SECTION 4. TRANSLATION OF mRNA INTO PROTEINS

LEARNING OBJECTIVES

By the end of this section, you will be able to do the following:

- Describe how protein is synthesized from mRNA
- Differentiate the mechanisms of translation in eukaryotes and prokaryotes

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Translation involves the conversion of an mRNA sequence to a corresponding polypeptide sequence, and is the last step in the utilization of genetic information encoded in the DNA.

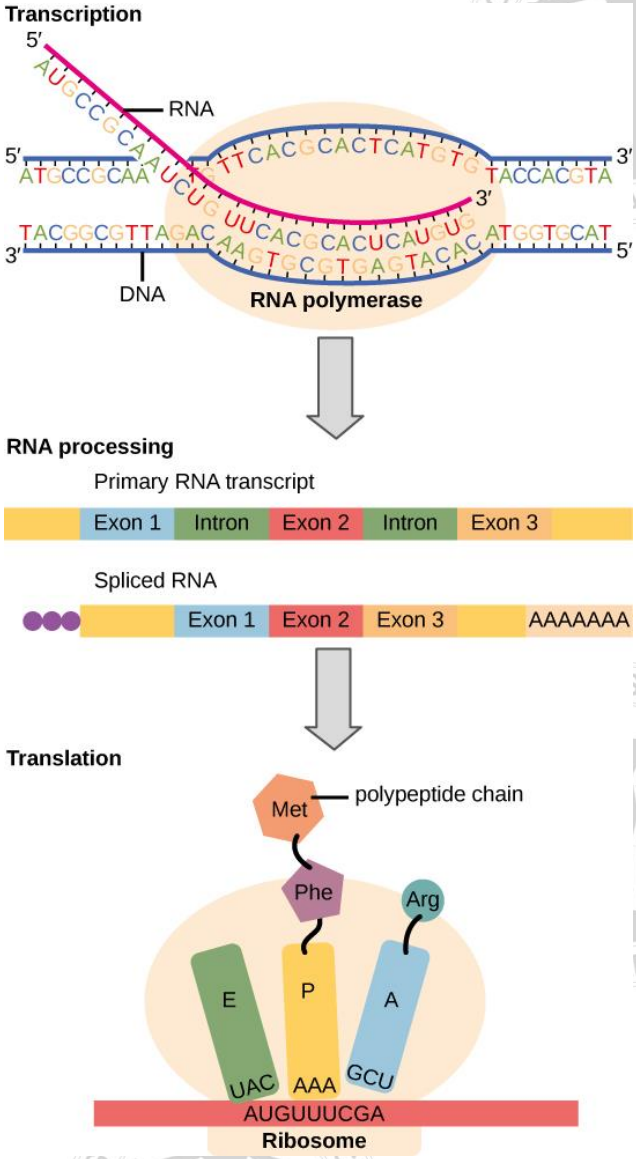


Figure 1: Instructions on DNA are transcribed onto messenger RNA. Ribosomes are able to read the genetic information inscribed on a strand of messenger RNA and use this information to string amino acids together into a protein. From https://openstax.org/books/microbiology/pages/11-4-protein-synthesis-translation

The mRNA is read in segments of 3 nucleotides, called codons, starting from the 5' end. Each codon corresponds to an amino acid. The correspondence between codons formed from the four RNA nucleotides and the 20 naturally occurring amino acids is given by the **genetic code**.

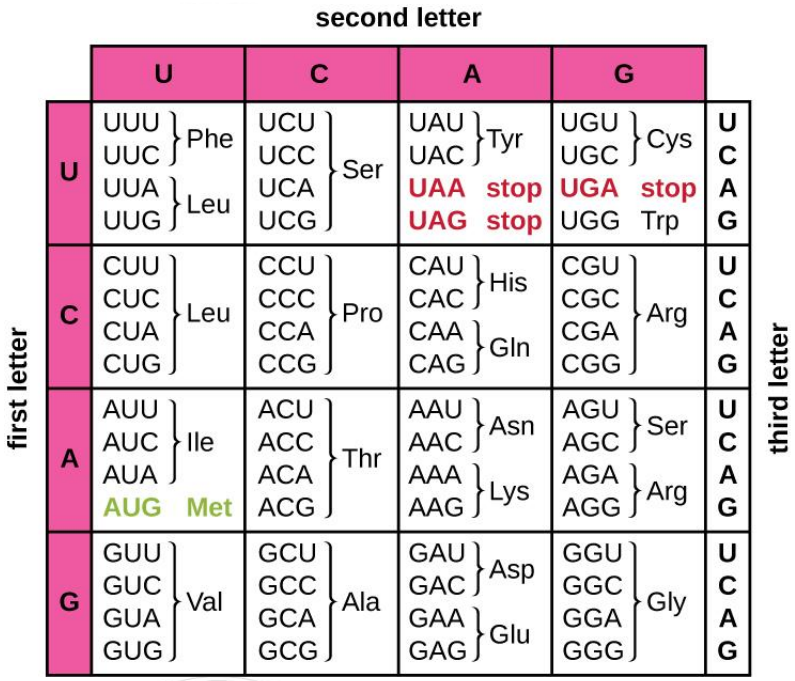


Figure 2: The genetic code translates each nucleotide triplet, or codon, in mRNA into an amino acid or a termination signal in a nascent protein. The first letter of a codon is shown vertically on the left, the second letter of a codon is shown horizontally across the top, and the third letter of a codon is shown vertically on the right. (credit: modification of work by National Institutes of Health)

https://openstax.org/books/microbiology/pages/11-4-protein-synthesis-translation

Features of the genetic code

1. *It is quasi – universal.* With a few minor exceptions, virtually all species use the same genetic code for protein synthesis.
2. *It is degenerate.* A single amino acid may be encoded by multiple codons. Degeneracy is believed to be a cellular mechanism that can reduce the negative impact of random mutations. Codons that specify the same amino acid typically only differ by one nucleotide.
3. *It is comma-less and non-overlapping.* mRNA sequences are read as discrete segments of codons which do not overlap.

Sixty-four different combinations exist for the 3 nucleotide positions in a codon. Of these, 61 code for 20 different amino acids, while three do not correspond to any amino acid. These codons (UAG, UGA and UAA) are referred to as stop codons and serve to terminate protein synthesis. Another codon, AUG, also has a special function; in addition to specifying the amino acid methionine, it also serves as the start codon to initiate translation.

There are 3 possible reading frames for an mRNA strand in synthesizing a protein, if we consider any nucleotide as the starting point for translation. However, translation almost always starts at an AUG codon. An **open reading frame (ORF) is a** long stretch of codons in an mRNA sequence that starts with an AUG and ends with a stop codon. Since this is unlikely to occur by chance, the ORF usually encodes a protein.

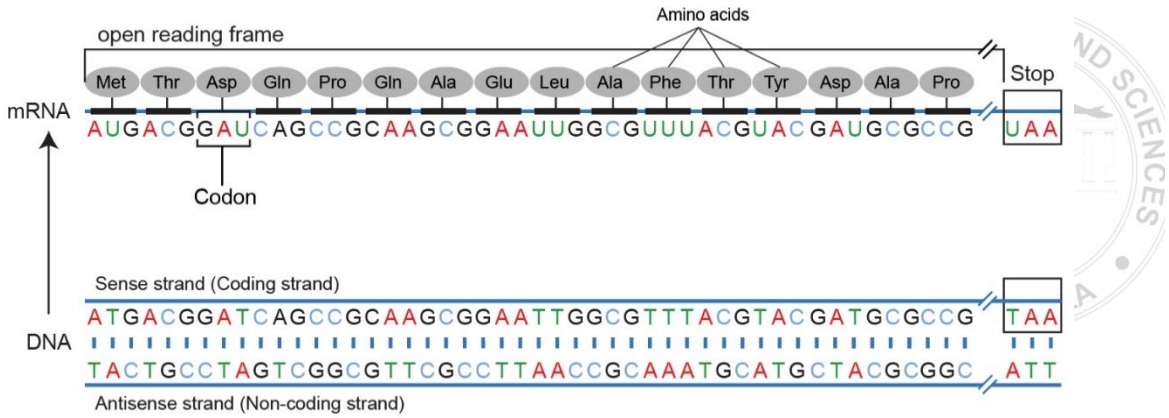


Figure 3: An open reading frame is a continuous stretch of codons that begins with a start codon (usually AUG) and ends at a stop codon (usually UAA, UAG or UGA).
From https://www.genome.gov/genetics-glossary/Open-Reading-Frame

The basics of translation are similar in prokaryotes and eukaryotes. Prokaryotic translation was first elucidated in *E. coli*, a representative prokaryote.

Components of translation

- mRNA – produced during transcription
- Ribosomes – cellular organelle made up of a protein-RNA complex
- tRNA – includes amino acid
- Elongation factors (EFs)
- Release factors (RFs)

Ribosomes

The site of protein synthesis is the ribosome, a complex macromolecular organelle that is made up of catalytic rRNAs (called ribozymes) and structural rRNAs, as well as many distinct polypeptides. It is quite abundant; an *E. coli* cell contains ~15,0000 ribosomes.

Ribosomes dissociate into large and small subunits when they are not synthesizing proteins, and reassociate during the **initiation of translation**. The intact ribosome in prokaryotes is known as the 70S ribosomes, and is made up of a 50S large subunit and a 30S small subunit, and several ribosomal RNAs, including 16S rRNA. Eukaryotic ribosomes are similar, but somewhat more complex in structure: the intact 80S ribosome has 60S and 40S subunits. The 60S subunit has three rRNAs: 28S, 5.8S, and 5S and 50 proteins. The 40S subunit has an 18S rRNA and 33 proteins.

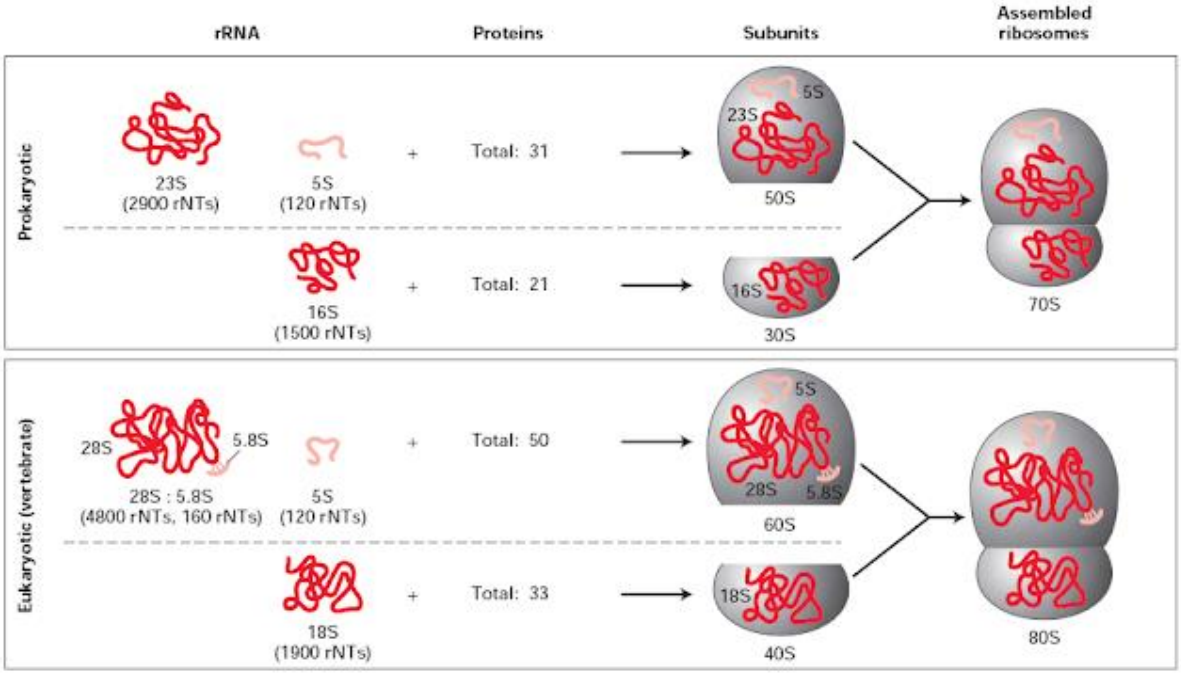
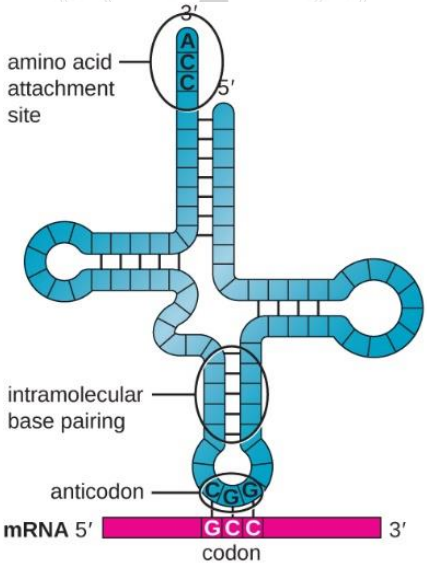


Figure 4. The composition of the prokaryotic and eukaryotic ribosomes. Both are composed of small and large protein subunits, plus a variety of RNAs, that combine to form the intact ribosome.

Transfer RNAs



Transfer RNA (tRNA) is the key link between transcribing RNA and translating that RNA into protein. The tRNA specific for a given amino acid matches up with a codon specifying that amino acid in the mRNA via complementary basepairing with its anticodon (Fig.), and adds the corresponding amino acid to the polypeptide chain.

Figure 5. After folding caused by intramolecular base pairing, a tRNA molecule has one end that contains the anticodon, which interacts with the mRNA codon, and the CCA amino acid binding end. From https://openstax.org/books/microbiology/pages/11-4-protein-synthesis-translation

A single tRNA can base pair with more than one codon. This is due to the fact that codon – anticodon interactions do not always strictly follow classic Watson – crick basepairing. The 5' base of the anticodon is referred to as the "wobble" base, and can form non – standard H-bonds with the 3' end of the mRNA codon. For example, U may basepair not only with an A but also with a G. The anticodon may also contain non – standard bases, such as inosine, which can form H-bonds with multiple bases. (Fig. 4).

Base-Pairing Combinations	
Base at 5′ End of Anticodon	Base at 3′ End of Codon
I*	A, C, or U
G	C or U
U	A or G
A	U
C	G

* I = hypoxanthine.

Note that there are no variations in base pairing when the wobble position is occupied by A or C.

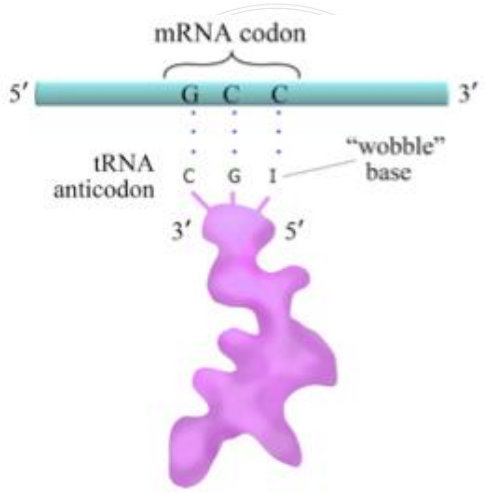


Figure 6. Base-pairing combinations between the 5' base of the anticodon and the 3' end of the codon.

An amino acid is added to the end of a tRNA molecule through the process of tRNA "charging," during which each tRNA molecule is linked to its correct **amino acid** by **aminoacyl tRNA synthetase**s. At least one type of aminoacyl tRNA synthetase exists for each amino acid. During this process, the amino acid is first activated by the addition of adenosine monophosphate (AMP) and then transferred to the tRNA, making it a **charged tRNA**, and AMP is released. Once the tRNA is charged, a ribosome can transfer the amino acid from the tRNA onto a growing peptide

$$\text{Amino acid} + \text{ATP} \rightarrow \text{aminoacyl-AMP} + \text{PP}_i$$
$$\text{Aminoacyl-AMP} + \text{tRNA} \rightarrow \text{aminoacyl-tRNA} + \text{AMP}$$
$$\text{Amino acid} + \text{ATP} + \text{tRNA} \rightarrow \text{aminoacyl-tRNA} + \text{AMP} + \text{PP}_i$$

Figure 7. The tRNA charging reaction catalyzed by aminoacyl tRNA synthetase.

Initiation of Translation

The initiation of prokaryotic translation begins with the assembly of the ribosome complex. First, three initiation factor proteins (IF1, IF2, and IF3) bind to the small subunit of the ribosome. This preinitiation complex and a methionine-carrying tRNA then bind to the mRNA, near the AUG start codon, forming the initiation complexIn prokaryotes, the assembled ribosome binds to specific sequences upstream of AUG codon.

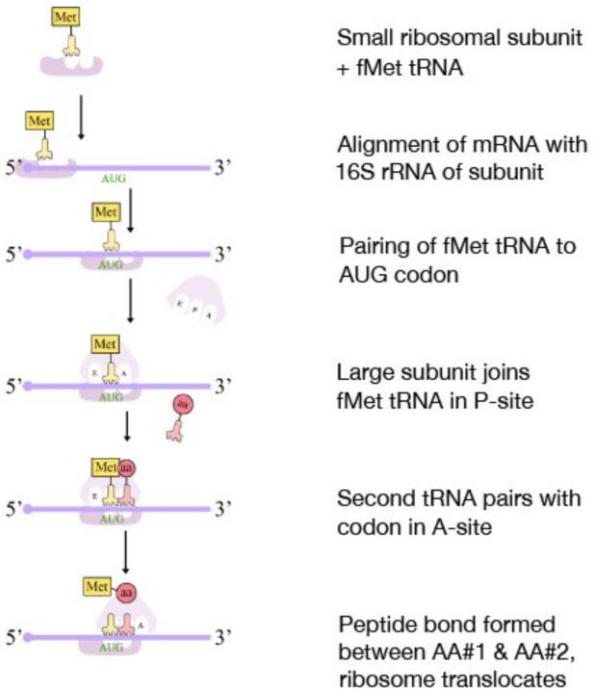


Figure 8. Assembly of the prokaryotic ribosomal complex. From https://biochem.oregonstate.edu/node/392

The specificity of the ribosome – mRNA interaction is due to base-pairing interactions between the 16S rRNA base-pairs and a 8-nt sequence called the ribosome binding sequence (RBS), also known as the Shine-Dalgarno sequence. This is centered ~10 bases upstream of the start codon (AUG) in prokaryotes.

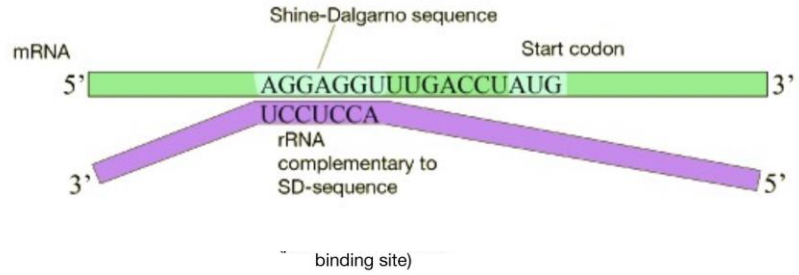


Figure 9. The ribosome binding site (RBS), also known as the Shine-Dalgarno sequence, basepairs with 16S rRNA.

Translation commences from the AUG codon that is the nearest downstream from the RBS. The initiator tRNA interacts with this AUG is unique in that it carries a formylated methionine (fMet). However, AUG codons downstream of the first one will be recognized by tRNA charged with standard methionine.

In eukaryotes, initiation complex formation is similar, with the following differences:

- The tRNA for both the first methionine codon and any other ones downstream carry regular methionine, called Met-tRNAi
- Instead of binding to the mRNA at the RBS, the eukaryotic initiation complex recognizes the 5′ cap of the eukaryotic mRNA, then scans the mRNA in the 5′ to 3′ direction until it finds an AUG start codon, at which point, the 60S subunit binds to the complex of Met-tRNAi, mRNA, and the 40S subunit.
- Most eukaryotic mRNAs start being translated at the first AUG from the 5′ cap.

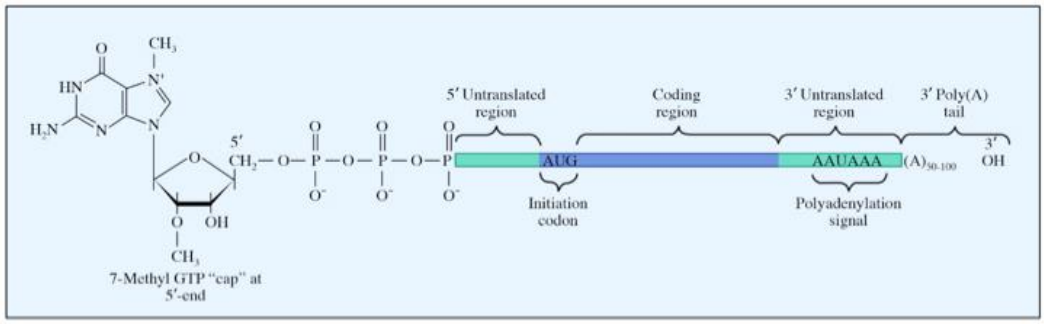


Figure 10. The eukaryotic ribosome loads at the 5′ cap of a eukaryotic mRNA and slides towards the 3′ end, scanning for the first AUG codon, from which it will initiate transcription. From https://biochem.oregonstate.edu/node/392

Although methionine (Met) is the first amino acid incorporated into any new protein in both prokaryotes and eukaryotes, it is not always the first amino acid in mature proteins. In many proteins, methionine is removed after translation.

In the translation complex formed, the tRNA binding region of the ribosome consists of three compartments:

1. A (aminoacyl) site – binds incoming charged aminoacyl tRNAs.

2. P (peptidyl) site – binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA.
3. E (exit) site releases dissociated tRNAs so that they can be recharged with free amino acids.

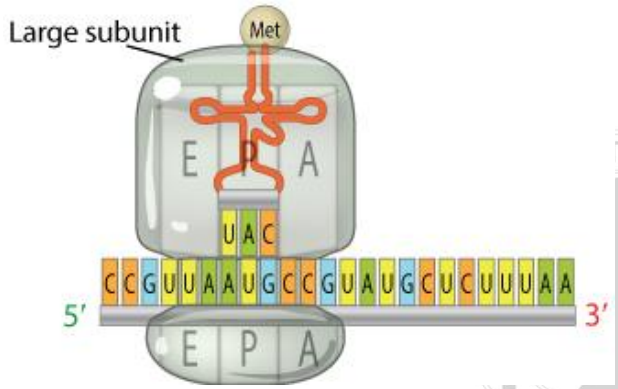


Figure 11. The large ribosomal subunit binds to the small ribosomal subunit to complete the initiation complex. The initiator tRNA molecule, carrying the methionine amino acid that will serve as the first amino acid of the polypeptide chain, is bound to the P site on the ribosome. The A site is aligned with the next codon, which will be bound by the anticodon of the next incoming tRNA. © 2013 Nature Education

The initiating methionyl-tRNA occupies the P site at the beginning of the elongation phase of translation in both prokaryotes and eukaryotes.

Elongation of the Polypeptide Chain

In both prokaryotes and eukaryotes, polypeptide chain growth requires additional protein factors called elongation factors (EFs). A peptide bond is formed between amino acids in the A and P sites. Another elongation factor is involved in translocation of the mRNA relative to the ribosome after the peptide bond is formed.

The elongation phase steps are as follows:

1. The ribosome moves along the mRNA in the 5'-to-3'direction
2. The tRNA that corresponds to the 2nd codon binds to the A site
   - ❑ in *E. coli,* requires elongation factors EF-Tu and EF-Ts, as well as GTP as an energy source
3. Upon binding of the tRNA-amino acid complex in the A site, GTP is cleaved to form GDP
   - ❑ released along with EF-Tu
4. Peptide bonds between the 1st and 2nd amino acids are formed by peptidyl transferase enzyme
5. After peptide bond is formed, the ribosome shifts, or translocates, again, and the amino acid-less tRNA tRNA now occupies the E site
6. The empty tRNA is released; the A site is now empty and ready to receive the tRNA for the next codon

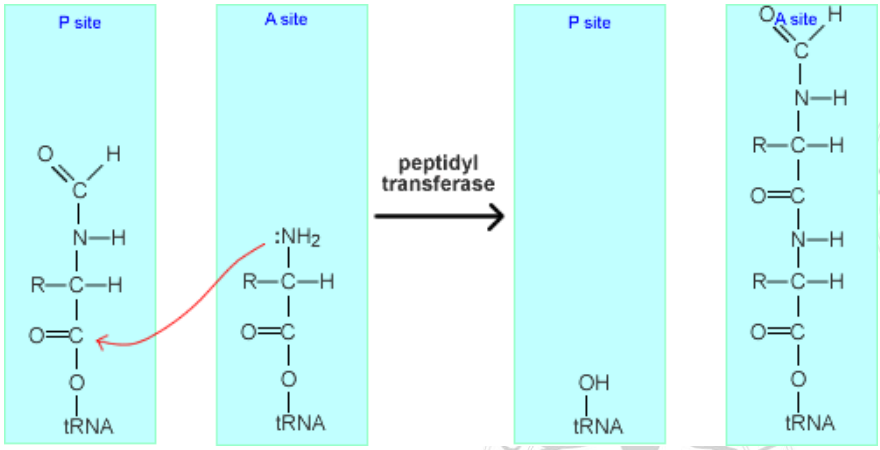


Figure 12. Peptidyl transferase catalyzes the formation of a peptide bond between amino acids in the P site and the A site.

This process is repeated until all the codons in the mRNA have been read by tRNA molecules, and the amino acids attached to the tRNAs have been linked together in the growing polypeptide chain in the appropriate order.

Termination of Protein Synthesis

Termination of translation occurs when the ribosome reaches a stop codon (UAA, UAG, or UGA). These codons do not have any corresponding tRNAs, but are recognized by *release factors* (RFs) that resemble tRNAs. The RFs facilitate the cleavage of the growing aminoacyl chain from its tRNA attachment, and the newly made protein is released. The small and large ribosomal subunits dissociate from the mRNA and are recruited almost immediately into another translation initiation complex.

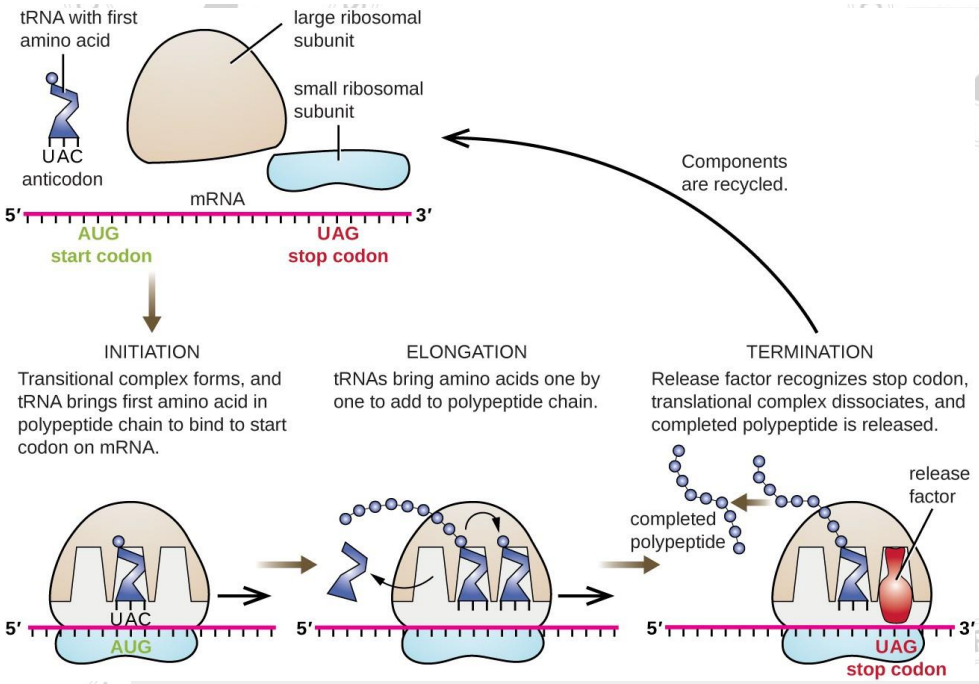


Figure 13. Translation in bacteria begins with the formation of the initiation complex, which includes the small ribosomal subunit, the mRNA, the initiator tRNA carrying N-formyl-methionine, and initiation factors. Then the 50S subunit binds, forming an intact ribosome. From https://courses.lumenlearning.com/microbiology/chapter/protein-synthesis-translation/

Each mRNA molecule is simultaneously translated by multiple ribosomes synthesizing protein in the same direction: reading the mRNA from 5' to 3' and synthesizing the polypeptide from

the N terminus to the C terminus. The complete structure containing an mRNA with multiple associated ribosomes is called a **polyribosome** (or **polysome**).

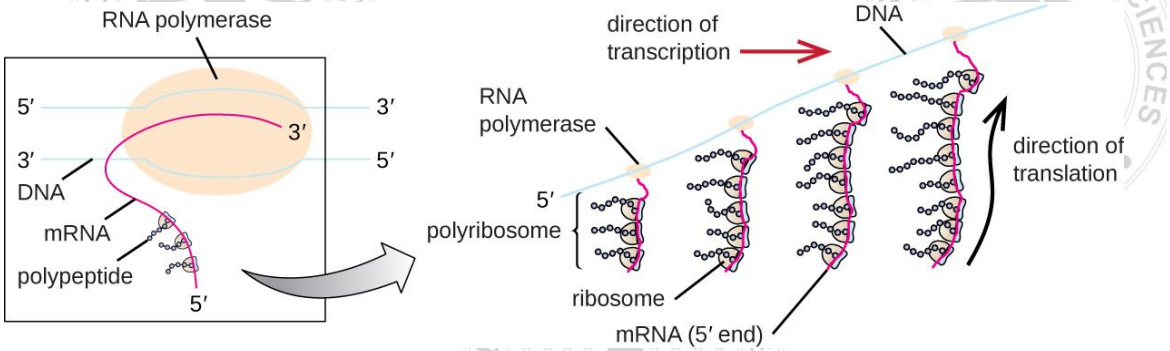


Figure 14. In prokaryotes, multiple RNA polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell. https://openstax.org/books/microbiology/pages/11-4-protein-synthesis-translation

In prokaryotes, the absence of organellar compartmentalization also means that transcription and translation can happen simultaneously.

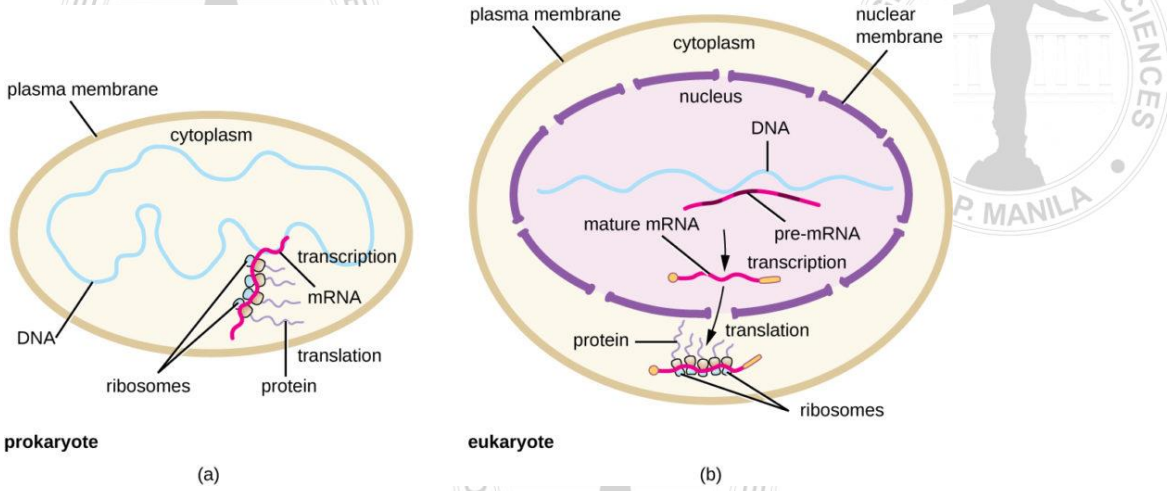


Figure 15. (a) In prokaryotes, the processes of transcription and translation occur simultaneously in the cytoplasm, allowing for a rapid cellular response to an environmental cue. (b) In eukaryotes, transcription is localized to the nucleus and translation is localized to the cytoplasm, separating these processes and necessitating RNA processing for stability.From https://courses.lumenlearning.com/wm-biology1/chapter/prokaryotic-translation/

Post-translational Modifications of Proteins

After a protein is synthesized, the protein may be further modified by cutting off parts of the polypeptide chain (at either end or internally) or by attaching fats (lipids), sugars (carbohydrates), or small chemical groups (such as phosphate). These modifications may be important for the action and stability of the protein, for its localization in the cell, or for signaling.

The cytoplasmic **ribosomes** found in animal cells (80S) are structurally distinct from those found in bacterial cells (70S), making protein biosynthesis a good selective target for antibacterial drugs. Several types of protein biosynthesis inhibitors are shown in Fig. 10.

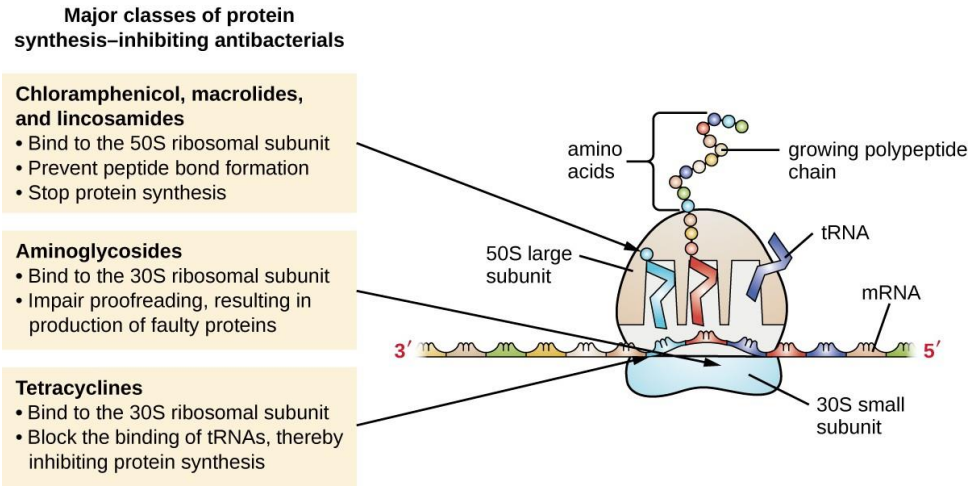


Figure 16. The major classes of protein synthesis inhibitors target the 30S or 50S subunits of cytoplasmic ribosomes. From https://courses.lumenlearning.com/microbiology/chapter/mechanisms-of-antibacterial-drugs/

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https://biochem.oregonstate.edu/node/392

https://courses.lumenlearning.com/wm-biology1/chapter/reading-steps-of-translation/

ADDITIONAL VIDEO RESOURCES

- How Translation Works HD Animation https://www.youtube.com/watch?v=0IiF6SIY7_4
- Translation Initiation HD Animation https://www.youtube.com/watch?v=ybtNK4pHi4o&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=7
- Aminoacyl tRNA synthetase HD Animation https://www.youtube.com/watch?v=igVWV8vzxYo&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=85