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Bi4025en
Molecular Biology

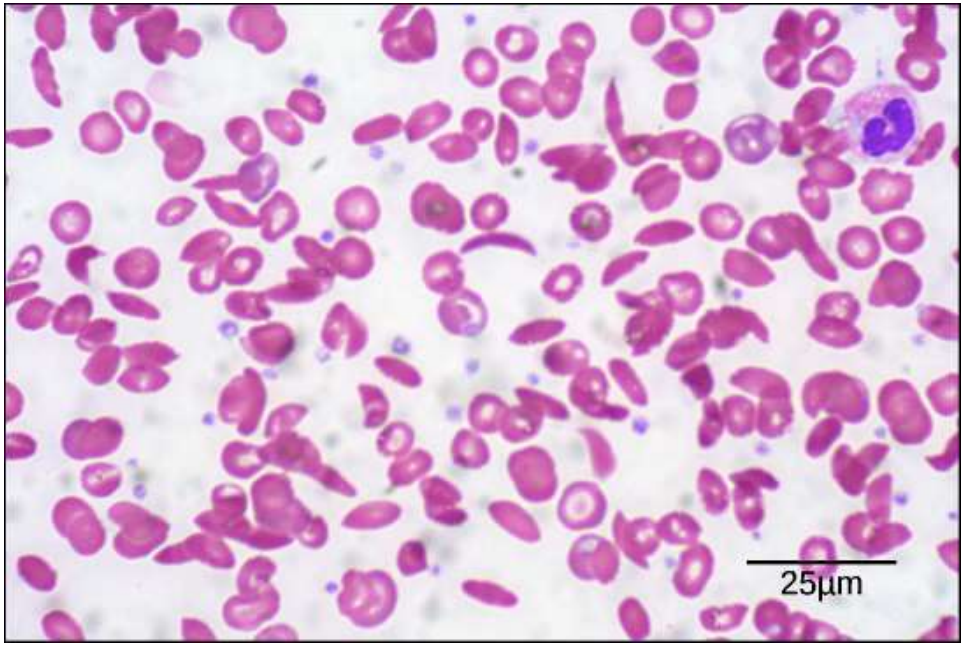
Mgr. Jiří Kohoutek, Ph.D.

Lecture 5

- Translation of prokaryotic and eukaryotic mRNAs.

Sickle cell anemia

- Substitution of one amino acid in the molecule of Hemoglobin.
- Loss of function protein/cell/organism.



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Sickle cell disease – anemia

- In year 1904:
- Doctor James Herrick's office in Chicago visited by a patient of African origin with an enlarged heart.
- Complains of weakness, fatigue and dizziness.
- Herrick diagnoses an anemia.
- In the blood sample erythrocytes of unusual sickle-shaped appeared.
- For the first time hypothesizes that sickle cell red blood cells are the cause of anemia.

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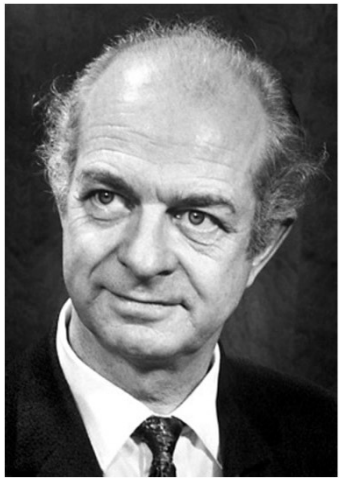
Sickle cell anemia – middle age

- In 1927: evidence that the change in shape of normal red blood cells to sickle cell is related to their impaired function – oxygen transport in the body.
- In 1949: Linus Pauling, formulates a hypothesis, that the cause of sickle cell anemia could be abnormal form of hemoglobin.
- Hypothesis successfully verified by electrophoretic techniques.
- In 1954 he was awareded by the Nobel Prize in Chemistry.



Linus Pauling
(1901 – 1994)

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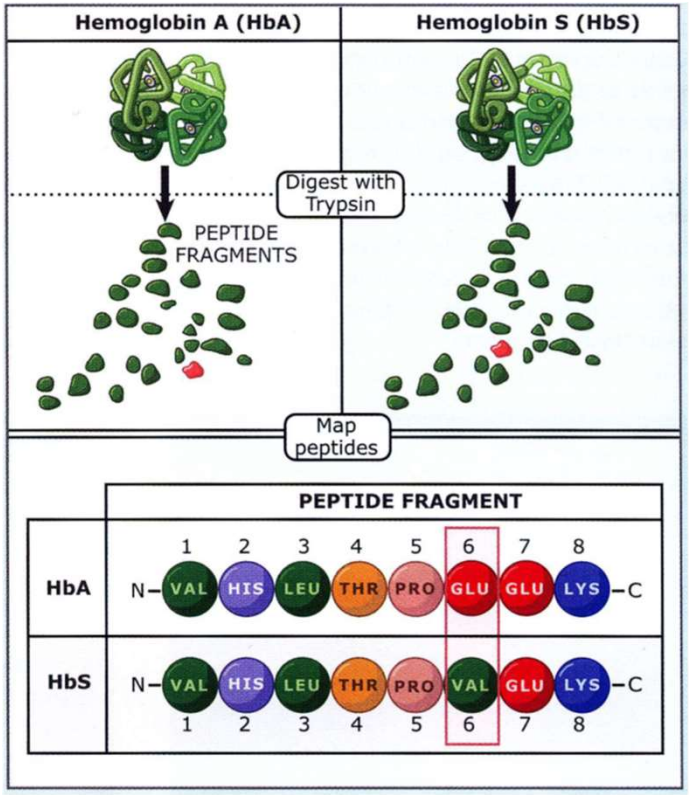
Sickle cell anemia – middle age

- In 1954: Vernon Ingram (1924-2006).
- Hemoglobin from healthy (A) and sickle cell (S) cells cleaved by trypsin into fragments that have been divided by electrophoresis and peptide mapping found one different peptide.
- Trypsin breaks down proteins after lysine and arginine, unless they are followed by proline.
- In the AA sequence of ß-chains of hemoglobin the single mismatch was found: valine (hydrophobic) replaced glutamic acid (sixth AA from N-ends). Hydrophobic valine causes clumping of molecules hemoglobin and deformation of red blood cells.



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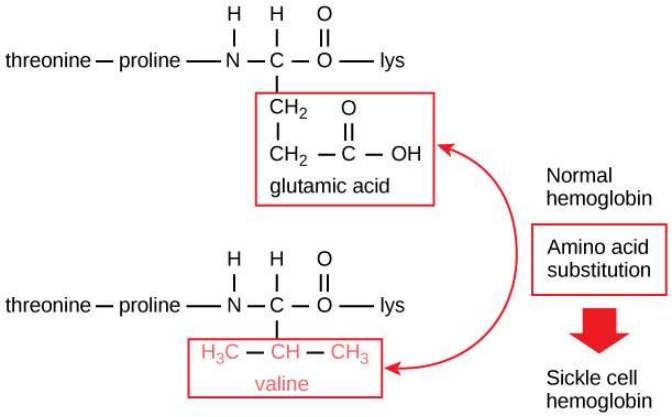
One mutation can severely impact protein function

	U		C		A		G	
U	UUU	fenylalanin	UCU	serin	UAU	tyrosin	UGU	cystein
	UUC	fenylalanin	UCC	serin	UAC	tyrosin	UGC	cystein
	UUA	leucin	UCA	serin	UAA	**stop**	UGA	**stop**
	UUG	leucin	UCG	serin	UAG	**stop**	UGG	tryptofan
C	CUU	leucin	CCU	prolin	CAU	histidin	CGU	arginin
	CUC	leucin	CCC	prolin	CAC	histidin	CGC	arginin
	CUA	leucin	CCA	prolin	CAA	glutamin	CGA	arginin
	CUG	leucin	CCG	prolin	CAG	glutamin	CGG	arginin
A	AUU	izoleucin	ACU	treonin	AAU	asparagin	AGU	serin
	AUC	izoleucin	ACC	treonin	AAC	asparagin	AGC	serin
	AUA	izoleucin	ACA	treonin	AAA	lysin	AGA	arginin
	AUG	**metionin**	ACG	treonin	AAG	lysin	AGG	arginin
G	GUU	valin	GCU	alanin	GAU	kys.	GGU	glycin
	GUC	valin	GCC	alanin	GAC	asparagová	GGC	glycin
	GUA	valin	GCA	alanin	GAA	kys.	GGA	glycin
	GUG	valin	GCG	alanin	GAG	glutamová	GGG	glycin



https://www.khanacademy.org/science/biology/macromolecules/proteins-and-amino-acids/a/orders-of-protein-structure

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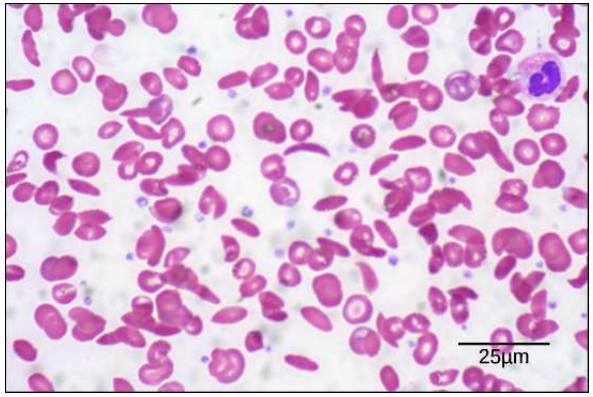
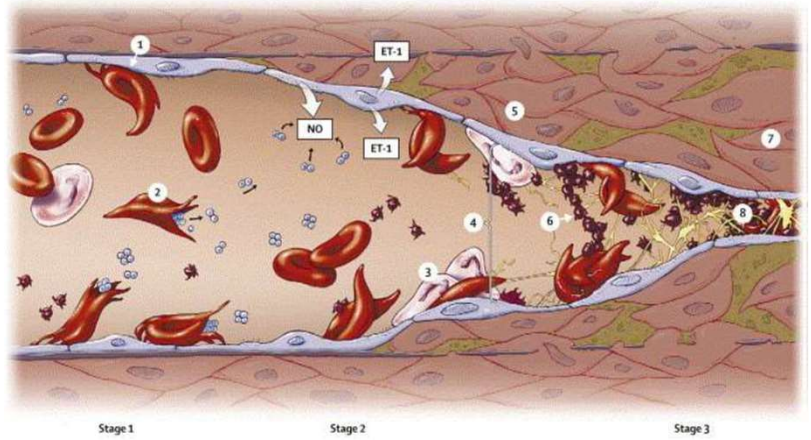
Sickle cell disease – pathophysiology

- Because the glutamic acid-to-valine amino acid change makes the hemoglobin molecules assemble into long fibers.
- The fibers distort disc-shaped red blood cells into crescent shapes.
- The sickled cells get stuck as they try to pass through blood vessels, which impairs blood flow leading to serious health problems for patients with sickle cell anemia, including breathlessness, dizziness, headaches, and abdominal pain.





https://www.khanacademy.org/science/biology/macromolecules/proteins-and-amino-acids/a/orders-of-protein-structure

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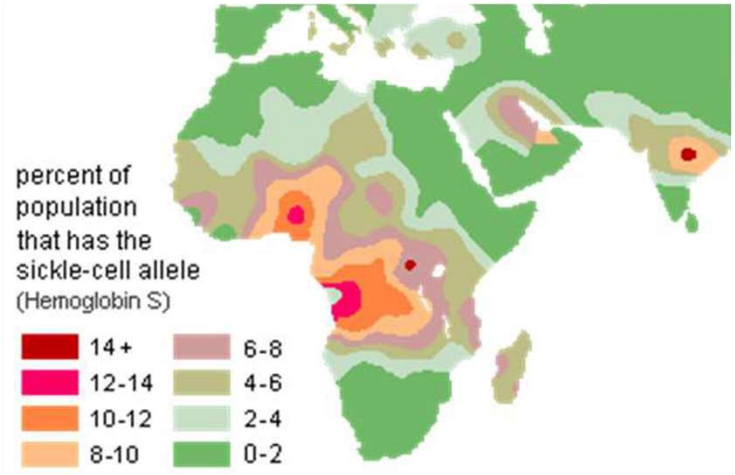
Sickle cell disease – genetics



- Why has sickle cell anemia not been eliminated by selection pressure?
- Because the selection pressure contributes to the maintenance of the mutant gene in the population against another factor.
- Heterozygous individuals, one "healthy" and one "sickle cell" from allele manifest a weaker form of anemia.
- At the same time they show a higher degree of resistance to the malaria agent (protozoan plasmodium) than healthy homozygotes.
- The highest incidence of sickle cell anemia is in malarial areas.

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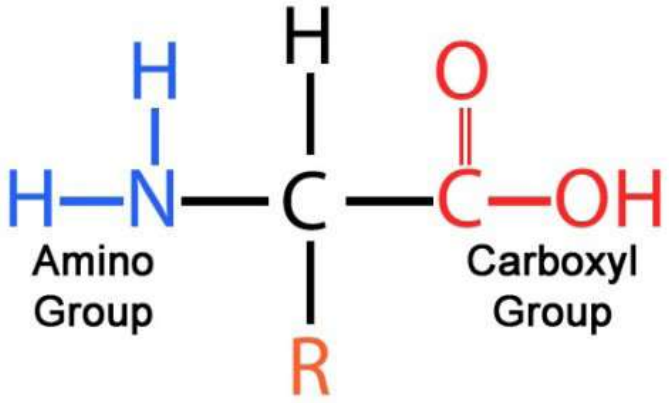
Protein structure

- Proteins are composed of 20 different essential amino acids.
- Accounts for 15% of the native weight of cells.
- With the exception of water, constitute the main structural component of living organisms.
- Participate in the structure and function of their bodies.

Amino acids

- Proteins consist of polypeptides, polypeptides of amino acids (AA).
- In the polypeptide amino acids are joined into strand by covalent bonds.
- There are 20 amino acids; all have a free amino group (NH_2) and free carboxyl group (COOH).
- Amino acids are distinguished from each other by lateral groups (R).
- The nature of the substituent R varies considerably, it could be a hydrocarbon group, OH, SH, SCH_3, COOH, or NH_2.
- The number of AA combinations in different polypeptides is huge (e.g. for a peptide composed of 7 AK, there are 20^7 combinations).



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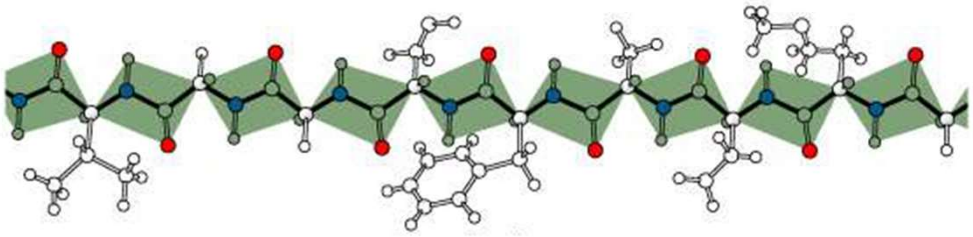
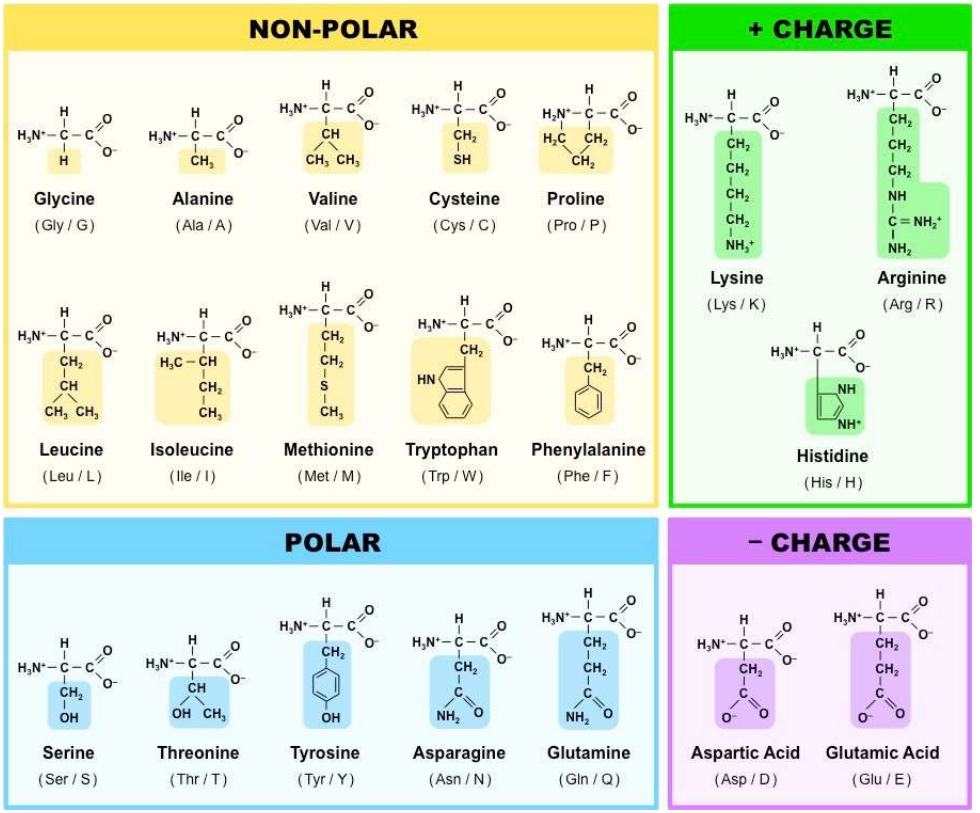
Chemical proterties of lateral groups od AA

- Lateral groups of amino acids are source of their structural and functional diversity.

4 types:

- hydrophobic (non-polar),
- hydrophilic (polar),
- acidic (negatively charged),
- basic (positively charged).





https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/24-proteins/amino-acids.html

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Chemical proterties of lateral groups od AA

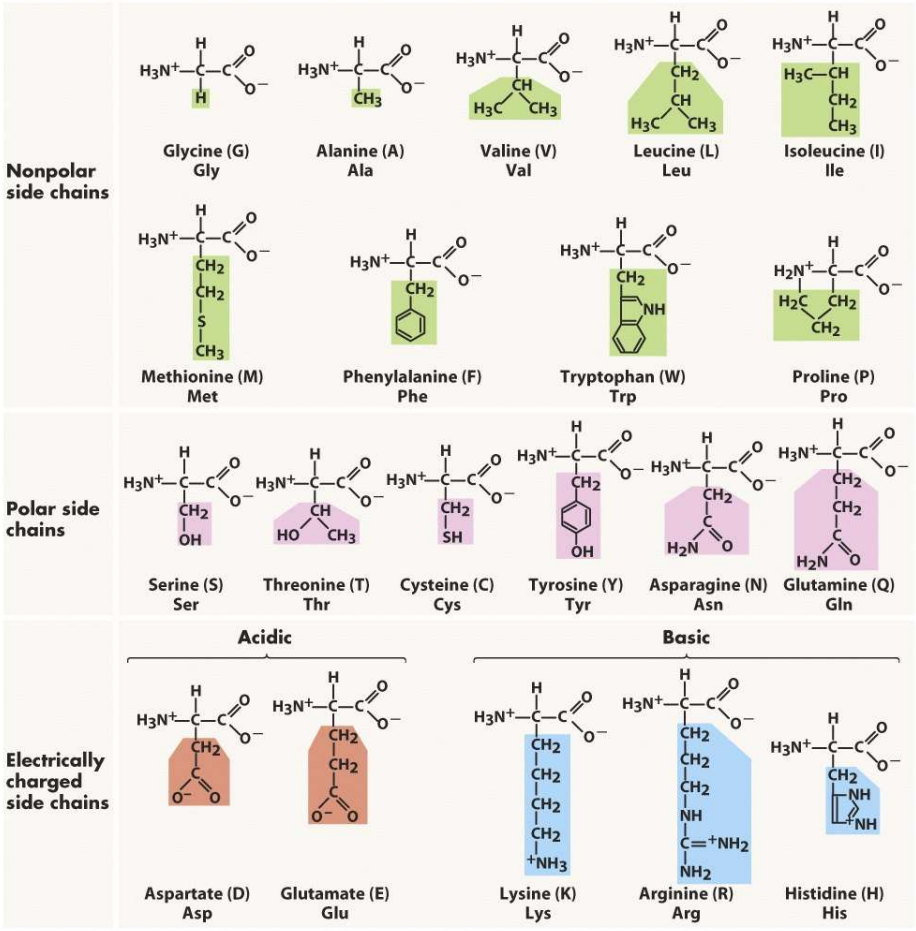
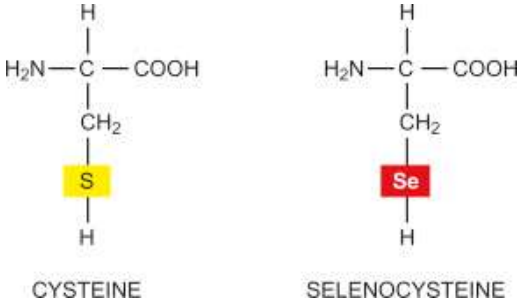
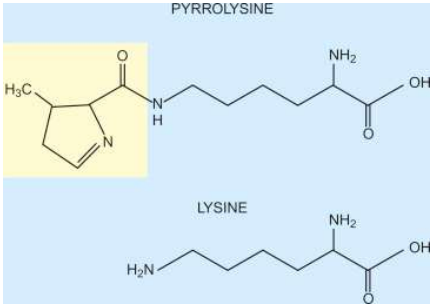


Figure 3-5 Biological Science, 2/e

- In addition, there are two AA
- Selenocysteine - has selenium instead of sulfur.



- Pyrrolysine - derivative of lysine with an attached pyrroline ring.



https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/24-proteins/amino-acids.html

https://kevintbeel.com/2017/04/19/three-acronym-mnemonics-for-remembering-the-amino-acids/

https://www.sciencedirect.com/topics/medicine-and-dentistry/selenocysteine

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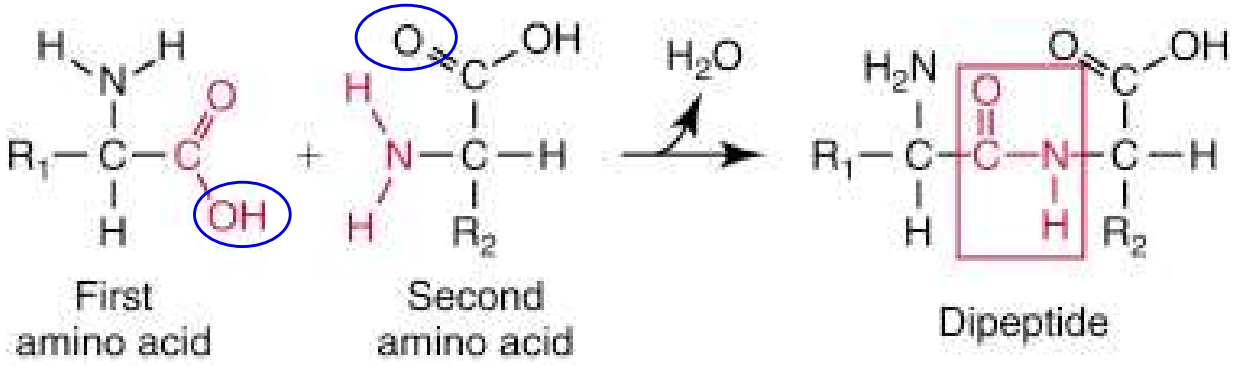
Peptide bond

- Amino acids are linked in peptides by covalent peptide bonds.

- Peptide bond - also referred to as an amide bond, is formed between the amino group (α-nitrogen atom) of one amino acid and the carboxyl group (carbonyl carbon) of its neighbour, releasing water molecules at the same time. The process is also called – condensation.



https://www.sciencedirect.com/topics/engineering/peptide-bond

Levels of protein structure

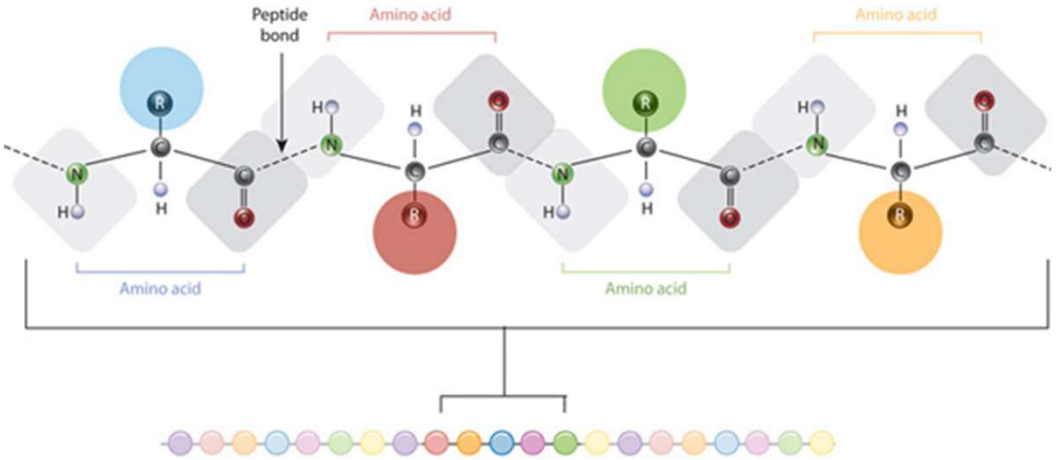
4 levels of organization of protein structure:

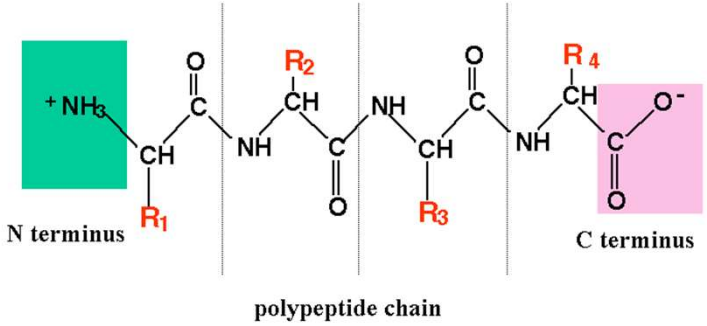
- Primary structure: determined by amino acid sequence (encoded by gene).
- Secondary structure: refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone.
- Tertiary structure: the assembly of the polypeptide in three-dimensional space.
- Quaternary structure: results from the union of two or more polypeptides into a multi-subunit protein.

https://quizizz.com/admin/quiz/606682e1825246001f488abd/dna-structure-function-and-replication-basics

Primary structure of protein

- The linear sequence of amino acids within a protein is considered the primary structure of the protein.





- In the polypeptide chain-protein the left hand amino acid is called the N-terminus, and the right hand amino acid is called the C-terminus.

https://www.nature.com/scitable/topicpage/protein-structure-14122136/
https://socratic.org/questions/57c278efb72cff0d7b97f247

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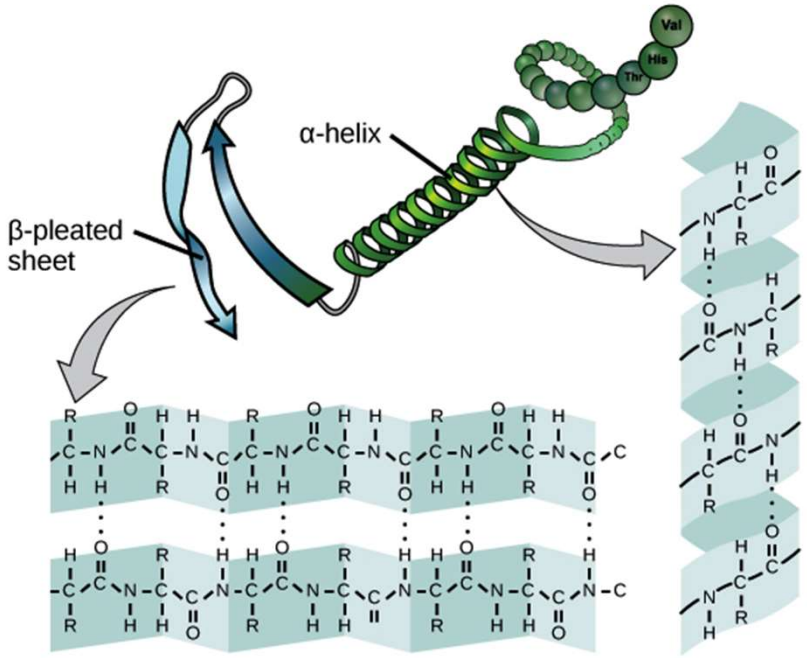
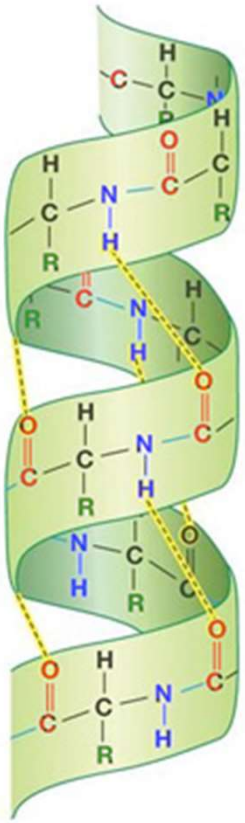
Secondary structure of protein

- Secondary structure, refers to local folded structures that form within a polypeptide due to peptide bonds between neighbor amino acis. interactions between atoms of the backbone.
- The secondary structure does not involve R group atoms.
- The most common types of protein secondary structures are the α helix and the β pleated sheet. Both structures are held in shape by hydrogen bonds, which form between the carbonyl O of one amino acid and the amino H of another.



https://www.khanacademy.org/science/biology/macromolecules/proteins-and-amino-acids/a/orders-of-protein-structure

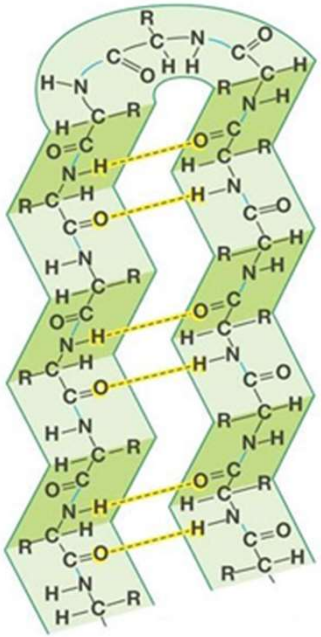
Secondary structure of protein – α – helix



- α helix - the carbonyl (C=O) of one amino acid is hydrogen bonded to the amino H (N-H) of an amino acid that is four down the chain. (E.g., the carbonyl of amino acid 1 would form a hydrogen bond to the N-H of amino acid 5.) This pattern of bonding pulls the polypeptide chain into a helical structure that resembles a curled ribbon.
- Each turn of the helix containing 3.6 amino acids.
- The R groups of the amino acids stick outward from the α helix, where they are free to interact.

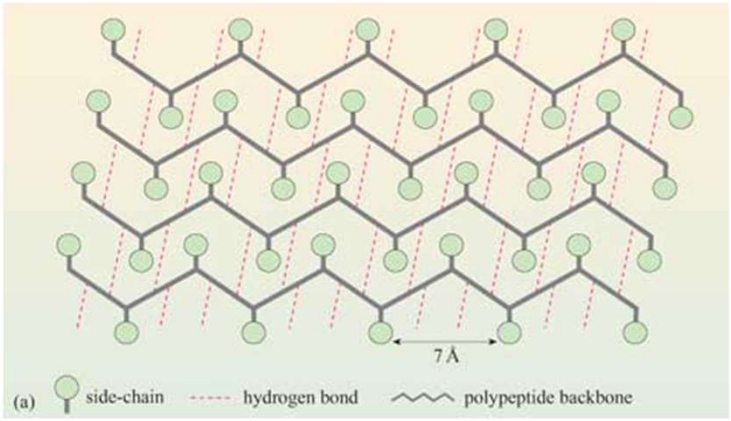
https://www.khanacademy.org/science/biology/macromolecules/proteins-and-amino-acids/a/orders-of-protein-structure
https://cbm.msoe.edu/teachingResources/proteinStructure/secondary.html

Secondary structure of protein – β pleated sheet



- β pleated sheet - two or more segments of a polypeptide chain line up next to each other, forming a sheet-like structure held together by hydrogen bonds.

- The hydrogen bonds form between carbonyl and amino groups of backbone, while the R groups extend above and below the plane of the sheet.



https://www.mun.ca/biology/scarr/iGen3_03-01.html
https://www.open.edu/openlearn/science-maths-technology/science/biology/proteins/content-section-1.3.2l

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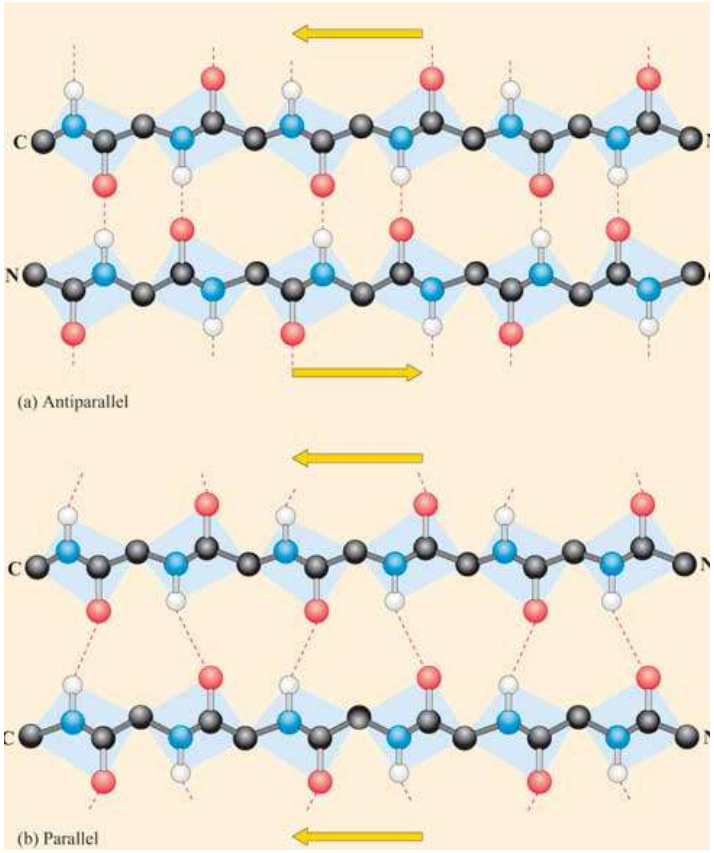
Secondary structure of protein – β pleated sheet

- In parallel β pleated sheet, polypeptide strands run in the same direction (i.e. from N- to C-terminus).
- In antiparallel β pleated sheet, neighbouring strands extend in opposite directions.



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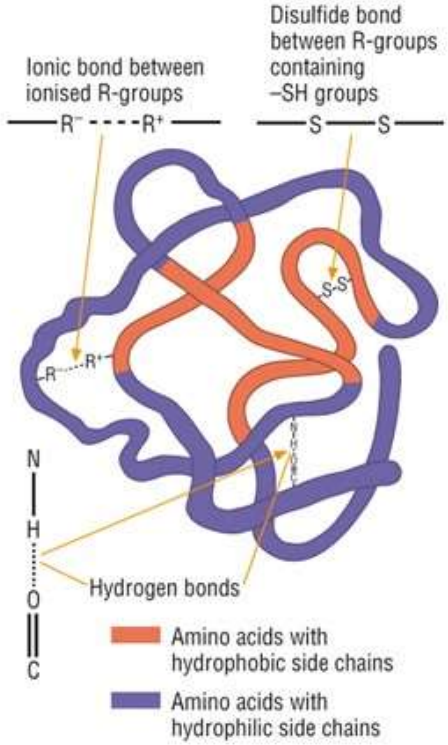
Tertiary structure of protein

- Tertiary structure is the complex looping and folding that occurs as a result of interactions and bonding between portions, amino acids, of the protein that are farther apart.
- Tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein.
- Amino acids with nonpolar, hydrophobic R groups cluster together on the inside of the protein,
- The hydrophilic amino acids cluster on the outside, in order to interact with surrounding water molecules.



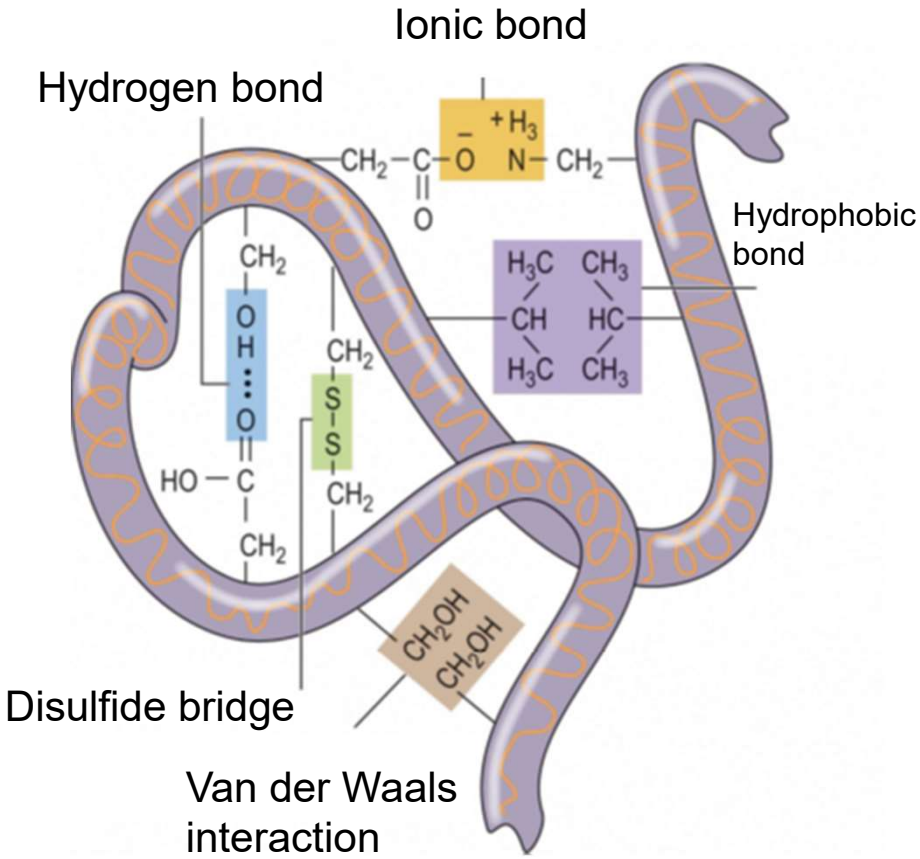
https://www.lecturio.com/concepts/post-translational-protein-processing/
https://slideplayer.com/slide/10879273/

Tertiary structure of protein

- Tertiary structure is mainly stabilized by non-covalent and covalent bonds.

- Ionic bond between chemical groups with opposite charges.
- Hydrogen bond: between partial electronegative atoms negative charge and hydrogen atoms.
- Hydrophobic interactions: between non-polar groups.
- Van der Waals interactions: between atoms in close proximity, weak (1/1000 of the covalent bond strength), but important for maintaining conformation.
- Metallic – Fe2+.

- Single type of covalent bond- disulfide bridges.



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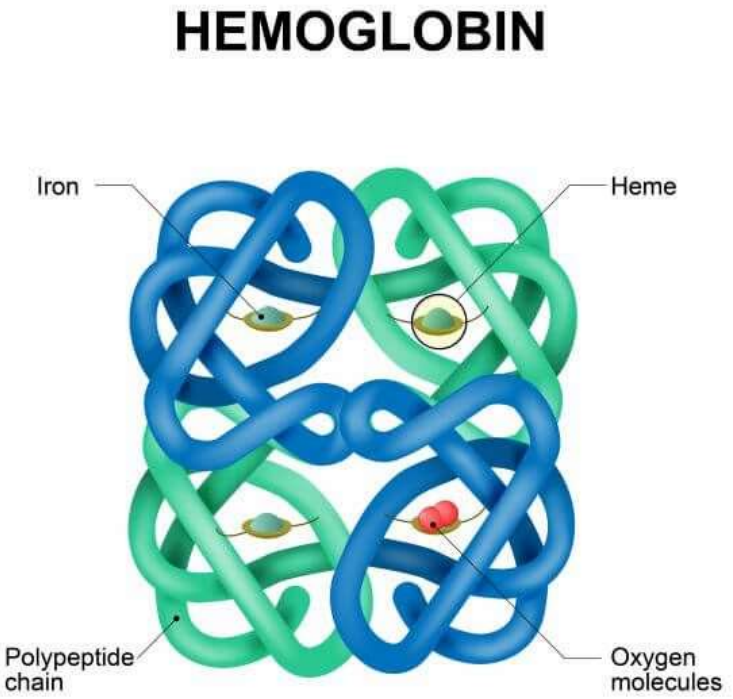
Quaternary structure of protein



- Many proteins are made of a single polypeptide chain and don't become any more complex than their tertiary structure.
- Some proteins are made up of multiple polypeptide chains, also known as subunits. When these subunits come together, they give the protein its quaternary structure.
- One example of a quaternary protein structure is hemoglobin. Hemoglobin is made up of four polypeptide chains, and is specially adapted to bind oxygen in the blood.

https://www.khanacademy.org/science/biology/macromolecules/proteins-and-amino-acids/a/orders-of-protein-structure

https://biologydictionary.net/protein-structure/

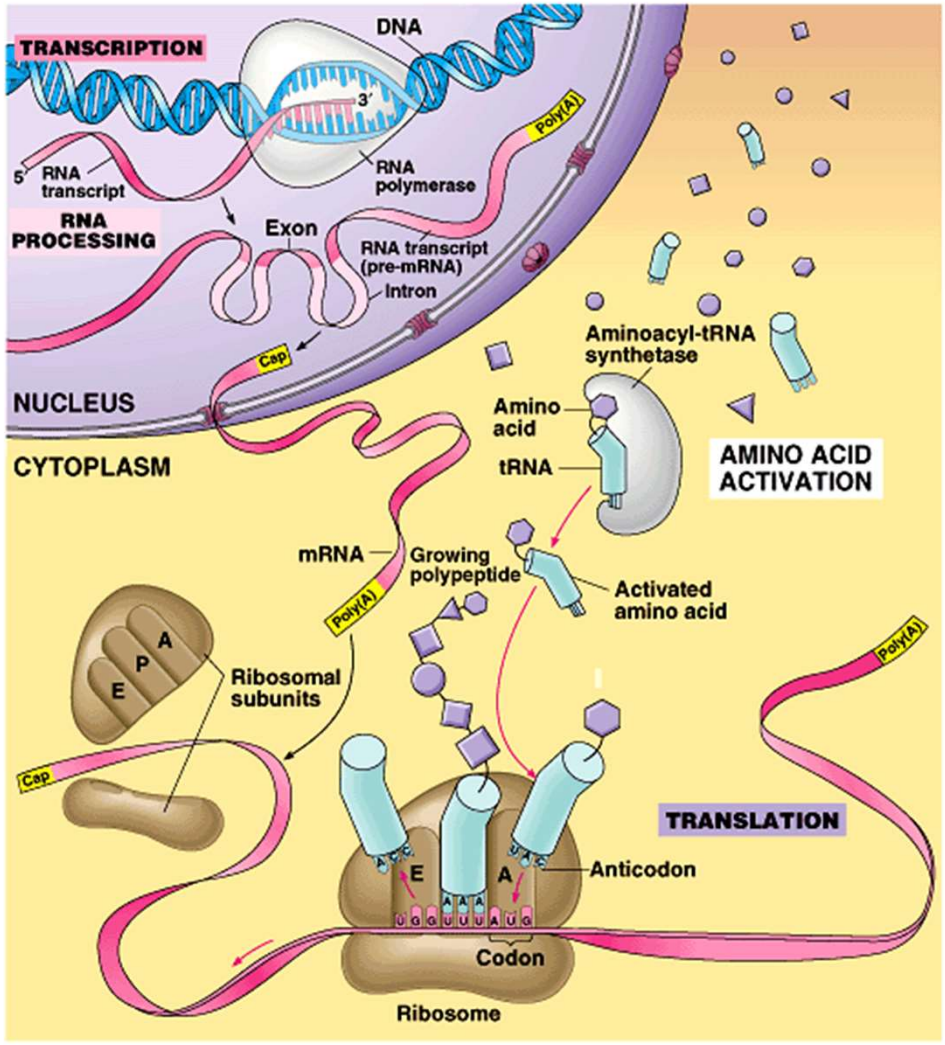
Translation

- Translation of genetic information from mRNA into amino acid sequence using the genetic code.

Components of the translational apparatus:

- mRNA
- 20 standard amino acids (+ selenocysteine, pyrrolysine)
- tRNA
- 20 enzymes for amino acid activation (aminoacyl-tRNA synthetase)
- Ribosomes
- soluble protein series: translational factors IF, EF, RF- ATP, GTP
- the translational system represents the dominant part of the metabolic apparatus of the cell.

Translation overwiev



http://uoitbiology12u2014.weebly.com/protein-synthesis-and-genetic-code.html

Genetic code

- The genetic code is a set of rules defining how the four-letter code of DNA is translated into the 20-letter code of amino acids, which are the building blocks of proteins.
- Each AK is determined by a codon in mRNA.
- Each codon is defined by a triplet of nucleotides.
- Triplets/codons do not overlap.
- 64 possible codons: 61 determine amino acids + 3 termination of translation.
- Codons in mRNA are recognized (and temporarily bound on the basis of base pairing) by complementary sequences, anticodons, in tRNA.
- Amino acids are carried by specific tRNAs.

https://www.nature.com/scitable/definition/genetic-code-13/

Genetic code

- Is degenerate – one amino acid can be encoded by multiple codons (redundance).
- These synonymous codons are included in codon families.
- Three codons are meaningless (stop codons, termination codons): UAA – ochre, UAG – amber, UGA – opal.
- 3 codons are bifunctional:
  - UGA – opal = meaningless or encodes selenocysteine
  - UAG – amber = meaningless or encodes pyrrolysine
  - AUG = initiating or encoding methionine
- Is universal – most codons have the same meaning in all living systems (prokaryotes, eukaryotes, viruses).

https://basicmedicalkey.com/s-phase-and-dna-replication/

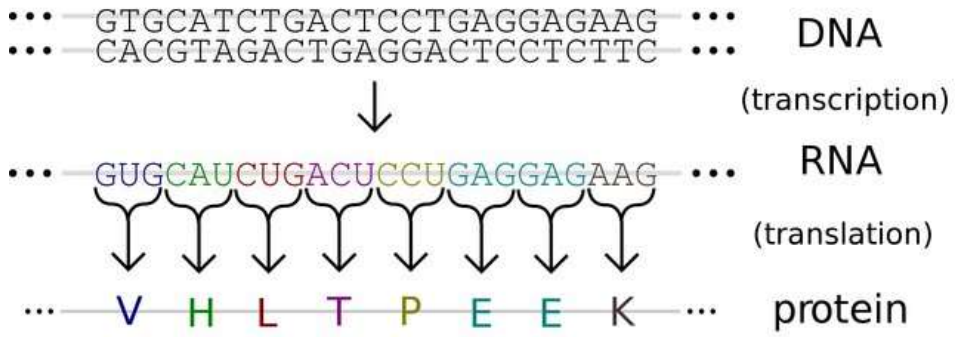
Cracking the genetic code

- Work by Crick and coworkers showed that the genetic code was based on non-overlapping triplets of bases, called codons.
- H. G. Khorana, R. Holley and M. Nirenberg and others deciphered the encoding the meaning of all codons in 1966.
- In 1968 H.G. Khorana R. Holley M. Nirenberg were awarded by the Nobel Prize in Physiology or Medicine.

H. G. Khorana

R. Holley

M. Nirenberg



https://www.researchgate.net/publication/282279062_Computational_Statistics_for_the_Identification_of_Transcriptional_Gene_Regulatory_Networks

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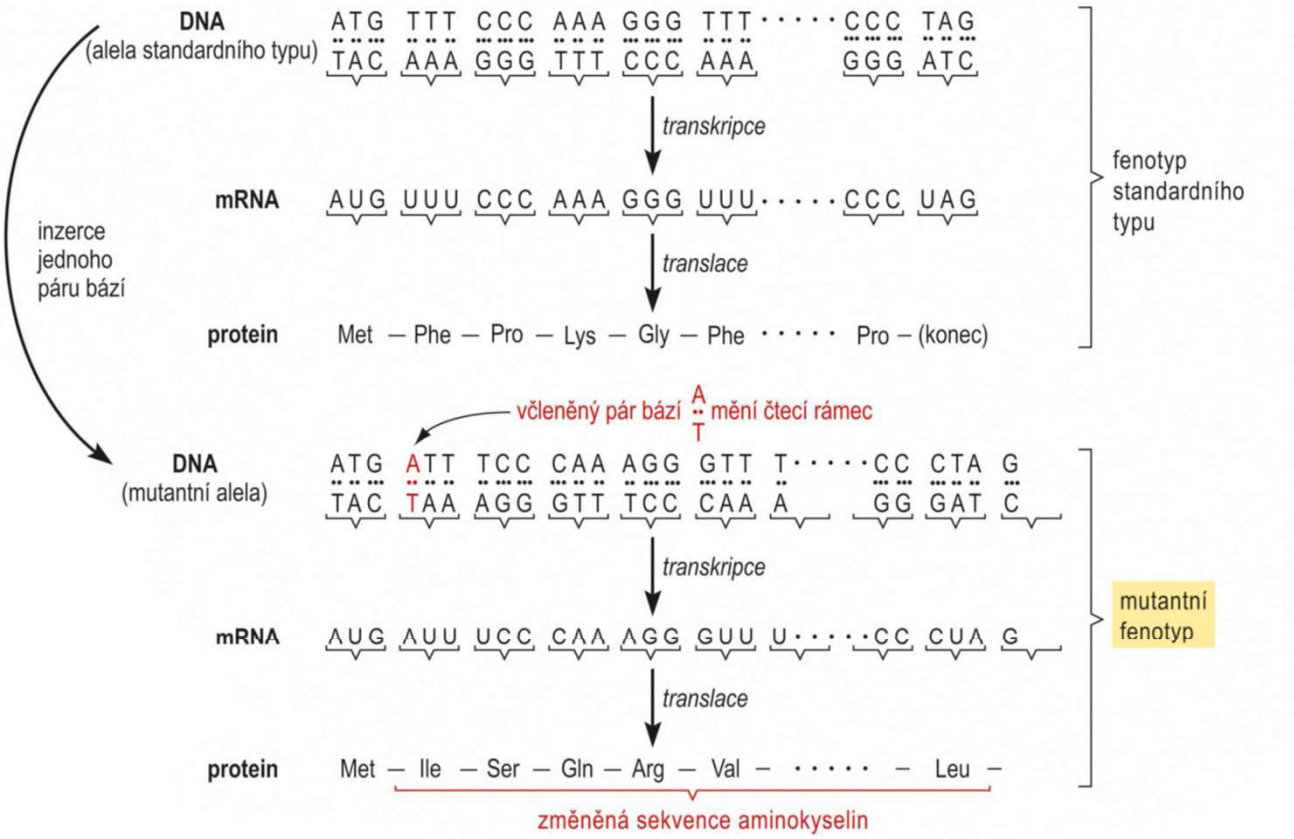
Genetic code is formed by triplets of codons

- Insertion/deletion of one/two base pairs changes the reading frame.



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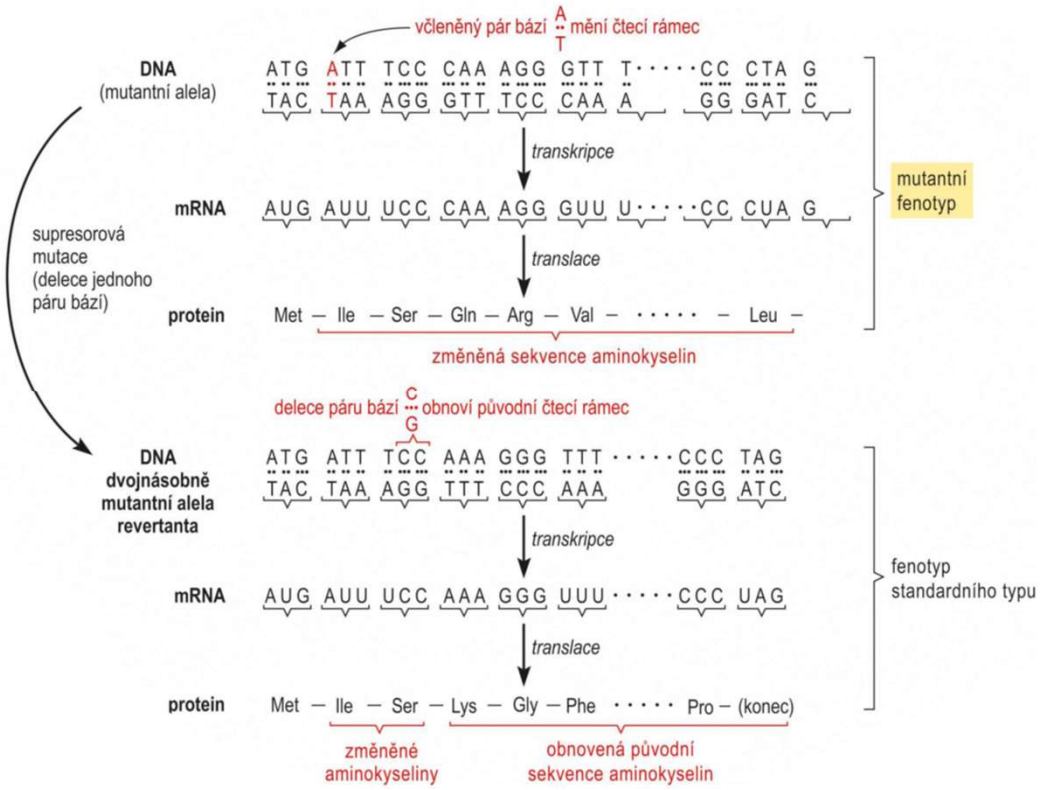
Genetic code is formed by triplets of codons

- The suppressor mutation cancels or reverts the effect of the original mutation and restores the reading frame.



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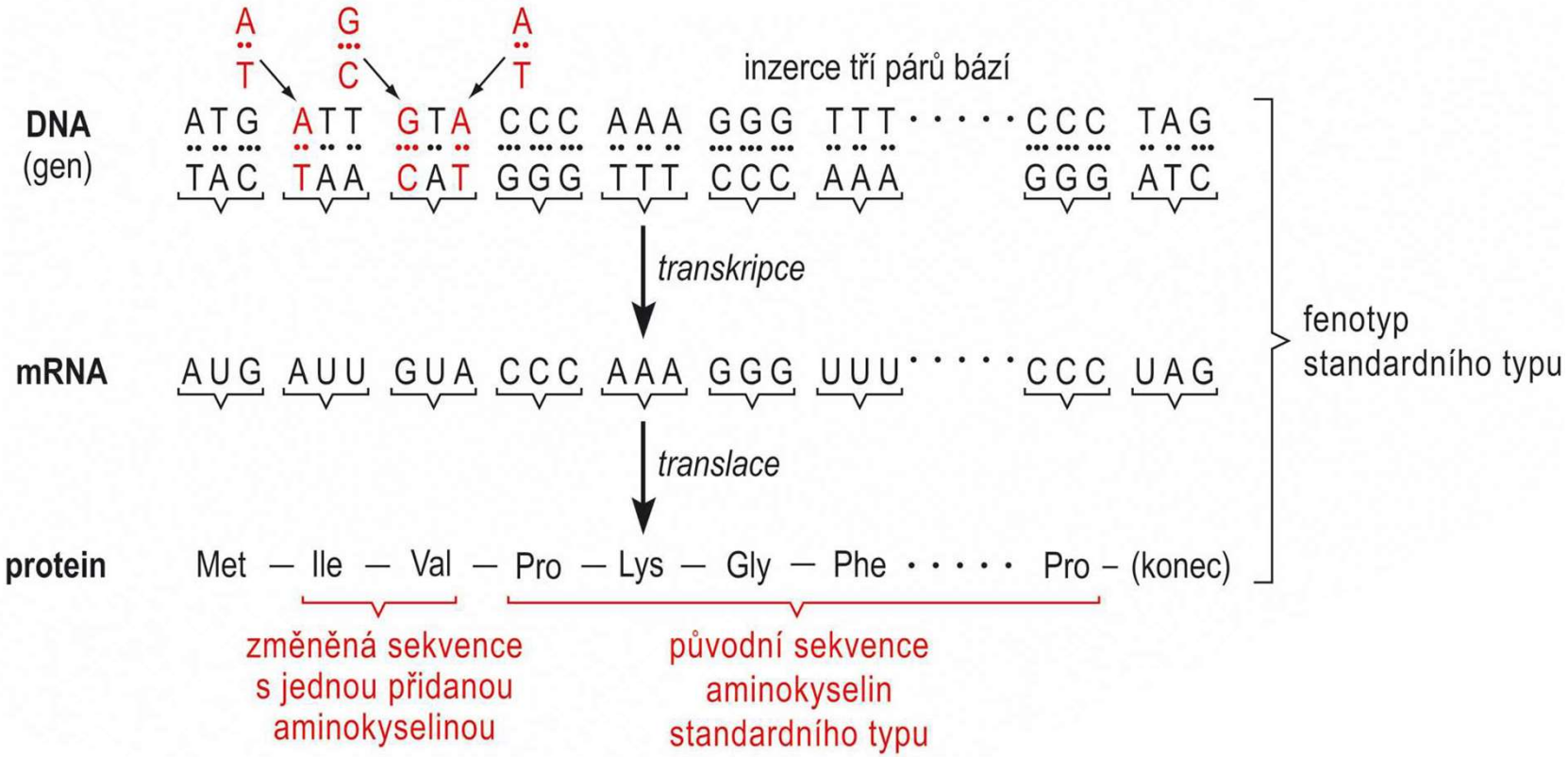
Genetic code is formed by triplets of codons

- Insertion/Deletion of three bases does not change the reading frame.



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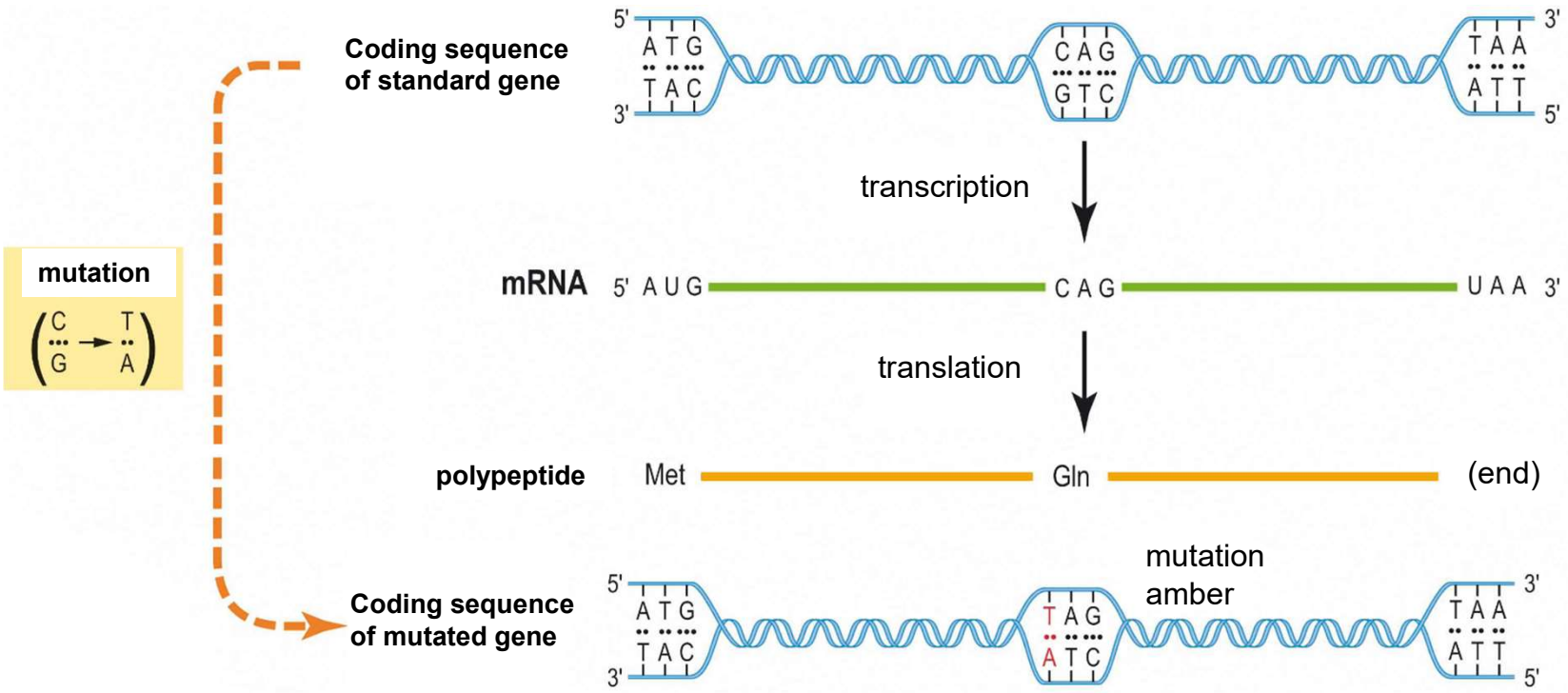
Suppressor mutations

- Some mutations in tRNA genes change the structure of anticodons and therefore lead to a misinterpretation of codons in mRNA.
- These mutations, originally found as suppressor mutations, have suppressive effect on other mutations.
- Example: tRNA mutations that suppress amber mutations causing termination of string translation in UAG.

Mutation Amber (UAG) termination of translation



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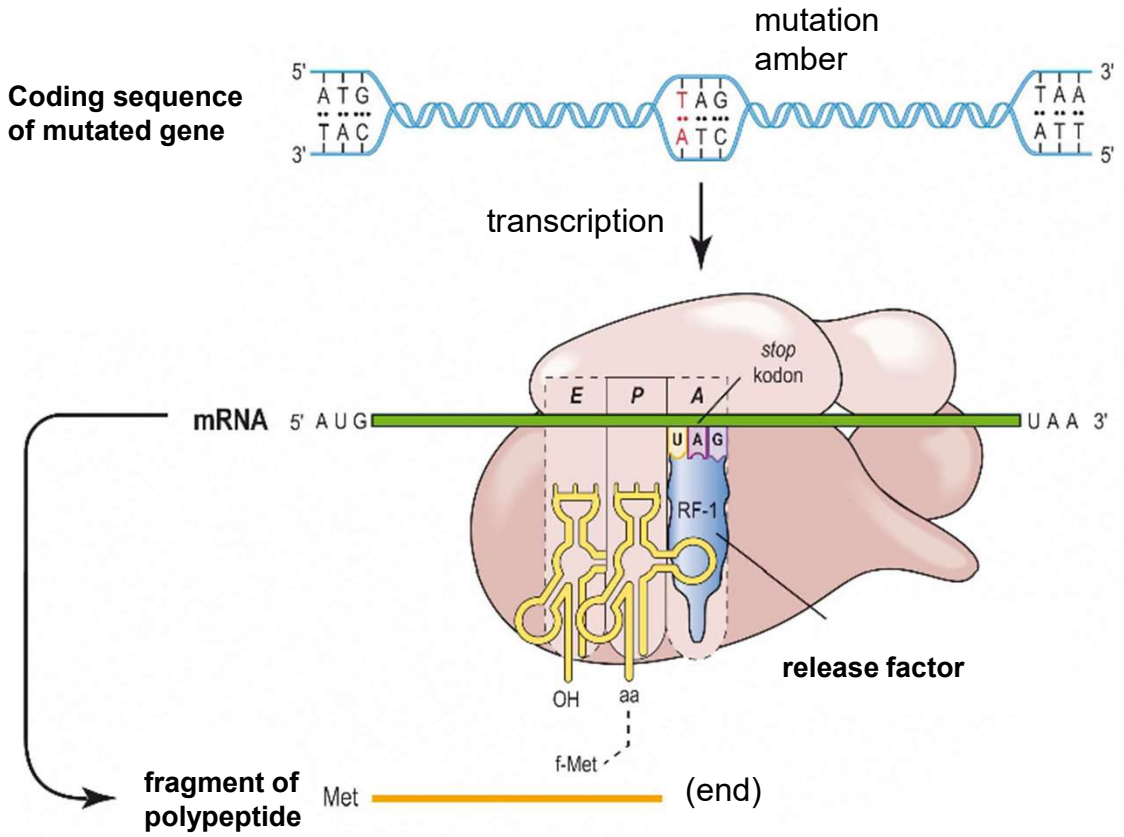
Mutation Amber in the absence of suppressor mutations



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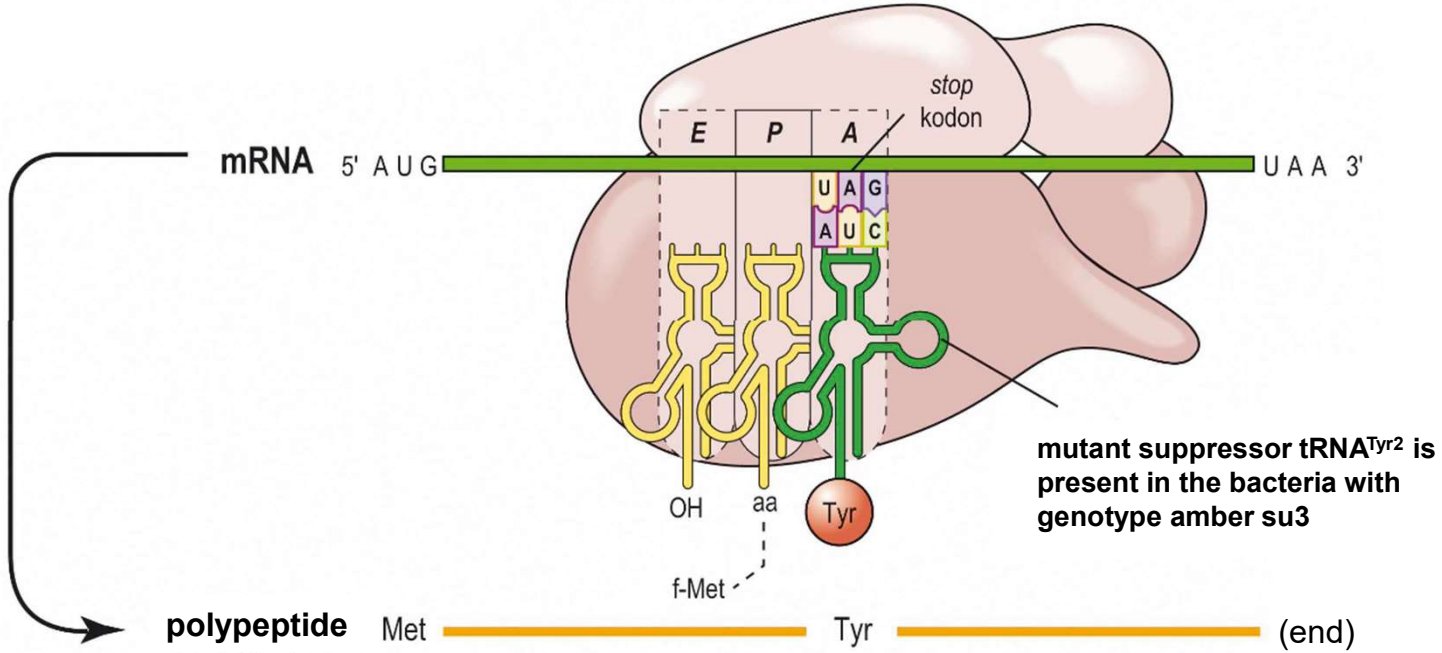
Mutation Amber in the presence of suppressor mutations

- Some suppressor mutations alter tRNA anticodons so that mutant tRNA pairs to termination codon.
- It leads to incorporation of an amino acid into the polypeptide (instead of termination of translation).

translation in the presence of suppressor tRNA



mutant suppressor $tRNA^{Tyr2}$ is present in the bacteria with genotype amber su3

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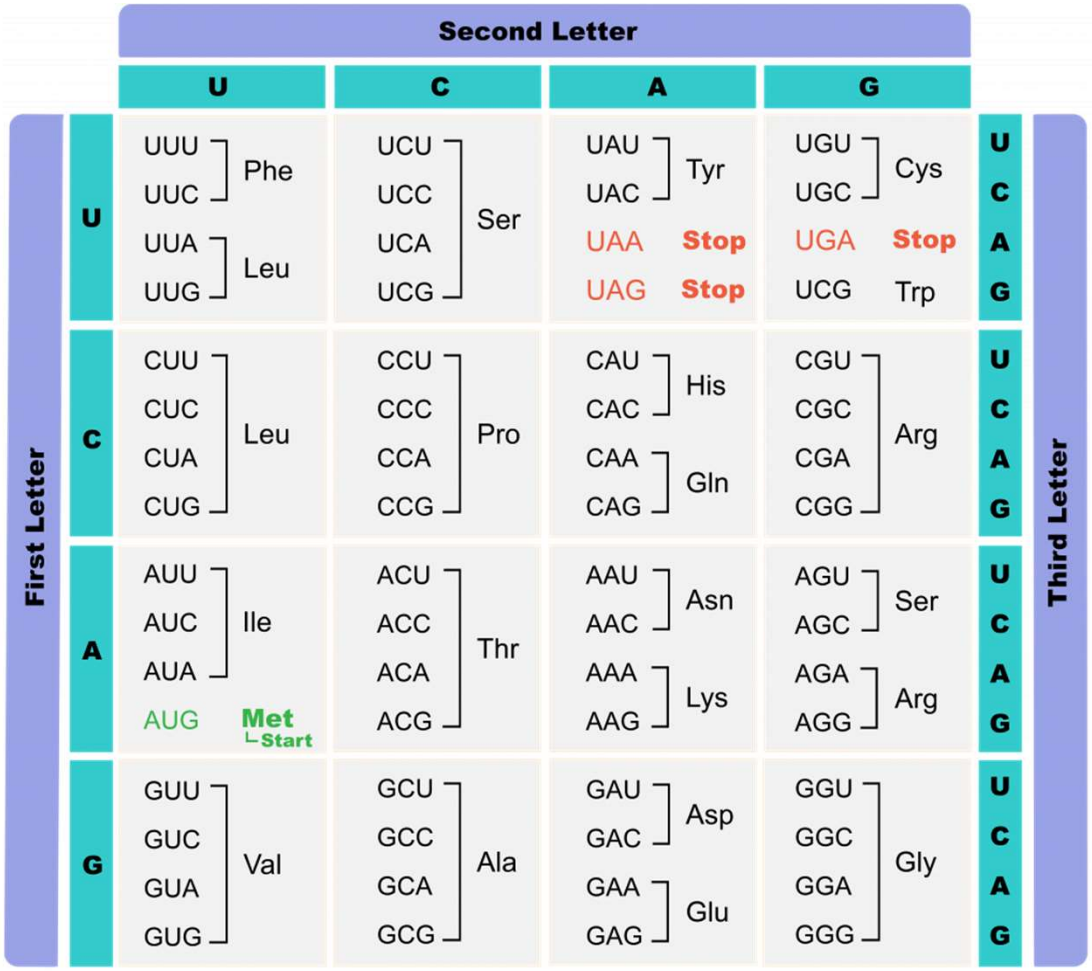
Standard genetic code



- UGA (opal) - Selenocysteine
- UAG (amber) - pyrrolysine

https://open.lib.umn.edu/evolutionbiology/chapter/5-8-using-the-genetic-code-2/

Nonstandard amino acids – selenocysteine

- Selenium = an important rare element necessary for catalytic activity of oxidoreductase, with the amino acid selenocysteine (Sec).

- Selenocysteine is 21st amino acid and is located in the active sites of enzymes that participate in oxidation–reduction reactions (glutathione peroxidases, thioredoxin reductases, formate dehydrogenases, some hydrogenases). Selenoproteins are rare (1‰ proteins).

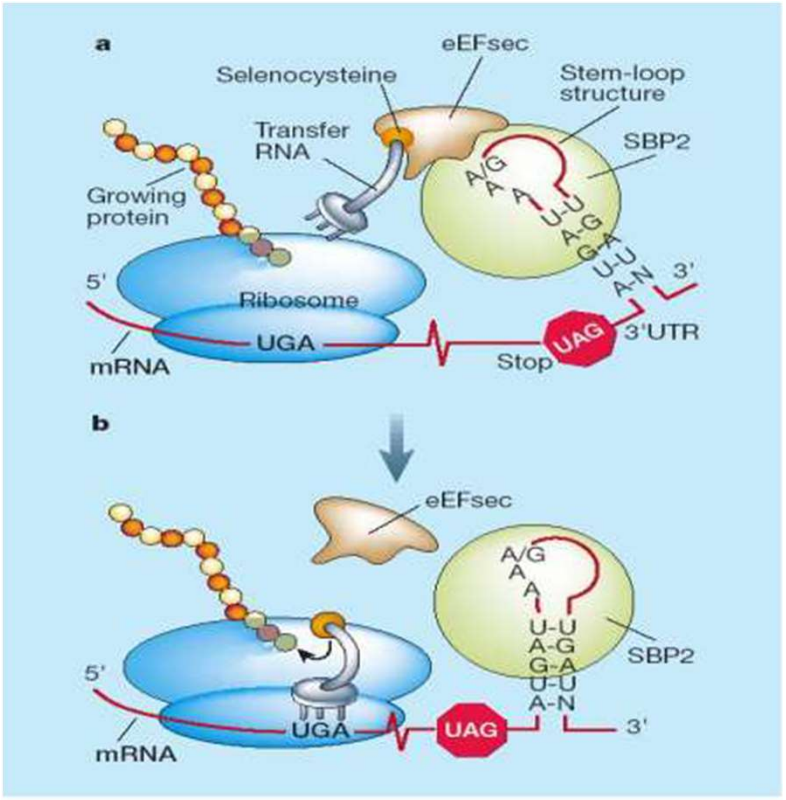
- Selenocysteine-tRNA is initially charged with serine. Then the attached serine is enzymatically modified to form selenocysteine.

Serine (Ser) Cysteine (Cys) Selenocysteine (Sec)

TOXICOLOGY COMMUNICATIONS, 2017VOL. 1, NO. 1, 24–28

Nonstandard amino acids – selenocysteine

- Selenocysteine is not produced by posttranslational modification, but by translation. Condition - alternative use of stop codon UGA.
- The gene in question must contain a specific signaling sequence SECIS (selenocysteine insertion sequence), which is transcribed into mRNA, but is not translated into the protein.
- SECIS element contains auto-complementary sequences, forming a hairpin structure with a loop.
- Special proteins associate with the hairpin and ensures recruitment of tRNA recognizing UGA codon carrying the selenocysteine to the ribosome.



Selenoprotein Synthesis

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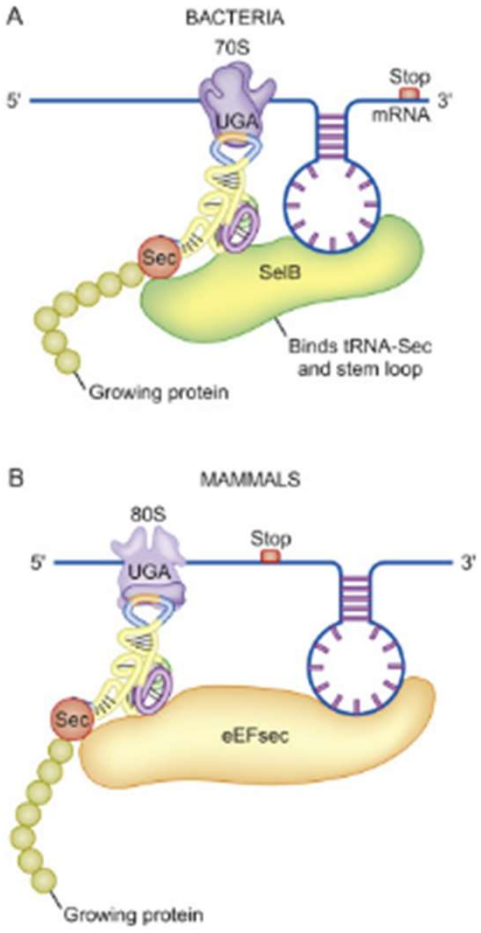
Nonstandard amino acids – selenocysteine

- The use of the codon is not accidental, but depends on the context of the given mRNA.
- In bacteria, the tRNA carrying selenocysteine (Sec) first binds to SelB and the complex then binds to a stem and loop in the mRNA. This aligns the tRNA-Sec with a UGA codon within the coding sequence on the mRNA. Selenocysteine is then inserted as part of the growing polypeptide.
- In mammals, the protein that binds the stem and loop and the tRNA-Sec is called eEFsec. In addition, the stem and loop are more distant, being found after the stop codon.



https://www.sciencedirect.com/topics/medicine-and-dentistry/selenocysteine

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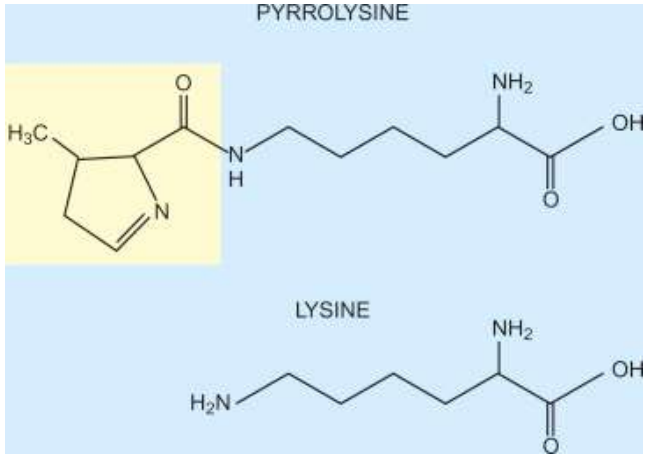
Nonstandard amino acids – pyrrolysine

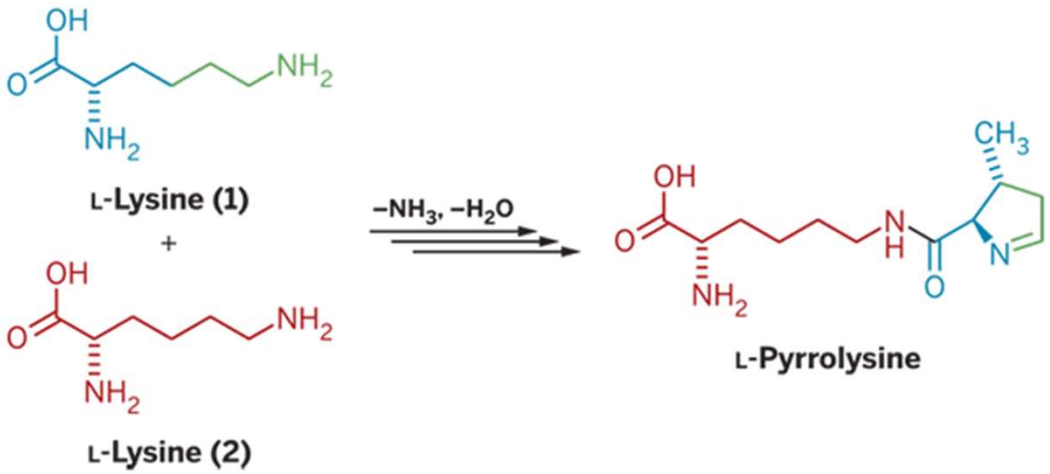
- Pyrrolysine (Pyl) is 22nd genetically encoded amino acid, it is a derivative of lysine with an attached pyrroline ring.
- Occurrence is limited to archaebacteria *Methanosarcinaceae* and on the bacterium *Desulfitobacterium hafniense*.
- The involvement of pyrroliethylene in them is associated with an exceptional ability use methylamines as an energy source using methylamine methyl-transferase.
- For the synthesis of these enzymes, one UAG termination triplet must be used for the inclusion of pyrrolysine.
- Molecular mechanism of alternative interpretation of stop codon is not yet fully known.



https://www.sciencedirect.com/topics/medicine-and-dentistry/selenocysteine

Nonstandard amino acids – pyrrolysine

- Pyrrolysine is made first as a free amino acid and then attached to tRNA-Pyl.
- There is no pyrrolysine specific elongation factor.

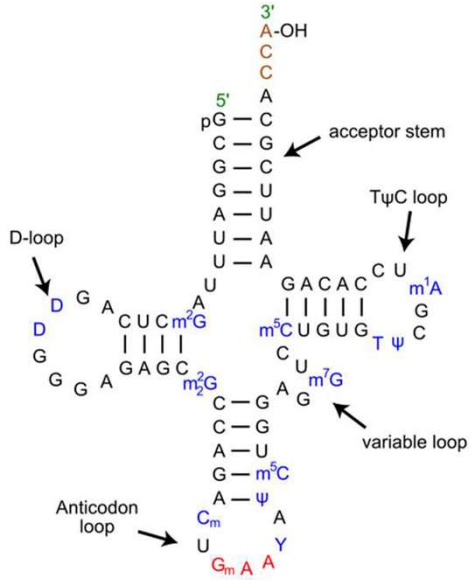


- Moreover, the sequence determinants that specify which UAG codons should be used for pyrrolysine insertion are unclear.
- Genome sequencing has found genes homologous to those for the pyrrolysine system in occasional *Eubacteria*, yet, pyrrolysine itself has not yet been identified directly in these organisms.

https://cen.acs.org/articles/89/web/2011/04/Pyrrolysine-Synthesis-Revealed.html

Transfer RNA – tRNA

- Transfer ribonucleic acid (tRNA) is a type of RNA molecule that helps decode a messenger RNA (mRNA) sequence into a protein.
- 70-95 nucleotides.
- Internal base pairing creates partially ds sections -folding tRNA into a three-dimensional structure.



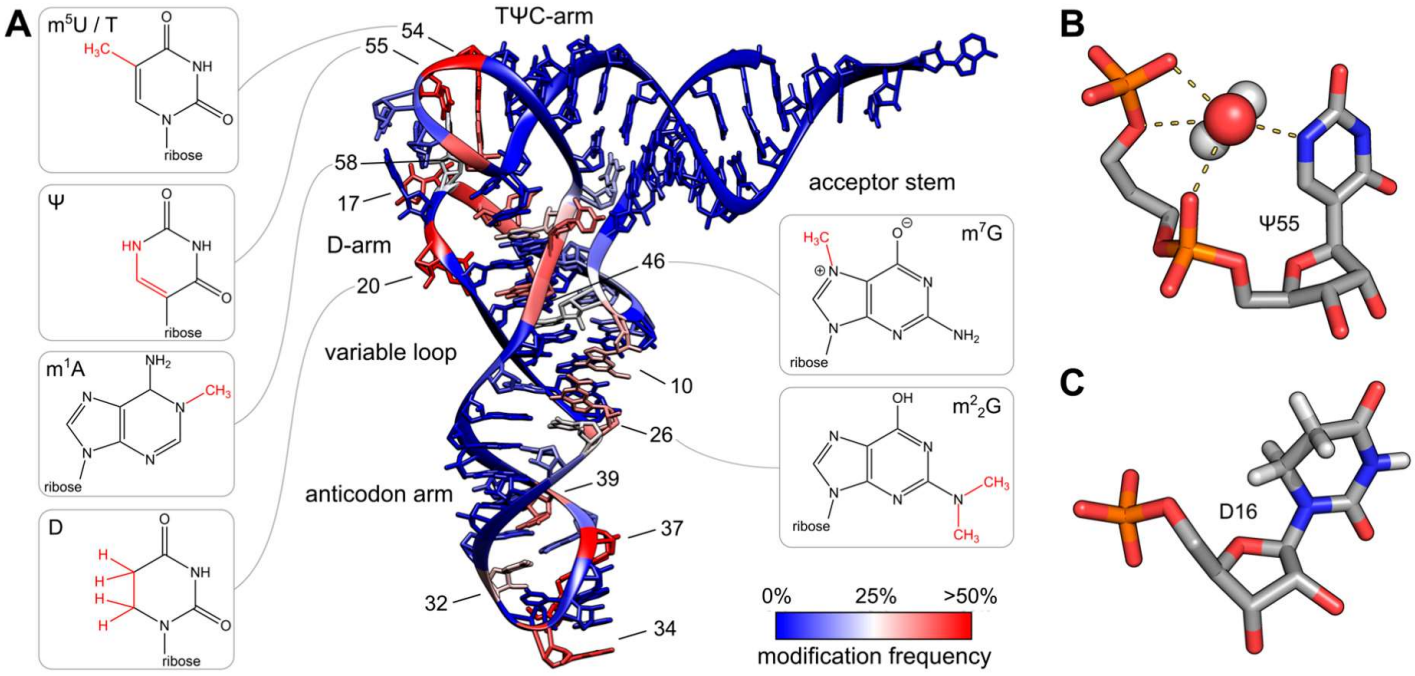
- Contain anticodon - a sequence of three nucleotides complementary to the codon in mRNA.
- The amino acid is covalently (ester) attached to 3'-OH end of tRNA.
- Dihydrouridine and pseudouridine arms stabilize the secondary structure of the clover leaf.

https://www.nature.com/scitable/definition/trna-transfer-rna-256/

Transfer RNA – tRNA

- Modified nucleotides often appear in tRNA and they help correct folding of tRNA - pseudouridine, methylguanosine, dimethylguanosine, methylinosine, dihydrouridine.

- Modified nucleotides are introduced post-transcriptionally.



Biomolecules 2017, 7(2), 35; https://doi.org/10.3390/biom7020035

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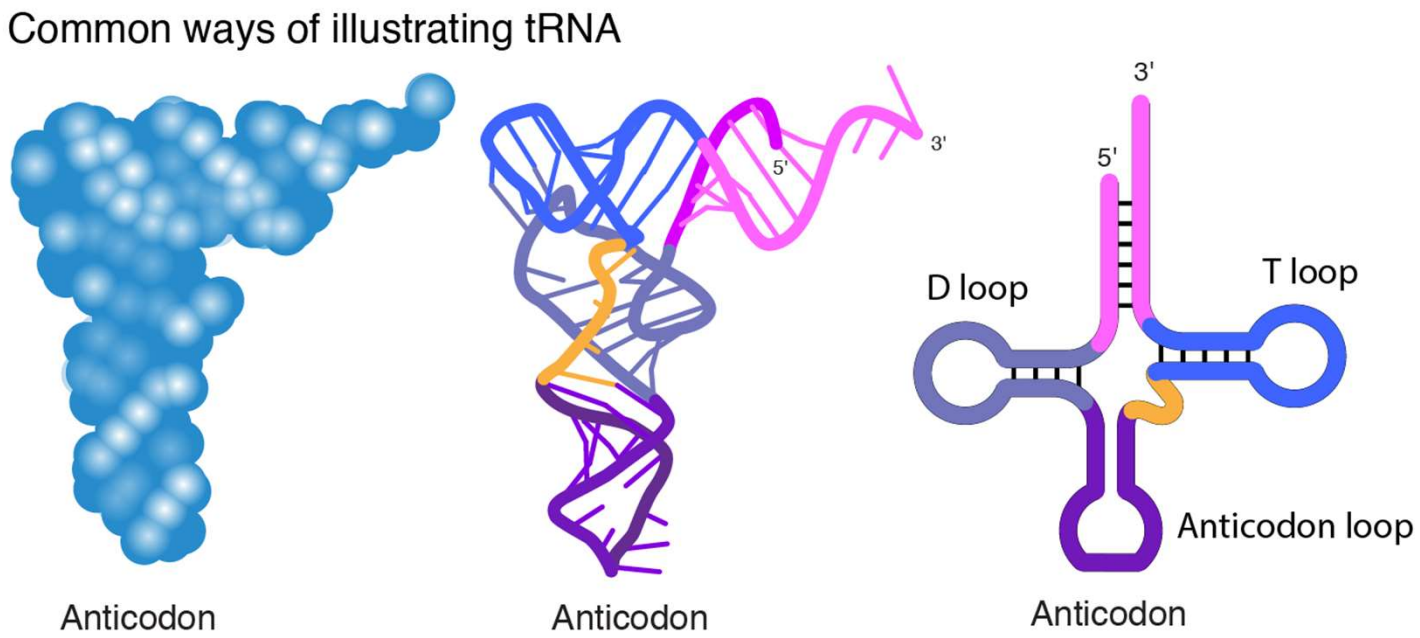
Structure of tRNA molecule

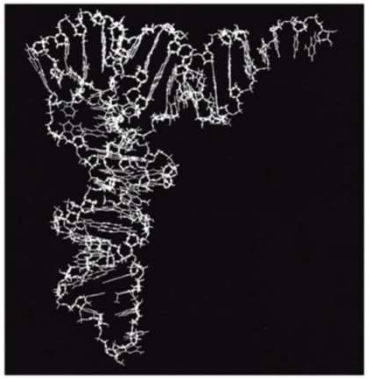
- Clover leaf is folded into an L-shaped structure due to hydrogen bonds between different domains.

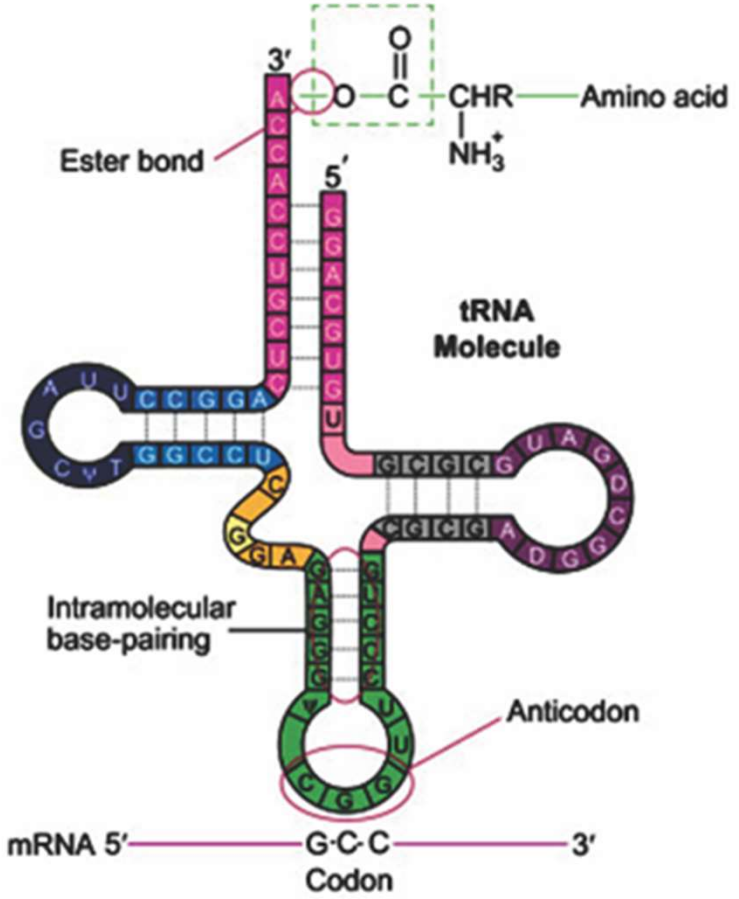


https://www.genome.gov/genetics-glossary/Transfer-RNA

Structure of tRNA molecule

- At opposite ends of the L-shaped tRNA structure there are two regions of unpaired nucleotides:
- Anticodon composed of three nucleotides complementary to the codon.
- Short single-string area at the 3´-end, where a specific AK is connected.





https://www.chegg.com/learn/biology/introduction-to-biology/peptidyl-trna-and-transfer-rna

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tRNA and redundancy of genetic code

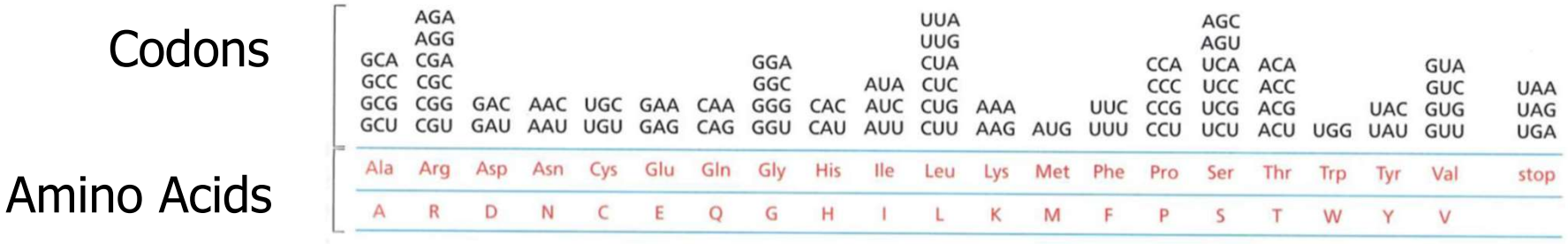
- The genetic code is redundant: several codons determine the same amino acid.

Possible explanations:

- There is more than one tRNA for certain amino acids.
- Certain tRNAs can pair with multiple codons.
- Both variants are true.

tRNA and redundancy of genetic code

- Precise pairing between codon and anticodoe is required only between nucleotides in the first two positions of the codon, in the third position alternatives are tolerated jittering/"wobble“.



- Thanks to fluctuating base pairing, 20 amino acids and 61 codons are covered only by 31 variants of tRNA molecules.

- The exact number of tRNA species is not the same for different species.

https://microbenotes.com/dna-polymerase/#dna-polymerase-iii

FEMS Microbiol Rev. 2012 Nov; 36(6): 1105–1121.

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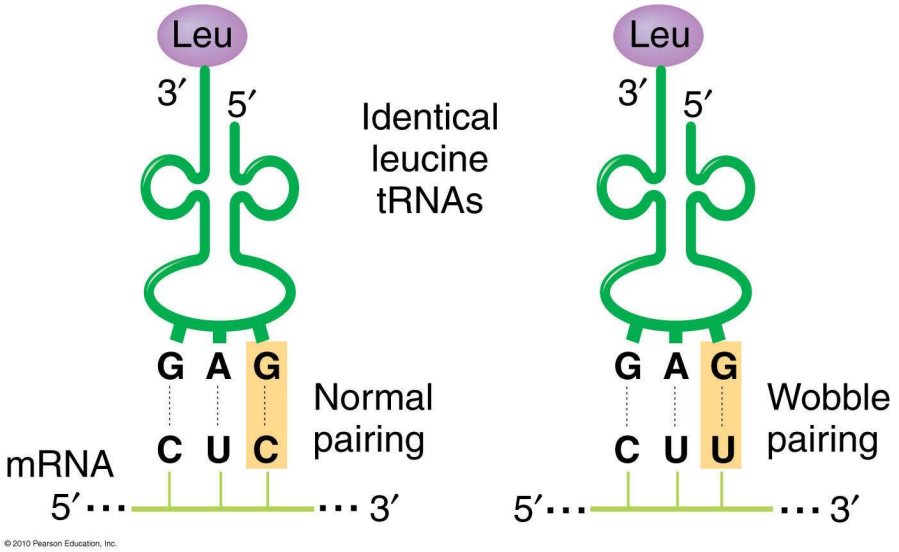
tRNA and redundancy of genetic code

- Pairing of the tRNA anticodon with the mRNA codon proceeds from the 5' end of the codon.
- Once the first two positions are paired, exact base pairing of the third position is less critical. The first (5') base of the anticodon can typically pair with either member of the purine or pyrimidine pair in the codon as appropriate: it "wobbles".
- In this example, the double-ringed G can pair with either a single-ringed U or C.
- This allows mRNA to be translated with fewer than the 64 tRNAs that would be required without wobble. Some wobble positions can pair with any of the four bases.



GAG (anticodon)

CUC CUU

Two codons

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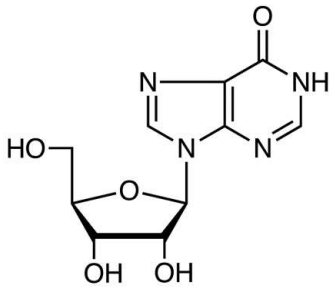
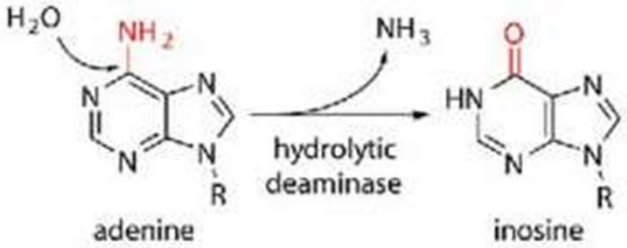
Alternative base pairing between anticodon CGI in tRNA and three different (synonymous) codons

- I = Inosine





- Inosine = nucleoside composed of ribose and hypoxanthin.
- In eukaryotes the modification is present in eight different tRNAs.
- The modification of adenosine to inosine in tRNA anticodons has a profound impact upon codon-anticodon recognition.

RNA Biol. 2018; 15(4-5): 500–507.

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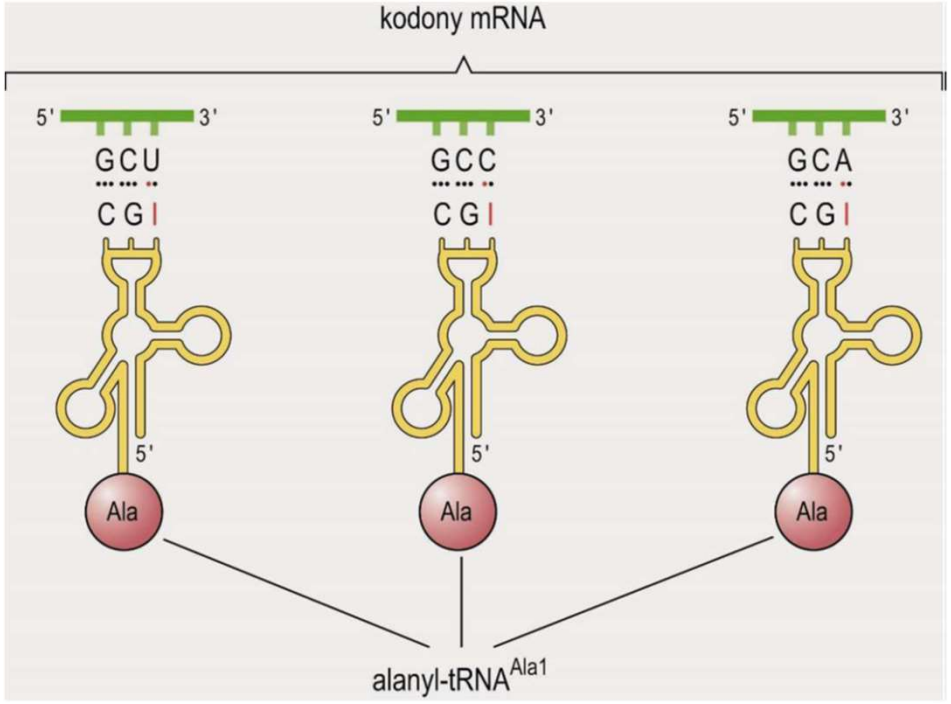
Alternative base pairing between anticodon CGI in tRNA and three different (synonymous) codons

- It can pairs with uracile, cytosine and adenine.
- Modified tRNAs with inosine are preferred for the translation of highly repetitive coding sequences.



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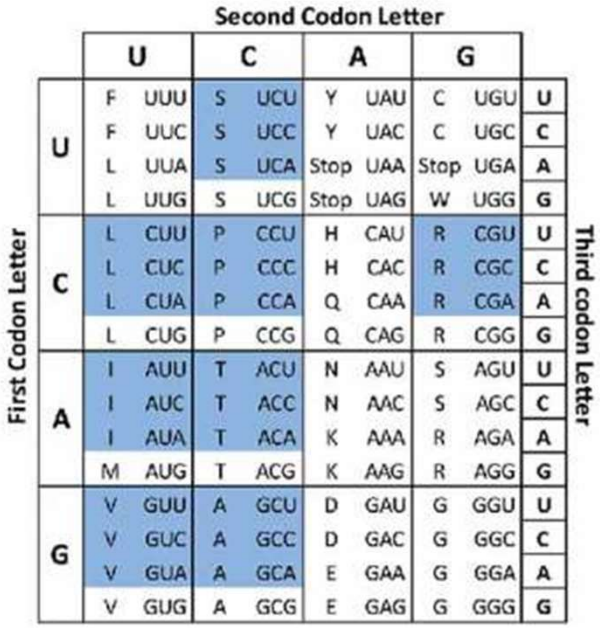
Isoacceptor tRNA

- Isoacceptor tRNAs are tRNAs with different anticodons but incorporating the same amino acid in protein synthesis.

Codon	tRNA	Antikodon
UCU UCC	$tRNA^{Ser1}$	AGG + wobbling
UCA UCG	RNA^{Ser2}	AGU + wobbling
AGU AGC	$tRNA^{Ser3}$	UCG + wobbling



RNA Biol. 2018; 15(4-5): 500–507.

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Speficity of tRNA

tRNA

- Must have the correct anticodon sequence (to respond to the correct codon).
- Must be recognized by correct aminoacyl-tRNA synthetases (to carry the correct AA).
- Must bind to the correct place of the ribosome (so that it can realize its adapter function).

Aminoacyl tRNA synthetase

Aminoacyl tRNA synthetase

- Enzymes that covalently attach specific AA to specific tRNA.
- There are 20 AA-tRNA synthetases, one for each AA.
- For instance, one AA-tRNA synthetase attaches glycine to all tRNAs, which recognize codons for glycine, etc.
- Each AA-tRNA synthetase must recognize:
  - relevant AA
  - unpaired nucleotides in the anticodon arm
  - sequence in AK-tRNA arm.
- The exact function of AA-tRNA synthetases is equally important for translation accuracy, as a codon-anticodon bond!

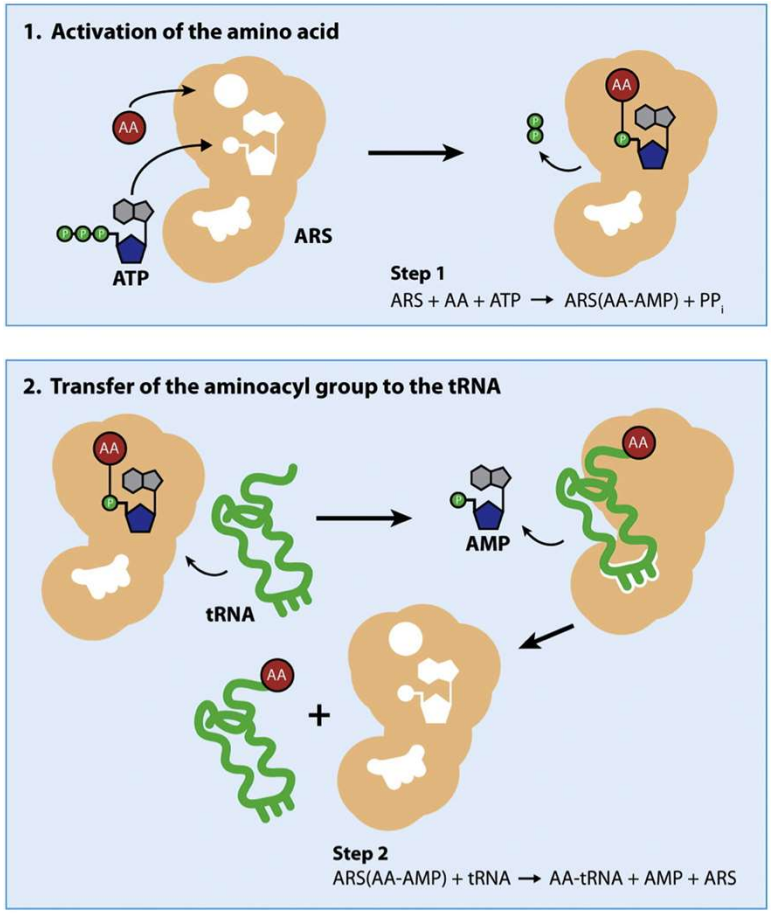
Two phases of translation

- Extra-ribosomal: attachment of an amino acid to its tRNA by activity of aminoacyl-tRNA-synthetase.
- Ribosomal: amino acids, bound to appropriate tRNA, are assembled to polypeptide on ribosome based on sequence of codons.

The ribosomal phase has three stages:

- translation initiation
- polypeptide chain elongation
- translation termination.

Extra-ribosomal phase of translation



- Extra-ribosomal phase is sometimes called "charging" or "loading" the tRNA with an AA.
- The synthetase first binds ATP and the corresponding amino acid (or its precursor) to form an aminoacyl-adenylate, releasing inorganic pyrophosphate (PPi).
- The adenylate-aa ARS complex then binds the appropriate tRNA molecule's D arm, and the amino acid is transferred from the aa-AMP to either the 2'- or the 3'-OH of the last tRNA nucleotide at the 3'-end.
- 1. Amino Acid + ATP → Aminoacyl-AMP + PPi
- 2. Aminoacyl-AMP + tRNA → Aminoacyl-tRNA + AMP

International Journal of Biological Macromolecules, Volume 111, May 2018, Pages 400-414
https://en.wikipedia.org/wiki/Aminoacyl_tRNA_synthetase

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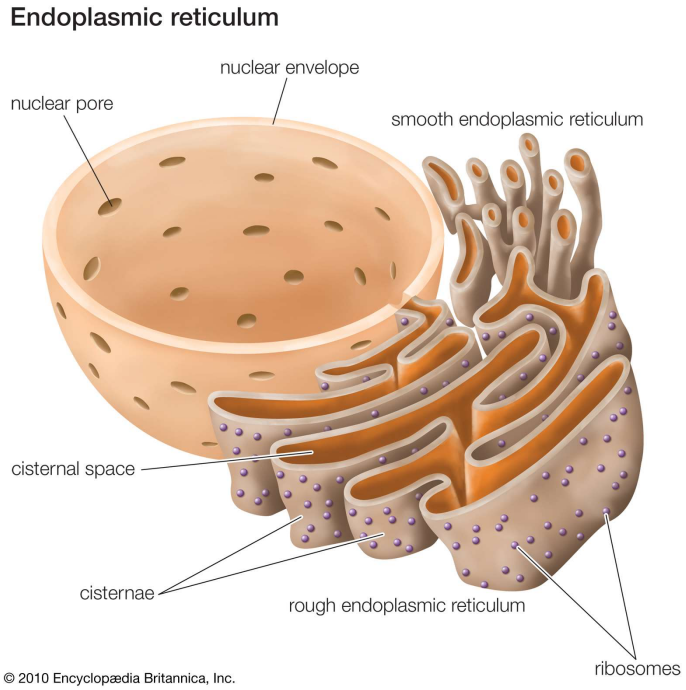
Ribosomes

- Cellular particle made of RNA and protein that serves as the site for protein synthesis in the cell.
- The ribosome reads the sequence of the messenger RNA (mRNA) and, using the genetic code, translates the sequence of RNA bases into a sequence of amino acids.
- It is the place where codons meet anticodons according to the base pairing rules.
- It can assemble onto mRNA, capture and correctly direct tRNA molecules in order to covalently connect amino acids into the polypeptide chain.
- Work non-specifically: they synthesize any peptide properly encoded by any mRNA.

https://www.genome.gov/genetics-glossary/Ribosome

Ribosomes

- Ribosomes are present in large numbers in all living cells.
- Ribosomes occur
  - free particles in prokaryotic and eukaryotic cells
  - particles attached to the membranes of the endoplasmic reticulum in eukaryotic cells.
- There are about 200,000 ribosomes in the *E. coli* cell (i.e. 25% of the dry mass of the cell).
- A typical eukaryotic cell in cytoplasm contains millions of ribosomes.
- All molecules involved in protein synthesis together forms 1/3 of the dry weight of cells, high energy consumption.



https://www.britannica.com/science/ribosome

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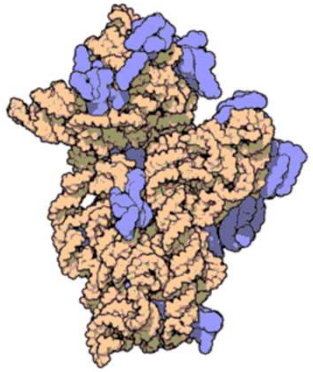
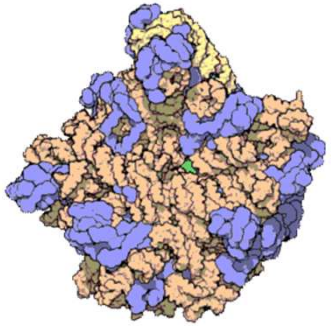
Ribosomes

Small 30S subunit



Large 50S subunit



- Ribosomes consist of two major components: the small and large ribosomal subunits.
- Each subunit consists of one or more ribosomal RNA (rRNA) molecules and many ribosomal proteins (RPs or r-proteins).
- rRNA sequences are very evolutionarily conserved.
- Ribosome sizes are often expressed according to Svedberg's units "S„ the Svedberg unit, a measure of the rate of sedimentation in centrifugation rather than size.

https://commons.wikimedia.org/wiki/File:010_small_subunit-1FKA.gif
https://commons.wikimedia.org/wiki/File:010_large_subunit-1FFK.gif

Ribosomes

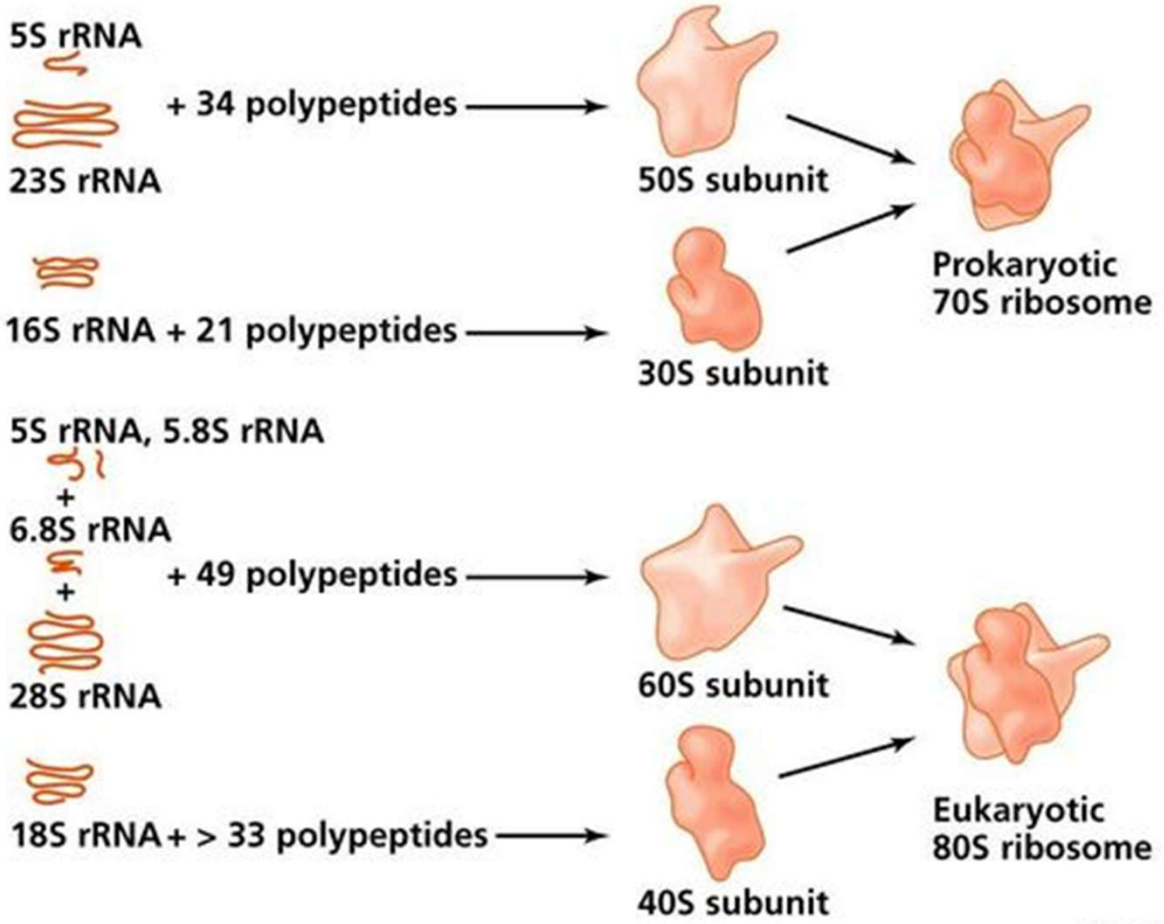
Prokaryotes:

- Size 70S (20 x 25 nm)
- Small subunit 30S: rRNA 16S + 21 proteins
- Large subunit 50S: rRNA 5S and 23S + 34 proteins.

Eukaryotes:

- Usual size 80S (some interspecies variability)
- In mitochondria and chloroplasts 60S
- Small subunit 40S: rRNA 18S + 33 proteins
- Large subunit 60S: rRNA 5S, 5.8S, 28S + 49 proteins

Ribosomes in prokaryotes and eukaryotes



https://nl.pinterest.com/pin/503418064572288990/?amp_client_id=CLIENT_ID(_)&mweb_unauth_id={{default.session}}&simplified=true

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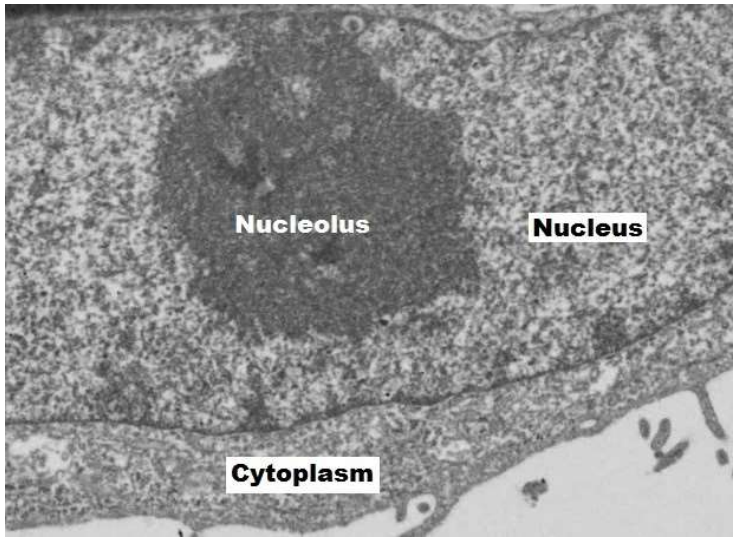
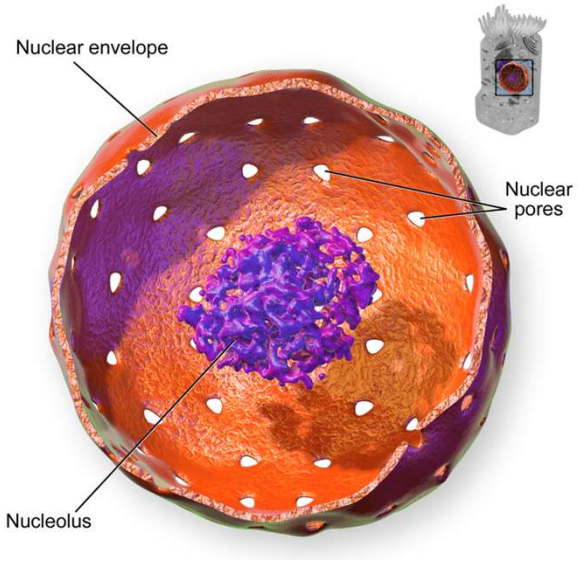
rRNAs come from the nucleolus

- rRNAs are produced by DNA transcription.
- In eukaryotes rRNA is produced by RNA-polymerase I in the nucleolus.





https://sciencetrends.com/what-is-the-nucleolus/

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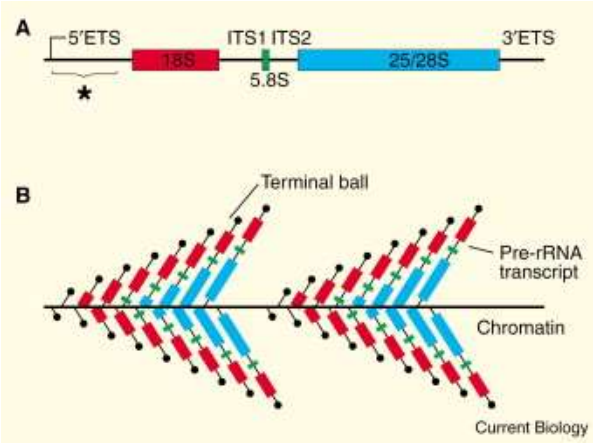
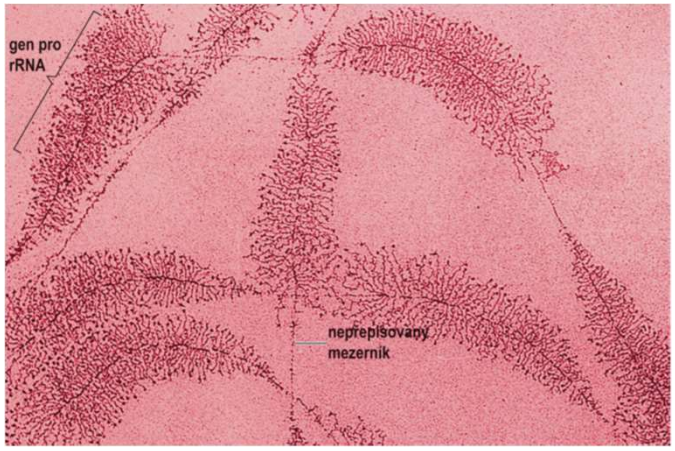
rRNA genes

- The rRNA genes are arranged in succession in many copies.
- Separated by intergene areas (spacebars).
- Transcription of these genes is very effective.





https://www.cell.com/fulltext/S0960-9822(02)01135-1

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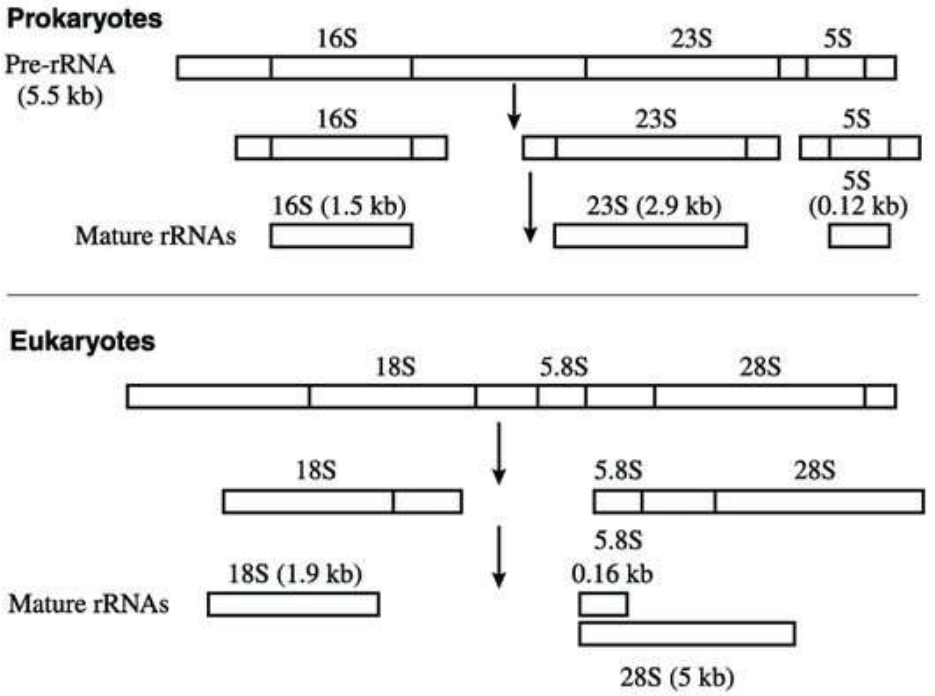
rRNA genes

- The genes for rRNA are transcribed as a longer precursors, which are spliced and modified by post-transcriptional modifications.



Canadian journal of gastroenterology = Journal canadien de gastroenterologie 14(2):99-110

Small and Large subunit of ribosome

Unique function

- Small subunit
- correct assembly with respect to mRNA and tRNA.

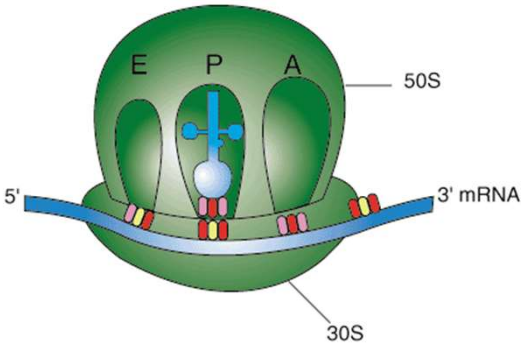
- Large subunit
- formation of peptide bonds.

- Both subunits are assembled into one complex near the 5´- end of the mRNA, where the translation begins.

- mRNA gradually stretches through the ribosome and allows translation one codon at a time.

- After completion of translation, the complex disintegrates and the two subunits are separated from each other.

Structure of the ribosome



- Four interacting sites are located at the ribosome:
- Binding site for mRNAs.

Three binding sites for tRNA, designated A, P and E:

- A (aminoacyl) - binds an aminoacyl-tRNA or termination release factors.

- P (peptidyl) - binds a peptidyl-tRNA (a tRNA bound to the poly-peptide chain), when a stop codon is reached, the peptidyl-tRNA bond of the tRNA located in the P-site is cleaved releasing the newly synthesized protein.

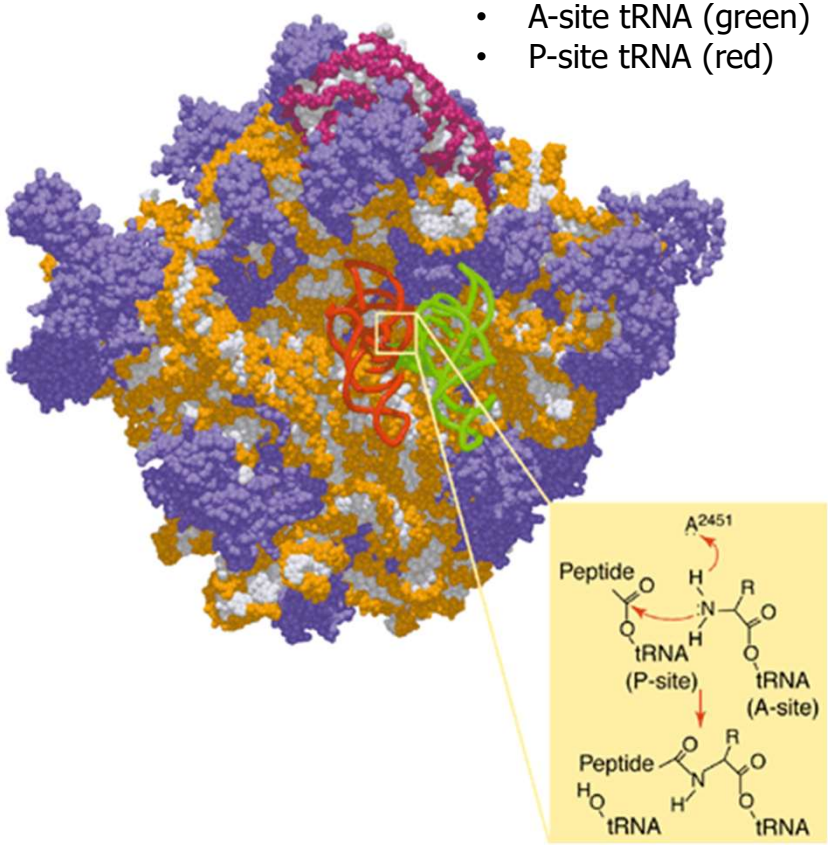
- E (exit) - binds tRNA, without AA, before leaving the ribosome.

https://www.sparknotes.com/biology/molecular/translation/section3/

Ribosome is Ribozyme

- Very complex structure, composition: 2/3 rRNA a 1/3 protein.
- The three-dimensional structure of the ribosome in 2000 was considered a triumph of the modern biology.
- Confirmed that rRNA (not proteins) provide the main structural and functional parameters of this complex, including catalysis of protein synthesis!

- proteins – purple
- 23S rRNA in orange/white
- 5S rRNA in burgundy/white
- A-site tRNA (green)
- P-site tRNA (red)



SCIENCE • 11 Aug 2000 • Vol 289, Issue 5481 • pp. 878-879

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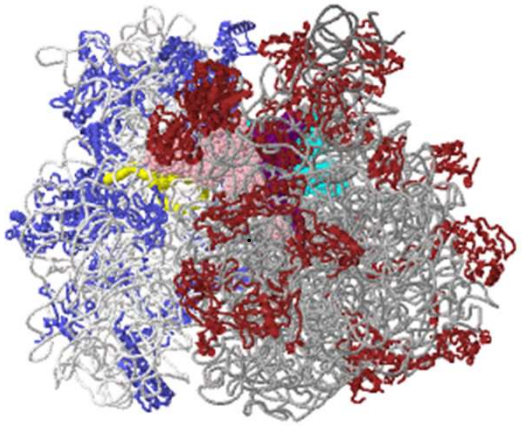
Ribosome is Ribozyme

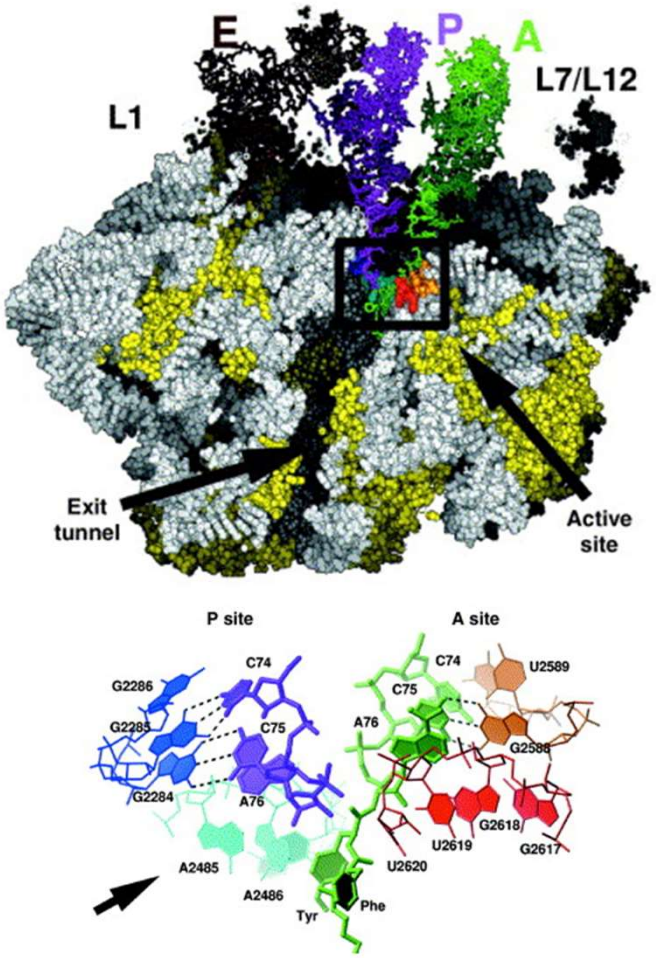
3D structure:

- Very precisely defined, compact structure.
- rRNA forms the core of the ribosome.
- Proteins located on the surface overlap gaps and fissures between folded rRNAs.
- The main role of proteins:
  - Correctly fold and stabilize the RNA nucleus
  - Participate in conformational changes in rRNA conformation related to catalysis of protein synthesis



https://teaching.ncl.ac.uk/bms/wiki/index.php/Rolling_circle_replication

Ribosome is Ribozyme



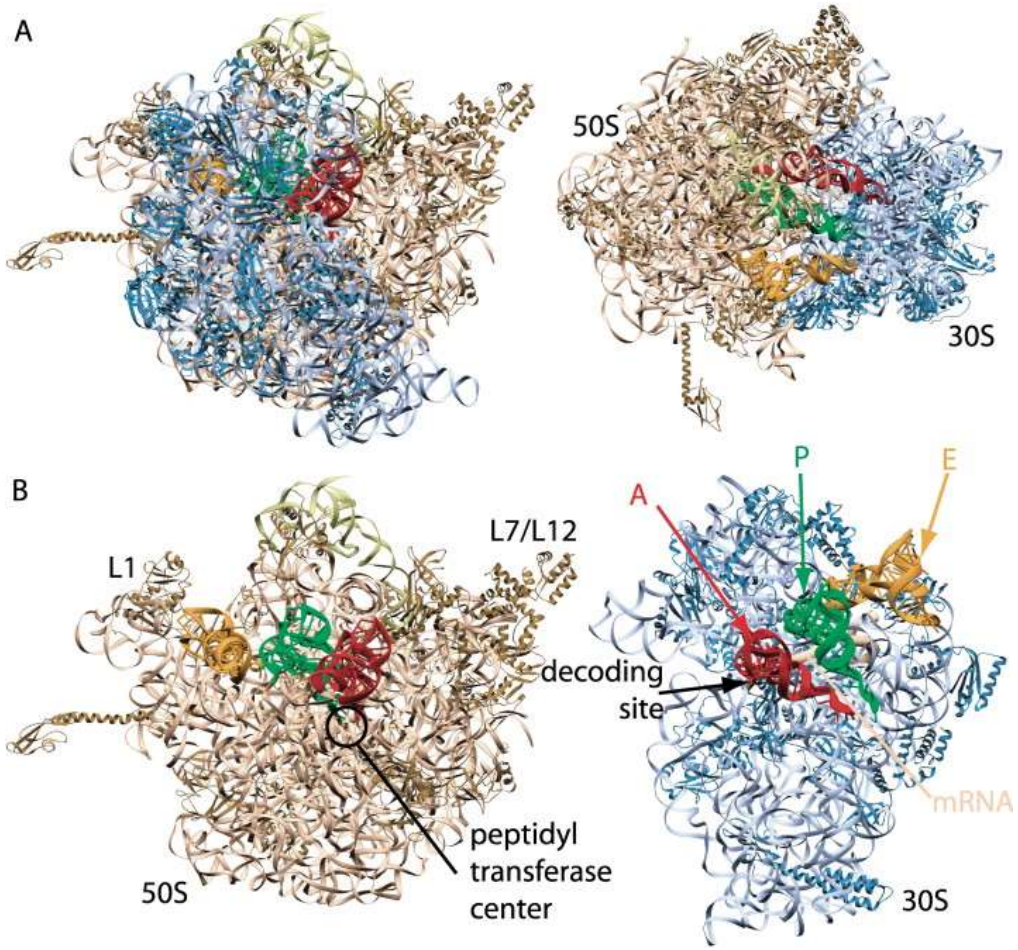
- rRNA forms all 3 binding sites for tRNA (A, P, E).
- 23S rRNAs of large subunits form a catalytic site for the formation of peptide bond.
- Catalytic RNA site with peptidyl-transferase activity with structurally protein-like: it forms a distinct pocket in which it precisely orients both substrates to each other – elongated polypeptide and tRNA with AA.
- This significantly facilitates the course of the reaction.
- RNA molecules with catalytic activity are referred to as ribozymes.
- A relic of the ancient world of RNA?

Trends in Biochemical Sciences, Volume 28, Issue 8, August 2003, Pages 411-418

Structure of the Large subuit of the ribosome

rRNA is responsible for:

- ribosome structure
- interaction of tRNA with mRNA in translation
- catalysis of peptide bond.



CELL, VOLUME 108, ISSUE 4, P557-572, FEBRUARY 22, 2002

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Translation – protein synthesis

Translation has three phases:

- Initiation of translation – binding of mRNA and first aa~tRNA to ribosome.
- Polypeptide chain elongation – continuous integration of amino acids into a growing polypeptide chain according to codons in mRNA.
- Termination of translation – termination of polypeptide chain synthesis, detachment of mRNA from the ribosome and its disintegration into subunits.

https://slideplayer.com/slide/5746723/

Translation – Initiation phase is critical

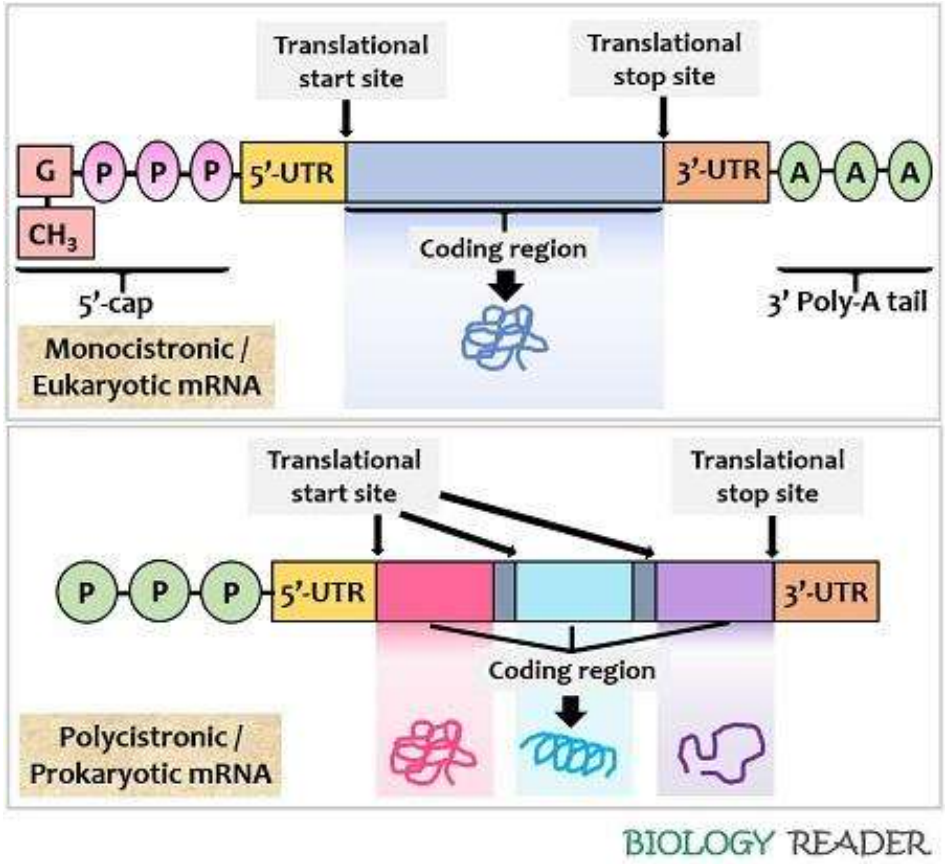
- Defines the reading frame.
- Shift of the start of mRNA translation just by a single nucleotide: incorrect reading of the entire transcript and the formation of a non-functional protein.
- Translation begins at the AUG initiation codon, for which a special initiator tRNA must be available charged with methionine (or, in bacteria, N-formylmethionine).
- All newly formed proteins have an AA methionine at the N-end.
- Usually N-terminal methionine is later cleaved by a specific protease.

Translation – Initiation phase is critical

- Bacterial, prokaryptic, transcripts are not modified by a cap, which helps the eukaryote apparatus to find the place of the beginning of translation.
- This role is fulfilled by ribosome binding sites (RBS) about 6 nucleotides long, that are several nucleotides away from AUG.
- RBS can be found in one transcript repeatedly.
- Thanks to RBS, bacterial initiation of translation from AUG codons inside the transcript is appropriately located – 1 mRNA molecule can encode multiple proteins (polycistrontic transcript).

Translation – Monocistronic versus polycistronic

- The monocistronic transcription unit contains a structural gene coding for only one polypeptide (mostly in eukaryotic cells).
- The polycistronic transcription unit contains structural genes coding for more than one polypeptides (mostly in prokaryotic cells).



https://biologyreader.com/wp-content/uploads/2019/11/

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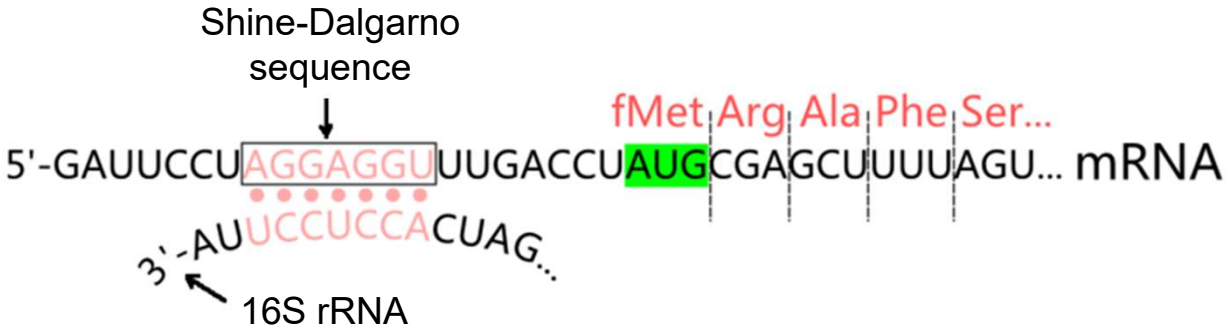
Shine – Dalgarno sequence

- Ribosome binding site (RBS) to prokaryotic mRNA defining start of translation and placing the ribosome in the correct position relative to the AUG.
- Consensus **AGGAGGU**
- Placed 7 (5-10) nucleotides against the direction of translation from the initiation codon AUG.
- Is the complementary sequence of the 3´-end of the 16S rRNA of a small subunit of the ribosome.



https://commons.wikimedia.org/wiki/File:Shine-Dalgarno_sequence-es.png

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Translation – Initiation phase – prokaryotes

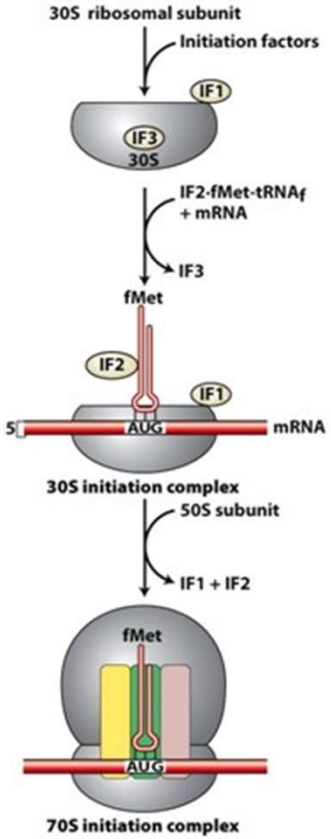

Figure 9-14
Introduction to Genetic Analysis, Tenth Edition
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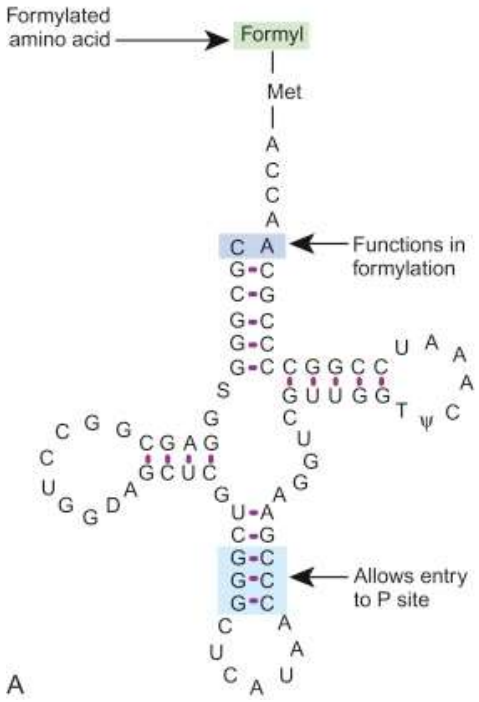
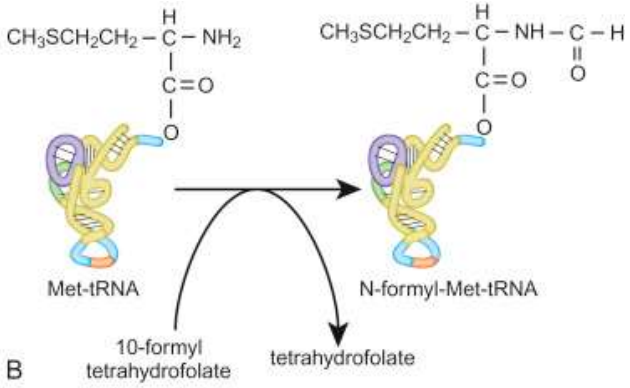
- Shine-Dalgarno sequence pairs with 16S rRNA of 30S subunit.
- IF3 keeps 30S subunit dissociated from 50S subunit.
- Formyl group is added to methionine when associated with the initiator tRNA.
- IF1 and IF2 allows only initiator tRNA to enter P site.
- Initiation factors are released when two ribosomal subunits associate.

https://slideplayer.com/slide/5746723/

Translation – Initiation phase – prokaryotes

- A special tRNA, the initiator tRNA, is charged with methionine and binds to the AUG start codon.
- In prokaryotes, chemically-tagged methionine, N-formyl-methionine (fMet), is attached to the initiator tRNA
- Formyl protects NH2 group of methionine.





https://www.sciencedirect.com/topics/nursing-and-health-professions/n-formylmethionine

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Translation – Elongation phase – prokaryotes

- EF-Tu associates with aminoacyl-tRNA to form a ternary complex.
- Correct match of ternary complex with codon in A site (decoding center) changes conformation of ribosome.
- EF-Tu leaves ternary complex, and peptide bond is formed between amino acids as amino acids are positioned together in peptidyltransferase center.
- Amino acid in P site is transferred to amino acid in E site.
- Translocation requires GTP and EF-G. EF-G enters A site, shifting tRNAs. When EF-G leaves, A site is open for a new ternary complex. A new ternary complex associates with A site, and deacylated tRNA leaves from E site.

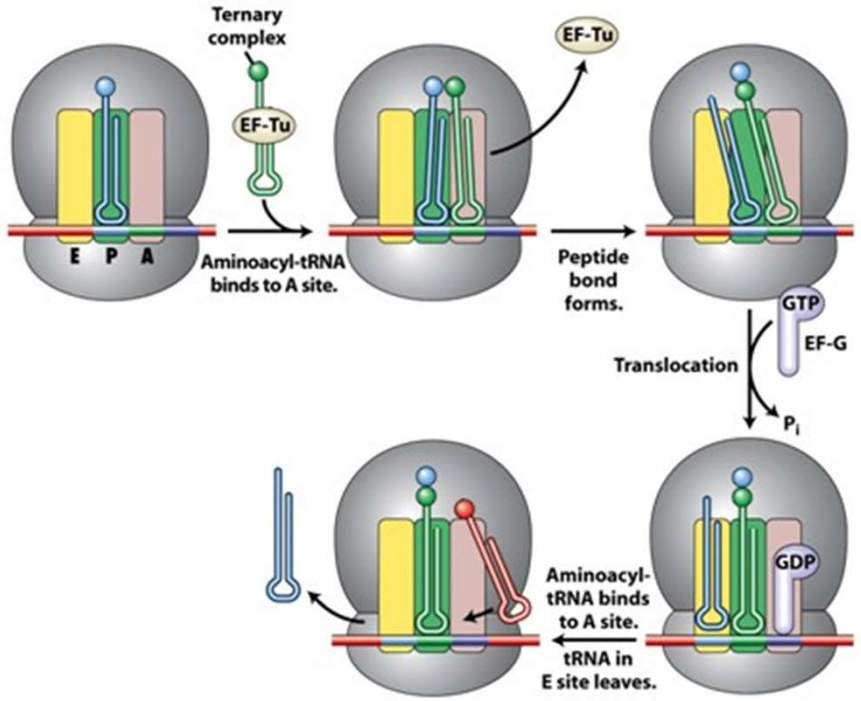

Figure 9-16
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Translation – Termination phase – prokaryotes

- tRNA molecules do not recognize stop codons.
- Termination codons are recognized by release factors. (RF1, RF2, RF3 in bacteria)
- UAA and UAG are recognized by RF1.
- UAA and UGA are recognized by RF2.
- RF3 assists in release activity.
- Release factors bind to a stop codon in the A site by association between codon and tripeptide of RF.
- Polypeptide is released from P site when RF fits into A site.
- Release of polypeptide is followed by dissociation of ribosomal subunits.

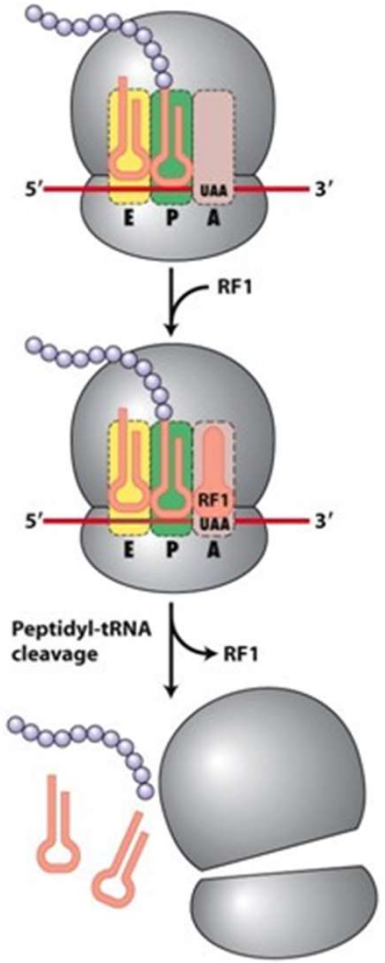

Figure 9-17
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Kozak sequence

- The Kozak consensus sequence is a nucleic acid motif that functions as the protein translation initiation site in most eukaryotic mRNA transcripts.
- The Kozak consensus sequence for initiation of translation in vertebrates is (GCC) GCCRCCATGG, where R is a purine (A or G).
- It ensures that a protein is correctly translated from the genetic message, mediating ribosome assembly and translation initiation.

Marilyn Kozak

https://en.wikipedia.org/wiki/Kozak_consensus_sequence

https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/kozak-consensus-sequence

Translation – Initiation phase – eukaryotes

- eIF4A, eIF4B, and eIF4G associates with 5’ end, then with 40S subunit and initiator tRNA.
- mRNA is unwound by movement of this complex in 5’ -> 3’ direction.
- 60S subunit associates with initiation complex when start codon is recognized.
- Initiation factors are released when the two ribosomal subunits associate.

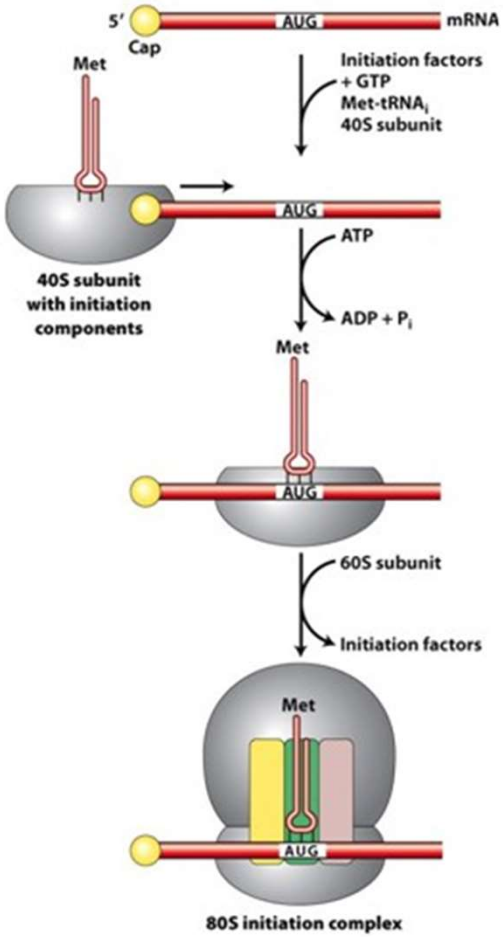

Figure 9-15
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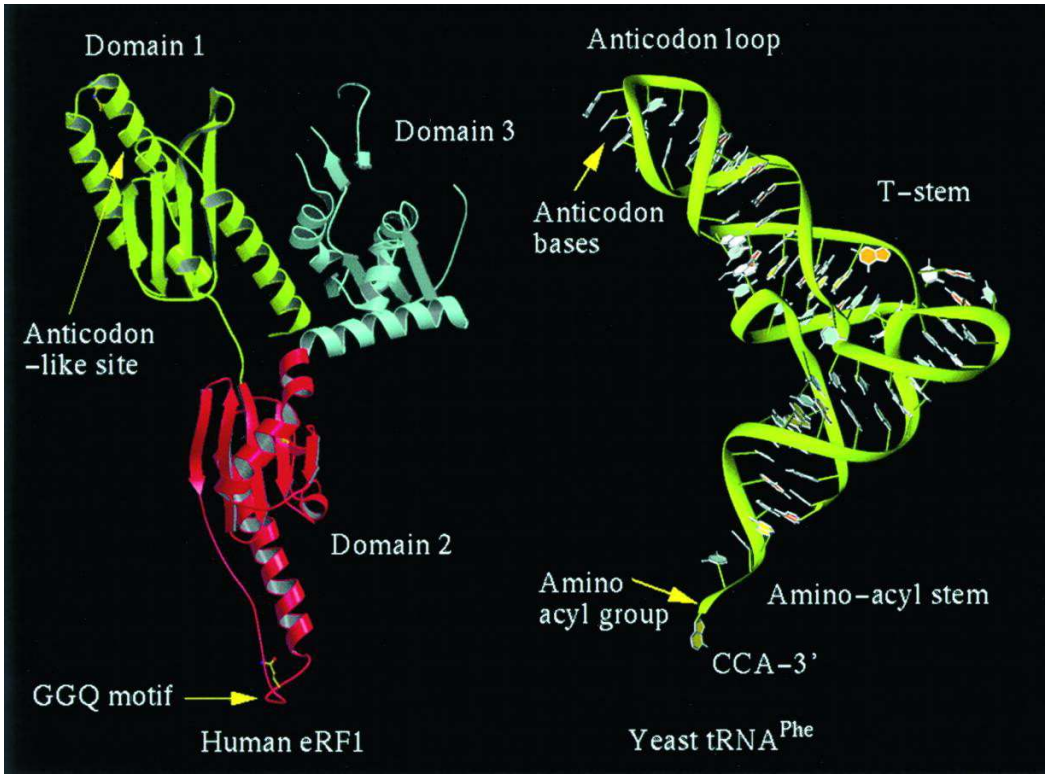
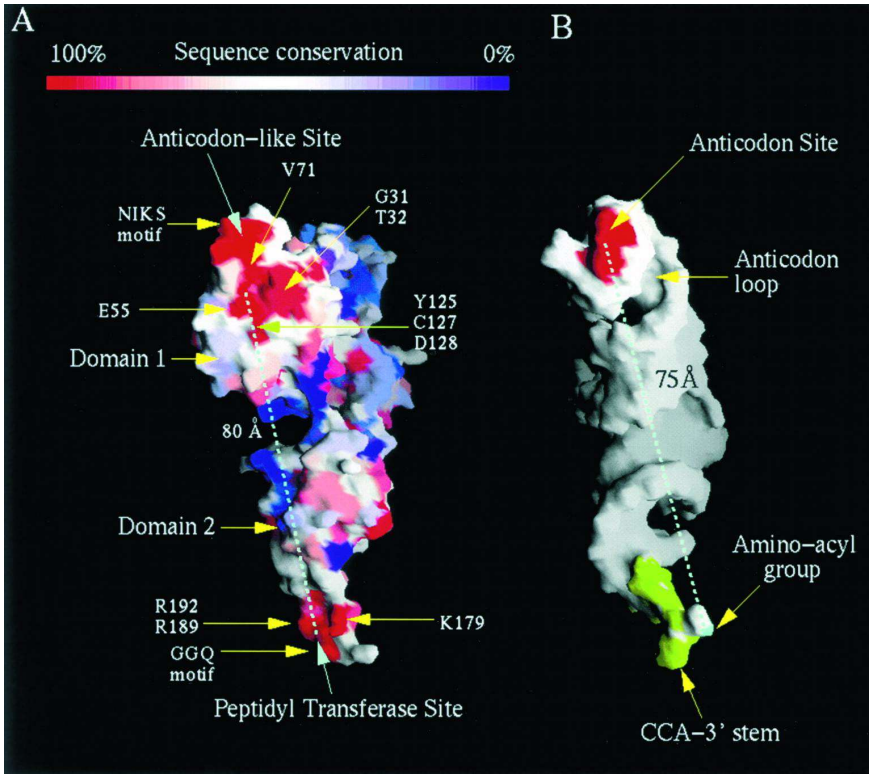
Mimicry of tRNA Molecules





CELL, VOLUME 100, ISSUE 3, P311-321, FEBRUARY 04, 2000

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Translation – prokaryotes versus eukaryotes

prokaryotic	eukaryotic	function
Initiation factor		
IF1 IF3	eIF3 eIF4c eIF6 eIF4B eIF4F	Bind to ribosome submits Bind to mRNA
IF2	eIF2B eIF2 eIF5	Initiator tRNA delivery Displacement of other factors
Elongation factor		
EF-Tu	eEF1α	Aminoacyl tRNA delivery
EF-Ts	eEF1βγ	Recycling of EF-Tu or eEF1α
EF-g	eEF2	Translocation
Termination factors		
RF1, RF2, RF3	eRF	Polypeptides Chain release

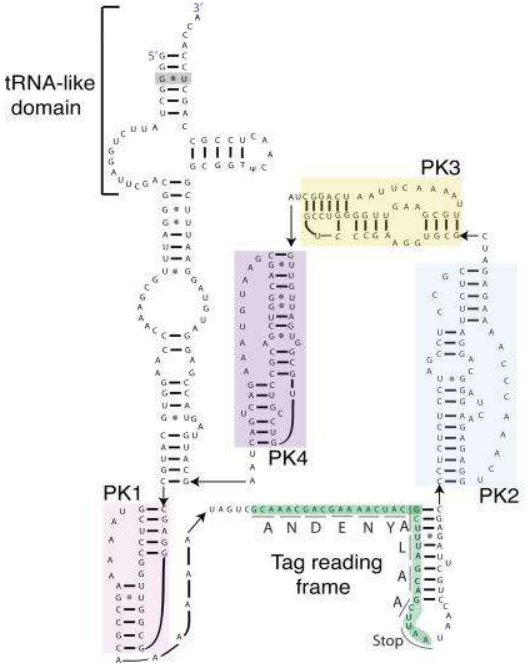
https://slideplayer.com/slide/3330529/

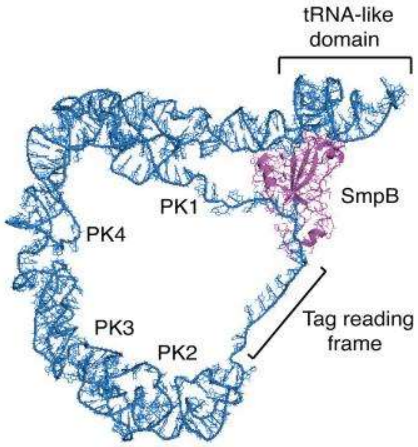
Termination of translation by tmRNA

- Transfer-messenger RNA (tmRNA) is a bifunctional RNA that has properties of a tRNA and an mRNA.
- tmRNA uses these two functions to release ribosomes stalled during translation and target the nascent polypeptides for degradation.
- This concerted reaction, known as trans-translation, contributes to translational quality control and regulation of gene expression in bacteria, when ribosome fails to recognize stop codon or RF can not bind to ribosome.
- tmRNA is conserved throughout bacteria, and is one of the most abundant RNAs in the cell, suggesting that trans-translation is of fundamental importance for bacterial fitness.

Biochimie. 2011 Nov; 93(11): 1993–1997.

Termination of translation by tmRNA

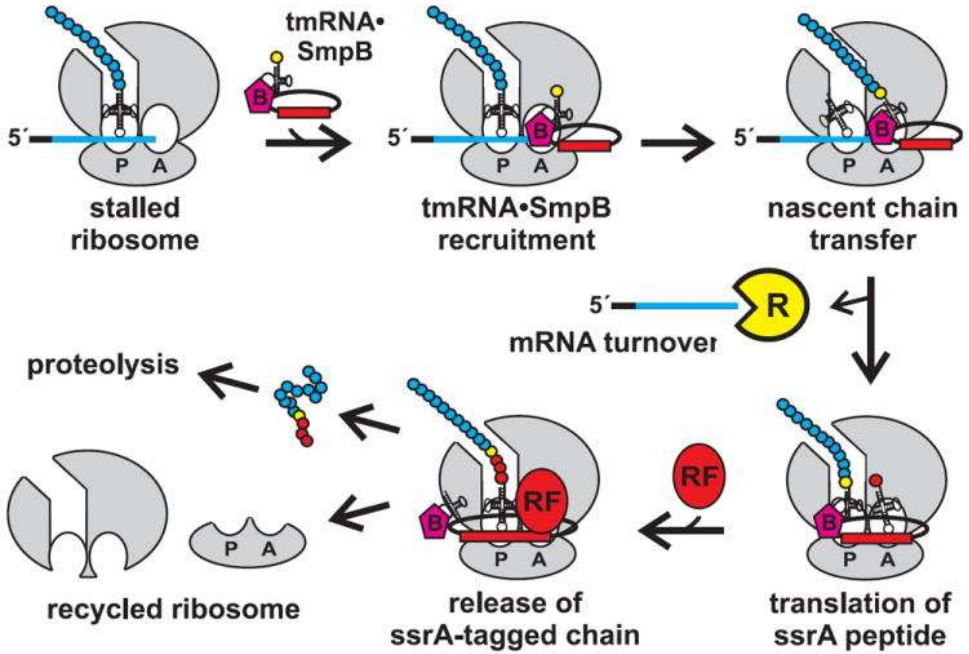




- Longer than tRNA.
- Does not have a clover leaf structure.
- Alanine bound to the 3´-end.
- Carries a short sequence encoding 10 AA, followed by a stop codon.

Biochimie. 2011 Nov; 93(11): 1993–1997.

Termination of translation by tmRNA



- tmRNA•mpB binds the A site of stalled ribosomes and accepts the nascent chain.
- The non-stop mRNA is released and preferentially degraded by RNase R.
- Translation then resumes using the open reading frame found within tmRNA.
- After synthesis of the ssrA peptide, release factors (RF) terminate translation and the ribosome is recycled into large and small subunits.
- The ssrA-tagged chain is degraded by a number of proteases.

Adv Protein Chem Struct Biol. 2012; 86: 151–191.

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Polysomes

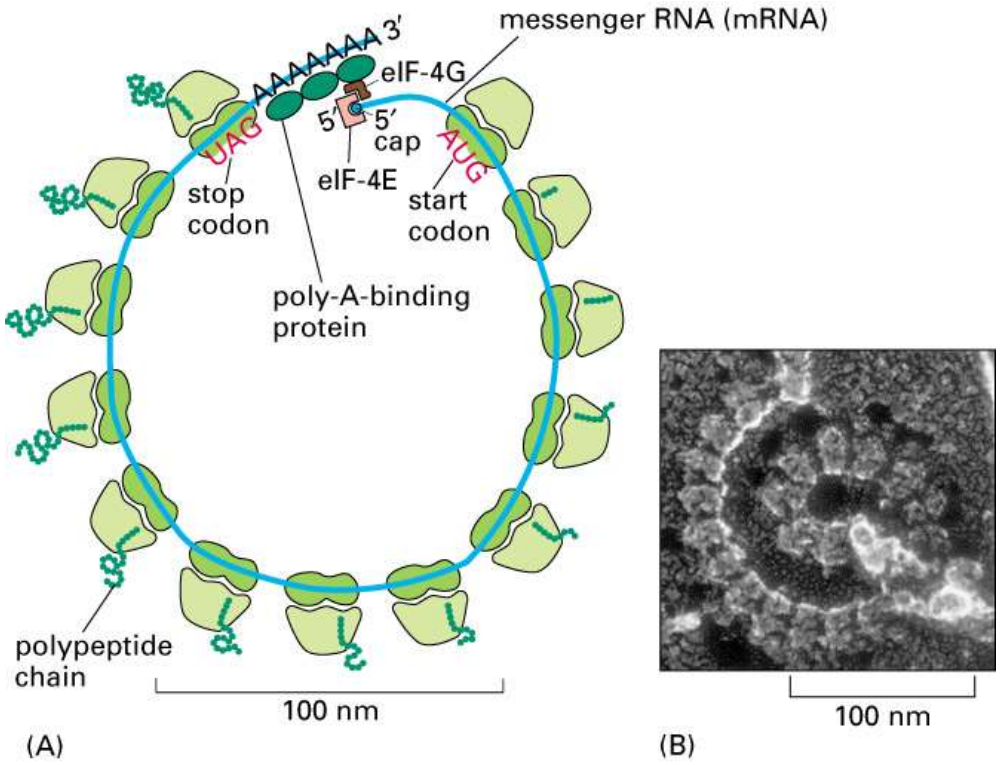


Figure 6–75. Molecular Biology of the Cell, 4th Edition.

- The synthesis of most proteins takes from 20 sec to a few minutes.
- Even during this short period of time, multiple ribosomes bind to mRNA.
- For intensively translated transcripts, the ribosome mounts on the 5'-end of the mRNA immediately as soon as the previous ribosome frees up enough space for him.
- The resulting polysomes contain many ribosomes bound to the same mRNA at a distance of about 80 nucleotides.
- High efficiency of the creation of the given Protein.
- In bacteria ribosomes to mRNA immediately during its creation by transcription.

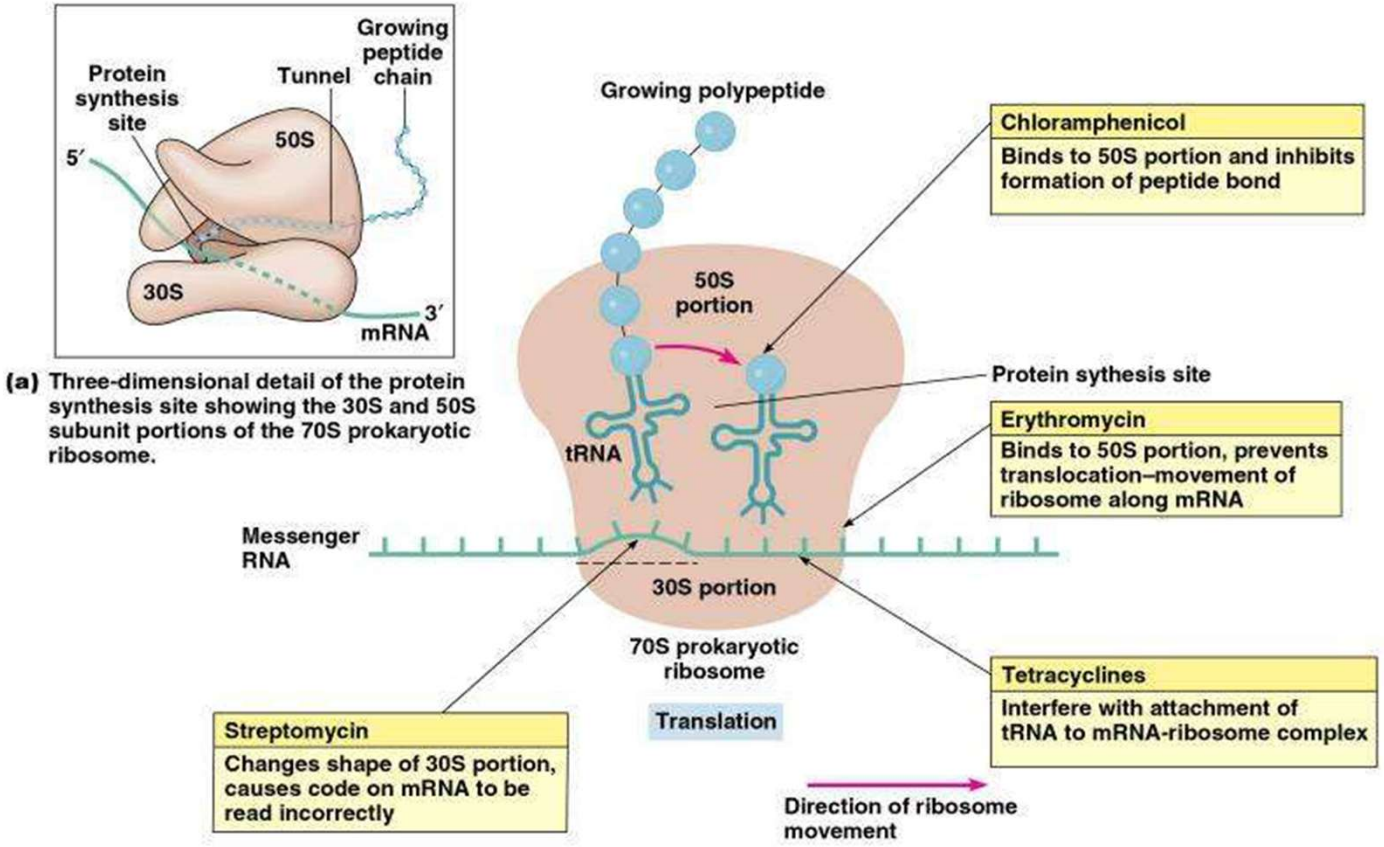
SCIENCE OF AGING KNOWLEDGE ENVIRONMENT • 26 Feb 2003 • Vol 2003, Issue 8 • p. re3 • DOI: 10.1126/sageke.2003.8.re3

Translation machinery is often target of antibiotics

- Successful protein synthesis is a condition of life.
- In the process of translation of prokaryotes and eukaryotes there are subtle differences.
- Most antibiotics inhibit the translation of bacteria (not eukaryotes).
- Often uses structural differences of ribosomes.
- A number of antibiotics have been isolated from fungi that often inhabit the same habitant as bacteria.
- In order to withstand evolution, they have developed the ability to produce toxins that kill bacteria that do not harm them.
- Fungi are eukaryotic organisms – more closely related to humans than bacteria, their products are useful for protecting humans.

https://wou.edu/chemistry/courses/online-chemistry-textbooks/ch450-and-ch451-biochemistry-defining-life-at-the-molecular-level/chapter-9-dna-replication-and-repair-2/

Translation machinery is often target of antibiotics



(a) Three-dimensional detail of the protein synthesis site showing the 30S and 50S subunit portions of the 70S prokaryotic ribosome.

(b) In the diagram the black arrows indicate the different points at which chloramphenicol, erythromycin, the tetracyclines, and streptomycin exert their activities.

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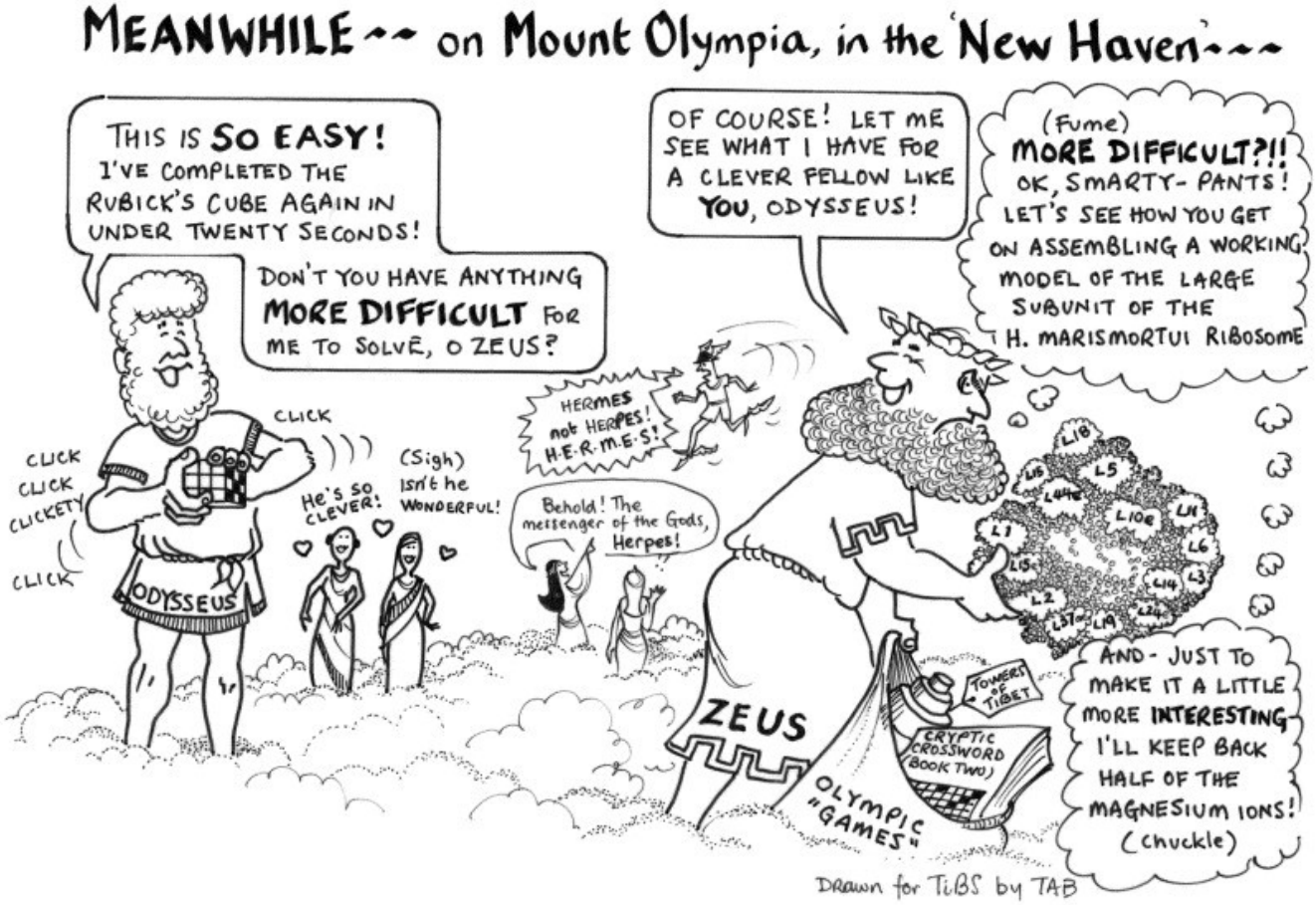
Differences between prokaryotic and eukaryotic translation

- In bacteria, the initiation complex is formed directly on the sequences near AUG.
- In eukaryotes, 40S first recognizes the 5' end of mRNA and then moves to the initiation point where it connects with the 60S.
- eIF-4G is bound to polyA-end RNA and to eIF-4E bound to cap = only mRNA with full length translated.
- N-formlymethinine in prokaryotic 5´end of proteins.

THANK YOU FOR YOUR ATTENTION



Trends in Biochemical Sciences, Volume 28, Issue 8, August 2003, Pages 411-418

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