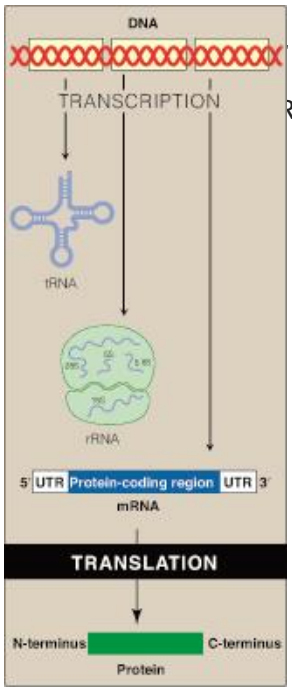
32: Protein Synthesis

Overview

Genetic information, stored in the chromosomes and transmitted to daughter cells through DNA replication, is expressed through transcription to RNA and, in the case of messenger RNA (mRNA), subsequent translation into proteins (polypeptides) as shown in Figure 32.1. (Note: The proteome is the complete set of proteins expressed in a cell.) The process of protein synthesis is called translation because the “language” of the nucleotide sequence on the mRNA is translated into the language of an amino acid sequence. Translation requires a genetic code, through which the information contained in the nucleotide sequence is expressed to produce a specific amino acid sequence. Any alteration in the nucleotide sequence may result in an incorrect amino acid being inserted into the protein, potentially causing disease or even death of the organism. Newly made immature (nascent) proteins undergo a number of processes to achieve their functional form. They must fold properly, otherwise misfolding can result in aggregation or degradation of the protein. Many proteins are covalently modified to alter their activities. Lastly, proteins are targeted to their final intra- or extracellular destinations by signals present in the proteins themselves.

FIGURE 32.1



translation.

RNA = ribosomal RNA; mRNA = messenger RNA; UTR = untranslated region.

The Genetic Code

Listen

The genetic code is a “dictionary” that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids. Each individual “word” in the code is composed of three nucleotide bases. These genetic words are called codons.

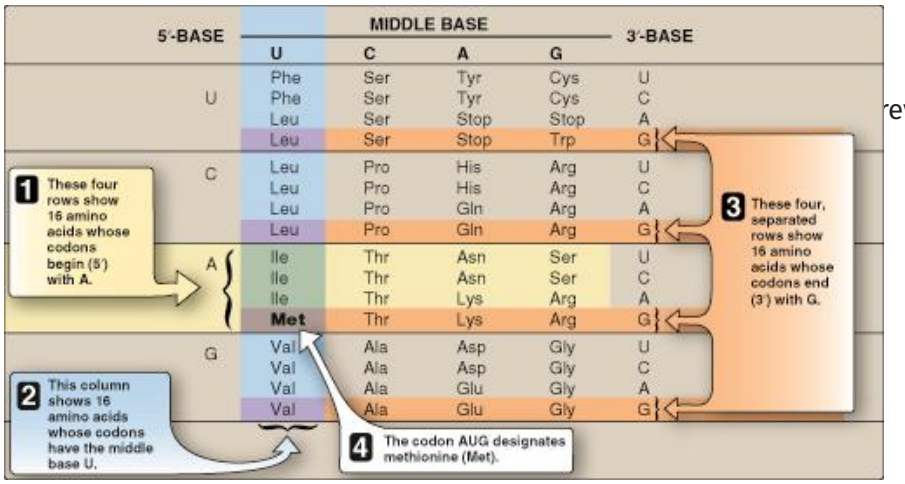
Codons

Codons are presented in the mRNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5′ end to the 3′ end. The four nucleotide bases are used to produce the three-base codons. Therefore, 64 different combinations of bases exist, taken three at a time (a triplet code), as shown in the table in Figure 32.2.

How to translate a codon

This table can be used to translate any codon and, thus, to determine which amino acids are coded for by an mRNA sequence. For example, the codon AUG codes for methionine ([Met], see Fig. 32.2). (Note: AUG is the initiation [start] codon for translation.) Sixty-one of the 64 codons code for the 20 standard amino acids (see p. 1).

FIGURE 32.2



reviations for many common amino acids

Termination codons

Three of the codons, UAA, UAG, and UGA, do not code for amino acids but, rather, are termination (also called stop, or nonsense) codons. When one of these codons appears in an mRNA sequence, synthesis of the polypeptide coded for by that mRNA stops.

Characteristics

Usage of the genetic code is remarkably consistent throughout all living organisms. It is assumed that once the standard genetic code evolved in primitive organisms, any mutation (a permanent change in DNA sequence) that altered its meaning would have caused the alteration of most, if not all, protein sequences, resulting in lethality. Characteristics of the genetic code include the following.

Specificity

The genetic code is specific (unambiguous), because a particular codon always codes for the same amino acid.

Universality

The genetic code is virtually universal insofar as its specificity has been conserved from very early stages of evolution, with only slight differences in the manner in which the code is translated. (Note: An exception occurs in mitochondria, in which a few codons have meanings different than those shown in Figure 32.2; e.g., UGA codes for tryptophan [Trp].)

Degeneracy

The genetic code is degenerate (sometimes called redundant). Although each codon corresponds to a single amino acid, a given amino acid may have more than one triplet coding for it. For example, arginine (Arg) is specified by six different codons (see Fig. 32.2). Only Met and Trp have just one coding triplet. Most codons that code for the same amino acid differ only in the last base of the triplet.

Nonoverlapping and commaless

The genetic code is nonoverlapping and commaless, meaning that the code is read from a fixed starting point as a continuous sequence of bases, taken three at a time without any punctuation between codons. For example, AGCUGGAUACAU is read as AGC UGG AUA CAU. The order of the codons that produces the correct sequence of amino acids in a protein is called the reading frame.

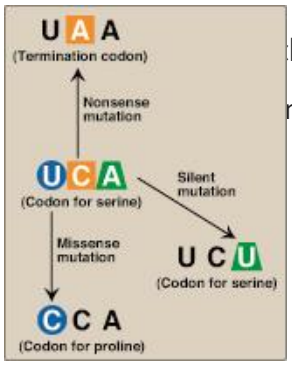
Consequences of altering the nucleotide sequence

Changing a single nucleotide base (a point mutation) in the coding region of an mRNA can lead to any one of three results (Fig. 32.3).

Silent mutation

The codon containing the changed base may code for the same amino acid. For example, if the serine (Ser) codon UCA is changed at the third base and becomes UCU, it still codes for Ser. This is termed a silent mutation.

FIGURE 32.3



hanging a single nucleotide base in the coding region of a messenger RNA.

ne; U = uracil.

Missense mutation

The codon containing the changed base may code for a different amino acid. For example, if the Ser codon UCA is changed at the first base and becomes CCA, it will code for a different amino acid (in this case, proline [Pro]). This is termed a missense mutation.

Nonsense mutation

The codon containing the changed base may become a termination codon. For example, if the Ser codon UCA is changed at the second base and becomes UAA, the new codon causes premature termination of translation at that point and the production of a shortened (truncated) protein. This is termed a nonsense mutation. (Note: The nonsense-mediated degradation pathway can degrade mRNA containing premature stops.)

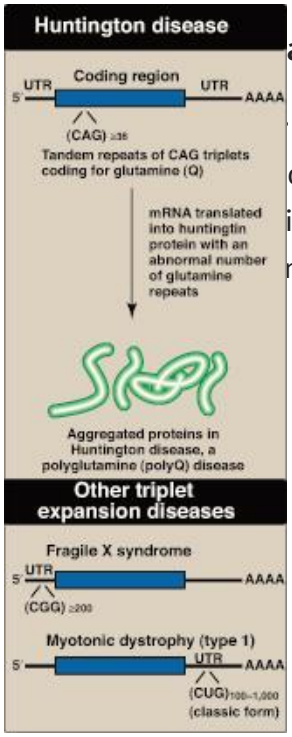
Other mutations

These can alter the amount or structure of the protein produced by translation.

Trinucleotide repeat expansion

Occasionally, a sequence of three bases that is repeated in tandem will become amplified in number so that too many copies of the triplet occur. If this happens within the coding region of a gene, the protein will contain many extra copies of one amino acid. For example, expansion of the CAG codon in exon 1 of the gene for huntingtin protein leads to the insertion of many extra glutamine residues in the protein, causing the neurodegenerative disorder Huntington disease (Fig. 32.4). The additional glutamines result in an abnormally long protein that is cleaved, producing toxic fragments that aggregate in neurons. If the trinucleotide repeat expansion occurs in an untranslated region (UTR) of a gene, the result can be a decrease in the amount of protein produced, as seen in fragile X syndrome and myotonic dystrophy. Over 20 triplet expansion diseases are known. (Note: In fragile X syndrome, the most common cause of intellectual disability in males, the expansion results in gene silencing through DNA hypermethylation [see p. 526].)

FIGURE 32.4



ats in messenger RNA (mRNA) causing Huntington disease and other triplet

dividuals, the number of repeats in the huntingtin protein is <27; in fragile X mental is 5–44; and in myotonic dystrophy protein kinase, it is 5–34.) UTR = untranslated region; ne; G = guanine; U = uracil; Q = single-letter abbreviation for glutamine.

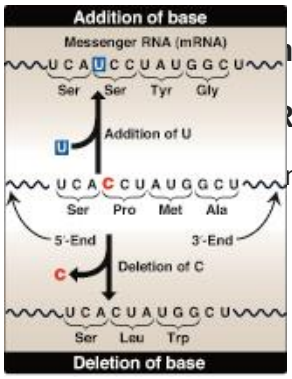
Splice site mutations

Mutations at splice sites (see p. 492) can alter the way in which introns are removed from pre-mRNA molecules, producing aberrant proteins. (Note: In myotonic dystrophy, a muscle disorder, gene silencing is the result of splicing alterations due to triplet expansion.)

Frameshift mutations

If one or two nucleotides are either deleted from or added to the coding region of an mRNA, a frameshift mutation occurs, altering the reading frame. This can result in a product with a radically different amino acid sequence or a truncated product due to the eventual creation of a termination codon (Fig. 32.5). If three nucleotides are added or deleted, the effect on the protein depends on where the changes occur. If the three nucleotides are added within an existing codon sequence or are deleted from two adjacent codons, then a frameshift happens. If three nucleotides are added between two codons, either a new amino acid is added into the protein or a stop is generated that shortens the product. The deletion of a codon causes the loss of an amino acid. Loss or addition of three nucleotides may maintain the reading frame but can result in serious pathology. For example, cystic fibrosis (CF), a chronic, progressive, inherited disease that primarily affects the pulmonary and digestive systems, is most commonly caused by deletion of three nucleotides from the coding region of a gene, resulting in the loss of phenylalanine (Phe, or F; see p. 5) at the 508th position (ΔF508) in the CF transmembrane conductance regulator (CFTR) protein encoded by that gene. This ΔF508 mutation prevents normal folding of CFTR, leading to its destruction by the proteasome (see p. 273). CFTR normally functions as a chloride channel in epithelial cells, and its loss results in the production of thick, sticky secretions in the lungs and pancreas, leading to lung damage and a digestive deficiency known as pancreatic insufficiency (see p. 192). The incidence of CF is highest (1 in 3,300) in those of Northern European origin. In >70% of individuals with CF, the ΔF508 mutation is the cause of the disease.

FIGURE 32.5



…s as a result of addition or deletion of a base can cause an alteration in the …RNA.

…ne; G = guanine; U = uracil.

Components Required for Translation

Listen

A large number of components are required for the synthesis of a protein. These include all the amino acids that are found in the finished product, the mRNA to be translated, transfer RNA (tRNA) for each of the amino acids, functional ribosomes, energy sources, and enzymes as well as noncatalytic protein factors needed for the initiation, elongation, and termination steps of polypeptide chain synthesis.

Amino acids

All the amino acids that eventually appear in the finished protein must be present at the time of protein synthesis. If one amino acid is missing, translation stops at the codon specifying that amino acid. (Note: This demonstrates the importance of having all the essential amino acids [see p. 291] in sufficient quantities in the diet to ensure continued protein synthesis.)

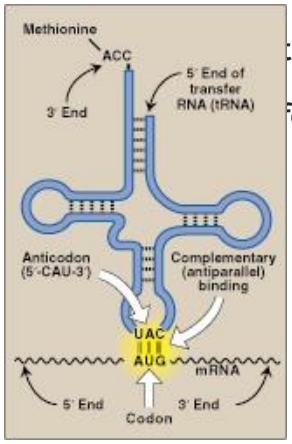
Transfer RNA

At least one specific type of tRNA is required for each amino acid. In humans, there are at least 50 species of tRNA, whereas bacteria contain at least 30 species. Because there are only 20 different amino acids commonly carried by tRNA, some amino acids have more than one specific tRNA molecule. This is particularly true of those amino acids that are coded for by several codons.

Amino acid attachment site

Each tRNA molecule has an attachment site for a specific (cognate) amino acid at its 3′ end (Fig. 32.6). The carboxyl group of the amino acid is in an ester linkage with the 3′ hydroxyl of the ribose portion of the A nucleotide in the –CCA sequence at the 3′ end of the tRNA. (Note: A tRNA with a covalently attached [activated] amino acid is charged. Without an attached amino acid, it is uncharged.)

FIGURE 32.6



tiparallel binding of the anticodon for methionyl-tRNA (CAU) to the messenger for methionine (AUG), the initiation codon for translation.

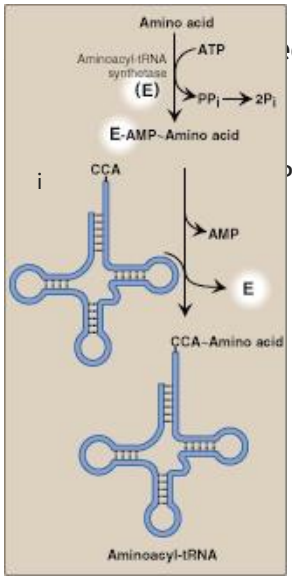
Anticodon

Each tRNA molecule also contains a three-base nucleotide sequence, the anticodon, which pairs with a specific codon on the mRNA (see Fig. 32.6). This codon specifies the insertion into the growing polypeptide chain of the amino acid carried by that tRNA.

Aminoacyl-tRNA synthetases

This family of 20 different enzymes is required for attachment of amino acids to their corresponding tRNA. Each member of this family recognizes a specific amino acid and all the tRNA that correspond to that amino acid (isoaccepting tRNA, up to five per amino acid). Aminoacyl-tRNA synthetases catalyze a two-step reaction that results in the covalent attachment of the α-carboxyl group of an amino acid to the A in the –CCA sequence at the 3′ end of its corresponding tRNA. The overall reaction requires ATP, which is cleaved to adenosine monophosphate and inorganic pyrophosphate (PP_i), as shown in Figure 32.7. The extreme specificity of the synthetases in recognizing both the amino acid and its cognate tRNA contributes to the high fidelity of translation of the genetic message. In addition to their synthetic activity, the aminoacyl-tRNA synthetases have a proofreading, or editing activity that can remove an incorrect amino acid from the enzyme or the tRNA molecule.

FIGURE 32.7



cific amino acid to its corresponding transfer RNA (tRNA) by an aminoacyl-

i P_i = inorganic phosphate; A = adenine; C = cytosine; AMP = adenosine monophosphate; ~

Messenger RNA

The specific mRNA required as a template for the synthesis of the desired polypeptide must be present.

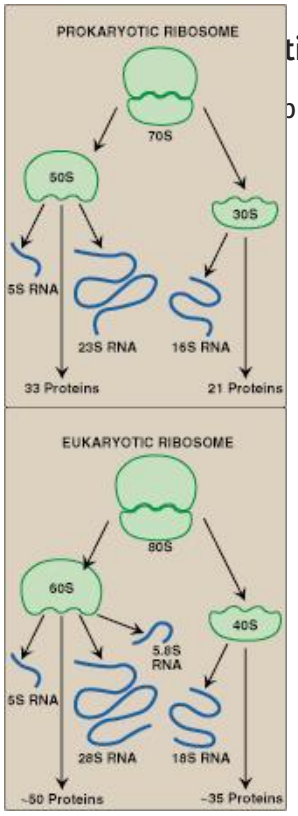
Functionally competent ribosomes

As shown in Figure 32.8, ribosomes are large complexes of protein and ribosomal RNA (rRNA), in which rRNA predominates. They consist of two subunits (one large and one small) whose relative sizes are given in terms of their sedimentation coefficients, or Svedberg (S) values. (Note: Because the S values are determined by both shape and size, their numeric values are not strictly additive; e.g., the prokaryotic 50S and 30S ribosomal subunits together form a 70S ribosome. The eukaryotic 60S and 40S subunits form an 80S ribosome.) Prokaryotic and eukaryotic ribosomes are similar in structure and serve the same function, namely, as the macromolecular complexes in which the synthesis of proteins occurs.

Ribosomal RNA

As discussed on p. 483, prokaryotic ribosomes contain three size species of rRNA, whereas eukaryotic ribosomes contain four (see Fig. 32.8). The rRNA are generated from a single pre-rRNA by the action of ribonucleases, and some bases and ribose sugars are modified.

FIGURE 32.8



tion.

proteins in the eukaryotic ribosomal subunits varies somewhat from species to species.)

The small ribosomal subunit binds mRNA and determines the accuracy of translation by ensuring correct base pairing between the mRNA codon and the tRNA anticodon. The large ribosomal subunit catalyzes formation of the peptide bonds that link amino acid residues in a protein.

Ribosomal proteins

Ribosomal proteins are present in greater numbers in eukaryotic ribosomes than in prokaryotic ribosomes. These proteins play a variety of roles in the structure and function of the ribosome and its interactions with other components of the translation system.

A, P, and E sites

The ribosome has three binding sites for tRNA molecules: the A, P, and E sites, each of which extends over both subunits. Together, they cover three neighboring codons. During translation, the A site binds an incoming aminoacyl-tRNA as directed by the codon currently occupying this site. This codon specifies the next amino acid to be added to the growing peptide chain. The P site is occupied by peptidyl-tRNA. This tRNA carries the chain of amino acids that has already been synthesized. The E site is occupied by the empty tRNA as it is about to exit the ribosome. (See Fig. 32.13 for an illustration of the role of the A, P, and E sites in translation.)

Cellular location

In eukaryotic cells, the ribosomes either are free in the cytosol or are in close association with the endoplasmic reticulum (which is then known as the rough endoplasmic reticulum or RER). RER-associated ribosomes are responsible for synthesizing proteins (including glycoproteins; see p. 182) that are to be exported from the cell, incorporated into membranes, or imported into lysosomes (see p. 185 for an overview of the latter process). Cytosolic ribosomes synthesize proteins required in the cytosol itself or destined for the nucleus, mitochondria, or peroxisomes. (Note: Mitochondria contain their own ribosomes [55S] and their own unique, circular DNA. Most mitochondrial proteins, however, are encoded by nuclear DNA, synthesized completely in the cytosol, and then targeted to mitochondria.)

Protein factors

Initiation, elongation, and termination (or, release) factors are required for polypeptide synthesis. Some of these protein factors perform a catalytic function, whereas others appear to stabilize the synthetic machinery. (Note: A number of the factors are small, cytosolic G proteins and thus are active when bound to guanosine triphosphate [GTP] and inactive when bound to guanosine diphosphate [GDP]. See p. 104 for a discussion of the membrane-associated G proteins.)

Energy sources

Cleavage of four high-energy bonds is required for the addition of one amino acid to the growing polypeptide chain: two from ATP in the aminoacyl-tRNA synthetase reaction, one in the removal of PP_i and one in the subsequent hydrolysis of the PP_i, to two P_i by pyrophosphatase, and two from GTP, one for binding the aminoacyl-tRNA to the A site and one for the translocation step (see Fig. 32.13). (Note: Additional ATP and GTP molecules are required for initiation in eukaryotes, whereas an additional GTP molecule is required for termination in both eukaryotes and prokaryotes.) Translation, then, is a major consumer of energy.

Codon Recognition by Transfer RNA

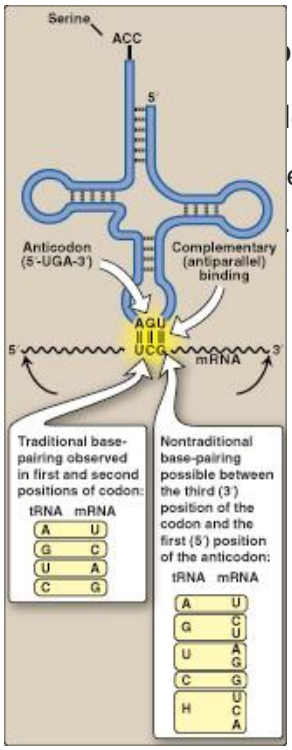
Listen

Correct pairing of the codon in the mRNA with the anticodon of the tRNA is essential for accurate translation (see Fig. 32.6). Most tRNA (isoaccepting tRNA) recognize more than one codon for a given amino acid.

Antiparallel binding between codon and anticodon

Binding of the tRNA anticodon to the mRNA codon follows the rules of complementary and antiparallel binding, that is, the mRNA codon is read 5′→3′ by an anticodon pairing in the opposite (3′→5′) orientation (Fig. 32.9). (Note: Nucleotide sequences are always written in the 5′ to 3′ direction unless otherwise noted. Two nucleotide sequences orient in an antiparallel manner.)

FIGURE 32.9



nal base pairing between the 5′ nucleotide (first nucleotide) of the anticodon

e (last nucleotide) of the codon.

e product of adenine deamination and the base in the nucleotide inosine

. A = adenine; G = guanine; C = cytosine; U = uracil; tRNA = transfer RNA; mRNA =

Wobble hypothesis

The mechanism by which a tRNA can recognize more than one codon for a specific amino acid is described by the wobble hypothesis, which states that codon–anticodon pairing follows the traditional Watson–Crick rules (G pairs with C and A pairs with U) for the first two bases of the codon but can be less stringent for the last base. The base at the 5′ end of the anticodon (the first base of the anticodon) is not as spatially defined as the other two bases. Movement of that first base allows nontraditional base pairing with the 3′ base of the codon (the last base of the codon). This movement is called wobble and allows a single tRNA to recognize more than one codon. Examples of these flexible pairings are shown in Figure 32.9. The result of wobble is that 61 tRNA species are not required to read the 61 codons that code for amino acids.

Steps in Translation

Listen

The process of protein synthesis translates the 3-letter alphabet of nucleotide sequences on mRNA into the 20-letter alphabet of amino acids that constitute proteins. The mRNA is translated from its 5′ end to its 3′ end, producing a protein synthesized from its amino (N)-terminal end to its carboxyl (C)-terminal end. Prokaryotic mRNA often have several coding regions (i.e., they are polycistronic). Each coding region has its own initiation and termination codon and produces a separate species of polypeptide. In contrast, each eukaryotic mRNA has only one coding region (i.e., it is monocistronic). The process of translation is divided into three separate steps: initiation, elongation, and termination. Eukaryotic translation resembles that of prokaryotes in most aspects. Individual differences are noted in the text.

One important difference is that translation and transcription are temporally linked in prokaryotes, with translation starting before transcription is completed as a consequence of the lack of a nuclear membrane in prokaryotes.

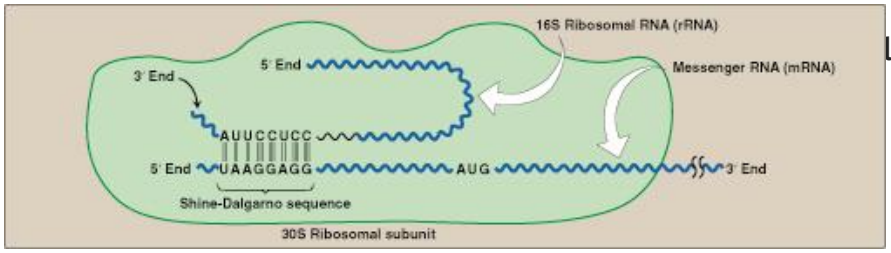
Initiation

Initiation of protein synthesis involves the assembly of the components of the translation system before peptide-bond formation occurs. These components include the two ribosomal subunits, the mRNA to be translated, the aminoacyl-tRNA specified by the first codon in the message, GTP, and initiation factors (IFs) that facilitate the assembly of this initiation complex (see Fig. 32.13). (Note: In prokaryotes, three IFs are known [IF-1, IF-2, and IF-3], whereas in eukaryotes, there are many [designated eIF to indicate eukaryotic origin]. Eukaryotes also require ATP for initiation.) The following are two mechanisms by which the ribosome recognizes the nucleotide sequence (AUG) that initiates translation.

Shine–Dalgarno sequence

In *Escherichia coli (E. coli*), a purine-rich sequence of nucleotide bases, known as the Shine–Dalgarno (SD) sequence, is located 6 to 10 bases upstream of the initiating AUG codon on the mRNA molecule (i.e., near its 5′ end). The 16S rRNA component of the small (30S) ribosomal subunit has a nucleotide sequence near its 3′ end that is complementary to all or part of the SD sequence. Therefore, the 5′ end of the mRNA and the 3′ end of the 16S rRNA can form complementary base pairs, facilitating the positioning of the 30S subunit on the mRNA in close proximity to the initiating AUG codon (Fig. 32.10).

FIGURE 32.10



lgarno sequence and 16S rRNA.

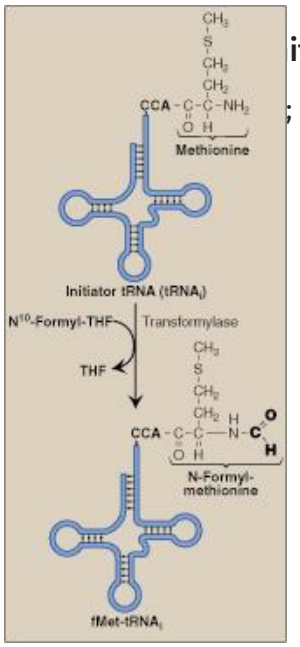
5′ Cap

Eukaryotic mRNA do not have SD sequences. In eukaryotes, the small (40S) ribosomal subunit (aided by members of the eIF-4 family of proteins) binds close to the cap structure at the 5′ end of the mRNA and moves 5′→3′ along the mRNA until it encounters the initiator AUG. This scanning process requires ATP. Cap-independent initiation can occur if the 40S subunit binds to an internal ribosome entry site close to the start codon. (Note: Interactions between the cap-binding eIF-4 proteins and the poly-A tail–binding proteins on eukaryotic mRNA mediate circularization of the mRNA and likely prevent the use of incompletely processed mRNA in translation.)

Initiation codon

The initiating AUG is recognized by a special initiator tRNA ($tRNA_i$). Recognition is facilitated by IF-2-GTP in prokaryotes and eIF-2-GTP (plus additional eIF) in eukaryotes. The charged $tRNA_i$ is the only tRNA recognized by (e)IF-2 and the only tRNA to go directly to the P site on the small subunit. (Note: Base modifications distinguish $tRNA_i$ from the tRNA used for internal AUG codons.) In bacteria and mitochondria, $tRNA_i$ carries an N-formylated methionine (fMet), as shown in Figure 32.11. After Met is attached to $tRNA_i$, the formyl group is added by the enzyme transformylase, which uses N^{10}-formyl tetrahydrofolate (see p. 296) as the carbon donor. In eukaryotes, the cytosolic $tRNA_i$ carries a Met that is not formylated. In both prokaryotic and eukaryotic cells, this N-terminal Met is usually removed before translation is completed. The large ribosomal subunit then joins the complex, and a functional ribosome is formed with the charged $tRNA_i$ in the P site. The A site is empty. (Note: Specific [e]IF function as antiassociation factors and prevent premature addition of the large subunit.) The GTP on (e)IF-2 gets hydrolyzed to GDP. In eukaryotes, the G nucleotide exchange factor eIF-2B facilitates the reactivation of eIF-2-GDP through replacement of GDP by GTP.

FIGURE 32.11



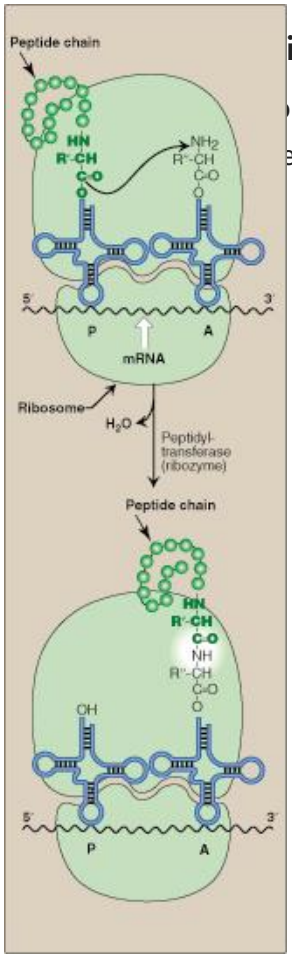
itiator N-formylmethionyl-transfer RNA ($fMet\text{-}tRNA_i$).

; C = cytosine; A = adenine.

Elongation

Elongation of the polypeptide involves the addition of amino acids to the carboxyl end of the growing chain. Delivery of the aminoacyl-tRNA whose codon appears next on the mRNA template in the ribosomal A site (a process known as decoding) is facilitated in *E. coli* by elongation factors EF-Tu-GTP and EF-Ts and requires GTP hydrolysis. (Note: In eukaryotes, comparable elongation factors are EF-1α-GTP and EF-1βγ. Both EF-Ts and EF-1βγ function in guanine nucleotide exchange.) Peptide bond formation between the α-carboxyl group of the amino acid in the P site and the α-amino group of the amino acid in the A site is catalyzed by peptidyl transferase, an activity intrinsic to an rRNA of the large subunit (Fig. 32.12). (Note: Because this rRNA catalyzes the reaction, it is a ribozyme.) After the peptide bond has been formed, the peptide on the tRNA at the P site is transferred to the amino acid on the tRNA at the A site, a process known as transpeptidation. The ribosome then advances three nucleotides toward the 3′ end of the mRNA. This process is known as translocation and, in prokaryotes, requires the participation of EF-G-GTP (eukaryotes use EF-2-GTP) and GTP hydrolysis. Translocation causes movement of the uncharged tRNA from the P to the E site for release and movement of the peptidyl-tRNA from the A to the P site. The process is repeated until a termination codon is encountered. (Note: Because of the length of most mRNA, more than one ribosome at a time can translate a message. Such a complex of one mRNA and a number of ribosomes is called a polysome, or polyribosome.)

FIGURE 32.12



ide bond.

n results in transfer of the peptide on the transfer RNA (tRNA) in the P site to the amino e A site (transpeptidation). mRNA = messenger RNA; R′, R″ = different amino acid side

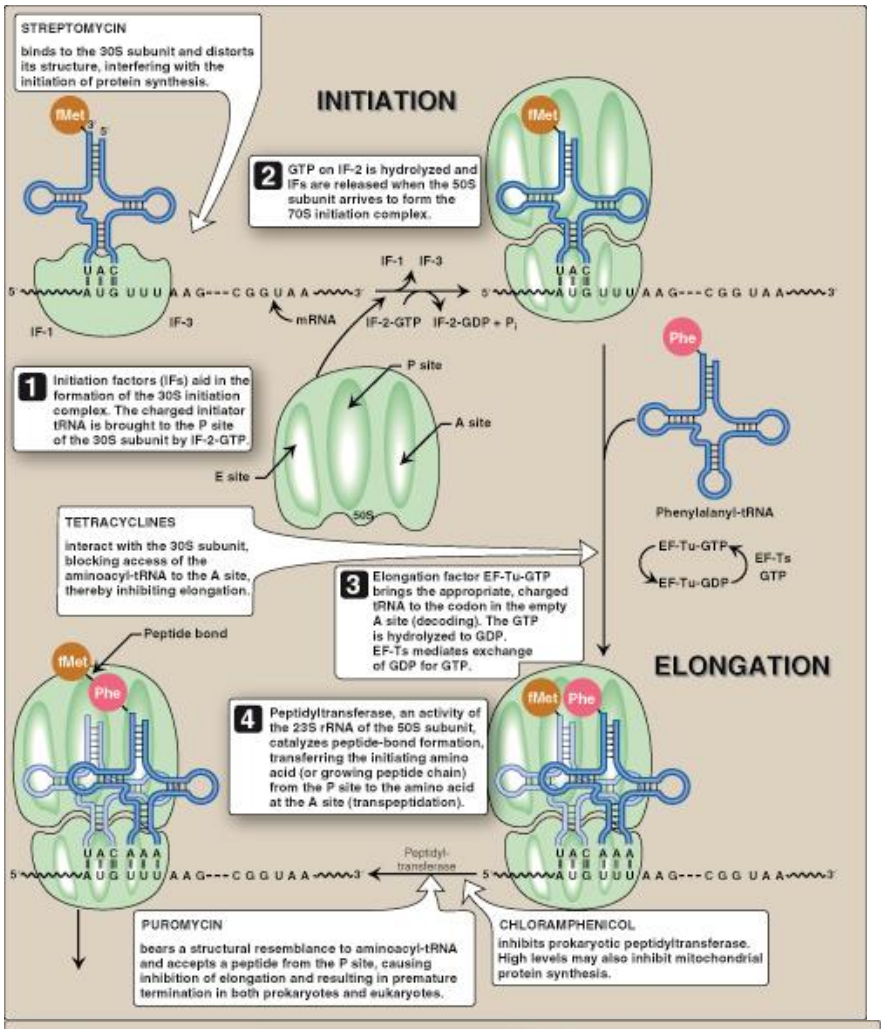
Termination

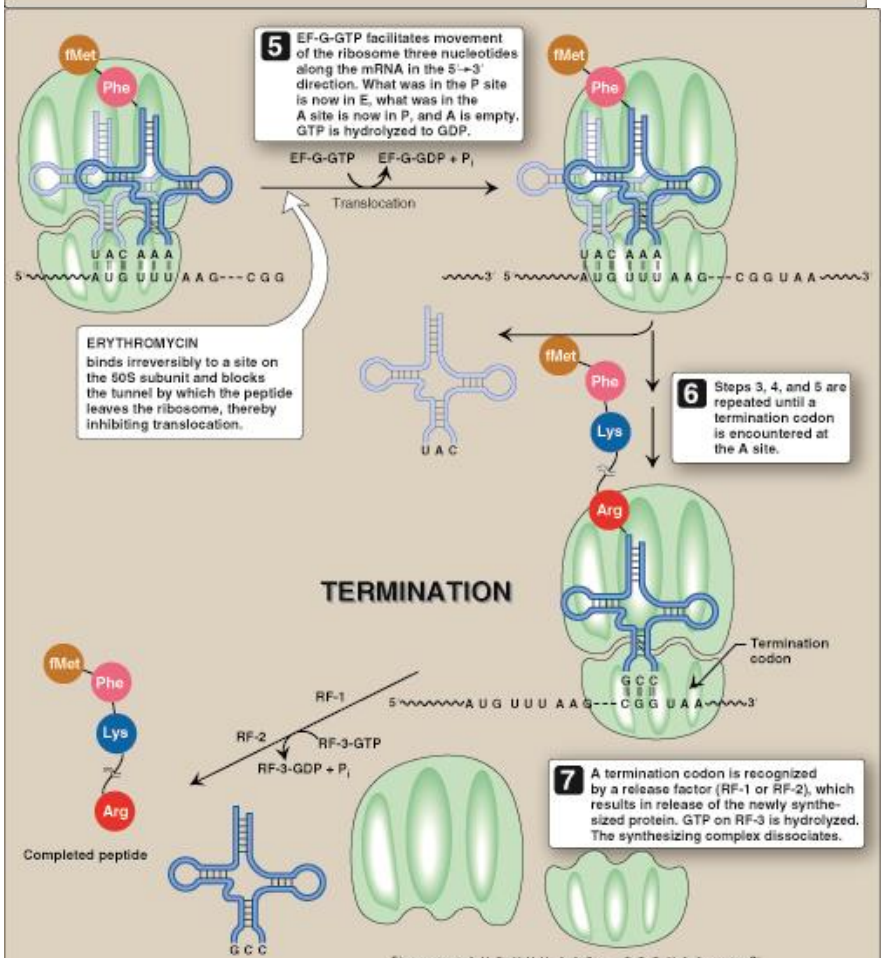
Termination occurs when one of the three termination codons moves into the A site. These codons are recognized in *E. coli* by release factors: RF-1, which recognizes UAA and UAG, and RF-2, which recognizes UGA and UAA. The binding of these release factors results in hydrolysis of the bond linking the peptide to the tRNA at the P site, causing the nascent protein to be released from the ribosome. A third release factor, RF-3-GTP, then causes the release of RF-1 or RF-2 as GTP is hydrolyzed (see Fig. 32.13). (Note: Eukaryotes have a single release factor, eRF, which recognizes all three termination codons. A second factor, eRF-3, functions like the prokaryotic RF-3. See Figure 32.14 for a summary of the factors used in translation.) The steps in prokaryotic protein synthesis, as well as some antibiotic inhibitors of the process, are summarized in Figure 32.13. The newly synthesized polypeptide may undergo further modification as described below, and the ribosomal subunits, mRNA, tRNA, and protein factors can be recycled and used to synthesize another polypeptide. (Note: In prokaryotes, ribosome recycling factors mediate separation of the subunits. In eukaryotes, eRF and ATP hydrolysis are required.)

FIGURE 32.13

Steps in prokaryotic protein synthesis (translation), and their inhibition by antibiotics.

(Note: EF-Ts is a guanine nucleotide exchange factor. It facilitates the removal of guanosine diphosphate (GDP) from EF-Tu, allowing its replacement by guanosine triphosphate [GTP]. The eukaryotic equivalent is EF-1βγ.) fMet = formylated methionine; S = Svedberg unit; Phe = phenylalanine; Lys = lysine; Arg = arginine; tRNA = transfer RNA; mRNA = messenger RNA. (Note: In eukaryotes, diphtheria toxin inactivates EF-2 [the equivalent of prokaryotic EF-G], thereby inhibiting the translocation phase of elongation. Ricin, a toxin from castor beans, removes a specific A from the 28S ribosomal RNA [rRNA] in the large subunit of eukaryotic ribosomes, thereby inhibiting ribosomal function.)


STREPTOMYCIN
binds to the 30S subunit and distorts its structure, interfering with the initiation of protein synthesis.
INITIATION
fMet
2 GTP on IF-2 is hydrolyzed and IFs are released when the 50S subunit arrives to form the 70S initiation complex.
IF-1 IF-3
mRNA
IF-2-GTP IF-2-GDP + Pi
1 Initiation factors (IFs) aid in the formation of the 30S initiation complex. The charged initiator tRNA is brought to the P site of the 30S subunit by IF-2-GTP.
P site
A site
E site
50S
Phe
Phenylalanyl-tRNA
TETRACYCLINES
interact with the 30S subunit, blocking access of the aminoacyl-tRNA to the A site, thereby inhibiting elongation.
EF-Tu-GTP
EF-Tu-GDP
EF-Ts
GTP
3 Elongation factor EF-Tu-GTP brings the appropriate, charged tRNA to the codon in the empty A site (decoding). The GTP is hydrolyzed to GDP. EF-Ts mediates exchange of GDP for GTP.
Peptide bond
ELONGATION
4 Peptidyltransferase, an activity of the 23S rRNA of the 50S subunit, catalyzes peptide-bond formation, transferring the initiating amino acid (or growing peptide chain) from the P site to the amino acid at the A site (transpeptidation).
Peptidyl-transferase
PUROMYCIN
bears a structural resemblance to aminoacyl-tRNA and accepts a peptide from the P site, causing inhibition of elongation and resulting in premature termination in both prokaryotes and eukaryotes.
CHLORAMPHENICOL
inhibits prokaryotic peptidyltransferase. High levels may also inhibit mitochondrial protein synthesis.


5 EF-G-GTP facilitates movement of the ribosome three nucleotides along the mRNA in the 5'→3' direction. What was in the P site is now in E, what was in the A site is now in P, and A is empty. GTP is hydrolyzed to GDP.
EF-G-GTP EF-G-GDP + Pi
Translocation
ERYTHROMYCIN
binds irreversibly to a site on the 50S subunit and blocks the tunnel by which the peptide leaves the ribosome, thereby inhibiting translocation.
6 Steps 3, 4, and 5 are repeated until a termination codon is encountered at the A site.
Lys
Arg
TERMINATION
Termination codon
RF-1
RF-2
RF-3-GTP
RF-3-GDP + Pi
7 A termination codon is recognized by a release factor (RF-1 or RF-2), which results in release of the newly synthesized protein. GTP on RF-3 is hydrolyzed. The synthesizing complex dissociates.
Completed peptide

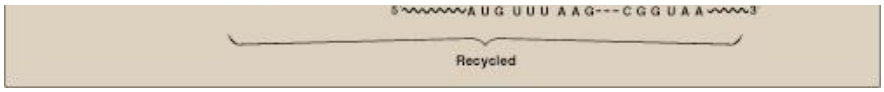


FIGURE 32.14

Cell	Factor	Function
Initiation		
Prok Euk	IF-2-GTP eIF-2-GTP	Bring charged initiating tRNA to P site
Prok Euk	IF-3 eIF-3	Prevent association of subunits
Elongation		
Prok Euk	EF-Tu-GTP EF1α-GTP	Bring all other charged tRNA to A site
Prok Euk	EF-Ts EF-1βγ	Guanine nucleotide exchange factors
Prok Euk	EF-G-GTP EF-2-GTP	Translocation
Termination		
Prok Euk	RF-1, 2 eRF	Recognize stop codons
Prok Euk	RF-3-GTP eRF-3-GTP	Release of other RF

e three stages of translation.

k = eukaryotes; tRNA = transfer RNA; IF = initiation factor; EF = elongation factor; RF = uanosine triphosphate.

Translation regulation

Gene expression is most commonly regulated at the transcriptional level, but translation may also be regulated. An important mechanism by which this is achieved in eukaryotes is by covalent modification of eIF-2: Phosphorylated eIF-2 is inactive (see p. 526). In both eukaryotes and prokaryotes, regulation can also be achieved through proteins that bind mRNA and inhibit its use by blocking translation.

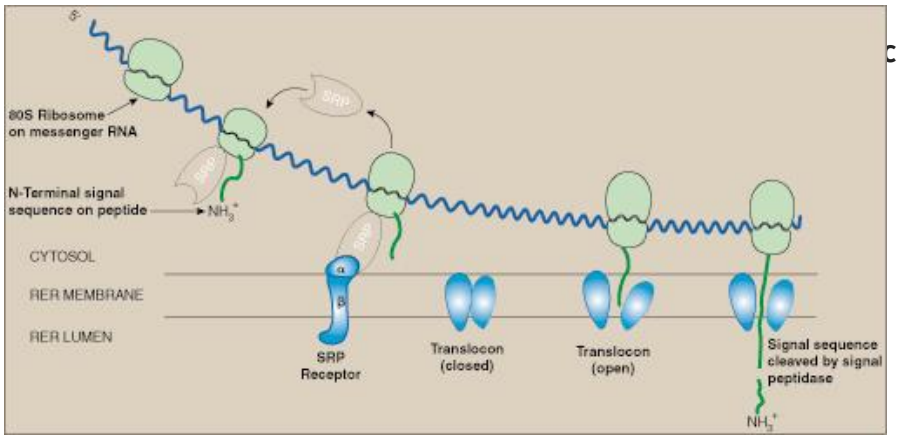
Protein folding

Proteins must fold to assume their functional, native state. Folding can be spontaneous (as a result of the primary structure) or facilitated by proteins known as chaperones (see p. 21).

Protein targeting

Although most protein synthesis in eukaryotes is initiated in the cytoplasm, many proteins perform their functions within subcellular organelles or outside of the cell. Such proteins normally contain amino acid sequences that direct the proteins to their final locations. For example, secreted proteins are targeted during synthesis (cotranslational targeting) to the RER by the presence of an N-terminal hydrophobic signal sequence. The sequence is recognized by the signal recognition particle (SRP), a ribonucleoprotein that binds the ribosome, halts elongation, and delivers the ribosome–peptide complex to an RER membrane channel (the translocon) via interaction with the SRP receptor. Translation resumes, the protein enters the RER lumen, and its signal sequence is cleaved (Fig. 32.15). The protein moves through the RER and the Golgi, is processed, packaged into vesicles, and secreted. Proteins targeted after synthesis (posttranslational) include nuclear proteins that contain an internal, short, basic nuclear localization signal; mitochondrial matrix proteins that contain an N-terminal, amphipathic, α-helical mitochondrial entry sequence; and peroxisomal proteins that contain a C-terminal tripeptide signal.

FIGURE 32.15



c reticulum (RER).

Co- and Posttranslational Modifications

Listen

Many polypeptides are covalently modified, either while they are still attached to the ribosome (cotranslational) or after their synthesis has been completed (posttranslational). These modifications may include removal of part of the translated sequence or the covalent addition of one or more chemical groups required for protein activity.

Trimming

Many proteins destined for secretion are initially made as large, precursor molecules that are not functionally active. Portions of the protein must be removed by specialized endoproteases, resulting in the release of an active molecule. The cellular site of the cleavage reaction depends on the protein to be modified. Some precursor proteins are cleaved in the RER or the Golgi; others are cleaved in developing secretory vesicles (e.g., insulin; see Fig. 23.4, p. 343); and still others, such as collagen (see p. 49), are cleaved after secretion.

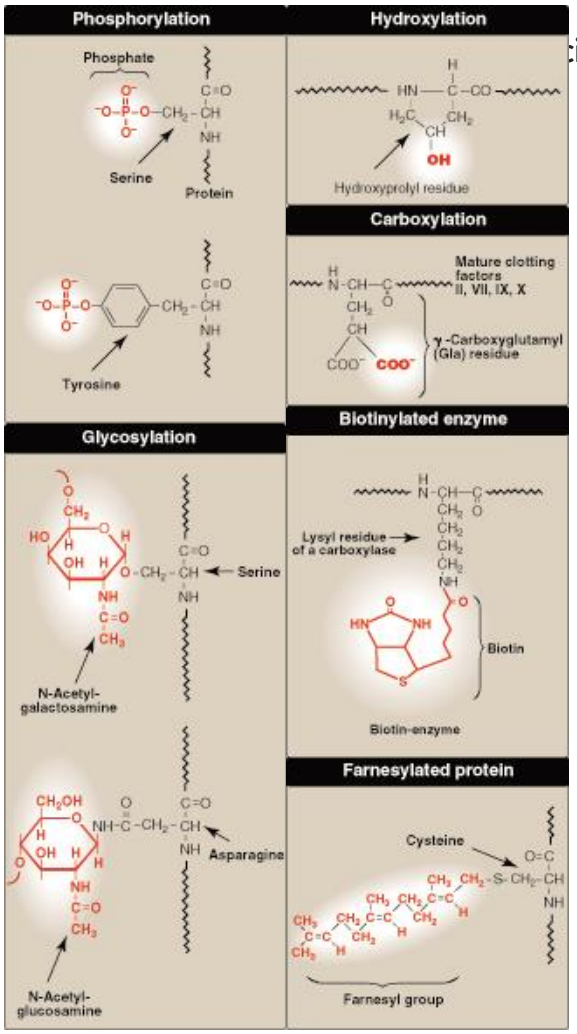
Covalent attachments

Protein function can be affected by the covalent attachment of a variety of chemical groups (Fig. 32.16). Examples include the following.

Phosphorylation

Phosphorylation occurs on the hydroxyl groups of Ser, threonine, or, less frequently, tyrosine residues in a protein. It is catalyzed by one of a family of protein kinases and may be reversed by the action of protein phosphatases. The phosphorylation may increase or decrease the functional activity of the protein. Several examples of phosphorylation reactions have been previously discussed (e.g., see Chapter 11, p. 144, for the regulation of glycogen synthesis and degradation).

FIGURE 32.16



id residues.

Glycosylation

Many of the proteins that are destined to become part of a membrane or to be secreted from a cell have carbohydrate chains added *en bloc* to the amide nitrogen of an asparagine (N linked) or built sequentially on the hydroxyl groups of a Ser, threonine, or hydroxylysine (O linked). N-glycosylation occurs in the RER and O-glycosylation in the Golgi. (The process of producing such glycoproteins was discussed on p. 181.) N-glycosylated acid hydrolases are targeted to the matrix of lysosomes by the phosphorylation of mannose residues at carbon 6 (see p. 185).

Hydroxylation

Pro and lysine residues of the α-chains of collagen are extensively hydroxylated by vitamin C–dependent hydroxylases in the RER (see p. 49).

Other covalent modifications

These may be required for the functional activity of a protein. For example, additional carboxyl groups can be added to glutamate residues by vitamin K–dependent carboxylation (see p. 440). The resulting γ-carboxyglutamate (Gla) residues are essential for the activity of several of the blood-clotting proteins (see Chapter 35). Biotin is covalently bound to the ε-amino groups of lysine residues of biotin-dependent enzymes that catalyze carboxylation reactions such as pyruvate carboxylase (see Fig. 10.3 on p. 130). Attachment of lipids, such as farnesyl groups, can help anchor proteins to membranes (see p. 221). Many eukaryotic proteins are cotranslationally acetylated at the N end. (Note: Reversible acetylation of histone proteins influences gene expression [see p. 526].)

Protein degradation

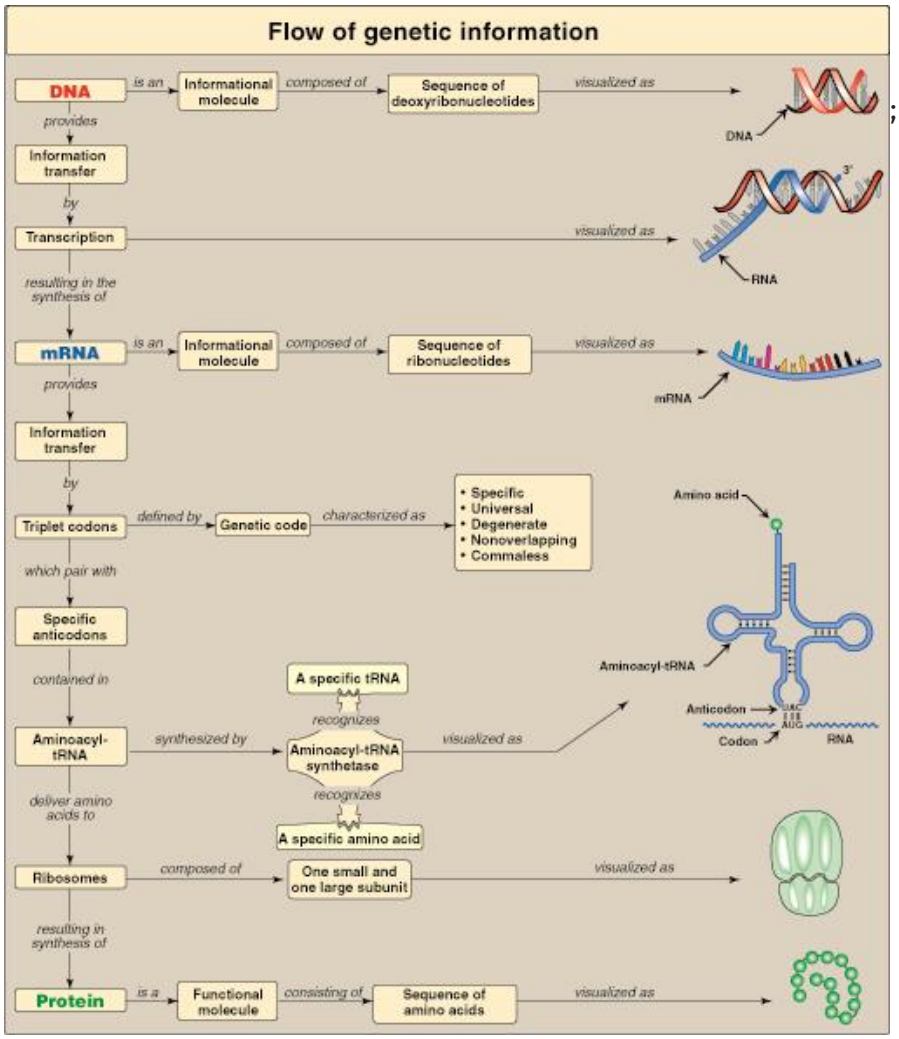
Proteins that are defective (e.g., misfolded) or destined for rapid turnover are often marked for destruction by ubiquitination, the covalent attachment of chains of a small, highly conserved protein called ubiquitin (see Fig. 19.3 on p. 273). Proteins marked in this way are rapidly degraded by the proteasome, which is a macromolecular, ATP-dependent, proteolytic system located in the cytosol. For example, misfolding of the CFTR protein (see p. 499) results in its proteasomal degradation. (Note: If folding is impeded, unfolded proteins accumulate in the RER causing stress that triggers the unfolded protein response, in which the expression of chaperones is increased; global translation is decreased by eIF-2 phosphorylation; and the unfolded proteins are sent to the cytosol, ubiquitinated, and degraded in the proteasome by a process called ER-associated degradation.)

Chapter Summary

- **Codons** in are composed of three nucleotides in mRNA, which contains the bases **A**, **G**, **C**, and **U**. Codons are always written **5′→3′**.
- Of the 64 possible three-base combinations, 61 code for the 20 standard amino acids and three signal for the termination of protein synthesis (**translation**). In an organism, the genetic code is specific (each codon produces one amino acid) and degenerate (more than one codon can code for each amino acid).
- Altering the nucleotide sequence in a codon can cause **silent mutations** (the altered codon codes for the original amino acid), **missense mutations** (the altered codon codes for a different amino acid), or **nonsense mutations** (the altered codon is a termination codon). Frameshift mutations that result from the addition or deletion of a base can cause an alteration in the reading frame of mRNA.
- **Translation** of a protein requires all of the **amino acids** in the protein; the **tRNA** and **aminoacyl-tRNA synthetase** for each amino acid; the **mRNA** coding for the protein; fully competent **ribosomes** (70S in prokaryotes, 80S in eukaryotes); **protein factors** needed for initiation, elongation, and termination of protein synthesis; and **ATP** and **GTP** as energy sources.
- Ribosomes are large complexes of **protein** and **rRNA**. They consist of **two subunits**, 30S and 50S in prokaryotes and 40S and 60S in eukaryotes. Each ribosome has three tRNA binding sites: the A, P, and E sites that cover three neighboring codons. The **A site** binds an **incoming aminoacyl-tRNA**, the **P site** is occupied by **peptidyl-tRNA**, and the **E site** is occupied by the **empty tRNA**.
- An mRNA codon is recognized by a tRNA **anticodon** following the rules of **complementarity** and **antiparallel** binding. The **wobble hypothesis** states that the first (5′) base of the anticodon is not as spatially constrained as the other two bases. Nontraditional base pairing may occur between the first (5′) anticodon base and the last (3′) base of the codon, thus allowing a single tRNA to recognize more than one codon for a specific amino acid.
- For the initiation of **translation**, an mRNA must associate with the small ribosomal subunit. The process requires **IFs**. In **prokaryotes**, a purine-rich region of the mRNA (the **SD sequence**) base pairs with a complementary sequence on 16S rRNA, resulting in the positioning of the small subunit on the mRNA. In eukaryotes, this positioning is guided by the **5′ cap** of the mRNA, which is bound by proteins of the eIF-4 family. The **initiation codon** is **AUG**. **N-formylmethionine** is the initiating amino acid in prokaryotes, whereas **Met** is used in eukaryotes. The charged initiator tRNA ($tRNA_i$) is brought to the P site by (**e)IF-2**.
- **Elongation** (lengthening) of the polypeptide chain occurs by the addition of amino acids to its carboxyl end. **Elongation factors** facilitate the binding of the aminoacyl-tRNA to the A site as well as the movement of the ribosome along the mRNA. The formation of the peptide bond is catalyzed by **peptidyl transferase**, which is an activity intrinsic to the rRNA of the large subunit and, therefore, is a **ribozyme**. Following peptide bond formation, the ribosome advances along the mRNA in the **5′→3′ direction** to the next codon (**translocation**). Because of the length of most mRNA, more than one ribosome at a time can translate a message, forming a **polysome.**
- **Termination** begins when a termination codon moves into the A site and is recognized by **release factors**. The newly synthesized protein is released from the ribosomal complex, and the ribosome is dissociated from the mRNA.

- Numerous **antibiotics** interfere with the process of protein synthesis in prokaryotes.
- Polypeptide chains may be covalently modified during or after translation. Such modifications include amino acid **removal**; **phosphorylation,** which may activate or inactivate the protein; **glycosylation**, which plays a role in **protein targeting**; and **hydroxylation,** such as that seen in collagen.
- Protein targeting can be either **cotranslational** (as with secreted proteins) or **posttranslational** (as with mitochondrial matrix proteins).
- Proteins must **fold** to achieve their functional form. Folding can be spontaneous or facilitated by **chaperones**. Proteins that are defective (e.g., misfolded) or destined for rapid turnover are marked for destruction by the attachment of chains of a small, highly conserved protein called **ubiquitin**. Ubiquitinated proteins are rapidly degraded by a cytosolic complex known as the **proteasome** (Fig. 32.17).

FIGURE 32.17



; C = cytosine; U = uracil.

Study Questions

Listen

Choose the ONE best answer.

32.1. A 20-year-old male with a microcytic anemia is found to have an abnormal form of β-globin (Hemoglobin Constant Spring) that is 172 amino acids long, rather than the 141 found in the normal protein. Which of the following point mutations is consistent with this abnormality? Use Figure 32.2 to answer the question.

A. CGA → UGA

B. GAU → GAC

C. GCA → GAA

D. UAA → CAA

E. UAA → UAG

Correct answer = D. Mutating the normal termination (stop) codon from UAA to CAA in β-globin messenger RNA causes the ribosome to insert a glutamine at that point. It will continue extending the protein chain until it comes upon the next stop codon farther down the message, resulting in an abnormally long protein. The replacement of CGA (arginine) with UGA (stop) would cause the protein to be too short. GAU and GAC both code for aspartate and would cause no change in the protein. Changing GCA (alanine) to GAA (glutamate) would not change the size of the protein product. A change from UAA to UAG would simply change one termination codon for another and would have no effect on the protein.

32.2. A pharmaceutical company is studying a new antibiotic that inhibits bacterial protein synthesis. When this antibiotic is added to an *in vitro* protein synthesis system that is translating mRNA sequence AUGUUUUUUUAG, the only product formed is the dipeptide fMet-Phe. What step in protein synthesis is most likely inhibited by the antibiotic?

A. Initiation

B. Binding of tRNA to the ribosomal A site

C. Peptidyl transferase activity

D. Ribosomal translocation

E. Termination

Correct answer = D. Because fMet-Phe (formylated methionyl-phenylalanine) is made, the ribosomes must be able to complete initiation, bind Phe-tRNA to the A site, and use peptidyl transferase activity to form the first peptide bond. Because the ribosome is not able to proceed any further, ribosomal movement (translocation) is most likely the inhibited step. Therefore, the ribosome is stopped before it reaches the termination codon of this message.

32.3. A tRNA molecule that is supposed to carry cysteine ($tRNA^{Cys}$) is mischarged, so that it actually carries alanine ($Ala\text{-}tRNA^{Cys}$). Assuming no correction occurs, what would be the most likely fate of this alanine residue during protein synthesis?

A. Alanine is incorporated into a protein.

B. Cysteine is incorporated into a protein.

C. Alanine is transferred to a $tRNA^{Ala}$ in the E site of the ribosome.

D. No protein synthesis occurs as alanine remains attached to the tRNA.

E. Alanine is chemically converted to cysteine by cellular enzymes.

Correct answer = A. Once an amino acid is attached to a tRNA molecule, only the anticodon of that tRNA determines the specificity of incorporation. Therefore, the incorrectly activated alanine will be incorporated into the protein at a position determined by a cysteine codon. A mischarged tRNA will cause a change in the protein that is not due to a mutation in the DNA.

32.4. In a patient with cystic fibrosis (CF) caused by the ΔF508 mutation, the mutant CF transmembrane conductance regulator (CFTR) protein folds incorrectly. The patient's cells modify this abnormal protein by attaching ubiquitin molecules to it. What is the fate of this modified CFTR protein?

A. It is degraded by the proteasome.

B. It is placed into storage vesicles.

C. It is repaired by cellular enzymes.

D. It is targeted to the lysosome.

E. It is secreted from the cell.

Correct answer = A. Ubiquitination usually marks old, damaged, or misfolded proteins for destruction by the cytosolic proteasome. There is no known cellular mechanism for repair of damaged proteins. Proteins are targeted to the matrix of the lysosome by a mannose 6-phosphate residue.

32.5. Many antimicrobials inhibit translation. Which of the following antimicrobials is correctly paired with its mechanism of action?

A. Chloramphenicol inhibits transformylase.

B. Erythromycin binds to the 60S ribosomal subunit.

C. Puromycin inactivates elongation factor-2.

D. Streptomycin binds to the 30S ribosomal subunit.

E. Tetracyclines inhibit peptidyl transferase.

Correct answer = D. Streptomycin binds the 30S subunit and inhibits translation initiation. Chloramphenicol inhibits the peptidyl transferase activity of the 23S rRNA (ribozyme) of the 50S subunit. Erythromycin binds the 50S ribosomal subunit (60S denotes a eukaryote) and blocks the tunnel through which the peptide leaves the ribosome. Puromycin has structural similarity to aminoacyl-transfer RNA. It is incorporated into the growing chain, inhibits elongation, and results in premature termination in both prokaryotes and eukaryotes. Tetracyclines bind the 30S ribosomal subunit and block access to the A site, inhibiting elongation.

32.6. Translation of a synthetic polyribonucleotide containing the repeating sequence CAA in a cell-free protein-synthesizing system produces three homopolypeptides: polyglutamine, polyasparagine, and polythreonine. If the codons for glutamine and asparagine are CAA and AAC, respectively, which of the following triplets is the codon for threonine?

A. AAC

B. ACA

C. CAA

D. CAC

E. CCA

Correct answer = B. The synthetic polynucleotide sequence of CAACAACAACAA ... could be read by the *in vitro* protein-synthesizing system starting at the first C, the first A, or the second A (i.e., in any one of three reading frames). In the first case, the first triplet codon would be CAA, which codes glutamine; in the second case, the first triplet codon would be AAC, which codes for asparagine; in the last case, the first triplet codon would be ACA, which codes for threonine.

32.7. Which of the following is required for both prokaryotic and eukaryotic protein synthesis?

A. Binding of the small ribosomal subunit to the Shine–Dalgarno sequence

B. Formylated methionyl-transfer (t)RNA

C. Movement of the messenger RNA out of the nucleus and into the cytoplasm

D. Recognition of the 5′ cap by initiation factors

E. Translocation of the peptidyl-tRNA from the A site to the P site

Correct answer = E. In both prokaryotes and eukaryotes, continued translation (elongation) requires movement of the peptidyl-tRNA from the A to the P site to allow the next aminoacyl-tRNA to enter the A site. Only prokaryotes have a Shine–Dalgarno sequence and use formylated methionine and only eukaryotes have a nucleus and co- and posttranscriptionally process their mRNA.

32.8. α1-Antitrypsin (AAT) deficiency can result in emphysema, a lung pathology, because the action of elastase, a serine protease, is unopposed. Deficiency of AAT in the lungs is the consequence of impaired secretion from the liver, the site of its synthesis. Proteins such as AAT that are destined to be secreted are best characterized by which of the following statements?

A. Their synthesis is initiated on the smooth endoplasmic reticulum.

B. They contain a mannose 6-phosphate targeting signal.

C. They always contain methionine as the N-terminal amino acid.

D. They are produced from translation products that have an N-terminal hydrophobic signal sequence.

E. They contain no sugars with O-glycosidic linkages because their synthesis does not involve the Golgi.

Correct answer = D. Synthesis of secreted proteins is begun on free (cytosolic) ribosomes. As the N-terminal signal sequence of the peptide emerges from the ribosome, it is bound by the signal recognition particle, taken to the rough endoplasmic reticulum (RER), threaded into the lumen, and cleaved as translation continues. The proteins move through the RER and the Golgi and undergo processing such as N-glycosylation (RER) and O-glycosylation (Golgi). In the Golgi, they are packaged in secretory vesicles and released from the cell. The smooth endoplasmic reticulum is associated with synthesis of lipids, not proteins, and has no ribosomes attached. Phosphorylation at carbon 6 of terminal mannose residues in glycoproteins targets these proteins (acid hydrolases) to lysosomes. The N-terminal methionine is removed from most proteins during processing.

32.9. Why is the genetic code described as both degenerate and unambiguous?

A given amino acid can be coded for by more than one codon (degenerate code), but a given codon codes for just one particular amino acid (unambiguous code).