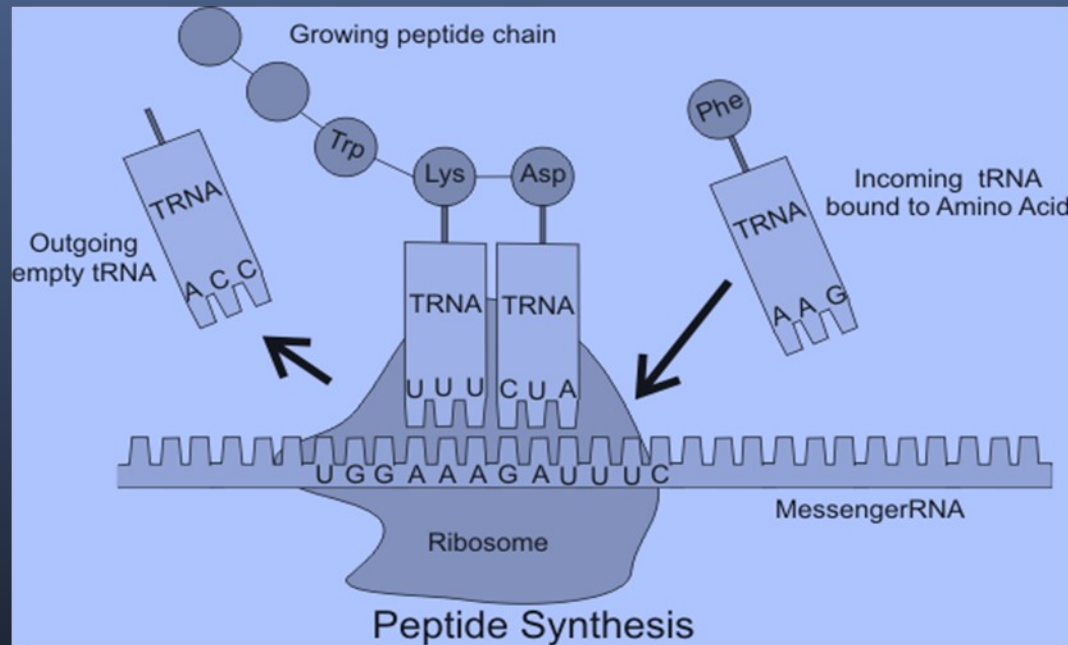


Translation- An overview



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Translation-Introduction

- The pathway of protein synthesis is called **Translation** because the 'language' of the nucleotide sequence on the mRNA is translated into the language of the amino acid sequence.
- The mRNA is translated from its 5' end to its 3' end, producing a protein synthesized from its amino terminal end to its carboxyl terminal end.



Components required for Translation-

- Amino acids
- Transfer RNA
- Messenger RNA
- Aminoacyl t RNA synthetase
- Functionally competent ribosomes
- Protein factors
- ATP and GTP as a source of energy



Steps of Protein Synthesis

The process of protein synthesis is divided into
3 stages-

- Initiation
- Elongation
- Termination

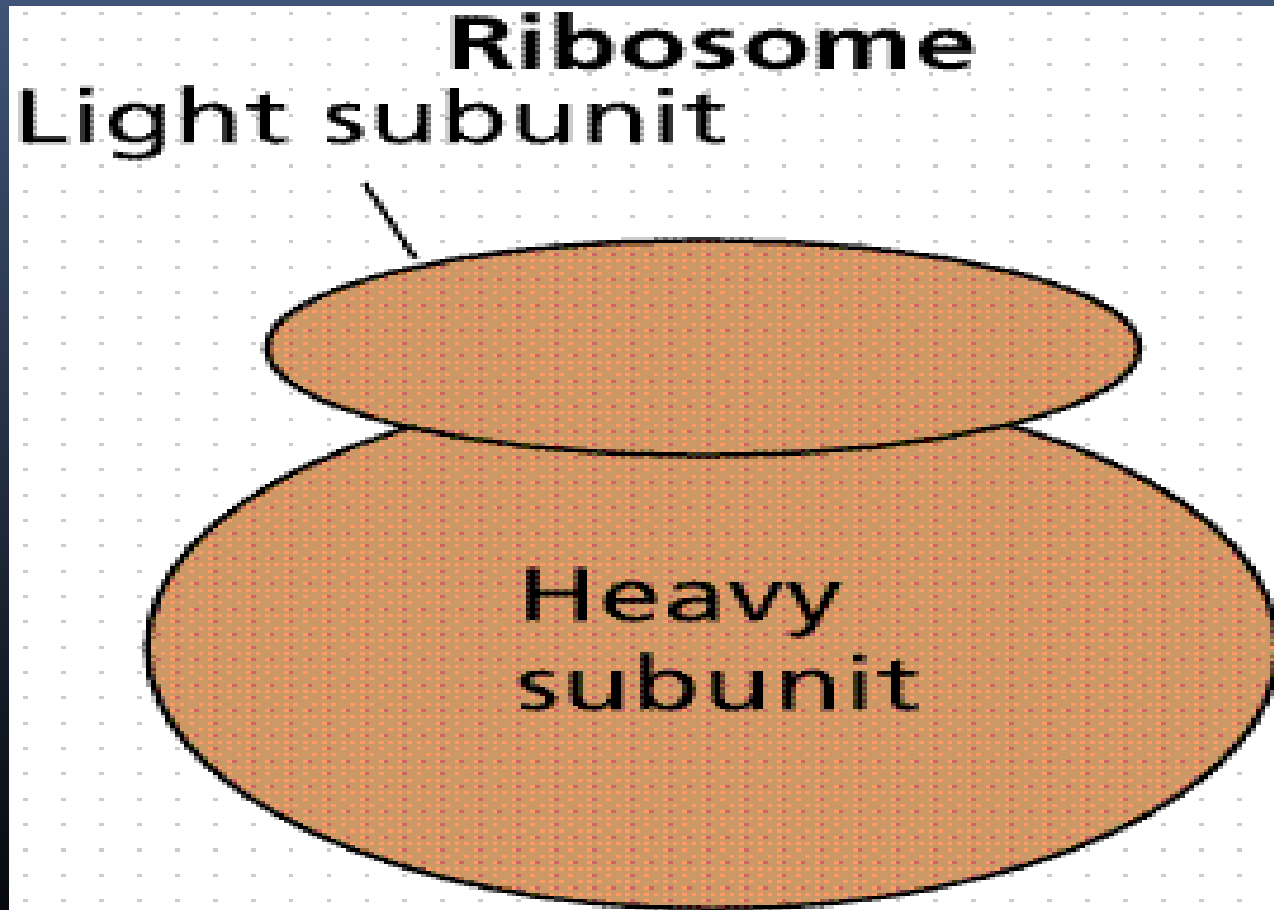
Initiation

- Initiation of the protein synthesis involves the **assembly of the components of the translation** system before the peptide bond formation occurs. These components include-
- Two **ribosomal subunits**
- **m RNA, Aminoacyl tRNA specified by the codons in the message, GTP and initiation factors** that facilitate the assembly of this initiation complex.
- In prokaryotes three initiation factors are known (**IF-1, IF-2 and IF-3**) while in eukaryotes there are at least 9, designated as **e IF**, to indicate the eukaryotic origin.

Ribosomes

- Ribosomes are large **complexes of protein and r RNA**. They consist of two subunits- one large and one small whose relative sizes are generally given in terms of their **sedimentation coefficients or S (Svedberg) values**.
- The S values are determined by the shape as well as by the molecular mass, their numerical values are strictly not additive.
- The **prokaryotic 50S and 30S ribosomal subunits together form a ribosome with an S value of 70**.

Structure of Ribosome



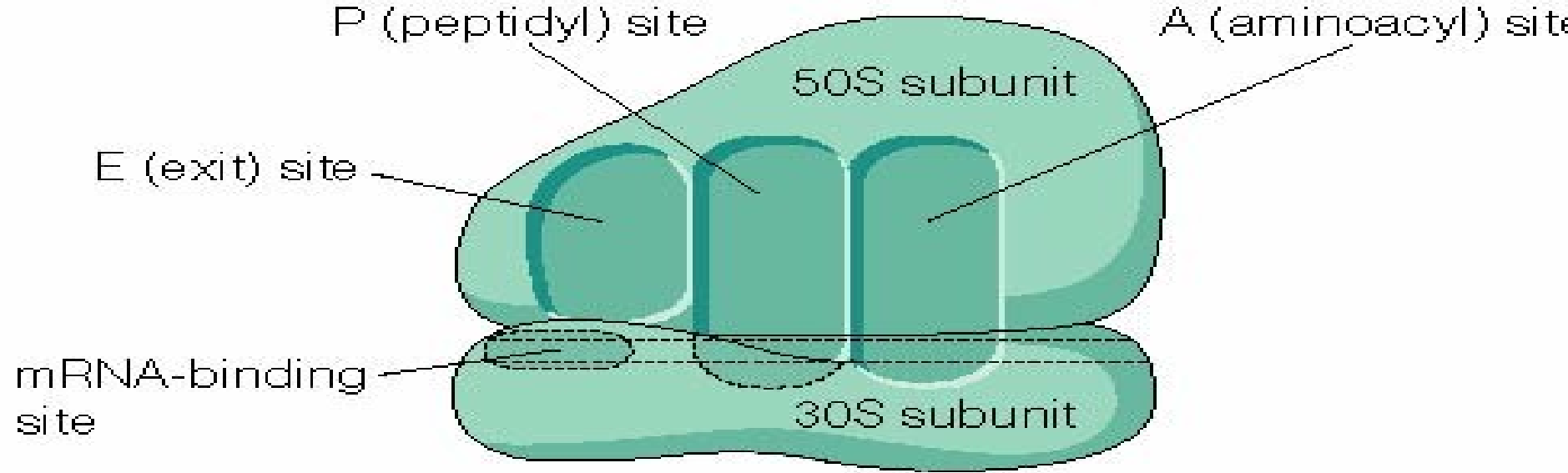
A and P sites on Ribosome

The ribosome has two binding sites for t RNA molecules, each of which extends over both subunits. Together they cover the neighboring codons. During translation,

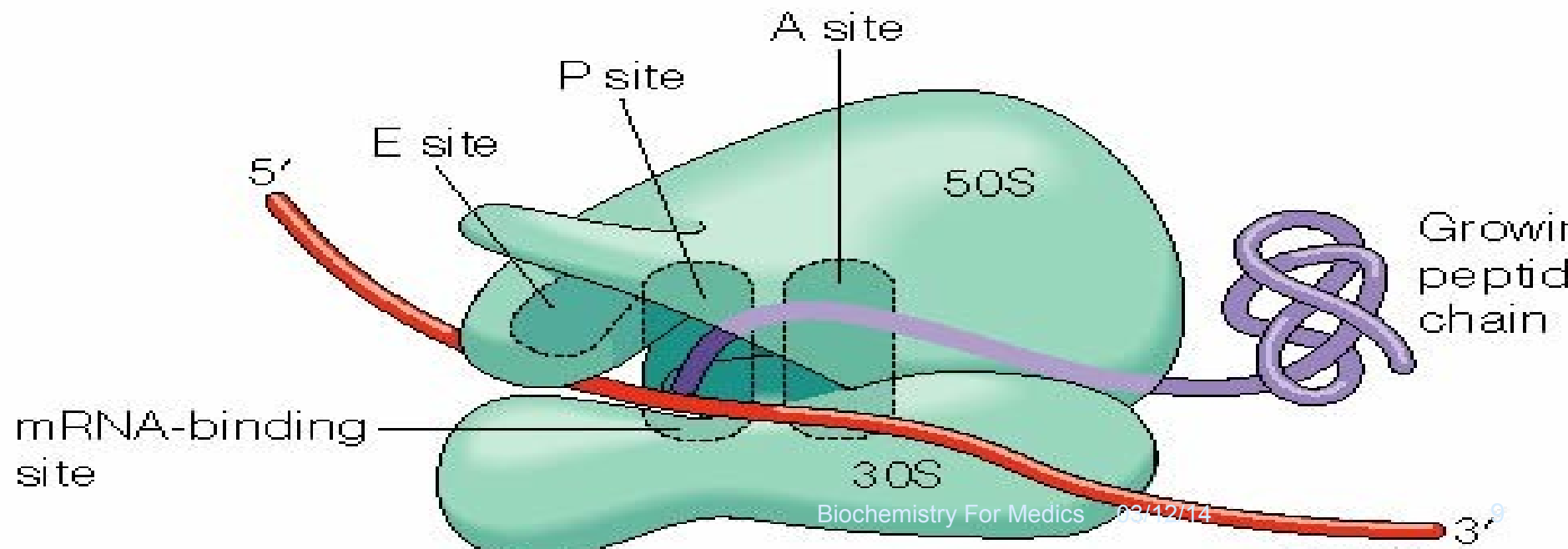
The A site binds an incoming Aminoacyl t RNA as directed by the Codon currently occupying the site. This Codon specifies the next amino acid to be added to the growing peptide chain.

The P site codon is occupied by the peptidyl-t RNA. This t RNA carries the chain of amino acids that has already been synthesized.

An E site is also there that is occupied by the empty t RNA that is about to exit the ribosome



(a)



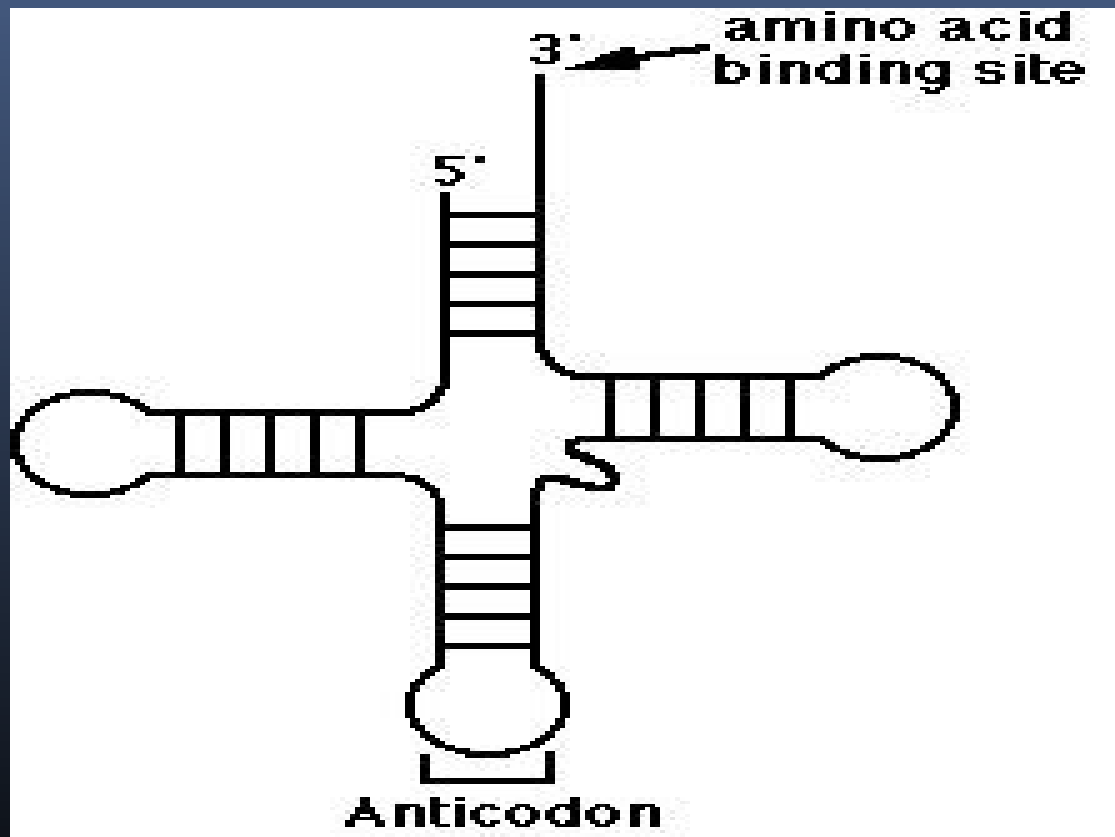
Transfer RNA

At least one specific type of t RNA is required per amino acid.

Two sites are important, one **Amino acid attachment site** the other is **Anticodon site**.

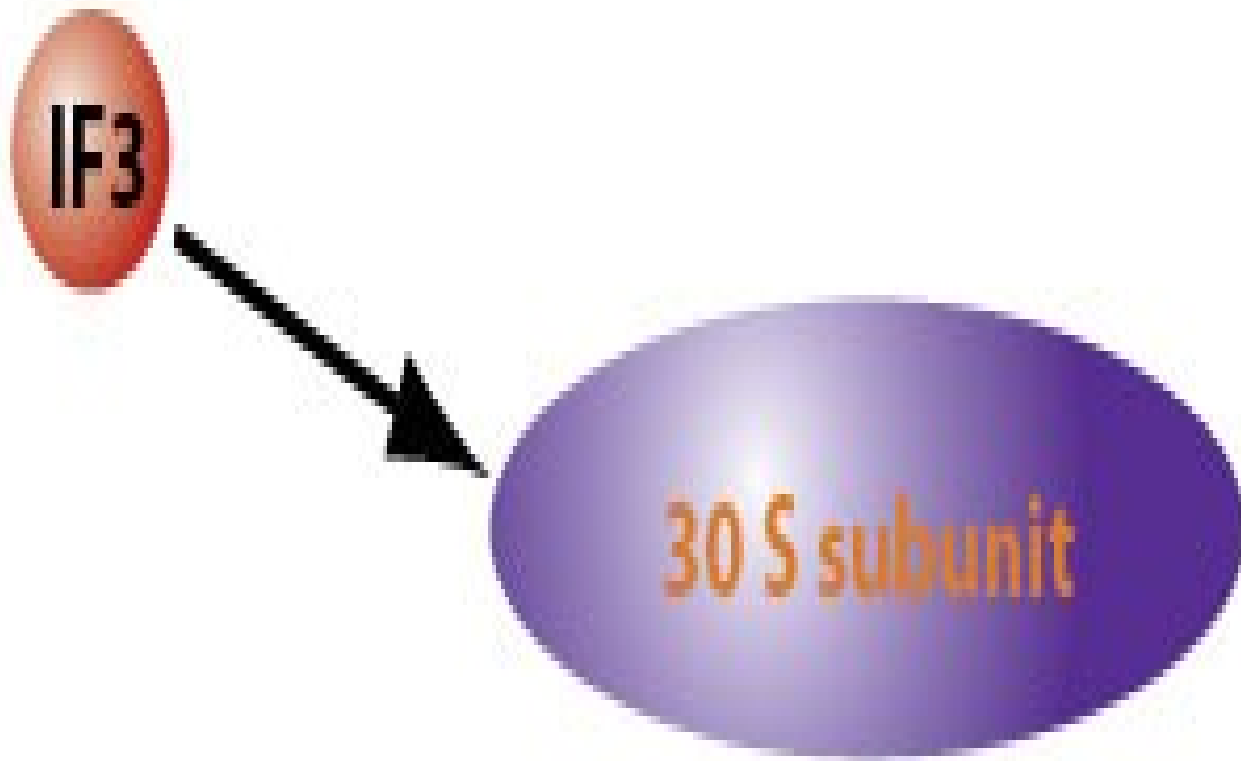
Because of their ability to carry a specific amino acid and to recognize the codons for that amino acid, t RNA are called the **adapter molecules**.

Structure of Transfer RNA



Initiation-

The small ribosomal subunit binds to Initiation Factor 3 (IF3).



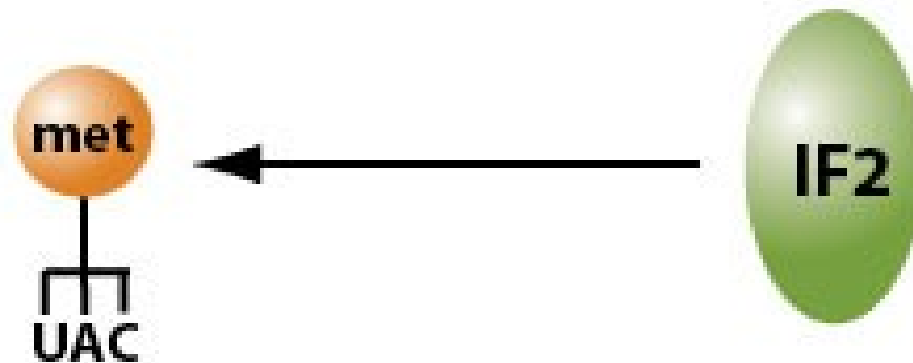
5'-CUAUCGCGCAGGAGGCCUAUGCCAUCGAACUUUUAA-3'

The small subunit/IF3 complex binds to the mRNA. Specifically, it binds to the sequence AGGAGG, known as the **Shine-Delgarno** sequence, which is found in all prokaryotic mRNAs.

5'-CUAUCCGCGCAGGAGGCCUAUGCCAUCGAACUUUUAA-3'



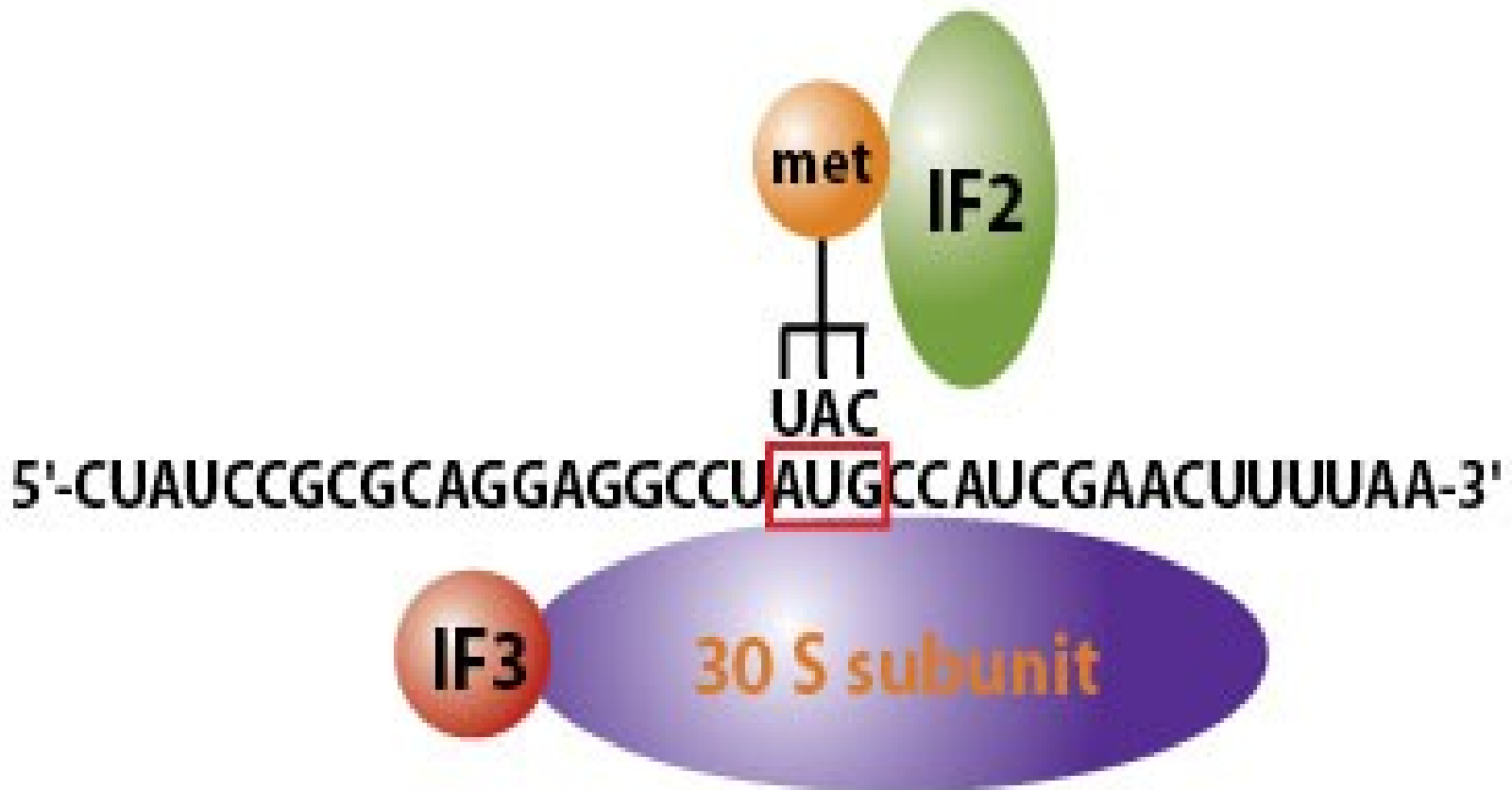
Meanwhile, the fmet tRNA binds to Initiation Factor 2 (IF2), which promotes binding of the tRNA to the start codon.



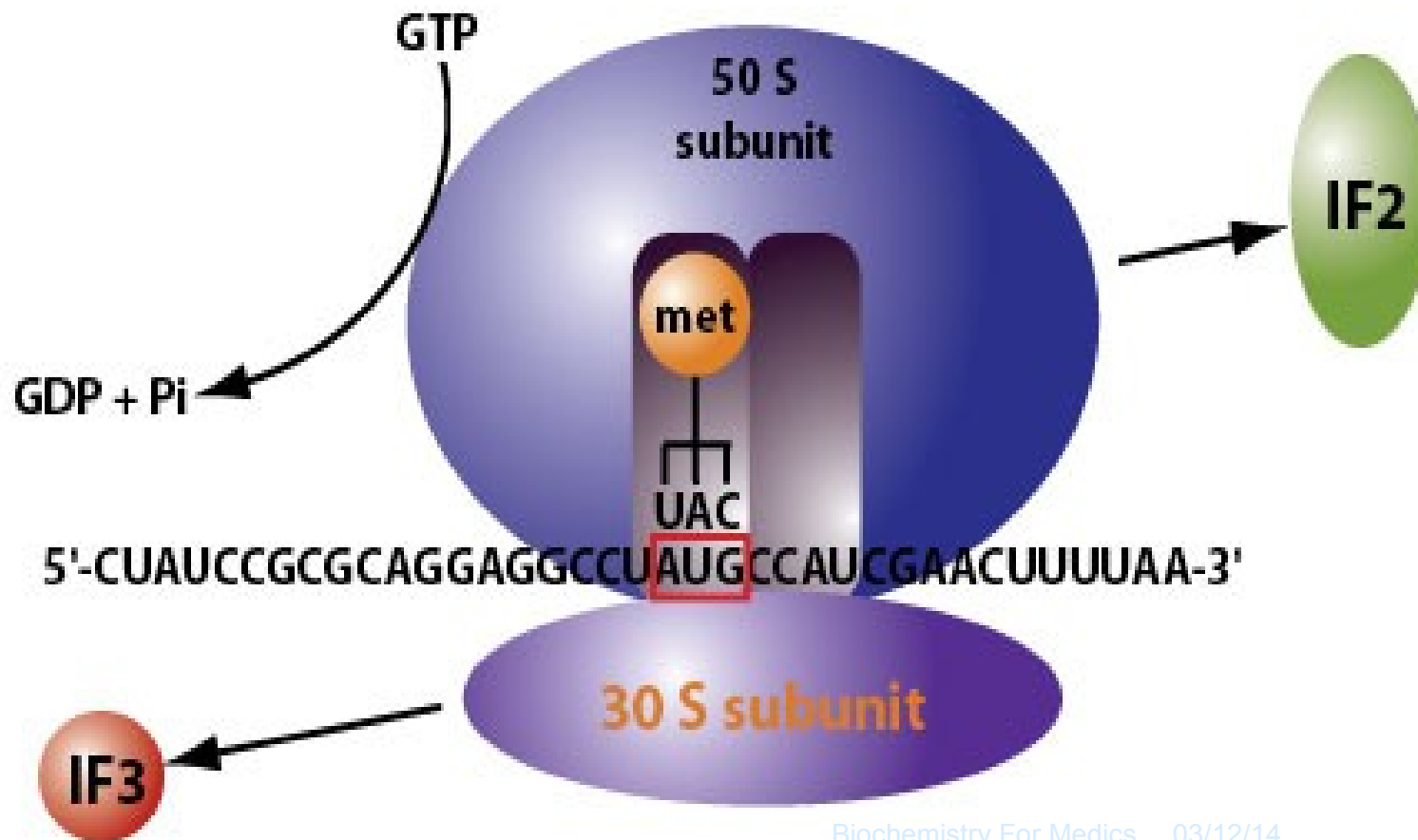
5'-CUAUCCGCGCAGGAGGCCUAUGCCAUCGAACUUUUAA-3'



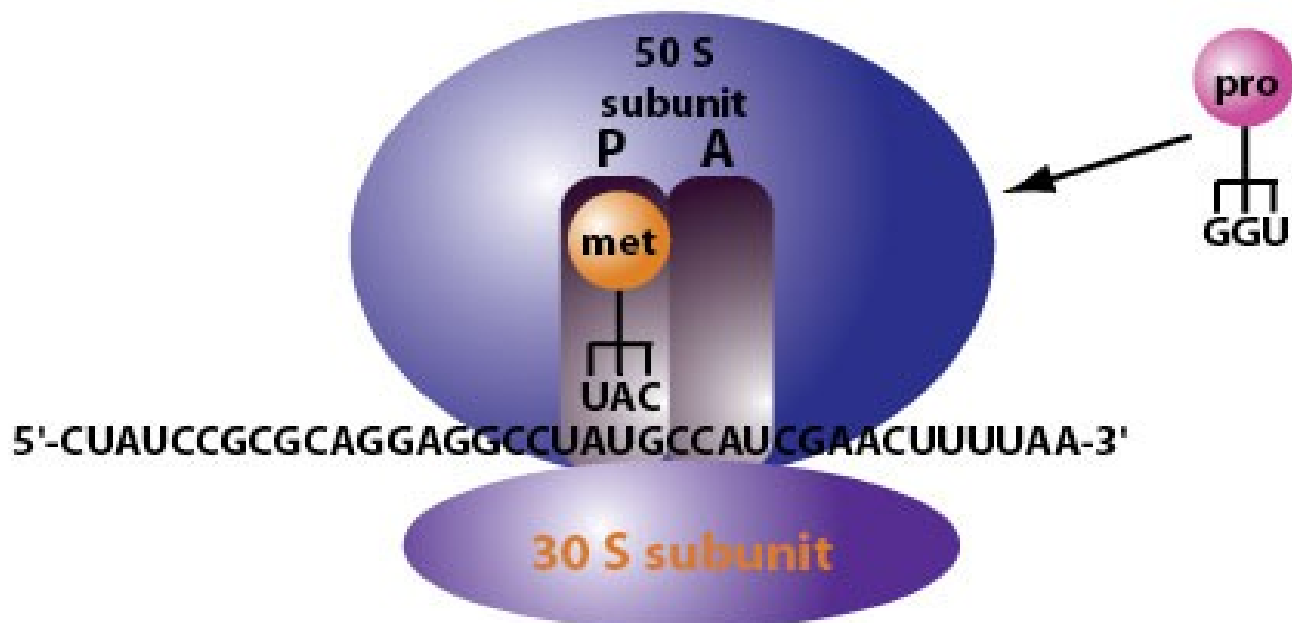
The small subunit/IF3 complex scans along the mRNA until it encounters the start codon. The tRNA/IF2 complex also binds to the start codon. This complex of the small ribosomal subunit, IF3, initiator tRNA, and IF2 is called the initiation complex.



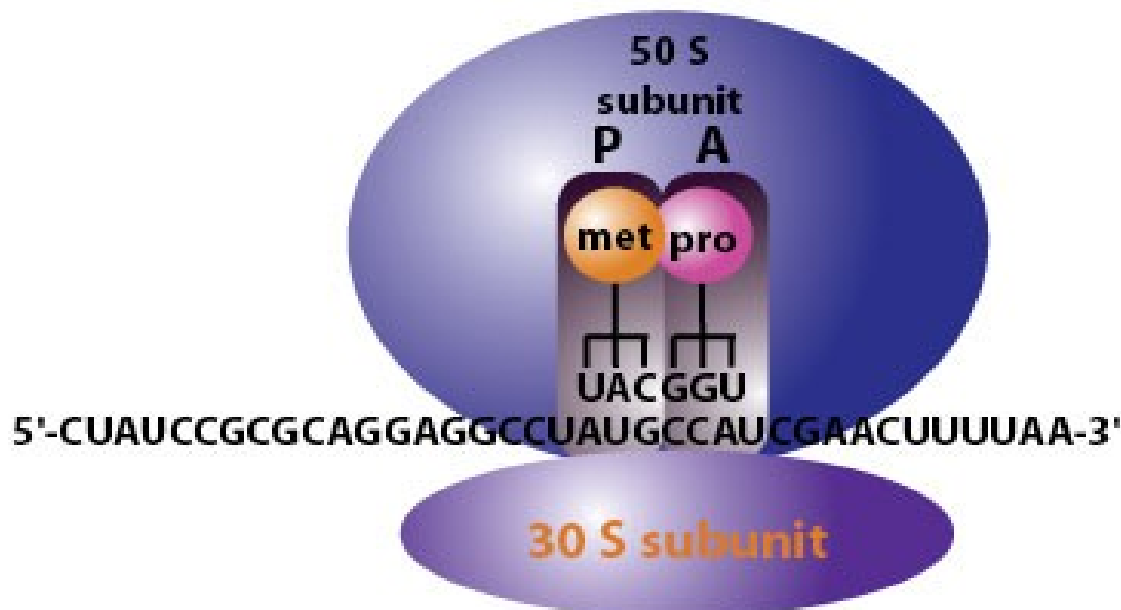
At this point, the large ribosomal subunit joins in. A molecule of GTP is hydrolyzed, and the initiation factors are released. The ribosomal complex is now ready for protein synthesis.



When the ribosome is assembled, two tRNA binding sites are created; these are designated 'P' and 'A' (P stands for peptidyl, A stands for amino acyl). The initiator tRNA is in the P site, and the A site will be filled by the tRNA with the anticodon that is complementary to the codon next to the start. (In this case, it is the tRNA that binds proline.)

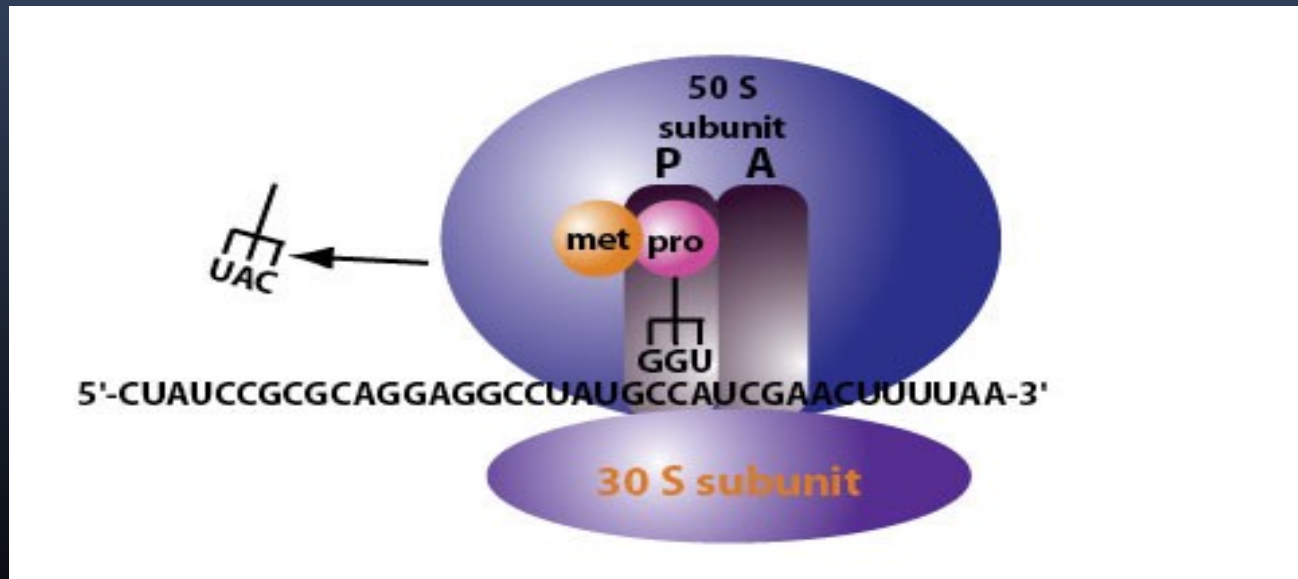


When the second tRNA base pairs with the appropriate codon in the mRNA, an enzyme called **peptidyl transferase** catalyzes the formation of a **peptide bond** between the two amino acids present (while breaking the bond between fmet and its tRNA). This activity is intrinsic to the 23S rRNA found in the large subunit. Since the rRNA catalyzes this process, it is referred to as the Ribozyme

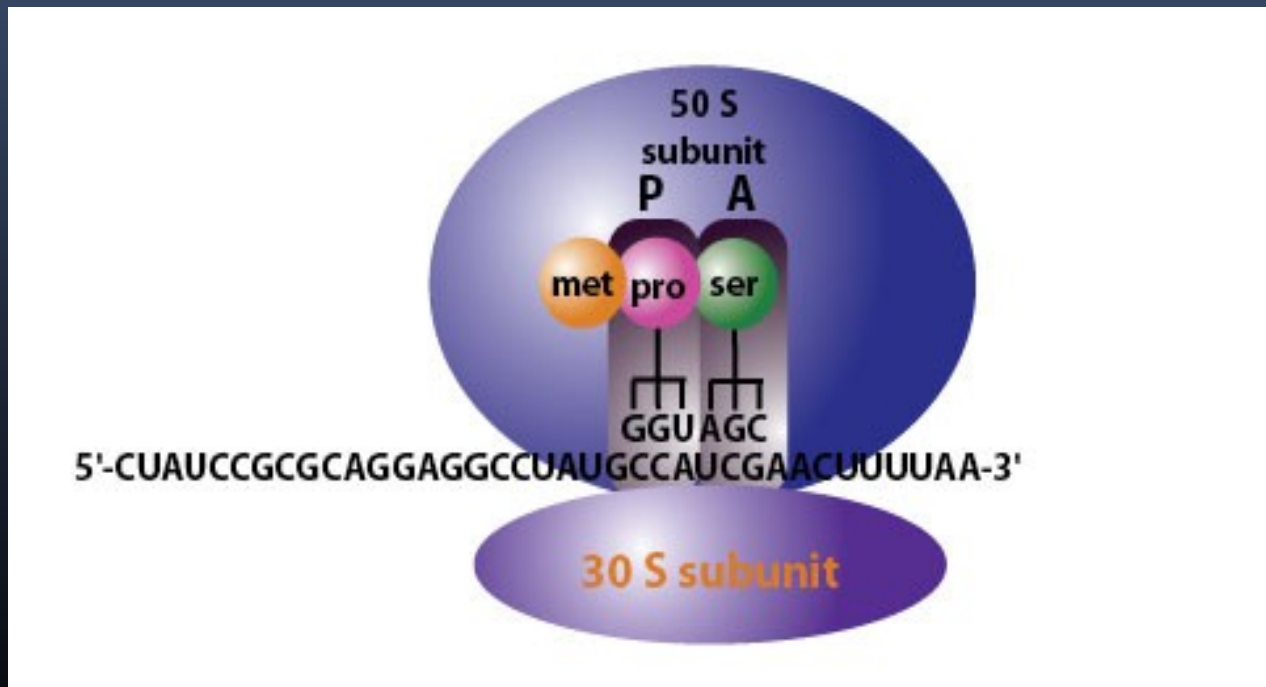


Elongation-

At this point, the whole ribosome shifts over one codon. This shift requires several elongation factors (not shown) and energy from the hydrolysis of GTP. The result of the shift is that the uncharged tRNA that was in the P site is ejected, and the tRNA that was in the A site is now in the P site. The A site is free to accept the tRNA molecule with the appropriate anticodon for the next codon in the mRNA.



The next tRNA base pairs with the next codon, and peptidyl transferase catalyzes the formation of a peptide bond between the new amino acid and the growing peptide chain.



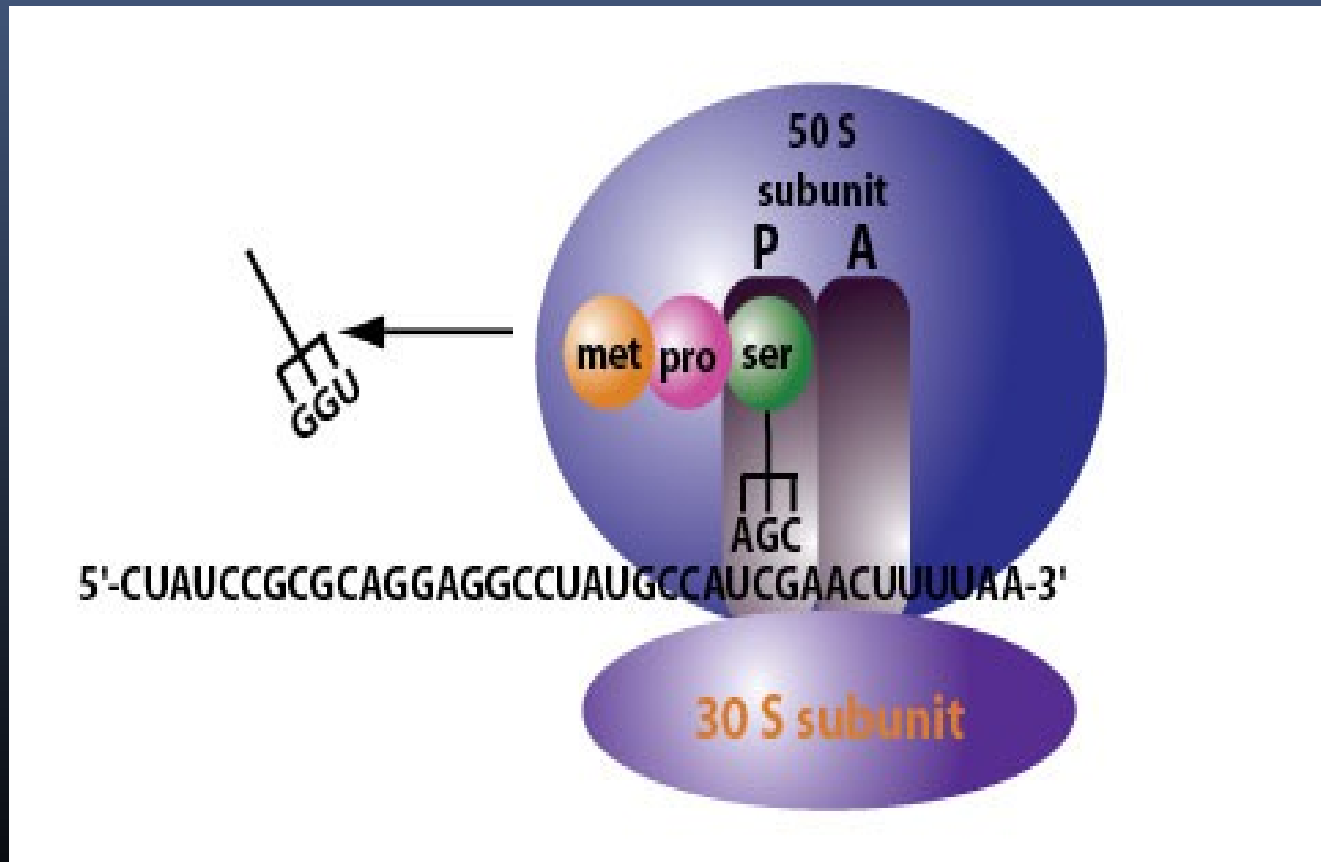
Elongation (Contd.)

Once again, the ribosome shifts over, so that the uncharged tRNA is expelled, and the tRNA with the peptide chain occupies the P site. (This is why this site is called the '**peptidyl**' **site** - after the shift, it contains the tRNA with the growing peptide chain. The other site will accept a tRNA with an amino acid, hence the name '**aminoacyl**' **site**) .

The process of shifting and peptide bond formation continues over and over until a termination codon is encountered. The elongation process is fairly rapid, with prokaryotic ribosomes able to add 15 amino acids to the growing polypeptide every second.

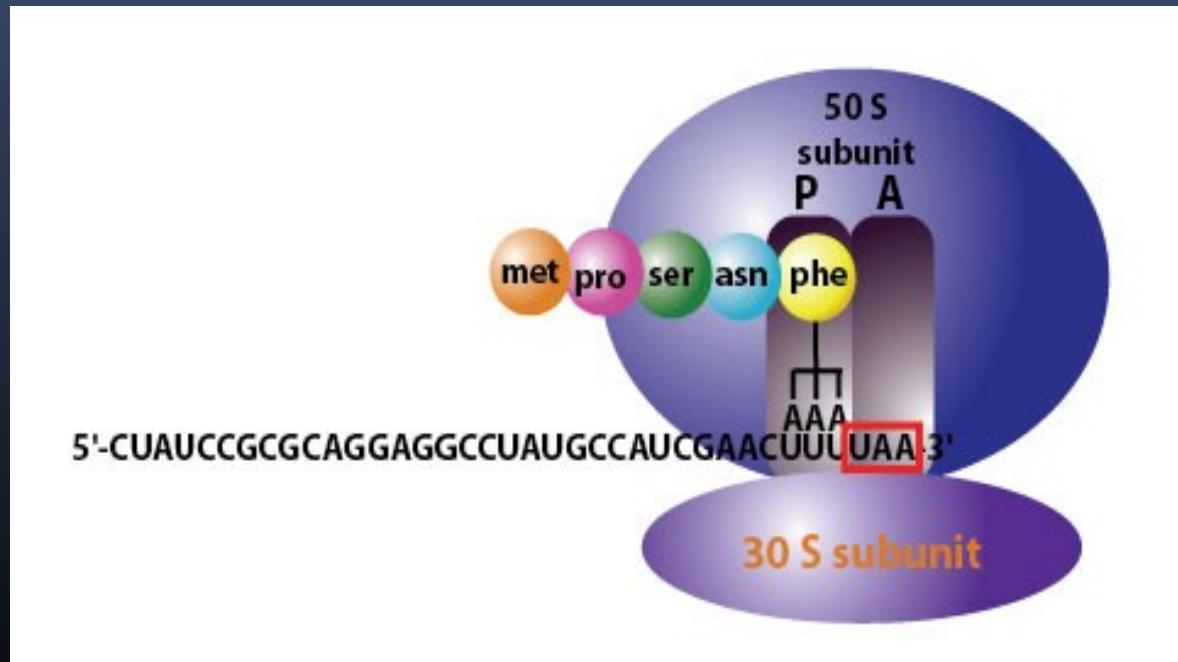
The process is also relatively error-free. Only one mistake is made every 10,000 amino acids. For large proteins of 1000 amino acids, that would mean one wrong amino acid in every 10 polypeptides.


Elongation (Contd.)



Termination-


When a termination codon enters the A site, translation halts. This is because there is no tRNA with an anti codon that is complementary to any of the **stop codons**.





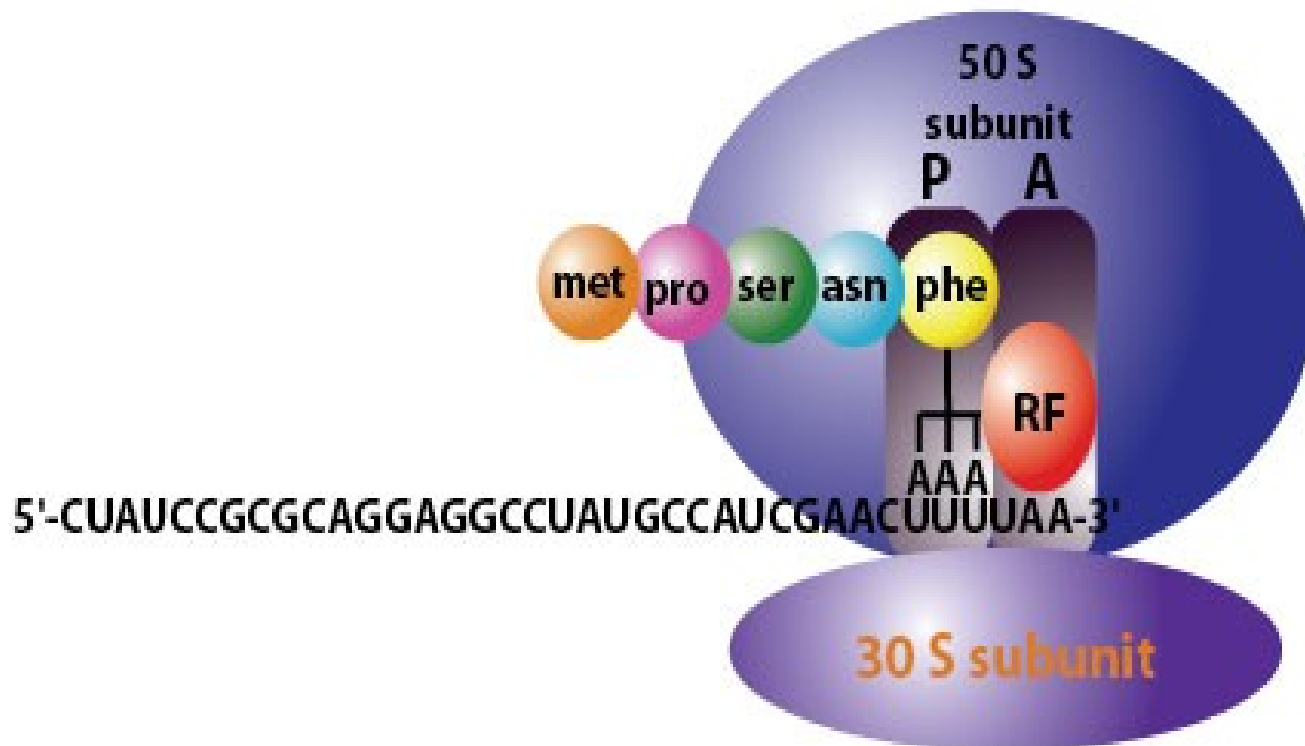
The release factor causes the translation complex to fall apart, and cleaves the polypeptide from the final tRNA. The polypeptide product is now free to function in the cell. The mRNA molecule is now available to be translated again.

Very often, more than one ribosome will translate a single mRNA at the same time. One ribosome will initiate translation, and after it moves down the mRNA a bit, another ribosome will initiate, then another, and so on.



The structure consisting of multiple ribosomes translating a single mRNA molecule is called a **polysome**. Eventually, the mRNA is degraded, and translation of that particular message will cease.

Termination



Eukaryotic Translation

Eukaryotic translation is very similar overall to prokaryotic translation. There are a few notable differences, These include the followings:

Eukaryotic mRNAs do not contain a **Shine-Delgarno** sequence. Instead, ribosomal subunits recognize and bind to the 5' cap of eukaryotic mRNAs. In other words, the 5' cap takes the place of the Shine-Delgarno sequence.

Eukaryotes **do not use formyl methionine** as the first amino acid in every polypeptide; ordinary methionine is used.

Eukaryotes do have a specific initiator tRNA, however. Eukaryotic translation involves many more protein factors than prokaryotic translation (For example, eukaryotic initiation involves at least 10 factors, instead of the 3 in prokaryotes.)

Inhibitors of protein synthesis

- The **tetracyclines** (tetracycline, doxycycline, demeclocycline, minocycline, etc.) block bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodons of the charged tRNAs cannot align properly with the codons of the mRNA.
- **Puromycin** structurally binds to the amino acyl t RNA and becomes incorporated into the growing peptide chain thus causing inhibition of the further elongation.

Inhibitors of protein synthesis

- **Choramphenicol** inhibits prokaryotic peptidyl Transferase
- **Clindamycin and Erythromycin** bind irreversibly to a site on the 50 s subunit of the bacterial ribosome thus inhibit translocation.
- **Diphtheria toxin** inactivates the eukaryotic elongation factors thus prevent translocation.

Post Translational Modifications

The newly synthesized protein is modified to become functionally active. The various post translational modifications are as follows-

-Trimming

-Covalent Alterations

- a) Phosphorylation
- b) Glycosylation
- c) Hydroxylation
- d) Gamma carboxylation
- e) Isoprenylation

-Protein degradation

Post Translational Modifications

Trimming removes excess amino acids.

Phosphorylation may activate or inactivate the protein

Glycosylation targets a protein to become a part of the plasma membrane , or lysosomes or be secreted out of the cell

Hydroxylation such as seen in collagen is required for acquiring the three dimensional structure and for imparting strength

Defective proteins or destined for turn over are marked for destruction by attachment of a **Ubiquitin** protein. Proteins marked in this way are degraded by a cellular component known as the **Proteasome**.

Translation: Summary of Key Points

- Translation is the synthesis of a polypeptide using the information encoded in an mRNA molecule. The process involves mRNA, tRNA, and ribosomes.
- tRNA has a unique structure that exposes an anticodon, which binds to codons in an mRNA, and an opposite end that binds to a specific amino acid. Binding of an amino acid to a tRNA is carried out by an enzyme called aminoacyl tRNA synthetase in a process called charging.
- Translation consists of three basic steps: initiation, elongation, and termination. Initiation involves the formation of the ribosome/mRNA/initiator tRNA complex.
- Elongation is the actual synthesis of the polypeptide chain, by formation of peptide bonds between amino acids.
- Termination dissociates the translation complex and releases the finished polypeptide chain. Each of these steps requires the activity of a specific set of protein factors in addition to the ribosome, tRNA, and mRNA.

Summary of Translation

