Proteins are produced by the process of translation, which occurs on ribosomes and is directed by mRNA. The genetic message encoded in DNA is first transcribed into mRNA, and the nucleotide sequence in the coding region of the mRNA is then translated into the amino acid sequence of the protein.

**Translation of the code.** The portion of mRNA that specifies the amino acid sequence of the protein is read in codons, which are sets of three nucleotides that specify individual amino acids (Fig 15.1). The codons on mRNA are read sequentially in the 5’ to 3’ direction, starting with the 5’-AUG (or “start” codon) that specifies methionine and sets the reading frame and ending with a 3’-termination (or “stop”) codon (UAG, UGA, or UAA). The protein is synthesized from its N-terminus to its C-terminus.

Each amino acid is carried to the ribosome by an aminoacyl-tRNA (i.e., a tRNA with an amino acid covalently attached). Base-pairing between the anticodon of the tRNA and the codon on the mRNA ensures that each amino acid is inserted into the growing polypeptide at the appropriate position.

**Synthesis of the protein.** Initiation involves formation of a complex containing the initial methionyl-tRNA bound to the AUG “start” codon of the mRNA and to the “P” site of the ribosome. It requires GTP and proteins known as eukaryotic initiation factors (eIFs).

Elongation of the polypeptide involves three steps: (a) binding of an aminoacyl-tRNA to the “A” site on the ribosome where it base-pairs with the second codon on the mRNA; (b) formation of a peptide bond between the first and second amino acids; and (c) translocation, movement of the mRNA relative to the ribosome, so that the third mRNA codon moves into the “A” site. These three elongation steps are repeated until a termination codon aligns with the site on the ribosome where the next aminoacyl-tRNA would normally bind. Release factors bind instead, causing the completed protein to be released from the ribosome.

After one ribosome binds and moves along the mRNA, translating the polypeptide, another ribosome can bind and begin translation. The complex of a single mRNA with multiple ribosomes is known as a polysome.

**Folding and modification and targeting of the protein.** Folding of the polypeptide into its three-dimensional configuration occurs as the polypeptide is being translated. This process involves proteins called chaperones. Modification of amino acid residues in a protein occurs during or after translation. Proteins synthesized on cytosolic ribosomes are released into the cytosol or transported into mitochondria, peroxisomes, and nucleus. Proteins synthesized on ribosomes attached to the rough endoplasmic reticulum (RER) are destined for lysosomes, cell membranes, or secretion from the cell. These proteins are transferred to the Golgi complex, where they are modified and targeted to their ultimate locations.
THE WAITING ROOM

Anne Niemick, a 4-year old patient with β*-thalassemia intermedia (see Chapter 14), showed no improvement in her symptoms at her second visit. Her hemoglobin level was 7.0 g/dL (reference range for females = 12–16 g/dL).

Jay Sakz is a 9-month-old male infant of Ashkenazi Jewish parentage. His growth and development were normal until age 5 months, when he began to exhibit mild, generalized muscle weakness. By 7 months, he had poor head control, slowed development of motor skills, and was increasingly inattentive to his surroundings. His parents also noted unusual eye movements and staring episodes. On careful examination of his retinae, his pediatrician observed a “cherry-red” spot within a pale macula. The physician suspected Tay-Sachs disease and sent whole blood samples to the molecular biology-genetics laboratory.

Neu Moania returned to his physician’s office after 1 week of erythromycin therapy (see Chapter 12). The sputum sample from his previous visit had been cultured. The results confirmed that his respiratory infection was caused by *Streptococcus pneumoniae* and that the organism was sensitive to penicillin, macrolides (e.g., erythromycin, clarithromycin), tetracycline, and other antibiotics.

Erna Nemdy, a 25-year-old junior medical student (“earn an M.D.”), brings her healthy 4-month-old daughter, Beverly, to the pediatrician for her second diphtheria, pertussis, tetanus (DPT-2) immunization. Erna tells the doctor that her great, great aunt had died of diphtheria during an epidemic many years ago.

I. THE GENETIC CODE

Transcription, the transfer of the genetic message from DNA to RNA, and translation, the transfer of the genetic message from the nucleotide language of nucleic acids to the amino acid language of proteins, both depend on base-pairing. In the late 1950s and early 1960s, molecular biologists attempting to decipher the process of translation recognized two problems. The first involved decoding the relationship between the “language” of the nucleic acids and the “language” of the proteins, and the second involved determining the molecular mechanism by which translation between these two languages occurs.

Twenty different amino acids are commonly incorporated into proteins, and, therefore, the protein “alphabet” has 20 characters. The nucleic acid alphabet, however, has only four characters, corresponding to the four nucleotides of mRNA (A, G, C, and U). If two nucleotides constituted the code for an amino acid, then only $4^2$ or 16 amino acids could be specified. Therefore, the number of nucleotides that code for an amino acid has to be three, providing $4^3$ or 64 possible combinations or “codons,” more than required, but not overly excessive.

Scientists set out to determine the specific codons for each amino acid. In 1961, Marshall Nirenberg produced the first crack in the genetic code (the collection of codons that specify all the amino acids found in proteins). He showed that poly(U), a polynucleotide in which all the bases are uracil, produced polyphenylalanine in a cell-free protein-synthesizing system. Thus, UUU must be
As a result of experiments using synthetic polynucleotides in place of mRNA, other codons were identified. The pioneering molecular biologists recognized that, because amino acids cannot bind directly to the sets of three nucleotides that form their codons, “adapters” are required. The “adapters” were found to be tRNA molecules. Each tRNA molecule contains an anticodon and covalently binds a specific amino acid at its 3′-end (see Chapters 12 and 14). The anticodon of a tRNA molecule is a set of three nucleotides that can interact with a codon on mRNA (Fig. 15.2). To interact, the codon and anticodon must be complementary (i.e., they must be able to form base pairs in an antiparallel orientation). Thus, the anticodon of a tRNA serves as the link between an mRNA codon and the amino acid that the codon specifies.

Obviously, each codon present within mRNA must correspond to a specific amino acid. Nirenberg found that trinucleotides of known base sequence could bind to ribosomes and induce the binding of specific aminoacyl-tRNAs (i.e., tRNAs with amino acids covalently attached). As a result of these and the earlier experiments, the relationship between all 64 codons and the amino acids they specify (the entire genetic code) was determined by the mid-1960s (Table 15.1).

Three of the 64 possible codons (UGA, UAG, and UAA) terminate protein synthesis and are known as “stop” or nonsense codons. The remaining 61 codons specify amino acids. Two amino acids each have only one codon (AUG = methionine; UGG = tryptophan). The remaining amino acids have multiple codons.

A. The Code Is Degenerate, But Unambiguous

Because many amino acids are specified by more than one codon, the genetic code is described as “degenerate,” which means that an amino acid may have more than one codon. However, each codon specifies only one amino acid, and the genetic code is, thus, unambiguous.

Inspection of a codon table shows that in most instances of multiple codons for a single amino acid, the variation occurs in the third base of the codon (see Table 15.1). Crick noted that the pairing between the 3′-base of the codon and the 5′-base of the anticodon does not always follow the strict base-pairing rules that he and Watson had previously discovered (i.e., A pairs with U, and G with C). This observation resulted in the “wobble” hypothesis.

At the third base of the codon (the 3′-position of the codon and the 5′-position of the anticodon), the base pairs can “wobble”, e.g., G can pair with U; and A, G, or U can pair with the unusual base hypoxanthine (I) found in tRNA. Thus, three of

<table>
<thead>
<tr>
<th>First Base</th>
<th>Second Base</th>
<th>Third Base</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 15.1. The Genetic Code

<table>
<thead>
<tr>
<th>First Base</th>
<th>Second Base</th>
<th>Third Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>Phe Ser</td>
<td>Tyr Cys</td>
</tr>
<tr>
<td>C</td>
<td>Leu Pro</td>
<td>His Arg</td>
</tr>
<tr>
<td>A</td>
<td>Ile Thr</td>
<td>Asn Ser</td>
</tr>
<tr>
<td>G</td>
<td>Val Ala</td>
<td>Asp Gly</td>
</tr>
</tbody>
</table>

Hypoxanthine is the base attached to ribose in the nucleoside inosine. The single-letter abbreviation for hypoxanthine is “I,” in reference to the nucleoside inosine. (In other cases, the first letter of the base is also the first letter of the nucleoside and the single letter abbreviation. For example, A is the base adenine and the nucleoside adenosine.)
the four codons for alanine (GCU, GCC, and GCA) can pair with a single tRNA that contains the anticodon 5'-IGC-3' (see Fig. 15.2). If each of the 61 codons for amino acids required a distinct tRNA, cells would contain 61 tRNAs. However, because of "wobble" between the codon and anticodon, fewer than 61 tRNAs are required to translate the genetic code.

B. The Code Is Almost Universal

All organisms studied so far use the same genetic code, with some rare exceptions. One exception occurs in human mitochondrial mRNA, where UGA codes for tryptophan instead of serving as a stop codon, AUA codes for methionine instead of isoleucine, and CUA codes for threonine instead of leucine.

C. The Code Is Nonoverlapping and without Punctuation

mRNA does not contain punctuation to separate one codon from the next and the codons do not overlap. Each nucleotide is read only once. Beginning with a start codon (AUG) near the 5'-end of the mRNA, the codons are read sequentially, ending with a stop codon (UGA, UAG, or UAA) near the 3'-end of the mRNA.

II. RELATIONSHIP BETWEEN mRNA AND THE PROTEIN PRODUCT

The start codon (AUG) sets the reading frame, the order in which the sequence of bases in the mRNA is sorted into codons (Fig. 15.3). The order of the codons in the mRNA determines the sequence in which amino acids are added to the growing polypeptide chain. Thus, the order of the codons in the mRNA determines the linear sequence of amino acids in the protein.

III. EFFECTS OF MUTATIONS

Mutations that result from damage to the nucleotides of DNA molecules or from unrepaired errors during replication (see Chapter 13) can be transcribed into mRNA, and, therefore, can result in the translation of a protein with an abnormal amino acid sequence. Various types of mutations can occur that have different effects on the encoded protein (Table 15.2).

Fig. 15.3. Reading frame of mRNA. A. For any given mRNA sequence, there are three possible reading frames (1, 2, and 3). B. An AUG near the 5'-end of the mRNA (the start codon) sets the reading frame for translation of a protein from the mRNA. The codons are read in linear order, starting with this AUG. (The other potential reading frames are not used. They would give proteins with different amino acid sequences.)
A. Point Mutations

Point mutations occur when only one base in DNA is altered, producing a change in a single base of an mRNA codon. There are three basic types of point mutations: silent mutations, missense mutations, and nonsense mutations. Point mutations are said to be “silent” when they do not affect the amino acid sequence of the protein. For example, a codon change from CGA to CGG does not affect the protein because both of these codons specify arginine (see Table 15.1). In missense mutations, one amino acid in the protein is replaced by a different amino acid. For example, a change from CGA to CCA causes arginine to be replaced by proline. A “nonsense” mutation causes the premature termination of a polypeptide chain. For example, a codon change from CGA to UGA causes a codon for arginine to be replaced by a stop codon and synthesis of the mutant protein terminates at this point.

B. Insertions, Deletions, and Frameshift Mutations

An insertion occurs when one or more nucleotides are added to DNA. If the insertion does not generate a stop codon, a protein with more amino acids than normal could be produced.

When one or more nucleotides are removed from DNA, the mutation is known as a deletion. If the deletion does not affect the normal start and stop codons, a protein with fewer than the normal number of amino acids could be produced.

A frameshift mutation occurs when the number of inserted or deleted nucleotides is not a multiple of three (Fig. 15.4). The reading frame shifts at the point where the insertion or deletion begins. Beyond that point, the amino acid sequence of the protein translated from the mRNA differs from the normal protein.

IV. FORMATION OF AMINOACYL-tRNA

A tRNA that contains an amino acid covalently attached to its 3’-end is called an aminoacyl-tRNA and is said to be “charged.” Aminoacyl-tRNAs are named both for the amino acid and the tRNA that carries the amino acid. For example, the tRNA

![Fig. 15.4. A frameshift mutation. The insertion of a single nucleotide (the A in the dotted box) causes the reading frame to shift, so that the amino acid sequence of the protein translated from the mRNA is different after the point of insertion. A similar effect can result from the insertion or deletion of nucleotides if the number inserted or deleted is not a multiple of 3.](image-url)
for alanine (tRNA\textsubscript{Ala}) acquires alanine to become alanyl-tRNA\textsubscript{Ala}. A particular tRNA recognizes only the AUG start codon that initiates protein synthesis and not other AUG codons that specify insertion of methionine within the polypeptide chain. This initiator methionyl-tRNA\textsubscript{Met} is denoted by the subscript “i” in methionyl-tRNA\textsubscript{iMet}.

Amino acids are attached to their tRNAs by highly specific enzymes known as aminoacyl-tRNA synthetases. Twenty different synthetases exist, one for each amino acid. Each synthetase recognizes a particular amino acid and all of the tRNAs that carry that amino acid.

The formation of the ester bond that links the amino acid to the tRNA by an aminoacyl-tRNA synthetase is an energy-requiring process that occurs in two steps. The amino acid is activated in the first step when its carboxyl group reacts with adenosine triphosphate (ATP) to form an enzyme/aminoacyl–AMP complex and pyrophosphate (Fig. 15.5). The cleavage of a high-energy bond of ATP in this reaction provides energy, and the subsequent cleavage of pyrophosphate by a pyrophosphatase helps to drive the reaction by removing one of the products. In the second step, the activated amino acid is transferred to the 2’- or 3’-hydroxyl group (depending on the type of aminoacyl-tRNA synthetase catalyzing the reaction) of the 3’ terminal A residue of the tRNA, and AMP is released (recall that all tRNAs have a CCA added to their 3’ end posttranscriptionally). The energy in the aminoacyl-tRNA ester bond is subsequently used in the formation of a peptide bond during the process of protein synthesis.

Some aminoacyl-tRNA synthetases use the anticodon of the tRNA as a recognition site as they attach the amino acid to the hydroxyl group at the 3’-end of the tRNA (Fig. 15.6). However, other synthetases do not use the anticodon but recognize only bases located at other positions in the tRNA. Nevertheless, insertion of the amino acid into a growing polypeptide chain depends solely on the bases of the anticodon, through complementary base-pairing with the mRNA codon.

V. PROCESS OF TRANSLATION

Translation of a protein involves three steps: initiation, elongation, and termination. It begins with the formation of the initiation complex. Subsequently, synthesis of the polypeptide occurs by a series of elongation steps that are repeated as each A nonsense mutation at codon 17 would cause premature termination of translation. A nonfunctional peptide containing only 16 amino acids would result, producing a β\textsuperscript{0}-thalassemia if the mutation occurred in both alleles. A large deletion in the coding region of the gene could also produce a truncated protein.

If Anne Niemick has a nonsense mutation or a large deletion, it could only be in one allele. The mutation in the other allele must be milder, since she produces some normal β-globin. Her hemoglobin is 7 g/dL, typical of thalassemia intermedia (a β\textsuperscript{–}-thalassemia).
amino acid is added to the growing chain (Fig. 15.7). Termination occurs where the mRNA contains an in-frame stop codon, and the completed polypeptide chain is released.

### A. Initiation of Translation

In eukaryotes, initiation of translation involves formation of a complex composed of methionyl-tRNA\textsubscript{Met}, mRNA, and a ribosome (Fig. 15.8). Methionyl-tRNA\textsubscript{Met} (also known as Met-tRNA\textsubscript{Met}) initially forms a complex with the protein eukaryotic initiation factor 2 (eIF2), which binds GTP. This complex then binds to the small (40S) ribosomal subunit. The cap at the 5′-end of the mRNA binds an initiation factor known as the cap binding protein (CBP). CBP contains a number of subunits, including eIF4E. Several other eIFs join, and the mRNA then binds to the eIFs-Met-tRNA\textsubscript{Met} – 40S ribosome complex. In a reaction requiring hydrolysis of ATP (due to the helicase activity of an eIF subunit), this complex unwinds a hairpin loop in the mRNA and scans the mRNA until it locates the AUG start codon (usually the
first AUG). GTP is hydrolyzed, the initiation factors are released, and the large ribosomal (60S) subunit binds. The ribosome is now complete. It contains one small and one large subunit, and has two binding sites for tRNA, known as the P (peptidyl) and A (aminoacyl) sites. During initiation, Met-tRNA\textsubscript{Met} binds to the ribosome at the P site.

The initiation process differs for prokaryotes and eukaryotes (Table 15.3). In bacteria, the initiating methionyl-tRNA is formylated, producing a formyl-methionyl-tRNA\textsubscript{fMet} that participates in formation of the initiation complex (Fig. 15.9). Only three initiation factors (IFs) are required to generate this complex in prokaryotes, compared with the dozen or more required by eukaryotes. The ribosomes also differ in size. Prokaryotes have 70S ribosomes, composed of 30S and 50S subunits, and eukaryotes have 80S ribosomes, composed of 40S and 60S subunits. Unlike eukaryotic mRNA, bacterial mRNA is not capped. Identification of the initiating AUG triplet in prokaryotes occurs when a sequence in the mRNA (known as the Shine–Dalgarno sequence) binds to a complementary sequence near the 3\textsuperscript{'}-end of the 16S rRNA of the small ribosomal subunit.

### B. Elongation of Polypeptide Chains

After the initiation complex is formed, addition of each amino acid to the growing polypeptide chain involves binding of an aminoacyl-tRNA to the A site on the ribosome, formation of a peptide bond, and translocation of the peptidyl-tRNA to the P site (Fig. 15.10). The peptidyl-tRNA contains the growing polypeptide chain.

#### 1. BINDING OF AMINOACYL-tRNA TO THE A SITE

When Met-tRNA\textsubscript{Met} (or a peptidyl-tRNA) is bound to the P site, the mRNA codon in the A site determines which aminoacyl-tRNA will bind to that site. An aminoacyl-tRNA binds when its anticodon is antiparallel and complementary to the mRNA codon. In eukaryotes, the incoming aminoacyl-tRNA first combines with elongation

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**Table 15.3. Differences between Eukaryotes and Prokaryotes in the Initiation of Protein Synthesis**

<table>
<thead>
<tr>
<th></th>
<th>Eukaryotes</th>
<th>Prokaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding of mRNA to small ribosomal subunit</td>
<td>Cap at 5' end of mRNA binds elfs and tRNA\textsubscript{Met}. mRNA is scanned for AUG start codon upstream of initiating AUG binds to complementary sequence in 16S rRNA</td>
<td></td>
</tr>
<tr>
<td>First amino acid</td>
<td>Methionine</td>
<td>Formyl-methionine</td>
</tr>
<tr>
<td>Initiation factors</td>
<td>elfs (12 or more)</td>
<td>IFs (3)</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>80S (40S and 60S subunits)</td>
<td>70S (30S and 50S subunits)</td>
</tr>
</tbody>
</table>

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**Fig. 15.9.** Bacterial tRNA containing formyl-methionine. The initial methionine is not formylated in eukaryotic protein synthesis.

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**Fig. 15.10.**
factor EF1α containing bound GTP before binding to the mRNA–ribosome complex (EF1α is the GTP-binding α subunit of a heterotrimeric G protein, which is activated for association with other proteins when it contains GTP; see Chapter 11). When the aminoacyl-tRNA-EF1α-GTP complex binds to the A site, GTP is hydrolyzed to GDP. This prompts dissociation of EF1α-GDP from the aminoacyl-tRNA ribosomal complex, thereby allowing protein synthesis to continue (Fig. 15.11).

Fig. 15.10. Elongation of a polypeptide chain. 1. Binding of valyl-tRNAVal to the A site. 2. Formation of a peptide bond. 3. Translocation. After step 3, step 1 is repeated using the aminoacyl-tRNA for the new codon in the A site. Steps 2 and 3 follow. These three steps keep repeating until termination occurs. (In prokaryotes, an exit site called the E site binds the t-RNA after it is displaced from the P site). EF = elongation factor.

Fig. 15.11. Recycling of EF1 in eukaryotes. Note that EF1 is a heterotrimeric G protein. Its α-subunit binds GTP and activates the process whereby an aminoacyl-tRNA binds to the A site of the ribosome. GTP is hydrolyzed, and EF1α binds to the EF1βγ subunits, releasing GDP. GTP binds to the α subunit, the βγ subunits are released, and EF1α GTP is ready for another round. In prokaryotes, EF1α is EF-Tu and the protein corresponding to βγ is EF-Ts.
The free EF1α-GDP reassociates with the EF1βγ subunits, and GDP is released. Subsequently, GTP binds and the βγ subunits dissociate. Thus, EF1α-GTP is ready to bind another aminoacyl-tRNA molecule.

The process of elongation is very similar in prokaryotes, except that the corresponding factor for EF1α is named EF-Tu and the associating elongation factors are called EF-Ts instead of EF1βγ.

2. FORMATION OF A PEPTIDE BOND

In the first round of elongation, the amino acid on the tRNA in the A site forms a peptide bond with the methionine on the tRNA in the P site. In subsequent rounds of elongation, the amino acid on the tRNA in the A site forms a peptide bond with the peptide on the tRNA in the P site (see Fig. 15.10). Peptidyltransferase, which is not a protein but the rRNA of the large ribosomal subunit, catalyzes the formation of the peptide bond. The tRNA in the A site now contains the growing polypeptide chain, and the tRNA in the P site is uncharged (i.e., it no longer contains an amino acid or peptide).

3. TRANSLOCATION

Translocation in eukaryotes involves another G protein, elongation factor EF2 (EF-G in prokaryotes) that complexes with GTP and binds to the ribosome, causing a conformational change that moves the mRNA and its base-paired tRNAs with respect to the ribosome. The uncharged tRNA moves from the P site and is released from the ribosome. The peptidyl-tRNA moves into the P site, and the next codon of the mRNA occupies the A site. During translocation, GTP is hydrolyzed to GDP, which is released from the ribosome along with the elongation factor (see Fig. 15.10).

C. Termination of Translation

The three elongation steps are repeated until a termination (stop) codon moves into the A site on the ribosome. Because no tRNAs with anticodons that can pair with stop codons normally exist in cells, release factors bind to the ribosome instead, causing peptidyltransferase to hydrolyze the bond between the peptide chain and tRNA. The newly synthesized polypeptide is released from the ribosome, which dissociates into its individual subunits, releasing the mRNA.
VI. POLYSOMES

As one ribosome moves along the mRNA, producing a polypeptide chain, a second ribosome can bind to the vacant 5′-end of the mRNA. Many ribosomes can simultaneously translate a single mRNA, forming a complex known as a polysome (Fig. 15.12). A single ribosome covers approximately 80 nucleotides of mRNA. Therefore, ribosomes are positioned on mRNA at intervals of approximately 100 nucleotides. The growing polypeptide chains attached to the ribosomes become longer as each ribosome moves from the 5′-end toward the 3′-end of the mRNA.

VII. PROCESSING OF PROTEINS

Nascent polypeptide chains (i.e., polypeptides that are in the process of being synthesized) are processed. As they are being produced, they travel through a tunnel in the ribosome, which can hold roughly 30 amino acid residues. As polymerization of the chain progresses, the amino acid residues at the N-terminal end begin to emerge from this protected region within the ribosome and to fold and refold into the three-dimensional conformation of the polypeptide. Proteins bind to the nascent polypeptide and mediate the folding process. These mediators are called chaperones (see Chapter 7) because they prevent improper interactions from occurring. Disulfide bond formation between cysteine residues is catalyzed by disulfide isomerases and may also be involved in producing the three-dimensional structure of the polypeptide.

VIII. POSTTRANSLATIONAL MODIFICATIONS

After proteins emerge from the ribosome, they may undergo posttranslational modifications. The initial methionine is removed by specific proteases; methionine is not the N-terminal amino acid of all proteins. Subsequently, other specific cleavages also may occur that convert proteins to more active forms (e.g., the conversion of proinsulin to insulin). In addition, amino acid residues within the peptide chain can be enzymatically modified to alter the activity or stability of the proteins, direct it to a subcellular compartment, or prepare it for secretion from the cell.

Amino acid residues are enzymatically modified by the addition of various types of functional groups. (Box 15.1) For example, the N-terminal amino acid is sometimes acetylated, and methyl groups can be added to lysine residues. These changes alter the charge on the protein. Proline and lysine residues can be modified by hydroxylation. In collagen, hydroxylations lead to stabilization of the protein. Carboxylations are important, especially for the function of proteins involved in blood coagulation. Formation of γ-carboxyglutamate allows these proteins to chelate Ca²⁺, a step in clot formation. Fatty acids or other hydrophobic groups (e.g., prenyl groups) anchor the protein in membranes. An ADP-ribose group can be transferred from NAD⁺ to certain proteins. The addition and removal of phosphate groups (which bind covalently to serine, threonine, or tyrosine residues) serve to regulate the activity of many proteins (e.g., the enzymes of glycogen degradation and regulators of gene transcription.) Glycosylation, the addition of carbohydrate groups, is a common modification that occurs mainly on proteins that are destined to be secreted or incorporated into lysosomes or cellular membranes.

IX. TARGETING OF PROTEINS TO SUBCELLULAR AND EXTRACELLULAR LOCATIONS

Many proteins are synthesized on polysomes in the cytosol. After they are released from ribosomes, they remain in the cytosol, where they carry out their functions. Other proteins synthesized on cytosolic ribosomes enter organelles,
such as mitochondria or nuclei. These proteins contain amino acid sequences called targeting sequences or signal sequences that facilitate their transport into a certain organelle. Another group of proteins are synthesized on ribosomes bound to the RER. These proteins are destined for secretion or for incorporation into various subcellular organelles (e.g., lysosomes, endoplasmic reticulum [ER], Golgi complex) or cellular membranes, including the plasma membrane. Proteins that enter the RER as they are being synthesized have signal peptides near their N-termini that do not have a common amino acid sequence. However, they do contain a number of hydrophobic residues and are 15 to 30 amino acids in length (Fig. 15.13). A signal recognition particle (SRP) binds to the ribosome and to the signal peptide as the nascent polypeptide emerges from the tunnel in the ribosome, and translation ceases. When the SRP subsequently binds to an SRP receptor (docking protein) on the RER, translation resumes, and the polypeptide begins to enter the lumen of the RER. The signal peptide is removed by the signal peptidase, and the remainder of the newly synthesized protein enters the lumen of the RER. These proteins are transferred in small vesicles to the Golgi complex.

The Golgi complex serves to process the proteins it receives from the RER and to sort them so that they are delivered to their appropriate destinations (Fig. 15.14). Processing, which can be initiated in the endoplasmic reticulum, involves glycosylation, the addition of carbohydrate groups, and modification of existing carbohydrate chains. Sorting signals permit delivery of proteins to their target locations. For example, glycosylation of enzymes destined to become lysosomal enzymes results in the presence of a mannose 6-phosphate residue on an oligosaccharide attached to the enzyme. This residue is recognized by the mannose 6-phosphate receptor protein, which incorporates the enzyme into a clathrin-coated vesicle. The vesicle travels to endosomes, and is eventually incorporated into lysosomes. Other proteins containing a KDEL (lys-asp-glu-leu) sequence at their carboxyl terminal are returned to the ER from the Golgi. Proteins with hydrophobic regions can embed in various membranes. Some proteins, whose sorting signals have not yet been determined, enter secretory vesicles and travel to the cell membrane, where they are secreted by the process of exocytosis.

I-cell disease (Mucolipidosis II) is a disorder of protein targeting. Lysosomal proteins are not sorted properly from the Golgi to the lysosomes, and lysosomal enzymes end up secreted from the cell. This is because of a mutation in the enzyme N-acetylglucosamine phosphotransferase, which is a required first step for attaching the lysosomal targeting signal, mannose-6-phosphate, to lysosomal proteins. Thus, lysosomal proteins cannot be targeted to the lysosomes, and these organelles become clogged with materials that cannot be digested, destroying overall lysosomal function. This leads to a lysosomal storage disease of severe consequence, with death before the age of 8.
attached to the ER travel in vesicles to the RER. Proteins synthesized on ribosomes bud from the complex, and some return to the RER. Others mine their fate. Some remain in the Golgi plex. Structural features of the proteins determine fusion, the proteins enter the Golgi complex. After the membrane proteins. See Chapter 10 for descriptions of the endoplasmic reticulum, Golgi complex, and the cell membrane (exocytosis). Proteins with hydrophobic regions embedded in the membrane proteins become cell membrane proteins. See Chapter 10 for descriptions of the endoplasmic reticulum, Golgi complex, lysosomes, and the cell membrane, and also for an explanation of the process of exocytosis.

**CLINICAL COMMENTS**

**Ann Niemick** has a $\beta^+$ thalassemia classified clinically as $\beta$-thalassemia intermedia. She produces an intermediate amount of functional $\beta$ globin chains (her hemoglobin is 7 g/dL; normal is 12–16). In $\beta^0$-thalassemia, little or none of the hemoglobin $\beta$ chain is produced. $\beta$-thalassemia intermedia is usually the result of two different mutations (one that mildly affects the rate of synthesis of $\beta$-globin and one severely affecting its rate of synthesis), or, less frequently, homozygosity for a mild mutation in the rate of synthesis, or a complex combination of mutations. The mutations that cause the thalassemias have been studied extensively and are summarized in Table 15.4. For each of these mutations, you should now be able to explain whether it is most likely to result in a $\beta^+$ or $\beta^0$ thalassemia.

**Jay Sakz.** The molecular biology genetics laboratory’s report on Jay Sakz’s white blood cells indicated that he had a deficiency of hexosaminidase A caused by a defect in the gene encoding the $\alpha$ subunit of this enzyme (variant B, Tay-Sachs disease). Hexosaminidases are lysosomal enzymes necessary for the normal degradation of glycosphingolipids, such as the gangliosides. Gangliosides are found in high concentrations in neural ganglia, although they are produced in many areas of the nervous system. When the activity of these degradative enzymes is absent or subnormal, partially degraded gangliosides accumulate in lysosomes in various cells of the central nervous system, causing a wide array of neurologic disorders known collectively as gangliosidoses.

### Table 15.4 Some Examples of Mutations in $\beta$-Thalassemia

<table>
<thead>
<tr>
<th>Type of Mutation</th>
<th>Phenotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsense</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codon 17 (A $\rightarrow$ T)</td>
<td>$\beta^0$</td>
<td>Chinese</td>
</tr>
<tr>
<td>Codon 39 (C $\rightarrow$ T)</td>
<td>$\beta^0$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Codon 121 (A $\rightarrow$ T)</td>
<td>$\beta^0$</td>
<td>Polish</td>
</tr>
<tr>
<td>Frameshift</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codon 6 (-1 bp)</td>
<td>$\beta^0$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Codon 16 (-1 bp)</td>
<td>$\beta^0$</td>
<td>Asian Indian</td>
</tr>
<tr>
<td>Codon 41/42 (-4 bp)</td>
<td>$\beta^0$</td>
<td>Asian Indian, Chinese</td>
</tr>
<tr>
<td>Codon 71/72 (+1 bp)</td>
<td>$\beta^0$</td>
<td>Chinese</td>
</tr>
<tr>
<td>Promoter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Position -88 (C $\rightarrow$ T)</td>
<td>$\beta^+$</td>
<td>African American</td>
</tr>
<tr>
<td>Position -31 (A $\rightarrow$ G)</td>
<td>$\beta^+$</td>
<td>Japanese</td>
</tr>
<tr>
<td>Position -28 (A $\rightarrow$ C)</td>
<td>$\beta^+$</td>
<td>Kurdish</td>
</tr>
<tr>
<td>Cap Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Position +1 (A $\rightarrow$ C)</td>
<td>$\beta^+$</td>
<td>Asian Indian</td>
</tr>
<tr>
<td>Splice Junction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intron 1, position 1 (G $\rightarrow$ A)</td>
<td>$\beta^0$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Intron 1, 3’-end (-25 bp)</td>
<td>$\beta^0$</td>
<td>Asian Indian</td>
</tr>
<tr>
<td>Intron 2, position 1 (G $\rightarrow$ A)</td>
<td>$\beta^0$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Intron 2, 3’-end (A $\rightarrow$ G)</td>
<td>$\beta^0$</td>
<td>African American</td>
</tr>
<tr>
<td>Intron, internal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intron 1, position 5 (G $\rightarrow$ T)</td>
<td>$\beta^+$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Intron 1, position 6 (T $\rightarrow$ C)</td>
<td>$\beta^+$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Intron 2, position 110 (G $\rightarrow$ A)</td>
<td>$\beta^+$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Intron 2, position 654 (C $\rightarrow$ T)</td>
<td>$\beta^0$</td>
<td>Chinese</td>
</tr>
<tr>
<td>Intron 2, position 745 (C $\rightarrow$ G)</td>
<td>$\beta^+$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>Exon, internal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codon 24 (T $\rightarrow$ A)</td>
<td>$\beta^+$</td>
<td>African American</td>
</tr>
<tr>
<td>Codon 26 (G $\rightarrow$ A)</td>
<td>$\beta^+$</td>
<td>Southeast Asian</td>
</tr>
<tr>
<td>Codon 27 (G $\rightarrow$ T)</td>
<td>$\beta^{G620A}$</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>RNA cleavage/polyadenylation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AATAAA $\rightarrow$ AACAAA</td>
<td>$\beta^+$</td>
<td>African American</td>
</tr>
</tbody>
</table>

When the enzyme deficiency is severe, symptoms appear within the first 3–5 months of life. Eventually symptoms include upper and lower motor neuron deficits, visual difficulties that can progress to blindness, seizures, and increasing cognitive dysfunction. By the second year of life, the patient may regress into a completely vegetative state, often succumbing to bronchopneumonia caused by aspiration or an inability to cough.

**Erna Nemdy.** With the availability of diphtheria toxoid as part of the almost universal DPT immunization practices in the United States, fatalities due to infection by the Gram-positive bacillus *C. diphtheriae* are rare. Most children, as is the case with Erna Nemdy’s daughter Beverly, are immunized. In unimmunized individuals, however, symptoms are caused by a bacterial exotoxin encoded by a phage that infects the bacterial cells. The toxin enters human cells, inhibiting protein synthesis and, ultimately, causing cell death. Complications related to cardiac and nervous system involvement are the major cause of morbidity and mortality. Patients for whom a definitive diagnosis of diphtheria is established are treated with equine diphtheria antitoxin.

### BIOCHEMICAL COMMENTS

**Antibiotics That Inhibit Protein Synthesis.** The processes of translation on bacterial ribosomes and on the cytoplasmic ribosomes of eukaryotic cells have many similarities, but there are a number of subtle differences. Antibiotics act at steps at which these differences occur, and different antibiotics target each of the major steps of protein synthesis (Table 15.5). Therefore, these compounds can be used selectively to prevent bacterial protein synthesis and inhibit bacterial proliferation, while having little or no effect on human cells. Caution must be exercised in their use, however, because some of the antibiotics affect human mitochondria, which have a protein-synthesizing system similar to that of bacteria. Another problem with these drugs is that bacteria can become resistant to their action. Mutations in genes encoding the proteins or RNA of bacterial ribosomes can cause resistance. Resistance also results when bacteria take up plasmids carrying genes for inactivation of the antibiotic. Because of the widespread and often indiscriminate use of antibiotics, strains of bacteria are rapidly developing that are resistant to all known antibiotics.

**Streptomycin.** Streptomycin inhibits initiation by binding to three proteins and probably the 16S rRNA of the 30S ribosomal subunit of bacteria. Abnormal initiation complexes, known as streptomycin monosomes, accumulate. Streptomycin can also cause misreading of mRNA, resulting in premature termination of translation or in the incorporation of incorrect amino acids into polypeptide chains that already have been initiated. The use of this antibiotic is limited because it causes ototoxicity that can result in loss of hearing.

**Table 15.5 Inhibitors of Protein Synthesis in Prokaryotes**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>Binds to the 30S ribosomal subunit of prokaryotes, thereby preventing formation of the initiation complex. It also causes misreading of mRNA</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Binds to the 30S ribosomal subunit and inhibits binding of aminoacyl-tRNA to the A site</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Binds to the 50S ribosomal subunit and inhibits peptidyltransferase</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Binds to the 50S ribosomal subunit and prevents translocation</td>
</tr>
</tbody>
</table>
**Tetracycline.** Tetracycline binds to the 30S ribosomal subunit of bacteria and prevents an aminoacyl-tRNA from binding to the A site on the ribosome. This effect of the drug is reversible; thus, when the drug is removed, bacteria resume protein synthesis and growth, resulting in a rekindling of the infection. Furthermore, tetracycline is not absorbed well from the intestine, and its concentration can become elevated in the contents of the gut, leading to changes in the intestinal flora. Because it has been used to treat human infections and added to animal feed to prevent animal infections, humans have had extensive exposure to tetracycline. As a result, resistant strains of bacteria have developed.

**Chloramphenicol.** Chloramphenicol binds to the 50S ribosomal subunit of bacteria and prevents binding of the amino acid portion of the aminoacyl-tRNA, effectively inhibiting peptidyltransferase action. This antibiotic is used only for certain extremely serious infections, such as meningitis and typhoid fever. Chloramphenicol readily enters human mitochondria, where it inhibits protein synthesis. Cells of the bone marrow often fail to develop in patients treated with chloramphenicol, and use of this antibiotic has been linked to fatal blood dyscrasias, including an aplastic anemia.

**Erythromycin.** Erythromycin and the other macrolide antibiotics bind to the 50S ribosomal subunit of bacteria near the binding site for chloramphenicol. They prevent the translocation step, the movement of the peptidyl-tRNA from the “A” to the “P” site on the ribosome. Because the side effects are less severe and more readily reversible than those of many other antibiotics, the macrolides are often used to treat infections in persons who are allergic to penicillin, an antibiotic that inhibits bacterial cell wall synthesis. However, bacterial resistance to erythromycin is increasing. Therefore, its close relative, clarithromycin, is often used.

### Suggested References

**Translation:**

**Tay-Sachs:**

**Thalassemia:**

**Antibiotics that inhibit protein synthesis:**

### REVIEW QUESTIONS—CHAPTER 15

1. In the read-out of the genetic code in prokaryotes, which one of the following processes acts before any of the others?
   (A) tRNAi alignment with mRNA
   (B) Termination of transcription
   (C) Movement of the ribosome from one codon to the next
   (D) Recruitment of termination factors to the A site
   (E) Export of mRNA from the nucleus
2. tRNA charged with cysteine can be chemically treated so that the amino acid changes its identity to alanine. If some of this charged tRNA is added to a protein-synthesizing extract that contains ALL the normal components required for translation, which of the following statements represents THE MOST LIKELY OUTCOME after adding an mRNA that has both cys and ala codons in the normal reading frame?

(A) Cysteine would be added each time the alanine codon was translated.
(B) Alanine would be added each time the cysteine codon was translated.
(C) The protein would have a deficiency of cysteine residues.
(D) The protein would have a deficiency of alanine residues.
(E) The protein would be entirely normal.

3. The genetic code is said to be degenerate because of which of the following?

(A) Many codons have pairs of identical bases next to each other.
(B) Some triplets are made up of repeating purines or pyrimidines.
(C) Many of the amino acids have more than one triplet code.
(D) There is wobble in the bond between the first base of the anticodon and the third base of the codon.
(E) All triplets seem to have at least one uracil.

4. Which of the following is responsible for retaining the enzyme protein disulfide isomerase in the ER?

(A) Glycosylation
(B) The presence of a KDEL sequence at the C-terminal
(C) Retention of the signal peptide
(D) Phosphorylation of Ser 22
(E) Attachment of a fatty acid

5. The reason there are 64 possible codons is which of the following?

(A) There are 64 aminoacyl tRNA synthetases.
(B) Each base is able to participate in wobbling.
(C) All possible reading frames can be used this way.
(D) There are four possible bases at each of three codon positions.
(E) The more codons, the faster protein synthesis can be accomplished.