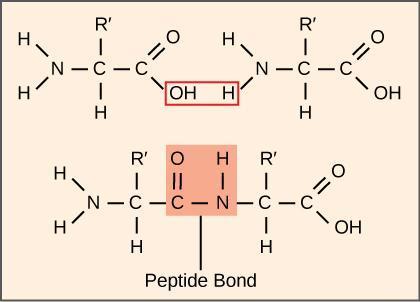
Chapter 4: Translation

We will learn about all of the different molecules, mostly RNAs, and a couple of proteins that are important players in translation. Interestingly, the fact that so many RNA molecules play such central roles in the conversion of the genetic information, DNA, into the functional workhorses of the cell, proteins, is one of the supporting pieces of evidence that RNAs were likely the first significant biomolecule of life. The fact that RNAs remain as an intermediary in the processes of transcription and translation is likely due to its ancient and central role as both genetic material and life’s first enzymes (or as we more commonly refer to catalytic RNAs as ribozymes).

## Protein Synthesis

The process of translation, or protein synthesis, involves the decoding of an mRNA message into a polypeptide product. Amino acids are covalently strung together by interlinking peptide bonds. Polypeptides are formed when the amino group of one amino acid forms a covalent peptide bond with the carboxyl group of another amino acid (Figure 4-1).



**Figure 4-1: Peptide bond formation**. A peptide bond is formed after a dehydration synthesis/condensation reaction links two individual amino acids together via a covalent peptide bond.

In addition to the mRNA template, many molecules and macromolecules contribute to the process of translation. The composition of each component may vary across species; for instance, ribosomes may consist of different numbers of rRNAs (ribosomal RNAs) and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs (transfer RNAs), and various enzymatic factors.

## mRNA

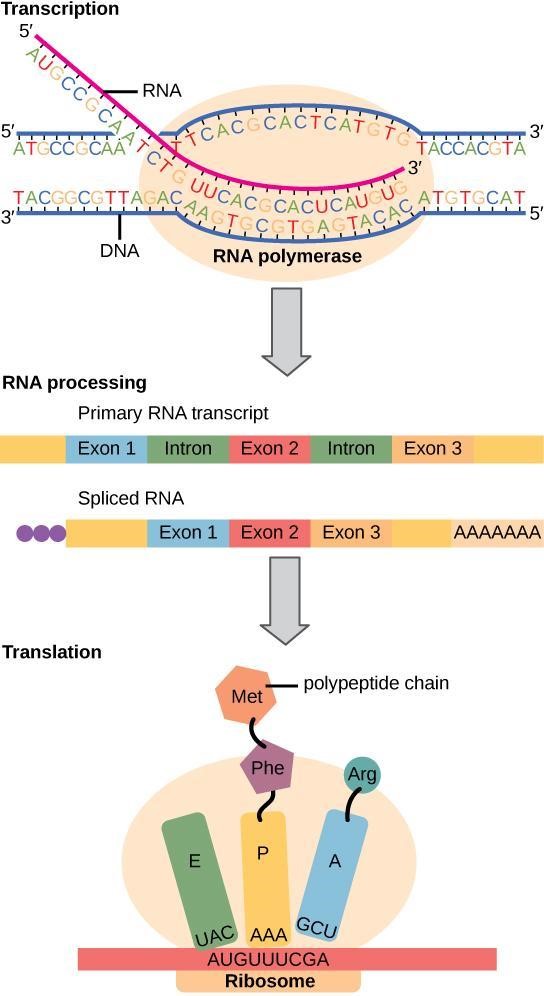
As previously discussed, a fully processed, mature mRNA can be exported from the nucleus to undergo translation in the cytoplasm (Fig 4-2).



**Figure 4-2: A fully processed mRNA transcript.** After all RNA modifications have been completed, a mature mRNA contains a 5’ cap, a poly-A tail, and untranslated regions (UTRs) at the 5’ and 3’ ends.

**mRNA Codons**

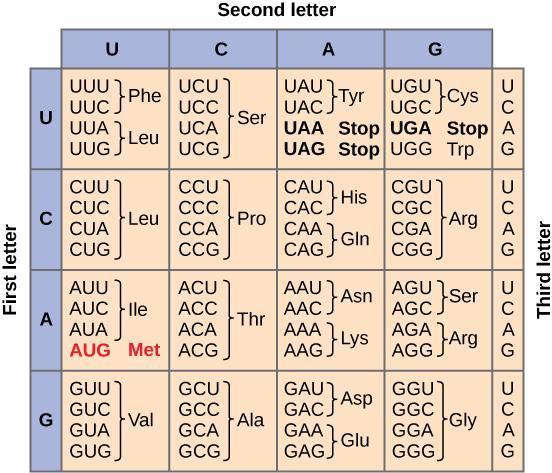
The copying of DNA to RNA is relatively straightforward, with one nucleotide being added to the mRNA strand for every nucleotide read in the DNA strand. The translation to protein is a bit more complicated because three mRNA nucleotides correspond to one amino acid in the polypeptide sequence. A group of three mRNA nucleotides that code for an amino acid is called a **codon**. Figure 4-3 shows the overall process of transcription and translation, demonstrating how one codon can code for an amino acid, using a tRNA to recognize the codon and deliver the correct amino acid to the ribosome.



**Figure 4-3: Overview of transcription and translation.** The image shows the transcription, post-transcriptional processing, and translation of a gene. Note that a codon (a sequence of three nucleotides within an mRNA molecule) codes for a single amino acid.

**The Genetic Code is Degenerate and Universal**

There are 64 possible nucleotide triplets (43), which is far more than the number of amino acids. Scientists theorized that nucleotide triplets encoded amino acids, and that the genetic code was **degenerate**. In other words, a given amino acid could be encoded by more than one nucleotide triplet. These nucleotide triplets are called codons. Scientists painstakingly solved the genetic code by translating synthetic mRNAs in vitro and sequencing the proteins they specified, the result of this work is called a **codon table** (Fig 4-4). Codon tables list each possible codon (in the 5’ to 3’ direction) and if they code for an amino acid or represent a stop codon (discussed below).

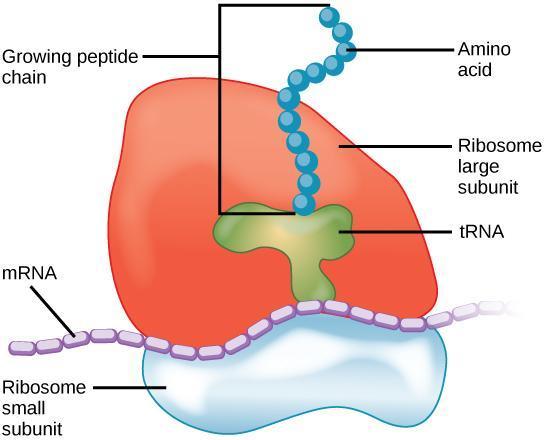


**Figure 4-4: Codon table.** The codon table listing all possible codons and the amino acids that they code for.

In addition to instructing the addition of a specific amino acid to a polypeptide chain, three of the 64 codons terminate protein synthesis and release the polypeptide from the translation machinery. These triplets are called **stop codons**. Another codon, AUG, also has a unique function. In addition to specifying the amino acid methionine, it also serves as the **start codon** to initiate translation. The AUG start codon sets the reading frame for translation near the 5' end of the mRNA.

## Ribosomes

A **ribosome** is a complex macromolecule composed of structural and catalytic ribosomal RNAs (rRNAs) and many distinct polypeptides. The rRNAs in the ribosome are involved in the formation of the peptide bonds. They are called **ribozymes** because the RNA is catalyzing the covalent bond, not a protein enzyme. In eukaryotes, the nucleolus is a location in the nucleus specialized for the synthesis and assembly of rRNAs. Ribosomes exist in the cytoplasm and may be moved to the surface of the rough endoplasmic reticulum in eukaryotes during the translation of specific types of proteins. Ribosomes dissociate into large and small subunits when they are not synthesizing proteins and re-associate during the initiation of translation. Mammalian ribosomes have a small 40S subunit and a large 60S subunit, for a total of 80S. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs that deliver the correct amino acid to the growing polypeptide chain (Fig 4-5). Each mRNA molecule is simultaneously translated by many ribosomes, all 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 resulting mRNA and all of the attached ribosomes is called a **polyribosome**.

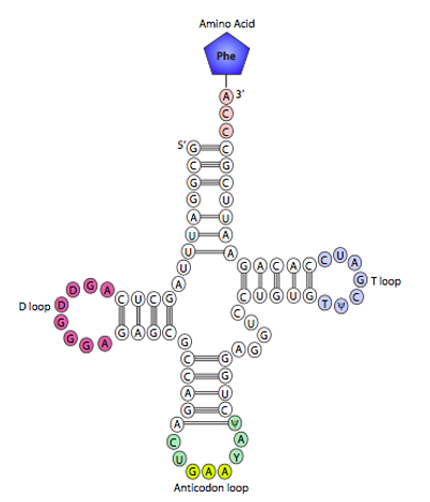


**Figure 4-5: Translation machinery**. Ribosomes form peptide bonds between amino acids during translation. Ribosomes are made of large and small subunits and “sandwich” the mRNA molecule. The large subunit has binding pockets for tRNA molecules.

**Transfer RNA (tRNA)**

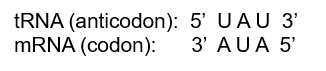
The tRNAs are structural RNA molecules that were transcribed from genes by RNA polymerase III. tRNAs exist in the cytoplasm. tRNAs bind to specific codon sequences on the mRNA template and deliver the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually “translate” the language of RNA into the language of proteins.

Although RNA is similar to DNA on many counts, it is single-stranded, and that property, combined with the opportunity for complementary base pairing within a strand, allows it to do something far different than double-stranded DNA: it can form highly complex secondary structures. One of the simplest and clearest examples of this is tRNA, which depends on its conformation to accomplish its cellular function. The prototypical cloverleaf-form of a tRNA is shown in Figure 4-6. As you can see, the fully-splayed-out shape has four stem-loop “arms” with the amino acid attached to the acceptor arm. This is on the opposite side of the tRNA from the anticodon arm, which is where the **anticodon** on the tRNA must match with the mRNA codon during translation, In general terms. The arms are used to properly position the tRNA within the ribosome as well as recognizing the mRNA codon and bringing in the correct amino acid.



**Figure 4-6: tRNA structure.** tRNA molecules have a cloverleaf structure with the anticodon loop on one end and the amino acid linked to the 3’ end of the tRNA.

When it comes time for the tRNA to match its anticodon with the codon on the mRNA, following the convention of nucleic acid sequences, the mRNA sequence is always written 5’ to 3’, even though in the case of codon-anticodon matching, the strands of mRNA and tRNA are antiparallel (Fig 4-7).

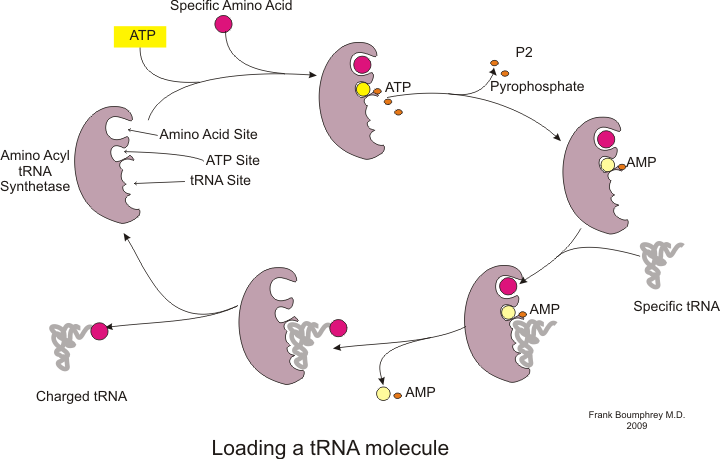


**Figure 4-7: Codon and anti-codon interactions.** Nucleotides on the anticodon loop of the tRNA bind non-covalently to the codon on the mRNA, resulting in nucleotides from each molecule that are antiparallel and complementary upon binding.

Of the 64 possible mRNA codons—or triplet combinations of A, U, G, and C—three specify the termination of protein synthesis, and 61 determine the addition of amino acids to the polypeptide chain. Of these 61, one codon (AUG) is the start codon and is responsible for the initiation of translation. Each tRNA anticodon can base pair with one of the mRNA codons and add an amino acid or terminate translation, according to the genetic code. For instance, if the sequence CUA occurred on an mRNA template in the proper reading frame, it would bind a tRNA expressing the complementary sequence, GAU, which would be linked to the amino acid leucine.

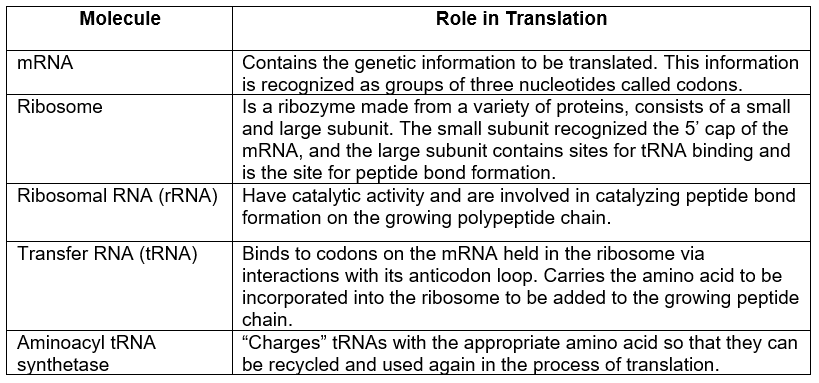
## Charging the tRNA

The knowledge of the genetic code begs the question: how is the correct amino acid attached to any given tRNA? A class of enzymes called the **aminoacyl tRNA synthetases**are responsible for recognizing both a specific tRNA and a specific amino acid, binding an ATP for energy and then joining them together, the addition of an amino acid to the tRNA molecule is called "**charging**” the tRNA. Charging a tRNA with its amino acid requires energy. The aminoacyl tRNA synthetase first binds a molecule of ATP and the appropriate amino acid. These enzymes first bind and hydrolyze ATP to catalyze a high-energy bond between an amino acid and adenosine monophosphate (AMP). The activated amino acid is then transferred to the bound tRNA, and AMP is released (Fig 4-8).



**Figure 4-8: Charging of the tRNA.** The animoacyl tRNA synthetase used the energy supplied by ATP to charge the tRNA with the correct amino acid so that it can be used again in the process of translation.

In summary, Table 4-1 shows the various players involved in translation and their individual roles in the process.

**Table 4-1: Players in translation.**

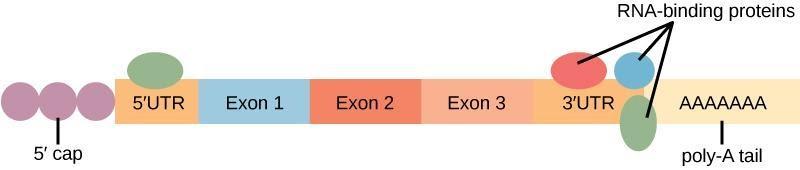
# Translation is how mRNA is converted to protein

In the previous section, we learned about all of the different molecules, mostly RNAs, and a couple of proteins that are important players in translation. Interestingly, the fact that so many RNA molecules play such central roles in the conversion of the genetic information, DNA, into the functional workhorses of the cell, proteins, is one of the supporting pieces of evidence that RNAs were likely the first significant biomolecule of life. The fact that RNAs remain as an intermediary in the processes of transcription and translation is likely due to its ancient and central role as both genetic material and life’s first enzymes (or as we more commonly reference catalytic RNAs, ribozymes).

In Eukaryotes, mature mRNA molecules are exported from the nucleus through the nuclear pore and enter the cytoplasm. Ribosomes that are floating in the cytoplasm then recognize the 5’-cap, find the start codon, and begin translation using charged tRNA molecules. Some proteins need to be modified within the endoplasmic reticulum and are thus quickly moved by the ribosome and other associated proteins to the ER. More on this process can be found in chapter 9. This chapter details the process of translation. We will begin with a quick discussion about two regions of the mRNA that are very important for mRNA stability and the process of translation but are not, themselves, translated.

## The Untranslated Regions of mature mRNA

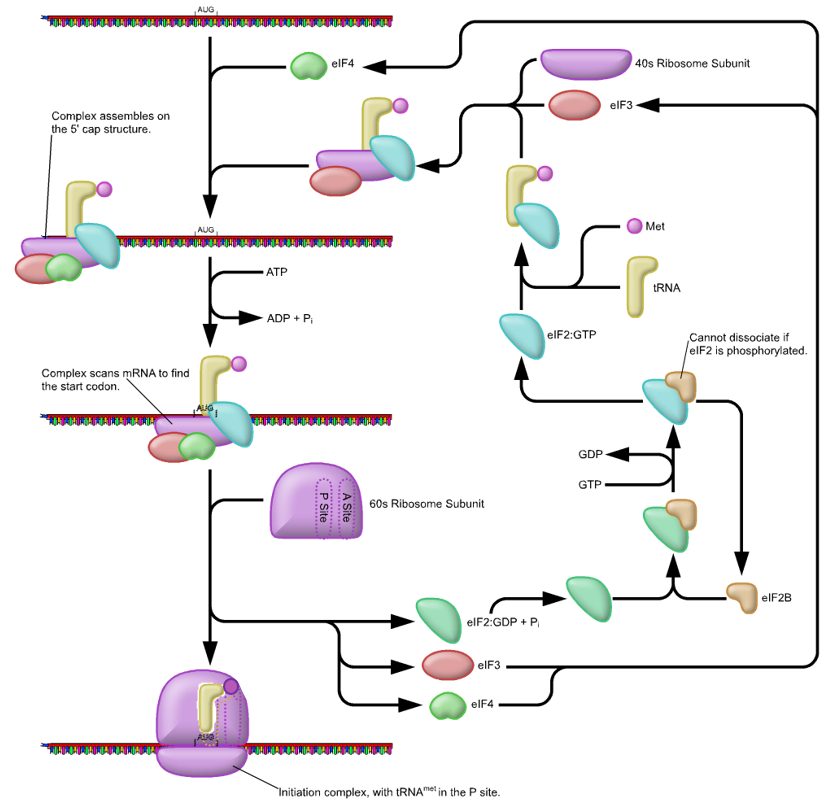
Two regions within the mRNA are not translated introns and the very ends of the mRNA are termed the 5’ and 3’-untranslated regions (UTR). Introns were removed in the nucleus when RNA modifications were completed prior to export of a mature mRNA. The 5’-UTR includes the 5’-cap at the most 5’ end of the mRNA until the nucleotide before the A of the AUG in the start codon (Figure 4-9). The 5'-UTR is important in translation initiation, you can think of it as a landing pad for the ribosome. Additionally, proteins called RNA-binding proteins, or RBPs, can bind to the 5’ and 3'-UTRs. The binding of RBPs to these regions can increase or decrease the stability of an RNA molecule, depending on the specific RBP that binds. Thereby regulating the length of time the mRNA persists in the cell. If the RBPs increase the mRNA’s stability, then it will persist longer in the cell and likely be translated more (making more protein). Alternatively, if it decreases its stability, then the mRNA will be degraded sooner and likely be used fewer times and produce less protein. The 3’-UTR contains the sequence necessary for adding the poly A tail, and therefore, it is important in transcription termination (chapter 3). The 3’-UTR starts right after the stop codon and includes the subsequent nucleotides until the 3’ end of the mRNA molecule. The 3’-UTR is often a site for translation regulation. Special RNAs called microRNAs and regulatory proteins called repressors can bind to an mRNA’s 3’-UTR and regulate the amount of translation that will occur with a particular mRNA.



**Figure 4-9: RNA-binding proteins.** The protein-coding region of this processed mRNA is flanked by 5' and 3' untranslated regions (UTRs). The presence of RNA-binding proteins at the 5' or 3' UTR influences the stability of the RNA molecule.

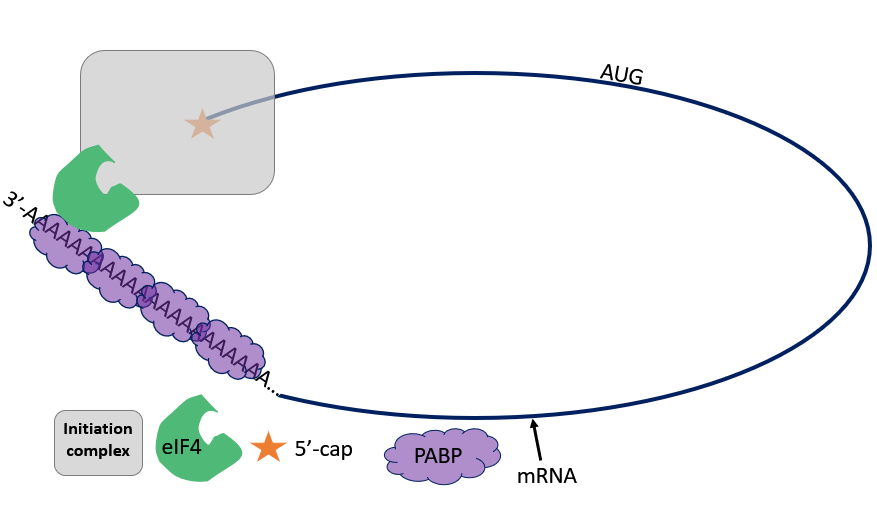
## Translation initiation

The initiation of translation usually involves the interaction of certain key proteins, initiation factors, with the 5'- cap, as well as the 5' UTR (Figure 4-10). Some initiation factors bind the small (40S) ribosomal subunit and hold the mRNA in place. Initiation factors such as eIF1, eIF3, and eIF6 (e=eukaryotic; IF=initiation factor) are associated with the 40S ribosomal subunit and play a role in keeping the large (60S) ribosomal subunit from binding too soon. eIF3 also interacts with the eIF4F complex, which associates with the 5’-cap. This 43S preinitiation complex along with the protein factors moves along the mRNA toward its 3'-end, in a process known as 'scanning', to reach the start codon (typically AUG). The start codon is at the beginning of the first exon. In eukaryotes, the amino acid encoded by the start codon is methionine. The Met-charged initiator tRNA (Met-tRNAiMet) is brought to the P-site of the small ribosomal subunit by eIF2. eIF2 hydrolyzes GTP, and signals for the dissociation of several initiation factors from the small ribosomal subunit, leading to the binding of the large (60S) subunit. The complete ribosome (80S) then commences translation elongation.



**Figure 4-10: Initiation of translation in eukaryotes.** A number of proteins, including the small subunit of the ribosome, and the initiator tRNA (Met) assemble at the 5’ cap of the mRNA. They then ‘scan’ the mRNA for the first AUG. The large subunit of the ribosome binds with the small subunit and the initiation factors are released. The first tRNA is bound in the P-site leaving the A-site open for the next tRNA to bind.

It is interesting to note that the poly(A)-binding protein (PABP) also associates with the eIF4F complex and binds the poly-A tail of most eukaryotic mRNA molecules. The interaction between PABP and eIF4F is thought to play a role in circularization of the mRNA during translation, which ultimately allows an increase in translation efficiency (Figure 4-11).



**Figure 4-11: Interaction between eIF4 and PABP.** eIF4 (green) is a member of the initiation complex (simplified and shown in grey) in translation that is associated with the 5’-cap (orange star). It has interactions with the Poly A Binding Protein (PABP-shown in purple) and helps to circularize the mRNA. This is thought to speed up the time between translation termination and re-initiation.

## Translation Elongation

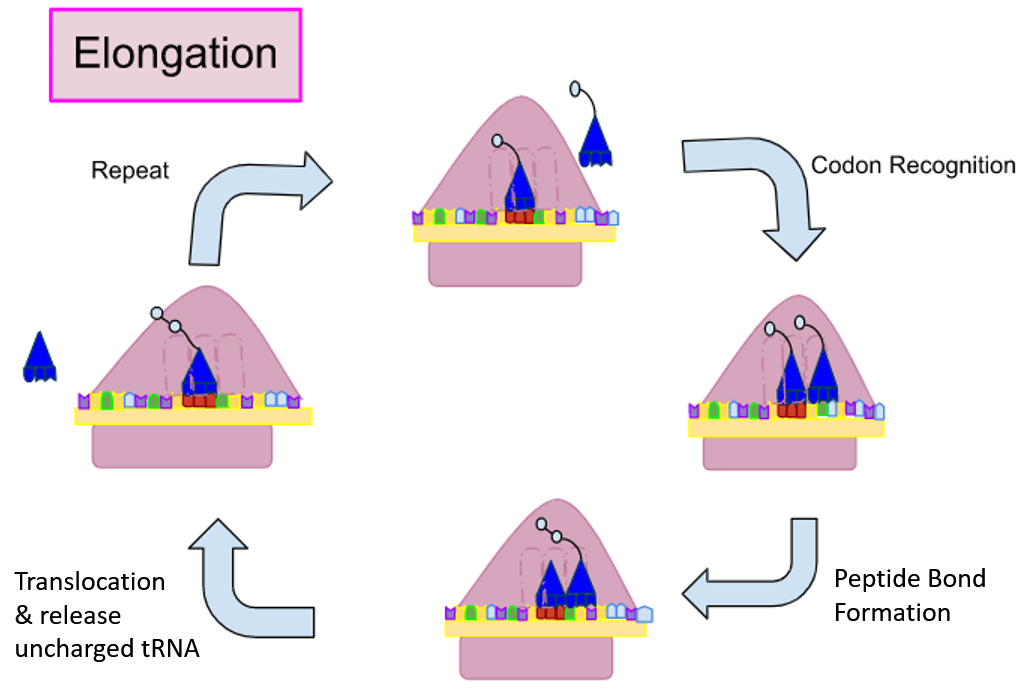
Remember that translation occurs in the ribosome and the ribosome consists of ribosomal RNA (rRNA) and proteins. Interestingly and importantly, it is the rRNA that is doing the catalysis of translation. The proteins of the ribosome are ONLY serving to help stabilize the structure of the ribosome and do NOT catalyze any part of translation! Scientists like to talk about the compartments of the ribosome because each “compartment” has a unique function. The intact ribosome has three compartments: the A-site binds incoming aminoacyl tRNAs; the P-site (location of the peptidyl transferase activity) binds tRNAs carrying the growing polypeptide chain (this is where the peptide bond is formed); the E-site releases dissociated, uncharged tRNAs so that they can be recharged with amino acids by the aminoacyl tRNA synthetase.

The first tRNA (tRNAiMet) begins in the P-site of the ribosome, and this creates an initiation complex with a free A-site ready to accept the next aminoacyl-tRNA (aka ‘charged’ tRNA) that matches the first codon after the start codon. Every new, charged tRNA after the initiator tRNA enters the ribosome through the A-site.

The aminoacyl-tRNA with an anticodon complementary to the A-site codon lands in the A-site. A peptide bond is formed between the amino group of the A-site amino acid and the carboxyl group of the amino acid in the P-site. The formation of the peptide bond is catalyzed by **peptidyl transferase**, an RNA-based enzyme (ribozyme) that is part of the large rRNA subunit. The energy for the peptide bond formation comes from GTP hydrolysis, which is catalyzed by a separate elongation factor.

Catalyzing the formation of a peptide bond removes the covalent bond connecting the growing polypeptide chain to the tRNA in the P-site. The growing polypeptide chain is then transferred to the amino end of the amino acid that is connected to the tRNA that was in the A-site. The A-site tRNA becomes translocated or moved into the P-site, and the tRNA that was in the P-site is now uncharged and it exits the ribosome through the E-site (Fig 4-12).

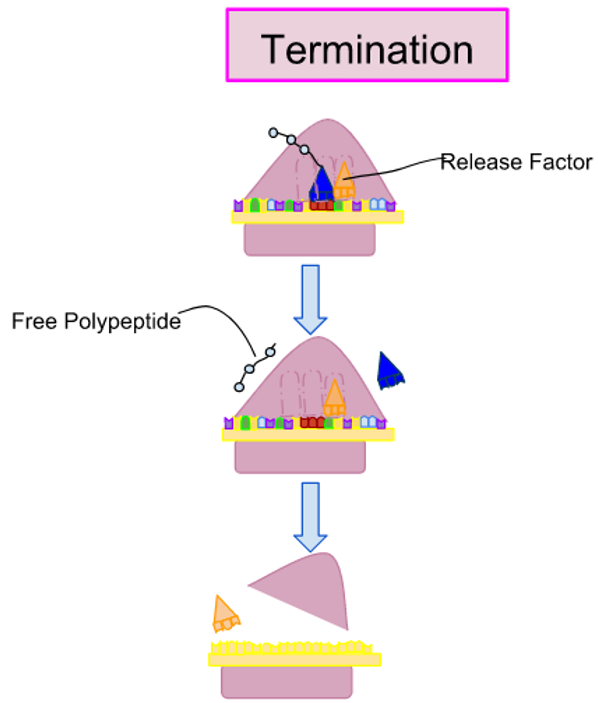
The ribosome’s A-site is now shifted three nucleotides down the mRNA. A new aminoacyl-tRNA with an anticodon complementary to the new A-site codon enters the ribosome at the A-site, and the elongation process repeats itself. The energy for each step of the ribosome is donated by an elongation factor that hydrolyzes GTP to GDP.



**Figure 4-12:  Elongation of a polypeptide.** A tRNA will enter into the A site of the ribosome and be checked by base pairing between the codon and anticodon. The amino acid in the P-site will be covalently attached to the amino acid in the A-site. The ribosome will translocate and the newly uncharged amino acid will leave out of the E-site, the tRNA that was in the A-site will now be in the P-site and a new, charged-tRNA can enter into the A-site. The cycle repeats.

# Translation Termination

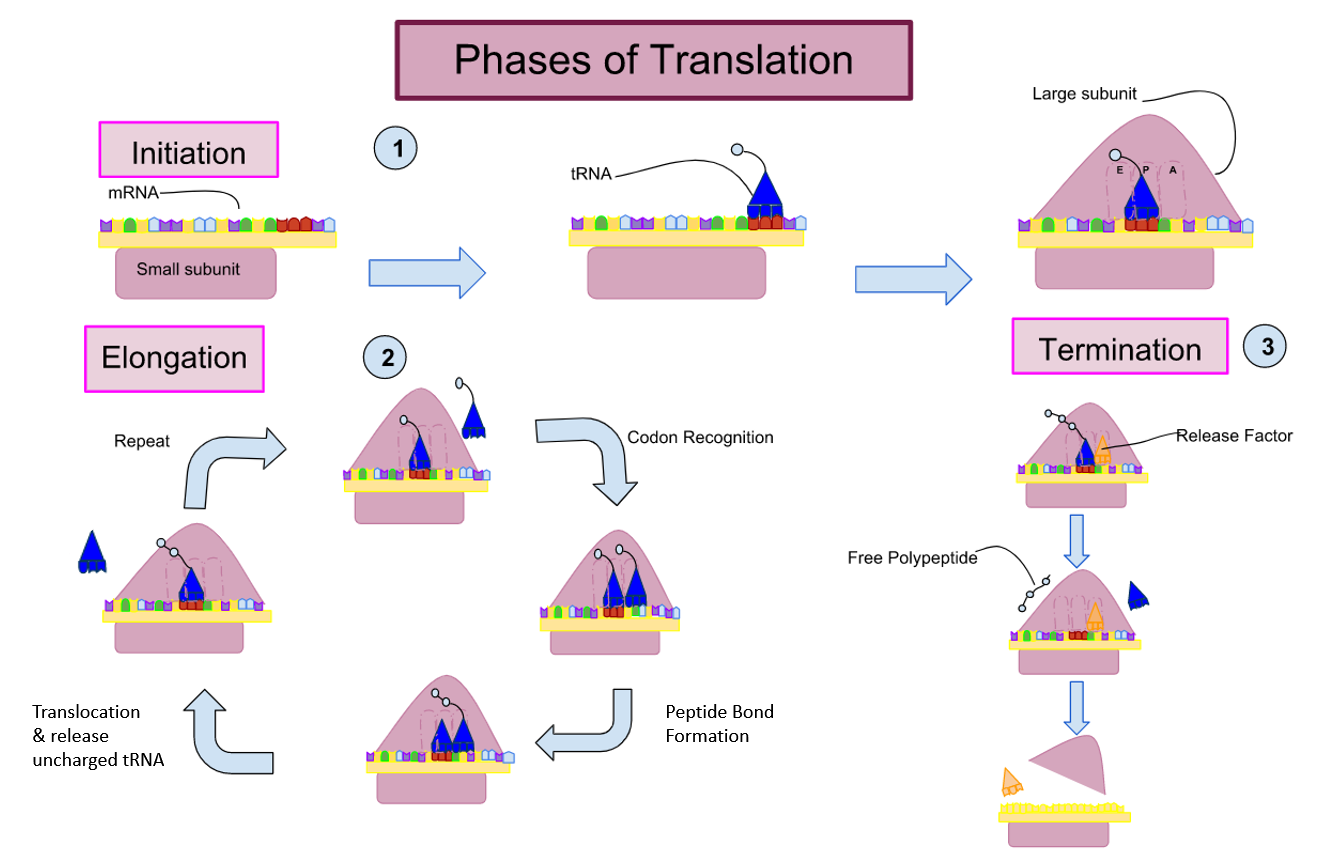
Interestingly, termination does not require tRNAs; there is no such thing as a ‘stop’ tRNA; therefore, there are no tRNAs that bind to the three stop codons. Instead, the cell provides proteins called Release Factors (RF) to terminate translation (Fig 4-13). Termination occurs at the end of the last exon. When the ribosome comes to a stop codon (UAA, UAG, or UGA) an RF recognizes the sequence.  The binding of the RF causes the ribosome peptidyl transferase to add a water molecule to the carboxyl end of the most recently added amino acid. This causes the covalent bond between the last amino acid and its tRNA to be broken, and the newly-made polypeptide is released. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation initiation complex. After the mRNA has been translated many times, the mRNA is degraded so the nucleotides can be reused in another transcription reaction.



**Figure 4-13: Termination of translation.** A release factor enters the A-site causing the bond between the last tRNA and its amino acid to be broken, releasing the polypeptide and the tRNA. The ribosome disassociates and can be reused in a new process of translation.

## Summary of Translation

Translation in eukaryotes begins after the mRNA is exported from the nucleus into the cytoplasm. A number of initiation factors, the small subunit of the ribosome, and initiator tRNA assemble at the 5’-cap of the mRNA. Together these scan the mRNA for the first AUG. Binding of the AUG helps to assemble the large subunit of the ribosome. The initiation factors release and translation elongation commences. During initiation, the first tRNA binds to the P-site of the ribosome, which allows tRNA that binds to the second codon to enter into the A-site. A condensation reaction occurs in the active site of the ribosome in the P-site that connects the amino acid in the P and A-sites together. The first tRNA no longer has the methionine amino acid attached and is considered ‘uncharged’. The ribosome translocates (or moves down) one codon. This allows the uncharged tRNA to leave the ribosome through the E-site; the tRNA that was in the A-site moves into the P-site and the A-site is open once again for a new ‘charged’ tRNA to enter. This cycle repeats until the stop codon is reached. Termination occurs when the ribosome encounters a stop codon. A protein called a release factor interacts with the ribosome and recognizes the stop codon. It then breaks the covalent bond between the last tRNA and its amino acid releasing both from the ribosome and helping the subunits of the ribosome to dissociate. Figure 4-14 illustrates the entire process.



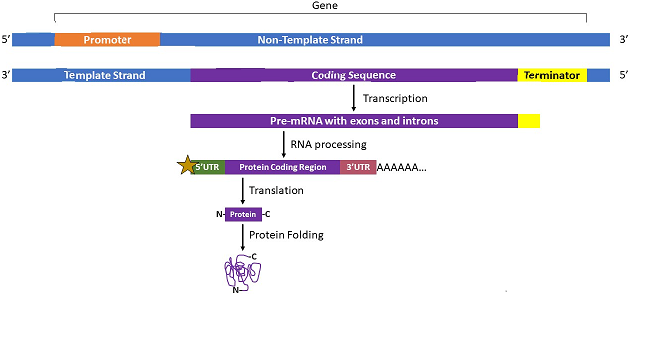
**Figure 4-14: Basic overview of the phases of translation.** Translation consists of 1) initiation, 2) elongation, and 3) termination. The basic steps of all three processes are represented.

## DNA to RNA to Protein one last time

After translation is completed we have a protein which came from mRNA that was transcribed from DNA. This protein product will eventually be trafficked to the location where it does its job. More information on protein trafficking will be covered in chapter 9.

Figure 4-15 illustrates the flow of this information and how the size of the genetic product gets smaller and smaller after each step. It is important to note that nucleic acids are relatively large in comparison to the typical protein. It is a misconception to think proteins, because they do most of the functions of the cell, are larger than the DNA or RNA molecules that make them.

Overall, in eukaryotes, a large amount of DNA is transcribed into mRNA. The mRNA is processed to add a 5’-cap, remove introns, and add a poly-A tail. Removal of intronic sequences greatly reduces the size of the mature mRNA (remember introns are typically much larger than exons). The mature mRNA is exported to the cytoplasm from the nucleus. In the cytoplasm the mRNA is translated, but two regions of the mRNA, the 3’-UTR and 5’-UTR remain untranslated. This doesn’t mean these sequences aren’t important; it has been noted these regions are critical for the process of export out of the nucleus, translation, and the mRNA’s stability. Only the region that incorporates the start and stop codons in the mRNA are translated. Overall this means the protein product is much smaller than the mRNA for multiple reasons: 1) there are regions of the mRNA that are not translated (mentioned above); 2) it takes three RNA nucleotides (a codon) to get one amino acid and 3) amino acids are typically about much smaller (fewer atoms) than a ribonucleotide. This size difference is roughly illustrated in Figure 4-15.



**Figure 4-15: Flow of genetic information from DNA to protein.** A gene is transcribed into mRNA, which is processed. Notice the relative size of the gene compared to the mature mRNA (not to scale). After the mRNA is processed it is much smaller than the pre-mRNA, which is smaller in size compared to the original DNA sequence. The protein created after translation in the cytoplasm is even smaller and finally the protein folds into its three-dimensional structure.

Associated Videos:

[Charging the tRNA](https://vimeo.com/138715154)