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# Lecture – 19

## Translation and Protein synthesis

by

**Dr. Praveen Kumar**

*Asst. Prof. **GPB***

MSSSoA, CUTM, Odisha



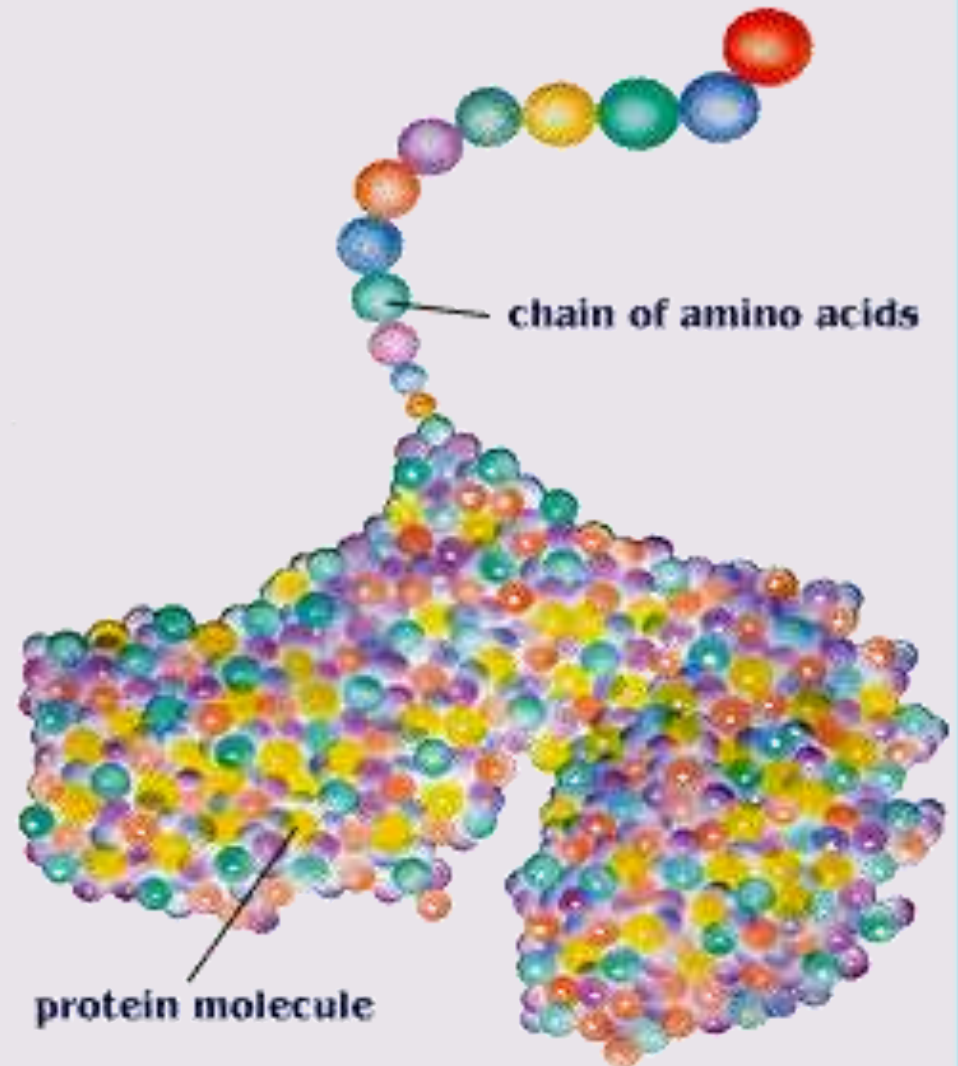
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# TRANSLATION-

## Assembling proteins- in the cytoplasm

- Proteins are composed of amino acids – there are 20 different amino acids
- Different proteins are made by combining these 20 amino acids in different combinations

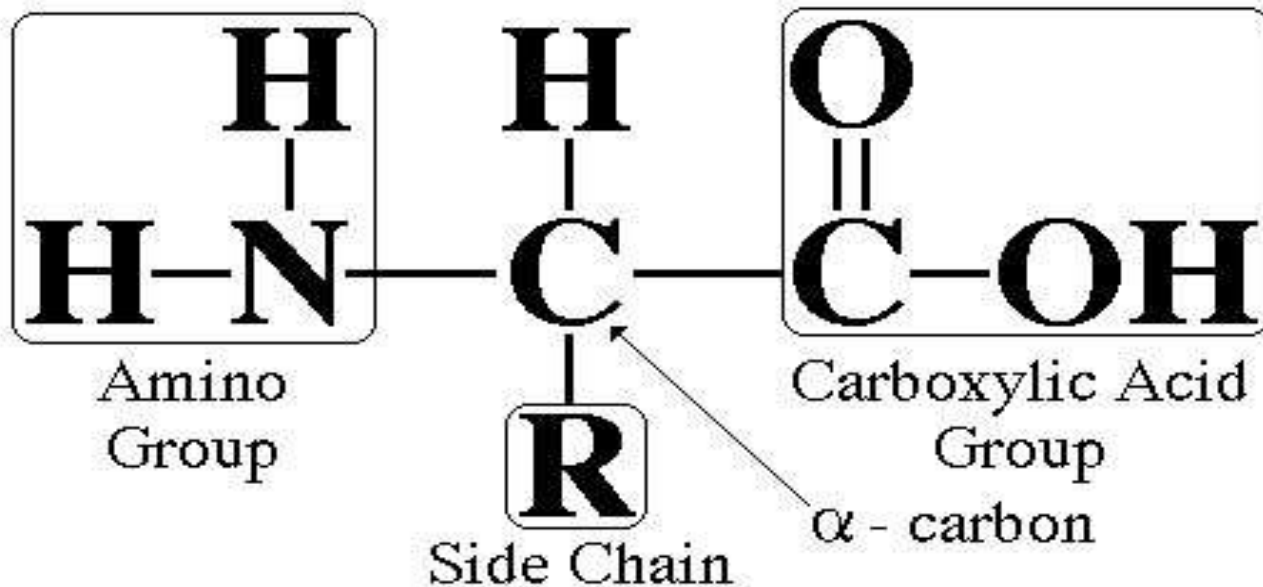


These amino acids come from the **food we eat**. Proteins we eat are broken down into individual **amino acids** and then simply **rearranged** into new **proteins** according to the needs and directions of our **DNA**.



# Amino Acid Structure

## Amino Acid Structure





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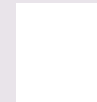
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# Why not DNA directly undergoes in protein synthesis ?

DNA has the **genetic code** for the **protein** that needs to be made, but proteins are made by the ribosomes—ribosomes are outside the **nucleus** in the **cytoplasm**.

DNA is too **large** to leave the nucleus (**double** stranded), but RNA **can leave** the nucleus (**single** stranded).

DNA consist of a number of non-coding sequences (INTRON)





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# Requirements of protein synthesis

The translation process requires

- ❖ mRNA,
- ❖ rRNA,
- ❖ Ribosomes,
- ❖ 20 kinds of amino acids and their
- ❖ tRNAs specific to each amino acid
- ❖ Many translation factors.






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# Steps of translation (protein synthesis)

**consists of five major steps viz.,**

- (1) activation of amino acids
- (2) transfer of amino acids to tRNA
- (3) chain initiation
- (4) chain elongation and
- (5) chain termination.

Each step is governed by specific enzymes and cofactors.



# Ribosome: Serves as work benches for protein synthesis.

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- ❖ The ribosome consists of rRNAs and different proteins.
- ❖ Ribosome contains two subunits; the large subunit and the small subunit.
- ❖ The process of translation starts when an initiating aminoacylated tRNA base pairs with an initiation codon of an **mRNA molecule that has been located in the small subunit of ribosome.**
- ❖ Then the larger subunit joins.
- ❖ Two separate and distinct sites are available in the ribosome to which the tRNAs can bind; **A (acceptor or aminoacyl attachment) site and P (peptidyl) site.**

**P or Peptidyl site-**

Bind the t-RNA to which polypeptide growing polypeptide is attached

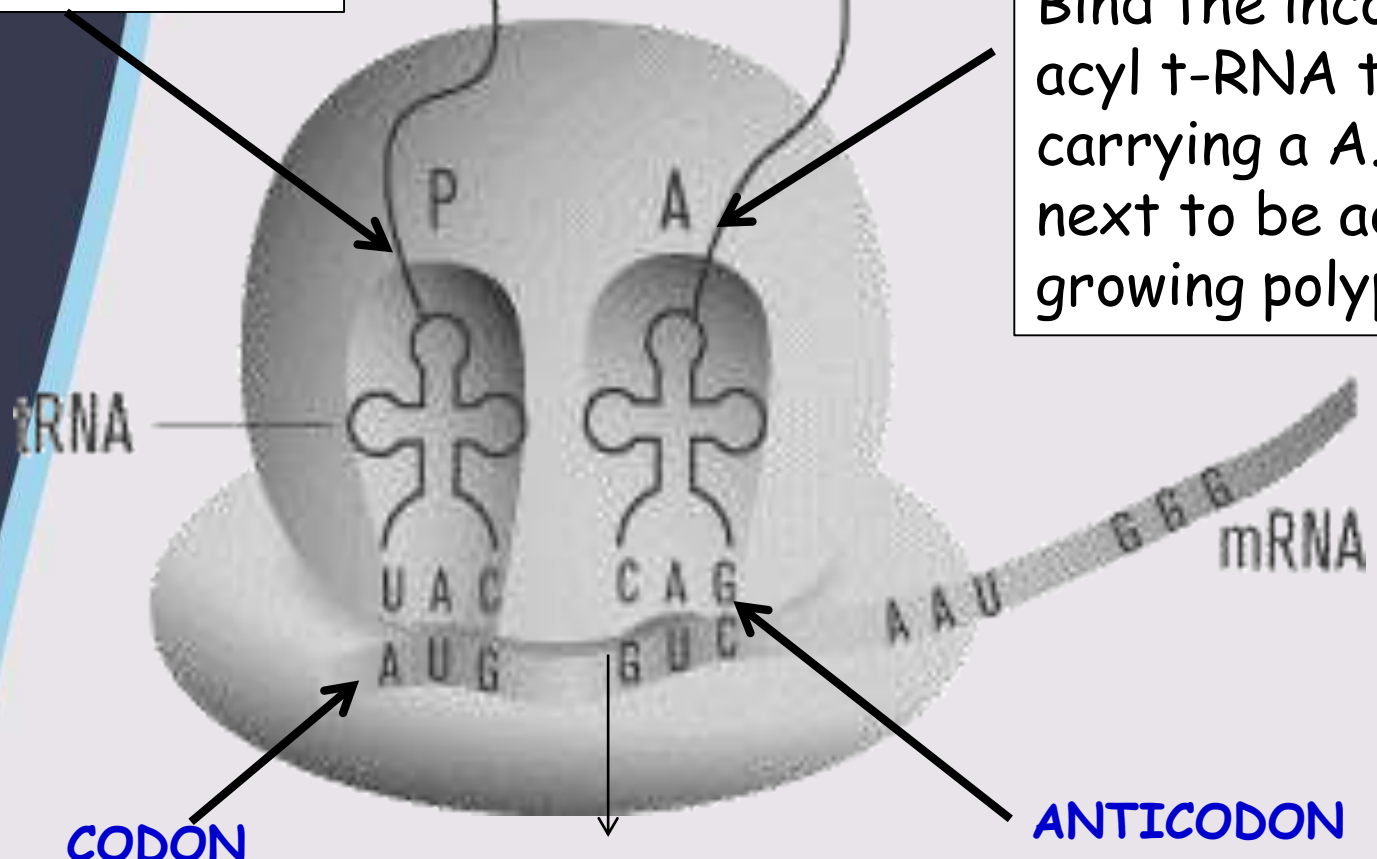
peptide bond

Methionine

Valine

**A or Amino acyl site-**

Bind the incoming amino acyl t-RNA that is t-RNA carrying a A. A. which is next to be added in growing polypeptide chain.



**EXAMPLE:** The tRNA molecule for methionine (an amino acid) has the “anticodon” UAC which matches to the “codon” AUG.

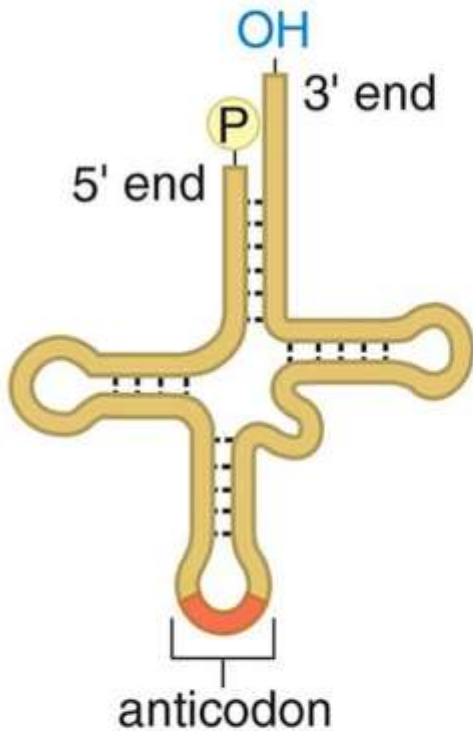




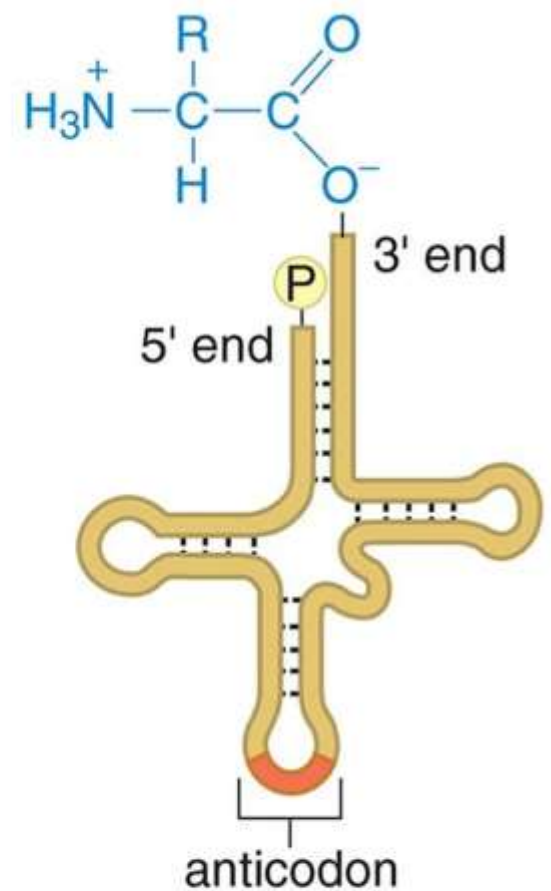
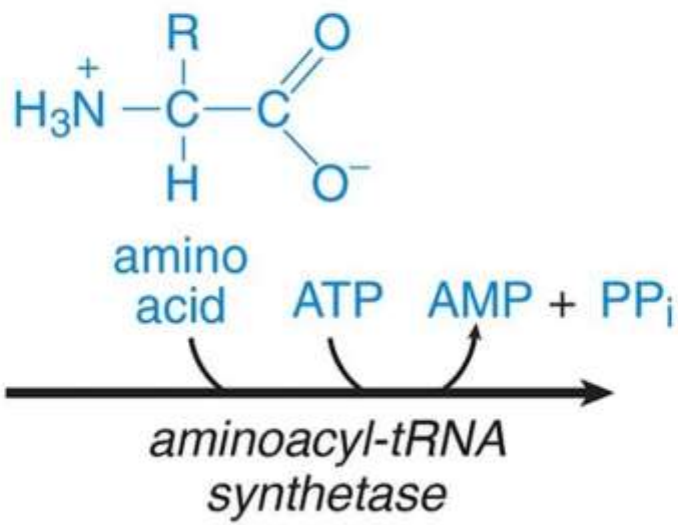
# 1. Activation of amino acids

Each amino acid has a specific tRNA

There is specific aminoacyl-tRNA-synthetase for each AA



tRNA



aminoacyl-tRNA

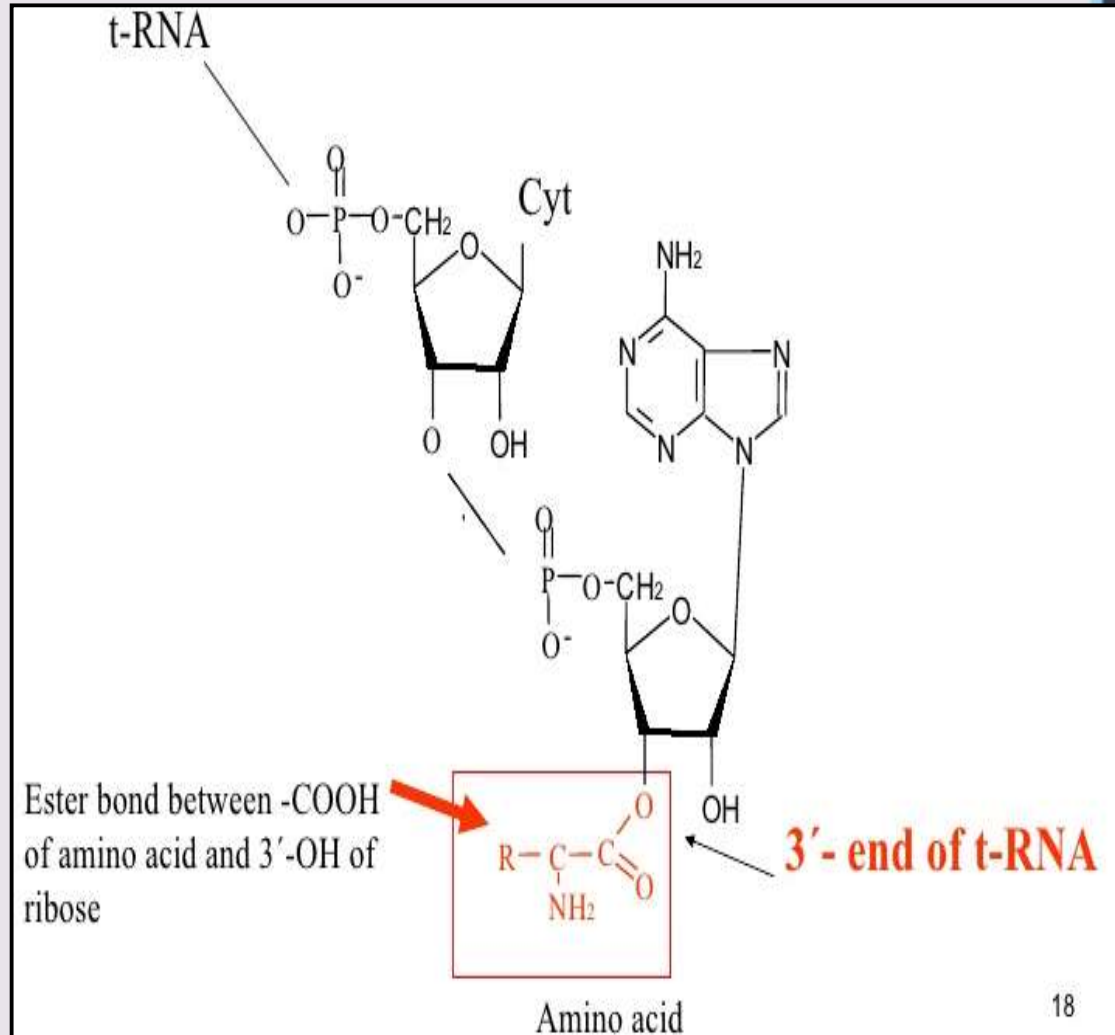
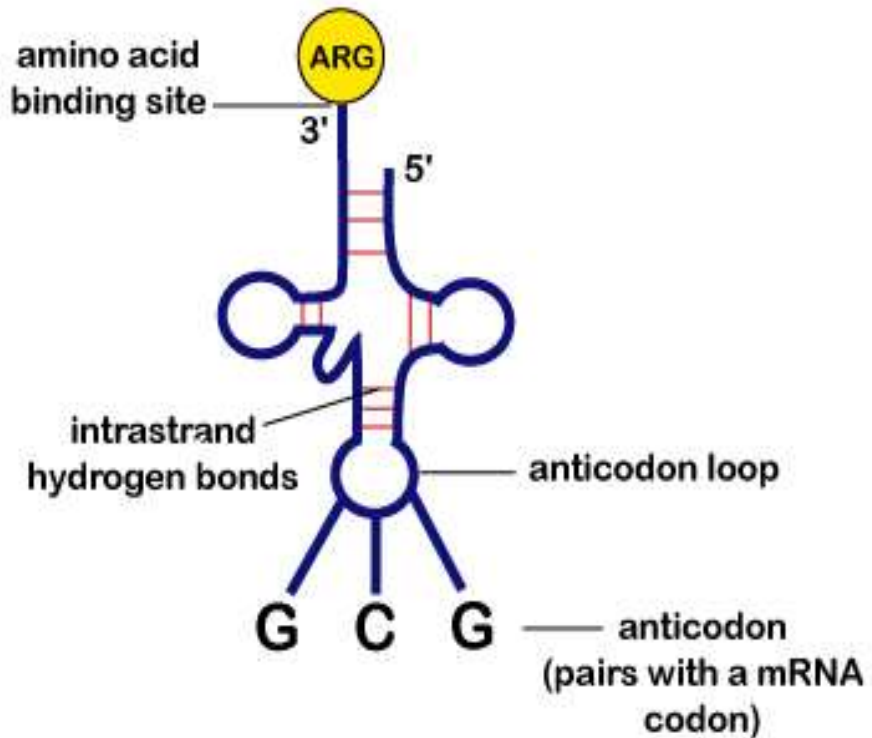


# 2. Transfer of amino acids to t-RNA

Three nucleotides at the 3' end are CCA in all t-RNA molecules, this segment is the amino acid acceptor region and the A.A. bind to 3' -OH group of ribose of terminal adanine nucleotide.

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Fig. 2: Charged Transfer RNA (tRNA)





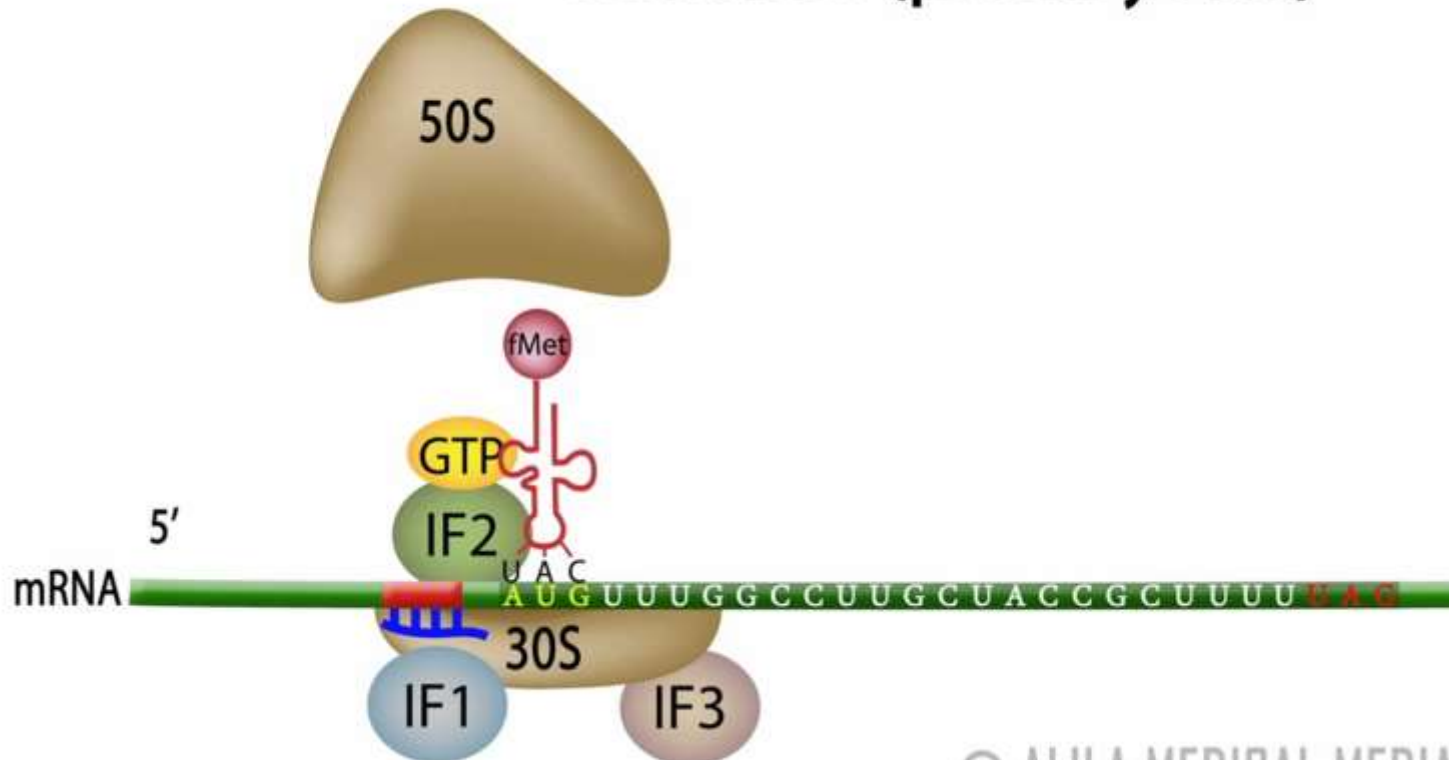
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## 3. Chain initiation

- ❖ Protein synthesis is initiated by a special initiator t-RNA designated as **t-RNA<sup>met</sup>** it means that all polypeptide begin with methionine.
- ❖ An aminoacyl-t-RNA first attaches to site A and the chain initiation occur with formation of **complex between m-RNA, methionyl t-RNA<sub>f</sub><sup>met</sup> and the small sub unit of ribosome.**

### Initiation (prokaryotes)



- ❖ For this 3 **protein initiation factor (IF1, IF2 and IF3)** and **GTP** are required

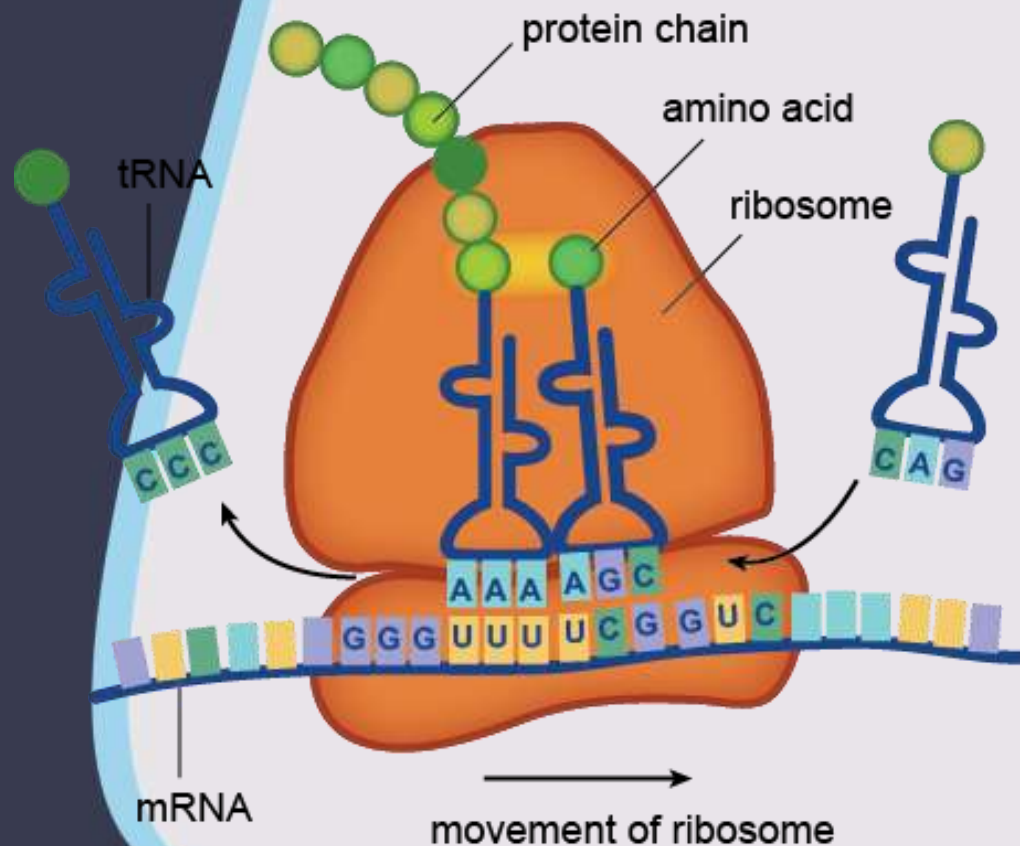


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## 4. chain elongation

❖ The peptide bonds are formed between the amino acids which is catalysed by the enzyme **peptidyl transferase**.

❖ The peptidyl tRNA along with the mRNA codon moves to the P (peptidyl) site making the A site available for the attachment of a new aminoacyl-tRNA. The elongation proceeds till the stop codon recognize.



❖ During the elongation process either m-RNA move (in eukaryotes) left side or Ribosome move (in prokaryotes) right side by one codon length to incorporate another t-RNA amino acyl in A site

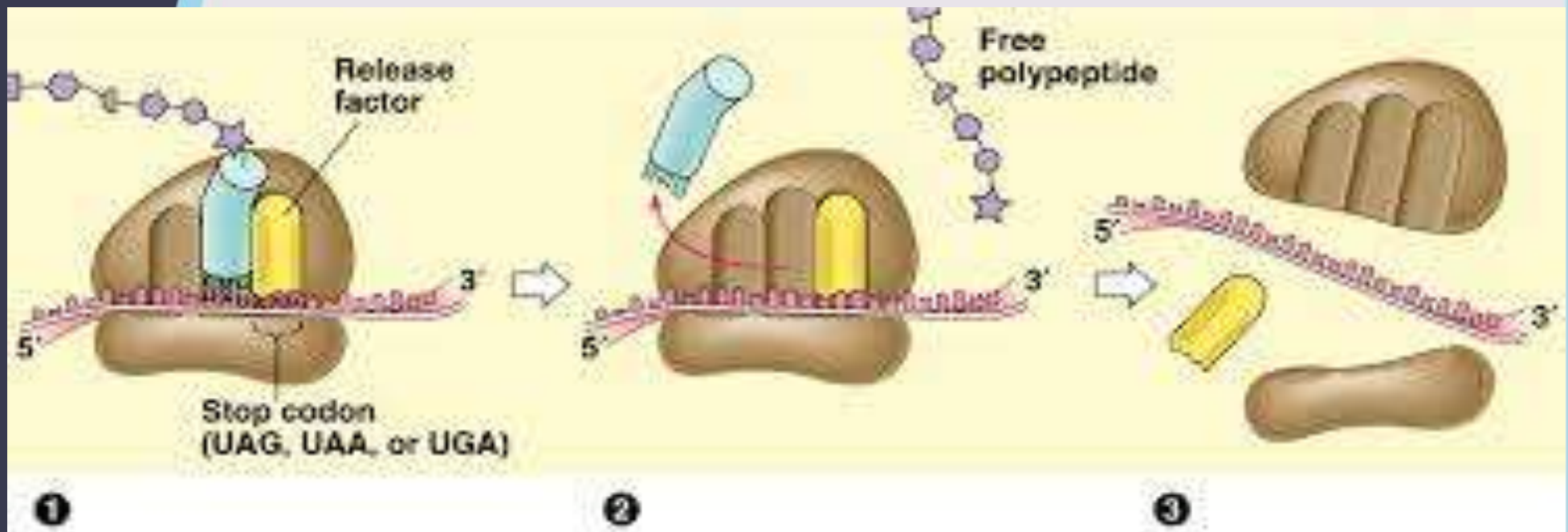




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## 5. Chain termination

- ❖ The translation proceeds and at the end a releasing factor binds to the stop codon terminating the translation. Two protein RF1 and Rf2 (eRF1 & eRF2 in eukaryotes) required.
- ❖ The ribosome releases the polypeptide and mRNA and subsequently dissociates into two subunits.







- ❖ Further processing of polypeptide chain into proteins and enzymes is done in the cytoplasm itself and depends upon the bonding properties of the amino acids joined in them.
- ❖ Most of the mRNA molecules are unstable and degraded after the release of polypeptide chain, but some mRNAs such as those coding for hemoglobin may be stable.
- ❖ When the cell needs large quantities of a particular enzyme or protein, more number of mRNA molecules coding for the same protein are produced to meet the demand.



**TABLE 24.1. Comparison of protein synthesis in Prokaryotes and Eukaryotes**

S.No.	Particulars	Prokaryotes	Eukaryotes
1.	Polymerase used	One	Several
2.	Ribosomes used	70S with 30S and 50S subunits	80S with 40S and 60S subunits
3.	Transcription and translation	Can overlap	Do not overlap
4.	Starting amino acid	N-formyl methionine	Methionine
5.	Initiation and termination sites	Several	Single
6.	Initiation factors used	IF 1, IF 2 and IF 3	eIF2, eIF3, eIF 4A, eIF 4E, eIF 4F and eIF 4G
7.	Chain elongation factors	EF-T4, EF-TS and EF-G	EF1 and EF2
8.	Release Factors	RF 1, RF 2 and RF 3	RF
9.	Synthesis of mRNA	From DNA or RNA	From RNA only

**Table 34.1. Differences between Prokaryotes and eukaryotes in the mechanism of translation.**

Prokaryotes	Eukaryotes
1. Initiating amino acid (methionine) needs to be formylated (therefore two tRNAs for methionine i.e. $tRNA_f^{met}$ and $tRNA_m^{met}$ are found)	1. Initiating amino acid (methionine) is not formylated (only one tRNA for methionine i.e. $tRNA_i^{met}$ is found)
2. Ribosomes enter the mRNA at AUG codon or at nearby Shine-Delgarno site.	2. Ribosomes enter at the capped 5' end of mRNA and then advance to AUG codon by linear scanning.
3. No initiation factors or co-factors are required for initial contact between ribosomes and mRNA.	3. ATP and a number of protein factors are needed for ribosomes to engage the mRNA.
4. Small ribosomal sub-unit (30S) can engage mRNA before binding of initiator met- $tRNA_f^{met}$ .	4. Small ribosomal subunit (40S) binds stably to mRNA only after initiator met-tRNA has bound.

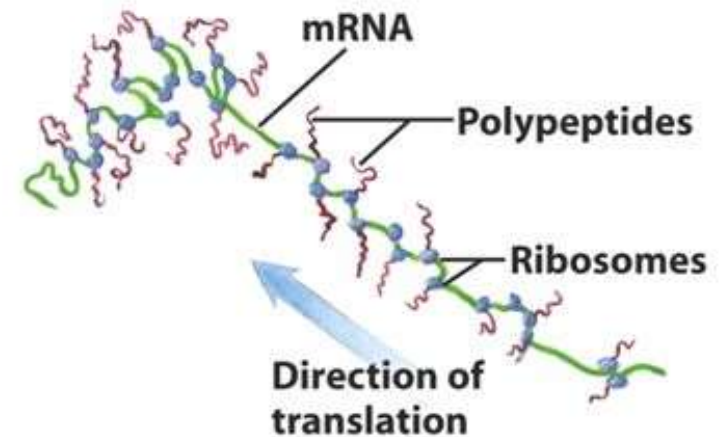
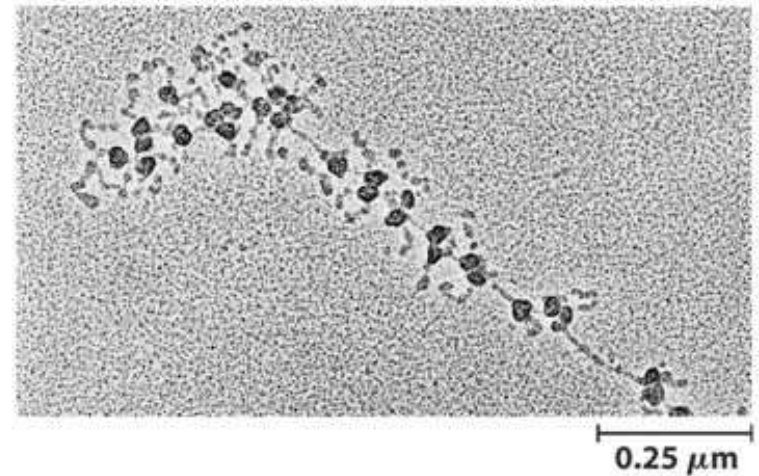


# Polysomes (Poly-ribosomes)

Multiple ribosomes simultaneously translating a single mRNA provides:

- Efficient protein synthesis
  - 10 to 100 ribosomes producing protein from each mRNA
- Stability of mRNA
  - Close spacing of ribosomes protect mRNA from ribonucleases

Polysomes occur in both prokaryotic and eukaryotic translation





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