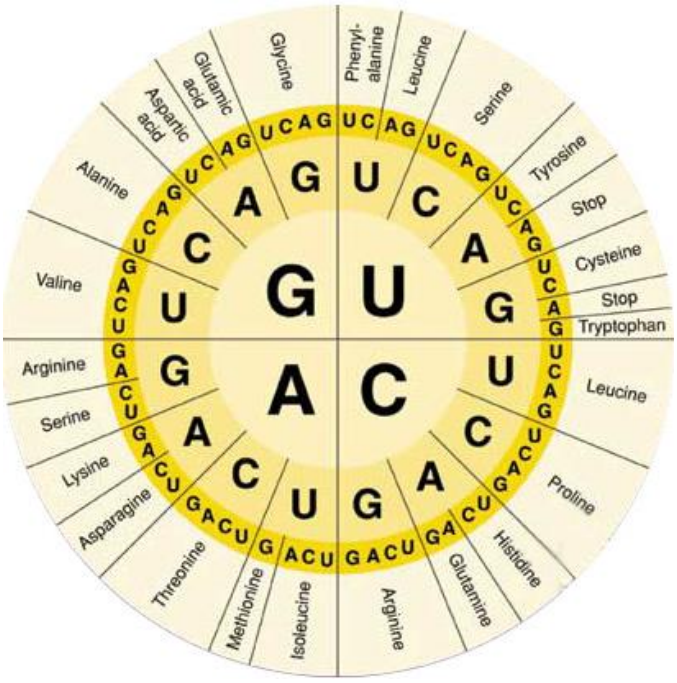
Translation

Genetic code

If genes are segments of DNA and if DNA is just a string of nucleotide pairs, then how does the sequence of nucleotide pairs dictate the sequence of amino acids in proteins?

Simple logic tells us that, if nucleotide pairs are the “letters” in a code, then a combination of letters can form “words” representing different amino acids. We must ask how the code is read. How many letters in the RNA make up a word, or codon, and which specific codon or codons represent each specific amino acid.

The logic is that the nucleotide code must be able to specify the placement of 20 amino acids. Since there are only four nucleotides, a code of single nucleotides would only represent four amino acids, such that A, C, G and U could be translated to encode amino acids. A doublet code could code for 16 amino acids (4 x 4). A triplet code could make a genetic code for 64 different combinations (4 X 4 X 4) genetic code and provide plenty of information in the DNA molecule to specify the placement of all 20 amino acids.



Also, this genetic code is redundant in nature. After the start and stop codons, the remaining 60 codons are far more than enough to code for the other 19 amino acids— and indeed there are repeats (Figure 1). Thus we say that the genetic code is redundant; that is, an amino acid may be represented by more than one codon.

Figure 1- Table of genetic code. Three letter code for amino acids.

Deciphering the genetic code:

In 1961, Marshall Nirenberg and Heinrich Matthaei mixed poly(U) with the protein synthesizing machinery of E. coli in vitro and observed the formation of a protein! The main excitement centered on the question of the amino acid sequence of this protein. It proved to be polyphenylalanine—a string of phenylalanine molecules attached to form a polypeptide. This clearly meant that "words" consisting purely of U somehow caused the incorporation of phenylalanine.

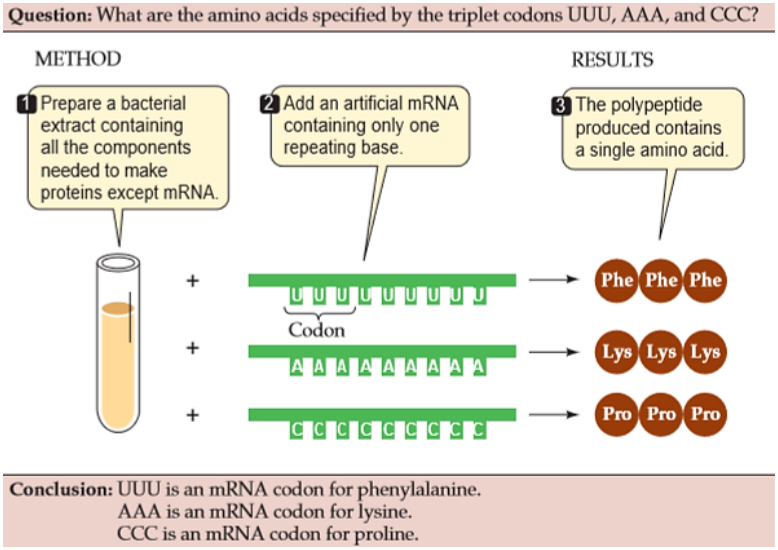


Figure 2- Nirenberg and Matthaei used a test-tube protein synthesis system to determine the amino acids specified by synthetic mRNAs of known codon composition. Image courtesy- Sadava et al, Life: The science of Biology, 7th edition.

H. Gobind Khorana then conceived and carried out the experiment that decisively revealed the nature of the genetic code. He synthesized artificial messages more complex than Nierenberg's and analyzed the resulting polypeptides. His data are shown below. "$(XY)_n$" means "XYXYXY …", and the resulting amino-acid couplet also repeats indefinitely (e.g., Ser-Leu-Ser-Leu-Ser-Leu ...).

Preparation for Translation: Linking RNAs, Amino Acids and Ribosomes

The translation of mRNA into proteins requires a molecule that links the information contained in mRNA codons with specific amino acids in proteins. That function is performed by tRNA. Two key events must take place to ensure that the protein made is the one specified by mRNA:

- tRNA must read mRNA correctly.
- tRNA must carry the amino acid that is correct for its reading of the mRNA.

Transfer RNAs carry specific amino acids and bind to specific codons

The codon in mRNA and the amino acid in a protein are related by way of an adapter—a specific tRNA with an attached amino acid. For each of the 20 amino acids, there is at least one specific type (species) of tRNA molecule.

The tRNA molecule has three functions:

It carries ("charged") an amino acid, it associates with mRNA molecules, and it interacts with ribosomes.

At the 3' end of every tRNA molecule is a site to which its specific amino acid binds covalently. The charging of each tRNA with its correct amino acid is achieved by a family of activating enzymes, known more formally as aminoacyl-tRNA synthetases At about the midpoint of tRNA is a group of three bases, called the anticodon that constitutes the site of complementary base pairing (hydrogen bonding) with mRNA. Each tRNA species has a unique anticodon, which is complementary to the mRNA codon for that tRNAs amino acid. At contact, the codon and the anticodon are antiparallel to each other. As an example of this process, consider the amino acid arginine:

- The DNA coding region for arginine is 3'-GCC-5', which is transcribed, by complementary base pairing, to the mRNA codon 5'-CGG-3'.
- That mRNA codon binds by complementary base pairing to a tRNA with the anticodon 3'-GCC-5', which is charged with arginine.

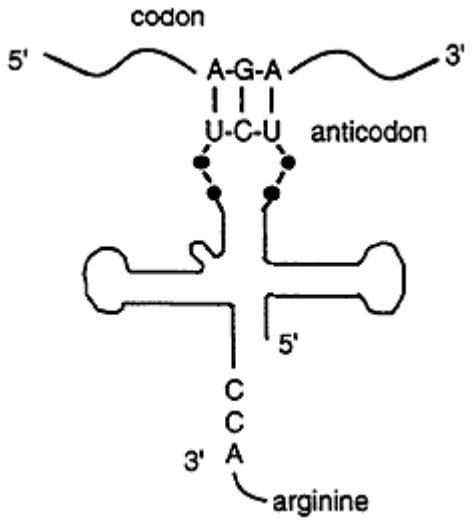


Figure 3- tRNA with a specific anticodon for arginine interacts with one arginine amino acid. This is called an activated tRNA. Activated tRNA specifically recognizes the codon on mRNA and produces hydrogen bonds at the site of anticodon-codon interaction. (Note the anticodon and codon are complementary to each other).

Ribosomes act as the workbench for translation:

Ribosomes are required for the translation of the genetic information in mRNA into a polypeptide chain. Each ribosome consists of two subunits, a large one and a small one. In eukaryotes, the large subunit consists of three different molecules of rRNA and about 45 different protein molecules, arranged in a precise pattern. The ribosomes of prokaryotes are somewhat smaller than those of eukaryotes, and their ribosomal proteins and RNAs are different.

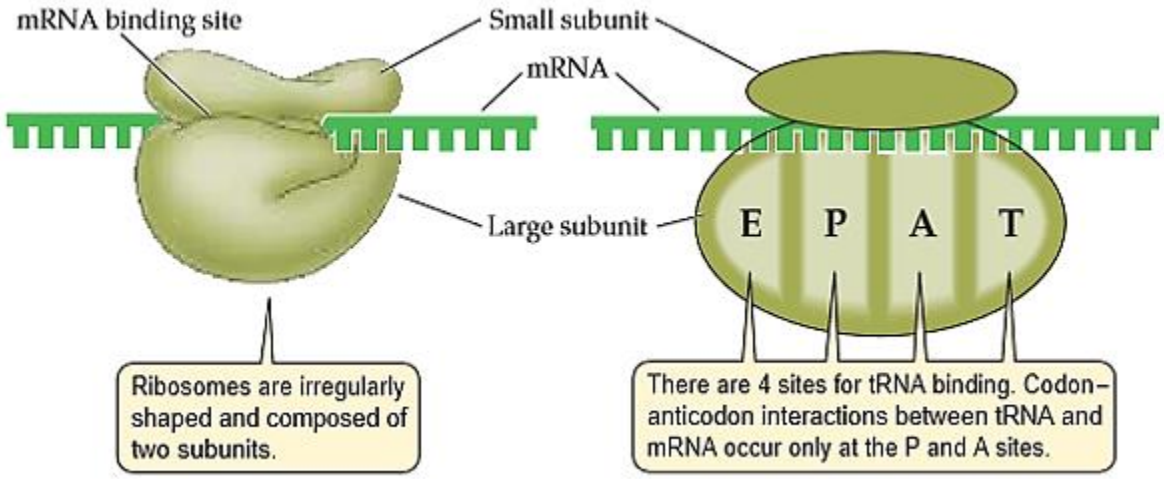


Figure 4- Ribosome Structure Each ribosome consists of a large and a small subunit. The subunits remain separate when they are not in use for protein synthesis. Its structure enables it to hold the mRNA and charged tRNAs in the right positions, thus allowing the growing polypeptide to be assembled efficiently. Image courtesy- Sadava et al, Life: The science of Biology, 7th edition.

A given ribosome does not specifically produce just one kind of protein. A ribosome can use any mRNA and all species of charged tRNAs, and thus can be used to make many different polypeptide products.

On the large subunit of the ribosome are four sites to which tRNA binds (Figure 4). A charged tRNA traverses these four sites:

- The T (transfer) site
- The A (amino acid) site
- The P (polypeptide) site
- The E (exit) site

An important role of the ribosome is to make sure that the mRNA–tRNA interactions are precise: that is, that a charged tRNA with the correct anticodon (e.g., 3'-UAC-5') binds to the appropriate codon in mRNA (e.g., 5'-AUG-3'). When this occurs, hydrogen bonds form between the base pairs. But these hydrogen bonds are not enough to hold the tRNA in place. The rRNA of the small ribosomal subunit plays a role in validating the three-base-pair match. If hydrogen bonds have not formed between all three base pairs, the tRNA must be the wrong one for that mRNA codon, and that tRNA is ejected from the ribosome.

Translation Process: RNA-Directed Polypeptide Synthesis

Like transcription, translation occurs in three steps: initiation, elongation, and termination.

Initiation:

The translation of mRNA begins with the formation of an initiation complex, which consists of a charged tRNA bearing what will be the first amino acid of the polypeptide chain and a small ribosomal subunit, both bound to the mRNA. The rRNA of the small ribosomal subunit binds to a complementary ribosome recognition sequence on the mRNA. This sequence is "upstream" (toward the 5' end) of the actual start codon that begins translation.

The mRNA start codon in the genetic code is AUG. The anticodon of a methionine charged tRNA binds to this start codon by complementary base pairing to form the initiation complex. Thus the first amino acid in the chain is always methionine.

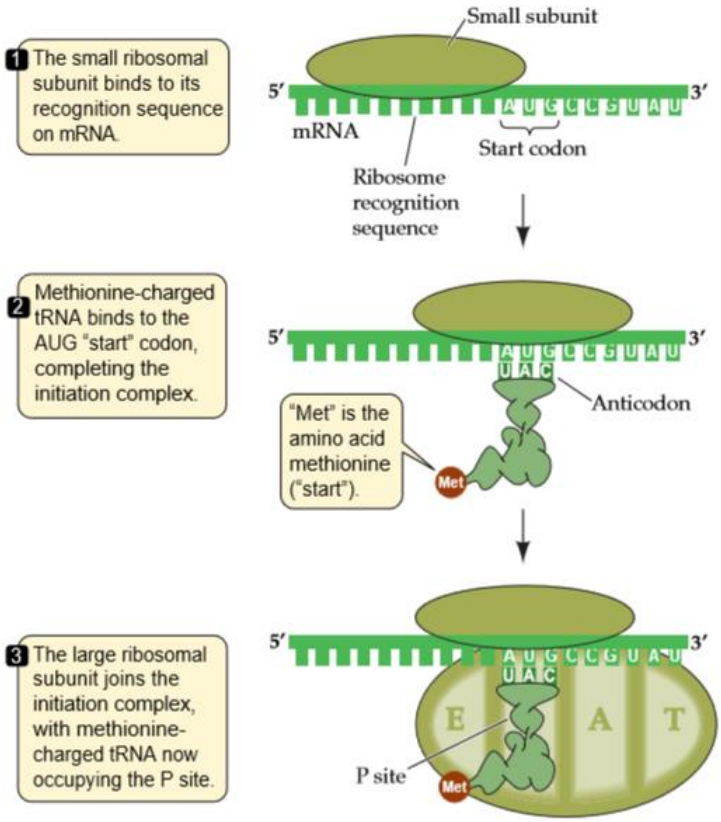


Figure 5- Initiation of translation begins with formation of an initiation complex (step 2). Image courtesy- Sadava et al, Life: The science of Biology, 7th edition.

Elongation:

The polypeptide elongates from the N terminus. A charged tRNA whose anticodon is complementary to the second codon on the mRNA now enters the open A site of the large ribosomal subunit. The large subunit then catalyzes two reactions:

- It breaks the bond between the tRNA in the P site and its amino acid.
- It catalyzes the formation of a peptide bond between that amino acid and the one attached to the tRNA in the A site.

Because the large subunit performs these two actions, it is said to have peptidyl transferase activity. In this way, methionine (the amino acid in the P site) becomes the N terminus of the new protein. The second amino acid is now bound to methionine, but remains attached to its tRNA by its carboxyl group (—COOH) in the A site.

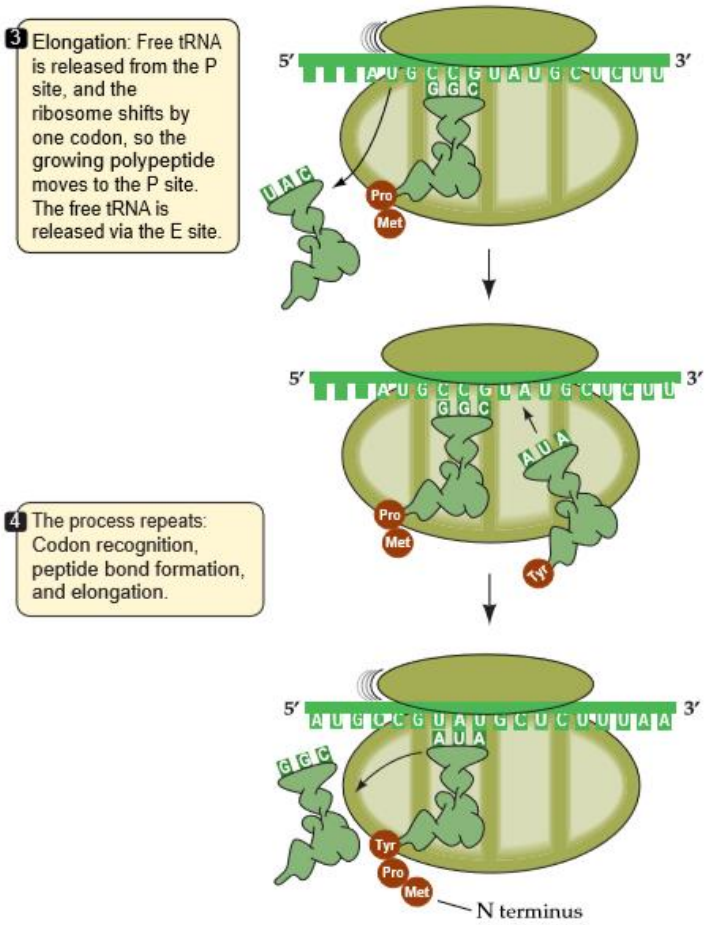


Figure 6- The incoming charged tRNA recognizes the specific codon on the mRNA and enters into the A site. Peptidyl transferase activity of the large subunit causes the transfer of amino acid on the first tRNA (at P site) on the second tRNA (at A site) and a peptide bond is formed between these amino acids. Image courtesy- Sadava et al, Life: The science of Biology, 7th edition.

After the first tRNA releases its methionine, it dissociates from the ribosome, returning to the cytosol to become charged with another methionine. The second tRNA, now bearing a dipeptide, is shifted to the P site as the ribosome moves one codon along the mRNA in the 5′-to-3′ direction. The elongation process continues, and the polypeptide chain grows, as the steps are repeated. Elongation factors assist the elongation of a polypeptide chain.

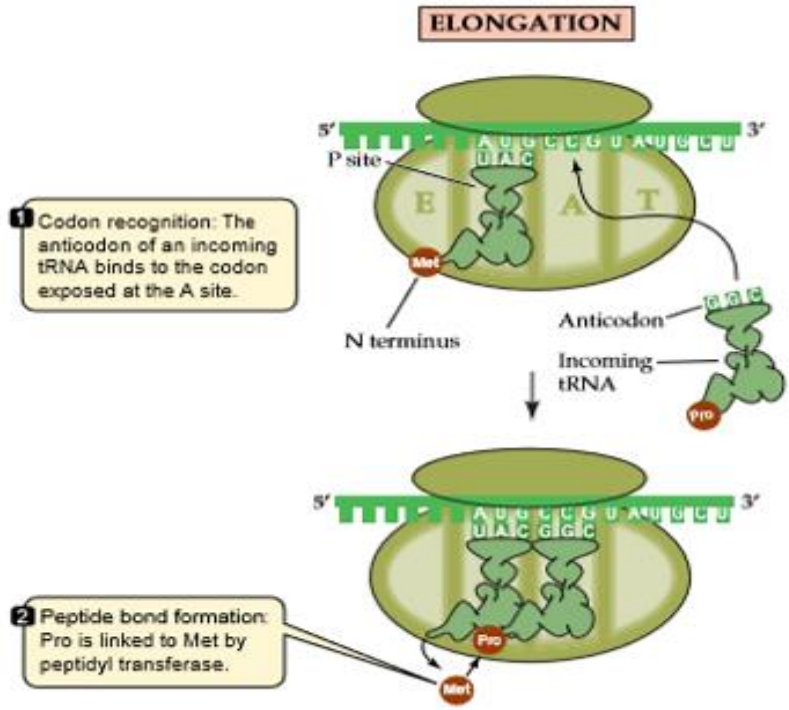


Figure 7- Elongation of polypeptide: (a) The next charged tRNA enters the open A site. (b) Its amino acid forms a peptide bond with the amino acid chain in the P site, so that it picks up the growing polypeptide chain from the tRNA in the P site. (c) The tRNA in the P site. Image courtesy- Sadava et al, Life: The science of Biology, 7th edition.

Termination:

The elongation cycle ends, and translation is terminated, when a stop codon—UAA, UAG, or UGA—enters the A site. These codons encode no amino acids, nor they bind tRNAs. Rather, they bind a protein release factor, which hydrolyzes the bond between the polypeptide and the tRNA in the P site. The newly completed protein thereupon separates from the ribosome. Its C terminus is the last amino acid to join the chain. Its N terminus, at least initially, is methionine, as a consequence of the AUG start codon. In its amino acid sequence, it contains information specifying its conformation, as well as its ultimate cellular destination.

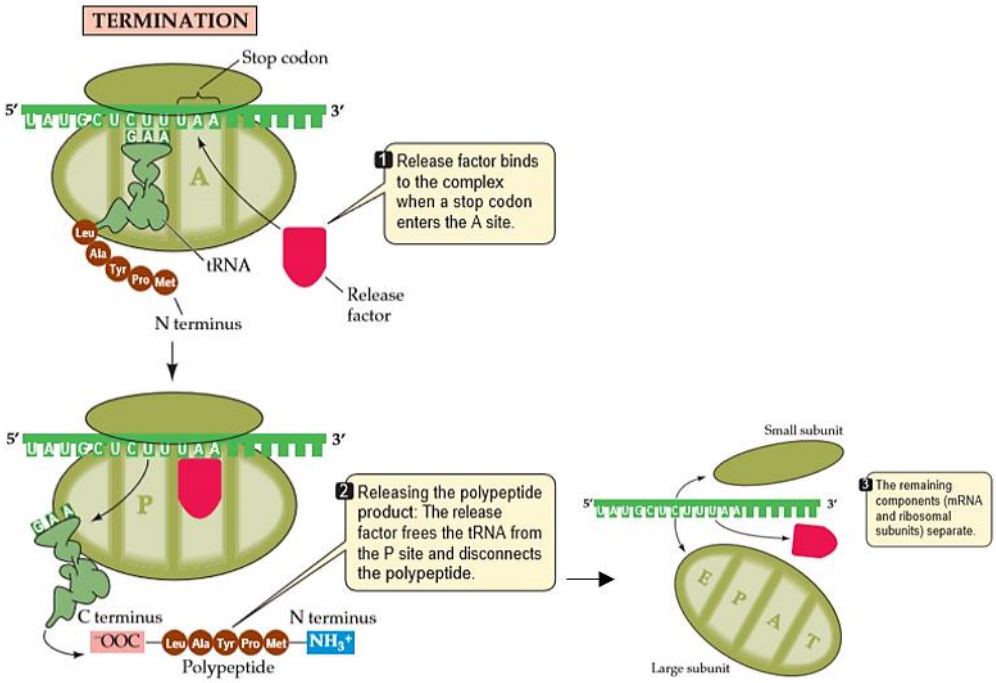


Figure 8- Events of termination of polypeptide chain. Image courtesy- Sadava et al, Life: The science of Biology, 7th edition.

	TRANSCRIPTION	TRANSLATION
Initiation	Promoter sequence in DNA	AUG start codon in mRNA
Termination	Terminator sequence in DNA	UAA, UAG, or UGA stop codon in mRNA

Figure 9- Signals that start and stop transcription and Translation. These act as a boundary for the mRNA and protein synthesis. Image courtesy- Sadava et al, Life: The science of Biology, 7th edition.