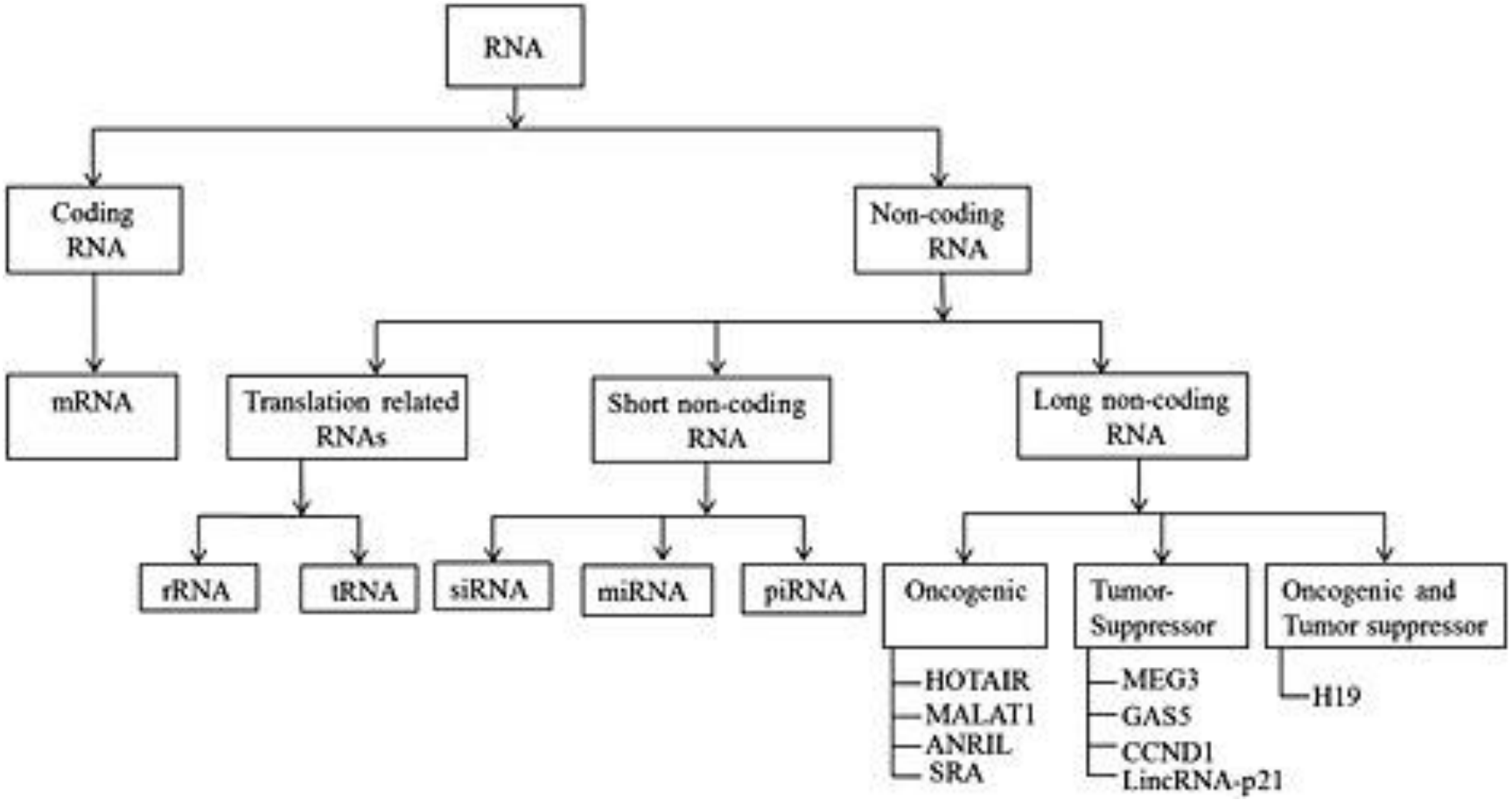
RNA structure, mRNA processing and Protein Synthesis

RNA

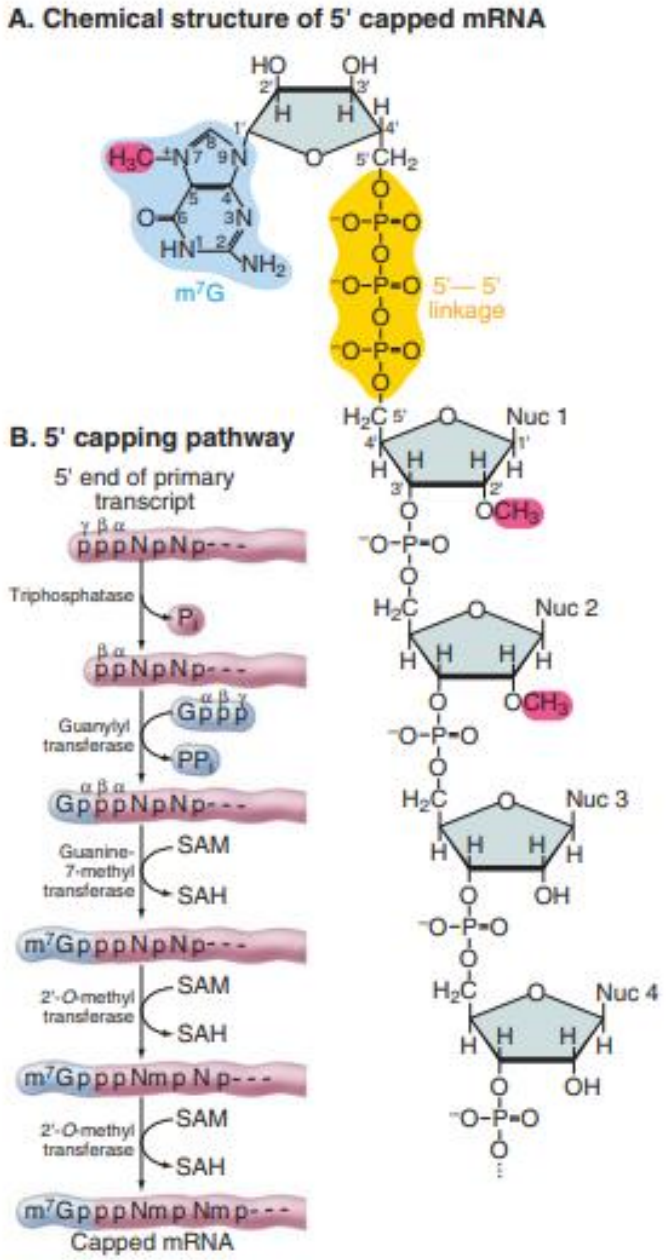
- **RNA**, abbreviation of **ribonucleic acid**, complex compound of high molecular weight that functions in cellular protein synthesis and replaces DNA (deoxyribonucleic acid) as a carrier of genetic codes in some viruses. RNA consists of ribose nucleotides (nitrogenous bases appended to a ribose sugar) attached by phosphodiester bonds, forming strands of varying lengths.
- The nitrogenous bases in RNA are adenine, guanine, cytosine, and uracil, which replaces thymine in DNA. The ribose sugar of RNA is a cyclical structure consisting of five carbons and one oxygen. The presence of a chemically reactive hydroxyl (−OH) group attached to the second carbon group in the ribose sugar molecule makes RNA prone to hydrolysis. This chemical lability of RNA, compared with DNA, which does not have a reactive −OH group in the same position on the sugar moiety (deoxyribose), is thought to be one reason why DNA evolved to be the preferred carrier of genetic information in most organisms.
- Different types of RNA exist in cells: messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). In addition, some RNAs are involved in regulating gene expression. Certain viruses use RNA as their genomic material.

RNA types


RNA
Coding RNA
Non-coding RNA
mRNA
Translation related RNAs
Short non-coding RNA
Long non-coding RNA
rRNA
tRNA
siRNA
miRNA
piRNA
Oncogenic
Tumor-Suppressor
Oncogenic and Tumor suppressor
HOTAIR
MALAT1
ANRIL
SRA
MEG3
GAS5
CCND1
LincRNA-p21
H19

Messenger RNA Processing: Capping and Polyadenylation

- Messenger RNA Capping and Polyadenylation Two distinguishing features set mRNA apart from other RNAs: a 5′ cap structure and a 3′ poly(A) tail. These elements help protect the mRNA against degradation and act synergistically to promote translation in the cytoplasm. The mRNA cap is an unusual structure. It consists of an inverted 7-methylguanosine residue, which is joined onto the body of the mRNA by a 5′-triphosphate–5′ linkage (Fig.).
- Cap addition involves three enzymatic activities: (a) a 5′ RNA triphosphatase cleaves the 5′ triphosphate on the nascent transcript to a diphosphate; (b) RNA guanylyltransferase forms a covalent enzyme–guanosine monophosphate (GMP) complex and then caps the RNA by transferring this to the diphosphate; and (c) RNA (guanine-7) methyltransferase covalently alters the guanosine base by methylation, generating m7 G. In addition, the first encoded nucleotides are frequently modified by methylation of the 2′ hydroxyl position on the ribose group, but the functional significance of these internal modifications is currently unclear.
- During 3′ end processing, the nascent pre-mRNA is cleaved by an endonuclease, and a tail of adenosine residues is added by poly(A) polymerase. Approximately 200 to 250 A residues are added to mRNAs in human cells, while 70 to 90 are added in yeast. Cleavage and polyadenylation are performed by a large complex containing approximately 20 proteins that recognizes sequences in the mRNA, of which the best defined is a highly conserved AAUAAA motif located upstream of the site of polyadenylation (Fig.).



MESSENGER RNAS HAVE A DISTINCTIVE 5′ CAP STRUCTURE. A, The 5′ ends of messenger RNAs (mRNAs) are blocked by an inverted guanosine residue that is attached to the body of the mRNA by a 5′–5′ triphosphate linkage. The N7 position of the guanosine is methylated (red). The first encoded nucleotide of the mRNA (Nuc 1) is also methylated on the 2′-hydroxyl of the ribose ring. The second nucleotide (Nuc 2) may also be methylated. B, Capping of mRNAs is a multistep process

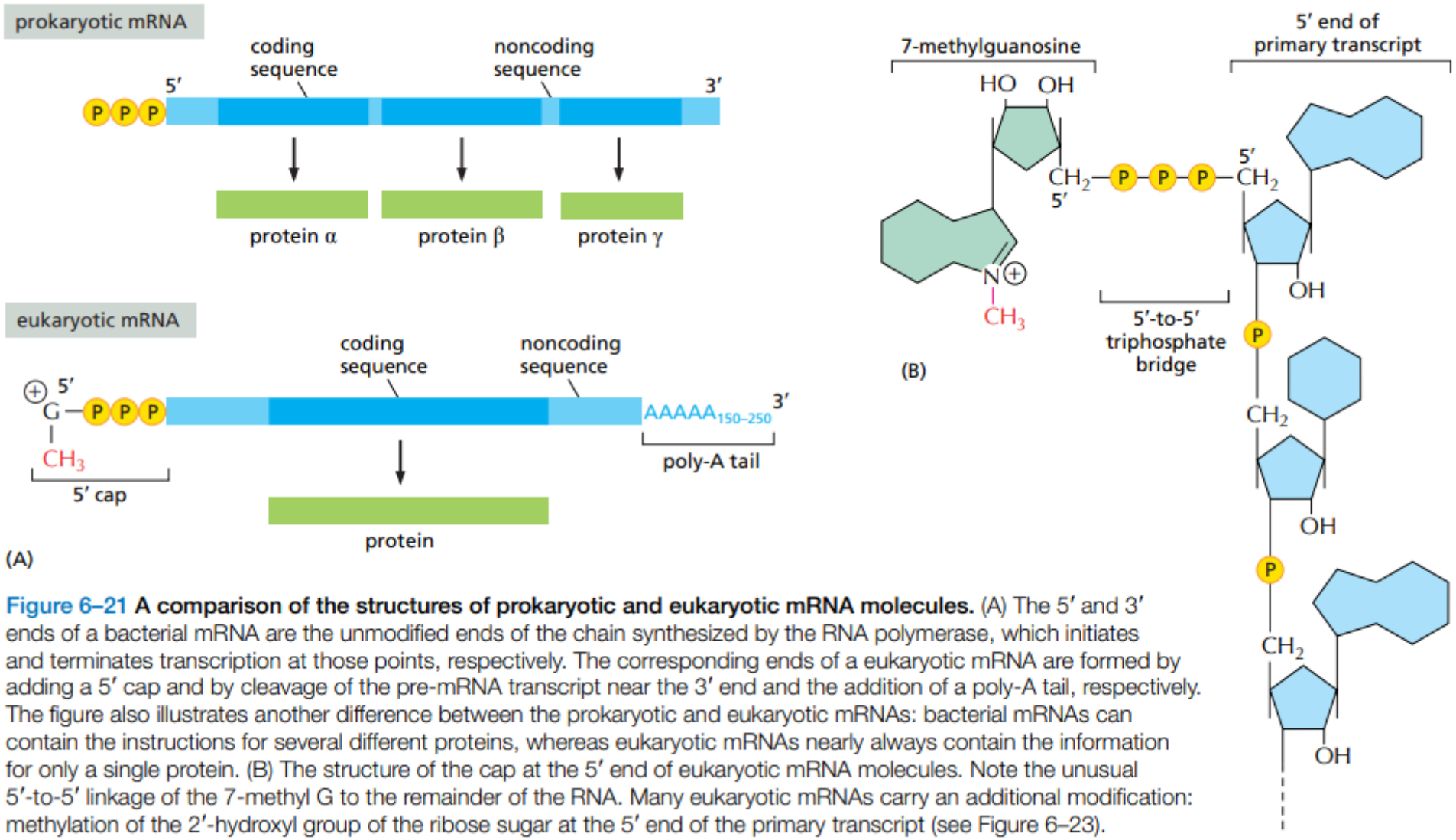
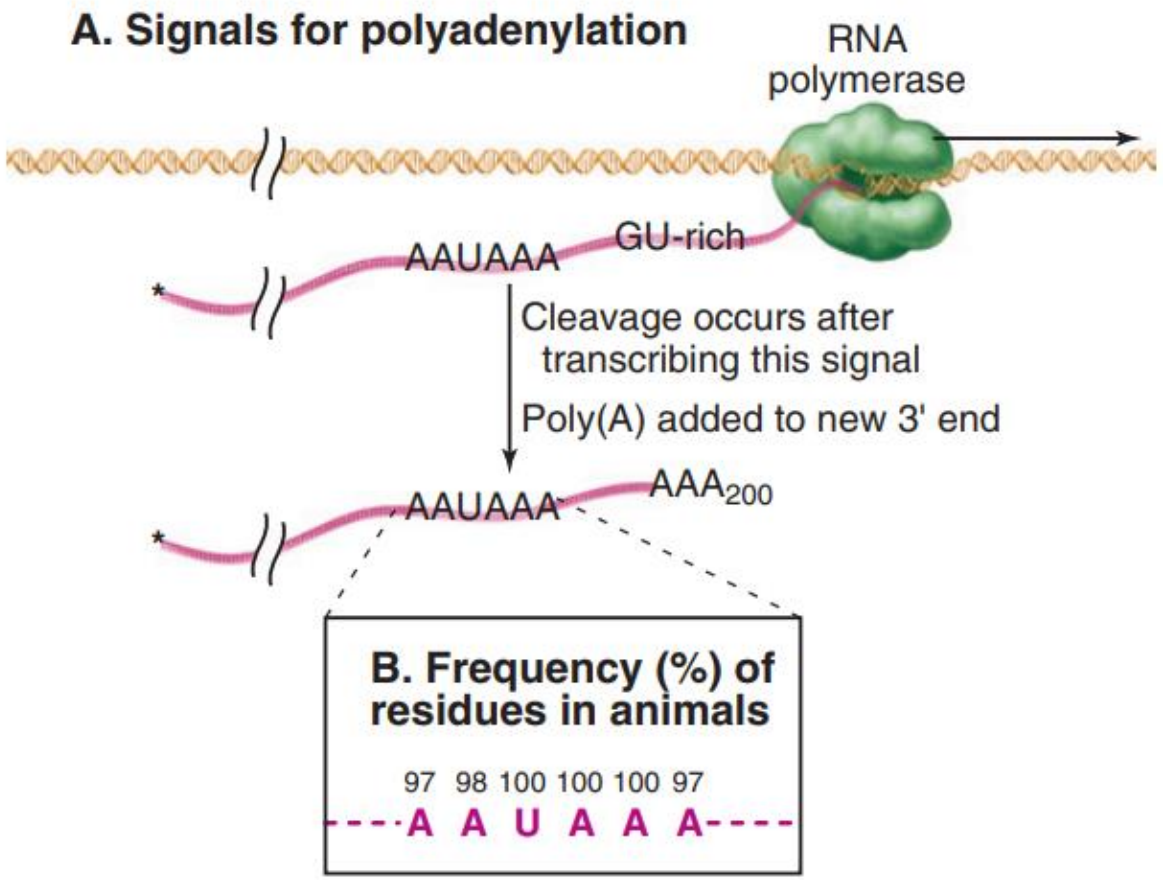
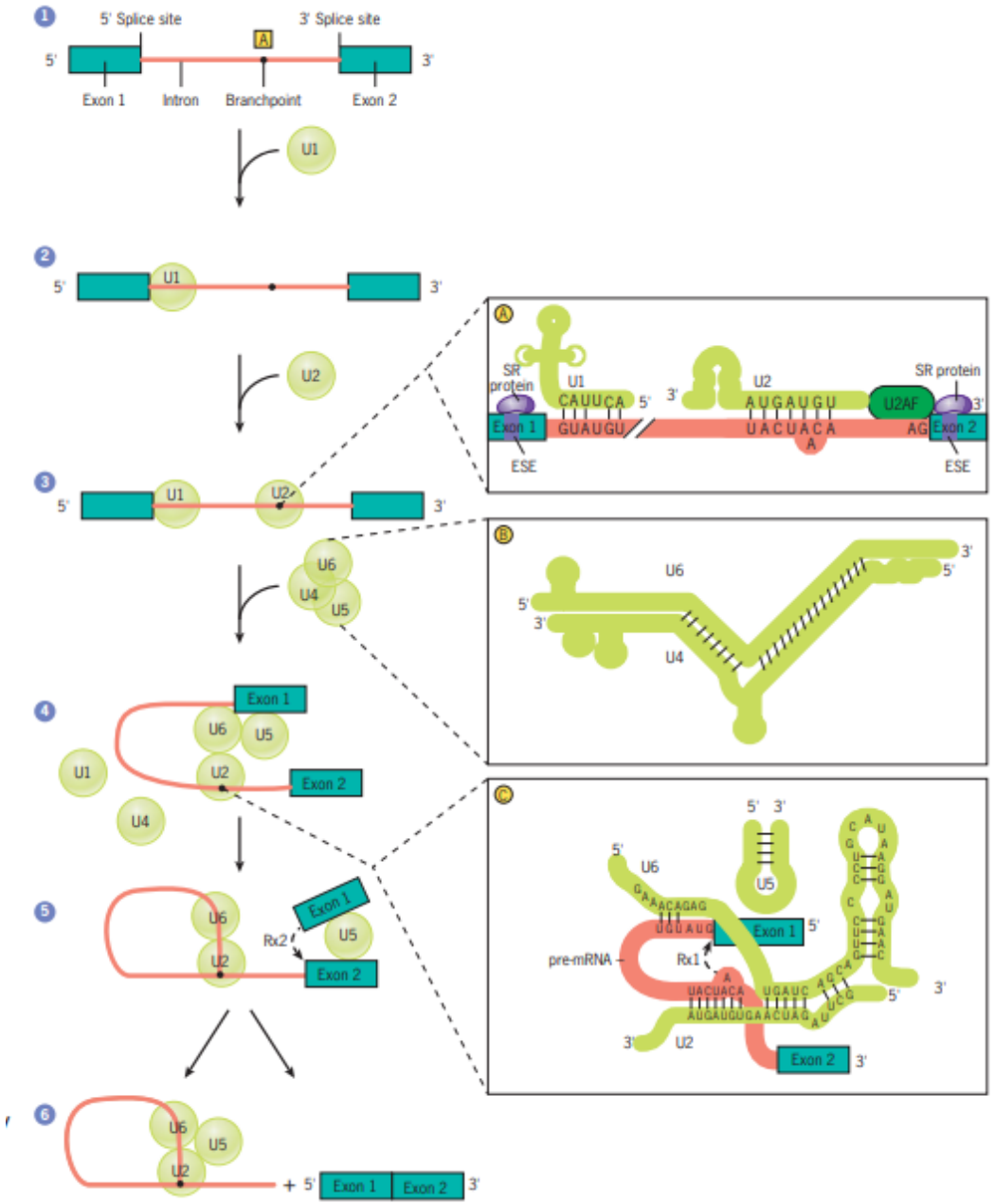


Figure 6–21 A comparison of the structures of prokaryotic and eukaryotic mRNA molecules. (A) The 5′ and 3′ ends of a bacterial mRNA are the unmodified ends of the chain synthesized by the RNA polymerase, which initiates and terminates transcription at those points, respectively. The corresponding ends of a eukaryotic mRNA are formed by adding a 5′ cap and by cleavage of the pre-mRNA transcript near the 3′ end and the addition of a poly-A tail, respectively. The figure also illustrates another difference between the prokaryotic and eukaryotic mRNAs: bacterial mRNAs can contain the instructions for several different proteins, whereas eukaryotic mRNAs nearly always contain the information for only a single protein. (B) The structure of the cap at the 5′ end of eukaryotic mRNA molecules. Note the unusual 5′-to-5′ linkage of the 7-methyl G to the remainder of the RNA. Many eukaryotic mRNAs carry an additional modification: methylation of the 2′-hydroxyl group of the ribose sugar at the 5′ end of the primary transcript (see Figure 6–23).



SIGNALS FOR PRE–MESSENGER RNA POLYADENYLATION. A, Poly(A) tails are added to pre–messenger RNAs (mRNAs) following transcription. After pol II transcribes the protein coding region of the mRNA, it encounters two sequence elements: AAUAAA and a GU-rich element. These act as signals for the assembly of a large 3′ processing complex that cleaves the nascent pre-mRNA, releasing it from the transcription complex, and adds a tail of up to 200 adenosine residues. B, The poly(A) signal is highly conserved in vertebrates.



Schematic model of the assembly of the splicing machinery and some of the steps that occur during pre-mRNA splicing. Step 1 shows the portion of the pre-mRNA to be spliced. In step 2, the first of the splicing components, U1 snRNP, has become attached at the 5 ′ splice site of the intron. The nucleotide sequence of U1 snRNA is complementary to the 5 ′ splice site of the pre-mRNA, and evidence indicates that U1 snRNP initially binds to the 5 ′ side of the intron by the formation of specific base pairs between the splice site and U1 snRNA (see inset A). The U2 snRNP is next to enter the splicing complex, binding to the pre-mRNA (as shown in inset A) in a way that causes a specific adenosine residue (dot) to bulge out of the surrounding helix (step 3). This is the site that later becomes the branch point of the lariat. U2 is thought to be recruited by the protein U2AF, which binds to the polypyrimidine tract near the 3 ′ splice site. U2AF also interacts with SR proteins that bind to the exonic splicing enhancers (ESEs). These interactions play an important role in recognizing intron/exon borders. The next step is the binding of the U4/U6 and U5 snRNPs to the pre-mRNA with the accompanying displacement of U1 (step 4). The assembly of a spliceosome involves a series of dynamic interactions between the pre-mRNA and specific snRNAs and among the snRNAs themselves. As they enter the complex with the pre-mRNA, the U4 and U6 snRNAs are extensively base-paired to one another (inset B). The U4 snRNA is subsequently stripped away from the duplex, and the regions of U6 that were paired with U4 become base-paired to a portion of the U2 snRNA (inset C). Another portion of the U6 snRNA is situated at the 5 ′ splice site (inset C), having displaced the U1 snRNA that was previously bound there (inset A). It is proposed that U6 is a ribozyme and that U4 is an inhibitor of its catalytic activity. According to this hypothesis, once the U1 and U4 snRNA have been displaced, the U6 snRNA is in position to catalyze the two chemical reactions required for intron removal. According to an alternate view, the reactions are catalyzed by the combined activity of U6 snRNA and a protein of the U5 snRNP. Regardless of the mechanism, the first reaction (indicated by the arrow in inset C) results in the cleavage of the 5 ′ splice site, forming a free 5 ′ exon and a lariat intron–3 ′ exon intermediate (step 5). The free 5 ′ exon is thought to be held in place by its association with the U5 snRNA of the spliceosome, which also interacts with the 3 ′ exon (step 5). The first cleavage reaction at the 5 ′ splice site is followed by a second cleavage reaction at the 3 ′ splice site (arrow, step 5), which excises the lariat intron and simultaneously joins the ends of the two neighboring exons (step 6). Following splicing, the snRNPs must be released from the pre-mRNA, the original associations between snRNAs must be restored,and the snRNPs must be reassembled at the sites of other introns.

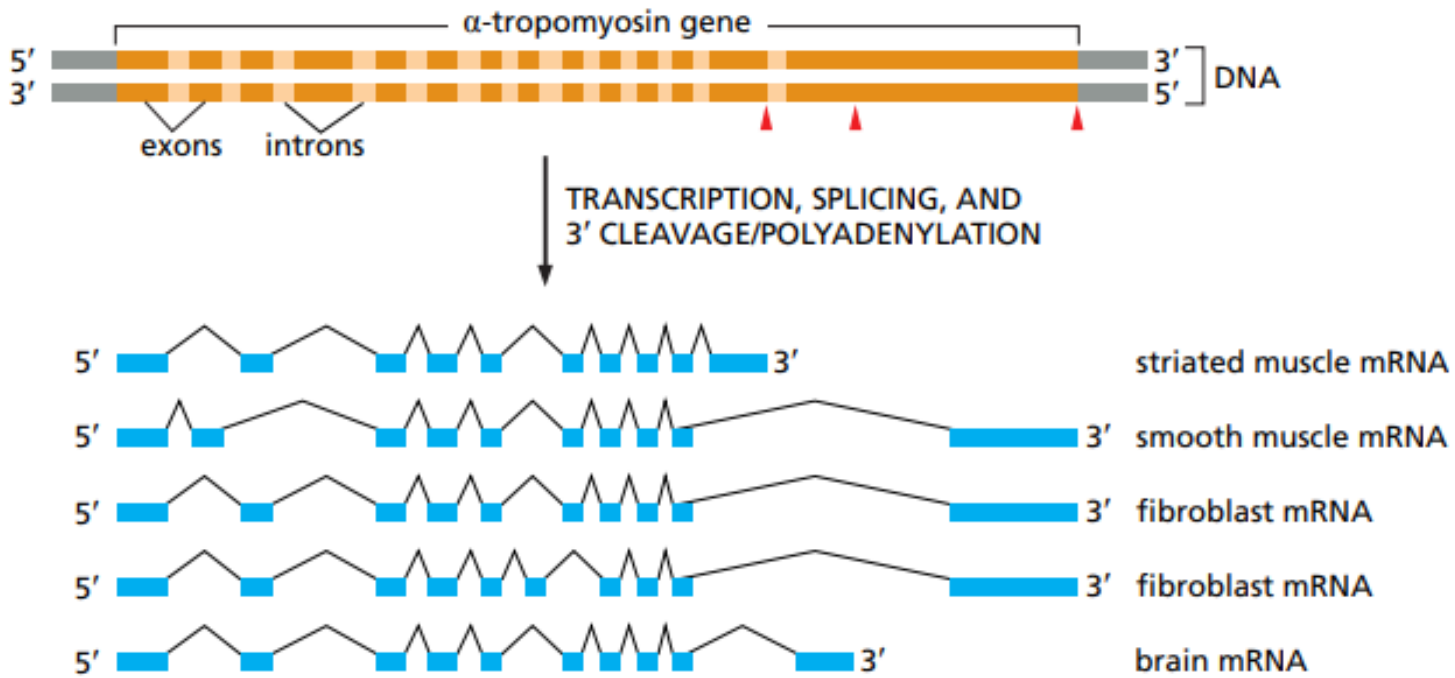


Figure 6–26 Alternative splicing of the α-tropomyosin gene from rat. α-Tropomyosin is a coiled-coil protein (see Figure 3–9) that carries out several tasks, most notably the regulation of contraction in muscle cells. The primary transcript can be spliced in different ways, as indicated in the figure, to produce distinct mRNAs, which then give rise to variant proteins. Some of the splicing patterns are specific for certain types of cells. For example, the α-tropomyosin made in striated muscle is different from that made from the same gene in smooth muscle. The arrowheads in the top part of the figure mark the sites where cleavage and poly-A addition form the 3′ ends of the mature mRNAs.

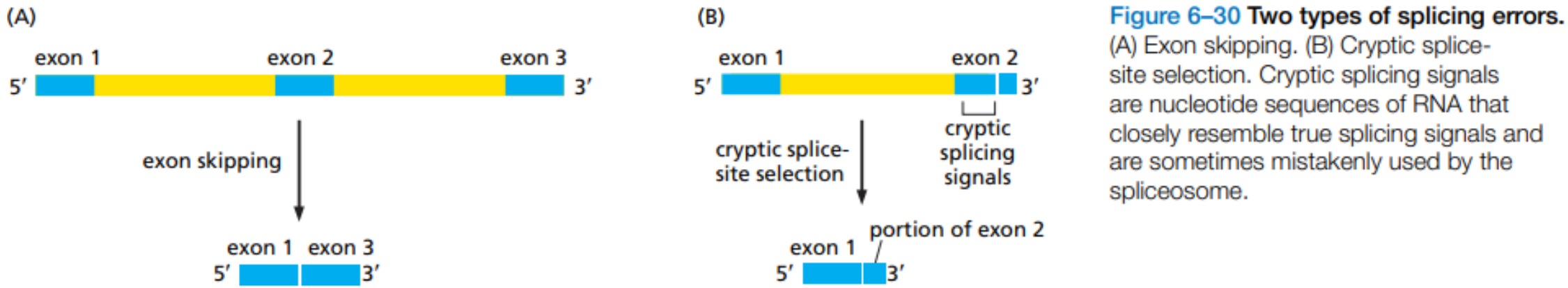
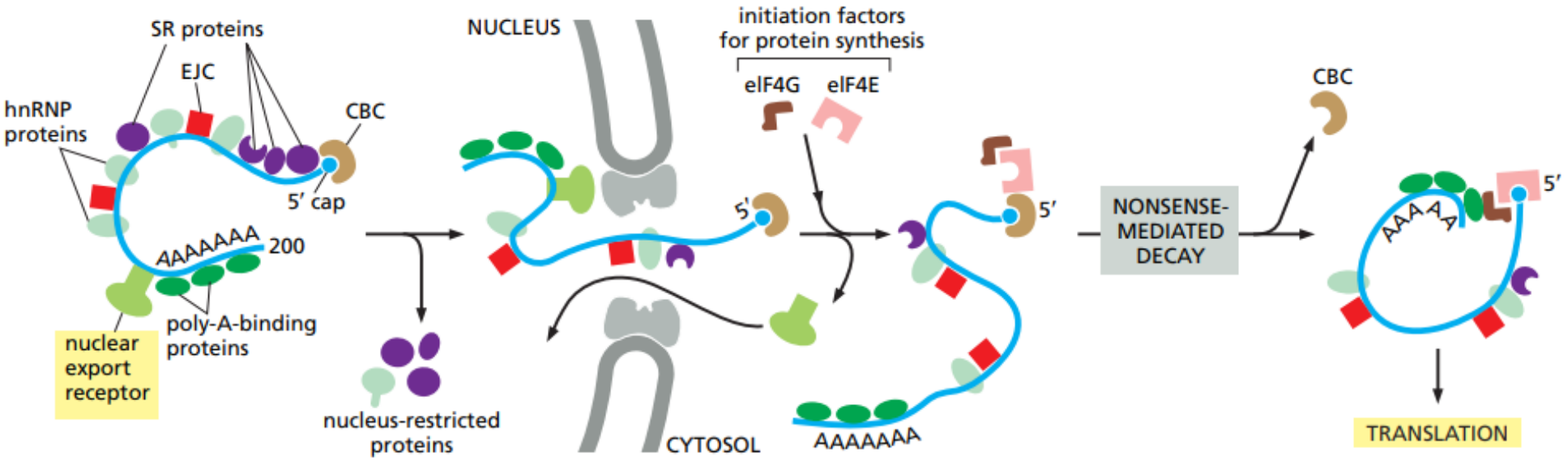
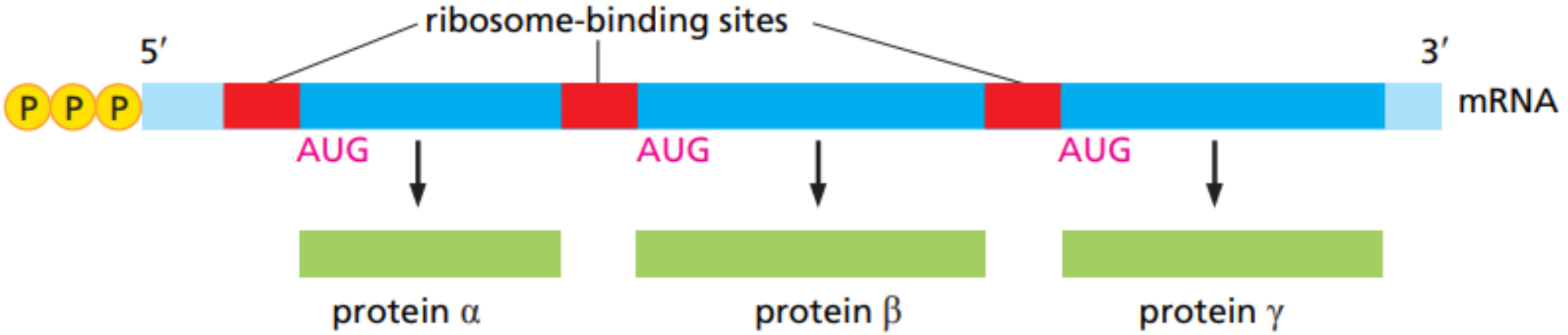


Figure 6–30 Two types of splicing errors. (A) Exon skipping. (B) Cryptic splice-site selection. Cryptic splicing signals are nucleotide sequences of RNA that closely resemble true splicing signals and are sometimes mistakenly used by the spliceosome.



Schematic illustration of an export-ready mRNA molecule and its transport through the nuclear pore. As indicated, some proteins travel with the mRNA as it moves through the pore, whereas others remain in the nucleus. The nuclear export receptor for mRNAs is a complex of proteins that binds to an mRNA molecule once it has been correctly spliced and polyadenylated. After the mRNA has been exported to the cytosol, this export receptor dissociates from the mRNA and is re-imported into the nucleus, where it can be used again. The final check indicated here, called nonsense-mediated decay, will be described later in the chapter.

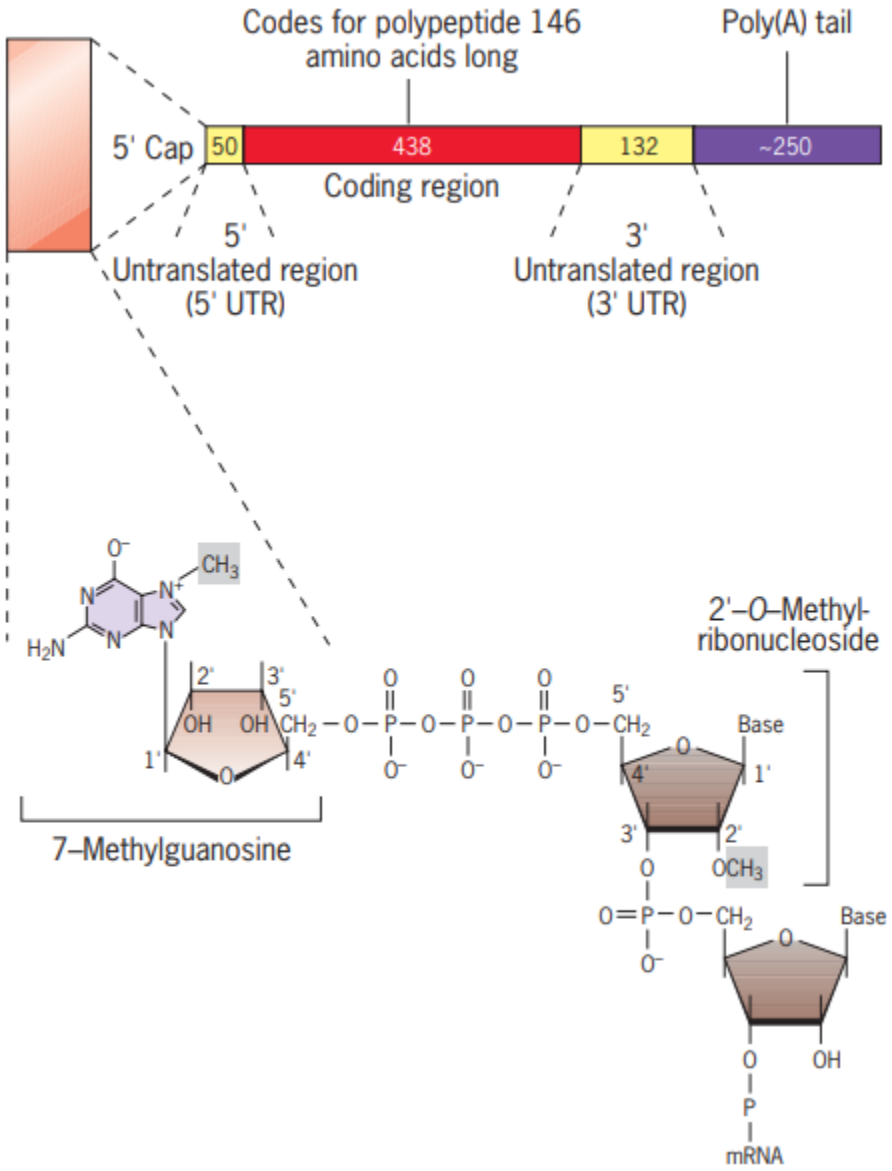


Structure of a typical bacterial mRNA molecule. Unlike eukaryotic ribosomes, which typically require a capped 5′ end on the mRNA, prokaryotic ribosomes initiate translation at ribosome-binding sites (Shine–Dalgarno sequences), which can be located anywhere along an mRNA molecule. This property of their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

Structure of eukaryotic mRNA

The Structure of mRNAs Messenger RNAs share certain properties:

- 1. They contain a continuous sequence of nucleotides encoding a specific polypeptide.
- 2. They are found in the cytoplasm.
- 3. They are attached to ribosomes when they are translated.
- 4. Most mRNAs contain a significant noncoding segment, that is, a portion that does not direct the assembly of amino acids. For example, approximately 25 percent of each globin mRNA consists of noncoding, non translated regions (Figure). Noncoding portions are found at both the 5′ and 3′ ends of a messenger RNA and contain sequences that have important regulatory roles.
- 5. Eukaryotic mRNAs have special modifications at their 5′ and 3′ termini that are not found on either bacterial mRNAs or on tRNAs or rRNAs. The 3′ end of nearly all eukaryotic mRNAs has a string of 50 to 250 adenosine residues that form a poly(A) tail, whereas the 5′ end has a methylated guanosine cap (Figure).



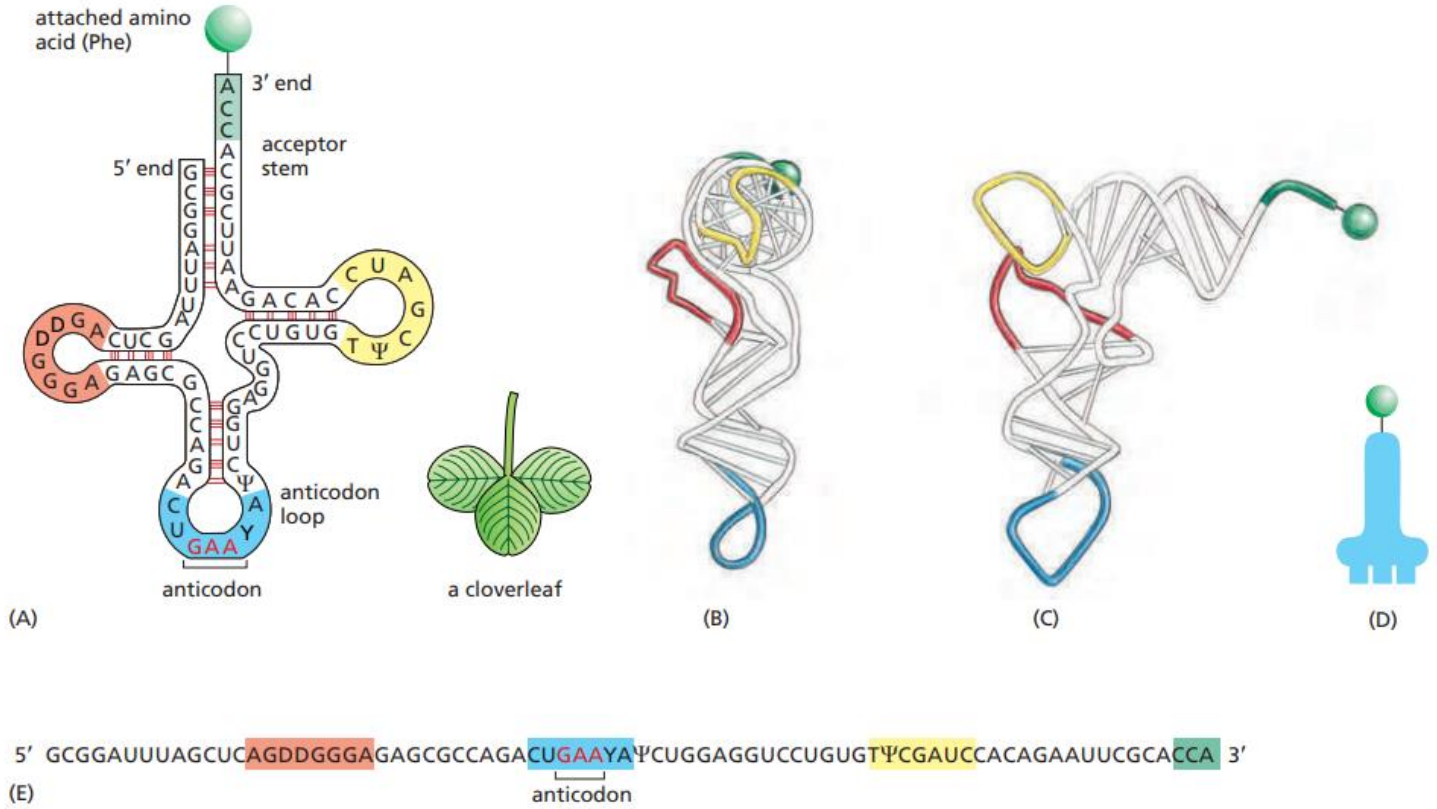
Structure of the human β-globin mRNA. The mRNA contains a 5 ′ methylguanosine cap, a 5 ′ and 3 ′ noncoding region that flanks the coding segment, and a 3 ′ poly(A) tail. The lengths of each segment are given in numbers of nucleotides. The length of the poly(A) tail is variable. It typically begins at a length of about 250 nucleotides and is gradually reduced in length, as shown in Figure. The structure of the 5 ′ cap is shown.

Transfer RNAs

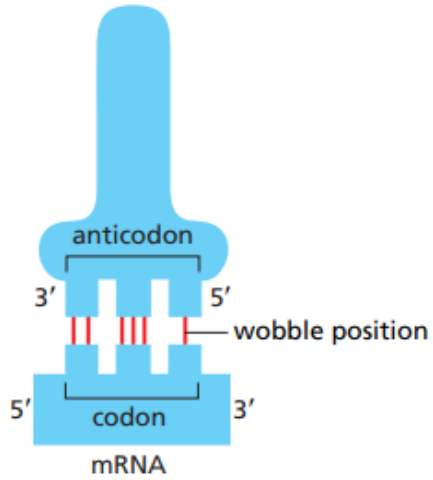
- Transfer RNAs (tRNAs) are structural RNA molecules and, depending on the species, many different types of tRNAs exist in the cytoplasm. Bacterial species typically have between 60 and 90 types. Serving as adaptors, each **tRNA** type binds to a specific codon on the mRNA template and adds the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually "translate" the language of RNA into the language of proteins. As the adaptor molecules of translation, it is surprising that tRNAs can fit so much specificity into such a small package. The tRNA molecule interacts with three factors: aminoacyl tRNA synthetases, ribosomes, and mRNA.

  Mature tRNAs take on a three-dimensional structure when complementary bases exposed in the single-stranded RNA molecule hydrogen bond with each other (Figure 3). This shape positions the amino-acid binding site, called the **CCA amino acid binding end**, which is a cytosine-cytosine-adenine sequence at the 3′ end of the tRNA, and the **anticodon** at the other end. The anticodon is a three-nucleotide sequence that bonds with an mRNA codon through complementary base pairing.

  An amino acid is added to the end of a tRNA molecule through the process of tRNA "charging," during which each tRNA molecule is linked to its correct or **cognate amino acid** by a group of enzymes called **aminoacyl tRNA synthetases**. At least one type of aminoacyl tRNA synthetase exists for each of the 20 amino acids. During this process, the amino acid is first activated by the addition of adenosine monophosphate (AMP) and then transferred to the tRNA, making it a **charged tRNA**, and AMP is released.



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 6–41) and D (dihydrouridine—see Figure 6–53) 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. (D) The tRNA icon we use in this book. (E) The linear nucleotide sequence of the molecule, color-coded to match (A), (B), and (C).



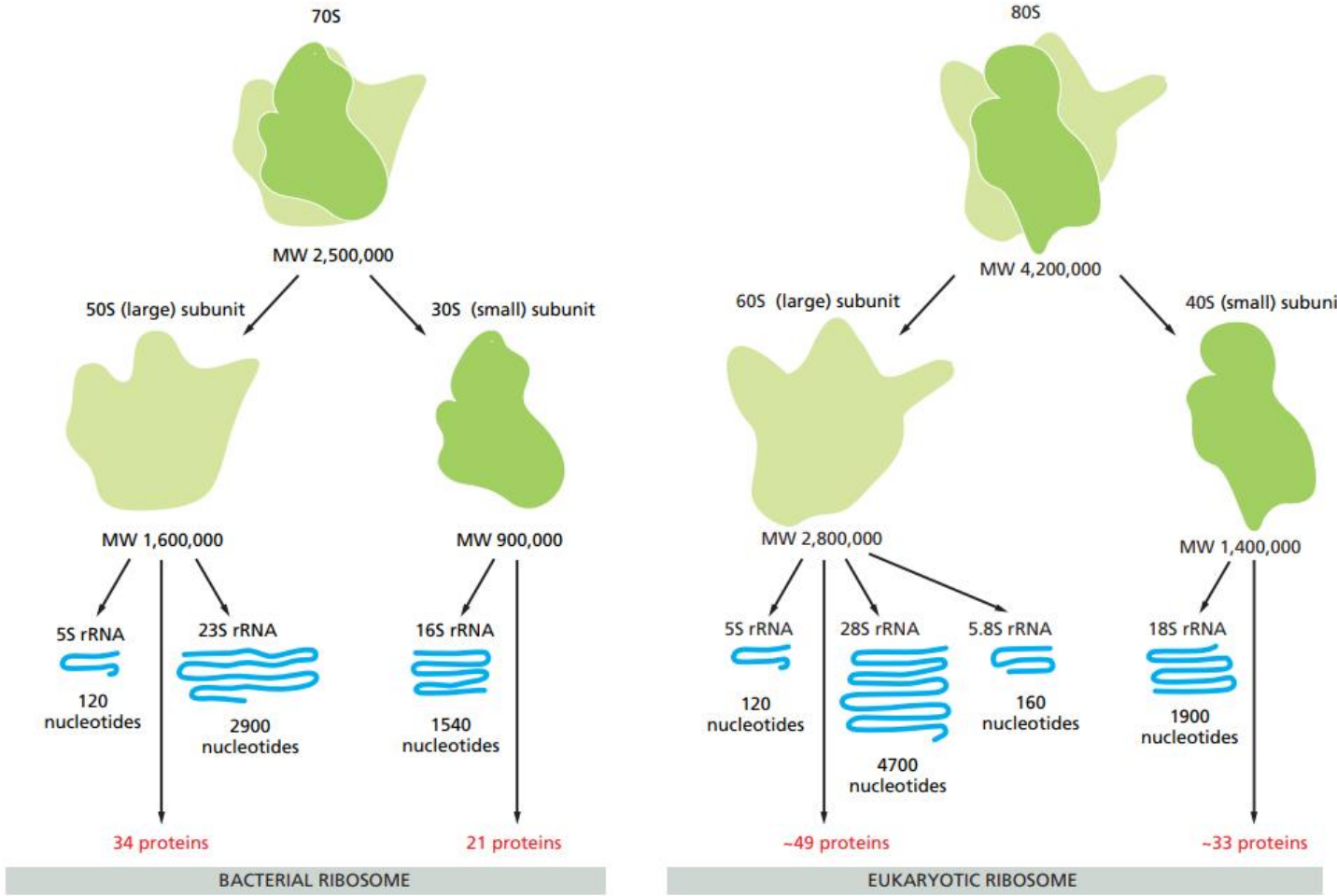
bacteria

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

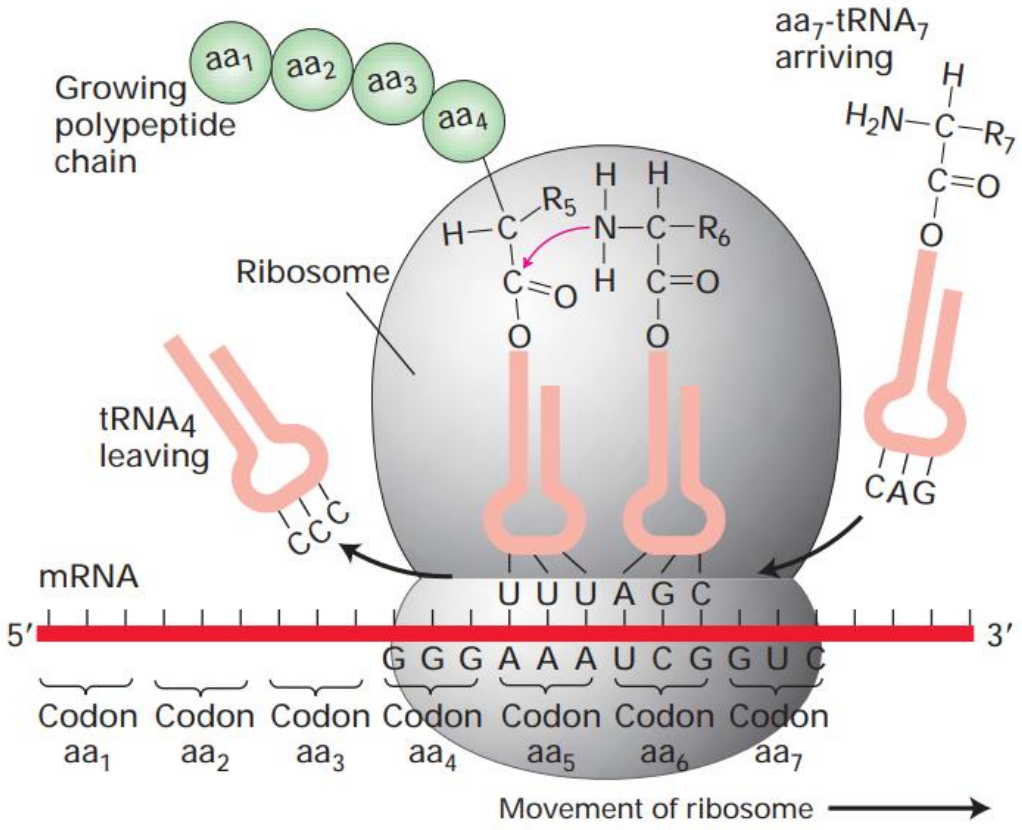
eukaryotes

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

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 eukaryotes. The inosine in tRNAs is formed from the deamination of adenosine (see Figure 6–53), 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. Codon–anticodon base-pairing is more stringent at positions 1 and 2 of the codon, where only conventional base pairs are permitted. The differences in wobble base-pairing interactions between bacteria and eukaryotes presumably result from subtle structural differences between bacterial and eukaryotic 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



A comparison of bacterial and eukaryotic ribosomes. Despite differences in the number and size of their rRNA and protein components, both bacterial and eukaryotic ribosomes have nearly the same structure and they function similarly. Although the 18S and 28S rRNAs of the eukaryotic 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 the rRNA largely unchanged.

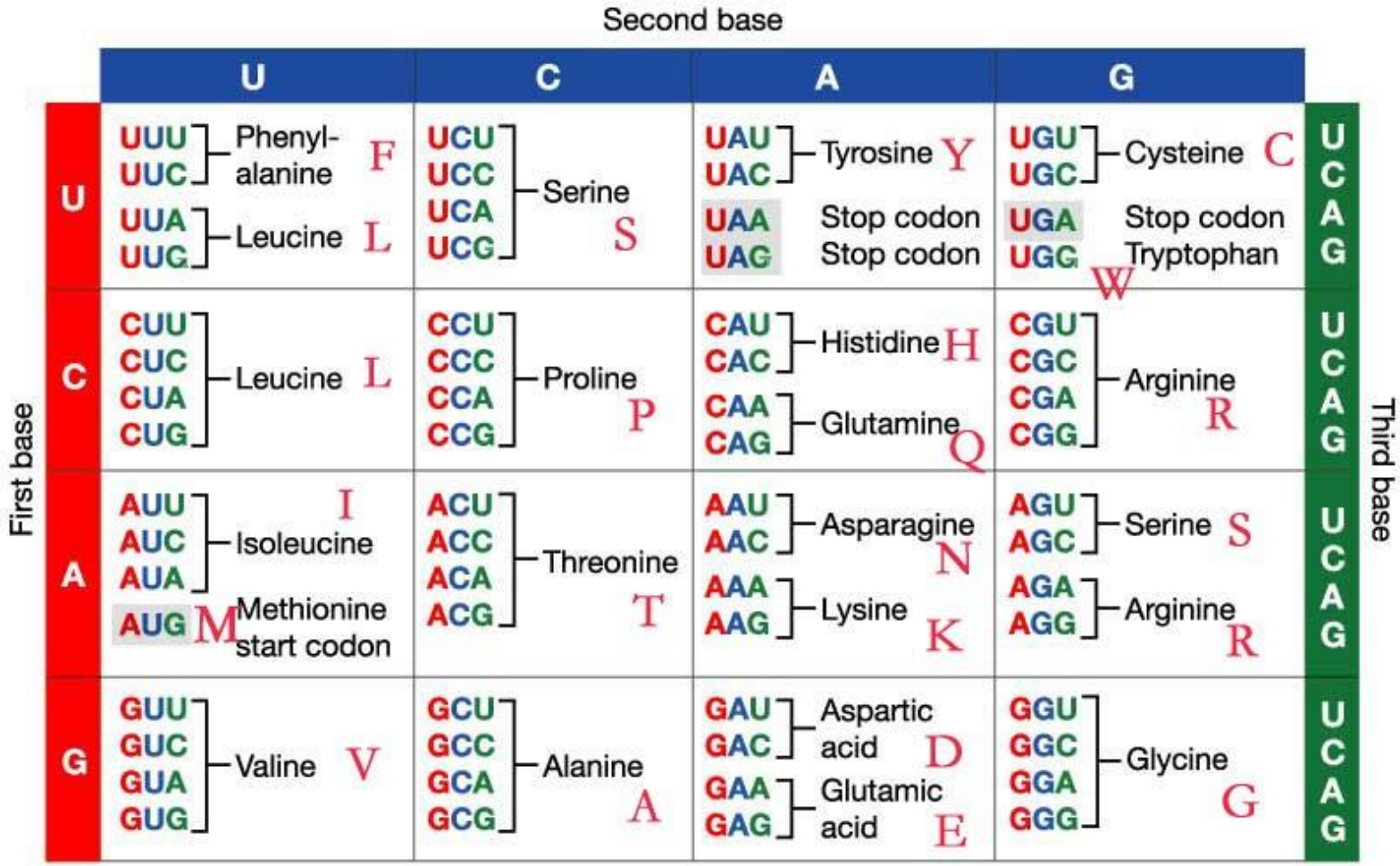


The three roles of RNA in protein synthesis. Messenger RNA (mRNA) is translated into protein by the joint action of transfer RNA (tRNA) and the ribosome, which is composed of numerous proteins and two major ribosomal RNA (rRNA) molecules (not shown). Note the base pairing between tRNA anticodons and complementary codons in the mRNA. Formation of a peptide bond between the amino group N on the incoming aa-tRNA and the carboxyl-terminal C on the growing protein chain (purple) is catalyzed by one of the rRNAs. aa amino acid; R side group. [Adapted from A. J. F. Griffiths et al., 1999, Modern Genetic Analysis, W. H. Freeman and Company.]

Genetic code

- **genetic code**, the sequence of nucleotides in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that determines the amino acid sequence of proteins. Though the linear sequence of nucleotides in DNA contains the information for protein sequences, proteins are not made directly from DNA. Instead, a messenger RNA (mRNA) molecule is synthesized from the DNA and directs the formation of the protein. RNA is composed of four nucleotides: adenine (A), guanine (G), cytosine (C), and uracil (U). Three adjacent nucleotides constitute a unit known as the codon, which codes for an amino acid. For example, the sequence AUG is a codon that specifies the amino acid methionine. There are 64 possible codons, three of which do not code for amino acids but indicate the end of a protein. The remaining 61 codons specify the 20 amino acids that make up proteins. The AUG codon, in addition to coding for methionine, is found at the beginning of every mRNA and indicates the start of a protein. Methionine and tryptophan are the only two amino acids that are coded for by just a single codon (AUG and UGG, respectively). The other 18 amino acids are coded for by two to six codons. Because most of the 20 amino acids are coded for by more than one codon, the code is called degenerate.
- The genetic code, once thought to be identical in all forms of life, has been found to diverge slightly in certain organisms and in the mitochondria of some eukaryotes. Nevertheless, these differences are rare, and the genetic code is identical in almost all species, with the same codons specifying the same amino acids. The deciphering of the genetic code was accomplished by American biochemists Marshall W. Nirenberg, Robert W. Holley, and Har Gobind Khorana in the early 1960s.

Triplate Codon



Known Deviations from the Universal Genetic Code

Codon	Universal Code	Unusual Code*	Occurrence
UGA	Stop	Trp	*Mycoplasma, Spiroplasma,* mitochondria of many species
CUG	Leu	Thr	Mitochondria in yeasts
UAA, UAG	Stop	Gln	*Acetabularia, Tetrahymena,* Paramecium, etc.
UGA	Stop	Cys	*Euplotes*

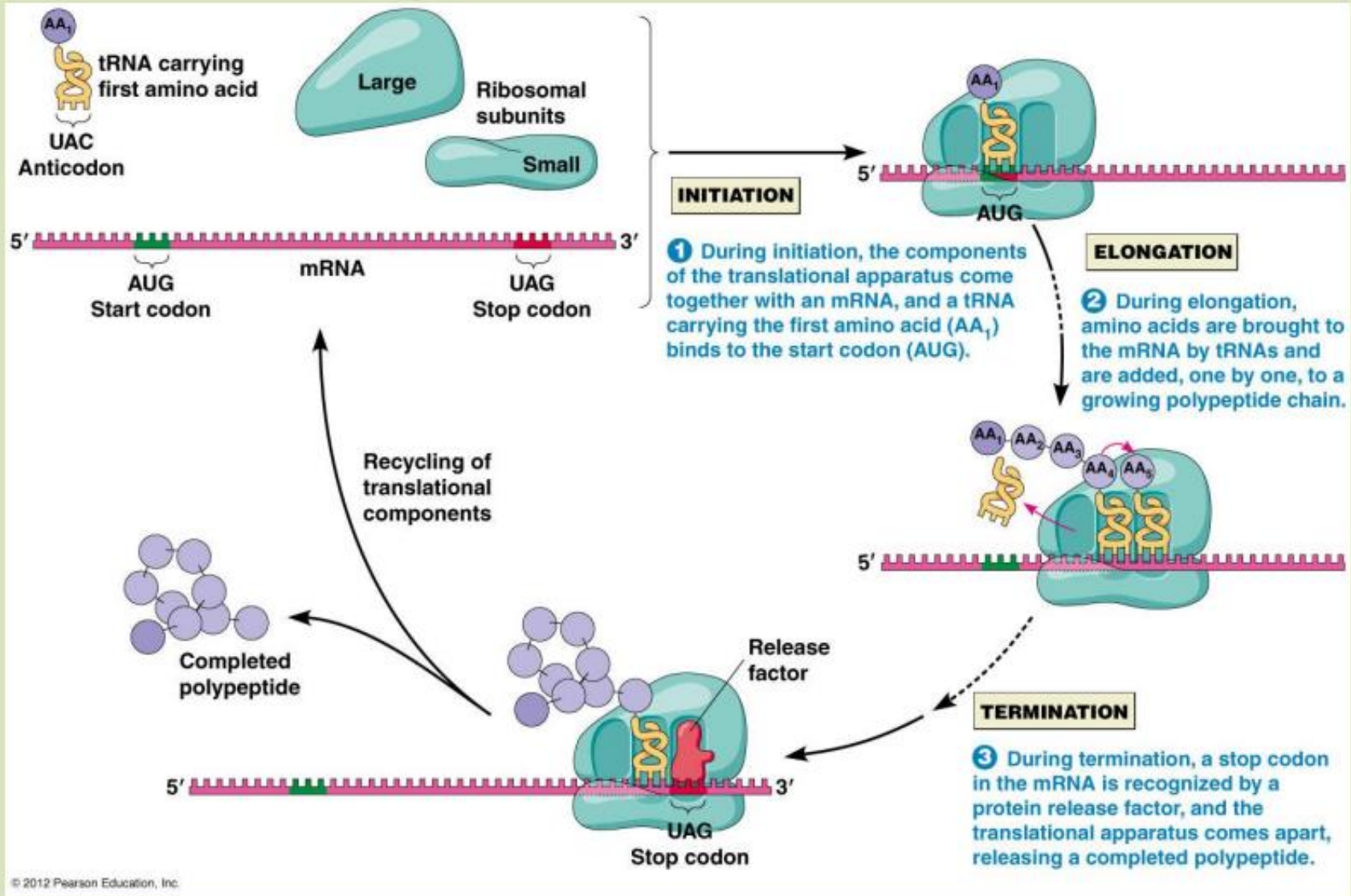
*"Unusual code" is used in nuclear genes of the listed organisms and in mitochondrial genes as indicated.
SOURCE: S. Osawa et al., 1992, *Microbiol. Rev.* **56**:229.

Protein synthesis

- Within all cells, the translation machinery resides within a specialized organelle called the ribosome. In eukaryotes, mature mRNA molecules must leave the nucleus and travel to the cytoplasm, where the ribosomes are located. On the other hand, in prokaryotic organisms, ribosomes can attach to mRNA while it is still being transcribed. In this situation, translation begins at the 5' end of the mRNA while the 3' end is still attached to DNA.

- In prokaryotic cells, the ribosome is composed of two subunits: the large (50S) subunit and the small (30S) subunit, and 60S and 40S subunits in eukaryotic cells(S, for svedberg unit, is a measure of sedimentation velocity and, therefore, mass). Each subunit exists separately in the cytoplasm, but the two join together on the mRNA molecule. The ribosomal subunits contain proteins and specialized RNA molecules—specifically, ribosomal RNA (rRNA) and transfer RNA (tRNA).The tRNA molecules are adaptor molecules—they have one end that can read the triplet code in the mRNA through complementary base-pairing, and another end that attaches to a specific amino acid. Within the ribosome, the mRNA and aminoacyl-tRNA complexes are held together closely, which facilitates base-pairing. The rRNA catalyzes the attachment of each new amino acid to the growing chain.

Protein Synthesis



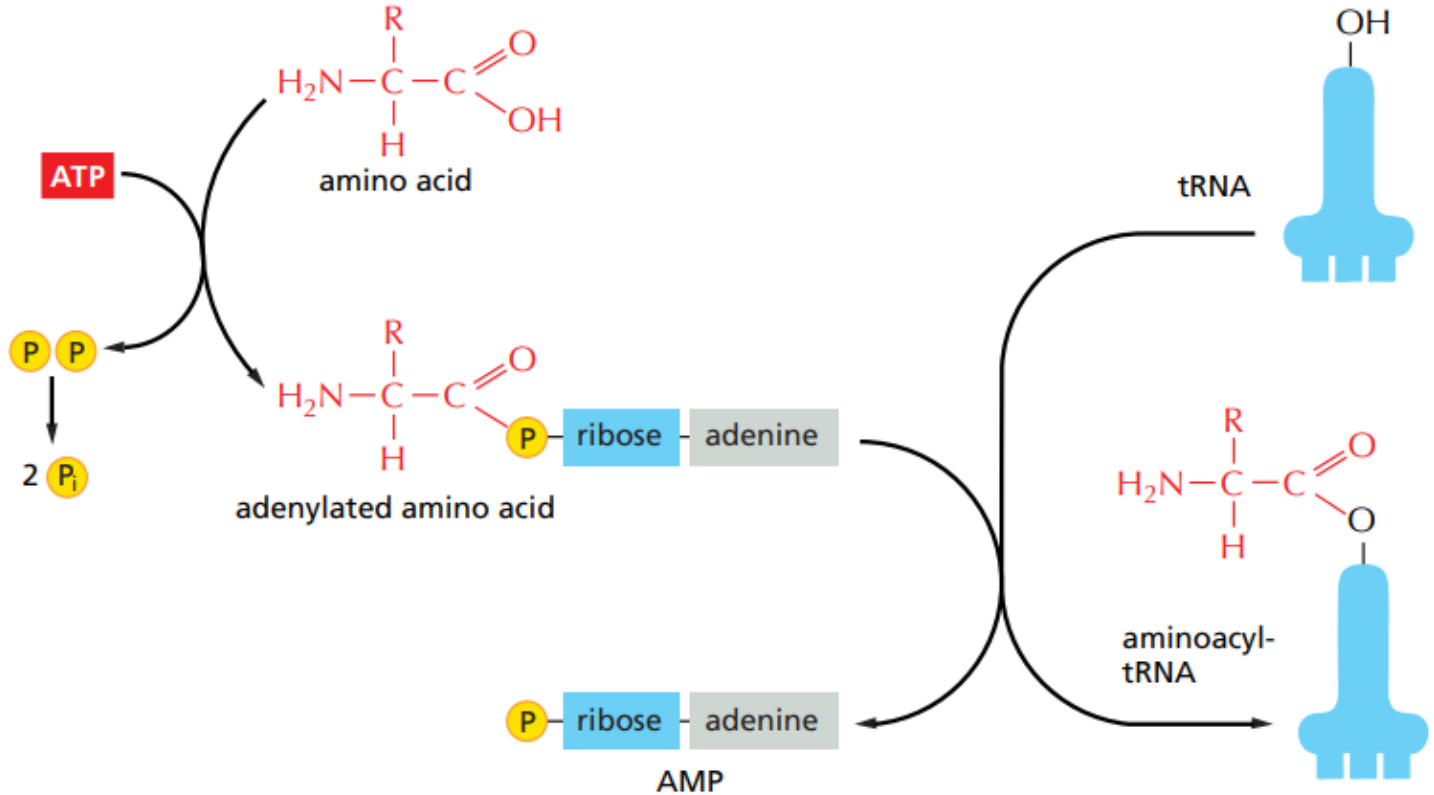
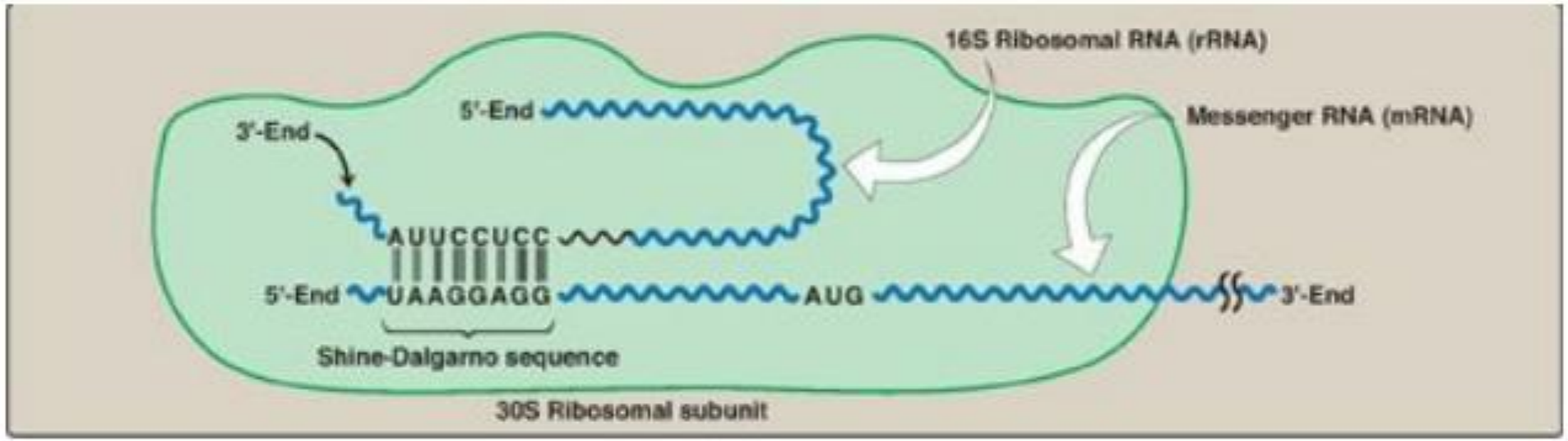


Figure 6–54 Amino acid activation by synthetase enzymes. An amino acid is activated for protein synthesis by an aminoacyl-tRNA synthetase enzyme in two steps. As indicated, the energy of ATP hydrolysis is used to attach each amino acid to its tRNA molecule in a high-energy linkage. The amino acid is first activated through the linkage of its carboxyl group directly to AMP, 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.

Components Required for Each Step of Protein Synthesis in *Escherichia coli*	
Step	**Components**
Amino acid activation	Amino acids
	tRNAs
	Aminoacyl-tRNA synthetases
	ATP, Mg^{2+}
Chain initiation	fmet-$tRNA^{fmet}$
	Initiation codon (AUG) of mRNA
	30S ribosomal subunit
	50S ribosomal subunit
	Initiation factors (IF-1, IF-2, and IF-3)
	GTP, Mg^{2+}
Chain elongation	70S ribosome
	Codons of mRNA
	Aminoacyl-tRNAs
	Elongation factors (EF-Tu, EF-Ts, and EF-G)
	GTP, Mg^{2+}
Chain termination	70S ribosome
	Termination codons (UAA, UAG, and UGA) of mRNA
	Release factors (RF-1, RF-2, and RF-3)
	GTP, Mg^{2+}

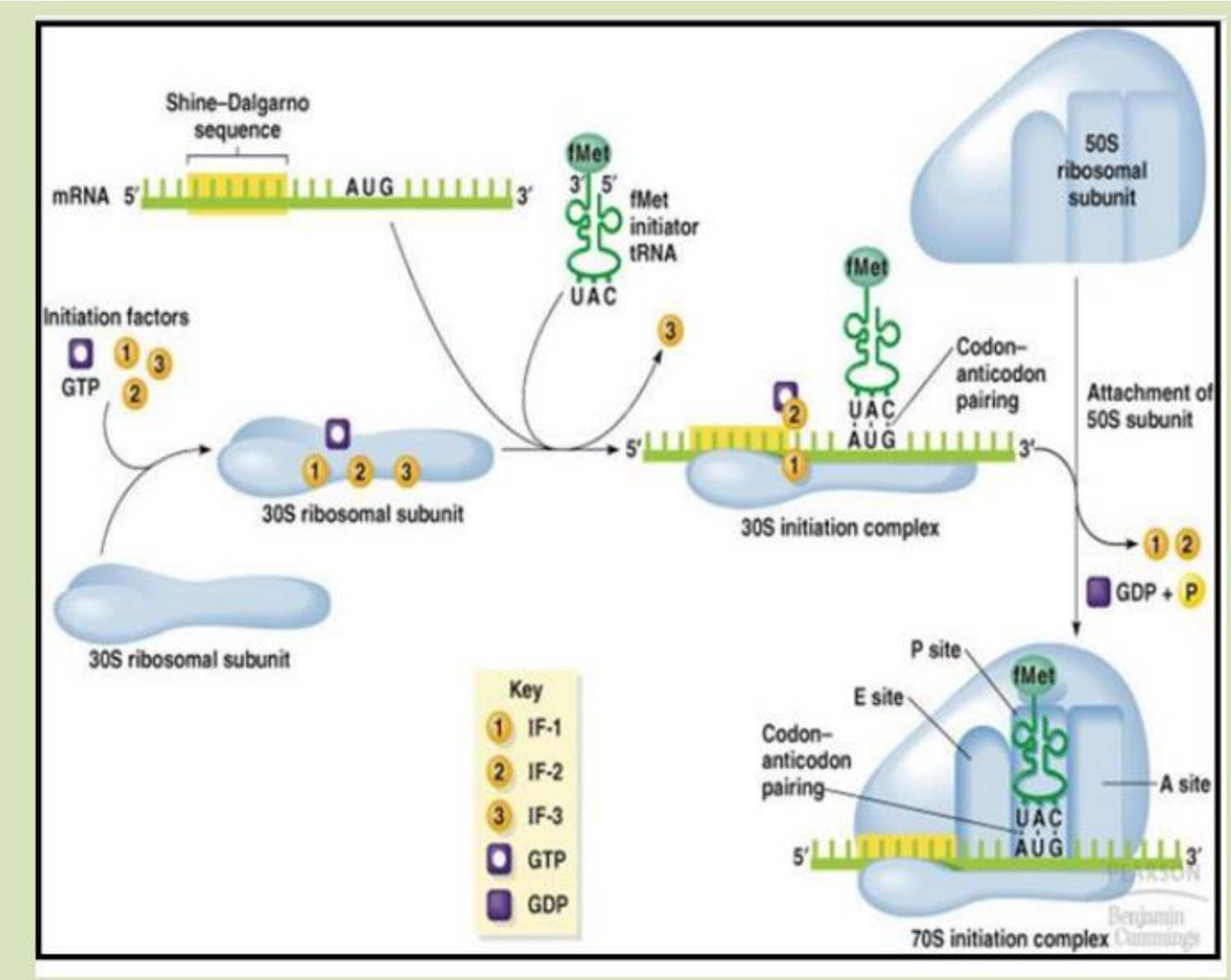
Protein synthesis in prokaryotes

- The **initiation of protein synthesis** begins with the formation of an initiation complex. In ***E. coli***, this complex involves the small 30S ribosome, the mRNA template, three **initiation factors** that help the ribosome assemble correctly, guanosine triphosphate (GTP) that acts as an energy source, and a special initiator tRNA carrying ***N*-formyl-methionine** (fMet-tRNA^{fMet}) (Figure).
- The initiator tRNA interacts with the start codon AUG of the mRNA and carries a formylated methionine (fMet). Because of its involvement in initiation, fMet is inserted at the beginning (N terminus) of every polypeptide chain synthesized by *E. coli*. In *E. coli* mRNA, a leader sequence upstream of the first AUG codon, called the **Shine-Dalgarno sequence** (also known as the ribosomal binding site AGGAGG), interacts through complementary base pairing with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. At this point, the 50S ribosomal subunit then binds to the initiation complex, forming an intact ribosome.



Complementary binding between prokaryotic mRNA Shine-Dalgarno sequence and 16S rRNA. S = Svedberg unit.

Protein synthesis in prokaryotes



Summary of Prokaryotic translation

- The unique and elegant structure of the ribosome allows the binding of aminoacyl-tRNA molecules and mRNA. The ribosome catalyzes the nucleophilic attack of one amino acid on the next, allowing for protein synthesis.
- Protein synthesis begins at an AUG codon on the mRNA. The ribosome and mRNA forms an initiation complex that includes the two main ribosomal subunits, the mRNA, GTP, and three initiation factors, IF1, IF2, and IF3.
- The ribosome locates the correct AUG to start translation by binding to a consensus sequence called the Shine–Dalgarno sequence.
- The first aminoacyl-tRNA bound to the ribosome carries *N*-formylmethionine, and it is initially bound to the P site of the ribosome.
- In chain elongation, the second amino acyl-tRNA binds to the A site. This amino acid's α-amino group performs a nucleophilic attack on the carbonyl group of the *N*-formylmethionine in the peptidyl transfer reaction. In a translocation step, the ribosome then moves one codon, leaving a dipeptidyl-tRNA in the A site and moving the uncharged tRNA to the exit site. The process continues with a new aminoacyl-tRNA entering the P site. The uncharged tRNA is then ejected from the E site.
- When the ribosome encounters a stop codon, the chain is terminated in a process requiring GTP and three protein release factors.
- The ribosome is actually a ribozyme. There are no amino acids at the active site where the peptidyl transferase reaction occurs. Specific bases on the rRNA are believed to catalyze the reaction.

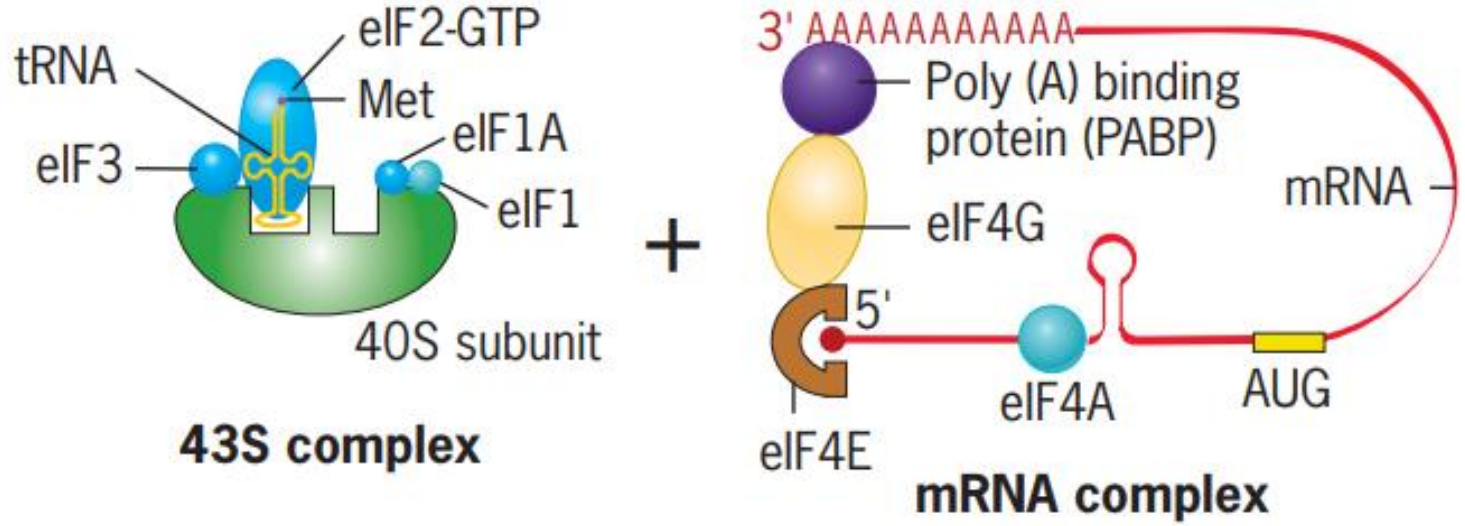
Protein synthesis in eukaryotes

- The overall mechanism of protein synthesis in eukaryotes is basically the same as in **prokaryotes**.
- However, there are some significant differences:
- Whereas a prokaryotic ribosome has a sedimentation coefficient of the 70S and subunits of 30S and 50S, a eukaryotic ribosome has a sedimentation coefficient of 80S with subunits of 40S and 60S.
- The composition of eukaryotic ribosomal subunits is also more complex than prokaryotic subunits but the function of each subunit is essentially the same as in prokaryotes.
- In eukaryotes, each mRNA is monocistronic that is, discounting any subsequent post-translational cleavage reactions that may occur; the mRNA encodes a single protein. In prokaryotes, many mRNAs are polycistronic that is they encode several proteins. Each coding sequence in a prokaryotic mRNA has its own initiation and termination codons.
- Initiation of protein synthesis in eukaryotes requires at least nine distinct eukaryotic initiation factors (eIFs) compared with the three initiation factors (IFs) in prokaryotes.
- In eukaryotes, the initiating amino acid is methionine, not N-formylmethionine as in prokaryotes.
- As in prokaryotes, a special initiator tRNA is required for initiation and is distinct from the tRNA that recognizes and binds to codons for methionine at internal positions in the mRNA. When charged with methionine ready to begin initiation, this is known as Met-tRNA$_i^{met}$
- The main difference between initiation of translation in prokaryotes and eukaryotes is that in bacteria, a Shine–Dalgarno sequence lies 5’ to the AUG initiation codon and is the binding site for the 30S ribosomal subunit.
- In contrast, most eukaryotic mRNAs do not contain Shine–Dalgarno sequences. Instead, a 40S ribosomal subunit attaches at the 5’ end of the mRNA and moves downstream (i.e. in a 5’ to 3’ direction) until it finds the AUG initiation codon. This process is called scanning.
- Prokaryotic translation requires no helicase, presumably because protein synthesis in bacteria can start even as the mRNA is still being synthesized whereas, in eukaryotes, transcription in the nucleus and translation in the cytoplasm are separate events that allow time for mRNA secondary structure to form.

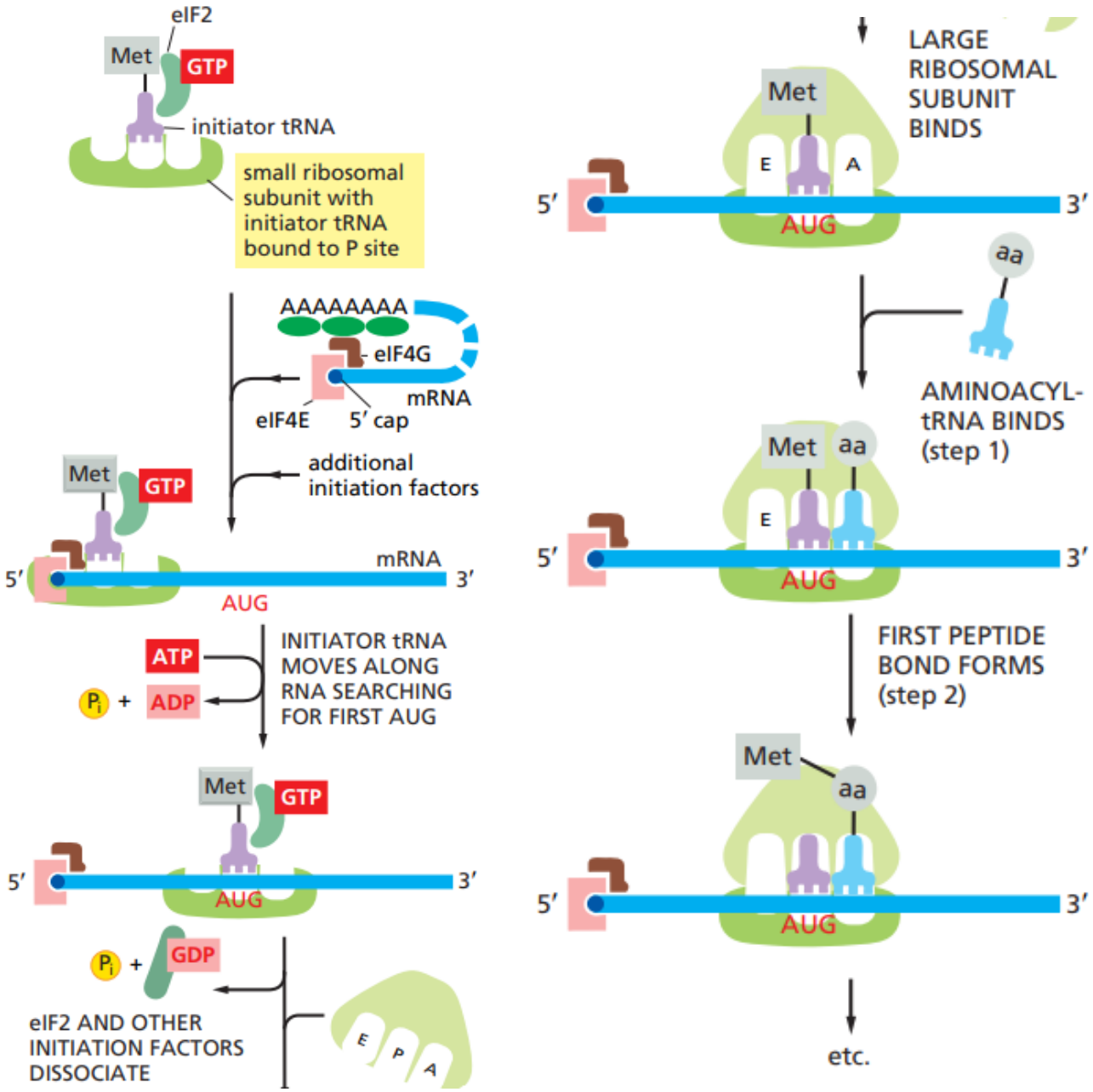
Protein synthesis stages:

1. Initiation
2. Elongation and
3. Termination

- The first step is the formation of a pre-initiation complex consisting of the 40S small ribosomal subunit, Met-$tRNA_i^{met}$, eIF-2, and GTP.
- The pre-initiation complex binds to the 5’ end of the eukaryotic mRNA, a step that requires eIF-4F (also called cap-binding complex) and eIF-3.
- The eIF-4F complex consists of eIF-4A, eIF-4E, and eIF-4G; eIF-4E binds to the 5’ cap on the mRNA whilst eIF-4G interacts with the poly (A) binding protein on the poly (A) tail.
- The eIF-4A is an ATP-dependent RNA helicase that unwinds any secondary structures in the mRNA, preparing it for translation.
- The complex then moves along the mRNA in a 5’ to 3’ direction until it locates the AUG initiation codon (i.e. scanning).
- The 5’ untranslated regions of eukaryotic mRNAs vary in length but can be several hundred nucleotides long and may contain secondary structures such as hairpin loops. These secondary structures are probably removed by initiation factors of the scanning complex.
- The initiation codon is usually recognizable because it is often (but not always) contained in a short sequence called the **Kozak consensus** (5’-ACCAUGG-3’).
- Once the complex is positioned over the initiation codon, the 60S large ribosomal subunit binds to form an 80S initiation complex, a step that requires the hydrolysis of GTP and leads to the release of several initiation factors.



Initiation of protein synthesis in eukaryotes. As discussed in the text, initiation begins with the union of two complexes. One (called the 43S complex) contains the 40S ribosomal subunit bound to several initiation factors (eIFs) and the initiator tRNA, whereas the other contains the mRNA bound to a separate group of initiation factors. This union is mediated by an interaction between eIF3 on the 43S complex and eIF4G on the mRNA complex. eIF1 and eIF1A are thought to induce a conformational change in the small ribosomal subunit that opens a “latch” to accommodate the entry of the mRNA. Once the 43S complex has bound to the 5 ′ end of the mRNA, it scans along the message until it reaches the appropriate AUG initiation codon.



The initiation of protein synthesis in eukaryotes. 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 (see Figure). In this way, the translation apparatus ascertains that both ends of the mRNA are intact before initiating protein synthesis. 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. In the last two steps shown in the figure, the ribosome has begun the standard elongation cycle

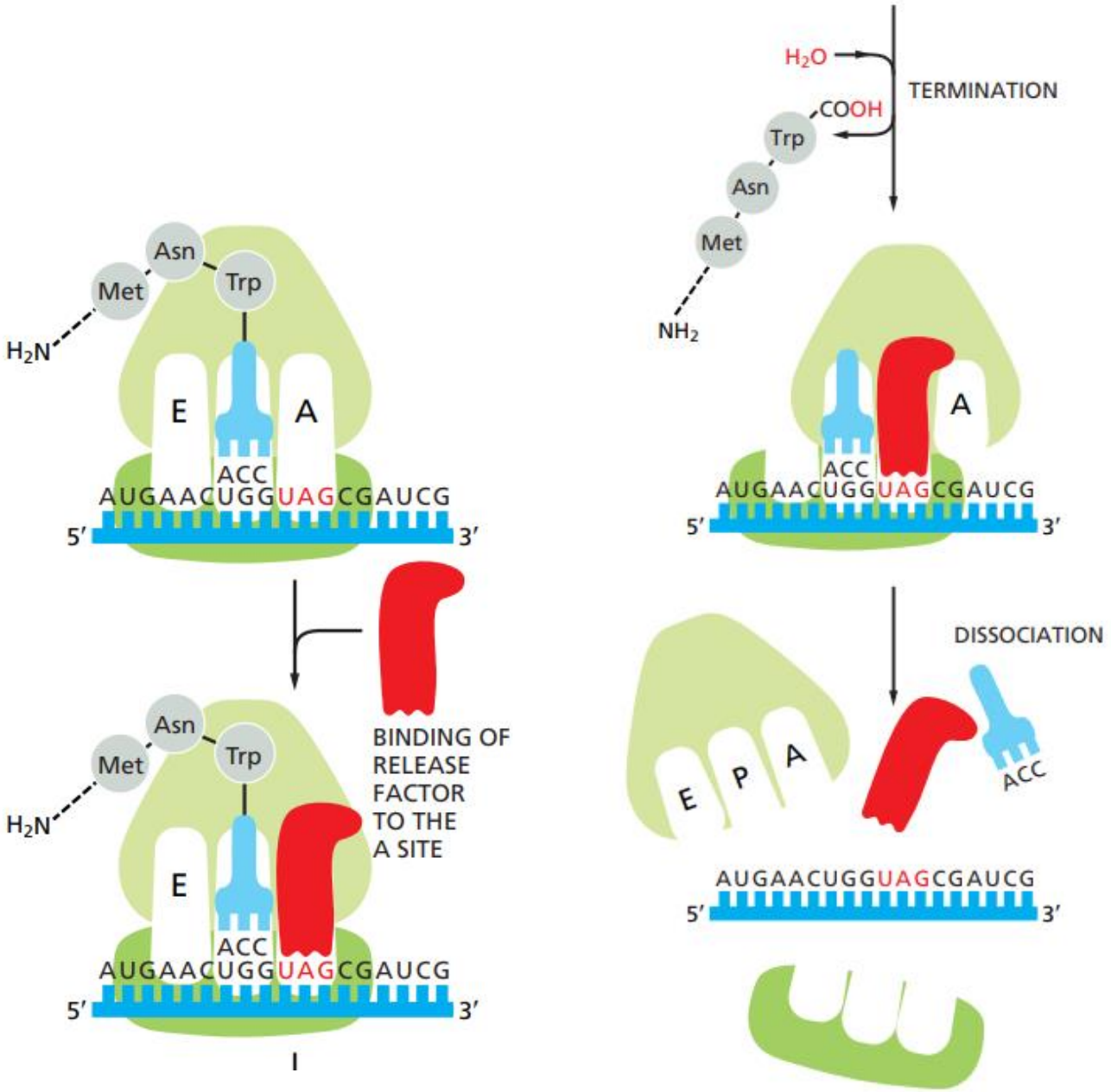
- Elongation depends on eukaryotic elongation factors.
- Three elongation factors, eEF-1A, eEF-IB, and eEF-2, are involved which have similar functions to their prokaryotic counterparts EF-Tu, EF-Ts and EF-G.
- At the end of the initiation step, the mRNA is positioned so that the next codon can be translated during the elongation stage of protein synthesis.
- The initiator tRNA occupies the P site in the ribosome, and the A site is ready to receive an aminoacyl-tRNA.
- During chain elongation, each additional amino acid is added to the nascent polypeptide chain in a three-step microcycle.
- The steps in this microcycle are:

1. Positioning the correct aminoacyl-tRNA in the A site of the ribosome,
2. Forming the peptide bond and
3. Shifting the mRNA by one codon relative to the ribosome.

- Although most codons encode the same amino acids in both prokaryotes and eukaryotes, the mRNAs synthesized within the organelles of some eukaryotes use a variant of the genetic code.
- During elongation in bacteria, the deacylated tRNA in the P site moves to the E site prior to leaving the ribosome. In contrast, although the situation is still not completely clear, in eukaryotes the deacylated tRNA appears to be ejected directly from the ribosome.

Termination mechanism in Protein synthesis

- Termination of elongation depends on eukaryotic release factors.
- In eukaryotes, eukaryotic release factor eRF-1 recognizes all three termination codons (UAA, UAG, and UGA) and, with the help of protein eRF-3, terminates translation.
- Upon termination, the ribosome is disassembled and the completed polypeptide is released.

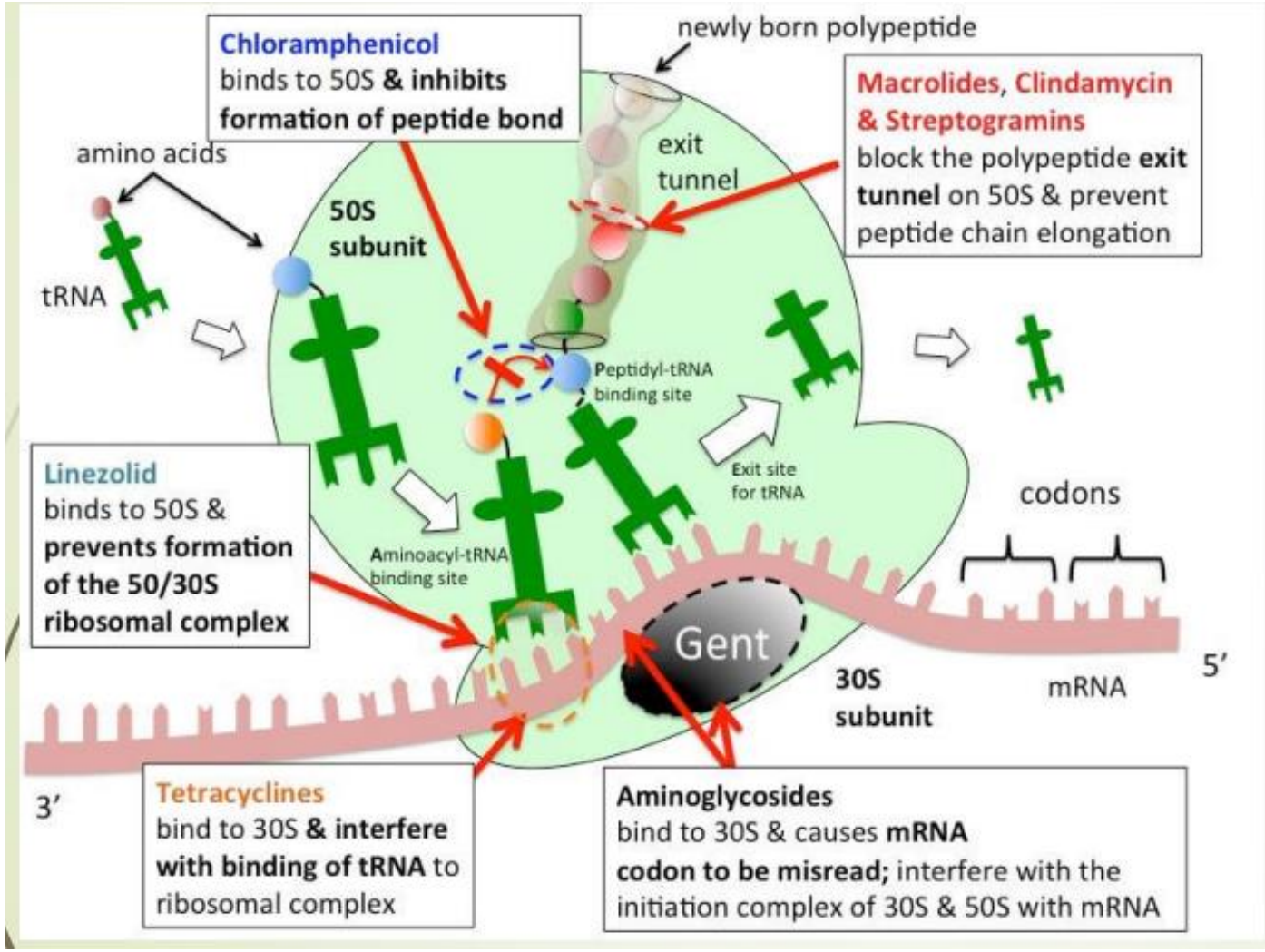


The final phase of protein synthesis. The binding of a release factor to an A site bearing a stop codon terminates translation. The completed polypeptide is released and, in a series of reactions that requires additional proteins and GTP hydrolysis (not shown), the ribosome dissociates into its two separate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

Protein synthesis inhibitors

Protein synthesis is a complex, multi-step process involving many enzymes as well as conformational alignment. However, the majority of antibiotics that block bacterial protein synthesis interfere with the processes at the 30S subunit or 50S subunit of the 70S bacterial ribosome. The aminoacyltRNA synthetases that activate each amino acid required for peptide synthesis are not antibiotic targets. Instead, the primary steps in the process that are attacked are (1) the formation of the 30S initiation complex (made up of mRNA, the 30S ribosomal subunit, and formyl-methionyl-transfer RNA), (2) the formation of the 70S ribosome by the 30S initiation complex and the 50S ribosome, and (3) the elongation process of assembling amino acids into a polypeptide. Tetracyclines, including doxycycline, prevent the binding of aminoacyl-tRNA by blocking the A (aminoacyl) site of the 30S ribosome. They are capable of inhibiting protein synthesis in both 70S and 80S (eukaryotic) ribosomes, but they preferentially bind to bacterial ribosomes due to structural differences in RNA subunits. Additionally, tetracyclines are effective against bacteria by exploiting the bacterial transport system and increasing the concentration of the antibiotic within the cell to be significantly higher than the environmental concentration. Aminoglycoside antibiotics have an affinity for the 30S ribosome subunit. Streptomycin, one of the most commonly used aminoglycosides, interferes with the creation of the 30S initiation complex. Kanamycin and tobramycin also bind to the 30S ribosome and block the formation of the larger 70S initiation complex. Erythromycin, a macrolide, binds to the 23S rRNA component of the 50S ribosome and interferes with the assembly of 50S subunits. Erythromycin, roxithromycin, and clarithromycin all prevent elongation at the transpeptidation step of synthesis by blocking the 50S polypeptide export tunnel. Elongation is prematurely terminated after a small peptide has been formed but cannot move past the macrolide roadblock. Peptidyl transferase is a key enzyme involved in translocation, the final step in the peptide elongation cycle. Lincomycin and clindamycin are specific inhibitors of peptidyl transferase, while macrolides do not directly inhibit the enzyme. Puromycin does not inhibit the enzymatic process, but instead competes by acting as an analog of the 3’-terminal end of aminoacyl-tRNA, disrupting synthesis and causing premature chain termination. Hygromycin B is an aminoglycoside that specifically binds to a single site within the 30S subunit in a region that contains the A, P, and E sites of tRNA. It has been theorized that this binding distorts the ribosomal A site and may be the cause of the ability of hygromycin to induce misreading of aminoacyl-tRNAs as well as prevent the translocation of peptide elongation.

Protein synthesis inhibitors



- **Oxazolidinone:** Linezolid
- **Tetracycline:** Doxycycline
- **Macrolide:** Azithromycin, Erythromycin
- **Chloramphenicol:** Chloromycetin
- **Aminoglycosides:** Amikacin, Kanamycin

References:

- Molecular biology of the cell, Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter ; with problems by John Wilson, Tim Hunt. -- Sixth edition.
- Cell biology, Thomas D. Pollard, William C. Earnshaw, Jennifer Lippincott-Schwartz, Graham T. Johnson, 3rd edition.