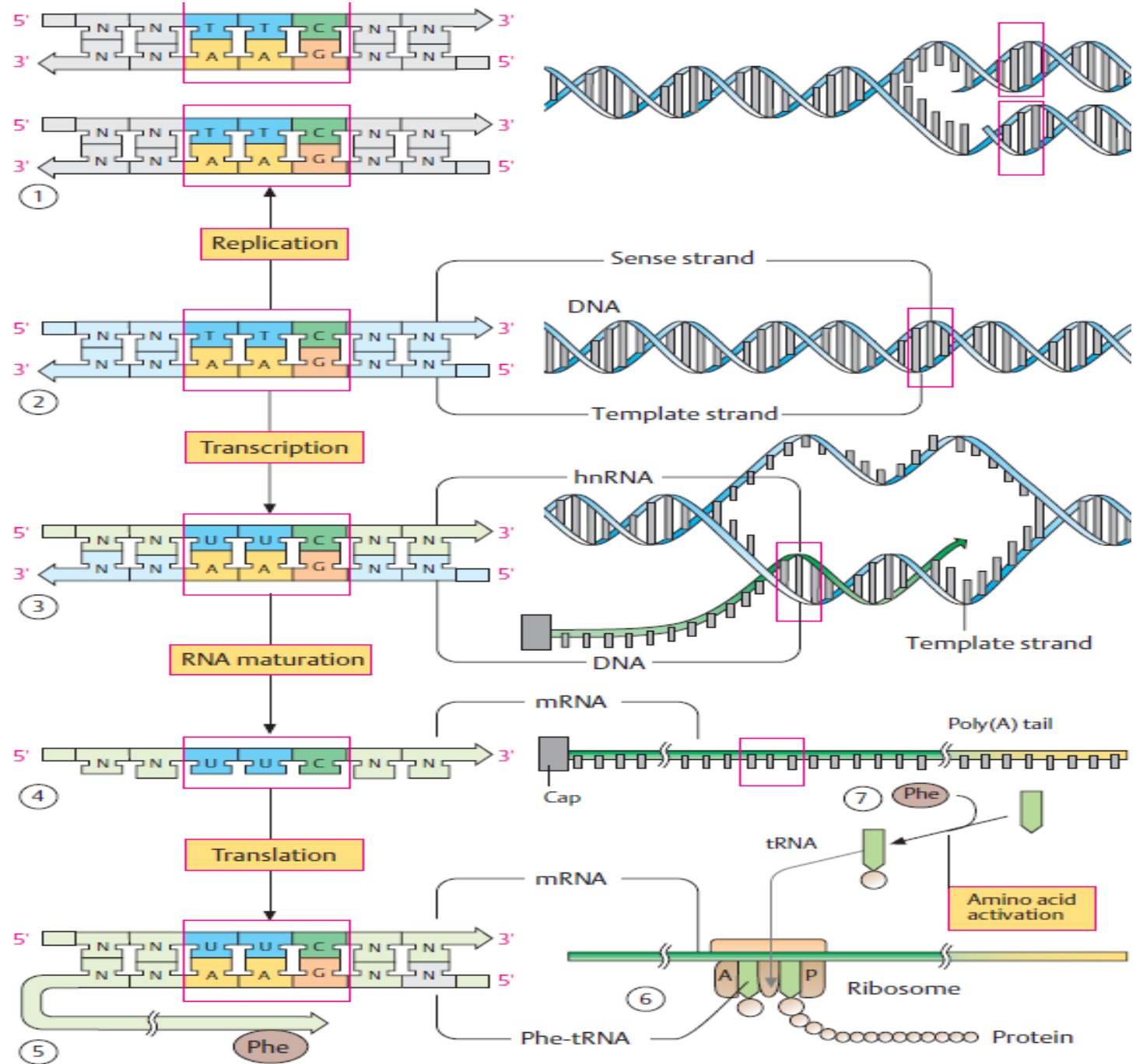




Protein Synthesis (Translation)

Nebras Melhem

Dr. Walaa Bayoumie El Gazzar



Steps of protein synthesis

- **Activation of amino acid (AA) and synthesis of aminoacyl-tRNA:**

Aminoacyl t-RNA synthetase belongs to the enzyme family which is required for attachment of AAs to their corresponding tRNA.

Each member of this family recognizes a specific AA and all the tRNAs that correspond to that AA.

The enzymes check their work, and if the incorrect amino acid has been linked to a particular tRNA, the enzyme will remove the amino acid from the tRNA and try again utilizing the correct amino acid.

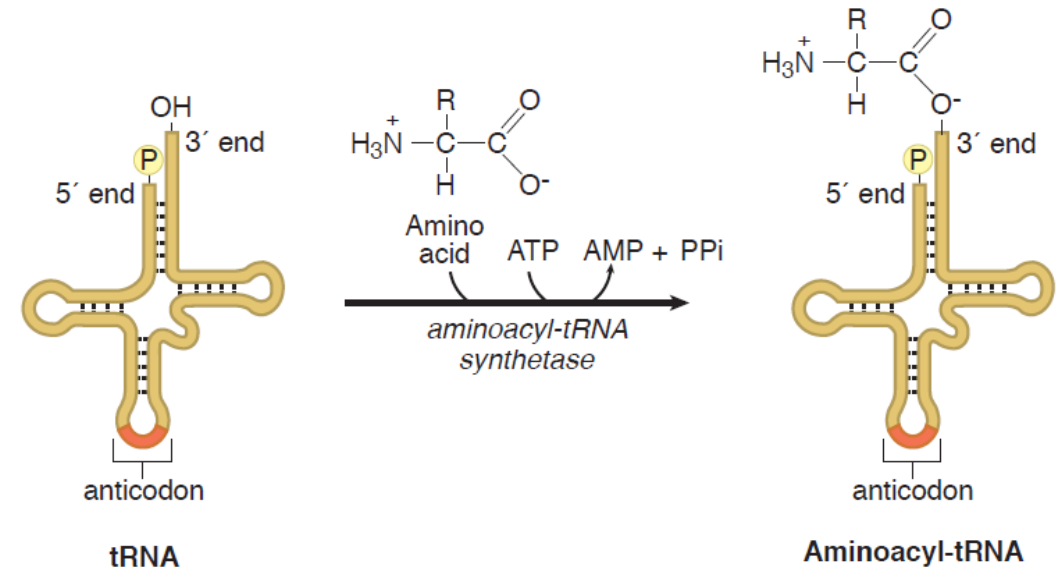
Steps of protein synthesis

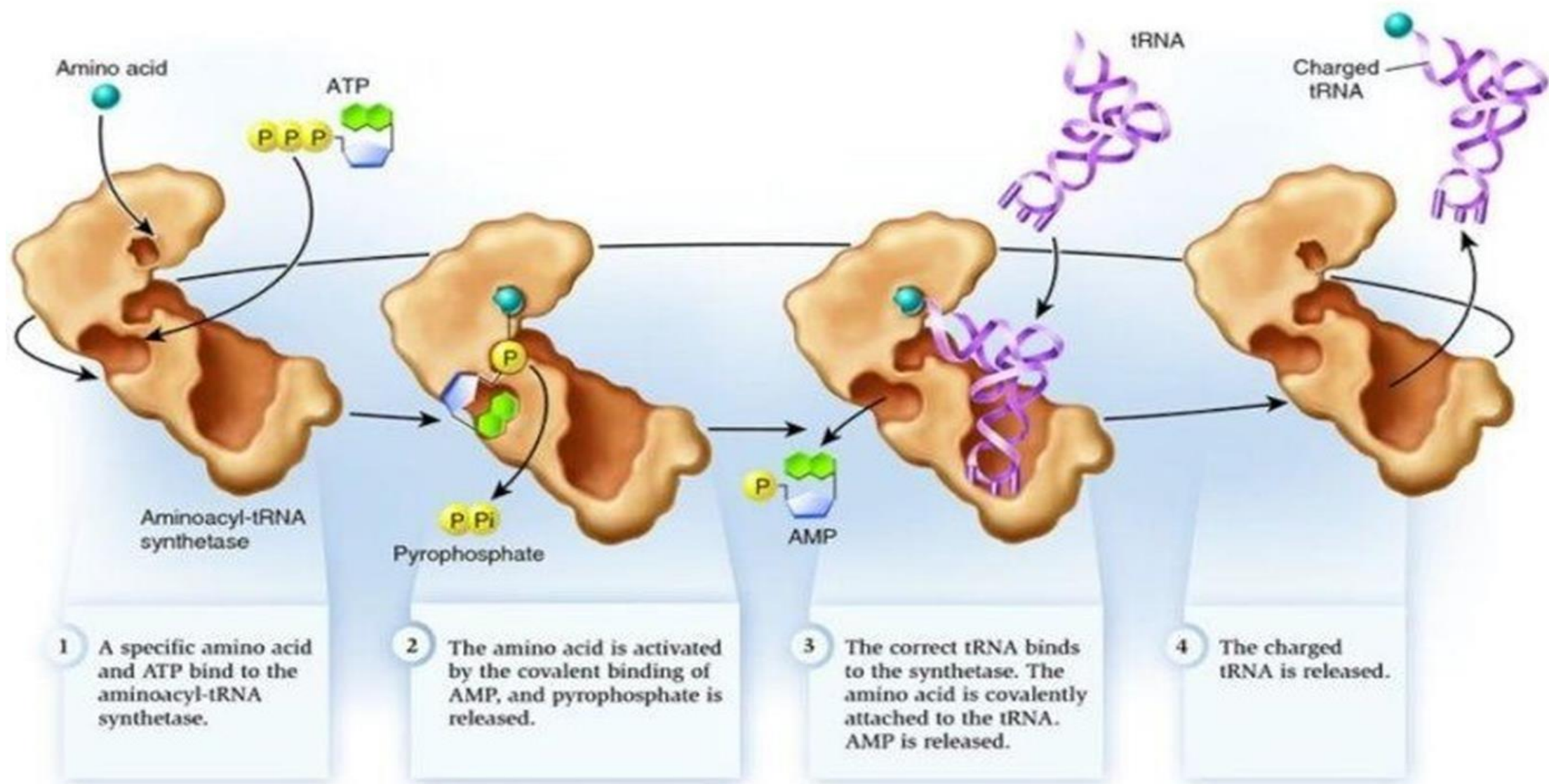
- The enzyme recognizes the **-R radical** of the amino acid and recognizes the **anticodon** of the tRNA.
- Transfer-RNAs for different amino acids differ not only in their anticodon but also at other points, giving them slightly different overall configurations. The aminoacyl-tRNA synthetases recognize the correct tRNAs primarily through their overall configuration, not just through their anticodon.
- In the cytosol, there are 20 species for this enzyme, one specific for each of the 20 amino acids required for protein synthesis.
- This enzyme connects the carboxyl group of the amino acid to the 3'-OH of the specific tRNA.

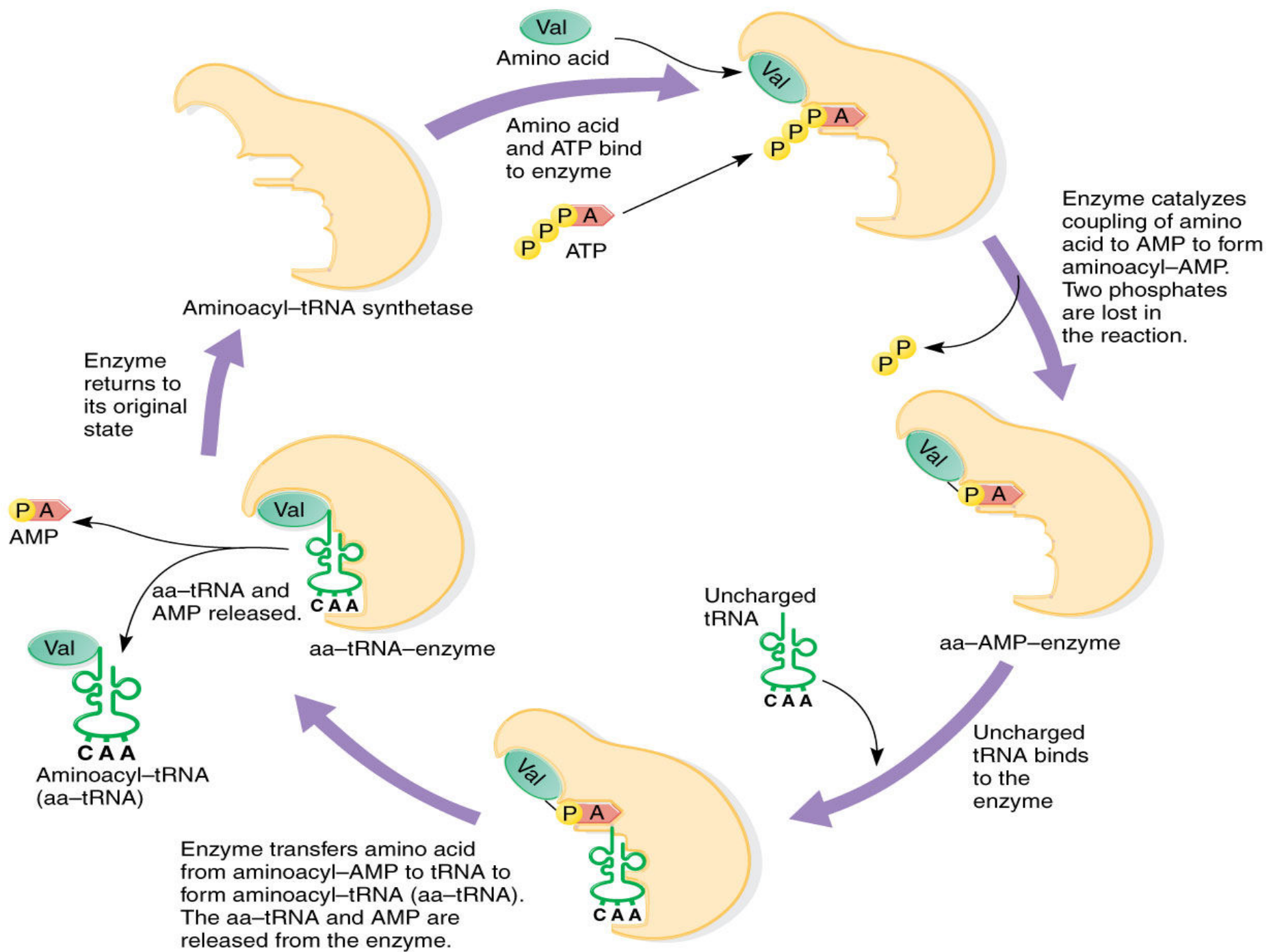
Steps of protein synthesis

- Each type of amino acid is activated by a different aminoacyl tRNA synthetase.
- Two high-energy bonds from an ATP are required.
- The aminoacyl tRNA synthetase transfers the activated amino acid to the 3' end of the correct tRNA.
- The amino acid is linked to its cognate tRNA with an energy-rich bond.

This bond will later supply energy to make a peptide bond linking the amino acid into a protein.









Translation phases

I. Initiation

II. Elongation

III. Termination

- Special protein factors for initiation (IF), elongation (EF), and termination (release factors), as well as GTP, are required for each stage.
-

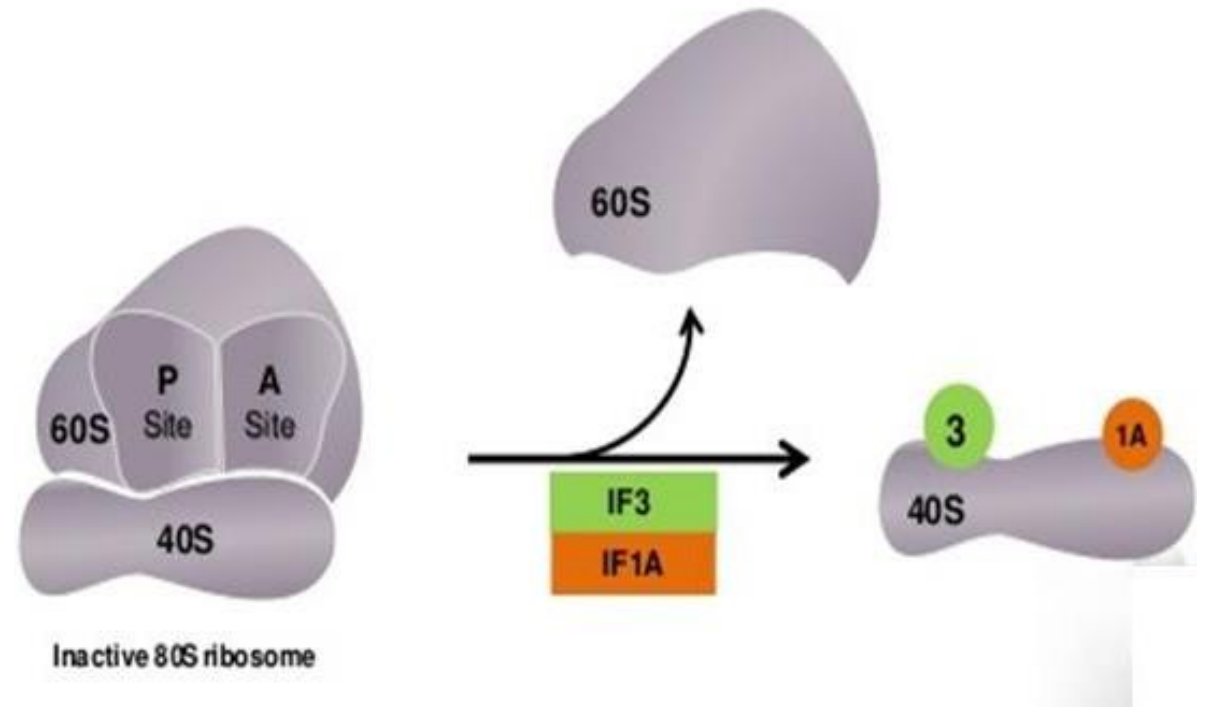


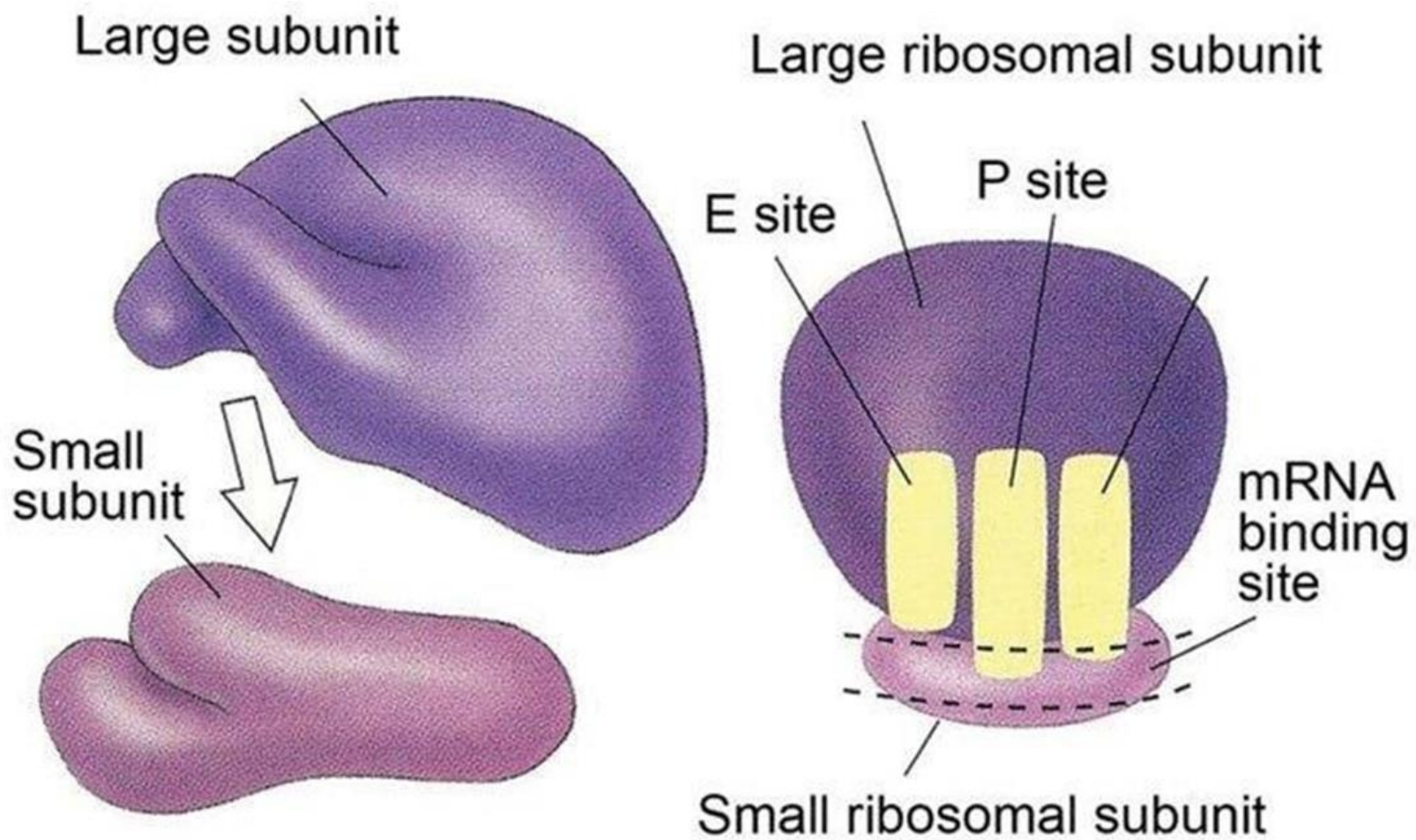
I. Initiation

- Ribosomal dissociation
 - Formation of 43S preinitiation complex
 - Formation of 48S initiation complex
 - Formation of 80S initiation complex
-

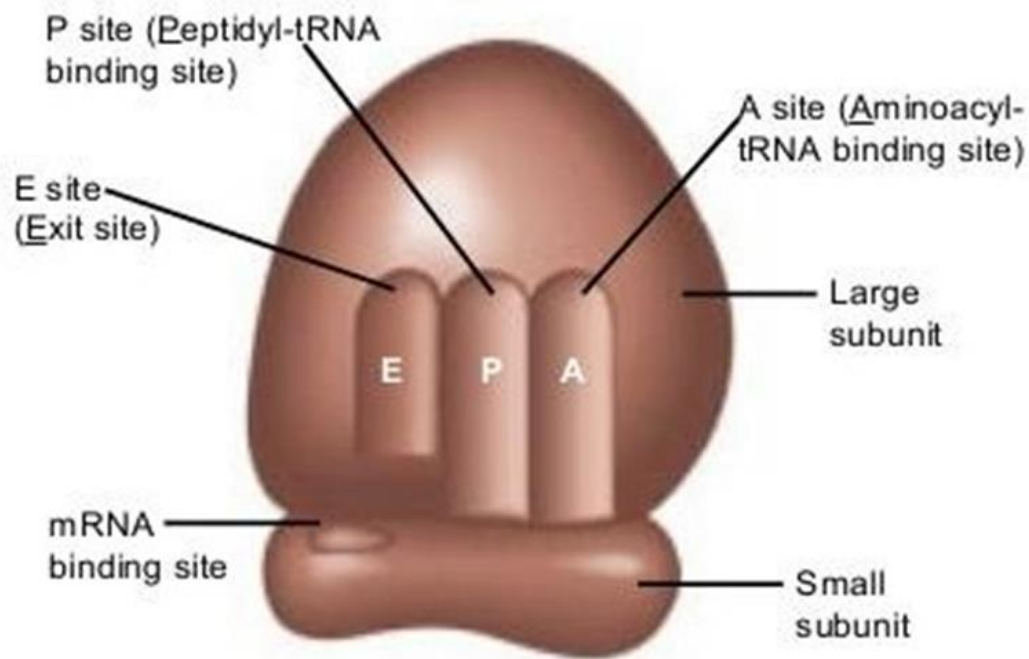
Ribosomal dissociation

Two initiation factors (**IF-1A** and **IF3**) bind to the 40S subunit of the 80S ribosome and produce its dissociation to 40S and 60S ribosomal subunits.





- The ribosome has three binding sites for tRNA
 - The P site
 - The A site
 - The E site

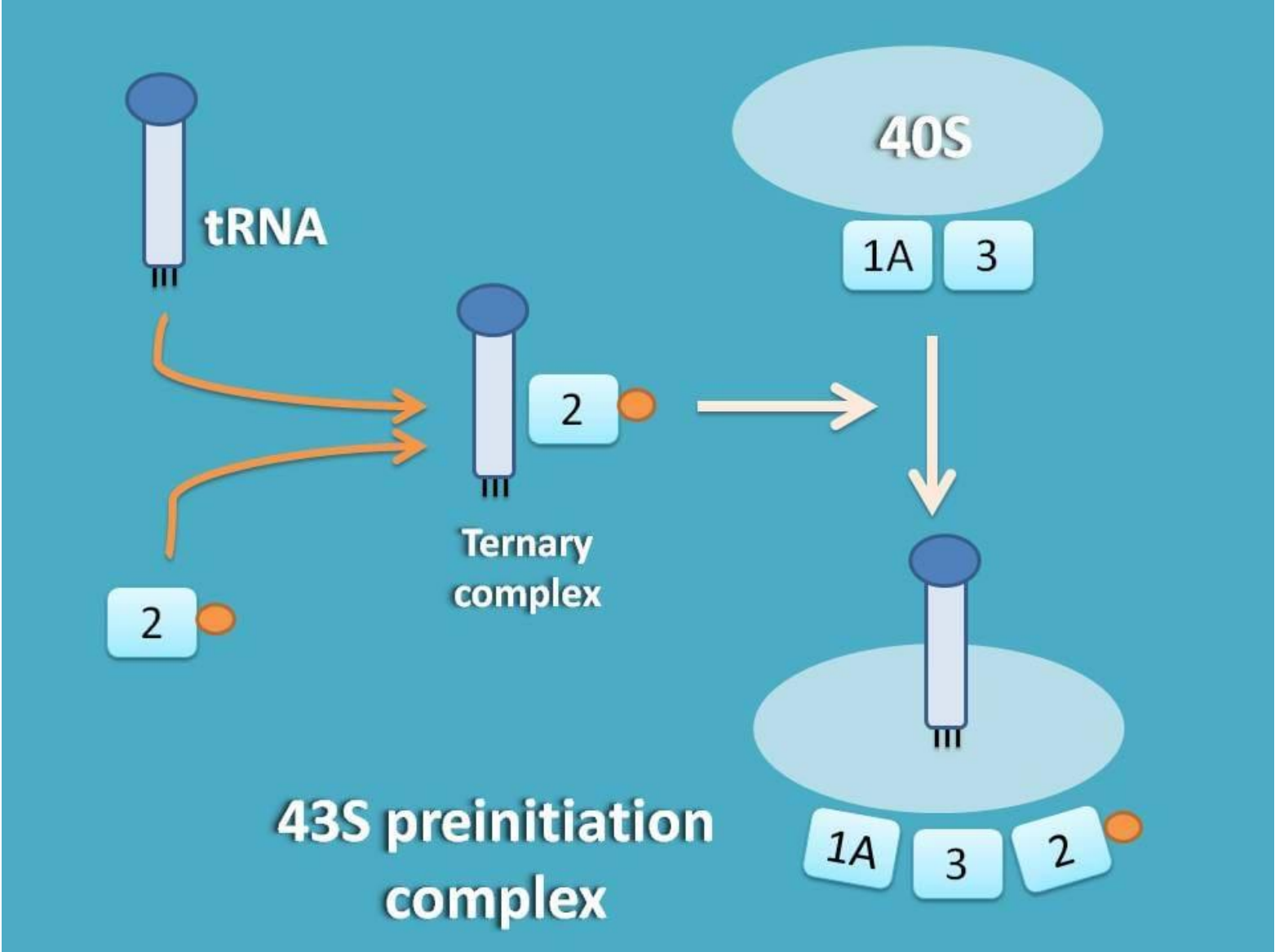


(b) Schematic model showing binding sites. A ribosome has an mRNA binding site and three tRNA binding sites, known as the A, P, and E sites. This schematic ribosome will appear in later diagrams.

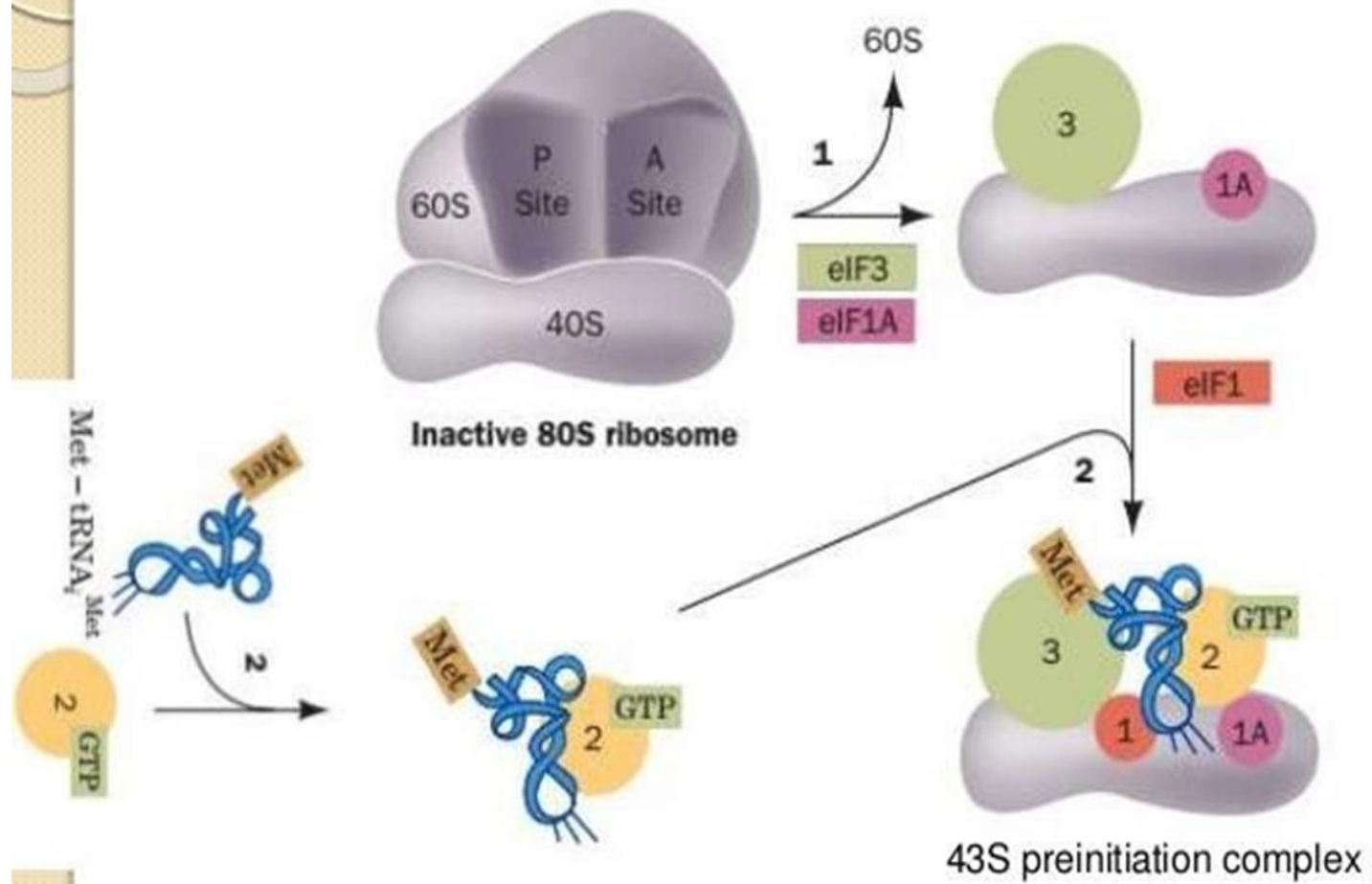
Figure 17.16b

Formation of 43S preinitiation complex

- This step requires :
 - **IF-2** complexed with **GTP**
 - Methionine tRNA (met-tRNA)
- The **eIF-2** binds **GTP** then binds to met-tRNA_i (initiating methionyl-tRNA) to form a complex that binds to the *P-site of the 40S* ribosomal subunit, forming the 43S preinitiation complex.



Initiation in Eukaryotes



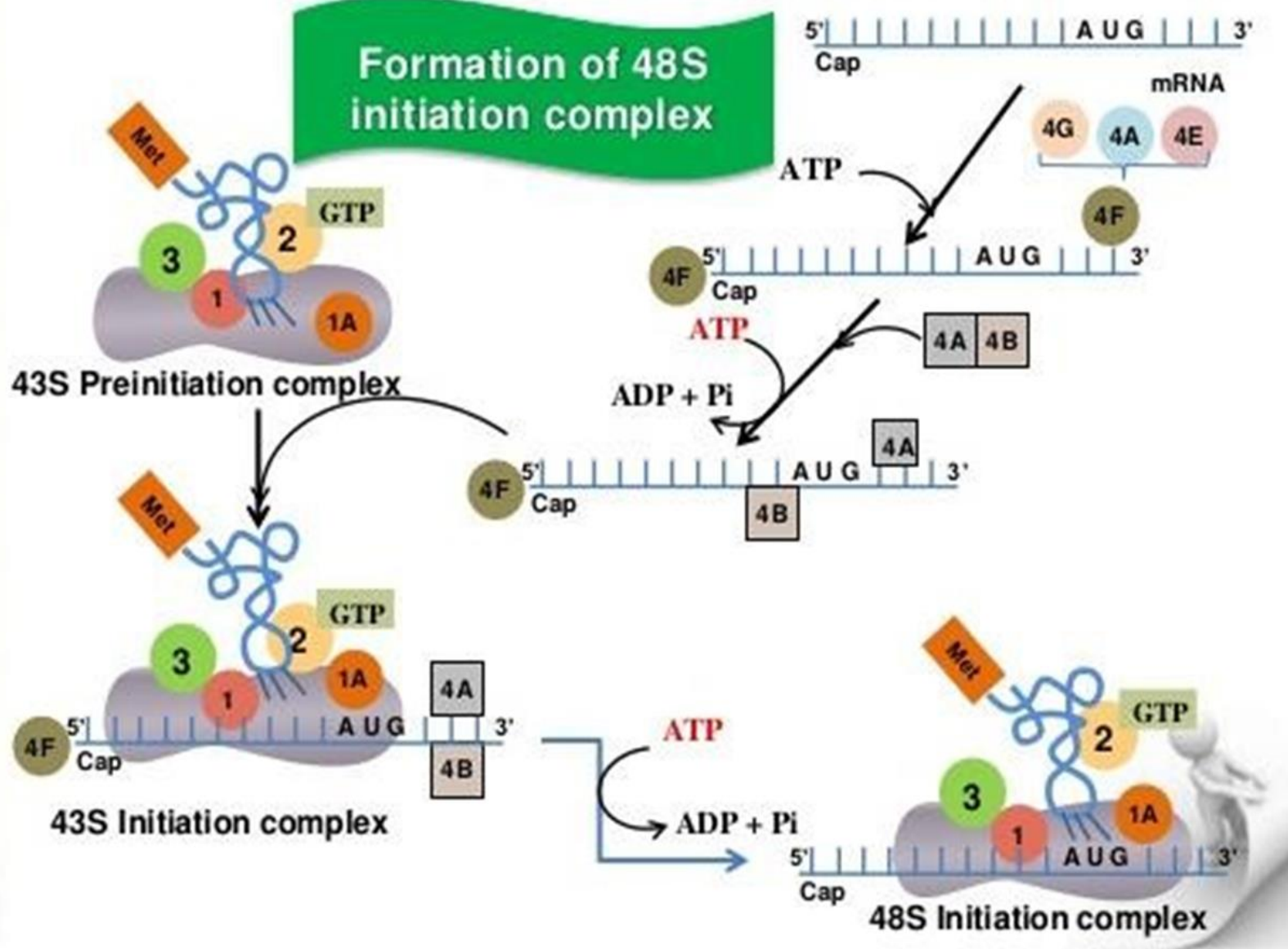
Formation of 48S initiation complex

- The mRNA is **activated** by several initiation factors (**eIF-4**), a process associated with hydrolysis of ATP.
- The 43S preinitiation complex becomes associated with the 5` capped end of the activated mRNA forming the 48S initiation complex .

Formation of 48S initiation complex

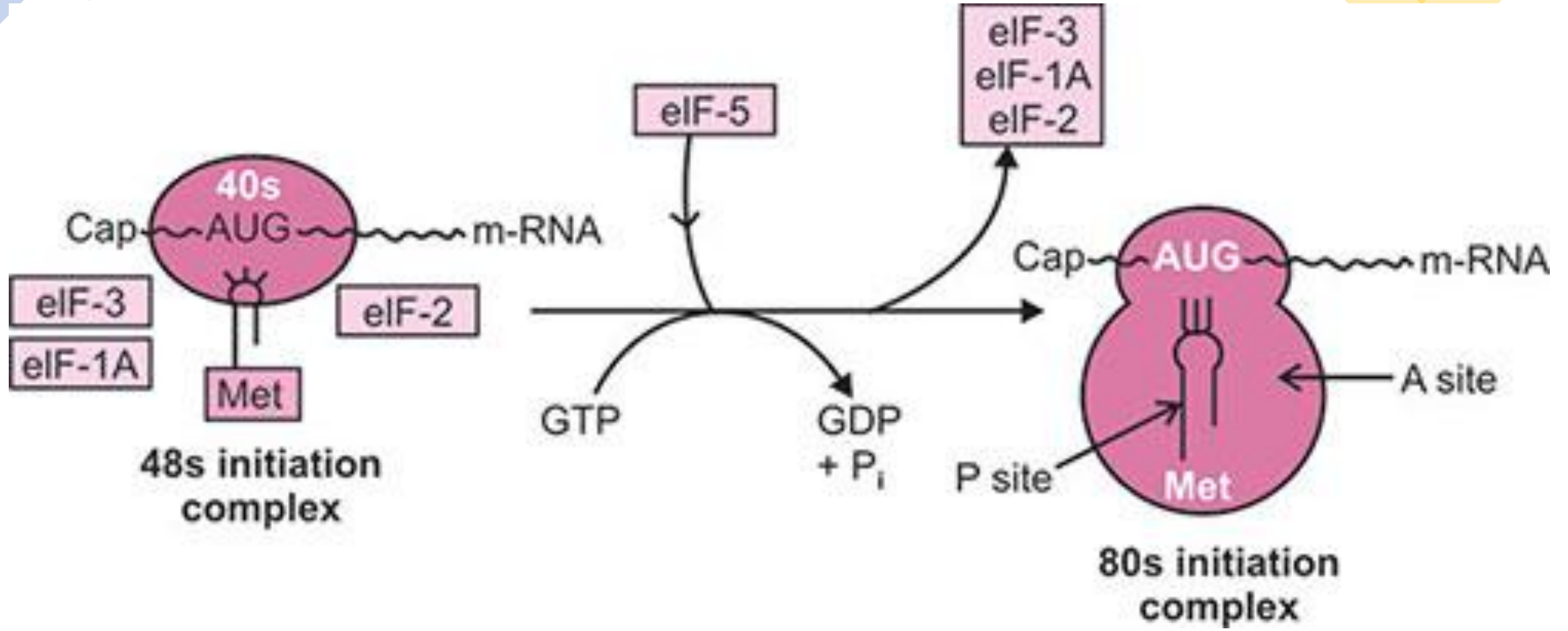
- The 43S preinitiation complex scans the activated mRNA for the initiating codon, which is usually the 1st AUG on the 5' side.
 - The preinitiation complex stops moving on the mRNA when the initiating codon is in the P- site opposite the anticodon of the met-tRNA_i.
-

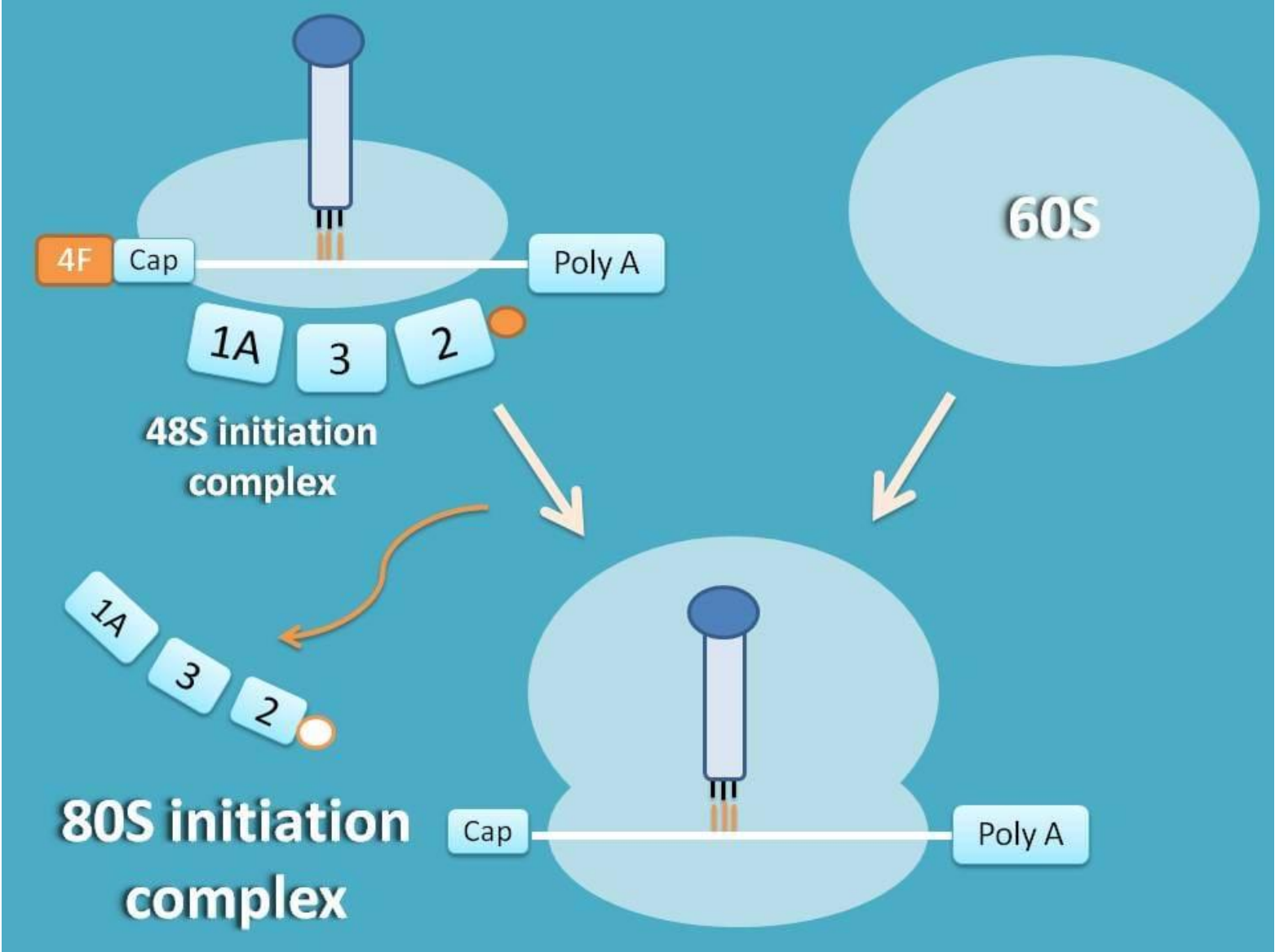
Formation of 48S initiation complex



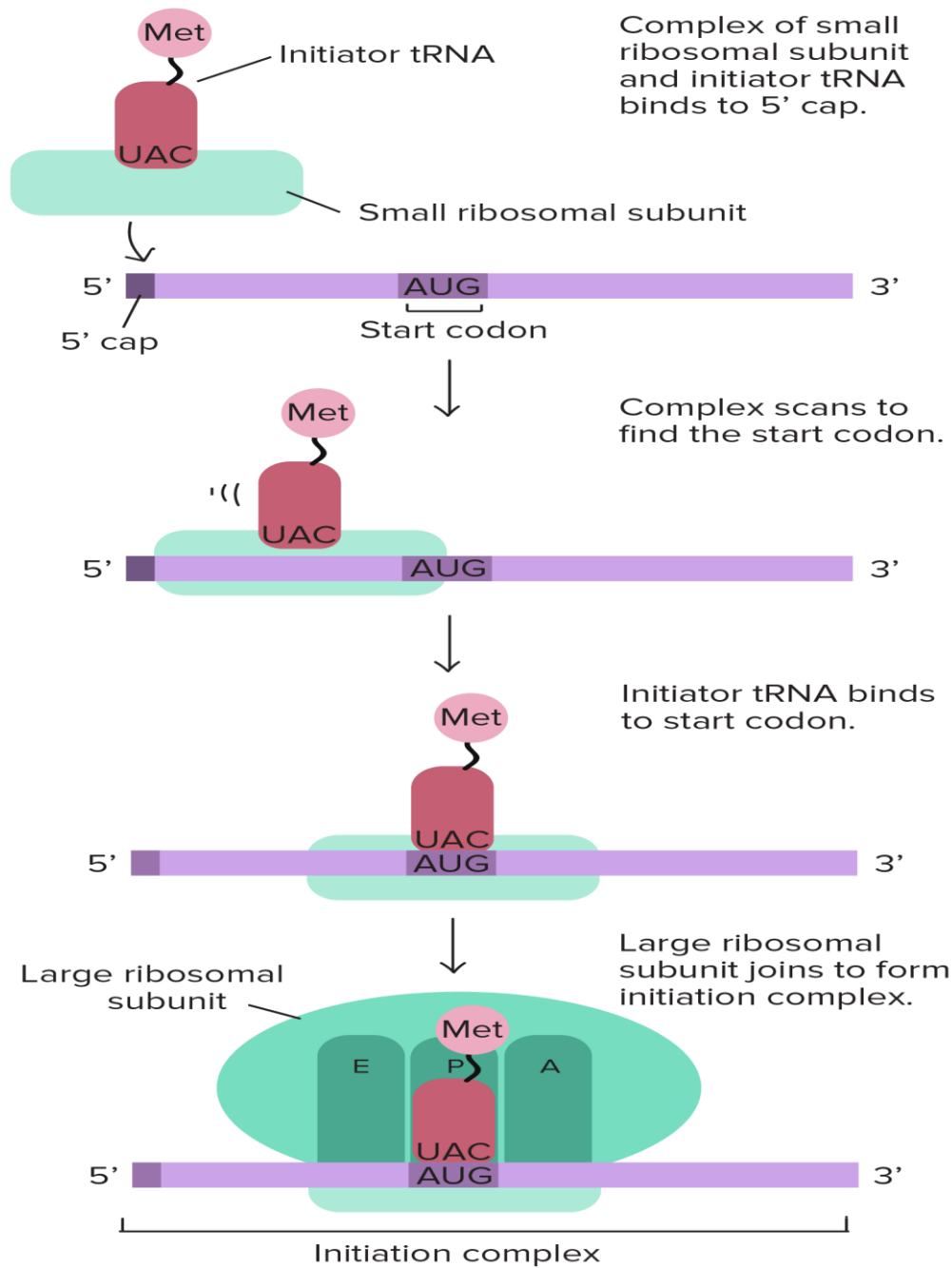
Formation of 80S initiation complex

- With the help of the **eIF-5**, the 60S ribosomal subunit binds to the 48S initiation complex, forming the 80S ribosome.
 - This is associated with the release of all eIFs as well as the hydrolysis of GTP to GDP and Pi.
 - At this point, met-tRNA is on the P-site (peptidyl site) of the ribosome ready for elongation & the A-site is free ready to accept the next aminoacyl-tRNA.
-





Eukaryotic translation initiation





II. Elongation

- The ribosome moves along the mRNA in the 5` to 3` direction, translating the successive codons. It adds amino acids one by one to the **carboxyl end** of the growing peptide chain.
 - **Elongation requires:**
 - The 80S initiation complex
 - Aminoacyl-tRNAs, as sources of amino acids
 - GTP, as source of energy
 - Elongation factors
-

1. Binding of aminoacyl-tRNA to the A site of the ribosome

- Initiation ends in the formation of 80S ribosome with the P site containing the initiation codon and occupied by met-tRNA.
 - The **A site** contains the codon for the second amino acid and is waiting to be occupied by the aminoacyl-tRNA with a complementary anticodon.
 - eEF-1 binds GTP & forms a complex with aminoacyl-tRNA. This complex delivers aminoacyl-tRNA to the A site, with the release of eEF-1 & hydrolysis of GTP to GDP and Pi.
-

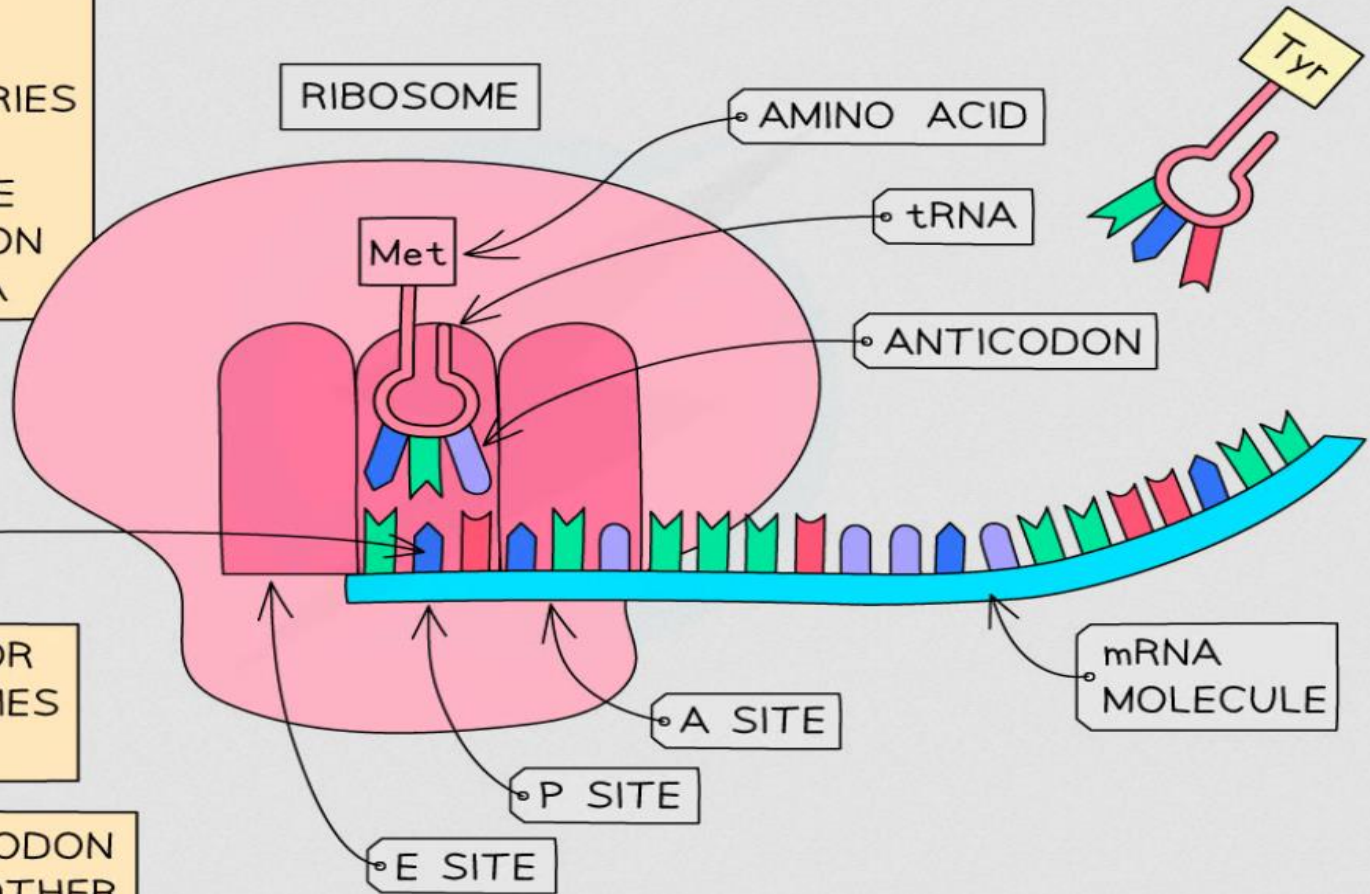
mRNA BINDS TO THE RIBOSOME

AN INITIATOR tRNA (WHICH ALWAYS CARRIES METHIONINE) BINDS AT THE 'START' CODON ON THE mRNA

START CODON

THE INITIATOR tRNA OCCUPIES THE P SITE

THE NEXT CODON SIGNALS ANOTHER tRNA TO BIND

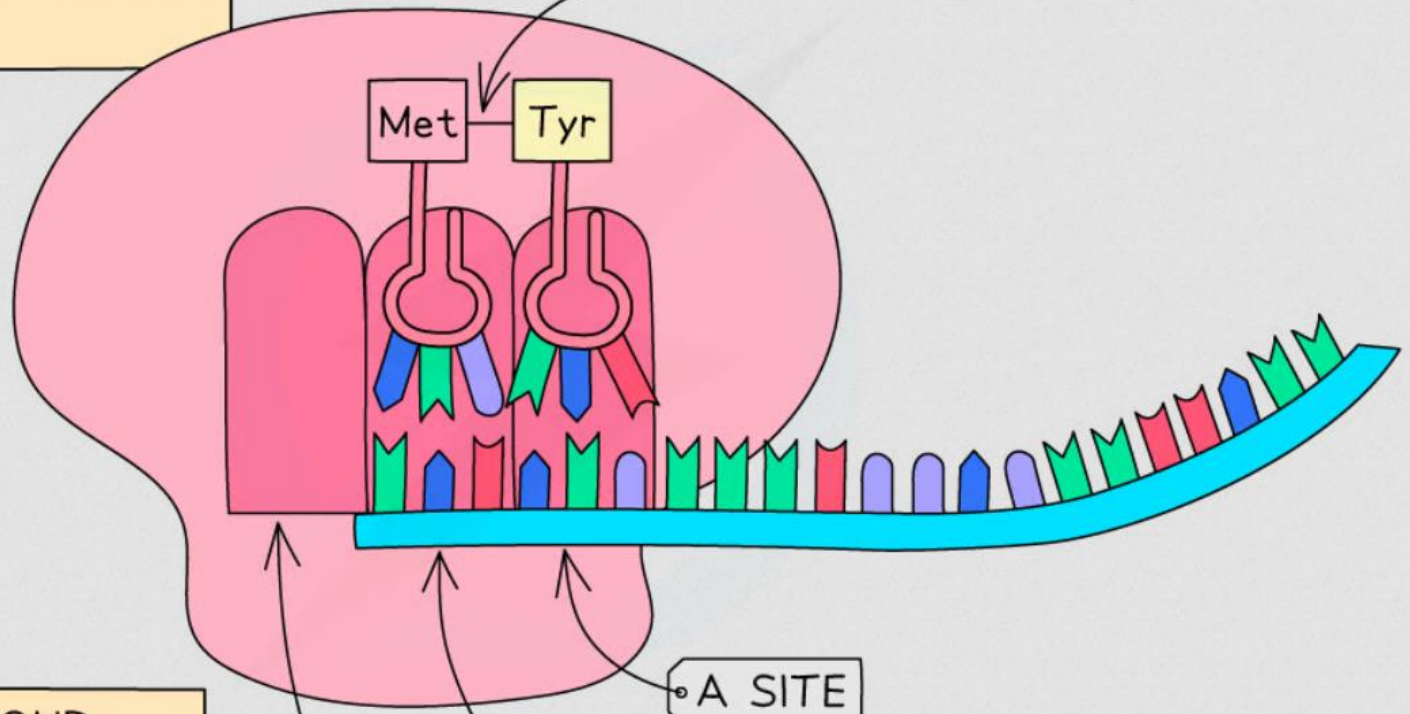


Formation of peptide bond

- The 60S ribosomal subunit contains the enzyme peptidyltransferase (an RNA enzyme or ribozyme).
- It connects the carboxyl group of the 1st amino acid to the amino group of the 2nd amino acid, forming the 1st peptide bond in the peptide chain
- This enzyme transfers the 1st amino acid from its tRNA to the 2nd amino acid.
- Now a dipeptide is connected to the 2nd tRNA in the A site. The 1st tRNA is now free & vacates the P site.

A tRNA (ATTACHED TO AN AMINO ACID) BINDS TO THE NEXT CODON AND OCCUPIES THE A SITE

PEPTIDE BOND



A PEPTIDE BOND IS FORMED BETWEEN THE AMINO ACIDS

A SITE

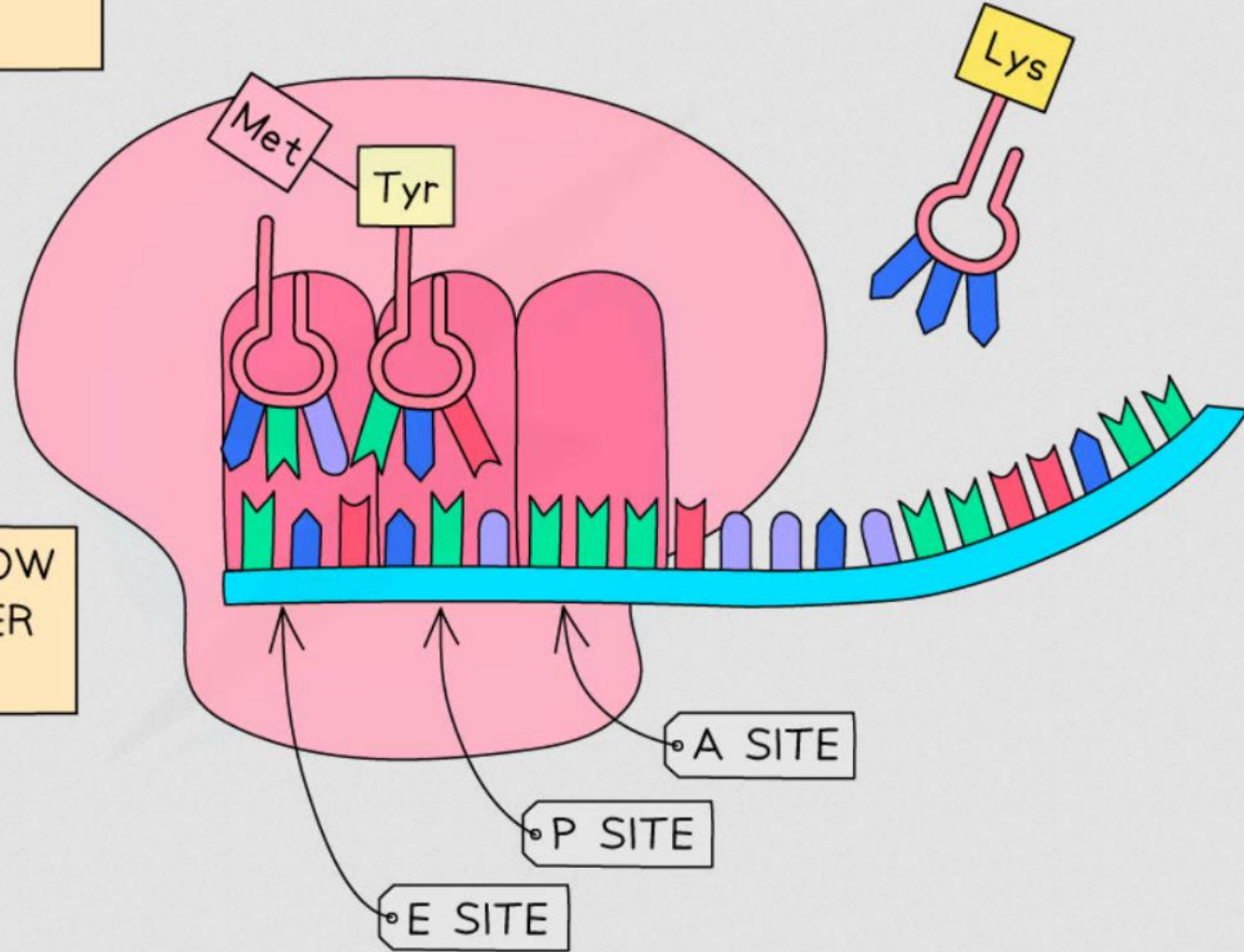
P SITE


E SITE

THE RIBOSOME MOVES
THREE BASES ALONG
THE mRNA

THE INITIATOR
tRNA NOW
OCCUPIES THE
E SITE AND
RELEASED THE
AMINO ACID

THE A SITE IS NOW
FREE FOR ANOTHER
tRNA





Translocation of the peptidyl-tRNA from the A site to the P site

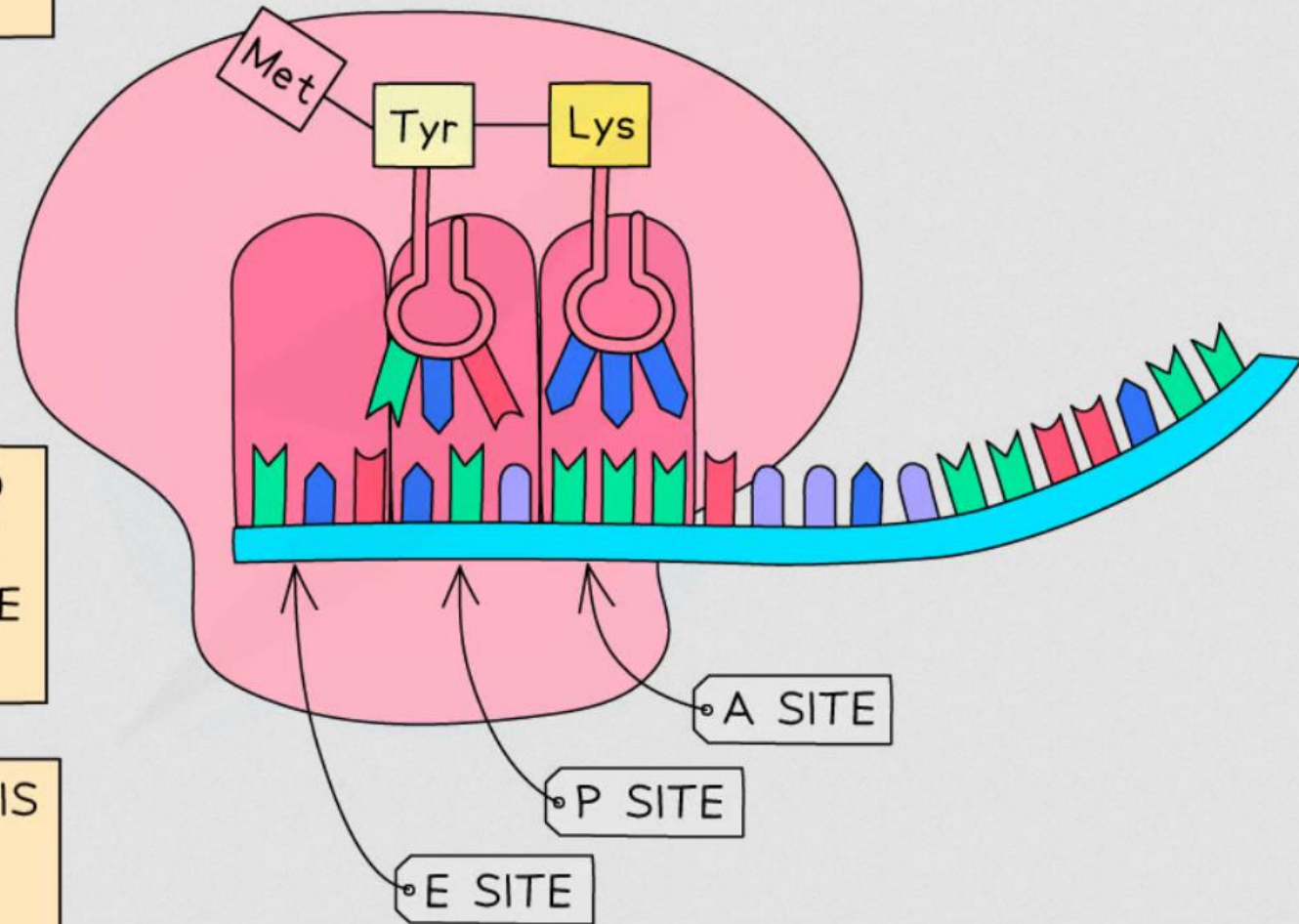
- The newly formed peptidyl-tRNA is translocated, together with its codon, from the A site to the P site.
 - This requires **eEF-2** & **GTP**, which is hydrolyzed to GDP and Pi.
 - Now the A site contains the codon for the 3rd amino acid & can accept the aminoacyl-tRNA with the proper anticodon & a new cycle of elongation occurs.
-

THE INITIATOR tRNA IS RELEASED FROM THE E SITE

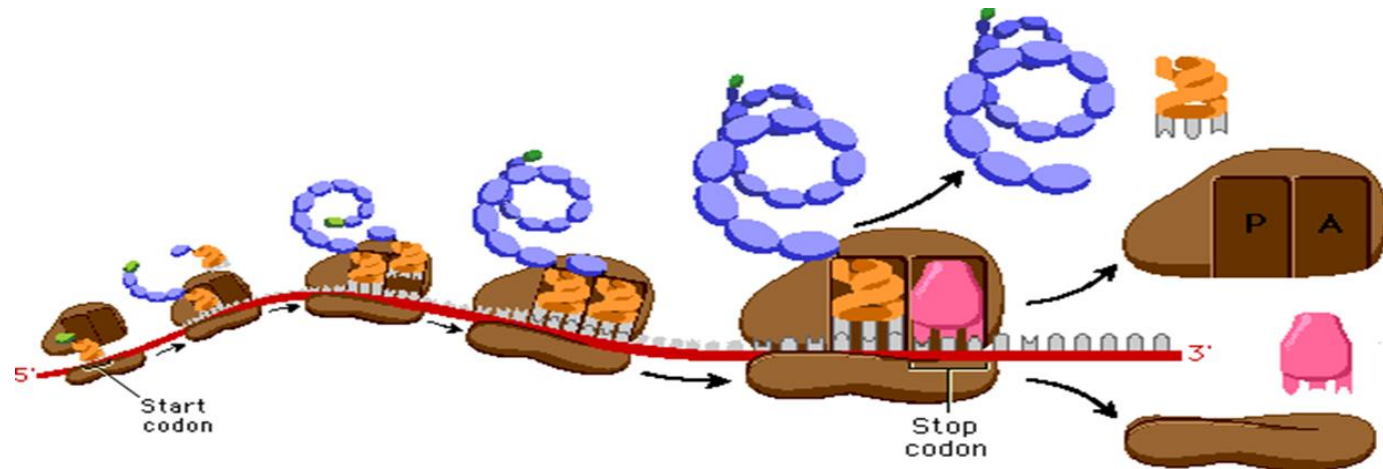


A tRNA BINDS TO THE NEXT CODON AND OCCUPIES THE A SITE

A PEPTIDE BOND IS FORMED BETWEEN THE AMINO ACIDS

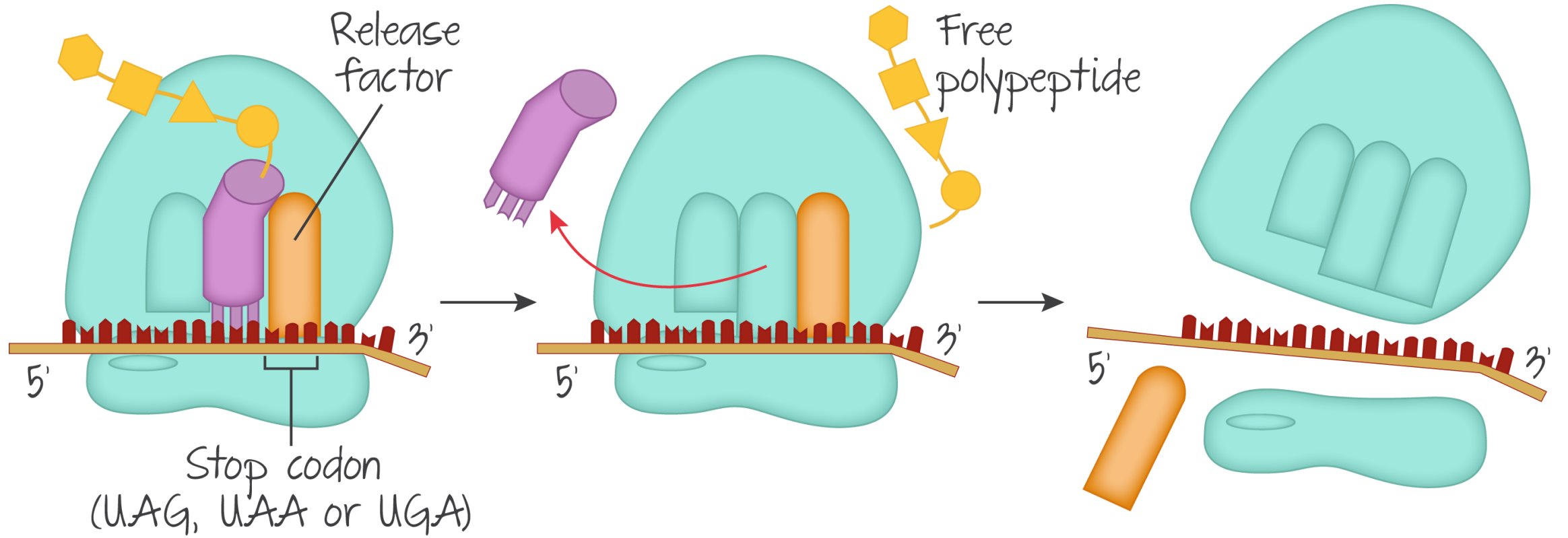


- As the ribosome moves along the mRNA, a new ribosome can attach to the initiating codon (with a distance of 80 nucleotides in between), starting the synthesis of a new peptide chain .
- Thus, several ribosomes may translate the same mRNA simultaneously. They form together a polyribosome or polysome.



III. Termination

- Termination occurs when one of the three termination stop codons appears into the **A site**.
 - These codons are recognized by releasing factors (**eRF**). It also requires **GTP**.
 - The binding of these release factors induces peptidyl transferase to hydrolyze the bond linking the peptide to the tRNA at the P site, causing the nascent protein to be released from the ribosome.
 - The ribosomal subunits, mRNA, tRNA and protein factors are dissociated and can be recycled and used to synthesize another polypeptide.
-



Inhibitors of translation

- **Tetracycline:** an antibiotic that combines with the **30S ribosomal subunit** of bacteria, preventing access of aminoacyl-tRNA (block A site).
- **Chloramphenicol:** an antibiotic that inhibits peptidyltransferase in bacterial **50S ribosomal subunit**.
- **Clindamycin and erythromycin:** two antibiotics bind to the 50S ribosomal subunit of bacteria, inhibiting translocation of peptidyl-tRNA (inhibits the movement of ribosome along the mRNA).

Post-translational processing of polypeptide chains

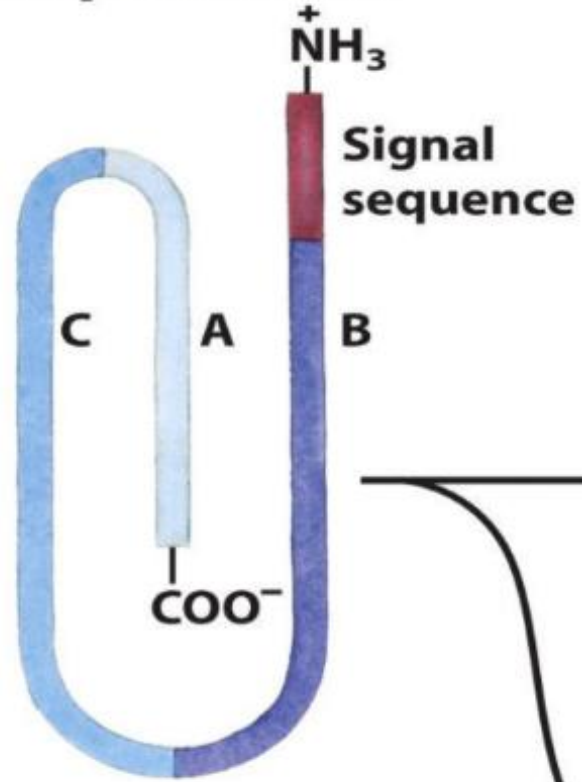
Trimming: many proteins are formed as large **precursor molecules** that are functionally inactive, and part of their chains must be removed to release the active molecules.

- Trimming means removal of part of the peptide chain.
- **Insulin** is translated as a protein containing 109 amino acids known as **preproinsulin**.

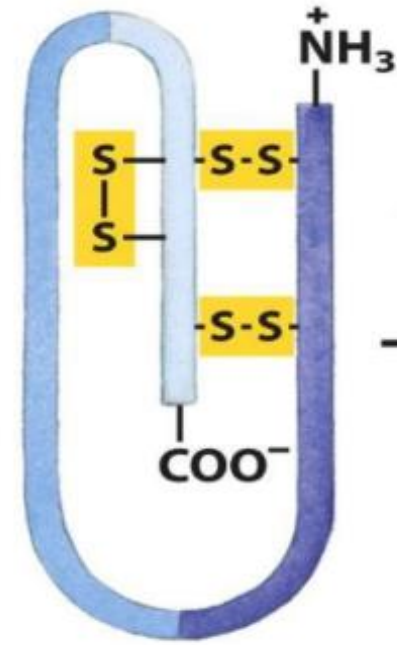
A signal peptide of 23 aa is removed, forming **proinsulin**.

A further 35 aa are removed, forming insulin that contains only 51 aa

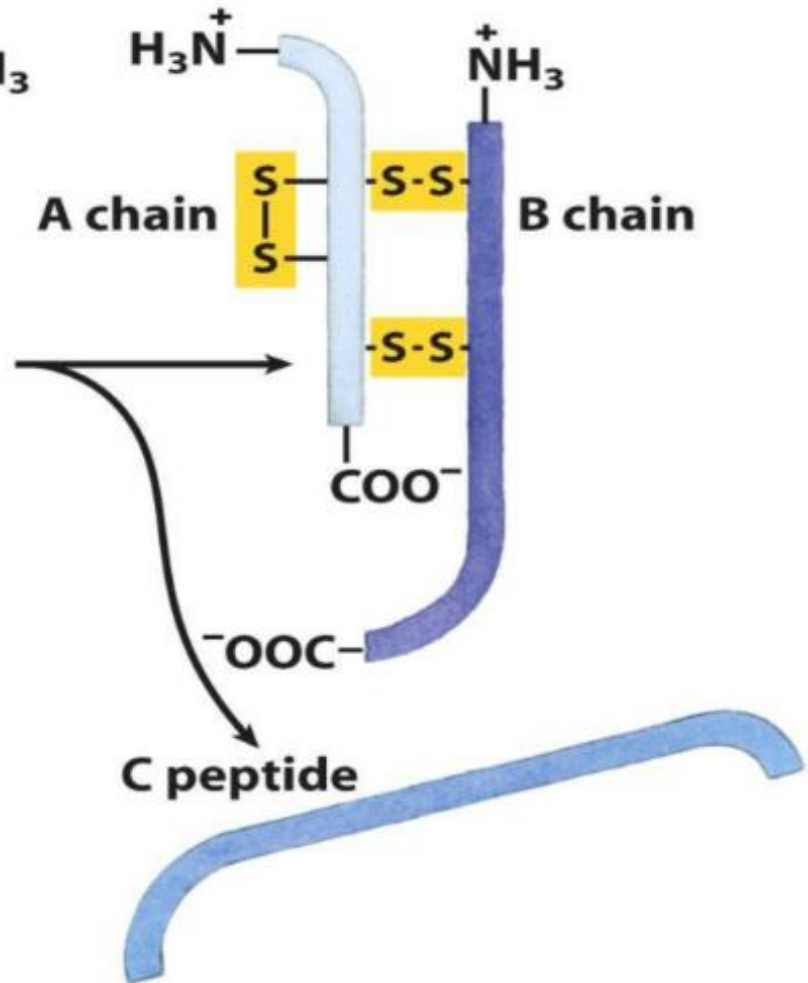
Preproinsulin



Proinsulin



Mature insulin



Signal sequence

C peptide

Covalent modification of the polypeptide chains

- It means addition of chemical groups which may activate or inactivate the proteins. These chemical groups are:

1. **Phosphorylation**: It means the addition of phosphate group to the enzyme which may activate or inactivate this enzyme.

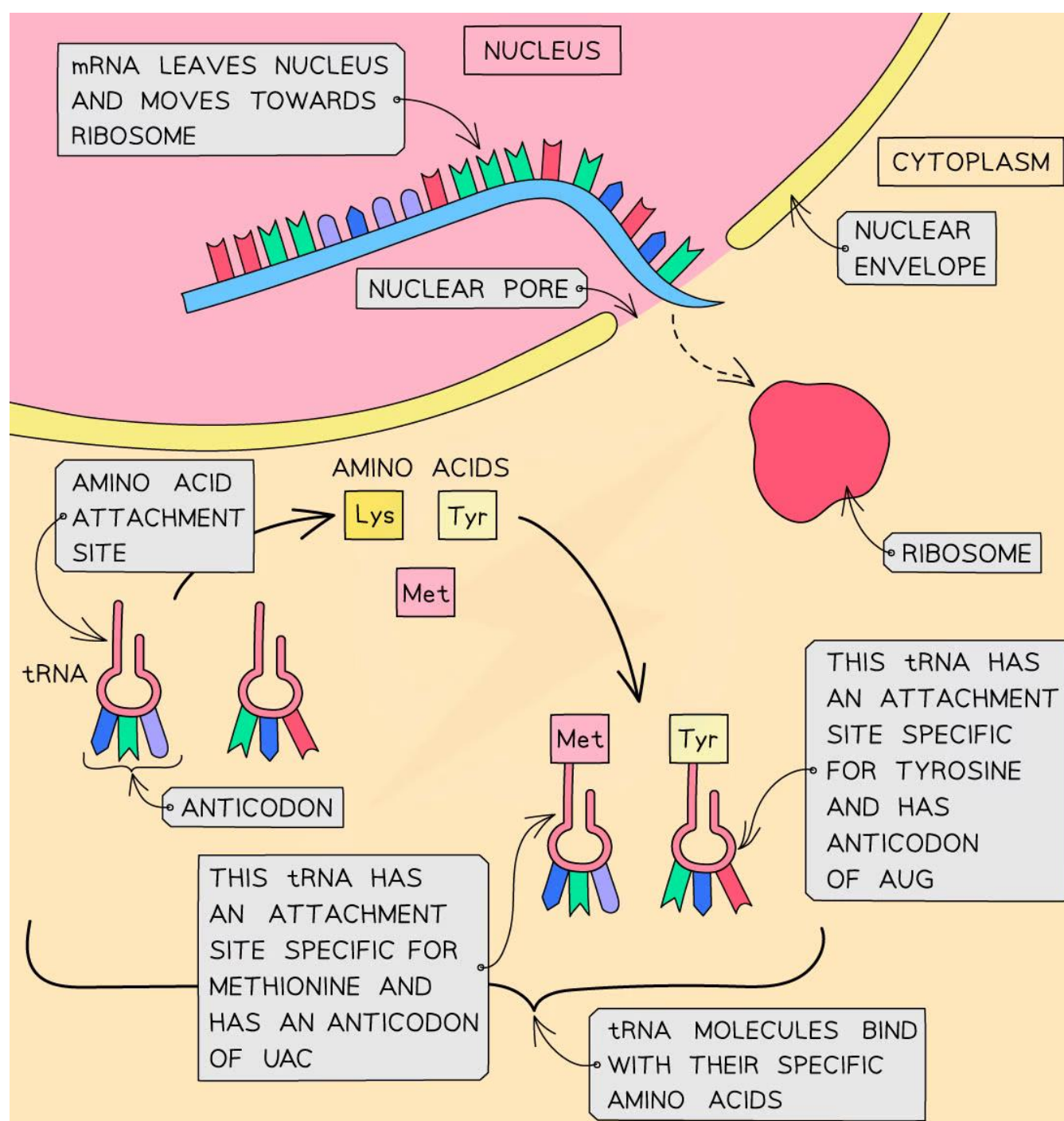
occurs on hydroxyl groups of **serine, threonine** or **tyrosine** residues of proteins. This Phosphorylation is catalyzed by **protein kinases** & reversed by **protein phosphatases** e.g. phosphorylation of enzymes & receptors.

Covalent modification of the polypeptide chains

- 2. Glycosylation:** It means **addition** of carbohydrates chain to the protein to form glycoproteins. The carbohydrates chains may be attached to the hydroxyl group of serine or threonine (O-linked) or the amide group of asparagine (N-linked).
- 3. Hydroxylation:** It means **addition** of hydroxyl group to the protein. e.g. in collagen fibers
Proline and lysine amino acids are hydroxylated to form hydroxyproline and hydroxylysine. Important for formation of strong collagen.

Covalent modification of the polypeptide chains

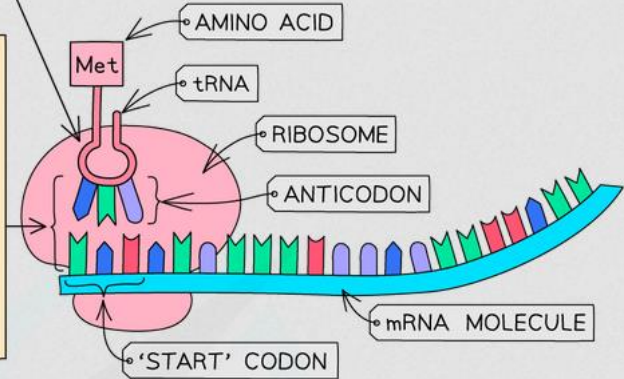
- 4. Carboxylation:** It means **addition** of carboxylic group (COO^-) to the protein e.g. carboxylation of glutamic acid residues in some clotting factors to help them to bind calcium and formation of blood clot.
- 5. Acetylation:** It means **addition** of acetyl group to the proteins. Acetyl radicals may be connected to the ϵ amino group of lysine. This is very important in **histones** as it leads to separation from DNA, which becomes transcriptionally active.



1 IN THE CYTOPLASM THE mRNA ATTACHES TO A RIBOSOME

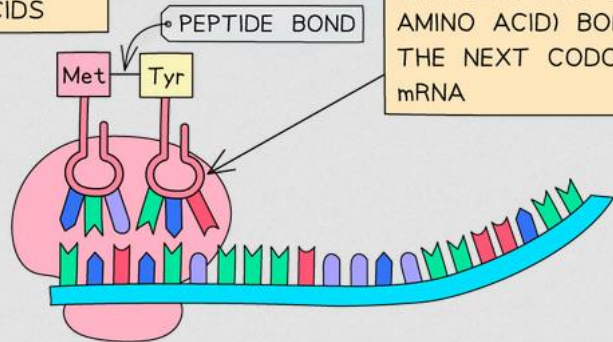
2 EACH tRNA HAS THE COMPLEMENTARY ANTICODON TO THE CODON ON THE mRNA

3 THE FIRST tRNA (WHICH ALWAYS CARRIES THE METHIONINE AMINO ACID) FORMS HYDROGEN BONDS WITH THE FIRST OR 'START' CODON (AUG) ON THE mRNA.

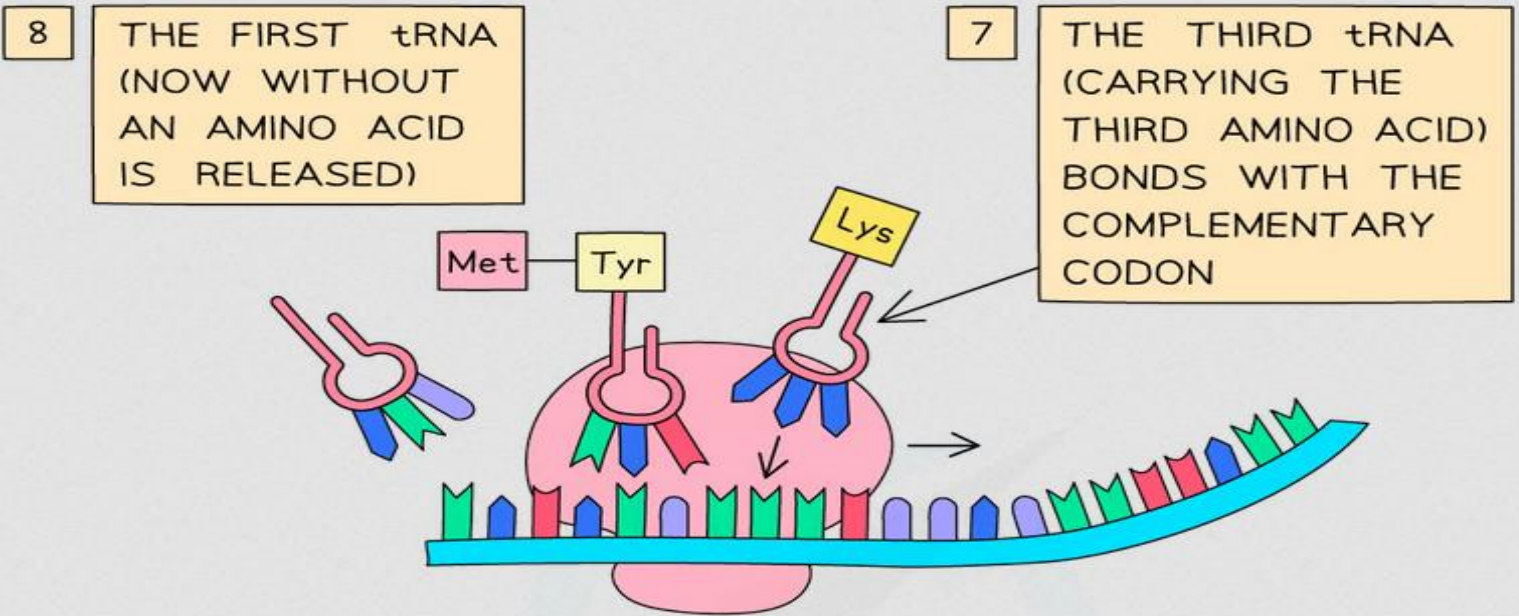


5 A PEPTIDE BOND FORMS BETWEEN THE AMINO ACIDS

4 THE SECOND tRNA (BRINGING THE SECOND AMINO ACID) BOND WITH THE NEXT CODON ON THE mRNA



6 THE RIBOSOME MOVES ALONG THE mRNA (IN A 5' TO 3' DIRECTION) 'READING' THE NEXT CODON



9 THE RIBOSOME CONTINUES TO 'READ' THE mRNA MOLECULE BUILDING A POLYPEPTIDE CHAIN UNTIL IT REACHES A 'STOP' CODON

