm and attaches to ribosomes, where the machinery for protein synthesis is found. In prokaryotes, ribosomes can attach to mRNA even while it is being synthesized, and translation can begin immediately.

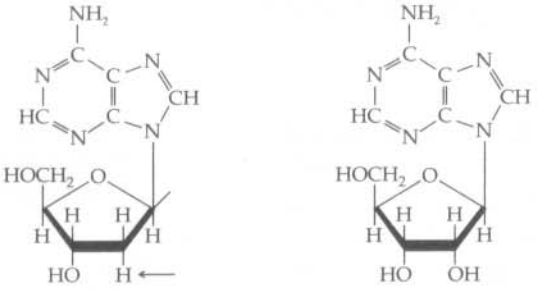
Objectives

- ☐ Differentiate between the chemical structures of RNA and DNA.
- ☐ Distinguish between the structures and roles of tRNA, rRNA, and mRNA.
- ☐ Describe the process of transcription.
- ☐ Describe the role of aminoacyl-tRNA synthetase enzymes.
- ☐ Distinguish between the functions of amino acid activation and charging of tRNAs.
- ☐ Define codon and explain why the genetic code is called a triplet code.
- ☐ Read the genetic code and identify the amino acid that corresponds to a given codon.
- ☐ List, in sequence, the steps involved in translation.
- ☐ Describe the events of elongation, translocation, and termination.
- ☐ Indicate the role of AUG, UGA, UAA, and UAG codons.

✔ PART I Transcription—RNA Synthesis

Using the I′ strand of blue DNA made from the I strand of white DNA, you will now synthesize a molecule of messenger RNA (mRNA) using the green ribonucleotides (Figure 17B-1). *Note:* You will be transcribing only one strand of the double-stranded DNA molecule. This strand serves as the template for mRNA synthesis. The messenger RNA made complementary to this strand of the DNA encodes the same information (sequence of nucleotides), with U substituted for T, as the nontranscribed strand of the original double-stranded DNA (often called the "sense" strand because it is the information in the nontranscribed DNA strand that makes "sense" and will ultimately determine the sequence of amino acids in the protein). The enzyme **RNA polymerase** is responsible for the polymerization reactions of transcription.

Figure 17B-1 *You will notice that the green nucleotides have the same shape as the blue nucleotides. The only difference in their structure is that* deoxyribonucleotides *are missing an oxygen on carbon number 2 (carbon 2′) of the sugar molecule (a). The sugar is deoxyribose in the nucleotides of DNA (a) and ribose in the nucleotides of RNA (b).*



deoxyadenosine (a) adenosine (b)

Procedure

1. Line up the green ribonucleotides complementary to the I′ template DNA strand and tape them together, as in Exercise A, to form a polynucleotide strand. Like DNA, mRNA is always

synthesized in a 5 → 3′ direction. Make sure that you tape the nucleotides together in the proper direction to simulate the process of polymerization.

2. Label the 5′ and 3′ ends of the messenger RNA you have made.

   a. What do 5′ and 3′ refer to? _____

3. Record the nucleotide sequence of your mRNA.

   5′ end _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 3′ end

4. Each group of three nucleotides in a messenger RNA is called a **codon.** Using brackets, identify the codons of your mRNA sequence as written above. (Assume the first codon begins with the first nucleotide at the 5′end.)

   b. What is the importance of these codons? _____

   _____

PART 2 Translation—Protein Synthesis

You are now ready to use your mRNA molecule to synthesize a protein. The first step in this process of translation is the **activation** of the amino acids, the addition of adenosine monophosphate (AMP) to amino acids so that they can be attached to tRNA molecules. (AMP is one part of the two-part orange ATP molecule in your kit.) Special enzymes called **aminoacyl-tRNA synthetases** (the large green molecules in your kit) then "charge" or bind specific "activated" amino acids onto the proper transfer RNAs (tRNAs)—one for each amino acid. Charged transfer RNAs carry amino acids to the ribosomes and serve as "adapters" between the code built into the nucleotide sequence of the mRNA and the sequence of amino acids in the protein. (Keep in mind that amino acids and nucleotides are two very different kinds of molecules that must be paired during the process of protein synthesis. Just as you use an adapter to put a three-pronged plug into a two-pronged outlet, a tRNA molecule pairs amino acids and nucleotides.)

Each tRNA (there are four blue tRNA molecules in your kit) has a nucleotide triplet, an **anticodon,** at a specific site in the molecule's three-dimensional structure. Eventually, this anticodon sequence will pair with a specific codon (also a nucleotide triplet) on an mRNA molecule that is being translated. Amino acids are attached to another specific site at one end of the tRNA molecule. As the anticodons of tRNA molecules pair, one at a time, with the sequence of codons in mRNA, the proper amino acids are aligned in the order dictated by the sequence of mRNA nucleotide triplets. Peptide bonds are formed between the amino acids to synthesize the polypeptide chain coded for by the mRNA.

Procedure

1. Find the four green aminoacyl-tRNA synthetase enzymes in your kit (see Figure 17B-2). Place one of the enzymes on your desk.

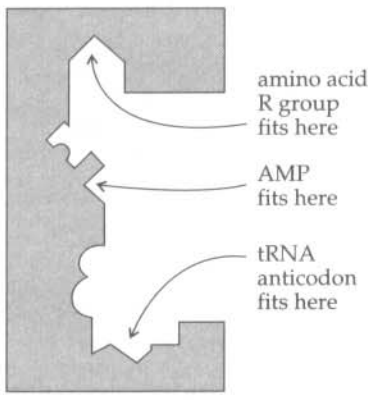


Figure 17B-2 *Each aminoacyl-tRNA synthetase enzyme contains a binding site for the unique R group of a specific amino acid and a binding site for the anticodon of a specific tRNA. In this way, a specific amino acid will be attached to a prescribed tRNA. A third binding site for the adenosine portion of AMP is also present.*

2. Find the proper amino acid that fits into the enzyme. The enzyme is shaped so that the anticodon bases of a tRNA fit into one end and the R group of a specific amino acid fits into the other. This is how a particular tRNA molecule ends up carrying its specific amino acid: each aminoacyl-tRNA synthetase enzyme contains binding sites for a specific amino acid and a specific tRNA (Figure 17B-2). Do *not*, however, insert the amino acid into the enzyme at this time.

ATP is used to "activate" the amino acid—that is, to convert it to a higher-energy form—so that it *can* be inserted into the enzyme and then attached to its tRNA. When this happens, ATP is split to form AMP and inorganic pyrophosphate (PP_i). The AMP, attached to the amino acid, fits into a groove on the enzyme (Figure 17B-3).

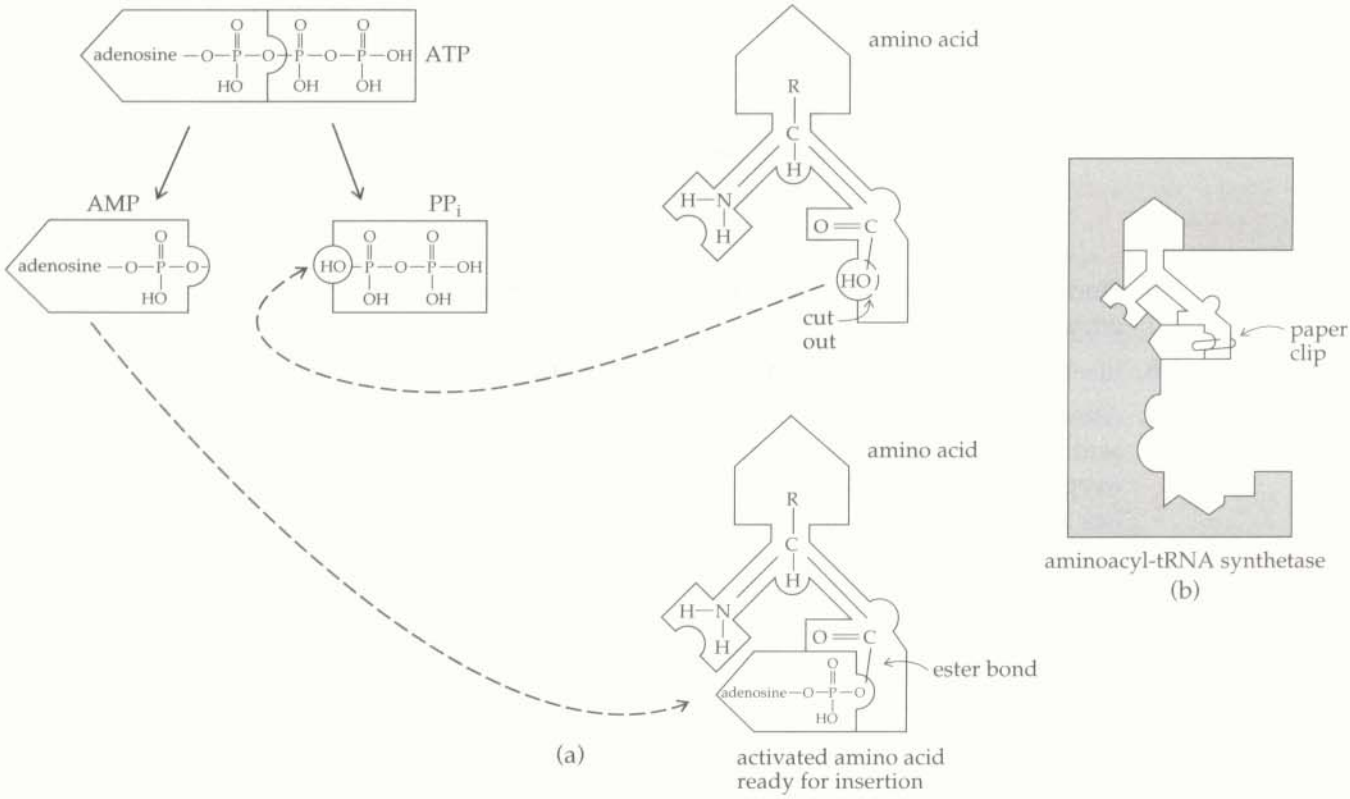
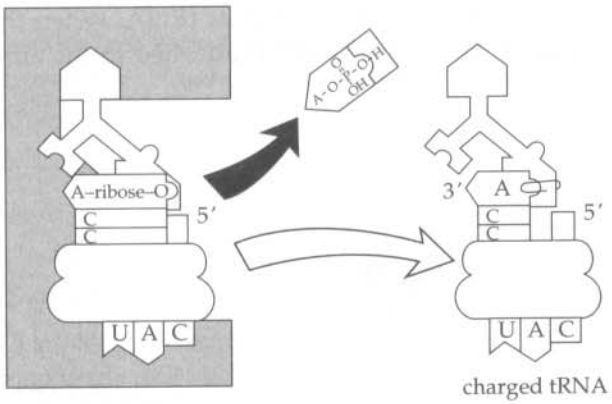


Figure 17B-3 *(a) An amino acid is activated by the splitting of ATP, resulting in the formation of AMP, inorganic pyrophosphate (PP_i), and the energy required to attach the activated amino acid to the aminoacyl-tRNA synthetase enzyme (b). During its activation, the amino acid loses an —OH group, which is incorporated into the pyrophosphate. (The nonionized forms of the amino acid, ATP, AMP, and PP_i are shown here.)*

3. Find the ATP molecule (orange). Cut the —OH group from the carboxylic acid group on the end of the amino acid you are working with. Use a paper clip to attach AMP at the same site (Figure 17B-3). Note that an ester bond is formed between the carbonyl group (C=O) of the amino acid and the phosphate group of AMP. The amino acid is now activated.
4. Insert the amino acid into the aminoacyl-tRNA synthetase enzyme.

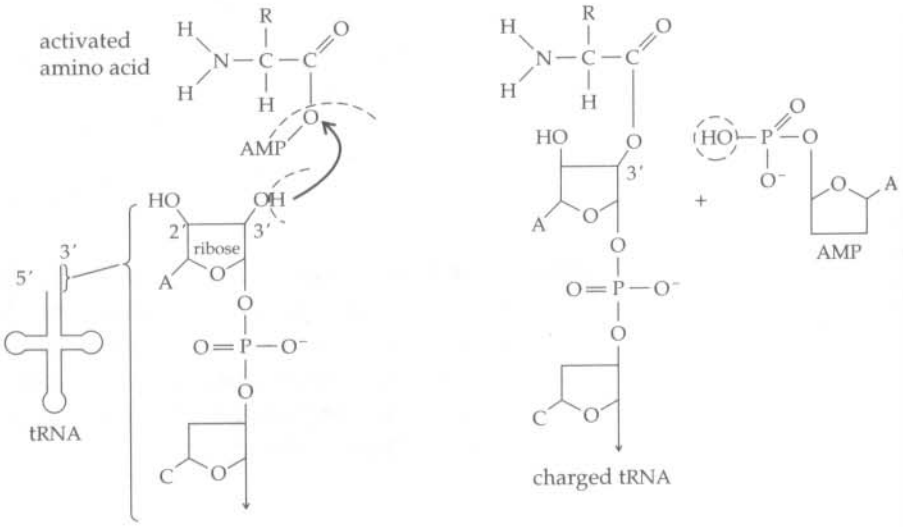
Now tRNA can enter the synthetase enzyme (Figure 17B-4). Each tRNA has the same set of three nucleotides (CCA) on its 3′ end. This is where the amino acid will be attached. Since each aminoacyl-tRNA synthetase enzyme has a special binding site for a particular anticodon, only one tRNA can enter the enzyme to bind with the already-bound amino acid. The adenine nucleotide (A) of the 3′ CCA of tRNA fits into the same groove in the synthetase enzyme as does the adenosine of AMP. When AMP is released, the A residue of the CCA can be attached to the amino acid (Figure 17B-4).

Figure 17B-4 *The amino acid attaches to the 3′ hydroxyl of the A nucleotide at the CCA end of tRNA.*



5. Find a tRNA bearing an anticodon that will fit into the proper binding site of the synthetase enzyme.
6. Insert the tRNA into the synthetase enzyme and remove the AMP molecule.
7. Attach the amino acid to the tRNA using a paper clip. The tRNA now has the correct amino acid hooked to it and is said to be a **charged** tRNA. Note that the amino acid is bound to the oxygen on the 3′ carbon of the ribose of the 3′-terminal A nucleotide of tRNA. An ester bond has been formed (Figure 17B-5).

Figure 17B-5 *Formation of an ester bond linking an amino acid to the 3′ hydroxyl of the A nucleotide at the CCA end of tRNA.*



8. The tRNA carrying its appropriate amino acid now breaks loose from the aminoacyl-tRNA synthetase molecule to participate in the process of protein synthesis. The synthetase enzyme can be used repeatedly. Remove the charged tRNA from the enzyme.
9. Now charge your three other tRNAs with amino acids, as in steps 3–8. Once all four tRNA molecules are charged, you are ready to begin the process of protein synthesis.

During protein synthesis, messenger RNA attaches to a small ribosomal subunit. In most bacteria, a short sequence of nucleotides in the 16s RNA of the small ribosomal subunit binds to a special sequence of nucleotides (the Shine-Dalgarno sequence) in the mRNA near the "start" site for protein synthesis. In eukaryotes, the 5′ cap present on all mRNAs is involved in recognition of and binding to the small ribosomal subunit.

10. Attach your messenger RNA molecule to the ribosome by sliding it up through the right-hand slit. (For convenience, the large and small ribosomal subunits are already attached to one another, but this is not the case in the living cell.) Position the first two codons between the two slits, with the **AUG** or **initiation codon** on the left and the second mRNA codon on the right. (Although the AUG codon is the first one in the mRNA you are using, this is not usually the case in cells. The nucleotides to the 5′ side of the AUG—to its left, or upstream—represent the **leader sequence,** much like the leader on a movie film or VCR tape. Once the mRNA is bound, the AUG codon will be in register at the correct site for translation.) You will translate the message in the familiar 5′ → 3′ direction (Figure 17B-6).

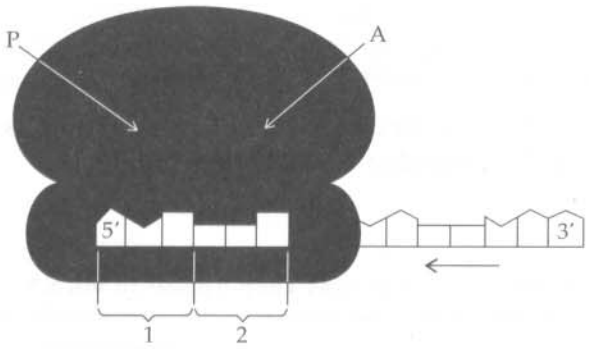


Figure 17B-6 *Attaching mRNA to the ribosome. When you first attach the mRNA, codon 1 is AUG, the initiation codon.*

Now, a charged tRNA, carrying an amino acid, will pair with the AUG initiation codon of mRNA. The 3′ end of the tRNA anticodon will pair with the 5′ nucleotide of mRNA—the tRNA and mRNA are antiparallel. (Two nucleotide strands, no matter how short, can interact only when they are antiparallel.) The combination of tRNA + mRNA + small ribosomal subunit is called the **initiation complex** (Figure 17B-7).

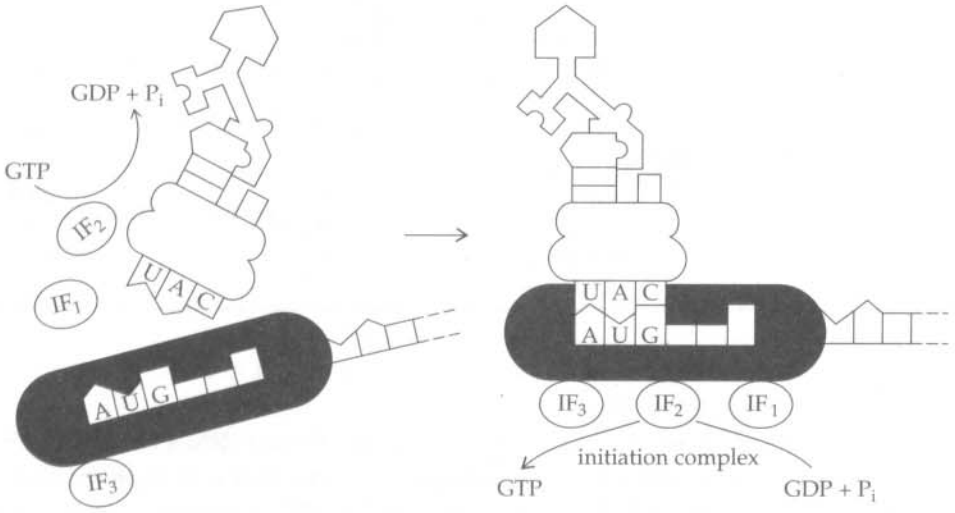


Figure 17B-7 *Formation of the initiation complex.*

A protein "factor" (small protein molecule) IF_3 is involved in binding AUG and in the attachment of mRNA to the small subunit during formation of this initiation complex. Factors IF_1 and IF_2-GTP are also involved in the attachment of the first tRNA molecule. (GTP, guanosine triphosphate, is used as a source of energy for most steps in protein synthesis.)

11. Find the blue tRNA that has an anticodon complementary to the AUG initiation codon. Pair it to the mRNA on the ribosome.

The large ribosomal subunit now attaches to the small ribosomal subunit of the initiation complex. Hydrolysis of the GTP of IF_2-GTP is required for this step. The large subunit is configured to form two major sites of activity: the **P site** (for peptidyl-tRNA), where new peptide bonds are formed, on the left, and the **A site** (for amino-acyl-tRNA), where new charged tRNAs arrive, on the right (Figure 17B-6). A third site, the **E site** (not shown), is occupied for a short time by the CCA end of the tRNA about to be ejected from the P site following removal of its amino acid during peptide bond synthesis.

12. Using the genetic code table (Table 17B-1), look up the initiation codon, AUG. Note that both methionine (Met) and formylmethionine (fMet) are specified for this codon. In prokaryotes, the initiation codon, AUG, always specifies fMet; Met is used in response to internal AUG codons in the mRNA. This means that, in prokaryotes, all proteins originally begin with fMet (which can be removed at a later time). In eukaryotes, methionine is also used for initiation, but it is not formulated. Write fMet on the yellow amino acid attached to the blue tRNA.

Important: **The genetic code specifies amino acids for codons in messenger RNA. Never look up the anticodon in the genetic code table.**

Table 17B-1 The Genetic Code: Codons as They Appear in mRNA

First Nucleotide	Second Nucleotide: U	Second Nucleotide: C	Second Nucleotide: A	Second Nucleotide: G	Third Nucleotide
U	UUU, UUC — phenylalanine UUA, UUG — leucine	UCU, UCC, UCA, UCG — serine	UAU, UAC — tyrosine UAA — stop UAG — stop	UGU, UGC — cysteine UGA — stop UGG — tryptophan	U C A G
C	CUU, CUC, CUA, CUG — leucine	CCU, CCC, CCA, CCG — proline	CAU, CAC — histidine CAA, CAG — glutamine	CGU, CGC, CGA, CGG — arginine	U C A G
A	AUU, AUC — isoleucine AUA — methionine AUG — methionine (start); formylmethionine (start)	ACU, ACC, ACA, ACG — threonine	AAU, AAC — asparagine AAA, AAG — lysine	AGU, AGC — serine AGA, AGG — arginine	U C A G
G	GUU, GUC, GUA, GUG — valine	GCU, GCC, GCA, GCG — alanine	GAU, GAC — aspartic acid GAA, GAG — glutamic acid	GGU, GGC, GGA, GGG — glycine	U C A G

13. A second tRNA now attaches to the ribosome-tRNA-mRNA complex. This tRNA fits into the A site of the large ribosomal subunit and its anticodon is complementary to the second mRNA codon (Figure 17B-8). Insert the second tRNA, carrying amino acid 2, into the A site on the mRNA-ribosome complex. Which amino acid is carried by this second tRNA? Write the name on the amino acid.

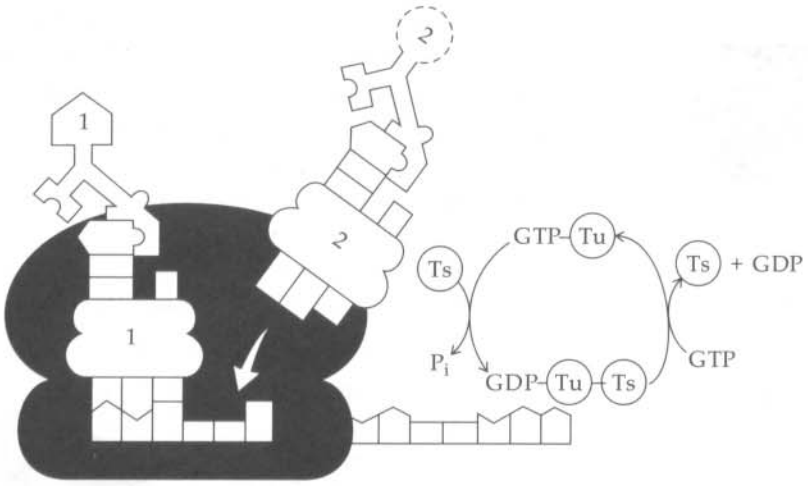


Figure 17B-8 *The large ribosomal subunit attaches to the initiation complex. Note the P and A sites in the large subunit (see Figure 17B-6). A second charged tRNA pairs with the codon located at the A site. The elongation factors shown here (Tu, Ts) are those present in bacteria (prokaryotes).*

A **peptide bond** is now formed between the —C(=O)—O— of amino acid 1 and the $—NH_2$ of amino acid 2. When this happens, the bond between the first tRNA (in the P site) and its amino acid breaks. Amino acid 1 is now held by the peptide bond to amino acid 2 on the second tRNA (Figure 17B-9). This step is called **peptidyl transfer.**

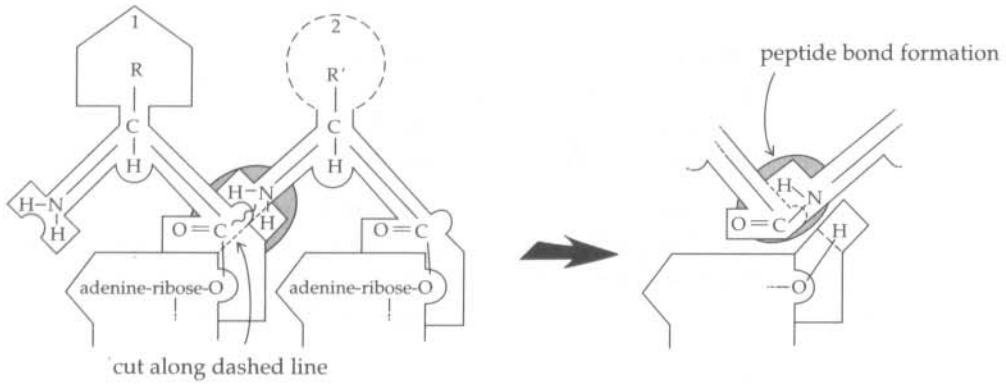


Figure 17B-9 *Formation of a peptide bond between two amino acids. The enzyme peptidyl transferase catalyzes the reaction. The H from $—NH_2$ of amino acid 2 is transferred to the oxygen in the 3′ position on the A residue of the first tRNA, restoring it to a 3′ —OH group.*

14. Attach the two amino acids together with a piece of tape. Using your scissors, remove the extra H on $—NH_2$. A peptide bond has been formed and the protein chain is now two amino acids long.

Each time a tRNA carrying an amino acid is added and a peptide bond is formed, the chain gets longer. Thus, this process is known as **elongation.** A protein elongation factor complexed with GTP aids in insertion of charged tRNAs into the A site on the ribosome. In prokaryotes, the elongation factor is a protein complex of Tu-GTP and Ts (which reactivates Tu after hydrolysis of GTP). In eukaryotes, elongation factors EF_1 and $EF_{1\beta}$ are used. Hydrolysis of GTP to GDP and P_i provides the energy for elongation (Figure 17B-8). The peptidyl transfer reaction is accomplished by an enzyme complex (**peptidyl transferase**) which is part of the large ribosomal subunit. However, recent evidence suggests that rather than ribosomal proteins possessing the enzymatic activity necessary for this reaction, it is RNA that is responsible: the RNA acts as a **ribozyme** (an RNA molecule with enzymatic activity).

15. The ribosome now moves along the message in a 5′ → 3′ direction. Place your fingers on the mRNA and move the ribosome to the right. The tRNA associated with the first codon is released since it is no longer bound to its amino acid. The process of ribosome movement from one codon to the next is known as **translocation** (Figure 17B-10). Another elongation factor (G in prokaryotes, EF_2 in eukaryotes) complexed with GTP is involved in the movement of the ribosome. Once again, GTP is hydrolyzed to provide energy for this movement.

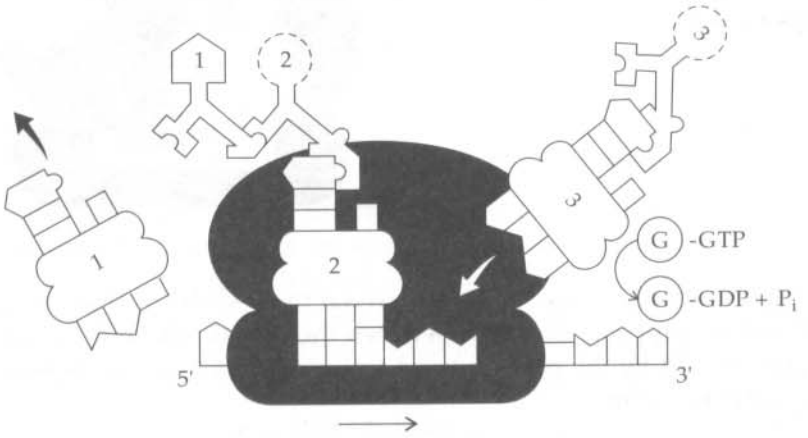


Figure 17B-10 *Translocation and insertion of a new charged tRNA. The elongation factor (G) is that present in prokaryotes.*

16. Now, tRNA 2 is on your left with the first two amino acids attached to it. Match the anticodon of tRNA 3, carrying amino acid 3, to the next codon and repeat steps 14 and 15. You should now have three amino acids attached to tRNA 3. Which amino acid has been added in this third position? Write the name on the amino acid.

17. Repeat steps 14–16 until the last mRNA codon is in the A site. There is no tRNA having an anticodon to match this mRNA codon. The codons UAA, UAG, and UGA are **termination** or "stop" **codons.** The bond between the tRNA in the P site and the protein chain attached to it is hydrolyzed with the addition of H_2O (Figure 17B-11). This releases the newly synthesized

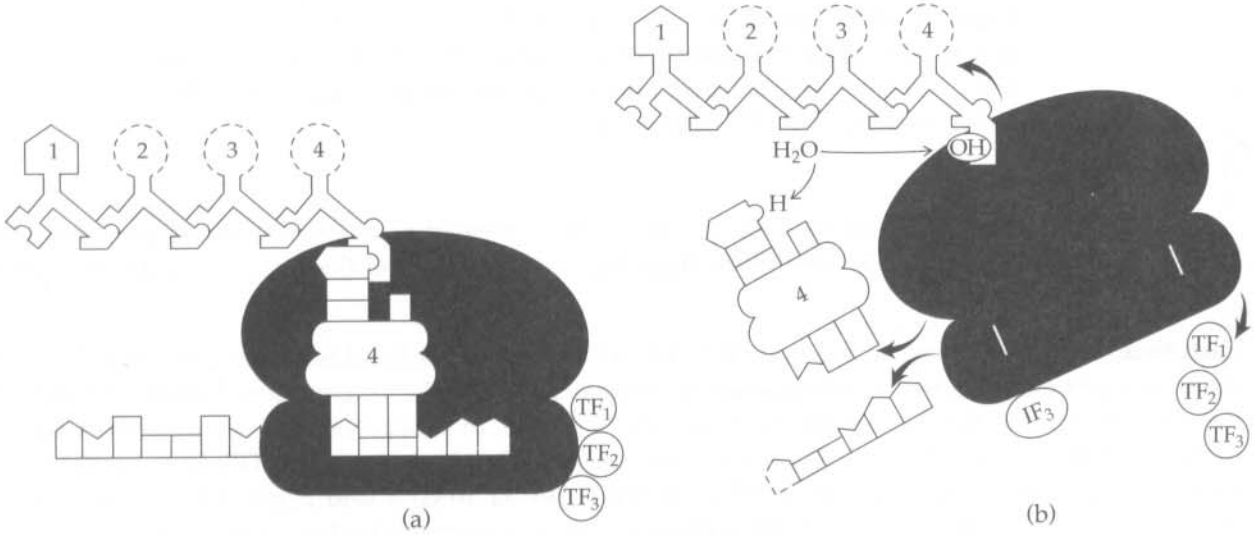


Figure 17B-11 *Chain termination. A termination or "stop" codon on mRNA is located in the A site and peptidyl transferase hydrolyzes the bond between the last amino acid of the protein and tRNA.*

protein. Several protein termination factors (TF) aid in the recognition of "stop" codons and termination of the peptide chain.

18. Release your protein chain, which should now be four amino acids long. The tRNA in the P site is also released, and the ribosomal subunits and mRNA separate.

Note that as a ribosome moves across a message, additional ribosomes can attach to the freed codons (codon 1, 2, 3, etc.). Each of these ribosomes can then serve as a site to start the synthesis of a protein. In this way, several molecules of a protein can be made simultaneously from one message. A complex of several ribosomes attached to a messenger RNA is called a **polysome.**

19. Make sure that you have identified all amino acids. Have all peptide bonds been formed correctly? Your laboratory instructor will check your work to see that you have completed the peptide chain correctly.

EXERCISE C Point Mutations in DNA

Point mutations are small changes in the DNA, such as base substitutions, base additions, and base deletions, but they may have profound effects on the protein formed by the gene, depending on where the mutations occur.

Objectives

- ☐ Determine the effect on the amino acid sequence of a point mutation (base substitution, base addition, or base deletion) in DNA.

PART 1 Base Substitutions—Possible Effects

Procedure

Refer back to the blue DNA strand that you used to make the messenger RNA for your model (Exercise B, Part 1).

a. Assume that a base substitution has occurred such that the ninth nucleotide has been changed from an A to a G. What is the sequence of the nucleotides in the third codon of the mRNA now?

_____

b. What amino acid, if any, does this codon specify? _____

c. What effect will this have on the protein formed from the DNA? _____

_____

d. If the ninth nucleotide had been changed from an A to a C, what effect would this have had on the protein formed? _____

e. If the ninth nucleotide had been changed from an A to a T, what effect would this have had on the protein formed? _____

f. Which of the base substitutions specified in questions a, d, *and* e *would be most likely to cause the production of a defective protein?* _____

PART 2 Base Substitution Resulting in Sickle-Cell Anemia

Sickle-cell anemia is a genetic disease caused by a base substitution in the DNA of the gene coding for one of the polypeptides that makes up hemoglobin, the oxygen-carrying molecule of the red blood cells.

A normal individual has two alleles for the production of normal hemoglobin. The red blood cells of this individual will have the typical "doughnut shape." An individual with **sickle-cell anemia** has two

alleles (i.e., is homozygous) for the production of sickle-cell hemoglobin. The presence of sickle-cell hemoglobin causes red blood cells to take on sickle shapes, causing difficulty in passing through small blood vessels. These cells may clump and clog the blood vessels to the internal organs, thus depriving them of needed oxygen. Individuals with sickle-cell anemia may often be pale, tired, and short of breath. They may have pain in their arms, legs, back, and abdomen, and their joints may swell. Their low resistance to infections can trigger severe worsening of their condition and may eventually be fatal. (The anemia results from the fragility of the red blood cells.)

Procedure

1. Examine the prepared slide of normal human blood that is on demonstration. Draw a representative area of the slide in the space below.

2. Examine the prepared slide of blood from a person with sickle-cell anemia. Draw a representative area of the slide in the space below.

An individual who has one allele for sickle-cell hemoglobin and one normal allele produces both normal and sickle-cell hemoglobin; the amount of normal hemoglobin is enough to prevent the individual from becoming anemic. This individual is classified as a **carrier** since he or she can pass the recessive allele to offspring. In most ways, a carrier is normal and healthy. However, when the individual is in an environment that is low in oxygen, some red blood cells will undergo sickling. Such individuals are said to have **sickle-cell trait** (*not* sickle-cell anemia).

Sickle-cell hemoglobin differs from normal hemoglobin by only one amino acid in each of two β chains. Normal hemoglobin contains glutamic acid but sickle-cell hemoglobin contains valine.

a. What are the codons for glutamic acid? _____

b. What are the codons for valine? _____

c. Based on this information, what base substitution had to occur in the DNA in order to produce the allele for sickle-cell hemoglobin? _____

Valine is a nonpolar amino acid, whereas glutamic acid is an acidic amino acid. Valine produces hydrophobic areas on the β chains that interact with hydrophobic areas on the β chains of other hemoglobin molecules, causing the hemoglobin molecules to clump. This produces the deformed red blood cells characteristic of sickle-cell anemia.

PART 3 Frame-Shift Mutations: Base Additions and Deletions

Refer back to the blue DNA strand that you used to make your mRNA (Exercise B, Part I). Assume that an A has been added between the fourth and fifth nucleotides. Indicate the sequence of the bases in the mRNA which will be transcribed from the DNA.

5′_ 3′

In the space below, indicate the sequence of amino acids in the protein coded for by this mRNA.

a. How does the above sequence of amino acids compare with the protein formed from the original DNA?

b. What effect would the removal *of a nucleotide from the original DNA have on the protein formed?*

Laboratory Review Questions and Problems

1. If DNA contained only the bases adenine and thymine, how long a code word would be necessary to enable coding for each of 20 different amino acids?

2. A particular DNA base sequence transcribed into messenger RNA is TTATCTTCGGGAGAGAAAACA. (a) If reading begins at the left, what amino acids are coded by this sequence? (*Note:* The initiation sequence is disregarded in this example.)

(b) If proflavine treatment caused the deletion of the first adenine nucleotide on the left, what changes would occur in the first six amino acids coded by this sequence?

3. Streisinger and co-workers studied amino acid sequences in the lysozyme protein produced by the T4 phage. One sequence is Lys-Ser-Pro-Ser-Leu-Asn-Ala, but as a result of a deletion of a single nucleotide and subsequent insertion of another nucleotide, this amino acid sequence was found to change to Lys-Val-His-His-Leu-Met-Ala. Using the codons in Table 17B-1, determine the nucleotide sequences that produced (a) the original amino acid sequence and (b) the subsequent changes.

4. A single (+) strand of DNA (base composition: A, 21 percent; G, 29 percent; C, 29 percent, T, 21 percent) is replicated by DNA polymerase to yield a complementary (−) strand. The resulting duplex DNA is then used as a template by RNA polymerase, which transcribes the (−) strand. Indicate the base composition of the RNA formed.

5. a. Given the following DNA molecule, write the sequence of the messenger RNA synthesized from the upper strand.

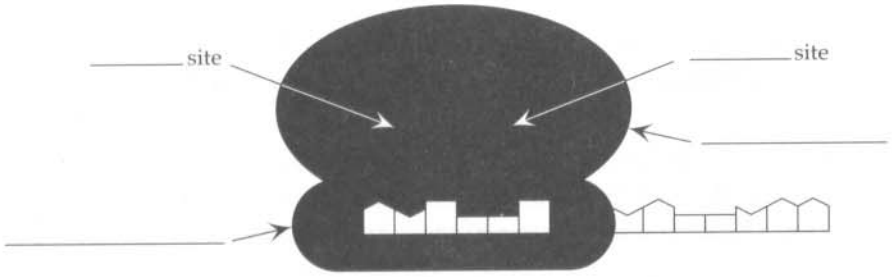
b. Indicate the 5′ and 3′ ends of the message.

c. Groups of three letters on the mRNA molecule are called _____.

d. What is the special significance of the *first* group of three letters in the message?

e. What is the special significance of the *last* group of three letters in the message?

6. a. In the diagram, label the indicated parts.



b. Label the 5′ and 3′ ends of the messenger RNA in the diagram.

c. The triplet of tRNA nucleotides responsible for insertion of the correct amino acid into a protein chain by complementing with a triplet of mRNA is known as the _____.

d. What are the last three nucleotides always found at the 3′ end of tRNA?

e. Use the space below to draw two tRNA molecules with attached amino acids. Two amino acids will be joined by a _____ bond to form a dipeptide. The enzyme responsible for this reaction is _____. Explain how the bond between the two amino acids is formed and show the final result by drawing an additional diagram.

7. You have the following DNA strand. Synthesize a protein from this strand. (Recall that a leader sequence may precede the AUG initiation codon.)

3′ AGATTACTCGAGCCGGGTAATCGGC 5′

mRNA

Protein

8. Make a strand of DNA complementary to the DNA strand in question 7. Mark the new strand's 5′ and 3′ ends. Now synthesize mRNA and a protein from this strand. Is the message the same as in question 7? Is the protein the same? (Recall that mRNA is read in a 5′ → 3′ direction!)

Complementary DNA

mRNA

Protein

9. You have synthesized the following protein: fMet-Pro-Asp-Gly-Thr. You accomplished this in a cell-free system containing tRNA molecules with the anticodons listed below:

3′ CCG 5′

5′ UGU 3′

5′ CGG 3′

5′ CAU 3′

3′ CUG 5′

mRNA

Construct the double-stranded DNA molecule from which this protein was synthesized. Show all of your reasoning.

DNA (2 strands)