CONCEPT MAP

TRANSLATION

Translation is the process by which a protein or polypeptide is synthesised inside the living cell using *m*RNA as a template. This biochemical process is called translation because the information present in the form of four letter alphabet of nucleic acid is translated into twenty letter alphabets of proteins.

Translational Machinery

- Basic translational machinery is same in eukaryotes and prokaryotes, however few differences occur. It is composed of five components, *i.e.*, *m*RNA, *t*RNA, amino acid, enzymes (aminoacyl *t*RNA synthetase, peptidyl transferase) and ribosome.
- In eukaryotes, each *m*RNA is **monocistronic** (encodes for only one polypeptide) whereas in prokaryotes, many *m*RNAs are **polycistronic** (encodes for two or more different polypeptides).
- **Ribosome** is the **site of protein synthesis**. **70S** (30S + 50S) ribosomes are involved in prokaryotic translation while **80S** (40S + 60S) ribosomes are involved in eukaryotic translation. The two subunits of ribosomes associate at the time of protein synthesis and then separate after the completion of process. Ribosomes have three sites; **the peptidyl (P) site**, **aminoacyl (A) site** and **exit (E) site**. Eukaryotic ribosomes do not have E-site.
- *t*RNAs pick up particular amino acids (at CCA or 3′ end) and take the same to *m*RNA over particular codons corresponding to their anticodons. Each *t*RNA contact with ribosome at T Ψ C loop and the enzyme aminoacyl *t*RNA synthetase at DHU loop. Eukaryotic *m*RNAs have 5′- cap and poly A tail at 3′ end.

MECHANISM OF TRANSLATION

- The steps of translation are common in both prokaryotes and eukaryotes. Three main steps involved in translation are initiation, elongation and termination. Before initiation amino acids are activated and attached to *t*RNAs in two steps called activation of amino acids and charging or aminoacylation of *t*RNA respectively.
- In eukaryotes, the initiating amino acid is methionine, not N-formylmethionine (*f*Met) as in prokaryotes.
- The main difference between initiation of translation in prokaryotes and eukaryotes is that in bacteria, a Shine-Dalgarno sequence (4 to 9 purine residues, 8 to 13 base pairs to the 5′ side of initiation codon) guides correct initiation codon (5′AUG) and is the binding site for the 30S ribosomal subunit.
- In contrast, most eukaryotic *m*RNAs do not contain Shine-Dalgarno sequences. Instead, a 40S ribosomal subunit attaches at the 5′ end of the *m*RNA and moves downstream (*i.e.*, in a 5′ to 3′ direction) until it finds the AUG initiation codon. This process is called **scanning**.

1 Activation of amino acid and Charging of *t*RNA

- Amino acids are activated by activating enzymes, aminoacyl *t*RNA synthetases in presence of ATP to produce aminoacyl-adenylate-enzyme complex.

$$\underset{\text{Amino acid}}{AA} + ATP + Enzyme \xrightarrow{Mg^{2+}} \underset{\text{Aminoacyl-adenylate enzyme-complex}}{AA \sim AMP - E} + PPi$$

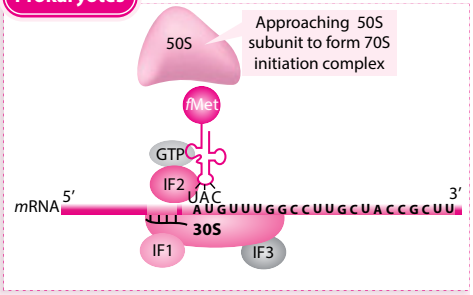
- This complex reacts with *t*RNA specific for the amino acid. Amino acid links to 3′ – OH end of *t*RNA through its –COOH group to form aminoacyl *t*RNA complex.

$$\underset{\text{Aminoacyl adenylate enzyme}}{AA \sim AMP - E} + tRNA \rightarrow \underset{\text{Charged } t\text{RNA}}{AA - tRNA} + AMP + E$$

Prokaryotes

2 Initiation

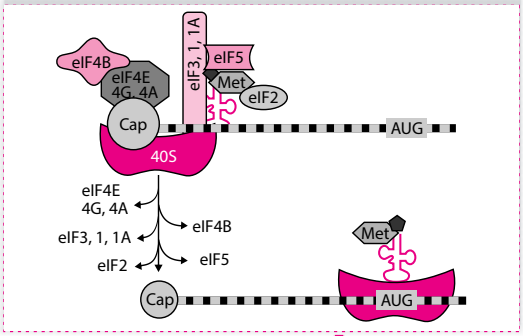
- 3 initiation factors are required *i.e.*, **IF1, IF2** and **IF3**.
- Initiation begins with the binding of **IF1** and **IF3** to the small (30S) ribosomal subunit.
- The small subunit then binds to the *m*RNA *via* complementary pyrimidine rich sequences close to 3′end of 16S *r*RNA guided by Shine-Dalgarno sequence and moves 3′ along the *m*RNA until it locates the AUG initiation codon.
- The initiator *t*RNA charged with N-formlymethionine and a complex of IF2 and GTP (*f*Met-$tRNA_f^{Met}$/IF2/GTP) now binds to *m*RNA and 30S subunit.
- The complex of *m*RNA, *f*Met-$tRNA_f^{Met}$, IF1, IF2 and the 30S ribosomal subunit is called the **30S initiation complex**.
- Structural changes then lead to the ejection of IF1 and IF3 and IF2 now stimulates the association of 50S subunit of ribosomes. Simultaneously, the GTP bound to IF2 is hydrolysed to GDP and Pi and leading to release of IF2. This forms **70S initiation complex**.
- When this complex is formed, the ribosome is ready for the elongation phase.



Eukaryotes

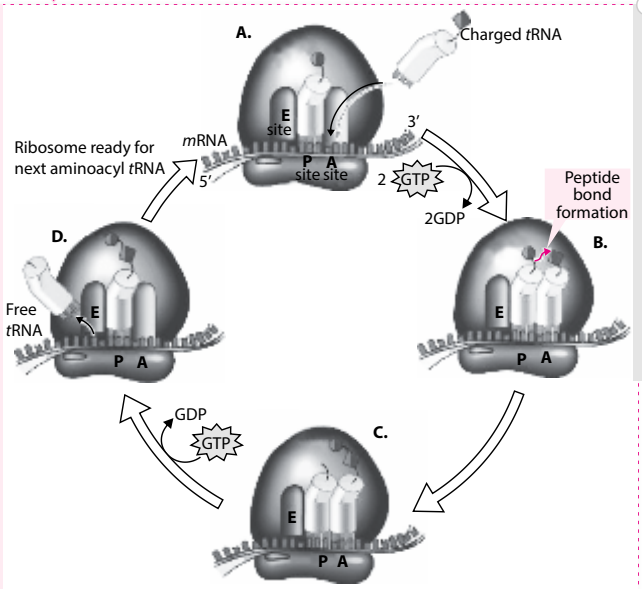
2 Initiation

- Eukaryotic cells have atleast nine initiation factors, *i.e.*, **eIF2, eIF2B, eIF3, eIF4A, eIF4B, eIF4E, eIF4G, eIF5** and **eIF6**.
- The first step is the formation of a **pre-initiation complex** consisting of the 40S small ribosomal subunit, Met-$tRNA_i^{Met}$, eIF2 and GTP;
- The pre-initiation complex now binds to the 5′end of the eukaryotic *m*RNA, a step that requires **eIF4F** complex (eIF4A, eIF4E, eIF4G, also called **cap binding complex**) and **eIF3**. Thus, this complex intact both the 5′and 3′ends of the *m*RNA.
- The eIF4A is a RNA helicase that unwinds any secondary structure of *m*RNA, preparing it for translation.
- The complex now moves along the *m*RNA in a 5′to 3′direction until it locates the AUG initiation codon (*i.e.*, scanning of *m*RNA).
- Once the complex is positioned over the initiation codon, the 60S large ribosomal subunit binds to form an **80S initiation complex**, a step that requires the hydrolysis of GTP and leads to the release of several initiation factors.



3 Elongation

- Elongation requires three factors, *i.e.*, **EF – Tu, EF – Ts** and **EF – G** and enzyme **peptidyl transferase**.
- The *f*Met-$tRNA_f^{Met}$ occupies the P site and another aminoacyl *t*RNA complex (aminoacyl *t*RNA – EF – Tu – GTP) reach at A site depending upon the anticodon present on *m*RNA. EF – Ts and GTP are required for the regeneration of **EF – Tu – GTP complex**.
- First peptide linkage is now established between –COOH group of amino acids at P site and $-NH_2$ group of amino acid at A site catalysed by the ezyme peptidyl transferase.
- This produces a dipeptidyl – *t*RNA in the A site and now uncharged $tRNA_f^{Met}$ remains bound to the P site. With the help of EF – G (translocase) dipeptidyl - *t*RNA moves from A site to P site. The ribosome moves one codon toward the 3′ end of *m*RNA (called translocation). Free *t*RNA slips to E site and from there to outside in the cytoplasm.
- New codon exposed at A site attract new aminoacyl *t*RNA complex and thus peptide chain elongates.



3 Elongation

- The elongation stage of translation in eukaryotes is quite similar to the prokaryotes.
- It requires three elongation factors, **eEF1**α, **eEF1**$\beta\gamma$ and **eEF2** as counterparts of prokaryotic EF – Tu, EF – Ts and EF – G respectively.
- The GTP form of EF1α delivers aminoacyl *t*RNA to the A site of the ribosome and EF1$\beta\gamma$ catalyses the exchange of GTP for bound GDP. eEF2 mediates GTP driven translocation similar to prokaryotic EF – G.
- As eukaryotic ribosome do not have E site, uncharged *t*RNAs are expelled directly from the P site.
- The elongated peptide chain or polypeptide lies in the groove of the larger subunit of ribosome.

4 Termination

- Termination occurs when a **non sense** or **stop codon** (UAA, UAG, UGA) reaches A site.
- Stop codons are recognised by 2 release factors **RF1** and **RF2**. A third factor **RF3** mediates interaction between RF1 or RF2 with the ribosome.
- RF1 is specific to UAG and UAA.
- RF2 is specific to UAA and UGA.
- RFs hydrolyse the terminal peptidyl-*t*RNA bond, release polypeptide and last *t*RNA from the P site and dissociates two subunits of ribosomes to start new cycle of translation.

4 Termination

- Termination in eukaryotes is similar to that in prokaryotes.
- In eukaryotes, a single factor **eRF1** recognises all three termination codons and with the help of eRF3, ribosomal subunits are released.
- eRF3 prevents the reassociation of ribosomal subunits in the absence of an initiation complex.