N-formylmethionine. The answer lies about ten nucleotides upstream of the AUG, where the ribosome binding site (Shine-Delgarno box) is located. Ribosome binding sites can vary in strength and are an important consideration in genetic engineering. The initiation of polymerization in procaryotes requires an *initiation complex* composed of a 30s ribosomal unit with an *N*-formylmethionine bound to its initiation region, a 50s ribosomal unit, three proteins called initiation factors (IF1, IF2, and IF3), and the phosphate bond energy from GTP.

The elongation of the amino acid chain uses *t*-RNAs as decoders. One end of the *t*-RNA contains the *anticodon*, which is complementary to the codon on the *m*-RNA. The other end of the *t*-RNA binds a specific amino acid. The *t*-RNA is called *charged* when it is carrying an amino acid. The binding of an amino acid to the *t*-RNA molecule requires the energy from two phosphate bonds and enzymes known as *aminoacyl-t-RNA synthetases*. Figure 2.19 depicts a *t*-RNA molecule.

The actual formation of the peptide bond between the two amino acids occurs on adjacent sites on the ribosome: the P or *peptidyl* site and the A or *aminoacyl* site (see Fig. 4.7). The growing protein occupies the P site, while the next amino acid to be added occupies the A site. As the peptide bond is formed, the *t*-RNA associated with the P site is released, and a rachet mechanism moves the *m*-RNA down one codon so as to cause the *t*-RNA that was in the A site to be in the P site. Then a charged *t*-RNA with the correct anticodon can be recognized and inserted into the A site. The whole process is then repeated. The cell expends a total of four phosphate bonds to add one amino acid to each growing polypeptide (two to charge the *t*-RNA and two in the process of elongation), and this accounts for most of the cellular energy expenditure in bacteria.

When a nonsense or stop codon is reached, the protein is released from the ribosome with the aid of a protein *release factor* (RF). The 70S ribosome then dissociates into 30S and 50S subunits. An *m*-RNA typically is being read by many (for example, 10 to 20) ribosomes at once; as soon as one ribosome has moved sufficiently far along the message that the ribosome binding site is not physically blocked, another ribosome can bind and initiate synthesis of a new polypeptide chain.

4.5.3. Posttranslational Processing: Making the Product Useful

Often the polypeptide formed from the ribosome must undergo further processing before it can become truly useful. First, the newly formed chain must fold into the proper structure; in some cases, several different chains must associate to form a particular enzyme or structural protein. Additionally, *chaperones* are an important class of proteins that assist in the proper folding of peptides. There are distinct pathways to assist in folding polypeptides. The level of chaperones in a cell increases in response to environmental stresses such as high temperature. Misfolded proteins are subject to degradation if they remain soluble. Often misfolded proteins aggregate and form insoluble particles (i.e., inclusion bodies). High levels of expression of foreign proteins through recombinant DNA technology in *E. coli* often overwhelm the processing machinery, resulting in inclusion bodies. The formation of proteins in inclusion bodies greatly complicates any bioprocess, since in vitro methods to unfold and refold the protein product must be employed. Even when a