

Translation in prokaryotes

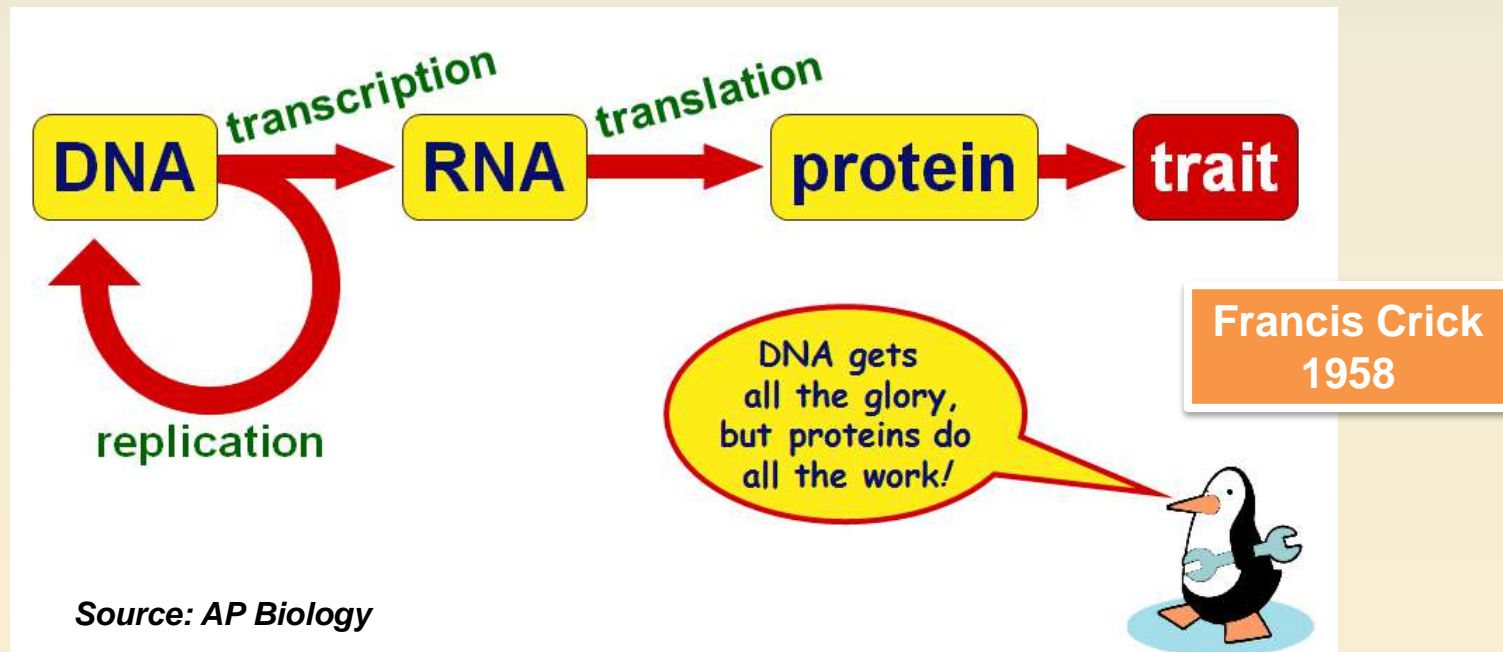
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Jehanabad

Introduction

- ❑ It is the process of synthesis of proteins from messenger RNA transcripts (mRNA) after the process of transcription of DNA to RNA.
- ❑ It takes place in the cytoplasm by specialized organelle known as ribosomes.
- ❑ There are no endoplasmic reticulum in the prokaryotes and ribosomes are suspended in the cytoplasm, whereas endoplasmic reticulum are present in eukaryotes which harbors ribosomes – translation takes place on rough endoplasmic reticulum (RER) in eukaryotes, whereas translation occurs freely in cytoplasm in the prokaryotes.
- ❑ The codons on the mRNA are translated into amino acid sequence which leads to the synthesis of protein.
- ❑ Translation requires a variety of cellular components, such as proteins, RNAs and different small molecules.
- ❑ It has also three main steps:
 - **Initiation** – Formation of mRNA-ribosome complex
 - **Elongation** – Formation of polypeptide chain complimentary to the mRNA
 - **Termination** – Termination of polypeptide chain

The “Central Dogma”

- Flow of genetic information in a cell
 - How do we move information from DNA to proteins?



“It states that genetic information is transmitted from DNA to RNA to protein and this information cannot be transferred back from protein to either protein or nucleic acid”.

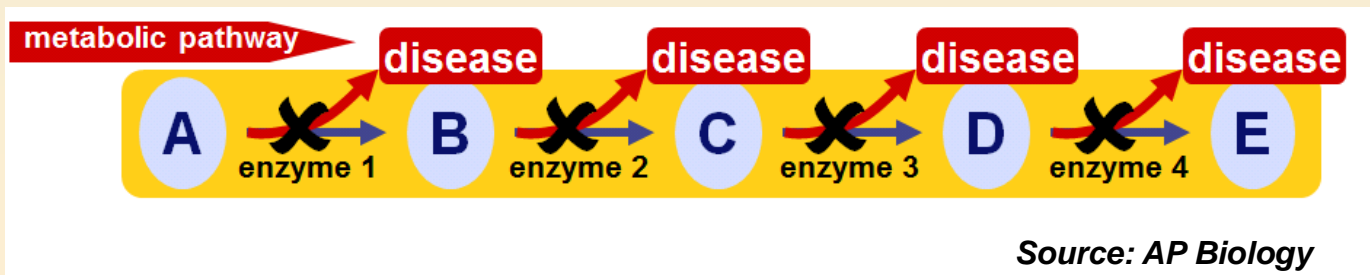
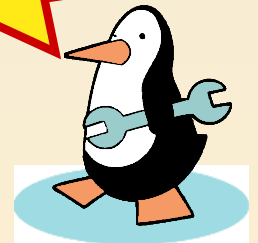
The “Central Dogma”

Inheritance of metabolic disease

- It suggests that genes code for enzymes.
- Each disease, i.e., phenotype, is caused by non-functional gene product, which may be
 - lack of an enzyme
 - Tay Sachs disease - **hexosaminidase A**, causes a fatty substance to collect .
 - Phenylketonuria (PKA) - absence or deficiency of an enzyme called **phenylalanine hydroxylase (PAH)**, responsible for processing the amino acid phenylalanine.
 - Albinism - absence or defect of **tyrosinase**, a copper-containing enzyme involved in the production of melanin.

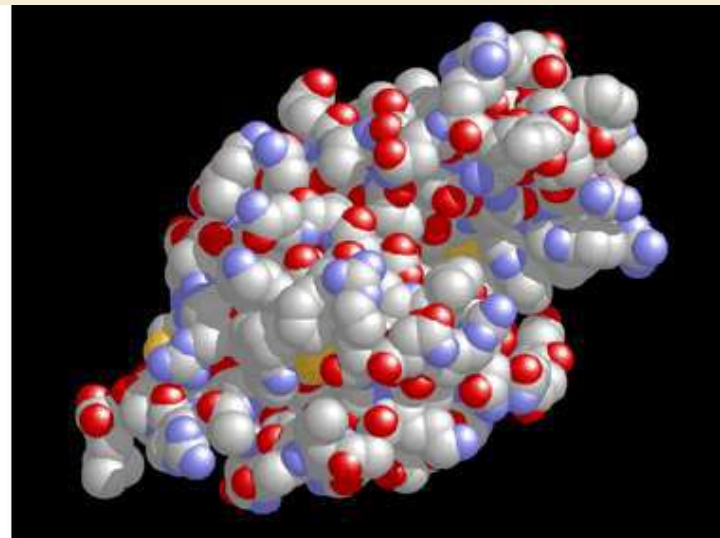
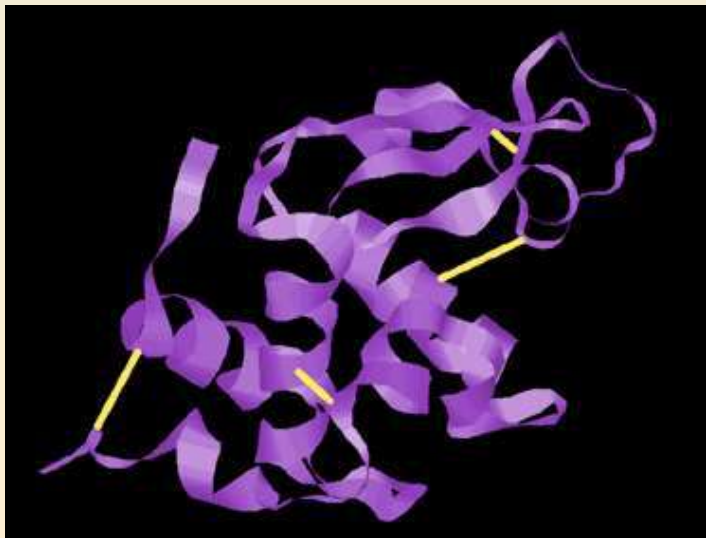
Proteome - between 80,000 and 400,000 proteins.

Am I just the sum of my proteins?



Proteins

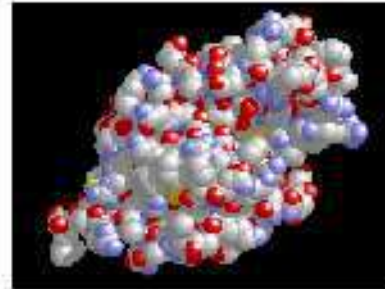
- ❑ Proteins are the “workhorse” molecule found in organisms, which determine phenotype of an organism, i.e. what we look like.
- ❑ Proteins are made of polypeptide chains that have primary, secondary, tertiary and quaternary structure.
- ❑ The polypeptide chains are made of 20 amino acids - an average polypeptide chain is 400 amino acid long (can be shorter than this value).
- ❑ *The part of the DNA that codes for a particular polypeptide chain is called as a gene.*



Uses of Proteins



Carrying gases

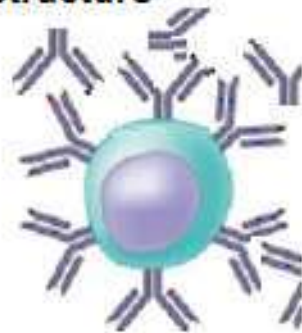


Enzymes

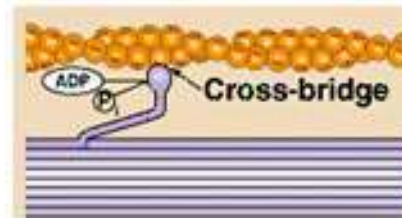


Uses of Proteins

Structure



Antibodies



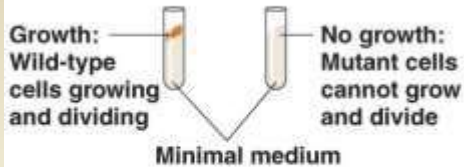
Movement



Storage of protein

Work of Beadle and Tatum

EXPERIMENT

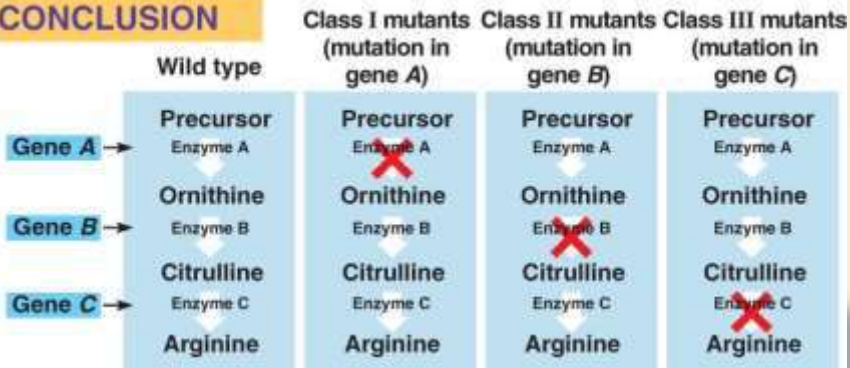


RESULTS

Classes of *Neurospora crassa*

	Wild type	Class I mutants	Class II mutants	Class III mutants
Minimal medium (MM) (control)				
MM + ornithine				
MM + citrulline				
MM + arginine (control)				

CONCLUSION

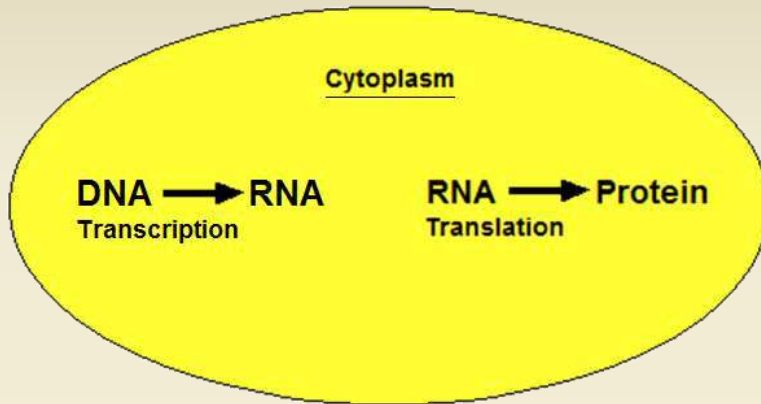


George Beadle and Edward Tatum established the link between genes and enzymes in studying bread mold, *Neurospora crassa*.

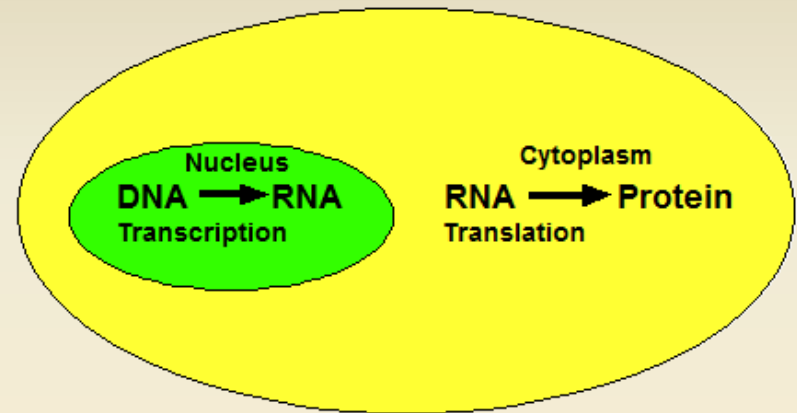
Conclusion

- One gene produces one enzyme.
- Later it was modified
- One gene produces one protein.
- One gene produces one polypeptide chain.

Overview of Protein Synthesis



In prokaryotes



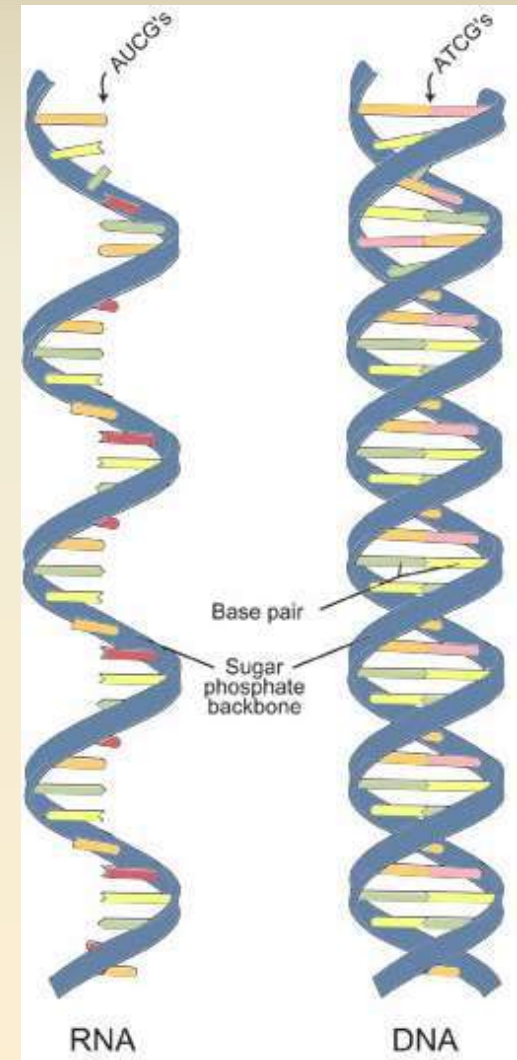
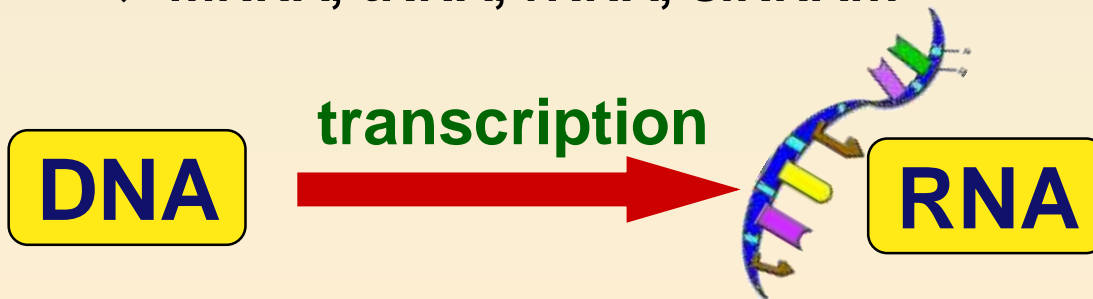
In eukaryotes

- ❑ DNA is transcribed to mRNA in the cytoplasm in prokaryotes as it has no nucleus, and in the nucleus in eukaryotes.
- ❑ With mRNA and help of other RNA as well as protein molecules, mRNA is translated into specific proteins in the cytoplasm
- ❑ Therefore, RNA is the intermediate between the DNA code and the actual synthesis of a protein

RNAs in the Protein Synthesis

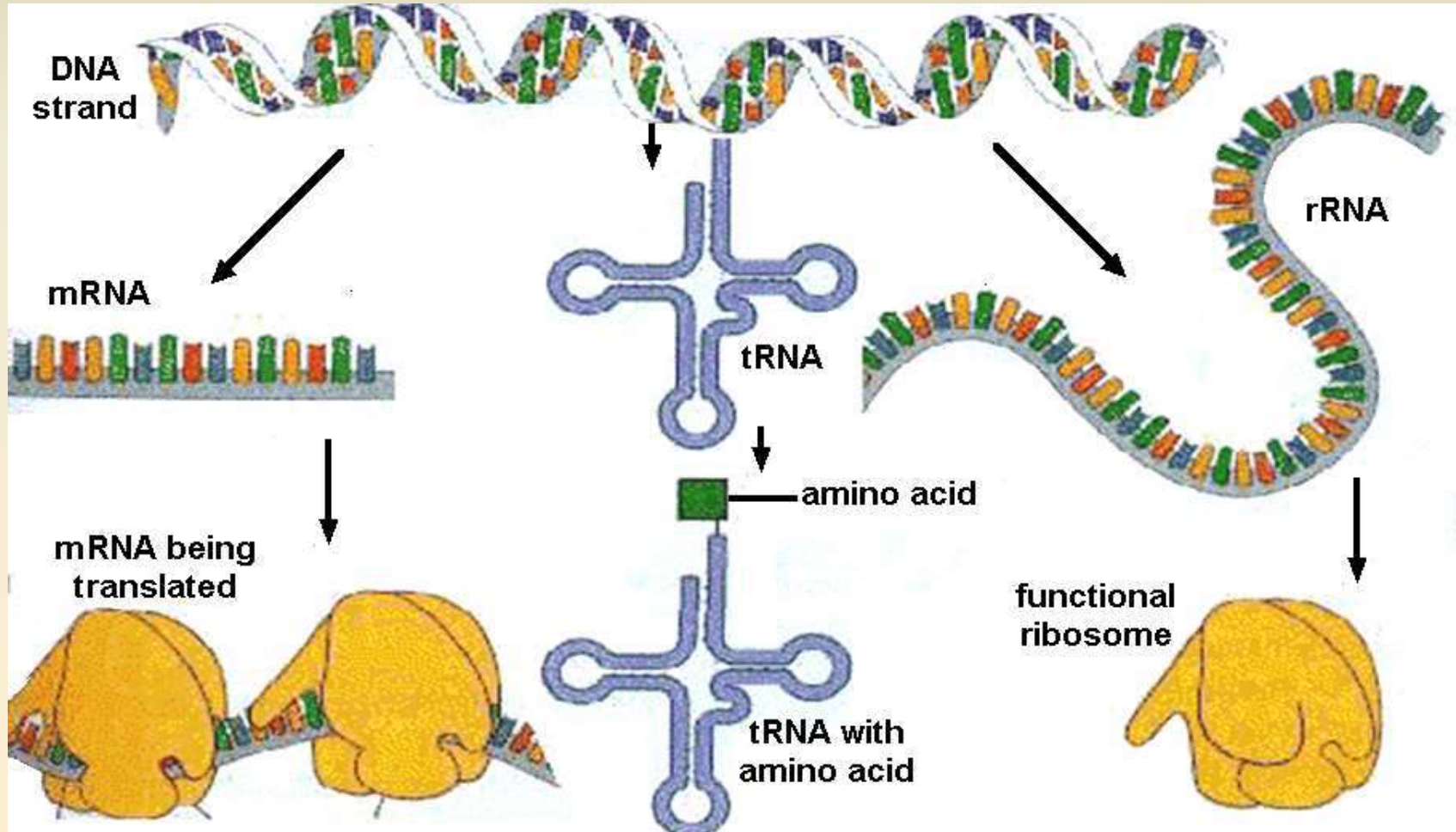
RNA

- ribose sugar
- N-bases
 - ◆ uracil instead of thymine
 - ◆ U : A
 - ◆ C : G
- single stranded
- lots of RNAs
 - ◆ mRNA, tRNA, rRNA, siRNA...



RNAs in the Protein Synthesis

Types of RNA in protein synthesis



RNAs in the Protein Synthesis

3 types of RNA help in protein synthesis

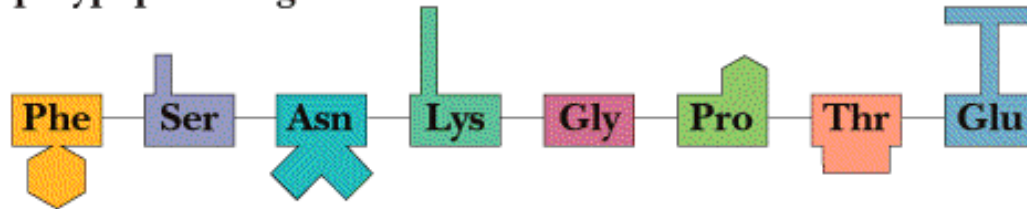
Messenger RNA (mRNA)

- It carries code from DNA to ribosomes. It actually acts as a transporter of genetic information to protein synthesis machinery.
- It is specific for a particular protein.

A strand of DNA



A polypeptide segment

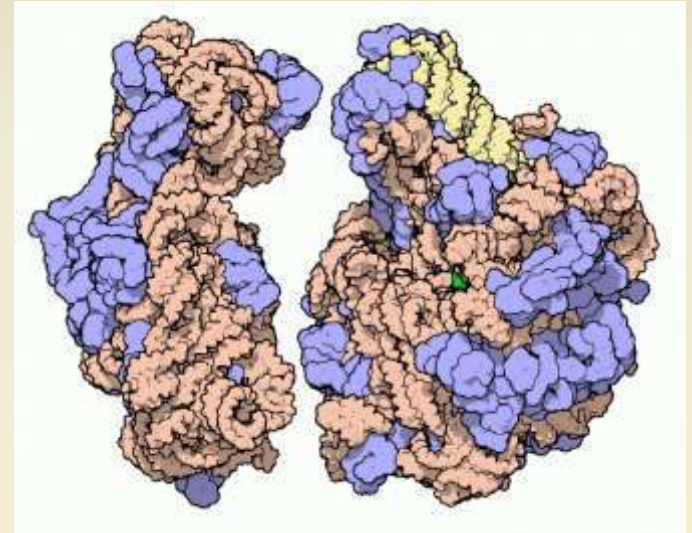


RNAs in the Protein Synthesis

3 types of RNA help in protein synthesis

RIBOSOMAL RNA (rRNA)

- Made in nucleolus in eukaryotes, while it is made in the cytoplasm in prokaryotes.
- It has 2 subunits (large & small)
- It combines with proteins to form ribosomes
- Prokaryotic ribosomes are of different size than eukaryotic ribosomes (30S and 50S subunits in prokaryotes and 40S and 60S subunits in eukaryotes).
- Medically significant – some antibiotics target bacterial ribosomes in the host



Ribosomal subunits – RNA in orange and yellow and proteins in blue.

Source: <https://pdb101.rcsb.org/motm/10>

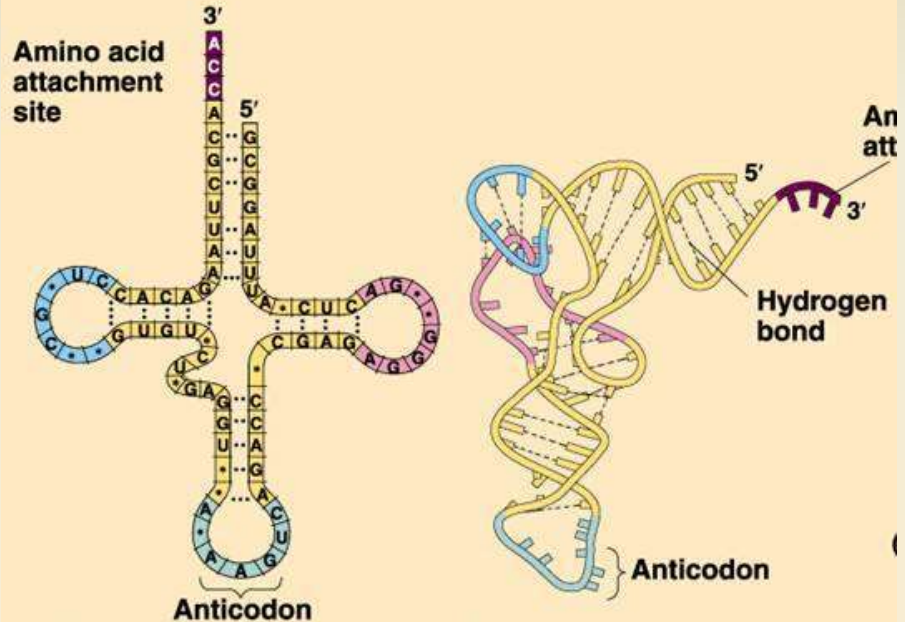
Endosymbiotic theory: It states that some of the organelles in eukaryotic cells were once prokaryotic microbes.

RNAs in the Protein Synthesis

3 types of RNA help in protein synthesis

Transfer RNA (tRNA)

- Anticodon sequence are present.
- **Anticodon** sequence matches **codon** on mRNA to add correct amino acid during protein synthesis.
- **Aminoacyl-tRNA synthetase** enzyme attaches a specific amino acid using energy from ATP.

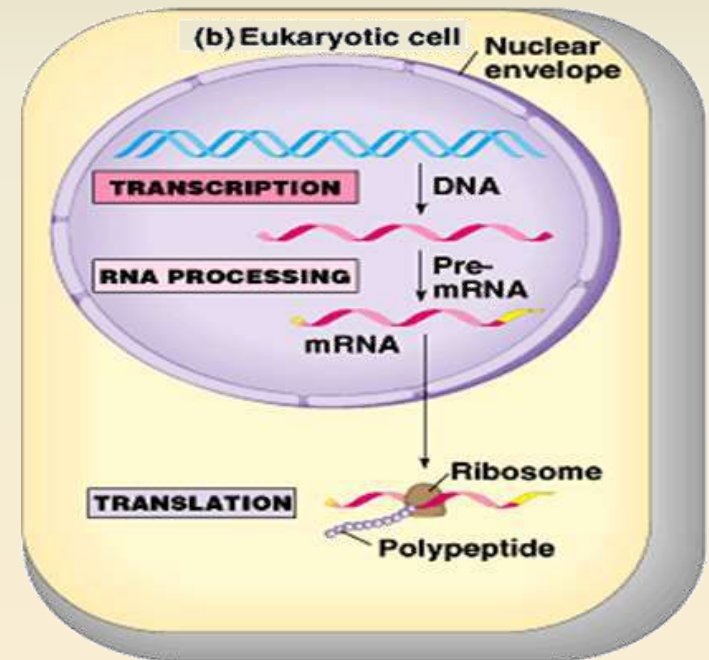
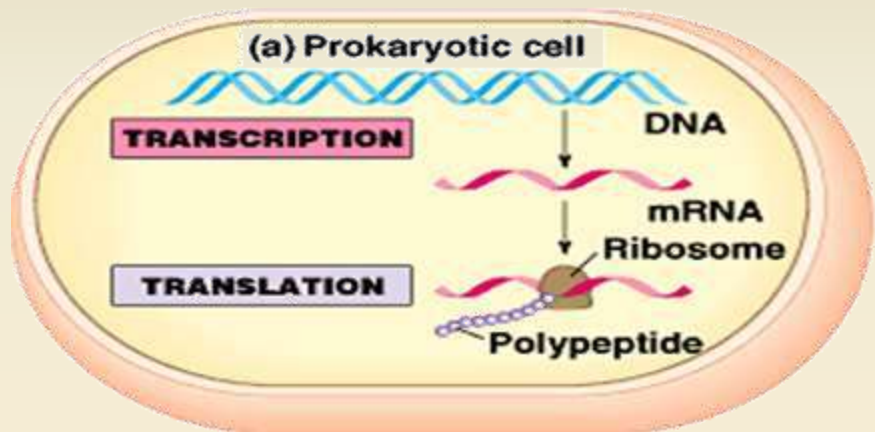


http://www-math.mit.edu/~lippert/18.417/lectures/01_Intro/

What is the anticodon for the codon **AUG**?
UAC

Protein Synthesis in Prokaryotes and Eukaryotes

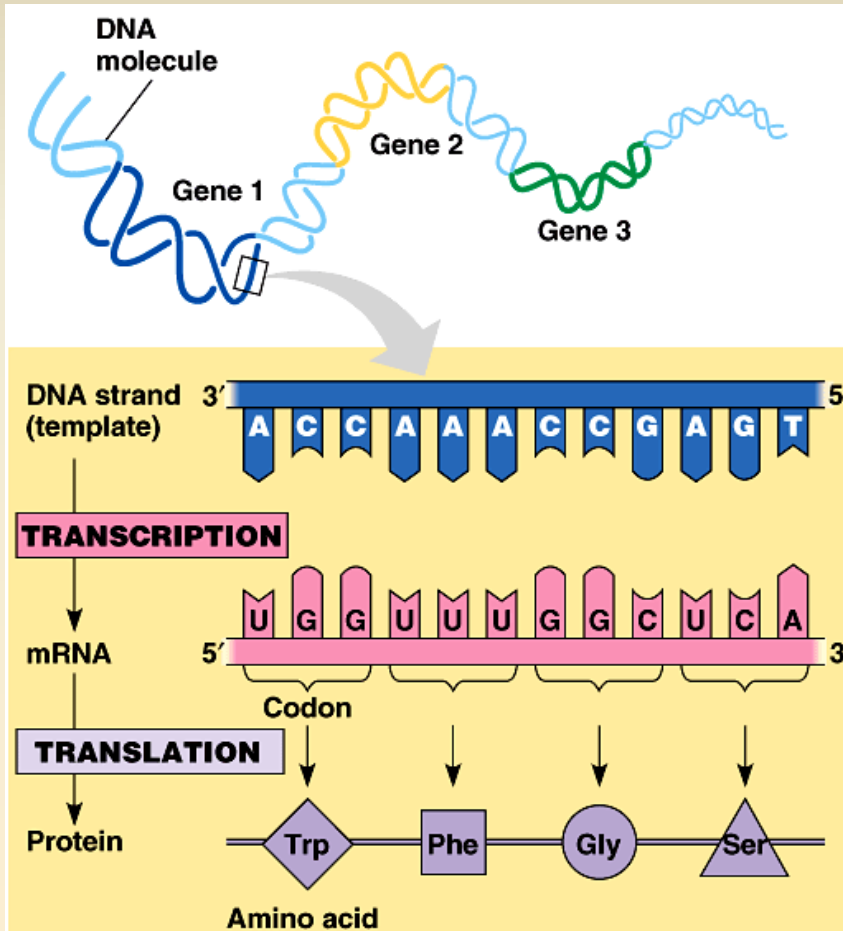
Difference between Protein Synthesis in Prokaryotes and Eukaryotes



- Prokaryotes do not have introns like eukaryotes.
- There is no or very little RNA processing in prokaryotes.
- Transcription and translation can be simultaneous in prokaryotes.

Protein synthesis requires genetic information as genetic code contained in the DNA.

Genetic Code



Amino acids are coded by triplets of DNA nucleotides, called codons.

There are 64 codons.

61 code for amino acids, 3 code for STOP, and one codes for START and also for Methionine.

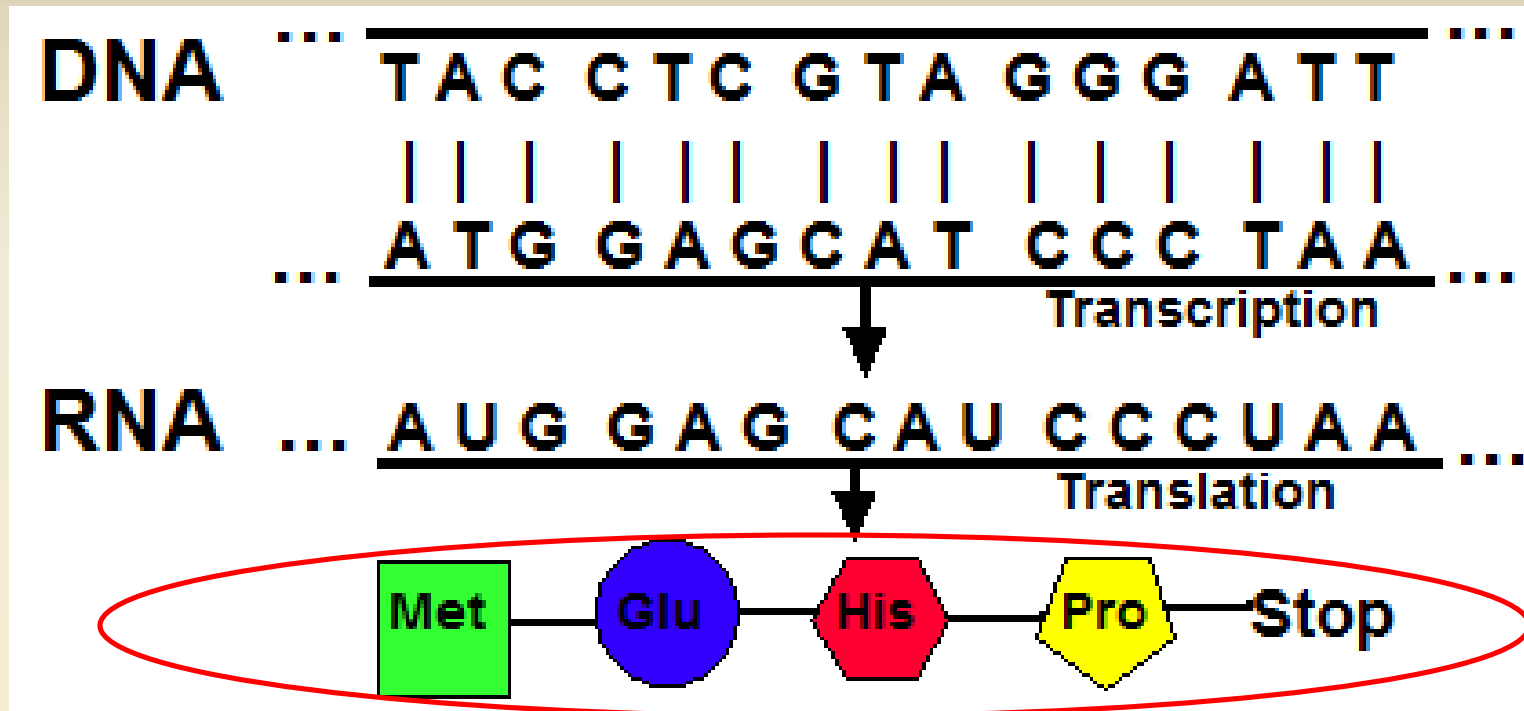
Marshall Nirenberg and Heinrich Matthaei determined the first codon for an amino acid, which was UUU that coded for the amino acid phenylalanine, by creating mRNA entirely of uracil.

Genetic Code

		Second base					
		U	C	A	G		
First base (5' end)	U	UUU	UCU	UAU	UGU	Third base (3' end)	U
		UUC	UCC	UAC	UGC		C
		UUA	UCA	UAA Stop	UGA Stop		A
		UUG	UCG	UAG Stop	UGG Trp		G
	C	CUU	CCU	CAU	CGU		U
		CUC	CCC	CAC	CGC		C
		CUA	CCA	CAA	CGA		A
		CUG	CCG	CAG	CGG		G
	A	AUU	ACU	AAU	AGU		U
		AUC	ACC	AAC	AGC		C
		AUA	ACA	AAA	AGA		A
		AUG Met or start	ACG	AAG	AGG		G
	G	GUU	GCU	GAU	GGU		U
		GUC	GCC	GAC	GGC		C
		GUA	GCA	GAA	GGA		A
		GUG	GCG	GAG	GGG		G

- The code has redundancy (GGU, GGC, GGA, and GGG); all code for the amino acid glycine.
- Each codon only codes for one amino acid.
- The code is a universal code meaning almost all cells use the same code. A eukaryotic gene can be expressed in a prokaryotic cell.
- Code for ALL life (common origin for all life..)

Translation of Codons into a Polypeptide



The gene designates that the following peptide chain be made with the amino acids in this particular order.

■ Start codon

◆ AUG

■ Stop codon

◆ UGA, UAA, UAG

Why is genetic code triplate?

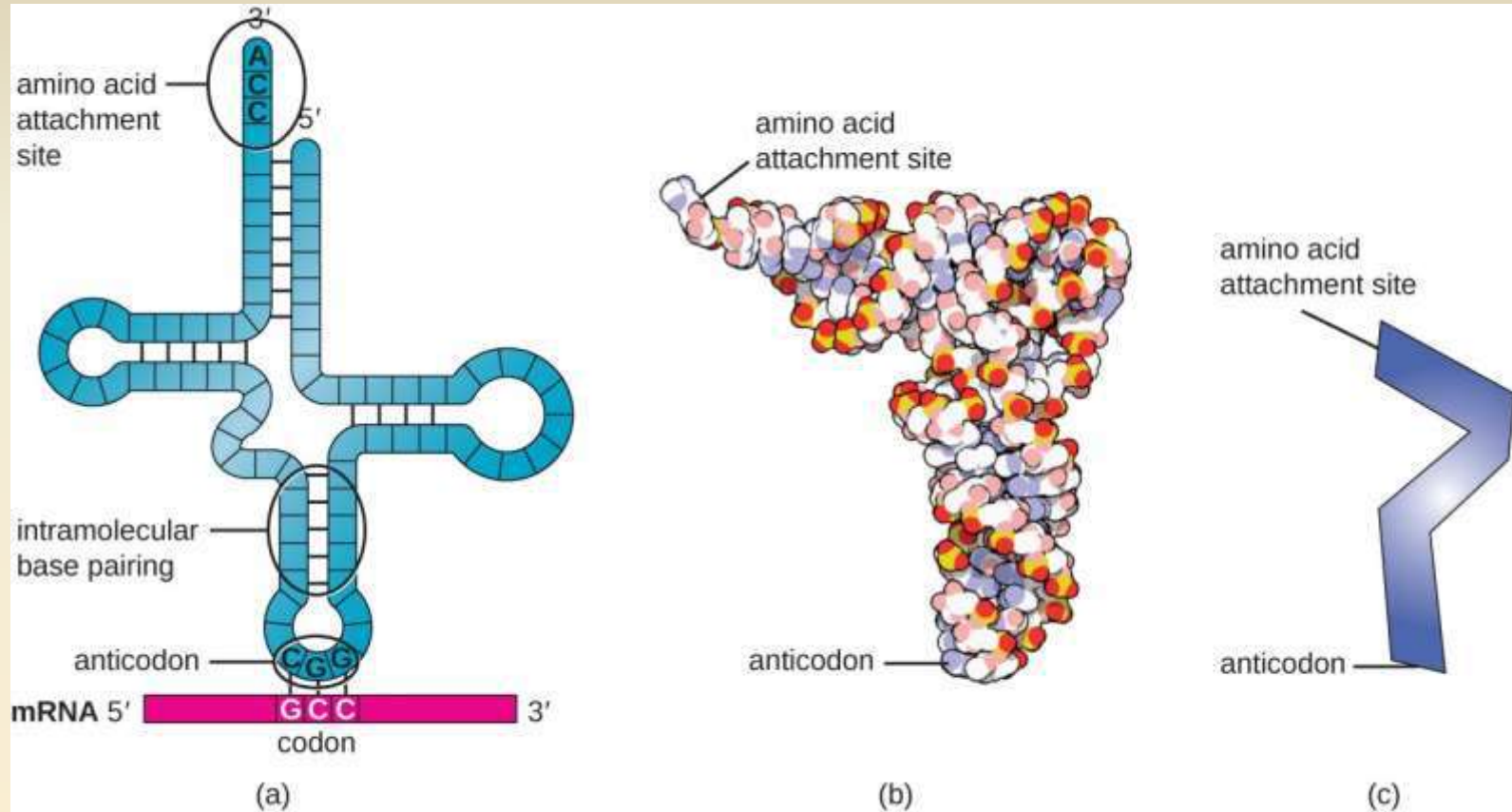
Given that there are four bases in DNA, and these code for 20 amino acids, what is the basis for the genetic code?

- If **one** base = one amino acid,
possible amino acids = **4**
- If **two** bases = one amino acid,
possible amino acids = **16** (4×4)
- If **three** bases = one amino acid,
possible amino acids = **64** ($4 \times 4 \times 4$)



The existence of a three-base (**triplet**) code was confirmed by experiments by Francis Crick and his colleagues in 1961. The triplet code is **degenerate**, which means that each amino acid is coded for by more than one triplet.

How are the codons matched to amino acid?



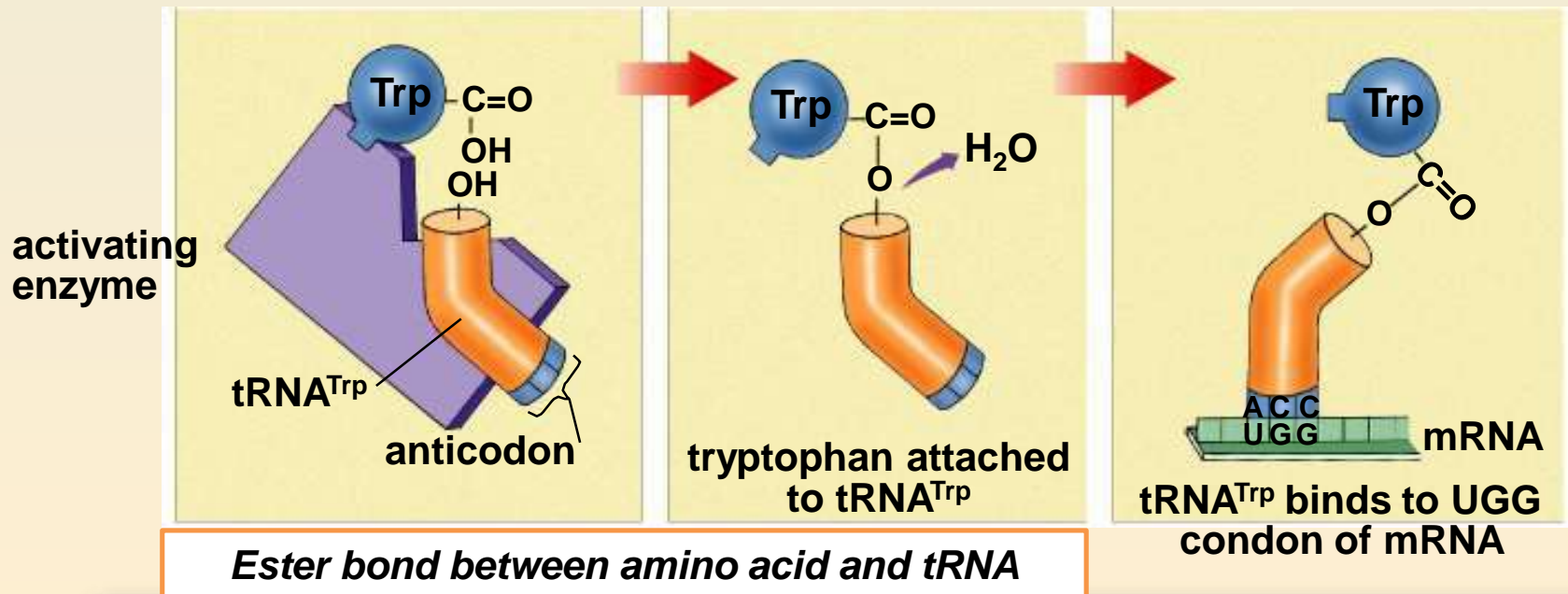
Charging of tRNA by an enzyme called aminoacyl tRNA synthetase also called tRNA-ligase (one aminoacyl tRNA synthetase is present for each standard amino acid, i.e., 20 aminoacyl tRNA synthetase are present in most of the cell).

Charging of tRNA

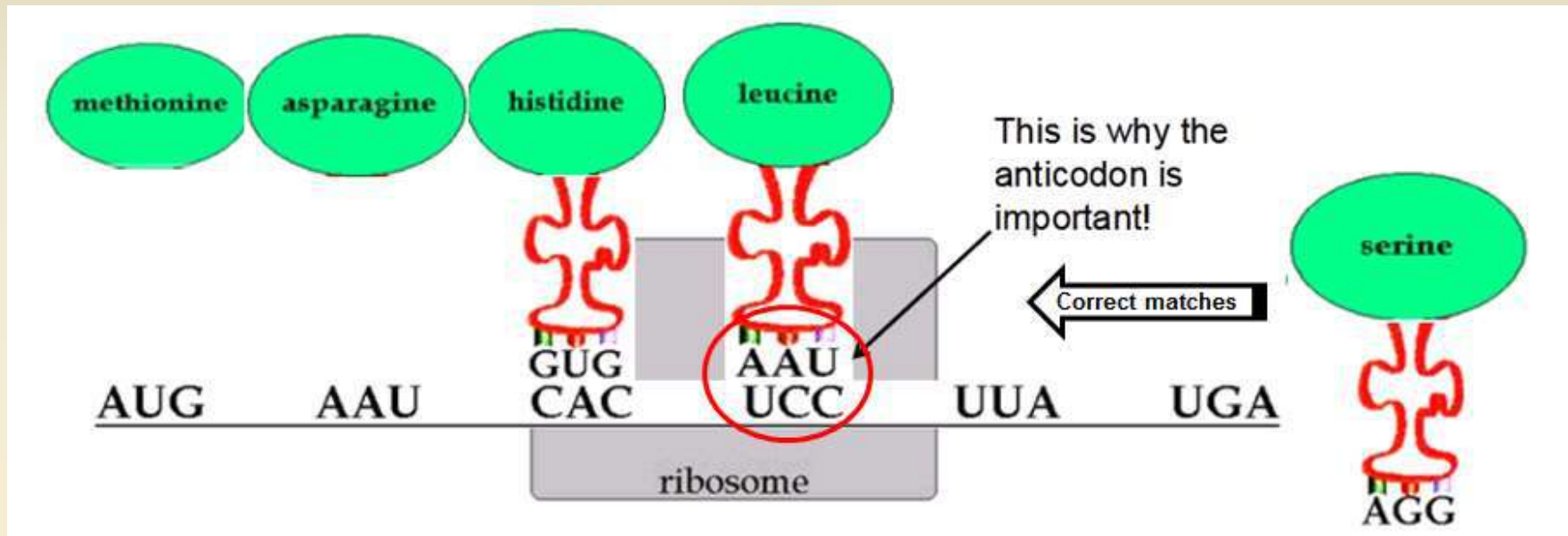
Charging of tRNA is accomplished by an enzyme aminoacyl tRNA synthetase

■ Aminoacyl tRNA synthetase

- ◆ enzyme which bonds amino acid to tRNA
- ◆ bond requires energy
 - $ATP \rightarrow AMP$
 - bond is unstable, so it can release amino acid at ribosome easily



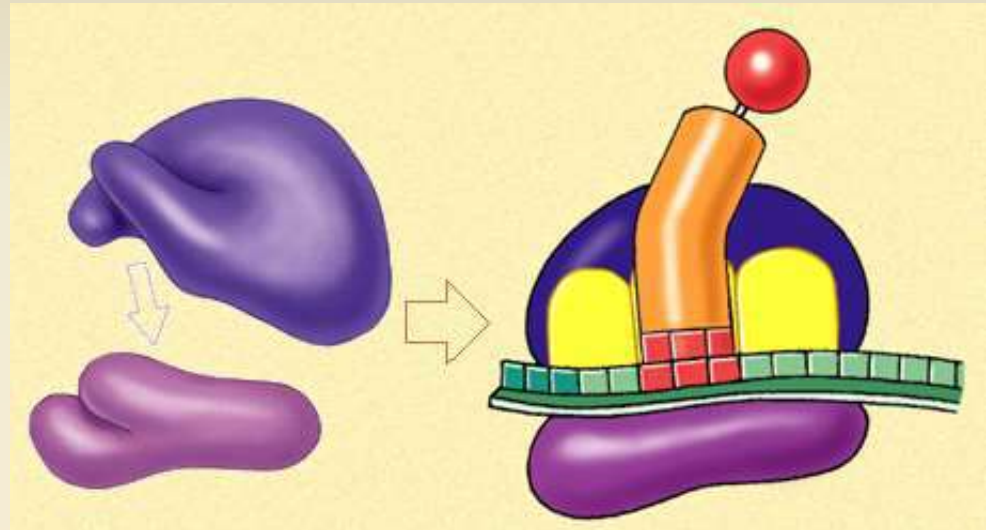
How are the codons matched to amino acid?



- ***Each codon represents a particular amino acid, and each codon is recognized by a specific tRNA.***
- ***Only codon specific anticodon containing tRNA interacts with codon, otherwise tRNA of wrong anticodon cannot interact with codon site.***

Ribosomes

- ❑ Ribosomes are a complex of ribosomal RNA (rRNA) and proteins.
- ❑ It is composed of two subunits of unequal size;
 - Large subunit
 - small subunit
- ❑ It facilitates coupling of tRNA anticodon to mRNA
- ❑ It has many sites meant for specialized function, which are:



→ **A site** (aminoacyl-tRNA site)

- *It holds tRNA carrying next amino acid to be added to the polypeptide chain.*

→ **P site** (peptidyl-tRNA site)

- *It holds tRNA carrying growing polypeptide chain.*

→ **E site** (exit site)

- *It ensures empty tRNA leaves ribosomes from exit site*

Protein Synthesis

Three steps

□ Initiation

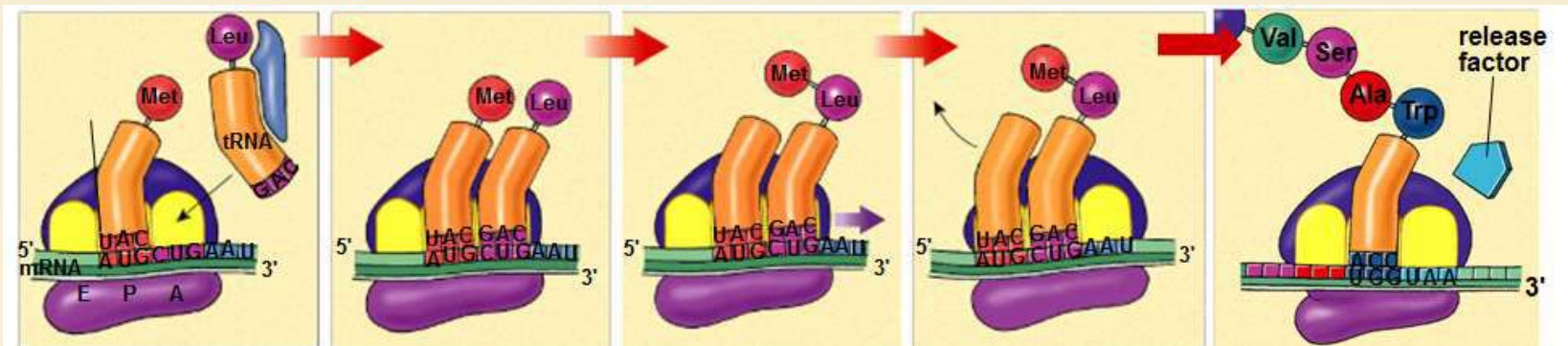
- It brings together mRNA, ribosomal subunits and initiator tRNA.

□ Elongation

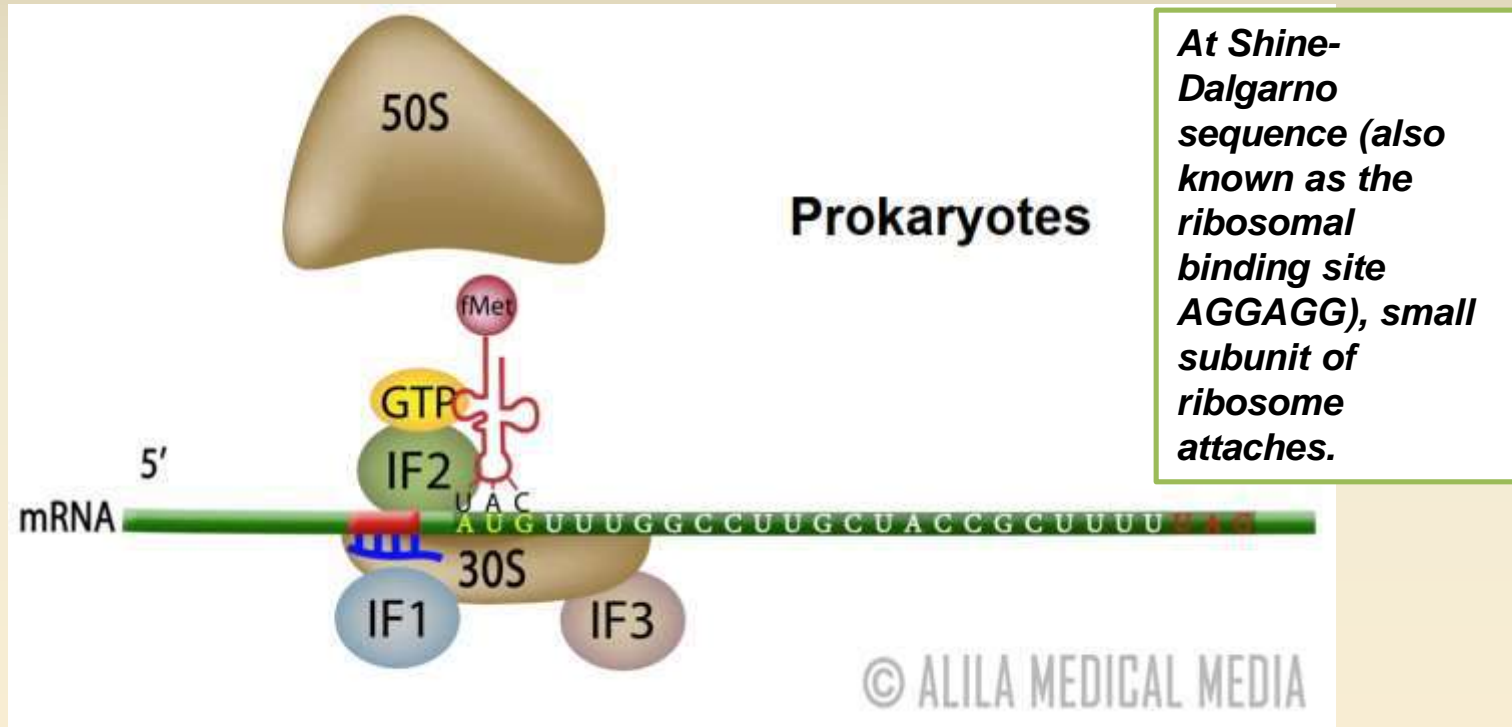
- In this phase, adding of amino acids take place based on codon sequence.

□ Termination

- Here codon ends and translation stops



Initiation of Translation

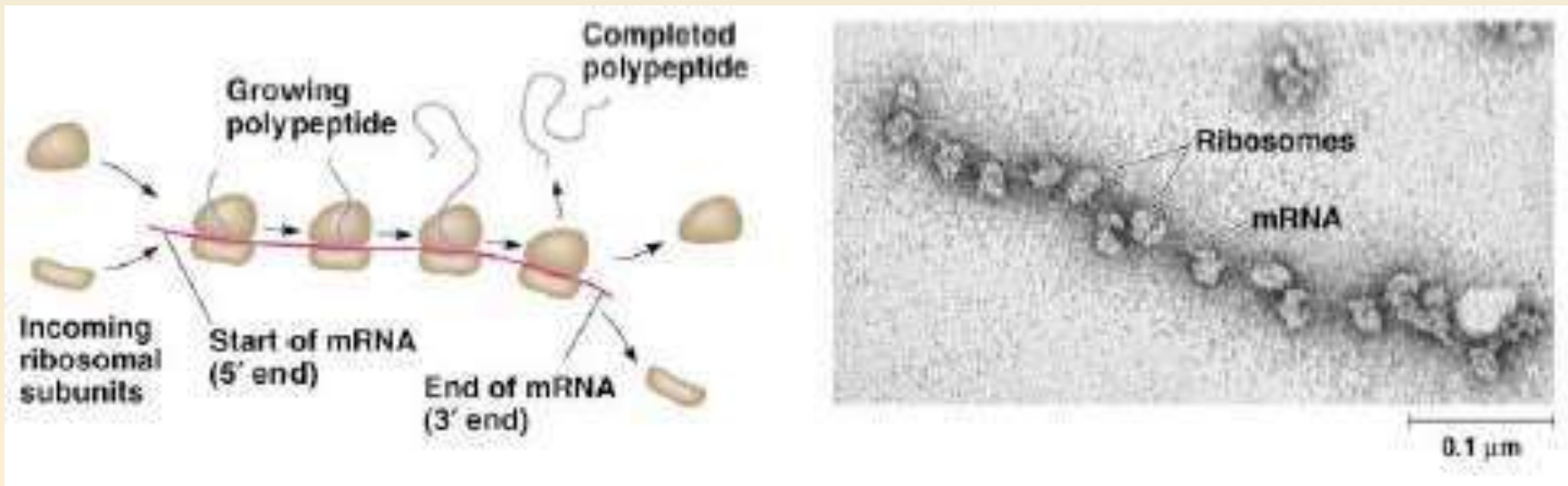


In prokaryotes, protein synthesis begins with the formation of an initiator complex which involves the small 30S ribosome, the mRNA template, 3 initiation factors that helps the ribosome assemble correctly, guanosine triphosphate (GTP) that acts as a energy source, and a special initiator tRNA carrying N-formyl-methionine (fMet-tRNA^{fMet})

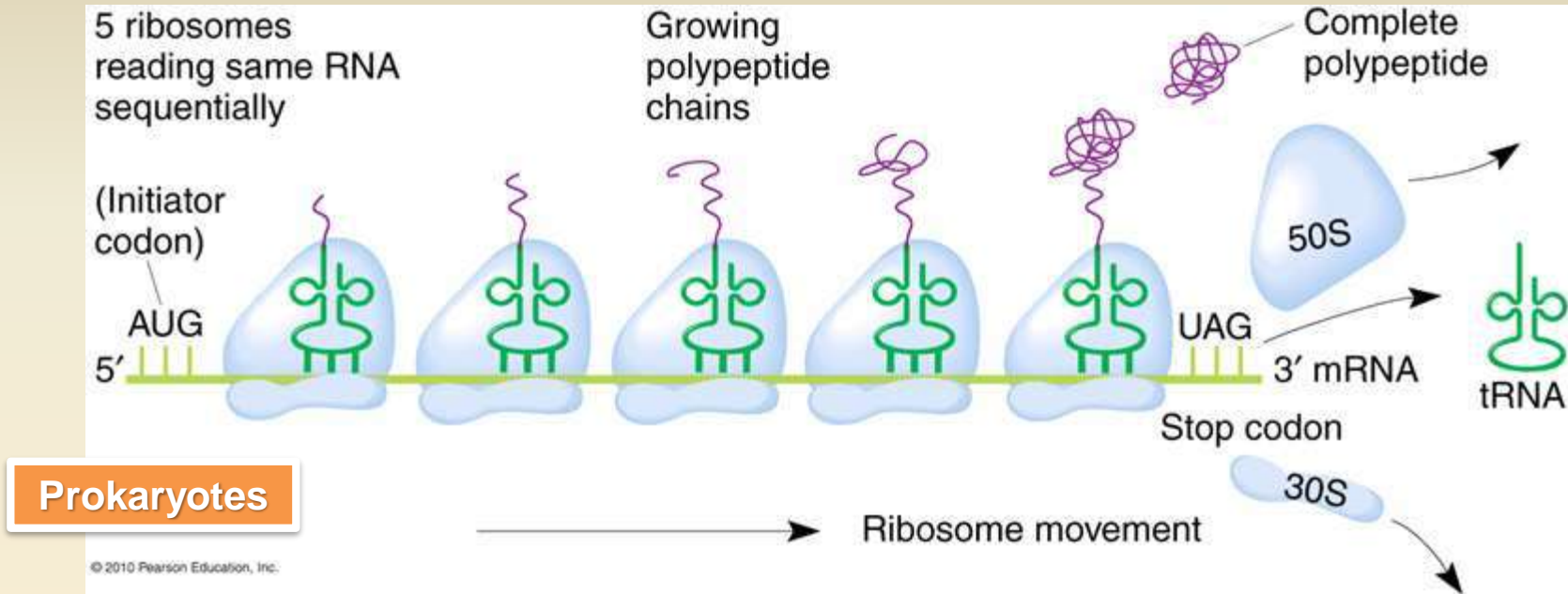
Elongation of Polypeptide Chain

■ Polyribosomes (Polysomes)

- ◆ Numerous ribosomes translate same mRNA at same time
- ◆ 3-D folding (1', 2', 3' structure)
- ◆ **Chaparonins:** provide essential kinetic assistance to protein folding by binding non-native proteins.

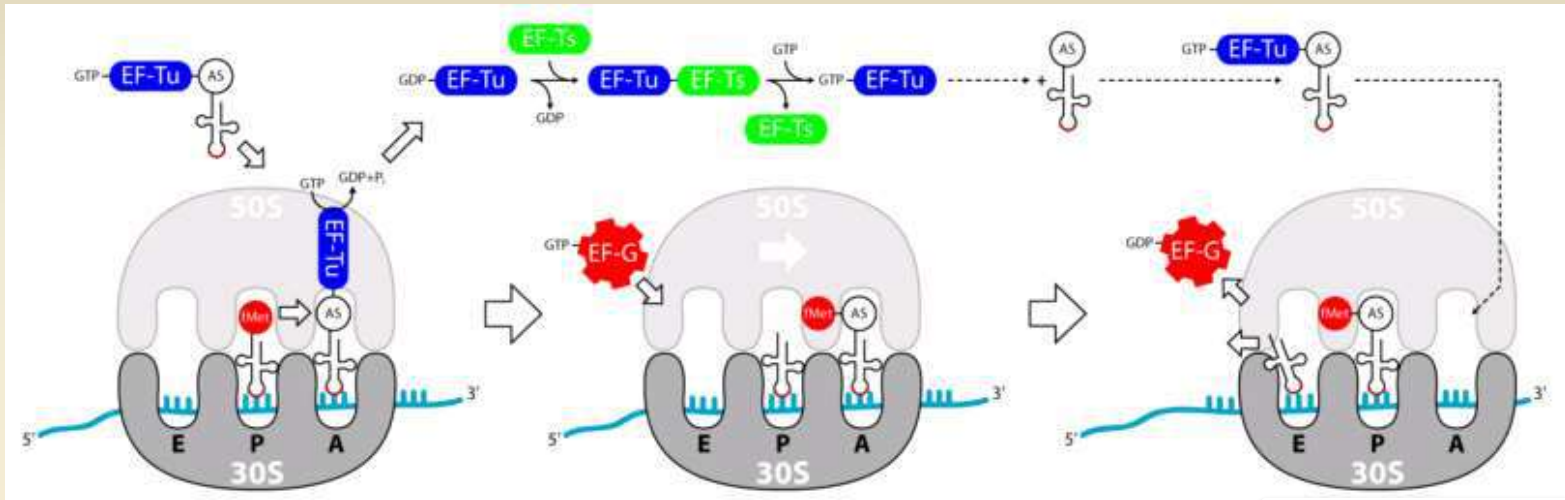


Elongation of Polypeptide Chain



- ❑ Elongation starts with the occupation of methionyl-tRNA to the ribosome P – site after recognizing a start codon on mRNA bound to small ribosomal subunit.
- ❑ The large subunit then joins the small subunit, and a second tRNA is recruited.
- ❑ As the mRNA moves relative to the ribosome, successive tRNAs move through the ribosome and the polypeptide chain is formed.
- ❑ In this way, elongation proceeds with charged tRNAs sequentially entering and leaving the ribosome as each new amino acid is added to the polypeptide chain.

Elongation of Polypeptide Chain

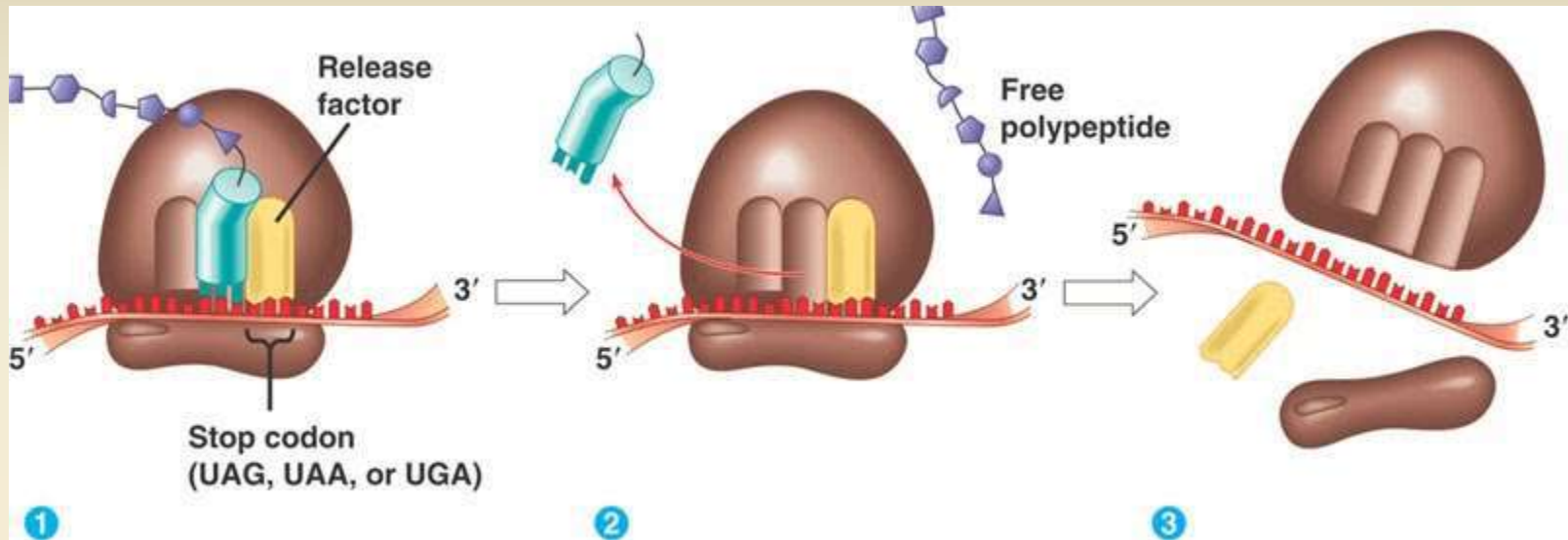


Souli et. al. *International Journal of Molecular Sciences* 2013; 14(3):4525-44

Prokaryotes

- ❑ This movement of a tRNA from A to P to E site is induced by conformational changes that advance the ribosome by 3 bases in 3' direction.
- ❑ The energy for each step, such as binding of a new aminoacyl-tRNA to the A site and for its translocation to the P site after formation of the peptide bond along the ribosome is donated by elongation factors that hydrolyze GTP.
- ❑ Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA by an enzyme peptidyl transferase integrated into large subunit..

Termination of Translation

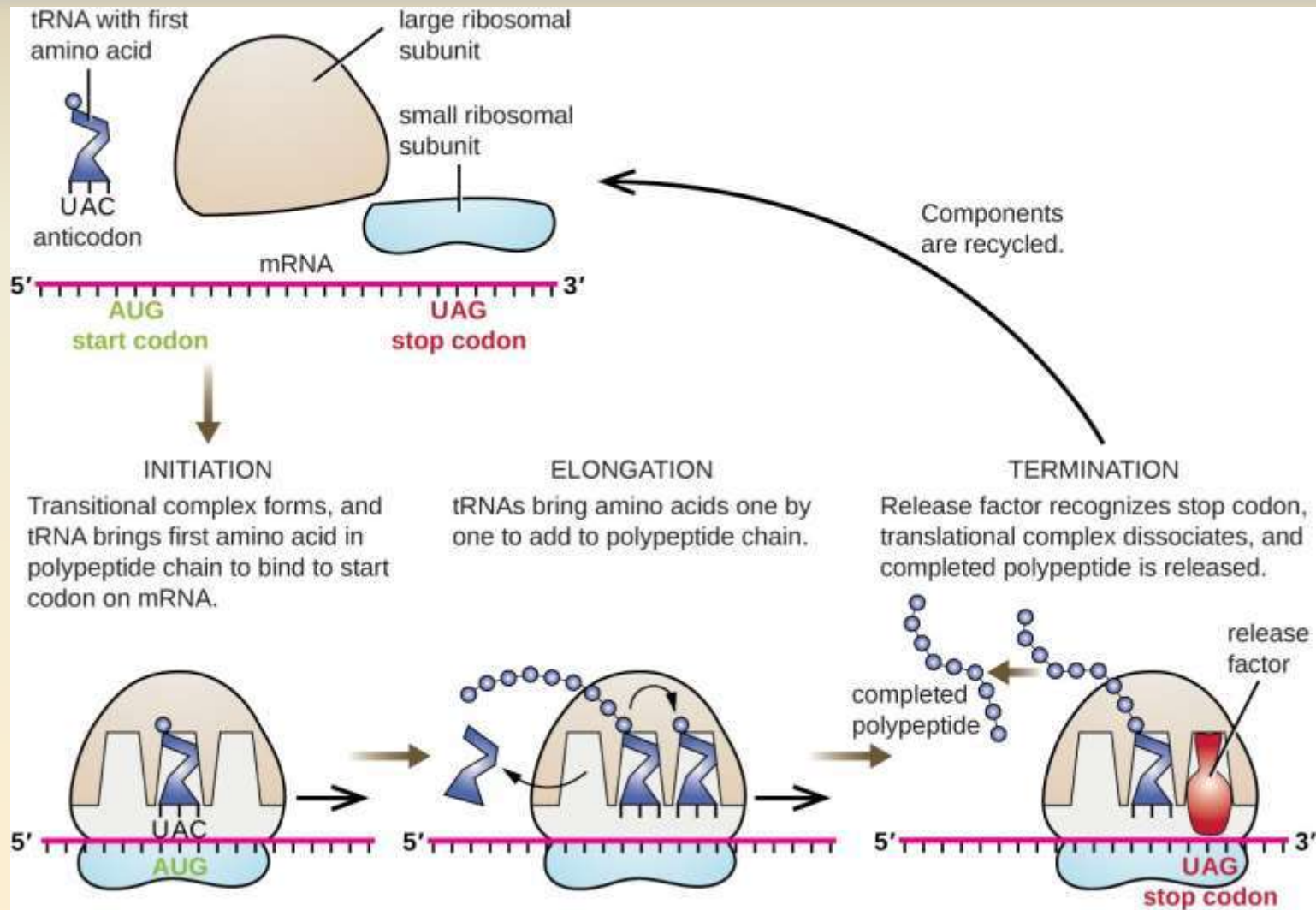


Step 1 – After the amino acids needed to make the protein required are properly bonded to each other a release factor binds to the complex when a stop codon enters the A site.

Step 2 – The release factor then disconnects the polypeptide from the tRNA in the P site.

Step 3 – The remaining components mRNA, ribosomal subunit, and the protein are released and the process of translation ends.

Overview of Translation



Overview of Translation

Comparison of translation in Prokaryotes and Eukaryotes (& Archea)		
Ribosomes	70S <ul style="list-style-type: none">▪ 30S (small subunit) with 16S rRNA subunit▪ 50S (large subunit) with 5S and 23S rRNA subunits	80S <ul style="list-style-type: none">▪ 40S (small subunit) with 18S rRNA subunit▪ 60S (large subunit) with 5S, 5.8S and 28S rRNA subunits
Amino acid carried by initiator tRNA	fMet	Met
Shine-Dalgarno sequence in mRNA	Present	Absent
Simultaneous transcription and translation	Yes	No

In prokaryotes, Met-tRNA is modified by the addition of a formyl group and it is fMet-tRNA in prokaryotes.

Brainteaser

Do the same DNA sequences lead to the same proteins in all organisms?

- No, due to differential mRNA splicing identical DNA sequences may lead to non-identical proteins.
- There are about 150 known cases of deviations from the universal genetic code with 1 or 2 codons being re-assigned to different amino acids; typically in small (organelle) genomes, but occasionally in nuclear genomes as well.

Further reading

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