

Protein synthesis (translation)

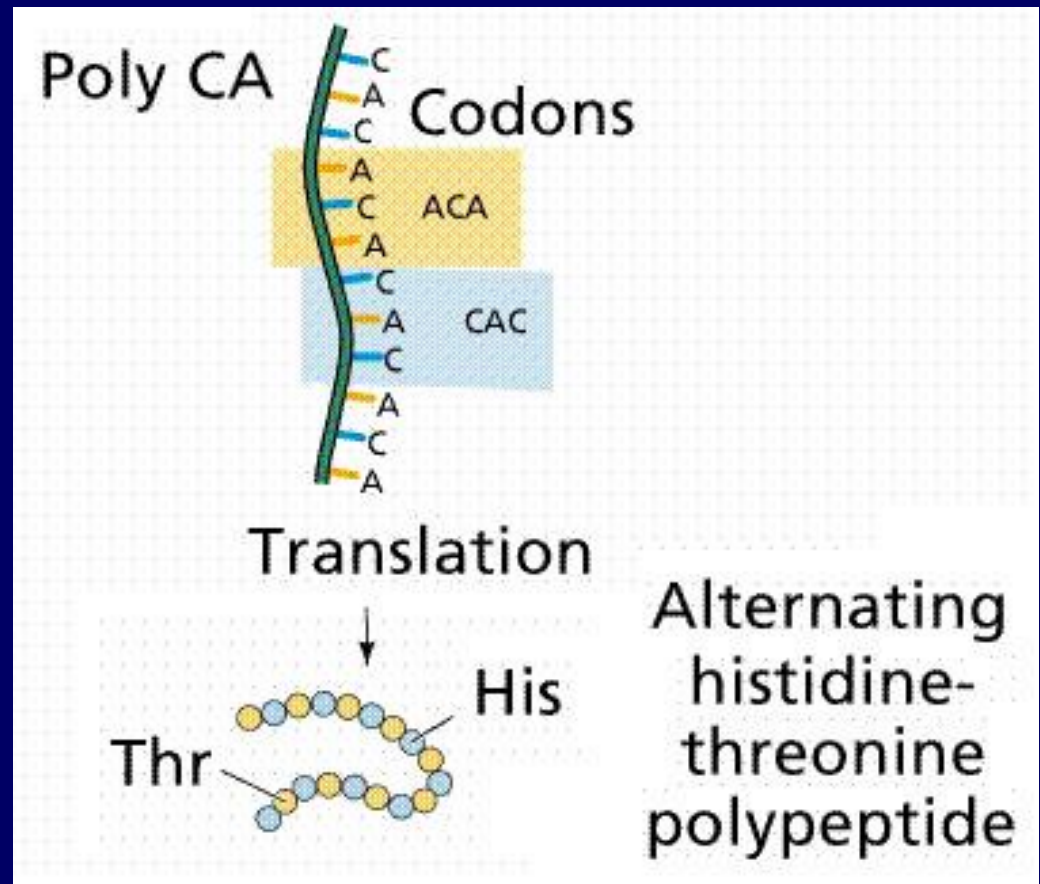
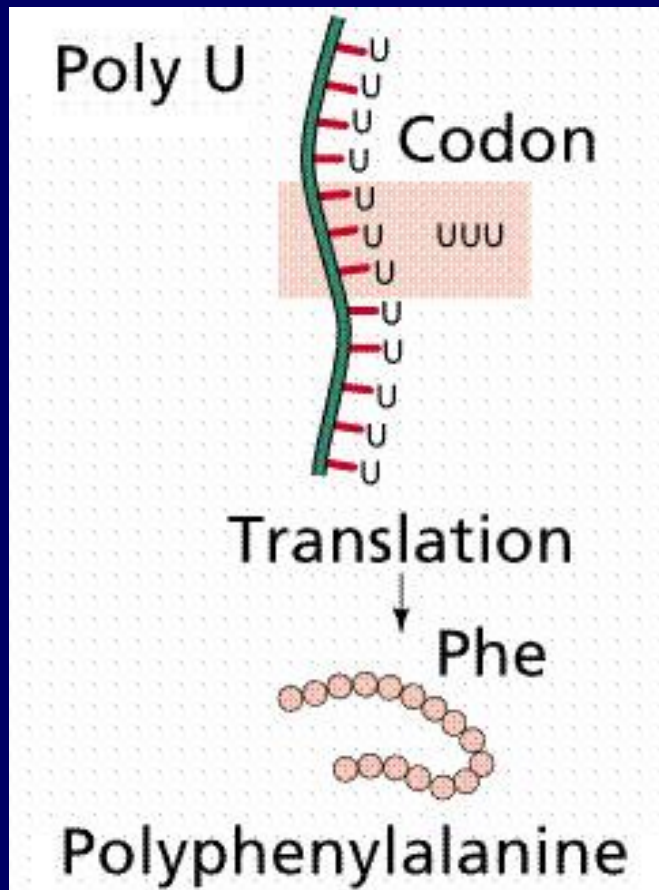
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Translation is a process of protein synthesis. During translation genetic information in a messenger RNA specifies arrangement of the amino acids in a polypeptide chain. The process is called translation, because genetic information is translated from a nucleotide sequence to an amino acid sequence. Translation occurs in the cytoplasm of the cell. Like replication and transcription, this process also requires energy, has a template and only one possible direction of progress.

The three types of RNA – mRNA, tRNA and rRNA, produced during transcription, are used in the cytoplasm for protein synthesis. Energy, required for the process, is supplied by the amino acids which are charged with ATP before participating in translation. Amino acids are activated by special enzyme adding ATP to them.

To decode genetic information, encoded in mRNA, is necessary to have special rules how the nucleotide sequence specifies the place of each amino acid in the protein. Now we know it is made of the genetic code. By the rules of simple Mathematics, scientists discover genetic code consists of triplets – three nucleotides specify the place of one amino acids. If the code was singlet – one nucleotide – one amino acid – only four amino acid would be coded by A, G, T and C. If the code was doublet, the number of coded amino acids would be $4^2 = 16$, again insufficient for all 20 amino acids used in proteins. Triplet code means $4^3 = 64$ possible combinations of nucleotides, totally enough to encode all of amino acids.

Deciphering the genetic code



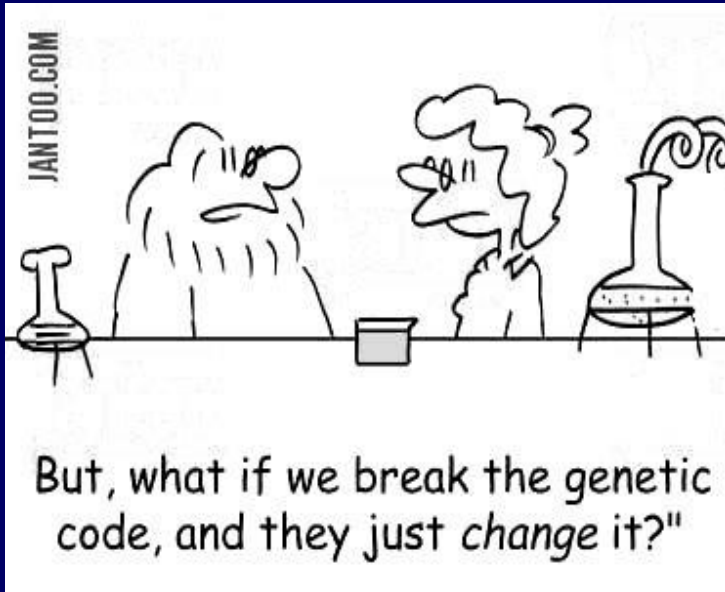
Key to the standard genetic code

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U C A G
		UUC } Leu	UCC } Ser	UAC } Tyr	UGC } Cys	
		UUA } Leu	UCA } Ser	UAA Stop	UGA Stop	
		UUG } Leu	UCG } Ser	UAG Stop	UGG Trp	
	C	CUU } Leu	CCU } Pro	CAU } His	CGU } Arg	U C A G
		CUC } Leu	CCC } Pro	CAC } His	CGC } Arg	
		CUA } Leu	CCA } Pro	CAA } Gln	CGA } Arg	
		CUG } Leu	CCG } Pro	CAG } Gln	CGG } Arg	
	A	AUU } Ile	ACU } Thr	AAU } Asn	AGU } Ser	U C A G
		AUC } Ile	ACC } Thr	AAC } Asn	AGC } Ser	
		AUA } Ile	ACA } Thr	AAA } Lys	AGA } Arg	
		AUG Met	ACG } Thr	AAG } Lys	AGG } Arg	
	G	GUU } Val	GCU } Ala	GAU } Asp	GGU } Gly	U C A G
		GUC } Val	GCC } Ala	GAC } Asp	GGC } Gly	
		GUA } Val	GCA } Ala	GAA } Glu	GGA } Gly	
		GUG } Val	GCG } Ala	GAG } Glu	GGG } Gly	
		Third letter				

Main characteristic of genetic code

1. Triplet nature – three nucleotides specify the place of one amino acid;
2. Degeneracy (synonymous) – codons are 64, but amino acids are 20. That's why some amino acids are coded by more than one codon. Of the 64 codons, 61 encode amino acids. The other three (UAA, UAG and UGA) are signals to stop protein synthesis. They are called stop, terminal or nonsense codons;
3. Non overlapping – the adjacent codons do not overlap;
4. Comma-free - there is no intermediary nucleotides between the codons;
5. Non-ambiguous – while the same amino acid can be coded by more than one codon, the same codon shall not code for two or more different amino acids;
6. Universality – the same triplets code the same amino acids in all of life forms – from simple microorganism to complex multicellular organism like a human. Nowadays is known that some genetic systems like mitochondrial DNA and DNA in the chloroplasts use alternative versions of genetic code – the code has dialects. That's why the code which more of genetic systems use is defined as standard;
7. Polarity – the code always read in a fixed direction – from 5' to 3' end of mRNA.

Genetic code “dialects”



Compiled by A. Elzanowski & J. Ostell

Reading frames

...heyhasshegottheteacupandthesugar...

- 1 ...hey has she got the tea cup and the sug ar...
- 2 ...h eyh ass heg ott het eac upa ndt hes uga r...
- 3 ...he yha ssh ego tth ete acu pan dth esu gar...

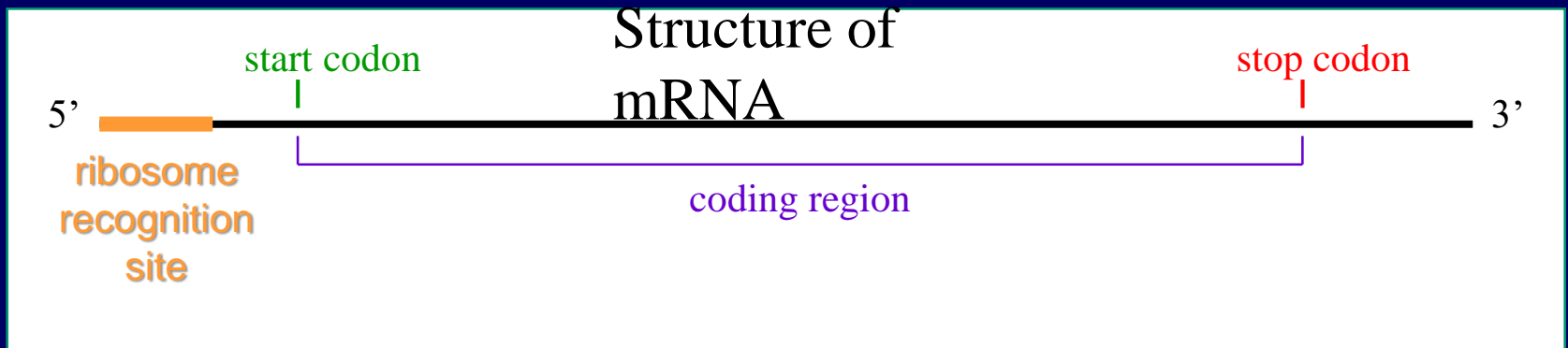
The three reading frames have absolutely different meanings, and only one (No. 1) is open.

Participants in translation

Messenger RNA (mRNA)

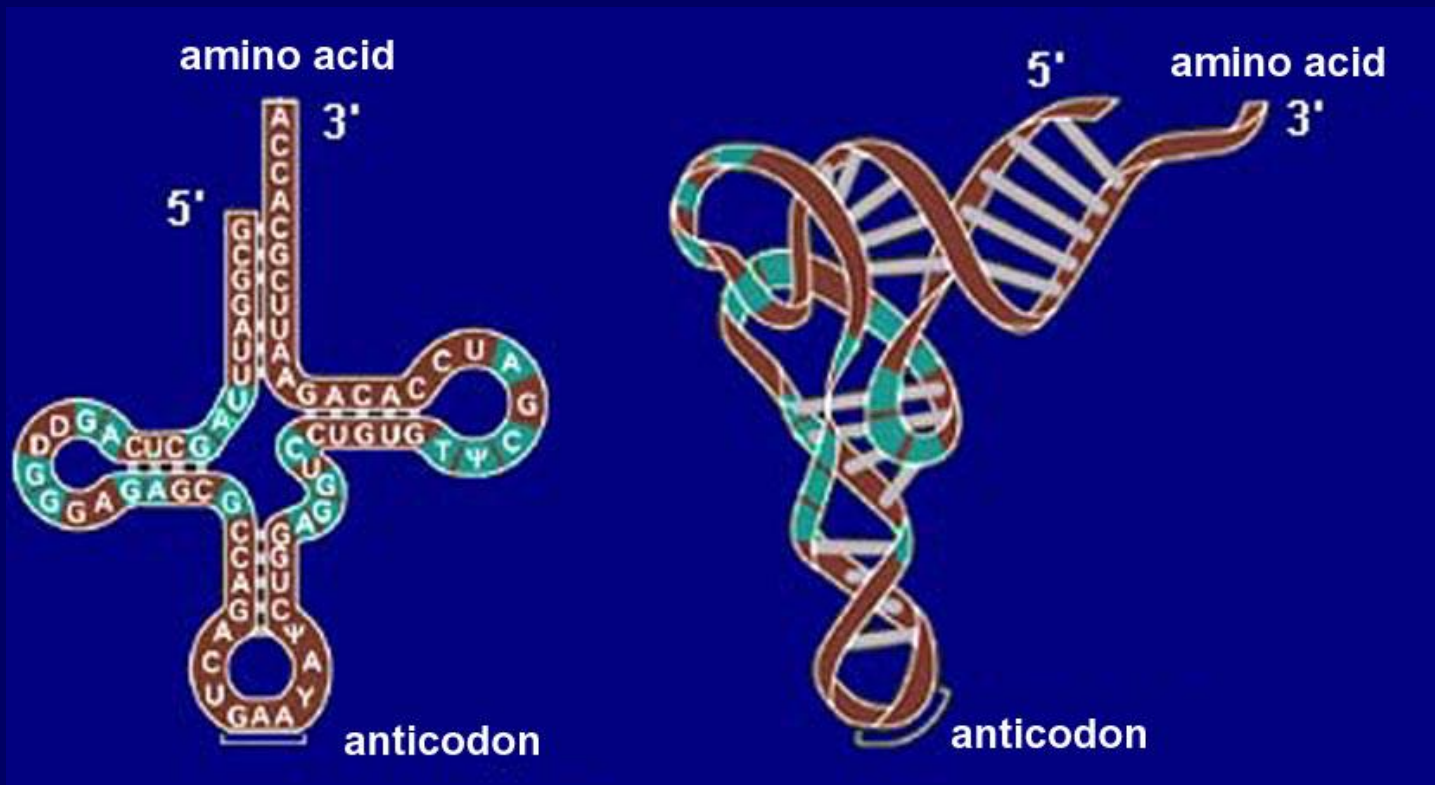
Messenger RNA is a linear sequence which is a copy of the corresponding gene. It is the message coming from the nucleus to the cytoplasm to carry the information for amino acids arrangement in the proteins. Messenger RNA is 2% of cellular RNA. It has no specific secondary structure. For mRNA the primary structures – linear arrangement of nucleotides – is only important.

It consists of coding region – where each three nucleotides form a codon. The codons in mRNA specify the places of the amino acids. There is only one start codon which starts translation every time. Three codons are not corresponding to amino acids – stop (nonsense) codons. Their appearance gives a signal to stop translation. On the 5' end of mRNA has a place for recognition of the ribosomes. In eukaryotes it is noted as 5' cap and orientates the ribosome to start translation from the beginning of mRNA. In the 3' end mRNA has poly A tail which protects it of degradation.



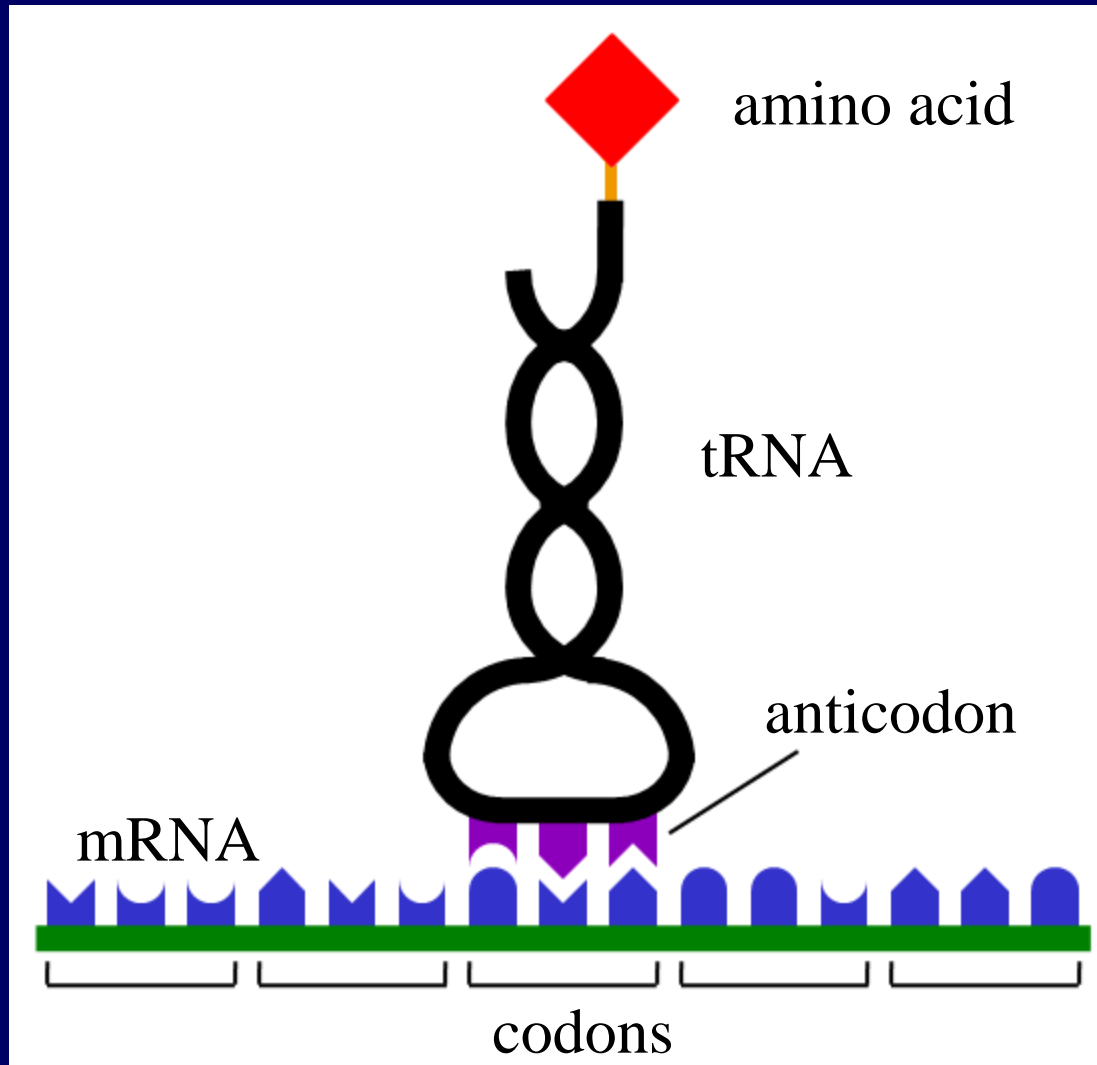
Transfer RNA (tRNA)

The function of tRNA is matching amino acids to codons. Its molecule has 4 loops. Three of them are large, leading to a “cloverleaf” secondary structure. The 3' end has a single-stranded CCA where the amino acid binds. Opposite to it, a large loop carries the anticodon – a triplet complementary to the codon. The 3D structure is L-shaped.

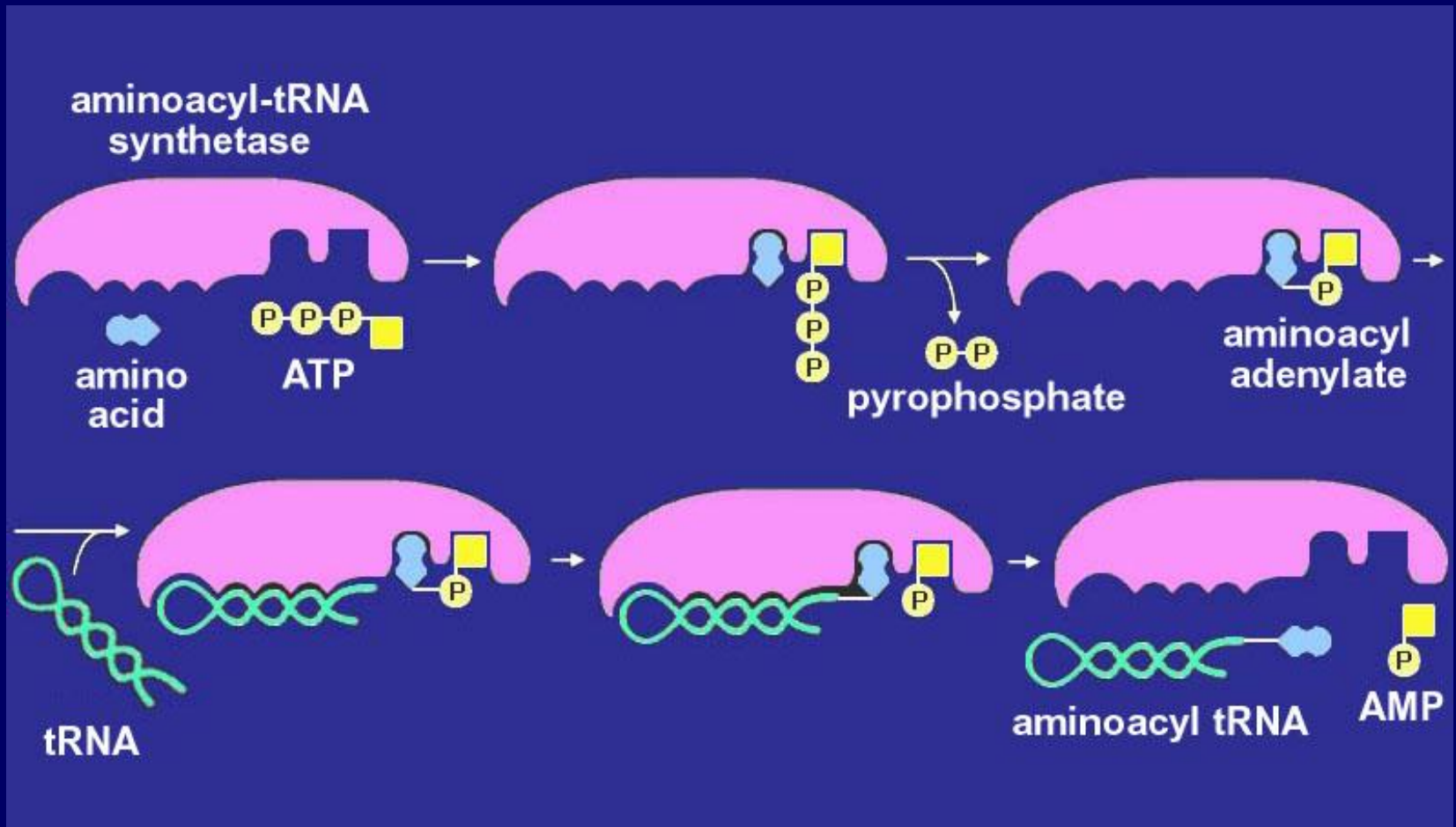


From Alberts et al., Molecular Biology of the Cell

The adaptor role of tRNA

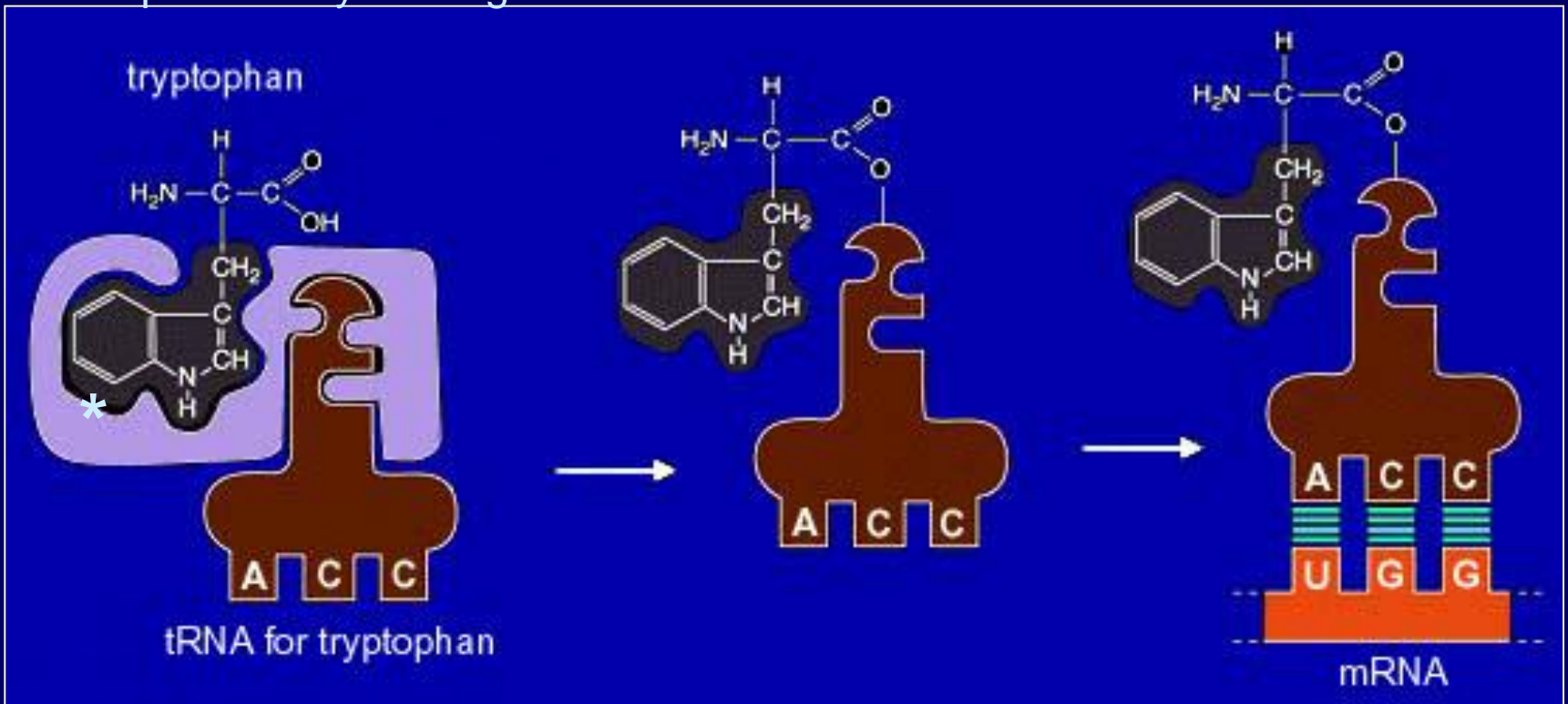


Amino acid activation and binding to its specific tRNA

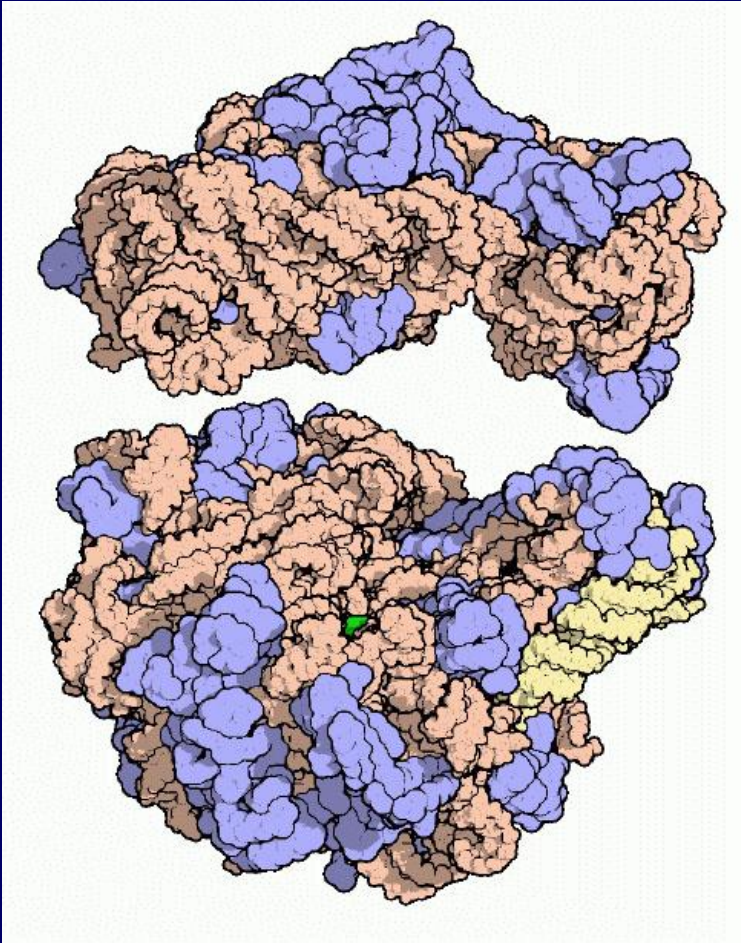


The adaptor of translation

Transfer RNA acts as an adaptor because the amino acids have no spatial affinity to codons. The connection between tRNA and its amino acid is made by a special enzyme – aminoacyl-tRNA synthetase. Each amino acid requires specific shape of the enzyme. The enzyme joins the amino acid and its tRNA. The same enzyme binds ATP to the amino acid to activate it. Later the anticodon of tRNA recognizes corresponding codon of mRNA because of complementary binding.

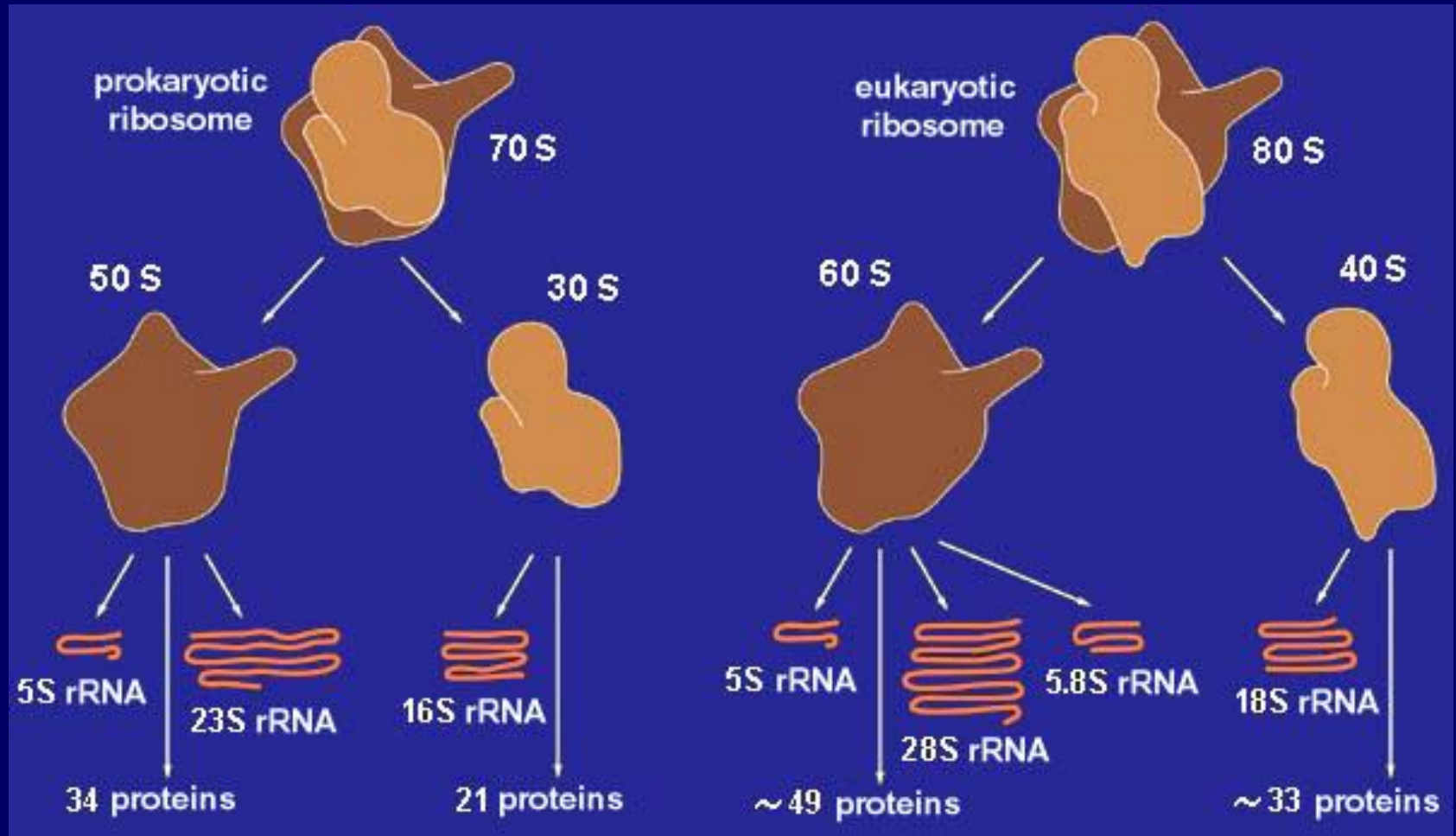


The ribosome, the construction site for proteins



Ribosomes consist of ribosomal RNA and proteins. Ribosomal RNA is 80% of cellular RNA. It is produced in the nucleolus – special compartment of the nucleus. In the nucleolus ribosomal subunits start to assemble. When is synthesized, rRNA binds to specific proteins to form large and small ribosomal subunit. These subunits leave the nucleus by nuclear pores. Only in the cytoplasm intact ribosomes assemble to participate in protein synthesis.

Composition of the ribosome

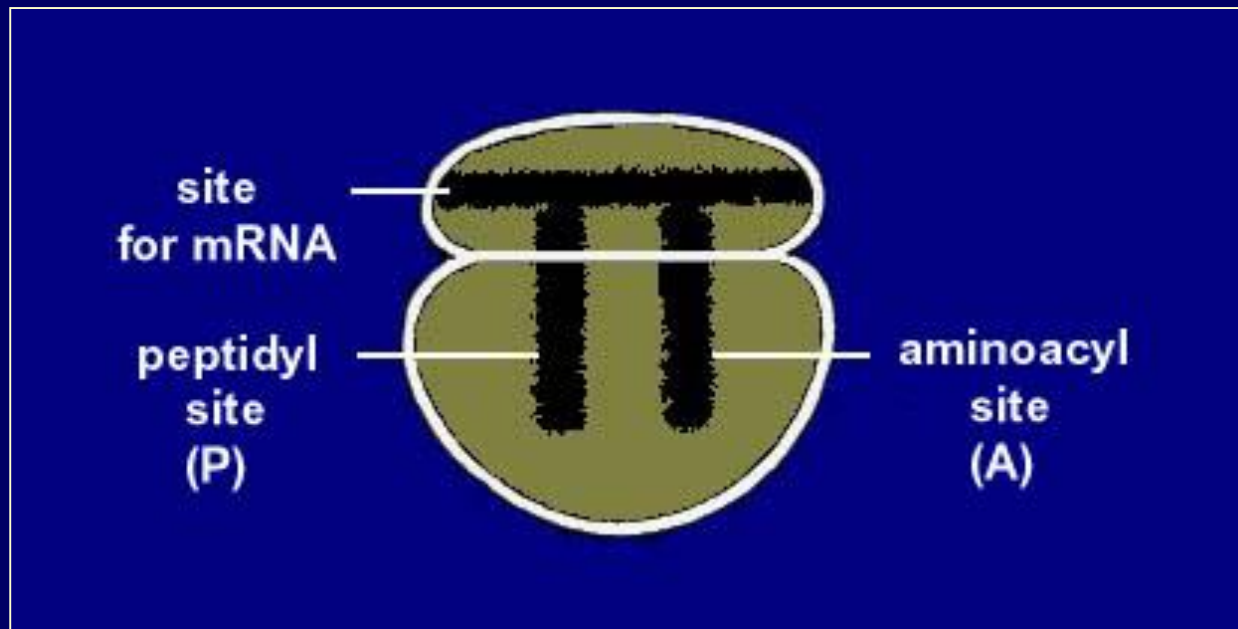


From Alberts et al., Molecular Biology of the Cell

RNA binding sites in the ribosome

The ribosome has 3 binding sites for RNA. One of them is for the mRNA template and is located entirely in the small subunit. The other two are for tRNAs and span both subunits, but mostly the large. One of these sites for tRNAs is called peptidyl site and is designated by P. The other one is called aminoacyl site and is designated by A.

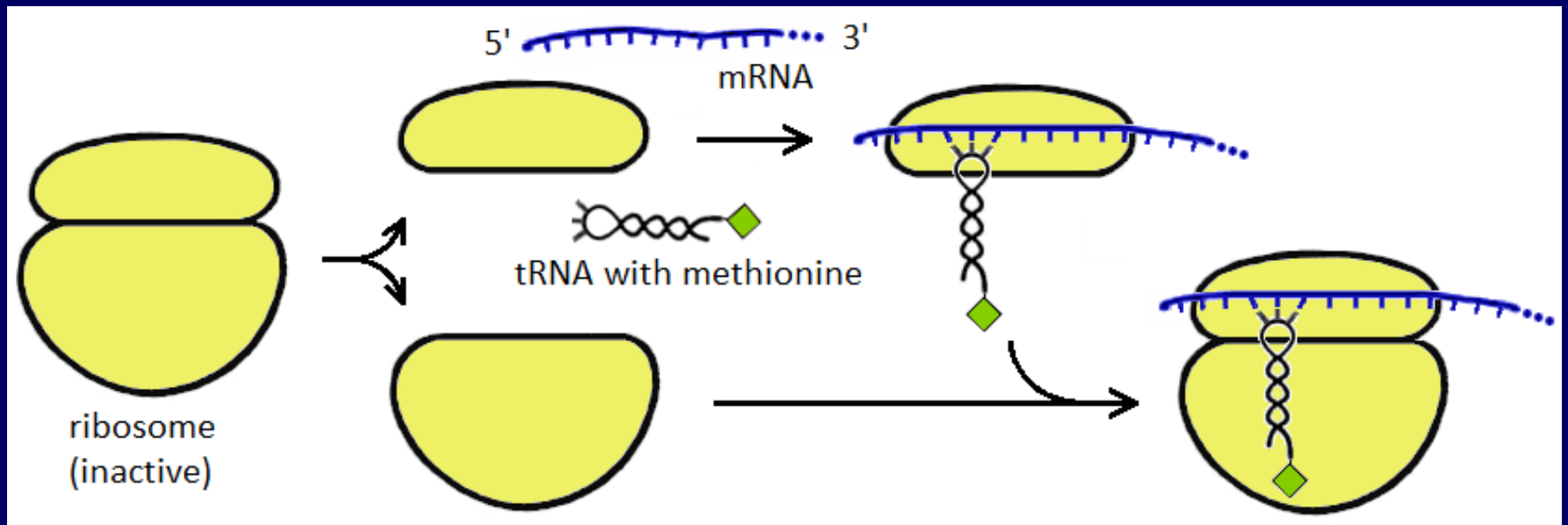
The ribosome below is drawn mushroom-like, which is a good approximation of its real shape. When the two subunits are bound together without mRNA and tRNAs, as shown here, the ribosome is inactive.



Stages of translation

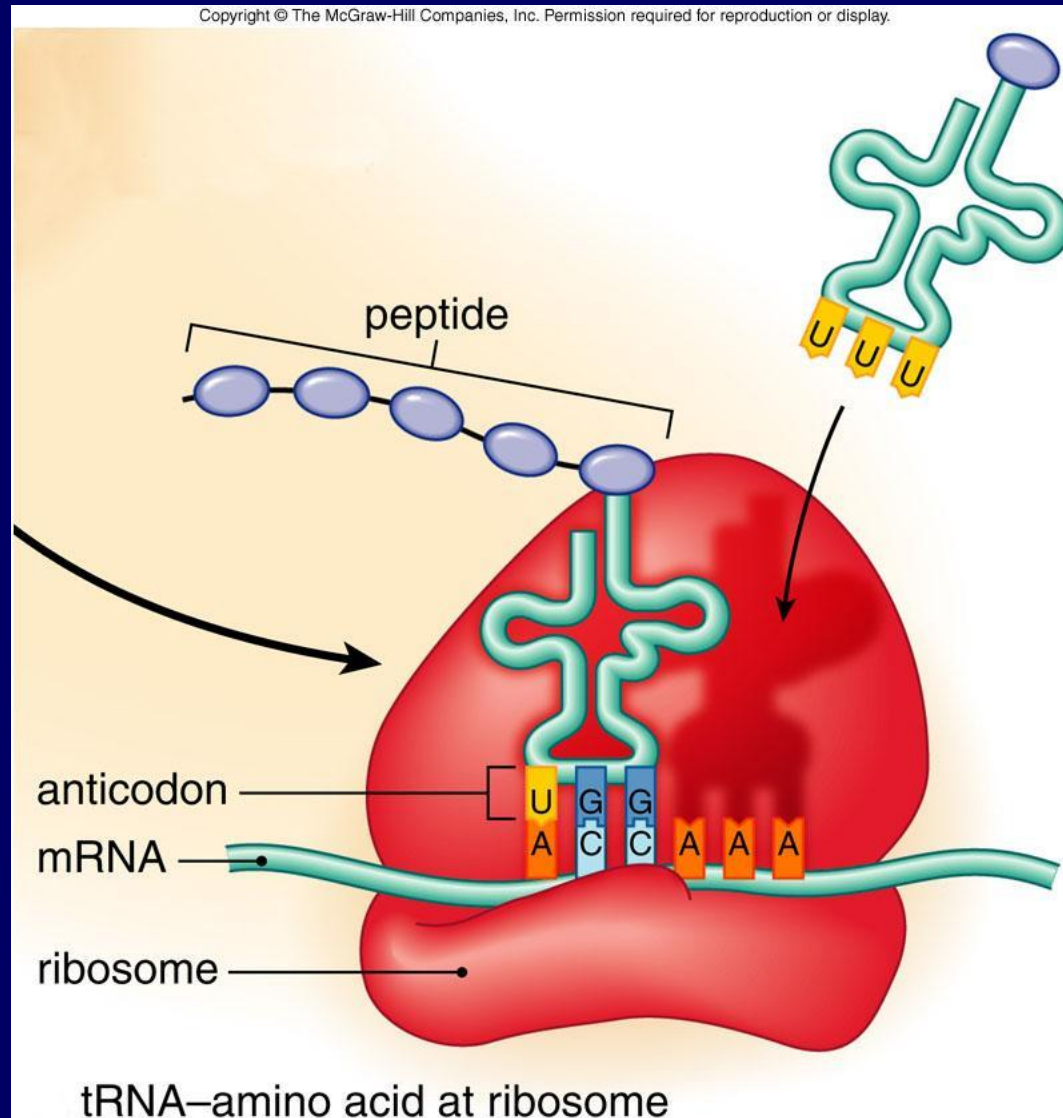
Initiation

When the two subunits are joined without mRNA and tRNA, the ribosome is inactive. They first separate...



...then, the small subunit binds mRNA and the first aminoacyl-tRNA carrying methionine, forming the so called initiation complex.

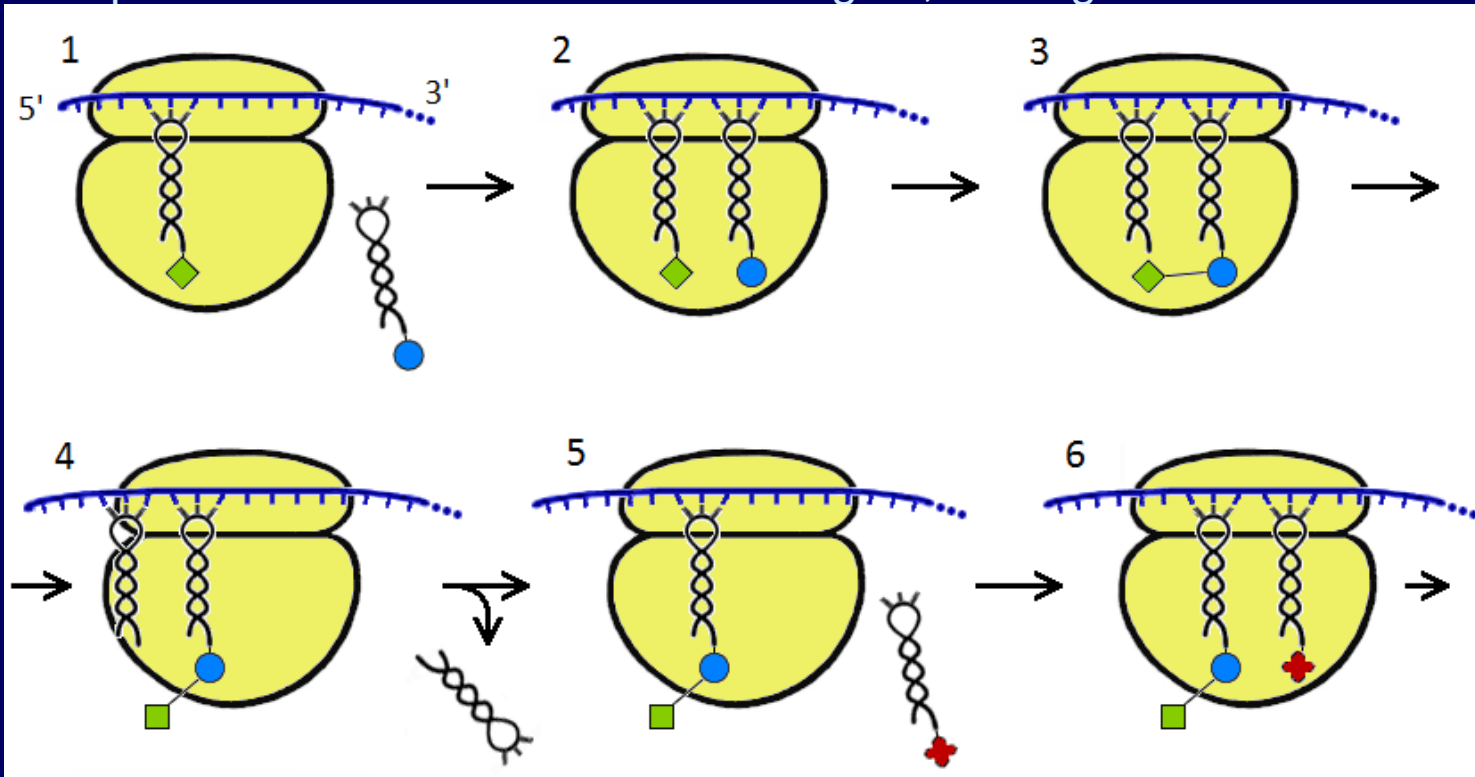
Binding the mRNA and tRNA during initiation



Elongation

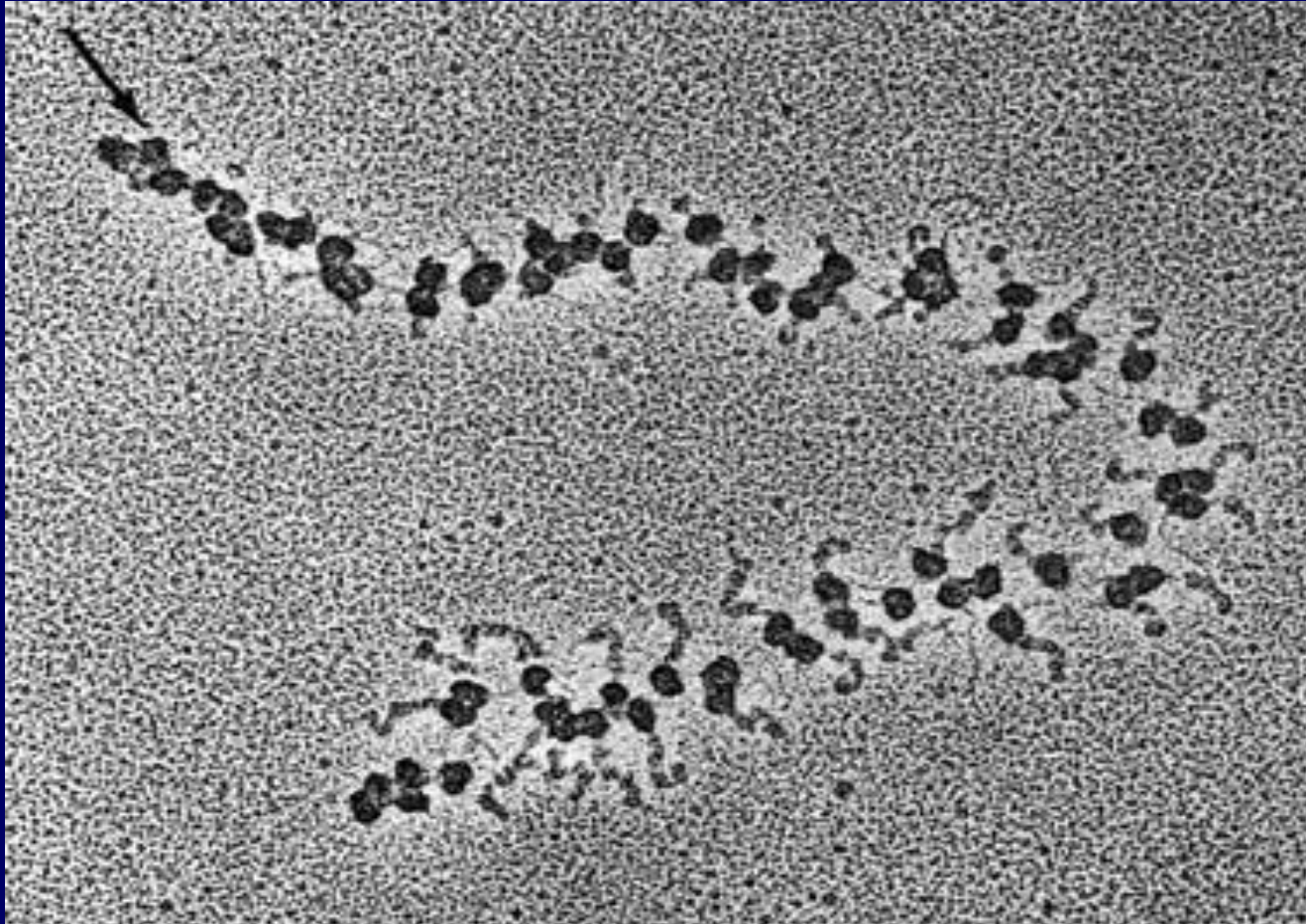
Another aminoacyl tRNA enters the A site. If the anticodon matches the codon, tRNA will remain there and a peptide bond will form. Peptide bond is made by one rRNA in the large ribosomal subunit. This rRNA has catalytic activity – it is a ribozyme.

When the peptide bond is made, the first amino acid is bound together with the second to the second tRNA. The first tRNA is already free and it leaves the ribosome. The ribosome moves with a codon forward and tRNA with dipeptide occupies the P site. The A site is free again, waiting for a new tRNA.



Polysomes

When the ribosome moves away from the 5' end of mRNA, another ribosome can bind there and initiate translation. This way, large “strings” of ribosomes on mRNA are formed, called polyribosomes or polysomes.



From Francke et al. (1982)

Part of a polysome, very high magnification. The ribosomes look mushroom-like, attached with their small subunits to the mRNA. The polypeptides protruding from the large subunits look like zig zags. This is due to the angles in the peptide bond.

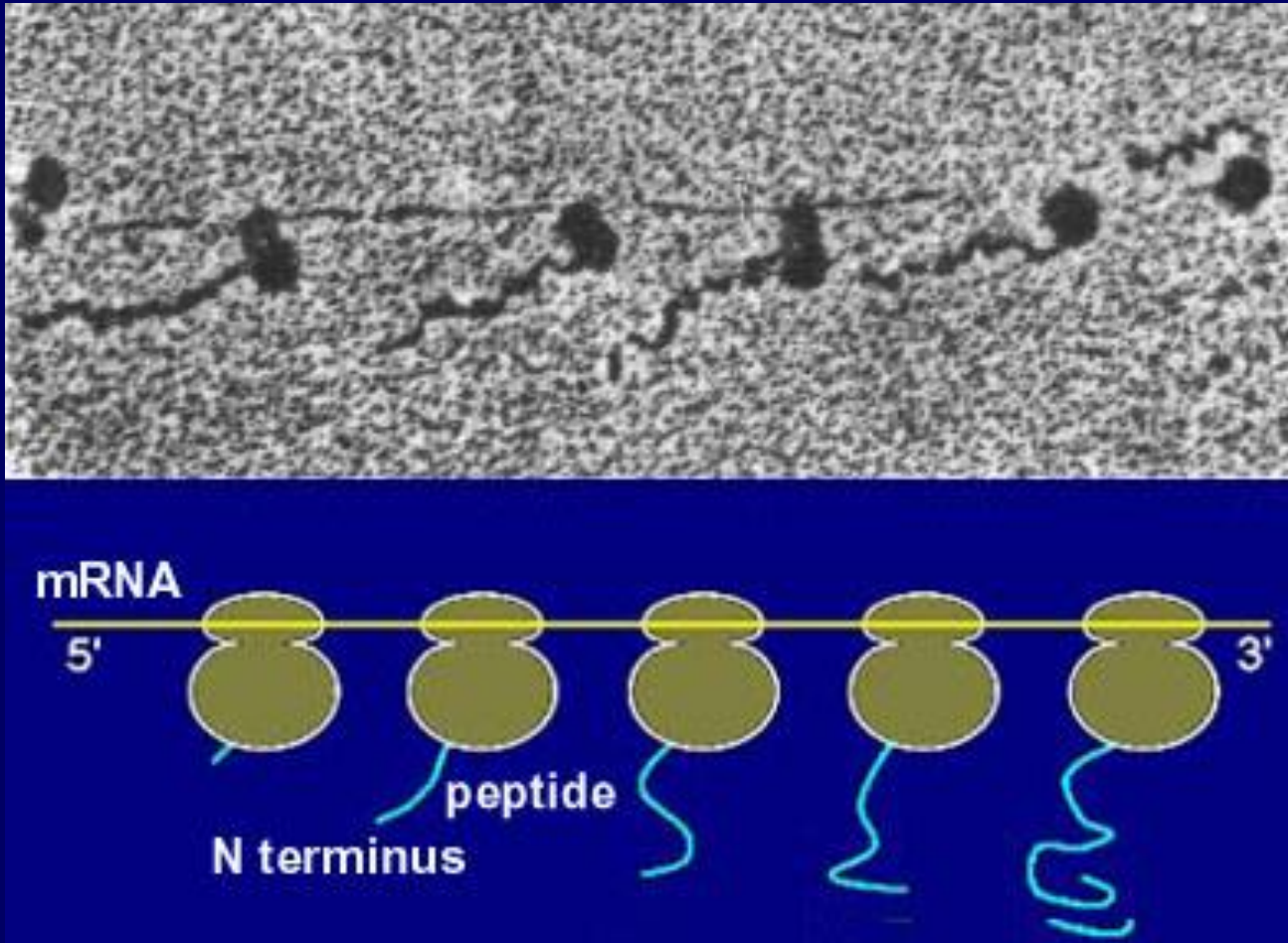


Photo Francke et al. (1982)



Cold Spring Harbor Laboratory, New York.

Termination

During elongation ribosome moves on mRNA and it reaches a signal to stop translation. The signal is a stop codon. This codon has no complementary tRNA and release factors bind to the ribosome. This release factor initiates the ribosome to bind growing polypeptide to water molecule. This binding makes COOH - group at the end of the protein and the polypeptide is liberated. Release factors provoke ribosomal disassembly. Small and large subunit separate and ribosome can start new translation

