Given the importance of nuclear subdomains in RNA processing, it might have been expected that pre-mRNA splicing would occur in a particular location in the nucleus, as it requires numerous RNA and protein components. However, the assembly of splicing components on pre-mRNA is co-transcriptional; thus, splicing must occur at many locations along chromosomes. Although a typical mammalian cell may be expressing on the order of 15,000 genes, transcription and RNA splicing may be localized to only several thousand sites in the nucleus. These sites themselves are highly dynamic and probably result from the association of transcription and splicing components to create small "assembly lines" with a high local concentration of these components. Interchromatin granule clusters—which contain stockpiles of RNA-processing components—are often observed next to sites of transcription, as though poised to replenish supplies. Thus, the nucleus seems to be highly organized into subdomains, with snRNPs, snoRNPs, and other nuclear components moving between them in an orderly fashion according to the needs of the cell (see Figure 6–48; also see Figure 4–69).

Summary

Before the synthesis of a particular protein can begin, the corresponding mRNA molecule must be produced by transcription. Bacteria contain a single type of RNA polymerase (the enzyme that carries out the transcription of DNA into RNA). An mRNA molecule is produced when this enzyme initiates transcription at a promoter, synthesizes the RNA by chain elongation, stops transcription at a terminator, and releases both the DNA template and the completed mRNA molecule. In eucaryotic cells, the process of transcription is much more complex, and there are three RNA polymerases—polymerase I, II, and III—that are related evolutionarily to one another and to the bacterial polymerase.

RNA polymerase II synthesizes eucaryotic mRNA. This enzyme requires a series of additional proteins, the general transcription factors, to initiate transcription on a purified DNA template, and still more proteins (including chromatin-remodeling complexes and histone-modifying enzymes) to initiate transcription on its chromatin templates inside the cell.

During the elongation phase of transcription, the nascent RNA undergoes three types of processing events: a special nucleotide is added to its 5′ end (capping), intron sequences are removed from the middle of the RNA molecule (splicing), and the 3′ end of the RNA is generated (cleavage and polyadenylation). Each of these processes is initiated by proteins that travel along with RNA polymerase II by binding to sites on its long, extended C-terminal tail. Splicing is unusual in that many of its key steps are carried out by specialized RNA molecules rather than proteins. Properly processed mRNAs are passed through nuclear pore complexes into the cytosol, where they are translated into protein.

For some genes, RNA is the final product. In eucaryotes, these genes are usually transcribed by either RNA polymerase I or RNA polymerase III. RNA polymerase I makes the ribosomal RNAs. After their synthesis as a large precursor, the rRNAs are chemically modified, cleaved, and assembled into the two ribosomal subunits in the nucleolus—a distinct subnuclear structure that also helps to process some smaller RNA–protein complexes in the cell. Additional subnuclear structures (including Cajal bodies and interchromatin granule clusters) are sites where components involved in RNA processing are assembled, stored, and recycled.

FROM RNA TO PROTEIN

In the preceding section we have seen that the final product of some genes is an RNA molecule itself, such as those present in the snRNPs and in ribosomes. However, most genes in a cell produce mRNA molecules that serve as intermediaries on the pathway to proteins. In this section we examine how the cell converts the information carried in an mRNA molecule into a protein molecule. This feat of translation was a focus of attention of biologists in the late 1950s, when it

was posed as the "coding problem": how is the information in a linear sequence of nucleotides in RNA translated into the linear sequence of a chemically quite different set of units—the amino acids in proteins? This fascinating question stimulated great excitement among scientists at the time. Here was a cryptogram set up by nature that, after more than 3 billion years of evolution, could finally be solved by one of the products of evolution—human beings. And indeed, not only has the code been cracked step by step, but in the year 2000 the structure of the elaborate machinery by which cells read this code—the ribosome—was finally revealed in atomic detail.

An mRNA Sequence Is Decoded in Sets of Three Nucleotides

Once an mRNA has been produced by transcription and processing, the information present in its nucleotide sequence is used to synthesize a protein. Transcription is simple to understand as a means of information transfer: since DNA and RNA are chemically and structurally similar, the DNA can act as a direct template for the synthesis of RNA by complementary base-pairing. As the term *transcription* signifies, it is as if a message written out by hand is being converted, say, into a typewritten text. The language itself and the form of the message do not change, and the symbols used are closely related.

In contrast, the conversion of the information in RNA into protein represents a **translation** of the information into another language that uses quite different symbols. Moreover, since there are only 4 different nucleotides in mRNA and 20 different types of amino acids in a protein, this translation cannot be accounted for by a direct one-to-one correspondence between a nucleotide in RNA and an amino acid in protein. The nucleotide sequence of a gene, through the intermediary of mRNA, is translated into the amino acid sequence of a protein by rules that are known as the **genetic code**. This code was deciphered in the early 1960s.

The sequence of nucleotides in the mRNA molecule is read in consecutive groups of three. RNA is a linear polymer of four different nucleotides, so there are $4 \times 4 \times 4 = 64$ possible combinations of three nucleotides: the triplets AAA, AUA, AUG, and so on. However, only 20 different amino acids are commonly found in proteins. Either some nucleotide triplets are never used, or the code is redundant and some amino acids are specified by more than one triplet. The second possibility is, in fact, the correct one, as shown by the completely deciphered genetic code in **Figure 6–50**. Each group of three consecutive nucleotides in RNA is called a **codon**, and each codon specifies either one amino acid or a stop to the translation process.

This genetic code is used universally in all present-day organisms. Although a few slight differences in the code have been found, these are chiefly in the DNA of mitochondria. Mitochondria have their own transcription and protein synthesis systems that operate quite independently from those of the rest of the cell, and it is understandable that their small genomes have been able to accommodate minor changes to the code (discussed in Chapter 14).

	AGA AGG									UUA UUG					AGC AGU					
GCA	CGA						GGA			CUA				CCA	UCA	ACA			GUA	
GCC	CGC						GGC		AUA	CUC				CCC	UCC	ACC			GUC	UAA
GCG	CGG	GAC	AAC	UGC	GAA	CAA	GGG	CAC	AUC	CUG	AAA		UUC	CCG	UCG	ACG		UAC	GUG	UAG
GCU	CGU	GAU	AAU	UGU	GAG	CAG	GGU	CAU	AUU	CUU	AAG	AUG	UUU	CCU	UCU	ACU	UGG	UAU	GUU	UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Figure 6–50 The genetic code. The standard one-letter abbreviation for each amino acid is presented below its three-letter abbreviation (see Panel 3–1, pp. 128–129, for the full name of each amino acid and its structure). By convention, codons are always written with the 5′-terminal nucleotide to the left. Note that most amino acids are represented by more than one codon, and that there are some regularities in the set of codons that specifies each amino acid. Codons for the same amino acid tend to contain the same nucleotides at the first and second positions, and vary at the third position. Three codons do not specify any amino acid but act as termination sites (stop codons), signaling the end of the protein-coding sequence. One codon—AUG—acts both as an initiation codon, signaling the start of a protein-coding message, and also as the codon that specifies methionine.

In principle, an RNA sequence can be translated in any one of three different **reading frames**, depending on where the decoding process begins (**Figure 6–51**). However, only one of the three possible reading frames in an mRNA encodes the required protein. We see later how a special punctuation signal at the beginning of each RNA message sets the correct reading frame at the start of protein synthesis.

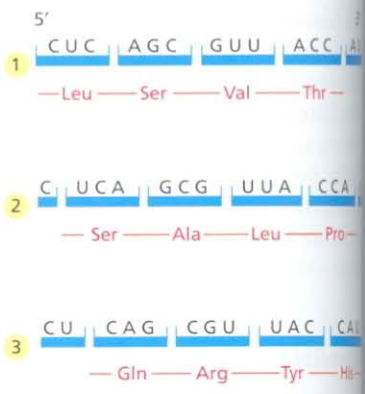


Figure 6–51 The three possible reading frames in protein synthesis. In the process of translating a nucleotide sequence *(blue)* into an amino acid sequence *(red)*, the sequence of nucleotides in an mRNA molecule is read from the 5′ end to the 3′ end in consecutive sets of three nucleotides. In principle, therefore, the same RNA sequence can specify three completely different amino acid sequences, depending on the reading frame. In reality, however, only one of these reading frames contains the actual message.

tRNA Molecules Match Amino Acids to Codons in mRNA

The codons in an mRNA molecule do not directly recognize the amino acids they specify: the group of three nucleotides does not, for example, bind directly to the amino acid. Rather, the translation of mRNA into protein depends on adaptor molecules that can recognize and bind both to the codon and, at another site on their surface, to the amino acid. These adaptors consist of a set of small RNA molecules known as **transfer RNAs** (**tRNAs**), each about 80 nucleotides in length.

We saw earlier in this chapter that RNA molecules can fold into precise three-dimensional structures, and the tRNA molecules provide a striking example. Four short segments of the folded tRNA are double-helical, producing a molecule that looks like a cloverleaf when drawn schematically (**Figure 6–52**). For example, a 5′-GCUC-3′ sequence in one part of a polynucleotide chain can form a relatively strong association with a 5′-GAGC-3′ sequence in another region of the same molecule. The cloverleaf undergoes further folding to form a compact L-shaped structure that is held together by additional hydrogen bonds between different regions of the molecule.

Two regions of unpaired nucleotides situated at either end of the L-shaped molecule are crucial to the function of tRNA in protein synthesis. One of these

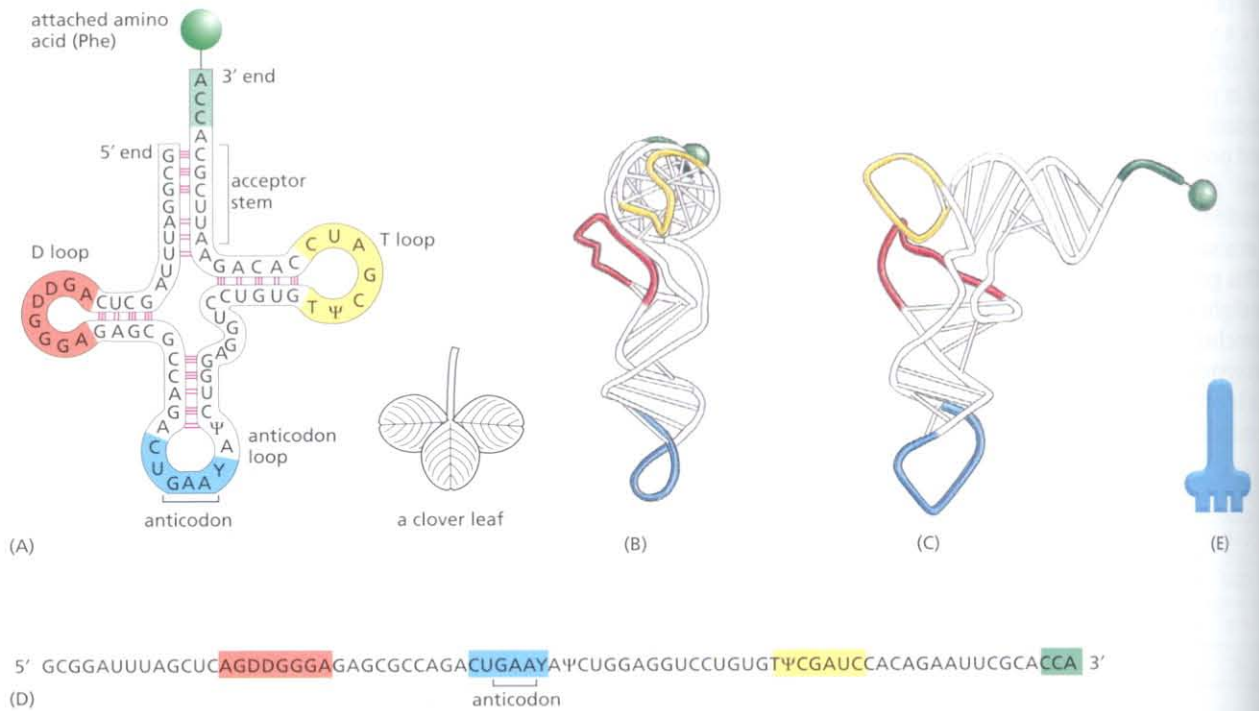


Figure 6–52 A tRNA molecule. A tRNA specific for the amino acid phenylalanine (Phe) is depicted in various ways. (A) The cloverleaf structure showing the complementary base-pairing *(red lines)* that creates the double-helical regions of the molecule. The anticodon is the sequence of three nucleotides that base-pairs with a codon in mRNA. The amino acid matching the codon/anticodon pair is attached at the 3′ end of the tRNA. tRNAs contain some unusual bases, which are produced by chemical modification after the tRNA has been synthesized. For example, the bases denoted ψ (pseudouridine—see Figure 6–43) and D (dihydrouridine—see Figure 6–55) are derived from uracil. (B and C) Views of the L-shaped molecule, based on x-ray diffraction analysis. Although this diagram shows the tRNA for the amino acid phenylalanine, all other tRNAs have similar structures. <CGCA> (D) The linear nucleotide sequence of the molecule, color-coded to match (A), (B), and (C). (E) The tRNA icon we use in this book.

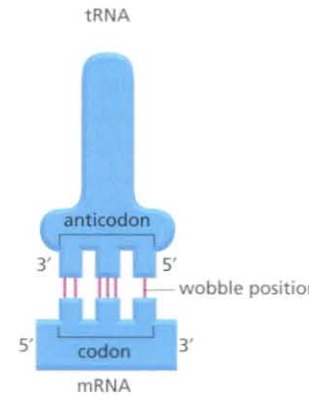
regions forms the **anticodon**, a set of three consecutive nucleotides that pairs with the complementary codon in an mRNA molecule. The other is a short single-stranded region at the 3′ end of the molecule; this is the site where the amino acid that matches the codon is attached to the tRNA.

We have seen in the previous section that the genetic code is redundant; that is, several different codons can specify a single amino acid (see Figure 6–50). This redundancy implies either that there is more than one tRNA for many of the amino acids or that some tRNA molecules can base-pair with more than one codon. In fact, both situations occur. Some amino acids have more than one tRNA, and some tRNAs are constructed so that they require accurate base-pairing only at the first two positions of the codon and can tolerate a mismatch (or *wobble*) at the third position (**Figure 6–53**). This wobble base-pairing explains why so many of the alternative codons for an amino acid differ only in their third nucleotide (see Figure 6–50). In bacteria, wobble base-pairings make it possible to fit the 20 amino acids to their 61 codons with as few as 31 kinds of tRNA molecules. The exact number of different kinds of tRNAs, however, differs from one species to the next. For example, humans have nearly 500 tRNA genes but, among them, only 48 different anticodons are represented.

tRNAs Are Covalently Modified Before They Exit from the Nucleus

Like most other eucaryotic RNAs, tRNAs are covalently modified before they are allowed to exit from the nucleus. Eucaryotic tRNAs are synthesized by RNA polymerase III. Both bacterial and eucaryotic tRNAs are typically synthesized as larger precursor tRNAs, which are then trimmed to produce the mature tRNA. In addition, some tRNA precursors (from both bacteria and eucaryotes) contain introns that must be spliced out. This splicing reaction differs chemically from pre-mRNA splicing; rather than generating a lariat intermediate, tRNA splicing uses a cut-and-paste mechanism that is catalyzed by proteins (**Figure 6–54**). Trimming and splicing both require the precursor tRNA to be correctly folded in its cloverleaf configuration. Because misfolded tRNA precursors will not be processed properly, the trimming and splicing reactions are thought to act as quality-control steps in the generation of tRNAs.

All tRNAs are modified chemically—nearly 1 in 10 nucleotides in each mature tRNA molecule is an altered version of a standard G, U, C, or A ribonucleotide. Over 50 different types of tRNA modifications are known; a few are shown in **Figure 6–55**. Some of the modified nucleotides—most notably inosine, produced by the deamination of adenosine—affect the conformation and base-pairing of the anticodon and thereby facilitate the recognition of the appropriate mRNA codon by the tRNA molecule (see Figure 6–53). Others affect the accuracy with which the tRNA is attached to the correct amino acid.



bacteria

wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U or I
G	C or U

eucaryotes

wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U
G	C

Figure 6–53 Wobble base-pairing between codons and anticodons. If the nucleotide listed in the first column is present at the third, or wobble, position of the codon, it can base-pair with any of the nucleotides listed in the second column. Thus, for example, when inosine (I) is present in the wobble position of the tRNA anticodon, the tRNA can recognize any one of three different codons in bacteria and either of two codons in eucaryotes. The inosine in tRNAs is formed from the deamination of guanine (see Figure 6–55), a chemical modification that takes place after the tRNA has been synthesized. The nonstandard base pairs, including those made with inosine, are generally weaker than conventional base pairs. Note that codon–anticodon base pairing is more stringent at positions 1 and 2 of the codon: here only conventional base pairs are permitted. The differences in wobble base-pairing interactions between bacteria and eucaryotes presumably result from subtle structural differences between bacterial and eucaryotic ribosomes, the molecular machines that perform protein synthesis. (Adapted from C. Guthrie and J. Abelson, in The Molecular Biology of the Yeast *Saccharomyces*: Metabolism and Gene Expression, pp. 487–528. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1982.)

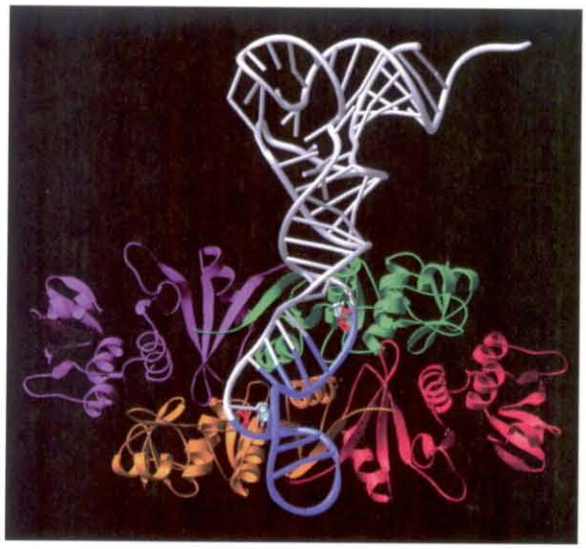


Figure 6–54 Structure of a tRNA-splicing endonuclease docked to a precursor tRNA. The endonuclease (a four-subunit enzyme) removes the tRNA intron (blue). A second enzyme, a multifunctional tRNA ligase (not shown), then joins the two tRNA halves together. (Courtesy of Hong Li, Christopher Trotta and John Abelson.)

Specific Enzymes Couple Each Amino Acid to Its Appropriate tRNA Molecule

We have seen that, to read the genetic code in DNA, cells make a series of different tRNAs. We now consider how each tRNA molecule becomes linked to the one amino acid in 20 that is its appropriate partner. Recognition and attachment of the correct amino acid depends on enzymes called **aminoacyl-tRNA synthetases**, which covalently couple each amino acid to its appropriate set of tRNA molecules (**Figure 6–56** and **Figure 6–57**). Most cells have a different synthetase enzyme for each amino acid (that is, 20 synthetases in all); one attaches glycine to all tRNAs that recognize codons for glycine, another attaches alanine to all tRNAs that recognize codons for alanine, and so on. Many bacteria, however, have fewer than 20 synthetases, and the same synthetase enzyme is responsible for coupling more than one amino acid to the appropriate tRNAs. In these cases, a single synthetase places the identical amino acid on two different types of tRNAs, only one of which has an anticodon that matches the amino acid. A second enzyme then chemically modifies each "incorrectly" attached amino acid so that it now corresponds to the anticodon displayed by its covalently linked tRNA.

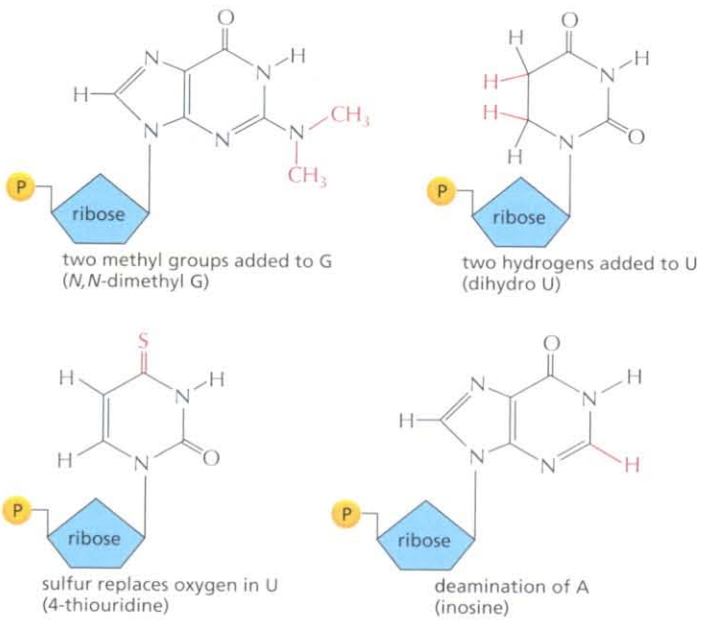


Figure 6–55 A few of the unusual nucleotides found in tRNA molecules. These nucleotides are produced by covalent modification of a normal nucleotide after it has been incorporated into an RNA chain. Two other types of modified nucleotides are shown in Figure 6–43. In most tRNA molecules about 10% of the nucleotides are modified (see Figure 6–52).

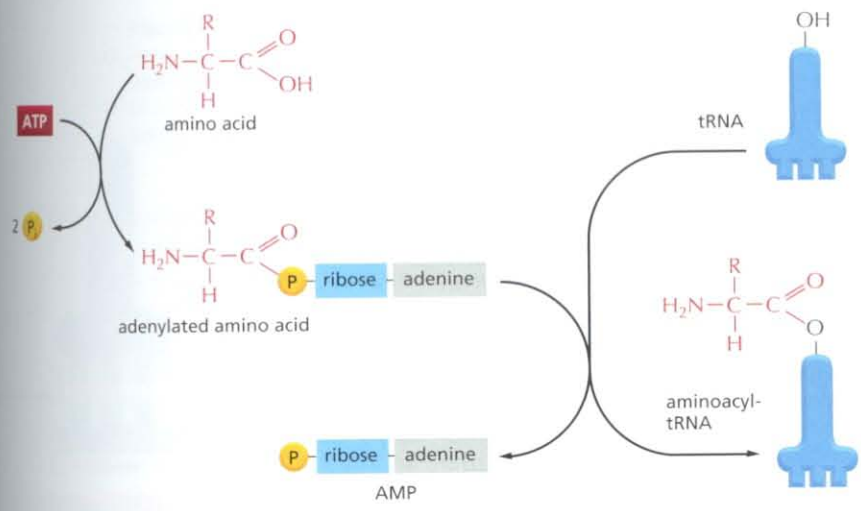


Figure 6–56 Amino acid activation. An amino acid is activated for protein synthesis by an aminoacyl-tRNA synthetase enzyme in two steps. As indicated, the energy of ATP hydrolysis is used to attach each amino acid to its tRNA molecule in a high-energy linkage. The amino acid is first activated through the linkage of its carboxyl group directly to an AMP moiety, forming an *adenylated amino acid*; the linkage of the AMP, normally an unfavorable reaction, is driven by the hydrolysis of the ATP molecule that donates the AMP. Without leaving the synthetase enzyme, the AMP-linked carboxyl group on the amino acid is then transferred to a hydroxyl group on the sugar at the 3′ end of the tRNA molecule. This transfer joins the amino acid by an activated ester linkage to the tRNA and forms the final aminoacyl-tRNA molecule. The synthetase enzyme is not shown in this diagram.

The synthetase-catalyzed reaction that attaches the amino acid to the 3′ end of the tRNA is one of many reactions coupled to the energy-releasing hydrolysis of ATP (see pp. 79–81), and it produces a high-energy bond between the tRNA and the amino acid. The energy of this bond is used at a later stage in protein synthesis to link the amino acid covalently to the growing polypeptide chain.

The aminoacyl-tRNA synthetase enzymes and the tRNAs are equally important in the decoding process (**Figure 6–58**). This was established by an experiment in which one amino acid (cysteine) was chemically converted into a different amino acid (alanine) after it already had been attached to its specific tRNA. When such "hybrid" aminoacyl-tRNA molecules were used for protein synthesis in a cell-free system, the wrong amino acid was inserted at every point in the protein chain where that tRNA was used. Although, as we shall see, cells have several quality control mechanisms to avoid this type of mishap, the experiment establishes that the genetic code is translated by two sets of adaptors that act sequentially. Each matches one molecular surface to another with great specificity, and it is their combined action that associates each sequence of three nucleotides in the mRNA molecule—that is, each codon—with its particular amino acid.

Editing by tRNA Synthetases Ensures Accuracy

Several mechanisms working together ensure that the tRNA synthetase links the correct amino acid to each tRNA. The synthetase must first select the correct amino acid, and most synthetases do so by a two-step mechanism. First, the correct amino acid has the highest affinity for the active-site pocket of its synthetase

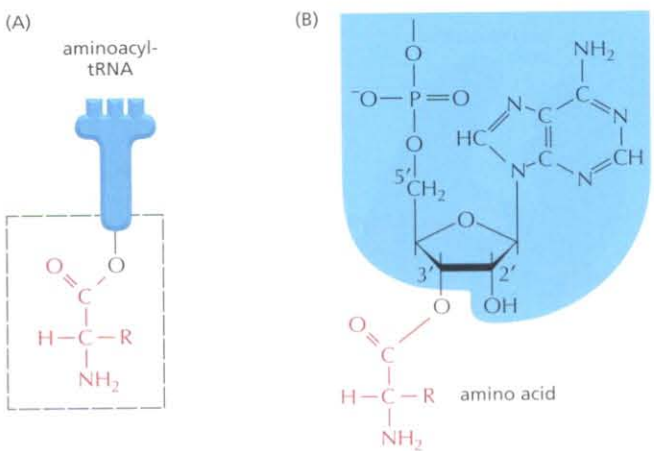
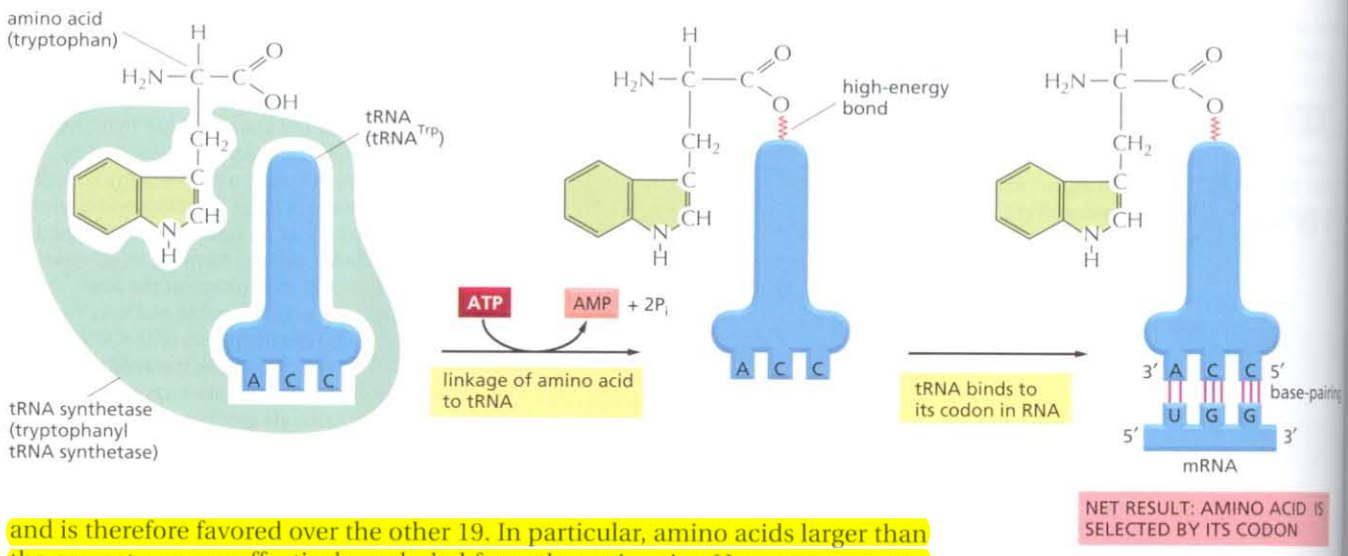


Figure 6–57 The structure of the aminoacyl-tRNA linkage. The carboxyl end of the amino acid forms an ester bond to ribose. Because the hydrolysis of this ester bond is associated with a large favorable change in free energy, an amino acid held in this way is said to be activated. (A) Schematic drawing of the structure. The amino acid is linked to the nucleotide at the 3′ end of the tRNA (see Figure 6–52). (B) Actual structure corresponding to the boxed region in (A). There are two major classes of synthetase enzymes: one links the amino acid directly to the 3′-OH group of the ribose, and the other links it initially to the 2′-OH group. In the latter case, a subsequent transesterification reaction shifts the amino acid to the 3′ position. As in Figure 6–56, the "R group" indicates the side chain of the amino acid.



and is therefore favored over the other 19. In particular, amino acids larger than the correct one are effectively excluded from the active site. However, accurate discrimination between two similar amino acids, such as isoleucine and valine (which differ by only a methyl group), is very difficult to achieve by a one-step recognition mechanism. A second discrimination step occurs after the amino acid has been covalently linked to AMP (see Figure 6–56). When tRNA binds the synthetase, it tries to force the amino acid into a second pocket in the synthetase, the precise dimensions of which exclude the correct amino acid but allow access by closely related amino acids. Once an amino acid enters this editing pocket, it is hydrolyzed from the AMP (or from the tRNA itself if the aminoacyl-tRNA bond has already formed), and is released from the enzyme. This hydrolytic editing, which is analogous to the exonucleolytic proofreading by DNA polymerases (**Figure 6–59**), raises the overall accuracy of tRNA charging to approximately one mistake in 40,000 couplings.

Figure 6–58 The genetic code is translated by means of two adaptors that act one after another. The first adaptor is the aminoacyl-tRNA synthetase, which couples a particular amino acid to its corresponding tRNA; the second adaptor is the tRNA molecule itself, whose *anticodon* forms base pairs with the appropriate *codon* on the mRNA. An error in either step would cause the wrong amino acid to be incorporated into a protein chain. In the sequence of events shown, the amino acid tryptophan (Trp) is selected by the codon UGG on the mRNA.

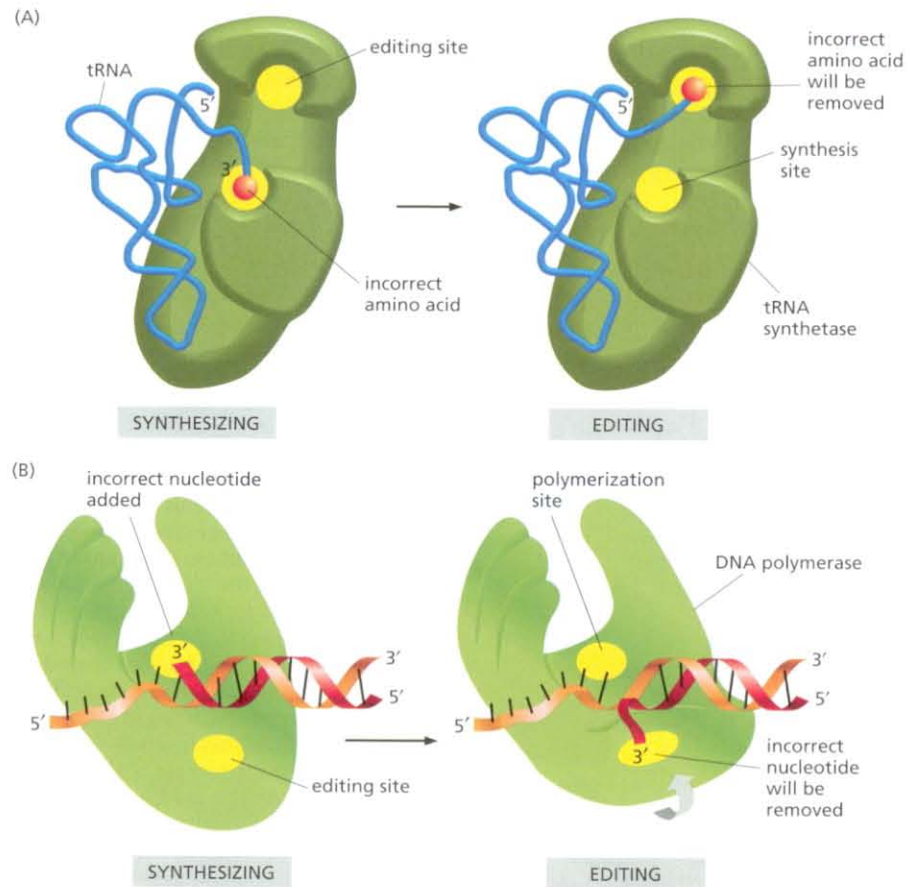


Figure 6–59 Hydrolytic editing. (A) tRNA synthetases remove their own coupling errors through hydrolytic editing of incorrectly attached amino acids. As described in the text, the correct amino acid is rejected by the editing site. (B) The error-correction process performed by DNA polymerase shows some similarities; however, it differs in so far as the removal process depends strongly on a mispairing with the template (see Figure 5–8).

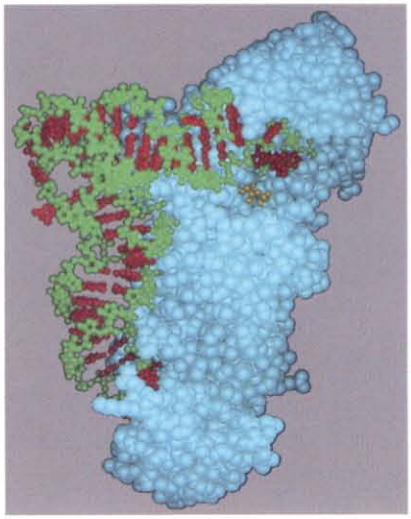


Figure 6–60 The recognition of a tRNA molecule by its aminoacyl-tRNA synthetase. For this tRNA ($tRNA^{Gln}$), specific nucleotides in both the anticodon *(bottom)* and the amino acid-accepting arm allow the correct tRNA to be recognized by the synthetase enzyme *(blue)*. A bound ATP molecule is *yellow*. (Courtesy of Tom Steitz.)

The tRNA synthetase must also recognize the correct set of tRNAs, and extensive structural and chemical complementarity between the synthetase and the tRNA allows the synthetase to probe various features of the tRNA (**Figure 6-60**). Most tRNA synthetases directly recognize the matching tRNA anticodon; these synthetases contain three adjacent nucleotide-binding pockets, each of which is complementary in shape and charge to a nucleotide in the anticodon. For other synthetases, the nucleotide sequence of the acceptor stem is the key recognition determinant. In most cases, however, the synthetase "reads" the nucleotides at several different positions on the tRNA.

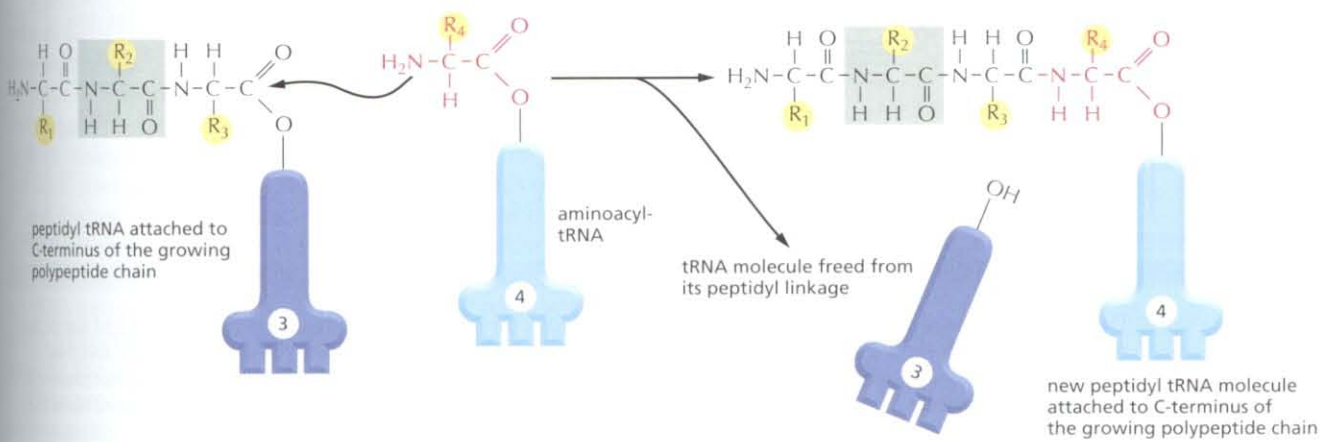
Amino Acids Are Added to the C-terminal End of a Growing Polypeptide Chain

Having seen that amino acids are first coupled to tRNA molecules, we now turn to the mechanism that joins amino acids together to form proteins. The fundamental reaction of protein synthesis is the formation of a peptide bond between the carboxyl group at the end of a growing polypeptide chain and a free amino group on an incoming amino acid. Consequently, a protein is synthesized stepwise from its N-terminal end to its C-terminal end. Throughout the entire process the growing carboxyl end of the polypeptide chain remains activated by its covalent attachment to a tRNA molecule (forming a peptidyl-tRNA). Each addition disrupts this high-energy covalent linkage, but immediately replaces it with an identical linkage on the most recently added amino acid (**Figure 6–61**). In this way, each amino acid added carries with it the activation energy for the addition of the next amino acid rather than the energy for its own addition—an example of the "head growth" type of polymerization described in Figure 2–68.

Figure 6–61 The incorporation of an amino acid into a protein. A polypeptide chain grows by the stepwise addition of amino acids to its C-terminal end. The formation of each peptide bond is energetically favorable because the growing C-terminus has been activated by the covalent attachment of a tRNA molecule. The peptidyl-tRNA linkage that activates the growing end is regenerated during each addition. The amino acid side chains have been abbreviated as R_1, R_2, R_3, and R_4; as a reference point, all of the atoms in the second amino acid in the polypeptide chain are shaded *gray*. The figure shows the addition of the fourth amino acid *(red)* to the growing chain.

The RNA Message Is Decoded in Ribosomes

The synthesis of proteins is guided by information carried by mRNA molecules. To maintain the correct reading frame and to ensure accuracy (about 1 mistake every 10,000 amino acids), protein synthesis is performed in the **ribosome**, a complex catalytic machine made from more than 50 different proteins (the *ribosomal proteins*) and several RNA molecules, the **ribosomal RNAs** (**rRNAs**). <CGC> A typical eucaryotic cell contains millions of ribosomes in its cytoplasm (**Figure 6–62**). Eucaryotic ribosome subunits are assembled at the nucleolus, when newly transcribed and modified rRNAs associate with ribosomal



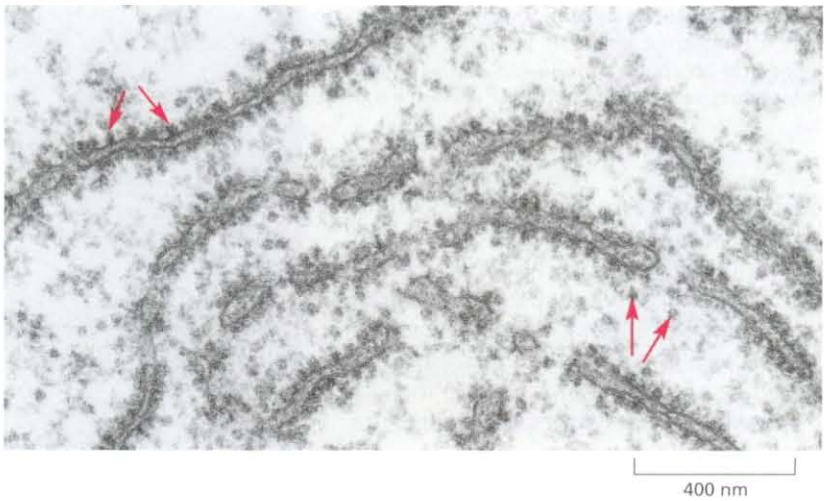


Figure 6–62 Ribosomes in the cytoplasm of a eucaryotic cell. This electron micrograph shows a thin section of a small region of cytoplasm. The ribosomes appear as black dots *(red arrows)*. Some are free in the cytosol; others are attached to membranes of the endoplasmic reticulum. (Courtesy of Daniel S. Friend.)

proteins, which have been transported into the nucleus after their synthesis in the cytoplasm. The two ribosomal subunits are then exported to the cytoplasm, where they join together to synthesize proteins.

Eucaryotic and procaryotic ribosomes have similar designs and functions. Both are composed of one large and one small subunit that fit together to form a complete ribosome with a mass of several million daltons (**Figure 6–63**). The small subunit provides the framework on which the tRNAs can be accurately

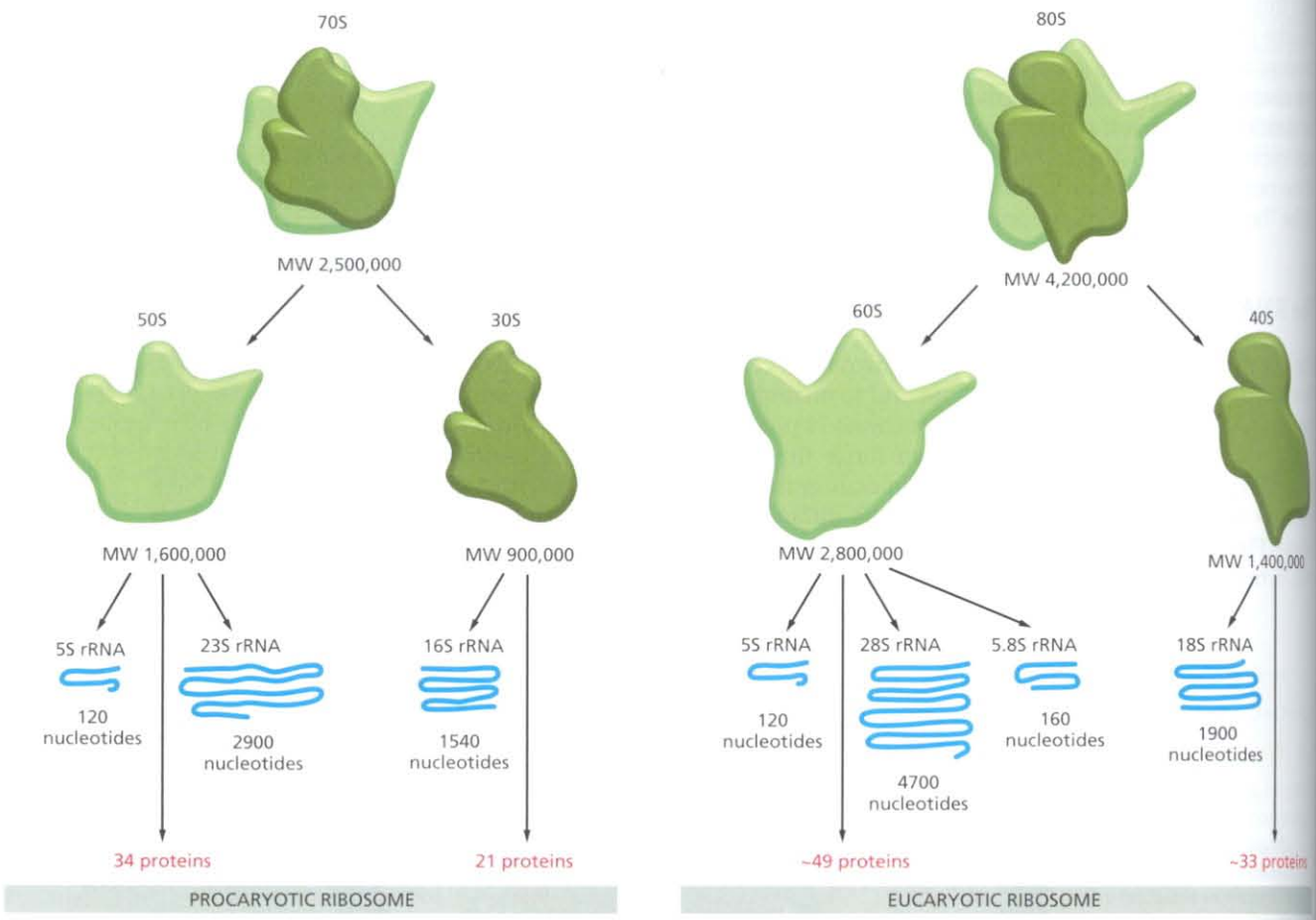


Figure 6–63 A comparison of procaryotic and eucaryotic ribosomes. Despite differences in the number and size of their rRNA and protein components, both procaryotic and eucaryotic ribosomes have nearly the same structure and they function similarly. Although the 18S and 28S rRNAs of the eucaryotic ribosome contain many nucleotides not present in their bacterial counterparts, these nucleotides are present as multiple insertions that form extra domains and leave the basic structure of each rRNA largely unchanged.

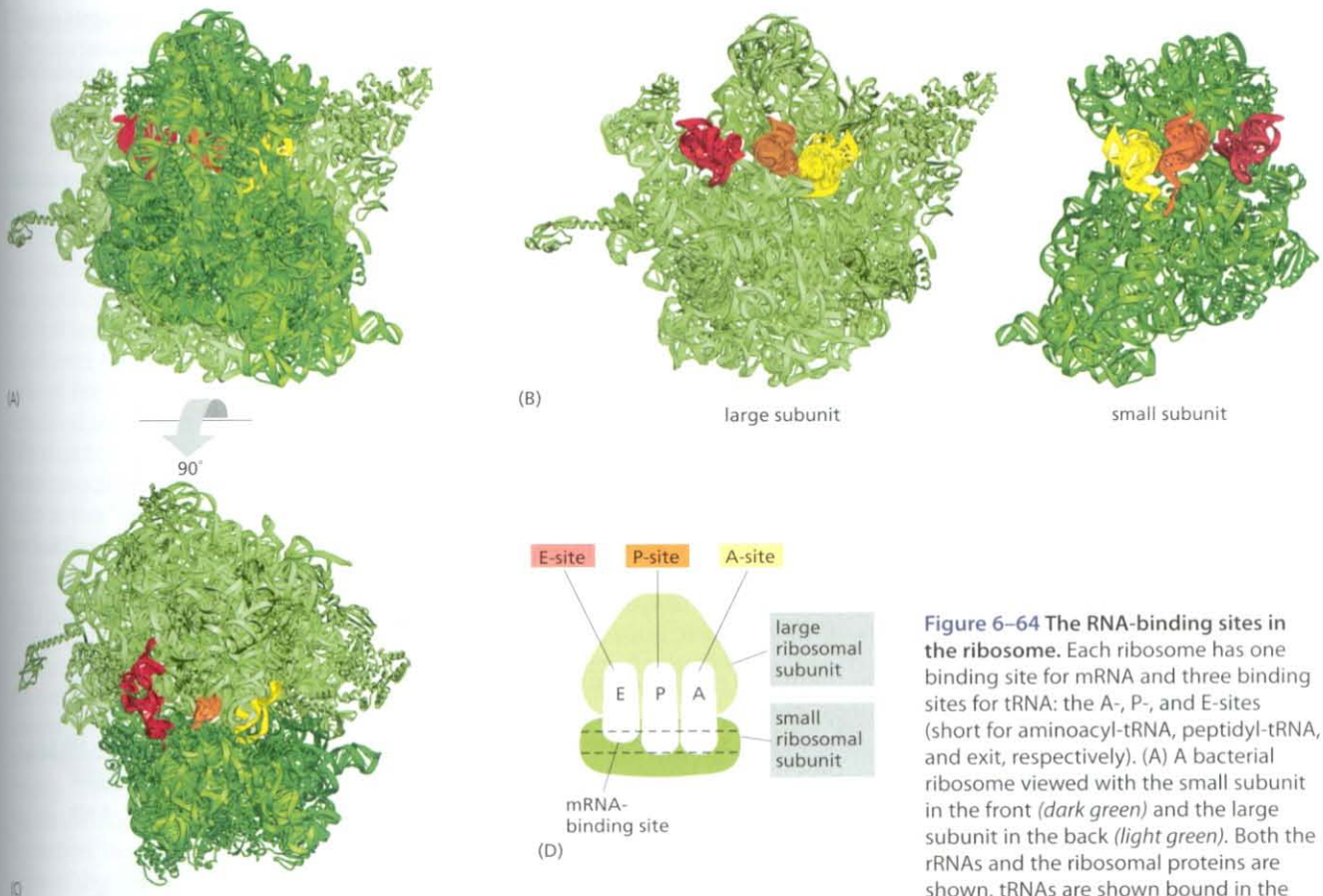


Figure 6–64 The RNA-binding sites in the ribosome. Each ribosome has one binding site for mRNA and three binding sites for tRNA: the A-, P-, and E-sites (short for aminoacyl-tRNA, peptidyl-tRNA, and exit, respectively). (A) A bacterial ribosome viewed with the small subunit in the front *(dark green)* and the large subunit in the back *(light green)*. Both the rRNAs and the ribosomal proteins are shown. tRNAs are shown bound in the E-site *(red)*, the P-site *(orange)* and the A-site *(yellow)*. Although all three tRNA sites are shown occupied here, during the process of protein synthesis not more than two of these sites are thought to contain tRNA molecules at any one time (see Figure 6–66). (B) Large and small ribosomal subunits arranged as though the ribosome in (A) were opened like a book. (C) The ribosome in (A) rotated through 90° and viewed with the large subunit on top and small subunit on the bottom. (D) Schematic representation of a ribosome (in the same orientation as C), which will be used in subsequent figures. (A, B, and C, adapted from M.M. Yusupov et al., *Science* 292:883–896, 2001. With permission from AAAS; courtesy of Albion Baucom and Harry Noller.)

matched to the codons of the mRNA (see Figure 6–58), while the large subunit catalyzes the formation of the peptide bonds that link the amino acids together into a polypeptide chain (see Figure 6–61).

When not actively synthesizing proteins, the two subunits of the ribosome are separate. They join together on an mRNA molecule, usually near its 5′ end, to initiate the synthesis of a protein. The mRNA is then pulled through the ribosome; as its codons enter the core of the ribosome, the mRNA nucleotide sequence is translated into an amino acid sequence using the tRNAs as adaptors to add each amino acid in the correct sequence to the end of the growing polypeptide chain. When a stop codon is encountered, the ribosome releases the finished protein, and its two subunits separate again. These subunits can then be used to start the synthesis of another protein on another mRNA molecule.

Ribosomes operate with remarkable efficiency: in one second, a single ribosome of a eucaryotic cell adds about 2 amino acids to a polypeptide chain; the ribosomes of bacterial cells operate even faster, at a rate of about 20 amino acids per second. How does the ribosome choreograph the many coordinated movements required for efficient translation? A ribosome contains four binding sites for RNA molecules: one is for the mRNA and three (called the A-site, the P-site, and the E-site) are for tRNAs (**Figure 6–64**). A tRNA molecule is held tightly at the A- and P-sites only if its anticodon forms base pairs with a complementary codon (allowing for wobble) on the mRNA molecule that is threaded through the ribosome (**Figure 6–65**). The A- and P-sites are close enough together for their two tRNA molecules to be forced to form base pairs with adjacent codons on the mRNA molecule. This feature of the ribosome maintains the correct reading frame on the mRNA.

Once protein synthesis has been initiated, each new amino acid is added to the elongating chain in a cycle of reactions containing four major steps: tRNA

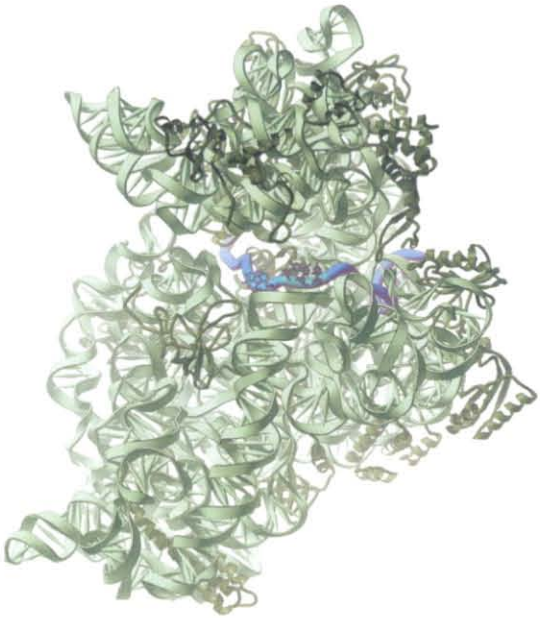


Figure 6–65 The path of mRNA *(blue)* through the small ribosomal subunit. The orientation is the same as that in the right-hand panel of Figure 6–64B. (Courtesy of Harry F. Noller, based on data in G.Z. Yusopova et al., *Cell* 106:233–241, 2001. With permission from Elsevier.)

binding, peptide bond formation, large subunit and small subunit translocation. As a result of the two translocation steps, the entire ribosome moves three nucleotides along the mRNA and is positioned to start the next cycle. (**Figure 6–66**). Our description of the chain elongation process begins at a point at which some amino acids have already been linked together and there is a tRNA molecule in the P-site on the ribosome, covalently joined to the end of the growing polypeptide. In step 1, a tRNA carrying the next amino acid in the chain binds to the ribosomal A-site by forming base pairs with the mRNA codon positioned there, so that the P-site and the A-site contain adjacent bound tRNAs. In step 2, the carboxyl end of the polypeptide chain is released from the tRNA at the P-site (by breakage of the high-energy bond between the tRNA and its amino acid) and joined to the free amino group of the amino acid linked to the tRNA at the A-site, forming a new peptide bond. This central reaction of protein synthesis is catalyzed by a *peptidyl transferase* contained in the large ribosomal subunit. In step 3, the large subunit moves relative to the mRNA held by the small subunit, thereby shifting the acceptor stems of the two tRNAs to the E- and P-sites of the large subunit. In step 4, another series of conformational changes moves the small subunit and its bound mRNA exactly three nucleotides, resetting the ribosome so it is ready to receive the next aminoacyl-tRNA. Step 1 is then repeated with a new incoming aminoacyl-tRNA, and so on. <CGTT>

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This four-step cycle is repeated each time an amino acid is added to the polypeptide chain, as the chain grows from its amino to its carboxyl end.

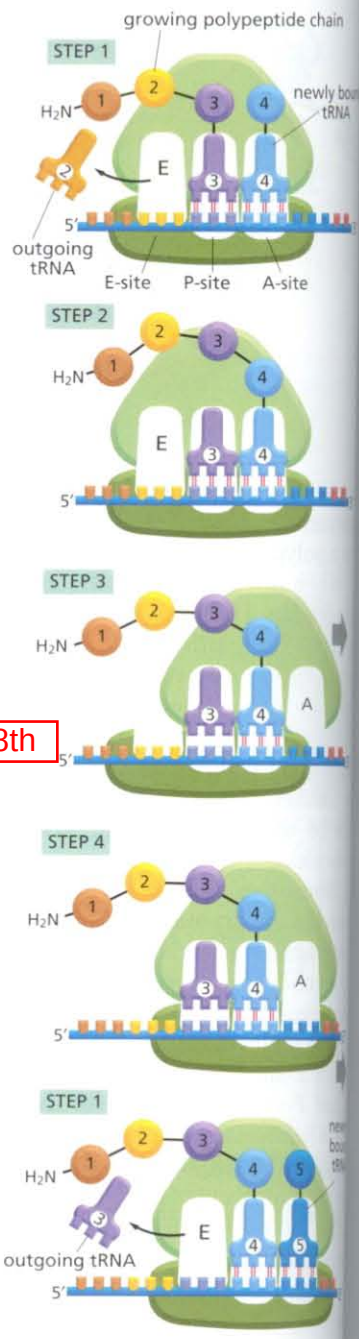

Figure 6–66 Translating an mRNA molecule. Each amino acid added to the growing end of a polypeptide chain is selected by complementary base-pairing between the anticodon on its attached tRNA molecule and the next codon on the mRNA chain. Because only one of the many types of tRNA molecules in a cell can base-pair with each codon, the codon determines the specific amino acid to be added to the growing polypeptide chain. The four-step cycle shown is repeated over and over during the synthesis of a protein. In step 1, an aminoacyl-tRNA molecule binds to a vacant A-site on the ribosome and a spent tRNA molecule dissociates from the E-site. In step 2, a new peptide bond is formed. In step 3, the large subunit translocates relative to the small subunit, leaving the two tRNAs in hybrid sites: P on the large subunit and A on the small, for one; E on the large subunit and P on the small, for the other. In step 4, the small subunit translocates carrying its mRNA a distance of three nucleotides through the ribosome. This "resets" the ribosome with a fully empty A-site, ready for the next aminoacyl-tRNA molecule to bind. As indicated, the mRNA is translated in the 5′-to-3′ direction, and the N-terminal end of a protein is made first, with each cycle adding one amino acid to the C-terminus of the polypeptide chain.

Elongation Factors Drive Translation Forward and Improve Its Accuracy

The basic cycle of polypeptide elongation shown in outline in Figure 6–66 has an additional feature that makes translation especially efficient and accurate. Two *elongation factors* enter and leave the ribosome during each cycle, each hydrolyzing GTP to GDP and undergoing conformational changes in the process. These factors are called EF-Tu and EF-G in bacteria, and EF1 and EF2 in eucaryotes. Under some conditions *in vitro*, ribosomes can be forced to synthesize proteins without the aid of these elongation factors and GTP hydrolysis, but this synthesis is very slow, inefficient, and inaccurate. Coupling the GTP hydrolysis-driven changes in the elongation factors to transitions between different states of the ribosome speeds up protein synthesis enormously. Although these ribosomal states are not yet understood in detail, they almost certainly involve RNA structure rearrangements in the ribosome core. The cycles of elongation factor association, GTP hydrolysis, and dissociation ensure that all such changes occur in the "forward" direction so that translation can proceed efficiently (**Figure 6–67**).

As shown previously, EF-Tu simultaneously binds GTP and aminoacyl-tRNAs (see Figure 3–74). In addition to helping move translation forward, EF-Tu (EF1 in eucaryotes) increases the accuracy of translation in several ways. First, as it escorts an incoming aminoacyl-tRNA to the ribosome, EF-Tu checks whether the tRNA-amino acid match is corrrect. Exactly how this is accomplished is not well understood. According to one idea, correct tRNA–amino acid matches have a narrowly defined affinity for EF-Tu, which allows EF-Tu to discriminate, albeit crudely, among many different amino acid–tRNA combinations, selectively bringing the correct ones with it into the ribosome. Second, EF-Tu monitors the initial interaction between the anticodon of an incoming aminoacyl-tRNA and the codon of the mRNA in the A-site. Aminoacyl-tRNAs are "bent" when bound to the GTP-form of EF-Tu; this bent conformation allows codon pairing but prevents incorporation of the amino acid into the growing polypeptide chain. However, if the codon–anticodon match is correct, the ribosome rapidly triggers the hydrolysis of the GTP molecule, whereupon EF-Tu releases its grip on the tRNA and dissociates from the ribosome, allowing the tRNA to donate its amino acid for protein synthesis. But how is the "correctness" of the codon–anticodon match assessed? This feat is carried out by the ribosome itself through an RNA-based mechanism. The rRNA in the small subunit of the ribosome forms a series of hydrogen bonds with the codon–anticodon pair that allows determination of its correctness (**Figure 6–68**). In essence, the rRNA folds around the codon–anticodon pair, and its final closure—which occurs only when the correct anticodon is in place—triggers GTP hydrolysis. Remarkably, this induced fit mechanism can distinguish correct from incorrect codon–anticodon interactions despite the rules for wobble base-pairing summarized in Figure 6–53. From this example, as for RNA splicing, one gets a sense of the highly sophisticated forms of molecular recognition that can be achieved solely by RNA.

The interactions of EF-Tu, tRNA, and the ribosome just described introduce critical proofreading steps into protein synthesis at the initial tRNA selection stage. But after GTP is hydrolyzed and EF-Tu dissociates from the ribosome, there is an additional opportunity for the ribosome to prevent an incorrect amino acid from being added to the growing chain. Following GTP hydrolysis, there is a short time delay as the amino acid carried by the tRNA moves into position on the ribosome. This time delay is shorter for correct than incorrect codon–anticodon pairs. Moreover, incorrectly matched tRNAs dissociate more

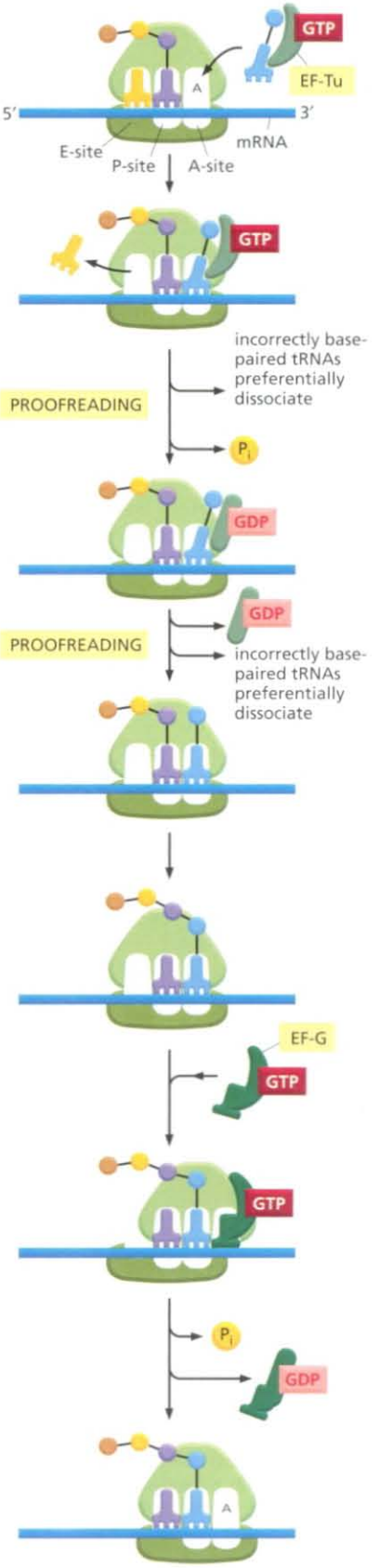

Figure 6–67 Detailed view of the translation cycle. The outline of translation presented in Figure 6–66 has been expanded to show the roles of two elongation factors EF-Tu and EF-G, which drive translation in the forward direction. As explained in the text, EF-Tu also provides two opportunities for proofreading of the codon–anticodon match. In this way, incorrectly paired tRNAs are selectively rejected, and the accuracy of translation is improved.

rapidly than those correctly bound because their interaction with the codon is weaker. Thus, most incorrectly bound tRNA molecules (as well as a significant number of correctly bound molecules) will leave the ribosome without being used for protein synthesis. All of these proofreading steps, taken together, are largely responsible for the 99.99% accuracy of the ribosome in translating RNA into protein.

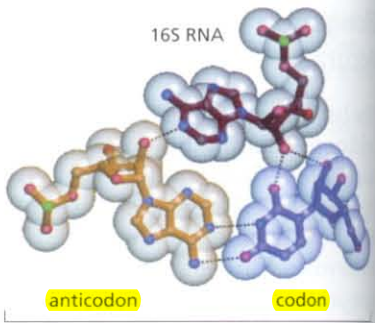

Figure 6–68 Recognition of correct codon–anticodon matches by the small subunit rRNA of the ribosome. Shown is the interaction between a nucleotide of the small subunit rRNA and the first nucleotide pair of a correctly paired codon–anticodon; similar interactions occur between other nucleotides of the rRNA and the second and third positions of the codon–anticodon pair. The small subunit rRNA can form this network of hydrogen bonds only with correctly matched codon–anticodon pairs. As explained in the text, this codon–anticodon monitoring by the small subunit rRNA increases the accuracy of protein synthesis. (From J.M. Ogle et al., *Science* 292:897–902, 2001. With permission from AAAS.)

The Ribosome Is a Ribozyme

The ribosome is a large complex composed of two-thirds RNA and one-third protein. The determination, in 2000, of the entire three-dimensional conformation of its large and small subunits is a major triumph of modern structural biology. The findings confirm earlier evidence that rRNAs—and not proteins—are responsible for the ribosome's overall structure, its ability to position tRNAs on the mRNA, and its catalytic activity in forming covalent peptide bonds. The ribosomal RNAs are folded into highly compact, precise three-dimensional structures that form the compact core of the ribosome and determine its overall shape (**Figure 6–69**).

In marked contrast to the central positions of the rRNAs, the ribosomal proteins are generally located on the surface and fill in the gaps and crevices of the folded RNA (**Figure 6–70**). Some of these proteins send out extended regions of polypeptide chain that penetrate short distances into holes in the RNA core (**Figure 6–71**). The main role of the ribosomal proteins seems to be to stabilize the RNA core, while permitting the changes in rRNA conformation that are necessary for this RNA to catalyze efficient protein synthesis. The proteins probably also aid in the initial assembly of the rRNAs that make up the core of the ribosome.

Not only are the A-, P-, and E-binding sites for tRNAs formed primarily by ribosomal RNAs, but the catalytic site for peptide bond formation is also formed by RNA, as the nearest amino acid is located more than 1.8 nm away.

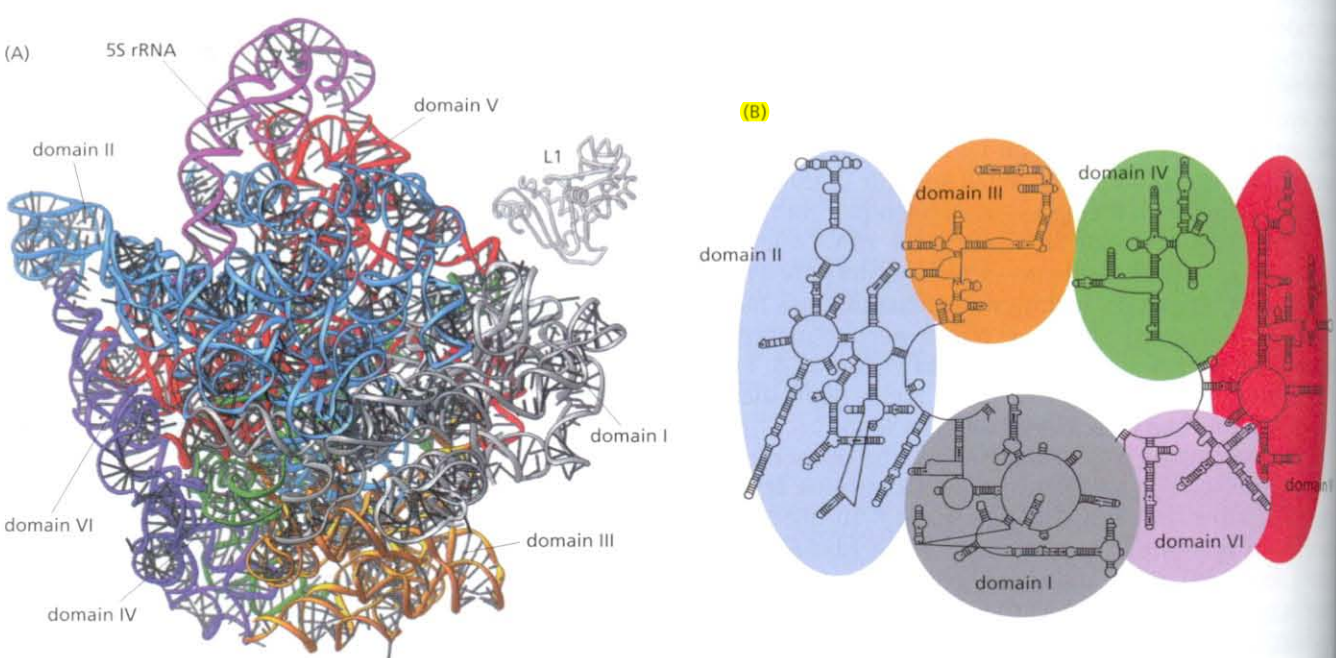

Figure 6–69 Structure of the rRNAs in the large subunit of a bacterial ribosome, as determined by x-ray crystallography. (A) Three-dimensional conformations of the large-subunit rRNAs (5S and 23S) as they appear in the ribosome. One of the protein subunits of the ribosome (L1) is also shown as a reference point, since it forms a characteristic protrusion on the ribosome. (B) Schematic diagram of the secondary structure of the 23S rRNA, showing the extensive network of base-pairing. The structure has been divided into six "domains" whose colors correspond to those in (A). The secondary-structure diagram is highly schematized to represent as much of the structure as possible in two dimensions. To do this, several discontinuities in the RNA chain have been introduced, although in reality the 23S RNA is a single RNA molecule. For example, the base of Domain III is continuous with the base of Domain IV even though a gap appears in the diagram. (Adapted from N. Ban et al., *Science* 289:905–920, 2000. With permission from AAAS.)

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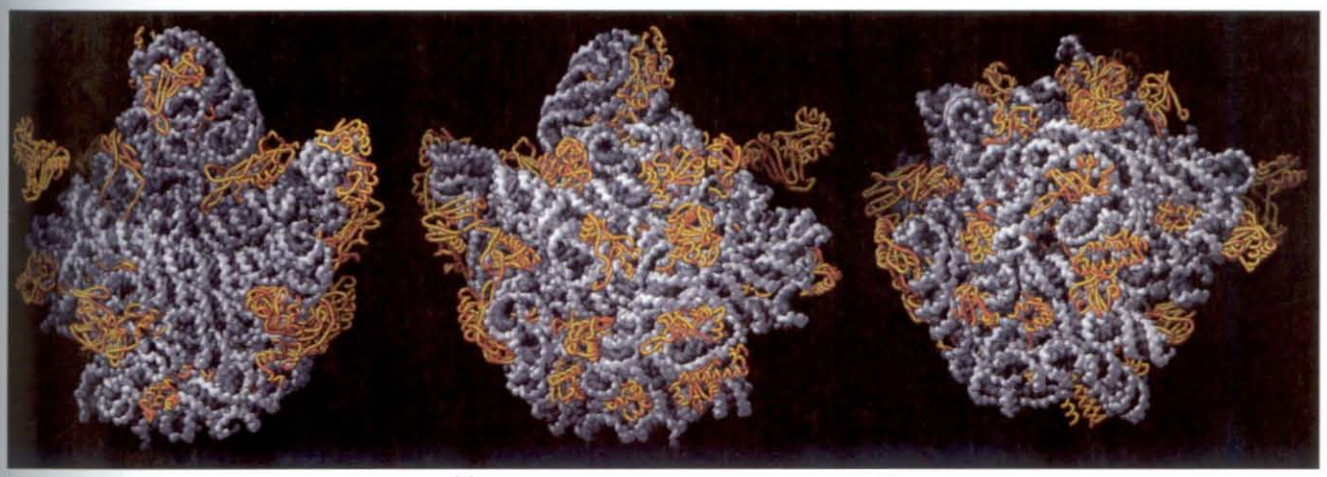


(A) (B) (C)

Figure 6–70 Location of the protein components of the bacterial large ribosomal subunit. The rRNAs (5S and 23S) are shown in *gray* and the large-subunit proteins (27 of the 31 total) in *gold*. For convenience, the protein structures depict only the polypeptide backbones. (A) Interface with the small subunit, the same view shown in Figure 6–64B. (B) Side opposite to that shown in (A), obtained by rotating (A) by 180° around a vertical axis. (C) Further slight rotation of (B) through a diagonal axis, allowing a view into the peptide exit channel in the center of the structure. (From N. Ban et al., *Science* 289:905–920, 2000. With permission from AAAS.)

This discovery came as a surprise to biologists because, unlike proteins, RNA does not contain easily ionizable functional groups that can be used to catalyze sophisticated reactions like peptide bond formation. Moreover, metal ions, which are often used by RNA molecules to catalyze chemical reactions (as discussed later in the chapter), were not observed at the active site of the ribosome. Instead, it is believed that the 23S rRNA forms a highly structured pocket that, through a network of hydrogen bonds, precisely orients the two reactants (the growing peptide chain and an aminoacyl-tRNA) and thereby greatly accelerates their covalent joining. In addition, the tRNA in the P site contributes to the active site, perhaps supplying a functional OH group that participates directly in the catalysis. This mechanism may ensure that catalysis occurs only when the tRNA is properly positioned in the ribosome.

RNA molecules that possess catalytic activity are known as **ribozymes**. We saw earlier in this chapter how other ribozymes function in self-splicing reactions (for example, see Figure 6–36). In the final section of this chapter, we consider what the ability of RNA molecules to function as catalysts for a wide variety of different reactions might mean for the early evolution of living cells. For now, we merely note that there is good reason to suspect that RNA rather than protein molecules served as the first catalysts for living cells. If so, the ribosome, with its RNA core, may be a relic of an earlier time in life's history—when protein synthesis evolved in cells that were run almost entirely by ribozymes.

Figure 6–71 Structure of the L15 protein in the large subunit of the bacterial ribosome. The globular domain of the protein lies on the surface of the ribosome and an extended region penetrates deeply into the RNA core of the ribosome. The L15 protein is shown in *yellow* and a portion of the ribosomal RNA core is shown in *red*. (From D. Klein, P.B. Moore and T.A. Steitz, *J. Mol. Biol.* 340:141–147, 2004. With permission from Academic Press.)

Nucleotide Sequences in mRNA Signal Where to Start Protein Synthesis

The initiation and termination of translation share features of the translation elongation cycle described above. The site at which protein synthesis begins on the mRNA is especially crucial, since it sets the reading frame for the whole length of the message. An error of one nucleotide either way at this stage would cause every subsequent codon in the message to be misread, resulting in a nonfunctional protein with a garbled sequence of amino acids. The initiation step is also important because for most genes it is the last point at which the cell can decide whether the mRNA is to be translated and the protein synthesized; the rate of initiation is thus one determinant of the rate at which any protein is synthesized. We shall see in Chapter 7 that cells use several mechanisms to regulate translation initiation.

The translation of an mRNA begins with the codon AUG, and a special tRNA is required to start translation. This **initiator tRNA** always carries the amino acid methionine (in bacteria, a modified form of methionine—formylmethionine—is used), with the result that all newly made proteins have methionine as the first amino acid at their N-terminus, the end of a protein that is synthesized first. This methionine is usually removed later by a specific protease. The initiator tRNA can be specially recognized by initiation factors because it has a nucleotide sequence distinct from that of the tRNA that normally carries methionine.

In eucaryotes, the initiator tRNA–methionine complex (Met–tRNAi) is first loaded into the small ribosomal subunit along with additional proteins called **eucaryotic initiation factors**, or **eIFs** (**Figure 6–72**). Of all the aminoacyl-tRNAs in the cell, only the methionine-charged initiator tRNA is capable of tightly binding the small ribosome subunit without the complete ribosome being present and it binds directly to the P-site. Next, the small ribosomal subunit binds to the 5′ end of an mRNA molecule, which is recognized by virtue of its 5′ cap and its two bound initiation factors, eIF4E (which directly binds the cap) and eIF4G (see Figure 6–40). The small ribosomal subunit then moves forward (5′ to 3′) along the mRNA, searching for the first AUG. Additional initiation factors that act as ATP-powered helicases facilitate the ribosome's movement through RNA secondary structure. In 90% of mRNAs, translation begins at the first AUG encountered by the small subunit. At this point, the initiation factors dissociate, allowing the large ribosomal subunit to assemble with the complex and complete the ribosome. The initiator tRNA is still bound to the P-site, leaving the A-site vacant. Protein synthesis is therefore ready to begin (see Figure 6–72).

The nucleotides immediately surrounding the start site in eucaryotic mRNAs influence the efficiency of AUG recognition during the above scanning process. If this recognition site differs substantially from the consensus recognition sequence (5′-ACCAUGG-3′), scanning ribosomal subunits will sometimes ignore the first AUG codon in the mRNA and skip to the second or third AUG codon instead. Cells frequently use this phenomenon, known as "leaky scanning," to produce two or more proteins, differing in their N-termini, from the same mRNA molecule. It allows some genes to produce the same protein with and without a signal sequence attached at its N-terminus, for example, so that the protein is directed to two different compartments in the cell.

The mechanism for selecting a start codon in bacteria is different. Bacterial mRNAs have no 5′ caps to signal the ribosome where to begin searching for the start of translation. Instead, each bacterial mRNA contains a specific ribosome-binding site (called the Shine–Dalgarno sequence, named after its discoverers) that is located a few nucleotides upstream of the AUG at which translation is to begin. This nucleotide sequence, with the consensus 5′-AGGAGGU-3′, forms base pairs with the 16S rRNA of the small ribosomal subunit to position the initiating AUG codon in the ribosome. A set of translation initiation factors orchestrates this interaction, as well as the subsequent assembly of the large ribosomal subunit to complete the ribosome.

Unlike a eucaryotic ribosome, a bacterial ribosome can therefore readily assemble directly on a start codon that lies in the interior of an mRNA molecule, so long as a ribosome-binding site precedes it by several nucleotides. As a result, bacterial mRNAs are often *polycistronic*—that is, they encode several different proteins, each of which is translated from the same mRNA molecule (**Figure 6–73**). In contrast, a eucaryotic mRNA generally encodes only a single protein.

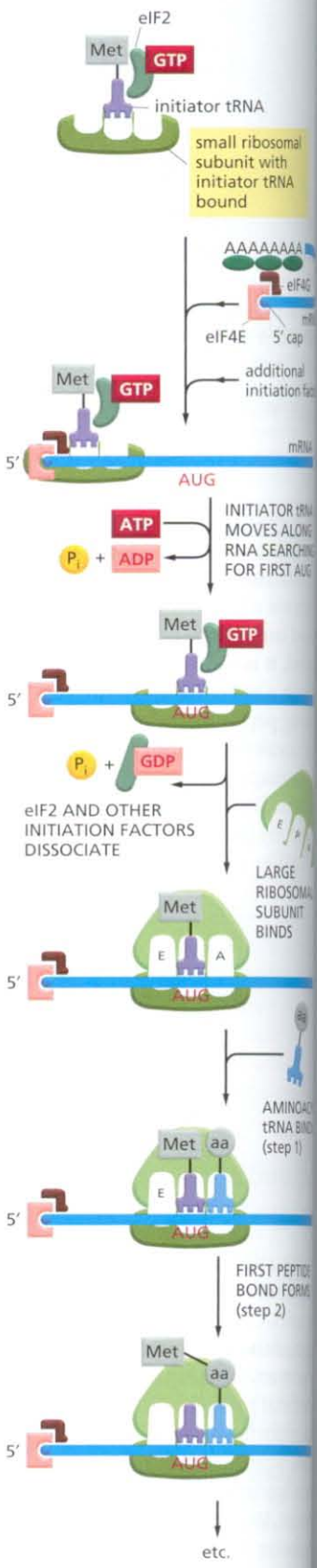


Figure 6–72 The initiation of protein synthesis in eucaryotes. Only three of the many translation initiation factors required for this process are shown. Efficient translation initiation also requires the poly-A tail of the mRNA bound by poly-A-binding proteins which, in turn, interact with eIF4G. In this way, the translation apparatus ascertains that both ends of the mRNA are intact before initiating protein synthesis (see Figure 6–40). Although only one GTP hydrolysis event is shown in the figure, a second is known to occur just before the large and small ribosomal subunits join.

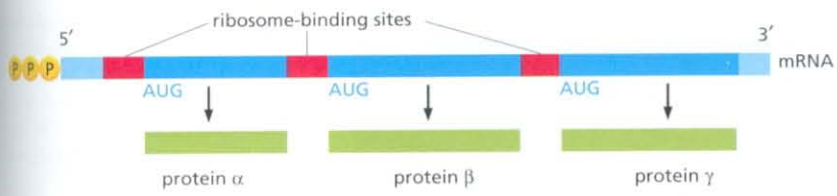


Figure 6–73 Structure of a typical bacterial mRNA molecule. Unlike eucaryotic ribosomes, which typically require a capped 5′ end, procaryotic ribosomes initiate transcription at ribosome-binding sites (Shine–Dalgarno sequences), which can be located anywhere along an mRNA molecule. This property of ribosomes permits bacteria to synthesize more than one type of protein from a single mRNA molecule.

Stop Codons Mark the End of Translation

The end of the protein-coding message is signaled by the presence of one of three *stop codons* (UAA, UAG, or UGA) (see Figure 6–50). These are not recognized by a tRNA and do not specify an amino acid, but instead signal to the ribosome to stop translation. Proteins known as *release factors* bind to any ribosome with a stop codon positioned in the A site, forcing the peptidyl transferase in the ribosome to catalyze the addition of a water molecule instead of an amino acid to the peptidyl-tRNA (**Figure 6–74**). This reaction frees the carboxyl end of the growing polypeptide chain from its attachment to a tRNA molecule, and since only this attachment normally holds the growing polypeptide to the ribosome, the completed protein chain is immediately released into the cytoplasm. The ribosome then releases the mRNA and separates into the large and small subunits, which can assemble on this or another mRNA molecule to begin a new round of protein synthesis.

Release factors are an example of *molecular mimicry*, whereby one type of macromolecule resembles the shape of a chemically unrelated molecule. In this case, the three-dimensional structure of release factors (made entirely of protein) resembles the shape and charge distribution of a tRNA molecule (**Figure 6–75**). This shape and charge mimicry helps them enter the A-site on the ribosome and cause translation termination.

During translation, the nascent polypeptide moves through a large, water-filled tunnel (approximately 10 nm × 1.5 nm) in the large subunit of the ribosome (see Figure 6–70C). The walls of this tunnel, made primarily of 23S rRNA, are a patchwork of tiny hydrophobic surfaces embedded in a more extensive hydrophilic surface. This structure is not complementary to any peptide, and thus provides a "Teflon" coating through which a polypeptide chain can easily slide. The dimensions of the tunnel suggest that nascent proteins are largely unstructured as they pass through the ribosome, although some α-helical regions of the protein can form before leaving the ribosome tunnel. As it leaves the ribosome, a newly synthesized protein must fold into its proper three-dimensional conformation to be useful to the cell, and later in this chapter we discuss how this folding occurs. First, however, we describe several additional aspects of the translation process itself.

Proteins Are Made on Polyribosomes

The synthesis of most protein molecules takes between 20 seconds and several minutes. During this very short period, however, it is usual for multiple initiations to take place on each mRNA molecule being translated. As soon as the preceding ribosome has translated enough of the nucleotide sequence to move out

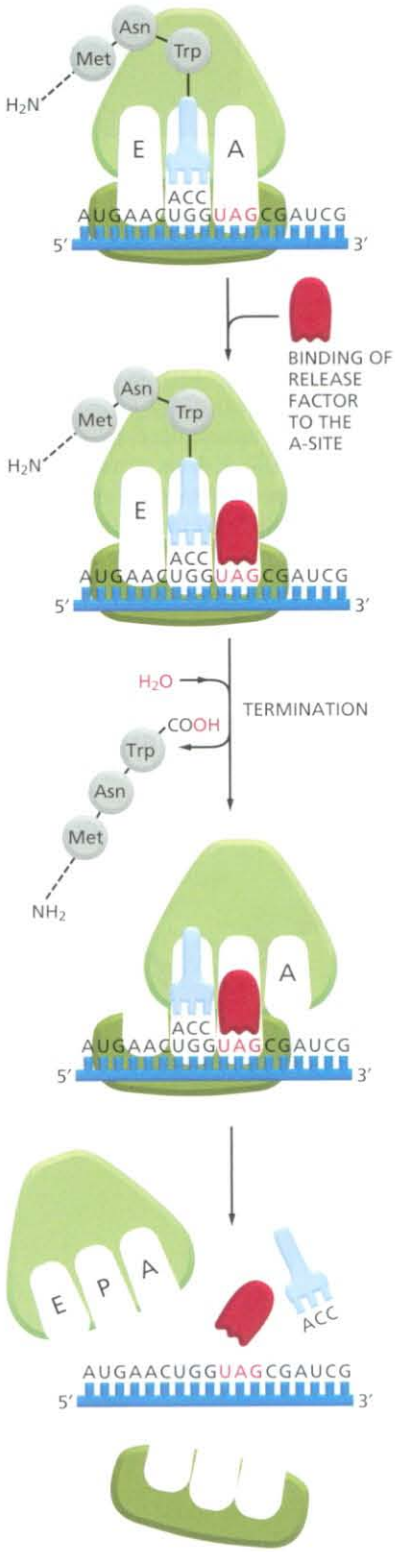


Figure 6–74 The final phase of protein synthesis. The binding of a release factor to an A-site bearing a stop codon terminates translation. The completed polypeptide is released and, in a series of reactions that requires additional proteins and GTP hydrolysis (not shown), the ribosome dissociates into its two separate subunits.

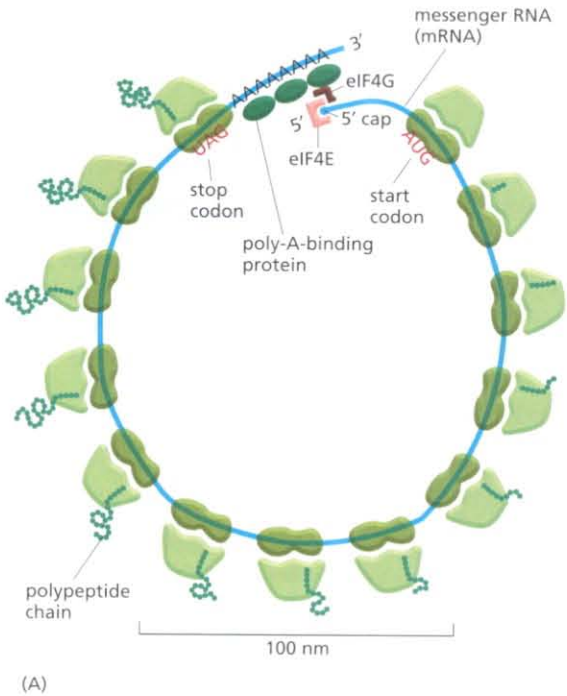
Figure 6–75 The structure of a human translation release factor (eRF1) and its resemblance to a tRNA molecule. The protein is on the *left* and the tRNA on the *right*. (From H. Song et al., *Cell* 100:311–2000. With permission from Elsevier.)

of the way, the 5′ end of the mRNA is threaded into a new ribosome. The mRNA molecules being translated are therefore usually found in the form of *polyribosomes* (or *polysomes*): large cytoplasmic assemblies made up of several ribosomes spaced as close as 80 nucleotides apart along a single mRNA molecule (**Figure 6–76**). These multiple initiations allow the cell to make many more protein molecules in a given time than would be possible if each had to be completed before the next could start. <GAAG>

Both bacteria and eucaryotes use polysomes, and both employ additional strategies to speed up the overall rate of protein synthesis even further. Because bacterial mRNA does not need to be processed and is accessible to ribosomes while it is being made, ribosomes can attach to the free end of a bacterial mRNA molecule and start translating it even before the transcription of that RNA is complete, following closely behind the RNA polymerase as it moves along DNA. In eucaryotes, as we have seen, the 5′ and 3′ ends of the mRNA interact (see Figures 6–40 and 6–76A); therefore, as soon as a ribosome dissociates, its two subunits are in an optimal position to reinitiate translation on the same mRNA molecule.

There Are Minor Variations in the Standard Genetic Code

As discussed in Chapter 1, the genetic code (shown in Figure 6–50) applies to all three major branches of life, providing important evidence for the common



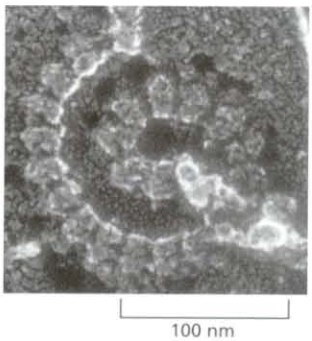


Figure 6–76 A polyribosome. (A) Schematic drawing showing how a series of ribosomes can simultaneously translate the same eucaryotic mRNA molecule. (B) Electron micrograph of a polyribosome from a eucaryotic cell. (B, courtesy of John Heuser.)

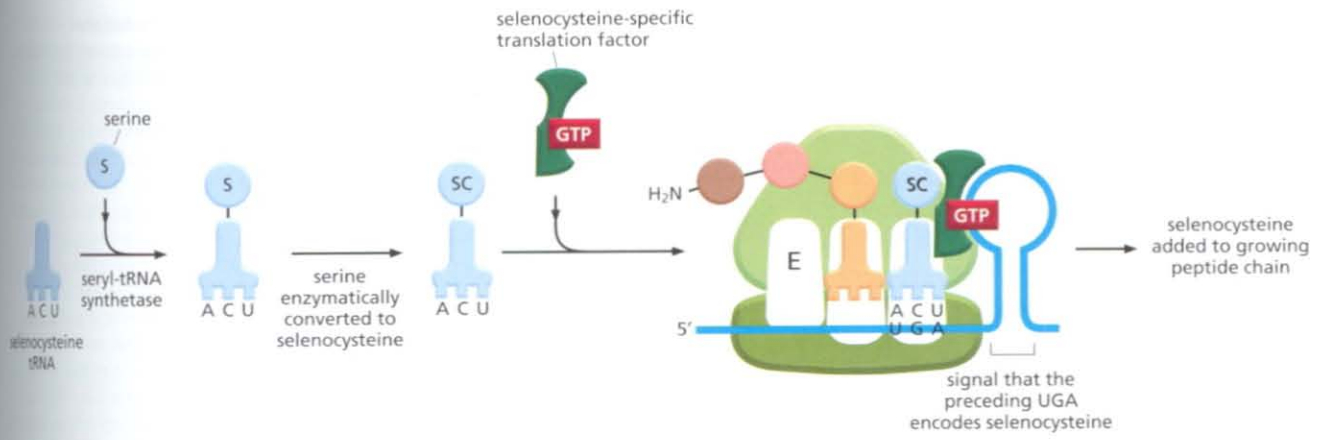


Figure 6–77 Incorporation of selenocysteine into a growing polypeptide chain. A specialized tRNA is charged with serine by the normal seryl-tRNA synthetase, and the serine is subsequently converted enzymatically to selenocysteine. A specific RNA structure in the mRNA (a stem and loop structure with a particular nucleotide sequence) signals that selenocysteine is to be inserted at the neighboring UGA codon. As indicated, this event requires the participation of a selenocysteine-specific translation factor.

ancestry of all life on Earth. Although rare, there are exceptions to this code. For example, *Candida albicans*, the most prevalent human fungal pathogen, translates the codon CUG as serine, whereas nearly all other organisms translate it as leucine. Mitochondria (which have their own genomes and encode much of their translational apparatus) often deviate from the standard code. For example, in mammalian mitochondria AUA is translated as methionine, whereas in the cytosol of the cell it is translated as isoleucine (see Table 14–3, p. 862). This type of deviation in the genetic code is "hardwired" into the organisms or the organelles in which it occurs.

A different type of variation, sometimes called *translation recoding*, occurs in many cells. In this case, other nucleotide sequence information present in an mRNA can change the meaning of the genetic code at a particular site in the mRNA molecule. The standard code allows cells to manufacture proteins using only 20 amino acids. However, bacteria, archaea, and eucaryotes have available to them a twenty-first amino acid that can be incorporated directly into a growing polypeptide chain through translation recoding. Selenocysteine, which is essential for the efficient function of a variety of enzymes, contains a selenium atom in place of the sulfur atom of cysteine. Selenocysteine is enzymatically produced from a serine attached to a special tRNA molecule that base-pairs with the UGA codon, a codon normally used to signal a translation stop. The mRNAs for proteins in which selenocysteine is to be inserted at a UGA codon carry an additional nucleotide sequence in the mRNA nearby that causes this recoding event (**Figure 6–77**).

Another form of recoding, *translational frameshifting*, allows more than one protein to be synthesized from a single mRNA. Retroviruses, members of a large group of eucaryotic-infecting pathogens, commonly use translational frameshifting to make both the capsid proteins *(Gag proteins)* and the viral reverse transcriptase and integrase *(Pol proteins)* from the same RNA transcript (see Figure 5–73). The virus needs many more copies of the Gag proteins than it does of the Pol proteins. This quantitative adjustment is achieved by encoding the *Pol* genes just after the *Gag* genes but in a different reading frame. Small amounts of the Pol gene products are made because, on occasion, an upstream translational frameshift allows the Gag protein stop codon to be bypassed. This frameshift occurs at a particular codon in the mRNA and requires a specific *recoding signal*, which seems to be a structural feature of the RNA sequence downstream of this site (**Figure 6–78**).

Inhibitors of Procaryotic Protein Synthesis Are Useful as Antibiotics

Many of the most effective antibiotics used in modern medicine are compounds made by fungi that inhibit bacterial protein synthesis. Fungi and bacteria compete for many of the same environmental niches, and millions of years of coevolution has resulted in fungi producing potent bacterial inhibitors. Some of these

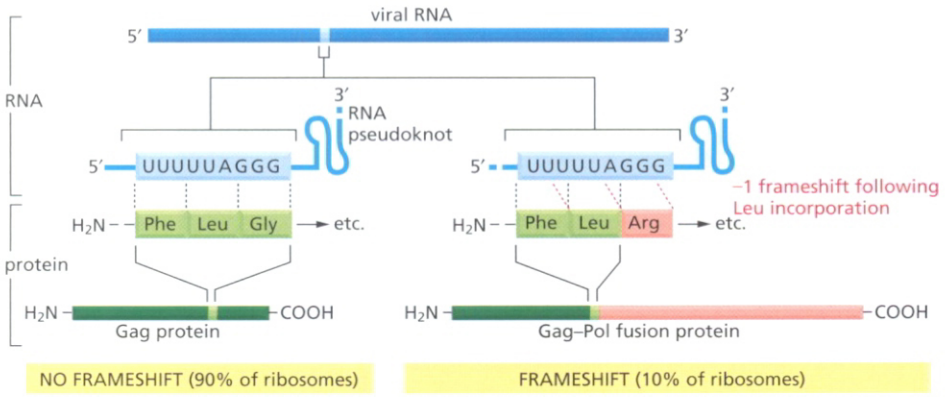


Figure 6–78 The translational frameshifting that produces the reverse transcriptase and integrase of a retrovirus. The viral reverse transcriptase and integrase are produced by proteolytic processing of a large protein (the Gag-Pol fusion protein) consisting of both the Gag and Pol amino acid sequences. Proteolytic processing of the more abundant Gag protein produces the viral capsid protein. Both the Gag and the Gag–Pol fusion proteins start with identical mRNA, but whereas the Gag protein terminates at a stop codon downstream of the sequence shown, translation of the Gag–Pol fusion protein bypasses this stop codon, allowing the synthesis of the longer Gag–Pol fusion protein. The stop-codon-bypass is made possible by a controlled translational frameshift, as illustrated. Features in the local RNA structure (including the RNA loop shown) cause the $tRNA^{Leu}$ attached to the C-terminus of the growing polypeptide chain occasionally to slip backward by one nucleotide on the ribosome, so that it pairs with a UUU codon instead of the UUA codon that initially specified its incorporation; the next codon (AGG) in the new reading frame specifies an arginine rather than a glycine. This controlled slippage is due in part to a *pseudoknot* that forms in the viral mRNA (see Figure 6–102). The sequence shown is from the human AIDS virus, HIV. (Adapted from T. Jacks et al., *Nature* 331:280–283, 1988. With permission from Macmillan Publishers Ltd.)

drugs exploit the structural and functional differences between bacterial and eucaryotic ribosomes so as to interfere preferentially with the function of bacterial ribosomes. Thus humans can take high dosages of some of these compounds without undue toxicity. Many antibiotics lodge in pockets in the ribosomal RNAs and simply interfere with the smooth operation of the ribosome (**Figure 6–79**). **Table 6–4** lists some of the more common antibiotics of this kind along with several other inhibitors of protein synthesis, some of which act on eucaryotic cells and therefore cannot be used as antibiotics.

Because they block specific steps in the processes that lead from DNA to protein, many of the compounds listed in Table 6–4 are useful for cell biological studies. Among the most commonly used drugs in such investigations are *chloramphenicol*, *cycloheximide*, and *puromycin*, all of which specifically inhibit protein synthesis. In a eucaryotic cell, for example, chloramphenicol inhibits protein synthesis on ribosomes only in mitochondria (and in chloroplasts in plants), presumably reflecting the procaryotic origins of these organelles (discussed in Chapter 14). Cycloheximide, in contrast, affects only ribosomes in the cytosol. Puromycin is especially interesting because it is a structural analog of a tRNA molecule linked to an amino acid and is therefore another example of molecular mimicry; the ribosome mistakes it for an authentic amino acid and covalently incorporates it at the C-terminus of the growing peptide chain, thereby causing the premature termination and release of the polypeptide. As might be expected, puromycin inhibits protein synthesis in both procaryotes and eucaryotes.

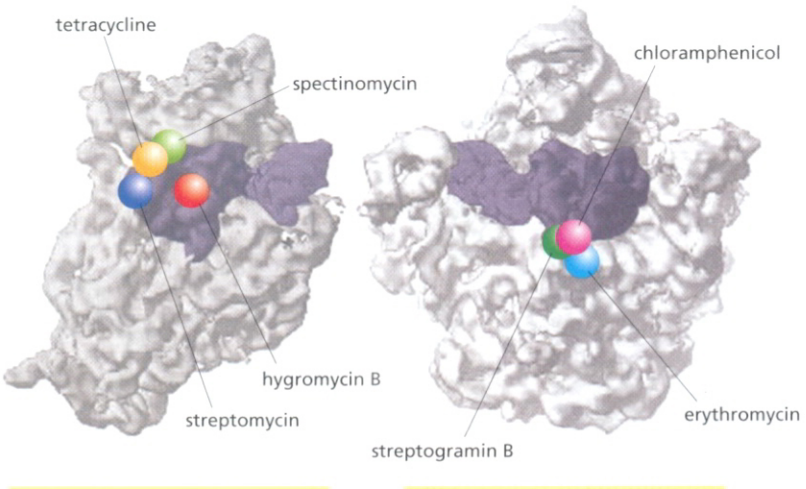


Figure 6–79 Binding sites for antibiotics on the bacterial ribosome. The small (*left*) and large (*right*) subunits of the ribosome are arranged as though the ribosome had been opened like a book; the bound tRNA molecules are shown in *purple* (see Figure 6–64). Most of the antibiotics shown bind directly to pockets formed by the ribosomal RNA molecules. Hygromycin B induces errors in translation, spectinomycin blocks the translocation of the peptidyl-tRNA from the A-site to the P-site, and streptogramin B prevents elongation of nascent peptides. Table 6–4 lists the inhibitory mechanisms of the other antibiotics shown in the figure. (Adapted from J. Poehlsgaard and S. Douthwaite, *Nat. Rev. Microbiol.* 3:870–881, 2005. With permission from Macmillan Publishers Ltd.)

Table 6–4 Inhibitors of Protein or RNA Synthesis

INHIBITOR	SPECIFIC EFFECT
Acting only on bacteria	
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from translation initiation to chain elongation and also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
Erythromycin	binds in the exit channel of the ribosome and thereby inhibits elongation of the peptide chain
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
Acting on bacteria and eucaryotes	
Puromycin	causes the premature release of nascent polypeptide chains by its addition to the growing chain end
Actinomycin D	binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
Acting on eucaryotes but not bacteria	
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 6–66)
Anisomycin	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
α-Amanitin	blocks mRNA synthesis by binding preferentially to RNA polymerase II

The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.

Accuracy in Translation Requires the Expenditure of Free Energy

Translation by the ribosome is a compromise between the opposing constraints of accuracy and speed. We have seen, for example, that the accuracy of translation (1 mistake per 10^4 amino acids joined) requires time delays each time a new amino acid is added to a growing polypeptide chain, producing an overall speed of translation of 20 amino acids incorporated per second in bacteria. Mutant bacteria with a specific alteration in the small ribosomal subunit have longer delays and translate mRNA into protein with an accuracy considerably higher than this; however, protein synthesis is so slow in these mutants that the bacteria are barely able to survive.

We have also seen that attaining the observed accuracy of protein synthesis requires the expenditure of a great deal of free energy; this is expected, since, as discussed in Chapter 2, there is a price to be paid for any increase in order in the cell. In most cells, protein synthesis consumes more energy than any other biosynthetic process. At least four high-energy phosphate bonds are split to make each new peptide bond: two are consumed in charging a tRNA molecule with an amino acid (see Figure 6–56), and two more drive steps in the cycle of reactions occurring on the ribosome during synthesis itself (see Figure 6–67). In addition, extra energy is consumed each time that an incorrect amino acid linkage is hydrolyzed by a tRNA synthetase (see Figure 6–59) and each time that an incorrect tRNA enters the ribosome, triggers GTP hydrolysis, and is rejected (see Figure 6–67). To be effective, these proofreading mechanisms must also allow an appreciable fraction of correct interactions to be removed; for this reason, proofreading is even more costly in energy than it might seem.

Quality Control Mechanisms Act to Prevent Translation of Damaged mRNAs

In eucaryotes, mRNA production involves both transcription and a series of elaborate RNA-processing steps; these take place in the nucleus, segregated from ribosomes, and only when the processing is complete are the mRNAs transported to the cytoplasm to be translated (see Figure 6–40). However, this scheme is not foolproof, and some incorrectly processed mRNAs are inadvertently sent to the cytoplasm. In addition, mRNAs that were flawless when they left the nucleus can become broken or otherwise damaged in the cytosol. The danger of translating damaged or incompletely processed mRNAs (which would produce truncated or otherwise aberrant proteins) is apparently so great that the cell has several backup measures to prevent this from happening.

To avoid translating broken mRNAs, the 5′ cap and the poly-A tail are both recognized by the translation-initiation machinery before translation begins (see Figure 6–72). To help ensure that mRNAs are properly spliced before they are translated, the exon junction complex (EJC), which is deposited on the mRNA following splicing (see Figure 6–40), stimulates the subsequent translation of the mRNA.

But the most powerful mRNA surveillance system, called **nonsense-mediated mRNA decay**, eliminates defective mRNAs before they can be efficiently translated into protein. This mechanism is brought into play when the cell determines that an mRNA molecule has a nonsense (stop) codon (UAA, UAG, or UGA) in the "wrong" place—a situation likely to arise in an mRNA molecule that has been improperly spliced. Aberrant splicing will usually result in the random introduction of a nonsense codon into the reading frame of the mRNA, especially in organisms, such as humans, that have a large average intron size (see Figure 6–32B).

This surveillance mechanism begins as an mRNA molecule is being transported from the nucleus to the cytosol. As its 5′ end emerges from the nuclear pore, the mRNA is met by a ribosome, which begins to translate it. As translation proceeds, the exon junction complexes (EJC) bound to the mRNA at each splice-site are apparently displaced by the moving ribosome. The normal stop codon will be within the last exon, so by the time the ribosome reaches it and stalls, no more EJCs should be bound to the mRNA. If this is the case, the mRNA "passes inspection" and is released to the cytosol where it can be translated in earnest (**Figure 6–80**). However, if the ribosome reaches a premature stop codon and stalls, it senses that EJCs remain and the bound mRNA molecule is rapidly degraded. In this way, the first round of translation allows the cell to test the fitness of each mRNA molecule as it exits the nucleus.

Nonsense-mediated decay may have been especially important in evolution, allowing eucaryotic cells to more easily explore new genes formed by DNA rearrangements, mutations, or alternative patterns of splicing—by selecting only those mRNAs for translation that can produce a full-length protein. Nonsense-mediated decay is also important in cells of the developing immune system, where the extensive DNA rearrangements that occur (see Figure 25–36) often generate premature termination codons. The surveillance system degrades the mRNAs produced from such rearranged genes, thereby avoiding the potential toxic effects of truncated proteins.

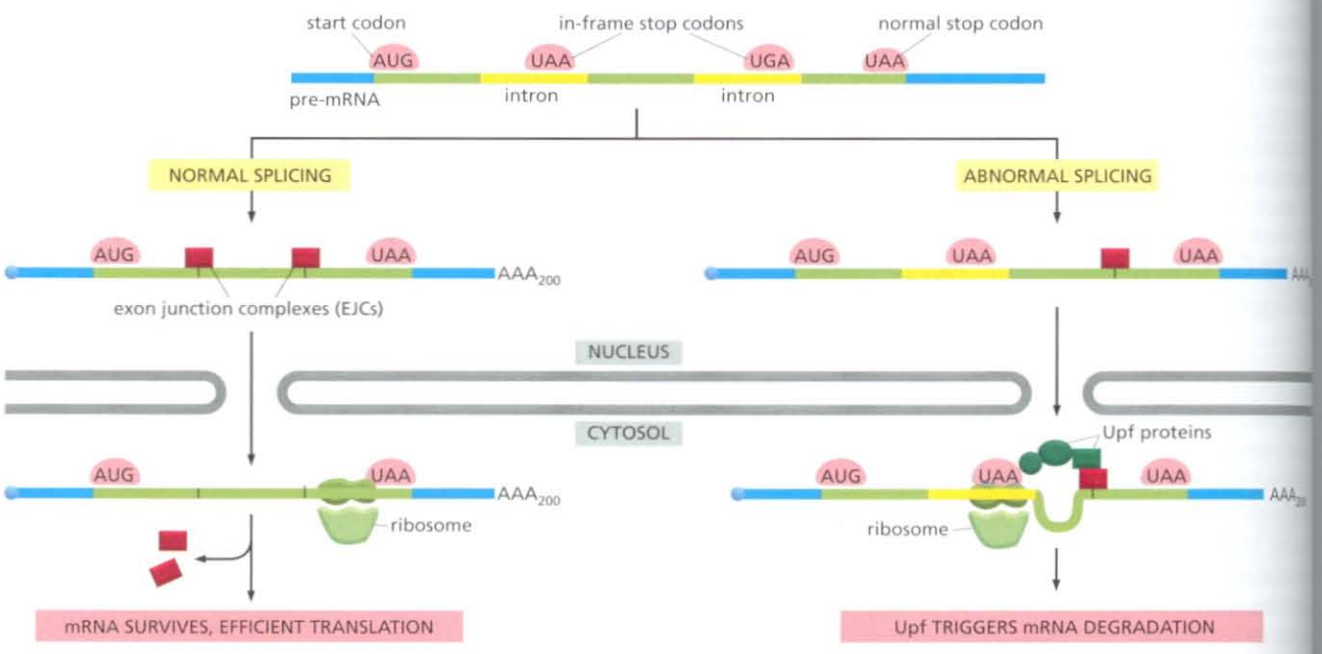


Figure 6–80 Nonsense-mediated mRNA decay. As shown on the right, the failure to correctly splice a pre-mRNA often introduces a premature stop codon into the reading frame for the protein. The introduction of such an "in-frame" stop codon is particularly likely to occur in mammals, where the introns tend to be very long. When translated, these abnormal mRNAs produce aberrant proteins, which could damage the cell. However, as shown at the bottom right of the figure, these abnormal RNAs are destroyed by the nonsense-mediated decay mechanism. According to one model, an mRNA molecule, bearing exon junction complexes (EJCs) to mark successfully completed splices, is first met by a ribosome that performs a "test" round of translation. As the mRNA passes through the tight channel of the ribosome, the EJCs are stripped off, and successful mRNAs are released to undergo multiple rounds of translation *(left side)*. However, if an in-frame stop codon is encountered before the final exon junction complex is reached *(right side)*, the mRNA undergoes nonsense-mediated decay, which is triggered by the Upf proteins (*green*) that bind to each EJC. Note that, to trigger nonsense-mediated decay, the premature stop codon must be in the same reading frame as that of the normal protein. (Adapted from J. Lykke-Andersen et al., *Cell* 103:1121–1131, 2000. With permission from Elsevier.)

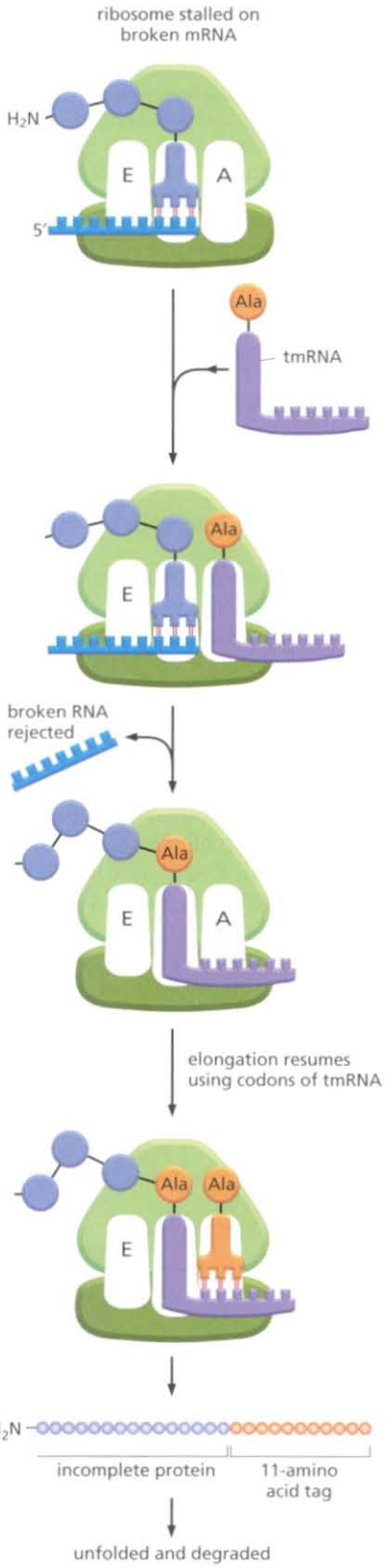


Figure 6–81 The rescue of a bacterial ribosome stalled on an incomplete mRNA molecule. The tmRNA shown is a 363-nucleotide RNA with both tRNA and mRNA functions, hence its name. It carries an alanine and can enter the vacant A-site of a stalled ribosome to add this alanine to a polypeptide chain, mimicking a tRNA although no codon is present to guide it. The ribosome then translates 10 codons from the tmRNA, completing an 11 amino acid tag on the protein. Proteases recognize this tag and degrade the entire protein. Although the example shown in the figure is from bacteria, eucaryotes can employ a similar strategy.

Finally, the nonsense-mediated surveillance pathway plays an important role in mitigating the symptoms of many inherited human diseases. As we have seen, inherited diseases are usually caused by mutations that spoil the function of a key protein, such as hemoglobin or one of the blood clotting factors. Approximately one-third of all genetic disorders in humans result from nonsense mutations or mutations (such as frameshift mutations or splice-site mutations) that place nonsense mutations into the gene's reading frame. In individuals that carry one mutant and one functional gene, nonsense-mediated decay eliminates the aberrant mRNA and thereby prevents a potentially toxic protein from being made. Without this safeguard, individuals with one functional and one mutant "disease gene" would likely suffer much more severe symptoms.

We saw earlier in this chapter that bacteria lack the elaborate mRNA processing found in eucaryotes and that translation often begins before the synthesis of the RNA molecule is completed. Yet bacteria also have quality control mechanisms to deal with incompletely synthesized and broken mRNAs. When the bacterial ribosome translates to the end of an incomplete RNA it stalls and does not release the RNA. Rescue comes in the form of a special RNA (called tmRNA), which enters the A-site of the ribosome and is itself translated, releasing the ribosome. The special 11 amino acid tag thus added to the C-terminus of the truncated protein signals to proteases that the entire protein is to be degraded (**Figure 6–81**).

Some Proteins Begin to Fold While Still Being Synthesized

The process of gene expression is not over when the genetic code has been used to create the sequence of amino acids that constitutes a protein. To be useful to the cell, this new polypeptide chain must fold up into its unique three-dimensional conformation, bind any small-molecule cofactors required for its activity, be appropriately modified by protein kinases or other protein-modifying enzymes, and assemble correctly with the other protein subunits with which it functions (**Figure 6–82**).

The information needed for all of the steps listed above is ultimately contained in the sequence of linked amino acids that the ribosome produces when it translates an mRNA molecule into a polypeptide chain. As discussed in Chapter 3, when a protein folds into a compact structure, it buries most of its hydrophobic residues in an interior core. In addition, large numbers of noncovalent interactions form between various parts of the molecule. It is the sum of all of these energetically favorable arrangements that determines the final folding pattern of the polypeptide chain—as the conformation of lowest free energy (see p. 130).

Through many millions of years of evolution, the amino acid sequence of each protein has been selected not only for the conformation that it adopts but also for an ability to fold rapidly. For some proteins, this folding begins immediately, as the protein spins out of the ribosome, starting from the N-terminal end. In these cases, as each protein domain emerges from the ribosome, within a few seconds it forms a compact structure that contains most of the final secondary features (α helices and β sheets) aligned in roughly the right conformation (**Figure 6–83**). For many protein domains, this unusually dynamic and flexible state called a *molten globule*, is the starting point for a relatively slow process in which many side-chain adjustments occur that eventually form the correct tertiary

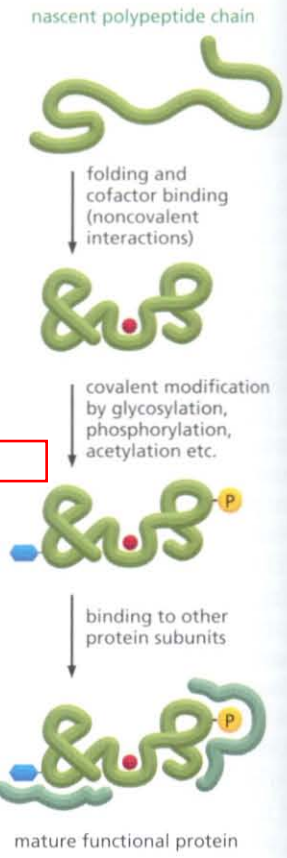


Figure 6–82 Steps in the creation of a functional protein. As indicated, translation of an mRNA sequence into an amino acid sequence on the ribosome is not the end of the process of forming a protein. To function, the completed polypeptide chain must fold correctly into its three-dimensional conformation, bind any cofactors required, and assemble with its partner protein chains (if any). Noncovalent bond formation drives these changes. As indicated, many proteins also require covalent modifications of selected amino acids. Although the most frequent modifications are protein glycosylation and protein phosphorylation, more than 100 different types of covalent modifications are known (see, for example, Figure 3–81).

structure. It takes several minutes to synthesize a protein of average size, and for some proteins much of the folding process is complete by the time the ribosome releases the C-terminal end of a protein (**Figure 6–84**).

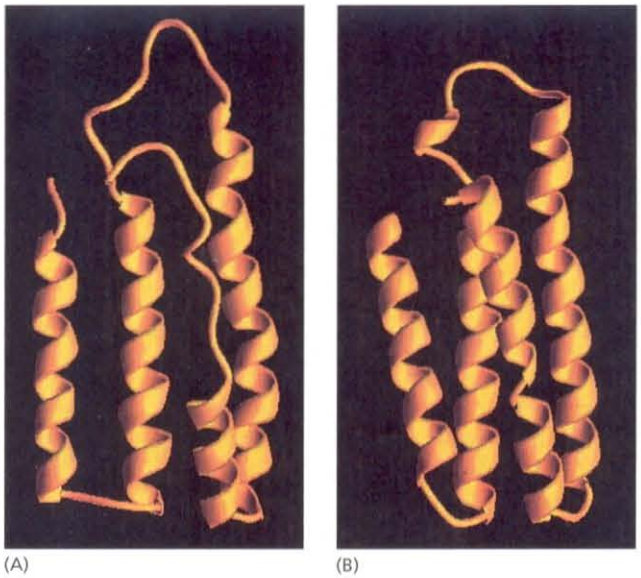
10th

Molecular Chaperones Help Guide the Folding of Most Proteins

Most proteins probably do not begin to fold during their synthesis. Instead, they are met at the ribosome by a special class of proteins called **molecular chaperones**. Molecular chaperones are useful for cells because there are many different paths that can be taken to convert an unfolded or partially folded protein to its final compact conformation. For many proteins, some of the intermediates formed along the way would aggregate and be left as off-pathway dead ends without the intervention of a chaperone (**Figure 6–85**).

Many molecular chaperones are called *heat-shock proteins* (designated *Hsp*), because they are synthesized in dramatically increased amounts after a brief exposure of cells to an elevated temperature (for example, 42°C for cells that normally live at 37°C). This reflects the operation of a feedback system that responds to an increase in misfolded proteins (such as those produced by elevated temperatures) by boosting the synthesis of the chaperones that help these proteins refold.

There are several major families of eucaryotic molecular chaperones, including the Hsp60 and Hsp70 proteins. Different family members function in different organelles. Thus, as discussed in Chapter 12, mitochondria contain their own Hsp60 and Hsp70 molecules that are distinct from those that function in the cytosol; and a special Hsp70 (called *BIP*) helps to fold proteins in the endoplasmic reticulum.



(A) (B)

Figure 6–83 The structure of a molten globule. (A) A molten globule form of cytochrome b_{562} is more open and less highly ordered than the final folded form of the protein, shown in (B). Note that the molten globule contains most of the secondary structure of the final form, although the ends of the α helices are unravelled and one of the helices is only partly formed. (Courtesy of Joshua Wand from Y. Feng et al., *Nat. Struct. Biol.* 1:30–35, 1994. With permission from Macmillan Publishers Ltd.)

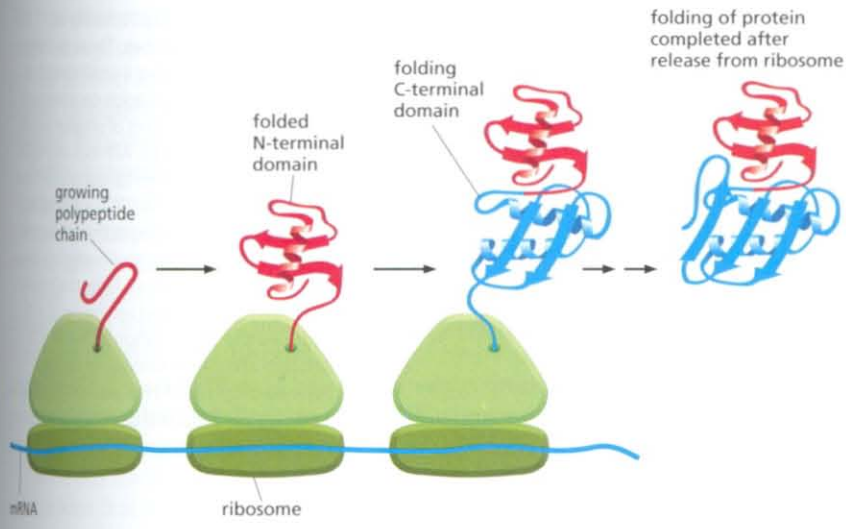


Figure 6–84 Co-translational protein folding. A growing polypeptide chain is shown acquiring its secondary and tertiary structure as it emerges from a ribosome. The N-terminal domain folds first, while the C-terminal domain is still being synthesized. This protein has not achieved its final conformation at the time it is released from the ribosome. (Modified from A.N. Federov and T.O. Baldwin, *J. Biol. Chem.* 272:32715–32718, 1997.)

The Hsp60 and Hsp70 proteins each work with their own small set of associated proteins when they help other proteins to fold. Hsps share an affinity for the exposed hydrophobic patches on incompletely folded proteins, and they hydrolyze ATP, often binding and releasing their protein substrate with each cycle of ATP hydrolysis. In other respects, the two types of Hsp proteins function differently. The Hsp70 machinery acts early in the life of many proteins, binding to a string of about seven hydrophobic amino acids before the protein leaves the ribosome (**Figure 6–86**). In contrast, Hsp60-like proteins form a large barrel-shaped structure that acts after a protein has been fully synthesized. This type of chaperone, sometimes called a *chaperonin*, forms an "isolation chamber" into which misfolded proteins are fed, preventing their aggregation and providing them with a favorable environment in which to attempt to refold (**Figure 6–87**).

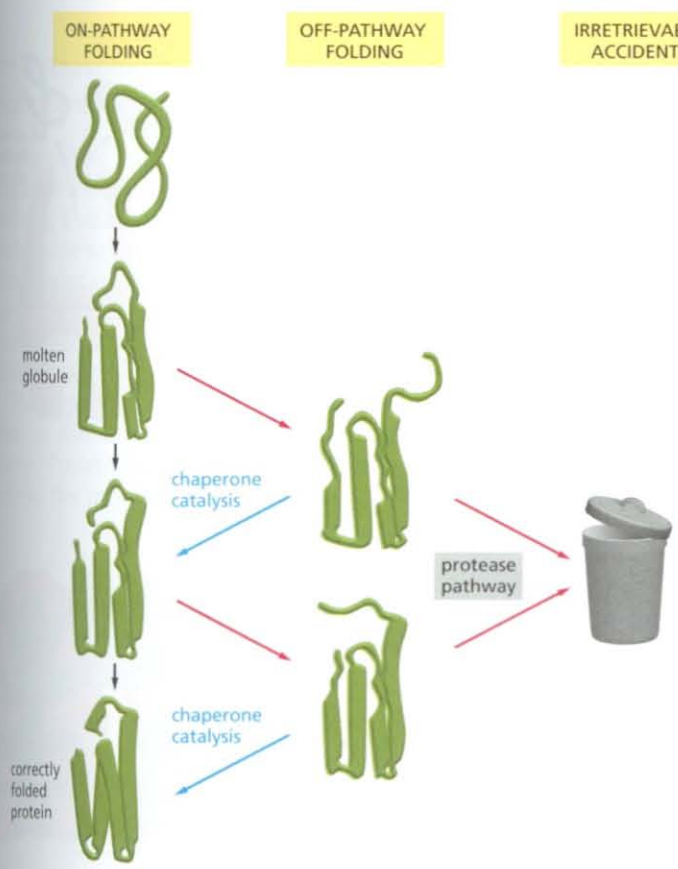


Figure 6–85 A current view of protein folding. Each domain of a newly synthesized protein rapidly attains a "molten globule" state. Subsequent folding occurs more slowly and by multiple pathways, often involving the help of a molecular chaperone. Some molecules may still fail to fold correctly; as explained in the text, specific proteases recognize and degrade these molecules.

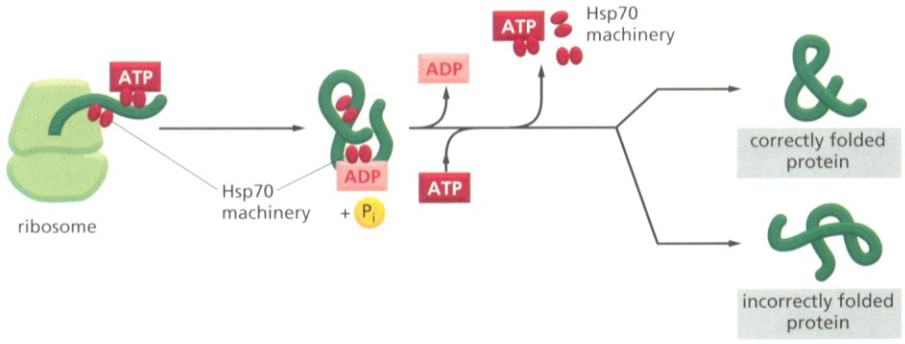


Figure 6–86 The Hsp70 family of molecular chaperones. These proteins act early, recognizing a small stretch of hydrophobic amino acids on a protein's surface. Aided by a set of smaller Hsp40 proteins (not shown), ATP-bound Hsp70 molecules grasp their target protein and then hydrolyze ATP to ADP, undergoing conformational changes that cause the Hsp70 molecules to associate even more tightly with the target. After the Hsp40 dissociates, the rapid rebinding of ATP induces the dissociation of the Hsp70 protein after ADP release. In reality, repeated cycles of Hsp protein binding and release help the target protein to refold, as schematically illustrated in Figure 6–85.

The chaperones shown in Figures 6–86 and 6–87 often use many cycles of ATP hydrolysis to fold a single polypeptide chain correctly. Although some of this energy expenditure is used to perform mechanical work, probably much more is expended to ensure that protein folding is accurate. Just as we saw for transcription, splicing, and translation, the expenditure of free energy can be used by cells to improve the accuracy of a biological process. In the case of protein folding, ATP hydrolysis allows chaperones to recognize a wide variety of misfolded structures, to halt any further misfolding and to recommence folding of a protein in an orderly way.

Although our discussion focuses on only two types of chaperones, the cell has a variety of others. The enormous diversity of proteins in cells presumably requires a wide range of chaperones with versatile surveillance and correction capabilities.

Exposed Hydrophobic Regions Provide Critical Signals for Protein Quality Control

If radioactive amino acids are added to cells for a brief period, the newly synthesized proteins can be followed as they mature into their final functional form.

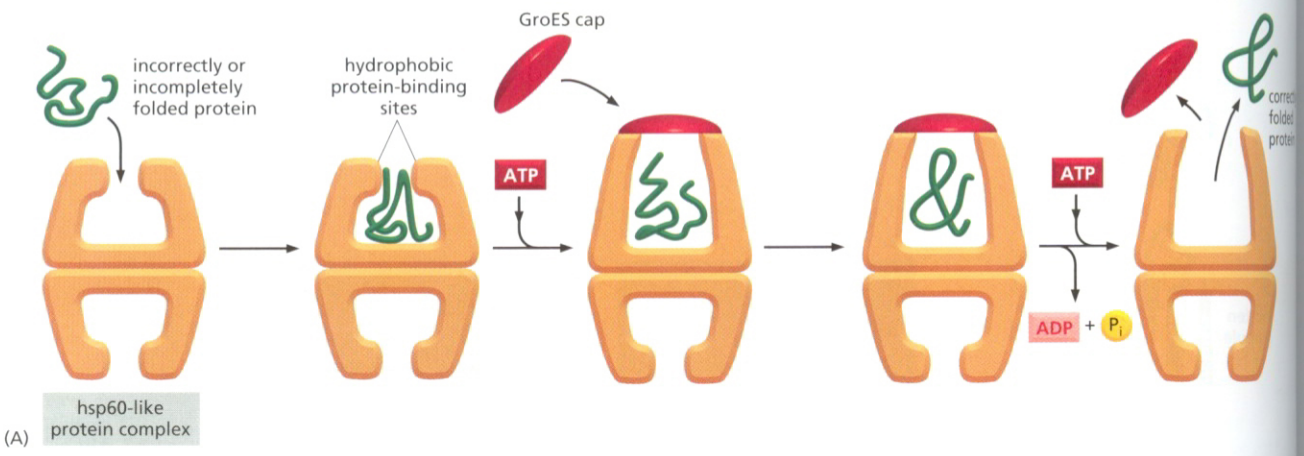
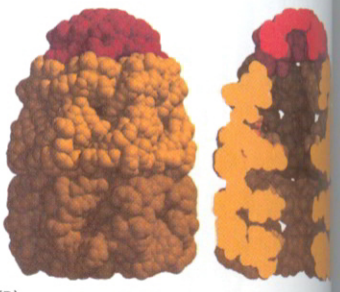


Figure 6–87 The structure and function of the Hsp60 family of molecular chaperones. (A) The catalysis of protein refolding. A misfolded protein is initially captured by hydrophobic interactions along one rim of the barrel. The subsequent binding of ATP plus a protein cap increases the diameter of the barrel rim, which may transiently stretch (partly unfold) the client protein. This also confines the protein in an enclosed space, where it has a new opportunity to fold. After about 15 seconds, ATP hydrolysis occurs, weakening the complex. Subsequent binding of another ATP molecule ejects the protein, whether folded or not, and the cycle repeats. This type of molecular chaperone is also known as a chaperonin; it is designated as Hsp60 in mitochondria, TCP1 in the cytosol of vertebrate cells, and GroEL in bacteria. As indicated, only half of the symmetrical barrel operates on a client protein at any one time. (B) The structure of GroEL bound to its GroES cap, as determined by X-ray crystallography. On the *left* is shown the outside of the barrel-like structure and on the *right* a cross section through its center. (B, adapted from B. Bukau and A.L. Horwich, *Cell* 92:351–366, 1998. With permission from Elsevier.)



This type of experiment demonstrates that the Hsp70 proteins act first, beginning when a protein is still being synthesized on a ribosome, and the Hsp60-like proteins act only later to help fold completed proteins. But how does the cell distinguish misfolded proteins, which require additional rounds of ATP-catalyzed refolding, from those with correct structures?

Before answering, we need to pause to consider the post-translational fate of proteins more broadly. Usually, if a protein has a sizable exposed patch of hydrophobic amino acids on its surface, it is abnormal: it has either failed to fold correctly after leaving the ribosome, suffered an accident that partly unfolded it at a later time, or failed to find its normal partner subunit in a larger protein complex. Such a protein is not merely useless to the cell, it can be dangerous. Many proteins with an abnormally exposed hydrophobic region can form large aggregates in the cell. We shall see that, in rare cases, such aggregates do form and cause severe human diseases. Normally, however, powerful protein quality control mechanisms prevent such disasters.

Given this background, it is not surprising that cells have evolved elaborate mechanisms that recognize the hydrophobic patches on proteins and minimize the damage they cause. Two of these mechanisms depend on the molecular chaperones just discussed, which bind to the patch and attempt to repair the defective protein by giving it another chance to fold. At the same time, by covering the hydrophobic patches, these chaperones transiently prevent protein aggregation. Proteins that very rapidly fold correctly on their own do not display such patches and the chaperones bypass them.

Figure 6–88 outlines all of the quality control choices that a cell makes for a difficult-to-fold, newly synthesized protein. As indicated, when attempts to refold a protein fail, a third mechanism is called into play that completely destroys the protein by proteolysis. The proteolytic pathway begins with the recognition of an abnormal hydrophobic patch on a protein's surface, and it ends with the delivery of the entire protein to a protein destruction machine, a complex protease known as the *proteasome*. As described next, this process depends on an elaborate protein-marking system that also carries out other central functions in the cell by destroying selected normal proteins.

The Proteasome Is a Compartmentalized Protease with Sequestered Active Sites

The proteolytic machinery and the chaperones compete with one another to reorganize a misfolded protein. If a newly synthesized protein folds rapidly, at most only a small fraction of it is degraded. In contrast, a slowly folding protein is vulnerable to the proteolytic machinery for a longer time, and many more of its molecules are destroyed before the remainder attain the proper folded state. Due to mutations or to errors in transcription, RNA splicing, and translation, some proteins never fold properly. It is particularly important that the cell destroy these potentially harmful proteins.

The apparatus that deliberately destroys aberrant proteins is the **proteasome**, an abundant ATP-dependent protease that constitutes nearly 1% of cell protein. Present in many copies dispersed throughout the cytosol and the nucleus, the proteasome also destroys aberrant proteins of the endoplasmic

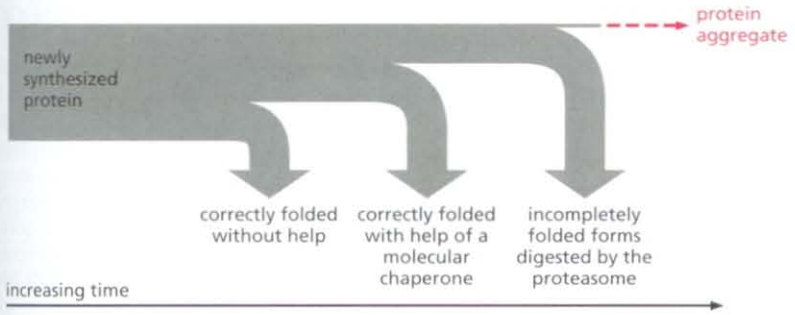


Figure 6–88 The processes that monitor protein quality following protein synthesis. A newly synthesized protein sometimes folds correctly and assembles on its own with its partner proteins, in which case the quality control mechanisms leave it alone. Incompletely folded proteins are helped to refold by molecular chaperones: first by a family of Hsp70 proteins, and then in some cases, by Hsp60-like proteins. For both types of chaperones, the client proteins are recognized by an abnormally exposed patch of hydrophobic amino acids on their surface. These "protein-rescue" processes compete with another mechanism that, upon recognizing an abnormally exposed patch, marks the protein for destruction by the proteasome. The combined activity of all of these processes is needed to prevent massive protein aggregation in a cell, which can occur when many hydrophobic regions on proteins clump together nonspecifically.

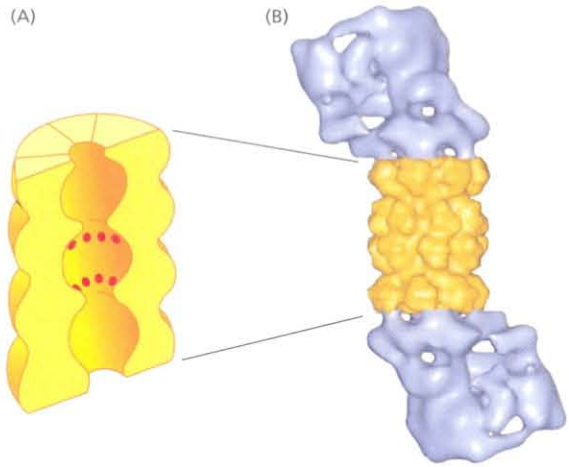


Figure 6–89 The proteasome. (A) A cut-away view of the structure of the central 20S cylinder, as determined by x-ray crystallography, with the active sites of the proteases indicated by *red dots*. (B) The entire proteasome, in which the central cylinder *(yellow)* is supplemented by a 19S cap *(blue)* at each end. The cap structure has been determined by computer processing of electron microscope images. The complex cap (also called the regulatory particle) selectively binds proteins that have been marked by ubiquitin for destruction; it then uses ATP hydrolysis to unfold their polypeptide chains and feed them through a narrow channel (see Figure 6–91) into the inner chamber of the 20S cylinder for digestion to short peptides. (B, from W. Baumeister et al., *Cell* 92:367–380, 1998. With permission from Elsevier.)

reticulum (ER). An ER-based surveillance system detects proteins that fail either to fold or to be assembled properly after they enter the ER, and *retrotranslocates* them back to the cytosol for degradation (discussed in Chapter 12).

Each proteasome consists of a central hollow cylinder (the 20S core proteasome) formed from multiple protein subunits that assemble as a quasi-cylindrical stack of four heptameric rings (**Figure 6–89**). Some of the subunits are distinct proteases whose active sites face the cylinder's inner chamber. The design prevents these highly efficient proteases from running rampant through the cell. Each end of the cylinder is normally associated with a large protein complex (the 19S cap), which contains a six-subunit protein ring, through which target proteins are threaded into the proteasome core where they are degraded (**Figure 6–90**). The threading reaction, driven by ATP hydrolysis, unfolds the target proteins as they move through the cap, exposing them to the proteases lining the proteasome core (**Figure 6–91**). The proteins that make up the ring structure in the proteasome cap belong to a large class of protein "unfoldases" known as *AAA proteins*. Many of them function as hexamers, and it is possible that they share mechanistic features with the ATP-dependent unwinding of DNA by DNA helicases (see Figure 5–15).

A crucial property of the proteasome, and one reason for the complexity of its design, is the *processivity* of its mechanism: in contrast to a "simple" protease that cleaves a substrate's polypeptide chain just once before dissociating, the proteasome keeps the entire substrate bound until all of it is converted into short peptides.

The 19S caps also act as regulated "gates" at the entrances to the inner proteolytic chamber, and they are responsible for binding a targeted protein substrate to the proteasome. With a few exceptions, the proteasomes act on proteins that have been specifically marked for destruction by the covalent attachment of a recognition tag formed from a small protein called *ubiquitin* (**Figure 6–92**A). Ubiquitin exists in cells either free or covalently linked to

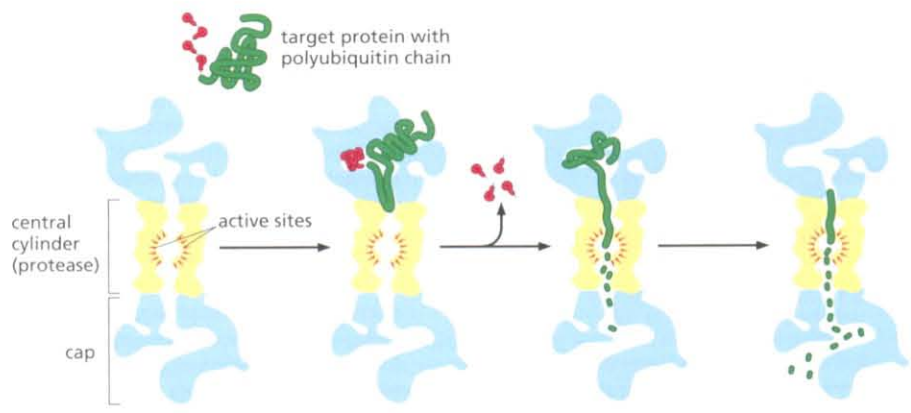


Figure 6–90 Processive protein digestion by the proteasome. The proteasome cap recognizes a substrate protein, in this case marked by a polyubiquitin chain (see Figure 6–92), and subsequently translocates it into the proteasome core, where it is digested. At an early stage, the ubiquitin is cleaved from the substrate protein and is recycled. Translocation into the core of the proteasome is mediated by a ring of ATP-dependent proteins that unfold the substrate protein as it is threaded through the ring and into the proteasome core (see Figure 6–91). (From S. Prakash and A. Matouschek, *Trends Biochem. Sci.* 29:593–600, 2004. With permission from Elsevier.)

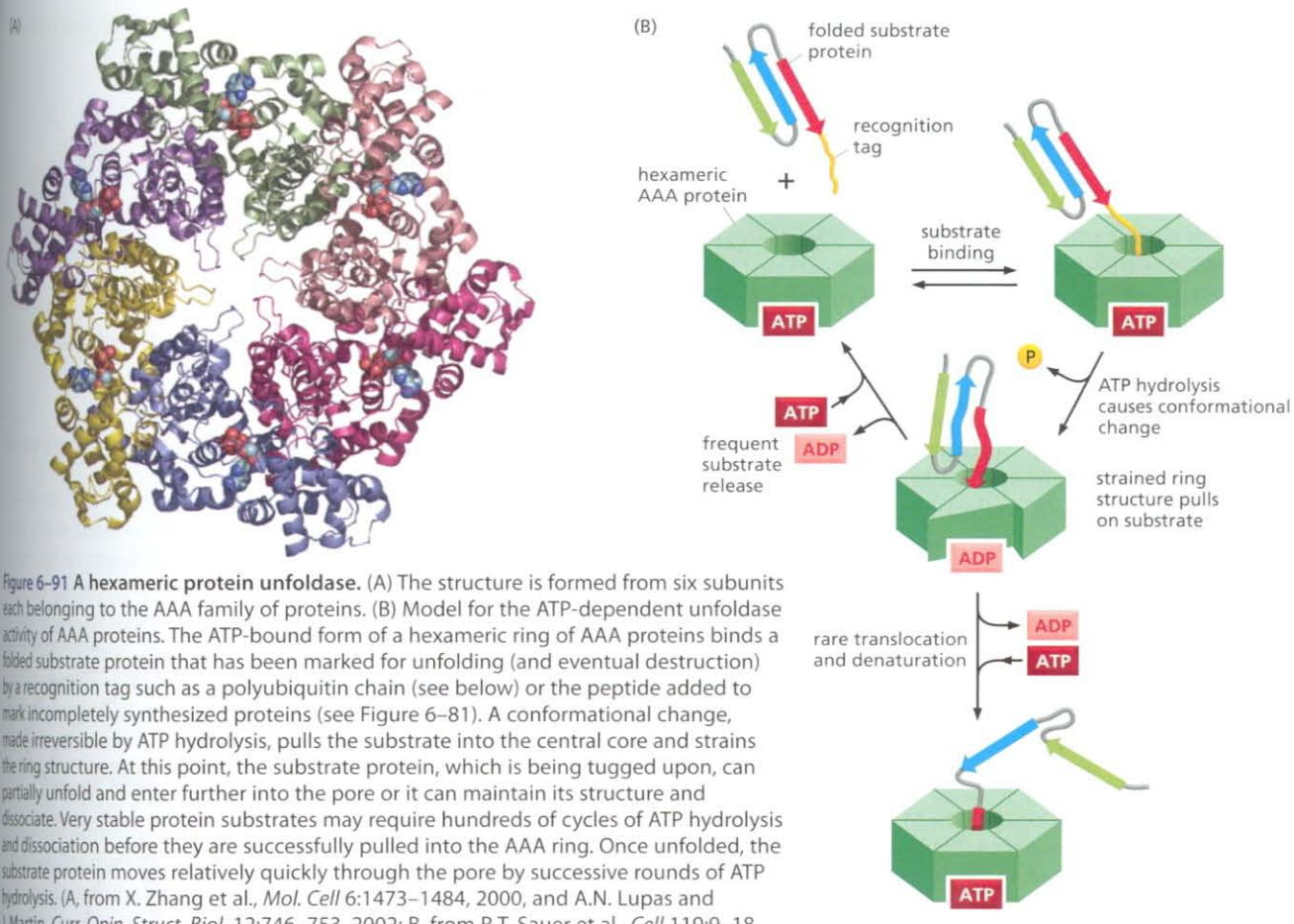


Figure 6–91 A hexameric protein unfoldase. (A) The structure is formed from six subunits each belonging to the AAA family of proteins. (B) Model for the ATP-dependent unfoldase activity of AAA proteins. The ATP-bound form of a hexameric ring of AAA proteins binds a folded substrate protein that has been marked for unfolding (and eventual destruction) by a recognition tag such as a polyubiquitin chain (see below) or the peptide added to mark incompletely synthesized proteins (see Figure 6–81). A conformational change, made irreversible by ATP hydrolysis, pulls the substrate into the central core and strains the ring structure. At this point, the substrate protein, which is being tugged upon, can partially unfold and enter further into the pore or it can maintain its structure and dissociate. Very stable protein substrates may require hundreds of cycles of ATP hydrolysis and dissociation before they are successfully pulled into the AAA ring. Once unfolded, the substrate protein moves relatively quickly through the pore by successive rounds of ATP hydrolysis. (A, from X. Zhang et al., *Mol. Cell* 6:1473–1484, 2000, and A.N. Lupas and J. Martin, *Curr. Opin. Struct. Biol.* 12:746–753, 2002; B, from R.T. Sauer et al., *Cell* 119:9–18, 2004. All with permission from Elsevier.)

many different intracellular proteins. For many proteins, tagging by ubiquitin results in their destruction by the proteasome. However, in other cases, ubiquitin tagging has an entirely different meaning. Ultimately, it is the number of ubiquitin molecules added and the way in which they are linked together that determines how the cell interprets the ubiquitin message (**Figure 6–93**). In the following sections, we emphasize the role of ubiquitylation in signifying protein degradation.

11th

An Elaborate Ubiquitin-Conjugating System Marks Proteins for Destruction

Ubiquitin is prepared for conjugation to other proteins by the ATP-dependent *ubiquitin-activating enzyme* (E1), which creates an activated, E1-bound ubiquitin that is subsequently transferred to one of a set of *ubiquitin-conjugating* (E2) enzymes (Figure 6–92B). The E2 enzymes act in conjunction with accessory (E3) proteins. In the E2–E3 complex, called *ubiquitin ligase*, the E3 component binds to specific degradation signals, called degrons, in protein substrates, helping E2 to form a *polyubiquitin* chain linked to a lysine of the substrate protein. In this chain, the C-terminal residue of each ubiquitin is linked to a specific lysine of the preceding ubiquitin molecule (see Figure 6–93), producing a linear series of ubiquitin–ubiquitin conjugates (Figure 6–92C). It is this polyubiquitin chain on a target protein that is recognized by a specific receptor in the proteasome.

There are roughly 30 structurally similar but distinct E2 enzymes in mammals, and hundreds of different E3 proteins that form complexes with specific E2 enzymes. The ubiquitin–proteasome system thus consists of many distinct but similarly organized proteolytic pathways, which have in common both the

E1 enzyme at the "top" and the proteasome at the "bottom," and differ by the compositions of their E2–E3 ubiquitin ligases and accessory factors. Distinct ubiquitin ligases recognize different degradation signals, and therefore target distinct subsets of intracellular proteins for destruction.

Denatured or otherwise misfolded proteins, as well as proteins containing oxidized or other abnormal amino acids, are recognized and destroyed because abnormal proteins tend to present on their surface amino acid sequences or conformational motifs that are recognized as degradation signals by a set of E3 molecules in the ubiquitin–proteasome system; these sequences must of course be buried and therefore inaccessible in the normal counterparts of these proteins. However, a proteolytic pathway that recognizes and destroys abnormal proteins must be able to distinguish between *completed* proteins that have "wrong" conformations and the many growing polypeptides on ribosomes (as well as polypeptides just released from ribosomes) that have not yet achieved their normal folded conformation. This is not a trivial problem; the ubiquitin–proteasome system is thought to destroy many of the nascent and newly formed protein molecules not because these proteins are abnormal as such, but because they transiently expose degradation signals that are buried in their mature (folded) state.

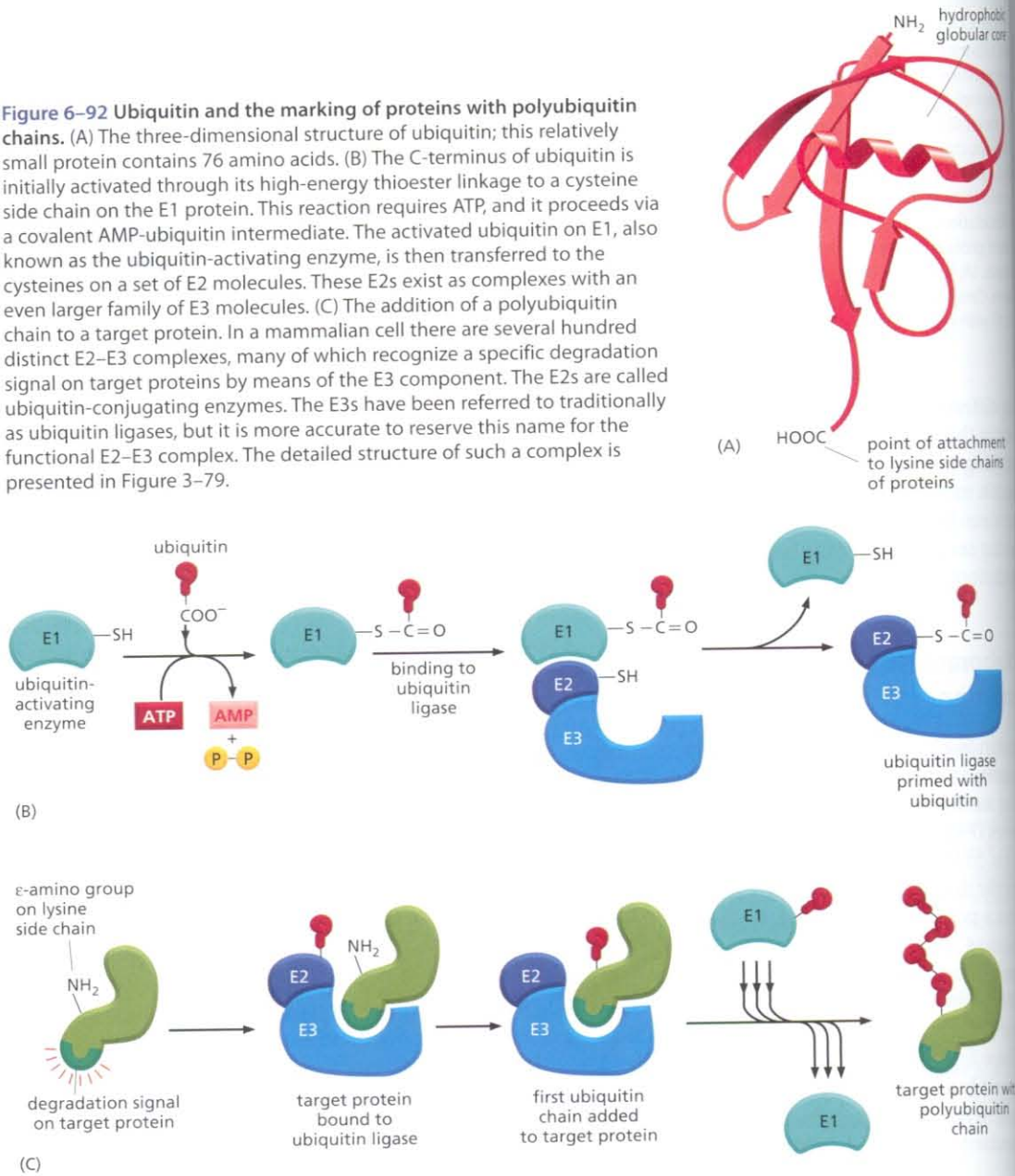


Figure 6–92 Ubiquitin and the marking of proteins with polyubiquitin chains. (A) The three-dimensional structure of ubiquitin; this relatively small protein contains 76 amino acids. (B) The C-terminus of ubiquitin is initially activated through its high-energy thioester linkage to a cysteine side chain on the E1 protein. This reaction requires ATP, and it proceeds via a covalent AMP-ubiquitin intermediate. The activated ubiquitin on E1, also known as the ubiquitin-activating enzyme, is then transferred to the cysteines on a set of E2 molecules. These E2s exist as complexes with an even larger family of E3 molecules. (C) The addition of a polyubiquitin chain to a target protein. In a mammalian cell there are several hundred distinct E2–E3 complexes, many of which recognize a specific degradation signal on target proteins by means of the E3 component. The E2s are called ubiquitin-conjugating enzymes. The E3s have been referred to traditionally as ubiquitin ligases, but it is more accurate to reserve this name for the functional E2–E3 complex. The detailed structure of such a complex is presented in Figure 3–79.

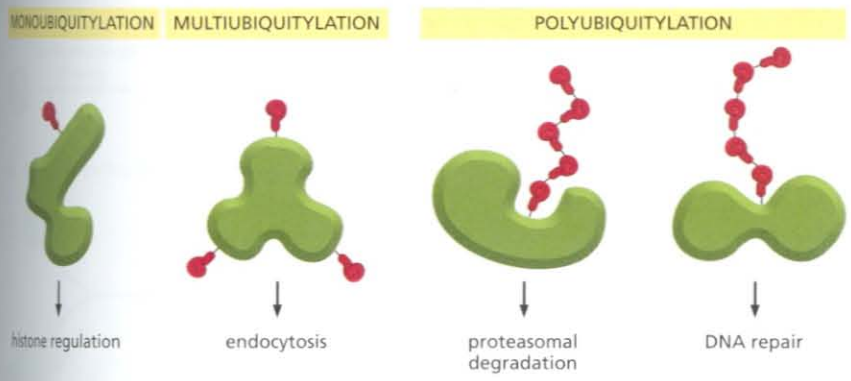


Figure 6–93 The marking of proteins by ubiquitin. Each modification pattern shown can have a specific meaning to the cell. The two types of polyubiquitylation differ in the way the ubiquitin molecules are linked together. Linkage through Lys48 signifies degradation by the proteasome whereas that through Lys63 has other meanings. Ubiquitin markings are "read" by proteins that specifically recognize each type of modification.

Many Proteins Are Controlled by Regulated Destruction

One function of intracellular proteolytic mechanisms is to recognize and eliminate misfolded or otherwise abnormal proteins, as just described. Yet another function of these proteolytic pathways is to confer short lifetimes on specific normal proteins whose concentrations must change promptly with alterations in the state of a cell. Some of these short-lived proteins are degraded rapidly at all times, while many others are *conditionally* short-lived, that is, they are metabolically stable under some conditions but become unstable upon a change in the cell's state. For example, mitotic cyclins are long-lived throughout the cell cycle until their sudden degradation at the end of mitosis, as explained in Chapter 17.

How is such a regulated destruction of a protein controlled? Several mechanisms are illustrated through specific examples that appear later in this book. In one general class of mechanism (**Figure 6–94**A), the activity of a ubiquitin ligase is turned on either by E3 phosphorylation or by an allosteric transition in an E3 protein caused by its binding to a specific small or large molecule. For example, the anaphase-promoting complex (APC) is a multisubunit ubiquitin ligase that is activated by a cell-cycle-timed subunit addition at mitosis. The activated APC then causes the degradation of mitotic cyclins and several other regulators of the metaphase–anaphase transition (see Figure 17–44).

Alternatively, in response either to intracellular signals or to signals from the environment, a degradation signal can be created in a protein, causing its rapid ubiquitylation and destruction by the proteasome. One common way to create such a signal is to phosphorylate a specific site on a protein that unmasks a normally hidden degradation signal. Another way to unmask such a signal is by the regulated dissociation of a protein subunit. Finally, powerful degradation signals can be created by cleaving a single peptide bond, provided that this cleavage creates a new N-terminus that is recognized by a specific E3 as a "destabilizing" N-terminal residue (Figure 6–94B).

The N-terminal type of degradation signal arises because of the "N-end rule," which relates the lifetime of a protein *in vivo* to the identity of its N-terminal residue. There are 12 destabilizing residues in the N-end rule of the yeast *S. cerevisiae* (Arg, Lys, His, Phe, Leu, Tyr, Trp, Ile, Asp, Glu, Asn, and Gln), out of the 20 standard amino acids. The destabilizing N-terminal residues are recognized by a special ubiquitin ligase that is conserved from yeast to humans.

As we have seen, all proteins are initially synthesized bearing methionine (or formylmethionine in bacteria), as their N-terminal residue, which is a stabilizing residue in the N-end rule. Special proteases, called methionine aminopeptidases, will often remove the first methionine of a nascent protein, but they will do so only if the second residue is also stabilizing according to N-end rule. Therefore, it was initially unclear how N-end rule substrates form *in vivo*. However, it is now understood that these substrates are formed by site-specific proteases. For example, a subunit of cohesin, a protein complex that holds sister chromatids together, is cleaved by a highly specific protease during the metaphase–anaphase transition. This cell-cycle-regulated cleavage allows separation of the sister chromatids and leads to the completion of mitosis (see

Figure 17–44). The C-terminal fragment of the cleaved subunit bears an N-terminal arginine, a destabilizing residue in the N-end rule. Mutant cells lacking the N-end rule pathway exhibit a greatly increased frequency of chromosome loss, presumably because a failure to degrade this fragment of the cohesin subunit interferes with the formation of new chromatid-associated cohesin complexes in the next cell cycle.

Abnormally Folded Proteins Can Aggregate to Cause Destructive Human Diseases

Many inherited human diseases (for example, sickle-cell anemia (see p. 1495) and α-1-antitrypsin deficiency, a condition that often leads to liver disease and emphysema) result from mutant proteins that escape the cell's quality controls, fold abnormally, and form aggregates. By absorbing critical macromolecules, these aggregates can severely damage cells and even cause cell death. Often, the inheritance of a single mutant allele of a gene can cause disease, since the normal copy of the gene cannot protect the cell from the destructive properties of the aggregate.

In normal humans, the gradual decline of the cell's protein quality controls can also cause disease by permitting normal proteins to form aggregates (**Figure 6–95**). In some cases, the protein aggregates are released from dead cells and accumulate in the extracellular matrix that surrounds the cells in a tissue, and in extreme cases they can also damage tissues. Because the brain is composed of a highly organized collection of nerve cells, it is especially vulnerable.

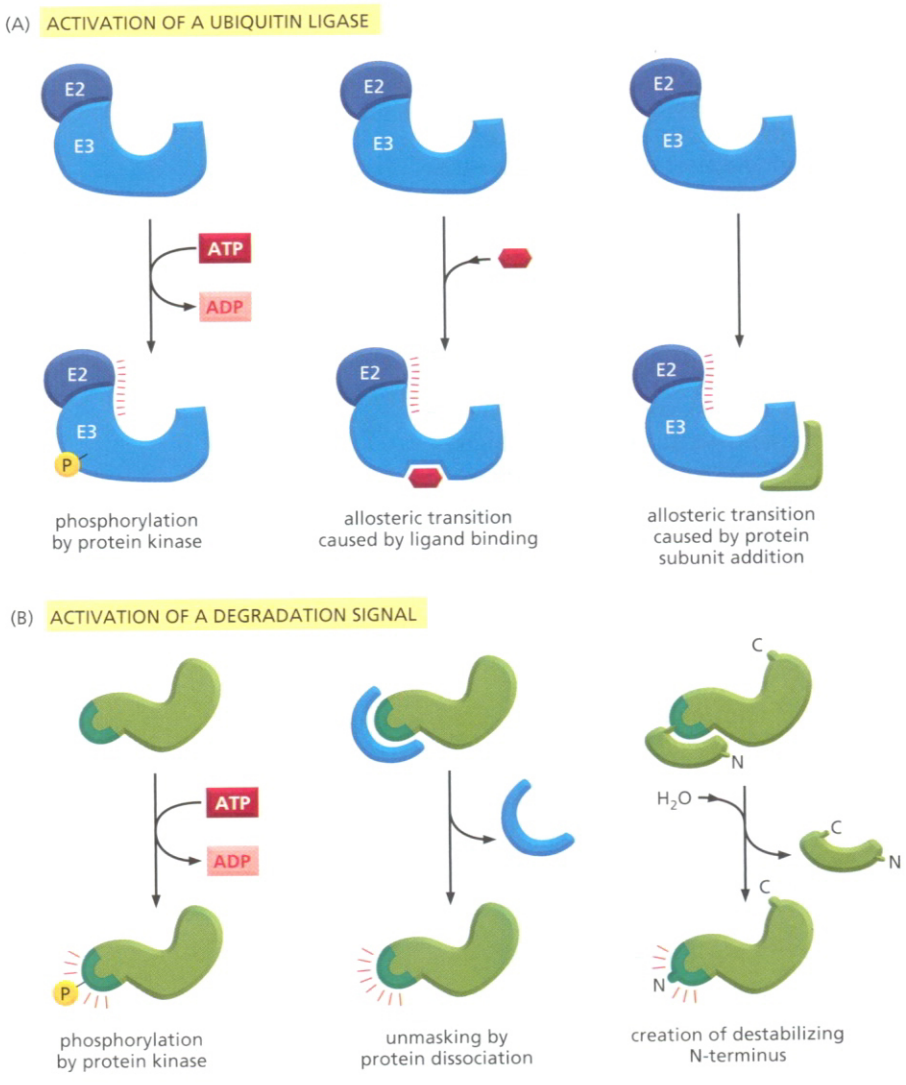


Figure 6–94 Two general ways of inducing the degradation of a specific protein. (A) Activation of a specific E3 molecule creates a new ubiquitin ligase. (B) Creation of an exposed degradation signal in the protein to be degraded. This signal binds a ubiquitin ligase, causing the addition of a polyubiquitin chain to a nearby lysine on the target protein. All the pathways shown are known to be used by cells to induce the movement of selected proteins into the proteasome.

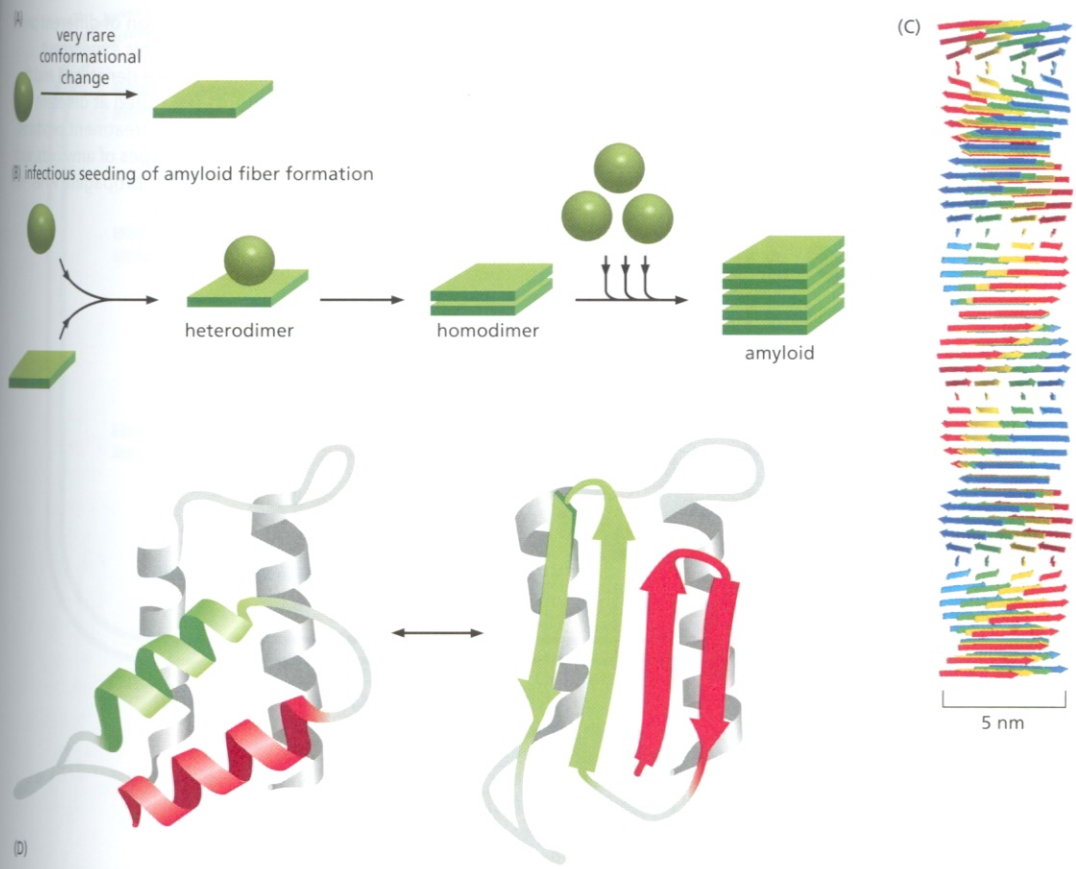


Figure 6–95 Protein aggregates that cause human disease. (A) Schematic illustration of the type of conformational change in a protein that produces material for a cross-beta filament. (B) Diagram illustrating the self-infectious nature of the protein aggregation that is central to prion diseases. PrP (prion protein) is highly unusual because the misfolded version of the protein, called PrP*, induces the normal PrP protein it contacts to change its conformation, as shown. Most of the human diseases caused by protein aggregation are caused by the overproduction of a variant protein that is especially prone to aggregation, but the protein aggregate cannot spread from one animal to another. (C) Drawing of a cross-beta filament, a common type of protease-resistant protein aggregate found in many human neurological diseases. Because the hydrogen-bond interactions in a β sheet form between polypeptide backbone atoms (see Figure 3–9), a number of different abnormally folded proteins can produce this structure. (D) One of several possible models for the conversion of PrP to PrP*, showing the likely change of two α helices into four β strands. Although the structure of the normal protein has been determined accurately, the structure of the infectious form is not yet known with certainty because the aggregation has prevented the use of standard structural techniques. (C, courtesy of Louise Serpell, adapted from M. Sunde et al., *J. Mol. Biol.* 273:729–739, 1997. With permission from Academic Press; D, adapted from S.B. Prusiner, *Trends Biochem. Sci.* 21:482–487, 1996. With permission from Elsevier.)

Not surprisingly, therefore, protein aggregates primarily cause neurodegenerative diseases. Prominent among these are Huntington's disease and Alzheimer's disease—the latter causing age-related dementia in more than 20 million people in today's world.

For a particular type of protein aggregate to survive, grow, and damage an organism, it must be highly resistant to proteolysis both inside and outside the cell. Many of the protein aggregates that cause problems form fibrils built from a series of polypeptide chains that are layered one over the other as a continuous stack of β sheets. This so-called *cross-beta filament* (Figure 6–95C), a structure particularly resistant to proteolysis, is observed in many of the neurological disorders caused by protein aggregates, where it produces distinctly staining deposits known as *amyloids*.

One particular variety of these pathologies has attained special notoriety. These are the **prion diseases**. Unlike Huntington's or Alzheimer's, prion diseases can spread from one organism to another, providing that the second organism eats a tissue containing the protein aggregate. A set of diseases—called scrapie in sheep, Creutzfeldt–Jacob disease (CJD) in humans, and bovine spongiform encephalopathy (BSE) in cattle—are caused by a misfolded, aggregated form of a protein called PrP (for prion protein). The PrP is normally located on the outer surface of the plasma membrane, most prominently in neurons. Its normal

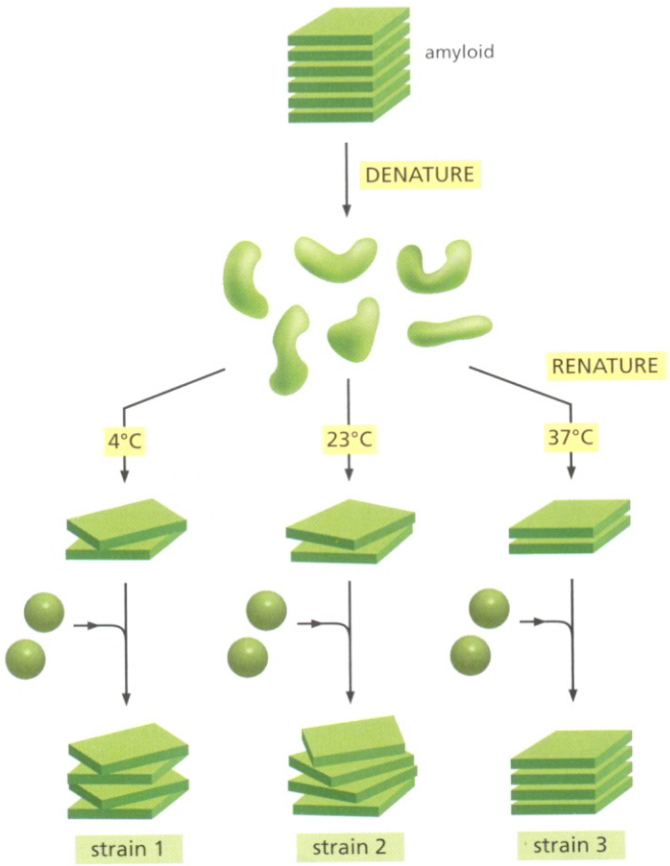


Figure 6–96 Creation of different prion strains *in vitro*. In this experiment, amyloid fibers were denatured and the components renatured at different temperatures. This treatment produced three distinctive types of amyloids, each of which could self-propagate when new subunits are added.

function is not known. However, PrP has the unfortunate property of being convertible to a very special abnormal conformation (see Figure 6–95A). This conformation not only forms protease-resistant, cross-beta filaments; it is also "infectious" because it converts normally folded molecules of PrP to the same pathological form. This property creates a positive feedback loop that propagates the abnormal form of PrP, called PrP* (see Figure 6–95B) and thereby allows the pathological conformation to spread rapidly from cell to cell in the brain, eventually causing death in both animals and humans. It can be dangerous to eat the tissues of animals that contain PrP*, as witnessed by the spread of BSE (commonly referred to as "mad cow disease") from cattle to humans in Great Britain. Fortunately, in the absence of PrP*, PrP is extraordinarily difficult to convert to its abnormal form.

Although very few proteins have the potential to misfold into an infectious conformation, another example causes an otherwise mysterious "protein-only inheritance" observed in yeast cells. The ability to study infectious proteins in yeast has clarified another remarkable feature of prions. These protein molecules can form several distinctively different types of aggregates from the same polypeptide chain. Moreover, each type of aggregate can be infectious, forcing normal protein molecules to adopt the same type of abnormal structure. Thus, several different "strains" of infectious particles can arise from the same polypeptide chain (**Figure 6–96**). How a single polypeptide sequence can adopt multiple aggregate forms is not fully understood; it is possible that all prion aggregates resemble cross-beta filaments (see Figure 6–95C) where the structure is held together predominantly with main peptide chain interactions. This would leave the amino acid side chains free to adopt different conformations and, if the structures are self-propagating, the existence of different strains could be explained.

Finally, although prions were discovered because they cause disease, they also appear to have some positive roles in the cell. For example, some species of fungi use prion transformations to establish different types of cells. Although the idea is controversial, it has even been proposed that prions have a role in consolidating memories in complex, multicellular organisms like ourselves.

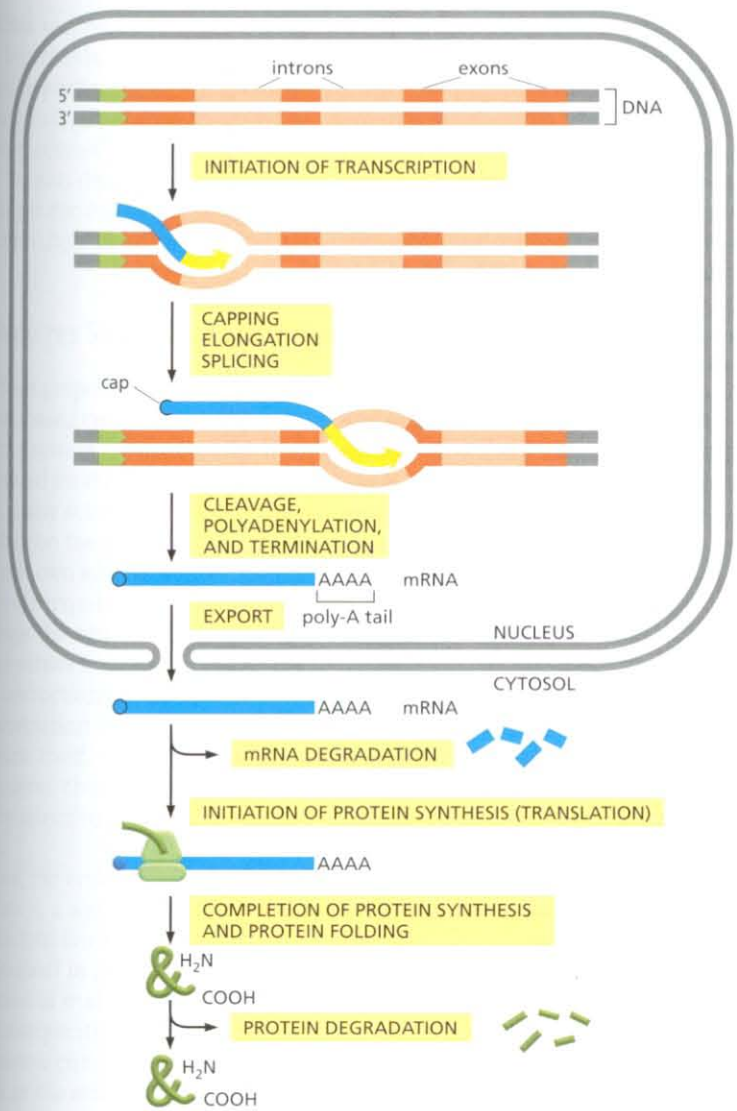


Figure 6–97 The production of a protein by a eucaryotic cell. The final level of each protein in a eucaryotic cell depends upon the efficiency of each step depicted.

There Are Many Steps From DNA to Protein

We have seen so far in this chapter that many different types of chemical reactions are required to produce a properly folded protein from the information contained in a gene (**Figure 6–97**). The final level of a properly folded protein in a cell therefore depends upon the efficiency with which each of the many steps is performed.

In the following chapter, we shall see that cells have the ability to change the levels of their proteins according to their needs. In principle, any or all of the steps in Figure 6–97 could be regulated for each individual protein. As we shall see in Chapter 7, there are examples of regulation at each step from gene to protein. However, the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes. This makes sense, inasmuch as the most efficient way to keep a gene from being expressed is to block the very first step—the transcription of its DNA sequence into an RNA molecule.

Summary

The translation of the nucleotide sequence of an mRNA molecule into protein takes place in the cytoplasm on a large ribonucleoprotein assembly called a ribosome. The amino acids used for protein synthesis are first attached to a family of tRNA molecules, each of which recognizes, by complementary base-pair interactions, particular sets of three nucleotides in the mRNA (codons). The sequence of nucleotides in the mRNA is then read from one end to the other in sets of three according to the genetic code.

To initiate translation, a small ribosomal subunit binds to the mRNA molecule at a start codon (AUG) that is recognized by a unique initiator tRNA molecule. A large ribosomal subunit binds to complete the ribosome and begin protein synthesis. During this phase, aminoacyl-tRNAs—each bearing a specific amino acid—bind sequentially to the appropriate codons in mRNA through complementary base pairing between tRNA anticodons and mRNA codons. Each amino acid is added to the C-terminal end of the growing polypeptide in four sequential steps: aminoacyl-tRNA binding, followed by peptide bond formation, followed by two ribosome translocation steps. Elongation factors use GTP hydrolysis to drive these reactions forward and to improve the accuracy of amino acid selection. The mRNA molecule progresses codon by codon through the ribosome in the 5′-to-3′ direction until it reaches one of three stop codons. A release factor then binds to the ribosome, terminating translation and releasing the completed polypeptide.

Eucaryotic and bacterial ribosomes are closely related, despite differences in the number and size of their rRNA and protein components. The rRNA has the dominant role in translation, determining the overall structure of the ribosome, forming the binding sites for the tRNAs, matching the tRNAs to codons in the mRNA, and creating the active site of the peptidyl transferase enzyme that links amino acids together during translation.

In the final steps of protein synthesis, two distinct types of molecular chaperones guide the folding of polypeptide chains. These chaperones, known as Hsp60 and Hsp70, recognize exposed hydrophobic patches on proteins and serve to prevent the protein aggregation that would otherwise compete with the folding of newly synthesized proteins into their correct three-dimensional conformations. This protein folding process must also compete with an elaborate quality control mechanism that destroys proteins with abnormally exposed hydrophobic patches. In this case, ubiquitin is covalently added to a misfolded protein by a ubiquitin ligase, and the resulting polyubiquitin chain is recognized by the cap on a proteasome that moves the entire protein to the interior of the proteasome for proteolytic degradation. A closely related proteolytic mechanism, based on special degradation signals recognized by ubiquitin ligases, is used to determine the lifetimes of many normally folded proteins. By this method, selected normal proteins are removed from the cell in response to specific signals.

THE RNA WORLD AND THE ORIGINS OF LIFE

We have seen that the expression of hereditary information requires extraordinarily complex machinery and proceeds from DNA to protein through an RNA intermediate. This machinery presents a central paradox: if nucleic acids are required to synthesize proteins and proteins are required, in turn, to synthesize nucleic acids, how did such a system of interdependent components ever arise? One view is that an *RNA world* existed on Earth before modern cells arose (**Figure 6–98**). According to this hypothesis, RNA both stored genetic information and catalyzed the chemical reactions in primitive cells. Only later in evolutionary time did DNA take over as the genetic material and proteins become the major catalyst and structural component of cells. If this idea is correct, then the transition out of the RNA world was never complete; as we have seen in this chapter, RNA still catalyzes several fundamental reactions in modern-day cells, which can be viewed as molecular fossils of an earlier world.

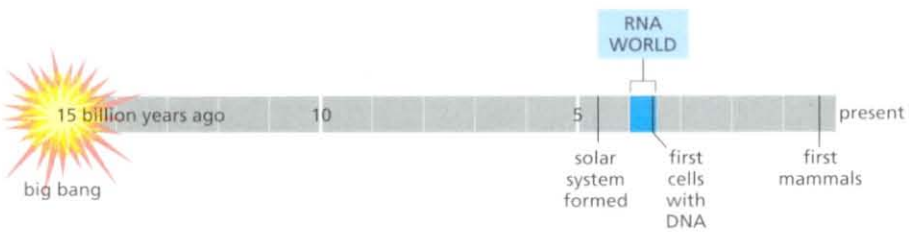


Figure 6–98 Time line for the universe suggesting the early existence of an RNA world of living systems.

In this section we present some of the arguments in support of the RNA world hypothesis. We will see that several of the more surprising features of modern-day cells, such as the ribosome and the pre-mRNA splicing machinery, are most easily explained by viewing them as descendants of a complex network of RNA-mediated interactions that dominated cell metabolism in the RNA world. We also discuss how DNA may have taken over as the genetic material, how the genetic code may have arisen, and how proteins may have eclipsed RNA to perform the bulk of biochemical catalysis in modern-day cells.

Life Requires Stored Information

It has been proposed that the first "biological" molecules on Earth were formed by metal-based catalysis on the crystalline surfaces of minerals. In principle, an elaborate system of molecular synthesis and breakdown (metabolism) could have existed on these surfaces long before the first cells arose. Although controversial, many scientists believe that an extensive phase of "chemical evolution" took place on the prebiotic Earth, during which small molecules that could catalyze their own synthesis competed with each other for raw materials.

But life requires much more than this. As described in Chapter 1, *heredity* is perhaps the central feature of life. Not only must a cell use raw materials to create a network of catalyzed reactions, it must do so according to an elaborate set of instructions encoded in the hereditary information. The replication of this information ensures that the complex metabolism of cells can accurately reproduce itself. Another crucial feature of life is the genetic variability that results from changes in the hereditary information. This variability, acted upon by selective pressures, is responsible for the great diversity of life on our planet.

Thus, the emergence of life requires a way to store information, a way to duplicate it, a way to change it, and a way to convert the information through catalysis into favorable chemical reactions. But how could such a system begin to be formed? In present-day cells the most versatile catalysts are polypeptides, composed of many different amino acids with chemically diverse side chains and, consequently, able to adopt diverse three-dimensional forms that bristle with reactive chemical groups. Polypeptides also carry information, in the order of their amino acid subunits. But there is no known way in which a polypeptide can reproduce itself by directly specifying the formation of another of precisely the same sequence.

Polynucleotides Can Both Store Information and Catalyze Chemical Reactions

Polynucleotides have one property that contrasts with those of polypeptides: they can directly guide the formation of copies of their own sequence. This capacity depends on complementary base pairing of nucleotide subunits, which enables one polynucleotide to act as a template for the formation of another. As we have seen in this and the preceding chapter, such complementary templating mechanisms lie at the heart of DNA replication and transcription in modern-day cells.

But the efficient synthesis of polynucleotides by such complementary templating mechanisms requires catalysts to promote the polymerization reaction: without catalysts, polymer formation is slow, error-prone, and inefficient. Today, template-based nucleotide polymerization is rapidly catalyzed by protein enzymes—such as the DNA and RNA polymerases. How could such polymerization be catalyzed before proteins with the appropriate enzymatic specificity existed? The beginnings of an answer to this question came from the discovery in 1982 that RNA molecules themselves can act as catalysts. We have seen in this chapter, for example, that a molecule of RNA catalyzes one of the central reactions in the cell, the covalent joining of amino acids to form proteins. The unique potential of RNA molecules to act both as information carrier and as catalyst forms the basis of the RNA world hypothesis.

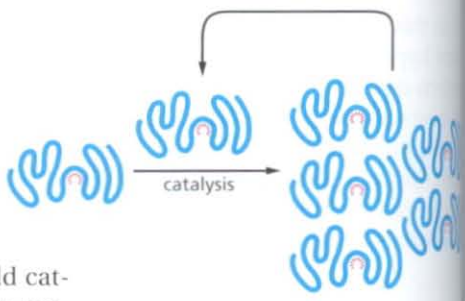


Figure 6–99 An RNA molecule that can catalyze its own synthesis. This hypothetical process would require catalysis of the production of both a second RNA strand of complementary nucleotide sequence and the use of this second RNA molecule as a template to form many molecules of RNA with the original sequence. The *red* rays represent the active site of this hypothetical RNA enzyme.

RNA therefore has all the properties required of a molecule that could catalyze a variety of chemical reactions, including those that lead to its own synthesis (**Figure 6–99**). Although self-replicating systems of RNA molecules have not been found in nature, scientists are confident that they can be constructed in the laboratory. While this demonstration would not prove that self-replicating RNA molecules were essential in the origin of life on Earth, it would certainly indicate that such a scenario is possible.

A Pre-RNA World May Predate the RNA World

Although RNA seems well suited to form the basis for a self-replicating set of biochemical catalysts, it is not clear that RNA was the first kind of molecule to do so. From a purely chemical standpoint, it is difficult to imagine how long RNA molecules could be formed initially by purely nonenzymatic means. For one thing, the precursors of RNA, the ribonucleotides, are difficult to form nonenzymatically. Moreover, the formation of RNA requires that a long series of 3′-to-5′ phosphodiester linkages assemble in the face of a set of competing reactions, including hydrolysis, 2′-to-5′ linkages, and 5′-to-5′ linkages. Given these problems, it has been suggested that the first molecules to possess both catalytic activity and information storage capabilities may have been polymers that resemble RNA but are chemically simpler (**Figure 6–100**). We do not have any remnants of these compounds in present-day cells, nor do such compounds leave fossil records. Nonetheless, the relative simplicity of these "RNA-like polymers" suggests that one of them, rather than RNA itself, may have been the first biopolymer on Earth capable of both information storage and catalytic activity.

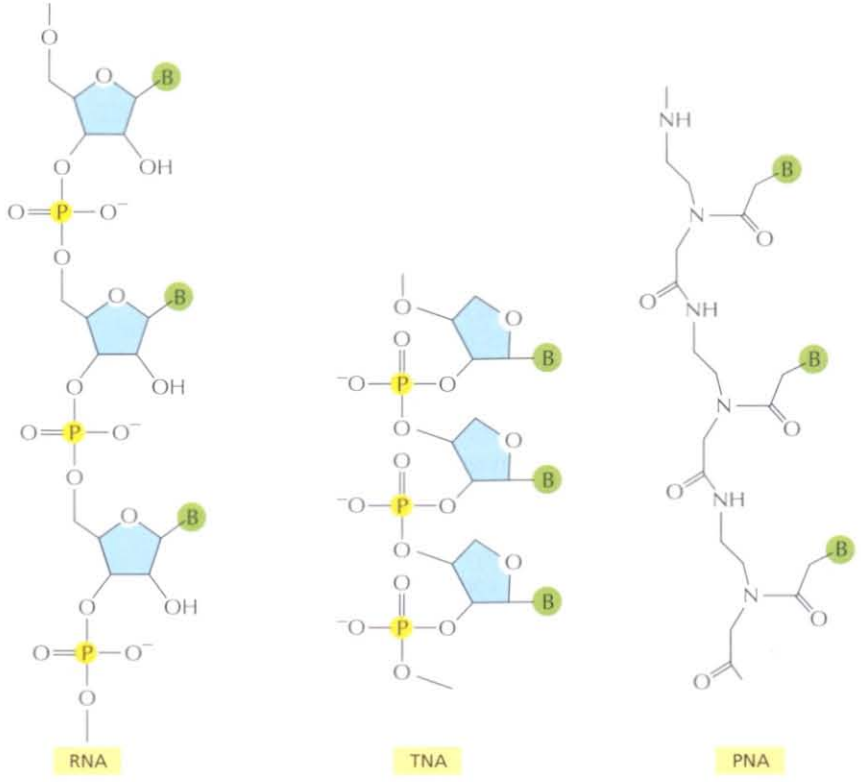


Figure 6–100 Structures of RNA and two related information-carrying polymers. In each case, B indicates a purine or pyrimidine base. The polymer TNA (threose nucleic acid) has a 4-carbon sugar unit in contrast to the 5-carbon ribose in RNA. In PNA (peptide nucleic acid), the ribose phosphate backbone of RNA has been replaced by the peptide backbone found in proteins. Like RNA, TNA and PNA can form double helices through complementary base-pairing and each could therefore in principle serve as a template for its own synthesis.

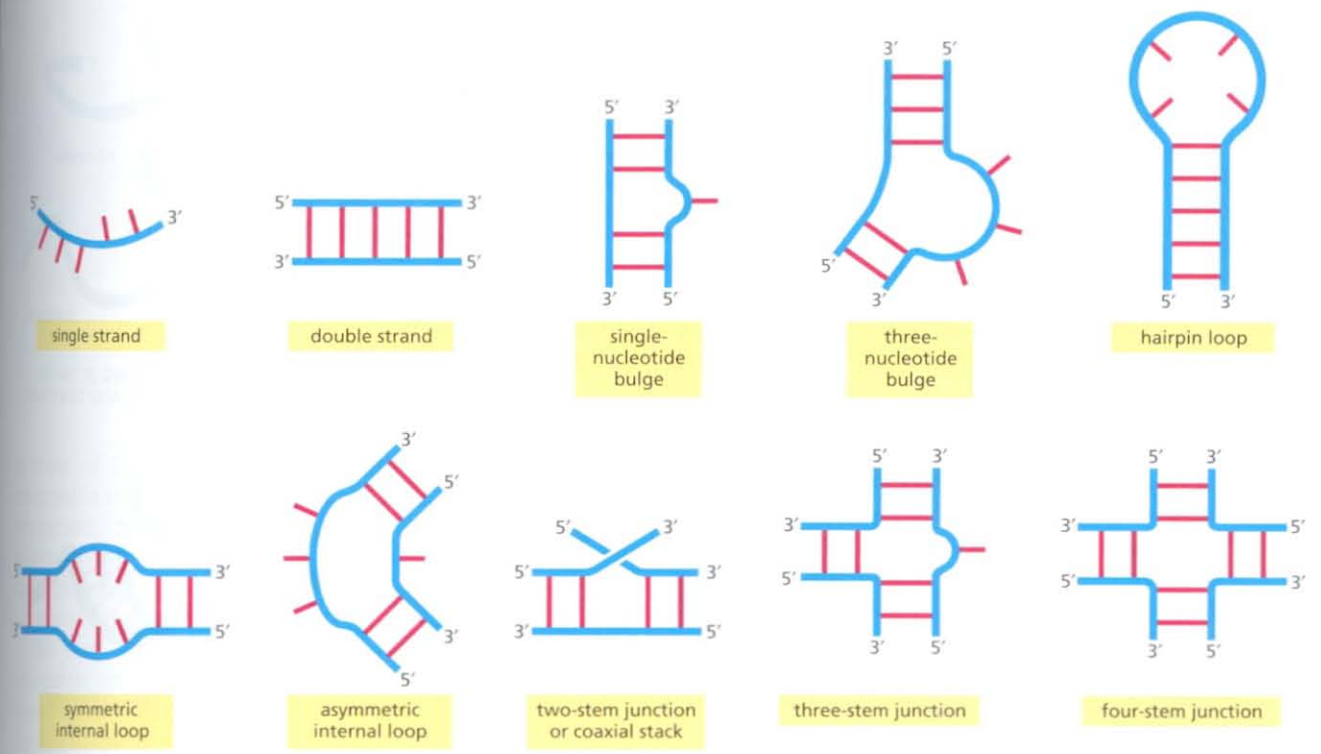


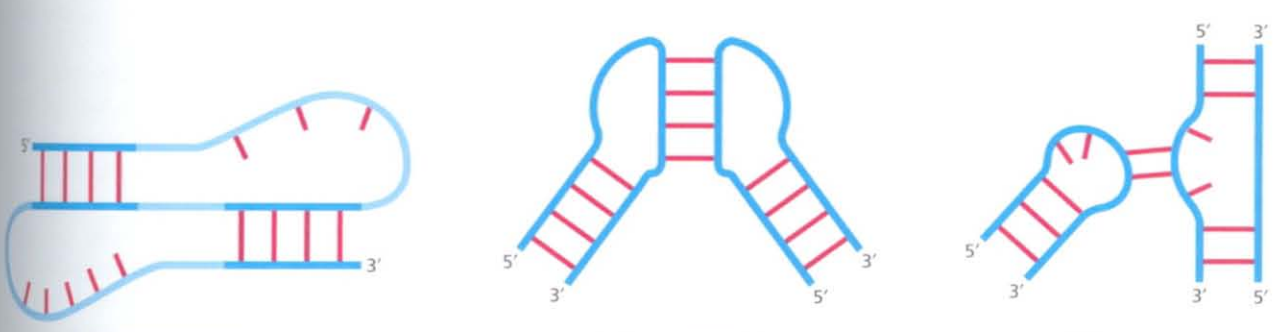
Figure 6–101 **Common elements of RNA secondary structure.** Conventional, complementary base-pairing interactions are indicated by *red* "rungs" in double-helical portions of the RNA.

If the pre-RNA world hypothesis is correct, then a transition to the RNA world must have occurred, presumably through the synthesis of RNA using one of these simpler polymers as both template and catalyst. While the details of the pre-RNA and RNA worlds will likely remain unknown, we know for certain that RNA molecules can catalyze a wide variety of chemical reactions, and we now turn to the properties of RNA that make this possible.

Single-Stranded RNA Molecules Can Fold into Highly Elaborate Structures

We have seen that complementary base-pairing and other types of hydrogen bonds can occur between nucleotides in the same chain, causing an RNA molecule to fold up in a unique way determined by its nucleotide sequence (see, for example, Figures 6–6, 6–52, and 6–69). Comparisons of many RNA structures have revealed conserved motifs, short structural elements that are used over and over again as parts of larger structures. **Figure 6–101** shows some of these RNA secondary structural motifs, and **Figure 6–102** shows a few common examples of more complex and often longer-range interactions, known as RNA tertiary interactions.

Figure 6–102 **Examples of RNA tertiary interactions.** Some of these interactions can join distant parts of the same RNA molecule or bring two separate RNA molecules together.



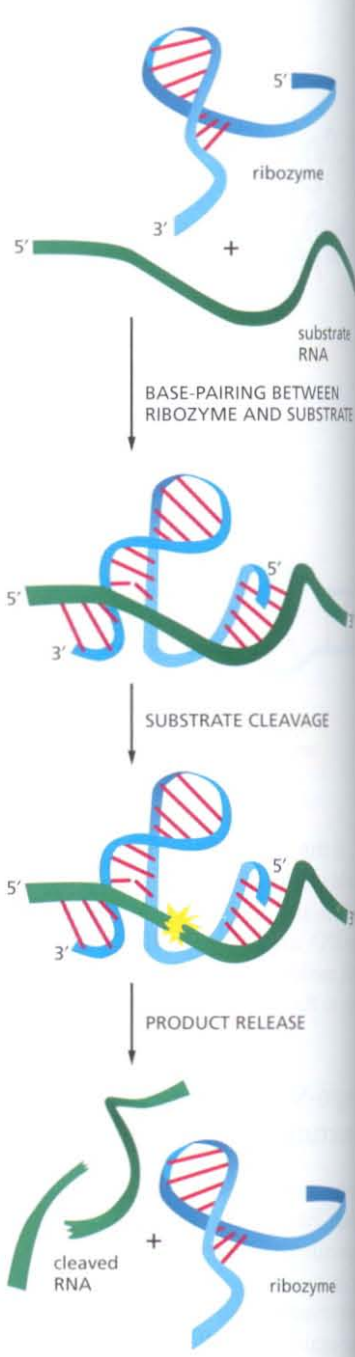


Figure 6–103 A ribozyme. This simple RNA molecule catalyzes the cleavage of a second RNA at a specific site. This ribozyme is found embedded in larger RNA genomes—called viroids—which infect plants. The cleavage, which occurs in nature at a distant location on the same RNA molecule that contains the ribozyme, is a step in the replication of the viroid genome. Although not shown in the figure, the reaction requires a Mg molecule positioned at the active site. (Adapted from T.R. Cech and O.C. Uhlenbeck, *Nature* 372:39–40, 1994. With permission from Macmillan Publishers Ltd.)

Protein catalysts require a surface with unique contours and chemical properties on which a given set of substrates can react (discussed in Chapter 3). In exactly the same way, an RNA molecule with an appropriately folded shape can serve as an enzyme (**Figure 6–103**). Like some proteins, many of these ribozymes work by positioning metal ions at their active sites. This feature gives them a wider range of catalytic activities than the limited chemical groups of the polynucleotide chain.

Relatively few catalytic RNAs are known to exist in modern-day cells, however, and much of our inference about the RNA world has come from experiments in which large pools of RNA molecules of random nucleotide sequences are generated in the laboratory. Those rare RNA molecules with a property specified by the experimenter are then selected out and studied (**Figure 6–104**). Such experiments have created RNAs that can catalyze a wide variety of biochemical reactions (**Table 6–5**), with reaction rate enhancements approaching those of proteins. Given these findings, it is not clear why protein catalysts greatly outnumber ribozymes in modern cells. Experiments have shown that RNA molecules may have more difficulty than proteins in binding to flexible, hydrophobic substrates; moreover, the availability of 20 types of amino acids over four types of bases may provide proteins with a greater number of catalytic strategies.

Like proteins, RNAs can undergo conformational changes, either in response to small molecules or to other RNAs. We saw several examples of this in the ribosome and the spliceosome, and we will see others in Chapter 7 when we discuss *riboswitches*. One of the most dramatic RNA conformational changes has been observed with an artificial ribozyme which can exist in two entirely different conformations, each with a different catalytic activity (**Figure 6–105**). Since the discovery of catalysis by RNA, it has become clear that RNA is an enormously versatile molecule, and it is therefore not unreasonable to contemplate the past existence of an RNA world with a very high level of biochemical sophistication.

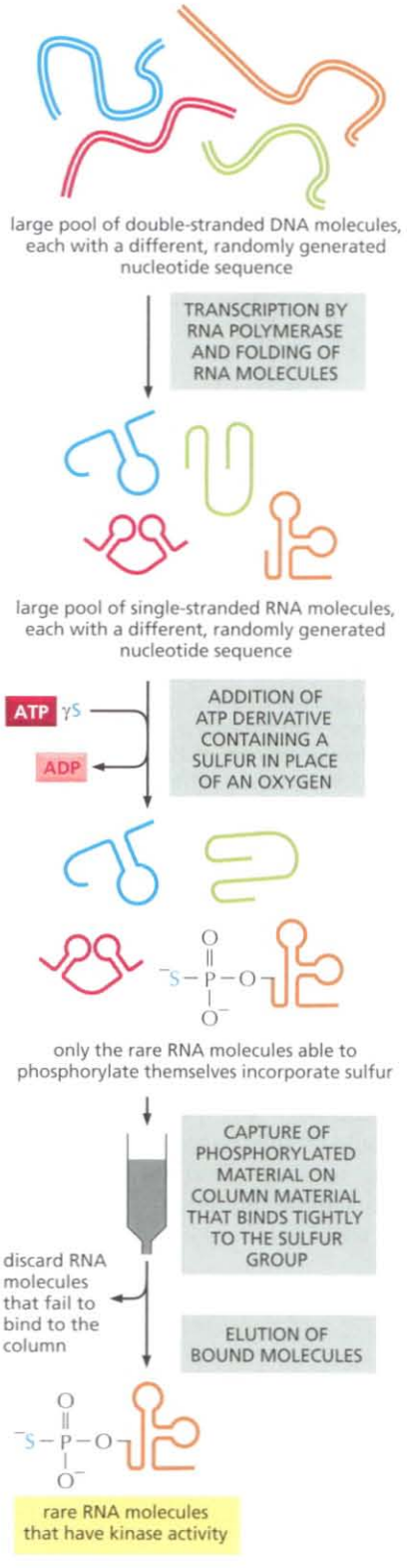
Self-Replicating Molecules Undergo Natural Selection

The three-dimensional folded structure of a polynucleotide affects its stability, its actions on other molecules, and its ability to replicate. Therefore, certain polynucleotides will be especially successful in any self-replicating mixture. Because errors inevitably occur in any copying process, new variant sequences of these polynucleotides will be generated over time.

Certain catalytic activities would have had a cardinal importance in the early evolution of life. Consider in particular an RNA molecule that helps to catalyze the process of templated polymerization, taking any given RNA molecule as a template (**Figure 6–106**). Such a molecule, by acting on copies of itself, can replicate. At the same time, it can promote the replication of other types of RNA molecules in its neighborhood (**Figure 6–107**). If some of these neighboring RNAs have catalytic actions that help the survival of RNA in other ways (catalyzing ribonucleotide production, for example), a set of different types of RNA molecules, each specialized for a different activity, could evolve into a cooperative system that replicates with unusually great efficiency.

But for any of these cooperative systems to evolve, they must be present together in a compartment. For example, a set of mutually beneficial RNAs (such

Figure 6–104 *In vitro* selection of a synthetic ribozyme. Beginning with a large pool of nucleic acid molecules synthesized in the laboratory, those rare RNA molecules that possess a specified catalytic activity can be isolated and studied. Although a specific example (that of an autophosphorylating ribozyme) is shown, variations of this procedure have been used to generate many of the ribozymes listed in Table 6–5. During the autophosphorylation step, the RNA molecules are kept sufficiently dilute to prevent the "cross"-phosphorylation of additional RNA molecules. In reality, several repetitions of this procedure are necessary to select the very rare RNA molecules with this catalytic activity. Thus, the material initially eluted from the column is converted back into DNA, amplified many fold (using reverse transcriptase and PCR as explained in Chapter 8), transcribed back into RNA, and subjected to repeated rounds of selection. (Adapted from J.R. Lorsch and J.W. Szostak, *Nature* 371:31–36, 1994. With permission from Macmillan Publishers Ltd.)



as those of Figure 6–107) could replicate themselves only if all the RNAs remained in the neighborhood of the RNA that is specialized for templated polymerization. Moreover, compartmentalization would bar parasitic RNA molecules from entering the system. Selection of a set of RNA molecules according to the quality of the self-replicating systems they generated could not therefore occur efficiently until some form of compartment evolved to contain them.

An early, crude form of compartmentalization may have been simple adsorption on surfaces or particles. The need for more sophisticated types of containment is easily fulfilled by a class of small molecules that has the simple physicochemical property of being *amphiphilic*, that is, consisting of one part that is hydrophobic (water insoluble) and another part that is hydrophilic (water soluble). When such molecules are placed in water, they aggregate, arranging their hydrophobic portions as much in contact with one another as possible and their hydrophilic portions in contact with the water. Amphiphilic molecules of appropriate shape aggregate spontaneously to form *bilayers*, creating small closed vesicles whose aqueous contents are isolated from the external medium (**Figure 6–108**). The phenomenon can be demonstrated in a test tube by simply mixing phospholipids and water together: under appropriate conditions, small vesicles will form. All present-day cells are surrounded by a *plasma membrane* consisting of amphiphilic molecules—mainly phospholipids—in this configuration; we discuss these molecules in detail in Chapter 10.

The spontaneous assembly of a set of amphiphilic molecules, enclosing a self-replicating mixture of RNAs (or pre-RNAs) and other molecules (**Figure**

Table 6–5 Some Biochemical Reactions That Can Be Catalyzed by Ribozymes

ACTIVITY	RIBOZYMES
Peptide bond formation in protein synthesis	ribosomal RNA
RNA cleavage, RNA ligation	self-splicing RNAs; RNase P; also *in vitro* selected RNA
DNA cleavage	self-splicing RNAs
RNA splicing	self-splicing RNAs, perhaps RNAs of the spliceosome
RNA polymerizaton	*in vitro* selected RNA
RNA and DNA phosphorylation	*in vitro* selected RNA
RNA aminoacylation	*in vitro* selected RNA
RNA alkylation	*in vitro* selected RNA
Amide bond formation	*in vitro* selected RNA
Glycosidic bond formation	*in vitro* selected RNA
Oxidation/reduction reactions	*in vitro* selected RNA
Carbon–carbon bond formation	*in vitro* selected RNA
Phosphoamide bond formation	*in vitro* selected RNA
Disulfide exchange	*in vitro* selected RNA

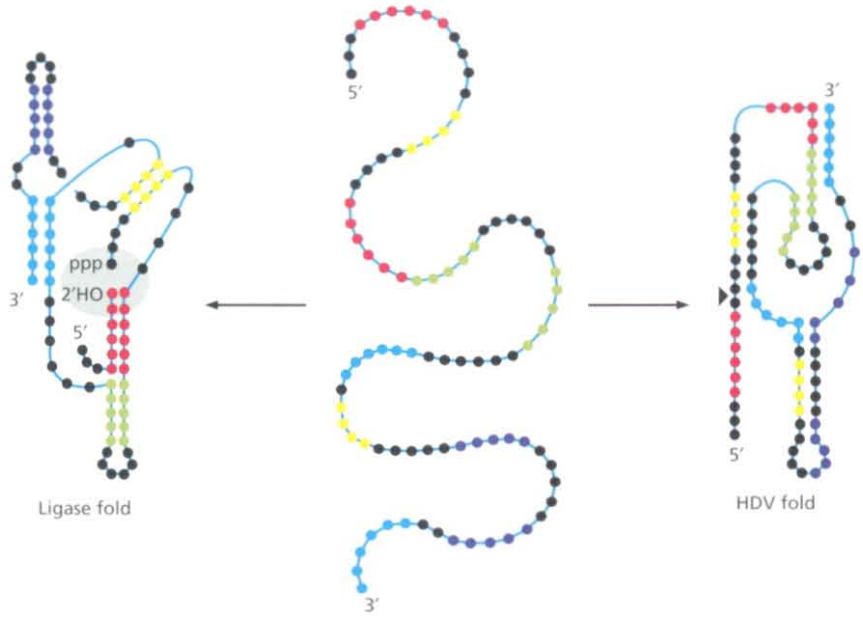


Figure 6–105 An RNA molecule that folds into two different ribozymes. This 88-nucleotide RNA, created in the laboratory, can fold into a ribozyme that carries out a self-ligation reaction *(left)* or a self-cleavage reaction *(right)*. The ligation reaction forms a 2′,5′ phosphodiester linkage with the release of pyrophosphate. This reaction seals the gap *(gray* shading), which was experimentally introduced into the RNA molecule. In the reaction carried out by the HDV fold, the RNA is cleaved at this same position, indicated by the *arrowhead*. This cleavage resembles that used in the life cycle of HDV, a hepatitis B satellite virus, hence the name of the fold. Each nucleotide is represented by a *colored dot*, with the colors used simply to clarify the two different folding patterns. The folded structures illustrate the secondary structures of the two ribozymes with regions of base-pairing indicated by close oppositions of the *colored dots*. Note that the two ribozyme folds have no secondary structure in common. (Adapted from E.A. Schultes and D.P. Bartel, *Science* 289:448–452, 2000. With permission from AAAS.)

6–109), presumably formed the first membrane-bounded cells. Although it is not clear at what point in the evolution of biological catalysts this might have occurred, once RNA molecules were sealed within a closed membrane they could begin to evolve in earnest as carriers of genetic instructions: new variants could be selected not merely on the basis of their own structure, but also according to their effect on the other molecules in the same compartment. The nucleotide sequences of the RNA molecules could now be expressed in the character of a unitary living cell.

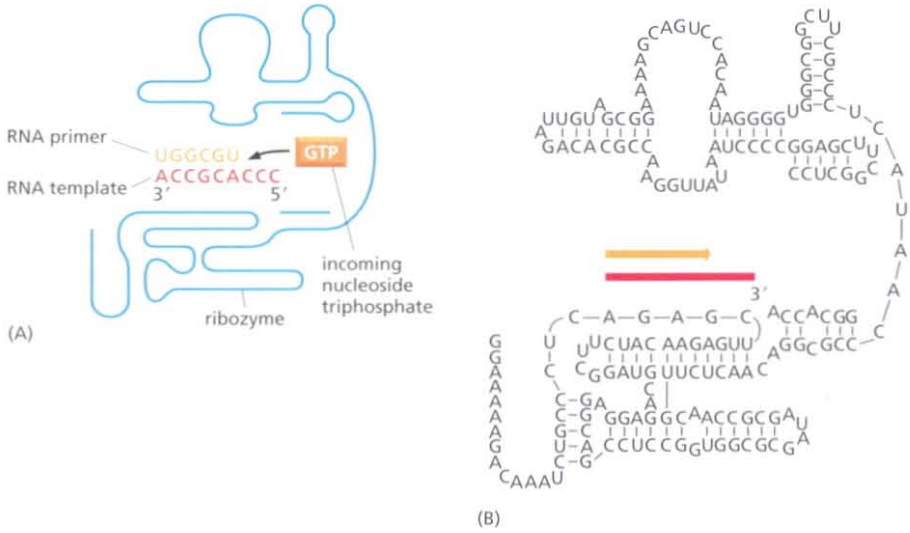


Figure 6–106 A ribozyme created in the laboratory that can catalyze templated synthesis of RNA from nucleoside triphosphates. (A) Schematic diagram of the ribozyme showing one step of the templated polymerization reaction it catalyzes. (B) Nucleotide sequence of the ribozyme with base pairings indicated. Although relatively inefficient (it can only synthesize short lengths of RNA), this ribozyme adds the correct base, as specified by the template, over 95% of the time. (From W.K. Johnston et al., *Science* 292:1319–1325, 2001. With permission from AAAS.)

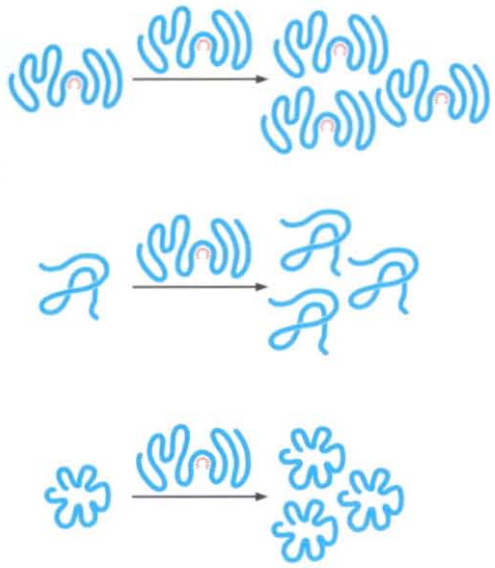


Figure 6–107 A family of mutually supportive RNA molecules. One molecule is a ribozyme that replicates itself as well as the other RNA molecules. The other molecules would catalyze secondary tasks needed for the survival of the cooperative system, for example, by synthesizing ribonucleotides for RNA synthesis or phospholipids for compartmentalization.

How Did Protein Synthesis Evolve?

The molecular processes underlying protein synthesis in present-day cells seem inextricably complex. Although we understand most of them, they do not make conceptual sense in the way that DNA transcription, DNA repair, and DNA replication do. It is especially difficult to imagine how protein synthesis evolved because it is now performed by a complex interlocking system of protein and RNA molecules; obviously the proteins could not have existed until an early version of the translation apparatus was already in place. The RNA world hypothesis is especially appealing because the use of RNA in both information and catalysis seems both economic and conceptually simple. As attractive as this idea is for envisioning early life, it does not explain how the modern-day system of protein synthesis arose. Although we can only speculate on the origins of modern protein synthesis and the genetic code, several experimental observations have provided plausible scenarios.

In modern cells, some short peptides (such as antibiotics) are synthesized without the ribosome; peptide synthetase enzymes assemble these peptides, with their proper sequence of amino acids, without mRNAs to guide their synthesis. It is plausible that this non-coded, primitive version of protein synthesis first developed during the RNA world where it would have been catalyzed by RNA molecules. This idea presents no conceptual difficulties because, as we have seen, rRNA catalyzes peptide bond formation in present-day cells. We also know that ribozymes created in the laboratory can perform specific aminoacylation reactions; that is, they can match specific amino acids to specific tRNAs. It is therefore possible that tRNA-like adapters, each matched to a specific amino acid, could have arisen in the RNA world, marking the beginnings of a genetic code.

In principle, other RNAs (the precursors to mRNAs) could have served as crude templates to direct the nonrandom polymerization of a few different amino acids. Any RNA that helped guide the synthesis of a useful polypeptide would have a great advantage in the evolutionary struggle for survival. We can envision a relatively nonspecific peptidyl transferase ribozyme, which, over time, grew larger and acquired the ability to position charged tRNAs accurately on RNA templates—leading eventually to the modern ribosome. Once protein

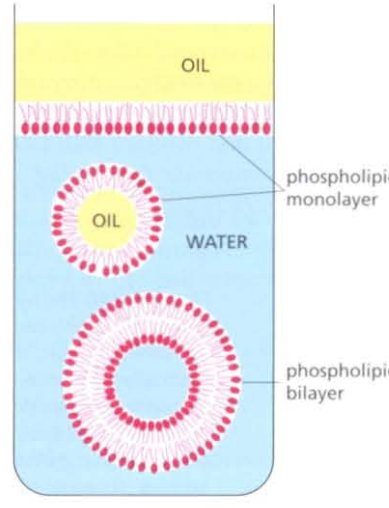


Figure 6–108 Formation of membrane by phospholipids. Because these molecules have hydrophilic heads and lipophilic tails, they align themselves at an oil/water interface with their heads in the water and their tails in the oil. In the water they associate to form closed bilayer vesicles in which the lipophilic tails are in contact with one another and the hydrophilic heads are exposed to the water.

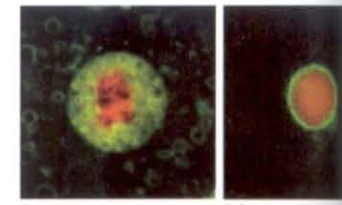


Figure 6–109 Encapsulation of RNA by simple amphiphilic molecules. For these experiments, the clay mineral montmorillonite was used to bring together RNA and fatty acids. (A) A montmorillonite particle, coated by RNA (*red*) has become trapped inside a fatty acid vesicle (*green*). (B) RNA *(red)* in solution has been encapsulated by fatty acids *(green)*. These experiments show that montmorillonite can greatly accelerate the spontaneous generation of vesicles from amphiphilic molecules and trap RNA inside them. It has been hypothesized that conceptually similar actions may have led to the first primitive cells on Earth. (From M.M. Hanczyc et al., *Science* 302:618–622, 2003. With permission from AAAS.)

synthesis evolved, the transition to a protein-dominated world could proceed, with proteins eventually taking over the majority of catalytic and structural tasks because of their greater versatility, with 20 rather than 4 different subunits. Although the scenarios just discussed are highly speculative, the known properties of RNA molecules are consistent with these ideas.

All Present-Day Cells Use DNA as Their Hereditary Material

If the evolutionary speculations embodied in the RNA world hypothesis are correct, early cells would have differed fundamentally from the cells we know today in having their hereditary information stored in RNA rather than in DNA (**Figure 6–110**). Evidence that RNA arose before DNA in evolution can be found in the chemical differences between them. Ribose, like glucose and other simple carbohydrates, can be formed from formaldehyde (HCHO), a simple chemical which is readily produced in laboratory experiments that attempt to simulate conditions on the primitive Earth. The sugar deoxyribose is harder to make, and in present-day cells it is produced from ribose in a reaction catalyzed by a protein enzyme, suggesting that ribose predates deoxyribose in cells. Presumably, DNA appeared on the scene later, but then proved more suitable than RNA as a permanent repository of genetic information. In particular, the deoxyribose in its sugar-phosphate backbone makes chains of DNA chemically more stable than chains of RNA, so that much greater lengths of DNA can be maintained without breakage.

12th

The other differences between RNA and DNA—the double-helical structure of DNA and the use of thymine rather than uracil—further enhance DNA stability by making the many unavoidable accidents that occur to the molecule much easier to repair, as discussed in detail in Chapter 5 (see pp. 296–297 and 300–301).

Summary

From our knowledge of present-day organisms and the molecules they contain, it seems likely that the development of the directly autocatalytic mechanisms fundamental to living systems began with the evolution of families of molecules that could catalyze their own replication. With time, a family of cooperating RNA catalysts probably developed the ability to direct the synthesis of polypeptides. DNA is likely to have been a late addition: as the accumulation of additional protein catalysts allowed more efficient and complex cells to evolve, the DNA double helix replaced RNA as a more stable molecule for storing the increased amounts of genetic information required by such cells.

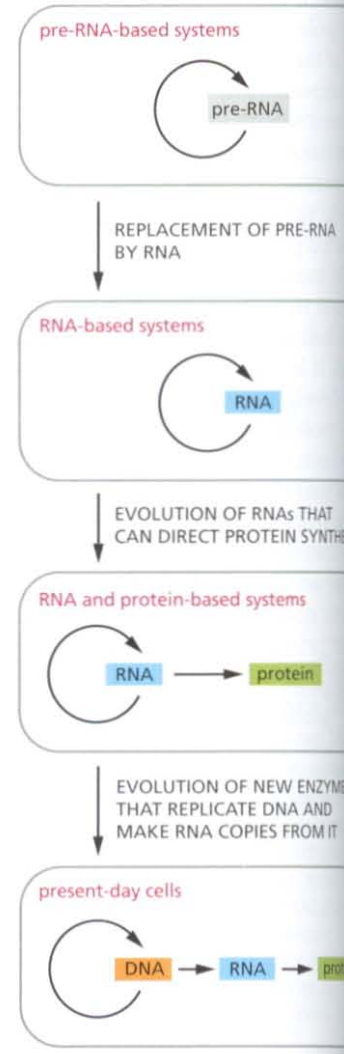


Figure 6–110 The hypothesis that RNA preceded DNA and proteins in evolution. In the earliest cells, pre-RNA molecules would have had combined genetic, structural, and catalytic functions and RNA would have gradually taken over these functions. In present-day cells, DNA is the repository of genetic information, and proteins perform the vast majority of catalytic functions in cells. RNA primarily functions today as a go-between in protein synthesis, although it remains a catalyst for a small number of crucial reactions.