Protein Biosynthesis
or
Translation

Protein Synthesis

- DNA transcribed to m RNA → Translated into protein with the help of ribosome
- Cytoplasmic process

language of nucleotides in DNA →
language of amino acids in proteins

mediated through

- **mRNA – Messenger RNA**
- **tRNA – Transfer RNA**
- **rRNA – Ribosomal RNA**

Cellular Machinery for protein Synthesis

- Specialized apparatus –*ribosome*

- Ribosomes are made of a *small subunit* and a *large subunit*.

Ribosomal assembly

30S

Prokaryotes -70S

50S

40S

Eukaryotes-80S

60S

Ribosomal assembly – rRNA + Proteins

- Eukaryotic ribosomes - 4rRNAs & 80 different proteins.

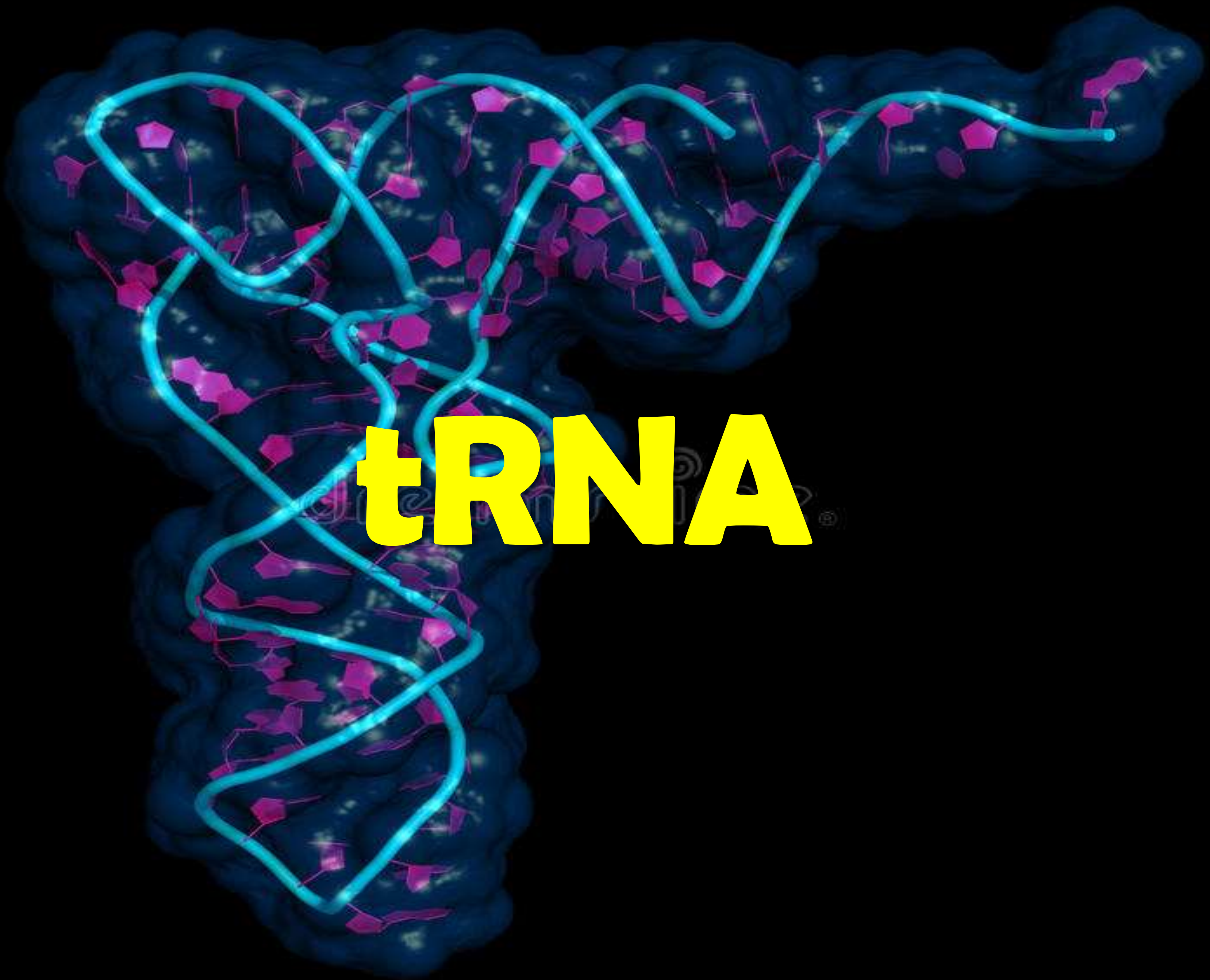
- 28S + 5.8S + 5S + ~ 50 Proteins →60S
- 18S + ~30 Proteins → 40S

1Ribosome can synthesize ~100 peptide bonds/mt.

- Nucleolus- rRNA synthesize and ribosomal assembly
- rRNA –catalytic activity
- Peptidyl transferase-28SrRNA
- RNAP -I

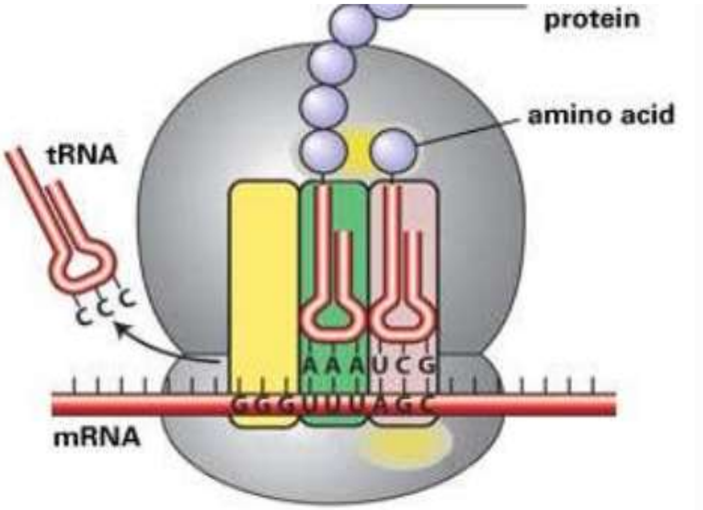
BACTERIAL RIBOSOME

- 70S -30S+ 50S
- 50S- 23S,5S
- 30S-16S rRNA
- Antibiotic inhibit bacterial protein synthesis

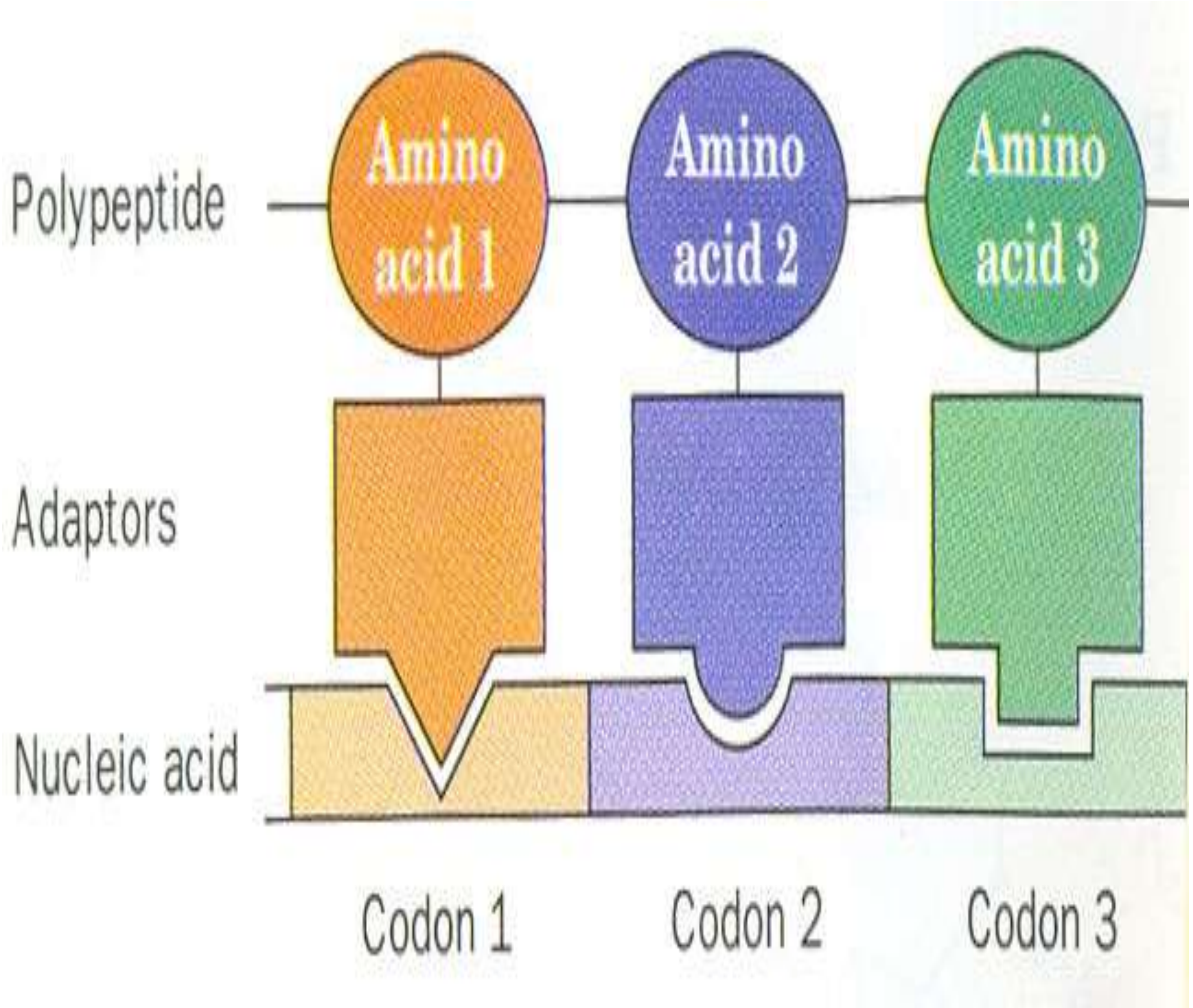


tRNA

- **Transfer RNA–** transfer amino acid from cytoplasm to ribosomal protein synthesising machinery
- Soluble RNA/ sRNA– easily soluble



- Adapter molecules for translation
- 20% of total cellular RNA
- 74-95 nucleotides long


Polypeptide
Amino acid 1
Amino acid 2
Amino acid 3
Adaptors
Nucleic acid
Codon 1
Codon 2
Codon 3

UNIQUE FEATURE OF tRNA

- **Presence of unusual bases**
- They protect tRNA from dehydration by Rnase.
- Hypoxanthine
- Pseudouridine
- Dihydrouracil

ROLE OF tRNA

- Recognizing codons in mRNA
- Activating amino acid

- There is atleast one kind of tRNA for each amino acid.
- some amino acids there are 2 or more specific tRNAs
- Atleast 31 tRNAs are required to recognise all aminoacid codons
- Each tRNA presents only one amino acid to ribosome

Synthesis and processing of tRNA

- RNA polymerase III transcribes tRNA.
- Undergo post transcriptional modification
- 1. Trimmed by ribonuclease P, Ribozyme
- 2. CCA sequence is added at 3’end
- 3. bases modified

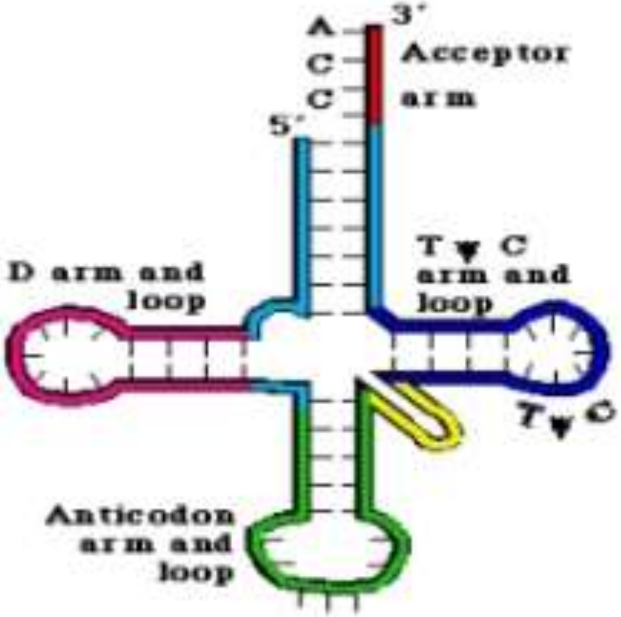
STRUCTURE OF tRNA

- Nucleotide sequence of all tRNA molecules allows extensive folding

→secondary structure

- Intrastrand complementarity
  in 2D like a clover leaf

Secondary structure/ clover leaf model



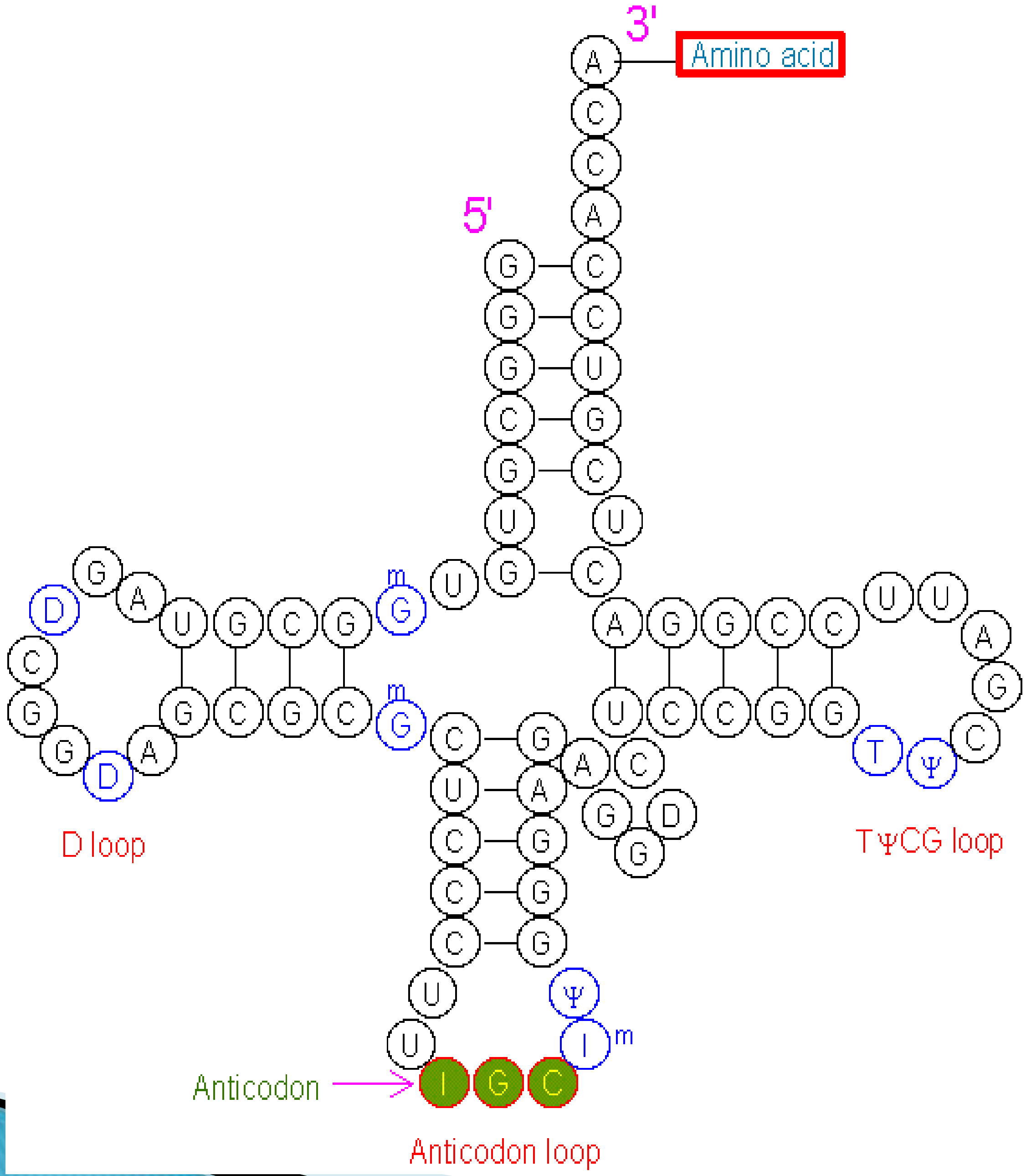
- Robert Holley proposed clover leaf model for the first time in 1968.
- It is a two dimensional description of the t-RNA.

CLOVER LEAF Structure of tRNA

4 main arms

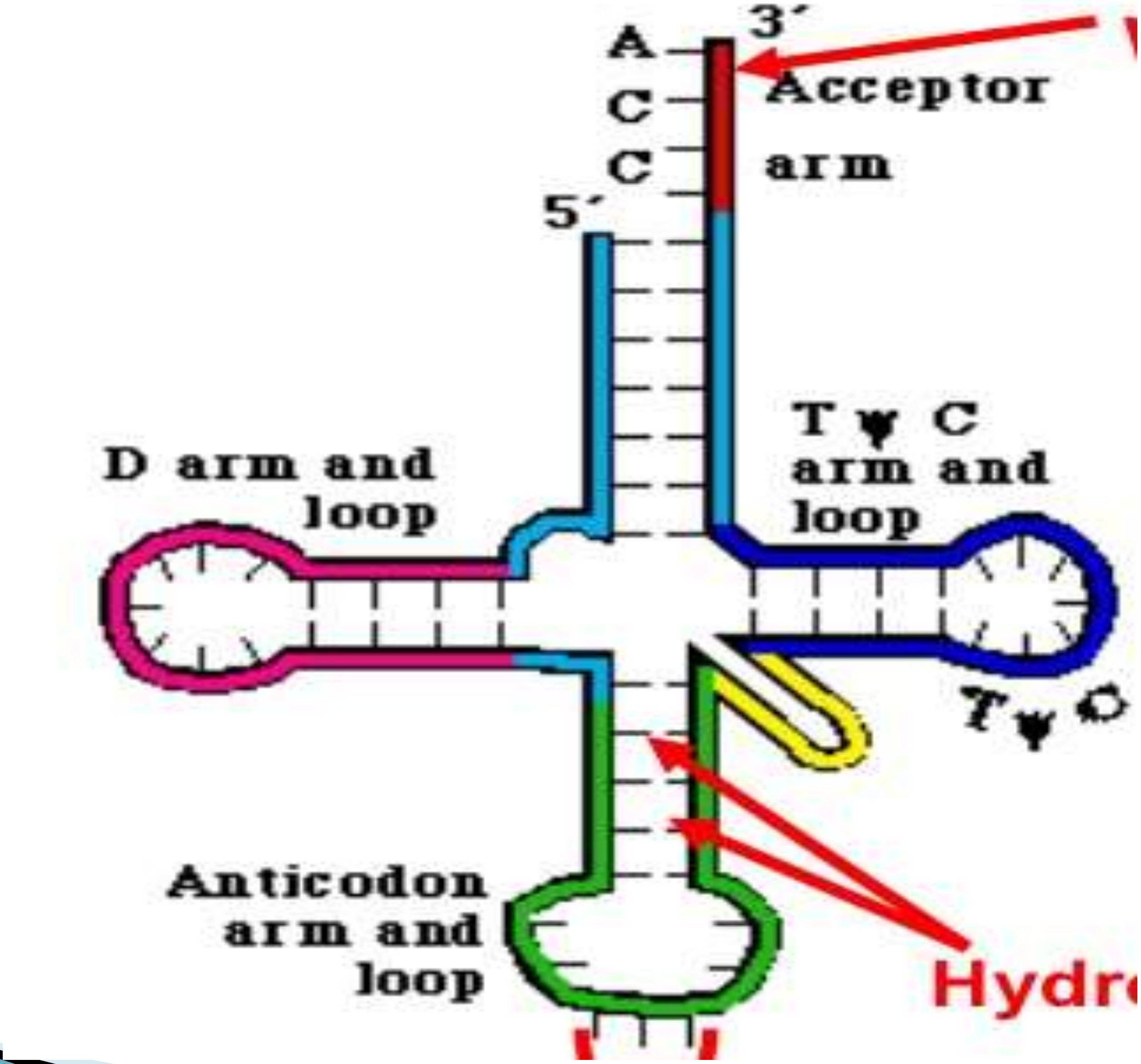
- Acceptor Arm
- anticodon arm
- D arm
- TψC arm

- Extra arm


3'
Amino acid
5'
D loop
TΨCG loop
Anticodon
Anticodon loop
m
I
G
C

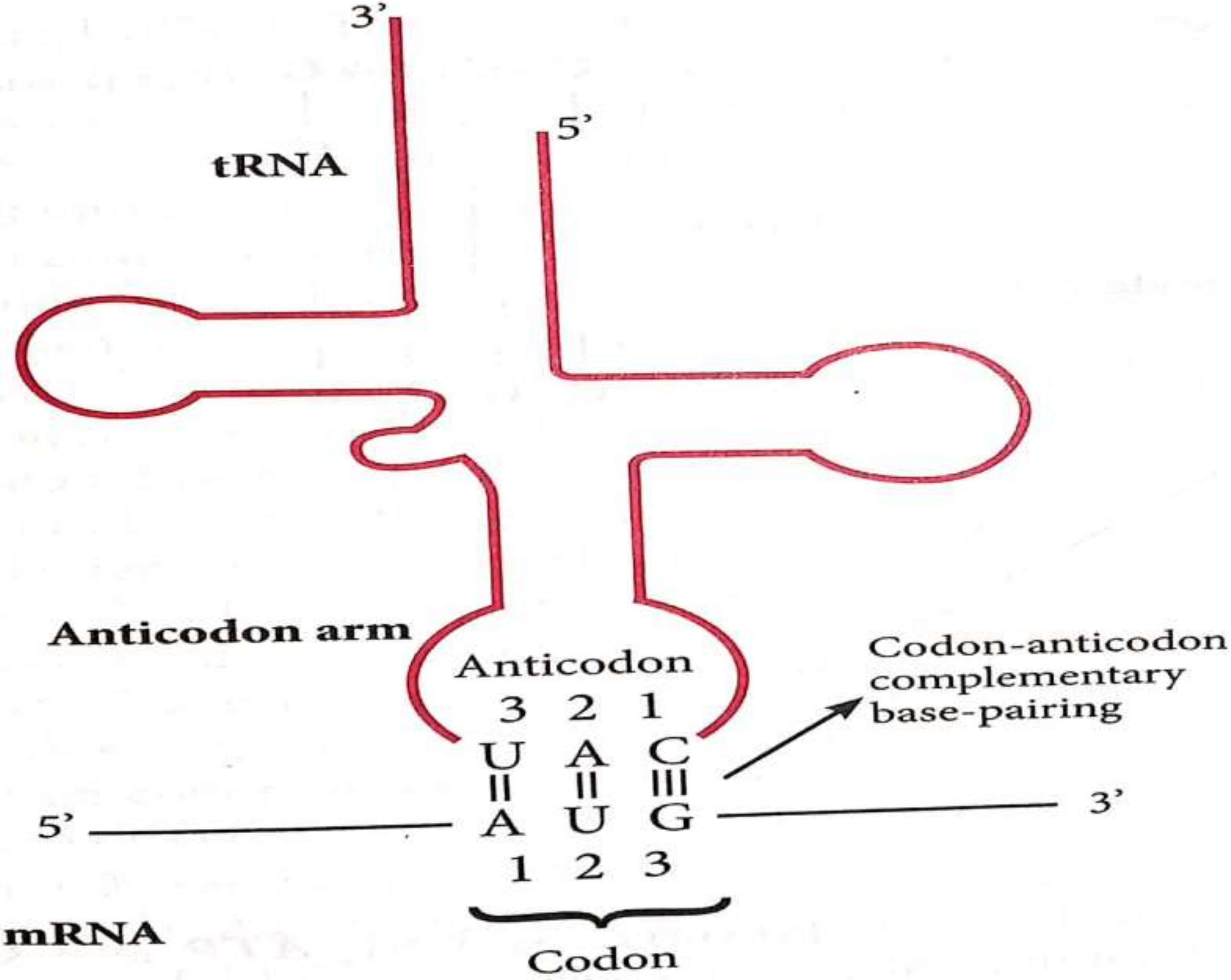
Acceptor arm

- Terminates in the nucleotides CCA-3’
- 7 BASE PAIRS
- CCA triplet is not base paired
- appropriate AA is attached to 3’ OH of A moiety by amino acyl- tRNA synthetase
- Each tRNA can be charged with only 1 specific amino acid

3´
A
C
C
5´
Acceptor arm
D arm and loop
T ψ C arm and loop
T ψ C
Anticodon arm and loop
Hydr

Anticodon arm

- opposite side of acceptor arm
- recognize triplet NT codon present on m-RNA
- Specificity of t RNA
- Base sequence complimentary to that of m-RNA codon


3’
5’
tRNA
Anticodon arm
Anticodon
3 2 1
U A C
Codon-anticodon
complementary
base-pairing
5’
A U G
3’
1 2 3
mRNA
Codon

- For a given codon in mRNA, only a single species of tRNA molecule possess the proper anticodon

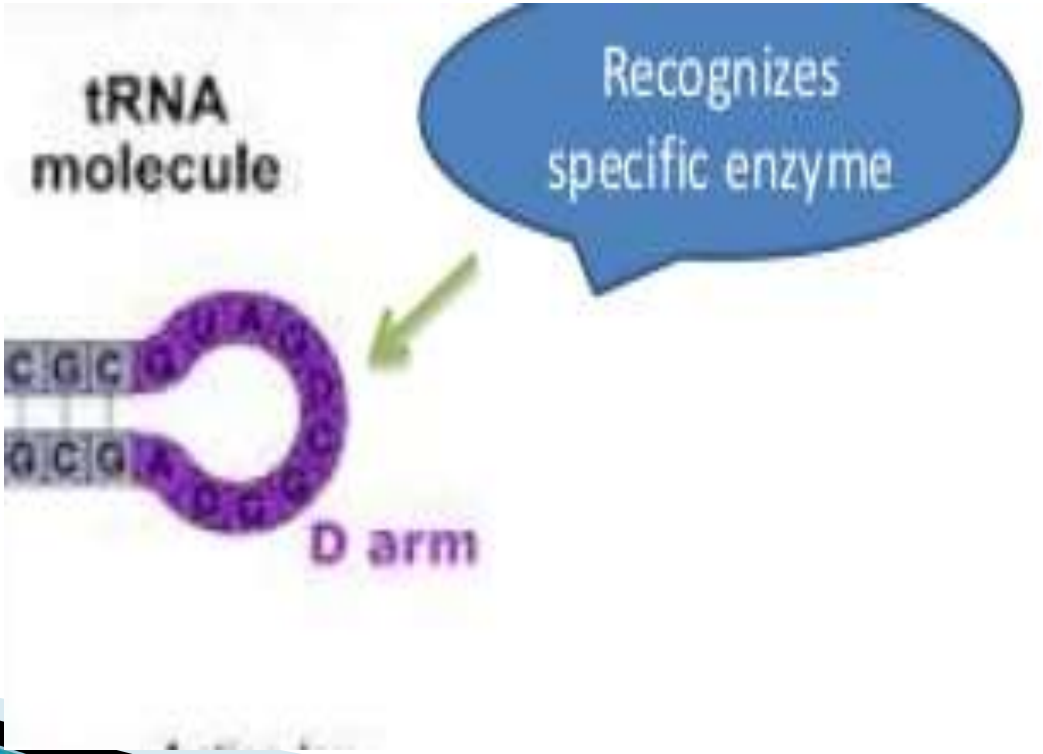
SPECIFICITY IN BOTH ASPECTS

- Recognizing m-RNA codon
- Accepting specific AA coded by that codon
- Amino acid attachement by aminoacyl t-RNA synthetase

- adapter molecule b/w m-RNA and amino acid coded by it

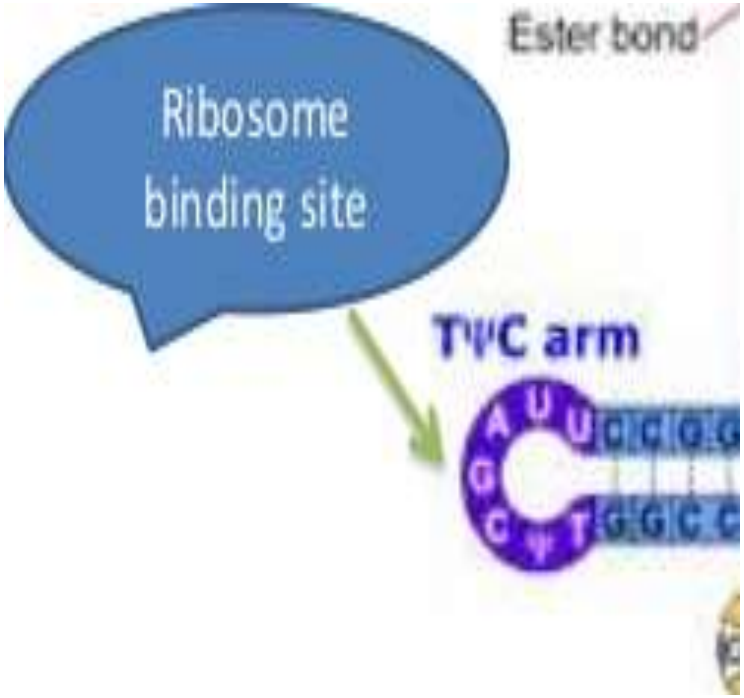
D ARM/ Dihydrouracil arm

- Contains dihydrouridine
- recognition site for the enzyme which adds amino acid



TψC loop

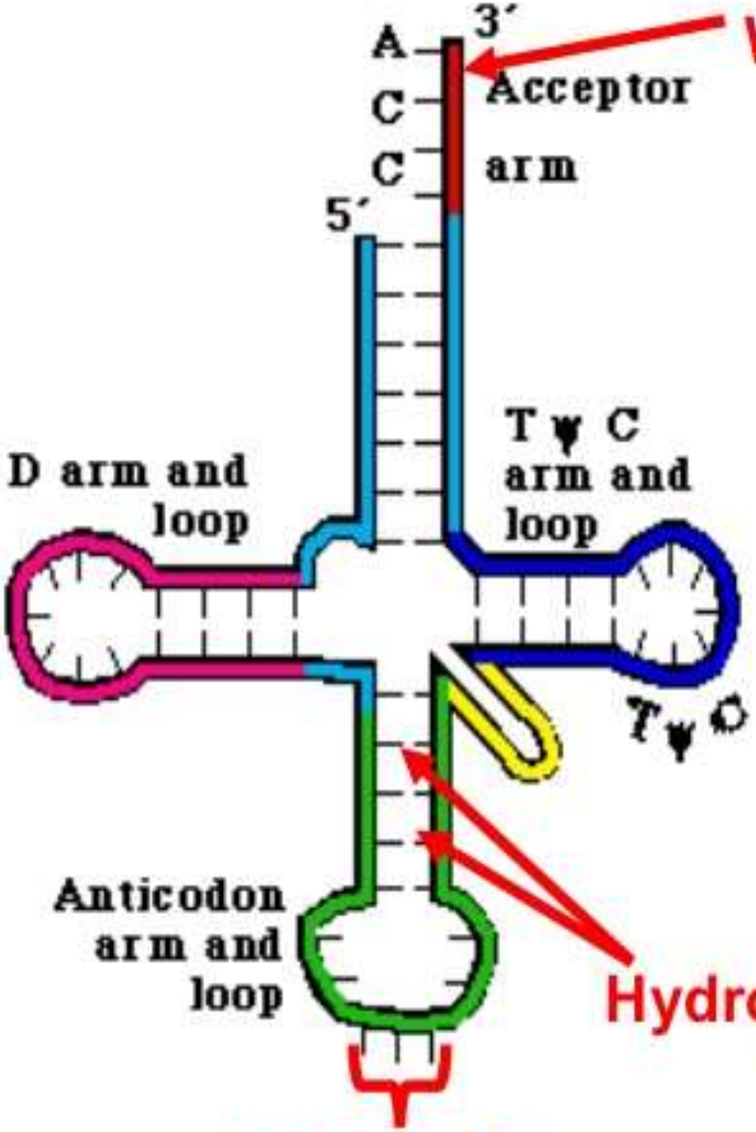
- Contains thymine- pseudouridine-cytosine sequence
- binds tRNA-aa to the ribosomal surface



Variable loop

Class I- 3-5 base pairs long

Class II- 13-21 base pairs long

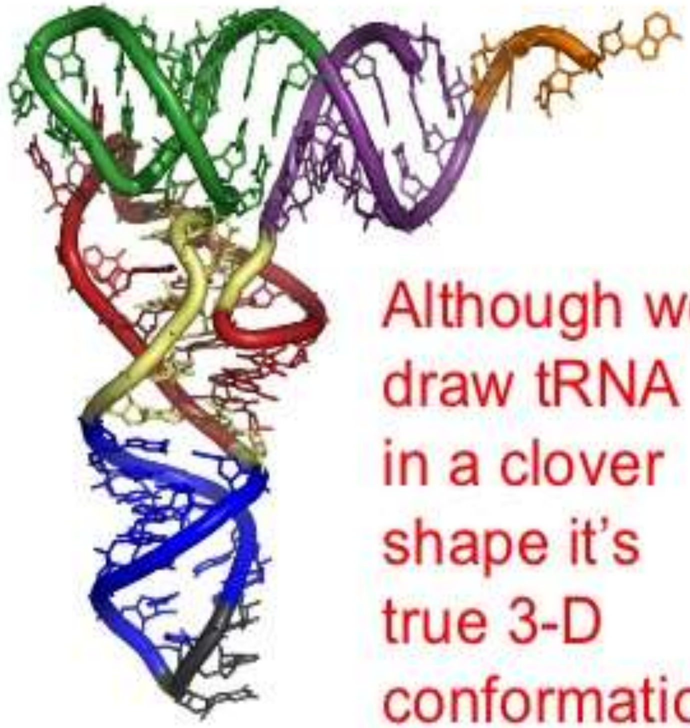


Where the a.a. attaches

Hydrogen bonds

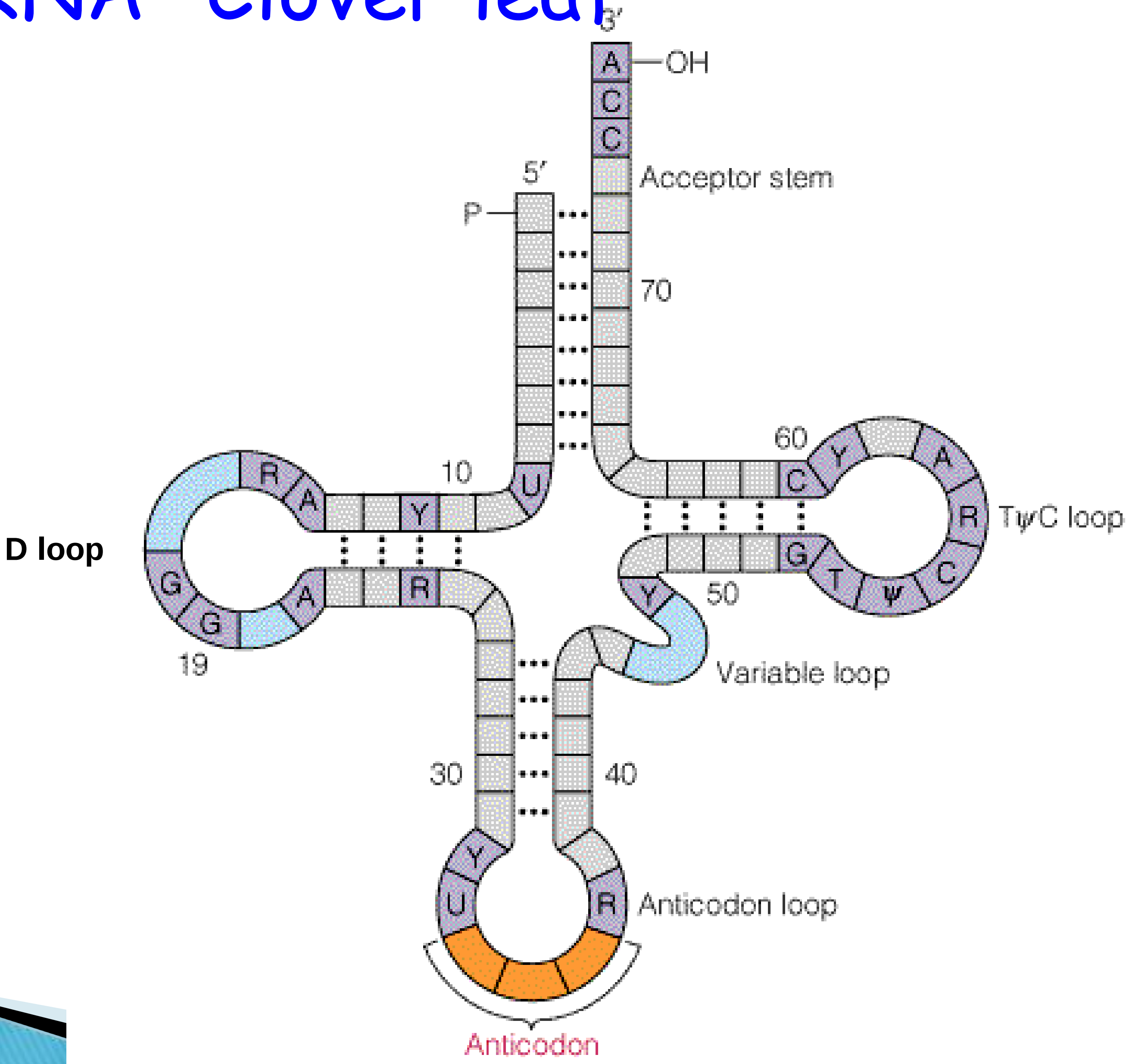
Anticodon

=



Although we draw tRNA in a clover shape it's true 3-D conformation is L-shaped.

t RNA- Clover leaf



- ***Psedouridine arm*** -Binds tRNA to ribosome

- ***Anticodon arm-*** Recognises the codon on Mrna

- ***Acceptor arm-***Binds the specific amino acid.

- ***DHU arm -*** Recognises the specific amino acyl tRNA synthetase enzyme.

- Reduction of number of tRNAs is possible due to Wobbling.

- The phenomenon of Wobbling was put forward by Watson and Crick.

Protein synthesis-
Eukaryotes and Prokaryotes

- The steps are same in eukaryotes and prokaryotes

- In eukaryotic cell-in the cytoplasm ,while transcription and RNA processing takes place in the nucleus

- In bacteria- two processes coupled; protein synthesis starts even before transcription is finished.

- mRNA synthesized in the nucleus transported to the cytoplasm
- 5’-3’direction
- First AA is amino terminal one
- Chain growth –amino terminal to carboxy terminal

- initiating amino acid in eukaryotes-methionine
- prokaryotes -N-Formyl Methionine.

- Eukaryotic mRNA is capped–the recognition feature for ribosome binding.

Requirement of the components

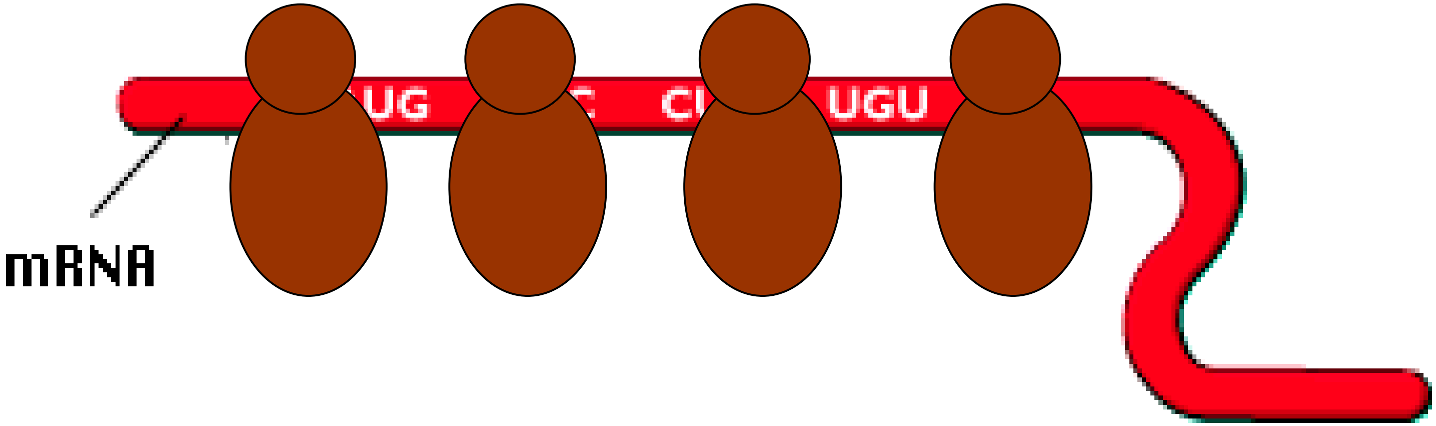
Amino acids

- 20 aa
- Essential should be supplied
- Deficiency –translation will stops
- Prokaryotes-no requirement
- Synthesized from inorganic components

Ribosomes

- A site-binding aminoacyl t-RNA
- P site-binding peptidyl tRNA
- Eukaryotes, E /Exit site

Several ribosomes simultaneously translate on a single Mrna → **Polyribosomes.**



mRNA

- Information required for synthesize of protein
- Polycistronic-single mRNA has many coding region
- Eukaryote-monocistronic

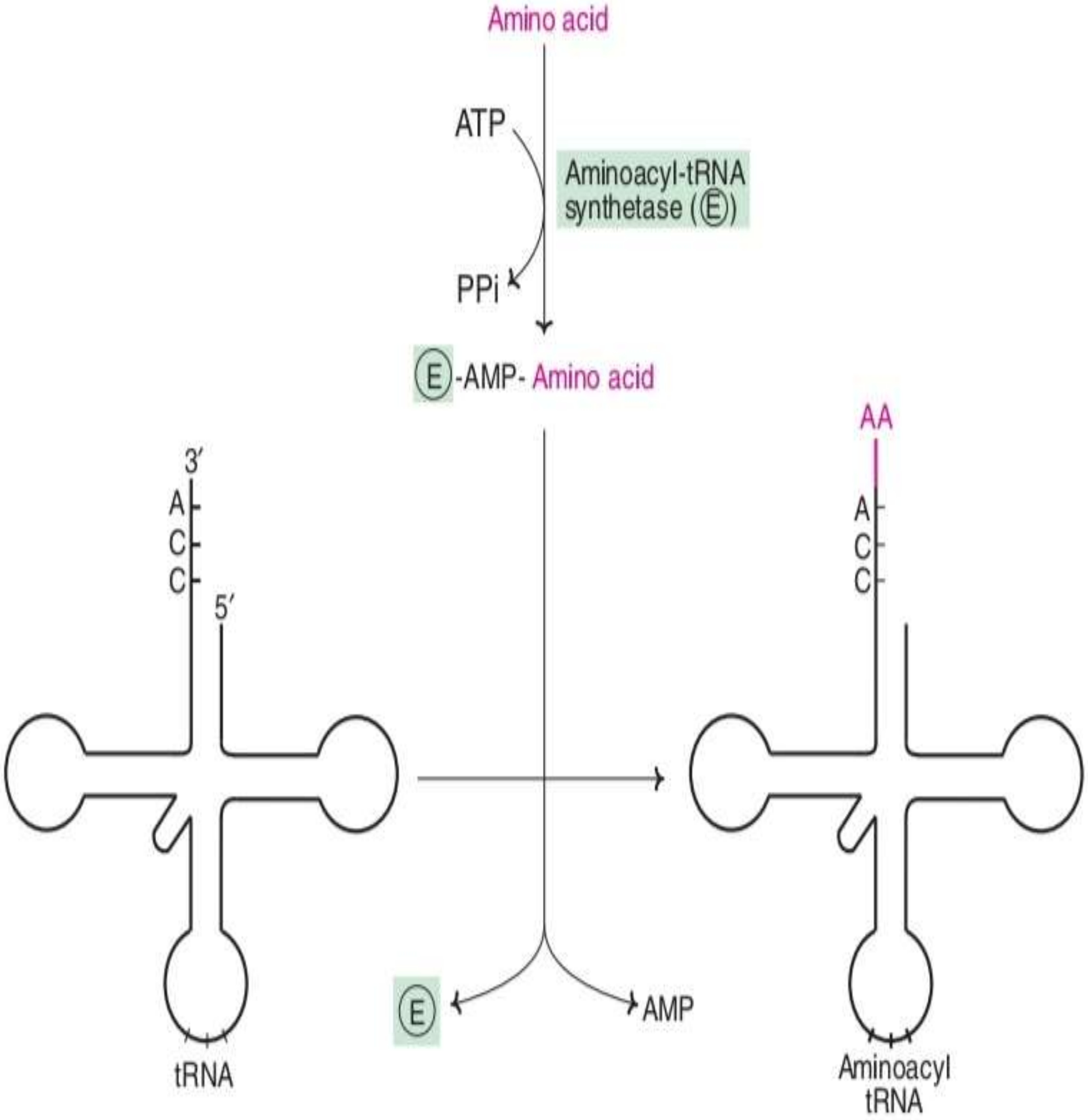
- **tRNA**
- **Energy** -ATP,GTP
- **Protein factors**

Translation

- Activation of amino acid
- Initiation
- Elongation
- Termination
- Post translational processing

Activation of aminoacid (Charging)

- AA activated and attached to t RNA
- 2 step
- Enzyme- **Aminoacyl tRNA synthetases**
- Highly specific for AA & corresponding tRNA


Amino acid
ATP
Aminoacyl-tRNA synthetase (E)
PPi
E-AMP- Amino acid
3′
A
C
C
5′
tRNA
E
AMP
AA
A
C
C
Aminoacyl tRNA

- Requires energy

$aa + tRNA + ATP \rightarrow tRNA^{aa} + AMP$

- Specific enzyme is identified by DHU arm.
- Amino acid is attached to acceptor arm.

Initiation of protein Synthesis

Involves eukaryotic initiation factors(eIFs)

Initiation factors: eIF

- The initiation phase of protein synthesis requires >10 eukaryotic Initiation Factors (eIFs) .

Initiation factors: eIF

eIF	Functions
eIF-1	**Forms a ternary complex with 40S ribosome and eIF-3**
eIF-2	**Binding of tRNA^{met} to 40S**
eIF-3	**Prevents reassociation of ribosomes**
eIF4A,4B,4G,4F	**Binding of mRNA to ribosomes**
eIF-4E	**Recognition of mRNA cap**
eIF-5	**Reassembly of 80S ribosomes**

Initiation of protein Synthesis

Involves 4 steps

- Ribosomal dissociation
- Formation of **43 S Pre - initiation** complex
- Formation of **48S initiation complex**
- Formation of **80S initiation complex**.

Ribosomal dissociation

- 80S→ 40S+60S
- eIF-3& eIF 1A binds to 40S
- block reassociation with 60S
- eIF-3 –anti-association factor

Formation of 43S PIC

- Ternary complex containing met-tRNA^i & eIF-2 bound to GTP attaches to 40S
- eIF-3 & eIF -1A stabilize the complex

43 S Pre–initiation complex–

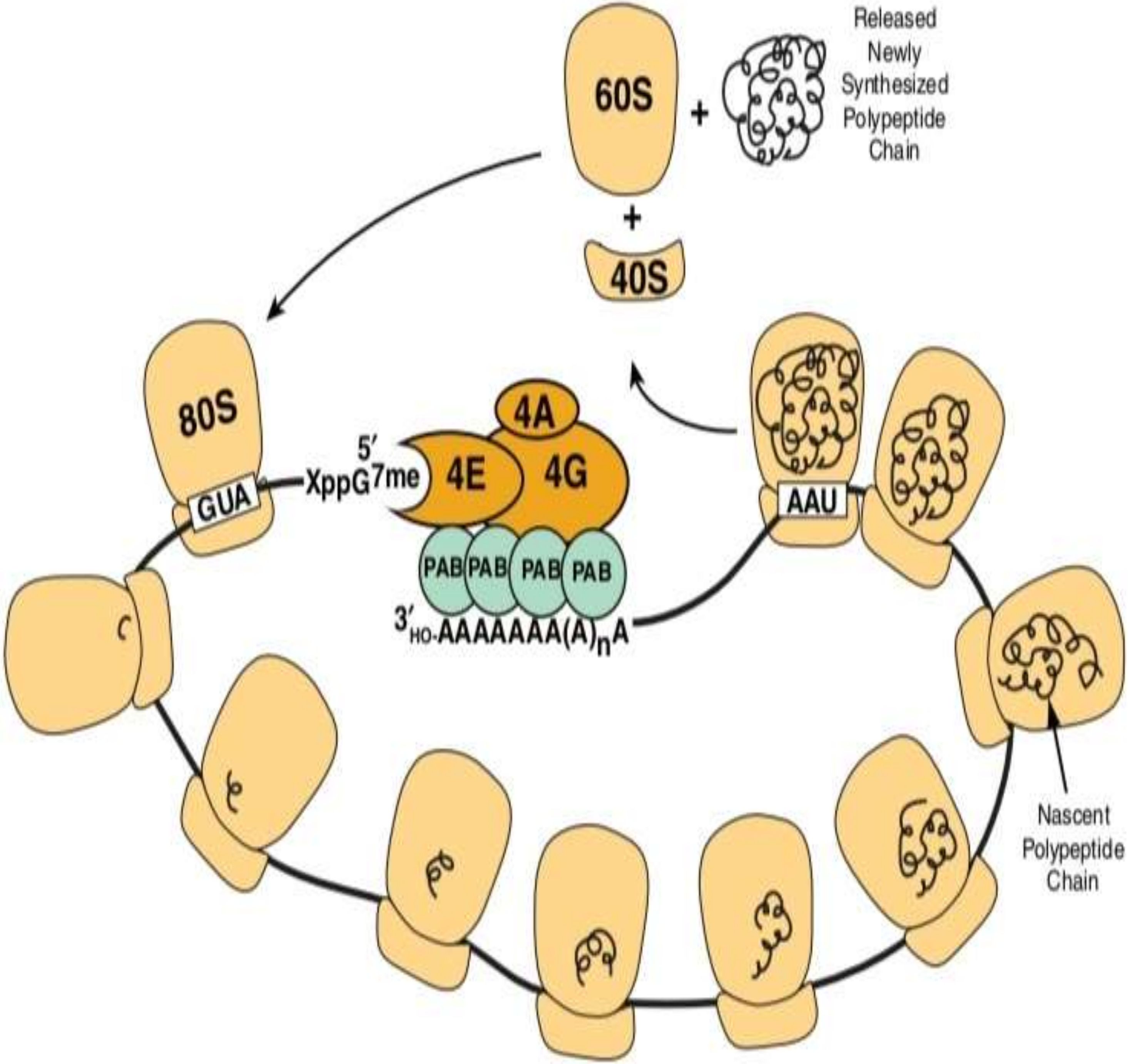
Ternary complex –GTP+elF-2+ met-tRNA

Ternary complex+ 40S ribosome

Formation of 48S IC

- binding of mRNA to 43S PIC→48S IC (intermediate 43S IC)
- EIF -4F complex involved
- EIF-4F→ 4G, 4A, 4E
- Cap binding protein
- Then eIF4A& 4B binds to mRNA
- This mRNA transferred to 43S IC
- Energy-ATP

48 S IC = *PI complex +mRNA* + eIF4 (4A,4B,4E,4G,4F) + ATP


60S
+
Released
Newly
Synthesized
Polypeptide
Chain
+
40S
80S
4A
4E
4G
5′
XppG7me
GUA
AAU
PAB
PAB
PAB
PAB
3′HO-AAAAAAA(A)nA
Nascent
Polypeptide
Chain

Identification of the initiator Codon:

- Ribosomal IC complex scans the m RNA
- The first AUG sequence after the marker sequence →start codon
- **Marker sequence:**

Prokaryotes :Shine Dalgarno sequence

Eukaryotes: Kozak consensus sequence

- AUG - met in eukaryotes and N-Formyl methionine in prokaryotes.
- This forms the N- terminal end of amino acid in the newly synthesized polypeptide chain.

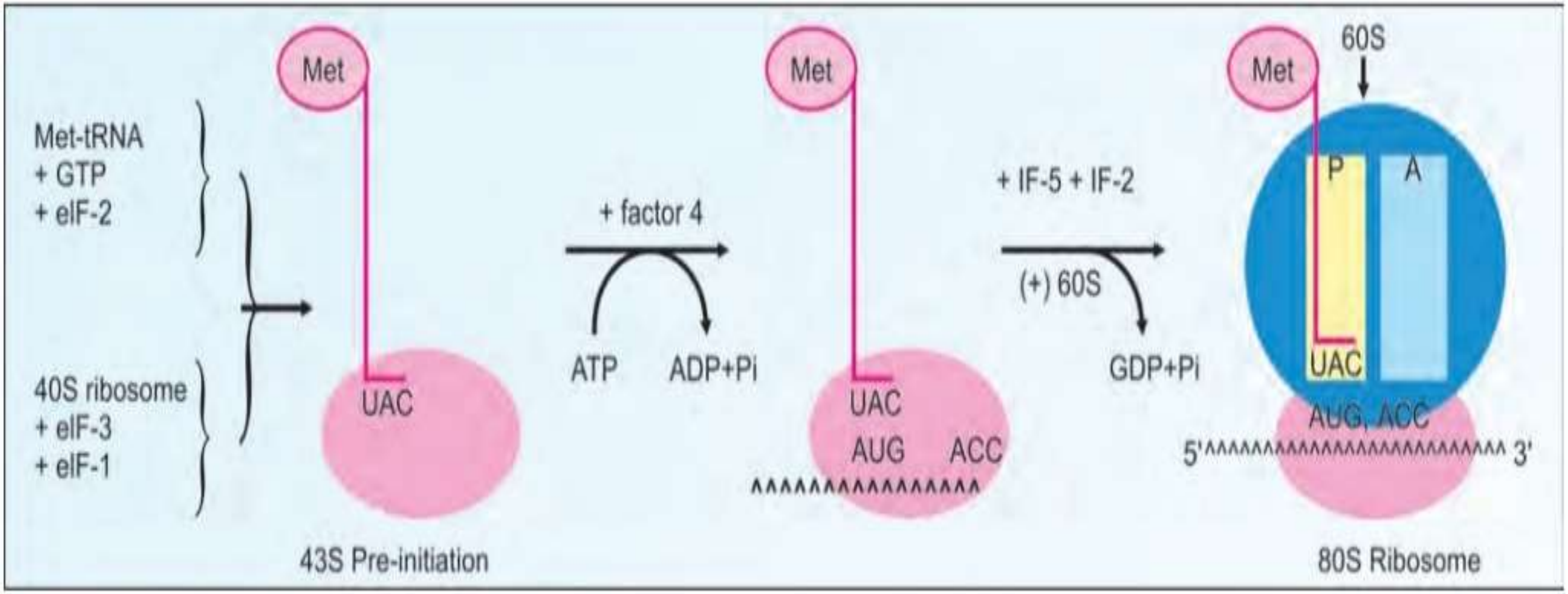
Formation of 80S IC

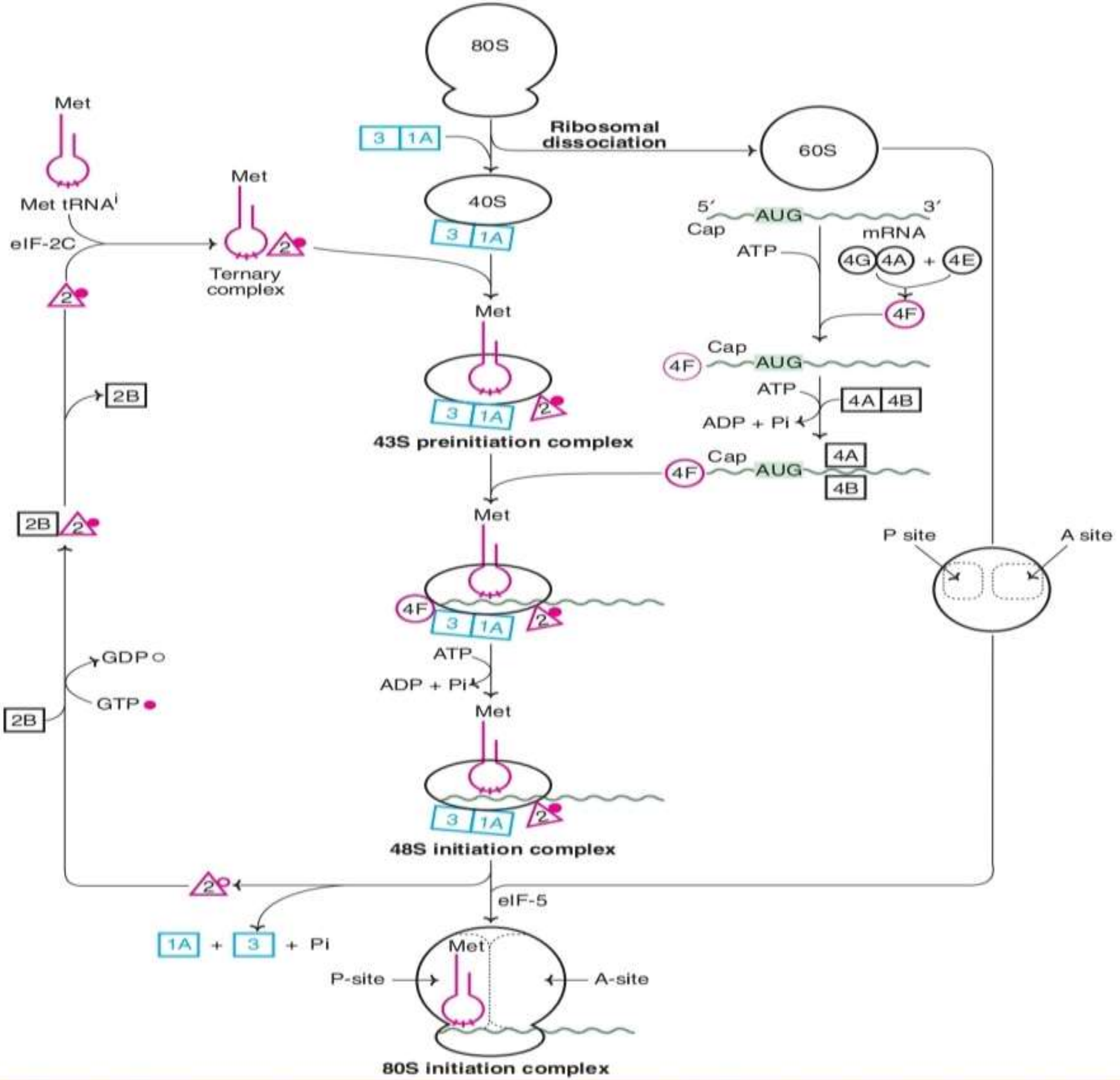
- 48S IC binds to 60S ribosome
- Involves hydrolysis of GTP (Bound to eIF 2)
- eIF 5

80S IC – ***48 S IC+60S*****+eIF2+eIF5** +GTP

80S complex formed

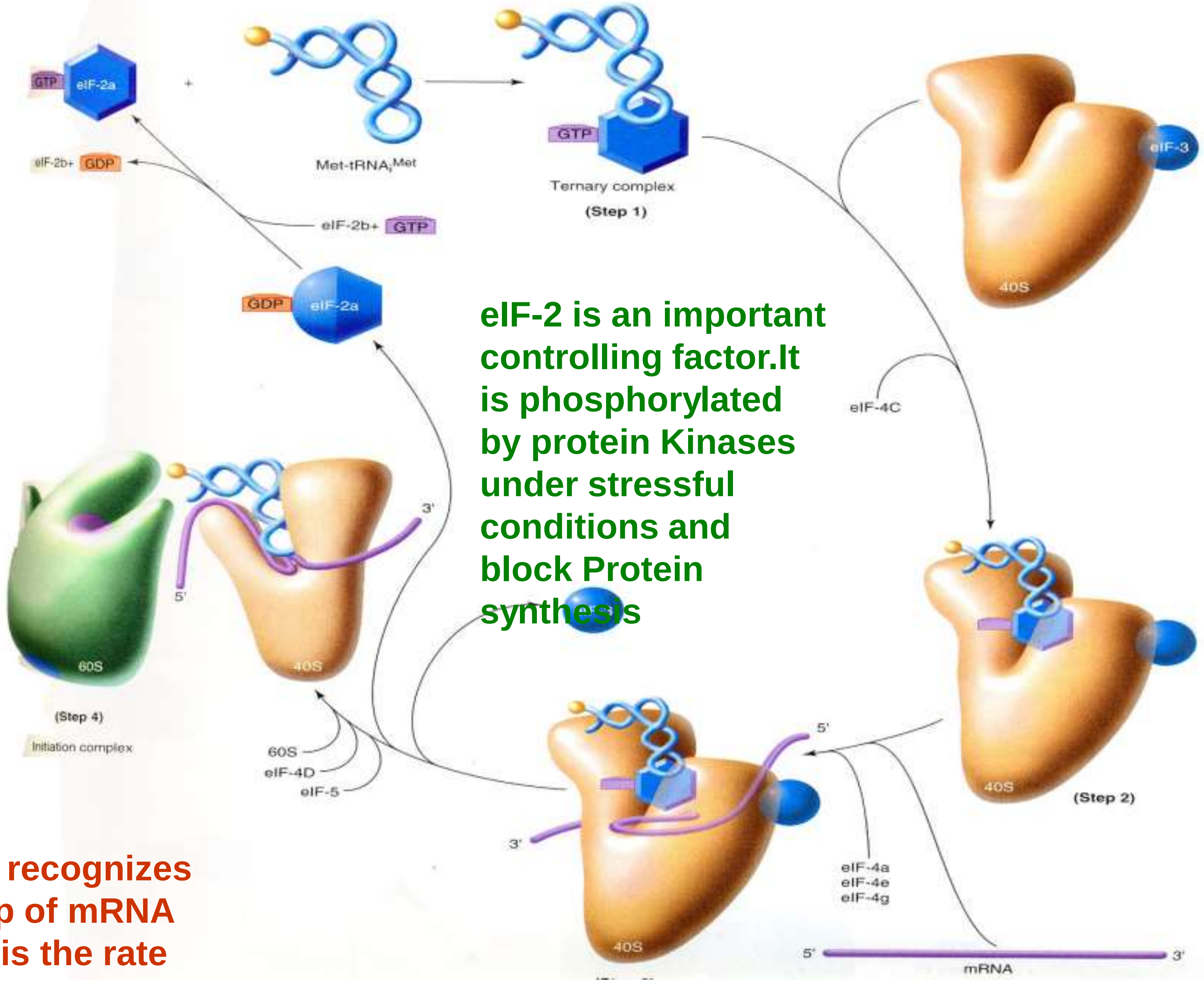
- IF released & recycled
- Activation of IF 2 require eIF2B (guanine NT exchange factor) & GTP
- Activated eIF2 require eIF2C to form ternary complex


Met-tRNA
+ GTP
+ eIF-2
40S ribosome
+ eIF-3
+ eIF-1
Met
UAC
43S Pre-initiation
+ factor 4
ATP
ADP+Pi
Met
UAC
AUG
ACC
^^^^^^^^^^^^^^^^
+ IF-5 + IF-2
(+) 60S
GDP+Pi
Met
60S
P
A
UAC
AUG, ACC
5'^^^^^^^^^^^^^^^^^^^^^^^^^^ 3'
80S Ribosome


80S
3
1A
Ribosomal dissociation
60S
Met
Met tRNA^i
eIF-2C
Met
2
Ternary complex
40S
3
1A
5′
AUG
3′
Cap
mRNA
ATP
4G
4A
+
4E
4F
Met
2
Cap
4F
AUG
ATP
4A
4B
ADP + Pi
2B
3
1A
2
43S preinitiation complex
Cap
4F
AUG
4A
4B
2B
2
Met
P site
A site
4F
3
1A
2
ATP
ADP + Pi
GDP ○
GTP ●
2B
Met
3
1A
2
48S initiation complex
2
eIF-5
1A
+
3
+ Pi
Met
P-site
A-site
80S initiation complex

Regulation of initiation

- eIF-4F complex
- 4E responsible for recognition of mRNA cap
- Rate limiting
- eIF 2 also has a control



eIF-2 is an important controlling factor.It is phosphorylated by protein Kinases under stressful conditions and block Protein synthesis

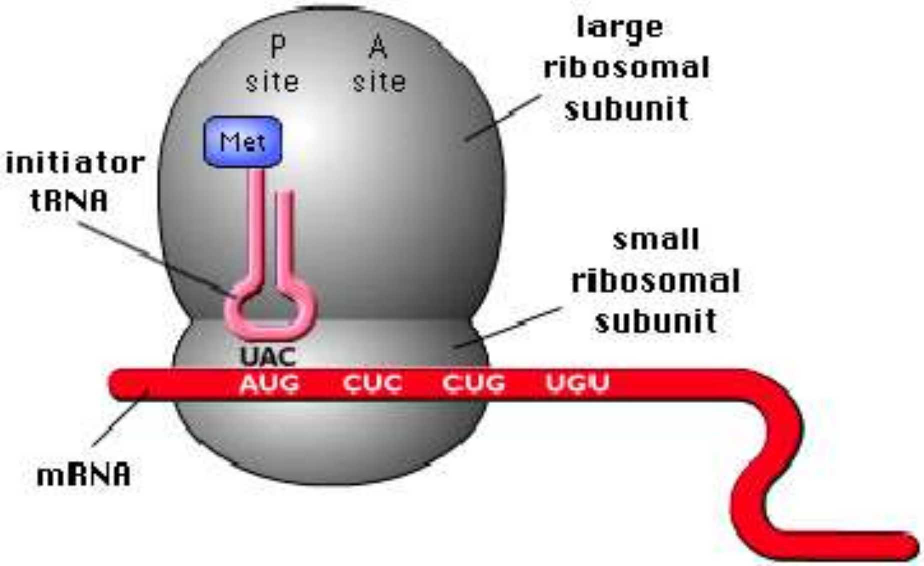
eIF-4E recognizes the cap of mRNA which is the rate limiting step.

Initiation of translation in prokaryotes

- 30S ribosomal subunit bounds to IF -3
- attaches to ternary complex of IF 2, formyl met t RNA & GTP
- IF -1

2 receptor sites for tRNA on ribosomes

- P Site (peptidyl site)
- A Site (amino acyl site)



Now the $\mathrm{tRNA}^{\mathrm{met}}$ is on the peptidyl site. A site is free.

ELONGATION OF TRANSLATION

- By sequential addition of amino acids
- Sequence is determined by the order of the codon in the specific m RNA
- Involves elongation factors

3 steps

- Binding of aminoacyl t-RNA to A site
- Peptide bond formation
- translocation

Binding of aminoacyl t-RNA to A site

- 80S IC complex contain met t-RNA^i in the P site
- A site is free
- Another aminoacyl t-RNA placed in the A site
- EF-1a
- GTP

- As aminoacyl t-RNA is placed in the A site,EF-1α & GDP recycled to bring another aminoacyl t-RNA

Peptide bond formation

- Peptidyl transferase
- 28Sr-RNA
- AA is already activated , no additional energy

- Net result of peptide bond formation → attachment of growing peptide chain to the t-RNA in the A site

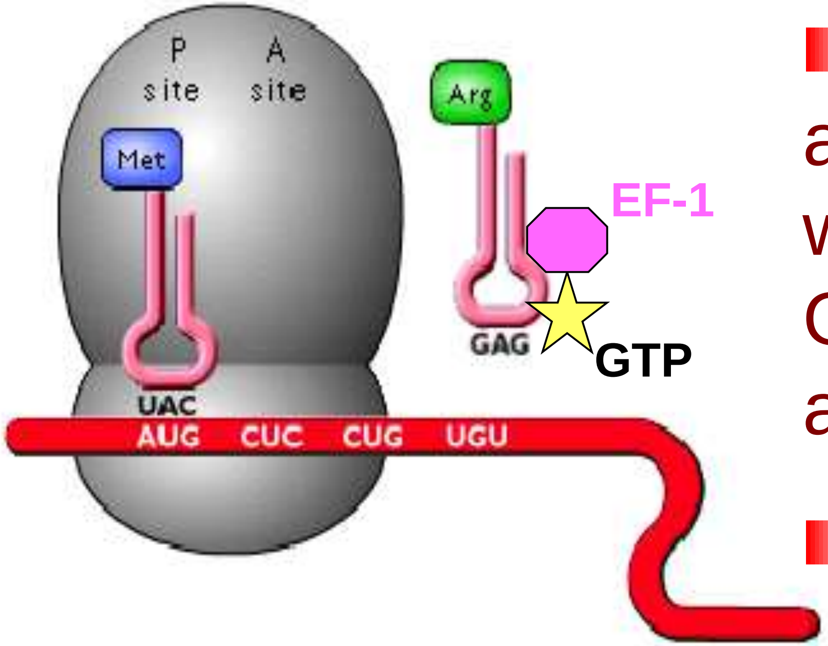
TRANSLOCATION

- As the peptide bond formation occurs, the ribosome moves to the next codon of m-RNA
- TRANSLOCATION
- Involves movement of growing from A Site to P site

- Requires EF-2 & GTP complex

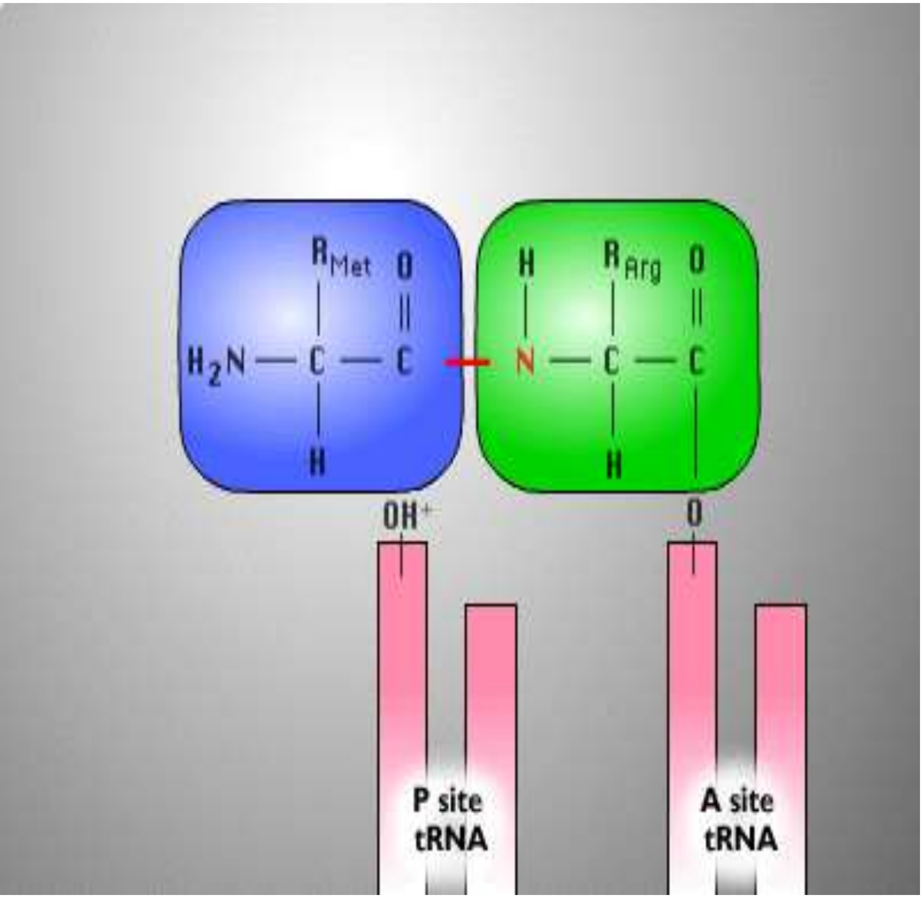
- Deacylated t- RNA moves into the E site
- It leaves the ribosome

Elongation



- Next codon in mRNA determines the incoming amino acid.
- Elongation factor EF-1 and GTP are complexed with the incoming tRNA^{aa} . GTP is hydrolysed to GDP and tRNA^{aa} binds to A site.
- EF-1 is released.

Elongation – Peptide bond formation

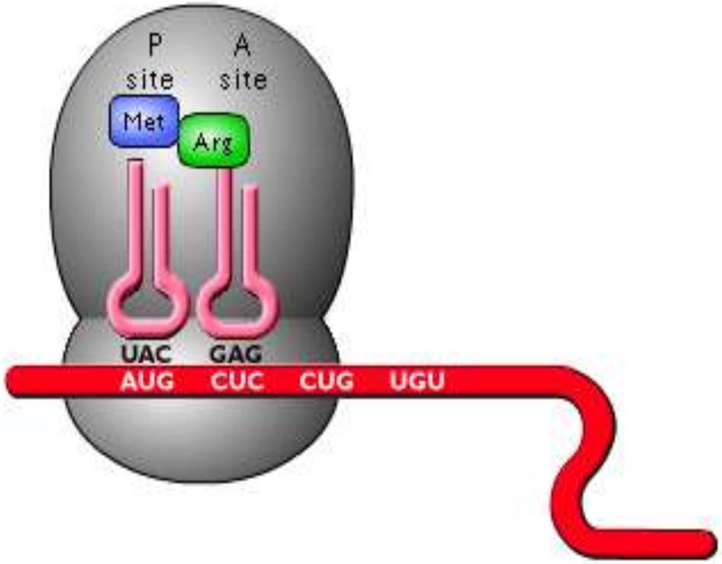


- The alpha amino gp of the incoming amino acid in the A site forms peptide bond with the COOH gp of the peptidyl tRNA in the P site.
- Enzyme is Peptidyl transferase.

Peptidyl Transferase- Ribozyme

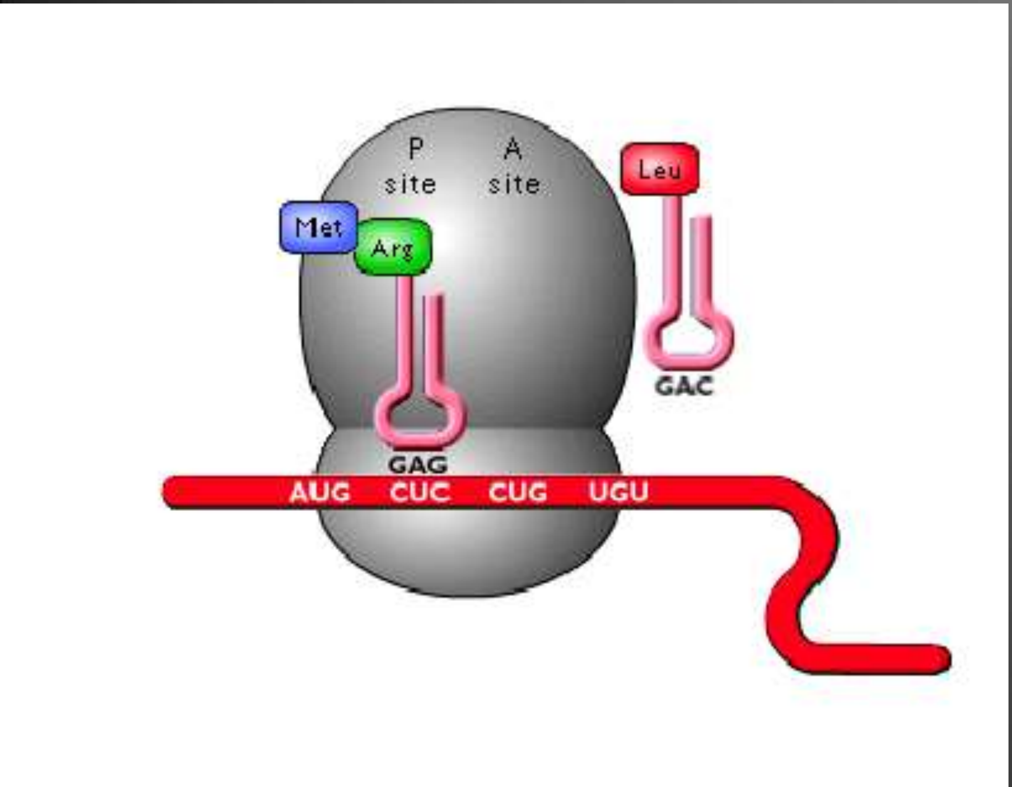
- Enzyme is a ribozyme.
- 28S rRNA, a sub unit of 60S Ribosomal subunit.
- Since $tRNA^{aa}$ is already activated no need of energy for peptide bond formation.

Translocation

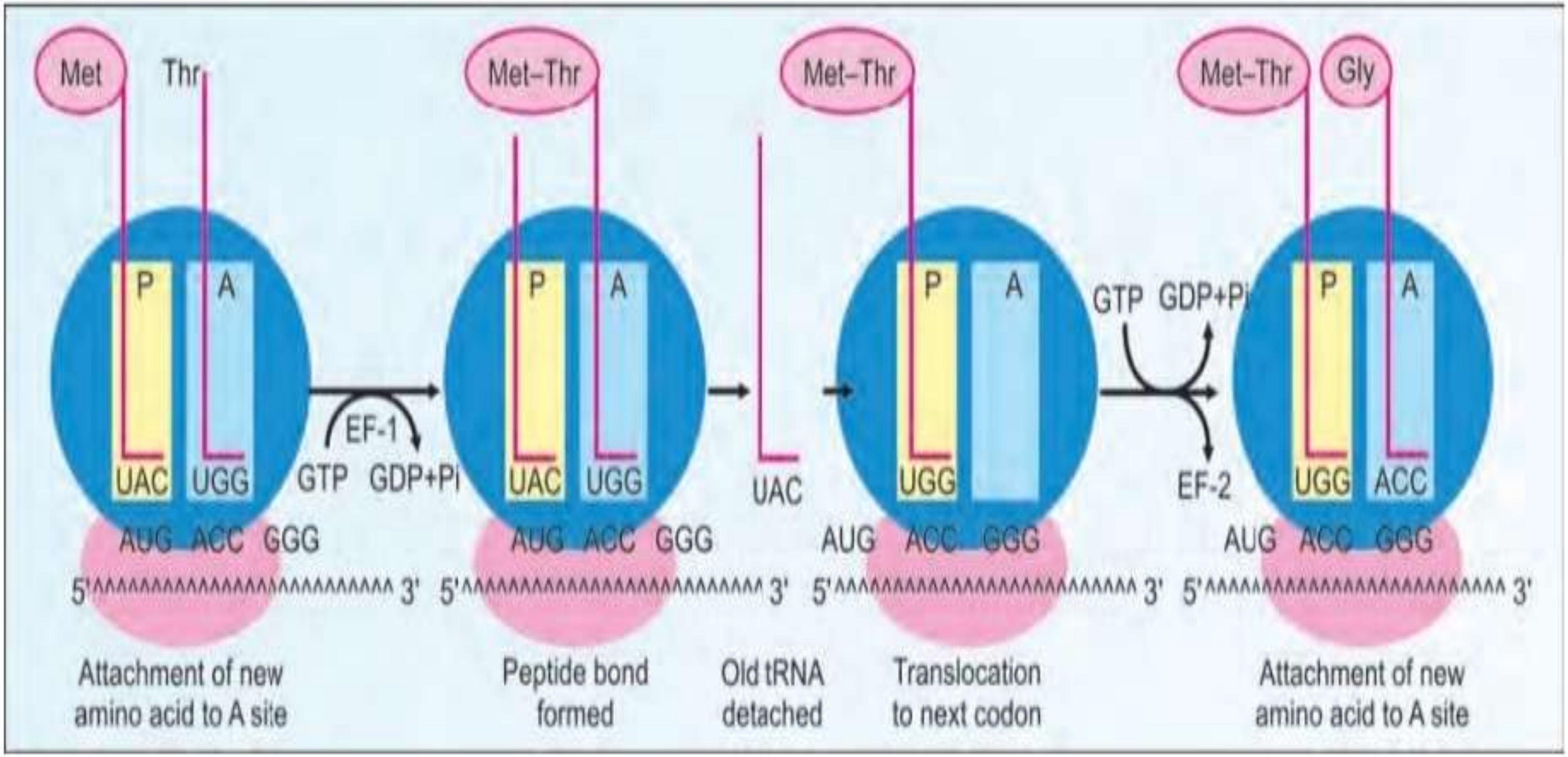


- Now the growing peptide chain is in the A site.
- The uncharged tRNA is released from the ribosomes.
- P site is empty.
- Whole ribosome moves over mRNA through a distance of one codon.

Translocation



- Peptidyl tRNA is translocated to P site.
- A site is free to receive the next incoming tRNA^{aa}.
- Translocation requires EF-2 and GTP.


Met
Thr
P
A
UAC
UGG
AUG ACC GGG
5'^^^^^^^^^^^^^^^^^^^^^^^^^^ 3'
Attachment of new amino acid to A site
EF-1
GTP
GDP+Pi
Met–Thr
P
A
UAC
UGG
AUG ACC GGG
5'^^^^^^^^^^^^^^^^^^^^^^^^^^ 3'
Peptide bond formed
UAC
Old tRNA detached
Met–Thr
P
A
UGG
AUG ACC GGG
5'^^^^^^^^^^^^^^^^^^^^^^^^^^ 3'
Translocation to next codon
GTP
GDP+Pi
EF-2
Met–Thr
Gly
P
A
UGG
ACC
AUG ACC GGG
5'^^^^^^^^^^^^^^^^^^^^^^^^^^ 3'
Attachment of new amino acid to A site

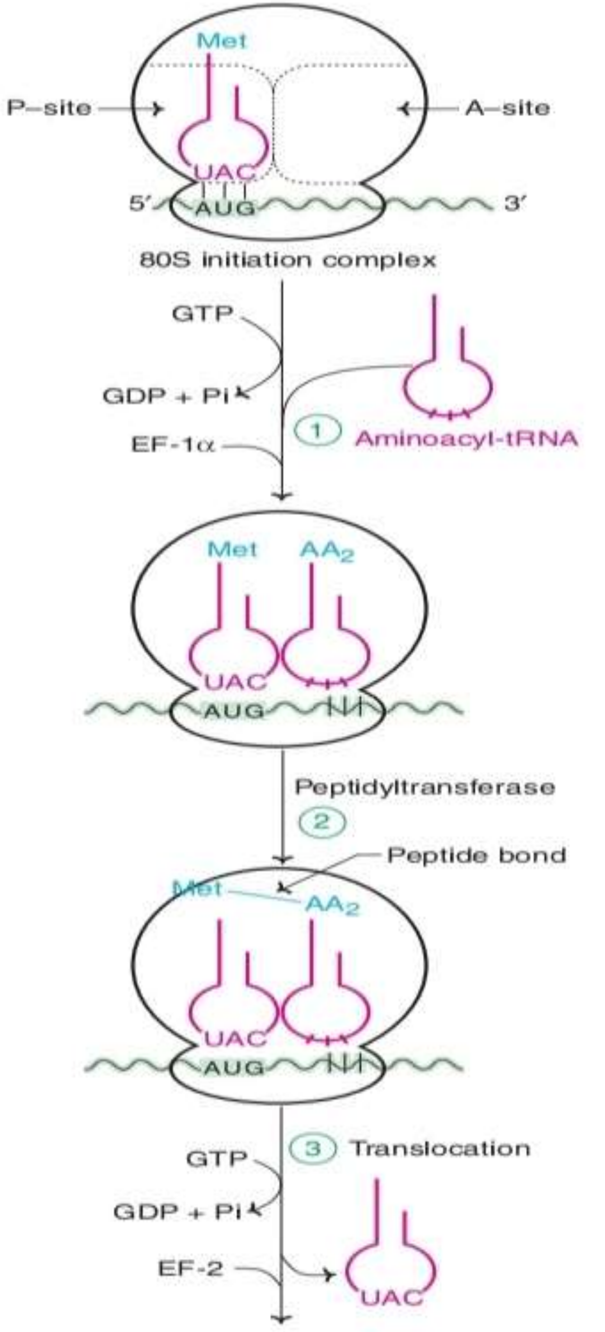
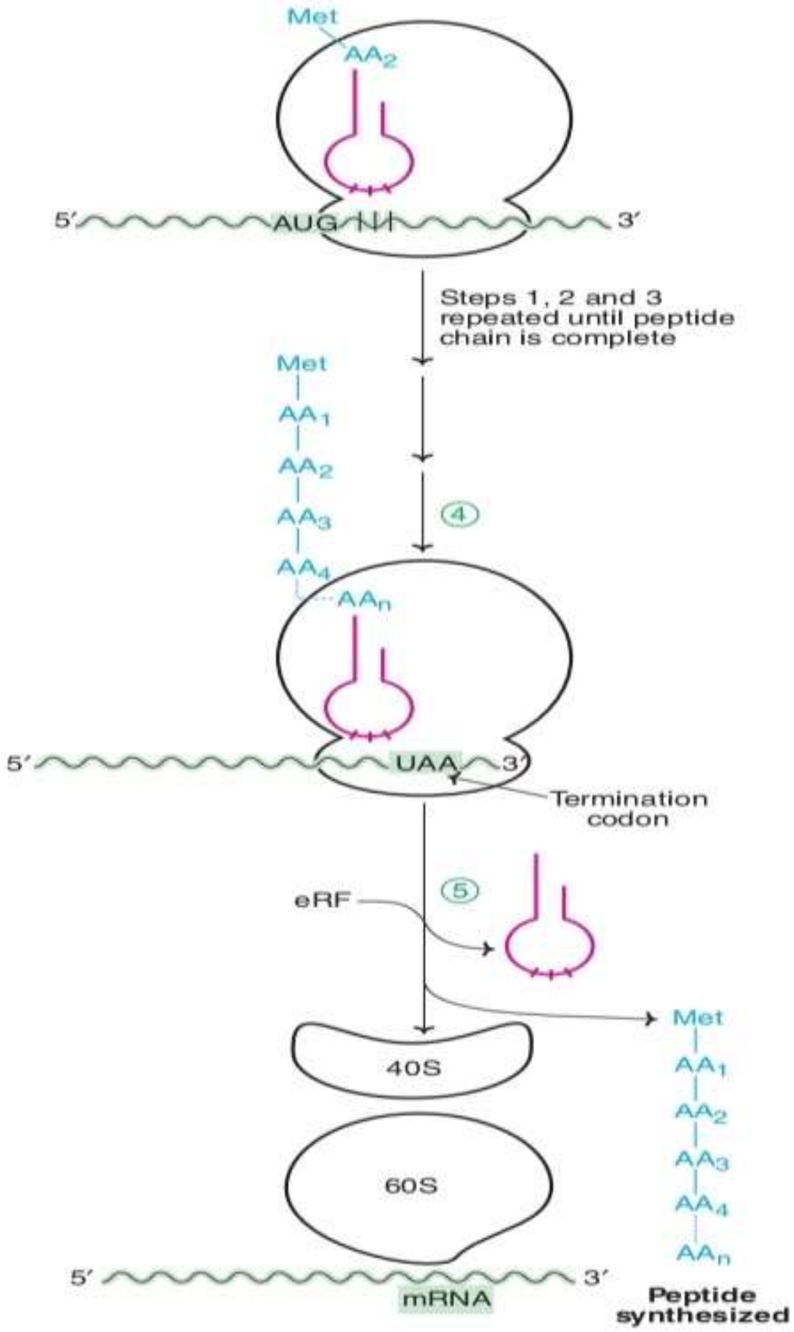
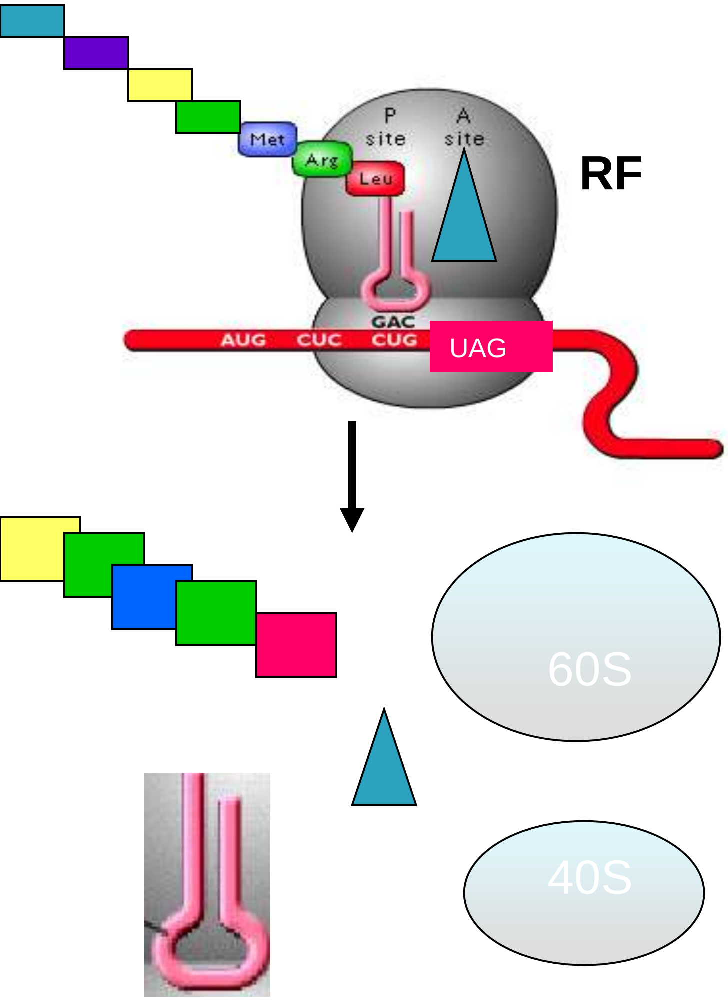


Fig. 25.18 contd. next column



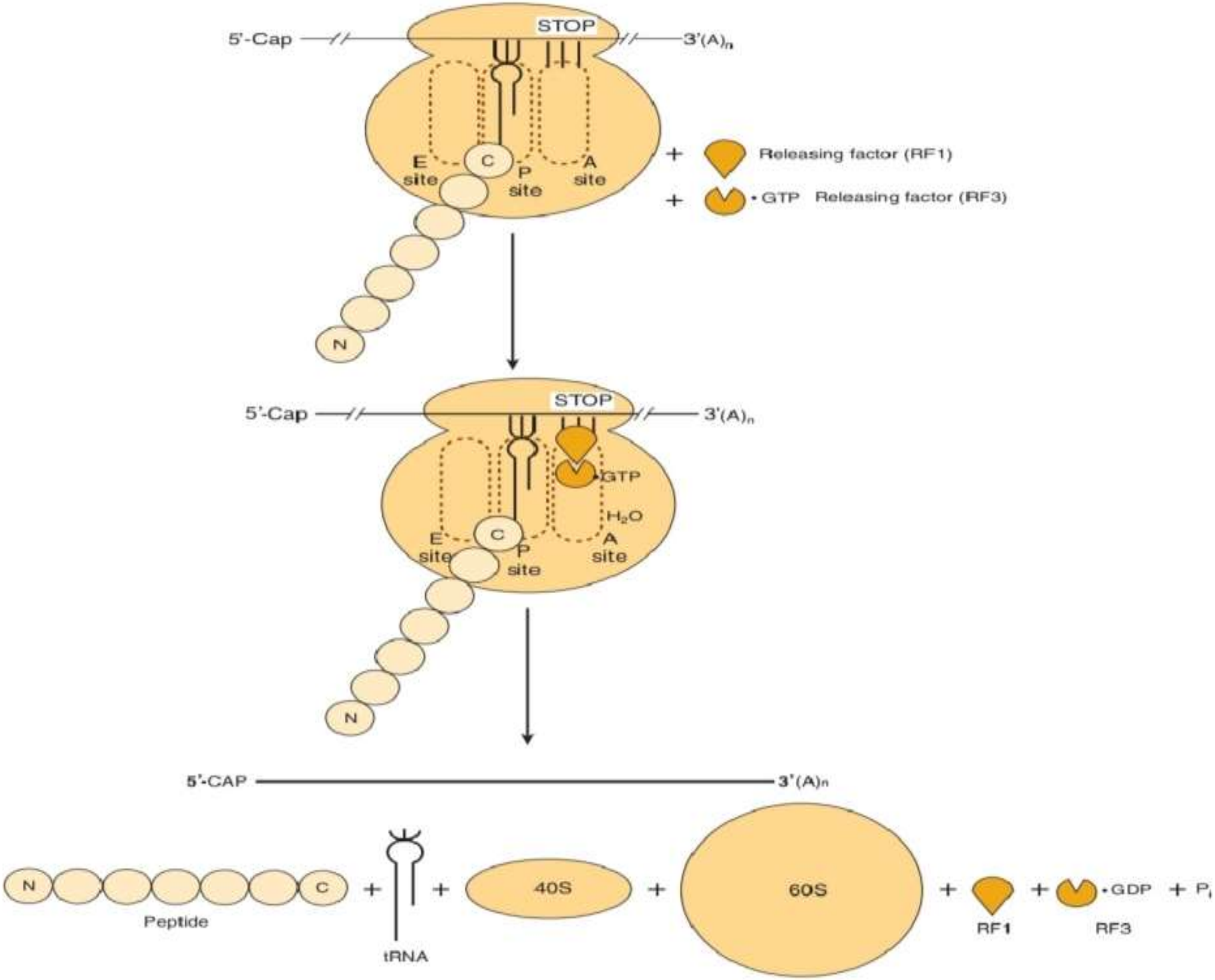
Termination

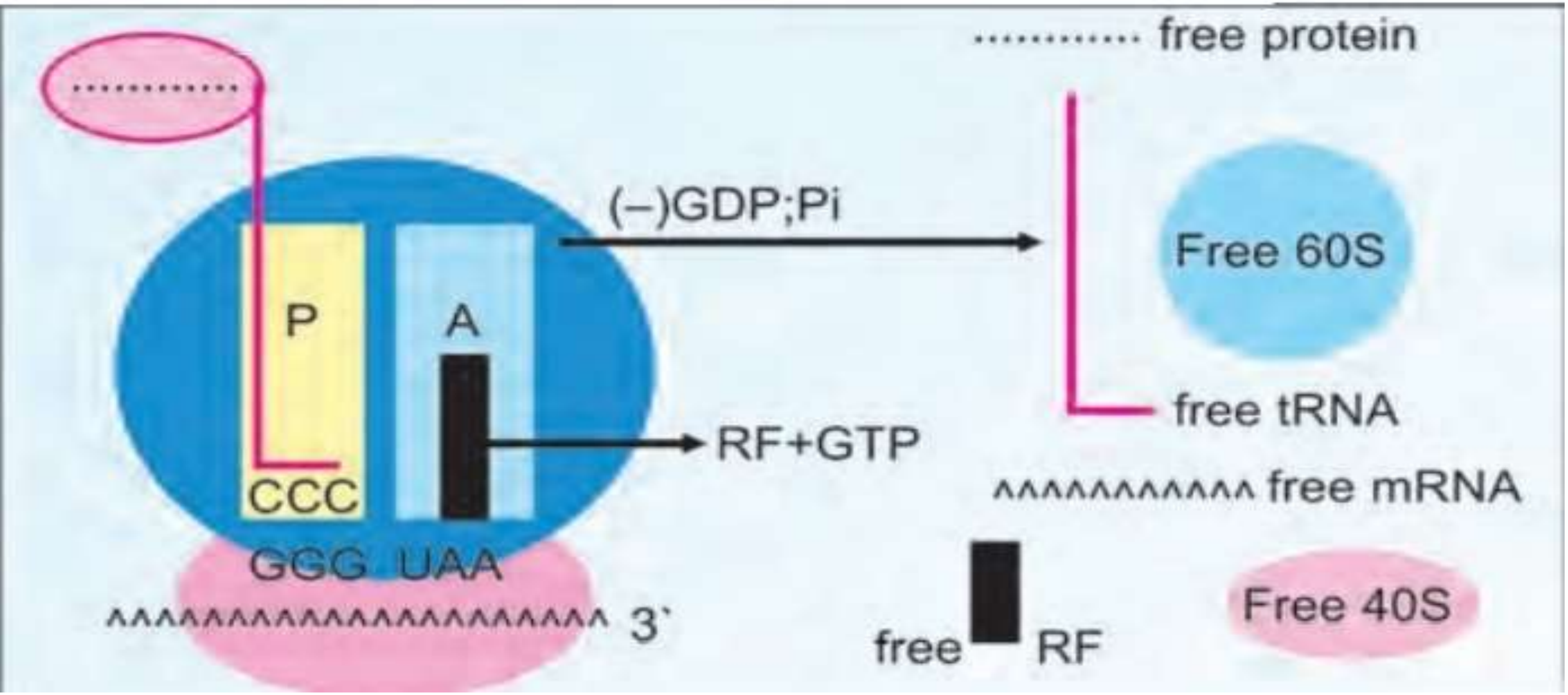


- Next codon is a stop Codon.
- A site is empty, but no tRNA anticodon corresponding to the stop codon.
- Releasing factor enters A site. Binds with ribosome with hydrolysis of GTP to GDP.

.

- RF hydrolyses the peptide bond on peptidyl tRNA at the P site and release the polypeptide chain
- A water molecule instead of aminoacid is added
- Releases protein
- Uncharged tRNA at P site is also released.
- Ribosomes dissociate to 40 S and 60S units.


5'-Cap
STOP
3'(A)n
E site
C
P site
A site
N
+ Releasing factor (RF1)
+ • GTP Releasing factor (RF3)
5'-Cap
STOP
3'(A)n
• GTP
H_2O
E site
C
P site
A site
N
5'-CAP
3'(A)n
N
C
Peptide
+
tRNA
+
40S
+
60S
+
RF1
+
• GDP
RF3
+ P_i


free protein
P
A
(–)GDP;Pi
Free 60S
free tRNA
RF+GTP
CCC
GGG UAA
free mRNA
3`
free RF
Free 40S

Energetics

- Charging of tRNA→ $tRNA^{aa}$ – 2 ~P
- EF1– binding of $tRNA^{aa}$ to A Site– 1GTP
- EF2– Translocation –1GTP

Hence

- For each peptide bond formation – 4~P

1ATP–IC formation

1GTP– 80S ribosome

1GTP– termination

Inhibitors of protein Synthesis

Reversible: Bacteriostatic

- Tetracyclins: Bind with 30S ribosomal unit and inhibit binding of $tRNA^{aa}$ to A site of ribosomes.
- Chloramphenicol: Inhibit peptidyl transferase activity of bacterial ribosomes.
- Erythromycin and Clindamycin :Inhibit translocation process.

Irreversible Inhibitors- Bacteriocidal

- Streptomycin and other aminoglycosides:
  Bind to 30S subunit .

At low concentrations cause misreading of the codons and produce useless proteins.

At pharmacological dose, they inhibit formation of initiation complex.

Mammalian protein Synthesis Inhibitors

- Puromycin: Structural similarity to tRNA^{tyr}.
- Cycloheximide: Inhibit peptidyl transferase of 60S ribosomes.
- Diphtheria toxin: Inactivate EF2.
- Ricin – a highly toxic protein from castor bean inactivate eukaryotic 28 S ribosomal unit.

THANK YOU