Processing of tRNAs and rRNAs

The tRNAs and rRNAs are structural molecules that have roles in protein synthesis; however, these RNAs are not themselves translated. Pre-rRNAs are transcribed, processed, and assembled into ribosomes in the nucleolus. Pre-tRNAs are transcribed and processed in the nucleus and then released into the cytoplasm where they are linked to free amino acids for protein synthesis.

Most of the tRNAs and rRNAs in eukaryotes and prokaryotes are first transcribed as a long precursor molecule that spans multiple rRNAs or tRNAs. Enzymes then cleave the precursors into subunits corresponding to each structural RNA. Some of the bases of pre-rRNAs are *methylated*; that is, a $-CH_3$ methyl functional group is added for stability. Pre-tRNA molecules also undergo methylation. As with pre-mRNAs, subunit excision occurs in eukaryotic pre-RNAs destined to become tRNAs or rRNAs.

Mature rRNAs make up approximately 50 percent of each ribosome. Some of a ribosome's RNA molecules are purely structural, whereas others have catalytic or binding activities. Mature tRNAs take on a three-dimensional structure through local regions of base pairing stabilized by intramolecular hydrogen bonding. The tRNA folds to position the amino acid binding site at one end and the **anticodon** at the other end (Figure 15.14). The anticodon is a three-nucleotide sequence in a tRNA that interacts with an mRNA codon through complementary base pairing.

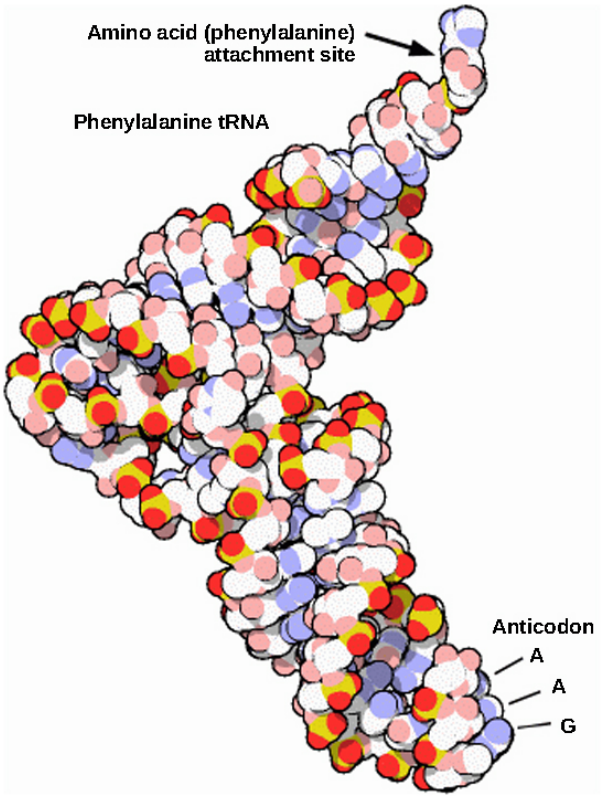

Figure 15.14 This is a space-filling model of a tRNA molecule that adds the amino acid phenylalanine to a growing polypeptide chain. The anticodon AAG binds the Codon UUC on the mRNA. The amino acid phenylalanine is attached to the other end of the tRNA.

15.5 Ribosomes and Protein Synthesis

By the end of this section, you will be able to do the following:

- Describe the different steps in protein synthesis
- Discuss the role of ribosomes in protein synthesis

The synthesis of proteins consumes more of a cell's energy than any other metabolic process. In turn, proteins account for more mass than any other component of living organisms (with the exception of water), and proteins perform virtually every function of a cell. The process of translation, or protein synthesis, involves the decoding of an mRNA message into a polypeptide product. Amino acids are covalently strung together by interlinking peptide bonds in lengths ranging from approximately 50 to more

than 1000 amino acid residues. Each individual amino acid has an amino group (NH_2) and a carboxyl (COOH) group. Polypeptides are formed when the amino group of one amino acid forms an amide (i.e., peptide) bond with the carboxyl group of another amino acid (Figure 15.15). This reaction is catalyzed by ribosomes and generates one water molecule.

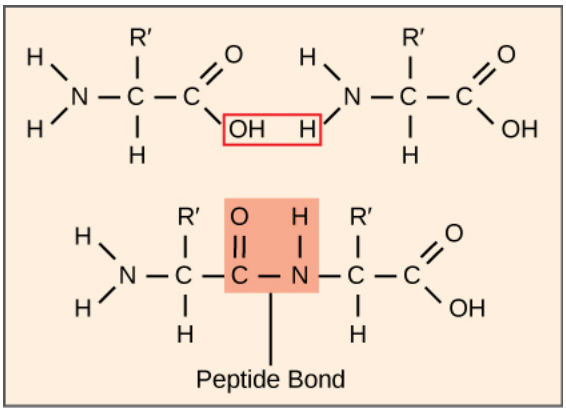

Figure 15.15 A peptide bond links the carboxyl end of one amino acid with the amino end of another, producing one water molecule during the process. For simplicity in this image, only the functional groups involved in the peptide bond are shown. The R and R' designations refer to the rest of each amino acid structure.

The Protein Synthesis Machinery

In addition to the mRNA template, many molecules and macromolecules contribute to the process of translation. The composition of each component may vary across species; for example, ribosomes may consist of different numbers of rRNAs and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors. (Note: A ribosome can be thought of as an enzyme whose amino acid binding sites are specified by mRNA.)

LINK TO LEARNING

Click through the steps of this PBS interactive (http://openstax.org/l/prokary_protein) to see protein synthesis in action.

Ribosomes

Even before an mRNA is translated, a cell must invest energy to build each of its ribosomes. In *E. coli*, there are between 10,000 and 70,000 ribosomes present in each cell at any given time. A **ribosome** is a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides. In eukaryotes, the nucleolus is completely specialized for the synthesis and assembly of rRNAs.

Ribosomes exist in the cytoplasm of prokaryotes and in the cytoplasm and rough endoplasmic reticulum of eukaryotes. Mitochondria and chloroplasts also have their own ribosomes in the matrix and stroma, which look more similar to prokaryotic ribosomes (and have similar drug sensitivities) than the ribosomes just outside their outer membranes in the cytoplasm. Ribosomes dissociate into large and small subunits when they are not synthesizing proteins and reassociate during the initiation of translation. *In E. coli, the small subunit is described as 30S, and the large subunit is 50S, for a total of 70S (recall that Svedberg units are not additive).* Mammalian ribosomes have a small 40S subunit and a large 60S subunit, for a total of 80S. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs. Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction: reading the mRNA from 5′ to 3′ and synthesizing the polypeptide from the N terminus to the C terminus. The complete mRNA/poly-ribosome structure is called a **polysome**.

tRNAs

The tRNAs are structural RNA molecules that were transcribed from genes by RNA polymerase III. Depending on the species, 40 to 60 types of tRNAs exist in the cytoplasm. Transfer RNAs serve as adaptor molecules. Each tRNA carries a specific amino acid and recognizes one or more of the mRNA codons that define the order of amino acids in a protein. Aminoacyl-tRNAs bind to the ribosome and add the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually

"translate" the language of RNA into the language of proteins.

Of the 64 possible mRNA codons—or triplet combinations of A, U, G, and C—three specify the termination of protein synthesis and 61 specify the addition of amino acids to the polypeptide chain. Of these 61, one codon (AUG) also encodes the initiation of translation. Each tRNA anticodon can base pair with one or more of the mRNA codons for its amino acid. For instance, if the sequence CUA occurred on an mRNA template in the proper reading frame, it would bind a leucine tRNA expressing the complementary sequence, GAU. The ability of some tRNAs to match more than one codon is what gives the genetic code its blocky structure.

As the adaptor molecules of translation, it is surprising that tRNAs can fit so much specificity into such a small package. Consider that tRNAs need to interact with three factors: 1) they must be recognized by the correct aminoacyl synthetase (see below); 2) they must be recognized by ribosomes; and 3) they must bind to the correct sequence in mRNA.

Aminoacyl tRNA Synthetases

The process of pre-tRNA synthesis by RNA polymerase III only creates the RNA portion of the adaptor molecule. The corresponding amino acid must be added later, once the tRNA is processed and exported to the cytoplasm. Through the process of tRNA "charging," each tRNA molecule is linked to its correct amino acid by one of a group of enzymes called **aminoacyl tRNA synthetases**. At least one type of aminoacyl tRNA synthetase exists for each of the 20 amino acids; the exact number of aminoacyl tRNA synthetases varies by species. These enzymes first bind and hydrolyze ATP to catalyze a high-energy bond between an amino acid and adenosine monophosphate (AMP); a pyrophosphate molecule is expelled in this reaction. The activated amino acid is then transferred to the tRNA, and AMP is released. The term "charging" is appropriate, since the high-energy bond that attaches an amino acid to its tRNA is later used to drive the formation of the peptide bond. Each tRNA is named for its amino acid.

The Mechanism of Protein Synthesis

As with mRNA synthesis, protein synthesis can be divided into three phases: *initiation, elongation, and termination*. The process of translation is similar in prokaryotes and eukaryotes. Here we'll explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between prokaryotic and eukaryotic translation.

Initiation of Translation

Protein synthesis begins with the formation of an **initiation complex**. In *E. coli*, this complex involves the small 30S ribosome, the mRNA template, three initiation factors (IFs; IF-1, IF-2, and IF-3), and a special **initiator tRNA**, called $tRNA^{Metf}$.

In *E. coli* mRNA, a sequence upstream of the first AUG codon, called the Shine-Dalgarno sequence (AGGAGG), interacts with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. Guanosine triphosphate (GTP), which is a purine nucleotide triphosphate, acts as an energy source during translation—both at the start of elongation and during the ribosome's translocation. Binding of the mRNA to the 30S ribosome also requires IF-III.

The initiator tRNA then interacts with the **start codon** AUG (or rarely, GUG). This tRNA carries the amino acid methionine, which is formylated after its attachment to the tRNA. The formylation creates a "faux" peptide bond between the formyl carboxyl group and the amino group of the methionine. Binding of the $fMet\text{-}tRNA^{Metf}$ is mediated by the initiation factor IF-2. The fMet begins every polypeptide chain synthesized by *E. coli*, but it is usually removed after translation is complete. When an in-frame AUG is encountered during translation elongation, a non-formylated methionine is inserted by a regular $Met\text{-}tRNA^{Met}$. After the formation of the initiation complex, the 30S ribosomal subunit is joined by the 50S subunit to form the translation complex. In eukaryotes, a similar initiation complex forms, comprising mRNA, the 40S small ribosomal subunit, eukaryotic IFs, and nucleoside triphosphates (GTP and ATP). The methionine on the charged initiator tRNA, called $Met\text{-}tRNA_i$, is not formylated. However, $Met\text{-}tRNA_i$ is distinct from other Met-tRNAs in that it can bind IFs.

Instead of depositing at the Shine-Dalgarno sequence, the eukaryotic initiation complex recognizes the 7-methylguanosine cap at the 5' end of the mRNA. A cap-binding protein (CBP) and several other IFs assist the movement of the ribosome to the 5' cap. Once at the cap, the initiation complex tracks along the mRNA in the 5' to 3' direction, searching for the AUG start codon. Many eukaryotic mRNAs are translated from the first AUG, but this is not always the case. According to **Kozak's rules**, the nucleotides around the AUG indicate whether it is the correct start codon. Kozak's rules state that the following consensus sequence must appear around the AUG of vertebrate genes: 5'-gccRccAUGG-3'. The R (for purine) indicates a site that can be either A or G, but cannot be C or U. Essentially, the closer the sequence is to this consensus, the higher the efficiency of translation.

Once the appropriate AUG is identified, the other proteins and CBP dissociate, and the 60S subunit binds to the complex of Met-$tRNA_i$, mRNA, and the 40S subunit. This step completes the initiation of translation in eukaryotes.

Translation, Elongation, and Termination

In prokaryotes and eukaryotes, the basics of elongation are the same, so we will review elongation from the perspective of *E. coli*. When the translation complex is formed, the tRNA binding region of the ribosome consists of three compartments. The A (aminoacyl) site binds incoming charged aminoacyl tRNAs. The P (peptidyl) site binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The E (exit) site releases dissociated tRNAs so that they can be recharged with free amino acids. The initiating methionyl-tRNA, however, occupies the P site at the beginning of the elongation phase of translation in both prokaryotes and eukaryotes.

During translation elongation, the mRNA template provides tRNA binding specificity. As the ribosome moves along the mRNA, each mRNA codon comes into register, and specific binding with the corresponding charged tRNA anticodon is ensured. If mRNA were not present in the elongation complex, the ribosome would bind tRNAs nonspecifically and randomly (?).

Elongation proceeds with charged tRNAs sequentially entering and leaving the ribosome as each new amino acid is added to the polypeptide chain. Movement of a tRNA from A to P to E site is induced by conformational changes that advance the ribosome by three bases in the 3′ direction. The energy for each step along the ribosome is donated by elongation factors that hydrolyze GTP. GTP energy is required both for the binding of a new aminoacyl-tRNA to the A site and for its translocation to the P site after formation of the peptide bond. Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA. The formation of each peptide bond is catalyzed by **peptidyl transferase**, an RNA-based enzyme that is integrated into the 50S ribosomal subunit. The energy for each peptide bond formation is derived from the high-energy bond linking each amino acid to its tRNA. After peptide bond formation, the A-site tRNA that now holds the growing peptide chain moves to the P site, and the P-site tRNA that is now empty moves to the E site and is expelled from the ribosome (Figure 15.16). Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200-amino-acid protein can be translated in just 10 seconds.

VISUAL CONNECTION

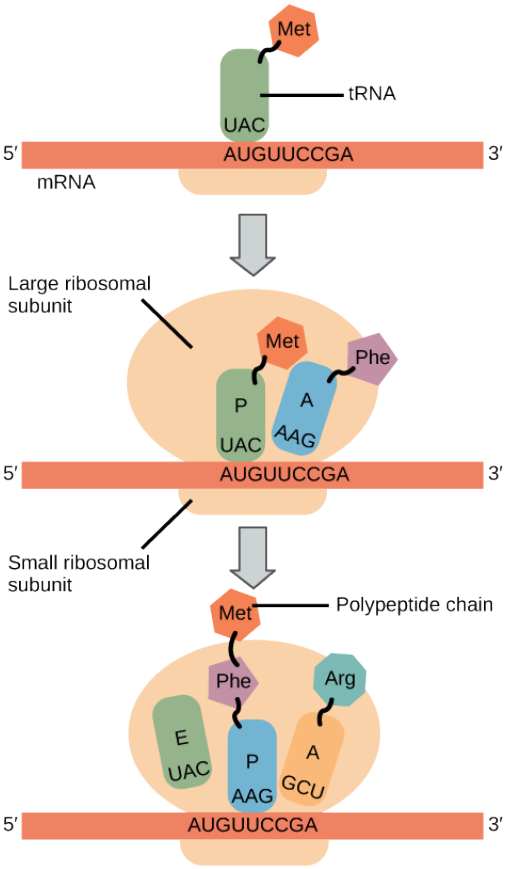

Figure 15.16 Translation begins when an initiator tRNA anticodon recognizes a start codon on mRNA bound to a small ribosomal subunit. The large ribosomal subunit joins the small subunit, and a second tRNA is recruited. As the mRNA moves relative to the ribosome, successive tRNAs move through the ribosome and the polypeptide chain is formed. Entry of a release factor into the A site terminates translation and the components dissociate.

Many antibiotics inhibit bacterial protein synthesis. For example, tetracycline blocks the A site on the bacterial ribosome, and chloramphenicol blocks peptidyl transfer. What specific effect would you expect each of these antibiotics to have on protein synthesis?

Tetracycline would directly affect:

a. tRNA binding to the ribosome
b. ribosome assembly
c. growth of the protein chain

Chloramphenicol would directly affect:

a. tRNA binding to the ribosome
b. ribosome assembly
c. growth of the protein chain

Termination of translation occurs when a nonsense codon (UAA, UAG, or UGA) is encountered. Upon aligning with the A site, these nonsense codons are recognized by *protein release factors* that resemble tRNAs. The releasing factors in both prokaryotes and eukaryotes instruct peptidyl transferase to add a water molecule to the carboxyl end of the P-site amino acid. This reaction forces the P-site amino acid to detach from its tRNA, and the newly made protein is released. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation

initiation complex. After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction.

Protein Folding, Modification, and Targeting

During and after translation, individual amino acids may be chemically modified, signal sequences appended, and the new protein "folded" into a distinct three-dimensional structure as a result of intramolecular interactions. A **signal sequence** is a short sequence at the amino end of a protein that directs it to a specific cellular compartment. These sequences can be thought of as the protein's "train ticket" to its ultimate destination, and are recognized by signal-recognition proteins that act as conductors. For instance, a specific signal sequence terminus will direct a protein to the mitochondria or chloroplasts (in plants). Once the protein reaches its cellular destination, the signal sequence is usually clipped off.

Many proteins fold spontaneously, but some proteins require helper molecules, called *chaperones*, to prevent them from aggregating during the complicated process of folding. Even if a protein is properly specified by its corresponding mRNA, it could take on a completely dysfunctional shape if abnormal temperature or pH conditions prevent it from folding correctly.