CLASS XII BIOLOGICAL SCIENCES (D.S)

PROTEIN SYNTHESIS machinery consists of ribosomes, amino acids, mRNA, tRNAs & amino acyl synthetases. mRNA functions as a template having genetic information. Ribosome is the site of protein synthesis. tRNA brings the desired amino acid, reads the genetic information & places the amino acid at proper place. RNAs are formed over DNA during transcription, while protein synthesis occurs in the cytoplasm over ribosomes.

TRANSLATION : This step involved the translation of the language of nucleic acids (available in the form of mRNA) into the language of proteins. This part is much more complex than transcription, & involves the following steps:

1. **ACTIVATION OF AMINO ACID :** It is carried out by activating enzymes, known as <u>amino acyl tRNA synthetase</u> In the presence of ATP, an amino acid combines with its specific amino acyl – tRNA synthetase (amino acyl RNA synthetases are specific with respect to amino acids) Mg^{2+} is required. It produces amino-acyl adenylate-enzyme complex [AA (amino acid) + ATP + E (amino acyl tRNA synthetase) $\xrightarrow{Mg^{2+}}$ AA–AMP–E + PPi (Pyrophosphate)]

(Hydrolysis of pyrophosphate with the help of enzyme pyrophosphatase provides energy for driving the initial reactions).

2. **CHARGING OR AMINOACYLATION OF tRNA :** The Amino-acyl adenylate enzyme complex reacts with tRNA specific for the amino acid to form amino acyl – tRNA complex. Enzymes

and AMP are released. tRNA complexed with amino acid is sometimes called charged tRNA.

3. INITIATION : It requires factors called initiation factors. There are 3 initiation factors in procaryotes — IF3, IF2 & IF1. Encaryotes have 9 initiation factors — eIF2, eIF3, eIF1, eIF4A, eIF4B, eIF4C, eIF4D, eIF5, eIF6.

The initiating methionine accepting tRNA is charged with non-formylated methionine ($tRNA_m^{Met}$) in the cytoplasm of eucaryotes & formylated methionine ($tRNA_f^{Met}$) in procaryotes. tRNA engaged in transferring formylated methionine is different from the one that transfers non-formylated methionine.

The initiation of polypeptide chain in procaryotes is always brought about by the amino acid methionine, which is regularly coded by the codon AUG, but rarely also by GUG as the initiating codon. [The methionine molecule carried by $tRNA_m^{met}$ can't be formylated, while that carried by $tRNA_f^{met}$ can be formylated].

In procaryotes, formylation of initiating amino acid methionine is essential requirement, so that $tRNA_f^{met}$ is meant for depositing methionine as the first amino acid, while $tRNA_m^{met}$ deposits methionine at the intercalary positions only.

In encaryotes, formylation of initiating methionine is not brought about due to the absence of $tRNA_f^{met}$ in plants, & due to the absence of enzyme transformylase in animals. Initiation in higher organisms will, therefore, take place

(2) without formylation.

Initiation in procaryotes involves the foll. steps :

(i) The binding of methionine with $tRNA_f^{met}$. Later met - $tRNA_f^{met}$ undergoes formylation with the help of transformylase enzyme in the presence of a formate source (formyl - tetrahydrofolic acid) ⟶ formation of formyl-methionyl $tRNA_f^{met}$ (f - met $tRNA_f^{met}$).

(ii) Ribosomes are found dissociated into subunits (30S & 50S) while not taking part in protein synthesis. The 30S subunit attaches at the 5' end of mRNA carrying AUG codon, that is facilitated by an initiation protein factor IF3 [IF3 - 30S - mRNA].

(iii) Binding of f - met - $tRNA_f^{met}$ with IF3 - 30S - mRNA complex, with the help of initiation factor IF2. [IF3 - 30S - mRNA - IF2 - f - met - $tRNA_f^{met}$].

(iv) The initiation complex formed in step (iii) then associates with 50S subunit to form a complete ribosome, that requires initiation factor IF2. Coming together of the two subunits of ribosomes is called <u>association</u>. The intact ribosome encloses the mRNA - tRNA complex present at the P-site but keeps the A-site exposed.

Initiation of translation in eucaryotes is similar to that of procaryotes except the following minor differences -

(a) In eucaryotes, there are nine initiation factors instead of three in procaryotes, which are distinguised by putting a prefix e to signify their eucaryotic origin. Of these eIF2 & eIF3 are analogous to IF2 & IF3 of procaryotes.

(b) In eucaryotes, formylation of methionine, does not take place.

(c) In eucaryotes, smaller subunit (40S) of ribosome associates with initiator tRNA known as $tRNA_f^{met}$ (because methionine is not formylated) without the help of mRNA, while in procaryotes only 30S – mRNA complex can associate with f-met – $tRNA_f^{met}$.

4. Elongation of polypeptide: This is brought about by the regular addition of amino acids by the following 3 steps:

(i) Each ribosome has 2 cavities, in which tRNA can be inserted. These are 'P' site (peptidyl site) & 'A' site (amino acyl site). f-met – $tRNA_f^{met}$ has to be present on 'P' site, to make 'A' available for the next amino acyl tRNA (AA-tRNA). After 50S subunit joins the initiation complex, the first amino acyl tRNA enters 'A' site. Factor responsible for this entry was originally called T Factor or TF1 (transfer factor). Later this was separated into two components, the elongation factor EF-Tu (unstable when heated) & EF-TS (S means stable when heated). Proper binding of AA-tRNA at site 'A' of ribosome occurs only in the presence of EF-Tu.

(ii) Formation of peptide bond: during which a peptide bond is formed between the free carboxyl group ($-COOH$) of the peptidyl tRNA at the 'P' site & the free amino ($-NH_2$) group present with amino acyl tRNA at the 'A' site. The enzyme involved in this reaction is peptidyl transferase. After the formation of peptide bond, the tRNA at 'P' site is deacylated & the tRNA at 'A' site now carries the polypeptide.

(iii) The peptidyl tRNA present at 'A' site is now translocated to 'P' site, during which, deacylated tRNA is liberated from 'P' site. The next amino acyl tRNA now occupies the 'A' site (in the form of AA-tRNA-EF-Tu-GTP complex) & the cycle continues till the ribosome comes across a termination codon.

5. Termination of polypeptide : Termination of polypeptide chain is brought about due to the presence of one of the three terminating codons, namely UAA, UAG & UGA. These terminating codons are recognized by one of the two release factors, RF1 & RF2, which are stimulated by RF3. In eucaryotes, only one release factor eRF1 is known. These release factors help in splitting of the carboxyl end of polypeptide & the last tRNA carrying the chain. The polypeptide is thus released in the cytoplasm, & the ribosome dissociates into two subunits, with the help of the initiation factor, IF3. The two subunits of ribosome separate or undergo dissociation in the presence of dissociation factor (DF).

—

Please note : students are requested to do the project work in any file (covered with any paper) in brief, but with adequate pictures.

—