The Initiation of Translation

The second stage in the process of protein synthesis is initiation. During initiation, all the components necessary for protein synthesis assemble: (1) mRNA; (2) the small and large subunits of the ribosome; (3) a set of three proteins called initiation factors; (4) initiator tRNA with *N*-formylmethionine attached (fMet-tRNA^{fMet}); and (5) guanosine triphosphate (GTP). Initiation comprises three major steps. First, mRNA binds to the small subunit of the ribosome. Second, initiator tRNA binds to the mRNA through base pairing between the codon and anticodon. Third, the large ribosome joins the initiation complex. Let's look at each of these steps more closely.

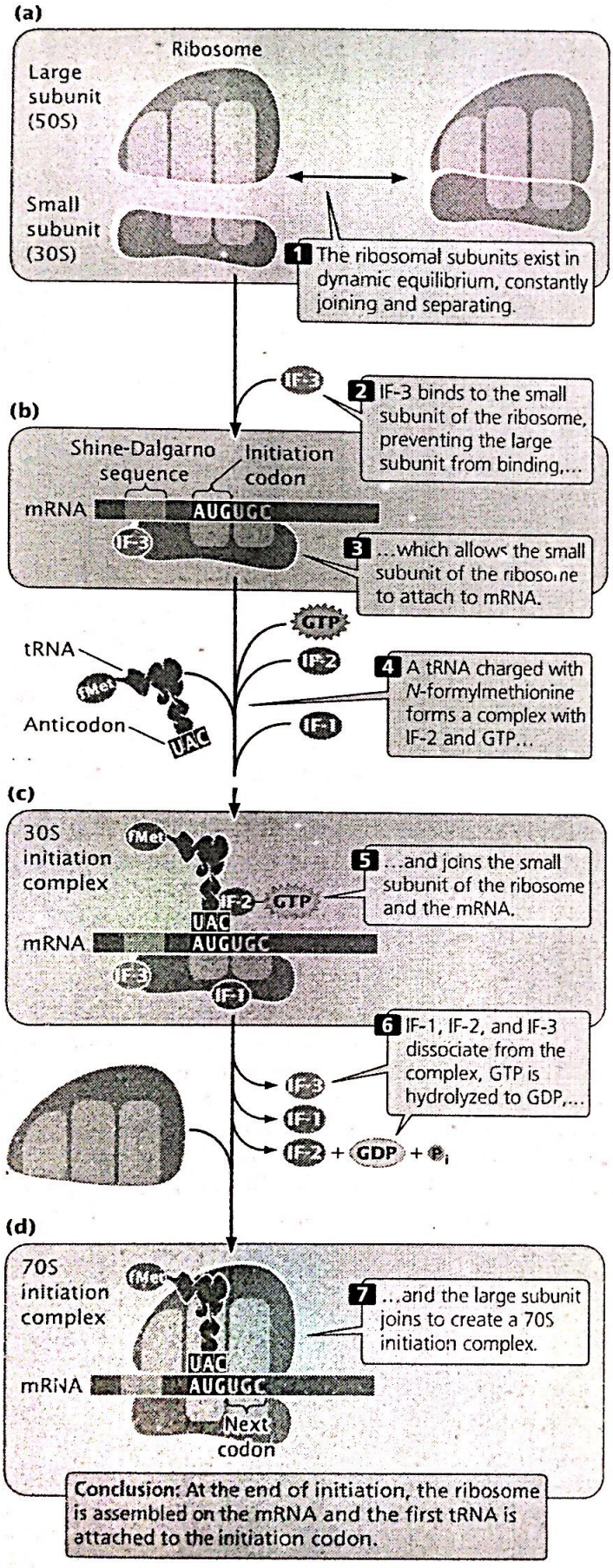
A functional ribosome exists as two subunits, the small 30S subunit and the large 50S subunit (in bacterial cells). When not actively translating, the two subunits exist in dynamic equilibrium, in which they are constantly joining

and separating (◀ FIGURE 15.19). An mRNA molecule can bind to the small ribosome subunit only when the subunits are separate. **Initiation factor 3** (IF-3) binds to the small subunit of the ribosome and prevents the large subunit from binding during initiation (see Figure 15.19b).

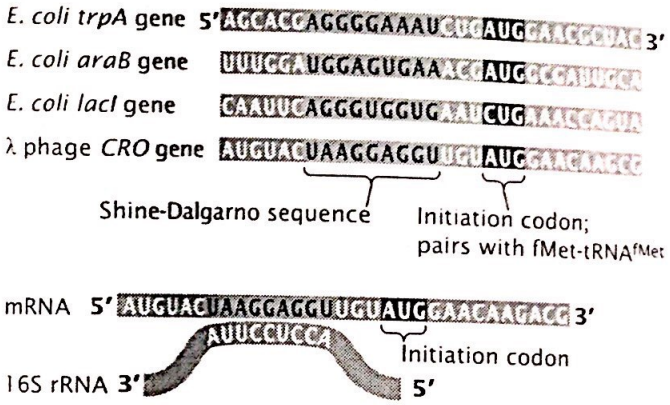
Key sequences on the mRNA required for ribosome binding have been identified in experiments in which the ribosome is allowed to bind to mRNA under conditions that allow initiation but prevent later stages of protein synthesis, thereby stalling the ribosome at the initiation site. After the ribosome has attached to the mRNA in these experiments, ribonuclease is added, which degrades all the mRNA except the region covered by the ribosome. The remaining mRNA can be separated from the ribosome and studied. The sequence covered by the ribosome during initiation is from 30 to 40 nucleotides long and includes the AUG initiation codon. Within the ribosome-binding site is the Shine-Dalgarno consensus sequence (◀ FIGURE 15.20) (see Chapter 14), which is complementary to a sequence of nucleotides at the 3′ end of 16S rRNA (part of the small subunit of the ribosome). During initiation, the nucleotides in the Shine-Dalgarno sequence pair with their complementary nucleotides in the 16S rRNA, allowing the small subunit of the ribosome to attach to the mRNA and positioning the ribosome directly over the initiation codon.

Next, the initiator fMet-tRNA^{fMet} attaches to the initiation codon (see Figure 15.19c). This step requires **initiation factor 2** (IF-2), which forms a complex with GTP. A third factor, **initiation factor 1** (IF-1), enhances the dissociation of the large and small ribosomal subunits.

At this point, the initiation complex consists of (1) the small subunit of the ribosome; (2) the mRNA; (3) the initiator tRNA with its amino acid (fMet-tRNA^{fMet}); (4) one molecule of GTP; and (5) IF-3, IF-2, and IF-1. These components are collectively known as the **30S initiation complex** (see Figure 15.19c). In the final step of initiation, IF-3 dissociates from the small subunit, allowing the large



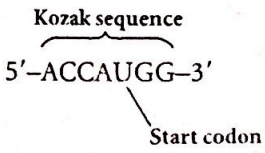
15.19 The initiation of translation in bacterial cells requires several initiation factors and GTP.



15.20 Shine-Dalgarno consensus sequences in mRNA are required for the attachment of the small subunit of the ribosome. The Shine-Dalgarno sequences are complementary to a sequence of nucleotides found near the 3′ end of 16S rRNA in the small subunit of the ribosome. These complementary nucleotides base pair during the initiation of translation.

subunit of the ribosome to join the initiation complex. The molecule of GTP (provided by IF-2) is hydrolyzed to guanosine diphosphate (GDP), and IF-1 and IF-2 depart (see Figure 15.19d). When the large subunit has joined the initiation complex, it is called the **70S initiation complex.**

Similar events take place in the initiation of translation in eukaryotic cells, but there are some important differences. In bacterial cells, sequences in 16S rRNA of the small subunit of the ribosome bind to the Shine-Dalgarno sequence in mRNA; this binding positions the ribosome over the start codon. No analogous consensus sequence exists in eukaryotic mRNA. Instead, the cap at the 5′ end of eukaryotic mRNA plays a critical role in the initiation of translation. The small subunit of the eukaryotic ribosome, with the help of initiation factors, recognizes the cap and binds there; the small subunit then migrates along (scans) the mRNA until it locates the first AUG codon. The identification of the start codon is facilitated by the presence of a consensus sequence (called the Kozak sequence) that surrounds the start codon:



Another important difference is that eukaryotic initiation requires more initiation factors. Some factors keep the ribosomal subunits separated, just as IF-3 does in bacterial cells. Others recognize the 5′ cap on mRNA and allow the small subunit of the ribosome to bind there. Still others possess RNA helicase activity, which is used to unwind secondary structures that may exist in the 5′ untranslated region of mRNA, allowing the small subunit to move down

the mRNA until the initiation codon is reached. Other initiation factors help bring the initiator tRNA and methionine ($Met\text{-}tRNA^{fMet}$) to the initiation complex.

The poly(A) tail at the 3′ end of eukaryotic mRNA also plays a role in the initiation of translation. Proteins that attach to the poly(A) tail interact with proteins that bind to the 5′ cap, enhancing the binding of the small subunit of the ribosome to the 5′ end of the mRNA. This interaction between the 5′ cap and the 3′ tail suggests that the mRNA bends backward during the initiation of translation, forming a circular structure (◀ FIGURE 15.21). A few eukaryotic mRNAs contain internal ribosome entry sites, where ribosomes can bind directly without first attaching to the 5′ cap.