

Genetic Code and Translation

GENETIC CODE

- The information needed to direct the synthesis of protein is contained in the mRNA in the form of a **genetic code**.
- The genetic code is the **system of nucleotide** sequences of mRNA that determines the sequence of amino acids in protein.
- Codons are a group of **three adjacent bases** that specify the amino acids of protein.

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

*Termination code

Dictionary of amino acid code words in mRNAs.

Characteristics of Genetic Code

- Universal
- Degenerate
- Unambiguous
- Nonoverlapping and commaless.

The code is degenerate but unambiguous:

- As there are 61 codons for 20 amino acids, one amino acid has more than one codon and the code is referred to as **degenerate**.
- Although an amino acid may have more than one codon, each codon specifies only one amino acid. Thus, the genetic code is unambiguous.

: Degeneracy of the genetic code.

<i>Amino acids</i>	<i>Number of codons</i>	<i>Amino acids</i>	<i>Number of codons</i>
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6

The code is almost universal

Exceptions to the universality of the genetic code are found in human mitochondria

- UGA codes for tryptophan instead of stop codon
- AUA codes for methionine instead of isoleucine
- CUA codes for threonine instead of leucine.

Code is non-overlapping and without punctuation

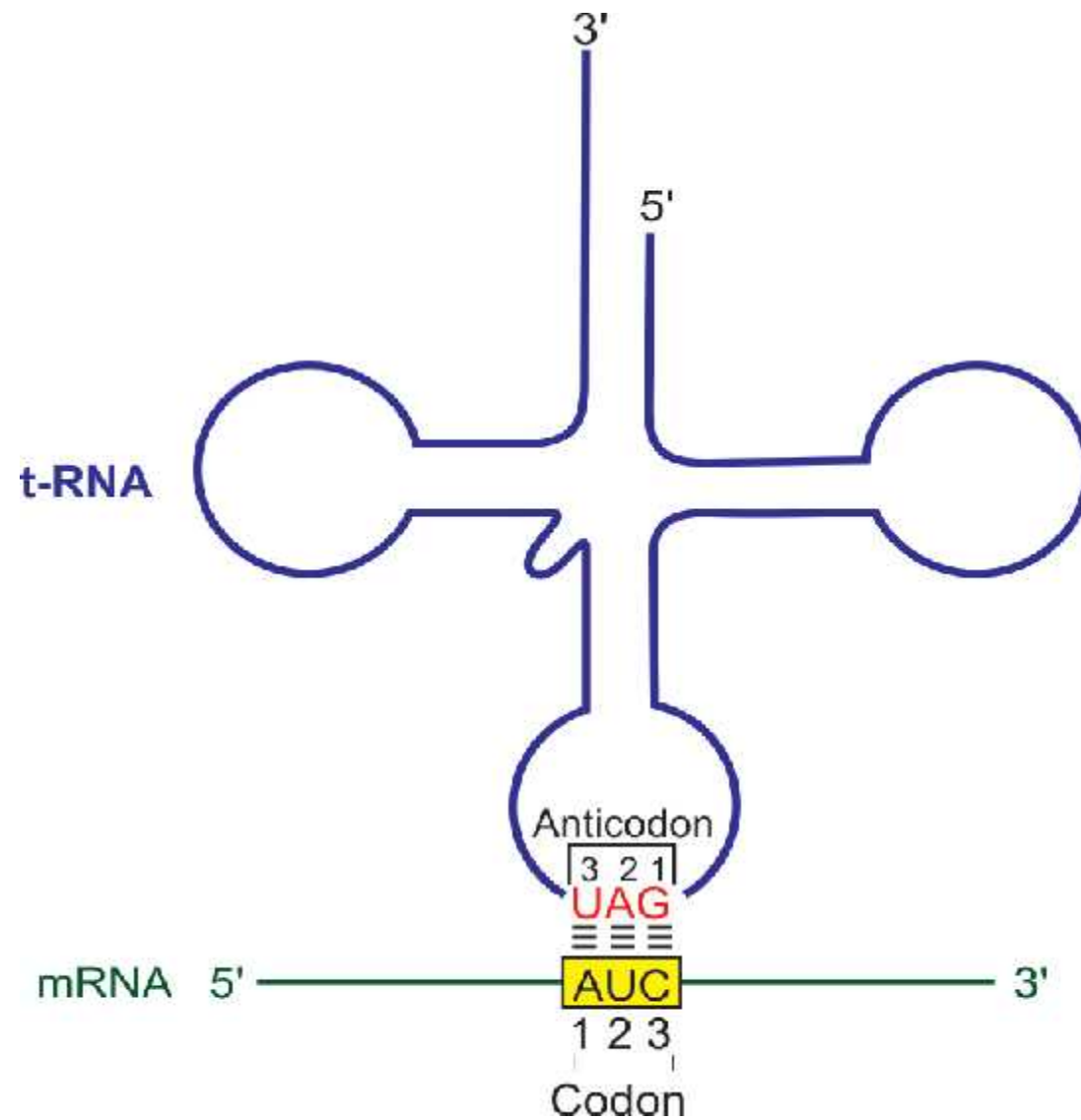
During translation, code is read sequentially,
without spacer bases, taken 3 at a time, e.g.

A U G C U A G A C U U U is read as:

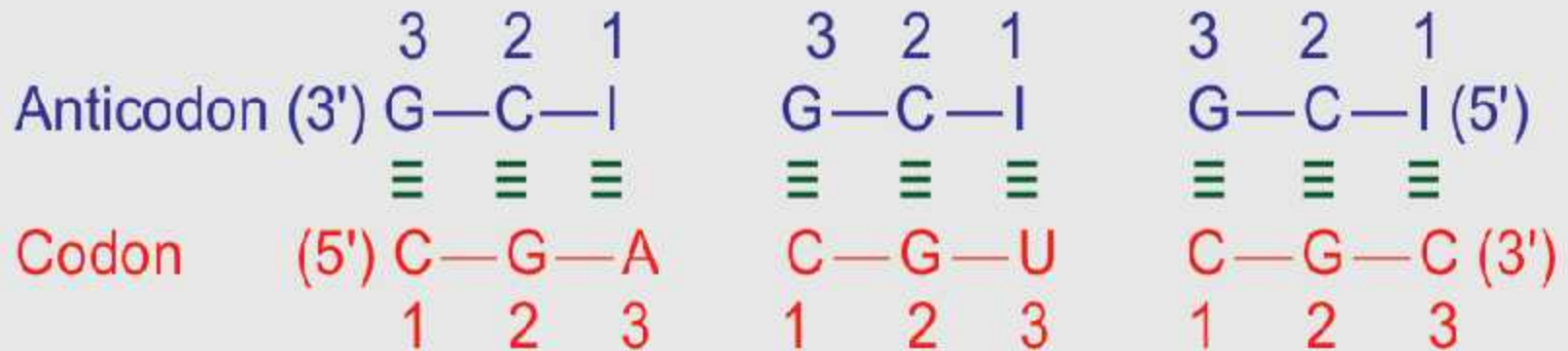
AUG / CUA / GAC / UUU

Wobble Hypothesis for Codon-anticodon Interactions

- The third base of most codons pairs rather loosely with the corresponding base of its anticodon, the third base of such codons (and the first base of their corresponding anticodons) “wobbles”.
- Crick proposed an assumption to explain these data on the basis of set of four relationships called the “**wobble hypothesis**”.



Pairing relationship of codon and anticodon.



Three different codon pairing of arginine is possible when the tRNA anticodon contains inosine (I).

TRANSLATION

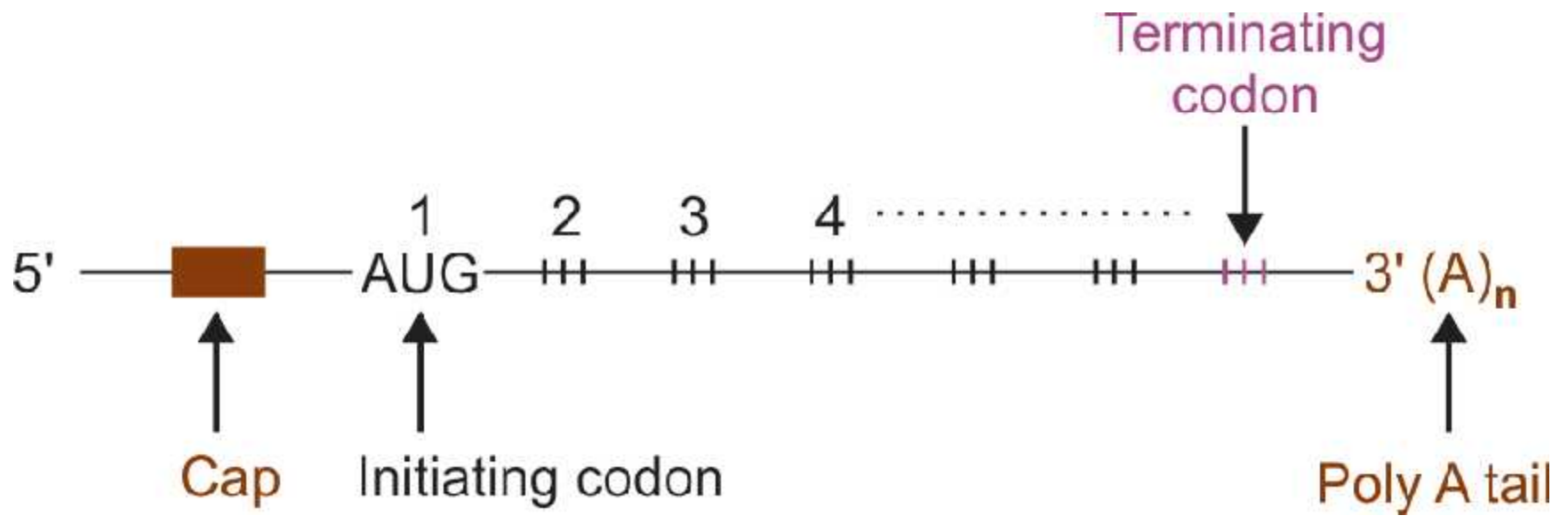
(PROTEIN BIOSYNTHESIS)

- The pathway of protein Biosynthesis is called *translation*.
- Translation is the process by which ribosomes convert the information carried by mRNA in the form of genetic code to the synthesis of new protein.

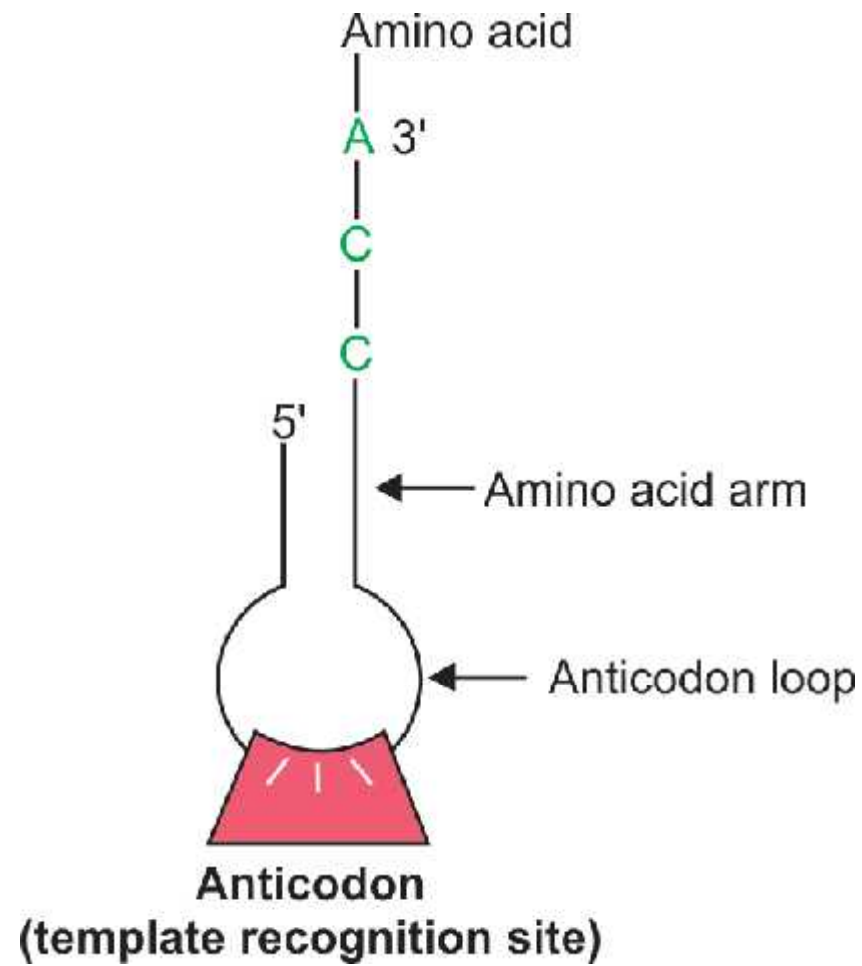
- Translation occurs in **cytosol on ribosomes** and is guided by mRNA.
- Basic plan of protein synthesis in eukaryotes is similar to that in prokaryotes.

Basic Requirements for the Translation

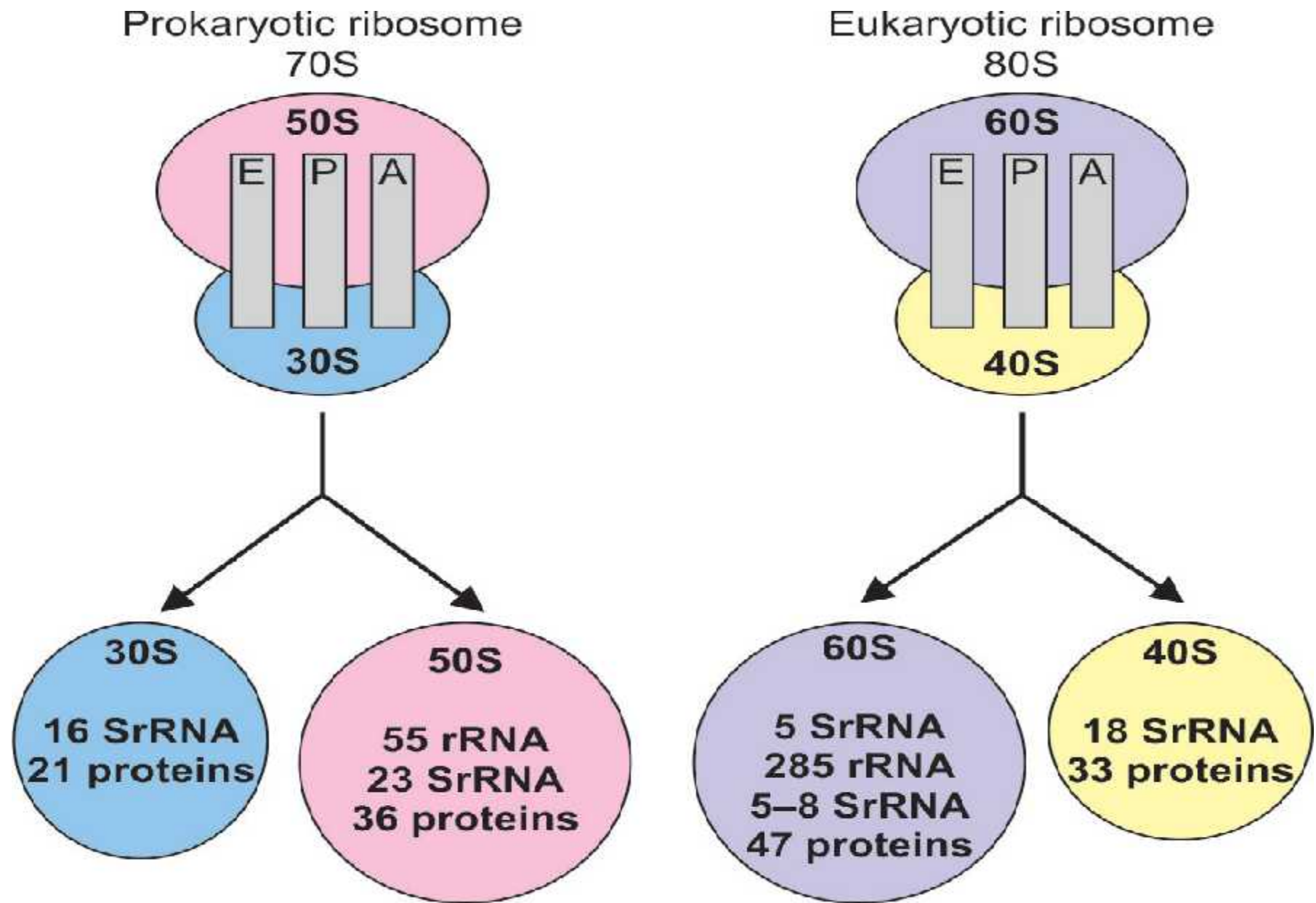
- mRNA
- tRNAs
- Ribosomes
- Energy in the form of ATP and GTP
- Enzymes and specific protein factors.



Symbolic diagram of mRNA.



Symbolic diagram of an aminoacyl-tRNA.



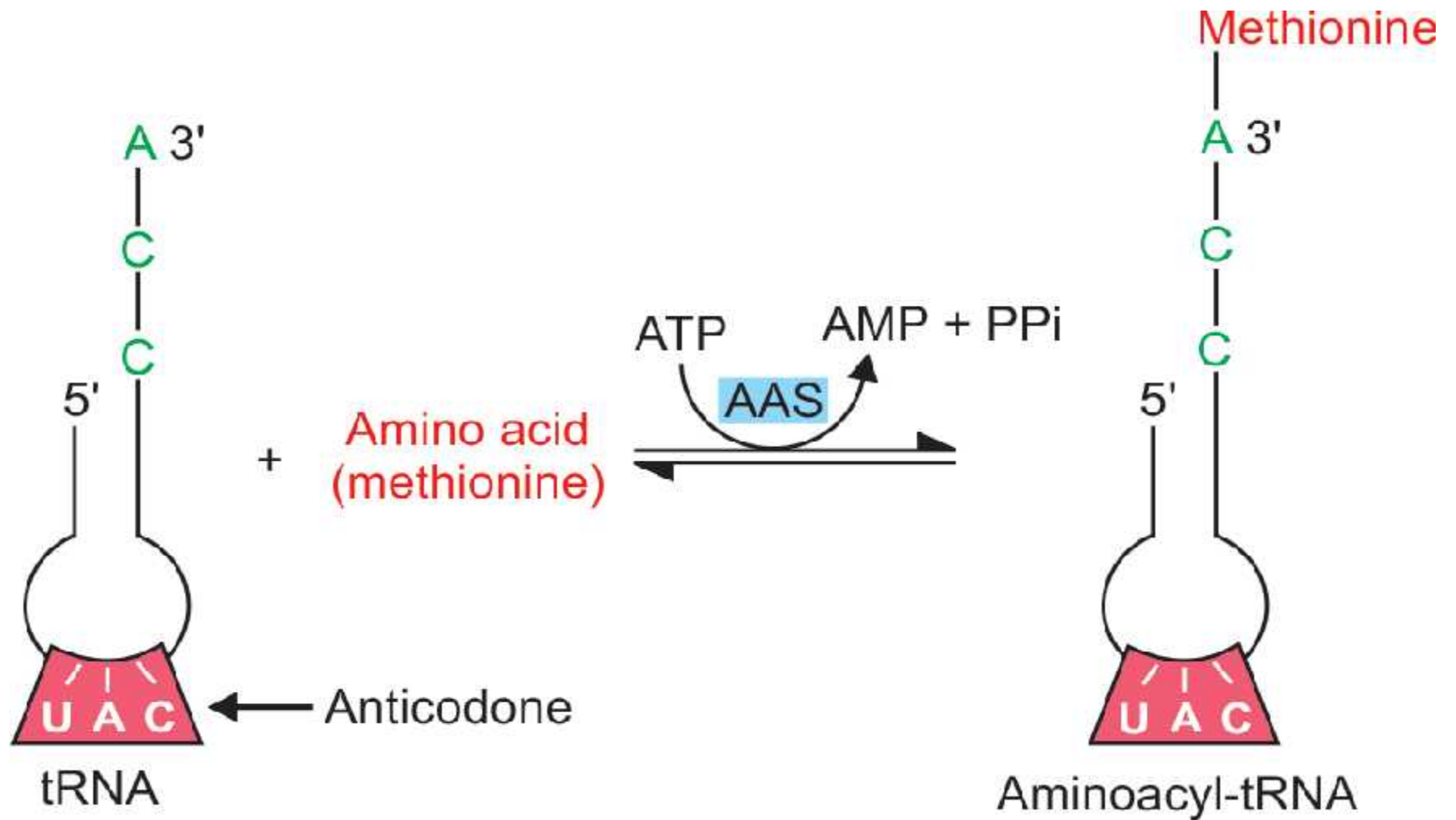
Ribosomes of prokaryotes and eukaryotes.

Stages of Eukaryotic Translation

1. Activation of amino acids
2. Initiation
3. Elongation
4. Termination

Activation of Amino Acid

- Activation of amino acid takes place in the cytosol.
- In activation of amino acid each of the 20 amino acids, is covalently attached to their respective tRNA, at the expense of ATP, by **aminoacyl tRNA synthases (AAS)** enzyme

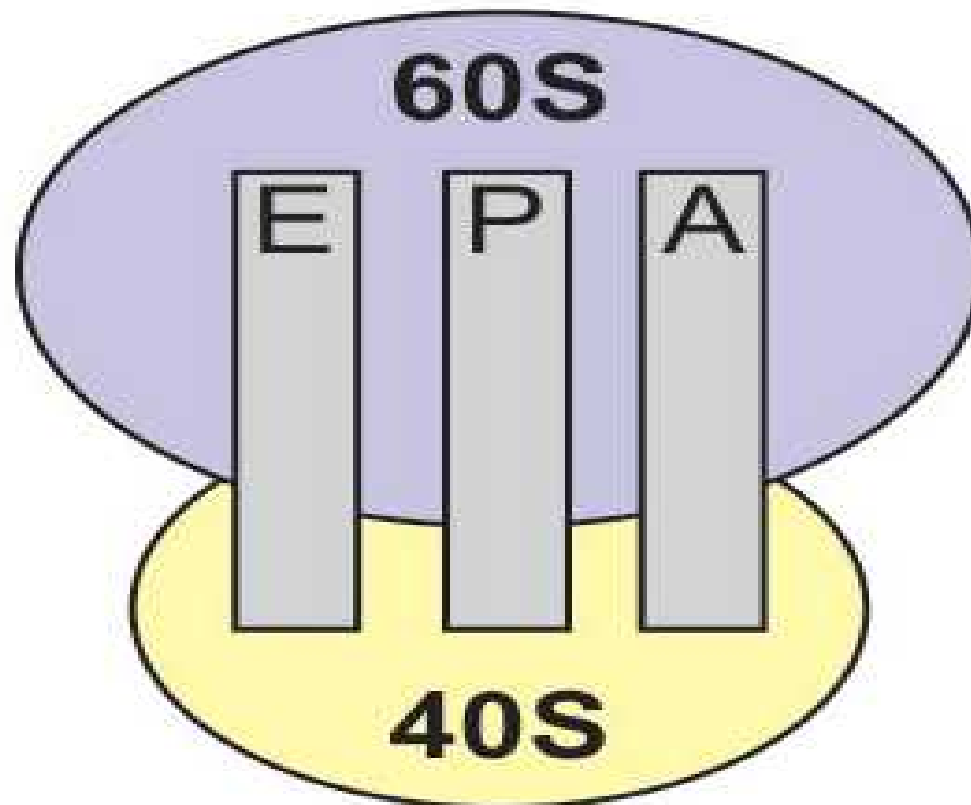


Activation of amino acid.
(AAS: aminoacyl-tRNA synthetase)

Initiation

1. Ribosomal dissociation
2. Formation of 43S pre-initiation complex.
3. Formation of 48S initiation complex
4. Formation of 80S initiation complex.

Eukaryotic ribosome 80S



Ribosomes have three sites:

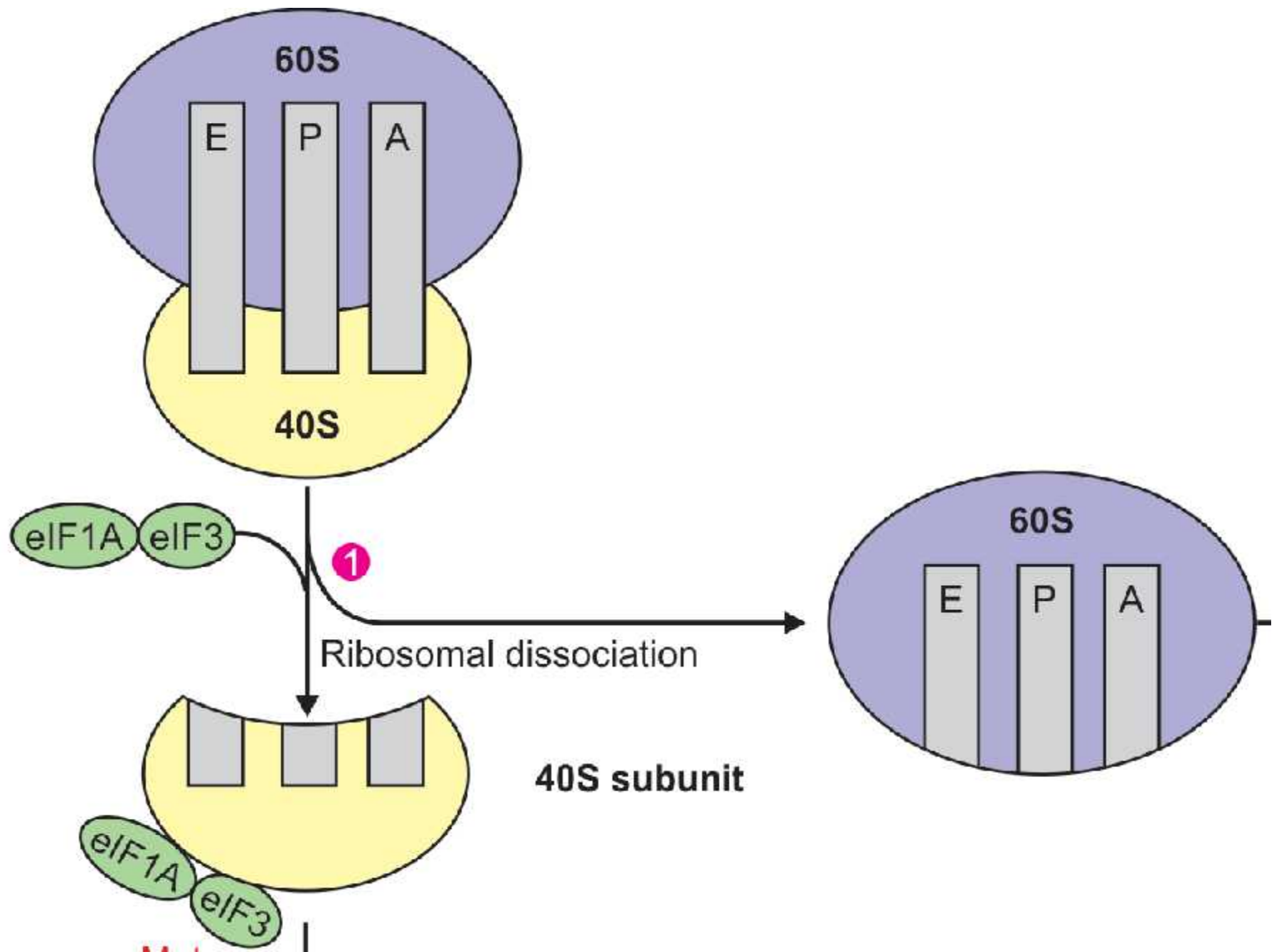
1. Aminoacyl site (A site)

2. Peptidyl (P) site

3. Exit (E) site.

- A and P sites bind to aminoacyl–tRNAs,
- E site binds only to uncharged tRNAs

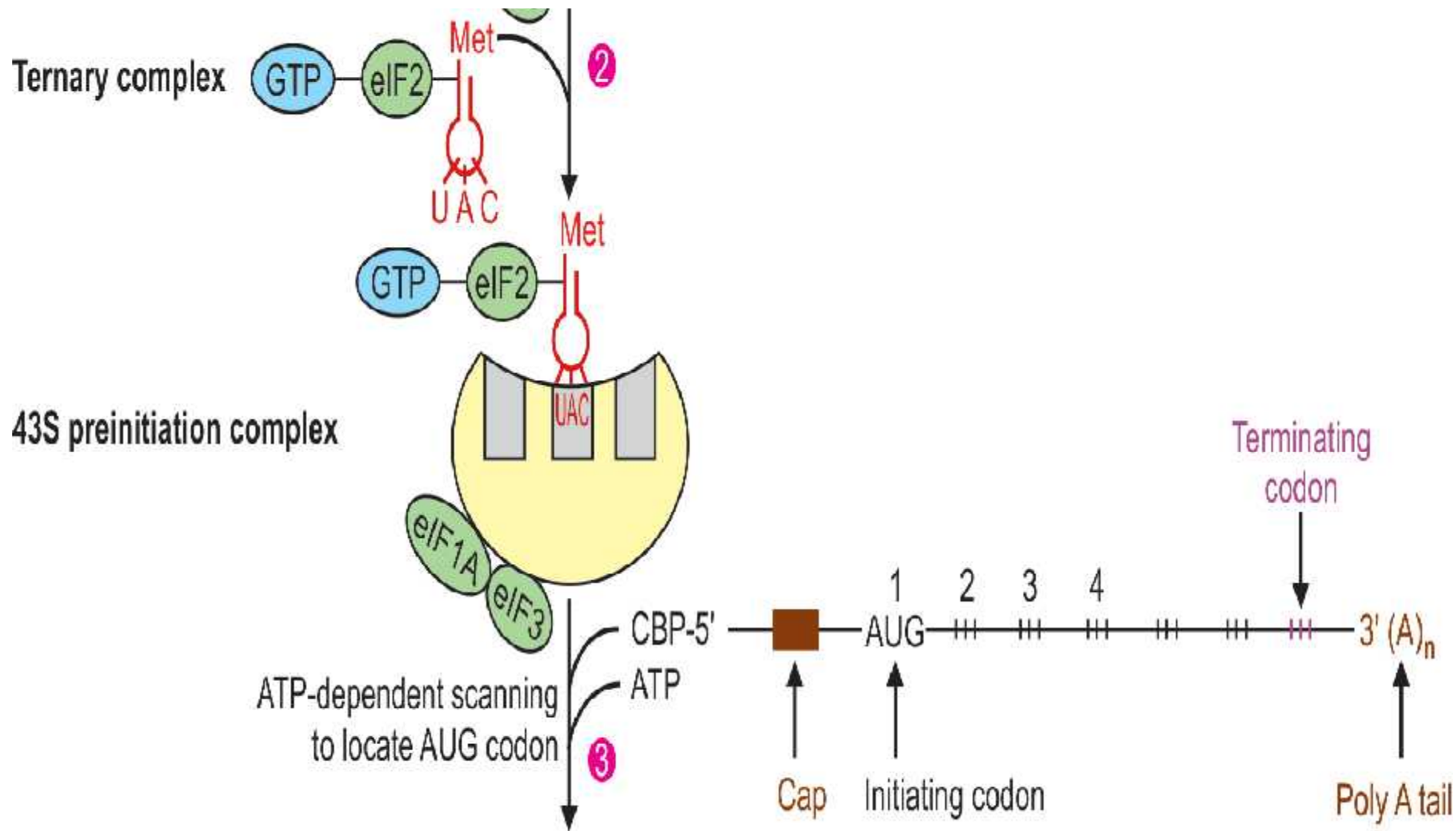
40S subunit of ribosome binds two initiation factors eIF3 and eIF-1A and dissociates 80S into two subunits 40S and 60S



Formation of 43S pre-initiation complex

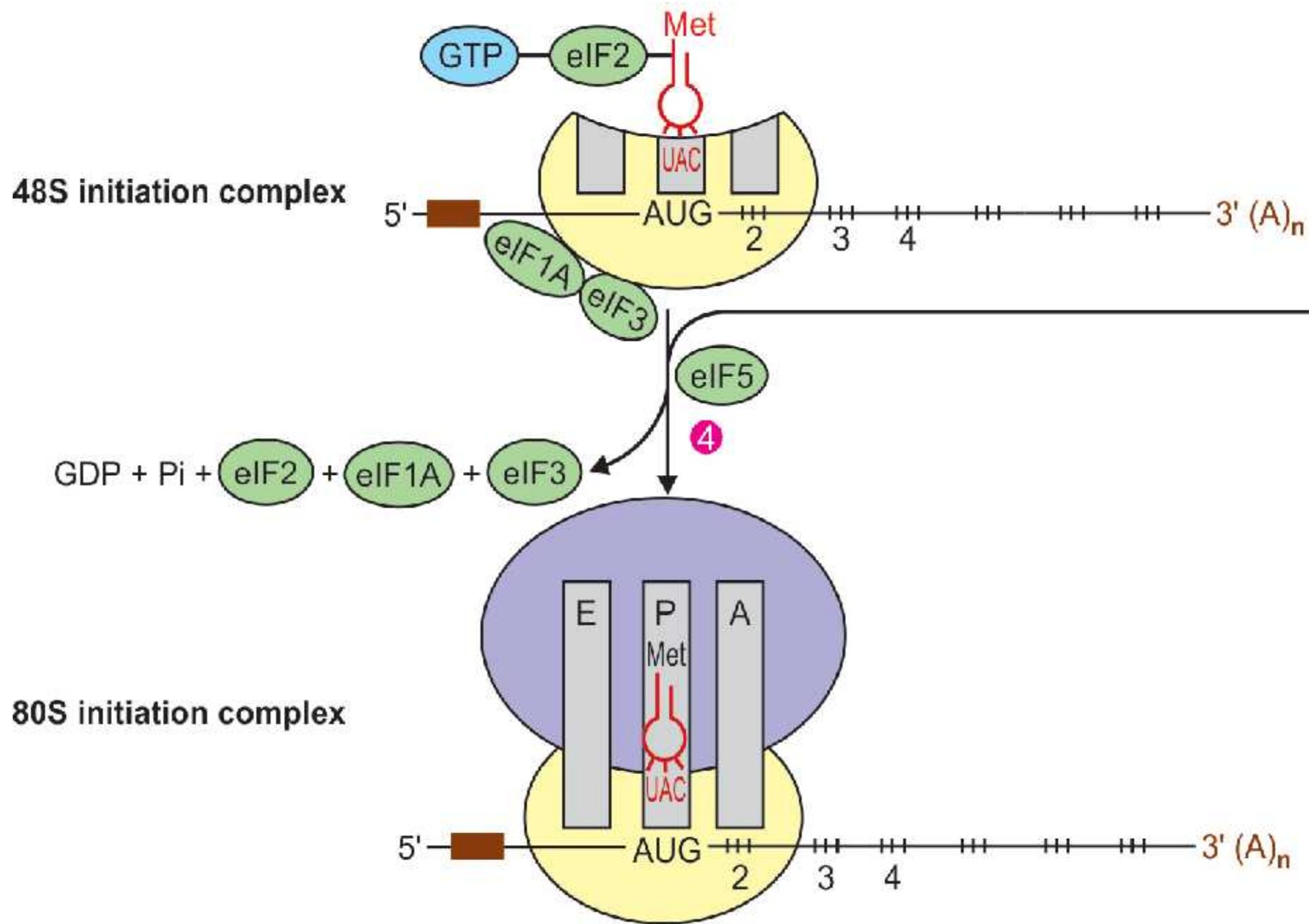
- Binding of GTP with initiation factors eIF2.
- GTP-eIF2 binds to Met-tRNA^{Met}
- GTP-eIF2-tRNA a ternary complex binds to 40S ribosomal subunit to form 43S pre-initiation complex
- 43S pre-initiation complex is stabilized by association with eIF3 and eIF-1A

In prokaryotes, the first amino acid methionine is modified by formylation of its amino group to N-formyl-methionine (f Met) and initiator tRNA is fmet-tRNA^{fMet}.



Formation of 48S initiation complex

- Binding of mRNA to the 43S pre-initiation complex forms 48S initiation complex.
- The association of mRNA with 43S initiation complex requires cap binding protein (CBP) or eIF4 and ATP



Formation of 80S initiation complex

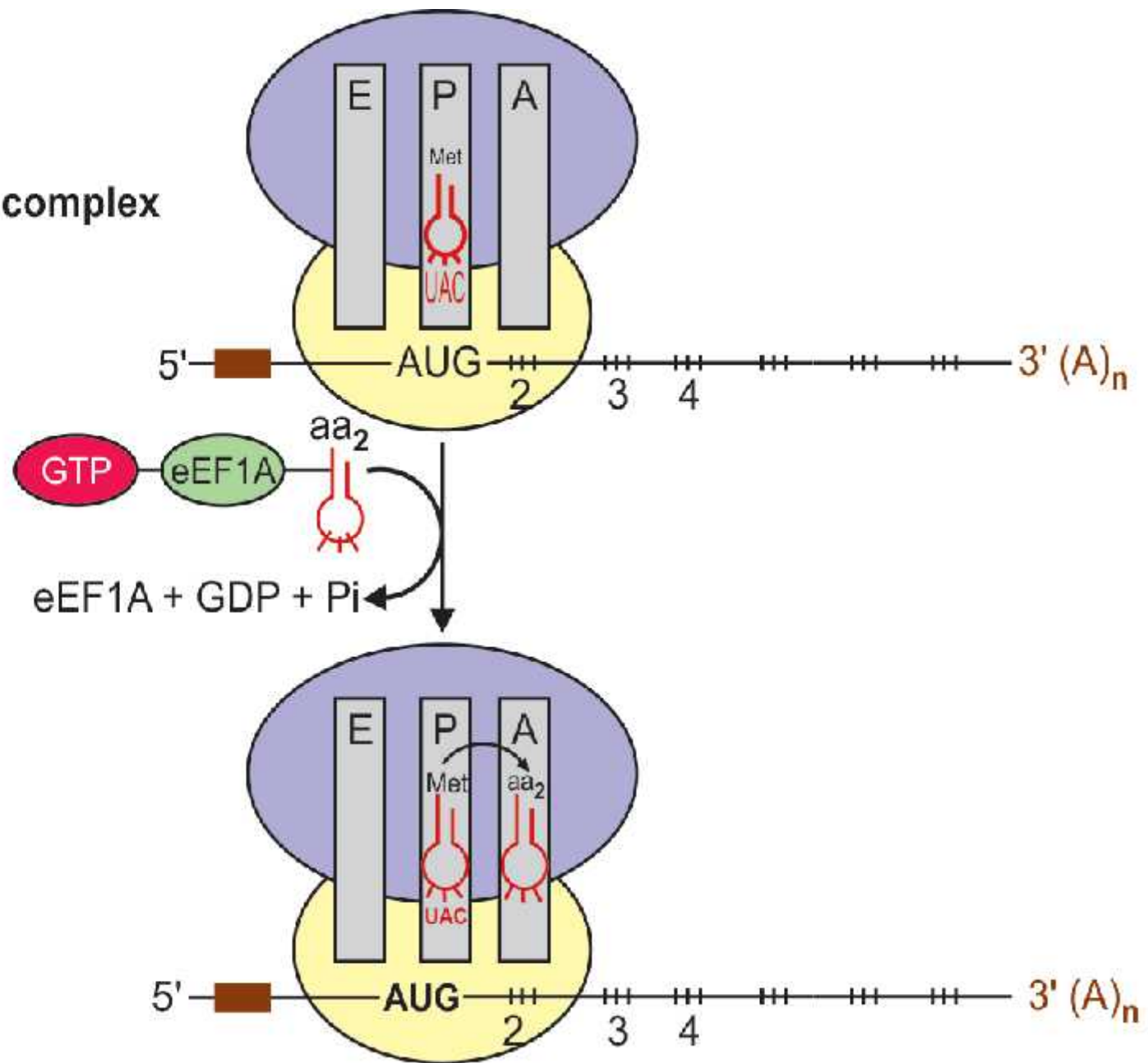
- Combination of the 48S initiation complex with 60S ribosomal subunit forms 80S initiation complex.
- Hydrolysis of the GTP bound to eIF2, by eIF5
- Release of the initiation factors bound to the 48S initiation complex.
- At this stage, the Met-tRNA^{Met}, is on the P site of the ribosome so that its anticodon pairs with the initiating AUG codon on the mRNA and is now ready for the elongation process

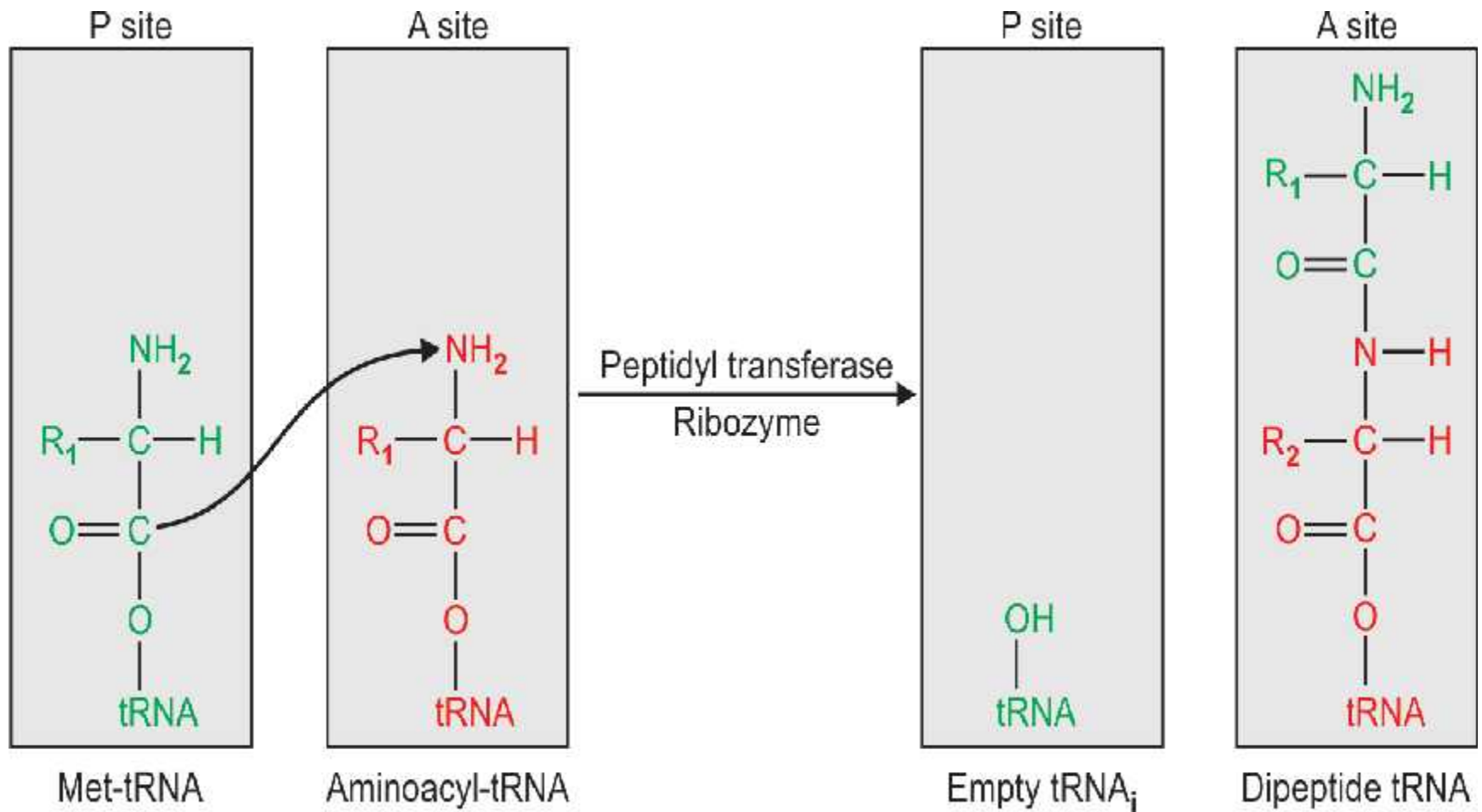
Elongation

Elongation can be divided into following steps

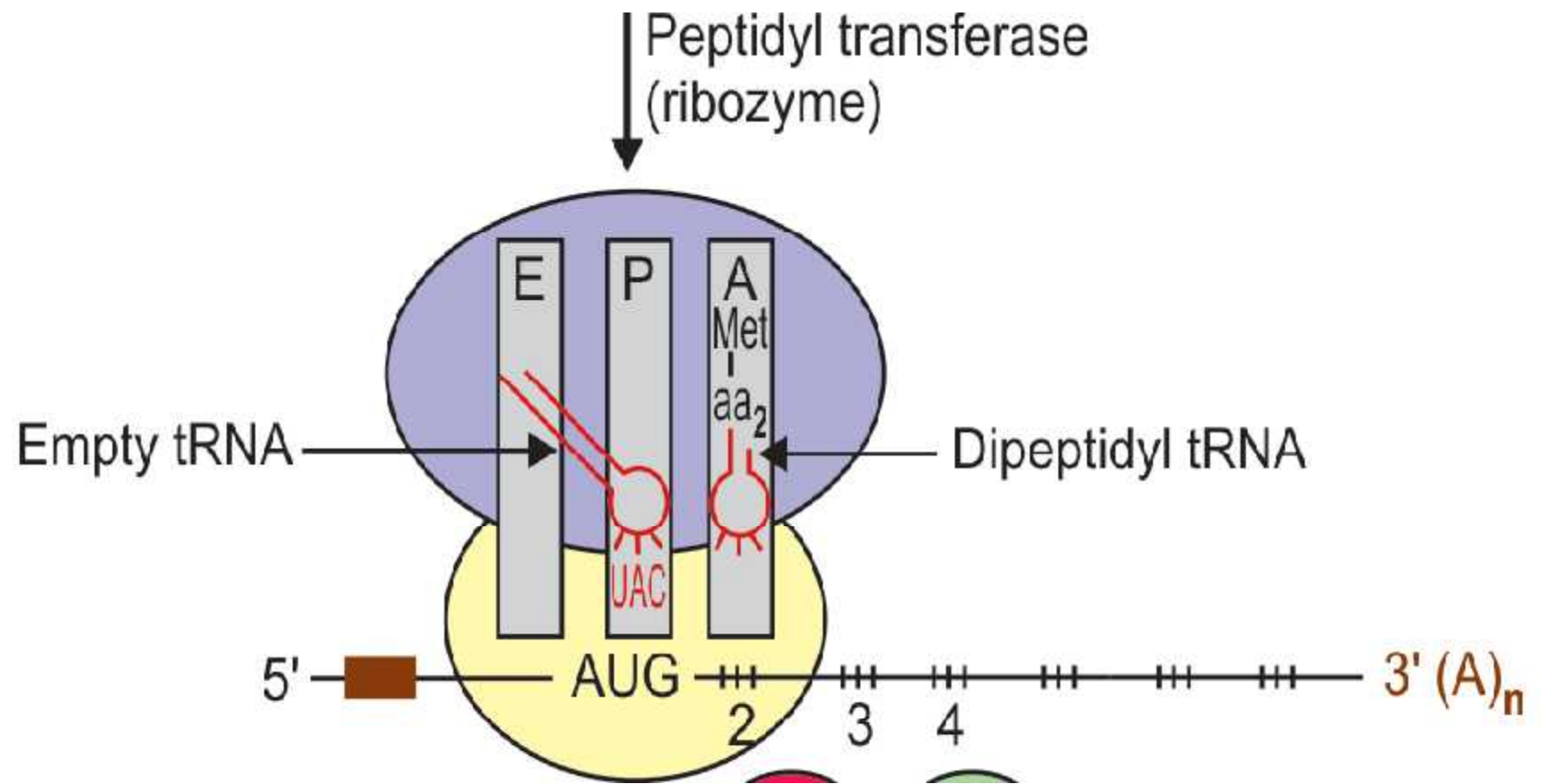
1. Binding of aminoacyl-tRNA specified by the next codon in the mRNA to the A site
2. Formation of peptide bond between amino acids present in P and A site of ribosome
3. Translocation of the ribosome along the mRNA
4. Elimination of deacylated tRNA from the P and E sites.

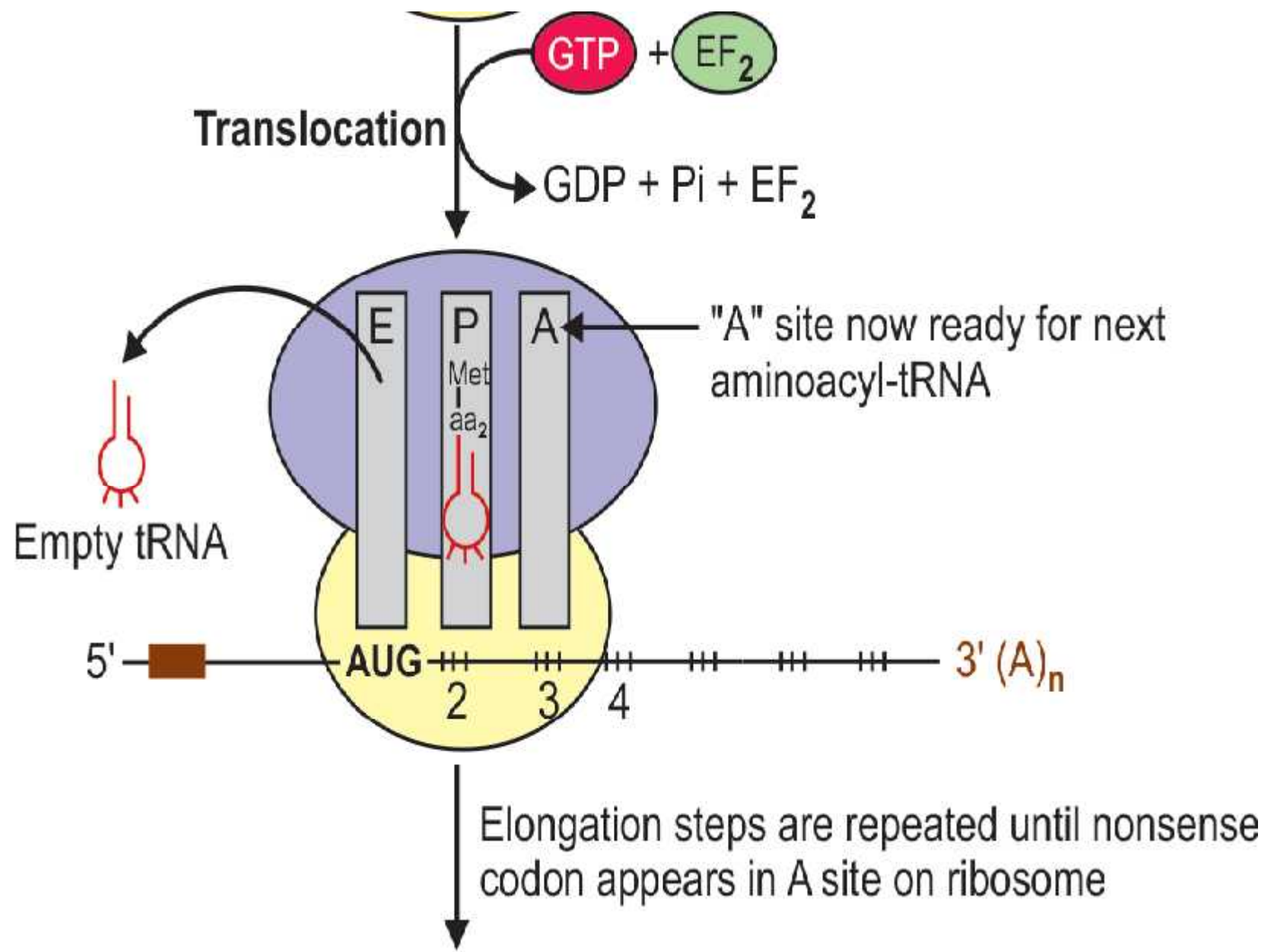
80S initiation complex

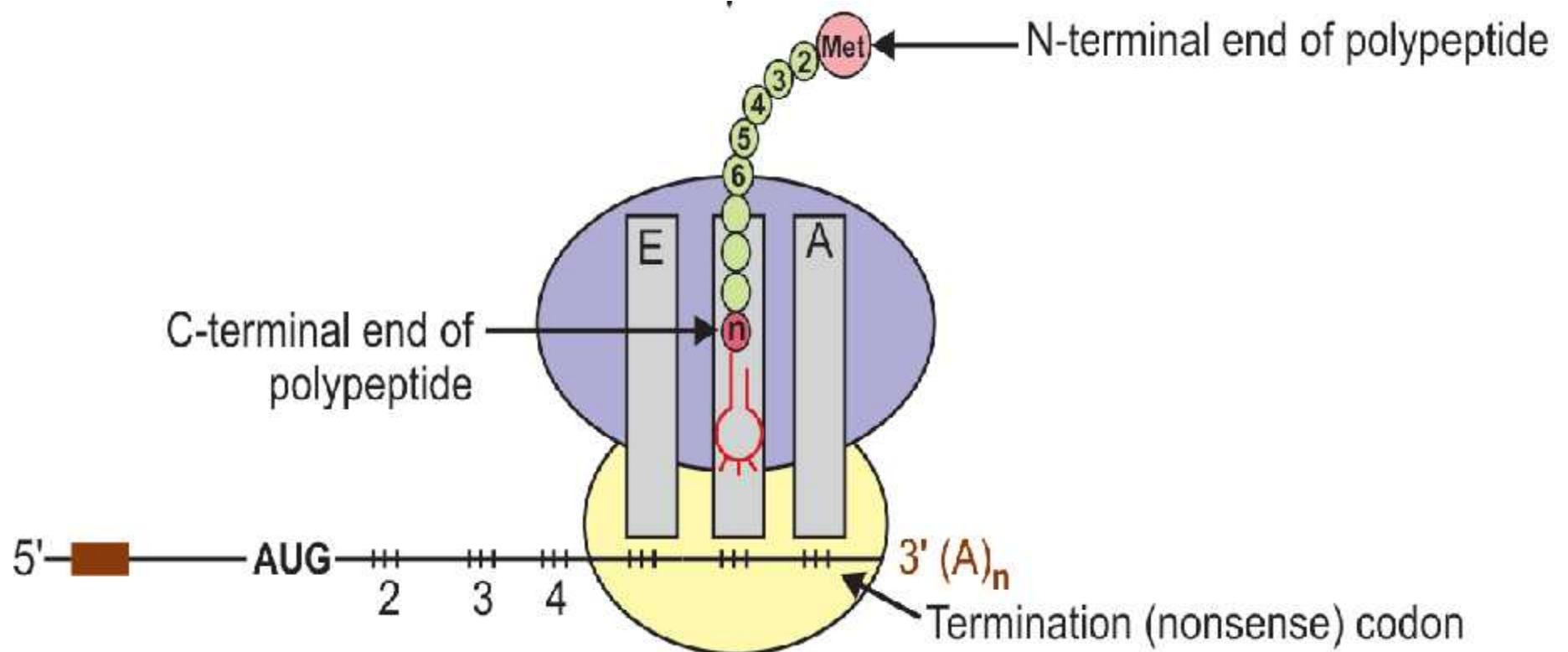




Formation of peptide bond at site of the ribosome.







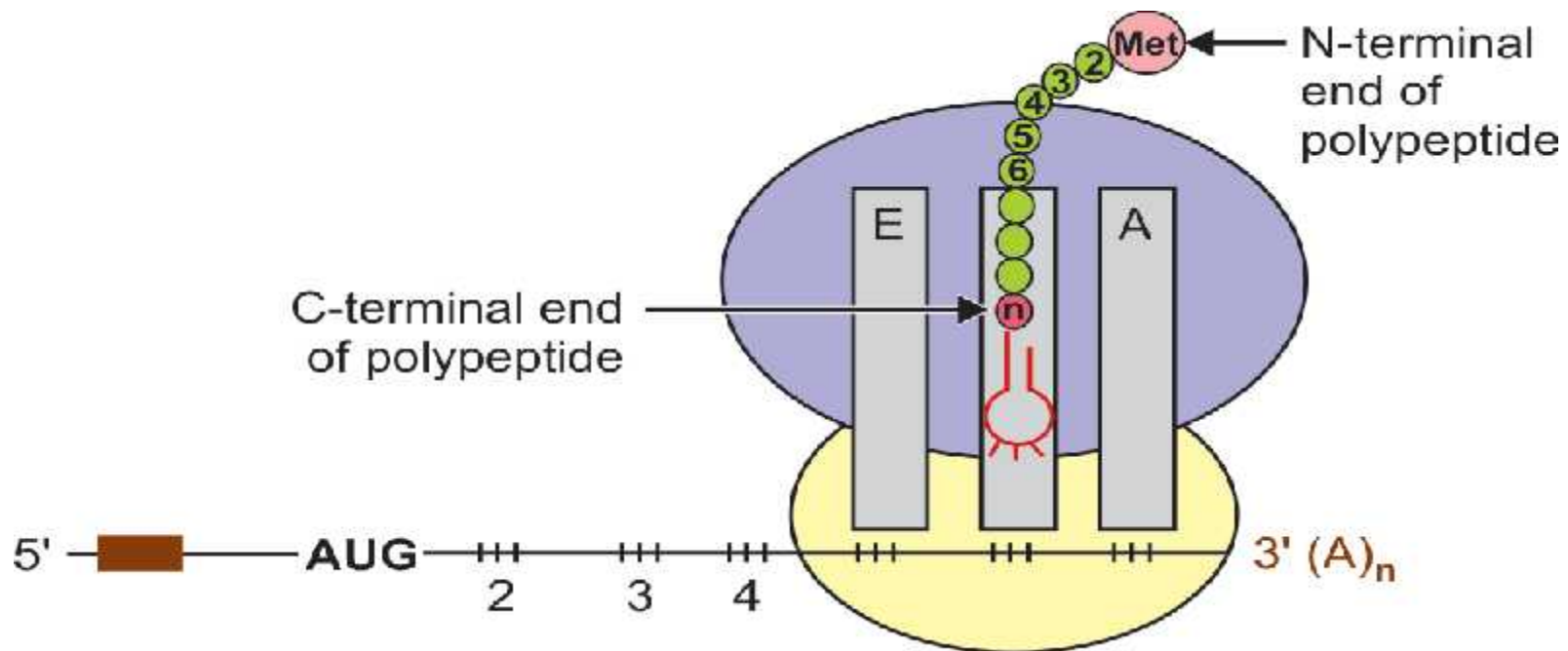
Ribosomal complex with elongated polypeptide and appearance of termination codon of mRNA in A site

Termination

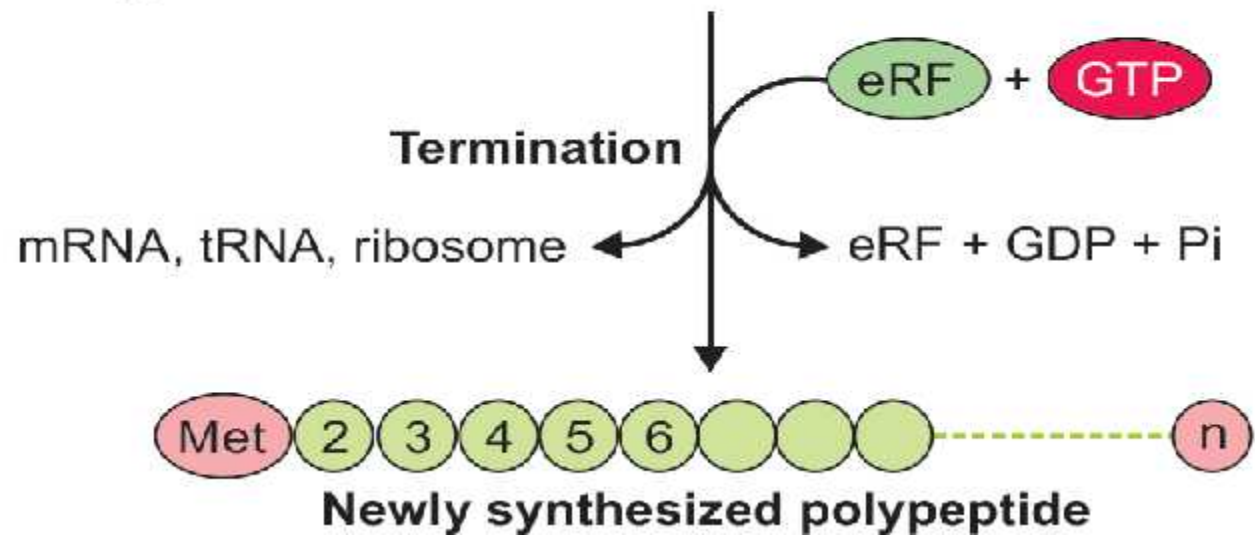
- Elongation steps are repeated until one of the three (UAA, UAG, UGA) termination or nonsense codon of mRNA appears in A site.
- Once the ribosome reaches a termination codon, releasing factors are capable of recognizing the termination signal present in the A site.

- Prokaryotes have three release factors, RF-1, RF-2, and RF-3 but eukaryotes have only single release factor, eRF.
- Releasing factors, GTP, and peptidyl transferase hydrolyzes the peptidyl tRNA bond
- This hydrolysis releases the polypeptide and tRNA from P site.

- Upon hydrolysis and release of polypeptide the mRNA is then released from the ribosome.
- Ribosome then dissociates into 60S and 40S subunits, which are then recycled.



Ribosomal complex with elongated polypeptide and appearance of termination codon of mRNA in A site



(POST-TRANSLATIONAL MODIFICATION)

Types of post-translational modifications are

- 1 Amino Terminal and Carboxyl Terminal Modifications
- 2 Loss of Signal Sequence
- 3 Attachment of Carbohydrate Side Chains

4. Modification of Individual Amino Acids

- Glycosylation
- Phosphorylation
- Carboxylation
- Hydroxylation
- Methylation
- Addition of prosthetic group

5. Proteolytic Processing

6. Formation of Disulfide Cross Links

Inhibitors Of Protein Synthesis

TABLE 21.4: Inhibitors of protein synthesis.

<i>Antibiotic</i>	<i>Action</i>
Streptomycin	Inhibits initiation and causes misreading of mRNA in prokaryotes. It binds to the 30S subunit at the A site, thereby causing a cessation of chain elongation. It's binding also distorts the A site and may result in the inseration of improper amino acids
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl-tRNA to mRNA in prokaryotes
Chloramphenicol	Binds to the 50S ribosomal subunit and blocks the peptidyl transferase reaction in prokaryotes

Erythromycin	Binds to the 50S ribosomal subunit that inhibits the translocation reaction in prokaryotes
Lincomycin and clindamycin	Binds to the 50S subunit and inhibits peptidyl transferase, thereby preventing peptide bond formation in prokaryotes
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA in both prokaryotes and eukaryotes
Cycloheximide	Inhibits peptidyl transferase activity of the 60S ribosomal subunit in eukaryotes

THANK YOU