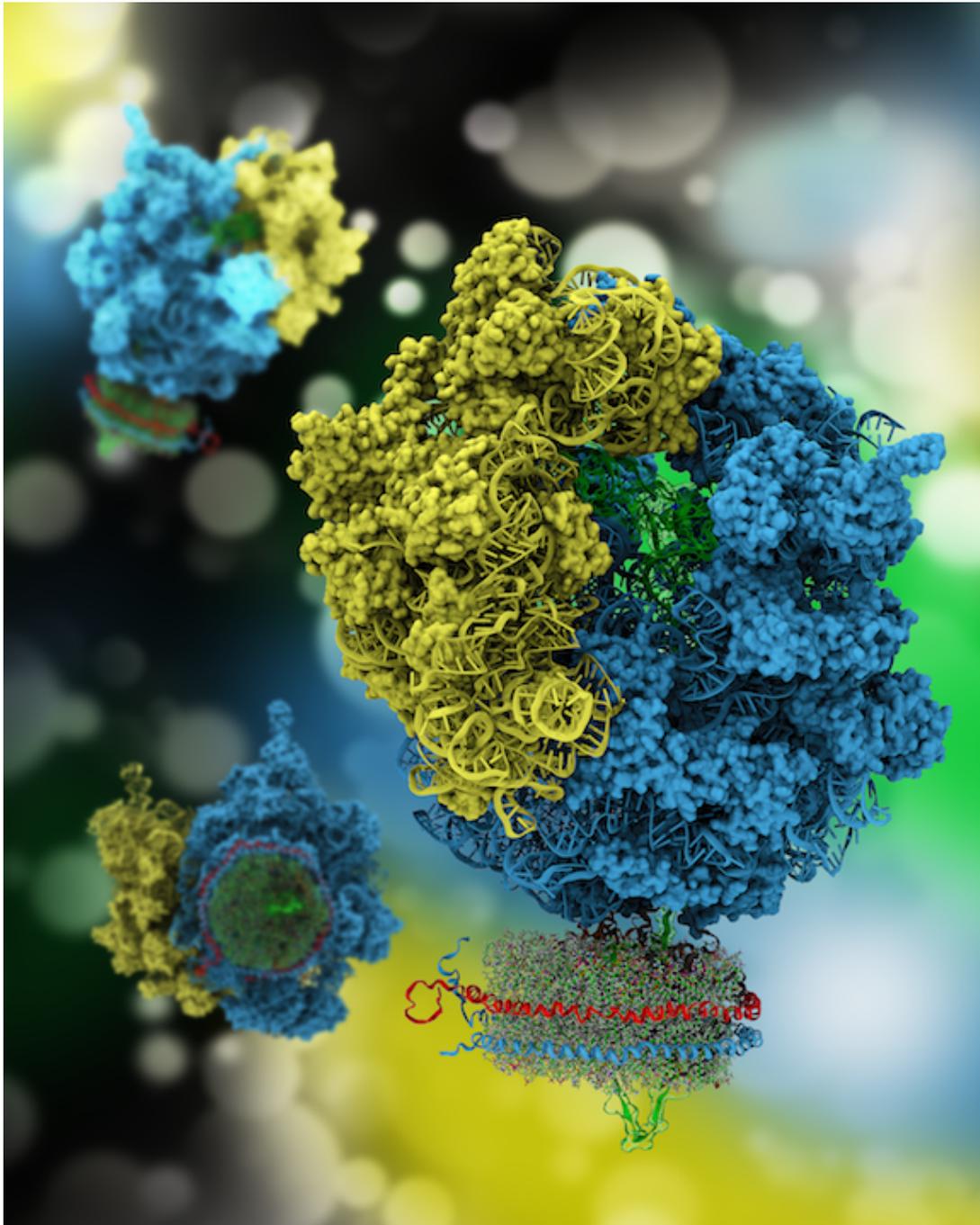


Case Study: Ribosome

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A colored version of this Case Study is available at
<http://www.ks.uiuc.edu/Training/CaseStudies/pdfs/ribosome.pdf>.



Ribosome - the birth place of proteins in living cells

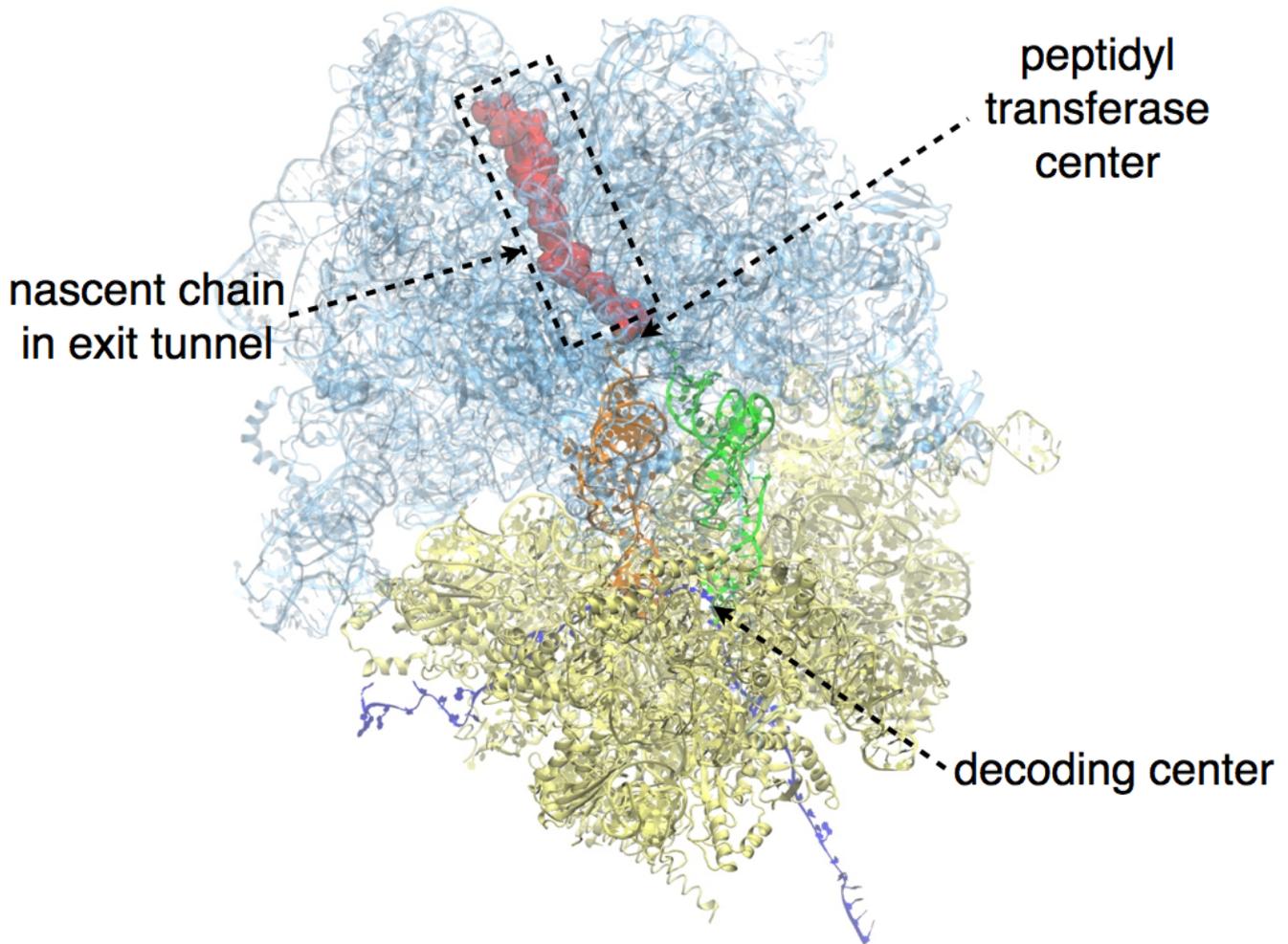


Figure 1: A translating ribosome decodes the genetic sequence on the mRNA (blue) in the decoding center in the small subunit (yellow). The peptide bond formation takes place at the peptidyl transferase center in the large subunit (cyan) to elongate the nascent chain (red). The nascent chain leaves the ribosome through the exit tunnel. The tRNAs are colored orange and green.

bond formation between the nascent chain and the new amino acid, elongating the nascent chain by one amino acid at a time. The elongation process continues until the whole nascent protein is synthesized. During the synthesis the nascent chain migrates through a tunnel in the large subunit, the so-called exit tunnel, to exit the ribosome. When all amino acids of the nascent protein are linked as a polypeptide, the one encoded in the gene of that specific protein, the termination step kicks in to release the nascent polypeptide from the ribosome; this step is triggered by the stop codon on the mRNA. Finally, in the recycling step, the small and large ribosome subunits separate and are reused again to assemble with other mRNA strands in order to synthesize new proteins.

The ribosome does not work alone; throughout the four stages of protein synthesis, the ribosome works jointly with various binding ligands and factors to carry out the processes in an accurate and efficient manner. Also often more than one ribosome work on a single, long mRNA at a time in a complex called polysome.

In the present case study, you will learn about three critical issues of ribosomal translation,

namely the decoding step, the role of the ribosome in peptide-bond formation at the so-called peptidyl transferase center (PTC), the place in the ribosome where amino acids are joined together through peptide bonds, as well as the role of the ribosome in exiting, folding and subsequent localization (delivery to the right place in the cell) of the nascent polypeptide. The bacterial ribosome will be used as the example model. For translation in higher-level organisms, variations in the mechanism exists but the main ideas remain valid.

Exercise 1: Visualizing the ribosome in VMD

In this exercise you will use VMD to visualize a bacterial ribosome. You are required to know the basics of VMD, if not, please consult the VMD tutorial (<http://www.ks.uiuc.edu/Training/Tutorials/vmd-index.html>). Load the provided file 2WDG-2WDI.pdb into VMD.

A. Identifying the RNAs in the ribosome. The three RNAs are called 5S, 16S and 23S respectively. Make a graphical representation for each of them and explore their structure. (Hint: type `segname 5S` in the selection text box for selecting the 5S RNA.) How many bases are there in the 5S, 16S and 23S RNA respectively?

B. Identifying the proteins in the ribosome. The proteins in the large subunit are assigned names L1, L2, etc. and the proteins in the small subunit are assigned names S1, S2, etc. Make ONE graphical representation that includes all the proteins in the large subunit. Do the same for the small subunit. (Hint: type `segname "L.*"` in the selection text box for selecting all proteins in the large subunit. Similarly use `segname "S.*"` for selecting proteins in the small subunit.) Are the proteins mainly in the core or at the exterior of the ribosome?

C. Electrostatics inside the ribosome. The ribosome is a highly negatively-charged system. Why? (Hint: consider the charges on the backbone of RNAs.) How does the ribosome stabilize itself given the highly negative charges? (Hint: Consider the type and number of ions in the pdb file. One can use the selection text `ion` to select ions in VMD.)

D. Antibiotics targeting the ribosome. In the provided structure, there is an antibiotic bound to the ribosome. Locate the antibiotic and make a figure of it. (Hint: Use the selection texts `resname PAR` to locate it in VMD.) The antibiotic is called Paromomycin. Is it bound to the large or the small subunit? Having this information, guess how Paromomycin stops the bacterial ribosome from functioning. (Hint: What are the major functions of the two subunits of the ribosome?)

2 Decoding of mRNA in the ribosome

Decoding is the first step of the protein elongation cycle. In this step the sequence of nucleotide bases of mRNA is decoded such that a protein's amino acid sequence is recognized and the corresponding protein can be synthesized. The units of the genetic information on the mRNA are called codons. Codons are sets of three nucleotides, chosen among four types: adenine (A), uracil (U), cytosine (C), and guanine (G). Each codon gets decoded and translated into an amino acid during translation. The relationship between codons and amino acids is shown in Table 1. One can recognize in Table 1 that some codons correspond rather to stop signals, namely the codons UAA, UGA and UAG.

Table 1: The Genetic Code Table

		2nd letter												
		U	C	A	G									
1st letter	U	UUU	UCU } UCC } UCA } UCG }	UUA } UUG }	UAA } UAG }	UGU } UGC } UGA } UGG }	U C A G							
		UUC						}Phe	}Leu	}Tyr	}Cys			
		UUA										}ser	}stop	}stop
		UUG												
	C	CUU	CCU } CCC } CCA } CCG }	CUA } CUC }	CAU } CAC }	CGU } CGC }	U C A G							
		CUC						}Leu	}Pro	}His	}Arg			
		CUA										}Gln	}Arg	
		CUG												
	A	AUU	ACU } ACC } ACA } ACG }	AUA } AUG }	AAU } AAC }	AGU } AGC }	U C A G							
		AUC						}Ile	}Thr	}Asn	}Ser			
		AUA										}Lys	}Arg	
		AUG												}Met
	G	GUU	GCU } GCC } GCA } GCG }	GUA } GUG }	GAU } GAC }	GGU } GGC }	U C A G							
		GUC						}Val	}Ala	}Asp	}Gly			
		GUA										}Glu		
		GUG												

This table could be viewed as a dictionary that connects the nucleotide sequence on one hand and its corresponding protein sequence, which is synthesized by the ribosome, on the other hand.

The decoding step starts with the delivery of a ternary complex, consisting of an aminoacyl-tRNA, a GTP molecule and elongation factor Tu (EF-Tu), to the decoding center of the ribosome (Fig. 2-(a)). One end of the tRNA, namely the one which carries the amino acid, is surrounded by the protein factor EF-Tu. The other end of the tRNA binds to the mRNA through its so-called anticodon loop, a set of three nucleotides which corresponds to the amino acid charged.

Two cases of binding between the ternary complex and the mRNA are possible:

(1) The codon-anticodon pair matches, or in other words, the three nucleotides on the anticodon form Watson-Crick interactions (see Fig. 2-(b) and (c)) with the corresponding nucleotides on the codon, meaning the amino acid charged is the correct one. Once the codon-anticodon pair matches, a signal is transduced over a distance of about 70 Å to the other end of the ternary complex which results in the hydrolysis of the GTP molecule. The GTP hydrolysis is followed by conformational rearrangements in EF-Tu and then detachment of the factor from the ribosome. The tRNA is then freed to move its aminoacyl end to the peptidyl transferase center into a position ready for peptide bond formation. This bond formation will be discussed in the next section.

(2) The anticodon on the tRNA does not match with the codon of the mRNA. In this case hydrogen bonds do not form between all three basepairs of the codon-anticodon pair, no message is sent to the EF-Tu and GTP hydrolysis will not take place. As a result the ternary complex leaves the ribosome. New ternary complexes keep coming in until a codon-anticodon pair matches.

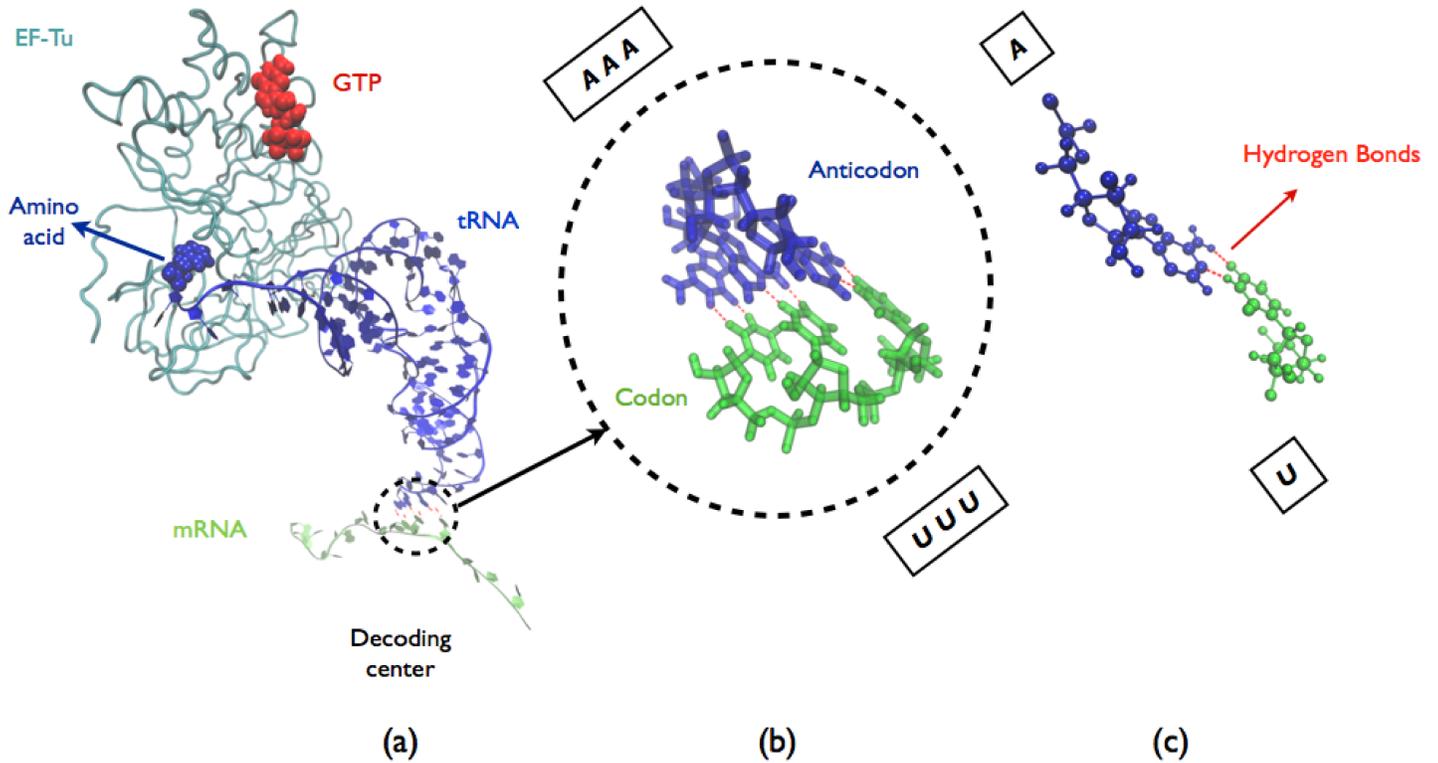


Figure 2: (a) The ternary complex, including an aminacyl-tRNA, a GTP molecule and an elongation factor-Tu (EF-Tu), binds to the mRNA through tRNA's anticodon, located at the bottom end of the tRNA in the anticodon loop. (b) A closer view of the decoding center and the codon-anticodon matching. The particular codon shown has three uracils (U) and the matching anticodon is made of three adenines (A). (c) Only one A-U basepair is shown and the hydrogen bonds between these two nucleotides are drawn as red dotted lines. Such base pairing through multiple hydrogen bonds is called a Watson-Crick interaction as it is the same interaction as the one arising in double-stranded DNA where it leads to the Watson-Crick DNA structure.

Exercise 2: The decoding pathway

As you read above, once the codon-anticodon pair matches, the hydrolysis of the GTP molecule occurs. The nature of the communication between the decoding center (where the codon-anticodon pair forms) and the GTP binding site is unknown despite decades of intense studies. Start a VMD session and load the provided decoding.pdb file. Verify the distance between the decoding center and the GTP molecule. You can pick the middle nucleotide of the anticodon at the decoding center and determine with VMD its distance from the GTP molecule.

Exercise 3: What if the ribosome was not there?

While the genetic code has some redundancy built in, the ribosome still needs to be able to discriminate between some tRNAs differing by only one base in the anticodon when matching them to the mRNA codon (cognate vs. near-cognate). A mismatch of one base pair (for example, CUU vs. UUU) results in the loss of a single hydrogen bond; let us assume the resulting free energy difference is 2.5 kcal/mol. What is the minimum error rate possible for this free energy difference? Is codon-anticodon recognition sufficient to explain a typically measured error rate of about 5×10^{-3} ?

The process of selecting the correct amino acid among the pool of other amino acids has to take place with both speed and accuracy. However as you have realized in Exercise 3, the energy difference between cognate and near-cognate codon-anticodon pairs cannot provide this level of accuracy. Today the ribosome is known to be responsible for the enhanced accuracy in favoring the cognate codon-anticodon pair over the near-cognate pairs through what is the so-called ribosomal induced fit. Once the cognate codon-anticodon pair is recognized, the ribosome undergoes local rearrangements around the decoding center and then brings about a non-local, i.e., spatially more extended, conformational change which involves domain-wise motions of the small subunit. The resulting conformation favors the GTP hydrolysis significantly for the cognate pair compared to a near-cognate pair. In other words the ribosome exploits the small energy difference between the cognate and near-cognate pairs to trigger a series of processes that eventually give rise to the low error rate. You can think about the induced fit mechanism as a domino effect initiated by a small energy difference. A realization of such effect is not possible without the complexity of the ribosome. The free energy involved in the pre-triggering conformation is provided by the binding of the ternary complex.

3 Peptide-bond formation and peptidyl transferase center (PTC)

The ribosome is also called a ribozyme because it is the molecular complex in living cells responsible for catalyzing the peptide-bond formation of nascent proteins. In this section, we will learn about the structural basis underlying the primary enzymatic function of the ribosome, namely to connect adjacent amino acids using tRNAs during the translation process of protein biosynthesis.

3.1 The ribosome is an entropy trap

Proteins are made of amino acids, which are building blocks that can be linked into a chain polypeptide. Individual amino acids in a polypeptide are held together by peptide bonds (see Figure 3). The peptide bond is a covalent chemical bond formed between the carboxyl group (C=O) of one amino acid and the amino group (N-H) of the other amino acid. The peptide-bond formation is a dehydration process, as shown in Figure 3 in the chemical formulas describing the reaction.

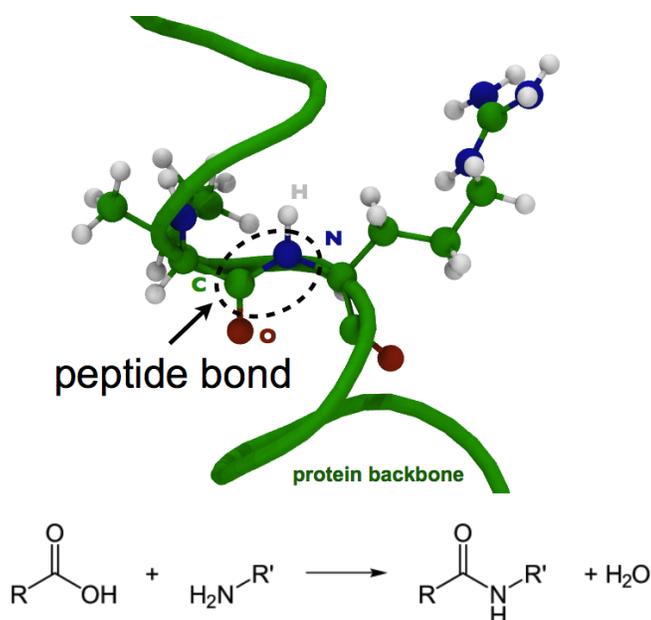


Figure 3: Peptide bond in proteins. This protein segment is from ribosomal protein L1.

Compared with non-catalyzed conditions, the ribosome can speed up the peptide-bond formation by a factor of 2×10^7 , which is extraordinarily efficient. However, the peptide-bond formation inside the ribosome is slightly less favorable in regard to reaction enthalpy than in solution [3]. This means that the ribosome enhances peptide-bond formation completely by lowering the entropy of activation. The ribosome does this by properly aligning substrates at the ribosomal peptidyl transferase center (PTC), which is the core region of the ribozyme. The peptide bond could just form spontaneously inside the ribosome. In this sense, the ribosome is called an entropy trap, which traps substrates into conformations that are optimal for peptide-bond formation.

3.2 The peptidyl transferase center (PTC)

PTC is the region where peptide-bond formation occurs. PTC is highly conserved and is entirely constructed by ribosomal RNAs, not protein; the closest ribosomal protein is about 20 Å away

from the PTC and is not involved in the peptidyl transferase reaction. This RNA relic, namely the PTC, is the most significant piece of evidence suggesting the RNA world hypothesis ². Now, let us take a closer look at the PTC, again, using *E. coli* ribosome as the model system.

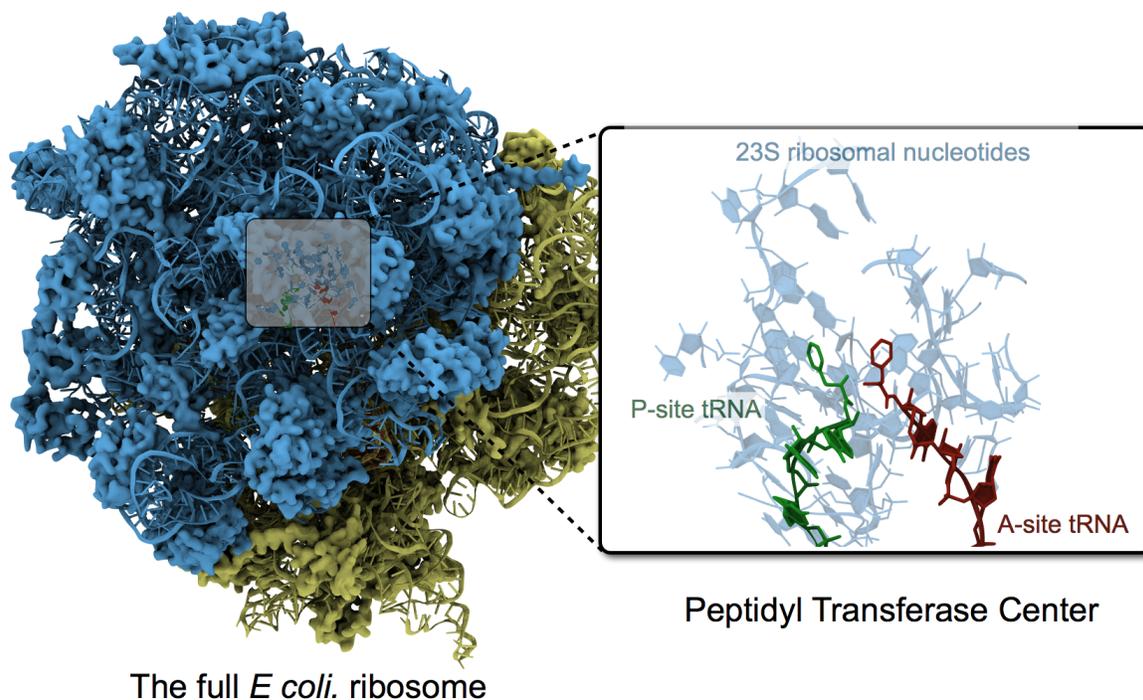


Figure 4: Peptidyl Transferase Center of the ribosome. The structure shown was determined by crystallography (PDB code: 2wdl, 2wdk) [4]

. The large subunit is shown in Cyan, the small subunit is shown in yellow. The inset figure shows the atomic detail of the PTC, where the A-site tRNA is shown in red, the P-site tRNA is shown in green and structure of the 23S ribosome RNA is shown in light Cyan.

Just before peptide-bond formation, the nascent peptide is carried on the P-site tRNA and the new amino acid to be added to the nascent peptide is carried on the A-site tRNA. The final three nucleotides of any tRNA on the 3' end are always a cytosine-cytosine-adenine (CCA) sequence. This characteristic of the tRNAs is the "handle" for the ribosome to properly position the P-site tRNA and the A-site tRNA in a conformation which is favorable for peptide-bond formation. In this reaction, the carbon atom of carboxyl group (C=O) on the P-site tRNA is linked to the nitrogen atom of the amino group (N-H) on the A-site tRNA.

As Figure 4 shows, about 30 nucleotides from the ribosomal 23S form an RNA environment in which the P-site tRNA CCA end and the A-site tRNA CCA end are placed very close to each other. Figure 5 shows the ribosomal 23S nucleotides G2251 and G2252 at the P-site form hydrogen bonds with the P-site CCA and U2555 G2553 G2583 U2584 at the A-site forming hydrogen-bond networks with the A-site CCA. Those two groups of ribosomal nucleotides are the PTC scaffolds for precisely aligning the tRNA and nascent peptide substrates in the PTC to assume proper orientations for the peptide-bond formation.

²http://en.wikipedia.org/wiki/RNA_world_hypothesis

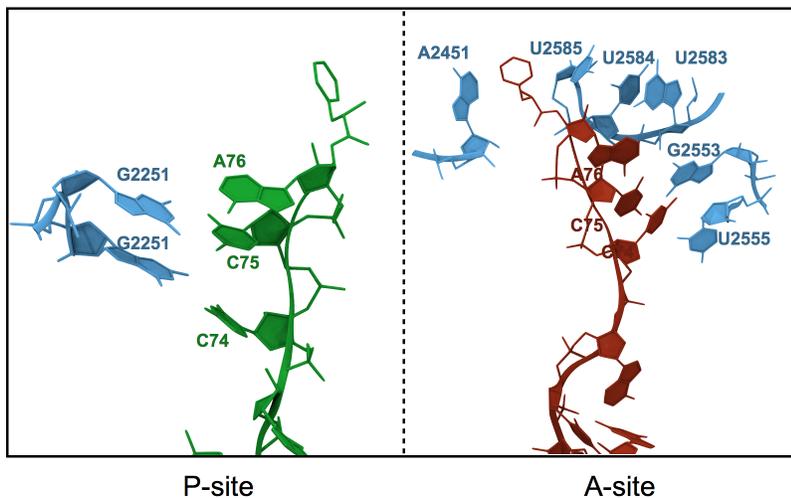


Figure 5: Ribosomal nucleotides in the PTC align substrates to assume proper conformation for peptide-bond formation. The structure shown was determined by crystallography (PDB code: 2wdl, 2wdk)

Several other nucleotides in the PTC are as important as the scaffold nucleotides. For example, A2451 is sitting right between the P-site nascent peptide and the A-site amino acid. Study has shown that the base of A2451 is not important for the ribosome's function [5], however, the 2'-OH group on the nucleoside of A2451 is very critical for both aligning the substrates and for participating in the peptide-bond formation process through a proton-shuttling mechanism [6] (see below). The U2585 is sitting at the flank side of A2451, forming a gate for the linkage of the nascent peptide and the P-site tRNA. There are experimental results suggesting that U2585 is critical for protecting the peptidyl-tRNA at the P-site from accidental hydrolysis by water molecules [7], which would otherwise result in pre-mature release of the nascent peptide.

3.3 The quantum nature of the peptide-bond formation

The mechanism of peptide-bond formation is best studied by quantum mechanics methods. Many plausible mechanisms have been attributed to peptide-bond formation inside the ribosome. We will not talk about the details of different mechanisms, and instead, refer the reader to an article [6] that offers a comprehensive view of the quantum process during peptide-bond formation inside the ribosome. Despite of all the different mechanisms suggested by quantum mechanics, one thing is for sure, namely the ribosome brings together and aligns the substrates for bond formation to occur rapidly, which means the ribosome acts as an entropy trap.

3.4 The role of water

Water molecules might be also involved in the peptide-bond formation process in the PTC. Crystallographic structures have confirmed the presence of two water molecules in the vicinity of the 2'-OH group of the P-site tRNA A76. The spatial arrangement suggests that water molecules could participate a proton shuttle process which facilitates the transition from a reaction intermediate to the completion of peptide-bond formation [8].

3.5 After peptide-bond formation

After peptide-bond formation, the nascent peptide is one amino acid longer and on the A-site tRNA linked to the nascent chain. The ribosome can undergo a process called translocation which brings the P-site tRNA to the E site and the A-site peptidyl-tRNA to the P-site. After the translocation, the E-site tRNA will eventually leave the ribosome, the P-site ribosome carries the nascent peptide and the ribosomal A site will accommodate a new aminoacyl-tRNA through the decoding process which had been discussed in the previous section.

Exercise 4: Identify A-minor motifs in the ribosome

A-minor motifs are a key presence in the ribosome to help maintain tertiary structures of ribosomal RNAs. The motifs may consist of adenine (A) rich edges on the minor groove of RNA inserted into neighboring minor grooves usually rich in Cytosine (C) and Guanine (G). Hydrogen bonds and van der Waals interactions are dominant driving forces to keep the motifs together. The authors in [9] have summarized four subtypes of A-minor motifs, depending on the spatial arrangement of neighboring nucleotides. Read [9] and identify at least two A-minor motifs around the PTC. For this purpose, you can use the PDB structures cited in the paper [9], however, we recommend using PDB structures 2wdk and 2wdl for educational purposes because they are more recent and more complete.

Hint We have learned that the major function of the PTC is to align substrates for the peptide-bond formation, so there is a high possibility that you will find A-minor motifs around the substrates, which are the P-site and A-site tRNAs

4 Exiting, folding and translocation of protein nascent chains

4.1 The ribosomal exit tunnel

While the protein nascent chain is being elongated at the PTC during the elongation cycle, the nascent chain leaves the ribosome through the so-called ribosomal exit tunnel, a channel initiating from the PTC and reaching the outer surface of the large subunit (see Fig. 6). Exercise 5 will guide you to study the structure of the exit tunnel. For a long time the exit tunnel was considered to be a passive conduction channel for the nascent protein to migrate through, however, an increasing number of studies [10–12] have shown that the exit tunnel is actually involved in many co-translational activities of the nascent peptides, such as folding of the nascent chain inside the exit tunnel [12] (see Exercise 5).

Exercise 5: Exploring the structure of the ribosomal exit tunnel

In this exercise you will employ VMD to explore the exit tunnel of a bacterial ribosome. For this purpose load the provided file 2WDG-2WDI.pdb into VMD.

A. Locating the exit tunnel. Source the script `macro.tcl` by typing `'source macro.tcl'` in the VMD Tk console. The script defines a macro called `exitunnel` which includes all the residues on the wall of the exit tunnel. Now use this macro to produce a figure of the exit tunnel and the P-site tRNA. (Use the macro by typing `exitunnel` in the selection text box in the graphical representation interface. P-site tRNA can be selected by `segname PRNA`.) Is the wall of the exit tunnel mainly composed of nucleic acids or mainly of proteins?

B. Measuring the size of the exit tunnel. What is the length of the exit tunnel in Å, measuring from the PTC to the outer surface of the ribosome? Given this length, how many amino acids of a nascent protein chain, in an extended conformation, can reside inside the exit tunnel? Next consider the width of the exit tunnel. What is the width of the exit tunnel at the widest and the narrowest parts? Do you think folding of the nascent peptide is possible within the tunnel, and why? (Hint: Is there enough space for any secondary structure to form in the wider part of the tunnel?)

C. Antibiotics and antibiotic resistance. Erythromycin is an antibiotic which binds to nucleotides 2058 and 2059 of the 23S RNA on the wall of the exit tunnel. Locate the two nucleotides. (Hint: Use the selection texts `segname 23S` and `resid 2058 2059`.) What are the types of the two nucleotides (A, U, C or G)? Given that erythromycin binds to the ribosomal exit tunnel, make a guess about how erythromycin kills the bacteria. Methylation of the 2058 nucleotide, i.e., addition of a methyl group to the nucleotide, has been found in some bacterial ribosomes. Why is this happening? (Hint: Consider antibiotic resistance.)

The exit tunnel is designed to allow smooth passage of a wide range of protein sequences, hence the wall is expected to interact indifferently to protein chains of various sequences. However it is found that the exit tunnel can differentiate nascent chains of certain sequences. One famous example is the recognition of nascent peptides with the so-called stalling sequences by the exit tunnel, which will stop the peptidyl transferase reaction at the PTC and stall the ribosome [10].

Translational stalling of the ribosome by stalling sequences, such as TnaC [10] and SecM [13], is employed for translational control, i.e., for the regulation of the rate of protein synthesis.

4.2 Outside ribosome folding and translocation of nascent peptide

Upon leaving the ribosome, proper folding of the protein chain into its functional form has to be ensured. Moreover the protein chain has to be transported to the destined cellular compartment at which the protein performs its function, a process called localization in cell biology. Both folding and localization of the protein chain are reported to be initiated co-translationally, i.e., occur while the nascent peptide is still being elongated inside the ribosome. To achieve proper folding and localization of nascent chains, the ribosome-nascent chain complex binds and works with different ligand partners during translation.

As discussed in Exercise 4, the nascent peptide can fold inside the exit tunnel [12]. In contrast to folding inside the exit tunnel, folding of nascent peptide on the ribosomal surface can be highly error-prone if the partially translated protein chain is allowed to fold in open space. The ribosome avoids mis-folding events by recruiting a protein called the trigger factor, which binds to the exit site of the nascent chain on the ribosomal surface and covers up the nascent chain (see Fig. 6). Folding spaces are restricted by the trigger factor and, hence, mis-folding can be prevented [14].

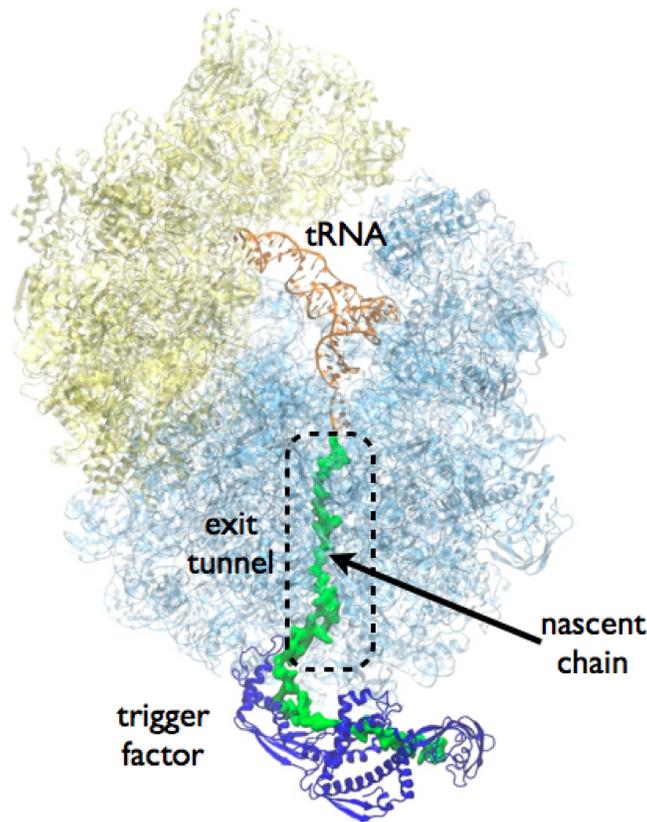


Figure 6: Nascent chain (green) inside the exit tunnel of a translating ribosome (cyan and yellow for large and small subunits, respectively) with trigger factor (blue) bound.

Not all translating ribosomes, but only those synthesizing cytosolic protein chains (proteins that function in the cytoplasm), recruit trigger factors. Indeed, many ribosomes in a living cell

are found to be bound on the membranes instead. These membrane-bound ribosomes dock, for example, with a protein channel called the translocon, through which the nascent peptide is either secreted across or inserted into the membrane during translation, a process called co-translational translocation (see Fig. 7). The translocon only opens to nascent chains with a specific N-terminal sequence called the signal anchor sequence, such that only proteins that need to be translocated can get through. The 1999 Nobel Prize in Physiology or Medicine was awarded to Günter Blobel for his discovery of signal sequence recognition by the translocon to regulate protein translocation.

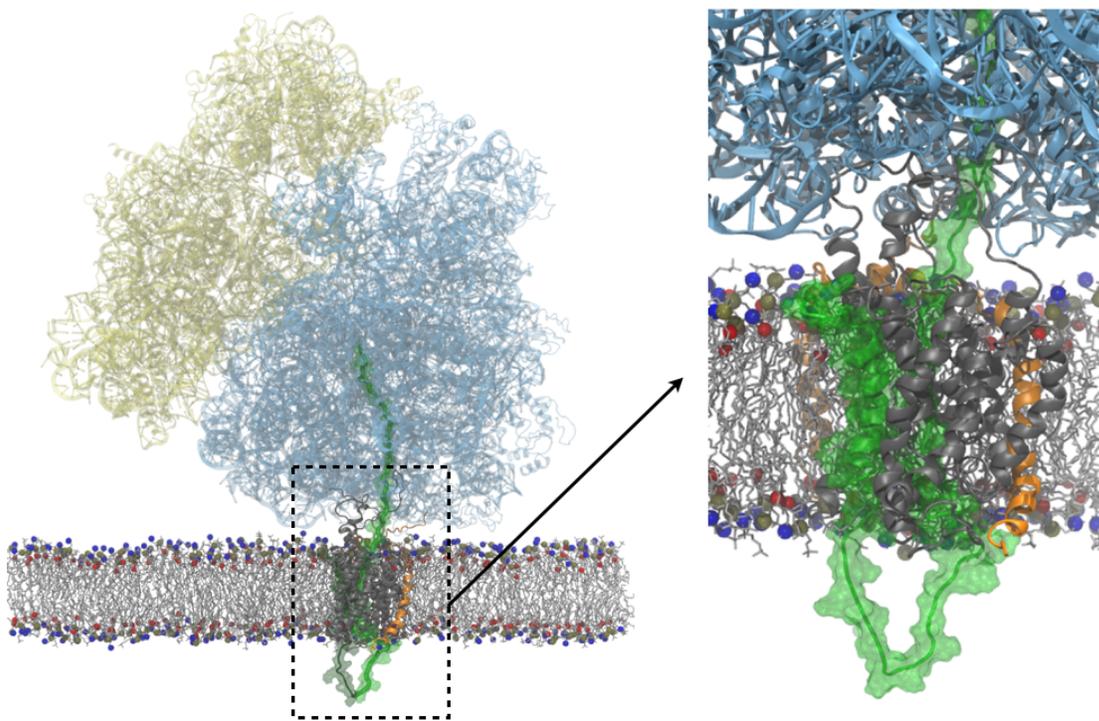


Figure 7: A translating ribosome, docked to the translocon, is feeding a membrane protein nascent chain (green) into the translocon. A closed-up view shows how the nascent peptide is being inserted into the membrane by the translocon. (Model [15] and figures by James Gumbart.)

As mentioned above, the translocon inserts the nascent peptide into the membrane if the chain is destined to function in the membrane. The insertion is done through a so-called lateral gate in the translocon, which is a side opening of the channel to allow passage into the lipid layers. The ribosome plays a role in aiding the insertion process. For example, simulation of a ribosome-translocon-nascent-chain complex [15] suggested interactions between a ribosomal RNA helix with the lipids which assist the insertion of the nascent peptide into the lipids (see movie at <http://youtu.be/5Z6rTkC8vs0>).

5 Concluding remarks

In the present case study we focused on three aspects of ribosomal translation, namely decoding process, peptide bond formation and localization and folding of nascent peptides. Indeed, the ribosome plays many further roles in the translation process.

During the termination step, the stop codon is recognized by a so-called termination factor instead of ternary complex (amino-acid-loaded tRNAs in complex with elongation factor EF-Tu). The termination factor induces cleavage of the fully synthesized protein chain from the last tRNA as well as the dissociation of the two (small and large) ribosomal subunits.

The ribosome can also prevent so-called frame shifting events, in which the mRNA strand does not move by one codon (triplet of nucleotides) during the elongation, but rather by a shorter (e.g., just 2) or longer (e.g., 4) number of bases resulting in a devastating subsequent misreading of the genetic message on the mRNA. The ribosome is able to prevent frame-shifted mRNA from further translation.

The rate of protein synthesis is regulated by the ribosome according to the cellular environment, e.g., in case of cell starvation, the ribosome senses the low level of ATP-molecules, the fuel driving many cellular processes, and avoids in this case translation of a new mRNA to be initiated.

Besides the translocon, there are other membrane proteins that work with the ribosome during localization, such as YidC, working by itself or jointly with the translocon. Often not even translocon and YidC suffice, but further chaperones join the complex with the ribosome, which assists insertion of the nascent chain into the membrane.

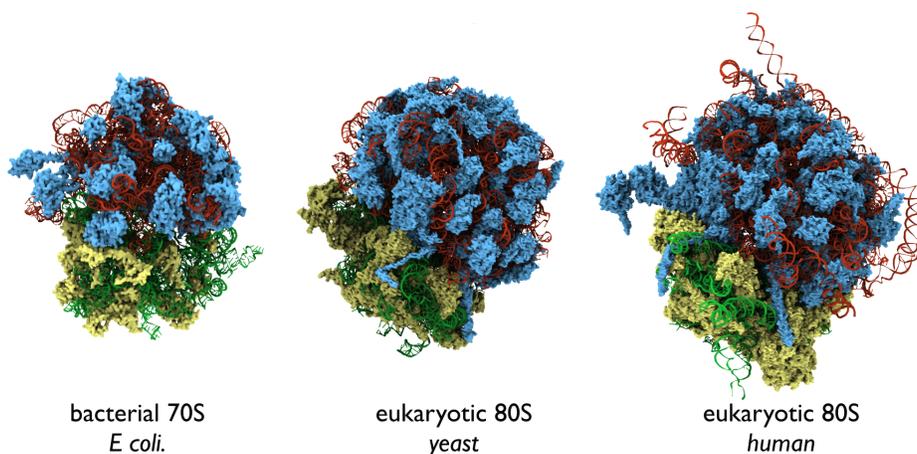


Figure 8: Overall structural comparison of ribosomes from different species.

Finally, although the core structure of the ribosome responsible for translation is highly conserved across all domains of life, the overall ribosomal assemblies from different species can be very different. For example, the yeast ribosome is approximately 30% bigger than bacterial ribosomes, in terms of molecular weight. The human ribosome is even bigger, as the structure has a significant amount of RNA and protein extensions on its outer surface (see Figure 8).

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