TRANSLATION IN PROKARYOTES AND EUKARYOTES

1. DNA in nucleus serves as a template.

2. mRNA is processed before leaving the nucleus.

3. When mRNA is formed it has codons.

4. mRNA moves into cytoplasm and becomes associated with ribosomes.

5. tRNA with anticodon carries amino acid to mRNA.

6. Anticodon-codon complementary base pairing occurs.

7. Peptide chain is transferred from resident tRNA to incoming tRNA.

8. tRNA departs and will soon pick up another amino acid.

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ROLL NUMBER - 1784
BIOCHEMISTRY
1. Introduction
2. Messenger RNA (mRNA)
3. Transfer RNA (tRNA)
4. Amino acyl tRNA synthetase
5. Ribosome
6. Initiation of translation
7. Elongation of translation: Peptidyl transferase reaction
8. Termination of translation
9. Antibiotics: Target and consequences
Translation is the process in which a sequence of nucleotide triplets in a messenger RNA give rise to a specific sequence of amino acids during the synthesis of a polypeptide chain or protein.

Translation is among the most highly conserved across all organisms.

Protein synthesis requires the coordinated action of well over 100 proteins and RNAs.
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8. tRNA departs and will soon pick up another amino acid.
1.1 Translational machinery

- The machinery required for translating the language of messenger RNAs into the language of proteins is composed of four primary components:
  
  i. mRNAs
  
  ii. tRNAs
  
  iii. Aminoacyl tRNA synthetase
  
  iv. Ribosome.

- Together these components accomplish the extraordinary task of translating the code written in a four base alphabet (A,T,G,C) into a second code written in language of 20 amino acids.
2. MESSENGER RNA (mRNA)

- Messenger RNA (mRNA) provides an intermediate that carries the copy of a DNA sequence that represents protein.
- Protein coding region of mRNA is composed of a contiguous, non-overlapping string of codons called **Open reading frame (ORF)**.
- mRNA containing single ORF is **Monocistronic mRNA**.
- mRNA containing multiple ORRFs is **Polycistronic mRNA**.
- **Codons** – ordered series of three nucleotides specific for amino acids.
• **Start codon** — first codon of an ORF present at the 5’ end

<table>
<thead>
<tr>
<th>Prokaryotic start codon</th>
<th>Eukaryotic start codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’-AUG-3’</td>
<td>5’-AUG-3’</td>
</tr>
<tr>
<td>5’-GUG-3’</td>
<td>-</td>
</tr>
<tr>
<td>5’-UUG-3’</td>
<td>-</td>
</tr>
</tbody>
</table>

• **Stop codon** — last codon of an ORF at the 3’ end which define the signal termination of polypeptide synthesis.

• 3 stop codons are — 5’-UAG-3’
  5’-UGA-3’
  5’-UAA-3’
<table>
<thead>
<tr>
<th>First base in codon</th>
<th>second base in codon</th>
<th>third base in codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>UUU Phe</td>
<td>UCU Ser</td>
<td>UAU Tyr</td>
</tr>
<tr>
<td>UUC Phe</td>
<td>UCC Ser</td>
<td>UAC Tyr</td>
</tr>
<tr>
<td>UUA Leu</td>
<td>UCA Ser</td>
<td>UAA stop</td>
</tr>
<tr>
<td>UUG Leu</td>
<td>UCG Ser</td>
<td>UAG stop</td>
</tr>
<tr>
<td>CUU Leu</td>
<td>CCU Pro</td>
<td>CAU His</td>
</tr>
<tr>
<td>CUC Leu</td>
<td>CCC Pro</td>
<td>CAC His</td>
</tr>
<tr>
<td>CUA Leu</td>
<td>CCA Pro</td>
<td>CAA Gln</td>
</tr>
<tr>
<td>CUG Leu</td>
<td>CCG Pro</td>
<td>CAG Gln</td>
</tr>
<tr>
<td>AUU Ile</td>
<td>ACU Thr</td>
<td>AAU Asn</td>
</tr>
<tr>
<td>AUC Ile</td>
<td>ACC Thr</td>
<td>AAC Asn</td>
</tr>
<tr>
<td>AUA Ile</td>
<td>ACA Thr</td>
<td>AAA Lys</td>
</tr>
<tr>
<td>AUG Met</td>
<td>ACG Thr</td>
<td>AAG Lys</td>
</tr>
<tr>
<td>GUU Val</td>
<td>GCU Ala</td>
<td>GAU Asp</td>
</tr>
<tr>
<td>GUC Val</td>
<td>GCC Ala</td>
<td>GGU Gly</td>
</tr>
<tr>
<td>GUA Val</td>
<td>GCA Ala</td>
<td>GGA Gly</td>
</tr>
<tr>
<td>GUG Val</td>
<td>GCG Ala</td>
<td>GGG Gly</td>
</tr>
</tbody>
</table>
2.1 PROKARYOTIC mRNA

- Prokaryotic ORFs contain short sequences, (3-9bp) upstream of start codon called Ribosome binding site (RBS) or Shine Dalgarno Sequence.

- The core of the sequence is mostly a subset of sequence 5’-AGGAGG-3’ which binds to the 5’-CCUCCU-3’ of 16S rRNA.

- Some prokaryotes that lack RBS undergo Translational coupling as they contain overlapping open reading frames.
2.2 EUKARYOTIC mRNA

3 modifications for the recruitment of ribosome to mRNA

1) **Methylated guanine cap** at the 5’ end

2) **Kozak sequences** :- presence of a purine 3 bp upstream of AUG and presence of a guanine immediately downstream (5’-G/ANNAUGG-3’)

3) Presence of a **poly A tail** at the extreme 3’ end
3. TRANSFER RNA (tRNA)

- tRNA acts as an *adaptor* between the codons and the amino acids they specify.
- There are many types of tRNA molecules, but each is attached to a specific amino acid and each recognize a particular codon.
- 75-95 ribonucleotides in length.
- Terminus is **5′-CCA-3′** which is the binding site of amino acid to tRNA.
- Contains unusual or modified bases- uridine, thymine, pseudouridine, methylguanine, hypoxanthine etc.
- They are not essential for tRNA but those lacking these show reduced rate of growth.
Modified bases in tRNA

- Uridine
- Pseudouridine
- Dihydouridine
Structure of tRNA

- tRNA share a common secondary structure that resembles a cloverleaf
  - **Acceptor arm** - 5’-CCA-3’ which is the binding site of amino acid to tRNA
  - **ΨU loop** – contains Pseudouridin (ΨU)
  - **D loop** - contains Dihydouridine
  - **Anticodon loop** – binds to codons in mRNA
  - **Variable loop** – varies from 3 to 21 bases
Structure of tRNA
• Also called charging of tRNA
• Catalyzed by Amino acyl tRNA synthetase
• 2 classes of amino acyl tRNA synthetase
  • Class 1 - monomeric which attach amino acid at 2’ OH of tRNA
  • Class 2 - Dimeric or tetrameric which attach aa. at 2’ OH of tRNA
Amino acyl tRNA synthetase
Attachment of amino acids to tRNA

Amino acids are attached to tRNA in two steps

1) Adenylation – Amino acid with the ATP to become adenylated. carbonyl group of amino acid is ionized to phosphate group of AMP by releasing PPi from ATP.

2) Charging – Carbonyl group of adenylated aa react with 3’OH of tRNA. A high energy bond and a concommittent release of AMP.
Attachment of amino acids to tRNA
5. RIBOSOME

- It is the macromolecular complex that directs the synthesis of proteins.
- Composed of a large subunit and a small subunit
- Large subunit contains the peptidyl transferase center which is responsible for the formation of peptide bonds
- Small subunit contains the decoding center in which charged tRNAs read or decode the codons of mRNA
- Large and small subunits undergo association and dissociation during each cycle of translation
ROLE OF rRNA in RIBOSOME

• Ribosomes are made up of 50% RNA and 50% proteins.
• RNA in ribosomes are called **rRNA** which have extensive secondary structures and associate with proteins to form ribosomes.
• **16 S rRNA** in the small subunit of ribosomes is the binding site for the mRNA.
• **23 S rRNA** of the large subunit is the ribozyme which catalyzes the peptidyl transferase reaction.
• An mRNA bearing multiple ribosome is known as **polyribosome or polysome**.
• Ribosome has three tRNA binding sites
  1) **A site** – binding site for first **aminocylated tRNA**
  2) **P site** – binding site for the **peptidyl tRNA**
  3) **E site** – binding site for the **uncharged tRNA**

• These sites are present at the interface between the small and the large subunit of ribosome.
Channels in ribosome

- Small subunit of ribosome has two narrow tunnels
- **Entry channel** for mRNA
- **Exit channel** for mRNA
- Large subunit has an **exit channel** for newly synthesized polypeptide chain
Protein synthesis uses three types of RNA

mRNA has a sequence of bases that represent protein
size range 500-10,000 bases

tRNA is a small RNA with extensive secondary structure
size range 74 - 95 bases

major rRNAs have extensive secondary structure and associate with proteins to form the ribosome
size range 1500-1900 (small rRNA) and 2900-4700 (large rRNA)
6. INITIATION OF TRANSLATION

- It occurs in three steps
  1) Ribosome must be recruited to the mRNA
  2) Charged tRNA must be placed into the P site of ribosome
  3) Ribosome must be precisely positioned over the start codon
- The initiator tRNA is charged with **N-Formyl methionine** in prokaryotes and with **methionine** in eukaryotes.
- Three initiation factors direct the assembly of an initiation complex that contains mRNA and the initiator tRNA called translational initiation factors.
GENERAL OVERVIEW OF INITIATION
6.1 PROCESS OF INITIATION IN PROKARYOTES

1. **IF1** – prevents tRNA from binding to the portion of the small subunit that will become the part of a site.

2. **IF2** – a GTPase which interacts with small subunit, IF1, charged initiator tRNA and prevents other charged tRNAs to bind to the small subunit.

3. **IF3** – it binds to the small subunit and blocks it from reassociating with a large subunit, or from binding charged tRNA and in dissociation of 70S ribosome into large subunit and small subunit.

   - With all three initiation factors bound, the small subunit is prepared to bind to the mRNA and the initiator which can bind in either order.
   - This results in the formation of 70S initiation complex.
INITIATION IN PROKARYOTES
6.2 PROCESS OF INITIATION IN EUKARYOTES

Involves 4 general steps:

- Binding of tRNA precedes binding of mRNA
- mRNA is recruited separately
- Small subunit bound to tRNA scans mRNA for AUG
- Large subunit is recruited after tRNA base pairs with the start codon
a. Binding of itRNA to the P site

- 4 initiation factors are involved - eIF1, eIF1A, eIF2, eIF5.
- They bind to small subunit
- eIF1, eIF1A, eIF5 act in analogous manner to prokaryotic initiation factors of IF3 & IF1 to prevent both large subunit binding and tRNA binding to the A site
- itRNA is escorted by the GTP binding protein - eIF2
- This is Ternary complex
- eIF2 positions itRNA at P site
b. Binding of mRNA to 43S initiation complex

- 4 initiation Factors involved are eIF4E, eIF4G, eIF4B, eIF4A.
- Recognition by 5’ cap by a 3 subunit complex eIF4E
- eIF4G binds to eIF4E and mRNA, to which binds eIF4A
- It is then joined by eIF4B which activates an RNA helicase activity of eIF4A that unwinds any secondary structure
- This eIF4F-eIF4B complex is then recruits the 43S preinitiation complex to the mRNA by interactions between eIF4F and eIF3.
- This 43S complex with mRNA is called 48S preinitiation complex.
c. Scanning for AUG

• After assembling at the 5’ end of mRNA, the small subunit scans the mRNA for the start codon in 5’ – 3’ direction in an ATP dependent process.

• Correct base pairing between the initiator tRNA and start codon releases eIF3 and eIF2 which allows the large subunit to bind to the small subunit.

• Binding of large subunit leads to loss of eIF5B by GTP hydrolysis and binding of initiator tRNA to P site and formation of 80S complex.
d. Association of small and large subunit

• Correct base pairing changes the conformation of 48S complex leading to the release of eIF1 and a change in conformation of eIF5

• Both these events hydrolyze eIF2GTP into eIF2 GDP

• Loss of eIF2GDP stimulates the loss of eIF5B which stimulate the correct base pairing of large and small subunit of ribosome.
7. ELONGATION OF TRANSLATION

• Three key events for correct addition of each amino acids:
  1) Correct aminoacyl-tRNA is loaded to the A site of the ribosome as dictated by the A site codon
  2) Peptidyl transferase reaction
  3) Translocation of peptidyl tRNA from A site of ribosome to the P site of ribosome.

• Two proteins control these events called Elongation factors, both of which use energy from GTP binding and hydrolysis
  • EF-Tu – It “escorts” aminoacyl tRNAs to the ribosomes
  • EF-G – it derieves translocation of tRNA and mRNA
Elongation in Prokaryotes
7.2 Peptidyl transferase reaction

- Once the correctly charged tRNA has been placed in the A site and has rotated in the peptidyl transferase centre, peptide bond formation takes place.
- This reaction is catalysed by the 23S rRNA component of the large subunit.
- So ribosome is called a Ribozyme.
- Base pairing between the 23S rRNA and the CCA end of tRNA in the A site and the P sites help to position the alpha amino group of the aminoacyl tRNA to attack the carbonyl group of the growing polypeptide chain attached to the peptidyl tRNA.
Role of EF- G

• **EF-G** drives the translocation by displacing the tRNA bound to the A site.

• **EF-G GTP** binds to the *factor binding center* on the large subunit of ribosome.

• It then dephosphorylates to form **EF-G GDP** and there is a structural change in it and it binds to mRNA at the A site.

• **EF-G GDP** displaces the A site tRNA to the P site and essentially the mRNA is moved along with this movement.

• After translocation, changes in the structure of large subunit allow **EF-G GDP** to be released.
Role of EF G in Elongation
8. TERMINATION OF TRANSLATION

- Stop codons are recognized by proteins called release factors (RF).
- These activate the hydrolysis of polypeptide from the peptidyl tRNA.
- There are 2 classes of RF:
  - **Class 1 RFs** – recognize stop codon and trigger the hydrolysis of polypeptide chain from the peptidyl tRNA.
  - **Class 2 RFs** – stimulate the dissociation of class 1 RFs from the ribosome after the release of polypeptide.

<table>
<thead>
<tr>
<th>RF CLASS</th>
<th>PROKARYOTES</th>
<th>EUKARYOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLASS 1</td>
<td>RF 1 - UAG</td>
<td></td>
</tr>
<tr>
<td>CLASS 1</td>
<td>RF 2 - UGA</td>
<td>eRF 1- All stop codon</td>
</tr>
<tr>
<td>CLASS 1</td>
<td>RF `1 &amp; RF 2 - UAA</td>
<td></td>
</tr>
<tr>
<td>CLASS 2</td>
<td>RF 3</td>
<td>eRF 3</td>
</tr>
</tbody>
</table>
• All class 1 factors share a conserved three amino acid sequence (glycine, glycine, glutamine- GGQ) that is essential for polypeptide release.

• Studies have led to the hypothesis that class 1 RFs functionally mimic tRNA; having a peptide anticodon that binds to the stop codon and GGQ motif reaches the peptidyl transferase center.

• Steps involved are:
  1) After RFs bind to the A site and recognize stop codon, there is a conformational change in RF which releases the polypeptide.
  2) RF 3 GDP binds on class 1 RFs after release of polypeptide.
  3) Change in conformation of ribosome and RFs stimulates RF3 to exchange its bound GDP to GTP.
  4) This RF3 GTP forms a high affinity interaction with ribosome that displaces class 1 RFs and concurrent hydrolysis of GTP into GDP.
  5) Now this RF-GDP has a low affinity for ribosome and is released.
TERMINATION OF TRANSLATION IN PROKARYOTES
TERMINATION OF TRANSLATION IN EUKARYOTES
8.2 Ribosome Recycling

• After the release of polypeptide and the release factors the ribosome is still bound to the mRNA and is left with two deacylated tRNA (in the P and E sites).
• To participate in a new round of polypeptide synthesis, these mRNA and the tRNA must be released and the ribosome must dissociate into small subunit and large subunit.
• Collectively these events are termed as ribosome recycling
• In prokaryotes, a factor called ribosome recycling factor (RRF) cooperates with EF-G and 1F3 to recycle ribosome
• RRF binds to the empty A site of the ribosome where it mimics tRNA.
• RRF also recruits EF-G to the ribosome which stimulate the release of uncharged tRNA.
• After the release of tRNA, 1F3 may also participate in the release of the mRNA and is required to separate the two subunits of ribosomes.
RIBOSOME RECYCLING

1. RRF binds in A site
2. EF-G-GTP
3. GTP hydrolysis
4. Release of P<sub>i</sub>
5. EF-G-GDP
6. RRF and EF-G-GDP release
7. mRNA and ribosome release
Antibiotics arrest cell division by blocking the steps of translation.

<table>
<thead>
<tr>
<th>Antibiotic/Toxin</th>
<th>Target Cells</th>
<th>Molecular Target</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>Prokaryotic cells</td>
<td>A site of 30S subunit</td>
<td>Inhibits aminoacyl-tRNA binding to the A site</td>
</tr>
<tr>
<td>Hygromycin B</td>
<td>Prokaryotic and eukaryotic cells</td>
<td>Near the A site of 30S subunit</td>
<td>Prevents translocation of A-site tRNA to P site</td>
</tr>
<tr>
<td>Paromycin</td>
<td>Prokaryotic cells</td>
<td>Adjacent to the A site codon-anticodon interaction site in 30S subunit</td>
<td>Increases error rate during translation by decreasing selectivity of codon-anticodon pairing</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Prokaryotic cells</td>
<td>Peptidyl transferase center of 50S subunit</td>
<td>Blocks correct positioning of the A site aminoacyl-tRNA for peptidyl transfer reaction</td>
</tr>
<tr>
<td>Puromycin</td>
<td>Prokaryotic and eukaryotic cells</td>
<td>Peptidyl transferase center of large ribosomal subunit</td>
<td>Chain terminator; mimics the 3' end of aminoacyl-tRNA in A site and acts as acceptor for the nascent polypeptide chain</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Prokaryotic cells</td>
<td>Peptide exit tunnel of 50S subunit</td>
<td>Blocks exit of the growing polypeptide chain from the ribosome; arrests translation</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>Prokaryotic cells</td>
<td>EF-G</td>
<td>Prevents release of EF-G-GDP from the ribosome</td>
</tr>
<tr>
<td>Thiotrepton</td>
<td>Prokaryotic cells</td>
<td>Factor binding center of the 50S subunit</td>
<td>Interferes with the association of IF2 and EF-G with factor binding center</td>
</tr>
<tr>
<td>Kpomycin</td>
<td>Prokaryotic cells</td>
<td>EF-Tu</td>
<td>Prevents the conformational changes associated with GTP hydrolysis and, therefore, EF-Tu release</td>
</tr>
<tr>
<td>Ricin and α-Sarcin (protein toxins)</td>
<td>Prokaryotic and eukaryotic cells</td>
<td>Chemically modifies the RNA in the factor binding center of large ribosomal subunit</td>
<td>Prevents activation of translation factor GTPases</td>
</tr>
<tr>
<td>Diptheria Toxin</td>
<td>Eukaryotic cells</td>
<td>Chemically modifies EF-Tu</td>
<td>Inhibits EF-Tu function</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>Eukaryotic cells</td>
<td>Peptidyl transferase center of the 60S subunit</td>
<td>Inhibits peptidyl transferase activity</td>
</tr>
</tbody>
</table>