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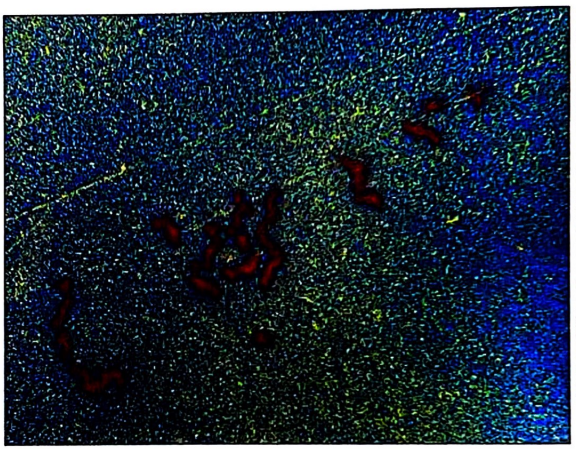
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16 Transcription and Translation

KEY CONCEPTS

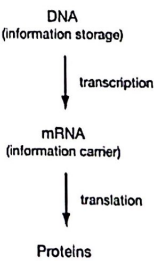
- After the enzyme RNA polymerase binds to a specific site in DNA with the help of other proteins, it catalyzes the production of an RNA molecule. The base sequence of the RNA produced is complementary to the base sequence of the DNA template strand.
- In eukaryotes, genes consist of coding regions called exons interspersed with noncoding regions called introns. After a eukaryotic mRNA is produced, introns must be spliced out. The ends of the molecule receive a cap and tail.
- Inside ribosomes, mRNAs are translated to proteins via intermediary molecules called transfer RNAs. Transfer RNAs carry an amino acid and have a three-base-pair anticodon, which binds to an mRNA codon. The amino acid carried by the transfer RNA is then added to the growing protein via formation of a peptide bond.
- Many mutations produce changes in the phenotype.



A micrograph showing transcription and translation occurring simultaneously in a bacterial cell. The yellow lines are strands of DNA, and the red structures are ribosomes.

Proteins are the stuff of life. They give cells their shape and control the chemical reactions that go on inside them. No one knows exactly how many different proteins can be made in each of your body's cells, but 100,000 is a reasonable guess. While some of those 100,000 different proteins may not be manufactured at all in certain types of cells, other proteins may be present in quantities ranging from millions of copies to fewer than a dozen. If even one of those types of protein is defective, the cell may sicken or even die.

A cell builds the proteins it needs from instructions encoded in its genome. Chapter 15 explored the basic strategy that cells use to retrieve these instructions and convert them into action in the form of proteins. The first step in the process is the transcription of a gene and the production of a messenger RNA, or mRNA. The RNA message is a short-lived copy of the archived instructions in DNA. The sequence of ribonucleotides in the mRNA is then translated into a sequence of amino acids. This series of events defines the flow of information in the cell:



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The discovery of the relationship between DNA and proteins was one of the great scientific advances of the twentieth century. But once the relationship between transcription and translation had been firmly established, biologists puzzled over how cells actually accomplish the feat of converting genetic information into protein. How do transcription and translation work? For example, how does RNA polymerase know where to start transcribing a gene and where to end? Once an RNA message is produced, how is the linear sequence of ribonucleotides translated into the linear sequence of amino acids in a protein?

This chapter explores the experiments that answered these questions. In addition to providing a deeper understanding of how cells work, the results summarized here have had a significant impact on biomedicine and drug development. Many of the antibiotics prescribed today work by disrupting translation in bacteria that cause disease. In addition, research on transcription and translation set the stage for an explosion of work, introduced in Chapters 17 and 18, on how the expression of specific genes is regulated. Most genes are transcribed and translated only at certain times or in certain types of cells.

Let's begin by examining research on the mechanisms of transcription in bacteria and eukaryotes, then delve into the mechanics of translation. The chapter concludes by examining how changes in DNA can lead to changes in protein structure and function.

16.1 Transcription in Bacteria

The first step in converting genetic information into proteins is to synthesize a messenger RNA version of the instructions archived in DNA. The enzyme RNA polymerase, introduced in Chapters 14 and 15, is responsible for synthesizing mRNA. Like the DNA polymerases introduced in Chapter 14, RNA polymerase performs a template-directed synthesis in the 5′ → 3′ direction. (Recall that 5′ and 3′ refer to carbons on the sugar subunits of ribonucleotides.) But unlike DNA polymerases, RNA polymerase does not require a primer to begin transcription.

Transcription occurs when RNA polymerase matches the base in a ribonucleotide triphosphate with the complementary base in a gene—a section of DNA. Once a matching ribonucleotide is in place, RNA polymerase catalyzes the formation of a phosphodiester bond between the 3′ end of the growing mRNA chain and the new ribonucleotide. As this matching-and-catalysis process continues, an RNA that is complementary to the gene is synthesized (**Figure 16.1**). Note that only one of the two DNA strands is used as a template and transcribed, or "read," by RNA polymerase. The strand that is read by the enzyme is called the **template strand**; the other strand is called the **non-template strand**. Because RNA has uracil (U) rather than thymine (T), an adenine (A) in the DNA template strand specifies a U in the complementary RNA strand.

Biologists reached these conclusions by studying RNA synthesis in cell-free, or in vitro, systems. Once the basic chemical

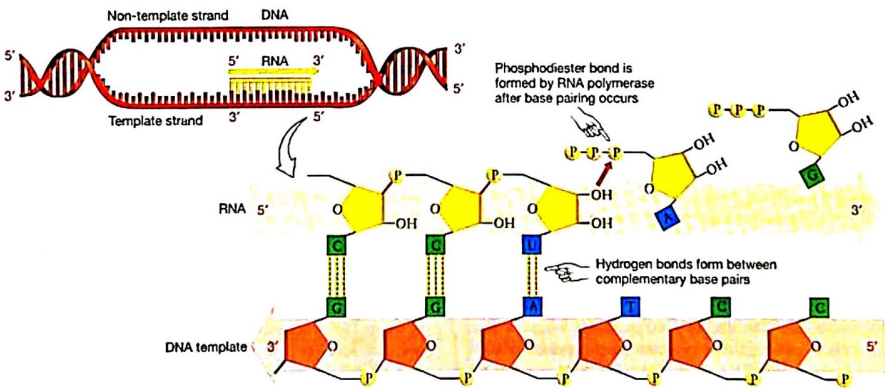


FIGURE 16.1 Transcription Is the Synthesis of RNA from a DNA Template
The reaction catalyzed by RNA polymerase results in the formation of a phosphodiester bond between ribonucleotides. RNA polymerase produces an RNA strand whose sequence is complementary to the bases in the DNA template. QUESTION In which direction is RNA synthesized, 5′ → 3′ or 3′ → 5′? In which direction is the DNA template "read"?

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reaction was understood, an entirely new set of questions arose:

- What does RNA polymerase look like?
- Are any other proteins or factors involved in transcription, or does RNA polymerase act alone?
- How does the enzyme know where to start transcription on the DNA template?

RNA Polymerase Structure and Function

To understand the structure of RNA polymerase, researchers have employed the technique called X-ray crystallography introduced in Chapter 4. Recall that this procedure allows biologists to obtain information about the three-dimensional structure of large, complex molecules.

The most recent models of bacterial RNA polymerase indicate that the enzyme is large and globular and has several prominent channels that run through the interior. The enzyme's active site, where phosphodiester bonds form, is located where several of these channels intersect. The active site contains a strategically placed magnesium atom (Mg^{2+}), which plays a critical role in stabilizing the transition state as the polymerization reaction takes place.

How does this structure correlate with the enzyme's function? It is logical to predict that DNA fits into one of the enzyme's channels and that the two strands in the double helix separate inside the enzyme to expose a single-stranded template at the active site. If so, how do the enzyme and DNA come together?

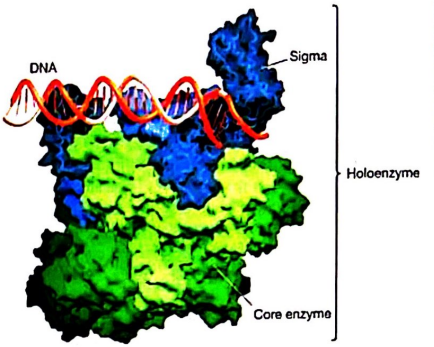


FIGURE 16.2 The Three-Dimensional Structure of RNA Polymerase Bacterial RNA polymerase is a globular enzyme. Sigma is a regulatory component, and the core enzyme contains the active site.

Initiation: How Does Transcription Begin in Bacteria?

WEB TUTORIAL 16.1 Transcription

Soon after the discovery of RNA polymerase, researchers realized that the enzyme cannot initiate transcription on its own. Instead, a detachable protein subunit called **sigma** must bind to RNA polymerase before transcription can begin. RNA polymerase and sigma form what biologists call a **holoenzyme** ("whole enzyme"). A holoenzyme consists of a **core enzyme**, which contains the active site for catalysis, and other required proteins (Figure 16.2).

If RNA polymerase is the core enzyme of this holoenzyme, what does sigma do? When researchers mixed RNA polymerase, sigma, and DNA together, they found that the holoenzyme bound tightly to specific sections of DNA. These binding sites were named **promoters**, because they are sections of DNA where transcription begins. The discovery of promoters suggested that sigma's function is regulatory in nature. Sigma appeared to be responsible for guiding RNA polymerase to specific locations where transcription should begin. What is the nature of these specific locations? What do promoters look like, and what do they do?

David Pribnow offered an initial answer to these questions in the mid-1970s. When Pribnow analyzed the base sequence of promoters from a variety of bacteria and from viruses that infect bacteria, he found that the promoters are all 40–50 base pairs long and that a particular section in each promoter looked similar. This similar segment of DNA had a series of bases identical or similar to TATAAT. This six-base-pair sequence is now known as the **−10 box**, because it occurs about 10 bases from the point where RNA polymerase starts transcription. (DNA that is located in the direction that RNA polymerase moves during transcription is said to be **downstream**; DNA in the opposite direction is said to be **upstream**. Thus, the −10 box is 10 bases upstream from the transcription start site.) Soon after, researchers recognized that the sequence TTGACA occurred in these same promoters about 35 bases upstream from the start of the mRNA. This second key sequence is called the **−35 box**.

Follow-up work showed that transcription begins when sigma binds to the −35 and −10 boxes (Figure 16.3, step 1). This is a key point: Sigma, and not RNA polymerase, makes the initial contact with DNA that starts transcription in bacteria. This finding supports the hypothesis that sigma is a regulatory protein. Sigma tells RNA polymerase where and when to start synthesizing RNA.

Once sigma binds to a promoter, the DNA helix opens and creates two strands of single-stranded DNA. As shown in step 2 of Figure 16.3, the template strand is threaded through a channel that leads to the active site inside RNA polymerase. Monomers known as *ribonucleoside triphosphates*, or NTPs, enter a channel at the bottom of the enzyme and diffuse to the active site. When an incoming NTP pairs with a complementary base on the template strand of DNA, RNA polymerization begins. The reaction catalyzed by RNA polymerase is exergonic and spontaneous because NTPs have so much potential energy, owing to their three phosphate groups. As step 3 of Figure 16.3

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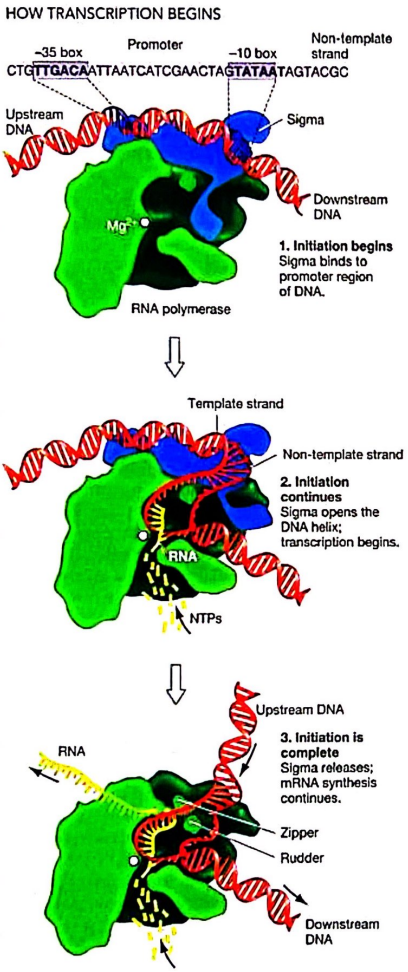


FIGURE 16.3 In Bacteria, Sigma Plays a Key Role in Initiating Transcription

When sigma attaches to RNA polymerase, the holoenzyme is able to bind to the −35 box and the −10 box of a promoter. Once it has bound to a promoter, sigma opens the DNA helix and threads the template strand through the enzyme's active site. When transcription is under way, sigma disengages from RNA polymerase. EXERCISE Circle and label RNA polymerase's active site.

shows, sigma is released once RNA synthesis is under way. The **initiation phase** of transcription is complete.

Recent work has shown that most bacteria have several types of sigma proteins, each with a distinctive structure and function. *Escherichia coli* has seven different sigma proteins, for example, while *Streptomyces coelicolour* has more than 60. Each of these proteins binds to promoters with slightly different DNA base sequences. Although all promoters have a −10 box and a −35 box, the sequences within each box vary somewhat. These variations in DNA sequences affect the ability of different sigma proteins to bind. As a result, each type of sigma protein allows RNA polymerase to bind to a different type of gene. The identity of the sigma protein in the RNA polymerase holoenzyme determines which types of genes will be transcribed. Thus by controlling which sigma proteins are active, bacterial cells control which genes are expressed.

Elongation and Termination

During the **elongation phase** of transcription, RNA polymerase moves along the DNA template in the 3′ → 5′ direction of the template strand, synthesizing RNA in the 5′ → 3′ direction. In the interior of the enzyme, a group of projecting amino acids called the enzyme's *zipper* helps open the double helix at the upstream end, and a nearby group of amino acids called the *rudder* helps steer the template and non-template strands through channels inside the enzyme. Meanwhile, the enzyme's active site catalyzes the addition of nucleotides to the 3′ end of the growing RNA molecule at the rate of about 50 nucleotides per second.

Note that during the elongation phase of transcription, three prominent channels or grooves in the enzyme are filled. Double-stranded DNA goes into and out of one groove; ribonucleoside triphosphates enter another; and the growing RNA strand exits to the rear. In this way, the enzyme's structure correlates closely with its function.

Transcription ends with a **termination phase.** In most cases, transcription stops when RNA polymerase reaches a stretch of DNA sequence that functions as a **transcription termination signal.** The bases that make up the termination signal code for a stretch of RNA with an unusual property: As soon as it is synthesized, the RNA sequence folds back on itself and forms a short double helix that is held together by complementary base pairing. The secondary structure that results is called a *hairpin* (see Chapter 4). The formation of the hairpin structure is thought to disrupt the interaction between RNA polymerase and the RNA transcript, resulting in the physical separation of the enzyme and its product.

To summarize, transcription begins when sigma binds to the promoter at the start of a gene. Once binding occurs, RNA polymerase begins to synthesize mRNA. Transcription ends when a termination signal at the end of the gene leads to the formation of a hairpin in the mRNA, disrupting the transcription complex.

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16.2 Transcription in Eukaryotes

The data reviewed in Section 16.1 established how transcription occurs in bacteria. How do these results compare with research on the mechanism of transcription in eukaryotes? What similarities and differences have come to light?

One of the first conclusions about transcription in eukaryotes is that it is similar to bacterial transcription in an important respect: RNA polymerase does not bind directly to promoter sequences by itself. Instead, proteins that came to be called **basal transcription factors** initiate eukaryotic transcription by matching the enzyme with the appropriate promoter region in DNA. The function of basal transcription factors observed in eukaryotes is analogous to the function of the sigma proteins in bacteria.

The discovery of basal transcription factors emerged from studies with cell-free, or in vitro, systems. The basic approach was to purify RNA polymerase from human cells, add template DNA, and analyze the RNAs that were produced. But in early experiments, researchers found that RNA polymerase began copying at random locations on the template DNA instead of specifically at promoter regions. In addition, when RNA polymerase was alone, both strands of the DNA were transcribed instead of just one. This observation inspired the hypothesis that eukaryotic cells must contain sigma-like proteins that are required for normal transcription. To test this hypothesis, biologists added proteins isolated from human cells to the cell-free reaction system. By adding one or a few proteins at a time and recording which ones enabled RNA polymerase to bind to promoters and correctly transcribe the template strand, researchers were gradually able to characterize the suite of proteins that came to be called basal transcription factors.

Follow-up work confirmed several important distinctions about how transcription works in bacteria and eukaryotes:

- In bacteria a single sigma protein binds to a promoter and initiates transcription, but in eukaryotes many basal transcription factors are required to initiate transcription.
- Eukaryotes have three distinct types of RNA polymerase (abbreviated RNA pol) instead of just one. As Table 16.1 shows, RNA pol I, pol II, and pol III each transcribe a discrete class of RNA. Only RNA pol II transcribes the genes that code for proteins. Stated another way, RNA pol II produces mRNA. RNA pol I, in contrast, makes the large RNA molecules that are found in ribosomes. RNA pol III manufactures both the small RNAs that are found in ribosomes and the molecules called transfer RNAs, which are required for translation. **Box 16.1** explains how research on toxic mushrooms led to the discovery that eukaryotes have three different RNA polymerases.
- Although eukaryotic genomes contain promoters that signal where transcription should begin, just as bacteria do, the promoters in eukaryotic DNA are much more diverse and complex than bacterial promoters. Many of the eukaryotic promoters recognized by RNA polymerase II include a unique sequence called the **TATA box**, located 30 base pairs upstream of the transcription start site. Some of the promoters recognized by pol II do not contain a TATA box, however. In addition, RNA pol I and pol III interact with entirely different promoters.

TABLE 16.1 Eukaryotic RNA Polymerases

Name of Enzyme	Type of Gene Transcribed
RNA polymerase I (RNA pol I)	Genes that code for most of the large RNA molecules (rRNAs) found in ribosomes (see Section 16.3)
RNA polymerase II (RNA pol II)	Protein-coding genes (produce mRNAs)
RNA polymerase III (RNA pol III)	Genes that code for transfer RNAs (see Section 16.4), and genes that code for one of the small RNA molecules (rRNAs) found in ribosomes
RNA pol II and RNA pol III	RNA molecules found in snRNPs (see Section 16.2)

The overall message of this research is that the molecular mechanisms involved in transcription are much more complex in eukaryotes than in bacteria. An even more striking contrast between bacterial and eukaryotic gene expression emerged when researchers discovered that eukaryotic genes do not consist of one continuous DNA sequence that codes for a product, as do bacterial genes. Instead, the regions of eukaryotic genes that code for proteins come in pieces that are separated by hundreds or many thousands of intervening DNA bases. Although these intervening bases are part of the gene, they do not code for a product.

The Startling Discovery of Eukaryotic Genes in Pieces

When researchers realized that the protein-coding regions of eukaryotic genes are interrupted by stretches of noncoding DNA, it became clear that the nature of the gene and information processing are different in bacteria and eukaryotes. In eukaryotes, the conversion of information in DNA sequences to mRNA sequences does not occur directly. To make a functional mRNA, eukaryotic cells must dispose of certain sequences inside genes and then combine the separated coding sections into an integrated whole.

What sort of data would provoke such a startling claim? To answer this question, consider work that Phillip Sharp and colleagues carried out in the late 1970s to determine how DNA templates are transcribed. They began one of their experiments by heating DNA molecules enough to break the hydrogen bonds between complementary bases. This treatment separated the two strands. The single-stranded DNA was then incubated with the mRNA encoded by the sequence. The team's idea was to promote base pairing between the mRNA and the single-

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BOX 16.1 Toxins and Transcription

Although wild mushrooms can be delicious, dining on certain species may be lethal. For example, in late summer and fall the reproductive structures of the death cap mushroom (*Amanita phalloides*) appear in forests, fields, and backyards in North America and Europe (Figure 16.4). The death cap is among the most poisonous of all mushrooms. Worldwide, it is thought to account for 90 percent of mushroom poisoning deaths. Eating a single death cap can kill a healthy adult.



FIGURE 16.4 The Death Cap Mushroom These mushrooms contain the toxin α-amanitin, which at low concentrations poisons the eukaryotic enzyme RNA polymerase II. QUESTION State a hypothesis to explain why the presence of α-amanitin increases the fitness of death cap mushrooms.

The active agent in death caps is a toxin called α-amanitin. By happenstance, researchers who were investigating α-amanitin's mode of action made a crucial discovery about transcription in eukaryotes. The researchers observed that mRNA synthesis stopped when eukaryotic cells were treated with low concentrations of α-amanitin. The production of other types of RNAs—specifically, transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs)—continued, however. At very high concentrations of the toxin, tRNA production also stopped but transcription of rRNA genes continued. These observations eventually led to the discovery that eukaryotes have three RNA polymerases, each with a distinct role in the transcription of mRNA, tRNA, and rRNA. Low concentrations of α-amanitin inhibit RNA polymerase II, which synthesizes mRNAs. As a result, low concentrations of the toxin halt protein synthesis. High concentrations of this poison block RNA polymerase III, which synthesizes tRNAs. RNA polymerase I is not affected by α-amanitin at any concentration.

Toxins such as α-amanitin are useful in research on transcription and translation for the same reason that knock-out mutations, which completely disable genes, are useful in genetics. Toxins allow researchers to understand what happens when a molecule or a particular stage in a process does *not* work. This is similar, in concept, to learning how a car works by removing a part or a system and carefully recording the machine's response; or to learning how the brain works by studying the behavior of people whose brains have been damaged in specific, quantifiable ways by accidents. Toxins and knock-out mutations provide a molecular dissecting kit. They let biologists explore the intricacies of cell function by inactivating key components.

stranded DNA. Under these conditions, the research team expected the mRNA to form base pairs with the DNA sequences that act as the template for its synthesis. When the researchers examined the DNA-RNA hybrid molecules with the electron microscope, however, they observed the structure shown in Figure 16.5a. Instead of matching up exactly, parts of the DNA formed loops. As Figure 16.5b shows, Sharp and his coworkers interpreted these loops as stretches of nucleotides that are present in the DNA template strand but are *not* in the corresponding mRNA.

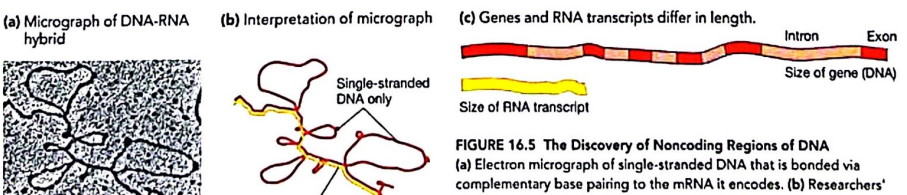


FIGURE 16.5 The Discovery of Noncoding Regions of DNA **(a)** Electron micrograph of single-stranded DNA that is bonded via complementary base pairing to the mRNA it encodes. **(b)** Researchers' interpretation of the photograph in part (a). The loops represent regions of DNA that do not have an equivalent sequence in the mRNA. These intervening regions are "extra" DNA compared with the sequences in the mRNA. **(c)** Because eukaryotic genes contain both introns and exons, these genes are much larger than the corresponding mature mRNA.

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To make sense of these and other findings, Sharp's group and a team headed by Richard Roberts proposed that there is *not* a one-to-one correspondence between the nucleotide sequence of a eukaryotic gene and its mRNA. By analogy, eukaryotic genes do not carry messages such as "Biology is my favorite course of all time." Instead, eukaryotic genes carry messages that read something like "Biolτηεπρστειν χοδιψγρεγιονσοφγενεσogy is my favor αρειντερρνπτεδβψνονχοδινγδite course οfανδηαω̈ετοβειπλιχεδτογετηερall time." The sections of noncoding sequence must be removed from the mRNA before it can carry an intelligible message to the translation machinery.

When it became clear that the genes-in-pieces hypothesis was correct, Walter Gilbert suggested that regions of eukaryotic genes that are part of the final mRNA be referred to as **exons** (because they are expressed) and the untranslated stretches as **introns** (because they are *int*ervening). Introns are sections of genes that are not represented in the final mRNA product. As a result, eukaryotic genes are much larger than their corresponding mature RNA transcripts (Figure 16.5c, page 343).

Exons, Introns, and RNA Splicing

The transcription of eukaryotic genes by RNA polymerase generates a **primary RNA transcript** that contains both the exon and intron regions (Figure 16.6a). As transcription proceeds, the introns are removed from the growing mRNA strand by a process known as **splicing**. In this phase of information processing, pieces of the primary transcript are removed and the remaining segments are joined together. Splicing occurs while transcription is still under way and results in an mRNA that contains an uninterrupted genetic message.

Figure 16.6b provides more detail about how introns are removed from genes. In the case illustrated here, splicing is catalyzed by a complex of proteins and small RNAs known as small nuclear ribonucleoproteins, or **snRNPs** (pronounced "snurps"). The process begins when snRNPs bind to specific sequences on the primary RNA transcript that define the exon-intron boundaries. Once the initial snRNPs are in place, other snRNPs arrive to form a multipart complex called a **spliceosome**. Recent data have shown that the spliceosomes found in human cells contain about 145 different proteins and RNAs, making them the most complex molecular machines known. Once the spliceosome forms, the intron forms a loop with an adenine ribonucleotide at its base (step 1, Figure 16.6b). The loop breaks when the 2′ hydroxyl group on the adenine attacks a phosphodiester bond at the 5′ end of the intron.

Once the initial cut in the RNA is made, the free 5′ end of the intron becomes attached to the adenine nucleotide and forms a "lariat" of RNA (step 2, Figure 16.6b). Then the free 3′ end of the first exon reacts with the 5′ end of the second exon. This reaction

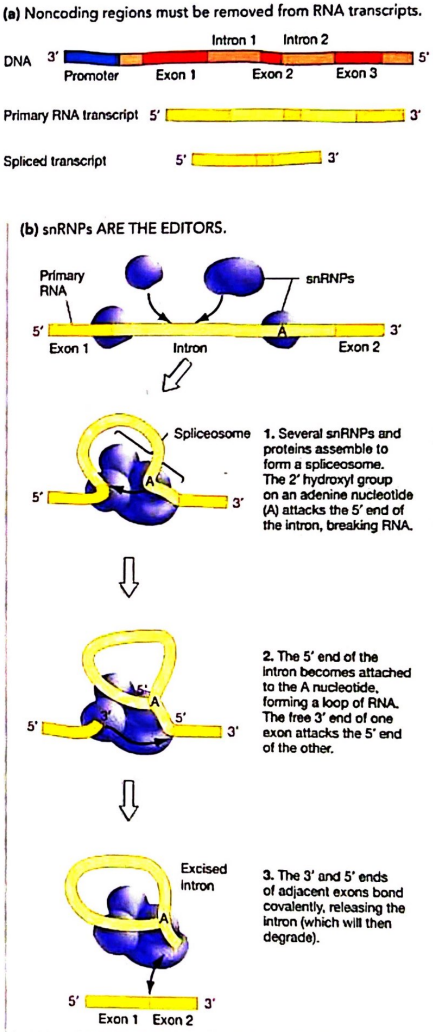


FIGURE 16.6 Introns Are Spliced Out of the Original mRNA **(a)** RNA polymerase produces a primary RNA transcript that contains exons and introns. Subsequent processing generates a mature transcript. **(b)** snRNPs are responsible for the splicing reactions that take place in the nucleus of eukaryotes.

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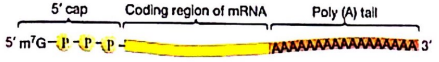


FIGURE 16.7 In Eukaryotes, mRNAs Are Given a Cap and a Tail Eukaryotic mRNAs have a cap consisting of a molecule called 7-methylguanylate (symbolized as m^7G) bonded to three phosphate groups; the tail is made up of a long series of adenine residues.

breaks the 3′ end of the intron and covalently joins the two exons into a contiguous coding sequence (step 3, Figure 16.6b). Splicing is now complete. In most cases, the excised intron is degraded to ribonucleotide monophosphates.

Current data suggest that both the cutting and rejoining reactions that occur during splicing are catalyzed by RNA molecules in the spliceosome. Recall from Chapter 4 that RNA molecules with catalytic ability are called ribozymes. In eukaryotes, ribozymes play a key role in the production of proteins.

Other Aspects of Transcript Processing: Caps and Tails

Intron splicing is not the only way that primary mRNA transcripts are processed in eukaryotes. As soon as the 5′ end of a eukaryotic mRNA emerges from RNA polymerase, enzymes add a structure called the **5′ cap** (**Figure 16.7**). The cap consists of the molecule 7-methylguanylate and three phosphate groups. When transcription is complete, an enzyme cleaves the 3′ end of most mRNAs, and other enzymes add a long tract of 100–250 adenine nucleotides. This sequence is known as the **poly (A) tail**.

Not long after the caps and tails on eukaryotic mRNAs were described, evidence began to accumulate that they protect mRNAs from degradation by ribonucleases and that they enhance the efficiency of translation. For example, experimental mRNAs that have a cap and a tail last longer when they are introduced into cells than do experimental mRNAs that lack a cap, a tail, or both a cap and a tail. The mRNAs with caps and tails also produce more proteins than do mRNAs without caps and tails.

Recent work has shown why the cap and tail are so critical to mRNAs. The 5′ cap serves as a recognition signal for the translation machinery, and the poly (A) tail extends the life span of an mRNA by protecting the message from degradation by ribonucleases in the cytoplasm.

✓CHECK YOUR UNDERSTANDING

Transcription occurs when RNA polymerase catalyzes the 5′ → 3′ synthesis of an RNA molecule. RNA synthesis is driven by the potential energy stored in nucleotide triphosphates and is based on matching complementary base pairs to the sequence in a template DNA strand. **Table 16.2** summarizes some of the similarities and differences observed in transcription in bacteria versus eukaryotes. You should be able to diagram (1) how transcription initiation, elongation, and termination occur in bacteria and eukaryotes and (2) how mRNAs are processed in eukaryotes.

16.3 An Introduction to Translation

In translation, the sequence of bases in a messenger RNA molecule is converted into a sequence of amino acids in a polypeptide. The genetic code specifies the relationship between the bases of a triplet codon in mRNA and the amino acid it codes for (see Chapter 15). But how are the amino acids assembled into a polypeptide according to the information in messenger RNA?

Studying how translation occurred in cell-free systems proved to be an extremely productive approach to answering this question. Once in vitro translation systems had been developed from human cells, *E. coli*, and a variety of other organisms, it became clear that the basic mechanisms of translation are fundamentally the same throughout the tree of life. The sequence of events that occurs during protein synthesis is similar in bacteria and eukaryotes.

TABLE 16.2 Comparing Transcription in Bacteria and Eukaryotes

Aspect	Bacteria	Eukaryotes
RNA polymerase	One	Three; each produces a different class of RNA
Promoter structure	Typically contains a −35 box and a −10 box	Complex and variable; often includes a TATA box −30 from start of gene
Protein(s) involved in contacting promoter	Sigma; different versions of sigma bind to different promoters	Many basal transcription factors
RNA processing steps	None; translation occurs while transcription is still under way	Extensive; several processing steps occur in nucleus before RNA is exported to cytoplasm for translation: 1. Enzyme-catalyzed addition of 5′ cap 2. Splicing (intron removal) by spliceosome 3. Enzyme-catalyzed addition of 3′ poly (A) tail

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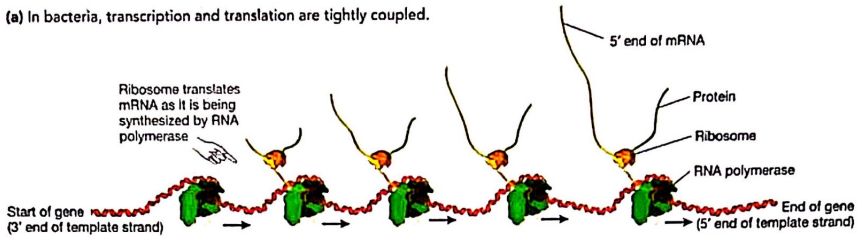


FIGURE 16.8 Transcription and Translation Occur Simultaneously in Bacteria but Not in Eukaryotes
(a) In bacteria, ribosomes attach to mRNA transcripts and begin translation while RNA polymerase is still transcribing the DNA template strand. (b) In eukaryotes, mRNA transcripts are processed inside the nucleus before they are exported to the cytoplasm. There, they are translated by ribosomes. EXERCISE In bacteria, many ribosomes attach to an mRNA transcript at the same time—so many copies of the protein product are made simultaneously. Add two ribosomes to the longest mRNA transcript in part (a), along with the protein each is producing.

Ribosomes Are the Site of Protein Synthesis

The first question that biologists answered about translation concerned where it occurs. The answer was inspired by a simple observation: There is a strong, positive correlation between the number of small cellular structures known as **ribosomes** in a given type of cell and the rate at which that cell synthesizes proteins. For example, immature human red blood cells divide rapidly, synthesize millions of copies of the protein hemoglobin, and contain large numbers of ribosomes. In contrast, the same cells at maturity have low rates of protein synthesis and very few ribosomes. Based on this correlation, investigators proposed that ribosomes are the site of protein synthesis in the cell.

To test this hypothesis, Roy Britten and collaborators did a pulse-chase experiment similar in design to experiments introduced in Chapter 7. Recall that the goal of a pulse-chase experiment is to label a population of molecules as they are being produced. The location of the tagged molecules is then followed through time. The tagging is done by supplying a pulse of radioactive atoms that are incorporated into the molecule as it is being synthesized, followed by a chase of unlabeled atoms. Britten's group wanted to label amino acids with radioactive atoms and then track the molecules as translation occurred.

The biologists began by feeding a pulse of radioactive sulfate ($^{35}SO_4^{-2}$) to growing cultures of *E. coli*. They expected the cells to incorporate the radioactive sulfur into the amino acids methionine and cysteine—which contain sulfur (see Chapter 3)—and then into newly synthesized proteins. Fifteen seconds after adding the radioactive sulfate, the researchers "chased" the label by adding a large excess of nonradioactive sulfate to the culture medium. The chase vastly reduced the incorporation of radioactive sulfur into proteins. If the ribosome hypothesis was correct, then the radioactive signal should be associated with ribosomes for a short period of time. Later, all of the radioactivity should be found in proteins independently of the ribosomes.

This is exactly what the researchers found. Soon after the pulse of labeled sulfate ended, the radioactive atoms were found in free amino acids or in ribosomes. Later, all of the radioactive atoms were found on completed proteins. Based on these data, biologists concluded that proteins are synthesized at ribosomes and then released.

About a decade after the ribosome hypothesis was confirmed, electron micrographs like this chapter's opening photograph showed bacterial ribosomes in action. The images confirmed that, in bacteria, ribosomes attach to mRNAs and begin synthesizing proteins even before transcription is complete (Figure 16.8a). Transcription and translation can occur concurrently in bacteria because there is no nuclear envelope to

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separate the two processes. In bacteria, transcription and translation are physically connected. But in eukaryotes, mRNAs are spliced in the nucleus and then exported to the cytoplasm. Once RNA messages are outside the nucleus, ribosomes attach to them and begin translation (Figure 16.8b). In eukaryotes, transcription and translation are separated in time and space.

How Does an mRNA Triplet Specify an Amino Acid?

During translation, genetic information is converted from nucleic acids to proteins. In effect, the hereditary instructions are translated from one distinctive chemical language into another. The discovery of the genetic code revealed that each triplet codon in mRNA specifies a particular amino acid in a protein. How does this conversion happen?

One early hypothesis was that mRNA codons and amino acids interact directly. The proposal was that the bases in a particular codon were complementary in shape or charge to the side group of a particular amino acid (Figure 16.9a). But Francis Crick pointed out that the chemistry involved didn't make sense. For example, how could the nucleic acid bases interact with a hydrophobic amino acid side group, which does not form hydrogen bonds?

Crick proposed an alternative hypothesis. As Figure 16.9b shows, he suggested that some sort of adapter molecule holds amino acids in place while interacting directly and specifically with a codon in mRNA via hydrogen bonding. In essence, Crick predicted the existence of a chemical go-between that produced a physical connection between the two types of molecules. Crick, as it turns out, was right.

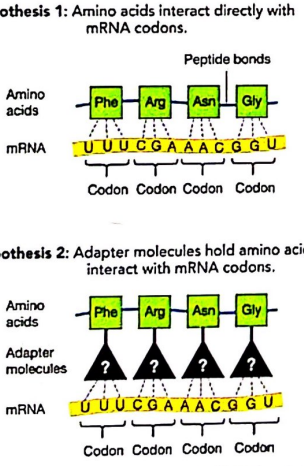


FIGURE 16.9 Two Hypotheses for How mRNA Codons Interact with Amino Acids
(a) An early hypothesis suggested that amino acids and mRNA codons interact directly. (b) The adapter-molecule hypothesis predicts that mRNA codons do not interact directly with the amino acids being added to a growing polypeptide. Instead, an intermediary adapter molecule carries an amino acid and aligns it with the appropriate mRNA codon.

16.4 The Role of Transfer RNA

Crick's adapter molecule was discovered by accident. Biologists were trying to work out a cell-free protein synthesis system derived from mammalian liver cells and had discovered that ribosomes, mRNA, amino acids, ATP, and a molecule called guanosine triphosphate, or GTP, had to be present for translation to occur. (GTP is similar to ATP but contains guanosine instead of adenosine.) These results were logical—ribosomes provide the catalytic machinery, mRNAs contribute the message to be translated, amino acids are the building blocks of proteins, and ATP and GTP supply potential energy to drive the endergonic polymerization reactions responsible for forming proteins. But in addition, a cellular fraction that contained a previously unknown type of RNA turned out to be indispensable. If this type of RNA is missing, protein synthesis does not occur. What is this mysterious RNA, and why is it essential to translation?

The novel class of RNAs eventually became known as **transfer RNA (tRNA)**. The role of tRNA in translation was a mystery until some researchers happened to add a radioactive amino acid—leucine—to an in vitro protein synthesis system. The treatment was actually done as a control for an unrelated experiment. To the researchers' amazement, some of the radioactive leucine attached to tRNA molecules. Follow-up experiments showed that the attachment of an amino acid to a tRNA requires an input of energy in the form of ATP. A tRNA molecule that becomes covalently linked to an amino acid, such as any of the molecules observed in the lucky experiment, is called an **aminoacyl tRNA**. More recent research has shown that enzymes called **aminoacyl tRNA synthetases** are responsible for catalyzing the addition of amino acids to tRNAs. For each of the 20 major amino acids, there is a different aminoacyl synthetase and one or more tRNAs. Figure 16.10, page 348, summarizes how amino acids are transferred from aminoacyl tRNA synthetases to tRNAs.

What happens to the amino acid that is carried by an aminoacyl tRNA? Biologists who tracked the fate, over time, of radioactive leucine molecules that were attached to tRNAs discovered that the amino acids are transferred from aminoacyl tRNAs to proteins. The data that support this conclusion are

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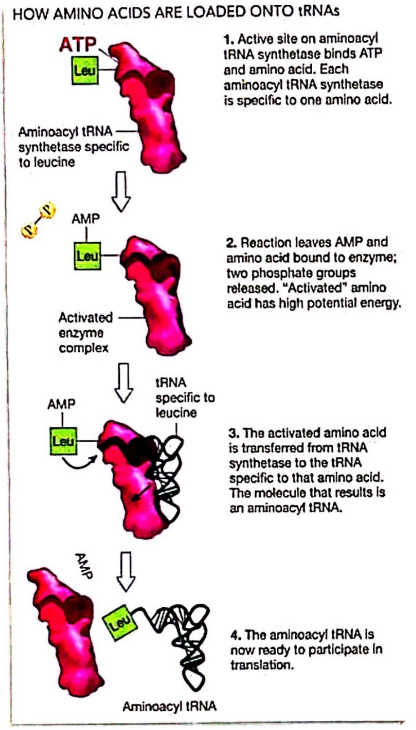


FIGURE 16.10 Aminoacyl tRNA Synthetases Load Amino Acids onto the Appropriate tRNA
An aminoacyl tRNA synthetase—in this case, for leucine—becomes activated in a reaction that requires ATP. Activated aminoacyl tRNA synthetases transfer an amino acid to a tRNA, producing an aminoacyl tRNA.

shown in Figure 16.11. The graph's message is that radioactive amino acids are lost from tRNAs and incorporated into polypeptides synthesized in ribosomes. These results inspired the use of *transfer* in tRNA's name. The experiment also confirmed that aminoacyl tRNAs act as the interpreter in the translation process. tRNAs are Crick's adapter molecules.

Connecting Structure with Function

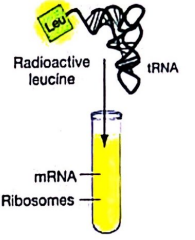
Transfer RNAs serve as the chemical go-betweens that allow amino acids to interact with an mRNA template. But precisely how does this connection occur?

Question: Are amino acids that are attached to tRNA molecules transferred to proteins?

Hypothesis: Aminoacyl tRNAs transfer amino acids to growing polypeptides.

Null hypothesis: The amino acids held on tRNAs are not involved in protein synthesis.

Experimental setup:

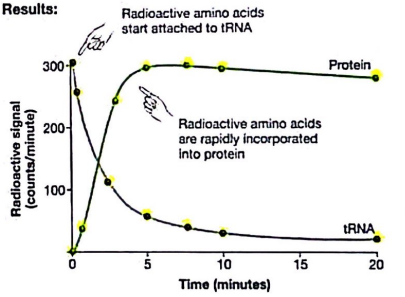


1. Attach radioactive leucine molecules to tRNAs.
2. Add these aminoacyl tRNAs to in vitro translation system. Follow the fate of the radioactive amino acids.

Prediction: Radioactive amino acids will be found in proteins.

Prediction of null hypothesis: Radioactive amino acids will not be found in proteins.

Results:



Conclusion: Aminoacyl tRNAs transfer amino acids to growing polypeptides.

FIGURE 16.11 Experimental Evidence That Amino Acids Are Transferred from tRNAs to Proteins

This question was answered by research on tRNA's molecular structure. The initial studies established the sequence of nucleotides in various tRNAs, or what is termed their primary structure. Transfer RNAs are relatively short, ranging from 75 to 85 nucleotides in length. When biologists studied the primary sequence closely, however, they noticed that certain parts of the molecules can form secondary structures. Specifically, some of the bases in the tRNA molecule can form hydrogen bonds with complementary bases in a different region of the

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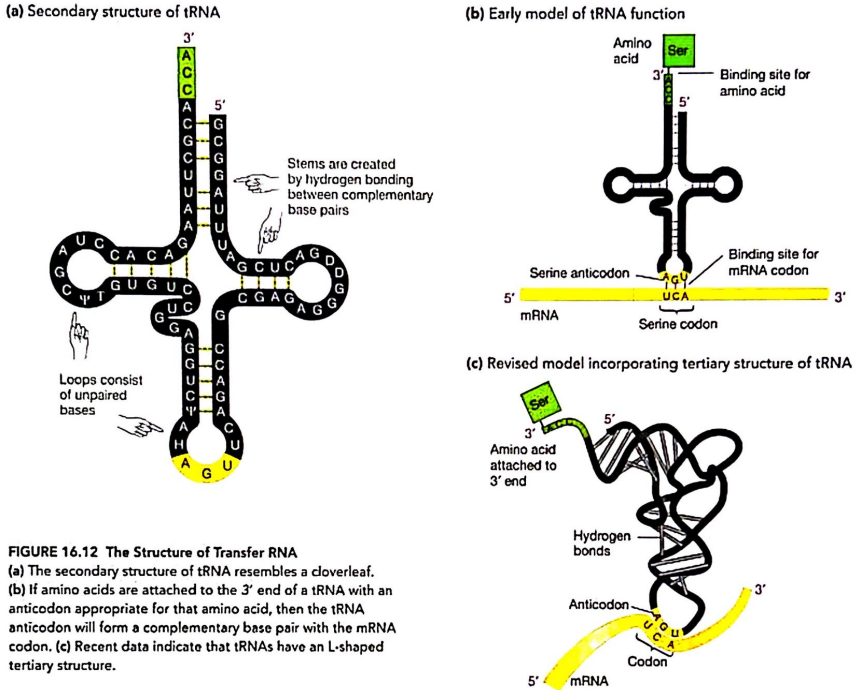


FIGURE 16.12 The Structure of Transfer RNA
(a) The secondary structure of tRNA resembles a cloverleaf. **(b)** If amino acids are attached to the 3′ end of a tRNA with an anticodon appropriate for that amino acid, then the tRNA anticodon will form a complementary base pair with the mRNA codon. **(c)** Recent data indicate that tRNAs have an L-shaped tertiary structure.

same molecule. As a result, the entire tRNA molecule could assume the cloverleaf shape illustrated in Figure 16.12a. The stems in the cloverleaf are produced by complementary base pairing between different portions of the molecule, while the loops consist of single-stranded RNA.

Two aspects of this secondary structure proved especially interesting. The CCA sequence at the 3′ end of the molecule offered a binding site for amino acids, while the triplet on a loop on the opposite end of the cloverleaf could serve as an anticodon. An **anticodon** is a set of three nucleotides that forms base pairs with the mRNA codon. Figure 16.12b depicts an early model of how the anticodon of a tRNA connects an mRNA codon with the appropriate amino acid.

This model had to be modified, however, when X-ray crystallography studies revealed the tertiary structure of tRNAs. Recall from Chapter 3 that the tertiary structure of a molecule is defined by the three-dimensional arrangement of its atoms and is usually a product of folding. According to the X-ray crystallography data, the cloverleaf structure folds over to produce an L-shaped molecule (Figure 16.12c). All of the tRNAs in a cell have the same L-shaped structure, but each has a distinct anticodon and attached amino acid. The tertiary structure of tRNAs is important because it results in a precise separation between the anticodon and the attached amino acid. As we'll see, this separation is key to the positioning of the amino acid and the anticodon in the ribosome.

How Many tRNAs Are There?

When research succeeded in characterizing all of the different types of tRNAs available in cells, a paradox arose. According to the genetic code introduced in Chapter 15, the 20 amino acids found in proteins are specified by 61 different mRNA codons. Instead of containing 61 different tRNAs with 61 different anticodons, though, most cells contain only about 40. How can all 61 mRNA codons in the genome be translated with only two-thirds of the tRNAs required?

To resolve this paradox, Francis Crick proposed what is known as the **wobble hypothesis.** To understand his logic, recall from Chapter 15 that many amino acids are specified by more than one codon. Further, recollect that codons for the same

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amino acid tend to have the same nucleotides at the first and second positions but a different nucleotide at the third position. For example, both of the codons CAA and CAG code for the amino acid glutamine. (Codons are always written in the 5′ → 3′ direction.) Surprisingly, experimental data have shown that a tRNA with an anticodon of GUU can base pair with both CAA and CAG in mRNA. (Anticodons are always written in the 3′ → 5′ direction.) The GUU anticodon matches the first two bases (C and A) in both codons, but the U in the third position forms a nonstandard base pair with a G in the CAG codon.

Crick proposed that inside the ribosome, tRNAs with nonstandard base pairing at the third position can still bind successfully to an anticodon. If so, it would allow a limited flexibility, or "wobbling," in the base pairing. According to the wobble hypothesis, a nonstandard base pair in the third position—such as G-U—is acceptable as long as it does not change the amino acid for which the codon codes.

Follow-up research has shown that certain tRNAs are especially prone to pairing with more than one mRNA codon. These tRNAs have a nitrogen-containing base called *inosine* in the third position of their anticodon. (Inosine is derived from adenine by a chemical modification that occurs after the tRNA is produced.) During protein synthesis, an inosine in the anticodon can base pair with a A, U, or C in an mRNA codon. For example, the tRNA with the anticodon GAI carries the amino acid leucine and binds to four of the six codons that specify leucine: CUA, CUC, CUU, and UUA. (The UUA is possible because the G in the anticodon sometimes pairs with U in the mRNA. This interaction between anticodons and codons is one of the only known exceptions to the standard base-pairing rules.) In this way, "wobble" in the third position of a codon allows just 40 or so tRNAs to bind to all 61 mRNA codons.

16.5 Ribosomes and the Mechanism of Translation

During translation, the sequence of bases in an RNA message is converted to a sequence of amino acids in a polypeptide. The conversion begins when the anticodon of a tRNA that carries an amino acid binds to a codon in mRNA. The conversion is complete when a peptide bond forms between that amino acid and the growing polypeptide chain.

Both of these events occur inside a ribosome. Biologists have known since the 1930s that ribosomes contain a considerable amount of protein along with a great deal of **ribosomal RNA (rRNA)**. When high-speed centrifuges became available, researchers found that ribosomes could also be separated into two major substructures, called the *large subunit* and *small subunit*. Each subunit consists of a complex of RNA molecules and proteins. For example, the large subunit in *E. coli* contains two different rRNA molecules and 34 proteins. More recent work has shown that the small subunit holds the mRNA in place during translation and that peptide bond formation takes place in the large subunit.

The three-dimensional model of the ribosome in Figure 16.13a shows how all of the molecules and reactions required for translation fit together. Note that during protein synthesis,

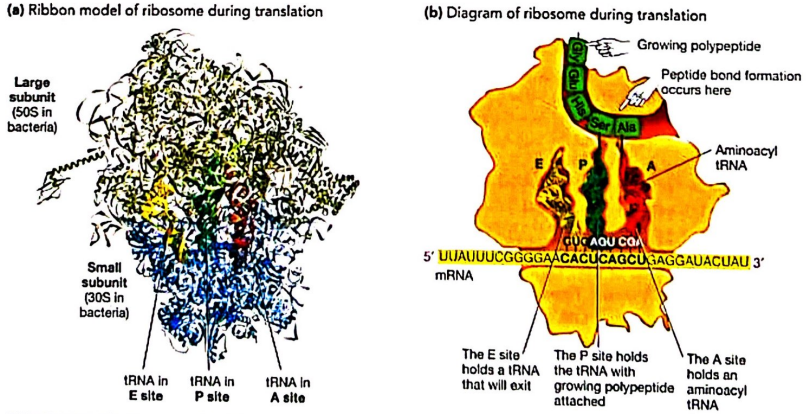


FIGURE 16.13 The Structure of the Ribosome
(a) During translation, three tRNAs line up side by side inside the ribosome. (b) Different events occur at each of the three sites inside the ribosome where tRNAs are found. EXERCISE Circle and label the active site in the ribosome.

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three distinct tRNAs are lined up inside the ribosome. All three are bound to their corresponding mRNA codon at the base of the structure. The tRNA that is on the right (colored red) in the figure carries an amino acid. This tRNA's position in the ribosome is called the **A site**, for acceptor or aminoacyl. The tRNA that is in the middle (green) holds the growing polypeptide chain and occupies the **P site**, for peptidyl, inside the ribosome. (Think of "P" for "peptide-bond formation.") The left-hand (yellow) tRNA no longer has an amino acid attached and is about to leave the ribosome. It occupies the ribosome's **E site**, for exit. Figure 16.13b summarizes what happens to each tRNA inside the ribosome. Because all tRNAs have the same secondary and tertiary structure, they all fit equally well in the A, P, and E sites.

The ribosome is a molecular machine that synthesizes proteins in a three-step sequence:

1. An aminoacyl tRNA diffuses into the A site; its anticodon binds to a codon in mRNA.
2. A peptide bond forms between the amino acid held by the aminoacyl tRNA in the A site and the existing polypeptide, which is held by a tRNA in the P site.
3. The ribosome moves ahead, and all three tRNAs move one position down the line. The tRNA in the E site exits; the tRNA in the P site moves to the E site; and the tRNA in the A site switches to the P site.

The protein that is being synthesized grows by one amino acid each time this three-step sequence repeats. The process occurs up to 20 times per second in bacterial ribosomes and about 2 times per second in eukaryotic ribosomes.

This introduction to how tRNAs, mRNAs, and ribosomes interact during protein synthesis leaves a number of key questions unanswered, however. How do mRNAs and ribosomes get together to start the process? Once protein synthesis is under way, how is peptide bond formation catalyzed inside the ribosome? And how does protein synthesis conclude when the ribosome reaches the end of the message? Let's consider each question in turn.

Initiation

To translate an mRNA, a ribosome must begin at a specific point in the message, translate the mRNA up to the message's termination codon, and then stop. Biologists call these three phases of protein synthesis **initiation**, **elongation**, and **termination**, respectively. One key to understanding initiation is to recall, from Chapter 15, that the codon AUG is found near the *5′* end of all mRNAs and codes for the amino acid methionine. The presence of this start codon is an aspect of initiation that is common to both bacteria and eukaryotes.

Figure 16.14 shows how translation gets under way in bacteria. The process begins when a section of rRNA in the small subunit of a ribosome binds to a complementary sequence on an mRNA. The mRNA region is called the **ribosome binding site** or **Shine-Dalgarno sequence**, after the biologists who discovered it. The site is about six nucleotides upstream from the AUG start codon. It consists of all or part of the bases *5′* AGGAGGU *3′*. The complementary sequence in the rRNA of the small subunit reads *3′* UCCUCCA *5′*. The interaction between the small subunit and the message is mediated by proteins called **initiation factors**. In eukaryotes, initiation factors bind to the *5′* cap on mRNAs and guide it to the ribosome.

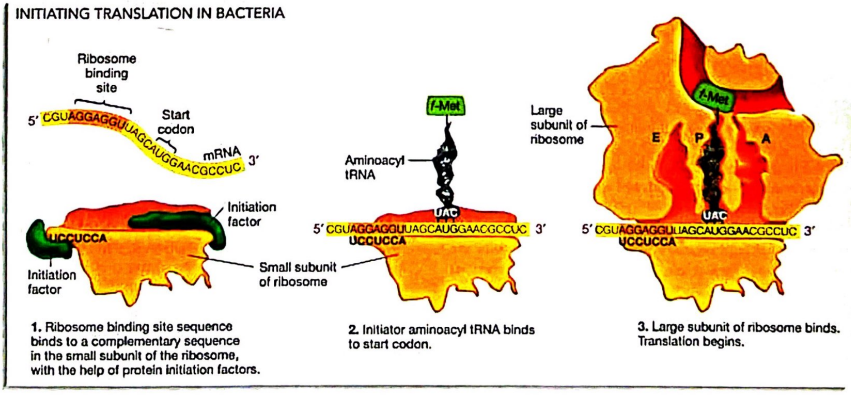


FIGURE 16.14 The Initiation Phase of Translation in Bacteria

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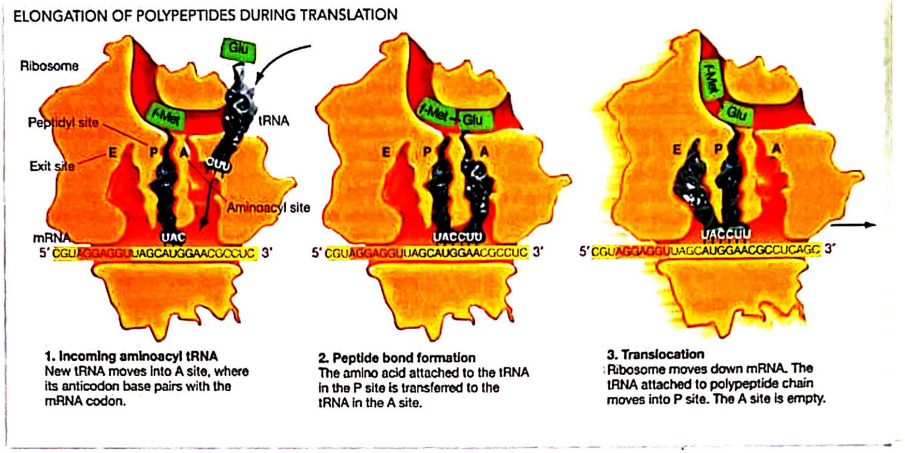


FIGURE 16.15 The Elongation Phase of Translation

Once the Shine-Dalgarno sequence has attached to the small ribosomal subunit, an aminoacyl tRNA bearing a modified form of methionine called *N*-formylmethionine (abbreviated *f*-met) binds to the AUG start codon. Initiation is complete when the large subunit joins the complex. When the ribosome is completely assembled, the tRNA bearing *f*-met occupies the P site.

To summarize, initiation is a three-step process in bacteria: (1) The mRNA binds to the small subunit of the ribosome, (2) the initiator aminoacyl tRNA binds to the start codon, and (3) the large subunit of the ribosome completes the complex.

It's important to note that in bacteria a single mRNA may contain several start codons, termination codons, and ribosome binding sites and thus code for several distinct proteins. Such mRNAs are said to be **polycistronic**. In polycistronic messages, an AUG start sequence near the 5′ end of the mRNA is followed by a termination signal farther along, which in turn is followed by an AUG start sequence for a second protein and then a second termination signal, and so on.

Elongation

At the start of elongation, the E and A sites in the ribosome are empty of tRNAs. As a result, an mRNA codon is exposed at the base of the A site. As step 1 in Figure 16.15 illustrates, elongation proceeds when an aminoacyl tRNA binds to the codon in the A site via complementary base pairing between anticodon and codon.

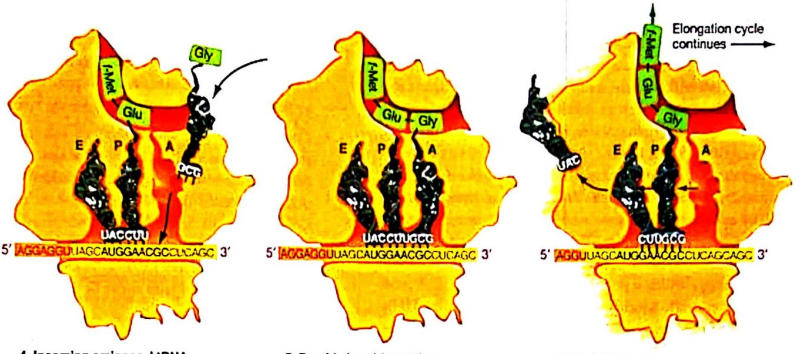
When a tRNA occupies both the P site and A site, the amino acids they hold are placed in the ribosome's active site. This is where peptide bond formation—the essence of protein synthesis—occurs. Peptide bond formation is considered one of the most important reactions that takes place in cells, because manufacturing proteins is among the most fundamental of all cell processes. The question is, how does it happen?

Is the Ribosome an Enzyme or a Ribozyme? Because ribosomes contain both proteins and RNA, researchers had argued for decades over whether the active site consisted of protein or RNA. The debate was not resolved until the year 2000, when researchers completed three-dimensional models that were detailed enough to view the structure of the active site. These models confirmed that the active site consists entirely of ribosomal RNA. Based on these results, biologists are now convinced that protein synthesis is catalyzed by RNA. The ribosome is a ribozyme—not an enzyme.

The observation that protein synthesis is catalyzed by RNA is important, because it supports the RNA world hypothesis introduced in Chapter 4. Recall that proponents of this hypothesis claim that life began with RNA molecules and that the presence of DNA and proteins in cells evolved later. If the RNA world hypothesis is correct, then it is logical to observe that the production of proteins is catalyzed by RNA.

Moving Down the mRNA What happens after a peptide bond forms? Step 2 in Figure 16.15 shows that when peptide bond formation is complete, the polypeptide chain is transferred from the tRNA in the P site to the amino acid held by the tRNA in the A site. Step 3 shows the process called **translocation**, which occurs when the ribosome moves down

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4. Incoming aminoacyl tRNA
New tRNA moves into A site, where its anticodon base pairs with the mRNA codon.

5. Peptide bond formation
The polypeptide chain attached to the tRNA in the P site is transferred to the tRNA in the A site.

6. Translocation
Ribosome moves down mRNA. The tRNA attached to polypeptide chain moves into P site. Empty tRNA from P site moves to E site, where tRNA is ejected. The A site is empty again.

the mRNA in the 5′ → 3′ direction. Translocation does several things: It moves the empty tRNA into the E site; it moves the tRNA containing the growing polypeptide into the P site; and it opens the A site and exposes a new mRNA codon. If the E site is occupied when translocation occurs, the tRNA there is ejected into the cytoplasm.

The three steps in elongation—(1) arrival of aminoacyl tRNA, (2) peptide bond formation, and (3) translocation—repeat down the length of the mRNA. By adding specific molecules to in vitro translation systems, researchers have confirmed that each elongation cycle depends on an input of energy from several GTP molecules as well as assistance from proteins called **elongation factors**. Further, recent three-dimensional models of ribosomes in various stages of the translation sequence show that the machine as a whole is highly dynamic during the process. The ribosome constantly changes shape as tRNAs come and go and catalysis and translocation occur. Its structure changes in conjunction with its multipart function: coordinating interactions between tRNAs and an mRNA, and catalyzing peptide bond formation.

Electron microscopy has also confirmed that once elongation is under way, additional ribosomes bind to the start site in the same message and initiate translation. As this process continues, strings of ribosomes called **polyribosomes** assemble along an mRNA. Each ribosome synthesizes a copy of the protein (Figure 16.16). Even though it may take a minute or more for a single ribosome to produce a large polypeptide, the presence of polyribosomes can quicken the overall pace of protein production. Polyribosomes are observed routinely in both bacteria and eukaryotes.

Termination

How does protein synthesis end? To answer this question, recall from Chapter 15 that the genetic code includes three stop codons: UAA, UAG, and UGA. In most cells, no aminoacyl tRNA has an anticodon that binds to these sequences. When

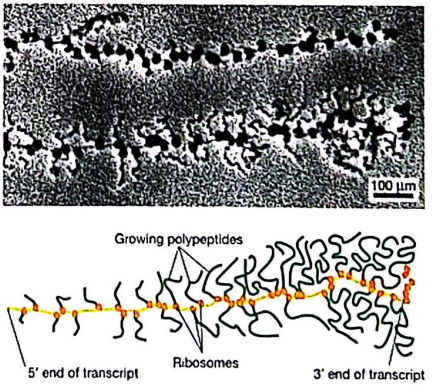


FIGURE 16.16 Polyribosomes
In both bacteria and eukaryotes, many ribosomes synthesize proteins from the same mRNA at the same time. The resulting structures are called polyribosomes.

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translocation opens the A site and exposes one of the stop codons, a protein called a **release factor** fills the A site (Figure 16.17). Release factors are proteins that fit tightly into the A site because their size, shape, and electrical charge are extremely similar to the same properties in a tRNA. Release factors do not carry an amino acid, however. When a release factor occupies the A site, the active site catalyzes the hydrolysis of the bond linking the tRNA in the P site with the polypeptide chain. This reaction frees the polypeptide, and it is released from the ribosome. The ribosome then separates from the mRNA, and the two ribosomal subunits dissociate. The subunits are ready to attach to the start codon of another message and start translation anew.

Post-Translational Modifications Proteins are not fully formed and functional when termination occurs. From earlier chapters, it should be clear that most proteins go through an extensive series of processing steps, collectively called **post-translational modification**, before they are ready to go to work in a cell:

- Recall from Chapter 3 that a protein's function depends on its shape and that a protein's shape depends on how it folds. Folding actually begins during elongation, long before termination occurs and ribosomes disassemble. Although it occurs spontaneously, in the sense that no energy input is required, folding is frequently speeded up by proteins called **molecular chaperones.** Recent data have shown that, in some bacteria, chaperone proteins actually bind to the ribosome near the "tunnel" where the growing polypeptide emerges from the ribosome. This result suggests that folding occurs as the polypeptide is emerging from the ribosome.
- Chapter 7 pointed out that many eukaryotic proteins are extensively modified after they are synthesized. For example, small chemical groups may be added to proteins in the organelles called the rough endoplasmic reticulum and the Golgi apparatus. Some proteins receive a sorting signal that serves as an address and ensures that the molecule will be carried to the correct location in the cell. Certain proteins are also augmented with sugar or lipid groups that are critical for normal functioning.
- Many proteins are altered by enzymes that add or remove a phosphate group. Phosphorylation (addition of phosphate) and dephosphorylation (removal of phosphate) of proteins were introduced in Chapter 3 and Chapter 8. Recall that because a phosphate group has two negative charges, adding or removing a phosphate group causes major changes in the shape and chemical reactivity of proteins. These changes have a dramatic effect on the protein's activity—often switching it from an inactive state to an active state or vice versa.

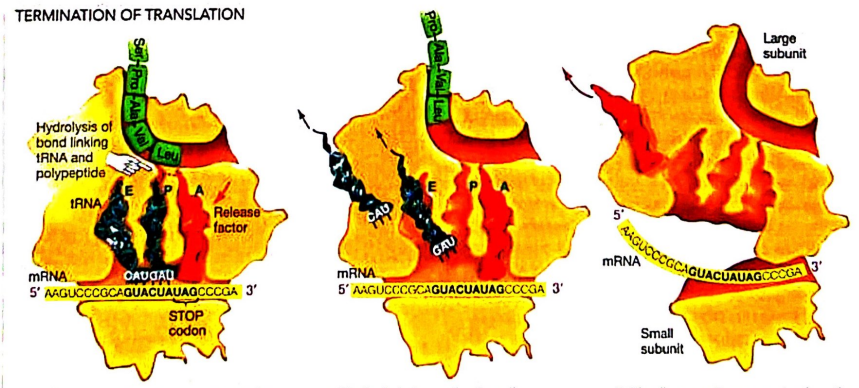


FIGURE 16.17 The Termination Phase of Translation

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The overall message is that the manufacture of a completely functional protein depends not only on translation in the ribosome but also on a wide array of other molecules and events that take place throughout the cell.

✓CHECK YOUR UNDERSTANDING

In bacteria, translation begins when the ribosome binding site on an mRNA binds to an rRNA sequence in the small subunit of the ribosome. The initiator aminoacyl tRNA binds to the start codon in the mRNA, and the large subunit of the ribosome attaches to the small subunit to complete the ribosome.

In both bacteria and eukaryotes, polypeptides elongate when the appropriate aminoacyl tRNA enters the A site. A peptide bond forms between the amino acid held by that tRNA in the A site and the polypeptide held by the tRNA in the P site. The ribosome then moves down the mRNA one codon, and the process occurs again. Translation ends when the ribosome reaches a stop codon. You should be able to (1) diagram a ribosome during translation initiation, elongation, and termination; (2) label each major structure in the diagrams; and (3) describe the function of each major structure.

16.6 The Molecular Basis of Mutation

This chapter has focused on how the information in DNA is transcribed to RNA and then translated into a functioning protein.

Although biologists continue to work on issues such as how basal transcription factors in eukaryotes interact with RNA polymerase and how ribosomes change shape during translation, the molecular mechanisms responsible for information flow in the cell, from DNA to RNA to proteins, are now considered fairly well understood.

Before we go on to consider how transcription and translation are regulated in bacteria and eukaryotes in the next two chapters, it will be helpful to tie together some major points covered in the last three chapters by analyzing the molecular basis of mutation. A **mutation** is any change in an organism's DNA. It is a change in a cell's information archive—a change in its genotype. What are the consequences of mutation?

The molecular machinery introduced in this chapter faithfully transcribes and translates mutations in DNA sequences. The result is the production of novel types of proteins. In this way, changes in DNA lead to changes in molecules that affect the organism's phenotype.

Figure 16.18 shows how a common type of mutation occurs. If DNA polymerase mistakenly inserts the wrong base as it synthesizes a new strand of DNA, and if proofreading by DNA polymerase and the mismatch repair system fail to correct the mismatched base, a change in the sequence of bases in DNA results. A single base change such as this is called a **point mutation.**

What happens when point mutations are transcribed and translated? To answer this question, consider the first point mutation ever described. This mutation occurs in the human gene for the protein hemoglobin. Recall that hemoglobin is abundant in red blood cells and that it carries oxygen to tissues.

Figure 16.19a, page 356, shows a small section of DNA sequence from the normal gene for hemoglobin, along with the same sequence from the mutant allele found in individuals who suffer from sickle-cell disease. The mutant form has a thymine in place of an adenine at the second position in the sixth codon specified by this gene. A glance back at the genetic code in Chapter 15 confirms why this point mutation is significant: During protein synthesis, the mutant codon specifies valine instead of glutamic acid in the amino acid chain of hemoglobin. Point mutations that cause changes in the amino acid sequence of proteins are called **missense mutations** or **replacement mutations.** In the case of hemoglobin, the single change in primary structure causes the protein to crystallize when oxygen levels in the blood are low. When hemoglobin crystallizes, it causes red blood cells to become sickle shaped (Figure 16.19b). The misshapen cells get stuck in blood vessels, so nearby cells are starved for oxygen. The result is intense pain and anemia.

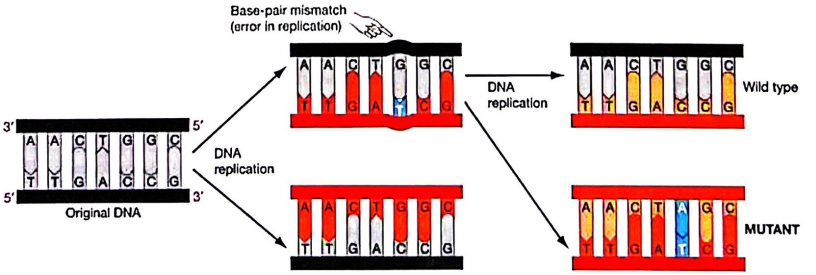


FIGURE 16.18 Unrepaired Mistakes in DNA Synthesis Lead to Point Mutations
QUESTION Why is it logical that the type of mutation illustrated here is termed a point mutation?

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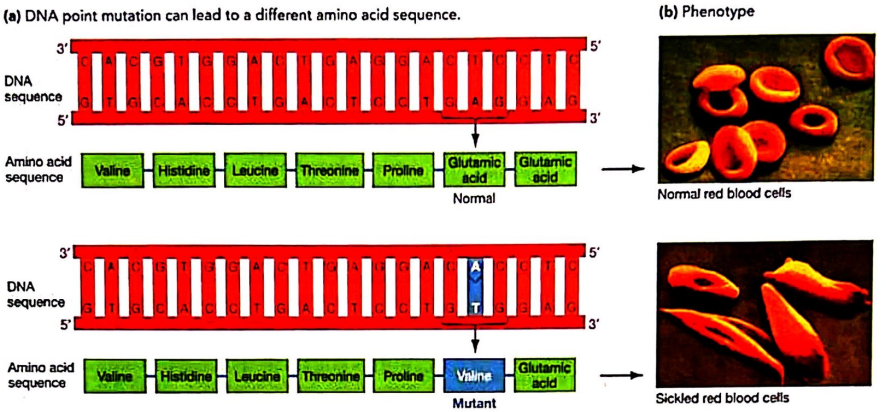


FIGURE 16.19 Sickle-Cell Disease Results from a Point Mutation in the Gene for Hemoglobin
(a) These normal and mutant genes that code for hemoglobin differ by a single nucleotide. The sequences shown are just a small portion of the gene. **(b)** The mutation in part (a) changes the primary sequence of the gene product. The change in amino acid sequence causes hemoglobin molecules to crystallize when oxygen levels in the blood are low. As a result, red blood cells sickle and get stuck in small blood vessels. EXERCISE The mRNA codon GUG specifies the valine at the start (left) of this protein. Label the template strand and the non-template strand of DNA, and indicate their 5′ and 3′ ends.

Missense mutations such as that found in the sickling allele can be **deleterious**, meaning they reduce an individual's fitness—the ability to survive and reproduce. But missense mutations may also be beneficial. For example, the sickling allele confers a fitness advantage in regions of the world where the life-threatening disease malaria is common. The advantageous effect is possible because the parasite that causes malaria infects red blood cells. If an individual has one normal hemoglobin allele and one sickling allele, red blood cells that become infected with the parasite tend to sickle while others function normally. The deformed cells are quickly destroyed by the body, eliminating the parasitic cells inside the blood cells. As a result, individuals who have one copy of the mutant gene have lower parasite loads and tend to be much healthier than people who have two normal copies of the gene.

In contrast to having a negative or positive effect on the organism, many point mutations have virtually no effect at all. To understand why, suppose that at the next site in the hemoglobin gene of Figure 16.19 (the third position in the same sixth codon), a thymine had been substituted for the cytosine. This point mutation would have no consequence. Why? Both GAA and GAG specify glutamic acid. In this case, the amino acid sequence of the gene product does not change even though the DNA sequence is altered due to mutation. This type of alteration in the base sequence is called a **silent mutation**. Silent mutations are said to be **neutral** in their effect on an individual's fitness. It is possible for point mutations to be silent and neutral with respect to fitness because of the redundancy in the genetic code.

The take-home message from this analysis of hemoglobin, sickle-cell disease, and malaria is simple: It is possible for point mutations to be deleterious, beneficial, or neutral. **Figure 16.20a** summarizes the types of point mutations that have been documented and reviews their consequences for the amino acid sequences of proteins.

In addition to documenting various types of point mutations, biologists study mutations that involve larger-scale changes in the composition of chromosomes. Chapter 9 introduced the phenomena called polyploidy and aneuploidy, which involve changes in the number of chromosome complements present and the addition or deletion of a chromosome, respectively. Just as point mutations are caused by random errors made by DNA polymerase, chromosome changes result from chance mistakes in the partitioning of chromosomes during meiosis or mitosis. In addition, errors that occur in crossing over during prophase of meiosis I (Chapter 12) can lead to gene duplications or deletions, as illustrated in **Figure 16.20b**. Finally, when accidental breaks in

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(a) Types of point mutations

	Definition	Example	Consequence
		Original DNA sequence – TAT TGG CTA GTA CAT Original polypeptide – Tyr Trp Leu Val His	
Silent	Change in nucleotide that does not change amino acid specified by codon	TAC TGG CTA GTA CAT Tyr Trp Leu Val His	Change in genotype but no change in phenotype
Missense (Replacement)	Change in nucleotide that changes amino acid specified by codon	TAT TGT CTA GTA CAT Tyr Cys Leu Val His	Change in primary structure of protein
Nonsense	Change in nucleotide that results in early stop codon	TAT TGA CTA GTA CAT Tyr STOP	Premature termination—polypeptide is truncated
Frameshift	Addition or deletion of a nucleotide	TAT TCG GCT AGT ACA T Tyr Ser Ala Ser Thr	Reading frame is shifted—massive missense

(b) Other types of mutations

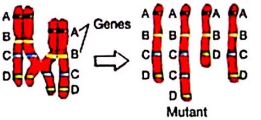
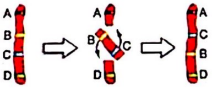
	Definition	Example	Consequence
Gene duplication	Addition of a small chromosome segment due to an error during crossing over at meiosis I—homologs do not align correctly		Produces an extra copy or deletion of one or more genes. Families of related genes arise by gene duplication.
Chromosome inversion	Change in a chromosome segment when DNA breaks in two places, flips, and rejoins		Changes gene order along chromosome. Other types of chromosome breaks can lead to deletion or addition of chromosome segments.

FIGURE 16.20 A Mutation Is a Change in an Organism's DNA
(a) Point mutations in DNA produce an array of effects on the amino acid sequences of proteins, ranging from no change to extensive change. **(b)** Many mutations involve large-scale changes in the composition of chromosomes. In addition to gene duplication and chromosome inversions, Chapter 9 introduced polyploidy (multiple chromosome complements), trisomy (additional chromosomes), and aneuploidy (loss or gain of a chromosome).

chromosomes occur, the chromosome segments may become flipped and rejoin—a phenomenon known as a **chromosome inversion** (Figure 16.20b).

When changes in an organism's genotype are transcribed and translated, they may result in changes in its phenotype. In this way, mutations produce new alleles and new traits. At the level of populations, mutations furnish the heritable variation that Mendel and Morgan analyzed and that makes evolution possible. At the level of individuals, mutations can cause disease or death or lead to increases in fitness.

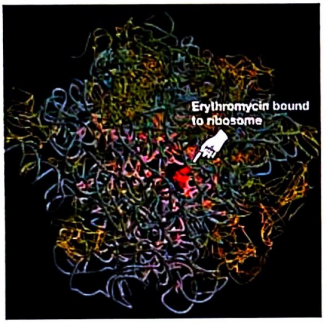
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ESSAY Antibiotics That Poison the Ribosome

Antibiotics are molecules that kill bacteria or slow their growth. Since their initial discovery by biomedical researchers in the 1930s, their use has saved tens of millions of human lives that were threatened by bacterial infections. It is no exaggeration to claim that the era of modern medicine began with the widespread availability of inexpensive antibiotics.

(a) Erythromycin binds to ribosomes and plugs the exit tunnel for the polypeptide.



(b) Mode of action of some antibiotics that poison translation

Antibiotic	Mode of Action
Chloramphenicol	Binds at the active site in the large subunit of ribosome
Clindamycin	Binds to A and P sites and prevents correct positioning of tRNAs
Erythromycin, carbomycin A, clarithromycin, roxithromycin, spiramycin, tylosin, azithromycin	Plugs the tunnel where growing polypeptide exits ribosome
Puromycin	Enters A site and bonds to growing polypeptide, but blocks addition of more amino acids
Spectinomycin	Inhibits translocation of ribosome
Streptomycin, parmomycin	Binds to the small subunit of ribosome and prevents accurate reading of the mRNA
Tetracycline	Prevents aminoacyl tRNAs from entering A site in ribosome

FIGURE 16.21 Many Antibiotics Work by Disrupting Translation in Bacteria
[(a) "Structural basis for the interaction of antibiotics with peptidyl transferase centre in eubacteria: by Schluenzen et al." Figure 5 in *Nature* (2001) 413: 814–821 by Schluenzen et al.]

Many of the antibiotics that physicians prescribe today are produced naturally by soil-dwelling fungi. These organisms compete with bacterial cells for space and resources. For a fungus, the ability to synthesize antibiotics is an adaptation that helps individuals destroy competitors and increase their access to nutrients. Antibiotics are weapons in chemical warfare that has gone on among competing soil-dwelling organisms for millions of years. Pharmaceutical companies still search for new antibiotics by studying naturally occurring fungi in the soil.

Recent research on antibiotics has focused on two seemingly distinct topics: the evolution of bacteria that resist them and the molecular mechanism of drug action.

Recent research on antibiotics has focused on two seemingly distinct topics: the evolution of bacteria that resist them and the molecular mechanism of drug action. **Antibiotic resistance** is the ability to grow and reproduce efficiently in an environment containing antibiotics. But as often happens in biological science, efforts to answer questions that seem unrelated produce closely connected answers.

How are evolutionary and molecular studies linked? Early research showed that several medically important antibiotics work by inhibiting translation in bacterial cells. In the presence of certain drugs, bacterial ribosomes stop working, protein synthesis ceases, and the targeted cell dies. Fortunately, bacterial and eukaryotic ribosomes are different enough at the molecular level that ribosome-poisoning antibiotics tend not to disrupt protein synthesis in humans. This is why antibiotics that target bacterial ribosomes have few side effects when administered to humans as a drug.

To see how evolutionary and molecular studies of ribosomes intersect, consider the latest three-dimensional models of bacterial ribosomes from bacteria treated with the antibiotic erythromycin. As **Figure 16.21a** shows, the drug binds to the tunnel where growing polypeptide chains leave the ribosome. When erythromycin binds to this site, it inhibits translation and kills the cell. A number of bacterial strains have evolved resistance to this antibiotic, however. When researchers analyzed the structure of ribosomes from drug-resistant populations of bacteria, they found that the ribosomes contained novel amino acids and rRNA sequences at the site where erythromycin binds. The altered amino acids and bases make erythromycin much less likely to bind to the ribosome. As a result, the drug no longer inhibits translation effectively.

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Similar studies have now been done on a wide array of antibiotics. The results have documented the binding site and mode of action of many commonly prescribed drugs (Figure 16.21b). In many cases, investigators have also been able to confirm that drug-resistant strains of bacteria have mutations precisely at these binding sites.

To make sense of these findings, biomedical researchers acknowledge that mutations are constantly creating variations in the makeup of ribosomes within bacterial populations. The researchers hypothesize that extensive use of antibiotics has led to intensive natural selection for cells with mutations that make them resistant to one or more drugs. In areas where antibiotic use is widespread, the mutant bacteria have higher fitness and come to dominate their population.

Antibiotic resistance is now so pervasive among disease-causing bacteria that some physicians claim that we have entered a post-antibiotic era. Researchers and physicians have already spent several decades in a race to develop new antibiotics that are effective against drug-resistant strains of bacteria. Research on ribosome structure and the mechanisms of translation has taken on an urgent tone. The more we know about the molecular mechanisms of protein synthesis, the better we can run the race against drug-resistant bacteria.

CHAPTER REVIEW

Summary of Key Concepts

The instructions for making and operating a cell are archived in DNA, transcribed into messenger RNA, and then translated into protein.

After the enzyme RNA polymerase binds to a specific site in DNA with the help of other proteins, it catalyzes the production of an RNA molecule. The base sequence of the RNA produced is complementary to the base sequence of the DNA template strand.

Early experiments established that RNA polymerase begins transcription by binding to promoter sequences in DNA. In bacteria, this binding occurs in conjunction with a regulatory protein called sigma. Sigma recognizes particular sequences within promoters that are located 10 bases and 35 bases upstream from the start of the actual genetic message. These binding sites ensure that the correct DNA strand is transcribed in the correct direction. Eukaryotic promoters are more complex and variable than bacterial promoters, and interact with many more transcription factors.

Web Tutorial 16.1 Transcription

In eukaryotes, genes consist of coding regions called exons interspersed with noncoding regions called introns. After a eukaryotic mRNA is produced, introns must be spliced out. The ends of the molecule receive a cap and tail.

After transcription of a eukaryotic mRNA is complete, several key events occur. Stretches of noncoding RNA called introns are spliced out by complex molecular machines called spliceosomes, a "cap" signal is added to the 5′ end, and a poly (A) tail is added to the 3′ end. Experiments with modified mRNAs established that both the 5′ cap and the poly (A) tail serve as recognition signals for the translation machinery and protect the message from degradation by ribonucleases.

Inside ribosomes, mRNAs are translated to proteins via intermediary molecules called transfer RNAs. Transfer RNAs carry an amino acid and have a three-base-pair anticodon, which binds to an mRNA codon. The amino acid carried by the transfer RNA is then added to the growing protein via formation of a peptide bond.

Experiments with radioactively labeled amino acids confirmed that ribosomes are the site of protein synthesis, and that transfer RNAs (tRNAs) serve as the chemical bridge between the RNA message and the polypeptide product. tRNAs have a predicted secondary structure that resembles a cloverleaf and an L-shaped tertiary structure. One leg of the L contains the anticodon, which forms a base pair with the mRNA codon, while the other leg holds the amino acid appropriate for that codon. Because imprecise pairing—or "wobbling"—is allowed in the third positions of anticodons, only about 40 different tRNAs are required to translate the 61 codons that code for amino acids.

Through intensive study of ribosome structure and function, researchers established that the process of elongating a polypeptide involves three steps: (1) an incoming aminoacyl tRNA occupies a position in the ribosome called the A site; (2) the growing polypeptide chain is transferred from a peptidyl tRNA in the ribosome's P site to the amino acid bound to the tRNA in the A site, and a peptide bond is formed; and (3) the ribosome is translocated to the next codon on the mRNA, accompanied by ejection of the empty tRNA from the E site. Recent data on the three-dimensional structure of the ribosome has confirmed that peptide bond formation is catalyzed by a ribozyme, not an enyzme.

While translation is in progress, proteins fold into their three-dimensional conformation (tertiary structure), sometimes with the aid of chaperone proteins. Some proteins are targeted to specific locations in the cell by the presence of signal sequences, while others remain inactive until modified by phosphorylation or the removal of certain amino acids.

Web Tutorial 16.2 Translation

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Many mutations produce changes in the phenotype.

When mutations occur in DNA, the altered sequences are faithfully transcribed and translated into gene products that are part of the organism's phenotype. Depending on the location and type of alteration in DNA and its impact on the resulting RNA or protein product, a mutation can be beneficial, deleterious, or neutral with respect to fitness. Mutations produce novel proteins and RNAs. They are the source of the heritable variation that makes evolution possible.

Questions

Content Review

1. How did the A site of the ribosome get its name?
   a. It is where amino acids are affixed to tRNAs, producing aminoacyl tRNAs.
   b. It is where the amino group on the growing polypeptide chain is available for peptide bond formation.
   c. It is the site occupied by incoming aminoacyl tRNAs.
   d. It is surrounded by α-helices of ribosomal proteins.
2. How did the P site of the ribosome get its name?
   a. It is where the promoter resides.
   b. It is the site where peptide bond formation takes place.
   c. It is where peptidyl tRNAs reside.
   d. It is the site where the growing polypeptide chain is phosphorylated.
3. What is a molecular chaperone?
   a. a protein that recognizes the promoter and guides the binding of RNA polymerase
   b. a protein that activates or deactivates another protein by adding or removing a phosphate group
   c. a protein that is a component of the large ribosomal subunit and that assists with peptide bond formation
   d. a protein that helps newly translated proteins fold into their proper three-dimensional configuration
4. The three types of RNA polymerase found in eukaryotic cells transcribe different types of genes. What does RNA polymerase II produce?
   a. rRNAs
   b. tRNAs
   c. mRNAs
   d. spliceosomes
5. What is an anticodon?
   a. the part of an RNA message that signals the termination of translation
   b. the part of an RNA message that signals the start of translation
   c. the part of a tRNA that binds to a complementary codon in mRNA
   d. the part of a tRNA that accepts an amino acid, via a reaction catalyzed by tRNA synthetase
6. Which of the following questions about transcription was *not* answered by the discovery of the −35 and −10 sequences in bacterial promoters?
   a. Does RNA polymerase act alone to initiate transcription, or are other proteins involved?
   b. How do sigma proteins bind to RNA polymerase?
   c. How does RNA polymerase "know" where to initiate transcription?
   d. How does RNA polymerase "know" which strand of DNA acts as the template?

Conceptual Review

1. Explain the relationship among eukaryotic promoter sequences, basal transcription factors, and RNA polymerase. Explain the relationship among bacterial promoter sequences, sigma, and RNA polymerase.
2. According to the wobble rules, the correct amino acid can be added to a growing polypeptide chain even if the third base in an mRNA codon does not correctly match the corresponding base in a tRNA anticodon. How do the wobble rules relate to the redundancy of the genetic code?
3. Why does splicing occur in eukaryotic mRNAs? Where does it occur, and how are snRNPs involved?
4. Sketch the structure of a tRNA molecule. Label the anticodon and the CCA sequence where amino acids bind. Explain how a tRNA interacts with tRNA synthetase. Sketch the structure of an aminoacyl tRNA. Explain how an aminoacyl tRNA interacts with an mRNA in the ribosome.
5. Explain the sequence of events that occurs during translation as a protein elongates by one amino acid. At each step, specify what is happening in the ribosome's A site, P site, and E site.
6. What evidence supports the hypothesis that peptide bond formation is catalyzed by a ribozyme?

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