Stage 1: Aminoacyl-tRNA Synthetases Attach the Correct Amino Acids to Their tRNAs

During the first stage of protein synthesis, taking place in the cytosol, aminoacyl-tRNA synthetases esterify the 20 amino acids to their corresponding tRNAs. Each enzyme is specific for one amino acid and one or more corresponding tRNAs. Most organisms have one aminoacyl-tRNA synthetase for each amino acid. For amino acids with two or more corresponding tRNAs, the same enzyme usually aminoacylates all of them.

The structures of all the aminoacyl-tRNA synthetases of *E. coli* have been determined. Researchers have divided them into two classes (Table 27-7), based on substantial differences in primary and tertiary structure and in reaction mechanism **(Fig. 27-19)**; these two classes are the same in all organisms. There is no evidence that the two classes share a common ancestor, and the biological, chemical, or evolutionary reasons for two enzyme classes for essentially identical processes remain obscure.

The reaction catalyzed by an aminoacyl-tRNA synthetase is

$$\text{Amino acid} + \text{tRNA} + \text{ATP} \xrightarrow{Mg^{2+}} \text{aminoacyl} - \text{tRNA} + \text{AMP} + PP_i$$

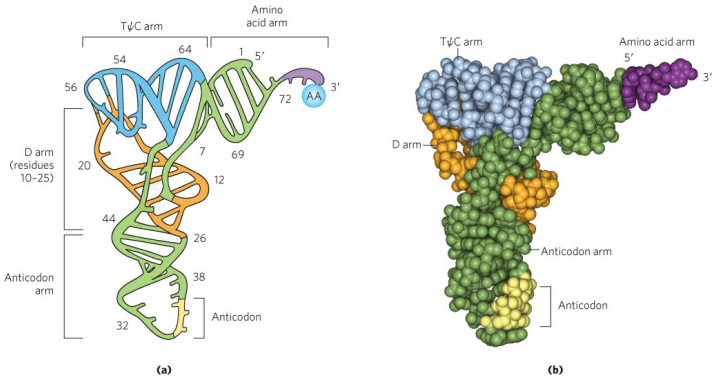


FIGURE 27-18 Three-dimensional structure of yeast $tRNA^{Phe}$ deduced from x-ray diffraction analysis. The shape resembles a twisted L. **(a)** Schematic diagram with the various arms identified in Figure 27-17 shaded in different colors. **(b)** A space-filling model, with the same color coding. The CCA sequence at the 3′ end (purple) is the attachment point for the amino acid. [Source: (b) PDB ID 4TRA, E. Westhof et al., *Acta Crystallogr. A* 44:112, 1988.]

This reaction occurs in two steps in the enzyme's active site. In step 1 (Fig. 27-19), an enzyme-bound intermediate, aminoacyl adenylate (aminoacyl-AMP), is formed. In the second step, the aminoacyl group is transferred from enzyme-bound aminoacyl-AMP to its corresponding specific tRNA. The course of this second step depends on the class to which the enzyme belongs, as shown by pathways 2a and 2b in Figure 27-19. The resulting ester linkage between the amino acid and the tRNA **(Fig. 27-20)** has a highly negative standard free energy of hydrolysis ($\Delta G'^\circ$ = –29 kJ/mol). The pyrophosphate formed in the activation reaction undergoes hydrolysis to phosphate by inorganic pyrophosphatase. Thus *two* high-energy phosphate bonds are ultimately expended for each amino acid molecule activated, rendering the overall reaction for amino acid activation essentially irreversible:

$$\text{Amino acid} + \text{tRNA} + \text{ATP} \xrightarrow{Mg^{2+}} \text{aminoacyl} - \text{tRNA} + \text{AMP} + 2P_i \qquad \Delta G'^\circ \approx -29\ \text{kJ/mol}$$

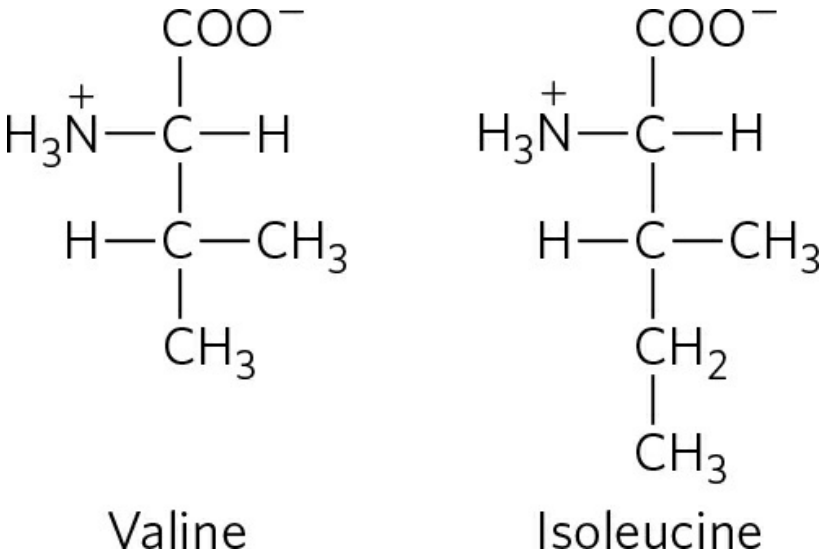
TABLE 27-7 The Two Classes of Aminoacyl-tRNA Synthetases

Class I		Class II	
Arg	Leu	Ala	Lys
Cys	Met	Asn	Phe
Gln	Trp	Asp	Pro
Glu	Tyr	Gly	Ser
Ile	Val	His	Thr

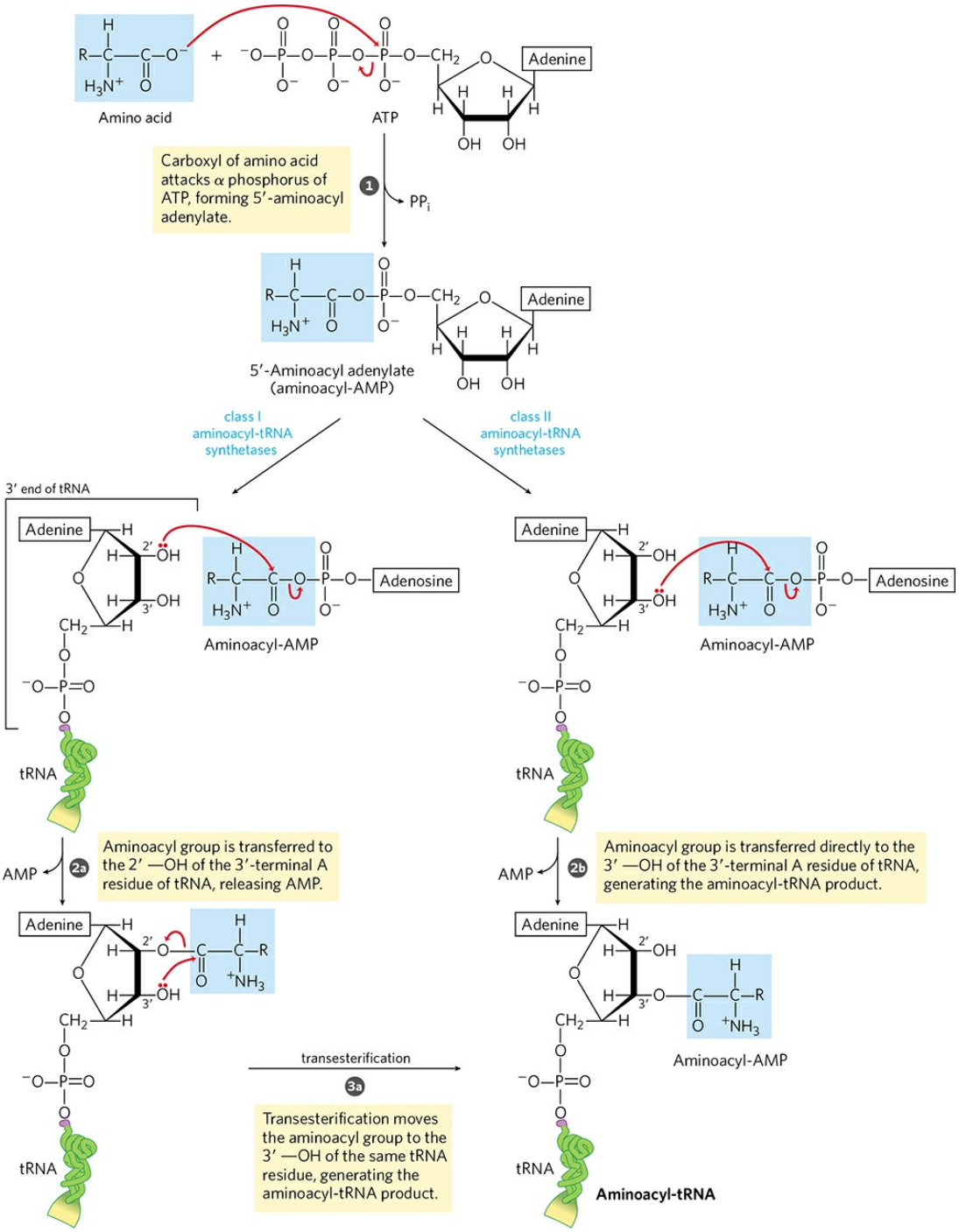
Note: Here, Arg represents arginyl-tRNA synthetase, and so forth. The classification applies to all organisms for which tRNA synthetases have been analyzed and is based on protein structural distinctions and on the mechanistic distinction outlined in Figure 27-19 .

Proofreading by Aminoacyl-tRNA Synthetases The aminoacylation of tRNA accomplishes two ends: (1) it activates an amino acid for peptide bond formation and (2) it ensures appropriate placement of the amino acid in a growing polypeptide. The identity of the amino acid attached to a tRNA is not checked on the ribosome, so attachment of the correct amino acid to the tRNA is essential to the fidelity of protein synthesis.

As you will recall from Chapter 6, enzyme specificity is limited by the binding energy available from enzyme-substrate interactions. Discrimination between two similar amino acid substrates has been studied in detail in the case of Ile-tRNA synthetase, which distinguishes between valine and isoleucine, amino acids that differ by only a single methylene group (—CH_2—):



Valine Isoleucine



MECHANISM FIGURE 27-19 Aminoacylation of tRNA by aminoacyl-tRNA synthetases. Step 1 is formation of an aminoacyl adenylate, which remains bound to the active site. In the second step, the aminoacyl group is transferred to the tRNA. The mechanism of this step is somewhat different for the two classes of aminoacyl-tRNA synthetases (see Table 27-7). For class I enzymes, 2a the aminoacyl group is transferred first to the 2′-hydroxyl group of the 3′-terminal A residue, then 3a to the 3′-hydroxyl group by a

transesterification reaction. For class II enzymes, 2b the aminoacyl group is transferred directly to the 3′-hydroxyl group of the terminal adenylate.

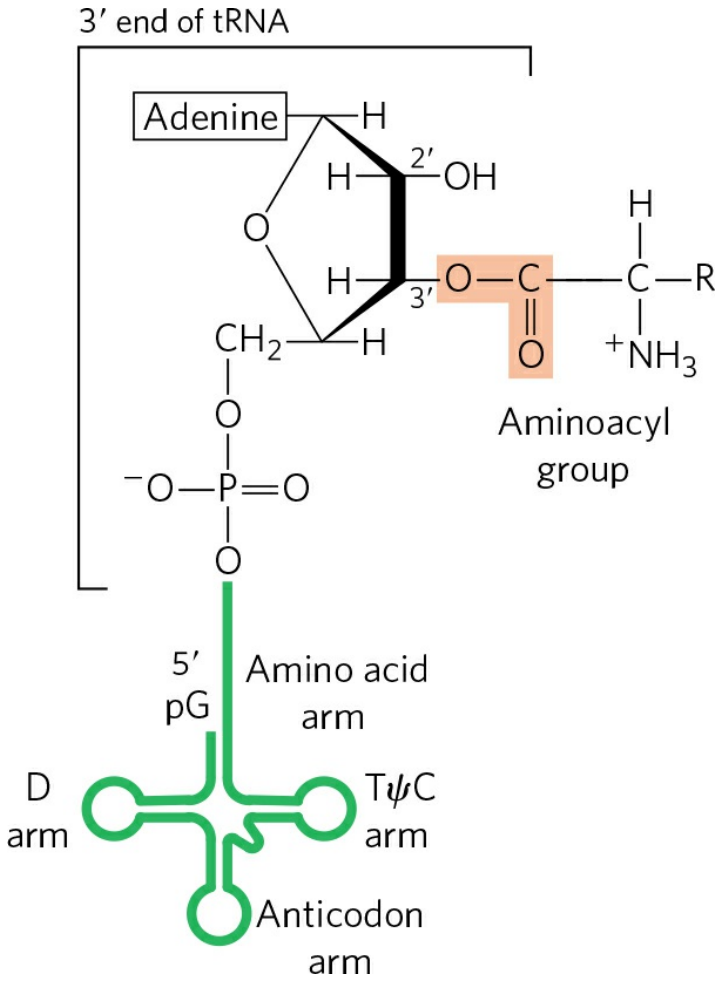


FIGURE 27-20 General structure of aminoacyl-tRNAs. The aminoacyl group is esterified to the 3′ position of the terminal A residue. The ester linkage that both activates the amino acid and joins it to the tRNA is shaded light red.

Ile-tRNA synthetase favors activation of isoleucine (to form Ile-AMP) over valine by a factor of 200—as we would expect, given the amount by which a methylene group (in Ile) could enhance substrate binding. Yet valine is erroneously incorporated into proteins in positions normally occupied by an Ile residue at a frequency of only about 1 in 3,000. How is this greater than 10-fold increase in accuracy brought about? Ile-tRNA synthetase, like some other aminoacyl-tRNA synthetases, has a proofreading function.

Recall a general principle from the discussion of proofreading by DNA polymerases (see Fig. 25-7): if available binding interactions do not provide sufficient discrimination between two substrates, the necessary specificity

can be achieved by substrate-specific binding in *two successive* steps. The effect of forcing the system through two successive filters is multiplicative. In the case of Ile-tRNA synthetase, the first filter is the initial binding of the amino acid to the enzyme and its activation to aminoacyl-AMP. The second is the binding of any *incorrect* aminoacyl-AMP products to a separate active site on the enzyme; a substrate that binds in this second active site is hydrolyzed. The R group of valine is slightly smaller than that of isoleucine, so Val-AMP fits the hydrolytic (proofreading) site of the Ile-tRNA synthetase, but Ile-AMP does not. Thus Val-AMP is hydrolyzed to valine and AMP in the proofreading active site, and tRNA bound to the synthetase does not become aminoacylated to the wrong amino acid.

In addition to proofreading after formation of the aminoacyl-AMP intermediate, most aminoacyl-tRNA synthetases can hydrolyze the ester linkage between amino acids and tRNAs in the aminoacyl-tRNAs. This hydrolysis is greatly accelerated for incorrectly charged tRNAs, providing yet a third filter to enhance the fidelity of the overall process. The few aminoacyl-tRNA synthetases that activate amino acids with no close structural relatives (Cys-tRNA synthetase, for example) demonstrate little or no proofreading activity; in these cases, the active site for aminoacylation can sufficiently discriminate between the proper substrate and any incorrect amino acid.

The overall error rate of protein synthesis (~1 mistake per 10^4 amino acids incorporated) is not nearly as low as that of DNA replication. Because flaws in a protein are eliminated when the protein is degraded and are not passed on to future generations, they have less biological significance. The degree of fidelity in protein synthesis is sufficient to ensure that most proteins contain no mistakes and that the large amount of energy required to synthesize a protein is rarely wasted. One defective protein molecule is usually unimportant when many correct copies of the same protein are present.

Interaction between an Aminoacyl-tRNA Synthetase and a tRNA: A "Second Genetic Code" An individual aminoacyl-tRNA synthetase must be specific not only for a single amino acid but for certain tRNAs as well. Discriminating among dozens of tRNAs is just as important for the overall fidelity of protein biosynthesis as is distinguishing among amino acids. The interaction between aminoacyl-tRNA synthetases and tRNAs has been

referred to as the "second genetic code," reflecting its critical role in maintaining the accuracy of protein synthesis. The "coding" rules appear to be more complex than those in the "first" code.

Figure 27-21 summarizes what we know about the nucleotides involved in recognition by some aminoacyl-tRNA synthetases. Some nucleotides are conserved in all tRNAs and therefore cannot be used for discrimination. By observing changes in nucleotides that alter substrate specificity, researchers have identified nucleotide positions necessary for discrimination by the aminoacyl-tRNA synthetases. These nucleotide positions seem to be concentrated in the amino acid arm and the anticodon arm, including the nucleotides of the anticodon itself, but are also located in other parts of the tRNA molecule. Determination of the crystal structures of aminoacyl-tRNA synthetases complexed with their cognate tRNAs and ATP has added a great deal to our understanding of these interactions **(Fig. 27-22)**.

Ten or more specific nucleotides may be involved in recognition of a tRNA by its specific aminoacyl-tRNA synthetase. But in a few cases the recognition mechanism is quite simple. Across a range of organisms from bacteria to humans, the primary determinant of tRNA recognition by the Ala-tRNA synthetases is a single G=U base pair in the amino acid arm of $tRNA^{Ala}$ **(Fig. 27-23a)**. A short synthetic RNA with as few as 7 bp arranged in a simple hairpin minihelix is efficiently aminoacylated by the Ala-tRNA synthetase, as long as the RNA contains the critical G=U (Fig. 27-23b). This relatively simple alanine system may be an evolutionary relic of a period when RNA oligonucleotides, ancestors to tRNA, were aminoacylated in a primitive system for protein synthesis.

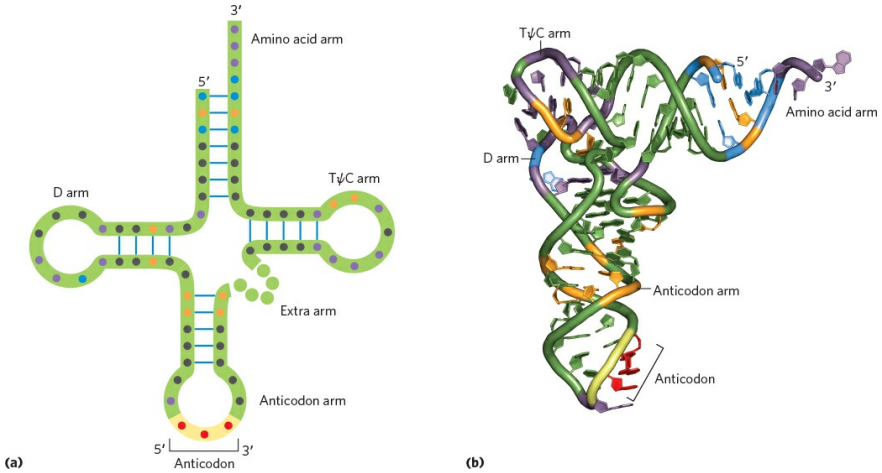


FIGURE 27-21 Nucleotide positions in a tRNA that are recognized by aminoacyl-tRNA synthetases. (a) Some positions (purple dots) are the same in all tRNAs and therefore cannot be used to discriminate one from another. Other positions are known recognition points for one (orange) or more (blue) aminoacyl-tRNA synthetases. Structural features other than sequence are important for recognition by some of the synthetases. **(b)** The same structural features are shown in three dimensions, with the orange and blue residues again representing positions recognized by one or more aminoacyl-tRNA synthetases, respectively.
[Source: PDB ID 1EHZ, H. Shi and P. B. Moore, *RNA* 6:1091, 2000.]

The interaction of aminoacyl-tRNA synthetases and their cognate tRNAs is critical to accurate reading of the genetic code. Any expansion of the code to include new amino acids would necessarily require a new aminoacyl-tRNA synthetase–tRNA pair. A limited expansion of the genetic code has been observed in nature; a more extensive expansion has been accomplished in the laboratory (Box 27-2).

Stage 2: A Specific Amino Acid Initiates Protein Synthesis

Protein synthesis begins at the amino-terminal end and proceeds by the stepwise addition of amino acids to the carboxyl-terminal end of the growing polypeptide, as determined by Howard Dintzis in 1961 **(Fig. 27-24)**. The AUG initiation codon thus specifies an *amino-terminal* methionine residue. Although methionine has only one codon, (5′)AUG, all organisms have two tRNAs for methionine. One is used exclusively when (5′)AUG is the initiation codon for protein synthesis. The other is used to code for a Met residue in an internal position in a polypeptide.

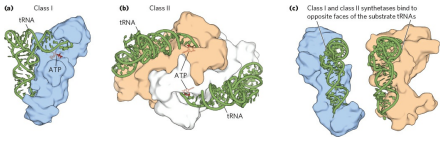


FIGURE 27-22 Aminoacyl-tRNA synthetases. The synthetases are complexed with their cognate tRNAs (green). Bound ATP (red) pinpoints the active site near the end of the aminoacyl arm. **(a)** Gln-tRNA synthetase of *E. coli*, a typical monomeric class I synthetase. **(b)** Asp-tRNA synthetase of yeast, a typical dimeric class II synthetase. **(c)** The two classes of aminoacyl-tRNA synthetases recognize different faces of their tRNA substrates. [Sources: (a, c

(left)) PDB ID 1QRT, J. G. Arnez and T. A. Steitz, *Biochemistry* 35:14,725, 1996. (b, c (right)) PDB ID 1ASZ, J. Cavarelli et al., *EMBO J.* 13:327, 1994.]

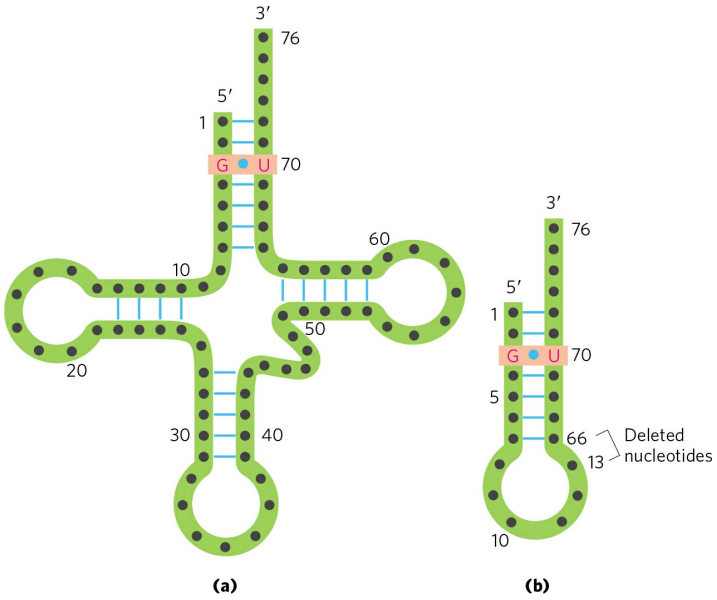


FIGURE 27-23 Structural elements of $tRNA^{Ala}$ that are required for recognition by Ala-tRNA synthetase. (a) The $tRNA^{Ala}$ structural elements recognized by the Ala-tRNA synthetase are unusually simple. A single G=U base pair (light red) is the only element needed for specific binding and aminoacylation. **(b)** A short synthetic RNA minihelix, with the critical G=U base pair but lacking most of the remaining tRNA structure. This is aminoacylated specifically with alanine almost as efficiently as the complete $tRNA^{Ala}$.

The distinction between an initiating (5′)AUG and an internal one is straightforward. In bacteria, the two types of tRNA specific for methionine are designated $tRNA^{Met}$ and $tRNA^{fMet}$. The amino acid incorporated in response to the (5′)AUG initiation codon is *N*-formylmethionine (fMet). It arrives at the ribosome as *N*-formylmethionyl-$tRNA^{fMet}$ (fMet-$tRNA^{fMet}$), which is formed in two successive reactions. First, methionine is attached to $tRNA^{fMet}$ by the Met-tRNA synthetase (which in *E. coli* aminoacylates both $tRNA^{fMet}$ and $tRNA^{Met}$):

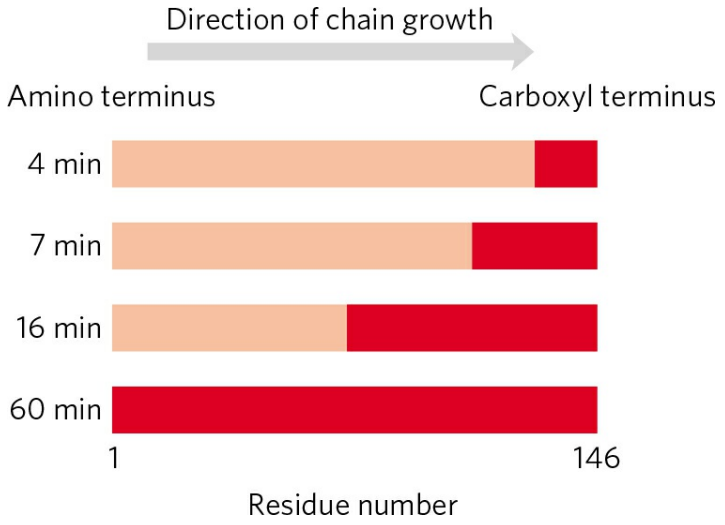


FIGURE 27-24 Proof that polypeptides grow by addition of amino acid residues to the carboxyl end: the Dintzis experiment. Reticulocytes (immature erythrocytes) actively synthesizing hemoglobin were incubated with radioactive leucine (selected because it occurs frequently in both the α and β-globin chains). Samples of completed α chains were isolated from the incubating reticulocytes at various times, and the distribution of radioactivity determined. The dark red zones show the portions of completed α-globin chains containing radioactive Leu residues. At 4 min, only a few residues at the carboxyl end of α-globin were labeled, because the only *complete* globin chains with incorporated label after 4 min were those that had nearly completed synthesis at the time the label was added. With longer incubation times, successively longer segments of the polypeptide contained labeled residues, always in a block at the carboxyl end of the chain. The unlabeled end of the polypeptide (the amino terminus) was thus defined as the initiating end, which means that polypeptides grow by successive addition of amino acids to the carboxyl end.

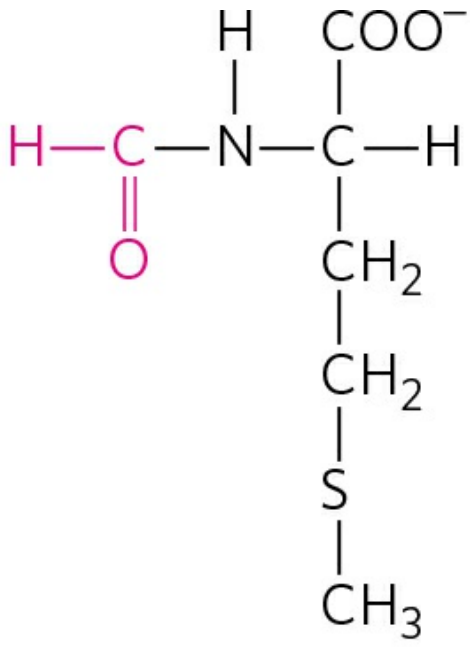
$$\text{Methionine} + \text{tRNA}^{\text{fMet}} + \text{ATP} \rightarrow \text{Met-tRNA}^{\text{fMet}} + \text{AMP} + \text{PP}_\text{i}$$

Next, a transformylase transfers a formyl group from N^{10}-formyltetrahydrofolate to the amino group of the Met residue:

$$N^{10}\text{-Formyltetrahydrofolate} + \text{Met-tRNA}^{\text{fMet}} \rightarrow \text{tetrahydrofolate} + \text{fMet-tRNA}^{\text{fMet}}$$

The transformylase is more selective than the Met-tRNA synthetase; it is specific for Met residues attached to $\text{tRNA}^{\text{fMet}}$, presumably recognizing some

unique structural feature of that tRNA. By contrast, Met-tRNA^{Met} inserts methionine in interior positions in polypeptides.



N-Formylmethionine

Addition of the *N*-formyl group to the amino group of methionine by the transformylase prevents fMet from entering interior positions in a polypeptide while also allowing fMet-tRNA^{fMet} to be bound at a specific ribosomal initiation site that accepts neither Met-tRNA^{Met} nor any other aminoacyl-tRNA.

The Three Steps of Initiation The **initiation** of polypeptide synthesis in bacteria requires (1) the 30S ribosomal subunit, (2) the mRNA coding for the polypeptide to be made, (3) the initiating fMet-tRNA^{fMet}, (4) a set of three proteins called initiation factors (IF1, IF2, and IF3), (5) GTP, (6) the 50S ribosomal subunit, and (7) Mg^{2+}. Formation of the initiation complex takes place in three steps **(Fig. 27-25)**.

In step , the 30S ribosomal subunit binds two initiation factors, IF1 and IF3. Factor IF3 prevents the 30S and 50S subunits from combining prematurely. The mRNA then binds to the 30S subunit. The initiating (5′)AUG is guided to its correct position by the **Shine-Dalgarno sequence** (named for Australian researchers John Shine and Lynn Dalgarno, who identified it) in the mRNA. This consensus sequence is an initiation signal of four to nine purine residues, 8 to 13 bp to the 5′ side of the initiation codon **(Fig. 27-26a)**. The sequence base-pairs with a complementary pyrimidine-rich sequence near the 3′ end of the 16S rRNA of the 30S ribosomal subunit (Fig. 27-26b). This mRNA-rRNA interaction positions the initiating (5′)AUG sequence of the mRNA in the precise position on the 30S subunit where it is required for initiation of translation. The particular (5′)AUG where fMet-tRNA^{fMet} is to be bound is distinguished from other methionine codons by its proximity to the Shine-Dalgarno sequence in the mRNA.

Bacterial ribosomes have three sites that bind tRNAs, the **aminoacyl (A) site**, the **peptidyl (P) site**, and the **exit (E) site**. The A and P sites bind aminoacyl-tRNAs, whereas the E site binds only uncharged tRNAs that have completed their task on the ribosome. Both the 30S and the 50S subunits contribute to the characteristics of the A and P sites, whereas the E site is largely confined to the 50S subunit. The initiating (5′)AUG is positioned at the P site, the only site to which fMet-tRNA^{fMet} can bind (Fig. 27-25). The fMet-tRNA^{fMet} is the only aminoacyl-tRNA that binds first to the P site; during the subsequent elongation stage, all other incoming aminoacyl-tRNAs

(including the Met-tRNA^{Met} that binds to interior AUG codons) bind first to the A site and only subsequently to the P and E sites. The E site is the site from which the "uncharged" tRNAs leave during elongation. Factor IF1 binds at the A site and prevents tRNA binding at this site during initiation.

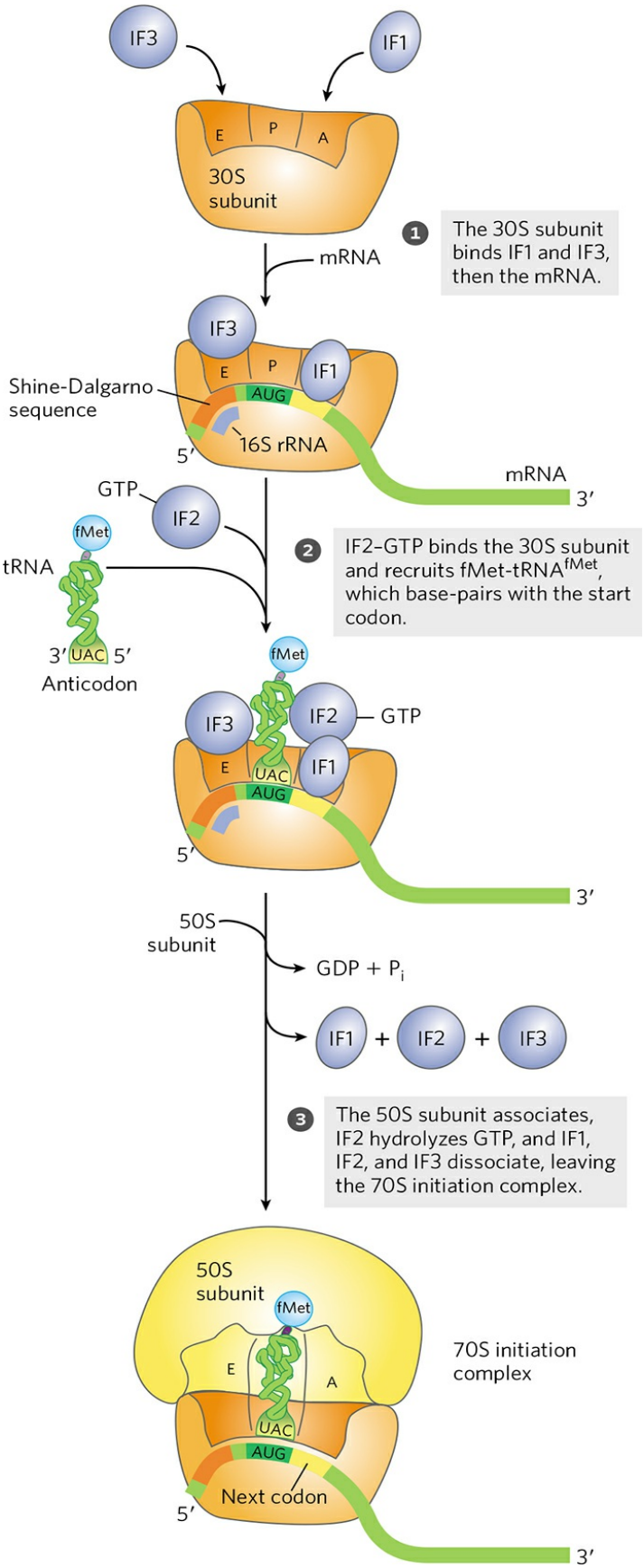

IF3
IF1
E
P
A
30S subunit
1 The 30S subunit binds IF1 and IF3, then the mRNA.
mRNA
IF3
E
P
IF1
Shine-Dalgarno sequence
AUG
16S rRNA
5′
mRNA
3′
GTP
IF2
fMet
tRNA
2 IF2–GTP binds the 30S subunit and recruits fMet-tRNA^{fMet}, which base-pairs with the start codon.
3′ UAC 5′
Anticodon
fMet
IF3
IF2
GTP
E
UAC
IF1
AUG
5′
3′
50S subunit
GDP + P_i
IF1 + IF2 + IF3
3 The 50S subunit associates, IF2 hydrolyzes GTP, and IF1, IF2, and IF3 dissociate, leaving the 70S initiation complex.
50S subunit
fMet
E
A
UAC
AUG
Next codon
5′
3′
70S initiation complex

FIGURE 27-25 Formation of the initiation complex in bacteria. The complex forms in three steps (described in the text) at the expense of the hydrolysis of GTP to GDP and P_i. IF1, IF2, and IF3 are initiation factors. E designates the exit site; P, the peptidyl site; and A, the aminoacyl site. Here the anticodon of the tRNA is oriented 3′ to 5′, left to right, as in Figure 27-8 but opposite to the orientation in Figures 27-21 and 27-23.

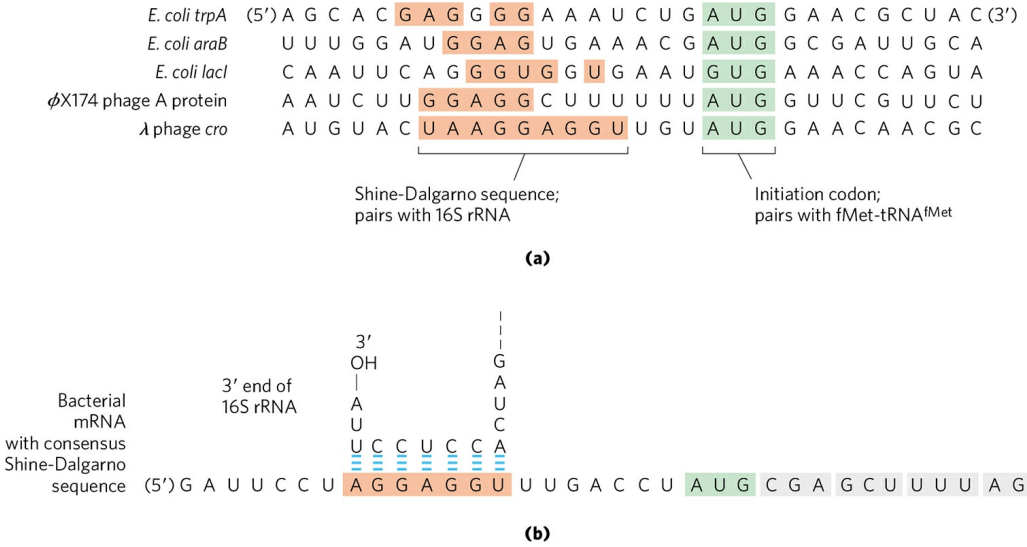


FIGURE 27-26 Messenger RNA sequences that serve as signals for initiation of protein synthesis in bacteria. (a) Alignment of the initiating AUG (shaded in green) at its correct location on the 30S ribosomal subunit depends in part on upstream Shine-Dalgarno sequences (light red). Portions of the mRNA transcripts of five bacterial genes are shown. Note the unusual example of the *E. coli* LacI protein, which initiates with a GUG (Val) codon (see Box 27-1). In *E. coli*, AUG is the start codon in approximately 91% of genes, with GUG (7%) and UUG (2%) assuming this role more rarely. **(b)** The Shine-Dalgarno sequence of the mRNA pairs with a sequence near the 3′ end of the 16S rRNA.

In step ❷ of the initiation process (Fig. 27-25), the complex consisting of the 30S ribosomal subunit, IF3, and mRNA is joined by both GTP-bound IF2 and the initiating fMet-tRNA^{fMet}. The anticodon of this tRNA now pairs correctly with the mRNA's initiation codon.

In step ❸, this large complex combines with the 50S ribosomal subunit; simultaneously, the GTP bound to IF2 is hydrolyzed to GDP and P_i, which are released from the complex. All three initiation factors leave the ribosome at this point.

Completion of the steps in Figure 27-25 produces a functional 70S ribosome called the **initiation complex**, containing the mRNA and the initiating fMet-tRNA^{fMet}. The correct binding of the fMet-tRNA^{fMet} to the P site in the complete 70S initiation complex is ensured by at least three points of recognition and attachment: the codon-anticodon interaction involving the initiation AUG fixed in the P site, the interaction between the Shine-Dalgarno sequence in the mRNA and the 16S rRNA, and the binding interactions between the ribosomal P site and the fMet-tRNA^{fMet}. The initiation complex is now ready for elongation.

Stage 3: Peptide Bonds Are Formed in the Elongation Stage

The third stage of protein synthesis is **elongation**. Again, we begin with bacterial cells. Elongation requires (1) the initiation complex described above, (2) aminoacyl-tRNAs, (3) a set of three soluble cytosolic proteins called **elongation factors** (EF-Tu, EF-Ts, and EF-G in bacteria), and (4) GTP. Cells use three steps to add each amino acid residue, and the steps are repeated as many times as there are residues to be added.

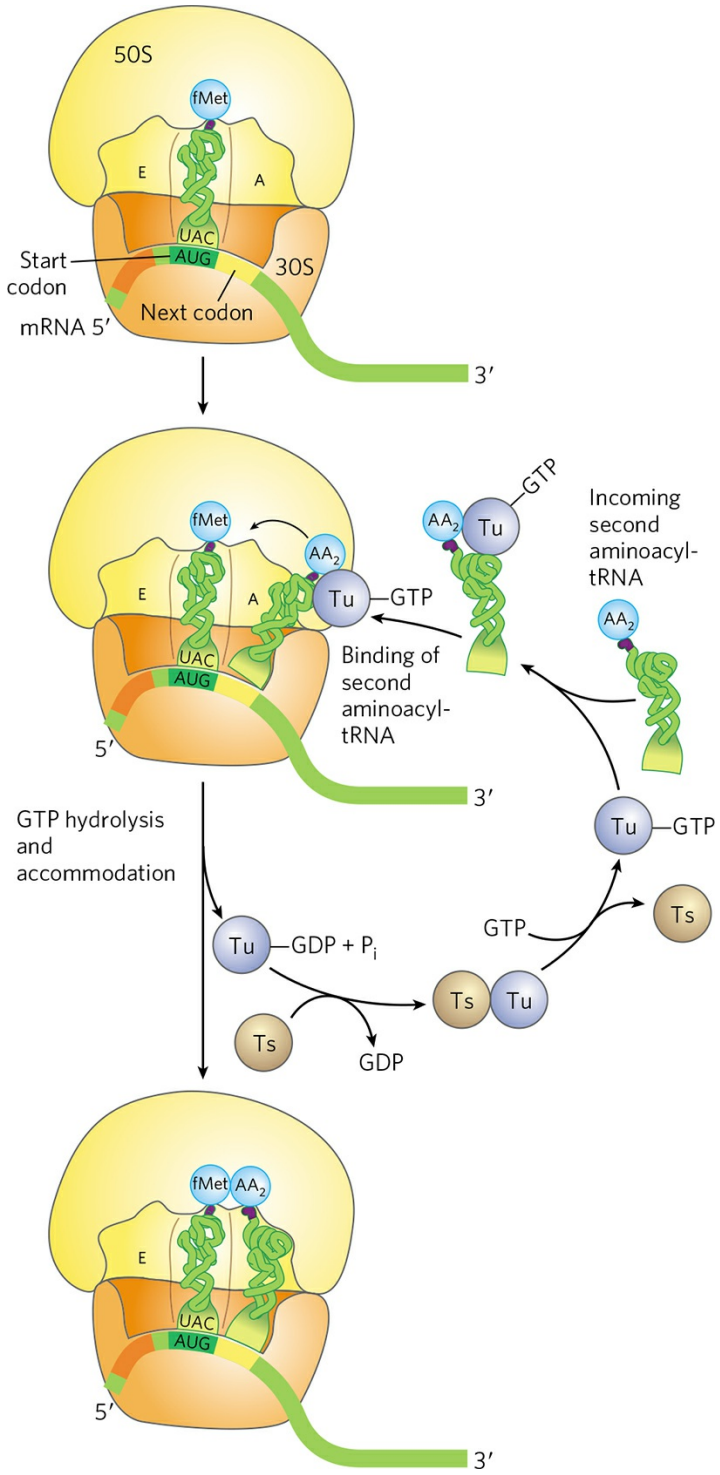


FIGURE 27-29 First elongation step in bacteria: binding of the second aminoacyl-tRNA. The second aminoacyl-tRNA (AA_2) enters the A site of the ribosome bound to GTP-bound EF-Tu (shown here as Tu). Binding of the second aminoacyl-tRNA to the A site is accompanied by hydrolysis of the GTP to GDP and P_i and release of the EF-Tu–GDP complex from the ribosome. The

bound GDP is released when the EF-Tu–GDP complex binds to EF-Ts, and EF-Ts is subsequently released when another molecule of GTP binds to EF-Tu. This recycles EF-Tu and makes it available to repeat the cycle. "Accommodation" involves a change in the conformation of the second tRNA that pulls its aminoacyl end into the peptidyl transferase site.

Elongation Step 1: Binding of an Incoming Aminoacyl-tRNA In the first step of the elongation cycle **(Fig. 27-29)**, the appropriate incoming aminoacyl-tRNA binds to a complex of GTP-bound EF-Tu. The resulting aminoacyl-tRNA–EF-Tu–GTP complex binds to the A site of the 70S initiation complex. The GTP is hydrolyzed and an EF-Tu–GDP complex is released from the 70S ribosome. The EF-Tu–GTP complex is regenerated in a process requiring EF-Ts and GTP.

Elongation Step 2: Peptide Bond Formation A peptide bond is now formed between the two amino acids bound by their tRNAs to the A and P sites on the ribosome. This occurs by transfer of the initiating *N*-formylmethionyl group from its tRNA to the amino group of the second amino acid, now in the A site **(Fig. 27-30)**. The α-amino group of the amino acid in the A site acts as a nucleophile, displacing the tRNA in the P site to form a peptide bond. This reaction produces a dipeptidyl-tRNA in the A site, and the now "uncharged" (deacylated) $tRNA^{fMet}$ remains bound to the P site. The tRNAs then shift to a hybrid binding state, with elements of each spanning two different sites on the ribosome, as shown in Figure 27-30.

The enzymatic activity that catalyzes peptide bond formation has historically been referred to as **peptidyl transferase** and was widely assumed to be intrinsic to one or more of the proteins in the large ribosomal subunit. We now know that this reaction is catalyzed by the 23S rRNA, adding to the known catalytic repertoire of ribozymes. This discovery has interesting implications for the evolution of life (Chapter 26).

Elongation Step 3: Translocation In the final step of the elongation cycle, **translocation**, the ribosome moves one codon toward the 3′ end of the mRNA **(Fig. 27-31a)**. This movement shifts the anticodon of the dipeptidyl-tRNA, which is still attached to the second codon of the mRNA, from the A site to the P site, and shifts the deacylated tRNA from the P site to the E site, from where the tRNA is released into the cytosol. The third codon

of the mRNA now lies in the A site and the second codon in the P site. Movement of the ribosome along the mRNA requires EF-G (also known as translocase) and the energy provided by hydrolysis of another molecule of GTP. A change in the three-dimensional conformation of the entire ribosome results in its movement along the mRNA. Because the structure of EF-G mimics the structure of the EF-Tu–tRNA complex (Fig. 27-31b), EF-G can bind the A site and, presumably, displace the peptidyl-tRNA.

After translocation, the ribosome, with its attached dipeptidyl-tRNA and mRNA, is ready for the next elongation cycle and attachment of a third amino acid residue. This process occurs in the same way as addition of the second residue (as shown in Figs 27-29, 27-30, and 27-31). For each amino acid residue correctly added to the growing polypeptide, two GTPs are hydrolyzed to GDP and P_i as the ribosome moves from codon to codon along the mRNA toward the 3′ end.

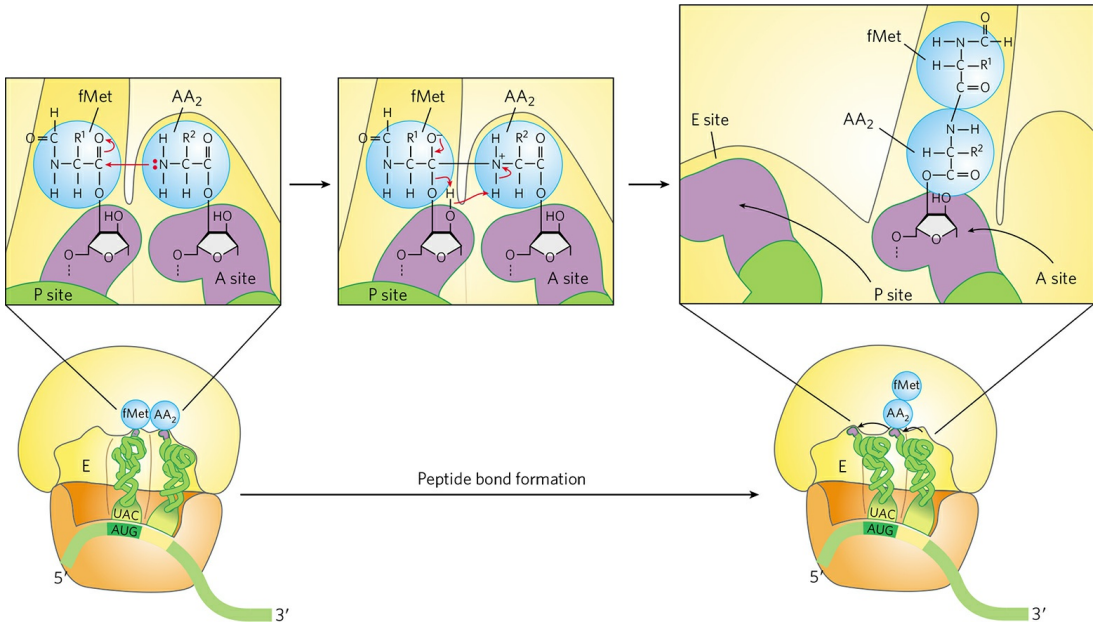


FIGURE 27-30 Second elongation step in bacteria: formation of the first peptide bond. The peptidyl transferase catalyzing this reaction is the 23S rRNA ribozyme. The *N*-formylmethionyl group is transferred to the amino group of the second aminoacyl-tRNA in the A site, forming a dipeptidyl-tRNA. At this stage, both tRNAs bound to the ribosome shift position in the 50S subunit to take up a hybrid binding state. The uncharged tRNA shifts so that its 3′ and 5′ ends are in the E site. Similarly, the 3′ and 5′ ends of the peptidyl-tRNA shift to the P site. The anticodons remain in the P and A sites. Note the involvement of the 2′-hydroxyl group of the 3′-terminal adenosine as a general acid-base catalyst in this reaction.

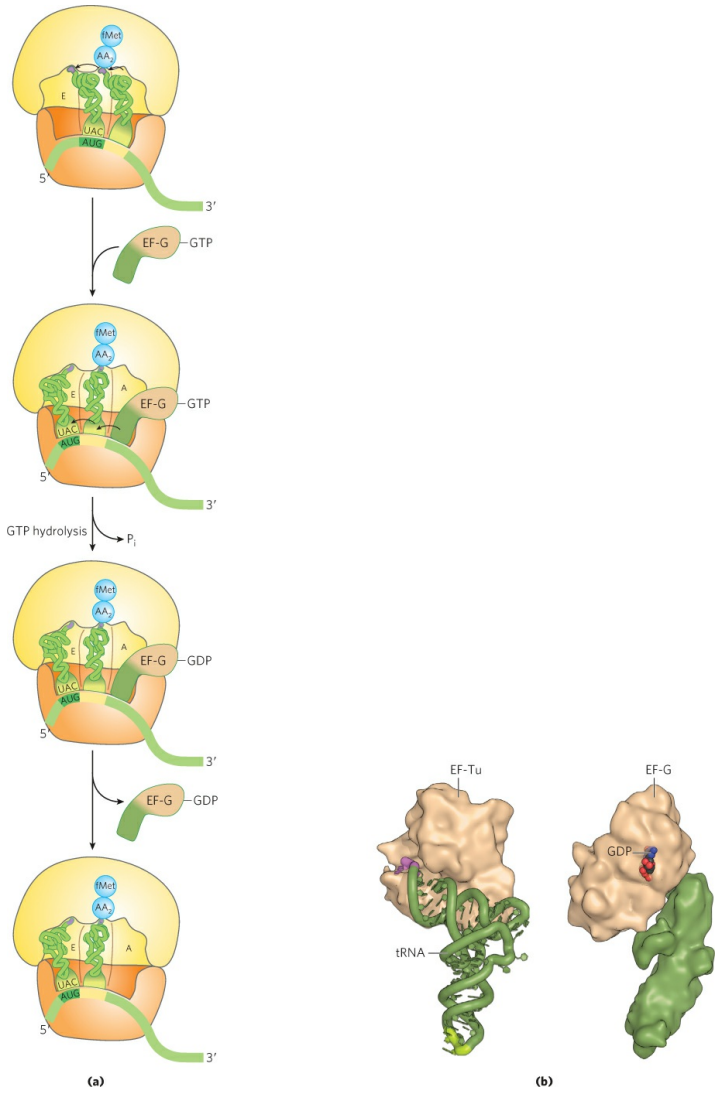


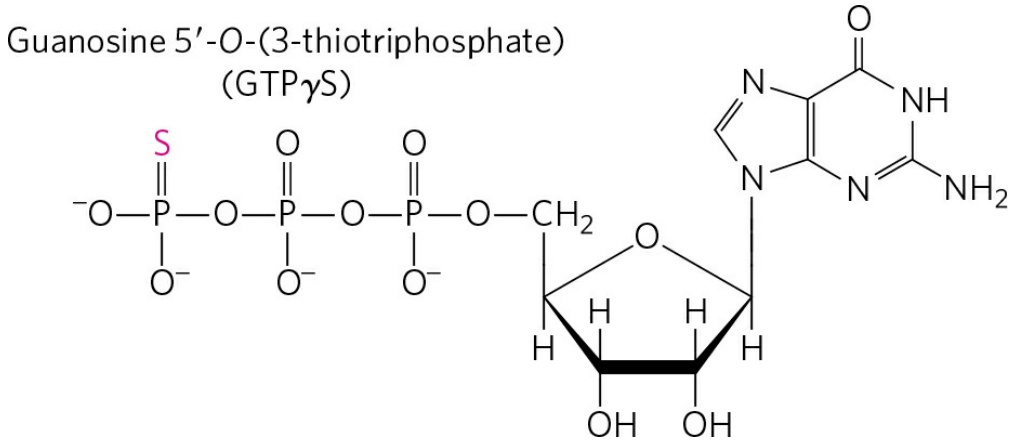
FIGURE 27-31 Third elongation step in bacteria: translocation. (a) The ribosome moves one codon toward the 3′ end of the mRNA, using energy provided by hydrolysis of GTP bound to EF-G (translocase). The dipeptidyl-tRNA is now entirely in the P site, leaving the A site open for an incoming (third) aminoacyl-tRNA. The uncharged tRNA later dissociates from the E site, and the elongation cycle begins again. **(b)** The structure of EF-G mimics the structure of EF-Tu complexed with tRNA. Shown here are (left) EF-Tu complexed with tRNA and (right) EF-G complexed with GDP. The carboxyl-terminal part of EF-G mimics the structure of the anticodon loop of tRNA in both shape and charge distribution. [Sources: (b) (left) PDB ID 1B23, P. Nissen et al., *Structure* 7:143, 1999; (right) PDB ID 1DAR, S. al-Karadaghi et al., *Structure* 4:555, 1996.]

The polypeptide remains attached to the tRNA of the most recent amino acid to be inserted. This association maintains the functional connection

between the information in the mRNA and its decoded polypeptide output. At the same time, the ester linkage between this tRNA and the carboxyl terminus of the growing polypeptide activates the terminal carboxyl group for nucleophilic attack by the incoming amino acid to form a new peptide bond (Fig. 27-30). As the existing ester linkage between the polypeptide and tRNA is broken during peptide bond formation, the linkage between the polypeptide and the information in the mRNA persists, because each newly added amino acid is still attached to its tRNA.

The elongation cycle in eukaryotes is similar to that in bacteria. Three eukaryotic elongation factors (eEF1α, eEF1$\beta\gamma$, and eEF2) have functions analogous to those of the bacterial elongation factors (EF-Tu, EF-Ts, and EF-G, respectively). When a new aminoacyl-tRNA binds to the A site, an allosteric interaction leads to ejection of the uncharged tRNA from the E site.

Proofreading on the Ribosome The GTPase activity of EF-Tu during the first step of elongation in bacterial cells (Fig. 27-29) makes an important contribution to the rate and fidelity of the overall biosynthetic process. Both the EF-Tu–GTP and EF-Tu–GDP complexes exist for a few milliseconds before they dissociate. These two intervals provide opportunities for the codon-anticodon interactions to be proofread. Incorrect aminoacyl-tRNAs normally dissociate from the A site during one of these periods. If the GTP analog guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) is used in place of GTP, hydrolysis is slowed, improving the fidelity (by increasing the proofreading intervals) but reducing the rate of protein synthesis.



The process of protein synthesis (including the characteristics of codon-anticodon pairing already described) has clearly been optimized through

evolution to balance the requirements for speed and fidelity. Improved fidelity might diminish speed, whereas increases in speed would probably compromise fidelity. And, recall that the proofreading mechanism on the ribosome establishes only that the proper codon-anticodon pairing has taken place, not that the correct amino acid is attached to the tRNA. If a tRNA is successfully aminoacylated with the wrong amino acid (as can be done experimentally), this incorrect amino acid is efficiently incorporated into a protein in response to whatever codon is normally recognized by the tRNA.

Stage 4: Termination of Polypeptide Synthesis Requires a Special Signal

Elongation continues until the ribosome adds the last amino acid coded by the mRNA. **Termination**, the fourth stage of polypeptide synthesis, is signaled by the presence of one of three termination codons in the mRNA (UAA, UAG, UGA), immediately following the final coded amino acid. Mutations in a tRNA anticodon that allow an amino acid to be inserted at a termination codon are generally deleterious to the cell (Box 27-3). In bacteria, once a termination codon occupies the ribosomal A site, three **termination factors**, or **release factors**—the proteins RF1, RF2, and RF3—contribute to (1) hydrolysis of the terminal peptidyl-tRNA bond; (2) release of the free polypeptide and the last tRNA, now uncharged, from the P site; and (3) dissociation of the 70S ribosome into its 30S and 50S subunits, ready to start a new cycle of polypeptide synthesis **(Fig. 27-32)**. RF1 recognizes the termination codons UAG and UAA, and RF2 recognizes UGA and UAA. Either RF1 or RF2 (depending on which codon is present) binds at a termination codon and induces peptidyl transferase to transfer the growing polypeptide to a water molecule rather than to another amino acid. The release factors have domains thought to mimic the structure of tRNA, as shown for the elongation factor EF-G in Figure 27-31b. The specific function of RF3 has not been firmly established, although it is thought to release the ribosomal subunit. In eukaryotes, a single release factor, eRF, recognizes all three termination codons.

Ribosome recycling leads to dissociation of the translation components. The release factors dissociate from the posttermination complex (with an uncharged tRNA in the P site) and are replaced by EF-G and a protein called ribosome recycling factor (RRF; M_r 20,300). Hydrolysis of GTP by EF-G leads to dissociation of the 50S subunit from the 30S–tRNA–mRNA complex. EF-G and RRF are replaced by IF3, which promotes dissociation of the tRNA. The mRNA is then released. The complex of IF3 and the 30S subunit is then ready to initiate another round of protein synthesis (Fig. 27-25).

Ribosome Rescue Ribosomes may stall during protein biosynthesis, especially while translating an mRNA that is damaged or incomplete. When the ribosome encounters the end of an mRNA before encountering a stop codon, the translocation step leads to formation of a stable "non-stop complex," in which the A site has no mRNA that can interact with a new charged tRNA. The non-stop complex cannot be recycled by the normal termination factors. Instead, the ribosome is rescued by a process called trans-translation **(Fig. 27-33)**. In virtually all bacteria, the rescue system consists of an RNA called transfer-messenger RNA (tmRNA) and a very small protein, small protein B (SmpB). These bind to the stalled complex in such a way that the tmRNA is positioned in the empty A site so that the ribosome can continue translation until it encounters a stop codon embedded in the tmRNA. The ribosome is then recycled, and both the defective mRNA and the polypeptide translated from it are degraded. Similar systems exist in eukaryotes.

Energy Cost of Fidelity in Protein Synthesis Synthesis of a protein true to the information specified in its mRNA requires energy. Formation of each aminoacyl-tRNA uses two high-energy phosphate groups. An additional ATP is consumed each time an incorrectly activated amino

acid is hydrolyzed by the deacylation activity of an aminoacyl-tRNA synthetase as part of its proofreading activity. A GTP is cleaved to GDP and P_i during the first elongation step, and another during the translocation step. Thus, on average, the energy derived from the hydrolysis of more than four NTPs to NDPs is required for the formation of each peptide bond of a polypeptide.

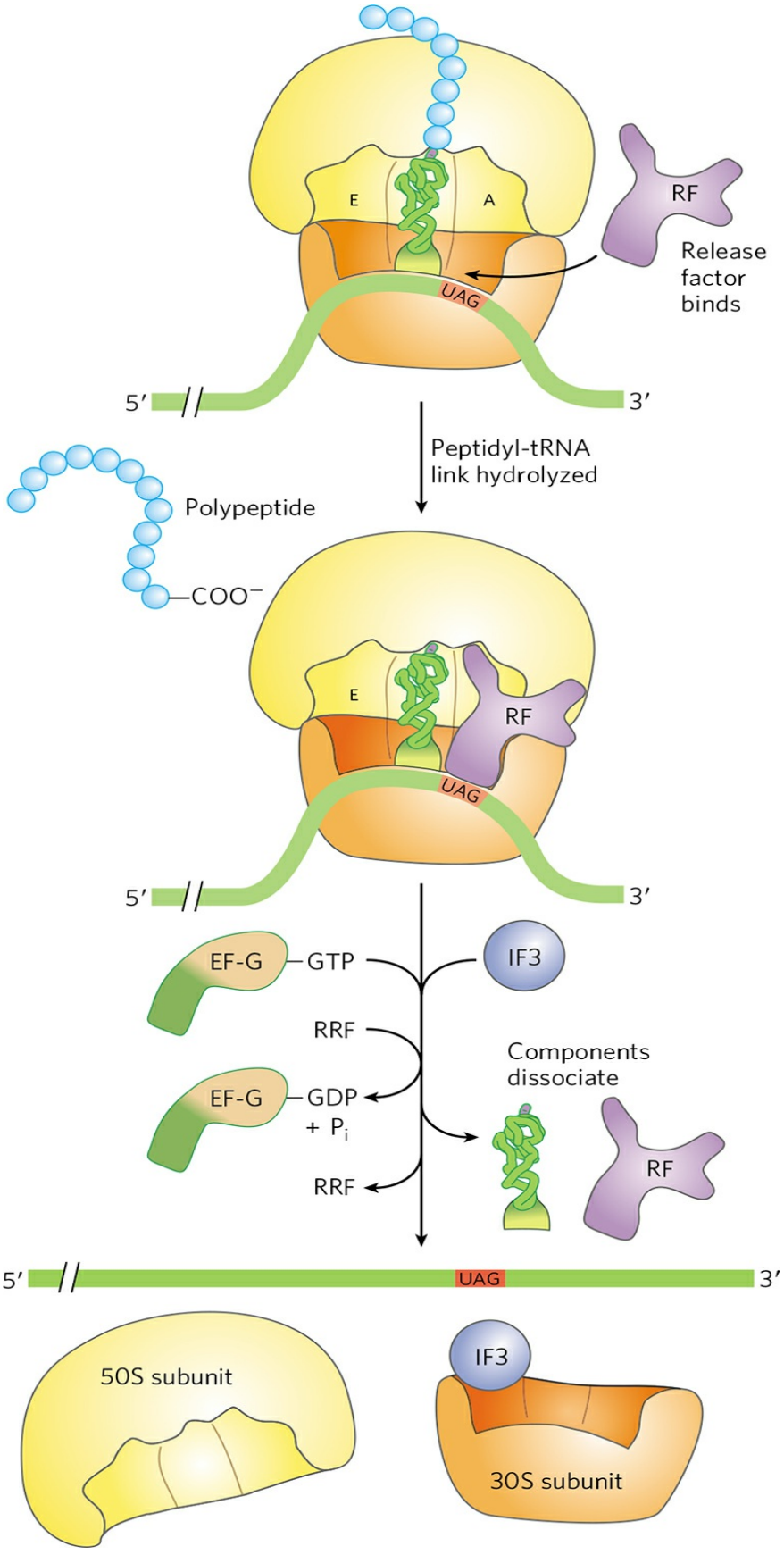

E
A
RF
Release factor binds
UAG
5′
3′
Peptidyl-tRNA link hydrolyzed
Polypeptide
—COO⁻
E
RF
UAG
5′
3′
EF-G
GTP
IF3
RRF
Components dissociate
EF-G
GDP + Pi
RF
RRF
5′
UAG
3′
50S subunit
IF3
30S subunit

FIGURE 27-32 Termination of protein synthesis in bacteria. Synthesis is terminated in response to a termination codon in the A site. First, a release factor, RF (RF1 or RF2, depending on which termination codon is present), binds to the A site. This leads to hydrolysis of the ester linkage between the nascent polypeptide and the tRNA in the P site and release of the completed polypeptide. Finally, the mRNA, deacylated tRNA, and release factor leave the ribosome, which dissociates into its 30S and 50S subunits, aided by ribosome recycling factor (RRF), IF3, and energy provided by EF-G–mediated GTP hydrolysis. The 30S subunit complex with IF3 is ready to begin another cycle of translation (see Fig. 27-25).

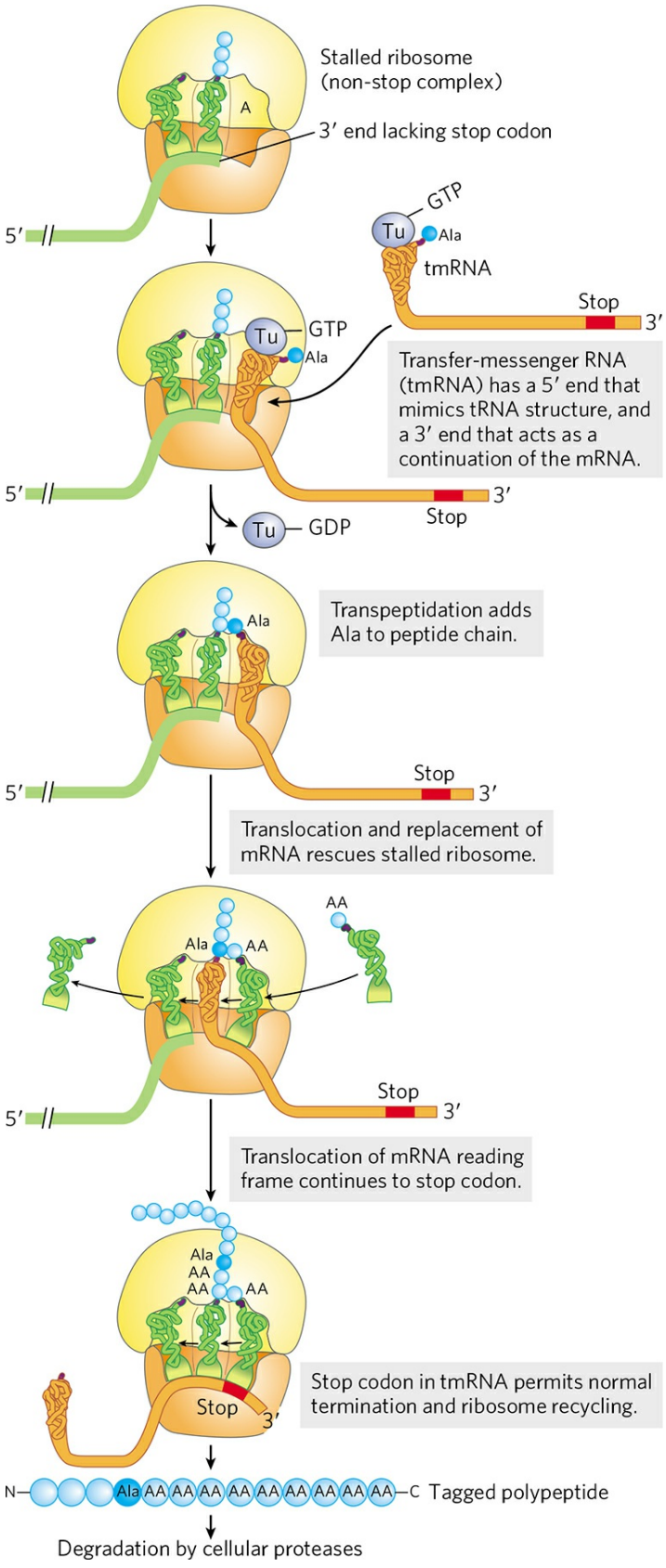

Stalled ribosome
(non-stop complex)
A
3′ end lacking stop codon
5′
GTP
Tu
Ala
tmRNA
Stop
3′
Tu
GTP
Ala
Transfer-messenger RNA (tmRNA) has a 5′ end that mimics tRNA structure, and a 3′ end that acts as a continuation of the mRNA.
5′
3′
Stop
Tu
GDP
Ala
Transpeptidation adds Ala to peptide chain.
Stop
5′
3′
Translocation and replacement of mRNA rescues stalled ribosome.
AA
Ala
AA
Stop
5′
3′
Translocation of mRNA reading frame continues to stop codon.
Ala
AA
AA
AA
AA
Stop
3′
Stop codon in tmRNA permits normal termination and ribosome recycling.
N
Ala AA AA AA AA AA AA AA AA AA AA
C
Tagged polypeptide
Degradation by cellular proteases

FIGURE 27-33 Rescue of stalled bacterial ribosomes by tmRNA. In bacteria, tmRNA rescues stalled ribosomes by mimicking both tRNA and mRNA. The multistep pathway permits release and degradation of the damaged mRNA and marks the truncated polypeptide for degradation.

This represents an exceedingly large thermodynamic "push" in the direction of synthesis: at least 4 × 30.5 kJ/mol = 122 kJ/mol of phosphodiester bond energy to generate a peptide bond, which has a standard free energy of hydrolysis of only about −21 kJ/mol. The net free-energy change during peptide bond synthesis is thus −101 kJ/mol. Proteins are information-containing polymers. The biochemical goal is not simply the formation of a peptide bond but the formation of a peptide bond between two *specified* amino acids. Each of the high-energy phosphate compoundsexpended in this process plays a critical role in maintaining proper alignmentbetween each new codon in the mRNA and its associated amino acid at thegrowing end of the polypeptide. This energy permits very high fidelity in thebiological translation of the genetic message of mRNA into the amino acidsequence of proteins.