Protein synthesis

- A messenger RNA (mRNA) transcript carries 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 mRNA template, engaging in rapid cycles of peptide bond synthesis to build a polypeptide. Aminoacyl-tRNAs shoot into the ribosome at an incredibly fast rate to deposit amino acids, and elongation factor proteins cyclically associate with and dissociate from the ribosome

Ribosome

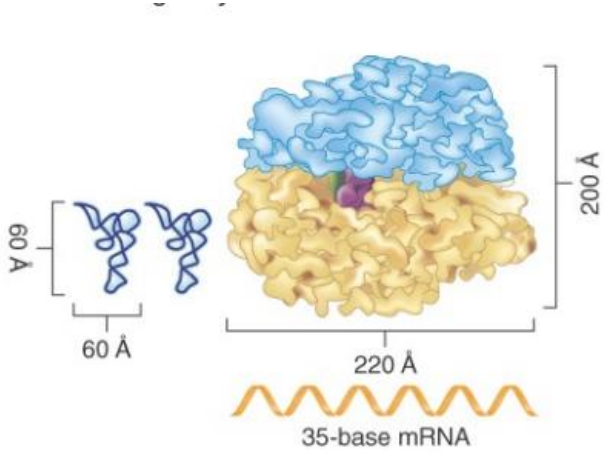


FIGURE 22.1 The ribosome is large enough to bind several tRNAs and an mRNA.

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.

TABLE 30.5 Structural Organization of *E. coli* Ribosomes

	Ribosome	Small Subunit	Large Subunit
Sedimentation coefficient	70S	30S	50S
Mass (kD)	2520	930	1590
Major RNAs		16S = 1542 bases	23S = 2904 bases
Minor RNAs			5S = 120 bases
RNA mass (kD)	1664	560	1104
RNA proportion	66%	60%	70%
Protein number		21 polypeptides*	31 polypeptides†
Protein mass (kD)	857	370	487
Protein proportion	34%	40%	30%

*The S proteins
†The L proteins

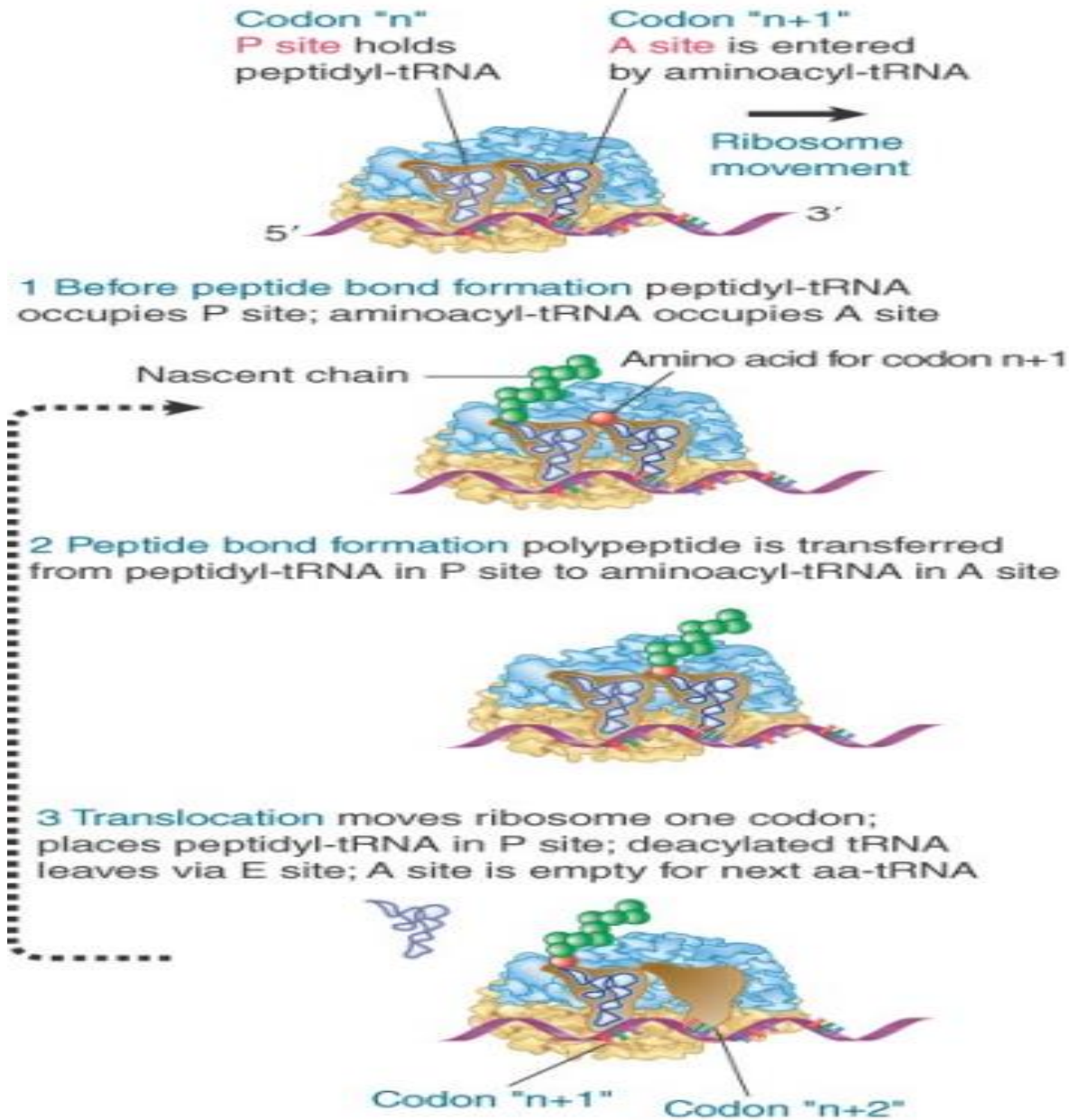


FIGURE 22.3 The ribosome has two sites for binding charged tRNA.

Translation is divided into the three stages

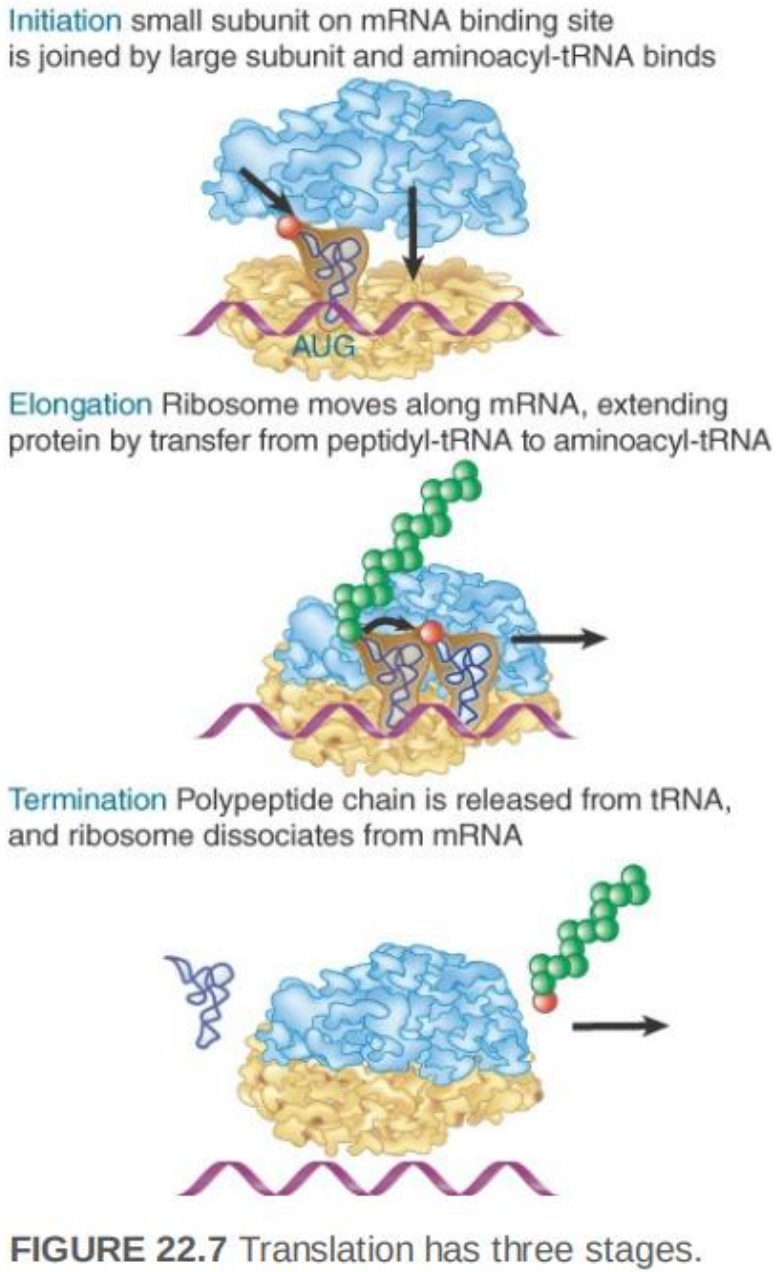


FIGURE 22.7 Translation has three stages.

Initiatic

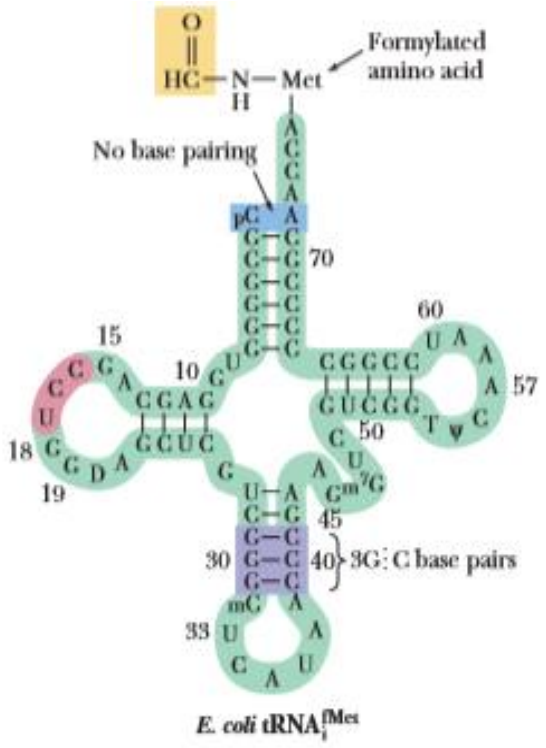
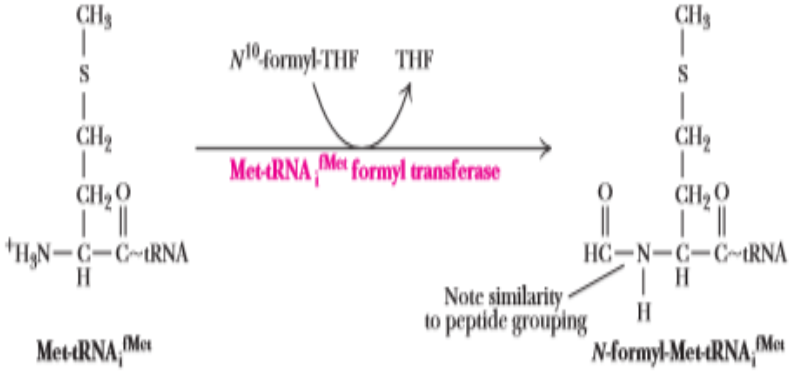


FIGURE 30.16 The secondary structure of *E. coli* N-formyl-methionyl-tRNA$_i^{fMet}$. The features distinguishing it from noninitiator tRNAs are highlighted.

- The components required for peptide chain initiation include (1) mRNA; (2) 30S and 50S ribosomal subunits; (3) a set of proteins known as initiation factors; (4) GTP; and (5) a specific charged tRNA, f-Met-tRNAifMet.
- Initiator tRNA tRNAifMet is a particular tRNA for reading an AUG (or GUG, or even UUG) codon that signals the start site, or N-terminus, of a polypeptide cha[illegible] not read int[illegible]



The methionine contributed in peptide chain initiation by tRNAifMet is unique in that its amino group has been formylated. This reaction is catalyzed by a specific enzyme, methionyl-tRNAifMet formyl transferase

Base pairing between a pyrimidine-rich sequence at the 3-end of 16S rRNA and complementary purine-rich tracts at the 5-end of prokaryotic mRNAs positions the 30S ribosomal subunit in proper alignment with an initiation codon on the mRNA. The purine-rich mRNA sequence, the ribosome-binding site, is often called the Shine–Dalgarno sequence

Initiation Factors

Initiation Factors Initiation involves interaction of the initiation factors (IFs) with GTP, N-formyl-Met-tRNAifMet, mRNA, and the 30S subunit to give a 30S initiation complex to which the 50S subunit then adds to form a 70S initiation complex. The initiation factors are soluble proteins required for assembly of proper initiation complexes.

Events in Initiation Initiation begins when a 30S subunit(IF-3IF-1) complex binds mRNA and a complex of IF-2, GTP, and f-Met-tRNAifMet

The presence of IF-3 on 30S subunits also prevents them from reassociating with 50S subunits.

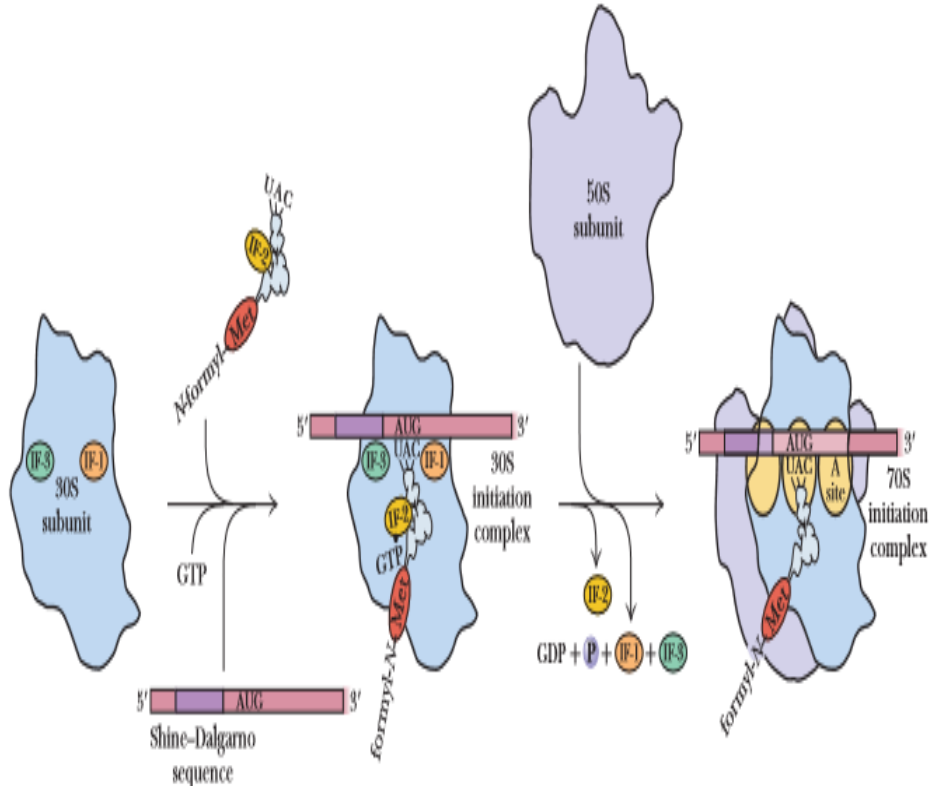
IF-2 delivers the initiator f-Met-tRNAifMet in a GTP-dependent process.

GTP hydrolysis is necessary to form an active 70S ribosome. GTP hydrolysis is triggered when the 50S subunit joins and is accompanied by IF-1 and IF-2 release.

The A site of the 70S initiation complex is ready to accept an incoming aminoacyl-tRNA.

TABLE 30.7 Properties of *E. coli* Initiation Factors

Factor	Mass (kD)	Molecules/ Ribosome	Function
IF-1	9	0.15	Binds to 30S A site and prevents tRNA binding
IF-2	97		G-protein that binds fMet-tRNA$_i^{fMet}$; interacts with IF-1
IF-3	23	0.25	Binds to 30S E site; prevents 50S binding



Elongation

- Aminoacyl-tRNA Binding EF-Tu binds aminoacyl-tRNA and GTP. There is only one EF-Tu species serving all the different aminoacyl-tRNAs, and aminoacyl-tRNAs are accessible to the A site of active 70S ribosomes only in the form of aminoacyl-tRNAEF-TuGTP complexes.
- Once correct base pairing between codon and anticodon has been established within the A site, the GTP is hydrolyzed to GDP and Pi by EF-Tu.
- The aminoacyl end of the tRNA is properly oriented in the peptidyl transferase site of the 50S subunit, and the EF-Tu molecule is released as a EF-TuGDP complex
- Elongation factor Ts (EF-Ts) is a guanine-nucleotide exchange factor (GEF) that catalyzes the recycling of EF-Tu by mediating the displacement of GDP and its replacement by GTP. EF-Ts forms a transient complex with EF-Tu by displacing GDP, whereupon GTP displaces EF-Ts from EF-Tu
- This decoding center, where anticodon loops of the A- and P-site tRNAs and the codons of the mRNA are matched up, is primarily a property of 16S rRNA.

TABLE 30.8 Properties of *E. coli* Elongation Factors

Factor	Mass (kD)	Molecules/ Cell	Function
EF-Tu	43	70,000	G protein that binds aminoacyl-tRNA and delivers it to the A site
EF-Ts	74	10,000	Guanine-nucleotide exchange factor (GEF) that replaces GDP on EF-Tu with GTP
EF-G	77	20,000	G protein that promotes translocation of mRNA

• **Peptidyl transferase**, the activity catalyzing peptide bond formation, is associated with the 50S ribosomal subunit. Indeed, this reaction is a property of the 23S rRNA in the 50S subunit.

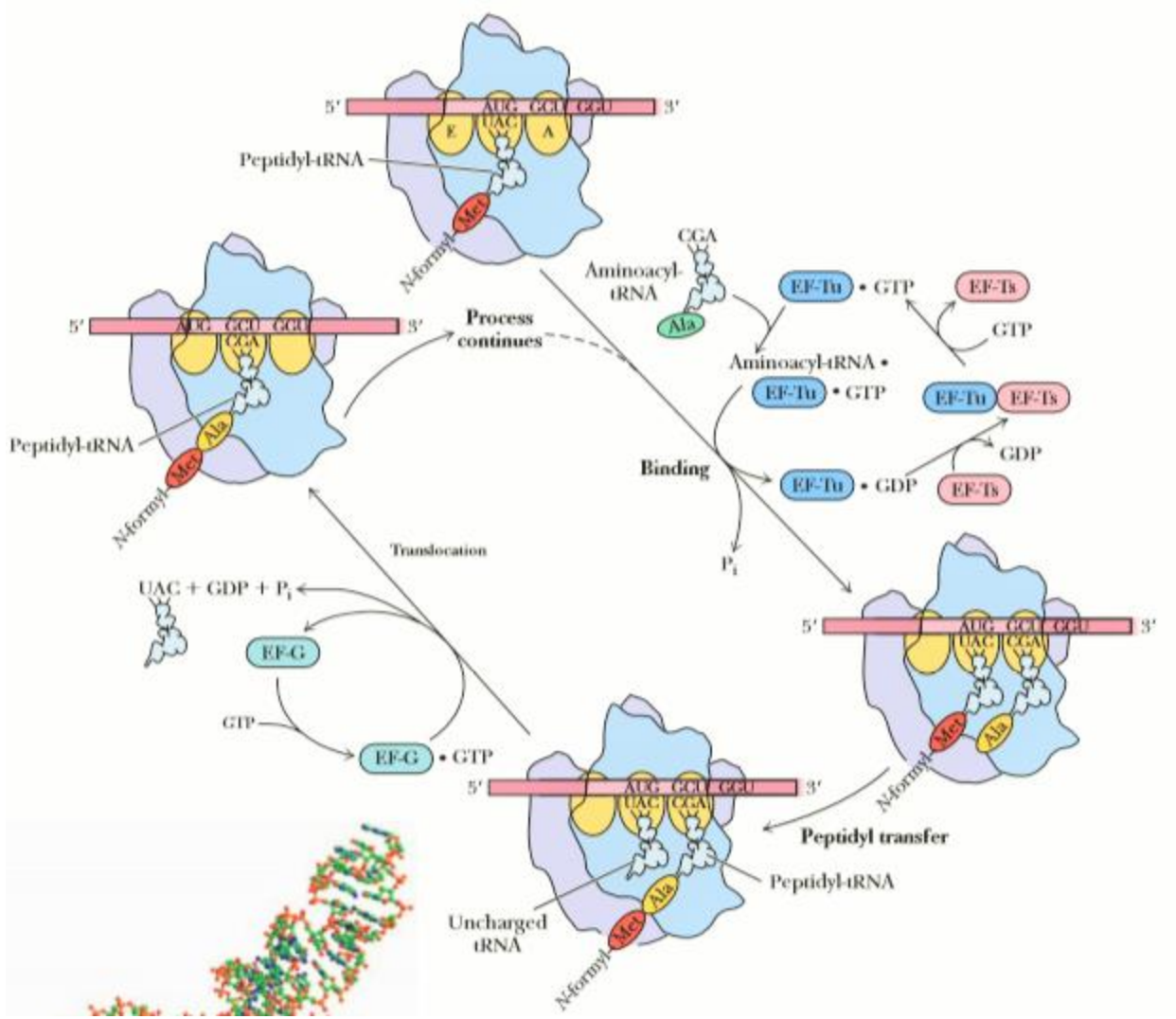
•23S rRNA Is the Peptidyl Transferase Enzyme Peptide bond formation is catalyzed by the large rRNA in the large ribosomal subunit (e.g., the 23S rRNA in prokaryotic 50S ribosomal subunits)

Translocation Three things remain to be accomplished in order to return the active 70S ribosome mRNA complex to the starting point in the elongation cycle:

1. The deacylated tRNA must be removed from the P site.
2. The peptidyl-tRNA must be moved (translocated) from the A site to the P site.
3. The ribosome must move one codon down the mRNA so that the next codon is positioned in the A site.

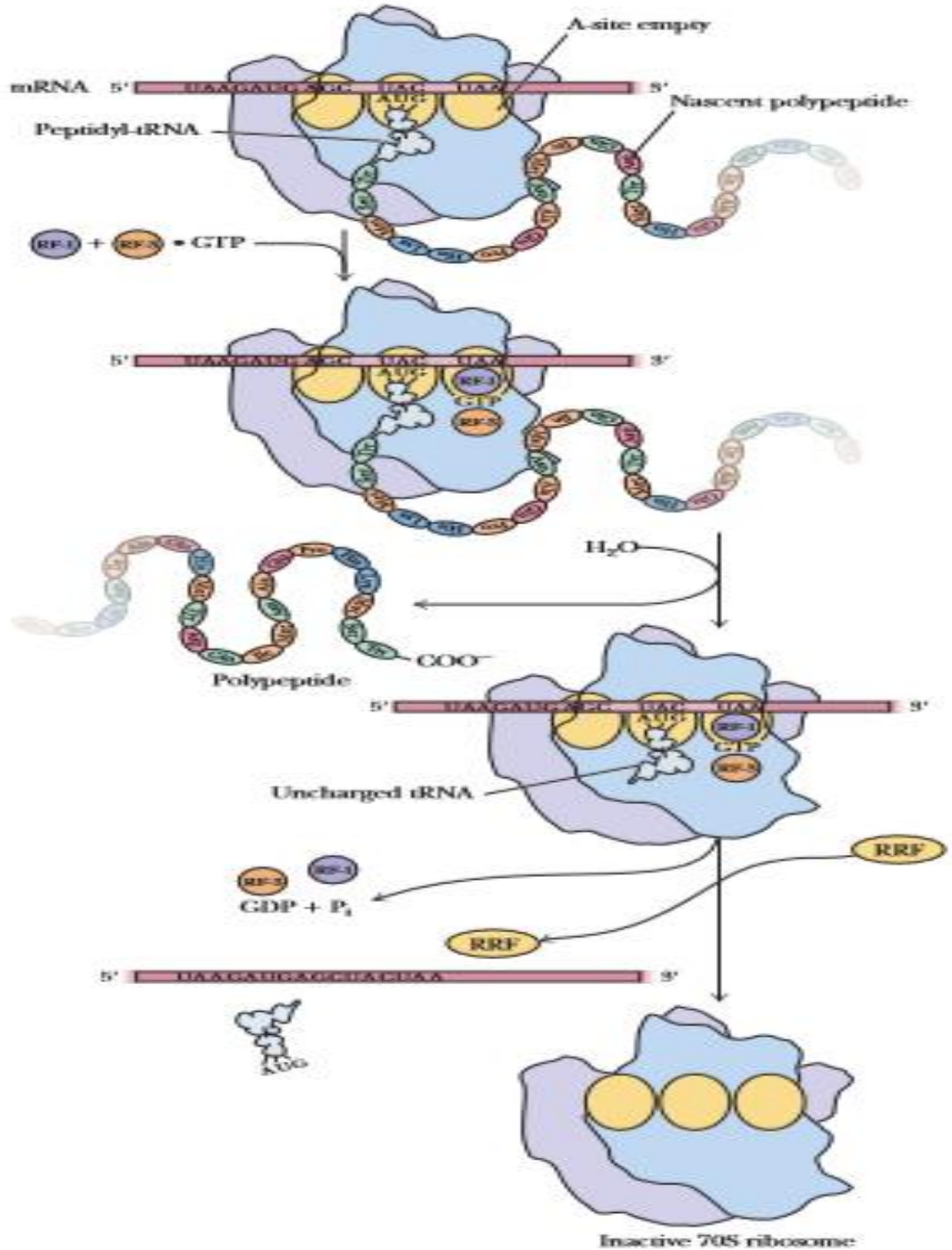
These ratchetlike movements of the 30S subunit relative to the 50S subunit are catalyzed by the translocation protein elongation factor G (EF-G)

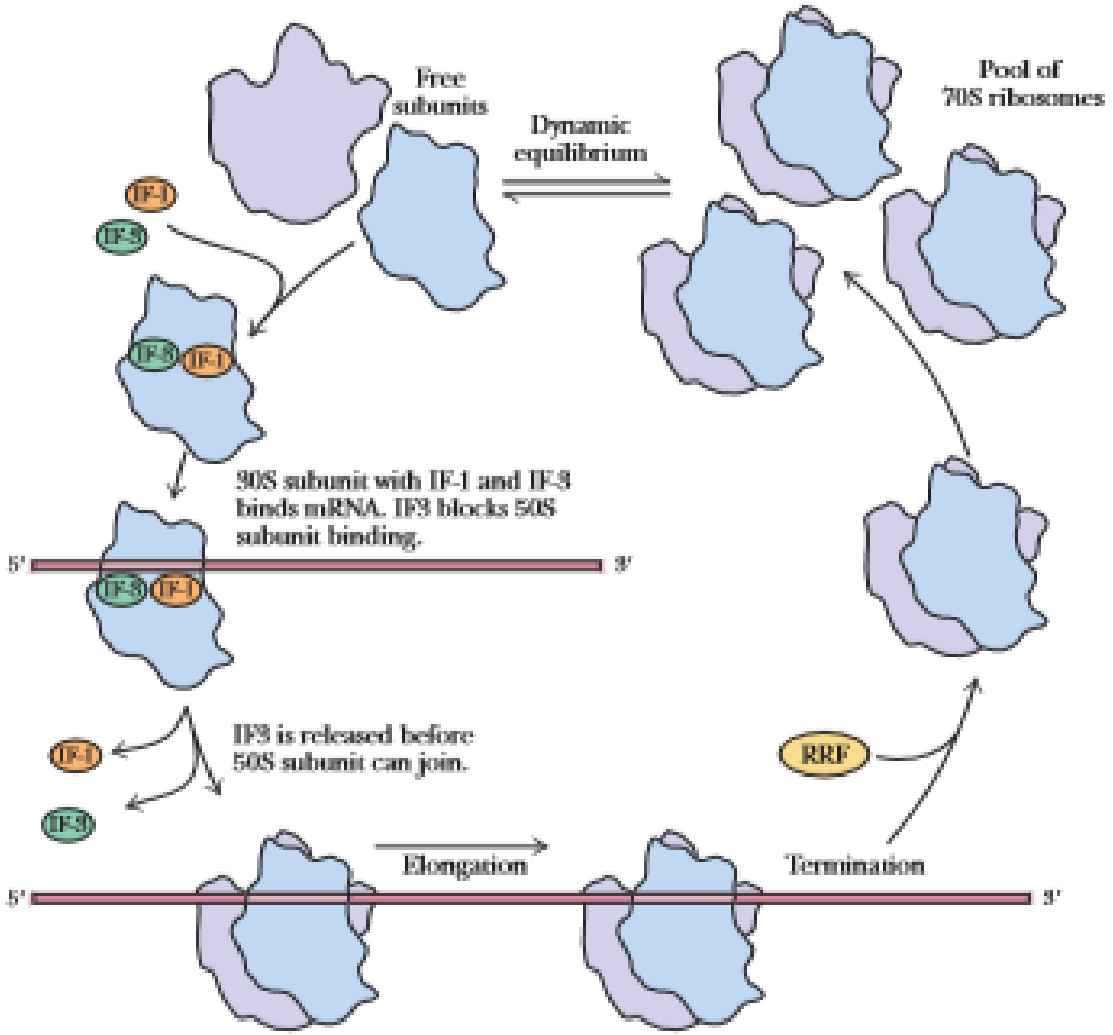
EF-G binds to the ribosome as an EF-GGTP complex. GTP hydrolysis is essential not only for translocation but also for subsequent EF-G dissociation.


5′
AUG GCU CGU
3′
E
UAC
A
Peptidyl-tRNA
Met
N-formyl-
CGA
Aminoacyl-
tRNA
Ala
EF-Tu • GTP
EF-Ts
GTP
Aminoacyl-tRNA •
EF-Tu • GTP
EF-Tu EF-Ts
GDP
Process
continues
Binding
EF-Tu • GDP
EF-Ts
P_i
Translocation
UAC + GDP + P_i
EF-G
GTP
EF-G • GTP
Peptidyl transfer
Uncharged
tRNA

Termination

- The elongation cycle of polypeptide synthesis continues until the 70S ribosome encounters a “stop” codon. At this point, polypeptidyl-tRNA occupies the P site and the arrival of a “stop” or nonsense codon in the A site signals that the end of the polypeptide chain has been reached .
- These nonsense codons are not “read” by any “terminator tRNAs” but instead are recognized by specific proteins known as release factors, so named because they promote polypeptide release from the ribosome. The release factors bind at the A site. RF-1 recognizes UAA and UAG, whereas RF-2 recognizes UAA and UGA. RF-1 and RF-2 are members of the guanine nucleotide exchange factor (GEF) family of proteins, which includes EF-Ts. Like EF-G, RF-1 and RF-2 interact well with the ribosomal A-site structure.
- Ribosomal binding of RF-1 or RF-2 is competitive with EF-G. RF-1 or RF-2 recruit a third release factor, RF-3, complexed with GTP; this protein is a structural mimic of tRNA. RF-3 is the fourth G-protein family member (the other three are IF-2, EF-Tu, and EF-G) involved in protein synthesis.
- The presence of release factors with a nonsense codon in the A site creates a 70S ribosomeRF-1 (or RF-2)RF-3-GTPtermination signal complex that transforms the ribosomal peptidyl transferase into a hydrolase.


A-site empty
mRNA
5'
3'
Nascent polypeptide
Peptidyl-tRNA
RF-1 + RF-3 • GTP
H_2O
COO⁻
Polypeptide
Uncharged tRNA
RRF
RRF
GDP + P_i
Inactive 70S ribosome


Free subunits
Dynamic equilibrium
Pool of 70S ribosomes
IF-1
IF-3
30S subunit with IF-1 and IF-3 binds mRNA. IF3 blocks 50S subunit binding.
5'
3'
IF3 is released before 50S subunit can join.
RRF
Elongation
Termination