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SEMESTER: IV

TOPIC: TRANSLATION

FACULTY: MS. HENA NAZ

EMAIL ID: Henanaz64@gmail.com

TRANSLATION: SYNTHESIS OF PROTEIN

- The conversion of language of nucleotide in mRNA into the language of amino acid to form polypeptide is called as **translation.**
- The process during which genetic information which is stored in the sequence of nucleotide in mRNA molecule is translated into the sequence of amino acid in polypeptide gene product. This process is called **protein synthesis**.

MACHINERY FOR PROTEIN SYNTHESIS

1. **mRNA**:
   - It carries all the codons which needs to be converted into language of protein.
   - The mRNA carries the coded information of one or more cistron for transalation over
   - ribosomes.
   - It also carries nucleotide sequences which are not translated and are called untranslated
   - or non coding regions.

2. **tRNA:**
   - These are the smallest units of RNA which posses a loop for attachment to ribosomes.
   - It's the site of binding of amino acid to tRNA.

3. **RIBOSOMES:**
   - They are ribonuleoprotein particles which function as the site for polypeptide synthesis.
   - They are called as protien factories.
   - They occur in cytoplasm.
   - Cytoplasmic ribosomes are 70s (30s+50s) in prokaryotes and 80s (40s+60s) in eukaryotes.
   - Each ribosome has 2 parts, a smaller subunit and a larger subunit.

4. **AMINO ACIDS:**
   - Proteins are the polymers of amino acids. Therefore, amino acids form the raw material for
   - protein synthesis
   - The proteins of living organisms need about 20 amino acids as building blocks or monomers.
   - These are available in the cytoplasmic matrix as an amino acid pool.

5. **ENERGY SOURCES:**
   - ATP as an energy source.
   - GTP for synthesis of peptide bonds.

6. **ENZYMES:**
   - Amino acid activating system (eg. Aminoacyl-tRNA- synthetase)
   - Peptide polymerase system

7. **TRANSLATION FACTORS:**
   a) **Initiation factors**: IF1, IF2, IF3.
   b) **Elongation factors**: EF-Tu, EF-G, EF-Ts.
   c) **Termination factors (TF).**
   d) **Release factors**: RF1, RF2, RF3.

MECHANISM OF PROTEIN SYNTHESIS

The mechanism of protein synthesis involves following 4 stages:

1. **Formation of Aminoacyl-tRNA**
2. **Initiation**
3. **Elongation**
4. **Termination**

(A). FORMATION OF AMINOACYL-tRNA:

Involves following two steps:

a). Activation of amino acids:

- Each of 20 amino acids occur in inactive state.
- Each of them before binding to specifc tRNA needs to be activated by specific enzyme known as aminoacyl-synthetase and ATP in the presence of Mg^2+.
- As a result of the reaction, amino acid-AMP-enzyme complex and pyrophosphate are formed.

AA + ATP —(aminoacyl synthetase)→ AA ~ AMP-ENZYME COMPLEX + PP
aminoacyl adenylate enzyme complex

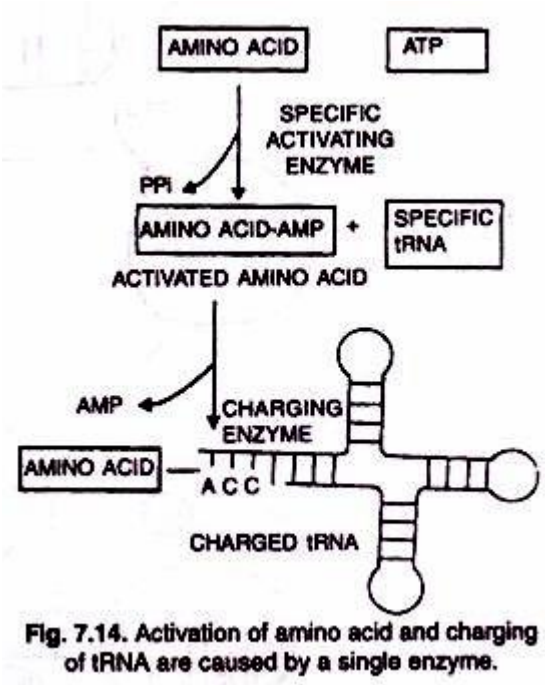


Fig. 7.14. Activation of amino acid and charging of tRNA are caused by a single enzyme.

b). Aminoacylation of tRNA:

- AA ~ AMP-Enzyme Complex reacts with tRNA and transfers its amino acid to tRNA at the
- acceptor site of tRNA.
- The resulting tRNA-amino acid complex is called a charged tRNA
- AMP and enzyme are freed
- The tRNA-amino acid complex moves to the site of protein synthesis, the ribosome.

AA ~ AMP-ENZYME COMPLEX + tRNA ⟶ AA-tRNA + AMP + Enzyme

(Aminoacyl tRNA)

(B). INITIATION:

- Specific initiating tRNA molecule is employed.
- In prokaryotes this tRNA molecule is acylated with modified amino acid N-formyl methione
- (f MET) and is designated as tRNA^f Met which recognises the codon AUG.
- In eukaryotes formylation does not occur due to the absence of formylase enzyme.

Formylation reaction: Formylation of Met tRNA^f Met takes place with the help of transformylase enzyme in the presence of source formyltetrahydrofolic acid.

Met tRNA^fmet + formate ⟶ (transformylase) f Met tRNA^f Met

formyltetrahydrofolic acid — (Formylmethionine tRNA^f Met)

- Steps involved in initiation of polypeptide:

i). <u>Binding of 30s subunit of ribosome with 5'end of mRNA in the presence of initiation factor IF3.</u>

70s ⟶ 50s + 30s

↓

30s + IF3 + mRNA ⟶ 30s-IF3-mRNA complex

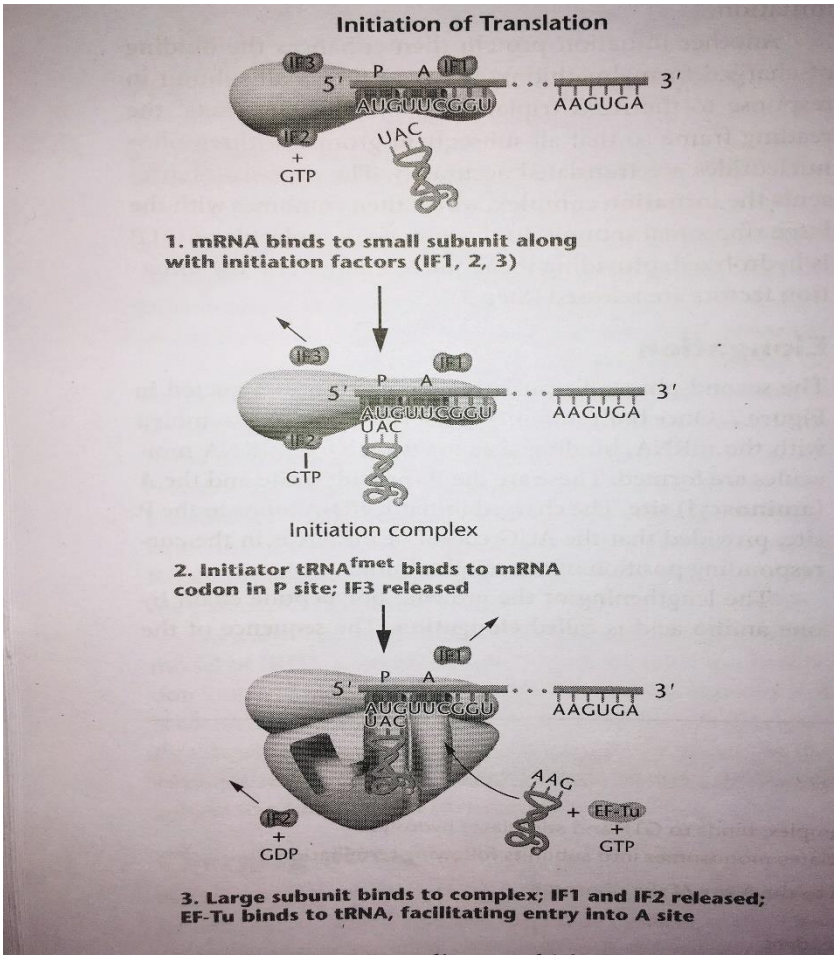
ii). <u>Binding of f Met tRNA^f Met with above complex in the presence IF2.</u>

30s-IF3-mRNA complex + f Met tRNA^f Met + IF2 ⟶ 30s- mRNA- f Met tRNA^f Met-IF3-IF2 complex

iii). <u>Binding of 50s with above complex in the presence of GTP and Mg^2+.</u>

30s- mRNA- f Met tRNA^f Met- IF3-IF2 complex + 50s +GTP + Mg^2+

⟶ 70s-mRNA- f Met tRNA^f Met-IF3-IF2-GTP-Mg^2+ complex



Main properties of initiation factors:

Sl. No.	INITIATION FACTORS	MASS	FUNCTION
1).	IF1	8100	Stimulates activities of IF2 and IF3. Increase the affinity of 30s subunit of ribosome with other factors.
2).	IF2	97300	Favors binding of + f Met tRNA^f Met with 30s-mRNA-IF3 complex.
3).	IF3	10700	Favors binding of mRNA with 30s subunit of ribosome.

C). ELONGATION:

- Elongation of peptide chain is brought about by regular addition of amino acids.
- It requires GTP as an energy source and elongation factors such as:

a). **EF-Tu**: Elongation factor temperature unstable.
b). **EF-Ts**: Elongation factor temperature stable.

- Elongation is achieved by following two steps:

i). Binding of aminoacyl tRNA at site of ribosome.

- Once both subunits of ribosomes are assembled with mRNA, binding sites for two charged tRNA molecules are formed. These are:

a). P (peptidyl) site
b). A (aminoacyl) site

- f Met tRNA^f Met enters directly into P site of ribosome while all other aminoacyl tRNA enter A
- site.
- Second charged tRNA binds to A site with the help of EF-Tu.

ii). Formation of peptide bonds between two amino acids.

- First amino acid is removed from its attachment to tRNA and transferred to free -NH terminus of
- The second amino acid.
- Peptide bond is formed between -COOH of first amino acid with -NH2 group of second amino acid. The resulting compund is called **dipeptide.**
- The reaction is catalysed by an enzyme associated with 50s subunit and called peptidyl transferase.
- Formation of peptide bond takes place in the presence of EF-Tu, EF-Ts and GTP.

iii) Translocation:

- It's the movement of entire ribosome relative to mRNA strand.
- Three important movements occur:

(1). The fmet-tRNA which is now uncharged leaves the P site,

(2). The second tRNA with bound dipeptide is moved to the P site, and

(3). mRNA moves a distance of three nucleotides. Occurs in the presence of EF-G or translocase and GTP.

D). TERMINATION:

- Two conditions are necessary for termination of protein synthesis.
- One is the presence of a stop code such as UAG, UGA and UAG that signals the chain elongation to
- terminate as no tRNA exist for these codons.
- And the other is the presence of release factors (RF) such as RF1, RF2 and RF3 which recognise the which recognise the chain terminating signal.

Various Protein Factors Involved during Translation in *E. coli*

Process	Factor	Role
Initiation of translation	IF1	Stabilizes 30*S* subunit
	IF2	Binds f-met-tRNA to 30*S*-mRNA complex; binds to GTP and stimulates hydrolysis
	IF3	Binds 30*S* subunit to mRNA; dissociates monosomes into subunits following termination
Elongation of polypeptide	EF-Tu	Binds GTP; brings aminoacyl-tRNA to the A site of the ribosome
	EF-Ts	Generates active EF-Tu
	EF-G	Stimulates translocation; GTP-dependent
Termination of translation and release of polypeptide	RF1	Catalyzes release of the polypeptide chain from tRNA and dissociation of the translocation complex; specific for UAA and UAG termination codons
	RF2	Behaves like RF1; specific for UGA and UAA codons
	RF3	Stimulates RF1 and RF2

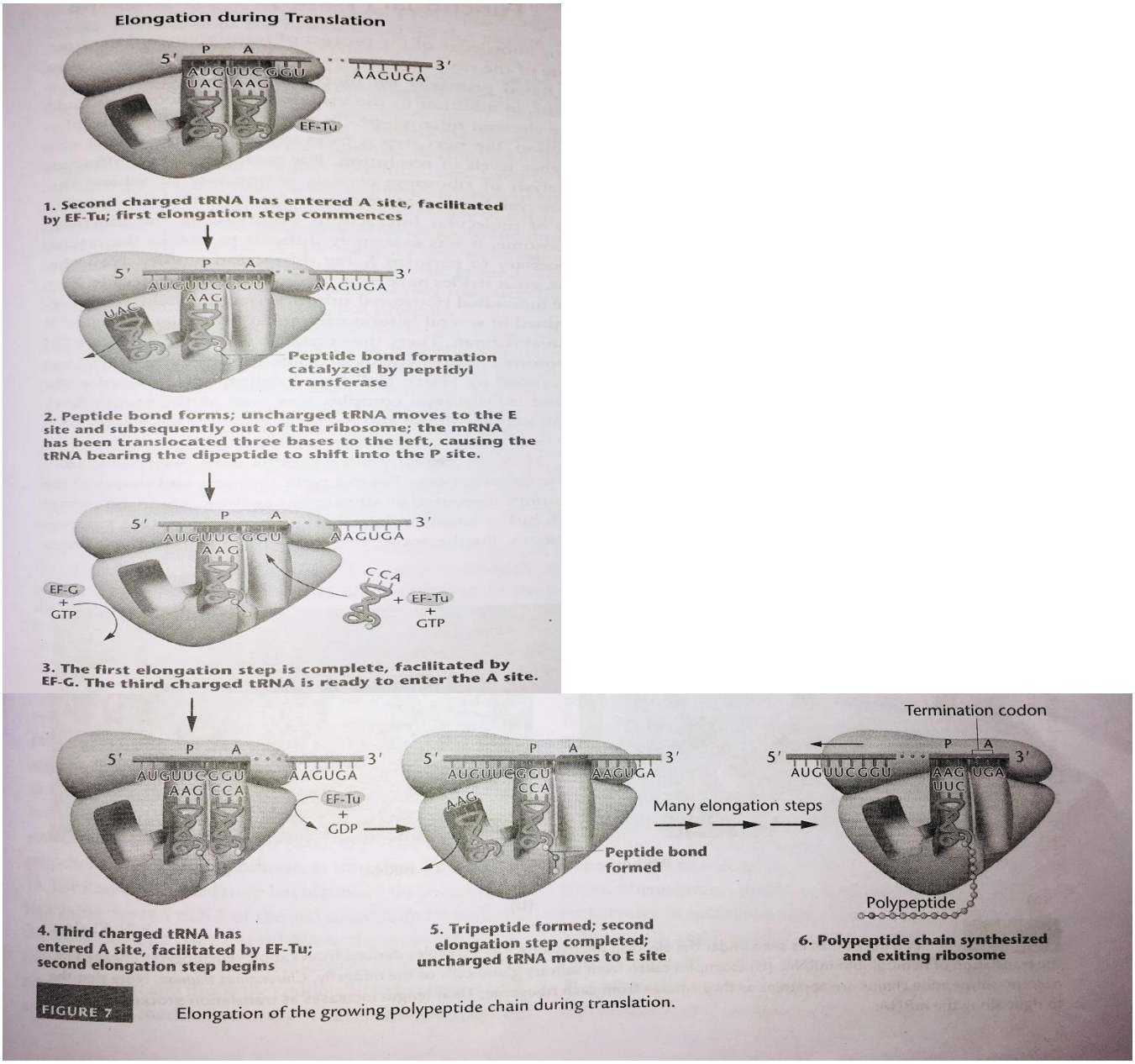


FIGURE 7 Elongation of the growing polypeptide chain during translation.

MODIFICATION OF RELEASED POLYPEPTIDES

- The just released polypeptide has primary structure, i.e., it is a straight, linear molecule. It is often called as nascent polypeptide.
- It may lose some amino acids from the end with the help of an exopeptidase enzyme, and then coil and fold on itself to acquire secondary and tertiary structure.
- It may combine with other polypeptides to have quaternary structure.
- The proteins synthesized on free polysomes are released into the cytoplasm and function as structural and enzymatic proteins.
- The proteins formed on the polysomes attached to ER pass into the ER channels and are exported as cell secretions by exocytosis after packaging in the Golgi apparatus