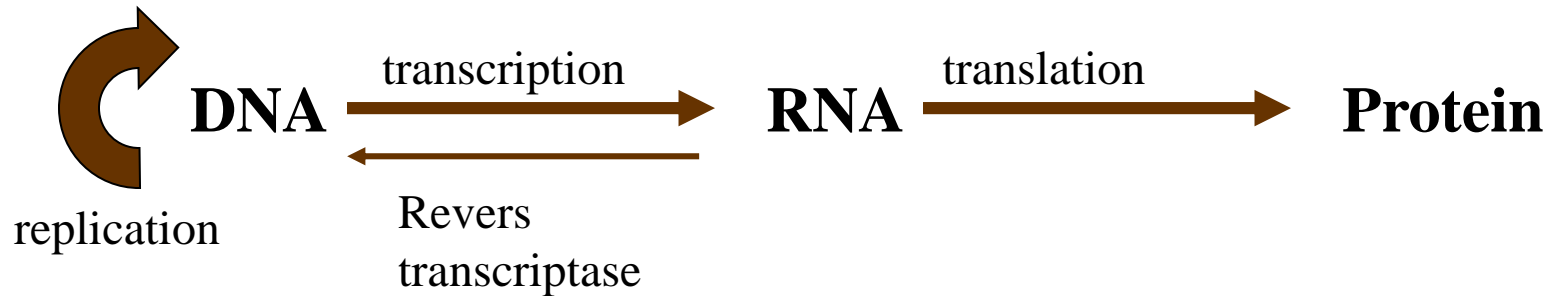


# Translation: the synthesis of polypeptides

The central dogma of molecular biology



**Proteins are essential for every cell in all living systems. A typical cell requires thousands of different proteins to catalyze metabolic reactions, transport molecules or replicate DNA.**

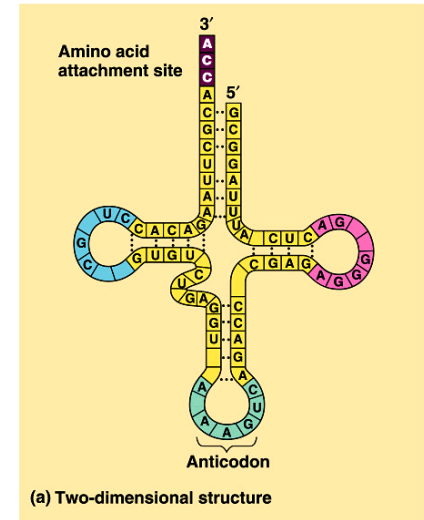
**This process uses up to 90% of the chemical energy used by a cell for all biosynthetic reactions.**

**the genetic information is encoded in four-letter language of nucleic acids and it could be translated into the 20-letter language of proteins**

**The nucleotide sequence of an mRNA is translated into amino acid sequence of a polypeptide with help of tRNAs.**

**mRNA:** carries the genetic information from DNA to the place of protein synthesis (ribosomes).

**rRNA:** a component of the protein synthesizing machinery (ribosomes).



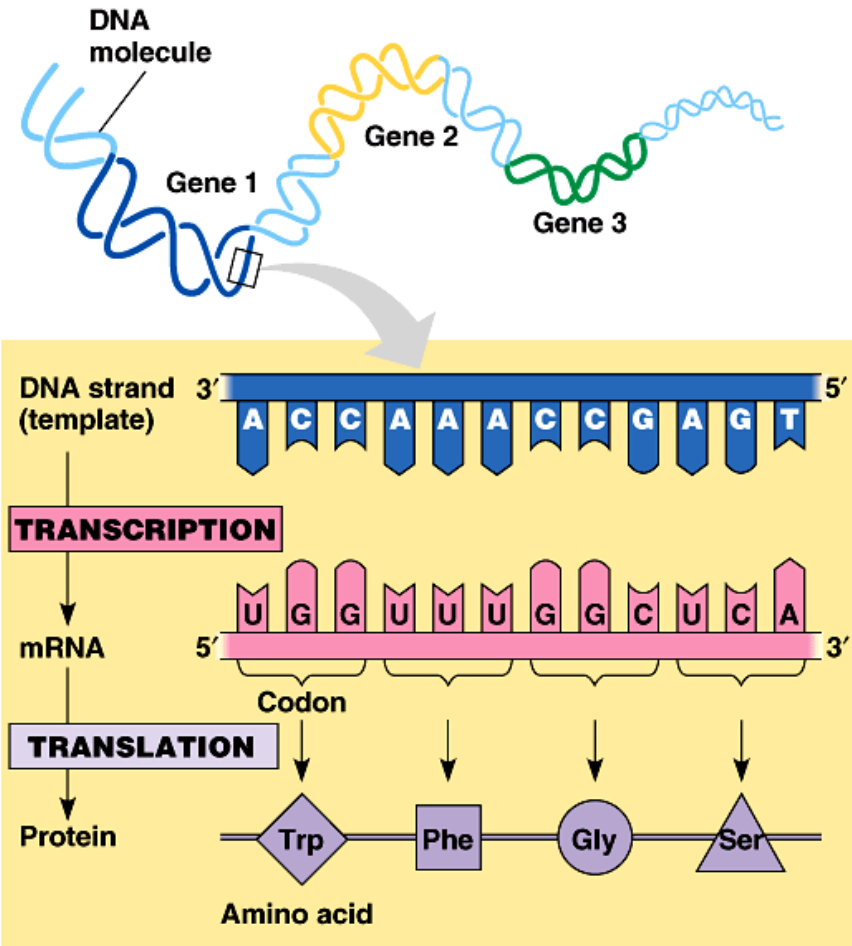
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It was obvious that four nucleotide bases in groups of two can yield only  $4^2=16$  different combinations, which is not enough for the 20 amino acids.

Groups of three yield  $4^3=64$  combinations, so a triplet of nucleotides (codon) codes an amino acid.

Genetic code in all living systems is known to be **nonoverlapping** which means that **codons do not share nucleotids**.

There is **no punctuation between the codons**, so the mRNA is read without pauses. A specific first codon in the sequence establishes the reading frame, in which a new codon begins every three nucleotide residues.



61 of the 64 possible codons encode amino acids.

The remaining three codons do not encode any amino acid, these are

termination codons.

In the presence of these codons the synthesizing process would stop.

**AUG is the initiation codon** which is responsible for the beginning of the translation. It also encodes methionine residues in internal positions.

Codons do not share nucleotids.

The genetic code is nearly **universal**

These exceptions mostly occur in mitochondrial RNA

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

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

An amino acid may be encoded by **more than one codon**, so the code is described as **degenerate**. Although an amino acid may have two or more codons, each codon specifies only one amino acid.

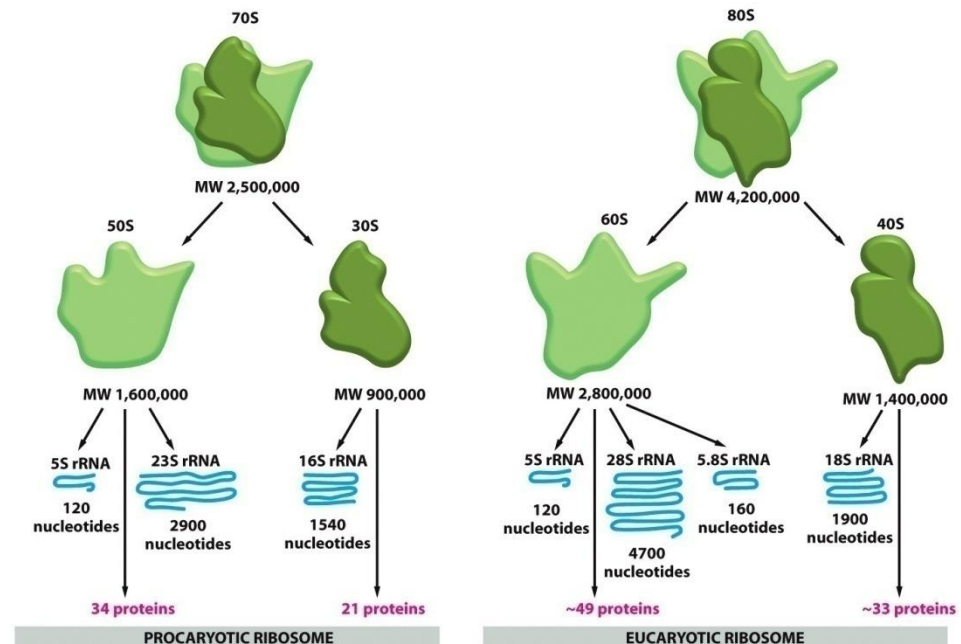
Protein synthesis follows the same pattern as DNA or RNA synthesis; the main stages are **initiation, elongation and termination**.

Before these processes the activation of precursors is needed

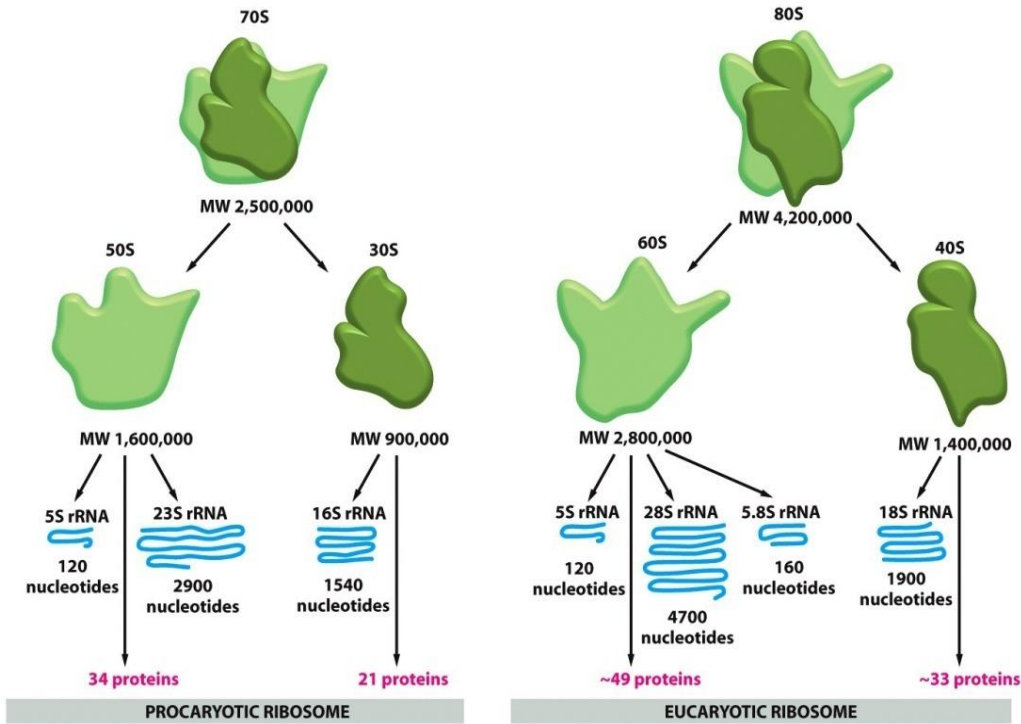
## The structure and role of the ribosome and tRNA

The ribosome is a complex supramolecular unit which contain about **65% rRNA and 35% protein**.

Bacterial ribosomes are composed of two different subunits with sedimentation coefficients of **30S** (Svedberg unit) and **50S** and a combined sedimentation coefficient of **70S**.



The two subunits fit together to form a cleft through which mRNA passes during translation.



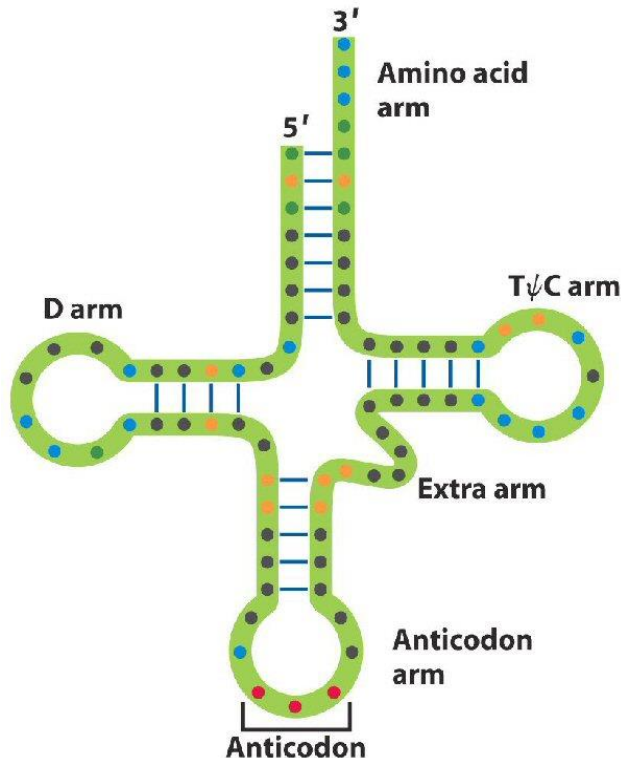
The eukaryotic ribosomes are **larger and more complex** than bacterial ones with **80S** total sedimentation coefficient. They also have two subunits (**60S** and **40S**) and the function is similar.



# Transfer RNAs

tRNAs serve as adaptors in **translating the language of nucleic acids into the language of proteins**

In every cell there is at least one kind of tRNA for each amino acid.



**The 2D structure of the tRNA forms a cloverleaf**

The amino acid arm can carry a specific amino acid esterified by its carboxyl group the 2'- or 3'-hydroxyl group of the A at the 3' end of the tRNA.

The anticodon arm contains the anticodons.

The other two main arms are responsible for the folding of tRNA and they interact with the rRNA.



# Stages of the translation process

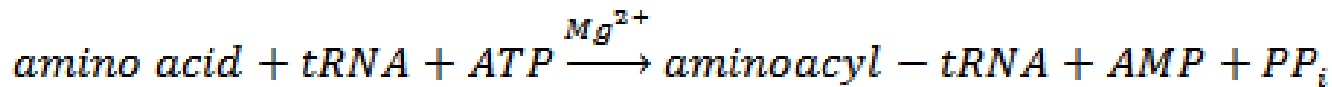
Before these processes the activation of precursors is needed

## Activation of amino acids

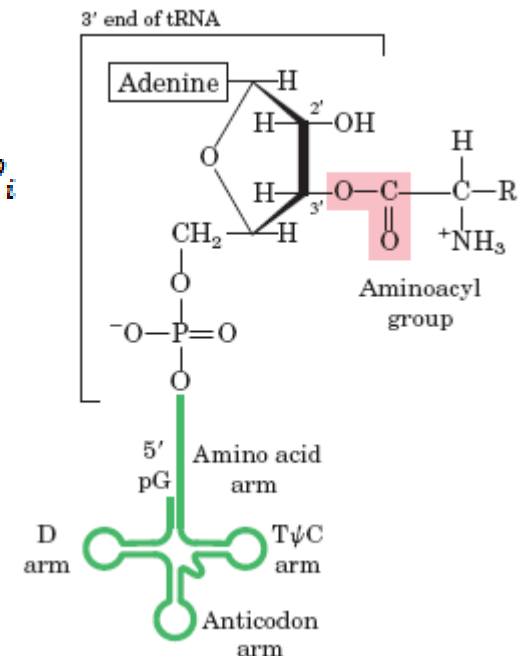
It takes place in the cytosol.

The aminoacyl-tRNA synthetases esterify the 20 amino acids to their corresponding tRNAs.

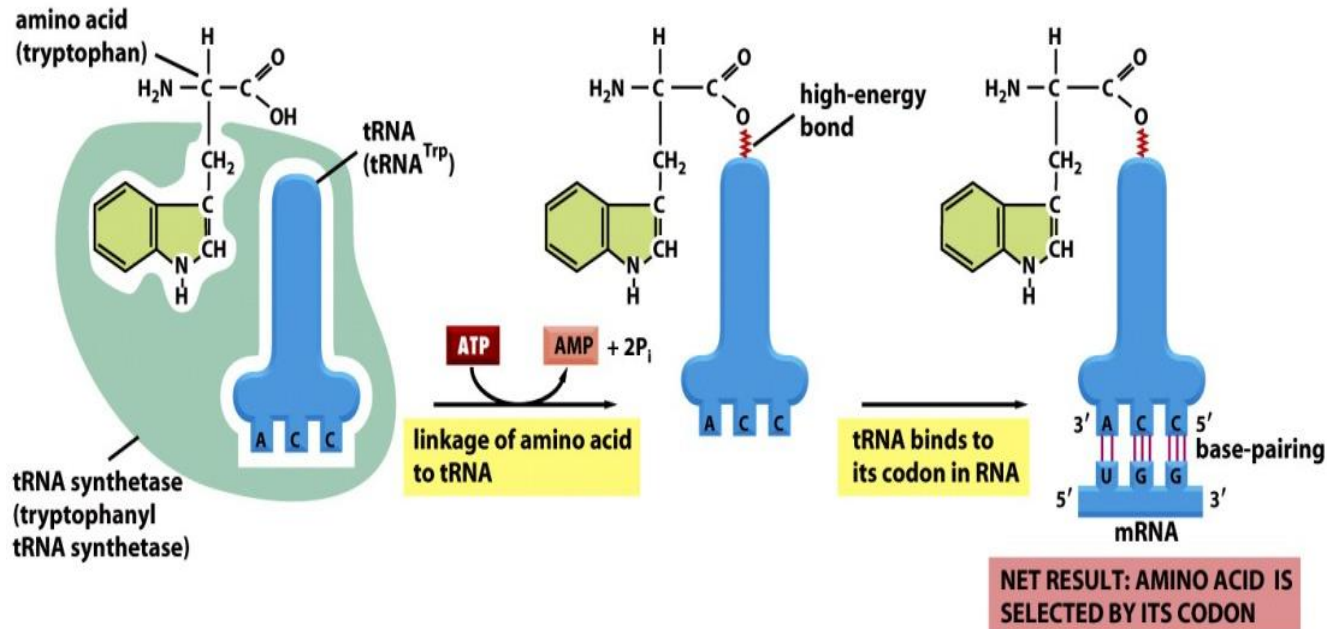
Most organisms have one aminoacyl-tRNA synthetase for each amino acid. Each enzyme is specific for one amino acid and one or more corresponding tRNAs



The hydrolysis of two phosphate group provides the necessary energy for the reaction and makes it irreversible



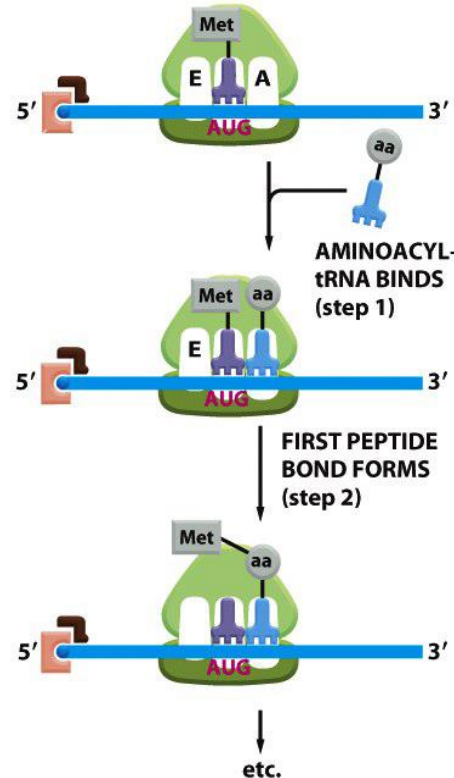
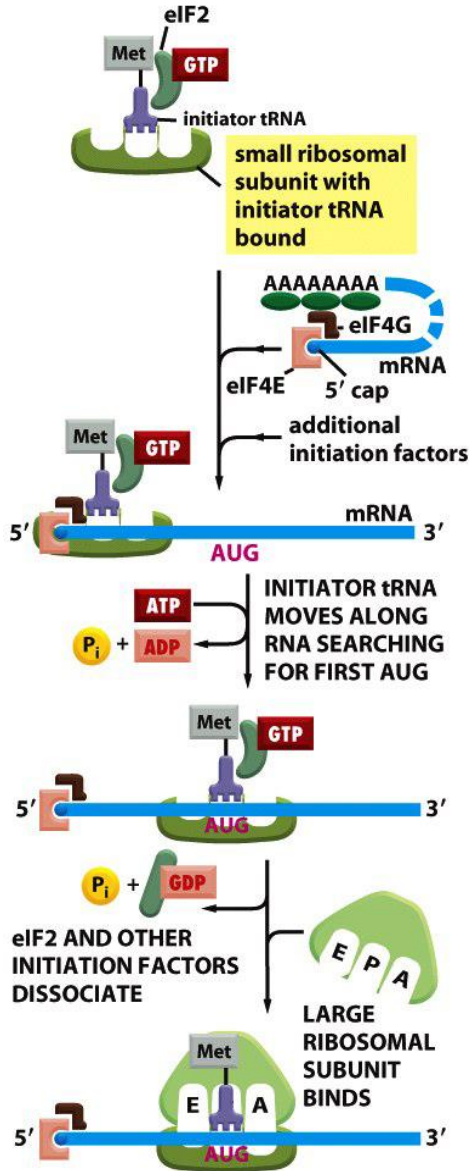
# An aminoacyl-tRNA synthetase must be specific not only for a single amino acid but for certain tRNAs as well



# Initiation

Protein synthesis begins at the amino-terminal end with the codon of AUG.

The formation of the initiation complex in bacteria requires the 30S ribosomal subunit, the mRNA, the initiating fMet-tRNA, 3 initiation factors, GTP, the 50S subunit and  $Mg^{2+}$ .



Every ribosome has three sites that bind tRNA: the aminoacyl site (A), the peptidyl site (P) and an exit site (E)

In the next step the fMet-tRNA and IF-2-GTP bind to the P site of the 30S subunit. In the last step the complex combines with the 50S subunit

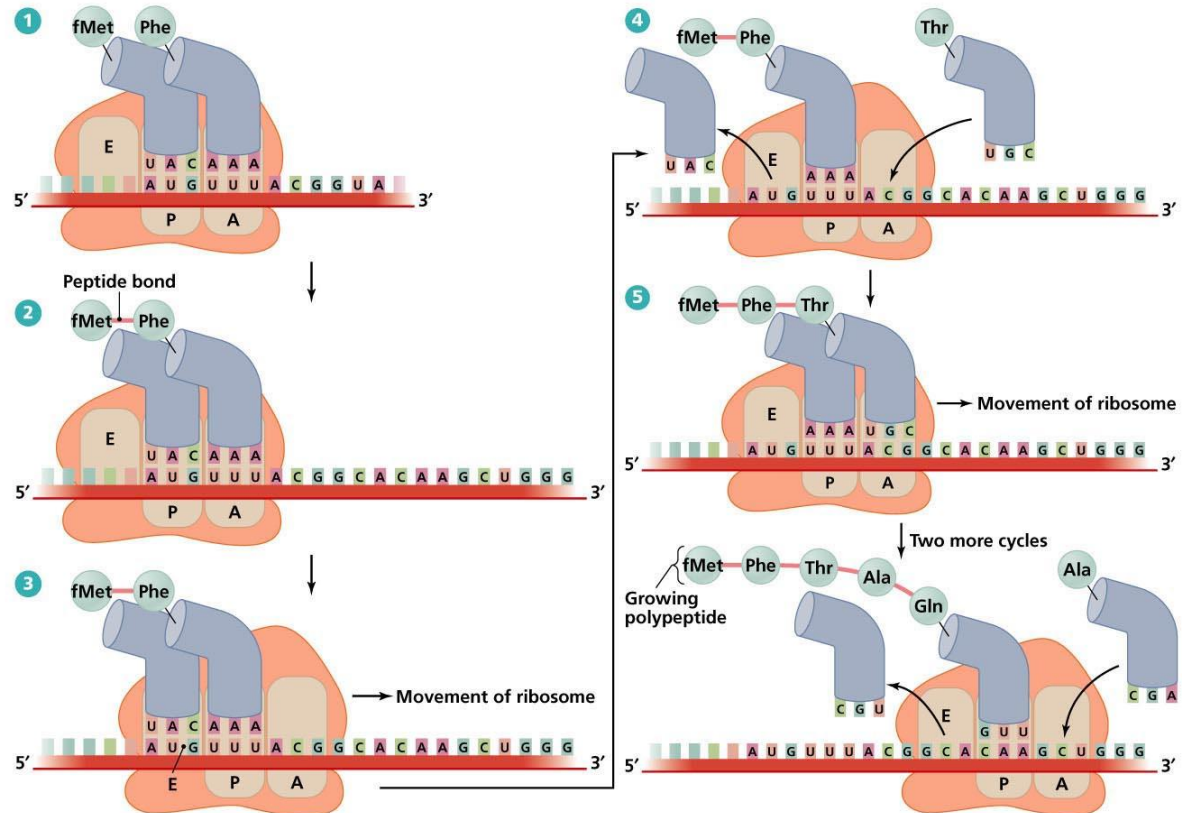
# Elongation

Elongation in bacterial cells requires the initiation complex, aminoacyl-tRNAs, three elongation factors and GTP.

Three steps are repeated for adding each amino acid residues.

**1. The appropriate incoming aminoacyl tRNA binds to the A site of the ribosome with the help of elongation factors and the energy of the GTP hydrolysis.**

The regeneration of GTP needs several milliseconds and the ribosome uses this time to check the codon-anticodon pairing.

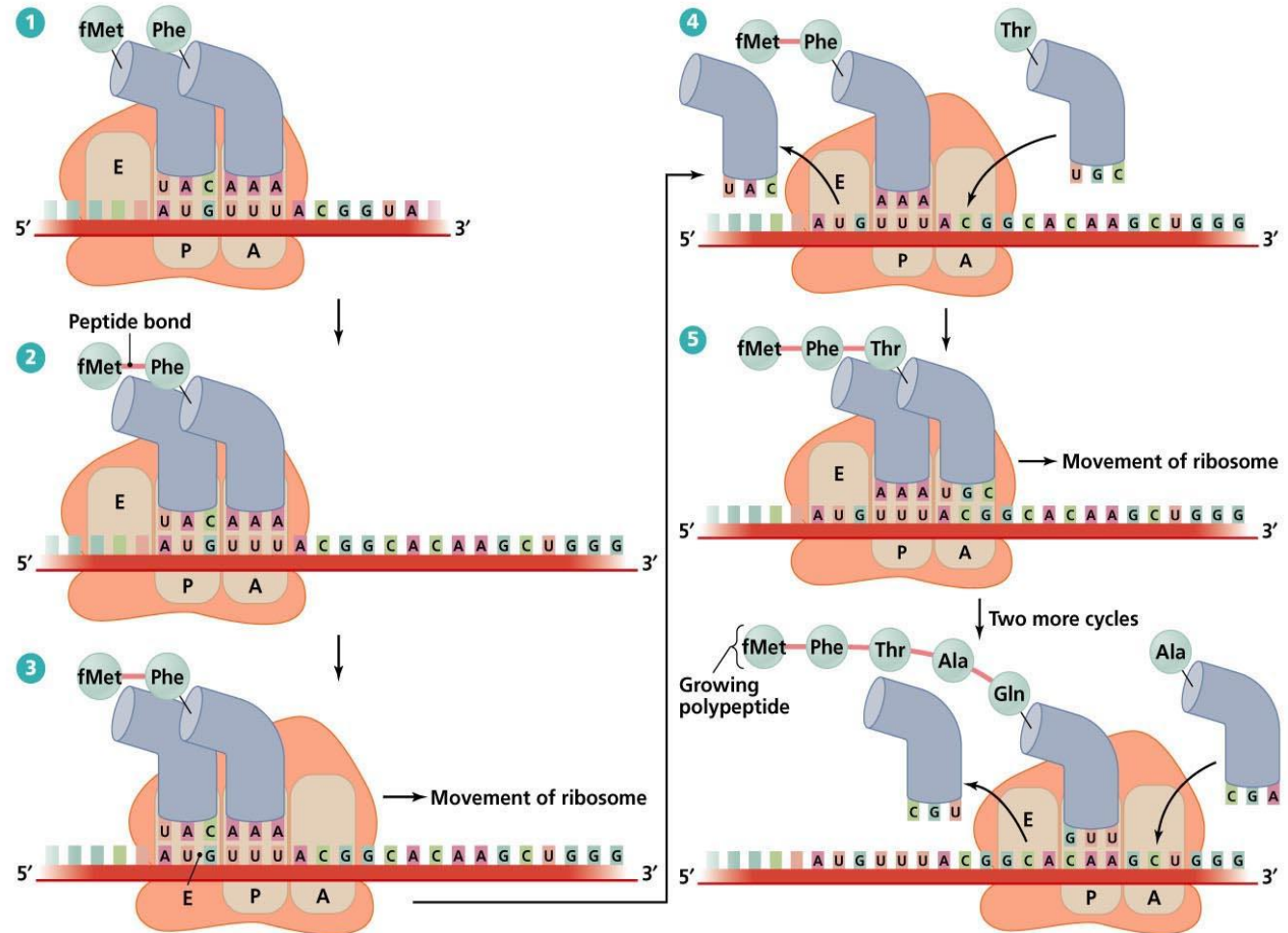


**2. The peptide bond formation**, the  $\alpha$ -amino group of the amino acid in the A site displace the tRNA in the P site to form a peptide bond.

**3. The translocation**, the ribosome moves one codon toward the 3' end of the mRNA.

This shifts the anticodon of the peptidyl-tRNA to the P site. The uncharged tRNA moves from the P site to the E site.

This movement requires GTP and an elongation factor.

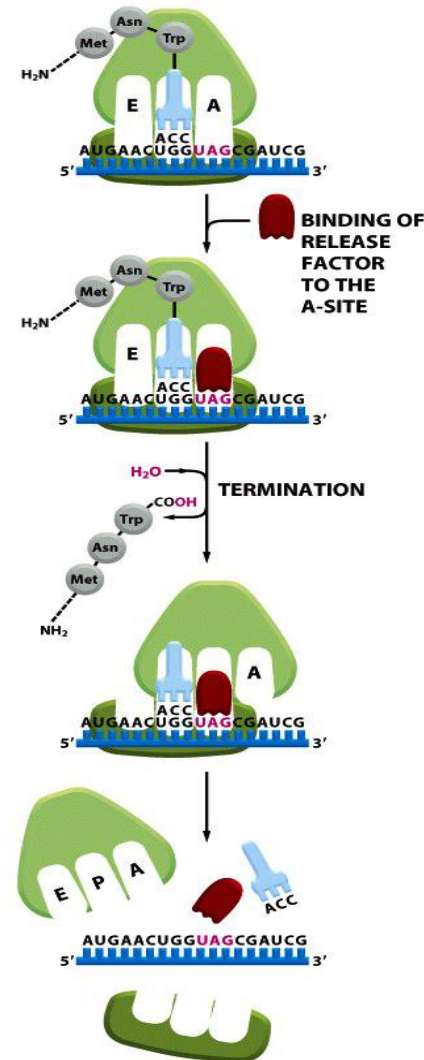




# Termination

Termination is signaled by one of the three termination codons: UAA, UAG, UGA

- Three termination factors hydrolyse the terminal peptidyl-tRNA bond
- Release the free polypeptide and the last tRNA from the P site
- Make the ribosome dissociate into the two subunits



# The energetics of peptide bond formation

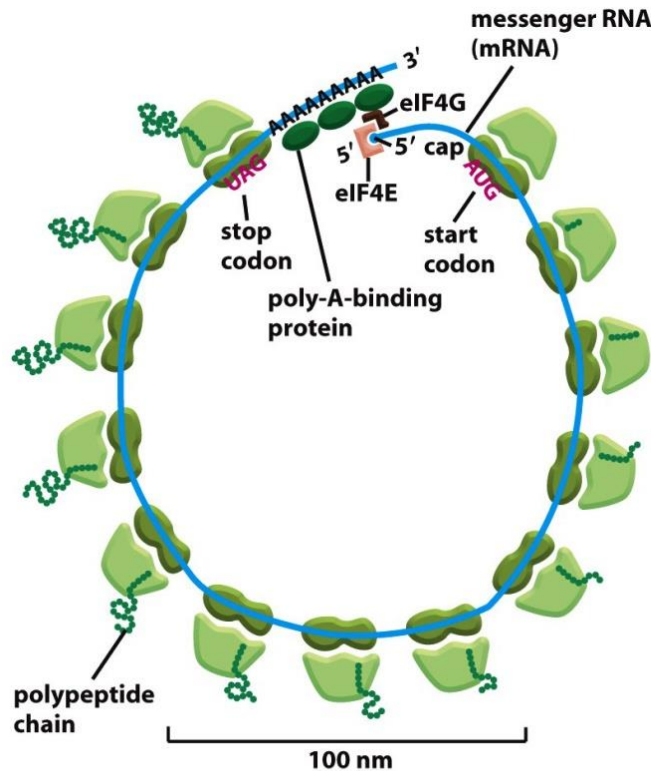
1. Formation of each aminoacyl-tRNA requires two high energy phosphate groups
2. Elongation needs two  $\text{GTP} \rightarrow \text{GDP} + \text{P}_i$  hydrolysis.
3. An additional ATP is consumed each time an incorrect amino acid is hydrolysed by an aminoacyl-tRNA synthetase.

**This is at least 122 kJ/mol (4x30,5 kJ/mol) of phosphodiester bond energy to generate a peptide bond**

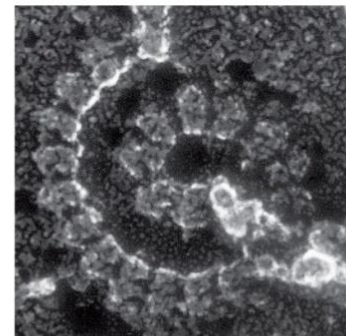


# Polysomes

Large clusters of 10 to 100 ribosomes can be observed in cells which are called **polysomes**. These are ribosomes which read the same mRNA and allow the rapid production of many copies of the same protein



(A)



(B)

# Folding and Posttranslational Processing

**The polypeptide chain is folded and processed into its biologically active form**

- amino-terminal and carboxyl-terminal modifications
- loss of signal sequences
- modification of individual amino acids
- attachment of carbohydrate side chains
- addition of isoprenyl groups
- addition of prosthetic groups
- proteolytic processing
- formation of disulfide cross-links

Protein synthesis is one of the primary targets of many antibiotics and toxins. These antibiotics usually inhibit protein synthesis in bacteria and are **relatively harmless to eukaryotic cells** because they exploit the **differences between eukaryotic and bacterial translation**.



**SMALL RIBOSOMAL SUBUNIT**