Information Processing: Translation

Translation is the process by which information in mRNAs is used to direct the synthesis of proteins. As you have learned in introductory biology, in eukaryotic cells, this process is carried out in the cytoplasm of the cell, by large RNA-protein machines called ribosomes. Ribosomes contain ribosomal RNA (rRNA) and proteins. The proteins and rRNA are organized into two subunits, a large and a small. Ribosomes function by binding to mRNAs and holding them in a way that allows the amino acids encoded by the RNA to be joined sequentially to form a polypeptide. Transfer RNAs are the carriers of the appropriate amino acids to the ribosome.

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The genetic code

We speak of genes (i.e., DNA) coding for proteins and the central dogma, which states that DNA makes RNA makes protein. What does this actually mean? A code can be thought of as a system for storing or communicating information. A familiar ex-

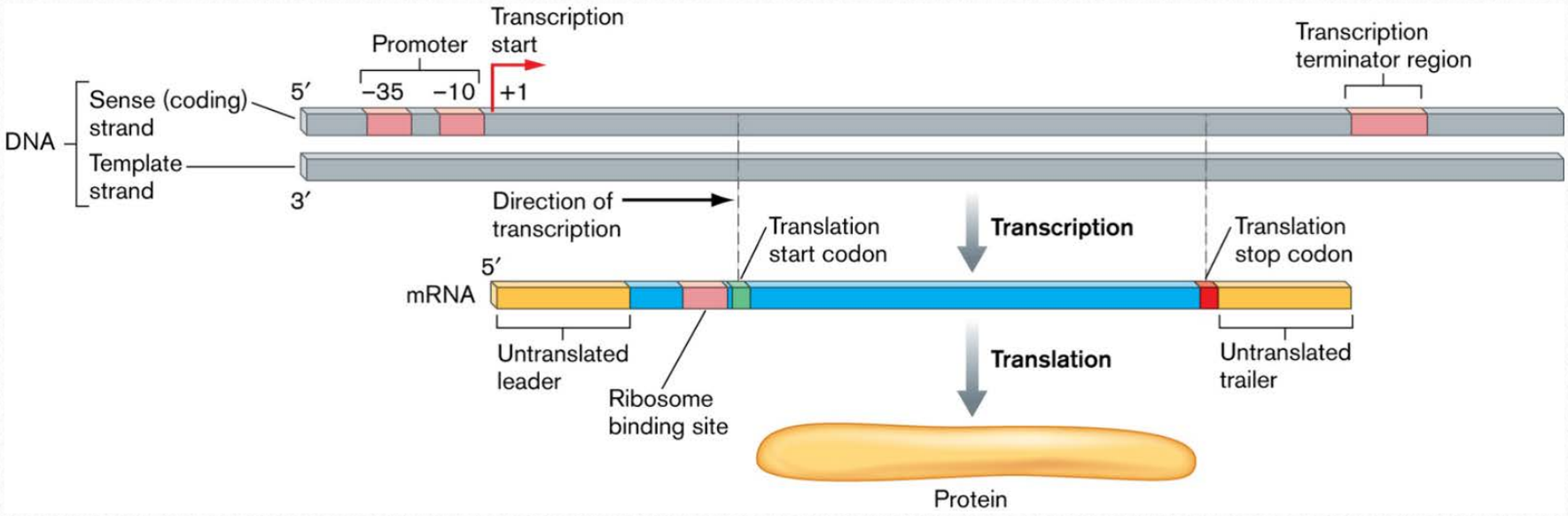


Figure 7.80 - The central dogma in a bacterial cell

Wikipedia

ample is the use of letters to represent the names of airports (e.g., PDX for Portland, Oregon and ORD for Chicago's O'Hare). When a tag on your luggage shows PDX as the destina-

nonpolar | polar | basic | acidic | (stop codon)

Standard genetic code

1st base	2nd base								3rd base
	U		C		A		G		
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

Figure 7.81 - The standard genetic code

Wikipedia

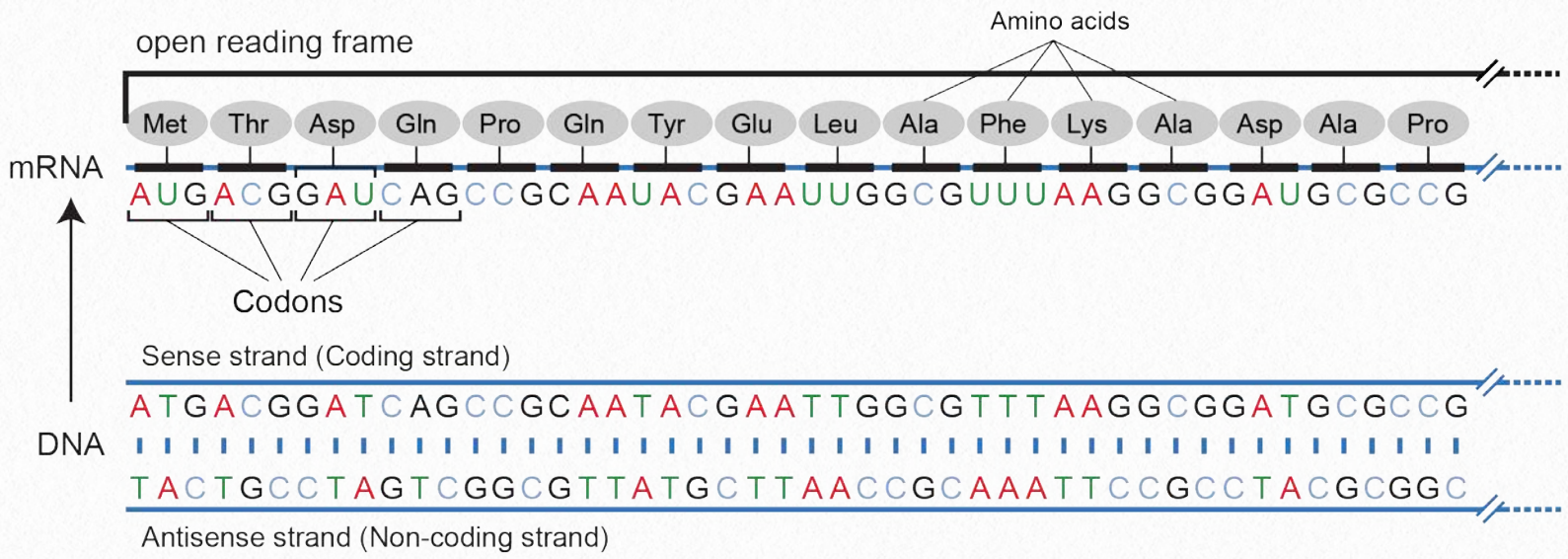


Figure 7.82 - Coding in DNA, transcribed to RNA, translated to protein

tion, it conveys information that your bag should be sent to Portland, Oregon. To function well, such a set-up must have unique identifiers for each airport and people who can decode the identifiers correctly. That is, PDX must stand only for Portland, Oregon and no other airport. Also, luggage handlers must be able to correctly recognize what PDX stands for, so that your luggage doesn't land in Phoenix, instead.

How does this relate to genes and the proteins they encode?

Genes are first transcribed into mRNA, as we have already discussed. The sequence of an mRNA, copied from a gene, directly specifies the sequence of amino acids in the protein it encodes. Each amino acid in the protein is specified by a sequence of 3 bases called a codon in the mRNA (Figure 7.81). For example, the amino acid tryptophan is encoded by the sequence UGG on an mRNA. All of the twenty amino acids used to build proteins have, likewise, 3-base sequences that encode them.

Degeneracy

Given that there are 4 bases in RNA, the number of different 3-base combinations that are possible is 4^3, or 64. There are, however, only 20 amino acids that are used in building proteins in cells. This discrepancy in the number of possible codons and the actual number of amino acids they specify is explained by the fact that the same amino acid may be specified by more than one codon. In fact, with the exception of the amino acids methionine and tryptophan, all the other amino acids are encoded by multiple codons. Codons for the same amino acid are often related, with the first two bases the same and the third being variable. An example would be the codons for alanine: GCU, GCA, GCC and GCG all stand for alanine. This sort of re-

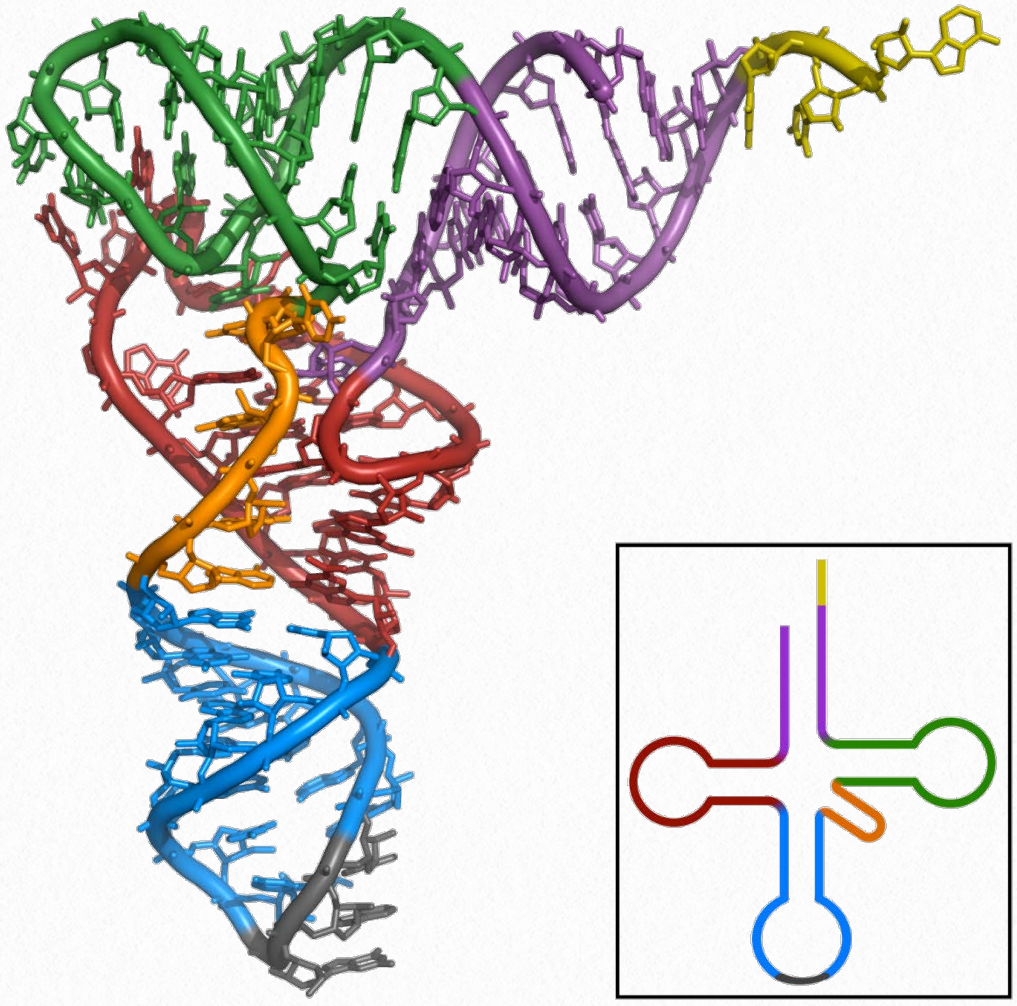


Figure 7.83 - tRNA - 3D projection (left) and 2D projection (inset)
Wikipedia

dundancy in the genetic code is termed degeneracy.

Stop and start codons

Three of the 64 codons are what are known as termination or stop codons, and as their name suggests, indicate the end of a protein coding sequence. The codon for methionine, AUG, is used as the initiation or start codon for the majority of proteins. This ingenious system is used to direct the assembly of a protein in the same way that you might string together colored beads in a particular order using instructions that used symbols like UGG for a red bead, followed by UUU for a green bead, CAC for yellow, and so on, till you came to UGA, indicating that you should stop stringing beads.

Translating the code

While the ribosomes are literally the factories that join amino acids together using the instructions in mRNAs, another class of RNA molecules, the transfer RNAs (tRNAs) are also needed for translation (Figure 7.83 and Interactive 7.1). Transfer RNAs are small RNA molecules, about 75-90 nucleotides long, that function to 'interpret' the instructions in the mRNA during protein synthesis. Transfer RNAs are extensively modified post-transcriptionally and contain a large number of unusual bases. The sequences of tRNAs have several self complementary regions, where the single-stranded tRNA folds on itself and base-pairs to form what is sometimes described as a clover leaf structure.

This structure is crucial to the function of the tRNA, providing both the sites for attachment of the appropriate amino acid and for recognition of codons in the mRNA. In terms of the bead analogy above, someone or something has to be able to bring a red bead in when the instructions indicate UGG, and a green bead when the instructions say UUU. This, then, is the function of the tRNAs. They must be able to bring the amino acid corre-

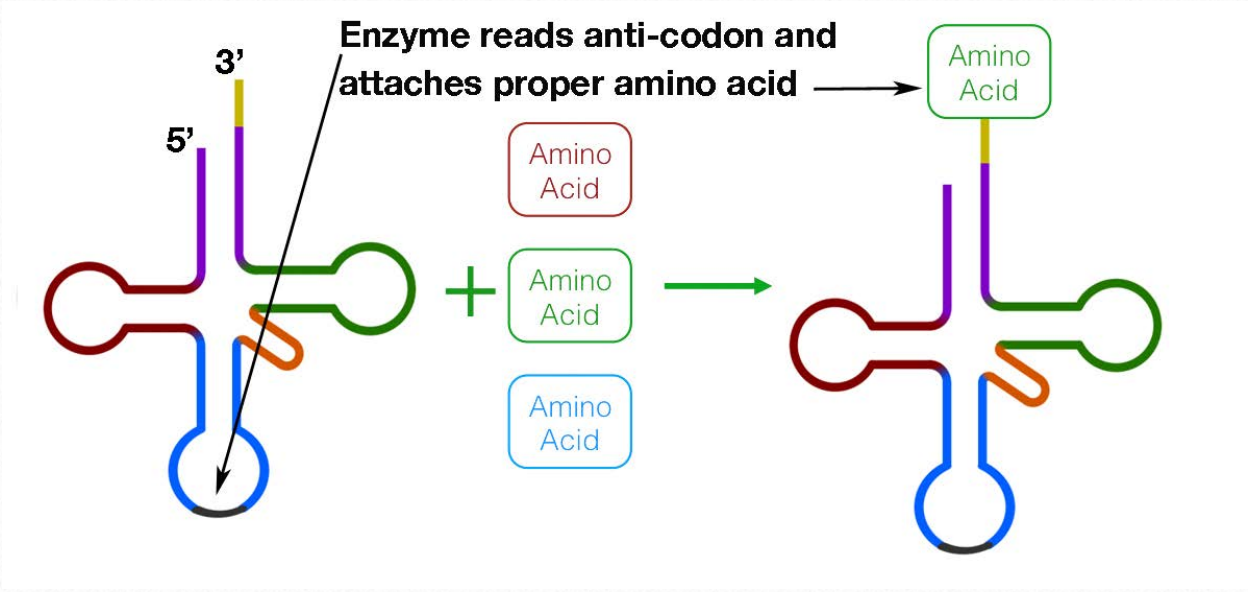


Figure 7.84 - Charging of a tRNA by aminoacyl tRNA synthetase

Wikipedia

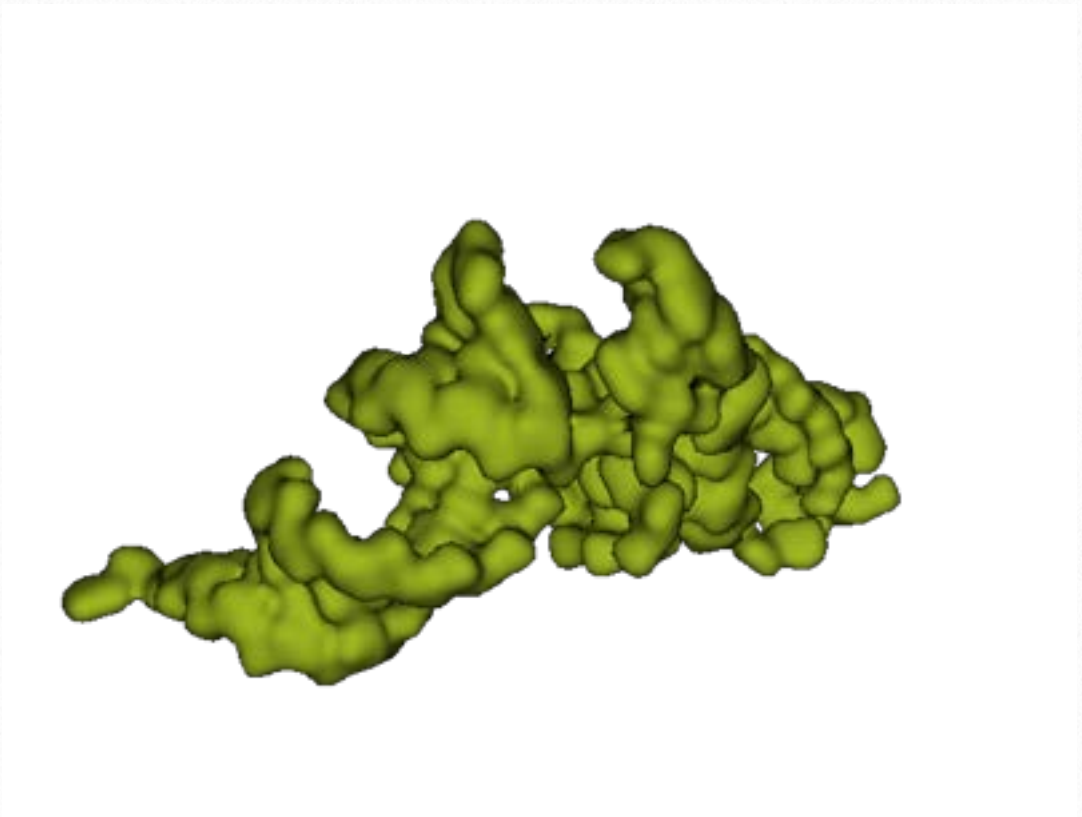
sponding to the instructions to the ribosome.

t-RNA specificity

A given transfer RNA is specific for a particular amino acid. It is linked covalently at its 3' end to the appropriate amino acid by an enzyme called aminoacyl tRNA synthetase. For example, there is a transfer RNA that is specific to the amino acid tryptophan, and a corresponding aminoacyl tRNA synthetase, called a tryptophanyl tRNA synthetase, that can attach the tryptophan specifically to this tRNA. Likewise, there is an aminoacyl tRNA synthetase specific for each amino acid. A tRNA with an amino acid attached to it is said to be *charged* (Figure 7.84). A pool of charged tRNAs is necessary to carry out protein synthesis. How do these tRNAs, carrying specific amino acids assist the ribosome in stringing together the correct amino acids, as specified by the sequence of the mRNA?

Codon recognition

As we already know, the amino acid sequence of the protein is determined by the order of the codons in the mRNA. We also have charged tRNAs carrying the various amino acids present. How are the amino acids attached to each other in the order indicated by the base se-



Interactive 7.1 - Phenylalanyl-tRNA

PDB

quence of the mRNA? This requires recognition of the codons on the mRNA by the appropriate charged tRNAs. The amino acid tryptophan, as we noted, is specified by the codon UGG in the mRNA. This codon must be recognized by a tRNA charged with tryptophan. Every tRNA has a a sequence of 3 bases, the anticodon, that is complementary to the codon for the amino acid it is carrying. When the tRNA encounters the codon for its amino acid on the messenger RNA, the anticodon will base-pair with the codon. For the tryptophan tRNA this is what it would look like:

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Sequence of tryptophan codon in mRNA:

5’ UGG 3’

Anticodon sequence in tryptophan tRNA:

5’ CCA 3’

Note that the sequences are both written, by convention, in the 5’ to 3’ direction. To base pair, though, they must be oriented in opposite directions (anti-parallel). The codon-anticodon basepair in the antiparallel orientation then would be:

5’ UGG 3’
3’ ACC 5’

The base-pairing of the anticodon on a charged tRNA with the codon on the mRNA is what brings the correct amino acids in to the ribosome to be added on to the growing protein chain (Figure 7.85).

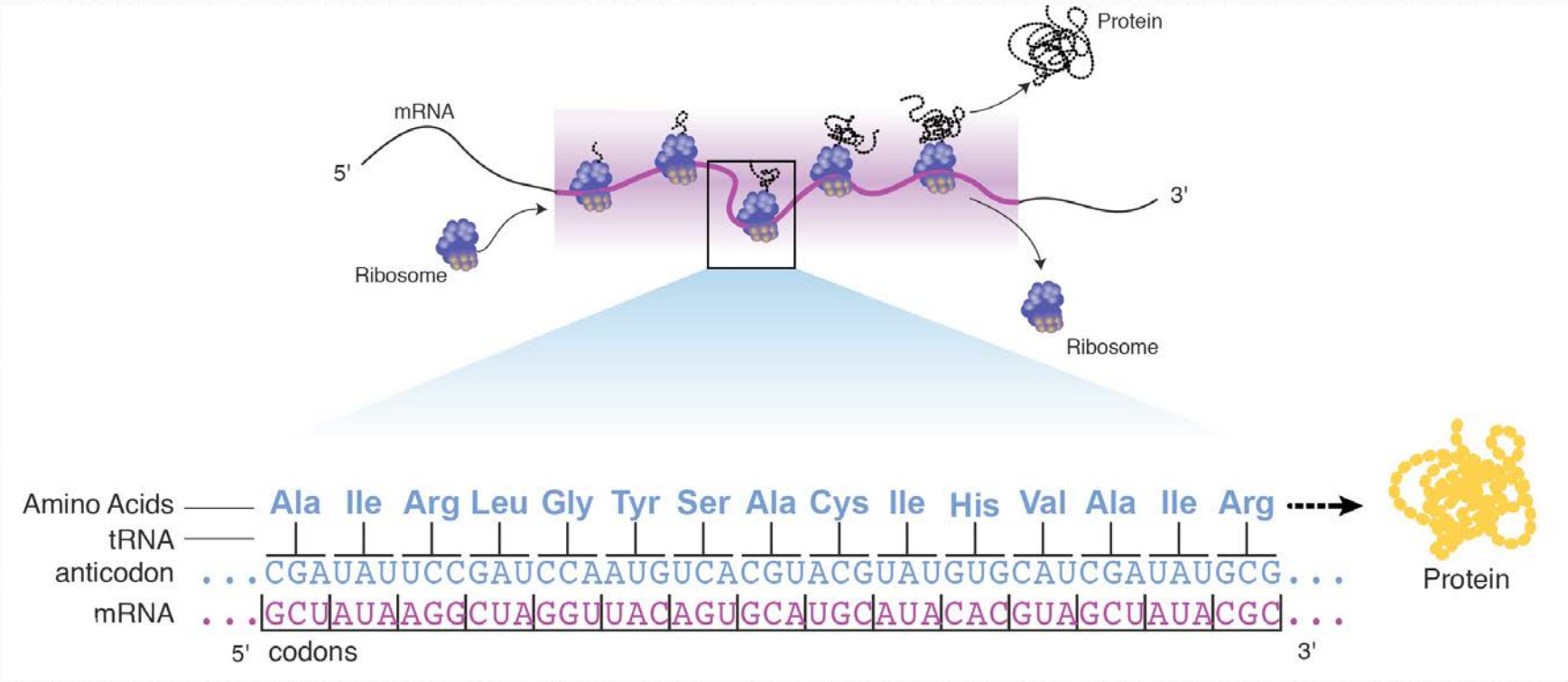

Figure 7.85 - Codons in mRNA pair with anticodons on tRNA to bring the appropriate amino acid to the ribosome for polypeptide assembly

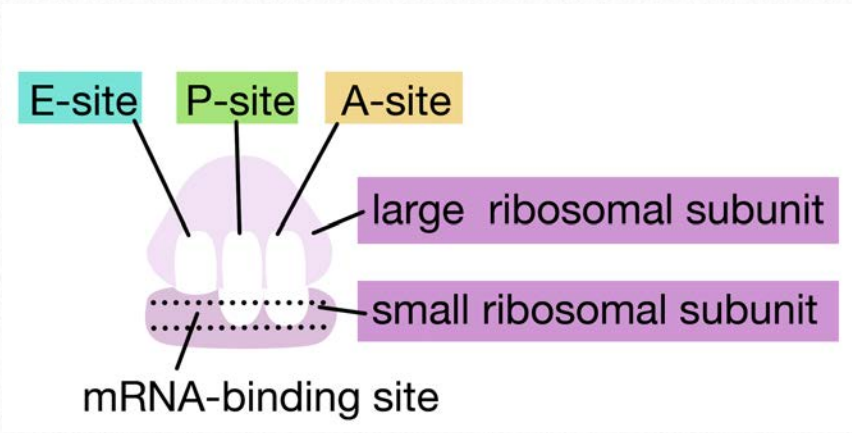

Figure 7.86 - The A, P, and E sites in a ribosome

Image by Martha Baker

Making a polypeptide

With an idea of the various components necessary for translation and how they work, we can now take a look at the process of protein synthesis. The main steps in the process are similar in prokaryotes and eukaryotes. As we already noted, ribosomes bind to mRNAs and facilitate the interaction between the codons in the mRNA and the anticodons on charged tRNAs.

Ribosomes have two sites (P-site and A-site) for binding and positioning charged tRNAs so each can form base pairs between their anticodon and a codon from the mRNA. The start codon (AUG) is positioned to base pair with the tRNA in the P-site (peptidyl site). Next, the charged tRNA complementary to the codon adjacent to the start codon binds and occupies the A-site (aminoacyl site) in the ribosome (Figure 7.86).

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At this point, the ribosome joins the amino acids carried on each tRNA by making a peptide bond. The bond between the amino acid and the tRNA in the P-site is broken and the dipeptide is joined to the tRNA on the A-site.

The initiator tRNA without its amino acid is then released, moving into a site known as the Exit or E-site, while the second tRNA carrying the dipeptide (and the codon it is base paired to) moves into the P-site. The A-site now is ready with a new codon for the next incoming charged tRNA.

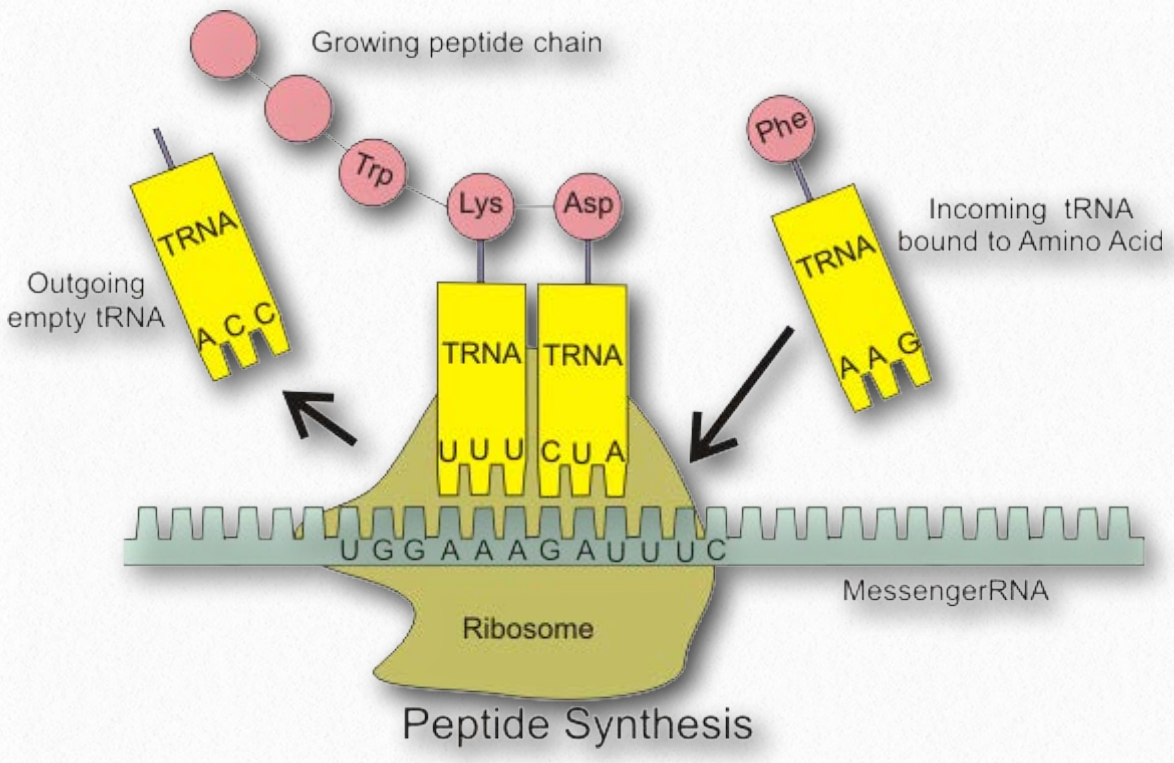


Figure 7.87 - Overview of elongation

Wikipedia

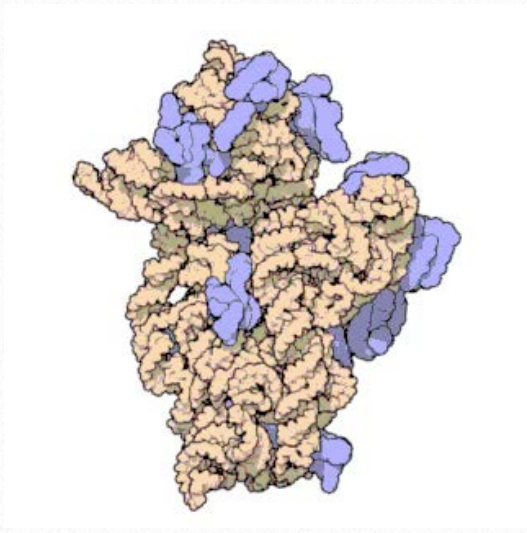
This process is repeated, with the ribosome moving on the mRNA one codon at a time, until the stop codon reaches the A-site. At this point, a release factor binds at the A-site, and helps to free the completed polypeptide from the ribosome. The ribosome then dissociates into the small and large subunits, once more.

rRNA Name	Prokaryotes	Eukaryotes	Function
5S	Large Subunit	Large Subunit	tRNA binding?
5.8S		Large Subunit	Translocation?
16S	Small Subunit		mRNA alignment
18S		Large Subunit	mRNA alignment
23S	Large Subunit		Peptide bond formation
28S		Large Subunit	Peptide bond formation

Table 7.1 - Location and function of rRNAs.

Three steps

Having considered the steps of translation in broader terms, we can now look at them in greater detail. We will consider the three steps of translation (bel0w) individually.



Movie 7.1 - 30S ribosomal subunit

Wikipedia

- Initiation (binding of the ribosomal subunits to the transcript and initiator tRNA)
- Elongation (repeated addition of amino acids to the growing polypeptide, based on the sequence of the mRNA - Figure 7.87)
- Termination (release of the completed polypeptide and dissociation of the ribosome into its subunits).

We already know that processed mRNAs are sent from the nucleus to the cytoplasm in eukaryotic cells, while in prokaryotic cells, transcription and translation occur in a single cellular compartment. The small and large subunits of ribosomes, each composed of characteristic rRNAs and proteins, are found in the cytoplasm and assemble on mRNAs to form complete ribosomes that carry out translation. Both prokaryotic

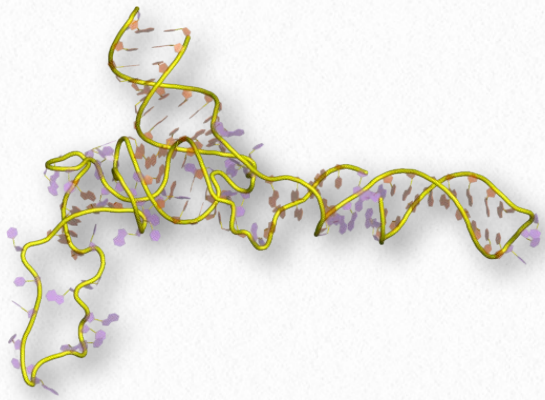


Figure 7.88 - Structure of 5S rRNA

and eukaryotic ribosomal subunits are made up of one or more major rRNAs together with a large number of ribosomal proteins. The small subunits of prokaryotic cells are called the 30S ribosomal subunits, while their counterparts in eukaryotes are the 40S subunits. The large ribosomal subunits in prokaryotes are the 50S subunits, while those in eukaryotic cells are 60S. These differences reflect the larger mass of eukaryotic ribosomes. The rRNA components of ribosomes are important for the recognition of the 5' end of the mRNA, and also play a catalytic role in the formation of peptide bonds.

Initiation

Messenger RNAs have non-coding sequences both at their 5' and 3' ends, with the actual protein-coding region sandwiched in between these untranslated regions (called the 5' UTR and 3' UTR, respectively). The ribosome must be able to recognize the 5' end of the mRNA and bind to it, then determine where the start codon is located. It is important to note that both in prokaryotes and eukaryotes, ribosomes assemble at the 5' end of the transcript by the stepwise binding of the small and large subunits. The small subunit first binds to the mRNA at specific sequences in the 5' UTR. The large subunit then binds to the complex of the mRNA and small subunit, to form the complete ribosome.

Initiator tRNA

Initiation also requires the binding of the first tRNA to the ribosome. As we have noted earlier, the initiation, or start codon is usually AUG, which codes for the amino acid methionine. Thus, the initiator tRNA is one that carries methionine and is designated as $tRNA^{met}$ or methionyl $tRNA^{met}$. In bacteria, the methionine on the initiator tRNA is modified by the addition of a formyl group, and is designated $tRNA^{fmet}$. The initiator tRNA carrying methionine to the AUG is different from the tRNAs that carry methionine

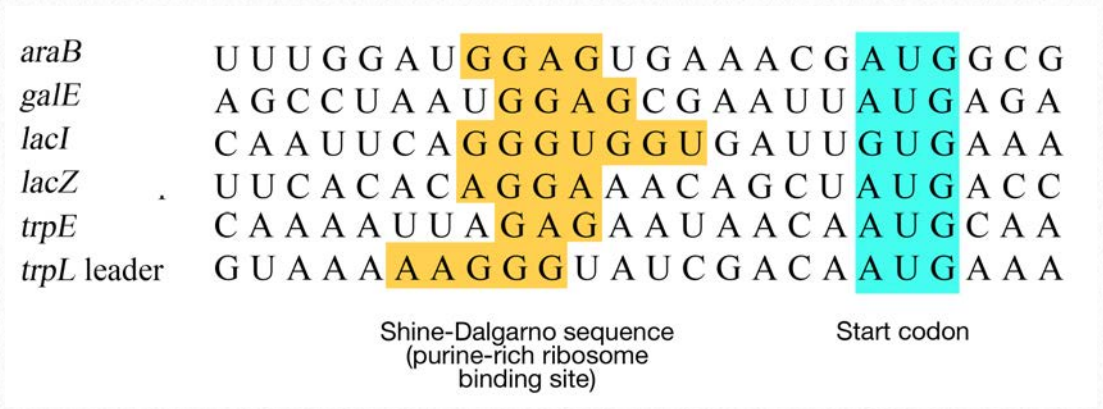
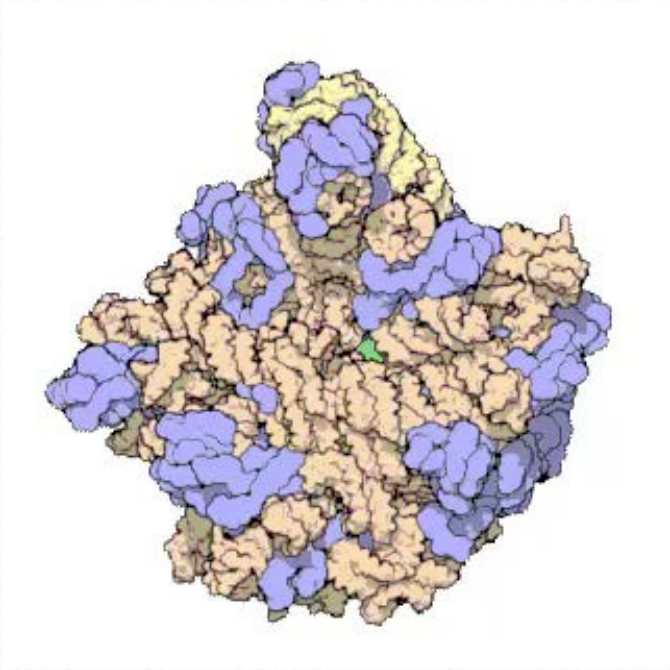


Figure 7.89 - Conserved sequences adjacent to start codons for various bacterial genes

Image by Martha Baker



Movie 7.2 - Large ribosomal subunit
Wikipedia

intended for other positions in proteins. As such, the initiator tRNA is sometimes referred to as $tRNA^{imet}$.

Prokaryotic initiation

In prokaryotes, the 5' end of the mRNA is the only free end available, as transcription is tightly coupled to translation and the entire mRNA is not transcribed before translation begins. Nevertheless, the ribosome must be correctly positioned at the 5' end of the messenger RNA in order to initiate translation. How does the ribosome "know" exactly where to bind in the 5'UTR of the mRNA?

Shine-Dalgarno sequence

Examination of the sequences upstream of the start codon in prokaryotic mRNAs reveals that there is a short purine-rich sequence ahead of the start codon that is crucial to recognition and binding by the small ribosomal subunit (Figure 7.89). This sequence, called the Shine-Dalgarno sequence, is complementary to a stretch of pyrimidines at the 3' end of the 16S rRNA component of the small ribosomal subunit (Figure 7.90). Base-pairing between these complementary sequences positions the small ribosomal subunit at the right spot on the mRNA, with the AUG start codon at the P-site.

Initiation factors

The binding of the small ribosomal subunit to the mRNA requires the assistance of three protein factors called Initiation Factors 1, 2 and 3 (IF1, IF2, IF3). These proteins, which are associated with the small ribosomal subunit, are necessary for its bind-

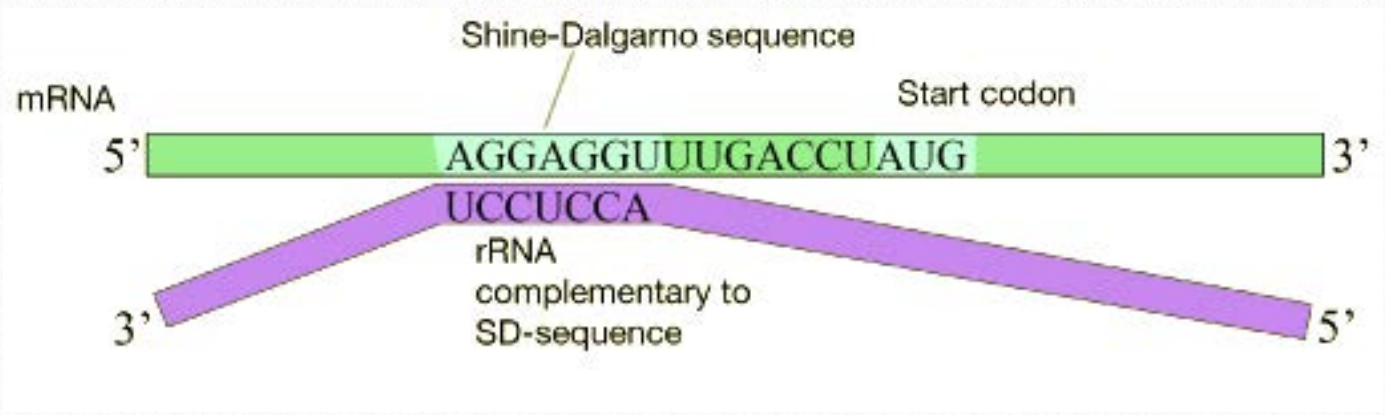


Figure 7.90 - Base pairing between the Shine-Dalgarno sequence in the mRNA and the 16S rRNA

Image by Martha Baker

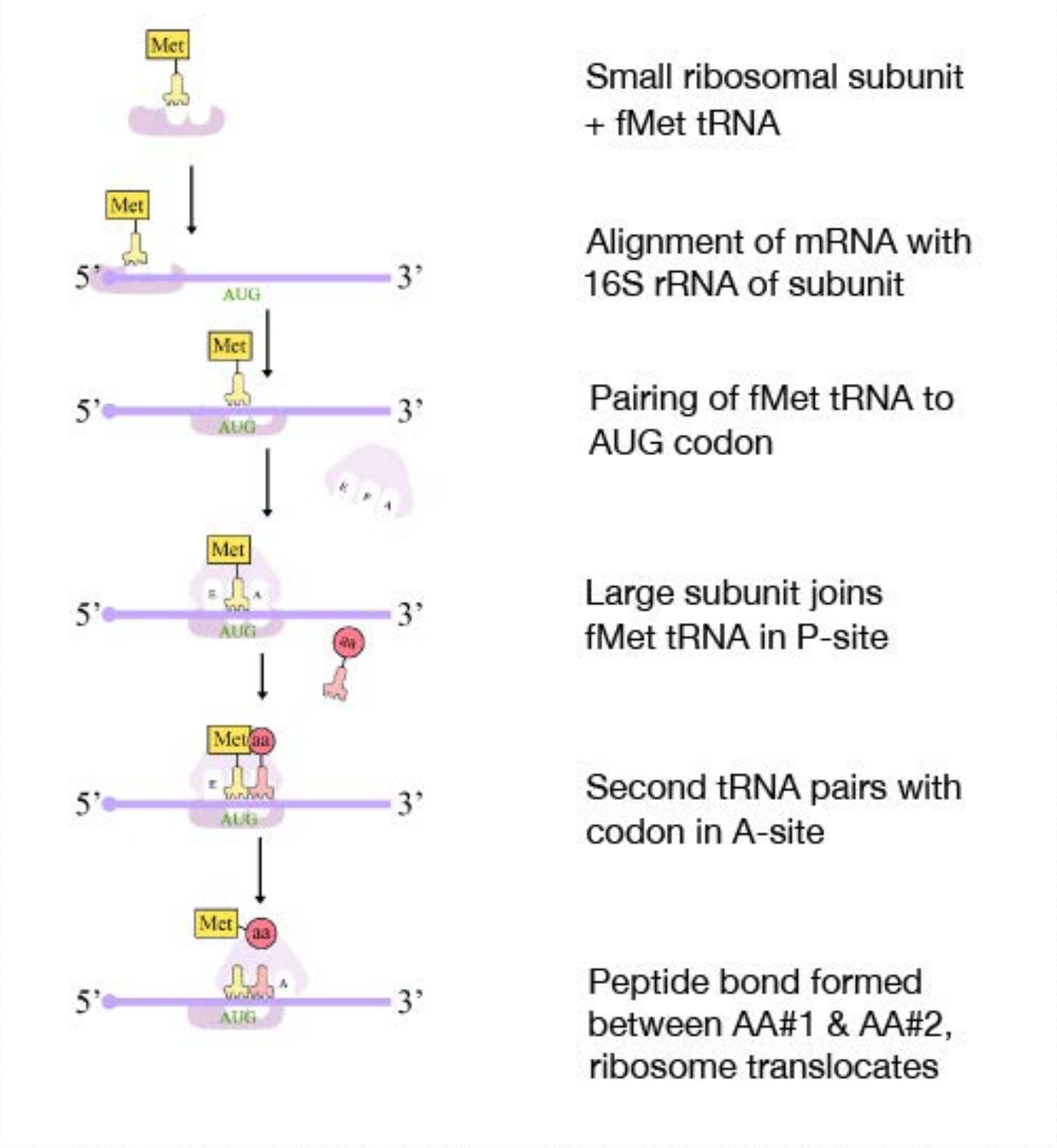


Figure 7.91 - Initiation - assembly of the ribosomal translation complex

Image by Martha Baker

ing to mRNA, but dissociate from it when the 50S ribosomal subunit binds. Of these proteins, IF3 is important for the binding of the small subunit to the mRNA, while IF2 is involved in bringing the initiator tRNA to the partial P-site of the small ribosomal subunit. IF1 occupies the A-site, preventing the binding of the initiator tRNA at that site.

Once the small ribosomal subunit is bound to the mRNA and the initiator tRNA is positioned at the P-site, the large ribosomal subunit is recruited and the initiation complex is formed. Binding of the 50S ribosomal subunit is accompanied by the dissociation of all three initiation factors. The removal of IF1 from the A-site on the ribosome frees up the site for the binding of the charged tRNA corresponding to the second codon (Figure 7.91).

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Eukaryotic initiation

In eukaryotes, initiation follows a similar pattern, although the order of events and the specific initiation factors are different.

Eukaryotes have a large number of

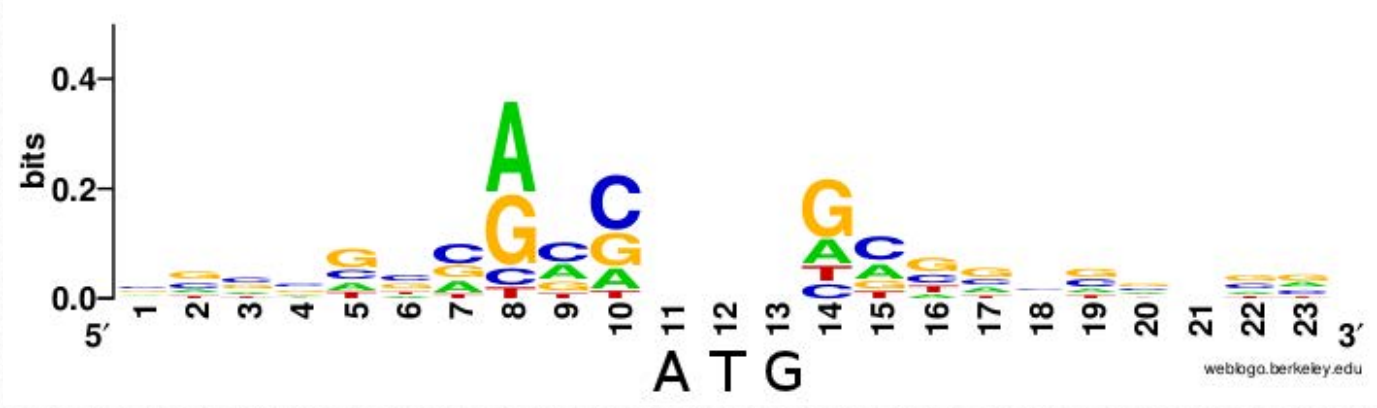

Figure 7.92 - Kozak sequence plot showing relative abundance of bases surrounding the AUG (ATG) start codon of human genes

Kozak sequences

Specific sequences surrounding the AUG, called Kozak sequences for the scientist who defined them, have been shown to be necessary for the binding of the 40S subunit, with the bases at -4 and +1 relative to the AUG being especially important.

IFs that are known as eIFs (eukaryotic initiation factors). These initiation factors are involved in the binding of the initiator tRNA to the small subunit, as well as in association of the small subunit with mRNA and subsequent attachment of the large subunit.

Ribosome assembly

The assembly of the translation machinery in eukaryotes begins with the binding of the initiator tRNA to the 40S (small) subunit. This step requires the assistance of eIF2 and eIF3. Next the small subunit with the initiator tRNA binds to the 7-methyl G cap on the 5'end of the mRNA. The 40S subunit then moves along the mRNA, scanning for a a start codon. Binding of the ribosomal subunit to the mRNA is dependent not just on finding an AUG, but on the sequences surrounding the codon.

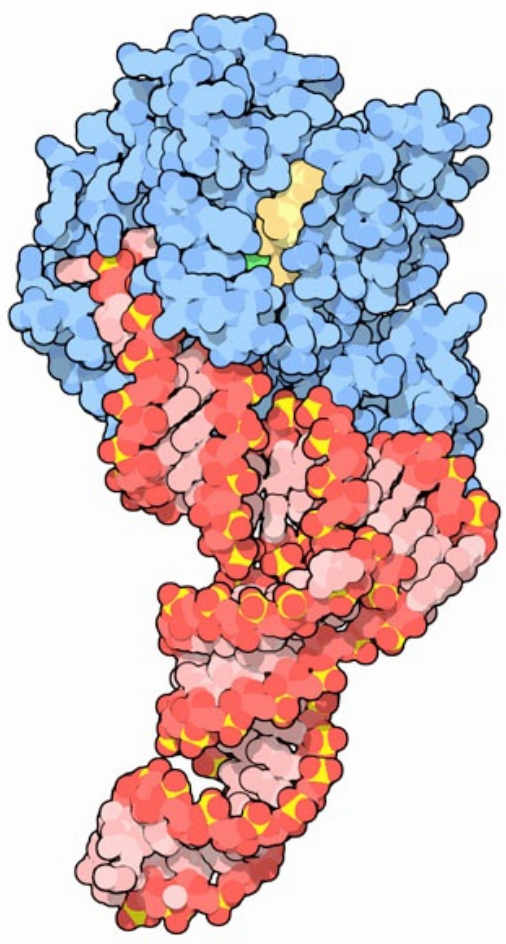

Figure 7.93 - EF-Tu (blue) bound to tRNA (red) and GTP (yellow)

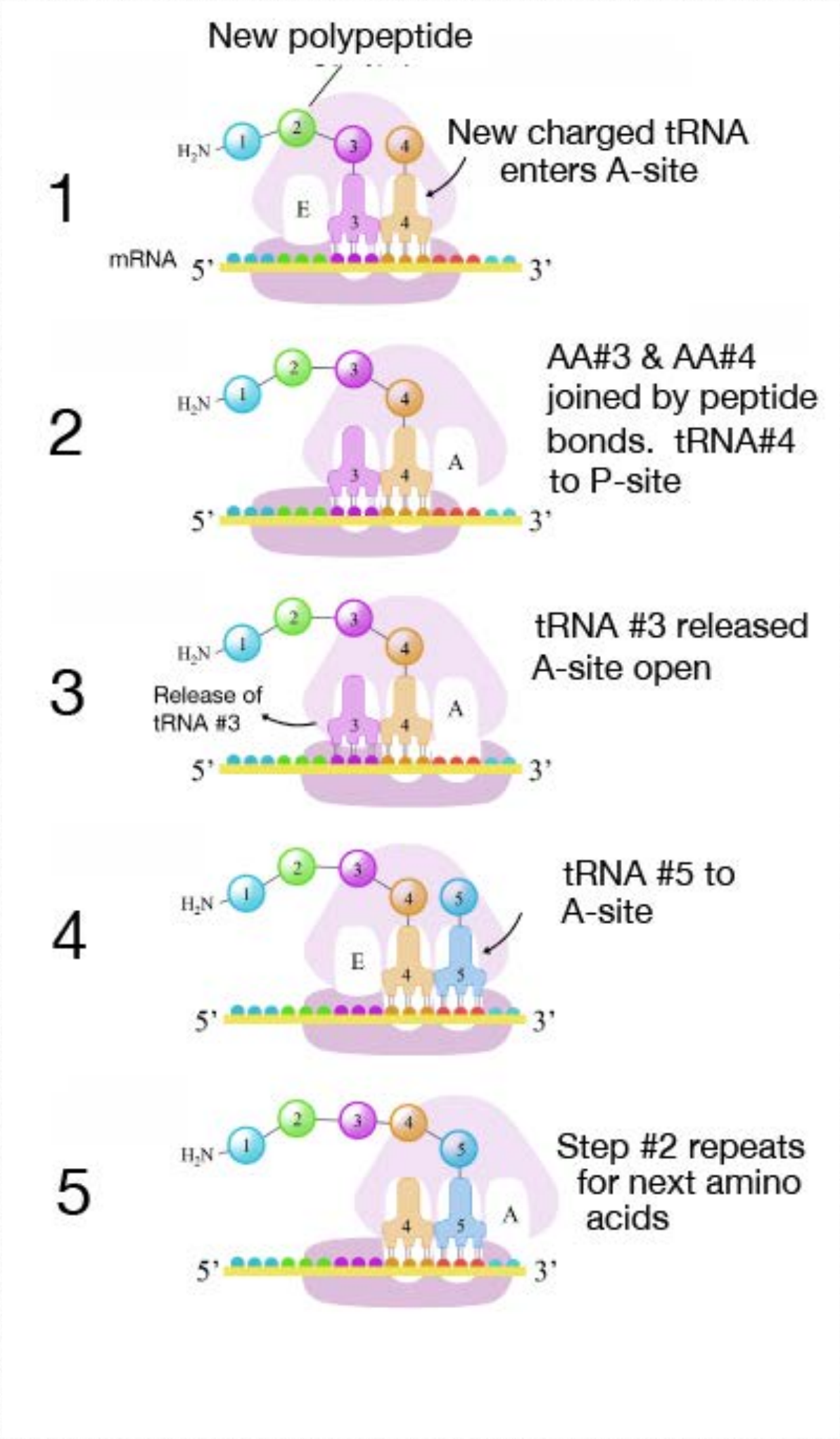

Figure 7.94 - The process of elongation

Image by Martha Baker

tant (Figure 7.92). Once the small subunit is properly positioned, the large ribosomal subunit (60S) binds, forming the initiation complex.

Elongation

After the ribosome is assembled with the initiator tRNA positioned at the AUG in the P-site, the addition of further amino acids can begin. In both prokaryotes and eukaryotes, the elongation of the polypeptide chain requires the assistance of elongation factors. In bacteria, the binding of the second charged tRNA at the A-site requires the elongation factor EF-Tu complexed with GTP (Figure 7.93). When the charged tRNA has been loaded at the A-site, EF-Tu hydrolyzes the GTP to GDP and dissociates from the ribosome. The free EF-Tu can then work with another charged tRNA to help position it at the A-site (Figure 7.94), after exchanging its GDP for a new GTP.

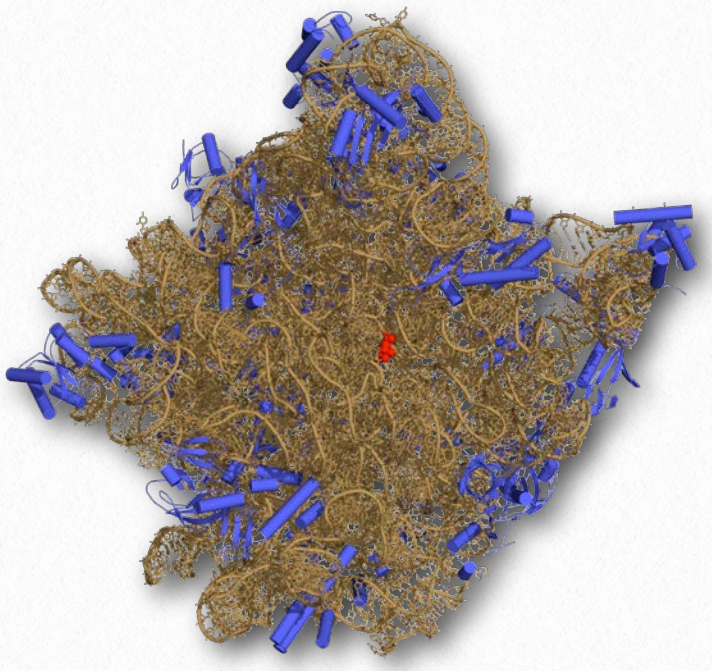

Figure 7.95 - 50S ribosomal subunit. RNA in brown. Protein in blue. Peptidyl transferase site in red.
Wikipedia

The corresponding step in eukaryotic cells is dependent on the elongation factor eEF1α.GTP. Once both P-site and A-site are occupied, the methionine carried by the tRNA in the P-site is joined to the amino acid carried by tRNA in the A-site, forming a peptide bond.

The reaction that joins the amino acids occurs in the ribosomal peptidyl transferase center, which is part of the large ribosomal subunit (Figure 7.95).

Ribozyme

Interestingly, there is strong evidence that this reaction is catalyzed by rRNA components of the large subunit, making the formation of peptide bonds an example of the activity of RNA enzymes, or ribozymes. The result of the peptidyl transferase activity is that the tRNA in the A-site now has two amino acids attached to it, while the tRNA at the P-site has none. This "empty" or deacylated tRNA is moved to the E-site on the ribosome, from which it can exit. The tRNA in the A-site, then moves to occupy the vacated P-site, leaving the A-site open for the next incoming charged tRNA. Yet another elongation factor, EF-G complexed with GTP, is required for the translocation of the ribosome along the mRNA in bacteria, while in eukaryotes, this role is played by eEF2.GTP. Repeated cycles of these steps result in the elongation of the polypeptide by one amino acid per cycle, until a termination, or stop codon is in the A-site.

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Termination

When a stop codon is in the A-site, proteins called release factors (RFs) are needed to recognize the stop codon and cleave and release the newly made polypeptide. In bacteria, RF1 is a release factor that can recognize the stop codon UAG, while RF2 recognizes UGA. Both RF1 and RF2 can recognize UAA. A third release factor, RF3, works with RF1 and RF2 to hydrolyze the linkage between the polypeptide and the final tRNA, to release the newly synthesized protein. This is followed by the dissociation of the ribosomal subunits from the mRNA, ending the process of translation.

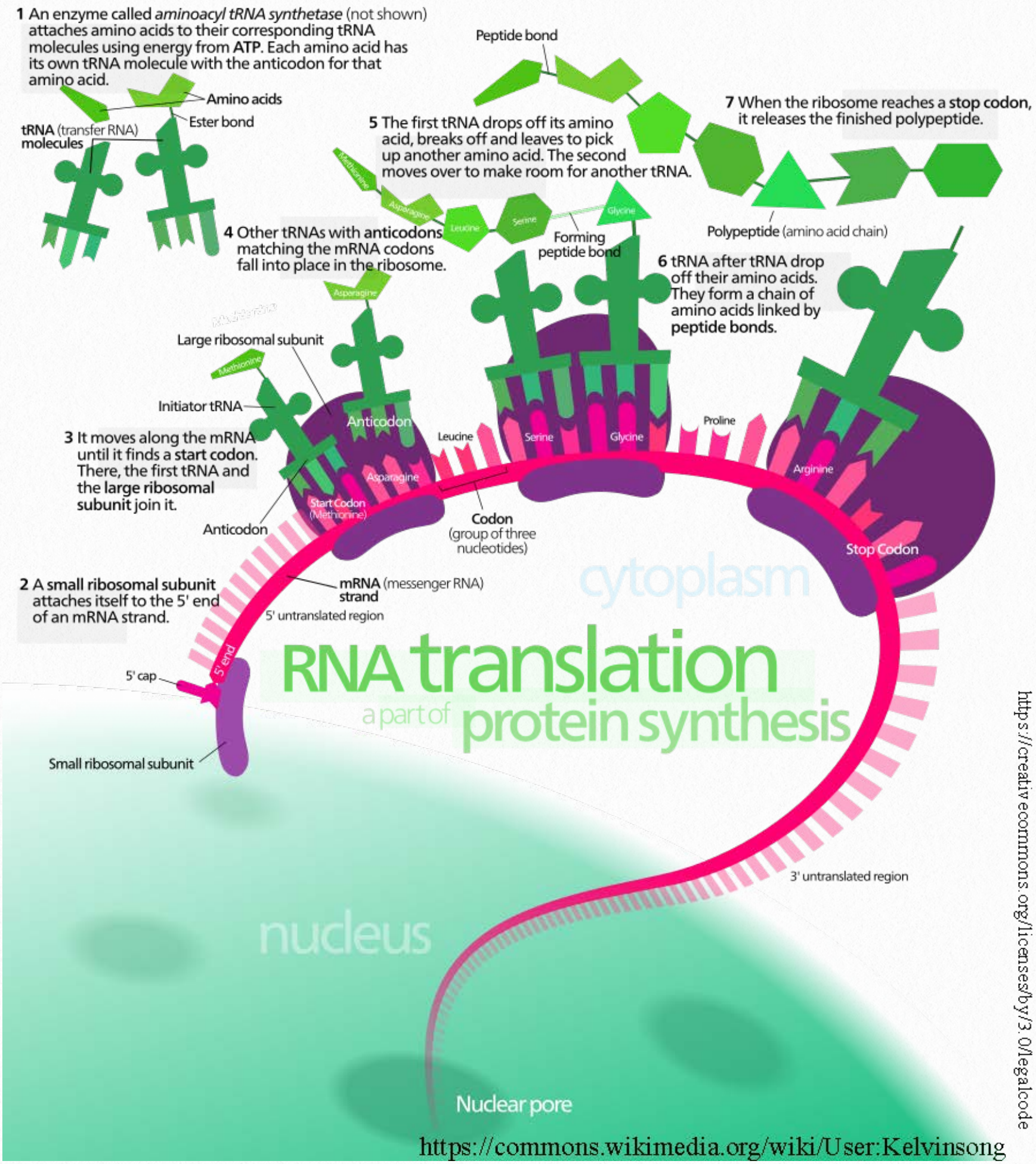

Figure 7.96 - The process of translation

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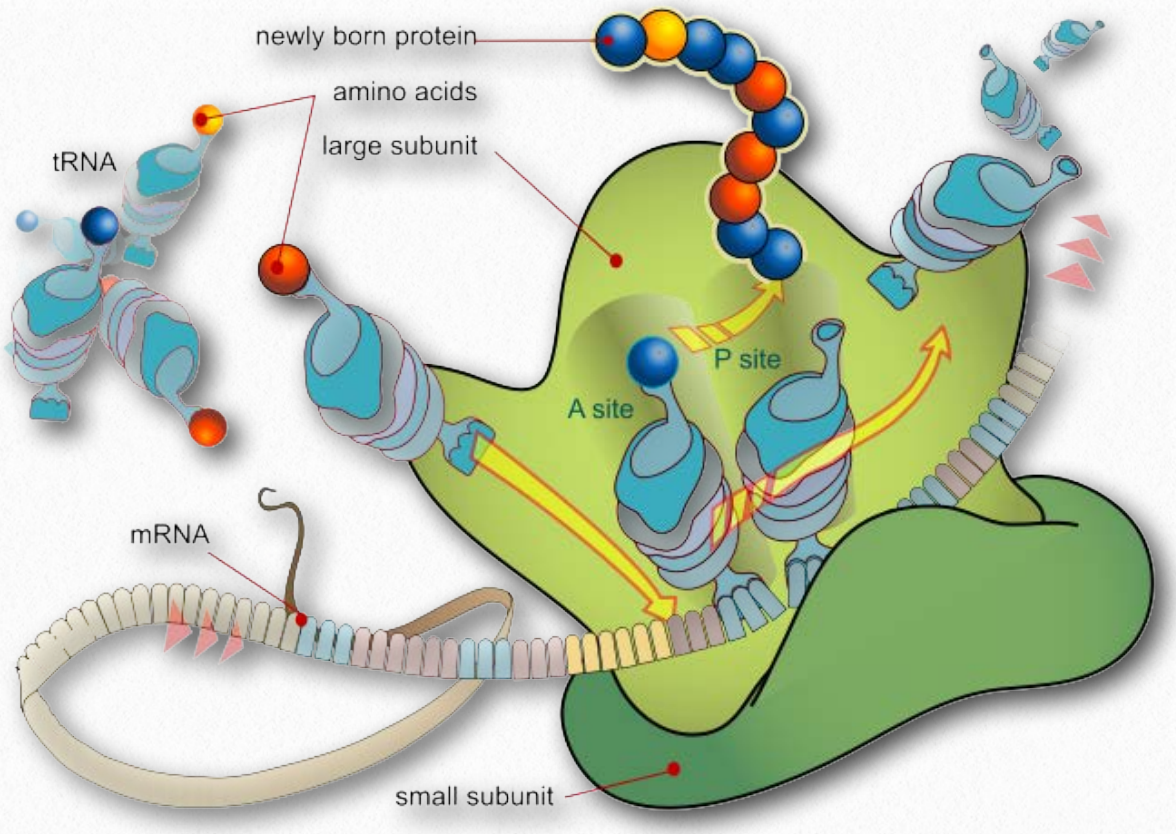


Figure 7.97 - Another perspective of translation. The 3' end of the mRNA is on the left and the ribosome is moving from right to left

But the vast majority of proteins in eukaryotic cells are made by ribosomes, free or membrane-bound, in the cytoplasm of the cell (the exceptions are a handful of proteins made within mitochondria and chloroplasts).

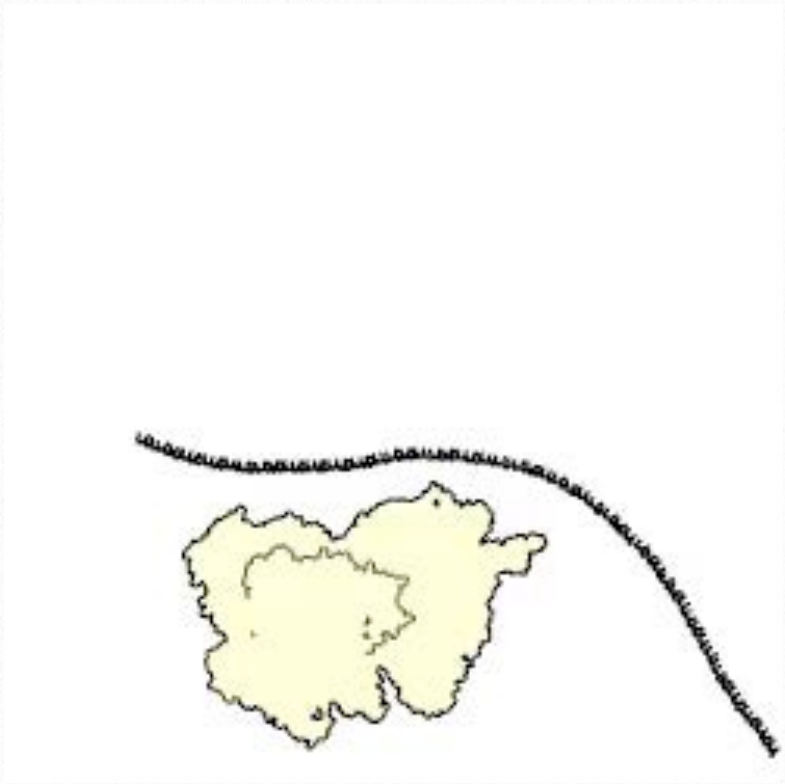
Delivery

Each of the thousands of proteins made in the cytoplasm must, therefore, be delivered to the appropriate cellular compartment in which it functions.

Polypeptide processing

What happens to the newly synthesized polypeptide after it is released from the ribosome? As you know, functional proteins are not simply strings of amino acids. The polypeptide must fold properly in order to perform its function in the cell. It may also undergo a variety of modifications such as the addition of phosphate groups, sugars, lipids, etc. Some proteins are produced as inactive precursors that must be cleaved by proteases to be functional.

An additional challenge in eukaryotic cells is the presence of internal, membrane-bounded compartments. Each compartment contains different proteins with different functions.



Movie 7.3 Translation of a protein secreted into the endoplasmic reticulum. Small subunit in yellow. Large subunit in green. tRNAs in blue.

Wikipedia

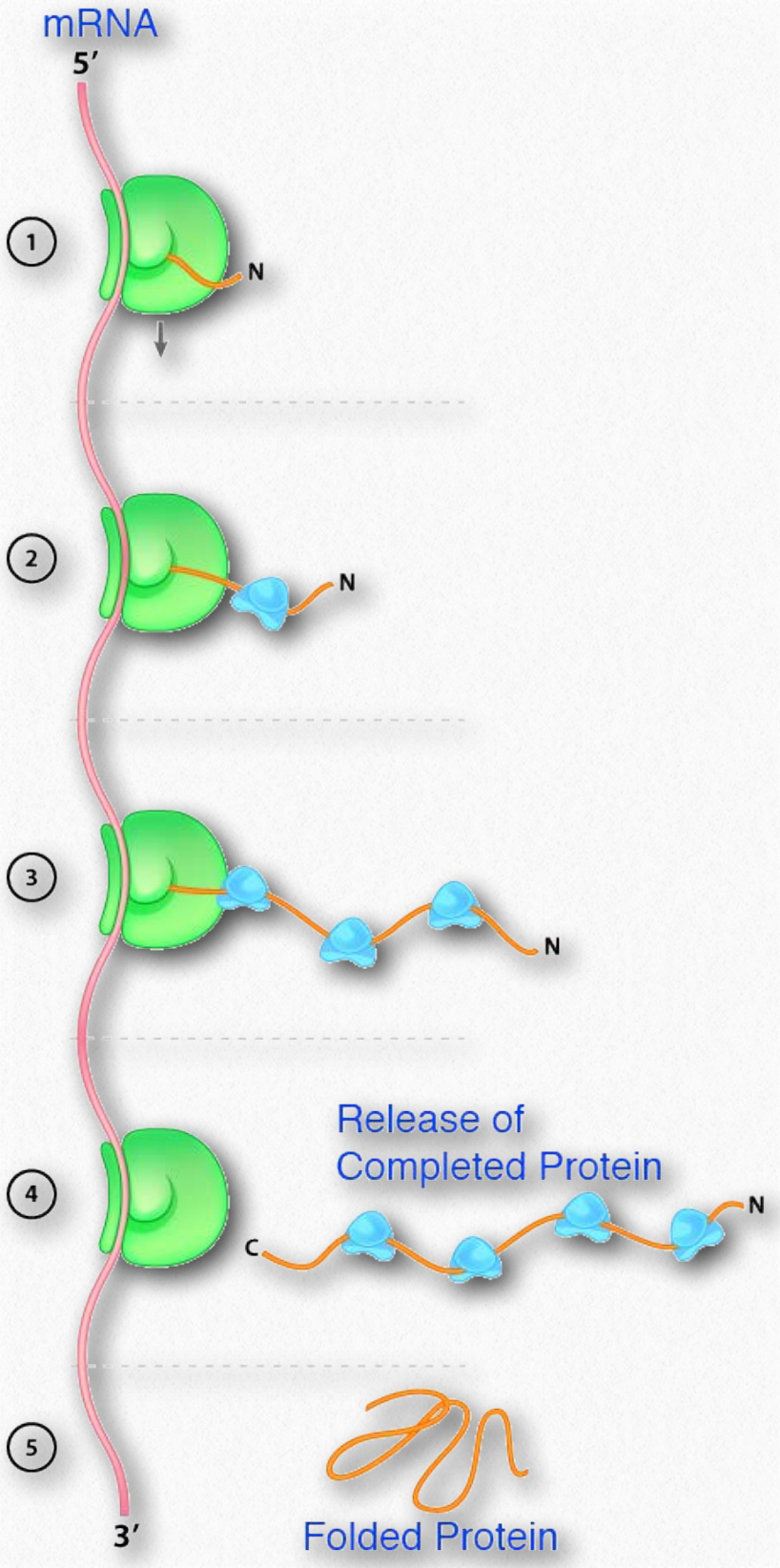


Figure 7.98 - Action of chaperone to facilitate proper folding of a protein (orange)

Image by Aleia Kim

Some proteins are delivered to their destinations in an unfolded state, and are folded within the compartment in which they function. Others are fully folded and may be post-translationally modified before they are sent to their cellular (or extracellular) destinations.

Some proteins are delivered as they are being synthesized (co-translationally - see Movie 7.3) while others are sorted to their compartments post-translationally. But, with the exception of cytosolic proteins, all proteins must cross membrane barriers, through membrane channels or other "gates", or by transport within membrane vesicles that fuse with the membrane of the target organelle to deliver their contents.

YouTube Lectures by Kevin HERE & HERE

Folding and post-translational modifications

Proper folding of a protein into its 3-dimensional conformation is necessary for it to function effectively. As described in an earlier chapter (HERE), the folding of a protein is largely influenced by hydrophobic interactions that result in folding of the protein in such a way as to position hydrophobic residues in the interior, or core, of the protein, away from the aqueous environment of the cell.

Proper folding may also involve the interaction of regions of the polypeptide that are distant from each other, so that portions of the N-terminal region of the polypeptide may be in close proximity to parts of the C-terminus of the final folded mole-

cule.

As a polypeptide emerges from the ribosome, however, the N-terminal region of the polypeptide may begin to fold on itself, with adjacent parts of the chain interacting in inappropriate ways, before the entire protein has been made. This can result in misfolding of the protein and consequent malfunction. To prevent misfolding, cells have protein chaperones, whose function is to bind to and shield regions of polypeptides as they emerge from the ribosome, and keep them from improperly interacting with one another or with other proteins in the vicinity, until they can fold into their correct final shape (Figure 7.98). In addition, there are classes of chaperones that are able sequester proteins in such a way as to permit unfolding and refolding of misfolded polypeptides. These proteins ensure that the vast majority of proteins in cells are folded into their correct, functional 3-dimensional shapes.

Protein sorting

The process by which proteins are identified as belonging to a particular compartment and then correctly delivered to that destination is known as protein sorting. How does a cell know where a particular protein should be sent?

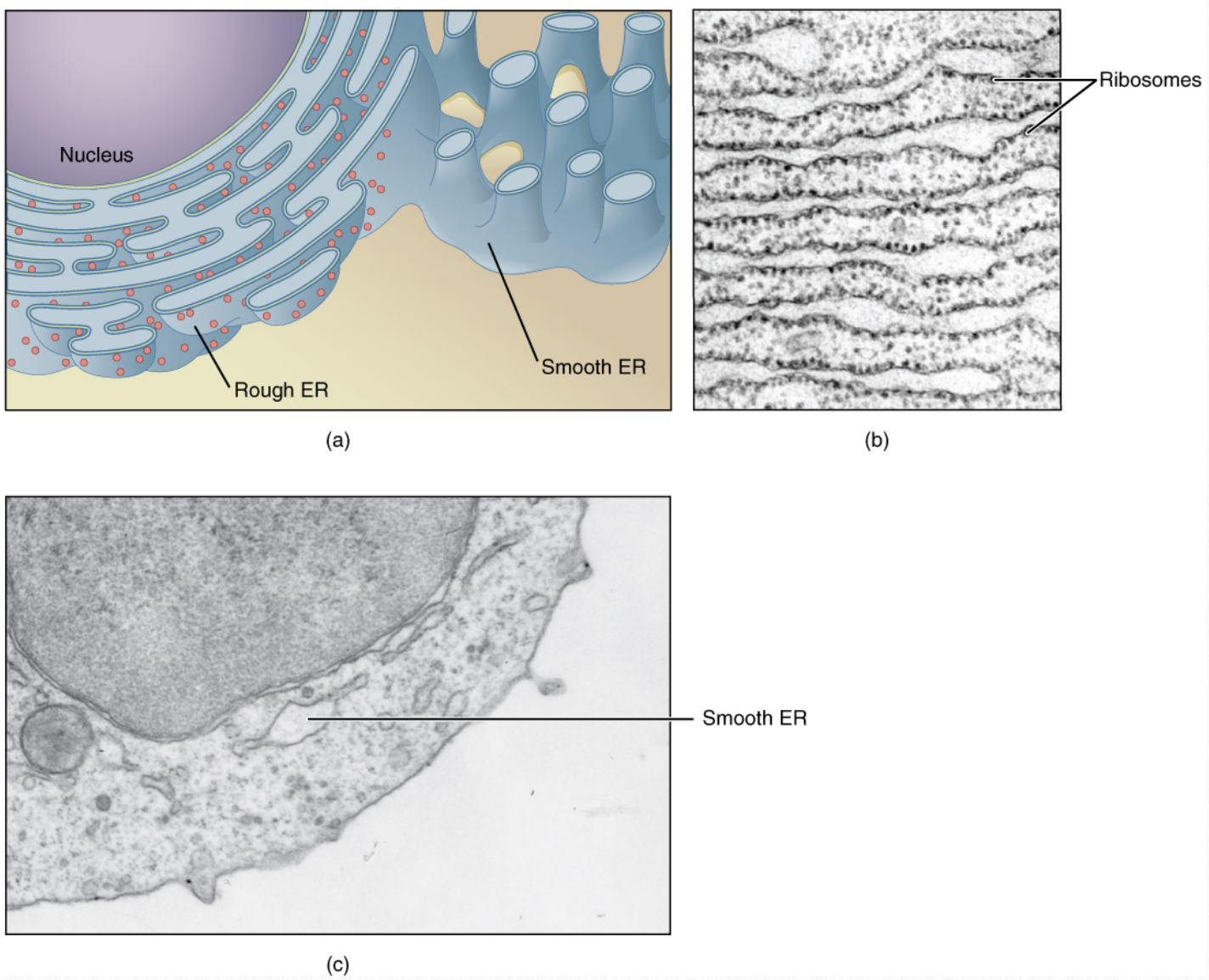


Figure 7.99 - Rough (ribosome bound) and smooth endoplasmic reticulum

Wikipedia

Proteins have "address labels" or sorting signals that indicate which cellular compartment they are destined for. Characteristic sorting signals are found on proteins that are sent to the nucleus, the ER (Figure 7.99), the mitochondria, etc.

Signal sequences

What do these sorting signals look like? Most sorting signals (also called signal sequences) are short stretches of amino acid sequence (that is, they are part of the amino acid sequence of the protein). Different cellular compartments have different "address labels".

Signal sequences may be found at the N-terminal or C-terminal region of proteins, or they may be within the amino acid sequence of the proteins. The location of the signal sequence for any given protein is fixed, however. Signal sequences for proteins to be delivered to the endoplasmic reticulum (ER) are found at the N-terminus of the protein. Mitochondrial and chloroplast proteins encoded by nuclear genes also have N-terminal signal sequences. Signal sequences for nuclear proteins, by contrast, are internal to the polypeptide, and may consist of one or more stretches of amino acids that will be displayed on the surface of these proteins once they are folded.

Figure 7.100 - N-terminal signal sequence (green) emerging from the ribosome.

Free and membrane-bound ribosomes

Proteins are synthesized by ribosomes in the cytoplasm or by those that associate with membranes temporarily (membrane-bound ribosomes). The free ribosomes make proteins that are destined for the nucleus, as well as those going to chloroplasts, mitochondria and peroxisomes. Nuclear proteins are delivered in their folded state, while chloroplast and mitochondrial proteins are threaded through translocation channels in the membranes of these organelles, to be folded at their destination.

Proteins that are destined for the ER, the Golgi apparatus, lysosomes as well as those that are to be secreted from the cell are first delivered to the ER by ribosomes that associate with the membrane of the rough ER and synthesize the protein directly into the ER. Proteins delivered by this manner into the lumen of the ER undergo folding and modification in the ER. All proteins delivered to the ER, regardless of their final destination, have an N-

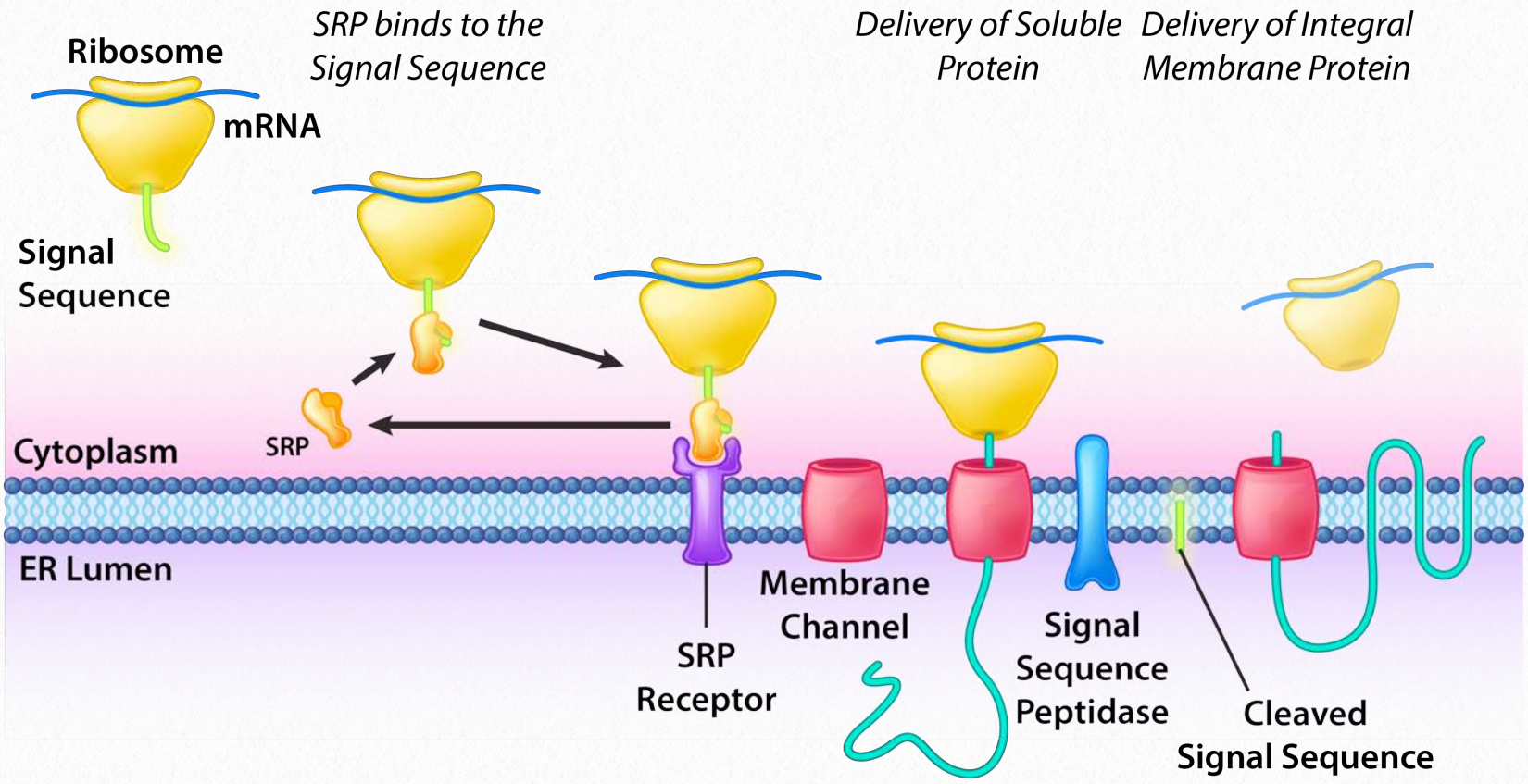


Figure 7.101 - Translation of a protein into the endoplasmic reticulum

Image by Aleia Kim

terminal ER signal sequence of 15-30 amino acids.

Protein delivery into the endoplasmic reticulum

The N-terminal part of a protein is the first part of a nascent polypeptide that emerges from the ribosome (Figure 7.100). The sequence of amino acids in this region, if it is an ER signal, will be recognized and bound by a ribonucleoprotein complex called the Signal Recognition Particle (SRP). Binding of the SRP to the N-terminal signal sequence causes translation to pause. The SRP, in turn, is bound by an SRP receptor in the ER membrane (Movie 7.3 & Figure 7.101), effectively anchoring the ribosome to the membrane.

The location of SRP receptors near membrane channels in the ER positions the ribosome over a translocation channel. Once the ribosome is docked over the channel, the SRP releases the signal sequence, which is threaded through the channel, with its hydrophobic residues interacting with the hydrophobic interior of the membrane. Translation resumes at this point and the rest of the protein is delivered into the lumen of the ER as it is made. The ribosome remains associated with the ER membrane till translation is completed, at which point it dissociates. The signal sequence, which is no longer needed once the protein has been delivered, is cleaved off by a membrane associated signal peptidase, releasing the completed protein into the ER lumen.

While soluble proteins are delivered into the ER, integral membrane proteins do not pass all the way through, but, instead, are anchored in the membrane of the ER by hydrophobic stop transfer sequences.

Folding and modification

Proteins in the lumen of the ER are folded with the help of numerous chaperones resident in the endoplasmic reticulum. The environment within the ER lumen is also more oxidizing than the cytosol, and permits the formation of disulfide bonds to stabilize the folded proteins. Protein disulfide isomerase, an enzyme active in the ER lumen both helps to make disulfide bonds and removes bonds that were incorrectly made during the folding process. In addition, proteins in the ER undergo modifications such as glycosylation and addition of glycolipids. Multimeric proteins are also assembled from their subunits in the ER.

Proteins that have been correctly folded and modified are transported from the ER, in membrane vesicles, to their final destinations. Improperly folded proteins are recognized by a surveillance mechanism in the ER and are sent back to the cytoplasm to be degraded in proteasomes.

Graphic images in this book were products of the work of several talented students. Links to their Web pages are below

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Click HERE for Pehr Jacobson's Web Page

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Email **Kevin Ahern** / **Indira Rajagopal** / **Taralyn Tan**

Translation

To the tune of "*Maria*" (from "West Side Story")

Metabolic Melodies Website HERE

Translation
The most intricate thing I ever saw

From five prime to three prime, translation, translation
The final step that we know about the central dog-ma

Amino, carboxyl, translation, translation. . . .
Translation, translation, translation . .
Translation!

I just learned the steps of translation
And all the things they say
About tRNA
Are true

Translation!
To form peptide bonds in translation
The ribosomal cleft
Must bind to an E-F
tee-you!

Translation!
A-U-G binds the f-met's cargo
16S lines up Shine and Dalgarno
Translation
I'll never stop needing translation
The most intricate thing I ever saw
Translationnnnnnnnnnnnnnnn

Recording by Tim Karplus
Lyrics by Kevin Ahern

Good Protein Synthesis

To the tune of "*Good King Wenceslaus*"

Metabolic Melodies Website HERE

Amino acids cannot join
By themselves together
They require ribosomes
To create the tether

All the protein chains get made
'Cording to instruction
Carried by m-R-N-A
In peptide bond construction

Small subunit starts it all
With initiation
Pairing up two RNAs
At the docking station

Shine Dalgarno's complement
In the 16 esses
Lines the A-U-G up so
Synthesis commences

Elongation happens in
Ribosomic insides
Where rRNA creates
Bonds for polypeptides

These depart the ribosome
Passing right straight through it
In the tiny channels there
Of the large subunit

Finally when the sequence of
One of the stop codons
Parks itself in the A site
Synthesis can't go on

P-site RNA lets go
Of what it was holding
So the polypeptide can
Get on with its folding

Recording by David Simmons
Lyrics by Kevin Ahern