TRANSLATION IN EUKARYOTES

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TRANSLATION FACTORS IN EUKARYOTES

Eukaryotic Initiation Factors:

1. eIF1
2. eIF1A
3. eIF2
4. eIF3
5. eIF4A
6. eIF5
7. IF5B
8. eIF4E
9. eIF4F
10. eIF4G

Elongation Factor:

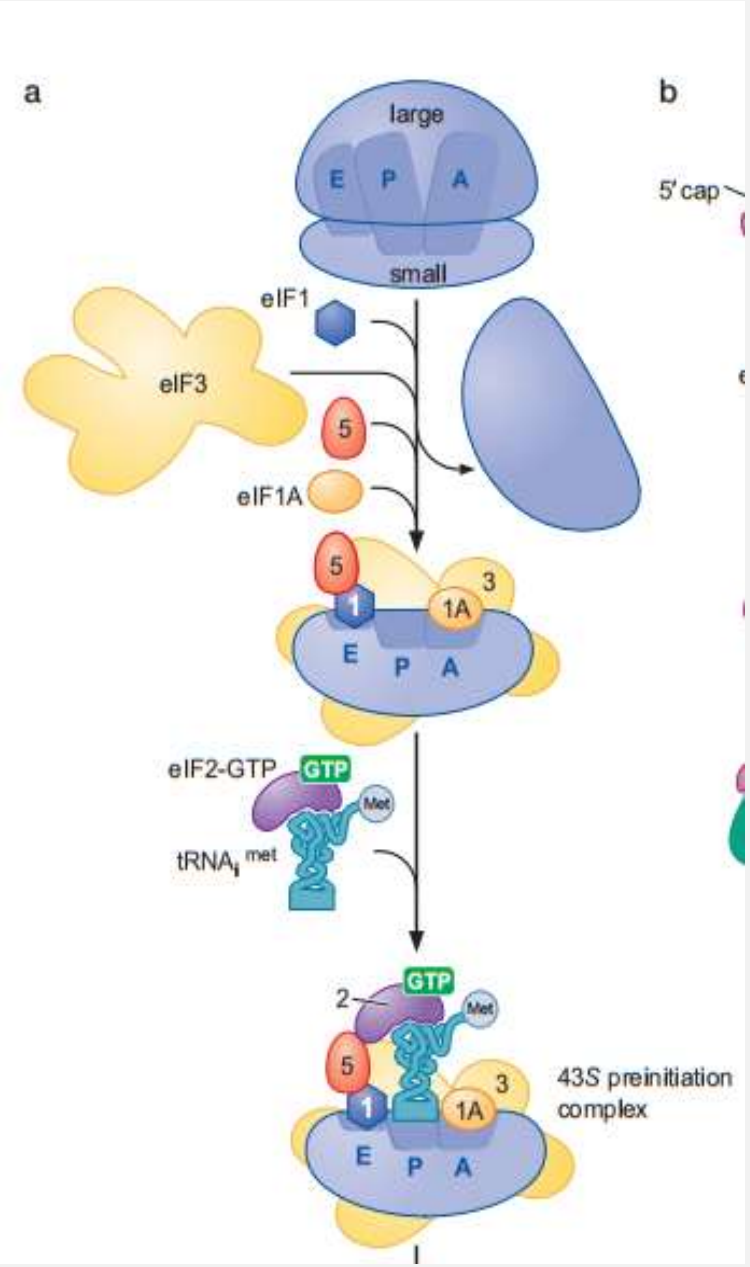
eEF

1. EF-Tu
2. EF-G

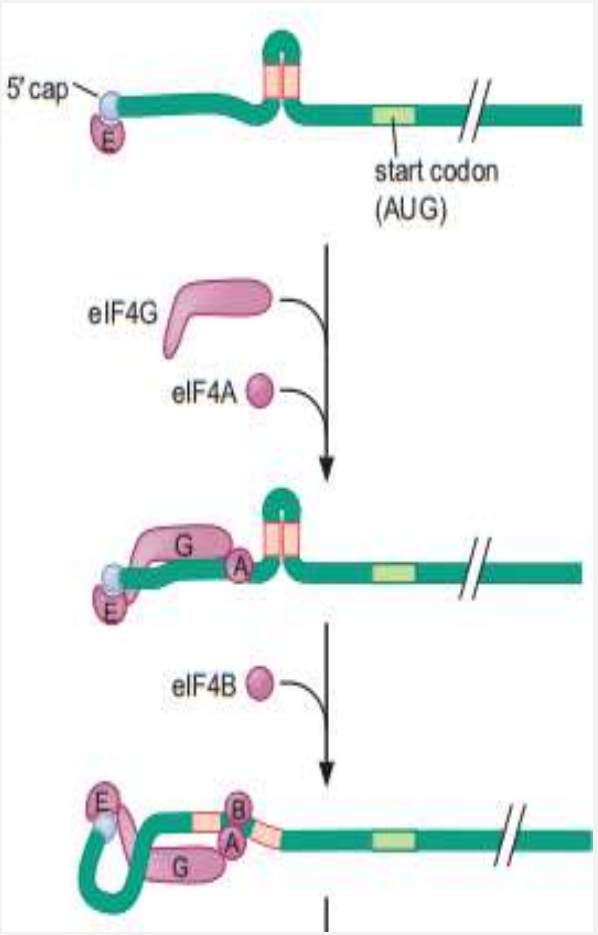
Termination Factors:

1. eRF1
2. eRF2
3. eRF3

- In eukaryotes, the small subunit is already associated with an initiator tRNA when it is recruited to the capped 5’ end of the mRNA.
- It then “scans” along the mRNA in a 5’→3’ direction until it reaches the first **5’-AUG-3’**, which it recognizes as the start codon.
- Eukaryotic cells require more auxiliary proteins to drive the initiation process as compared to prokaryotes.
- The events of initiation can be broken down into four steps.
    1) In contrast to the situation in prokaryotes, in eukaryotic cells, binding of the initiator tRNA to the small subunit always precedes association with the mRNA.
    2) A separate set of auxiliary factors mediates the recognition of the mRNA.
    3) The small ribosomal subunit bound to the initiator tRNA scans the mRNA for the first AUG sequence.
    4) The large subunit of the ribosome is recruited after the initiator tRNA base-pairs with the start codon.
- As the eukaryotic ribosome completes a cycle of translation, it dissociates into free large and small subunits, and four initiation factors—**eIF1, eIF1A, eIF3, and eIF5**—bind to the small subunit.



- Initiation factors—eIF1, eIF1A, eIF3, and eIF5—bind to the small subunit.
- eIF1, eIF1A and eIF5 act together to prevent both large subunit binding and tRNA binding to the A-site.
- The initiator tRNA is escorted to the small subunit by the three subunit GTP-binding protein eIF2.
- eIF2 binds the initiator tRNA only in the GTP-bound state. The complex between the initiator tRNA and EIF2 is called the **ternary complex (TC)**.
- the initiator tRNA is charged with methionine, not N-formyl methionine, and is referred to as Met-tRNAi Met.
- eIF2 positions the Met-tRNAi Met in the P-site of the initiation factor–bound small subunit, resulting in the formation of the **43S preinitiation complex (43S PIC)**.



- In a separate series of reactions, the mRNA is prepared for recognition by the small subunit. This process begins with recognition of the 5’ cap by the cap-binding protein **eIF4E**. A series of additional initiation factors is then recruited.
- **eIF4G** binds to both **eIF4E and the mRNA**, whereas **eIF4A binds eIF4G and the mRNA** .
- The association of IF4G with eIF4E is particularly important—the overall level of translation in the cell is controlled at this step by a family of proteins that compete with eIF4G binding called eIF4E-binding proteins.
- This complex is joined by **eIF4B**, which activates the **RNA helicase activity of eIF4A**.
- The helicase unwinds any secondary structures (such as hairpins) that may have formed at the end of the mRNA.
- Removal of secondary structures is critical because the 5’ end of the mRNA must be unstructured to bind to the small subunit.
- Finally, interactions between the **eIF4G bound to the unstructured mRNA** and the initiation factors (particularly **eIF3**) bound to the small sub-unit recruit the **43S preinitiation complex to the mRNA** to form the **48S preinitiation complex**.

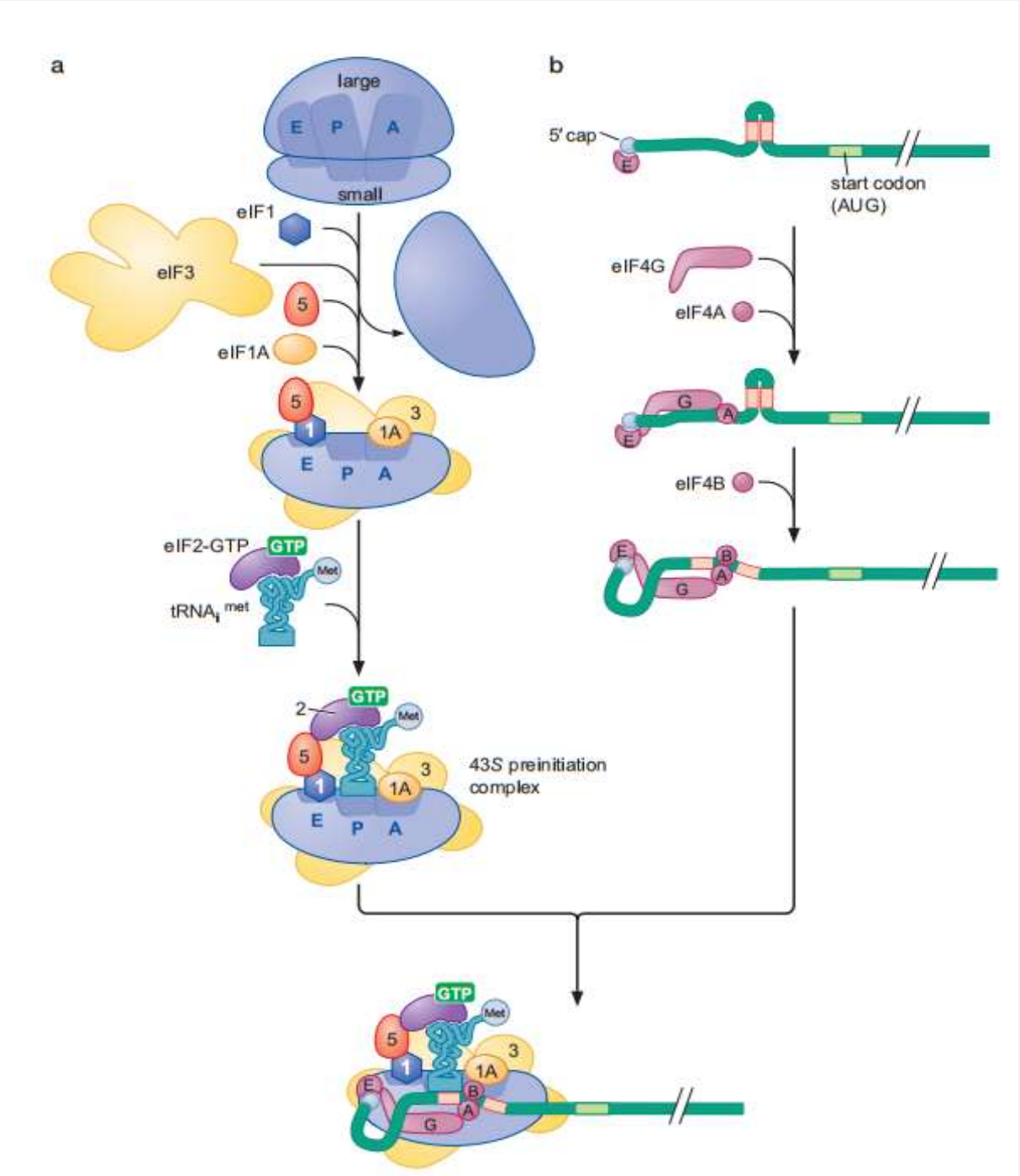
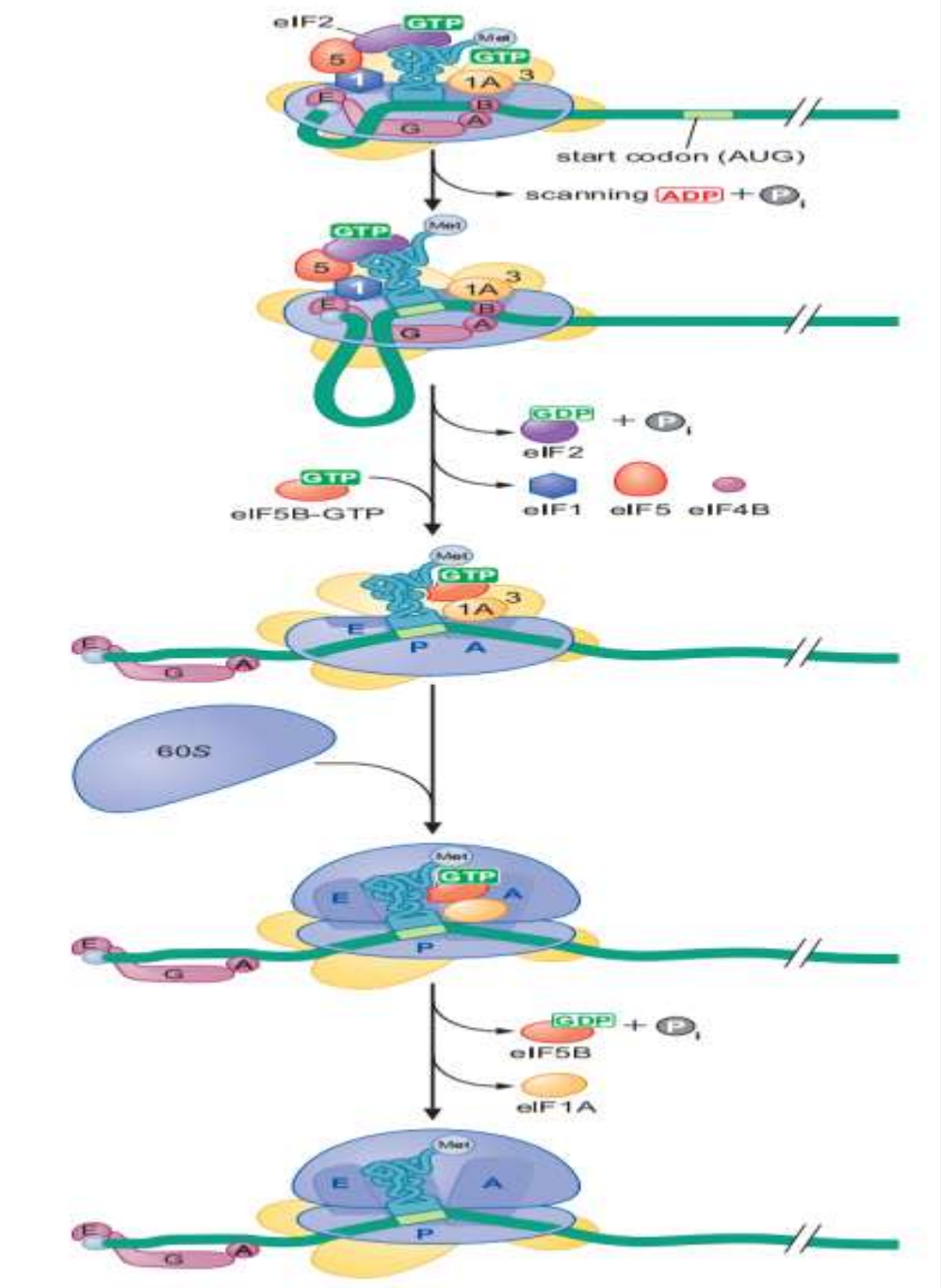


Fig: Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA



- Once assembled at the 5’end of the mRNA, the small subunit and its associated factors move along the mRNA in a 5’→3’ direction in an ATP-dependent process that is stimulated by the eIF4A/B-associated RNA helicase . During this movement, the small subunit “scans” the mRNA for the first start codon.
- The start codon is recognized through base pairing between the anticodon of the initiator tRNA and the start codon.
- The importance of this interaction in identifying the start codon shows why it is critical that the initiator tRNA bind to the small subunit before it binds to the mRNA.
- Correct base pairing changes the conformation of the 48S complex, leading to release of eIF1 and a change in conformation of eIF5. Both of these events stimulate eIF2 to hydrolyze its associated GTP.

Identification of the initiating AUG by the 48S PIC and large subunit joining during eukaryotic translation initiation.

- In its GDP-bound state, eIF2 no longer binds the initiator tRNA and is released from the small subunit along with eIF5.
- Loss of eIF2 allows the binding of a second GTP-regulated, initiator tRNA–binding protein called eIF5B.
- Upon binding the initiator tRNA, eIF5B, GTP stimulates the association of the 60S subunit with the correctly positioned 40S subunit.
- This association is possible because the factors that previously prevented this association (eIF1 and eIF5) have been released.
- As in the prokaryotic situation, binding of the large subunit leads to the release of the remaining initiation factors by stimulating GTP hydrolysis by eIF5B.
- As a result of these events, the Met-tRNAi Met is placed in the P-site of the resulting 80S initiation complex and the ribosome is now ready to accept a charged tRNA into its A-site and form the first peptide bond.

ELONGATION

- Once the peptidyl transferase reaction has occurred, the tRNA in the P-site is deacetylated (no longer attached to an amino acid), and the growing polypeptide chain is linked to the tRNA in the A-site.

- For a new round of peptide chain elongation to occur, the P-site tRNA must move to the E-site and the A-site tRNA must move to the P-site. At the same time, the mRNA must move by three nucleotides to expose the next codon. These movements are coordinated within the ribosome and are collectively referred to as **translocation.**

- The initial steps of translocation are coupled to the peptidyl transferase reaction.

- Once the growing peptide chain has been transferred to the A-site tRNA, the A- and P-site tRNAs have a preference to occupy new positions in the large subunit.

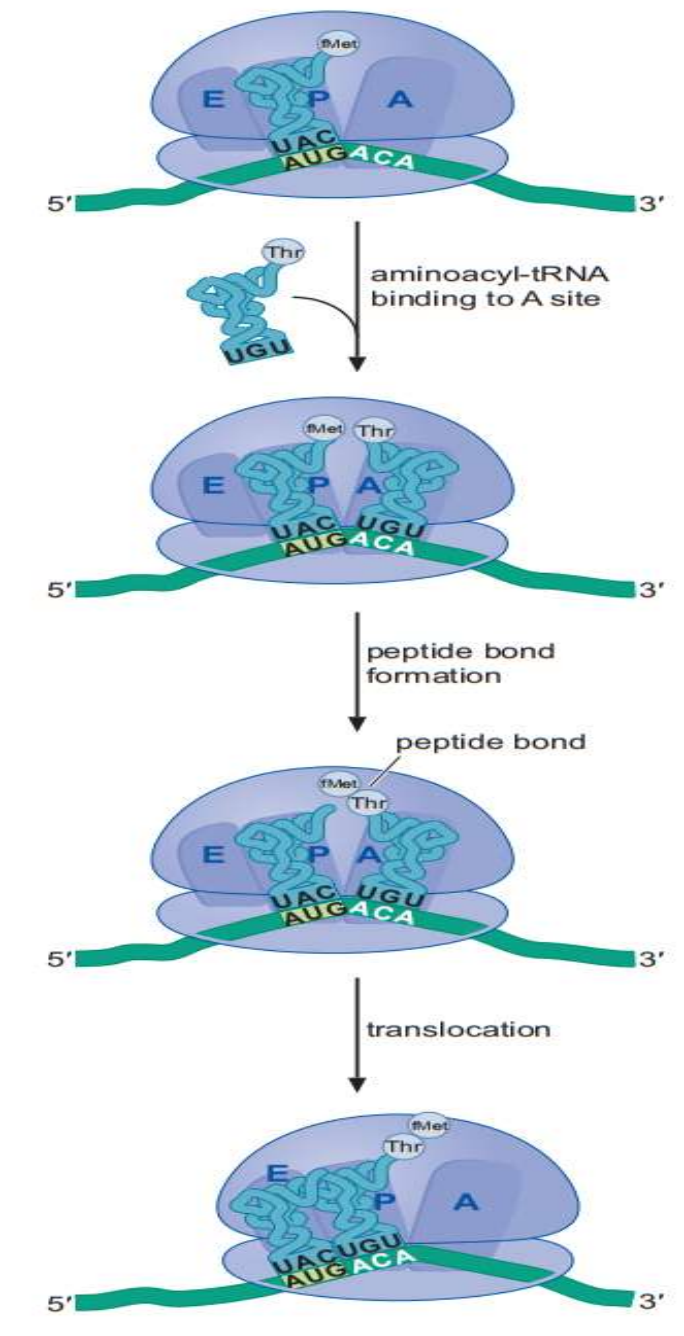
- The 3’end of the A-site tRNA is bound to the growing polypeptide chain and prefers to bind in the P-site of the large subunit.

- The now deacetylated P-site tRNA is no longer attached to the growing polypeptide chain and prefers to bind in the E-site of the large subunit.

- In contrast, at this time, the anticodons of these tRNAs remain in their initial location in the small subunit bound to the mRNA.

- Thus, translocation is initiated in the large subunit before the small subunit, and the tRNAs are said to be in “hybrid states.” Their 3’ ends have shifted into a new location, but their anticodon ends are still in their pre–peptidyl transfer position .

- As with the original positioning of the mRNA, this shift must occur precisely to maintain the correct reading frame of the message.
- Two auxiliary proteins known as elongation factors control these events.
- Both of these factors use the energy of GTP binding and hydrolysis to enhance the rate and accuracy of ribosome function.
- The mechanism of elongation is highly conserved between prokaryotic and eukaryotic cells.
- The events that occur in eukaryotic cells are similar to those in prokaryotes, both in the factors involved and in their mechanism of action.



Once the ribosome is assembled with the charged initiator tRNA in the P-site, polypeptide synthesis can begin. There are three key events that must occur for the correct addition of each amino acid.

1. The correct aminoacyl-tRNA is loaded into the A-site of the ribosome as dictated by the A-site codon.
2. Apeptide bond is formed between the aminoacyl-tRNA in the A-site and the peptide chain that is attached to the peptidyl-tRNA in theP-site. This peptidyl transferase reaction, aswe have seen, results in the transfer of the growing polypeptide from the tRNA in the P-site to the amino acid moiety of the charged tRNA in the A-site.
3. The resulting peptidyl-tRNA in theA-site and its associated codonmust be translocatedto the P-site so that the ribosome is poised for another cycle of codon recognition and peptide bond formation.

Summary of steps of translation elongation

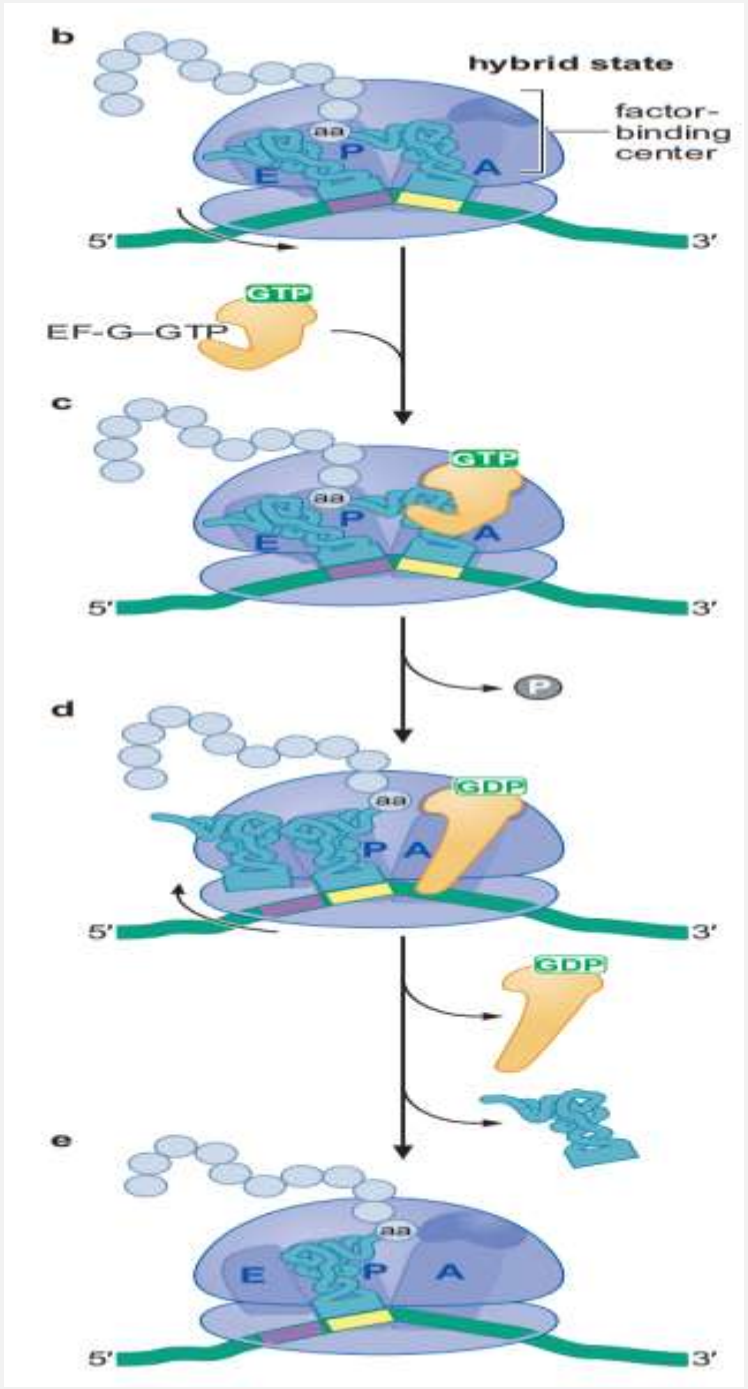


Fig: EF-G stimulation of translocation requires GTP hydrolysis.

TERMINATION

- There are class I and II release factors in eukaryotic cells.
- Like RF1 and RF2, eRF1 recognizes all three stop codons and brings a GGQ motif into the peptidyl transferase center leading to polypeptide release.
- Unlike the prokaryotic RF3 that catalyzes RF1/RF2 release, eRF3 delivers eRF1 to the ribosome.
- eRF3.GTP binds to eRF1 away from the ribosome and, like EF-Tu and a charged tRNA, escorts eRF1 to the ribosome .
- Also like EF-Tu, if eRF1 recognizes a stop codon, eRF3 . GTP binds the factor-binding center, stimulating GTP hydrolysis. eRF3.GDP is rapidly released from the ribosome, and eRF1moves into the peptidyl-transferase center in a manner thought to be analogous to tRNA accommodation.
- Current models suggest that after stimulating peptide hydrolysis from the P-site tRNA, eRF1 (in conjunction with an ATPase called Rli1) also participates in ribosome recycling based on the similarity of eRF1 and eRF3 to two proteins shown to stimulate ribosome disassembly at stalled ribosomes called Dom34 and Hbs1.

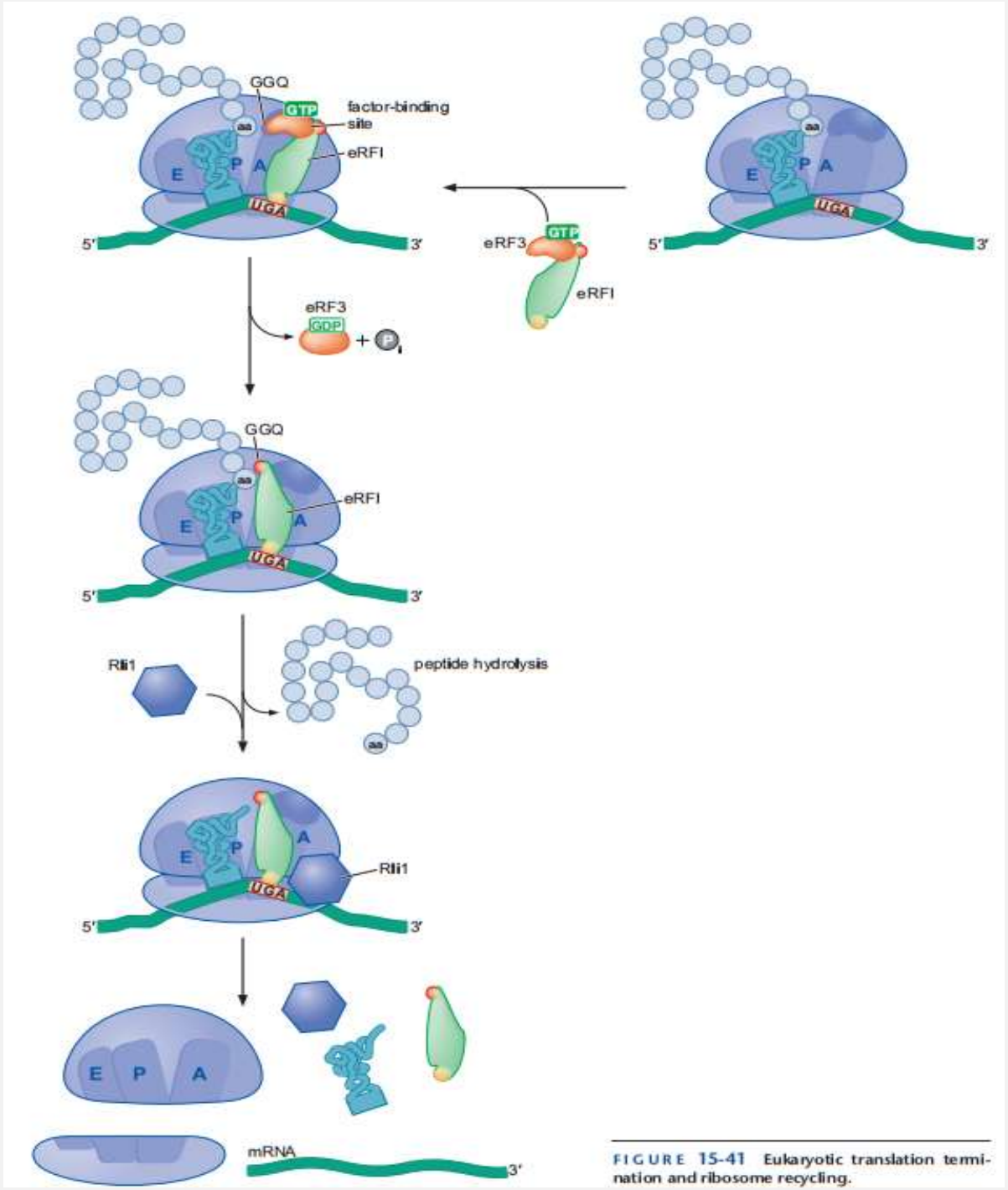


FIGURE 15-41 Eukaryotic translation termination and ribosome recycling.

Eukaryotic translation termination and ribosome recycling

THANK YOU