# Cell and molecular biology

## Assignment

# Ribosomes and protein synthesis

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# RIBOSOMES AND PROTEIN SYNTHESIS

## INTRODUCTION:

### *CENTRAL DOGMA OF THE LIFE:*

The DNA is organized in to genes, the fundamental units of genetic information. The genes control the protein synthesis through the mediation of RNA, as shown below

DNA $\xrightarrow{\text{transcription}}$ RNA $\xrightarrow{\text{translation}}$ PROTEINS

The inter relationship of these three classes of biomolecules (DNA, RNA, Proteins) constitute the central dogma of molecular biology or more commonly the central dogma of life. Transcription is a process in which RNA synthesised from DNA. The genetic information stored in DNA is passed on to RNA (transcription), and ultimately expressed in the language of proteins. The biosynthesis of a protein or a polypeptide in a living cell is referred to as translation.

The functionally active ribosomes are the centres or factories for protein synthesis purely a cytoplasmic process, while transcription is nuclear process. Here detail illustration of the ribosomes and protein synthesis.

## Ribosomes

Ribosomes are remarkable organelles of cell, both prokaryotic and eukaryotic cells. They are small, dense, rounded and granular particles of the ribonucleoprotein. They occur either freely in the matrix of mitochondria, chloroplast and cytoplasm (cytoplasmic matrix) or remain attached with the membranes of the endoplasmic reticulum and nucleus. They occur in most prokaryotic and eukaryotic cells and are known to provide a scaffold for the ordered interaction of all the molecules involved in protein synthesis.

In prokaryotic cells the ribosomes often occur freely in the cytoplasm. In eukaryotic cells the ribosomes either occur freely in the cytoplasm or

remain attached to the outer surface of the membrane of endoplasmic reticulum. The cells in which active protein synthesis take place as pancreatic cells, plasma cells, hepatic parenchymal cells, Nissles bodies, osteoblasts, serous cells, chief cells of the glandular stomach, thyroid cells and mammary gland cells, the ribosomes remain attached with the membranes of endoplasmic reticulum. The cells which synthesize specific proteins for the intracellular utilization and storage as erythroblasts, developing muscle cells, skin and hair often contain large number of free ribosomes.

## ***TYPES OF RIBOSOMES:***

The ribosomes are usually isolated from the cell by the deferential centrifugation method. The sedimentation coefficient of the ribosomes is determined by the various optical and electronic techniques. The sedimentation coefficient is expressed in the Svedberg unit. That is denoted as S. The S related with the size and molecular weight of the ribosomal particles. Recently according to the size and the sedimentation coefficient (S), two types of ribosomes have been recognised.

1. 70S ribosomes: They occur in the prokaryotic cells of the blue green algae and bacteria and also in mitochondria and chloroplast of eukaryotic cells. These are comparatively smaller in size and have sedimentation coefficient 70S and the MW $2.7*10^6$ Daltons.
2. 80S ribosomes: They occur in eukaryotic cells of the plants and animals. These ribosomes have the sedimentation coefficient of 80S and the MW $40*10^6$ Daltons.

## ***STRUCTURE OF RIBOSOMES:***

The ribosomes are oblate spheroid structure of 150 to 250 A° in diameter. Each ribosome is porous, hydrated and composed of two sub units. One ribosomal subunit is large in size and has a dome like shape, while the other ribosomal sub unit is smaller in size and occurring above the large sub unit and forming a cap like structure**.**

The 70S ribosome consists of two sub units as 50S and 30S. The 50S ribosomal subunit is larger in size and the 30S ribosomal sub unit is smaller in size and occurs above the 50S sub unit like a cap. The 80S ribosomes also consist of two sub units as 60S and 40S. The 60S ribosomal sub unit is dome shaped and larger in size. In the case of bound ribosomes the 60S subunit remains attached with the membrane. The 40S subunit is smaller in size and occurs above the 60S sub unit as a cap like structure. Both the sub units are separated by a narrow cleft.

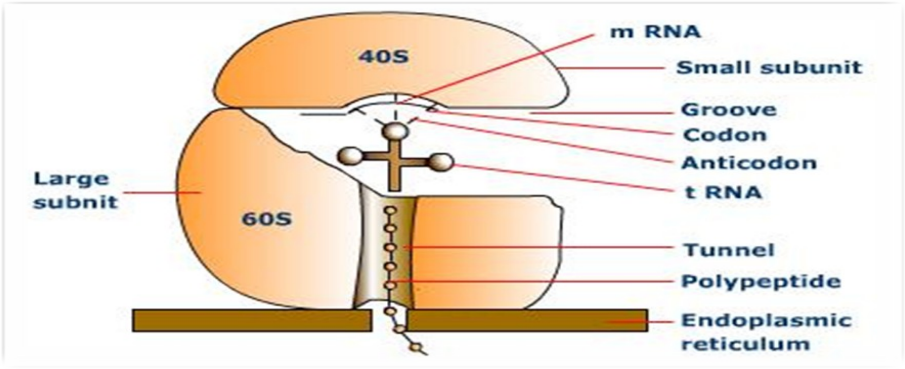



Fig 2: The ribosome ultra structure

Two ribosomal sub units united with each other due to high concentration of $Mg^{2+}$ ions. When the concentration of the $Mg^{2+}$ ions reduced in the matrix, both sub units are seperated. Actually in bacterial cells the two sub units are occur freely in the cytoplasm and they unite only during process of protein synthesis. The two ribosomal sub units called monomers become associated with each other and known as the dimer. During protei synthesis many ribosomes are aggregated deu to common mRNA and form the polyribosomes or polysomes. The ribosomes are chemically composed of RNA and proteins as their major constituents. Eukaryotic 80S ribosomes differ from prokaryotic 70S ribosomes due to :

1. They are considerably larger
2. They contain large number proteins (70-80 types of proteins instead of 53)
3. They have four types of RNA instead of three types
4. Their proteins and neuclic acids are larger
5. The RNA- Protein ratio is ~1:1 instead of 2:1
6. Several antibiotics such as Chloramphenicol inhibits bacterial but not eukaryotic.

Molecular organization and functions of ribosomes have been studied more in prokaryotes than eukaryotes. Fine or ultra structure of 70S ribosome is very complex. Eukaryotic ribosomes do not differ functionally from prokaryotes, they perform the same functions by the same set of reactions.

Hybrid ribosomes containing one bacterial subunit and one subunit from the chloroplast ribosomes are found fullyactive in protein synthesis, but the two sub units each one subunit from bacteria and other from eukaryote, they are found to be inactive.

| Characteristics of ribosomes of various organisms | | | | |
|---|---|---|---|---|
| **Source** | **Intact Ribosomes** | **Ribosome Subunits** | **rRNA in Subunits** | **No: of Proteins in Subunits** |
| **PROKARYOTES** | **70S** | **30S<br>50S** | **16S<br>23S, 5S** | **21<br>32-34** |
| **EUKARYOTES** | **80S** | **40S<br>60S** | | **~30<br>~50** |
| **Animals** | | **40S<br>60S** | **18S<br>28S, 5S, 5.8S** | |
| **Plants** | | **40S<br>60S** | **18S<br>25-26S, 5S, 5.8S** | |
| **Fungi** | | **40S<br>60S** | | |
| **Protozoa** | | **40S<br>60S** | | |

Table 1: some characteristic of ribosomes of various organisms ((Avers 1976)

# Protein synthesis

DNA with its own correct mechanism of replication, serves to carry genetic information from cell to cell and from generation to generation. This information is translated in to proteins that determine the phenotype. Proteins are composed of one or more long linear polymer of aminoacid residues - polypeptide chains - that are synthesized almost exclusively in the cytoplasm. In this assignment for how the information present in the sequence of bases – triplet codons – of the mRNA is translated in to sequence of aminoacids in proteins.

There are wide variations in the cells with respect to the quality and quandity of protein synthesised. This largely depends on the need and ability of the cells. Erythrocytes lack the machinary for traslation and cannot synthesize proteins. The normal liver cells are very rich in th eprotein biosynthetic machinary, so the liver regarded as the protein factory in the human body.

***TRIPLET CODONS:***

The three nucleotide (triplet) base sequence in mRNA that act as code for amino acids in protein constitute the genetic code ar simply *codons.* The

codons are composed of four neicleotide bases, namely the purines - adenine A and guanin G, and pyramidines – cytosine C and uracil U.

These four bases produce 64 different combinations ($4^3$) of three base codons. The nucleotide sequence of the codon on mRNA is written from the 5’ end to 3’ end. 61 codons code for the 20 aminoacids found in protein. The three codons *UAA, UAG,* and *UGA* do not code for amino acids. They act as stop signals in protein synthesis and collectively known as *termination codons*. The codons *AUG* and some times *GUG* are the *chain initiating codons.*

## ***MINIMUM NECESSARY MATERIALS:***

The success in polypeptide synthesis the minimum necessary materials are found to be,

1. Amino acids (pool of 20 amino acids in the cytoplasm)
2. Ribosomes (exist as seperate subunits prior to the transalation of mRNA and contain three tRNA binding sites: P or peptidyl site, A or aminoacyl site and E or exit site)
3. mRNA
4. tRNA of several kinds
5. Enzymes
    a. Amino acid activating system (eg. Aminoacyl tRNA synthetase)
    b. Peptide polymerase system
6. ATP as an energy source
7. GTP for synthesis of peptide bonds
8. Soluble protein initiation and transfer factors
9. Various inorganic cations (eg. $K^+$, $NH_2^+$, $Mg^{2+}$).

| First base | Second base: U | Second base: C | Second base: A | Second base: G | Third base |
|---|---|---|---|---|---|
| U | UUU Phe | UCU Ser | UAU Tyr | UGU Cys | U |
| | UUC Phe | UCC Ser | UAC Tyr | UGC Cys | C |
| | UUA Leu | UCA Ser | UAA Stop | UGA Stop | A |
| | UUG Leu | UCG Ser | UAG Stop | UGG Trp | G |
| C | CUU Leu | CCU Pro | CAU His | CGU Arg | U |
| | CUC Leu | CCC Pro | CAC His | CGC Arg | C |
| | CUA Leu | CCA Pro | CAA Gln | CGA Arg | A |
| | CUG Leu | CCG Pro | CAG Gln | CGG Arg | G |
| A | AUU Ile | ACU Thr | AAU Asn | AGU Ser | U |
| | AUC Ile | ACC Thr | AAC Asn | AGC Ser | C |
| | AUA Ile | ACA Thr | AAA Lys | AGA Arg | A |
| | AUG Met | ACG Thr | AAG Lys | AGG Arg | G |
| G | GUU Val | GCU Ala | GAU Asp | GGU Gly | U |
| | GUC Val | GCC Ala | GAC Asp | GGC Gly | C |
| | GUA Val | GCA Ala | GAA Glu | GGA Gly | A |
| | GUG Val | GCG Ala | GAG Glu | GGG Gly | G |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AGA | | | | | | | | | UUA | | | | | AGC | | | | |
| | AGG | | | | | | | | | UUG | | | | | AGU | | | | |
| GCA | CGA | | | | | | GGA | | | CUA | | | | CCA | UCA | ACA | | GUA | |
| GCC | CGC | | | | | | GGC | | AUA | CUC | | | | CCC | UCC | ACC | | GUC | UAA |
| GCG | CGG | GAC | AAC | UGC | GAA | CAA | GGG | CAC | AUC | CUG | AAA | | UUC | CCG | UCG | ACG | UAC | GUG | UAG |
| GCU | CGU | GAU | AAU | UGU | GAG | CAG | GGU | CAU | AUU | CUU | AAG | AUG | UUU | CCU | UCU | ACU | UGG | UAU | GUU | UGA |
| Ala | Arg | Asp | Asn | Cys | Glu | Gln | Gly | His | Ile | Leu | Lys | Met | Phe | Pro | Ser | Thr | Trp | Tyr | Val | stop |
| A | R | D | N | C | E | Q | G | H | I | L | K | M | F | P | S | T | W | Y | V | |

Fig 4: The genetic code along with their respective aminoacids

## ***MECHANISM OF PROTEIN SYNTHESIS:***

Protein synthesis is the most complx biochemical transformation in cells and atleast 200 different proteins are required for the protein synthesis. Both in pro- eukaryotes, the mechanism of protein synthesis can be divided into three main steps;

1. Initiation: The main features of this step are,
   a. The binding of mRNA to the ribosomes
   b. Selection of the initiation codon
   c. Amino acid activation
   d. Transfer activated aminoacid to tRNA
   e. Binding of acylated tRNA bearing the first aminoacid

2. Elongation: includes two processes
   a. Joining together of two amino acids by peptide bond formation
   b. Moving the mRNA and ribosomes with respect to one another so that the codon can be translated.
   c. Translocations
3. Termination: The completed polypeptide or protein is dissociated from the synthetic machinery and the ribosomes are released.

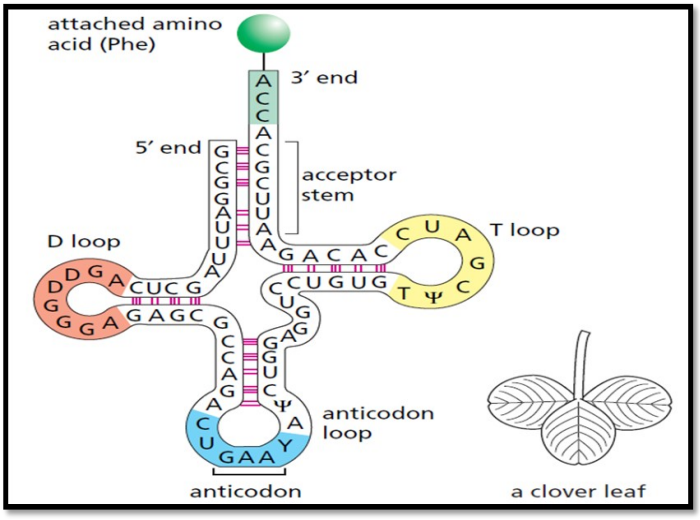
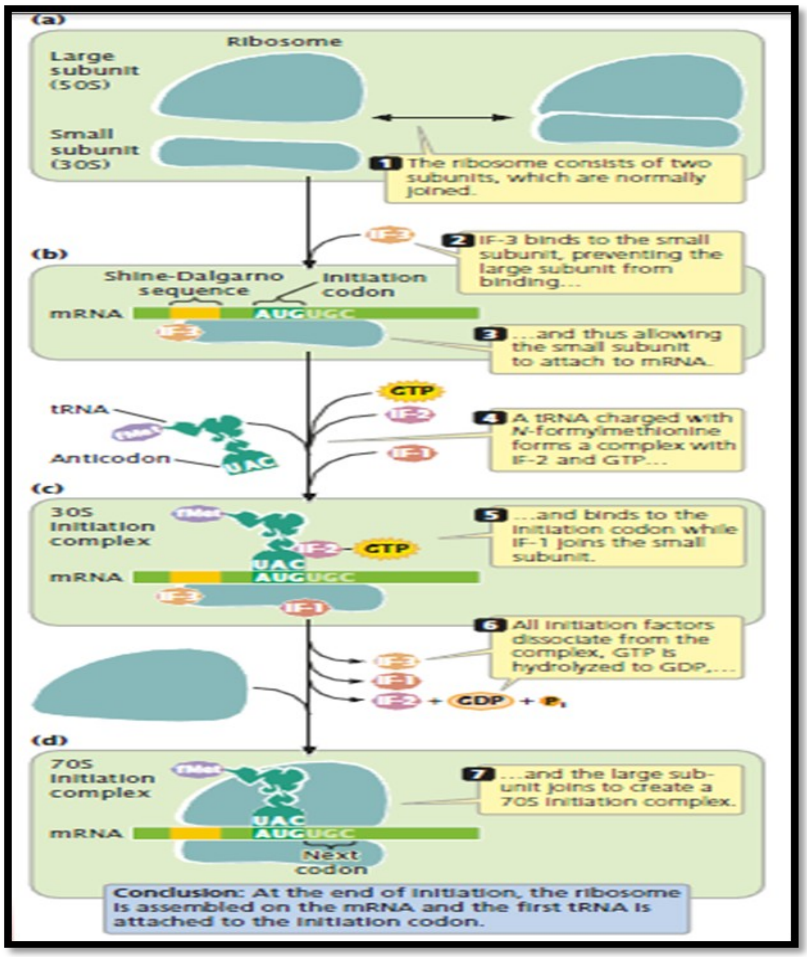
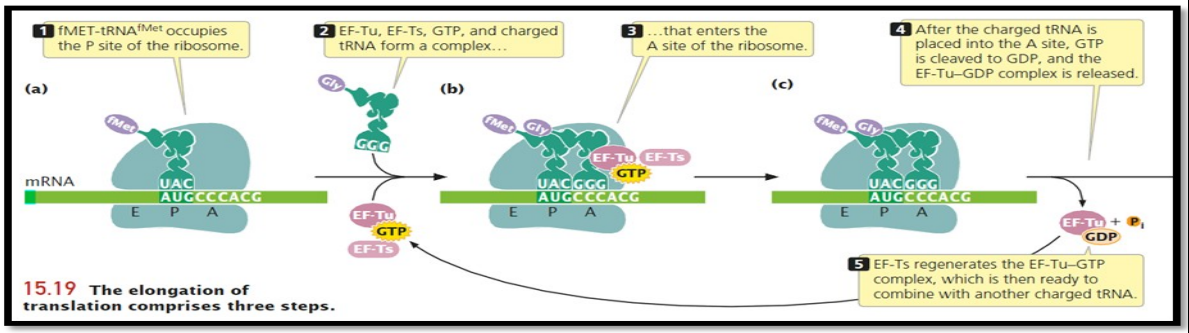



Fig 7: The structure of Trna (Benjamin Pierce)




15.19 **The elongation of translation comprises three steps.**

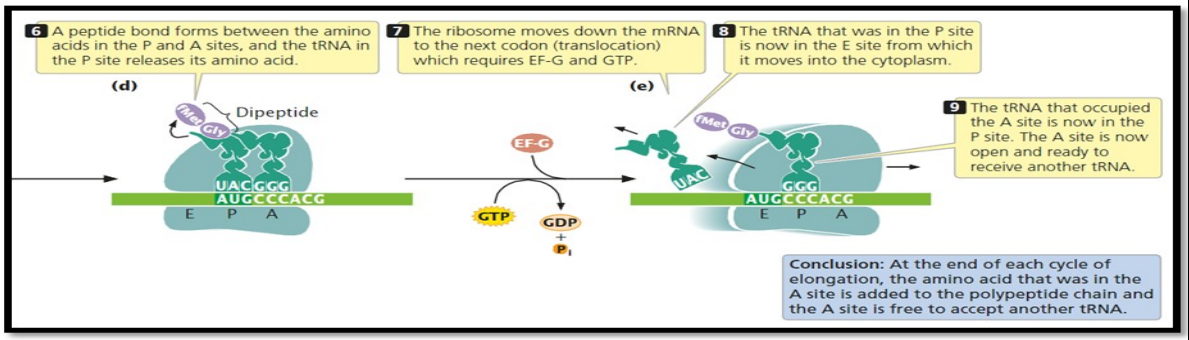



Fig 6: initiation and elongation process in protein synthesis

(Benjamin A Pierce)

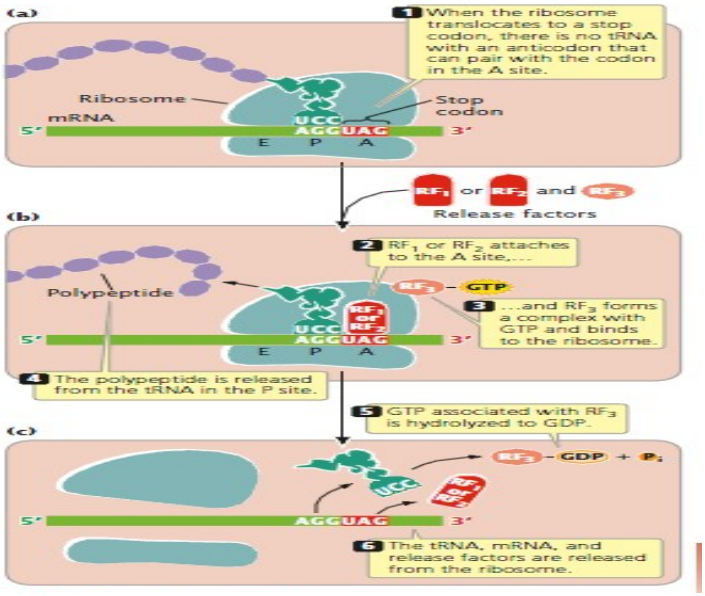



Fig 9: Translation ends when stop codon encountered (Benjamin A Pierce)

**The various events of protein synthesis can be studied under the following heads:**

1. An mRNA sequence is decoded in set three nucleotides: triple codon coding described above tRNA molecules match amino acids to codon in mRNA

2. amino acylation of tRNA (formation of aminoacyl-tRNA)
   a. activation of amino acids: Each aminoacid before its attachment with its specific tRNA is activated by a specific activating enzyme known as aminoacyl synthetase and ATP. The free aminoacids react with ATP, resulting in the production of aminoacyl adenylate and pyrophosphate. This aminoacyl adenylate or aminoacylAMP is bound to the enzyme in the form of a monocovalent bond. This enzyme complex then esterifies to specific tRNA molecule.

   b. Attachment of activated aminoacid to tRNA: The aminoacylAMP collide with the specific tRNA molecule and its synthetase is recognised by dihydrouridine (DHU) loop of specific tRNA. As a result AMP and enzyme are released and a final product aminoacyl tRNA is formed. It is the only stepin protein synthesis in which the identity of the aminoacid ( ie. R group) plays a part.

3. Aminoacids are added to the C terminal end of a growing poly peptide chain: a polypeptide chain grows by the stepwise addition of aminoacids to its C terminal end. The formation of each peptide bond is energetically favourable because the C terminus has been activated by the covalent attachment of tRNA molecule. The peptidyl tRNA linkage that activates the growing end is regenerated during each addition.
4. Translation ends when stop codon encountered: proteins called release factors bind to the ribosome. E coli has three release factors, $RF_1$, $RF_2$ and $RF_3$. $RF_1$ recognize the termination codon UAA and UAG and $RF_2$ recognizes UGA and UAA. $RF_3$ forming complex with GTP and bind to the ribosome. It then promotes the cleavage of tRNA in the Psite from the poly peptide chain.

**Properties of the three initiation factors IFs of e.coli ( prokaryote)**

| Factors | Mass(Dalton) | Functions |
|---|---|---|
| $IF_1$ | 8100 | Stimulates activity of$IF_2$ and $IF_3$. Increases the affinity of the 30s subunit for the factors. |
| $IF_2$ | 97300 | KINETIC effector of 30s initiation complex formation. Favours binding of aminoacyl Trnas with blocked $\alpha NH_2$ groups. Positioning of fMet tRNA $^{fMet}$ in P site ribosome. |
| $IF_3$ | 20700 | Kinetic effector of 30S initiation complex formation. Ensuring provision of free 30S ribosomal subunit for initiation. |

**Properties of ten initiation factors (eIFs) of reticulate cell ( an eukaryotic cell)**

| S No: | Factors | Structure & mass (Dalton) | functions |
|---|---|---|---|
| 1 | eIF3 | Multimer, 7500000 | Binding mRNA |
| 2 | eIF4F | Multimer, 200000 | Binding mRNA 5’ end; unwinding |
| 3 | eIF1 | Monomer, 15000 | Assists mRNA binding |
| 4 | eIF4B | Monomer, 15000 | Assists mRNA binding and unbinding |
| 5 | eIF4A | Multimer, 15000 | Assists mRNA binding, binds ATP |
| 6 | eIF6 | Monomer,23000 | Prevents 40S – 60S joining |
| 7 | eIF5 | Monomer, 150000 | Releasing eIF2 & eIF3 |
| 8 | eIF4C | Monomer, 15000 | Binding 60S subunits |

| | | | |
|---|---|---|---|
| 9 | eIF2 | Trimer containing 3 chains<br>αChain – 35000<br>β Chain – 38000<br>γ Chain – 55000 | Binding Met tRNA<br>Binds to GTP<br>Recycling factor<br>Binds to Met tRNA |
| 10 | eIF4D | Monomer | Unknown |

| DIFFERENCES BETWEEN PROKARYOTES AND EUKARYOTES IN THE MECHANISMS OF TRASLATION | | |
|---|---|---|
| **S No:** | **Prokaryotes** | **Eukaryotes** |
| **I** | Initiating aminoacid methionine needs to be formylated (due to this reason there are present two tRNAs for methionine i.e. $tRNA^{fMet}$ & $tRNA^{Met)}$. | Initiating aminoacid, methionine is not formylated (this occur only one tRNA for methionine, i.e. $tRNA^{Met}$). |
| **2** | Ribosomes enter the mRNA at AUG codon or at nearby Shine Delgarno site. | Ribosomes enter at the capped 5' end of mRNA and then advance to AUG CODON BY linear scanning |
| **3** | No initiation factors needed for the initial contact between ribosomes and mRNA | ATP and many protein factors are needed for ribosomes to engage the mRNA |
| **4** | Small 30S ribosomal subunit can engage mRNA before binding of initiator Met $tRNA^{fMet}$. | Small 40S ribosomal subunits bind stably to mRNA only after initiator Met $tRNA^{Met}$ has bound to it. |

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4. **CELL BIOLOGY, GENETICS, MOLECULAR BIOLOGY, EVOLUTION AND ECOLOGY; 10th edition; Page 280 – 292 and 3/ 77 – 90; P S Verma; V K Agarwal.**

5. **GENETICS; 3RD Edition; Page 540 – 562; Monroe W Strickberger.**