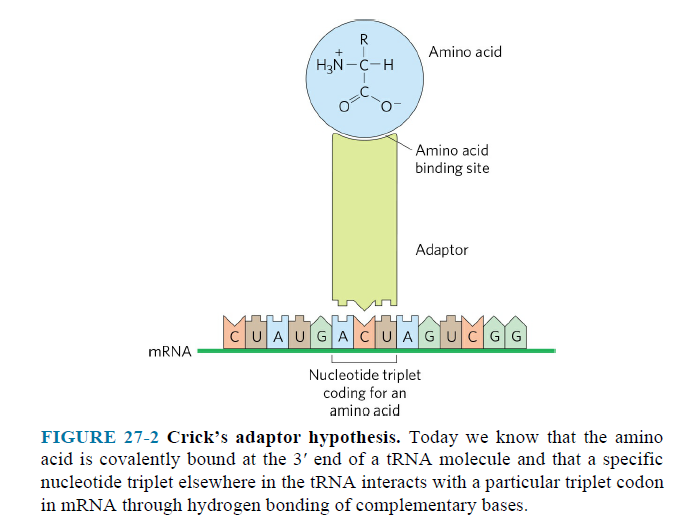
**Chapter 27 Protein Metabolism**

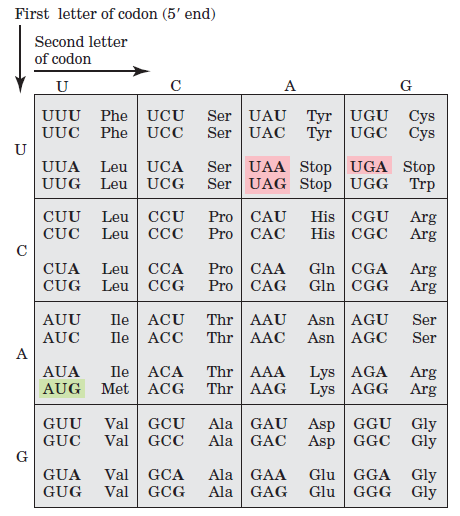
* The particular amino acid sequence of a protein is constructed through the **translation** of information encoded in mRNA. This process is carried out by ribosomes.
* A polypeptide of 100 residues is synthesized in an *E. coli* cell (at 37 oC) in about 5 seconds.

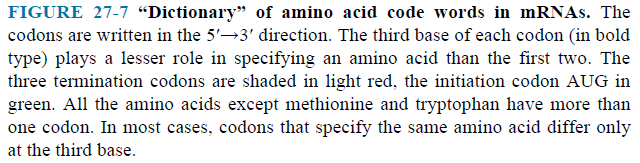
**27.1 The Genetic Code**

* The amino acids attach to transfer RNA (tRNA) to form **aminoacyl-tRNAs**.
* The genetic information encoded in the 4 letter language of nucleic acids is translated into the 20-letter language of proteins.
* The tRNA adaptor “translates” the nucleotide sequence of an mRNA into the amino acid sequence of a polypeptide **(Fig. 27–2)**.

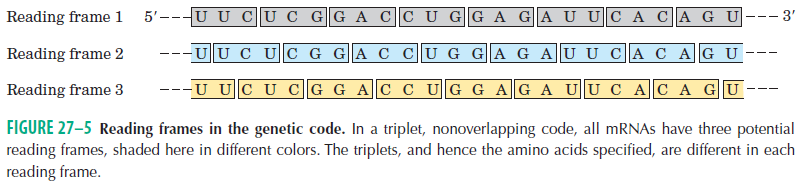


* A **codon** is a triplet of nucleotides that codes for a speciﬁc amino acid.
* There are 64 different codons **(Fig. 27–7)**.

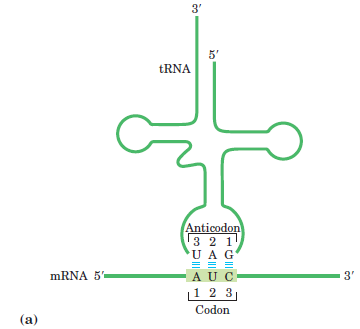


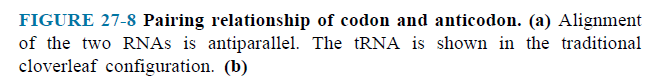


* The genetic code is nearly universal.
* Several codons serve special functions.
* The **initiation codon** AUG is the most common signal for the beginning of a polypeptide in all cells, in addition to coding for Met residues in internal positions of polypeptides.
* The **termination codons** (UAA, UAG, and UGA), also called stop codons or nonsense codons, normally signal the end of polypeptide synthesis and do not code for any known amino acids.
* A speciﬁc ﬁrst codon in the sequence establishes the **reading frame**.
* Each reading frame gives a different sequence of codons, but only one is likely to encode a given protein **(Fig. 27–5)**.



* A reading frame without a termination codon is referred to as an **open reading frame (ORF)**.
* Long open reading frames usually correspond to genes that encode proteins.
* When several different codons specify one amino acid, the difference between them usually lies at the third base position (at the 3’ end).
* Transfer RNAs base-pair with mRNA codons at a three-base sequence on the tRNA called the **anticodon**.
* The ﬁrst base of the codon in mRNA (read in the 5’→3’ direction) pairs with the third base of the anticodon **(Fig. 27–8a)**.

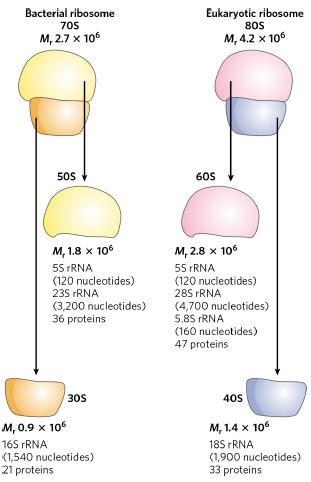


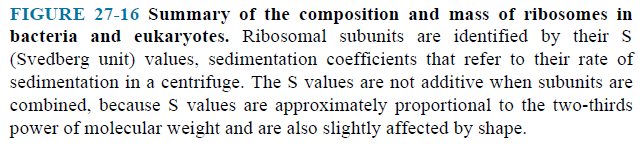


**27.2 Protein Synthesis**

**The Ribosome Is a Complex Supramolecular Machine**

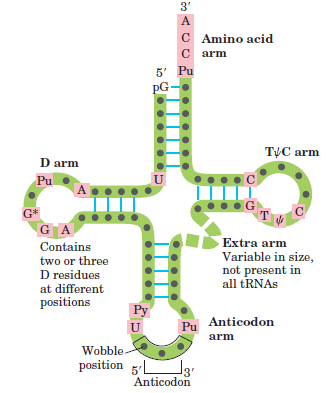
* Each *E. coli* cell contains 15,000 or more ribosomes, accounting for almost a quarter of the dry weight of the cell.
* Bacterial ribosomes contain about 65% rRNA and 35% protein.
* They are composed of two unequal subunits with sedimentation coefﬁcients of 30S and 50S and a combined sedimentation coefficient of 70S.
* Both subunits contain dozens of ribosomal proteins and at least one large rRNA.
* The ribosomes of eukaryotic cells are larger and more complex than bacterial ribosomes **(Fig. 27–16).**

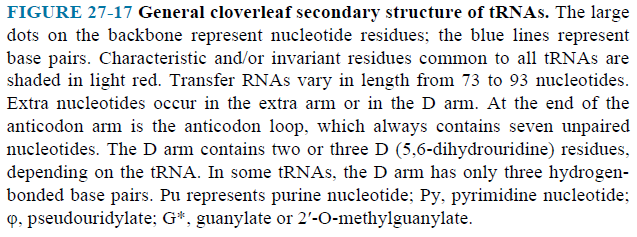




**Transfer RNAs Have Characteristic Structural Features**

* The tRNAs have between 73 and 93 nucleotide residues **(Fig. 27–17)**.





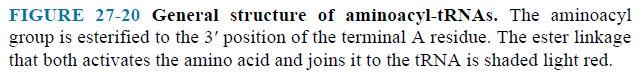
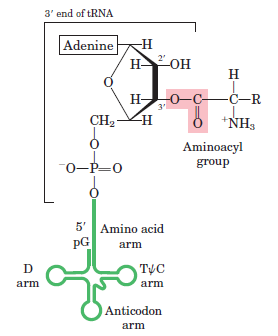
* Cells have at least one kind of tRNA for each amino acid; at least 32 tRNAs are required to recognize all the amino acid codons (some recognize more than one codon), but some cells use more than 32.
* The **amino acid arm** can carry a speciﬁc amino acid esteriﬁed by its carboxyl group to the 2’ or 3’-hydroxyl group of the A residue at the 3’ end of the tRNA.
* The **anticodon arm** contains the anticodon.

**Protein Biosynthesis in *E. coli* Takes Place in Five Stages**

***Stage 1: Activation of Amino Acid***

* **Aminoacyl-tRNA synthetases** attach the correct amino acids to their tRNAs in cytosol.
* Most organisms have one aminoacyl-tRNA synthetase for each amino acid.
* The reaction catalyzed by an aminoacyl-tRNA synthetase is

Amino acid + tRNA + ATP aminoacyl-tRNA + AMP + 2 Pi



***Stage 2: Initiation***

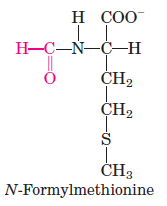
* A speciﬁc amino acid initiates protein synthesis.
* Protein synthesis begins at the amino-terminal end and proceeds by the stepwise addition of amino acids to the carboxyl-terminal end of the growing polypeptide.
* The AUG initiation codon specifies an *amino-terminal* methionine residue.
* Although methionine has only one codon, (5’)AUG, all organisms have two tRNAs for methionine.
* One is used for the initiation codon and the other is used an internal position in a polypeptide.
* In bacteria, the two types of tRNA speciﬁc for methionine are designated tRNAMet and tRNAfMet.
* First, methionine is attached to tRNAfMet by the **Met-tRNA synthetase** (which in E. coli aminoacylates both tRNAfMet and tRNAMet).



* Next, a **transformylase** transfers a formyl group from N10-formyltetrahydrofolate to the amino group of the Met residue.



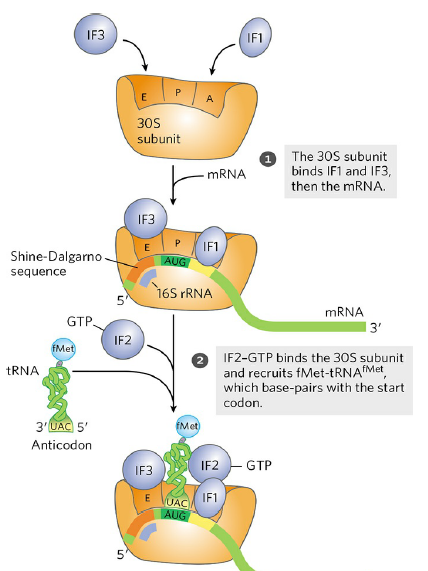
* Product is fMet-tRNAfMet.

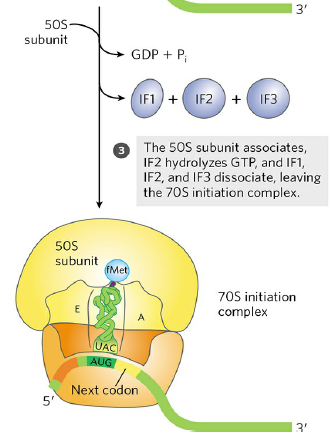


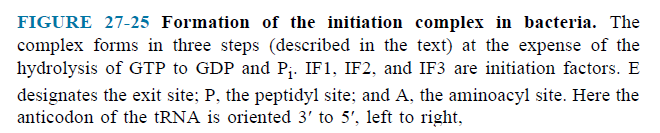
* The transformylase is speciﬁc for Met residues attached to tRNAfMet.

**The Three Steps of Initiation**

* In step 1 **(Fig. 27–25)**,







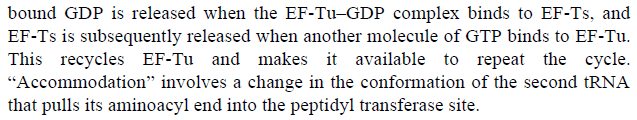
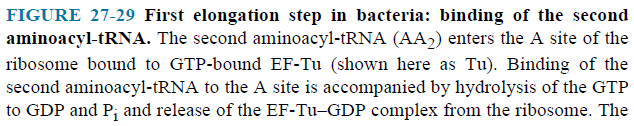
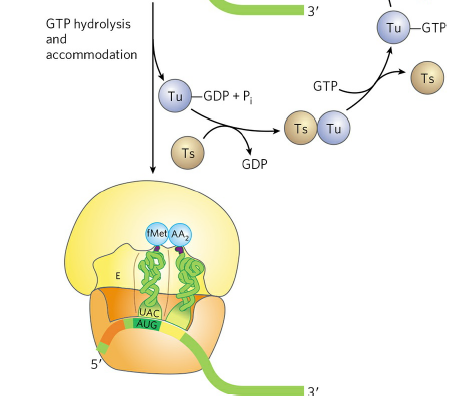
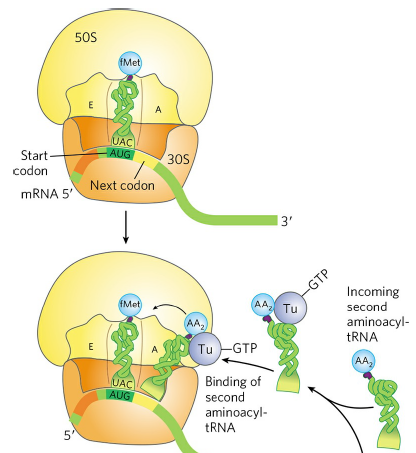
* 30S ribosomal subunit binds two initiation factors, IF-1 and IF-3.
* IF-3 prevents the 30S and 50S subunits from combining prematurely.
* The mRNA then binds to the 30S subunit.
* The initiating (5’)AUG is guided to its correct position by the **Shine-Dalgarno sequence** in the mRNA.
* The sequence base-pairs with a complementary sequence near the 3’end of the 16S rRNA of the 30S ribosomal subunit.
* Bacterial ribosomes have three sites that bind tRNAs, the **aminoacyl (A) site**, the **peptidyl (P) site**, and the **exit (E) site**.
* The fMet-tRNAfMet is the only aminoacyl-tRNA that binds ﬁrst to the P site.
* Factor IF-1 binds at the A site and prevents tRNA binding at this site during initiation.
* In step 2 **(Fig. 27–25)**,
* GTP-bound IF-2 facilitates binding of fMet-tRNAfMet to 30S ribosomal subunit.
* In step 3 **(Fig. 27–25)**,
* 50S subunit associates,
* IF-2 hydrolyzes to GTP,
* IF-1, IF-2, and IF-3 dissociate, leaving the initiation complex.
* Completion of the steps produces a functional 70S ribosome called the **initiation complex**.

***Stage 3: Elongation***

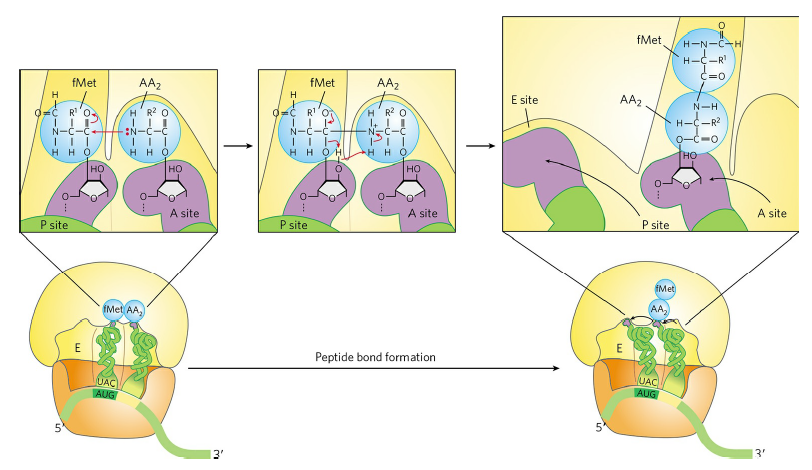
* Peptide bonds are formed.

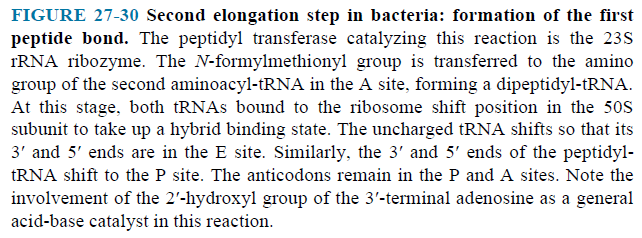
**The Three Steps of Elongation**

* In step 1 **(Fig. 27–29)**,

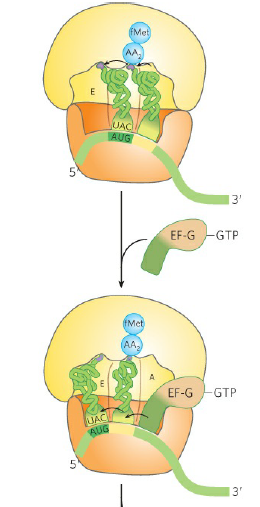


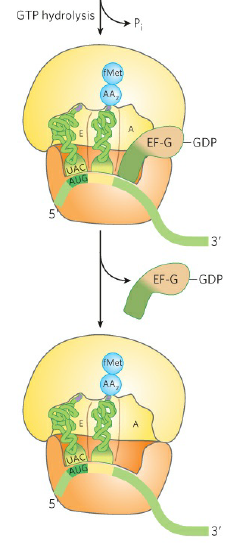
* Aminoacyl-tRNA binds to a complex of GTP-bound EF-Tu (Elongation Factor).
* Aminoacyl-tRNA–EF-Tu–GTP complex binds to the A site.
* GTP is hydrolyzed and an EF-Tu–GDP complex is released.
* EF-Tu–GTP complex is regenerated in a process involving EF-Ts and GTP.
* In step 2 **(Fig. 27–30)**,
* Peptide bonds are formed by **peptidyl transferase** in the large ribosomal subunit.

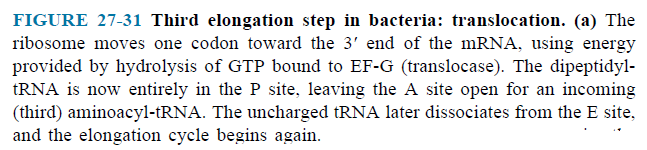




* In step 3 **(Fig. 27–31)**,
* Ribosome moves one codon toward the 3’ end of the mRNA, **translocation**.
* Movement of the ribosome along the mRNA requires EF-G and GTP.
* EF-G can bind the A site and displace the peptidyl-tRNA.
* Deacylated tRNA dissociates from the ribosomal E site.
* This process occurs in the same way for the next elongation cycles.

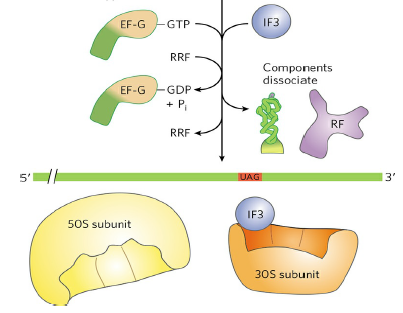
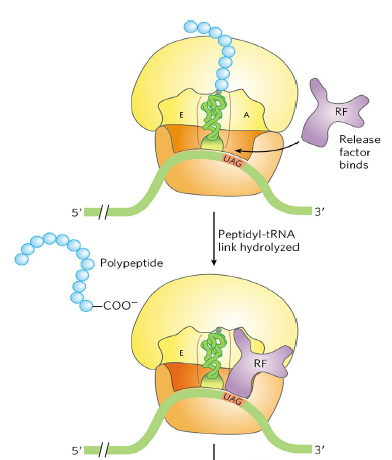


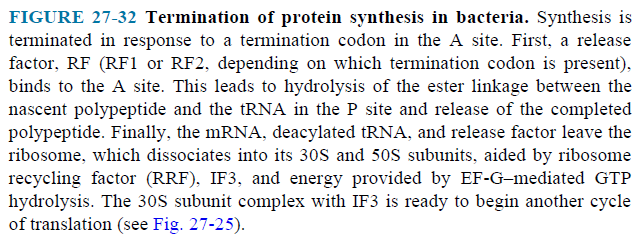




***Stage 4: Termination and Ribosome Recycling***

* Elongation continues until the ribosome adds the last amino acid coded by the mRNA.
* Termination is signaled by the presence of one of three termination codons in the mRNA.
* Once a termination codon occupies the ribosomal A site, three **termination factors**, or **release factors** (RF-1, RF-2, and RF-3) contribute to termination **(Fig. 27–32)**.
* RF-1 recognizes UAG and UAA, and RF-2 recognizes UGA and UAA.
* Either RF-1 or RF-2 binds at a termination codon and induces peptidyl transferase to hydrolyze the growing polypeptide. The polypeptide is released.
* The release factors are replaced by EF-G and ribosome recycling factor (RF-3).
* Hydrolysis of GTP by EF-G leads to dissociation of the 50S subunit from the 30S tRNA–mRNA complex.
* EF-G and RRF are replaced by IF-3, which promotes the dissociation of the tRNA.
* The mRNA is released.
* The complex of IF-3 and the 30S subunit is ready to initiate another round of protein synthesis.





***Stage 5: Folding and Posttranslational Processing***

* The polypeptide chain is folded and processed into its biologically active form.