ZO502 CELL AND MOLECULAR BIOLOGY

Genetic Code and Protein Synthesis

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GENETIC CODE

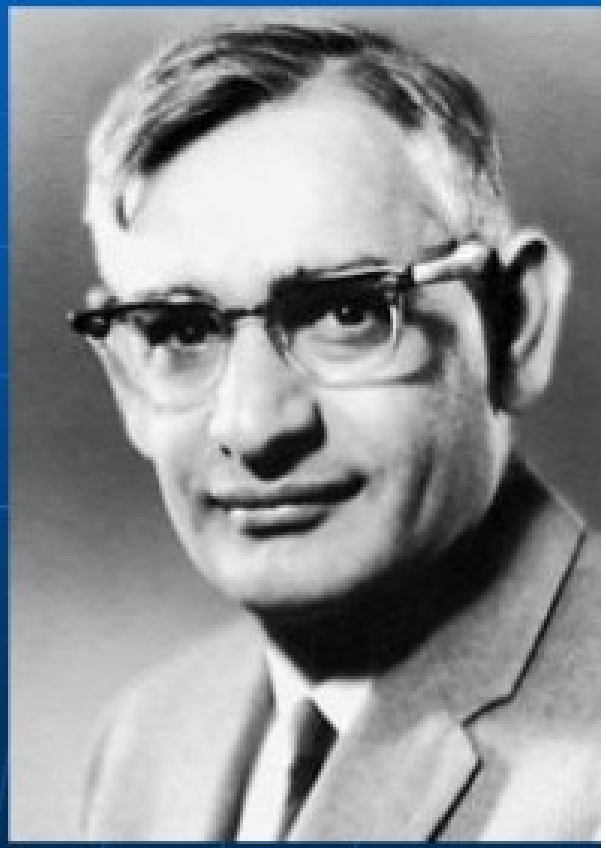
Definition: System of nucleotide sequences of mRNA that designates particular amino acid sequences in the process of translation.

- Genetic code is the relation between the sequence bases in DNA and the sequence of amino acids in protein
- Code words for amino acids--**Codons**

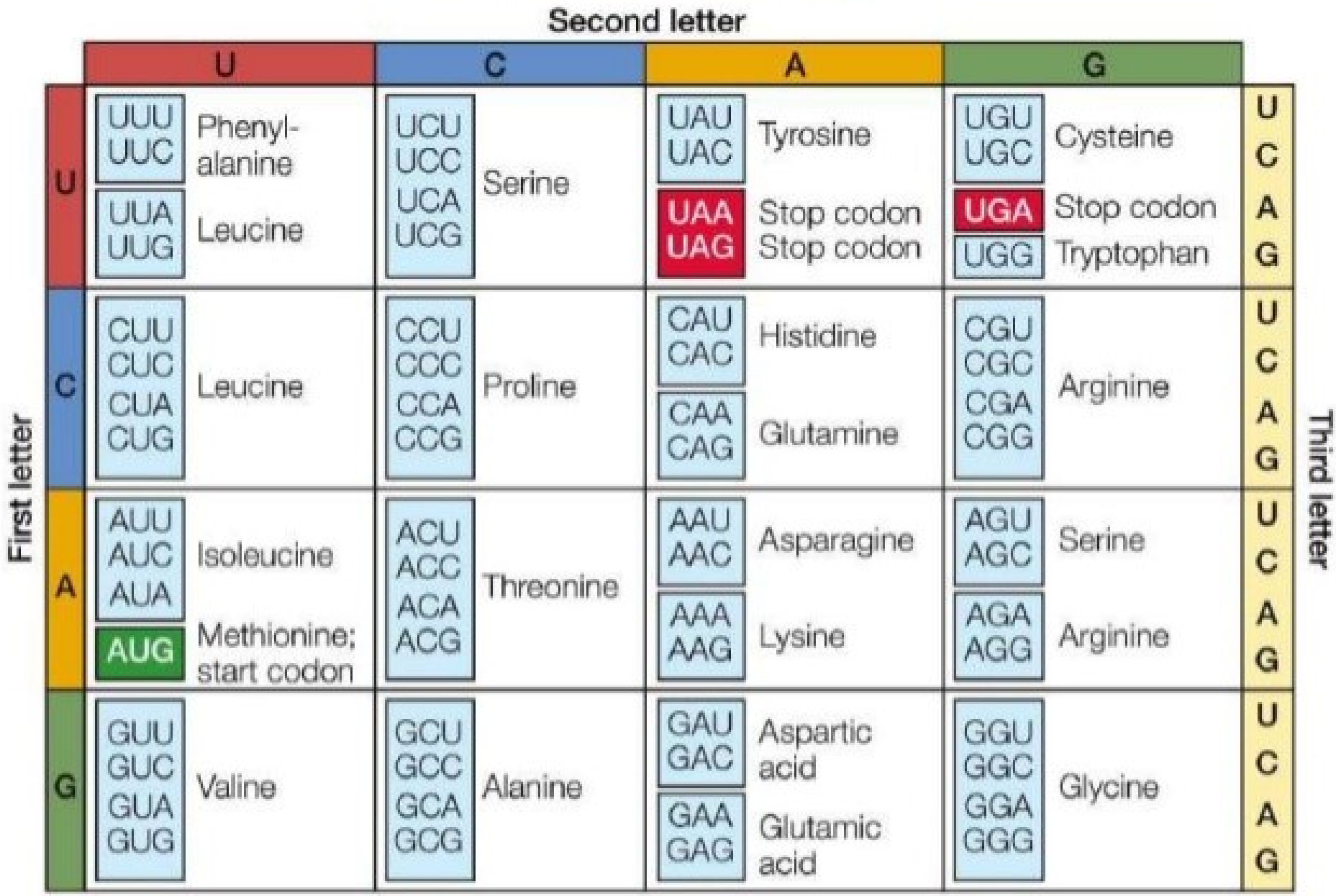
Indian-American scientist

He showed the order of nucleotides in nucleic acids, which carry the genetic code of the cell and control the cell's synthesis of proteins.

Shared NP with Nirenberg and Holley



THE GENETIC CODE



Characteristics

1. **64** possible codons

4 nucleotides– A,G,C,U

Triplet base codons

$4^3=64$

2. **UNIVERSAL**

Few exceptions– AUA codes for Met in mitochondria

AUA– Ile in cytoplasm

UGA– stop codon in Cytoplasm

UGA– Trp in Mt

Bacteria– GUG,UUG, AUU,CUG initiating codons

3. **Stop codon and Initiating codon**

AUG– Start codon

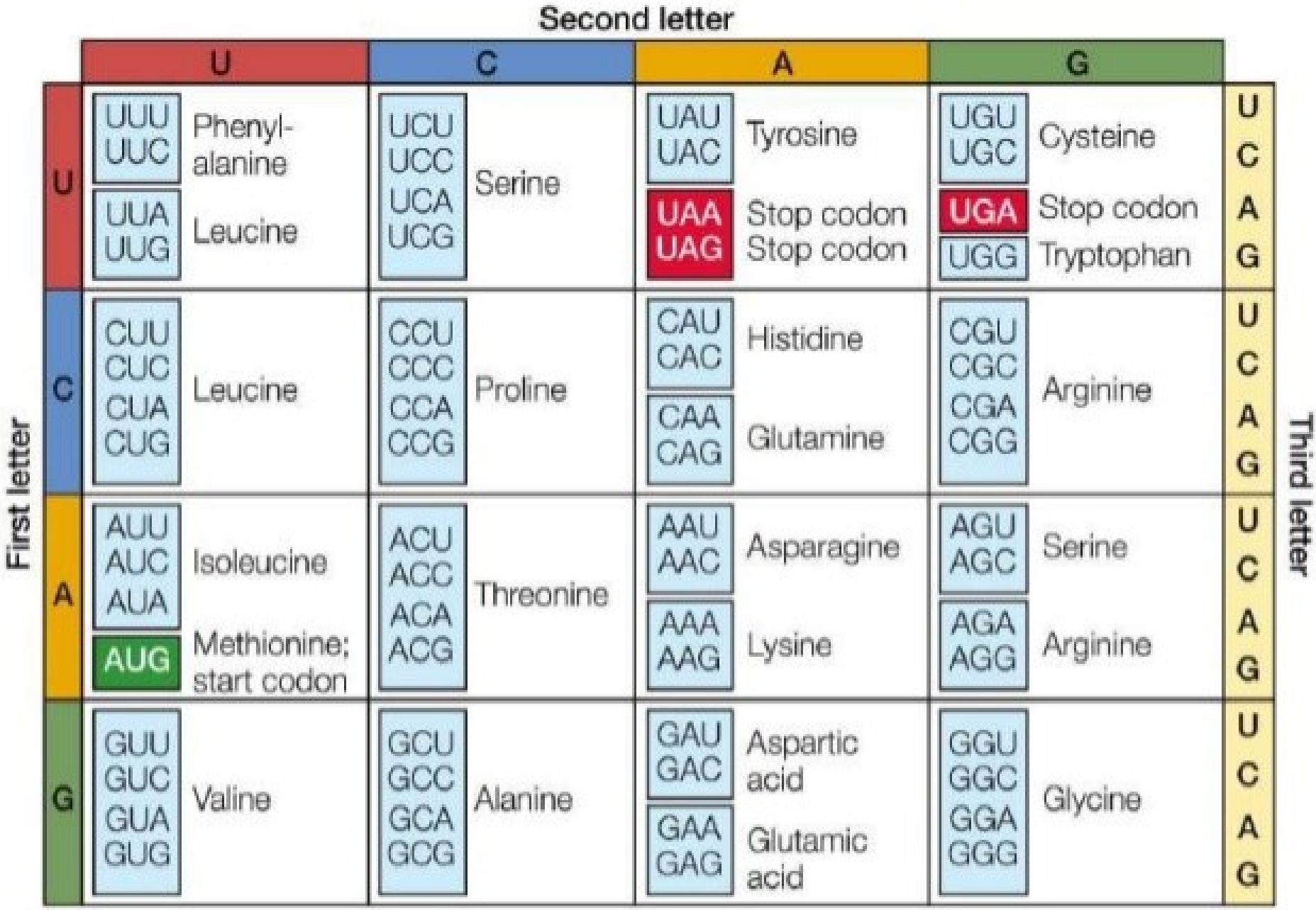
UAA,UGA and UAG– stop/ non-sense codon

Exception– 21st AA Selenocystine--UGA

4.Genetic code is **degenerate**

5. It is **unambiguous**

THE GENETIC CODE



6. Code is **non-overlapping** and **without punctuation**

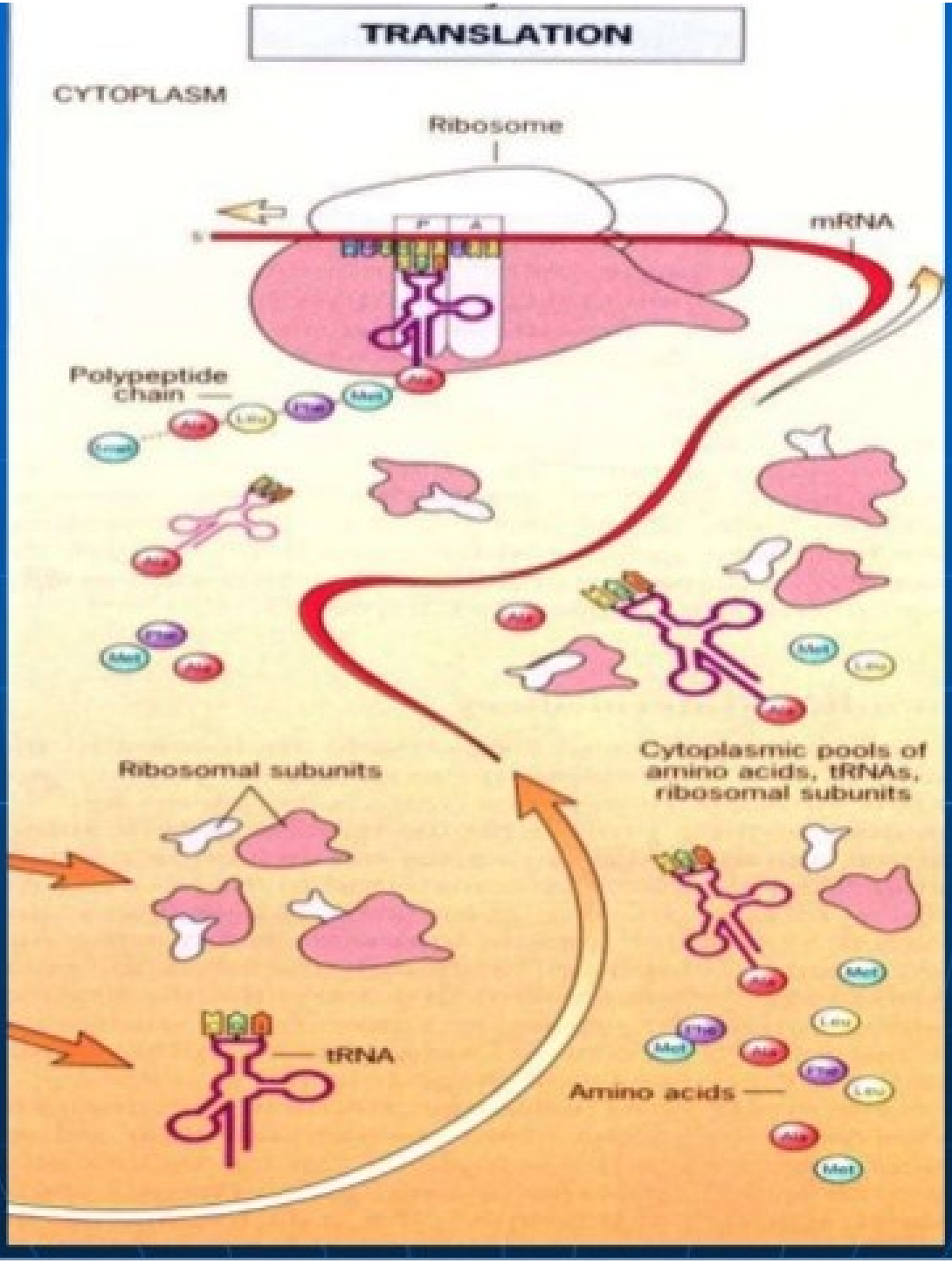
7. **Wobbling phenomenon** —reduced stringency between the 3rd base of codon and complementary base of anticodon

Mitochondria have different codes

TRANSLATION PROCESS

Translation (An Overview)

- Translation is defined as protein synthesis.
- Occurs on **ribosomes**—mRNA → Potein
- mRNA is translated in the 5′ to 3′ direction.
- Highly regulated
- Very fast– **20AA/sec**
- Amino acids are brought to the ribosome bound to a specific tRNA molecule.
- The mRNA and tRNA are responsible for the correct recognition of each amino acid in the growing polypeptide


TRANSLATION
CYTOPLASM
Ribosome
mRNA
Polypeptide chain
Ribosomal subunits
tRNA
Cytoplasmic pools of amino acids, tRNAs, ribosomal subunits
Amino acids

- Template - mRNA
- tRNAs (transfer RNAs)
  - Linked to amino acids
- Ribosomes
- Many accessory proteins
- Some energy (GTP hydrolysis)

Functions of the Types of RNA

- **mRNA**- serves as a template code
- **tRNA-** serves as an adapter molecule
- **rRNA-** holds molecules in the correct position, protein portion also catalyze reactions

Translation Is the Most Complicated Biological Process Known

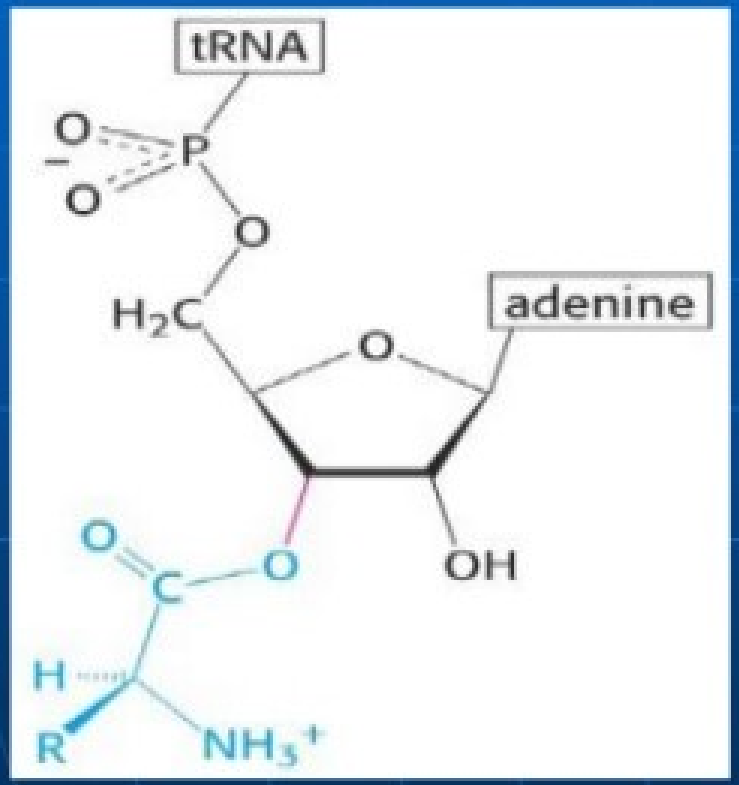
In eukaryotes,

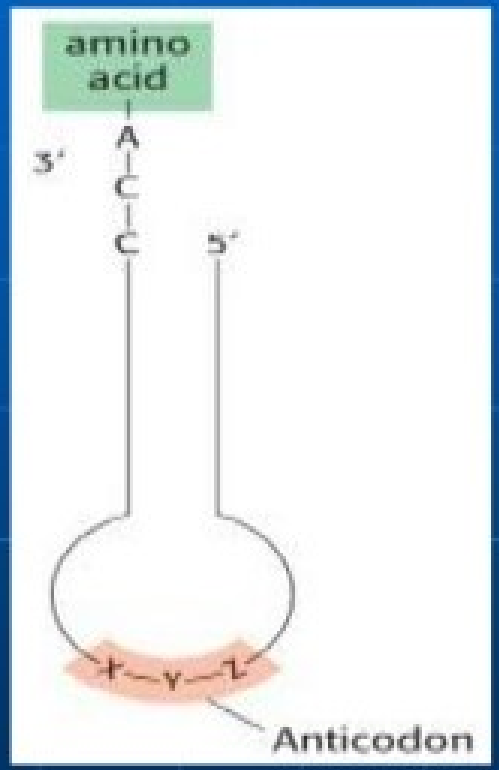
- >70 ribosomal proteins
- 20 (more) proteins to activate aa's
- 12 (more) auxiliary enzymes
- ≥100 proteins for processing
- ≈40 tRNA and rRNAs (minimum)
- Other specific proteins
- ~300 molecules

Five stages

- Preinitiation
- Initiation
- Elongation
- Termination
- Post-translational modification

Pre-initiation/Charging of AA

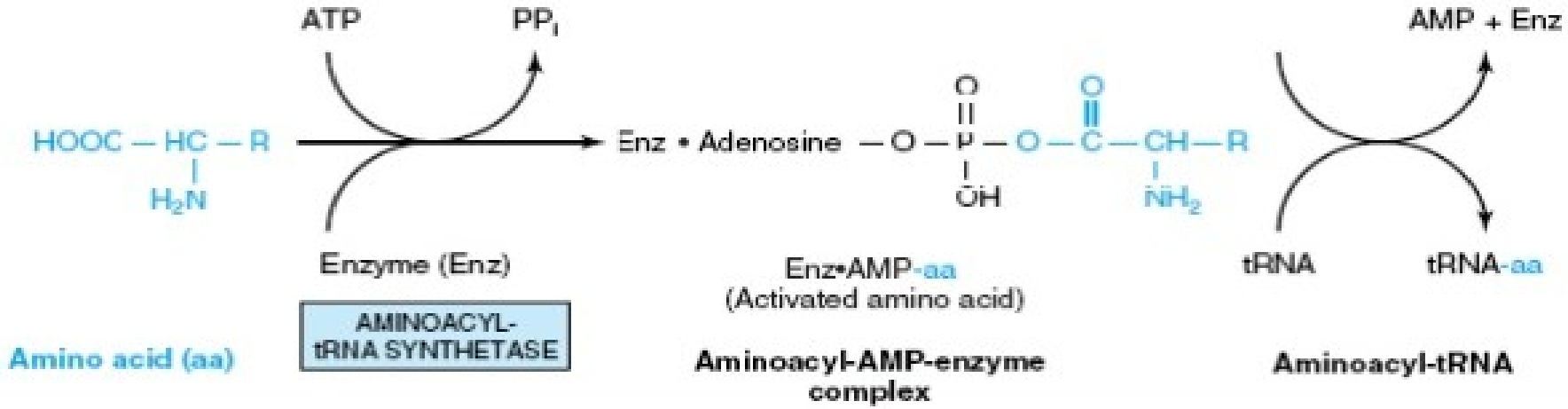


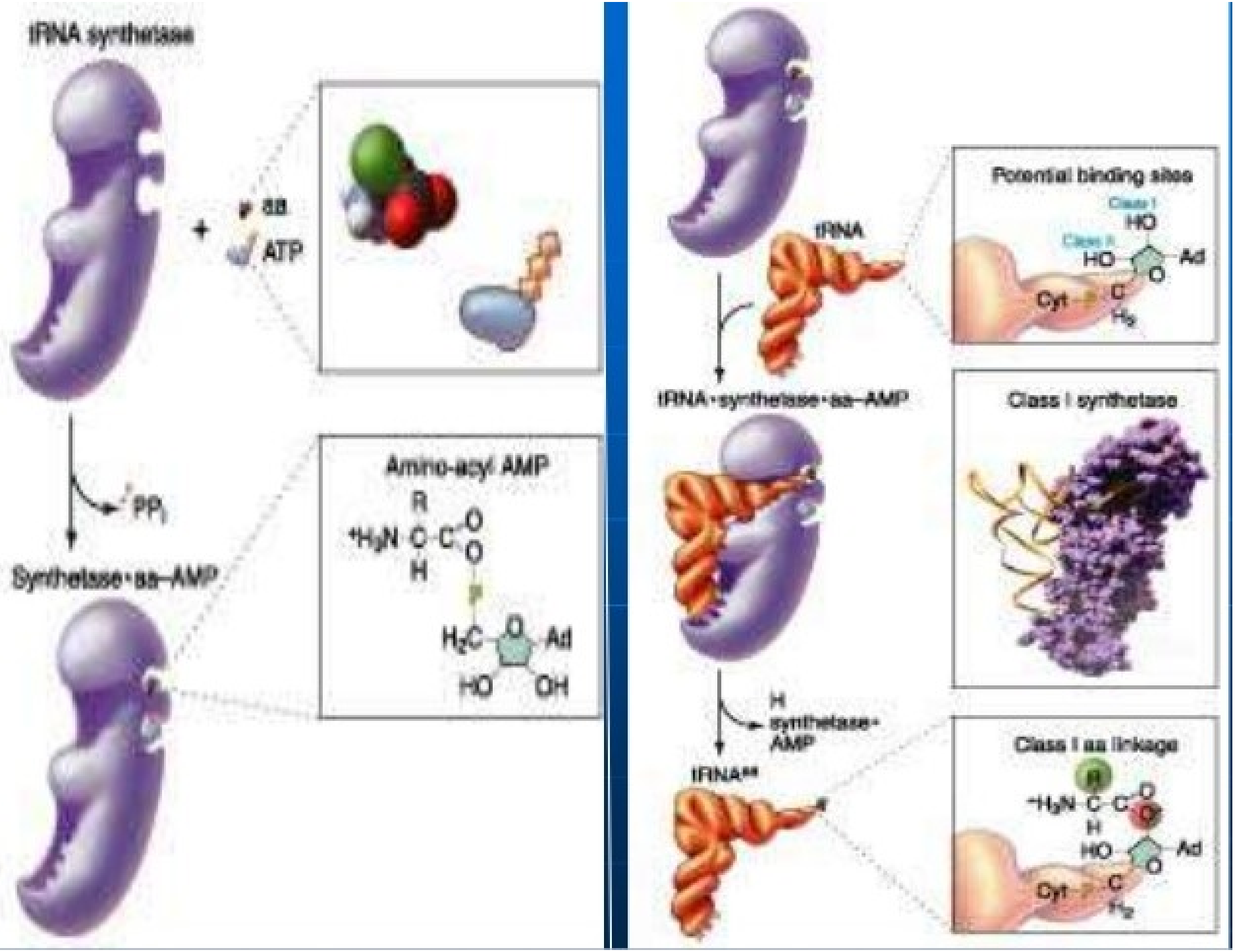


Aminoacyl t-RNA

Charging of tRNA

- Linking amino acids to correct t-RNAs
- Catalyzed by aminoacyl-tRNA synthetase (aa-tRNA)
- Couples an amino acid to its cognate tRNA
- Fidelity of coupling – 20 different synthetases
- Two steps
  - Activation of amino acid
  - Transfer of amino acid to tRNA




tRNA synthetase
aa
ATP
PPi
Synthetase·aa–AMP
Amino-acyl AMP
tRNA
tRNA·synthetase·aa–AMP
Potential binding sites
Class I
Class II
Cyt
Class I synthetase
synthetase·AMP
tRNAaa
Class I aa linkage

Initiation

- Attachment of initiator tRNA (Met-tRNA) to start codon on mRNA and assembly of ribosomal subunits

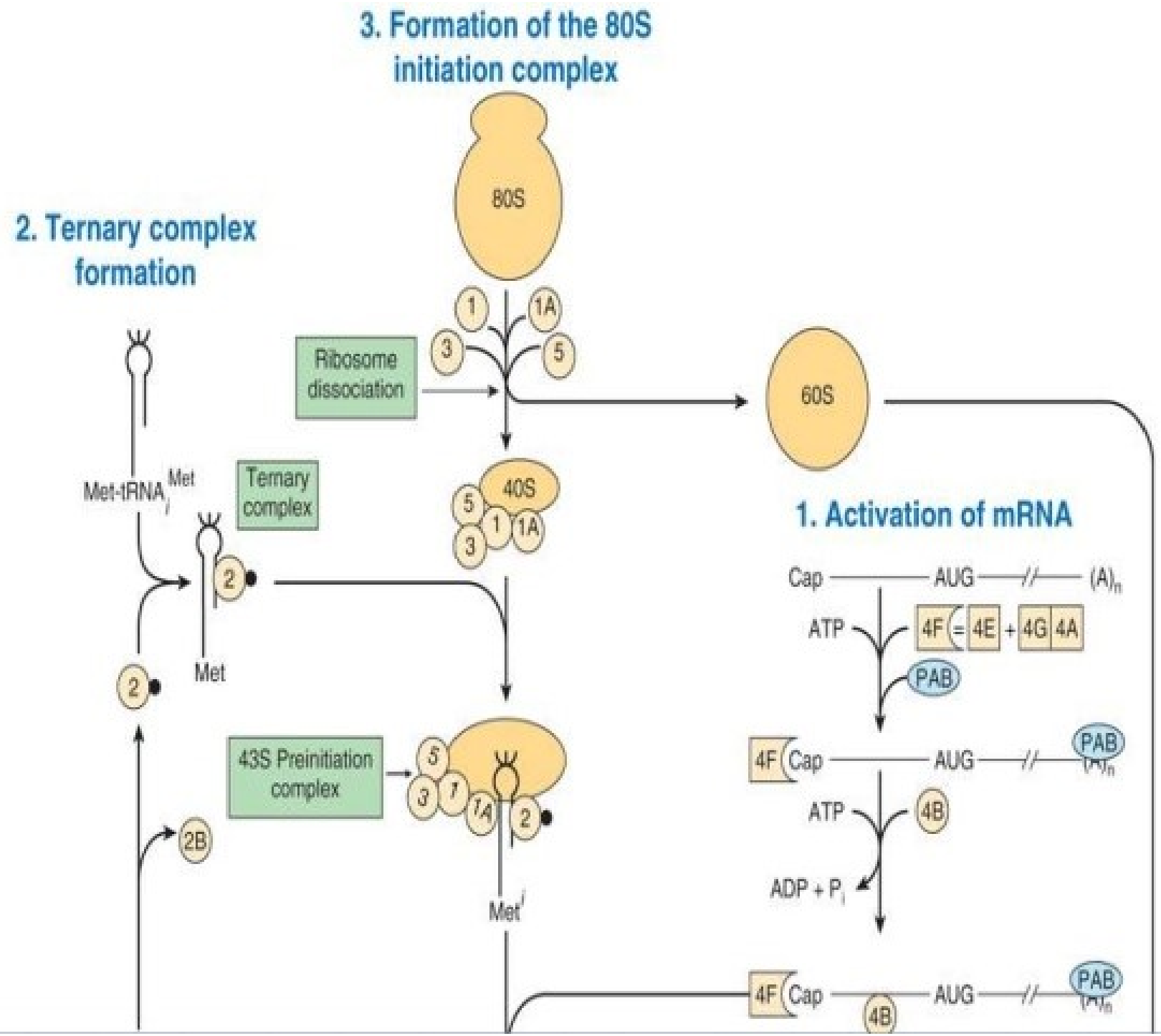
i) **Dissociation of ribosomes –**

80s ribosomes→40s+60s

eIF-3 & eIF-1A →Bind to newly dissociated 40s unit

↓

- Allows other translation factors to bind
- Delays reassociation of 40s with 60s


3. Formation of the 80S initiation complex
80S
2. Ternary complex formation
Ribosome dissociation
60S
1
1A
3
5
Met-tRNA$_i^{Met}$
Ternary complex
40S
5
1
1A
3
2
Met
2
1. Activation of mRNA
Cap
AUG
(A)$_n$
ATP
4F
4E
4G
4A
PAB
43S Preinitiation complex
5
3
1
1A
2
4F Cap
AUG
PAB
ATP
4B
ADP + P$_i$
2B
Met$_i$
4F Cap
AUG
PAB
4B

ii) Formation of 43s pre-initiation complex

GTP + eIF-2

↓

Bind to met-t-RNA^{i} **(Ternary complex)**

↓

43s pre-initiation complex formed

(40s ribosome+ eIF-2+GTP+eIF-3+eIF-1A)

eIF-2 is a control point

- α, β, γ subunits
- eIF 2α – phosphorylated by 4 different protein kinases- **HCR, PKR, PERK, and GCN2**

  Cell under stress (AA/Glucose starvation, viral infection, mis-folded protein, serum deprivation, hyperosmolality, heat shock)

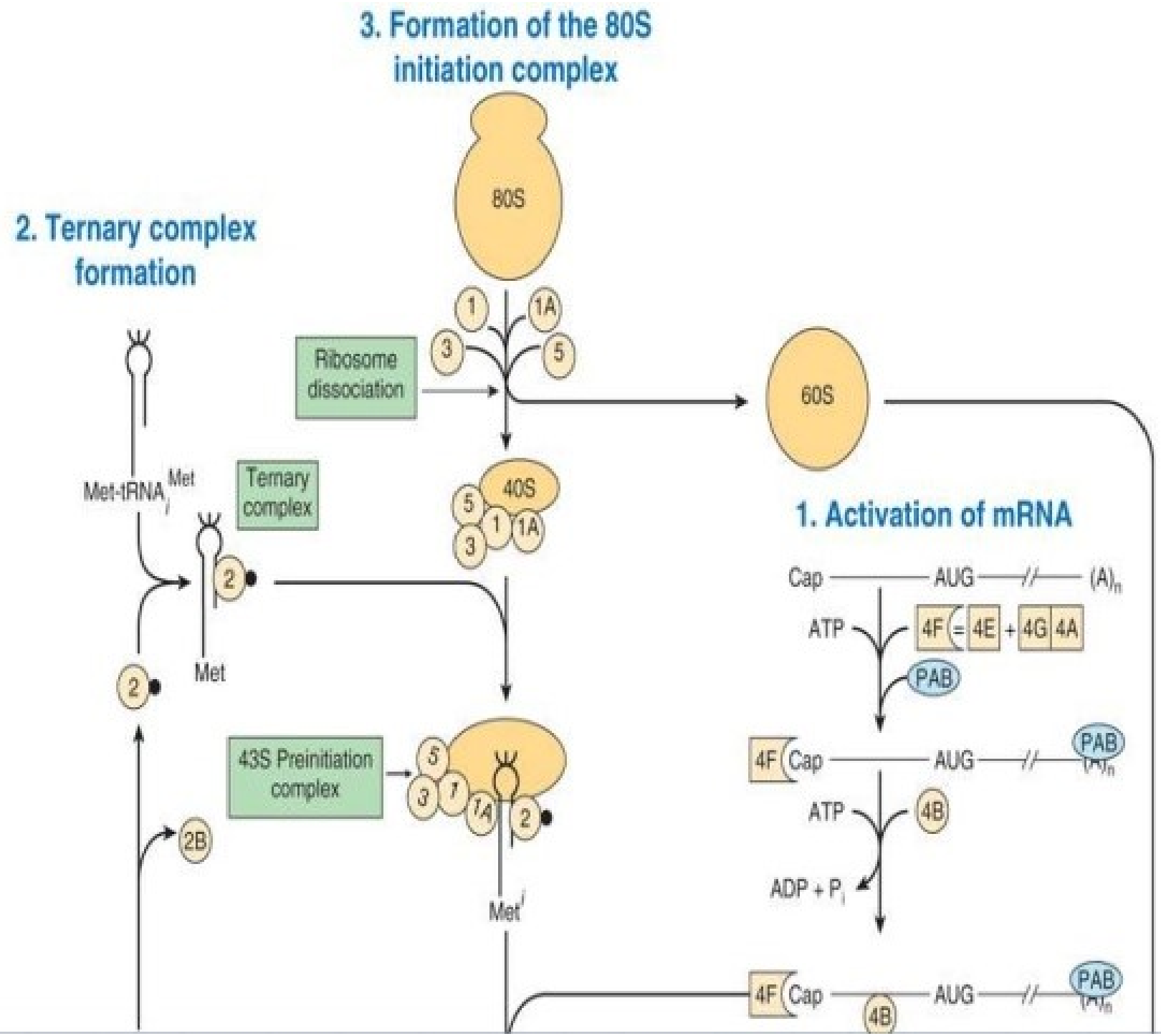
  ↓

  Kinase activated

  ↓

  Translation inactivated

- PKR- actvated in viral infection
- eIF2α binds tightly to and deactivates GTP-GDP recycling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4B
ADP + P$_i$
2B
Met$_i$
4F Cap
AUG
PAB
4B

iii) Activation of mRNA & Formation of 48s initiation complex

- Binding of mRNA to 43s pre-initiation complex
- eIF-4F= eIF-4E + eIF-4G—eIF-4A
- **PAB**- Poly A binding protein
- 4F binds to 5' cap of mRNA through 4E

↓

4B come and bind (helicase activity)

↓**ATP**

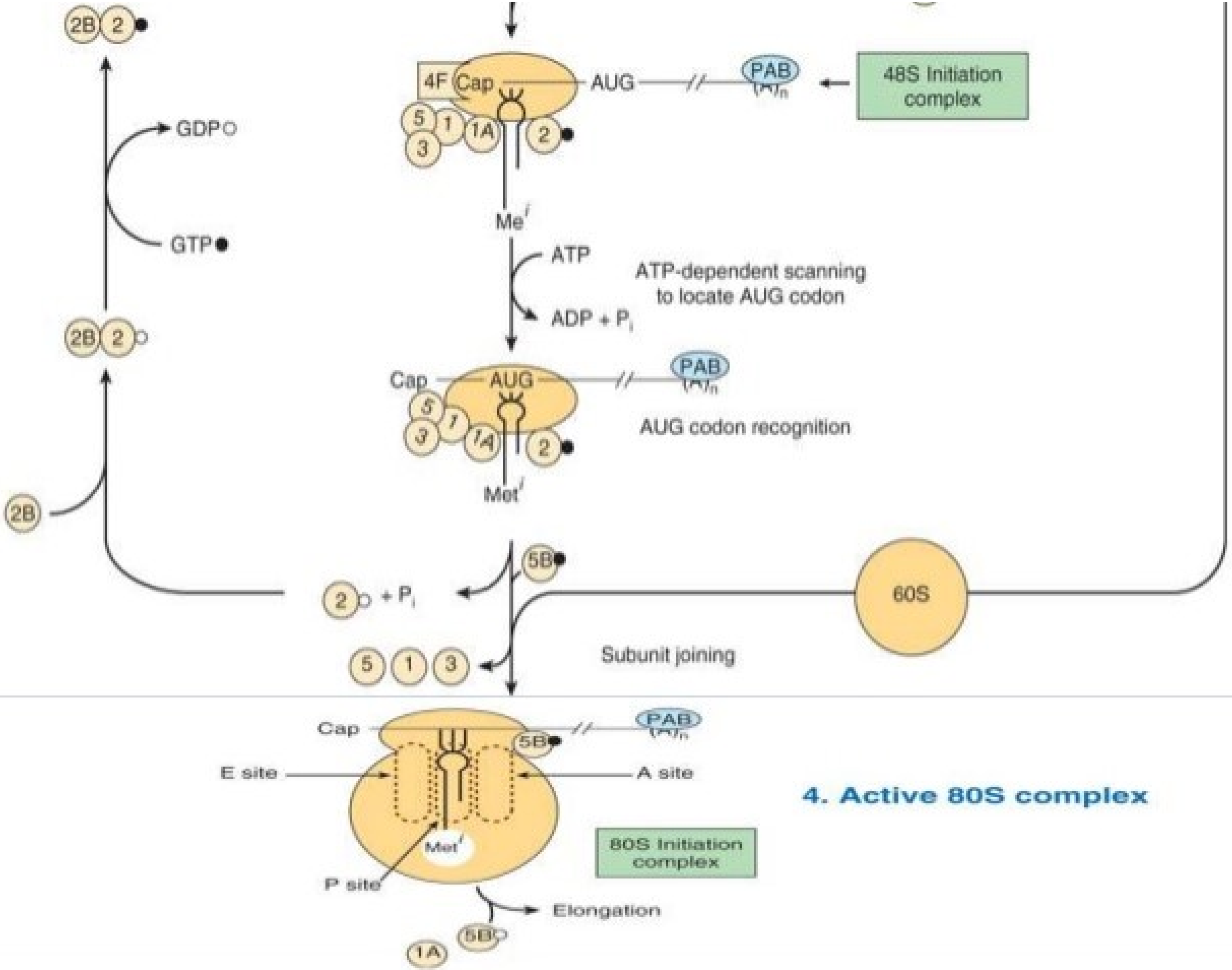
mRNA binds to 43s PIC

↓

48s initiation complex formed

(40s ribosome+ eIF-3,1A,2+ GTP +mRNA + eIF-4F)

eIF3 is main factor for binding



4. Active 80S complex

- Usually to 5′ most AUG is chosen but it is decided by Kozak consensus sequences- surrounding AUG codon
- Prokaryotes– **Shine-Dalgarno sequence--**a sequence of nucleotide bases on m-RNA that facilitates m-RNA binding to the pre-initiation complex

Eukaryotes– **Kozak consensus sequence**

- A purine present at -3 and +4 positions of the initiating codon

- Formation of PIC

↓

Melting of secondary str at 5’ end

↓

Complex scans m-RNA for a suitable initiating codon **(5’ most AUG)**

- PAB1 & Poly A tail initiate translation and also protect mRNA from exonucleolytic degradation.

iv) Formation of 80s initiation complex—

48s IC combines with 60s ribosome

↓

Hydrolysis of GTP bound to eIF-2 by eIF-5

↓

Release of initiation factors and recycled

↓

Rapid reassociation of 40s and 60s to form 80s ribosome

- At this stage Met-tRNA$_i^{met}$ on the P site

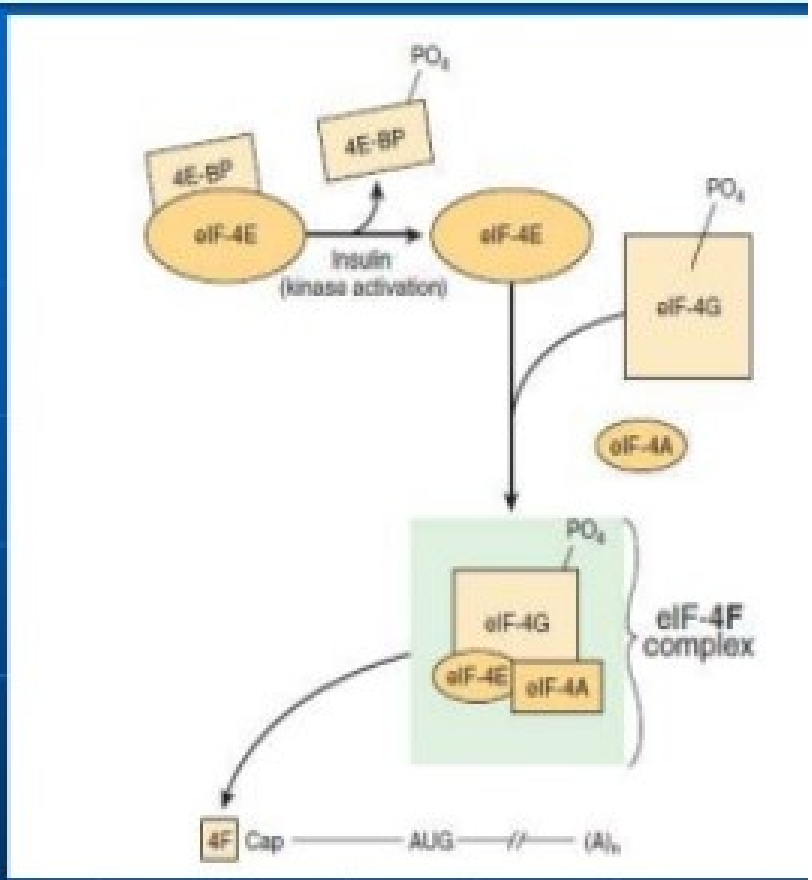
Regulation of initiation

- eIF-4F– rate of translation
- 4E– recognises the cap of mRNA
- 4G– Scaffolding protein

  -- binds to helicase complex that helps in unwinding the RNA
- eIF-2– one of the key regulators
- PAB regulates the initiation of translation

Phosphorylated 4E binds more avidly to mRNA cap structure

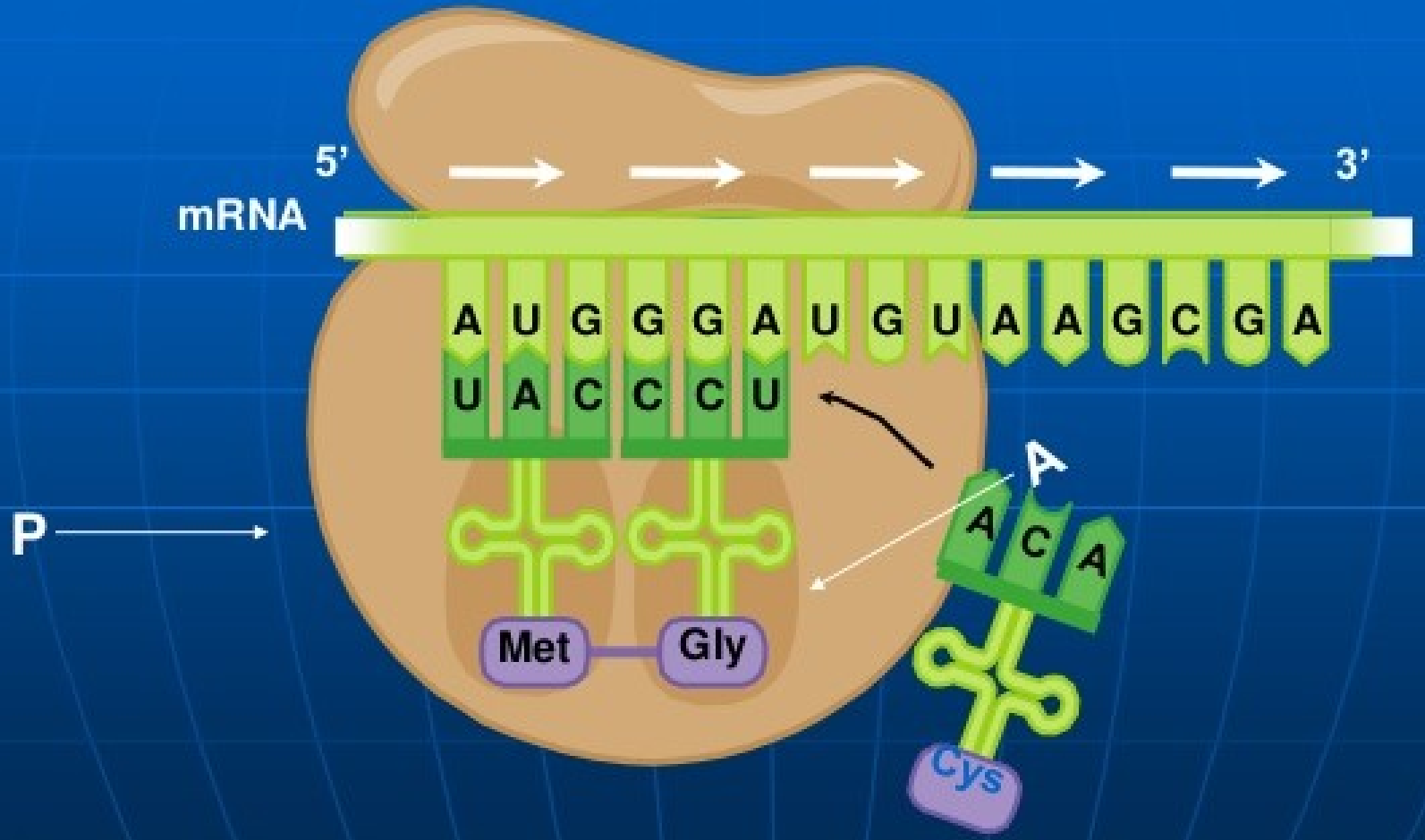
- 1. Phosphorylation of 4E (Ser 209 or Thr 210)is controlled by insulin and mitogenic factors
- Components of MAP kinase, mTOR, PI3K, RAS, s6 kinase pathways- involved in regulation
- 2nd path of regulation- Binding of binding proteins keep 4E inactivated
- Insulin & GF- cause phosphorylation of BP and its release from 4E

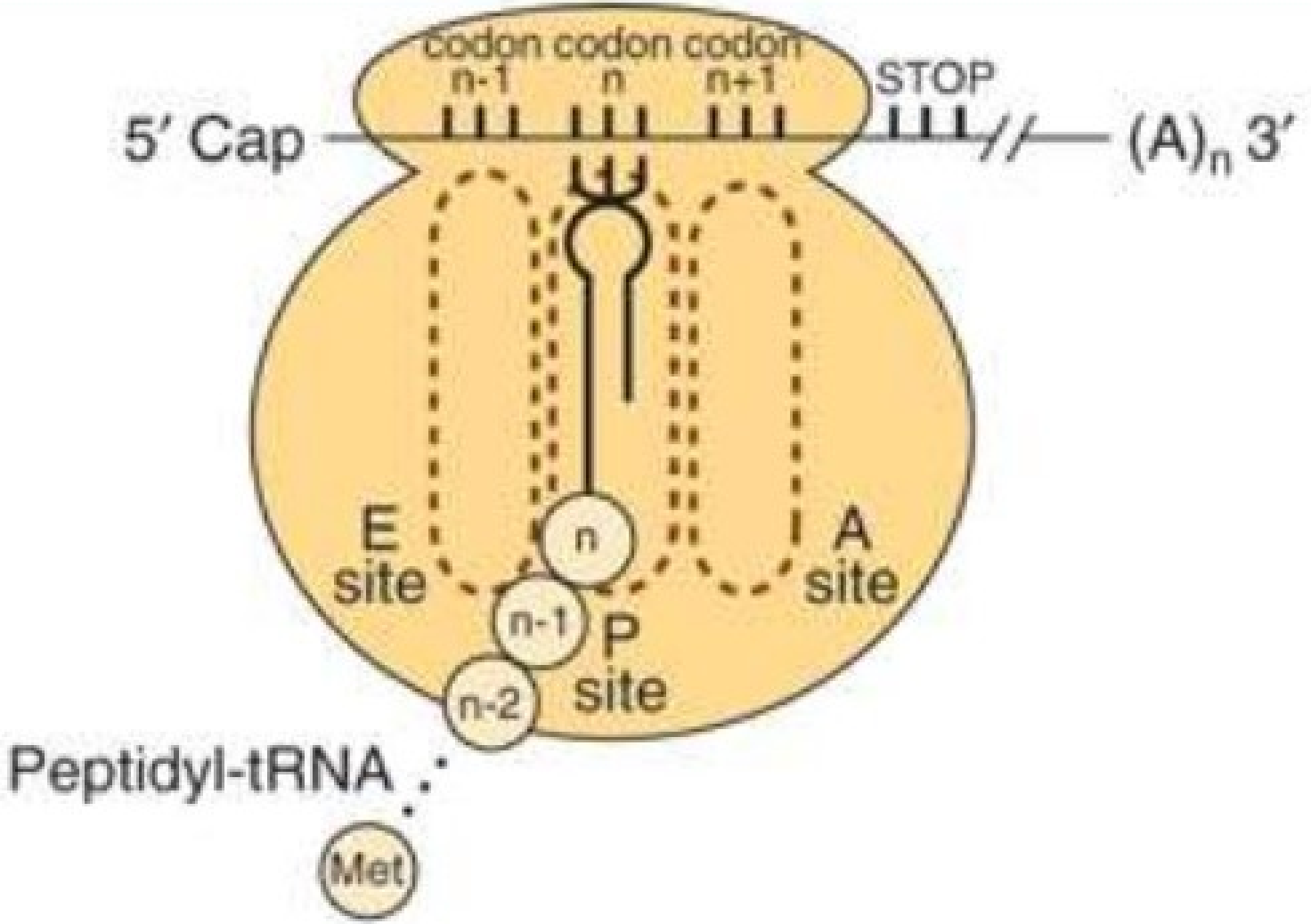


Insulin and GF enhance post-transcriptional protein synthesis in liver, muscle and adipose tissues

Elongation

- One AA added at a time




codon codon codon
n-1 n n+1
STOP
5′ Cap
(A)n 3′
E site
n
A site
n-1
P site
n-2
Peptidyl-tRNA
Met

i) **Binding of aminoacyl t-RNA to the A-site**

A site empty

Binding of proper AA-t-RNA requires codon recognition

EF-1A (Elongation factor)+ GTP+ Aminoacyl t-RNA

↓

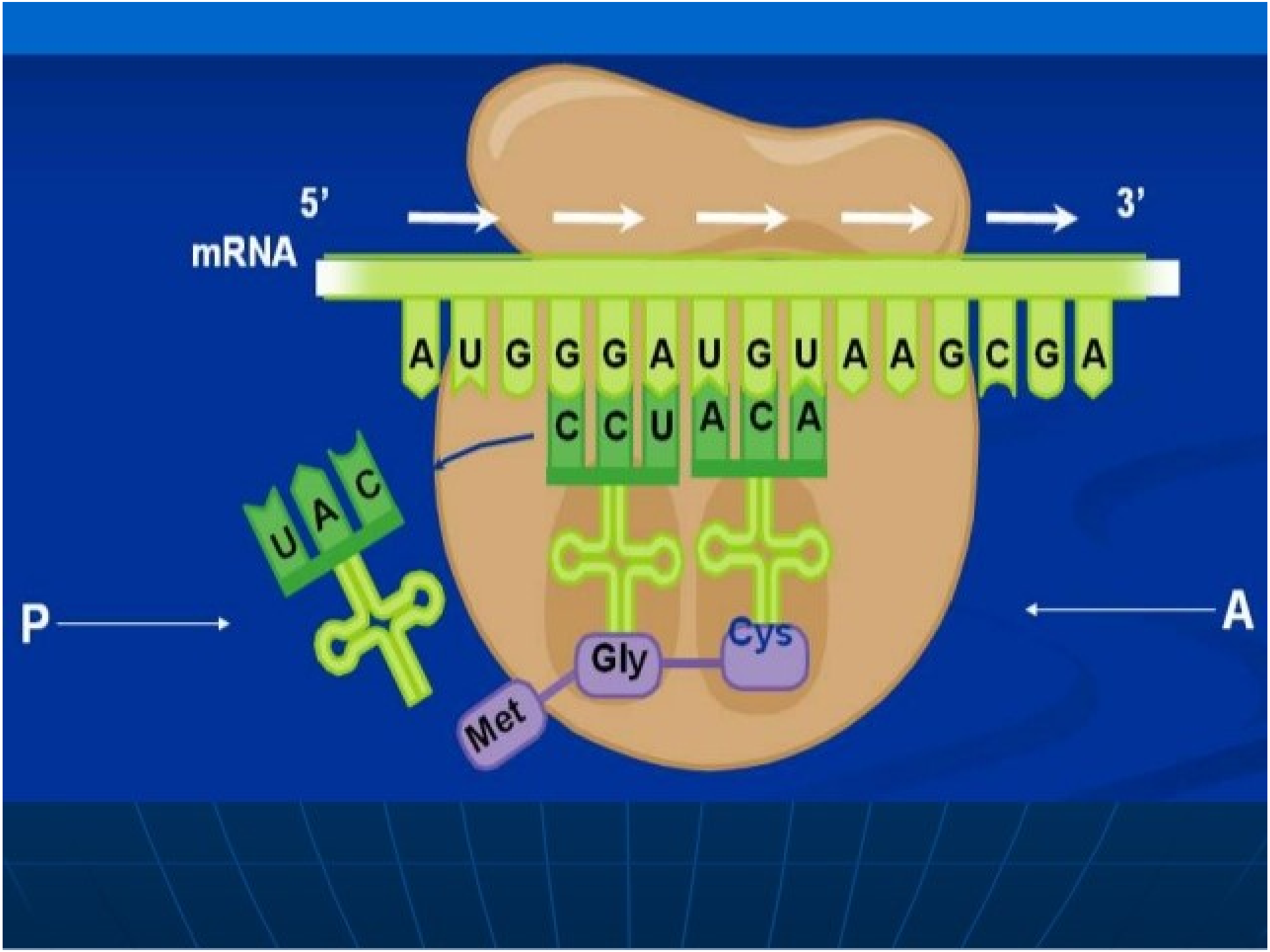
Ternary complex

↓

Enters A site

↓ EF-1A,GDP+Pi

Aminoacyl-tRNA

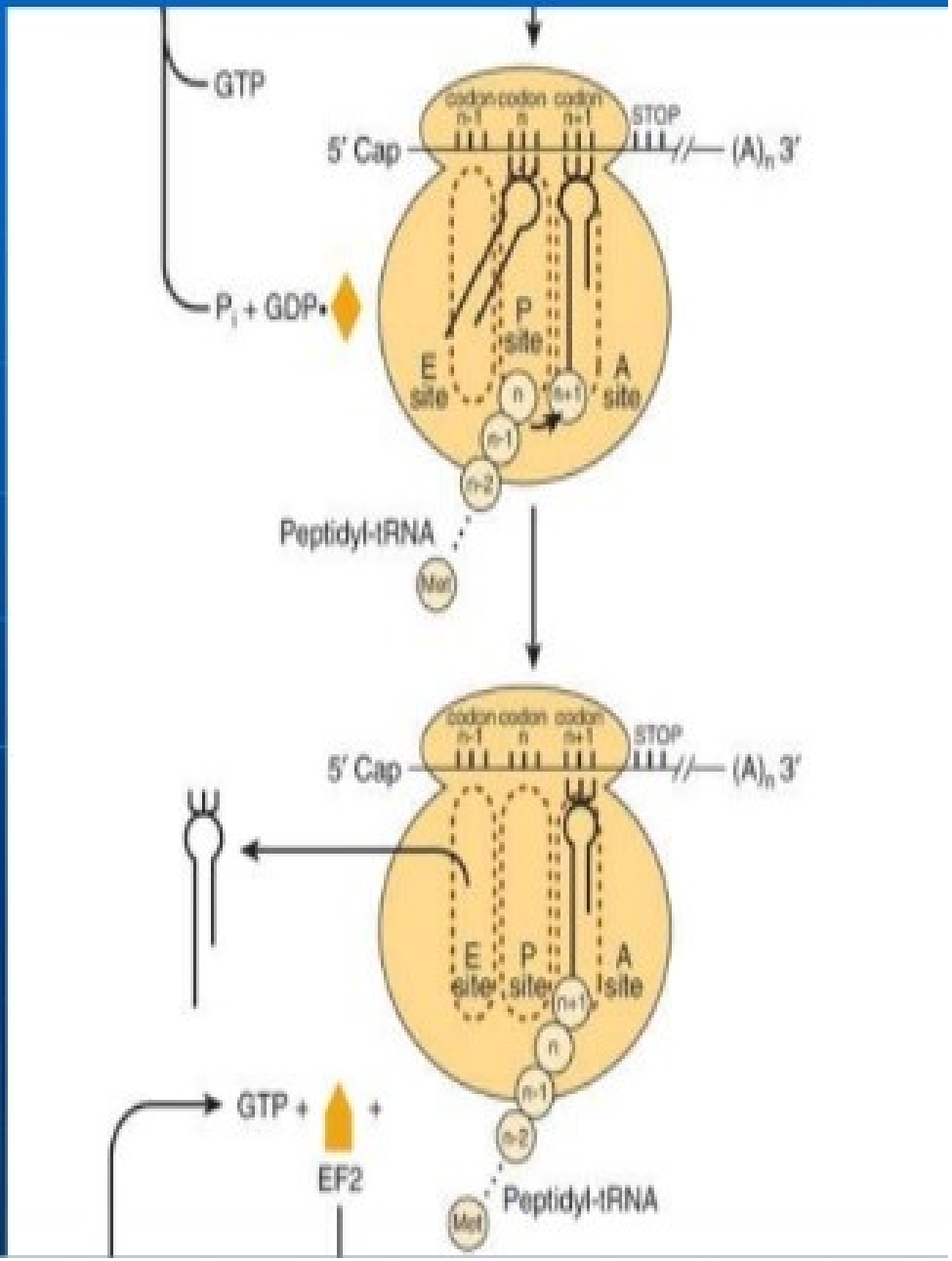

5'
3'
mRNA
A U G G G A U G U A A G C G A
C C U A C A
U A C
P
A
Met
Gly
Cys

ii) Peptide bond formation

- Peptidyl transferase– catalyses peptide bond formation
- Found on 28s rRNA
- Ribozyme
- No energy required

iii) Translocation

- Growing peptide chain moves from A site to P site
- Deacylated tRNA moves out from P site to E site (Exit)



- EF-2 factor binds

↓

Displaces peptidyl tRNA from A site to P site

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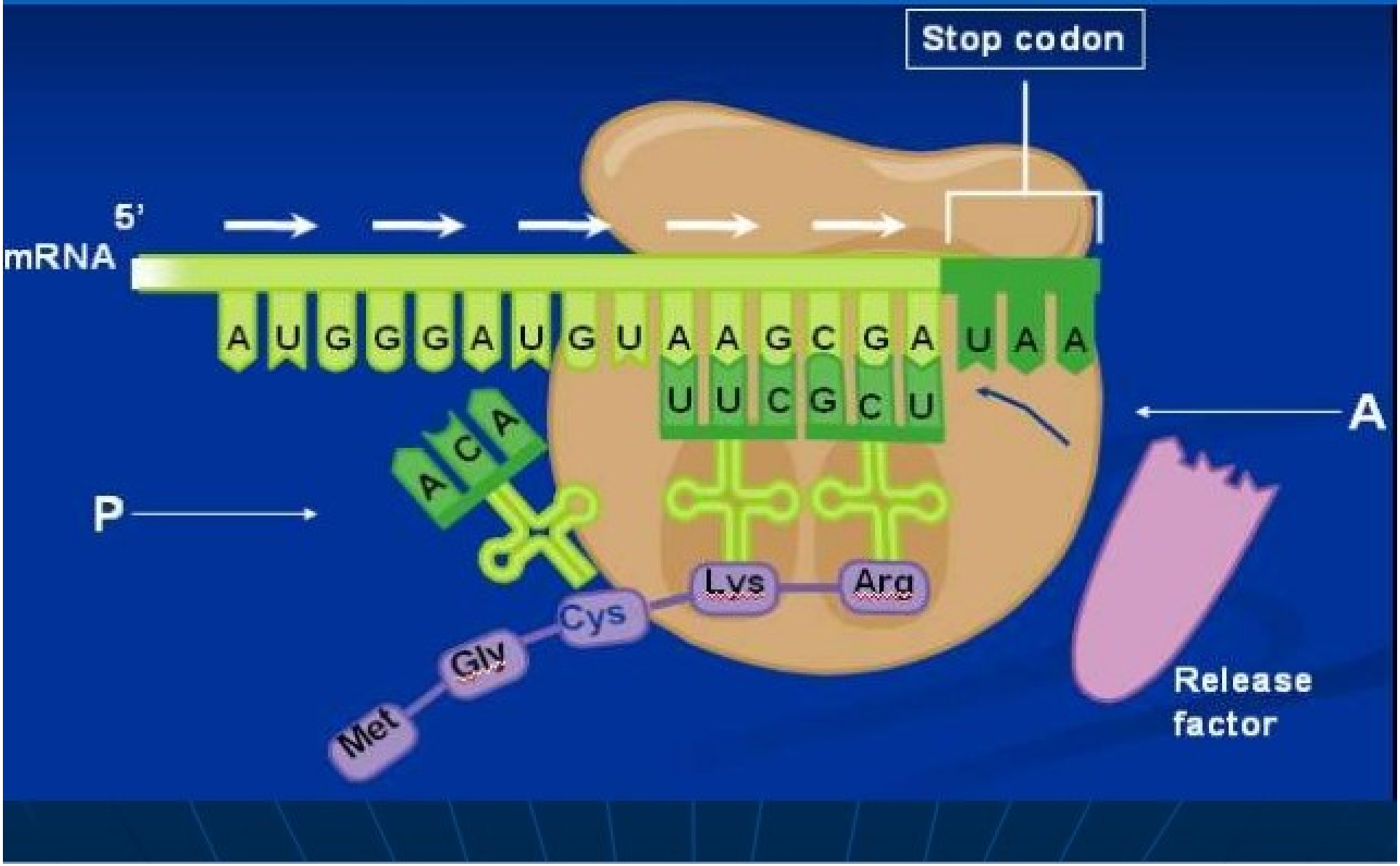
Energy from GTP; mRNA moves by one codon

- Process of peptide synthesis occurs until a termination codon is reached

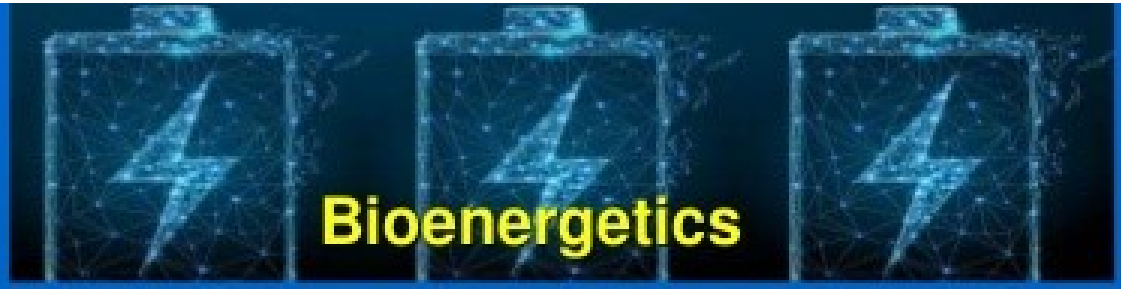
Termination



- Stop codon appears on A site
- No tRNA available
- Releasing factor **(RF-1)** recognises its presence in A site
- RF-1+RF-3+GTP→ Hydrolysis of bond between peptide and tRNA occupying P site (a water molecule is added)

↓

Protein, tRNA, 60s & 40s ribosomes, GDP released



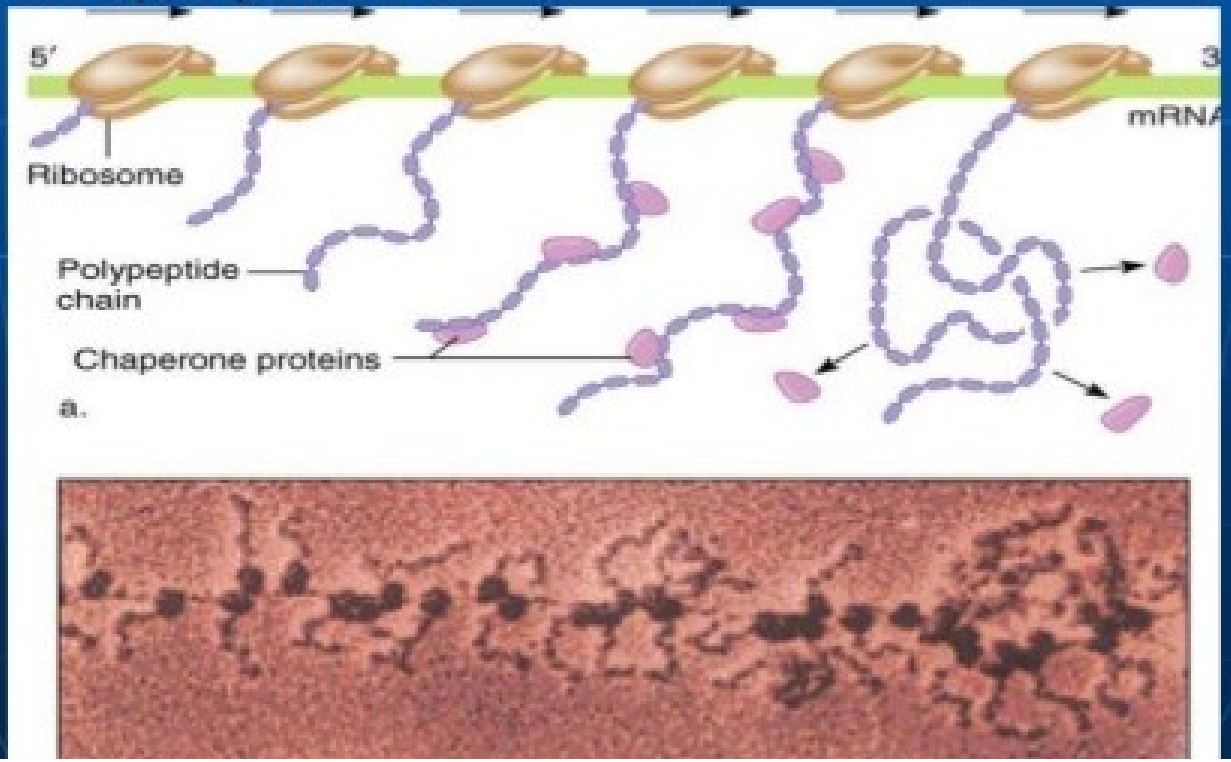
- Activation of AA- 2 high energy bonds
- Entry of aminoacyl t-RNA to A site- 1 GTP
- Translocation of peptidyl t-RNA from A site to P site
- Release- 1 GTP
- 5 High energy bonds

Speed

- Prokaryotes- 18/sec
- Eukaryotes- 6/sec

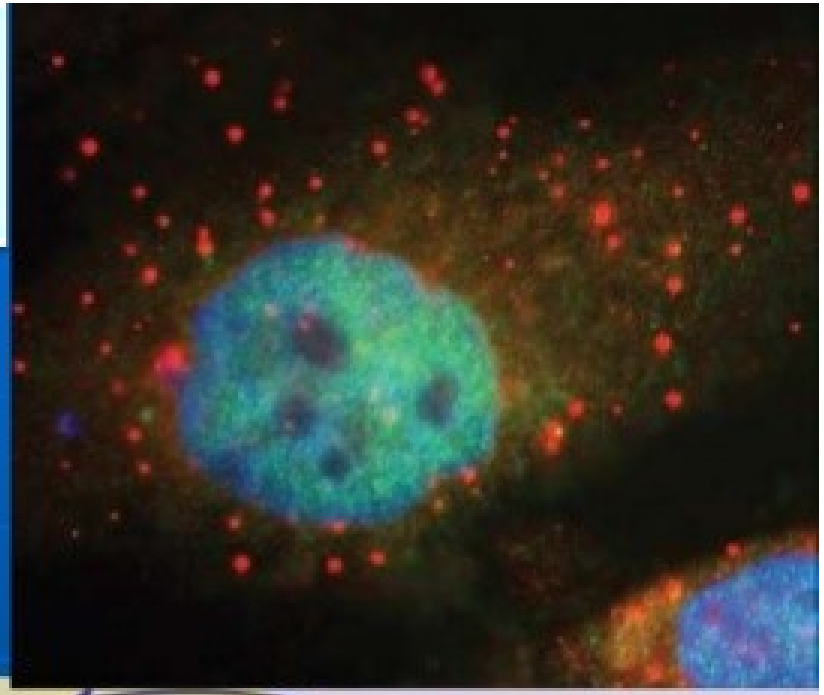
Polysomes

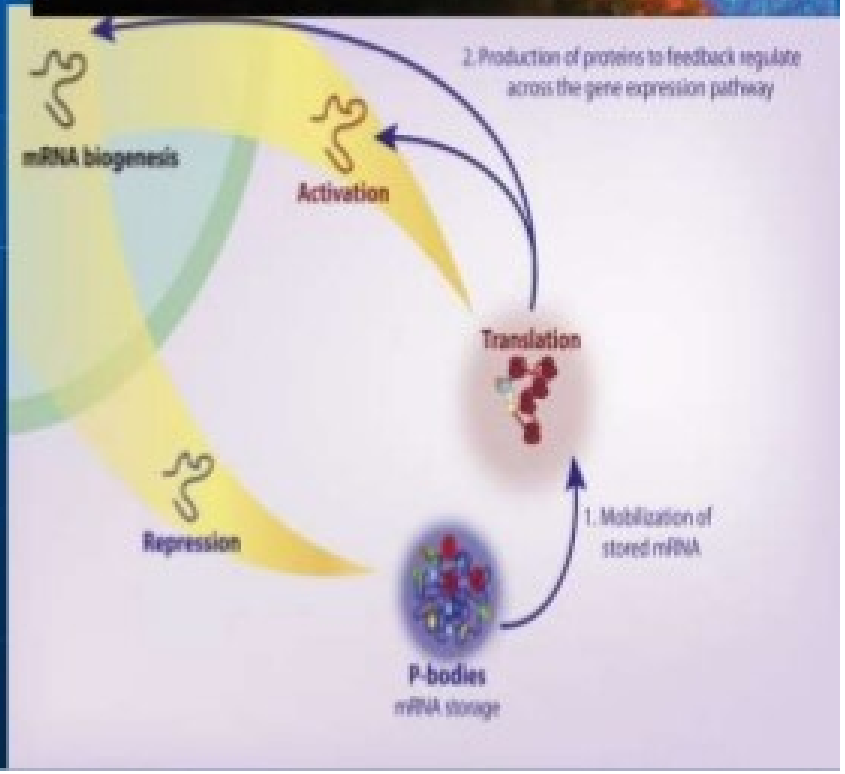
- In eukaryotes the same molecule of mRNA can be simultaneously translated several times
- Each emerging peptide is synthesized on a separate ribosome
- Many ribosomes on the same "string" of mRNA are called polysomes



P bodies

- Non-translating mRNAs can form Ribonucleoprotein particles (mRNPs)- accumulate in Cytoplasmic organelles as P bodies
- **P bodies-** sites for translation repression and mRNA decay
- **35** distinct proteins are found in P bodies like mRNA decapping enzyem, RNA helicases, exonucleases etc
- Few mRNAs are retrieved at the time of need

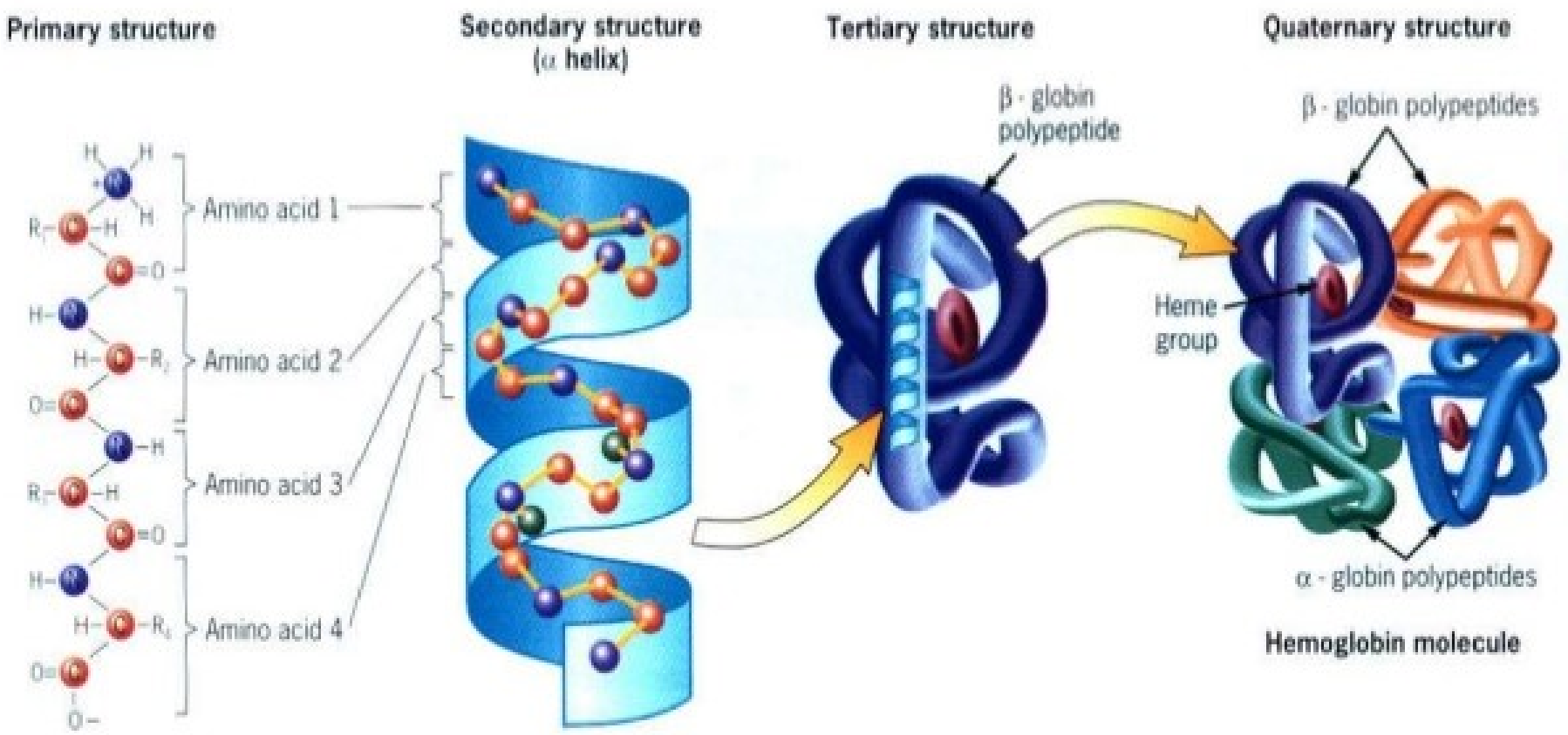




Environmental factors regulate translation

- Iron excess- stimulates ferritin synthesis
- Viruses also utilise host cell translation machinery for their growth- encephalomyocarditis virus
- Certain viruses inhibit host cell machinery by binding to 40s ribosome- polioviruses and picornaviruses

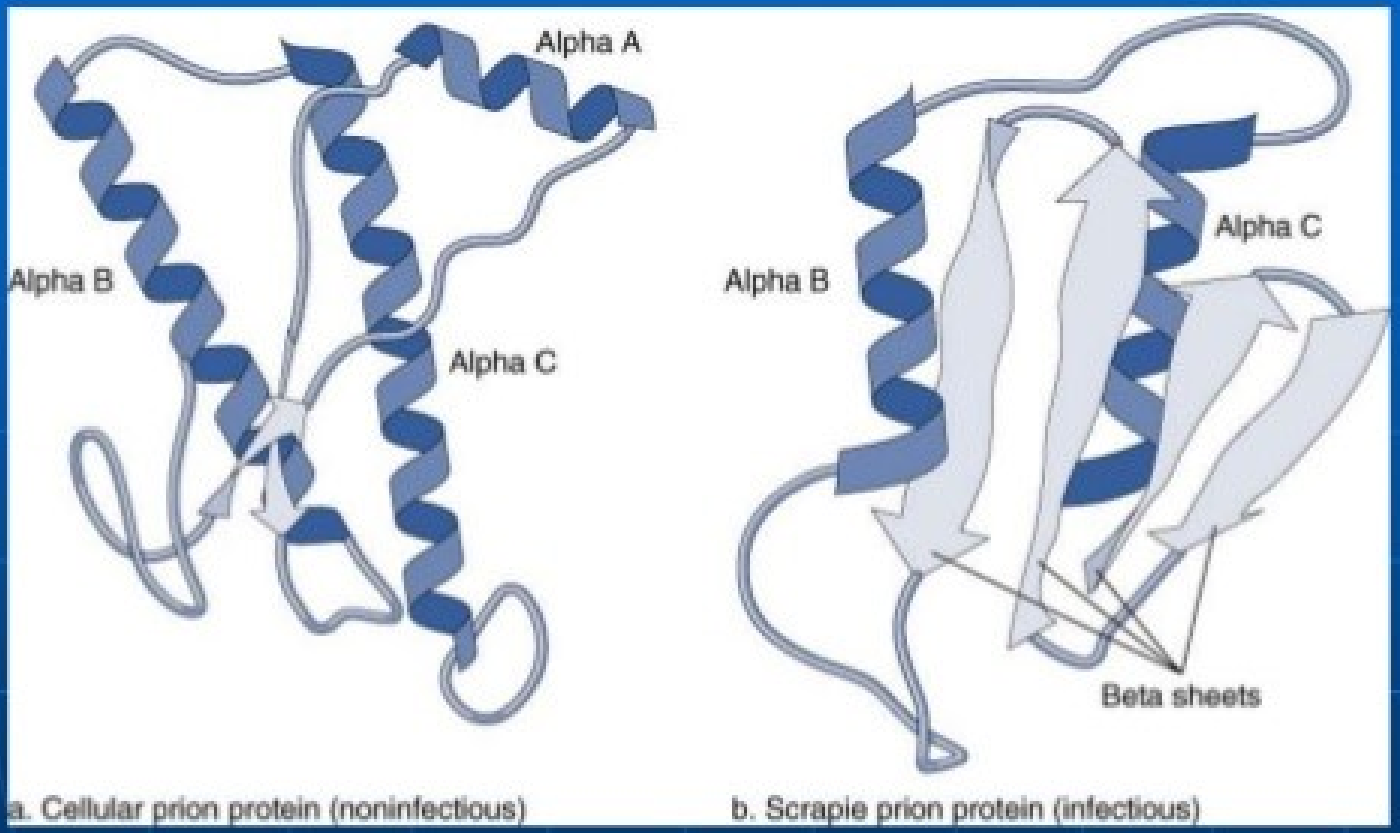
Four levels of protein structure



Chaperones and protein folding

- Heat shock proteins
  - Hsp70
  - Chaperonin (Hsp60)
- Prevent aggregation
- Put all the hydrophobic ends inside
- Misfolding causes diseases e.g. Cystic fibrosis, Prion diseases

Misfolding of protein impairs function



- **Misfolded prion protein disrupts functions of other normally folded prion proteins.**
- **Aberrant conformation can propagate like an "infectious" agent**

Inhibitors of Translation

A. Reversible inhibitor

a. Tetracyclin– Binds to 30s ribosome

↓

Inhibit attachment of aminoacyl tRNA to the A site

b. Chloramphenicol– Inhibits peptidyl transferase

c. Erythromycin & Clindamycin– Prevent translocation

B. Irreversible inhibitor

Streptomycin and Aminoglycosides—Bind to 30s ribosomal sub-unit

Low conc.- Misreading of protein

↓

Useless bacterial proteins

Pharma. Conc.- Inhibit IC synthesis

↓

Protein synthesis inhibited

C. Inhibitors in mammals

1. Puromycin– Structural analogue of tyrosinyl t-RNA
2. Cycloheximide– Inhibits peptidyl transferase
3. Diphtheria toxin—Inactivation of EF-2 by attachment of ADP to EF-2
4. Ricin– Inactivates 28s rRNA


THANK YOU