TRANSLATION

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TRANSLATION OF m RNA

- In the process of protein synthesis also called translation of mRNA, the amino acids are added sequentially in specific number and sequence, determined by the sequence of codons in the genetic code of the relevant mRNA.

MATERIALS REQUIRED FOR PROTEIN SYNTHESIS

- Amino acids
- Enzymes
- Ribosomes
- mRNA
- tRNA
- Energy Source
- Protein Factors

AMINO ACIDS

- Protein synthesis can occur only when all the amino acids needed for particular protein are available
- If there is a deficiency in the dietary supply of anyone of the essential amino acids, the translation stops
- It is, therefore, necessary that a regular dietary supply of essential amino acids, in sufficient quantities, is maintained for protein synthesis

ENZYMES

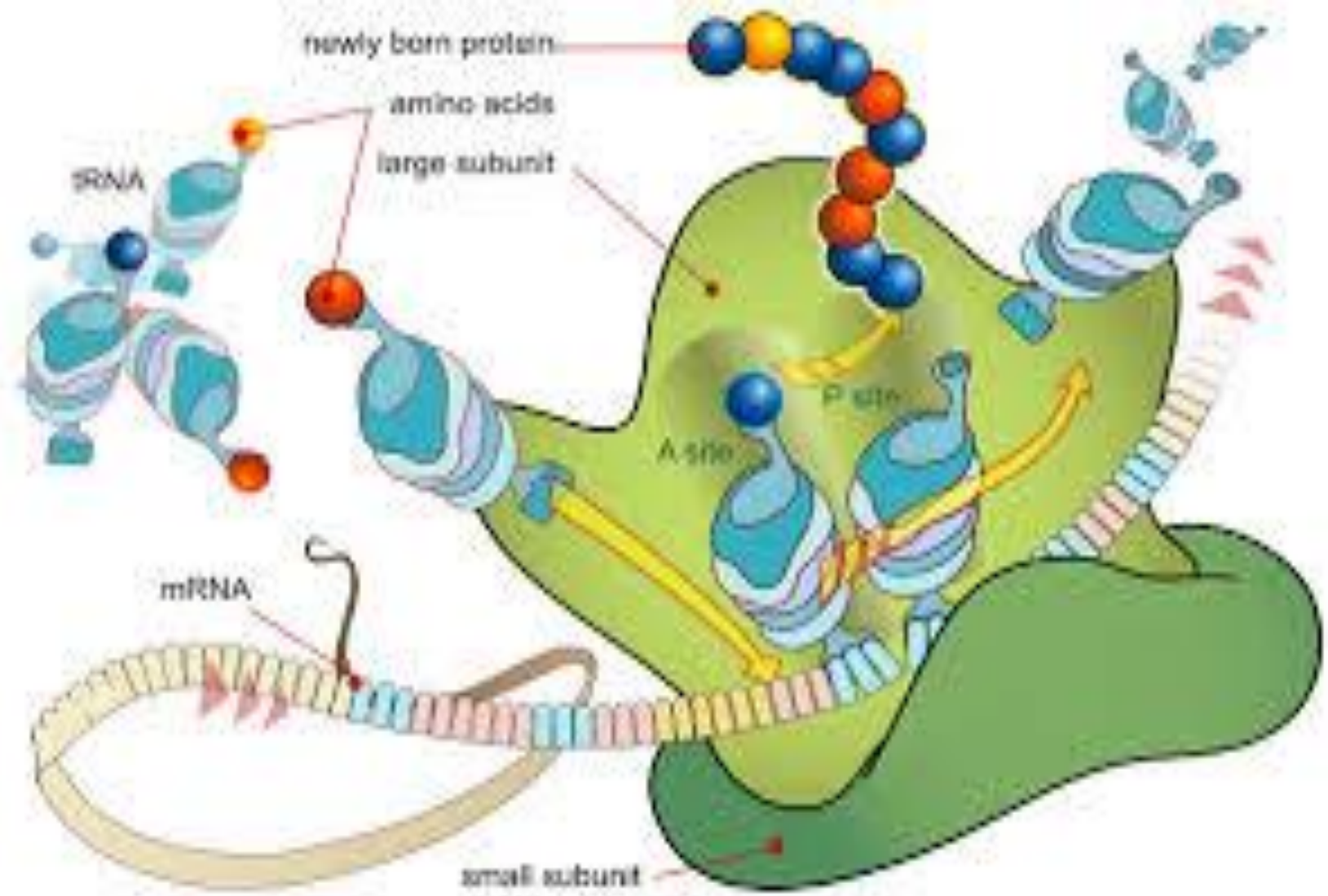
- Amino acyl-t-RNA synthetase: enzyme required for activation of amino acids

- Peptide synthetase (peptidyl transferase) for the peptide bond formation

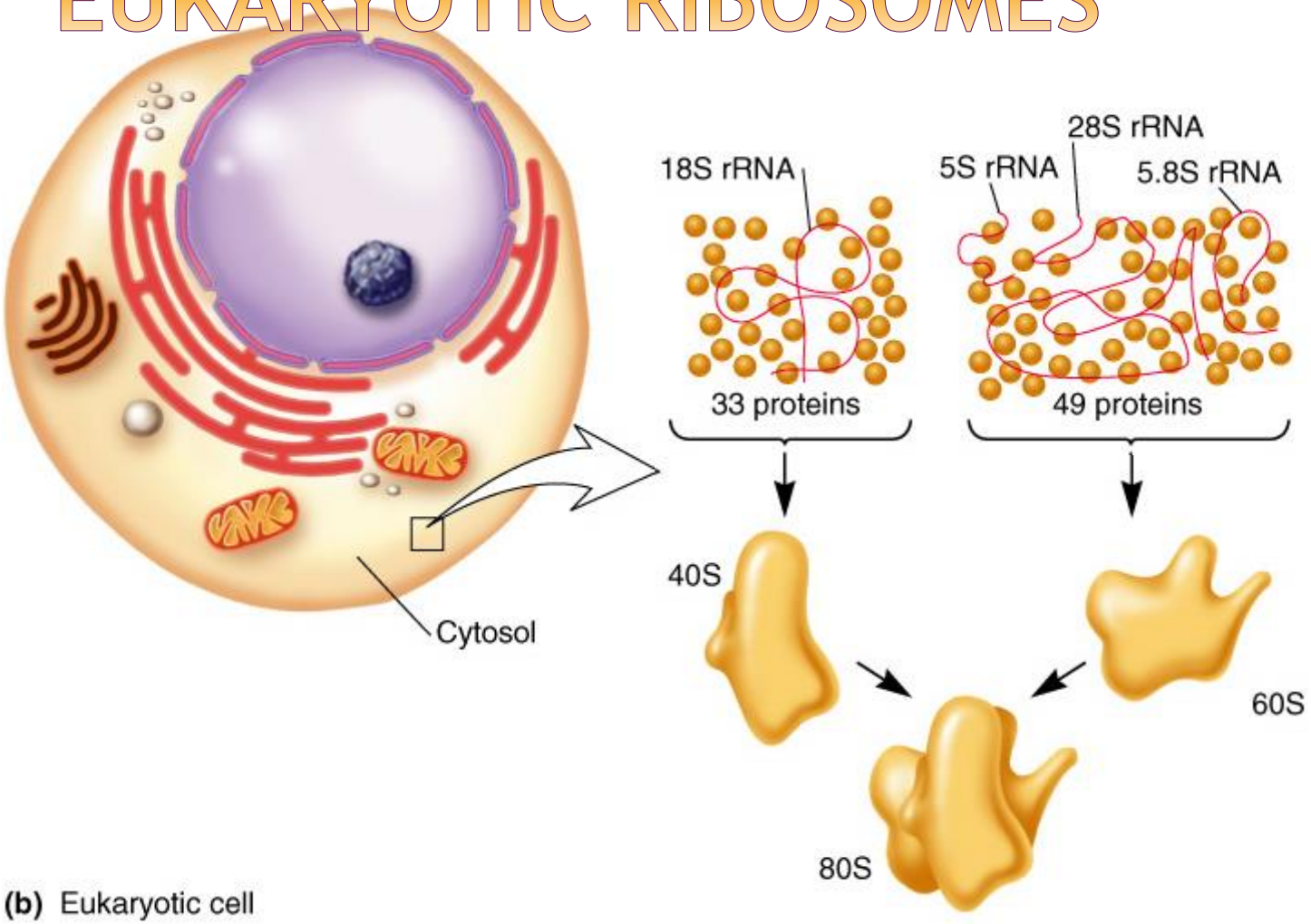
RIBOSOMES

- Protein synthesis takes place on ribosomes which is a nucleoprotein and contain 65% r-RNA and 35% protein
- The two subunits in prokaryotes are 50s and 30s and in eukaryotes are 60s and 40s**(sedimentation coefficient in Svedberg units)**
- A **polysome** or **polyribosome** is a beaded string like linear cluster of 5-8 ribosomes on mRNA
- Each ribosome has **peptidyl (P), amino acyl (A) and an exit (E) site**

RIBOSOMES


newly born protein
amino acids
large subunit
tRNA
P site
A site
mRNA
small subunit

EUKARYOTIC RIBOSOMES



(b) Eukaryotic cell

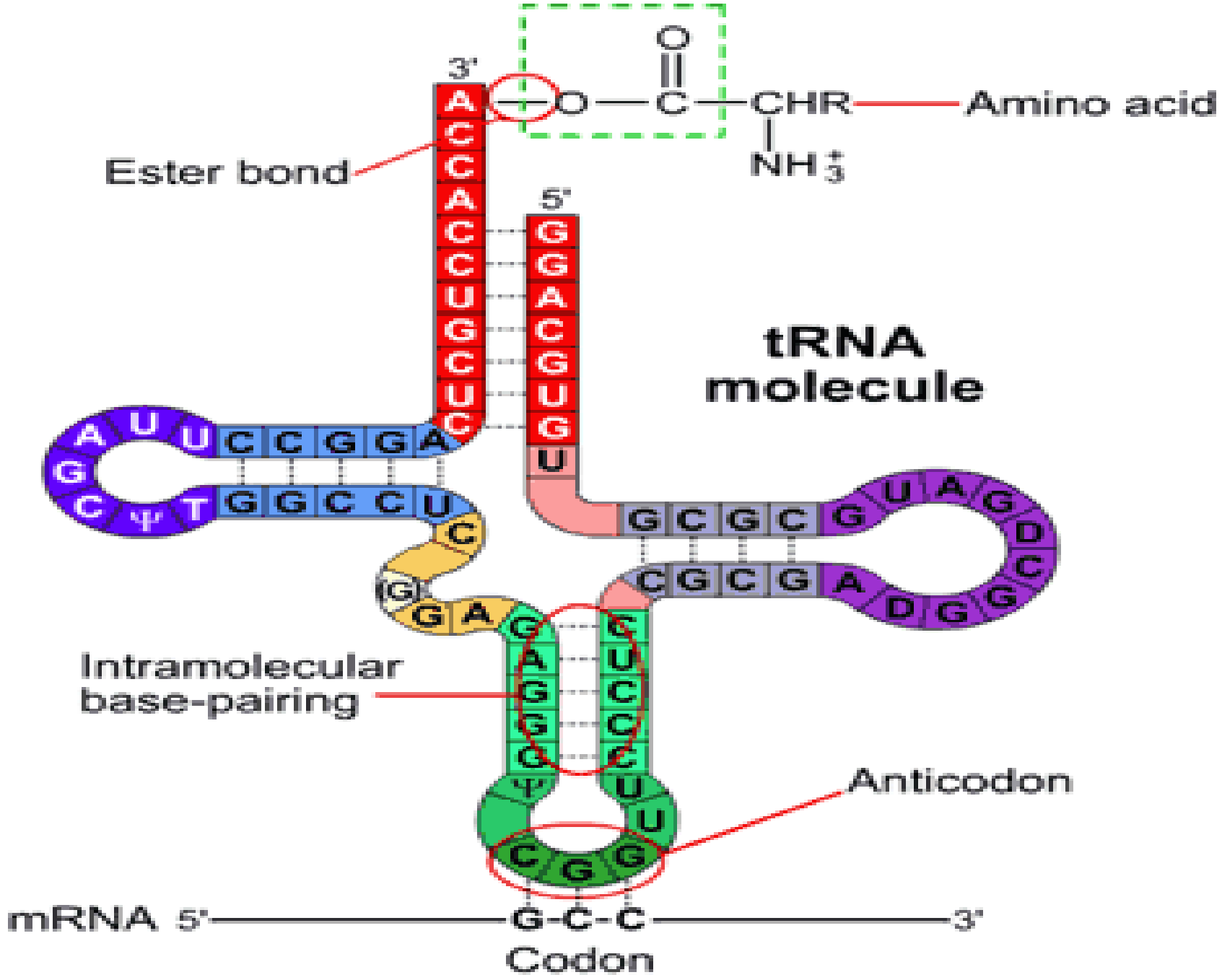
Figure 13.13

m RNA

- The specific information required for the synthesis of given protein is present on the mRNA.
- The DNA has passed on the genetic information to the mRNA to be translated into a protein sequence.

t RNA

- They carry the amino acids and hand them over to the growing peptide chain.
- The amino acid is bound to tRNA at the 3’ end by ester bonding.
- Each tRNA has three nucleotide base sequence– the **anti codon** which is responsible to recognize the codon of mRNA for protein synthesis.


3'
5'
Ester bond
O
C
CHR
Amino acid
NH3+
tRNA molecule
Intramolecular base-pairing
Anticodon
mRNA 5'
G-C-C
3'
Codon

PROTEIN FACTORS

The process of translation involves a number of protein factors

- **Initiation factors**: eIF-1,eIF-2, eIF-3, eIF-4A, eIF-4B, eIF-4G, eIF4E, elf-5
- **Elongation factor**: EF1 and EF2
- **Release factor**: RF-1 and RF-3

ENERGY SOURCES

- ATP and GTP are required for the supply of energy in protein synthesis
- Total energy requirement is

  ATP=4

  GTP=5
- **ATP**: i)It is required for the activation of amino acids and formation of t-RNA-amino acid complex

  ii) as one molecule of ATP is converted to AMP, it can be considered equivalent to utilization of 2 ATPs (two high energy PO4 bonds used).

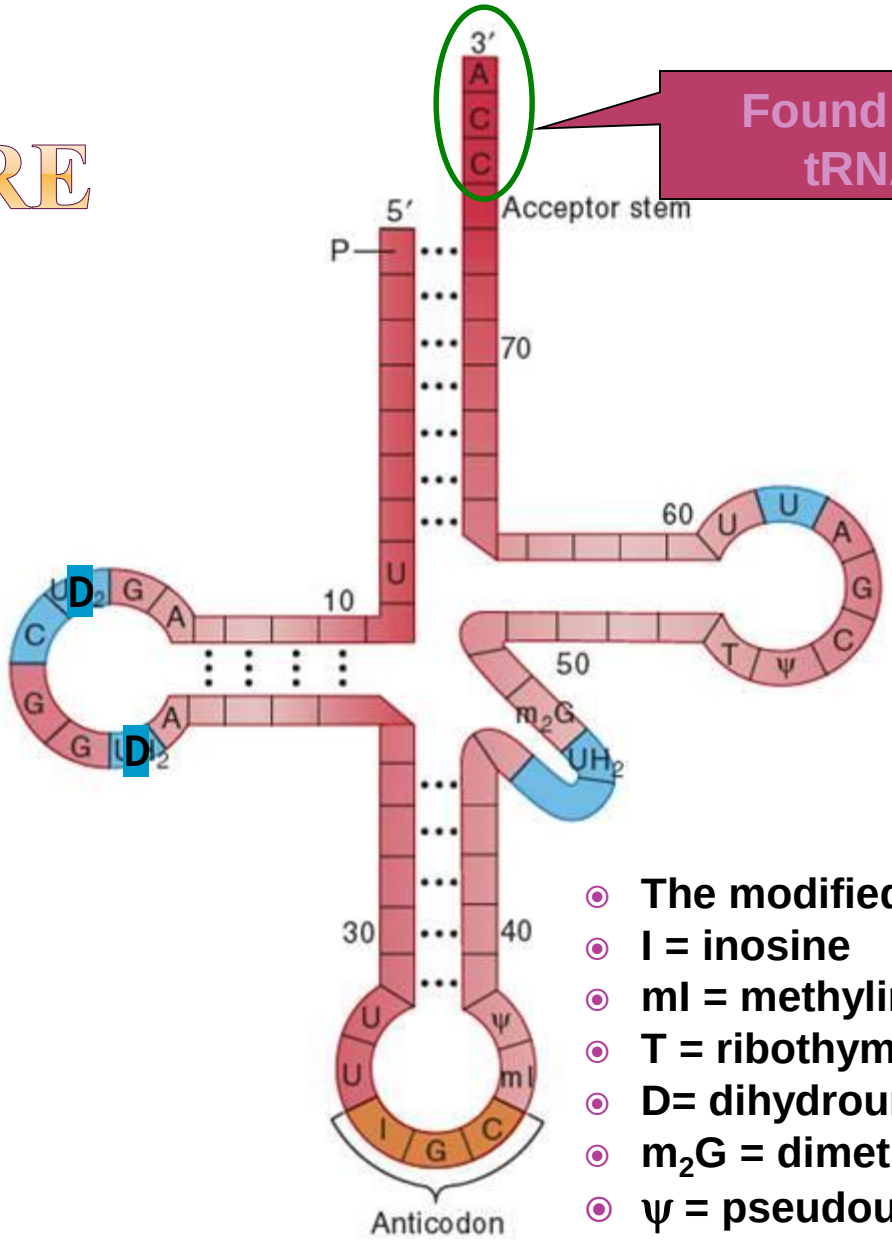
- iii)One ATP is required for formation of initiation complex.
- iv) One ATP required in formation of 48S initiation complex.
- **GTP** i)is required for ***binding with eIF-2 for forming*** a binary complex required in formation of 43S pre initiation complex
- ii)GTP hydrolysis provides energy ***for formation of 80S initiation complex***
- iii)GTP is required in binding of amino-acyl-tRNA to 'A' site
- iv)for translocation
- v)for *termination*

STEPS OF PROTEIN SYNTHESIS

The process of protein synthesis can be divided in following steps

- Activation of Amino acids
- Initiation
- Elongation
- Termination

TRNA 2° STRUCTURE



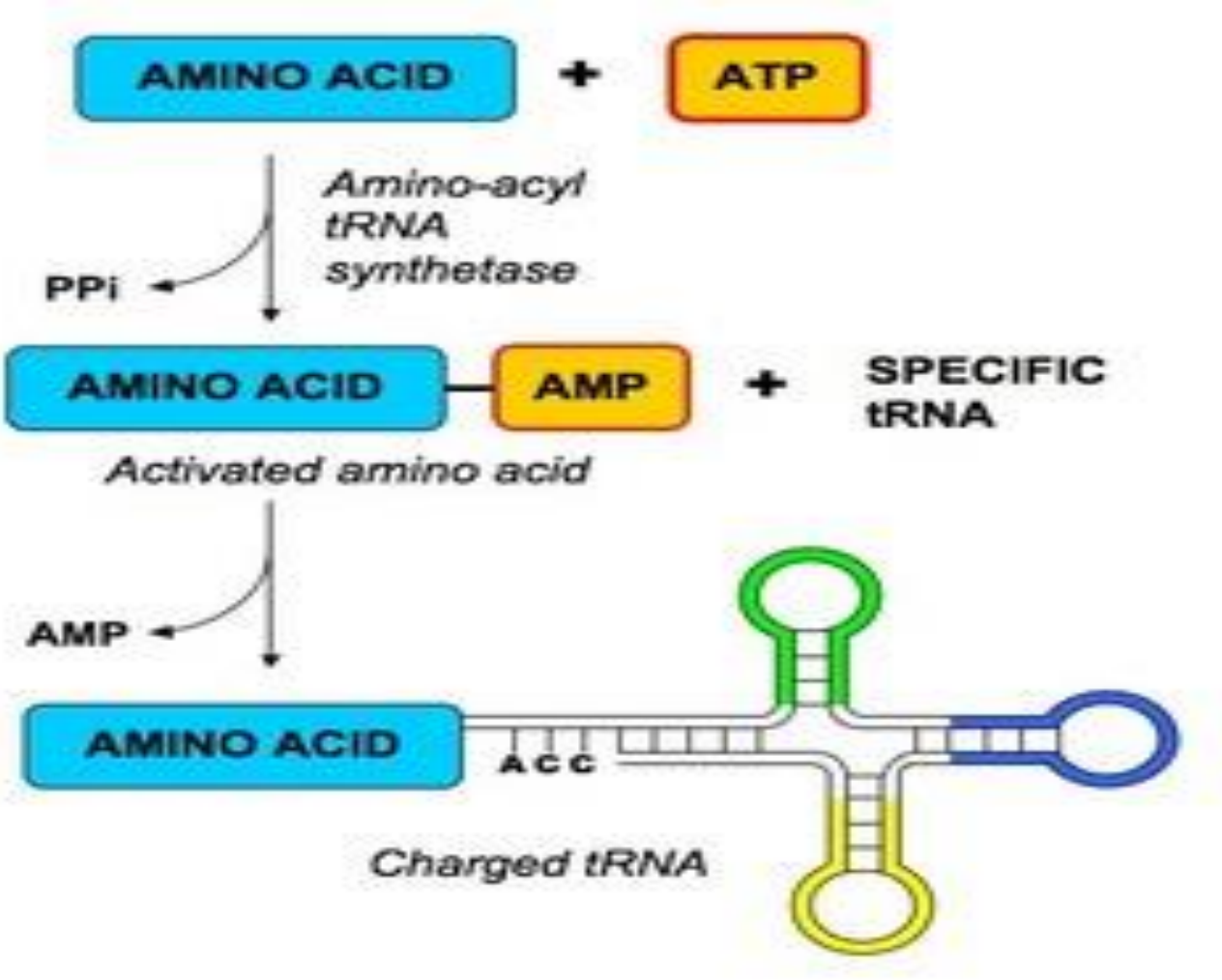
- **The modified bases are:**
- **I = inosine**
- **mI = methylinosine**
- **T = ribothymidine**
- **D= dihydrouridine**
- **m_2G = dimethylguanosine**
- **ψ = pseudouridine**

CHARGING OF t RNAS

- aminoacyl-tRNA synthetases
  - The enzymes that attach amino acids to t RNAs
  - There are >20 types one for each amino acid

- Aminoacyl-tRNA synthetases catalyze a two-step reaction
  - 1- adenylation of amino acid
  - 2- amino acylation of tRNA

ACTIVATION OF AMINO ACID



INITIATION

The initiation may be divided into the following 4 steps

- Dissociation of ribosomes 80s into 60s and 40s subunits
- Formation of 43s pre initiation complex
- Formation of 48s initiation complex
- Formation of 80s initiation complex

RIBOSOME STRUCTURE AND ASSEMBLY

- Translation occurs on the surface of a large macromolecular complex termed the ribosome

- Prokaryotic cells
  - 1 type of ribosome located in the cytoplasm

- Eukaryotic cells
  - 2 types of ribosomes

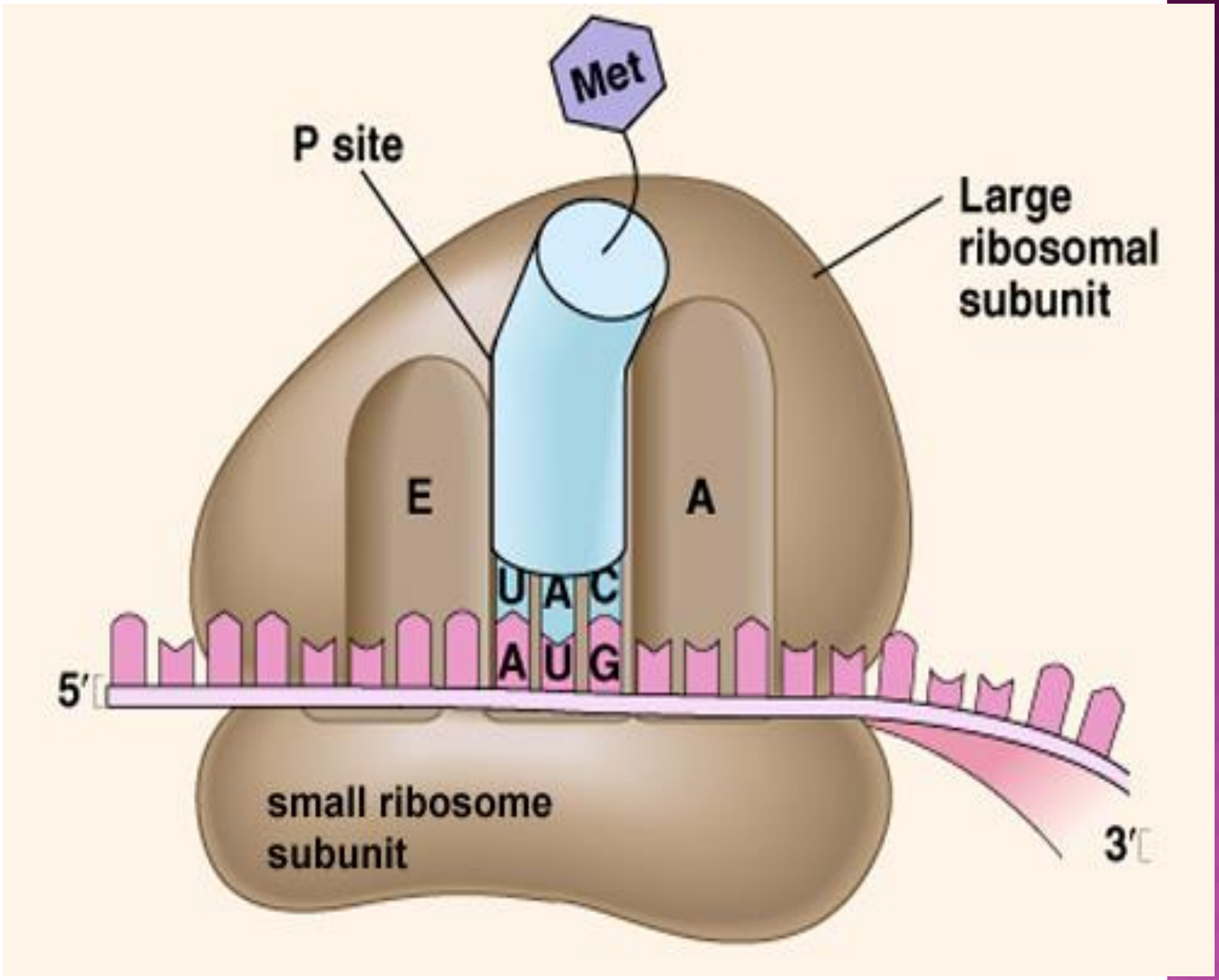
  1st found in the cytoplasm as free cellular particles

  2nd found attached to the ER called rough ER

FUNCTIONAL SITES OF RIBOSOMES

- During bacterial translation, the mRNA lies on the surface of the 30S subunit
- As a polypeptide is being synthesized, it exits through a hole within the 50S subunit

- Ribosomes contain three discrete sites
  - Peptidyl site (P site)
  - Aminoacyl site (A site)
  - Exit site (E site)


Met
P site
Large ribosomal subunit
E
A
U A C
A U G
5′
3′
small ribosome subunit

RIBOSOMAL DISSOCIATION

- Before initiation starts, the 80s ribosome dissociates to form 40s and 60s subunits
- Two initiation factors **eIF-3** and **eIF-1A** binds to the newly formed 40s subunit
- The binding of initiation factors with 40S subunit will prevent its re association with 60S and will also allows other translation factors to get attach to 40S subunit & prepare it for formation of **80S initiation complex**.

Formation of 43S pre initiation complex

- This process involves the binding of GTP with **eIF-2** forming **binary complex**
- The binary complex then get attach to Met-t-RNA forming **ternary complex**
- The ternary complex then binds to 40S ribosomal unit and form **43s pre initiation complex**
- This complex is stabilized by **eIF-3 and eIF-1A**
- In eukaryotes **elf-2** is the controlling factor in protein synthesis.

INITIATION:

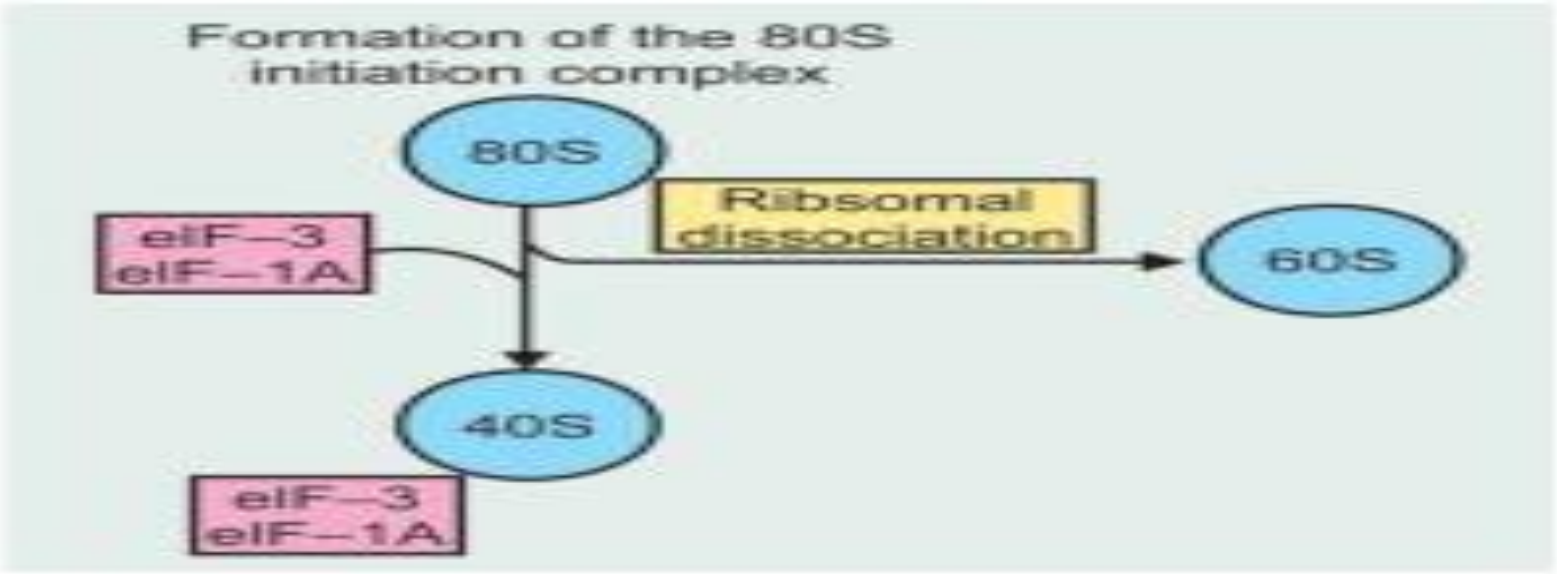


Fig. 17.5: Dissociation of 80S ribosome

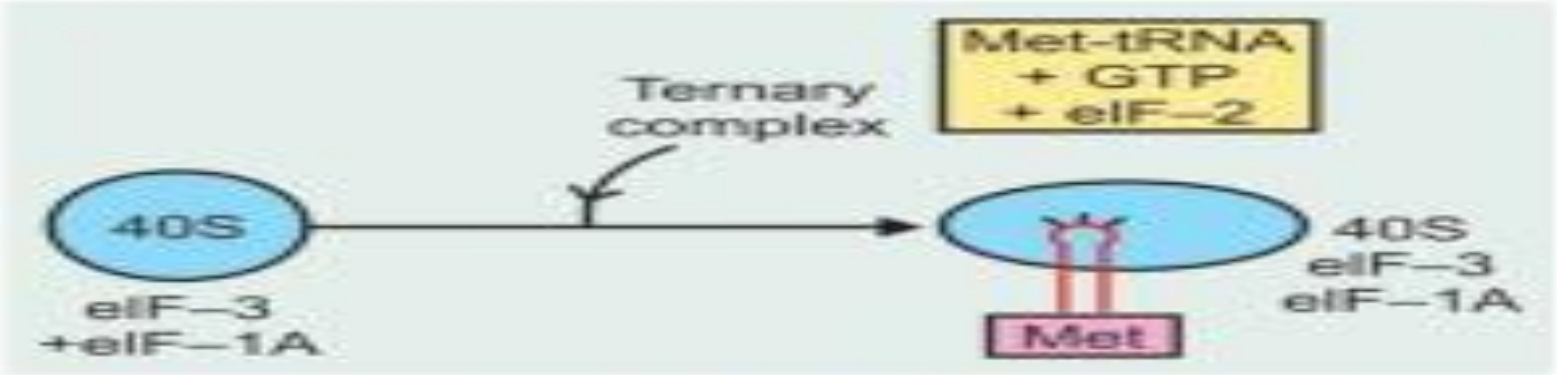


Fig. 17.6: Formation of 43S pre-initiation complex

Formation of 48S initiation complex

- The binding of mRNA to 43S pre initiation complex results in the formation of **48S initiation complex**
- Before the attachment many initiation factors like **eIF-4F**, **eIF-4G,eIF- 4A** and **eIF- 4E** get attached to mRNA

INITIATION:

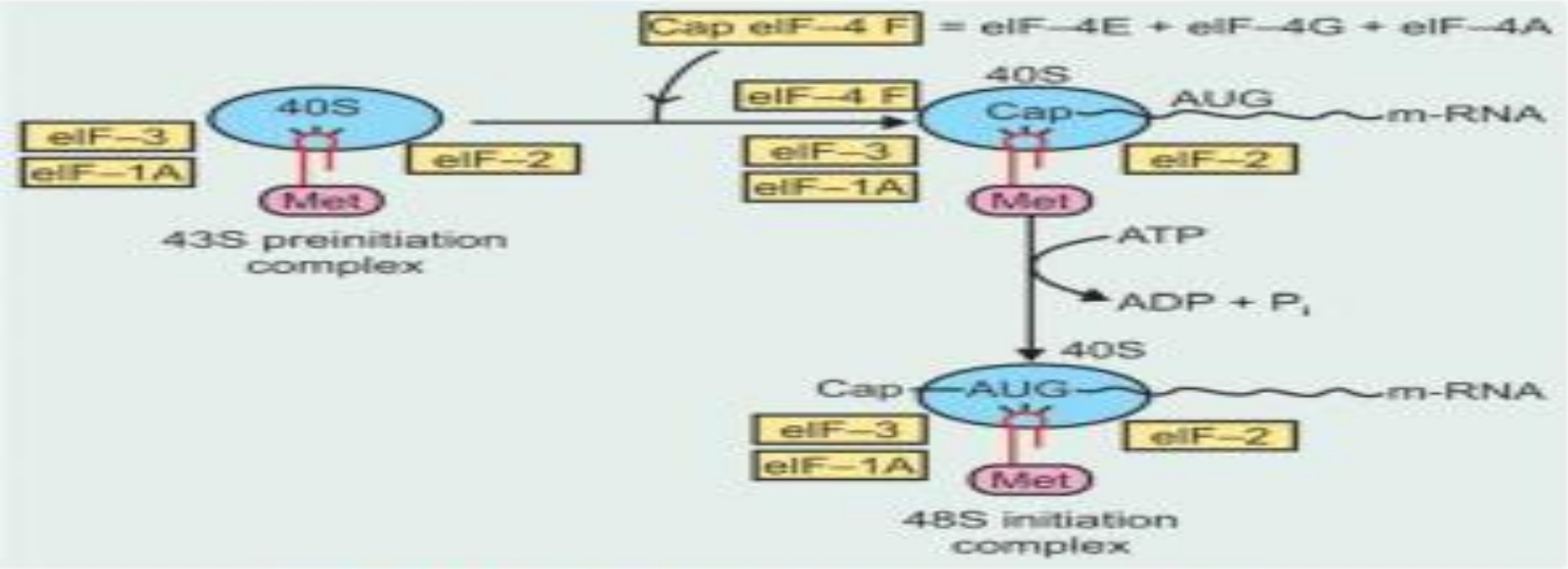


Fig. 17.7: Formation of initiation complex

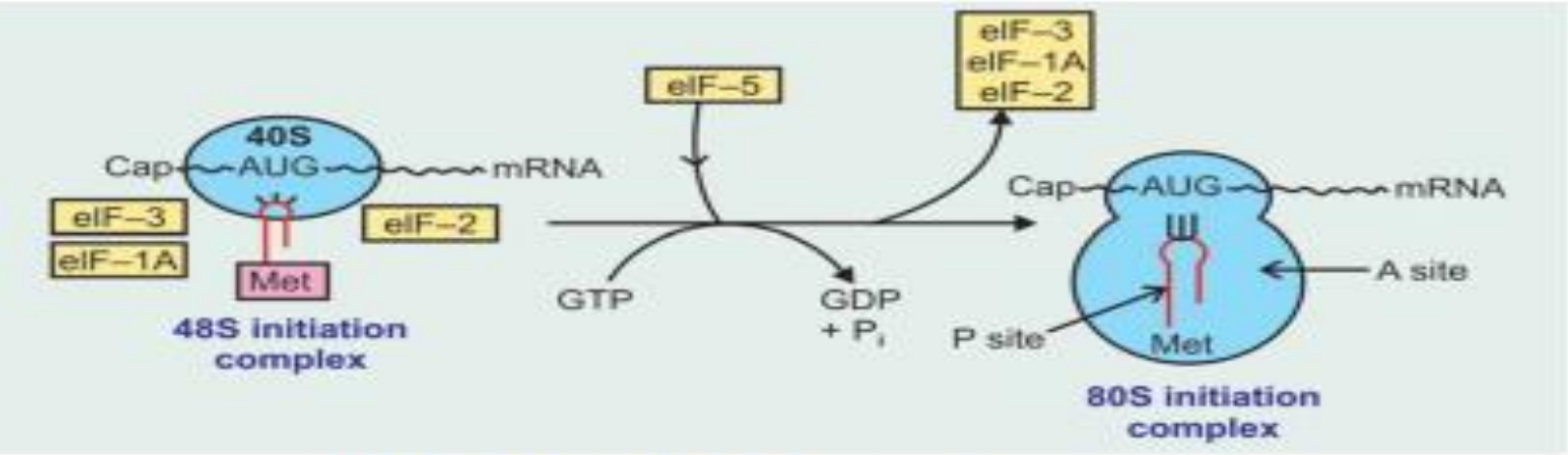


Fig. 17.8: Formation of 80S ribosomal initiation complex

FORMATION OF 80S INITIATION COMPLEX

- 48S initiation complex binds to 60S ribosomal subunit to form 80 S initiation complex
- The binding involves the hydrolysis of GTP
- As the 80S complex is formed the initiation factors are released.
- **eIF-2** carries GTP & **eIF-5** GTP ase activity
- This 80S ribosomal complex is ready for protein synthesis.

STAGES OF TRANSLATION

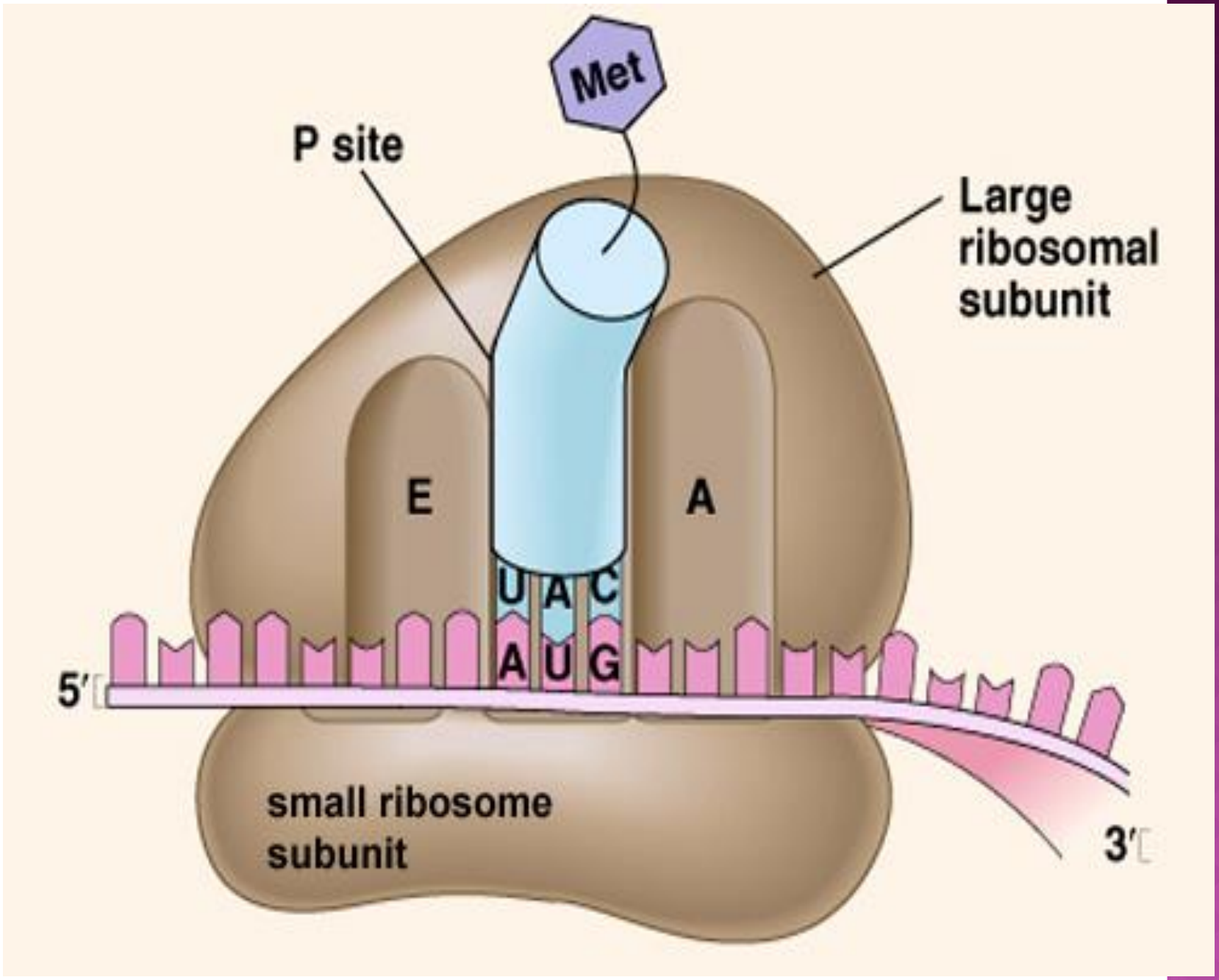
- Initiation
- Elongation
- Termination

TRANSLATION INITIATION

- Components
  - mRNA,
  - initiator tRNA,
  - Initiation factors
  - ribosomal subunits
- The initiator tRNA
  - In prokaryotes, this tRNA is designated $tRNA^{fmet}$
    - It carries a methionine modified to N-formylmethionine
  - In eukaryotes, this tRNA is designated $tRNA_i^{met}$
    - It carries an unmodified methionine
  - In both cases the initiator tRNA is different from a $tRNA^{fmet}$ that reads an internal AUG codon

80S RIBOSOMAL COMPLEX

- The 80s complex has three receptor sites
- **P' site or peptidyl site:** at this point the met-tRNA is on the p site. On this site the growing peptide chain will grow
- **A' site or aminoacyl site**: At this point it is free, the new incoming tRNA with the amino acid to be added next is taken up, at this site
- '**E'site or exit site**: After the addition of amino acid the tRNA will exit from this site.


Met
P site
Large ribosomal subunit
E
A
U A C
A U G
5′
3′
small ribosome subunit

ELONGATION

Elongation process involves three following steps

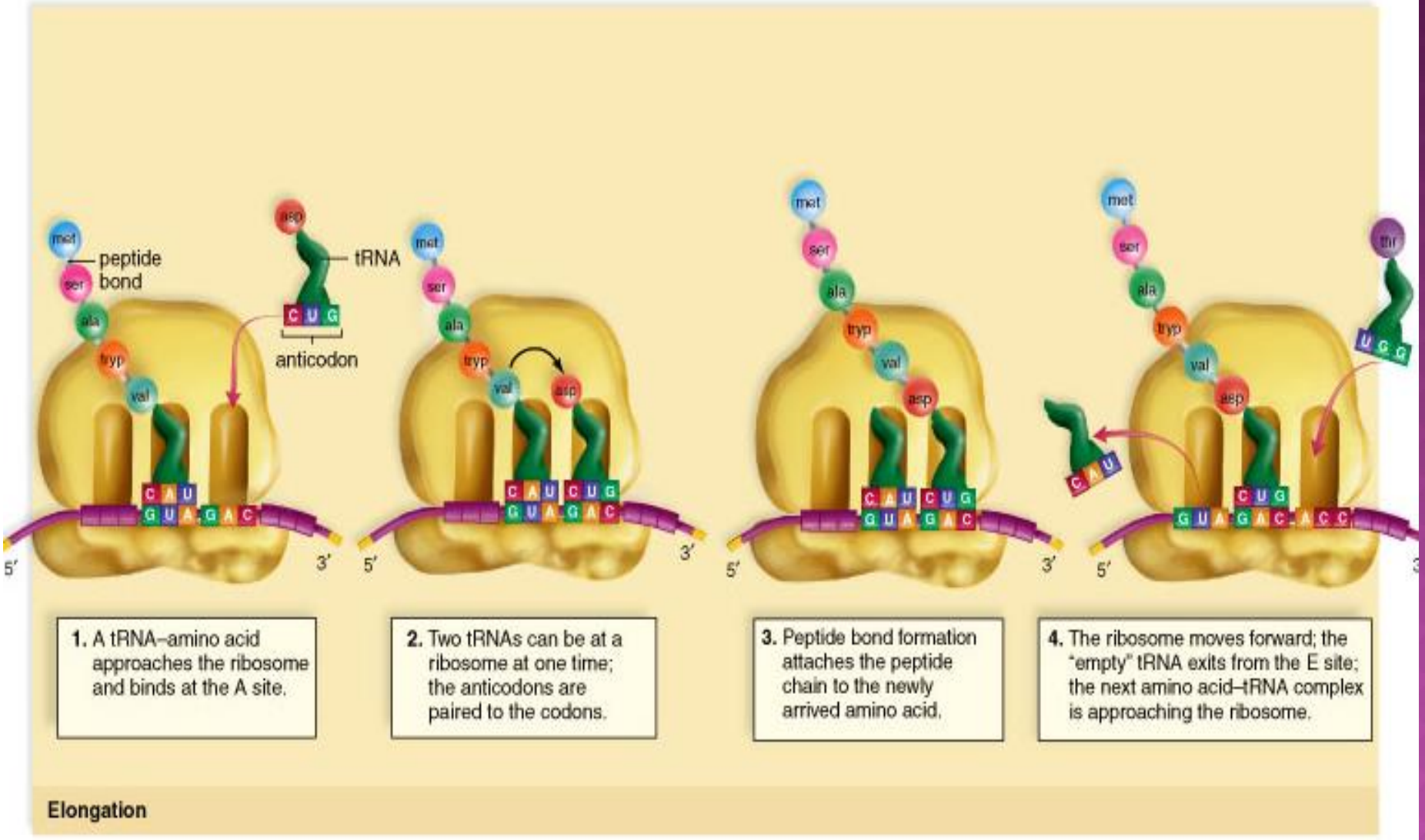
- The binding of new amino acyl-tRNA
- Peptide bond formation
- Translocation process

TRANSLATION ELONGATION

- During this stage, the amino acids are added to the polypeptide chain, one at a time

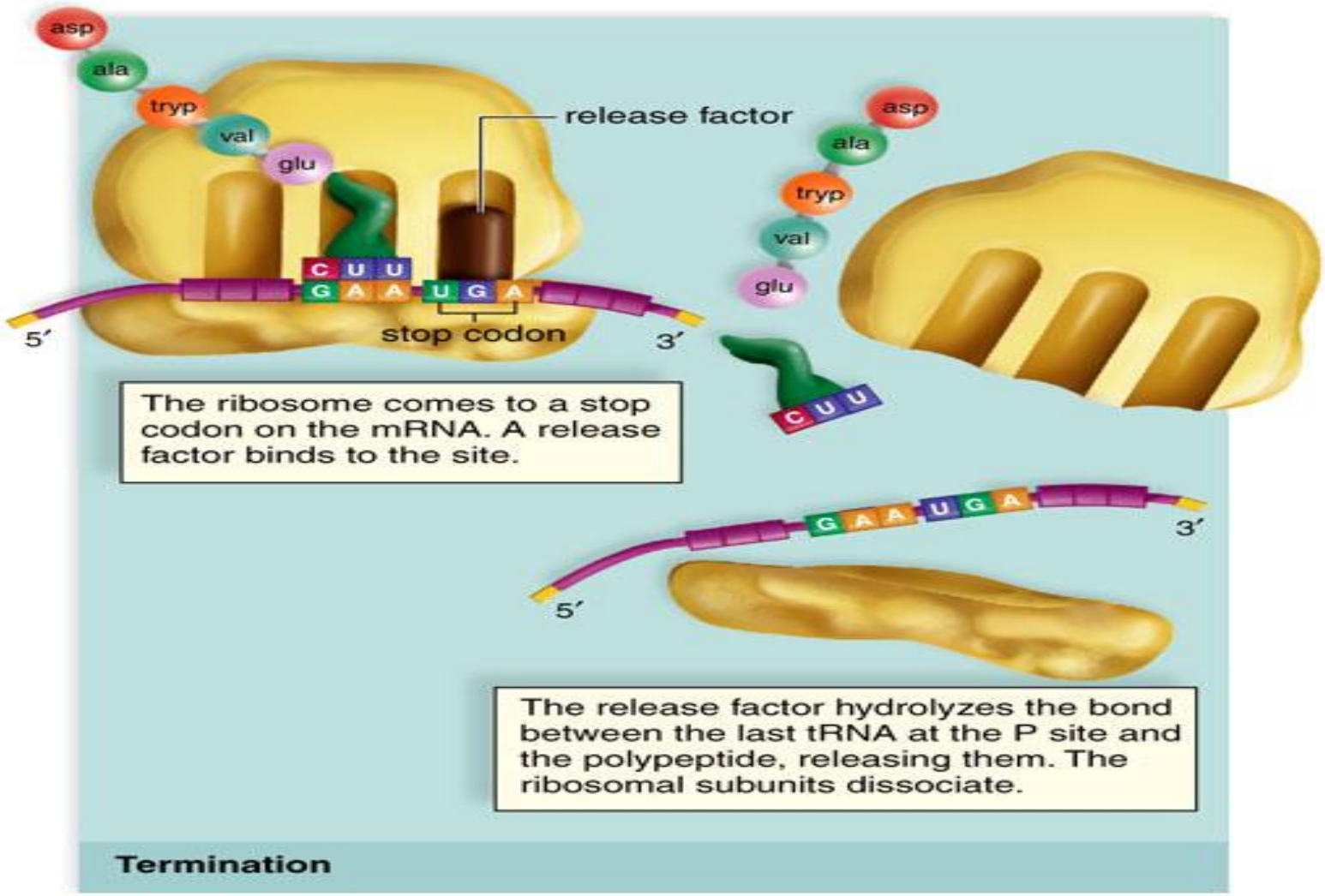
- The addition of each amino acid occurs via a series of steps
- This process, though complex, can occur at a remarkable rate
  - In bacteria → 15-18 amino acids per second
  - In eukaryotes → 6 amino acids per second

ELONGATION



Elongation

TERMINATION PROCESS

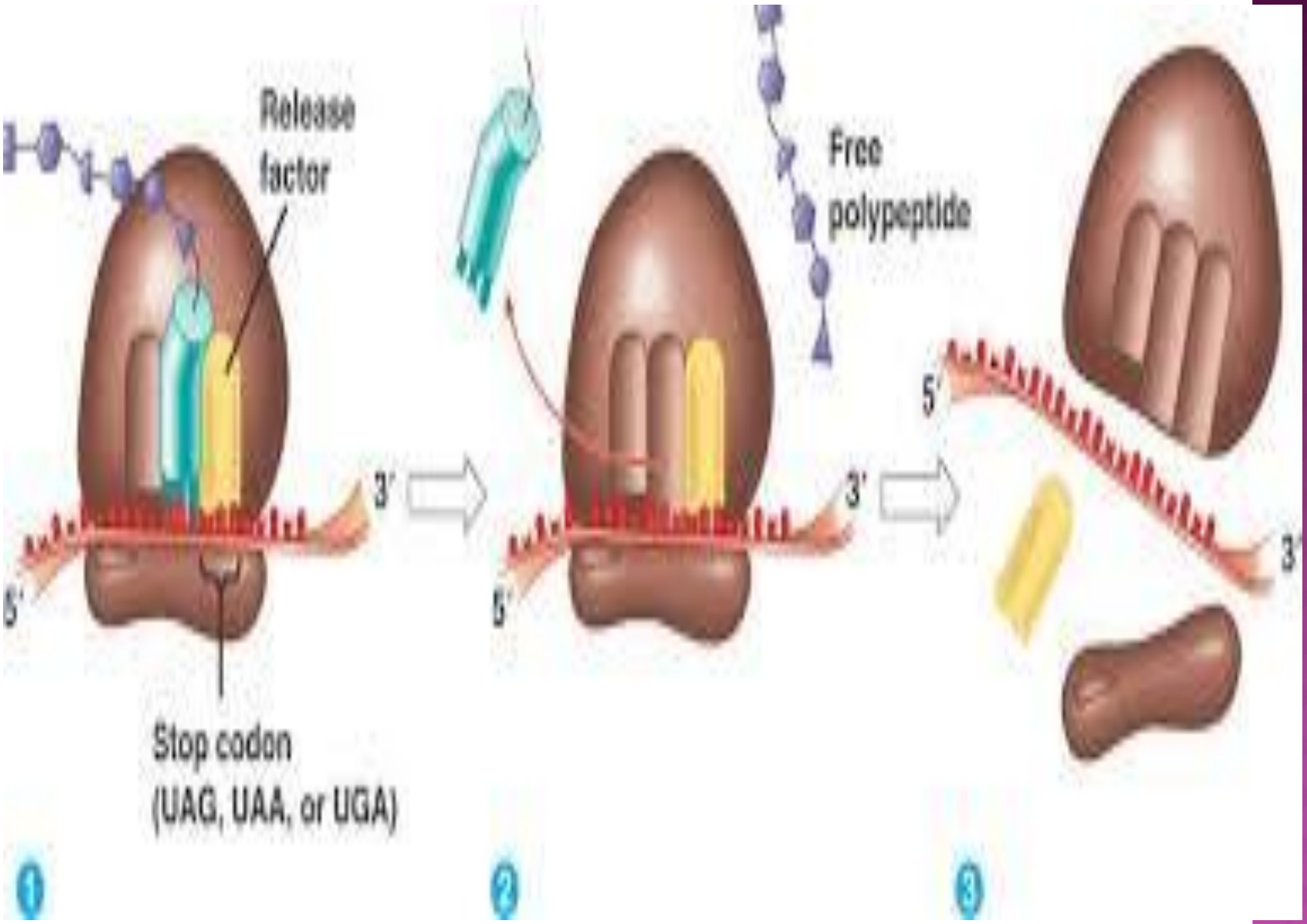


TRANSLATION TERMINATION

- Occurs when a stop codon is reached in the mRNA
- Three stop or nonsense codons
  - UAG
  - UAA
  - UGA
- Recognized by proteins called release factors NOT tRNAs

TRANSLATION TERMINATION

- Bacteria have three release factors
  - RF1 - recognizes UAA and UAG
  - RF2 - recognizes UAA and UGA
  - RF3 - binds GTP and facilitates termination process
- Eukaryotes only have one release factor
  - eRF1 - recognizes all three stop codons


Release factor
3'
5'
Stop codon (UAG, UAA, or UGA)
1
Free polypeptide
3'
5'
2
5'
3'
3

INHIBITORS OF TRANSLATION

- **Streptomycin**: causes misreading of mRNA and interfere with the normal pairing between codons and anticodons
- **Tetracycline**: It inhibits the binding of aminoacyl tRNA to the ribosomal complex
- **Puromycin**: Puromycin enters the A site and gets incorporated into the growing peptide chain and causes its release

INHIBITORS OF TRANSLATION

- **Chloramphenicol**: It acts as a competitive inhibitor of the enzyme peptidyl transferase and thus interfere with elongation of peptide chain
- **Erythromycin**: It inhibits the translocation process

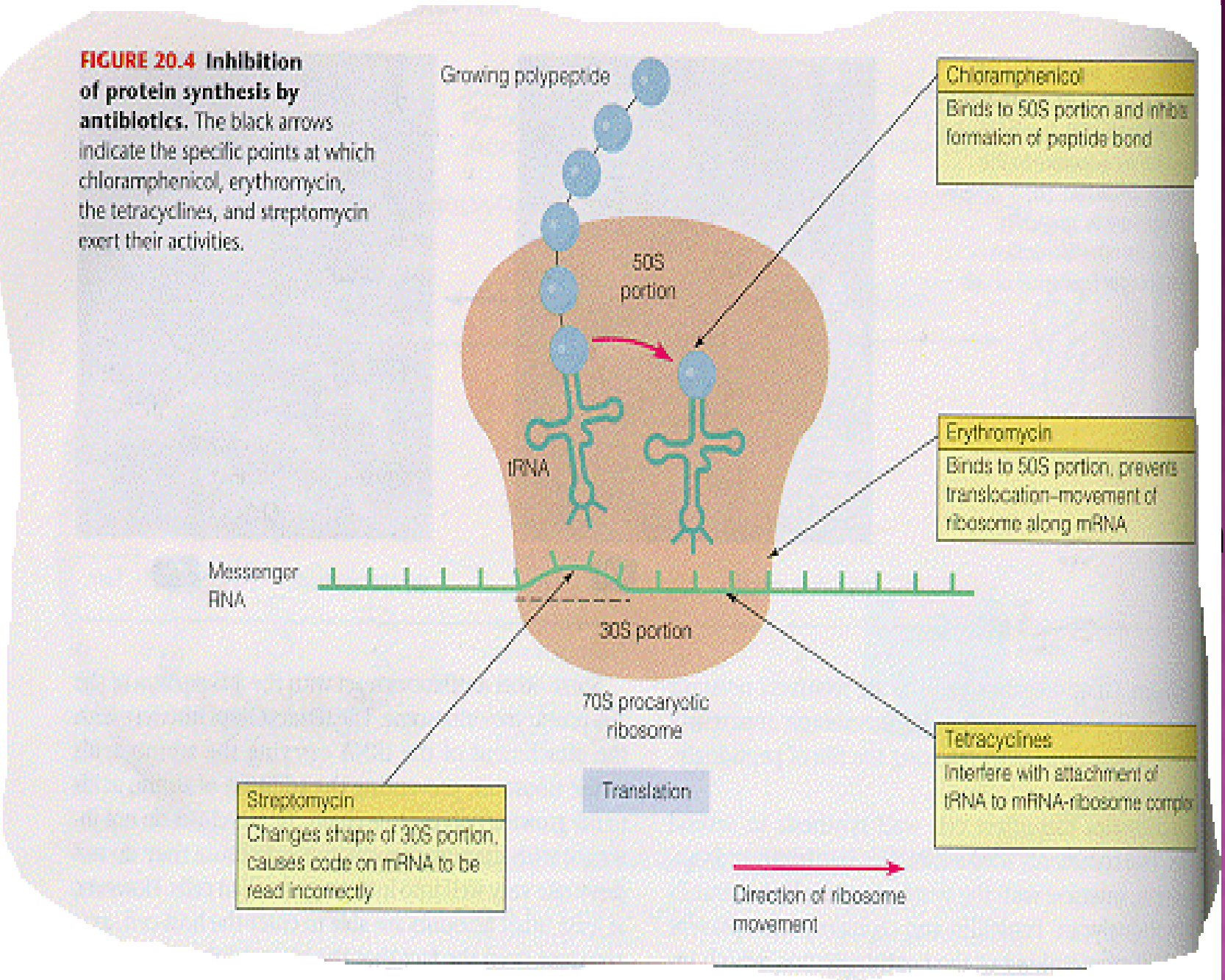


FIGURE 20.4 Inhibition of protein synthesis by antibiotics. The black arrows indicate the specific points at which chloramphenicol, erythromycin, the tetracyclines, and streptomycin exert their activities.
Growing polypeptide
50S portion
tRNA
Messenger RNA
30S portion
70S procaryotic ribosome
Translation
Direction of ribosome movement
Chloramphenicol
Binds to 50S portion and inhibits formation of peptide bond
Erythromycin
Binds to 50S portion, prevents translocation–movement of ribosome along mRNA
Tetracyclines
Interfere with attachment of tRNA to mRNA-ribosome complex
Streptomycin
Changes shape of 30S portion, causes code on mRNA to be read incorrectly

POST TRANSLATIONAL MODIFICATION

These modification include

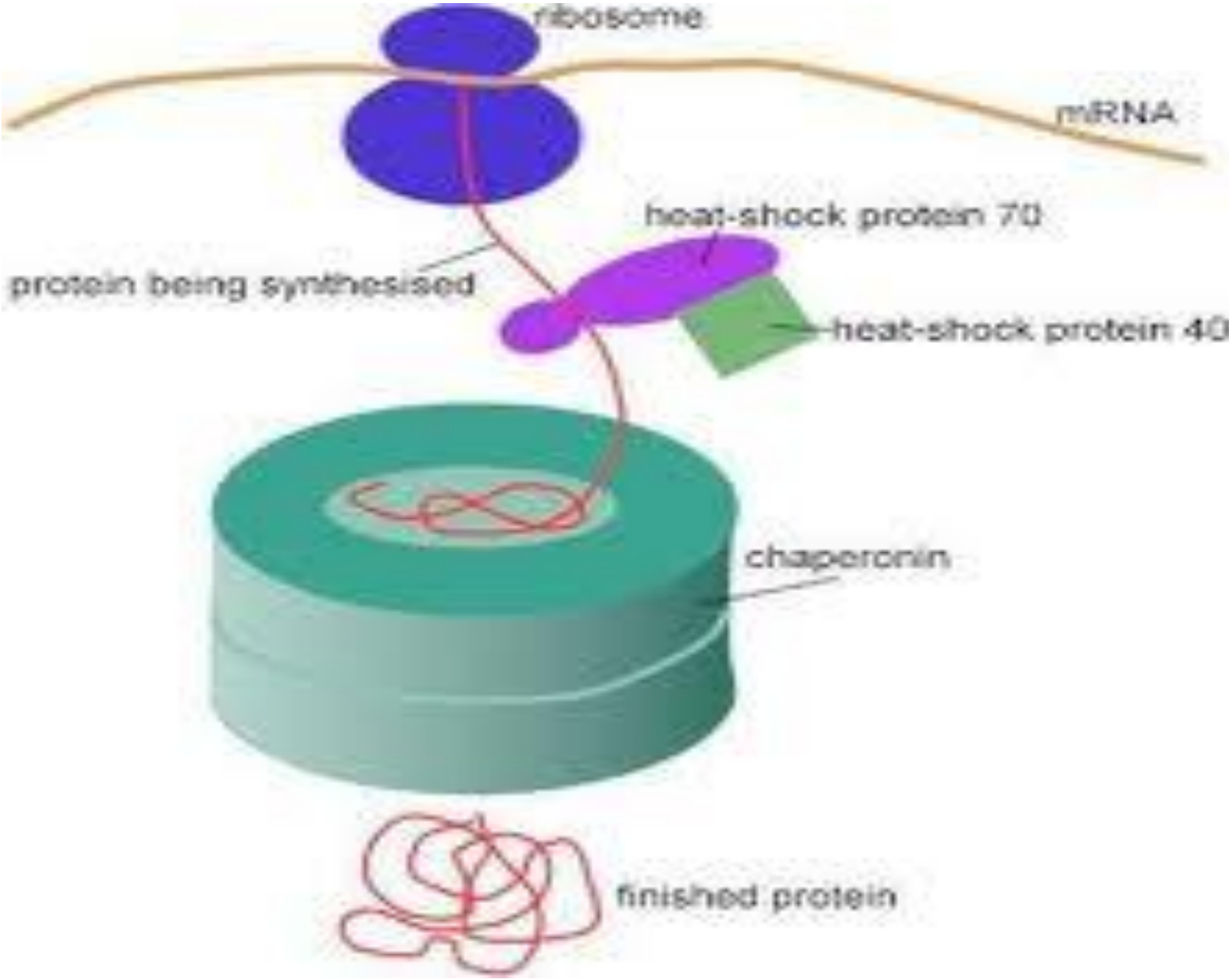
- Protein Folding
- Trimming by proteolytic degradation
- Intein splicing
- Covalent changes

1. PROTEIN FOLDING

- The three dimensional conformation of proteins is important for their biological function
- Some of the protein can spontaneously generate the correct functionally active conformation
- A vast majority of proteins can attain correct conformation only through the assistance of certain proteins called **chaperons**

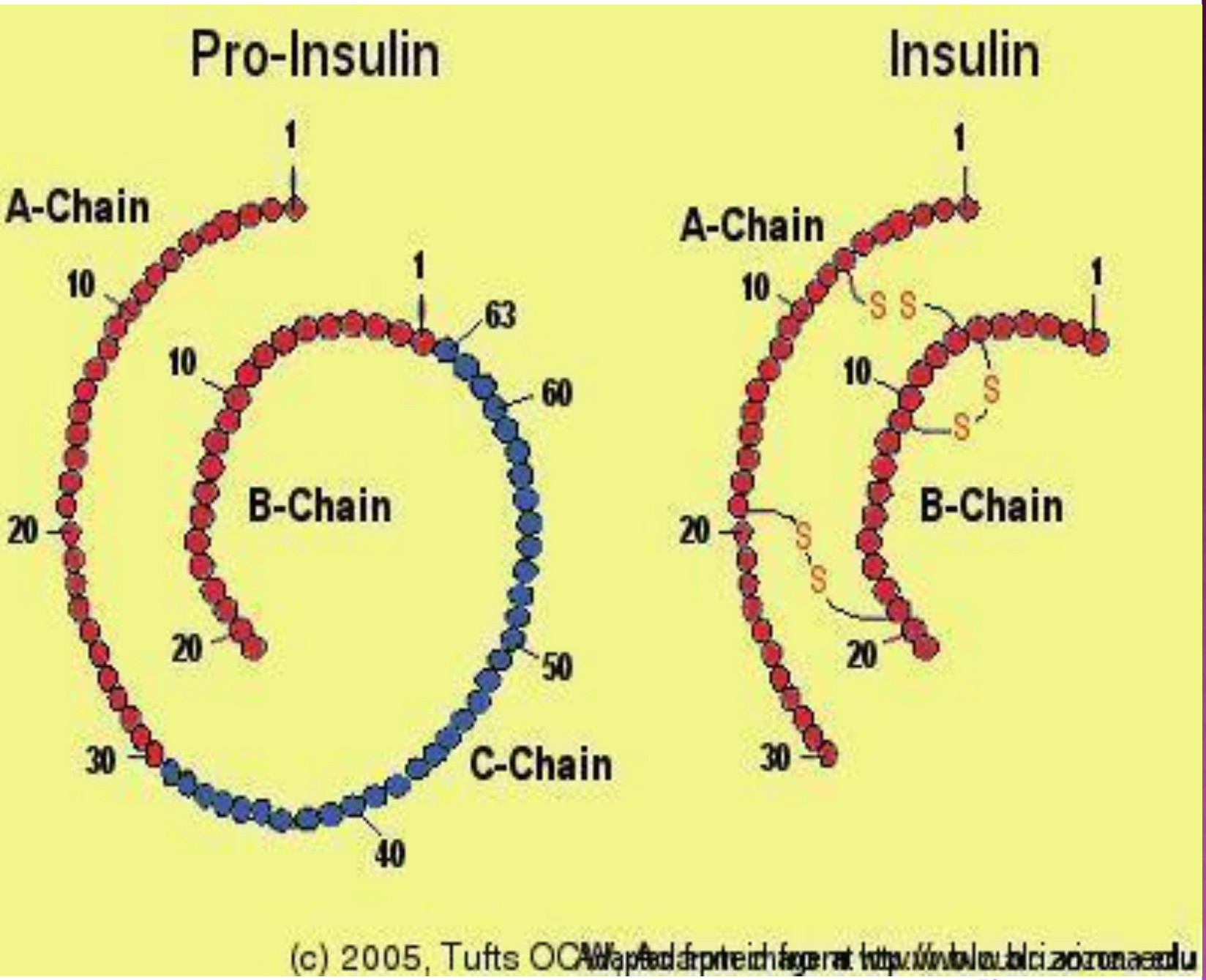
CHAPERONS AND PROTEIN FOLDING

- Chaperons binds to the hydrophobic regions of unfolded proteins and folding intermediates
- They can **stabilize** intermediates, **prevent formation of incorrect intermediates** and also **prevent undesirable interactions with other proteins**
- All these activities of chaperons help the protein to attain compact and biological active conformation



2. PROTEOLYTIC DEGRADATION

- Many proteins are synthesized as the precursor which are much bigger in size than the functional protein
- Some portions of precursor molecules are removed by proteolysis to liberate active protein
- This process is also called **trimming**
- Example: Formation of insulin from pre pro insulin


Pro-Insulin
Insulin
A-Chain
B-Chain
C-Chain
A-Chain
B-Chain
S S
S
S
S
S
1
10
20
30
40
50
60
63
1
10
20
1
10
20
30
(c) 2005, Tufts OC

3. INTEIN SPLICING

- Inteins are intervening sequences in certain protein
- These are comparable to introns in mRNAs
- Inteins have to be removed and exteins ligated in appropriate order for the protein to become active

4. COVALENT MODIFICATION