TRANSLATION

B.Sc. SEM 3

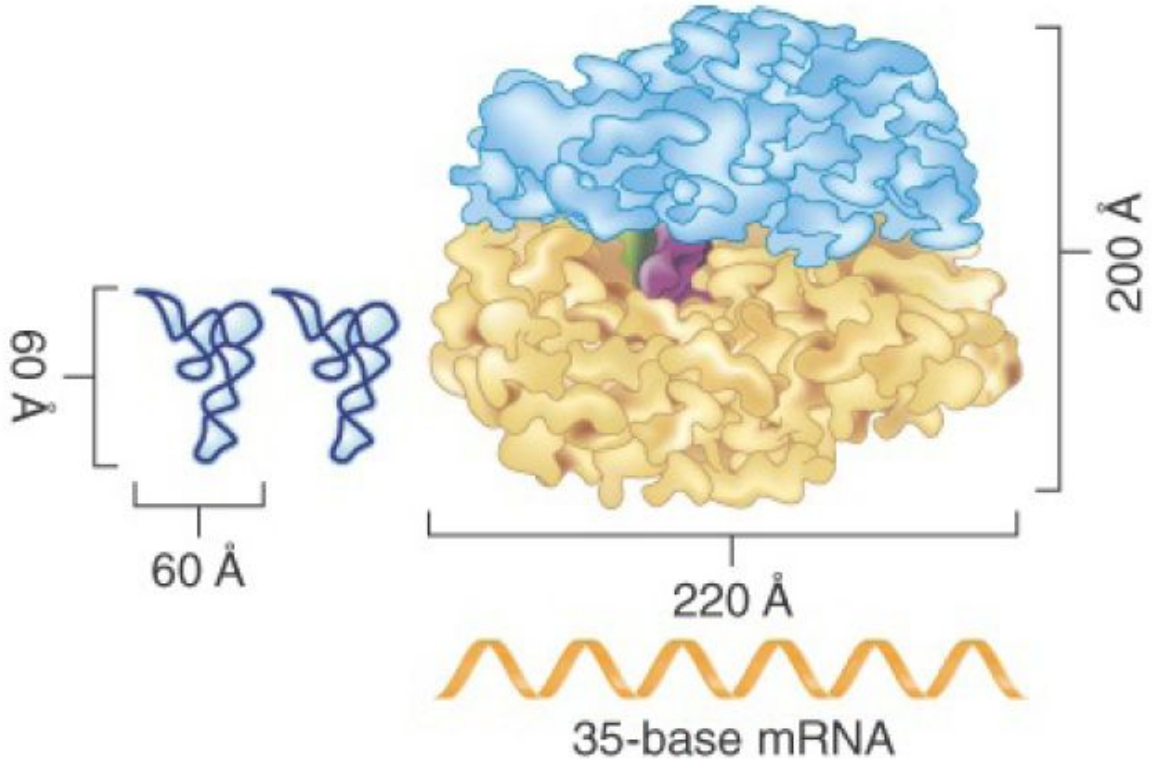


FIGURE 22.1 The ribosome is large enough to bind several tRNAs and an mRNA.

Ribosomes		rRNAs	r-proteins
Bacterial (70S) mass: 2.5 MDa 66% RNA	50S	23S = 2,904 bases 5S = 120 bases	31
	30S	16S = 1,542 bases	21
Mammalian (80S) mass: 4.2 MDa 60% RNA	60S	28S = 4,718 bases 5.8S = 160 bases 5S = 120 bases	49
	40S	18S = 1,874 bases	33

FIGURE 22.2 Ribosomes are large ribonucleoprotein particles that contain more RNA than protein and are composed of a large and a small subunit.

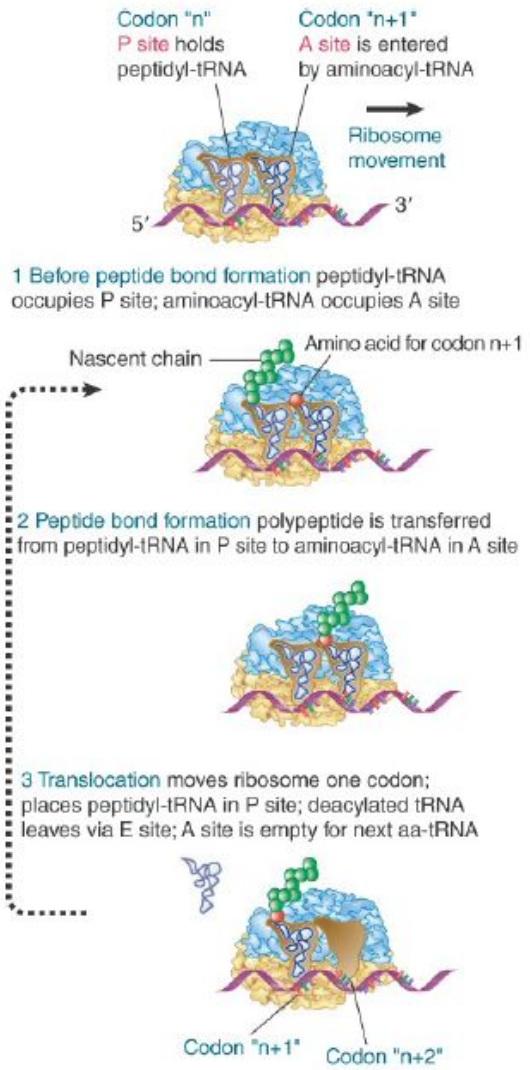
Translation Occurs by Initiation,Elongation, and Termination

- The ribosome has three 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.

- Except for the initiator tRNA, an incoming aminoacyl-tRNA binds to the A site. Prior to the entry of aminoacyl-tRNA, the site exposes the mRNA codon representing the next amino acid 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 22.3 The ribosome has two sites for binding charged tRNA.



The deacylated tRNA, lacking any amino acids, lies in the P site, and a new peptidyl-tRNA is in the A site. The peptide on this peptidyl-tRNA is one amino acid residue longer than the one that was carried on the peptidyl-tRNA that had been in the P site in step 1.
The ribosome now moves one triplet along the messenger RNA. This stage is called translocation.

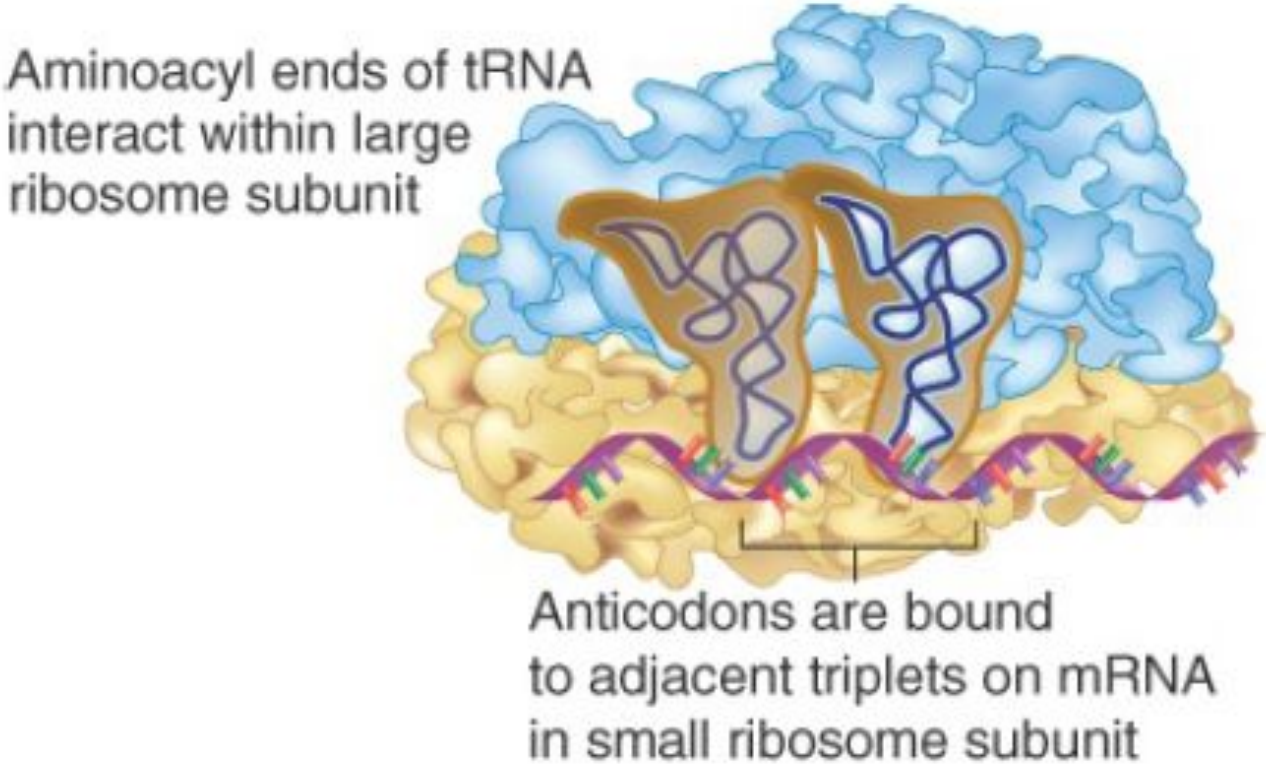


FIGURE 22.4 The P and A sites position the two bound tRNAs across both ribosomal subunits.

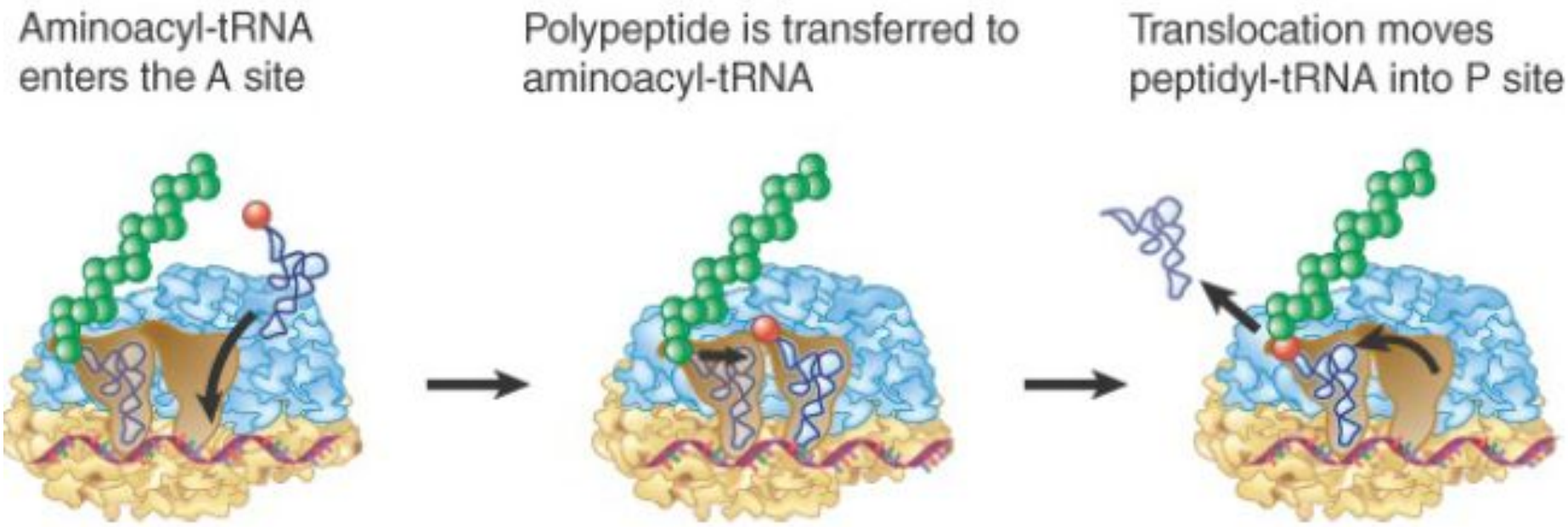


FIGURE 22.5 Aminoacyl-tRNA enters the A site, receives the polypeptide chain from peptidyl-tRNA, and is transferred into the P site for the next cycle of elongation.

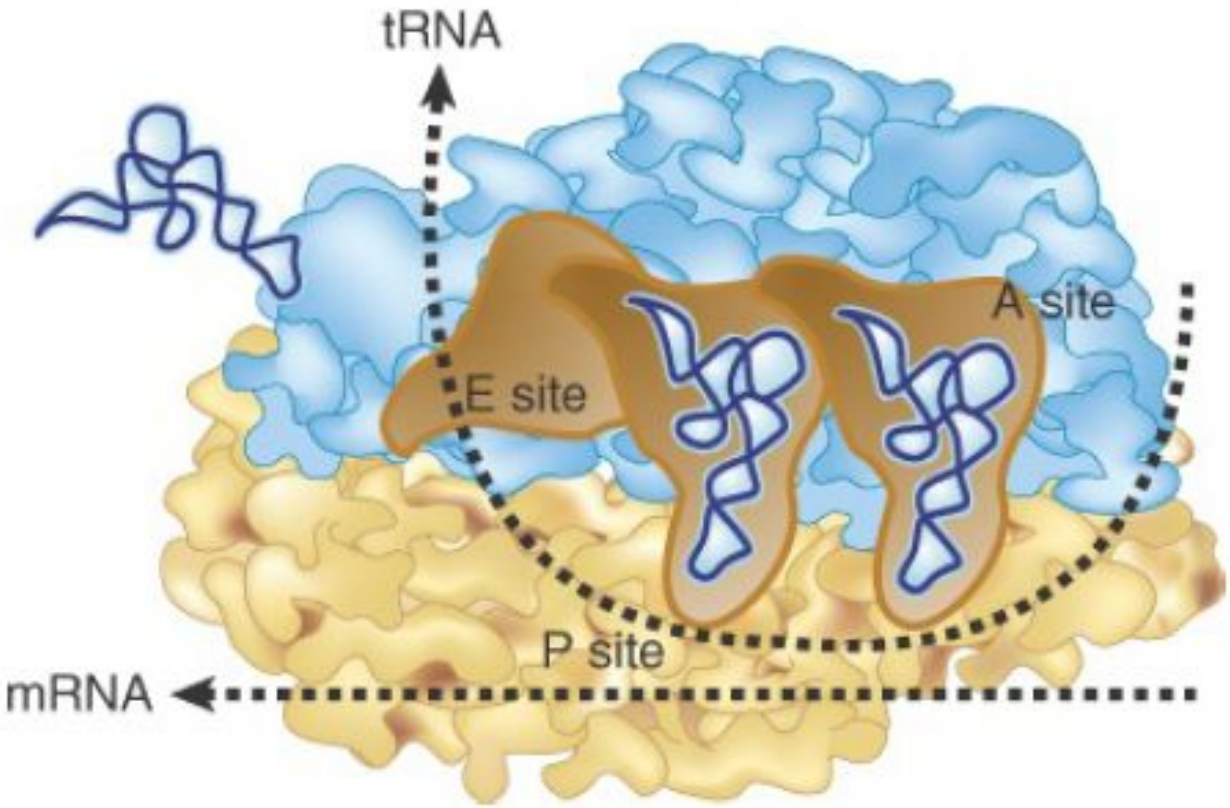


FIGURE 22.6 tRNA and mRNA move through the ribosome in the same direction.

- **Initiation** involves the reactions that precede formation of the peptide bond between the first two amino acids of the polypeptide. It requires the ribosome to bind to the mRNA, which forms an initiation complex that contains the first aminoacyl-tRNA. This is a relatively slow step in translation and usually determines the rate at which an mRNA is translated.
- **Elongation** includes all the reactions from the formation of the first peptide bond to the 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 translation.
- **Termination** encompasses the steps that are needed to release the completed polypeptide chain; at the same time, the ribosome dissociates from the mRNA.

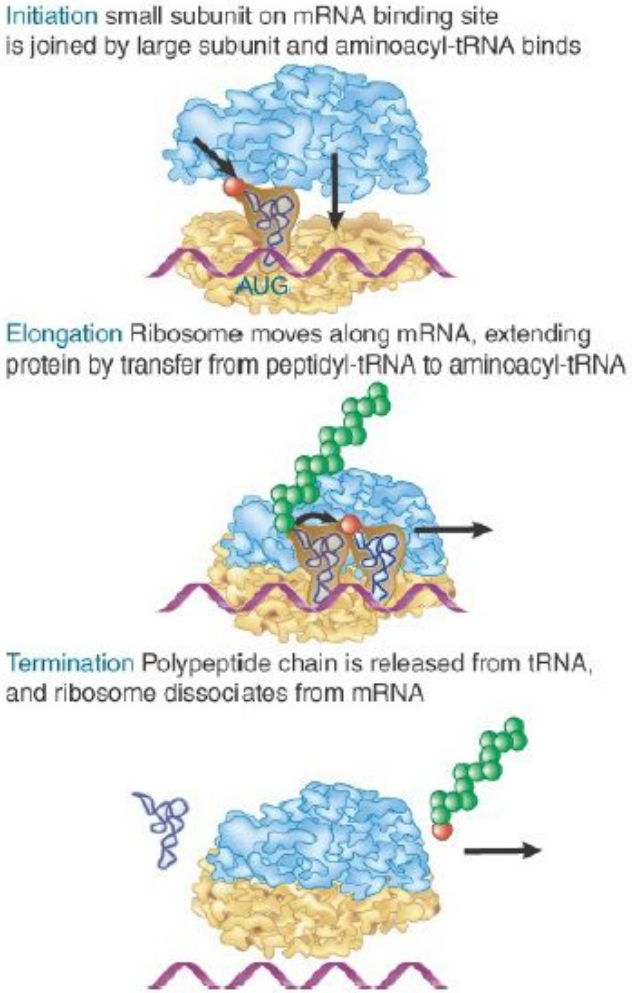


FIGURE 22.7 Translation has three stages.

Initiation in Bacteria Needs 30S Subunits and Accessory Factors

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

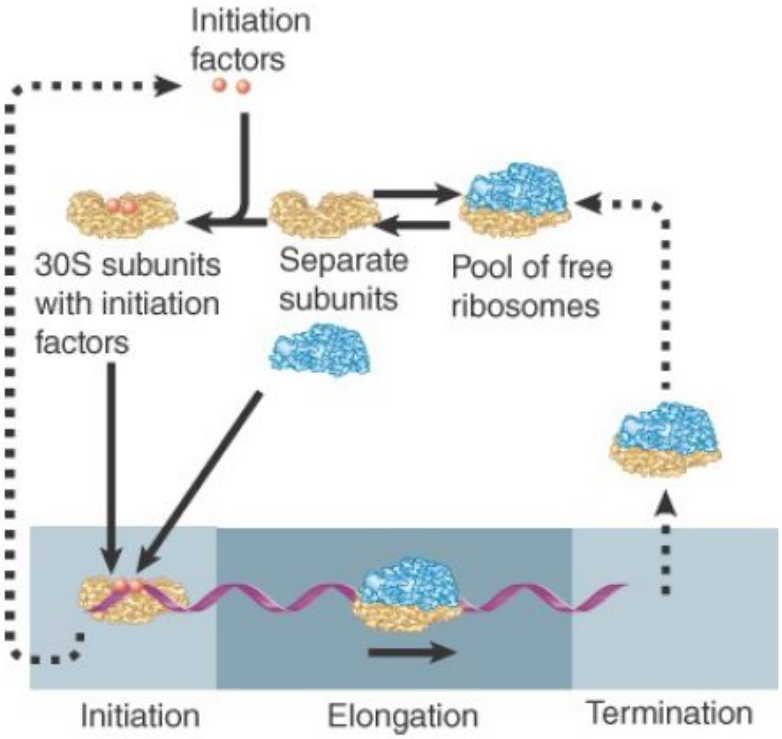


FIGURE 22.9 Initiation requires free ribosome subunits. When ribosomes are released at termination, the 30S subunits bind initiation factors and dissociate to generate free subunits. When subunits reassociate to produce a functional ribosome at initiation, they release these factors.

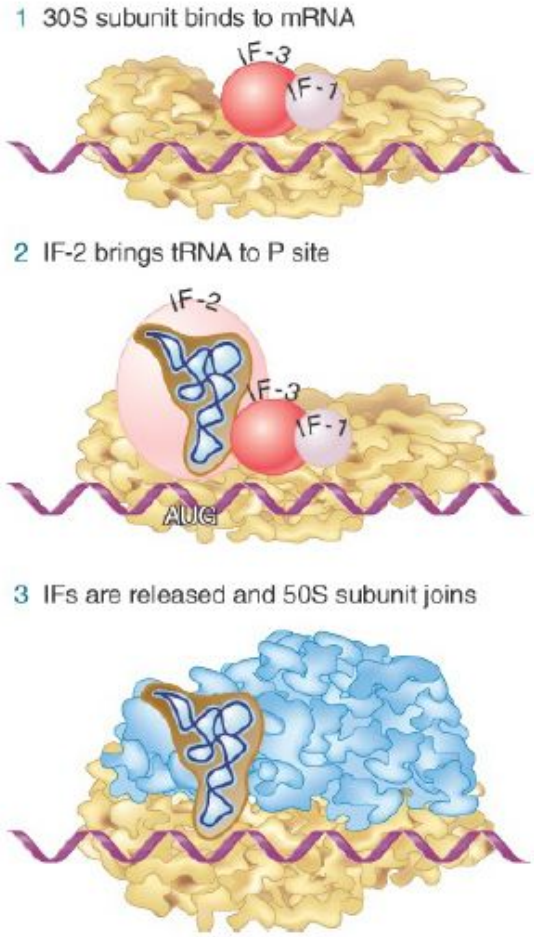


FIGURE 22.10 Initiation factors stabilize free 30S subunits and bind initiator tRNA to the 30S–mRNA complex.

- IF-3 has multiple functions: It is needed to stabilize (free) 30S subunits and to inhibit the premature binding of the 50S subunit; it enables 30S subunits to bind to initiation sites in mRNA; and, as part of the 30S-mRNA complex, it checks the accuracy of recognition of the first aminoacyl-tRNA.
- IF-2 binds a special initiator tRNA and controls its entry into the ribosome.
- IF-1 binds to 30S subunits as a part of the complete initiation complex. It binds in the vicinity of the A site and prevents aminoacyl-tRNA from entering. Its location also may impede the 30S subunit from binding to the 50S subunit.

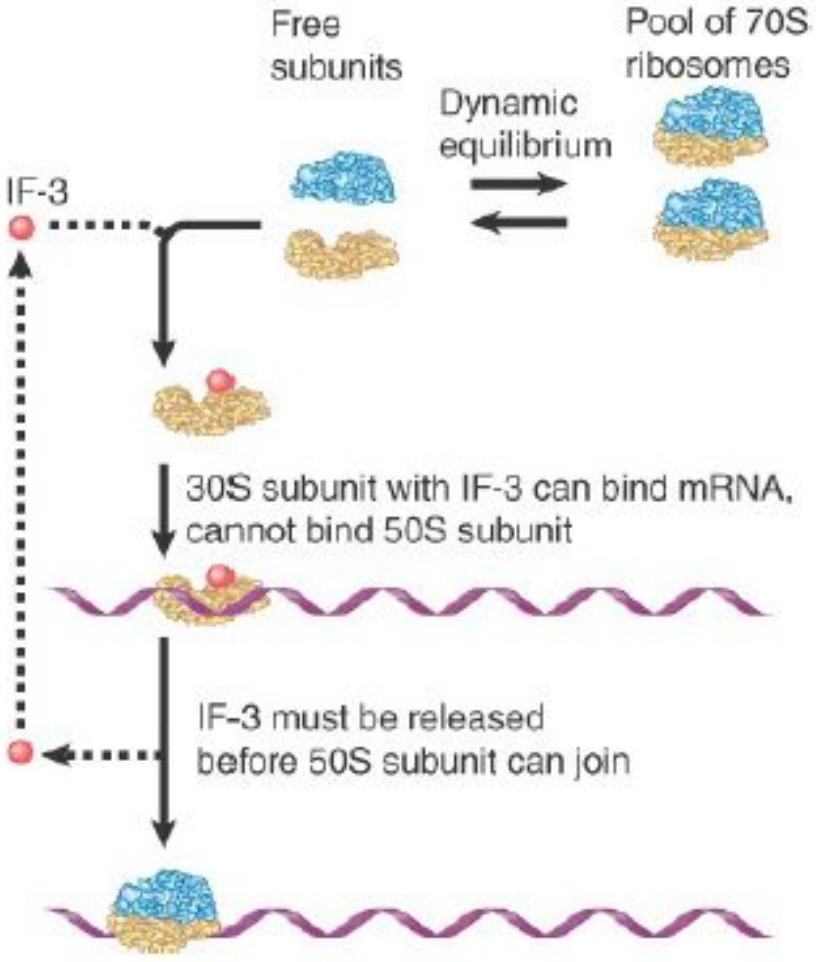


FIGURE 22.11 Initiation requires 30S subunits that carry IF-3.

Initiation Involves Base Pairing Between mRNA and rRNA

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

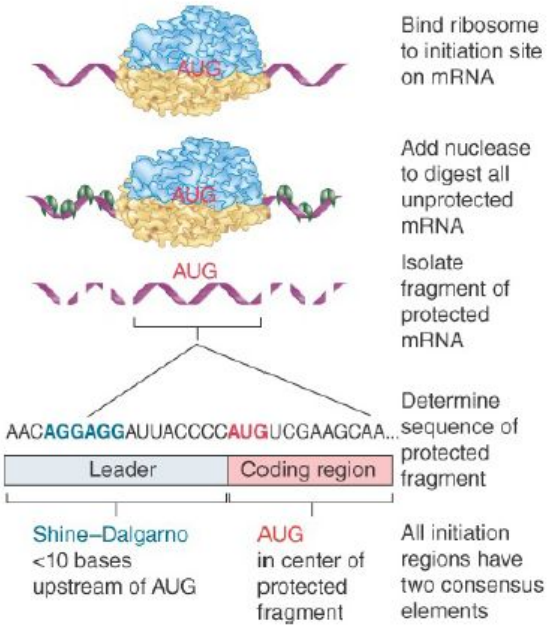


FIGURE 22.12 Ribosome-binding sites on mRNA can be identified by studying initiation complexes. They include the upstream Shine–Dalgarno sequence and the initiation codon.

➔ The AUG (or less often, GUG or UUG) initiation codon is always included within the protected sequence.

➔ Approximately 10 bases upstream of the initiation codon 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 the 16S rRNA.

A Special Initiator tRNA Starts the Polypeptide Chain

- Translation starts with a methionine amino acid usually encoded 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 amino group of the methionine bound to the bacterial initiator tRNA is formylated.

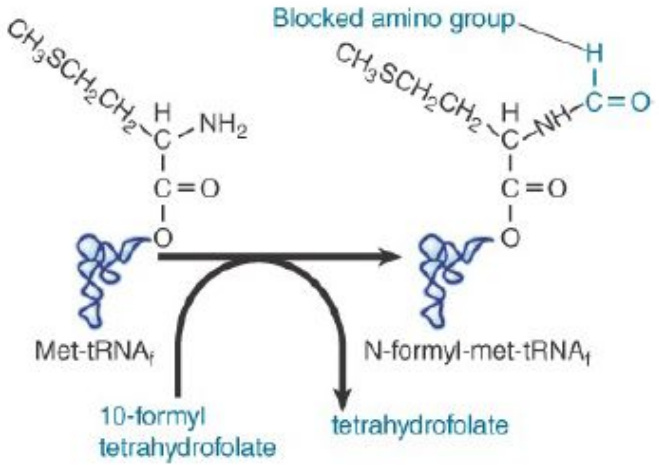


FIGURE 22.14 The initiator N-formyl-methionyl-tRNA (fMet-tRNA$_f$) is generated by formylation of methionyl-tRNA using formyl-tetrahydrofolate as a cofactor.

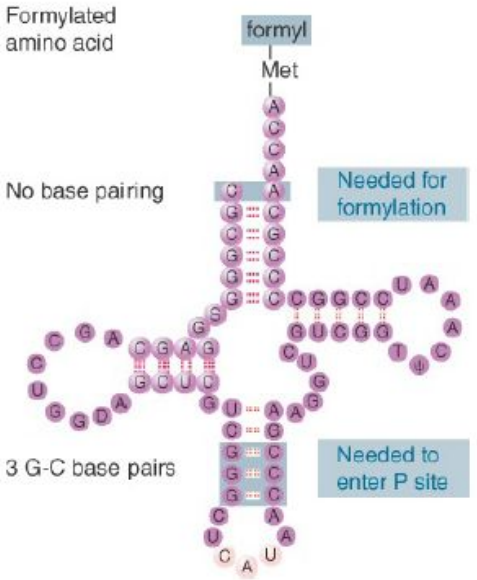


FIGURE 22.15 fMet-tRNA$_f$ has unique features that distinguish it as the initiator tRNA.

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

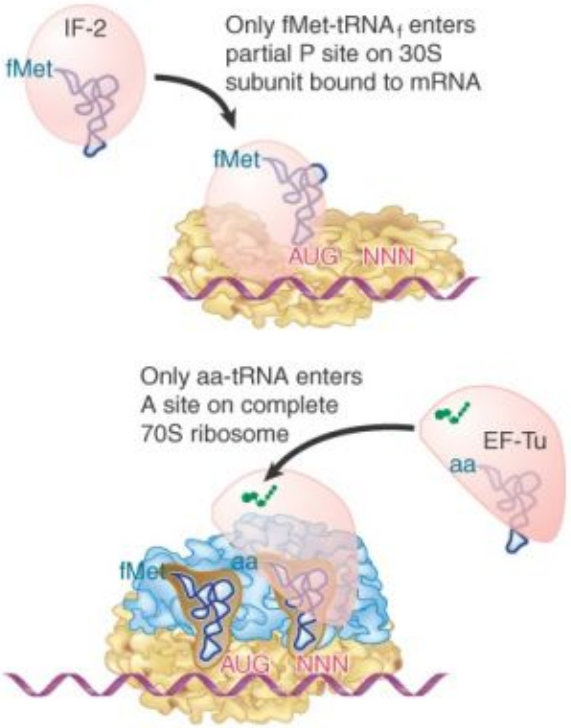


FIGURE 22.16 Only fMet-tRNA$_f$ can be used for initiation by 30S subunits; other aminoacyl-tRNAs (aa-tRNAs) must be used for elongation by 70S ribosomes.

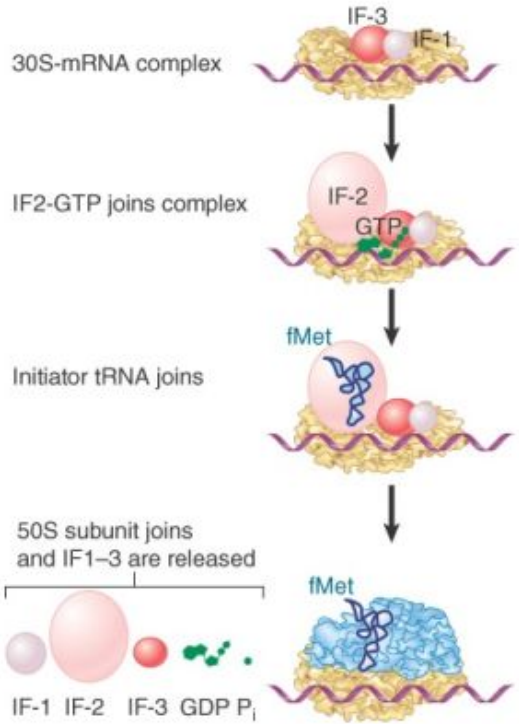


FIGURE 22.17 IF-2 is needed to bind fMet-tRNA$_f$ to the 30S–mRNA complex. After 50S binding, all IFs are released and GTP is cleaved.

Small Subunits Scan for Initiation Sites on Eukaryotic mRNA

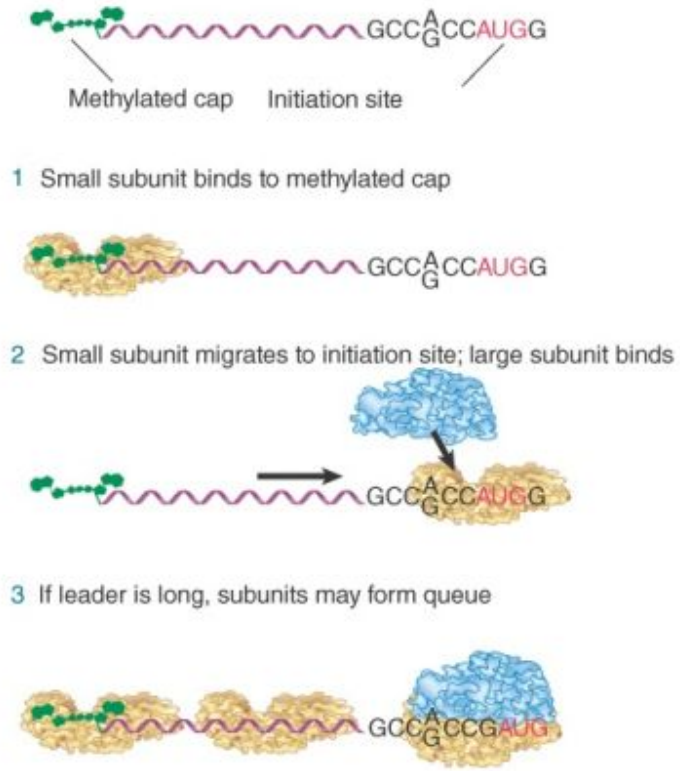


FIGURE 22.18 Eukaryotic ribosomes migrate from the 5′ end of mRNA to the ribosome binding site, which includes an AUG initiation codon.

- 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

Eukaryotes Use a Complex of Many Initiation Factors

- Initiation factors are required for all stages of initiation, including binding of the initiator tRNA, attachment of the 40S subunit to the mRNA, joining of the 60S subunit, and movement of the ribosome along the mRNA.
- Eukaryotic initiator tRNA is a Met-tRNA that is different from the Met-tRNA used in elongation, but the methionine is not formylated as it is for the prokaryotic initiator tRNA.
- eIF2 binds the initiator Met-tRNA and GTP, forming a ternary complex that binds to the 40S subunit before it associates with mRNA.
- A cap-binding complex binds to the 5′ end of mRNA prior to association of the mRNA with the 40S subunit.

Eukaryotic cells have more initiation factors than prokaryotic cells do. The factors are named similarly to those in prokaryotes (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
- Binding the mRNA-factor complex to the Met-tRNA-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

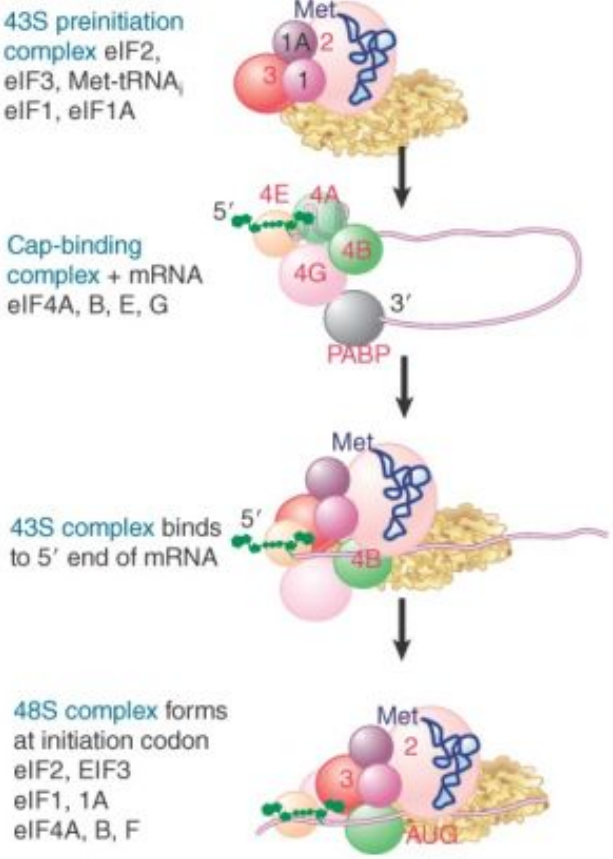


FIGURE 22.19 Some eukaryotic initiation factors bind to the 40S ribosome subunit to form the 43S preinitiation complex; others bind to mRNA. When the 43S complex binds to mRNA, it scans for the initiation codon and can be isolated as the 48S complex.

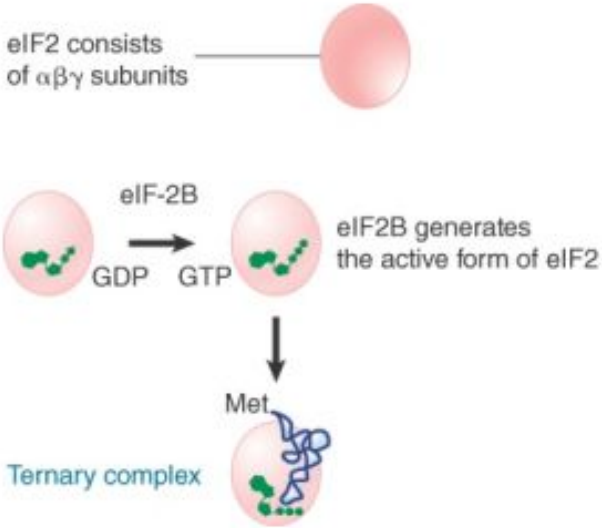
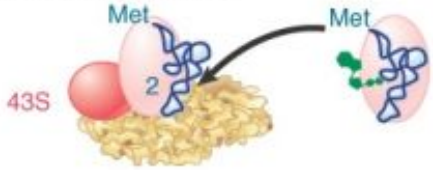


FIGURE 22.20 In eukaryotic initiation, eIF-2 forms a ternary complex with Met-tRNA$_i$ and GTP. The ternary complex binds to free 40S subunits, which attach to the 5′ end of mRNA.

eIF3 maintains free 40S subunits

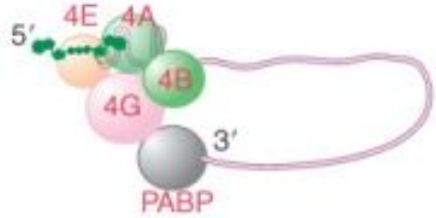
eIF2 binds Met-tRNA to 40S



eIF2 is a GTPase
eIF2B is the exchange factor

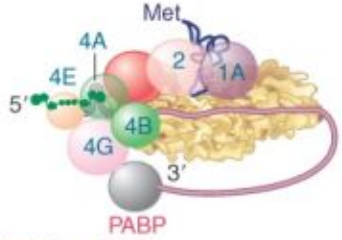
FIGURE 22.21 Initiation factors bind the initiator Met-tRNA to the 40S subunit to form a 43S complex. Later in the reaction, GTP is hydrolyzed and eIF2 is released in the form of eIF2-GDP. eIF2B regenerates the active form.

eIF4F is a heterotrimer consisting of:
eIF4G is a scaffold protein
eiF4E binds the 5' methyl cap
eIF4A is a helicase that unwinds the 5′ structure



eIF4G binds two further factors
eIF4B stimulates eIF4A helicase
PABP binds 3′ poly(A)

FIGURE 22.22 The heterotrimer eIF4F binds to the 5′ end of mRNA as well as to other factors.



Possible interactions:
eIF4G binds to eIF3
mRNA binds eIF4G, eIF3, and 40S subunit

FIGURE 22.23 Interactions involving initiation factors are important when mRNA binds to the 43S complex.

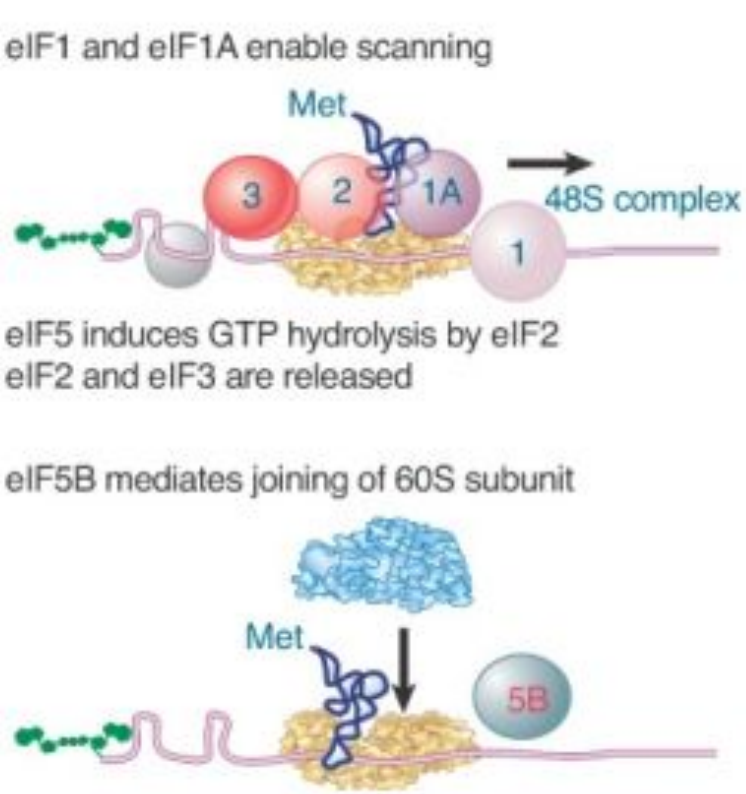


FIGURE 22.24 eIF1 and eIF1A help the 43S initiation complex to "scan" the mRNA until it reaches an AUG codon. eIF2 hydrolyzes its GTP to enable its release together with IF3. eIF5B mediates joining of the 60S and 40S subunits.

Elongation Factor Tu Loads aminoacyl-tRNA into the A Site

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

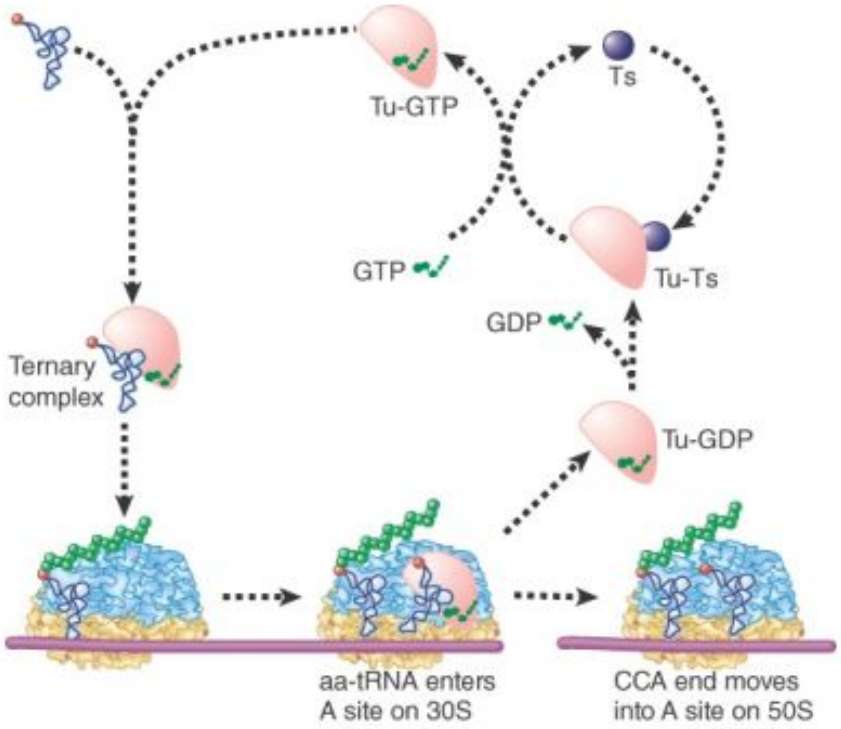


FIGURE 22.25 EF-Tu–GTP places aminoacyl-tRNA on the A site of ribosome and then is released as EF-Tu–GDP. EF-Ts is required to mediate the replacement of GDP by GTP. The reaction consumes GTP and releases GDP. The only aminoacyl-tRNA that cannot be recognized by EF-Tu–GTP is fMet-tRNA$_f$, whose failure to bind prevents it from responding to internal AUG or GUG codons.

The Polypeptide Chain Is Transferred to Aminoacyl-tRNA

- The 50S subunit has peptidyl transferas activity, as provided by an rRNA ribozyme.
- 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.

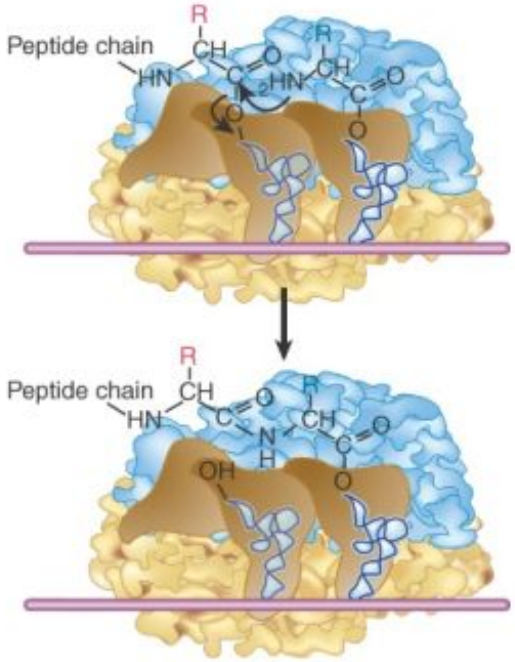


FIGURE 22.26 Peptide bond formation takes place by a reaction between the polypeptide of peptidyl-tRNA in the P site and the amino acid of aminoacyl-tRNA in the A site.

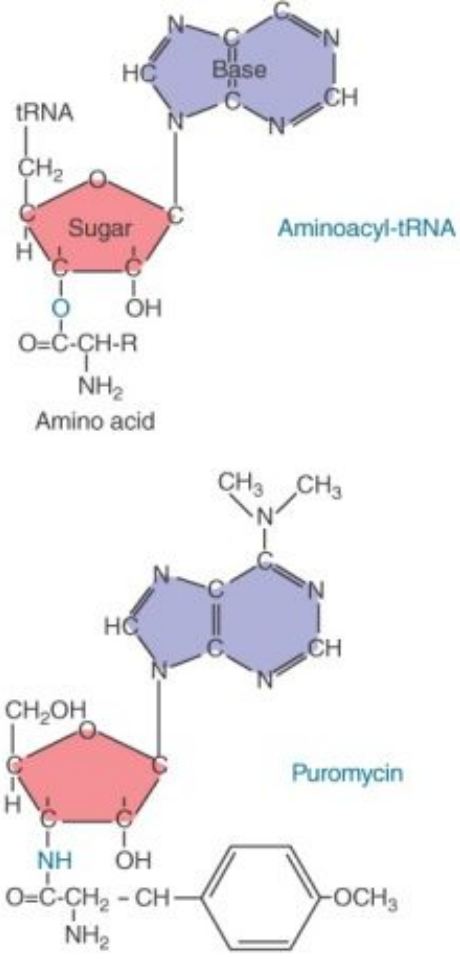


FIGURE 22.27 Puromycin mimics aminoacyl-tRNA because it resembles an aromatic amino acid linked to a sugar-base moiety.

Translocation Moves the Ribosome

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

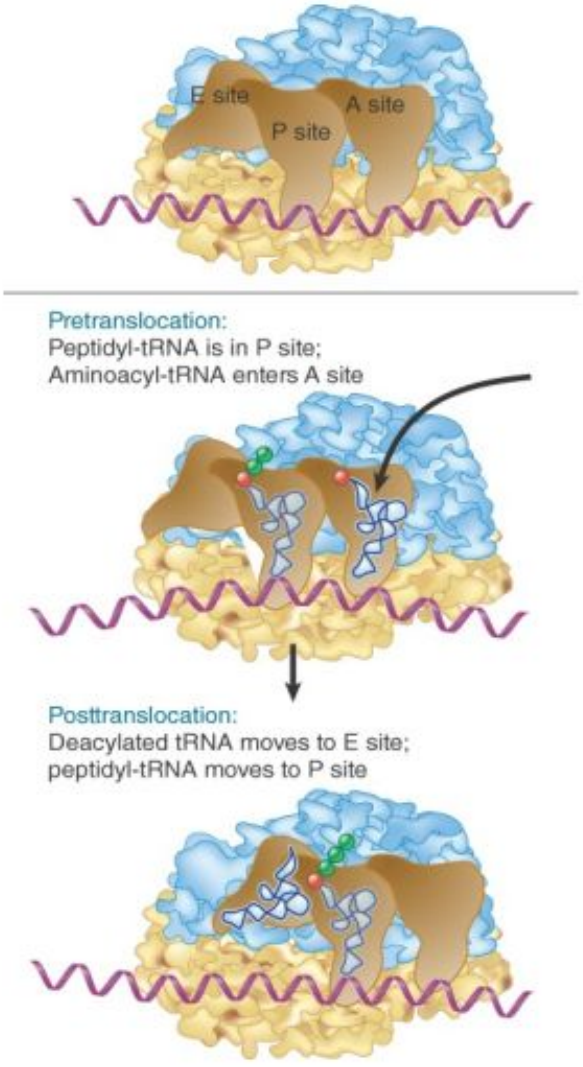


FIGURE 22.28 A bacterial ribosome has three tRNA-binding sites. Aminoacyl-tRNA enters the A site of a ribosome that has peptidyl-tRNA in the P site. Peptide bond synthesis deacylates the P site tRNA and generates peptidyl-tRNA in the A site. Translocation moves the deacylated tRNA into the E site and moves peptidyl-tRNA into the P site.

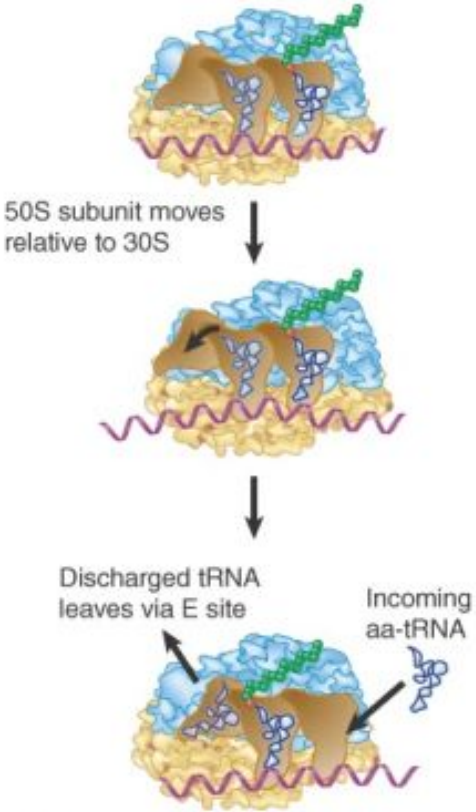


FIGURE 22.29 The hybrid state model for translocation involves two stages. First, at peptide bond formation the aminoacyl end of the tRNA in the A site becomes relocated in the P site. Second, the anticodon end of the tRNA becomes relocated in the P site.

Elongation Factors Bind Alternately to the Ribosome

- ★ 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.

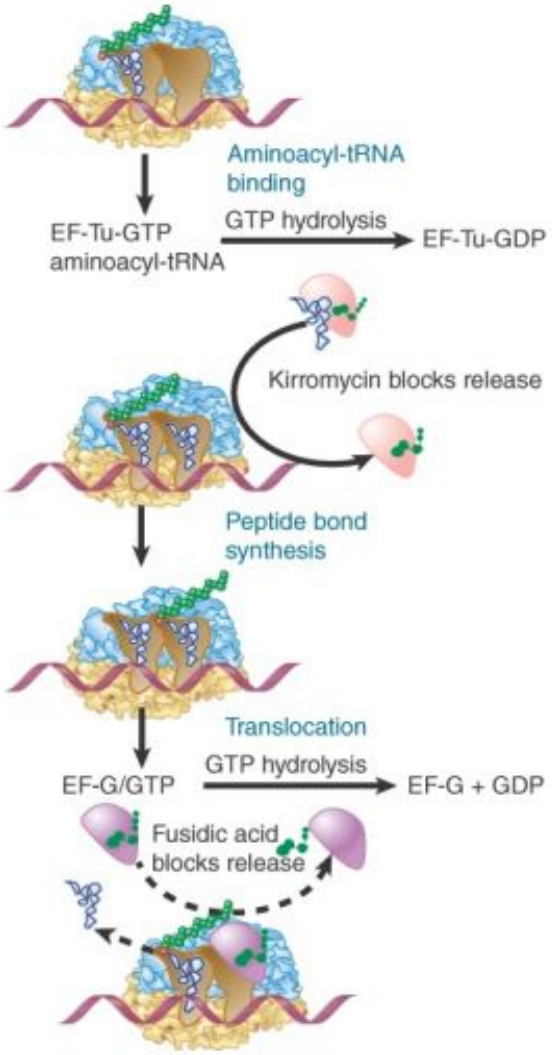


FIGURE 22.30 Binding of factors EF-Tu and EF-G alternates as ribosomes accept new aminoacyl-tRNAs, form peptide bonds, and translocate.

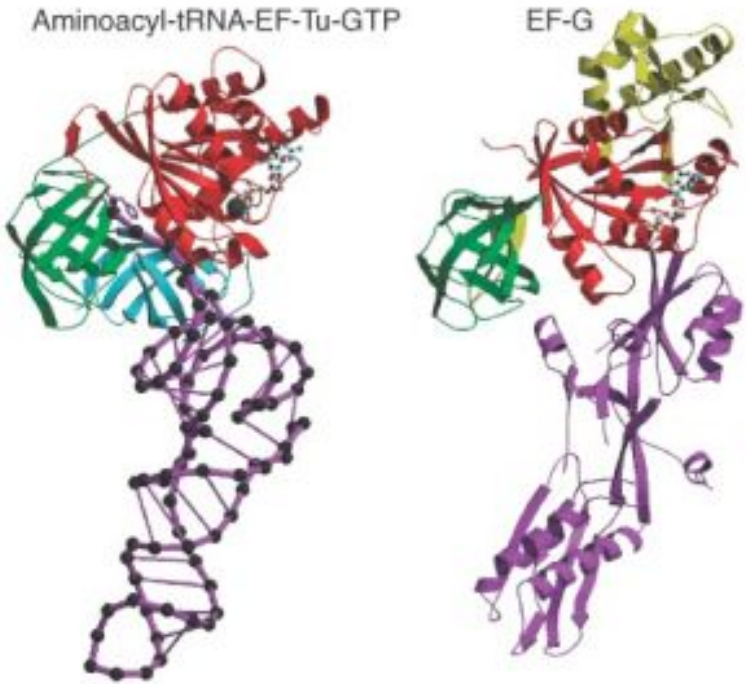


FIGURE 22.31 The structure of the ternary complex of aminoacyl-tRNA–EF-Tu–GTP (left) resembles the structure of EF-G (right). Structurally conserved domains of EF-Tu and EF-G are in red and green; the tRNA and the domain resembling it in EF-G are in purple.

Three Codons Terminate Translation

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

Termination Codons Are Recognized by Protein Factors

- Termination codons are recognized by protein release factors, not by aminoacyl-tRNAs.
- The structures of the class 1 release factors (RF1 and RF2 in E. coli) resemble aminoacyl-tRNA–EF-Tu and EFG.
- 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 (such as RF3) that depend on GTP.
- The mechanism of termination in bacteria (which have two types of class 1 release factors) is similar to that of eukaryotes (which have only one class 1 release factor).

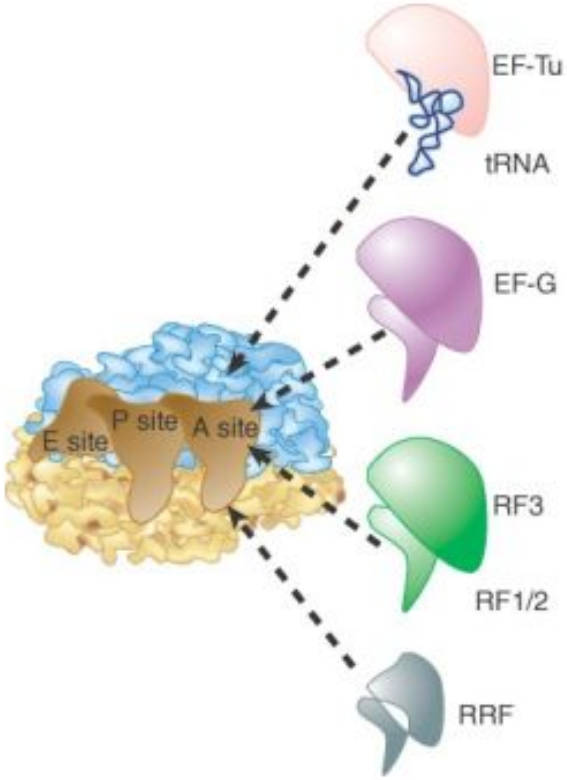


FIGURE 22.32 Molecular mimicry enables the EF-Tu–tRNA complex, the translocation factor EF-G, and the release factors RF1/2-RF3 to bind to the same ribosomal site. RRF is the ribosome recycling factor.

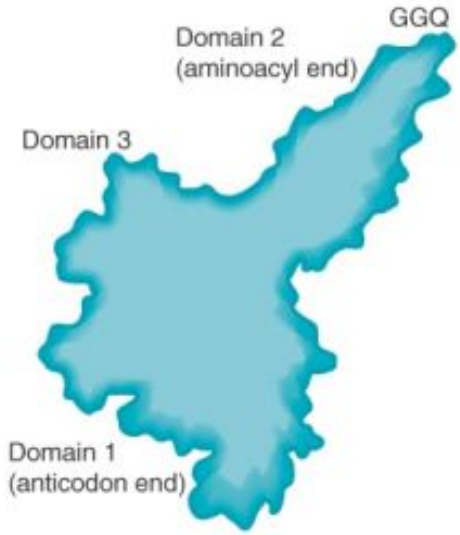


FIGURE 22.33 The eukaryotic termination factor eRF1 has a structure that mimics tRNA. The motif GGQ at the tip of domain 2 is essential for hydrolyzing the polypeptide chain from tRNA.

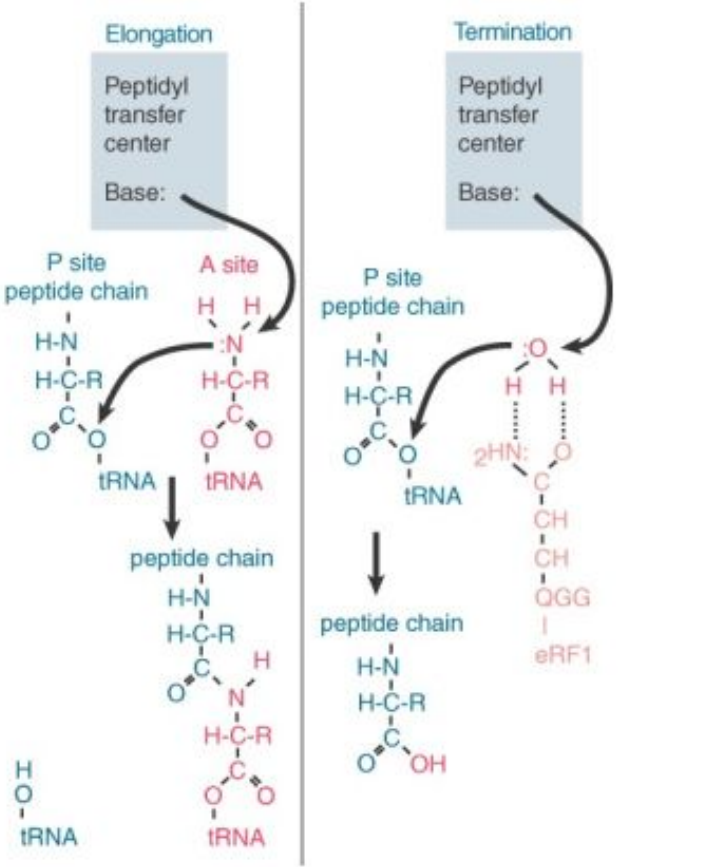


FIGURE 22.34 Peptide transfer and termination are similar reactions in which a base in the peptidyl transfer center triggers a transesterification reaction by attacking an N–H or O–H bond, releasing the N or O to attack the link to tRNA.

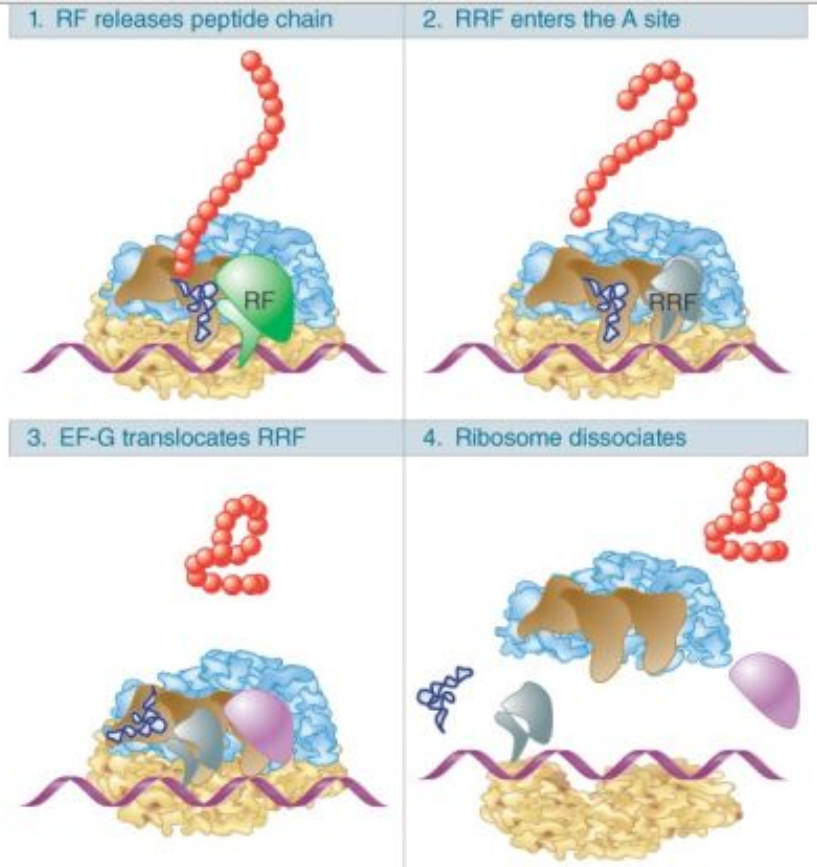


FIGURE 22.35 The RF (release factor) terminates translation by releasing the polypeptide chain. The RRF (ribosome recycling factor) releases the last tRNA, and EF-G releases RRF, causing the ribosome to dissociate.

TABLE 22.1 Functional homologies of prokaryotic and eukaryotic translation factors.

Initiation Factors			
Prokaryotic	**Eukaryotic**	**General Function**	**Notes**
IF-1	eIF1A	Blocks A site	eIF1A assists eIF2 in promoting Met-$tRNA_i^{Met}$ to bind to 40S; also promotes subunit dissociation.
IF-2*†	eIF2, eIF3, eIF5B*	Entry of initiator tRNA	eIF2 is a GTPase. eIF3 stimulates formation of the ternary complex, its binding to 40S, and binding and scanning of mRNA. eIF5B is involved in initiator tRNA entry and is a GTPase.
IF-3	eIF1, eIF4 complex, eIF3	Small subunit binding to mRNA	eIF4 complex functions in cap binding.

Elongation Factors

Prokaryotic	Eukaryotic	General Function
EF-Tu†‡, EF-G†	eEF1α‡	GTP-binding
EF-Ts	eEF1β, eEF1γ	GDP-exchanging
EF-G§	eEF2§	Ribosome translocation

Release Factors

Prokaryotic	Eukaryotic	General Function
RF1	eRF1	UAA/UAG recognition
RF2	eRF1	UAA/UGA recognition
RF3†	eRF3	Stimulation of other RF(s)

* IF-2 and eIF5B have sequence homology.

† IF-2, EF-Tu, EF-G, and RF3 have sequence homology.

‡ EF-Tu and eEF1α have sequence homology.

§ EF-G and eEF2 have sequence homology.

Ribosomal RNA Is Found Throughout Both Ribosomal Subunits

- 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.

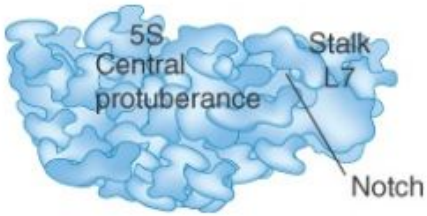


FIGURE 22.37 The 50S subunit has a central protuberance where 5S rRNA is located, separated by a notch from a stalk made of copies of the protein L7.

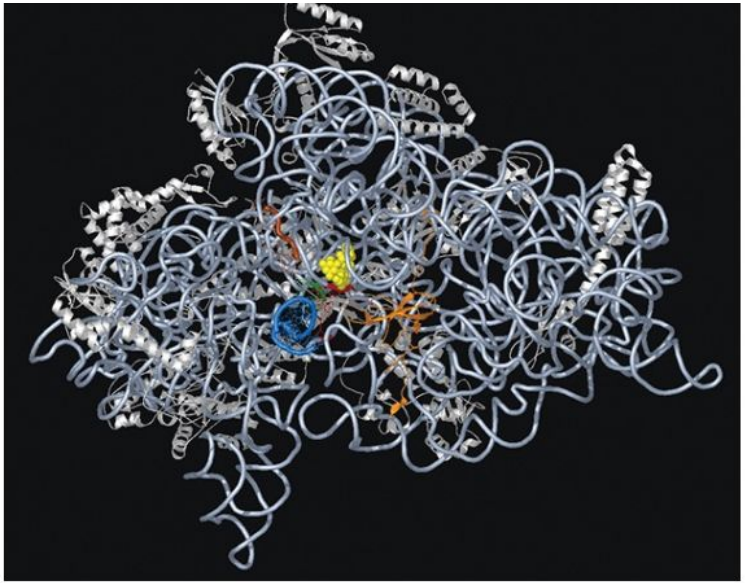


FIGURE 22.39 The 30S ribosomal subunit is a ribonucleoprotein particle. Ribosomal proteins are white and rRNA is light blue.

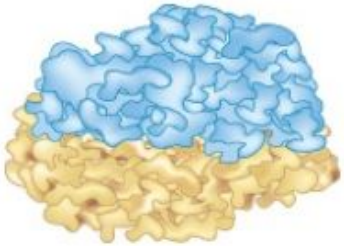


FIGURE 22.38 The platform of the 30S subunit fits into the notch of the 50S subunit to form the 70S ribosome.

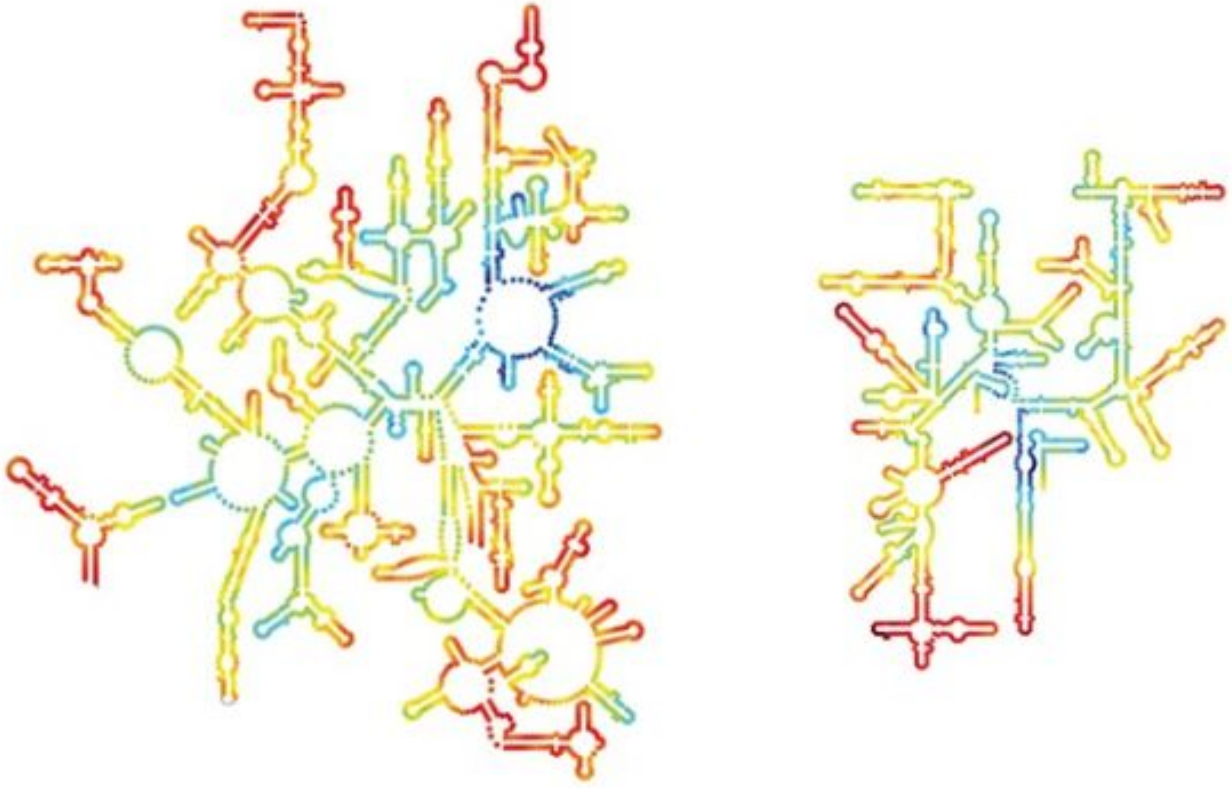


FIGURE 22.40 Contact points between the rRNAs are located in two domains of 16S rRNA and one domain of 23S rRNA.

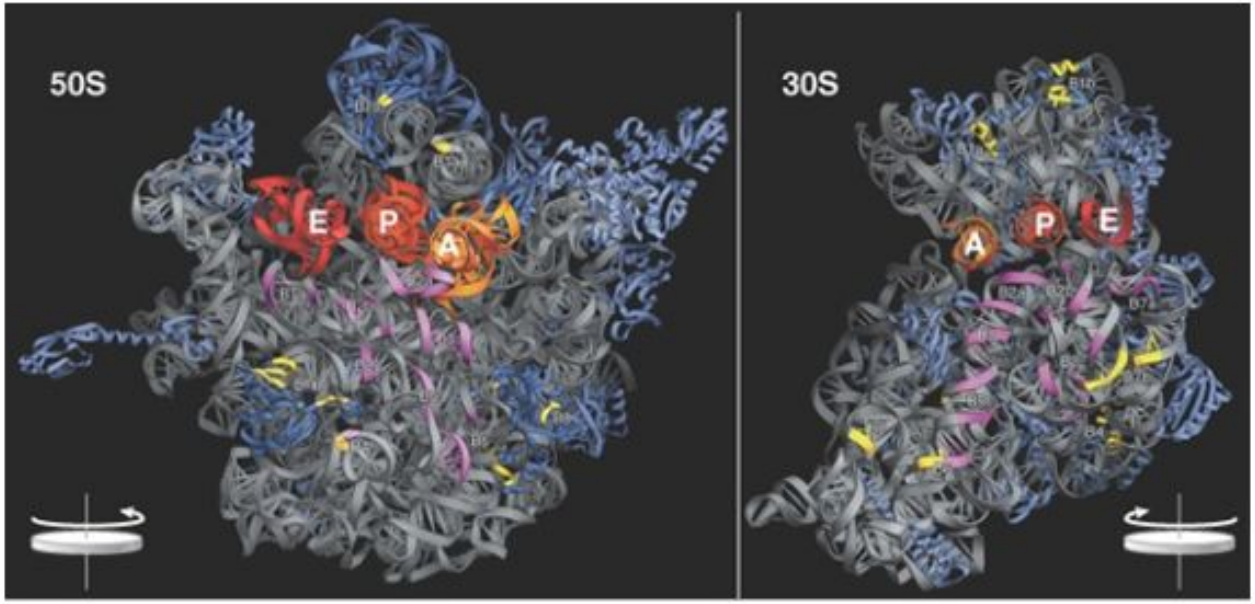


FIGURE 22.41 Contacts between the ribosomal subunits are mostly made by RNA (shown in purple). Contacts involving proteins are shown in yellow. The two subunits are rotated away from one another to show the faces where contacts are made; from a plane of contact perpendicular to the screen, the 50S subunit is rotated 90° counterclockwise, and the 30S is rotated 90° clockwise (this shows it in the reverse of the usual orientation).

Ribosomes Have Several Active Centers

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

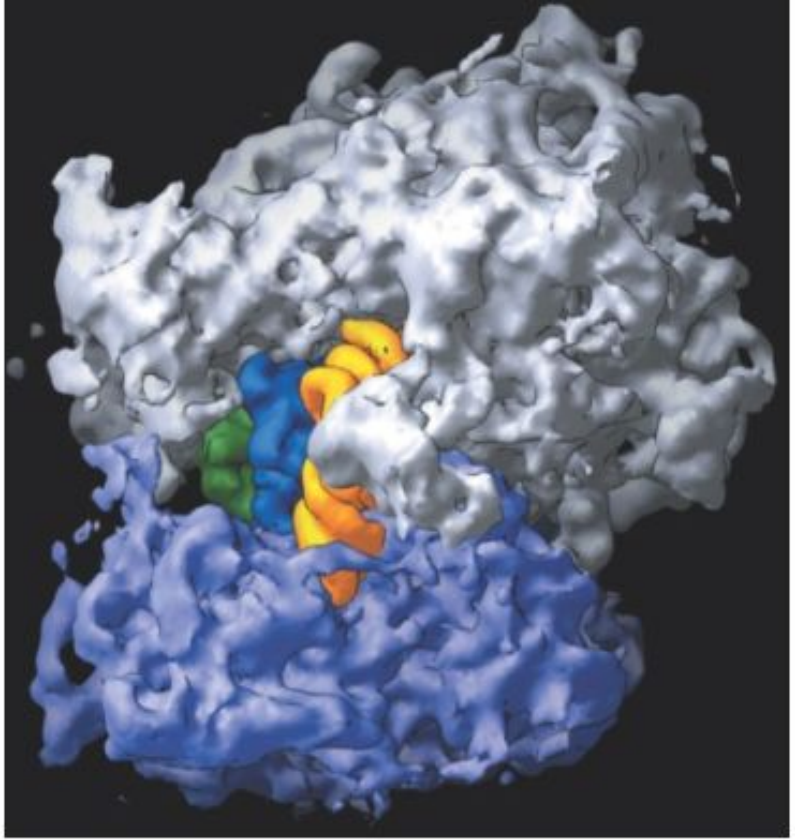


FIGURE 22.42 The 70S ribosome consists of the 50S subunit (white) and the 30S subunit (purple), with three tRNAs located superficially: yellow in the A site, blue in the P site, and green in the E site.

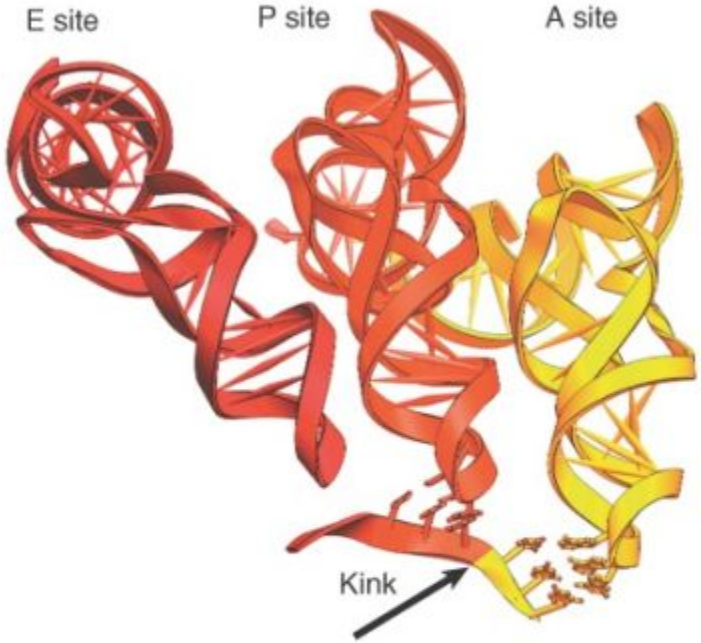


FIGURE 22.43 Three tRNAs have different orientations on the ribosome. mRNA turns between the P and A sites to allow aminoacyl-tRNAs to bind adjacent codons.

The crystal structure shows a 45° kink in the mRNA between the P and A sites, which allows the tRNAs to fit.

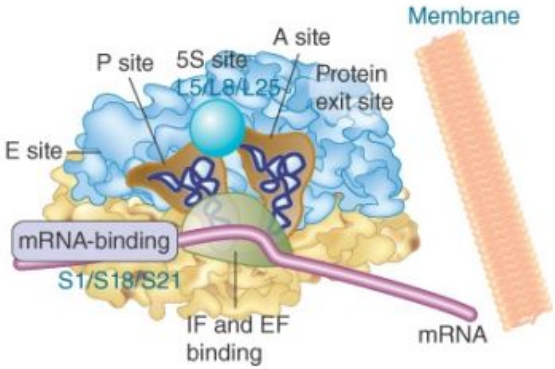


FIGURE 22.44 The ribosome has several active centers. It may be associated with a membrane. mRNA takes a turn as it passes through the A and P sites, which are angled with regard to each other. The E site lies beyond the P site. The peptidyl transferase site (not shown) stretches across the tops of the A and P sites. Part of the site bound by EF-Tu/G lies at the base of the A and P sites.

16S rRNA Plays an Active Role in Translation

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

Several interactions involve specific regions of rRNA:

- The 3′ terminus of the 16S 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.

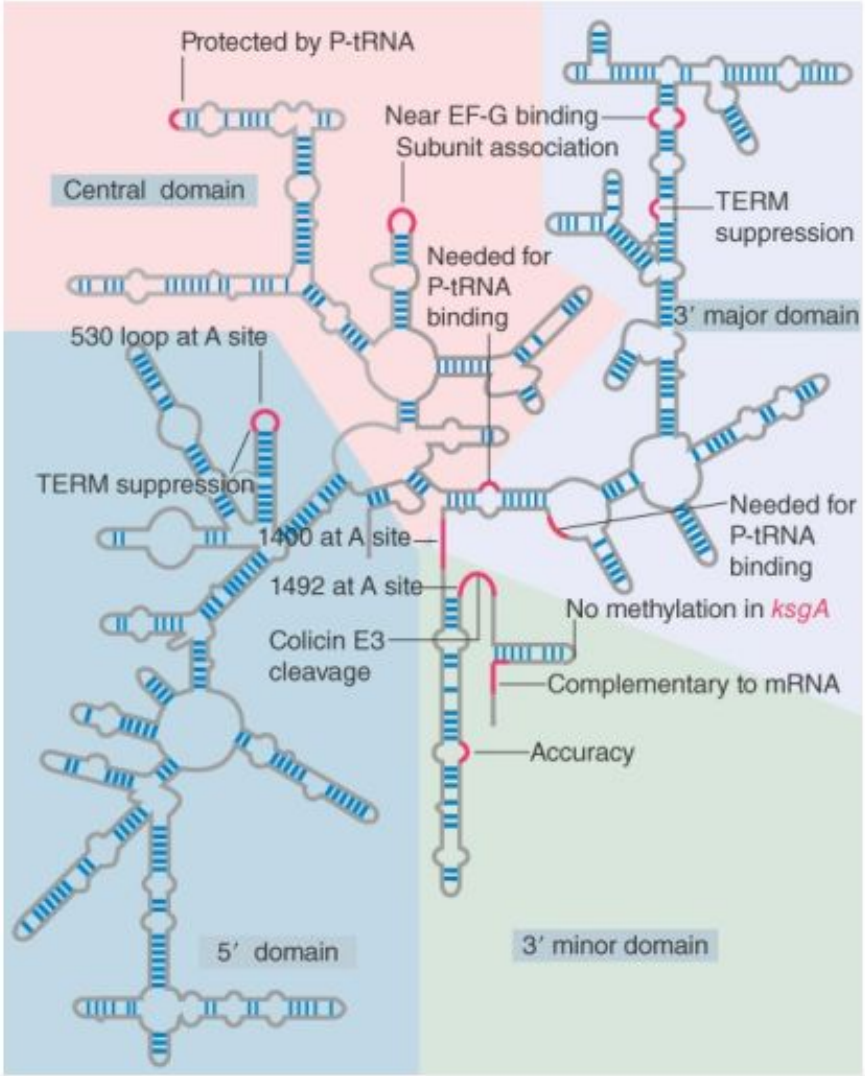


FIGURE 22.45 Some sites in 16S rRNA are protected from chemical probes when 50S subunits join 30S subunits or when aminoacyl-tRNA binds to the A site. Others are the sites of mutations that affect translation. TEM suppression sites may affect termination at some or several termination codons. The large colored blocks indicate the four domains of the rRNA.

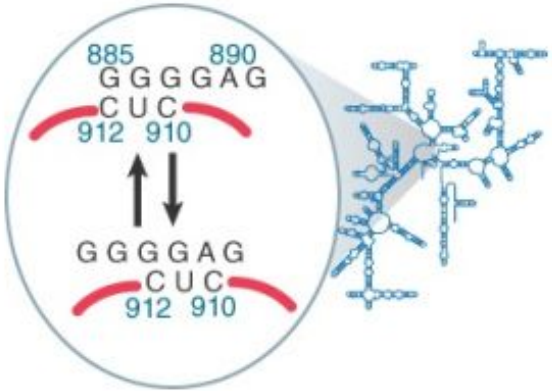


FIGURE 22.46 A change in conformation of 16S rRNA may occur during translation.

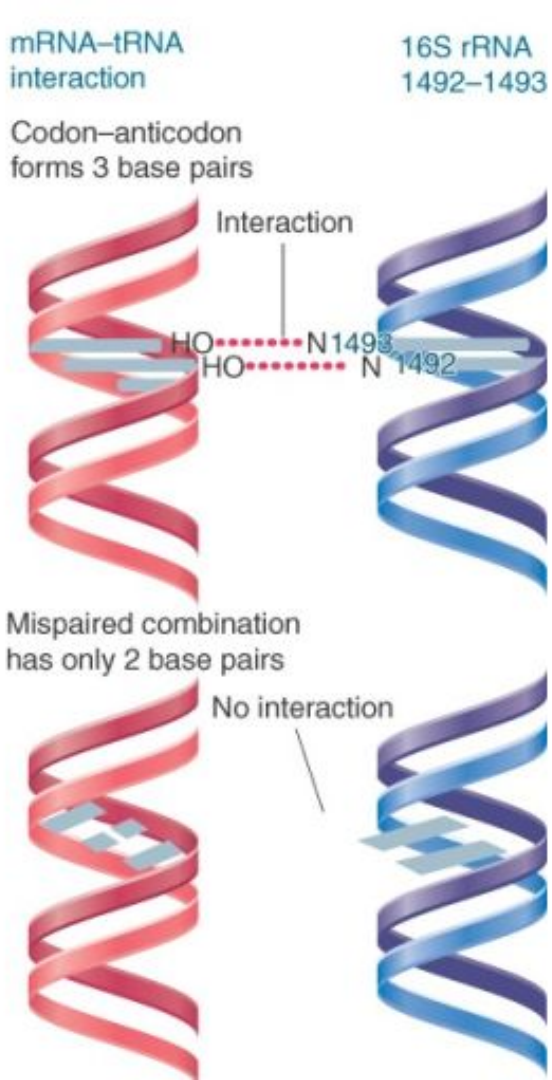


FIGURE 22.47 Codon–anticodon pairing supports interaction with adenines 1492 and 1493 of 16S rRNA, but mispaired tRNA–mRNA cannot interact.

Peptidyl transferase activity resides exclusively in the 23S rRNA.

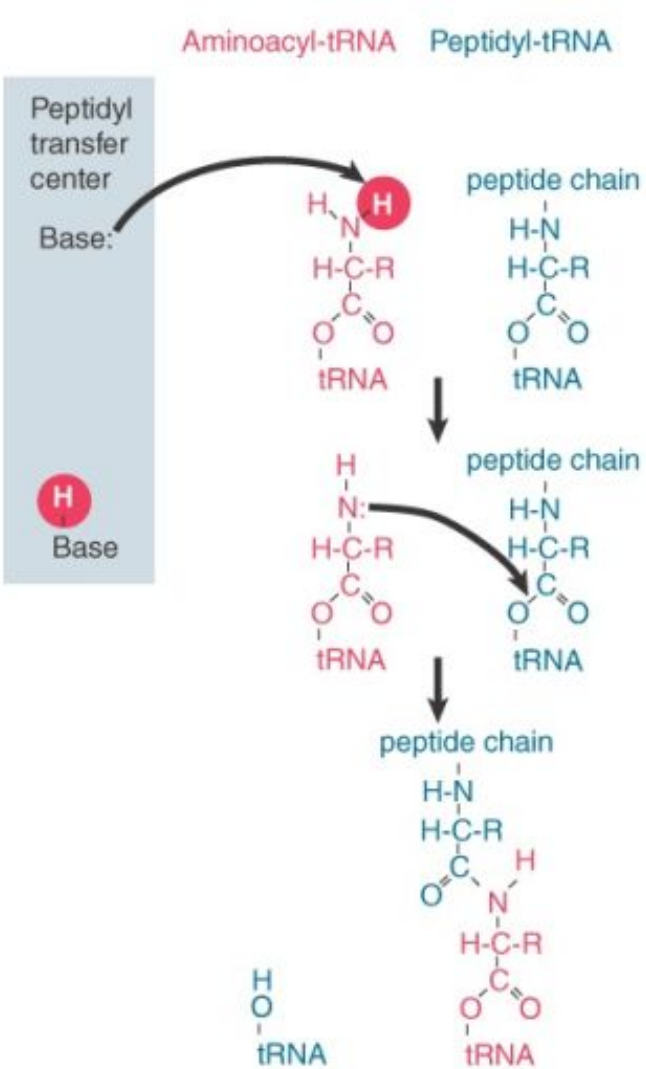


FIGURE 22.48 Peptide bond formation requires acid–base catalysis in which an H atom is transferred to a basic residue.

Translation Can Be Regulated

- Translation can be regulated by the 5′ untranslated region (UTR) of the mRNA.
- Translation may be regulated by the abundance of various tRNAs.
- A repressor protein can regulate translation by preventing a ribosome from binding to an initiation codon.
- Accessibility of initiation codons in a polycistronic mRNA can be controlled by changes in the structure of the mRNA that occur as the result of translation.

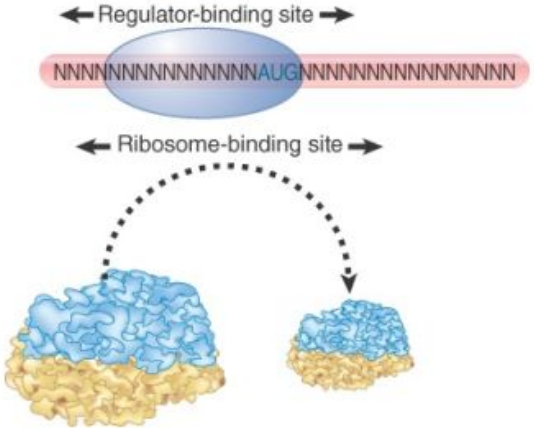
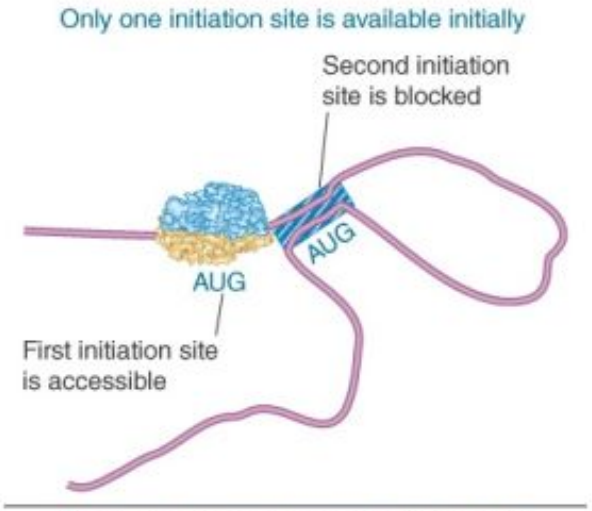


FIGURE 22.49 A regulator protein may block translation by binding to a site on mRNA that overlaps the ribosome-binding site at the initiation codon.

TABLE 22.2 Proteins that bind to sequences within the initiation regions of mRNAs may function as translational repressors.

Repressor	Target Gene	Site of Action
R17 coat protein	R17 replicase	Hairpin that includes ribosome-binding site
T4 RegA	Early T4 mRNAs	Various sequences, including initiation codon
T4 DNA polymerase	T4 DNA polymerase	Shine–Dalgarno sequence
T4 p32	Gene 32	Singe-stranded 5′ leader



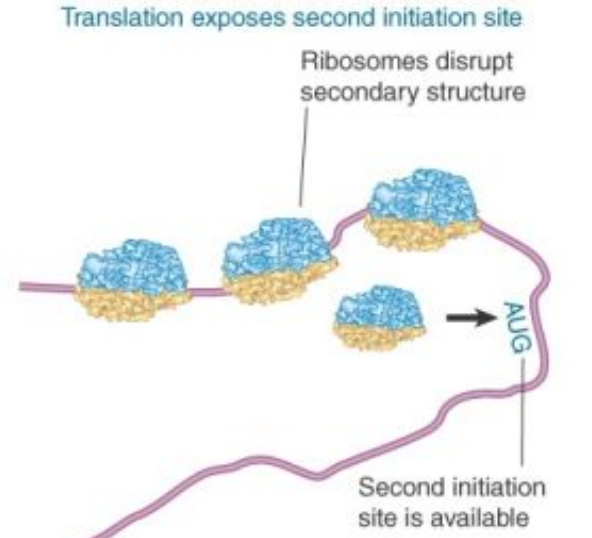


FIGURE 22.50 Secondary structure can control initiation. Only one initiation site is available in the RNA phage, but translation of the first gene changes the conformation of the RNA so that other initiation site(s) become available.

The Cycle of Bacterial Messenger RNA

- Transcription and translation occur simultaneously in bacteria (called coupled transcription/translation) as ribosomes begin translating an mRNA before its synthesis has been completed.
- Bacterial mRNA is unstable and has a half-life of only a few minutes.
- A bacterial mRNA may be polycistronic in having several coding regions that represent different cistrons.

In **bacteria**, mRNA is transcribed and translated in the single cellular compartment; the two processes are so closely linked that they occur simultaneously. Ribosomes attach to bacterial mRNA even before its transcription has been completed so the polysome is likely to still be attached to DNA. Bacterial mRNA is usually unstable and is therefore translated into polypeptides for only a few minutes. This process is called coupled transcription/translation.

In a **eukaryotic cell**, synthesis and maturation of mRNA occur exclusively in the nucleus. Only after these events are completed is the mRNA exported to the cytoplasm, where it is translated by ribosomes. A typical eukaryotic mRNA is often intrinsically stable and continues to be translated for several hours, though there is a great deal of variation in the stability of specific mRNAs, in some cases due to stability or instability sequences in the 5′ or 3′ UTRs.

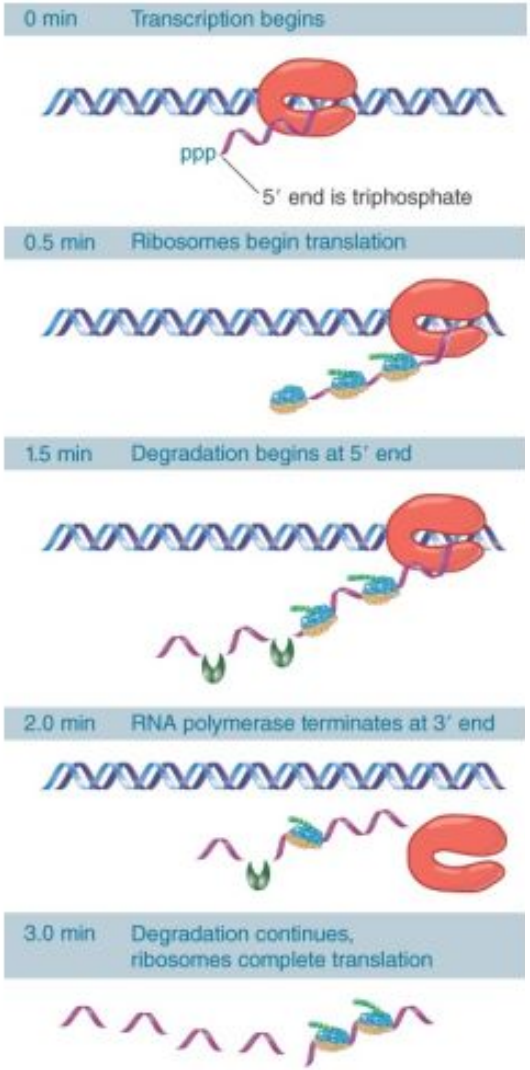


FIGURE 22.51 mRNA is transcribed, translated, and degraded simultaneously in bacteria.

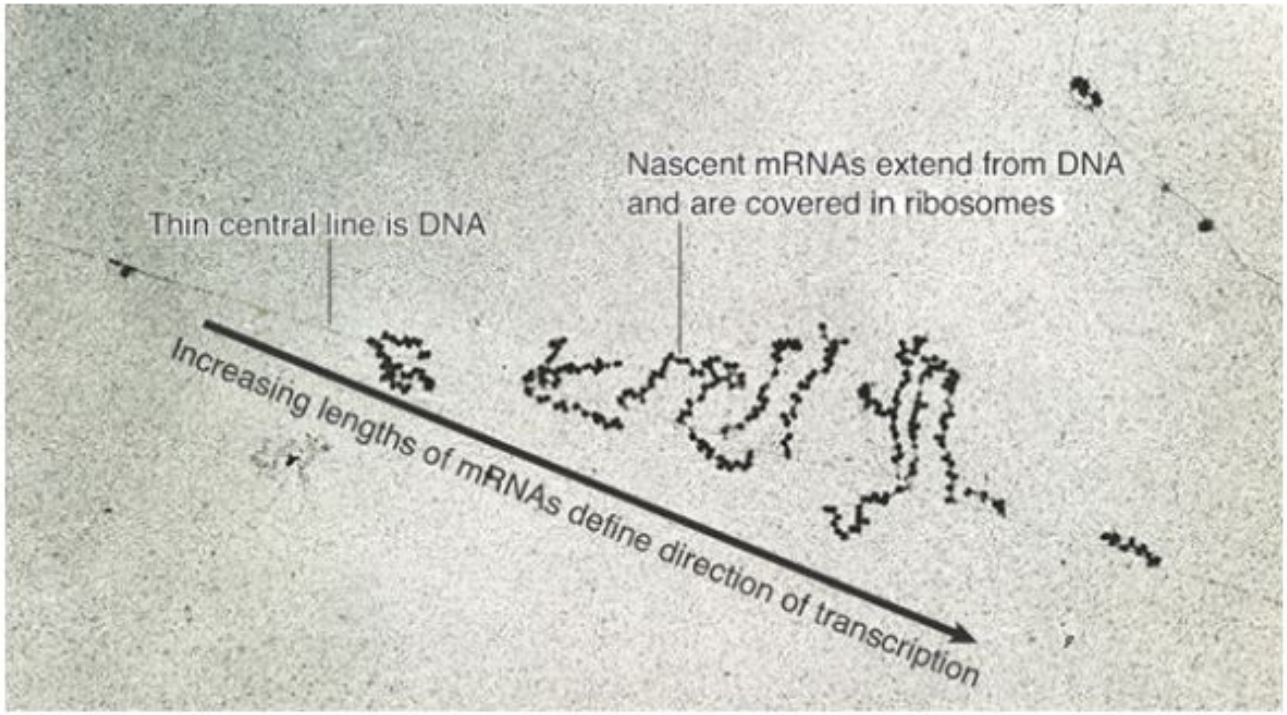


FIGURE 22.52 Transcription units can be visualized in bacteria.

URE 22.53 Bacterial mRNA includes untranslated as well as slated regions. Each coding region has its own initiation and ination signals. A typical mRNA may have several coding ons (ORFs).