

BERG/STRYER V STUDY GUIDE

CHAPTER 29

1. HOMEWORK 3, 4, 13. The complete structure of the ribosome was recently determined, and Berg's interest in the subject makes this chapter look rather like an Italian cookbook with many illustrations of pasta. Don't be discouraged by all of the complex figures here. You should know the **structure of tRNA** with specific loops etc. (815-7) and be familiar with the items listed on 816-7. The **first** stage of protein synthesis is called "**activation**" and it involves connecting the correct tRNA molecules to the correct amino acids. Obviously the enzymes which do this job (**amino-acyl tRNA synthetases**) are responsible for observance of the genetic code. Even though there are 61 different codons which code for amino acids, and at least 30 different tRNA's, you should know that there are **only 20** different aa-tRNA synthetases, one for each amino acid, in prokaryotes. Each amino acid is attached to **AMP** (adenylate) before being attached to the 3' end of the proper tRNA. Understand Fig. 29.10, the 3' end of the tRNA can "flip" between the activation site and an editing site. "Hard" distinctions, like that between Valine and Isoleucine, require proofreading but distinctive amino acids like Tyrosine are easier to recognize and don't require it. There is no further discrimination after this step.

2. Know that prokaryotic **ribosomes** are 70S, and have subunits of 50S and 30S (823). The large subunit has 34 kinds of protein (L1 to L34), 23S rRNA and 5S rRNA. The small subunit has 21 kinds of protein (S1 to S21) and 16S rRNA. The 3' end of prokaryotic 16S rRNA has a sequence CUCCU which binds to the **ribosome binding site** of mRNA's AGGAG in what is called the "**Shine-Dalgarno interaction**" (827). The 23S rRNA contains the active site for peptidyl synthase, and the 5S rRNA "greet" incoming tRNA molecules. The **second** stage of protein synthesis is "**initiation**." The normal enzyme, Met-tRNA synthase, connects Methionine to the initiator tRNA, called tRNA_f. Then the Met is formylated to produce fMet-tRNA_f (Fig 29.21). The formation of the **initiation complex** is shown in Fig 29.27. First the 30S subunit complexes with IF-1 and IF-3. Then IF-2 brings in GTP and fMet-tRNA_f, which pairs with the newly introduced mRNA. When the GTP hydrolyzes, the 3 IF proteins depart and the 50S subunit clamps down, trapping the mRNA and the first aa-tRNA.

3. The **third** stage of protein synthesis is "**elongation**" which is a cyclic process. We start with aa-tRNA in the "P" site (peptidyl tRNA site). There are three steps in elongation – **introduction, peptide synthesis, and translocation**. For introduction, **EF-Tu** brings in GTP and an aa-tRNA (834). When the aa-tRNA is accepted into the "A" site (aminoacyl tRNA), the GTP is hydrolyzed, and the GDP "sticks" to EF-Tu. Another factor, **EF-Ts** has to come "scrape off" the GDP so that EF-Tu can bind another aa-tRNA. Peptide synthesis occurs spontaneously, without GTP or factors, catalyzed by 23S rRNA. Then **EF-G** comes in with GTP and "shoves" the peptidyl tRNA into the "P" site, which moves the empty tRNA into the "E" (exit) site (translocation). Thus each cycle of elongation uses up two GTP's. The **fourth** stage of translation is "**termination**." There are three **releasing factors**, which are proteins that resemble tRNA in size and shape. RF1 recognizes UAA and UAG, RF2 recognizes UAA and UGA.

4. Know the inhibitors in Table 29.4. **Streptomycin** blocks initiation, **Tetracycline** blocks the introduction step of elongation, and **Chloramphenicol** inhibits peptide synthesis. **Erythromycin** blocks translocation, and **Puromycin** mimics aa-tRNA to cause abortive chain termination. (838)