Translation

The process

from

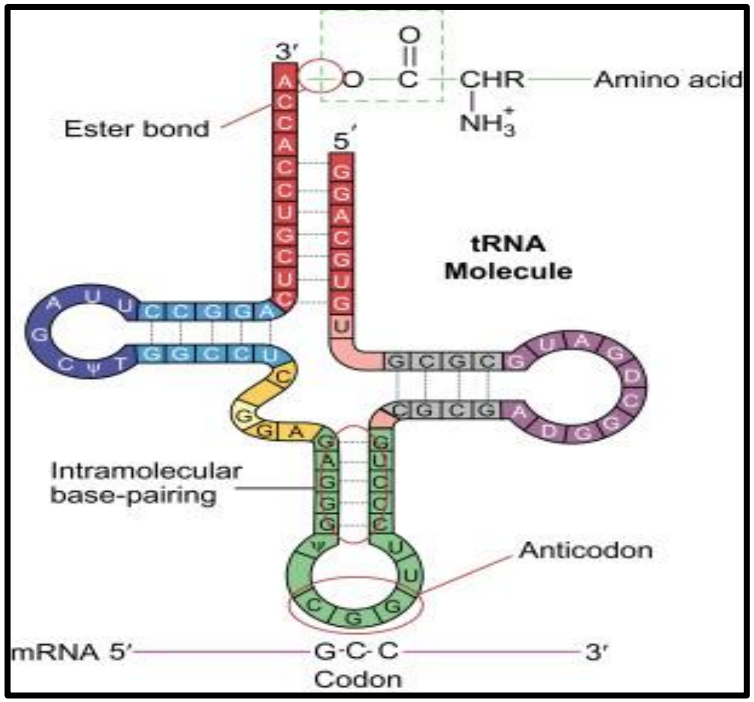
m-RNA **polypeptide chain**

Accessories required: -

- **m-RNA**
- **Ribosomes**
- **t-RNA**
- **Aminoacids**
- **Aminoacyl t-RNA synthetases**
- **Different factors (IF, EF, RF) and Enzymes**
- **Energy (ATP, GTP)**

Aminoacylation

Attachment of amino acids to t-RNA



GCC
GCU
GCA
GCG
Alanine

Enzyme required

Aminoacyl t-RNA synthetase

(20)

Due to degeneracy of Genetic code, All the t-RNA that are specific for a particular amino acid must have a common recognition site for the respective aminoacyl t-RNA synthetase enzyme.

Translation comprises of 3 steps:

- **Initiation**
- **Elongation**
- **Termination**

- Almost same in both prokaryotes and eukaryotes

Initiation requires special signals :

In prokaryotes – Shine-Dalgarno sequence (purine rich; 8-12 nucleotides upstream from initiation codon)

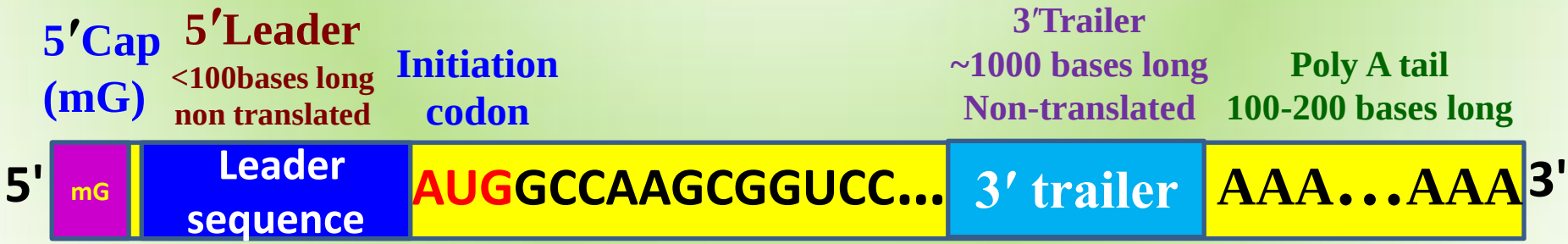
Initiation requires special signals :

In eukaryotes – No Shine-Dalgarno sequence; have

1) 5′ cap

2) Leader sequence (between 5′ cap and initiating AUG; usually < 100 bases)

3) 3′ trailer sequence (~1000 bases) and poly A tail (recently known tail and tail binding protein may take part)



In Prokaryotes

3 Initiation Factors (IF1, IF2 and IF3)

In eukaryotes

probably 9 Initiation factors (including eIF-4A and eIF-4B)

Initiation codon: AUG codes for methionine

In prokaryotes

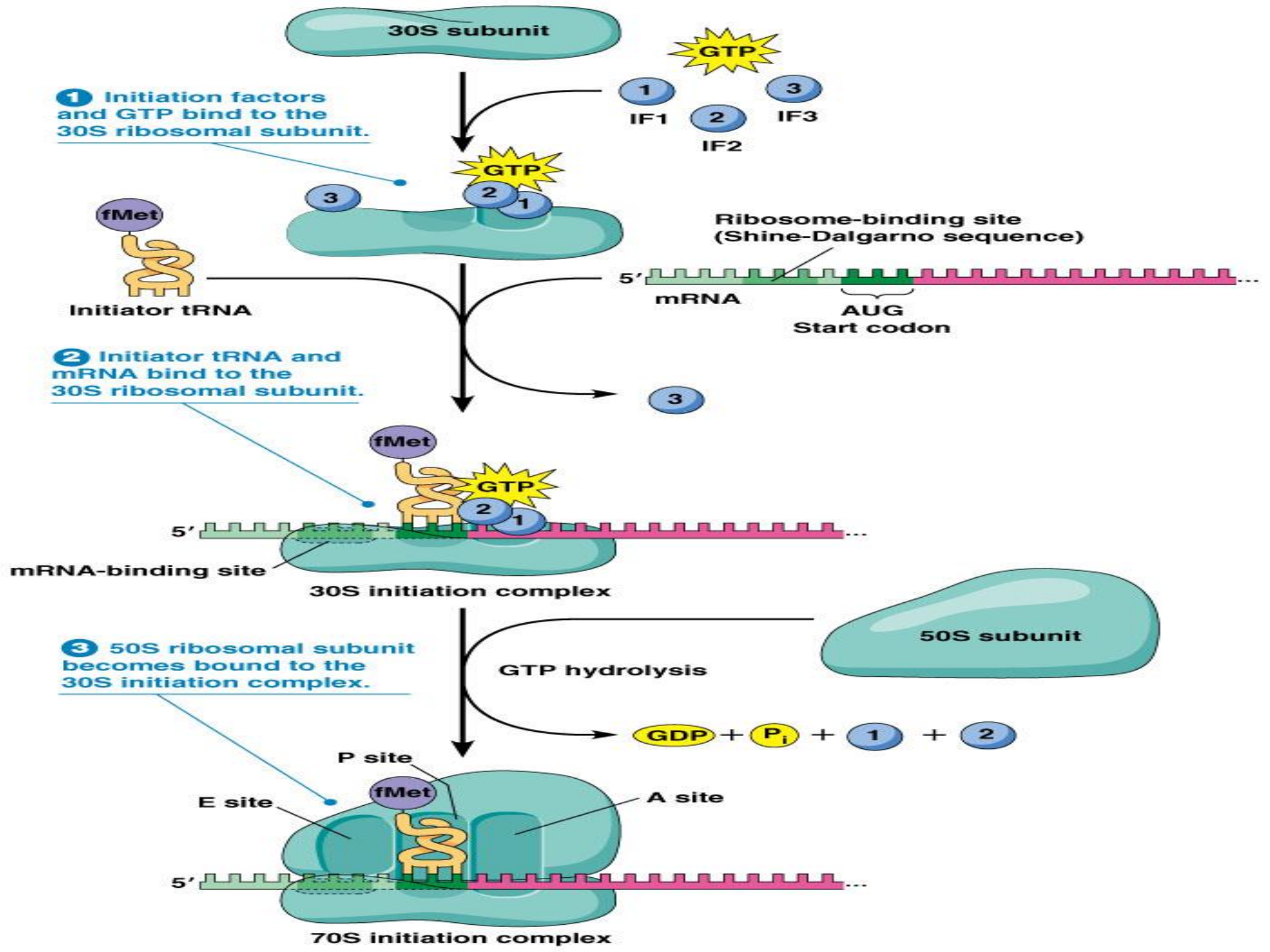
Methionine is of special form → **formyl methionine**

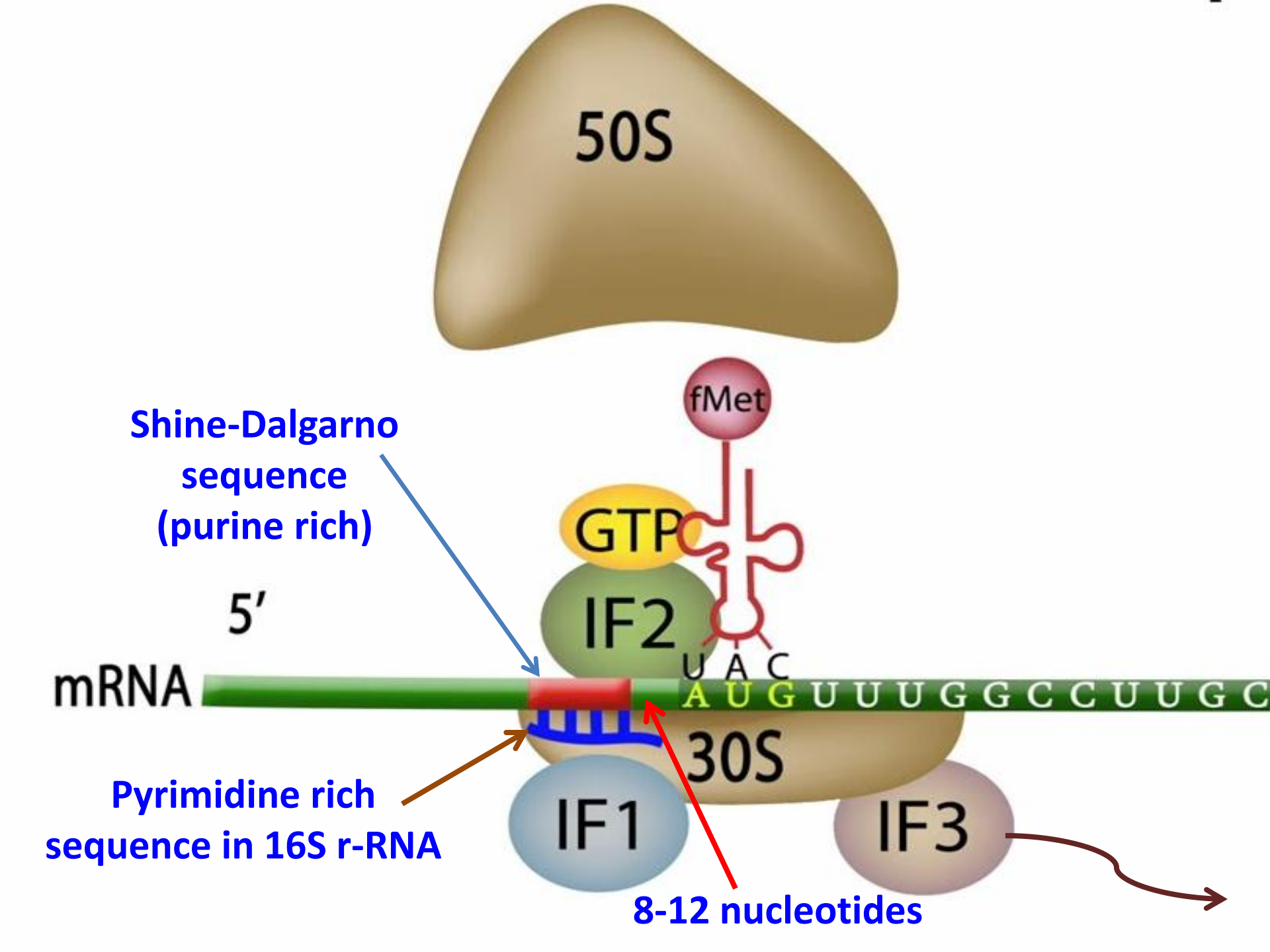
1) t-RNA + methionine —(**Methionyl t-RNA synthetase**)→ methionyl t-RNA

2) methionyl t-RNA —(**Transformylase**)→ **f-met-t-RNA$_f^{met}$**

In eukaryotes, **no transformylase enzyme**,

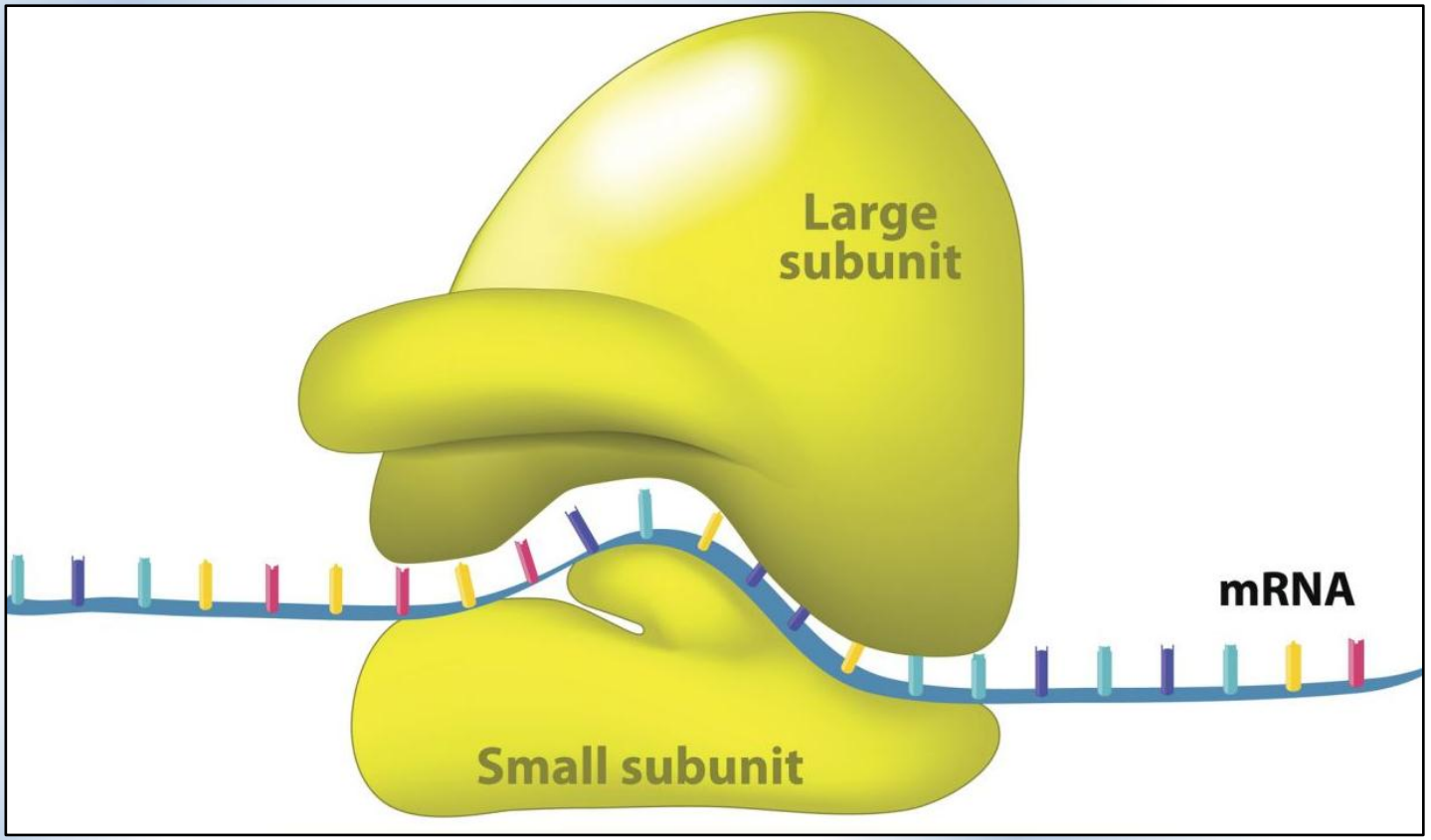
so, methionine is not formylated


30S subunit
GTP
1
3
IF1
2
IF3
IF2
1 Initiation factors and GTP bind to the 30S ribosomal subunit.
GTP
3
2
1
fMet
Ribosome-binding site (Shine-Dalgarno sequence)
5′
mRNA
AUG
Start codon
Initiator tRNA
2 Initiator tRNA and mRNA bind to the 30S ribosomal subunit.
3
fMet
GTP
2
1
5′
mRNA-binding site
30S initiation complex
50S subunit
3 50S ribosomal subunit becomes bound to the 30S initiation complex.
GTP hydrolysis
GDP + Pi + 1 + 2
P site
fMet
E site
A site
5′
70S initiation complex


50S
fMet
Shine-Dalgarno
sequence
(purine rich)
GTP
IF2
5′
U A C
mRNA
A U G U U U G G C C U U G C
30S
Pyrimidine rich
sequence in 16S r-RNA
IF1
IF3
8-12 nucleotides

Exactly where the m-RNA lies in intact ribosome ?

not known



could be:

fits into the space between 2 subunits of ribosome

or

lies slightly embedded in the smaller subunit

It must lie in a manner that the codons are sufficiently exposed for aminoacyl t-RNA to recognize and bind

Ribosome dimension (~250A°) enough to cover 30-40 bases of m-RNA i.e., 10-13 codons lie inside

Out of these only two codons are at any given time involved in protein synthesis

Initiating t-RNA is also special

In prokaryotes it is t-RNA$_f^{met}$

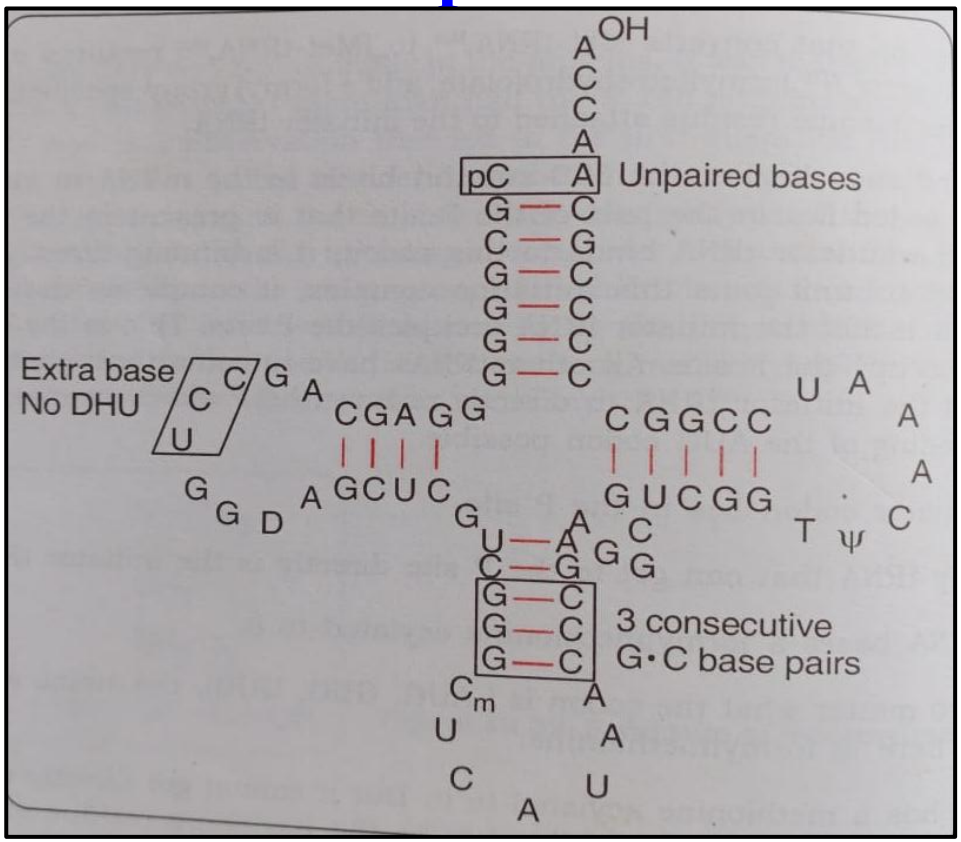
In eukaryotes it is t-RNA$_i^{met}$

Both are structurally different from t-RNA^{met}

that is specific for internal AUG

t-RNA$_f^{met}$ have

1) a series of 3 G-C pairs in anticodon arm and
2) 5 unpaired bases in acceptor arm

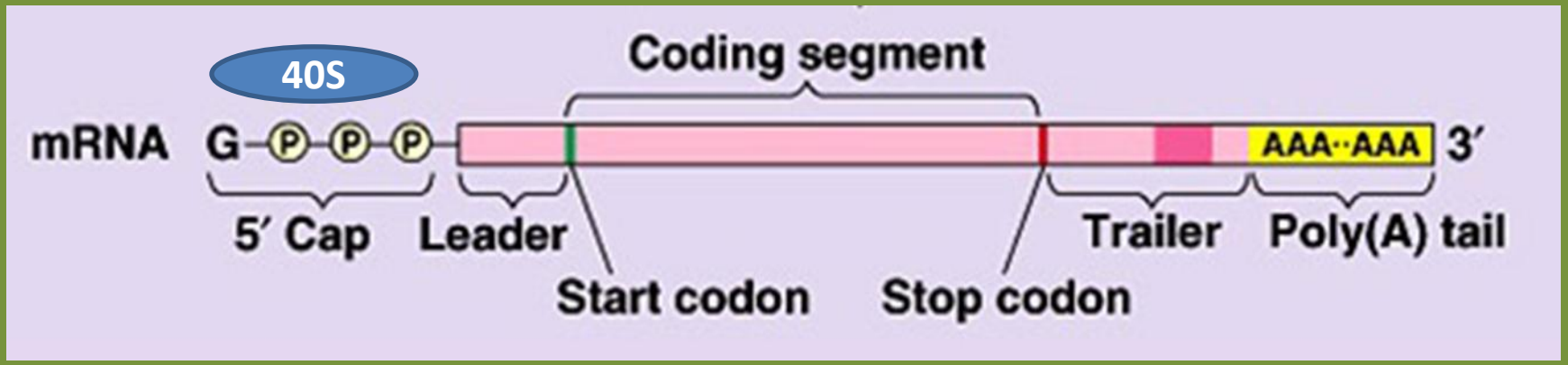


t-RNA$_i^{met}$ have

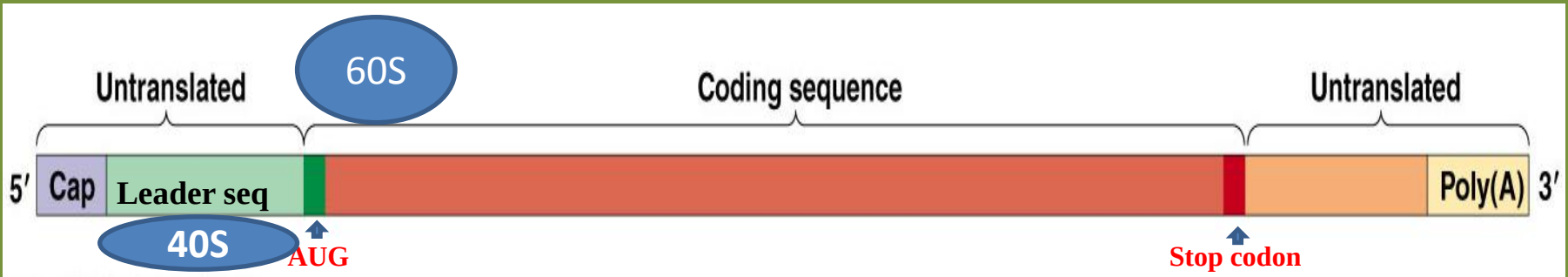
1) unusual tertiary structure and
2) phosphorylated 2′ ribose of base 64

In eukaryotes -

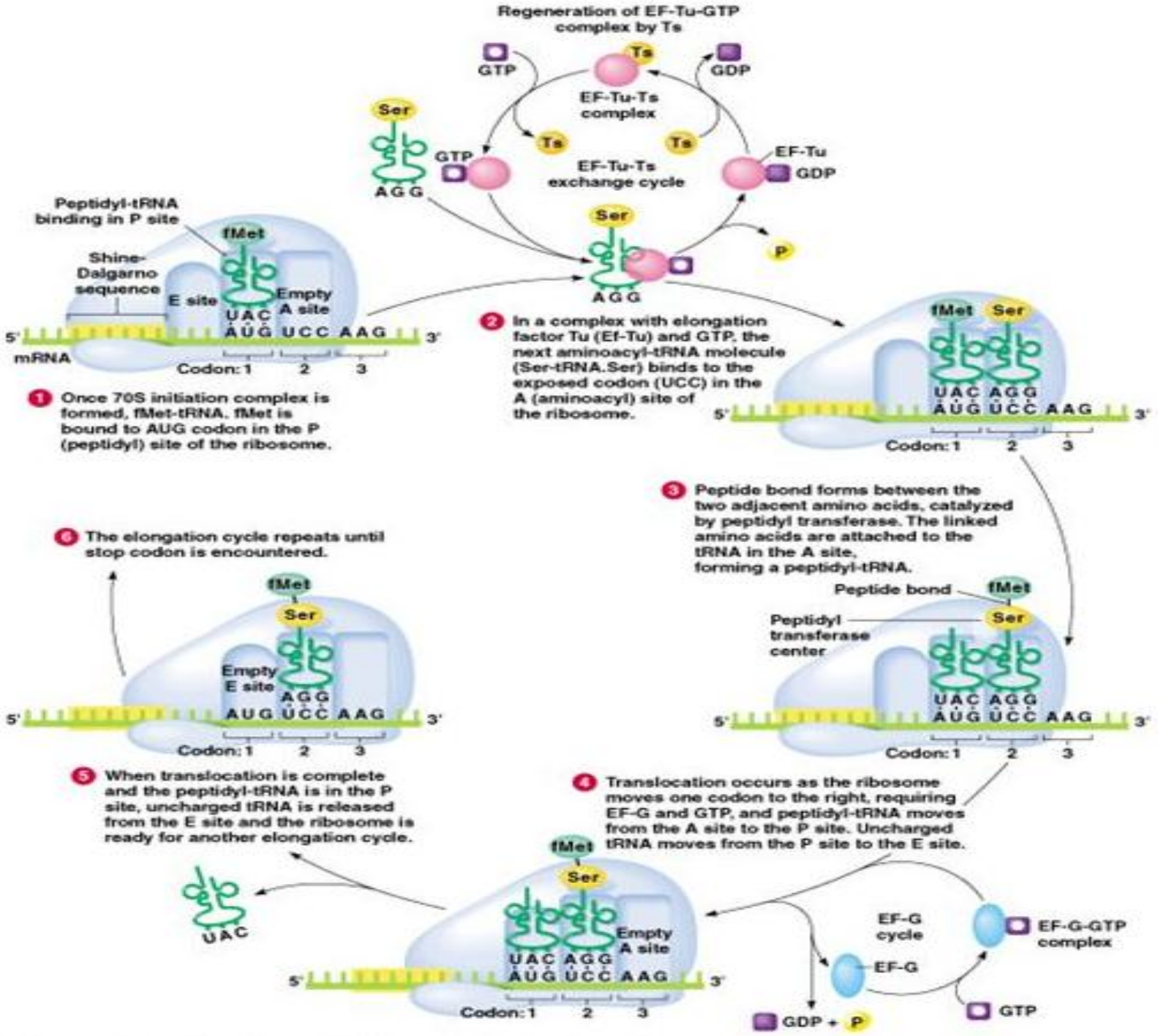
- Small subunit is involved in recognition and binding of mRNA and tRNA. Large subuint is involved in enzymatic reactions.
- 40S subuint recognise 5′ cap, bind at or near it and then migrate towards first AUG

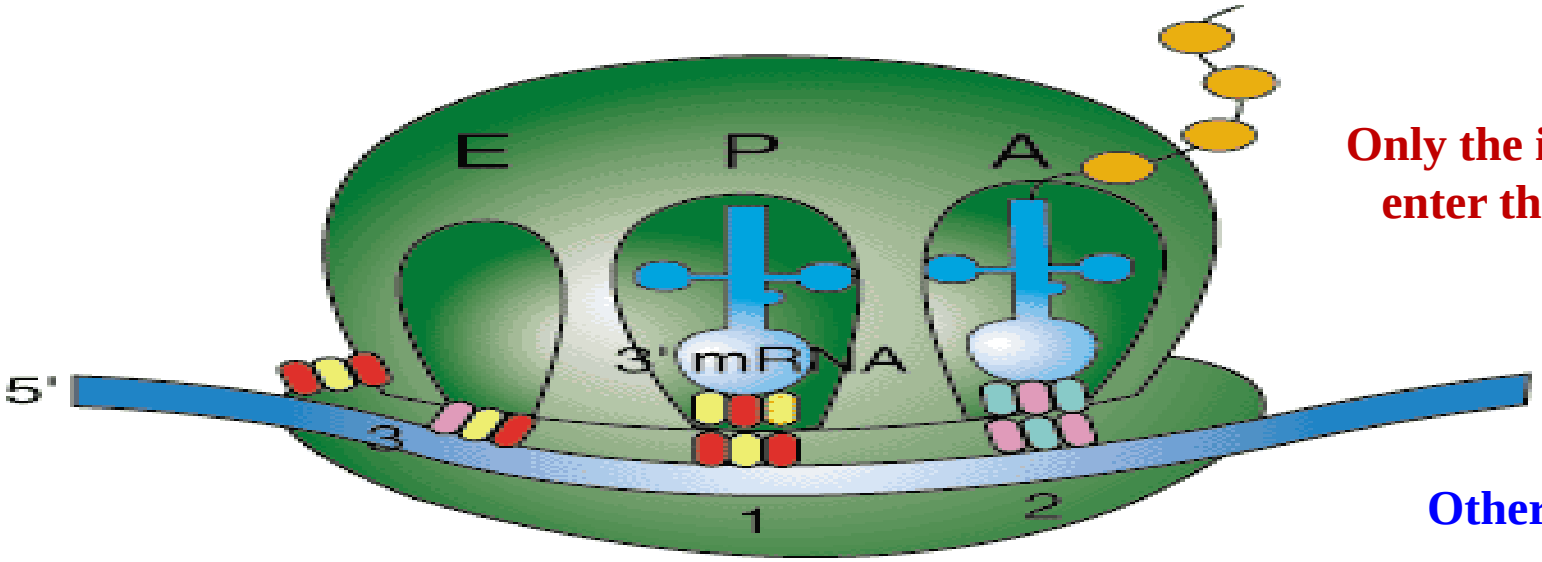


Eukaryotic processed m-RNA for translation

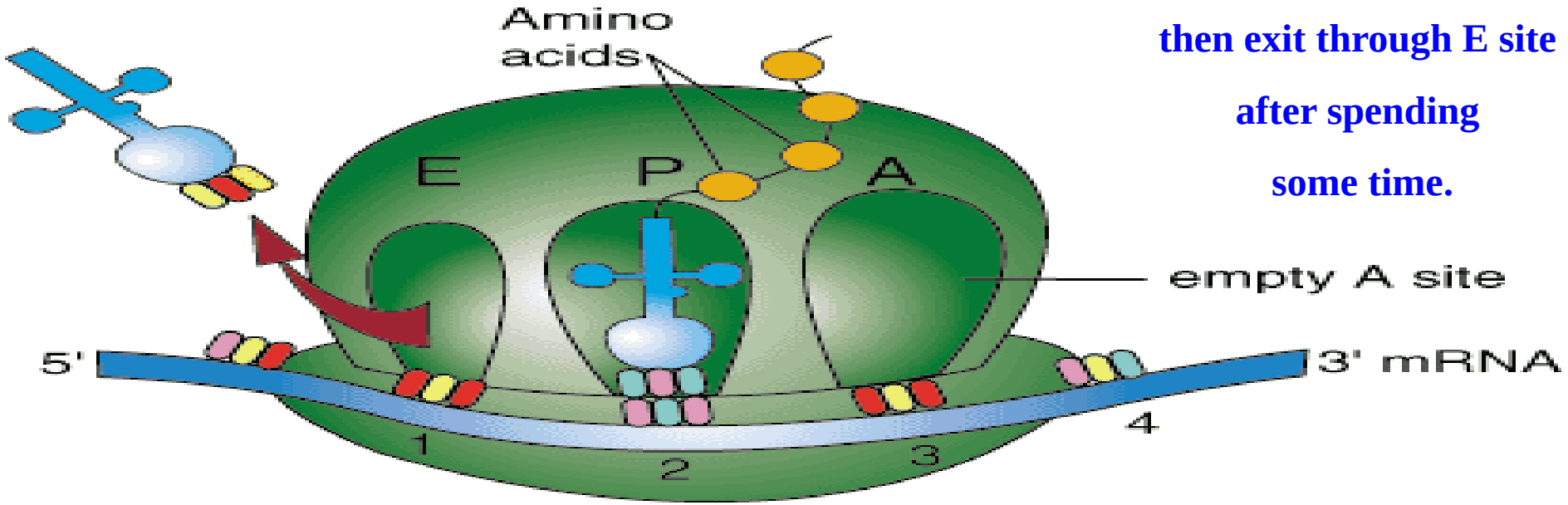


- Leader sequence (variable in length) if within ~40 bases from 5′ cap, the initiating codon automatically covered by ribosome binding at 5′ end.
- Oterwise ribosome have to move till it reaches initiating codon.
- Protein factor eIF-4A binds to 5′ cap, it unwinds the secondary structure of mRNA
- Further away from cap, eIF-4A needs eIF-4B. ATP is required for unwinding.
- Only 40S subunit migrates. 60S joins at the initiating codon
- A sequence GCCPuCCAUGG is required for cessation of migration


Regeneration of EF-Tu-GTP complex by Ts
GTP
Ts
GDP
EF-Tu-Ts complex
Ts
Ts
Ser
GTP
EF-Tu-Ts exchange cycle
EF-Tu
GDP
AGG
Peptidyl-tRNA binding in P site
fMet
Shine-Dalgarno sequence
E site
Empty A site
UAC
AUG UCC AAG
5'
3'
mRNA
Codon: 1 2 3
Ser
P
AGG
1 Once 70S initiation complex is formed, fMet-tRNA. fMet is bound to AUG codon in the P (peptidyl) site of the ribosome.
2 In a complex with elongation factor Tu (Ef-Tu) and GTP, the next aminoacyl-tRNA molecule (Ser-tRNA.Ser) binds to the exposed codon (UCC) in the A (aminoacyl) site of the ribosome.
fMet
Ser
UAC AGG
AUG UCC AAG
Codon: 1 2 3
3 Peptide bond forms between the two adjacent amino acids, catalyzed by peptidyl transferase. The linked amino acids are attached to the tRNA in the A site, forming a peptidyl-tRNA.
Peptide bond
Peptidyl transferase center
4 Translocation occurs as the ribosome moves one codon to the right, requiring EF-G and GTP, and peptidyl-tRNA moves from the A site to the P site. Uncharged tRNA moves from the P site to the E site.
Empty A site
EF-G cycle
EF-G
EF-G-GTP complex
GTP
GDP + P
5 When translocation is complete and the peptidyl-tRNA is in the P site, uncharged tRNA is released from the E site and the ribosome is ready for another elongation cycle.
UAC
6 The elongation cycle repeats until stop codon is encountered.
Empty E site
AGG



Translocation



Only the initiator tRNA enter through P site.

Other incoming aminoacyl tRNA is welcomed at A site, then carried to P site, and then exit through E site after spending some time.

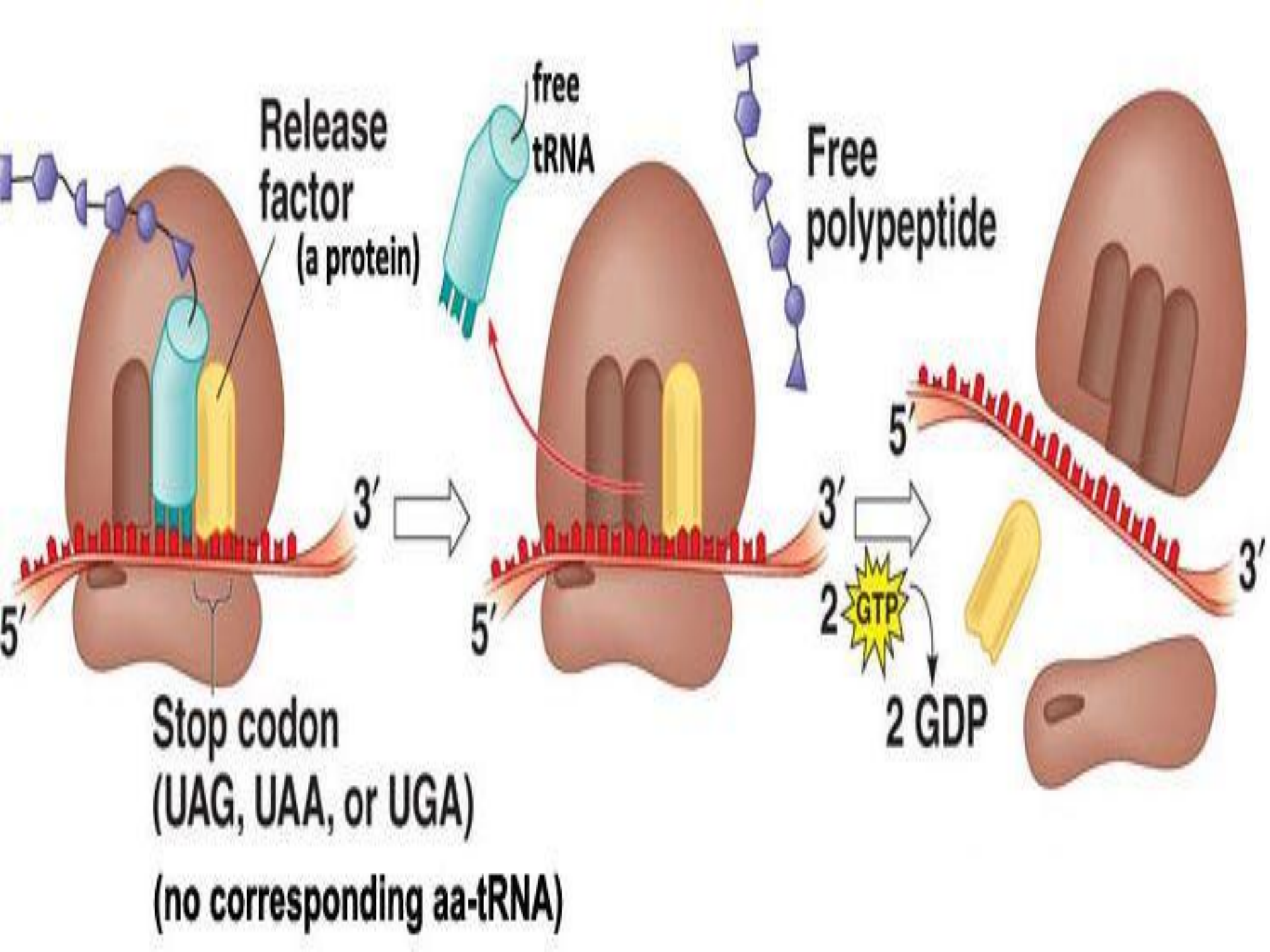
7. ELONGATION OF TRANSLATION

- Three key events for correct addition of each amino acids:

1) Correct aminoacyl-tRNA is loaded to the A site of the ribosome as dictated by the A site codon
2) Peptidyl transferase reaction
3) Translocation of peptidyl tRNA from A site of ribosome to the P site of ribosome.

- Two proteins control these events called Elongation factors, both of which use energy from GTP binding and hydrolysis

- **EF-Tu** – It "escorts" aminoacyl tRNAs to the ribosomes
- **EF-G** – it derieves translocation of tRNA and mRNA


Release factor
(a protein)
free tRNA
Free polypeptide
5′
3′
5′
3′
5′
3′
2 GTP
2 GDP
Stop codon
(UAG, UAA, or UGA)
(no corresponding aa-tRNA)

In Prokaryotes

3 Release Factors (RF1, RF2 and RF3)

RF1 recognizes **UAA** and **UAG**

RF2 recognizes **UAA** and **UGA**

RF3 plays stimulatory role

In eukaryotes

Only 1 release factor: eRF