TRANSLATION.

It is the process by which the base sequence of mRNA is translated into amino acid sequence of protein

OR

The process of protein synthesis based on the information present in mRNA.

→ The language of nucleotide translated into the language of amino acids.

Genetic code. (6 mark)

→ Informations present in mRNA is used for synthesis proteins by translation.

→ These informations are present in mRNA as triplet nucleotide sequence is called codons.

→ Arrangement of codons in mRNA is called genetic code.

→ Each codon codes for one aminoacid.

→ Alterations in the nucleotide sequence of codon will result in incorporating a wrong amino acid into the polypeptide being synthesised.

→ The codons are on the mRNA.

→ Each codon is a consecutive sequence of three bases on the mRNA - triplet code.

UUU - phenylalanine.
AUG - Methionine.

→ There are only 4 bases (A, G, C, U)

→ Each codon consists of 3 nucleotide bases.
∴ total 4³ different codons
i.e. 64 codons.

Among the 64 codons, 3 are stop codons.
(terminator codons — 1 mark
UAG, UAA, UGA

→ Remaining 61 codons represent 20 amino acids.

Features of genetic code

(1) Triplet codons.

Each codon is a consecutive sequence of three nucleotide bases on the mRNA

UUU - phenylalanine
AUG - Methionine.

② Universal.

the codons are the same for the same amino acid in all the species.

" E coli to Elephant "

③ Specific (Unambiguous)

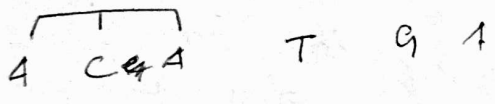
1 codon always code for the same amino acid.

UUU - phenylalanine

AUG - Methionine.

④ Genetic code is non-overlapping, continuous and non-punctuated

codons are read one after another



→ The codons are read in a continuous manner from 5' → 3'.

→ Genetic code is non-punctuated There is no coma or separation in between

⑤ Degeneracy (1 mark)

→ Some aminoacids have more than one codon. This is called degeneracy of codons.

→ Difference between these codons usually will be on the 3rd base (wobbled base)

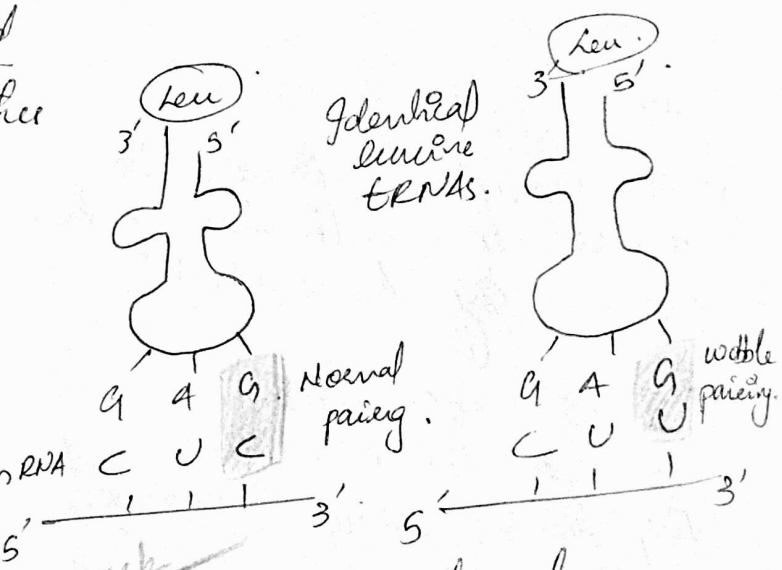
of the triplet sequence

Eg: Codon for Phe are UUU and UUC.

⑥ Wobbling Hypothesis

→ The base that fit in the 3rd position of the codon is termed as wobbled base.

→ When tRNA pairs with mRNA, there is a reduced stringency between the third base of the codon and the complementary base in the anticodon - WOBBLING.



1 mark

→ Degeneracy of codon and wobbling phenomenon will reduce the effect of mutations.

⑦ Initiator codon.

→ AUG is the initiator codon which codes for methionine in eukaryotes. In prokaryotes, it is N-formyl methionine.

(8) Terminator codons (Stop codons)

There are 3 codons which do not code for any aminoacid.

– UAG, UAA, UGA

→ Translation stops at area of mRNA where these codons are present. (terminator codons)

→ Also called as non-sense codons.

→ Under special circumstances, a stop codon may code for aminoacid.

Eg: UGA code for selenocystine.

→ In prokaryotes, translation starts as soon as transcription has finished since the newly synthesized mRNA is fully functional.

→ In eukaryote, hn RNA (primary transcript) has to undergo post transcriptional modification to form mature RNA. Then only it enters cytosol for translation.

Components required for Translation

1. Aminoacids.
2. tRNA
3. Aminoacyl tRNA synthetase
4. mRNA.
5. Ribosomes.
6. Protein factors
7. ATP & GTP

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(1) Aminoacids

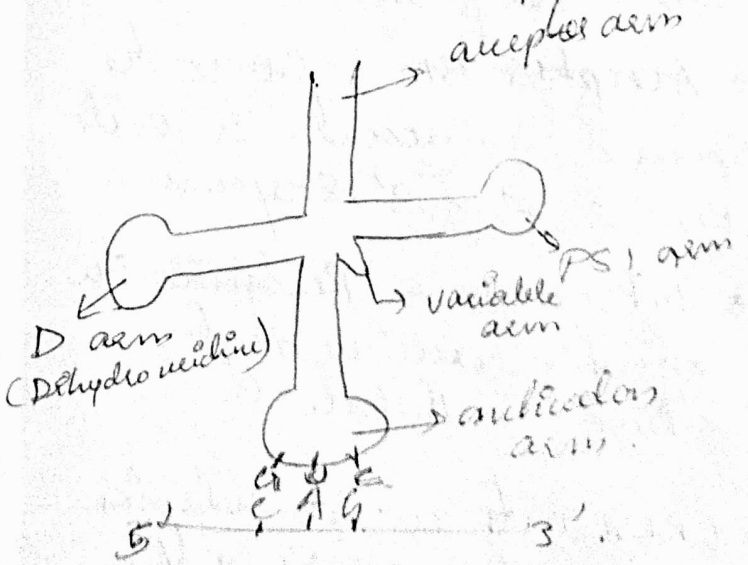
All the aminoacids required for synthesizing a protein should be present at the time of translation.

→ If one aminoacid is altered, translation stops at the codon for that aminoacid.

(2) Transfer RNA (tRNA)

Transfers amino acids from cytoplasm to ribosome for protein synthesis.

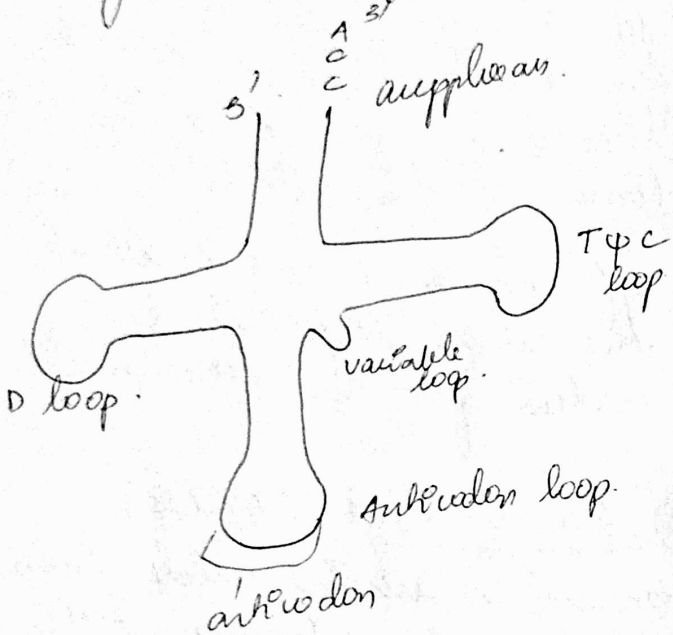
→ There is atleast one specific type of tRNA for each of 20 aminoacids in proteins.



tRNA structure

Clover leaf structure:
Due to extensive intrachain base pairing.

Contains unusual bases like pseudouridine (Ψ), Dihydrouracil, hypoxanthine.



→ Pseudouridine (TψC) arm - Binds tRNA to ribosome.

→ Anticodon arm - recognises the codon on mRNA

→ Acceptor arm - Binds the specific aminoacid - It ends in CCA - 3' sequence.

→ DHU arm - Recognises the specific amino acyl tRNA synthetase.

→ tRNA acts as an adaptor between the mRNA & the amino acid coded by it.

→ Specific tRNA is required for an aminoacid. Some aminoacids have more than on tRNA as they are coded by more than one codon.

→ Acceptor arm ends in CCA - 3' sequence.

→ Aminoacid binds to 3'OH of the adenosine of CCA sequence at the acceptor arm.

→ When the tRNA & attached to the aminoacid it is said to be charged.

→ The aminoacid attached to tRNA is said to be activated.

→ This attachment requires an enzyme - Aminoacyl tRNA synthetase.

~~Activ~~ (3) Aminoacyl tRNA synthetase :-

Enzyme required for activation of amino acid.

→ ATP dependent.

(4) mRNA.

→ mRNA carries the genetic message from DNA to the protein synthesising machinery on the ribosome.

→ genetic code is present in the structure of mRNA.

→ Nucleotide sequence of mRNA is translated into a a

→ Prokaryotic mRNA is polycistronic as a single mRNA codes for more than one protein.

→ Eukaryotic mRNA is monocistronic because it codes for a single polypeptide.

→ mRNA is translated (read) from 5' to 3' end and polypeptide chain is synthesized from N terminal to C terminal end.

(5) Ribosomes (Short Note)

→ Site of protein synthesis

→ Ribosome along with RER provide the platform for protein synthesis

→ Ribosomes are large complexes of protein and rRNA.

→ During translation, several ribosomes (polyribosomes) operate together on a single mRNA.

→ Site of protein biosynthesis

→ Actual sites are the ribosomes on RER in the cytoplasm (in eukaryote)

→ consists of 2 subunits
one large subunit
one small subunit

→ 3 molecules of ribosomal RNA (r-RNA) are present in prokaryote (5S, 16S, 23S)

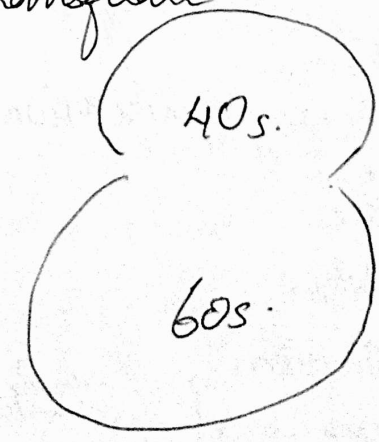
→ 4 molecules of ribosomal RNA (r-RNA) are present in eukaryote (5S, 18S, 28S, 5.8S).

1 mark

Peptidyl transferase → Ribozyme

Ribosomal RNA - rRNA

It act as a ribozyme (RNA with catalytic activity)
eg: peptidyl transferase.
(28S rRNA has peptidyl transferase activity).



Eukaryotes 80S.

Functions of Subunits.

→ Large subunit catalyses the formation of peptide bond on the protein.

→ Small sub unit binds mRNA

→ Ribosomal proteins play important role in structure and function of ribosomes.

⑥ Protein factors.

→ They act as initiation, elongation and termination factors.

→ Eukaryotic initiation factors (eIF)

→ Eukaryotic elongation factors (eEF)

→ Releasing Factors (RF)

→ stabilize translation process.

⑦ ATP & GTP.

→ Energy source.

STEPS IN TRANSLATION

I. Initiation

II. Elongation

III. Termination

• Post translational modifications

I. Initiation

2 steps:

1. Activation of amino acid
2. Formation of ribosomal assembly.

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① Activation of amino acid

Enzyme for amino acid activation —

Aminoacyl tRNA synthetase.

→ 2 high energy bonds are used for activation.

→ tRNA acts as an adaptor molecule b/w the aa and the corresponding codon in mRNA.

→ DHU arm of tRNA recognises the specific aminoacyl tRNA synthetase.

Amino acid + tRNA + ATP —(aminoacyl tRNA synthetase)→ Amino Acyl tRNA + AMP + PPi

② formation of ribosomal assembly

• Requires 4 specific steps:

- Ribosomal dissociation.
- Formation of Pre-initiation complex (43S)
- Formation of initiation complex (48S)
- Ribosomal assembly.

Requires many protein factors - Eukaryotic initiation factors (eIF)

a) Ribosomal dissociation.

→ 80s ribosome splits into 40s and 60s subunits

→ eIF-3 and eIF-1A bind to the 40s subunit

→ This prevent reassociation with 60s subunits & allows other initiation factors to bind with 40s.

b) Formation of 43s pre-initiation complex.

→ GTP with eIF-2 bind to Met-tRNA.

→ This complex of GTP, eIF-2, Met-tRNA. (Ternary complex) binds to 40s forming 43s pre-initiation complex.

→ eIF-3 and 1A stabilizes it.

c) Formation of 48s initiation complex.

→ mRNA cap facilitates binding of mRNA to 43s pre initiation complex (4F = 4A, 4B & 4E)

→ A protein complex called eIF-4F binds to the cap.

→ This complex helps PIC (pre initiation complex) to scan mRNA for a suitable initiator codon.

→ First AUG after the marker sequence is taken as initiator codon.

eIF-4F complex.

→ eIF-4F complex consists of eIF-4A, eIF-4G and eIF-4E.

→ Additional protein factors are also present such as eIF-4B, PABP (poly A tail binding protein).

→ eIF-4E binds to the 5' cap of mRNA

→ eIF-4A binds to 5'UTR (Untranslated region) and has helicase activity.

→ eIF-4A along with 4B reduces the complex secondary structure of 5' end of mRNA through ATP dependent helicase activity

→ PABP binds to poly A tail.

→ Binding of PABP to poly A tail will result in circularisation of mRNA with the help of eIF-4G (a scaffolding protein), forming a circular loop structure.

i.e. PABP binds to poly A tail and interacts with eIF-4G, which in turn binds to eIF-4E attached to the 5' cap.

→ So 5' cap and poly A tail are brought close and this will enhance the initiation of translation, which help in recruiting 40S subunit to mRNA

Rate of Initiation

→ Regulation of eIF-4E controls the rate of initiation.

→ eIF-4E is responsible for the recognition of mRNA cap which is considered to be the rate limiting step in translation.

Recognition of Translation Site - Marker sequence. (4 mark)

→ First AUG after the marker sequence is taken as initiator codon.

→ In prokaryotes, the marker sequence is purine rich, known as Shine-Dalgarno sequence (1 mark)

→ It is located 6-10 bases upstream of the initiator codon. It helps in binding of 30S ribosomal subunit to mRNA

→ In eukaryotes, the marker sequence is known as Kozak sequence (1 mark).

→ It helps in binding of 40S subunit to mRNA

→ In eukaryotes, the initiator AUG is recognized by a Met-tRNA.

→ In prokaryote and in mitochondria, this is N-formyl Met-tRNA.

→ ~~Thus~~ This charged Met-tRNA, in eukaryotes, enters the P-site of ribosomal assembly

d) Formation of 80S initiation complex (Ribosomal assembly)

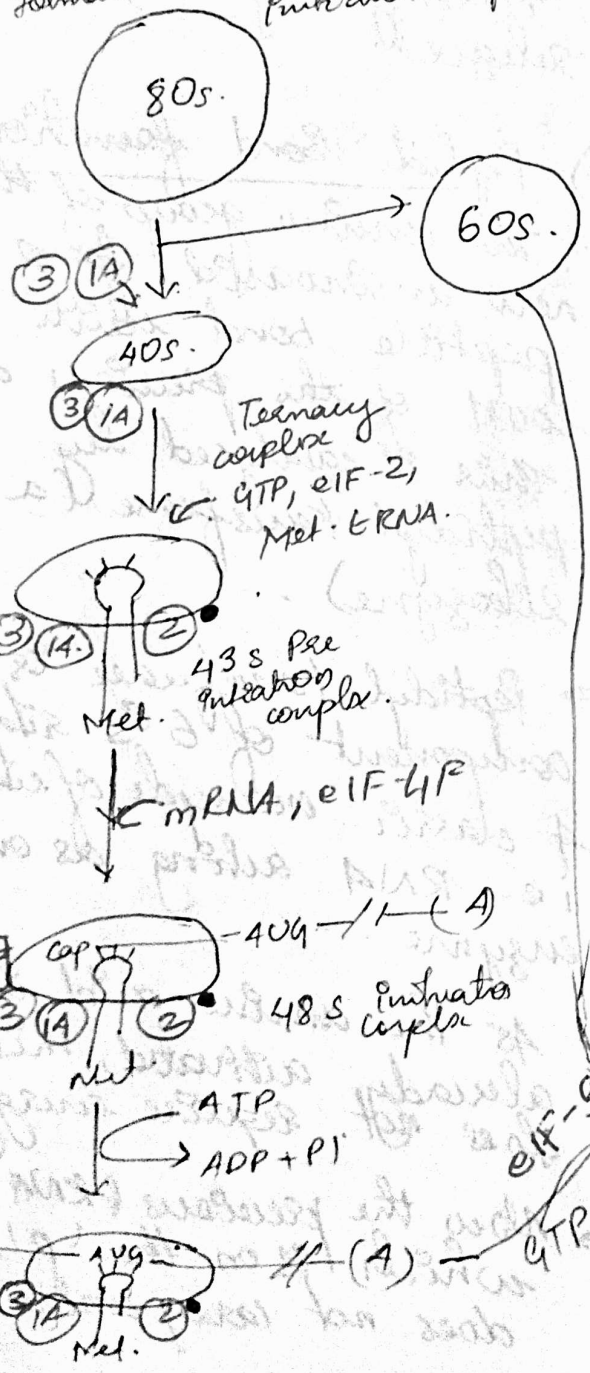
→ 60S ribosomal subunit binds to 48S initiation complex with the help of eIF-5 (a GTPase)

→ eIF-5 hydrolyses GTP attached to eIF-2 and this energy is utilised for attaching 60s subunit to 40S subunit.

→ All initiation factors bound to 48s are released.

→ 40s and 60s sub unit assemble to form 80s ribosomal assembly.

Formation of the 80S initiation complex.



→ Once the ribosomal association occurs, all initiation factors are released.

→ As a result, circularisation of mRNA is also converted to linear form.

→ And translation continues through elongation.

11. Elongation.

3 steps:

- A) Binding of new amino acyl tRNA
- B) Peptide bond formation
- C) translocation.

Binding site on ribosome.

A site: binds incoming aminoacyl tRNA.

P site: carries tRNA with growing peptide chain.

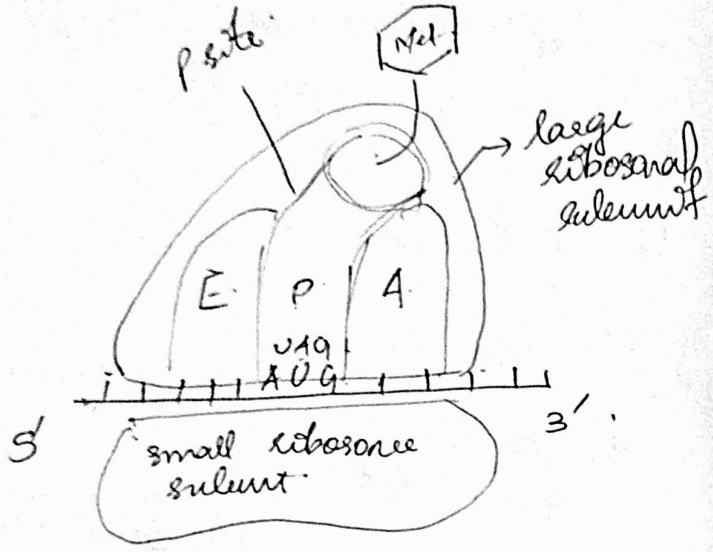
E site: empty tRNA about to exit from ribosome.

→ A ribosome has an mRNA binding site and three tRNA

binding sites.

→ The tRNA binding sites are A, P, E:

- A: aminoacyl-tRNA.
- P: peptidyl-tRNA.
- E: exit.



~~Too~~ → mRNA is translated from 5' to 3' end & polypeptide chain is synthesized from N terminal to C terminal end.

→ 1st amino acid incorporated will be the N terminal.

→ The last amino acid added will be the C terminal.

→ Once the process of translation is initiated, the polypeptide chain is expanded in length by sequential addition of amino acids.

1) Binding of new amino acyl-tRNA to 'A' site.

→ The codon after the initiator codon decides which aminoacyl tRNA is to be added next.

→ The new aminoacyl-tRNA comes to the 'A' site: This requires elongation factor EF-1 and GTP.

→ GTP hydrolysed; EF-1 released.

B) Peptide Bond formation.

→ The amino group of the new aminoacid forms a peptide bond with the COOH of the previous aa. This is catalysed by peptidyl transferase (a ribozyme).

→ Peptidyl transferase is a component of 60S ribosome. A classic example of ribozyme i.e. RNA acting as an enzyme.

→ As the amino acid is already activated, this does not require energy.

→ Now the previous tRNA, which is on the 'P' site does not carry amino acid.

c) Translocation

→ Ribosome moves in 5' ⟶ 3' direction of the mRNA, to the next codon.

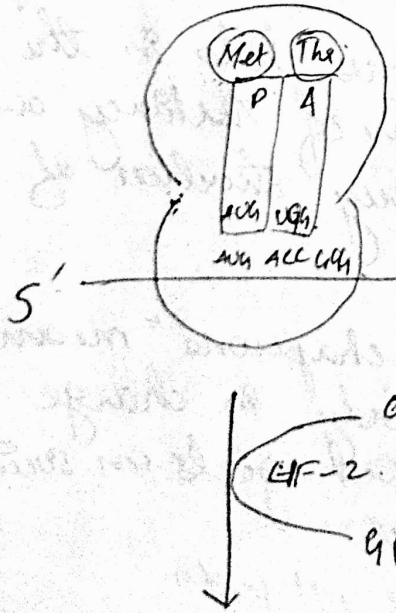
→ Now the empty tRNA reaches the 'E' site and is expelled from ribosome.

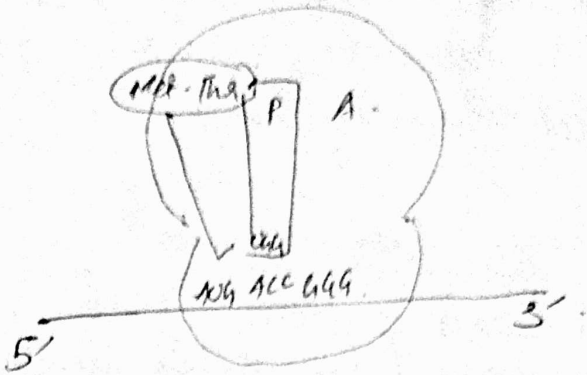
→ tRNA which carries the growing peptide reaches the 'P' site.

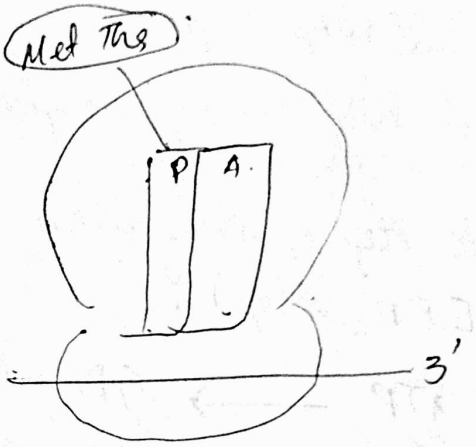
→ New incoming aminoacyl tRNA as guided by the next codon comes to the 'A' site.

→ This requires EF-2 and GTP.

→ Elongation proves continuation till the polypeptide chain synthesis is completed or a stop codon is reached.







III Termination

→ When the A site of ribosome reaches a terminator codon sequence in mRNA, translation stops.

→ eRF recognises terminator codon (In prokaryote, it is by RF-1 and RF-2).

→ eRF induces peptidyl transferase to hydrolyse the polypeptide from tRNA, which is GTP mediated.

→ mRNA is freed from the ribosome.

→ 80S ribosomal assembly

dissociates into 60S and 40S subunits, which are later re-cycled.

Energy Requirements

for each peptide bond formation, 4 high energy phosphate bonds are used.

- Amino acid activation step.

1 ATP → AMP + PPi

2 high energy phosphate bonds.

- First step of elongation (eEF1 step).

1 GTP ⟶ GDP + Pi

1 high energy phosphate bond.

- Translocation (eEF_2 - step).

1 GTP ⟶ GDP + Pi

1 high energy phosphate bond.

In addition to this,

- 1 ATP for ~~form~~ formation of 43S initiation complex.
- 1 GTP for formation of 80S ribosomal assembly
- 1 GTP for termination.

Protein folding, the final step

→ Newly synthesized polypeptide chain is folded and processed into its biologically active form (higher orders of structure).

→ As the polypeptide is being synthesized by ribosome, the initial segment of protein starts to fold.

→ This initiates favours folding of other parts of protein

→ Hydrophobic regions aggregate into the interior of protein molecule.

disease associated with abnormal protein : MAD COW DISEASE

Chaperons

→ Chaperons, the heat shock proteins will help in the correct folding of proteins

→ The attach to nascent polypeptide chains and prevent wrong foldings.

→ So, folding is allowed in correct direction only.

→ Chaperones help in the assembly of tertiary and quaternary structure of proteins.

→ Word "chaperone" means elderly lady in charge of unmarried girls on social occasions.

eg: Hsp 60, Hsp 70

Inhibitors of protein synthesis

In prokaryotic

1. Tetracyclins: Bind with 30S ribosomal unit and inhibit attachment of amino acyl tRNA to A site of ribosome.
2. Chloramphenicol: Inhibit peptidyl transferase activity of bacteria.
3. Erythromycin: Inhibits translocation.
4. Streptomycin: Binds to 30S subunit, prevents the complex formation.

In eukaryote

1. Puromycin: Structurally similar to tyrosine-tRNA inhibits peptide chain elongation.
2. Cycloheximide: Inhibit peptidyl transferase in 60S ribosomes.
3. Diphtheria toxin: Inactivates (eukaryotic) eEF2 and blocks translocation.

Post translational modifications

- Proteolytic cleavage
- Modification of amino acids
  - Hydroxylation
  - Gamma carboxylation
  - phosphorylation
  - Glycosylation
  - Acetylation
- Subunit aggregation
- Protein folding

→ Most of the post translational modifications occur in Golgi complex and endoplasmic reticulum.

1. Proteolytic cleavage

Many proteins are converted into further proteins after proteolytic cleavage.

a) Pepsinogen ⟶ Pepsin
b) Proinsulin ⟶ Insulin

2. Modification of amino acids

Hydroxylation:
hydroxylation of proline and lysine residues in the formation of mature collagen.

Gamma carboxylation:
gamma carboxylation of glutamic acid residues of clotting factors II, VII, IX and X. Vitamin K plays an important role in this.

Phosphorylation:

→ Phosphorylation of hydroxy groups in glycogen phosphorylase enzyme makes it active.

→ Phosphorylation of hydroxy groups in Glycogen synthase enzyme makes it inactive. i.e. phosphorylation and dephosphorylation makes protein either active or inactive.

Glycosylation:

→ carbohydrate side chains are added to proteins to form glycoproteins.

eg: Formation of glycated hemoglobin - HbA1c.

Acetylation.

→ In some proteins, N-terminal aminoacids are acetylated after translation.

3. Subunit Aggregation

Various polypeptides or a protein aggregate into a subunit for a functional protein.

eg. Synthesis of HbA (aggregation of 4 polypeptide chains)

formation of Immunoglobulin (aggregation of heavy and light chains)

Protein targetting

→ After synthesis on ribosomes, proteins are directed to their destined locations to exhibit their biological functions.

This is called protein targetting.

→ Proteins reach their target locations through ER and golgi complex embedded in transport vesicles.

Protein targetting defect

eg: Primary hyperoxaluria