PROTEIN SYNTHESIS

2.6.1 Introduction

An mRNA contains a series of codons that interact with the anticodons of aminoacyl-tRNAs so that a corresponding series of amino acids is incorporated into a polypeptide chain. The ribosome provides the environment for controlling the interaction between mRNA and aminoacyl-tRNA. The ribosome behaves like a small migrating factory that travels along the template engaging in rapid cycles of peptide bond synthesis. Aminoacyl-tRNAs shoot in and out of the particle at a fearsome rate, depositing amino acids; and elongation factors cyclically associate with and dissociate from the ribosome. Together with its accessory factors, the ribosome provides the full range of activities required for all the steps of protein synthesis.

Figure 6.1 shows the relative dimensions of the components of the protein synthetic apparatus. The ribosome consists of two subunits that have specific roles in protein synthesis. Messenger RNA is associated with the small subunit; ~30 bases of the mRNA are bound at any time. The mRNA threads its way along the surface close to the junction of the subunits. Two tRNA molecules are active in protein synthesis at any moment; so polypeptide elongation involves reactions taking place at just two of the (roughly) 10 codons covered by the ribosome. The two tRNAs are inserted into internal sites that stretch across the subunits. A third tRNA may remain present on the ribosome after it has been used in protein synthesis, before being recycled.

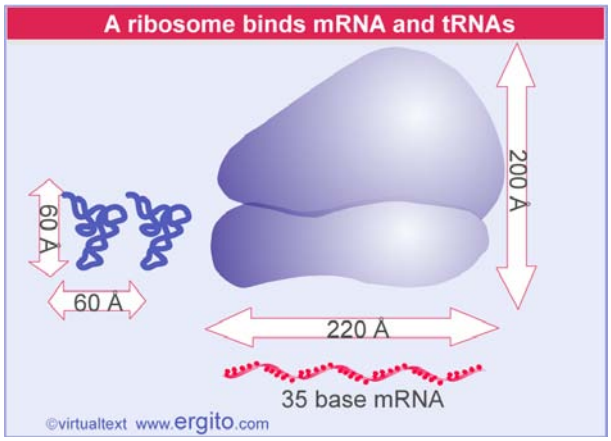

Figure 6.1 Size comparisons show that the ribosome is large enough to bind tRNAs and mRNA.

The basic form of the ribosome has been conserved in evolution, but there are appreciable variations in the overall size and proportions of RNA and protein in the ribosomes of bacteria, eukaryotic cytoplasm, and organelles. **Figure 6.2** compares the components of bacterial and mammalian ribosomes. Both are ribonucleoprotein particles that contain more RNA than protein. The ribosomal proteins are known as *r-protein*s.

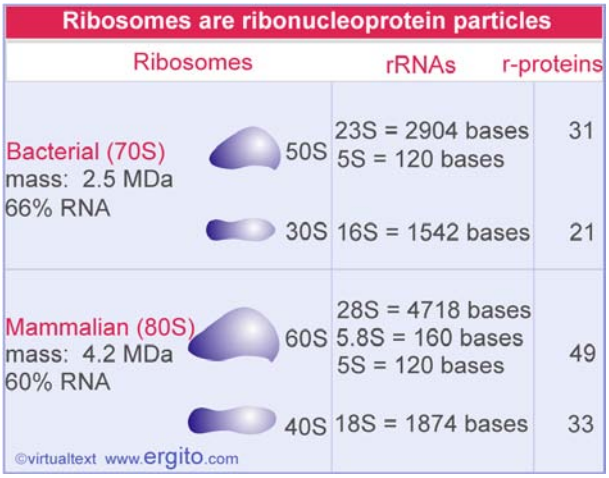

Figure 6.2 Ribosomes are large ribonucleoprotein particles that contain more RNA than protein and dissociate into large and small subunits.

Each of the ribosome subunits contains a major rRNA and a number of small proteins. The large subunit may also contain smaller RNA(s). In *E. coli*, the small (30S) subunit consists of the 16S rRNA and 21 r-proteins. The large (50S) subunit contains 23S rRNA, the small 5S RNA, and 31 proteins. With the exception of one protein present at four copies per ribosome, there is one copy of each protein. The major RNAs constitute the major part of the mass of the bacterial ribosome. Their presence is pervasive, and probably most or all of the ribosomal proteins actually contact rRNA. So the major rRNAs form what is sometimes thought of as the backbone of each subunit, a continuous thread whose presence dominates the structure, and which determines the positions of the ribosomal proteins.

The ribosomes of higher eukaryotic cytoplasm are larger than those of bacteria. The total content of both RNA and protein is greater; the major RNA molecules are longer (called 18S and 28S rRNAs), and there are more proteins. Probably most or all of the proteins are present in stoichiometric amounts. RNA is still the predominant component by mass.

Organelle ribosomes are distinct from the ribosomes of the cytosol, and take varied forms. In some cases, they are almost the size of bacterial ribosomes and have 70% RNA; in other cases, they are only 60S and have <30% RNA.

The ribosome possesses several active centers, each of which is constructed from a group of proteins associated with a region of ribosomal RNA. The active centers require the direct participation of rRNA in a structural or even catalytic role. Some catalytic functions require individual proteins, but none of the activities can be reproduced by isolated proteins or groups of proteins; they function only in the context of the ribosome.

Two types of information are important in analyzing the ribosome. Mutations implicate particular ribosomal proteins or bases in rRNA in participating in particular reactions. Structural analysis, including direct modification of components of the ribosome and comparisons to identify conserved features in rRNA, identifies the physical locations of components involved in particular functions.

PROTEIN SYNTHESIS

2.6.2 Protein synthesis occurs by initiation, elongation, and termination

Key Terms

The **A site** of the ribosome is the site that an aminoacyl-tRNA enters to base pair with the codon.

The **P site** of the ribosome is the site that is occupied by peptidyl-tRNA, the tRNA carrying the nascent polypeptide chain, still paired with the codon to which it bound in the A site.

Peptidyl-tRNA is the tRNA to which the nascent polypeptide chain has been transferred following peptide bond synthesis during protein synthesis.

Deacylated tRNA has no amino acid or polypeptide chain attached because it has completed its role in protein synthesis and is ready to be released from the ribosome.

Translocation is the movement of the ribosome one codon along mRNA after the addition of each amino acid to the polypeptide chain.

Elongation is the stage in a macromolecular synthesis reaction (replication, transcription, or translation) when the nucleotide or polypeptide chain is being extended by the addition of individual subunits.

Termination is a separate reaction that ends a macromolecular synthesis reaction (replication, transcription, or translation), by stopping the addition of subunits, and (typically) causing disassembly of the synthetic apparatus.

Key Concepts

- The ribosome has 3 tRNA-binding sites.
- An aminoacyl-tRNA enters the A site.
- Peptidyl-tRNA is bound in the P site.
- Deacylated tRNA exits via the E site.
- An amino acid is added to the polypeptide chain by transferring the polypeptide from peptidyl-tRNA in the P site to aminoacyl-tRNA in the A site.

An amino acid is brought to the ribosome by an aminoacyl-tRNA. Its addition to the growing protein chain occurs by an interaction with the tRNA that brought the previous amino acid. Each of these tRNA lies in a distinct site on the ribosome. **Figure 6.3** shows that the two sites have different features:

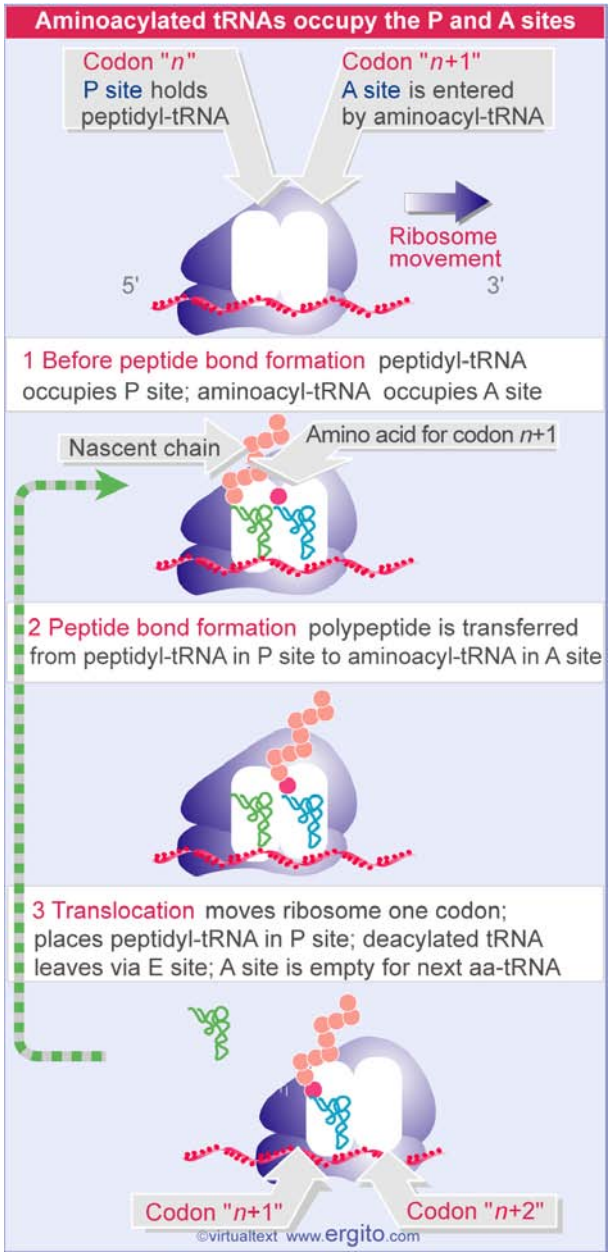

Figure 6.3 The ribosome has two sites for binding charged tRNA.

- An incoming aminoacyl-tRNA binds to the **A site**. Prior to the entry of aminoacyl-tRNA, the site exposes the codon representing the next amino acid due to be added to the chain.

- The codon representing the most recent amino acid to have been added to the nascent polypeptide chain lies in the **P site**. This site is occupied by **peptidyl-tRNA**, a tRNA carrying the nascent polypeptide chain.

Figure 6.4 shows that the aminoacyl end of the tRNA is located on the large subunit,

while the anticodon at the other end interacts with the mRNA bound by the small subunit. So the P and A sites each extend across both ribosomal subunits.

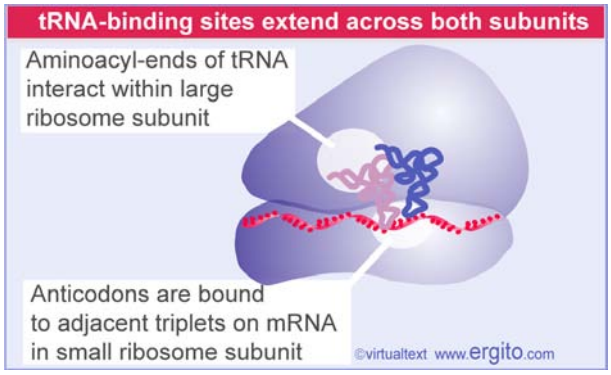

Figure 6.4 The P and A sites position the two interacting tRNAs across both ribosome subunits.

For a ribosome to synthesize a peptide bond, it must be in the state shown in step 1 in **Figure 6.3**, when peptidyl-tRNA is in the P site and aminoacyl-tRNA is in the A site. Then peptide bond formation occurs when the polypeptide carried by the peptidyl-tRNA is transferred to the amino acid carried by the aminoacyl-tRNA. This reaction is catalyzed by the large subunit of the ribosome.

Transfer of the polypeptide generates the ribosome shown in step 2, in which the **deacylated tRNA**, lacking any amino acid, lies in the P site, while a new peptidyl-tRNA has been created in the A site. This peptidyl-tRNA is one amino acid residue longer than the peptidyl-tRNA that had been in the P site in step 1.

Then the ribosome moves one triplet along the messenger. This stage is called **translocation**. The movement transfers the deacylated tRNA out of the P site, and moves the peptidyl-tRNA into the P site (see step 3). The next codon to be translated now lies in the A site, ready for a new aminoacyl-tRNA to enter, when the cycle will be repeated. **Figure 6.5** summarizes the interaction between tRNAs and the ribosome.

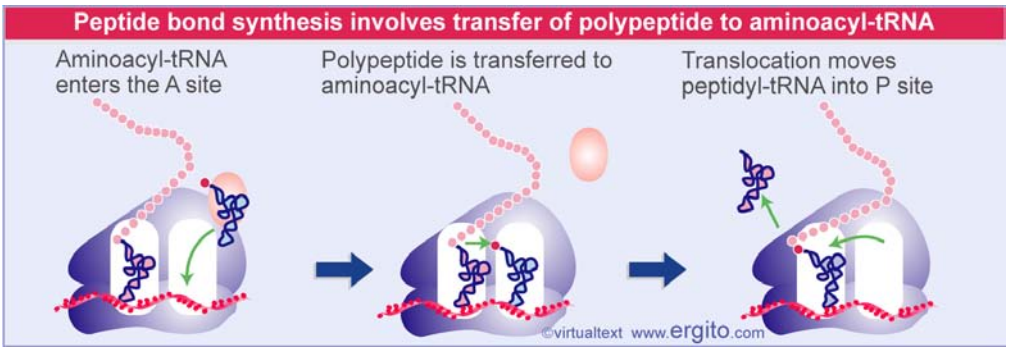

Figure 6.5 Aminoacyl-tRNA enters the A site, receives the polypeptide chain from peptidyl-tRNA, and is transferred into the P site for the next cycle of elongation.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.2 to view properly.

The deacylated tRNA leaves the ribosome via another tRNA-binding site, the E site. This site is transiently occupied by the tRNA en route between leaving the P site and being released from the ribosome into the cytosol. So the flow of tRNA is into the A site, through the P site, and out through the E site (see also **Figure 6.28**). **Figure 6.6** compares the movement of tRNA and mRNA, which may be thought of as a sort of ratchet in which the reaction is driven by the codon-anticodon interaction.

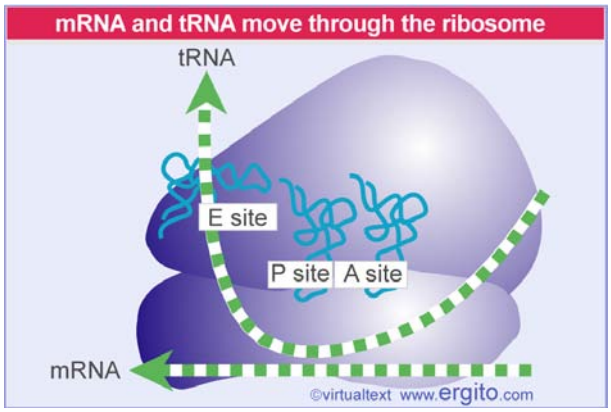

Figure 6.6 tRNA and mRNA move through the ribosome in the same direction.

Protein synthesis falls into the three stages shown in **Figure 6.7**:

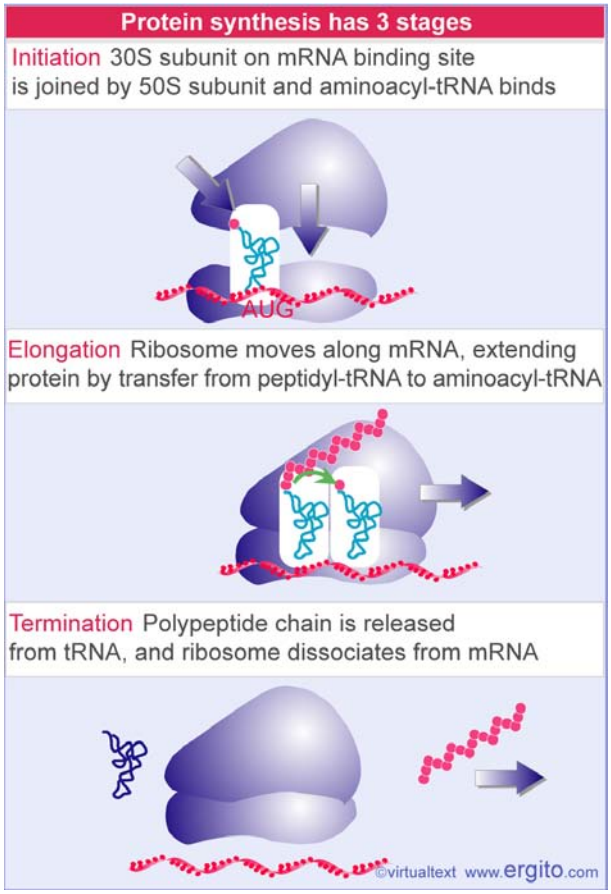

Figure 6.7 Protein synthesis falls into three stages.

- Initiation involves the reactions that precede formation of the peptide bond between the first two amino acids of the protein. It requires the ribosome to bind to the mRNA, forming an initiation complex that contains the first aminoacyl-tRNA. This is a relatively slow step in protein synthesis, and usually determines the rate at which an mRNA is translated.

- **Elongation** includes all the reactions from synthesis of the first peptide bond to addition of the last amino acid. Amino acids are added to the chain one at a time; the addition of an amino acid is the most rapid step in protein synthesis.

- **Termination** encompasses the steps that are needed to release the completed polypeptide chain; at the same time, the ribosome dissociates from the mRNA.

Different sets of accessory factors assist the ribosome at each stage. Energy is provided at various stages by the hydrolysis of GTP.

During initiation, the small ribosomal subunit binds to mRNA and then is joined by the 50S subunit. During elongation, the mRNA moves through the ribosome and is translated in triplets. (Although we usually talk about the ribosome moving along mRNA, it is more realistic to think in terms of the mRNA being pulled through the ribosome.) At termination, the protein is released, mRNA is released, and the

individual ribosomal subunits dissociate in order to be used again.

PROTEIN SYNTHESIS

2.6.3 Special mechanisms control the accuracy of protein synthesis

Key Concepts

- The accuracy of protein synthesis is controlled by specific mechanisms at each stage.

We know that protein synthesis is generally accurate, because of the consistency that is found when we determine the sequence of a protein. There are few detailed measurements of the error rate *in vivo*, but it is generally thought to lie in the range of 1 error for every 10^4 - 10^5 amino acids incorporated. Considering that most proteins are produced in large quantities, this means that the error rate is too low to have any effect on the phenotype of the cell.

It is not immediately obvious how such a low error rate is achieved. In fact, the nature of discriminatory events is a general issue raised by several steps in gene expression. How do synthetases recognize just the corresponding tRNAs and amino acids? How does a ribosome recognize only the tRNA corresponding to the codon in the A site? How do the enzymes that synthesize DNA or RNA recognize only the base complementary to the template? Each case poses a similar problem: how to distinguish one particular member from the entire set, all of which share the same general features.

Probably any member initially can contact the active center by a random-hit process, but then the wrong members are rejected and only the appropriate one is accepted. The appropriate member is always in a minority (1 of 20 amino acids, 1 of ~40 tRNAs, 1 of 4 bases), so the criteria for discrimination must be strict. The point is that the enzyme must have some mechanism for increasing discrimination from the level that would be achieved merely by making contacts with the available surfaces of the substrates.

Figure 6.8 summarizes the error rates at the steps that can affect the accuracy of protein synthesis.

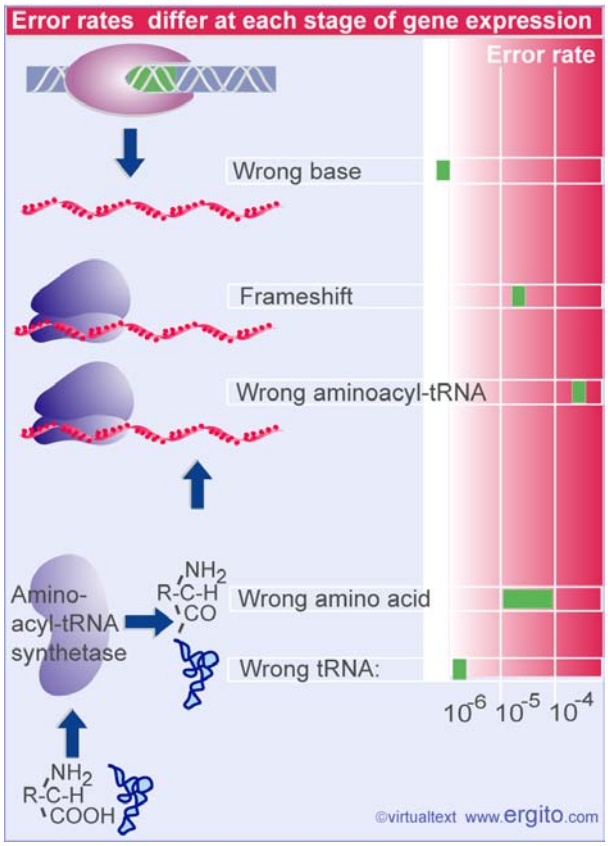

Figure 6.8 Errors occur at rates from 10^{-6} to 5×10^{-4} at different stages of protein synthesis.

Errors in transcribing mRNA are rare – probably $<10^{-6}$. This is an important stage to control, because a single mRNA molecule is translated into many protein copies. We do not know very much about the mechanisms.

The ribosome can make two types of errors in protein synthesis. It may cause a frameshift by skipping a base when it reads the mRNA (or in the reverse direction by reading a base twice, once as the last base of one codon and then again as the first base of the next codon). These errors are rare, $\sim 10^{-5}$. Or it may allow an incorrect aminoacyl-tRNA to (mis)pair with a codon, so that the wrong amino acid is incorporated. This is probably the most common error in protein synthesis, $\sim 5 \times 10^{-4}$. It is controlled by ribosome structure and velocity (see *Molecular Biology 2.7.15 The ribosome influences the accuracy of translation*).

A tRNA synthetase can make two types of error. It can place the wrong amino acid on its tRNA; or it can charge its amino acid with the wrong tRNA. The incorporation of the wrong amino acid is more common, probably because the tRNA offers a larger surface with which the enzyme can make many more contacts to ensure specificity. Aminoacyl-tRNA synthetases have specific mechanisms to correct errors before a mischarged tRNA is released (see *Molecular Biology 2.7.11 Synthetases use proofreading to improve accuracy*).

This content is available online at http://www.ergito.com/main.jsp?bcs=MBIO.2.6.3

PROTEIN SYNTHESIS

2.6.4 Initiation in bacteria needs 30S subunits and accessory factors

Key Terms

A **ribosome-binding site** is a sequence on bacterial mRNA that includes an initiation codon that is bound by a 30S subunit in the initiation phase of protein synthesis.

An **initiation complex** in bacterial protein synthesis contains a small ribosome subunit, initiation factors, and initiator aminoacyl-tRNA bound to mRNA at an AUG initiation codon.

Initiation factors (IF) (IF in prokaryotes, eIF in eukaryotes) are proteins that associate with the small subunit of the ribosome specifically at the stage of initiation of protein synthesis.

IF-1 is a bacterial initiation factor that stabilizes the initiation complex.

IF-2 is a bacterial initiation factor that binds the initiator tRNA to the initiation complex.

IF-3 is a bacterial initiation factor required for 30S subunits to bind to initiation sites in mRNA. It also prevents 30S subunits from binding to 50S subunits.

Key Concepts

- Initiation of protein synthesis requires separate 30S and 50S ribosome subunits.
- Initiation factors (IF-1,2,3), which bind to 30S subunits, are also required.
- A 30S subunit carrying initiation factors binds to an initiation site on mRNA to form an initiation complex.
- IF-3 must be released to allow 50S subunits to join the 30S-mRNA complex.

Bacterial ribosomes engaged in elongating a polypeptide chain exist as 70S particles. At termination, they are released from the mRNA as free ribosomes. In growing bacteria, the majority of ribosomes are synthesizing proteins; the free pool is likely to contain ~20% of the ribosomes.

Ribosomes in the free pool can dissociate into separate subunits; so 70S ribosomes are in dynamic equilibrium with 30S and 50S subunits. *Initiation of protein synthesis is not a function of intact ribosomes, but is undertaken by the separate subunits,* which reassociate during the initiation reaction. **Figure 6.9** summarizes the ribosomal subunit cycle during protein synthesis in bacteria.

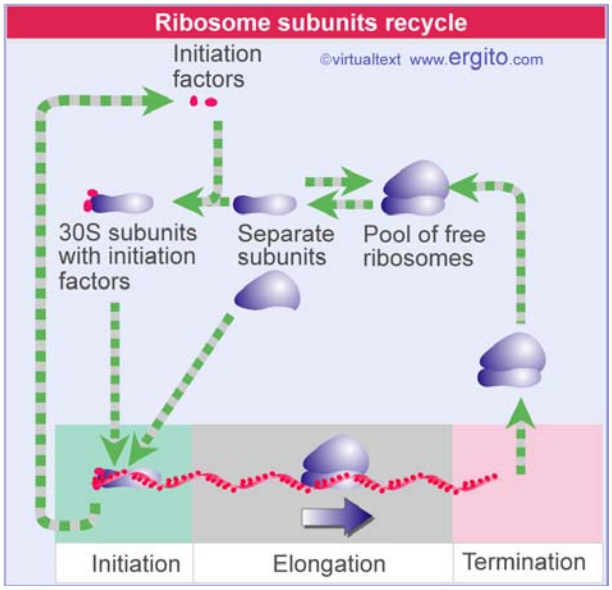

Figure 6.9 Initiation requires free ribosome subunits. When ribosomes are released at termination, the 30S subunits bind initiation factors, and dissociate to generate free subunits. When subunits reassociate to give a functional ribosome at initiation, they release the factors.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.4 to view properly.

Initiation occurs at a special sequence on mRNA called the **ribosome-binding site**. This is a short sequence of bases that precedes the coding region (see **Figure 6.16**). The small and large subunits associate at the ribosome-binding site to form an intact ribosome. The reaction occurs in two steps:

- Recognition of mRNA occurs when a small subunit binds to form an **initiation complex** at the ribosome-binding site.
- Then a large subunit joins the complex to generate a complete ribosome.

Although the 30S subunit is involved in initiation, it is not by itself competent to undertake the reactions of binding mRNA and tRNA. It requires additional proteins called **initiation factors** (**IF**). These factors are found only on 30S subunits, and they are released when the 30S subunits associate with 50S subunits to generate 70S ribosomes. This behavior distinguishes initiation factors from the structural proteins of the ribosome. The initiation factors are concerned solely with formation of the initiation complex, they are absent from 70S ribosomes, and they play no part in the stages of elongation (for review see 428). **Figure 6.10** summarizes the stages of initiation.

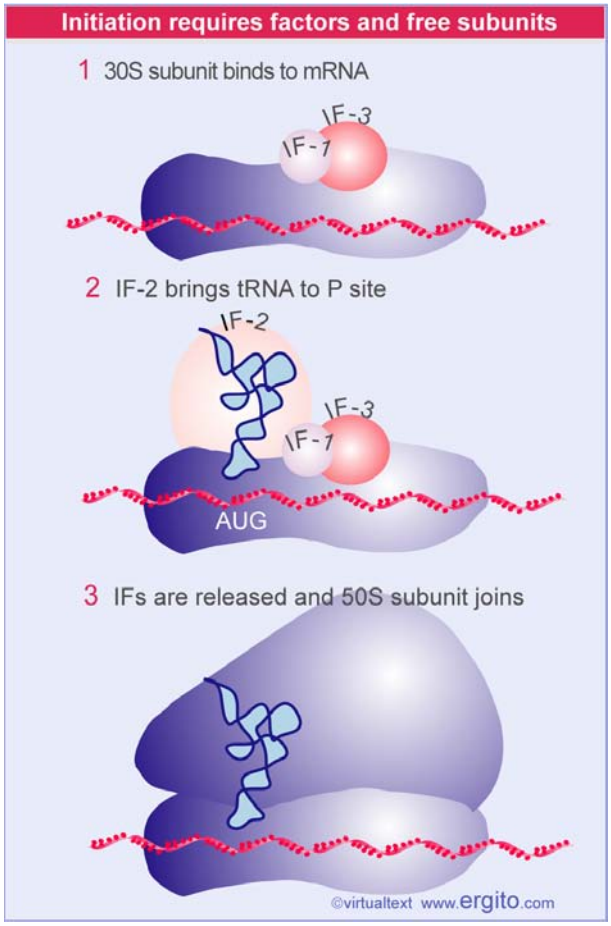

Figure 6.10 Initiation factors stabilize free 30S subunits and bind initiator tRNA to the 30S-mRNA complex.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.4 to view properly.

Bacteria use three initiation factors, numbered **IF-1**, **IF-2**, and **IF-3**. They are needed for both mRNA and tRNA to enter the initiation complex:

- IF-3 is needed for 30S subunits to bind specifically to initiation sites in mRNA.
- IF-2 binds a special initiator tRNA and controls its entry into the ribosome.
- IF-1 binds to 30S subunits only as a part of the complete initiation complex. It binds to the A site and prevents aminoacyl-tRNA from entering (3053). Its location also may impede the 30S subunit from binding to the 50S subunit (2391).

IF-3 has multiple functions: it is needed first to stabilize (free) 30S subunits; then it enables them to bind to mRNA; and as part of the 30S-mRNA complex it checks the accuracy of recognition of the first aminoacyl-tRNA (see *Molecular Biology 2.6.6 Use of fMet-tRNA$_f$ is controlled by IF-2 and the ribosome*.

The first function of IF-3 controls the equilibrium between ribosomal states, as shown in **Figure 6.11**. IF-3 binds to free 30S subunits that are released from the pool of 70S ribosomes. The presence of IF-3 prevents the 30S subunit from reassociating with a 50S subunit. The reaction between IF-3 and the 30S subunit is stoichiometric: one molecule of IF-3 binds per subunit. There is a relatively small amount of IF-3, so its availability determines the number of free 30S subunits.

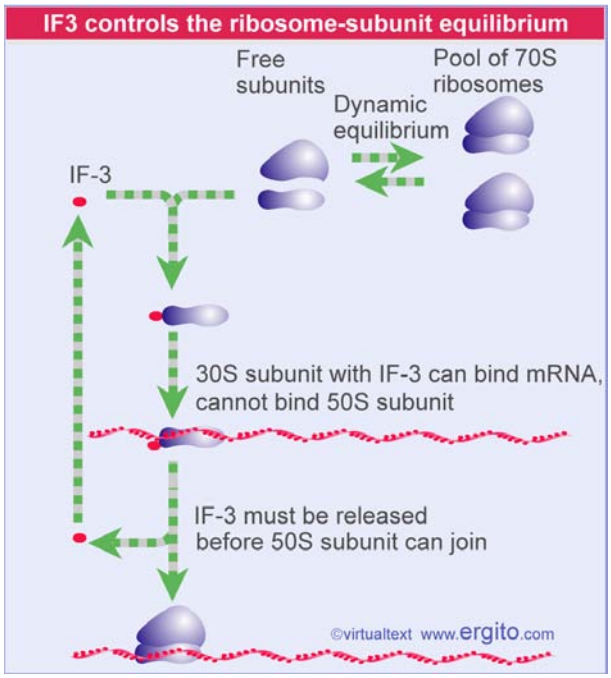

Figure 6.11 Initiation requires 30S subunits that carry IF-3.

IF-3 binds to the surface of the 30S subunit in the vicinity of the A site. There is significant overlap between the bases in 16S rRNA protected by IF-3 and those protected by binding of the 50S subunit, suggesting that it physically prevents junction of the subunits (3054). IF-3 therefore behaves as an anti-association factor that causes a 30S subunit to remain in the pool of free subunits.

The second function of IF-3 controls the ability of 30S subunits to bind to mRNA. Small subunits must have IF-3 in order to form initiation complexes with mRNA. IF-3 must be released from the 30S·mRNA complex in order to enable the 50S subunit to join. On its release, IF-3 immediately recycles by finding another 30S subunit.

IF-2 has a ribosome-dependent GTPase activity: it sponsors the hydrolysis of GTP in the presence of ribosomes, releasing the energy stored in the high-energy bond. The GTP is hydrolyzed when the 50S subunit joins to generate a complete ribosome. The GTP cleavage could be involved in changing the conformation of the ribosome, so that the joined subunits are converted into an active 70S ribosome.

Last updated on January 6, 2004

Reviews

428. Maitra, U. et al. (1982). *Initiation factors in protein biosynthesis.* Annu. Rev. Biochem. 51, 869-900.

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PROTEIN SYNTHESIS

2.6.5 A special initiator tRNA starts the polypeptide chain

Key Terms

The **initiation codon** is a special codon (usually AUG) used to start synthesis of a protein.

N-formyl-methionyl-tRNA ($tRNA_f^{Met}$) is the aminoacyl-tRNA that initiates bacterial protein synthesis. The amino group of the methionine is formylated.

$tRNA_f^{Met}$ is the special RNA that is to initiate protein synthesis in bacteria. It mostly uses AUG, but can also respond to GUG and CUG.

$tRNA_m^{Met}$ inserts methionine at internal AUG codons.

Key Concepts

- Protein synthesis starts with a methionine amino acid usually coded by AUG.
- Different methionine tRNAs are involved in initiation and elongation.
- The initiator tRNA has unique structural features that distinguish it from all other tRNAs.
- The NH_2 group of the methionine bound to bacterial initiator tRNA is formylated.

Synthesis of all proteins starts with the same amino acid: methionine. The signal for initiating a polypeptide chain is a special **initiation codon** that marks the start of the reading frame. Usually the initiation codon is the triplet AUG, but in bacteria, GUG or UUG are also used.

The AUG codon represents methionine, and two types of tRNA can carry this amino acid. One is used for initiation, the other for recognizing AUG codons during elongation.

In bacteria and in eukaryotic organelles, the initiator tRNA carries a methionine residue that has been formylated on its amino group, forming a molecule of **N-formyl-methionyl-tRNA**. The tRNA is known as **$tRNA_f^{Met}$**. The name of the aminoacyl-tRNA is usually abbreviated to fMet-$tRNA_f$ (3057).

The initiator tRNA gains its modified amino acid in a two stage reaction. First, it is charged with the amino acid to generate Met-$tRNA_f$; then the formylation reaction shown in **Figure 6.12** blocks the free NH_2 group. Although the blocked amino acid group would prevent the initiator from participating in chain elongation, it does not interfere with the ability to initiate a protein.

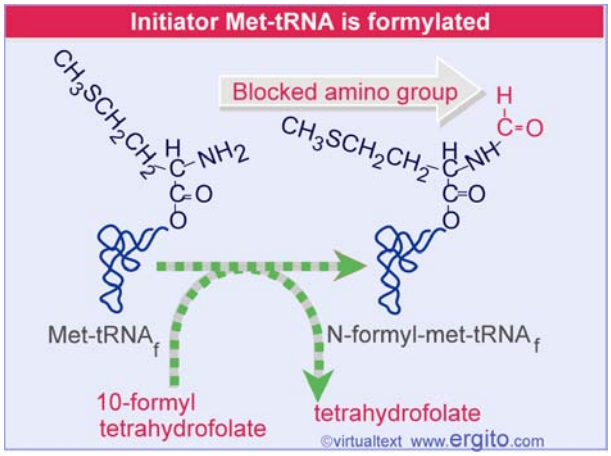

Figure 6.12 The initiator N-formyl-methionyl-tRNA (fMet-tRNA$_f$) is generated by formylation of methionyl-tRNA, using formyl-tetrahydrofolate as cofactor.

This tRNA is used only for initiation. It recognizes the codons AUG or GUG (occasionally UUG). The codons are not recognized equally well: the extent of initiation declines about half when AUG is replaced by GUG, and declines by about half again when UUG is employed.

The species responsible for recognizing AUG codons in internal locations is **tRNA$_m^{Met}$**. This tRNA responds only to internal AUG codons. Its methionine cannot be formylated.

What features distinguish the fMet-tRNA$_f$ initiator and the Met-tRNA$_m$ elongator? Some characteristic features of the tRNA sequence are important, as summarized in **Figure 6.13**. Some of these features are needed to prevent the initiator from being used in elongation, others are necessary for it to function in initiation:

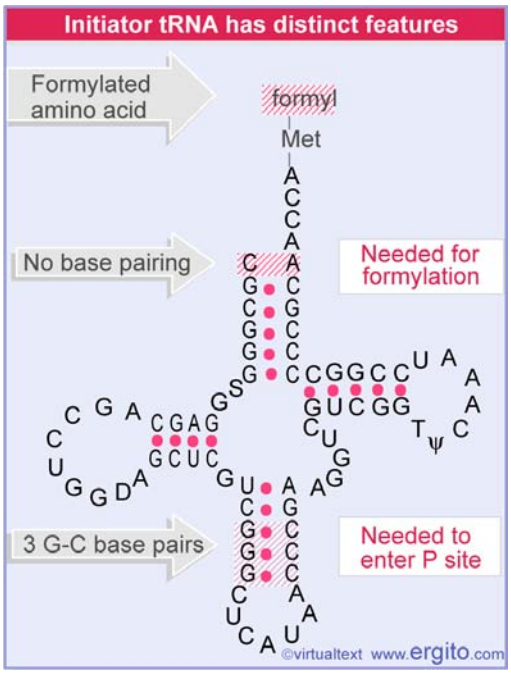

Figure 6.13 fMet-tRNA$_f$ has unique features that distinguish it as the initiator tRNA.

- Formylation is not strictly necessary, because nonformylated Met-tRNA$_f$ can function as an initiator, but it improves the efficiency with which the Met-tRNA$_f$ is used, because it is one of the features recognized by the factor IF-2 that binds the initiator tRNA (3056).

- The bases that face one another at the last position of the stem to which the amino acid is connected are paired in all tRNAs except tRNA$_f^{Met}$. Mutations that create a base pair in this position of tRNA$_f^{Met}$ allow it to function in elongation. The absence of this pair is therefore important in preventing tRNA$_f^{Met}$ from being used in elongation. It is also needed for the formylation reaction (3058).

- A series of 3 G·C pairs in the stem that precedes the loop containing the anticodon is unique to tRNA$_f^{Met}$. These base pairs are required to allow the fMet-tRNA$_f$ to be inserted directly into the P site.

In bacteria and mitochondria, the formyl residue on the initiator methionine is removed by a specific deformylase enzyme to generate a normal NH_2 terminus. If methionine is to be the N-terminal amino acid of the protein, this is the only necessary step. In about half the proteins, the methionine at the terminus is removed by an aminopeptidase, creating a new terminus from R_2 (originally the second amino acid incorporated into the chain). When both steps are necessary, they occur sequentially. The removal reaction(s) occur rather rapidly, probably when the nascent polypeptide chain has reached a length of 15 amino acids.

Last updated on 8-12-2002

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PROTEIN SYNTHESIS

2.6.6 Use of fMet-tRNA$_f$ is controlled by IF-2 and the ribosome

Key Terms

The **context** of a codon in mRNA refers to the fact that neighboring sequences may change the efficiency with which a codon is recognized by its aminoacyl-tRNA or is used to terminate protein synthesis.

Key Concepts

- IF-2 binds the initiator fMet-tRNA$_f$ and allows it to enter the partial P site on the 30S subunit.

The meaning of the AUG and GUG codons depends on their **context**. When the AUG codon is used for initiation, it is read as formyl-methionine; when used within the coding region, it represents methionine. The meaning of the GUG codon is even more dependent on its location. When present as the first codon, it is read via the initiation reaction as formyl-methionine. Yet when present within a gene, it is read by Val-tRNA, one of the regular members of the tRNA set, to provide valine as required by the genetic code.

How is the context of AUG and GUG codons interpreted? **Figure 6.14** illustrates the decisive role of the ribosome, in conjunction with accessory factors.

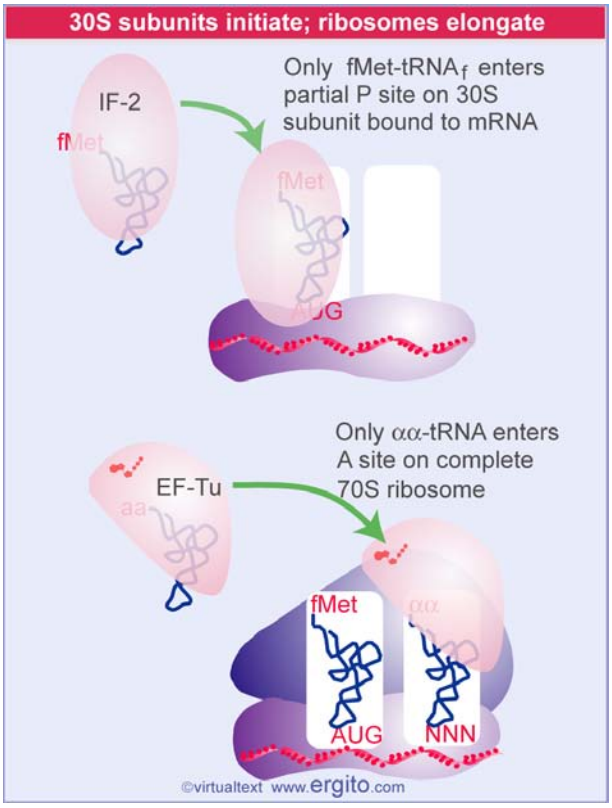

Figure 6.14 Only fMet-tRNA$_f$ can be used for initiation by 30S subunits; only other aminoacyl-tRNAs (α α -tRNA) can be used for elongation by 70S ribosomes.

In an initiation complex, the small subunit alone is bound to mRNA. The initiation codon lies within the part of the P site carried by the small subunit. The only aminoacyl-tRNA that can become part of the initiation complex is the initiator, which has the unique property of being able to enter directly into the partial P site to recognize its codon.

When the large subunit joins the complex, the partial tRNA-binding sites are converted into the intact P and A sites. The initiator fMet-tRNA$_f$ occupies the P site. and the A site is available for entry of the aminoacyl-tRNA complementary to the second codon of the gene. The first peptide bond forms between the initiator and the next aminoacyl-tRNA.

Initiation prevails when an AUG (or GUG) codon lies within a ribosome-binding site, because only the initiator tRNA can enter the partial P site generated when the 30S subunit binds *de novo* to the mRNA. Internal reading prevails subsequently, when the codons are encountered by a ribosome that is continuing to translate an mRNA, because only the regular aminoacyl-tRNAs can enter the (complete) A site.

Accessory factors are critical in controlling the usage of aminoacyl-tRNAs. All aminoacyl-tRNAs associate with the ribosome by binding to an accessory factor. The factor used in initiation is IF-2 (see *Molecular Biology 2.6.4 Initiation in bacteria needs 30S subunits and accessory factors*), and the corresponding factor used at elongation is EF-Tu (see *Molecular Biology 2.6.10 Elongation factor Tu loads*

aminoacyl-tRNA into the A site).

The initiation factor IF-2 places the initiator tRNA into the P site. By forming a complex specifically with fMet-tRNA$_f$, IF-2 ensures that only the initiator tRNA, and none of the regular aminoacyl-tRNAs, participates in the initiation reaction. Conversely, EF-Tu, which places aminoacyl-tRNAs in the A site cannot bind fMet-tRNA$_f$, which is therefore excluded from use during elongation.

An additional check on accuracy is made by IF-3, which stabilizes binding of the initiator tRNA by recognizing correct base pairing with the second and third bases of the AUG initiation codon.

Figure 6.15 details the series of events by which IF-2 places the fMet-tRNA$_f$ initiator in the P site. IF-2, bound to GTP, associates with the P site of the 30S subunit. At this point, the 30S subunit carries all the initiation factors. fMet-tRNA$_f$. then binds to the IF-2 on the 30S subunit. IF-2 then transfers the tRNA into the partial P site.

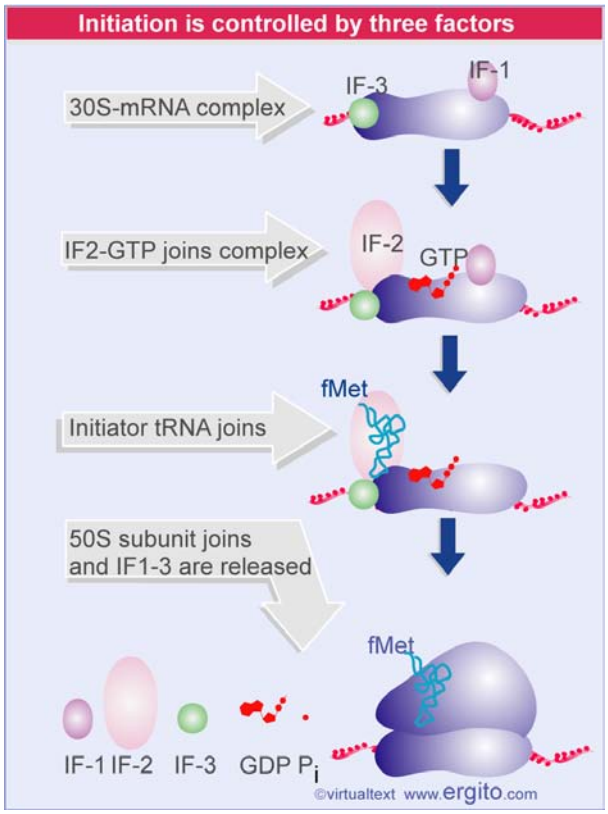

Figure 6.15 IF-2 is needed to bind fMet-tRNA$_f$ to the 30S-mRNA complex. After 50S binding, all IF factors are released and GTP is cleaved.

Last updated on January 6, 2004

This content is available online at http://www.ergito.com/main.jsp?bcs=MBIO.2.6.6

PROTEIN SYNTHESIS

2.6.7 Initiation involves base pairing between mRNA and rRNA

Key Terms

The **Shine-Dalgarno** sequence is the polypurine sequence AGGAGG centered about 10 bp before the AUG initiation codon on bacterial mRNA. It is complementary to the sequence at the 3′ end of 16S rRNA.

Key Concepts

- An initiation site on bacterial mRNA consists of the AUG initiation codon preceded with a gap of ~10 bases by the Shine-Dalgarno polypurine hexamer.
- The rRNA of the 30S bacterial ribosomal subunit has a complementary sequence that base pairs with the Shine-Dalgarno sequence during initiation.

An mRNA contains many AUG triplets: how is the initiation codon recognized as providing the starting point for translation? The sites on mRNA where protein synthesis is initiated can be identified by binding the ribosome to mRNA under conditions that block elongation. Then the ribosome remains at the initiation site. When ribonuclease is added to the blocked initiation complex, all the regions of mRNA outside the ribosome are degraded, but those actually bound to it are protected, as illustrated in **Figure 6.16**. The protected fragments can be recovered and characterized.

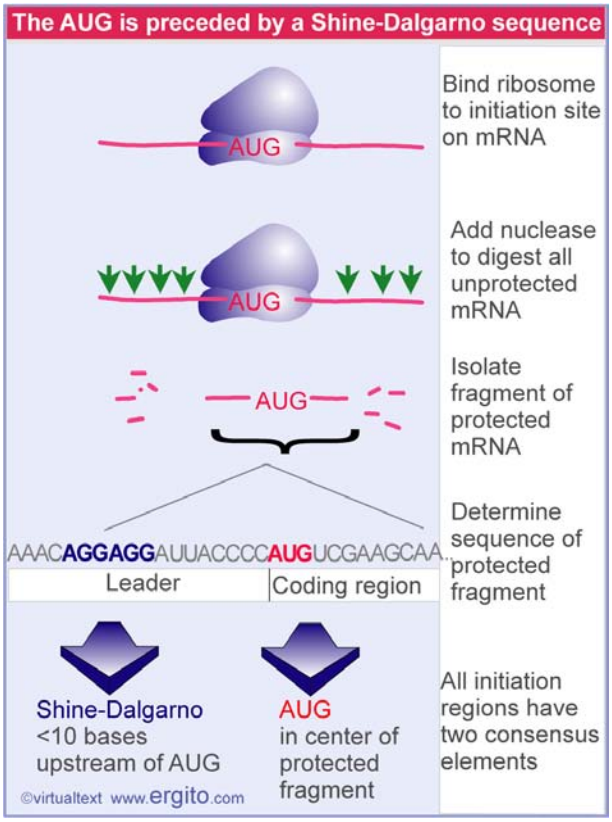

Figure 6.16 Ribosome-binding sites on mRNA can be recovered from initiation complexes. They include the upstream Shine-Dalgarno sequence and the initiation codon.

The initiation sequences protected by bacterial ribosomes are ~30 bases long. The ribosome-binding sites of different bacterial mRNAs display two common features:

- The AUG (or less often, GUG or UUG) initiation codon is always included within the protected sequence.
- Within 10 bases upstream of the AUG is a sequence that corresponds to part or all of the hexamer.

5′ ... A G G A G G ... 3′

This polypurine stretch is known as the **Shine-Dalgarno** sequence. It is complementary to a highly conserved sequence close to the 3′ end of 16S rRNA. (The extent of complementarity differs with individual mRNAs, and may extend from a 4-base core sequence GAGG to a 9-base sequence extending beyond each end of the hexamer.) Written in reverse direction, the rRNA sequence is the hexamer:

3′ ... U C C U C C ... 5′

Does the Shine-Dalgarno sequence pair with its complement in rRNA during mRNA-ribosome binding? Mutations of both partners in this reaction demonstrate its

importance in initiation. Point mutations in the Shine-Dalgarno sequence can prevent an mRNA from being translated. And the introduction of mutations into the complementary sequence in rRNA is deleterious to the cell and changes the pattern of protein synthesis. The decisive confirmation of the base pairing reaction is that a mutation in the Shine-Dalgarno sequence of an mRNA can be suppressed by a mutation in the rRNA that restores base pairing (see *Great Experiments 4.8 rRNA-mRNA base pairing selects translational initiator regions in bacteria*).

The sequence at the 3′ end of rRNA is conserved between prokaryotes and eukaryotes except that in all eukaryotes there is a deletion of the five-base sequence CCUCC that is the principal complement to the Shine-Dalgarno sequence. There does not appear to be base pairing between eukaryotic mRNA and 18S rRNA. This is a significant difference in the mechanism of initiation.

In bacteria, a 30S subunit binds directly to a ribosome-binding site. As a result, the initiation complex forms at a sequence surrounding the AUG initiation codon. When the mRNA is polycistronic, each coding region starts with a ribosome-binding site.

The nature of bacterial gene expression means that translation of a bacterial mRNA proceeds sequentially through its cistrons. At the time when ribosomes attach to the first coding region, the subsequent coding regions have not yet even been transcribed. By the time the second ribosome site is available, translation is well under way through the first cistron.

What happens between the coding regions depends on the individual mRNA. Probably in most cases the ribosomes bind independently at the beginning of each cistron. The most common series of events is illustrated in **Figure 6.17**. When synthesis of the first protein terminates, the ribosomes leave the mRNA and dissociate into subunits. Then a new ribosome must assemble at the next coding region, and set out to translate the next cistron.

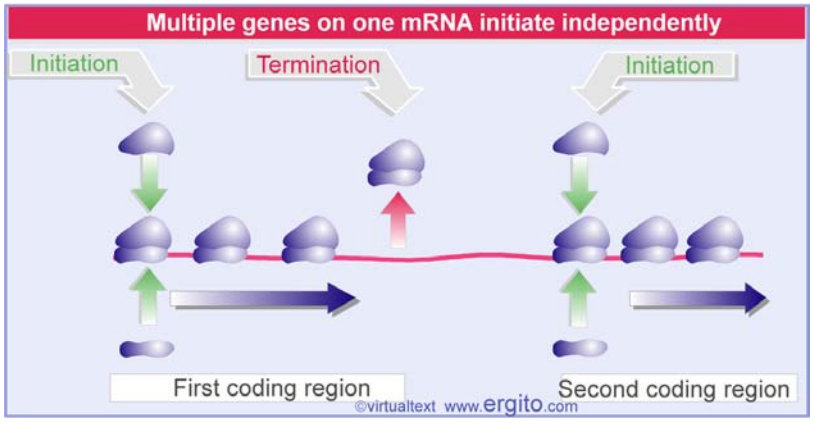

Figure 6.17 Initiation occurs independently at each cistron in a polycistronic mRNA. When the intercistronic region is longer than the span of the ribosome, dissociation at the termination site is followed by independent reinitiation at the next cistron.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.7 to view properly.

In some bacterial mRNAs, translation between adjacent cistrons is directly linked, because ribosomes gain access to the initiation codon of the second cistron as they complete translation of the first cistron. This effect requires the space between the two coding regions to be small. It may depend on the high local density of ribosomes; or the juxtaposition of termination and initiation sites could allow some of the usual intercistronic events to be bypassed. A ribosome physically spans ~30 bases of mRNA, so that it could simultaneously contact a termination codon and the next initiation site if they are separated by only a few bases.

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PROTEIN SYNTHESIS

2.6.8 Small subunits scan for initiation sites on eukaryotic mRNA

Key Concepts

- Eukaryotic 40S ribosomal subunits bind to the 5′ end of mRNA and scan the mRNA until they reach an initiation site.
- A eukaryotic initiation site consists of a 10 nucleotide sequence that includes an AUG codon.
- 60S ribosomal subunits join the complex at the initiation site.

Initiation of protein synthesis in eukaryotic cytoplasm resembles the process in bacteria, but the order of events is different, and the number of accessory factors is greater. Some of the differences in initiation are related to a difference in the way that bacterial 30S and eukaryotic 40S subunits find their binding sites for initiating protein synthesis on mRNA. In eukaryotes, small subunits first recognize the 5′ end of the mRNA, and then move to the initiation site, where they are joined by large subunits. (In prokaryotes, small subunits bind directly to the initiation site.)

Virtually all eukaryotic mRNAs are monocistronic, but each mRNA usually is substantially longer than necessary just to code for its protein. The average mRNA in eukaryotic cytoplasm is 1000-2000 bases long, has a methylated cap at the 5′ terminus, and carries 100-200 bases of poly(A) at the 3′ terminus.

The nontranslated 5′ leader is relatively short, usually <100 bases. The length of the coding region is determined by the size of the protein. The nontranslated 3′ trailer is often rather long, sometimes ~1000 bases.

The first feature to be recognized during translation of a eukaryotic mRNA is the methylated cap that marks the 5′ end. Messengers whose caps have been removed are not translated efficiently *in vitro*. Binding of 40S subunits to mRNA requires several initiation factors, including proteins that recognize the structure of the cap.

Modification at the 5′ end occurs to almost all cellular or viral mRNAs, and is essential for their translation in eukaryotic cytoplasm (although it is not needed in organelles). The sole exception to this rule is provided by a few viral mRNAs (such as poliovirus) that are not capped; only these exceptional viral mRNAs can be translated *in vitro* without caps. They use an alternative pathway that bypasses the need for the cap.

Some viruses take advantage of this difference. Poliovirus infection inhibits the translation of host mRNAs. This is accomplished by interfering with the cap binding proteins that are needed for initiation of cellular mRNAs, but that are superfluous for the noncapped poliovirus mRNA.

We have dealt with the process of initiation as though the ribosome-binding site is always freely available. However, its availability may be impeded by secondary structure. The recognition of mRNA requires several additional factors; an important part of their function is to remove any secondary structure in the mRNA (see **Figure 6.20**).

Sometimes the AUG initiation codon lies within 40 bases of the 5′ terminus of the mRNA, so that both the cap and AUG lie within the span of ribosome binding. But in many mRNAs the cap and AUG are farther apart, in extreme cases ~1000 bases distant. Yet the presence of the cap still is necessary for a stable complex to be formed at the initiation codon. How can the ribosome rely on two sites so far apart?

Figure 6.18 illustrates the "scanning" model, which supposes that the 40S subunit initially recognizes the 5′ cap and then "migrates" along the mRNA. Scanning from the 5′ end is a linear process. When 40S subunits scan the leader region, they can melt secondary structure hairpins with stabilities <–30 kcal, but hairpins of greater stability impede or prevent migration.

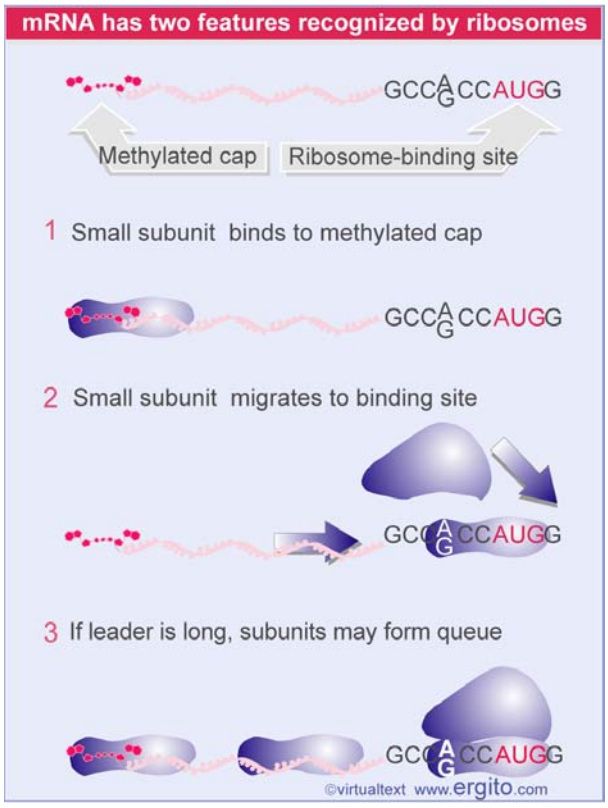

Figure 6.18 Eukaryotic ribosomes migrate from the 5′ end of mRNA to the ribosome binding site, which includes an AUG initiation codon.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.8 to view properly.

Migration stops when the 40S subunit encounters the AUG initiation codon. Usually, although not always, the first AUG triplet sequence to be encountered will be the

initiation codon. However, the AUG triplet by itself is not sufficient to halt migration; it is recognized efficiently as an initiation codon only when it is in the right context. The most important determinants of context are the bases in positions –4 and +1. An initiation codon may be recognized in the sequence NNNPuNN*AUG*G. The purine (A or G) 3 bases before the AUG codon, and the G immediately following it, can influence the efficiency of translation by 10×. When the leader sequence is long, further 40S subunits can recognize the 5′ end before the first has left the initiation site, creating a queue of subunits proceeding along the leader to the initiation site (427; for review see 429).

It is probably true that the initiation codon is the first AUG to be encountered in the most efficiently translated mRNAs. But what happens when there is an AUG triplet in the 5′ nontranslated region? There are two possible escape mechanisms for a ribosome that starts scanning at the 5′ end. The most common is that scanning is leaky, that is, a ribosome may continue past a non-initiation AUG because it is not in the right context. In the rare case that it does recognize the AUG, it may initiate translation but terminate before the proper initiation codon, after which it resumes scanning.

The vast majority of eukaryotic initiation events involve scanning from the 5′ cap, but there is an alternative means of initiation, used especially by certain viral RNAs, in which a 40S subunit associates directly with an internal site called an IRES (for review see 2242). (This entirely bypasses any AUG codons that may be in the 5′ nontranslated region.) There are few sequence homologies between known IRES elements. We can distinguish three types on the basis of their interaction with the 40S subunit:

- One type of IRES includes the AUG initiation codon at its upstream boundary. The 40S subunit binds directly to it, using a subset of the same factors that are required for initiation at 5′ ends (2244; 2245).
- Another is located as much as 100 nucleotides upstream of the AUG, requiring a 40S subunit to migrate, again probably by a scanning mechanism.
- An exceptional type of IRES in hepatitis C virus can bind a 40S subunit directly, without requiring any initiation factors (2243). The order of events is different from all other eukaryotic initiation. Following 40S - mRNA binding, a complex containing initiator factors and the initiator tRNA binds.

Use of the IRES is especially important in picornavirus infection, where it was first discovered, because the virus inhibits host protein synthesis by destroying cap structures and inhibiting the initiation factors that bind them (see *Molecular Biology 2.6.9 Eukaryotes use a complex of many initiation factors*)(995).

Binding is stabilized at the initiation site. When the 40S subunit is joined by a 60S subunit, the intact ribosome is located at the site identified by the protection assay. A 40S subunit protects a region of up to 60 bases; when the 60S subunits join the complex, the protected region contracts to about the same length of 30-40 bases seen in prokaryotes.

Last updated on 8-12-2002

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PROTEIN SYNTHESIS

2.6.9 Eukaryotes use a complex of many initiation factors

Key Terms

$tRNA_i^{Met}$ is the special tRNA used to respond to initiation codons in eukaryotes.

Key Concepts

- Initiation factors are required for all stages of initiation, including binding the initiator tRNA, 40S subunit attachment to mRNA, movement along the mRNA, and joining of the 60S subunit.
- Eukaryotic initiator tRNA is a Met-tRNA that is different from the Met-tRNA used in elongation, but the methionine is not formylated.
- eIF2 binds the initiator Met-$tRNA_i$ and GTP, and the complex binds to the 40S subunit before it associates with mRNA.

Initiation in eukaryotes has the same general features as in bacteria in using a specific initiation codon and initiator tRNA. Initiation in eukaryotic cytoplasm uses AUG as the initiator. The initiator tRNA is a distinct species, but its methionine does not become formylated. It is called **$tRNA_i^{Met}$**. So the difference between the initiating and elongating Met-tRNAs lies solely in the tRNA moiety, with Met-$tRNA_i$ used for initiation and Met-$tRNA_m$ used for elongation.

At least two features are unique to the initiator $tRNA_i^{Met}$ in yeast; it has an unusual tertiary structure; and it is modified by phosphorylation of the 2′ ribose position on base 64 (if this modification is prevented, the initiator can be used in elongation). So the principle of a distinction between initiator and elongator Met-tRNAs is maintained in eukaryotes, but its structural basis is different from that in bacteria (for comparison see **Figure 6.13**).

Eukaryotic cells have more initiation factors than bacteria – the current list includes 12 factors that are directly or indirectly required for initiation (for review see 2393). The factors are named similarly to those in bacteria, sometimes by analogy with the bacterial factors, and are given the prefix "e" to indicate their eukaryotic origin. They act at all stages of the process, including:

- forming an initiation complex with the 5′ end of mRNA
- forming a complex with Met-$tRNA_i$
- binding the mRNA-factor complex to the Met-$tRNA_i$-factor complex
- enabling the ribosome to scan mRNA from the 5′ end to the first AUG

- detecting binding of initiator tRNA to AUG at the start site
- mediating joining of the 60S subunit.

Figure 6.19 summarizes the stages of initiation, and shows which initiation factors are involved at each stage. eIF2 and eIF3 bind to the 40S ribosome subunit. eIF4A, eIF4B, eIF4F bind to the mRNA. eIF1 and eIF1A bind to the ribosome subunit-mRNA complex (for review see 2840).

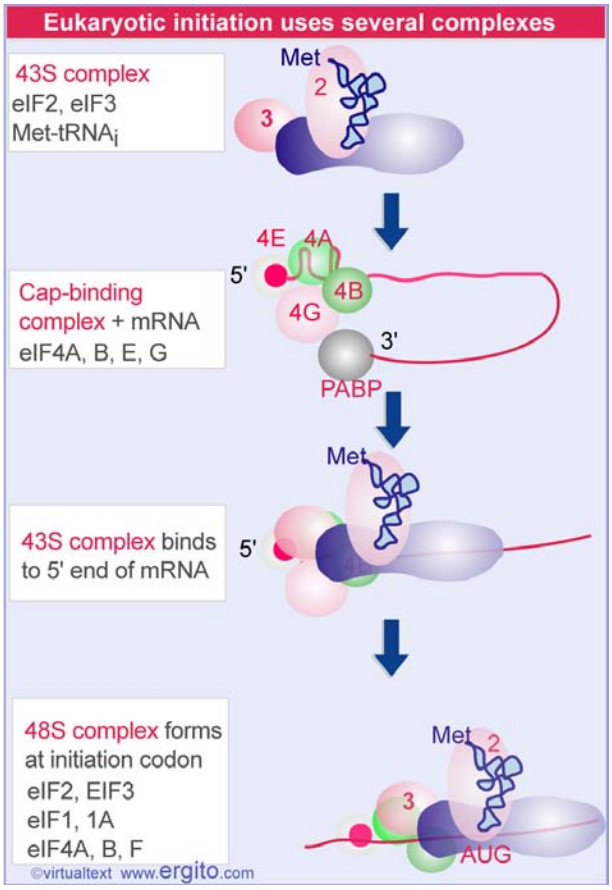

Figure 6.19 Some initiation factors bind to the 40S ribosome subunit to form the 43S complex; others bind to mRNA. When the 43S complex binds to mRNA, it scans for the initiation codon and can be isolated as the 48S complex.

Figure 6.20 shows the group of factors that bind to the 5′ end of mRNA. The factor eIF4F is a protein complex that contains three of the initiation factors (for review see 994). It is not clear whether it preassembles as a complex before binding to mRNA or whether the individual subunits are added individually to form the complex on mRNA. It includes the cap-binding subunit eIF4E, the helicase eIF4A, and the "scaffolding" subunit eIF4G. After eIF4E binds the cap, eIF4A unwinds any secondary structure that exists in the first 15 bases of the mRNA. Energy for the unwinding is provided by hydrolysis of ATP. Unwinding of structure farther along the mRNA is accomplished by eIF4A together with another factor, eIF4B. The main role of eIF4G is to link other components of the initiation complex.

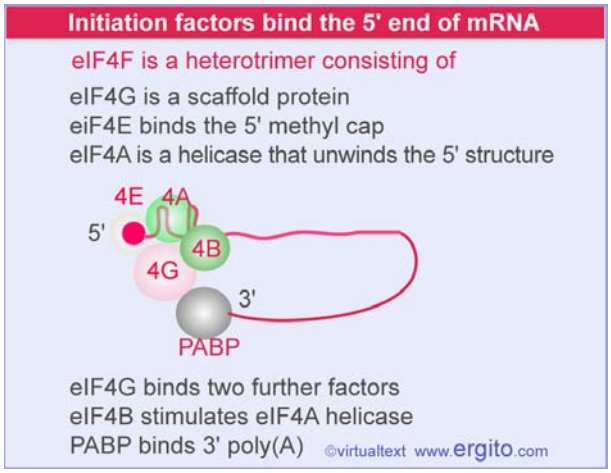

Figure 6.20 The heterotrimer eIF4F binds the 5′ end of mRNA and also binds further factors.

eIF4E is a focus for regulation. Its activity is increased by phosphorylation, which is triggered by stimuli that increase protein synthesis, and reversed by stimuli that repress protein synthesis. eIF4F has a kinase activity that phosphorylates eIF4E. The availability of eIF4E is also controlled by proteins that bind to it (called 4E-BP1,2,3), to prevent it from functioning in initiation. eIF4G is also a target for degradation during picornavirus infection, as part of the destruction of the capacity to initiate at 5′ cap structures (see *Molecular Biology 2.6.8 Small subunits scan for initiation sites on eukaryotic mRNA*).

The presence of poly(A) on the 3′ tail of an mRNA stimulates the formation of an initiation complex at the 5′ end. The poly(A)-binding protein (Pab1p in yeast) is required for this effect. Pab1p binds to the eIF4G scaffolding protein(2309). This implies that the mRNA will have a circular organization so long as eIFG is bound, with both the 5′ and 3′ ends held in this complex (see **Figure 6.20**) (for review see 439). The significance of the formation of this closed loop is not clear, although it could have several effects, such as:

- stimulating initiation of translation;
- promoting reinitiation of ribosomes, so that when they terminate at the 3′ end, the released subunits are already in the vicinity of the 5′ end;
- stabilizing the mRNA against degradation;
- allowing factors that bind to the 3′ end to regulate the initiation of translation.

eIF2 is the key factor in binding Met-tRNA$_i$. It is a typical monomeric GTP-binding protein that is active when bound to GTP, and inactive when bound to GDP (see *Molecular Biology Supplement 32.10 G proteins*). **Figure 6.21** shows that the eIF2-GTP binds to Met-tRNA$_i$ (1865). The product is sometimes called the ternary complex (after its three components, eIF2, GTP, Met-tRNA$_i$).

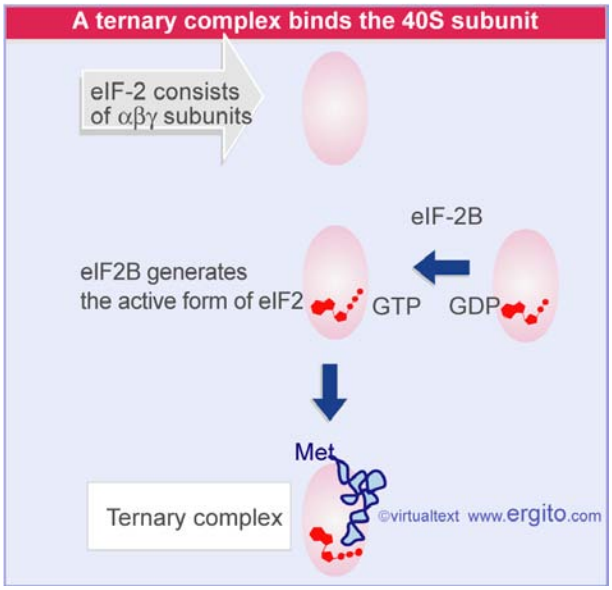

Figure 6.21 In eukaryotic initiation, eIF-2 forms a ternary complex with Met-tRNA$_i$. The ternary complex binds to free 40S subunits, which attach to the 5′ end of mRNA. Later in the reaction, GTP is hydrolyzed when eIF-2 is released in the form of eIF2-GDP. eIF-2B regenerates the active form.

Figure 6.22 shows that the ternary complex places Met-tRNA$_i$ onto the 40S subunit. This generates the 43S initiation complex. The reaction is independent of the presence of mRNA. In fact, the Met-tRNA$_i$ initiator must be present in order for the 40S subunit to bind to mRNA (for review see 435; 438). One of the factors in this complex is eIF3, which is required to maintain 40S subunits in their dissociated state. eIF3 is a very large factor, with 8-10 subunits.

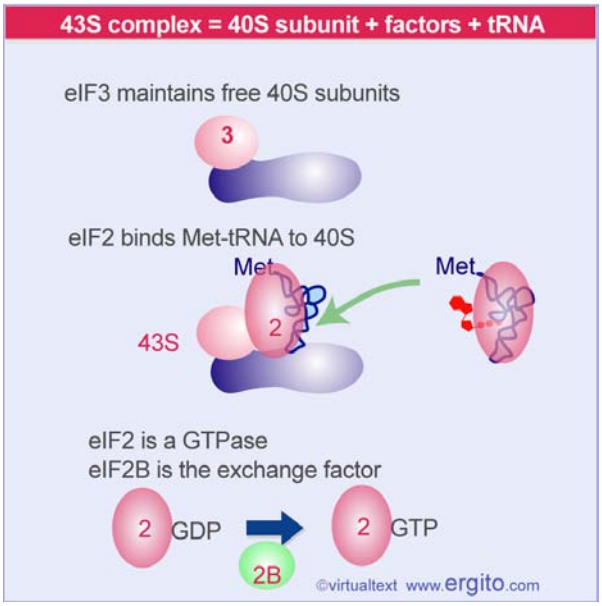

Figure 6.22 Initiation factors bind the initiator Met-tRNA to the 40S subunit to form a 43S complex. Later in the reaction, GTP is hydrolyzed when eIF-2 is released in the form of eIF2-GDP. eIF-2B regenerates the active form.

The next step is for the 43S complex to bind to the 5′ end of the mRNA.**Figure 6.23** shows that the interactions involved at this stage are not completely defined, but probably involve eIF4G and eIF3 as well as the mRNA and 40S subunit. eIF4G binds to eIF3. This provides the means by which the 40S ribosomal subunit binds to eIF4F, and thus is recruited to the complex. In effect, eIF4F functions to get eIF4G in place so that it can attract the small ribosomal subunit.

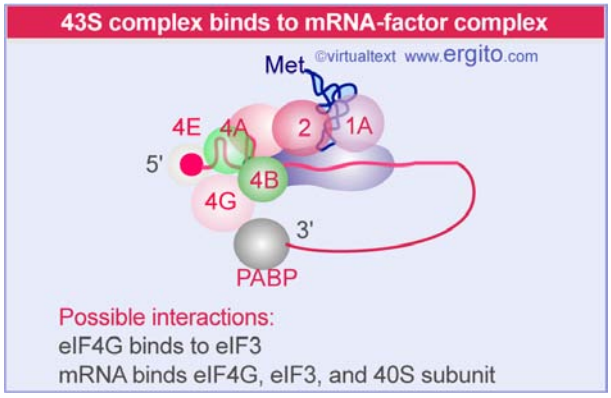

Figure 6.23 Interactions involving initiation factors are important when mRNA binds to the 43S complex.

When the small subunit has bound mRNA, it migrates to (usually) the first AUG codon. This requires expenditure of energy in the form of ATP. It is assisted by the factors eIF1 and eIF1A. **Figure 6.24** shows that the small subunit stops when it reaches the initiation site, forming a 48S complex.

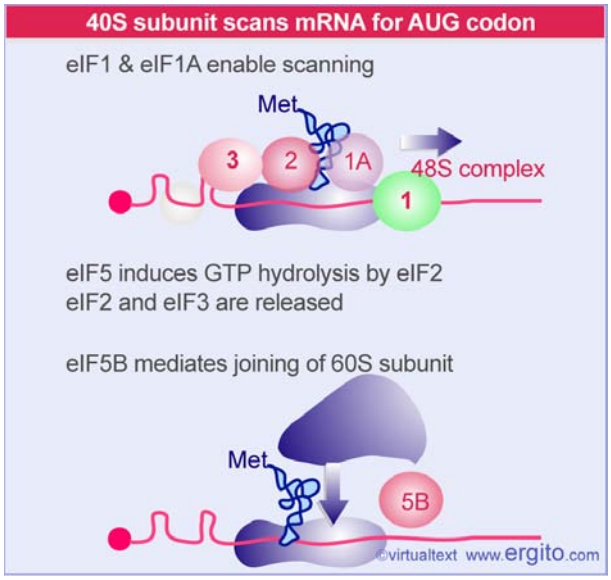

Figure 6.24 eIF1 and eIF1A help the 43S initiation complex to scan the mRNA until it reaches an AUG codon. eIF2 hydrolyzes its GTP to enable its release together with IF3. eIF5B mediates 60S-40S joining.

Junction of the 60S subunits with the initiation complex cannot occur until eIF2 and eIF3 have been released from the initiation complex. This is mediated by eIF5, and causes eIF2 to hydrolyze its GTP. The reaction occurs on the small ribosome subunit, and requires the initiator tRNA to be base paired with the AUG initiation codon (2839). Probably all of the remaining factors are released when the complete 80S ribosome is formed.

Finally the factor eIF5B enables the 60S subunit to join the complex, forming an intact ribosome that is ready to start elongation (2241). eIF5B has a similar sequence to the prokaryotic factor IF2, which has a similar role in hydrolyzing GTP (in addition to its role in binding the initiator tRNA).

Once the factors have been released, they can associate with the initiator tRNA and ribosomal subunits in another initiation cycle. Because eIF2 has hydrolyzed its GTP, the active form must be regenerated. This is accomplished by another factor, eIF2B, which displaces the GDP so that it can be replaced by GTP.

eIF2 is a target for regulation. Several regulatory kinases act on the α subunit of eIF2. Phosphorylation prevents eIF2B from regenerating the active form. This limits the action of eIF2B to one cycle of initiation, and thereby inhibits protein synthesis (for review see 2393).

Last updated on 8-12-2002

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PROTEIN SYNTHESIS

2.6.10 Elongation factor Tu loads aminoacyl-tRNA into the A site

Key Terms

Elongation factors (EF in prokaryotes, eEF in eukaryotes) are proteins that associate with ribosomes cyclically, during addition of each amino acid to the polypeptide chain.

EF-Tu is the elongation factor that binds aminoacyl-tRNA and places it into the A site of a bacterial ribosome.

GMP-PCP is an analog of GTP that cannot be hydrolyzed. It is used to test which stage in a reaction requires hydrolysis of GTP.

Kirromycin is an antibiotic that inhibits protein synthesis by acting on EF-Tu.

Key Concepts

- EF-Tu is a monomeric G protein whose active form (bound to GTP) binds aminoacyl-tRNA.
- The EF-Tu·GTP·aminoacyl-tRNA complex binds to the ribosome A site.

Once the complete ribosome is formed at the initiation codon, the stage is set for a cycle in which aminoacyl-tRNA enters the A site of a ribosome whose P site is occupied by peptidyl-tRNA. Any aminoacyl-tRNA except the initiator can enter the A site. Its entry is mediated by an **elongation factor** (**EF-Tu** in bacteria). The process is similar in eukaryotes. EF-Tu is a highly conserved protein throughout bacteria and mitochondria, and is homologous to its eukaryotic counterpart.

Just like its counterpart in initiation (IF-2), EF-Tu is associated with the ribosome only during the process of aminoacyl-tRNA entry. Once the aminoacyl-tRNA is in place, EF-Tu leaves the ribosome, to work again with another aminoacyl-tRNA. So it displays the cyclic association with, and dissociation from, the ribosome that is the hallmark of the accessory factors.

The pathway for aminoacyl-tRNA entry to the A site is illustrated in **Figure 6.25**. EF-Tu carries a guanine nucleotide. The factor is a monomeric G protein whose activity is controlled by the state of the guanine nucleotide (see introduction on *Molecular Biology Supplement 32.10 G proteins*):

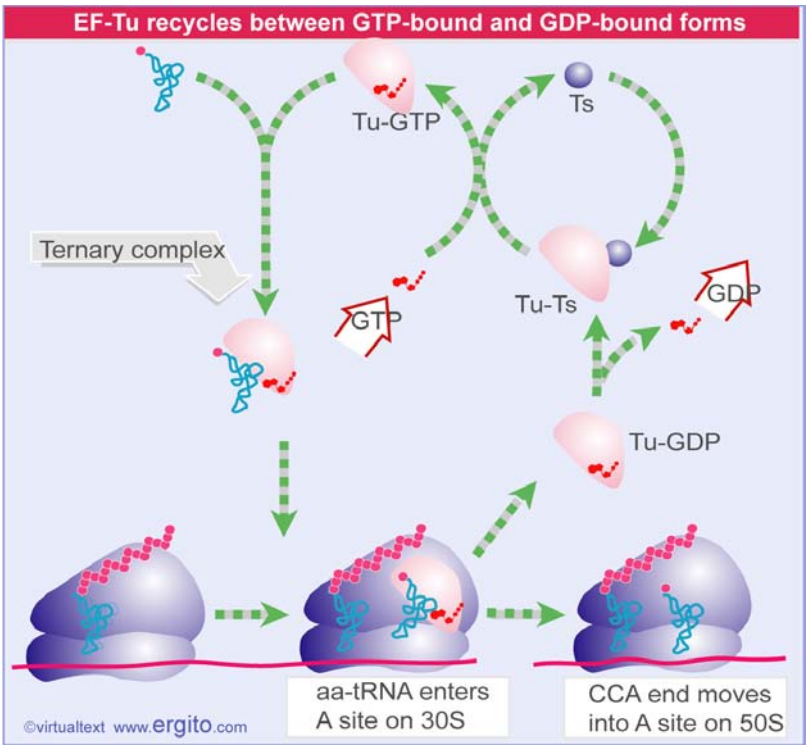

Figure 6.25 EF-Tu-GTP places aminoacyl-tRNA on the ribosome and then is released as EF-Tu-GDP. EF-Ts is required to mediate the replacement of GDP by GTP. The reaction consumes GTP and releases GDP. The only aminoacyl-tRNA that cannot be recognized by EF-Tu-GTP is fMet-tRNA$_f$, whose failure to bind prevents it from responding to internal AUG or GUG codons.

- When GTP is present, the factor is in its active state.
- When the GTP is hydrolyzed to GDP, the factor becomes inactive.
- Activity is restored when the GDP is replaced by GTP.

The binary complex of EF-Tu·GTP binds aminoacyl-tRNA to form a ternary complex of aminoacyl-tRNA·EF-Tu·GTP. The ternary complex binds only to the A site of ribosomes whose P site is already occupied by peptidyl-tRNA. This is the critical reaction in ensuring that the aminoacyl-tRNA and peptidyl-tRNA are correctly positioned for peptide bond formation.

Aminoacyl-tRNA is loaded into the A site in two stages. First the anticodon end binds to the A site of the 30S subunit. Then codon-anticodon recognition triggers a change in the conformation of the ribosome. This stabilizes tRNA binding and causes EF-Tu to hydrolyze its GTP. The CCA end of the tRNA now moves into the A site on the 50S subunit. The binary complex EF-Tu·GDP is released. This form of EF-Tu is inactive and does not bind aminoacyl-tRNA effectively.

Another factor, EF-Ts, mediates the regeneration of the used form, EF-Tu·GDP, into the active form, EF-Tu·GTP. First, EF-Ts displaces the GDP from EF-Tu, forming the combined factor EF-Tu·EF-Ts. Then the EF-Ts is in turn displaced by GTP,

reforming EF-Tu·GTP. The active binary complex binds aminoacyl-tRNA; and the released EF-Ts can recycle.

There are ~70,000 molecules of EF-Tu per bacterium (~5% of the total bacterial protein), which approaches the number of aminoacyl-tRNA molecules. This implies that most aminoacyl-tRNAs are likely to be present in ternary complexes. There are only ~10,000 molecules of EF-Ts per cell (about the same as the number of ribosomes). The kinetics of the interaction between EF-Tu and EF-Ts suggest that the EF-Tu·EF-Ts complex exists only transiently, so that the EF-Tu is very rapidly converted to the GTP-bound form, and then to a ternary complex.

The role of GTP in the ternary complex has been studied by substituting an analog that cannot be hydrolyzed. The compound **GMP-PCP** has a methylene bridge in place of the oxygen that links the β and γ phosphates in GTP. In the presence of GMP-PCP, a ternary complex can be formed that binds aminoacyl-tRNA to the ribosome. But the peptide bond cannot be formed. So the presence of GTP is needed for aminoacyl-tRNA to be bound at the A site; but the hydrolysis is not required until later.

Kirromycin is an antibiotic that inhibits the function of EF-Tu. When EF-Tu is bound by kirromycin, it remains able to bind aminoacyl-tRNA to the A site. But the EF-Tu·GDP complex cannot be released from the ribosome. Its continued presence prevents formation of the peptide bond between the peptidyl-tRNA and the aminoacyl-tRNA. As a result, the ribosome becomes "stalled" on mRNA, bringing protein synthesis to a halt.

This effect of kirromycin demonstrates that inhibiting one step in protein synthesis blocks the next step. The reason is that the continued presence of EF-Tu prevents the aminoacyl end of aminoacyl-tRNA from entering the A site on the 50S subunit (see **Figure 6.31**). So the release of EF-Tu·GDP is needed for the ribosome to undertake peptide bond formation. The same principle is seen at other stages of protein synthesis: one reaction must be completed properly before the next can occur.

The interaction with EF-Tu also plays a role in quality control. Aminoacyl-tRNAs are brought into the A site without knowing whether their anticodons will fit the codon. The hydrolysis of EF-Tu·GTP is relatively slow: because it takes longer than the time required for an incorrect aminoacyl-tRNA to dissociate from the A site, most incorrect species are removed at this stage. The release of EF-Tu·GDP after hydrolysis also is slow, so any surviving incorrect aminoacyl-tRNAs may dissociate at this stage. The basic principle is that the reactions involving EF-Tu occur slowly enough to allow incorrect aminoacyl-tRNAs to dissociate before they become trapped in protein synthesis.

In eukaryotes, the factor *eEF1* α is responsible for bringing aminoacyl-tRNA to the ribosome, again in a reaction that involves cleavage of a high-energy bond in GTP. Like its prokaryotic homologue (EF-Tu), it is an abundant protein. After hydrolysis of GTP, the active form is regenerated by the factor eEF1 β γ, a counterpart to EF-Ts.

Last updated on 3-21-2002

PROTEIN SYNTHESIS

2.6.11 The polypeptide chain is transferred to aminoacyl-tRNA

Key Terms

Peptidyl transferase is the activity of the ribosomal 50S subunit that synthesizes a peptide bond when an amino acid is added to a growing polypeptide chain. The actual catalytic activity is a property of the rRNA.

Puromycin is an antibiotic that terminates protein synthesis by mimicking a tRNA and becoming linked to the nascent protein chain.

Key Concepts

- The 50S subunit has peptidyl transferase activity.
- The nascent polypeptide chain is transferred from peptidyl-tRNA in the P site to aminoacyl-tRNA in the A site.
- Peptide bond synthesis generates deacylated tRNA in the P site and peptidyl-tRNA in the A site.

The ribosome remains in place while the polypeptide chain is elongated by transferring the polypeptide attached to the tRNA in the P site to the aminoacyl-tRNA in the A site. The reaction is shown in **Figure 6.26**. The activity responsible for synthesis of the peptide bond is called **peptidyl transferase**.

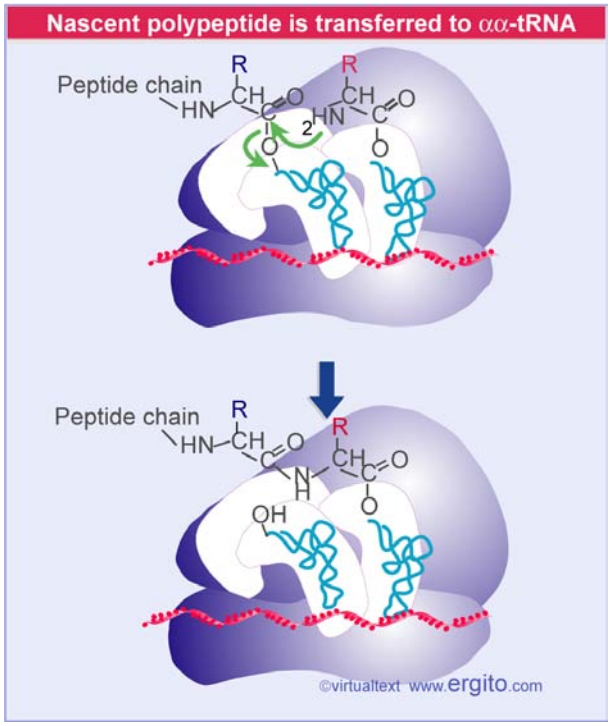

Figure 6.26 Peptide bond formation takes place by reaction between the polypeptide of peptidyl-tRNA in the P site and the amino acid of aminoacyl-tRNA in the A site.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.11 to view properly.

The nature of the transfer reaction is revealed by the ability of the antibiotic **puromycin** to inhibit protein synthesis. Puromycin resembles an amino acid attached to the terminal adenosine of tRNA. **Figure 6.27** shows that puromycin has an N instead of the O that joins an amino acid to tRNA. The antibiotic is treated by the ribosome as though it were an incoming aminoacyl-tRNA. Then the polypeptide attached to peptidyl-tRNA is transferred to the NH_2 group of the puromycin.

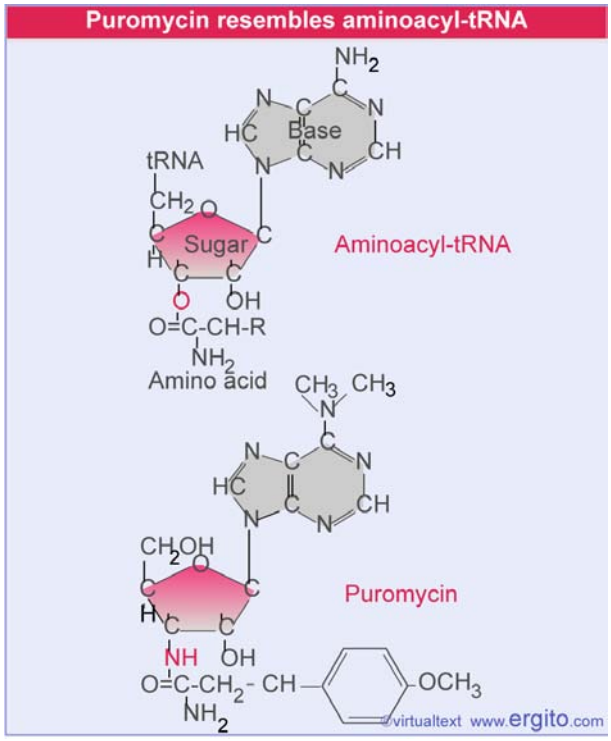

Figure 6.27 Puromycin mimics aminoacyl-tRNA because it resembles an aromatic amino acid linked to a sugar-base moiety.

Because the puromycin moiety is not anchored to the A site of the ribosome, the polypeptidyl-puromycin adduct is released from the ribosome in the form of polypeptidyl-puromycin. This premature termination of protein synthesis is responsible for the lethal action of the antibiotic.

Peptidyl transferase is a function of the large (50S or 60S) ribosomal subunit. The reaction is triggered when EF-Tu releases the aminoacyl end of its tRNA. The aminoacyl end then swings into a location close to the end of the peptidyl-tRNA. This site has a peptidyl transferase activity that essentially ensures a rapid transfer of the peptide chain to the aminoacyl-tRNA . Both rRNA and 50S subunit proteins are necessary for this activity, but the actual act of catalysis is a property of the ribosomal RNA of the 50S subunit (see *Molecular Biology 2.6.19 23S rRNA has peptidyl transferase activity*).

Last updated on 3-25-2002

This content is available online at http://www.ergito.com/main.jsp?bcs=MBIO.2.6.11

PROTEIN SYNTHESIS

2.6.12 Translocation moves the ribosome

Key Terms

Translocation is the movement of the ribosome one codon along mRNA after the addition of each amino acid to the polypeptide chain.

Key Concepts

- Ribosomal translocation moves the mRNA through the ribosome by 3 bases.
- Translocation moves deacylated tRNA into the E site, peptidyl-tRNA into the P site, and empties the A site.
- The hybrid state model proposes that translocation occurs in two stages, in which the 50S moves relative to the 30S, and then the 30S moves along mRNA to restore the original conformation.

The cycle of addition of amino acids to the growing polypeptide chain is completed by **translocation**, when the ribosome advances three nucleotides along the mRNA. **Figure 6.28** shows that translocation expels the uncharged tRNA from the P site, so that the new peptidyl-tRNA can enter. The ribosome then has an empty A site ready for entry of the aminoacyl-tRNA corresponding to the next codon. As the figure shows, in bacteria the discharged tRNA is transferred from the P site to the E site (from which it is then expelled into the cytoplasm). In eukaryotes it is expelled directly into the cytosol. The A and P sites straddle both the large and small subunits; the E site (in bacteria) is located largely on the 50S subunit, but has some contacts in the 30S subunit.

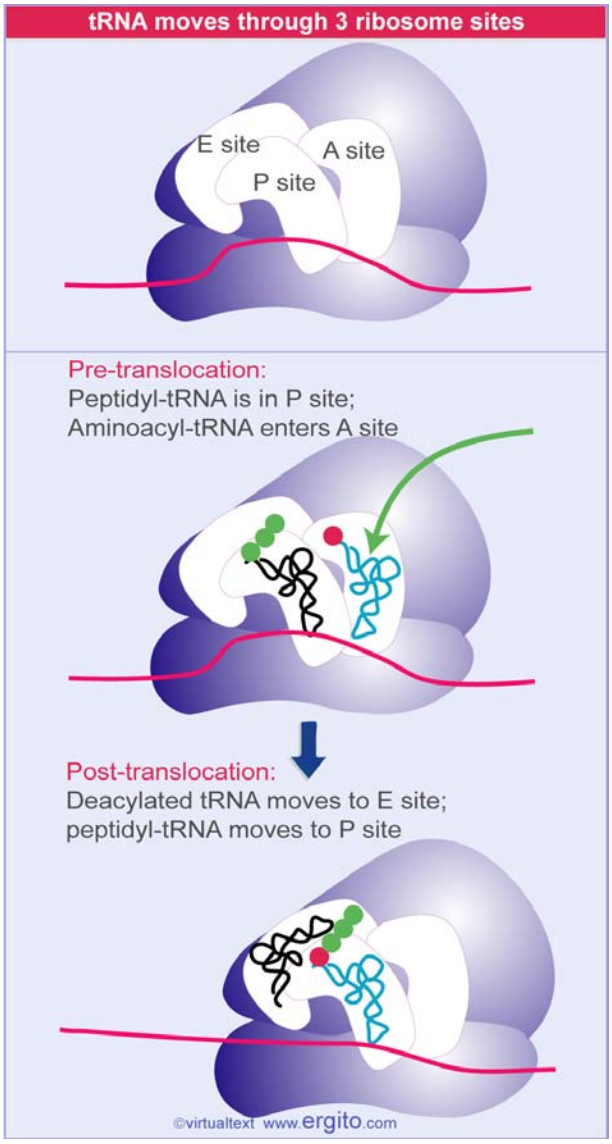

Figure 6.28 A bacterial ribosome has 3 tRNA-binding sites. Aminoacyl-tRNA enters the A site of a ribosome that has peptidyl-tRNA in the P site. Peptide bond synthesis deacylates the P site tRNA and generates peptidyl-tRNA in the A site. Translocation moves the deacylated tRNA into the E site and moves peptidyl-tRNA into the P site.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.12 to view properly.

Most thinking about translocation follows the hybrid state model, which proposes that translocation occurs in two stages (for review see 2390). **Figure 6.29** shows that first there is a shift of the 50S subunit relative to the 30S subunit; then a second shift occurs when the 30S subunit moves along mRNA to restore the original conformation. The basis for this model was the observation that the pattern of contacts that tRNA makes with the ribosome (measured by chemical footprinting) changes in two stages (934). When puromycin is added to a ribosome that has an aminoacylated tRNA in the P site, the contacts of tRNA on the 50S subunit change

from the P site to the E site, but the contacts on the 30S subunit do not change. This suggests that the 50S subunit has moved to a post-transfer state, but the 30S subunit has not changed.

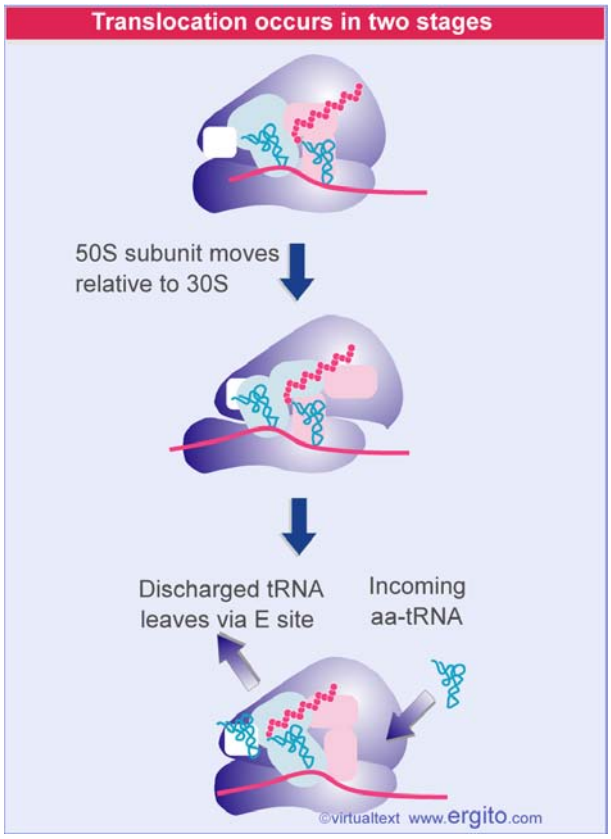

Figure 6.29 Models for translocation involve two stages. First, at peptide bond formation the aminoacyl end of the tRNA in the A site becomes located in the P site. Second, the anticodon end of the tRNA becomes located in the P site.

The interpretation of these results is that first the aminoacyl ends of the tRNAs (located in the 50S subunit) move into the new sites (while the anticodon ends remain bound to their anticodons in the 30S subunit). At this stage, the tRNAs are effectively bound in hybrid sites, consisting of the 50S E/ 30S P and the 50S P/ 30S A sites. Then movement is extended to the 30S subunits, so that the anticodon-codon pairing region finds itself in the right site. The most likely means of creating the hybrid state is by a movement of one ribosomal subunit relative to the other, so that translocation in effect involves two stages, the normal structure of the ribosome being restored by the second stage (444).

The ribosome faces an interesting dilemma at translocation. It needs to break many of its contacts with tRNA in order to allow movement. But at the same time it must maintain pairing between tRNA and the anticodon (breaking the pairing of the deacylated tRNA only at the right moment). One possibility is that the ribosome switches between alternative, discrete conformations. The switch could consist of changes in rRNA base pairing. The accuracy of translation is influenced by certain mutations that influence alternative base pairing arrangements. The most likely

interpretation is that the effect is mediated by the tightness of binding to tRNA of the alternative conformations (440).

Last updated on 3-22-2002

Reviews

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PROTEIN SYNTHESIS

2.6.13 Elongation factors bind alternately to the ribosome

Key Concepts

- Translocation requires EF-G, whose structure resembles the aminoacyl-tRNA·EF-Tu·GTP complex.
- Binding of EF-Tu and EF-G to the ribosome is mutually exclusive.
- Translocation requires GTP hydrolysis, which triggers a change in EF-G, which in turn triggers a change in ribosome structure.

Translocation requires GTP and another elongation factor, EF-G. This factor is a major constituent of the cell; it is present at a level of ~1 copy per ribosome (20,000 molecules per cell).

Ribosomes cannot bind EF-Tu and EF-G simultaneously, so protein synthesis follows the cycle illustrated in Figure 151 in which the factors are alternately bound to, and released from, the ribosome. So EF-Tu·GDP must be released before EF-G can bind; and then EF-G must be released before aminoacyl-tRNA·EF-Tu·GTP can bind.

Does the ability of each elongation factor to exclude the other rely on an allosteric effect on the overall conformation of the ribosome or on direct competition for overlapping binding sites? **Figure 6.30** shows an extraordinary similarity between the structures of the ternary complex of aminoacyl-tRNA·EF-Tu·GDP and EF-G (928). The structure of EF-G mimics the overall structure of EF-Tu bound to the amino acceptor stem of aminoacyl-tRNA. This creates the immediate assumption that they compete for the same binding site (presumably in the vicinity of the A site). The need for each factor to be released before the other can bind ensures that the events of protein synthesis proceed in an orderly manner (928).

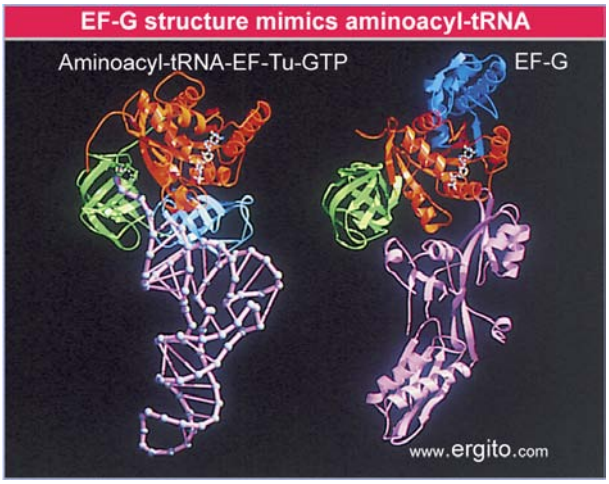

Figure 6.30 The structure of the ternary complex of aminoacyl-tRNA·EF-Tu·GTP (left) resembles the structure of EF-G (right). Structurally conserved domains of EF-Tu and EF-G are in red and green; the tRNA and the domain resembling it in EF-G are in purple. Photograph kindly provided by Poul Nissen.

Both elongation factors are monomeric GTP-binding proteins that are active when bound to GTP but inactive when bound to GDP. The triphosphate form is required for binding to the ribosome, which ensures that each factor obtains access to the ribosome only in the company of the GTP that it needs to fulfill its function.

EF-G binds to the ribosome to sponsor translocation; and then is released following ribosome movement. EF-G can still bind to the ribosome when GMP-PCP is substituted for GTP; thus the presence of a guanine nucleotide is needed for binding, but its hydrolysis is not absolutely essential for translocation (although translocation is much slower in the absence of GTP hydrolysis). The hydrolysis of GTP is needed to release EF-G.

The need for EF-G release was discovered by the effects of the steroid antibiotic fusidic acid, which "jams" the ribosome in its post-translocation state (see **Figure 6.31**). In the presence of fusidic acid, one round of translocation occurs: EF-G binds to the ribosome, GTP is hydrolyzed, and the ribosome moves three nucleotides. But fusidic acid stabilizes the ribosome·EF-G·GDP complex, so that EF-G and GDP remain on the ribosome instead of being released. Because the ribosome then cannot bind aminoacyl-tRNA, no further amino acids can be added to the chain.

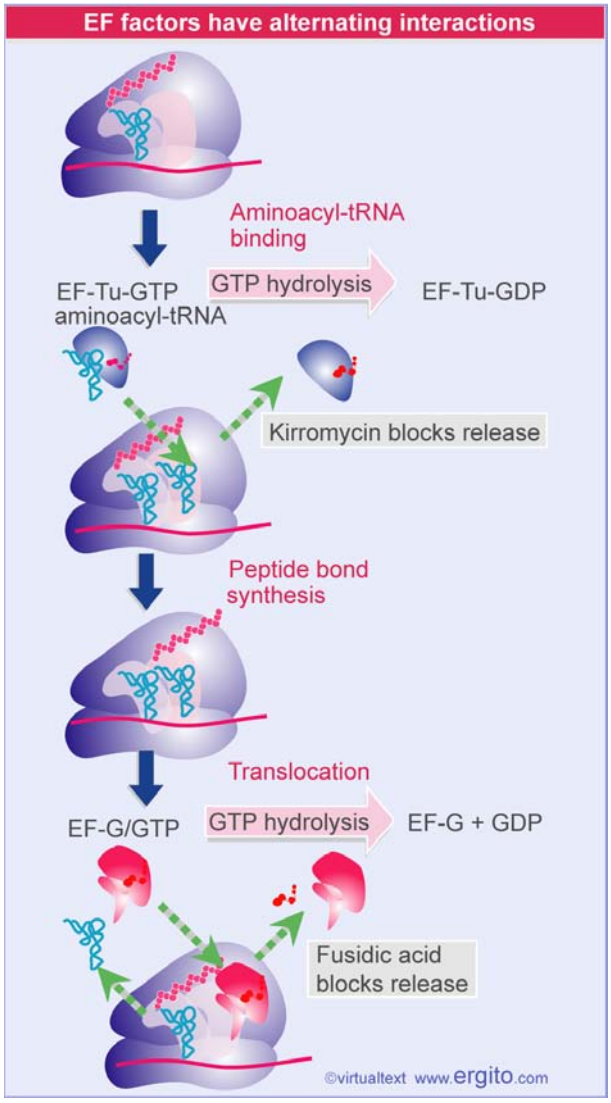

Figure 6.31 Binding of factors EF-Tu and EF-G alternates as ribosomes accept new aminoacyl-tRNA, form peptide bonds, and translocate.

Translocation is an intrinsic property of the ribosome that requires a major change in structure (see *Molecular Biology 2.6.17 Ribosomes have several active centers*). However, its activated by EF-G in conjunction with GTP hydrolysis, which occurs before translocation and accelerates the ribosome movement. The most likely mechanism is that GTP hydrolysis causes a change in the structure of EF-G, which in turn forces a change in the ribosome structure. An extensive reorientation of EF-G occurs at translocation (947). Before translocation, it is bound across the two ribosomal subunits. Most of its contacts with the 30S subunit are made by a region called domain 4, which is inserted into the A site. This domain could be responsible for displacing the tRNA. After translocation, domain 4 is instead oriented toward the 50S subunit.

The eukaryotic counterpart to EF-G is the protein eEF2, which functions in a similar manner, as a translocase dependent on GTP hydrolysis. Its action also is inhibited by

fusidic acid. A stable complex of eEF2 with GTP can be isolated; and the complex can bind to ribosomes with consequent hydrolysis of its GTP.

A unique reaction of eEF2 is its susceptibility to diphtheria toxin. The toxin uses NAD (nicotinamide adenine dinucleotide) as a cofactor to transfer an ADPR moiety (adenosine diphosphate ribosyl) on to the eEF2. The ADPR-eEF2 conjugate is inactive in protein synthesis. The substrate for the attachment is an unusual amino acid, produced by modifying a histidine; it is common to the eEF2 of many species.

The ADP-ribosylation is responsible for the lethal effects of diphtheria toxin. The reaction is extremely effective: a single molecule of toxin can modify sufficient eEF2 molecules to kill a cell.

Last updated on 7-21-2003

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PROTEIN SYNTHESIS

2.6.14 Three codons terminate protein synthesis

Key Terms

The **amber** codon is the triplet UAG, one of the three termination codons that end protein synthesis.

The **ochre** codon is the triplet UAA, one of the three termination codons that end protein synthesis.

The **opal** codon is the triplet UGA, one of the three termination codons that end protein synthesis. It has evolved to code for an amino acid in a small number of organisms or organelles.

Missense mutations change a single codon so as to cause the replacement of one amino acid by another in a protein sequence.

Premature termination describes the termination of protein or of RNA synthesis before the chain has been completed. In protein synthesis it can be caused by mutations that create termination codons within the coding region. In RNA synthesis it is caused by various events that act on RNA polymerase.

A **nonsense mutation** is any change in DNA that replaces a codon specifying an amino acid with a translation-termination codon (UAG, UGA, or UAA).

A **stop codon (Termination codon)** is one of three triplets (UAG, UAA, UGA) that causes protein synthesis to terminate. They are also known historically as *nonsense codons*. The UAA codon is called ochre, and the UAA codon is called amber, after the names of the nonsense mutations by which they were originally identified.

Key Concepts

- The codons UAA (ochre), UAG (amber) and UGA terminate protein synthesis.
- In bacteria they are used most often with relative frequencies UAA>UGA>UAG.

Only 61 triplets are assigned to amino acids. The other three triplets are **termination codons** (or stop codons) that end protein synthesis. They have casual names from the history of their discovery. The UAG triplet is called the **amber** codon; UAA is the **ochre** codon; and UGA is sometimes called the **opal** codon.

The nature of these triplets was originally shown by a genetic test that distinguished two types of point mutation:

- A point mutation that changes a codon to represent a different amino acid is called a **missense** mutation. One amino acid replaces the other in the protein; the effect on protein function depends on the site of mutation and the nature of the amino acid replacement.

- When a point mutation creates one of the three termination codons, it causes **premature termination** of protein synthesis at the mutant codon. Only the first part of the protein is made in the mutant cell. This is likely to abolish protein function (depending, of course, on how far along the protein the mutant site is located). A change of this sort is called a **nonsense mutation**.

(Sometimes the term *nonsense codon* is used to describe the termination triplets. "Nonsense" is really a misnomer, since the codons do have meaning, albeit a disruptive one in a mutant gene. A better term is **stop codon**.)

In every gene that has been sequenced, one of the termination codons lies immediately after the codon representing the C-terminal amino acid of the wild-type sequence. Nonsense mutations show that any one of the three codons is sufficient to terminate protein synthesis within a gene. The UAG, UAA, and UGA triplet sequences are therefore necessary and sufficient to end protein synthesis, whether occurring naturally at the end of a gene or created by mutation within a coding sequence.

In bacterial genes, UAA is the most commonly used termination codon. UGA is used more heavily than UAG, although there appear to be more errors reading UGA. (An error in reading a termination codon, when an aminoacyl-tRNA improperly responds to it, results in the continuation of protein synthesis until another termination codon is encountered.)

PROTEIN SYNTHESIS

2.6.15 Termination codons are recognized by protein factors

Key Terms

A **release factor (RF)** is required to terminate protein synthesis to cause release of the completed polypeptide chain and the ribosome from mRNA. Individual factors are numbered. Eukaryotic factors are called eRF.

RF1 is the bacterial release factor that recognizes UAA and UAG as signals to terminate protein synthesis.

RF2 is the bacterial release factor that recognizes UAA and UGA as signals to terminate protein synthesis.

RF3 is a protein synthesis termination factor related to the elongation factor EF-G. It functions to release the factors RF1 or RF2 from the ribosome when they act to terminate protein synthesis.

Key Concepts

- Termination codons are recognized by protein release factors, not by aminoacyl-tRNAs.
- The structures of the class 1 release factors resemble aminoacyl-tRNA·EF-Tu and EF-G.
- The class 1 release factors respond to specific termination codons and hydrolyze the polypeptide-tRNA linkage.
- The class 1 release factors are assisted by class 2 release factors that depend on GTP.
- The mechanism is similar in bacteria (which have two types of class 1 release factors) and eukaryotes (which have only one class 1 release factor).

Two stages are involved in ending translation. The *termination reaction* itself involves release of the protein chain from the last tRNA. The *post-termination reaction* involves release of the tRNA and mRNA, and dissociation of the ribosome into its subunits.

None of the termination codons is represented by a tRNA. They function in an entirely different manner from other codons, and are recognized directly by protein factors. (Since the reaction does not depend on codon-anticodon recognition, there seems to be no particular reason why it should require a triplet sequence. Presumably this reflects the evolution of the genetic code.)

Termination codons are recognized by class 1 **release factors** (**RF**). In *E. coli* two class 1 release factors are specific for different sequences (927). **RF1** recognizes UAA and UAG; **RF2** recognizes UGA and UAA. The factors act at the ribosomal A

site and require polypeptidyl-tRNA in the P site. The release factors are present at much lower levels than initiation or elongation factors; there are ~600 molecules of each per cell, equivalent to 1 RF per 10 ribosomes. Probably at one time there was only a single release factor, recognizing all termination codons, and later it evolved into two factors with specificities for particular codons. In eukaryotes, there is only a single class 1 release factor, called eRF. The efficiency with which the bacterial factors recognize their target codons is influenced by the bases on the 3′ side.

The class 1 release factors are assisted by class 2 release factors, which are not codon-specific. The class 2 factors are GTP-binding proteins. In *E. coli*, the role of the class 2 factor is to release the class 1 factor from the ribosome.

Although the general mechanism of termination is similar in prokaryotes and eukaryotes, the interactions between the class 1 and class 2 factors have some differences.

The class 1 factors RF1 and RF2 recognize the termination codons and activate the ribosome to hydrolyze the peptidyl tRNA. Cleavage of polypeptide from tRNA takes place by a reaction analogous to the usual peptidyl transfer, except that the acceptor is H_2O instead of aminoacyl-tRNA (see **Figure 6.34**).

Then RF1 or RF2 is released from the ribosome by the class 2 factor **RF3**, which is related to EF-G (1161; 1162). RF3-GDP binds to the ribosome before the termination reaction occurs. The GDP is replaced by GTP. This enables RF3 to contact the ribosome GTPase center, where it causes RF1/2 to be released when the polypeptide chain is terminated (4766).

RF3 resembles the GTP-binding domains of EF-Tu and EF-G, and RF1/2 resemble the C-terminal domain of EF-G, which mimics tRNA. This suggests that the release factors utilize the same site that is used by the elongation factors. **Figure 6.32** illustrates the basic idea that these factors all have the same general shape and bind to the ribosome successively at the same site (basically the A site or a region extensively overlapping with it) (2231; for review see 3050).

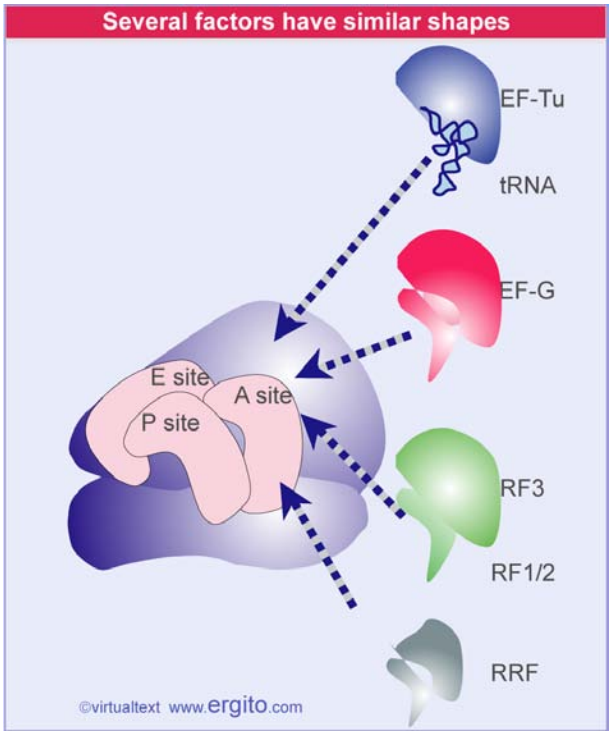

Figure 6.32 Molecular mimicry enables the elongation factor Tu-tRNA complex, the translocation factor EF-G, and the release factors RF1/2-RF3 to bind to the same ribosomal site.

The eukaryotic class 1 release factor, eRF1, is a single protein that recognizes all three termination codons (2229). Its sequence is unrelated to the bacterial factors. It can terminate protein synthesis *in vitro* without the class 3 factor, eRF3, although eRF3 is essential in yeast *in vivo*. The structure of eRF1 follows a familiar theme: **Figure 6.33** shows that it consists of three domains that mimic the structure of tRNA (2230).

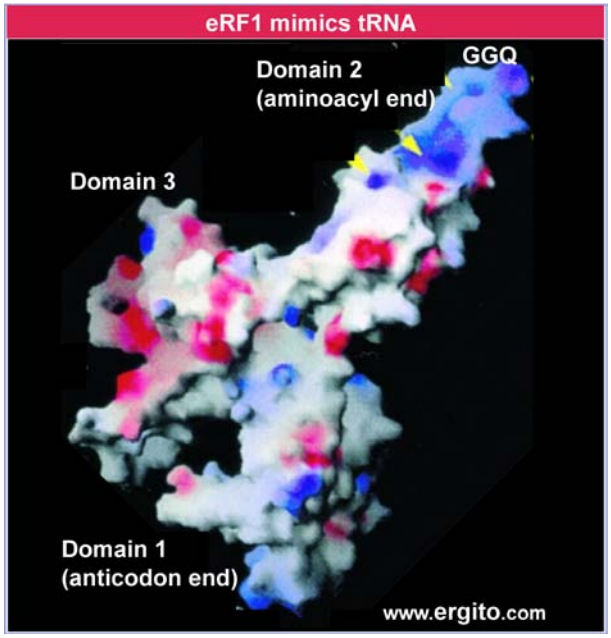

Figure 6.33 The eukaryotic termination factor eRF1 has a structure that mimics tRNA. The motif GGQ at the tip of domain 2 is essential for hydrolyzing the polypeptide chain from tRNA. Photograph kindly provided by David Barford (see 2230).

An essential motif of three amino acids, GGQ, is exposed at the top of domain 2. Its position in the A site corresponds to the usual location of an amino acid on an aminoacyl-tRNA. This positions it to use the glutamine (Q) to position a water molecule to substitute for the amino acid of aminoacyl-tRNA in the peptidyl transfer reaction. **Figure 6.34** compares the termination reaction with the usual peptide transfer reaction. Termination transfers a hydroxyl group from the water, thus effectively hydrolyzing the peptide-tRNA bond (and see **Figure 6.48** for discussion of how the peptidyl transferase center works).

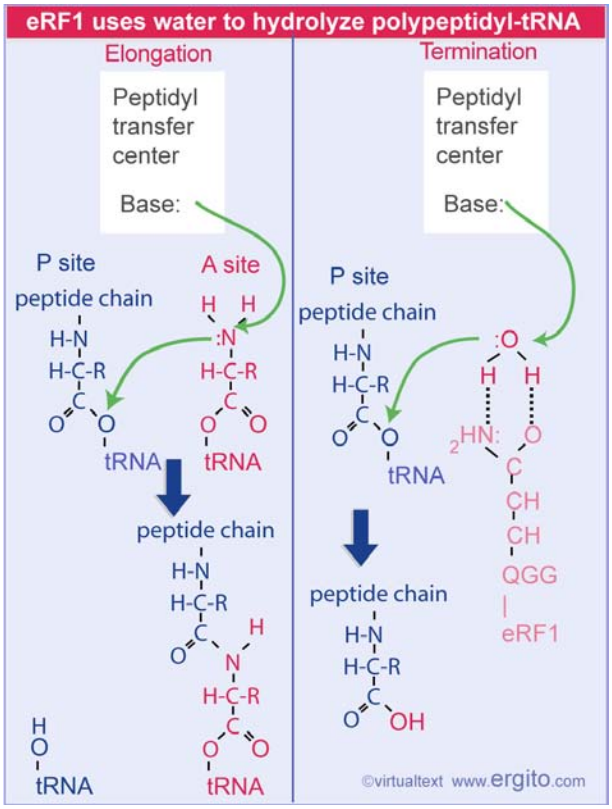

Figure 6.34 Peptide transfer and termination are similar reactions in which a base in the peptidyl transfer center triggers a transesterification reaction by attacking an N-H or O-H bond, releasing the N or O to attack the link to tRNA.

Mutations in the RF genes reduce the efficiency of termination, as seen by an increased ability to continue protein synthesis past the termination codon. Overexpression of RF1 or RF2 increases the efficiency of termination at the codons on which it acts. This suggests that codon recognition by RF1 or RF2 competes with aminoacyl-tRNAs that erroneously recognize the termination codons (for review see 36). The release factors recognize their target sequences very efficiently (1163).

The termination reaction involves release of the completed polypeptide, but leaves a deacylated tRNA and the mRNA still associated with the ribosome. **Figure 6.35** shows that the dissociation of the remaining components (tRNA, mRNA, 30S and 50S subunits) requires the factor RRF, ribosome recycling factor. This acts together with EF-G in a reaction that uses hydrolysis of GTP. Like the other factors involved in release, RRF has a structure that mimics tRNA, except that it lacks an equivalent for the 3′ amino acid-binding region (926). IF-3 is also required, which brings the wheel full circle to its original discovery, when it was proposed to be a dissociation factor! RRF acts on the 50S subunit, and IF-3 acts to remove deacylated tRNA from the 30S subunit. Once the subunits have separated, IF-3 remains necessary, of course, to prevent their reassociation.

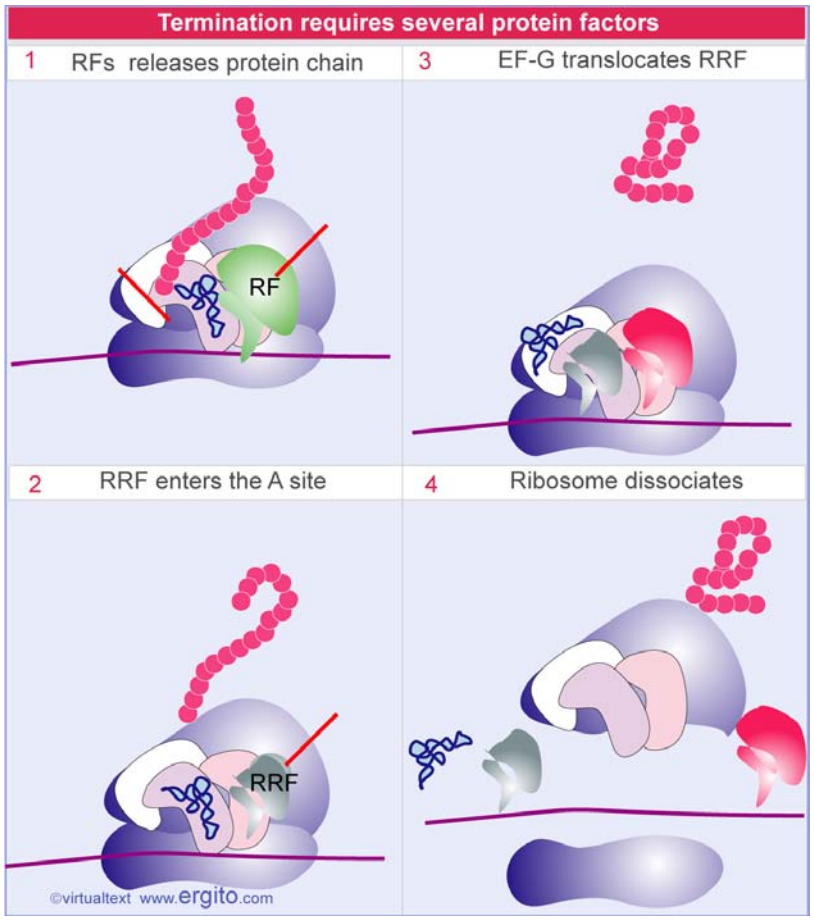

Figure 6.35 The RF (release factor) terminates protein synthesis by releasing the protein chain. The RRF (ribosome recycling factor) releases the last tRNA, and EF-G releases RRF, causing the ribosome to dissociate.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.6.15 to view properly.

Last updated on March 4, 2004

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PROTEIN SYNTHESIS

2.6.16 Ribosomal RNA pervades both ribosomal subunits

Key Terms

5S RNA is a 120 base RNA that is a component of the large subunit of the ribosome.

5.8S RNA is an independent small RNA present on the large subunit of eukaryotic ribosomes. It is homologous to the 5′ end of bacterial 23S rRNA.

Key Concepts

- Each rRNA has several distinct domains that fold independently.
- Virtually all ribosomal proteins are in contact with rRNA.
- Most of the contacts between ribosomal subunits are made between the 16S and 23S rRNAs.

Two thirds of the mass of the bacterial ribosome is made up of rRNA. The most penetrating approach to analyzing secondary structure of large RNAs is to compare the sequences of corresponding rRNAs in related organisms. Those regions that are important in the secondary structure retain the ability to interact by base pairing. So if a base pair is required, it can form at the same relative position in each rRNA. This approach has enabled detailed models to be constructed for both 16S and 23S rRNA.

Each of the major rRNAs can be drawn in a secondary structure with several discrete domains. 16S rRNA forms four general domains, in which just under half of the sequence is base paired (see **Figure 6.45**). 23S rRNA forms six general domains. The individual double-helical regions tend to be short (<8 bp). Often the duplex regions are not perfect, but contain bulges of unpaired bases. Comparable models have been drawn for mitochondrial rRNAs (which are shorter and have fewer domains) and for eukaryotic cytosolic rRNAs (which are longer and have more domains). The increase in length in eukaryotic rRNAs is due largely to the acquisition of sequences representing additional domains (for review see 431). The crystal structure of the ribosome shows that in each subunit the domains of the major rRNA fold independently and have a discrete location in the subunit (1670).

Differences in the ability of 16S rRNA to react with chemical agents are found when 30S subunits are compared with 70S ribosomes; also there are differences between free ribosomes and those engaged in protein synthesis. Changes in the reactivity of the rRNA occur when mRNA is bound, when the subunits associate, or when tRNA is bound. Some changes reflect a direct interaction of the rRNA with mRNA or tRNA, while others are caused indirectly by other changes in ribosome structure. The main point is that ribosome conformation is flexible during protein synthesis.

A feature of the primary structure of rRNA is the presence of methylated residues. There are ~10 methyl groups in 16S rRNA (located mostly toward the 3′ end of the

molecule) and ~20 in 23S rRNA. In mammalian cells, the 18S and 28S rRNAs carry 43 and 74 methyl groups, respectively, so ~2% of the nucleotides are methylated (about three times the proportion methylated in bacteria).

The large ribosomal subunit also contains a molecule of a 120 base **5S RNA** (in all ribosomes except those of mitochondria). The sequence of 5S RNA is less well conserved than those of the major rRNAs. All 5S RNA molecules display a highly base-paired structure.

In eukaryotic cytosolic ribosomes, another small RNA is present in the large subunit. This is the **5.8S RNA**. Its sequence corresponds to the 5′ end of the prokaryotic 23S rRNA.

Some ribosomal proteins bind strongly to isolated rRNA. Some do not bind to free rRNA, but can bind after other proteins have bound. This suggests that the conformation of the rRNA is important in determining whether binding sites exist for some proteins. As each protein binds, it induces conformational changes in the rRNA that make it possible for other proteins to bind. In *E. coli*, virtually all the 30S ribosomal proteins interact (albeit to varying degrees) with 16S rRNA. The binding sites on the proteins show a wide variety of structural features, suggesting that protein-RNA recognition mechanisms may be diverse.

The 70S ribosome has an asymmetric construction. **Figure 6.36** shows a schematic of the structure of the 30S subunit, which is divided into four regions: the head, neck, body, and platform. **Figure 6.37** shows a similar representation of the 50S subunit, where two prominent features are the central protuberance (where 5S rRNA is located) and the stalk (made of multiple copies of protein L7). **Figure 6.38** shows that the platform of the small subunit fits into the notch of the large subunit. There is a cavity between the subunits which contains some of the important sites (for review see 430; 433; 434).

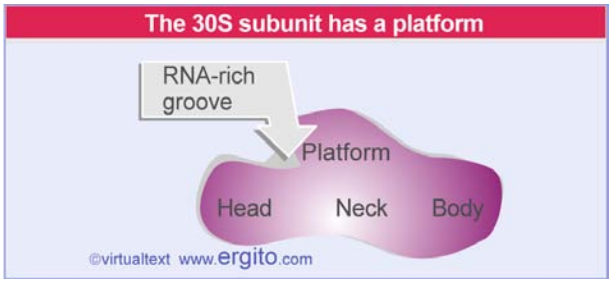

Figure 6.36 The 30S subunit has a head separated by a neck from the body, with a protruding platform.

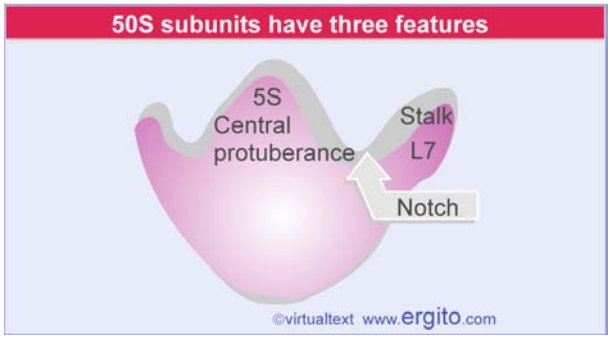

Figure 6.37 The 50S subunit has a central protuberance where 5S rRNA is located, separated by a notch from a stalk made of copies of the protein L7.

Figure 6.38 The platform of the 30S subunit fits into the notch of the 50S subunit to form the 70S ribosome.

The structure of the 30S subunit follows the organization of 16S rRNA, with each structural feature corresponding to a domain of the rRNA. The body is based on the 5′ domain, the platform on the central domain, and the head on the 3′ region. **Figure 6.39** shows that the 30S subunit has an asymmetrical distribution of RNA and protein (931; 1184). One important feature is that the platform of the 30S subunit that provides the interface with the 50S subunit is composed almost entirely of RNA. Only two proteins (a small part of S7 and possibly part of S12) lie near the interface. This means that the association and dissociation of ribosomal subunits must depend on interactions with the 16S rRNA. Subunit association is affected by a mutation in a loop of 16S rRNA (at position 791) that is located at the subunit interface, and other nucleotides in 16S rRNA have been shown to be involved by modification/interference experiments. This behavior supports the idea that the evolutionary origin of the ribosome may have been as a particle consisting of RNA rather than protein.

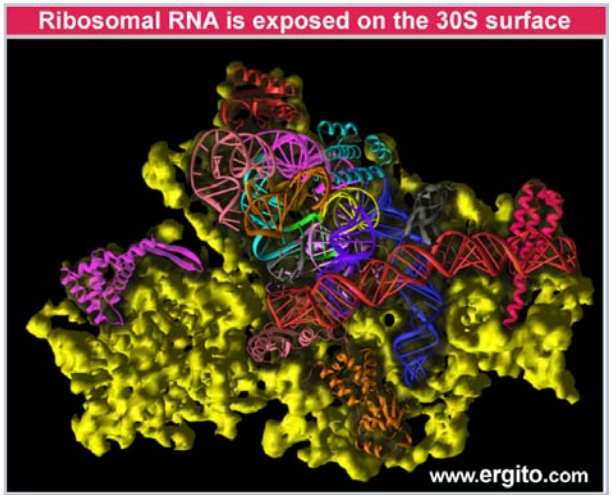

Figure 6.39 The 30S ribosomal subunit is a ribonucleoprotein particle. Proteins are in yellow. Photograph kindly provided by Venkitaraman Ramakrishnan.

The 50S subunit has a more even distribution of components than the 30S, with long rods of double-stranded RNA crisscrossing the structure (932; 1086). The RNA forms a mass of tightly packed helices. The exterior surface largely consists of protein, except for the peptidyl transferase center (see *Molecular Biology 2.6.19 23S rRNA has peptidyl transferase activity*). Almost all segments of the 23S rRNA interact with protein, but many of the proteins are relatively unstructured.

The junction of subunits in the 70S ribosome involves contacts between 16S rRNA (many in the platform region) with 23S rRNA. There are also some interactions between rRNA of each subunit with proteins in the other, and a few protein-protein contacts. **Figure 6.40** identifies the contact points on the rRNA structures. **Figure 6.41** opens out the structure (imagine the 50S subunit rotated counterclockwise and the 30S subunit rotated clockwise around the axis shown in the figure) to show the locations of the contact points on the face of each subunit.

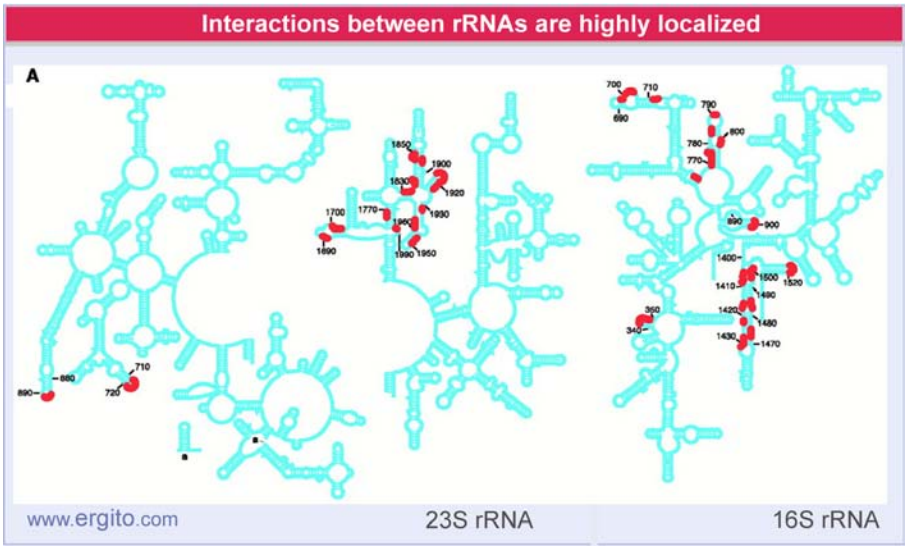

Figure 6.40 Contact points between the rRNAs are located in two domains of 16S rRNA and one domain of 23S rRNA. Photograph kindly provided by Harry Noller (see 1670).

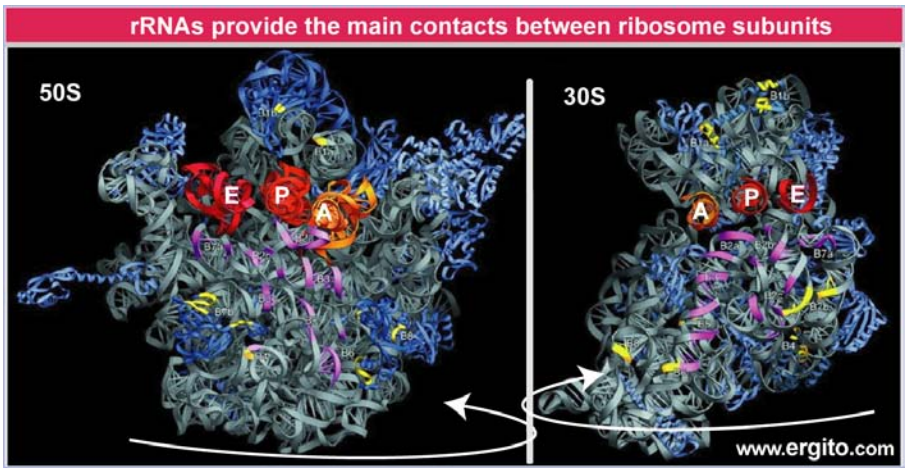

Figure 6.41 Contacts between the ribosomal subunits are mostly made by RNA (shown in purple). Contacts involving proteins are shown in yellow. The two subunits are rotated away from one another to show the faces where contacts are made; from a plane of contact perpendicular to the screen, the 50S subunit is rotated 90°counter-clockwise, and the 30S is rotated 90° clockwise (this shows it in the reverse of the usual orientation). Photograph kindly provided by Harry Noller (see 1670).

Last updated on 4-9-2001

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PROTEIN SYNTHESIS

2.6.17 Ribosomes have several active centers

Key Concepts

- Interactions involving rRNA are a key part of ribosome function.
- The environment of the tRNA-binding sites is largely determined by rRNA.

The basic message to remember about the ribosome is that it is a cooperative structure that depends on changes in the relationships among its active sites during protein synthesis. The active sites are not small, discrete regions like the active centers of enzymes. They are large regions whose construction and activities may depend just as much on the rRNA as on the ribosomal proteins. The crystal structures of the individual subunits and bacterial ribosomes give us a good impression of the overall organization and emphasize the role of the rRNA. The most recent structure, at 5.5 Å resolution, clearly identifies the locations of the tRNAs and the functional sites (1670; for review see 2390). We can now account for many ribosomal functions in terms of its structure (for review see 3447, 4529).).

Ribosomal functions are centered around the interaction with tRNAs. **Figure 6.42** shows the 70S ribosome with the positions of tRNAs in the three binding sites (933). The tRNAs in the A and P sites are nearly parallel to one another. All three tRNAs are aligned with their anticodon loops bound to the mRNA in the groove on the 30S subunit. The rest of each tRNA is bound to the 50S subunit. The environment surrounding each tRNA is mostly provided by rRNA. In each site, the rRNA contacts the tRNA at parts of the structure that are universally conserved.

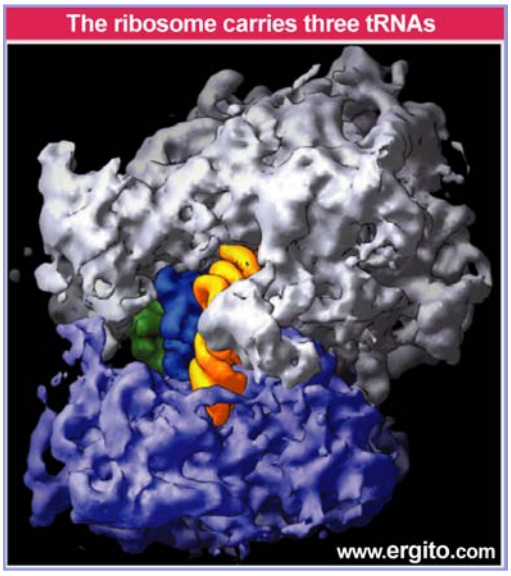

Figure 6.42 The 70S ribosome consists of the 50S subunit (blue) and the 30S subunit (purple) with three tRNAs located superficially: yellow in the A site, blue in the P site, and green in the E site. Photograph kindly provided by Harry Noller (see 933

It has always been a big puzzle to understand how two bulky tRNAs can fit next to one another in reading adjacent codons. The crystal structure shows a 45° kink in the mRNA between the P and A sites, which allows the tRNAs to fit as shown in the expansion of **Figure 6.43**. The tRNAs in the P and A sites are angled at 26° relative to each other at their anticodons. The closest approach between the backbones of the tRNAs occurs at the 3′ ends, where they converge to within 5 Å (perpendicular to the plane of the screen). This allows the peptide chain to be transferred from the peptidyl-tRNA in the A site to the aminoacyl-tRNA in the A site.

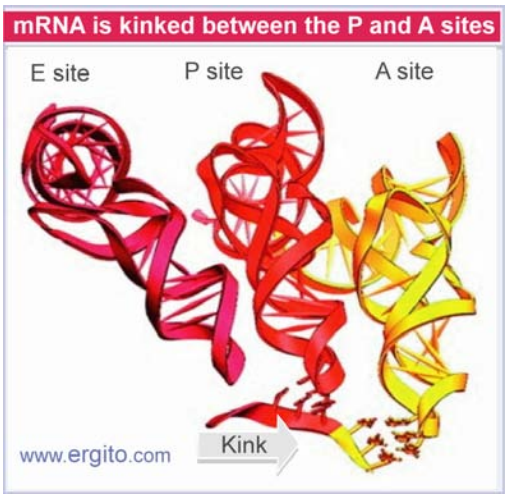

Figure 6.43 Three tRNAs have different orientations on the ribosome. mRNA turns between the P and A sites to allow aminoacyl-tRNAs to bind adjacent codons. Photograph kindly provided by Harry Noller (see 1670).

Aminoacyl-tRNA is inserted into the A site by EF-Tu, and its pairing with the codon is necessary for EF-Tu to hydrolyze GTP and be released from the ribosome (see *Molecular Biology 2.6.10 Elongation factor Tu loads aminoacyl-tRNA into the A site*). EF-Tu initially places the aminoacyl-tRNA into the small subunit, where the anticodon pairs with the codon. Movement of the tRNA is required to bring it fully into the A site, when its 3′ end enters the peptidyl transferase center on the large subunit. There are different models for how this process may occur. One calls for the entire tRNA to swivel, so that the elbow in the L-shaped structure made by the D and T Ψ C arms moves into the ribosome, enabling the T Ψ C arm to pair with rRNA (2846). Another calls for the internal structure of the tRNA to change, using the anticodon loop as a hinge, with the rest of the tRNA rotating from a position in which it is stacked on the 3′ side of the anticodon loop to one in which it is stacked on the 5′ side (3060). Following the transition, EF-Tu hydrolyzes GTP, allowing peptide synthesis to proceed.

Translocation involves large movements in the positions of the tRNAs within the ribosome. The anticodon end of tRNA moves ~28 Å from the A site to the P site, and then a further 20 Å from the P site to the E site. Because of the angle of each tRNA relative to the anticodon, the bulk of the tRNA moves much larger distances, 40 Å from A to P site, and 55 Å from P site to E site. This suggests that translocation requires a major reorganization of structure.

For many years, it was thought that translocation could occur only in the presence of the factor EF-G. However, the antibiotic sparsomyicn (which inhibits the peptidyl transferase activity) triggers translocation (3998). This suggests that the energy to drive translocation actually is stored in the ribosome after peptide bond formation has occurred. Usually EF-G acts on the ribosome to release this energy and enable it to drive translocation, but sparsomycin can have the same role. Sparsomycin inhibits peptidyl transferase by binding to the peptidyl-tRNA, blocking its interaction with aminoacyl-tRNA. It probably creates a conformation that resembles the usual post-translocation conformation, which in turn promotes movement of the peptidyl-tRNA. The important point is that translocation is an intrinsic property of the ribosome.

The hybrid states model suggests that translocation may take place in two stages, with one ribosomal subunit moving relative to the other to create an intermediate stage in which there are hybrid tRNA-binding sites (50S E/30S P and 50S P/ 30S A) (see **Figure 6.29**). Comparisons of the ribosome structure between pre- and post-translocation states, and comparisons in 16S rRNA conformation between free 30S subunits and 70S ribosomes, suggest that mobility of structure is especially marked in the head and platform regions of the 30S subunit. An interesting insight on the hybrid states model is cast by the fact that many bases in rRNA involved in subunit association are close to bases involved in interacting with tRNA. This suggests that tRNA-binding sites are close to the interface between subunits, and carries the implication that changes in subunit interaction could be connected with movement of tRNA.

Much of the structure of the ribosome is occupied by its active centers. The schematic view of the ribosomal sites in **Figure 6.44** shows they comprise about two thirds of the ribosomal structure. A tRNA enters the A site, is transferred by translocation into the P site, and then leaves the (bacterial) ribosome by the E site. The A and P sites extend across both ribosome subunits; tRNA is paired with mRNA

in the 30S subunit, but peptide transfer takes place in the 50S subunit. The A and P sites are adjacent, enabling translocation to move the tRNA from one site into the other. The E site is located near the P site (representing a position en route to the surface of the 50S subunit). The peptidyl transferase center is located on the 50S subunit, close to the aminoacyl ends of the tRNAs in the A and P sites (see *Molecular Biology 2.6.18 16S rRNA plays an active role in protein synthesis*).

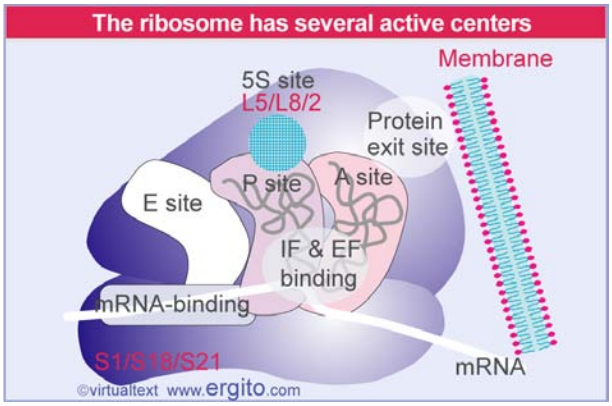

Figure 6.44 The ribosome has several active centers. It may be associated with a membrane. mRNA takes a turn as it passes through the A and P sites, which are angled with regard to each other. The E site lies beyond the P site. The peptidyl transferase site (not shown) stretches across the tops of the A and P sites. Part of the site bound by EF-Tu/G lies at the base of the A and P sites.

All of the GTP-binding proteins that function in protein synthesis (EF-Tu, EF-G, IF-2, RF1,2,3) bind to the same factor-binding site (sometimes called the GTPase center), which probably triggers their hydrolysis of GTP. It is located at the base of the stalk of the large subunit, which consists of the proteins L7/L12. (L7 is a modification of L12, and has an acetyl group on the N-terminus.) In addition to this region, the complex of protein L11 with a 58 base stretch of 23S rRNA provides the binding site for some antibiotics that affect GTPase activity. Neither of these ribosomal structures actually possesses GTPase activity, but they are both necessary for it. The role of the ribosome is to trigger GTP hydrolysis by factors bound in the factor-binding site.

Initial binding of 30S subunits to mRNA requires protein S1, which has a strong affinity for single-stranded nucleic acid. It is responsible for maintaining the single-stranded state in mRNA that is bound to the 30S subunit (2200). This action is necessary to prevent the mRNA from taking up a base-paired conformation that would be unsuitable for translation. S1 has an extremely elongated structure and associates with S18 and S21. The three proteins constitute a domain that is involved in the initial binding of mRNA and in binding initiator tRNA. This locates the mRNA-binding site in the vicinity of the cleft of the small subunit (see **Figure 6.38**). The 3′ end of rRNA, which pairs with the mRNA initiation site, is located in this region.

The initiation factors bind in the same region of the ribosome. IF-3 can be crosslinked to the 3′ end of the rRNA, as well as to several ribosomal proteins, including those probably involved in binding mRNA. The role of IF-3 could be to

stabilize mRNA·30S subunit binding; then it would be displaced when the 50S subunit joins.

The incorporation of 5S RNA into 50S subunits that are assembled in vitro depends on the ability of three proteins, L5, L8, and L25, to form a stoichiometric complex with it. The complex can bind to 23S rRNA, although none of the isolated components can do so. It lies in the vicinity of the P and A sites.

A nascent protein debouches through the ribosome, away from the active sites, into the region in which ribosomes may be attached to membranes (see *Molecular Biology 2.8 Protein localization*). A polypeptide chain emerges from the ribosome through an exit channel, which leads from the peptidyl transferase site to the surface of the 50S subunit. The tunnel is composed mostly of rRNA. It is quite narrow, only 1-2 nm wide, and ~10 nm long. The nascent polypeptide emerges from the ribosome ~15 Å away from the peptidyl transferase site. The tunnel can hold ~50 amino acids, and probably constrains the polypeptide chain so that it cannot fold until it leaves the exit domain.

Last updated on 7-21-2003

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PROTEIN SYNTHESIS

2.6.18 16S rRNA plays an active role in protein synthesis

Key Concepts

- 16S rRNA plays an active role in the functions of the 30S subunit. It interacts directly with mRNA, with the 50S subunit, and with the anticodons of tRNAs in the P and A sites.

The ribosome was originally viewed as a collection of proteins with various catalytic activities, held together by protein-protein interactions and by binding to rRNA. But the discovery of RNA molecules with catalytic activities (see *Molecular Biology 5.24 RNA splicing and processing*) immediately suggests that rRNA might play a more active role in ribosome function. There is now evidence that rRNA interacts with mRNA or tRNA at each stage of translation, and that the proteins are necessary to maintain the rRNA in a structure in which it can perform the catalytic functions. Several interactions involve specific regions of rRNA:

- The 3′ terminus of the rRNA interacts directly with mRNA at initiation.
- Specific regions of 16S rRNA interact directly with the anticodon regions of tRNAs in both the A site and the P site. Similarly, 23S rRNA interacts with the CCA terminus of peptidyl-tRNA in both the P site and A site.
- Subunit interaction involves interactions between 16S and 23S rRNAs (see *Molecular Biology 2.6.16 Ribosomal RNA pervades both ribosomal subunits*).

Much information about the individual steps of bacterial protein synthesis has been obtained by using antibiotics that inhibit the process at particular stages. The target for the antibiotic can be identified by the component in which resistant mutations occur. Some antibiotics act on individual ribosomal proteins, but several act on rRNA, which suggests that the rRNA is involved with many or even all of the functions of the ribosome.

The functions of rRNA have been investigated by two types of approach. Structural studies show that particular regions of rRNA are located in important sites of the ribosome, and that chemical modifications of these bases impede particular ribosomal functions. And mutations identify bases in rRNA that are required for particular ribosomal functions. **Figure 6.45** summarizes the sites in 16S rRNA that have been identified by these means (for review see 436).

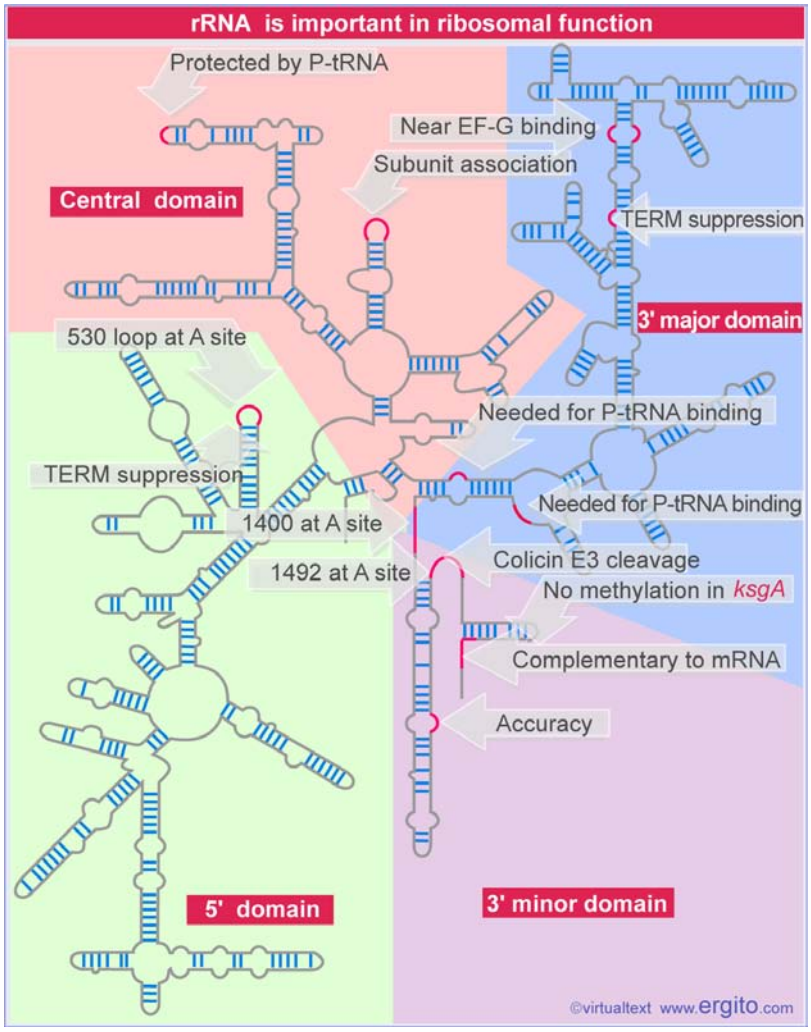

Figure 6.45 Some sites in 16S rRNA are protected from chemical probes when 50S subunits join 30S subunits or when aminoacyl-tRNA binds to the A site. Others are the sites of mutations that affect protein synthesis. TERM suppression sites may affect termination at some or several termination codons. The large colored blocks indicate the four domains of the rRNA.

An indication of the importance of the 3′ end of 16S rRNA is given by its susceptibility to the lethal agent colicin E3. Produced by some bacteria, the colicin cleaves ~50 nucleotides from the 3′ end of the 16S rRNA of *E. coli*. The cleavage entirely abolishes initiation of protein synthesis. Several important functions require the region that is cleaved: binding the factor IF-3; recognition of mRNA; and binding of tRNA.

The 3′ end of the 16S rRNA is directly involved in the initiation reaction by pairing with the Shine-Dalgarno sequence in the ribosome-binding site of mRNA (see **Figure 6.16** and *Great Experiments 4.8 rRNA-mRNA base pairing selects translational initiator regions in bacteria*). Another direct role for the 3′ end of 16S rRNA in protein synthesis is shown by the properties of kasugamycin-resistant mutants, which lack certain modifications in 16S rRNA. Kasugamycin blocks initiation of protein synthesis. Resistant mutants of the type *ksgA* lack a methylase

enzyme that introduces four methyl groups into two adjacent adenines at a site near the 3′ terminus of the 16S rRNA. The methylation generates the highly conserved sequence G–m_2^6A–m_2^6A, found in both prokaryotic and eukaryotic small rRNA. The methylated sequence is involved in the joining of the 30S and 50S subunits, which in turn is connected also with the retention of initiator tRNA in the complete ribosome. Kasugamycin causes fMet-$tRNA_f$ to be released from the sensitive (methylated) ribosomes, but the resistant ribosomes are able to retain the initiator.

Changes in the structure of 16S rRNA occur when ribosomes are engaged in protein synthesis, as seen by protection of particular bases against chemical attack. The individual sites fall into a few groups, concentrated in the 3′ minor and central domains. Although the locations are dispersed in the linear sequence of 16S rRNA, it seems likely that base positions involved in the same function are actually close together in the tertiary structure.

Some of the changes in 16S rRNA are triggered by joining with 50S subunits, binding of mRNA, or binding of tRNA. They indicate that these events are associated with changes in ribosome conformation that affect the exposure of rRNA. They do not necessarily indicate direct participation of rRNA in these functions. One change that occurs during protein synthesis is shown in **Figure 6.46**; it involves a local movement to change the nature of a short duplex sequence.

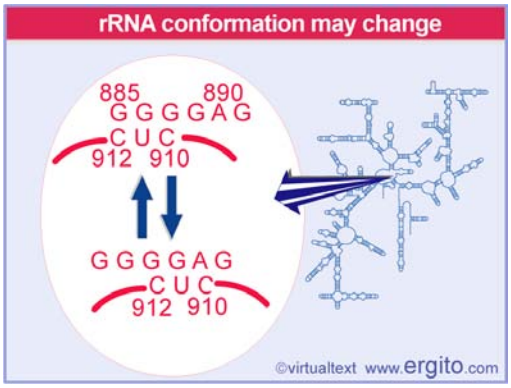

Figure 6.46 A change in conformation of 16S rRNA may occur during protein synthesis.

The 16S rRNA is involved in both A site and P site function, and significant changes in its structure occur when these sites are occupied (934). Certain distinct regions are protected by tRNA bound in the A site (see **Figure 6.45**). One is the 530 loop (which is also is the site of a mutation that prevents termination at the UAA, UAG, and UGA codons). The other is the 1400-1500 region (so-called because bases 1399-1492 and the adenines at 1492-1493 are two single-stranded stretches that are connected by a long hairpin). All of the effects that tRNA binding has on 16S rRNA can be produced by the isolated oligonucleotide of the anticodon stem-loop, so that tRNA-30S subunit binding must involve this region (443).

The adenines at 1492-1493 provide a mechanism for detecting properly paired codon-anticodon complexes (935). The principle of the interaction is that the structure of the 16S rRNA responds to the structure of the first two bases pairs in the minor groove of the duplex formed by the codon-anticodon interaction. Modification

of the N1 position of either base 1492 or 1493 in rRNA prevents tRNA from binding in the A site. However, mutations at 1492 or 1493 can be suppressed by the introduction of fluorine at the 2′ position of the corresponding bases in mRNA (which restores the interaction). **Figure 6.47** shows that codon-anticodon pairing allows the N1 of each adenine to interact with the 2′–OH in the mRNA backbone. When an incorrect tRNA enters the A site, the structure of the codon-anticodon complex is distorted, and this interaction cannot occur. The interaction stabilizes the association of tRNA with the A site.

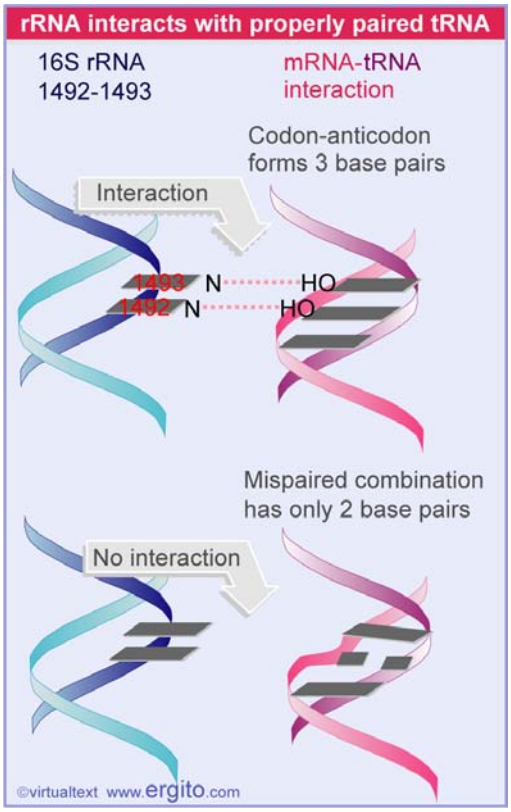

Figure 6.47 Codon-anticodon pairing supports interaction with adenines 1492-1493 of 16S rRNA, but mispaired tRNA-mRNA cannot interact.

A variety of bases in different positions of 16S rRNA are protected by tRNA in the P site; probably the bases lie near one another in the tertiary structure. In fact, there are more contacts with tRNA when it is in the P site than when it is in the A site. This may be responsible for the increased stability of peptidyl-tRNA compared with aminoacyl-tRNA. This makes sense, because once the tRNA has reached the P site, the ribosome has decided that it is correctly bound, whereas in the A site, the assessment of binding is being made. The 1400 region can be directly cross-linked to peptidyl-tRNA, which suggests that this region is a structural component of the P site.

The basic conclusion to be drawn from these results is that rRNA has many interactions with both tRNA and mRNA, and that these interactions recur in each cycle of peptide bond formation.

Reviews

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PROTEIN SYNTHESIS

2.6.19 23S rRNA has peptidyl transferase activity

Key Concepts

- Peptidyl transferase activity resides exclusively in the 23S rRNA.

The sites involved in the functions of 23S rRNA are less well identified than those of 16S rRNA, but the same general pattern is observed: bases at certain positions affect specific functions. Bases at some positions in 23S rRNA are affected by the conformation of the A site or P site. In particular, oligonucleotides derived from the 3′CCA terminus of tRNA protect a set of bases in 23S rRNA which essentially are the same as those protected by peptidyl-tRNA. This suggests that the major interaction of 23S rRNA with peptidyl-tRNA in the P site involves the 3′ end of the tRNA.

The tRNA makes contacts with the 23S rRNA in both the P and A sites. At the P site, G2552 of 23S rRNA base pairs with C74 of the peptidyl tRNA. A mutation in the G in the rRNA prevents interaction with tRNA, but interaction is restored by a compensating mutation in the C of the amino acceptor end of the tRNA. At the A site, G2553 of the 23S rRNA base pairs with C75 of the aminoacyl-tRNA. So there is a close role for rRNA in both the tRNA-binding sites. Indeed, when we have a clearer structural view of the region, we should be able to understand the movements of tRNA between the A and P sites in terms of making and breaking contacts with rRNA (442).

Another site that binds tRNA is the E site, which is localized almost exclusively on the 50S subunit. Bases affected by its conformation can be identified in 23S rRNA.

What is the nature of the site on the 50S subunit that provides peptidyl transferase function? The involvement of rRNA was first indicated because a region of the 23S rRNA is the site of mutations that confer resistance to antibiotics that inhibit peptidyl transferase.

A long search for ribosomal proteins that might possess the catalytic activity has been unsuccessful. More recent results suggest that the ribosomal RNA of the large subunit has the catalytic activity. Extraction of almost all the protein content of 50S subunits leaves the 23S rRNA associated largely with fragments of proteins, amounting to <5% of the mass of the ribosomal proteins. This preparation retains peptidyl transferase activity. Treatments that damage the RNA abolish the catalytic activity (441).

Following from these results, 23S rRNA prepared by transcription *in vitro* can catalyze the formation of a peptide bond between Ac-Phe-tRNA and Phe-tRNA. The yield of Ac-Phe-Phe is very low, suggesting that the 23S rRNA requires proteins in order to function at a high efficiency. But since the rRNA has the basic catalytic activity, the role of the proteins must be indirect, serving to fold the rRNA properly or to present the substrates to it. The reaction also works, although less effectively, if

the domains of 23S rRNA are synthesized separately and then combined. In fact, some activity is shown by domain V alone, which has the catalytic center. Activity is abolished by mutations in position 2252 of domain V that lies in the P site.

The crystal structure of an archaeal 50S subunit shows that the peptidyl transferase site basically consists of 23S rRNA (1086). There is no protein within 18Å of the active site where the transfer reaction occurs between peptidyl-tRNA and aminoacyl-tRNA!

Peptide bond synthesis requires an attack by the amino group of one amino acid on the carboxyl group of another amino acid. Catalysis requires a basic residue to accept the hydrogen atom that is released from the amino group, as shown in **Figure 6.48**. If rRNA is the catalyst, it must provide this residue, but we do not know how this happens. The purine and pyrimidine bases are not basic at physiological pH. A highly conserved base (at position 2451 in *E. coli*) had been implicated, but appears now neither to have the right properties nor to be crucial for peptidyl transferase activity (2001; 2002).

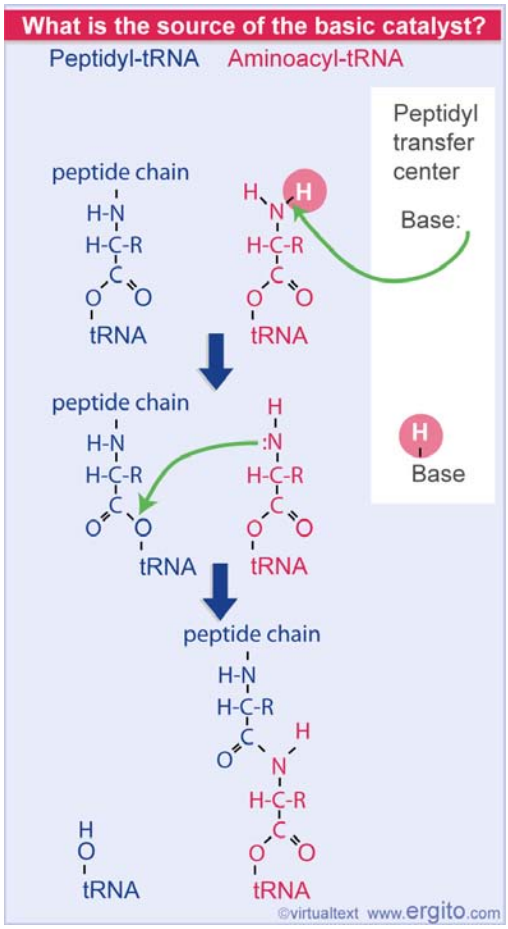

Figure 6.48 Peptide bond formation requires acid-base catalysis in which an H atom is transferred to a basic residue.

Proteins that are bound to the 23S rRNA outside of the peptidyl transfer region are

almost certainly required to enable the rRNA to form the proper structure *in vivo*. The idea that rRNA is the catalytic component is consistent with the results discussed in *Molecular Biology 5.24 RNA splicing and processing*that identify catalytic properties in RNA that are involved with several RNA processing reactions. It fits with the notion that the ribosome evolved from functions originally possessed by RNA.

Last updated on 8-29-2000

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PROTEIN SYNTHESIS

2.6.20 Summary

Ribosomes are ribonucleoprotein particles in which a majority of the mass is provided by rRNA. The shapes of all ribosomes are generally similar, but only those of bacteria (70S) have been characterized in detail. The small (30S) subunit has a squashed shape, with a "body" containing about two-thirds of the mass divided from the "head" by a cleft. The large (50S) subunit is more spherical, with a prominent "stalk" on the right and a "central protuberance." Locations of all proteins are known approximately in the small subunit.

Each subunit contains a single major rRNA, 16S and 23S in prokaryotes, 18S and 28S in eukaryotic cytosol. There are also minor rRNAs, most notably 5S rRNA in the large subunit. Both major rRNAs have extensive base pairing, mostly in the form of short, imperfectly paired duplex stems with single-stranded loops. Conserved features in the rRNA can be identified by comparing sequences and the secondary structures that can be drawn for rRNA of a variety of organisms. The 16S rRNA has four distinct domains; the 23S rRNA has six distinct domains. Eukaryotic rRNAs have additional domains.

The crystal structure shows that the 30S subunit has an asymmetrical distribution of RNA and protein. RNA is concentrated at the interface with the 50S subunit. The 50S subunit has a surface of protein, with long rods of double-stranded RNA crisscrossing the structure. 30S-50S joining involves contacts between 16S rRNA and 23S rRNA.

Each subunit has several active centers, concentrated in the translational domain of the ribosome where proteins are synthesized. Proteins leave the ribosome through the exit domain, which can associate with a membrane. The major active sites are the P and A sites, the E site, the EF-Tu and EF-G binding sites, peptidyl transferase, and mRNA-binding site. Ribosome conformation may change at stages during protein synthesis; differences in the accessibility of particular regions of the major rRNAs have been detected.

The tRNAs in the A and P sites are parallel to one another. The anticodon loops are bound to mRNA in a groove on the 30S subunit. The rest of each tRNA is bound to the 50S subunit. A conformational shift of tRNA within the A site is required to bring its aminoacyl end into juxtaposition with the end of the peptidyl-tRNA in the P site. The peptidyl transferase site that links the P and A binding sites is made of 23S rRNA, which has the peptidyl transferase catalytic activity, although proteins are probably needed to acquire the right structure.

An active role for the rRNAs in protein synthesis is indicated by mutations that affect ribosomal function, interactions with mRNA or tRNA that can be detected by chemical crosslinking, and the requirement to maintain individual base pairing interactions with the tRNA or mRNA. The 3′ terminal region of the rRNA base pairs with mRNA at initiation. Internal regions make individual contacts with the tRNAs in both the P and A sites. Ribosomal RNA is the target for some antibiotics or other agents that inhibit protein synthesis

A codon in mRNA is recognized by an aminoacyl-tRNA, which has an anticodon complementary to the codon and carries the amino acid corresponding to the codon. A special initiator tRNA (fMet-tRNA$_f$ in prokaryotes or Met-tRNA$_i$ in eukaryotes) recognizes the AUG codon, which is used to start all coding sequences. In prokaryotes, GUG is also used. Only the termination (nonsense) codons UAA, UAG, and UGA are not recognized by aminoacyl-tRNAs.

Ribosomes are released from protein synthesis to enter a pool of free ribosomes that are in equilibrium with separate small and large subunits. Small subunits bind to mRNA and then are joined by large subunits to generate an intact ribosome that undertakes protein synthesis. Recognition of a prokaryotic initiation site involves binding of a sequence at the 3′ end of rRNA to the Shine-Dalgarno motif which precedes the AUG (or GUG) codon in the mRNA. Recognition of a eukaryotic mRNA involves binding to the 5′ cap; the small subunit then migrates to the initiation site by scanning for AUG codons. When it recognizes an appropriate AUG codon (usually but not always the first it encounters), it is joined by a large subunit.

A ribosome can carry two aminoacyl-tRNAs simultaneously: its P site is occupied by a polypeptidyl-tRNA, which carries the polypeptide chain synthesized so far, while the A site is used for entry by an aminoacyl-tRNA carrying the next amino acid to be added to the chain. Bacterial ribosomes also have an E site, through which deacylated tRNA passes before it is released after being used in protein synthesis. The polypeptide chain in the P site is transferred to the aminoacyl-tRNA in the A site, creating a deacylated tRNA in the P site and a peptidyl-tRNA in the A site.

Following peptide bond synthesis, the ribosome translocates one codon along the mRNA, moving deacylated tRNA into the E site, and peptidyl tRNA from the A site into the P site. Translocation is catalyzed by the elongation factor EF-G, and like several other stages of ribosome function, requires hydrolysis of GTP. During translocation, the ribosome passes through a hybrid stage in which the 50S subunit moves relative to the 30S subunit.

Protein synthesis is an expensive process. ATP is used to provide energy at several stages, including the charging of tRNA with its amino acid, and the unwinding of mRNA. It has been estimated that up to 90% of all the ATP molecules synthesized in a rapidly growing bacterium are consumed in assembling amino acids into protein!

Additional factors are required at each stage of protein synthesis. They are defined by their cyclic association with, and dissociation from, the ribosome. IF factors are involved in prokaryotic initiation. IF-3 is needed for 30S subunits to bind to mRNA and also is responsible for maintaining the 30S subunit in a free form. IF-2 is needed for fMet-tRNA$_f$ to bind to the 30S subunit and is responsible for excluding other aminoacyl-tRNAs from the initiation reaction. GTP is hydrolyzed after the initiator tRNA has been bound to the initiation complex. The initiation factors must be released in order to allow a large subunit to join the initiation complex.

Eukaryotic initiation involves a greater number of factors. Some of them are involved in the initial binding of the 40S subunit to the capped 5′ end of the mRNA. Then the initiator tRNA is bound by another group of factors. After this initial binding, the small subunit scans the mRNA until it recognizes the correct AUG codon. At this point, initiation factors are released and the 60S subunit joins the

complex.

Prokaryotic EF factors are involved in elongation. EF-Tu binds aminoacyl-tRNA to the 70S ribosome. GTP is hydrolyzed when EF-Tu is released, and EF-Ts is required to regenerate the active form of EF-Tu. EF-G is required for translocation. Binding of the EF-Tu and EF-G factors to ribosomes is mutually exclusive, which ensures that each step must be completed before the next can be started.

Termination occurs at any one of the three special codons, UAA, UAG, UGA. Class 1 RF factors that specifically recognize the termination codons activate the ribosome to hydrolyze the peptidyl-tRNA. A class 2 RF factor is required to release the class 1 RF factor from the ribosome. The GTP-binding factors IF-2, EF-Tu, EF-G, RF3 all have similar structures, with the latter two mimicking the RNA-protein structure of the first two when they are bound to tRNA; they all bind to the same ribosomal site, the G-factor binding site.